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Lead optimization to improve the antiviral potency of 2-aminobenzamide derivatives targeting HIV-1 Vif-A3G axis

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ABSTRACT

The viral infectivity factor (Vif)–apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) axis has been recognized as a valid target for developing novel small-molecule therapies for acquired immune deficiency syndrome (AIDS) or for enhancing innate immunity against viruses. Our previous work reported the novel Vif antagonist 2-amino-*N*-(2-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**2**) with strong antiviral activity. In this work, through optimizations of ring C of **2**, we discovered the more potent compound **6m** with an EC_{50} of 0.07 μM in non-permissive H9 cells, reflecting an approximately 5-fold enhancement of antiviral activity compared to that of **2**. Western blotting indicated that **6m** more strongly suppressed the defensive protein Vif than **2** at the same concentration. Furthermore, **6m** suppressed the replication of various clinical drug-resistant HIV strains (FI, NRTI, NNRTI, IN and PI) with relatively high efficacy. These results suggested that compound **6m** is a more potent candidate for treating AIDS.

1. Introduction

Acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus-1 (HIV-1) infection has been a serious public health problem for more than 30 years [1,2]. According to statistics reported by UNAIDS on July 16th, 2019, approximately 74.9 million people have been infected by HIV globally, and 32.0 million people have died from AIDS-related illnesses over the past 30 years [3]. Despite the remarkable benefit of highly active antiretroviral therapy, toxicity and frequent drug resistance caused by rapid viral replication have limited its clinical applications [4–6]. Therefore, novel therapeutics targeting other viral proteins in HIV-1 are urgently needed.

Human apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G (Human APOBEC3G, hA3G), a DNA-editing cytidine deaminase expressed in "non-permissive" host cells, acts as an innate weapon against retroviruses [7]. During viral infection, hA3G converts cytosine to uracil in newly synthesized viral DNA, thus causing a G to A hyper-mutation [8–10] and inactivation of the new viral genome [11,12]. To neutralize the function of A3G, HIV-1 produces the protein viral infectivity factor (Vif) to overcome the effective antiviral function of hA3G. Vif binds to hA3G and recruits an E3 ubiquitin ligase complex consisting of Elongin B and C, Cullin 5, Rbx2 and CBF-β, followed by the polyubiquitination and subsequent proteasomal degradation of hA3G [13–19]. Because of the absolute dependence on Vif for viral replication in the host and non-existing cellular homologues, the development of small-molecule inhibitors that inhibit Vif-mediated degradation of A3G is an extremely attractive approach for anti-HIV drug discovery.

Early studies had demonstrated that targeting Vif-A3G axis is an effective approach for implementing anti-HIV-1 activity, and the reported lead compounds included **RN-18** [20], **IMB-26** [21], 1,2,3-triazole derivatives [22], **Zif-15** [23], and **CV-3** [24]. In our previous work, a potent Vif antagonist **12c** (compound **2** in this work) was derived from the first Vif antagonist **RN-18** (compound **1** in this work) [25]. The 150-fold greater antiviral activity of compound **2** was attributed to the introduction of an amino group on the central ring of **RN-18**, which might form an H-bond interaction with

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Gln136 of Vif. Further structure-activity relationship (SAR) studies of compound **2** indicated that the replacement of the nitro group with other groups (e.g., COOH, F, CF_3) can only reduce cytotoxicity without further enhancing antiviral activity [26]. However, the moderate activity of compound **2** (EC_{50} = 0.34 μM in H9 cells in this work) is still inadequate for further development as a clinical candidate. Analysis of the binding mode of compound **2** with Vif revealed that the hydrophobic pocket formed by Phe115, Cys 133, Tyr 148, Ile155, and Pro157 was sufficient to accommodate ring C of compound **2** [25]; in other words, ring C of compound **2** has not adequately occupied the pocket. Therefore, further optimization of ring C could lead to the development of better Vif inhibitors. Furthermore, the introduction of heteroatoms into ring B was attempted to reduce the potential toxicity caused by the aniline group [27] (Fig. 1).

2. Results and discussion

2.1. Synthetic chemistry

The general synthetic steps of **5f**, **5m**, **5n**, **5o**, **6a**–**6p**, and **7a**–**7c** are outlined in Scheme 1. Briefly, commercially available 2-amino-6-bromobenzoic acid (**3**) was reacted with 4-nitrobenzenethiol via a thiolation reaction using Cu/Cu_2O as the catalyst to give the intermediate **4**, which was subsequently condensed with different substituted anilines to provide **5a**–**5p**. Then, oxidation of **5a**–**5p** by hydrogen peroxide generated the sulfones **6a**–**6p**. In addition, nucleophilic substitution of the phenol **5m** with different alkyl halides under basic conditions yielded compounds **7a**–**7c**.

13a–**13d** were prepared in four steps starting from 4,6-dichloropyrimidine-5-carbaldehyde (**8**), as presented in Scheme 2. Firstly, amination of **8** in the presence of ammonia afforded **9**. Oxidation of the aldehyde **9** with $NaClO_2$ followed by condensation with different substituted anilines led to **11a**–**11d**. The final compounds **13a**–**13d** were obtained through a S_NAr reaction between **11a**–**11d** and 4-nitrothiophenol under basic conditions.

2.2. SAR study

The SARs of compound **2** in our previous investigation revealed that the formation of a hydrogen bond between the amino group on ring B and Gln136 is essential for its antiviral activity [25], and replacement of the nitro group of ring A with other electro-withdrawing groups would decrease the antiviral activity [26]. Furthermore, after observing the binding mode of compound **2** with Vif, we found that the *ortho*-methoxyl substituted ring C did not sufficiently match the hydrophobic pocket formed by Phe115, Cys133, Tyr148, Ile155, and Pro157 [25]. Based on these findings (Fig. 1), we introduced different substitutions to ring C to generate a series of novel derivatives and investigated their activities.

Firstly, the methoxyl group of ring C of compound **2** was moved from *ortho* position to *meta* and *para* position to yield compound **6a** and **6b**, respectively. However, they all showed decreased antiviral activity in nonpermissive H9 cells (reduced HIV-1 p24 antigen production by 50%, Table 1). Then the methoxyl group of ring C of **6a** and **6b** was substituted with hydroxyl group to obtain **6c** and **6d**, respectively. **6c** still showed reduced activity, while the *para*-hydroxyl substituted **6d** displayed moderate antiviral potency with an EC_{50} of 4.59 μM. Next, to expand the hydrophobic volume of ring C, some disubstituted groups including methoxyl, methyl, halogen, hydroxyl and their cross were introduced on ring C to produce 2,4-dimethoxyl substituted **6e**, 2,5-dimethoxyl substituted **6f**, 3,4-dimethoxyl substituted **6g**, 3,5-dimethoxyl substituted **6h**, 3-hydroxy-4-methoxyl substituted **6i**, 2-methyl-3-bromo substituted **6j**, 3,5-dimethyl substituted **6k** and 2-fluoro-4-chloro substituted **6l**, respectively. Among these disubstituted derivatives, only **6e** and **6f** bearing 2-methoxyl substitution on ring C displayed good antiviral effect with EC_{50} values of 3.58 and 3.97 μM, respectively, and other compounds exhibited sharply reduced efficacy.

Therefore, the 2-methoxyl group on ring C was retained in our following optimization. When an additional hydroxyl group was introduced to the C-5 position of ring C, compound **6m** exhibited remarkable improvement in antiviral potency with an EC_{50} of 0.07 μM, meanwhile, it also possesses low toxicity with CC_{50} at 258.69 μM and therapeutic index up to 3695.57. Inspired by this result, a carboxyl group (**6n**) and fluorine (**6o**) were introduced at the same position. However, **6n** showed reduced activity and the activity of **6o** decreased about 28 fold when compared with **6m**. Besides, moving the hydroxyl group from C-5 to C-4 position (**6p**) also resulted in an apparent loss in potency. At the same time, we retrospectively tested the antiviral potency of non-oxidized

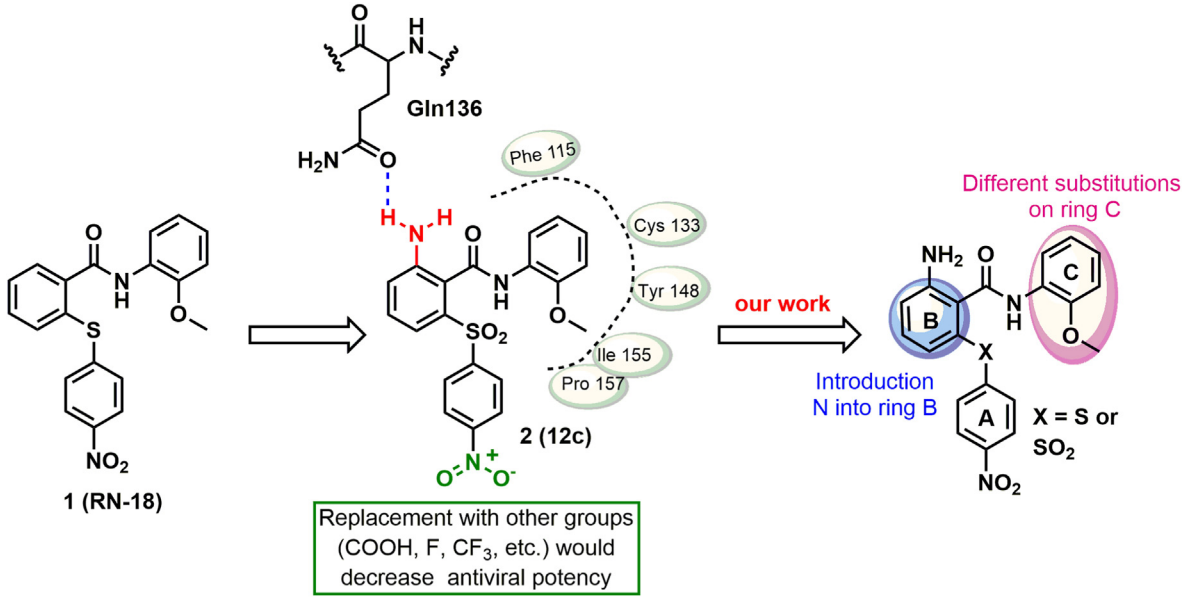
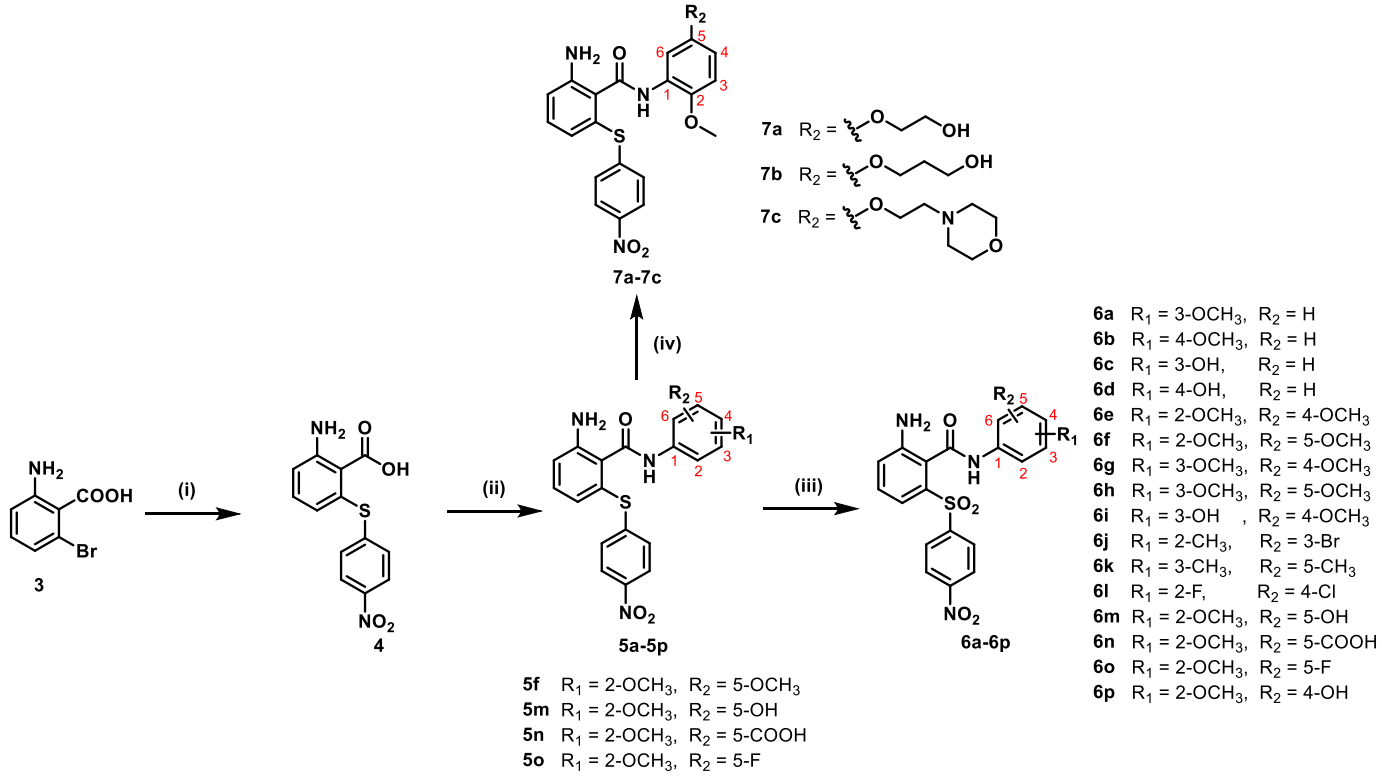
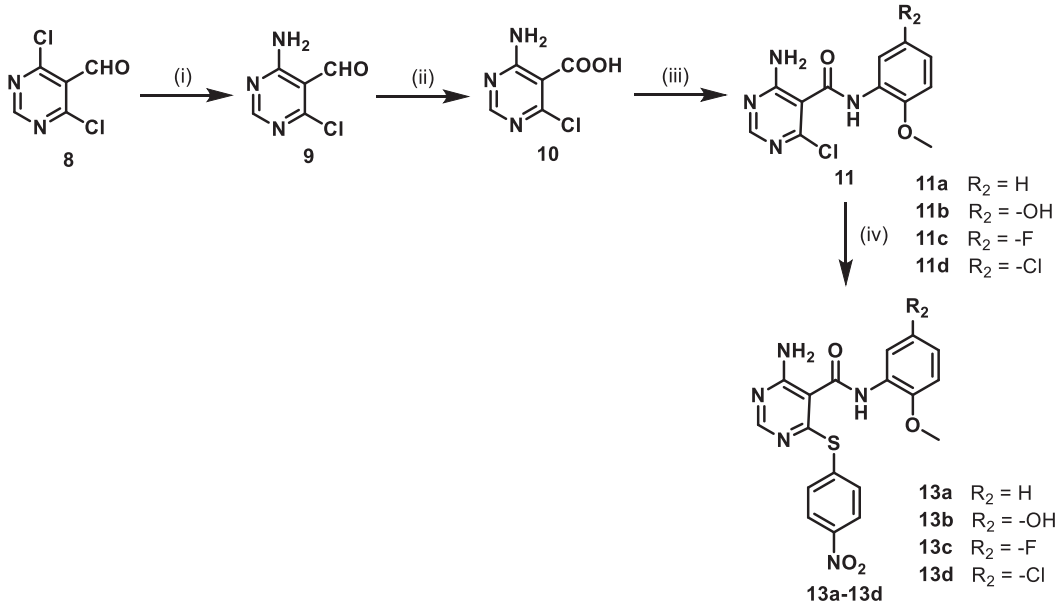


Fig. 1. Design strategies for compound **2** focusing on ring C with different substitutions and ring B featuring heteroatoms.



Scheme 1. Reagents and conditions: (i) 4-Nitrothiophenol, K_2CO_3, Cu/Cu_2O, 2-ethoxyethanol, 95 °C, overnight; (ii) substituted Ar-NH_2, EDCI, THF, rt, 5 h; (iii) 30% H_2O_2, AcOH, 55 °C, 6 h; (iv) alkyl halide, K_2CO_3, DMF, 60 °C, 5 h.

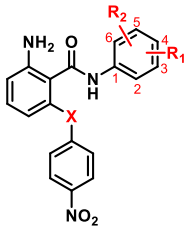


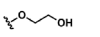
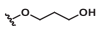
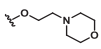
Scheme 2. Reagents and conditions: (i) NH_3 (7 M solution in MeOH), toluene, 60 °C, 4 h; (ii) aminosulfonic acid, $NaClO_2$, THF, 0 °C–rt; (iii) substituted Ar-NH_2, EDCI, THF, rt, 5 h; (iv) 4-Nitrothiophenol, Et_3N, *n*-butanol, 120 °C.

compounds **5f**, **5m**, **5n** and **5o** which bearing both 2-methoxyl and C-5 substitution on ring C, and most of them (**5f**, **5m** and **5o**) manifested slightly lower antiviral activity compared with the oxidized counterpart (**6f**, **6m** and **6o**) with EC_{50} values of 0.42–4.05 μM, which is coincident with our previous QSAR research [25]. In addition, to increase aqueous solubility, some hydrophilic groups were linked to compound **6m** via the 5-hydroxyl bridge. Nevertheless, the resulting compounds **7a**, **7b** and **7c** exhibited a dramatic loss in antiviral activity, which might be ascribed to the bulky volume of hydrophilic substituent.

To reduce the potential toxicity of aniline, a heteroatom nitrogen was incorporated into ring B. Although the newly synthesized

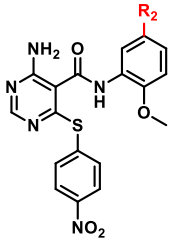
Table 1
In vitro antiviral activity of **2**, **5f**, **5m**, **5n**, **5o**, **6a**–**6p**, and **7a**–**7c** in H9 cells.



Compd	X	R_1	R_2	EC_{50} (μM)	CC_{50} (μM)	TI[a]
2	SO_2	2-OCH_3	H	0.34	>468	>1376.47
6a	SO_2	3-OCH_3	H	>100	N.A[b]	N.A.
6b	SO_2	4-OCH_3	H	>100	N.A.	N.A.
6c	SO_2	3-OH	H	>100	N.A.	N.A.
6d	SO_2	4-OH	H	4.59	32.53	21.79
6e	SO_2	2-OCH_3	4-OCH_3	3.58	>100	>27.93
6f	SO_2	2-OCH_3	5-OCH_3	3.97	>200	>50.44
6g	SO_2	3-OCH_3	4-OCH_3	15.51	>100	>6.45
6h	SO_2	3-OCH_3	5-OCH_3	>100	N.A.	N.A.
6i	SO_2	3-OH	4-OCH_3	>100	N.A.	N.A.
6j	SO_2	2-CH_3	3-Br	>100	N.A.	N.A.
6k	SO_2	3-CH_3	5-CH_3	26.37	>100	>3.79
6l	SO_2	2-F	4-Cl	10.3	>100	>9.71
6m	SO_2	2-OCH_3	5-OH	0.07	258.69	3695.57
6n	SO_2	2-OCH_3	5-COOH	>100	N.A.	N.A.
6o	SO_2	2-OCH_3	5-F	1.93	89.63	46.44
6p	SO_2	2-OCH_3	4-OH	5.77	54.71	9.48
5f	S	2-OCH_3	5-OCH_3	4.05	>200	>49.38
5m	S	2-OCH_3	5-OH	0.47	>100	>212.77
5n	S	2-OCH_3	5-COOH	0.42	>100	>238.10
5o	S	2-OCH_3	5-F	2.12	>100	>47.17
7a	S	2-OCH_3		>100	N.A.	N.A.
7b	S	2-OCH_3		>100	N.A.	N.A.
7c	S	2-OCH_3		>100	N.A.	N.A.
Lamivudine				0.21	>200	>975.61

[a] TI: therapeutic index, TI = CC_{50}/EC_{50}.
[b] NA: a CC_{50} values could not be obtained at the tested concentration range.

Table 2
In vitro antiviral activity of **13a**–**13d** in H9 cells.



Compd	R_2	EC_{50} (μM)	CC_{50} (μM)	TI[a]
13a	H	9.73	32.91	3.38
13b	-OH	40.61	35.83	0.88
13c	-F	41.13	26.57	0.65
13d	-Cl	20.04	2.22	0.11
Lamivudine		0.21	>200	>975.61

[a] TI: therapeutic index, TI = CC_{50}/EC_{50}.

amino-pyrimidine compounds **13a**–**13d** maintained moderate antiviral activities, remarkable cytotoxicity (CC_{50} = 2.22–35.83 μM) was also observed, leading to low therapeutic indexes (TI = 0.11–3.38, Table 2). Hence, these analogues were not conducted for further investigation.

Based on its highest antiviral activity and acceptable therapeutic index, compound **6m** was chosen for further evaluation.

2.3. Binding mode analysis

To elucidate the interaction mode of **6m** with Vif, we conducted molecular docking analysis using a reported crystal structure of HIV-1 Vif (PDB ID: 4N9F) [28]. The binding site selection referenced our previous research [25]. As illustrated in Fig. 2, compound **6m** adopted a similar binding conformation with **2** and maintained the key H-bonding interaction of amino group with Vif. The original *o*-methoxyl group formed hydrogen bond with Tyr 148. Apart from that, the extra hydroxyl group on ring C of compound **6m** forms two new hydrogen bonds with Cys133 and Lys156, which increased the binding affinity and can interpret the phenomenon that **6m** bearing more potent antiviral activity than compound **2**.

2.4. *6m* protects A3G from Vif-mediated degradation

Because Vif was reported to mediate the degradation of A3G, western blotting was performed to measure the effect of **6m** on Vif-mediated A3G degradation (Fig. 3). The results indicated that **6m** induced the decrease of Vif protein in a dose-dependent manner, and the reduction of Vif level in the presence of 50 μM **6m** was more obvious than that in the presence of the same concentration of compound **2**. Meanwhile, the degradation of A3G mediated by Vif was reversed by the addition of **6m** in a concentration-dependent manner. Unexpectedly, the recovery of A3G in the presence of 50 μM **6m** was merely equivalent to that in the presence of compound **2**, which not matched with the distinct reduction of Vif expression. The possible reason for this observation was that Vif can also bind to other proteins and mediated their degradation, such as the reported substrate protein phosphatase 2A (PP2A) [29]. All these results suggested that **6m** more potently inhibited Vif-mediated A3G degradation than the lead compound **2**.

2.5. Inhibitory effects of *6m* on drug-resistant HIV-1 strains

A common problem in current anti-HIV therapy is the development of drug resistant. Subsequently, we determined the antiviral activity of **6m** in TZM-bl cells infected with different clinical drug-resistant HIV strains including $pNL_{4\text{-}3gp41(36G)V38A,N42T}$ (FI-resistant), $HIV\text{-}1_{4755\text{-}5}$ (NRTI-resistant), $HIV\text{-}1_{A17}$ (NNRTI-resistant), $pYU2_{G140S/Q148H}$ (IN-resistant) and $HIV\text{-}1_{L10R/M46I/L63P/V82T/I84V}$ (PI-resistant). As presented in Table 3, **6m** inhibited the replication of different drug-resistant HIV-1 strains effectively with EC_{50} values of 3.02–8.40 μM, although the compound only slightly inhibited the replication of the NNRTI-resistant strain $HIV\text{-}1_{A17}$ (EC_{50} = 46.43 μM).

3. Conclusion

Through further structural optimization of compound **2**, we developed the novel HIV-1 Vif antagonist **6m** bearing additional 5-hydroxyl group on ring C. Compared to **2**, **6m** displayed 5-fold greater antiviral activity with an EC_{50} of 0.07 μM in non-permissive H9 cells. Molecular docking studies revealed that the extra 5-hydroxyl group might form two new hydrogen bonds with Cys133 and Lys156, which reinforces the ligand and Vif interaction. Meanwhile, **6m** more strongly reduced Vif expression than **2** at the same concentration in Western blot analysis. Further biological activity assays illustrated the promising inhibitory effects of **6m** against various clinical drug-resistant HIV strains. Thus, its

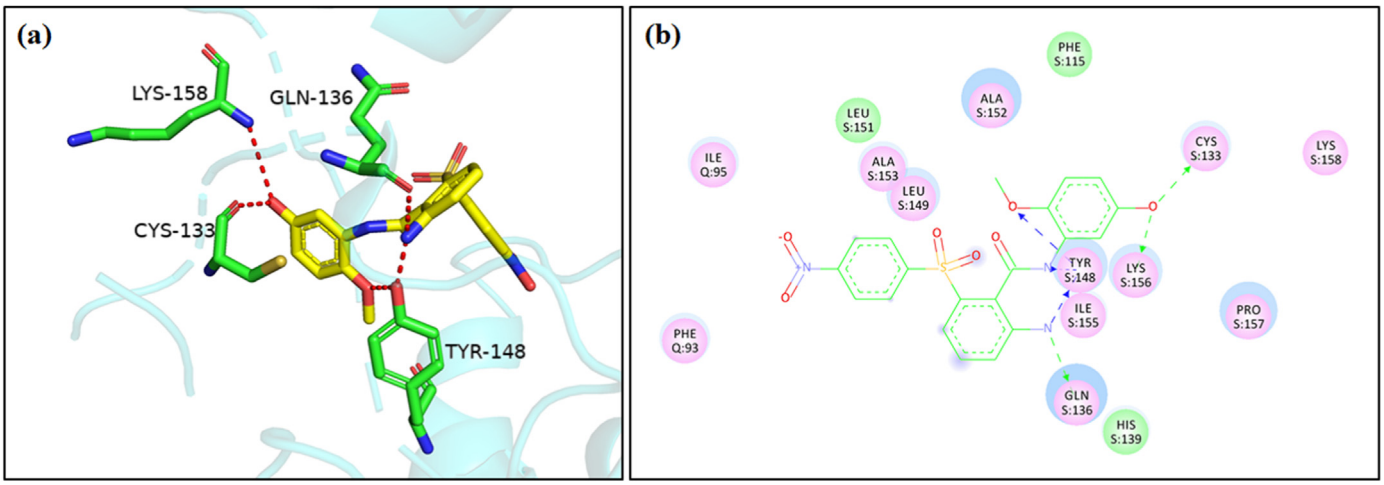


Fig. 2. Binding mode analysis of **6m** with Vif (PDB ID: 4N9F). (**a**) The possible binding pose of **6m** with Vif. (**b**) 2D diagram of **6m** bound to Vif.

improved antiviral activity makes compound **6m** a more effective candidate for treating HIV.

4. Experimental

4.1. Chemistry

All reagents and drugs are commercially available analytical reagents, which can be used directly without treatment. Purity of the intermediates and compounds was monitored using thin layer chromatography (TLC) on precoated silica gel 60 F254 plates (0.25 mm, Qingdao Haiyang Inc.) and components were visualized under ultraviolet light (254 nm). The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance spectrometer in DMSO-d_6 or $CDCl_3$, and analyzed by MestReNova Software. High resolution mass spectrometry (HRMS) was performed on an Agilent LC/MSD TOF system G3250AA. Silicycle silica gel 300–400 (particle size 40–63 μm) mesh was used for all column chromatography experiments. All final compounds were purified to >95% purity as determined by HPLC analyses were carried out on an Agilent 1260 Infinity II LC system (Waldbronn, Germany) with a fixed wavelength UV detector at 254 nm. An InertSustain®C18 Column (Waldbronn, Germany) (250 mm × 4.6 mm, 5 μM) was used with a flow rate of 1.0 mL/min for the sample. The injection volume was 5 μL. Mobile phase was consisted of methanol (C) and water with 0.1% of formic acid (D) with gradient elution timing programs: 0–20 min (50% C and 50% D).

*4.1.1. Synthesis of 2-amino-6-((4-nitrophenyl)thio)benzoic acid (**4**)*

A mixture of commercially available 2-amino-6-bromobenzoic acid (**3**, 2370 mg, 11 mmol), 4-nitrobenzenethiol (2050 mg, 13.2 mmol), Cu (130 mg, 2 mmol), Cu_2O (140 mg, 1 mmol) and K_2CO_3 (3036 mg, 22 mmol) in 2-ethoxyethanol (50 mL) was stirred at 95 °C for 12 h. Upon completion, the reaction mixture was cooled to room temperature, and the resulting precipitate was filtered off, while the filter cake was washed with ethyl acetate. Then the filtrate was acidified with 5 N HCl aqueous solution, extracted with ethyl acetate twice. The combined extracts were washed with bine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using ethyl acetate/hexanes as eluent to give compound **4**.

*4.1.2. General procedure A for the preparation of **5a-5p***

A mixture of **4** (50 mg, 0.13 mmol), different substituted aniline (0.13 mmol) and EDCI (30 mg, 0.16 mol) in THF (10 mL) was stirred at room temperature for 5 h. The reaction was quenched with water and extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the desired compound **5a-5p**.

*4.1.2.1. 2-amino-N-(2,5-dimethoxyphenyl)-6-((4-nitrophenyl)thio) benzamide (**5f**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.09 (d, J = 8.9 Hz, 2H), 7.51 (d, J = 2.9 Hz, 1H), 7.31 (d, J = 8.9 Hz, 2H), 7.23 (t, J = 7.9 Hz, 1H), 6.91 (dd, J = 8.5, 3.5 Hz, 2H), 6.77 (d, J = 7.5 Hz, 1H), 6.66 (dd, J = 8.9, 3.0 Hz, 1H), 5.41 (s, 2H), 3.67 (s, 3H), 3.63 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.65, 153.37, 148.86, 147.50, 145.09, 131.26, 128.05, 127.27, 123.91, 123.39, 117.54, 112.54, 109.67, 56.28. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_5S$ $[M+H]^+$ 426.1119, found: 426.1120; Purity > 96%.

*4.1.2.2. 2-amino-N-(5-hydroxy-2-methoxyphenyl)-6-((4-nitrophenyl)thio)benzamide (**5m**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 9.19 (s, 1H), 8.07 (d, J = 13.0 Hz, 2H), 7.30 (d, J = 6.7, 2H), 7.26 (d, J = 8.5 Hz, 1H), 7.20 (t, J = 7.9 Hz, 1H), 6.88 (d, J = 7.7 Hz, 1H), 6.75 (d, J = 7.0 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 6.30 (dd, J = 8.5, 2.4 Hz, 1H), 5.36 (s, 2H), 3.65 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.47, 151.35, 148.93, 147.41, 145.25, 143.75, 131.22, 127.59, 124.44, 123.44, 117.53, 112.86, 111.33, 110.69, 56.70. HRMS (ESI) calcd for $C_{20}H_{17}N_3O_5S$ $[M+H]^+$ 412.0963, found: 412.0960; Purity > 97%.

*4.1.2.3. 2-amino-N-(5-hydroxy-2-methoxyphenyl)-6-((4-nitrophenyl)thio)benzamide (**5n**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.24 (s, 1H), 8.07 (d, J = 13.8 Hz, 2H), 7.69 (d, J = 8.2 Hz, 1H), 7.32 (d, J = 8.9 Hz, 2H), 7.22 (t, J = 7.9 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.90 (d, J = 8.1 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 5.40 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.72, 154.11, 148.89, 147.40, 145.26, 131.23, 127.66, 127.46, 127.30, 126.64, 124.98, 124.43, 123.36, 117.46, 111.06, 56.36. HRMS (ESI) calcd for $C_{21}H_{17}N_3O_6S$ $[M+Na]^+$ 462.0730, found: 462.0729; Purity > 99%.

*4.1.2.4. 2-amino-N-(5-fluoro-2-methoxyphenyl)-6-((4-nitrophenyl) thio)benzamide (**5o**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.08 (d, J = 8.1 Hz, 2H), 7.83 (dd, J = 10.6, 3.0 Hz, 1H), 7.31 (d, J = 8.9 Hz, 2H), 7.23 (t, J = 7.9 Hz, 1H), 6.98 (dd, J = 16.9, 8.5 Hz, 1H), 6.94–6.84 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 7.5 Hz, 1H), 5.43 (s, 2H), 3.67 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.94, 157.27, 154.94, 148.82, 147.49, 146.77, 145.27, 131.34, 127.42, 126.93, 124.43, 123.32, 117.55, 112.50, 109.63, 56.67. HRMS (ESI) calcd for $C_{20}H_{16}FN_3O_4S$

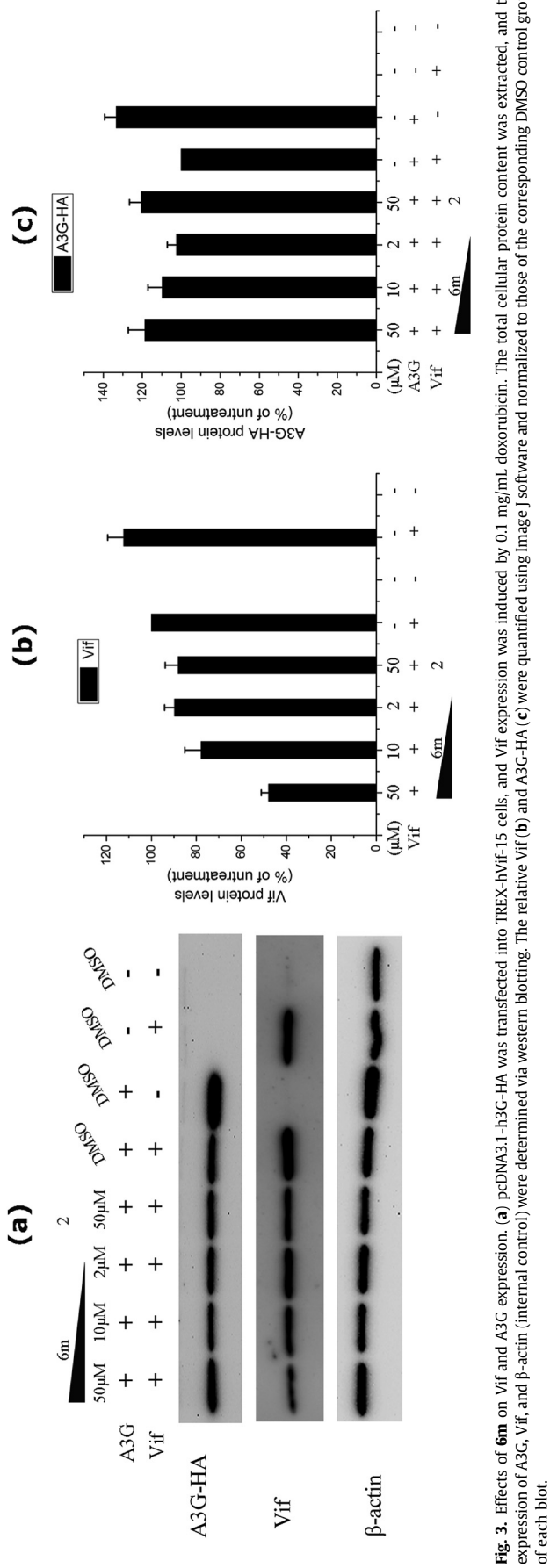


Fig. 3. Effects of **6m** on Vif and A3G expression. (**a**) pcDNA3.1-h3G-HA was transfected into TREX-hVif-15 cells, and Vif expression was induced by 0.1 mg/mL doxorubicin. The total cellular protein content was extracted, and the expression of A3G, Vif, and β-actin (internal control) were determined via western blotting. The relative Vif (**b**) and A3G-HA (**c**) were quantified using Image J software and normalized to those of the corresponding DMSO control group of each blot.

Table 3
Anti-HIV-1 activities of **6m** in drug-resistant strains[a].

Cell	Virus	Subtype	$EC_{50}(\mu M)$[b]
TZM-bl	$pNL4\text{-}3_{gp41(36G)V38A,N42T}$	FI-resistant	3.02 ± 1.07
	$HIV\text{-}1_{4755\text{-}5}$	NRTI-resistant	8.40 ± 0.74
	$HIV\text{-}1_{A17}$	NNRTI-resistant	46.43 ± 2.70
	$pYU2_{G140S/Q148H}$	IN-resistant	3.74 ± 0.32
	$HIV\text{-}1_{L10R/M46I/L63P/V82T/I84V}$	PI-resistant	4.39 ± 2.66

[a] All data represent means ± SD of three separate experiments.
[b] EC_{50}, 50% effective concentration.

$[M+Na]^+$ 436.0738, found: 436.0737; Purity > 98%.

*4.1.3. General procedure B for the preparation of compounds **6a-6p***

To a solution of compounds **5a-5p** (0.15 mmol) in acetic acid (10 mL) was added 30% H_2O_2 (0.75 mmol) slowly. The reaction mixture was heated in an oil bath at 55 °C and monitored using TLC. Next, the reaction was cooled to room temperature and quenched with MnO_2 (0.5 mmol). The insoluble solid was filtered off. The filtrate was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using ethyl acetate/hexanes as eluent to afford compound **6a-6p**.

*4.1.3.1. 2-amino-N-(3-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl) benzamide (**6a**).* ^1H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 8.36 (d, J = 8.8 Hz, 2H), 8.13–8.08 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 9.1, Hz, 2H), 7.29 (dd, J = 9.0, 2.0 Hz, 2H), 7.08 (d, J = 2.5 Hz, 1H), 6.83 (d, J = 7.8 Hz, 1H), 6.76 (t, J = 7.5 Hz, 1H), 5.47 (s, 2H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.42, 159.89, 150.46, 147.37, 140.79, 136.98, 130.45, 129.68, 124.89, 121.54, 117.13, 112.83, 109.37, 106.53, 55.51. HRMS (ESI) calcd for $C_{20}H_{17}N_3O_6S$ $[M+Na]^+$ 450.0730, found: 450.0732; Purity > 97%.

*4.1.3.2. 2-amino-N-(4-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl) benzamide (**6b**).* ^1H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.37 (d, J = 8.3 Hz, 2H), 8.19–8.07 (d, J = 8.8 Hz, 2H), 7.57 (m, 2H), 7.41–7.25 (m, 2H), 7.08 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 15.7 Hz, 2H), 5.45 (s, 2H), 3.77–3.74 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.88, 156.08, 150.45, 147.39, 147.16, 137.01, 132.77, 130.36, 129.61, 126.77, 124.87, 121.85, 121.59, 117.15, 115.27, 114.16, 55.72. HRMS (ESI) calcd for $C_{20}H_{17}N_3O_6S$ $[M+Na]^+$ 450.0730, found: 450.0731; Purity > 98%.

*4.1.3.3. 2-amino-N-(3-hydroxyphenyl)-6-((4-nitrophenyl)sulfonyl) benzamide (**6c**).* ^1H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 9.39 (s, 1H), 8.39–8.33 (d, J = 9.1 Hz, 2H), 8.16–8.10 (d, J = 9.1 Hz, 2H), 7.35 (t, J = 7.9 Hz, 1H), 7.32–7.25 (m, 2H), 7.13–7.06 (m, 2H), 7.04–7.00 (d, J = 8.1 Hz, 1H), 6.54–6.49 (dd, J = 8.5, 1.8 Hz, 1H), 5.45 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.25, 157.96, 150.46, 147.62, 147.12, 140.62, 136.94, 130.39, 129.59, 124.86, 121.63, 117.18, 111.24, 107.70. HRMS (ESI) calcd for $C_{19}H_{15}N_3O_6S$ $[M+Na]^+$ 436.0574, found: 436.0579; Purity > 96%.

*4.1.3.4. 2-amino-N-(4-hydroxyphenyl)-6-((4-nitrophenyl)sulfonyl) benzamide (**6d**).* ^1H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.32–8.24 (d, J = 9.1 Hz, 2H), 7.99–7.90 (d, J = 9.0 Hz, 2H), 7.67 (d, J = 14.0 Hz, 1H), 7.61 (t, J = 7.9 Hz, 1H), 7.54 (dd, J = 7.7, 1.0 Hz, 1H), 7.22 (dd, J = 10.0, 3.1 Hz, 1H), 6.96–6.87 (m, 2H), 6.15 (dt, J = 6.9, 3.4 Hz, 1H), 6.07 (dd, J = 10.0, 2.1 Hz, 1H), 5.75 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 184.58, 162.48, 155.17, 148.83, 147.71, 147.57, 147.10, 134.85, 128.50, 127.78, 126.66, 124.57, 118.18, 113.75, 109.48, 64.47. HRMS (ESI) calcd for $C_{19}H_{15}N_3O_5S$ $[M+K]^+$436.0545, found: 436.0543; Purity > 97%.

*4.1.3.5. 2-amino-N-(2,4-dimethoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6e**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (s, 1H), 8.39–8.32 (d, J = 8.9 Hz, 2H), 8.20–8.11 (d, J = 8.9 Hz, 2H), 7.48 (d, J = 8.6 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.28 (dd, J = 7.8, 1.1 Hz, 1H), 6.69–6.62 (m, 2H), 6.61–6.58 (d, J = 2.8 Hz, 1H), 5.57 (s, 2H), 3.80–3.77 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.79, 158.86, 154.24, 150.42, 147.76, 147.12, 137.07, 129.67, 127.13, 126.50, 124.80, 121.66, 119.66, 117.22, 112.52, 105.10, 99.51, 56.24, 55.90. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_7S$ [M+Na]$^+$ 480.0836, found: 480.0840; Purity > 98%.

*4.1.3.6. 2-amino-N-(2,5-dimethoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6f**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H), 8.36 (d, J = 8.9 Hz, 2H), 8.15 (d, J = 8.9 Hz, 2H), 7.49 (d, J = 3.1 Hz, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.29 (d, J = 7.1 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 7.00 (d, J = 9.0 Hz, 1H), 6.76 (dd, J = 9.3, 4.7 Hz, 1H), 5.55 (s, 2H), 3.75 (s, 3H), 3.72 (s, J = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.71, 153.52, 150.44, 147.67, 147.18, 146.04, 137.00, 130.48, 129.57, 127.96, 124.86, 121.43, 117.22, 112.72, 111.35, 110.11, 56.34. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_7S$ [M+Na]$^+$ 480.0836, found: 480.0840; Purity > 97%.

*4.1.3.7. 2-amino-N-(3,4-dimethoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6g**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.44–8.29 (d, J = 9.1 Hz, 2H), 8.10 (d, J = 9.1 Hz, 2H), 7.41–7.32 (t, J = 7.8 Hz, 2H), 7.31 (d, J = 7.3 Hz, 1H), 7.22 (dd, J = 13.9, 7.0 Hz, 1H), 7.09 (t, J = 8.6 Hz, 1H), 6.97–6.93 (m, 1H), 5.45 (s, 2H), 3.75 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.87, 150.44, 148.95, 147.58, 147.19, 145.75, 136.97, 133.27, 130.35, 129.62, 124.87, 121.62, 117.12, 112.60, 105.89, 56.36, 55.94. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_7S$ [M+Na]$^+$ 480.0836, found: 480.0840; Purity > 99%.

*4.1.3.8. 2-amino-N-(3,5-dimethoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6h**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.40–8.34 (d,J = 8.8 Hz, 2H), 8.13–8.10 (d, J = 8.8 Hz, 2H), 7.36 (t, J = 7.9 Hz, 1H), 7.30 (dd, J = 7.7, 0.9 Hz, 1H), 7.09 (d, J = 3.7 Hz, 1H), 6.95 (d, J = 7.2 Hz, 2H), 6.28 (d, J = 2.2 Hz, 1H), 5.46 (s, 2H), 3.74 (d, J = 1.4 Hz, 6H).^{13}C NMR (101 MHz, DMSO-d_6) δ 165.11, 150.51, 147.50, 147.14, 137.90, 137.00, 132.84, 131.90, 129.84, 129.71, 127.55, 126.41, 125.03, 124.99, 121.74, 121.38, 117.40, 49.07. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_7S$ [M+Na]$^+$ 480.0836, found: 480.0840; Purity > 97%.

*4.1.3.9. 2-amino-N-(3-hydroxy-4-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6i**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 9.03 (s, 1H), 8.38–8.32 (d, J = 8.8 Hz, 2H), 8.16–8.08 (d, J = 8.9 Hz, 2H), 7.35 (t, J = 7.9 Hz, 1H), 7.29 (dd, J = 7.8, 1.5 Hz, 2H), 6.95–6.87 (m, 2H), 5.40 (s, 2H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.74, 150.45, 147.64, 147.12, 146.81, 144.64, 136.95, 133.25, 129.60, 126.73, 124.84, 121.68, 112.96, 111.39, 109.13, 56.52. HRMS (ESI) calcd for $C_{20}H_{17}N_3O_7S$ [M+Na]$^+$ 466.0679, found: 466.0678; Purity > 99%.

*4.1.3.10. 2-amino-N-(3-bromo-2-methylphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6j**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.39 (d, J = 8.9 Hz, 2H), 8.19 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 7.9 Hz, 1H), 7.53–7.45 (d, J = 7.8 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 7.2 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.08 (d, J = 11.1 Hz, 1H), 5.64–5.39 (s, 2H), 2.40 (s, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 165.11, 150.51, 147.78, 147.22, 137.90, 137.00, 132.84, 130.51, 129.84, 129.58, 127.55, 126.41, 125.03, 124.95, 121.74, 121.38, 117.40, 113.30, 18.55. HRMS (ESI) calcd for $C_{20}H_{16}BrN_3O_5S$ [M+Na]$^+$ 511.9886, found: 511.9890; Purity > 98%.

*4.1.3.11. 2-amino-N-(3,5-dimethylphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6k**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.39–8.33 (d, J = 8.8 Hz, 2H), 8.16–8.09 (d, J = 8.7 Hz, 2H), 7.35 (t, J = 7.9 Hz, 1H), 7.31–7.22 (m, 3H), 7.08 (d, J = 6.0 Hz, 1H), 6.74 (s, 1H), 5.43 (s, 2H), 2.27 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.26, 150.43, 147.56, 147.17, 139.48, 137.88, 136.93, 129.98, 129.59, 125.60, 124.90, 121.63, 117.86, 117.12, 21.62. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_5S$ [M+H]$^+$ 426.1119, found: 426.1121; Purity > 98%.

*4.1.3.12. 2-amino-N-(4-chloro-2-fluorophenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6l**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.39 (d, J = 8.5 Hz, 2H), 8.16 (d, J = 8.8 Hz, 2H), 8.04 (t, J = 8.6 Hz, 1H), 7.50 (dd, J = 8.9, 4.4 Hz, 1H), 7.36 (t, J = 8.0 Hz, 2H), 7.27 (d, J = 7.6 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 5.51 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.26, 155.82, 153.33, 150.50, 147.68, 147.11, 137.07, 130.62, 129.56, 126.82, 126.38, 124.93, 121.60, 120.81, 117.23, 114.19. HRMS (ESI) calcd for $C_{19}H_{13}ClFN_3O_5S$ [M+Na]$^+$ 472.0140, found: 472.0141; Purity > 97%.

*4.1.3.13. 2-amino-N-(5-hydroxy-2-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6m**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.15–8.98 (s, 1H), 8.36 (d, J = 8.6 Hz, 2H), 8.11 (d, J = 8.3 Hz, 2H), 7.35 (dd, J = 9.3, 5.5 Hz, 2H), 7.31–7.20 (d, J = 7.5 Hz, 1H), 7.06 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.58 (dd, J = 8.6, 2.5 Hz, 1H), 5.55 (s, 2H), 3.77–3.55 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.54, 151.48, 150.45, 147.70, 147.18, 145.03, 136.98, 130.45, 129.57, 127.60, 124.83, 121.47, 117.23, 113.07, 112.27, 56.73. HRMS (ESI) calcd for $C_{20}H_{17}N_3O_7S$ [M+Na]$^+$ 466.0679, found: 466.0678; Purity > 98%.

*4.1.3.14. 3-(2-amino-6-((4-nitrophenyl)sulfonyl)benzamido)-4-methoxybenzoic acid (**6n**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.42 (d, J = 2.1 Hz, 1H), 8.36 (d, J = 8.9 Hz, 2H), 8.16 (d, J = 8.9 Hz, 2H), 7.83 (dd, J = 8.5, 2.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.27 (d, J = 7.7 Hz, 1H), 7.18 (d, J = 8.7 Hz, 1H), 7.10–7.05 (d,J = 7.9 Hz, 1H), 5.56 (s, 2H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.48, 168.94, 167.25, 156.58, 151.97, 138.31, 128.66, 127.84, 126.37, 124.78, 123.82, 118.90, 114.70, 113.87, 112.47, 56.41. HRMS (ESI) calcd for $C_{21}H_{17}N_3O_8S$ [M+Na]$^+$ 494.0628, found: 494.0634; Purity > 99%.

*4.1.3.15. 2-amino-N-(5-fluoro-2-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6o**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.40–8.33 (d, J = 8.9 Hz, 2H), 8.18–8.12 (d, J = 8.9 Hz, 2H), 7.86 (dd, J = 10.4, 3.1 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.31–7.24 (dd, J = 7.9, 1.1 Hz, 1H), 7.09–7.03 (m, 2H), 7.01 (d, J = 3.2 Hz, 1H), 5.53 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.01, 150.46, 147.59, 147.19, 136.99, 130.55, 130.17, 129.56, 124.90, 121.60, 121.02, 117.20, 112.75, 110.77, 56.77. HRMS (ESI) calcd for $C_{20}H_{16}FN_3O_6S$ [M+H]$^+$ 446.0636, found: 446.0634; Purity > 98%.

*4.1.3.16. 2-amino-N-(4-hydroxy-2-methoxyphenyl)-6-((4-nitrophenyl)sulfonyl)benzamide (**6p**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.40–8.32 (d, J = 8.8 Hz, 2H), 8.21–8.14 (d, J = 8.9 Hz, 2H), 7.84 (d, J = 6.1 Hz, 1H), 7.32 (d, J = 3.2 Hz, 1H), 7.30–7.27 (m, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.51 (d, J = 2.4 Hz, 1H), 6.42 (dd, J = 8.5, 2.4 Hz, 1H), 5.58 (s, 2H), 3.72 (s, 3H). HRMS (ESI) calcd for $C_{20}H_{16}FN_3O_6S$ [M+H]$^+$ 446.0679, found: 446.0678; Purity > 97%.

*4.1.4. General procedure C for the preparation of compounds **7a-7c***

To a stirring mixture of compound **6m** (0.2 mmol) and anhydrous potassium carbonate (0.2 mmol) in DMF (10 mL) was added different kinds of alkyl halide (0.24 mmol). The reaction was heated at 60 °C for 8 h and monitored using TLC. After cooling, the mixture was extracted with ethyl acetate twice. The combined extracts were washed with bine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography

using ethyl acetate/hexanes as eluent to give compound **7a-7c**.

*4.1.4.1. 2-amino-N-(5-(2-hydroxyethoxy)-2-methoxyphenyl)-6-((4-nitrophenyl)thio)benzamide (**7a**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 9.0 Hz, 2H), 7.37 (d, J = 8.9 Hz, 2H), 6.97 (m, 2H), 6.85–6.58 (m, 2H), 6.54 (d, J = 7.6 Hz, 1H), 6.37 (d, J = 8.0 Hz, 1H), 5.16 (s, 2H), 3.94–3.62 (s, 3H), 3.57 (t, J = 15.5 Hz, 2H), 2.98 (d, J = 16.0 Hz, 2H). HRMS (ESI) calcd for $C_{22}H_{21}N_3O_6S$ $[M+Na]^+$ 478.1043, found: 478.1044; Purity > 99%.

*4.1.4.2. 2-amino-N-(5-(3-hydroxypropoxy)-2-methoxyphenyl)-6-((4-nitrophenyl)thio)benzamide (**7b**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.03 (d, J = 9.1 Hz, 2H), 7.37 (d, J = 9.3 Hz, 2H), 6.91 (m, 4H), 6.51 (d, J = 7.6 Hz, 1H), 6.38 (d, J = 8.1 Hz, 1H), 5.26 (s, 2H), 4.83–4.17 (m, 2H), 4.10–3.85 (m, 1H), 3.67 (s, 3H), 3.51 (t, J = 5.8 Hz, 2H), 2.94 (s, 2H), 1.79–1.63 (t, J = 6.5 Hz, 2H). HRMS (ESI) calcd for $C_{23}H_{23}N_3O_6S$ $[M+H]^+$ 492.1200, found: 492.1205; Purity > 97%.

*4.1.4.3. 2-amino-N-(2-methoxy-5-(2-morpholinoethoxy)phenyl)-6-((4-nitrophenyl)thio)benzamide (**7c**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.03 (d, J = 9.1 Hz,2H), 7.36 (d, J = 9.3 Hz, 2H), 6.95 (d, J = 16.8 Hz, 2H), 6.76 (s, 2H), 6.54 (d, J = 7.7 Hz, 1H), 6.40 (d, J = 8.1 Hz, 1H), 5.26 (s, 2H), 4.19 (m, 1H), 3.86–3.59 (s, 3H), 3.59–3.53 (t, J = 4.6 Hz 4H), 3.17 (d, J = 3.6 Hz, 1H), 3.11–2.86 (t, J = 7.3 Hz, 2H), 2.43–2.33 (t, J = 4.1 Hz, 4H). HRMS (ESI) calcd for $C_{26}H_{28}N_4O_6S$ $[M+H]^+$ 525.1804, found: 525.1806; Purity > 98%.

*4.1.5. General procedure D for the preparation of compounds **13a-13d***

*4.1.5.1. Synthesis of 4-amino-6-chloropyrimidine-5-carbaldehyde (**9**).* A mixture of 4,6-dichloro-5-pyrimidine formaldehyde (**8**, 1050 mg, 6 mmol) and ammonia solution (7 M in methanol, 12 mmol) in toluene was heated 60 °C for 4 h. The reaction mixture was concentrated under vacuum to give the crude product **9** (800 mg), which was used in next step without further purification.

*4.1.5.2. Synthesis of 4-amino-6-chloropyrimidine-5-carboxylic acid (**10**).* To a solution of **9** (800 mg, 5.0 mmol) in THF (15 mL) at 0 °C was added aminosulfonic acid (593 mg, 6 mmol) and $NaClO_2$ (900 mg, 10.2 mmol) sequentially and slowly. After being stirred at 0 °C for 0.5 h, the reaction was warmed to room temperature and monitored using TLC. Upon completion, the reaction mixture was quenched with water, then extracted with dichloromethane (3 × 25 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography using ethyl acetate/hexanes as eluent to afford compound **10** (600 mg, yield = 67%).

*4.1.5.3. General procedure D for the preparation of compounds **13a-13d**.* A mixture of 4-amino-6-chloropyrimidine-5-carboxylic acid **10** (52 mg, 0.3 mmol), different substituted Ar-NH_2 (0.3 mmol) and EDCI (71 mg, 0.36 mmol) in THF (10 mL) was stirred at room temperature for 5 h. Upon completion, the reaction mixture was quenched with water, and then extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using ethyl acetate/hexanes as eluent to give compound **11a-11d**.

A mixture of **11a-11d** (0.2 mmol), 4-nitrobenzenethiol (31 mg, 0.2 mmol) and Et_3N (40 mg, 0.4 mmol) in *n*-butanol (10 mL) was heated in oil bath at 120 °C for 4 h and monitored using TLC. After cooling, the reaction mixture was poured in to water and extracted with ethyl acetate (3 × 10 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography using ethyl acetate/hexanes as eluent to give compound **13a-13d**.

*4.1.5.3.1. 4-amino-N-(2-methoxyphenyl)-6-((4-nitrophenyl)thio) pyrimidine-5-carboxamide (**13a**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.23 (s, 1H), 8.20 (d, J = 2.5 Hz, 2H), 7.99 (m, 1H), 7.74 (d, J = 8.3 Hz, 2H), 7.15 (dd, J = 11.3, 4.2 Hz, 1H), 7.07 (d, J = 7.5 Hz, 2H), 6.96 (t, J = 7.5 Hz, 1H), 3.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.46, 157.79, 147.31, 140.52, 134.27, 127.14, 125.88, 124.17, 123.83, 120.70, 113.62, 112.00, 56.30. HRMS (ESI) calcd for $C_{18}H_{15}N_5O_4S$ $[M+H]^+$ 398.0917, found: 398.0919; Purity > 96%.

*4.1.5.3.2. 4-amino-N-(5-hydroxy-2-methoxyphenyl)-6-((4-nitrophenyl)thio)pyrimidine-5-carboxamide (**13b**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 9.02 (s, 1H), 8.19 (d, J = 12.0 Hz, 3H), 7.74 (d, J = 8.7 Hz, 2H), 7.62 (s, 1H), 7.07 (s, 2H), 6.87 (d, J = 8.8 Hz, 1H), 6.52 (dd, J = 8.8, 2.9 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.99, 160.44, 157.77, 151.41, 147.30, 140.56, 134.23, 127.94, 127.27, 125.04, 124.16, 113.71, 113.21, 111.47, 110.66, 57.05. HRMS (ESI) calcd for $C_{18}H_{15}N_5O_5S$ $[M+H]^+$ 414.0868, found: 414.0873; Purity > 97%.

*4.1.5.3.3. 4-amino-N-(5-fluoro-2-methoxyphenyl)-6-((4-nitrophenyl)thio)pyrimidine-5-carboxamide (**13c**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, J = 4.5 Hz, 1H), 8.28–8.16 (m, 3H), 8.03 (d, J = 10.0 Hz, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.60–7.46 (m, 1H), 7.06 (dd, J = 9.0, 5.2 Hz, 1H), 6.95 (dd, J = 8.6, 3.0 Hz, 1H), 3.80 (s, 3H). HRMS (ESI) calcd for $C_{18}H_{14}FN_5O_4S$ $[M+H]^+$ 416.0825, found: 416.0834; Purity > 98%.

*4.1.5.3.4. 4-amino-N-(5-chloro-2-methoxyphenyl)-6-((4-nitrophenyl)thio)pyrimidine-5-carboxamide (**13d**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.20 (d, J = 7.0 Hz, 3H), 7.73 (d, J = 8.7 Hz, 2H), 7.19 (dd, J = 8.8, 2.6 Hz, 1H), 7.14 (s, 1H), 7.08 (d, J = 8.8 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.75, 160.76, 160.39, 157.86, 149.50, 147.29, 140.54, 134.13, 128.63, 124.88, 124.19, 122.55, 113.74, 113.28, 56.69. HRMS (ESI) calcd for $C_{18}H_{14}ClN_5O_4S$ $[M+H]^+$ 432.0529, found: 432.0534; Purity > 97%.

4.2. In vitro antiviral assays

The antiviral activities of the compounds were determined by the HIV-1 p24 assay. H9 cells were infected with HIV−1_{IIIB} at a multiplicity of infection (MOI) of 0.03 for 2 h, then washed three times to remove free virus and resuspended with RPMI-1640. Serial diluted compounds and 4×10^4 cells/well infected cells were added into 96-well cell culture plates and incubated at 37 °C with 5% CO_2 for 7 days. The p24 level of the culture supernatant was measured by in-house ELISA assay [30]. The 50% effective concentrations (EC_{50}) were calculated.

4.3. Cytotoxicity assays

The cytotoxicity of the compounds on H9 were determined by MTT colorimetric assay described previously [31]. The prepared compounds with different concentrations were added into 96 well culture plate and maintained in RPMI-1640 complete medium (containing 10% FBS). 2×10^5 cell/well H9 were added and co-incubated at 37 °C, 5% CO_2 for seven days. The cell viability was determined by using MTT, and the 50% cytotoxicity concentration (CC_{50}) was calculated.

4.4. Molecular docking

The crystal structure of HIV-1 Vif was gained from Protein Data Bank (PDB ID 4N9F), and the binding site (X = 72.881, Y = −69.580, Z = −181.951) prepared for docking was chosen based on our previous work [25]. Docking studies were performed with the AutoDock (4.2) suite of programs. Binding sites were defined from

receptor cavities by using “define and edit binding site” method of Discovery Studio 3.1.

4.5. Western blot assays

Cells were treated with **6m** at the concentrations of 50, 10, 2 μM at 37 °C and cultured for 48 h. After that, the cells were harvested, washed with ice-cold PBS, and lysed with RIPA buffer to extract the total protein (Beyotime, China). The expression of A3G-HA and Vif protein was detected by Western blotting. The target proteins were separated by SDS polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. The PVDF membranes with proteins were incubated overnight with primary antibodies at 4 °C. Membranes were probed with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 2 h. The membranes were washed thoroughly, stained with Chemiluminescent HRP Substrate (Millipore, USA), and exposed to X-ray film.

The information of the antibodies was as follow: anti-HIV-1 Vif antibody [319] (ab66643, Abcam, UK); anti-APOBEC3GP antibody (sc-130689, Santa Cruz Biotechnology, Inc, USA); anti-HA antibody [HA-7] (H3663, Sigma Aldrich, USA); anti-β-actin antibody (cw0096a, CWBIO, China).

4.6. Drug resistant virus strains assays

2×10^5 cells/well TZM-bl cells were placed in 96-well cell and cultured overnight at 37 °C with 5% CO_2. Next day, compounds with different concentrations and virus dilutions were added into each well: pNL4-$3_{gp41(36G)V38A,N42T}$ (MOI = 0.03), HIV-$1_{4755\text{-}5}$ (MOI = 0.06), HIV-1_{A17} (MOI = 0.03), HIV-$1_{L10R/M46I/L63P/V82T/I84V}$ (MOI = 0.03), pYU$2_{G140S/Q148H}$ (MOI = 0.03). After 48 h, the supernatant was removed, cells were washed with 200 μL PBS twice and lysed with a lysis buffer at 4 °C for 30 min. Then the cell lysate was transferred to 96 well and the prepared luciferase substrate was added. The relative fluorescence unit (RFU) of each well was determined by a microplate reader (FLEX Station 3) and the 50% effective concentrations (EC_{50}) were calculated.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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