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ARTICLE

Identification of Benzenesulfonamide quinoline derivatives as potent HIV-1 replication inhibitors targeting Rev protein

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Human immunodeficiency virus type 1 (HIV-1) Rev protein facilitates the export of viral RNA from nucleus to cytoplasm, which is a key step in HIV-1 pathogenesis and transmission. In this study, we screened a commercial library and identified a hit compound **1** bearing benzenesulfonamide quinoline scaffold that inhibited Rev activity and HIV-1 infectivity. Compounds bearing this scaffold were synthesized and the SAR was studied. We identified compound **20** with low toxicity and potent activity to inhibit HIV-1 replication through affecting Rev function.

Introduction

AIDS has been a worldwide epidemic for more than 30 years and it has caused 1.6 million deaths every year. The agents in high active antiretroviral therapy (HAART) protocol target several HIV-1 enzymes which play key roles in the virus life cycle and HAART has become the standard therapy for AIDS. However, the serious side effects, the fast mutation of HIV-1 virus and high cost of life-long HAART have raised the requirement of new therapeutics targeting other viral proteins other than integrase, protease or reverse transcriptase. Rev is a regulatory protein encoded by HIV-1 which plays a key role in the nuclear export of unspliced or incompletely spliced HIV-1 mRNA from nucleus to cytoplasm.[1-3] Rev specifically binds to an RNA stem-loop structure termed RRE which locates in all unspliced or incompletely spliced HIV-1 transcripts.[4, 5] Rev also contains a leucine-rich nuclear export signal (NES) that allows the RNA-protein complex to access a nuclear export pathway.[6] Multiple lines of evidence indicate that Rev-RRE transport system requires cellular co-factors such as crm-1, Ran, DDX1, DDX3, Nup214 and Sam68.[7-10] The crucial role of Rev in viral replication and activation makes it a good target to develop new chemotherapy methods against HIV-1. There have been reports of Rev inhibitors from natural products, peptide mimics and synthesized compounds.[11-17] These reported Rev inhibitors showed the IC_{50} of anti-HIV-1 activity at hundreds of micromolar to submicromolar level. Given that no anti-HIV-1 drug targeting Rev is in the clinical trial, the discovery and development of new chemical compounds targeting Rev with potent anti-HIV-1 activity and low toxicity are of great significance.

Results and discussion

Hit Identification by High-throughput Screening (HTS) and Anti-HIV-1 Replication Assay

In our study, High-throughput screening (HTS) system was developed in 293T cells to test the anti-Rev activity and it was applied to screen a commercial available drug-like chemical library of 20155 compounds (Enamine). In the HTS system, PDM628 and PCDNA3.1-Rev plasmids were co-transfected into 293T cells, followed by the treatment of compounds in 50 μM concentration for 48 h and then the luciferase expression was recorded. [18] The HTS results was summarized in Table 1 and 2,4-Difluoro-*N*-8-quinolinyl-benzenesulfonamide (1) was identified as a hit compound to inhibit Rev function (Figure 1, A).

This compound was further tested in the anti-HIV-1 infectivity assay. In brief, In brief, H9 and SupT1 cells (1×10^6) were infected with the equivalent of 5 ng HIV-1 p24 for 3 h. HIV-1 replication was monitored by the quantification of p24 antigen in supernatant by ELISA after 4 days. The IC_{50} value of

compound **1** was 0.23 μM (Figure 2). Although compound **1** showed potent anti-HIV-1 replication activity, it also showed high toxicity with the CC_{50} value of 5.1 μM in MTT test in 293T cells (Table 2).

Table 1. Results of the screening of compound libraries

Parameter	No. (%)
Compounds screened	20,155
Primary hits (inhibition of Rev/RRE system in the HTS screen)[a]	346 (1.7)
Primary hits inhibiting wide type HIV-1[b]	79 (0.4)
Specific hits displaying a inhibition at 25 μM[c]	12 (0.06)

[a] Rev/RRE system was generated by transfection of 293T cells with PDM628 and PCDNA3.1-Rev plasmids. [b] A total of 346 primary hits inhibited wide type HIV-1 by more than 50% at 50 μM. [c] Some compound hits inhibited wide type HIV-1 by more than 50% at 25 μM.

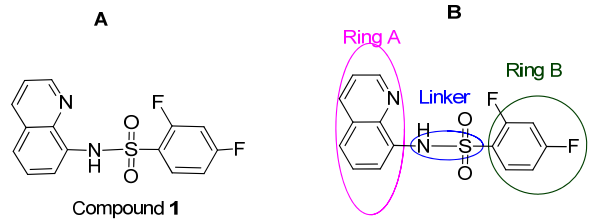


Figure 1. (A) Hit compound (**1**) identified from HTS. (B) Hit substructures for SAR studies.

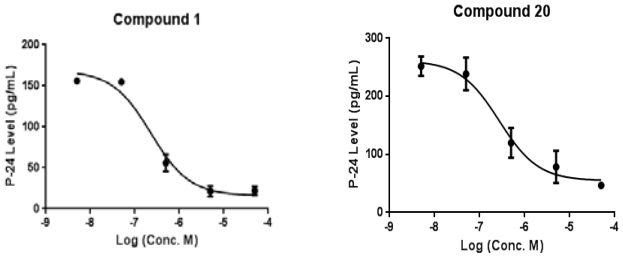
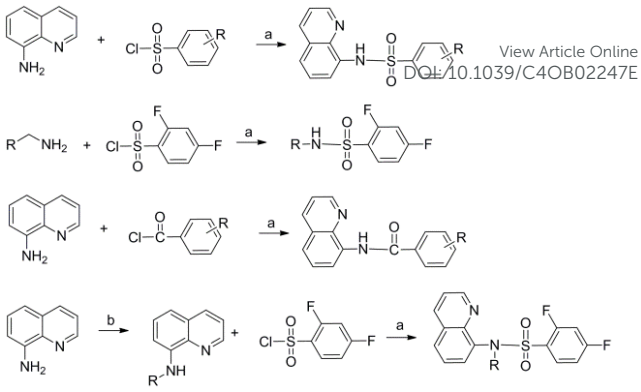


Figure 2. Dose–response curve for hit compound **1** and compound **20** yielded IC_{50} values of 0.23 μM and 0.27μM respectively.

Chemical Synthesis

The synthesis of series of compounds bearing the benzenesulfonamide quinoline scaffold or similar structures was carried out as outlined in Scheme 1. The derivatives of 8-aminoquinoline or neucleobases were coupled with aromatic sulfonamide compounds in pyridine. The alkylation of benzenesulfonamide quinoline was achieved with alkyl iodide with reflux in ethanol. The alkyl substitution on nitrogen of sulfonamide linker did not obviously affect the followed reactions.



Scheme 1. Reagents and conditions: (a) 25 ºC -60 ºC, pyridine. (b) Methyl iodide /ethyl iodide, ethanol, reflux

SAR

In SAR studies, synthesized compounds were first tested for their anti-Rev activity with the protocol similar with HTS. The active compounds with low toxicity (CC_{50}>50 μM) were then tested their anti-HIV infectivity activities. The structure of compound **1** was divided into substituted quinoline (A), benzne ring (B), and the linker (Figure 1, B). These substructures were investigated their influences on the activities in both assays.

Firstly, the effects of ring A's modifications on the anti-Rev activity was studied. The quinoline was replaced with naphthalene, benzene or pyridine to test the effects of the size and the H-bond acceptor atom of the quinoline structure (compound **2-4**, Table 2). The nucleobases or their analogues have been widely used in drugs which target RNA, DNA or their interactions with proteins. Therefore compounds 5-7 bearing adenine, cytosine or guanine were synthesized to replace quinoline substructure. However, these modifications led to the loss of activities. These results showed that the quinoline substructure was required for their anti-Rev and anti-HIV-1 replication activities.

It was thought that the 2,4-difluoride substitution on ring B could be replaced with other halogen atoms that reserved the electron withdrawing property. Compound **8** with 2,4-dichloro substitution showed similar activity with compound **1**. However, compound **9-11** with other mono- or di-halogen substitutions resulted in a loss of the activities, as did the compounds (**12-14)** with non-halogen electron-withdrawing groups on ring B (Table 2). Then the compounds with no substitution (**15**) and with electron-donating groups (**16-17**) were synthesized and tested but these modifications abolished the activities (Table 2). These results indicated that the 2,4-difluoro substitution was vital for the anti-Rev activity and the exact mechanism should be more complicated than their electron withdrawing property.

The influences of the linker were then studied (Table 3). Firstly, the sulfonamide linker was replaced by amide structure (**18**). Compound **18** was active in anti-Rev assay, but it showed

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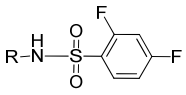
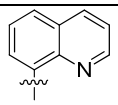
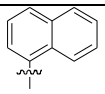
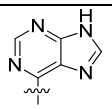
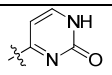
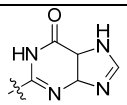
lower activity in anti-HIV-1 infectivity assay than compound **1**. Interestingly, compounds (**19**, **20**) with methyl or ethyl substitutions on the nitrogen atom of sulfonamide were active in both anti-Rev assay and anti-HIV-1 infectivity assay. The compound **20** showed potent anti-HIV-1 activity (IC_{50}= 0.27 μM, Figure 2) and more than 10 fold lower toxicity (CC_{50}>50 μM) than hit compound **1**.

Table 2. Influence of modifications of ring A on anti-Rev activity.

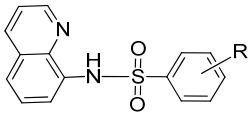


Compd No.	R	Anti-Rev Assay	CC_{50} (μM)[a]	IC_{50} (μM)[b]
1		+	5.1	0.23
2		+	>30	7.1
3		–[c]	>20	>20
4		–	>20	>20
5		–	>50	>20
6		–	>50	>20
7		–	>50	>20

CC_{50} (μM)[a] : median (50%) cytotoxic concentration ;IC_{50}(μM)[b]: half maximal (50%) inhibitory concentration

–[c]: test result did not show activity

Table 3. Influence of modifications of ring B on anti-Rev activity.



Compd No.	R	Anti-Rev Assay	CC_{50} (μM)	IC_{50} (μM)
8	2-Cl,4-Cl	+[c]	>50	0.47[d]
9	2-F	–	>50	>20

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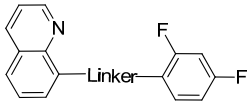
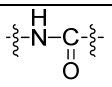
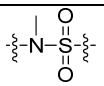
10	4-F	–	>50	>20
11	4-Cl	–	>50	>20
12	4-CF_3	–	>50	>20
13	3-NO_2	–	>30	>20
14	4-NO_2	–	>30	>20
15	H	–	>50	>20
16	4-Me	–	>50	>20
17	4-OMe	–	>50	>20

Table4. Influence of modifications of linker on anti-Rev activity.



Compd No.	Linker	Anti-Rev Assay	CC_{50} (μM)	IC_{50} (μM)
18		+	>50	48
19		+	>50	1.18
20		+	>50	0.27

Compound 1 and 20 affected the Rev function

The substructures of both 8-aminoquinoline and sulfonamide are distributed in drugs and natural products with potent biological activities. Therefore it is crucial to further clarify the anti-HIV-1 mechanism and the corresponding targets of compound **1** and **20**. Firstly, the quantitative changes of relative RRE between nucleus and cytoplasm after the treatment of compound **1** and **20** were measured by real-time PCR. In Brief, the 293T cells were transfected with vector PDM628 or co-transfected with Rev, and then treated with compound **1**. Fractionation of cytoplasmic and nuclear components was isolated from 293T cells and RNA was then extracted from each of the fractions. Reverse transcription reactions were performed with RT reagent and the cytoplasmic/nuclear ratios of RRE were detected with real-time PCR.As shown in Figure 3, compound **1** (A) and **20** (B) caused about 3-fold decrease of RRE-containing mRNA in cytoplasm while 2-fold increase in nucleus. This result showed that the potent activity of compound **1** and **20** was related with the inhibition of Rev function of nuclear export of HIV-1 mRNA from nucleus to cytoplasm.

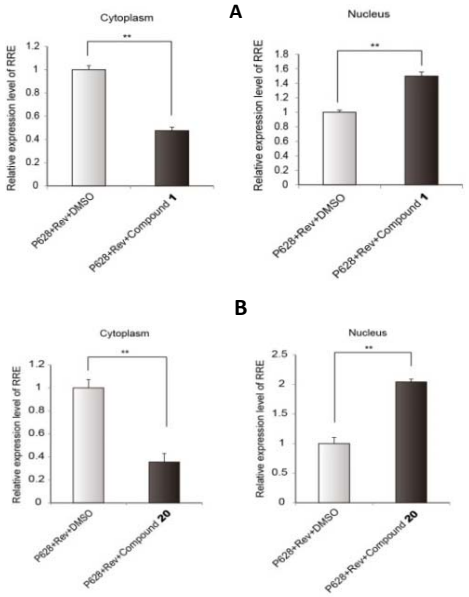


Figure 3. (A) The expression of RRE in the cytoplasm (left) and nucleus (right) was changed by compound **1**. (B) The similar test

was applied to compound **20**. Data represent three independent expriments. ** P<0.01 using a student's *t* test.

To test whether compound **20** inhibited HIV-1 activity in a Rev dependent way, the RRE sequence in the plasmid pMDLg/RRE was replaced by a constitutive transport element (CTE) of the type D Mason-Pfizer monkey virus (MPMV) to construct the plasmid pMDLg/CTE, which expresses protein in a Rev-independent manner. [19] The 293T cells were co-transfected with pMDLg/RRE and Rev or pMDLg/CTE and Rev, and treated with compound **20**. The p-24 level showed that compound **20** totally lost its activity to block the nuclear export of viral RNA in the CTE/REV system (Figure 4 A) but inhibited the nuclear export of viral RNA in the RRE/Rev system (Figure 4 B). These data demonstrated that the compound **20** could inhibit HIV-1 replication by targeting the export of Rev/RRE from nucleus.

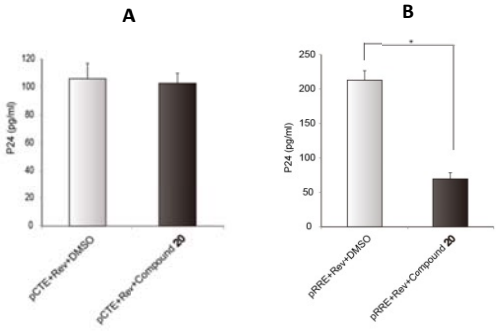


Figure 4. The compound **20** affects the export of Rev/RRE but not CTE-dependent gene expression. (A) The 293T cells were co-transfected with pMDLg/CTE and Rev, and treated with compound **20**. The expression of P24 was detected by ELISA. (B) The 293T cells were co-transfected with pMDLg/RRE and Rev and treated with compound **20**. The expression of P24 was detected by ELISA. Data represent three independent expriments. *P<0.05 using a student's *t* test.

Conclusions

From the HTS hit (**1**) with potent anti-HIV-1 activity but high toxicity, we identified compound **20** to inhibit HIV-1 infectivity by SAR studies. For compound **20**, the toxicity was decreased more than 10 fold, while the potent anti-HIV-1 activity was still similar to compound **1**. Moreover, their anti-HIV-1 mechanism was clarified by measuring the quantitative change of RRE between nucleus and cytoplasm in a Rev/RRE dependent way. Together, compound **20** could be a good starting point to develop new chemical compounds with potent anti-HIV-1 activity through specifically targeting the Rev function.

Experimental

Chemistry

General Chemistry Methods.

All reagents and solvents were purchased from Sigma-Aldrich, J&K Chemical and Aladdin. Analytical thin-layer plates were obtained from Qingdao Haiyang Chemical. Flash chromatography was performed with silica gel 60. NMR spectra were recorded on a Bruker Avance 400 with the solvents indicated. Purity was analyzed by reverse-phase HPLC performed on Agilent 6130 Quadrupole and Agilent 1200 equipment, or on Agilent ProStar 218 system. HRMS were recorded at the Instrumental Analysis & Research Center in Sun Yat-sen University using a Thermo Scientific LTQ-Orbitrap Elite mass spectrometer.

2,4-Difluoro-*N*-(quinolin-8-yl)benzenesulfonamide (1). To the solution of 8-aminoquinoline (119 mg, 0.83 mmol) and 2,4-Difluorobenzenesulfonyl chloride (93 μL, 0.69 mmol) in pyridine (3 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was washed with HCl solution (2N) (3×20 mL) and extracted with hexane. The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was purified by silica gel chromatography using cyclohexane-ethyl acetate (6:1) to give **1** as white powder (204 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.50 (s, 1H), 8.81 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.11 (dd, *J*= 8.3, 1.6 Hz, 1H), 8.00 (td, *J*= 8.4, 6.2 Hz, 1H), 7.75 (dd, *J*= 7.6, 1.1 Hz, 1H), 7.52–7.37 (m, 3H), 6.94–6.86 (m, 1H), 6.78 (ddd, *J*= 10.0, 8.5, 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.29 (dd, *J* = 257.9, 11.3 Hz), 161.23 (dd, *J* = 259.8, 13.0 Hz), 150.27,139.93, 137.55, 134.57, 134.03 (d, *J* = 10.6 Hz), 129.58, 128.09, 125.06 (d, *J* = 14.0 Hz), 123.81, 123.48, 116.09, 112.94 (dd, *J* = 22.0, 3.4 Hz), 107.05 (t, *J* = 25.4 Hz). HRMS (ESI-) m/z: calcd for C15H9O2N2F2S [M - H]-: 319.03583. Found: 319.03584 (Δ = 0.04 ppm).

2,4-Difluoro-*N*-(naphthalen-1-yl)benzenesulfonamide (2). The title compound was synthesized according to the procedure of preparing compound **1** except using 8-aminonaphthalene instead of 4-methylbenzaldehyde.White powder (42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, *J*= 8.3 Hz, 1H), 7.83 (d, *J*= 7.5 Hz, 1H), 7.79–7.69 (m, 2H), 7.58–7.47 (m, 2H), 7.44–7.31 (m, 2H), 7.10 (s, 1H), 6.89 (dddd, J= 10.1, 9.0, 8.2, 1.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d6) δ 165.53 (dd, *J* = 254.1, 11.8 Hz), 159.54 (dd, *J* = 256.9, 13.3 Hz), 134.32, 132.54 (d, *J* = 10.8 Hz), 132.05, 130.11, 128.51, 127.69, 126.75 (d, *J* = 6.2 Hz), 126.02, 125.06 (d, *J* = 14.3 Hz), 124.35, 123.19, 112.67 (d, *J* = 22.2 Hz), 106.43 (t, *J* = 26.1 Hz). HRMS (ESI+) m/z: calcd for C16H11O2NF2NaS [M + Na]+ : 342.03708. Found: 342.03715 (Δ = 0.21 ppm).

2,4-Difluoro-*N*-phenylbenzenesulfonamide (3). The title compound was synthesized according to the procedure of preparing compound 1 except using aniline instead of 4-methylbenzaldehyde.White powder (75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (ddd, *J*= 8.3, 7.2, 3.9 Hz, 1H), 7.31–7.19 (m, 2H), 7.17–7.07 (m, 3H), 6.99–6.89 (m, 2H), 6.80 (s, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 167.40 (dd, *J* = 258.3, 11.6 Hz), 160.87 (dd, *J* = 257.3, 12.9 Hz), 136.97, 134.15 (d, *J* = 10.5 Hz), 2×130.84, 127.28, 124.60 (d, *J* = 13.5 Hz), 2×122.86 , 113.38 (dd, *J* = 21.9, 3.3 Hz), 106.90 (t, *J* = 25.6 Hz). HRMS (ESI^+) m/z: calcd for C12H9O2NF2NaS $[M + Na]^+$: 292.02143. Found: 292.02169 (Δ = 0.9 ppm).

2,4-Difluoro-*N*-(pyridin-2-ylmethyl)benzenesulfonamide (4). The title compound was synthesized according to the procedure of preparing compound **1** except using pyridin-2-yl-methylamine instead of 4-methylbenzaldehyde.White powder (38% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, *J*= 4.4 Hz, 1H), 7.88 (td, *J*= 8.5, 6.2 Hz, 1H), 7.60 (td, *J*= 7.7, 1.8 Hz, 1H), 7.16 (dd, *J*= 11.5, 6.5 Hz, 2H), 6.98–6.90 (m, 1H), 6.89–6.81 (m, 1H), 6.21 (s, 1H), 4.33 (d, *J*= 5.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.04 (dd, *J* = 256.8, 11.8 Hz), 161.04 (dd, *J* = 257.7, 13.0 Hz), 155.84, 150.53, 138.12, 133.32 (d, *J* = 10.3 Hz), 126.00, 124.08, 123.18, 112.94 (d, *J* = 18.5 Hz), 106.79 (t, *J* = 25.6 Hz), 48.93.HRMS (ESI$^+$) m/z: calcd for $C_{12}H_{11}O_2N_2F_2S$ $[M + H]^+$: 285.05038. Found: 285.05045 (Δ = 0.24 ppm).

2,4-Difluoro-*N*-(7*H*-purin-6-yl)benzenesulfonamide (5). The title compound was synthesized according to the procedure of preparing compound **1** except using adenine instead of 4-methylbenzaldehyde.White powder (36% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.67 (s, 1H), 8.33 (td, *J*= 8.7, 6.0 Hz, 1H), 8.12 (s, 1H), 7.70 (s, 2H), 7.62 (ddd, *J*= 11.2, 9.1, 2.4 Hz, 1H), 7.46 (td, *J*= 8.4, 2.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 168.81 (dd, *J* = 258.7, 12.3 Hz), 161.33 (dd, *J* = 259.7, 14.1 Hz), 158.08, 156.20, 150.18, 140.10, 135.85 (d, *J* = 11.7 Hz), 122.79 (d, *J* = 16.1 Hz), 120.41, 115.11 (d, *J* = 25.6 Hz), 108.65 (t, *J* = 25.9 Hz). HRMS (ESI$^+$) m/z: calcd for $C_{11}H_8O_2N_5F_2S$ $[M + H]^+$: 312.03613. Found: 312.03640 (Δ = 0.87 ppm).

2,4-Difluoro-*N*-(2-oxo-1,2-dihydropyrimidin-4-yl)benzenesulfonamide (6). The title compound was synthesized according to the procedure of preparing compound **1** except using cytosine instead of 4-methylbenzaldehyde.White powder (36% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.16–7.95 (m, 4H), 7.71–7.56 (m, 1H), 7.40 (td, *J*= 8.6, 2.1 Hz, 1H), 6.00 (d, *J*= 7.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.99 (dd, *J* = 256.8, 12.3 Hz),167.82, 160.68 (dd, *J* = 257.6, 13.9 Hz), 152.52, 141.14, 136.34 (d, *J* = 11.3 Hz), 123.79 (d, *J* = 13.0 Hz), 114.53 (d, *J* = 20.2 Hz), 107.96 (t, *J* = 26.1 Hz), 99.61. HRMS (ESI$^+$) m/z: calcd for $C_{10}H_8O_3N_3F_2S$ $[M + H]^+$: 288.02489. Found: 288.02523 (Δ = 1.16 ppm).

2,4-Difluoro-*N*-(6-oxo-6,7-dihydro-1*H*-purin-2-yl)benzenesulfonamide (7). The title compound was synthesized according to the procedure of preparing compound **1** except using guanine instead of 4-methylbenzaldehyde.White powder (6% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.97 (s, 1H), 8.22 (td, *J*= 8.7, 6.1 Hz, 1H), 8.16 (s, 1H), 7.65 (ddd, *J*= 11.3, 9.2, 2.4 Hz, 1H), 7.43 (td, *J*= 8.4, 2.2 Hz, 1H), 6.73 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) 167.33 (dd, *J* = 258.5, 12.1 Hz), 159.86 (dd, *J* = 259.9, 14.1 Hz), 156.60 , 155.14, 150.94, 134.87, 134.37 (d, *J* = 11.5 Hz), 121.52 (d, *J* = 12.6 Hz), 117.28, 113.56 (d, *J* = 20.6 Hz), 107.25 (t, *J* = 25.8 Hz). HRMS (ESI$^+$) m/z: calcd for $C_{11}H_8O_3N_5F_2S$ $[M + H]^+$: 328.03104. Found: 328.03148 (Δ = 1.33 ppm).

2,4-Dichloro-*N*-(quinolin-8-yl)benzenesulfonamide (8). The title compound was synthesized according to the procedure of preparing compound **1** except using 2,4-dichloro-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (56% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.81 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.20–8.05 (m, 2H), 7.70 (dd, *J*= 7.6, 1.2 Hz, 1H), 7.45 (dd, *J*= 8.3, 4.2 Hz, 2H), 7.37 (dd, *J*= 12.8, 4.8 Hz, 2H), 7.32 (dd, *J*= 8.5, 2.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.17, 141.16, 139.72, 137.52, 136.54, 134.52, 134.22, 133.00, 129.64, 128.44, 128.09, 123.56 (d, *J* = 8.3 Hz), 115.31. HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{11}O_2N_2Cl_2S$ $[M + H]^+$: 352.99128. Found: 352.99136 (Δ = 0.23 ppm).

2-Fluoro-*N*-(quinolin-8-yl)benzenesulfonamide (9). The title compound was synthesized according to the procedure of preparing compound **1** except using 2-fluoro-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (56% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.81 (dd, *J*= 4.1, 1.2 Hz, 1H), 8.13 (dd, *J*= 8.2, 1.0 Hz, 1H), 8.01 (td, *J*= 7.8, 1.6 Hz, 1H), 7.8 –7.73 (m, 1H), 7.52–7.38 (m, 4H), 7.20 (t, *J*= 7.6 Hz, 1H), 7.04 (t, *J*= 9.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.10 (d, *J* = 254.5 Hz), 151.37, 140.70, 138.58, 137.95 (d, *J* = 8.7 Hz), 134.65, 132.27, 129.93, 128.63, 128.50, 126.67, 125.57, 124.30, 119.38, 119.06 (d, *J* = 20.9 Hz). HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{12}O_2N_2FS$ $[M + H]^+$: 303.05980. Found: 303.06004 (Δ = 0.78 ppm).

4-Fluoro-*N*-(quinolin-8-yl)benzenesulfonamide (10). The title compound was synthesized according to the procedure of preparing compound **1** except using 4-fluoro-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (30% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.76 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.11 (dd, *J*= 8.3, 1.6 Hz, 1H), 7.98–7.88 (m, 2H), 7.83 (dd, *J*= 7.3, 1.6 Hz, 1H), 7.50–7.38 (m, 3H), 7.05–6.93 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.53 (d, *J* = 255.1 Hz), 150.19, 140.02, 137.65, 136.80, 134.94, 131.35, 131.26, 129.60, 128.17 , 123.86, 123.40, 117.58, 117.35, 116.86. HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{12}O_2N_2FS$ $[M + H]^+$: 303.05980. Found: 303.06008 (Δ = 0.91 ppm).

4-Chloro-*N*-(quinolin-8-yl)benzenesulfonamide (11). The title compound was synthesized according to the procedure of preparing compound **1** except using 4-chloro-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.76 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.11 (dd, *J*= 8.3, 1.5 Hz, 1H), 7.89–7.75 (m, 3H), 7.53–7.38 (m, 3H), 7.39–7.29 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.27, 140.96 , 140.32, 139.76, 138.34, 135.22, 2×131.05, 2×130.70, 130.00, 128.48, 125.37, 124.15, 119.56. HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{12}O_2N_2ClS$ $[M + H]^+$: 319.03025. Found: 319.03057 (Δ = 1 ppm).

N-(quinolin-8-yl)-4-(trifluoromethyl)benzenesulfonamide (12). The title compound was synthesized according to the procedure of preparing compound **1** except using 4-trifluoromethyl-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (72% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.55 (s, 1H), 8.79 (dd, *J*= 4.2, 1.7 Hz, 1H), 8.34 (dd, *J*= 8.3, 1.6 Hz, 1H), 8.31–8.25 (m, 2H), 8.15–8.09 (m, 2H), 7.71 (ddd, *J*= 11.8, 8.0, 1.2 Hz,2H), 7.55 (dd, *J*= 8.2, 4.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.30, 144.33, 139.96,

137.72, 136.05, 135.73, 134.57 , 129.64, 129.05, 128.18, 127.40 (d, *J* = 3.5 Hz), 125.80 , 124.18, 123.49, 123.09, 116.94. HRMS (ESI$^+$) m/z: calcd for $C_{16}H_{12}O_2N_2F_3S$ [M + H]$^+$: 353.05661. Found: 353.05700 (Δ = 1.11 ppm).

3-Nitro-*N*-(quinolin-8-yl)benzenesulfonamide (13). The title compound was synthesized according to the procedure of preparing compound **1** except using 3-nitro-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (36% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) *δ* 8.78 (dd, *J*= 4.2, 1.7 Hz, 1H), 8.68 (t, *J*= 1.9 Hz, 1H), 8.40–8.31 (m, 2H), 8.24 (ddd, *J*= 7.9, 1.7, 1.0 Hz, 1H), 7.79–7.68 (m, 3H), 7.60–7.49 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ* 151.21, 149.51, 143.29, 141.46, 138.85, 134.64, 132.83 , 130.20, 129.26, 128.63, 126.52, 124.10, 123.76, 122.46 . HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{12}O_4N_3S$ [M + H]$^+$: 330.05430. Found: 330.05464 (Δ = 1.02 ppm).

4-Nitro-*N*-(quinolin-8-yl)benzenesulfonamide (14). The title compound was synthesized according to the procedure of preparing compound **1** except using 4-nitrro-benzenesulfonylchloride instead of 2,4-difluoro-benzenesulfonyl chloride. Gray powder (36% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) *δ* 10.40 (s, 1H), 8.79 (dd, *J*= 4.2, 1.7 Hz, 1H), 8.35 (dd, *J*= 8.3, 1.6 Hz, 1H), 8.08 (d, *J*= 8.2 Hz, 2H), 7.86 (d, *J*= 8.3 Hz, 2H), 7.76–7.67 (m, 2H), 7.62–7.49 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*151.65, 151.36, 147.22, 141.43, 138.34, 134.95, 2×130.35, 130.10, 128.48, 2×126.16, 126.03, 124.13, 120.99. HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{12}O_4N_3S$ [M + H]$^+$: 330.05430. Found: 330.05438 (Δ = 0.23 ppm).

N-(quinolin-8-yl)benzenesulfonamide (15). The title compound was synthesized according to the procedure of preparing compound **1** except using benzenesulfonylchloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 9.24 (s, 1H), 8.75 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.09 (dd, *J*= 8.3, 1.6 Hz, 1H), 8.01–7.89 (m, 2H), 7.83 (dd, *J*= 6.8, 2.0 Hz, 1H), 7.47–7.39 (m, 4H), 7.36 (dd, *J*= 10.5, 4.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 150.08, 140.83, 139.94, 137.58, 135.15, 134.21,2× 130.22, 129.56, 2×128.55, 128.19, 123.53, 123.31, 116.49. HRMS (ESI$^+$) m/z: calcd for $C_{15}H_{13}O_2N_3S$ [M + H]$^+$: 285.06922. Found: 285.06932 (Δ = 0.33 ppm).

4-Methyl-*N*-(quinolin-8-yl)benzenesulfonamide (16). The title compound was synthesized according to the procedure of preparing compound **1** except using 4-methyl-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (69% yield). ^{1}H NMR (500 MHz, DMSO-*d6*) *δ* 9.86 (s, 1H), 8.87 (dd, *J*= 4.2, 1.7 Hz, 1H), 8.35 (dd, *J*= 8.3, 1.6 Hz, 1H), 7.80 (d, *J*= 8.3 Hz, 2H), 7.66 (ddd, *J*= 15.0, 7.9, 1.1 Hz, 2H), 7.59 (dd, *J*= 8.3, 4.2 Hz, 1H), 7.55–7.48 (m, 1H), 7.28 (d, *J*= 8.0 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO) *δ* 151.18,145.42,140.40,138.37,135.45,2×131.43, 129.91,2×128.78,128.51,124.66,124.19,117.98,22.72. HRMS (ESI$^+$) m/z: calcd for $C_{16}H_{15}O_2N_2S$ [M + H]$^+$: 299.15497. Found: 299.15503 (Δ = 0.21 ppm).

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4-Methoxy-*N*-(quinolin-8-yl)benzenesulfonamide (17). The title compound was synthesized according to the procedure of preparing compound **1** except using 4-methoxy-benzenesulfonyl chloride instead of 2,4-difluoro-benzenesulfonyl chloride. White powder (91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 9.19 (s, 1H), 8.75 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.08 (dd, *J*= 8.3, 1.6 Hz, 1H), 7.87–7.83 (m, 2H), 7.81 (dd, *J*= 6.4, 2.5 Hz, 1H), 7.49–7.38 (m, 3H), 6.86–6.76 (m, 2H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 164.39,150.03, 139.96,137.56,135.36,132.46,2×130.75,129.56,128.19,123.30 ,116.31,2×115.41,56.83. HRMS (ESI$^+$) m/z: calcd for $C_{16}H_{15}O_3N_2S$ [M + H]$^+$: 315.07979. Found: 315.08006 (Δ = 0.86 ppm).

2,4-Difluoro-*N*-(quinolin-8-yl)benzamide (18). To the solution of 8-Aminoquinoline (280 mg, 1.94 mmol) and 2,4-Difluorobenzoyl chloride (200 L, 1.61 mmol) in pyridine (3 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was washed with HCl solution (2N) (3×20 mL) and extracted with dichloromethane (20 mL). The organic phase was collected and concentrated under vacuum. The product was purified by silica gel chromatography using cyclohexane-ethyl acetate (10:1) as eluate to give **18** as white powder (311 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 11.11 (d, *J*= 11.8 Hz, 1H), 8.95 (dd, *J*= 7.2, 1.7 Hz, 1H), 8.87 (dd, *J*= 4.2, 1.6 Hz, 1H), 8.26 (td, *J*= 8.8, 6.6 Hz, 1H), 8.19 (dd, *J*= 8.3, 1.6 Hz, 1H), 7.63–7.53 (m, 2H), 7.48 (dd, *J*= 8.2, 4.2 Hz, 1H), 7.12–7.04 (m, 1H), 6.99 (ddd, *J*= 11.2, 8.5, 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 166.42 (dd, *J* = 255.5, 12.6 Hz), 162.35 (dd, *J* = 251.8, 12.2 Hz), 162.04, 149.88, 140.19, 137.66, 136.11, 135.22 (d, *J* = 10.2 Hz), 129.38, 128.76, 123.50, 123.08, 119.97 (d, *J* = 11.8 Hz), 118.67 , 113.81 (dd, *J* = 21.3, 2.9 Hz), 105.89 (t, *J* = 28.2 Hz). HRMS (ESI$^+$) m/z: calcd for $C_{16}H_{11}ON_2F_2$ [M + H]$^+$: 285.08340. Found: 285.08371 (Δ = 1.1 ppm).

N-methylquinolin-8-amine (19-0). To the solution of 8-Aminoquinoline (500 mg, 3.5 mmol) was added iodomethane (199 *μ*L, 3.2 mmol) in ethanol (3 mL). The mixture was refluxed for 20h. The reaction mixture was concentrated under vacuum and purified by silica gel chromatography using cyclohexane-ethyl acetate (20:1) as eluate to give **19-0** as yellow oil (310 mg, 61%). ^{1}H NMR (400 MHz, DMSO-*d6*) *δ* 8.69 (dd, *J*= 4.1, 1.7 Hz, 1H), 8.17 (dd, *J*= 8.3, 1.6 Hz, 1H), 7.46 (dd, *J*= 8.3, 4.2 Hz, 1H), 7.35 (t, *J*= 7.9 Hz, 1H), 7.02 (dd, *J*= 8.1, 0.9 Hz, 1H), 6.56 (t, *J*= 7.4 Hz, 2H), 2.89 (d, *J*= 5.2 Hz, 3H).

2,4-Difluoro-*N*-methyl-*N*-(quinolin-8-yl)benzenesulfonamide (19). To the solution of N-methylquinolin-8-amine (142 mg, 0.90 mmol) and 2,4-Difluorobenzenesulfonyl chloride (101 mL, 0.75 mmol) in pyridine (3 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was washed with HCl solution (2N) (3 × 20 mL) and extracted with dichloromethane (20 mL). The organic phase was collected and concentrated under vacuum. The product was purified by

silica gel chromatography using cyclohexane-ethyl acetate (6:1) as eluate to give **19** as white powder (60 mg, 24%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.53 (dd, *J*= 4.2, 1.7 Hz, 1H), 8.35 (dd, *J*= 8.3, 1.7 Hz, 1H), 7.98 (dd, *J*= 8.2, 1.3 Hz, 1H), 7.72 (dd, *J*= 7.4, 1.4 Hz, 1H), 7.65–7.57 (m, 1H), 7.57–7.49 (m, 1H), 7.48–7.38 (m, 2H), 7.02 (d, *J*= 2.4 Hz, 1H), 3.50 (d, *J*= 1.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.81 (dd, *J* = 255.7, 11.4 Hz), 161.89 (dd, *J* = 258.6, 12.5 Hz), 150.94, 146.37, 138.94, 137.49, 135.71, 133.86 (d, *J* = 10.5 Hz), 133.64, 130.72, 130.21, 127.82, 122.63, 112.10 (dd, *J* = 21.6, 3.3 Hz), 106.38 (t, *J* = 25.8 Hz), 41.18 (s). HRMS (ESI$^+$) m/z: calcd for $C_{16}H_{13}O_2N_2F_2S$ $[M + H]^+$: 335.06603. Found: 335.06622 (Δ = 0.56 ppm).

***N*-ethylquinolin-8-amine (20-0).** To the solution of 8-Aminoquinoline (500 mg, 3.5 mmol) and Iodoethane (256 μL, 3.2 mmol) in ethanol (3 mL). The mixture was refluxed for 19h. The reaction mixture was concentrated under vacuum and purified by silica gel chromatography using cyclohexane-ethyl acetate (30:1) as eluate to give **20-0** as yellow oil (232 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (dd, *J*= 4.1, 1.6 Hz, 1H), 8.05 (dd, *J*= 8.3, 1.6 Hz, 1H), 7.37 (dt, *J*= 8.3, 6.0 Hz, 2H), 7.26 (s, 1H), 7.03 (d, *J*= 8.2 Hz, 1H), 6.67 (d, *J*= 7.6 Hz, 1H), 3.36 (d, *J*= 6.6 Hz, 2H), 0.88 (dd, *J*= 12.0, 5.0 Hz, 3H).

***N*-ethyl-2,4-difluoro-*N*-(quinolin-8-yl)benzenesulfonamide (20).** To the solution of N-ethylquinolin-8-amine (232 mg, 1.35 mmol) and 2,4-Difluorobenzenesulfonyl chloride (165 L, 1.23 mmol) in pyridine (3 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was washed with HCl solution (2N) (3×20 mL) and extracted with dichloromethane (20 mL). The organic phase was collected and concentrated under vacuum.The product was purified by silica gel chromatography using cyclohexane-ethyl acetate (30:1) as eluate to give **20** as white powder (135 mg, 32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (dd, *J*= 4.1, 1.7 Hz, 1H), 8.10 (dd, *J*= 8.3, 1.7 Hz, 1H), 7.81 (d, *J*= 7.4 Hz, 2H), 7.61–7.44 (m, 2H), 7.27 (dd, *J*= 8.3, 4.1 Hz, 1H), 6.84 (ddd, *J*= 10.0, 8.8, 2.4 Hz, 1H), 6.69 – 6.61 (m, 1H), 4.16 (s, 2H), 1.10 (t, *J*= 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.36 (dd, *J* = 255.5, 11.3 Hz), 160.51 (dd, *J* = 258.6, 12.7 Hz), 149.59, 145.12 , 136.18, 134.63, 134.37, 132.35 (d, *J* = 10.1 Hz), 129.34, 129.03, 126.30, 125.28 (dd, *J* = 14.9, 3.4 Hz), 121.24, 110.63 (dd, *J* = 21.7, 3.2 Hz), 104.94 (t, *J* = 25.9 Hz), 46.71 (d, *J* = 2.4 Hz), 14.98 (s).HRMS (ESI$^+$) m/z: calcd for $C_{17}H_{15}O_2N_2F_2S$ $[M + H]^+$: 349.08168. Found: 349.08198 (Δ = 0.86 ppm).

Biology

High-Throughput Screen.

PDM628 and PCDNA3.1-Rev plasmidswere co-transfected into 293T cells in 96-well plate (Corning, Costar) and Lipofectamine 2000 (Invitrogen) by following the instructions of manufacturer. After transfection, 20155 drug-like set compounds (Enamine, Monmouth Jct, NJ) were added using a Tecan Freedom EVO150 (Tecan, Männedorf, Schweiz) with a final concentration of 50 μM. Column 12 received only DMSO instead of any compounds. In addition, column 1 was only transfected with the plasmid PDM628 as a control. The luciferase expression was detected with a Promega Reader at 48 hour post-transfection. [20]

HIV-1 Production, Infection, and IC_{50} Assay.

H9 cells were infected with the HIV-1 virus (pNL4-3) in accordance with the amount of 10ng p24/1*10^6 cells. After 3 hours, the infected cells were washed by PBS 3 times. Then the cells were plated in 96-well plates (10^4 cells/well) and incubated with the compounds by 10-fold gradient concentrations. 96h later all the supernatant were conducted to p24 ELISA assay. The readout of p24 antigen level in the control group (infected cells and DMSO) should higher than 1. The IC50 values were determined by plotting the logarithm of compound concentration versus p24 amount to obtain concentration that produced 50% inhibition.[21]

Cell Toxicity Assay.

Cell toxicity assay was performed with the CellTiter-Glo Luminescent Cell Viability Assay Kit which was purchased from Promega Company. The instructions of manufacturer were followed. Luminescence was recorded with a Promega plate reader.[22, 23]

Cell Fractionation and RNA Isolation

Cytoplasm and nuclear fractions were isolated by following the manufacturer's instructions of PARIS kit (Ambion). 293T cells were washed with PBS and collected to 1.5mL tube. The fractionation buffer was added to the cells. The cells were gently agitated on ice for 5min. The lysate was then centrifuged for 5min at 500g. The cytoplasmic fraction was collected from the upper 400μL. The 400μL ice-cold disruption buffer was added to the nuclear fraction and vortex vigorously to lysate the nuclei. RNA was then isolated from each of the fractions using the supplied buffers and columns. Quantity of total RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).[24]

ELISA assay

The 293T cells were co-transfected with pMDLg/RRE and Rev, or pMDLg/CTE and Rev, and treated with compound 20 for 2 days. The supernatant was collected for ELISA assay. The p24 ELISA assays were performed according to manufacturer's protocol. [5]

RT-PCR

We used about 1000ng total RNA for reverse transcriptions by PrimeScript RT ragent kit (TaKaRa) according to the manufacturer's instructions, followed by real-time amplification using SYBR Premix Ex Taq (TaKaRa). The cytoplasm fractions were normalized by actin and the nuclear fractions were normalized by U6.

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Notes

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