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Probing Mercaptobenzamides as HIV Inactivators via Nucleocapsid Protein 7

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Abstract

Human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein 7 (NCp7), a zinc finger protein, plays critical roles in viral replication and maturation and represents an attractive target for drug development. However, development of drug-like molecules that inhibit NCp7 has been a significant challenge. In this report, a series of novel 2-mercaptobenzamide prodrugs have been investigated for anti-HIV activity in the context of NCp7 inactivation. The molecules are synthesized from the corresponding thiosalicylic acids, and they are all crystalline solids and stable at room temperature. Derivatives with a range of amide side chains and aromatic substituents were synthesized and screened for anti-HIV activity. Wide ranges of antiviral activity were observed, with IC₅₀ values ranging from 1 to 100 μ M depending on subtle changes to the substituents on the aromatic ring and the sidechain. Results from these structure-activity relationships were fit to a probable mode of intracellular activation and interaction with NCp7 to explain variations in antiviral activity. Our strategy to make a series of mercaptobenzamide prodrugs represents a general new direction to make libraries that can be screened for anti-HIV activity.

Graphical Abstract

Mercaptobenzamide derivatives could become the next generation of drugs to treat HIV infection by targeting and inactivating nucleocapsid protein 7 (NCp7). This protein contains two zinc-coordinated knuckles that bind to HIV RNA and are essential for viral replication. Certain

mercaptobenzamide derivatives promote the acetylation of NCp7, loss of zinc coordination, and eliminate the ability of NCp7 to bind HIV RNA. This manuscript describes how to quickly make mercaptobenzamide analogs to screen for antiviral activity.

Keywords

HIV; Nucleocapsid Protein 7; Mercaptobenzamide; Prodrug; Antiviral

HIV continues to be a major public health issue with approximately 37 million people affected globally.^[1,2] According to the WHO, 1.1 million people died from HIV-related causes in 2015.^[3] The therapies being used to manage HIV infection inhibit the essential viral enzymes reverse transcriptase, protease, or integrase.^[4] The standard treatment for HIV is HAART (highly active antiretroviral therapy) in which combinations of drugs simultaneously target these viral enzymes.^[5] Infected individuals who adhere to HAART can expect a normal life span. However, HAART requires regular and lifelong access to costly medication that often impedes many HIV-infected people from receiving proper treatment. Furthermore, HAART is hampered by viral resistance^[6] and may also predispose patients to cardiovascular and neurological diseases.^[7,8] Therefore, it is essential to continue development of affordable and mutation-resistant small molecule inhibitors of HIV.

Nucleocapsid protein 7 (NCp7, Figure 1), a 55-amino acid protein containing two highly conserved zinc-knuckle motifs,^[9] is an attractive target for antiretroviral drugs due to its essential role in viral replication and maturation.^[10] NCp7 facilitates essential replication steps, such as strand transfers during reverse transcription of viral RNA and genome packaging during virion assembly.^[11] The principle role of NCp7 is to bind viral RNA and protect it from degradation. The NCp7 protein is part of the larger HIV polyprotein Gag which contains matrix and capsid proteins. To successfully mature, HIV protease must cleave peptide bonds at specific locations within Gag to liberate the matrix, capsid, and NCp7 proteins.^[11]

Inhibition of NCp7 yields immature virions that are non-infectious. A number of compounds in the literature are known to inhibit NCp7,^[12,13] and these molecules typically have electrophilic groups that react with the most nucleophilic cysteine in NCp7 (shown to be Cys₄₉). These inhibitors are also generally toxic due to non-specific reactions with other cellular targets. In general, NCp7 has proven to be a difficult drug target^[14] as there is no distinct three-dimensional structure for the protein other than the zinc-coordinated regions. Furthermore, traditional medicinal chemistry approaches to target the active sites of enzymes are not applicable to a protein like NCp7. As a result, there are currently no NCp7 inhibitors that are used clinically to treat HIV infection.^[15]

Our lab is interested in the potential of mercaptobenzamide (**MB**)-based molecules to inhibit HIV-1 through the inactivation of NCp7. Previously, we have shown that the S-acetyl forms of these molecules (**MB1**, Figure 2) are NCp7 inactivators that promote the unfolding of the protein.^[16] In contrast to other electrophilic inhibitors of NCp7, **MB1** reacts selectively at Cys₃₆ (in the C-terminal knuckle) of the protein (Figure 2).¹⁷ In this mechanism, the sulfur of Cys₃₆ attacks **MB1** as a nucleophile at the acetyl group of the thioester. This results in an

intermolecular acetyl transfer from **MB1** to the cysteine and release of a free thiol (**MB2**). Next, an intramolecular acetyl transfer takes place between acetylated Cys₃₆ and an epsilon nitrogen on the sidechain of a proximal lysine residue (typically Lys₃₈). The sulfur to nitrogen acetyl transfer creates a very stable amide and is likely irreversible. Once a lysine is acetylated, zinc coordination is disrupted, allowing further modification of the protein and zinc ejection. As the binding of zinc is specifically required for the function and stability of NCp7, loss of zinc causes disruption of the NCp7 structure, loss of function, and ultimately the formation of immature non-infectious virions.

The molecule **MB2** also has the same anti-HIV activity as **MB1** in cell-based assays due to a unique intracellular mechanism where **MB2** is acetylated in cells by acetyl coenzyme A to generate **MB1**. The **MB1** generated by activation inside cells then inactivates NCp7 by the same mechanism described previously.^[16,18] Both **MB1** and **MB2** are too chemically unstable to develop into drugs due to hydrolysis of the thioester and oxidation of the free thiol. In addition, these molecules display EC₅₀ values in the low micromolar range in cell-based assays that test for anti-HIV activity. Testing different analogs of **MB1** and **MB2** could lead to the discovery of new mercaptobenzamides with better anti-HIV activity. However, the chemical instabilities of the thioester and thiol complicate the synthesis of analogs. Recently, we found that introduction of a prodrug group improves the chemical stability without compromising anti-viral activity. Attaching an *S*-methyl butyrate prodrug to the sulfur of **MB2** affords **MB3**, which has exactly the same anti-HIV activity in cell-based assays.^[19] The prodrug of **MB3** is sensitive to esterase enzyme-promoted hydrolysis that yields butyric acid, formaldehyde, and **MB2**. The toxicity of **MB3** is remarkably low.^[19] Attaching the *S*-methyl butyrate group to a series of prodrugs facilitates the chemical synthesis of stable analogs to screen for anti-HIV activity. Herein, we report a series of 2-mercaptobenzamide prodrugs and their corresponding anti-HIV activity in cell-based assays. By making a series of mercaptobenzamide prodrugs,^[20] it was easier to isolate and purify analogs compared to their corresponding free thiols or *S*-acetyl forms. The results from this screen allowed us to probe the influence of mercaptobenzamide substitution on anti-HIV activity, and how it may be rationalized by interaction with the NCp7 target.

The general synthetic route for the 2-mercaptobenzamide prodrugs is shown in Scheme 1. The mercaptobenzamides were synthesized in two steps from thiol **1**. In most cases, the carboxylic acid **1** was first coupled with amine **2**, using suitable peptide coupling reagents to form the amide bond.^[21] Then, the free thiol intermediate was protected with chloromethyl butyrate (**3**) in the same pot to provide the desired prodrug **4**. The sequence of reactions had to be reversed when electron withdrawing groups were attached to the aromatic ring. The prodrugs are mostly crystalline white solids that are purified by column chromatography and can be stored at room temperature without any special precautions.

If the thiols were not commercially available, the precursor thiols for the mercaptobenzamide prodrugs were synthesized in three steps (Scheme 2).^[22] Amine **5** was first diazotized and the diazo compound was treated with freshly prepared Na₂S₂ (from Na₂S·9H₂O and elemental sulfur) to give disulfide **7**. The disulfide bond in **7** was then reduced with either NaBH₄ or TCEP to produce the mercapto-benzoic acid **1**.

Table 1 describes the anti-HIV data of 2-mercaptobenzamide prodrugs containing different functional groups on the aromatic ring. These assays employ CEMSS cells infected with a laboratory-adapted strain of HIV to provide an initial gauge of anti-HIV activity relative to cellular toxicity.^[19] For comparison, the activity of **MB3** is listed in entry 1. Adding an electron donating methoxy group (OMe, entry 2) group had little effect on EC₅₀ values of anti-HIV activity and also did not influence the toxicity. However, a *p*-hydroxy (OH, entry 3) was not active nor toxic. Adding electron-withdrawing groups (entries 4 to 8) at the para position of the aromatic ring improves the anti-HIV activity slightly, but only at the expense of increasing toxicity to the cells in the assay. An additional electron-withdrawing group at the 4 position (entry 6) does not further improve the activity. An *o*-methyl group to sulfur (entry 9) eliminated all anti-HIV activity, but remained non-toxic. We envisioned that the *o*-methyl group could obstruct the acetyl group transfer from the sulfur of the mercaptobenzamide to Cys₃₆ of NCp7 or perhaps interfere with acetylation at sulfur by acetyl CoA. Therefore, we added a relatively smaller *o*-fluoro group to sulfur (entry 10) expecting a different result. However, we found the *o*-fluoro group also has no anti-HIV activity and displays moderate toxicity that is consistent with the other derivatives with electron withdrawing groups.

Next, we moved our attention to functionalizing the amide side chain on the 2-mercaptobenzamide prodrugs with different geminal substitutions on the α position of the amides (Table 2). The anti-HIV activity of the mercaptobenzamide with a β -alanine amide side chain (**MB3**) is in entry 1, table 1.^[19] The glycineamide side chain also showed similar activity to **MB3** (table 2, entry 1). Geminal bis-substitution was tolerated for an α -methylalanine amide (entry 2) and up to ring size of 5 (entry 3–5). Within this series it is interesting that the EC₅₀ values gradually increase as the ring size increases from a three-, to four-, to five-membered ring. At the same time, the cellular toxicity remains about the same within this series. Then there is a dramatic change for the derivative with a ring size of 6: this molecule has no antiviral activity and displays a significant increase in cellular toxicity (entry 7). We also briefly evaluated the anti-HIV activity of two prodrugs with the α -methylalanine amide chain and substitution on the aromatic ring (entry 7–8). Neither the electron-donating nor the electron-withdrawing group affected the EC₅₀ values compared to the non-substituted form (entry 2). Cellular toxicity was increased with the electron-withdrawing group in a manner consistent with the results in Table 1. Except for the geminal cyclohexyl amide and aromatic OCF₃ groups, the rest of the compounds in Table 2 are non-toxic.

The assays based on CEMSS cells were used to provide an initial gauge of antiviral activity and cellular toxicity. At the same time, we tested a selection of compounds in an assay that more closely represents HIV infection in which human peripheral blood mononuclear cells (PBMCs) are infected with a clinical isolate of HIV-1.^[24] The selected compounds (**MB4**, **MB15**, **MB19** and **MB21**) were tested in both CEMSS and PBMC assays and a comparison of data between the two assays is in Table 3. We also included tests of a mercaptobenzamide with a pyridine ring (compound **MB21**). All the compounds showed no toxicity (TC₅₀ >100 μ M) in PBMCs. The EC₅₀ values were comparable for all the prodrugs, with only slightly weaker antiviral activity in the PMBC assay compared to the CEMSS. Interestingly, **MB21**,

which has a pyridine as the core aromatic building block, showed significantly weaker antiviral activity in the PMBC assay.

From the antiviral data, there is clearly a subtle interplay between substituents on the aromatic ring and the overall antiviral activity and toxicity. Substituents ortho to the sulfur atom effectively eliminate activity. For instance, **MB11** (which has an ortho methyl group) did not have any antiviral activity or toxicity. Electron withdrawing groups on the aromatic ring consistently increase the toxicity of these molecules. Based on our prior mechanistic work, the thiol of the mercaptobenzamides must be acetylated by acetyl coenzyme A for antiviral activity (Figure 3). It is likely that there is a distinct equilibrium between the free thiol **MB2** and the acetylated derivative **MB1** that exists within cells, mediated by the interaction of **MB2** with acetyl CoA. A possible explanation for the poor activity of **MB11** is that the acetylation at sulfur is blocked due to steric hindrance from the ortho methyl group, and that without acetylation there is no antiviral activity. The introduction of electron withdrawing groups on the aromatic ring may also interfere with acetylation by decreasing the nucleophilicity at the sulfur atom. This situation would perturb the equilibrium between free thiol and S-acetyl forms in favor of the free thiol, possibly increasing the general toxicity.

The correlation of lowered antiviral activity with increases in the steric size of sidechains as seen with compounds **MB13**, **MB15**, **MB16**, **MB17** and **MB18** indicated that there could be steric interaction with the NCp7 protein. Molecular models were generated to probe the interaction of this series of compounds with the C-terminal finger of NCp7 (Figure 4). As described earlier, the prodrugs are first removed by esterases to afford the free thiol forms of these molecules, followed by S-acetylation by acetyl CoA. For this comparison, we assumed that the S-acetyl thioester compounds **8–12** all form in the cell and we modeled their potential to inhibit NCp7. In the model, the primary amide of the glycine side chain of thioester **8** may form hydrogen-bonds with Lys₃₄ and His₄₄ of the C-terminal knuckle. At the same time the aromatic ring of **8** may engage in a π -stacking interaction with Trp₃₇. This arrangement positions the carbonyl carbon of **8** for nucleophilic attack by the sulfur of Cys₃₆. Another hydrogen-bonding interaction between the oxygen of the thioester carbonyl of **8** and Met₄₆ may also help to facilitate the nucleophilic attack by increasing the electrophilicity of the carbonyl. A similar model could also be constructed for geminal cyclopropyl (**9**), cyclobutyl (**10**) and cyclopentyl (**11**) glycine side chains, but as the sidechains increase in size they sterically clash with the carbonyl oxygen of Lys₃₄ and Gln₄₅. These increases in steric hindrance between mercaptobenzamide and NCp7 may explain why the antiviral activity decreases as the size of the ring increase from 3, to 4, to 5. A model with compound **12** shows that the cyclohexyl group clearly cannot interact with NCp7 in the same manner as the previous compounds as the ring sterically clashes with Lys₃₄, Gln₄₅, and His₄₄. This steric interaction probably eliminates the antiviral activity of compound **12** in the cell-based assays.

In conclusion, a series of novel 2-mercaptobenzamide prodrugs was synthesized and evaluated for their anti-viral activity. Cell viability studies in the same cell line indicate that most of these molecules are non-toxic (TC₅₀>100 μ M). These prodrugs are stable at room temperature, crystalline, and easily synthesized in two steps from the corresponding

aromatic substituted thiols. The substituted thiols were synthesized from inexpensive starting materials. We speculate that ortho substitution next to the sulfur atom may hinder acetylation of the thiol by acetyl CoA. It is possible that electron withdrawing groups decrease the nucleophilicity of the sulfur atom and lead to alternative modes of reactivity that are toxic. We found that the introduction of gem-disubstitution on the amide side chain may be tolerated to an extent, but large rings like cyclohexyl likely block interaction with NCp7. Further improvement and applications of the mercaptobenzamide prodrugs will continue.

Experimental Section

¹H NMR spectra were recorded in deuterated solvents and are reported in ppm relative to tetramethylsilane and referenced internally to the residually protonated solvent. ¹³C NMR spectra were recorded in deuterated solvents and are reported in ppm relative to tetramethylsilane and referenced internally to the residually protonated solvent. Routine monitoring of reactions was performed using EM Science DC-Alufolien silica gel TLC plates. Flash chromatography was performed with the indicated eluents on EM Science Gedurian 230–400 mesh silica gel. Air and/or moisture sensitive reactions were performed under usual inert atmosphere conditions. Reactions requiring anhydrous conditions were performed under a blanket of nitrogen, in glassware dried in an oven at 130 °C or by flame, then cooled under nitrogen. Dry acetonitrile, DMF, THF and DCM were obtained via a solvent purification system. All other solvents and commercially available reagents were either purified via literature procedures or used without further purification.

General Procedure for Synthesis of Prodrugs 4

Method A—To a stirred solution of thiosalicylic acid **1** in DMF (0.2 M) at rt was added HBTU or HATU or HCTU (1 equiv.) and ^tPr₂NEt (3.5 equiv.) sequentially. After 10 min, the amine **2** (1.05 equiv.) was added to the previous solution. After stirring for 16–24 h, chloromethyl butyrate **3** (0.99 equiv.) was added to the reaction mixture and stirred for 12–18 h. Then, the reaction was concentrated *in vacuo* and treated with H₂O. The aq. layer was extracted with EtOAc (3×) and the combined organic layer was washed with 1 M HCl (2x), sat. NaHCO₃ (2x) and brine. The dried (Na₂SO₄) extract was then concentrated *in vacuo* and the crude product was purified by chromatography over silica gel, eluting with EtOAc/hexanes or MeOH/EtOAc or MeOH/CH₂Cl₂, to give **4**.

Method B—To a stirred solution of thiosalicylic acid **1** in DMF (0.2 M) at rt was added ^tPr₂NEt (2.5 equiv.) and chloromethyl butyrate **3** (0.99 equiv.). After stirring for 18–24 h, HBTU or HATU or HCTU (1 equiv.), amine **2** (1.05 equiv.) and ^tPr₂NEt (1 equiv.) were added to the previous solution sequentially and stirred for 16–24 h. Then, the reaction was concentrated *in vacuo* and treated with H₂O. The aq. layer was extracted with EtOAc (3×) and the combined organic layer was washed with 1 M HCl (2x), sat. NaHCO₃ (2x) and brine. The dried (Na₂SO₄) extract was then concentrated *in vacuo* and the crude product was purified by chromatography over silica gel, eluting with EtOAc/hexanes or MeOH/EtOAc or MeOH/CH₂Cl₂, to give **4**.

MB4: Method B; 21 mg, 44% yield; white solid; ^1H NMR (400 MHz, DMSO- d_6) δ 8.34 (t, J = 5.7 Hz, 1H), 7.47 (d, J = 8.6 Hz, 1H), 7.37 (s, 1H), 7.01 (m, 1H), 6.95 (d, J = 2.9 Hz, 1H), 6.85 (s, 1H), 5.33 (s, 2H), 3.78 (s, 3H), 3.39 – 3.35 (m, 2H), 2.35–2.29 (m, 4H), 1.59–1.19 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, DMSO- d_6) δ 172.39, 172.23, 167.31, 158.57, 141.19, 133.69, 122.44, 115.69, 113.32, 68.01, 55.47, 35.76, 35.37, 34.83, 17.81, 13.34 ppm; HRMS (ES+) calcd. for $\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_5\text{NaS}$ (M+Na) 377.1147, found 377.1141.

MB5: Method B; 10 mg, 20% yield from **SI-2**; white solid; ^1H NMR (400 MHz, CD_3CN) δ 7.43 (dd, J = 0.8, 8.0 Hz, 1H), 7.01 (s, 1H), 6.99 – 6.84 (m, 2H), 6.25 (s, 1H), 5.70 (s, 1H), 5.27 (s, 2H), 3.54 – 3.49 (m, 2H), 2.44 (t, J = 6.5 Hz, 2H), 2.29 (t, J = 7.3 Hz, 3H), 1.63 – 1.53 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3CN) δ 174.20, 173.60, 168.81, 158.07, 143.02, 136.71, 121.78, 118.08, 115.67, 69.68, 36.63, 36.58, 35.40, 18.93, 13.75 ppm; HRMS (ES+) calcd. for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_5\text{NaS}$ (M+Na) 363.0991, found 363.0989.

MB6: Method A, 61 mg, 32% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.61 (d, J = 8.6 Hz, 1H), 7.36 (dq, J = 0.9, 2.8 Hz, 1H), 7.30 – 7.20 (m, 1H), 7.01 (t, J = 5.6 Hz, 1H), 5.83 (s, 1H), 5.59 (s, 1H), 5.37 (s, 2H), 3.71 (dddd, J = 2.0, 4.5, 6.5, 7.8 Hz, 3H), 2.58 (t, J = 5.8 Hz, 2H), 2.33 (t, J = 7.4 Hz, 2H), 1.65 (dt, J = 7.4, 14.8 Hz, 2H), 0.93 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (Some carbons are not resolved due to extensive C-F coupling. Major peaks are listed.) (100 MHz, CDCl_3) δ 173.83, 173.03, 166.88, 148.54, 139.89, 133.46, 131.46, 124.28, 122.97, 121.71, 120.83, 119.14, 67.71, 36.18, 35.91, 34.61, 18.34, 13.69 ppm; ^{19}F NMR (376 MHz, CDCl_3) δ –57.87 ppm; HRMS (ES+) calcd. for $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_5\text{S}$ (M+H) 409.1045, found 409.1039.

MB8: Method B; 31 mg, 61% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.45 – 7.38 (m, 2H), 7.01 (m, 1H), 5.69 (s, 1H), 5.46 (s, 1H), 5.35 (s, 2H), 3.71 (q, J = 6.0 Hz, 2H), 2.58 (m, 2H), 2.35 (t, J = 7.4 Hz, 2H), 1.70 – 1.61 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 173.77, 172.96, 166.19, 134.87, 129.67, 121.23, 121.04, 118.05, 117.86, 67.78, 36.18, 35.90, 34.56, 18.36, 13.71 ppm; ^{19}F NMR (376 MHz, CDCl_3) δ –133.1 (d, J = 21.7 Hz, 1F), –137.4 (d, J = 21.7 Hz, 1F) ppm; HRMS (ES+) calcd. for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_4\text{F}_2\text{NaS}$ (M+Na) 383.0853, found 383.0851.

MB9: Method B, 151 mg, 23% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.72 – 7.60 (m, 3H), 7.07 (t, J = 5.6 Hz, 1H), 5.76 (s, 1H), 5.61 (s, 1H), 5.46 (s, 2H), 3.72 (q, J = 5.9 Hz, 2H), 2.60 (t, J = 5.8 Hz, 2H), 2.34 (t, J = 7.4 Hz, 2H), 1.66 (sex, J = 7.5 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 176.03, 174.93, 168.37, 143.96, 138.85, 135.88, 133.42, 130.93, 120.02, 112.24, 67.69, 38.28, 38.07, 36.57, 20.50, 15.83 ppm; HRMS (ES+) calcd. for $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{SNa}$ (M+Na) 372.0994, found 372.0994.

MB10: Method B, 129 mg, 25% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.53 – 7.42 (m, 2H), 7.33 (dd, J = 2.4, 8.4 Hz, 1H), 7.11 (t, J = 6.0 Hz, 1H), 6.10 (s, 1H), 5.83 (s, 1H), 5.34 (s, 2H), 3.68 (q, J = 6.0 Hz, 2H), 2.57 (t, J = 5.8 Hz, 2H), 2.31 (t, J = 7.4 Hz, 2H), 1.62 (q, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 176.29, 175.22, 169.32, 141.59, 135.94, 135.02, 133.73, 132.92, 130.42, 69.81, 38.35,

38.14, 36.87, 20.50, 15.86 ppm; HRMS (ES+) calcd. for $C_{15}H_{19}ClN_2O_4SNa$ (M+Na) 381.0652, found 381.0659.

MB11: Method B, 31 mg, 61% yield; white solid; 1H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 3H), 6.56 (s, 1H), 5.94 (s, 1H), 5.45 (s, 1H), 5.25 (s, 2H), 3.71 (q, J = 6.0 Hz, 2H), 2.59 (m, 2H), 2.53 (s, 3H), 2.32 (t, J = 7.4 Hz, 2H), 1.66–1.57 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.01, 173.50, 169.80, 144.07, 144.00, 131.83, 129.69, 128.59, 125.71, 69.52, 36.17, 35.86, 34.87, 21.73, 18.26, 13.78 ppm; HRMS (ES+) calcd. for $C_{16}H_{22}N_2O_4NaS$ (M+Na) 361.1198, found 361.1200.

MB12: Method B, 59 mg, 35% yield; white solid; 1H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.34 (m, 1H), 7.26 (d, J = 6.2 Hz, 1H), 7.16 (td, J = 1.3, 8.4 Hz, 1H), 6.96 – 6.89 (m, 1H), 6.07 (s, 1H), 5.69 (s, 1H), 5.27 (s, 2H), 3.67 (dd, J = 5.1, 5.5 Hz, 2H), 2.57 (t, J = 5.7 Hz, 2H), 2.30 (t, J = 7.4 Hz, 2H), 1.66 (sex, J = 7.4 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.20, 175.57, 171.10, 169.83, 166.48, 164.02, 162.06, 146.19, 133.55, 133.46, 133.10, 125.93, 122.16, 119.66, 119.42, 114.44, 70.17, 38.25, 38.12, 36.92, 20.35, 15.85 ppm; ^{19}F NMR (376 MHz, $CDCl_3$) δ –104.62 (dd, J = 5.1, 8.7 Hz, 1F) ppm; HRMS (ES+) calcd. for $C_{15}H_{19}FN_2O_4SNa$ (M+Na) 365.0947, found 365.0952.

MB13: Method A, 228 mg, 33% yield; white solid; 1H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 18.4 Hz, 1H), 7.55 (dd, J = 1.2, 7.6 Hz, 1H), 7.42 (td, J = 1.6, 7.7 Hz, 1H), 7.31 (td, J = 1.2, 7.5 Hz, 1H), 7.09 (t, J = 5.4 Hz, 1H), 6.60 (s, 1H), 5.79 (s, 1H), 5.39 (s, 2H), 4.15 (d, J = 5.2 Hz, 2H), 2.31 (t, J = 7.4 Hz, 2H), 1.62 (sex, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.08, 171.21, 168.64, 136.95, 133.37, 131.46, 131.28, 128.44, 127.70, 77.47, 77.35, 77.15, 76.83, 67.74, 43.42, 36.20, 18.34, 13.70. ppm; HRMS (ES+) calcd. for $C_{14}H_{18}N_2O_4NaS$ (M+Na) 333.0885, found 333.0883.

MB14: Method A, 310 mg, 39% yield; white solid; 1H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, J = 0.8, 6.8 Hz, 1H), 7.54 (dd, J = 1.5, 7.2 Hz, 1H), 7.43 (td, J = 1.5, 7.5 Hz, 1H), 7.35 (td, J = 1.1, 7.5 Hz, 1H), 6.71 (s, 1H), 6.57 (s, 1H), 5.41 (s, 2H), 2.31 (t, J = 7.3 Hz, 2H), 1.69 (s, 6H), 1.63 (q, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.84, 173.25, 168.33, 139.02, 132.52, 132.23, 131.27, 128.56, 128.36, 68.24, 58.30, 36.41, 25.72, 18.55, 13.92 ppm; HRMS (ES+) calcd. for $C_{16}H_{22}N_2O_4SNa$ (M+Na) 361.1198, found 361.1193.

MB15: Method A, 68 mg, 37% yield; white solid; 1H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 1H), 7.49 – 7.44 (m, 2H), 7.42 – 7.40 (m, 1H), 7.01 (s, 1H), 6.59 (s, 1H), 5.88 (s, 1H), 5.36 (s, 2H), 2.29 (t, J = 7.4 Hz, 2H), 1.65 – 1.57 (m, 4H), 1.12 (dd, J = 4.5, 7.7 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.63, 169.62, 164.97, 133.13, 132.30, 131.05, 130.88, 127.47, 126.58, 68.62, 43.73, 36.11, 18.26, 17.62, 13.69 ppm; HRMS (ES+) calcd. for $C_{16}H_{20}N_2O_4SNa$ (M+Na) 359.1041, found 359.1046.

MB16: Method A, 71 mg, 46% yield; white solid; 1H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, J = 1.0, 7.8 Hz, 1H), 7.54 (dd, J = 1.4, 7.6 Hz, 1H), 7.43 (td, J = 1.6, 7.4 Hz, 1H), 7.35 (td, J = 1.3, 7.6 Hz, 1H), 7.07 (s, 1H), 6.82 (s, 1H), 5.41 (s, 2H), 2.84 – 2.77 (m, 2H), 2.40 – 2.25

(m, 4H), 2.12 – 1.99 (m, 2H), 1.63 (sex, $J = 7.4$ Hz, 2H), 0.92 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 175.41, 172.99, 168.31, 137.90, 132.45, 132.32, 131.28, 128.57, 128.12, 68.07, 60.18, 36.17, 31.50, 18.33, 15.86, 13.70 ppm; HRMS (ES+) calcd. for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_4\text{SNa}$ (M+Na) 373.1198, found 373.1195.

MB17: Method A; 16 mg, 6% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.61 (dd, $J = 1.2, 7.9$ Hz, 1H), 7.51 (dd, $J = 1.8, 7.5$ Hz, 1H), 7.44 (td, $J = 1.8, 7.6$ Hz, 1H), 7.37 (td, $J = 1.2, 7.6$ Hz, 1H), 7.08 (s, 1H), 6.35 (s, 1H), 5.41 (m, 3H), 2.43 – 2.39 (m, 2H), 2.31 (t, $J = 7.4$ Hz, 2H), 2.14 – 2.09 (m, 2H), 1.86 – 1.81 (m, 4H), 1.67–1.58 (m, 2H), 0.92 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 176.16, 173.03, 168.65, 138.93, 132.80, 131.75, 131.20, 128.44, 128.42, 68.25, 68.21, 37.24, 36.19, 24.35, 18.34, 13.72 ppm; HRMS (ES+) calcd. for $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_4\text{NaS}$ (M+Na) 387.1354, found 387.1358.

MB18: Method A, 31 mg, 12% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.61 (dd, $J = 1.5, 7.7$ Hz, 1H), 7.54 (ddd, $J = 1.6, 7.6$ Hz, 1H), 7.43 (td, $J = 1.6, 7.4$ Hz, 1H), 7.36 (td, $J = 1.2, 7.5$ Hz, 1H), 7.03 (s, 1H), 6.18 (s, 1H), 5.41 (s, 2H), 2.30 (t, $J = 7.4$ Hz, 2H), 2.25 (d, $J = 14.3$ Hz, 2H), 2.03 – 1.91 (m, 2H), 1.74 – 1.59 (m, 6H), 1.54 – 1.47 (m, 2H), 0.92 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 176.54, 173.00, 168.49, 139.00, 132.66, 131.07, 128.30, 68.09, 61.18, 36.17, 32.30, 25.27, 21.68, 18.33, 13.71 ppm; HRMS (ES+) calcd. for $\text{C}_{19}\text{H}_{26}\text{N}_2\text{O}_4\text{SNa}$ (M+Na) 401.1511, found 401.1515.

MB19: Method B; 6 mg, 13% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.51 (d, $J = 8.6$ Hz, 1H), 7.10 (d, $J = 2.9$ Hz, 1H), 6.95 (dd, $J = 2.9, 8.6$ Hz, 1H), 6.80 (s, 1H), 6.75 (s, 1H), 5.59 (s, 1H), 5.30 (s, 2H), 3.84 (s, 3H), 2.30 (t, $J = 7.4$ Hz, 2H), 1.67 (s, 6H), 1.66 – 1.56 (m, 2H), 0.91 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 176.93, 173.11, 167.84, 160.42, 141.74, 136.92, 120.62, 117.35, 113.74, 69.41, 58.08, 55.79, 36.19, 25.59, 18.32, 13.74 ppm; HRMS (ES+) calcd. for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_5\text{S}$ (M+H) 369.1484, found 369.1487.

MB20: Method A, 65 mg, 51% yield; white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.63 (d, $J = 8.6$ Hz, 2H), 7.38 (d, $J = 2.6$ Hz, 1H), 7.28 – 7.22 (m, 1H), 6.86 (s, 2H), 6.48 (s, 1H), 5.50 (s, 1H), 5.39 (s, 2H), 2.32 (t, $J = 7.4$ Hz, 2H), 1.70 (s, 6H), 1.61 (q, $J = 7.4$ Hz, 2H), 0.92 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 176.36, 172.91, 166.32, 148.87, 140.52, 134.18, 131.32, 130.61, 123.08, 121.69, 120.94, 119.12, 115.83, 111.53, 67.89, 58.12, 36.14, 25.28, 18.32, 13.68 ppm; ^{19}F NMR (376 MHz, CDCl_3) δ –57.83 ppm; HRMS (ES+) calcd. For $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_5\text{F}_3\text{S}$ (M+H) 423.1202, found 423.1201.

MB21: Method A; 25 mg, 6% yield; white solid; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.73 (t, $J = 5.9$ Hz, 1H), 8.43 (dd, $J = 1.3, 4.5$ Hz, 1H), 8.04 (dd, $J = 1.4, 8.3$ Hz, 1H), 7.59 (dd, $J = 4.5, 8.3$ Hz, 1H), 7.38 (s, 1H), 6.87 (s, 1H), 5.53 (s, 2H), 3.45 – 3.41 (m, 2H), 2.36–2.29 (m, 4H), 1.52 (m, 2H), 0.84 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 173.64, 172.83, 165.69, 144.49, 144.23, 136.31, 134.19, 126.20, 64.29, 36.22, 35.48, 35.35, 18.42, 13.71 ppm; HRMS (ES+) calcd. for $\text{C}_{14}\text{H}_{19}\text{N}_3\text{O}_4\text{NaS}$ (M+Na) 348.0994, found 348.0092.

General Procedure for Syntheses of Disulfides 7

An Erlenmeyer flask was charged with 2-aminobenzoic acid **5** (11.5 mmol) and 25 mL water. The mixture was cooled to $\sim 0^{\circ}\text{C}$ with an ice bath, and 4.5 mL of concentrated hydrochloric acid was added. A blast shield was installed and then a solution of sodium nitrite (1.61 g, 23.3 mmol) in 5 mL water was carefully added drop wise (CAUTION: Diazonium salts are potentially explosive; the use of a blast shield and appropriate personal protective gear is required.) The reaction was stirred for 1 h and carefully maintained at $\sim 0^{\circ}\text{C}$. In the meantime, a solution of disodium disulfide was prepared under a stream of N_2 by mixing sodium sulfide nonahydrate ($\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$) (5.93 g, 24.6 mmol) with 12.5 mL water at 72°C . Elemental sulfur was added (780 mg, 24.3 mmol) and the mixture was stirred at 72°C for 40 min. The resulting dark orange solution was cooled to $\sim 0^{\circ}\text{C}$ and treated with a solution of sodium hydroxide (900 mg, 22.5 mmol) in 7.5 mL water. The mix was stirred for an additional 5 min before being used in the next step. The freshly prepared disodium disulfide (Na_2S_2) solution was poured into a large Erlenmeyer flask maintained at $\sim 0^{\circ}\text{C}$ via an external ice water bath. A handful of ice chips were added, and a blast shield placed in front of the flask. Using the utmost caution, the cooled (0°C) diazonium solution was carefully and slowly poured into the cooled (0°C) solution of Na_2S_2 and the reaction was allowed to stir at 0° to rt overnight. The reaction was then acidified to $\sim \text{pH } 1$ with concentrated HCl, and the resulting precipitate was filtered and washed thoroughly with cold water. The solids were then transferred to an Erlenmeyer flask, combined with saturated aqueous potassium carbonate and heated gently to produce a heterogeneous mixture of dark brown liquor (basic to pH paper) and cream-colored solids. The mixture was passed through a pad of celite, and the filtrate was carefully acidified to $\sim \text{pH } 1$ with drops of concentrated HCl. The resulting precipitate was isolated by filtration, washed with cold water and dried *in vacuo* to give **7** of a crude tan solid that was carried forward to the next step without further purification.

General Procedure for Syntheses of Thiols

Method A—Disulfide **2** was suspended in MeOH (0.1 M) under N_2 atmosphere at rt and sodium borohydride (4 equiv.) was added in small portions over the course of 30 min. Then, the reaction mixture was stirred for 2–12 h and the yellow slurry became a clear solution during the reaction. The reaction was carefully quenched with drops of water and the solvents were removed under reduced pressure. The residue was combined with water and acidified to $\text{pH} = \sim 1$ with drops of concentrated HCl to give a pale yellow precipitate. The precipitate was filtered, washed with ice cold water (5X) and dried on a Büchner funnel overnight under nitrogen atmosphere to give **1** as a crude yellowish white solid that was used immediately in the next step.

General Procedure for Syntheses of Thiols

Method B—To a solution of disulfide **2** in 9:1 DMF/ H_2O (0.1 M) was added TCEP hydrochloride (1.5 equiv.) and Et_3N (3 equiv.) at rt. After stirring for 18 h, the reaction was concentrated *in vacuo* at 45°C and treated with cold water. The mixture was stirred vigorously for 2h and acidified ($\text{pH} = \sim 1$) with conc. HCl to give yellow precipitate. The precipitate was filtered, washed with ice cold water (5X) and dried on a Büchner funnel

overnight under nitrogen atmosphere to give **1** as a crude yellowish white solid that was used immediately in the next step.

MB7: To a stirred solution of thiophenol **SI-7** (54 mg, 0.206 mmol) in DMF (0.25 mL) at rt was added triethylamine (57 μ L, 0.309 mmol). After 3 min, chloromethyl butyrate **3** (40 μ L, 0.309 mmol) was added and the mixture was stirred for 18 h. The reaction was quenched with 5 mL of water and extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (EtOAc/hexanes) to give **MB7** (24 mg, 34% yield) as a light tan solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.46 (t, $J = 5.7$ Hz, 1H), 7.61 (dd, $J = 5.3, 8.7$ Hz, 1H), 7.37–7.26 (m, 3H), 6.85 (s, 1H), 5.41 (s, 2H), 3.41–3.36 (m, 2H), 2.32 (td, $J = 4.7, 7.4$ Hz, 4H), 1.59–1.49 (m, 2H), 0.87 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 172.32, 172.23, 166.20, 161.96, 159.51, 140.42, 140.36, 132.69, 132.61, 128.56, 128.53, 117.24, 117.02, 114.98, 114.75, 66.99, 35.82, 35.31, 34.77, 17.80, 13.31 ppm; ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ –114.71 ppm; HRMS calcd. for $\text{C}_{15}\text{H}_{19}\text{N}_2\text{O}_4\text{FNaS}$ ($\text{M}+\text{Na}$) 365.0947, found 365.0940.

Anti-HIV Cytoprotection Evaluation - Assay Methodology

Inhibition of virus-induced cytopathic effects (CPE) and cell viability following HIV replication in CEM-SS cells was measured by XTT tetrazolium dye. CEM-SS cells (2.5×10^3 cells per well) were seeded in 96-well U-bottomed tissue culture plates in RPMI medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin. Serially diluted compounds (6 concentrations) and HIV-1_{RF} diluted to a pre-determined titer to yield 85 to 95% cell killing at 6 days post-infection were added to the plate. AZT was evaluated in parallel as a positive control. Following incubation at 37°C, 5% CO_2 for six days, cell viability was measured by XTT staining. The optical density of the cell culture plate will be determined spectrophotometrically at 450 and 650 nm using Softmax Pro 4.6 software. Percent CPE reduction of the virus-infected wells and the percent cell viability of uninfected drug control wells were calculated to define the EC_{50} , TC_{50} and therapeutic index (TI_{50}) using Microsoft Excel Xlfit4.

Anti-HIV Evaluation in Human PBMCs – Assay Methodology

PHA-P stimulated PBMCs from three donors were pooled together and resuspended in fresh tissue culture medium 1×10^6 cells/mL and plated in the interior wells of a 96 well round bottom microplate at 50 $\mu\text{L}/\text{well}$. A 100 μL volume of 9 concentrations of compound serially diluted were transferred to the round-bottom 96 well plate containing the cells in triplicate. 50 μL of HIV-1 at a pre-determined dilution was added. Each plate contained cell control wells and virus control wells in parallel with experimental wells. After 7 days in culture, efficacy was evaluated by measuring the reverse transcriptase in the culture supernatants and the cells were stained with the tetrazolium dye XTT to evaluate cytotoxicity.

General Method for Docking

Models of the different inhibitor compounds were developed with the CHARMM software package.^[25] The structure of the C-terminal finger of NCp7 was obtained from the NMR-

derived ensemble determined by Lee *et al.* (PDB: 1MFS).^[26] Images of the “manually”-docked complexes were developed with the UCSF Chimera software program.^[27]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Primary sequence of NCp7 with Zn coordinating residues specified with spherical shapes. N-terminal knuckle represented with blue color and C-terminal knuckle represented with brown color. Zn atoms are represented with green spheres.

Figure 2.

Illustration of how **MB1** acetylates NCp7. The molecules **MB1**, **MB2**, and **MB3** all display similar anti-HIV activity in cell-based assays.

Figure 3. Illustration of how mercaptobenzamide substitution may affect reaction with acetyl coenzyme.

Figure 4. Models of NCp7 interacting with the series of inactivators with different geminal-disubstituted side chains (glycinamide (**8**), cyclopropyl (**9**), cyclobutyl (**10**), cyclopentyl (**11**), cyclohexyl (**12**) glycinamide side chains). NCp7 is shown as grey surface with oxygens in red, nitrogen in blue, hydrogen in white, and sulfur in yellow. The skeleton of the compounds (**8** – **12**) are portrayed as magenta sticks with blue, red, yellow and white representing nitrogen, oxygen, sulfur and hydrogen atoms respectively. Key amino acid residues in NCp7 are labeled in the model with compound **8**. Green X's represent steric clashes that may inhibit interactions.

Scheme 1. Synthesis of Mercaptobenzamides^a

^aReagents and conditions: **1**, HATU, HBTU or HCTU, DIPEA, DMF, rt, 10–15 min; **2**, 16–24 h; **3**, rt, 12–24 h (X = Cl or TFA)

Scheme 2. Synthesis of Thiols^b

^bReagents and conditions: (i) NaNO_2 , Conc. HCl , H_2O , 0°C , 1h, (ii) $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, S_8 , $70-80^\circ\text{C}$, ~30 min; add **6**, 0°C to rt, (iii) TCEP, $\text{DMF}/\text{H}_2\text{O}$ (9:1), rt, 14–18h, (iv) NaBH_4 , MeOH or THF/MeOH , rt, 2–12 h.

Table 1

Anti-HIV activity (EC₅₀) data for substituents on the aromatic ring.^[c]

Entry	Compound	R ¹	R ²	R ³	CEMSS EC ₅₀ (μM)	CEMSS TC ₅₀ (μM)
1	MB3	H	H	H	2.38 ± 2.23	>100
2	MB4	OMe	H	H	1.50 ± 0.33	>100
3 ²³	MB5	OH	H	H	>100	>100
4	MB6	OCF ₃	H	H	1.11 ± 0.60	32.1 ± 7.9
5	MB7	F	H	H	1.16 ± 0.95	>5
6	MB8	F	F	H	1.26 ± 0.53	39 ± 28
7	MB9	CN	H	H	1.64 ± 0.35	14.8 ± 5.7
8	MB10	Cl	H	H	2.60 ± 1.44	14.5 ± 0.2
9	MB11	H	H	Me	>100	>100
10	MB12	H	H	F	>100	>6

^[c] Inhibition of HIV₁RF in CEMSS cells were performed using published procedures. In each individual antiviral assay, efficacy and toxicity values are derived from a minimum of three replicate wells.^[19]

Table 2Anti-HIV activity and toxicity data with variable amide side chains.^[d]

Entry	Compound	R ¹	R ²	CEMSS EC ₅₀ (μM)	CEMSS TC ₅₀ (μM)
1	MB13	H		2.93 ± 1.92	>100
2	MB14	H		2.24 ± 2.05	>100
3	MB15	H		2.18 ± 1.43	>100
4	MB16	H		3.60 ± 0.95	>100
5	MB17	H		8.20 ± 4.20	>100

Entry	Compound	R ¹	R ²	CEMSS EC ₅₀ (μM)	CEMSS TC ₅₀ (μM)
6	MB18	H		>100	34.8 ± 23.7
7	MB19	OMe		2.20 ± 0.89	>100
8	MB20	OCF ₃		3.7 ± 1.7	56.1 ± 0.8

d) Inhibition of HIV^{RT} in CEMSS cells were performed using published procedures. In each individual antiviral assay, efficacy and toxicity values are derived from a minimum of three replicate wells. [19]

Table 3

Comparison of cytopathic effect inhibition assay (inhibition of cell lysis in CEM-SS cells) to full active HIV assay (PBMC/HIV-1_{92HT599})^[e]

Compound	CEMSS EC ₅₀ (μM)	CEMSS TC ₅₀ (μM)	PBMC HIV-1 EC ₅₀ (μM)	PBMC HIV-1 TC ₅₀ (μM)
MB4	1.50 ± 0.33	>100	4.23 ± 2.89	>100
MB15	2.18 ± 1.43	>100	6.80 ± 2.59	>100
MB19	2.20 ± 0.89	>100	14.1 ± 6.8	>100
MB21	2.37 ± 0.34	>100	67.8 ± 23.5	>100

^[e] Inhibition of HIV_{RF} in CEMSS cells and inhibition HIV-1_{92HT599} in human PBMCs were performed using previously published procedures. In each individual antiviral assay, efficacy and toxicity values are derived from a minimum of three replicate wells.^[19]