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Preliminary SAR and biological evaluation of potent HIV-1 protease inhibitors with pyrimidine bases as novel P2 ligands to enhance activity against DRV-resistant HIV-1 variants

Mei Zhu [a], Ling Ma [a], Huiyu Zhou [a], Biao Dong [a], Yujia Wang [a], Zhen Wang [b], Jinming Zhou [a], Guoning Zhang [a], Juxian Wang [a], Chen Liang [b], Shan Cen [a, *], and Yucheng Wang [a, **]

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ABSTRACT: Introducing pyrimidine bases, the basic components of nucleic acid, to P2 ligands might enhance the potency of Human Immunodeficiency Virus-1 (HIV-1) protease inhibitors because of the carbonyl and amino groups promoting the formation of extensive hydrogen bonding interactions. In this work, we provide evidence that inhibitor **10e**, with *N*-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl) acetamide as the P2 ligand and a 4-methoxylphenylsulfonamide as the P2' ligand, displayed remarkable enzyme inhibitory and antiviral activity, with the IC_{50} 2.53 nM *in vitro* and a promising inhibition ratio with 68% against wild-type HIV-1 *in vivo*, with low cytotoxicity. This inhibitor also exhibited appreciable antiviral activity against DRV-resistant HIV-1 variants, which was of great value for further study.

Keywords: HIV-1 protease inhibitors, pyrimidine bases, antiviral activity, DRV-resistant HIV-1 variants

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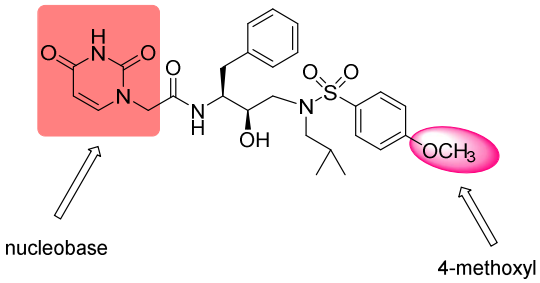
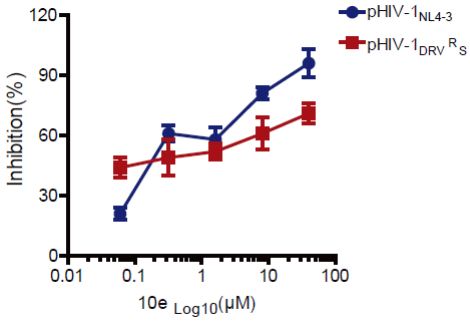


10e
IC_{50} = 2.53 nM (*in vitro*)
inhibiton ratio = 68% (*in vivo*)



1. Introduction

The appearance of HIV-1 protease inhibitors (HIV-1 PIs) in the mid-1990's and their combination with reverse transcriptase inhibitors marked the beginning of highly active antiretroviral therapy (HAART), which had dramatically reduced the mortality and morbidity rates of HIV/AIDS [1-4]. However, there still exist severe problems, such as the emergence of extensively cross-resistant strains of HIV-1, as well as adverse effects [5-7]. In addition, protease inhibitors regimens suffer from a number of other drawbacks including high pill burden, poor ADMET properties, and so on [8]. Even worse, the most egregious issue is the growing emergence of drug-resistant strains of HIV which has rendered the long-term therapy options. Moreover, the newest PI, Darunavir (DRV), with the highest bioavailability, has emerged highly DRV-resistant HIV-1 variants. Up to now, more than ten HIV-1 protease mutations associated with DRV resistance have been identified in highly treatment-experienced patients; notably, the virologic efficacy of DRV was compromised in the presence of three or more of these mutations [9, 10].

To address the growing problem of PI resistance, great effort has been made to discover new compounds through modification of proved PIs including DRV. Some

of these PIs displayed remarkable enzyme inhibitory in the low nanomolar range (32 − 0.027 nM) and exhibited excellent antiviral activity against a panel of multidrug-resistant HIV-1 variants in recent studies [11-18]. For instance, the representative PIs that mainly contains a new P2 ligands in DRV (Figure 1) exhibits potent activity against several HIV-1 isolates resistant to ATV, LPV or APV. However, none of them was found to effectively inhibit DRV-resistant HIV-1 variants [19, 20]. In particular, these PIs failed to block the replication of HIV-$1_{DRV}{}^{R}{}_{P51}$ that is highly resistant to most PIs found thus far. Hence, there is an urgent need for novel PIs with high genetic barrier against multidrug-resistant HIV-1 variants, especially against DRV-resistant HIV-1 variants.

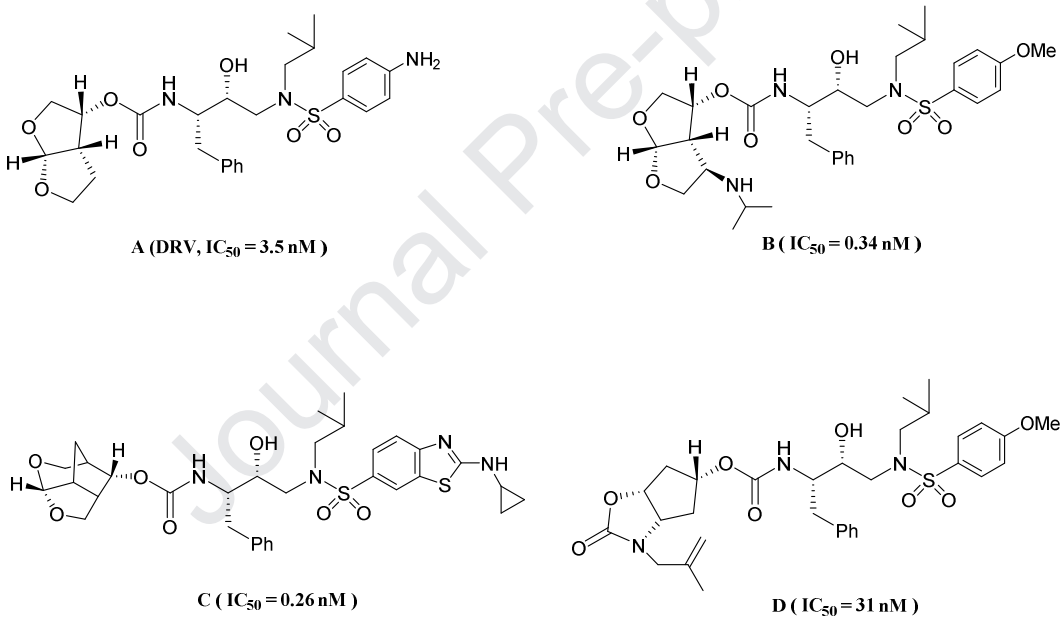


Figure 1. Antiviral activity of protease inhibitors against highly DRV-resistant HIV-1 variants.

In an effort to explore potent inhibitors active on resistant strains especially on DRV-resistant HIV-1 variants and optimize ligand-binding site interactions in the active site of HIV-1 protease, we introduced pyrimidine bases with flexible heterocyclic moieties, as well as carbonyl and/or amino groups as the P2 ligands instead of the bis-THF structural template in the lead compound DRV (see in Figure 2), aiming at increasing enzyme inhibitory *in vivo* on the one hand, as nucleobases represent an important kind of kinetophore which might affect the absorption and improve the bioavailability when they were introduced into the new designed

compounds [21, 22]. On the other hand, according to the strategy to overcome drug resistance through increasing interactions between inhibitors and PR, carbonyl and/or amino groups involved in newly introduced P2 moieties can promote extensive hydrogen bonding interactions involved directly or water-mediated with the backbone amino groups of residues Asp29 and Asp30 of PR in the corresponding S2 subsite [13, 23-27]. Herein, we report our studies on pyrimidine bases-derived protease inhibitors in design, synthesis, and biological evaluation. A number of inhibitors displayed potent enzyme inhibitor activity.

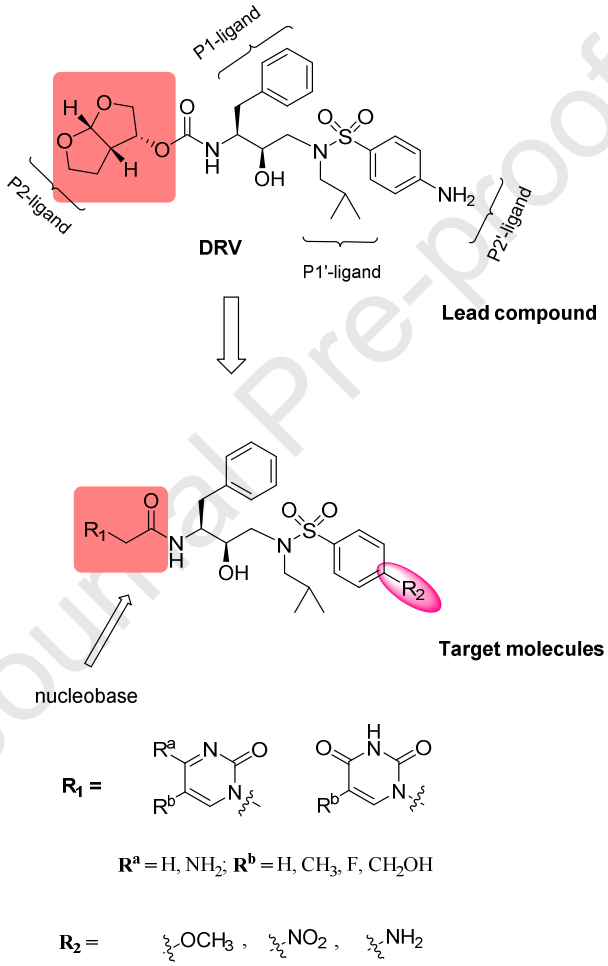


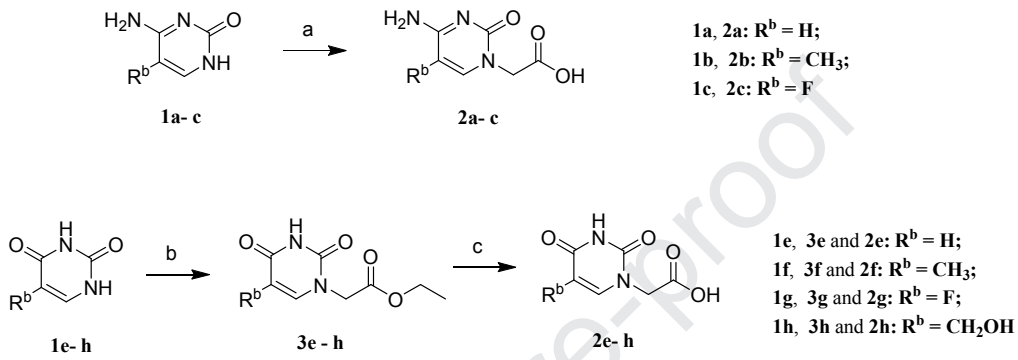
Figure 2. Design and general structure of target molecules.

2. Results and discussion

2.1.Chemistry

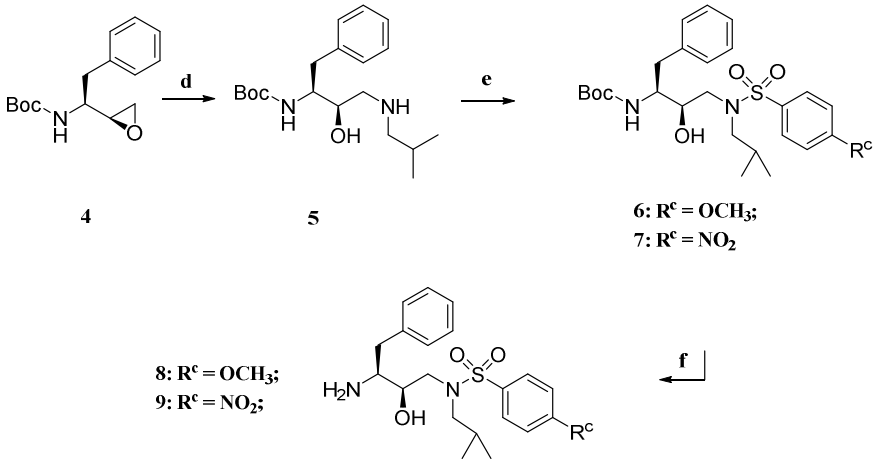
The syntheses of intermediates of substituted 4-amino-2-oxopyrimidin-1(2*H*)-yl) acetic acids **2a−c** and 2, 4-dioxopyrimidin-1(2*H*)-yl acetic acids **2e−h** are outlined in

Scheme 1. Acids **2a−c** were synthesized directly by saponification undergoing a two-step one-pot reaction after the *N*-alkylation of **1a−c** with ethyl bromoacetate in 43-65% yields [28]. *N*-Alkylation of **1e-h** with ethyl bromoacetate proceeded using potassium carbonate as the base in anhydrous DMF to give corresponding esters **3e−h** in 30-60% yields. Subsequent saponification was finished by sodium hydroxide to give acetic acids **2e−h** in yields of 61-75%.



Scheme 1. Syntheses of Substituted 2-(4-amino-2-oxopyrimidin-1(2*H*)-yl)acetic Acids and Substituted 2-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetic Acids **2a-h**. Reagents and conditions: (a) (i) Ethyl bromoacetate, K_2CO_3, anhydrous DMF, Argon, r.t, overnight; (ii) NaOH, H_2O, r.t, 1 h; (iii) 4 M HCl, 0 °C, 0.5 h; (b) Ethyl bromoacetate, K_2CO_3, anhydrous DMF, Argon, r.t, overnight; (c) (i) NaOH, H_2O, r.t, 1 h; (ii) 4 M HCl, 0 °C, 0.5 h.

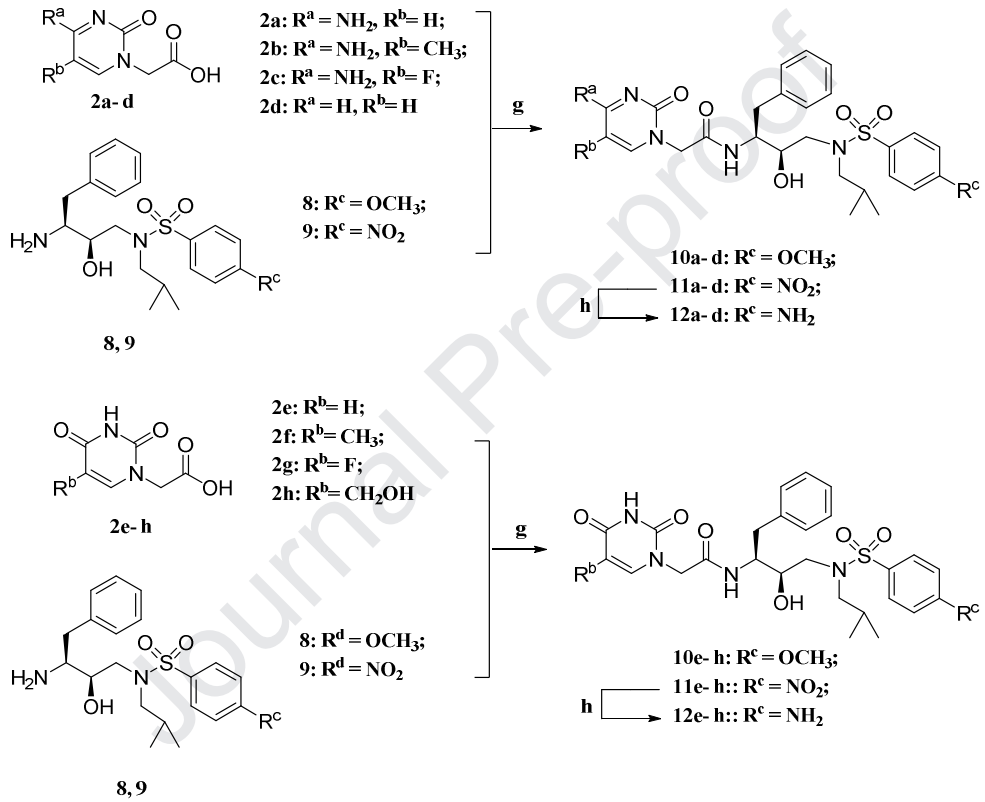
Compounds **8−9** were prepared from the commercially available material (2*S*, 3*S*)-1,2-epoxy-3-(boc-amino)-4-phenylbutane (**4**), as reported in the literature and shown in **Scheme 2** [29, 30].



Scheme 2. Syntheses of Amines **8-9**. Reagents and conditions: (d) *i*-BuNH_2, CH_3CN, 80 °C, 6 h;

(e) Aryl sulfonyl chloride, DIEA, DMAP(Cat.), THF, 0 °C ~ r.t, 3-5 h; (f) CH_2Cl_2-CF_3COOH (1:1), 0 °C~r.t, 3 h.

The syntheses of inhibitors **10−12** shown in **Scheme 3** were carried out by coupling acids **2a−h** with amines **8−9** under an EDCI/HOBt/DMAP-mediated coupling method. The inhibitors **12a−h** were obtained from **11a−h** by refluxing with ammonium formate and 10% Pd/C [31]. The inhibitor structures are shown in **Scheme 3**.



Scheme 3. Syntheses of Inhibitors **10-12**. Reagents and conditions: (g) EDCI, HOBt, DMAP, anhydrous DMF, Argon, 0 °C~r.t, 3 h; (h) $HCOONH_4$, 10% Pd/C, CH_3OH, reflux, 1 h.

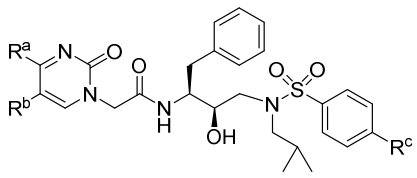
2.2. Structure activity relationships

The inhibitory activity of the synthetic compounds against HIV-1 wild-type protease was evaluated *in vitro* using a fluorescence resonance energy transfer (FRET) method [32, 33]. The enzyme inhibitory of these compounds was compared to clinically available PI, DRV. Pyrimidine bases as the P2 ligands were investigated in combination with other phenylsulfonamide substituents as the P2' ligands in the protease S2' subsite. The inhibitors with pyrimidine base amine-acetamide P2 ligands

showed impressive activities of nanomolar inhibitory potency. In particular, compounds **10e**, **10f**, **10g**, **10h** and **10a** displayed the most potent inhibitory activities (IC_{50} = 1.95−9.07 nM).

These inhibitors exhibited nanomolar inhibitory potency as shown in **Tables 1** and **2**. Moreover, the activity of 2,4-dioxopyrimidin-1(2*H*)-yl derivatives with more oxygen atoms as shown in **Table 2** is usually superior to that of the 4-amino-2-oxopyrimidin-1 (2*H*)-yl) derivatives in **Table 1**. This consideration suggests that the uracil moiety of 2,4-dioxopyrimidin-1(2*H*)-yl derivatives as P2 ligands can make more additional interactions with the backbone atoms and residues through oxygen atoms (supporting by the docking in Figure 4), which is similar to the cyclic ether (*S*)-THF or *bis*-THF scaffolds as P2 ligands reported by Ghosh *et al* [14]. Similarly, phenylsulfonamide derivatives with 4-methoxy (**10a-h**) and 4-amino (**12a-h**) groups displayed generally higher potency than the corresponding substituted compounds with 4-nitro (**11a-h**) groups. The P2' oxygen atom of methoxyl could form hydrogen bonds (O⋯H-N) with the main-chain amide of Asp30' in the protease S2' subsite, similar to inhibitors GRL-0489A [21], TMC-126 [21], GRL-0467 [6] and GRL-0519A [25]. Moreover, the strong electron-withdrawing group 4-nitro would reduce the electron density of not only the oxygen atom itself via inductive effects but also the oxygen atom on the sulfonyl group via conjugative effects, reducing the ability of hydrogen to bond with the amide of Asp30' (phenyl-O_{inh}⋯N-H) [13, 14, 23] or water-mediated interactions with the amide of Ile50' (SO_{2inh}⋯H_2O⋯N-H) in the generally conserved protease S2' subsite.

Table 1. Enzymatic Inhibitory Activities and Cytotoxicity of Inhibitors with Pyrimidine Base Amine-acetamide P2 Ligands (1)

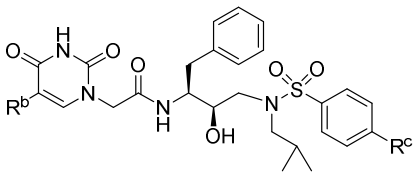


Compd.	R^a	R^b	R^c	IC_{50}(nM) [a]	CC_{50}(μM) [a]

10a	NH_2	H	OMe	9.04 ± 2.72	＞100
11a	NH_2	H	NO_2	411.3 ± 65.9	＞100
12a	NH_2	H	NH_2	153.2 ± 14.6	＞100
10b	NH_2	Me	OMe	147 ± 57.2	＞100
11b	NH_2	Me	NO_2	1191 ± 472.9	＞100
12b	NH_2	Me	NH_2	375.4 ± 79.1	＞100
10c	NH_2	F	OMe	36.03 ± 10.37	＞100
11c	NH_2	F	NO_2	482.1 ± 167.4	＞100
12c	NH_2	F	NH_2	104.8 ± 17.4	＞100
10d	H	H	OMe	10.35 ± 2.45	＞100
11d	H	H	NO_2	245 ± 96.4	＞100
12d	H	H	NH_2	126.1 ± 37.2	＞100
DRV*	-	-	-	1.12 ± 0.59	＞100

[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.

Table 2. Enzymatic Inhibitory Activities and Cytotoxicity of Inhibitors with Pyrimidine Base Amine-acetamide P2 Ligands (2)



Compd.	R^b	R^c	IC_{50}(nM) [a]	CC_{50}(μM) [a]
10e	H	OMe	2.53 ± 0.42	＞100
11e	H	NO_2	20.06 ± 7.50	＞100

12e	H	NH_2	20.07 ± 2.09	＞100
10f	Me	OMe	2.78 ± 0.63	＞100
11f	Me	NO_2	44.31 ± 14.71	＞100
12f	Me	NH_2	19.65 ± 4.03	＞100
10g	F	OMe	1.95 ± 0.32	＞100
11g	F	NO_2	27.64 ± 9.44	32.14
12g	F	NH_2	16.28 ± 2.80	8.80
10h	CH_2OH	OMe	9.07 ± 1.87	＞100
11h	CH_2OH	NO_2	405.3 ± 28.2	＞100
12h	CH_2OH	NH_2	240.6 ± 36.7	＞100
DRV	-	-	1.12 ± 0.59	＞100

[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.

2.3.HIV-1 infectivity assay

On the basis of these results above, selected inhibitors were further evaluated using a single-round infection assay with HIV-1 pseudotyped with vesicular stomatitis virus G protein (VSVg). In the assay, virus-producing cells were treated with the compounds, and the infectivity of the resultant virus was determined [34]. Notably, the most active compounds **10e** and **10g** were equipotent as the reference compound DRV. It is noteworthy that although equipotent in biochemical assays *in vitro*, inhibitors of uracil analogues are more active in cell-based assays than that of cytosine analogues (10h *vs* 10a), as shown in **Table 3** and **Figure 3**.

Table 3. Enzymatic Inhibitory Activities and Inhibition of Inhibitors with Pyrimidine Base Amine-acetamide P2 Ligands

Compd.	IC_{50}(nM) [a]	Inhibition [b]	Compd.	IC_{50}(nM) [a]	Inhibition [b]

		(%) (10 μM)			(%) (10 μM)
10e	2.53±0.42	80.99	11g	27.64 ± 9.44	32.76
11e	20.06±7.50	51.03	12g	16.28 ± 2.80	62.98
12e	20.07±2.09	48.43	10a	9.04 ± 2.72	33.22
10d	10.35±2.45	50.27	10h	9.07 ± 1.87	59.77
10f	2.78±0.63	72.69	DRV	1.12 ± 0.59	83.21
12f	19.65±4.03	53.99	DMSO	-	0
10g	1.95±0.32	83.23			

[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 assays were conducted in quadruplicate.

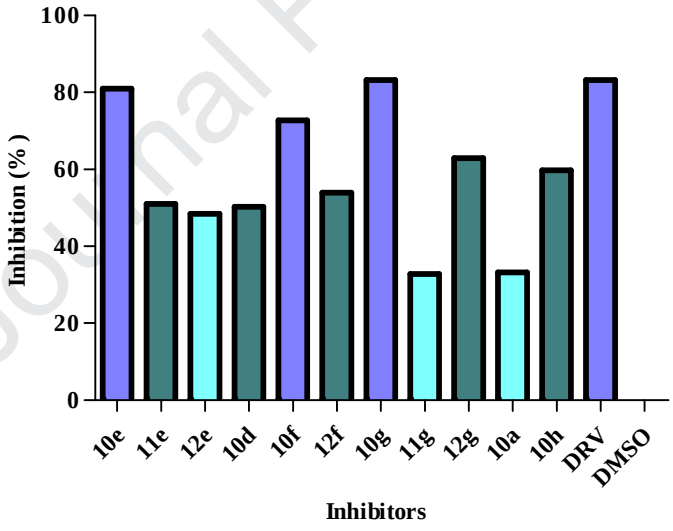


Figure 3. Inhibition of Inhibitors with Pyrimidine Base Amine-acetamide P2 Ligands.

In order to better evaluate the newly identified uracil isosteres, an *in vivo* infection assay with wild-type HIV-1 was performed. Notably, inhibitors **10e** and **10g** exhibited a promising inhibition ratio against wild-type HIV-1 at a concentration of 100 nM *in vivo*, with values of 68% and 51%, respectively, as shown in **Figure 4** [35]. It is noteworthy that compound **10e** exhibited active antiviral inhibition if compared with

the reference compound Nevirapine (NVP) with 77% inhibition (the two compounds were tested with other kinds of compounds together, so Nevirapine was chosen as the positive control drug), which might be a potent HIV-1 inhibitor worthy for in-depth study.

A

Compd.	Inhibition(%) (100 nM)
10e	68
10g	51
NVP[a]	76
DMSO	0

B

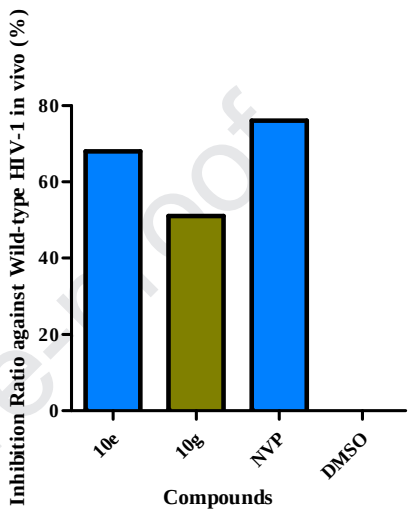


Figure 4. Inhibition Ratio against Wild-type HIV-1 of Compounds *in vivo*.[a] The two compounds were tested with other kinds of compounds together, so Nevirapine (NVP) was chosen as the positive control drug.

2.4 Antiviral activity against DRV-resistant HIV-1 variants

We next investigated if these compounds are able to overcome drug resistance through increasing interactions between inhibitors and PR. Four amino acid substitutions (V32I, L33F, I54M, and I84V) in HIV-1 PR were reported previously to confer high-level resistance to DRV [10], and were introduced into pNL4-3-E$^-$R$^-$ (pHIV-1_{NL4-3}), resulting in DRV-resistant HIV-1 proviral DNA pHIV-$1_{DRV}{}^{R}{}_{S.}$ The compounds **10e**, **10f** and **10g** were tested for antiviral activity against DRV-sensitive or resistant pseudotyped HIV-1 using a single-round infection assay. As shown in **Table 4** and **Figure 5**, the compounds **10e**, **10f** and **10g** exhibited similar potency against DRV-sensitive or resistant HIV-1 in a dose-response assay, and DRV-resistant mutations only cause 1-2 fold increase in EC_{50}. However, more than 16 fold increase

in EC_{50} was observed for mutated virus treated with DRV compared with that of wild type virus. This suggests the ability of these compounds to overcome DRV resistance and a broad prospect for further study.

Table 4. Antiviral Activity of 10e, 10f and 10g against Multidrug Resistant HIV-1 Variants

	mean EC_{50} μM ± SD [a]		fold resistance[b]
Compd.	HIV-1_{NL4-3}	$HIV_{DRV}{}^{R}S$	
10e	0.27±0.09	0.59 ± 0.11	2.19
10f	2.50±0.67	2.87 ± 0.42	1.15
10g	1.07±0.21	2.70 ± 0.36	2.52
DRV	0.006±0.004	0.10 ± 0.010	16.67

[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 $EC_{50(mutant)}/EC_{50(WT)}$.

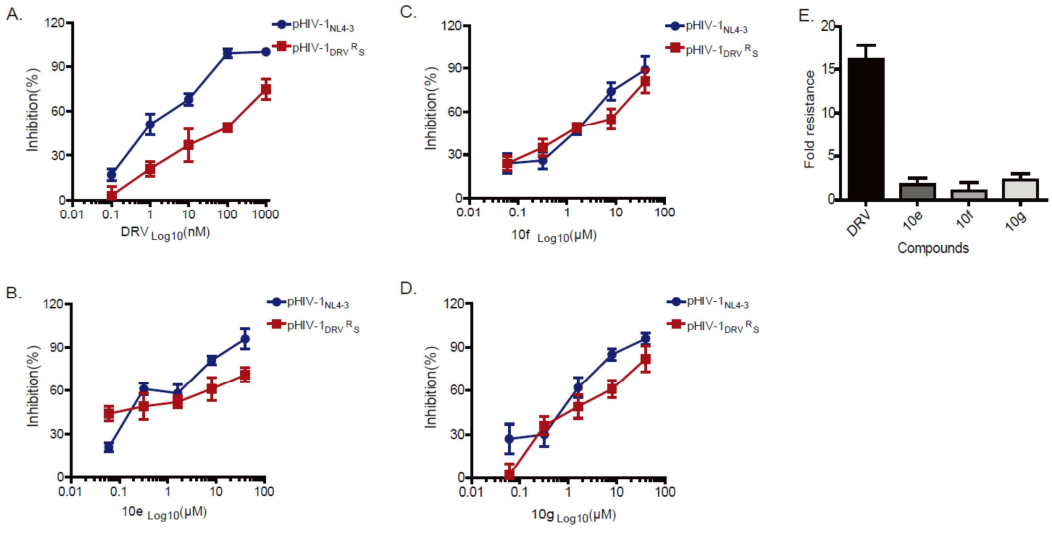


Figure 5. Antiviral Activity of **10e**, **10f** and **10g** against Multidrug Resistant HIV-1 Variants. (A-D) Dose-response of compounds DRV, 10e, 10f and 10g against wild type HIV-1 and DRV-resistant mutant. (E) Fold resistance is defined by $EC_{50(mutant)}/EC_{50(WT)}$.

2.5. Molecular docking

The common mode of inhibitory binding was explored through molecular docking

using a HIV PR crystal structure (PDB-ID: 4mc9) [36]. Remarkably, the inhibitor **10e** fits perfectly into the PR binding site and several hydrogen-bonding interactions are possible between the residues Asp_{29}, Gly_{48}, Ile_{A50} and Ile_{B50}, as well as van der Waals interactions with the outer enzyme atoms, both of which might account for the promising HIV-1 PR inhibitory activity (**Figure 6**). Also as can be seen, the uracil moiety of 2,4-dioxopyrimidin-1(2*H*)-yl derivative in compound **10e** could produce interactions with the PR active site via the two oxygen atoms, and this could account for why inhibitors of uracil analogues exhibited higher potency than that of cytosine analogues, which contain only one oxygen atom.

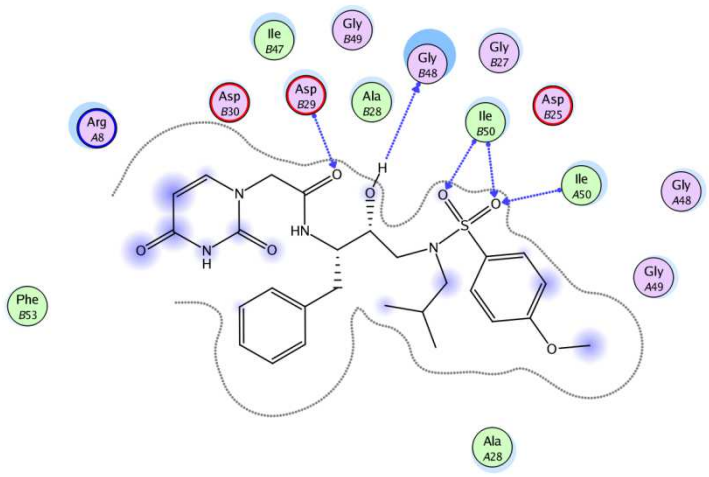


Figure 6. Docking of inhibitor **10e** in HIV-1 PR. Ligand exposures are represented as purple spheres. Amino acid side chains important for the ligand binding are depicted as blue arrows.

2.6.Correlation for 2,4-dioxo-3,4-dihydropyrimidine analogues

Further validation was achieved by correlation of the SAR of docked inhibitors **10e**, **12e**, **11f**, **12f**, **16q**, **11g**, **12g** and **10h**, as shown in **Figure 7**. The correlation observed between these two sets of IC_{50} data (expt *vs* calcd, correlation coefficiency = 0.78) supports our docking model with a common mode of binding as a valid platform for PR inhibitor design.

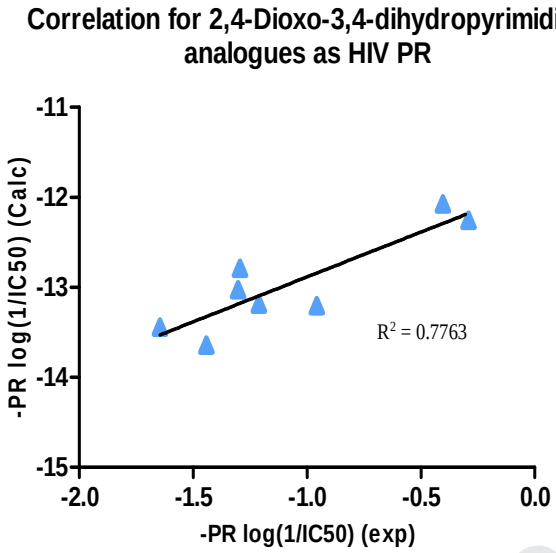


Figure 7. Strong correlation of docked 2,4-Dioxo-3,4-dihydropyrimidine analogues supports a common mode of binding for HIV PR

3. Conclusion

We have designed, synthesized, and evaluated a series of inhibitors containing the pyrimidine base moiety as the P2 ligand. Inhibitor **10e** incorporating *N*-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamide as the P2 ligand and 4-methoxyphenylsulfonamide isostere as the P2' ligand displayed remarkable enzyme inhibitory and antiviral activity: **10e** exhibited the closest enzyme inhibitory potency (IC_{50} = 2.53 nM) and inhibition of infectivity in single round infection assay comparable to DRV, as well as a promising inhibition ratio against wild-type HIV-1 *in vivo* (68% inhibition) and an appreciable antiviral activity against DRV-resistant HIV-1 variants. SAR studies indicated that carbonyl and amino groups, as well as flexible heterocyclic moieties in the introduced P2 ligand, were critical to the ligand's high enzyme affinity. Both P2 and P2' ligands were involved in hydrogen bonding interactions with the backbone of both S2 and S2' subsites.

Furthermore, nucleobases represent an important kind of kinetophore, which might be in favor of enhancing cell membrance permeability and improving the bioavailability when they were introduced into the new designed compounds, deserving further study.

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, $(CD_3)_2CO$ 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; $(CD_3)_2CO$: ^{1}H, δ = 2.05 ppm, ^{13}C, δ = 30, 205 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. 2-(4-Amino-2-oxopyrimidin-1(2H)-yl)acetic acid (**2a**)*

To a dry flask was added cytosine (**1a**, 0.22 g, 2.0 mmol), K_2CO_3 (0.55 g, 4.0 mmol) and anhydrous DMF (3 mL). The mixture was stirred vigorously under an argon atmosphere at room temperature for 1 hour, and then ethyl bromoacetate (0.27 mL, 2.4 mmol) was added dropwise via syringe and stirred overnight. Sodium hydroxide (0.24 g, 6.0 mmol) dissolved in 5 mL water was added dropwise to the reaction mixture and stirred for 1 hour at room temperature, which was then cooled to 0 °C to acidified to pH 4.0 with 4 M HCl (aqueous) and stirred additional 0.5 hour. The precipitate separated out was isolated by filtration and dried, *in vacuo*, over P_2O_5

to give **2a** as brown powder: yield 0.22 g (65%); mp319-321 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M + H]^+$ m/z 170.4.

*4.1.2. 2-(4-Amino-5-methyl-2-oxopyrimidin-1(2H)-yl)acetic acid (**2b**)*

The title compound was obtained from 5-methyl cytosine (**1b)** in 43% yield as pink powder as described for **2a**: mp315-318 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M - H]^-$ m/z 182.4.

*4.1.3. 2-(4-Amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)acetic acid (**2c**)*

The title compound was obtained from 5-fluorocytosine (**1c)** in 63% yield as white powder as described for **2a**: mp303-305 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M - H]^-$ m/z 186.4.

*4.1.4. Ethyl 2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**3e**)*

To a dry flask was added uracil (**1e**, 0.56 g, 5.0 mmol), K_2CO_3 (1.38 g, 10.0 mmol) and anhydrous DMF (6 mL). The mixture was stirred vigorously under an argon atmosphere at room temperature for 1 hour, and then ethyl bromoacetate (0.67 mL, 6.0 mmol) was added dropwise via syringe and stirred overnight. Water (10 mL) was added to the residue and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, and removed under reduced pressure. Silica gel column chromatography (hexanes/ethyl acetate, 2: 1, v: v) afforded the product as white acicular crystal: yield 0.38 g (38%); mp118-120 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.56 (d, J = 8.0 Hz, 1H), 5.71 (d, J = 8.0 Hz, 1H), 4.55 (s, 2H), 4.26 (q, J = 7.0 Hz, 2H), 1.31 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CH_3OD) δ 168.1, 165.3, 151.4, 146.0, 101.1, 61.6, 48.7, 13.0; LC-MS (ESI) $[M + H]^+$ m/z 199.3.

*4.1.5. Ethyl 2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**3f**)*

This compound was prepared analogously to **3e** from thymine (**1f)** in 60% yield as white powder: mp 138-140 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.38 (s, 1H), 4.49 (d, J = 8.0 Hz, 2H), 4.22 (q, J = 7.0 Hz, 2H), 1.87 (s, 3H), 1.28 (t, J = 7.0 Hz, 3H); ^{13}C

NMR (151 MHz, CD_3OD) δ 169.8, 167.0, 153.1, 143.3, 111.4, 63.0, 50.0, 14.5, 12.3; LC-MS (ESI) $[M + H]^+$ m/z 213.4.

*4.1.6. Ethyl 2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**3g**)*

This compound was prepared analogously to **3e** from 5-fluorouracil (**1g)** in 40% yield as white acicular crystal: mp 149-151 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.85 (d, J = 6.0 Hz, 1H), 4.53 (s, 2H), 4.28 (q, J = 7.0 Hz, 2H), 1.33 (t, J = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.5, 160.0, 159.8, 151.6, 142.5, 140.9, 131.5, 131.3, 63.1, 50.1, 14.5; LC-MS (ESI) $[M + H]^+$ m/z 217.4.

*4.1.7. Ethyl 2-(5-hydroxymethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**3h**)*

This compound was prepared analogously to **3e** from 5-hydroxy methyl uracil (**1h)** in 30% yield as white powder: mp 156-158 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.59 (s, 1H), 4.59 (s, 2H), 4.38 (s, 2H), 4.28 (q, J = 7.0 Hz, 2H), 1.34 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CH_3OD) δ 168.21 (s), 164.18 (s), 151.43 (s), 142.77 (s), 113.79 (s), 61.55 (s), 56.29 (s), 48.76 (s), 13.00 (s); LC-MS (ESI) $[M - H]^-$ m/z 227.5.

*4.1.8. 2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**2e**)*

Sodium hydroxide (0.12 g, 3.0 mmol) dissolved in 2 mL water was added dropwise to the flask contained ethyl 2-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetate (**3e**, 0.20 g, 1.0 mmol). The reaction mixture was stirred for 1 hour at room temperature and then cooled to 0 °C. The solution was acidified to pH 4.0 with 4 M HCl (aqueous) and stirred for another 0.5 hour at 0 °C. The precipitate was isolated by filtration and dried, *in vacuo*, over P_2O_5 to give **2e** as white powder: yield 0.10 g (61%); mp296-301 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M - H]^-$ m/z 169.4.

*4.1.9. 2-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**2f**)*

The title compound was obtained from **3f** in 64% yield as white powder as

described for **2e**: mp270-272 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M - H]^-$ m/z 183.4.

*4.1.10. 2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**2g**)*

The title compound was obtained from **3g** in 69% yield as white powder as described for **2e**: mp264-266 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M - H]^-$ m/z 187.4.

*4.1.11. 2-(5-(Hydroxymethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**2h**)*

The title compound was obtained from **3h** in 75% yield as white powder as described for **2e**: mp195-198 °C; The powder wasn't dissolved in any deuterated solvents; LC-MS (ESI) $[M - H]^-$ m/z 199.4.

*4.1.12. 2-(4-Amino-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**10a**)*

N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI, 0.29 g, 1.5 mmol) and 1-hydroxybenzotriazole (HOBt, 0.15 g, 1.1 mmol) were sequentially added in batches to a stirring solution of 2-(4-amino-2-oxopyrimidin-1(2*H*)-yl)acetic acid (**2a**, 0.17 g, 1.0 mmol) and *N*-((2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyl)-*N*-isobutyl-4-methoxybenzenesulfonamide (**8**, 0.43 g, 1.05 mmol) in dry DMF (3 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 10 min at 0 °C and then additional 1 hour at room temperature. 4-Dimethylaminopyridine (DMAP, 0.024 g, 0.20 mmol) was added and the reaction mixture was stirred for another 2 hours at room temperature. The solvent was removed under reduced pressure. Water (6 mL) was added to the residue and extracted with ethyl acetate (3 × 6 mL). The combined organic layers were dried over Na_2SO_4, and evaporated, i*n vacuo*. The residue was purified by chromatography on a silica gel column (30 × 6 cm). Elution with 1:3 to 1:4 hexanes-ethyl acetate gave **10a** as colorless oil: yield 0.17 g (30%);

mp185-187 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (d, J = 8.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 4H), 7.30 (d, J = 4.5 Hz, 1H), 7.26-7.20 (m, 1H), 7.12 (d, J = 8.5 Hz, 2H), 5.84 (d, J = 6.0 Hz, 1H), 4.49 ((d, J = 16.0 Hz, 1H), 4.21 (d, J = 16.0 Hz, 1H), 4.16-4.11 (m, 1H), 3.91 (s, 3H), 3.49 (d, J = 16.0 Hz, 1H), 3.39 (d, J = 16.0 Hz, 1H), 3.20-3.16 (m, 1H), 3.08-3.02 (m, 2H), 2.92-2.88 (m, 1H), 2.77-2.72 (m, 1H), 2.06-2.01(m, 1H), 0.95 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.6, 168.2, 164.5, 158.9, 147.8, 140.0, 132.1, 130.7, 130.5, 129.4, 127.3, 115.4, 95.7, 73.5, 58.9, 56.2, 55.8, 54.0, 52.4, 36.3, 28.1, 20.6, 20.5; HRMS (ESI) m/z calcd. for $C_{27}H_{35}N_5O_6S$ ([M - H]$^-$): 556.2224, found 556.2211.

4.1.13.

*2-(4-Amino-5-methyl-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**10b**)*

The title compound was obtained by **2b** which was coupled with **8** through EDCI/HOBt/DMAP coupling procedure in 27% yield (white powder) as described for **10a**: mp168-170 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.02 (s, 2H), 7.72 (d, J = 9.0 Hz, 2H), 7.23 – 7.19 (m, 4H), 7.15 – 7.13 (m, 1H), 7.05 (d, J = 9.0 Hz, 2H), 7.03 (d, J = 1.2 Hz, 1H), 4.35 (d, J = 15.6 Hz, 1H), 4.14 (d, J = 15.6 Hz, 1H), 4.05 – 4.01 (m, 1H), 3.84 (s, 3H), 3.82 – 3.79 (m, 1H), 3.42 (dd, J = 15.0, 3.6 Hz, 1H), 3.10 (dd, J = 14.0, 4.0 Hz, 1H), 3.00 (dd, J = 13.8, 8.4 Hz, 1H), 2.91 (dd, J = 15.0, 8.4 Hz, 1H), 2.80 (dd, J = 13.8, 7.2 Hz, 1H), 2.64 (dd, J = 13.8, 10.8 Hz, 1H), 2.00 – 1.93 (m, 1H), 1.85 (d, J = 1.2 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 168.2, 164.6, 163.1, 157.5, 143.8, 138.5, 130.6, 129.2, 129.0, 127.9, 125.8, 114.0, 102.5, 72.1, 57.5, 54.8, 54.3, 52.5, 50.8, 34.8, 26.6, 19.1, 19.0, 11.6; HRMS (ESI) m/z calcd. for $C_{28}H_{37}N_5O_6S$ ([M - H]$^-$): 570.2381, found 570.2345.

4.1.14.

*2-(4-Amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**10c**)*

The title compound was obtained by **2c** which was coupled with **8** through

EDCI/HOBt/DMAP coupling procedure in 65% yield (white powder) as described for **10a**: mp139-141 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.72 (d, J = 9.0 Hz, 2H), 7.38 (d, J = 6.0 Hz, 1H), 7.22 – 7.20 (m, 4H), 7.15 – 7.12 (m, 1H), 7.04 (d, J = 9.0 Hz, 2H), 4.36 (d, J = 15.6 Hz, 1H), 4.14 (d, J = 15.6 Hz, 1H), 4.06 – 4.03 (m, 1H), 3.83 (s, 3H), 3.82 – 3.80 (m, 1H), 3.40 (dd, J = 15.0, 3.6 Hz, 1H), 3.10 (dd, J = 14.0, 4.2 Hz, 1H), 2.99 (dd, J = 13.8, 8.4 Hz, 1H), 2.93 (dd, J = 15.0, 8.4 Hz, 1H), 2.81 (dd, J = 13.8, 7.2 Hz, 1H), 2.65 (dd, J = 14.0, 10.8 Hz, 1H), 1.98 – 1.94 (m, 1H), 0.86 (d, J = 6.6 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 167.9, 164.7, 163.1, 155.9, 138.5, 130.6, 130.4, 129.2, 129.0, 127.9, 125.9, 114.0, 72.1, 57.5, 54.8, 54.4, 52.5, 51.0, 34.8, 26.6, 19.1, 19.0; HRMS (ESI) m/z calcd. for $C_{27}H_{34}FN_5O_6S$ ([M - H]$^-$): 574.2130, found 574.2138.

4.1.15.

*N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)-2-(2-oxopyrimidin-1(2H)-yl)acetamide (**10d**)*

The title compound was obtained by 2-(2-oxopyrimidin-1(2*H*)-yl)acetic acid (**2d**) which was coupled with **8** through EDCI/HOBt/DMAP coupling procedure in 74% yield (white powder) as described for **10a**: mp172-174 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.61 – 8.60 (m, 1H), 7.88 (dd, J = 6.5, 2.5 Hz, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 4.0 Hz, 4H), 7.22 (dd, J = 8.5, 4.0 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 6.51 (dd, J = 6.0, 4.5 Hz, 1H), 4.66 (d, J = 15.5 Hz, 1H), 4.38 (d, J = 15.5 Hz, 1H), 4.11 – 4.07 (m, 1H), 3.91 (s, 4H), 3.49 (dd, J = 15.0, 3.0 Hz, 1H), 3.21 (dd, J = 14.0, 3.5 Hz, 1H), 3.08 – 2.99 (m, 2H), 2.89 (dd, J = 13.5, 7.0 Hz, 1H), 2.73 (dd, J = 14.0, 10.5 Hz, 1H), 2.08 – 2.00 (m, 1H), 0.93 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 168.3, 168.1, 164.6, 158.1, 152.3, 140.0, 132.2, 130.8, 130.5, 129.5, 127.4, 115.5, 106.0, 73.8, 59.1, 56.3, 56.1, 54.2, 54.1, 36.5, 28.2, 20.6, 20.5; HRMS (ESI) m/z calcd. for $C_{27}H_{34}N_4O_6S$ ([M - H]$^-$): 541.2115, found 541.2128.

4.1.16.

*2-(4-Amino-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**11a**)*

The title compound was obtained by **2a** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 24% yield (colorless oil) as described for **10a**: mp172-174 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.40 (d, J = 8.8 Hz, 2H), 8.08 (d, J = 8.8 Hz, 2H), 7.28-7.22 (m, 5H), 7.19-7.16 (m, 1H), 5.80 (d, J = 7.2 Hz, 1H), 4.42 (d, J = 15.6 Hz, 1H), 4.12 (d, J = 15.6 Hz, 1H), 4.00 (ddd, J = 10.4, 6.4, 4.0 Hz, 1H), 3.83 – 3.79 (m, 1H), 3.51 (dd, J = 15.0, 2.8 Hz, 1H), 3.20- 3.10 (m, 3H), 3.02-2.96 (m, 1H), 2.68 (dd, J = 13.8, 10.4 Hz, 1H), 2.07-1.99 (m, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.6, 168.0, 158.6, 151.4, 148.1, 146.9, 139.8, 130.4, 129.9, 129.4, 127.3, 125.4, 95.7, 73.0, 58.0, 55.9, 53.2, 52.6, 36.4, 27.8, 20.4; HRMS (ESI) m/z calcd. for $C_{26}H_{32}N_6O_7S$ ([M - H]$^-$): 571.1969, found 571.1946.

4.1.17.

*2-(4-Amino-5-methyl-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobuty-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**11b**)*

The title compound was obtained by **2b** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 58% yield (colorless oil) as described for **10a**: mp212-214 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H), 7.24-7.17 (m, 4H), 7.15-7.12 (m, 1H), 7.07 (s, 1H), 4.36 (d, J = 15.8 Hz, 1H), 4.09 (d, J = 15.8 Hz, 1H), 3.99-3.94 (m, 1H), 3.79-3.74 (m, 1H), 3.47 (dd, J = 15.0, 2.9 Hz, 1H), 3.16-3.05 (m, 3H), 2.98-2.93 (m, 1H), 2.63 (dd, J = 13.8, 10.4 Hz, 1H), 1.99 (dt, J = 14.0, 6.8 Hz, 1H), 1.86 (s, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.7, 167.7, 158.8, 151.4, 146.9, 145.4, 139.8, 130.4, 129.9, 129.4, 127.3, 125.4, 103.9, 73.0, 58.0, 55.9, 53.2, 52.5, 36.4, 27.8, 20.4, 13.1; HRMS (ESI) m/z calcd. for $C_{27}H_{34}N_6O_7S$ ([M - H]$^-$): 585.2126, found 585.2116.

4.1.18.

2-(4-Amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)acetamide ***(11c)***

The title compound was obtained by **2c** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 60% yield (white powder) as described for **10a**: mp230-232 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 6.0 Hz, 1H), 7.24-7.18 (m, 4H), 7.16-7.12 (m, 1H), 4.36 (d, J = 15.8 Hz, 1H), 4.09 (d, J = 15.8 Hz, 1H), 3.97 (ddd, J = 10.4, 6.4, 4.0 Hz, 1H), 3.80 – 3.75 (m, 1H), 3.47 (dd, J = 15.0, 2.8 Hz, 1H), 3.16-3.06 (m, 3H), 2.95 (dd, J = 13.6, 7.2 Hz, 1H), 2.64 (dd, J = 13.6, 10.4 Hz, 1H), 2.04-1.94 (m, 1H), 0.88 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.4, 160.0, 157.2, 151.4, 146.8, 139.8, 132.2, 131.9, 130.4, 129.9, 129.4, 127.3, 125.4, 73.0, 58.0, 55.9, 53.2, 52.6, 36.4, 27.8, 20.4; HRMS (ESI) m/z calcd. for $C_{26}H_{31}FN_6O_7S$ ([M - H]$^-$): 589.1875, found 589.1886.

4.1.19.

N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)-2-(2-oxopyrimidin-1(2H)-yl)acetamide ***(11d)***

The title compound was obtained by **2d** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 46% yield (white powder) as described for **10a**: mp168-170 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.56 (dd, J = 4.2, 2.8 Hz, 1H), 8.40 (d, J = 9.0 Hz, 2H), 8.13 (d, J = 9.0 Hz, 2H), 7.96 (dd, J = 6.6, 3.0 Hz, 1H), 7.27-7.21 (m, 4H), 7.17 (dt, J = 8.4, 1.2 Hz, 1H), 6.41 (dd, J = 6.6, 4.2 Hz, 1H), 4.53 (d, J = 15.0 Hz, 1H), 4.23 (d, J = 15.0 Hz, 1H), 3.75 (td, J = 10.8, 3.6 Hz, 1H), 3.62 (ddd, J = 17.0, 7.2, 3.0 Hz, 1H), 3.44 (dd, J = 14.8, 2.4 Hz, 1H), 3.10-3.05 (m, 2H), 2.95 (dd, J = 15.0, 9.6 Hz, 1H), 2.87 (dd, J = 13.8, 6.0 Hz, 1H), 2.55 (dd, J = 13.8, 10.8 Hz, 1H), 2.00-1.95 (m, 1H), 0.83 (d, J = 6.6 Hz, 3H), 0.76 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.5, 166.1, 155.6, 150.9, 149.5, 145.1, 139.1, 129.1, 128.7, 1281, 125.9, 124.5, 103.4, 71.5, 56.2, 54.3, 52.5, 52.0, 35.4, 25.8, 19.9, 19.8; HRMS (ESI) m/z calcd. for $C_{26}H_{31}N_5O_7S$ ([M - H]$^-$): 556.1860, found 556.1858.

*4.1.20. 2-(4-Amino-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl)acetamide (**12a**)*

To a flask was added **11a** (0.050 g, 0.087 mmol) 10% d/C (humidity 37%) (0.050 g, w/w 0.37:1), ammonium formate (0.034 g, 0.52 mmol) and methanol (2 mL). The reaction mixture was refluxed for 1 hour, and then filtered using a pad of Celite 545® followed by washing with a little methanol. The solvent was removed under reduced pressure. The residue was purified by silica gel preparation thin layer chromatography with the developing solvent ethyl acetate/methanol 6: 1. **12a** was obtained as yellow powder: yield 0.038 g (81%); mp206-208 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.49 (d, J = 8.8 Hz, 2H), 7.26-7.25 (m, 4H), 7.20 (d, J = 7.2 Hz, 1H), 7.18 (dd, J = 8.8, 4.8 Hz, 1H), 6.71 (d, J = 8.8 Hz, 2H), 5.79 (d, J = 7.2 Hz, 1H), 4.40 (d, J = 15.8 Hz, 1H), 4.19 (d, J = 15.8 Hz, 1H), 4.10 -4.07 (m, 1H), 3.87-3.84 (m, 1H), 3.40 (dd, J = 15.0, 3.6 Hz, 1H), 3.14 (dd, J = 13.8, 3.6 Hz, 1H),3.00-2.90 (m, 2H), 2.80 (dd, J = 13.8, 7.2 Hz, 1H), 2.69 (dd, J = 13.8, 10.8 Hz, 1H), 1.99 (dt, J = 13.8, 6.6 Hz, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.0, 167.6, 158.4, 153.8, 147.3, 139.5, 130.0, 128.8, 126.7, 125.5, 114.0, 95.3, 73.1, 58.7, 55.3, 53.7, 51.9, 35.7, 27.7, 20.1, 20.0; HRMS (ESI) m/z calcd. for $C_{26}H_{34}N_6O_5S$ ([M - H]$^-$): 541.2228, found 541.2210.

*4.1.21. 2-(4-Amino-5-methyl-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl)acetamide (**12b**)*

The title compound was obtained by hydrogenation of **11b** in 75% yield (yellow powder) as described for **12a**: mp145-147 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (d, J = 8.8 Hz, 2H), 7.22- 7.20 (m, 4H), 7.16-7.11 (m, 1H), 7.02 (s, 1H), 6.67 (d, J = 8.8 Hz, 2H), 4.34 (d, J = 15.8 Hz, 1H), 4.17 (d, J = 15.8 Hz, 1H), 4.08-4.03 (m, 1H), 3.83-3.79 (m, 1H), 3.37 (dd, J = 14.8, 3.6 Hz, 1H), 3.10 (dd, J = 13.6, 4.0 Hz, 1H), 2.97-2.84 (m, 2H), 2.75 (dd, J = 13.6, 7.0 Hz, 1H), 2.64 (dd, J = 13.6, 10.4 Hz, 1H), 1.98-1.89 (m, 1H), 1.85 (s, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.6, 167.7, 158.9, 154.3, 145.2, 140.0, 130.5, 129.3, 127.2, 125.9, 114.5, 104.0, 73.6, 59.2, 55.7, 54.2, 52.2, 36.2, 28.2, 20.6, 20.5, 13.1;

HRMS (ESI) m/z calcd. for $C_{27}H_{36}N_6O_5S$ ([M - H]$^-$): 555.2384, found 555.2362.

*4.1.22. 2-(4-Amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl)acetamide (**12c**)*

The title compound was obtained by hydrogenation of **11c** in 48% yield (yellow powder) as described for **12a**: mp179-181 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.52 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 6.0 Hz, 1H), 7.31 – 7.28 (m, 4H), 7.22 – 7.17 (m, 1H), 6.74 (d, J = 8.5 Hz, 2H), 4.40 (d, J = 16.0 Hz, 1H), 4.23 (d, J = 16.0 Hz, 1H), 4.14 – 4.10 (m, 1H), 3.88 (t, J = 8.5 Hz, 1H), 3.43 (dd, J = 15.0, 3.5 Hz, 1H), 3.17 (dd, J = 14.0, 3.5 Hz, 1H), 3.01 (dd, J = 13.5, 8.0 Hz, 1H), 2.94 (dd, J = 15.0, 8.0 Hz, 1H), 2.83 (dd, J = 13.5, 7.0 Hz, 1H), 2.71 (dd, J = 13.5, 10.5 Hz, 1H), 2.06 – 1.98 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.4, 160.1, 160.0, 157.4, 154.4, 140.1, 136.9, 132.1, 131.8, 130.6, 129.4, 127.3, 126.0, 114.6, 73.7 59.3, 55.9, 54.3, 52.4, 36.3, 28.3, 20.7, 20.6; HRMS (ESI) m/z calcd. for $C_{26}H_{33}FN_6O_5S$ ([M - H]$^-$): 559.1875, found 559.2108.

*4.1.23. N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl) -2-(2-oxopyrimidin-1(2H)-yl)acetamide (**12d**)*

The title compound was obtained by hydrogenation of **11d** in 85% yield (yellow powder) as described for **12a**: mp171-173 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.54 (dd, J = 4.2, 3.0 Hz, 1H), 7.80 (dd, J = 6.6, 3.0 Hz, 1H), 7.46 (d, J = 9.0 Hz, 2H), 7.24 – 7.21 (m, 4H), 7.15 – 7.13 (m, 1H), 6.67 (d, J = 9.0 Hz, 2H), 6.44 (dd, J = 6.6, 4.2 Hz, 1H), 4.57 (d, J = 15.6 Hz, 1H), 4.34 (d, J = 15.6 Hz, 1H), 4.06 – 4.02 (m, 1H), 3.83 – 3.80 (m, 1H), 3.37 (dd, J = 15.0, 3.6 Hz, 1H), 3.13 (dd, J = 13.8, 4.0 Hz, 1H), 2.93 (dd, J = 13.2, 7.2 Hz, 1H), 2.89 (dd, J = 14.4, 7.8 Hz, 1H), 2.77 (dd, J = 13.8, 7.2 Hz, 1H), 2.66 (dd, J = 13.8, 10.2 Hz, 1H), 1.97 – 1.93 (m, 1H), 0.87 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 166.7, 166.5, 156.6, 152.8, 150.8, 138.5, 129.4, 129.0, 127.9, 125.8, 113.1, 104.4, 72.3, 57.9, 54.5, 52.8, 52.5, 34.9, 26.7, 19.2, 19.1; HRMS (ESI) m/z calcd. for $C_{26}H_{33}N_5O_5S$ ([M - H]$^-$):

526.2119, found 526.2151.

4.1.24.

*2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**10e**)*

The title compound was obtained by **2e** which was coupled with **8** through EDCI/HOBt/DMAP coupling procedure in 98% yield (white powder) as described for **10a**: mp125-127 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, J = 8.5 Hz, 2H), 7.31 – 7.27 (m, 4H), 7.22 – 7.21 (m, 2H), 7.12 (d, J = 8.5 Hz, 2H), 5.62 (d, J = 8.0 Hz, 1H), 4.42 (d, J = 16.0 Hz, 1H), 4.19 (d, J = 16.0 Hz, 1H), 4.10 – 4.06 (m, 1H), 3.90 (s, 3H), 3.89 – 3.85 (m, 1H), 3.47 (dd, J = 15.0, 3.0 Hz, 1H), 3.21 (dd, J = 14.0, 3.5 Hz, 1H), 3.06 (dd, J = 13.5, 8.0 Hz, 1H), 2.98 (dd, J = 15.0, 8.5 Hz, 1H), 2.88 (dd, J = 13.5, 7.0 Hz, 1H), 2.70 (dd, J = 13.5, 1.0 Hz, 1H), 2.08 – 2.00 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.1, 166.9, 164.7, 152.8, 147.6, 140.0, 132.2, 130.8, 130.5, 129.5, 127.4, 115.6, 102.3, 74.0, 59.2, 56.3, 56.0, 54.3, 51.0, 36.7, 28.2, 20.7, 20.6; HRMS (ESI) m/z calcd. for $C_{27}H_{34}N_4O_7S$ ([M - H]$^-$): 557.2064, found 557.2060.

4.1.25.

*N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**10f**)*

The title compound was obtained by **2f** which was coupled with **8** through EDCI/HOBt/DMAP coupling procedure in 83% yield (white powder) as described for **10a**: mp188-190 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.72 (d, J = 9.0 Hz, 2H), 7.24 – 7.19 (m, 4H), 7.16 – 7.13 (m, 1H), 7.04 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 1.2 Hz, 1H), 4.32 (d, J = 16.2 Hz, 1H), 4.11 (d, J = 16.2 Hz, 1H), 4.03 – 4.00 (m, 1H), 3.83 (s, 3H), 3.82 – 3.79 (m, 1H), 3.41 (dd, J = 15.0, 3.6 Hz, 1H), 3.14 (dd, J = 14.0, 4.2 Hz, 1H), 3.00 (dd, J = 13.8, 8.4 Hz, 1H), 2.90 (dd, J = 15.0, 8.4 Hz, 1H), 2.80 (dd, J = 13.8, 7.2 Hz, 1H), 2.63 (dd, J = 13.8, 10.8 Hz, 1H), 2.00 – 1.95 (m, 1H), 1.77 (d, J = 1.2 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD)

δ 167.7, 165.5, 163.1, 151.4, 141.8, 138.5, 130.6, 129.2, 129.0, 127.9, 125.9, 114.0, 109.6, 72.5, 57.6, 54.8, 54.4, 52.7, 49.2, 35.1, 26.6, 19.1, 19.0, 10.8; HRMS (ESI) m/z calcd. for $C_{28}H_{36}N_4O_7S$ ([M - H]$^-$): 571.2221, found 571.2188.

4.1.26.

*2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**10g**)*

The title compound was obtained by **2g** which was coupled with **8** through EDCI/HOBt/DMAP coupling procedure in 99% yield (white powder) as described for **10a**: mp138-140 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 6.0 Hz, 1H), 7.32 – 7.27 (m, 4H), 7.22 (t, J = 7.0 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 4.38 (d, J = 16.0 Hz, 1H), 4.19 (d, J = 16.0 Hz, 1H), 4.11 – 4.07 (m, 1H), 3.91 (s, 3H), 3.89 – 3.86 (m, 1H), 3.47 (dd, J = 15.0, 3.0 Hz, 1H), 3.21 (dd, J = 14.0, 3.5 Hz, 1H), 3.07 (dd, J = 13.5, 8.0 Hz, 1H), 2.98 (dd, J = 15.0, 8.5 Hz, 1H), 2.88 (dd, J = 13.5, 7.0 Hz, 1H), 2.70 (dd, J = 13.5, 11.0 Hz, 1H), 2.07 – 2.00 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 168.9, 164.6, 160.0, 159.9, 151.5, 142.3, 140.7, 140.0, 132.1, 131.5, 131.3, 130.7, 130.5, 129.4, 127.4, 115.5, 74.0, 59.1, 56.3, 55.9, 54.2, 51.0, 36.6, 28.1, 20.6, 20.5; HRMS (ESI) m/z calcd. for $C_{27}H_{33}FN_4O_7S$ ([M - H]$^-$): 575.1970, found 575.1996.

4.1.27.

*N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)-2-(5-hydroxymethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**10h**)*

The title compound was obtained by **2h** which was coupled with **8** through EDCI/HOBt/DMAP coupling procedure in 99% yield (white powder) as described for **10a**: mp171-173 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.66 (d, J = 9.0 Hz, 2H), 7.18-7.12 (m, 5H), 7.09-7.06 (m, 1H), 6.98 (d, J = 9.0 Hz, 2H), 4.30 (d, J = 16.2 Hz, 1H), 4.17 (t, J = 1.2 Hz, 2H), 4.08 (d, J = 16.2 Hz, 1H), 3.96-3.92 (m, 1H), 3.77 (s, 3H), 3.75-3.71 (m, 1H), 3.33 (dd, J = 15.0, 3.6 Hz, 1H), 3.07 (dd, J = 14.0, 3.6 Hz, 1H), 2.92 (dd, J = 13.6, 8.4 Hz, 1H), 2.83 (dd, J = 15.0, 8.4 Hz, 1H), 2.73 (dd, J =

13.8, 7.0 Hz, 1H), 2.56 (dd, *J* = 13.8, 10.8 Hz, 1H), 1.90-1.85 (m, 1H), 0.80 (d, *J* = 6.6 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.1, 165.6, 164.6, 152.7, 144.4, 139.9, 132.0, 130.7, 130.4, 129.4, 127.3, 115.4, 114.8, 73.94 (s), 59.1, 57.7, 56.2, 55.9, 54.2, 50.9, 36.6, 28.0, 20.5, 20.4; HRMS (ESI) m/z calcd. for $C_{28}H_{36}N_4O_8S$ ([M - H]$^-$): 587.2170, found 587.2192.

4.1.28. 2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)acetamide ***(11e)***

The title compound was obtained by **2e** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 85% yield (colorless acicular crystal) as described for **10a**: mp157-159 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.41 (d, *J* = 9.0 Hz, 2H), 8.18 (d, *J* = 9.0 Hz, 1H), 8.09 (d, *J* = 9.0 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.26 (t, *J* = 7.2 Hz, 2H), 7.21-7.16 (m, 3H), 5.53 (dd, *J* = 7.8, 2.4 Hz, 1H), 4.28 (d, *J* = 16.2 Hz, 1H), 4.14 (d, *J* = 16.2 Hz, 1H), 3.82-3.77 (m, 1H), 3.61 (m, 1H), 3.41 (dd, *J* = 15.0, 2.8 Hz, 1H), 3.13 (dd, *J* = 13.8, 8.4 Hz, 1H), 3.03-2.98 (m, 2H), 2.91 (d, *J* = 6.6 Hz, 1H), 2.56 (dd, *J* = 14.0, 10.2 Hz, 1H), 2.00-1.92 (m, 1H), 0.84 (d, *J* = 6.6 Hz, 3H), 0.78 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.5, 163.9, 149.6, 146.4, 145.3, 139.0, 129.2, 128.6, 125.9, 124.5, 100.5, 71.2, 55.8, 54.0, 51.5, 49.5, 35.2, 25.8, 19.9, 19.7; HRMS (ESI) m/z calcd. for $C_{26}H_{31}N_5O_8S$ ([M - H]$^-$): 572.1810, found 572.1776.

4.1.29.

N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide ***(11f)***

The title compound was obtained by **2f** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 86% yield (white powder) as described for **10a**: mp172-174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.40 (d, *J* = 8.8 Hz, 2H), 8.15 (d, *J* = 9.0 Hz, 1H), 8.08 (d, *J* = 8.8 Hz, 2H), 7.27-7.24 (m, 2H), 7.21-7.17 (m, 3H), 7.16 (s, 1H), 5.11 (d, *J* = 7.0 Hz, 1H), 4.25 (d, *J* = 16.4 Hz, 1H), 4.07 (d, *J* = 16.4 Hz, 1H), 3.78 (td, *J* = 10.4, 3.2 Hz, 1H), 3.60 (td, *J* = 9.6, 2.8 Hz,

1H), 3.42-3.39 (m, 1H), 3.13 (dd, *J* = 13.6, 8.8 Hz, 1H), 3.04-2.95 (m, 2H), 2.91 (d, *J* = 6.4 Hz, 1H), 2.58-2.55 (m, 1H), 2.00-1.93 (m, 1H), 1.73 (s, 3H), 0.84 (d, *J* = 6.6 Hz, 3H), 0.77 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.6, 164.4, 150.9, 149.5, 145.2, 142.0, 139.0, 129.1, 128.6, 128.0, 125.9, 124.5, 108.0, 71.3, 55.8, 54.0, 51.5, 49.3, 35.2, 25.8, 19.9, 19.7, 11.9; HRMS (ESI) m/z calcd. for $C_{27}H_{33}N_5O_8S$ ([M - H]$^-$): 586.1966, found 586.1991.

4.1.30.

*2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)acetamide (**11g**)*

The title compound was obtained by **2g** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 76% yield (white powder) as described for **10a**: mp146-148 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.44 (d, *J* = 8.5 Hz, 2H), 8.12 (d, *J* = 8.5 Hz, 2H), 7.49 (d, *J* = 6.0 Hz, 1H), 7.32 – 7.27 (m, 2H), 7.26 (d, *J* = 7.0 Hz, 2H), 7.22 (t, *J* = 7.0 Hz, 1H), 4.39 (d, *J* = 16.5 Hz, 1H), 4.16 (d, *J* = 16.5 Hz, 1H), 4.06 – 4.02 (m, 1H), 3.86 – 3.83 (m, 1H), 3.55 (dd, *J* = 15.0, 2.0 Hz, 1H), 3.24 – 3.12 (m, 3H), 3.02 (dd, *J* = 13.5, 6.5 Hz, 1H), 2.69 (dd, *J* = 14.0, 11.0 Hz, 1H), 2.11 – 2.03 (m, 1H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 168.9, 160.1, 159.8, 151.4, 146.8, 142.6, 140.3, 139.8, 131.7, 131.3, 130.4, 129.9, 129.4, 127.4, 125.4, 73.3, 58.1, 55.9, 53.3, 51.1, 36.7, 27.7, 20.4; HRMS (ESI) m/z calcd. for $C_{26}H_{30}FN_5O_8S$ ([M - H]$^-$): 590.1715, found 590.1746.

4.1.31.

*N-((2S,3R)-3-hydroxy-4-(N-isobutyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)-2-(5-hydroxymethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**11h**)*

The title compound was obtained by **2h** which was coupled with **9** through EDCI/HOBt/DMAP coupling procedure in 54% yield (colorless oil) as described for **10a**: mp194-196 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.29 (d, *J* = 9.0 Hz, 2H), 7.97 (d, *J* = 9.0 Hz, 2H), 7.15 (dd, *J* = 13.2, 6.0 Hz, 3H), 7.13-7.11 (m, 2H), 7.09-7.06 (m, 1H), 4.29 (d, *J* = 16.2 Hz, 1H), 4.18 (dd, *J* = 3.0, 1.2 Hz, 2H), 4.06 (d, *J* = 16.2 Hz, 1H),

3.89 (ddd, *J* = 10.8, 7.2, 4.2 Hz, 1H), 3.70 (ddd, *J* = 9.6, 7.2, 2.8 Hz, 1H), 3.41 (dd, *J* = 15.0, 2.8 Hz, 1H), 3.09-2.98 (m, 3H), 2.89-2.85 (m, 1H), 2.55 (dd, *J* = 14.0, 10.8 Hz, 1H), 1.95-1.90 (m, 1H), 0.80 (d, *J* = 6.6 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.1, 165.6, 152.7, 151.4, 146.8, 144.34, 139.8, 130.3, 129.9, 129.4, 127.4, 125.4, 114.8, 73.4, 58.1, 57.7, 55.9, 53.3, 51.1, 36.7, 27.7, 20.3; HRMS (ESI) m/z calcd. for $C_{27}H_{33}N_5O_9S$ ([M - H]$^-$): 602.1915, found 602.1893.

4.1.32.

*N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl) -2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**12e**)*

The title compound was obtained by hydrogenation of **11e** in 95% yield (colorless oil) as described for **12a**: mp156-158 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.53 (d, *J* = 8.5 Hz, 2H), 7.31 – 7.28 (m, 4H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.74 (d, *J* = 8.5 Hz, 2H), 5.62 (d, *J* = 8.0 Hz, 1H), 4.39 (d, *J* = 16.0 Hz, 1H), 4.22 (d, *J* = 16.0 Hz, 1H), 4.12 – 4.08 (m, 1H), 3.89 – 3.85 (m, 1H), 3.43 (d, *J* = 3.0 Hz, 1H), 3.21 (dd, *J* = 14.0, 3.5 Hz, 1H), 3.00 (dd, *J* = 13.5, 8.0 Hz, 1H), 2.95 (dd, *J* = 15.0, 8.5 Hz, 1H), 2.84 (dd, *J* = 13.5, 7.0 Hz, 1H), 2.70 (dd, *J* = 13.5, 11.0 Hz, 1H), 2.06 – 1.98 (m, 1H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.0, 166.8, 154.3, 152.7, 147.6, 140.0, 130.6, 130.5, 129.4, 127.4, 126.1, 114.6, 102.3, 74.0, 59.4, 55.9, 54.4, 50.9, 36.6, 28.2, 20.7, 20.6; HRMS (ESI) m/z calcd. for $C_{26}H_{33}N_5O_6S$ ([M - H]$^-$): 542.2068, found 542.2103.

4.1.33.

*N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl) -2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**12f**)*

The title compound was obtained by hydrogenation of **11f** in 77% yield (faint yellow powder) as described for **12a**: mp186-188 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.45 (d, *J* = 9.0 Hz, 2H), 7.24 – 7.19 (m, 4H), 7.16 – 7.13 (m, 1H), 6.98 (d, *J* = 1.2 Hz, 1H), 6.67 (d, *J* = 9.0 Hz, 2H), 4.29 (d, *J* = 16.2 Hz, 1H), 4.14 (d, *J* = 16.2 Hz, 1H), 4.05 – 4.01 (m, 1H), 3.81 – 3.78 m, 1H), 3.37 (dd, *J* = 15.0, 3.6 Hz, 1H), 3.14 (dd, *J* =

13.8, 4.2 Hz, 1H), 2.94 (dd, *J* = 13.8, 8.4 Hz, 1H), 2.86 (dd, *J* = 15.0, 8.4 Hz, 1H), 2.75 (dd, *J* = 13.8, 7.2 Hz, 1H), 2.63 (dd, *J* = 13.8, 10.8 Hz, 1H), 1.98 – 1.93 (m, 1H), 1.77 (d, *J* = 1.2 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 167.7, 165.5, 152.8, 151.4, 141.8, 138.5, 129.0, 127.9, 125.8, 124.5, 113.1, 109.6, 72.6, 57.9, 54.3, 52.9, 49.2, 35.1, 26.7, 19.2, 19.1, 10.8; HRMS (ESI) m/z calcd. for $C_{27}H_{35}N_5O_6S$ ([M - H]$^-$): 556.2224, found 556.2251.

4.1.34.

*N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl) -2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**12g**)*

The title compound was obtained by hydrogenation of **11g** in 53% yield (colorless oil) as described for **12a**: mp163-165 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 6.0 Hz, 1H), 7.29-7.24 (m, 4H), 7.21-7.17 (m, 1H), 6.72 (d, *J* = 8.8 Hz, 2H), 4.33 (d, *J* = 16.4 Hz, 1H), 4.18 (d, *J* = 16.4 Hz, 1H), 4.10-4.06 (m, 1H), 3.87-3.82 (m, 1H), 3.40 (dd, *J* = 14.8, 3.6 Hz, 1H), 3.19 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.99-2.89 (m, 2H), 2.81 (dd, *J* = 13.6, 7.2 Hz, 1H), 2.67 (dd, *J* = 13.6, 10.8 Hz, 1H), 2.03-1.96 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 168.8, 159.8, 154.2, 151.4, 140.3, 139.9, 131.2, 130.5, 130.4, 129.4, 127.3, 126.0, 114.6, 74.0, 59.3, 55.8, 54.3, 50.9, 36.5, 28.1, 20.6, 20.5; HRMS (ESI) m/z calcd. for $C_{26}H_{32}FN_5O_6S$ ([M - H]$^-$): 560.1947, found 560.1962.

4.1.35.

*N-((2S,3R)-4-(4-amino-N-isobutylphenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl) -2-(5-hydroxymethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (**12h**)*

The title compound was obtained by hydrogenation of **11h** in 69% yield (white powder) as described for **12a**: mp186-188 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.39 (d, *J* = 8.8 Hz, 2H), 7.17-7.13 (m, 5H), 7.09-7.06 (m, 1H), 6.60 (d, *J* = 8.8 Hz, 2H), 4.26 (d, *J* = 16.4 Hz, 1H), 4.17 (d, *J* = 0.6 Hz, 2H), 4.10 (d, *J* = 16.4 Hz, 1H), 3.96-3.93 (m, 1H), 3.72 (ddd, *J* = 8.4, 6.6, 3.6 Hz, 1H), 3.28 (dd, *J* = 15.0, 3.6 Hz, 1H), 3.07 (dd, *J* =

13.8, 3.6 Hz, 1H), 2.89-2.84 (m, 1H), 2.82-2.76 (m, 1H), 2.69 (dd, *J* = 13.8, 7.2 Hz, 1H), 2.56 (dd, *J* = 13.8, 10.8 Hz, 1H), 1.88 (dt, *J* = 14.0, 7.0 Hz, 1H), 0.80 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 169.1, 165.7, 154.3, 152.7, 144.5, 140.0, 130.5, 130.4, 129.4, 127.3, 126.0, 114.8, 114.5, 74.0, 59.3, 57.8, 55.9, 54.3, 50.9, 36.5, 28.2, 20.6, 20.5; HRMS (ESI) m/z calcd. for $C_{27}H_{35}N_5O_7S$ ($[M - H]^-$): 572.2173, found 572.2178.

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 [13, 23]. 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 mins at room temperature and then the substrate was added. Each reaction was recorded for about 10 mins.

4.3. HIV-1 Infectivity Assay

The inhibitory effect of compounds on HIV-1 infectivity were determined using a single-round HIV-1 infectivity assay [34]. 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-$1_{DRV}{}^{R}{}_{S}$) 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 hours of post-transfection. After

incubating for 48 hours at temperature 37 °C, pseudotyped viruses in 10 μL of supernatant were used to infect SupT1 cells for 48 hours, followed by measuring luciferase activity of newly infected cells using Centro LB960 (Berthold).

For the assay using wild type HIV-1, 1×10^6 SupT1 cells were infected with 100 μL HIV-1 NL4-3 in the presence of 100 nM chemicals and 10 μg/mL polybrene, keeping a total volume of 500 μL (Spin infection at 1800rpm, 45min) [35]. Cells were washed once in the next morning and medium were replaced with fresh medium containing 100 nM chemicals. At 48 hpi, viruses were harvested and 50 μL of viruses were used to infect TZM-bl cells, followed by measuring luciferase activity in the infected cells.

4.4. Construction of DRV-resistant pNL4-3-E-R- cloning (pHIV-$1_{DRV}^{R}{}_{S}$)

To generate HIV-1 clones carrying the intended mutations, site-directed mutagenesis kit (*SBS Genetech*) was used [10]. V32I, L33F, I54M, and I84V in the protease were introduced into pNL4-3-E-R- according to the manual from the manufacturer. The primers used for mutations were 32/33 (F'-ACAGGAGCA GATGATACAATATTTGAAGAAAT GAATTTGCCA, R'-TGGCAAATTCATTTC TTCAAATATTGTATCATCTGC TCCTGT), 54(F'-GGGAATTGGAGGTTTTATG AAAGTAAGACAGTATGAT, R'-ATCATACTGTCTTACTTTCATAAAACCTCCAATTCCC) and 84(F'- GGA CCTACACCTGTCAACGTAATTGGAAGAA 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*).

4.5. Cytotoxicity Assay

Selected inhibitors were further evaluated in cytotoxicity assay using a cell counting kit-8 assay [37]. Plates were prepared with 20 000 293T cells per well. After 24h of culture, 1μL 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.

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Supplementary data

Experimental details for the syntheses and spectroscopic characterization of the compounds in this paper and details related to enzyme, cellular and in vivo studies.

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In this manuscript, inhibitor **10e** exhibited the closest enzyme inhibitory potency (IC_{50} = 2.53 nM) and inhibition of infectivity in a single-round infection assay comparable to DRV, as well as a promising inhibition ratio against wild-type HIV-1 *in vivo* (68% inhibition), with low cytotoxicity.

Compounds **10e**, **10f** and **10g** exhibited similar potency against DRV-sensitive or resistant HIV-1 in a dose-response assay, and DRV-resistant mutations only cause 1-2 fold increase in EC_{50}, compared with more than 16 fold increase of DRV in EC_{50}.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: