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 IC_{50} 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 regarding the discovery of CCR2 antagonists have been published to date.[12−24]

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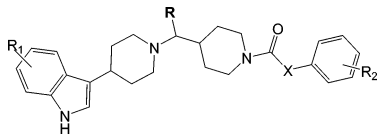
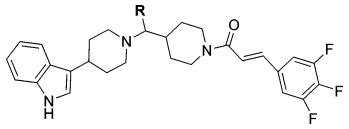


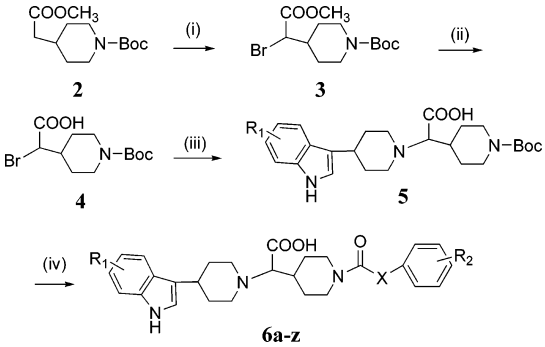
Figure 1.

Table 1. Functional Group Effect on CCR2 Binding Affinity



compd	R	CCR2 IC_{50} (nM)
1a	CO_2Me	3300 ± 400
1b	$CONH_2$	1800 ± 100
1c	H	470 ± 170
1d	CO_2H	5 ± 2

Scheme 1. Synthesis of Carboxylic Acid Analogues[a]



[a] (i) LHMDS, TMSCl, −78 °C, then Br_2, 77%; (ii) LiOH, 66%; (iii) substituted indolepiperidine, CH_3CN, TEA, reflux, 27−70%; (iv) (a) HCl, (b) $ArCH_n$ = CH_nCOCl or ArNCO, 60−90%.

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 CH_2 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-bromoacid **4** through bromination (LHMDS/TMSCl, Br_2) 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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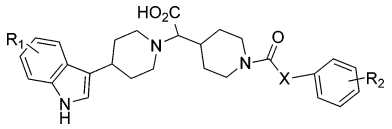
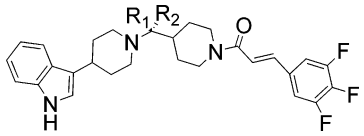
Table 2. CCR2 Binding Affinities of Carboxylic Acid Analogues



compd	R_1	X[a]	R_2	IC_{50} [b]
6a	H	CH=CH	4-CH_3O	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_2H	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_3O	CH=CH	3,4,5-triF	16.7 ± 3.3
6m	5-F	CH=CH	3,4,5-triF	50 ± 10
6n	5-CH_3SO_2NH	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_3O	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_3CO	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_2CH_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_2H	4 ± 2
7b	CO_2H	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 CH_3CN/CH_3OH). 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 IC_{50} of 2 nM. A similar IC_{50} was observed when the MCP-1-induced flux of Ca^{2+} 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 IC_{50} 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 ^{125}I-mouse MCP-1 and either mouse peripheral blood monocytes or a mouse monocytic cell line (WEHI-265.1). Compound **7a** had IC_{50} of 2 μM in these assays, much higher than its IC_{50} in the hCCR2 membrane binding assay and the human cellular-based assays. A similar phenomenon was observed in the rat assay (IC_{50} = 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 $NaHCO_3$).

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 noninvasive 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 $NaHCO_3$) 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.

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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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