Comparative Docking Study of Anibamine as the First Natural Product CCR5 Antagonist in CCR5 Homology Models

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Anibamine, a novel pyridine quaternary alkaloid recently isolated from *Aniba sp*., has been found to effectively bind to the chemokine receptor CCR5 with an IC_{50} at 1 μM in competition with ^{125}I-gp120, an HIV viral envelope protein binding to CCR5 with high affinity. Since CCR5, a G-protein-coupled receptor, is an essential coreceptor for the human immunodeficiency virus type I (HIV-1) entry to host cells, a CCR5 antagonist that inhibits the cellular entry of HIV-1 provides a new therapy choice for the treatment of HIV. Anibamine provides a novel structural skeleton that is remarkably different from all lead compounds previously identified as CCR5 antagonists. Here, we report comparative docking studies of anibamine with several other known CCR5 antagonists in two CCR5 homology models built based on the crystal structures of bovine rhodopsin and human β_2-adrenergic receptor. The binding pocket of anibamine has some common features shared with other high affinity CCR5 antagonists, suggesting that they may bind in similar binding sites and/or modes. At the same time, several unique binding features of anibamine were identified, and it will likely prove beneficial in future molecular design of novel CCR5 antagonists based on the anibamine scaffold.

INTRODUCTION

According to UNAIDS Report 2006,[1] an estimated 38.6 million people worldwide were living with HIV at the end of 2005. In the United States about one million people are currently living with HIV.[2] Since the AIDS pandemic remains one of the leading global public health threats, the continued discovery and development of new antiretroviral drugs with reduced toxicity, enhanced potency, novel mechanisms of action, and reduced prevalence of adverse drug-drug interactions remains a very high priority. The chemokine receptors CCR5 and CXCR4 are essential coreceptors required for the attachment of HIV-1 to the host cell membrane[3−5] suggesting that CCR5 antagonists may lead to a novel therapeutic strategy in blocking the entry of HIV into host cells, especially when utilized in combination with the classical reverse transcriptase and protease inhibitors.[6,7] At the same time, the CCR5-Δ32 homo- and heterozygous genotype carriers have been shown to confer either resistance to HIV-1 infection or delayed progression of the disease.[8,9] Therefore, it is clear that CCR5 plays an essential role in HIV pathogenesis.

The chemokine receptor CCR5 (Figure 1) belongs to the extensively exploited family of G-protein-coupled receptors (GPCRs). Chemokines and chemokine receptors play a crucial role in the trafficking of leukocyte populations in the body and are involved in the development of a wide variety of human diseases.[10] Despite the fact that it has been more than ten years since the chemokine receptor CCR5 was identified as one of the essential coreceptors utilized by HIV-1 for entry into human CD4+ cells,[3−5] there are currently only a few CCR5 antagonists (Figure 2) being investigated as anti-HIV-1 agents in human clinical trials.[11−14] In fact, only one drug (Maraviroc or Selzentry) was approved by the FDA on August 6, 2007[14d] even though the FDA raised concerns that Maraviroc could be associated with an increased risk of liver damage, lymphoma, infections, and increased risk of heart attack.[14c,d] Also, clinical trials of Aplaviroc were halted due to severe liver side effects in both treatment-naive and treatment-experienced patients.[13b] It should be noted that the lead compounds for all of these CCR5 antagonists were discovered through high-throughput screening campaigns targeting the disruption of the virus attachment to the coreceptor. While this is often a valid approach that yields interesting lead compounds, natural products can often be more diverse, having novel structure skeletons with wider ranges of chemical shape and structural features. It is well-known[15] that natural products offer unmatched structural variety compared with combinatorial chemistry libraries, and their high diversity and specific biological activities very often make them useful resources for drug discovery.

Recently, anibamine, a novel pyridine quaternary alkaloid that was isolated from *Aniba sp.*, has been found to effectively bind to CCR5 with an IC_{50} of 1 μM in competitive binding assays with ^{125}I-gp120, a viral envelope protein that binds to CCR5 with high affinity.[16] As the first and only natural product to date showing CCR5 antagonism, anibamine has a novel structure quite different from those other known classes of CCR5 antagonists. Recently, we reported the total synthesis of anibamine[17] applying a flexible synthetic scheme that will allow the production of a wide variety of analogs.

It should be noted that the 1 μM activity of anibamine is in line with those of other "lead" compounds (0.39−2 μM)

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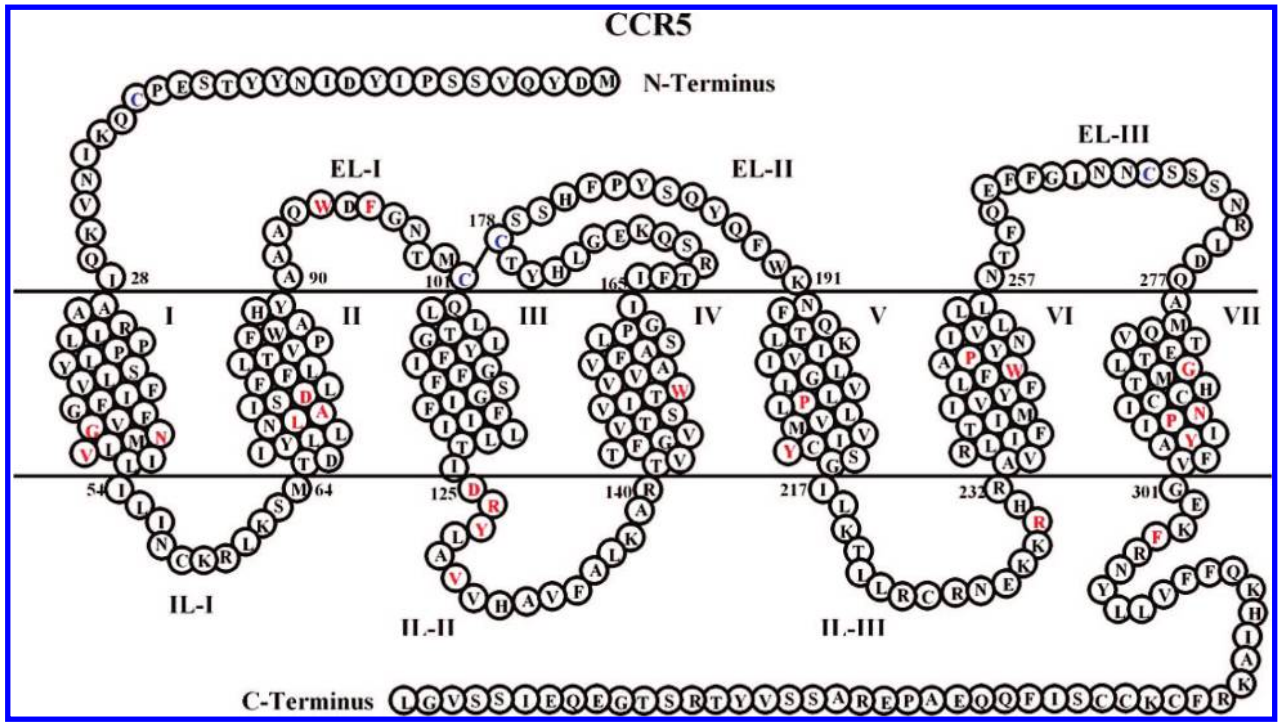

Figure 1. The schematic representation of the CCR5 sequence: EL, extracellular loop; IL, intracellular loop; I−VII, transmembrane helices.

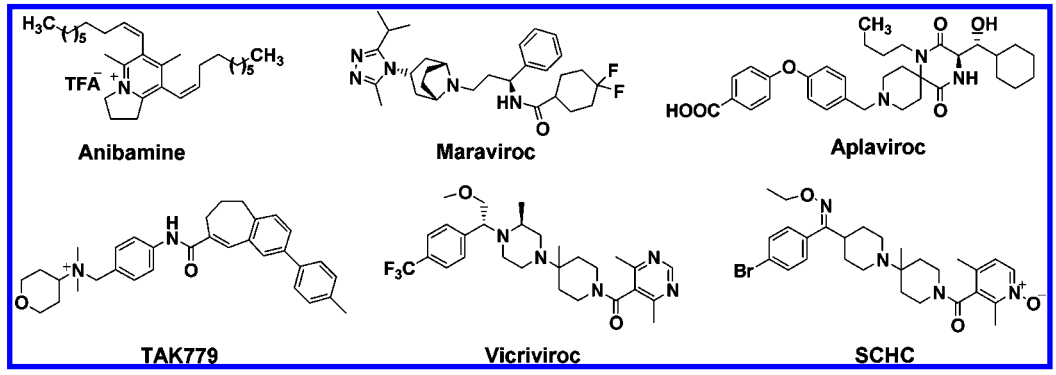

Figure 2. Chemical structures of anibamine and several known potential CCR5 antagonists.

that were developed into the antagonists illustrated in Figure 2.[17−21] Anibamine also has structural similarities to these compounds, in particular a potentially charged nitrogenic central moiety with two or more hydrophobic side chains attached. However, because of its structural novelty, we cannot dismiss the possibility that anibamine may possess a different binding modality than that proposed for the other CCR5 antagonists.[22] In the absence of experimental structural data for the CCR5 receptor, one of the few ways to investigate the binding modes of these compounds is through molecular modeling. An understanding of anibamine and its binding mode in CCR5 may assist the design of novel derivatives that are CCR5 antagonists with higher affinity. Since anibamine has a unique chemical structural skeleton with a potentially novel molecular design template, comparing its binding modality with those of other known CCR5 antagonists should be instructive regarding its activity and further validate it as a lead compound for next generation molecular design.

In general, computational and mathematical approaches, such as structural bioinformatics,[23] molecular docking,[24] molecular packing,[25] pharmacophore modeling,[26] Monte Carlo simulated annealing approach,[27] graph/diagram approach,[28] diffusion-controlled reaction simulation,[29] biomacromolecular internal collective motion simulation,[30] QSAR,[31] protein subcellular location prediction,[32] identification of membrane proteins and their types,[33] identification of enzymes and their functional classes,[34] identification of GPCR and their types,[35] identification of proteases and their types,[36] protein cleavage site prediction,[37] and signal peptide prediction[38] can provide useful information and insights for both basic research and drug design and hence are widely welcome by the science community.

More specifically, in the absence of specific structural data, molecular modeling studies that build and refine homology models of the receptor and dock the various ligands into these models are the most accessible approaches to studying binding modes for ligands. Like nearly all GPCRs, a crystal structure for the CCR5 receptor is not currently available, and a template-derived homology model has to be used to gain insight into the receptor structure. In fact, a homology modeling method based on the crystal structure of bovine rhodopsin[39] has been successfully applied to other GPCRs,[40] including the CCR5 receptor,[22] to further understand the

ligand-protein interactions and to identify new and potent ligands. For example, a homology model of Angiotensin II type 1 (AT1) receptor was used to explore the binding sites of several nonpeptide AT1 receptor antagonists,[41] and a homology model of M1 muscarinic acetylcholine receptor was applied to understand the mechanism by which the agonist-receptor complex activates the G proteins.[42] More relevant, the models of CCR5 developed by several groups helped characterize the binding mode of known CCR5 agonists and antagonists.[22] It is believed that with all the lessons learned from previous experience, homology modeling of GPCRs based on bovine rhodopsin crystal structure will help in structure-based drug design and virtual screening for therapeutic applications at many GPCR targets. The recent seminal resolution of two human β_2-adrenergic receptor (β_2AR) crystal structures[43] led us to question which crystal structure would be the more reasonable template for building the homology model of the CCR5 receptor. Both β_2AR structures have a bound inverse agonist carazolol, and presumably these structures were closer to the inactive state of β_2AR. However, the broken ionic locks in the reported structures and the increased affinity of β_2AR-T4 chimera for agonists compared to the wild type β_2AR suggest that these β_2AR crystal structures seemed closer to the active state of the β_2AR receptor or, possibly, a state between active and inactive.[44] Thus, a homology model based on the β_2AR structure would also be worth exploring for valuable information of CCR5 receptor.

In this study, the binding mode of anibamine in the antagonist locus of CCR5 has been studied by building and analyzing two complete (i.e., including both the extracellular and intracellular loops) homology models of the CCR5 receptor. As in nearly all homology models of GPCRs, one of our models was based on the crystal structure of bovine rhodopsin in the dark state (PDB code 1F88), which was, up until very recently, the only available crystal structure for a GPCR. The other homology model was constructed based on the crystal structure of β_2AR (PDB code 2RH1). The docking studies of known CCR5 antagonists into each of these two CCR5 homology models showed that the CCR5 homology model based on the bovine rhodopsin crystal structure seemed more compatible with the site-directed mutagenesis study results. The docking studies of anibamine in these two homology models showed that the anibamine binding mode shares some common binding features with other CCR5 antagonists. Interestingly, some unique intermolecular interactions between anibamine and the CCR5 receptor homology model may further direct structural modifications of anibamine as a lead for next generation CCR5 antagonist design.

RESULTS AND DISCUSSION

The computational analysis of anibamine was processed in several steps: first, we performed a conformational study of it and other known CCR5 antagonists. Next, homology models of the chemokine receptor CCR5, including transmembrane, extracellular and intracellular components, were built based on the crystal structure of bovine rhodopsin and human β_2AR, followed by molecular dynamics simulation/annealing to optimize the conformations of the models. Finally, anibamine and the other ligands were docked into these two CCR5 models, with particular emphasis on evaluating the CCR5 binding pocket interactions with anibamine and other known CCR5 antagonists.

Conformation Study of Anibamine and Other Known CCR5 Antagonists. In order to simulate the conformation of anibamine in a biological system as well as in the CCR5 binding site, a conformation study was conducted by minimization and molecular dynamics simulations. Other compounds reported to be potential CCR5 antagonists, including Aplaviroc, Maraviroc, and Vicriviroc that have entered clinical trials, were similarly analyzed. The lowest energy conformation obtained for each ligand was further simulated in a solvated system, and these results were then applied to the initial conformation to explore their binding modes in the CCR5 receptor antagonist binding locus. These conformations were only expected to provide a reasonable starting point for our docking studies; they were to endure some possibly dramatic alterations during docking in order to achieve the lowest energy conformations for the ligand−receptor complex (vide infra).

Homology Modeling of Chemokine Receptor CCR5. Chemokine receptors belong to the rhodopsin subfamily in the GPCR superfamily and are characterized by a heptapeptidic transmembrane helical fold (7-TM) spanning the plasma membrane (see Figure 1). Homology modeling studies of the CCR5 receptor, based on the crystal structures of the dark state bovine rhodopsin at 2.8 Å resolution (PDB code 1F88)[39b] and on the human β_2AR structure at 2.4 Å resolution (PDB code 2RH1),[43a] involved several steps: sequence alignment, assignment of Cartesian coordinates for amino acid residues in structurally conserved regions, assignment of coordinates for amino acid residues in structurally nonconserved regions, and refinement of coordinates of side chains of the entire protein.

The initial, and likely most essential, step in homology modeling is sequence alignment among the CCR5, the bovine rhodopsin, and the human β_2AR. This was performed for the entire protein sequences by using the InsightII/Homology module (Figure 3). In order to confirm the alignment automatically generated by the software, manual alignment of the amino acid residues was also performed by comparing the chemical structures of the autoaligned residues. While the entire protein sequences homology level was approximately 20% among all three proteins, their transmembrane domains gave 58% homology identity between the bovine rhodopsin and CCR5 while 56% between human β_2AR and CCR5 (Table 1). This further validated applying these two crystal structures as templates to construct homology models of the CCR5 receptor. It is important to note that CCR5 receptor's sequence contains all of the conserved amino acid residues found in most GPCRs, and this provides an important guideline for sequence alignment.

The overlapping amino acid sequences between rhodopsin, β_2AR, and the CCR5 receptor, i.e., structurally conserved regions, were divided into different lengths separated by gaps (most of which are in the loop domains). The coordinates for the amino acid residues in these regions of rhodopsin or β_2AR were assigned to the corresponding residues in the CCR5 receptor.

The remaining amino acid residues of the CCR5 receptor constitute the structurally nonconserved regions, while most of them are in the extracellular and intracellular loop regions.

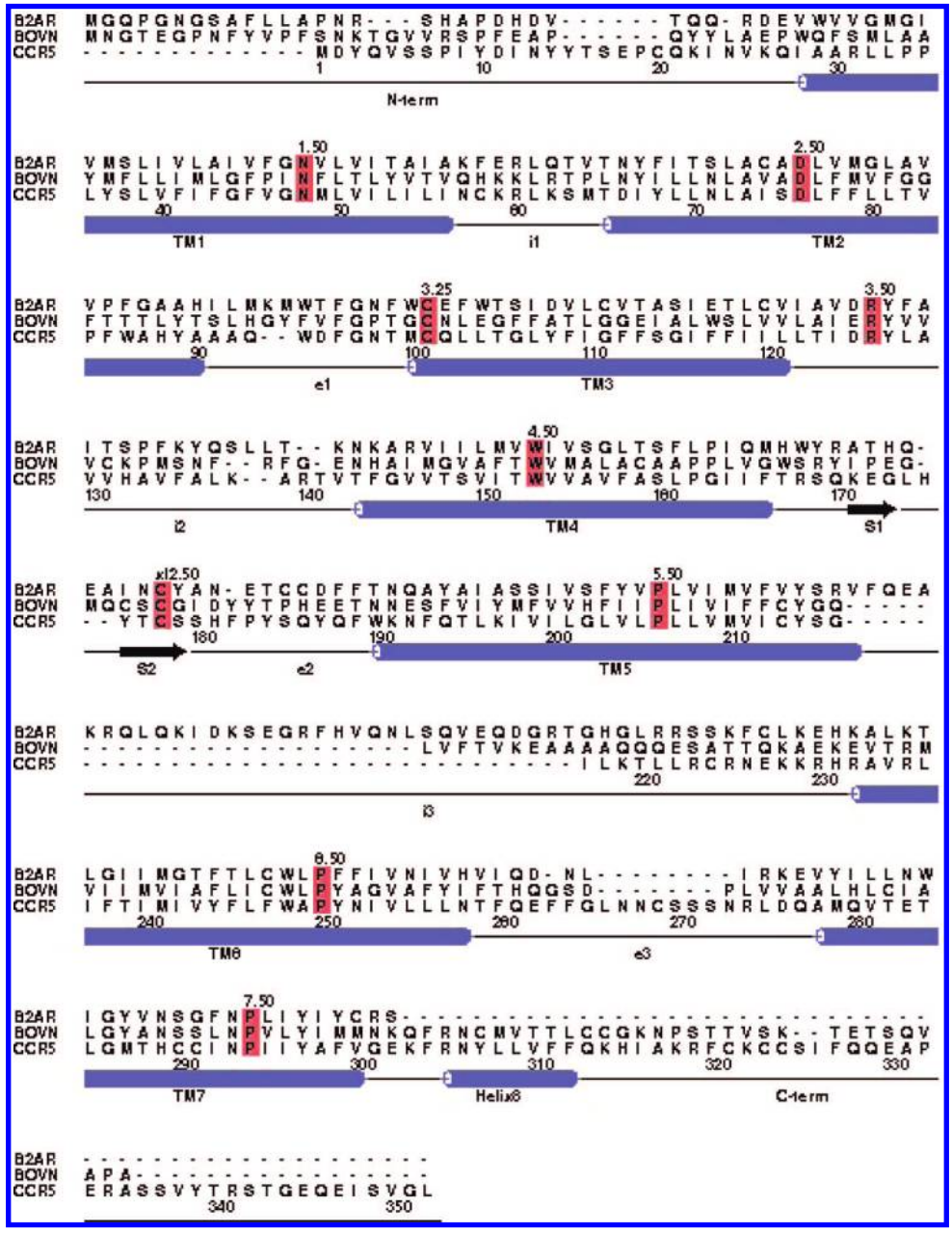

Figure 3. Sequence alignment of human CCR5 (CCR5), bovine rhodopsin (BOVN) (PDB code 1F88), and human β_2AR (B2AR) (PDB code 2RH1). The Ballesteros-Weinstein numbering system was adopted to mark all the conserved amino acid residues among most of the GPCRs and colored in red. The CCR5 protein was numbered accordingly under its sequence. The secondary structure of the CCR5 receptor 3D conformation based on bovine rhodopsin crystal structure was marked out below the sequence.

Table 1. Sequence Homology Analysis of the CCR5 Receptor, Bovine Rhodopsin, and the β_2-Adrenergic Receptor

	CCR5						
homology level %	TM1	TM2	TM3	TM4	TM5	TM6	TM7
bovine rhodopsin (1F88)	53	63	61	57	65	58	43
β_2-adrenergic receptor (2RH1)	50	50	61	52	65	58	57

The coordinates for residues in these regions were obtained by searching for these sequences in the PDB. The adopted conformations of these nonconserved regions all have close similarity to the backbone orientation of the corresponding regions in rhodopsin or β_2AR.

The side chain conformational energies for the entire protein were minimized, while the backbone was constrained followed by a vigorous dynamics simulation to reach an energetically reasonable conformation. Then the local geometry of energy-minimized structure was checked using the InsightII/Homology program. All amino acid residues have reasonable bond lengths and bond angles. The analysis of φ, Ψ, χ_1, and χ_2 angles of the resulting protein conformations was further conducted with Procheck 4.1,[45] and the results were shown in Figure 4 and Table 2. While the model generated based on the β_2AR template has better parameters that that using bovine rhodopsin template, it mainly is because of the parameter difference from the two templates, rather than the modeling operation (see the Supporting Information).

The most significant difference between the crystal structures of bovine rhodopsin and β_2AR is the short α-helical structure of β_2AR in the EL2 loop compared to

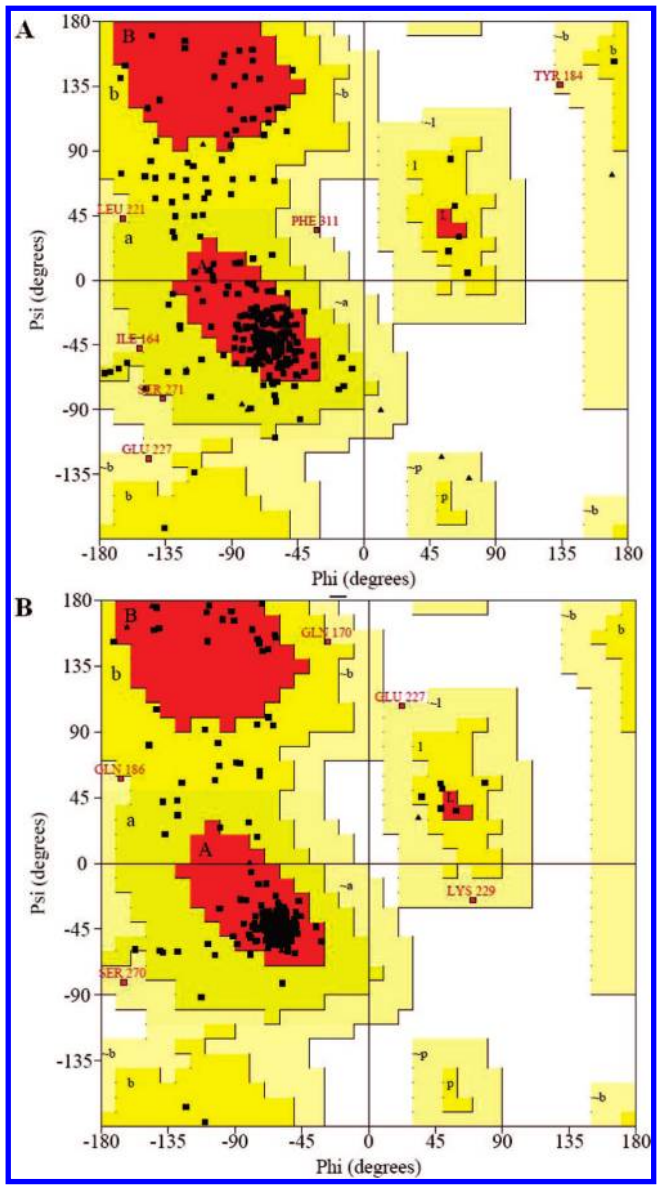

Figure 4. Ramachandran plots of A) rhodopsin-based and B) β_2AR-based CCR5 receptor homology models.

Table 2. Data from the Ramachandran Plot of the Rhodopsin- and β_2-Adrenergic Receptor-Based CCR5 Homology Models

	MFR[a]		AAR[b]		GAR[c]			number of	
model	n	%	n	%	n	%	subtotal[d]	Gly and Pro	total[e]
Rho-based	184	69.7	74	28.0	6	2.3	264	24	288
β_2AR-based	221	83.7	38	14.4	5	1.9	264	24	288

[a] The most favored regions (A, B, and L). [b] The additional allowed regions (a, b, l, and p). [c] The generously allowed regions (-a, -b, -l, and -p). [d] The total number of residues evaluated except glycines and prolines. [e] The total number of amino acids in the receptor models.

the β-sheet structure in bovine rhodopsin. For most GPCRs, the EL2 loop part is very important for ligand recognition with their receptor.[46−54] Thus, we were concerned if an α-helix structure in the EL2 of CCR5 receptor is a reasonable conformation. This possibility (forming an α-helix in the EL2 loop of the CCR5 receptor) was checked with nnpredict (available online from the Cohen group at Department of Cellular and Molecular Pharmacology, University of California, San Francisco) by predicting the secondary structure

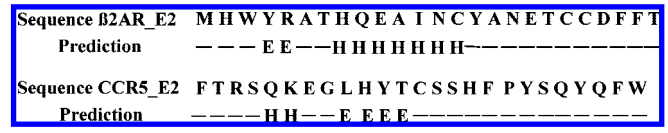

Figure 5. β_2AR and CCR5 EL2 secondary structure prediction (H = helix, E = strand, - = no prediction).

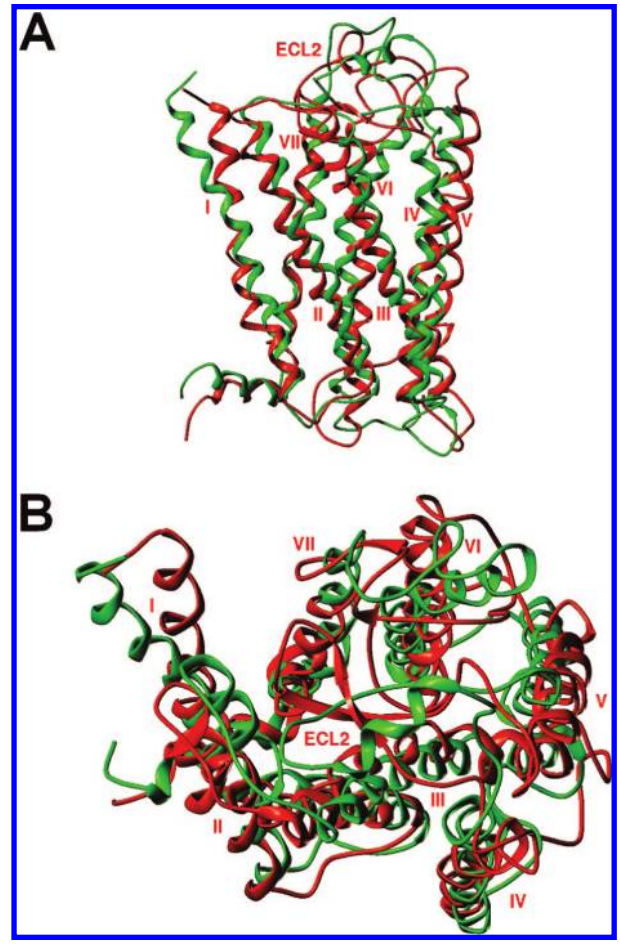

Figure 6. Homology models of CCR5 overlapped in ribbon. The model based on bovine rhodopsin crystal structure is in red, while the one based on β_2AR is in green.

of the EL2 loop. Comparing the nnpredict results for the β_2AR EL2 loop with that of the CCR5 EL2 loop suggests a much lower probability of forming an α-helix structure in CCR5 (Figure 5). Therefore, this domain in the homology model of CCR5 constructed from the β_2AR template may need to be further scrutinized, e.g., by site-directed mutagenesis data.

The differences between the two models were assessed (Figure 6). The overall weighted Root Mean Square Distance (rmsd) value between them was 5.79 Å. In contrast to the rhodopsin-derived model, the TM1 domain of the β_2AR-derived model has a more straight extension. The TM2 to TM7 domains of the two models basically adopted fairly similar orientations. As expected from the discussion above, the EL2 domains were significantly different; i.e., the rhodopsin-derived model presented a β-sheet, while the β_2AR-derived model presented an α-helix conformation. In addition to these differences that largely resulted from their parent templates, different orientations of amino acid residues in these two models transmembrane regions were also observed. These differences may result in each specific binding pocket having different spatial distributions of

Table 3. Top GoldScores for Anibamine and Three Other Known Antagonists Docked in Both Homology Models of CCR5

fitness	anibamine	Aplaviroc	Maraviroc	Vicriviroc
model based on bovine rhodopsin (1F88)	51.57	66.71	54.01	48.83
model based on β_2-adrenergic receptor (2RH1)	50.53	47.64	49.22	43.61

hydrophilic and hydrophobic regions. This will be discussed with the docking study results below.

Binding Site Study of Anibamine As an Antagonist to CCR5. The lowest energy conformation of anibamine and the other antagonists (Aplaviroc, Vicriviroc, and Maraviroc) from the conformation study were first docked interactively into the antagonist binding locus of CCR5 at the upper part of the transmembrane region of the receptor followed by minimization and dynamics simulation of the resulting CCR5-ligand complex. This operation was a necessity because the preliminary automated docking studies revealed no reasonable binding loci in either model due to the stringently steric arrangement of the amino acid residues in the potential binding pocket. By conducting the interactive docking, the ligand binding locus in the receptor was thus identified for the following automated docking study. The orientation of the molecule skeleton in the binding locus was directed by the following: first, the putative ionic interaction between the tertiary nitrogenic group in the ligand and the carboxylic group of Glu283 on TM helix 7 in the receptor; and second, facing the hydrophobic side chain portion of the ligand toward the hydrophobic TM helices. The dockings were validated with a short minimization (500 iterations) and dynamics simulation (1000 steps, 1000 fs total) followed by a more rigorous optimization (minimization of 5000 iterations and a dynamics simulation of 100,000 steps, 100 ps total). In both processes, the backbone of the receptor was fixed to prevent the disruption of the receptor α-helical bundle. The lowest energy conformation from the latter simulation was extracted and saved for analysis.

The automated docking study was conducted by using GOLD 3.1[55] (Genetic Optimization for Ligand Docking) with most default parameters. GOLD permits full ligand flexibility and partial protein flexibility (side chain and backbone flexibility for up to ten user-defined residues). The protein model used for docking each ligand was the lowest energy conformation resulting from the interactive docking studies. The oxygen atom OE1 in Glu283 defined a 10 Å active site radius in the CCR5 receptor. A number of trials were conducted, and the GOLDScores from each docking trial were recorded. The top ten solutions from the final trial were saved. The ligand−receptor complex from the docking result with the highest GOLDScore was extracted (Table 3). It seemed that there was no difference in the binding of anibamine in both models from the viewpoint of GOLDScores, while all other three ligands tended to bind better with relatively higher GOLDScores in the homology model of CCR5 based on bovine rhodopsin than the one based on β_2AR.

The final conformations (from the GOLD docking) of the receptor−ligand complexes are illustrated in Figure 7, and the major amino acid residues in the binding sites of the eight complexes are summarized in Table 4. Analyses of the binding pockets of anibamine and the other antagonists revealed that all the antagonists bound to similar hydrophobic pockets in the CCR5 models, while they may occupy different subloci leading to the differences in GOLDScores. For all four ligands studies, the binding pockets in both models were built mainly by TM2, TM3, TM5, TM6, and TM7. Note that residue Glu283 on TM7 is shared by all four antagonists in both models, which is in concordance with the existence and importance of a potentially positively charged core in the ligands.

As an amphiphilic molecule, anibamine carries a positive charge and two highly hydrophobic side chains. Thus, it is not surprising that its binding pocket is composed mainly of aliphatic hydrophobic amino acid residues in both models, while the positively charged nitrogen atom is in the vicinity of Glu283 to form a putative salt bridge. The GOLDScores for each model are not significantly different (Table 3). It should be noted that we do not observe significant interactions between anibamine and the extracellular loop II (EL2) of the protein although Ser179 and Ser180 from EL2 were in the vicinity of the binding locus of anibamine in both homology models of CCR5. Since this loop is believed to be involved in the binding of gp120 to the receptor,[46−51] this observation may explain why anibamine's inhibition affinity to CCR5 was not particularly outstanding. While in the rhodopsin-derived model (Figure 7A,C,E,G) Tyr108 seemed to play important roles in the binding of all four ligands, for anibamine it probably only contributed to the hydrophobic interactions between the ligand and the receptor. Residue Tyr251 seemed to be important to the binding of both anibamine and Aplaviroc. Some amino acid residues were unique for only anibamine; e.g., His289 not only interacted with one of the long aliphatic chains in the ligand molecule but also might confer cation-pi and/or pi-pi stacking interaction with the positively charged core ring system (as His289 was observed in the vicinity). In the β_2AR-derived model (Figure 7B), the two long aliphatic side chains of anibamine adopted a completely different orientation than they do in the rhodopsin-derived model, which lead to a different set of amino acid residues in the binding pocket. Thus, instead of Phe112, Phe109 and Ile164 were part of the hydrophobic pocket. Also, Trp190 and Met279 appeared to play some role in the recognition of anibamine by the CCR5 antagonist binding loci, while His289 was not observed in the binding pocket. Apparently the significant difference from the orientation of the two aliphatic side chains in the molecule provided useful information for future validation of the binding modes as well as for the anibamine derivative design.

In agreement with a very comprehensive site-directed mutagenesis study,[22e] Aplaviroc showed very strong interactions with ECL2 in the rhodopsin-derived model (Figure 7C). For example, Ser180 on EL2 may form hydrogen bond interaction with the molecule. In agreement with the same report, residues Tyr108, Phe112, Tyr251, and Glu283 were all observed in the binding pocket of Aplaviroc, while Asn252 seemed to contribute uniquely to the binding of the molecule. Besides, an ionic or hydrogen bonding interaction between the carboxylic acid moiety of the molecule and Lys197 was also observed in this model. On the other hand, we did not observe a similar interaction between this acidic

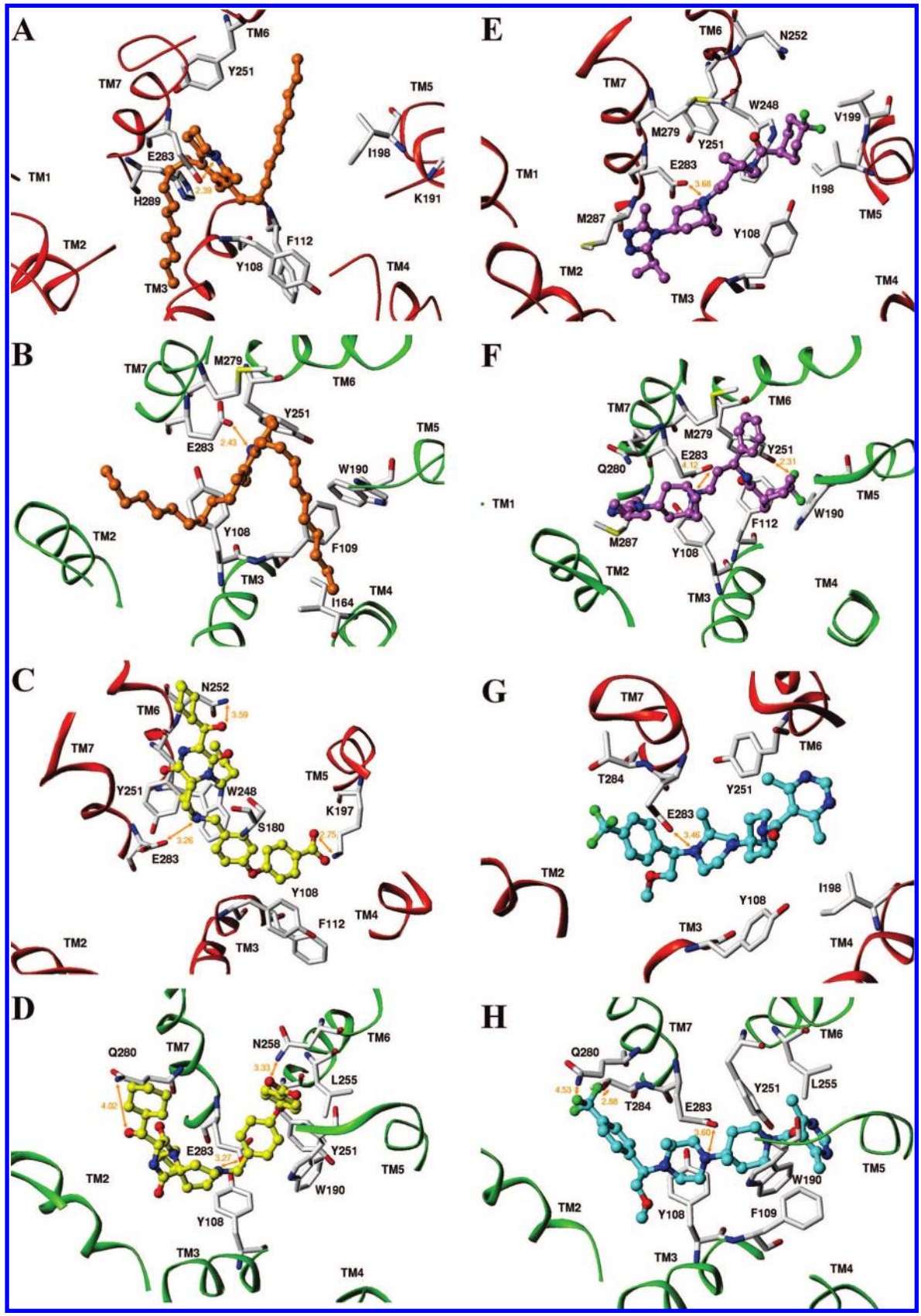

Figure 7. Binding modes of the CCR5 antagonists in the homology model of CCR5 constructed based on bovine rhodopsin (red) and human β_2AR (green). The amino acid residues of the binding pockets were as shown in capped sticks and the ligands were in ball and stick, while the receptor models were in ribbon. ELs and part of TMs were omitted for clarity. Oxygen atoms were in red, fluorine in green, and nitrogen in deep blue. Carbon atoms and the bonds were colored according to the ligands. A and B, anibamine (orange); C and D, Aplaviroc (yellow); E and F, Maraviroc (purple); and G and H, Vicriviroc (cyan).

moiety and Lys191 that was proposed in the literature, while Lys197 seemed to be less important in the same report.[22e]

In the β_2AR-derived CCR5 model (Figure 7D), the binding pocket for Aplaviroc was formed by a totally different set

Table 4. Major Amino Acid Residues in the Binding Pockets of CCR5 Antagonists in the Two Homology Models of the Receptor[a]

	model based on bovine rhodopsin (1F88)	model based on β_2-adrenergic receptor (2RH1)
anibamine	E283, Y251, H289, I198, Y108, F112	E283, Y251, M279, W190, Y108, F109, I164
Aplaviroc	**E283**, ***Y251***, **S180**, **K197**, ***Y108***, **F112,** W248, N252	**E283**, ***Y251***, ***Y108***, Q280, N258, L255, *W190*
Maraviroc	**E283**, **Y251**, **I198**, **Y108**, M279, N252, V199, W248, *M287*	**E283**, **Y251**, **Y108**, M279, Q280, W190, F112, *M287*
Vicriviroc	**E283**, **Y251**, **Y108**, **I198,** T284	**E283**, **Y251**, **Y108,** L255, T284, Q280, W190, *F109*

[a] The residues in **Bold** are consistent with the site-directed mutagenesis report. The residues in *Italics* are in contrary to the site-directed mutagenesis report. The residues in ***Bold/Italics*** are the ones that were reported controversially in the site-directed mutagenesis reports.

of amino acid residues, including Gln280, Asn258, Leu255, Trp190, Tyr251, and Tyr108, with Glu283 as the only exception. Among these residues, Gln280, Asn258, and Leu255 have not been tested in any site-directed mutagenesis studies, while Trp190, Tyr251, and Tyr108 were deemed not essential for ligand binding to CCR5 in another report.[22f] Besides, Ser180 was not observed in the binding locus since EL2 was not involved in the binding pocket formation in this model. This may explain the lower GOLDScore of Aplaviroc in the β_2AR-derived CCR5 model, while plausible hydrogen bonding between Ser180 and the Aplaviroc molecule may contribute significantly in the rhodopsin-derived model.

Maraviroc showed weak interactions with the EL2 region in the rhodopsin-derived model (Figure 7E). Similar to Vicriviroc, Glu283, Tyr108, and Ile198 were important in the binding pocket of Maraviroc in this model, and this was also supported by site-directed mutagenesis studies.[22f] Also, Tyr251 was part of the binding locus in this model, again as supported by site-directed mutagenesis.[22f] Moreover, Trp248 was in the vicinity of the ligand molecule in this model, while it was not supported by the same literature. In the β_2AR-derived CCR5 model (Figure 7F), the binding pocket for Maraviroc showed some similarity to the model based on rhodopsin, e.g. the presence of Tyr251 and Tyr108 in the binding locus. In addition, the involvement of Phe112 and Trp190 has not been tested, while the involvement of Met287 in the binding locus was not supported by site-directed mutagenesis.[22f] While the Goldscores in the two models did not show significant difference, the top score (54.01) for the rhodospin based model was slightly higher than that for the β_2AR based model. This might come from the weak interaction with the EL2 region in the rhodospin based model, which was not observed in the β_2AR based one.

As in anibamine, our rhodopsin-derived model did not indicate strong interactions between Vicriviroc and the EL2 of CCR5, while Glu283, Tyr108, and Ile198 played important roles in the binding pocket (Figure 7G) in agreement with recent site-directed mutagenesis results.[22f] In addition, Tyr251, described as an important residue in the binding pocket of Vicriviroc in the same report, also had significant interactions with the ligand in our model. Hydrophobic interactions of Thr284 with the ligand may be significant based on our observations although this has not been examined experimentally. In the β_2AR-derived CCR5 model (Figure 7H), the binding pocket for Vicriviroc shared some features with the rhodopsin-derived model with some differences arising from variations in orientation of the amino acid residues and the ligand. Again, the model indicates involvement of Tyr251 and Tyr108 with binding, as verified by site-directed mutagenesis.[22f] However, our observation of Phe109 in the binding pocket was contrary to the mutagenesis data. Similarly, the roles of Gln280, Trp190, Thr284, and Leu255 have not, as of yet, been verified. While the major component in the Goldscores was the van der Waals interactions between the ligand and the receptor, no apparent difference was observed for either model.

CONCLUSION

Two homology models of chemokine receptor CCR5 were constructed based on the crystal structures of bovine rhodopsin and the human β_2-adrenergic receptor, respectively. Anibamine, the first natural product CCR5 antagonist, along with other three well-known CCR5 antagonists were docked into these two models. The binding pockets for these ligands were characterized and compared with the reported site-directed mutagenesis studies. Our modeling studies indicate that the binding pockets for all four CCR5 antagonists are located in the transmembrane (TM) part of the protein near Glu283. This position allows a putative ionic interaction with the quaternary nitrogen that is a common feature of the CCR5 antagonists. While some ligands showed a very strong interaction with extracellular loop 2 in the rhodopsin template-based model, others did not, which may directly influence their GOLDScores (binding fitness). In both the rhodopsin-derived model and the β_2AR-derived model, all four ligands tended to adopt a similar binding locus, while for each ligand, the binding modes and orientations were somewhat different between these two models. This is certainly related to the different 3D conformations of the TM domains and orientations of the amino acid side chains in the crystal structure template the models were based on. Most importantly for our future work is that anibamine shares many common binding features with the other known CCR5 ligands in each model, thus suggesting that its inhibition activity may stem from binding to the CCR5 antagonist binding locus.

While from our studies, both the crystal structures of bovine rhodopsin and human β_2AR seemed to be reasonable templates to conduct homology modeling operation for CCR5 receptor, the rhodopsin-derived model received more support from the reported site-directed mutagenesis studies for the known CCR5 antagonists, while the binding pockets for those ligands identified from the β_2AR-derived model need to be further verified by more extensive experimental studies.

Anibamine is an interesting lead compound for anti-HIV drug discovery. It is the first and only natural product CCR5 antagonist identified such far, and because of its unique skeleton, the recently reported flexible total synthesis of anibamine opens up a number of research directions. In particular, the pursuing of the next generation molecular design based on the unique binding features of anibamine may yield a clinically relevant CCR5 antagonist. The binding

mode study of anibamine in CCR5 homology models reported herein will be beneficial to the future study in novel drug design and development.

COMPUTATIONAL METHODS

The sequence of human chemokine receptor CCR5 was obtained from http://www.expasy.org (Swiss-Prot and TrEMBL), and the crystal structures of bovine rhodopsin and human β_2AR were downloaded from http://www.rcsb.org. All computations were performed on SGI Octane2 workstations (Silicon Graphics, Inc., Mountain View, CA).

Homology modeling was carried out using the Homology and Discover modules of the InsightII package (Accelrys, San Diego, CA). The sequence alignment was conducted automatically by applying different scoring matrices, gap penalty, and gap length penalty parameters in order to achieve the highest sequence homology. Then the coordinates for the amino acid residues in the structurally conserved regions of CCR5 were assigned following the corresponding residues in the CCR5 receptor. The coordinates for the amino acid residues of the CCR5 receptor in the structurally nonconserved regions were obtained by searching the protein database with the InsightII/Homology/Loop_Search module. After the completion of the amino acid residue coordinates assignment, the conformation of the whole protein was minimized with the backbone constrained. A brief minimization (2,000 iterations) and dynamics simulation (10,000 step, 1 fs each step) was conducted to relax major steric clashes introduced during the coordinate assignment. Further vigorous simulation (minimization 10,000 iterations and dynamics 100,000 steps, 100 ps total) was conducted to produce an energetically reasonable conformation. The CFF91 force field was applied, while the minimization was terminated until the maximum derivative is less than 0.001 kcal/mol.

All the small (ligand) molecules were built using InsightII/Builder Module. Minimizations with steepest descent followed by conjugate gradient were performed to generate the lowest energy conformation for each ligand studied (CFF91 force field and default termination values were adopted). Then a molecular dynamics simulation was performed (an equilibration phase of 1000 fs at 300 K, followed by a collection phase of 5000 fs at the same temperature) to further study the small molecule conformation. We used the lowest energy conformation of the small molecules in water extracted from the 5 ps molecular dynamics calculations, as the initial configuration for docking into the proposed binding site of the CCR5 receptor. The ligand was modeled in its nitrogen-protonated form when applicable.

The interactive docking study was conducted in the Discover module of InsightII. The ligands were docked in the upper portion of transmembrane region of the receptor, following orientations reported in the literature.[22] Each ligand−receptor complex was minimized in the gas phase first with the backbone of the receptor fixed, but all the side chain atoms were left unconstrained. The optimized conformation was then used as the initial configuration for molecular dynamics simulations. For each complex, a short-term steepest descent energy minimization (500 iterations) and a dynamics simulation (1000 step, 1 fs each step) was conducted to validate the initial docking conformation. This was followed by another simulation minimization (5000 iterations), and 100,000 steps of dynamics were conducted with an initial 2000 steps equilibration. The total simulation time was 102 ps. Again the CFF91 force field and default termination value (0.01 kcal/mol) was adopted.

Automated docking was conducted with GOLD.[55] Default parameters were adopted except that the docking locus was defined as 10 Å around the OE1 of Glu283 on helix 7 of CCR5. Based on the fitness scores and binding orientation of the ligands, the best solution of GOLD binding (among top ten solutions that were recorded) was selected and merged into the structure of CCR5 receptor. The combined structures of the ligand and CCR5 receptor were minimized together in order to allowing the receptor to adopt the presence of ligand. After that, the ligand was removed from the complex, and the resulting structure was evaluated by PROCHECK again. The docking results were summarized in Table 3. Finally, all the models were optimized by molecular dynamics simulation, using SYBYL7.3 with default parameters except for the following: length - 100,000 fs, snapshot every 25 fs; time step - 1 fs, dielectric constant 4.0.

The PDB files of the homology models are available from the corresponding author upon request.

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Supporting Information Available: Goldscore summary: the top ten scores from the last trial in the automatic docking for all four ligands and the Ramachandran plots of bovine rhodopsin and β_2AR crystal structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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