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Original article

Design, synthesis and biological evaluation of 4-fluoropyrrolidine-2-carbonitrile and octahydrocyclopenta[*b*]pyrrole-2-carbonitrile derivatives as dipeptidyl peptidase IV inhibitors

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ABSTRACT

Based on the previous work in our group and the principle of computer-aided drug design, a series of novel β-amino pyrrole-2-carbonitrile derivatives was designed and synthesized. Compounds **8l** and **9l** were efficacious and selective DPP4 inhibitors resulting in decreased blood glucose *in vivo*. Compound **8l** had moderate DPP4 inhibitory activity (IC_{50} = 0.05 μM) and good oral bioavailability (F = 53.2%). Compound **9l** showed excellent DPP4 inhibitory activity (IC_{50} = 0.01 μM), good selectivity (selective ratio: DPP8/DPP4 = 898.00; DPP9/DPP4 = 566.00) against related peptidases, and good efficacy in an oral glucose tolerance tests in ICR mice and moderate PK profiles (F = 22.8%, $t_{1/2}$ = 2.74 h). Moreover, compound **9l** did not block hERG channel and exhibited no inhibition of liver metabolic enzymes such as CYP2C9.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease that is usually accompanied by the complications, such as cardiovascular disease, blindness, kidney disorders and amputation [1]. Current therapeutic strategies, including reducing insulin resistance using glitazones [2], increasing insulin secretion with sulfonylureas [3], and reducing hepatic glucose output with biguanides [4], are associated with undesirable side effects including hypoglycemia, β-cell apoptosis, and other gastrointestinal tract effects. Therefore, discovery of safe and effective agents to treat T2DM becomes more important. Evidence from numerous of studies shows that dipeptidyl peptidase IV (DPPIV, DPP4, also known as CD26) inhibitors represent a novel approach for treatment T2DM [5].

DPP4 is an ubiquitous yet specific serine protease that cleaves *N*-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position [6]. It exists as both a membrane-bound protein and a soluble protein in plasma [7]. The most important function of DPP4 is inactivation of glucagon-like peptide-1 (GLP-1) *in vivo*. GLP-1 (7–36) is an important incretin that regulates blood glucose levels [8]. After ingestion of nutrients, it is released from *L*-cells to stimulate insulin secretion and biosynthesis [9], inhibits glucose release [10], and delays gastric emptying, resulting in a reduced appetite [11]. However, active GLP-1 (7–36) can be rapidly degraded by DPP4, which cleaves the *N*-terminal two amino acids

Abbreviations: DPP4, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide-1; FAP, fibroblast activation protein; T2DM, type 2 diabetes mellitus; SARs, structure–activity relationships; OGTTs, oral glucose tolerance tests; PK, pharmacokinetics; AUC, area under curve; ICR, institute of cancer research; KKAy mice, the yellow obese gene (A^y) into mice.

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to generate the inactive GLP-1 (9–36). Hence, restoration of GLP-1 activity by inhibition of DPP4 represents a new approach for the treatment of T2DM.

Recently, several DPP4 inhibitors [12–20] such as **1** (sitagliptin, MK-0431) [21], **2** (vildagliptin, LAF-237) [22], **3** (saxagliptin, BMS-477118) [23], **4** (alogliptin, SYR-322) [24], and **5** (linagliptin, BI-1356) [25] (Fig. 1) have been approved. These agents have been demonstrated to be capable to lower glucose and HBA_{1c} levels and to improve glucose tolerance in type 2 diabetic patients [26]. In our group, we had presented aromatic substituted α-amino pyrrole-2-carbonitrile derivatives as DPP4 inhibitors [27]. And we found compound **6** showed excellent DPP4 inhibitory activity and good oral glucose tolerance tests (OGTTs) efficacy in institute of cancer research (ICR) mice and KKAy mice (the yellow obese gene (A^y) into mice). However, compound **6** showed moderate oral bioavailability. Therefore, compound **7** was designed and synthesized, and showed excellent DPP4 inhibitory activity and selectivity and good OGTTs efficacy in ICR mice. Moreover, the oral bioavailability was increased [28]. Herein, we report the other modification results based on compound **6**, which was design, synthesis, structure–activity relationships (SARs), OGTTs and pharmacokinetics (PK) evaluation of compound **9l** as a novel, potent, and selective DPP4 inhibitor for treatment of T2DM.

2. Results and discussion

2.1. Chemistry

2.1.1. Design of pyrrolidine-2-carbonitrile compounds

We had presented aromatic substituted α-amino pyrrole-2-carbonitrile derivatives as DPP4 inhibitors [27]. And we found compound **6** showed excellent DPP4 inhibitory activity and good OGTTs efficacy in ICR mice and KKAy mice. However, compound **6** showed moderate oral bioavailability. Therefore, the further modification was carried out, and compound **7** showed excellent DPP4 inhibitory activity and selectivity and good efficacy in OGTTs in ICR mice. Moreover, the oral bioavailability was increased [28]. Besides, the other strategy was carried out by introduction of β-amino group based on compound **6**. Moreover, 3-amino-4-phenylbutanoic acid moiety as predominant fragment was found in launched drugs, such as sitagliptin [21]. Therefore, a series of novel β-amino pyrrolidine-2-carbonitrile derivatives **8** was designed (Fig. 2). From the SARs of β-amino 4-fluoropyrrolidine-2-carbonitrile derivatives, compound **8l** showed moderate DPP4 inhibitory activity, however, the selectivity against DPP8/9 was very low. Therefore, a further design and modification was carried out.

From the binding mode of compound **8l**, the 4-fluoropyrrolidine-2-carbonitrile moiety occupied the S2 pocket and the nitrile group could form a hydrogen bond with residue Tyr547 *via* a water molecular bridge, and the nitrogen atom of 4-fluoropyrrolidine-2-carbonitrile moiety could form hydrogen bonds with residues Glu205 and Glu206 *via* water molecular bridge (Fig. 3a). However, the fluorine atom could not form any specific interaction with the enzyme. Furthermore, the S2 pocket still had more space to fill. Therefore, a further design and modification to enhance DPP4 inhibitory activities and selectivity was focused on the pyrrolidine-2-carbonitrile moiety. In addition, the 2-azabicyclo [3.1.0]hexane-3-carbonitrile fragment of saxagliptin [29] enhanced the inhibitory activity and chemical stability. Moreover, octahydrocyclopenta[*b*]pyrrole as the predominant fragment was found in launched drugs and active compounds, like ramipril [30] and retagliptin [18]. Based on these strategies, a five-cycle substituted pyrrolidine was introduced in subsequent design and modification (Fig. 2).

2.1.2. Synthesis of target compounds

Compounds **8a**–**m** were synthesized according to Scheme 1. Commercially available compound **10** was protected and then fluorinated with diethylaminosulfur trifluoride (DAST) to generate compound **12**. Compound **16** [31] was obtained from compound **12** *via* demethylation, amidation, dehydration, and deprotection, followed by a coupling reaction with *N*-Boc-β-amino acids (**17a**–**m**) to yield the compounds **18a**–**m**. Deprotection of **18a**–**m** with trifluoroacetic acid (TFA) produced the target compounds **8a**–**m**.

The synthetic route of compounds **9a**–**l** was shown in Scheme 2. The important intermediate **24** was generated from starting material **19** *via* protection, reduction, amidation, dehydration, and deprotection as previously described. Coupling compound **24** with various *N*-Boc-β-amino acids (**17a**–**l**) yielded compounds **25a**–**l**, which were deprotected with Et_2O–HCl to afford final compounds **9a**–**l**.

2.2. Biological evaluation

2.2.1. In vitro enzyme inhibition studies

All the synthesized compounds (**8a**–**m**) were evaluated *in vitro* for the capacity to inhibit DPP4 (Table 1). Due to the diversity of serine proteases, the inhibitory activities of the other members of the serine protease family (DPP7, DPP8, DPP9, and FAP) were also evaluated. Selectivity against DPP8/9 was of particular importance because inhibition of DPP8/9 had been associated with toxicity in animal studies [32]. As shown in Table 1, all the compounds (**8a**–**m**) demonstrated good DPP4 inhibitory activities

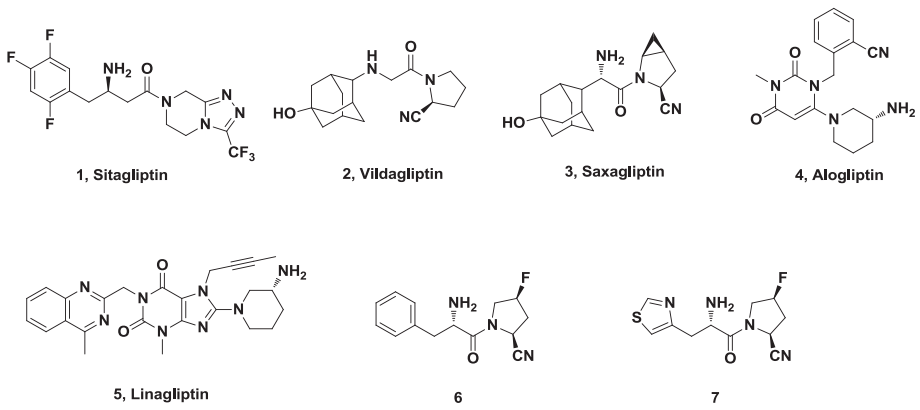


Fig. 1. Representative DPP4 inhibitors.

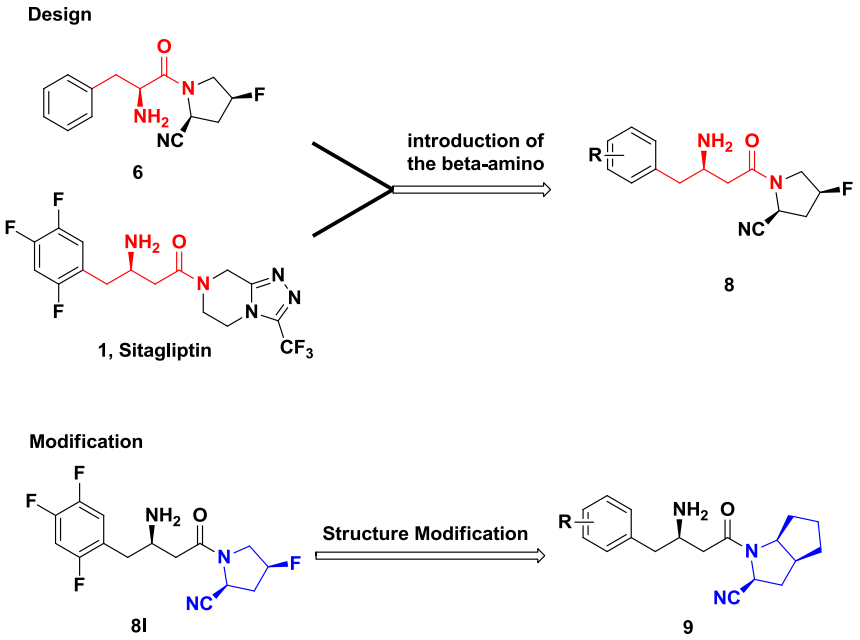


Fig. 2. Design and modification strategies of novel DPP4 inhibitors.

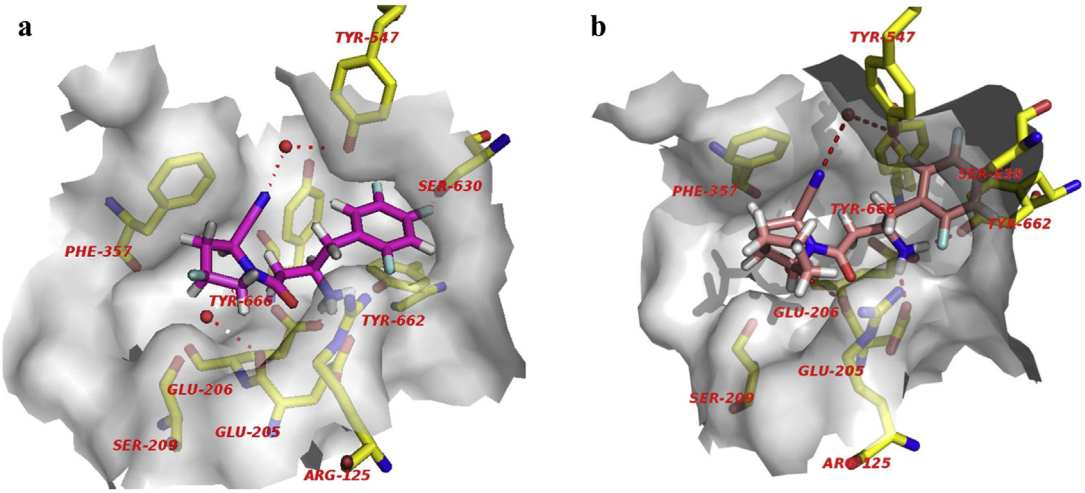
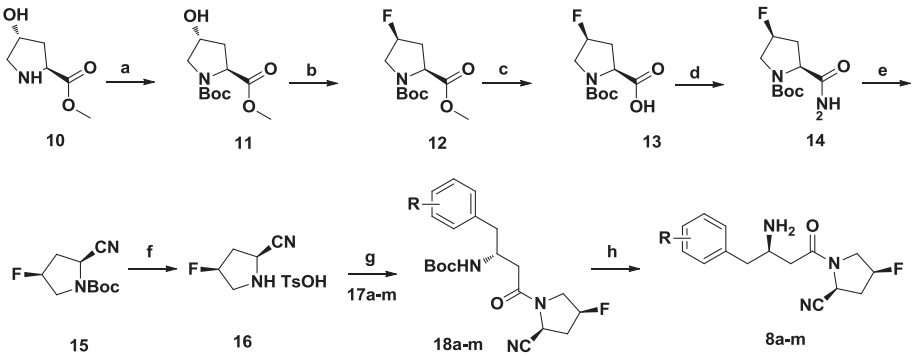
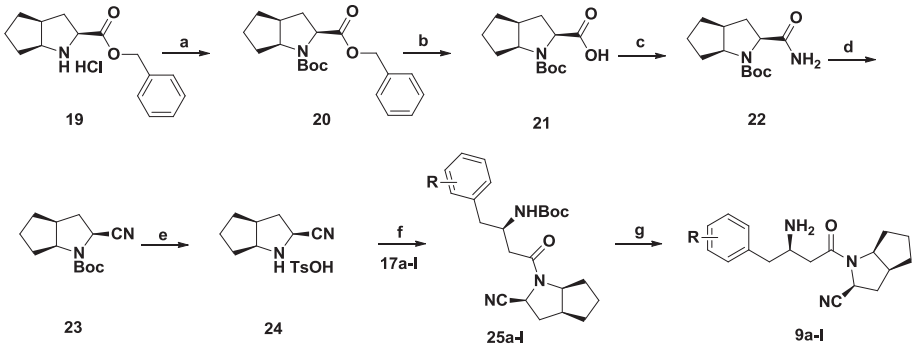


Fig. 3. Three-dimensional structural modes of inhibitors **8l** (a) and **9l** (b) to DPP4 (PDB ID: 2AJL) derived from the docking simulations. These two images were generated using the Pymol program.

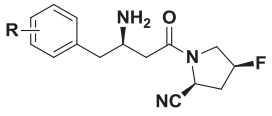


Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, $NaHCO_3$, dioxane, 24 h; (b) DAST, CH_2Cl_2, −78 °C to rt, 24 h; (c) LiOH, dioxane, H_2O, overnight; (d) $(Boc)_2O$, NH_4HCO_3, pyridine, dioxane, 6 h; (e) cyanuric chloride, DMF, 1 h; (f) TsOH, CH_3CN, rt, 24 h; (g) different group substituted *N*-Boc-β-amino acids, EDCI, HOBt, TEA, DMF, 20 h; (h) CH_2Cl_2, TFA, 0 °C to rt, 1 h.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, $NaHCO_3$, dioxane, 24 h; (b) Pd/C, H_2, CH_3OH, overnight; (c) $(Boc)_2O$, NH_4HCO_3, pyridine, dioxane, 6 h; (d) cyanuric chloride, DMF, 1 h; (e) TsOH, CH_3CN, rt, 24 h; (f) different group substituted *N*-Boc-β-amino acids, EDCI, HOBt, Et_3N, DMF, 20 h; (g) Et_2O–HCl, 0 °C to rt, 24 h.

Table 1
Inhibitory activities and selectivity of β-amino 4-fluoropyrrolidine-2-carbonitrile derivatives.



Compd.	R	IC_{50} (μM)[a]					SR[b]	
		DPP4	DPP7	DPP8	DPP9	FAP	DPP8/DPP4	DPP9/DPP4
8a	H	0.45 ± 0.01	NI[c]	0.60 ± 0.08	0.17 ± 0.04	5.90 ± 0.53	1.33	0.38
8b	2-Cl	0.22 ± 0.01	129.71 ± 24.4	0.49 ± 0.03	0.12 ± 0.02	3.12 ± 0.30	2.23	0.55
8c	2-Me	0.16 ± 0.01	NI	0.71 ± 0.17	0.03 ± 0.01	1.09 ± 0.09	4.44	0.19
8d	3-F	0.22 ± 0.02	59.10 ± 3.97	1.05 ± 0.09	0.62 ± 0.06	6.85 ± 2.14	4.77	2.82
8e	4-F	0.32 ± 0.01	192.79 ± 0.27	0.68 ± 0.05	0.19 ± 0.03	5.77 ± 1.05	2.13	0.59
8f	4-I	0.43 ± 0.03	9.55 ± 0.85	0.64 ± 0.10	0.14 ± 0.02	2.73 ± 0.37	1.49	0.33
8g	4-CF_3	0.64 ± 0.03	ND[d]	ND	ND	ND	/	/
8h	4-OMe	0.77 ± 0.05	ND	ND	ND	ND	/	/
8i	**2,4-*di*-Cl**	**0.04 ± 0.00**	**31.80 ± 2.20**	**0.67 ± 0.22**	**0.04 ± 0.01**	**1.69 ± 0.20**	**16.75**	**1.00**
8j	3,4-*di*-Cl	0.53 ± 0.05	9.34 ± 0.75	0.19 ± 0.04	0.23 ± 0.03	4.83 ± 1.46	0.36	0.43
8k	3,5-*di*-F	1.58 ± 0.05	ND	ND	ND	ND	/	/
8l	**2,4,5-*tri*-F**	**0.05 ± 0.01**	**50.62 ± 6.04**	**1.01 ± 0.11**	**0.33 ± 0.04**	**3.38 ± 0.12**	**20.20**	**6.60**
8m[e]	H	1.94 ± 1.11	ND	ND	ND	ND	/	/
MK-0431	/	0.02 ± 0.00	207.35 ± 6.04	67.72 ± 10.3	67.41 ± 9.3	30.39 ± 2.94	3386.00	3370.50
LAF-237	/	0.07 ± 0.00	>100	1.96 ± 0.23	0.20 ± 0.03	3.72 ± 0.31	28.00	2.86

[a] Mean values of at least two experiments.
[b] Selectivity Ratio (SR) = DPP8 IC_{50}/DPP4 IC_{50} and DPP9 IC_{50}/DPP4 IC_{50}.
[c] No inhibition.
[d] Not detected.
[e] The amino is *S* configuration.

(IC_{50} = 0.04–1.94 μM). Compound **8a**, which was non-substituted at the phenyl ring, showed moderate inhibitory activity against DPP4 (IC_{50} = 0.45 μM). In order to improve the DPP4 inhibitory activities *in vitro*, electron-withdrawing and electron-donating groups were introduced at the *ortho*-, *meta*-, and *para*-positions of the benzene ring. Introduction of chloro group (**8b**, IC_{50} = 0.22 μM), methyl group (**8c**, IC_{50} = 0.16 μM), and fluoro group (**8d**, IC_{50} = 0.22 μM and **8e**, IC_{50} = 0.32 μM) at the *ortho*-, *meta*-, and *para*-positions of the benzene ring resulted in slightly improvement in DPP4 inhibition (2.0-, 2.8-, 2.0-, and 1.4-fold, respectively). However, introduction of large groups at the *para*-position, the inhibitory activities of compounds **8f**–**g** were equipotent or reduced compared to compound **8a**. For example, compound **8f** (IC_{50} = 0.43 μM) which contained an iodo group at the *para*-position was equipotent with compound **8a**, and compounds **8g** (IC_{50} = 0.64 μM) and **8h** (IC_{50} = 0.77 μM) containing trifluoromethyl group and methoxyl group at the *para*-position resulted in reduced potency (1.4-fold and 1.7-fold, respectively). This indicated that introduction of a small group appeared to be essential for the *para*-position substituted compounds. For the *di*-substituted compounds, compound **8i** (IC_{50} = 0.04 μM), which was *di*-chloro substituted at the *ortho*- and *para*-positions showed 11.3-fold improvement in DPP4 inhibitory activity, and the selectivity against DPP8/9 was also improved compared with compound **8a**. However, compound **8j** (IC_{50} = 0.53 μM), which was *di*-chloro substituted at the *meta*- and *para*-positions, and compound **8k** (IC_{50} = 1.58 μM), which was *di*-fluoro substituted at the *meta*-positions, resulted in decreased inhibitory potency (1.2- and 3.5-fold, respectively). Therefore, the *meta*-position was shown to be important for potency, and the *meta*-position should not be substituted simultaneously. Interestingly, introduction of the 2,4,5-trifluoro group, resulted in 9.0-fold improvement in DPP4 inhibitory activity of compound **8l** (IC_{50} = 0.05 μM) compared with that of compound **8a**. Furthermore, investigation of the influence of the configuration revealed that the *S*-configuration of compound **8m** (IC_{50} = 1.94 μM) was 4.3-fold less potent than that of compound **8a**. This demonstrated that the *R*-configuration was particularly important for the β-amino compounds.

Compared with positive control compounds **MK-0431** and **LAF-237**, the inhibitory activity and selectivity of compound **8l** were the similar as **LAF-237**. However, the inhibitory activity and selectivity of compound **8l** were very lower than that of **MK-0431** (Table 1).

To enhance the inhibitory activities, octahydrocyclopenta[*b*]pyrrole-2-carbonitrile derivatives (**9a**–**l**) were designed, synthesized and evaluated. As shown in Table 2, Compound **9a** (IC_{50} = 0.44 μM) showed moderate inhibitory activity against DPP4 and excellent selectivity against other serine proteases. Introduction of the chloro group (**9b**, IC_{50} = 0.07 μM), methyl group (**9c**, IC_{50} = 0.36 μM), and fluoro group (**9d**, IC_{50} = 0.13 μM and **9e**, IC_{50} = 0.27 μM) at the *ortho*-, *meta*-, or *para*-positions of the benzene ring, resulted in moderate enhancement in inhibitory potency (6.3-, 1.2-, 3.4-, and 1.6-fold, respectively). Introduction of a large group such as iodo group (**9f**, IC_{50} = 8.80 μM) and trifluoromethyl group (**9g**, IC_{50} = 1.98 μM) at the *para*-position resulted in a dramatically decreased in inhibitory potency. Replacement of the trifluoromethyl group with the methoxyl group (**9h**) at the *para*-position resulted in no inhibitory activity. Therefore, for the octahydrocyclopenta[*b*]pyrrole-2-carbonitrile derivatives, the electron-donating group should not be substituted at the *para*-position, particularly in the case of large group. For the *di*-substituted compounds **9i**–**k** (compound **9i**, IC_{50} = 0.07 μM; compound **9j**, IC_{50} = 47.44 μM; and compound **9k**, IC_{50} = 9.65 μM), the *ortho*-, and *para*-position were beneficial while the *meta*-position was detrimental. Moreover, introduction of *di*-substituted groups at the *meta*-position simultaneously resulted in a sharply reduced potency. Interestingly, introduction of the 2,4,5-trifluoro group (**9l**, IC_{50} = 0.01 μM) resulted in a 44-fold enhancement in inhibitory activity compared to compound **9a**, and the selectivity was also improvement (SR: DPP8/DPP4 = 898.00; DPP9/DPP4 = 566.00).

In comparison of these two series of compounds **8a**–**m** and **9a**–**l**, the DPP4 inhibitory activities of compounds **8b**–**f** and **9b**–**f** showed only slightly improvement in DPP4 inhibitory activities compared with compounds **8a** and **9a**; while the inhibitory activities of compounds **8i** and **9i** showed 11.3- and 6.3-fold improvement, respectively. However, compared to compounds **8f**–**h** and **8j**–**k**, the DPP4 inhibitory activities of compounds **9f**–**h** and **9j**–**k** were very low. It indicated that the electronic effect and steric effect might have greater influence for the series of compounds **8a**–**l** than that of compounds **9a**–**m**. Moreover, as a consequence of the introduction of the fluorine atom at the *ortho*-, *meta*-, or *para*-positions of the benzene ring, the DPP4 inhibitory activity of compounds **8l** and **9l** were showed 9.0-fold and 44.0-fold improvement compared to compounds **8a** and **9a**, respectively. This could be ascribed to the fluorine atom attached to the benzene ring was capable to form a hydrogen bond with residue Ser630 (see binding modes section). Moreover, compared to compound **8l**, the DPP4 inhibitory activity of compound **9l** was 5.0-fold higher, and the corresponding selectivity was 44.5-fold (DPP8/DPP4) and 85.8-fold (DPP9/DPP4) greater, respectively. The probable reason was that the octahydrocyclopenta[*b*]pyrrole fragment of compound **9l** might occupy more space in the S2 pocket and form hydrophobic interaction with the side chains of Phe357, Ser209, and Arg125 (Fig. 3b) in comparison of the 4-fluoropyrrolidine-2-carbonitrile fragment of compound **8l**.

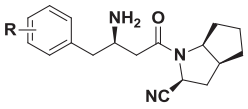
In summary, by introduction of the predominant fragment octahydrocyclopenta[*b*]pyrrole into the lead compound **8l**, the DPP4 inhibitory activities were improvement, such as compound **9l** (IC_{50} = 0.01 μM). The important was the selectivity of compounds **9a**–**l** was enhancement compared to compounds **8a**–**m**.

2.2.2. Binding modes of compounds *8l* and *9l*

To gain structural information for further optimization, the proposed 3D binding modes of compounds **8l** and **9l** to DPP4 were generated based on docking simulation (Fig. 3a and b).

The binding modes showed that compounds **8l** and **9l** were bound to the active site of DPP4 with the amide moiety, which was similar to the binding mode of sitagliptin with DPP4 [21]. The 2,4,5-trifluorophenyl moiety fully occupied the S1 hydrophobic pocket, and the fluorine atom formed a hydrogen bond with residue Ser630. The 4-fluoropyrrolidine-2-carbonitrile and octahydrocyclopenta[*b*]pyrrole-2-carbonitrile moieties occupied the S2 pocket. Water molecular bridged the cyano nitrogen and the hydroxyl of Tyr547, and also interacted with the nitrogen of the pyrrole-2-carbonitrile moiety and with the carbonyl of Glu205 and

Table 2
Inhibitory activities and selectivity of β-amino octahydrocyclopenta[*b*]pyrrole-2-carbonitrile derivatives.



Compd.	R	IC_{50} (μM)[a]					SR[b]	
		DPP4	DPP7	DPP8	DPP9	FAP	DPP8/DPP4	DPP9/DPP4
9a	H	0.44 ± 0.02	NI[c]	NI	107.66 ± 8.4	NI	–	244.68
9b	2-Cl	0.07 ± 0.00	NI	5.69 ± 1.06	22.27 ± 1.27	34.41 ± 2.87	81.29	318.14
9c	2-Me	0.36 ± 0.03	NI	76.72 ± 8.98	70.46 ± 5.74	109.88 ± 6.53	213.11	195.72
9d	3-F	0.13 ± 0.01	NI	36.53 ± 7.82	21.62 ± 3.03	NI	281.00	166.30
9e	4-F	0.27 ± 0.01	77.03 ± 2.99	51.60 ± 3.65	21.96 ± 2.77	153.70 ± 12.2	191.11	81.33
9f	4-I	8.80 ± 0.86	ND[d]	ND	ND	ND	/	/
9g	4-CF_3	1.98 ± 0.18	ND	ND	ND	ND	/	/
9h	4-OMe	NI	ND	ND	ND	ND	/	/
9i	2,4-*di*-Cl	0.07 ± 0.00	28.26 ± 1.02	2.12 ± 0.51	0.88 ± 0.16	3.85 ± 0.38	30.29	12.57
9j	3,4-*di*-Cl	47.44 ± 6.2	ND	ND	ND	ND	/	/
9k	3,5-*di*-F	9.65 ± 0.94	ND	ND	ND	ND	/	/
9l	**2,4,5-*tri*-F**	**0.01 ± 0.00**	**71.68 ± 2.13**	**8.98 ± 0.83**	**5.66 ± 0.44**	**12.46 ± 0.47**	**898.00**	**566.00**
MK-0431	/	0.02 ± 0.00	207.35 ± 6.0	67.72 ± 10.34	67.41 ± 9.26	30.39 ± 2.94	3386.00	3370.50
LAF-237	/	0.07 ± 0.00	>100	1.96 ± 0.23	0.20 ± 0.03	3.72 ± 0.31	28.00	2.86

[a] Mean values of at least two experiments.
[b] Selectivity Ratio (SR) = DPP8 IC_{50}/DPP4 IC_{50} and DPP9 IC_{50}/DPP4 IC_{50}.
[c] No inhibition.
[d] Not detected.

Glu206. Furthermore, the (*R*)-β-amino group of compounds **8l** and **9l** could form three hydrogen bonds with the side chains of a tyrosine (Tyr662) and two glutamate residues (Glu205 and Glu206) (Fig. 3a and b). Compared with the 4-fluoropyrrolidine-2-carbonitrile moiety of compound **8l**, the octahydrocyclopenta[*b*] pyrrole-2-carbonitrile moiety could occupy more space in the S2 pocket and form hydrophobic interaction with the side chains of Phe357, Ser209, and Arg125, which accounted for the increased inhibitory activity of compound **9l** compared with that of **8l**. Moreover, because of introduction of the fluorine atom, the inhibitory activities against DPP4 of compounds **8l** (IC_{50} = 0.05 μM) and **9l** (IC_{50} = 0.01 μM) showed 9.0-fold and 44.0-fold improvement compared to compounds **8a** (IC_{50} = 0.45 μM) and **9a** (IC_{50} = 0.44 μM), respectively. From the binding modes of compounds **8l** and **9l**, the fluorine atom [33] could form hydrogen bonds with Ser630 (Fig. 3a and b), and this was consistent with the observed enhancement in potency with this series compared to compounds **8a** and **9a**.

2.2.3. *In vivo studies*

On the basis of overall properties of *in vitro* potency and selectivity, compounds **8l** and **9l** were selected for evaluation in OGTTs in ICR mice. **LAF-237** was used as positive control.

Compounds **8l** and **9l** were assessed for the ability to improve glucose tolerance in ICR mice. Single dose administration (5 and 15 mg/kg) of compounds **8l** and **9l** to ICR mice at 30 min before an oral glucose challenge produced a significant decrease in glucose excursion, showed by the reduction of area under curve $(AUC)_{0-120}$. Compounds **8l** and **9l** reduced the blood glucose level significantly at time points of 15, 30, 60, and 90 min after glucose challenge, and showed in dose-dependent manner at the 15 min time point. Collectively, the efficacy of 15 mg/kg of **8l** (AUC_{0-120}, 795.0 ± 37.9) and **9l** (AUC_{0-120}, 847.3 ± 29.8) was comparable to **LAF-237** of 15 mg/kg (AUC_{0-120}, 904.1 ± 33.9) (Table 3), compounds **8l** and **9l** improved the glucose tolerance capacity at 15 min better than that of positive group **LAF-237**.

Considering to the inhibitory activities against DPP4 (compound **8l**, IC_{50} = 0.05 μM; compound **9l**, IC_{50} = 0.01 μM) and selectivity (compound **8l**, selective ratio: DPP8/DPP4 = 20.20; DPP9/DPP4 = 6.60; compound **9l**, selective ratio: DPP8/DPP4 = 898.00; DPP9/DPP4 = 566.00) *in vitro*, compound **9l** was selected to do the chronic effects experiment, although compound **8l** had better data in oral glucose tolerance tests than that of compound **9l** *in vivo*.

Chronic effects of compound **9l** were investigated in BKS *db*/*db* mice dosed with 15 mg/kg/day in 5-weeks (Fig. 4). **LAF-237** at the same dose was included as positive control. Fig. 4 showed that compound **9l** significantly improved the glucose excursion during the 60 min−120 min after oral gavage, as demonstrated by the reduction of the AUC (3246.3 ± 165.0 vs 3649.5 ± 91.5 of the vehicle group). The fasting blood glucose level and plasma triglyceride content decreased obviously following compound **9l** treatment, although the free fatty acid and total cholesterol content were unchanged. As expected, compound **9l** and **LAF-237** increased the basal plasma insulin content significantly by 2.2-fold and 1.6-fold, respectively (Table 4).

2.2.4. *Pharmacokinetic evaluation of compounds **8l** and **9l***

The pharmacokinetic (PK) profiles of the selected compounds **8l** and **9l** were assessed in SD rats (Table 5). Compound **8l** showed a high clearance (*i.v.*), high AUC and a high maximal concentration (C_{max}) when dosed orally. The half-life and the oral bioavailability of compound **8l** were 2.03 h and 53.2%, respectively (Table 5). Compound **9l** showed a lower AUC than that of compound **8l** due to the different dose administered. Compared to compound **8l**, compound **9l** showed lower clearance (*i.v.*) and a longer half-life ($t_{1/2}$ = 2.74 h). However, the oral bioavailability of compound **9l** decreased to 22.8%. Otherwise, compared to the ratios of C_{max}/Dose, AUC_{0-t}/Dose, and $AUC_{0-\infty}$/Dose of these two compounds **8l** and **9l**, compound **8l** had better PK properties than that of compound **9l** when dosage as *p.o.* However, compound **9l** had higher AUC value when dosage as *i.v.*

2.2.5. *hERG testing of compounds **8l** and **9l***

Blockade of the hERG channel was a significant hurdle encountered in the drug discovery [34]. Because of being efficacious *in vivo*, compounds **8l** and **9l** were chosen to hERG testing (Table 6). The IC_{50} values of compounds **8l** and **9l** on hERG were larger than 40.00 μM using Patch-clamp experiment, and the IC_{50} values of compounds **8l** and**9l** on hERG were 97.70 μM and 55.30 μM using Thallium assay, respectively.

2.2.6. *Liver metabolic enzymes P450 testing of compounds **8l** and **9l***

Both compounds **8l** and **9l** showed no inhibition with liver metabolic enzymes such as CYP2C9, and the inhibition activity of liver metabolic enzymes such as CYP3A4 of compounds **8l** and **9l** were 56.49 μM and 2.25 μM, respectively (Table 6).

3. Conclusions

A series of novel β-amino pyrrolidine-2-carbonitrile derivatives was designed, synthesized and evaluated as potent and selective DPP4 inhibitors. Compound **8l** demonstrated a moderate DPP4 inhibitory activity, a good PK properties, and good *in vivo* efficacy but poor selectivity. Then a further design and modification was undertaken, and compound **9l** showed excellent DPP4 inhibitory activity, high selectivity against other related enzymes, a moderate PK properties, and excellent *in vivo* efficacy in an OGTTs in ICR mice and BKS *db*/*db* mice. Moreover, compounds **8l** and **9l** did not block hERG channel and displayed no inhibition of liver metabolic enzymes such as CYP2C9. Further investigation of octahydrocyclopenta[*b*]pyrrole-2-carbonitrile derivatives is in progress.

Table 3
Single dose effect of compounds **8l** and **9l** on oral glucose tolerance tests in ICR mice.

Compd.	Dose (mg/kg)	Time (min)						AUC_{0-120} mmol/L/min
		0	15	30	60	90	120	
Veh	–	6.0 ± 1.1	13.2 ± 1.3	15.8 ± 3.5	11.5 ± 2.7	8.3 ± 1.8	6.0 ± 1.3	1175.5 ± 216.4
8l	5	5.8 ± 0.3	10.3 ± 0.5**	10.9 ± 0.6**	8.5 ± 0.3**	6.2 ± 0.3**	5.4 ± 0.3	876.6 ± 32.3**
	15	5.4 ± 0.2	8.5 ± 0.4^{**,¶,δ}	10.2 ± 0.7**	7.6 ± 0.5**	5.9 ± 0.5**	5.1 ± 0.3	795.0 ± 37.9**,¶
9l	5	6.0 ± 0.2	11.2 ± 0.5**	12.2 ± 0.7**	9.2 ± 0.6*	6.8 ± 0.5*	6.0 ± 0.4	961.7 ± 41.8*
	15	5.9 ± 0.3	9.4 ± 0.6^{**,¶,ϕ}	10.6 ± 0.5**	7.9 ± 0.3**	6.5 ± 0.4*	5.2 ± 0.4	847.3 ± 29.8^{**,ϕ}
LAF-237	15	5.6 ± 0.1	11.2 ± 0.6*	11.0 ± 0.5**	8.2 ± 0.4**	6.9 ± 0.4*	5.8 ± 0.3	904.1 ± 33.9**

$^{*}p < 0.05$ or $^{**}p < 0.01$ was compared to the vehicle control group, and regarded as statistically significant; $^{¶}p < 0.05$ was compared to the positive control **LAF-237** group; $^{δ}p < 0.05$ was compared to 5 mg/kg **8l** group; $^{ϕ}p < 0.05$ was compared to 5 mg/kg **9l** group.

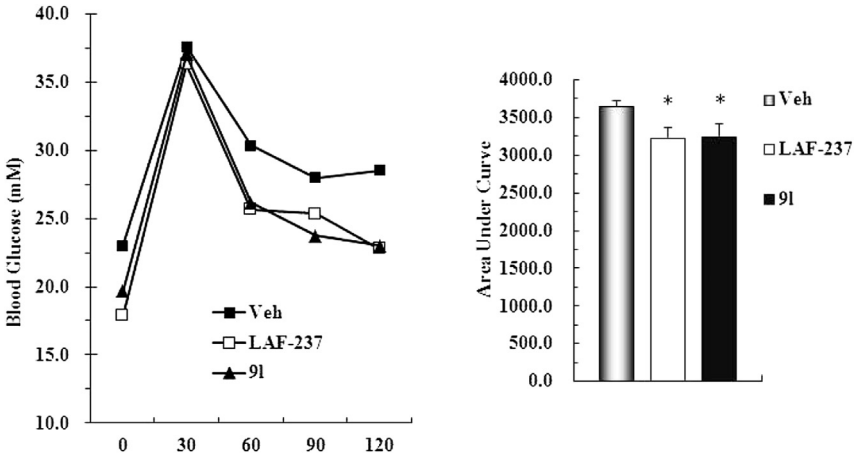


Fig. 4. Chronic effect of compound **9l** on OGTT in BKS *db*/*db* mice. C57BKS *db*/*db* mice with 15 mg/kg/day **9l** and **LAF-237** treatment, oral glucose tolerance tests (1.5 g/kg) was carried out after 6-h starvation of 5th-week treatment, the blood glucose level at 0, 30, 60, 90, and 120 min were recorded for the glucose tolerance capacity evaluation. The results are presented as the mean ± SE. $^{*}p < 0.05$ compared to vehicle group ($n = 5–8$/group).

4. Experimental section

4.1. Chemistry

The chemical reagents were purchased and used without further purification. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker AMX-400 and AMX-300 NMR (IS as TMS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray, and matrix-assisted laser desorption ionization (EI and ESI) produced by a Finnigan MAT-95, LCQ-DECA spectrometer and IonSpec 4.7 T. Optical rotations were reported as follows: $[\alpha]_D^{25}$ (c = g/100 mL, in solvent). Compounds **8a**–**m** and **9a**–**l** were confirmed ≥95% purity (Supporting Information, Table S1). The details for purity analyses of compounds **8a**–**m** and **9a**–**l** are described in the Supplementary material.

4.1.1. 1-tert-Butyl 2-methyl (2S,4R)-4-hydroxypyrrolidine-1,2-dicarboxylate (*11*)

A solution of (2*S*,4*R*)-methyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride (**10**) (0.5 g, 2.75 mmol) in dioxane (15 mL) was added $(Boc)_2O$ (721 mg, 3.3 mmol) and saturated $NaHCO_3$ (10.5 mL). The reaction was stirred at room temperature overnight. The solvent was then removed *in vacuo* and CH_2Cl_2 was added. The organics were washed with H_2O twice and saturated NaCl once, then dried, filtered and concentrated. The residue was purified by flash chromatography on silica gel, eluted with a mixture of EA/PE (1:5, v/v), to afford **11** (611 mg, 91%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.35–4.48 (m, 2H), 3.72 (s, 3H), 3.46–3.65 (m, 2H), 2.03–2.29 (m, 2H), 1.39 (s, 9H). MS (ESI) m/z 246 $[M+H]^+$.

4.1.2. 1-tert-Butyl 2-methyl (2S,4S)-4-fluoropyrrolidine-1,2-dicarboxylate (*12*)

Under the nitrogen protected, a solution of compound **11** (2 g, 8.2 mmol) in dry CH_2Cl_2 cooled to −78 °C, was added DAST (1.97 g, 12.2 mmol). After stirring 3 h, the reaction slowly warmed to room temperature overnight. Then the reaction solution was poured into 200 mL ice and $NaHCO_3$ mixture solution and stirred acutely until no CO_2 evolution. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2, dried, filtered and concentrated. The residue was purified by flash chromatography on silica gel, eluted with a mixture of EA/PE (1:4, v/v), to afford **12** (1.6 g, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.18–5.34 (m, 2H), 3.72 (s, 3H), 3.46–3.65 (m, 2H), 2.03–2.29 (m, 2H), 1.40 (s, 9H). MS (ESI) m/z 248 $[M+H]^+$.

4.1.3. (2S,4S)-1-[(tert-Butoxy)carbonyl]-4-fluoropyrrolidine-2-carboxylic acid (*13*)

A solution of compound **12** (2.46 g, 9.96 mmol) in dioxane (20 mL), was added 10 mL of H_2O followed by lithium hydroxide hydrate (2.09 g, 49.8 mmol) at room temperature, the reaction was stirred for 3 h (monitored by TLC). Then the solution was filtered removing the insoluble substance, and the filtrate was removed the solvent in *vacuo*, then the residue was added 10 mL of H_2O and acidified with concentrated HCl to pH 3–4, the product began to precipitate, filtered and dried to afford **13** (2.2 g, 95%) as a white solid. ^{1}H NMR (CD_3OD, 300 MHz): δ 5.12–5.30 (m, 1H), 4.38–4.42 (m, 1H), 3.59–3.72 (m, 2H), 2.39–2.46 (m, 2H), 1.47 (s, 9H). MS (ESI) m/z 232 $[M-H]^-$.

4.1.4. tert-Butyl (2S,4S)-2-carbamoyl-4-fluoropyrrolidine-1-carboxylate (*14*)

A mixture of compound **13** (0.5 g, 1.86 mmol), $(Boc)_2O$ (0.61 g, 2.79 mmol), NH_4HCO_3 (0.22 g, 2.79 mmol) and pyridine (2 mL) in dioxane (20 mL) was stirred at room temperature for 6 h. The product was extracted with CH_2Cl_2, washed with 1 M HCl and saturated NaCl, dried, filtrated, and concentrated. n-Hexane (100 mL) was added and the product **14** (0.37 g, 85%) began to precipitate using the ultrasound as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.59 (br, s, 1H), 5.13–5.31 (m, 1H), 4.36 (br, s, 1H), 3.52–3.81 (m, 2H), 2.31–2.78 (m, 2H), 1.48 (s, 9H). MS (ESI) m/z 233 $[M+H]^+$.

4.1.5. tert-Butyl (2S,4S)-2-cyano-4-fluoropyrrolidine-1-carboxylate (*15*)

A mixture of compound **14** (13 g, 56.0 mmol) and cyanuric chloride (6.19 g, 33.6 mmol) in DMF (10 mL) was stirred at room temperature for 2 h (monitored by TLC). After the reaction completed, the solution was extracted with EtOAc, washed, dried, concentrated, and purified by flash chromatography on silica gel, eluted with a mixture of PE/EA (1/1, v/v), to afford **15** (9.7 g, 76%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.21–5.41 (m, 1H),

Table 4
Chronic effect of **9l** on blood glucose and plasma indicators in BKS *db*/*db* mice.

Biochemical indicator	Veh	LAF-237	9l
Random blood glucose (mM)	23.3 ± 1.0	22.2 ± 1.8	21.3 ± 0.9
Fasting blood glucose (mM)	23.0 ± 1.0	18.5 ± 0.7*	19.7 ± 1.5*
Plasma insulin (ng/mL)	1.95 ± 0.14	3.16 ± 0.4*	4.31 ± 0.65*
Plasma triglyceride (mM)	1.01 ± 0.09	0.75 ± 0.06*	0.85 ± 0.05*
Plasma free fatty acid (mEq/L)	1.46 ± 0.10	1.07 ± 0.07*	1.43 ± 0.08
Plasma total cholesterol	2.22 ± 0.14	2.47 ± 0.11	2.40 ± 0.13
AUC_{0-120} of oGTT after 5-wks-treatment	3649.5 ± 91.5	3229.1 ± 139.7*	3246.3 ± 165.0*

C57BKS *db*/*db* mice with 15 mg/kg/day compound **9l** treatment, oral glucose tolerance tests (1.5 g/kg) was carried out after 6-h starvation of 5th-week treatment, the blood glucose level was recorded for the glucose tolerance capacity evaluation. The results are presented as the mean ± SE. *$p < 0.05$ compared to vehicle group ($n = 5–8$/group).

Table 5
Pharmacokinetic properties of compounds **8l** and **9l** in SD rats.

Compd.	Admini.	Dose mg/kg	T_{max} h	C_{max} ng/mL	AUC_{0-t} ng/mL*h	$AUC_{0-\infty}$ ng/mL*h	MRT h	$t_{1/2}$ h	CLz L/h/kg	F %
8l	*p.o.*	50	0.3	2692	8838	8935	3.03	2.03	/	53.2
	i.v.	20	0.3	2711	5843	5866	2.93	2.33	3.42	/
9l	*p.o.*	20	0.5	509	2040	2361	2.81	2.74	/	22.8
	i.v.	10	0.5	1121	4467	4974	2.71	2.39	2.01	/

p.o., oral administration; *i.v.*, intravenous injection.

Table 6
hERG and live metabolic enzymes P450 testing of compounds **8l** and **9l**.

Compd.	hERG (IC_{50},μM)		CYP450 (IC_{50}, μM)	
			CYP3A4	CYP2C9
8l	>40.00[a]	97.70[b]	56.49	NI[c]
9l	>40.00[a]	55.30[b]	2.25	NI

[a] Tested by Patch Clamp.
[b] Using Thallium assay.
[c] No inhibition.

4.62–4.76 (m, 1H), 3.49–3.93 (m, 2H), 2.63 (dd, J_1 = 15.0 Hz, J_2 = 15.3 Hz, 1H), 2.30–2.44 (m, 1H), 1.49 (s, 9H). MS (ESI) *m*/*z* 215 $[M+H]^+$.

*4.1.6. (2S,4S)-4-fluoropyrrolidine-2-carbonitrile: 4-methylbenzene-1-sulfonic acid (**16**)*

A solution of compound **15** (7.9 g, 36.9 mmol) in CH_3CN (50 mL) was added 4-methylbenzenesulfonic acid hydrate (10.5 g, 55.32 mmol) and stirred at room temperature for 24 h. After the reaction completed, the solution was removed *in vacuo*. The residual brown oil was dissolved in EtOAc (100 mL) and put into fridge overnight, the product **16** (7.4 g, 71%) was precipitated as a brown crystal. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.51 (dd, J_1 = 16.0 Hz, J_2 = 8.0 Hz, 2H), 7.09–7.15 (m, 2H), 5.45–5.62 (m, 1H), 4.99–5.04 (m, 1H), 3.42–3.67 (m, 2H), 2.30–2.70 (m, 3H), 3.17 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 143.3, 142.0, 130.0, 127.0, 116.4, 93.9, 92.5, 53.8 (d, J = 19.0 Hz), 46.8, 38.4 (d, J = 17.0 Hz), 21.4. MS (EI) *m*/*z* 114 $[M]^+$. HRMS (EI) *m*/*z* calcd $C_5H_7FN_2$ 114.0593 $[M]^+$, found 114.0668.

*4.1.7. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-4-oxo-1-phenylbutan-2-yl]carbamate (**18a**)*

A solution of (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-phenylbutanoic acid (compound **17a**, 108 mg, 0.385 mmol) in DMF (5 mL) was added HOBt (166 mg, 1.225 mmol) and EDCI (154 mg, 0.805 mmol). After stirring for 30 min compound **16** (100 mg, 0.35 mmol) and additional TEA (0.17 mL, 1.225 mmol) were added. This solution was allowed to stir at room temperature for 20 h and then the saturated $NaHCO_3$ was added. The mixture was extracted with EtOAc and washed with saturated NaCl, dried over Na_2SO_4 and concentrated. The residue was purified with flash chromatography on silica gel, eluted with a mixture of PE/EA (4/1, v/v) to afford **18a** (112 mg, 85%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28–7.32 (m, 2H), 7.20–7.24 (m, 3H), 4.90 (d, J = 9.1 Hz, 1H), 4.11–4.15 (m, 1H), 3.59 (br, s, 1H), 3.45–3.49 (m, 1H), 3.05–3.10 (m, 1H), 2.85–2.89 (m, 1H), 2.62–2.66 (m, 1H), 2.39 (d, J = 4.8 Hz, 2H), 2.16–2.33 (m, 2H), 1.41 (s, 9H). MS (ESI) *m*/*z* 398 $[M+Na]^+$.

*4.1.8. tert-Butyl N-[(2R)-1-(2-chlorophenyl)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-4-oxobutan-2-yl]carbamate (**18b**)*

In the same manner as described for **18a**, **18b** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2-chlorophenyl)butanoic acid (**17b**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28–7.36 (m, 2H), 7.12–7.19 (m, 2H), 5.27–5.46 (m, 2H), 4.95 (d, J = 9.0 Hz, 1H), 4.24 (br, s, 1H), 3.45–3.84 (m, 2H), 3.08–3.14 (m, 2H), 2.21–2.73 (m, 4H), 1.38 (s, 9H). MS (ESI) *m*/*z* 432 $[M+Na]^+$.

*4.1.9. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(2-methylphenyl)-4-oxobutan-2-yl]carbamate (**18c**)*

In the same manner as described for **18a**, **18c** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2-methylphenyl)butanoic acid (**17c**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.05–7.12 (m, 4H), 5.24–5.58 (m, 2H), 4.94 (d, J = 9.0 Hz, 1H), 4.18 (br, s, 1H), 3.30–3.77 (m, 2H), 2.90–2.96 (m, 2H), 2.18–2.66 (m, 7H), 1.37 (s, 9H). MS (ESI) *m*/*z* 412 $[M+Na]^+$.

*4.1.10. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(3-fluorophenyl)-4-oxobutan-2-yl]carbamate (**18d**)*

In the same manner as described for **18a**, **18d** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(3-fluorophenyl)butanoic acid **17d**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.22–7.28 (m, 1H), 6.88–6.93 (m, 3H), 5.27–5.44 (m, 2H), 4.95 (d, J = 9.0 Hz, 1H), 4.15 (br, s, 1H), 3.39–3.84 (m, 2H), 2.90–2.97 (m, 2H), 2.18–2.68 (m, 4H), 1.39 (s, 9H). MS (ESI) *m*/*z* 416 $[M+Na]^+$.

*4.1.11. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(4-fluorophenyl)-4-oxobutan-2-yl]carbamate (**18e**)*

In the same manner as described for **18a**, **18e** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-fluorophenyl)butanoic acid (**17e**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.11–7.19 (m, 2H), 6.92–6.99 (m, 2H), 5.28–5.46 (m, 2H), 4.93 (d, J = 9.0 Hz, 1H), 4.10–4.15 (m, 1H), 3.81–3.53 (m, 3H), 2.91–2.96 (m, 2H), 2.60–2.71 (m, 1H), 2.48–2.54 (m, 2H), 1.39 (s, 9H). MS (ESI) *m*/*z* 416 $[M+Na]^+$.

*4.1.12. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(4-iodophenyl)-4-oxobutan-2-yl]carbamate (**18f**)*

In the same manner as described for **18a**, **18f** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-iodophenyl)butanoic acid (**17f**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.56–7.63 (m, 2H),

6.89–6.96 (m, 2H), 5.28–5.46 (m, 2H), 4.92 (d, J = 9.0 Hz, 1H), 4.10 (br, s, 1H), 3.42–3.86 (m, 2H), 2.18–2.97 (m, 6H), 1.39 (s, 9H). MS (ESI) m/z 524 $[M+Na]^+$.

*4.1.13. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-4-oxo-1-[4-(trifluoromethyl)phenyl]butan-2-yl]carbamate (**18g**)*

In the same manner as described for **18a**, **18g** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-[4-(trifluoromethyl) phenyl]butanoic acid (**17g**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.50–7.58 (m, 2H), 7.31–7.37 (m, 2H), 5.27–5.46 (m, 2H), 4.93 (d, J = 9.0 Hz, 1H), 4.19 (br, s, 1H), 3.47–3.88 (m, 2H), 2.98–3.04 (m, 2H), 2.18–2.73 (m, 4H), 1.38 (s, 9H). MS (ESI) m/z 466 $[M+Na]^+$.

*4.1.14. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(4-methoxyphenyl)-4-oxobutan-2-yl]carbamate (**18h**)*

In the same manner as described for **18a**, **18h** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-methoxyphenyl)butanoic acid (**17h**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.05–7.14 (m, 2H), 6.78–6.83 (m, 2H), 5.27–5.40 (m, 2H), 4.93 (d, J = 9.0 Hz, 1H), 4.11 (br, s, 1H), 3.39–4.11 (m, 5H), 2.85–2.96 (m, 3H), 2.67 (t, J = 9.0 Hz, 1H), 2.18–2.45 (m, 2H), 1.40 (s, 9H). MS (ESI) m/z 428 $[M+Na]^+$.

*4.1.15. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(2,4-dichlorophenyl)-4-oxobutan-2-yl)carbamate (**18i**)*

In the same manner as described for **18a**, **18i** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2,4-dichlorophenyl) butanoic acid (**17i**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.39 (m, 1H), 7.18–7.22 (m, 2H), 5.31–5.63 (m, 2H), 4.93 (d, J = 9.0 Hz, 1H), 4.20 (br, s, 1H), 3.51–3.88 (m, 2H), 2.70–2.78 (m, 2H), 2.19–2.73 (m, 4H), 1.35 (s, 9H). MS (ESI) m/z 466 $[M+Na]^+$.

*4.1.16. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(3,4-dichlorophenyl)-4-oxobutan-2-yl)carbamate (**18j**)*

In the same manner as described for **18a**, **18j** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(3,4-dichlorophenyl) butanoic acid (**17j**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.39 (m, 1H), 7.20–7.29 (m, 1H), 7.00–7.09 (m, 1H), 5.30–5.46 (m, 2H), 4.95 (d, J = 9.0 Hz, 1H), 4.10 (br, s, 1H), 3.46–3.88 (m, 2H), 2.90–2.98 (m, 2H), 2.18–2.73 (m, 4H), 1.38 (s, 9H). MS (ESI) m/z 466 $[M+Na]^+$.

*4.1.17. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-1-(3,5-difluorophenyl)-4-oxobutan-2-yl)carbamate (**18k**)*

In the same manner as described for **18a**, **18k** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(3,5-difluorophenyl) butanoic acid (**17k**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.66–6.76 (m, 3H), 5.30–5.45 (m, 2H), 4.94 (d, J = 6.0 Hz, 1H), 4.11 (br, s, 1H), 3.47–3.86 (m, 2H), 2.90–2.96 (m, 2H), 2.17–2.73 (m, 1H), 1.40 (s, 9H). MS (ESI) m/z 434 $[M+Na]^+$.

*4.1.18. tert-Butyl N-[(2R)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-yl)carbamate (**18l**)*

In the same manner as described for **18a**, **18l** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2,4,5-trifluorophenyl) butanoic acid (**17l**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.05–7.14 (m, 1H), 6.85–6.94 (m, 1H), 5.33–5.50 (m, 2H), 4.95 (d, J = 16.0 Hz, 1H), 4.12 (br, s, 1H), 3.55–3.92 (m, 2H), 2.90–2.98 (m, 2H), 2.58–2.75 (m, 3H), 2.27–2.39 (m, 1H), 1.37 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.9, 162.2, 155.3, 121.7 (d, J = 18.6 Hz), 119.2 (dd, J_1 = 6.1 Hz, J_2 = 5.9 Hz), 117.4, 105.3 (dd, J_1 = 28.5 Hz, J_2 = 28.1 Hz), 92.8, 90.9, 79.6, 53.1 (d, J = 24.1 Hz), 48.0, 45.3, 44.5, 37.2, 36.1 (d, J = 21.2 Hz), 32.7, 28.2. MS (ESI) m/z 452 $[M+Na]^+$. HRMS (ESI) m/z calcd $C_{20}H_{23}N_3O_3F_4Na$ 452.1556 $[M+Na]^+$, found 452.1573.

*4.1.19. tert-Butyl N-[(2S)-4-[(2S,4S)-2-cyano-4-fluoropyrrolidin-1-yl]-4-oxo-1-phenylbutan-2-yl)carbamate (**18m**)*

In the same manner as described for **18a**, **18m** was prepared from (3*S*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-phenylbutanoic acid (**17m**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.20–7.31 (m, 5H), 5.22–5.60 (m, 2H), 4.91 (d, J = 9.0 Hz, 1H), 4.13 (br, s, 1H), 3.49–3.78 (m, 2H), 2.61–3.14 (m, 3H), 2.18–2.61 (m, 3H), 1.42 (s, 9H). MS (ESI) m/z 398 $[M+Na]^+$.

*4.1.20. 2-Benzyl 1-tert-butyl (2S,3aS,6aS)-hexahydrocyclopenta[b] pyrrole-1,2-dicarboxylate (**20**)*

A solution of benzyl (2*S*,3a*S*,6a*S*)-octahydrocyclopenta[*b*]pyrrole-2-carboxylate hydrochloride (**19**) (5 g, 17.74 mmol) in dioxane (20 mL) was added $(Boc)_2O$ (4.65 g, 21.29 mmol) and saturated $NaHCO_3$ (15 mL). The reaction was stirred at room temperature overnight. The solvent was then removed *in vacuo* and CH_2Cl_2 was added. The organics were washed with H_2O twice and saturated NaCl once, and then dried, filtered and concentrated. The compound **20** (6 g, 97%) was obtained as oil using in the next step without further purification. MS (ESI) m/z 268 $[M+Na]^+$.

*4.1.21. (2S,3aS,6aS)-1-[(tert-Butoxy)carbonyl]octahydrocyclopenta [b]pyrrole-2-carboxylic acid (**21**)*

A solution of compound **20** (6 g, 17.37 mmol) in CH_3OH (30 mL) was added 10% Pd/C. The mixture was stirred under H_2 at room temperature for 20 h, filtered, washed with CH_3OH twice, and concentrated to afford compound **21** as oil without further purification. MS (ESI) m/z 254 $[M-H]^-$.

*4.1.22. tert-Butyl (2S,3aS,6aS)-2-carbamoyl-octahydrocyclopenta [b]pyrrole-1-carboxylate (**22**)*

In the same manner as described for compound **14**, compound **22** was prepared from **21**. The compound 2**2** was used in the next step without further purification. MS (ESI) m/z 277 $[M+Na]^+$.

*4.1.23. tert-Butyl (2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b] pyrrole-1-carboxylate (**23**)*

In the same manner as described for compound **15**, compound **23** was prepared from **22**. The compound **23** was used in the next step without further purification. MS (ESI) m/z 259 $[M+Na]^+$.

*4.1.24. (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carbonitrile: 4-methylbenzene-1-sulfonic acid (**24**)*

In the same manner as described for compound **16**, compound **24** was prepared from **23**. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.67–7.79 (m, 2H), 7.21–7.28 (m, 2H), 4.89 (s, 3H), 4.54–4.67 (m, 1H), 4.12–4.24 (m, 1H), 2.93–3.03 (m, 1H), 2.70–2.76 (m, 1H), 1.66–1.96 (m, 9H). ^{13}C NMR (CD_3OD, 100 MHz): δ 143.4, 141.9, 129.9, 126.9, 115.6, 66.9, 48.2, 43.5, 36.9, 32.4, 31.5, 25.1, 21.4. MS (EI) m/z 136 $[M]^+$. HRMS (EI) m/z calcd $C_8H_{12}N_2$ 136.1000 $[M]^+$, found 136.1003. $[\alpha]_D^{25} = -20.3$ (c = 0.385 g/100 mL, CH_3OH).

*4.1.25. Tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-4-oxo-1-phenylbutan-2-yl] carbamate (**25a**)*

A solution of (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-phenylbutanoic acid (compound **17a**, 99.6 mg, 0.357 mmol) in DMF (5 mL) was added HOBt (153 mg, 1.134 mmol) and EDCI (160 mg, 0.81 mmol). After stirring for 30 min compound **24** (100 mg, 0.324 mmol) and TEA (0.16 mL, 1.134 mmol) were added. This solution was allowed to stir at room temperature for 20 h and then the saturated $NaHCO_3$ was added. The mixture was extracted with EtOAc and washed with saturated NaCl, dried over Na_2SO_4 and concentrated. The residue was purified with flash chromatography on silica gel, eluted with a mixture of PE/EA (4/1, v/v) to afford **25a**

(108 mg, 85%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.13–7.31 (m, 5H), 5.60–5.75 (m, 1H), 4.82–4.90 (m, 1H), 3.85–4.13 (m, 2H), 2.76–3.07 (m, 3H), 2.37–2.46 (m, 3H), 1.99–2.10 (m, 7H), 1.40 (s, 9H). MS (ESI) *m*/*z* 420 $[M+Na]^+$.

4.1.26. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(2-chlorophenyl)-4-oxobutan-2-yl]carbamate (**25b**)

In the same manner as described for **25a**, **25b** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2-chlorophenyl) butanoic acid (**17b**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29–7.32 (m, 1H), 7.20–7.25 (m, 1H), 7.11–7.18 (m, 2H), 5.65–5.92 (m, 1H), 4.80–4.91 (m, 1H), 4.01–4.54 (m, 2H), 3.07–3.17 (m, 2H), 2.79–2.86 (m, 1H), 2.29–2.62 (m, 3H), 1.52–2.11 (m, 7H), 1.34 (s, 9H). MS (ESI) *m*/*z* 332 $[M+H\text{-}Boc]^+$, 454 $[M+Na]^+$.

4.1.27. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-4-oxo-1-(2-methylphenyl) butan-2-yl]carbamate (**25c**)

In the same manner as described for **25a**, **25c** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2-methylphenyl)butanoic acid (**17c**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.00–7.15 (m, 4H), 5.71–5.88 (m, 1H), 4.86–4.92 (m, 1H), 3.87–4.19 (m, 2H), 2.80–3.06 (m, 3H), 2.29–2.52 (m, 6H), 1.47–2.12 (m, 7H), 1.37 (s, 9H). MS (ESI) *m*/*z* 312 $[M+H\text{-}Boc]^+$, 434 $[M+Na]^+$.

4.1.28. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(3-fluorophenyl)-4-oxobutan-2-yl]carbamate (**25d**)

In the same manner as described for **25a**, **25d** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(3-fluorophenyl) butanoic acid (**17d**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.19–7.24 (m, 1H), 6.88–6.97 (m, 3H), 5.59–5.71 (dd, $J_1 = 30.0$ Hz, $J_2 = 30.0$ Hz, 1H), 4.84–4.92 (m, 1H), 3.92–4.15 (m, 2H), 2.80–3.05 (m, 3H), 2.27–2.56 (m, 3H), 1.52–2.15 (m, 7H), 1.38 (s, 9H). MS (ESI) *m*/*z* 316 $[M+H\text{-}Boc]^+$, 438 $[M+Na]^+$.

4.1.29. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(4-fluorophenyl)-4-oxobutan-2-yl]carbamate (**25e**)

In the same manner as described for **25a**, **25e** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-fluorophenyl)butanoic acid (**17e**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10–7.19 (m, 2H), 6.98–7.07 (m, 2H), 5.52–5.63 (m, 1H), 4.81–4.88 (m, 1H), 3.98–4.07 (m, 2H), 2.81–3.00 (m, 3H), 2.29–2.52 (m, 3H), 1.52–1.87 (m, 7H), 1.35 (s, 9H). MS (ESI) *m*/*z* 438 $[M+Na]^+$.

4.1.30. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(4-iodophenyl)-4-oxobutan-2-yl]carbamate (**25f**)

In the same manner as described for **25a**, **25f** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-iodophenyl)butanoic acid (**17f**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.56–7.65 (m, 2H), 6.90–6.99 (m, 2H), 5.58–5.64 (m, 1H), 4.81–4.90 (m, 1H), 4.05–4.14 (m, 2H), 2.77–3.01 (m, 3H), 2.11–2.31 (m, 3H), 1.52–2.11 (m, 7H), 1.39 (s, 9H). MS (ESI) *m*/*z* 424 $[M+H\text{-}Boc]^+$, 546 $[M+Na]^+$.

4.1.31. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-4-oxo-1-(4-(trifluoromethyl) phenyl)butan-2-yl)carbamate (**25g**)

In the same manner as described for **25a**, **25g** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-(trifluoromethyl) phenyl)butanoic acid (**17g**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.50–7.57 (m, 2H), 7.28–7.34 (m, 2H), 5.61–5.70 (m, 1H), 4.84–4.90 (m, 1H), 4.10–4.16 (m, 2H), 2.81–3.18 (m, 3H), 2.29–2.57 (m, 3H), 1.46–2.11 (m, 7H), 1.34 (s, 9H). MS (ESI) *m*/*z* 366 $[M+H\text{-}Boc]^+$, 488 $[M+Na]^+$.

4.1.32. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(4-methoxyphenyl)-4-oxobutan-2-yl)carbamate (**25h**)

In the same manner as described for **25a**, **25h** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(4-methoxyphenyl) butanoic acid (**17h**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.03–7.14 (m, 2H), 6.78–6.84 (m, 2H), 5.57–5.72 (dd, $J_1 = 36.0$ Hz, $J_2 = 33.0$ Hz, 1H), 4.81–4.90 (m, 1H), 4.05–4.13 (m, 2H), 3.77 (s, 3H), 2.78–2.91 (m, 3H), 2.29–2.52 (m, 3H), 1.49–2.11 (m, 7H), 1.40 (s, 9H). MS (ESI) *m*/*z* 328 $[M+H\text{-}Boc]^+$, 450 $[M+Na]^+$.

4.1.33. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(2,4-dichlorophenyl)-4-oxobutan-2-yl)carbamate (**25i**)

In the same manner as described for **25a**, **25i** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2,4-dichlorophenyl) butanoic acid (**17i**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.38 (m, 1H), 7.16–7.21 (m, 2H), 5.64–5.85 (m, 1H), 4.88–4.96 (m, 1H), 4.12–4.44 (m, 2H), 2.85–3.13 (m, 3H), 2.31–2.61 (m, 3H), 1.56–2.17 (m, 7H), 1.37 (s, 9H). MS (ESI) *m*/*z* 366 $[M+H\text{-}Boc]^+$, 488 $[M+Na]^+$.

4.1.34. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(3,4-dichlorophenyl)-4-oxobutan-2-yl)carbamate (**25j**)

In the same manner as described for **25a**, **25j** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(3,4-dichlorophenyl) butanoic acid (**17j**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.39 (m, 2H), 7.07 (s, 1H), 5.57–5.71 (m, 1H), 4.88–4.92 (m, 1H), 4.10 (br, s, 2H), 2.81–3.05 (m, 3H), 2.32–2.59 (m, 3H), 1.61–2.14 (m, 7H), 1.39 (s, 9H). MS (ESI) *m*/*z* 366 $[M+H\text{-}Boc]^+$, 488 $[M+Na]^+$.

4.1.35. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-1-(3,5-difluorophenyl)-4-oxobutan-2-yl)carbamate (**25k**)

In the same manner as described for **25a**, **25k** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(3,5-difluorophenyl)butanoic acid (**17k**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.62–6.75 (m, 3H), 5.59–5.71 (dd, $J_1 = 30.0$ Hz, $J_2 = 27.0$ Hz, 1H), 4.84–4.91 (m, 1H), 4.10 (br, s, 2H), 2.74–3.09 (m, 3H), 2.29–2.62 (m, 3H), 1.46–2.17 (m, 7H), 1.38 (s, 9H). MS (ESI) *m*/*z* 334 $[M+H\text{-}Boc]^+$, 456 $[M+Na]^+$.

4.1.36. tert-Butyl N-[(2R)-4-[(2S,3aS,6aS)-2-cyano-octahydrocyclopenta[b]pyrrol-1-yl]-4-oxo-1-(2,4,5-trifluorophenyl) butan-2-yl)carbamate (**25l**)

In the same manner as described for **25a**, **25l** was prepared from (3*R*)-3-[[(*tert*-butoxy)carbonyl]amino]-4-(2,4,5-trifluorophenyl) butanoic acid (**17l**). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.00–7.10 (m, 1H), 6.81–6.90 (m, 1H), 5.59–5.74 (dd, $J_1 = 48.0$ Hz, $J_2 = 52.0$ Hz, 1H), 4.86–4.92 (m, 1H), 4.12 (br, s, 2H), 2.83–2.98 (m, 3H), 2.48–2.67 (m, 3H), 1.56–2.12 (m, 7H), 1.35 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz): δ 170.1, 157.1, 155.3, 147.5 (d, $J = 12.6$ Hz), 145.6 (d, $J = 9.8$ Hz), 121.9 (d, $J = 14.4$ Hz), 119.2, 119.0 (dd, $J_1 = 9.7$ Hz, $J_2 = 9.6$ Hz), 105.1 (dd, $J_1 = 22.9$ Hz, $J_2 = 22.8$ Hz), 79.3, 63.9, 48.1, 46.3, 44.2, 36.7, 35.1, 34.1, 32.2, 29.6, 28.2, 25.8. MS (ESI) *m*/*z* 352 $[M+H\text{-}Boc]^+$, 474 $[M+Na]^+$. HRMS (ESI) *m*/*z* calcd $C_{23}H_{29}N_3O_3F_3$ 452.2136 $[M+H]^+$, found 452.2161.

4.1.37. (2S,4S)-1-[(3R)-3-amino-4-phenylbutanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8a**)

A solution of **18a** (120 mg) in dry CH_2Cl_2 (2 mL) was added CF_3COOH (1.0 mL) at ice-bathe and warmed to room temperature.

After 1 h, the mixture was concentrated, and the residue was added 10 mL Et_2O. The white solid was precipitated, filtered and to afford **8a** as TFA salt (105 mg). Yield: 88.2%. HPLC: 97.33%, $t_R = 1.84$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.28–7.40 (m, 5H), 5.28–5.47 (m, 1H), 4.98–5.03 (m, 1H), 3.78–3.90 (m, 2H), 3.58–3.70 (m, 1H), 2.91–3.04 (m, 2H), 2.77–2.82 (m, 1H), 2.35–2.63 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.5, 169.1, 135.3, 129.0, 128.8, 127.3, 117.6, 92.5 (d, $J = 141.3$ Hz), 52.8 (d, $J = 18.9$ Hz), 49.5, 44.6, 37.9, 35.8 (d, $J = 16.7$ Hz), 34.6. MS (ESI) m/z 276 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{19}N_3OF$ 276.1512 $[M+H]^+$, found 276.1502. $[\alpha]_D^{25} = -80.9$ ($c = 0.11$ g/100 mL, CH_3OH).

4.1.38. (2S,4S)-1-[(3R)-3-amino-4-(2-chlorophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8b**)

In the same manner as described for **8a**, **8b** was prepared from **18b**. Yield: 80.5%. HPLC: 96.34%, $t_R = 1.59$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.42–7.51 (m, 1H), 7.30–7.38 (m, 3H), 5.29–5.49 (m, 1H), 4.98–5.03 (m, 1H), 3.91–3.98 (m, 1H), 3.57–3.89 (m, 2H), 3.18–3.24 (m, 2H), 2.79–2.96 (m, 1H), 2.39–2.63 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.3168.8, 133.6, 132.5, 131.5, 129.8, 129.2, 127.4, 116.8, 92.5 (d, $J = 141.4$ Hz), 52.8 (d, $J = 18.9$ Hz), 44.6, 35.8 (d, $J = 13.0$ Hz), 35.5, 34.4. MS (ESI) m/z 310 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{18}N_3OFCl$ 310.1122 $[M+H]^+$, found 310.1114. $[\alpha]_D^{25} = -72.0$ ($c = 0.132$ g/100 mL, CH_3OH).

4.1.39. (2S,4S)-1-[(3R)-3-amino-4-(2-menthylphenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8c**)

In the same manner as described for **8a**, **8c** was prepared from **18c**. Yield: 79.5%. HPLC: 95.32%, $t_R = 1.70$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.18–7.24 (m, 4H), 5.28–5.47 (m, 1H), 4.99 (d, $J = 12.0$ Hz, 1H), 3.78–3.89 (m, 2H), 3.56–3.70 (m, 1H), 3.01–3.09 (m, 2H), 2.77–2.93 (m, 1H), 2.41–2.63 (m, 3H), 2.37 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.5, 169.1, 136.7, 133.4, 130.7, 129.8, 127.5, 126.2, 117.6, 92.5 (d, $J = 141.4$ Hz), 52.8 (d, $J = 18.8$ Hz), 44.6, 35.8 (d, $J = 16.7$ Hz), 35.5, 34.7, 18.1. MS (ESI) m/z 290 $[M+H]^+$. HRMS (ESI) calcd $C_{16}H_{21}N_3OF$ 290.1669 $[M+H]^+$, found 290.1680. $[\alpha]_D^{25} = -65.4$ ($c = 0.104$ g/100 mL, CH_3OH).

4.1.40. (2S,4S)-1-[(3R)-3-amino-4-(3-fluorophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8d**)

In the same manner as described for **8a**, **8d** was prepared from **18d**. Yield: 75.2%. HPLC: 96.25%, $t_R = 1.72$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.35–7.45 (m, 1H), 7.00–7.08 (m, 3H), 5.30–5.49 (m, 1H), 4.98–5.07 (m, 1H), 3.82–3.91 (m, 2H), 3.60–3.74 (m, 1H), 3.04 (d, $J = 4.0$ Hz, 2H), 2.73–2.98 (m, 1H), 2.35–2.64 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 169.0, 163.1 (d, $J = 195.2$ Hz), 138.0 (d, $J = 5.9$ Hz), 130.6 (d, $J = 6.6$ Hz), 124.9, 117.6, 115.8 (d, $J = 17.3$ Hz), 114.1 (d, $J = 16.9$ Hz), 92.5 (d, $J = 141.4$ Hz), 52.8 (d, $J = 18.8$ Hz), 49.3, 44.6, 37.6, 35.8 (d, $J = 16.7$ Hz), 34.8. MS (ESI) m/z 294 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{18}N_3OF_2$ 294.1418$[M+H]^+$, found 294.1427. $[\alpha]_D^{25} = -66.0$ ($c = 0.156$ g/100 mL, CH_3OH).

4.1.41. (2S,4S)-1-[(3R)-3-amino-4-(4-fluorophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8e**)

In the same manner as described for **8a**, **8e** was prepared from **18e**. Yield: 84.4%. HPLC: 96.40%, $t_R = 1.66$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.28–7.32 (m, 2H), 7.08–7.13 (m, 2H), 5.30–5.47 (m, 1H), 4.90–5.06 (m, 1H), 3.82–3.91 (m, 2H), 3.58–3.73 (m, 1H), 2.72–3.01 (m, 3H), 2.35–2.64 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 169.0, 162.3 (d, $J = 194.5$ Hz), 131.2, 130.8 (d, $J = 6.5$ Hz), 117.6, 115.5 (d, $J = 17.2$ Hz), 92.5 (d, $J = 141.4$ Hz), 52.8 (d, $J = 18.8$ Hz), 49.5, 44.6, 37.2, 35.8 (d, $J = 16.7$ Hz), 34.7. MS (ESI) m/z 294 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{18}N_3OF_2$ 294.1418 $[M+H]^+$, found 294.1417. $[\alpha]_D^{25} = -70.0$ ($c = 0.13$ g/100 mL, CH_3OH).

4.1.42. (2S,4S)-1-[(3R)-3-amino-4-(4-iodophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8f**)

In the same manner as described for **8a**, **8f** was prepared from **18f**. Yield: 78.2%. HPLC: 95.88%, $t_R = 1.83$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.73 (d, $J = 8.0$ Hz, 2H), 7.00–7.13 (m, 1H), 5.29–5.49 (m, 1H), 4.97–5.05 (m, 1H), 3.82–3.91 (m, 2H), 3.60–3.73 (m, 1H), 2.98 (d, $J = 8.0$ Hz, 2H), 2.72–2.94 (m, 1H), 2.35–2.64 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 169.0, 138.0, 135.1, 131.2, 117.6, 92.5 (d, $J = 141.4$ Hz), 92.4, 52.8 (d, $J = 18.8$ Hz), 49.5, 44.6, 37.5, 35.8 (d, $J = 16.7$ Hz), 34.7. MS (ESI) m/z 402 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{18}N_3OFI$ 402.0479 $[M+H]^+$, found 402.0485. $[\alpha]_D^{25} = -55.6$ ($c = 0.18$ g/100 mL, CH_3OH).

4.1.43. (2S,4S)-1-[(3R)-3-amino-4-(4-(trifluoromethyl)phenyl) butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8g**)

In the same manner as described for **8a**, **8g** was prepared from **18g**. Yield: 81.2%. HPLC: 100.00%, $t_R = 1.89$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.68–7.70 (d, $J = 8.0$ Hz, 2H), 7.49–7.53 (m, 2H), 5.30–5.49 (m, 1H), 4.90–5.06 (m, 1H), 3.56–3.96 (m, 3H), 3.12 (d, $J = 8.0$ Hz, 2H), 2.76–2.96 (m, 1H), 2.37–2.64 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.3, 168.9, 161.6 (d, $J = 27.3$ Hz), 139.9, 129.8, 125.6, 117.6, 52.8 (d, $J = 18.8$ Hz), 44.6, 37.7, 35.8 (d, $J = 16.7$ Hz), 34.8. MS (ESI) m/z 344 $[M+H]^+$. HRMS (ESI) calcd $C_{16}H_{18}N_3OF_4$ 344.1386 $[M+H]^+$, found 344.1380. $[\alpha]_D^{25} = -68.3$ ($c = 0.12$ g/100 mL, CH_3OH).

4.1.44. (2S,4S)-1-[(3R)-3-amino-4-(4-methoxyphenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8h**)

In the same manner as described for **8a**, **8h** was prepared from **18h**. Yield: 79.7%. HPLC: 97.49%, $t_R = 1.80$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.18–7.22 (m, 3H), 6.91–6.94 (d, $J = 12.0$ Hz, 2H), 5.29–5.48 (m, 1H), 4.97–5.04 (m, 1H), 3.77–3.85 (m, 5H), 3.58–3.73 (m, 1H), 2.90–2.96 (m, 2H), 2.69–2.82 (m, 1H), 2.36–2.64 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.5, 169.2, 159.3, 130.1, 126.9, 117.6, 114.2, 92.5 (d, $J = 141.4$ Hz), 54.3, 52.8 (d, $J = 18.8$ Hz), 49.6, 44.6, 37.1, 35.8 (d, $J = 16.7$ Hz), 34.7. MS (ESI) m/z 306 $[M+H]^+$. HRMS (ESI) calcd $C_{16}H_{21}N_3O_2F$ 306.1618 $[M+H]^+$, found 306.1596. $[\alpha]_D^{25} = -75.0$ ($c = 0.10$ g/100 mL, CH_3OH).

4.1.45. (2S,4S)-1-[(3R)-3-amino-4-(2,4-dichlorophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8i**)

In the same manner as described for **8a**, **8i** was prepared from **18i**. Yield: 69.3%. HPLC: 95.43%, $t_R = 1.92$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.55 (s, 1H), 7.30–7.39 (m, 2H), 5.31–5.49 (m, 1H), 4.97–5.05 (m, 1H), 3.84–3.98 (m, 2H), 3.55–3.74 (m, 1H), 3.12–3.21 (m, 2H), 2.74–2.95 (m, 1H), 2.35–2.64 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.2, 168.9, 161.8, 134.9, 134.1, 132.5, 132.0, 129.5, 127.6, 117.6, 92.5 (d, $J = 141.4$ Hz), 52.8 (d, $J = 18.8$ Hz), 44.6, 35.8 (d, $J = 16.7$ Hz), 35.5, 34.5. MS (ESI) m/z 344 $[M+H]^+$, HRMS (ESI) calcd $C_{15}H_{17}N_3OFCl_2$ 344.0733 $[M+H]^+$, found 344.0721. $[\alpha]_D^{25} = -62.1$ ($c = 0.132$ g/100 mL, CH_3OH).

4.1.46. (2S,4S)-1-[(3R)-3-amino-4-(3,4-dichlorophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8j**)

In the same manner as described for **8a**, **8j** was prepared from **18j**. Yield: 80.1%. HPLC: 95.42%, $t_R = 1.84$ min. ^1H NMR (CD_3OD, 400 MHz): δ 7.49–7.55 (m, 2H), 7.22–7.27 (m, 1H), 5.30–5.49 (m, 1H), 4.98–5.08 (m, 1H), 3.85–3.94 (m, 2H), 3.62–3.74 (m, 1H), 2.97–3.06 (m, 2H), 2.74–2.94 (m, 1H), 2.35–2.62 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.3, 168.9, 161.5, 136.1, 132.5, 131.3, 131.2, 130.7, 128.9, 117.6, 92.5 (d, $J = 141.4$ Hz), 52.8 (d, $J = 18.8$ Hz), 49.2, 44.7, 37.0, 35.8 (d, $J = 16.7$ Hz), 34.7. MS (ESI) m/z 344 $[M+H]^+$, HRMS (ESI) calcd $C_{15}H_{17}N_3OFCl_2$ 344.0733 $[M+H]^+$, found 344.0715. $[\alpha]_D^{25} = -57.4$ ($c = 0.108$ g/100 mL, CH_3OH).

*4.1.47. (2S,4S)-1-[(3R)-3-amino-4-(3,5-difluorophenyl)butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8k**)*

In the same manner as described for **8a**, **8k** was prepared from **18k**. Yield: 68.2%. HPLC: 100.00%, t_R = 1.76 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 6.91–6.97 (m, 3H), 5.30–5.50 (m, 1H), 4.98–5.09 (m, 1H), 3.58–3.95 (m, 3H), 2.84–3.04 (m, 3H), 2.35–2.64 (m, 3H).^{13}C NMR (CD_3OD, 100 MHz): δ 169.3, 168.9, 164.4 (d, J = 10.3 Hz), 162.4 (d, J = 10.4 Hz), 161.8, 129.8, 125.6, 117.6, 112.1 (d, J = 20.5 Hz), 92.5 (d, J = 141.4 Hz), 52.8 (d, J = 18.8 Hz), 49.1, 44.6, 37.6, 35.8 (d, J = 16.7 Hz), 35.2. MS (ESI) m/z 312 $[M+H]^+$, HRMS (ESI) calcd $C_{15}H_{17}N_3OF_3$ 312.1324 $[M+H]^+$, found 312.1329. $[\alpha]_D^{25}$ = −72.0 (c = 0.10 g/100 mL, CH_3OH).

*4.1.48. (2S,4S)-1-[(3R)-3-amino-4-(2,4,5-trifluorophenyl) butanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8l**)*

In the same manner as described for **8a**, **8l** was prepared from **18l**. Yield: 85.6%. HPLC: 99.18%, t_R = 1.73 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.29–7.36 (m, 1H), 7.18–7.23 (m, 1H), 5.31–5.50 (m, 1H), 4.97–5.09 (m, 1H), 3.87–3.96 (m, 2H), 3.57–3.85 (m, 1H), 3.06 (d, J = 8.0 Hz, 2H), 2.75–3.00 (m, 1H), 2.39–2.66 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.1, 168.8, 161.6 (d, J = 27.2 Hz), 156.6 (d, J = 153.6 Hz), 149.8 (d, J = 198.8 Hz), 146.9 (d, J = 194.0 Hz), 119.1 (d, J = 16.0 Hz), 117.6, 105.7 (d, J = 23.0 Hz), 92.5 (d, J = 141.4 Hz), 52.8 (d, J = 18.8 Hz), 48.2, 44.6, 35.8 (d, J = 16.7 Hz), 34.8, 30.9. MS (ESI) m/z 330 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{16}N_3OF_4$330.1230 $[M+H]^+$, found 330.1221. $[\alpha]_D^{25}$ = −61.7 (c = 0.12 g/100 mL, CH_3OH).

*4.1.49. (2S,4S)-1-[(3S)-3-amino-4-phenylbutanoyl]-4-fluoropyrrolidine-2-carbonitrile (**8m**)*

In the same manner as described for **8a**, **8m** was prepared from **18m**. Yield: 62.2%. HPLC: 95.81%, t_R = 1.78 min. ^{1}H NMR (CD_3OD, 400 MHz), δ 7.26–7.37 (m, 5H), 5.32–5.45 (m, 1H), 4.89–4.93 (m, 1H), 3.56–3.80 (m, 3H), 2.87–3.04 (m, 2H), 2.30–2.65 (m, 4H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.8, 169.1, 135.9, 129.0, 128.7, 127.1, 117.7, 92.5 (d, J = 141.4 Hz), 52.8 (d, J = 18.8 Hz), 49.5, 44.6, 38.7, 35.8 (d, J = 16.7 Hz), 35.3. MS (ESI) m/z 276 $[M+H]^+$. HRMS (ESI) calcd $C_{15}H_{19}N_3OF$ 276.1512 $[M+H]^+$, found 276.1523. $[\alpha]_D^{25}$ = −12.8 (c = 0.125 g/100 mL, CH_3OH).

*4.1.50. (2S,3aS,6aS)-1-[(3R)-3-amino-4-phenylbutanoyl] octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9a**)*

Compound **25a** (108 mg) was suspended in Et_2O–HCl at ice-bathe, and warmed to room temperature. After stirring for 20 h, the solvent was removed. The residue was added 30 mL Et_2O, and the white solid was precipitated, filtered, and dried to afford compound **9a** (64 mg) as HCl salt. Yield: 70.5%. HPLC: 98.28%, t_R = 2.01 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.27–7.48 (m, 5H), 4.84–4.96 (m, 1H), 4.14–4.19 (m, 1H), 3.82–3.90 (m, 1H), 3.03–3.14 (m, 1H), 2.77–3.00 (m, 3H), 2.51–2.73 (m, 1H), 2.32–2.44 (m, 1H), 1.81–2.07 (m, 4H), 1.47–1.73 (m, 3H), 1.28–1.32 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.5, 135.4, 129.0, 128.8, 127.3, 117.1, 63.9, 49.6, 46.4, 44.0, 37.9, 34.4, 34.1, 33.6, 31.7, 25.3. MS (ESI) m/z 298 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{18}H_{24}N_3O$ 298.1919 $[M+H]^+$, found 298.1914.

*4.1.51. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(2-chlorophenyl)butanoyl] octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9b**)*

In the same manner as described for **9a**, **9b** was prepared from **25b**. Yield: 75.2%. HPLC: 95.76%, t_R = 2.15 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.43–7.52 (m, 1H), 7.29–7.35 (m, 3H), 4.83–4.96 (m, 1H), 4.12–4.21 (m, 1H), 3.91–4.01 (m, 1H), 3.12–3.24 (m, 2H), 2.34–2.92 (m, 4H), 1.83–2.08 (m, 4H), 1.47–1.72 (m, 3H), 1.29–1.34 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 134.2, 133.2, 131.7, 129.7, 129.2, 127.4, 119.1, 63.9, 48.3, 46.4, 44.0, 35.6, 34.4, 34.1, 33.7, 31.7, 25.2. MS (ESI) m/z 332 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{18}H_{23}N_3OCl$ 332.1530 $[M+H]^+$, found 332.1535.

*4.1.52. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(2-methylphenyl)butanoyl] octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9c**)*

In the same manner as described for **9a**, **9c** was prepared from **25c**. Yield: 68.9%. HPLC: 95.09%, t_R = 2.01 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.18–7.23 (m, 4H), 4.79–4.96 (m, 1H), 4.11–4.18 (m, 1H), 3.80–3.89 (m, 1H), 2.76–3.11 (m, 4H), 2.32–2.37 (m, 5H), 1.81–2.04 (m, 4H), 1.66–1.73 (m, 3H), 1.30 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 136.8, 133.5, 130.7, 129.9, 127.3, 126.1, 119.1, 63.9, 48.5, 46.4, 44.0, 35.4, 34.4, 34.1, 33.7, 31.7, 25.3, 18.1. MS (ESI) m/z 312 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{19}H_{26}N_3O$ 312.2076 $[M+H]^+$, found 312.2076.

*4.1.53. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(3-fluorophenyl)butanoyl] octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9d**)*

In the same manner as described for **9a**, **9d** was prepared from **25d**. Yield: 76.5%. HPLC: 99.26%, t_R = 1.98 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.36–7.41 (m, 1H), 7.03–7.11 (m, 3H), 4.86–4.96 (m, 1H), 4.17–4.23 (m, 1H), 3.82–3.90 (m, 1H), 2.88–3.11 (m, 3H), 2.32–2.85 (m, 3H), 1.83–2.09 (m, 4H), 1.50–1.73 (m, 3H), 1.29–1.32 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 163.1 (d, J = 195.2 Hz), 138.2, 130.6, 125.0, 119.1, 115.8 (d, J = 17.3 Hz), 114.0 (d, J = 9.1 Hz), 63.9, 48.1, 46.4, 44.0, 37.6, 34.5, 33.7, 31.7, 25.2. MS (ESI) m/z 316 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{18}H_{23}N_3OF$ 316.1825 $[M+H]^+$, found 316.1816.

*4.1.54. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(4-fluorophenyl)butanoyl] octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9e**)*

In the same manner as described for **9a**, **9e** was prepared from **25e**. Yield: 78.6%. HPLC: 97.36%, t_R = 2.17 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.27–7.32 (m, 2H), 7.07–7.13 (m, 2H), 4.86–4.96 (m, 1H), 4.18–4.24 (m, 1H), 3.80–3.89 (m, 1H), 2.89–3.08 (m, 3H), 2.32–2.83 (m, 3H), 1.84–2.07 (m, 4H), 1.51–1.73 (m, 3H), 1.28–1.34 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 162.4 (d, J = 218.8 Hz), 131.6, 130.9, 119.1, 115.4 (d, J = 16.3 Hz), 63.9, 49.6, 46.4, 44.0, 37.1, 34.5, 34.3, 33.7, 31.7, 25.2. MS (ESI) m/z 316 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{18}H_{23}N_3OF$ 316.1825 $[M+H]^+$, found 316.1843.

*4.1.55. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(4-iodophenyl)butanoyl] octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9f**)*

In the same manner as described for **9a**, **9f** was prepared from **25f**. Yield: 80.2%. HPLC: 95.87%, t_R = 2.23 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.70–7.76 (m, 2H), 7.00–7.10 (m, 2H), 4.90–4.98 (m, 1H), 4.13–4.19 (m, 1H), 3.82–3.89 (m, 1H), 2.89–3.02 (m, 3H), 2.33–2.77 (m, 3H), 1.85–2.07 (m, 4H), 1.47–1.73 (m, 3H), 1.26–1.32 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.4, 160.9, 137.9, 135.2, 131.2, 119.1, 63.9, 49.4, 46.4, 44.0, 37.5, 34.5, 34.3, 33.6, 31.7, 25.3. MS (ESI) m/z 424 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{18}H_{23}N_3OI$ 424.0886 $[M+H]^+$, found 424.0897.

*4.1.56. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(4-(trifluoromethyl) phenyl)butanoyl]octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9g**)*

In the same manner as described for **9a**, **9g** was prepared from **25g**. Yield: 78.2%. HPLC: 97.95%, t_R = 2.12 min. ^{1}H NMR(CD_3OD, 400 MHz): δ 7.65–7.74 (m, 2H), 7.42–7.49 (m, 2H), 4.86–4.96 (m, 1H), 4.18–4.25 (m, 1H), 3.89–3.95 (m, 1H), 3.06–3.15 (m, 2H), 2.54–2.90 (m, 3H), 2.35–2.60 (m, 1H), 1.83–2.08 (m, 4H), 1.48–1.72 (m, 3H), 1.29–1.34 (m, 1H).^{13}C NMR (CD_3OD, 100 MHz): δ 169.3, 160.9, 140.1, 129.8, 125.6, 123.7, 119.1, 63.9, 48.1, 46.4, 44.0, 37.7, 34.4, 34.2, 33.7, 31.7, 25.2. MS (ESI) m/z 366 $[M+H]^+$. HRMS (ESI) m/z calcd $C_{19}H_{23}N_3OF_3$ 366.1793 $[M+H]^+$, found 366.1785.

4.1.57. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(4-methoxyphenyl) butanoyl]octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9h**)

In the same manner as described for **9a**, **9h** was prepared from **25h**. Yield: 68.7%. HPLC: 96.06%, t_R = 1.99 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.12–7.21 (m, 2H), 6.89–6.95 (m, 2H), 4.84–4.96 (m, 1H), 4.12–4.23 (m, 1H), 3.76–3.81 (m, 4H), 2.94–3.02 (m, 1H), 2.68–2.92 (m, 3H), 2.34–2.63 (m, 2H), 1.83–2.08 (m, 4H), 1.53–1.72 (m, 3H), 1.29–1.34 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.5, 159.3, 130.1, 127.0, 119.1, 114.1, 63.9, 54.3, 49.6, 46.4, 44.0, 37.3, 34.5, 34.1, 33.7, 31.7, 25.2. MS (ESI) *m/z* 328 $[M+H]^+$. HRMS (ESI) *m/z* calcd $C_{19}H_{26}N_3O_2$ 328.2025 $[M+H]^+$, found 328.2024.

4.1.58. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(2,4-dichlorophenyl) butanoyl)octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9i**)

In the same manner as described for **9a**, **9i** was prepared from **25i**. Yield: 74.8%. HPLC: 95.61%, t_R = 2.24 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.50–7.61 (m, 1H), 7.31–7.39 (m, 2H), 4.84–4.96 (m, 1H), 4.18–4.24 (m, 1H), 3.90–3.99 (m, 1H), 3.12–3.24 (m, 2H), 2.71–2.92 (m, 2H), 2.35–2.61 (m, 2H), 1.86–2.09 (m, 4H), 1.55–1.73 (m, 3H), 1.28–1.35 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.3, 135.0, 134.1, 132.7, 132.2, 129.3, 127.6, 119.1, 63.9, 46.4, 44.0, 35.3, 34.4, 34.1, 33.6, 31.7, 25.3. MS (ESI) *m/z* 366 $[M+H]^+$. HRMS (ESI) *m/z* calcd $C_{18}H_{22}N_3OCl_2$ 366.1140 $[M+H]^+$, found 366.1127.

4.1.59. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(3,4-dichlorophenyl) butanoyl]octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9j**)

In the same manner as described for **9a**, **9j** was prepared from **25j**. Yield: 68.3%. HPLC: 98.32%, t_R = 1.96 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.49–7.54 (m, 2H), 7.18–7.23 (m, 1H), 4.86–4.94 (m, 1H), 4.20–4.28 (m, 1H), 3.82–3.90 (m, 1H), 2.77–3.04 (m, 5H), 2.36–2.62 (m, 2H), 1.87–2.06 (m, 4H), 1.56–1.73 (m, 3H), 1.29–1.34 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.2, 161.0, 136.3, 132.4, 131.2, 130.7, 129.0, 119.0, 63.9, 49.4, 46.5, 44.0, 37.1, 34.5, 34.4, 33.7, 31.7, 25.3. MS (ESI) *m/z* 366 $[M+H]^+$. HRMS (ESI) *m/z* calcd $C_{18}H_{22}N_3OCl_2$ 366.1140 $[M+H]^+$, found 366.1125.

4.1.60. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(3,5-difluorophenyl) butanoyl]octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9k**)

In the same manner as described for **9a**, **9k** was prepared from **25k**. Yield: 71.2%. HPLC: 96.08%, t_R = 2.16 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 6.90–6.99 (m, 3H), 4.91–4.97 (m, 1H), 4.22–4.29 (m, 1H), 3.89 (br, 1H), 3.00–3.08 (m, 1H), 2.57–2.94 (m, 3H), 2.35–2.45 (m, 1H), 1.85–2.09 (m, 4H), 1.57–1.76 (m, 3H), 1.28–1.32 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.3, 164.3 (d, *J* = 10.4 Hz), 132.4 (d, *J* = 10.3 Hz), 139.8, 119.1, 112.1, 102.5, 63.9, 49.2, 46.4, 44.1, 37.5, 34.4, 34.1, 33.8, 31.7, 25.2. MS (ESI) *m/z* 334 $[M+H]^+$. HRMS (ESI) *m/z* calcd $C_{18}H_{22}N_3OF_2$ 334.1731 $[M+H]^+$, found 334.1726.

4.1.61. (2S,3aS,6aS)-1-[(3R)-3-amino-4-(2,4,5-trifluorophenyl) butanoyl]octahydrocyclopenta[b]pyrrole-2-carbonitrile (**9l**)

In the same manner as described for **9a**, **9l** was prepared from **25l**. Yield: 84.1%. HPLC: 96.67%, t_R = 1.96 min. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.22–7.34 (m, 2H), 4.90–4.96 (m, 1H), 4.23–4.29 (m, 1H), 3.82–3.90 (m, 1H), 3.12–3.21 (m, 1H), 3.01–3.08 (m, 2H), 2.57–2.76 (m, 3H), 2.37–2.42 (m, 1H), 1.88–2.09 (m, 4H), 1.62–1.73 (m, 3H), 1.28–1.31 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 169.1, 161.6 (d, *J* = 27.0 Hz), 156.6 (d, *J* = 187.0 Hz), 149.6 (d, *J* = 198.7 Hz), 146.7 (d, *J* = 194.2 Hz), 119.2 (d, *J* = 15.5 Hz), 119.1, 105.7 (d, *J* = 22.8 Hz), 63.9, 48.3, 46.4, 44.0, 35.7, 34.6, 34.4, 33.7, 31.7, 30.8. MS (ESI) *m/z* 352 $[M+H]^+$. HRMS (ESI) *m/z* calcd $C_{18}H_{21}N_3OF_3$ 352.1673 $[M+H]^+$, found 352.1619. $[\alpha]_D^{25}$ = +25.2 (*c* = 0.23 g/100 mL, CH_3OH).

4.2. In vitro DPP4, DPP7, DPP8, DPP9 and FAP enzyme assay

4.2.1. Preparation of the DPPs enzyme

The DPP4, DPP7, DPP8, DPP9 and FAP enzymes were expressed in high five cells using a baculoviral system (Bac-To-Bac; Life Technologies) according to the literature [35], and his6-tagged recombinant proteins were purified by Ni-NTA resin individually.

4.2.2. Enzyme-based assay of DPP4

To measure the activity of DPP4, a continuous fluorometric assay was employed using Ala-Pro-AMC, which is cleaved by the enzyme to release the fluorescent aminomethylcoumarin (AMC). Liberation of AMC was monitored using an excitation wavelength of 355 nm and an emission wavelength of 460 nm using Envision microplate reader (PerkinElmer). A typical reaction contained 50 pmol/L enzyme, 10 μmol/L Ala-Pro-AMC, different concentrations of the compounds synthesized in this work, and assay buffer (100 mmol/L HEPES, pH 7.5, 0.1 mg/mL BSA) in a total reaction volume of 50 μL. The DPP4 enzyme used in these studies was soluble human recombinant protein produced in a baculovirus expression system (Bac-To-Bac; Life Technologies). The dose response of inhibition test was carried out in duplicate. And the IC_{50} data was calculated using the software GraphPad Prism 5, and chosen the equation "sigmoidal dose–response (variable slope)" for curve fitting.

4.2.3. Enzyme-based assay of DPPs inhibition selectivity

All DPPs proteins were expressed in high five cells using a baculoviral system, and the activities of DPPs were assayed by continuous fluorometric method. We used Nle-Pro-AMC as substrate to measure the activities of DPP7 and FAP, and Ala-Pro-AMC for DPP8 and DPP9 in the optimized pH (5.5 for DPP7 and 8.0 for other members) assay system. The selective dose response of inhibition test on DPPs and data analysis is the same as DPP4 assay system.

4.2.4. Pharmacokinetic profiles in SD rats

Compounds **8l** and **9l** were administered to SD rats. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with methanol containing an internal standard. After centrifugation, the supernatant was diluted with methanol and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

4.2.5. Oral glucose tolerance tests in ICR mice and chronic study in C57BKS db/db mice

All animal experiments were approved by the Animal Care and Use Committee of Shanghai Institute of Materia Medica. For the acute single dose study, vehicle (0.5% methylcellulose, 10 mL/kg), compounds **8l** (5 and 15 mg/kg), **9l** (5 and 15 mg/kg) and **LAF-237** (15 mg/kg) were administered to ICR mice after 16-h starvation, then the oral glucose tolerance test (2.5 g/kg) was conducted after 30 min of the single dose, the blood glucose level at 0, 15, 30, 60, 90 and 120 min were recorded for area under curve calculation. For the chronic study in C57BKS *db*/*db* mice with 15 mg/kg/day **9l** treatment, oral glucose tolerance test (1.5 g/kg) was carried out after 6-h starvation of 5th-week treatment, the blood glucose level was recorded for the glucose excursion capacity evaluation. Vehicle (0.5% methylcellulose, 10 mL/kg/day) and **LAF-237** (15 mg/kg/day) were included as negative and positive control, respectively. At the end of study, blood samples were collected and plasma insulin was measured using ELISA kits (Linco Research), plasma triglyceride and total cholesterol were assayed using kits from Shanghai Fudanzhangjiang, free fatty acid was determined by Wako Diagnostics (for NEFA).

The results are presented as the mean ± SE. Differences between the groups were analyzed with the Student's *t*-test. *, $p < 0.05$ or **, $p < 0.01$ was regarded as statistically significant.

4.2.6. hERG testing using FluxOR™ thallium assay

Step1: Growing cells. CHO-hERG-ZG cells are grown in 75 cm^2 flask with complete medium with 100 μg/mL G418 and 100 μg/mL Hygromycin B until 80–90% confluency. Wash cells with PBS once. Incubate cells with 1 mL 0.25% Trypsin until all cells are rounded and can be easily dislodged from the surface. Add 10 mL complete medium to stop Trypsin activity. Disassociate cells by thoroughly, repetitively pipetting. Transfer them to 50 mL Falcon tube and spin down at 1000 rpm for 5 min. Aspirate medium and resuspend cells using a small volume of complete medium, like 0.5 mL. Count cell density. Step 2: Cell seeding. CHO-hERG-ZG cells are plated into 96-well plates and after plating, tap plates on sides to separate cells and let plates sit in the dark at RT for 30 min before incubation at 37 °C for 16–18 h. Cells will reach 80% confluency. After overnight incubation the cells are media changed in loading buffer (old media is tapped out) and incubated in the dark at RT for 90 min. Remove the loading buffer and replace with assay buffer. Compounds **8l** and **9l** were added to the cell plate. The cell plate is incubated with compounds for 20 min in the dark at RT. Load the cell plates on FDSS. Fluorescent signals will be recorded every 2 s till 10 s. At 10 s, stimulus buffer will be added to cells. Then fluorescent signals will be recorded every second till 180 s, data QC on FDSS.

4.3. Binding studies

The DPP4 protein was extracted from RCSB Protein Data Bank (PDB ID: 2AJL). Compounds were generated using Sybyl program. Gasteiger-Hückel charge was used and the conformation of each compound was minimized using default parameters. Docking studies were performed using Glide program. The DPP4 protein was processed by minimal minimization with OPLS2005 force field. The grid was sized to 15 Å in each direction at the center of the binding pocket. Compounds were prepared for docking using Ligprep. Ligand docking was performed in XP mode and flexible option, with up to 100 poses saved per molecule. Glide score was consulted for results analyzing.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.059.

References

[1] B. Isomaa, A major health hazard: the metabolic syndrome, Life Sci. 73 (2003) 2395–2411.
[2] (a) S.A. Ross, E.A. Gulve, M. Wang, Chemistry and biochemistry of type 2 diabetes, Chem. Rev. 104 (2004) 1255–1282;
(b) M.L. Mohler, Y. He, Z. Wu, D.J. Hwang, D.D. Miller, Recent and emerging anti-diabetes targets, Med. Res. Rev. 29 (2009) 125–195;
(c) M. Virally, J.F. Blicklé, J. Girard, S. Halimi, D. Simon, P.J. Guillausseau, Type 2 diabetes mellitus: epidemiology, pathophysiology, unmet needs and therapeutical perspectives, Diabetes Metab. 33 (2007) 231–244.
[3] UK Prospective Diabetes Study 7: response of fasting plasma glucose to diet therapy in newly presenting type II diabetic patients, UKPDS Group, Metabolism 39 (1990) 905–912.
[4] L.A. Witters, The blooming of the French lilac, J. Clin. Investig 108 (2001) 1105–1107.
[5] For recent reviews, see: (a) A.E. Weber, Dipeptidyl peptidase IV inhibitors for the treatment of diabetes, J. Med. Chem. 47 (2004) 4135–4141;
(b) K. Augustyns, P.V. der Veken, K. Senten, A. Haemers, The therapeutic potential of inhibitors of dipeptidyl peptidase IV (DPP IV) and related proline-specific dipeptidyl aminopeptidases, Curr. Med. Chem. 12 (2005) 971–998;
(c) P. Van der Veken, A. Soroka, I. Brandt, Y.S. Chen, M.B. Maes, A.M. Lambeir, X. Chen, A. Haemers, S. Scharpe, K. Augustyns, I. De Meester, Prolyl peptidases related to dipeptidyl peptidase IV: potential of specific inhibitors in drug discovery, Curr. Top. Med. Chem. 7 (2007) 621–635.
[6] (a) A. Yaron, F. Nadier, Proline-dependent structural and biological properties of peptides and proteins, Crit. Rev. Biochem. Mol. Biol. 28 (1993) 31–81;
(b) A.M. Lambeir, C. Durnx, S. Scharpe, I. Meester, Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV, Crit. Rev. Clin. Lab. Sci. 40 (2003) 209–294;
(c) R. Mentlein, Dipeptidyl-peptidase IV (CD26)–role in the inactivation of regulatory peptides, Regul. Pept. 85 (1999) 9–24.
[7] (a) M.D. Gorrell, V. Gysbers, G.W. McCaughan, CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes, Scand. J. Immunol. 54 (2001) 249–264;
(b) K. Aertgeerts, S. Ye, M.G. Tennant, M.L. Kraus, J. Rogers, B.C. Sang, R.J. Skene, D.R. Webb, G.S. Prasad, Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation, Protein Sci. 13 (2004) 412–421.
[8] (a) J.F. Gautier, S. Fetita, E. Sobngwi, C. Salaün-Martin, Biological actions of the incretins GIP and GLP-1 and therapeutic perspectives in patients with type 2 diabetes, Diabetes Metab. 31 (2005) 233–242;
(b) C.F. Deacon, M.A. Nauck, J. Meier, K. Hücking, J.J. Holst, Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide, J. Clin. Endocrinol. Metab. 85 (2000) 3575–3581.
[9] S. Mojsov, G.C. Weir, J.F. Habener, Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas, J. Clin. Investig. 79 (1987) 616–619.
[10] M.A. Nauck, M.M. Heimesaat, K. Behle, J.J. Holst, M.S. Nauck, R. Ritzel, M. Hufner, W.H. Schmiegel, Effects of glucagon-like peptide 1 on counter-regulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers, J. Clin. Endocrinol. Metab. 87 (2002) 1239–1246.
[11] A. Wettergren, B. Schjoldager, P.E. Mortensen, J. Myhre, J. Christiansen, J.J. Holst, Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man, Dig. Dis. Sci. 38 (1993) 665–673.
[12] S.W. Wright, M.J. Ammirati, K.M. Andrews, A.M. Brodeur, D.E. Danley, S.D. Doran, J.S. Lillquist, L.D. McClure, R.K. McPherson, S.J. Orena, J.C. Parker, J. Polivkova, X. Qiu, W.C. Soeller, C.B. Soglia, J.L. Treadway, M.A. VanVolkenburg, H. Wang, D.C. Wilder, T.V. Olson, *cis*-2,5-Dicyanopyrrolidine inhibitors of dipeptidyl peptidase IV: synthesis and in vitro, in vivo, and X-ray crystallographic characterization, J. Med. Chem. 49 (2006) 3068–3076.
[13] D.J. Madar, H. Kopecka, D. Pireh, H. Yong, Z. Pei, X. Li, P.E. Wiedeman, S.W. Djuric, T.W. Von Geldern, M.G. Fickes, L. Bhagavatula, T. McDermott, S. Wittenberger, S.J. Richards, K.L. Longenecker, K.D. Stewart, T.H. Lubben, S.J. Ballaron, M.A. Stashko, M.A. Long, H. Wells, B.A. Zinker, A.K. Mika, D.W. Beno, A.J. Kempf-Grote, J. Polakowski, J. Segreti, G.A. Reinhart, R.M. Fryer, H.L. Sham, J.M. Trevillyan, Discovery of 2-[4-{{2-(2*S*,5*R*)-2-cyano-5-ethynyl-1-pyrrolidinyl]-2-oxoethyl]amino]- 4-methyl-1-piperidinyl]-4-pyridinecarboxylic acid (ABT-279): a very potent, selective, effective, and well-tolerated inhibitor of dipeptidyl peptidase-IV, useful for the treatment of diabetes, J. Med. Chem. 49 (2006) 6416–6420.
[14] H. Sakashita, H. Kitajima, M. Nakamura, F. Akahoshi, Y. Hayashi, 1-((*S*)-γ-substituted prolyl)-(*S*)-2-cyanopyrrolidine as a novel series of highly potent DPP-IV inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 2441–2445.
[15] H. Fukushima, A. Hiratate, M. Takahashi, A. Mikami, M. Saito-Hori, E. Munetomo, K. Kitano, S. Chonan, H. Saito, A. Suzuki, Y. Takaoka, K. Yamamoto, Synthesis and structure-activity relationships of potent 4-fluoro-2-cyanopyrrolidine dipeptidyl peptidase IV inhibitors, Bioorg. Med. Chem. 16 (2008) 4093–4106.
[16] P. Mattei, M. Boehringer, P. Di Giorgio, H. Fischer, M. Hennig, J. Huwyler, B. Kocer, B. Kuhn, B.M. Loeffler, A. Macdonald, R. Narquizian, E. Rauber, E. Sebokova, U. Sprecher, Discovery of carmegliptin: a potent and long-acting dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, Bioorg. Med. Chem. Lett. 20 (2010) 1109–1113.
[17] H.M. Pattzi, S. Pitale, M. Alpizar, C. Bennett, A.M. O'Farrell, J. Li, J.M. Cherrington, H.P. Guler, PHX1149-PROT202 Study Group, Dutogliptin, a selective DPP4 inhibitor, improves glycaemic control in patients with type 2 diabetes: a 12-week, double-blind, randomized, placebo-controlled, multi-centre trial, Diabetes Obes. Metab. 12 (2010) 348–355.
[18] T.P. Cho, Y.F. Long, L.Z. Gang, W. Yang, L.H. Jun, S.G. Yuan, F.J. Hong, W. Lin, G.D. Liang, Z. Lei, L.J. Jing, G.A. Shen, S.G. Hong, W. Dan, F. Ying, Y.P. Ke, L. Ying,

F. Jun, M.X. Tai, Synthesis and biological evaluation of azobicyclo[3.3.0] octane derivatives as dipeptidyl peptidase 4 inhibitors for the treatment of type 2 diabetes, Bioorg. Med. Chem. Lett. 20 (2010) 3565–3568.

[19] Y. Nishio, H. Kimura, N. Sawada, E. Sugaru, M. Horiguchi, M. Ono, Y. Furuta, M. Sakai, Y. Masui, M. Otani, T. Hashizuka, Y. Honda, J. Deguchi, T. Nakagawa, H. Nakahira, 2-({6-[(3R)-3-amino-3-methylpiperidine-1-yl]-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydro-5*H*-pyrrolo[3,2-d]pyrimidine-5-yl}methyl)-4-fluorobenzonitrile (DSR-12727): a potent, orally active dipeptidyl peptidase IV inhibitor without mechanism-based inactivation of CYP3A, Bioorg. Med. Chem. 19 (2011) 5490–5499.

[20] (a) J. Xu, H.O. Ok, E.J. Gonzalez, L.F. Colwell Jr., B. Habulihaz, H. He, B. Leiting, K.A. Lyons, F. Marsilio, R.A. Patel, J.K. Wu, N.A. Thornberry, A.E. Weber, E.R. Parmee, Discovery of potent and selective beta-homophenylalanine based dipeptidyl peptidase IV inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 4759–4762;
(b) L.L. Brockunier, J. He, L.F. Colwell Jr., B. Habulihaz, H. He, B. Leiting, K.A. Lyons, F. Marsilio, R.A. Patel, Y. Teffera, J.K. Wu, N.A. Thornberry, A.E. Weber, E.R. Parmee, Substituted piperazines as novel dipeptidyl peptidase IV inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 4763–4766.

[21] D. Kim, L. Wang, M. Beconi, G.J. Eiermann, M.H. Fisher, H. He, G.J. Hickey, J.E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M.E. McCann, R.A. Patel, A. Petrov, G. Scapin, S.B. Patel, R.S. Roy, J.K. Wu, M.J. Wyvratt, B.B. Zhang, L. Zhu, N.A. Thornberry, A.E. Weber, (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, J. Med. Chem. 48 (2005) 141–151.

[22] E.B. Villhauer, J.A. Brinkman, G.B. Naderi, B.F. Burkey, B.E. Dunning, K. Prasad, B.L. Mangold, M.E. Russell, T.E. Hughes, 1-[[(3-hydroxy-1-adamantyl)amino] acetyl]-2-cyano-*(S)*-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties, J. Med. Chem. 46 (2003) 2774–2789.

[23] D.J. Augeri, J.A. Robl, D.A. Betebenner, D.R. Magnin, A. Khanna, J.G. Robertson, A. Wang, L.M. Simpkins, P. Taunk, Q. Huang, S.P. Han, B. Abboa-Offei, M. Cap, L. Xin, L. Tao, E. Tozzo, G.E. Welzel, D.M. Egan, J. Marcinkeviciene, S.Y. Chang, S.A. Biller, M.S. Kirby, R.A. Parker, L.G. Hamann, Discovery and preclinical profile of saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, J. Med. Chem. 48 (2005) 5025–5037.

[24] J. Feng, Z. Zhang, M.B. Wallace, J.A. Stafford, S.W. Kaldor, D.B. Kassel, M. Navre, L. Shi, R.J. Skene, T. Asakawa, K. Takeuchi, R. Xu, D.R. Webb, S.L. Gwaltney 2nd, Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV, J. Med. Chem. 50 (2007) 2297–2300.

[25] M. Eckhardt, E. Langkopf, M. Mark, M. Tadayyon, L. Thomas, H. Nar, W. Pfrengle, B. Guth, R. Lotz, P. Sieger, H. Fuchs, F. Himmelsbach, 8-(3-(*R*)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes, J. Med. Chem. 50 (2007) 6450–6453.

[26] (a) B. Ahren, E. Simonsson, H. Larsson, M. Landin-Olsson, H. Torgeirsson, P.A. Jansson, M. Sandqvist, P. Bavenholm, S. Efendic, J.W. Eriksson, S. Dickinson, D. Holmes, Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes, Diabetes Care 25 (2002) 869–875;
(b) B. Ahren, R. Gomis, E. Standl, D. Mills, A. Schweizer, Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes, Diabetes Care 27 (2004) 2874–2880;
(c) B. Ahren, L. Landing-Olsson, P.A. Jansson, M. Sevensson, D. Holmes, A. Schweizer, Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes, J. Clin. Endocrinol. Metab. 89 (2004) 2078–2084;
(d) B. Ahrén, G. Pacini, J.E. Foley, A. Schweizer, Improved meal-related beta-cell function and insulin sensitivity by the dipeptidyl peptidase-IV inhibitor vildagliptin in metformin-treated patients with type 2 diabetes over 1 year, Diabetes Care 28 (2005) 1936–1940.

[27] J. Wang, Y. Feng, X. Ji, G. Deng, Y. Leng, H. Liu, Synthesis and biological evaluation of pyrrolidine-2-carbonitrile and 4-fluoropyrrolidine-2-carbonitrile derivatives as dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes, Bioorg. Med. Chem. 21 (2013) 7418–7429.

[28] X. Ji, M. Su, J. Wang, G. Deng, S. Deng, Z. Li, C. Tang, J. Li, J. Li, L. Zhao, H. Jiang, H. Liu, Design, synthesis and biological evaluation of hetero-aromatic moieties substituted pyrrole-2-carbonitrile derivatives as dipeptidyl peptidase IV inhibitors, Eur. J. Med. Chem. 75 (2014) 111–122.

[29] D.R. Magnin, J.A. Robl, R.B. Sulsky, D.J. Augeri, Y. Huang, L.M. Simpkins, P.C. Taunk, D.A. Betebenner, J.G. Robertson, B.E. Abboa-Offei, A. Wang, M. Cap, L. Xin, L. Tao, D.F. Sitkoff, M.F. Malley, J.Z. Gougoutas, A. Khanna, Q. Huang, S.P. Han, R.A. Parker, L.G. Hamann, Synthesis of novel potent dipeptidyl peptidase IV inhibitors with enhanced chemical stability: interplay between the N-terminal amino acid alkyl side chain and the cyclopropyl group of α-aminoacyl-L-*cis*-4,5-methanoprolinenitrile-based inhibitors, J. Med. Chem. 47 (2004) 2587–2598.

[30] (a) R.E. Schmieder, C. Delles, A. Mimran, J.P. Fauvel, L.M. Ruilope, Impact of telmisartan versus ramipril on renal endothelial function in patients with hypertension and type 2 diabetes, Diabetes Care 30 (2007) 1351–1356;
(b) J.E. Frampton, D.H. Peters, Ramipril, An updated review of its therapeutic use in essential hypertension and heart failure, Drugs 49 (1995) 440–466;
(c) S. Yusuf, K.K. Teo, J. Pogue, L. Dyal, I. Copland, H. Schumacher, G. Dagenais, P. Sleight, C. Anderson, Telmisartan, ramipril, or both in patients at high risk for vascular events, N. Engl. J. Med. 358 (2008) 1547–1559.

[31] (a) C.D. Haffner, D.L. McDougald, A.S. Randhawa, S.M. Reister, J.M. Lenhard, Fluoropyrrolidines as Dipeptidyl Peptidase Inhibitors. U.S. Pat. Appl. US 0171848 A1, 2004. (b) C.D. Haffner, D.L. McDougald, S.M. Reister, B.D. Thompson, C. Conlee, J. Fang, J. Bass, J.M. Lenhard, D. Croom, M.B. Secosky-Chang, T. Tomaszek, D. McConn, K. Wells-Knecht, P.R. Johnson, 2-Cyano-4-fluoro-1-thiovalylpyrrolidine analogues as potent inhibitors of DPP-IV, Bioorg. Med. Chem. Lett. 15 (2005) 5257–5261.

[32] G.R. Lankas, B. Leiting, R.S. Roy, G.J. Eiermann, M.G. Beconi, T. Biftu, C.C. Chan, S. Edmondson, W.P. Feeney, H. He, D.E. Ippolito, D. Kim, K.A. Lyons, H.O. Ok, R.A. Patel, A.N. Petrov, K.A. Pryor, X. Qian, L. Reigle, A. Woods, J.K. Wu, D. Zaller, X. Zhang, L. Zhu, A.E. Weber, N.A. Thornberry, Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9, Diabetes 54 (2005) 2988–2994.

[33] J. Wang, M. Sánchez-Roselló, J.L. Aceña, C. Del Pozo, A.E. Sorochinsky, S. Fustero, V.A. Soloshonok, H. Liu, Fluorine in pharmaceutical industry: fluorine-containing drugs introduced to the market in the last decade (2001–2011), Chem. Rev. 114 (2014) 2432–2506.

[34] C. Jamieson, E.M. Moir, Z. Rankovic, G. Wishart, Medicinal chemistry of hERG optimizations: highlights and hang-ups, J. Med. Chem. 49 (2006) 5029–5046.

[35] (a) J. Dobers, M. Zimmermann-Kordmann, M. Leddermann, T. Schewe, W. Reutter, H. Fan, Expression, purification, and characterization of human dipeptidyl peptidase IV/CD26 in Sf9 insect cells, Protein Expr. Purif. 25 (2002) 527–532;
(b) Y. Miyamoto, Y. Banno, T. Yamashita, T. Fujimoto, S. Oi, Y. Moritoh, T. Asakawa, O. Kataoka, H. Yashiro, K. Takeuchi, N. Suzuki, K. Ikedo, T. Kosaka, S. Tsubotani, A. Tani, M. Sasaki, M. Funami, M. Amano, Y. Yamamoto, K. Aertgeerts, J. Yano, H. Maezaki, Discovery of a 3-pyridylacetic acid derivative (TAK-100) as a potent, selective and orally active dipeptidyl peptidase IV (DPP-4) inhibitor, J. Med. Chem. 54 (2011) 831–850.