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# Possible differences in modes of agonist and antagonist binding at human 5-HT$_6$ receptors

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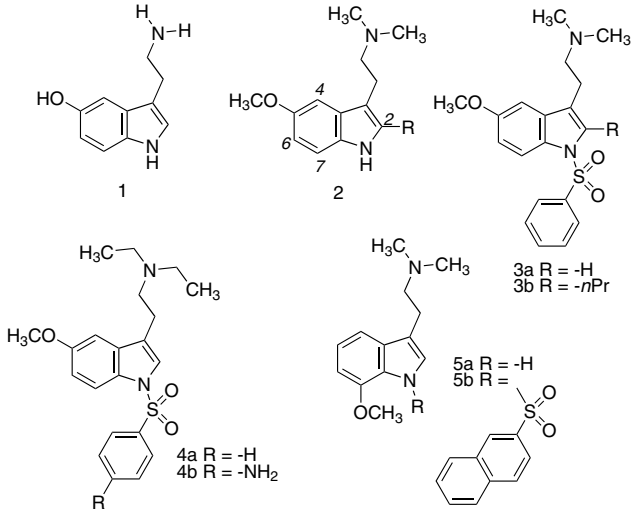
**Abstract**—A graphics model of the human 5-HT$_6$ receptor was constructed and automated docking studies were performed. The model suggests that 5-HT$_6$ antagonist arylsulfonyltryptamines might bind differently than that of the agonist serotonin. Furthermore, the model explains many of the empirical results from our previous structure–affinity studies.


## 1. Introduction

Although 5-HT$_6$ serotonin receptors were initially identified over a decade ago, only within the past few years have several selective ligands been reported (reviewed[1–6]). This receptor population is of interest due to its possible involvement in depression, psychosis, and cognition.[5,6]

The 5-HT$_6$ agonist serotonin (**1**; $K_i$ ca. 100 nM) and certain other closely related tryptamines (e.g., 5-methoxy-*N*,*N*-dimethyltryptamine, **2**, R = H) bind with similarly modest affinity at *h*5-HT$_6$ receptors, and have served as the basis for structure–affinity investigations.[7–11] These studies have revealed a number of inconsistencies depending upon whether or not an aryl-bearing moiety is attached to the indole 1-position. For example, incorporation of a benzenesulfonyl group at the tryptamine 1-position typically results in about 50-fold enhanced affinity and conversion of the ligand to a 5-HT$_6$ antagonist. Examples of the latter include MS-245 (**3a**; $K_i$ ca. 2 nM) and its diethyl homolog **4a** ($K_i$ = 6 nM).[9,11] The observed structure–affinity inconsistencies have led us to suggest that multiple modes of binding are possible.[8] Some of the observations leading to this conclusion are as follows. Introduction of a 2-methyl or 2-ethyl group (e.g., EMDT; **2**, R = Et, $K_i$ = 16 nM) results in 5-HT$_6$ agonists with somewhat enhanced affinity and increased selectivity;[9] however, further homologation of the 2-ethyl group to an *n*-propyl group is not well tolerated and results in decreased affinity (i.e., **2**, R = *n*-Pr, $K_i$ = 185 nM).[9] In contrast, introduction of either a 2-ethyl or 2-*n*-Pr group has no effect on the affinity of **3a** (e.g., **3b** $K_i$ = 2.3 nM).[10] The presence of a 5-methoxy group seems optimal for the binding of **2** (where R = H); for example, the 7-methoxy positional isomer (**5a**, $K_i$ = 19,600 nM) binds with significantly reduced affinity. Nevertheless, when an arylsulfonyl group is attached, 7-methoxy-substituted analogs can bind with high affinity (e.g., **5b**, $K_i$ = 5 nM).[8] In addition, certain substituents at the 5-position of simple tryptamines (e.g., $CF_3SO_2$–) decrease their affinity but have little effect on the affinity of arylsulfonyltryptamines.[10]

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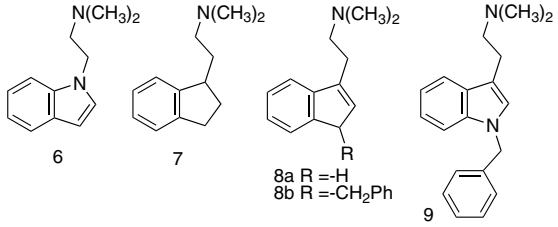
There is also evidence that the indolic NH is important to the binding of simple tryptamines. $N_1$-Methylation and $N_1$-ethylation decrease the affinity of tryptamines whereas, in contrast, the arylsulfonyltryptamines, which generally bind with high affinity, obviously possess an $N_1$ substituent. Simple tryptamine analogs that entirely lack the indolic NH (e.g., **6** and **7**, $K_i > 5000$ nM; **8a**, $K_i = 1900$ nM) typically display very low affinity.[7] Yet, compound **8b** ($K_i = 3$ nM), which lacks an indolic nitrogen atom (or NH), binds with an affinity similar to that of **9** ($K_i = 6$ nM).[11] Taken together, the results suggest different modes of binding are possible, and because the $N_1$-arylsulfonyl analogs display antagonist action, they further suggest that the observed differences might be related to the manner in which agonists bind relative to antagonists.




The purpose of the present investigation was to construct a graphics model of the human 5-HT$_6$ ($h$5-HT$_6$) receptor and to determine whether or not multiple modes of binding could be accounted for.

## 2. Results and discussion

In order to explore possible modes of binding we constructed a graphics model of the $h$5-HT$_6$ receptor. A 5-HT$_6$ receptor model was constructed following the same homology modeling procedure we reported for construction of a 5-HT$_{2A}$ receptor model.[12] Modeling studies, performed using SYBYL, were based on the high-resolution X-ray structure of bovine rhodopsin as the starting template.[13] Extra- and intracellular loops were incorporated, the structure was energy minimized, and automated docking was performed with FLEXX using lysergic acid diethylamide (LSD), 5-HT (**1**), and

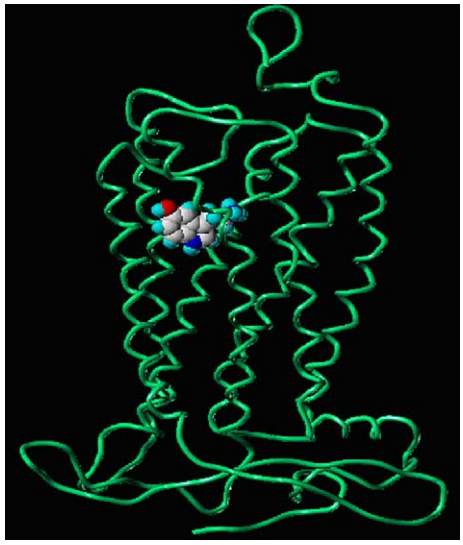


**Figure 1.** Graphics model of the $h$5-HT$_6$ receptor with 5-HT (**1**) docked to the TM3 aspartate moiety. See Figure 2 for a close-up view of the docked molecule.

examples of antagonist ligands, such as **3a**. Figure 1 shows a $h$5-HT$_6$ model with 5-HT docked.

One of the first major observations from the automated docking studies is that there appears to be two regions of steric accessibility, and that 5-HT (**1**) and other simple tryptamines bind in a different fashion than $N_1$-arylsulfonyltryptamines. For example, 5-HT (**1**) utilizes the TM3 aspartate moiety and was tucked into the TM3–TM6 region. In contrast, the arylsulfonyltryptamines utilized the same aspartate moiety, but were more closely flanked by the TM6, TM7, and TM1 region. This is shown in Figure 2, and schematically in Figure 3.

Consistent with the proposed binding mode of 5-HT (the indolic NH of which is within hydrogen bonding distance of the TM5 Thr$^{196}$ residue) is that the Asp$^{106}$ and Thr$^{196}$ residues have been previously implicated as being important for binding on the basis of mutagenesis studies.[14–16] The 5-hydroxyl group is within hydrogen bonding distance (ca. 3 Å) to the TM6 Asn$^{288}$. 6-Methoxy- and 7-methoxytryptamines (e.g., **5a**) display low affinity for

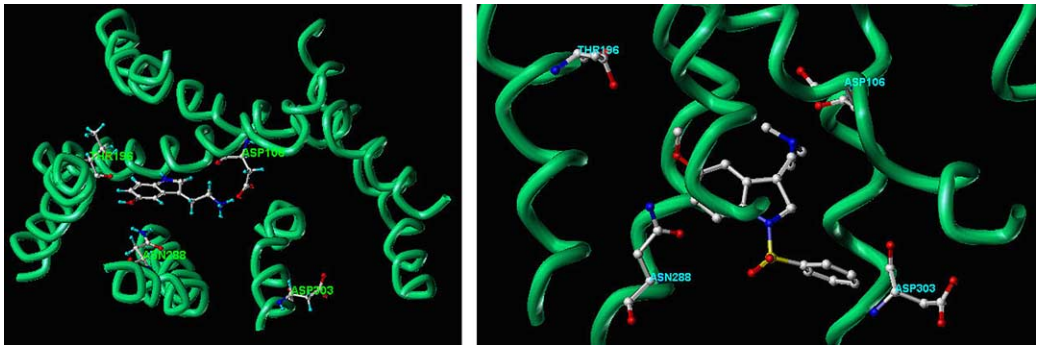



**Figure 2.** Graphics model (only helical segments shown, as viewed from the extracellular side) of the $h$5-HT$_6$ receptor with 5-HT bound (left). A close-up of a portion of the model with a benzenesulfonyltryptamine (i.e., **3a**) docked in a different binding region (right). Both use a common TM3-aspartate residue.

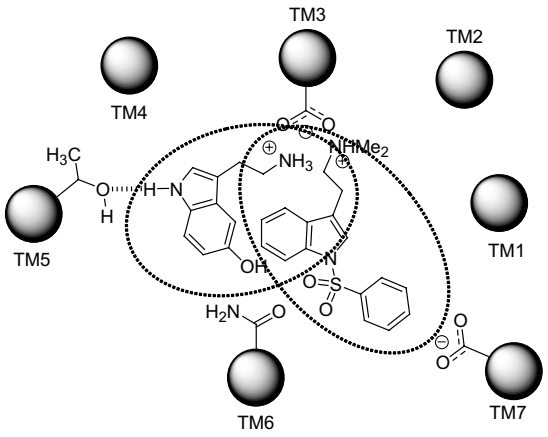
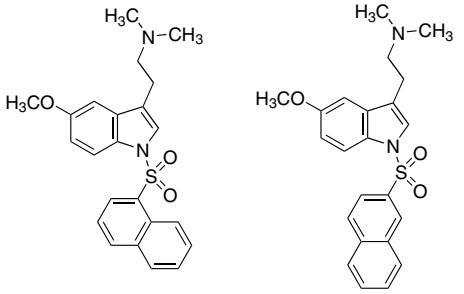


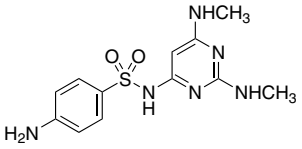

**Figure 3.** A two-dimensional representation of the *h*5-HT$_6$ receptor graphics model showing that, even though they share a common interaction with the TM3 aspartate moiety, agonists and arylsulfonyl-containing antagonists might bind in two different domains.

5-HT$_6$ receptors and the model suggests this could be due to a steric clash between the methoxy substituents and Ala[192] and Thr[196], respectively. Visual inspection suggests that the 2-position should sterically accommodate small alkyl substituents such as a methyl or ethyl group, but that larger substituents might not be as well tolerated. This is consistent with the reduced affinity of the *n*-Pr versus ethyl analog of **2**. In addition to the above mentioned residues, other amino acids featuring prominently in the potential 'agonist' binding pocket include the TM3 Val[107], Cys[110], and Ser[111], the TM5 Ala[192], and Ser[193], and the TM6 phenylalanines Phe[284] and Phe[285], and Asn[288].

With respect to the proposed binding of arylsulfonyltryptamines such as **3a**, the indolic nitrogen atom is no longer situated in the vicinity of the TM5 Thr[196] residue (Fig. 2). This could account for the high affinity of $N_1$-benzyltryptamine (**9**). Furthermore, this mode of binding would also explain the high-affinity binding of **8b** relative to **8a**. That is, the proposed model seems to account for the finding that whereas an indolic NH might be important for the binding of simple tryptamines due to formation of a hydrogen bond with Thr[196], the NH seems much less important when an aryl-bearing $N_1$-substituent is present. Associated with the aryl (i.e., indolic) portion of such compounds are aromatic residues Phe[284] and Phe[285], and the TM6 asparagine moiety (Asn[288]) is within hydrogen bonding distance (ca. 3.3 Å) to the methoxy oxygen function of **3a**. The benzenesulfonyl aryl ring is in the vicinity of the TM7 Phe[302] residue and projects into a cavity that appears to constitute a region of bulk tolerance bounded by TM2, TM3, and TM6. Construction of the (1-naphthyl)sulfonyl and (2-naphthyl)sulfonyl counterparts (i.e., **10** and **11**, respectively) of **3a**, from docked **3a**, followed by minimization of the resulting complex indicates that there is sufficient room to accommodate these larger groups. This is consistent with our previous report that both of these compounds bind with an affinity similar to that of **3a** (i.e., $K_i = 0.9$ and 1.6 nM for **10** and **11**, respectively).[8] The oxygen atoms of the –$SO_2$– function are within possible hydrogen bonding range (ca. 3–5 Å) of the TM6 Gln[291], and the e2 Arg[181] and the Leu[182] peptide NH. Another feature found on the side of the cavity and within <9 Å of the benzenesulfonyl centroid is TM7 Asp[303]. Perhaps this second aspartate moiety could account for the enhanced affinity of certain appropriately-substituted derivatives. For example, compound **4a** ($K_i = 6$ nM) binds with 10-fold enhanced affinity when a 4′-amino group is added to the ring (i.e., **4b**, $K_i = 0.6$ nM).[11]




The first reported 5-HT$_6$ receptor graphics model was that of Bromidge,[17] which was constructed on the basis of the electron microscopic α-carbon helix template of frog rhodopsin. In that study, an attempt was made to explain the binding of 5-HT$_6$ antagonists. An ionic interaction was proposed between a ligand–amine and Asp[106], and aromatic π-stacking interactions were proposed with Phe[277] and Trp[281]. In the present model, the latter residues are not sterically accessible. Shortly after our model was constructed,[18] Hirst et al.[19] described models for rat, mouse, and human (which is highly homologous to the rat) 5-HT$_6$ receptors, and the docking of LSD and several 5-HT$_6$ antagonists to mouse and rat 5-HT$_6$ receptors. Some of the docking features of their rat model are consistent with the present human model. For example, they propose that in the rat model the NH moiety of LSD interacts with Thr[196] and that the ring system is in a hydrophobic pocket lined by Val[107], Cys[110], Trp[281], Phe[284], and Phe[285]. Most of these same residues were implicated for the binding of 5-HT in the present investigation (vide supra). However, some of their docking studies with antagonists were complicated by the presence of multiple basic amine functions in the ligands examined. Ro 04-6790 (**12**), for example, possesses five protonatable nitrogen atoms and, in theory, any one of them might interact with the presumed amine binding site (Asp[106]) of the receptor. In the present study, 5-HT$_6$ antagonists such as MS-245 (**3a**) and its *N*,*N*-diethyl homolog **4a** possess only a single basic nitrogen atom, somewhat simplifying the problem. Nevertheless, with regard to antagonist **12**, the pyrimidine ring was found to be situated in a π-stacking orientation with Phe[284], and oxygen atoms of the –$SO_2$– were suggested to form a hydrogen bond with Asn[288] and Gln[216]. Neither residue (with Gln[216] being an arginine residue in the human receptor) was as readily accessible to the sulfonyl oxygen atoms in the present model as they were in the Hirst et al. model.



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In summary, a *h*5-HT$_6$ receptor graphics model was constructed and automated docking studies identified two sterically accessible regions where tryptamine-related analogs might bind. The overlapping but distinct domains seemingly account for the structure–affinity relationships that have been developed for these compounds, depending upon whether or not they possess an aryl-bearing $N_1$ substituent. In this model, which is the first to describe the binding of 5-HT at 5-HT$_6$ receptors, LSD binds in a manner similar to what was found by Hirst et al.[19] for a rat model. This mode of interaction seemingly explains the binding of simple tryptamine agonists, but does not readily allow for the binding of tryptamines with $N_1$ substituents. The binding of $N_1$-aryl-bearing 5-HT$_6$ antagonists is better described by their association with the receptor in a different fashion. The proposed mode of binding of the latter compounds is more consistent with their structure–affinity relationships. These models might also be of use in the development of novel 5-HT$_6$ ligands. Nevertheless, in the absence of specific empirical data (e.g., additional site-directed mutagenesis), the proposed modes of binding and the amino acid residues implicated are unproven, and will need to be investigated using appropriate experimental techniques.

## 3. Experimental

### 3.1. Model construction

Modeling studies were performed using SYBYL (version 6.8, 2001, Tripos Associates, Inc., St. Louis, MO). A 5-HT$_6$ receptor model was constructed as previously described for the 5-HT$_{2A}$ receptor.[12] An unambiguous alignment of the rhodopsin and 5-HT$_6$ receptor[20] sequences was performed manually by matching the highly conserved residues previously identified throughout the GPCR family.[21] The transmembrane (TM) helical segments were extracted from the experimental bovine rhodopsin structure (a chain of 1F88.pdb)[13] retaining the following segments (helix number, 5-HT$_6$ sequence range, rhodopsin sequence range in parentheses): TM1, 24–53 (35–64); TM2, 60–89 (71–100); TM3, 96–128 (107–139); TM4, 141–163 (151–173); TM5, 185–210 (200–225); TM6, 263–293 (247–277); TM7, 300–320 (286–306). Mutation of the rhodopsin sequence to that of the 5-HT$_6$ receptor was accomplished using BIOPOLYMER within SYBYL. Amino acid side-chain geometries for the 5-HT$_6$ receptor model were established from backbone-dependent libraries of rotamer preference using the program SCWRL.[13] The helix backbone geometry of rhodopsin was transferred without change in this procedure. PROCHECK, WHATIF,[22] and the PROTABLE facility within SYBYL were used to identify sites of unusual and sterically clashing side chain geometries which were interactively corrected as necessary.

The model loops were constructed using the LOOPSEARCH module in SYBYL. Loop fragment candidates were selected on the basis of end-to-end distance rmsd and sequence homology criteria from a database of high resolution experimental protein structures. In the extracellular loop 2 (e2), a cysteine residue is highly conserved in the GPCRs which forms a disulfide bond with another highly conserved cysteine residue in TM3. Hence, retaining the cysteine residue in e2, the loop search facility in SYBYL was used to replace the GIDYYTPHEETN (188–199) fragment of rhodopsin with the RLLA (181–184) fragment of the 5-HT$_6$ receptor. The solution with the lowest rmsd end-to-end distance and the highest homology was selected to construct the loop. Retaining the highly conserved cysteine residue, the remainder of the e2 loop (164–179) of the 5-HT$_6$ receptor was constructed using LOOPSEARCH. Intracellular loop 3 (i3) of the 5-HT$_6$ receptor is 31 residues longer than that of rhodopsin. Attempts to use the LOOPSEARCH facility to find a suitable 52 amino acid fragment (211–262) to replace the 21 amino acid fragment (226–246) of rhodopsin gave a few solutions all of which resulted in unusual geometries at the points of attachment of the fragment. Hence, the 52 amino acid i3 loop of the 5-HT$_6$ receptor was constructed in two steps. In the first step, a 26 amino acid fragment (211–236) of the 5-HT$_6$ receptor was constructed by replacing amino acids 226–236 of rhodopsin, and in the second step amino acids 237–246 of rhodopsin were replaced by another 26 amino acid fragment (237–262) of the 5-HT$_6$ receptor using the LOOPSEARCH facility.

Model minimizations were performed using the Tripos force field, Gasteiger–Huckel charges with distance-dependent dielectric constant = 8.0 and nonbonded cutoff of 8 Å. Minimizations were performed to a gradient of 0.05 kcal/mol Å. Receptor models were initially minimized with backbone atoms constrained and then with all constraints removed.

### 3.2. Docking

Docking of ligands was performed using the FLEXX program within SYBYL. LSD was manually docked into the receptor based on the mutagenesis studies available for the 5-HT$_6$ receptor. Site-directed mutagenesis studies have suggested formation of a hydrogen bond interaction between the indolic NH of LSD and the Thr$^{196}$ residue and an ionic interaction between the Asp$^{106}$ and the basic amine of LSD. Hence, the ligand was placed in the binding pocket to meet the distances for these proposed interactions. 5-Benzyloxytryptamine and ergopeptines displayed increased binding affinity for the mutated 5-HT$_6$ receptors A287L, N288S compared to the wild type receptor.[15] When LSD was placed in the binding pocket it was assumed that the 5-position of the indole ring of LSD faces toward the Asn$^{288}$. Once the ligand was manually placed in a probable position in the

binding-pocket, the ligand–receptor complex was minimized until a gradient of 0.05 kcal/mol Å. FLEXX was used for automated docking studies of the 5-$HT_6$ ligands. The radius around the ligand in the ligand–receptor complex was defined as 6.5 Å (for agonists) and 10.1 Å (for antagonists),[23] no core region was defined for the active site, formal charges were assigned, and the $Asp^{106}$ was considered charged to define the ionic interaction between the basic amine of 5-$HT_6$ ligands and the receptor. The LSD–receptor complex obtained with automated docking studies was used as the final model for further docking studies of 5-$HT_6$ ligands. The basic amine of all the ligands docked into the receptor was protonated.

## Acknowledgements

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23. A radius of 6.5 Å was used for agonists because when 5-HT was docked into the receptor using a 10.1 Å radius it yielded most solutions with the ligand placed in the other parts of the receptor. When 6.5 Å was used for antagonists it placed the ligand outside the binding pocket in most of the solutions. When 10.1 Å was used for agonists, 5-HT did not recognize $Asp^{303}$ as an amino acid for ionic interaction; although it placed 5-HT in that part of the pocket, the terminal amine of 5-HT was at least 5.5 Å away from the $Asp^{303}$ carboxylate group.