

Synthesis, Structure–Activity Relationship and in Vivo Antiinflammatory Efficacy of Substituted Dipiperidines as CCR2 Antagonists

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Abstract: A series of substituted dipiperidine compounds have been synthesized and identified as selective CCR2 antagonists. Combining the most favorable substituents led to the discovery of remarkably potent CCR2 antagonists displaying IC₅₀ values in the nanomolar range. Compound **7a** had outstanding selectivity over CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, and CCR8 and showed excellent efficacy in adjuvant-induced arthritis model, collagen-induced arthritis model, and allergic asthma model.

Monocyte chemoattractant protein-1 (MCP-1) is a major chemoattractant for monocytes and memory T cells through binding to its specific cell-surface receptor, CC-chemokine receptor-2 (CCR2). Animal model studies of chronic inflammatory diseases have demonstrated that inhibition of binding between MCP-1 and CCR2 by an antagonist suppresses the inflammatory response. MCP-1 and its receptor CCR2 have been implicated in inflammatory disease pathologies such as uveitis, rheumatoid arthritis, multiple sclerosis, allergic rhinitis, chronic obstructive pulmonary disease (COPD), allergic asthma, and solid tumors.^{1–8}

Monocyte migration is inhibited by MCP-1 antagonists (either antibodies or soluble, inactive fragments of MCP-1) that have been shown to inhibit the development of arthritis, asthma, and uveitis. MCP-1 and CCR2 knockout (KO) mice have demonstrated that monocyte infiltration into inflammatory lesions is significantly decreased.^{9,10} In addition, such knockout mice are resistant to the development of experimental allergic encephalomyelitis (EAE, a murine model of human multiple sclerosis), cockroach allergen-induced asthma, atherosclerosis, and uveitis. Rheumatoid arthritis and Crohn's disease patients have demonstrated a reduction in symptoms during the treatment with TNF- α antagonists (e.g., monoclonal antibodies and soluble receptors) at dose levels that correlated with decreases in MCP-1 expression and the number of infiltrating macrophages.¹¹

MCP-1 has been implicated in the pathogenesis of seasonal and chronic allergic rhinitis, having been found in the nasal mucosa of most patients with dust mite allergies. MCP-1 has also been found to induce histamine release from basophils in vitro. During allergic conditions, allergens and histamines have been shown to trigger (i.e., to up-regulate) the expression of MCP-1 and other chemokines in the nasal mucosa of people with allergic rhinitis, suggesting the presence of a positive feedback loop in such patients.

There remains a need for small-molecule CCR2 antagonists for preventing, treating, or ameliorating a CCR2-mediated inflammatory syndrome, disorder, or disease. Many reports

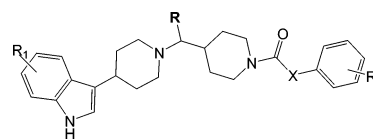
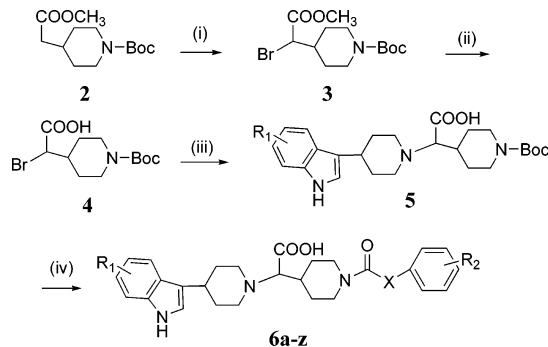


Figure 1.

Table 1. Functional Group Effect on CCR2 Binding Affinity

compd	R	CCR2 IC ₅₀ (nM)
1a	CO ₂ Me	3300 ± 400
1b	CONH ₂	1800 ± 100
1c	H	470 ± 170
1d	CO ₂ H	5 ± 2

Scheme 1. Synthesis of Carboxylic Acid Analogues^a



^a (i) LHMDS, TMSCl, –78 °C, then Br₂, 77%; (ii) LiOH, 66%; (iii) substituted indolepiperidine, CH₃CN, TEA, reflux, 27–70%; (iv) (a) HCl, (b) ArCH_n = CH_nCOCl or ArNCO, 60–90%.

regarding the discovery of CCR2 antagonists have been published to date.^{12–24}

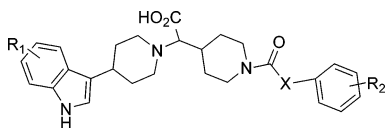
Recently, we disclosed a series of potent phenylpiperidine-based CCR2 antagonists. Those compounds demonstrated good selectivity over CCR1, CCR3, and 5-HT, an excellent cytochrome P450 profile, and reasonable pharmacokinetics.²⁵

Our search for more potent CCR2 antagonists led to the discovery of a series of substituted dipiperidines exemplified by the structure shown in Figure 1. In this communication, we report the synthesis, structure–activity relationships, and anti-inflammatory activities of these compounds.

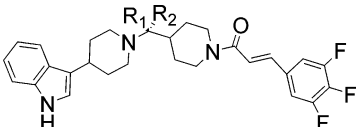
Initial SAR studies on the CH₂ linker between the two piperidine moieties revealed that carboxylic acid derivative **1d** showed a marked improvement in binding affinity to the human CCR2 receptor relative to the unsubstituted, amide, and ester analogues (Table 1). These results encouraged us to further explore the SAR surrounding the carboxylic acid–methylene-linked bispiperidine series. The synthesis of these carboxylic acid analogues is outlined in Scheme 1.

Boc-protected piperidin-4-ylacetic acid methyl ester **2** was converted to the α-bromo acid **4** through bromination (LHMDS/TMSCl, Br₂) and ester hydrolysis. Bromoacid **4** was refluxed with the desired substituted indole piperidine in acetonitrile to afford **5**, which was deprotected and then reacted with a suitable acid chloride or isocyanate to give substituted dipiperidineacetic acid analogues **6a–z**.

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Table 2. CCR2 Binding Affinities of Carboxylic Acid Analogues


compd	R ₁	X ^a	R ₂	IC ₅₀ ^b
6a	H	CH=CH	4-CH ₃ O	660 ± 230
6b	H	CH=CH	4-NO ₂	210 ± 90
6c	H	CH=CH	4-CH ₃	115 ± 55
6d	H	CH=CH	H	80 ± 9
6e	H	CH=CH	3,5-diF	20 ± 10
6f	H	CH=CH	4-CF ₃	15 ± 5
6g	H	CH=CH	3,4-diCl	4 ± 0.5
6h	5-OH	CH=CH	3,4,5-triF	15 ± 5
6i	2-CO ₂ H	CH=CH	3,4,5-triF	15% ^c
6j	6-F	CH=CH	3,4,5-triF	20 ± 6
6k	6-Cl	CH=CH	3,4,5-triF	7.7 ± 1.3
6l	5-CH ₃ O	CH=CH	3,4,5-triF	16.7 ± 3.3
6m	5-F	CH=CH	3,4,5-triF	50 ± 10
6n	5-CH ₃ SO ₂ NH	CH=CH	3,4,5-triF	6.3 ± 1.3
6o	5-NH ₂	CH=CH	3,4,5-triF	4 ± 0.6
6p	7-CH ₃ O	CH=CH	3,4,5-triF	2.5 ± 0.5
6q	2-CH ₃	CH=CH	3,4,5-triF	4 ± 0.5
6r	1-CH ₃	CH=CH	3,5-diF	44% ^c
6s	1-CH ₃ CO	CH=CH	3,5-diF	3400
6t	H	NH	2,3-diCl	6800
6u	H	NH	H	1100
6v	H	NH	3,5-diCl	720
6w	H	NH	4-CF ₃	120
6x	H	NH	3,4-diCl	25 ± 5
6y	H	NH	4-OCF ₃	10 ± 1
6z	H	CH ₂ CH ₂	3,4-diCl	320

^a All CH=CH are trans. ^b Data in nM. ^c At 25 μM.**Table 3.** CCR2 Binding Affinities of the Different Enantiomers


compd	R ₁	R ₂	CCR2 IC ₅₀ (nM)
7a	H	CO ₂ H	4 ± 2
7b	CO ₂ H	H	210 ± 100

Table 2 lists the CCR2 binding affinities for carboxylic acid analogues **6a–z** shown in Scheme 1. Substitution of the indole nitrogen (**6r**, **6s**) was not tolerated, while the 2-position tolerated a methyl group in preference to a carboxyl group (**6q**, **6i**). Generally, the 5, 6, and 7 positions on the indole ring tolerated various functional groups (**6j–p**). Halogen or trifluoromethyl substitution (**6e–h**) on the cinnamoylphenyl ring was preferred. Urea analogues (**6u**, **6x**) had lower affinity than the corresponding cinnamoyl compounds (**6d**, **6g**). Cinnamoyl (**6g**) and urea (**6x**) analogues were more potent than the corresponding phenylpropionamide compound (**6z**). The ortho position of the phenyl ring (**6t**) was much less tolerant of substitution than the meta or para position (**6v**, **6x**).

The carboxylic acid series had one chiral center. The racemates of key analogues were separated into enantiomers by chiral preparative HPLC. For example, racemate **1d** was separated into (*S*)-enantiomer **7a** and (*R*)-enantiomer **7b** with a Chiralpak AD column (eluent 85/15 CH₃CN/CH₃OH). The absolute configuration was determined by X-ray crystallography. Compound **7a** had higher binding affinity than compound **7b** (Table 3).

Compound **7a** was selected for further evaluation. In a chemotaxis assay using the THP-1 cell line, **7a** effectively

antagonized the MCP-1-induced effect with an IC₅₀ of 2 nM. A similar IC₅₀ was observed when the MCP-1-induced flux of Ca²⁺ ions was measured instead of chemotaxis. This compound did not significantly inhibit the binding of relevant chemokines to CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, or CCR8 at 25 μM and showed excellent specificity for CCR2. To further investigate the specificity, **7a** was tested for its ability to inhibit the binding of relevant ligands to many other GPCRs, and to various ion channels, in the Cerep panel. At the screening concentration of 1 μM (~250 times its IC₅₀ for CCR2), it did not inhibit binding to any receptor by >30% except for the H1 receptor (41%), the 5-HT1B receptor (39%), the 5-HT2A receptor (39%), and the NK3 receptor (31%).

Consistent with the relatively low degree of sequence homology between mouse and human MCP-1 and between mouse and human CCR2, **7a** was significantly less potent when tested in binding assays using ¹²⁵I-mouse MCP-1 and either mouse peripheral blood monocytes or a mouse monocytic cell line (WEHI-265.1). Compound **7a** had IC₅₀ of 2 μM in these assays, much higher than its IC₅₀ in the hCCR2 membrane binding assay and the human cellular-based assays. A similar phenomenon was observed in the rat assay (IC₅₀ = 2 μM).

These species differences became critical when the compound was used for in vivo studies in rodents. The compound was dosed ip rather than po in order to attain the high systemic drug levels necessary to overcome the relatively low affinity of the compound for mouse and rat CCR2. The bioavailability of **7a** in rats was 15% with po (*t*_{1/2} = 7 h) and 100% with ip at 10 mg/kg dosage (vehicle of 20% Solutol, 30% PEG400, and 50% 0.1 N NaHCO₃).

Compound **7a** was used for in vivo studies in the adjuvant-induced arthritis and the collagen-induced arthritis models. In the adjuvant-induced arthritis model, 7-week-old male Lewis rats were injected in the right hind footpad with a mixture of heat-killed *Mycobacterium butyricum* (0.5 mg) in liquid paraffin oil (50 μL). An increase in volume of the contralateral (noninjected) hind paw was used as a measure of arthritis severity. Body weight and hind paw volume (as measured by mercury plethysmography volume displacement) were typically recorded on days 0, 3, 7, 10, 12, 14, and 16. Rats were dosed with test compound **7a** (ip, bid, 100 mg/kg) or with vehicle alone from day 7 to day 14. Under these conditions, **7a** inhibited swelling of the contralateral paws by 94%. In a collagen-induced arthritis model in mice, DBA1 mice were immunized with bovine type II collagen on day 0, injected (sc) with lipopolysaccharide (LPS) on day 21, and dosed (ip, bid) with a test compound at 25, 50, or 100 mg/kg from day 20 to day 35. Body weight was monitored and clinical disease score recorded every 2–3 days starting on day 20. Compound **7a** inhibited the development of arthritis (clinical disease score on day 35) by 23%, 50%, and 79% at the 25, 50, and 100 mg/kg doses, respectively.

Compound **7a** was also tested in a mouse model of allergic asthma for therapeutic effect on asthmatic response as a function of airway inflammation and hyperresponsiveness. Airway responsiveness was measured in unrestrained mice by non-invasive whole body plethysmography using a BioSystem plethysmography instrument. Airway inflammation was measured in terms of total cellular influx in the bronchoalveolar lavage fluids (BALF). The result for the mice treated with **7a** (ip, 100 mg/kg, vehicle 20% of Solutol, 30% PEG400, and 50% 0.1 N NaHCO₃) represents an average of 36% reduction in airway hyperresponsiveness and 83% reduction in the total cellular influx in the BALF.

In summary, substituted dipiperidine compounds have been synthesized and identified as selective CCR2 antagonists. Carboxylic acid analogues **6a–z** exhibited remarkable affinity in a human CCR2 binding assay. They had much higher affinity than the corresponding ester (**1a**) or amide (**1b**). Compound **7a** had excellent selectivity for CCR2 over CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8 and showed significant in vivo efficacy in adjuvant-induced arthritis, collagen-induced arthritis, and allergic asthma models in rats and mice. An in depth biological profile of **7a** and systematic SAR studies of the dipiperidine scaffold will be reported in due course.

Acknowledgment. We thank Dr. William Murray for support and Dr. Mark Macielag, Dr. Peter Connolly, and Dr. Zhihua Sui for helpful discussions.

Supporting Information Available: Experimental details of the synthesis and characterization of representative CCR2 antagonists. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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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% CO₂ atmosphere at 37°C. The cell density was maintained at 0.5×10⁶ cells/mL.

THP-1 cells were incubated with 0.5 nM ¹²⁵I 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°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 10⁵ 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

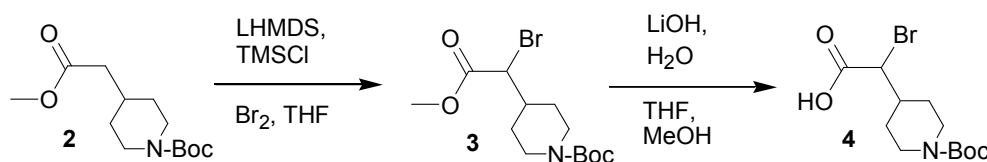
compound for 15 minutes. The change in calcium ion concentration upon addition of 0.2 μM MCP-1 was determined using FLIPR and compared to vehicle.

MCP-1 Induced Chemotaxis in THP-1 Cells

MCP-1 induced chemotaxis was run in a 24-well chemotaxis chamber. MCP-1 (0.01 $\mu\text{g/mL}$) was added to the lower chamber and 100 μL of THP-1 cells (1×10^7 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 $^{\circ}\text{C}$ 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

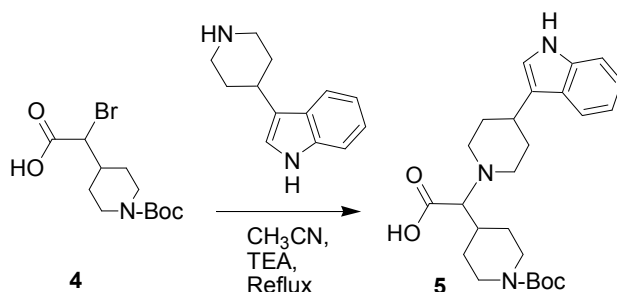
a) Synthesis of representative compound of carboxylic acid series -- Compound **1d**



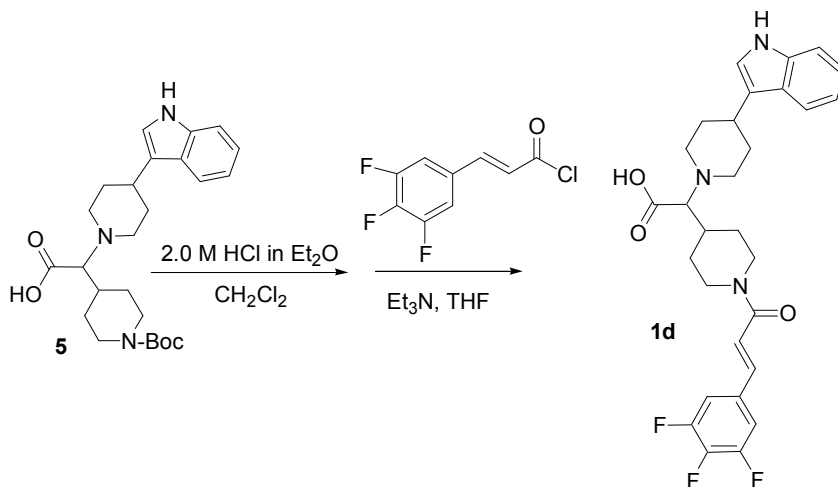
A solution of 4-methoxycarbonylmethyl-piperidine-1-carboxylic acid tert-butyl ester Compound **2** (1.0 g, 3.9 mmol) in THF (5 mL) was added to LHMDS (1.0 M in THF) (7.0 mL, 7.0 mmol) at -78°C and the reaction mixture was stirred at -78°C for 3 hrs. TMSCl (0.89 mL, 7.0 mmol) was added dropwise and the 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 ($\text{M}+\text{Na}^+$); ^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 Na₂SO₄. 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, CDCl₃) 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).



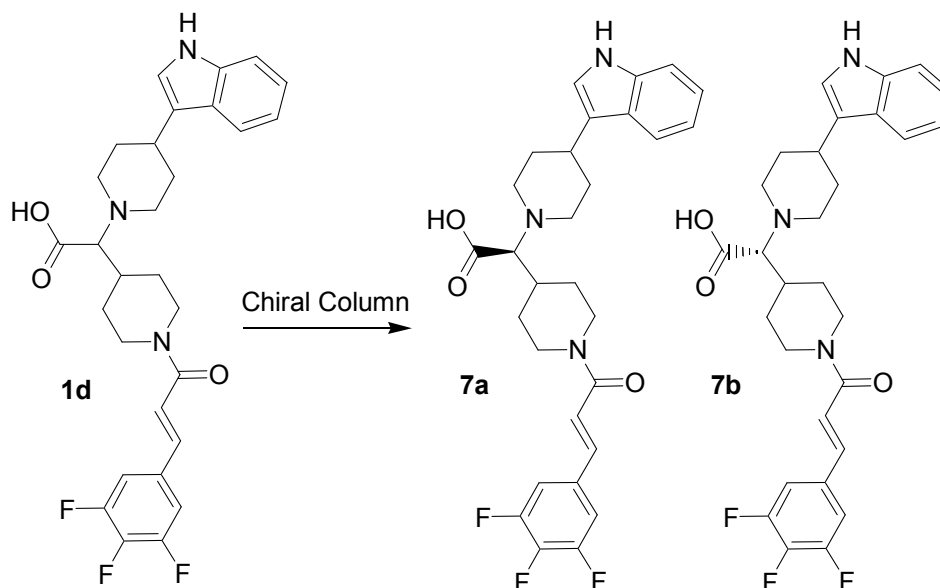
A solution of Compound **4** (0.335 g, 1.040 mmol), 3-piperidin-4-yl-1H-indole (0.208 g, 1.040 mmol) and TEA (0.29 mL, 2.080 mmol) in CH₃CN was refluxed for 5 hrs. The solvent was removed *in vacuo* to provide a yellow solid. The product was washed with a minimum amount of methanol to remove residual 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 Et₂O (5 mL, 10 mmol) was added to a solution of Compound **5** (0.441 g, 1.0 mmol) in CH₂Cl₂ (10 mL). The reaction mixture was stirred overnight at room temperature.

The solvent was removed *in vacuo* and the residue was washed with CH₂Cl₂ 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)⁺. ¹H 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. (C₂₉H₃₀F₃N₃O₃ • 2.5H₂O) 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 CH₃CN/CH₃OH 85 /15).

Compound **7a**: MS *m/z* 526 (M+H)⁺, 548 (M+Na)⁺. ¹H 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. (C₂₉H₃₀F₃N₃O₃ • 2.5 H₂O) C, H, N.

Compound **7b**: MS m/z 526 ($M+H$)⁺, 548 ($M+Na$)⁺. ¹H NMR (DMSO-*d*₆, 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. (C₂₉H₃₀F₃N₃O₃ • 2.5 H₂O) C, H, N.