# Receptor-Based Pharmacophores for Serotonin 5-HT$_7$R Antagonists—Implications to Selectivity

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A set of 31 diversified 5-HT$_7$ receptor antagonists was automatically docked to a conformational ensemble of rhodopsin-based 5-HT$_7$R models (flexible docking). It was found that ergolines, aporphines, and tricyclic psychotropic agents were always docked in a pocket formed by TMHs 4−6, and besides the main ionic bond with Asp3.32, they had specific interactions with Phe6.52, Phe6.51, Trp6.48, and Ser5.42. The arylpiperidine, arylpiperazine, or $\beta$-carboline fragment of the complex ligands occupied the same pocket, whereas the terminal amide/imide part of those compounds reached the second cavity formed by TMHs 7−3 and interacted with Phe3.28, Arg7.36, and Tyr7.43. A similar orientation of selective antagonists of the group of arylsulfonamidoalkylamines was observed, that is, the sulfonamide part was located in the second pocket. Coherent docking results allowed the generation of two receptor-based pharmacophores: first containing features necessary for high 5-HT$_7$R affinity and the other defining selectivity for this receptor subtype. The latter model indicated the importance of specific interactions with the residues of the TMHs 7−3 pocket (especially nonconserved Arg7.36) for selectivity over other monoamine GPCRs.

## Introduction

The 5-HT$_7$ receptor (5-HT$_7$R[a]), being the last member of the serotonin receptors family identified so far,[1−5] has been proposed to be involved in the regulation of body temperature,[6−8] circadian rhythms,[9−11] learning, and memory,[12−15] as well as neuronal excitability,[16,17] inflammatory processes in the brain,[18] and smooth muscle relaxation of cerebral arteries.[19] The high affinity of many well-known psychotropic drugs for 5-HT$_7$R sites (nonselective antagonists) strongly suggests its role in psychiatric disorders.[20−24] Recent studies conducted on knockout animals, and the lately introduced selective 5-HT$_7$R antagonists, have provided the first conclusive proof of the engagement of this receptor subtype in the pathomechanism of depression.[25,26] Hence, the development and investigation of 5-HT$_7$R antagonists lays down a new direction in the search for novel antidepressant agents.

As a result of the structural diversity of 5-HT$_7$R antagonists, their chemical classification is not clear-cut and may differ between authors. In the present study, we have divided 5-HT$_7$R antagonists into six following classes (see Chart 1): (1) ergolines, nonselective;[2,4,5,16,27,28] (2) aporphines, nonselective, with some exceptions;[29−31] (3) tricyclic psychotropic agents, nonselective;[1,4,5,32] (4) arylpiperidines and their bioisosters, arylpiperazines and $\beta$-carbolines (mostly long-chain arylpiperidines/piperazines [LCAPs] or long chain $\beta$-carbolines [LCBCs]), with different selectivity (e.g., derivatives with a tetrahydrobenzindole or a benzoazepinone as a terminal imide are predominantly selective);[5,33−40] (5) arylsulfonamidoalkylamines, a class of the most potent and selective antagonists;[41−43] (6) compounds with diverse structures, such as diaminopyridines, diaminopyrimidines, and diaminotriazines or 2-aminotetralins and 3-aminochromans, among which there are also selective antagonists.[44−47]

Because 5-HT$_7$R belongs to the family of membrane G-protein-coupled receptors (GPCRs), its tertiary structure remains unknown, and this significantly hampers the effective ligand design. In view of this fact, molecular modeling techniques have been employed to generalize the most specific features of ligands, as well as to describe their potential interactions with a biological target. First pharmacophore hypothesis for 5-HT$_7$R antagonism was presented in 2000[48] and optimized in 2003[49] by Lopez-Rodriguez et al. In 2004, Vermeulen et al. proposed pharmacophore as well as a CoMFA model for 5-HT$_7$R inverse agonism.[50] Those studies were based on the analysis of common structural features of ligands from different 5-HT$_7$R antagonist classes; the obtained pharmacophores or CoMFA fields were then mapped on a modeled receptor protein to propose a putative ligand binding mode. However, such approaches to pharmacophore generation, based exclusively on the analysis of ligand features, assume a common binding site for all the investigated compounds, whereas 5-HT$_7$R antagonists (which are structurally diversified) may have different binding modes and binding sites. Moreover, despite a sizable number of compounds used for developing pharmacophore hypotheses, studies into ligand−receptor interactions were limited to the manual docking of a few compounds to a single-receptor conformation.

Recently, we presented a rhodopsin-based model of the serotonin 5-HT$_{1A}$ receptor (5-HT$_{1A}$R), which was validated by the automated docking of conformationaly restricted arylpiperazine derivatives.[51] Such rigid, yet bioactive, compounds are believed to encode the information about the shape of a binding site and the spatial arrangement of specific interaction points within a binding pocket. The obtained model allowed a detailed description of the ligand binding mode; moreover, its validity was additionally confirmed by successful affinity prediction experiments. Considering similarities between 5-HT$_7$ and 5-HT$_{1A}$ receptors (high sequence homology in the putative binding site and the dual 5-HT$_7$R/5-HT$_{1A}$R affinity of many ligands[52]), we have presently created 5-HT$_7$R model on the basis of similar methodology. The primary goal of the present study is to

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[a] Abbreviations: 5-HT$_7$R, 5-HT$_7$ serotonin receptor; TMH, transmembrane helix; GPCR, G-protein coupled receptor; LCAP, long chain arylpiperazine or piperidine; LCBC, long chain $\beta$-carboline.

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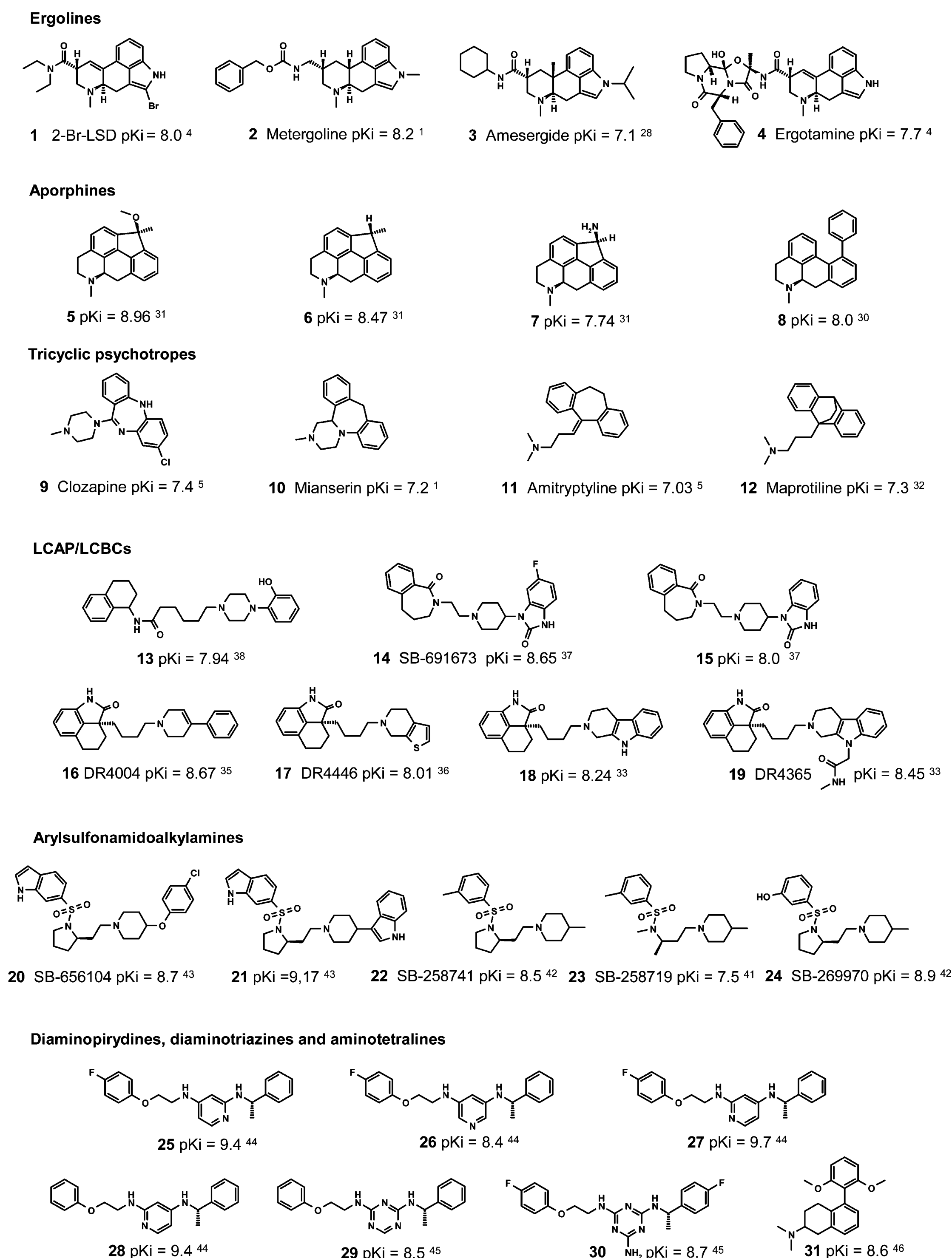
**Chart 1.** Compounds Used for the Binding Mode Determination and Generation of Pharmacophore Hypotheses for 5-HT$_7$R Antagonism[a]




[a] Affinity data come from several different laboratories, and they were obtained in experiments with membranes from various cell lines (COS-7, HEK-293, CHO) expressing 5-HT$_7$ receptors, and also different radioligands ([$^3$H]-5-CT, [$^3$H]-5-HT) were used.

comprehensively elucidate the ligand binding mode for all classes of 5-HT$_7$R antagonists by means of direct interactions with the receptor model. This is meant to help point out essential features pertinent to the affinity and/or selectivity of 5-HT$_7$R antagonists and, thus, to facilitate the search for new ligands with a desired pharmacological profile. Representatives of all classes of 5-HT$_7$R antagonists were automatically docked to the population of receptor conformations, reflecting its flexibility (flexible docking). The top-scored ligand–receptor complexes not only provided information about the binding site and specific interactions for individual classes of ligands, but also served as a basis for new pharmacophore hypotheses. The receptor protein model was used as a part of the "superimposing tool" so those hypotheses may be regarded as "receptor-based".

## Methodology

The methodology used here is based on the automated docking of a compound to a population of protein models, which explores the conformational space of amino acid side chains of the binding site (Figure 1A). Hence, the information about the

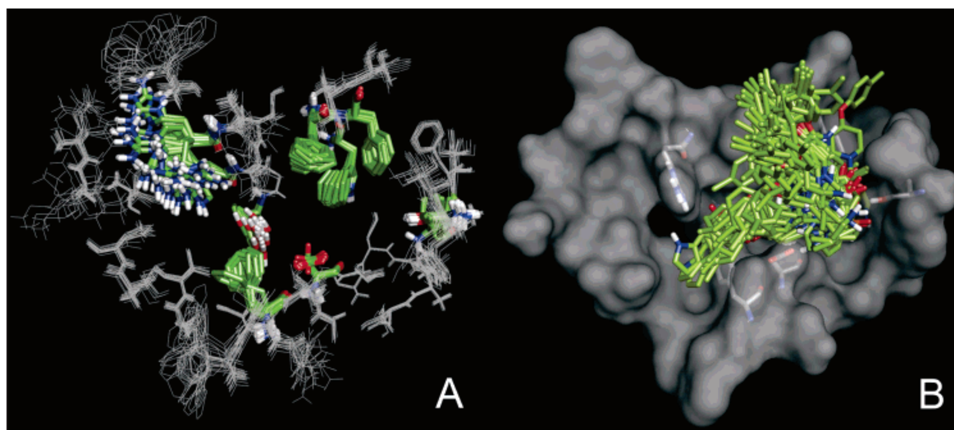



**Figure 1.** (A) The conformational space of 5-HT$_7$R binding site, sampled by Modeller. The residues shown define an "activesite" subset used in FlexX dockings. Amino acids entering into specific interactions with ligands are presented as "sticks". (B) The conformational and 3D spatial sampling of compound **20**, carried out by FlexX. The only constraint is a crucial ionic bond between the protonated nitrogen of the ligand and Asp3.32.

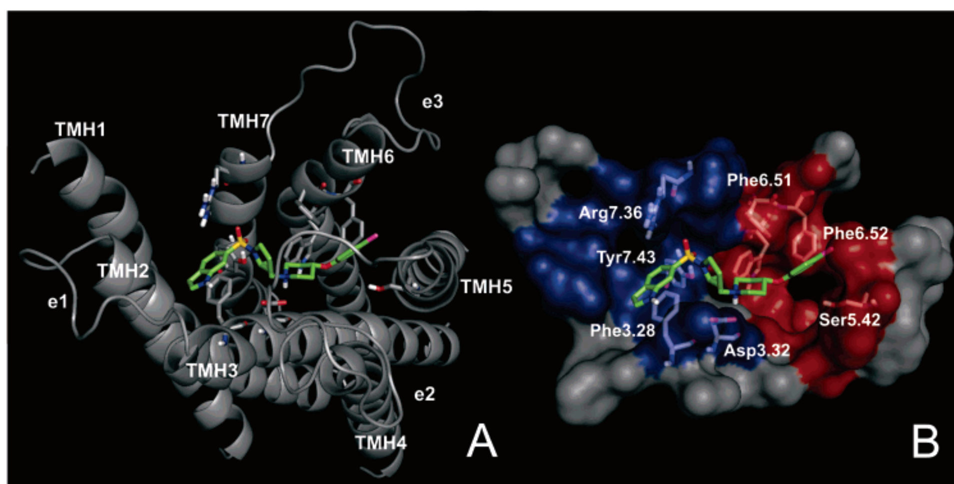



**Figure 2.** A binding site of 5-HT$_7$R with docked compound **20** (a view from the extracellular side); amino acids entering into specific interactions with ligands are presented as "sticks". (A) "Cartoon" helices; (B) surface of the binding site. Two binding pockets, one between TMHs 4−6 (red) and the other between TMHs 7−3, (blue) are indicated.

flexibility of the receptor and its potential induced fit to the bound ligand is included in the resulting model complexes. The flexible docking of a ligand ensures exhaustive sampling of its conformational space within the binding site (Figure 1B). Each obtained ligand−receptor complex is subjected to a consensus scoring procedure to select the results that are well-scored by five scoring functions simultaneously. Ligand poses having the highest PMF_scores of those scored "5" by the consensus scoring procedure (top scored) are used to predict the binding mode and to generate the pharmacophore hypotheses. The ligand−receptor complexes with obvious docking "decoys" (i.e., the H-bond between Asp3.32 and ligand OH or $NH_2$ groups, instead of an ionic bond with a protonated nitrogen) are excluded from the analysis.

## Results

**Binding Site Description.** The binding site of 5-HT$_7$R is located within the heptahelical bundle along the transmembrane helix (TMH) 3 with the centrally placed Asp3.32 (Ballesteros−Weinstein's nomenclature[53]), which provides the main anchoring point for the ligand. The site can be divided into two pockets: one between TMHs 4−6 (buried deeper in the receptor) and the other between TMHs 7−3 (more exposed to the extracellular side). Both of them have similar interaction points for aromatic moieties (Phe6.52/Phe6.51/Trp6.48 and Phe3.28/Arg7.36, respectively) and H-bond acceptors (Ser5.42 and Tyr7.43, respectively), which makes them fairly symmetrical and, thus, equivalent upon ligand docking (Figure 2).

**Ligand Binding Mode.** The binding modes for all classes of 5-HT$_7$R antagonists were proposed based on the analysis of mutual spatial arrangement of particular ligand fragments and receptor side chains. Besides the crucial ionic interaction with Asp3.32 (which was constrained during docking; see the Experimental Section), all the docked ligands had at least one specific, aromatic interaction (CH−$\pi$ or $\pi$−$\pi$) with one of the above-mentioned residues and one or more additional specific interactions, predominantly of the H-bond nature (Figure 3).

Ergoline derivatives were docked to the receptor model in a way enabling formation of the CH−$\pi$ interaction of their benzene ring with Phe6.51. In such a ligand position, the methyl substituent at the basic nitrogen penetrated the small hydrophobic cavity formed by TMHs 3, 6, and 7, while bulky amide moieties, substituted at the 8-position of the ergoline, were situated between TMHs 7−3, enabling amide carbonyl oxygen H-bonding to Tyr7.43 (Figure 3A).

In the case of aporphine derivatives, the whole ligand was placed in the pocket between TMHs 4−6. The two benzene rings of aporphine were prone to create a CH-$\pi$ interaction with Phe6.51 and Phe6.52 (Figure 3B). Like ergolines, the *N*-methyl substituent of aporphine penetrated the same small hydrophobic

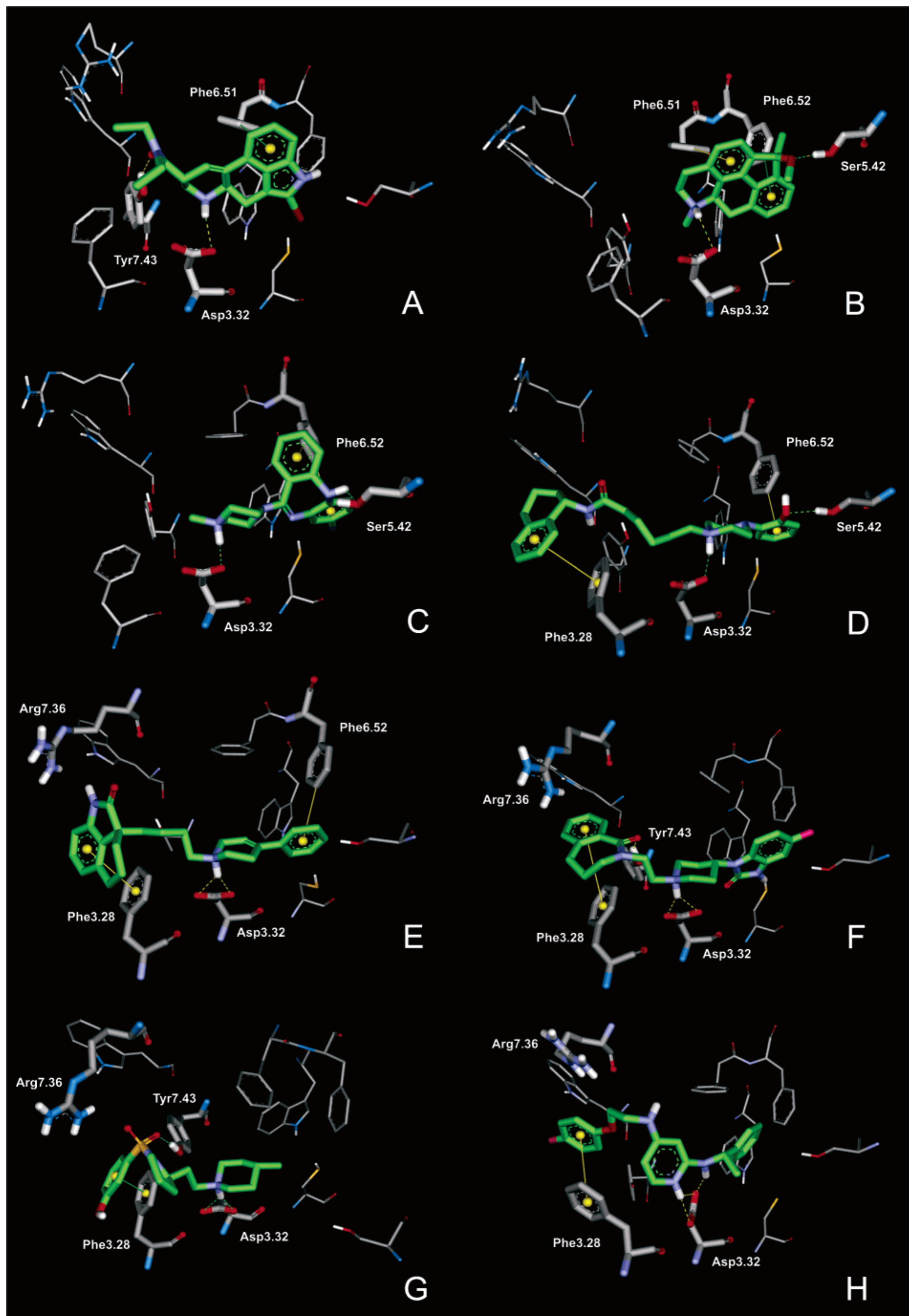



**Figure 3.** Top-scored ligand−receptor complexes of the selected compounds from each class of 5-HT$_7$R antagonists, showing their binding modes. Residues entering into specific interactions with ligands are presented as thick sticks. Dotted yellow lines represent H-bonds with Tyr7.43 or Ser5.43 and a salt bridge with Asp3.32. Solid yellow lines show CH−$\pi$ interactions with Phe6.52, Phe6.51, or Phe3.28 and $\pi$−$\pi$ stacking with Phe3.28. Compounds (A) **1**, (B) **5**, (C) **9**, (D) **13**, (E) **14**, (F) **16**, (G) **24**, and (H) **25**.

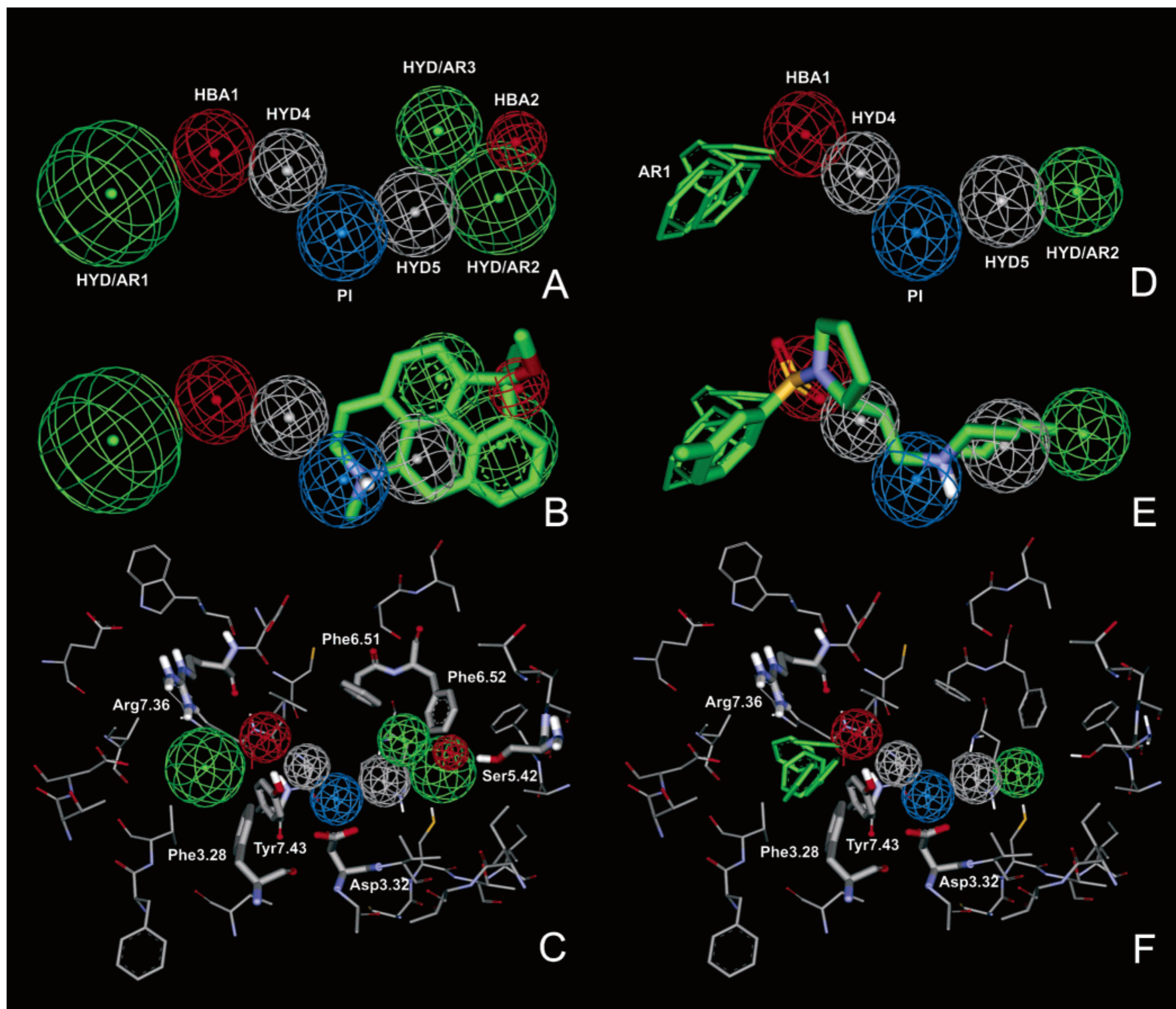



**Figure 4.** Pharmacophore models for 5-HT$_7$R antagonism. (A) A general "affinity" hypothesis; (B) superimposition of nonselective compound **5** on the affinity hypothesis; (C) the affinity hypothesis projected on the binding site model; (D) a "selectivity" hypothesis; (E) superimposition of the selective compound **22** on the selectivity hypothesis; and (F) the selectivity hypothesis projected on the binding site model.

cavity, whereas analogues with the H-bond accepting substituent could additionally form a hydrogen bond with Ser5.42.

The tricyclic moieties of psychotropic drugs were also accommodated by the pocket between TMHs 4−6. In such a pose, one (compounds **10** and **12**) or two (compound **9**) of the benzene rings formed CH−$\pi$ contacts with Phe6.52 or $\pi$−$\pi$ stacking with Trp6.48 (compound **11**). An additional H-bond was also observed between the NH group bridging benzene rings of compound **9** and Ser5.42 (Figure 3C).

Complex ligands of the LCAP/LCBC class consist of two pharmacophoric groups: the main arylpiperidine/arylpiperazine/$\beta$-carboline moiety (containing a crucial basic nitrogen) and the so-called "terminal amide/imide" of different structure (frequently containing another aryl ring), linked together by a flexible (2−5 unit) alkyl spacer. Compounds of that most numerous and diversified class of 5-HT$_7$R antagonists occupied both pockets of the binding site simultaneously, having different interactions due to their diversified structure. The aromatic ring of an arylpiperidine/arylpiperazine/$\beta$-carboline moiety penetrated the pocket between TMHs 4−6, forming a specific CH−$\pi$ interaction or having vdW contacts with one or more residues from the aromatic cluster of TMH6 (Phe6.52, Phe6.51, Trp6.48) (compounds **13**−**19**; Figures 3D−3F). The terminal amide/imide moiety occupied the pocket between TMHs 7−3 (compounds **13**−**19**; Figures 3D−3F), and the carbonyl oxygen could be H-bonded to Tyr7.43 (compound **14**; Figure 3F). Aromatic rings from terminal groups formed specific interactions with Phe3.28 and/or Arg7.36 (compounds **13**−**19**; Figures 3E and 3F). In the case of tetrahydrobenzindole as a terminal imide (compounds **16**−**19**), development of $\pi$−$\pi$ stacking with Phe3.28 and an ion−$\pi$ interaction with Arg7.36 were possible. That was due to the unique geometry of the tetrahydrobenzindole group, which positioned its benzene ring in a way that enabled those favorable interactions (Figure 3E). The benzene ring, condensed with an azepinone moiety (compounds **14** and **15**), stabilized the ligand−receptor complex by a concurrent ion−$\pi$ interaction with Arg7.36 and a CH−$\pi$ interaction with Phe3.28 (Figure 3F).

Being a class of the most potent and selective 5-HT$_7$R antagonists, arylsulfonamidoalkylamines can be divided into two subgroups: (1) compounds with a bulky aromatic substituent at the 4-position of piperidine, which could actually be also classified as complex arylpiperidines (LCAPs; **20** and **21**) and (2) compounds with a small methylpiperidine moiety only (**22**−**24**). The arylsulfonamide part of the molecule was situated in

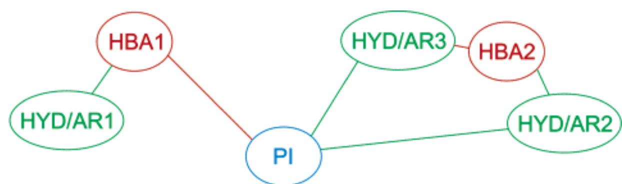



**Figure 5.** Pharmacophoric features representing specific interaction points in the structure of ligands, providing affinity and selectivity toward 5-HT$_7$R.

the pocket between TMHs 7−3, and the sulfone oxygen formed an H-bond with Tyr7.43, while the aromatic moiety was in an optimal position for the $\pi-\pi$ stacking and ion$-\pi$ interaction with Phe3.28 and Arg7.36, respectively. *p*-Cl-Phenoxy, indole, or methyl substituents at the piperidine ring interacted with the hydrophobic/aromatic residues in the pocket created by TMHs 4−6 (Figures 2 and 3G).

Diaminopyridine and diaminotriazine derivatives (**25**−**30**) interacted with Asp3.32, forming an ionic bond with the protonated nitrogen of the pyridine or triazine ring and also frequently hydrogen bonded with one of the amine groups substituted to the central heterocyclic ring. The phenoxyethyl substituent was located in a close vicinity of Phe3.28, which led to $\pi-\pi$ stacking between the benzene rings of the ligand and phenylalanine. Simultaneously, an ion$-\pi$ interaction between the ligand phenoxy moiety and the side-chain of Arg7.36 was observed in a certain number of obtained complexes. The phenyl substituent at the other end of the ligand penetrated the pocket between TMHs 4−6, having vdW contact with hydrophobic residues (Figure 3H). The aminotetralin derivative (**31**) was docked in a way analogous to the compounds from the aporfine class.

**Generation of a Pharmacophore Model.** The ligand poses used for constructing pharmacophores had the highest PMF_score values among those scored “5” in a consensus scoring procedure (top-scored). Because a number of ligands studied are selective for 5-HT$_7$R, the pharmacophore model for antagonists was divided into two distinct submodels: (1) a 5-HT$_7$R “affinity” pharmacophore and (2) a 5-HT$_7$R “selectivity” pharmacophore.

**Affinity Pharmacophore for 5-HT$_7$R Antagonists (Figures 4A−4C).** A general “affinity” hypothesis defines six features representing specific interaction points in the ligand structure, that is, a protonated nitrogen (positive ion, PI), three hydrophobic/aromatic regions (HYD/AR1−3), and two H-bond acceptors (HBA1,2; Figures 4A and 5 and Chart 2). For 5-HT$_7$R affinity, at least three of them must be present in a specific spatial arrangement. Two features are of strictly defined nature, that is, PI and one of ARs (capable of specific CH$-\pi$ or $\pi-\pi$ interaction), while the third may be HBA or another HYD/AR region (Figures 4A and 5 and Chart 2). The distances between these features are shown in Table 1. Each of the features has its counterpart in the receptor structure; PI is involved in the salt bridge formation with Asp3.32, AR1 interacts with Phe3.28 (CH$-\pi$ or $\pi-\pi$) and/or Arg7.36 (ion$-\pi$), AR2, and AR3 have a CH$-\pi$ contact with Phe6.52 and Phe6.51, while HBA1 and HBA2 form H-bonds with Tyr7.43 and Ser5.42, respectively (Figure 4C). HYD4 and HYD5 are additional, nonspecific, hydrophobic regions.

**Chart 2.** Assignment of Pharmacophoric Features to Specific Moieties of 5-HT$_7$R Antagonists. Asterisks Show Members of an Essential Pharmacophoric Triplet. The Shifting of Crucial Pharmacophoric Features from the Moieties Accommodated by TMHs 4−6 Cavity to That Interacting with the TMHs 7−3 Pocket Correlates with the Increase in Selectivity

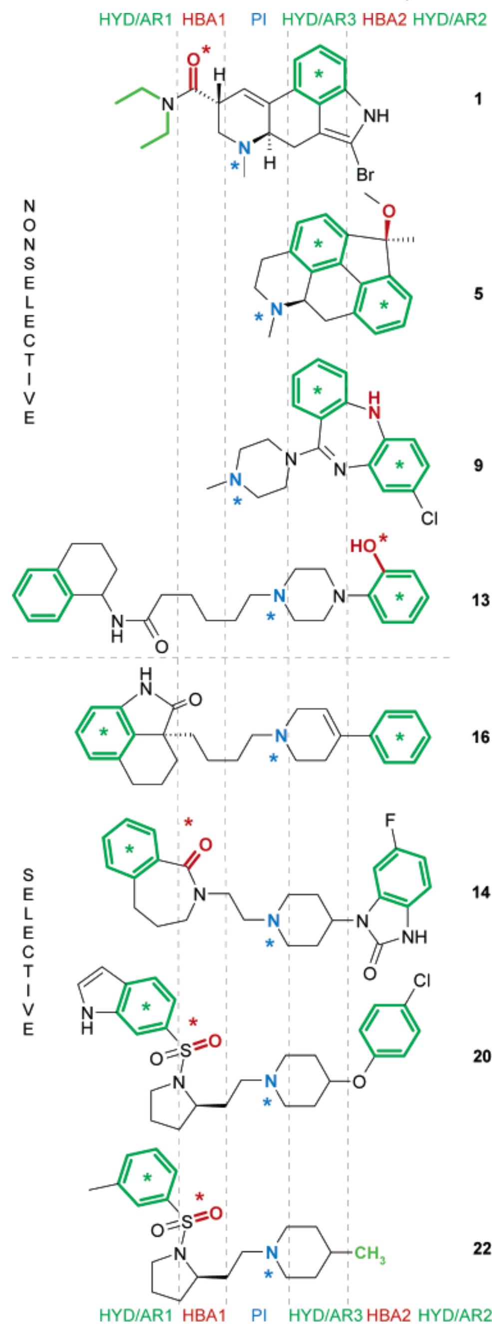



**Table 1.** Distances (Å) between Pharmacophoric Features in the General “Affinity” Hypothesis

| | PI | HYD/AR1 | HYD/AR2 | HYD/AR3 |
|---|---|---|---|---|
| HYD/AR1 | 6.9−9.4 | | | |
| HYD/AR2 | 4.3−7.8 | 11.1−14.4 | | |
| HYD/AR3 | 3.7−6.1 | 8.75 | 4.1−4.8 | |
| HBA1 | 3.7−5.1 | 3.7−3.8 | 8.3−10.1 | 6.9 |
| HBA2 | 5.1−6.9 | 14.2 | 2.8−3.7 | 2.8−3.7 |

**Table 2.** Distances (Å) between Pharmacophoric Features in the "Selectivity" Hypothesis

| | PI | HYD/AR1 | HYD/AR2 |
|---|---|---|---|
| HYD/AR1 | 6.9−7.7 | | |
| HYD/AR2 | 4.3−7.0 | 11.1−13.2 | |
| HBA1 | 3.7−5.1 | 3.7−3.8 | 8.3−10.1 |

**Selectivity Pharmacophore for 5-HT$_7$R Antagonists.** Based on the docked poses of selective 5-HT$_7$R antagonists (compounds **14**−**17**, **19**, **20**, **22**−**27**, **30**, **31**, see Supporting information, Table 3), a "selectivity" pharmacophore model consisting of three crucial features was also proposed (Figures 4D−4F, Table 2). Two of them are common for all the selective antagonists, that is, PI and an aromatic ring AR1, which form strong specific interactions with the residues in the pocket between TMHs 7−3 (especially Phe3.28 and Arg7.36). The third feature necessary for selectivity can be either (1) HBA1, an H-bond acceptor situated in the vicinity of Tyr7.43 or (2) HYD/AR2, a hydrophobic or an aromatic moiety penetrating the pocket between TMHs 4−6, but its interactions should not dominate that of the AR1 feature (Figure 5). Of crucial importance here is the geometry of a terminal moiety containing AR1 (aromatic imide/amide/sulfonamide), which should enable the formation of $\pi-\pi$ stacking with Phe3.28, ion−$\pi$ interaction with Arg7.36, or, optimally, both. The sp$^3$ hybridization of an atom connecting spacer with a terminal moiety (arylsulfonamides and tetrahydrobenzindoles) seems favorable for this purpose (compounds **16**−**24**; Figures 3E, 3G, and 3H).

## Discussion

In the present study, the ligand binding mode in 5-HT$_7$R was established for the first time by studying direct ligand−receptor interactions of representatives of all 5-HT$_7$R antagonist classes (31 compounds overall). The advantage of the fully flexible docking approach used here (both flexible ligand and receptor) lies in the fact that each docked ligand not only "found" its own best position and geometry in the receptor, but also "chose" the most convenient conformation of the binding site. The ligand binding mode was determined by studying numerous top-scored ligand−receptor complexes and was not biased by manual placement of a few compounds in an arbitrarily chosen site and direction (which, in fact, cannot significantly change even during molecular dynamics simulation). The superimposition of the ligands used for the generation of the pharmacophore hypothesis was determined by the shape and specific interaction points of the binding pocket, which is particularly beneficial in the case of a structurally diversified set of compounds with potentially different binding modes and binding sites. On the other hand, the results of receptor-based superimposition crucially depend on the quality of a receptor model, which makes this methodology complementary rather than fully alternative to a conventional, ligand-based approach.

The positioning of most of the ligands within a receptor binding site was analogous to the binding mode predicted in our earlier study for arylpiperazine derivatives and their structural analogues (ergolines and aporphines) in 5-HT$_{1A}$R.[51] However, a certain discrepancy can be observed between the ligand binding modes (and, therefore, ligand superimpositions), determined in the present study and proposed earlier for 5-HT$_7$R.[48−50] The binding modes of antagonist classes 1−3 are presumably similar, while those of classes 4 and 5 are different (class 6 was not studied before). The main difference lies in the placement of complex LCAP/LCBC and arylsulfonamidoalkylamine ligands in the binding pocket and their position in relation to ergolines, aporphines, and tricyclic agents. Our dockings superimposed an arylpiperidine/arylpiperazine or $\beta$-carboline moiety on an ergoline, aporphine, or tricyclic fragment and placed all of them in the pocket between TMHs 4−6, while amide/imide or sulfonamide moieties were located in the region occupied by an amide fragment of ergoline derivatives (a pocket between TMHs 7−3; Figures 3D−3G). In the other studies, an opposite placement was presented: the amide moieties of arylpiperazines[48,49] or arylsulfonamide fragments[50] were superimposed (without receptor structure) on an ergoline, aporphine, or tricyclic moiety and were then manually positioned in the cavity between TMHs 4−6. The above ambiguity may be due to the fact that ligand−ligand superimposition assumes one common binding pocket for all compounds and that there is a fair symmetry of the 5-HT$_7$R binding site (see Results, "binding site description" paragraph); the symmetry also occurs in the structure of the complex ligands of classes 4 and 5 (see Chart 2, compounds **13**−**20**). The ligand-based approach, guided by the ligand features only, would result in the superimposition of aryl and carbonyl groups of compound **24** (or **22**) directly on the respective moieties of compound **5**, while the superimposition obtained by the docking to a receptor model placed those compounds in different receptor pockets (Figures 3G, 3B, 4E and 4B). The concept of different binding modes and binding sites resulted from the applied receptor-based approach and substantially influenced the pharmacophore model for 5-HT$_7$R antagonism, especially the "selectivity" hypothesis.

The "affinity" pharmacophore presented in this study is fairly general and has a lot in common with other pharmacophores for monoamine receptors, as it was also generated using nonselective ligands showing a multireceptor profile. It defines features (and their combinations) that may afford 5-HT$_7$R antagonism, irrespective of the selectivity over other targets. On the other hand, the "selectivity" pharmacophore was based on selective antagonists only and is an attempt to describe the features providing exclusive affinity for 5-HT$_7$R. A key to selectivity lies in the differences between biological targets that are "recognized" by ligands upon binding. As mentioned in the introduction, the 5-HT$_7$R model was built using the methodology developed for 5-HT$_{1A}$R as a result of the high structural similarity and common ligands. The superimposition of both binding sites reveals that of the amino acids having vdW contacts with ligands, only three residues are different: Glu7.35 (5-HT$_7$R)−Gly (5-HT$_{1A}$R), Arg7.36−Ala, and Leu7.39−Asn. Sequence analysis of the putative binding sites of some other monoamine receptors, for which nonselective 5-HT$_7$R antagonists display affinity (5-HT$_{2A}$, D$_2$, $\alpha_1$), also indicates a certain similarity, especially in the binding cavity between TMHs 4−6. Like in the case of 5-HT$_{1A}$R, differences occur mainly in the pocket formed by TMHs 7−3 (residues 7.35, 7.36, 7.39 and 2.60, 2.61, 2.64, 2.65). This is in line with the observation that 5-HT$_7$R antagonists that enter into important, specific interactions with the residues from the TMHs 7−3 pocket (especially nonconserved Arg7.36; Figures 3E−3H) are predominantly more selective than those anchoring mainly between TMHs 4−6 (Figures 3A−3D). The presence and the quality of AR1 feature is of great importance here, because it assures specific interactions with Phe3.28 and Arg7.36. The geometry of the terminal amide/imide/sulfonamide fragment (a mutual arrangement of the aryl ring, AR1, and the carbonyl oxygen, HBA1) substantially influences 5-HT$_7$R affinity and/or selectivity by appropriately fitting the above-mentioned residues and Tyr7.43. Arylsulfonamidoalkylamines possess optimal geometry, which

allows their simultaneous $\pi-\pi$ stacking with Phe3.28, ion$-\pi$ interaction with Arg7.36, and H-bonding with Tyr7.43 (Figures 2 and 3G). These interactions (together with a salt bridge to Asp3.32) make them independent of specific interactions with the aromatic residues from TMHs 4−6 (e.g., compounds **22**−**24**; Figure 3G). Substitution of the aromatic moiety (AR2) in the 4-position of the piperidine ring (e.g., compounds **20** and **21**; Figure 2), allowing specific interactions with the pocket between TMHs 4−6, does not influence 5-HT$_7$R affinity, but increases (by 1−2 orders of magnitude) the affinity for some other monoamine receptors (see Supporting Information, Table 3), making these compounds less-selective than their methylpiperidine analogues. Benzoazepinone or tetrahydrobenzindole derivatives of the LCAP/LCBC class (e.g., compounds **14** and **16**; Figures 3E and 3F) are devoid of some of the optimal features of arylsulfonamidoalkylamines (benzoazepinones cannot form $\pi-\pi$ stacking with Phe3.28, while tetrahydrobenzindoles do not form an H-bond with Tyr7.43), which makes them dependent on the supporting interactions of the HYD/AR2 region with the TMHs 4−6 cavity. These groups do not possess such a level of selectivity for 5-HT$_7$R as do methylpiperidine derivatives of arylsulfonamides (compounds **22**−**24**). Ergolines, aporphines, and tricyclic psychotropic agents, which occupy exclusively (or predominantly) the TMHs 4−6 cavity, are generally nonselective. It should be stressed, however, that the ability of a ligand to enter into dominating interactions within the TMHs 7−3 pocket is not the only way to selective antagonism, because according to our docking experiments, some selective compounds (e.g., compound **31**) were accommodated by the cavity between TMHs 4−6. The selectivity, in this case, is possible probably due to spatial differences between the receptors, which cannot be explored by the model building methodology used in this study (a rigid, slightly modified, rhodopsin-based backbone in all the receptor subtypes; see Experimental Section). Nevertheless, we postulate that designing compounds that fit well the TMHs 7−3 pocket and are, therefore, independent of the interactions with the conserved TMHs 4−6 cavity is more likely to yield selective antagonists.

## Conclusions

The present paper shows homology modeling of the 5-HT$_7$ receptor and describes its interactions with representatives of six classes of its antagonists. The ligand binding mode was studied, regarding the flexibility of both ligand and receptor (automated docking to a multiconformational population of the receptor). Consistent results of the docking procedure allowed us to generate two receptor-based pharmacophore models describing features necessary for the affinity and selectivity of 5-HT7R antagonists. The "selectivity" hypothesis emphasized the role of the presence and geometry of aromatic (AR1) and H-bond accepting (HBA1) moieties interacting with a less-conserved part of the binding site localized between TMHs 7 and 3 in providing selectivity over other monoamine GPCRs.

## Experimental Section

A homology model of rat 5-HT$_7$ serotonin receptor was generated using MODELLER 7v7 (http://salilab.org/modeller) based on sequence alignment and guided by the most conserved residues in the GPCR family, as stated in the NIH GPCR database (http://mgddk1.niddk.nih.gov/GPCR.html; see Supporting Information, Figure 6). MODELLER implements comparative protein structure modeling by the satisfaction of spatial restraints, derived from the sequence alignment with a template and expressed as probability density functions for the features restrained.[54] Initially, we focused on the modeling of helical part of the receptor, so the crystal structure of the heptahelical bundle of bovine rhodopsin (inactive form, PDB code 1F88) was used as a template, because it was successfully applied in some previous studies on GPCR modeling.[51,55−57] In our earlier study on 5-HT$_{1A}$R modeling, we proved that certain modifications in the arrangement of transmembrane helices facilitated ligand docking and made specific interactions detectable by docking software.[51] Because the superimposition of the binding site models of 5-HT$_7$R and 5-HT$_{1A}$R reveals high similarity between the receptors (see discussion), and because a large number of ligands show high affinity for both receptors,[52] the same TMHs modifications were now incorporated in the template for the homology modeling of 5-HT$_7$R. TMH3 was translated 1.5 Å toward the cytoplasmic side of the receptor, and a −5° rotation of TMH6 on the $\varphi$ angle of Thr6.43 (64.1° → 59.4°) was introduced. Some other changes in the rhodopsin template, reflecting potential differences between the monoamine and the opsine GPCR families were also reported earlier.[49,58] In addition, conformational restraints were applied in certain side chains: the $\chi_1$ angles of Asp3.32, Phe6.52, and Ser5.42 were frozen in gauche(−), gauche(+), and trans conformations, respectively, as those residues in 5-HT$_{1A}$R were always found to interact with ligands in these very conformations. To explore the conformational space of the binding site, MODELLER was used to produce a population of 100 models, which differed significantly in their side-chain conformations, while the polypeptide backbone differed only insignificantly from the original template. A number between 10 and 100 models was reported to provide a satisfactory conformational sampling of the binding site.[59] The initial verification of models proved that the crucial residues proposed to be engaged in interactions with ligands on the basis of mutagenesis experiments with related receptors, that is, Asp3.32, Ser5.42, and Thr5.43, were located on the ligand-accessible surface of the receptor. Moreover, an interhelical salt bridge between Arg3.50 (E/DRY motif) and Glu6.30 or Thr6.33 (postulated to be present in inactive conformation of 5-HT$_{1A}$ receptor)[60] as well as a hydrogen bond between Asp2.50 and Asn7.49 (responsible for the allosteric regulation of agonist affinity)[61,62] were present in the obtained receptor models. Ligands were built with CORINA (www2.chemie.uni-erlangen.de/software/corina), and the construction of polycyclic, chiral molecules was guided by their crystal structures. Optimization of the ligands was performed using a PM5 quantum semiempirical method with the COnductorlike Screening MOdel (COSMO) approach to simulate water environment (MOPAC 2002, implemented in the CAChe Worksystem Pro 6.1; www.cachesoftware.com). That method was found to produce results, which corresponded to the geometries determined by 2D NOESY $^1$H NMR as well as crystallographic experiments (unpublished data). Gasteiger charges were assigned to the ligands, and a +1 formal charge was located on protonated nitrogen atoms.

The docking was carried out using FlexX (www.biosolveit.de), implemented as a part of SYBYL 7.1 (www.tripos.com) with default parameters. FlexX rapidly and exhaustively samples the conformational space of a ligand, using incremental construction algorithm that builds the ligand in the site.[63] An interaction constraint (the FlexX-Pharm module of FlexX) on a hydrogen bond between Asp3.32 and the protonated nitrogens of the ligands was applied in all dockings since that interaction was considered crucial for all the monoamine neurotransmitter receptors.[64] The obtained ligand−receptor complexes were scored using five scoring functions: F_score, D_score, G_score, Chem_score, and PMF_score, with a subsequent consensus scoring as implemented in the CScore module of SYBYL 7.1. Only complexes with the highest "5" CScore value were considered, and the ranking of compounds was based on the PMF_score because that scoring function was reported to provide the best enrichment factors in virtual screening experiments.[56]

Because we have recently shown that rigid, cyclohexyl analogs of LCAPs displayed very low affinity for 5-HT$_7$R sites,[65] we now used one such compound to perform "negative selection" of the generated 5-HT$_7$R models. MP349 (rigid arylpiperazine $K_i$ 5-HT$_7$R = 2045 nM) was docked to all the 100 receptor models, and only the models that did not dock the ligand were considered valid. Verification yielded a population of 62 models, which were

subjected to further docking of all 5-HT$_7$R antagonists. Top-scored ligand−receptor complexes ("5" CScore value and the best PMF_score, one for each ligand) were then used to construct extracellular loops (e1, e2, e3), which may have contact with the ligand. The loops were added to each receptor model containing a ligand in the active site using MODELLER. For each complex, 100 new models differing in the conformation of extracellular loops were produced, of which 10 with the best objective function were selected, yielding 300 new receptor models. Finally, all 5-HT$_7$R antagonists were docked again to a new population of models. Over 400 000 ligand−receptor complexes were obtained and subjected to a CScore procedure. Top-scored complexes were used for the analyzing of the ligand binding mode and the generation of pharmacophoric hypotheses. The docking scores and numbers of receptor models present in best complexes (both initial and with loops) are given in the Supporting Information (Table 4). The most important feature of the models that were most frequently found in the top-scored complexes (e.g., 184, 150, 53) is a side-chain of Arg7.36, which is directed toward the binding site and thus offers a possibility of, for example, ion−$\pi$ interactions with ligands. These findings support our hypothesis about the putative role of Arg7.36 in binding selective 5-HT$_7$R antagonists.

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**Supporting Information Available:** Sequence alignment (5-HT$_7$R vs rhodopsin), a table of available affinities for monoamine GPCRs of selective 5-HT$_7$R antagonists, a table of docking scores, and numbers of receptor models present in best complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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