DOI: 10.1002/cmdc.200800074

Multi-Receptor Binding Profile of Clozapine and Olanzapine: A Structural Study Based on the New β_2 Adrenergic Receptor Template

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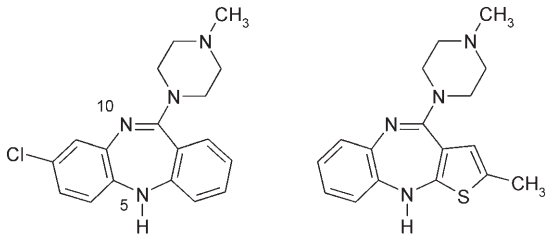
Schizophrenia is a devastating mental disorder that has a large impact on the quality of life for those who are afflicted and is very costly for families and society.[1] Although the etiology of schizophrenia is still unknown and no cure has yet been found, it is treatable, and pharmacological therapy often produces satisfactory results. Among the various antipsychotic drugs in use, clozapine is widely recognized as one of the most clinically effective agents, even if it elicits significant side effects such as metabolic disorders and agranulocytosis. Clozapine and the closely related compound olanzapine are good examples of drugs with a complex multi-receptor profile;[2] they have affinities toward serotonin, dopamine, α adrenergic, muscarinic, and histamine receptors, among others.



Experimental evidence suggests that a complex binding profile is linked to the clinical efficacy of antipsychotic drugs, and indeed, some of the latest efforts in the development of novel antipsychotic drugs[3] are aimed at obtaining compounds with clozapine-like binding affinities for a certain number of receptors: D_2, D_3, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6. Unfortunately, our current understanding of which receptors are relevant for the clinical efficacy of antipsychotic agents is based only on the study of a handful of drugs. At this stage, a clear discrimination between clinically useful receptors and those responsible for adverse effects is not possible, as this would require a more thorough understanding of subtle modulating effects, and this is still obscure. Even if the ideal multi-receptor binding profile was known, the problem of how to obtain ligands with such binding specificity would still remain open. A good starting point is to improve our understanding of the structural features associated with binding profiles, thus leading to clinically useful drugs.

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Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.

In this work, we made use of the recently reported structure of the human β_2 adrenergic receptor as a template to build models for a set of receptors that are putatively important for the pharmacological properties of antipsychotic drugs. The aim of this study is to identify characteristics of the complexes of such receptors with clozapine and olanzapine that can explain the excellent clinical behavior of these two drugs. Remarkably, docking studies with homology models based on the new template reveal a binding complex that is different from previously reported complexes for clozapine-like ligands.[4,5] In the first step, we studied the binding affinities of both olanzapine and clozapine for all the receptors in order to identify their common binding profile and the structural features associated with this profile. We then studied the structural differences between the drug–receptor complexes for both antipsychotic drugs that could be responsible for the observed differences in their pharmacological behavior.

For this, structural models for a set of 14 receptors that are potentially involved in the pharmacological profile of antipsychotic drugs (Table 1) were generated by homology modeling, using the recently reported structure of the human β_2 adrenergic receptor (PDB code: 2RH1)[6,7] as a template (Figure 1 a). This new structure presents many advantages over the structure of bovine rhodopsin (PDB code: 1F88),[8] the only structural template available until recently for homology mod-

Table 1. Monoaminergic receptors studied in this work.

Receptor	Rhodopsin homology[a]	β_2 Adrenergic homology[a]	Res. 3.36	pK_i olanzapine[13]	pK_i clozapine[13]
5-HT_{2A}	10.7	60.7	S	8.8	8.3
5-HT_{2B}	10.7	57.1	S	8.2	8.5
5-HT_{2C}	10.7	60.7	S	8.3	8.1
M_1	17.8	25	S	8.0	8.2
M_4	17.8	25	S	7.9	7.9
H_1	14.2	46.4	S	8.2	8.1
5-HT_{1A}	17.8	53.5	C	5.0[b]	7.0
5-HT_6	14.2	60.7	C	8.1	8.1
5-HT_7	10.7	53.5	C	7.1	7.7
D_2[c]	14.2	57.1	C	7.7	6.9
D_3	14.2	53.5	C	7.7	7.0
D_4[d]	14.2	53.5	C	7.7	7.4
α_1	14.2	57.1	C	7.6	8.0
α_2	14.2	50	C	6.5 (6.55)[e]	7.1 (7.82)[e]

[a] Percentage of pairwise sequence identity for the binding site residues (4.5 Å radius). [b] Inactive compound; a value of 5.0 was arbitrarily assigned for the computation of the Student *t* test and the box plot representation shown in Figure 3. [c] Binding data obtained for the D_{2s} variant (short form) of the D_2 receptor. [d] Binding data obtained for the $D_{4.4}$ variant of the D_4 receptor. [e] Binding data obtained for the rat receptor, which differs in primary sequence from the human isoform in the active site; in brackets: binding data for the human receptor taken from the PDSP database.[14]

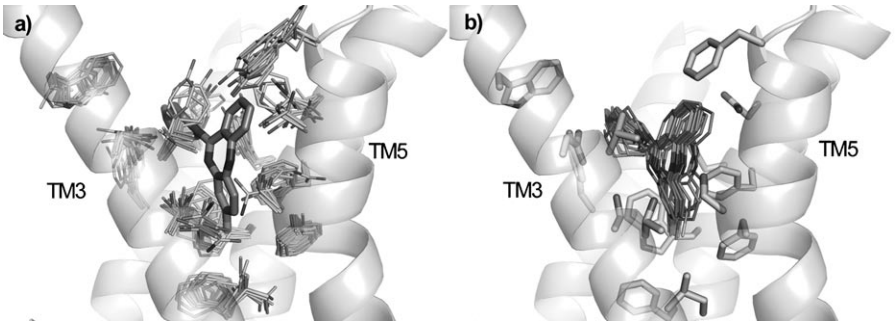

Figure 1. a) Superimposition of the 14 GPCR homology models with a consistent arrangement of the side chains lining the binding site. b) Superimposition of the conserved docking positions of clozapine obtained for the 14 receptor–ligand complexes.

eling of G-protein-coupled receptors (GPCRs). In the first place, the sequence homology of the studied receptors with the new template is much higher than with bovine rhodopsin. As listed in Table 1, for most of the receptors considered, the sequence identity within the binding site is as high as 60% and more than fourfold higher than the identity with bovine rhodopsin. Secondly, the newly available structure contains a noncovalently bound ligand, carazolol, which bears structural similarity to antipsychotic drugs. Because the aim of this study is to obtain structural models suitable for comparing the binding sites of diverse receptors, we developed a modeling protocol particularly oriented to produce consistent results for all the receptors. An initial inspection of the binding site structures obtained for the receptor set allows identification of some common structural features (Figure 2): 1) the well-known aspartic acid residue at position 3.32 (D3.32), essential for agonist and antagonist binding; 2) conserved hydrophilic regions in TM3 and TM5; and 3) hydrophobic regions such as the aromatic cluster in TM5 and TM6 (probably involved in the receptor activation process) and the aliphatic residues in TM1 and TM2. Among all these regions, the conserved hydrophilic regions of TM3 and TM5 show high sequence variability, suggesting their preeminent role in binding selectivity. Indeed, position C/S3.36 in TM3 was previously described as important for modulating ligand binding affinity.[9] The diversity present at TM5 positions 5.42, 5.43, and 5.46 was also suggested to play a major role in binding selectivity toward the aromatic moieties of endogenous ligands (phenol, catechol, imidazole, indole).[10] Furthermore, mutagenesis data corroborate that position 6.55 is involved in altering agonist/antagonist binding affinity.[11,12]

Table 1 lists experimentally measured binding affinities of clozapine and olanzapine for the receptors studied.[13,14] A close inspection of these affinity values shows that they are significantly higher for the receptors with a serine residue at position 3.36 than those with cysteine at the same position (Student *t* test, $p < 0.001$), as shown in Figure 3. For clozapine, the average decrease in affinity associated with the S3.36C substitution is 0.8 log units, whereas this decrease is 1.0 for olanzapine. A particularly relevant receptor couple belonging to these families is the 5-HT_{2A} (S3.36 family) and D_2 (C3.36 family) couple. The ratio between the binding affinities for both receptors (5-HT_{2A}/D_2 pK_i ratio, called the Meltzer index) takes values consistently greater than 1.12 as those of 'atypical' antipsychotic drugs (such as clozapine and olanzapine); this has become such a stable characteristic that its use has been suggested as a criterion for the classification of antipsychotic drugs as 'typical' or 'atypical'.[15] Interestingly, the binding sites of D_2 and 5-HT_{2A} are highly conserved, and only three positions show variability: 3.36 (C/S), 5.42 (G/S), and 6.55 (H/N) (Figure 2). The docking of clozapine and olanzapine into the binding sites of our D_2 and 5-HT_{2A} receptor models results in a ligand arrangement very similar to the co-crystallized inverse agonist carazolol present in the β_2 adrenergic receptor template and different from previously reported complexes with similar compounds.[4,5] The main differences observed in the binding position of our complexes seem to be a consequence of the slightly narrower binding site of the new template (PDB code: 2RH1) produced by the displacement of TM5 toward the receptor's main axis.

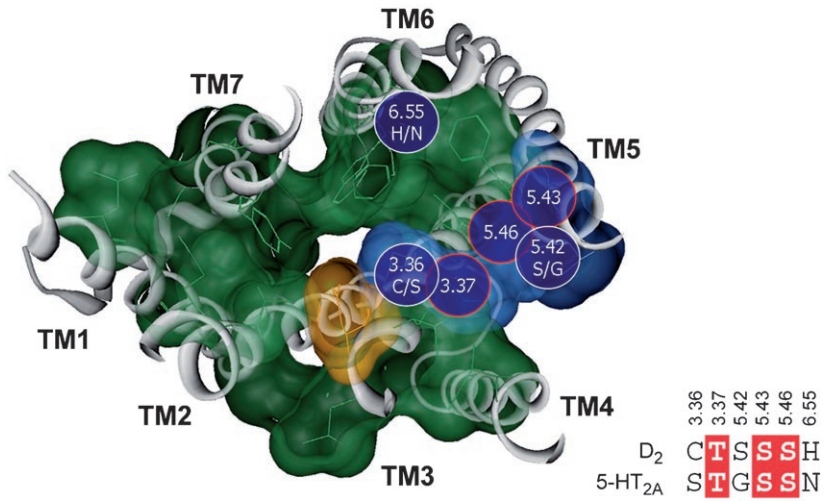

Figure 2. Homologous residues in the binding site of the studied receptor set (at left): conserved D3.32 in TM3 (orange); hydrophobic regions (green): aromatic cluster in TM5, TM6, and aliphatic residues in TM1, TM2; hydrophilic regions (blue): positions 3.36 and 3.37 (TM3), and 5.42, 5.43, and 5.46 (TM5). Sequence alignment of the putative binding site of 5-HT_{2A} and D_2 receptors (at right): conserved residues are highlighted in red.

The fused tricyclic system of the ligands adopts a position

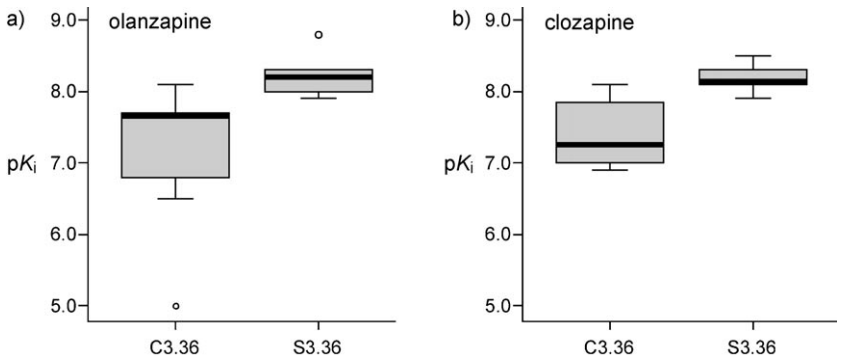

Figure 3. Box plot summarizing the binding affinities of a) olanzapine and b) clozapine for receptors with C3.36 (D_2, D_3, D_4, 5-HT_{1A}, 5-HT_6, 5-HT_7, α_1, and α_2) and for receptors with S3.36 (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, M_1, M_4, and H_1). For both compounds, the binding affinities are higher for the S3.36 family of receptors than for the C3.36 receptors.

perpendicular to the plane of the membrane, and the position of the piperazine ring is nearly parallel (Figure 4). The following key interactions were found for both the D_2 and 5-HT_{2A} receptors: 1) a salt bridge between the protonated ligand nitrogen atom and D3.32, 2) a hydrophobic sandwich of the ligand between F6.52 and V3.33, 3) a hydrogen bond between N5 and S5.46, and 4) an aromatic interaction (edge to face) between the tricyclic system and W6.48. All of these interactions are in agreement with site-directed mutagenesis data.[9,16–21] In the modeled structures, one of the major differences between the binding of clozapine and olanzapine with the 5-HT_{2A} and D_2 receptors is the interaction with the residue at position 3.36. In the 5-HT_{2A} receptor the cyclic nitrogen atom (N10) of clozapine and olanzapine forms a strong H bond with serine 3.36, which is replaced by cysteine 3.36 in the D_2 receptor, producing a much weaker H bond as supported by previous studies.[22] This finding is in agreement with the experimental binding affinities, which are higher for 5-HT_{2A} than for D_2, suggesting that the missing or weaker H bond between C3.36 and the ligand is responsible for the observed lower affinity toward D_2 than for 5-HT_{2A}. This supports the validity of the proposed structures for the ligand–receptor complexes. Moreover, provided that the clozapine and olanzapine binding positions are conserved in the entire receptor set, as supported by the docking studies (Figure 1 b), the effect of the aforementioned H bond between the ligand and the residue at position 3.36 can be extended to other receptors and not only to 5-HT_{2A} and D_2 (Table 1), thus helping to explain the multi-receptor profile of clozapine and olanzapine.

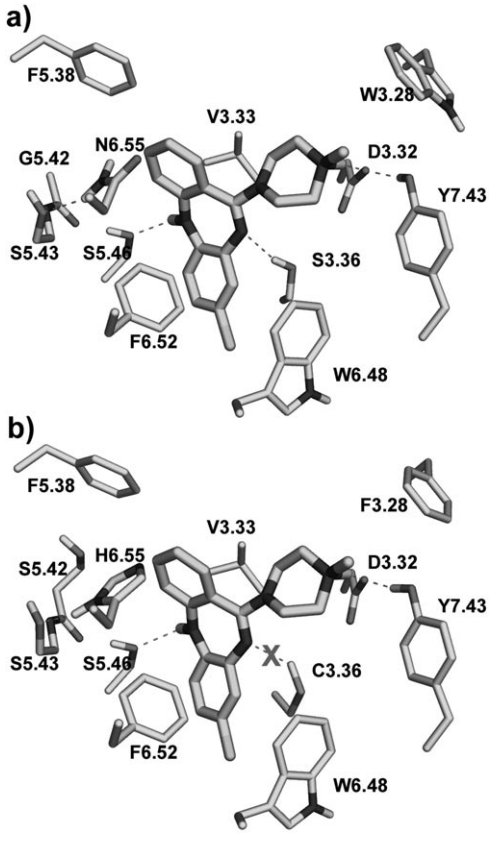

Figure 4. a) Clozapine–5-HT_{2A} receptor complex and b) clozapine–D_2 receptor complex, for which 'X' highlights the weaker or missing hydrogen bond to C3.36. Key interactions: 1) a salt bridge between the protonated nitrogen atom of clozapine with D3.32, 2) a hydrophobic sandwich of the ligand between F6.52 and V3.33, 3) a hydrogen bond between N5 and S5.46, and 4) an aromatic interaction (edge to face) between the fused tricyclic system and W6.48.

To rationalize the observed differences in the binding affinity of clozapine and olanzapine toward different receptors, we analyzed the structural differences observed for both complexes. Olanzapine and clozapine are closely related atypical antipsychotic drugs, with rather similar chemical structures and binding affinity profiles. The main structural difference between olanzapine and clozapine is the bioisosteric replacement of the phenyl ring by a thiophene ring in the fused tricyclic system. The differences in binding affinity for all the receptors studied are shown in Figure 5. The analysis of the structural differences of the binding complexes was focused on the receptors that exhibit the greatest differences in binding affinity ($\Delta pK_i > 0.3$). From these, 5-HT_{2A}, D_2, D_3, and D_4 make up cluster 1, having higher affinity for olanzapine, whereas α_1, α_2, 5-HT_{1A}, 5-HT_{2B}, and 5-HT_7 form receptor cluster 2, showing higher affinity for clozapine. The multiple sequence alignment of the residues present in the binding site (determined by proximity < 4.5 Å from the ligand) for all aforementioned receptors shows remarkable commonalities within the clusters, and marked differences between clusters. As shown in Figure 5, receptors in cluster 2 lack a conserved double-serine at positions 5.43 and 5.46, suggesting that these residues modulate the differential affinity for clozapine or olanzapine. Moreover, a residue with H bond acceptor/donor properties (asparagine N or histidine H) is conserved at position 6.55 for receptors in cluster 1, which show preference for olanzapine.

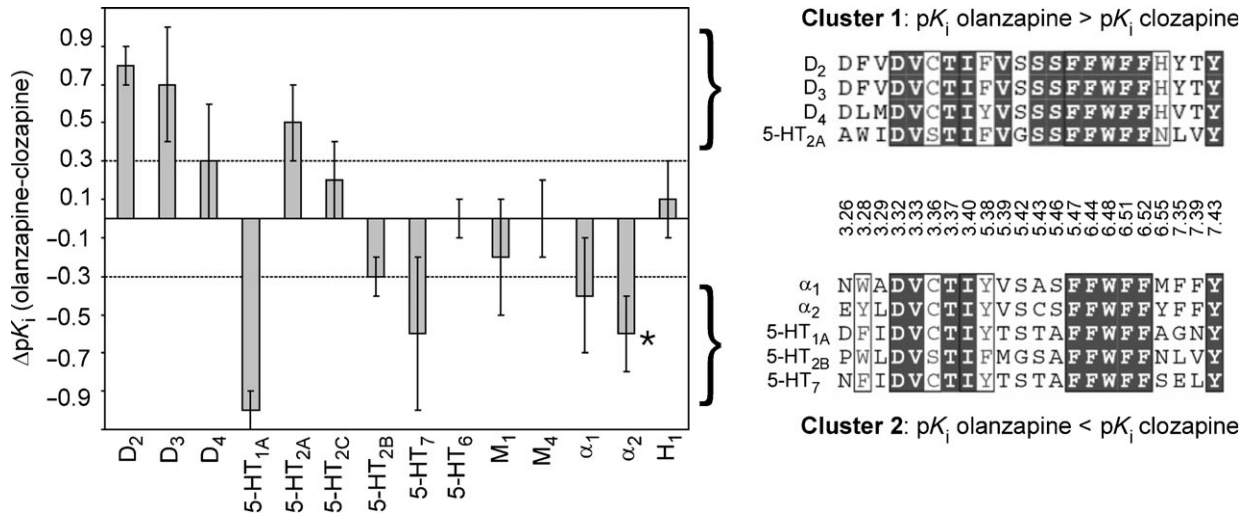

Figure 5. Differences in clozapine and olanzapine binding affinities for the receptors studied (± greatest SD). Receptors showing differences of >0.3 log units were assigned to one of the following clusters: cluster 1 (D_2, D_3, D_4, 5-HT_{2A}) contains receptors with pK_i olanzapine $>$ pK_i clozapine; cluster 2 (α_1, α_2, 5-HT_{1A}, 5-HT_{2B}, 5-HT_7) contains receptors with pK_i olanzapine $<$ pK_i clozapine (left); multiple sequence alignment of cluster 1 and cluster 2 (right). *The ΔpK_i olanzapine−clozapine value of -0.7 shown was determined using rat α_2 receptor. Therefore, slight sequence differences present in the binding site with respect to the human receptor render this value not directly comparable, but data from other sources (ΔpK_i olanzapine−clozapine for the human receptor found at the PDSP K_i Database[14]) suggest even greater differences.

In our complexes, olanzapine and clozapine are located approximately in the same position, with the olanzapine inserted slightly deeper owing to the absence of the chloro group at position 8. Interestingly, the three positions mentioned above that represent the main sequence differences between members of clusters 1 and 2 (S5.43, S5.46, N/H6.55) are very close to the thiophene ring of olanzapine which constitutes the main structural difference between olanzapine and clozapine (Figure 6). Based on the structures of the complexes, we can interpret the results as follows: in the case of olanzapine, the receptors in cluster 1 place S5.46 just in front of the ligand N and S heteroatoms located in the distal border of the tricyclic system thus producing favorably polar interactions. In this way, S5.43 interacts with the H-bond donor residue (N/H) at position 6.55 which forms additional polar interactions with the thiophene ring of olanzapine, stabilizing this conformational arrangement. Moreover, the slightly deeper insertion of olanzapine in the binding site probably makes a more favorable forked interaction with the more polar TM5 residues (the conserved double serines S5.43 and S5.46). Conversely, receptors in cluster 2 have at most one serine group at this location, are less polar, and as a consequence they interact better with the phenyl moiety of clozapine than with the slightly more polar thiophene moiety present in olanzapine.

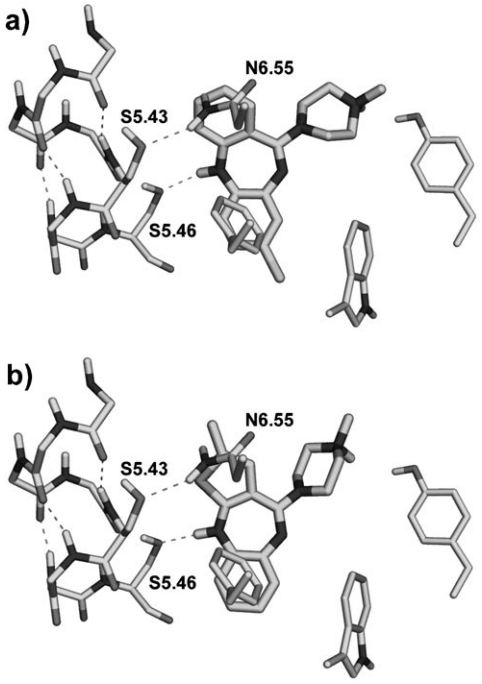

Figure 6. Complex of a) clozapine and b) olanzapine with the 5-HT_{2A} receptor. The residues of the SSH/N motif, characteristic for cluster 1, are labeled.

In conclusion, the binding profiles of both clozapine and olanzapine are highly influenced by the interaction between the N at position 10 and S3.36, mediating a tight ligand binding. The analysis reported herein of the differences between the binding profiles of the closely related olanzapine and clozapine structures is a good example of how small structural differences can produce relevant pharmacological changes. In this latest analysis, we were able to define two clusters in the set of receptors which bind both compounds on the basis of common elements in their sequence and three-dimensional features. According to the obtained complexes, binding differences between olanzapine and clozapine can be ascribed to diversity in TM5 and TM6. These results also emphasize the importance of multi-receptor treatment. Any structural changes in the ligands are likely to affect their ability to bind multiple receptors, some associated with therapeutic effects and others responsible for adverse side effects. This fact cannot be ignor-

ed in drug-design methodologies. This work represents a further step in our efforts to gain a deeper understanding of the therapeutic effect of antipsychotic drugs at a molecular level. We hope that the recent availability of better structural templates and more reliable experimental data will lead, with the help of appropriate analytical tools, to the design of more useful and safe antipsychotic drugs.

Experimental Section

A detailed description of all computational procedures, including homology modeling and docking simulation, is provided in the Supporting Information. The homology models of the GPCRs were generated using a recently published protocol[23] designed to ensure consistency and comparable results for all receptors. The receptor models were built starting from the new template (PDB code: 2RH1),[6,7] by applying the MODELLER suite of programs.[24] Optimization of the receptor structures was based on the Amber99 force field[25] as implemented in the molecular modeling suite MOE (Molecular Operating Environment; Chemical Computing Group). PROCHECK software[26] was used to assess the quality of the minimized structures. The binding mode of clozapine and olanzapine with the structural models obtained for 5-HT_{2A} and D_2 were explored by using docking simulations with the GOLD 3.1.1 program.[27]

Acknowledgements

We thank the Spanish Ministerio de Educación y Ciencia (project SAF2005-08025-C03) and the Instituto de Salud Carlos III (Red HERACLES RD06/0009) for financial support and grants for two of the authors (J.S. and L.L.).

Keywords: antipsychotic agents · binding selectivity · G-protein-coupled receptors · homology modeling · multireceptor profile

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Received: March 5, 2008
Revised: April 16, 2008
Published online on May 8, 2008

