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(*R*)-3-Amino-1-((3a*S*,7a*S*)-octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one derivatives as potent inhibitors of dipeptidyl peptidase-4: Design, synthesis, biological evaluation, and molecular modeling

Sinan Wang [†], Mingbo Su [†], Jiang Wang [†], Zeng Li, Lei Zhang, Xun Ji, Jingya Li, Jia Li [*], Hong Liu [*]

CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, People's Republic of China

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ABSTRACT

A series of (*R*)-3-amino-1-((3a*S*,7a*S*)-octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one derivatives was designed, synthesized, and evaluated as novel inhibitors of dipeptidyl peptidase-4 (DPP-4) for the treatment of type 2 diabetes. Most of the synthesized compounds demonstrated good inhibition activities against DPP-4. Among these, compounds **3e**, **4c**, **4l**, and **4n** exhibited prominent inhibition activities against DPP-4, with IC_{50}s of 0.07, 0.07, 0.14, and 0.17 μM, respectively. The possible binding modes of compounds **3e** and **4n** with dipeptidyl peptidase-4 were also explored by molecular docking simulation. These potent DPP-4 inhibitors were optimized for the absorption, distribution, metabolism, and excretion (ADME) properties, and compound **4n** displayed an attractive pharmacokinetic profile (F = 96.3%, $t_{1/2}$ = 10.5 h).

1. Introduction

Type 2 diabetes is a progressive metabolic disorder characterized by high blood glucose in the presence of high endogenous insulin levels, causing serious vascular complications, heart disease, renal failure, blindness, significant morbidity, and mortality.[1,2] This metabolic disease has become one of the fastest growing health concerns in the world.[3] Glucagon-like peptide-1 (GLP-1) is a potent antihyperglycemic hormone, inducing glucose-dependent stimulation of insulin secretion, while inhibiting glucagon secretion.[4–7] However, active GLP-1 is rapidly degraded by the serine protease dipeptidyl peptidase-4 (DPP-4) which selectively cleaves a dipeptide from the N-terminus of GLP-1 to produce the inactive GLP-1[9–36] amide.[8,9] Consequently, inhibition of DPP-4 has emerged as a new potential approach for the treatment of type 2 diabetes.[10–13] To date, a large number of DPP-4 inhibitors have been reported.[14–18]

In our previous work, we described a series of 4-fluoropyrrolidine-2-carbonitrile and octahydrocyclopenta[*b*]pyrrole-2-carbonitrile derivatives (**1** and **2**).[19] Early examples were found to be potent and selective inhibitors of DPP-4. However, their low oral bioavailability and short half-life raised concerns regarding their pharmacokinetic suitability for oral administration. As discussed previously,[19] we hypothesized that a larger hydration sphere carried by the adjacent amides might account for this issue. In our search for structural modifications to overcome this, while maintaining favorable properties, we envisaged to replace the side chain amide with a bioisosteric (3a*S*,7a*S*)-octahydro-1*H*-indol (**3a–f** and **4a–n**, Fig. 1). Herein, we report our efforts towards the design, synthesis, biological evaluation, and molecular modeling of (*R*)-3-amino-1-((3a*S*,7a*S*)-octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one derivatives as a novel series of DPP-4 inhibitors.

2. Results and discussion

2.1. Design

The X-ray crystal study of various inhibitors in complex with DPP-4 revealed that the binding site of DPP-4 mainly comprises three parts: a S1 pocket, the N-terminal recognition region, and a S2 pocket (Fig. 2).[14,20] The hydrophobic S1 pocket is formed by residues of Ser630, Tyr631, Tyr547, Val711, Asn710, and Arg125. The N-terminal recognition region is formed by Glu205, Glu206, and

* Corresponding authors. Tel.: +86 21 50801552; fax: +86 21 50800721 (J.L.); tel./fax: +86 21 50807042 (H.L.).
E-mail addresses: jli@simm.ac.cn (J. Li), hliu@simm.ac.cn (H. Liu).
† These authors contributed equally to this work.

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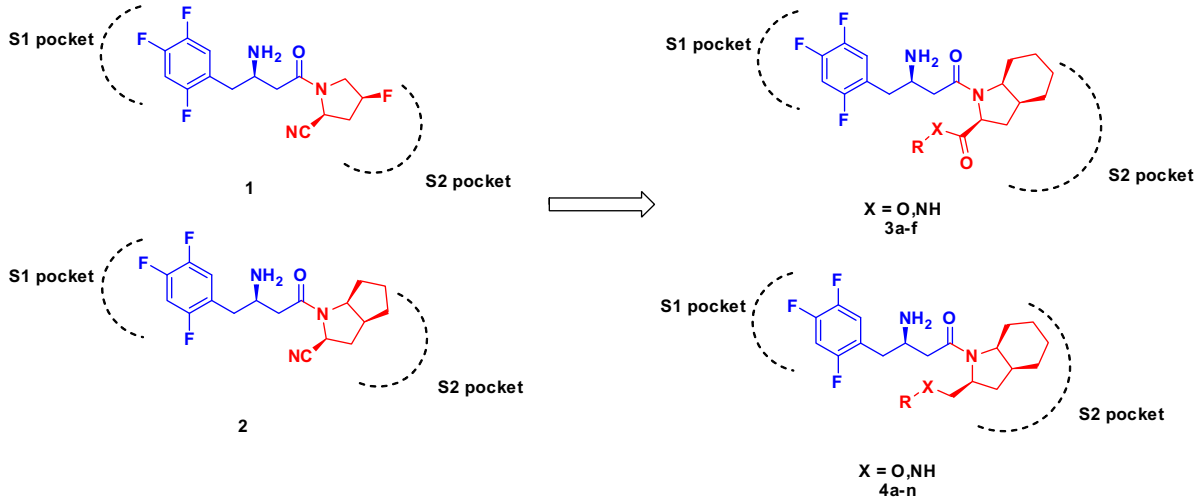


Figure 1. Design of novel (*R*)-3-amino-1-((3a*S*,7a*S*)-octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one derivatives as DPP-4 inhibitors.

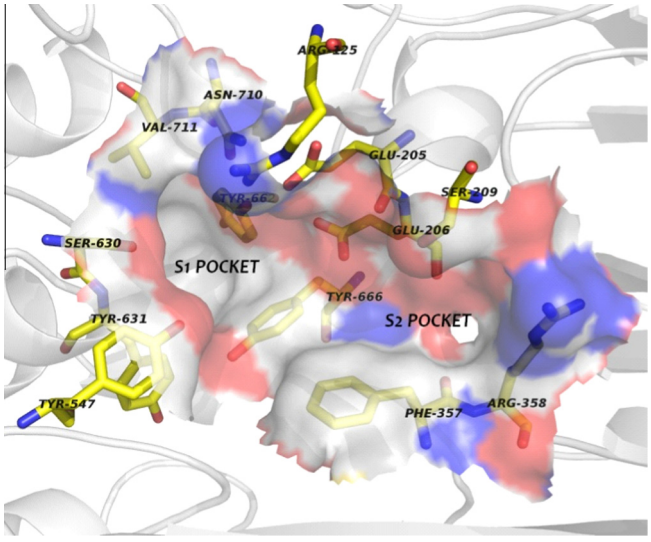


Figure 2. The binding pocket of DPP-4 (PDB ID: 1X70). This image was generated using the Pymol program.[20]

Tyr662. The hydrophobic S2 pocket, which is bigger compared with S1, is surrounded by residues Phe357, Arg358, Ser209, and Tyr666. Investigation of the structure–activity relationships (SARs) of the DPP-4 inhibitors previously reported,[14,21,22] indicated that the S2 pocket can tolerate different segments, thus we selected an (3a*S*,7a*S*)-octahydro-1*H*-indole moiety to fill in the S2 pocket and introduced various functional groups on it. To improve pharmacokinetic properties of DPP-4 inhibitors,[19] a novel series of (*R*)-3-amino-1-((3a*S*,7a*S*)-octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one derivatives (**3a–f** and **4a–n**) was designed (Fig. 1).

2.2. Chemistry

The synthesis of compounds **3a–f** is outlined in Scheme 1. Protection of the commercially available compound **5** formed compound **6**. Condensation of compound **6** with various amines (X = N) or alcohols (X = O) provided **7a–f**, followed by deprotection using trifluoroacetic acid (TFA) to form compounds **8a–f**, which were condensed with (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid **9** to provide compounds **10a–f** with a variety of substituents X and R. Deprotection of compounds **10a–f** using TFA in dichloromethane afforded target compounds **3a–f**.

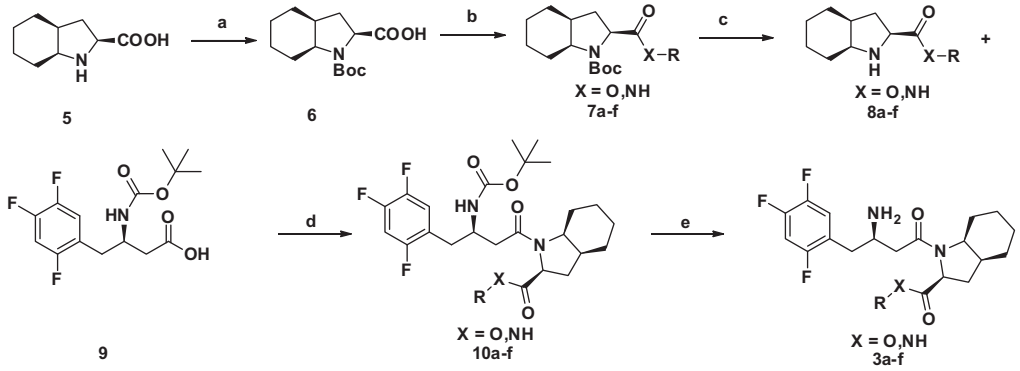
Scheme 2 depicts the sequence of reactions that led to the preparation of compounds **4a–f** using (2*S*,3a*S*,7a*S*)-1-(*tert*-butoxycarbonyl)octahydro-1*H*-indole-2-carboxylic acid (**6**) as the starting material. In general, reducing the carboxyl group in compound **6** to hydroxyl group using $LiAlH_4$ in tetrahydrofuran (THF) afforded derivative **11**, which was then reacted with alkyl or aryl acyl chloride to give compounds **12a–f**, respectively. The target compounds **13a–f** were prepared from **12a–f** and (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid **9** using standard coupling conditions followed by deprotection of the amines **14a–f**, which afforded target compounds **4a–f**.

Compounds **4g–n** were synthesized according to Scheme 3. Compound **11** was transformed to **16** using *p*-toluenesulfonyl chloride followed by NaN_3. After deprotection, condensation of **17** with (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid **9** provided compound **18**, which was then reduced by H_2, Pd/C in MeOH to give compound **19**. Condensation of compound **19** with various acyl chloride or sulfonyl chloride afforded compounds **20g–n**. Deprotection of Boc derivatives **20g–n** by treatment with TFA in CH_2Cl_2 provided target compounds **4g–n**.

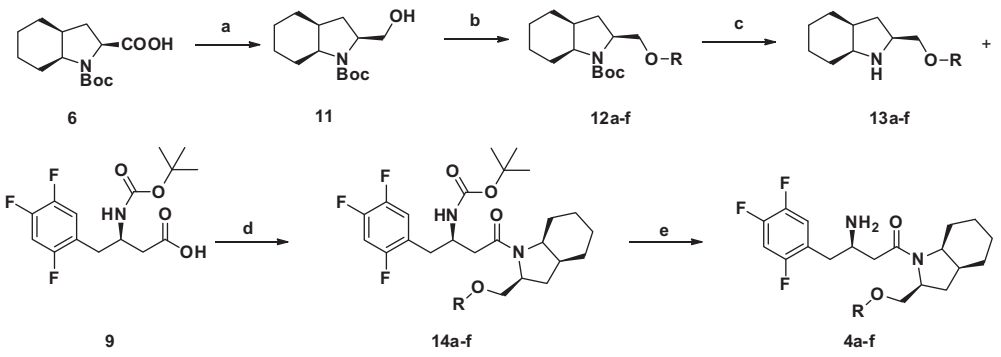
2.3. In vitro enzyme inhibition studies and structure–activity relationships

All the synthesized compounds were evaluated in vitro for inhibition of human recombinant DPP-4. Inhibitory potencies were measured by monitoring the hydrolytic reaction of Ala-Pro-AMC by human DPP-4 with **Sitagliptin** as the positive control. The results were reported as concentrations for 50% inhibition (IC_{50}). DPP-7, DPP-8, DPP-9, and fibroblast activation protein (FAP) data were also presented because the importance of selectivity over DPP-8/9 has been frequently mentioned in animal toxicity studies.[23–27] SARs for these compounds are summarized in Tables 1–3.

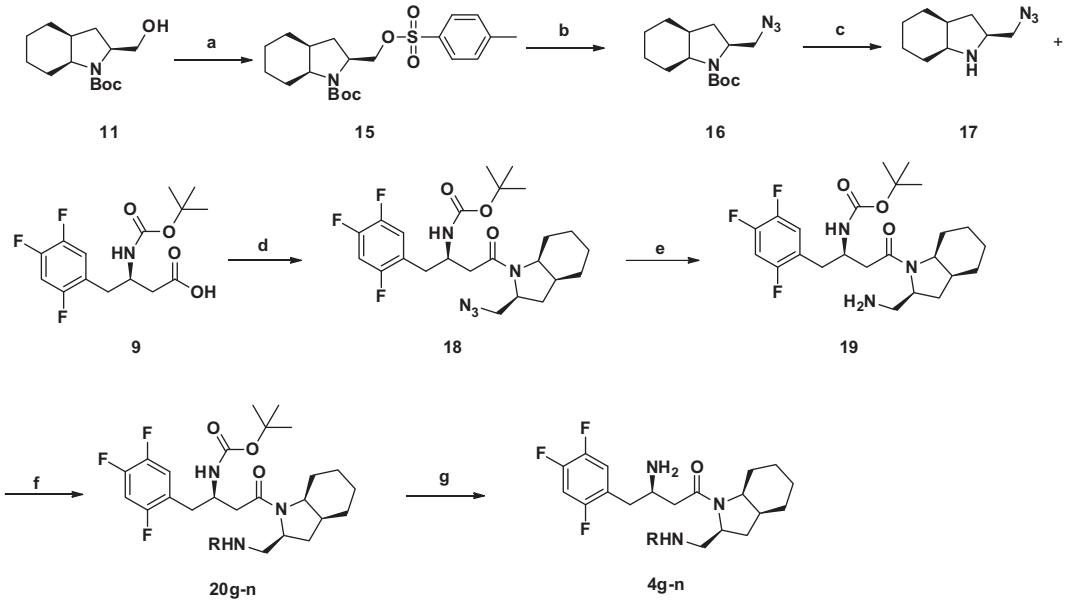
To obtain good inhibitory activity against DPP-4, various amides and esters were prepared. As shown in Table 1, introduction of the simple *N*-phenyl-acetamide **3a** resulted in DPP-4 inhibitory activity with an IC_{50} value of 5.81 μM. Substitution of *N*-benzyl-acetamide **3b** increased the activity (IC_{50} = 0.34 μM) by approximately 17-fold compared to that of **3a**. In case of three-membered *N*-cyclopropyl-acetamide **3c** and *N*-(cyclopropylmethyl)-acetamide **3d** increased potency by 15.3 and 7.6 fold compared with *N*-phenyl-acetamide **3a**. Generally, introduction of an aliphatic ring



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, TEA, CH_2Cl_2; (b) HOBT, EDCI, DMAP, TEA, CH_2Cl_2, overnight; (c) CF_3COOH, CH_2Cl_2, overnight; (d) EDCI, DMAP, TEA, CH_2Cl_2, overnight; and (e) CF_3COOH, CH_2Cl_2, overnight.



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, overnight; (b) acyl chloride, TEA, CH_2Cl_2, overnight; (c) CF_3COOH, CH_2Cl_2, overnight; (d) EDCI, DMAP, TEA, CH_2Cl_2, overnight; (e) CF_3COOH, CH_2Cl_2, overnight.

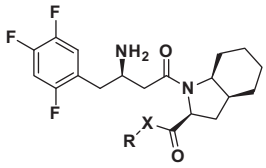


Scheme 3. Reagents and conditions: (a) *p*-toluenesulfonyl chloride, pyridine, overnight; (b) NaN_3, DMF, 65 °C, overnight; (c) CF_3COOH, CH_2Cl_2, overnight; (d) EDCI, DMAP, TEA, CH_2Cl_2, overnight; (e) H_2, Pd/C, MeOH, 2 h; (f) acyl chloride or sulfonyl chloride, TEA, CH_2Cl_2, overnight; and (g) CF_3COOH, CH_2Cl_2, overnight.

performed better than introduction of an aromatic group. Surprisingly, the ethyl ester **3e** significantly increased DPP-4 inhibitory activity (IC_{50} = 0.07 μM) being 5-fold more active than **3d**. Compound **3f**, which was a carboxylic acid lost its activity against DPP-4 (IC_{50} = 3.66 μM).

Among compounds **4a**–**f**, introduction of an acetyl group (**4a**) showed excellent DPP-4 inhibitory activity (IC_{50} = 0.07 μM). The DPP-4 inhibitory activities of hydroxyl derivatives (**4b**, **4c**, and **4d**) were directly correlated with the number of the ring size (the IC_{50} values of cyclopropyl, cyclobutyl, and cyclopentyl were

Table 1
Enzymatic activities of compounds **3a–f** against DPP-4



Compd	X	R	DPP-4 IC_{50}[a] (μM)
3a	NH		5.81
3b	NH		0.34
3c	NH		0.38
3d	NH		0.76
3e	O		0.07
3f	O	H	3.66
Sitagliptin	—	—	0.02

[a] In vitro activity.

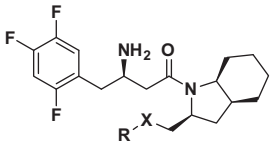
0.08, 0.07, and 0.18 μM, respectively). In the SAR, we found compounds **4e** (benzoyl moiety) and **4f** (carboxylic acid) were less potent with IC_{50} values of 0.32 and 0.51 μM. The activity decreased with the expansion of the ring and compounds with aromatic groups were relatively less potent. Replacement of oxygen in the hydroxyl linker to nitrogen showed a similar activity pattern, which was dependent on the bulkiness of direct substituent to nitrogen (**4g**, **4h**, **4i**, **4j**, and **4k**). Generally, the amide series were less potent than the ester series. Finally, incorporating a sulfonyl group instead of the acyl resulted in highly potent DPP-4 inhibitory activity (**4l**, **4m**, and **4n**). Investigation of the inhibitory activities of compounds **3a–f** and **4a–n** showed that compounds **3e**, **4a**, **4b**, **4c**, **4l**, and **4n** exhibited good activity. However, the inhibitory activity was lower than that of **Sitagliptin** (IC_{50} = 0.02 μM).

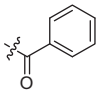
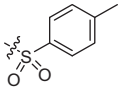
Inhibition of DPP-8 and DPP-9 might be connected with toxicity, selected compounds were tested for their selectivity profiles against the DPP-4 homologs DPP-7, DPP-8, DPP-9, and fibroblast activation protein (FAP). Data are presented in Table 3. Compound **3e** exhibited good selectivity (SR: DPP-8/DPP-4 = 53; DPP-9/DPP-4 = 246). The other selected compounds exhibited moderate selectivities against DPP-8 and DPP-9.

2.4. Binding mode of compounds 3e, 4n, and Sitagliptin

To gain structural information for further optimization, the 3D binding modes of compounds **3e**, **4n**, and **Sitagliptin** to DPP-4 (PDB ID: 1X70) were generated based on docking simulations (Fig. 3). The binding modes indicated that the 2,4,5-trifluorophenyl moiety of compounds **3e**, **4n**, and **Sitagliptin** effectively binds to DPP-4 in the S1 hydrophobic pocket. The β-amino group forms three hydrogen bonds with the side chains of Glu205, Glu206, and Tyr662. The triazolopiperazine of **Sitagliptin** is stacked against the side chain of Phe357 and the trifluoromethyl substituent interacts with the side chains of Arg358 and Ser209 in the S2 pocket. Meanwhile, the simulations indicated that the (3a*S*,7a*S*)-octahydro-1*H*-indole moiety of compounds **3e** and **4n** could not be stacked against the side chain of Phe357, respectively, compared with **Sitagliptin**, which could be the main reason for the decrease in inhibitory activity.

Table 2
Enzymatic activities of compounds **4a–n** against DPP-4



Compd	X	R	DPP-4 IC_{50}[a] (μM)
4a	O		0.07
4b	O		0.08
4c	O		0.07
4d	O		0.18
4e	O		0.32
4f	OH	—	0.51
4g	NH		0.29
4h	NH		0.54
4i	NH		0.33
4j	NH		0.43
4k	NH		0.61
4l	NH		0.14
4m	NH		0.43
4n	NH		0.17
Sitagliptin	—	—	0.02

[a] In vitro activity.

2.5. Pharmacokinetic evaluation of compounds 3e, 4l, and 4n

The pharmacokinetic (PK) profiles of the selected compounds **3e**, **4l**, and **4n** were assessed in Sprague–Dawley (SD) rats (Table 4). The C_{max} of compound **3e** at 1 h was 194 ng/mL, with an AUC_{0-t} 621.6 ng/mL*h at a dose of 20 mg/kg. Moreover, compound **3e** demonstrated high clearance in SD rats. The absolute oral bioavailability for compounds **3e** and **4l** were moderate (22.4% and 43.7%), while compound **4l** had a lower half-life than compound **3e** (2.52 h vs 2.80 h, respectively). Compound **4n** exhibited an attractive pharmacokinetic profile likely suitable for once daily dosing in humans. High oral bioavailability (96.3%) and exposure (5518.9 ng/mL*h) was accompanied by a reasonable half-life (10.5 h), which was much better than **Sitagliptin**.

Table 3
Potencies and selectivities of selected DPP-4 inhibitors

Compd	IC_{50} (μM)[a]					SR[b]	
	DPP-4	DPP-7	DPP-8	DPP-9	FAP	DPP-8/DPP-4	DPP-9/DPP-4
3e	0.07	NI[c]	3.42	15.96	3.35	53	246
4a	0.07	59.83	1.82	3.06	5.83	25	42
4b	0.08	31.94	0.75	2.18	3.29	10	28
4c	0.07	23.10	0.88	3.30	3.92	13	49
4d	0.18	24.34	1.75	1.96	1.71	10	11
4g	0.29	NI	4.17	6.27	6.36	14	22
4l	0.14	6.85	1.47	0.32	2.06	11	2
4n	0.17	14.00	2.90	5.26	4.03	17	32
Sitagliptin	0.02	207.35	56.33	56.72	34.18	2817	2836

[a] In vitro activity.
[b] Selectivity Ratio.
[c] NI: no inhibition.

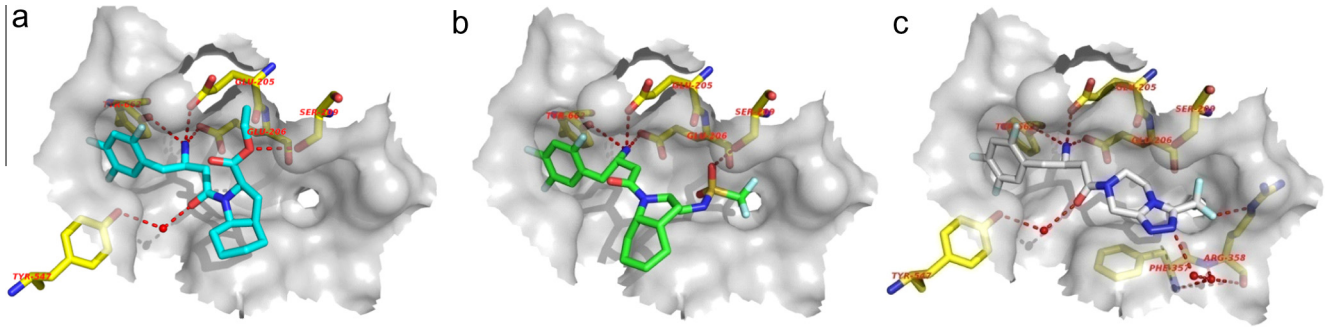


Figure 3. Three-dimensional structural modes of inhibitors **3e** (a), **4n** (b), and **Sitagliptin** (c) to DPP-4 (PDB ID: 1X70) derived from the docking simulations. These three images were generated using the Pymol program.[20]

Table 4
Pharmacokinetic properties of compounds **3e**, **4l**, **4n**, and **Sitagliptin** in SD rats

Compd	Admin	Dose mg/kg	T_{max} h	C_{max} ng/mL	AUC_{0-t} ng/mL*h	MRT h	$t_{1/2}$ h	CLz L/h/kg	*F* %
3e	p.o.[a]	20	1	194	621.6	2.67	2.80	/	22.4
	i.v. [a]	10	0.25	618.7	1387.4	1.90	1.56	7.01	/
4l	p.o.	20	2	360.3	1791.5	3.27	2.52	/	43.7
	i.v.	10	0.25	620.4	2049.4	2.57	1.95	4.52	/
4n	p.o.	20	2	851.7	5518.9	4.13	10.5	/	96.3
	i.v.	10	0.25	629.1	2865.0	3.28	4.51	2.42	/
Sitagliptin[b]	p.o.	2	/	/	/	/	1.7	/	76
	i.v.	1	/	/	/	/	/	/	/

[a] p.o., oral administration; i.v., intravenous injection.
[b] Ref. 14.

3. Conclusion

In conclusion, we designed, synthesized, and evaluated a series of (*R*)-3-amino-1-((3a*S*,7a*S*)-octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-ones as novel DPP-4 inhibitors. In vitro evaluation found that most of the compounds exhibited good in vitro potency against DPP-4 over a low micromolar range. Among these compounds, **3e** (IC_{50} = 0.07 μM), **4c** (IC_{50} = 0.07 μM), **4l** (IC_{50} = 0.14 μM), and **4n** (IC_{50} = 0.17 μM) showed good DPP-4 potency. The pharmacokinetic profiles of compounds **3e**, **4l**, and **4n** are suitable for clinical use. In particular, compound **4n** exhibited an attractive PK profile (*F* = 96.3%, $t_{1/2}$ = 10.5 h). These potent compounds represent a novel scaffold for the development of DPP-4 inhibitors. Current efforts are aimed at further modifying these compounds to achieve more potent and selective DPP-4 inhibitors.

4. Experiments

4.1. Chemistry

The reagents (chemicals) were purchased from commercial sources, and used without further purification. Analytical-thin layer chromatography was HSGF254 (0.15–0.2 mm thickness). Yields were not optimized. Nuclear magnetic resonance (NMR) spectroscopy were given on a Brucker AMX-400 and AMX-300 instruments (using tetramethylsilane as internal standard). Chemical shifts were reported in parts per million (ppm, delta) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low-resolution mass spectroscopy were carried out on an electric ionization (ESI) electrospray and a LCQ-DECA spectrometer produced by Finnigan MAT-95.

4.1.1. General procedures for the synthesis of compounds 3a–f

(a) To a solution of compound **5** in CH_2Cl_2 was added triethylamine and $(Boc)_2O$. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was diluted with CH_2Cl_2, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated to afford compound **6** as oil (94% yield).

(b) To a solution of compound **6** in CH_2Cl_2 was added HOBt, EDCI, DMAP, and triethylamine. After stirring for 30 min aniline was added. The reaction mixture was stirred overnight. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was diluted with CH_2Cl_2, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by a flash column chromatography (CH_2Cl_2 as an eluent) to afford compound **7a** as a white solid (68% yield).

(c) To a solution of **7a** in dry CH_2Cl_2 was added CF_3COOH. After stirring the reaction overnight, the solvent was removed under reduced pressure. The mixture was diluted with ethyl acetate, washed sequentially with saturated Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **8a** as a yellow solid (85% yield).

(d) To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid **9** in CH_2Cl_2 was added HOBt, EDCI, DMAP, and triethylamine. After stirring for 30 min **8a** was added. The reaction mixture was stirred overnight. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was diluted with CH_2Cl_2, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by a flash column chromatography (petroleum ether/ethyl acetate = 4:1, v/v, as an eluent) to afford compound **10a** as a white solid (72% yield).

(e) To a solution of **10a** in dry CH_2Cl_2 was added CF_3COOH. After stirring the reaction overnight, the solvent was removed under reduced pressure. The mixture was diluted with ethyl acetate, washed sequentially with saturated Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **3a** as oil (80% yield).

4.1.2. (2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)-*N*-phenyloctahydro-1*H*-indole-2-carboxamide (3a)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.67 (d, J = 8.1 Hz, 2H), 7.28–7.27 (m, 1H), 7.23–7.22 (m, 1H), 7.19–7.11 (m, 1H), 7.05–7.00 (m, 1H), 6.95–6.87 (m, 1H), 4.71–4.65 (m, 1H), 3.66–3.61 (m, 2H), 2.97–2.76 (m, 2H), 2.55–2.49 (m, 1H), 2.42–2.34 (m, 1H), 2.18–2.15 (m, 2H), 1.76–1.46 (m, 8H), 1.14–1.09 (m, 1H); ^{13}C NMR (MeOD-d_4, 75 MHz): δ 173.1, 170.4, 159.4, 159.4, 157.5, 157.4, 152.5, 152.4, 152.3, 150.5, 150.4, 150.3, 149.8, 149.6, 147.8, 147.7, 140.4, 130.4, 125.8, 121.8, 121.7, 121.3, 121.2, 121.1, 121.1, 107.7, 107.5, 107.5, 107.3, 62.8, 60.3, 50.8, 39.2, 35.8, 32.7, 32.7, 29.3, 27.1, 25.2, 21.6; ESI-MS m/z calcd for $C_{25}H_{29}F_3N_3O_2$ $[M+H]^+$ 460.2, found 460.1.

4.1.3. (2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)-*N*-benzyloctahydro-1*H*-indole-2-carboxamide (3b)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.26 (m, 4H), 7.23–7.20 (m, 1H), 7.06–6.98 (m, 1H), 6.94–6.86 (m, 1H), 4.51–4.41 (m, 3H), 3.76–3.68 (m, 1H), 3.50–3.42 (m, 1H), 2.75–2.69 (m, 1H), 2.63–2.56 (m, 1H), 2.45–2.39 (m, 2H), 2.34–2.25 (m, 1H), 1.99–1.90 (m, 4H), 1.75–1.61 (m, 4H), 1.52–1.36 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.8, 170.3, 157.4, 157.3, 154.9, 154.8, 150.2, 150.1, 149.9, 147.9, 147.8, 147.7, 147.6, 147.4, 145.5, 145.4, 138.5, 128.5, 127.5, 127.2, 121.8, 121.6, 119.2, 119.1, 119.0, 119.0, 105.7, 105.5, 105.4, 105.2, 59.8, 58.9, 48.9, 43.4, 39.8, 37.1, 35.6, 29.5, 27.7, 25.6, 23.8, 19.8; ESI-MS m/z calcd for $C_{26}H_{31}F_3N_3O_2$ $[M+H]^+$ 474.2, found 474.1.

4.1.4. (2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)-*N*-cyclopropyloctahydro-1*H*-indole-2-carboxamide (3c)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.26–7.14 (m, 1H), 6.96–6.88 (m, 1H), 4.38–4.35 (m, 1H), 3.71–3.59 (m, 2H), 3.15–3.11 (m, 2H), 2.79–2.72 (m, 2H), 2.35–2.33 (m, 1H), 2.09–2.06 (m, 3H), 1.72–1.25 (m, 8H), 0.46–0.44 (m, 2H), 0.18–0.16 (m, 2H); ^{13}C NMR (MeOD-d_4, 75 MHz): δ 172.8, 169.5, 157.5, 157.4, 155.0, 154.9, 150.8, 150.7, 150.6, 148.4, 148.3, 148.2, 148.2, 148.0, 145.4, 145.3, 119.5, 119.4, 119.3, 119.2, 119.0, 118.9, 105.8, 105.7, 105.6, 105.5, 61.7, 59.9, 49.5, 36.8, 35.3, 35.0, 33.4, 30.6, 26.6, 25.8, 25.4, 23.6, 6.2, 6.2; ESI-MS m/z calcd for $C_{22}H_{29}F_3N_3O_2$ $[M+H]^+$ 424.2, found 424.1.

4.1.5. (2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)-*N*-(cyclopropylmethyl)octahydro-1*H*-indole-2-carboxamide (3d)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.22–7.13 (m, 1H), 6.96–6.88 (m, 1H), 4.41–4.30 (m, 1H), 3.72–3.68 (m, 1H), 3.61–3.56 (m, 1H), 3.18–3.11 (m, 2H), 3.05–2.88 (m, 2H), 2.79–2.72 (m, 1H), 2.35–2.31 (m, 1H), 2.11–2.04 (m, 3H), 1.72–1.48 (m, 6H), 1.31–1.09 (m, 2H), 0.94–0.88 (m, 1H), 0.46–0.44 (m, 2H), 0.18–0.16 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.8, 169.0, 157.5, 157.4, 155.0, 154.9, 151.0, 150.8, 150.6, 148.4, 148.3, 148.2, 148.2, 148.1, 145.7, 145.6, 119.6, 119.4, 119.2, 119.1, 119.0, 118.9, 106.0, 105.7, 105.6, 105.5, 60.3, 59.0, 49.2, 44.2, 37.0, 33.6, 31.4, 31.0, 27.1, 25.4, 23.6, 19.8, 10.4, 3.2, 3.2; ESI-MS m/z calcd for $C_{23}H_{31}F_3N_3O_2$ $[M+H]^+$ 438.2, found 438.1.

4.1.6. (2*S*,3a*S*,7a*S*)-Ethyl-1-((*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indole-2-carboxylate (3e)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.09–7.04 (m, 1H), 6.91–6.82 (m, 1H), 4.36–4.30 (m, 1H), 4.15 (q, J = 7.5 Hz, 2H), 3.75–3.67 (m, 1H), 3.52–3.52 (m, 1H), 2.77–2.60 (m, 2H), 2.37–2.32 (m, 2H), 2.12–2.05 (m, 1H), 1.98–1.87 (m, 1H), 1.74–1.45 (m, 6H), 1.25–1.09 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.5, 169.2, 157.5, 157.3, 155.0, 154.9, 150.5, 150.2, 150.1, 148.0, 147.9, 147.8, 147.6, 147.4, 145.6, 145.4, 121.0, 120.8, 119.4, 119.3, 119.1, 118.9, 105.7, 105.5, 105.2, 104.9, 61.2, 59.0, 58.2, 48.9, 37.4, 34.7, 33.8, 30.5, 27.7, 25.6, 23.6, 19.9, 14.1; ESI-MS m/z calcd for $C_{21}H_{28}F_3N_2O_3$ $[M+H]^+$ 413.2, found 413.1.

4.1.7. (2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indole-2-carboxylic acid (3f)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14–7.06 (m, 1H), 6.96–6.87 (m, 1H), 4.41–4.35 (m, 1H), 3.80–3.72 (m, 1H), 3.60–3.57 (m, 1H), 2.78–2.65 (m, 2H), 2.45–2.42 (m, 2H), 2.15–2.10 (m, 1H), 2.03–1.92 (m, 1H), 1.79–1.50 (m, 6H), 1.30–1.25 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.9, 170.2, 157.4, 157.2, 155.0, 154.9, 150.3, 150.2, 150.1, 148.1, 148.0, 147.9, 147.5, 147.4, 145.5, 145.4, 121.0, 120.8, 119.2, 119.1, 119.0, 118.9, 105.5, 105.4, 105.2, 105.0, 62.3, 57.9, 50.4, 37.4, 36.2, 35.5, 32.6, 30.2, 26.7, 24.8, 23.0; ESI-MS m/z calcd for $C_{19}H_{24}F_3N_2O_3$ $[M+H]^+$ 385.2, found 385.1.

4.1.8. General procedures for the synthesis of compounds 4a–f

(a) Compound **6** in dry THF was added 1 N $LiAlH_4$ slowly at 0 °C. The reaction mixture was stirred overnight at room temperature. After the starting materials were completely consumed, the mixture was diluted with EA, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **11** as oil (60% yield).

(b) To a solution of **11** and triethylamine in CH_2Cl_2 was added acetyl chloride. The reaction mixture was stirred overnight. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was purified by a flash column chromatography (petroleum ether/ethyl acetate = 1:1, v/v, as an eluent) to afford compound **12a** as a white solid (79% yield).

(c) To a solution of **12a** in dry CH_2Cl_2 was added CF_3COOH. After stirring the reaction overnight, the solvent was removed under reduced pressure. The mixture was diluted with ethyl acetate, washed sequentially with saturated Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **13a** as a yellow solid (88% yield).

(d) To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid **9** in CH_2Cl_2 was added HOBt, EDCI, DMAP, and triethylamine. After stirring for 30 min **13a** was added. The reaction mixture was stirred overnight. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was diluted with CH_2Cl_2, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by a flash column chromatography to afford compound **14a** as a white solid (74% yield).

(e) To a solution of **14a** in dry CH_2Cl_2 was added CF_3COOH. After stirring the reaction overnight, the solvent was removed under reduced pressure. The mixture was diluted with ethyl acetate, washed sequentially with saturated Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **4a** as oil (85% yield).

4.1.9. ((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl acetate (4a)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.12–7.03 (m, 1H), 6.94–6.85 (m, 1H), 4.32–4.24 (m, 2H), 4.20–4.15 (m, 1H), 3.69–3.61 (m, 1H), 3.55–3.52 (m, 1H), 2.77–2.65 (m, 4H), 2.41–2.38 (m, 1H), 2.25–2.19 (m, 1H), 2.04 (s, 3H), 1.92–1.86 (m, 2H), 1.72–1.66 (m, 4H), 1.52–1.48 (m, 1H), 1.28–1.23 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.1, 169.8, 157.4, 157.3, 155.0, 154.9, 150.6, 150.5, 150.4, 148.1, 148.0, 148.0, 147.8, 147.7, 145.6, 145.5, 120.5, 120.3, 119.6, 119.5, 119.3, 119.1, 105.6, 105.5, 105.3, 105.2, 64.2, 61.6, 59.2, 49.0, 36.2, 36.0, 33.4, 29.7, 28.5, 25.7, 25.6, 23.8, 20.1; ESI-MS *m*/*z* calcd for $C_{21}H_{28}F_3N_2O_3$ $[M+H]^+$ 413.2, found 413.1.

4.1.10. ((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl cyclopropanecarboxylate (4b)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14–7.06 (m, 1H), 6.96–6.87 (m, 1H), 4.33–4.32 (m, 2H), 4.24–4.18 (m, 1H), 3.69–3.64 (m, 1H), 3.56–3.50 (m, 1H), 2.78–2.69 (m, 2H), 2.41–2.39 (m, 2H), 2.25–2.21 (m, 1H), 1.94–1.92 (m, 4H), 1.69–1.55 (m, 5H), 1.34–1.25 (m, 2H), 1.00–0.96 (m, 2H), 0.87–0.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.2, 169.5, 156.7, 156.6, 154.7, 154.7, 149.5, 149.4, 149.3, 147.4, 147.3, 147.2, 147.1, 147.0, 145.2, 145.0, 121.0, 120.9, 118.8, 118.8, 118.7, 118.6, 105.1, 105.0, 104.9, 104.8, 63.8, 58.0, 55.8, 48.6, 38.6, 36.0, 34.3, 29.0, 28.0, 25.2, 23.4, 19.6, 12.3, 8.0, 7.9; ESI-MS *m*/*z* calcd for $C_{23}H_{30}F_3N_2O_3$ $[M+H]^+$ 439.2, found 439.1.

4.1.11. ((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl cyclobutanecarboxylate (4c)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14–7.05 (m, 1H), 6.96–6.87 (m, 1H), 4.34–4.31 (m, 2H), 4.22–4.17 (m, 1H), 3.67–3.54 (m, 2H), 3.17–3.11 (m, 1H), 2.79–2.74 (m, 2H), 2.32–2.17 (m, 10H), 1.92–1.85 (m, 3H), 1.73–1.62 (m, 4H), 1.31–1.25 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.7, 169.2, 156.7, 156.6, 154.7, 154.7, 149.7, 149.6, 149.5, 147.6, 147.5, 147.4, 147.3, 147.2, 145.3, 145.2, 120.2, 120.0, 119.0, 118.9, 118.8, 118.8, 105.2, 105.1, 105.0, 104.8, 63.3, 57.9, 55.9, 48.6, 48.5, 37.5, 35.9, 33.0, 29.2, 28.8, 27.8, 25.0, 24.9, 24.6, 23.3, 19.6; ESI-MS *m*/*z* calcd for $C_{24}H_{32}F_3N_2O_3$ $[M+H]^+$ 453.2, found 453.1.

4.1.12. ((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl cyclopentanecarboxylate (4d)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.15–7.06 (m, 1H), 6.95–6.87 (m, 1H), 4.37–4.20 (m, 3H), 3.69–3.61 (m, 1H), 3.57–3.54 (m, 1H), 2.80–2.70 (m, 3H), 2.44–2.41 (m, 2H), 2.35–2.28 (m, 3H), 1.90–1.55 (m, 13H), 1.34–1.12 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 176.0, 169.4, 156.7, 156.6, 154.7, 154.7, 149.5, 149.4, 149.3, 147.5, 147.4, 147.3, 147.2, 147.1, 145.3, 145.2, 120.7, 120.6, 118.8, 118.8, 118.7, 118.6, 105.1, 105.0, 104.9, 104.8, 63.6, 57.9, 55.8, 48.5, 43.3, 38.0, 35.9, 33.9, 29.7, 29.6, 29.4, 28.9, 27.9, 25.3, 23.3, 19.6; ESI-MS *m*/*z* calcd for $C_{25}H_{34}F_3N_2O_3$ $[M+H]^+$ 467.3, found 467.1.

4.1.13. ((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl benzoate (4e)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.02 (d, J = 8.1 Hz, 2H), 7.59–7.54 (m, 1H), 7.46–7.41 (m, 2H), 7.13–7.05 (m, 1H), 6.94–6.86 (m, 1H), 4.67–4.62 (m, 1H), 4.55–4.50 (m, 1H), 4.38–4.32 (m, 1H), 3.74–3.66 (m, 1H), 3.57–3.51 (m, 1H), 2.82–2.65 (m, 2H), 2.42–2.40 (m, 2H), 2.31–2.24 (m, 1H), 2.01–1.91 (m, 5H), 1.75–1.63 (m, 3H), 1.38–1.25 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.8, 165.9, 156.7, 156.6, 154.7, 154.7, 149.4, 149.3, 149.2, 147.4, 147.3, 147.2, 147.1, 147.1, 145.2, 145.1, 132.5, 129.6, 129.0, 127.9, 121.4, 121.3, 118.7, 118.6, 118.5, 118.5, 105.1, 105.0, 104.9, 104.7, 64.7, 58.0, 55.7, 48.5, 39.8, 36.1, 35.1, 29.3, 28.4, 25.2, 23.4, 19.6; ESI-MS *m*/*z* calcd for $C_{26}H_{30}F_3N_2O_3$ $[M+H]^+$ 475.2, found 475.1.

4.1.14. (*R*)-3-Amino-1-((2*S*,3a*S*,7a*S*)-2-(hydroxymethyl)octahydro-1*H*-indol-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (4f)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14–7.05 (m, 1H), 6.93–6.87 (m, 1H), 4.19–4.09 (m, 1H), 3.75–3.67 (m, 2H), 3.57–3.50 (m, 2H), 2.80–2.74 (m, 2H), 2.46–2.42 (m, 2H), 2.28–2.22 (m, 1H), 1.94–1.88 (m, 2H), 1.71–1.33 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.4, 157.5, 157.4, 155.0, 154.9, 150.5, 150.4, 150.3, 148.1, 148.0, 147.9, 147.8, 147.7, 145.6, 145.5, 120.3, 120.2, 119.6, 119.5, 119.2, 119.1, 105.8, 105.7, 105.6, 105.5, 63.8, 63.7, 61.2, 49.5, 37.0, 35.9, 33.7, 31.6, 29.8, 27.4, 25.8, 21.8; ESI-MS *m*/*z* calcd for $C_{19}H_{26}F_3N_2O_2$ $[M+H]^+$ 371.2, found 371.1.

4.1.15. General procedures for the synthesis of compounds 4g–n

(a) Compound **11** was dissolved in pyridine, after a few minutes, *p*-toluenesulfonyl chloride was added. Stirring the reaction overnight, the mixture was diluted with EA, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **15** as oil (80% yield).

(b) The crude product **15** was dissolved in DMF, NaN_3 was added. The reaction mixture was stirred overnight at 65 °C. After the starting materials were completely consumed, the mixture was diluted with EA, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by a flash column chromatography (petroleum ether/ethyl acetate = 8:1, v/v, as an eluent) to afford compound **16** as a white solid (66% yield).

(c) To a solution of **16** in dry CH_2Cl_2 was added CF_3COOH. The reaction was monitored by TLC. After the starting materials were completely consumed, the solvent was removed under reduced pressure. The mixture was diluted with EA, washed sequentially with saturated Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **17** as a yellow solid (85% yield).

(d) To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid **9** in CH_2Cl_2 was added HOBt, EDCI, DMAP, and triethylamine. After stirring for 30 min **17** was added. The reaction mixture was stirred overnight. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was diluted with CH_2Cl_2, washed sequentially with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by a flash column chromatography (petroleum ether/ethyl acetate = 4:1, v/v, as an eluent) to afford compound **18** as a white solid (62% yield).

(e) Compound **18** was dissolved in MeOH and 10% Pd–C was added. The resulting mixture was stirred under hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered and evaporated in vacuo to give **19** as a white solid (94% yield).

(f) To a solution of compound **19** and triethylamine in CH_2Cl_2 was added various acetyl chloride and sulfonyl chloride. The reaction mixture was stirred overnight. The reaction was monitored by TLC. After the starting materials were completely consumed, the mixture was purified by a flash column chromatography (petroleum ether/ethyl acetate = 1:1, v/v, as an eluent) to afford compound **20**.

(g) To a solution of compound **20g–n** in dry CH_2Cl_2 was added CF_3COOH. The reaction was monitored by TLC. The reaction mixture was stirred overnight. After the starting materials were completely consumed, the solvent was removed under reduced pressure. The mixture was diluted with EA, washed sequentially with saturated Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compounds **4g–n**.

4.1.16. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)acetamide (4g)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76 (br s, 1H), 7.14–7.05 (m, 1H), 6.97–6.88 (m, 1H), 4.13–4.05 (m, 1H), 3.74–3.67 (m, 2H), 3.60–3.54 (m, 1H), 3.12–3.04 (m, 1H), 2.85–2.78 (m, 1H), 2.74–2.67 (m, 1H), 2.46–2.44 (m, 2H), 2.22–2.16 (m, 2H), 2.08–2.01 (m, 2H), 1.97 (s, 3H), 1.77–1.55 (m, 4H), 1.30–1.16 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.3, 172.4, 159.0, 159.0, 157.1, 157.0, 151.9, 151.8, 151.7, 149.9, 149.8, 149.7, 149.6, 149.5, 147.6, 147.5, 123.5, 123.4, 121.1, 121.0, 120.9, 120.9, 107.6, 107.5, 107.4, 107.2, 61.2, 59.4, 50.8, 48.5, 41.9, 38.2, 37.3, 33.2, 30.9, 27.4, 25.8, 25.1, 21.8; ESI-MS *m*/*z* calcd for $C_{21}H_{29}F_3N_3O_2$ $[M+H]^+$ 412.2, found 412.1.

4.1.17. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)cyclopropanecarboxamide (4h)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.81 (br s, 1H), 7.11–7.06 (m, 1H), 6.93–6.88 (m, 1H), 4.11–4.06 (m, 1H), 3.69–3.43 (m, 4H), 3.20–3.13 (m, 1H), 2.83–2.76 (m, 2H), 2.47–2.42 (m, 2H), 2.23–2.16 (m, 1H), 2.04–1.96 (m, 1H), 1.77–1.60 (m, 4H), 1.53–1.42 (m, 2H), 1.29–1.10 (m, 3H), 0.89–0.89 (m, 2H), 0.69–0.68 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.8, 173.1, 159.1, 159.0, 157.1, 157.1, 152.0, 151.9, 151.8, 150.0, 149.9, 149.8, 149.6, 149.5, 147.7, 147.6, 123.2, 123.1, 121.1, 121.1, 121.0, 120.9, 107.7, 107.5, 107.4, 107.3, 61.2, 59.7, 50.9, 48.1, 41.2, 38.2, 36.8, 33.1, 30.9, 27.4, 25.8, 21.8, 16.6, 8.9, 8.7; ESI-MS *m*/*z* calcd for $C_{23}H_{31}F_3N_3O_2$ $[M+H]^+$ 438.2, found 438.1.

4.1.18. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)cyclobutanecarboxamide (4i)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.64 (br s, 1H), 7.13–7.05 (m, 1H), 6.97–6.88 (m, 1H), 4.13–4.05 (m, 1H), 3.75–3.68 (m, 2H), 3.56–3.52 (m, 1H), 3.12–2.97 (m, 2H), 2.82–2.76 (m, 1H), 2.71–2.63 (m, 1H), 2.43–2.41 (m, 2H), 2.26–2.11 (m, 4H), 2.04–1.94 (m, 5H), 1.78–1.54 (m, 5H), 1.29–1.10 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.1, 173.4, 159.0, 159.0, 157.1, 157.0, 151.8, 151.7, 151.6, 149.8, 149.7, 149.6, 149.5, 147.6, 147.5, 123.8, 123.7, 121.0, 121.0, 120.9, 120.8, 107.6, 107.4, 107.4, 107.2, 61.2, 59.4, 50.9, 48.6, 42.7, 41.9, 38.2, 37.8, 33.2, 30.9, 27.4, 27.3, 27.2, 25.9, 21.8, 20.0; ESI-MS *m*/*z* calcd for $C_{24}H_{33}F_3N_3O_2$ $[M+H]^+$ 452.3, found 452.1.

4.1.19. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)cyclopentanecarboxamide (4j)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.58 (br s, 1H), 7.15–7.07 (m, 1H), 6.97–6.88 (m, 1H), 4.11–4.03 (m, 1H), 3.71–3.65 (m, 2H), 3.61–3.57 (m, 1H), 3.20–3.13 (m, 2H), 2.84–2.79 (m, 2H), 2.54–2.47 (m, 3H), 2.24–2.18 (m, 1H), 2.06–1.97 (m, 1H), 1.83–1.65 (m, 9H), 1.59–1.49 (m, 4H), 1.31–1.14 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 178.5, 173.1, 159.1, 159.0, 157.1, 157.1, 151.9, 151.8, 151.7, 149.9, 149.8, 149.7, 149.6, 149.5, 147.7, 147.6, 123.3, 123.2, 121.1, 121.1, 121.0, 120.9, 107.7, 107.5, 107.4, 107.3, 61.2, 59.6, 50.9, 48.1, 47.9, 41.5, 38.2, 37.0, 33.1, 32.3, 32.3, 30.9, 27.7, 27.4, 25.8, 21.8; ESI-MS *m*/*z* calcd for $C_{25}H_{35}F_3N_3O_2$ $[M+H]^+$ 466.3, found 466.1.

4.1.20. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)benzamide (4k)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (br s, 1H), 7.86 (d, *J* = 7.8 Hz, 2H), 7.46–7.42 (m, 3H), 7.15–7.06 (m, 1H), 6.94–6.86 (m, 1H), 4.29–4.21 (m, 1H), 3.94–3.87 (m, 1H), 3.74–3.68 (m, 1H), 3.65–3.61 (m, 1H), 3.33–3.26 (m, 1H), 3.12 (br s, 2H), 2.87–2.85 (m, 2H), 2.65–2.57 (m, 1H), 2.52–2.46 (m, 1H), 2.27–2.23 (m, 1H), 2.17–2.06 (m, 1H), 1.85–1.50 (m, 6H), 1.32–1.16 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.7, 169.0, 159.0, 159.0, 157.1, 157.1, 151.9, 151.8, 151.7, 149.9, 149.8, 149.7, 149.6, 149.5, 147.7, 147.5, 136.0, 133.2, 130.4, 129.0, 123.4, 123.3, 121.1, 121.1, 120.9, 120.9, 107.6, 107.5, 107.4, 107.2, 61.4, 59.2, 51.0, 49.9, 42.0, 38.2, 37.2, 33.3, 31.0, 27.4, 25.9, 21.8; ESI-MS *m*/*z* calcd for $C_{26}H_{31}F_3N_3O_2$ $[M+H]^+$ 474.2, found 474.1.

4.1.21. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)-4-methylbenzenesulfonamide (4l)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.72 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 6.6 Hz, 2H), 7.13–7.04 (m, 1H), 6.97–6.88 (m, 1H), 6.75 (br s, 1H), 4.00–3.92 (m, 1H), 3.63–3.57 (m, 1H), 3.54–3.48 (m, 1H), 3.31–3.27 (m, 1H), 2.96–2.90 (m, 1H), 2.81–2.75 (m, 1H), 2.72–2.65 (m, 1H), 2.39 (s, 3H), 2.37–2.34 (m, 2H), 2.19–2.13 (m, 3H), 1.95–1.89 (m, 1H), 1.73–1.47 (m, 6H), 1.25–1.10 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.4, 159.0, 159.0, 157.1, 157.0, 151.7, 151.6, 151.5, 149.8, 149.7, 149.5, 149.4, 147.6, 147.5, 144.9, 139.2, 131.4, 128.9, 124.0, 123.8, 121.1, 121.0, 120.9, 120.9, 107.6, 107.4, 107.3, 107.2, 60.9, 59.4, 50.9, 50.7, 42.6, 38.1, 37.9, 33.0, 30.7, 27.4, 25.8, 23.3, 21.8; ESI-MS m/z calcd for $C_{26}H_{33}F_3N_3O_3S$ $[M+H]^+$ 524.2, found 524.1.

4.1.22. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)methanesulfonamide (4m)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.20–7.12 (m, 1H), 6.99–6.90 (m, 1H), 4.07–4.01 (m, 1H), 3.83–3.78 (m, 1H), 3.57–3.52 (m, 1H), 3.37–3.32 (m, 1H), 3.23–3.19 (m, 1H), 2.92 (s, 3H), 2.85–2.71 (m, 3H), 2.62–2.56 (m, 1H), 2.28–2.23 (m, 1H), 2.03–2.00 (m, 1H), 1.84–1.48 (m, 6H), 1.25–1.08 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.6, 156.7, 156.7, 154.8, 154.7, 150.1, 150.0, 149.9, 148.1, 148.0, 147.9, 147.4, 147.3, 145.4, 145.3, 119.0, 118.9, 118.5, 118.3, 105.4, 105.3, 105.2, 105.0, 58.8, 57.9, 48.8, 46.6, 39.2, 35.5, 33.4, 30.8, 30.3, 27.9, 24.8, 23.2, 19.3; ESI-MS m/z calcd for $C_{20}H_{29}F_3N_3O_3S$ $[M+H]^+$ 448.2, found 448.1.

4.1.23. *N*-(((2*S*,3a*S*,7a*S*)-1-((*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)octahydro-1*H*-indol-2-yl)methyl)-1,1,1-trifluoromethanesulfonamide (4n)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.16–7.09 (m, 1H), 7.00–6.91 (m, 1H), 4.09–4.05 (m, 1H), 3.83–3.78 (m, 1H), 3.59–3.50 (m, 2H), 3.27–3.23 (m, 1H), 3.10–2.99 (m, 2H), 2.85–2.77 (m, 1H), 2.65–2.61 (m, 1H), 2.30–2.26 (m, 1H), 2.09–1.99 (m, 1H), 1.80–1.51 (m, 6H), 1.31–1.13 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.1, 161.6, 156.7, 156.7, 154.8, 154.7, 150.2, 150.1, 150.0, 148.2, 148.1, 148.0, 147.4, 147.3, 145.5, 145.4, 118.9, 118.7, 118.1, 118.0, 105.5, 105.4, 105.3, 105.2, 59.0, 58.1, 48.8, 48.3, 35.4, 33.4, 30.8, 30.2, 27.9, 24.7, 23.1, 19.3; ESI-MS m/z calcd for $C_{20}H_{26}F_6N_3O_3S$ $[M+H]^+$ 502.2, found 502.1.

4.2. In vitro DPP-4, DPP-7, DPP-8, DPP-9, and FAP enzyme assay

4.2.1. Preparation of the DPPs enzyme

The DPP-4, DPP-7, DPP-8, DPP-9, and FAP enzymes were expressed in high five cells using a baculoviral system (Bac-To-Bac; Life Technologies) according to the literature,[28] and his6-tagged recombinant proteins were purified by Ni-NTA resin individually.

4.2.2. Enzyme-based assay of DPP-4 inhibition

To measure the activity of DPP-4, 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. 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 inhibition selectivity against DPPs

The inhibitory effect of each compounds on DPPs were assayed by continuous fluorometric method. We used Nle-Pro-AMC as substrate to measure the activities of DPP-7 and FAP, and Ala-Pro-AMC for DPP-8 and DPP-9 in the optimized pH (5.5 for DPP-7 and 8.0 for other members) assay system.

4.3. Binding studies

The DPP-4 protein was extracted from RCSB Protein Data Bank (PDB ID: 1X70). 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 DPP-4 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.

4.4. Pharmacokinetic profile in SD rats

Compounds **3e**, **4l**, and **4n** 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.

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