

## Comparative Analysis of CCR2 and CCR5 Binding Sites to Facilitate the Development of Dual Antagonists: An in Silico Study

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### Abstract

Chemokine receptor antagonists have potential applications in field of drug discovery. Although the chemokine receptors are G-protein-coupled receptors, their cognate ligands are small proteins (8 to 12 kDa), and so inhibiting the ligand/receptor interaction has been challenging. In particular, CCR2 and CCR5 and their ligands have been implicated in the pathophysiology of a number of diseases, including rheumatoid arthritis and multiple sclerosis. Based on their roles in disease, they have been attractive targets for the pharmaceutical industry, targeting both CCR2 and CCR5 could be a useful strategy. Because of the importance of these receptors, providing information regarding the binding site is of prime importance. Herein, we report the comparison of CCR2 of CCR5 binding sites both sequentially as well as structurally. We also urged the importance of crucial residues in the binding site, to facilitate the development of dual antagonists targeting both the receptors. These results could also be useful for the design of novel and potent dual CCR2 and CCR5 antagonists using structure based drug design.

**Key words :** CCR2, CCR5, Drug Design

### 1. Introduction

The chemokine receptor family includes ~20 G-protein-coupled receptors that play a central role in leukocyte migration and activation<sup>[1]</sup>. Specific family members are also involved in viral entry and angiogenesis. Given this diverse range of important functions, they have been targeted as potential points of pharmaceutical intervention for blunting diseases as diverse as asthma, rheumatoid arthritis, multiple sclerosis, solid organ transplantation, atherosclerosis, cancer, and HIV infection<sup>[2]</sup>. Chemokines are relatively small proteins (8 to 12 kDa) that vary widely in sequence but exhibit similar tertiary structures. The typical chemokine structure consists of a disordered N-terminus (6 to 10 amino acids), the signature cysteine motif (C, CC, CXC, or CX3C), a loop region, a three-stranded beta-sheet, and a C-terminal alpha helix. Two disulfide bonds typically stabilize this tertiary structure<sup>[3]</sup>.

Among those, CC chemokines are the major family

which consists of the Monocyte Chemoattractant Protein-1 (MCP-1), the most characterized protein, also known as 'chemokine ligand CCL2'<sup>[4-6]</sup>. The CC family consists of several CC receptors (CCR 1-10); of which CCR2 is the primary receptor for MCP-1. Studies show that MCP-1 involves in the pathophysiology of the acute or chronic inflammatory conditions such as rheumatoid arthritis, atherosclerosis, asthma, obesity, and type-2 diabetes. Therefore, CCR2 receptor is an attractive target for the drug discovery<sup>[7-9]</sup>. CCR5 belongs to family of rhodopsin G-protein coupled receptor which is characterized by 7 transmembrane domains<sup>[10]</sup>. MIP-1 $\alpha$ , MIP-1 $\beta$  and RANTES are natural ligands available for CCR5<sup>[11]</sup>. Homozygous and heterozygous genotype carriers with CCR5-32 base pair deletion have shown either resistance or prolonged progression of HIV-1 infection<sup>[12,13]</sup>.

Because of their diverse range of important functions, chemokines have been targeted as potential points of pharmaceutical intervention for blunting diseases as diverse as asthma, rheumatoid arthritis, multiple sclerosis, solid organ transplantation, atherosclerosis, cancer, and HIV infection<sup>[14]</sup>. Since these chemokine receptors are G protein-coupled receptors and targeted for diverse diseases, many pharmaceutical and biotechnology com-

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panies have devoted enormous time, effort, and expense in developing potent small-molecule chemokine antagonists<sup>[15,16]</sup>. Accordingly, two such antagonists Maraviroc (a CCR5 antagonist) for the treatment of HIV/AIDS<sup>[17]</sup> and Plerixafor (a CXCR4 antagonist) used in combination with granulocyte-colony stimulating factor (G-CSF) to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma have been approved by the United States Food and Drug Administration (FDA)<sup>[18]</sup>.

But, for chronic inflammatory diseases, clinical trials with antagonists of a single chemokine receptor (e.g., CCR1, CCR2, or CCR5) have not proved successful<sup>[15,16]</sup>, which has been a major setback. Bearing in mind the difficulty of pathogenesis of these diseases and the potential for functional redundancy of chemokine receptors, targeting a single receptor may not be adequate for efficacy in these chronic conditions. CCR2 and CCR5 are two CC chemokine receptors that are important players in the trafficking of monocytes/macrophages and in the functions of other cell types relevant to disease pathogenesis.<sup>[19,20]</sup>

The main aim of this study was to provide adequate information regarding the binding site of these pharmaceutically important receptors. We compared the binding sites of these receptors both sequentially as well as structurally. Sequential studies were performed by aligning the residues using ClustalW. Whereas, structural analysis was done by homology modeling using the recently reported crystal structure of CXCR4 as the template<sup>[21]</sup>. The homology models of CCR2 and CCR5 were further superimposed and the varying residues in the binding site were compared and analyzed. Our results could also be useful for the design of novel and potent dual CCR2 and CCR5 antagonists using structure based drug design.

## 2. Experimental

### 2.1. Sequence Analysis of CCR2 and CCR5

The human sequences of CCR2 and CCR5 were retrieved from the Uniprot KB/TrEMBL database (accession numbers P41597 and P51681). In order to identify an adequate template for modeling of CCR2 and CCR5 chemokine receptors, Basic local alignment

search tool for protein (BLAST) algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was carried out against the protein data bank. After the search, finally the alignment between the template and the target sequences (CCR2 and CCR5) was performed using ClustalW 2.0 with default parameters.

### 2.2. Modeling of CCR2 and CCR5

A number of homologous structures were identified as templates in the protein data bank. However, with the availability of recently published CXCR4 (protein data base code: 3ODU; resolution-2.5 Å)<sup>[21]</sup> as the top template, comparative modeling was done. With the given identified hit as template structure, sequence alignments for query sequences (P41597 and P51681) were carried out. The structure of both CCR2 and CCR5 was generated using the Modeller9v4 program<sup>[22-25]</sup>. Modeller9v4 calculates a model composed of non-hydrogen atoms based on the alignment of the sequence to be modeled with known related structures. A 3D model was obtained by optimization of a molecular probability density function (PDF) using a variable target function procedure in Cartesian space that employs methods of conjugate gradients and molecular dynamics with simulated annealing.

## 3. Results and Discussion

### 3.1. Sequence Analysis

Sequence alignment is the prerequisite for homology modeling. The sequence conservation of the query (CCR2 and CCR5) with the template structure (PDB



Fig. 1. Alignment between the query (CCR2) and template sequence.



**Fig. 2.** Alignment between the query (CCR5) and template sequence.

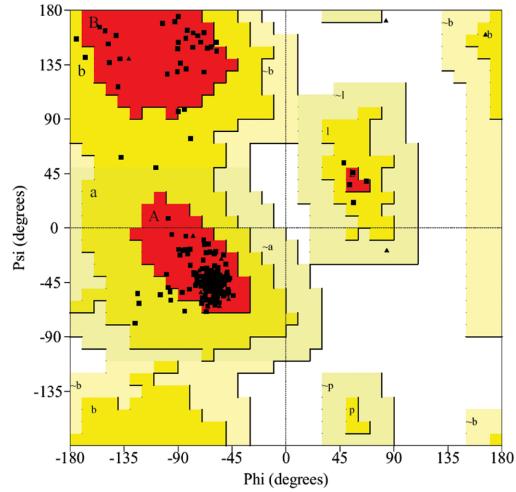
code: 3ODU) was examined with the alignment obtained using ClustalW. The alignment of query sequence with the template is shown in Fig. 1 and Fig. 2. The sequence identity between the query and the template was 35%. The sequences showed a high level of homology between the target and template sequences and were better than that of the traditional bovine rhodopsin and the more recent  $\beta$ 2-adrenergic receptor templates.

### 3.2. Homology Modeling

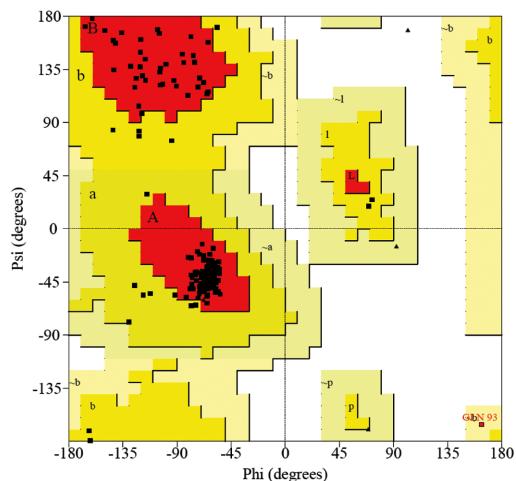
CXCR4 was used to develop the 3D models and a modeler program was used to derive 3D-models of CCR2 and CCR5. One hundred models were developed for both CCR2 and CCR5. Finally, a model (CCR2 and CCR5) with a lower MolPdf value and the one that displayed a lesser RMSD was selected for further computational analysis. More than 90% of the members of the GPCR super family have conserved disulfide bridges. As in CCR2, disulfide bridges were created between Cys32-Cys277 and Cys113-Cys190. In CCR5, the disulfide bridges were maintained between Cys20-Cys269 and Cys101-Cys178. The selected models were further validated stereo-chemically using additional parameters such as PROCHECK<sup>[26]</sup>. The Ramachandran plot for model is shown in the Fig. 3 and Fig. 4.

### 3.3. Comparative Analysis of CCR2 and CCR5

Sequences of CCR2 and CCR5 were compared and we found that it shares an identity of 66% between them. We also found that, they share 82% identity between them in their active sites. From the alignment



**Fig. 3.** Ramachandran plot of the CCR2 model obtained before MDS. The different color coding indicates most favored (red), generously allowed (dark yellow), additionally allowed (light yellow), and disallowed (white) regions.



**Fig. 4.** Ramachandran plot of the CCR5 model obtained before MDS. The different color coding indicates most favored (red), generously allowed (dark yellow), additionally allowed (light yellow), and disallowed (white) regions.

we found that the most of the residues are conserved and the alignment obtained is shown in Fig. 5.

As dual targeting of CCR2 and CCR5 is an important issue in current scenario of drug discovery, we concentrated on the binding site of these receptors to develop potent dual antagonists. The binding sites of both these receptors were superimposed and some residues were varied. Our analysis revealed that almost all the residues

Fig. 5. Alignment obtained between the CCR2 and CCR5 sequences for sequence analysis. Identical residues are marked as (\*), similar regions are marked as (:).

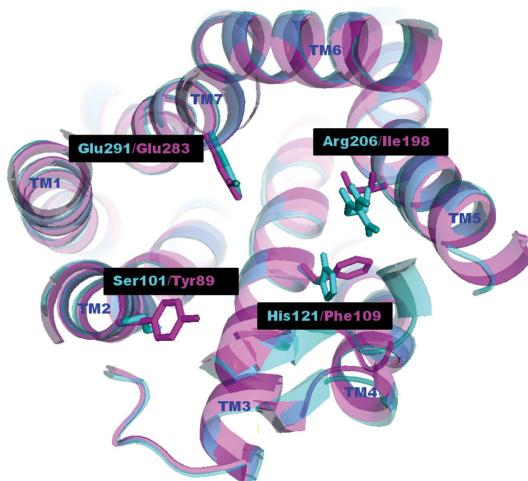


Fig. 6. Superposition of varying residues in the active sites of CCR2 (cyan) and CCR5 (magenta).

are identical except three residues. The varying residues in CCR2/CCR5 are Ser101/Tyr89, His121/Phe109, and Arg206/Ile198; these differ in their electrostatic properties. More specifically, Ser101 is hydrophilic and Tyr89 is hydrophobic in nature. Similarly, His121 and Arg206 are hydrophilic, whereas Phe109 and Ile198 are hydrophobic in nature. While designing dual inhibitors one may consider this variation of active sites residues for potent inhibition of dual targets. Superimposed binding site of CCR2 and CCR5 is shown in Fig. 6.

## 4. Conclusion

In this study, the binding sites of CCR2 and CCR5 were compared sequentially as well as structurally. Our

results showed that both the receptors shared immense similarity in terms of overall alignment and also in terms of binding site. We also found that the varying residues in CCR2/CCR5 are Ser101/Tyr89, His121/Phe109, and Arg206/Ile198. We conclude that, to design dual antagonists to target both these receptors the varied residues should be considered. Our results could be a starting point for further structure based drug design.

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