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Novel *trans*-2-aryl-cyclopropylamine analogues as potent and selective dipeptidyl peptidase IV inhibitors

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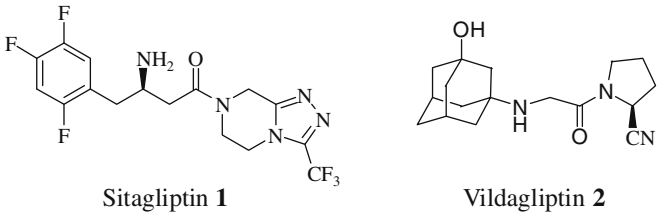
ABSTRACT

A series of *trans*-2-aryl-cyclopropylamine derived compounds were synthesized and evaluated their biological activities against DPP-IV. The structure-activity relationships (SAR) led to the discovery of novel series of DPP-IV inhibitors, having IC_{50} values of <100 nM with excellent selectivity over the closely related enzymes, DPP8, DPP-II and FAP. The studies identified a potent and selective DPP-IV inhibitor **24b**, which exhibited the ability to both significantly inhibit plasma DPP-IV activity in rats and improve glucose tolerance in lean mice and diet induced obese mice.

1. Introduction

Dipeptidyl peptidase IV (DPP-IV, also known as CD26) (EC 3.4.14.5) is a drug target for type II diabetes. It is a prolyl dipeptidase involved in the in vivo degradation of two insulin-sensing hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), by cleaving at the peptide bond of the penultimate position.[1,2] The incretin hormone GLP-1 stimulates insulin biosynthesis and secretion, inhibits glucagon release and slows gastric emptying; each a benefit in the control of glucose homeostasis.[3–7] DPP-IV knockout mice and rats have consistently displayed healthy phenotypes.[8–10] When challenged with a high concentration of glucose, these knockout animals show improved glucose tolerance, enhanced insulin secretion and increased circulating active GLP-1 peptide.[8–10] Small-molecular inhibitors of DPP-IV have been shown to prolong the beneficial effects of this incretin hormone in animal models.[5] Antidiabetic efficacy has been demonstrated clinically with DPP-IV inhibitors; sitagliptin **1** (MK-0431) was approved by the US Food and Drug Administration for the treatment of type 2 diabetes,[11] and vildagliptin **2** (LAF237) was approved for use in the European market (Fig. 1).[12]

Selectivity of the DPP-IV inhibitors for closely related prolyl peptidases such as DPP8/9, DPP-II and FAP (Fibroblast activation protein) is an important criterion for their further development as antidiabetic agents, since inhibition of DPP-II results in the apoptosis of quiescent T-cells[13] and also recent in vivo studies indicate that selective inhibition of DPP8/9 may be associated with profound toxicities.[14] The toxic effects of DPP8/9 inhibitors identified thus far in preclinical studies have included alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies, and animal mortality shown in rats.[14] Reports suggest that fibroblast activation protein (FAP) is involved in tumor growth and invasion.[15]



Sitagliptin **1** Vildagliptin **2**

Figure 1. DPP-IV inhibitors.

With few exceptions, most DPP-IV inhibitors resemble the P2-P1 dipeptidyl substrate cleavage product. As shown in Figure 1, inhibitor **2** is a covalent inhibitor. Often this electrophilic cyanopyrrolidine is able to bind covalently with the serine 630 in the S1 pocket of DPP-IV,[12] whereas the non-covalent inhibitor **1** depends on non-covalent protein–ligand interactions and the substituted phenyl group occupies very well the hydrophobic S1 pocket of the enzyme.[11] We are involved in the design and synthesis of

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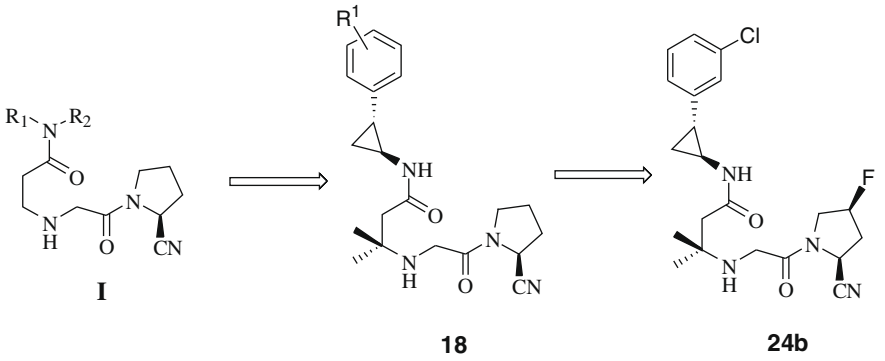


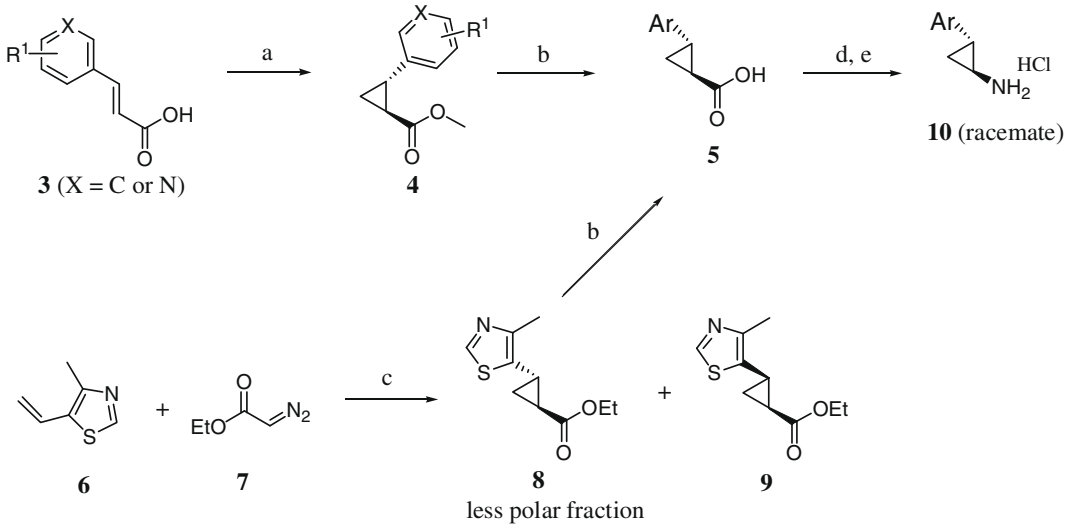
Figure 2. Design of phenylcyclopropylamine derivatives as DPP-IV inhibitors.

DPP-IV inhibitors and have reported potent and selective DPP-IV inhibitors.[16–18] Previous report described 2-[3-[[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-1-oxopropyl]-based (structure **I**) DPP-IV inhibitors (Fig. 2), the structure–activity relationships (SAR) of several series of structure **I**-based DPP-IV inhibitors were investigated, and these studies successfully identified potent, selective and orally available DPP-IV inhibitor with excellent chemical stability.[16] However, selected potent compounds in those series of structure **I**-based DPP-IV inhibitors suffered from low oral bioavailability (data not shown). In this paper, we described our extensive SAR studies regarding structure **I**-based derivatives to develop long-acting DPP-IV inhibitors with improved pharmacokinetic profile, we here report on the discovery of a novel series of 2-phenyl-cyclopropylamine derivatives **18** as DPP-IV inhibitors. To further improve the potency of this series of compounds, introduction of fluoro substitution at the 4-position of 2-cyanopyrrolidine led to the discovery of enantiopure compound **24b**, which was a potent DPP-IV inhibitor (IC_{50} = 15 nM) with excellent selectivity over DPP-II, DPP8 and FAP (>1300-fold) (Fig. 2). This compound was selected for extensive in vivo evaluation and demonstrated in vivo efficacy better than that of DPP-IV inhibitor **1**. In addition, pharmacokinetic studies are also presented.

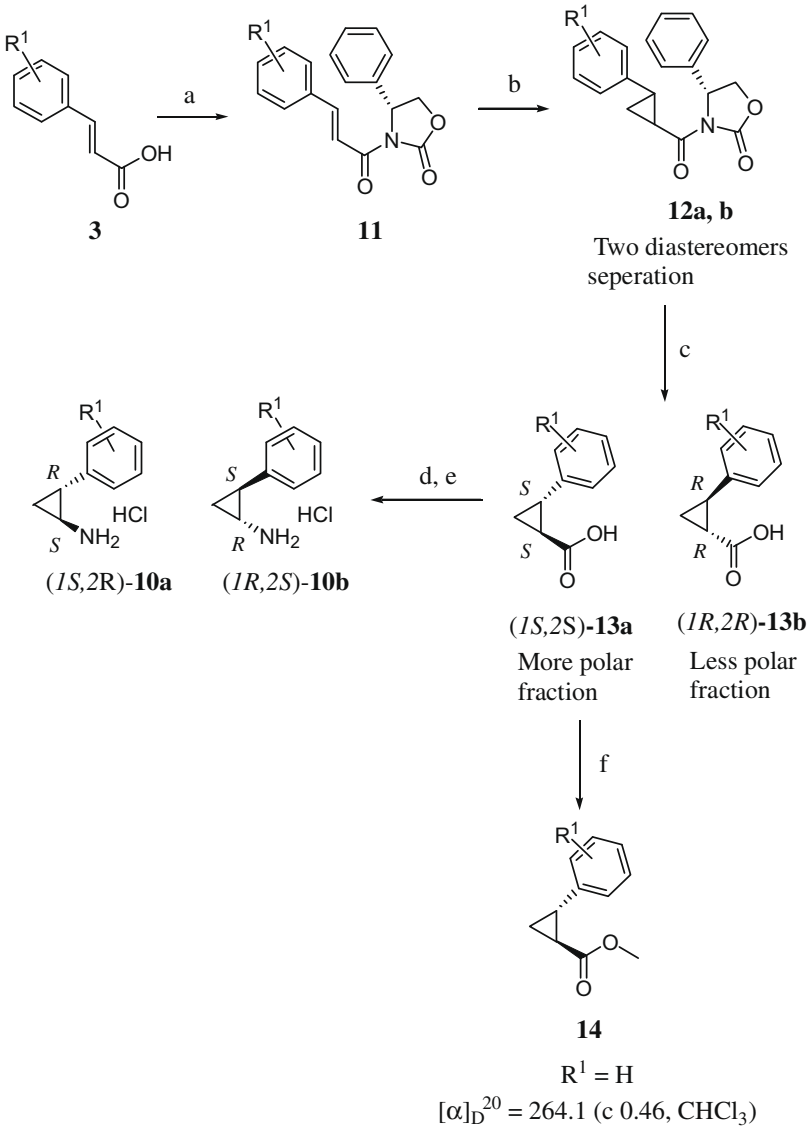
2. Chemistry

The general synthetic route for the preparation of racemic *trans*-2-aryl-cyclopropylamine derivatives **10** was illustrated in Scheme 1. The synthesis commonly began with commercially available *trans* arylated acrylic acid **3** as a starting material. Acid **3** was converted to its methyl ester followed by cyclopropanation of *E* alkenes employing diazomethane to afford *trans*-2-phenyl-cyclopropanecarboxylic acid methyl ester **4**. Ester **4** was saponified to yield the corresponding racemic acids **5**. To further our understanding of the SAR for hydrophilic compound, 2-(4-methyl-thiazol-5-yl)-cyclopropylamine was prepared. Thermal reaction of 4-methyl-thiazole **6** and ethyl diazoacetate **7** according to the method of Ivanskii and Maksimov afforded the racemate 2-(4-methyl-thiazol-5-yl)-cyclopropanecarboxylic acid ethyl esters *trans*-**8** and *cis*-**9**,[19] *trans/cis* isomers could be separated by flash column in a modest yield and approximately a 2:1 ratio. Alkaline hydrolysis gave the desired *trans*-2-aryl-cyclopropanecarboxylic acid **5**. These racemic acids **5** were transformed to the corresponding Boc-protected amines by Curtius rearrangement and then removal of the Boc group yielded racemic 2-aryl-cyclopropylamine **10**.

Enantiopure amines (*1R,2S*)-**14** and (*1S,2R*)-**14** were synthesized following the strategy illustrated in Scheme 2. Activation of the acid **3** with thionyl chloride followed by addition of lithiated (4*R*)-4-phenyl-2-oxazolidinone chiral auxiliary gave **11**. Cyclopropanation of *E* alkenes **11** employing diazomethane provided a separable mixture of diastereomeric cycloadducts *trans*-**12** in a 6:4 ratio. The chiral auxiliary of separated enantiopure **12** was removed by base hydrolysis to yield the corresponding enantiomeric acids **13**. To determine the absolute configuration of two isomers **13**, more polar fraction compound **13a** (R^1 = H) was converted into the *trans*-phenyl-cyclo-



Scheme 1. Reagents and conditions: (a) (i) CH_2N_2, CH_2Cl_2, (ii) $Pd(OAc)_2$, CH_2N_2, CH_2Cl_2/Et_2O (3:1), 0 °C, 1 h; (b) NaOH, MeOH/H_2O; (c) neat, 135 °C; (d) DPPA, $(Et)_3N$, *t*-BuOH, 90 °C, 8 h; (e) (i) TFA, rt, 0.5 h, (ii) HCl 1.0 M in diethylether.



Scheme 2. Reagents and conditions: (a) (i) $SOCl_2$, 55 °C, 4 h, (ii) (4*R*)-4-phenyl-2-oxazolidinone, NaH, THF, 0 °C→rt, 12 h; (b) CH_2N_2, $Pd(OAc)_2$, CH_2Cl_2/Et_2O (3:1), 0 °C, 1 h; (c) LiOH, H_2O_2, THF/H_2O (5:1), −5 °C→0 °C, 2 h; (d) DPPA, $(Et)_3N$, *t*-BuOH, 90 °C, 8 h; (e) (i) TFA, rt, 0.5 h, (ii) HCl 1.0 M in diethylether; (f) CH_2N_2, CH_2Cl_2.

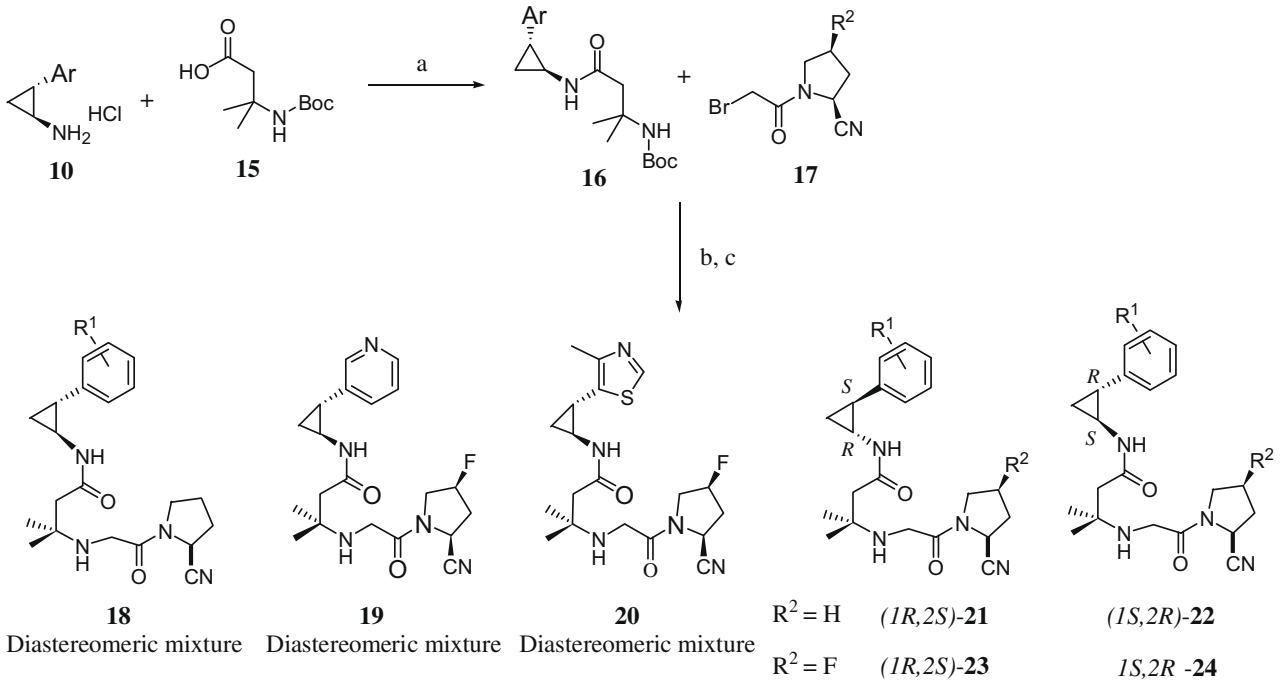
propanecarboxylic acid methyl ester **14** by esterification with diazomethane. When a comparative study of the specific rotation of ester **14** was performed, it was observed that specific rotation of compound **14** is 264.1 (*c* 0.46, $CHCl_3$) as literature value for enantiopure (1*S*,2*S*)-**14**.[20] Enantiopure (*1S*,*2S*)-**13** and (*1R*,*2R*)-**13** were transformed to the corresponding Boc-protected amines by Curtius rearrangement and then removal of the Boc group yielded primary amines (*1S*,*2R*)-**10** and (*1R*,*2S*)-**10**, respectively.

A series of *trans*-2-aryl-cyclopropylamine derivatives as DPP-IV inhibitors, compounds **18–24** were prepared as described in Scheme 3 and are listed in Tables 1–3. 3-Boc-amino-3-methylbutyric acid **15**[16] was EDC-coupled with various cyclopropyl amines to give **16**. Removal of the *N*-Boc group of **16** with trifluoroacetic acid followed by coupling with 1-bromoacetyl-2-cyano-(*S*)-pyrrolidine derivatives **17** to provide the desired 2-cyanopyrrolidine analogues **18–24**. The synthesis of bromo compounds **17** were carried out according to the literature procedure.[21]

3. Biological evaluation

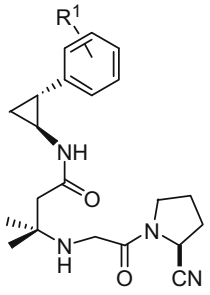
The DPP-IV inhibitory activity and selectivity of analogues in the present series were assayed against human DPP-IV and proline-specific enzymes DPP8/9, DPP-II and FAP. To speedily understand the effect of substituent at the phenyl ring attached on cyclopropylamine, easily prepared diastereomeric mixture **18** was firstly evaluated. As shown in Table 1, the lead compound, the unsubstituted 2-phenyl-cyclopropylamine **18a**, is a potent DPP-IV inhibitor with an IC_{50} value of 85 nM. However, substitution at the phenyl ring had little effect on DPP-IV activity and selectivity; compounds **18b-m** exhibited high potency ($IC_{50} \leqslant 100$ nM) against DPP-IV, with excellent selectivity versus the other peptidases ($IC_{50} > 20$ μM) except that **18a** had weak inhibition against DPP-II ($IC_{50} \sim 16$ μM). The most potent compound in Table 1 is 3-chloro substituted **18k**, which has an IC_{50} value of 42 nM for DPP-IV inhibition.

Since diastereomeric mixture 3-chloro **18k** is most potent compound in Table 1, we investigated the activity and selectivity of the two diastereomers (*1R*,*2S*)-**21** and (*1S*,*2R*)-**22** of compound **18k** (Table 2). Additionally, to further improve the potency of compounds **21** and **22**, introduction of fluoro substituent at the 4-position of 2-cyanopyrrolidine (*S* form) at the P1 site provided the diastereomers (*1R*,*2S*)-**23** and (*1S*,*2R*)-**24**,[22] and evaluated fluoro effects on both potency and selectivity. As shown in Table 2, the chiral centers at C1 and C2 of *trans*-2-phenylcyclopropylamines had



Scheme 3. Reagents and conditions: (a) (i) HOBT, EDC, $(Et)_3N$, CH_2Cl_2/1,4-dioxane, rt, 18 h; (b) (i) TFA, rt, 0.5 h, (ii) K_2CO_3, THF, rt, 1 h; (c) THF, rt, 18 h.

Table 1
Effects of substituent at the R^1 position

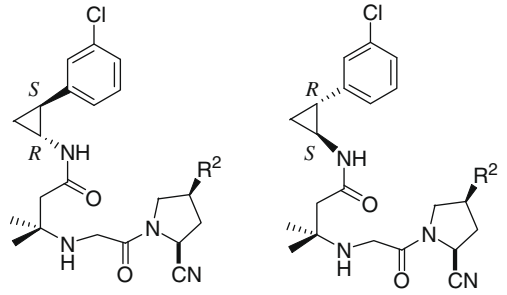


18
Diastereomeric mixture

Compd	R	IC_{50} (μM)			
		DPP-IV	DPP8	DPP-II	FAP
18a	H	0.085	>20	16	>20
18b	4-F	0.054	>20	>20	>20
18c	3-F	0.064	>20	>20	>20
18d	2,4-diF	0.084	>20	>20	>20
18e	3,5-diF	0.075	>20	>20	>20
18f	3,4-diF	0.062	>20	>20	>20
18g	4-CF_3	0.085	>20	>20	>20
18h	3-CF_3	0.060	>20	>20	>20
18i	3-OCF_3	0.073	>20	>20	>20
18j	4-Cl	0.086	>20	>20	>20
18k	3-Cl	0.042	>20	>20	>20
18l	3,4-Cl	0.068	>20	>20	>20
18m	3,4-OCH_3	0.101	>20	>20	>20

little effect on DPP-IV inhibition (**21** vs **22** and **23** vs **24b**). While (*1R,2S*) configuration was found to be detrimental to selectivity; (*1R,2S*)-**21** had weak inhibition against DPP-II (IC_{50} > 10 μM), (4*S*)-4-fluoro-2-cyanopyrrolidine (*1R,2S*)-**23** exhibited low micromolar activity against DPP-II (IC_{50} = 5.2 μM) and weak inhibition against DPP8 (IC_{50} = 11 μM). As expected, 4-fluoro-2-cyanopyrrolidine derivatives **23** and **24b** exhibited twofold and threefold better DPP-IV inhibitory activity than 4-unsubstituted **21** and **22**, respectively. In view of these facts, we focused our efforts on studying the (*1S,2R*) series of compounds with (4*S*)-4-fluoro-2-cyanopyrrolidine in the P1 site.

Table 2
Inhibitory properties of 2-phenyl-cyclopropylamine isomers



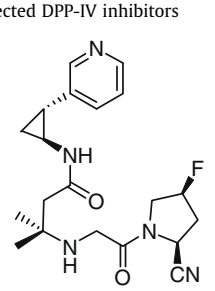
R^2 = H (*1R,2S*)-**21** (*1S,2R*)-**22**

R^2 = F (*1R,2S*)-**23** (*1S,2R*)-**24b**

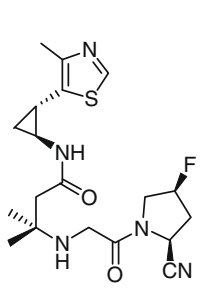
Compd	IC_{50} (μM)			
	DPP-IV	DPP8	DPP-II	FAP
21	0.056	>20	17	>20
22	0.047	>20	>20	>20
23	0.027	11	5.2	>20
24b	0.015	>20	>20	>20

The potent and selective ((*1S,2R*) isomer **24b** was then elaborated further by modifying the substitution at the phenyl ring. As we can see in Table 1, a similar SAR trend was observed in Table 3. The unsubstituted compound **24a**, 3-fluoro substituted **24c** and the alkoxy substituted compounds **24d–g** have very closely inhibitory activities of DPP-IV compared to lead **24b**. Compound **24a–g** also exhibited similar selectivity profiles except for **24c** and **24f**; compound **24c** showed moderate inhibition against

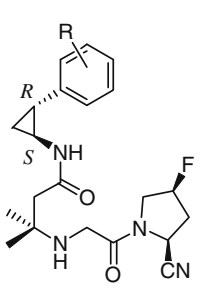
Table 3
Inhibitory properties of selected DPP-IV inhibitors



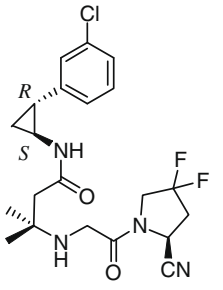
19
Diastereomeric mixture



20
Diastereomeric mixture



(*1S,2R*)-**24**



(*1S,2R*)-**25**

Compd	R	IC_{50} (μM)			
		DPP-IV	DPP8	DPP-II	FAP
24a	H	0.019	>20	>20	>20
24b	3-Cl	0.015	>20	>20	>20
24c	3-F	0.021	8.6	>20	>20
24d	4-OMe	0.022	>20	>20	>20
24e	3-OMe	0.026	>20	>20	>20
24f	3-OEt	0.017	>20	>20	13
24g	3-OCF_3	0.020	>20	>20	>20
19		0.024	>20	>20	>20
20		0.016	>20	>20	>20
25		0.026	12	>20	0.59
1		0.030	>20	>20	>20
2		0.051	14	>20	>20

DPP8 (IC_{50} = 8.6 μM) and compounds **24f** showed weak inhibition toward FAP (IC_{50} = 13 μM). Since Gao et al. reported that heteroatoms or polar substituents at the P2 site would help to increase the DPP8 IC_{50}/DPP-IV IC_{50} ratio, namely, to provide more selective DPP-IV inhibitors.[23] Alternative approach to improve potency and selectivity is replacement of the phenyl group present in cyclopropylamine with a polar heterocyclic ring. Two analogues with a pyridine moiety **19** and thiazole moiety **20** were prepared and evaluated their potency and selectivity. Both of them had potency toward DPP-IV similar to 3-chlorophenyl analogue **24b**; they did exhibit relatively high selectivity for inhibition of DPP-IV over DPP8 (17% and 23% inhibition at 20 μM for compounds **19** and **20**, respectively). Both **19** and **20** represents the most selective DPP-IV inhibitor among the *trans*-2-aryl-cyclopropylamine series of compounds. Interestingly, compound **25** with 3,3-difluoropyrrolidine in the P1 site exhibited submicromolar potency against DPP-II (IC_{50} = 588 nM). Generally, this series of inhibitors with 3,3-difluoropyrrolidine in the P1 site significantly inhibited DPP-II activity (data not shown). Each of the analogues shown in Table 3 was comparable to first-generation DPP-IV inhibitors **1** and **2** in terms of intrinsic potency and off-target selectivity profile.

Inhibitors that possessed excellent potency and selectivity profiles were chosen for extensive pharmacokinetic screening in rats, and the data are summarized in Table 4. 3-Chlorophenyl derivative **24b** displayed excellent half-life (4.8 h), moderate oral bioavailability (42%) and acceptable clearance (22 mL/min/kg). Replacing 3-chloro group with 3-fluoro group gave compound **24c**, which showed faster clearance (55 mL/min/kg) and shorter half-life ($t_{1/2}$= 1.2 h) compared to **24b**. The 4-OMe substituted analogue **24d** exhibited similar clearance and half-life to **24b**. Both compounds **24c** and **24d** displayed moderate oral bioavailability (F = 28% and 29%, respectively). Unlike 4-OMe analogue **24d**, 3-OMe analogue **24e** had very high clearance (124 mL/min/kg). Nevertheless, **24e** exhibited excellent half-life ($t_{1/2}$ = 6.8 h) and in excess of theoretical 100% for oral bioavailability. In comparison with 3-chlorophenyl **24b**, analogue **25** with 3,3-difluoropyrrolidine in the P1 site showed comparable half-life and oral bioavailability, but clearance is very high (189 mL/min/kg). Selected compounds **24b** and **25** did not block the hERG channel at a test concentration of 10 μM (Table 4).

Compound **24b** was selected for further in vivo evaluation based on its potency, off-target selectivity, pharmacokinetic profile and lack of binding affinity to the hERG channel. Although heterocyclic compound **20** is a diastereomeric mixture, it is equipotent with **24b** as DPP-IV inhibitor and shows excellent selectivity over DPP8; and further, compounds **20** is still very potent DPP-IV inhibitor at 50% human and rat serum, IC_{50} = 10 nM and 15 nM, respectively. For these reasons, compound **20** was chosen for more extensive studies. Firstly, compounds **20** and **24b** were assessed for their ability to inhibit plasma DPP-IV activity in rats (Fig. 3). For the plasma DPP-IV inhibition assay, the compounds were administered to Wistar rats by oral route at 10 mg/kg, blood samples were collected and analyzed for plasma DPP-IV activity. Compound **20** showed moderate plasma DPP-IV inhibition (60%) within 30 min after oral administration and provided only 30% inhibition of DPP-IV activity after 8 h. Compound **24b** is more potent and longer action at inhibiting the plasma DPP-IV level than **20**, compound **24b** inhibited plasma DPP-IV activity (>85%) within 30 min, and more than 70% inhibition of DPP-IV activity lasted for 8 h after oral dosing. The inhibition achieved by **24b** was more potent than inhibitor **1**, which is the first and only DPP-IV inhibitor available in US for the treatment of type 2 diabetes. The DPP-IV activity returned back to original values after around 24 h in both **24b**- and **1**-treated rats. 2-(4-Methyl-thiazol-5-yl)-cyclopropylamine analogue **20** showed unexpectedly less potent plasma DPP-IV inhibition in rats relative to in vitro potency (IC_{50} = 15 nM at 50% rat serum). The disconnection between in vivo efficacy and in vitro potency was most likely due to the decreased oral absorption for the increase in hydrophilicity.

Compound **24b** was chosen for further evaluation of its ability to improve glucose tolerance in C57BL/6j mice. In an oral glucose tolerance test (OGTT),[16] when compound **24b** was administered by oral route to C57BL/6j mice 30 min before glucose administration (3 g/kg), and then blood samples drawn and analyzed for glu-

Table 4
Pharmacokinetic properties of selected DPP-IV inhibitors in rats[a] and hERG binding

Compd	CLP (mL/min/kg)	PO C_{max} (ng/mL)	$T_{1/2}$ (po, h)	PO AUC_{nom} (ng/mL h)	F (%)	hERG[b,c] (%)
24b	22	621	4.8	1905	42	11
24c	55	175	1.2	449	29	ND
24d	20	347	5.3	1179	28	ND
24e	124	898	6.8	1345	194	ND
25	189	454	3.2	1195	53	15

[a] **24b**, **24c** and **24e** dosed at 5/10 mg/kg iv/po. **24d** and **25** dosed at 5/25 mg/kg iv/po.
[b] Inhibition at a test concentration of 10 μM.
[c] ND: not determined.

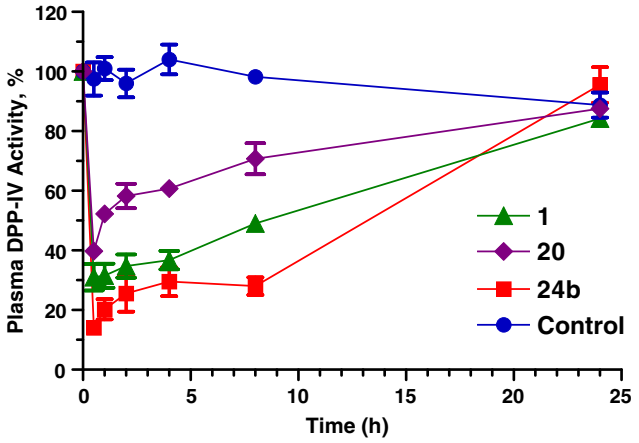


Figure 3. Effects of **1**, **20** and **24b** on the plasma DPP-IV activity in rats. Each compound was orally administered at a single dose of 10 mg/kg to rats at 0 h. Data are expressed as mean ± SEM (n = 4–6/group).

cose levels. The glucose AUC was determined from 0 to 120 min, and OGTT data on **24b** were summarized in Figure 4. The results shown in Figure 4 demonstrated that **24b** significantly reduced the blood glucose excursion in a dose-dependent manner from 1 mg/kg (50% reduction) to 10 mg/kg (73% reduction). The potency of **24b** on reduction of plasma glucose excursion during OGTT was almost equal to that of compound **1** at the dose of 3 mg/kg in mice (data not shown). Blood glucose lowering was also demonstrated in diet-induced obese (DIO) mice. In comparison to the control, glucose AUC as measured by the area under the curve was significantly reduced at all three doses from 1 mg/kg (72% reduction), 3 mg/kg (98% reduction) to 10 mg/kg (83% reduction) (Fig. 5).

Compound **24b** is a potent, selective and orally active inhibitor of DPP-IV; besides, **24b** also possesses an excellent safety pharmacology profile. Additional in vitro profiling of **24b** in an extensive panel of 167 enzyme assays showed no significant inhibition at a test concentration of 10 μM except endothelin converting enzyme-1 (ECE-1, 53% inhibition at 10 μM). Based on the data presented above, compound **24b** is a better candidate for further evaluation.

4. Conclusion

This report describes the SAR studies of optimizing 2-[3-[[2-[(2*S*)-2-cyano-1-pyrrolidinyl]-2-oxoethyl]amino]-1-oxopropyl]-based DPP-IV inhibitors by introducing the *trans*-2-aryl-cyclopropylamine building blocks. This approach led to develop a series of potent and selective inhibitors **18–24**. Notable among these is compound **24b**, which is a 15 nM DPP-IV inhibitor with high selectivity over DPP-II, DPP8 and FAP (>20 μM). Compound **24b** also possessed good pharmacokinetic profiles in rats. In addition, the in vivo effects of compound **24b**, including inhibition of plasma

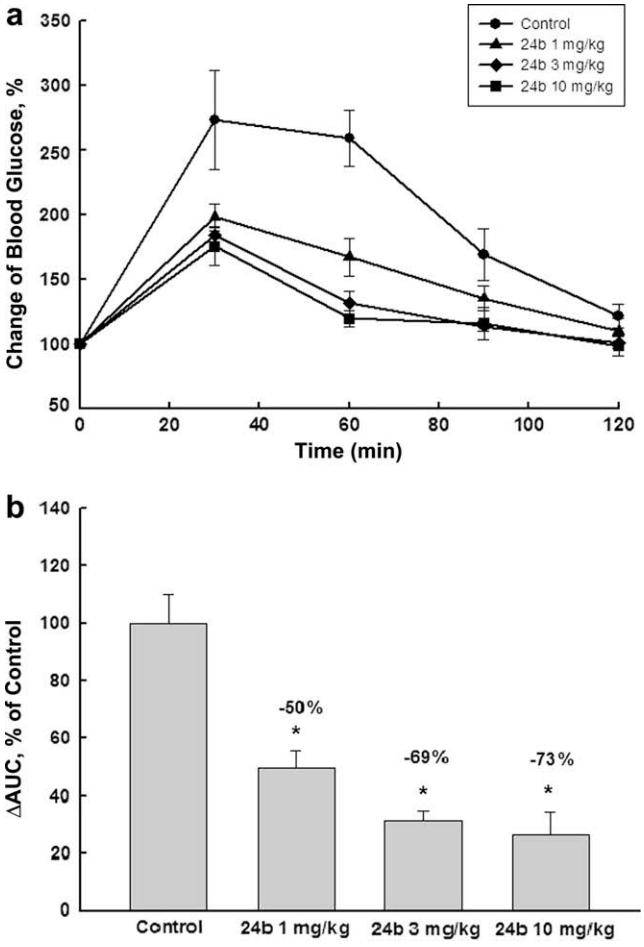


Figure 4. (a) Effects of **24b** (1, 3 and 10 mg/kg) on the glucose excursion levels of C57BL/6j mice during oral glucose tolerance test. All rats received 3 g/kg glucose orally at 0 min. Each of compounds was orally administered to mice at −30 min. (b) The suppression of glucose AUC was calculated from 0 to 120 min. Data are represented as mean ± SEM (n = 6/group).

DPP-IV activity in rats and suppression of blood glucose elevation in mice, were also demonstrated. The result of in vivo DPP-IV inhibition indicates that **24b** offers longer duration of action than the inhibitor **1**.

5. Experimental

5.1. Chemistry

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. ^{1}H NMR spectra were obtained with a Varian Mercury-300 or a Varian Mercury-400 spectrometer. Chemical shifts were recorded in parts

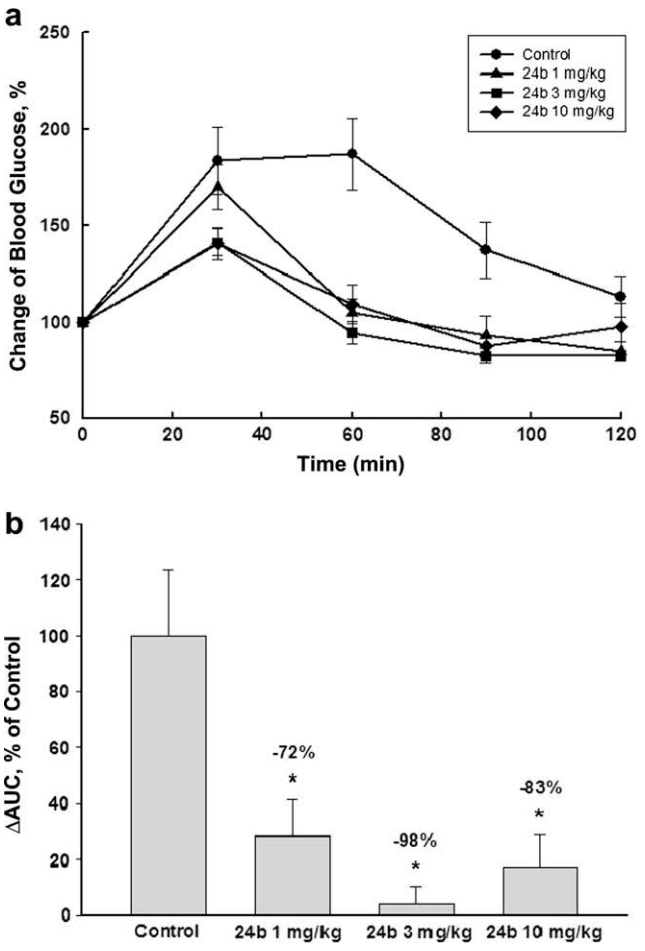


Figure 5. (a) Effects of **24b** (1, 3 and 10 mg/kg) on the glucose excursion level of DIO mice during oral glucose tolerance test. All rats received 3.0 g/kg glucose orally at 0 min. Each of compounds was orally administered to mice at −30 min. (b) The suppression of glucose AUC was calculated from 0 to 120 min. Data are represented as mean ± SEM (n = 6/group).

per million (ppm, δ) and were reported relative to the solvent peak or TMS. LC/MS data were measured on an Agilent MSD-1100 ESI-MS/MS System. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230–400 mesh ASTM). Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass backed plates (5 × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. All starting materials and amines were commercially available unless otherwise indicated.

5.2. General procedure for the preparation of compound 4

The ethereal solution of diazomethane (10 mmol, prepared by a Aldrich Mini Dazald apparatus) was continuously added into a stirred solution of **3** (2.0 mmol) in CH_2Cl_2/ether (10/5 mL) kept at 0 °C. After the addition of diazomethane ethereal solution was complete, the reaction was stirred at room temperature for 1 h. The excess diazomethane was quenched by dropwise addition of acetic acid. The mixture was diluted with CH_2Cl_2, washed sequentially with saturated sodium bicarbonate and brine, and then dried over $MgSO_4$. The organic layer was concentrated and purified by chromatography to give the desired ester, which was used without further purification. The ethereal solution of diazomethane (~16 mmol, prepared by a Aldrich Mini Dazald apparatus) was continuously added into a stirred solution of above ester and palladium(II) acetate (0.01 mmol) in CH_2Cl_2/ether (30/10 mL) kept at 0 °C. After the addition of diazomethane ethereal solution was complete, the reaction was stirred at room temperature for 1 h. The excess diazomethane was quenched by dropwise addition of acetic acid. The mixture was diluted with CH_2Cl_2, washed sequentially with saturated sodium bicarbonate and brine, and then dried over $MgSO_4$. The organic layer was concentrated and purified by chromatography using EtOAc/hexane (1:9) as an eluant to give the desired product **4** (>70%) as a colorless oil. Only the data of representative compounds are showed.

5.2.1. Methyl *trans*-2-(3-chlorophenyl)cyclopropanecarboxylate (4, X = C, R^1 = 3-Cl)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22–7.15 (m, 2H), 7.06 (t, J = 1.8 Hz, 1H), 6.98 (dt, J = 6.3, 1.8 Hz, 1H), 3.71 (s, 3H), 2.49 (ddd, J = 4.5, 6.3, 9.3 Hz, 1H), 1.90 (ddd, J = 4.5, 5.4, 8.4 Hz, 1H), 1.65–1.57 (m, 1H), 1.30 (ddd, J = 4.5, 6.3, 8.4 Hz, 1H). MS (ES^+) m/z calcd for $C_{11}H_{11}ClO_2$: 210.66; found: 211.1 (M+H), 233.0 (M+Na).

5.2.2. Methyl *trans*-2-(pyridin-3-yl)cyclopropanecarboxylate (4, X = N and R^1 = H)

The title compound was prepared from corresponding compound **3** in 78% yield as a colorless oil and used EtOAc/hexane (4:6) as an eluant. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (d, J = 1.8 Hz, 0.6H), 8.43 (d, J = 1.8 Hz, 1.4H), 7.35 (t, J = 1.8 Hz, 0.45H), 7.32 (t, J = 1.8 Hz, 0.55H), 7.19 (d, J = 5.1 Hz, 0.55H), 7.16 (d, J = 5.1 Hz, 0.45H), 3.71 (s, 3H), 2.51 (ddd, J = 4.5, 6.6, 9.3 Hz, 1H), 1.91 (ddd, J = 4.5, 5.4, 8.4 Hz, 1H), 1.68 – 1.59 (m, 1H), 1.32 (ddd, J = 4.5, 6.6, 8.4 Hz, 1H). MS (ES^+) m/z calcd for $C_{10}H_{11}NO_2$: 177.20; found: 178.1 (M+H).

5.3. Ethyl *trans*-2-(4-methyl-1,3-thiazol-5-yl)cyclopropanecarboxylate (8) and ethyl *cis*-2-(4-methyl-1,3-thiazol-5-yl)cyclopropanecarboxylate (9)

5-Ethenyl-4-methyl-1,3-thiazole **6** (2.5 g, 20 mmol) was stirred at 135 °C in an oil bath as ethyl diazoacetate **7** (1.14 g, 10 mmol) was added portionwise over a period of 2 h. After cooling, the mixture was by flash chromatography (silica gel, eluting with 15:85 EtOAc/hexanes) to give the less polar product **8** (1.29 g, 61%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 3.96 (q, J = 7.2 Hz, 2H), 2.45–2.37 (m, 4H, overlapped singlet at 2.40), 2.19–2.11 (m, 1H), 1.61–1.45 (m, 2H), 1.07 (t, J = 7.2 Hz, 3H). MS (ES^+) m/z calcd for $C_{10}H_{13}NO_2S$: 211.07; found: 212.1 (M+H), 234.1 (M+Na). The more polar product **9** (0.55 g, 26%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (s, 1H), 4.12 (q, J = 7.2 Hz, 2H), 2.50 (ddd, J = 4.2, 6.0, 9.0 Hz, 1H), 2.39 (s, 3H), 1.81 (ddd, J = 4.2, 5.1, 8.4 Hz, 1H), 1.64–1.58 (m, 1H), 1.23 (t, J = 7.2 Hz, 3H), 1.13 (ddd, J = 4.2, 6.0, 8.4 Hz, 1H). MS (ES^+) m/z calcd for $C_{10}H_{13}NO_2S$: 211.07; found: 212.1 (M+H), 234.1 (M+Na).

5.4. General procedure for the preparation of compound 5

A solution of compound **4** or **8** (10 mmol) in methanol (30 mL) was added 4 N NaOH aqueous solution (5 mL). The reaction was stirred at room temperature for 4 h. The resulting solution was neutralized by 1 N HCl aqueous solution and concentrated in vacuo. The crude product was added ethyl acetate (100 mL) and washed by 1 N HCl aqueous solution (100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the crude acid **5,** which was used for the next reaction without further purification.

5.5. General procedure for the preparation of compound 11

A solution of cinnamic acid **3** (10.0 mmol) in thionyl chloride (4.76 g, 40.0 mmol) was stirred at 55 °C under nitrogen for 4 h.

The volatiles were removed under reduced pressure to give the desired acid chloride, which was used without further purification. To a solution of sodium hydride (400 mg, 10.0 mmol, 60% dispersion in oil) in THF (10 mL) was added a solution of (*R*)-4-phenyl-2-oxazolidinone (1.63 g, 10.0 mmol) in THF (15 mL) at 0 °C. The mixture was stirred at room temperature for 1 h and the above acid chloride was added at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for additional 10 h. The reaction was quenched with 2 N HCl at 0 °C, extracted with CH_2Cl_2, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography with EtOAc/hexanes (1:9) to yield **11** (80–90%) as a white solid. Only the data of representative compound is showed.

5.5.1. (4*R*)-3-[(2*E*)-3-(3-Chlorophenyl)prop-2-enoyl]-4-phenyl-1,3-oxazolidin-2-one (11, R^1 = 3-Cl)

Mp 104–106 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 15.6 Hz, 1H), 7.69 (d, *J* = 15.6 Hz, 1H), 7.56–7.31 (m, 9H), 5.55 (dd, *J* = 3.9, 8.7 Hz, 1H), 4.75 (t, *J* = 8.7 Hz, 1H), 4.32 (dd, *J* = 3.9, 8.7 Hz, 1H). MS (ES^+) *m/z* calcd for $C_{18}H_{14}ClNO_3$: 327.07; found: 328.1 (M+H), 350.1 (M+Na).

5.6. General procedure for the preparation of compounds 12a and 12b

The ethereal solution of diazomethane (~16 mmol, prepared by a Aldrich Mini Dazald apparatus) was continuously added into a stirred solution of **11** (2.0 mmol) and palladium(II) acetate (2 mg, 0.01 mmol) in CH_2Cl_2/ether (56/20 mL) kept at 0 °C. After the addition of diazomethane ethereal solution was complete, the reaction was stirred at room temperature for 1 h. The excess diazomethane was quenched by dropwise addition of acetic acid. The mixture was diluted with CH_2Cl_2, washed sequentially with saturated sodium bicarbonate and brine, and then dried over $MgSO_4$. The organic layer was concentrated and purified by chromatography by using a Biotage system (FLASH12i, silica gel, eluting with 1:9 EtOAc/hexane) to give the less polar product **12b** (0.23 g, 33%) and the more polar product **12a** (0.46 g, 67%). Only the data of representative compounds are showed.

5.6.1. (4*R*)-3-{[(1*S*,2*S*)-2-(3-Chlorophenyl)cyclopropyl]carbonyl}-4-phenyl-1,3-oxazolidin-2-one (12a, R^1 = 3-Cl)

Mp 151–153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.29 (m, 5H), 7.21–7.16 (m, 2H), 7.10 (t, *J* = 1.6 Hz, 1H), 7.00 (dt, *J* = 6.8, 1.6 Hz, 1H), 5.44 (dd, *J* = 4.0, 8.0 Hz, 1H), 4.71 (t, *J* = 8.8 Hz, 1H), 4.27 (dd, *J* = 4.0, 8.8 Hz, 1H), 3.55 (ddd, *J* = 4.4, 5.2, 8.4 Hz, 1H), 2.51 (ddd, *J* = 4.4, 6.4, 9.2 Hz, 1H), 1.67 (ddd, *J* = 4.4, 5.2, 9.2 Hz, 1H), 1.40 (ddd, *J* = 4.4, 6.4, 8.4 Hz, 1H). MS (ES^+) *m/z* calcd for $C_{19}H_{16}ClNO_3$: 341.79; found: 342.1 (M+H), 364.1 (M+Na).

5.6.2. (4*R*)-3-{[(1*R*,2*R*)-2-(3-Chlorophenyl)cyclopropyl]carbonyl}-4-phenyl-1,3-oxazolidin-2-one (12b, R^1 = 3-Cl)

Mp 102–104 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.30 (m, 5H), 7.21–7.14 (m, 3H), 7.02 (dt, *J* = 6.6, 2.4 Hz, 1H), 5.44 (dd, *J* = 3.6, 8.7 Hz, 1H), 4.70 (t, *J* = 8.7 Hz, 1H), 4.29 (dd, *J* = 3.6, 8.7 Hz, 1H), 3.54 (ddd, *J* = 4.2, 5.1, 8.7 Hz, 1H), 2.56 (ddd, *J* = 4.2, 6.9, 9.6 Hz, 1H), 1.64 (ddd, *J* = 4.5, 5.1, 9.6 Hz, 1H), 1.40 (ddd, *J* = 4.5, 6.9, 8.7 Hz, 1H). MS (ES^+) *m/z* calcd for $C_{19}H_{16}ClNO_3$: 341.79; found: 342.1 (M+H), 364.1 (M+Na).

5.7. General procedure for the preparation of (1*S*,2*R*)-10a, (1*R*,2*S*)-10b or racemic form 10

To a stirred solution of **12** (2.0 mmol) in THF/H_2O (10/2 mL) at −5 °C was added hydrogen peroxide solution (35 wt. % in water, 0.9 mL). To the resulting slurry was added dropwise a solution of aqueous lithium hydroxide (1 N, 4 mL) within a period of 1 h by a syringe pump. The reaction was stirred at 0 °C for 2 h and a solution of aqueous sodium sulfite (1.5 N, 10 mL) was added. After THF was evaporated in vacuo, the residue was partitioned between CH_2Cl_2 and 1 N hydrochloric acid. The organic phase was dried over $MgSO_4$ and concentrated in vacuo to give **13** (67%) as a white solid, which was used for the next reaction without further purification. To a solution of (1*S*,2*R*)-**13a**, (1*R*,2*S*)-**13b** or racemic form **5** (5 mmol) in dry *tert*-butyl alcohol (10 mL) was added diphenylphosphoryl azide (1.38 g, 5 mmol) and triethylamine (0.60 g, 6 mmol). The mixture was stirred at 90 °C for 8 h. After concentration, the mixture was diluted with CH_2Cl_2, washed sequentially with saturated sodium bicarbonate and brine, and then dried over $MgSO_4$. The organic layer was concentrated and purified by flash chromatography (silica gel, eluting with 15:85 EtOAc/hexanes for phenyl series of analogues, and 4:6 EtOAc/hexanes for heterocyclic series of analogues) to give *N*-BOC protected compound **10** (45–60%) as a white solid. To a stirred solution of above *N*-BOC protected compound **10** (2.75 mmol) was added 2 mL of trifluoroacetic acid and the mixture was stirred at room temperature for 10 min. After concentration, the residue was diluted with 1N hydrochloric acid (10 mL) followed by lyophilization to give (1*S*,2*R*)-**10a,** (1*R*,2*S*)-**10b** or racemic form **10** as a white solid (>90%). Only the data of representative compounds are showed.

5.7.1. (1*S*,2*R*)-2-(3-Chlorophenyl)cyclopropanamine hydrochloride (10a, R^1 = 3-Cl)

Mp 210–212 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.34–7.28 (m, 2H), 7.23 (t, *J* = 2.0 Hz, 1H), 7.12 (dt, *J* = 6.8, 2.0 Hz, 1H), 2.90 (ddd, *J* = 3.6, 4.4. 8.0 Hz, 1H), 2.44 (ddd, *J* = 3.6, 6.8, 10.0 Hz, 1H), 1.47 (ddd, *J* = 4.4. 6.8, 10.0 Hz, 1H), 1.36 (q like, *J* = 3.8 Hz, 1H). MS (ES^+) *m/z* calcd for $C_9H_{10}ClN$: 167.05; found: 168.1 (M+H). The specific rotation of **10a** is $[\alpha]_D^{25} = +37.6$ (*c* 0.05, MeOH).

5.7.2. (1*R*,2*S*)-2-(3-Chlorophenyl)cyclopropanamine hydrochloride (10b, R^1 = 3-Cl)

^{1}H NMR spectrum of **10b** is the same as that of **10a**. The specific rotation of **10b** is $[\alpha]_D^{25} = -38.6$ (*c* 0.05, MeOH).

5.8. General procedure for EDC coupling reaction for compounds 16

A solution of *N*-BOC-protected carboxyl compound **15** (1.0 equiv) and HOBT (1.2 equiv) in 20 mL CH_2Cl_2/1,4-dioxane (2/1) was cooled in an ice-water bath. To this solution was added EDC (1.2 equiv) and stirred at room temperature for 1 h. To the resulting solution was added the appropriate aryl cyclopropylamine (**10**, 1.5 equiv, pretreated with 1.5 equiv Et_3N). After 18 h of stirring at room temperature, the precipitate was removed by filtration and washed by ethyl acetate. The filtrate and washings were combined, washed sequentially with 10% aqueous citric acid and saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (eluted with hexane/EA = 3/7 for phenyl series of analogues, hexane/EA = 6/4 for heterocyclic series of analogues) yielded the desired compound **16** (>70%) as a light yellow solid. Only the data of representative compound is showed.

5.8.1. *tert*-Butyl (2-methyl-4-{[(1*S*,2*R*)-2-(3-chlorophenyl)-cyclopropyl]amino}-4-oxobutan-2-yl)carbamate (16b, R^1 = 3-Cl)

Mp 118–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21–6.23 (m, 4H), 6.22 (s, 1H), 4.88 (s, 1H), 2.85 (ddd, *J* = 3.3, 4.6, 7.5 Hz, 1H), 2.57 (q like, *J* = 13.2 Hz, 2H), 1.96 (ddd, *J* = 3.3, 6.3, 9.6 Hz, 1H), 1.42 (s, 9H), 1.37 (s, 6H), 1.25–1.08 (m, 2H). MS (ES^+) *m/z* calcd for $C_{19}H_{27}ClN_2O_3$: 366.17; found: 389.2 (M+Na).

5.9. General procedure for the synthesis of compounds 18–25

A solution of compound **16** (1 equiv) in cool TFA was stirred at room temperature for 30 min and concentrated in vacuo for 3 h. The resultant yellowish oil was dissolved in THF, added K_2CO_3 (5 equiv) and stirred for a period of 1 h to liberate free amine. The solution was diluted with EA and filtered to remove potassium salts. The filtrate was concentrated in vacuo so as to obtain about 0.5 M free amine solution. To this solution was added bromide compound **17** (0.5 equiv), the reaction mixture was stirred 18 h at room temperature. The mixture was diluted with CH_2Cl_2, and then washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography (eluted with CH_2Cl_2/MeOH = 98/2–95/5) yielded desired compounds **18–25** (40–70%) as light yellow gums.

5.9.1. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-phenylcyclopropyl]butanamide (18a)

^{1}H NMR (400 MHz, $CDCl_3$) (4/1 mixture of *trans/cis* amide rotomers) δ 8.57 (br d, J = 10.4 Hz, 4/5H), 8.32–8.24 (m, 1/5H), 7.27–7.12 (m, 5H), 4.81–4.76 (m, 4/5H), 4.71–4.66 (m, 1/5H), 3.64–3.33 (m, 4H), 2.96–2.93 (m, 1H), 2.35–2.03 (m, 7H), 1.35–1.16 (m, 8H, overlapped singlet at 1.18). MS (ES+) *m/z* calcd for $C_{21}H_{28}N_4O_2$: 368.22; found: 369.2 (M+H).

5.9.2. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(4-fluorophenyl)cyclopropyl]butanamide (18b)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.60 (br s, 5/6H), 8.34–8.28 (m, 1/6H), 7.16–7.10 (m, 2H), 6.94 (t, *J* = 8.7 Hz, 2H), 4.79–4.76 (m, 5/6H), 4.69–4.64 (m, 1/6H), 3.62–3.36 (m, 4H), 2.88–2.84 (m, 1H), 2.35–2.0 (m, 7H), 1.17–1.13 (m, 8H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{21}H_{27}FN_4O_2$: 386.21; found: 387.2 (M+H).

5.9.3. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3-fluorophenyl)cyclopropyl]butanamide (18c)

^{1}H NMR (400 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.67 (br d, *J* = 7.2 Hz, 5/6H), 8.42 (br d, *J* = 7.2 Hz, 1/6H), 7.23–7.17 (m, 1H), 6.93 (t, *J* = 7.2 Hz, 1H), 6.86–6.82 (m, 2H), 4.78 (d, *J* = 7.6 Hz, 5/6H), 4.69–4.65 (m, 1/6H), 3.64–3.32 (m, 4H), 2.94–2.91 (m, 1H), 2.36–2.12 (m, 6H), 2.05 (dt, *J* = 3.2, 7.6 Hz, 1H), 1.25–1.18 (m, 8H, overlapped singlet at 1.18). MS (ES^+) *m/z* calcd for $C_{21}H_{27}FN_4O_2$: 386.21; found: 387.2 (M+H).

5.9.4. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3,5-di-fluorophenyl)cyclopropyl] butanamide (18e)

^{1}H NMR (400 MHz, $CDCl_3$) (6/1 mixture of *trans/cis* amide rotomers) δ 8.76–8.71 (m, 6/7H), 8.52–8.46 (m, 1/7H), 6.69–6.57 (m, 3H), 4.79–4.77 (m, 6/7H), 4.69–4.65 (m, 1/7H), 3.64–3.34 (m, 4H), 2.92–2.90 (m, 1H), 2.36–2.01 (m, 7H), 1.28–1.18 (m, 8H, overlapped singlet at 1.18). MS (ES^+) *m/z* calcd for $C_{21}H_{26}F_2N_4O_2$: 404.20; found: 405.2 (M+H).

5.9.5. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3,4-di-fluorophenyl)cyclopropyl] butanamide (18f)

^{1}H NMR (400 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.69 (br s, 5/6H), 8.43 (br s, 1/6H), 7.26–6.89 (m, 3H), 4.78 (br d, *J* = 7.2 Hz, 5/6H), 4.64–4.68 (m, 1/6H), 3.62–3.32 (m, 4H), 2.85–2.82 (m, 1H), 2.35–2.19 (m, 6H), 2.04–1.99 (m, 1H), 1.20–1.12 (m, 8H, overlapped singlet at 1.17). MS (ES^+) *m/z* calcd for $C_{21}H_{26}F_2N_4O_2$: 404.20; found: 405.2 (M+H).

5.9.6. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-{2-[4-(trifluoromethyl)phenyl]cyclopropyl} butanamide (18g)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.70 (br s, 5/6H), 8.45–8.40 (m, 1/6H), 7.52 (d, *J* = 8.1 Hz, 3H), 7.25 (d, *J* = 8.1 Hz, 2H), 4.81–4.76 (m, 5/6H), 4.69–4.64 (m, 1/6H), 3.64–3.34 (m, 4H), 2.97–2.92 (m, 1H), 2.33–2.03 (m, 7H), 1.32–1.19 (m, 8H, overlapped singlet at 1.19). MS (ES^+) *m/z* calcd for $C_{22}H_{27}F_3N_4O_2$: 436.21; found: 437.2 (M+H).

5.9.7. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-{2-[3-(trifluoromethyl)phenyl]cyclopropyl} butanamide (18h)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.74 (dd, *J* = 10.2, 3.3 Hz, 5/6H), 8.50 (br d, *J* = 10.2 Hz, 1/6H), 7.43–7.26 (m, 4H), 4.79–4.76 (m, 5/6H), 4.68–4.63 (m, 1/6H), 3.65–3.32 (m, 4H), 2.97–2.92 (m, 1H), 2.36–2.08 (m, 7H), 1.27–1.21 (m, 2H), 1.19(s, 6H). MS (ES^+) *m/z* calcd for $C_{22}H_{27}F_3N_4O_2$: 436.21; found: 437.2 (M+H).

5.9.8. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3-trifluoromethoxyphenyl)cyclopropyl] butanamide (18i)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.71 (dd, *J* = 9.6, 3.3 Hz, 5/6H), 8.48 (dd, *J* = 9.6, 3.3 Hz, 1/6H), 7.27 (t, *J* = 7.5 Hz, 1H), 7.11–6.99 (m, 3H), 4.79–4.76 (m, 5/6H), 4.68–4.65 (m, 1/6H), 3.65–3.32 (m, 4H), 2.97–2.90 (m, 1H), 2.36–1.98 (m, 7H), 1.27–1.17 (m, 8H, overlapped singlet at 1.18). MS (ES^+) *m/z* calcd for $C_{22}H_{27}F_3N_4O_3$: 452.20; found: 453.2 (M+H).

5.9.9. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(4-chlorophenyl)cyclopropyl]butanamide (18j)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.64 (br s, 5/6H), 8.36 (br d, *J* = 10.2 Hz, 1/6H), 7.23–7.20 (m, 2H), 7.11–7.06 (m, 2H), 4.80–4.75 (m, 5/6H), 4.69–4.64 (m, 1/6H), 3.66–3.36 (m, 4H), 2.90–2.85 (m, 1H), 2.34–1.99 (m, 7H), 1.25–1.16 (m, 8H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{21}H_{27}ClN_4O_2$: 402.18; found: 403.1 (M+H), 405.0 (M+3).

5.9.10. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3-chlorophenyl)cyclopropyl]butanamide (18k)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.65 (br d, *J* = 7.2 Hz, 5/6H), 8.42–8.38 (m, 1/6H), 7.20–7.13 (m, 3H), 7.03 (t, *J* = 6.6 Hz, 1H), 4.78 (d, *J* = 6.6 Hz, 5/6H), 4.65 (d, *J* = 7.5 Hz, 1/6H), 3.75–3.30 (m, 4H), 2.92–2.90 (m, 1H), 2.40–2.10 (m, 6H, overlapped singlet at 2.27), 2.05–1.99 (m, 1H), 1.24–1.16 (m, 8H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{21}H_{27}ClN_4O_2$: 402.18; found: 403.2 (M+H), 425.2 (M+Na).

5.9.11. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3,4-di-chlorophenyl)cyclopropyl] butanamide (18l)

^{1}H NMR (400 MHz, $CDCl_3$) (6/1 mixture of *trans/cis* amide rotomers) δ 8.74 (br s, 6/7H), 8.51 (br s, 1/7H), 7.31–7.24 (m, 2H), 7.01 (dt, J = 2.0, 8.4 Hz, 1H), 4.77 (br d, *J* = 6.8 Hz, 6/7H), 4.66 (br d, *J* = 6.8 Hz, 1/7H), 3.64–3.32 (m, 4H), 2.91–2.84 (m, 1H), 2.36–1.99 (m, 7H), 1.25–1.16 (m, 8H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{21}H_{26}Cl_2N_4O_2$: 436.14; found: 437.0 (M+H), 439.0 (M+3).

5.9.12. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(3,4-di-methoxyphenyl)cyclopropyl] butanamide (18m)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.56–8.52 (m, 5/6H), 8.24–8.16 (m, 1/6H), 6.81–6.67 (m, 3H), 4.79–4.64 (m, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.64–3.42 (m, 4H), 2.92–2.86 (m, 1H), 2.38–1.99 (m, 7H), 1.30–1.09 (m, 8H, overlapped singlet at 1.20). MS (ES^+) *m/z* calcd for $C_{23}H_{32}N_4O_4$: 428.24; found: 429.2 (M+H).

5.9.13. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[*trans*-2-(pyridin-3-yl) cyclopropyl]butanamide (19)

^{1}H NMR (400 MHz, DMSO-d_6) (5/1 mixture of *trans/cis* amide rotomers) δ 8.56 (dd, *J* = 4.0, 12.8 Hz, 1H), 8.41 (t, *J* = 2.8 Hz, 1H), 8.35 (dd, *J* = 0.8, 4.8 Hz, 1H), 8.35 (ddd, *J* = 2.0, 4.0 8.0 Hz, 1H), 7.26 (dd, *J* = 4.8, 8.0 Hz, 1H), 5.55 (t, *J* = 3.2 Hz, 5/12H), 5.48 (t, *J* = 3.2 Hz, 1/12H), 5.42 (t, *J* = 3.2 Hz, 5/12H), 5.38 (d, *J* = 8.4 Hz, 1/6H), 5.35 (t, *J* = 3.2 Hz, 1/12H), 4.97–4.95 (m, 5/6H), 3.98–3.27 (m, 4H, overlapped singlet at 3.33), 2.85–2.80 (m, 1H), 2.50–2.31 (m, 2H), 2.16 (dd, *J* = 15.2, 20.4 Hz, 2H), 1.96–1.91 (m, 1H), 1.24–1.18 (m, 2H), 1.06 (s, 6H). MS (ES^+) *m/z* calcd for $C_{20}H_{26}FN_5O_2$: 387.21; found: 388.2 (M+H), 410.2 (M+Na). HRMS (FAB) calcd for $C_{20}H_{27}FN_5O_2$: 388.2149; found: 388.2151.

5.9.14. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}-amino)-3-methyl-*N*-[*trans*-2-(4-methyl-1,3-thiazol-5-yl)cyclopropyl]butanamide (20)

^{1}H NMR (300 MHz, $CDCl_3$) (4/1 mixture of *trans/cis* amide rotomers) δ 8.58 (br s, 4/5H), 8.45 (s, 1H), 8.34 (br s, 1/5H), 5.52 (t, *J* = 3.3 Hz, 2/5H), 5.44 (t, *J* = 3.3 Hz, 1/10H), 5.35 (t, *J* = 3.3 Hz, 2/5H), 5.26 (t, *J* = 3.3 Hz, 1/10H), 4.94 (d, *J* = 9.3 Hz, 4/5H), 4.93 (d, *J* = 9.3 Hz, 1/5H), 3.99–3.49 (m, 2H), 3.40 (q like, *J* = 16.5 Hz, 2H), 2.91–2.89 (m, 1H), 2.76 (t, *J* = 15.9 Hz, 1/5H), 2.68 (t, *J* = 15.9 Hz, 4/5H), 2.55–2.22 (m, 6H, overlapped two singlet at 2.48, 2.27), 2.11–2.03 (m, 1H), 1.37–1.21 (m, 1H), 1.21–0.95 (m, 7H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{19}H_{26}FN_5O_2S$: 407.18; found: 408.2 (M+H). Anal. ($C_{19}H_{26}FN_5O_2S{\cdot}H_2O$) C, H, N.

5.9.15. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*R*,2*S*)-2-(3-chlorophenyl)cyclopropyl] butanamide (21)

^{1}H NMR (300 MHz, $CDCl_3$) (5/1 mixture of *trans/cis* amide rotomers) δ 8.65 (br s, 5/6H), 8.36 (br s, 1/6H), 7.39–7.11 (m, 3H), 7.02 (br d, *J* = 7.2 Hz, 1H), 4.78 (d, *J* = 6.3 Hz, 5/6H), 4.65 (d, *J* = 8.4 Hz, 1/6H), 3.73–3.30 (m, 4H), 2.95–2.90 (m, 1H), 2.33–2.17 (m, 6H, overlapped singlet at 2.27), 2.06–1.99 (m, 1H), 1.25–1.16 (m, 8H, overlapped singlet at 1.18). MS (ES^+) *m/z* calcd for $C_{21}H_{27}ClN_4O_2$: 402.18; found: 403.2 (M+H), 425.2 (M+Na).

5.9.16. 3-({2-[(2*S*)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-chlorophenyl)cyclopropyl] butanamide (22)

^{1}H NMR ($CDCl_3$): (5/1 mixture of *trans/cis* amide rotomers) δ 8.65 (d, *J* = 3.3 Hz, 5/6H), 8.45 (d, *J* = 3.3 Hz, 1/6H), 7.18–7.09 (m, 3H), 7.02–6.98 (m, 1H), 4.77–4.74 (m, 5/6H), 4.71 (d, *J* = 2.4 Hz, 1/6H), 3.63–3.38 (m, 4H, overlapped two singlet at 3.46, 3.44), 2.93–2.89 (m, 1H), 2.35–2.15 (m, 6H, overlapped singlet at 2.32), 2.06–1.99 (m, 1H), 1.26–1.43 (m, 8H, overlapped singlet at 1.20). MS (ES^+) *m/z* calcd for $C_{21}H_{27}ClN_4O_2$: 402.18; found: 403.2 (M+H), 425.2 (M+Na).

5.9.17. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*R*,2*S*)-2-(3-chlorophenyl) cyclopropyl]butanamide (23)

^{1}H NMR (300 MHz, $CDCl_3$) (4/1 mixture of *trans/cis* amide rotomers) δ 8.45 (br d, *J* = 3.0 Hz, 4/5H), 8.16 (br s, 1/5H), 7.21–7.00 (m, 4H), 5.51 (t, *J* = 3.3 Hz, 2/5H), 5.43 (t, *J* = 3.3 Hz, 1/10H), 5.34 (t, *J* = 3.3 Hz, 2/5H), 5.26 (t, *J* = 3.3 Hz, 1/10H), 4.98 (d, *J* = 9.3 Hz, 4/5H), 4.94 (d, *J* = 9.3 Hz, 1/5H), 4.02–3.61 (m, 2H), 3.39 (q like, *J* = 16.8 Hz, 2H), 2.96–2.87 (m, 1H), 2.74 (t, *J* = 15.3 Hz, 1/5H), 2.67 (t, *J* = 15.3 Hz, 4/5H), 2.51–2.22 (m, 3H, overlapped singlet at 2.28), 2.05–1.98 (m, 1H), 1.25–1.06 (m, 8H, overlapped singlet at 1.17). MS (ES^+) *m/z* calcd for $C_{21}H_{26}ClFN_4O_2$: 420.17; found: 421.2 (M+H).

5.9.18. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-phenylcyclopropyl] butanamide (24a)

^{1}H NMR ($CDCl_3$): (4/1 mixture of *trans/cis* amide rotomers) δ 8.35 (br d, *J* = 3.3 Hz, 4/5H), 8.18 (br d, *J* = 3.3 Hz, 1/5H), 7.27–7.10 (m, 5H), 5.46 (t, *J* = 3.3 Hz, 2/5H), 5.38 (t, *J* = 3.3 Hz, 1/10H), 5.29 (t, *J* = 3.3 Hz, 2/5H), 5.21 (t, *J* = 3.3 Hz, 1/10H), 5.00 (d, *J* = 9.0 Hz, 1/5H), 4.93 (d, *J* = 9.0 Hz, 4/5H), 4.03–3.55 (m, 2H), 3.39 (q like, *J* = 16.5 Hz, 2H), 2.96–2.89 (m, 1H), 2.69 (t, *J* = 15.3 Hz, 1/5H), 2.61 (t, *J* = 15.3 Hz, 4/5H), 2.52–2.19 (m, 3H), 2.08–2.01 (m, 1H), 1.25–0.96 (m, 8H, overlapped singlet at 1.17). MS (ES^+) *m/z* calcd for $C_{21}H_{27}FN_4O_2$: 386.46; found: 387.2 (M+H), 409.2 (M+Na). Anal. ($C_{21}H_{27}FN_4O_2{\cdot}0.33H_2O$) C, H, N.

5.9.19. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-chlorophenyl) cyclopropyl]butanamide (24b)

^{1}H NMR ($CDCl_3$): (4/1 mixture of *trans/cis* amide rotomers) δ 8.41 (br d, *J* = 3.0 Hz, 4/5H), 8.15 (br s, 1/5H), 7.17–6.99 (m, 4H), 5.52 (t, *J* = 3.3 Hz, 2/5H), 5.42 (t, *J* = 3.3 Hz, 1/10H), 5.35 (t, *J* = 3.3 Hz, 2/5H), 5.26 (t, *J* = 3.3 Hz, 1/10H), 4.96 (d, *J* = 9.3 Hz, 4/5H), 4.92 (d, *J* = 9.3 Hz, 1/5H), 3.91 (dd, *J* = 23.4, 23.1 Hz, 4/5H), 3.77 (d, *J* = 3.6 Hz, 1/5H), 3.73 (d, *J* = 3.9 Hz, 1/5H), 3.65–3.61 (m, 4/5H), 3.38 (q like, *J* = 16.5 Hz, 2H), 2.94–2.88 (m, 1H), 2.76 (t, *J* = 15.3 Hz, 1/5H), 2.69 (t, *J* = 15.3 Hz, 4/5H), 2.43–2.22 (m, 3H, overlapped singlet at 2.27), 2.05–1.99 (m, 1H), 1.24–1.16 (m, 8H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{21}H_{26}ClFN_4O_2$: 420.17; found: 421.2 (M+H). Anal. ($C_{21}H_{26}ClFN_4O_2{\cdot}0.33H_2O$) C, H, N.

5.9.20. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-fluorophenyl) cyclopropyl]butanamide (24c)

^{1}H NMR ($CDCl_3$): (3/1 mixture of *trans/cis* amide rotomers) δ 8.43 (br d, *J* = 3.3 Hz, 3/4H), 8.42 (br s, 1/4H), 7.20 (q like, *J* = 7.2 Hz, 1H), 6.94–6.81 (m, 3H), 5.51 (t, *J* = 3.3 Hz, 3/8H), 5.43 (t, *J* = 3.3 Hz, 1/8H), 5.34 (t, *J* = 3.3 Hz, 3/8H), 5.26 (t, *J* = 3.3 Hz, 1/8H), 4.95 (d, *J* = 9.3 Hz, 1H), 3.91 (dd, *J* = 23.7, 23.4 Hz, 3/4H), 3.78 (d, *J* = 3.6 Hz, 1/4H), 3.74 (d, *J* = 3.9 Hz, 1/4H), 3.66–3.61 (m, 3/4H), 3.39 (q like, *J* = 16.5 Hz, 2H), 2.95–2.88 (m, 1H), 2.74 (t, *J* = 15.3 Hz, 1/4H), 2.67 (t, *J* = 15.3 Hz, 3/4H), 2.45–2.22 (m, 3H, overlapped singlet at 2.27), 2.10–1.98 (m, 1H), 1.28–1.17 (m, 8H, overlapped singlet at 1.20). MS (ES^+) *m/z* calcd for $C_{21}H_{26}F_2N_4O_2$: 404.20; found: 405.2 (M+H), 427.2 (M+Na). HRMS (FAB) calcd for $C_{21}H_{27}FN_4O_2$: 405.2102; found: 405.2094.

5.9.21. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(4-methoxyphenyl) cyclopropyl]butanamide (24d)

^{1}H NMR ($CDCl_3$): (3/1 mixture of *trans/cis* amide rotomers) δ 8.29 (br d, *J* = 3.3 Hz, 3/4H), 8.00 (br s, 1/4H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 5.50 (t, *J* = 3.0 Hz, 3/8H), 5.42 (t, *J* = 3.0 Hz, 1/8H), 5.33 (t, *J* = 3.0 Hz, 3/8H), 5.24 (t, *J* = 3.0 Hz, 1/8H), 4.97 (d, *J* = 8.8 Hz, 1/4H), 4.95 (d, *J* = 8.8 Hz, 3/4H), 3.96–3.54 (m, 5H, overlapped singlet at 3.76), 3.40 (q like, *J* = 16.5 Hz, 2H), 2.88–2.82 (m, 1H), 2.73 (t, *J* = 15.6 Hz, 1/4H), 2.66 (t, *J* = 15.6 Hz, 3/4H), 2.45–2.23 (m, 3H, overlapped singlet at 2.28), 2.0–1.97 (m,

1H), 1.19–1.09 (m, 8H, overlapped singlet at 1.18). MS (ES^+) *m/z* calcd for $C_{22}H_{29}FN_4O_3$: 416.22; found: 417.2 (M+H), 439.2 (M+Na).

5.9.22. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-methoxyphenyl) cyclopropyl]butanamide (24e)

^1H NMR (300 MHz, $CDCl_3$) (3/1 mixture of *trans/cis* amide rotomers) δ 8.33 (br s, 3/4H), 8.05 (br s, 1/4H), 7.17 (t, J = 7.5 Hz, 1H), 6.74–6.67 (m, 3H), 5.53 (t, J = 3.3 Hz, 3/8H), 5.43 (t, J = 3.3 Hz, 1/8H), 5.36 (t, J = 3.3 Hz, 3/8H), 5.26 (t, J = 3.3 Hz, 1/8H), 4.97 (d, J = 9.3 Hz, 3/4H), 4.93 (d, J = 9.3 Hz, 1/4H), 4.12–3.62 (m, 5H, overlapped singlet at 3.78), 3.38 (q like, J = 16.5 Hz, 2H), 2.97–2.91 (m, 1H), 2.76 (t, J = 15.9 Hz, 1/4H), 2.70 (t, J = 15.9 Hz, 3/4H), 2.45–2.22 (m, 3H, overlapped singlet at 2.28), 2.06–2.00 (m, 1H), 1.33–1.11 (m, 8H, overlapped singlet at 1.17). MS (ES^+) *m/z* calcd for $C_{22}H_{29}FN_4O_3$: 416.22; found: 417.2 (M+H), 439.2 (M+Na). Anal. ($C_{22}H_{29}FN_4O_3 \cdot H_2O$) C, H, N.

5.9.23. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-ethoxyphenyl) cyclopropyl]butanamide (24f)

^1H NMR (400 MHz, DMSO-d_6) (4/1 mixture of *trans/cis* amide rotomers) δ 8.47 (br d, J = 4.4 Hz, 4/5H), 8.43 (br d, J = 4.4 Hz, 1/5H), 7.13 (t, J = 7.6 Hz, 1H), 6.70–6.61 (m, 3H), 5.55 (t, J = 2.8 Hz, 2/5H), 5.47 (t, J = 2.8 Hz, 1/10H), 5.42 (t, J = 2.8 Hz, 2/5H), 5.37 (d, J = 9.2 Hz, 1/5H), 5.34 (t, J = 2.8 Hz, 1/10H), 4.97–4.95 (m, 4/5H), 3.98 (q, J = 6.8 Hz, 2H), 3.92–3.27 (m, 4H, overlapped singlet at 3.33), 2.83–2.78 (m, 1H), 2.57–2.31 (m, 2H), 2.15 (dd, J = 14.0, 20.4 Hz, 2H), 1.90–1.85 (m, 1H), 1.30 (t, J = 7.2 Hz, 3H), 1.17–1.08 (m, 2H), 1.06 (s, 6H). MS (ES^+) *m/z* calcd for $C_{23}H_{31}FN_4O_3$: 430.24; found: 431.3 (M+H), 453.3 (M+Na). Anal. ($C_{21}H_{31}FN_4O_3 \cdot 0.33H_2O$) C, H, N.

5.9.24. 3-({2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-trifluoromethoxyphenyl)cyclopropyl]butanamide (24g)

^1H NMR (400 MHz, DMSO-d_6) (5/1 mixture of *trans/cis* amide rotomers) δ 8.54 (br d, J = 4.4 Hz, 5/6H), 8.50 (br d, J = 4.4 Hz, 1/6H), 7.37 (t, J = 8.0 Hz, 1H), 7.14–7.10 (m, 3H), 5.55 (t, J = 3.2 Hz, 5/12H), 5.47 (t, J = 3.2 Hz, 1/12H), 5.42 (t, J = 3.2 Hz, 5/12H), 5.37 (d, J = 8.4 Hz, 1/6H), 5.34 (t, J = 3.2 Hz, 1/12H), 4.97–4.94 (m, 5/6H), 3.99–3.51 (m, 2H), 3.35 (q like, J = 16.4 Hz, 2H), 2.87–2.80 (m, 1H), 2.57–2.31 (m, 2H), 2.15 (dd, J = 14.0, 22.8 Hz, 2H), 1.98–1.89 (m, 1H), 1.24–1.16 (m, 2H), 1.06 (s, 6H).MS (ES^+) *m/z* calcd for $C_{22}H_{26}F_4N_4O_3$: 470.19; found: 471.2 (M+H), 493.2 (M+Na). Anal. ($C_{22}H_{26}F_4N_4O_3 \cdot 0.33H_2O$) C, H, N.

5.9.25. 3-({2-[(2*S*)-2-Cyano-4,4-difluoropyrrolidin-1-yl]-2-oxoethyl}amino)-3-methyl-*N*-[(1*S*,2*R*)-2-(3-chlorophenyl) cyclopropyl]butanamide (25)

^1H NMR (300 MHz, $CDCl_3$) (4/1 mixture of *trans/cis* amide rotomers) δ 8.18 (d, J = 2.4 Hz, 4/5H), 7.81 (br s, 1/5H), 7.21–7.12 (m, 3H), 7.02 (d, J = 7.2 Hz, 1H), 5.21 (br d, J = 6.0 Hz, 1/5H), 4.97 (t, J = 606 Hz, 4/5H), 4.03–3.83 (m, 2H), 3.64–3.37 (m, 2H, overlapped singlet at 3.37), 2.94–2.87 (m, 1H), 2.80–2.70 (m, 2H), 2.26 (s, 2H), 2.02 (dt, J = 7.5, 3.3 Hz, 1H), 1.22–1.16 (m, 8H, overlapped singlet at 1.16). MS (ES^+) *m/z* calcd for $C_{21}H_{25}ClF_2N_4O_2$: 438.16; found: 439.2 (M+H), 461.2 (M+Na). Anal. Calcd for $C_{21}H_{25}ClF_2N_4O_2 \cdot 0.5H_2O$: C, 56.30; H, 5.75; N, 12.51. Found: C, 56.23; H, 6.03; N, 12.23.

5.10. Biological methods

5.10.1. Inhibition of DPP-IV, DPP8 and DPP-II in vitro[24,25]

IC_{50} determination is done as described in literature with modifications described below.[24] Sigma plot was used to obtain the IC_{50} values. DPP-IV, FAP and DPP8 were purified as described in our previously published method.[25] The purification of DPP-II was carried out as described in Ref. 25 with modifications. The detailed protocol for the cloning and expression of FAP will be described elsewhere. Briefly, cDNA of FAP was cloned into pBacPAC8-CD5 vector. The buffer for DPP-IV and DPP8 assays are 2 mM Tris–HCl, pH 8.0 and PBS buffer, respectively. The substrate used is Gly-Pro-pNA from Bachem at the concentrations of 500 μM, 6000 μM and 2500 μM for DPP-IV, FAP and DPP8 assays, respectively. DPP-II activity was assayed by 1.5 mM Gly-Pro-pNA in 50 mM potassium phosphate buffer, pH 5.5. The concentration of DPP-II is 10 nM. Reaction developed at 37 °C and OD_{405} was monitored.

5.10.2. DPP-IV inhibition in Wistar rats

Adult male Wistar rats (n = 4–6/group) were orally gavaged with the test compounds dissolved in 0.5% methyl cellulose at a single dose of 10 mg/kg. Blood samples of 25–50 μl were collected from the tail veins at the time points indicated in Figure 3 and the plasma fraction was kept frozen until DPP-IV activity measurement. The plasma DPP-IV activity was determined by cleavage rate of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; BACHEM). Plasma (10 μl) was mixed with 140 μl of 150 μM Gly-Pro-AMC in assay buffer that was composed of 25 mM tris(hydroxymethyl)-aminomethane HCl (pH 7.4), 140 mM NaCl, 10 mM KCl and 0.1% bovine serum albumin. The fluorescence was determined by using Fluoroskan Ascent FL (excitation at 390 nm and emission at 460 nm) (Thermo LabSystems; Thermo Electron Corporation). DPP-IV activity in plasma was described as unit per mL (U/mL). One unit of activity is defined as the amount of enzyme that produces 1 μM products per minute.

5.10.3. Oral glucose tolerance test in C57BL/6j lean mice and C57BL/6j DIO mice

Adult male C57BL/6 mice (20–25 g) (6–8 weeks of age, n = 6/group) or C57BL/6j DIO mice (40–50 g) (27 weeks of age, n = 6/group) were fasted overnight. Blood samples were obtained from the tail veins with needles of 27G and the blood glucose was measured with the Accu-Chek Compact System from Roche (Basel, Switzerland) and animals were grouped according to the glucose levels. The animals were then orally gavaged with the test compounds dissolved in distilled water at a dose indicated in Figure 4. Thirty minutes after the oral dosing of test compounds, the animals were orally gavaged with freshly prepared glucose solution of 400 mg/mL in distilled water at 3 g glucose/kg. Blood glucose levels of these dosed animals were monitored at 0, 15, 30, 60, and 120 min after the oral glucose challenge.

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