Supporting Information

Discovery of INCB9471, a High Affinity, Selective and Orally Bioavailable CCR5 Antagonist with Potent Anti-HIV-1 Activity

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Contents:

*1. Experimental Procedures for Compounds **6a-b**, **13a**, and **20a-22a***

5-Bromo-1-indanol (2). To a solution of 5-bromo-1-indanone (2.0 g, 9.5 mmol) in THF (20 mL) was added $NaBH_4$ (0.5 g, 12.8 mmol). After being stirred at room temperature overnight, the solution was quenched by addition of water. The resulting solution was extracted with EtOAc twice. The combined EtOAc layers were dried over Na_2SO_4 and concentrated under vacuum to give 2.0 g of the title compound as a solid. MS calculated for C_9H_9BrO: $(M+H)^+$ 212.9; found 194.9 $(M+H-H_2O)^+$, 197.0 $(M+H-H_2O)^+$.

***tert*-Butyl (3*S*)-4-(5-Bromo-2,3-dihydro-1H-inden-1-yl)-3-methylpiperazine-1-carboxylate (3a-b)**. 5-Bromo-1-indanol (1.0 g, 4.7 mmol) was dissolved in thionyl chloride (10 mL). After being stirred at room temperature for 2 hrs, the solution was concentrated under vacuum. The residue was taken up in DMF (10 mL). To it were added *tert*-butyl (3*S*)-3-methylpiperazine-1-carboxylate (0.94 g, 4.7 mmol), NaI (2 g, 13 mmol) and triethylamine (1.5 mL, 10 mmol). The resulting solution was stirred at 70 ºC overnight. After cooling to room temperature, water was added. The solution was extracted with EtOAc twice. The combined EtOAc layers were washed with brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica (50% EtOAc in hexanes) provided two isomers. Isomer 1 (fast moving isomer, **3a**): 0.36 g; ^{1}H NMR (500 MHz, $CDCl_3$): 7.32 (s, 1 H), 7.30 (d, J = 8.0 Hz, 1 H), 7.16 (d, J = 7.5 Hz, 1 H), 4.69 (dd, J^1 = 7.5 Hz, J^2 = 7.5 Hz, 1 H), 3.82 (m, 2 H), 2.89-2.70 (m, 3 H), 2.58 (m, 1 H), 2.54-2.46 (m, 1 H), 2.30 (d, J = 11.0 Hz, 1 H), 2.12-1.97 (m, 2 H), 1.92-1.84 (m, 1 H), 1.43 (s, 9 H), 1.16 (d, J = 6.0 Hz, 3 H). MS $(M+H)^+$: 395.1, 397.0. HRMS $(M+H)^+$: calcd 395.1300; found 395.1346. Anal. ($C_{19}H_{27}BrN_2O_2$): calcd C 57.72, H 6.88, Br 20.21, N 7.09; found C 57.49, H 7.01, Br 20.02, N 6.95. Isomer 2 (slow moving isomer, **3b**): 0.33 g; ^{1}H NMR (500 MHz, $CDCl_3$): 7.40 (s, 1 H), 7.32 (d, J = 8.0 Hz, 1 H), 7.19 (d, J = 7.5 Hz, 1 H), 4.60 (sb, 1 H), 3.40-3.75 (m, 2 H), 3.20 (m, 1 H), 2.98 (m, 3 H), 2.80 (m, 1 H), 2.60 (m, 1 H), 2.32 (m, 1 H), 2.00 (m, 2 H), 1.42 (s, 9 H), 1.20 (m, 3 H). MS $(M+H)^+$: 395.1, 397.0.

***tert*-Butyl 4-[(3*S*)-4-(5-Bromo-2,3-dihydro-1H-inden-1-yl)-3-methylpiperazin-1-yl]-4-cyanopiperidine-1-carboxylate (4a)**. Intermediate **3a** (0.33 g, 0.83 mmol) was

dissolved in 4 N HCl in dioxane (4 mL). After being stirred at room temperature for 2 h, the solution was concentrated. The residue was taken up in CH_2Cl_2 (5 mL). To it were added *tert*-butyl 4-oxo-1-piperidinecarboxylate (0.17 g, 0.85 mmol), $Ti(Oi\text{-}Pr)_4$ (0.87 mL) and triethylamine (0.6 mL). The mixture was stirred at room temperature overnight and the volatiles were removed under vacuum. The residue was dissolved in THF (5 mL). To it was added a 1.0 M solution of diethylaluminum cyanide (1 mL). The resulting solution was stirred at 30 ºC for 5 h and concentrated to provide the crude title compound (0.32 g) that was used for the next reaction without purification. MS $(M+H)^+$: 503.1, 505.1.

***tert*-Butyl 4-[(3*S*)-4-(5-Bromo-2,3-dihydro-1H-inden-1-yl)-3-methylpiperazin-1-yl]-4-methylpiperidine-1-carboxylate (5a)**. To a solution of *tert*-butyl 4-[(3*S*)-4-(5-bromo-2,3-dihydro-1H-inden-1-yl)-3-methylpiperazin-1-yl]-4-cyanopiperidine-1-carboxylate (0.32 g, 0.64 mmol) in THF (2 mL) was added a 3 M solution of methylmagnesium bromide (1.1 mL, 3.3 mmol). After being stirred at room temperature overnight, the solution was concentrated. Purification on silica (2:1 hexane/EtOAc) afforded the title compound (0.25 g). 1H NMR (500 MHz, CDCl3): d 7.34 (s, 1H), 7.32 (d, J=8.2 Hz, 1H), 7.20 (d, J=8.2 Hz, 1H), 4.71 (dd, J=7.6 Hz, 7.4 Hz, 1H), 3.37 (m, 4H), 2.81 (m, 2H), 2.68 (m, 1H), 2.61 (m, 1H), 2.56 (m, 1H), 2.31 (m, 1H), 2.16 (m, 2H), 2.10 (m, 1H), 2.03 (m, 1H), 1.86 (m, 1H), 1.72 (m, 2H), 1.42 (s, 9H), 1.31 (m, 2H), 1.13 (d, J=6.1 Hz, 3H), 0.93 (s, 3H). MS $(M+H)^+$: 491.2, 494.2. HRMS $(M+H)^+$: calcd 492.2147; found 492.2247. Anal. ($C_{25}H_{37}BrN_3O_2$): calcd C 60.97, H 7.78, Br 16.22, N 8.53; found C 60.87, H 7.98, Br 16.17, N 8.48.

5-({4-[(3*S*)-4-(5-Bromo-2,3-dihydro-1H-inden-1-yl)-3-methylpiperazin-1-yl]-4-methylpiperidin-1-yl}carbonyl)-4,6-dimethylpyrimidine (6a). Intermediate **5a** (0.23 g) was dissolved in a solution of 4 N HCl in dioxane (3 mL). After being stirred at room temperature for 2 h, the solution was concentrated to provide (2*S*)-1-(5-bromo-2,3-dihydro-1H-inden-1-yl)-2-methyl-4-(4-methylpiperidin-4-yl)piperazine as a trihydrochloride salt (0.23 g). MS calculated for $C_{20}H_{30}BrN_3$: $(M+H)^+$ 392; found 392.2, 394.2.

To a solution of (2*S*)-1-(5-bromo-2,3-dihydro-1H-inden-1-yl)-2-methyl-4-(4-methylpiperidin-4-yl)piperazine trihydrochloride (30 mg, 0.06 mmol) and 4,6-dimethyl-

pyrimidine-5-carboxylic acid (9 mg, 0.06 mmol) in DMF (2 mL) was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (30 mg, 0.06 mmol) followed by triethylamine (30 mg, 0.3 mmol). After being stirred at room temperature for 5 h, the mixture was diluted with EtOAc and a solution of Na_2CO_3 in water. The organic layer was separated, washed with water several times, dried over Na_2SO_4 and concentrated. Purification on reverse phase HPLC and lyophilization gave the title compound (20 mg) with 99.2% purity. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (s, 0.5 H), 8.90 (s, 0.5 H), 7.30 (m, 2 H), 7.15 (m, 1 H), 4.67 (m, 1 H), 4.13 (m, 1 H), 3.46 (m, 1 H), 3.33 (m, 1 H), 2.94 (m, 1 H), 2.82 (m, 2 H), 2.69 (m, 0.5 H), 2.63 (m, 1 H), 2.55 (m, 1 H), 2.54 (m, 0.5 H), 2.48 (m, 1 H), 2.45 (s, 1.5 H), 2.44 (s, 1.5 H), 2.43 (s, 1.5 H), 2.41 (s, 1.5 H), 2.33 (m, 1 H), 2.18 (m, 1 H), 2.09 (m, 1 H), 2.06 (m, 1 H), 1.94 (m, 1 H), 1.87 (m, 1 H), 1.76 (m, 1 H), 1.41 (m, 1 H), 1.24 (m, 1 H), 1.17 (d, *J*=6.2 Hz, 1.5 H), 1.12 (d, *J*=6.2 Hz, 1.5 H), 0.92 (s, 3H). MS $(M+H)^+$: 526.1, 528.1. Anal. ($C_{27}H_{36}BrN_5O\cdot 1/2H_2O$): calcd C 60.56, H 6.96, N 13.08; found C 60.19, H 6.96, N 12.78.

5-({4-[(3*S*)-4-(5-Bromo-2,3-dihydro-1H-inden-1-yl)-3-methylpiperazin-1-yl]-4-methylpiperidin-1-yl}carbonyl)-4,6-dimethylpyrimidine (6b). Starting from **3b**, the title compound was prepared following the procedures for **6a**. Purification by reverse phase HPLC provided the title compound with 99.4% purity. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.90 (s, 0.5 H), 8.89 (s, 0.5 H), 7.32 (m, 1 H), 7.27 (dd, *J*=5.7 Hz, 1.4 Hz, 0.5 H), 7.26 (dd, *J*=5.7 Hz, 1.4 Hz, 0.5 H), 7.14 (d, *J*=7.9 Hz, 0.5 H), 7.12 (d, *J*=7.9 Hz, 0.5 H), 4.56 (m, 1 H), 4.13 (m, 1 H), 3.39 (m, 1 H), 3.29 (m, 1 H), 2.93 (m, 1 H), 2.84 (m, 1 H), 2.77 (m, 1 H), 2.64 (m, 1.5 H), 2.63 (m, 1 H), 2.54 (m, 1.5 H), 2.44 (s, 1.5 H), 2.43 (s, 1.5 H), 2.41 (s, 1.5 H), 2.40 (s, 1.5 H), 2.39 (m, 1 H), 2.30 (m, 1 H), 2.29 (m, 1 H), 2.16 (m, 1 H), 2.01 (m, 2 H), 1.93 (m, 1 H), 1.74 (m, 1 H), 1.39 (m, 1 H), 1.19 (d, *J*=6.4 Hz, 1.5 H), 1.17 (d, *J*=6.4 Hz, 1.5 H), 0.92 (s, 3H). HRMS (M+H)+: calcd 526.2103; found 526.2186.

[2-Bromo-5-(trifluoromethyl)phenyl]methanol (8). To a solution of 2-bromo-5-(trifluoromethyl)benzonitrile (10.0 g, 40 mmol) in dichloromethane (100 mL) was dropwise added a 1.0 M solution of diisobutylaluminum hydride in hexane (48 mL). The resulting solution was stirred under nitrogen at ambient temperature for 1 h and was then diluted by addition of ether (100 mL). After cooling in an ice bath, a 3 N solution of HCl

was carefully added, and the mixture was vigorously stirred at ambient temperature for 15 min. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The resulting oil was purified by flash chromatography (5% EtOAc/hexane) affording 5 g of 2-bromo-5-trifluoromethylbenzaldehyde. ^{1}H NMR ($CDCl_3$) δ 10.39 (s, 1H), 8.18 (d, *J*=2 Hz, 1H), 7.82 (d, *J*=8.8 Hz, 1H), 7.70 (dd, *J*=8.5 Hz, 2 Hz, 1H).

To a mixture of 2-bromo-5-(trifluoromethyl)benzaldehyde (5 g, 20 mmol) in THF (20 mL) at 0 ºC was added sodium borohydride (0.8 g, 20 mmol). The resulting mixture was stirred at 0 ºC to ambient temperature for 1 h. The reaction was quenched by addition of an aqueous solution of $NaHCO_3$. The resulting solution was extracted with EtOAc twice. The combined extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated to give the desired alcohol as a white solid (4.4 g). ^{1}H NMR ($CDCl_3$) δ (ppm) 7.81 (s, 1H), 7.66 (d, *J*=8.3 Hz, 1H), 7.42 (dd, *J*=8.3 Hz, 2.0 Hz, 1H), 4.81 (d, *J*=6.3 Hz, 2H), 2.03 (m, 1H).

Diethyl [2-Bromo-5-(trifluoromethyl)benzyl]malonate (9). To [2-bromo-5-(trifluoromethyl)phenyl]methanol (4.4 g, 17 mmol) was added thionyl chloride (5 mL) and the resulting mixture was stirred at room temperature for 1 h. Evaporation *in vacuo* gave the crude product as an oil. ^{1}H NMR ($CDCl_3$) δ (ppm) 7.77 (d, *J*=8.3 Hz, 1 H), 7.73 (d, *J*=2.0 Hz, 1 H), 7.53 (dd, *J*=8.5, 2.2 Hz, 1 H), 5.66 (d, *J*=12.7 Hz, 1 H), 5.46 (d, *J*=12.2 Hz, 1 H).

To a solution of ethyl malonate (23 g, 140 mmol) in DMF (70 mL) at 0 ºC was added sodium hydride (3.9 g, 60% in mineral oil, 97 mmol), and the resulting mixture was stirred at ambient temperature for 30 min. To the mixture was added a solution of 1-bromo-2-(chloromethyl)-4-(trifluoromethyl)benzene (16 g, 60 mmol) in DMF (20 mL). The reaction mixture was stirred at room temperature for 3 h and quenched with ice water. The resulting solution was extracted with EtOAc twice. The extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. The crude material was purified by flash chromatography on silica eluting with 3% then 5% EtOAc/hexane to afford the desired product (15.2 g, 64%) as an oil. LC/MS calculated for $C_{15}H_{16}BrF_3O_4$: $(M+H)^+$ 397; found 397.1/399.1.

3-[2-Bromo-5-(trifluoromethyl)phenyl]propanoic Acid (10). To a solution of diethyl [2-bromo-5-(trifluoromethyl)benzyl]malonate (22.9 g, 57.6 mmol) in ethanol (100

mL) and water (50 mL) was added a 5 M solution of sodium hydroxide in water (30 mL). The mixture was heated to reflux for 2 h. Ethanol was removed by evaporation. The aqueous layer was extracted with ether and then acidified with concentrated HCl to pH 5 at which time a lot of white solid precipitated out. The solid was collected by filtration. The filtrate was extracted with ethyl acetate twice, and the extracts were washed with brine, dried ($MgSO_4$) and concentrated to give a white solid.

The combined solid was decarboxylated by heating in an oil bath to 180 ºC for about 1 h. The resulting yellow oil was cooled and pumped *in vacuo* to afford the desired mono-acid (11.5 g, 67%). LC/MS calculated for $C_{10}H_8BrF_3O_2$: $(M+H)^+$ 297; found 297.1/299.1.

5-(Trifluoromethyl)indan-1-one (11). To a solution of 3-[2-bromo-5-(trifluoromethyl)phenyl]propanoic acid (2.8 g, 9.4 mmol) in THF (100 mL) and hexane (20 mL) at –78 ºC was dropwise added a 2.5 M solution of *n*-butyllithium in hexane (8.3 mL). After the addition had been completed, the reaction was quenched with saturated NH_4Cl. The resulting solution was extracted with ethyl acetate twice. The extracts were washed with saturated $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated. The crude material was purified by flash chromatography on silica eluting with 10- 20% EtOAc/hexane to afford the desired product as a white solid (0.7 g, 37%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.82 (d, *J*=8.1 Hz, 1H), 7.74 (s, 1H), 7.60 (d, *J*=8.1 Hz, 1H), 3.20 (m, 2H), 2.75 (m, 2H). MS $(M+H)^+$: 201.1. HRMS: calcd 200.0400; found 223.0356 $(M+Na)^+$.

5-(Trifluoromethyl)indan-1-ol (12). To a solution of 5-(trifluoromethyl)indan-1-one (0.7 g, 3 mmol) in THF (5 mL) cooled in an ice bath was added sodium borohydride (0.1 g, 3 mmol) followed by MeOH (1 mL). After being stirred for 30 min, the reaction was quenched with aqueous $NaHCO_3$. The resulting solution was extracted with EtOAc twice. The extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. The crude material was purified by flash chromatography on silica eluting with 20% EtOAc/hexane to afford the desired product (0.65 g, 92%) as an oil. LC/MS calculated for $C_{10}H_9F_3O$: $(M+H)^+$ 203; found 185.1 $(M+H-H_2O)^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.43 (m, 3H), 5.13 (m, 1H), 3.60 (s, 1H), 2.98 (m, 1H), 2.80 (m, 1H), 2.40 (m, 1H), 1.85 (m, 1H).

4,6-Dimethyl-5-[(4-methyl-4-{(3*S*)-3-methyl-4-[5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]piperazin-1-yl}piperidin-1-yl)carbonyl]pyrimidine (13a). Starting from 5-(trifluoromethyl)indan-1-ol, the title compound was prepared following the procedures described for **6a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.94 (d, *J*=7.1 Hz, 1 H), 7.43 (m, 3 H), 4.79 (m, 1 H), 4.16 (m, 1 H), 3.41 (m, 2 H), 2.93 (m, 3 H), 2.65 (m, 2 H), 2.49 (s, 1.5 H), 2.47 (s, 1.5 H), 2.45 (s, 1.5 H), 2.44 (s, 1.5 H), 2.35 (m, 1 H), 2.18 (m, 4 H), 1.98 (m, 2 H), 1.80 (m, 2 H), 1.44 (m, 1 H), 1.28 (m, 1 H), 1.19 (d, *J*=6.1 Hz, 1.5 H), 1.17 (d, *J*=6.1 Hz, 1.5 H), 0.97 (s, 3 H). MS $(M+H)^+$ 516.2. HRMS $(M+H)^+$: calcd 516.2872; found 516.2957. Anal. ($C_{28}H_{36}F_3N_5O\cdot 1/2H_2O$): calcd C 64.10, H 7.11, F 10.86, N 13.35; found C 64.41, H 7.04, F 10.78, N 13.39.

(2*S*)-1-Benzyl-2-methylpiperazine (14). *tert*-Butyl (3S)-3-methylpiperazine-1-carboxylate (380.0 g, 1.897 mol) and benzyl bromide (248 mL, 2.09 mol) were mixed in acetonitrile (440 mL). Triethylamine (300.0 mL, 2.152 mol) was carefully added and the mixture was refluxed overnight. After the mixture was cooled down to room temperature, the solid was filtered out. The filtrate was concentrated. The residue was combined with the solid and dissolved in methylene chloride. The methylene chloride solution was washed with 1N NaOH and dried over magnesium sulfate. After the solvent was removed, the residue was directly treated with 6 N HCl at 0 °C, and 3 hours later, the solution was basified by slowly adding solid sodium hydroxide. The resulting mixture was extracted with methylene chloride and the extracts were dried over magnesium sulfate. After removal of the solvent, 330 g (91.4%) of product was obtained. The product was used directly for next step.^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.30 (m, 5 H), 4.05 (d, 1 H), 3.15 (d, 1 H), 2.92 (m, 1 H), 2.83 (m, 2 H), 2.67 (m, 1 H), 2.60 (m, 1 H), 2.38 (m, 1 H), 2.36 (bs, 2 H), 2.06 (m, 1 H), 1.14 (d, 3 H). MS (EI) 191.1 (M+1).

t-Butyl 4-[(3S)-4-Benzyl-3-methylpiperazin-1-yl]-4-methylpiperidine-1-carboxylate (15). In a 5 L flask, (2S)-1-benzyl-2-methylpiperazine (260.0 g, 1.366 mol), dichloromethane (1000 mL) , *t*-butyl 4-oxo-1-piperidinecarboxylate (272 g, 1.37 mol) and titanium tetraisopropoxide (480.0 mL, 1.626 mol) were mixed and the mixture was stirred at room temperature for 20 h. The mixture was cooled down to 0 °C and diethylaluminum cyanide in toluene (1.0 M, 1600 mL) was added dropwise. The resulting mixture was stirred at room temperature for 20 h. The reaction content was then

split into two 5 L flasks, To each flask, 1 L of ethyl acetate, 500 g of sodium bicarbonate, 150 g of celite were added before they were cooled down to -40 °C using dry ice/acetonitrile. To each falsk, 200 mL of saturated aqueous sodium sulfate was then slowly added with vigorous stirring. After the reaction mixture was slowly warmed up to room temperature and stirred for 4 hours, 1 L of methanol was added to each flask. After being stirred overnight, the reaction mixture was filtered through a thin layer of sand. The cake was taken back into a 5 L flask and stirred with 3 L of methanol for 5 hours, and insoluble solid was filtered off. The combined filtrates were concentrated to dryness, and 3 L of methylene chloride was added. Insoluble solid was filtered off. The filtrate was dried with magnesium sulfate, the solvent was removed to give 484 g (88.9%) of product as a slight yellow solid. The crude product was essentially pure and used directly for next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.32 (m, 5 H), 4.04 (d, 1 H), 3.95 (brs, 2 H), 3.15 (d, 1 H), 3.15 (brs, 2 H), 2.82 (m, 1 H), 2.73 (m, 2 H), 2.44 (m, 2 H), 2.25 (t, 1 H), 2.15 (m, 1 H), 2.05 (m, 1 H), 1.66 (m, 1 H), 1.46 (s, 9 H), 1.17 (d, 3 H); MS (EI) 399.2 (M+1).

A solution of *tert*-butyl 4-[(3*S*)-4-benzyl-3-methylpiperazin-1-yl]-4-cyanopiperidine-1-carboxylate (242 g, 0.605 mol) in tetrahydrofuran (1.5 L) in a 5 L flask was cooled down to -40 °C using dry ice/acetonitrile. Methylmagnesium bromide (3.0 M in tetrahydrofuran, 800 mL) was slowly added. After the addition, the reaction mixture was slowly warmed up to room temperature and stirred overnight. After cooling down to -40 °C using dry ice/acetonitrile, celite (200g), and then ethyl acetate (500 mL) were carefully added. After the addition, the mixture was stirred for 4 hours while the temperature slowly rose to room temperature. The reaction mixture was cooled back down to -40 °C again, and water (200 mL), and then methanol (1.5 L) were added. After being stirred at room temperature overnight, the mixture was filtered through a thin layer of sand. The cake was taken back into a 5 L flask and stirred with methanol (2 L) for 5 hours. Insoluble solid was filtered off. The combined filtrates were concentrated to dryness. Methylene chloride (3.5 L) was added. Insoluble solid was filtered off. The filtrate was dried with magnesium sulfate. After the solvent was removed, 436 g (92.6%) of product was obtained as a white sticky solid. The crude product was essentially pure and used directly for the next step. MS (EI) 388.3 (M+1).

t-Butyl 4-Methyl-4-[(3*S*)-3-methylpiperazin-1-yl]piperidine-1-carboxylate (16). A solution of *t*-butyl 4-[(3*S*)-4-benzyl-3-methylpiperazin-1-yl]-4-methylpiperidine-1-carboxylate (45.5 g, 0.118 mol) in methanol (320 mL) and acetic acid (35 mL, ~5 equiv) in a 2.25 L Parr bottle was charged with H_2 to 60 psi and the mixture was shaken for 18 hr. The reaction mixture was filtered through a pad of Celite and the pad was washed with methanol. The filtrate was concentrated under vacuum. The residual oil was dissolved in DCM (500 mL) and washed with aqueous sodium hydroxide (300 mL). The aqueous phase was back-extracted with methylene chloride (200 mL). The combined organic solution was washed with brine (500 mL), dried with sodium sulfate and the solvent was removed under vacuum to give 35 g (100%) of product as a pale yellow viscous oil that slowly crystallized. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.44 (m, 2 H), 3.36 (m, 2 H), 2.97 (dt, 1 H), 2.84 (dd, 1 H), 2.78 (m, 1 H), 2.71 (brd, 2 H), 2.16 (dt, 1 H), 1.81 (t, 2 H), 1.76 (m, 1 H), 1.45 (s, 9 H), 1.34 (m, 3 H), 1.03 (d, 3 H), 0.90 (s, 3 H); MS (EI) 298.2 (M+1). HRMS $(M+H)^+$: 298.2416; found 298.2492.

6-(Trifluoromethyl)-1H-indene (17). A mixture of 5-(trifluoromethyl)indan-1-ol (1.6 g, 7.9 mmol) and *p*-toluenesulfonic acid (0.02 g, 0.1 mmol) in toluene (20 mL) was refluxed through a Dean-Stark trap for about 3 h. The solution was concentrated *in vacuo* and the residue was purified by flash chromatography on silica eluting with 5% EtOAc/hexanes to afford the desired product as an oil (1.4 g, 96%).

4-(Trifluoromethyl)-6,6a-dihydro-1aH-indeno[1,2-b]oxirene (18). To a solution of 2 M sodium hypochlorite in water (200 mL) at 0 °C was added aqueous sodium hydroxide (1 M, 40 mL), 4-(3-phenylpropyl)pyridine N-oxide (6.0g, 0.03 mol) and a solution of (*S*,*S*)-(+)-N,N'-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediamino-manganese(III) chloride (4.13 g, 0.00651 mol) in dichloromethane (700 mL). The resulting brown solution was allowed to be stirred for 15 min at 0 °C. To the cold solution, a solution of 6-(trifluoromethyl)-1H-indene (51 g, 0.24 mol) in dichloromethane (700 mL) was added with simultaneous addition of aqueous sodium hypochlorite (2 M, 200 mL). The reaction was kept at 0 °C and the brown solution remained the same color upon addition of the indene. After 4 h, the organic phase was collected and dried over sodium sulfate. The mixture was plugged through silica gel using pentane. After removal of the solvent, 42 g (88%, 84% ee) of epoxide was

obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.72 (s, 1H), 7.55 (d, 1H), 7.48 (d, 1H), 6.93 (m, 1H), 6.74 (m, 1H), 3.46 (brs, 1H).

***t*-Butyl 4-(3*S*)-4-[(1*R*,2*R*)-2-hydroxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidine-1-carboxylate (19a).** A mixture of (1aS,6aR)-4-(trifluoromethyl)-6,6a-dihydro-1aH-indeno[1,2-b]oxirene (7.30 g, 36.5 mmol) and *t*-butyl 4-methyl-4-[(3S)-3-methylpiperazin-1-yl]piperidine-1-carboxylate (11.9 g, 40.1 mol) in ethanol (50 mL) was heated at 75 °C (oil bath temperature) over three days. The mixture was then concentrated, and the residue was chromatographed (10-70% EtOAc/hexanes) to give 9.3 g foamy solid (yield 51.2%). MS (EI) 498.2 (M+1).

5-[(4-(3S)-4-[(1*R*,2*R*)-2-hydroxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidin-1-yl)carbonyl]-4,6-dimethylpyrimidine (20a). *t*-Butyl 4-(3*S*)-4-[(1*R*,2*R*)-2-hydroxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidine-1-carboxylate (2.0 g, 4.0 mol) was dissolved in 20 mL of 4 N HCl in dioxane. After being stirred at room temperature for 2 hours, the solution was concentrated to give a residue. The residue was dissolved in DMF (10 mL). To it were added 4,6-dimethyl-pyrimidine-5-carboxylic acid (0.66 g, 4.4 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (1.94 g, 4.4 mmol) and triethylamine (2.54 mL, 18.3 mol). The resulted reaction mixture was stirred at room temperature overnight. The reaction was quenched with aq. $NaHCO_3$ and extracted with EtOAc three times. The combined extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. The crude material was purified by Combi-Flash, eluting with 60% EtOAc/hexanes to 100% EtOAc to afford the desired product. Further purification was conduct on reverse phase HPLC under basic condition to afford the product with > 99.4 % purity. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.96 (s, 0.5 H), 8.94 (s, 0.5 H), 7.42 (m, 1 H), 7.40 (m, 2 H), 4.82 (m, 1 H), 4.65 (m, 1 H), 4.19 (m, 1 H), 3.24-3.54 (m, 3 H), 2.98 (m, 2 H), 2.74-2.88 (m, 2 H), 2.50-2.68 (m, 2 H), 2.48 (s, 1.5 H), 2.47 (s, 1.5 H), 2.46 (s, 1.5 H), 2.44 (s, 1.5 H), 2.20-2.42 (m, 3 H), 2.10 (m, 1 H), 2.02 (s, 1 H), 1.70-2.00 (m, 2 H), 1.44 (m, 1 H), 1.30 (d, 1.5

H), 1.24 (d, 1.5 H), 0.96 (s, 3 H). HRMS $(M+H)^+$: calcd 532.2800; found 532.2892. Anal. ($C_{28}H_{36}F_3N_5O_2 \cdot H_2O$): calcd C 61.19, H 6.97, N 12.74; found 61.21, H 7.23, N 12.99.

5-[(4-(3*S*)-4-[(1*R*,2*R*)-2-ethoxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidin-1-yl)carbonyl]-4,6-dimethylpyrimidine (22a). Sodium hydride (257 mg, 6.43 mol) was mixed with dry DMF (20 mL) at 0 °C. Then a solution of *t*-butyl 4-(3*S*)-4-[(1*R*,2*R*)-2-hydroxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidine-1-carboxylate (2.0 g, 4.0 mol) in DMF (20 mL) was added dropwise at 0 °C over 20 min. After the addition, the mixture was stirred for another 20 min before iodoethane (0.42 mL, 5.22 mol) was added at one portion. The mixture was stirred for 1 h and HPLC indicated the completion of the reaction. The reaction content was then diluted with icy water and extracted with ethyl acetate. The combined organic layer was then washed with brine, and dried over $MgSO_4$. After the removal of the solvent, the residue was purified by chromatography on silica (10-150% EtOAc/hexanes) to give 1.5 g product (yield 51.2%). MS (EI) 526.2 (M+1).

t-Butyl 4-(3S)-4-[(1*R*,2*R*)-2-ethoxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidine-1-carboxylate (2.40 g, 4.56 mmol) was treated with 4.0 M of hydrogen chloride in 1,4-dioxane (20 mL, 80 mmol) at room temperature for 2 h. The reaction mixture was concentrated to dryness and the residue was further dried under high vacuum. The formed amine hydrochloride was then mixed with 4,6-dimethyl-pyrimidine-5-carboxylic acid (0.8 g, 5.3 mmol) in DMF (20 mL), and then benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (2.42 g, 5.48 mmol) and triethylamine (2.54 mL, 18.3 mol) were added. The resulting reaction mixture was stirred at room temperature overnight. The reaction was quenched with aq. $NaHCO_3$ and extracted with EtOAc three times. The combined extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. The crude material was purified by Combi-Flash, eluting with 50% EtOAc/hexanes to 100% EtOAc to afford the desired product (2.6 g). Further purification was conduct on reverse phase HPLC under basic condition to afford the product with > 99.5 % purity (1.8 g). ^1H NMR (400 MHz,

$CDCl_3$): δ (ppm) 8.94 (s, 0.5 H), 8.92 (s, 0.5 H), 7.46 (m, 1 H), 7.40 (m, 2 H), 4.72 (m, 1 H), 4.42 (m, 1 H), 4.12 (m, 1 H), 3.62 (m, 1.5 H), 3.52 (m, 1.5 H), 3.30-3.42 (m, 1 H), 3.20 (m, 1 H), 3.00 (m, 1 H), 2.74-2.88 (m, 2 H), 2.62 (m, 1 H), 2.50 (m, 1 H), 2.48 (s, 1.5 H), 2.47 (s, 1.5 H), 2.46 (s, 1.5 H), 2.44 (s, 1.5 H), 2.20-2.40 (m, 3 H), 2.10 (m, 1 H), 2.00 (m, 0.5 H), 1.92 (m, 0.5 H), 1.82 (m, 0.5 H), 1.75 (m, 0.5 H), 1.46 (m, 1 H), 1.30 (m, 1 H), 1.20-1.28 (m, 6 H), 0.96 (s, 3 H). MS (EI) 560.3 (M+1). HRMS $(M+H)^+$: calcd 560.3134; found 560.3207. Anal. ($C_{30}H_{40}F_3N_5O_2 \cdot 1/3H_2O$): C 63.70, H7.25, F 10.08, N 12.38; found C 63.90, H 7.36, F 9.91, N 12.32.

5-[(4-(3S)-4-[(1*R*,2*R*)-2-methoxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidin-1-yl)carbonyl]-4,6-dimethylpyrimidine (21a). This compound was prepared following the same procedures used for the preparation of compound **22a**, with *t*-butyl 4-(3*S*)-4-[(1*R*,2*R*)-2-hydroxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl-4-methylpiperidine-1-carboxylate as the starting material. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.94 (s, 0.5 H), 8.92 (s, 0.5 H), 7.45 (m, 1 H), 7.40 (m, 2 H), 4.70 (m, 1 H), 4.32 (m, 1 H), 4.12 (m, 1 H), 3.42-3.52 (m, 1 H), 3.41 (s, 1.5 H), 3.39 (s, 1.5 H), 3.30-3.39 (m, 1 H), 3.21 (m, 1 H), 3.00 (m, 1 H), 2.74-2.88 (m, 2 H), 2.50-2.68 (m, 2 H), 2.48 (s, 1.5 H), 2.47 (s, 1.5 H), 2.46 (s, 1.5 H), 2.44 (s, 1.5 H), 2.20-2.40 (m, 3 H), 2.10 (m, 1 H), 2.00 (m, 0.5 H), 1.92 (m, 0.5 H), 1.82 (m, 0.5 H), 1.75 (m, 0.5 H), 1.46 (m, 1 H), 1.30 (m, 1 H), 1.25 (d, 1.5 H), 1.20 (d, 1.5 H), 0.96 (s, 3 H). HRMS $(M+H)^+$: calcd 546.2977; found 546.3066. Anal. ($C_{29}H_{38}F_3N_5O_2 \cdot 1/3H_2O$): calcd C 63.14, H 7.06, F 10.33, N 12.70; found C 63.31, H 7.11, F 10.13, N 12.68.

2. Assay Protocols

CCR5 Binding Assay

In a 96 well MultiScreen^{TM} filter plate (Millipore Systems, Billerica, MA), 3×10^5 IL-10-treated monocytes in 150 μL RPMI (Invitrogen, Carlsbad, CA) with 20 mM HEPES (Invitrogen, Carlsbad, CA) and 0.3% BSA (Sigma, St Louis, MO) were incubated at room temperature for 1 hr. with 0.2 nM ^{125}I-MIP-1β (Perkin Elmer, Boston, MA) and a series concentrations of compound of the invention. Non-specific binding was

determined by incubating the cells with 0.3 μM MIP-1β (R&D Systems, Minneapolis, MN). The binding reaction was terminated by harvesting the cells onto the filter in the plate on a vacuum manifold (Millipore Systems, Billerica, MA). The filter was then washed 5 times with RPMI (Invitrogen, Carlsbad, CA) supplemented with 20 mM HEPES (Invitrogen, Carlsbad, CA), 0.3% BSA (Sigma, St Louis, MO) and 0.4 M NaCl on the vacuum manifold, air dried, and peeled from the plate. The filter dishes corresponding to the sample wells in a filter plate were punched out using the Millipore Punch System (Millipore Systems, Billerica, MA). The amount of bound radioactivity on each filter dish was determined by counting on a gamma counter. Specific binding was defined as the total binding minus the non-specific binding. The binding data were evaluated with Prism (GraphPad Software, San Diego, CA).

CCR5 Chemotaxis Assay

The assay utilizes human peripheral blood mononuclear cells, in a modified Boyden Chamber (Neuro Probe). 500,000 cells in serum free DMEM media (In Vitrogen) are incubated with or without the inhibitors and warmed to 37 ºC. The chemotaxis chamber (Neuro Probe) is also prewarmed. 400 μL of warmed 10 nM MCP-1 is added to the bottom chamber in all wells expect the negative control which has DMEM added. An 8 micron membrane filter (Neuro Probe) is place on top and the chamber lid is closed. Cells are then added to the holes in the chamber lid which are associated with the chamber wells below the filter membrane. The whole chamber is incubated at 37 ºC, 5% CO_2 for 30 minutes. The cells are then aspirated off, the chamber lid opened, and the filter gently removed. The top of the filter is washed 3 times with PBS and the bottom is left untouched. The filter is air dried and stained with Wright Geimsa stain (Sigma). Filters are counted by microscopy. The negative control wells serve as background and are subtracted from all values. Antagonist potency can be determined by comparing the number of cells that migrate to the bottom chamber in wells which contain antagonist, to the number of cells which migrate to the bottom chamber in MCP-1 control wells.

HIV-1 Infection Assay

The HIV-1 PBMC infection assays were performed by ImQuest BioSciences (Frederick, MD). Briefly, human PBMCs were purified from leukophoresed blood by Ficoll-Hypaque density centrifugation and stimulated for 48-72 hours in RPMI 1640 with 15% FBS, 2 mM L-glutamine, 2µg/mL PHA-P, 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C. After stimulation, PBMCs were maintained in tissue culture medium (RPMI 1640, 15% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 3.6 ng/mL recombinant human IL-2). For the PBMC infection assay, PHA-P stimulated PBMCs from three donors were pooled together to minimize the variability between individual donors and re-suspended in fresh tissue culture medium at $1x10^6$ cells/mL and plated in the interior wells of a 96-well round bottom microtiter plate at 50 µL/mL. Then, 100 µL of 2× concentrations of compound-containing medium was transferred to the round-bottom 96-well plate containing the cells in 50 µL of the medium. Immediately following test material addition to the wells, 50 µL of a predetermined dilution of HIV-1 virus (prepared at 4× of final in well concentration) was added, and fixed well. For infection, 50-150 $TCID_{50}$ of each virus was added per well (final MOI=0.05-0.1). PBMCs were exposed in triplicate to virus and cultured in the presence or absence of the test material at varying concentrations as described above in the 96-well microtiter plate. After 7 days in culture, HIV-1 replication was quantified by the measurement of cell free HIV-1 viral capsid protein p24 antigen in the tissue culture supernatant using a HIVp 24 ELISA kit (Coulter Retrovirology, Hialeah, FL).