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# Potent and selective CC-chemokine receptor-2 (CCR2) antagonists as a potential treatment for asthma

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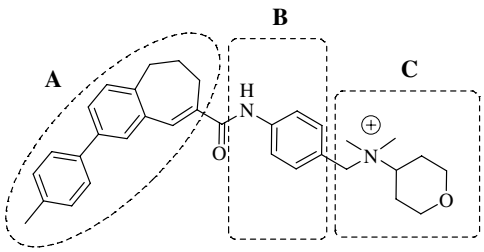
**Abstract**—A number of compounds bearing a quaternary ammonium moiety were found to be antagonists with nanomolar binding affinity for the chemokine receptor-2. The structure–activity relationships in the series are described herein along with some detailed characterization of the interesting compounds.


Monocyte chemotactic protein-1 (MCP-1) is an important member of a large family of chemokines that plays a significant role in directing the influx of mononuclear leukocytes into sites of inflammation (i.e., chemotaxis) and tissue injury.[1] MCP-1 is secreted by monocytes, memory T cells, macrophages, fibroblasts, endothelial cells, and mast cells, and stimulates the movement of responsive leukocytes along a chemotactic gradient following binding to its cognate cell-surface receptor, CC-chemokine receptor-2 (CCR2).[2] The binding of MCP-1 to the negatively charged extracellular loops of CCR2 is believed to initiate a signaling cascade which plays a critical role in both acute and chronic inflammatory processes.[3] Elevated expression of MCP-1 and CCR2 has been observed in various diseases characterized by chronic inflammation and large numbers of infiltrating monocytes including rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, atherosclerosis, asthma, and uveitis.[2–5] There is strong evidence, using both MCP-1 and CCR2 knockout mice, that the MCP-1/CCR2 ligand/receptor pair plays a critical role in inflammatory processes.[6,7] These observations have led to the hypothesis that an antagonist of the G-protein coupled receptor CCR2 would be an effective treatment for inflammatory disorders.

Structures of several CCR2 antagonists have been published by many research laboratories.[4,5,8] In general these compounds contain two lipophilic regions separated by a basic amine such as piperidine or pyrrolidine.[9–14] However, our attention was caught by a paper from Takeda Pharmaceuticals on a structurally unique CCR5 antagonist, TAK-779, which was in Phase I clinical trials as an anti-HIV treatment (Fig. 1).[15] In addition to its high affinity for the CCR5 receptor, this compound also showed affinity for the closely related CCR2 receptor ($IC_{50}$ for CCR5 = 1.4 nM, $IC_{50}$ for CCR2 = 27 nM). We were intrigued by the possibility of analoging out the (undesired) CCR5 activity of these types of compounds while maintaining or improving the binding affinity for CCR2. In order to test the hypothesis, the '**A**', '**B**', and '**C**' segments of the molecule were systematically modified (Fig. 1). The synthesis and structure–activity of these CCR2 antagonists is described in this letter.




**TAK-779**

**Figure 1.** Structure of TAK-779 and various segments targeted for CCR2 affinity.

*Keywords*: Chemokine receptor-2; CCR2 antagonist; Chemotaxis; MCP-1; Asthma; Monocytes; Quaternary ammonium salt.
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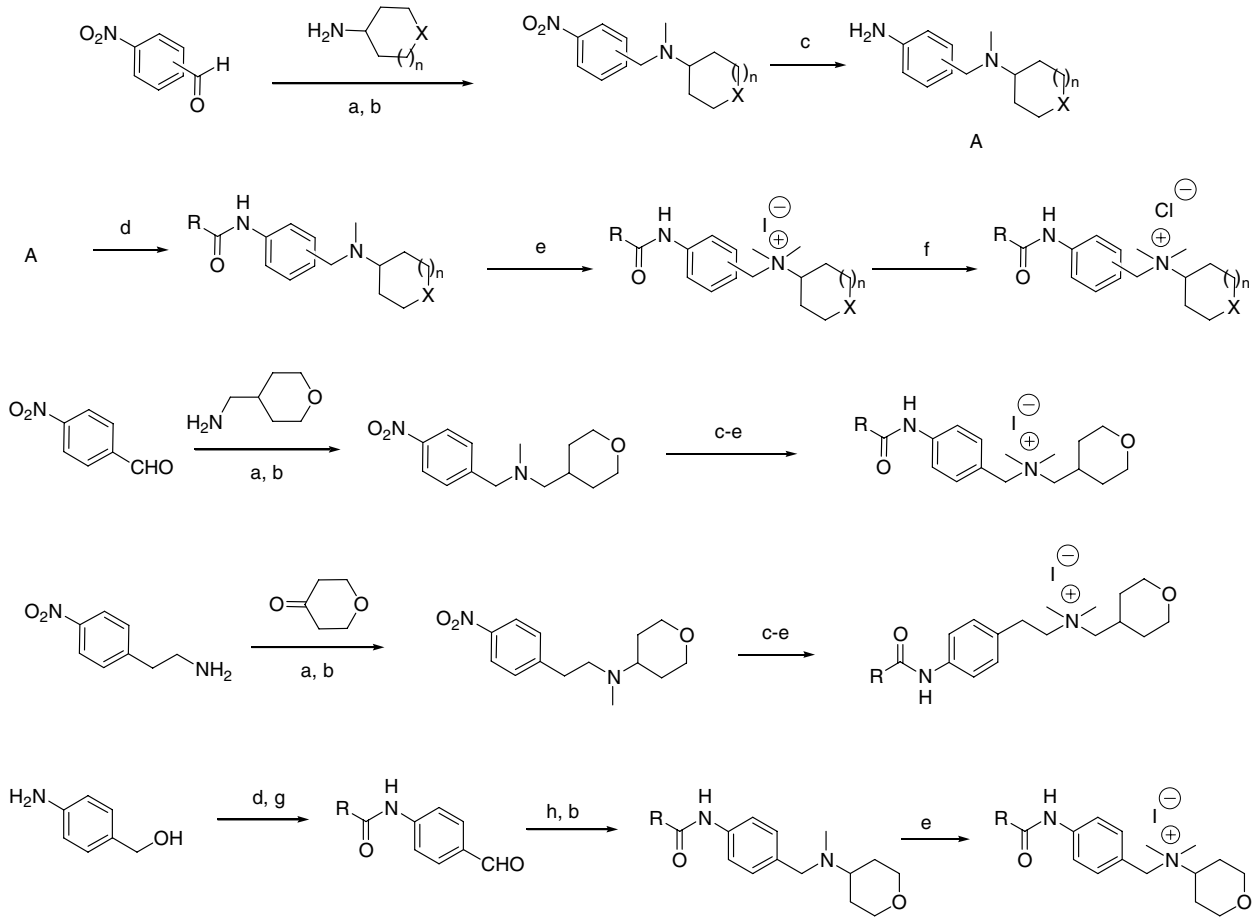
The compounds were synthesized in a straightforward manner using the synthetic routes described in Scheme 1.[15,16] The flexible nature of synthesis made the exploration of **A**, **B**, and **C** segments of the molecule in an efficient manner. The compounds were initially screened for the binding affinities to the CCR2 receptor using THP-1 cell line and $^{125}$I-labeled MCP-1.[17]

It soon became obvious that the '**B**' segment tolerated a number of modifications without deleteriously affecting the binding affinities for the CCR2 receptor as shown in Scheme 1. The modifications of the segment '**A**', on the other hand, had a significant impact on the binding affinities for the CCR2. Herein we describe the structure–activity relationship in a series of compounds that differed from TAK-779 in the '**A**' segment of the molecule. The binding affinities for the compounds possessing cinnamoyl, 2-H-chromenyl, and phenyl moieties in the segment '**A**' are summarized in Table 1.

In general, the compounds lacking the quaternary nitrogen showed significantly reduced (>10 μM) binding affinities for the CCR2 receptor as compared to the compounds that incorporate the quaternary nitrogen. Compounds **1a**–**b** containing the biphenyl moiety attached to a cinnamoyl group showed good binding affinity (0.01 μM) to the CCR2 receptor. The compounds devoid of the biphenyl group (**1c**–**d**) showed substantially decreased binding affinities for the receptor (>2 μM). Interestingly, the 3,4-dichlorocinnamoyl moiety is also present in the structures of CCR2 antagonists published from Glaxo–Smith Kline.[9,10]

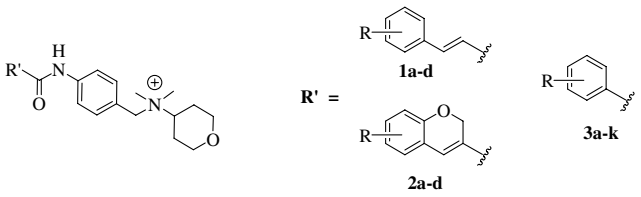
However, when the cinnamoyl moiety in segment '**A**' was replaced with a substituted 2H-chromene-3-carboxylic acid moiety, the compounds (**2a**–**e**) displayed submicromolar binding affinity (0.05–0.2 μM) for the CCR2 as shown in Table 1. The presence of chlorine at the 7-position on the 2H-chromene yielded compounds with good binding affinities (0.05–0.2 μM). When the segment '**A**' was changed to a substituted phenyl ring, an interesting SAR emerged for the resulting compounds.

In contrast to the compounds **1a** and **1b**, the biphenyl moiety-containing compound **3h** in this series of compounds did not show good binding affinity ($IC_{50}$ = 3.4 μM). The presence of strong electron donating groups (in **3e** and **3g**) or a presence of electron withdrawing groups (**3d** or **3f**) at the 3-position of the phenyl ring resulted in compounds with either the same or diminished binding affinity for the CCR2 as compared




**Scheme 1.** Reagents and conditions: (a) $NaB(OAc)_3H$, cat AcOH, $CH_2Cl_2$, rt, 6 h; (b) 37% HCHO, $NaB(OAc)_3H$, $CH_2Cl_2$, rt; (c) $SnCl_2{\cdot}2H_2O$, THF; (d) RCOOH, EDC, HOBt, $Et_3N$, DMF or RCOCl, $Et_3N$, THF; for the description of R groups, please see Table 1; (e) $CH_3I$, $CH_2Cl_2$ or acetone; (f) ion-exchange chromatography; (g) $MnO_2$, $CHCl_3$, rt, 12 h; (h) 4-aminotetrahydropyran, $NaB(OAc)_3H$, cat AcOH, $CH_2Cl_2$, rt, 6 h.

**Table 1.** Structure–activity relationship for CCR2 antagonists



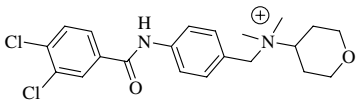
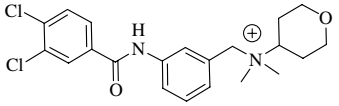
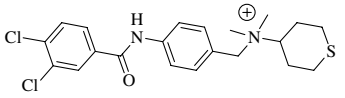
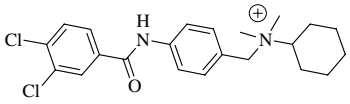
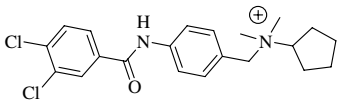
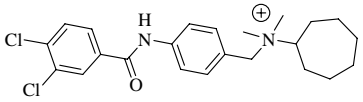
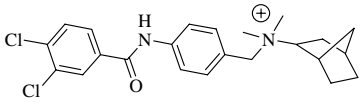
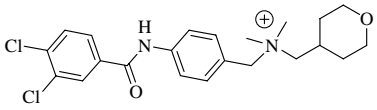
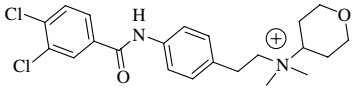
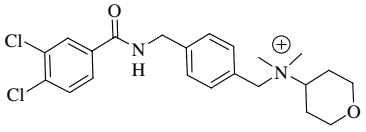

| Compound | R | $IC_{50}$ (μM)[a] |
|---|---|---|
| **1a** | 4-(*p*-toluyl) | 0.01 ± 0.01 |
| **1b** | 4-(*p*-$CF_3$)-Ph | 0.01 ± 0.01 |
| **1c** | 3-F | 1.81 ± 1.04 |
| **1d** | 3,4-di-Cl | 2.55 ± 0.95[b] |
| **2a** | 7-Br | 0.18 ± 0.10 |
| **2b** | 7-Cl | 0.12 ± 0.07 |
| **2c** | 7-Me | 0.17 ± 0.12 |
| **2d** | 9-Me | 0.22 ± 0.13 |
| **2e** | 7-Cl-9-Me | 0.05 ± 0.03 |
| **2f** | 6,8-di-Cl | 0.20 ± 0.10 |
| **2g** | 7,9-di-Cl | 0.16 ± 0.03[b] |
| **2h** | 4,5-benzo | 0.07 ± 0.03 |
| **3a** | H | 4.75 ± 0.20 |
| **3b** | 3-Br | 0.25 ± 0.14 |
| **3c** | 3-Cl | 0.23 ± 0.13 |
| **3d** | 3-CN | 3.25 ± 0.95[b] |
| **3e** | 3-OMe | 9.55 ± 1.85[b] |
| **3f** | 3-$CF_3$ | 2.05 ± 0.45[b] |
| **3g** | 3-Me | 4.25 ± 2.15[b] |
| **3h** | 3-(*p*-toluyl) | 3.44 ± 1.98 |
| **3i** | 4-Cl | 2.00 ± 0.40[b] |
| **3j** | 2-Cl | >10 |
| **3k** | 2,3-di-Cl | >10 |
| **3l** | 2,4-di-Cl | 7.80 ± 1.20[b] |
| **3m** | 3,5-di-Cl | 0.71 ± 0.35 |
| **3n** | 3,4-di-Cl | 0.02 ± 0.01 |
| **3p** | 3-Cl-4-F | 0.39 ± 0.23 |

[a] SEM shown for $n > 2$.
[b] An average with SD is shown for $n = 2$ mean number for $IC_{50}$ shown.

to the compound, **3a**. The presence of a chloro or a bromo group at the 3-position (**3b** and **3c**) yielded compounds with significantly improved binding affinities ($IC_{50} = 0.2$ μM). The position of the chlorine atom on the phenyl ring was crucial for the observed binding affinity (compounds **3i** and **3j**). Compound **3i**, possessing chloro group at the 4-position of the phenyl ring, had reduced binding affinity ($IC_{50} = 2$ μM), while compound **3j** with 2-chloro group showed significantly reduced binding affinity ($IC_{50} > 10$ μM) for CCR2. In the case of di-substituted phenyl analogs, compound **3n** bearing 3,4-di-chloro substituted phenyl ring showed a superior binding affinity for the CCR2 receptor as compared to the compounds **3l**, **3m**, and **3p** containing the other di-chloro substitutions.

Like segment '**A**', modifications in the segments '**B**' and '**C**' also have some influence on the binding affinities of the resulting compounds (Table 2). The *meta* substitution pattern in the di-substituted phenyl ring in the segment '**B**' leads to compound **4** with significantly reduced binding affinity ($IC_{50} = 11$ μM) as compared to the *para*-substituted **3n**. The replacement of the tetrahydropyran

**Table 2.** Structure–activity relationship for CCR2 antagonists

| Compound | R | $IC_{50}$ (μM)[a] |
|---|---|---|
| **3n** |  | 0.02 ± 0.01 |
| **4** |  | 11.35 ± 5.05[b] |
| **5** |  | 0.20 ± 0.04[b] |
| **6** |  | 0.03 ± 0.01 |
| **7** |  | 0.04 ± 0.01[b] |
| **8** |  | 0.29 ± 0.17 |
| **9** |  | 0.09 ± 0.04[b] |
| **10** |  | 1.02 ± 0.68[b] |
| **11** |  | >10 |
| **12** |  | 8.95 ± 2.55[b] |

[a] SEM shown for $n > 2$.
[b] An average with SD is shown for $n = 2$ mean number for $IC_{50}$ shown.

ring in the segment 'C' by 5, 6 or 7-membered cycloalkyl groups or norbornane (compounds **6**–**9**) does not affect the binding affinities (0.03–0.09 μM) in an adverse way. The replacement of the tetrahydropyran ring by a tetrahydrothiopyranyl group results in compound **5** with 10-fold lower binding affinity (0.2 μM vs 0.02 μM for **3n**).

The presence of an additional methylene group on either side of the phenyl ring (segment '**B**') leads to compounds **11** and **12** with substantially reduced binding affinities (>8 μM) for the CCR2. Similarly, compound **10** containing an additional methylene group between the tetrahydropyarn and the quaternary nitrogen resulted in the loss of binding affinity (1.0 μM). Compound **3n** was chosen for more detailed evaluation.

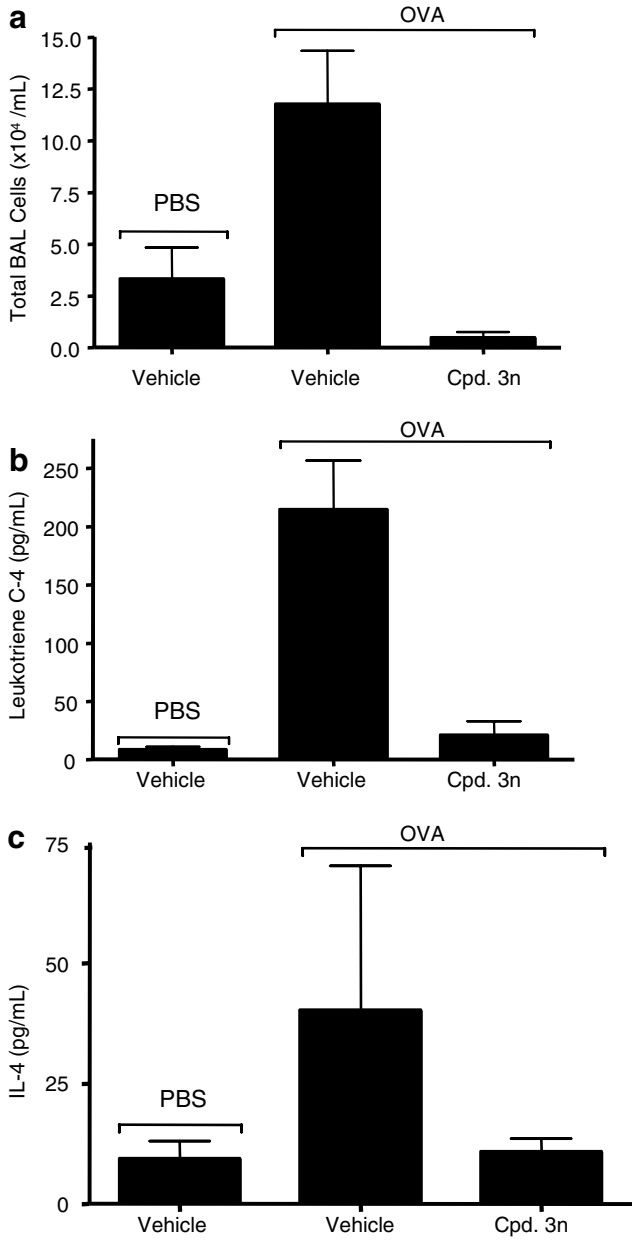



**Figure 2.** (a) Total cell count in bronchoalveolar lavage; (b) LTB4(pg/mL); (c) IL-4 (pg/mL).

The compound **3n** showed significantly reduced affinity (2 μM) for disrupting the binding of radiolabeled mouse MCP-1 to WEHI 265.1 cells (a mouse macrophage cell line). These data are not completely surprising given the fact that the human and mouse CCR2 proteins share only 83% sequence homology, and the human and mouse MCP-1 proteins share only 64% sequence identity. These species differences become critical when one examines the results from in vivo studies in rodents. In cell-based functional assays, the compound potently inhibited MCP-1 induced chemotaxis ($IC_{50}$ = 100 nM) of THP-1 cells (a human monocyte-like cell line). The compound also inhibited MCP-1 induced calcium mobilization ($IC_{50}$ = 100 nM) in both THP-1 cells and human peripheral blood mononuclear cells. The compound **3n** did not inhibit binding of the relevant CC-chemokines such as CCR1, CCR3, CCR4, CCR6, CCR7, and CCR8. Not surprisingly, the compound weakly inhibited binding of MIP-1α to the CCR5 receptor ($IC_{50} \sim 1$ μM). This observation underscores the fact that the original CCR5 antagonist (TAK-779) possessing low-nanomolar affinity for CCR5 and lower CCR2 affinity has been transformed into a compound that maintains a good binding affinity for the CCR2 receptor but has significantly reduced binding affinity for the CCR5 receptor. Such reversal of the chemokine receptor selectivity can be attributed to the changes made in the '**A**' region of TAK-779. Compound **3n** did not show any significant cross-reactivity when tested against a panel of more than 30 receptors and ion channels including some G-protein coupled receptors (e.g., 5-HT, alpha adrenergic receptors, $Ca^{2+}$ and $Cl^-$ ion channels). The compound has excellent aqueous solubility (>7 mg/mL) at pH 2 and 7. However, the compound **3n** showed poor permeability through Caco-2 cell line presumably due to its positive charge. The compound exhibited excellent metabolic stability following incubation with human liver microsomes for one hour (the % recovery was 100%). The compound does not show any oral bioavailability in rats (presumably due to poor absorption), but has 74% and 100% bioavailability upon intraperitoneal or subcutaneous administration, respectively.

The anti-inflammatory property of the compound was confirmed when compound **3n** showed 79% inhibition of the leukocyte recruitment in the zymosan-induced peritonitis model in mice at a dose of 30 mg/kg (subcutaneously).[18,19] Both CCR2 and MCP-1 have been postulated to play a role in the asthma.[20–23] Therefore, the compound was next evaluated in a mast cell-dependent model of ovalbumin-induced asthma in BALB/c mice.[24] The results are shown in Figure 2. A 95% inhibition of the cell influx into the bronchoalveolar lavage fluid was observed after dosing at 30 mpk (intraperitoneally). In addition, the compound also inhibited production of $LTC_4$ and IL-4 by 90% and 85%, respectively. Together, these beneficial effects on these critical markers of asthma after the administration of **3n** suggest that a potent CCR2 antagonist could potentially serve as treatment for asthma. The details of these experiments including histological evaluation of the relevant tissues will be published elsewhere.

In summary, a number of CCR2-selective antagonists were synthesized by modifications of the structure of a known CCR5 antagonist. One of the compounds, **3n**, showed excellent binding affinity and selectivity for the CCR2 receptor over a number of other GPCRs. When dosed parenterally, the compound showed good activity in two different models of inflammation. These observations suggest that CCR2 antagonists would be beneficial for treatment of immunoinflammatory diseases.

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18. In this model, mice are injected ip with zymosan, a polysaccharide derived from the cell walls of yeast (*Saccharomyces cerevisiae*). A rapid influx of neutrophils into the peritoneal cavity occurs, peaking at ~4 h, which is followed by a wave of monocyte infiltration which is maximal at 16–24 h. MCP-1 concentration in peritoneal lavage fluid peaks at ~4 h, and an antibody to MCP-1 significantly inhibits monocyte infiltration.
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