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Research paper

Design and evaluation of novel piperidine HIV-1 protease inhibitors with potency against DRV-resistant variants

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ABSTRACT

A novel class of HIV-1 protease inhibitors with flexible piperidine as the P2 ligand was designed with the aim of improving extensive interactions with the active subsites. Many inhibitors exhibited good to excellent inhibitory effect on enzymatic activity and viral infectivity. In particular, inhibitor **3a** with (*R*)-piperidine-3-carboxamide as the P2 ligand and 4-methoxybenzenesulfonamide as the P2' ligand showed an enzyme K_i value of 29 pM and antiviral IC_{50} value of 0.13 nM, more than six-fold enhancement of activity compared to DRV. Furthermore, there was no significant change in potency against DRV-resistant mutations and HIV-1_{NL4-3} variant for **3a**. Besides, inhibitor **3a** exhibited potent antiviral activity against subtype C variants with low nanomole EC_{50} values. In addition, the molecular modeling revealed important hydrogen bonds and other favorable van der Waals interactions with the backbone atoms of the protease and provided insight for designing and optimizing more potent HIV-1 protease inhibitors.

1. Introduction

Human immunodeficiency virus type 1 protease (HIV-1 PR) was identified as a treasure trove of biochemical target against the causative agent [1]. Inhibition of PR could interfere with HIV-1 maturation so as to generate immature and noninfectious virions [2]. Significantly, the approval of HIV-1 protease inhibitors (PIs), combing with reverse transcriptase inhibitors (RTIs), ushered in a new era of highly active antiretroviral therapy (HAART) [3–5], and the mortality and morbidity had reduced dramatically on account of multidrug treatment regimens in HAART. However, the constant emergence of virulent HIV-1 mutants presented a formidable challenge to treatment options [6]. Even worse, the latest HIV-1 PI Darunavir (DRV, shown in Table 1) had been reported to be proved powerless to several multidrug-resistant HIV-1 variants from DRV-experienced patients [7–10]. Therefore, potent PIs with high resistance barrier are urgently required.

According to the structure-based drug design (SBDD) strategy, maximizing van der Waals interactions, especially hydrogen bonding interactions between inhibitors and active site of HIV-1 protease, is of particularly important to combat drug resistance [11–13]. Indeed, we have focused much attention on the design of P2 ligands in HIV-1 PIs, which held an essential role in antiviral activity and drug resistance. Among which, compound **1** with *N*-2-(2, 4-Dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetamide as the P2 ligand in Fig. 1 displayed remarkable enzyme inhibitory ($IC_{50} = 2.53$ nM) and excellent inhibition of 81% [14]. However, the backbone conformations in mutants distorted minimally by altering several amino acid residues, which resulted in a decrease in affinity with inhibitors [15,16]. In order to be better fitted into the twisted active cave in S2 subsite of PR and keep hydrogen bonds at the same time, we got rid of double-bonds with steric hindrance and rotated hindrance in P2 ligand and substituted flexible heterocyclic morpholine for pyrimidine [17]. As a consequence, compound **2** with morpholine-4-carboxamide as the P2 ligand

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exhibited superior activity with enzyme K_i of 0.21 nM, antiviral IC_{50} value of 0.95 nM and inhibition of 91% [17]. However, it is worth noting that the exposed oxygen atom in morpholine could only form water-mediated hydrogen bond interaction, but the wrapped nitrogen atom could not form direct hydrogen bond with the residues of protease, which might put impact on the antiviral activity [18]. Thus, the conceiving of introducing flexible piperidine with exposed nitrogen atom into P2 ligand turned out to be reasonable. As exemplified by compound **3a**, it showed more than six-fold potent activity with enzyme K_i of 29 pM and IC_{50} value of 0.13 nM, compared to the control DRV. Moreover, **3a** exhibited prominent antiviral activity against DRV-resistant HIV-1 variants and subtype C variants.

2. Results and discussion

2.1. Chemistry

The syntheses of compounds **7a-d** were prepared as reported in the literature (Scheme 1) [17,19,20]. The commercial ethyl (*R*)-piperidine-3-carboxylate (**8e**) was reacted with formaldehyde (with 40% concentration) and formic acid in CH_3OH at 0 °C to reflux for 6 h to afford N-methylated ramification **9e** in 95.6% yield, which was hydrolyzed by aqueous sodium hydroxide to convert acid **10e** in yield of 99.1% over two steps [21]. Treatment of piperidine-carboxylic acids (**8a-d**) with $(Boc)_2O$ in the presence of $NaHCO_3$ in dual solvent system THF/H_2O (1:1) at 25 °C overnight obtained Boc-amino carboxylic acids (**10a-d**) in excellent yields (92.5–97.3%) [22]. Coupling of acids **10a-e** and amines **7a**, and **7c-d** under the catalysis of EDCI, HOBt and DMAP furnished amides **11a-l** and **3m-o** in good to excellent yields (53.9–95.4%) [23]. Removal of the Boc group in **11a-l** with hydrogen chloride gas in CH_2Cl_2 at 25 °C provided target compounds **3a-l** in yields of 66.0–97.4% [24].

Reaction of the commercially available chiral piperidin-3-ylmethanols **12a-b** with $(Boc)_2O$ in the presence of triethylamine in THF/H_2O (1:1) at 0–25 °C for 10 h provided Boc-aminoalcohols **13a-b** in yields of 97.9% and 99.0%, respectively [25]. Treatment of the alcohols with 4-nitrophenyl carbonochloridate and DMAP in anhydrous CH_2Cl_2 at 0–25 °C for 4 h afforded activated carbonates **14a** and **14b** in good yields (59.7–81.0%) [26]. Syntheses of Boc-derivatives **15p-u** were accomplished by coupling carbonates **14a** and **14b** with amines **7a**, and **7c-d** in the presence of DIEA in 68.5–98.8% yields. Compounds **3p-u** were removed the Boc-groups in **15p-u** by hydrogen chloride gas the same as **3a-l** in yields of 66.6–84.2% [24].

2.2. HIV-1 enzymatic inhibitory activity and antiviral activity

The fluorescence resonance energy transfer based assay of HIV-1 wild-type protease were utilized for all the target inhibitors, with DRV as a control [27]. Many of the inhibitors displayed impressive enzymatic inhibitory activity with K_i values of low nanomolar range and antiviral activity with IC_{50} values of nanomolar range in general, which demonstrated the superiority of the activity [28]. In particular, inhibitor **3a** with a (*R*)-piperidine-3-carboxamide P2 ligand and a 4-methoxybenzenesulfonamide P2' ligand showed more than six-fold enhancement of both enzyme inhibitory and antiviral activity compared to DRV, with low cytotoxicity.

As can be seen in Table 1 and Fig. 2, inhibitor **3a** with (*R*)-piperidine-3-carboxamide in P2 ligand exhibited more than 300-fold superior activity with enzyme K_i value of 29 pM and antiviral IC_{50} value of 0.13 nM compared to the corresponding inhibitor **3d** containing (*S*)-piperidine-3-carboxamide in the P2 ligand. This indicated that the configuration in P2 ligand was crucial for antiviral activity, and the same criteria applied to carbamate inhibitors such as **3p** *vs* **3s** (shown in Fig. 2 (B)). Furthermore, among the carboxamide inhibitors containing piperidine in P2 ligands, inhibitors with different positions of substitution exhibited discrepancy in inhibitory activity. As shown in Fig. 2 (C), inhibitors with meta-substitution of piperidine in P2 ligand exhibited significant enhancement of antiviral potency in comparison with those with ortho- and para-substitution. For instance, inhibitor **3a** displayed superior enzyme K_i value and antiviral IC_{50} value than **3g** and **3j**. In addition, it would cause slight reduction of inhibitory activity when the nitrogen atom was methylated in piperidine ligand, such as **3c** *vs* **3o** in Fig. 2 (D), for the reason that the active hydrogen in piperidine could form additional hydrogen bonding (N-H…N) with Gly48' in S2 subsite of the protease as shown in Fig. 7 (B). Moreover, the carboxamide inhibitors outperformed superior antiviral activity compared to carbamate inhibitors (**3a** *vs* **3p**), which might be attributed to both length and kinds of linkers between P2 ligand and the scaffold as shown in Fig. 2 (E).

In addition, the functional P2' ligands had prominent effect on the potency of inhibitors. In general, inhibitors with a 4-methoxybenzenesulfonamide as the P2' ligand showed improved activity than those containing a 4-amino or 4-trifluoromethylbenzenesulfonamide ligand in Fig. 2 (F). The oxygen of methoxy group formed strong hydrogen bond with Asp30' or Asp29' backbone aminde NHs, but the amino group formed weak or water-mediated hydrogen bonds with the side chain oxygen of Asp30' or Asp29' in S2' active subsite [29,30]. In addition, the electron-withdrawing inductive effect of trifluoromethyl group impaired the affinity between inhibitor and the protease S2' subsite, despite of the halogen interactions [31].

2.3. Infectivity assay on HIV-1 late stage

Some inhibitors with potent antiviral activity were further evaluated in the infectivity assay on the late stage of HIV-1 life cycle [32]. HIV-1 pseudotyped virus-producing cells were treated with the inhibitors, and the infectivity of the resultant virus was determined, which reflected the effect of inhibitors on the late stage of HIV-1 life cycle, including viral maturation by the protease. Obviously, almost all of these inhibitors exhibited prominent activity with inhibition above 80% at the concentration of 10 μM as shown in Table 2 and Fig. 3 (except for **3g** and **3q**), which were consistent with their antiviral activity *in vitro*. Particularly, **3a** showed the best inhibition of 99%, which shed light on further study.

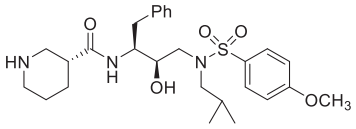
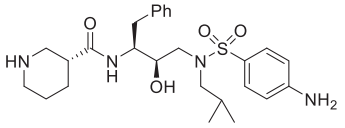
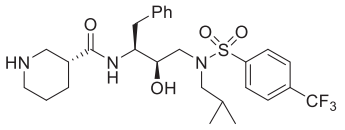
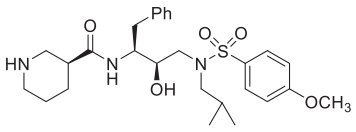
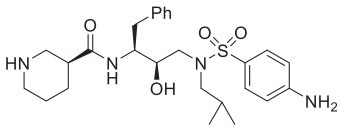
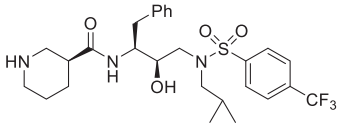
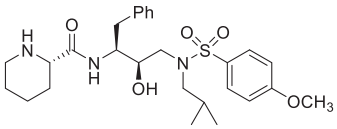
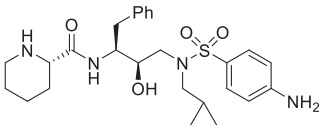
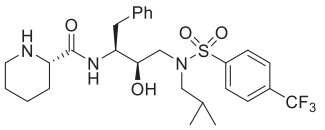
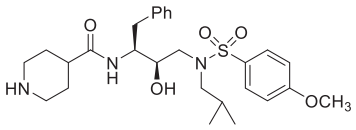
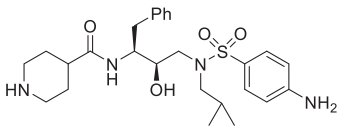
2.4. Infectivity assay on HIV-1 early stage

Meanwhile, in order to confirm the inhibitors selectively targeted the protease, we carried out an assay on the early stage of HIV-1 as exemplified by inhibitors **3a** and **3m** [33]. The inhibition on the late stage rather than on the early stage would reflect the effect of inhibitors on HIV-1 protease. So ideal PR inhibitors should be efficacious with high inhibitory rate on HIV-1 late stage and no inhibition on the early stage. Notably, both **3a** and **3m** exhibited almost no inhibitory effect in the infectivity assay on HIV-1 early stage with inhibition of only 3% and 5%, respectively, as shown in Table 3 and Fig. 4. The high selectivity makes these inhibitors worthy for further investigation.

2.5. Antiviral activity assay against DRV-resistant HIV-1 variants

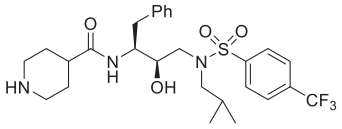
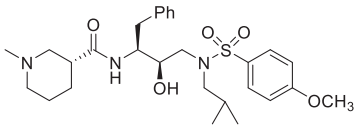
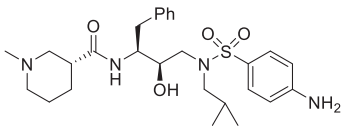
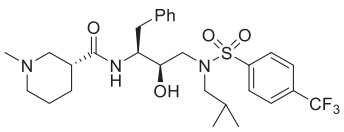
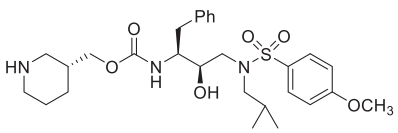
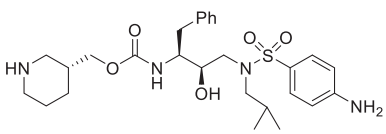
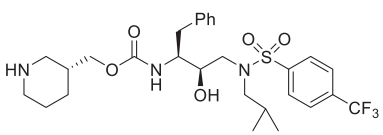
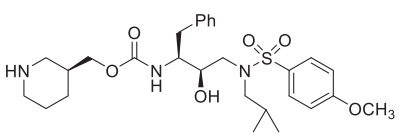
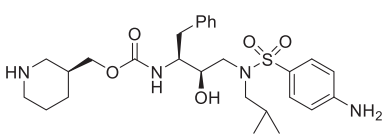
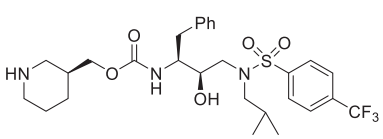
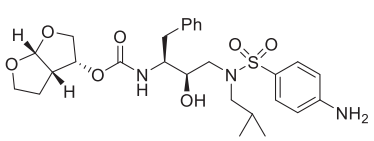
As the aim of this study was to discovery of potent HIV-1 PIs with high genetic barrier against the virulent mutants, we tested inhibitor **3a** for antiviral activity against DRV-sensitive or resistant pseudotyped HIV-1 in a single-round infection assay [10]. In the assay, DRV-resistant HIV-1 proviral DNA pHIV-1^{R}_{DRVS} was obtained

Table 1
HIV-1 protease inhibitory, antiviral activity and cytotoxicity of inhibitors.

Compd.	Structure	K_i (nM)[a]	IC_{50} (nM)[a]	CC_{50} (μM)[b]	SI[d]
3a		0.029	0.13 ± 0.01	>100	>769231
3b		0.27	1.21 ± 0.19	>100	>82645
3c		0.10	0.45 ± 0.18	>100	>222222
3d		9.99	44.60 ± 9.85	-[c]	–
3e		40.50	180.8 ± 10.9	–	–
3f		43.91	196.0 ± 16.3	–	–
3g		0.33	1.46 ± 0.46	>100	>68493
3h		2.03	9.04 ± 2.40	>100	>11062
3i		4.61	20.57 ± 4.47	–	–
3j		0.55	2.45 ± 0.75	>100	>40816
3k		5.52	24.64 ± 5.00	–	–

(*continued on next page*)

Table 1 (*continued*)

Compd.	Structure	K_i (nM)[a]	IC_{50} (nM)[a]	CC_{50} (μM)[b]	SI[d]
3l		5.93	26.48 ± 5.16	–	–
3m		0.040	0.18 ± 0.04	>100	>555556
3n		0.30	1.35 ± 0.31	>100	>74074
3o		0.19	0.84 ± 0.38	>100	>119048
3p		0.10	0.43 ± 0.15	>100	>232558
3q		0.28	1.27 ± 0.21	>100	>78740
3r		1.73	7.71 ± 1.85	>100	>12970
3s		0.30	1.35 ± 0.47	>100	>74074
3t		1.01	4.53 ± 0.80	>100	>22075
3u		2.61	11.66 ± 2.17	>100	>8576
DRV		0.18	0.82 ± 0.17	>100	>121951

[a] All assays were conducted in triplicate, and the data shown represent mean values (±1 standard deviation) derived from the results of three independent experiments.
[b] All cytotoxicity assays were conducted in triplicate.
[c] The cytotoxicity of inhibitors with IC_{50} values higher than 20 nM was not assayed.
[d] SI, namely selectivity index, denoted a ratio of CC_{50} to IC_{50}.

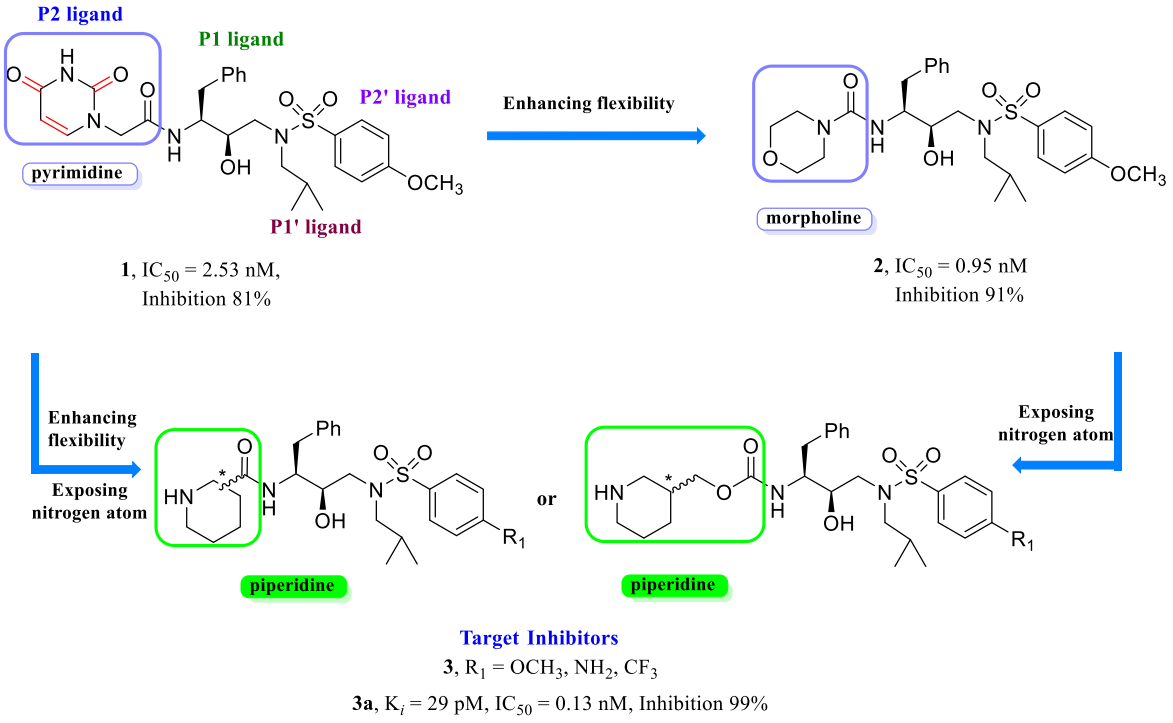


Fig. 1. Structures of novel HIV-1 protease inhibitors.

by changing four amino acid substitutions (V32I, L33F, I54 M, and I84V) in the protease. As can be seen in Fig. 5, there was no significant change in potency against DRV-resistant mutations and HIV-1_{NL4-3} variant for **3a**, with only two-fold increase in EC_{50}, comparing with DRV which exhibited 60-fold increment in EC_{50} against DRV-resistant mutations than that against wild type virus. However, the reduction of cellular potency of inhibitor **3a** might be due to its solubility or membrane permeability, for the reason that piperidine fragment might be easily protonated at physiological pH 7.4 which resulted in entering into an organic phase difficult [34,35]. Nevertheless, the speculation needs further confirmation in our following study. In brief, introducing flexible piperidine into the P2 ligand proved its positive effect on antiviral activity against DRV-resistant mutants and was worthy for exploring in more depth.

2.6. Antiviral activity assay against HIV-1 subtype C variants

Although C-HIV (Subtype C HIV-1) is the most prevalent HIV-1 variant that accounts for approximately 46% of global infections [36], little research has been focused on it [37]. In recent years, HIV-1 PIs which was designed originally against subtype B, have made available in many parts of the world with the epidemic subtype C [38]. Herein, we tested inhibitor **3a** for antiviral activity against four pseudotyped HIV-1 subtype C isolates, namely 11928, 11929, 11941 and plndie in the same way as done in infectivity assay on HIV-1 late stage [32,39]. Surprisingly, **3a** exhibited potent antiviral activity against subtype C variants with low nanomole EC_{50} values. Particularly, the activity against C-HIV isolate 11929 was only 4-fold decrement compared with the control DRV, with EC_{50} value of 14.76 nM as shown in Table 4. Furthermore, it showed robust antiviral activity against the isolate 11941 with EC_{50} value of 11.80 nM. However, compared with Figs. 5 and 6, there exhibited an obvious discrepancy in EC_{50} values between HIV-1 subtype B isolate NL4-3 and subtype C isolate 11941 or 11929. We speculated it might be attributed to the different active subsite structure and the specific susceptibility criteria of subtype C for inhibitors, apart from the factors on absorption or physical property of inhibitors. The results above might aid in further HIV-1 protease inhibitors design against subtype C.

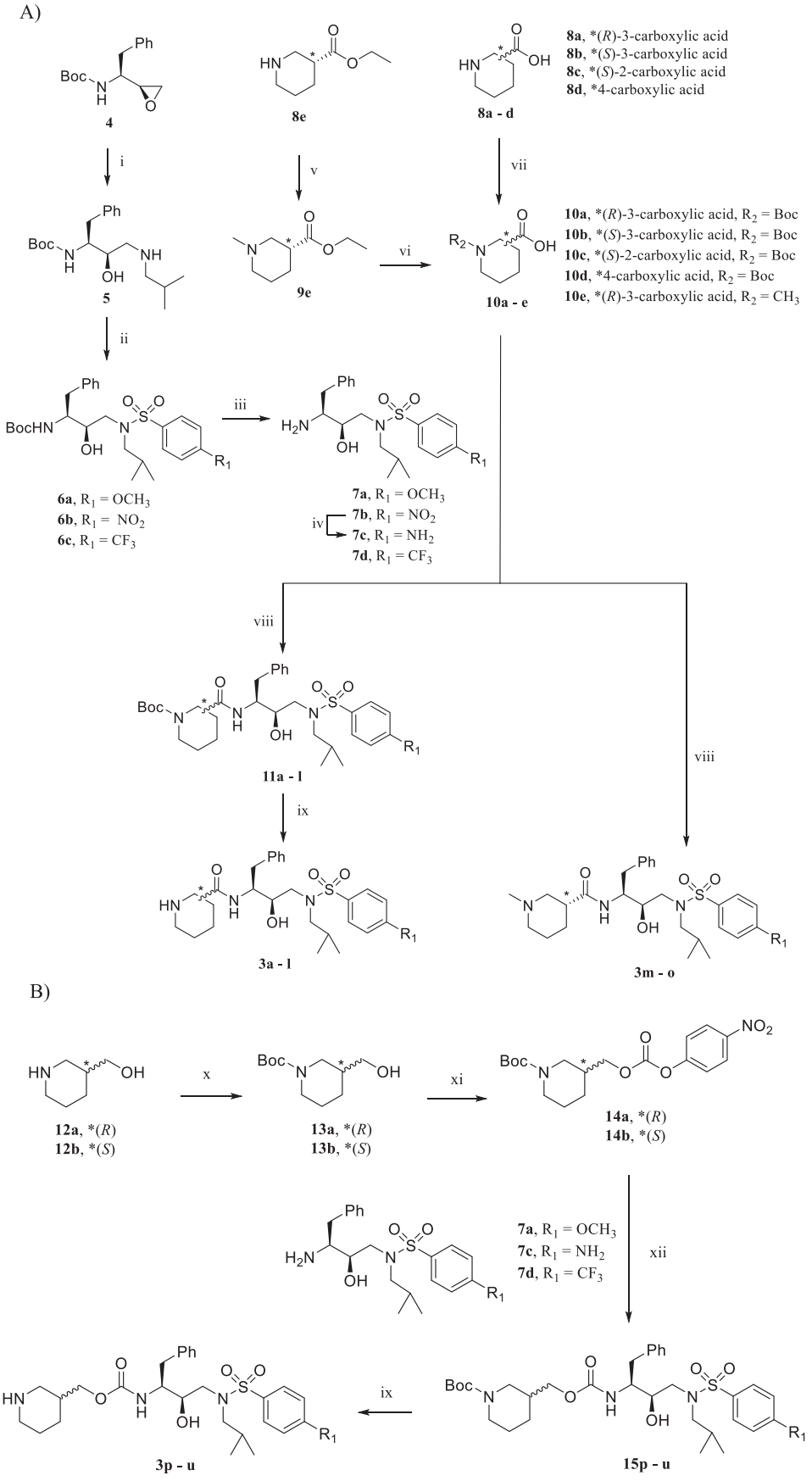
2.7. Molecular modeling studies

In order to investigate the possible ligand-binding site interactions, molecular modeling studies of **3a, 3c**, **3d** and **DRV** were done in the Molecular Operating Environment (MOE) (version 2009.06) with the protease structure (PDB-ID: 4mc9) from protein data bank [40,41]. As can be seen in Fig. 7, all the inhibitors were nicely accommodated into the cave of protease.

The extensive van der Waals interactions between piperidine P2 ligand and S2 subsite of HIV-1 protease in **3a** made important contributions to its excellent inhibitory potency. In addition, one of the sulfonamide oxygens formed hydrogen bonding interaction with the backbone NH group of Ile50, and the second oxygen also formed strong hydrogen bond with amide NHs of Ile50' located in the flaps [42]. Furthermore, hydrogen bond was observed between the oxygen of methoxy group in P2' ligand and Asp29' backbone amide NH.

In particular, taken inhibitor **3c** for example, introduction of piperidine in P2 ligand not only formed additional hydrogen bond with the backbone atom of Gly48' in the S2 subsite, but also made van der Waals contacts as shown in Fig. 7 (B). Besides, fluorine atoms in P2' ligand formed halogen bonds or favorable van der Waals interactions with the outer atoms of S2' subsite.

Although **3d** fitted into the protease binding site nicely and the scaffold formed hydrogen bonds with Asp25 of active pocket, its configuration of piperidine in P2 ligand might take negative effect in van der Waals interactions or hydrophobic contacts compared


A)
4
8e
8a - d
8a, *(R)-3-carboxylic acid
8b, *(S)-3-carboxylic acid
8c, *(S)-2-carboxylic acid
8d, *4-carboxylic acid
i
v
vii
5
9e
vi
10a - e
10a, *(R)-3-carboxylic acid, R2 = Boc
10b, *(S)-3-carboxylic acid, R2 = Boc
10c, *(S)-2-carboxylic acid, R2 = Boc
10d, *4-carboxylic acid, R2 = Boc
10e, *(R)-3-carboxylic acid, R2 = CH3
ii
iii
6a, R1 = OCH3
6b, R1 = NO2
6c, R1 = CF3
7a, R1 = OCH3
iv
7b, R1 = NO2
7c, R1 = NH2
7d, R1 = CF3
viii
11a - l
ix
3a - l
3m - o
B)
12a, *(R)
12b, *(S)
x
13a, *(R)
13b, *(S)
xi
14a, *(R)
14b, *(S)
7a, R1 = OCH3
7c, R1 = NH2
7d, R1 = CF3
xii
ix
3p - u
15p - u

with **3a**.

Perhaps the binding score of inhibitors could reflect the antiviral activity to some extent, on account of that higher negative binding score suggested better potency [43]. The binding scores of **3a, 3c** and **3d** were −14.24 kcal/mol, −12.93 kcal/mol and −11.57 kcal/mol, respectively, which were in accordance with their inhibitory activity.

In addition, both oxygens of the bis-tetrahydrofuran (bis-THF) in P2 ligand of **DRV** formed tight hydrogen bonds with the backbone NHs of Asp29 and Asp30 in Fig. 7 (D), which was of highly importance for its robust activity against a wide range of multidrug-resistant HIV-1 variants [15]. Further can be seen in the overlay structures of **3a** and **DRV** in Fig. 7 (E), the binding properties of **3a** and **DRV** in S2 subtitle of the protease were slightly different, although both piperidine group and bis-THF sitted nicely in the active pocket. The piperidine in **3a** could neither fold just as the polyether-like bis-THF of **DRV**, nor form strong hydrogen bonds with the active backbone which were responsible for the superb activity. So the key strategy of designing more potent PIs should still be focused on maximizing extensive interactions, particularly promoting hydrogen bonding interactions with the active subsites in the next study.

3. Conclusion

Novel HIV-1 PIs were designed and synthesized by introducing flexible piperidine as P2 ligand, for the aim of both accommodating to the twisted active cave in the S2 subsite of protease and forming additional hydrogen bonding interactions. In particular, **3a** with a (*R*)-piperidine-3-carboxamide P2 ligand and a 4-methoxybenzenesulfonamide P2' ligand showed an enzyme K_i value of 29 pM and antiviral IC_{50} value of 0.13 nM, more than six-fold enhancement of activity compared to DRV. Furthermore, there was no significant change in potency against DRV-resistant mutations and HIV-1_{NL4-3} variant for **3a**, which allowed further optimization of the promising inhibitors for better potency against highly resistant multidrug-resistant HIV-1 strains. Furthermore, **3a** exhibited potent antiviral activity against subtype C variants with low nanomole EC_{50} values. As can be seen in the molecular modeling studies, the new piperidine P2 ligand could form additional hydrogen bonding and other favorable van der Waals interactions in the S2 subsite. As it turned out, among these compounds, (*R*)-piperidine P2 ligand inhibitors exhibited better activity than those with (*S*)-piperidine. Moreover, inhibitors with methoxy group at the P2' moiety showed improved activity than those with amino or trifluoromethyl P2' ligands, for the reason that the oxygen atom formed strong hydrogen bond interaction with Asp29' backbone amide NH. Further design and modification of novel HIV-1 PIs are underway.

4. Experimental section+

4.1. Chemistry

All experiments requiring anhydrous conditions were conducted in flame-dried glassware fitted with rubber septa under a positive pressure of dry argon, unless otherwise noted. THF was distilled under argon from sodium-benzophenone ketyl and CH_2Cl_2 was distilled under argon from calcium hydride. All reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with the UV light. Flash column chromatography was performed on a CombiFlash®Rf 200 system employing silica gel (50–75 μm, Qingdao Haiyang Chemical Co.,Ltd). Melting points were taken on MP70 Melting Point System with revised. High resolution mass spectra were obtained on an Autospee Ultima-TOF spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD or DMSO-d_6 on a Bruker AVANCE III 400 MHz, 500 MHz or 600 MHz spectrometer (Bruker Inc) with tetramethylsilane (TMS) as an internal reference. The chemical shifts are given in δ (ppm) referenced to the respective solvent peak ($CDCl_3$: ^{1}H, δ = 7.26 ppm, ^{13}C, δ = 77.16 ppm; CD_3OD: ^{1}H, δ = 3.31 ppm, ^{13}C, δ = 49.00 ppm; DMSO-d_6: ^{1}H, δ = 2.49 ppm, ^{13}C, δ = 39.5 ppm), and coupling constants are reported in Hz. All the target compounds were characterized by ^{1}H and ^{13}C NMRs and HRMS spectra.

4.1.1. tert-Butyl ((2S, 3R)-3-hydroxy-4-(isobutylamino)-1-phenylbutan-2-yl)carbamate (5)

To a suspension of **4** (7.00 g, 26.6 mmol) in acetonitrile (32 mL) was added 2-methylpropan-1-amine (4.88 g, 66.5 mmol). The reaction was refluxed for 6 h, and the solvent was removed under reduced pressure. The solid product was then suspended in petroleum ether (35 mL) and stirred for 0.5 h. The precipitate was isolated by filtration and dried, *in vacuo*, over P_2O_5 to give **5** as a white powder: yield 8.17 g (91.5%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.29–7.16 (m, 5H), 3.71–3.59 (m, 2H), 3.16–3.08 (m, 1H), 2.83–2.74 (m, 1H), 2.68–2.56 (m, 2H), 2.54–2.48 (m, 1H), 2.46–2.39 (m, 1H), 1.88–1.77 (m, 1H), 1.32 (s, 9H), 0.96 (d, J = 6.4 Hz, 6H); LC-MS (ESI) $[M+H]^+$ *m/z* 337.2.

4.1.2. tert-Butyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (**6a**)

To a cold (0 °C) solution of **5** (3.37 g, 10.0 mmol) in THF (30 mL) was added DIEA (1.42 g, 11.0 mol) and DMAP (0.12 g, 1.00 mmol). 4-methoxybenzenesulfonyl chloride (2.27 g, 11.0 mmol) dissolved in 4 mL THF was added slowly to the reaction. The mixture was stirred at 25 °C for 4 h. The solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (100 mL) and washed with H_2O and saturated aqueous NaCl, and then dried over anhydrous Na_2SO_4. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to furnish **6a** as white amorphous solid: yield 4.94 g (97.7%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 6.9 Hz, 2H), 7.33–7.26 (m, 3H), 7.25–7.17 (m, 2H), 6.98 (d, J = 6.9 Hz, 2H), 4.65 (s, 1H), 3.87 (s, 3H), 3.84–3.74 (m, 2H), 3.13–2.80 (m, 6H), 1.92–1.80 (m, 1H), 1.35 (s, 9H), 0.89 (dd, J = 14.4, 5.2 Hz, 6H); LC-MS (ESI) $[M+Na]^+$ *m/z* 529.5.

4.1.3. tert-Butyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-nitrophenyl) sulfonamido)-1-phenylbutan-2-yl)carbamate (6b)

Compound **6b** was prepared from **5** (3.36 g, 10.0 mmol) and 4-nitrobenzenesulfonyl chloride (2.44 g, 11.0 mmol) by following the same procedure outlined for **6a** to give a yellow powder: yield 5.12 g (98.2%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.35–7.31 (m, 2H), 7.29–7.27 (m, 1H), 7.26–7.22 (m, 2H), 4.66 (d, J = 8.0 Hz, 1H), 3.85–3.76 (m, 2H), 3.25–3.20 (m, 2H), 3.03–2.96 (m, 3H), 2.94–2.87 (m, 1H), 1.95–1.86 (m, 1H), 1.38 (s, 9H), 0.90 (dd, J = 6.4, 4.8 Hz, 6H); LC-MS (ESI) $[M+Na]^+$ *m/z* 544.6.

Scheme 1. Syntheses of target inhibitors **3a-u**. Reagents and conditions: (i) *i*-$BuNH_2$, CH_3CN, 80 °C, 6 h; (ii) Aryl sulfonyl chloride, DIEA, DMAP(Cat.), THF, 0–25 °C, 3–5 h; (iii) CH_2Cl_2-CF_3COOH, 0 °C–r.t, 3 h; (iv) H_2 (gas), 50 psi, 10% Pd/C, CH_3OH, r.t, 2 h; (v) 40% formaldehyde, formic acid, CH_3OH, 0 °C–reflux, 6 h; (vi) NaOH (aq), 25 °C, 1 h; 1 N HCl, 0 °C, 0.5 h; (vii) $(Boc)_2O$, $NaHCO_3$, THF/H_2O (1:1), 25 °C, overnight; (viii) EDCI, HOBt, DMAP, anhydrous DMF, argon, 0–25 °C, 3–8 h; (ix) HCl (gas), CH_2Cl_2, 25 °C, 0.5 h; (x) $(Boc)_2O$, triethylamine, THF/H_2O (1:1), 0–25 °C, 10 h; (xi) 4-nitrophenyl carbonochloridate, DMAP, dry CH_2Cl_2, 0–25 °C, 4 h; (xii) DIEA, dry DMF, Argon, 0–25 °C, 5 h.

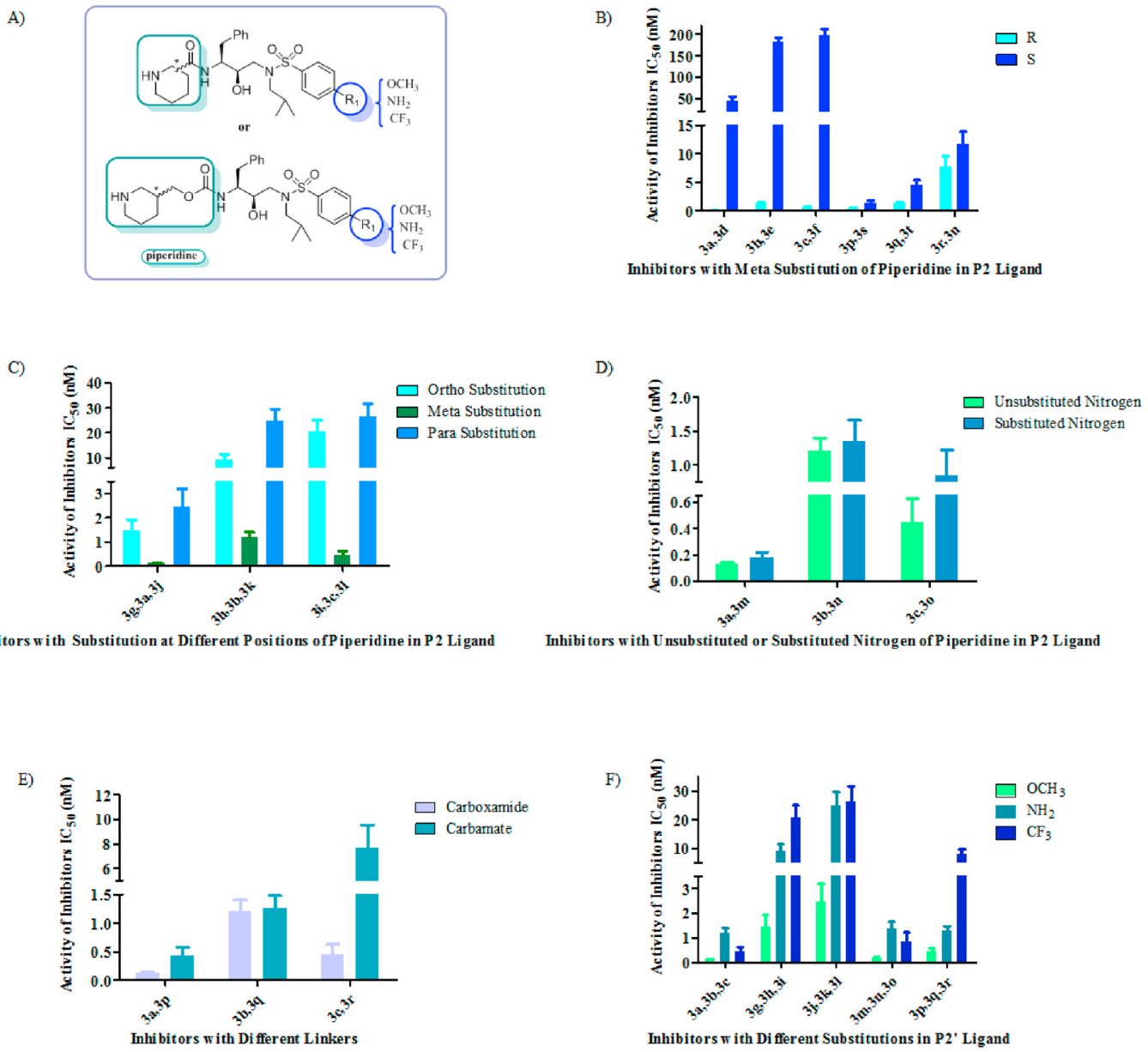


Fig. 2. Comparison on HIV-1 protease inhibitory activity of inhibitors. A) Two kinds of target inhibitors. B) Inhibitors with meta-substitution of piperidine in P2 ligand. C) Inhibitors with substitution at different positions of piperidine in P2 ligand. D) Inhibitors with unsubstituted or substituted nitrogen of piperidine in P2 ligand. E) Inhibitors with different linkers. F) Inhibitors with different substitutions in P2' ligand.

Table 2
Inhibition of selected inhibitors on HIV-1 late stage.

Compd.	Inhibition (%) (10 μM)[a]	Compd.	Inhibition (%) (10 μM)[a]
3a	99 ± 6	**3n**	83 ± 7
3b	81 ± 8	**3o**	88 ± 6
3c	93 ± 5	**3p**	94 ± 7
3g	79 ± 5	**3q**	71 ± 8
3m	98 ± 4	**DRV (100 nM)**	100

[a] All assays were conducted in quintuplicate.

4.1.4. tert-Butyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamate (6c)

Compound **6c** was prepared from **5** (0.99 g, 2.97 mmol) and 4-(trifluoromethyl)benzenesulfonyl chloride (0.8 g, 3.27 mmol) by following the same procedure outlined for **6a** to give a white powder: yield 1.59 g (98.4%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05–7.99 (m, 2H), 7.97–7.92 (m, 2H), 7.26–7.12 (m, 5H), 6.74–6.64 (m, 1H), 4.98 (s, 1H), 3.58–3.52 (m, 1H), 3.51–3.43 (m, 1H), 3.39–3.33 (m, 1H), 3.14–3.08 (m, 1H), 3.05–2.88 (m, 3H), 2.02–1.95 (m, 1H), 1.24 (s, 9H), 1.12–1.09 (m, 1H), 0.83 (d, J = 13.0 Hz, 6H); LC-MS (ESI) $[M+H]^+$ m/z 545.5.

4.1.5. N-((2R, 3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-isobutyl-4-methoxybenzenesulfonamide (7a)

To a stirred solution of **6a** (4.90 g, 9.68 mmol) in CH_2Cl_2 (20 mL) was added CF_3COOH (6.6 mL) dropwise. The reaction mixture was stirred at 25 °C for 2 h. The solvent was neutralized with saturated aqueous Na_2CO_3 to pH 7.0, and then extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed with saturated aqueous NaCl and dried over anhydrous Na_2SO_4, and then concentrated

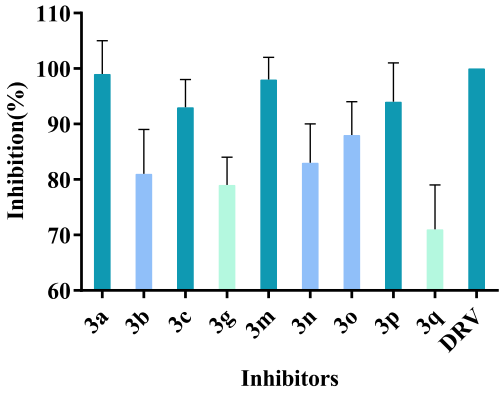


Fig. 3. Inhibition of inhibitors on HIV-1 late stage.

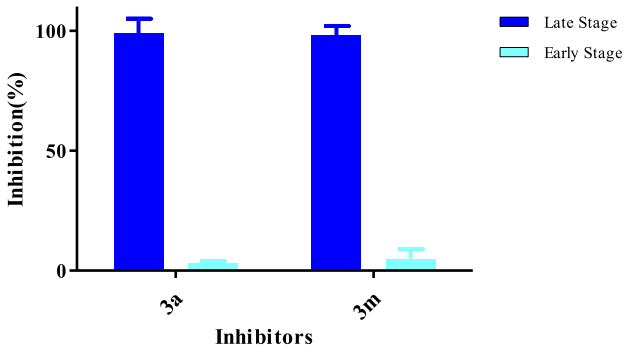


Fig. 4. Inhibition of inhibitors **3a** and **3m** on HIV-1 late stage compared with early stage.

under reduced pressure. The crude product was purified by silica gel column chromatography to furnish **7a** as a yellow solid: yield 3.18 g (80.9%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (d, J = 8.0 Hz, 2H), 7.38–7.25 (m, 5H), 7.12 (d, J = 8.0 Hz, 2H), 3.92 (s, 3H), 3.84–3.79 (m, 1H), 3.47–3.42 (m, 1H), 3.17–3.11 (m, 2H), 3.06–3.00 (m, 2H), 2.97–2.91 (m, 1H), 2.63–2.56 (m, 1H), 2.06–1.98 (m, 1H), 0.92 (dd, J = 16.5, 6.0 Hz, 6H); LC-MS (ESI) $[M+H]^+$ m/z 407.5.

4.1.6. *N-((2R, 3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-isobutyl-4-nitrobenzenesulfonamide (**7b**)*

Compound **7b** was prepared from **6b** (5.12 g, 9.82 mmol) by following the same procedure outlined for **7a** to give a yellow powder: yield 2.92 g (69.2%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.41 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 8.0 Hz, 2H), 7.38–7.24 (m, 5H), 3.74 (dd, J = 8.5, 4.5 Hz, 1H), 3.53 (d, J = 15.0 Hz, 1H), 3.30 (dd, J = 15.0, 9.0 Hz, 1H), 3.19 (dd, J = 14.0, 8.5 Hz, 1H), 3.13–3.02 (m, 2H), 2.97 (dd, J = 14.0, 5.0 Hz, 1H), 2.61 (dd, J = 13.5, 9.0 Hz, 1H), 2.08–1.98 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); LC-MS (ESI) $[M+H]^+$ m/z 422.5.

4.1.7. *4-Amino-N-((2R, 3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-isobutylbenzenesulfonamide (**7c**)*

To a solution of compound **7b** (1.26 g, 3.00 mmol) in ethyl acetate/methanol (5 mL/10 mL) was added 10% Pd/C (1.26 g). The mixture was stirred at 25 °C under the hydrogen atmosphere of 50 pis pressure for 7 h. The reaction solution was filtered with Celite 545® and washed with methanol. The filtrate was concentrated under reduced pressure to give **7c** as a white solid: yield 1.02 g (86.7%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, J = 8.4 Hz, 2H), 7.32–7.28 (m, 2H), 7.24–7.20 (m, 3H), 6.69 (d, J = 8.4 Hz, 2H), 4.13 (s, 2H), 3.78–3.75 (m, 1H), 3.29–3.24 (m, 1H), 3.18–3.14 (m, 2H), 3.01–2.95 (m, 2H), 2.84–2.80 (m, 1H), 2.55–2.49 (m, 1H), 1.90–1.84 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); LC-MS (ESI) $[M+H]^+$ m/z 392.5.

4.1.8. *N-((2R, 3S)-3-amino-2-hydroxy-4-phenylbutyl)-N-isobutyl-4-(trifluoromethyl)benzenesulfonamide (**7d**)*

Compound **7d** was prepared from **6c** (1.60 g, 2.90 mmol) by following the same procedure outlined for **7a** to give a white powder: yield 0.92 g (71.4%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H), 7.33–7.29 (m, 2H), 7.25–7.19 (m, 3H), 3.78–3.74 (m, 1H), 3.32–3.29 (m, 2H), 3.18–3.12 (m, 1H), 3.07–3.02 (m, 1H), 3.00–2.92 (m, 2H), 2.55–2.49 (m, 1H), 1.95–1.87 (m, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); LC-MS (ESI) $[M+H]^+$ m/z 445.5.

4.1.9. *Ethyl (R)-1-methylpiperidine-3-carboxylate (**9e**)*

Ehyl (*R*)-piperidine-3-carboxylate (**8e**, 0.25 g, 1.59 mmol) was dissolved in methanol (4 mL), 40% formaldehyde (0.60 mL, 7.95 mmol) and formic acid (0.12 mL, 3.18 mmol) were slowly added in sequence at 0 °C. The mixture was refluxed for 6 h, then cooled and brought to a pH in the region of 9 using saturated aqueous Na_2CO_3 solution. The solution was concentrated under reduced pressure, added H_2O (15 mL) and extracted with dichloromethane (3 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford **9e** in the form of a yellowish oil: yield 0.26 g (95.6%); ^{1}H NMR (500 MHz, CD_3OD) δ 4.15 (q, J = 7.0 Hz, 2H), 2.97 (d, J = 10.0 Hz, 1H), 2.76 (d, J = 10.0 Hz, 1H), 2.64–2.55 (m, 1H), 2.30 (s, 3H), 2.22–2.13 (m, 1H), 2.09–2.01 (m, 1H), 1.99–1.92 (m, 1H), 1.81–1.74 (m, 1H), 1.66–1.57 (m, 1H), 1.49–1.39 (m, 1H), 1.27 (t, J = 7.0 Hz, 3H); LC-MS (ESI) $[M+H]^+$ m/z 172.4.

4.1.10. *(R)-1-(tert-butoxycarbonyl)piperidine-3-carboxylic acid (**10a**)*

To a stirred solution of (*R*)-piperidine-3-carboxylic acid (**8a**, 0.5 g, 3.87 mmol) in THF/H_2O (10 mL/10 mL) was added $(Boc)_2O$ (1.18 g, 5.42 mmol) and $NaHCO_3$ (0.45 g, 5.42 mmol). The reaction was stirred overnight at 25 °C under argon. The mixture was diluted with H_2O (5 mL) and petroleum ether (5 mL), then the aqueous layer was acidified with 1 N HCl to pH 2 and the product was extracted with ethyl acetate (3 × 20 mL). The organic phase

Table 3
Inhibition of inhibitors **3a** and **3m** on HIV-1 late stage and early stage.

Compd.	Inhibition on late stage (%) (10 μM)[a]	Inhibition on early stage (%) (10 μM)[a]
3a	99 ± 6	3 ± 1
3m	98 ± 4	5 ± 4
DRV	100	–

[a] All assays were conducted in quintuplicate.Table 4

A)

Compd.	mean EC_{50} $\mu M \pm SD$ [a]		fold resistance [b]
	$HIV\text{-}1_{NL4-3}$	$HIV_{DRV}{}^{R}S$	
3a	0.50 ± 0.04	0.90 ± 0.07	1.80
DRV	0.0039 ± 0.0012	0.24 ± 0.021	60.26

[a] All assays were conducted in triplicate, and the data shown represent mean values (±1 standard deviation) derived from the results of three independent experiments.

[b] Fold resistance is defined by $EC_{50(mutant)}/EC_{50(WT)}$.

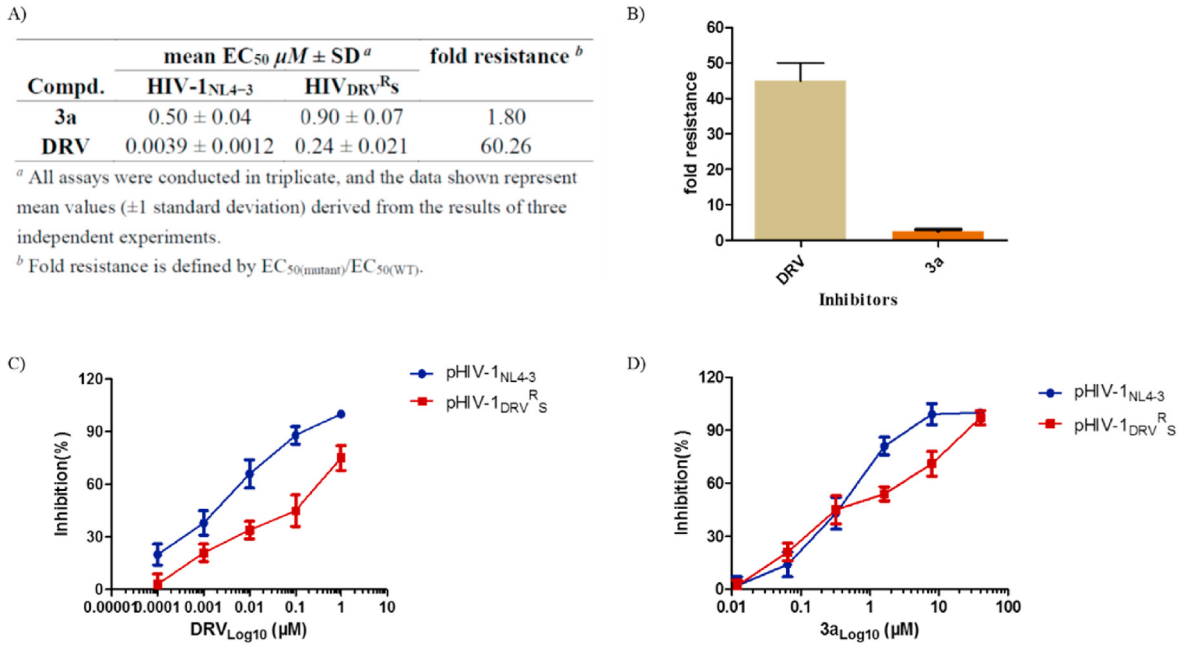


Fig. 5. Antiviral activity of inhibitor **3a** against multidrug resistant HIV-1 variants. A) Antiviral Activity of **3a** and DRV against Multidrug Resistant HIV-1 Variants. B) Fold resistance is defined by $EC_{50(mutant)}/EC_{50(WT)}$. (C–D) Dose-response of compounds DRV and 3a against wild type HIV-1 and DRV-resistant mutant.

Table 4
Antiviral activity of inhibitors **3a** and **DRV** against C-HIV.

Compd.	HIV-1 subtype C EC_{50} (nM)[a]			
	11928	11929	11941	plndie
3a	49.42 ± 1.84	14.76 ± 1.17	11.80 ± 1.08	26.27 ± 1.42
DRV	2.01 ± 0.30	3.63 ± 0.56	0.91 ± 0.02	1.26 ± 0.02

[a] All assays were conducted in triplicate, and the data shown represent mean values (±1 standard deviation) derived from the results of three independent experiments.

was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford **10a** as a white powder: yield 0.82 g (92.5%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.30–3.99 (m, 1H), 3.88 (d, J = 12.5 Hz, 1H), 3.16–2.94 (m, 1H), 2.85 (t, J = 11.5 Hz, 1H), 2.53–2.44 (m, 1H), 2.06 (d, J = 13.5 Hz, 1H), 1.71 (d, J = 11.5 Hz, 1H), 1.65 (d, J = 11.5 Hz, 1H), 1.53–1.47 (m, 1H), 1.45 (s, 9H); LC-MS (ESI) $[M - H]^-$ m/z 228.3.

4.1.11. (S)-1-(tert-butoxycarbonyl)piperidine-3-carboxylic acid (**10b**)

Compound **10b** was prepared from **8b** (0.5 g, 3.87 mmol) by following the same procedure outlined for **10a** to give a white powder: yield 0.84 g (94.8%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.27–3.96 (m, 1H), 3.88 (d, J = 13.0 Hz, 1H), 3.18–2.91 (m, 1H), 2.86 (t, J = 12.0 Hz, 1H), 2.54–2.42 (m, 1H), 2.06 (d, J = 12.0 Hz, 1H), 1.77–1.69 (m, 1H), 1.68–1.59 (m, 1H), 1.52–1.47 (m, 1H), 1.45 (s, 9H); LC-MS (ESI) $[M - H]^-$ m/z 228.3.

4.1.12. (S)-1-(tert-butoxycarbonyl)piperidine-2-carboxylic acid (**10c**)

Compound **10c** was prepared from **8c** (0.5 g, 3.87 mmol) by following the same procedure outlined for **10a** to give a white powder: yield 0.85 g (95.8%); ^{1}H NMR (500 MHz, CD_3OD) δ 4.80–4.64 (m, 1H), 3.95–3.89 (m, 1H), 3.06–2.88 (m, 1H), 2.26–2.17 (m, 1H), 1.72–1.62 (m, 3H), 1.44 (s, 9H), 1.42–1.36 (m, 1H), 1.33–1.25 (m, 1H).

4.1.13. 1-(tert-Butoxycarbonyl)piperidine-4-carboxylic acid (**10d**)

Compound **10d** was prepared from **8d** (0.45 g, 3.50 mmol) by

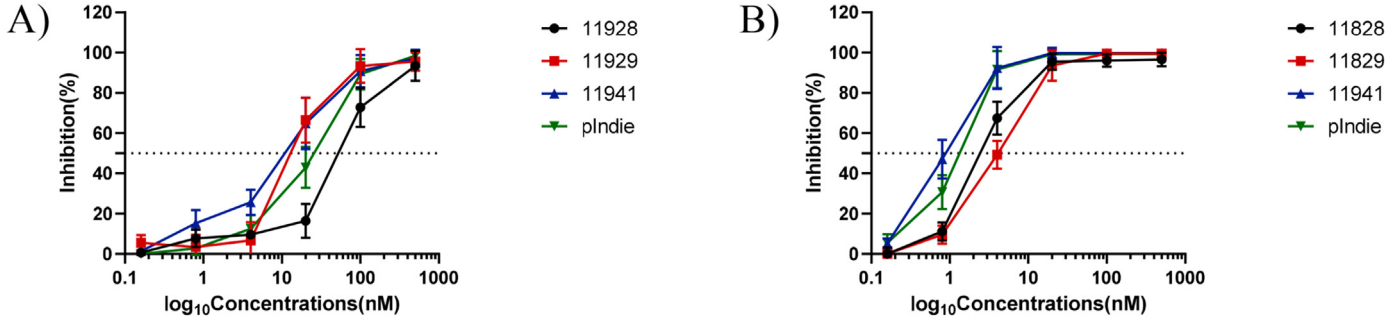


Fig. 6. Antiviral activity of inhibitor **3a** and **DRV** against C-HIV. A) Antiviral Activity of **3a** against four HIV-1 subtype C isolates. B) Antiviral Activity of **DRV** against four HIV-1 subtype C isolates.

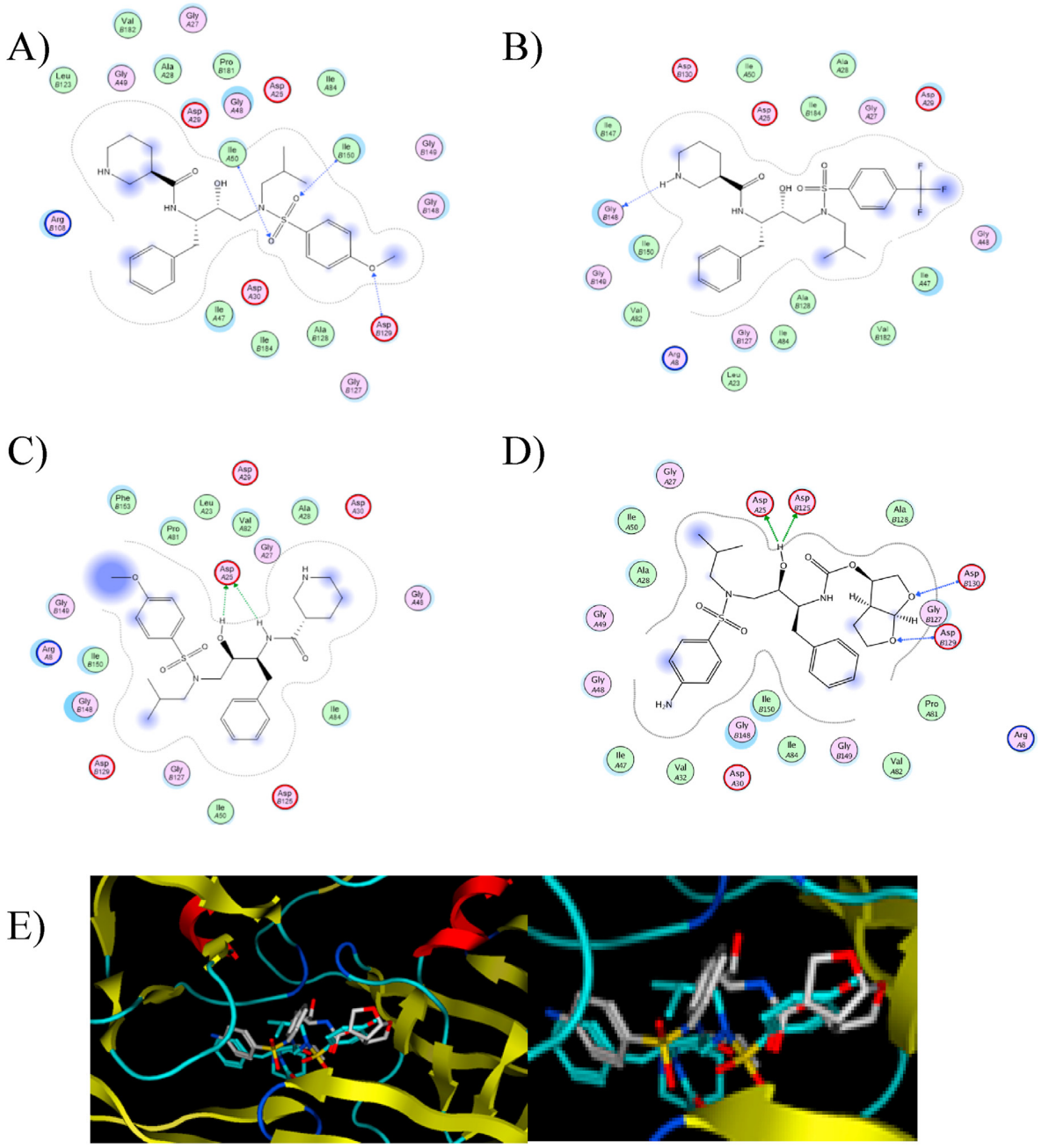


Fig. 7. The molecular modeling for inhibitors **3a**, **3c**, **3d** and **DRV**. A) Ligplot interaction of **3a**. B) Ligplot interaction of **3c**. C) Ligplot interaction of **3d**. D) Ligplot interaction of **DRV**. E) Stereoview of the overlay of **DRV** and **3a** at HIV-1 protease binding site. Ligand exposures were represented as purple spheres and hydrogen bonding was depicted as blue or green arrows.

following the same procedure outlined for **10a** to give a white powder: yield 0.78 g (97.3%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.02 (s, 2H), 2.85 (t, J = 12.5 Hz, 2H), 2.49 (t, J = 9.5 Hz, 1H), 1.90 (d, J = 13.0 Hz, 2H), 1.64 (q, J = 12.0 Hz, 2H), 1.45 (s, 9H); LC-MS (ESI) $[M - H]^-$ m/z 228.4.

*4.1.14. (R)-1-Methylpiperidine-3-carboxylic acid (**10e**)*

Sodium hydroxide (0.14 g, 3.60 mmol) was dissolved in H_2O (4 mL) and then added dropwise into **9e** (0.20 g, 1.20 mmol). The reaction was stirred for 1 h at 25 °C. The mixture was acidified to pH 2 with 1 N HCl and stirred for 0.5 h at 0 °C. After this period, the reaction mixture was washed with petroleum ether and concentrated under reduced pressure. The solid was dissolved with anhydrous methanol, filtered through Celite 545® and concentrated to afford **10e** in the form of a white powder: yield 0.17 g (99.1%); ^{1}H NMR (500 MHz, CD_3OD) δ 3.75–3.66 (m, 1H), 3.53–3.38 (m, 1H), 3.21–3.00 (m, 2H), 2.90 (s, 3H), 2.26–2.13 (m, 1H), 2.08–2.00 (m, 1H), 1.95–1.71 (m, 2H), 1.63–1.52 (m, 1H); LC-MS (ESI) $[M - H]^-$ m/z 142.4.

*4.1.15. (R)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-1-methylpiperidine-3-carboxamide (**3m**)*

To a stirred solution of **10e** (18.0 mg, 0.10 mmol) and **7a** (44.7 mg, 0.11 mmol) in anhydrous DMF (1 mL) was added EDCI (28.8 mg, 0.15 mmol), HOBt (14.9 mg, 0.11 mmol) and DMAP

(2.40 mg, 0.02 mmol) at 0 °C under argon atmosphere. The reaction mixture was warmed to 25 °C and stirred for 8 h. After this period, the reaction was diluted with 5 mL ethyl acetate, washed with H_2O and saturated aqueous NaCl and dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to furnish **3m** as a yellow powder: yield 30.2 mg (56.8%); mp 128.6–131.1 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.71 (d, J = 9.0 Hz, 2H), 7.22–7.17 (m, 4H), 7.15–7.10 (m, 1H), 7.03 (d, J = 9.0 Hz, 2H), 4.02–3.97 (m, 1H), 3.83 (s, 3H), 3.78–3.74 (m, 1H), 3.37–3.33 (m, 1H), 3.27 (s, 1H), 3.18–3.14 (m, 1H), 3.06–3.01 (m, 1H), 2.90–2.86 (m, 1H), 2.84–2.80 (m, 1H), 2.70–2.62 (m, 1H), 2.58–2.54 (m, 1H), 2.32–2.24 (m, 2H), 2.17 (s, 3H), 2.01–1.95 (m, 2H), 1.91–1.80 (m, 1H), 1.62–1.54 (m, 2H), 1.49–1.43 (m, 1H), 1.33–1.28 (m, 2H), 0.88 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 175.92, 164.51, 140.10, 132.12, 130.61, 130.43, 129.23, 127.24, 115.33, 74.27, 58.85, 58.36, 56.21, 54.95, 54.00, 49.85, 46.22, 43.91, 36.69, 28.03, 27.31, 24.67, 20.51, 20.46; HRMS (ESI) m/z calcd. for $C_{27}H_{37}N_3O_5S$ ([M − H]$^-$): 514.2376, found 514.2386.

4.1.16. (R)-N-((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-1-methylpiperidine-3-carboxamide (**3n**)

Compound **3n** was prepared from **10e** (13.4 mg, 0.07 mmol) and **7c** (30.8 mg, 0.08 mmol) by following the same procedure outlined for **3m** to give a yellowish powder: yield 24.4 mg (67.5%); mp 165.9–168.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 8.4 Hz, 2H), 7.30–7.26 (m, 3H), 7.26–7.24 (m, 1H), 7.21–7.17 (m, 1H), 6.67 (d, J = 8.4 Hz, 2H), 4.19–4.08 (m, 3H), 3.91–3.86 (m, 1H), 3.38–3.26 (m, 1H), 3.07–2.90 (m, 4H), 2.83–2.77 (m, 1H), 2.49–2.34 (m, 3H), 2.02 (s, 3H), 2.01–1.84 (m, 3H), 1.69–1.62 (m, 1H), 1.44–1.30 (m, 2H), 1.19–1.10 (m, 1H), 0.90–0.87 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.69, 150.72, 138.66, 129.57, 128.51, 126.82, 126.52, 114.22, 73.23, 58.92, 56.74, 55.52, 55.29, 53.27, 45.98, 41.67, 34.49, 27.40, 26.00, 21.95, 20.24; HRMS (ESI) m/z calcd. for $C_{26}H_{36}N_4O_4S$ ([M − H]$^-$): 499.2379, found 499.2385.

4.1.17. (R)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl)-1-methylpiperidine-3-carboxamide (**3o**)

Compound **3o** was prepared from **10e** (12.8 mg, 0.07 mmol) and **7d** (30 mg, 0.07 mmol) by following the same procedure outlined for **3m** to give a white power: yield 26.4 mg (68.2%); mp 114.2–116.9 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.31–7.27 (m, 2H), 7.26–7.19 (m, 3H), 4.14–4.09 (m, 1H), 3.92–3.88 (m, 1H), 3.47–3.37 (m, 1H), 3.10–3.01 (m, 3H), 2.94–2.89 (m, 2H), 2.59–2.39 (m, 3H), 2.12 (s, 3H), 2.06–1.89 (m, 2H), 1.73–1.64 (m, 1H), 1.52–1.27 (m, 3H), 1.24–1.02 (m, 1H), 0.90 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.57, 142.88, 138.26, 134.43, 129.45, 128.63, 127.94, 126.73, 126.36, 123.36, 72.85, 57.91, 56.59, 55.81, 55.44, 52.35, 45.81, 41.41, 34.91, 27.14, 25.89, 21.81, 20.12; HRMS (ESI) m/z calcd. for $C_{27}H_{34}F_3N_3O_4S$ ([M − H]$^-$): 552.2144, found 552.2150.

4.1.18. tert-Butyl (R)-3-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11a**)

Compound **11a** was prepared from **10a** (22.9 mg, 0.10 mmol) and **7a** (44.7 mg, 0.11 mmol) by following the same procedure outlined for **3m** to give a yellow oil: yield 58.4 mg (94.7%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (d, J = 8.0 Hz, 2H), 7.30–7.23 (m, 4H), 7.21–7.16 (m, 1H), 7.09 (d, J = 8.0 Hz, 2H), 4.05–3.96 (m, 2H), 3.89 (s, 3H), 3.83–3.75 (m, 2H), 3.40 (d, J = 14.5 Hz, 1H), 3.23 (d, J = 13.5 Hz, 1H), 3.12–3.06 (m, 1H), 2.96–2.86 (m, 2H), 2.70–2.50 (m, 3H), 2.22–2.16 (m, 1H), 2.07–2.01 (m, 1H), 1.81–1.76 (m, 1H), 1.66–1.61 (m, 1H), 1.57–1.52 (m, 1H), 1.48 (s, 9H), 1.44–1.33 (m, 2H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); LC-MS (APCI) [M+H]$^+$ m/z 618.6.

4.1.19. tert-Butyl (R)-3-(((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11b**)

Compound **11b** was prepared from **10a** (45.8 mg, 0.20 mmol) and **7c** (82.1 mg, 0.21 mmol) by following the same procedure outlined for **3m** to give a yellow oil: yield 95.6 mg (79.4%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.50 (d, J = 8.5 Hz, 2H), 7.30–7.24 (m, 4H), 7.22–7.16 (m, 1H), 6.71 (d, J = 8.5 Hz, 2H), 4.05–3.96 (m, 2H), 3.84–3.74 (m, 2H), 3.38–3.34 (m, 1H), 3.27–3.21 (m, 1H), 3.06–3.00 (m, 1H), 2.90–2.80 (m, 2H), 2.72–2.44 (m, 3H), 2.22–2.16 (m, 1H), 2.03–1.97 (m, 1H), 1.82–1.77 (m, 1H), 1.67–1.63 (m, 1H), 1.57–1.51 (m, 1H), 1.48 (s, 9H), 1.44–1.34 (m, 2H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); LC-MS (ESI) [M+Na]$^+$ m/z 625.8.

4.1.20. tert-Butyl (R)-3-(((2S,3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamoyl)piperidine-1-carboxylate (**11c**)

Compound **11c** was prepared from **10a** (34.4 mg, 0.15 mmol) and **7d** (71.1 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a yellowish oil: yield 67.3 mg (68.5%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.04 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 7.30–7.22 (m, 4H), 7.21–7.16 (m, 1H), 4.02–3.96 (m, 2H), 3.82–3.73 (m, 2H), 3.49–3.43 (m, 1H), 3.23–3.17 (m, 2H), 3.11–3.05 (m, 1H), 3.02–2.97 (m, 1H), 2.72–2.48 (m, 3H), 2.22–2.16 (m, 1H), 2.09–2.03 (m, 1H), 1.80–1.75 (m, 1H), 1.66–1.61 (m, 1H), 1.58–1.52 (m, 1H), 1.47 (s, 9H), 1.43–1.32 (m, 2H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); LC-MS (ESI) [M+Na]$^+$ m/z 678.7.

4.1.21. tert-Butyl (S)-3-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11d**)

Compound **11d** was prepared from **10b** (68.7 mg, 0.30 mmol) and **7a** (127 mg, 0.32 mmol) by following the same procedure outlined for **3m** to give a yellow oil: yield 175 mg (94.6%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.80–7.76 (m, 2H), 7.28–7.23 (m, 4H), 7.20–7.10 (m, 3H), 4.06–4.01 (m, 2H), 4.00–3.93 (m, 1H), 3.89 (s, 3H), 3.81 (s, 1H), 3.44 (s, 1H), 3.26–3.21 (m, 1H), 3.12–3.07 (m, 1H), 2.96–2.84 (m, 2H), 2.75 (s, 1H), 2.65–2.59 (m, 1H), 2.24–2.17 (m, 1H), 2.10–2.03 (m, 1H), 1.61–1.57 (m, 1H), 1.44 (s, 9H), 1.36–1.31 (m, 4H), 0.95 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); LC-MS (ESI) [M+H]$^+$ m/z 618.7.

4.1.22. tert-Butyl (S)-3-(((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11e**)

Compound **11e** was prepared from **10b** (45.8 mg, 0.20 mmol) and **7c** (82.1 mg, 0.21 mmol) by following the same procedure outlined for **3m** to give a yellow oil: yield 112 mg (93.4%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.51–7.48 (m, 2H), 7.26–7.22 (m, 4H), 7.19–7.15 (m, 1H), 6.77–6.69 (m, 2H), 4.05–3.93 (m, 2H), 3.85–3.78 (m, 1H), 3.25–3.21 (m, 1H), 3.16–3.03 (m, 2H), 3.01 (s, 1H), 2.89–2.82 (m, 2H), 2.82–2.77 (m, 1H), 2.68 (s, 1H), 2.62–2.57 (m, 1H), 2.23–2.13 (m, 1H), 2.06–2.01 (m, 1H), 1.66–1.53 (m, 2H), 1.44 (s, 9H), 1.35–1.28 (m, 3H), 0.96–0.93 (m, 3H), 0.90–0.87 (m, 3H); LC-MS (ESI) [M+Na]$^+$ m/z 625.8.

4.1.23. tert-Butyl (S)-3-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamoyl)piperidine-1-carboxylate (**11f**)

Compound **11f** was prepared from **10b** (34.4 mg, 0.15 mmol) and

7d (71.1 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a yellowish oil: yield 82.4 mg (83.3%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.08–8.02 (m, 2H), 7.98–7.90 (m, 2H), 7.27–7.21 (m, 4H), 7.21–7.16 (m, 1H), 4.04–3.94 (m, 2H), 3.83–3.73 (m, 1H), 3.64–3.38 (m, 1H), 3.23–3.18 (m, 2H), 3.14–3.02 (m, 2H), 3.00–2.95 (m, 1H), 2.72 (s, 1H), 2.64–2.58 (m, 1H), 2.24–2.14 (m, 1H), 2.10–2.04 (m, 1H), 1.63–1.57 (m, 1H), 1.44 (s, 9H), 1.36–1.27 (m, 4H), 0.95 (d, J = 6.0 Hz, 3H), 0.88 (d, J = 6.0 Hz, 3H); LC-MS (ESI) $[M+Na]^+$ m/z 678.7.

*4.1.24. tert-Butyl (S)-2-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11g**)*

Compound **11g** was prepared from **10c** (34.4 mg, 0.15 mmol) and **7a** (63.9 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a yellow oil: yield 49.9 mg (53.9%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (d, J = 7.0 Hz, 2H), 7.25 (s, 4H), 7.18 (s, 1H), 7.09 (d, J = 7.0 Hz, 2H), 4.47 (s, 1H), 4.23–4.03 (m, 1H), 3.89 (s, 3H), 3.83 (s, 2H), 3.45–3.38 (m, 1H), 3.29–3.20 (m, 1H), 3.10–2.91 (m, 4H), 2.64 (s, 1H), 2.07–2.00 (m, 1H), 1.79–1.64 (m, 1H), 1.47 (s, 2H), 1.40 (s, 9H), 1.35–1.23 (m, 3H), 0.93 (d, J = 5.5 Hz, 3H), 0.89 (d, J = 6.0 Hz, 3H); LC-MS (APCI) $[M+H]^+$ m/z 618.7.

*4.1.25. tert-Butyl (S)-2-(((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11h**)*

Compound **11h** was prepared from **10c** (27.5 mg, 0.12 mmol) and **7c** (49.2 mg, 0.13 mmol) by following the same procedure outlined for **3m** to give a yellow oil: yield 47.5 mg (65.8%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 8.5 Hz, 2H), 7.32–7.26 (m, 3H), 7.26–7.18 (m, 2H), 6.69 (d, J = 8.5 Hz, 2H), 6.30 (s, 1H), 4.61 (s, 1H), 4.16 (s, 1H), 4.05–3.80 (m, 2H), 3.13–3.02 (m, 3H), 2.97 (s, 2H), 2.90 (s, 2H), 2.86–2.82 (m, 1H), 2.66–2.36 (m, 1H), 2.12–2.01 (m, 1H), 1.91–1.81 (m, 1H), 1.59–1.53 (m, 1H), 1.52–1.46 (m, 2H), 1.44 (s, 9H), 1.35–1.27 (m, 3H), 0.92–0.87 (m, 6H); LC-MS (APCI) $[M+H]^+$ m/z 603.7.

*4.1.26. tert-Butyl (S)-2-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamoyl)piperidine-1-carboxylate (**11i**)*

Compound **11i** was prepared from **10c** (34.4 mg, 0.15 mmol) and **7d** (70.0 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a yellowish oil: yield 89.1 mg (90.6%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 7.28–7.22 (m, 4H), 7.21–7.16 (m, 1H), 4.52–4.46 (m, 1H), 4.18–3.99 (m, 1H), 3.90–3.74 (m, 2H), 3.53–3.45 (m, 1H), 3.29–3.11 (m, 3H), 3.01 (s, 2H), 2.68–2.57 (m, 1H), 2.09–2.03 (m, 1H), 1.79–1.65 (m, 1H), 1.52–1.45 (m, 2H), 1.40 (s, 9H), 1.34–1.22 (m, 3H), 0.93 (d, J = 6.0 Hz, 3H), 0.89 (d, J = 5.5 Hz, 3H); LC-MS (APCI) $[M+H]^+$ m/z 656.7.

*4.1.27. tert-Butyl 4-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11j**)*

Compound **11j** was prepared from **10d** (34.4 mg, 0.15 mmol) and **7a** (63.9 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a yellow powder: yield 88.3 mg (95.4%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.76 (d, J = 8.0 Hz, 2H), 7.27–7.21 (m, 4H), 7.20–7.15 (m, 1H), 7.09 (d, J = 8.0 Hz, 2H), 4.07–4.00 (m, 2H), 3.96–3.91 (m, 1H), 3.89 (s, 3H), 3.83–3.78 (m, 1H), 3.43–3.38 (m, 1H), 3.25–3.20 (m, 1H), 3.12–3.06 (m, 1H), 2.96–2.86 (m, 2H), 2.72 (s, 2H), 2.64–2.58 (m, 1H), 2.26–2.20 (m, 1H), 2.08–2.00 (m, 1H), 1.61–1.55 (m, 1H), 1.48–1.43 (m, 10H), 1.35–1.32 (m, 1H), 1.28–1.19 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H).

*4.1.28. tert-Butyl 4-(((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamoyl) piperidine-1-carboxylate (**11k**)*

Compound **11k** was prepared from **10d** (34.4 mg, 0.15 mmol) and **7c** (61.6 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a white powder: yield 86.2 mg (95.4%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.49 (d, J = 8.5 Hz, 2H), 7.27–7.22 (m, 4H), 7.20–7.16 (m, 1H), 6.71 (d, J = 8.5 Hz, 2H), 4.06–4.01 (m, 2H), 3.95–3.90 (m, 1H), 3.84–3.80 (m, 1H), 3.39–3.34 (m, 1H), 3.26–3.22 (m, 1H), 3.06–3.01 (m, 1H), 2.89–2.79 (m, 2H), 2.72 (s, 2H), 2.64–2.58 (m, 1H), 2.25–2.20 (m, 1H), 2.06–1.99 (m, 1H), 1.60–1.56 (m, 1H), 1.46 (s, 9H), 1.35–1.31 (m, 2H), 1.26–1.17 (m, 1H), 0.95 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H).

*4.1.29. tert-Butyl 4-(((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamoyl)piperidine-1-carboxylate (**11l**)*

Compound **11l** was prepared from **10d** (34.4 mg, 0.15 mmol) and **7d** (70.0 mg, 0.16 mmol) by following the same procedure outlined for **3m** to give a white powder: yield 89.3 mg (90.7%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.04 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 7.28–7.21 (m, 4H), 7.20–7.17 (m, 1H), 4.05–4.00 (m, 2H), 3.93 (d, J = 13.5 Hz, 1H), 3.79–3.74 (m, 1H), 3.47 (d, J = 13.5 Hz, 1H), 3.22–3.18 (m, 2H), 3.11–3.06 (m, 1H), 3.01–2.97 (m, 1H), 2.72 (s, 2H), 2.64–2.58 (m, 1H), 2.26–2.20 (m, 1H), 2.10–2.01 (m, 1H), 1.60–1.54 (m, 1H), 1.46 (s, 9H), 1.37–1.30 (m, 2H), 1.28–1.19 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H).

*4.1.30. (R)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)piperidine-3-carboxamide (**3a**)*

To a stirred solution of **11a** (58.4 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was bubbled hydrochloric acid gas at 25 °C for 0.5 h. The reaction mixture was concentrated under reduced pressure, and the residue was neutralized with saturated aqueous $NaHCO_3$ to pH 7.0, extracted with CH_2Cl_2 (3 × 5 mL), and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give **3a** as a white powder: yield 41.1 mg (88.3%); mp 183.7–185.8 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.75 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 7.5 Hz, 2H), 7.25–7.12 (m, 5H), 7.09 (d, J = 7.5 Hz, 2H), 5.05 (s, 1H), 3.83 (s, 3H), 3.81–3.75 (m, 1H), 3.57 (s, 1H), 3.28 (d, J = 14.0 Hz, 1H), 3.09–2.97 (m, 2H), 2.82–2.68 (m, 3H), 2.57–2.52 (m, 1H), 2.33 (t, J = 9.5 Hz, 1H), 2.25 (t, J = 10.0 Hz, 1H), 2.11–2.03 (m, 1H), 2.01–1.93 (m, 1H), 1.61–1.53 (m, 1H), 1.44–1.31 (m, 2H), 1.23 (s, 2H), 0.84 (d, J = 5.0 Hz, 3H), 0.79 (d, J = 5.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.21, 162.78, 139.63, 131.01, 129.70, 129.60, 128.40, 126.37, 114.82, 72.65, 57.14, 56.11, 53.43, 52.73, 48.57, 45.78, 43.46, 35.64, 27.79, 26.71, 24.98, 20.39; HRMS (ESI) m/z calcd. for $C_{27}H_{39}N_3O_5S$ ($[M-H]^-$): 516.2532, found 516.2525.

*4.1.31. (R)-N-((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)piperidine-3-carboxamide (**3b**)*

Compound **3b** was prepared from **11b** (75.0 mg, 0.12 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 53.4 mg (88.6%); mp 220.1–222.4 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.50 (d, J = 8.5 Hz, 2H), 7.28–7.21 (m, 4H), 7.20–7.15 (m, 1H), 6.71 (d, J = 8.5 Hz, 2H), 4.07–4.01 (m, 1H), 3.84–3.79 (m, 1H), 3.38–3.35 (m, 1H), 3.26–3.20 (m, 1H), 3.07–2.99 (m, 1H), 2.93–2.80 (m, 3H), 2.66–2.51 (m, 3H), 2.46–2.40 (m, 1H), 2.29–2.20 (m, 1H), 2.09–1.89 (m, 1H), 1.81–1.74 (m, 1H), 1.70–1.60 (m, 1H), 1.59–1.41 (m, 2H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 176.19, 154.33, 140.15, 130.45, 130.41, 129.18, 127.20, 125.87, 114.38, 74.58, 59.28, 54.93, 54.38, 46.44, 44.35, 36.89, 28.39, 28.16, 25.42, 20.58,

20.52; HRMS (ESI) *m/z* calcd. for $C_{26}H_{38}N_4O_4S$ ([M − H]$^-$): 501.2536, found 501.2550.

4.1.32. (R)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) piperidine-3-carboxamide (**3c**)

Compound **3c** was prepared from **11c** (56.0 mg, 0.09 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 40.3 mg (85.4%); mp 199.8–202.2 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.30–7.26 (m, 2H), 7.25–7.19 (m, 2H), 4.17–4.13 (m, 1H), 3.91–3.87 (m, 1H), 3.40–3.35 (m, 1H), 3.13–3.02 (m, 3H), 2.96–2.84 (m, 3H), 2.73–2.69 (m, 2H), 2.61–2.55 (m, 1H), 2.32–2.28 (m, 1H), 1.97–1.91 (m, 1H), 1.74–1.69 (m, 1H), 1.58–1.52 (m, 1H), 1.36–1.27 (m, 1H), 1.24–1.20 (m, 1H), 1.13–1.04 (m, 1H), 0.91–0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.21, 142.90, 138.12, 134.45, 129.41, 128.65, 127.93, 126.78, 126.36, 123.35, 73.02, 57.80, 55.68, 52.32, 48.15, 46.52, 41.44, 35.20, 27.28, 27.12, 22.41, 20.12, 20.11; HRMS (ESI) *m/z* calcd. for $C_{27}H_{36}F_3N_3O_4S$ ([M − H]$^-$): 554.2301, found 554.2327.

4.1.33. (S)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)piperidine-3-carboxamide (**3d**)

Compound **3d** was prepared from **11d** (70.0 mg, 0.11 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 52.3 mg (91.9%); mp 142.6–144.9 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.29–7.27 (m, 1H), 7.26–7.22 (m, 3H), 7.22–7.18 (m, 1H), 6.97 (d, J = 8.4 Hz, 2H), 4.19–4.14 (m, 1H), 3.88–3.85 (m, 4H), 3.26–3.21 (m, 1H), 3.10–3.05 (m, 2H), 2.94–2.88 (m, 3H), 2.83–2.79 (m, 1H), 2.76–2.64 (m, 3H), 2.26–2.22 (m, 1H), 1.93–1.87 (m, 1H), 1.70–1.65 (m, 1H), 1.61–1.56 (m, 1H), 1.50–1.44 (m, 1H), 1.43–1.38 (m, 1H), 0.89 (t, J = 7.2 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.32, 163.06, 138.26, 130.50, 129.54, 129.46, 128.52, 126.61, 114.42, 73.18, 58.55, 55.75, 54.64, 53.33, 48.26, 46.45, 41.74, 35.55, 27.35, 27.31, 23.33, 20.23, 20.08; HRMS (ESI) *m/z* calcd. for $C_{27}H_{39}N_3O_5S$ ([M − H]$^-$): 516.2532, found 516.2527.

4.1.34. (S)-N-((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)piperidine-3-carboxamide (**3e**)

Compound **3e** was prepared from **11e** (48.2 mg, 0.08 mmol) by following the same procedure outlined for **3a** to give a yellow powder: yield 36.8 mg (91.6%); mp 106.0–108.6 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.47 (d, J = 8.4 Hz, 2H), 7.25–7.20 (m, 4H), 7.17–7.13 (m, 1H), 6.69 (d, J = 8.4 Hz, 2H), 4.03–3.99 (m, 1H), 3.80–3.77 (m, 1H), 3.23–3.19 (m, 1H), 3.03–2.99 (m, 1H), 2.88–2.76 (m, 4H), 2.62–2.56 (m, 2H), 2.54–2.49 (m, 1H), 2.24–2.20 (m, 1H), 2.04–1.96 (m, 1H), 1.52–1.47 (m, 2H), 1.41–1.26 (m, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.48, 154.31, 140.11, 130.46, 130.41, 129.18, 127.17, 125.85, 114.39, 74.66, 59.28, 54.85, 54.45, 49.85, 46.52, 44.25, 36.94, 28.78, 28.15, 25.58, 20.58, 20.52; HRMS (ESI) *m/z* calcd. for $C_{26}H_{38}N_4O_4S$ ([M − H]$^-$): 501.2536, found 501.2555.

4.1.35. (S)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) piperidine-3-carboxamide (**3f**)

Compound **3f** was prepared from **11f** (65.0 mg, 0.10 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 48.8 mg (88.6%); mp 171.2–173.6 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.02 (d, J = 7.8 Hz, 2H), 7.90 (d, J = 7.8 Hz, 2H), 7.26–7.20 (m, 4H), 7.19–7.14 (m, 1H), 4.02–3.98 (m, 1H), 3.76–3.72 (m, 1H), 3.49–3.45 (m, 1H), 3.21–3.17 (m, 2H), 3.09–3.05 (m, 1H), 2.99–2.95 (m, 1H), 2.89–2.84 (m, 2H), 2.63–2.57 (m, 2H), 2.53–2.48 (m, 1H), 2.25–2.20 (m, 1H), 2.08–2.01 (m, 1H), 1.54–1.49 (m, 2H), 1.42–1.31 (m, 2H), 0.93 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 176.65, 145.06, 139.99, 134.98, 130.35, 129.23, 127.35, 127.23, 124.94, 74.00, 58.02, 54.95, 53.42, 46.57, 44.41, 36.77, 28.86, 27.78, 25.68, 20.34; HRMS (ESI) *m/z* calcd. for $C_{27}H_{36}F_3N_3O_4S$ ([M − H]$^-$): 554.2301, found 554.2334.

4.1.36. (S)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)piperidine-2-carboxamide (**3g**)

Compound **3g** was prepared from **11g** (49.9 mg, 0.08 mmol) by following the same procedure outlined for **3a** to give a yellowish powder: yield 40.3 mg (97.4%); mp 75.2–77.8 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 8.0 Hz, 2H), 7.33–7.26 (m, 4H), 7.24–7.20 (m, 1H), 7.12 (d, J = 7.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 2H), 4.18–4.12 (m, 1H), 3.92–3.87 (m, 4H), 3.20–3.06 (m, 4H), 2.95–2.84 (m, 4H), 2.64–2.56 (m, 1H), 1.93–1.84 (m, 1H), 1.78–1.71 (m, 1H), 1.67–1.61 (m, 1H), 1.55–1.48 (m, 1H), 1.38–1.30 (m, 3H), 1.21–1.16 (m, 1H), 0.92–0.88 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.27, 163.10, 138.20, 130.23, 129.60, 129.51, 128.60, 126.61, 114.45, 72.93, 59.92, 58.68, 55.76, 54.05, 53.36, 45.44, 34.95, 29.36, 27.33, 25.57, 23.68, 20.26, 20.13; HRMS (ESI) *m/z* calcd. for $C_{27}H_{39}N_3O_5S$ ([M − H]$^-$): 516.2532, found 516.2547.

4.1.37. (S)-N-((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)piperidine-2-carboxamide (**3h**)

Compound **3h** was prepared from **11h** (47.5 mg, 0.08 mmol) by following the same procedure outlined for **3a** to give a yellowish powder: yield 30.8 mg (76.4%); mp 105.6–108.1 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 8.5 Hz, 2H), 7.30–7.27 (m, 1H), 7.25–7.23 (m, 2H), 7.19 (t, J = 6.9 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 8.5 Hz, 2H), 4.19–4.09 (m, 3H), 3.89–3.83 (m, 1H), 3.13–3.04 (m, 4H), 2.95 (s, 1H), 2.89–2.80 (m, 5H), 2.60–2.53 (m, 1H), 1.88–1.82 (m, 1H), 1.73–1.69 (m, 1H), 1.64–1.60 (m, 1H), 1.51–1.46 (m, 1H), 1.35–1.29 (m, 3H), 1.17–1.10 (m, 1H), 0.90–0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.16, 162.67, 150.76, 138.27, 129.62, 129.53, 128.56, 126.56, 114.24, 72.98, 59.93, 58.80, 53.89, 53.49, 45.44, 34.96, 29.37, 27.37, 25.57, 23.69, 20.29, 20.15; HRMS (ESI) *m/z* calcd. for $C_{26}H_{38}N_4O_4S$ ([M+H]$^+$): 501.2536, found 501.2540.

4.1.38. (S)-N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) piperidine-2-carboxamide (**3i**)

Compound **3i** was prepared from **11i** (89.1 mg, 0.14 mmol) by following the same procedure outlined for **3a** to give a yellowish powder: yield 51.3 mg (66.0%); mp 88.7–91.6 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.32–7.27 (m, 2H), 7.25–7.20 (m, 3H), 7.06 (d, J = 7.5 Hz, 1H), 4.11–4.05 (m, 1H), 3.90–3.85 (m, 1H), 3.26–3.21 (m, 1H), 3.16–3.05 (m, 3H), 2.99–2.93 (m, 2H), 2.92–2.86 (m, 1H), 2.83–2.77 (m, 1H), 2.60–2.53 (m, 1H), 1.92–1.86 (m, 1H), 1.79–1.68 (m, 2H), 1.65–1.61 (m, 1H), 1.51–1.46 (m, 1H), 1.39–1.27 (m, 3H), 1.19–1.12 (m, 1H), 0.89–0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.94, 142.57, 137.94, 134.53, 129.43, 128.71, 127.96, 126.78, 126.41, 123.34, 72.75, 59.86, 58.12, 54.51, 52.85, 45.46, 35.06, 29.46, 27.18, 25.76, 23.73, 20.15, 20.05; HRMS (ESI) *m/z* calcd. for $C_{27}H_{36}F_3N_3O_4S$ ([M − H]$^-$): 554.2301, found 554.2300.

4.1.39. N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl) sulfonamido)-1-phenylbutan-2-yl)piperidine-4-carboxamide (**3j**)

Compound **3j** was prepared from **11j** (70.0 mg, 0.11 mmol) by following the same procedure outlined for **3a** to give a yellowish

powder: yield 52.0 mg (91.4%); mp 226.7–229.5 °C; ^{1}H NMR (500 MHz,CD_3OD) δ 7.78 (d, J = 8.5 Hz, 2H), 7.29–7.24 (m, 4H), 7.21–7.16 (m, 1H), 7.11 (d, J = 8.5 Hz, 2H), 4.14–4.08 (m, 1H), 3.90 (s, 3H), 3.85–3.80 (m, 1H), 3.52–3.47 (m, 1H), 3.42–3.37 (m, 1H), 3.25–3.16 (m, 2H), 3.14–3.08 (m, 1H), 3.03–2.86 (m, 4H), 2.67–2.59 (m, 1H), 2.49–2.41 (m, 1H), 2.08–2.03 (m, 1H), 1.91–1.77 (m, 2H), 1.63–1.58 (m, 1H), 1.55–1.46 (m, 1H), 0.94 (d, J = 5.5 Hz, 3H), 0.88 (d, J = 5.5 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.32, 164.53, 140.14, 132.13, 130.62, 130.43, 129.23, 127.24, 115.35, 74.49, 58.81, 56.23, 54.89, 53.98, 44.06, 40.51, 36.57, 28.11, 28.04, 26.75, 26.12, 20.52, 20.45; HRMS (ESI) m/z calcd. for $C_{27}H_{39}N_3O_5S$ ([M+H]$^+$): 518.2688, found 518.2705.

4.1.40. N-((2S, 3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)piperidine-4-carboxamide (**3k**)

Compound **3k** was prepared from **11k** (70.0 mg, 0.12 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 51.0 mg (84.7%); mp 190.1–192.5 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.76 (d, J = 8.0 Hz, 2H), 7.28–7.23 (m, 4H), 7.18 (d, J = 7.0 Hz, 3H), 4.11–4.04 (m, 2H), 3.92 (s, 1H), 3.87–3.79 (m, 2H), 3.57–3.53 (m, 1H), 3.49–3.44 (m, 1H), 3.41–3.36 (m, 1H), 3.25–3.16 (m, 3H), 3.14–3.08 (m, 1H), 3.03–2.86 (m, 4H), 2.67–2.58 (m, 1H), 2.50–2.42 (m, 1H), 2.07–2.01 (m, 1H), 1.91–1.77 (m, 2H), 1.63–1.58 (m, 1H), 1.54–1.45 (m, 1H), 0.93 (d, J = 5.5 Hz, 3H), 0.88 (d, J = 5.5 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.38, 140.12, 130.50, 130.43, 129.23, 127.25, 119.65, 74.27, 64.80, 59.99, 58.60, 55.94, 54.97, 53.84, 53.25, 44.09, 44.07, 40.53, 36.64, 28.00, 26.75, 26.13, 20.49, 20.45; HRMS (ESI) m/z calcd. for $C_{26}H_{38}N_4O_4S$ ([M+H]$^+$): 503.2692, found 503.2703.

4.1.41. N-((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl)piperidine-4-carboxamide (**3l**)

Compound **3l** was prepared from **11l** (75.0 mg, 0.11 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 55.0 mg (90.0%); mp 263.2–265.7 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.05 (d, J = 7.5 Hz, 2H), 7.92 (d, J = 7.5 Hz, 2H), 7.29–7.23 (m, 4H), 7.21–7.15 (m, 1H), 4.11–4.05 (m, 1H), 3.83–3.77 (m, 1H), 3.57–3.51 (m, 1H), 3.42–3.36 (m, 1H), 3.23–3.16 (m, 3H), 3.12–3.06 (m, 1H), 3.02–2.89 (m, 3H), 2.66–2.59 (m, 1H), 2.49–2.42 (m, 1H), 2.10–2.04 (m, 1H), 1.90–1.78 (m, 2H), 1.64–1.58 (m, 1H), 1.55–1.46 (m, 1H), 0.94 (d, J = 6.0 Hz, 3H), 0.88 (d, J = 6.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.41, 145.03, 140.05, 134.95, 130.40, 129.26, 129.25, 127.38, 127.27, 124.94, 73.90, 58.01, 55.04, 53.34, 44.06, 40.53, 36.60, 27.81, 26.75, 26.13, 20.36, 20.35; HRMS (ESI) m/z calcd. for $C_{27}H_{36}F_3N_3O_4S$ ([M+H]$^+$): 556.2457, found 556.2546.

4.1.42. tert-Butyl (R)-3-(hydroxymethyl)piperidine-1-carboxylate (**13a**)

To a cold (0 °C) solution of **12a** (0.25 g, 2.20 mmol) in THF/H_2O (1 mL/1 mL) was added trimethylamine (0.24 g, 2.30 mmol) and $(Boc)_2O$ (0.57 g, 2.60 mmol). The reaction mixture was stirred for 10 h at 25 °C, and the solvent was diluted with H_2O (2 mL) and petroleum ether (3 mL). The aqueous layer was extracted with ethyl acetate (3 × 2 mL), the combined organic layer and washed with saturated aqueous $NaHCO_3$, 1 N HCl and saturated aqueous NaCl, and dried over anhydrous Na_2SO_4. The solvent was evaporated to afford **13a** as a white powder: yield 0.46 g (97.9%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90–3.54 (m, 2H), 3.49 (d, J = 5.5 Hz, 2H), 3.19–2.79 (m, 2H), 1.88–1.66 (m, 3H), 1.65–1.59 (m, 1H), 1.47–1.42 (m, 10H), 1.30–1.23 (m, 1H); LC-MS (ESI) [M+Na]$^+$ m/z 238.5.

4.1.43. tert-Butyl (S)-3-(hydroxymethyl)piperidine-1-carboxylate (**13b**)

Compound **13b** was prepared from **12b** (0.23 g, 2.00 mmol) by following the same procedure outlined for **13a** to give a white powder: yield 0.42 g (99.0%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.88–3.52 (m, 2H), 3.48 (d, J = 6.6 Hz, 2H), 3.17–2.76 (m, 2H), 2.27 (s, 1H), 1.79–1.75 (m, 1H), 1.74–1.67 (m, 1H), 1.64–1.58 (m, 1H), 1.46–1.39 (m, 10H), 1.27–1.23 (m, 1H); LC-MS (ESI) [M+Na]$^+$ m/z 238.5.

4.1.44. tert-Butyl (R)-3-((((4-nitrophenoxy)carbonyl)oxy)methyl)piperidine-1-carboxylate (**14a**)

A solution of **13a** (0.45 g, 2.10 mmol) in dry CH_2Cl_2 (4 mL) was added DMAP (0.28 g, 2.30 mmol) and 4-nitrophenyl carbonochloridate (0.51 g, 2.50 mmol) at 0 °C under argon atmosphere. The mixture was stirred at 25 °C for 4 h. After this period, the mixture was diluted with CH_2Cl_2 (8 mL) and washed with saturated aqueous NH_4Cl and saturated aqueous NaCl, and dried over anhydrous Na_2SO_4. The solvent was evaporated to afford **14a** as a yellowish oil: yield 0.65 g (81.0%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.34 (d, J = 8.7 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 4.26–4.21 (m, 1H), 4.21–4.15 (m, 1H), 3.99 (s, 1H), 3.85 (s, 1H), 3.03–2.98 (m, 1H), 2.82 (s, 1H), 2.02–1.93 (m, 1H), 1.92–1.85 (m, 1H), 1.76–1.70 (m, 1H), 1.56–1.50 (m, 1H), 1.48 (s, 9H), 1.43–1.36 (m, 1H); LC-MS (ESI) [M+Na]$^+$ m/z 403.5.

4.1.45. tert-Butyl (S)-3-((((4-nitrophenoxy)carbonyl)oxy)methyl)piperidine-1-carboxylate (**14b**)

Compound **14b** was prepared from **13b** (0.23 g, 2.00 mmol) by following the same procedure outlined for **14a** to give a yellowish oil: yield 0.41 g (59.7%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 (d, J = 9.0 Hz, 2H), 7.38 (d, J = 9.0 Hz, 2H), 4.19–4.15 (m, 2H), 4.00 (s, 1H), 3.87–3.83 (m, 1H), 2.95–2.90 (m, 1H), 2.80 (s, 1H), 2.01–1.96 (m, 1H), 1.88–1.83 (m, 1H), 1.71–1.67 (m, 1H), 1.48–1.45 (m, 10H), 1.35–1.30 (m, 1H); LC-MS (ESI) [M+Na]$^+$ m/z 403.5.

4.1.46. tert-Butyl (R)-3-(((((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamoyl)oxy)methyl)piperidine-1-carboxylate (**15p**)

To a stirred solution of **14a** (57.0 mg, 0.15 mmol) and **7a** (63.9 mg, 0.16 mmol) in anhydrous DMF (1 mL) was added DIEA (38.8 mg, 0.30 mmol) at 0 °C under argon atmosphere. The reaction mixture was warmed to 25 °C and stirred for 5 h. After this period, the reaction was diluted with 5 mL ethyl acetate, washed with H_2O and saturated aqueous NaCl and dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to furnish **15p** as a white powder: yield 85.9 mg (88.5%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 8.5 Hz, 2H), 7.31–7.27 (m, 2H), 7.25–7.19 (m, 3H), 6.97 (d, J = 8.5 Hz, 2H), 4.90–4.78 (m, 1H), 3.92–3.76 (m, 9H), 3.16–3.09 (m, 1H), 3.03–2.88 (m, 4H), 2.82–2.73 (m, 2H), 2.52 (s, 1H), 1.86–1.79 (m, 1H), 1.77–1.56 (m, 4H), 1.44 (s, 9H), 1.43–1.35 (m, 1H), 1.16–1.03 (m, 1H), 0.91 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H).

4.1.47. tert-Butyl (R)-3-(((((2S, 3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamoyl)oxy)methyl)piperidine-1-carboxylate (**15q**)

Compound **15q** was prepared from **14a** (45.6 mg, 0.12 mmol) and **7c** (49.3 mg, 0.13 mmol) by following the same procedure outlined for **15p** to give a white powder: yield 74.5 mg (98.2%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 8.5 Hz, 2H), 7.31–7.27 (m, 2H), 7.24–7.19 (m, 3H), 6.69 (d, J = 8.5 Hz, 2H), 4.89–4.78 (m, 1H), 3.93–3.70 (m, 6H), 3.14–3.07 (m, 1H), 3.04–2.86 (m, 7H), 2.81–2.73 (m, 2H), 2.52 (s, 1H), 1.85–1.77 (m, 1H), 1.74–1.60 (m, 3H), 1.44 (s, 9H), 1.42–1.37 (m, 1H), 1.14–1.07 (m, 1H), 0.91 (d,

J = 6.5 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H).

*4.1.48. tert-Butyl (R)-3-(((((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamoyl)oxy)methyl)piperidine-1-carboxylate (**15r**)*

Compound **15r** was prepared from **14a** (57.0 mg, 0.15 mmol) and **7d** (77.0 mg, 0.16 mmol) by following the same procedure outlined for **15p** to give a white powder: yield 70.4 mg (68.5%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.32–7.27 (m, 2H), 7.24–7.21 (m, 3H), 4.88–4.78 (m, 1H), 3.96–3.76 (m, 6H), 3.19–3.08 (m, 2H), 3.03–2.95 (m, 2H), 2.92–2.86 (m, 2H), 2.81–2.73 (m, 1H), 2.52 (s, 1H), 1.89–1.82 (m, 1H), 1.78–1.55 (m, 4H), 1.44 (s, 9H), 1.42–1.36 (m, 1H), 1.14–1.06 (m, 1H), 0.90 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H).

*4.1.49. tert-Butyl (S)-3-(((((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)carbamoyl)oxy) methyl)piperidine-1-carboxylate (**15s**)*

Compound **15s** was prepared from **14b** (80.0 mg, 0.21 mmol) and **7a** (89.7 mg, 0.22 mmol) by following the same procedure outlined for **15p** to give a white powder: yield 132 mg (97.1%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, J = 8.4 Hz, 2H), 7.30–7.27 (m, 2H), 7.25–7.19 (m, 3H), 6.97 (d, J = 8.4 Hz, 2H), 4.91–4.83 (m, 1H), 3.92–3.81 (m, 8H), 3.79–3.74 (m, 1H), 3.15–3.10 (m, 1H), 3.04–2.99 (m, 2H), 2.97–2.93 (m, 1H), 2.89–2.85 (m, 1H), 2.82–2.76 (m, 2H), 2.55–2.48 (m, 1H), 1.86–1.79 (m, 1H), 1.73–1.65 (m, 2H), 1.64–1.59 (m, 1H), 1.44 (s, 9H), 1.41–1.37 (m, 1H), 1.14–1.08 (m, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); LC-MS (ESI) $[M+Na]^+$ m/z 670.7.

*4.1.50. tert-Butyl (S)-3-(((((2S, 3R)-4-((4-amino-N-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamoyl)oxy) methyl)piperidine-1-carboxylate (**15t**)*

Compound **15t** was prepared from **14b** (57.0 mg, 0.15 mmol) and **7c** (61.6 mg, 0.16 mmol) by following the same procedure outlined for **15p** to give a colorless oil: yield 80.5 mg (84.9%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 (d, J = 7.8 Hz, 2H), 7.30–7.27 (m, 2H), 7.24–7.19 (m, 3H), 6.72 (d, J = 7.8 Hz, 2H), 4.91–4.83 (m, 1H), 3.88–3.73 (m, 6H), 3.13–3.07 (m, 1H), 3.03–2.95 (m, 3H), 2.93–2.84 (m, 3H), 2.81–2.75 (m, 2H), 2.50 (s, 1H), 1.85–1.79 (m, 1H), 1.73–1.66 (m, 2H), 1.63–1.60 (m, 1H), 1.44 (s, 9H), 1.42–1.37 (m, 1H), 1.14–1.07 (m, 1H), 0.90 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); LC-MS (APCI) $[M+H]^+$ m/z 633.6.

*4.1.51. tert-Butyl (S)-3-(((((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl) carbamoyl)oxy)methyl)piperidine-1-carboxylate (**15u**)*

Compound **15u** was prepared from **14b** (57.0 mg, 0.15 mmol) and **7d** (70.0 mg, 0.16 mmol) by following the same procedure outlined for **15p** to give a colorless oil: yield 102 mg (98.8%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.05 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 9.0 Hz, 2H), 7.29–7.24 (m, 4H), 7.21–7.15 (m, 1H), 3.95–3.83 (m, 3H), 3.81–3.77 (m, 1H), 3.75–3.67 (m, 2H), 3.52–3.46 (m, 1H), 3.23–3.09 (m, 3H), 3.03–2.97 (m, 1H), 2.89–2.76 (m, 1H), 2.64–2.48 (m, 2H), 2.09–2.03 (m, 1H), 1.70–1.60 (m, 3H), 1.47 (s, 9H), 1.42–1.34 (m, 2H), 1.23–1.15 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); LC-MS (APCI) $[M+H]^+$ m/z 686.6.

*4.1.52. ((R)-piperidin-3-yl)methyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl) carbamate (**3p**)*

Compound **3p** was prepared from **15p** (75.0 mg, 0.12 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 55.3 mg (84.2%); mp 159.8–162.4 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (d, J = 8.5 Hz, 2H), 7.25–7.18 (m, 4H), 7.17–7.13 (m, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 9.0 Hz, 1H), 5.04–4.97 (m, 1H), 3.84 (s, 3H), 3.70–3.57 (m, 3H), 3.56–3.50 (m, 1H), 3.37–3.32 (m, 2H), 3.02–2.96 (m, 2H), 2.85–2.71 (m, 4H), 2.39–2.31 (m, 1H), 2.15–2.06 (m, 1H), 1.99–1.93 (m, 1H), 1.61–1.46 (m, 3H), 1.29–1.23 (m, 2H), 0.99–0.92 (m, 1H), 0.84 (d, J = 6.5 Hz, 3H), 0.79 (d, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.23, 155.98, 139.50, 130.75, 129.20, 129.13, 127.87, 125.71, 114.30, 72.18, 66.19, 56.55, 55.68, 55.62, 52.22, 48.91, 46.12, 36.09, 35.19, 27.11, 26.17, 24.95, 19.96; HRMS (ESI) m/z calcd. for $C_{28}H_{41}N_3O_6S$ ($[M-H]^-$): 546.2638, found 546.2643.

*4.1.53. ((R)-piperidin-3-yl)methyl ((2S, 3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl) carbamate (**3q**)*

Compound **3q** was prepared from **15q** (60.0 mg, 0.09 mmol) by following the same procedure outlined for **3a** to give a yellow powder: yield 38.6 mg (72.6%); mp 192.8–194.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.38 (d, J = 8.5 Hz, 2H), 7.25–7.19 (m, 4H), 7.17–7.12 (m, 1H), 7.00–6.93 (m, 1H), 6.60 (d, J = 8.5 Hz, 2H), 5.97 (s, 2H), 4.97–4.91 (m, 1H), 3.68–3.58 (m, 3H), 3.56–3.50 (m, 1H), 3.31–3.24 (m, 2H), 3.03–2.97 (m, 1H), 2.94–2.88 (m, 1H), 2.82–2.73 (m, 2H), 2.71–2.62 (m, 2H), 2.40–2.30 (m, 1H), 2.15–2.05 (m, 1H), 2.03–1.85 (m, 2H), 1.62–1.44 (m, 3H), 1.32–1.24 (m, 1H), 1.00–0.91 (m, 1H), 0.84 (d, J = 6.0 Hz, 3H), 0.79 (d, J = 6.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.98, 152.69, 139.60, 129.16, 129.01, 127.84, 125.66, 123.61, 112.65, 72.50, 66.31, 57.12, 55.64, 52.66, 49.24, 46.38, 36.38, 35.11, 27.38, 26.34, 25.34, 20.06; HRMS (ESI) m/z calcd. for $C_{27}H_{40}N_4O_5S$ ($[M+H]^+$): 533.2798, found 533.2769.

*4.1.54. ((R)-piperidin-3-yl)methyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (**3r**)*

Compound **3r** was prepared from **15r** (60.4 mg, 0.10 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 44.1 mg (75.3%); mp 178.7–200.9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04–7.96 (m, 4H), 7.26–7.14 (m, 5H), 7.04 (d, J = 9.0 Hz, 1H), 5.07 (s, 1H), 3.69–3.50 (m, 4H), 3.41–3.34 (m, 2H), 3.15–3.09 (m, 1H), 3.06–2.96 (m, 2H), 2.93–2.87 (m, 1H), 2.83–2.74 (m, 2H), 2.40–2.31 (m, 1H), 2.14–2.07 (m, 1H), 2.06–1.88 (m, 2H), 1.62–1.43 (m, 3H), 1.32–1.25 (m, 1H), 1.01–0.91 (m, 1H), 0.87–0.80 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.02, 143.51, 139.38, 132.14, 129.10, 127.98, 127.90, 126.33, 125.74, 123.52, 71.49, 66.34, 55.73, 55.56, 51.45, 49.33, 46.42, 36.42, 35.35, 27.40, 25.87, 25.36, 19.83, 19.72; HRMS (ESI) m/z calcd. for $C_{28}H_{38}F_3N_3O_5S$ ($[M+H]^+$): 586.2563, found 586.2543.

*4.1.55. ((S)-piperidin-3-yl)methyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl) carbamate (**3s**)*

Compound **3s** was prepared from **15s** (105.6 mg, 0.16 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 62.6 mg (71.5%); mp 177.0–179.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, J = 8.4 Hz, 2H), 7.31–7.27 (m, 2H), 7.25–7.20 (m, 3H), 6.97 (d, J = 8.4 Hz, 2H), 5.01–4.92 (m, 1H), 3.87 (s, 3H), 3.86–3.80 (m, 3H), 3.78–3.74 (m, 1H), 3.13–3.08 (m, 1H), 3.04–2.98 (m, 4H), 2.95–2.87 (m, 2H), 2.80 (dd, J = 13.4, 6.8 Hz, 1H), 2.55–2.50 (m, 1H), 2.30–2.25 (m, 1H), 1.85–1.80 (m, 1H), 1.75–1.62 (m, 3H), 1.48–1.42 (m, 1H), 1.11–1.00 (m, 1H), 0.90 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.15, 156.81, 137.87, 130.04, 129.65, 129.61, 128.64, 126.63, 114.47, 72.74, 67.84, 58.85, 55.77, 55.17, 53.80, 49.58, 46.73, 36.66, 35.50, 27.56, 27.38, 25.67, 20.30, 20.05; HRMS (ESI) m/z calcd. for $C_{28}H_{41}N_3O_6S$ ($[M-H]^-$): 546.2638, found 546.2625.

*4.1.56. ((S)-piperidin-3-yl)methyl ((2S, 3R)-4-((4-amino-N-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl) carbamate (**3t**)*

Compound **3t** was prepared from **15t** (80.5 mg, 0.13 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 46.1 mg (66.6%); mp 148.7–160.3 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (d, J = 8.4 Hz, 2H), 7.30–7.27 (m, 2H), 7.24–7.19 (m, 3H), 6.68 (d, J = 8.4 Hz, 2H), 5.01 (s, 1H), 4.21 (s, 2H), 3.87–3.79 (m, 3H), 3.78–3.73 (m, 1H), 3.48 (s, 1H), 3.11–2.99 (m, 5H), 2.93–2.84 (m, 2H), 2.81–2.76 (m, 1H), 2.58–2.51 (m, 1H), 2.32–2.26 (m, 1H), 1.85–1.79 (m, 1H), 1.76 (s, 1H), 1.71–1.64 (m, 2H), 1.53–1.43 (m, 1H), 1.35–1.26 (m, 1H), 1.12–0.98 (m, 1H), 0.90 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.73, 150.88, 137.93, 129.65, 128.61, 126.59, 126.29, 114.23, 72.72, 67.69, 58.94, 55.14, 53.88, 50.98, 49.34, 46.53, 36.41, 35.65, 27.39, 25.37, 20.32, 20.09; HRMS (ESI) m/z calcd. for $C_{27}H_{40}N_4O_5S$ ([M − H]$^-$): 531.2641, found 531.2627.

*4.1.57. ((S)-piperidin-3-yl)methyl ((2S, 3R)-3-hydroxy-4-((N-isobutyl-4-(trifluoromethyl)phenyl)sulfonamido)-1-phenylbutan-2-yl)carbamate (**3u**)*

Compound **3u** was prepared from **15u** (101.6 mg, 0.15 mmol) by following the same procedure outlined for **3a** to give a white powder: yield 68.6 mg (78.1%); mp 176.8–178.9 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 (d, J = 7.8 Hz, 2H), 7.78 (d, J = 7.8 Hz, 2H), 7.31–7.27 (m, 2H), 7.24–7.21 (m, 3H), 5.09 (s, 1H), 3.89–3.81 (m, 3H), 3.79–3.74 (m, 1H), 3.18–3.12 (m, 2H), 3.06–2.99 (m, 3H), 2.98–2.94 (m, 1H), 2.92–2.86 (m, 2H), 2.57–2.53 (m, 1H), 2.36–2.26 (m, 1H), 1.89–1.83 (m, 1H), 1.77 (s, 1H), 1.72–1.64 (m, 2H), 1.53–1.45 (m, 1H), 1.36–1.29 (m, 1H), 1.11–1.03 (m, 1H), 0.89–0.85 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.89, 142.37, 137.72, 134.6, 129.54, 128.72, 127.98, 126.75, 126.43, 123.3, 72.55, 67.86, 58.33, 55.38, 53.30, 49.40, 46.56, 36.38, 35.46, 27.37, 27.24, 25.35, 20.19, 19.99; HRMS (ESI) m/z calcd. for $C_{28}H_{38}F_3N_3O_5S$ ([M − H]$^-$): 584.2406, found 584.2399.

4.2. In vitro assay for HIV-1 protease inhibition

The HIV-1 protease inhibitory activities of all new designed inhibitors were measured using fluorescence resonance energy transfer (FRET) method [27]. Peptide (Arg-Glu (EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg) purchased from AnaSpec was selected as the substrate. The energy transfer donor (EDANS) and acceptor (DABCYL) dyes are labeled at two ends of the peptide to perform FRET. Excitation and emission wavelengths were set at 340 nm and 490 nm. Inhibitors were dissolved in dimethylsulfoxide (DMSO) and diluted to appropriate concentrations. HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* and purified. The experiment was carried out in 96-well plates. The FRET assay reaction buffer contained 0.1 M sodium acetate, 1 M sodium chloride, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), 2% DMSO and 1 mg/mL bovine serum albumin (BSA) with an adjusted pH 4.7. Protease and inhibitor were mixed and incubated for 20–30 min at room temperature and then the substrate was added. Each reaction was recorded for about 10 min.

4.3. Converting IC_{50} to K_i values

The IC_{50} value depends on concentrations of the enzyme HIV-1 PR, the inhibitor, and the substrate along with other experimental conditions. While, comparisons of K_i values can be more readily made among different laboratories to characterize the inhibitors. What is required is an accurate determination of the K_i value, an intrinsic, thermodynamic quantity that is independent of the substrate but depends on the enzyme and inhibitor [28]. As all approved HIV-1 PIs are competitive inhibitors except for Tipranavir (TPV) and we designed this class of new HIV-1 protease inhibitors with a core scaffold similar to Darunavir (DRV), the equation for K_i is used to take into account the concentration of the substrate, the K_m of the enzyme-substrate reaction and the IC_{50} value for competitive inhibition [44]. The expression is

$$K_i = IC_{50} /(S/K_m + \quad 1$$

The concentration of the substrate in the assay is 6.20 μM, and the K_m of the enzyme-substrate reaction is 1.79 μM.

4.4. Cytotoxicity assay

Selected inhibitors were further evaluated in cytotoxicity assay using a cell counting kit-8 assay [45]. Plates were prepared with 20 000 293T cells per well. After 24h of culture, 1 μL of drugs were added to each well. After another 24h of culture, 10 μL of CCK-8 was added to each well. Absorbance was quantified at wavelength 450 nm using an EnVision multilabel reader (PerkinElmer) after 2h at room temperature.

4.5. Infectivity assay on HIV-1 late stage

The inhibitory effect of compounds on HIV-1 infectivity were determined using a single-round HIV-1 infectivity assay [32]. 293T cells were co-transfected with either plasmid pNL4-3-E$^-$R$^-$ (pHIV-1_{NL4-3}) or DRV-resistant pNL4-3-E$^-$R$^-$ variants (pHIV-1^R_{DRVS}) and pHCMV-G (VSV-G) to produce VSV-G pseudotyped HIV-1. Inhibitors dissolved in dimethylsulfoxide (DMSO) and diluted to appropriate concentrations, were added into culture medium at 5 h of post-transfection. After incubating for 48 h at temperature 37 °C, pseudotyped viruses in 10 μL of supernatant were used to infect SupT1 cells for 48 h, followed by measuring luciferase activity of newly infected cells using Centro LB960 (Berthold).

4.6. Infectivity assay on HIV-1 early stage

To assess the effect of the compounds on HIV-1 infectivity, the experiments were carried out using VSVG-pseudotyped HIV-1 [33]. SupT1 cells (1×10^5/ml) were infected with VSVG-pseudotyped HIV-1(NL4-3) in 96-well plates, and then the compounds were added at the concentration of 10 μM. Equal volumes of DMSO were added into the culture medium, in order to keep constant final DMSO concentration as 1% (v/v). 48 h later, SupT1 cells were lysed and firefly luciferase activities were determined using a firefly Luciferase Assay System (Promega).

4.7. Construction of DRV-resistant pNL4-3-E-R- cloning (pHIV-1^R_{DRVS})

To generate HIV-1 clones carrying the intended mutations, site-directed mutagenesis kit (*SBS Genetech*) was used. V32I, L33F, I54 M, and I84V in the protease were introduced into pNL4-3-E-R- according to the manual from the manufacturer [10]. The primers used for mutations were 32/33 (F′-ACAGGAGCA GATGATACAA-TATTTGAAGAAAT GAATTTGCCA, R′-TGGCAAATTCATTTC TTCAAA-TATTGTATCATCTGC TCCTGT), 54(F′-GGGAATTGGAGGTTTTATG AAAGTAAGACAGTATGAT, R′- ATCATACTGTCTTACTTTCA-TAAAACCTCCAATTCCC) and 84(F′- GGA CCTA-CACCTGTCAACGTAATTGGAAGAA ATCTGT, R′- ATCATACTG TCTTACTTTCATAAAACCTCCAATTCCC). Determination of the nucleotide sequences of plasmids confirmed that each clone had the desired mutations but no unintended mutations (*BBI Life Sciences*

Corporation).

4.8. Molecular modeling

The docking was performed through "DOCK" module in the Molecular Operating Environment (MOE) using the alpha triangle placement method. Refinement of the docked poses was carried out using the Forcefield refinement scheme and scored using both the affinity dG and london dG scoring system [40]. The HIV-1 protease crystal structure (PDB-ID: 4mc9) was obtained from the protein data bank [41]. The pose with the higher docking negative score implied better binding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113450.

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