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Design, Synthesis, and Pharmacological Evaluation of Highly Potent and Selective Dipeptidyl Peptidase-4 Inhibitors

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The optimization of a series of fused β-homophenylalanine inhibitors of dipeptidyl peptidase-4 (DPP-4) is described. Modification on the P2-binding moiety of **6** ($IC_{50} = 10$ nM) led to the discovery of β-homophenylalanine derivatives containing pyrrolidin-2-ylmethyl amides. The introduction of a sulfamine in the *meta* position of the phenyl ring improved the potency against DPP-4 (6–12-fold increase). Compound **14k** showed DPP-4 inhibitory activity with an IC_{50} value of 0.87 nM. Meanwhile, *in vivo* experiments exhibited that **14h** had an efficiency comparable to sitagliptin at the dose of 10 mg/kg.

Keywords: β-Homophenylalanine / DPP-4 / Drug design / Inhibitors / P2-binding moiety

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Introduction

Secreted from the L cells of the small intestine [1], GLP-1 increases glucose-induced insulin secretion while it decreases glucagon secretion [2]. In addition, it also inhibits acid secretion and the gastric emptying in the stomach, together effects provide benefits for glycemic control [3]. However, GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) *in vivo*, which cleaves a dipeptide from the N-terminus of GLP-1 to give the inactive form of GLP-1 [4]. Therefore, inhibitors of DPP-4 would prolong the half-life of active GLP-1 and enhance the glucose-lowering effects [5], contributing to their potential to be anti-diabetic agents.

To date, a range of structurally diverse DPP-4 inhibitors have been reported, some of which have now been launched onto the market. Among them are sitagliptin (**1**) [5], vildagliptin (**2**) [6], saxagliptin (**3**) [7], alogliptin (**4**) [8], and linagliptin (**5**) [9] (Fig. 1).

Compound **6**, a DPP-4 inhibitor which was discovered by structure-based drug design in our laboratory, showed potent inhibitory activity, excellent selectivity, and good efficacy in an oral glucose tolerance test (OGTT) in C57BL/6 mice. The three-dimensional (3D) structural mode of inhibitors **6** to DPP-4 (Fig. 2) showed that the fused phenyl subunit which occupied the S1 hydrophobic pocket and the β-amino group which stabilized the binding site of DPP-4 by forming four hydrogen bonding interactions are essential for their inhibitory activity against DPP-4 [1]. However, there is a large unoccupied space around the P2-binding moiety (the fused heterocyclic ring). We expected that modification on these parts could generate a new series of DPP-4 inhibitors. We initially performed a replacement of the fused heterocyclic ring with a chainlike *N*-substituted ethylenediamine to quickly explore the feasibility of modifying the P2-binding moiety of **6**. The inhibitory activity of this series was moderate, which indicated a high tolerance for various functional groups at that range of the binding site of DPP-4. Reducing the number of rotatable bonds and increasing the rigidity of the molecules would probably improve the PK/PD

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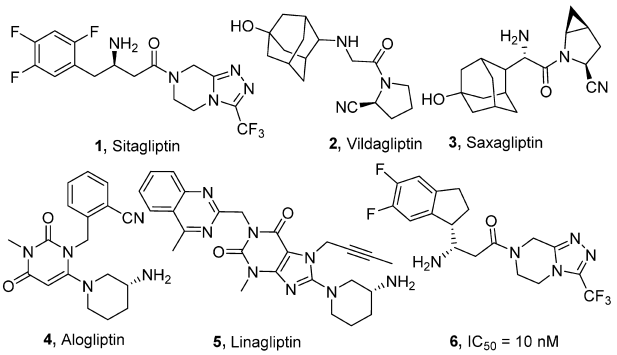


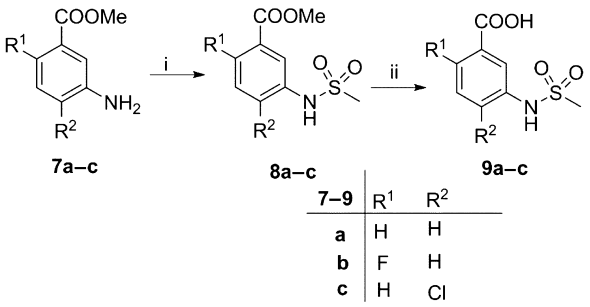
Figure 1. DPP-4 inhibitors.

profiles of molecules [10–12]. Meanwhile, inspired by the *N*-acylpyrrolidine moiety in the structure of vildagliptin (**2**) and saxagliptin (**3**), the chainlike linker was replaced with a pyrrolidine. Furthermore, to explore the substitution effects on the aromatic ring, the 3D binding modes of compounds **14d** and **14h** to DPP-4 (from 2AJL) were generated based on docking simulations. According to the information from 3D binding mode, various substituents were introduced on the aromatic ring. After optimization of this series, most of them emerged as showing excellent enzyme inhibitory activity and selectivity for DPP-4 over two dipeptidyl peptidase family homologues: DPP-8 and DPP-9. Inhibitors were then advanced to *in vivo* studies to assess the utility of series; **14h** and **14o** showed efficacy similar to that of sitagliptin in the OGTT.

Results and discussion

Chemistry

As shown in Scheme 1, commercially available compounds **7a–c** reacted with methanesulfonyl chloride in dichloromethane giving sulphonamides **8a–c**, which were respectively converted to **9a–c** by hydrolysis [13].



7–9	R^1	R^2
a	H	H
b	F	H
c	H	Cl

Scheme 1. Synthesis of intermediates **9a–c**. Reagents and conditions: (i) pyridine, MsCl, and CH_2Cl_2.

As shown in Scheme 2, sulfonylation of benzoic acids **7i–k** produced **8i–k**, respectively, which were coupled with according amines to give the corresponding sulfonamide derivatives **9i–r** [14].

Compounds **14a–r** were synthesized according to the routes described in Scheme 3. Corresponding amines were coupled with carboxylic acid derivatives **9a–r** which were commercially available or synthesized (Schemes 1 and 2) giving intermediates **10a–r**, respectively. Subsequently, removal of the *tert*-butoxycarbonyl group under standard conditions produced **11a–r**. Compound **12** coupled with **11a–r** gives **13a–r**, which was de-protected to yield target compounds **14a–r**.

In vitro DPP-4 inhibitory activity and selectivity

The inhibitory activity of compounds **14a–r** on DPP-4 was evaluated *in vitro* (Table 1). The chain-like ethylenediamine derivatives (**14a–c**) showed moderate inhibitory activity against DPP-4. When the chain-like linker was replaced with 2-aminomethyl pyrrolidine, **14d** and **14e** also exhibited moderate activity ($IC_{50} = 34$ and 26 nM, respectively). Within the 2-aminomethyl pyrrolidine analogs, replacing the phenyl ring with thiazolyl ring produced compound **14f**, which showed a slight increase in inhibitory activity over **14d** or **14e**; while this increase was remarkable in the pyridyl analog (**14g**). Such increase was also seen in compounds **14h** and **14i**, in which the aminosulfonyl was replaced by sulfone group or sulfonamide group. To explain this result, the 3D binding modes of **14d** and **14h** to DPP-4 were generated. The binding modes showed that the sulfone group of **14h** forms a hydrogen bonding interaction with the N–H atom of Q553 in DPP-4, while such hydrogen bond interaction cannot formed between the sulfone group of **14d** and the backbone N–H atom of Q553 (Figs. 3 and 4). As the sulfonyl group on the phenyl ring can form a hydrogen bonding interaction with DPP-4 (Fig. 4), we select sulfonyl group as our preferred substituent of the phenyl ring. In light of the findings noted above, a series of sulfonamide derivatives were synthesized according to Scheme 3. From comparison of **14i** versus **14j** versus **14k**, **14l** versus **14m** versus **14n**,

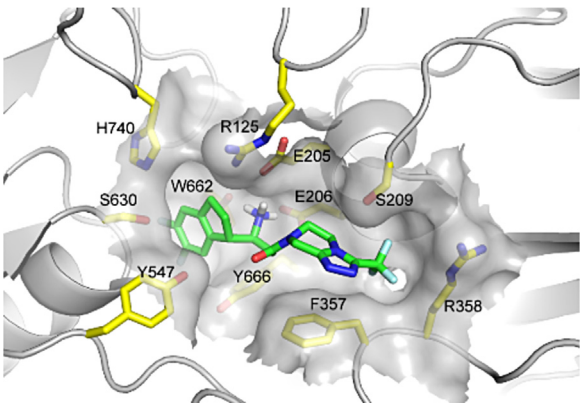
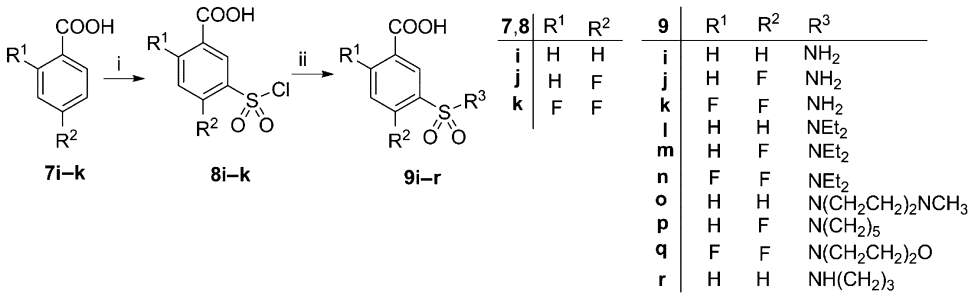


Figure 2. Three-dimensional structural mode of inhibitor **6** to DPP-4 derived from the docking simulations. This image was generated using the PyMOL program.



7,8	R^1	R^2
i	H	H
j	H	F
k	F	F

9	R^1	R^2	R^3
i	H	H	NH_2
j	H	F	NH_2
k	F	F	NH_2
l	H	H	NEt_2
m	H	F	NEt_2
n	F	F	NEt_2
o	H	H	$N(CH_2CH_2)_2NCH_3$
p	H	F	$N(CH_2)_5$
q	F	F	$N(CH_2CH_2)_2O$
r	H	H	$NH(CH_2)_3$

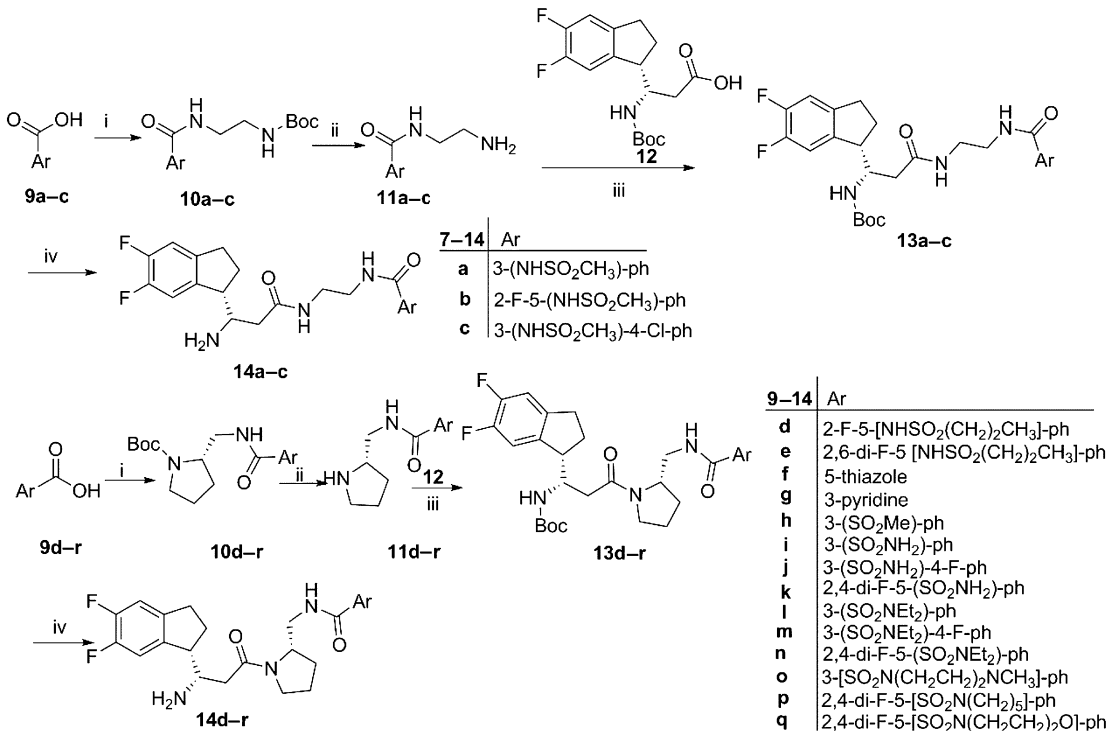
Scheme 2. Synthesis of intermediates **9i–r**. Reagents and conditions: (i) $ClSO_3H$, 150°C; (ii) for **8i–k**, NH_4OH, 0°C; for **8l–n**, diethylamine, EtOAc, 0°C; for **8o**, *N*-methyl piperazine, acetone, 0°C; for **8p**, piperidine, NEt_3, CH_2Cl_2, rt; for **8q**, morpholine, NEt_3, CH_2Cl_2, rt; for **8r**, cyclopropylamine, NEt_3, CH_2Cl_2, rt.

introduction of fluorine atom on the phenyl moiety was of influence on improving inhibitory activities. From comparison of **14i** versus **14l**, **14j** versus **14m**, and **14k** versus **14n**, ethylation of the sulfonamide decreases the inhibitory activities. Even so, all the sulfonamide derivatives exhibited excellent DPP4 inhibitory activity, with IC_{50} values ranging from sub- to single-digit nanomolar. Steric and electronic effects did not appear to affect the binding activity to any appreciable extent, as small alkyl amides (**14i–n**), cyclicamides (**14o–r**) represented in this group all displayed comparable activity.

From the selectivity profiles (Table 2), most of the compounds had more than 2500-fold selectivity for DPP-4 over DPP-8 and DPP-9. Meanwhile, compounds **14h–k** and **14o** had minimal hERG liabilities ($IC_{50} > 20\,\mu M$). High selectivity and good safety profiles improve the suitability of these compounds for further assessment in animal models.

In vivo evaluations

Based on *in vitro* potency, selectivity, and hERG liability analysis, compounds **14h**, **14k**, and **14o** were selected to evaluate the *in vivo* efficacy by acute OGTT in C57BL/6 mice.



7–14	Ar
a	3-($NHSO_2CH_3$)-ph
b	2-F-5-($NHSO_2CH_3$)-ph
c	3-($NHSO_2CH_3$)-4-Cl-ph

9–14	Ar
d	2-F-5-[$NHSO_2(CH_2)_2CH_3$]-ph
e	2,6-di-F-5 [$NHSO_2(CH_2)_2CH_3$]-ph
f	5-thiazole
g	3-pyridine
h	3-(SO_2Me)-ph
i	3-(SO_2NH_2)-ph
j	3-(SO_2NH_2)-4-F-ph
k	2,4-di-F-5-(SO_2NH_2)-ph
l	3-(SO_2NEt_2)-ph
m	3-(SO_2NEt_2)-4-F-ph
n	2,4-di-F-5-(SO_2NEt_2)-ph
o	3-[$SO_2N(CH_2CH_2)_2NCH_3$]-ph
p	2,4-di-F-5-[$SO_2N(CH_2)_5$]-ph
q	2,4-di-F-5-[$SO_2N(CH_2CH_2)_2O$]-ph

Scheme 3. Synthesis of compounds **14a–r**. Reagents and conditions: (i) for **9a–c**, *N*-Boc-ethylenediamine, HOBT, EDC, DIPEA, DMF, rt; for **9d–r**, (*S*)-2-(aminomethyl)-1-(*tert*-butoxycarbonyl)pyrrolidine, HOBT, EDC, DIPEA, DMF, rt; (ii) CF_3COOH, CH_2Cl_2, rt; (iii) HATU, NEt_3, DMF, rt; (iv) CF_3COOH, CH_2Cl_2, rt.

Table 1. DPP-4 inhibitory activity of compounds 14a–r.

Compounds	Ar	IC_{50} (nM)
14a	3-($NHSO_2CH_3$)-ph	41.1 ± 3.0%[a]
14b	2-F-5-($NHSO_2CH_3$)-ph	52.4 ± 3.4%[a]
14c	3-($NHSO_2CH_3$)-4-Cl-ph	57.4 ± 1.5%[a]
14d	2-F-5-[$NHSO_2(CH_2)_2CH_3$]-ph	33.9 ± 7.5
14e	2,6-di-F-5-[$NHSO_2(CH_2)_2CH_3$]-ph	26.3 ± 3.5
14f	5-thiazole	50.5 ± 13%[a]
14g	3-pyridine	5.13 ± 1.26
14h	3-(SO_2Me)-ph	1.06 ± 0.37
14i	3-(SO_2NH_2)-ph	1.72 ± 0.22
14j	3-(SO_2NH_2)-4-F-ph	1.40 ± 0.18
14k	2,4-di-F-5-(SO_2NH_2)-ph	0.87 ± 0.14
14l	3-(SO_2NEt_2)-ph	8.20 ± 1.34
14m	3-(SO_2NEt_2)-4-F-ph	6.99 ± 1.30
14n	2,4-di-F-5-(SO_2NEt_2)-ph	3.48 ± 0.99
14o	3-[$SO_2N(CH_2CH_2)_2NCH_3$]-ph	1.10 ± 0.24
14p	2,4-di-F-5-[$SO_2N(CH_2)_5$]-ph	4.26 ± 2.20
14q	2,4-di-F-5-[$SO_2N(CH_2CH_2)_2O$]-ph	3.95 ± 1.78
14r	2,4-di-F-5-[$SO_2NH(CH_2)_3$]-ph	5.52 ± 3.03
Sitagliptin	–	19.0

[a] Inhibition ratio (%) at 100 nM.

The compounds were administrated at a dose of 10 mg/kg in water 1 h prior to a glucose challenge (5 g/kg). Blood glucose was monitored at different time intervals from 0 to 80 min.

Compared to sitagliptin, compound **14k** showed almost no effect on glucose excursion, while compounds **14h** and **14o** showed comparable glucose lowering effect at 10 mg/kg dose: **14k** (12%), **14h** (40%), **14o** (34%) versus sitagliptin (41%, Fig. 5).

Conclusions

In summary, based on the analysis of large volume of crystal structure data available in the protein data bank (PDB), we designed, synthesized, and evaluated a series of novel fused β-homophenylalanine derivatives containing pyrrolidin-2-ylmethyl amides as potent and selective DPP-4 inhibitors. Most of them showed excellent inhibitory activity against DPP-4 and good selectivity over DPP-8 and DPP-9. Moreover, **14h** exhibited comparable *in vivo* efficiency with sitagliptin at the dose of 10 mg/kg.

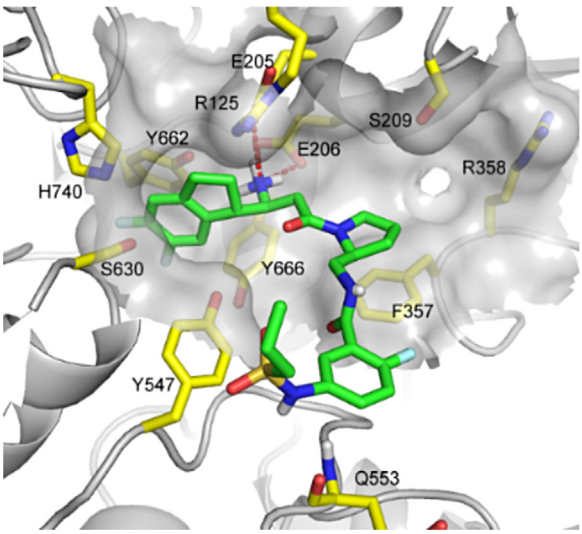


Figure 3. Three-dimensional structural modes of inhibitor **14d** to DPP-4 derived from the docking simulations. This image was generated using the PyMOL program.

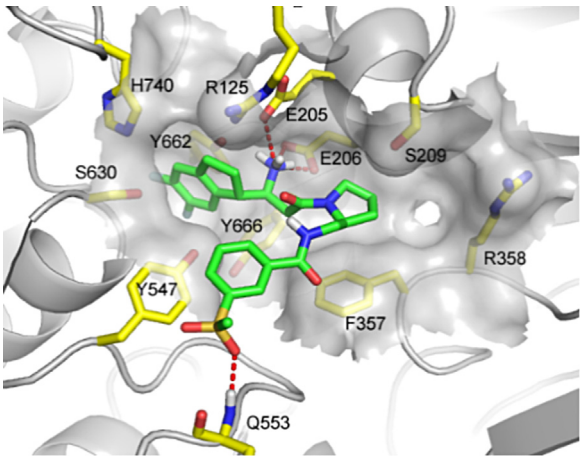


Figure 4. Three-dimensional structural modes of inhibitor **14h** to DPP-4 derived from the docking simulations. This image was generated using the PyMOL program.

Table 2. **Inhibitory activities of selected compounds against DPP-4, DPP-8, DPP-9, and hERG.**

Compounds	DPP-4 (IC_{50}, nM)	DPP-8[a)] (%)	DPP-9[a)] (%)	hERG (IC_{50}, μM)
14g	5.13 ± 1.26	32.7	36.2	NA
14h	1.06 ± 0.37	24.3	25.6	>20
14i	1.72 ± 0.22	34.3	50.5	>20
14j	1.40 ± 0.18	31.1	60.6	>20
14k	0.87 ± 0.14	28.2	50.2	>20
14l	8.20 ± 1.34	47.0	37.5	4.61
14m	6.99 ± 1.30	36.4	49.8	2.60
14n	3.48 ± 0.99	68.7	59.9	3.46
14o	1.10 ± 0.24	9.97	36.1	>20
14p	4.26 ± 2.20	2.19	30.1	NA
14q	3.95 ± 1.78	2.92	46.9	NA
14r	5.52 ± 3.03	NA	NA	NA

NA, not available.
[a)]Inhibition ratio (%) at 10 μM.

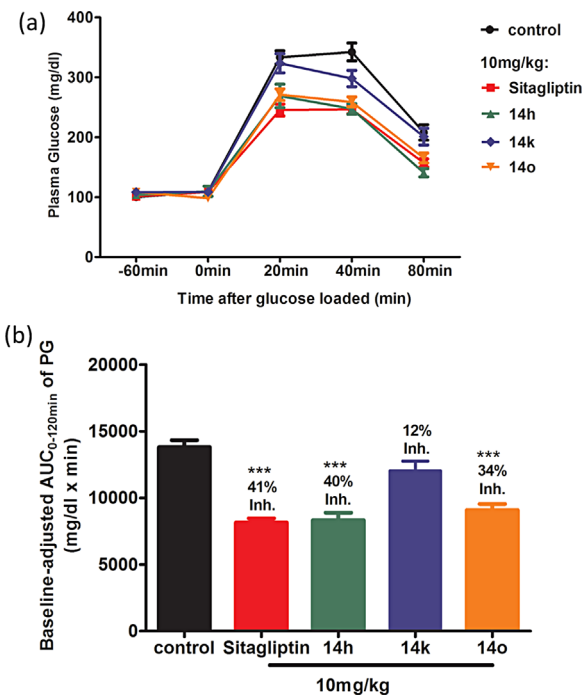


Figure 5. Effect of compounds on plasma glucose (a) and baseline (0 min)-adjusted $AUC_{0-80\,min}$ of plasma glucose (b) after an oral glucose load in glucose tolerance test for C57BL/6 mice. Compounds or water (control) was administered 1 h prior to an oral dextrose challenge (5 g/kg) after an overnight fast. The glucose AUC was determined from 0 to 80 min. Data are represented as mean ± SEM ($n = 6$–8), $^{***}p < 0.001$, Student's *t*-test.

Experimental

Chemistry

Compound synthesis

All reactions were carried out under a static atmosphere of nitrogen, and stirred magnetically unless otherwise noted. All solvents and reagents were obtained from commercial suppliers and used without further drying or purification. All reaction mixtures were monitored using thin-layer chromatography (TLC) on silica gel F-254 TLC plates. Nuclear magnetic resonance (NMR) spectroscopy was recorded on a Bruker AMX-300 or AMX-400 or AMX-500 NMR using Me_4Si as an internal standard. Chemical shifts (δ) are given in parts per million (ppm). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). EI-MS spectra were obtained on a Finnigan MAT95 spectrometer, and ESI-MS spectra were obtained on a Krats MS 80 mass spectrometer. All final compounds were purified to >95% purity by HPLC analysis.

All the final compounds exist as a mixture of amide rotamers (hindered rotation around the nitrogen–carbonyl bond). Severe overlap of signals does not permit unequivocal assignment of each rotamer. Complexity due to ^{19}F spin–spin coupling does not permit assignment of all ^{13}C resonances, therefore, ^{13}C data are not presented.

General procedure for the synthesis of sulphonamides ***9a–c***
Example: Synthesis of 3-(methylsulfonamido)benzoic acid (***9a***): To a solution of methyl 3-amino-benzoate (1.0 g, 6.6 mmol) and pyridine (1.15 g, 14.5 mmol) in dichloromethane (20 mL) at 0°C under a nitrogen atmosphere, methanesulfonyl chloride (1.1 mL, 14.5 mmol) was slowly added. After completion of the reaction as confirmed by TLC, the solution was diluted with dichloromethane, washed sequentially with 1 M hydrochloric acid aqueous solution and brine, dried over Na_2SO_4, and the solvent was removed under

vacuum. The crude product was used in the next step without further purification. A solution of the residue and lithium hydroxide (0.5 g, 12 mmol) in methanol (20 mL) and water (20 mL) was stirred for 18 h. The residue after removal of methanol was dissolved in 1 N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and removed under vacuum to get 8a as an oil (0.8 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.95 (s, 3H), 7.35–7.42 (m, 1H), 7.47–7.52 (m, 1H), 7.75–7.82 (m, 2H). MS *m/e* 214 [M−H].

*2-Fluoro-5-(methylsulfonamido)benzoic acid (**9b**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90 (s, 3H), 7.03–7.12 (m, 1H), 7.41–7.51 (m, 1H), 7.65–7.72 (m, 1H). MS *m/e* 232 [M−H].

*4-Chloro-3-(methylsulfonamido)benzoic acid (**9c**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.02 (s, 3H), 7.42–7.53 (m, 1H), 7.75–7.81 (m, 1H), 7.91–7.96 (m, 1H). MS *m/e* 250 [M−H].

*General procedure for the synthesis of intermediates **8i–k***
Example: Synthesis of 3-(chlorosulfonyl)benzoic acid (***8i***): A mixture of benzoic acid (2 g, 16.4 mmol) in chlorosulfonic acid (5 mL) was heated to 150°C in an oil bath for 2 h. After completion of the reaction as confirmed by TLC, the reaction mixture was slowly poured over ice and filtered. The solid was dried *in vacuo* to yield intermediates **8i** as white solid (3 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (t, 1H), 8.30 (dt, 1H), 8.49 (dt, 1H), 8.78 (t, 1H). MS *m/e* 219 [M−H].

*3-(Chlorosulfonyl)-4-fluorobenzoic acid (**8j**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48 (t, 1H), 8.47–8.54 (m, 1H), 8.74 (dd, 1H), 9.99 (br, 1H). MS *m/e* 237 [M−H].

*5-(Chlorosulfonyl)-2,4-difluorobenzoic acid (**8k**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17–7.29 (m, 1H), 8.69–8.80 (m, 1H). MS *m/e* 255 [M−H].

*General procedure for the synthesis of intermediates **9i–r***
Sulfonyl chloride derivative **8i** or **8j** or **8k** (3.99 mmol) was added gradually to a mixture of amine (12 mmol) in EtOAc with stirring at 0°C. The reaction mixture was stirred at room temperature. After completion of the reaction as confirmed by TLC, the reaction mixture was dissolved in dichloromethane and extracted with 10% NaOH. After the aqueous layer was acidified with 2 N HCl, the precipitate was collected by filtration, washed with H_2O, and dried *in vacuo* to give the desired products (**9i–r**), which were carried forward without further purification.

*3-Sulfamoylbenzoic acid (**9i**)*
^{1}H NMR (300 MHz, DMSO-d_6): δ 7.50 (s, 2H), 7.71 (t, 1H), 8.04 (dt, 1H), 8.13 (dt, 1H), 8.38 (t, 1H), 13.44 (br, 1H). MS *m/e* 200 [M−H].

*4-Fluoro-3-sulfamoylbenzoic acid (**9j**)*
^{1}H NMR (300 MHz, DMSO-d_6): δ 7.21–7.24 (m, 1H), 7.37–7.41 (m, 1H), 7.55–7.59 (m, 1H). MS *m/e* 218 [M–H].

*3-(N,N-Diethylsulfamoyl)benzoic acid (**9l**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15 (t, 6H), 3.29 (q, 4H), 7.64 (t, 1H), 8.07 (dt, 1H), 8.29 (dt, 1H), 8.54 (t, 1H). MS *m/e* 256 [M–H].

*3-(N,N-Diethylsulfamoyl)-4-fluorobenzoic acid (**9m**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, 6H), 3.38 (q, 4H), 7.29 (t, 1H), 8.26–8.32 (m, 1H), 8.66 (dd, 1H). MS *m/e* 274 [M−H].

*3-((4-Methylpiperazin-1-yl)sulfonyl)benzoic acid (**9o**)*
^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 3H), 2.43–2.48 (m, 4H), 2.90–2.98 (m, 4H), 7.79 (t, 1H), 7.96 (dt, 1H), 8.17 (t, 1H), 8.23 (dt, 1H). MS *m/e* 283 [M−H].

*2,4-Difluoro-5-(piperidin-1-ylsulfonyl)benzoic acid (**9p**)*
^{1}H NMR (300 MHz, DMSO-d_6): δ 1.35–1.52 (m, 6H), 3.00–3.08 (m, 4H), 7.73 (t, 1H), 8.21 (t, 1H). MS *m/e* 304 [M−H].

*2,4-Difluoro-5-(morpholinosulfonyl)benzoic acid (**9q**)*
^{1}H NMR (300 MHz, DMSO-d_6): δ 3.01–3.09 (m, 4H), 3.58–3.66 (m, 4H), 7.76 (t, 1H), 8.21 (t, 1H). MS *m/e* 306 [M−H].

*5-(N-Cyclopropylsulfamoyl)-2,4-difluorobenzoic acid (**9r**)*
^{1}H NMR (300 MHz, DMSO-d_6): δ 0.29–0.56 (m, 4H), 2.18–2.31 (m, 1H), 7.70 (t, 1H), 8.30 (t, 1H). MS *m/e* 276 [M−H].

*General procedure for the synthesis of compounds **10a–r***
A mixture of corresponding acid (**9a–r**, 1 mmol), EDCI (1.5 mmol), HOBT (1.5 mmol), and 4-methylmorpholine (3 mmol) in DMF was stirred at room temperature for 10 min, then a solution of corresponding amine (1 mmol) in DMF was added, and stirring was continued overnight. The solution was diluted with of ethyl acetate, washed sequentially with 1 M hydrochloric acid aqueous solution, saturated aqueous sodium bicarbonate solution and brine, dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was used in the next step without further purification.

*General procedure for the synthesis of compounds **11a–r***
TFA was added to a stirred solution of **10a–r** in CH_2Cl_2. The solution was stirred at room temperature for 0.5 h and the CH_2Cl_2/TFA solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and the solvent was removed once again under reduced pressure. The resultant amine was used in the next step without further purification.

*General procedure for the synthesis of compounds **13a–r***
To a solution of the intermediate **12** (0.35 mmol) in DMF, TEA (1.75 mmol) and HATU (0.53 mmol) were added. After 2 min, a solution of the resultant amine (**11a–r**, 0.42 mmol) obtained in the previous step in DMF was added. After the completion of the reaction as confirmed by TLC, water was added to the reaction mixture. The crude product was extracted with ethyl acetate. The aqueous layer was washed with ethyl acetate. The combined organic extract was dried over Na_2SO_4 and the solvents were removed *in vacuo* to afford the crude

compound, which was used in the next step without further purification.

*General procedure for the synthesis of compounds **14a–r***
To a stirred solution of **13a–r** in CH_2Cl_2, TFA was added. The solution was stirred at room temperature. After completion of the reaction, the CH_2Cl_2/TFA solvent was removed under reduced pressure. The residue was purified by column chromatography to afford target compounds.

*N-(2-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanamido)ethyl)-3-(methylsulfonamido)-benzamide (**14a**)*
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.98–2.19 (m, 2H), 2.65–2.92 (m, 4H), 2.98 (s, 3H), 3.18–3.44 (m, 5H), 3.86 (br, 1H), 7.23–7.43 (m, 4H), 7.56 (d, 1H), 7.65 (s, 1H), 7.98 (br, 3H), 8.40 (br, 1H), 8.59 (br, 1H), 9.89 (s, 1H). HRMS (ESI$^+$) calcd. for $C_{22}H_{27}F_2N_4O_4S$ $(M+H)^+$ *m/e*, 481.1716; found 481.1723.

*N-(2-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanamido)ethyl)-2-fluoro-5-(methylsulfonamido)benzamide (**14b**)*
^{1}H NMR (300 MHz, CD_3OD) δ 1.95–2.08 (m, 2H), 2.26–2.38 (m, 1H), 2.50–2.59 (m, 1H), 2.79–2.92 (m, 2H), 2.96 (s, 3H), 3.32–3.56 (m, 5H), 3.92–3.99 (m, 1H), 7.10–7.26 (m, 3H), 7.31–7.38 (m, 1H), 7.60–7.65 (m, 1H). HRMS (ESI$^+$) calcd. for $C_{22}H_{26}F_3N_4O_4S$ $(M+H)^+$ *m/e*, 499.1621; found 499.1623.

*N-(2-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanamido)ethyl)-4-chloro-3-(methylsulfonamido)benzamide (**14c**)*
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.98–2.19 (m, 2H), 2.65–2.92 (m, 3H), 2.98–3.05 (m, 1H), 3.06 (s, 3H), 3.18–3.44 (m, 5H), 3.86 (br, 1H), 7.27 (td, 1H), 7.37 (td, 1H), 7.57(d, 1H), 7.72 (dd, 1H), 7.88 (d, 1H), 7.98 (br, 3H), 8.41 (br, 1H), 8.74 (br, 1H), 9.59 (s, 1H). HRMS (ESI$^+$) calcd. for $C_{22}H_{26}ClF_2N_4O_4S$ $(M+H)^+$ *m/e*, 515.1326; found 515.1310.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-2-fluoro-5-(propylsulfonamido)benzamide (**14d**)*
^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (t, 3H), 1.68–1.78 (m, 2H), 1.82–2.16 (m, 6H), 2.31–2.42 (m, 1H), 2.65–2.96 (m, 4H), 3.08 (t, 2H), 3.19–3.56 (m, 4H), 3.73 (br, 1H), 4.15 (br, 1H), 7.16–7.55 (m, 5H), 8.59 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{27}H_{34}F_3N_4O_4S$ $(M+H)^+$ *m/e*, 567.2247; found 567.2247.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-2,6-difluoro-3-(propylsulfonamido)benzamide (**14e**)*
^{1}H NMR (300 MHz, CD_3OD) δ 1.03 (t, 3H), 1.78–1.89 (m, 2H), 1.92–2.16 (m, 6H), 2.26–2.42 (m, 1H), 2.57–2.67 (m, 1H), 2.74–2.82 (m, 1H), 2.82–3.04 (m, 2H), 3.08 (t, 2H), 3.45–3.56 (m, 4H), 3.95–4.03 (m, 1H), 4.25 (d, 1H), 6.99–7.35 (m, 3H), 7.50–7.84 (m, 1H). HRMS (ESI$^+$) calcd. for $C_{27}H_{33}F_4N_4O_4S$ $(M+H)^+$ *m/e*, 585.2153; found 585.2162.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)thiazole-5-carboxamide (**14f**)*
^{1}H NMR (300 MHz, CD_3OD) δ 1.88–2.15 (m, 6H), 2.26–2.43 (m, 1H), 2.60–3.12 (m, 4H), 3.42–3.67 (m, 5H), 3.99–4.42 (m, 1H), 7.06–7.30 (m, 2H), 8.38 (d, 1H), 9.13 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{21}H_{25}F_2N_4O_2S$ $(M+H)^+$ *m/e*, 435.1661; found 435.1663.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)nicotinamide (**14g**)*
^{1}H NMR (300 MHz, CD_3OD) δ 1.75–2.26 (m, 7H), 2.33–2.48 (m, 1H), 2.66–2.92 (m, 2H), 3.11–3.20 (m, 1H), 3.38–3.60 (m, 5H), 4.02–4.25 (m, 1H), 7.19–7.36 (m, 2H), 7.45–7.54 (m, 1H), 8.13–8.20 (m, 1H), 8.65–8.79 (m, 1H), 8.94–9.03 (m, 1H). HRMS (ESI$^+$) calcd. for $C_{23}H_{27}F_2N_4O_2$ $(M+H)^+$ *m/e*, 429.2097; found 429.2103.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-(methylsulfonyl)benzamide (**14h**)*
^{1}H NMR (300 MHz, CD_3OD) δ 1.88–2.22 (m, 5H), 2.26–2.43 (m, 1H), 2.60–2.76 (m, 1H), 2.79–3.09 (m, 3H), 3.16 (s, 3H), 3.20 (s, 1H), 3.41–3.70 (m, 4H), 4.02–4.25 (m, 1H), 4.42 (d, 1H), 7.10 (t, 1H), 7.27 (t, 1H), 7.68–7.80 (m, 1H), 8.07–8.21 (m, 2H), 8.39 (br, 1H). HRMS (ESI$^+$) calcd. for $C_{25}H_{30}F_2N_3O_4S$ $(M+H)^+$ *m/e*, 506.1920; found 506.1927.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-sulfamoylbenzamide (**14i**)*
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76–2.06 (m, 6H), 2.15–2.27 (m, 1H), 2.31–2.42 (m, 1H), 2.65–2.90 (m, 2H), 3.18 (br, 1H), 3.38–3.61 (m, 5H), 4.18 (d, 1H), 7.23 (t, 1H), 7.33 (t, 1H), 7.62–7.72 (m, 1H), 7.91–8.11 (m, 2H), 8.31 (d, 1H), 8.92 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{24}H_{29}F_2N_4O_4S$ $(M+H)^+$ *m/e*, 507.1872; found 507.1863.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-4-fluoro-3-sulfamoylbenzamide (**14j**)*
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76–2.15 (m, 6H), 2.19–2.27 (m, 1H), 2.34–2.42 (m, 1H), 2.68–2.90 (m, 2H), 3.18 (br, 1H), 3.38–3.65 (m, 5H), 4.15 (d, 1H), 7.24 (t, 1H), 7.34 (t, 1H), 7.50–7.63 (m, 1H), 8.12 (d, 1H), 8.31 (dd, 1H), 8.97 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{24}H_{28}F_3N_4O_4S$ $(M+H)^+$ *m/e*, 525.1778; found 525.1779.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-2,4-difluoro-5-sulfamoylbenzamide (**14k**)*
^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76–2.15 (m, 6H), 2.23–2.33 (m, 1H), 2.37–2.42 (m, 1H), 2.68–2.90 (m, 2H), 3.23 (br, 1H), 3.38–3.64 (m, 4H), 3.65 (br, 1H), 4.19 (d, 1H), 7.25 (t, 1H), 7.35 (t, 1H), 7.62–7.70 (d, 1H), 8.00–8.12 (m, 1H), 8.73 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{24}H_{27}F_4N_4O_4S$ $(M+H)^+$ *m/e*, 543.1684; found 543.1692.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-(N,N-diethylsulfamoyl)benzamide (**14l**)*
^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.04 (t, 6H), 1.76–2.06 (m, 6H), 2.15–2.27 (m, 1H), 2.31–2.42 (m, 1H), 2.65–2.90 (m, 2H), 3.14–3.22 (m, 5H), 3.38–3.54 (m, 4H), 3.60 (br, 1H), 4.15 (d, 1H), 7.23 (t, 1H), 7.33 (t, 1H), 7.72 (dt, 1H), 7.95 (dd, 1H), 8.11 (dd, 1H), 8.25 (d, 1H), 8.99 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{28}H_{37}F_2N_4O_4S$ $(M+H)^+$ *m/e*, 563.2498; found 563.2503.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-(N,N-diethylsulfamoyl)-4-fluorobenzamide (**14m**)*
^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.04 (t, 6H), 1.76–2.15 (m, 6H), 2.19–2.27 (m, 1H), 2.34–2.42 (m, 1H), 2.68–2.90 (m, 2H), 3.18 (br, 1H), 3.38–3.65 (m, 8H), 3.73–3.82 (m, 1H), 4.15 (d, 1H), 7.24 (t, 1H), 7.34 (t, 1H), 7.54–7.63 (m, 1H), 8.17 (dd, 1H), 8.31 (dd, 1H), 8.99 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{28}H_{36}F_3N_4O_4S$ $(M+H)^+$ *m/e*, 581.2404; found 581.2421.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanyl)pyrrolidin-2-yl)methyl)-5-(N,N-diethylsulfamoyl)-2,4-difluorobenzamide (**14n**)*
^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.04 (t, 6H), 1.76–2.15 (m, 6H), 2.19–2.27 (m, 1H), 2.34–2.42 (m, 1H), 2.68–2.90 (m, 2H), 3.18 (br, 1H), 3.38–3.65 (m, 8H), 3.73–3.81 (m, 1H), 4.01–4.25 (m, 1H), 7.26 (t, 1H), 7.36 (t, 1H), 7.64–7.77 (m, 1H), 8.01–8.11 (m, 1H), 8.74 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{28}H_{35}F_4N_4O_4S$ $(M+H)^+$ *m/e*, 599.2310; found 599.2312.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-((4-methylpiperazin-1-yl)sulfonyl)benzamide (**14o**)*
^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.75–2.08 (m, 6H), 2.11 (s, 3H), 2.18–2.28 (m, 1H), 2.30–2.39 (m, 5H), 2.53–3.00 (m, 6H), 3.15 (br, 1H), 3.37–3.63 (m, 5H), 4.15 (d, 1H), 7.17–7.38 (m, 2H), 7.71–7.91 (m, 2H), 8.11–8.24 (m, 2H), 8.99 (d, 1H). HRMS (ESI$^+$) calcd. for $C_{29}H_{38}F_2N_5O_4S$ $(M+H)^+$ *m/e*, 590.2607; found 590.2607.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-2,4-difluoro-5-(piperidin-1-ylsulfonyl)benzamide (**14p**)*
^{1}H NMR (300 MHz, CD$_3$OD) δ 1.47–1.66 (m, 6H), 1.85–2.11 (m, 5H), 2.29–2.41 (m, 1H), 2.64–2.75 (m, 1H), 2.79–3.06 (m, 3H), 3.12–3.20 (m, 5H), 3.37–3.64 (m, 4H), 4.15 (br, 1H), 4.42 (br, 1H), 7.12 (t, 1H), 7.24–7.44 (m, 2H), 8.19 (t, 1H). HRMS (ESI$^+$) calcd. for $C_{29}H_{35}F_4N_4O_4S$ $(M+H)^+$ *m/e*, 611.2310; found 611.2323.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-(morpholinosulfonyl)benzamide (**14q**)*
^{1}H NMR (300 MHz, CD$_3$OD) δ 1.85–2.21 (m, 6H), 2.29–2.41 (m, 1H), 2.62–2.72 (m, 1H), 2.84–3.06 (m, 3H), 3.16 (t, 4H), 3.37–3.62 (m, 4H), 3.71 (t, 4H), 4.11 (br, 1H), 4.42 (br, 1H), 7.15 (t, 1H), 7.24–7.44 (m, 2H), 8.22 (t, 1H). HRMS (ESI$^+$) calcd. for $C_{28}H_{33}F_4N_4O_5S$ $(M+H)^+$ *m/e*, 613.2102; found 613.2110.

*N-(((S)-1-((S)-3-Amino-3-((S)-5,6-difluoro-2,3-dihydro-1H-inden-1-yl)propanoyl)pyrrolidin-2-yl)methyl)-3-(N-cyclopropylsulfamoyl)-4-fluorobenzamide (**14r**)*
^{1}H NMR (300 MHz, CD$_3$OD) δ 0.46–0.62 (m, 4H), 0.83–0.93 (m, 1H), 1.84–2.46 (m, 8H), 2.83–3.11 (m, 3H), 3.46–3.67 (m, 4H), 4.18 (br, 1H), 4.42 (br, 1H), 7.14 (t, 1H), 7.26–7.42 (m, 2H), 8.30 (t, 1H). HRMS (ESI$^+$) calcd. for $C_{27}H_{31}F_4N_4O_4S$ $(M+H)^+$ *m/e*, 583.1997; found 583.1988.

In vitro studies

DPP-IV inhibition measurement in vitro: Caco-2 assay
DPP-4 was extracted from confluent Caco-2 cells as described previously [15]. Assays were performed by mixing 10 μL of appropriate compound dilutions with 40 μL of the substrate for the DPP-4 enzyme, H-Gly-Pro-AMC (AnaSpec; final concentration in the assay, 303 μM), and 50 μL of the Caco-2 cell extract (diluted 5× with 100 mM Tris-HCl, 100 mM NaCl, pH 7.8). Plates were incubated at room temperature for 10 min, and fluorescent absorbance was measured at excitation/emission wavelengths of 380/460 nm using FlexStation 3 microplate reader (Molecular Devices). Maximal reaction rates (fluorescence units/seconds 1000) in 3 min intervals were calculated using the SoftMax software of the FlexStation 3 and modified by the rate of an uninhibited reaction $[(v_{control} - v_{inhibitor})/v_{control}]$. All serial inhibitor dilutions were in DMSO and final solvent concentration did not exceed 0.1%.

DPP-8 and DPP-9 inhibition measurement in vitro
Recombinant human DPP8 (Abcam) and DPP9 (R&D Systems) enzyme activity were performed according to product activity assay protocols. Enzymes were diluted 1250× to a final volume of 50 μL in assay buffer (100 mM Tris-HCl, 100 mM NaCl, pH 7.8) and added to 96-well flat-bottom microtiter plates, followed by the addition of 10 μL inhibitor and 40 μL substrate (H-Gly-Pro-AMC, final concentration in the assay, 303 μM). The plates were incubated at 37°C for 10 min. After incubation, fluorescence was measured using FlexStation 3 microplate reader (Molecular Devices) (excitation 380 nm/emission 460 nm).

Oral glucose tolerance test in C57BL/6 mice

C57BL/6 male mice of 4–8 weeks-old were maintained under constant temperature and humidity conditions: a 12 h light–dark cycle, and free access to water and regular chow diet. After an overnight fasting period, the mice were orally administrated with vehicle (water) or DPP4 inhibitors. Blood samples were taken at −60 and 0 min as baselines. Glucose (5 g/kg) was then administered orally (at 0 min). Serial blood samples were collected at 20, 40, and 80 min for glucose determinations. Data represent the mean of 7–8 mice/group. Statistical analysis was performed using Student's *t*-test.

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References

[1] L. Zhu., Y. Li, L. Qiu, M. Su, X. Wang, C. Xia, Y. Qu, J. Li, J Li, J. Shen, *Chem. Med. Chem.* **2013**, *8*, 1104–1116.
[2] M. B. Toft-Nielsen, S. Madsbad, J. J. Holst, *J. Clin. Endocrinol. Metab.* **2001**, *86*, 3853–3860.
[3] Z. Pei, X. Li, K. Longenecker, T. W. Geldern, P. E. Wiedeman, T. H. Lubben, B. A. Zinker, K. Stewart, S. J. Ballaron, M. A. Stashko, A. K. Mika, D. W. A. Beno, M. Long, H. Wells, A. J. Kempf-Grote, D. J. Madar, T. S. McDermot, L. Bhagavatula, M. G. Fickes, D. Pireh, L. R. Solomon, M. R. Lake, R. Edalji, E. H. Fry, H. L. Sham, J. M. Trevillyan, *J. Med. Chem.* **2006**, *49*, 3520–3535.
[4] R. Mentlein, B. Gallwitz, W. E. Schmidt, *Eur. J. Biochem.* **1993**, *214*, 829–835.
[5] D. Kim, L. Wang, M. Beconi, G. J. Eiermann, M. H. Fisher, H. He, G. J. Hickey, J. E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, R. S. Roy, J. K. Wu, M. J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry, A. E. Weber, *J. Med. Chem.* **2005**, *48*, 141–151.
[6] E. B. Villhauer, J. A. Brinkman, G. B. Naderi, B. F. Burkey, B. E. Dunning, K. Prasad, B. L. Mangold, M. E. Russell, T. E. Hughes, *J. Med. Chem.* **2003**, *46*, 2774–2789.
[7] D. J. Augeri, J. A. Robl, D. A. Betebenner, D. R. Magnin, A. Khanna, J. G. Robertson, A. Wang, L. M. Simpkins, P. Taunk, Q. Huang, S.-P. Han, B. Abboa-Offei, M. Cap, L. Xin, L. Tao, E. Tozzo, G. E. Welzel, D. M. Egan, J. Marcinkeviciene, S. Y. Chang, S. A. Biller, M. S. Kirby, R. A. Parker, L. G. Hamann, *J. Med. Chem.* **2005**, *48*, 5025–5037.
[8] J. Feng, Z. Zhang, M. B. Wallace, J. A. Stafford, S. W. Kaldor, D. B. Kassel, M. Navre, L. Shi, R. J. Skene, T. Asakawa, K. Takeuchi, R. Xu, D. R. Webb, S. L. Gwaltney, *J. Med. Chem.* **2007**, *50*, 2297–2300.
[9] M. Eckhardt, E. Langkopf, M. Mark, M. Tadayyon, L. Thomas, H. Nar, W. Pfrengle, B. Guth, R. Lotz, P. Sieger, H. Fuchs, F. Himmelsbach, *J. Med. Chem.* **2007**, *50*, 6450–6453.
[10] D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615–2623.
[11] F. Okumu, G. Pauletti, D. Vander, Velde, T. Siahaan, R. Borchardt, *Pharma. Res.* **1997**, *14*, 169–175.
[12] M. C. Wenlock, R. P. Austin, P. Barton, A. M. Davis, P. D. Leeson, *J. Med. Chem.* **2003**, *46*, 1250–1256.
[13] E. Armani, G. Amari, A. Rizzi, R. De Fanti, E. Ghidini, C. Capaldi, L. Carzaniga, P. Caruso, M. Guala, I. Peretto, E. La Porta, P. T. Bolzoni, F. Facchinetti, C. Carnini, N. Moretto, R. Patacchini, F. Bassani, V. Cenacchi, R. Volta, F. Amadei, S. Capacchi, M. Delcanale, P. Puccini, S. Catinella, M. Civelli, G. Villetti, *J. Med. Chem.* **2014**, *54*, 793–816.
[14] M. A. Khanfar, L. Quinti, H. Wang, S. H. Choi, A. G. Kazantsev, R. B. Silverman, *Eur. J. Med. Chem.* **2014**, *76*, 414–426.
[15] L. Thomas, M. Eckhardt, E. Langkopf, M. Tadayyon, F. Himmelsbach, M. Mark, *J. Pharmacol. Exp. Ther.* **2008**, *325*, 175–182.