Supporting Information

Synthesis, Structure-Activity Relationship and In Vivo Anti-inflammatory Efficacy of Substituted Dipiperidines as CCR2 Antagonists

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1) Biological Assays

MCP-1 Receptor Binding Assay in THP-1 Cells

THP-1 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The THP-1 cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C. The cell density was maintained at 0.5×10^6 cells/mL.

THP-1 cells were incubated with 0.5 nM ^{125}I labeled MCP-1 (Perkin-Elmer Life Sciences, Inc. Boston, MA) in the presence of varying concentrations of either unlabeled MCP-1 (R & D Systems**,** Minneapolis, MN) or test compound for 2 hours at 30^{o}C in a 96 well plate. Cells were then harvested onto a filter plate, dried, and 20 μL of Microscint 20 was added to each well. Plates were counted in a TopCount NXT , Microplate Scintillation & Luminescence Counter (Perkin-Elmer Life Sciences, Inc. Boston, MA). Blank values (buffer only) were subtracted from all values and drug treated values were compared to vehicle treated values. 1 μM cold MCP-1 was used for nonspecific binding.

MCP-1 Induced Calcium Mobilization in THP-1 Cells

THP-1 cells were plated at a density of 8 x 10^5 cells/ mL (100 μL/well) into poly-D lysine coated clear bottom, black 96 well plates. The cells were loaded with 5 μM fluo-3 for 45 minutes. The fluo-3 was washed off and cells were incubated with varying concentrations of test

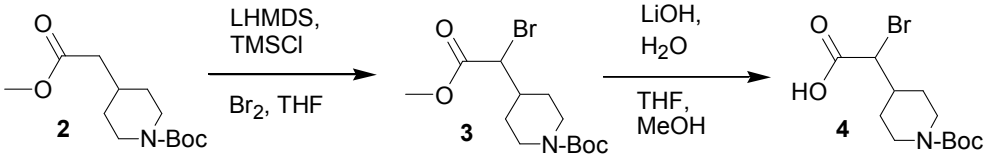
compound for 15 minutes. The change in calcium ion concentration upon addition of 0.2 μM MCP-1 was determined using FLIPR and compared to vehicle.

MCP-1 Induced Chemotaxis in THP-1 Cells

MCP-1 induced chemotaxis was run in a 24-well chemotaxis chamber. MCP-1 (0.01 μg/mL) was added to the lower chamber and 100 μL of THP-1 cells (1 x 10^7 cell/mL) was added to the top chamber. Varying concentrations of test compound were added to the top and bottom chambers. Cells were allowed to chemotax for 3 hours at 37 °C and 5% CO_2. An aliquot of the cells which had migrated to the bottom chamber was taken and counted then compared to vehicle.

2) **Synthesis of CCR2 Antagonists**

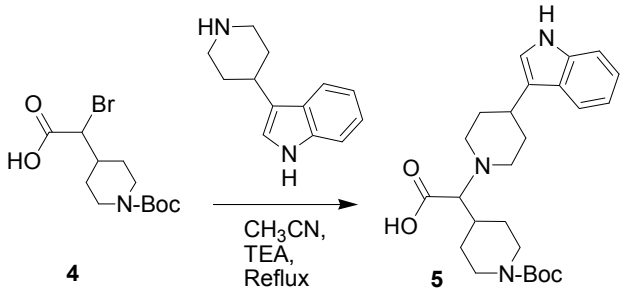
a) Synthesis of representative compound of carboxylic acid series -- Compound **1d**



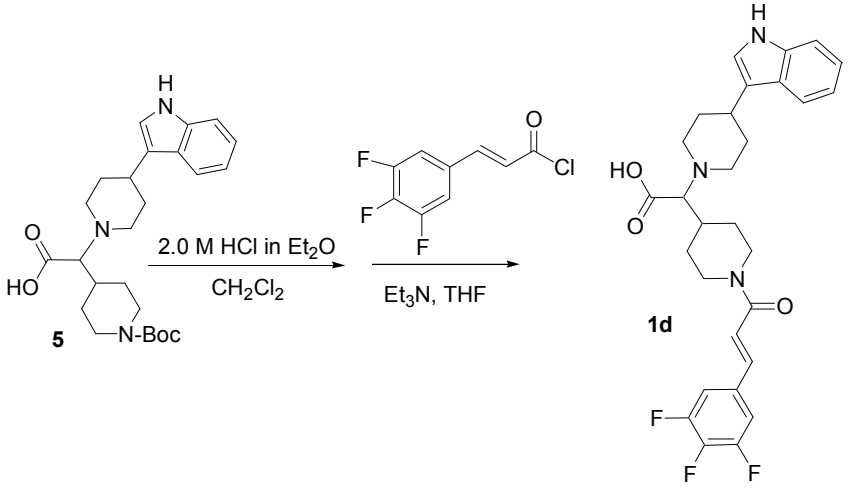
A solution of 4-methoxycarbonylmethyl-piperidine-1-carboxylic acid tert-butyl ester Compound **2** (1.0 g, 3.9 mmol) in THF (5 mL) was added to LHMDS (1.0 M in THF) (7.0 mL, 7.0 mmol) at -78°C and the reaction mixture was stirred at -78°C for 3 hrs. TMSCl (0.89 mL, 7.0 mmol) was added dropwise and the mixture was stirred for 1 hr at -78°C then Br_2 (0.24 mL, 4.7 mmol) was added dropwise. The mixture was stirred at -78°C for 2 hrs, then allowed to warm to 0°C and stirred for an additional 30 min. The mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ solution, then washed with H_2O. The organics were dried over Na_2SO_4, then the drying agent was filtered and solvent removed *in vacuo* to yield a yellow solid. The crude product was purified by flash column chromatography (50% EtOAc/hexane) to yield 4-(bromo-methoxycarbonyl-methyl)-piperidine-1-carboxylic acid tert-butyl ester Compound **3** as a pale yellow oil (1.0 g, 77%). MS *m/z* 358 $(M+Na)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 4.15 (br, 2H), 4.01 (d, J = 8.5 Hz, 1H), 3.80 (s, 3H), 2.65-2.78 (br s, 2H), 2.04 (m, 2H), 1.61 (m, 1H), 1.45 (s, 9H), 1.21 (m, 2H).

An aqueous LiOH solution (0.624 g, 14.87 mmol in 7 mL H_2O) was added to a solution of Compound **3** (1.0 g, 2.97 mmol) in MeOH (21 mL) and THF (7 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo to provide a white

solid, which was acidified with 1 N HCl. The crude product was extracted with ethyl acetate and the organics were washed with brine and dried over Na_2SO_4. The drying agent was filtered and the solvent removed *in vacuo*, yielding 4-(bromo-carboxy-methyl)-piperidine-1-carboxylic acid tert-butyl ester Compound **4** (0.663 g, 66%) as a white solid. The product was used in the next step without further purification. MS *m/z* 344; 346 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 4.05-4.20 (m, 3H), 2.60-2.80 (br s, 2H), 1.90 -2.10 (m, 2H), 1.64-1.75 (m, 1H), 1.45 (s, 9H), 1.20-1.30 (m, 2H).



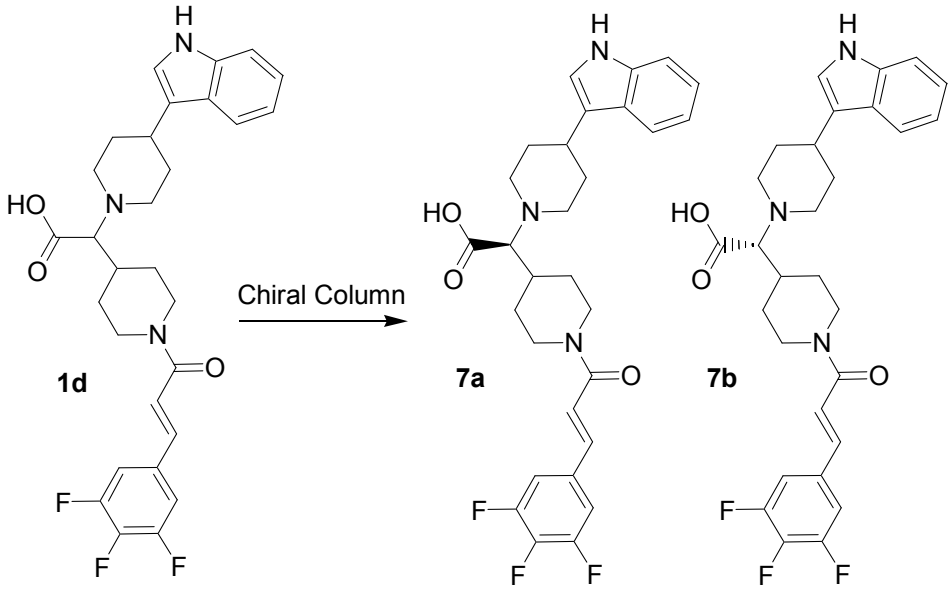
A solution of Compound **4** (0.335 g, 1.040 mmol), 3-piperidin-4-yl-1*H*-indole (0.208 g, 1.040 mmol) and TEA (0.29 mL, 2.080 mmol) in CH_3CN was refluxed for 5 hrs. The solvent was removed *in vacuo* to provide a yellow solid. The product was washed with a minimum amount of methanol to remove residual starting material, thus obtaining 4-{carboxy-[4-(1H-indol-3-yl)-piperidin-1-yl]-methyl}-piperidine-1-carboxylic acid tert-butyl ester Compound **5** (27%, 0.459 g) as a white solid. MS *m/z* 442 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d6) δ 10.73 (br s, 1H), 7.52 (m, 1H), 7.29 (m, 1H), 7.08 (m, 2H), 6.92 (m, 1H), 3.87 (m, 2H), 2.55-2.95 (m, 7H), 2.32 (m, 1H), 1.90 (m, 4H), 1.48-1.72 (m, 3H), 1.39 (s, 9H), 1.05 (m, 2H).



2.0 M HCl in Et_2O (5 mL, 10 mmol) was added to a solution of Compound **5** (0.441 g, 1.0 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred overnight at room temperature.

The solvent was removed *in vacuo* and the residue was washed with CH_2Cl_2 to give [4-(1*H*-indol-3-yl)-piperidin-1-yl]-piperidin-4-yl-acetic acid. It was dissolved in THF (10 mL). A solution of 3-(3,4,5-trifluoro-phenyl)-acryloyl chloride (0.220 g, 1.0 mmol) and triethylamine (0.6 mL) in THF (5 mL) was added. The resulting mixture was stirred for 4h, then concentrated under vacuum. The crude product was collected by vacuum filtration, washed with cold THF, slurried in hot water and suspended in MeOH. The product **1d** (0.38 g, 72%) was collected by vacuum filtration at room temperature. MS *m/z* 526 $(M+H)^+$. ^{1}H NMR (DMSO-d6, 400 MHz) δ 10.77 (s, 1H), 7.81 (m, 2H), 7.55 (d, J = 7.9 Hz, 1H), 7.39 (m, 2H), 7.32 (d, J = 8.1 Hz, 1H), 7.05 (m, 2H), 6.95 (q, J = 7.1 Hz, 1H), 4.47 (m, 1H), 4.31 (m, 1H), 3.10 (m, 1H), 2.90 (m, 3H), 2.65 (m, 3H), 2.35 (m, 1H), 2.06 (m, 1H), 1.94 (m, 3H), 1.68 (m, 1H), 1.62 (m, 2H), 1.10 (m, 2H). Anal. ($C_{29}H_{30}F_3N_3O_3$ •2.5H_2O) C, H, N.

b) Compounds **7a** and **7b**



The racemate [4-(1*H*-indol-3-yl)-piperidin-1-yl]-{1-[3-(3,4,5-trifluoro-phenyl)-acryloyl]-piperidin-4-yl}-acetic acid Compound **1d** (0.255 g, 0.49 mmol) was separated into two enantiomers Compound **7a** (0.11 g, 43%) and Compound **7b** (0.11g, 43%) with a Chiralpak AD column (eluted with CH_3CN/CH_3OH 85 /15).

Compound **7a**: MS *m/z* 526 $(M+H)^+$, 548 $(M+Na)^+$. ^{1}H NMR (DMSO-d6, 400 MHz) δ 11.95 (br s, 1H), 10.78 (s, 1H), 7.81 (m, 2H), 7.55 (d, J = 8.0 Hz, 1H), 7.37 (m, 2H), 7.32 (d, J = 8.0 Hz, 1H), 7.04 (m, 2H), 6.95 (q, J = 7.0 Hz, 1H), 4.47 (m, 1H), 4.31 (m, 1H), 3.10 (m, 1H), 2.90 (m, 3H), 2.65 (m, 3H), 2.35 (m, 1H), 2.06 (m, 1H), 1.94 (m, 3H), 1.69 (m, 1H), 1.61 (m, 2H), 1.09 (m, 2H). Anal. ($C_{29}H_{30}F_3N_3O_3$ •2.5 H_2O) C, H, N.

Compound **7b**: MS *m/z* 526 $(M+H)^+$, 548 $(M+Na)^+$. ^1H NMR (DMSO-d6, 400 MHz) δ 12.02 (br s, 1H), 10.73 (s, 1H), 7.81 (m, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.37 (m, 2H), 7.32 (d, J = 8.0 Hz, 1H), 7.04 (m, 2H), 6.95 (q, J = 7.0 Hz, 1H), 4.46 (m, 1H), 4.31 (m, 1H), 3.10 (m, 1H), 2.90 (m, 3H), 2.65 (m, 3H), 2.35 (m, 1H), 2.06 (m, 1H), 1.94 (m, 3H), 1.69 (m, 1H), 1.61 (m, 2H), 1.09 (m, 2H). Anal. ($C_{29}H_{30}F_3N_3O_3 \bullet 2.5\ H_2O$) C, H, N.