Binding of Serotonin and N_1-Benzenesulfonyltryptamine-Related Analogs at Human 5-HT_6 Serotonin Receptors: Receptor Modeling Studies

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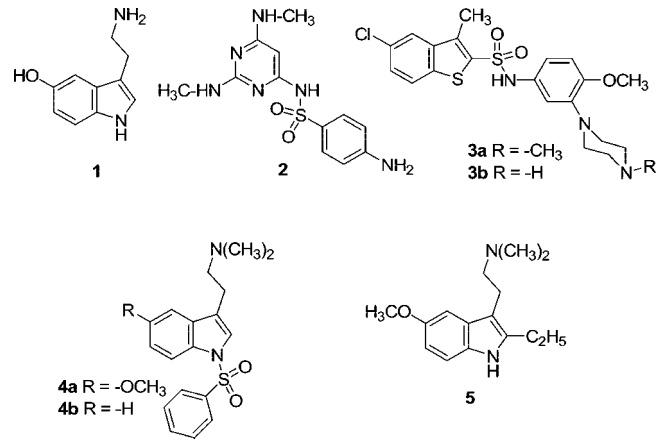
A population of 100 graphics models of the human 5-HT_6 serotonin receptor was constructed based on the structure of bovine rhodopsin. The endogenous tryptamine-based agonist serotonin (5-HT; **1**) and the benzenesulfonyl-containing tryptamine-derived 5-HT_6 receptor antagonist MS-245 (**4a**) were automatically docked with each of the 100 receptor models using a genetic algorithm approach. Similar studies were conducted with the more selective 5-HT_6 receptor agonist EMDT (**5**) and optical isomers of EMDT-related analog **8**, as well as with optical isomers of MS-245 (**4a**)-related and benzenesulfonyl-containing pyrrolidine **6** and aminotetralin **7**. Although associated with the same general aromatic/hydrophobic binding cluster, 5-HT (**1**) and MS-245 (**4a**) were found to preferentially bind with distinct receptor conformations, and did so with different binding orientations (i.e., poses). A 5-HT pose/model was found to be common to EMDT (**5**) and its analogs, whereas that identified for MS-245 (**4a**) was found common to benzenesulfonyl-containing compounds. Specific amino acid residues were identified that can participate in binding, and evaluation of a sulfenamide analog of MS-245 indicates for the first time that the presence of the sulfonyl oxygen atoms enhances receptor affinity. The results indicate that the presence or absence of an N_1-benzenesulfonyl group is a major determinant of the manner in which tryptamine-related agents bind at 5-HT_6 serotonin receptors.

Introduction

Many actions of the neurotransmitter substance serotonin (5-hydroxytryptamine; 5-HT, **1**) are mediated by its interaction with various populations (5-HT_1−5-HT_7) of 5-HT receptors.[1] Apart from ionotropic 5-HT_3 receptors, other members of this receptor family are class A rhodopsin-like G protein-coupled receptors (GPCRs).[1,2] Human 5-HT_6 receptors, first cloned in 1996,[3] are positively coupled to an adenylate cyclase second messenger system, and there is evidence that this receptor population is involved in cognition, obesity, and certain neurological and neuropsychiatric disorders, including schizophrenia and depression.[4] This receptor population is also of interest because clozapine and a number of other typical and atypical antipsychotic agents bind at 5-HT_6 receptors with high affinity.[5] Only within the last six or seven years have 5-HT_6-selective agents been identified, and among some of the first antagonists described were Ro 04-6790[a] (**2**),[6] SB-258510 (**3a**) and its *N*-desmethyl analog SB-271046 (**3b**),[7] MS-245[a] (**4a**),[8,9] and the agonist EMDT (**5**;[8] Chart 1; reviewed[4,10]).

Prior to the availability of selective agents, site-directed mutagenesis studies with rat 5-HT_6 receptors identified conserved aspartate (Asp106 [3.32]). (There is a high degree of homology between rat and human 5-HT_6 receptors, and their amino acid sequence numbering is identical for the amino acids described herein; for convenience, amino acids are numbered by their human sequence number followed, in brackets, by their numbering using the Ballesteros−Weinstein convention)[11] and threonine (Thr196 [5.46]) moieties as being directly or indirectly involved in the actions of serotonergic agonists and that their individual mutation substantially reduces both the affinity (radioligand binding) and the efficacy (stimulation of adenylate cyclase action) of the nonselective agonists 5-HT (**1**) and (+)-lysergic acid diethylamide (LSD).[12,13]

Chart 1. Structures of 5-HT (**1**), 5-HT_6 antagonists Ro 04-6790 (**2**), SB-258510 (**3a**), SB-271046 (**3b**), MS-245 (**4a**) and its des-methoxy analog **4b**, and the agonist EMDT (**5**)



To date, there have been four attempts to provide insight into how 5-HT_6 receptor–ligands interact with 5-HT_6 receptors using graphical models.[14–17] Bromidge[14] was first to describe a possible docking mode for a 5-HT_6 antagonist (i.e., SB-258510; **3a**) to a 5-HT_6 receptor model and his epigrammatic description implicated Phe277 [6.44] and Trp281 [6.48] as participating in possible $\pi-\pi$ stacking interactions (Table 1). Hirst et al.[16]

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[a]Abbreviations: MS-245, N_1-benzenesulfonyl-5-methoxy-*N*,*N*-dimethyltryptamine; EMDT, 2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine; LSD, lysergic acid diethylamide; SB-258510, 5-chloro-*N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-2-benzothiophenesulfonamide; Ro 04-6790, 4-amino-*N*-(2,6-bis-methylamino-pyrimidin-4-yl)benzenesulfonamide.

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Table 1. Proposed Modes of Interaction for Sulfonamide-Containing 5-HT_6 Antagonists

	Aryl′-SO_2-*N*-aryl-X-amine				
feature	Bromidge[14]	Pullagurla et al.[17]	Hirst et al.[16]	Lopez-Rodriguez et al.[15]	current[a]
amine	Asp106 [3.32]	Asp106 [3.32]	Asp106 [3.32]	Asp106 [3.32]	Asp106 [3.32]
aryl	Trp281 [6.48]	Phe284 [6.51]	Trp281[6.48]	Phe198 [5.48]	Val107 [3.33]
		Phe285 [6.52]	Phe284 [6.51]	Phe285 [6.52]	Leu182 [e2]
			Phe302 [7.35]		Trp281 [6.48]
					Phe284 [6.51]
SO_2		Leu182 [e2]	Asn288 [6.55]	Ser193 [5.43]	Ser111 [3.37]
		Gln291 [e3]	Gln216[b]	Asn288 [6.55]	Thr196 [5.46]
Aryl′	Phe277 [6.44]	Phe302 [7.35]	Phe188 [5.38]	Val107 [3.33]	Ala157 [4.56]
				Phe188 [5.38]	Ala192 [5.42]
				Ala192 [5.42]	Phe285 [6.52]

[a] Other residues within 5 Å of docked molecules, in 0.5 Å distance increments, are listed in the Supporting Information section. [b] Both the rat and human receptors contain a glutamine moiety at position 218, but an arginine residue at position 216. Hence, it is uncertain which of the two amino acids was actually identified in this study.

examined the docking of several antagonists including Ro 04-6790 (**2**) and SB-258510 (**3a**) at mouse, rat, and human 5-HT_6 receptor models to explain the reduced affinity of certain antagonists (in particular, Ro 04-6790) for mouse receptors. Implicated in possible $\pi-\pi$ stacking interactions (rat model) were Phe188 [5.38], Trp281 [6.48], Phe284 [6.51], and Phe302 [7.35]. Pullagurla et al.,[17] using a human 5-HT_6 receptor model, examined several MS-245 (**4a**) analogs and identified Phe284 [6.51], Phe285 [6.52], and Phe302 [7.35] as being involved in $\pi-\pi$ interactions. More recently, Lopez-Rodriguez et al.[15] studied the binding of a series of 5-HT_6 antagonists (including **2**, **3b**, and **4**) using a human 5-HT_6 receptor model. An aromatic/hydrophobic binding region was defined by Val107 [3.33], Phe188 [5.38], Ala192 [5.42], Phe198 [5.48], and Phe285 [6.52]. Despite using different approaches, each of the four studies identified certain common amino acids as possibly participating in the interaction of antagonists with 5-HT_6 receptors. That is, the studies all began with recognition that a role for Asp106 [3.32] is key to binding and identified a common binding pocket or "cluster" consisting of aromatic/hydrophobic residues in TM5 and TM6 (e.g., Trp281, Phe284, and Phe285). However, orientation of the ligands *within* this region differed considerably. For example, the SO_2 oxygen atoms of the sulfonyl-containing antagonists have been variously suggested to be within hydrogen bonding distance to, for example, Asn288 [6.55], Ser193 [5.43], Leu182 (in the e2 loop), and Gln291 (at the TM6-e3 junction) (see Table 1).[14,15]

Two investigations examined agonists,[16,17] and only one of these examined the possible binding modes of 5-HT at human 5-HT_6 receptors.[17] The two models implicated a role for Asp106 [3.32] and Thr196 [5.46] for interaction with the agonists 5-HT and/or LSD.[16,17]

Using graphics models of the receptor, attempts have been made to identify how 5-HT_6 receptor antagonist ligands interact with various receptor amino acid features. For the most part, however, these studies involved antagonists with multiple basic amine groups (with consequent and inherent uncertainty, and assumptions, as to which amine specifically interacts with the TM3 aspartate moiety Asp106), employed agents with substantial conformational flexibility, and have assumed that the sulfonyl moiety (common to nearly all 5-HT_6 antagonists) participates in binding. From what we can discern, only one of the prior studies explicitly states that a nonmanual docking method was utilized,[17] and only a single study examined a possible binding mode for 5-HT.[17]

The present investigation was designed to specifically examine the binding of tryptamine-related analogs; the binding of other 5-HT_6 ligands will be examined in due course. In this investigation we examined possible docking modes for the nonselective agonist 5-HT (**1**) and of the more 5-HT_6-selective agonist EMDT (**5**) at human 5-HT_6 receptors. We also examined the docking of the tryptamine-derived antagonist MS-245 (**4a**) and structurally related and stereochemically and conformationally defined benzenesulfonyl analog pyrrolidine **6** and aminotetralin **7**. In light of empirical evidence that N_1-unsubstituted and N_1-benzenesulfonyltryptamines likely bind in a dissimilar manner at 5-HT_6 receptors,[18,19] and with realization that agonists and antagonists might bind optimally with different receptor conformations (and that there might even be differences in the binding of different agonists at a given receptor),[20] we utilized a docking technique that should emphasize and account for such differences. Finally, we examined the binding of stereochemically defined optical isomers of EMDT analog **8** and also determined whether or not the sulfonyl oxygen atom(s) contributes to binding of benzenesulfonyltryptamines by examining sulfenamide **9** in comparison with its sulfonyl counterpart **4b**. As such, this investigation employed (a) 5-HT_6 receptor–ligands bearing a single basic amine, (b) stereochemically or (somewhat) conformationally defined analogs of the 5-HT_6 ligands MS-245 (**4a**) and EMDT (**5**), and (c) an automated and unbiased docking procedure that might account for differences in the binding of agonist versus antagonist analogs at human 5-HT_6 receptors.

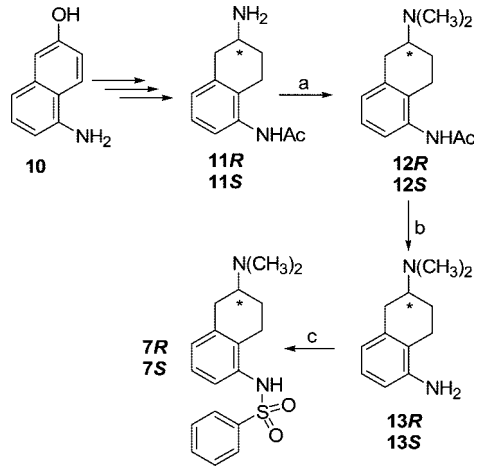
Chemistry. The synthesis of certain compounds required for this investigation has been previously reported by us, including MS-245 (**4a**),[8] **4b**,[21] EMDT (**5**),[8] *R*-(+)-**6**, and *S*-(−)-**6**.[22] The synthesis of compounds **7*R*** and **7*S*** employed the isomers of **11** (Scheme 1). Isomers **11** were prepared in five steps from 5-amino-2-naphthol (**10**) following a literature procedure[23] that involved protection of the amine, Birch reduction, and stereoselective synthesis of **11*R*** and **11*S*** from the resultant ketone. Dimethylation of the individual optical isomers of **11** afforded isomers **12**, which were deprotected to isomers **13** and then sulfonylated to isomers **7** by reaction with benzenesulfonyl chloride (Scheme 1). Compounds **12** and **13** have been previously reported in the patent literature as their racemates;[23] although optical isomers of **13** also were mentioned, synthesis was not described nor were physicochemical properties recorded.[23]

Isomers **8** were prepared from 2-ethyl-5-methoxyindole[24] in a manner that paralleled the synthesis of **6**[22] and related compounds.[25] Compound **9** was obtained by interaction of *N*,*N*-dimethyltryptamine (DMT) with *N*-(phenylthio)succinimide.[26]

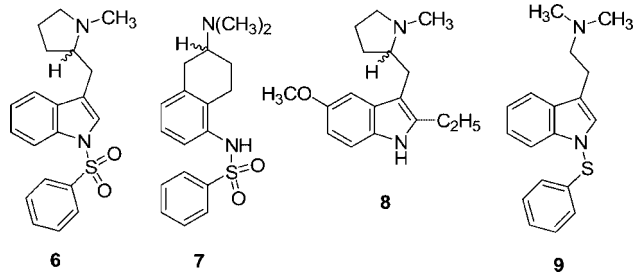
Results and Discussion

Human 5-HT_6 receptor affinities (K_i values) of certain compounds employed in this investigation already have been

Scheme 1[a]



[a] Reagents and conditions: (a) $H_2C{=}O$ (37%), $NaCNBH_3$, HOAc, MeOH/MeCN, rt, 3 h; (b) concd HCl, EtOH, Δ, 2 h; (c) $PhSO_2Cl$, CH_2Cl_2, 0 °C, 3 h.



reported from our laboratories: MS-245 and its des-methoxy counterpart (**4a** and **4b**; K_i = 2.1 and 4.1 nM, respectively),[21] EMDT (**5**; K_i = 16 nM),[8] *R*-(+)-**6** (K_i = 0.3 nM), and *S*-(−)-**6** (K_i = 1.7 nM).[22] Aminotetralin isomers **7*R*** and **7*S*** displayed little enantioselectivity (K_i = 49 ± 12 and 90 ± 20 nM, respectively), whereas isomers of the pyrrolidine counterpart of EMDT showed greater stereoselectivity of binding (**8*R*** K_i = 1.8 ± 0.2 nM; **8*S*** K_i = 220 ± 25 nM). For several structurally related chiral pyrrolidines, we have previously found greater enantioselectivity in the absence of the benzenesulfonyl group (up to 70-fold) than in its presence (<6-fold).[22] Sulfenamide **9** (K_i = 90 ± 10 nM) displayed >20-fold reduced affinity relative to its sulfonamide counterpart **4b** (K_i = 4.1 nM).

Model Construction and Docking Studies. Human 5-HT$_6$ receptor models were constructed and various docking solutions were explored for 5-HT (**1**), MS-245 (**4a**), EMDT (**5**), MS-245 analogs **6**, aminotetralins **7**, and EMDT-related optical isomers **8** such that both the receptor models and the ligands were allowed to interact in a fairly flexible manner. Receptor flexibility was explicitly addressed via the generation of a population of 100 h5-HT$_6$ receptor models whose conformational state varied among the members of the population. Automated docking routines were then used to flexibly place each ligand into each receptor model. The resulting receptor–ligand complexes were scored and subsequently ranked using the ChemScore[27,28] fitness function. Thus, each ligand was allowed to select an optimal receptor conformation from the population, reducing the bias that might occur when using a single receptor model to dock different ligands. Two common receptor conformations were identified: one for agents lacking a benzenesulfonyl group (i.e., 5-HT, EMDT and analogs) and another for agents possessing such a group (i.e., MS-245 and related analogs). The top-scoring receptor conformation for each

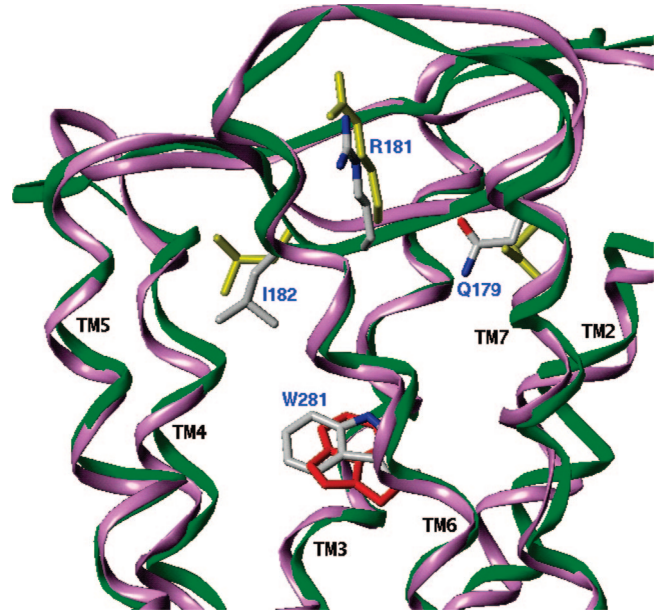


Figure 1. Overlap of the two selected receptor conformations highlighting those side chains in the binding site that differ most substantially between the two sites. The position of the receptors relative to one another was determined by calculating the minimum rmsd of the α carbon atoms of residues in the binding pocket. Side chains that are colored by atom type belong to the receptor preferred by ligands that do not contain a benzenesulfonyl group (green ribbon). Side chains that are colored either red (rmsd ≥ 2.0 Å) or yellow (1.0 Å ≥ rmsd > 2.0 Å) belong to the receptor selected for the ligands that do contain a benzenesulfonyl group (purple ribbon). The conformations of other side chains in the binding site differed between the two receptors but to a smaller degree. These have been omitted for clarity.

nonbenzenesulfonyl analog was identical. The top-scoring receptor conformation for benzenesulfonyl-containing analogs also was the same, except that for MS-245 (**4a**), the second highest top-scoring receptor conformation was selected. The latter (ChemScore = 39.32 relative to the top score of 41.20) was selected because of its commonality with the receptor conformation identified for the other benzenesulfonyl-containing analogs. In the top-scoring MS-245 receptor conformation, the hydrogen bonding component suffers at the expense of a greater lipophilic component, reflecting a somewhat different pose for the benzenesulfonyl moiety.

The primary differences in the two receptor models are depicted in Figure 1. The largest difference in side chain orientation occurs for Trp281 [6.48]. The phenyl portion of the indole nucleus of Trp281 is oriented toward transmembrane helix 5 (TM5) for the nonbenzenesulfonyl-containing compounds, and toward transmembrane helix 3 (TM3) for benzenesulfonyl-containing compounds. In addition, residues comprising the second extracellular loop (e2) also exhibited a significantly different position and orientation between the two receptor models. This is not surprising because the overall movement of the e2 loop is expected to be greater than that for the transmembrane helical segments. A complete list of rmsd differences for individual residues of the binding cavity may be found in the Supporting Information section. The two top receptor solutions are described in more detail in the following sections as they relate to docked ligands.

5-HT and EMDT Analogs. With the amine function docked within 2.7 Å of Asp106 [3.32], the indolic nucleus of 5-HT is situated in a cluster of amino acids consisting of Leu182 [e2], Trp281 [6.48], and Phe284 [6.51] (Figure 2). Previous models for docked 5-HT[17] and LSD[16] suggest that the indolic NH might

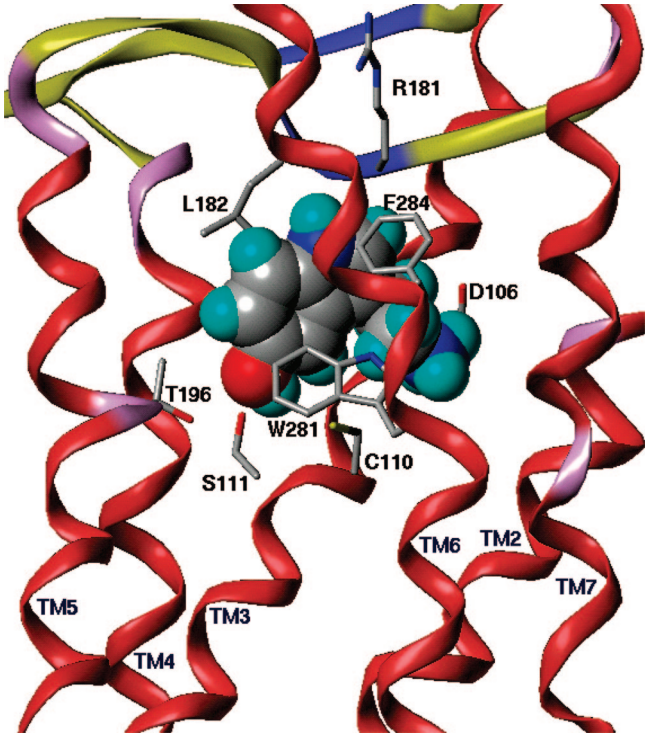


Figure 2. Proposed binding mode of serotonin (5-HT; **1**) in the h5-HT_6 receptor, looking through the extracellular side of the helical bundle. TM5, TM6, and TM7 are nearest to the viewer. The backbone ribbon trace is color-coded based on the assignment of secondary structure using the Kabsch−Sander[29] algorithm (red = helix, blue = sheet, violet = turn, and yellow = coil). 5-HT (**1**) is rendered as a CPK-style space-filling model, and the side chains of residues whose heavy atoms fall within 4.0 Å of the ligand's heavy atoms are rendered as capped sticks.

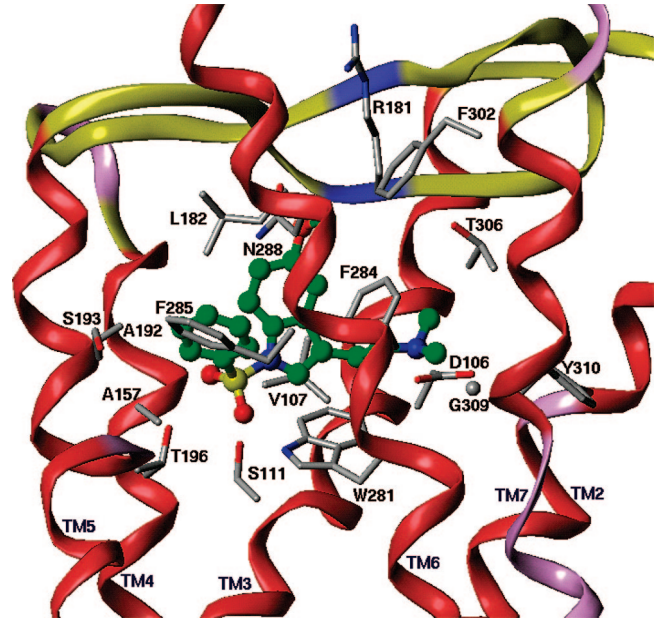


Figure 3. Proposed binding mode of MS-245 (**4a**) in the h5-HT_6 receptor, looking through the extracellular side of the helical bundle. TM5, TM6, and TM7 are nearest to the viewer. The MS-245 ligand is rendered as a ball-and-stick model with the carbon backbone in green, and the side chains of residues whose heavy atoms fall within 4.0 Å of the ligand's heavy atoms are rendered as capped sticks.

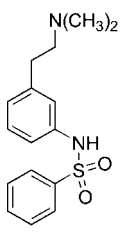
hydrogen bond with Thr196 [5.46]. In the present model, the indolic NH is directed toward Arg181 [e2], which is <4 Å distant. This virtually turns the indolic nucleus "upside down" relative to a prior binding motif described by us.[17] Both Ser111 [3.37] and Thr196 [5.46] (Figure 2) are within hydrogen bond distance of the 5-HT hydroxyl group (O−O distance 3.0–3.1 Å), indicating that the hydroxyl group can form a hydrogen bond with either one or, simultaneously, with both amino acids. This is consistent with the observation that removal of the 5-hydroxyl group of 5-HT results in several-fold decreased affinity.[30] These hydrogen bond interactions were stable during a 100-ps dynamics run (see Experimental Section). Similar results were obtained with EMDT (**5**) except that the methoxy oxygen atom is a little closer (2.6 Å) to Ser111 [3.37] and Thr196 [5.46].

Both optical isomers of EMDT analog **8** were also docked in a similar manner. Of the 100 docking solutions, the top solution (receptor conformation) for both isomers was the same docking model identified for 5-HT (**1**) and EMDT (**5**). The primary difference in docking modes for **8*R*** and **8*S*** is that the *N*-methyl group of **8*R*** was directed toward the extracellular region (opening) of the binding cavity, whereas for **8*S***, the *N*-methyl group faced the intracellular region (bottom) of the binding cavity. The pose distinctions for the isomers of **8** are reflected in their ChemScore values of 36.96 for **8*R*** and 35.13 for **8*S***. The ChemScore is larger for the higher-affinity **8*R***, which is consistent with the observed binding affinities for the two isomers. While the scores are similar in magnitude, there are some important but subtle differences in the components that contribute to the total ChemScore values. Analysis of the individual ChemScore components indicated that the hydrogen bond component was higher for the *R*-isomer (2.62) than for the *S*-isomer (2.40). This suggests that the ammonium group of **8*R*** is able to form a stronger ionic/hydrogen bond with Asp106 than can the ammonium group of **8*S***. Other differences in the ChemScore components occurred for the clash (**8*R*** = 3.84; **8*S*** = 3.30) and internal torsion (**8*R*** = 4.12; **8*S*** = 5.77) penalty terms. The clash penalty term, which is a measure of undesirable van der Waals overlap between atoms of the ligand and those of the receptor, was higher for **8*R***, whereas the internal torsion penalty, which is a measure of the deviation from ideality of the rotatable bonds (i.e., gauche vs eclipsed substituents), was higher for **8*S***. Analyses of molecular dynamics simulation data to explore more fully the significance of these terms as they relate to the binding modes of the two isomers are ongoing.

MS-245 and Analogs. A common binding model was identified for MS-245 (**4a**), pyrrolidine **6*R***, and aminotetralin **7*R*** (as illustrated in Figure 3 for MS-245). With the basic amine of each ligand within 2.7 Å of Asp106, their aryl moieties are situated in essentially the same hydrophobic pocket described above for 5-HT (**1**); however, they are positioned differently than 5-HT. For example, the benzenesulfonyl aryl groups (see Aryl′ in Table 1) are within 3.5 to 4.0 Å of Ala157, Ala192, and Phe285. Furthermore, the sulfonyl oxygen atoms are within hydrogen bond distance (O−O distance: 2.8–3.3 Å) to both Ser111 [3.37] and Thr196 [5.46], except for **6*R***, which is located within 3.0 Å of Ser111 and 3.8 Å away from Thr196. The model indicates that the sulfonyl oxygen atoms should be able to participate in hydrogen bond formation with at least one, if not both, of these amino acids. That the model is common to MS-245 (**4a**) and stereochemically or conformationally defined analogs **6*R*** and **7*R*** is telling and suggests that the conformation of MS-245, shown in Figure 3, reflects a conformation optimal for binding. Furthermore, as shown in Figure 3, the benzenesulfonyl group is nearly 90° out of the plane of the indole nucleus; this is a conformation we had previously predicted

would be optimal for binding on the basis of studies with conformationally constrained benzenesulfonyl analogs.[18]

The current model, like prior models, shows that the sulfonyl oxygen atoms of sulfonyl-containing antagonists are positioned for a productive interaction with certain receptor-associated features. This raises the obvious question: Are the sulfonyl oxygen atoms actually required for binding? To address this issue, we prepared and examined sulfenamide **9** (K_i = 90 nM), which was found to bind with 20-fold lower affinity than its sulfonyl counterpart **4b** (K_i = 4.1 nM). This provides the first evidence that at least one of the sulfonyl oxygen atoms of MS-245-type analogs is required for enhanced affinity. Moreover, that the sulfonyl oxygen atom(s), and not the methoxy group, of MS-245 (**4a**; K_i = 2.1 nM) interacts with Thr196 is consistent with the finding that removal of the methoxy group has little impact on affinity (i.e., **4b** K_i = 4.1 nM). Also, as shown in Figure 3, there are no amino acid residues within about 7 Å that could potentially hydrogen bond with this methoxy group. Indeed, even an intact indole nucleus is not required for tryptamines to bind at 5-HT$_6$ receptors. This is evident from the binding of phenylethylamine **15** (K_i = 52 nM)[31] and aminotetralins **7**. Compound **16** (K_i = 15 nM),[31] which lacks the benzenoid ring and methoxy substituent of MS-245 (**4a**), binds with only 4-fold lower affinity than **4b**. This further supports the notion that the methoxy substituent of MS-245 is unlikely to interact with a specific receptor-associated feature. The somewhat reduced affinity of **16** relative to **4** can be explained by the fewer aromatic/hydrophobic contacts possible with the abbreviated heterocyclic nucleus.



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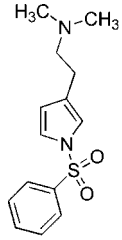

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Consistency with Site-Directed Mutagenesis Results. As already mentioned, Asp106 and Thr196 have been shown to be important for ligand binding at 5-HT$_6$ receptors. Two prior modeling studies with N$_1$-unsubstituted tryptamine analogs (i.e., 5-HT and LSD) have suggested that the indolic N$_1$-H moiety might be within hydrogen bonding distance to Thr196,[16,17] a finding not inconsistent with earlier site-directed mutagenesis studies that showed the importance of this residue for agonist binding and agonist efficacy. That is, on the basis of site-directed mutagenesis studies showing that the affinity of N$_1$-unsubstituted LSD-related ergolines (i.e., LSD, ergotamine, lisuride) is decreased and that the affinity of N$_1$-methyl ergolines (i.e., methysergide and mesulergine, but not metergoline) is enhanced when Thr196 is mutated to alanine, it was speculated that the indolic NH of 5-HT (**1**) might participate in hydrogen bond formation with Thr196.[13] This is certainly a possibility; however, it is not known how ergolines bind relative to 5-HT. In fact, comparing the affinities of various ergolines at wild type and several mutant 5-HT$_{2A}$ receptors, it has been suggested that there are differences in binding modes even among the ergolines.[32] Hence, there is no compelling or a priori reason to suspect, or assume, that 5-HT (**1**) and LSD-related ergolines must bind in a comparable manner to 5-HT receptors. Furthermore, we have found that N$_1$-substituted analogs of 5-HT-related tryptamines either display reduced or enhanced affinity for 5-HT$_6$ receptors (depending upon the nature of the N$_1$-substituent), and have previously attributed these affinity differences to different modes of binding.[9,18,19]

The present model shows that the hydroxyl group, not the N$_1$-H, of 5-HT (**1**) is situated within hydrogen bonding distance to Thr196 [5.46] (and Ser111 [3.37]). Interesting to note is that there are two conserved hydroxyl-containing amino acids (Ser204 [5.43] and Ser207 [5.46]) common to rhodopsin-like G-protein coupled receptors that bind catecholamine agonists (i.e., dopamine receptors, adrenergic receptors)[33] and that it has been shown on the basis of site-directed mutagenesis studies that these two residues are important for binding and agonist action and probably participate in hydrogen bond interactions with the hydroxyl groups of catecholaminergic agonists.[33–35]

Other than for Asp106 and Thr196, few other single amino acid mutations have been reported. Mutation of the rat Trp102 [3.28] (i.e., W102F mutant) had only a small effect on the affinity of 5-HT (<2-fold) or on several nonselective antagonists (<6-fold).[12] Although Trp102 is not within 5 Å of docked 5-HT, it is within 4.5−5.0 Å of EMDT (**5**) and the other agents examined (see Supporting Information). Given its distance from the docked molecules, Trp102 might not be expected to exert a major influence on binding. In the rat, mutation of Ala154 [4.53] to serine had no effect on the receptor affinity or agonist action of 5-HT;[12] Ala154 was not found within 5.0 Å of docked 5-HT in the current model. Teitler and co-workers[36] found that mutation of Ser267 [6.34] to lysine enhances the affinity of 5-HT at human 5-HT$_6$ receptors. However, it is unlikely that Ser267 is directly involved in the binding of 5-HT because it is near the intracellular margin of the receptor. Furthermore, it has been demonstrated that the Ser267 mutation results in a constitutively active receptor,[36,37] and it is known that constitutively active forms of 5-HT receptors characteristically display enhanced affinity for 5-HT.[38] Human 5-HT$_6$ receptor mutation constructs also have been examined in the intracellular loop regions.[39] On this basis, a particular residue (Lys265) has been shown instrumental in the coupling of 5-HT$_6$ receptors to Gα_s; however, there is no reason to suspect any direct involvement in ligand binding.

Taken together, new models are presented for the interaction of 5-HT and several tryptamine-related ligands at 5-HT$_6$ receptors. Docking solutions were identified for the interaction of agonist 5-HT (**1**), EMDT (**5**), and EMDT analog **8**, each at 100 different receptor conformations. In each case, their preferred (based on ChemScore) docking solution was the same. With this model, the basic amines interact with Asp106, the 5-hydroxy (or methoxy) group interacts with Thr196 and/or Ser111, and the indolic nucleus is located in an aromatic/hydrophobic pocket. Antagonist MS-245 (**4a**) binds differently; MS-245 utilizes the same aromatic/hydrophobic pocket but otherwise binds in an altogether different fashion than 5-HT. Specifically, the sulfonyl oxygen atoms are situated within hydrogen bond distance of Thr196 and Ser111, and the benzenesulfonyl aryl group (Aryl′; Table 1) interacts with Ala157, Ala192, and Phe285. (Note: other nearby amino acid residues are either shown in Figure 3 or are listed in Supporting Information.) Benzenesulfonyl compounds **6*R*** and **7*R*** were found to dock in a similar manner to this common model.

In summary, two relatively similar, but distinct, 5-HT$_6$ receptor binding models are described: one for the agonist 5-HT (**1**) and another for the antagonist MS-245 (**4a**). Both ligands utilize a common binding domain but are oriented quite differently within this region. There is consistency with the

Table 2. Results of Microanalysis (Atlantic Microlab)

	calculated/found		
	%C	%H	%N
7*R*	57.51	6.44	7.45
	57.87	6.21	7.46
7*S*	57.51	6.44	7.45
	57.45	6.10	7.36
8*R*	66.11	8.16	9.07
	66.29	8.13	9.18
8*S*	66.11	8.16	9.07
	65.83	8.12	8.88
9	62.16	5.74	7.25
	61.94	5.76	7.26

binding of other tryptamine-related analogs at one or the other of these two models, depending upon the presence or absence of an N_1-benzenesulfonyl group, and the models are also consistent with structure−affinity findings and the results of site-directed mutagenesis. We already have provided empirical evidence that tryptamine analogs likely bind differently at 5-HT_6 receptors, depending upon whether or not they possess an N_1-benzenesulfonyl group; thus, it is particularly gratifying that two distinct modes of binding were identified by the automated docking procedure employed herein. The models will now be employed to examine the binding of other 5-HT_6 receptor agonist and antagonist ligands to define pharmacophore models for 5-HT_6 receptor agonists and antagonists.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer, and peak position are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Microanalyses were performed by Atlantic Microlab (GA) for the indicated elements, and the results are within 0.4% of calculated values. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates, and chromatographic separations were performed on silica gel columns.

R*-(+)-5-Benzenesulfonamido-2-(dimethylamino)tetralin Hydrochloride (7*R*).** A solution of benzenesulfonyl chloride (0.08 g, 0.5 mmol) in dry CH_2Cl_2 (2 mL) was added in a dropwise manner at 0 °C to a solution of **13*R (0.09 g, 0.5 mmol) in dry CH_2Cl_2 (2 mL). The reaction mixture was allowed to stir at 0 °C for 3 h, solvent was evaporated under reduced pressure, and the residue was recrystallized from MeOH/Et_2O. The residue was converted to a salt by treatment of a dry methanolic solution with HCl-saturated anhydrous Et_2O. The precipitate was collected by filtration and recrystallized from MeOH/Et_2O to give **7*R*** (0.05 g, 28%) as light-pink crystals: mp 194–196 °C; $[\alpha]^{20}_D = +69.0°$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.50 (m, 1H, CH_2), 2.19 (m, 1H, CH_2), 2.47 (m, 1H, CH_2), 2.74–3.00 (m, 8H, CH_3, CH_2), 3.14 (d, 1H, CH_2), 3.36 (m, 1H, CH_2), 6.73 (d, 1H, ArH), 6.99–7.09 (m, 2H, ArH), 7.55–7.60 (m, 2H, ArH), 7.64–7.69 (m, 3H, ArH), 9.64 (bs, 1H, NH), 10.66 (bs, 1H, NH^+). Anal. Calcd for ($C_{18}H_{22}N_2O_2S \cdot HCl \cdot 0.5H_2O$) C, H, N (see Table 2).

S*-(−)-5-Benzenesulfonamido-2-(dimethylamino)tetralin Hydrochloride (7*S*).** Compound **7*S was prepared from **13*S*** in 32% yield in the same manner as **7*R***. The salt was obtained as light-pink crystals after recrystallization from MeOH/Et_2O: mp 195–197 °C; $[\alpha]^{20}_D = -75.7°$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.51 (m, 1H, CH_2), 2.19 (m, 1H, CH_2), 2.47 (m, 1H, CH_2), 2.75–3.01 (m, 8H, CH_3, CH_2), 3.13 (d, 1H, CH_2), 3.35 (m, 1H, CH_2), 6.73 (d, 1H, ArH), 6.99–7.09 (m, 2H, ArH), 7.54–7.60 (m, 2H, ArH), 7.64–7.69 (m, 3H, ArH), 9.64 (bs, 1H, NH), 10.65 (bs, 1H, NH^+). Anal. Calcd for ($C_{18}H_{22}N_2O_2S \cdot HCl \cdot 0.5H_2O$) C, H, N (see Table 2).

R*-(+)-2-Ethyl-5-methoxy-3-[(1-methylpyrrolidin-2-yl)methyl]-1*H*-indole Hydrochloride (8*R*).** A solution of **14*R (0.54 g, 1.38 mmol) in dry THF (7 mL) was added in a dropwise manner at 0 °C to a stirred suspension of $LiAlH_4$ (0.24 g, 6.21 mmol) in dry THF (7 mL). The resulting mixture was heated under a N_2 atmosphere for 4 h. The reaction mixture was cooled, and sodium sulfate decahydrate (7 g) was added very carefully, portionwise, followed by H_2O (0.24 mL) and EtOAc (7 mL). The resulting mixture was allowed to stir at room temperature under a N_2 atmosphere for 24 h. The reaction mixture was then filtered through Celite, and solvent was evaporated under reduced pressure. The residual oil was then chromatographed on a silica gel column (Aldrich silica gel 60) using first EtOAc (to remove benzyl alcohol) and then EtOAc/MeOH/NH_4OH (9:1:2 drops) as eluent to give a crude product (0.33 g, 88%) as an oil. The product was converted to its hydrochloride salt in anhydrous MeOH by addition of HCl/Et_2O to give an oil that solidified under high vacuum. The solid was recrystallized from CH_2Cl_2/Et_2O to give the product (0.27 g, 64%) as off-white crystals: mp 157–159 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, CH_3), 1.68–1.92 (m, 4H, CH_2), 2.72 (m, 2H, CH_2), 2.87 (s, 3H, CH_3), 2.92–3.06 (m, 2H, CH_2), 3.27 (dd, 1H, CH_2), 3.48–3.58 (m, 2H, CH_2), 3.77 (s, 3H, CH_3), 6.67 (d, 1H, ArH), 7.00 (s, 1H, ArH), 7.16 (d, 1H, ArH), 10.75 (s, 1H, NH); $[\alpha]^{20}_D = +19.6$ (*c* 0.5, MeOH). Anal. Calcd for ($C_{17}H_{24}N_2O \cdot HCl$) C, H, N (see Table 2).

S*-(−)-2-Ethyl-5-methoxy-3-[(1-methylpyrrolidin-2-yl)methyl]-1*H*-indole Hydrochloride (8*S*).** Beginning with the acid chloride of *N*-(benzyloxycarbonyl)-L-proline (i.e., **14*S), the target compound was prepared in 61% yield in the same manner as its *R*-(+)-enantiomer **8*R***, and isolated as off-white crystals after recrystallization from CH_2Cl_2/Et_2O: mp 156.5–158.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (t, 3H, CH_3), 1.68–1.94 (m, 4H, CH_2), 2.73 (m, 2H, CH_2), 2.86 (s, 3H, CH_3), 2.94–3.08 (m, 2H, CH_2), 3.27 (dd, 1H, CH_2), 3.48–3.58 (m, 2H, CH_2), 3.77 (s, 3H, CH_3), 6.67 (d, 1H, ArH), 7.00 (s, 1H, ArH), 7.16 (d, 1H, ArH), 10.75 (s, 1H, NH); $[\alpha]_D = -24.6$ (*c* 0.5, MeOH). Anal. Calcd for ($C_{17}H_{24}N_2O \cdot HCl$) C, H, N (see Table 2).

1-Phenylthio-*N*,*N*-dimethyltryptamine Oxalate (9). A solution of *N*-(phenylthio)succinimide[26] (0.21 g, 1.0 mmol) in CH_2Cl_2 was added to a vigorously stirred mixture of *N*,*N*-dimethyltryptamine (0.09 g, 0.5 mmol), tetrabutylammonium hydrogen sulfate (0.02 g, 0.05 mmol), and 50% aqueous KOH (0.5 mL) in CH_2Cl_2 (2.5 mL) over a 10−15 min period at room temperature. After half of the succinimide was added, additional tetrabutylammonium hydrogen sulfate (0.02 g, 0.05 mmol) was added to the reaction mixture. The reaction mixture was washed with H_2O (3 × 10 mL), the organic portion was separated and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give an oily yellow residue. Purification by chromatography on a silica gel column (Aldrich silica gel 60) using CH_2Cl_2/MeOH (9:1) as eluent gave **9** (free base; 0.10 g, 68%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H, CH_3), 2.67 (t, 2H, CH_2), 2.96 (t, 2H, CH_2), 6.92 (d, 2H, ArH), 7.06 (s, 1H, ArH), 7.13–7.31 (m, 5H, ArH), 7.58–7.65 (m, 2H, ArH). A small sample of the free base was converted to the oxalate salt and recrystallized from MeOH to give the product as white crystals: mp 191–192 °C. Anal. Calcd for ($C_{18}H_{20}N_2S \cdot C_2H_2O_4$) C, H, N (see Table 2).

R*-5-Acetamido-2-dimethylaminotetralin (12*R*).** A solution of aqueous H_2CO (37% w/w, 0.79 mL) was added in a dropwise manner to a cooled (ice bath) and stirred mixture of *R*-acetamido-2-aminotetralin[23] (**11*R; 0.51 g, 2.5 mmol), sodium cyanoborohydride (0.35 g, 5.6 mmol), and glacial HOAc (0.14 mL, 2.5 mmol) in a mixture of dry MeOH (30 mL) and MeCN (10 mL). The mixture was allowed to warm to room temperature and stirring was continued for 3 h before addition of saturated aqueous K_2CO_3 (25 mL). Organic solvent was removed under reduced pressure, and the residue was diluted with H_2O (25 mL) and extracted with EtOAc (3 × 30 mL). The combined organic portion was washed with brine and dried (Na_2SO_4). Solvent was removed under reduced pressure to give the product (0.53 g, 91%) as an oily residue that was used in the preparation of **13*R***: ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 1H, CH_2),

2.19–2.29 (m, 4H, CH_2, CH_3), 2.48–2.56 (m, 7H, CH_2, CH_3), 2.66 (m, 1H, CH_2), 2.82–2.94 (m, 2H, CH_2), 3.06 (d, 1H, CH_2), 6.97 (d, 1H, ArH), 7.14–7.19 (m, 2H, ArH, NH), 7.54 (d, 1H, ArH).

S*-5-Acetamido-2-*N*,*N*-dimethylaminotetralin (12*S*).** The compound was prepared in 87% yield from *S*-5-acetamido-2-aminotetralin (**11*S),[23] in the same manner as **12*R***, and isolated as an oily residue: ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 1H, CH_2), 2.19–2.29 (m, 4H, CH_2, CH_3), 2.48–2.56 (m, 7H, CH_2, CH_3), 2.66 (m, 1H, CH_2), 2.82–2.94 (m, 2H, CH_2), 3.06 (d, 1H, CH_2), 6.97 (d, 1H, ArH), 7.14–7.19 (m, 2H, ArH, NH), 7.54 (d, 1H, ArH).

R*-5-Amino-2-dimethylaminotetralin (13*R*).** Concentrated HCl (20 mL) was added to a solution of *R*-5-acetamido-2-dimethylaminotetralin (**12*R; 0.50 g, 2.2 mmol) in absolute EtOH (60 mL), and the reaction mixture was heated at reflux for 2 h. The mixture was cooled to 0 °C and basified with 40% NaOH to pH 12. The mixture was extracted with CH_2Cl_2 (3 × 50 mL), the combined organic portion was dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give the product (0.35 g, 86%) as a semisolid material: ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 1H, CH_2), 2.30 (m, 1H, CH_2), 2.41–2.56 (m, 7H, CH_3, CH_2), 2.68–2.86 (m, 3H, CH_2), 2.97 (d, 1H, CH_2), 3.60 (bs, 2H, NH_2), 6.56 (d, 1H, ArH), 6.59 (d, 1H, ArH), 6.99 (t, 1H, ArH). The compound was used without further purification in the synthesis of **7*R***.

S*-5-Amino-2-dimethylaminotetralin (13*S*).** Beginning with **12*S, compound **13*S*** was prepared in 78% yield in the same manner as **13*R*** and isolated as a semisolid material: ^{1}H NMR ($CDCl_3$) δ 1.71 (m, 1H, CH_2), 2.27 (m, 1H, CH_2), 2.44–2.55 (m, 7H, CH_3, CH_2), 2.63–2.84 (m, 3H, CH_2), 2.96 (d, 1H, CH_2), 3.60 (bs, 2H, NH_2), 6.55 (d, 1H, ArH), 6.59 (d, 1H, ArH), 6.98 (t, 1H, ArH).

***R*-3-[[*N*-(Benzyloxycarbonyl)pyrrolidin-2-yl]carbonyl]-2-ethyl-5-methoxy-1*H*-indole (14*R*).** Oxalyl chloride (0.19 mL, 2.21 mmol) was added to a stirred solution of *N*-(benzyloxycarbonyl)-D-proline (0.35 g, 1.47 mmol) in dry CH_2Cl_2 (5 mL) with a trace of DMF (1 drop). The resulting effervescent solution was allowed to stir at room temperature under a N_2 atmosphere for 3 h. Solvent was evaporated under reduced pressure, dry hexane (3 mL) was added, the resulting solution was again evaporated under reduced pressure to afford *N*-(benzyloxycarbonyl)-D-proline acid chloride, and the crude product was dissolved in dry benzene (3 mL).

Concomitantly, a solution of ethyl magnesium bromide (3.0 M in Et_2O, 1.07 mL, 3.09 mmol) was added in a dropwise manner to a stirred solution of 2-ethyl-5-methoxyindole[24] (0.52 g, 2.94 mmol) in benzene (10 mL) at 0 °C under a N_2 atmosphere. The resulting mixture was allowed to stir at the same temperature for 15 min, then the solution of *N*-(benzyloxycarbonyl)-D-proline acid chloride in benzene was added in a dropwise manner with vigorous stirring. The reaction mixture was allowed to stir at 0 °C under a N_2 atmosphere for 1 h, a saturated aqueous solution of $NaHCO_3$ (7 mL) and EtOAc (9 mL) were successively added, and the mixture was allowed to stir at room temperature for an additional 1 h. The organic layer was removed and the aqueous portion was extracted with EtOAc (3 × 10 mL). The organic extracts were combined and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column (Aldrich silica gel 60) using CH_2Cl_2/MeOH (195:5) as eluent to give a crude product (0.27 g, 47%) as a semisolid material. The product was used without further characterization in the preparation of **8*R***.

S*-3-[[*N*-(Benzyloxycarbonyl)pyrrolidin-2-yl]carbonyl]-2-ethyl-5-methoxy-1*H*-indole (14*S*).** Compound **14*S was prepared in 51% yield from *N*-(benzyloxycarbonyl)-L-proline in the same manner as **14*R*** and isolated as a yellow semisolid material. The product was used without further characterization in the preparation of **8*S***.

Radioligand Binding Assay. The h5-HT$_6$ radioligand binding assays were performed as previously described.[3] In brief, h5-HT$_6$ cDNA was transiently expressed in HEK-293 cells using Fugene6 according to the manufacturer's recommendations. At 24 h after transfection, the medium was replaced; 24 h later, medium containing dialyzed serum (to remove 5-HT) was added. At 75 h after transfection, cells were harvested by scraping and centrifugation. Cells were then washed by centrifugation and resuspension once in phosphate-buffered saline (pH = 7.40; PBS) and then frozen as tight pellets at −80 °C until use. Binding assays were performed at room temperature for 90 min in binding buffer (50 mM Tris-Cl, 10 mM $MgCl_2$, 0.1 mM EDTA, pH = 7.40) with [^{3}H]LSD (1 nM final concentration) using 10 μM clozapine for nonspecific binding. Various concentrations of unlabeled test agent were used for K_i determinations, with K_i values calculated using the program GraphPad Prism. Specific binding represented 80–90% of total binding. K_i values represent a minimum of triplicate determinations.

Computational Methods. Molecular modeling studies were conducted using AMD 64 Dual-Core Opteron-based HP xw9400 and Intel Dual-Core Xeon-based SGI VSS40 Linux workstations utilizing SYBYL (version 7.3; Tripos Associates, Inc., St. Louis, MO).

The amino acid sequence of the human 5-HT$_6$ receptor (accession code P50406) was retrieved from the SwissProt-ExPASY databank and aligned to the sequence of bovine rhodopsin (P02699) based on the Bissantz et al.[40] multiple alignment profile of several diverse type A human GPCRs: dopamine D_3 (P35462), muscarinic acetylcholine m_1 (P11229), vasopressin V_{1a} (P37288), β_2-adrenoceptor (P07550), and δ-opioid (P41143). The ClustalX[41,42] multiple alignment program was used; in short, (i) the amino acid sequences of the GPCR profile were aligned to the sequence of bovine rhodopsin in two steps starting from the first transmembrane helix (TM1) to the first five residues of the third intracellular loop (i3) and then from the last five residues of the i3 loop to the end of the seventh transmembrane helix (TM7) using the BLOSUM weight matrices and with a gap opening penalty of 15.0, and (ii) the alignment of extracellular loop e2 was manually adjusted to align the conserved disulfide bridge in the structure of bovine rhodopsin with the corresponding cysteines in all five aminergic receptors. This alignment provided an unambiguous mapping of residues in the TM regions containing no additions or deletions.

When the alignment described above was used, 3D models of the h5-HT$_6$ receptor were generated based on the 2.2 Å resolution X-ray crystal structure[43] of bovine rhodopsin ("A" chain of PDB code 1U19). Using MODELER[44] (version 9.1), a population of 100 models was generated whose members possessed varied side chain and, to a lesser extent, backbone conformations. The N- and C-termini were truncated prior to the first and after the seventh TM helix, respectively. The e3 loop (which varies considerably both in terms of length and sequence identity between bovine rhodopsin and 5-HT$_6$) was modeled as a simple poly Gly sequence whose initial backbone coordinates were taken from rhodopsin. Following the procedure of Xhaard et al.,[47] the variation in the side chain conformations of the MODELER-generated h5-HT$_6$ receptors was maximized by mutating to alanine the residues falling within 12.0 Å of the retinal ligand in the 1U19 MODELER rhodopsin template. After adding hydrogen atoms to the one hundred MODELER-generated h5-HT$_6$ models, the receptors were energy-minimized (Tripos Force Field, Powell minimization, termination criterion: energy gradient ≤ 0.05 kcal/(mol·Å), Gasteiger−Hückel charges with a distance-dependent dielectric constant = 4.0, and a nonbonded cutoff of 8 Å) using a SYBYL programming language script (written in-house).

The 3D structures of the ligands were generated using the SKETCH MOLECULE command within SYBYL. For chiral compounds, the individual isomers were sketched as separate structures. Basic amines were protonated. All ligand structures were then energy-minimized using the same parameters as described above for the receptor models.

The automated docking program GOLD [45,46] (Genetic Optimization for Ligand Docking, version 3.1, CCDC, Cambridge, U.K.) was employed to dock ligands into each of the one hundred h5-HT$_6$ receptor models. A 20 Å radius from the Asp106 [3.32] side chain C^γ atom was used to define the receptor binding cavity. A protein hydrogen bonding constraint was defined that included the oxygen atoms of the Asp106 [3.32] side chain. Docking simulations were not restricted to early termination and the ligands were allowed to rotate around single bonds and nonaromatic rings were free to

flip. A total of 10 genetic algorithm docking runs were performed for each ligand structure. The ChemScore fitness function (an estimation of the total free energy change that occurs on ligand binding) was used to rank the docked ligand–receptor complexes. Based on the resulting ChemScores, one of the 100 MODELER-generated receptor models was selected for each ligand. Two such receptors were consistently identified: one being common to ligands lacking the sulfonamide group and another common to ligands containing a sulfonamide moiety.

The PROTABLE facility within SYBYL and the PROCHECK program were employed to identify potential, unusual, and sterically unfavorable side chain geometries; these were iteratively corrected as necessary.

All receptor/ligand complexes were subjected to short molecular dynamics simulations using the DYNAMICS routine within SYBYL to provide additional evidence that the GOLD-docked poses were not artifactual (i.e., to assess the stability of the bound pose of the ligands in the h5-HT_6 receptor models). Molecular dynamics simulations were carried out for 100 ps and snapshots were taken every 25 fs. Otherwise, the default settings for the DYNAMICS were employed. The energy setup was analogous to that described for energy minimization. To maintain the integrity of the ligand–receptor complexes, all residues except the ligand and amino acids within an 8 Å radius of the ligand were maintained as an aggregate; these atoms did not move during the course of the simulation.

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Supporting Information Available: A table showing the root-mean-square distances (RMSD) between the heavy atoms of corresponding residues in the binding pocket of the two selected receptor models [i.e., binding models for 5-HT (**1**) and MS-245 (**4a**), as shown in Figure 1; Table S1] and of amino acids within various distances (in 0.5 Å distance increments from 3.0 to 5.0 Å) of the docked ligands (Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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