Supporting Information

Inhibition of Ebola Virus Infection: Identification of Host Cell Niemann-Pick C1 as the Molecular Target through Optimization of a Chemical Probe

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I. Chemistry general information

All reactions were carried out under nitrogen atmosphere unless otherwise stated. Solvents were purchased as anhydrous grade and used without further purification. Reagents were purchased from commercial sources and used as received. Reaction progress was monitored by TLC using pre-coated 250 μm silica gel plates or LC/MS using Agilent 1200/6130 LC/MS system. All chromatographic purifications were conducted using a parallel flash chromatography system (CombiFlash Companion 4x). ^{1}H NMR spectra were recorded on a Varian Inova 600 MHz spectrometer with chemical shifts reported in parts per million (ppm) relative to an internal standard (trimethylsilane). Coupling constants (*J*) are reported in hertz (Hz). Mass spectra were obtained on an Agilent 6130 Single Quad mass spectrometer in positive-mode electrospray ionization. HPLC was performed on an Agilent 1200 LC system using Phenomenex C18 column (4.6 x 100 mm, 5 μm) eluted with a 10 min gradient from 5 to 95% acetonitrile in water containing 0.1% formic acid.

II. Representative synthetic Procedures and Compound Characterization Data

1. Synthesis of 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-benzylpiperazin-1-yl)-2-oxoethyl)acetamide (1)

A mixture of *N*-Boc-glycine (26 mg, 0.15 mmol), BOP (98 mg, 0.23 mmol), 1-benzylpiperazine (29 mg, 0.17 mmol), and *N, N*-diisopropylethylamine (78 uL, 0.45 mmol) in DMF (1 mL) was stirred for 18h at room temperature. The mixture was diluted with water and extracted with EtOAc. The combined extracts were washed sequentially with 1N-NaOH, water, and brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient: 0-5% MeOH/ CH_2Cl_2) to give the desired amide (42 mg). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.26 (m, 5H), 5.52 (br s, 1H), 3.83 (d, 2H, *J*=3.6), 3.64 (br s, 2H), 3.60 (s, 2H), 3.41 (br s, 2H), 2.49 (br s, 4H), 1.43 (s, 9H); MS(ESI) m/z 334.2 $[M+H]^+$

To a solution of the amide above in CH_2Cl_2 (1 mL) was added TFA (0.1 mL) and the mixture was stirred for 6 hr at room temperature. The solvent and excess TFA were removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (1 mL) and cooled to 0 ^{o} C. *N, N*-diisopropylethylamine (0.13 mL, 0.72 mmol) and adamantaneacetyl chloride (26 mg, 0.12 mmol) were added. The reaction mixture was stirred for 6hr at room temperature and then partitioned between CH_2Cl_2 and water. The organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography (gradient: 0-5% MeOH/ CH_2Cl_2) to give 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-benzylpiperazin-1-yl)-2-oxoethyl)acetamide (1). ^{1}H NMR (600 MHz, CD_3OD) δ 7.86 (br s, 1H), 7.34-7.25 (m, 5H), 4.03 (s, 2H), 3.59 (app t, 2H, *J*=4.8), 3.57 (s, 2H), 3.52 (app t, 2H, *J*=4.8), 2.51 (app t, 2H, *J*=4.8), 2.47 (app t, 2H, *J*=4.8), 2.00 (s, 2H), 1.95 (br s, 3H), 1.74-1.66 (m, 12H) ; MS(ESI) m/z 410.2 $[M+H]^+$

The following compounds were synthesized using a procedure similar to that of 1.

N-(2-(4-benzylpiperazin-1-yl)-2-oxoethyl)-2-cyclohexylacetamide (4c): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34-7.26 (m, 5H), 6.57 (br s, 1H), 4.2 (d, 2H, *J*=4.2), 3.65 (app t, 2H, *J*=5.4), 3.53 (s, 2H), 3.41 (app t, 2H, *J*=5.4),2.45 (app t, 4H, *J*=5.4), 2.11 (d, 2H, *J*=7.2), 1.81-1.63 (m, 7H), 1.34-0.95 (m, 4H) ; ESI-MS [M+H]=358.2

N-(2-4-benzylpiperazin-1-yl)-oxoethyl)-2-phenylacetamide (4d): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38-7.22 (m, 5H), 4.01 (d, 2H, *J*=4.2), 3.61 (s, 2H), 3.59 (app t, 2H, *J*=5.4), 3.50 (s, 2H), 3.37 (app t, 2H, *J*=5.4), 2.41 and 2.39 (AB q, 4H, *J*=5.4) ; MS(ESI) m/z 352.2 $[M+H]^+$

N-(2-(4-benzylpiperazin-1-yl)-2-ocxoethyl)-3,3-dimethylbutanamide (4f): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34-7.26 (m, 5H), 6.51 (br s, 1H), 4.04 (d, 2H, *J*=4.2), 3.65 (app t, 2H,*J*=5.4), 3.53 (s, 2H), 3.41 (app t, 2H, *J*=5.4), 2.45 (app t, 4H, *J*=5.4), 2.13 (s, 2H), 0.05 (s, 9H) ; MS(ESI) m/z 332.2 $[M+H]^+$

(3*r*, 5*r*, 7*r*)-*N*-(2-(4-benzylpiperazin-1-yl)-2-oxoehtyl)adamantane-1-carboxamide (4g): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.27(m, 5H), 6.81 (br s, 1H), 4.01(d, 2H, *J*= 4.2), 3.64 (app t, 2H, *J*=5.4), 3.49 (s, 2H), 3.40 (app t, 2H, *J*= 5.4), 2.42 (app t, 4H, *J*=5.4), 2.01 (br s, 3H), 1.85 (br s, 6H), 1.75-1.71 (m, 6H) ; MS(ESI) m/z 396.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(1-(4-benzylpiperazin-1-yl)-1-oxopropan-2-yl)acetamide (5a): MS(ESI) m/z 424.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(3-(4-benzylpiperazin-1-yl)-3-oxopropyl)acetamide (5b): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34-7.26 (m, 5H), 6.27 (br s, 1H), 3.61 (app t, 2H, *J*=5.4), 3.53 (t, 2H, *J*=6.0), 3.51 (s, 2H), 3.42 (app t, 2H, *J*=5.4), 2.51 (t, 2H, *J*=6.0), 2.41 (app t, 4H, *J*=5.4), 1.96 (br s, 3H), 1.89 (s, 2H), 1.70-1.59 (m, 12H) ; MS(ESI) m/z 424.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(1-(4-benzylpiperazin-1-yl)-3-methyl-1-oxobutan-2-yl)acetamide (5c): MS(ESI) m/z 452.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(1-(4-benzylpiperazin-1-yl)-4-methyl-1-oxopentan-2-yl)acetamide (5d): MS(ESI) m/z 466.4 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(1-(4-benzylpiperazin-1-yl)-3-cyano-1-oxopropan-2-yl)acetamide (5e): MS(ESI) m/z 449.4 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(1-(4-benzylpiperazin-1-yl)-3-hydroxy-1-oxobutan-2-yl)acetamide (5f): MS(ESI) m/z 454.4 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(3-(benzyloxy)-1-(4-benzylpiperazin-1-yl)-1-oxobutan-2-yl)acetamide (5g): MS(ESI) m/z 544.3 $[M+H]^+$

4-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)-5-(4-benzylpiparazin-1-yl)-5-oxopentanoic acid (5h): MS(ESI) m/z 482.4 $[M+H]^+$

benzyl 4-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)-5-(4-benzylpiparazin-1-yl)-5-oxopentanoate (5i): MS(ESI) m/z 572.5 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-benzylpiparazin-1-yl)-2-oxoethyl)-*N*-methylacetamide (5j): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36-7.26 (m, 10H), 6.36 (d, 1H, *J*=8.4), 5.13 and 5.10 (AB q, 2H, *J*=6), 5.02 (dt, 1H, *J*=3, 8.4), 3.65-3.58 (m, 4H), 3.50 (s, 2H), 2.53-2.48 (m, 1H), 2.42-2.37 (m, 5H), 2.11-2.05 (m, 1H), 1.95 (s, 5H), 1.74-1.61 (m, 13H) ; MS(ESI) m/z 424.2 $[M+H]^+$

2-(3*r*, 5*r*, 7*r*)-adamantan-1-yl)-1-(2-(4-benzyl(piparazine-1-carbonyl)pyrrolidin-1-yl)ethanone (5k): MS(ESI) m/z 450.4 $[M+H]^+$

2. Synthesis of 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-methoxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10a)

A mixture of *N*-Boc-glycine (686 mg, 3.9 mmol) and BOP (2.59 g, 5.9 mmol), 1-Cbz-piperazine (863 mg, 3.9 mmol), and *N,N*-diisopropylethylamine (2.0 mL, 12 mmol) in DMF (8 mL) was stirred for 18 h at room temperature and the resulting mixture was partitioned between EtOAc and brine. The organic layer was washed sequentially with 1N-NaOH, water, and brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (gradient: 0-5% MeOH/ CH_2Cl_2) to give the desired amide (1.37g, 93 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.2691 (m, 5H), 5.47 (br s, 1H), 5.15 (s, 2H), 3.96 (d, 2H, *J*=4.2), 3.62 (br s, 2H), 3.55-3.50 (m, 4H), 3.38 (br s, 2H), 1.45 (s, 9H); MS(ESI) m/z 400.2 $[M+H]^+$

To a solution of the amide above in CH_2Cl_2 (8 mL) was added TFA (1.4 mL, 18 mmol) at 0 ^{o} C and the mixture was stirred for 3 hr at room temperature. The solvent and excess TFA were removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 ^{o} C. *N, N*-diisopropylethylamine (3.2 mL, 18 mmol) and adamantaneacetyl chloride (772 mg, 3.6 mmol) were added. The reaction mixture was stirred for 6hr at room temperature and then partitioned between CH_2Cl_2 and water. The organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography (gradient: 0-5% MeOH/ CH_2Cl_2) to give benzyl 4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piperazine-1-carboxylate (9) (1.2 g, 73 % for 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.32 (m, 5H), 6.42 (br s, 1H), 5.15 (s, 2H), 4.07 (d, 2H, *J*=4.2), 3.64 (br s, 2H), 3.55-3.48 (m, 4H), 3.40 (br s, 2H), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 454.1 $[M+H]^+$

A suspension of 9 (1.2 g, 2.6 mmol) and Palladium on carbon (10%, 120 mg) in MeOH (6 mL) was stirred under 1 atm of hydrogen atmosphere at room temperature for 18 hr. The catalyst was removed by filtered through celite and washed with methanol. The filtrate was concentrated under reduced pressure to give (1-(adamantan-1-yl)acetamido)acetyl)piperazine (797 mg) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.4 (br s, 1H), 4.06 (d, 2H, *J*=3.6), 3.67-3.65 (m, 2H), 3.44-3.42 (m, 2H), 2.94-2.90 (m, 4H), 2.01 (s, 2H), 1.97 (br s, 3H), 1.70-1.55 (m, 12H).

To a mixture of (1-(adamantan-1-yl)acetamido)acetyl)piperazine (10 mg, 0.031 mmol) and 2-methoxybenzaldehyde (0.19 mL of 0.2 M solution in toluene) in CH_2Cl_2 (1 mL) was added sodium triacetoxyborohydride (0.23 mL of 0.2 M solution in CH_2Cl_2) at 0 ^{o} C and the resulting mixture was stirred for 16h at ambient temperature. MeOH (0.5 mL) was added with vigorous stirring and then loaded onto a solid phase extraction (SPE) cartridge containing strong ion exchanger (SCX, 1g; UCT, CUBCX1M15). The cartridge was first washed with MeOH (6mL), which was discarded, and then the product was eluted with EtOAc-MeOH-Et_3N (20:2:1, 6 mL). The eluent was concentrated in vacuo to yield 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-methoxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (9 mg, 66%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (d, 1H, *J*=7.2), 7.25 (t, 1H, *J*=7.2), 6.94 (t, 1H, *J*=7.2), 6.87 (d, 1H, *J*=8.4), 6.48 (br s, 1H), 3.03 (d, 2H, *J*=4.2), 3.88 (s, 3H), 3.64 (app t, 2h, *J*=4.8), 3.59 (s, 2H), 3.41 (app t, 2H, *J*=4.8), 2.62 and 2.60 (AB q, 4H, *J*=4.8), 2.00 (s, 2H), 1.976 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 440.3 $[M+H]^+$

The following compounds were synthesized using a procedure similar to that of 10a.

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-(benzyloxy)benzylpiperazin-1-yl)-2-oxoethyl)acetamide (10b): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43-7.32 (m, 6H), 7.24 (t, 1H, *J*=7.8), 6.97-6.94 (m, 2H), 6.48 (br s, 1H), 5.08 (s, 2H), 4.03 (d, 2H, *J*=3.6), 3.64 (s, 4H), 3.39 (app t, 2H, *J*=4.8), 2.50 and 2.48(AB q, 4H, *J*=4.8), 2.00 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 516.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-(chlorobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10c): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (d, 1H, *J*=7.2), 7.36 (d, 1H, *J*=8.4), 7.26-7.20 (m, 2H), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=2.4), 3.66 (app t, 2H, *J*=3.6, 2H), 3.64 (s, 2H), 3.42 (app t, 2H, *J*=3.6), 2.53 (app t, 4H, *J*=3.6), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 444.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-(bromobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10d): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, 1H, *J*=7.8), 7.43 (d, 1H, *J*=7.8), 7.29 (t, 1H, *J*=7.8), 7.13(t, 1H, *J*=7.8), 6.50 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.66 (app t, 2H, *J*=4.8), 3.62 (s, 2H), 3.42 (app t, 2H, *J*=4.8), 2.54 and 2,52 (ABq, 4H, *J*=4.8), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 488.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-(cyanobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10e): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (d, 1H, *J*=7.8), 7.57 (t, 1H, *J*=7.8), 7.51 (d, 1H, *J*=8.4), 7.39 (d, 1H, *J*=7.8), 6.47 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.72 (s, 2H), 3.66 (app t, 2H, *J*=4.8), 3.42 (app t, 2H, *J*=4.8), 2.53 and 2.51 (AB q, 4H, *J*=4.8), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 435.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-(hydroxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10f): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20 (t, 1H, *J*=6.6), 6.98 (d, 1H, *J*=6.6), 6.84 (d, 1H, *J*=8.4), 6.81 (t, 1H, *J*=7.8), 6.44 (br s, 1H), 4.06 (d, 2H, *J*=3.6), 3.75 (br s, 2H), 3.74 (s, 2H), 3.48 (br s, 2H), 2.59 (br s, 4H), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 426.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-oxo-2-(4-(2-(trifluoromethyl)benzyl)piparazin-1-yl)ethyl)acetamide (10g): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, 1H, *J*=8.4), 7.64 (d, 1H, *J*=7.8), 7.53 (t, 1H, *J*=7.8), 7.36 (t, 1H, *J*=7.8), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=4.2), 3.69 (s, 2H), 3.66 (br s, 2H), 3.42 (app t, 2H, *J*=4.8), 2.49 (app t, 4H, *J*=4.8), 2.02 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 478.2 $[M+H]^+$

methyl 2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparazin-1-yl)methyl) benzoate (10h): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, 1H, *J*=7.8), 7.45-7.41 (m, 2H), 7.33 (t, 1H, *J*=7.2), 6.46 (br s, 1H), 4.03 (d, 2H, *J*=3.6), 3.88 (s, 3H), 3.80 (s, 2H), 3.59 (app t, 2H, *J*=4.8), 3.35 (app t , 2H, *J*=4.8), 2.44 and 2.42 (AB q, *J*=5.4), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 468.1 $[M+H]^+$

2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparazin-1-yl)methyl) benzoic acid (10i): MS(ESI) m/z 454.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-N-(2-oxo-2-(4-(pyridine-2-ylmethyl)piparazin-1-yl)ethyl)acetamide (10j): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (d, 1H, *J*=7.8), 7.68 (t, 1H, *J*=7.8), 7.38 (d, 1H, *J*=8.4), 7.22-7.19 (m, 1H), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.69 (s, 2H), 3.66 (br

s, 2H), 3.44 (app t, 2H, *J*=4.8), 2,53 and 2,51 (AB q, 4H, *J*=4.8), 2.01 (s, 2H), 1.99 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 411.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(3-methoxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10k): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.24 (t, 1H, *J*=7.8), 6.89 (d, 1H, *J*=8.4), 6.88 (s, 1H), 6.81 (d, 1H, *J*=7.8), 6.48 (br s, 1H), 4.04 (d, 2H, *J*=4.2), 3.81 (s, 3H), 3.65 (app t, 2H, *J*=4.8), 3.50 (s, 2H), 3.41 (app t, 2H, *J*=4.8), 2.46 and 2.44 (AB q, 4H, *J*=4.8), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.62(m, 12H) ; MS(ESI) m/z 440.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(3-(benzyloxy)benzylpiperazin-1-yl)-2-oxoethyl)acetamide (10l): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (d, 2H, *J*=7.8), 7.38 (t, 2H, *J*=7.2), 7.32 (t, 1H, *J*=7.8), 7.24 (t, 1H, *J*=7.8), 6.96 (s, 1H), 6.89 (d, 2H, *J*=7.2), 6.49 (br s, 1H), 5.07 (s, 2H), 4.03 (d, 2H, *J*=3.6), 3,63 (app t , 2H, *J*=4.2), 3.49 (s, 2H), 3.38 (app t, 2H, *J*=4.2), 2,43 and 2,41 (AB q, 4H, *J*=4.2), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 516.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(3-(bromobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10m): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.41 (d, 1H, *J*=7.8), 7.24-7.18 (m, 2H), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.66 (br s, 2H), 3.49 (s, 2H), 3.42 (br s, 2H), 2.45 (br s, 4H), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 488.1 $[M+H]^+$

10n: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.56 (d, 2H, *J*=7.8), 7.44 (d, 1H, *J*=8.4), 6.47 (br s, 1H), 4.05 (d, 2H, *J*=4.2), 3.66 (app t, 2H, *J*=4.8), 3.55 (s, 2H), 3.43 (app t, 2H, *J*=4.8), 2,45 and 2.43 (AB q, 4H, *J*=4.8), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 435.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(3-(methylbenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10o): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 (t, 1H, *J*=7.2), 7.12 (s, 1H), 7.09 (t, 2H, *J*=6.6), 6.48 (br s, 1H), 4.04 (d, 2H, *J*=4.2), 3.65 (app t, 2H, *J*=4.8), 3.49 (s, 2H), 3.40 (app t, 2H, *J*=4.8), 2.44 and 2.42 (AB q, 4H, *J*=4.8), 2.35 (s, 3H), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 424.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-oxo-2-(4-(3-(trifluoromethyl)benzyl)piparazin-1-yl)ethyl)acetamide (10p): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.54-7.51 (m, 2H), 7.45 (t, 1H, *J*=7.8), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=3), 3.66 (br s, 2H), 3.57 (s, 2H), 3.43 (app t, 2H, *J*=4.8), 2,46 (br s, 4H), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 478.2 $[M+H]^+$

methyl 3-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparazin-1-yl)methyl) benzoate (10q): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (d, 1H, *J*=7.2), 8.05 (s, 1H), 7.62(d, 1H, *J*=7.2), 7.54 (t, 1H, *J*=7.2), 6.33 (br s, 1H), 4.18 (s, 2H), 4.06 (br s, 2H), 3.94 (s, 3H), 3.79 (br s, 2H), 3.49 (s, 2H), 3.10 (br s, 4H), 2.01 (s, 2H), 1.97 (s, 3H), 1.71-1.61 (m, 12H) ; MS(ESI) m/z 468.1 $[M+H]^+$

3-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparazin-1-yl)methyl) benzoic acid (10r): MS(ESI) m/z 454.6 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-N-(2-oxo-2-(4-(pyridine-3-ylmethyl)piparazin-1-yl)ethyl)acetamide (10s): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.54 (d, 2H, *J*=11.4), 7.66 (d, 1H, *J*=7.8), 7.30-7.28 (m, 1H), 7.27 (s, 1H), 6.48 (br s, 1H), 4.05 (s, 2H), 3.65 (br s, 2H), 3.54 (s, 2H), 3.42 (br s, 2H), 2.46 (br s, 4H), 2.01 (s, 2H), 1.99 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 411.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-methoxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10t): MS(ESI) m/z 440.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-(chlorobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10u): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (d, 2H, *J*=8.4), 7.25 (d, 2H, *J*=7.8), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.64 (br s, 2H), 3.49 (s, 2H), 3.

40 (br s, 2H), 2.43 (br s, 4H), 2.01 (s, 2H), 1,98 (s, 3H), 1,70-1.63 (m, 12H) ; MS(ESI) m/z 444.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-(bromobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10v): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (d, 2H, *J*=8.4), 7.20 (d, 2H, *J*=7.8), 6.47 (br s, 1H), 4.04 (d, 2H, *J*=4.2), 3.64 (br s, 2H), 3.47 (s, 2H), 3.40 (br s, 2H), 2.43 (br s, 4H), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 488.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-(cyanobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10w): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, 2H, *J*=7.8), 7.45 (d, 2H, *J*=8.4), 6.46 (br s, 1H), 4.05 (d, 2H, *J*=4.2), 3.66 (app t, 2H, *J*=4.8), 3.57 (s, 2H), 3.42 (app t, 2H, *J*=4.8), 2.60 and 2.58 (AB q, 4H, *J*=4.8), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 435.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-(hydroxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10x): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (d, 2H, *J*=8.4), 6.78 (d, 2H, *J*=7.8), 6.53 (br s, 1H), 4.04 (d, 2H, *J*=3.6), 3.63 (app t, 2H, *J*=4.8), 3.43 (s, 2H), 3.36 (app t, 2H, *J*=4.8), 2.41 (app t, 4H, *J*=4.8), 2.02 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 426.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-(methylbenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10y): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.18 (d, 2H, *J*=7.8), 7.13 (d, 2H, *J*=8.4), 6.49 (br s), 4.03 (d, 2H, *J*=4.2), 3.64 (app t, 2H, *J*=4.8), 3.48 (s, 2H), 3.39 (app t, 2H, *J*=4.8), 2.44 and 2.42 (AB q, 4H, *J*=4.8), 2.34 (s, 3H), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 424.3 $[M+H]^+$

methyl 4-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparazin-1-yl)methyl) benzoate (10z): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (d, 2H, *J*=8.4), 7.48 (d, 2H, *J*=8.4), 6.35 (br s, 1H), 4.16 (s, 2H), 4.05 (br s, 2H), 3.94 (s, 3H), 3.78 (br s, 2H), 3.49 (s, 2H), 3.05 (br d, 4H), 2.00 (s, 2H), 1.97(s, 3H), 1.71-1.61 (m, 12H) ; MS(ESI) m/z 468.1 $[M+H]^+$

4-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparazin-1-yl)methyl) benzoic acid (10aa): MS(ESI) m/z 454.1 $[M+H]^+$

N-(2-(4-(4-acetamidobenzyl)piperazin-1-yl)-2-oxoethyl)-2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamide (10bb): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (br s, 1H), 7.46 (d, 2H, *J*=8.4), 7.25 (d, 2H, *J*=8.4), 6.49 (br s, 1H), 4.03 (d, 2H, *J*=4.2), 3.63 (app t, 2H, *J*=4.8), 3.47 (s, 2H), 3.37 (app t, 2H, *J*=4.8), 2.43 and 2.41 (AB q, 4H, *J*=4.8), 2.17 (s, 3H), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 467.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-oxo-2-(4-(pyridine-4-ylmethyl)piparazin-1-yl)ethyl)acetamide (10cc): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.56 (br s, 2H), 7.27 (d, 2H, *J*=4.8), 6.48 (br s, 1H), 4.05 (d, 2H, *J*=2.4), 3.67 (br s, 2H), 3.53(s, 2H), 3.43 (br s, 2H), 2.46 (br s, 4H), 2.01 (s, 2H), 1.99 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 411.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(5-bromo-2-hydroxybenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (10dd): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (d, 1H, *J*=9), 7.11 (s, 1H), 6.73 (d, 1H, *J*=9), 6.42 (br s, 1H), 4.07 (d, 2H, *J*=4.2), 3.70 (s, 2H), 3.66 (br s, 2H), 3.49 (br s, 2H), 2.58 (br s, 4H), 2.01 (s, 2H), 1.97 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 504.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2,4-dichlorobenzyl)piparazin-1-yl)-2-oxoethyl)acetamide (10ee): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, 2H, *J*=7.8), 7.23 (d, 1H, *J*=7.8), 6.49 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.65 (br s, 2H), 3.60 (s, 2H), 3.42 (app t, *J*=4.2), 2.50 (br s, 4H), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 478.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(3,4-dichlorobenzyl)piparazin-1-yl)-2-oxoethyl)acetamide (10ff): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.39 (d, 1H, *J*=8.4), 7.15 (d, 1H, *J*=8.4), 6.47 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.65 (br s, 2H), 3.47 (s, 2H), 3.42 (app t, 2H,

J=4.8), 2.44 (br s, 4H), 2.01 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 478.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(4-hydroxy-2,6-dimethylbenzyl)piparazin-1-yl)-2-oxoethyl)acetamide (10gg): ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.52 (s, 2H), 6.50 (br s, 1H), 4.05 (d, 2H, *J*=3.6), 3.66-3.57 (m, 2H), 3.42-31 (m, 2H), 2.87-2.86 (m, 2H), 2.42-2.40 (m, 2H), 2.30 (s, 6H), 2.02 (s, 2H), 1.98 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 454.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(naphthalen-1-ylmethyl)piparzain-1-yl)-2-oxoethyl)acetamide (10hh): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (d, 1H, *J*=7.8), 7.85 (d, 1H, *J*=7.8), 7.80 (d, 1H, *J*=7.8), 7.53-7.48 (m, 2H), 7.42-7.39 (m, 2H), 6.49 (br s, 1H), 4.04 (d, 2H, *J*=4.2), 3.92 (s, 2H), 3.63 (app t, 2H, *J*=4.8), 3.38 (app t, 2H, *J*=4.8), 2.51 (app t, 4H, *J*=4.8), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.63(m, 12H) ; MS(ESI) m/z 460.3 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(naphthalen-2-ylmethyl)piparzain-1-yl)-2-oxoethyl)acetamide (10ii): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84-7.80 (m, 3H), 7.72 (s, 1H), 7.49-7.45 (m, 3H), 6.49 (br s, 1H), 4.04 (d, 2H, *J*=3.6), 3.68 (s, 2H), 3.66 (app t, 2H, *J*=4.8), 3.41 (app t, 2H, *J*=5.4), 2.49 and 2.47 (AB q, 4H, *J*=4.8), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 460.3 $[M+H]^+$

3. Synthesis of compounds methyl 4-((2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparzain-1-yl)methyl)phenoxy)methyl)benzoate (2)

To a mixture of salicylaldehyde (0.4 mL, 3.8 mmol) and potassium carbonate (1.6 g, 11 mmol) in DMF (5 mL) was added methyl 4-(bromomethyl)benzoate (916 mg, 4.0 mmol) and the resulting mixture was stirred for 16 h at room temperature. The solvent was removed under reduced pressure and the residue was partitioned between H_2O (20 mL) and EtOAc (15 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography (gradient: 20-100% EtOAc/ hexanes) to give methyl 4- ((2-formylphenoxy)methyl)benzoate (3.1 g, 94 %) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.6 (s, 1H), 8.08 (d, 2H, *J*=7.8), 7.87 (d, 1H, *J*=7.2), 7.53 (t, 1H, *J*=7.8), 7.52 (d, 2H, *J*=7.8), 7.06 (t, 1H, *J*=7.8), 7.01 (d, 1H, *J*=8.4), 5.26 (s, 2H), 3.92 (s, 3H); MS(ESI) m/z 284.9 $[M+Na]^+$

To a mixture of methyl 4- ((2-formylphenoxy)methyl)benzoate (406 mg, 1.50 mmol) and (1-(adamantan-1-yl)acetamido)acetyl)piperazine (400 mg, 1.25 mmol) in CH_2Cl_2 (15 mL) was

added sodium triacetoxyborohydride (397 mg, 1.88 mmol) at 0 ^{o} C. After stirring for 4 h at room temperature, the mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography to give methyl 4-((2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparzain-1-yl)methyl)phenoxy)methyl)benzoate (517 mg, 72%) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 (d, 2H, *J*=8.4), 7.51 (d, 2H, *J*=7.8), 7.34 (d, 1H, *J*=7.8), 7.24 (t, 1H, *J*=7.8), 6.97 (t, 1H, *J*=7.8), 6.90 (d, 1H, *J*=8.4), 6.48 (br s, 1H), 5.15 (s, 2H), 4.03 (d, 2H, *J*=4.2), 3.93 (s, 3H), 3.65(br s, 4H), 3.40 (app t, 2H, *J*=4.8), 2.51 (app t, 4H, *J*=4.8), 2.01 (s, 2H), 1.98 (br s, 3H), 1.70-1.62 (m, 12H); MS(ESI) m/z 574.1 $[M+H]^{+}$

The following compounds were synthesized using a procedure similar to that of 2

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-4-(2-((2-nitrobenzyl)oxy)benzyl)piparazin-1-yl)-2-oxoethyl)acetamide (11a): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (d, 1H, *J*=7.8), 7.92 (d, 1H, *J*=7.8), 7.69 (t, 1H, *J*=7.8), 7.51 (t, 1H, *J*=7.2), 7.35 (d, 1H, J=7.2), 7.27 (t, 1H, *J*=7.8), 7.00 (t, 1H, *J*=7.8), 6.94 (d, 1H, *J*=8.4), 6.56 (br s, 1H), 5.50 (s, 2H), 4.05 (d, 2H, *J*=3.6), 3.68 (s, 2H), 3.65 (br s, 2H), 3.42 (app t, 2H, *J*=4.8), 2.53 (app t, 4H, *J*=4.8), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 561.1 $[M+H]^{+}$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-4-(2-((3-methylbenzyl)oxy)benzyl)piparazin-1-yl)-2-oxoethyl)acetamide (11b): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33-7.32 (t, 1H, *J*=7.8), 7.29 (d, 2H, *J*=7.8), 7.24 (t, 1H, *J*=7.8), 7.19 (d, 2H, *J*=7.8), 6.96-6.94 (m, 2H), 6.61 (br s, 1H), 5.03 (s, 2H), 4.03 (d, 2H, *J*=4.2), 3.67 (s, 2H), 3.64 (app t, 2H< *J*=4.8), 3.41 (app t, 2H, *J*=4.8), 2.52 (app t, 4H, *J*=4.8), 2.37 (s, 3H), 2.02 (s, 2H), 1.96 (s, 3H), 1.69-1.62 (m,12H) ; ESI-MS [M+H]=530.2

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-4-(2-((3-bromobenzyl)oxy)benzyl)piparazin-1-yl)-2-oxoethyl)acetamide (11c): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (s,1H), 7.34-7.28 (m, 4H), 7.25 (t, 1H, *J*=7.8), 6.97 (t, 1H, *J*=7.8), 6.90 (D, 1H, *J*=8.4), 6.91 (br s, 1H), 5.06 (s, 2H), 4.05 (d, 2H, *J*=3.6), 3.67 (s, 2H), 3.66 (br s, 2H), 3.43 (app t, 2H, *J*=4.8), 2.53 (app t, 4H, *J*=4.8), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 594 $[M+H]^{+}$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-4-(2-((3-methoxybenzyl)oxy)benzyl)piparazin-1-yl)-2-oxoethyl)acetamide (11d): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.2 (d, 1H, *J*=7.2), 7.30 (t, 1H, *J*=7.8), 7.27 (t, 1H, *J*=7.8), 7.00-6.93 (m, 4H), 6.86 (d, 1H, *J*=8.4), 6.57 (br s, 1H), 5.06 (s, 2H), 4.03 (d,

2H, *J*=3.6), 3.81 (s, 3H), 3.67 (s, 2H), 3.65 (app t, 2H, *J*=4.8), 3.41 (app t, 2H, *J*=4.8), 2.52 (app t, 4H, *J*=4.8), 2.01 (s, 2H), 1.96 (s, 3H), 1.69-1.62 (m, 12H) ; MS(ESI) m/z 346.2 $[M+H]^+$

methyl 3-((2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparzain-1-yl)methyl)phenoxy)methyl)benzoate (11e): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.00 (d, 1H, *J*=7.8), 7.62 (d, 1H, *J*=7.2), 7.47 (t, 1H, *J*=1H), 7.32 (d, 1H, *J*=1H), 7.26 (t, 1H, *J*=7.2), 6.97 (t, 1H, *J*=7.8), 6.93 (d, 1H, *J*=8.4), 6.62 (br s, 1H), 5.13 (s, 2H), 4.05 (d, 2H, *J*=3.6), 3.93 (s, 3H), 3.70 (s, 2H), 3.66 (app t, 2H, *J*=4.8), 3.44 (app t, 2H, *J*=4.8), 2.55 (br s, 4H), 2.01 (s, 2H), 1.96 (s, 3H), 1.69-1.61 (m, 12H) ; MS(ESI) m/z 574.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-oxo-(2-(4-(pyridine-3-ylmethoxy)benzyl)piparazin-1-yl)ethyl)acetamide (11f): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.60 (d, 1H, *J*=4.2), 7.79 (d, 1H, *J*=8.4), 7.36 (dd, 1H, *J*=5.4, 8.4), 7.34 (d, 1H, J=7.8), 7.27 (t, 1H, *J*=7.8), 7.01 (t, 1H, *J*=7.8), 6.95 (d, 1H, *J*=7.8), 6.56 (br s, 1H), 5.11 (s, 2H), 4.04 (d, 2H, *J*=4.2), 3.64 (br s, 2H), 3.63 (s, 2H), 3.41 (app t, 2H, *J*=5.4), 2.49 (app t, 2H, *J*=5.4), 2.02 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 517.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-4-(2-((4-nitrobenzyl)oxy)benzyl)piparazin-1-yl)-2-oxoethyl)acetamide (11g): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (d, 2H, *J*=8.4), 7.62 (d, 2H, *J*=8.4), 7.36 (d, 1H, *J*=7.8), 7.25 (t, 1H, *J*=7.8), 7.002 (t, 1H, *J*=7.8), 6.89 (d, 1H, *J*=8.4), 6.57 (br s, 1H), 5.20 (s, 2H), 4.05 (d, 2H, *J*=3.6), 3.68 (s, 2H), 3.66 (app t, 2H, *J*=4.8), 3.43 (app t, 2H, *J*=4.8), 2.53 (br s, 4H), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m, 12H) ; MS(ESI) m/z 561.2 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-4-(2-((4-cyanobenzyl)oxy)benzyl)piparazin-1-yl)-2-oxoethyl)acetamide (11h): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.67 (d, 1H, *J*=7.8), 7.62 (d, 1H, *J*=7.8), 7.51 (t, 1H, *J*=7.2), 7.35 (d, 1H, *J*=7.2), 7.26-7.24 (m, 1H), 6.99 (t, 1H, *J*=7.2), 6.89 (d, 1H, *J*=8.4), 6.47 (br s, 1H), 5.12 (s, 2H), 4.04 (d, 2H, *J*=3.6), 3.66 (app t, 2H, *J*=4.2), 3.63 (s, 2H), 3.41 (app t, 2H, *J*=4.2), 2.50 (app t, 4H, *J*=4.2), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.63 (m, 12H) ; MS(ESI) m/z 541.1 $[M+H]^+$

4-((2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparzain-1-yl)methyl)phenoxy)methyl)-*N*-methylbenzamide (11i): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, 2H, *J*=8.4), 7.44 (d, 2H, *J*=7.8), 7.40-7.38 (m, 2H), 7.04 (t, 1H, *J*=7.8), 7.02 (t, 1H, *J*=8.4), 6.73 (br s, 1H), 6,37 (br s, 1H), 5.14 (s, 2H), 4.12 (s, 2H), 4.07 (br s, 2H), 3.62 (s, 2H), 3.48 (s, 2H),

3.02 (d, 3H, *J*=4.8*),*2.88 (br s, 4H), 2.01 (s, 2H), 1.97 (s, 3H), 1.71-1.62 (m, 12H) ; MS(ESI) m/z 573.2 $[M+H]^+$

4-((2-((4-(2-(2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)acetamido)acetyl)piparzain-1-yl)methyl)phenoxy)methyl)-*N, N*-dimethylbenzamide (11j): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, 2H, *J*=7.8), 7.43-7.41 (m, 4H), 7.06 (t, 1H, *J*=7.8), 7.02 (d, 1H, *J*=8.4), 6.38 (br s, 1H), 5.12 (s, 2H), 4.29 (s, 2H), 4.01 (br s, 2H), 3.75 (s, 2H), 3.48 (s, 2H), 3.13 (s, 3H), 3.01 (s, 3H), 1.99 (s, 2H), 1.95 (s, 3H), 1.70-1.60 (m, 12H) ; MS(ESI) m/z 587.1 $[M+H]^+$

2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-oxo-(2-(4-(pyridine-4-ylmethoxy)benzyl)piparazin-1-yl)ethyl)acetamide (11k): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63 (d, 2H, *J*=6), 7.38 (d, 2H, *J*=6), 7.36 (d, 1H, *J*=7.8), 7.24 (t, 1H, *J*=7.8), 6.99 (t, 1H, *J*=7.8), 6.86(d, 1H, *J*=7.8), 6.55 (br s, 1H), 5.11 (s, 2H), 4.05 (d, 2H, *J*=3.6), 3.68 (s, 2H), 3.66 (app t, 2H, *J*=4.8), 3.42 (app t, 2H, *J*=4.8), 2.53 (app t, 4H, *J*=4.8), 2.01 (s, 2H), 1.96 (s, 3H), 1.70-1.62 (m,12H) ; MS(ESI) m/z 517.2 $[M+H]^+$

4. Synthesis of 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(5-azido-2-((4-ethynylbenzyl)oxy)benzyl)piperazin-1-yl)-2-oxoethyl)acetamide (3)

A solution of triphenylphosphine (3.9 g, 15 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a cold mixture of 4-ethynylbenzylalcohol (1.0 g, 7.6 mmol), tetrabromomethane (4.5 g, 14 mmol), and 2,6-lutidine (4.4 mL, 38 mmol) in CH_2Cl_2 (20 mL) at 0 ^{o} C and the mixture was stirred for 16h at room temperature. After concentration in vacuo, the residue was treated with ether (40 mL) and the resulting solid was removed by filtration. The filtrate was washed with 1% HCl solution and then water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography to give 4-ethynylbenzyl bromide (1.4 g, 95%) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (d, 2H, *J*=7.8), 7.34 (d, 2H, *J*=7.8), 4.47 (s, 2H), 3.10 (s, 1H).

A mixture of (4-ethynylphenyl)methanol (1.4 g, 7.2 mmol), 2-hydroxy-5-nitrobenzaldehyde (1.0 g, 6.6 mmol), and potassium carbonate (2.7 g, 20 mmol) in DMF (12 mL) was stirred overnight at room temperature. Then water (50 mL) was added and the mixture was extracted with EtOAc. The combined extracts were washed with saturated brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography to give 2-((4-ethynylbenzyl)oxy)-5-nitrobenzaldehyde (1.1 g, 60%) as a yellow oil. ^{1}H NMR (600 MHz,

$CDCl_3$) δ 10.5 (s, 1H), 8.73 (s, 1H), 8.41 (d, 1H, *J*=9.6), 7.56 (d, 2H, *J*=8.4), 7.40 (d, 2H, *J*=8.4), 7.14 (d, 1H, *J*=9), 5.34 (s, 2H), 3.11 (s, 1H).

To a mixture of 2-((4-ethynylbenzyl)oxy)-5-nitrobenzaldehyde (222 mg, 0.79 mmol) and 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-oxo-2-(piperazin-1-yl)ethyl)acetamide (210 mg, 0.66 mmol) in CH_2Cl_2 (12 mL) was added sodium triacetoxyborohydride (210 mg, 0.99 mmol) at 0 ^{o} C. After stirring for 4 h at room temperature, the mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography to give 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-((4-ethynylbenzyl)oxy)-5-nitrobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (178 mg, 46%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.15 (d, 1H, *J*=8.4), 7.53 (d, 2H, *J*=8.4), 7.37 (d, 2H, *J*=9), 6.96 (d, 1H, *J*=9), 6.47 (br s, 1H), 5.13 (s, 2H), 4.06 (s, 2H), 3.68 (m, 2H), 3.63 (s, 2H), 3.44 (m, 2H), 3.08 (s, 1H), 2.50 (m, 4H), 2.01(s, 2H), 1.98 (br s, 3H), 1.71-1.60 (m, 12H).

A mixture of 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(2-((4-ethynylbenzyl)oxy)-5-nitrobenzyl)piperazin-1-yl)-2-oxoethyl)acetamide (178 mg, 0.30 mmol) and tinchloride dehydrate (343 mg, 1.5 mmol) in methanol (12 mL) was heated at 90 ^{o} C for 6 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then treated with saturated $NaHCO_3$. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated to give the corresponding amine (46 mg; MS(ESI) m/z 555.0 $[M+H]^+$). A solution of sodium nitrite (17 mg, 0.25 mmol) in water (1 mL) was added to a solution of the crude amine above (46 mg, 0.083 mmol) in 6N-HCl (1 mL) at 0 ^{o}C under N_2 atmosphere. After stirring for 15 min, the mixture was then added dropwise to a stirred mixture of sodium azide (16 mg, 0.25 mmol) and sodium acetate (102 mg, 1.2 mmol) in water (5 mL) at 0 ^{o}C. After the addition was completed, the reaction mixture was warmed to room temperature, stirred for 4 h, and then extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by prep LCMS, using the predicted molecular weight to trigger fraction collection, to give 2-((3*r*, 5*r*, 7*r*)-adamantan-1-yl)-*N*-(2-(4-(5-azido-2-((4-ethynylbenzyl)oxy)benzyl)piperazin-1-yl)-2-oxoethyl)acetamide (5 mg). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, 1H, *J*=7.8), 7.51 (d, 1H, *J*=7.8), 7.38 (t, 1H, *J*=7.8), 7.30 (t, 1H, *J*=7.2), 7.07 (s, 1H), 6.88-6.94 (m, 2H), 6.47 (br s, 1H),

5.23 (s, 2H), 4.04 (d, 2H, *J*=3.6), 3.65 (br s, 2H), 3.62 (s, 2H), 3.40 (br s, 2H), 3.33 (s, 1H), 2.49 (br s, 4H), 2.01 (s, 2H), 1.97 (br s, 3H), 1.70-1.62 (m, 12H); MS(ESI) m/z 581.0 $[M+H]^+$

III Cell culture, virus production and measurement of infection

Vero and 293T cells were cultured in DMEM (Invitrogen) supplemented with penicillin/streptomycin (1000U/ml), L-glutamine (2mM, Invitrogen), FBS (5%) and FetalPlex (5%, Gemini). CHO_{null} and CHO_{NPC1} cells were cultured in equal mixture of F12 and DMEM media (Invitrogen) with penicillin/streptomycin, L-glutamine (Invitrogen) and FBS. Ectodomain of EboV GP was produced in 293T cells Vesicular stomatitis virus (VSV) particles encoding luciferase and pseudotyped with EboV GP, Lassa fever virus GP or VSV GP were produced in 293T cells as described previously[8]. Virus infection assays were performed in 384-well plate format using luciferase reporter.

IV Membrane binding assay

The membrane binding assay was performed as previously described.[8] Truncated EboV GP containing histidine affinity tag was expressed in 293T cells and isolated from supernatant using Ni-NTA beads (Qiagen). Purified EboV GP was cleaved with thermolysin protease (0.2mg/ml 37°C, 1 hour) to expose the NPC1 binding site. After protease inactivation (500 uM phosphoramidon, 1 mM PMSF, 5 mM EDTA, 1X Roche EDTA-free Complete Protease Inhibitor Cocktail), cleaved EboV GP was used as a ligand in a binding assay using LE/LY membranes expressing NPC1. CHO_{null} or CHO_{NPC1} cells were homogenized in sucrose (0.25M), EDTA (1mM) and HEPES (10mM, pH7.0) using a Dounce homogenizer. Nuclei were removed by centrifugation at 1,000 X g and LE/LY membranes were pelleted by centrifugation at 15,000 x g for 30 min, 4°C. LE/LY membranes were resuspended, further purified on a Percoll gradient and osmotically lysed by incubation in methyl-methionine ester (20 mM, Sigma) for 1 hour at room temperature. ELISA plates (Corning) were coated overnight with disrupted LE/LY membranes at 4°C. Unattached LE/LY membranes were removed and wells blocked with 5% FBS in PBS for 1 hour at room temperature. Small molecule inhibitors were incubated with adherent LE/LY membranes at the indicated concentration for 30 min prior to addition of

cleaved EboV GP in the continued presence of inhibitor. After one hour, wells were washed three times with PBS and bound EboV GP was recovered by dissolving LE/LY membranes in SDS (2%), glycerol (10%), Tris (62.5mM, pH 6.8), β-mercaptoethanol (1%). Lysates were boiled and analyzed by immunoblot probed with NPC1 and EboV GP1 antibodies.

V Photo-activation and click chemistry

CHO_{null} and CHO_{NPC1} cells were homogenized in HM buffer as described above. Membranes in the 15,000 x g pellet were used without further purification and protein concentration was determined using the BCA protein quantification assay (Pierce). Membranes (250μg of protein content) were incubated in **3** (25μM) for 10min at room temperature, exposed to UV light (305 nM) for 1 min on ice and centrifuged at 15,000 x g for 30 min at 4°C. Pelleted membranes were dissolved in Triton X-100 (1%), NP-40 (0.1%), NaCl (150mM) EDTA (0.4mM),Tris (50mM pH8.0) and protease inhibitors (Roche). Insoluble material was removed by centrifugation at 10,000 x g for 10 min at 4°C and supernatant was used in the click chemistry reaction.

For analysis of cross linking, lysate containing 50 μg of protein was incubated with Alexa Fluor 488 azide (20μM and covalently linked using the Click-iT Protein Buffer Reaction Kit (Invitrogen). Proteins were precipitated in methanol/chloroform/water (60:15:40), washed in methanol, dried and resuspended in sample buffer. Following resuspension, samples were analyzed by SDS/PAGE and immunoblot probed with Alexa Fluor 488 and NPC1 antibodies.

For direct analysis of NPC1 for cross-linking to **3**, lysate containing 200 ug of protein was incubated with Alexa Fluor 488 azide (80μM), ascorbic acid (7.5μM), copper sulfate (1.5μM) for 30 min, room temperature. NPC1 was purified by immunoprecipitation using NPC1 antibody (Abcam) and protein A-agarose beads (Sigma). The immunoprecipitate was eluted from the beads using glycine (0.1M, pH 3.5). The pH of eluted proteins was neutralized and NPC1 was analyzed by immunoblot probed with Alexa Fluor 488 (Invitrogen) and NPC1 antibodies.