Supporting Information

Aloperine and its Derivatives as a New Class of HIV-1 Entry Inhibitors

Zhao Dang,[†] Lei Zhu,[†] Weihong Lai,[†] Hal Bogerd,[§] Kuo-Hsiung Lee,[‡] Li Huang,*[,†] and Chin-Ho Chen*[,†]

[†]Surgical Science, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, United States

[§]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, United States

[‡]Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, United States, and Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan

EXPERIMENTAL SECTION

Multi-cycle viral replication in MT4 cell assay. HIV-1 NL4-3 Nanoluc-sec at a dose of 50 $TCID_{50}$/well was used to infect MT4 cells (1 x 10^5 cells/mL) in the presence of compounds at various concentrations in 96-well plates. The reporter virus, HIV-1 NL4-3 Nanoluc-sec, was created by inserting the secNluc sequence from pNL1.3[secNluc] (Promega Cat#: N1021) in place of the Nef sequence spanning nucleotide 8796-8892 of pNL4-3 plasmid (GenBank: AF324493.2) using Not I and Xho I restriction enzyme sites. Not I site was introduced into pNL4-3 by site directed mutagenesis and the Xho I site was a unique site in pNL4-3. On day 3 post-infection, supernatant samples were harvested and assayed for luciferase activity using the Promega Nano-Glo® Luciferase Assay System. The antiviral potency is defined as the drug concentration that reduces the luciferase activity by 50% (EC_{50}).

Cytotoxicity Assay. A CellTiter-Glo® Luminescent cytotoxicity assay (Promega) was used to determine the cytotoxicity of the synthesized aloperine derivatives. MT4 cells were cultured in the presence of various concentrations of the compounds for 3 days. Cytotoxicity of the compounds was determined by following the protocol provided by the manufacturer. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration that caused a 50% reduction of cell viability.

Fusion Assay. The fusion assay used in this study was previously described.[21] The fusion assay was performed by transfecting monkey kidney cells (COS) with an expression vector containing HIV-1 Env and tat genes. COS cells (1×10^6 cells/mL) were mixed with 5 μg of the Env-expressing vector and incubated on ice for 10 minutes. Electroporation was performed using a gene pulsar (Bio-Rad, Hercules, CA) with capacitance set at 950 μF and voltage at 150 V. The transfected COS cells were cultured for one day and were then mixed with TZM-bl cells. TZM-

bl cells were incubated with the Env-expressing COS cells in the presence of inhibitors in 96-well flat-bottom plates (Costar) overnight. Fusion was measured by quantifying luciferase activity in the fused cells using a Bright-Glow luciferase assay kit (Promega, Luis Obispo, CA). Inhibition of the Env-mediated membrane fusion was expressed as a percentage of the control (Env-mediated membrane fusion in the absence of inhibitors).

Chemistry.

General. Aloperine derivatives were synthesized and analyzed with positive or negative HR-FABMS on a Shimadzu LCMS-IT-TOF or a Joel SX-102 mass spectrometer. ^{1}H and ^{13}C (**7c** only) NMR spectra were measured on a Varian 400 or 500 MHz spectrometer as indicated. Samples were dissolved in methanol-d_4 unless specified. Biotage Initiator (Biotage) was used for microwave heating in synthesis. Silica gel chromatography was carried out on an ISCO CombiFlash Rf flash chromatograph system with a pre-packed Redi Sep Rf Si gel column (Teledyne ISCO) and mobile phase of EtOAc/MeOH/NH4OH in gradient of increased polarity. Compounds were purified with HPLC using a Varian ProStar HPLC system with a PDA detector and Agilent Zorbax C18 columns (5 μm particle size, 4.6 × 250 mm or 9.4 × 250 mm). The mobile phase used for the HPLC was ACN/MeOH/H_2O/TFA in a gradient of decreasing polarity. All synthesized compounds were confirmed to have a purity over 95% by HPLC.

Synthesis of compounds 5a – 5e. To a mixture of **1** (116 mg, 0.5 mmol) in 5 mL acetonitrile was added 2-(Boc-amino) ethyl bromide (112 mg, 0.5 mmol) and K_2CO_3 (210 mg, 1.5 mmol). The mixture was heated to 110 °C by microwave (Biotage Initiator) for 1 hour. After the solvent was removed under vacuum, the resultant residue was diluted with ethyl acetate, which was then washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was

chromatographed with Si-gel chromatography to give N-(N-boc-ethyl)aloperine intermediate. To this intermediate was added 55% TFA/DCM (1 mL). The mixture was stirred at rt for 20 minutes. After the solvent was removed in vacuum, the resultant residue was chromatographed with Si-gel chromatography to give **5a** (30% yield). Compounds **5b - 5e** were synthesized by same method, resulting in 45% - 74% yield.

Synthesis of compounds 6a - 6e, 7a - 7d, 8a - 8c, 9a - 9b, 10a - 10b, 11a - 11b, and 12a – 12f. To **5a** (20 mg, 0.07 mmol) and 4-trifluoromethoxybenzoic acid (31 mg, 0.15 mmol) in 2 mL THF was added EDC (30 mg, 0.15 mmol) and DIEA (52 μL, 0.3 mmol) at room temperature. The mixture was stirred under N_2 overnight. After the solvent was removed in vacuum, the resultant residue was dissolved in ethyl acetate, washed with water and brine, dried over $MgSO_4$, and concentrated to give a solid. The residue was chromatographed with Si-gel and then with HPLC to give **6a** (39% yield). Compounds **6b - 6e, 7a - 7d, 8a - 8c, 9a, 9b, 10a, 10b, 11a, 11b, and 12a - 12f** were synthesized by the same method with 9% - 98% yield.

Synthesis of compounds 13a – 13c. To **5c** (21 mg, 0.07 mmol) and 4-trifluoromethoxybenzoic acid (31 mg, 0.15 mmol) in 1 mL acetonitrile was added benzyl bromide (9.5 μL, 0.08 mmol), potassium carbonate (29 mg, 0.21 mmol). The mixture was heated to 110 °C by microwave (Biotage Initiator) for 1 hour. After the solvent was removed in vacuum, the resultant residue was dissolved in ethyl acetate, washed with water and brine, dried over $MgSO_4$, and concentrated to give a solid. The residue was chromatographed with Si-gel and then with HPLC to give **13a** (19% yield). Compounds **13b** and **13c** were synthesized by the same method resulting in 18% and 24% yield, respectively.

2-Aminoethyl-*N*^{12}-aloperine (5a): ^{1}H NMR (400 MHz) (CD_3OD) δ 5.97 (d, 1H, *J* = 6.0 Hz), 3.94 (d, 1H, *J* = 3.6 Hz), 3.91 (d, 1H, *J* = 4.0 Hz), 3.73 (d, 1H, *J* = 11.6 Hz), 3.52-3.61 (m, 5H) 3.38-3.39 (m, 2H), 3.12 (m, 1H), 2.93-3.01 (m, 2H), 2.59-2.63 (m, 2H), 2.38 (d, 2H, *J* = 9.6 Hz), 2.30 (ddd, 1H, *J* = 13.2 Hz, *J* = 3.2 Hz), 2.08 (d, 1H, *J* = 13.6 Hz), 1.86-2.03 (m, 6H), 1.72 (tt, 1H, *J* = 13.2 Hz, *J* = 3.6 Hz) . Calcd for $C_{17}H_{30}N_3$ $(M+H)^+$: 276.2434. Found: 276.2439.

3-Aminopropyl-*N*^{12}-aloperine (5b): ^{1}H NMR (400 MHz) (CD_3OD) δ 5.95 (bs, 1H), 4.15 (bs, 1H), 3.89 (bs, 1H), 3.86 (bs, 1H), 3.76 (d, 1H, *J* = 10.0 Hz), 3.40-3.45 (m, 5H), 3.25-3.28 (m, 2H), 3.00-3.02 (m, 2H), 2.90 (bs, 1H), 2.53-2.57 (m, 2H), 2.10-2.40 (m, 5H), 1.74-2.02 (m, 7H), 1.62 (t, 1H, *J* = 13.6 Hz). Calcd for $C_{18}H_{32}N_3$ $(M+H)^+$: 290.2591. Found: 290.2596.

4-Aminobutyl-*N*^{12}-aloperine (5c): ^{1}H NMR (400 MHz) (CD_3OD) δ 5.96 (d, 1H, *J* = 5.6 Hz), 4.24 (d, 1H, *J* = 4.8 Hz), 3.89 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.79 (d, 1H, *J* = 11.6 Hz), 3.47 (d, 1H, *J* = 12.0 Hz), 3.39 (dd, 1H, *J* = 13.2 Hz, *J* = 5.2 Hz), 3.56 (bs, 1H), 3.27 (d, 2H, *J* = 6.8 Hz), 3.09-3.22 (m, 2H), 3.01 (t, 2H, *J* =7.2 Hz), 2.92 (bs, 1H), 2.54-2.58 (m, 2H), 2.28-2.40 (m, 2H), 2.18 (ddd, 1H, *J* = 12.4 Hz, *J* = 4.0 Hz), 1.69-1.96 (m, 11H), 1.57-1.65 (m, 1H). Calcd for $C_{19}H_{34}N_3$ $(M+H)^+$: 304.2747. Found: 304.2750.

5-Aminopentyl-*N*^{12}-aloperine (5d): ^{1}H NMR (400 MHz) (CD_3OD) δ 5.95 (d, 1H, *J* = 4.8 Hz), 4.24 (d, 1H, *J* = 5.2 Hz), 3.90 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.78 (d, 1H, *J* = 12.0 Hz), 3.47 (d, 1H, *J* = 12.4 Hz), 3.26-3.38 (m, 4H), 3.15-3.22 (m, 1H), 3.06-3.13 (m, 1H), 2.96 (t, 2H, *J* = 8.0 Hz), 2.92 (bs, 1H), 2.53-2.57 (m, 2H), 2.13-2.40 (m, 3H), 1.70-1.96 (m, 11 H), 1.44-1.65 (m, 3H). Calcd for $C_{20}H_{36}N_3$ $(M+H)^+$: 318.2904. Found: 318.2906.

6-Aminohexyl-*N*^{12}-aloperine (5e): ^{1}H NMR (400 MHz) (CD_3OD) δ 5.95 (d, 1H, *J* = 5.6 Hz), 4.23 (d, 1H, *J* = 5.6 Hz), 3.89 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.78 (d, 1H, *J* = 11.6 Hz), 3.47 (d, 1H, *J* = 12.0 Hz), 3.27-3.37 (m, 4H), 3.14-3.23 (m, 1H), 3.08 (dt, 1H, *J* = 14.0 Hz, *J* = 5.2 Hz), 2.92-2.95 (m, 3H), 2.53-2.57 (m, 2H), 2.37 (dd, 1H, *J* = 9.2 Hz, *J* = 17.6 Hz), 2.29 (d, 1H, *J* = 15.6 Hz), 2.18 (ddd, 1H, *J* = 13.2 Hz, *J* = 4.0 Hz), 1.55-2.03 (m, 13 H), 1.43-1.50 (m, 3H). Calcd for $C_{21}H_{38}N_3$ $(M+H)^+$: 332.3060. Found: 332.3057.

4-Trifluoromethoxy-*N*-[2-(*N*^{12}-aloperine-yl)ethyl]benzamide (6a): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.98 (t, 1H, *J* = 2.4 Hz), 7.93 (t, 1H, *J* = 2.0 Hz), 7.38 (d, 2H, *J* = 8.0 Hz), 5.96 (d, 1H, *J* = 4.8 Hz), 4.31 (d, 1H, *J* = 4.4 Hz), 3.81-3.98 (m, 4H), 3.75 (p, 1H, *J* = 7.2 Hz), 3.46 (d, 1H, *J* = 12.8 Hz), 3.15-3.27 (m, 4H), 2.95-3.01 (m, 2H), 2.53-2.57 (m, 2H), 2.36-2.42 (m, 1H), 2.30 (d, 1H, *J* = 14.0 Hz), 2.17 (ddd, 1H, *J* = 12.4 Hz, *J* = 4.0 Hz), 1.93-1.98 (m, 3H), 1.68-1.84 (m, 4H), 1.59 (ddt, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz). Calcd for $C_{25}H_{33}F_3N_3O_2$ $(M+H)^+$: 464.2519. Found: 464.2514.

4-Trifluoromethoxy-*N*-[3-(*N*^{12}-aloperine-yl)propyl]benzamide (6b): ^{1}H NMR (400 MHz) ($CDCl_3$) δ 8.30 (bs, 1H), 7.97 (d, 2H, *J* = 8.4 Hz), 7.22 (d, 2H, *J* = 8.0 Hz), 5.87 (d, 1H, *J* = 6.4 Hz), 4.00 (bs, 1H), 3.50-3.74 (m, 5H), 3.27 (d, 1H, *J* = 12.4 Hz), 3.05-3.20 (m, 4H), 2.94-3.10 (m, 2H), 2.52 (d, 1H, *J* = 15.2 Hz), 2.41 (s, 1H), 1.35-2.25 (m, 13H). Calcd for $C_{26}H_{35}F_3N_3O_2$ $(M+H)^+$: 478.2676. Found: 478.2665.

4-Trifluoromethoxy-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (6c): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.94 (t, 1H, *J* = 2.4 Hz), 7.93 (t, 1H, *J* = 2.4 Hz), 7.37 (d, 2H, *J* = 8.0 Hz), 5.94 (d, 1H, *J* = 4.8 Hz), 4.23 (d, 1H, *J* = 4.4 Hz), 3.88 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.80 (d, 1H, *J* = 13.2 Hz), 3.55 (p, 1H, *J* = 6.8 Hz), 3.30-3.49 (m, 4H), 3.09-3.26 (m, 4H), 2.95 (bs, 1H), 2.52-2.56 (m, 2H), 2.28-2.39 (m, 2H), 2.18 (ddd, 1H, *J* = 12.4 Hz, *J* = 4.0 Hz), 1.74-2.00 (m, 11H), 1.60 (ddt, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz). Calcd for $C_{27}H_{37}F_3N_3O_2$ $(M+H)^+$: 492.2832. Found: 492.2831.

4-Trifluoromethoxy-*N*-[5-(*N*^{12}-aloperine-yl)pentyl]benzamide (6d): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.91-7.94 (m, 2H), 7.37 (d, 2H, *J* = 8.8 Hz), 5.95 (d, 1H, *J* = 5.6 Hz), 4.24 (d, 1H, *J* = 4.8 Hz), 3.88 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.77 (d, 1H, *J* = 12.0 Hz), 3.06-3.48 (m, 9H), 2.91 (bs, 1H), 2.53-2.57 (m, 2H), 2.13-2.40 (m, 3H), 1.45-1.99 (m, 14 H). Calcd for $C_{28}H_{39}F_3N_3O_2$ $(M+H)^+$: 506.2989. Found: 506.2987.

4-Trifluoromethoxy-*N*-[6-(*N*^{12}-aloperine-yl)hexyl]benzamide (6e): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.91-7.94 (m, 2H), 7.37 (d, 2H, *J* = 10.0 Hz), 5.94 (d, 1H, *J* = 5.6 Hz), 4.23 (d, 1H, *J* = 5.2 Hz), 3.87 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.76 (d, 1H, *J* = 12.4 Hz), 3.46 (d, 1H, *J* = 12.4 Hz), 3.40 (t, 2H, *J* = 6.8 Hz), 3.28-3.36 (m, 4H), 3.15-3.25 (m, 1H), 3.09 (dt, 1H, *J* = 13.2 Hz, *J* = 5.2 Hz), 2.89 (bs, 1H), 2.52-2.55 (m, 2H), 2.32-2.39 (m, 1H), 2.28 (d, 1H, *J* = 16.0 Hz), 2.17 (ddd, 1H, *J* = 13.2 Hz, *J* = 3.2 Hz), 1.48-2.02 (m, 16H). Calcd for $C_{29}H_{41}F_3N_3O_2$ $(M+H)^+$: 520.3145. Found: 520.3141.

4-Trifluoromethoxy-*N*-[2-(*N*^{12}-aloperine-yl)ethyl]benzeneacetamide (7a): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.39 (d, 2H, *J* = 8.8 Hz), 7.23 (d, 2H, *J* = 8.0 Hz), 5.94 (d, 1H, *J* = 4.8 Hz), 4.24 (d, 1H, *J* = 4.4 Hz), 3.86 (dd, 1H, *J* = 14.8 Hz, *J* = 3.6 Hz), 3.82 (d, 1H, *J* = 13.2 Hz), 3.67 (d, 1H, *J* = 7.2 Hz), 3.65 (d, 1H, *J* = 6.0 Hz), 3.61 (s, 2H), 3.57 (p, 1H, *J* = 7.2 Hz), 3.44 (d, 1H, *J* = 12.0 Hz), 3.24 (d, 2H, *J* = 13.6 Hz), 2.10-3.17 (m, 3H), 2.93 (bs, 1H), 2.51-2.55 (m, 2H), 2.30-2.37 (m, 1H), 2.27 (d, 1H, *J* = 14.4 Hz), 2.15 (ddd, 1H, *J* = 13.2 Hz, *J* = 4.0 Hz), 1.72-2.00 (m, 8H), 1.58 (ddt, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz). Calcd for $C_{26}H_{35}F_3N_3O_2$ $(M+H)^+$: 478.2676. Found: 478.2669.

2-(4-Trifluoromethoxyphenyl)-*N*-[3-(*N*^{12}-aloperine-yl)propyl]acetamide (7b): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.41 (dd, 2H, *J* = 6.4 Hz, *J* = 1.6 Hz), 7.23 (d, 2H, *J* = 8.4 Hz), 5.93 (d, 1H, *J* = 5.6 Hz), 4.20 (d, 1H, *J* = 5.2 Hz), 3.79 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.68 (d, 1H, *J* = 12.8 Hz), 3.56 (s, 2H), 3.45 (d, 1H, *J* = 12.8 Hz), 3.33-3.37 (m, 3H), 2.24-3.28 (m, 3H), 3.03-3.16 (m, 2H), 2.81 (bs, 1H), 2.51-2.55 (m, 2H), 2.28-3.38 (m, 1H), 2.26 (d, 1H, *J* = 11.6 Hz), 2.13 (ddd, 1H, *J* = 13.2 Hz, *J* = 4.0 Hz), 1.74-2.08 (m, 9H), 1.59 (qt, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz). Calcd for $C_{27}H_{37}F_3N_3O_2$ $(M+H)^+$: 492.2832. Found: 492.2824.

2-(4-Trifluoromethoxyphenyl)-*N*-[4-(*N*^{12}-aloperine-yl)butyl]acetamide (7c): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.39 (d, 2H, *J* = 8.8 Hz), 7.22 (d, 2H, *J* = 8.8 Hz), 5.93 (d, 1H, *J* = 5.6 Hz), 4.17 (d, 1H, *J* = 4.8 Hz), 3.83 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.70 (d, 1H, *J* = 11.6 Hz), 3.55 (s, 2H), 3.45 (d, 1H, *J* = 12.4 Hz), 3.34-3.47 (m, 2H), 3.21-3.30 (m, 4H), 3.04-3.12 (m, 2H), 2.87 (bs, 1H), 2.51-2.54 (m, 2H), 2.33 (dd, 1H, *J* = 9.6 Hz), 2.24 (d, 1H, *J* = 14.4 Hz), 2.15 (ddd, 1H, *J* = 13.2 Hz, *J* = 3.6 Hz), 1.72-1.97 (m, 9H), 1.54-1.65 (m, 3H). ^{13}C NMR (125 MHz) δ

172.3, 161.0, 148.1, 135.0, 134.8, 130.5, 130.5, 128.5, 120.7, 120.7, 63.7, 58.7, 53.8, 52.6, 51.7, 44.9, 41.6, 37.5, 33.1, 30.2, 27.5, 26.3, 22.8, 22.4, 22.0, 21.2, 19.3, 17.8. Calcd for $C_{28}H_{39}F_3N_3O_2$ $(M+H)^+$: 506.2989. Found: 506.2984.

2-(4-Trifluoromethoxyphenyl)-*N*-[5-(*N*^{12}-aloperine-yl)pentyl]acetamide (7d): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.39 (dd, 2H, *J* = 6.4 Hz, J = 2.0 Hz), 7.22 (d, 2H, *J* = 8.4 Hz), 5.93 (d, 1H, *J* = 5.2 Hz), 4.21 (d, 1H, *J* = 4.8 Hz), 3.83 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.73 (d, 1H, *J* = 12.4 Hz), 3.53 (s, 2H), 3.46 (d, 1H, *J* = 12.4 Hz), 3.14-3.27 (m, 7H), 3.05 (td, 1H, *J* = 13.2 Hz, *J* = 5.6 Hz), 2.86 (bs, 1H), 2.51-2.55 (m, 2H), 2.35 (dd, 1H, *J* = 8.8 Hz), 2.26 (d, 1H, *J* = 14.0 Hz), 2.16 (ddd, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz), 1.78-1.98 (m, 9H), 1.55-1.64 (m, 3H), 1.33-1.48 (m, 2H). Calcd for $C_{29}H_{41}F_3N_3O_2$ $(M+H)^+$: 424.2959. Found: 424.2954.

3-[3,4-(Methylenedioxy)phenyl)]-*N*-[2-(*N*^{12}-aloperine-yl)ethyl]acrylamide (8a): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.49 (d, 1H, *J* = 16.0 Hz), 7.09 (d, 1H, *J* = 1.2 Hz), 7.03 (dd, 1H, *J* = 8.0 Hz, *J* = 1.2 Hz), 6.84 (d, 1H, *J* = 8.0 Hz), 6.43 (d, 1H, *J* = 15.6 Hz), 6.00 (s, 2H), 5.97 (d, 1H, *J* = 6.0 Hz), 4.31 (d, 1H, *J* = 5.2 Hz), 3.94 (d, 1H, *J* = 6.8 Hz), 3.91 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.65-3.85 (m, 3H), 3.46 (d, 1H, *J* = 12.4 Hz), 3.14-3.26 (m, 5H), 2.99 (bs, 1H), 2.53-2.57 (m, 2H), 2.33-2.42 (m, 1H), 2.30 (d, 1H, *J* = 13.6 Hz), 2.17 (dq, 1H, *J* = 13.2 Hz, *J* = 3.6 Hz), 1.52-2.00 (m, 8H). Calcd for $C_{27}H_{36}N_3O_3$ $(M+H)^+$: 450.2751. Found: 450.2746.

3-[3,4-(Methylenedioxy)phenyl)]-*N*-[3-(*N*^{12}-aloperine-yl)propyl]acrylamide (8b): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.48 (d, 1H, *J* = 15.6 Hz), 7.11 (d, 1H, *J* = 1.2 Hz), 7.04 (dd, 1H, *J* = 8.4 Hz, *J* = 1.6 Hz), 6.84 (d, 1H, *J* = 8.0 Hz), 6.44 (d, 1H, *J* = 15.6 Hz), 6.00 (d, 2H, *J* = 1.2 Hz),

5.95 (d, 1H, *J* = 5.2 Hz), 4.24 (d, 1H, *J* = 4.8 Hz), 3.89 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.78 (d, 1H, *J* = 12.0 Hz), 3.43-3.48 (m, 5H), 3.26 (d, 2H, *J* = 6.8 Hz), 3.10-3.21 (m, 2H), 2.91 (bs, 1H), 2.52-2.56 (m, 2H), 2.33-2.39 (m, 1H), 2.29 (d, 1H, *J* = 13.6 Hz), 2.17 (ddd, 1H, *J* = 10.8 Hz, *J* = 4.0 Hz), 2.03-2.13 (m, 2H), 1.91-1.96 (m, 3H), 1.70-1.83 (m, 4H), 1.57 (dq, 1H, *J* = 13.2 Hz, *J* = 3.2 Hz). Calcd for $C_{28}H_{38}N_3O_3$ $(M+H)^+$: 464.2908. Found: 464.2900.

3-[3,4-(Methylenedioxy)phenyl)]-*N*-[4-(*N*^{12}-aloperine-yl)butyl]acrylamide (8c): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.45 (d, 1H, *J* = 16.0 Hz), 7.10 (d, 1H, *J* = 1.6 Hz), 7.03 (dd, 1H, *J* = 8.0 Hz, *J* = 2.0 Hz), 6.84 (d, 1H, *J* = 8.0 Hz), 6.44 (d, 1H, *J* = 15.6 Hz), 6.00 (s, 2H), 5.94 (d, 1H, *J* = 6.4 Hz), 4.23 (d, 1H, *J* = 4.8 Hz), 3.88 (dd, 1H, *J* = 16.0 Hz, *J* = 4.0 Hz), 3.78 (d, 1H, *J* = 12.4 Hz), 3.34-3.47 (m, 5H), 3.23-3.27 (m, 2H), 3.08-3.22 (m, 2H), 2.91 (bs, 1H), 2.52-2.55 (m, 2H), 2.35 (dd, 1H, *J* = 17.6 Hz, *J* = 8.0 Hz), 2.28 (d, 1H, *J* = 15.6 Hz), 2.15 (dq, 1H, *J* = 13.6 Hz, *J* = 3.6 Hz), 1.65-2.01 (m, 11H), 1.59 (ddt, 1H, *J* = 13.2 Hz, *J* = 3.6 Hz). Calcd for $C_{29}H_{40}N_3O_3$ $(M+H)^+$: 478.3064. Found: 478.3054.

3-[4-(Trifluoromethoxy)phenyl)]-*N*-[4-(*N*^{12}-aloperine-yl)butyl]acrylamide (9a): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.64 (d, 2H, *J* = 6.8 Hz), 7.53 (d, 1H, *J* = 16.0 Hz), 7.28 (d, 2H, *J* = 8.0 Hz), 6.60 (d, 1H, *J* = 12.0 Hz), 5.92 (d, 1H, *J* = 5.6 Hz), 4.21 (d, 1H, *J* = 4.8 Hz), 3.87 (dd, 1H, *J* = 14.0 Hz, *J* = 3.6 Hz), 3.76 (d, 1H, *J* = 11.6 Hz), 3.31-3.43 (m, 5H), 3.06-3.28 (m, 4H), 2.91 (bs, 1H), 2.50-2.53 (m, 2H), 2.30-2.37 (m, 1H), 2.27 (d, 1H, *J* = 14.0 Hz), 2.16 (ddd, 1H, *J* = 12.8 Hz, *J* = 3.2 Hz), 1.64-1.96 (m, 11H), 1.57 (dd, 1H, *J* = 12.8 Hz). Calcd for $C_{29}H_{39}F_3N_3O_2$ $(M+H)^+$: 512.2989. Found: 512.2983.

3-[4-(Trifluoromethoxy)phenyl)]-*N*-[5-(*N*^{12}-aloperine-yl)pentyl]acrylamide (9b): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.66 (dd, 2H, *J* = 6.8 Hz, *J* = 1.6 Hz), 7.53 (d, 1H, *J* = 16.0 Hz), 7.30 (d, 2H, *J* = 8.0 Hz), 6.62 (d, 1H, *J* = 16.0 Hz), 5.92 (d, 1H, *J* = 5.2 Hz), 4.24 (d, 1H, *J* = 4.8 Hz), 3.89 (dd, 1H, *J* = 14.8 Hz, *J* = 4.0 Hz), 3.77 (d, 1H, *J* = 12.0 Hz), 3.45 (d, 1H, *J* = 12.4 Hz), 3.33-3.37 (m, 4H), 3.17-3.28 (m, 3H), 3.10 (ddd, 1H, *J* = 13.2 Hz, *J* = 4.4 Hz), 2.90 (bs, 1H), 2.52-2.56 (m, 2H), 2.36 (dd, *J* = 9.6 Hz), 2.29 (d, 1H, *J* = 15.2 Hz), 2.17 (ddd, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz), 1.75-2.02 (m, 9H), 1.40-1.70 (m, 5H). Calcd for $C_{30}H_{41}F_3N_3O_2$ $(M+H)^+$: 532.3145. Found: 532.3137.

2-Fluoro-4-trifluoromethoxy-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (10a): ^{1}H NMR (400 MHz) (CD_3OD) δ 8.5 (bs, 1H), 7.82 (t, 1H, *J* = 8.4 Hz), 7.22-7.25 (m, 2H), 5.95 (d, 1H, *J* = 6.4 Hz), 4.24 (d, 1H, *J* = 4.8 Hz), 3.89 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.81 (d, 1H, *J* = 12.4 Hz), 3.35-3.58 (m, 5H), 3.10-3.27 (m, 4H), 2.96 (bs, 1H), 2.52-2.56 (m, 2H), 2.28-2.40 (m, 2H), 2.18 (ddd, 1H, *J* = 12.8 Hz, *J* = 4.0 Hz), 1.71-2.01 (m, 11 H), 1.59 (ddt, 1H, *J* = 12.8 Hz, *J* = 3.6 Hz). Calcd for $C_{27}H_{36}F_4N_3O_2$ $(M+H)^+$: 510.2738. Found: 510.2733.

2-Fluoro-4-trifluoromethoxy-*N*-[5-(*N*^{12}-aloperine-yl)pentyl]benzamide (10b): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.81 (t, 1H, *J* = 8.0 Hz), 7.24 (d, 2H, *J* = 9.2 Hz), 5.95 (d, 1H, *J* = 5.6 Hz), 4.24 (d, 1H, *J* = 4.4 Hz), 3.88 (dd, 1H, *J* = 14.8 Hz, *J* = 3.6 Hz), 3.77 (d, 1H, *J* = 10.4 Hz), 3.07-3.48 (m, 9H), 2.91 (bs, 1H), 2.53-2.57 (m, 2H), 2.17-2.40 (m, 3H), 1.45-1.99 (m, 14 H). Calcd for $C_{28}H_{38}F_4N_3O_2$ $(M+H)^+$: 524.2895. Found: 524.2889.

3-Trifluoromethoxy-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (11a): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.83 (d, 1H, *J* = 7.6 Hz), 7.75 (s, 1H), 7.58 (t, 1H, *J* = 8.0 Hz), 7.47 (d, 1H, *J* = 8.0 Hz), 5.95 (d, 1H, *J* = 6.0 Hz), 4.23 (d, 1H, *J* = 4.8 Hz), 3.89 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.81 (d, 1H, *J* = 12.4 Hz), 3.56 (p, 1H, *J* = 6.8 Hz), 3.35-3.51 (m, 4H), 3.24 (d, 2H, *J* = 6.8 Hz), 3.08-3.20 (m, 2H), 2.96 (bs, 1H), 2.52-2.56 (m, 2H), 2.32-2.39 (m, 1H), 2.30 (d, 1H, *J* = 13.6 Hz), 2.18 (dd, 1H, *J* = 12.8 Hz), 1.73-2.01 (m, 11H), 1.59 (ddt, 1H, *J* = 13.2 Hz, *J* = 4.0 Hz). Calcd for $C_{27}H_{37}F_3N_3O_2$ $(M+H)^+$: 492.2832. Found: 492.2829.

2-Trifluoromethoxy-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (11b): ^{1}H NMR (400 MHz) (CD_3OD) δ 8.62 (bs, 1H), 7.56-7.64 (m, 2H), 7.39-7.47 (m, 2H), 5.95 (d, 1H, *J* = 4.4 Hz), 4.23 (bs, 1H), 3.88 (dd, 1H, *J* = 14.8 Hz, *J* = 4.0 Hz), 3.80 (d, 1H, *J* = 12.0 Hz), 3.12-3.50 (m, 9H), 2.96 (bs, 1H), 2.53-2.57 (m, 2H), 2.13-2.40 (m, 3H), 1.72-1.99 (m, 11 H), 1.54-1.63 (m, 1H). Calcd for $C_{27}H_{37}F_3N_3O_2$ $(M+H)^+$: 492.2832. Found: 492.2830.

***N*-[4-(*N*^{12}-Aloperine-yl)butyl]benzamide (12a):** ^{1}H NMR (400 MHz) (CD_3OD) δ 7.80-7.84 (m, 2H), 7.53-7.57 (m, 1H), 7.45-4.49 (m, 2H), 5.93 (bs, 1H), 4.22 (bs, 1H), 3.79-3.88 (m, 2H), 3.55 (p, 1H, *J* = 6.8 Hz), 3.39-3.46 (m, 3H), 3.09-3.26 (m, 5H), 2.94 (bs, 1H), 2.51-2.55 (m, 2H), 2.30-2.39 (m, 1H), 2.27 (d, 1H, *J* = 14.0 Hz), 2.12-2.21 (m, 1H), 1.70-1.98 (m, 11H), 1.54-1.63 (m, 1H). Calcd for $C_{26}H_{38}N_3O$ $(M+H)^+$: 408.3009. Found: 408.3002.

4-Fluoro-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (12b): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.84-7.88 (m, 2H), 7.14-7.20 (m, 2H), 5.92 (bs, 1H), 4.19 (bs, 1H), 3.76-3.86 (m, 2H), 3.33-3.54 (m, 5H), 3.07-3.25 (m, 4H), 2.89-3.05 (m, 1H), 2.50-2.53 (m, 2H), 2.28-2.36 (m, 1H), 2.27 (d,

1H, *J* = 14.0 Hz), 2.15 (ddd, 1H, *J* = 13.4 Hz), 1.70-2.05 (m, 11H), 1.57 (ddd, 1H, *J* = 12.8 Hz). Calcd for $C_{26}H_{37}N_3O$ $(M+H)^+$: 426.2915. Found: 426.2907.

4-Methyl-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (12c): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.72 (dd, 2H, *J* = 6.8 Hz, *J* = 2.0 Hz), 7.27 (d, 2H, *J* = 8.0 Hz), 5.93 (d, 1H, *J* = 4.8 Hz), 4.22 (d, 1H, *J* = 4.8 Hz), 3.83 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.79 (d, 1H, *J* = 14.4 Hz), 3.53 (p, 1H, *J* = 6.8 Hz), 3.37-3.48 (m, 3H), 3.08-3.28 (m, 5H), 2.93 (bs, 1H), 2.51-2.55 (m, 2H), 2.39 (s, 3H), 2.32-2.38 (m, 1H), 2.27 (d, 1H, *J* = 16.0 Hz), 2.16 (ddd, 1H, *J* = 12.8 Hz, *J* = 4.0 Hz), 1.70-1.98 (m, 11H), 1.57 (qt, 1H, *J* = 13.2 Hz, *J* = 3.6 Hz). Calcd for $C_{27}H_{40}N_3O$ $(M+H)^+$: 422.3166. Found: 422.3156.

4-Trifluoromethyl-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (12d): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.99 (d, 2H, *J* = 8.4 Hz), 7.78 (d, 2H, *J* = 8.4 Hz), 5.94 (d, 1H, *J* = 5.6 Hz), 4.22 (d, 1H, *J* = 4.8 Hz), 3.87 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.80 (d, 1H, *J* = 12.0 Hz), 3.56 (p, 1H, *J* = 7.2 Hz), 3.42-3.50 (m, 4H), 3.08-3.26 (m, 4H), 2.95 (bs, 1H), 2.52-2.55 (m, 2H), 2.32-2.39 (m, 1H), 2.29 (d, 1H, *J* = 13.2 Hz), 2.18 (dd, 1H, *J* = 13.2 Hz), 1.72-1.98 (m, 11H), 1.58 (dd, 1H, *J* = 13.2 Hz). Calcd for $C_{27}H_{37}F_3N_3O$ $(M+H)^+$: 476.2883. Found: 476.2876.

4-Hydroxy-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (12e): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.71 (dd, 2H, *J* = 6.8 Hz, *J* = 2.0 Hz), 6.83 (dd, 2H, *J* = 6.8 Hz, *J* = 2.0 Hz), 5.94 (bs, 1H), 4.21 (bs, 1H), 3.78-3.83 (m, 2H), 3.34-3.55 (m, 4H), 3.07-3.29 (m, 5H), 2.91 (bs, 1H), 2.51-2.54 (m, 2H), 2.28-2.38 (m, 1H), 2.26 (d, 1H, *J* = 14.0 Hz), 2.15 (ddd, 1H, *J* = 12.4 Hz), 1.70-1.98 (m, 11H), 1.57 (ddd, 1H, *J* = 13.6 Hz). Calcd for $C_{26}H_{38}N_3O_2$ $(M+H)^+$: 424.2959. Found: 424.2954.

4-Methoxy-*N*-[4-(*N*^{12}-aloperine-yl)butyl]benzamide (12f): ^{1}H NMR (400 MHz) (CD_3OD) δ 7.81 (t, 1H, *J* = 2.0 Hz), 7.79 (t, 1H, *J* = 2.0 Hz), 6.99 (t, 1H, *J* = 2.0 Hz), 6.98 (t, 1H, *J* = 2.0 Hz), 5.94 (d, 1H, *J* = 5.2 Hz), 4.22 (d, 1H, *J* = 5.6 Hz), 3.78-3.86 (m, 5H), 3.52 (p, 1H, *J* = 6.8 Hz), 3.37-3.47 (m, 3H), 3.08-3.28 (m, 5H), 2.91 (bs, 1H), 2.51-2.55 (m, 2H), 2.35 (dd, 1H, *J* = 17.2 Hz, *J* = 8.4 Hz), 2.27 (d, 1H, *J* = 16.0 Hz), 2.16 (ddd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 1.73-2.06 (m, 11H), 1.58 (ddt, 1H, *J* = 13.2 Hz, *J* = 3.6 Hz). Calcd for $C_{27}H_{40}N_3O_2$ $(M+H)^+$: 438.3115. Found: 438.3114.

***N*-[4-(*N*^{12}-Aloperine-yl)butyl]benzylamine (13a):** ^{1}H NMR (400 MHz) (CD_3OD) δ 7.46-7.51 (m, 5H), 5.95 (d, 1H, *J* = 5.2 Hz), 4.23 (s, 3H), 3.88 (dd, 1H, *J* = 14.4 Hz, *J* = 4.0 Hz), 3.78 (d, 1H, *J* = 11.6 Hz), 3.46 (d, 1H, *J* = 11.6 Hz), 3.34-3.38 (m, 2H), 3.27 (d, 2H, *J* = 8.0 Hz), 3.08-3.21 (m, 4H), 2.91 (bs, 1H), 2.54-2.57 (m, 2H), 2.32-2.40 (m, 1H), 2.29 (d, 1H, *J* = 14.4 Hz), 2.18 (dd, 1H, *J* = 12.8 Hz), 1.74-2.03 (m, 11H), 1.60 (dd, 1H, *J* =12.8 Hz). Calcd for $C_{26}H_{40}N_3$ $(M+H)^+$: 394.3217. Found: 394.3212.

***N*-[5-(*N*^{12}-Aloperine-yl)pentyl]benzylamine (13b):** ^{1}H NMR (400 MHz) (CD_3OD) δ 7.44-7.50 (m, 5H), 5.96 (bs, 1H), 4.21 (s, 3H), 3.88 (dd, 1H, *J* = 14.0 Hz, *J* = 4.0 Hz), 3.78 (d, 1H, *J* = 12.8 Hz), 3.46 (d, 1H, *J* = 12.0 Hz), 3.34-3.38, 3.26-3.28 (m, 4H), 3.05-3.19 (m, 4H), 2.91 (bs, 1H), 2.53-2.57 (m, 2H), 2.32-2.39 (m, 1H), 2.29 (d, 1H, *J* = 15.6 Hz), 2.18 (dd, 1H, *J* = 12.4 Hz), 1.75-1.99 (m, 11H), 1.59 (dd, 1H, *J* =12.8 Hz), 1.42-1.56 (m, 2H). Calcd for $C_{27}H_{41}N_3$ $(M+H)^+$: 408.3373. Found: 408.3370.

***N*-[4-(*N*^{12}-Aloperine-yl)butyl]-4-(trifluoromethoxy)benzylamine (13c):** ^{1}H NMR (400 MHz) (CD_3OD) δ 7.63 (t, 1H, *J* = 2.4 Hz), 7.61 (t, 1H, *J* = 2.0 Hz), 7.38 (d, 2H, *J* = 7.6 Hz), 5.96 (d, 1H, *J* = 6.0 Hz), 4.27 (s, 2H), 4.22 (d, 1H, *J* = 4.4 Hz), 3.89 (dd, 1H, *J* = 14.4 Hz, *J* = 3.6 Hz), 3.78 (d, 1H, *J* = 11.6 Hz), 3.47 (d, 1H, *J* = 12.4 Hz), 3.39 (dd, 1H, *J* = 13.2 Hz, *J* = 4.4 Hz), 3.36 (bs, 1H), 3.27 (d, 2H, *J* = 6.4 Hz), 3.09-3.21 (m, 4H), 2.92 (bs, 1H), 2.54-2.57 (m, 2H), 2.32-2.40 (m, 1H), 2.29 (d, 1H, *J* = 16.0 Hz), 2.19 (ddd, 1H, *J* = 13.2 Hz, *J* = 1.2 Hz), 1.76-1.99 (m, 11H), 1.56-1.66 (m, 1H). Calcd for $C_{27}H_{39}F_3N_3O_2$ $(M+H)^+$: 478.3040. Found: 478.3041.

Supporting Results:

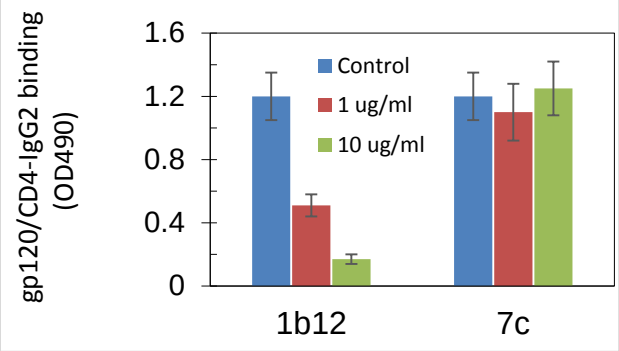

Figure S1. Compound 7c inhibited HIV-1 entry without affecting gp120/CD4 binding. HIV-1 IIIB gp120 (NIH AIDS Reagent Program, Catalog Number 11784) at 10 ug/ml in $(NH_4)_2CO_3$ was coated on ELISA plates. After blocking with 1% BSA in PBS, CD4-IgG2 (NIH AIDS Reagent Program) at 10 ug/ml was added in the presence of various concentrations of compounds (1 ug/ml and 10 ug/ml). Binding of CD4-IgG2 (NIH AIDS Reagent Program, Catalog Number 11780) to the gp120 was detected with HRP-anti-human IgG. "Control" in the figure denotes the binding of gp120 and CD4-IgG2 in the absence of 1b12 or **7c.** The data in the figure represent mean +/- SD from three independent assays.