Homology Modeling of the Serotonin 5-HT$_{1A}$ Receptor Using Automated Docking of Bioactive Compounds with Defined Geometry

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This paper describes a rhodopsin-based model of 5-HT$_{1A}$ serotonin receptor. The flexibility of the receptor was considered by using large number of models for ligand dockings. Rearrangements of the heptahelical bundle were introduced, which resulted in the improvement of correlation between computational results and experimental data. The model was validated by automated docking of conformationally restricted arylpiperazines. Specific interactions, responsible for the recognition of arylpiperazine derivatives, were identified. An ionic bond was formed between the protonated amine of ligands and Asp3.32. The aromatic moiety and its substituents specifically interacted with Phe6.52 and Ser5.42, respectively, while the carbonyl groups of imide part of ligands formed hydrogen bonds with Asn7.39 and Tyr7.43. The model reproduced the binding affinity of the test group of ligands (correlation $r = 0.8$ between pK_i and docking score). It also gave the enrichment in virtual screening-like experiment (100 compounds), in which 34 high-affinity compounds were found among 50 top-scored ligands.

Introduction

Although a serotonin 5-HT$_{1A}$ receptor is one of the most frequently modeled monoamine GPCRs, the binding mode of complex arylpiperazine ligands (the largest and the most thoroughly studied class of 5-HT$_{1A}$ receptor ligands) is still ambiguous. One of the main obstacles, the great conformational flexibility of arylpiperazines caused by the presence of a polymethylene linker, has recently been overcome by the development of rigid and highly potent 5-HT$_{1A}$ receptor agents.[1] In this study we present the modeling of the 5-HT$_{1A}$ receptor based on rhodopsin template and its further validation by means of conformationally constrained cyclohexylarylpiperazines.

Three complementary methodologies were used so far in the investigation of ligand binding to the 5-HT$_{1A}$ receptor. Ligand-based methods led to the construction of several pharmacophore models,[2,3] of which the most universal is that of Hibert et al.[4] It defines the relative position of two main pharmacophoric points, i.e., the protonated amine nitrogen atom and the aromatic ring, common to all classes of 5-HT$_{1A}$ receptor ligands. Directed mutagenesis experiments suggested that Asp3.32, Asn7.39, Ser5.42, and Thr5.43 (Ballesteros−Weinstein nomenclature[5]) may be involved in the ligand binding.[6−8] An ionic interaction between the protonated nitrogen of the ligand and Asp3.32 was considered crucial for all monoamine neurotransmitter receptors.[9] Eventually, molecular modeling techniques were employed to construct three-dimensional models of the receptor. The first attempts at modeling of the 5-HT$_{1A}$ receptor date back to the early 1990s, when bacteriorhodopsin was used as a template.[10−18] That template was later discarded due to the lack of sufficient amino acid sequence homology with GPCRs. Since the coordinates of the projection map of frog rhodopsin were published in 1997[19] and the crystal structure of bovine rhodopsin was resolved in 2000,[20] rhodopsin became a template of choice, being the first GPCR with the known 3D structure.

The published rhodopsin-based models of 5-HT$_{1A}$ are briefly reviewed below, our attention being mainly focused on the proposed binding modes of arylpiperazine derivatives, which are further discussed in the following chapters.

Since 1997, Sylte and co-workers have presented a series of 5-HT$_{1A}$ models first constructed on the template of the projection map of rhodopsin[21] and later based on rhodopsin crystal structure.[22] The models were obtained by means of homology modeling procedures and were then equilibrated in a series of MD simulations and energy minimizations. Two possible binding modes are proposed for arylpiperazines (buspirone analogues), based on a series of MD simulations of ligand−receptor complexes, both considering Asp3.32 as anchoring point for the protonated nitrogen of the ligand.[22] In the first binding mode a ligand is placed inside a deep cavity between TMHs 2, 3, and 7. This binding mode assumes the extended conformation of the studied arylpiperazines. In the second mode the conformation of buspirone analogues is folded due to the bent shape of the alkyl spacer joining the piperazine ring and the imide group. The aryl ring of the ligand reaches Phe6.61 and other amino acids in TMHs 5, 6, and 7.[22] Imide carboxyl oxygen can form a hydrogen bond with Ser7.46.[23] The second binding mode is proposed to correctly map the pharmacophore model for buspirone analogues developed by Chilmonczyk et al.[21]

In the 5-HT$_{1A}$ model presented by Lopez-Rodriguez and co-workers,[24] certain modifications are introduced in the structure of the 7TM helix bundle. In this model TMH3 is significantly bent toward TMH5. This modification reduces the distances Asp3.32-Ser5.42 and Asp3.32-Thr5.43, enabling concurrent interactions of ligand amine group with Asp3.32 and ligand amide with Ser5.42 and Thr5.43. The bent conformation of TMH3 was obtained by clustering the molecular dynamics trajectory and was the result of modification of the φ and ψ angles of residues 3.35−3.46. The rationale behind this modification comes from the observation that Ser, Thr, or Cys residues can change α-helix curvature due to the formation of an intrahelical H-bond between the side chain OH group and backbone carbonyl oxygen of residue in a preceding helical

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turn.[25] The binding mode of arylpiperazine analogues proposed by Lopez-Rodriguez et al. is different from those proposed by Sylte et al. and assumes that the arylpiperazine moiety is located between TMH3 and TMH7. The aromatic portion of the ligand may interact with Phe3.28, Trp7.40, and Tyr7.43,[26] while substituents in the aryl ring interact with Asn7.39.[24,27] The carbonyl groups of the ligand hydantoin moiety form hydrogen bonds with Ser5.42, Thr5.43, Thr3.37, and Trp6.48,[26,27] and the entire imide substituent is placed inside the cavity between TMH4−6.

The model published by Seeber and co-workers[28] was used in the molecular dynamics simulation studies of agonist- and antagonist-induced conformational changes. Ligand position in the receptor, determined for the arylpiperazine derivative WAY100635 by automated docking, is similar to that proposed by Lopez-Rodriguez et al., but ligand orientation, and thereby specific interactions, are opposite. The 2-methoxyphenylpiperazine part of the ligand is situated in the binding cavity formed by TMH4−6, while the nitrogen of pyridine ring substituted in the amide fragment forms an H-bond with the side chain nitrogen of Asn7.39. According to this binding mode, as well as to both previously discussed, Asp3.32 is considered as a counterpart for the protonated amine of the arylpiperazine moiety.

The 5-HT_{1A} model described in this paper is based on a large population of model structures covering the conformational space of the binding site. The verification of the model was conducted by means of the automated docking of rigid arylpiperazine derivatives. The decreased conformational flexibility of these compounds is believed to encode the information on the shape of the binding site and spatial arrangement of specific interaction points within the binding pocket. The primary goal of this work was to describe in detail the binding mode of arylpiperazines within the 5-HT_{1A} receptor. Additionally, the usefulness of that model as a tool for virtual screening experiments was tested.

Results

Construction of a 5-HT_{1A} Receptor Model. In general, our ligand-based receptor modeling approach consists of three basic elements: (1) generation of a large population of models exhaustively sampling the conformational space of the receptor; (2) automated docking of rigid ligands to the entire model population in order to select the best ligand-fitting conformation of the receptor ("inverse virtual screening"); (3) modification of the receptor (if necessary) aimed at developing a model that best describes the ligand binding in either a qualitative or a quantitative manner (see flowchart, Figure 1).

The above methodology is analogous to that published by Evers and co-workers[29] but has been developed independently.[30]

1. Model Building. The molecular model of the 5-HT_{1A} receptor was built by homology modeling using Modeler 7v7.[31] The crystal structure of bovine rhodopsin (PDB code 1F88[20]) was used as a template and the sequence alignment was based on 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). In the present study we focused entirely on the modeling of a transmembrane helical bundle (see Discussion). To explore the conformational space of the receptor, Modeler was used to produce 400 models, which differed significantly in side chains conformations, while polypeptide backbone varied only slightly from the original template (Figure 2). The initial verification of models proved, that the crucial residues proposed to be engaged in interactions with ligands, i.e., Asp3.32, Ser5.42, Thr5.43, and Asn7.39, 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)[28] as well as a hydrogen bond between Asp2.50 and Asn7.49 (responsible for the allosteric regulation of agonist affinity)[32,33] were present in the obtained receptor models.

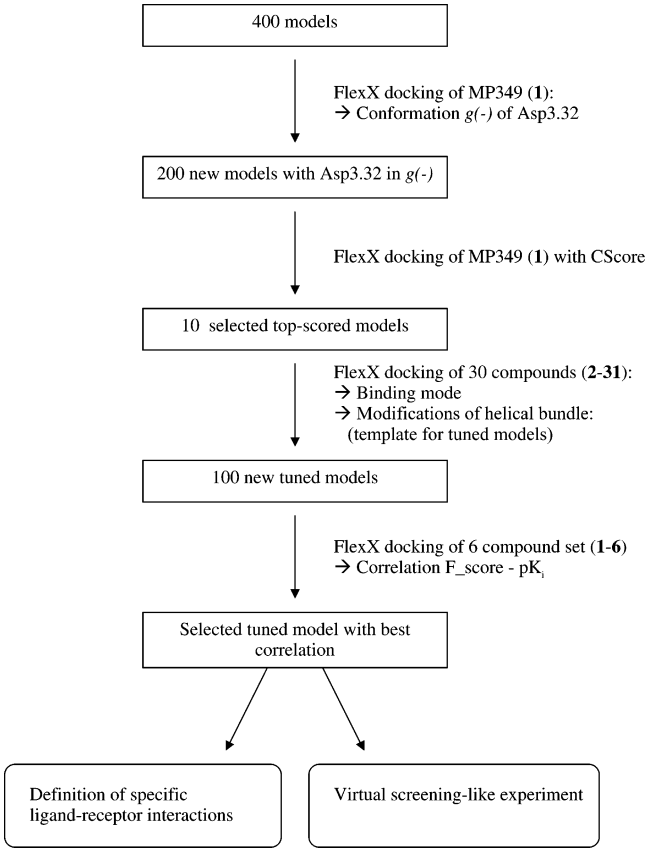


Figure 1. A flowchart of subsequent steps in 5-HT_{1A} receptor modeling.

2. Model Selection. In this step, the initial conformational population of the receptor was explored using test compounds in order to choose models showing a consistent binding mode for the largest possible number of arylpiperazines. In the first experiment, the test ligand MP349 (a cyclohexylarylpiperazine derivative) (Table 1, compound **1**) was docked to all the 400 receptor models using FlexX (www.biosolveit.de) implemented as a part of SYBYL 7.0 (www.tripos.com) with default parameters and without any constraints. Gasteiger charges were assigned to the ligand and a +1 formal charge was located on the protonated piperazine N4-nitrogen. The ligand poses obtained by docking covered the entire accessible surface of the defined active site, but in the majority of low-energy ligand−receptor complexes an interaction occurred between the protonated N4-nitrogen of the piperazine and Asp3.32. A detailed analysis showed that that crucial ionic bond was formed almost exclusively in the case of receptors with the gauche(−) conformation of the Asp3.32 χ_1 angle. Therefore 200 new models were produced using Modeler, with the χ_1 angle of Asp3.32 frozen in the gauche(−) conformation, and an interaction constraint (FlexX-Pharm module of FlexX) on a hydrogen bond between Asp3.32 and the protonated nitrogen of the ligand was applied in all further docking simulations. Then, MP349 was docked to all the new models, and the output ligand−receptor

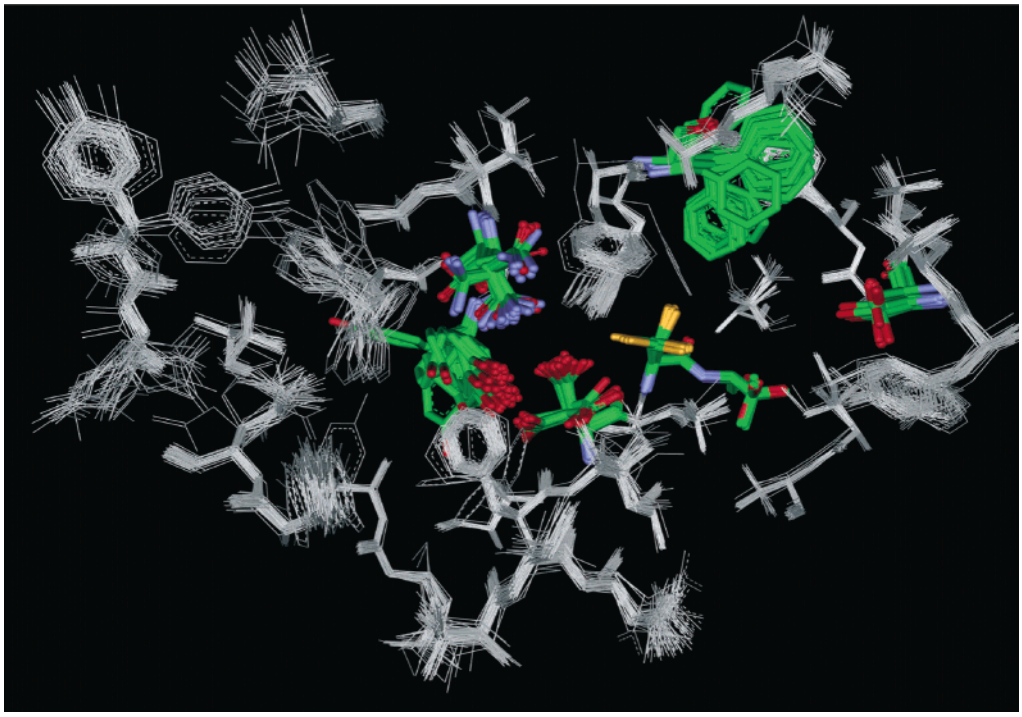


Figure 2. The conformational space of 5-HT$_{1A}$ binding site, sampled by Modeler. The shown residues define an "activesite" subset used in FlexX dockings. Amino acids forming specific interactions with arylpiperazines are represented as "sticks".

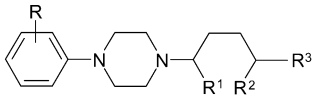
complexes were scored using five scoring functions: F_score, D_score, G_score, Chem_score, and PMF_score, with subsequent consensus scoring as implemented in the CScore module of SYBYL 7.0. Of the models that successfully accommodated the test ligand, 10 models with the highest number of complexes showing the best CScore value ("5") were selected for further experiments. A set of 30 rigid and flexible arylpiperazine derivatives with different substituents in the phenyl ring and various terminal imide fragments (Table 1, compounds **2**−**31**) were docked to the selected receptor models. Inspection of the top-scored ligand−receptor complexes led to the determination of a ligand binding mode but also suggested some modifications of the model, which would improve specific interactions.

3. Model Tuning. It was found that the phenyl ring of the arylpiperazine moiety was located between helices 4, 5, and 6, having van der Waals contact with the aromatic Phe6.52 residue, whereas the *o*-methoxy substituent was situated in the neighborhood of the hydrogen bond donating Ser5.42. The ligand pose in the receptor binding site was, however, not optimal due to the lack of specific interactions between the above-mentioned fragments, despite their vicinity. Moreover, there was still free space left in the region of para substituents of the aryl ring, while that region was reported to be sterically unfavorable in SAR studies for 5-HT$_{1A}$ ligands.[48] Such a nonoptimal ligand pose was caused by excessive distance between Asp3.32 and the above-mentioned residues. Asp3.32 and the ligand form a strong ionic bond which dominates other interactions and determines the ligand position. Some backbone modifications had to be introduced, since no changes in side chain conformations could make specific interactions with Ser5.42 and Phe6.52 detectable by docking software. At first, TMH3 was translated 1.5 Å toward the cytoplasmic side of the receptor, which resulted in a deeper penetration and considerably better ligand fitting to the receptor cavity, as well as in an optimal ligand placement for a hydrogen bonding with Ser5.42. The phenyl ring of the arylpiperazine moiety attained a closer contact with Phe6.52, but specific interaction between aromatic moieties was still not possible. Therefore, as a second step, a −5° rotation of TMH6 on the φ angle of Thr6.43 (64.1° → 59.4°) was introduced, facilitating the CH−π interaction (Figure 3). Modifications were made manually to the top scored "crude" receptor model. The modified receptor was then energy-optimized and used as a template for building 100 new "tuned" models with Modeler. While building tuned models, additional restraints were introduced on the conformations of two other important residues in binding site. The χ_1 angles of Phe6.52 and Ser5.42 were frozen in gauche(+) and trans conformations, respectively, since those residues were found to interact with ligands always in those conformations. It is noteworthy that the introduced modifications did not disrupt salt bridges (e.g. in the crucial E/DRY motif), the hydrogen bond network, or the side chain packing stabilizing protein tertiary structure, nor did they cause the rise in the potential energy of the tuned models compared to crude ones. On the other hand, they facilitated additional, specific interactions (suggested by the docking experiments on crude models), which resulted in better scores for the same ligands docked to tuned models and allowed application of an additional interaction constraint in the FlexX-Pharm module.

Ligand Binding Mode. Determination of the ligand binding mode in the 5-HT$_{1A}$ receptor was a primary objective of this study. It was achieved by an analysis of top-scored ligand−receptor complexes, obtained by the automated docking of rigid cyclohexylarylpiperazine ligands (Table 1, compounds **1**−**13**) and confirmed by docking of flexible compounds (**14**−**31**). At first, the position and orientation of a ligand in the binding site was established, being followed by a detailed description of the specific interactions determining ligand recognition.

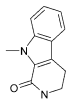
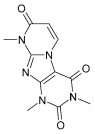
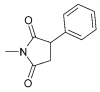
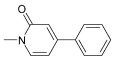
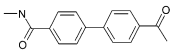
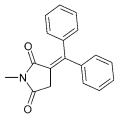
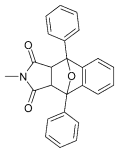
1. Ligand Position. In the proposed model of the ligand−receptor complex, arylpiperazine derivatives were placed within the heptahelical bundle, along TMH3. The crucial arylpiperazine moiety was located deep inside the receptor, between TMHs 4, 5, and 6, while the terminal imide was oriented toward TMHs 1, 2, 7, and the extracellular side. Such a ligand position was observed in most ligand−receptor complexes (all the top-scored

Table 1. Compounds Used in the Ligand-Based Modeling of 5-HT_{1A} Receptor



Compd.	R	R^1	R^2	R^3	5-HT_{1A}	Ref.
1 (MP349)	*o*-OMe	$-(CH_2)_2-$			15 ± 3.2	34
2	H	$-(CH_2)_2-$			43 ± 6	35
3	*m*-Cl	$-(CH_2)_2-$			5.4 ± 0.9	35
4	*m*-CF_3	$-(CH_2)_2-$			4 ± 0.5	35
5	*m*-OMe	$-(CH_2)_2-$			27 ± 2	35
6	*p*-OMe	$-(CH_2)_2-$			145 ± 15	35
7	*o*-OMe	$-(CH_2)_2-$			22 ± 4	36
8	*m*-Cl	$-(CH_2)_2-$			15 ± 4	36
9	*m*-CF_3	$-(CH_2)_2-$			9 ± 1	36
10	*o*-OMe	$-(CH_2)_2-$			8 ± 2	1
11	*o*-OMe	$-(CH_2)_2-$			72 ± 19	1
12	*o*-OMe	$-(CH_2)_2-$			52 ± 2	34
13	*o*-OMe	$-(CH_2)_2-$			44 ± 5	34
14 (MM77)	*o*-OMe	H	H		6.4 ± 0.3	37
15	H	H	H		7.4 ± 0.3	35
16	*m*-Cl	H	H		32 ± 2	35
17	*m*-CF_3	H	H		21 ± 1	35

Table 1 (Continued)

Compd.	R	R^1	R^2	R^3	5-HT$_{1A}$	Ref.
18	*o*-OMe	H	H		8 ± 2	36
19	m-Cl	H	H		7 ± 2	36
20	*m*-CF3	H	H		4 ± 1	36
21 (NAN190)	*o*-OMe	H	H		0.55 ± 0.14	38,1[a]
22	*o*-OMe	H	H		4 ± 2	1
23	*o*-OMe	H	H		7 ± 1	39,34[b]
24	*o*-OMe	H	H		4 ± 0.2	40,34[c]
25	*o*-OMe	H	H		0.3 ± 0.1	41
26	*o*-OMe	H	H		0.5 ± 0.3	42
27	*o*-OMe	H	H		6.8 ± 0.5	43
28	*o*-OMe	H	H		40 ± 2	44
29	*o*-OMe	H	H		3.2 ± N/A	45
30	*o*-OMe	H	H		37 ± N/A	46
31	*o*-OMe	H	H		170 ± N/A	47

[a] Binding data taken from ref 1. [b] Binding data taken from ref 34. [c] Binding data taken from ref 34.

complexes for rigid cyclohexylarylpiperazine derivatives) (Figures 4 and 5). The docking of arylpiperazine derivatives with a flexible butyl spacer gave convergent results; however, an alternative orientation with the arylpiperazine moiety located near TMH7 and the terminal imide buried between TMHs 4, 5, and 6 was also observed. Interestingly, in that alternative orientation, flexible ligands with more bulky imide moieties (Table 1, compounds **18**−**24**) interacted with the receptor predominantly in a nonlinear, "hockey-stick-shaped" or strongly bent conformation. Such a geometry is unattainable for their conformationally constrained yet bioactive cyclohexyl analogues (**7**−**13**). Moreover, a review of possible imide moieties found in the existing high-affinity arylpiperazine ligands shows that these groups may significantly vary in their bulkiness, reaching

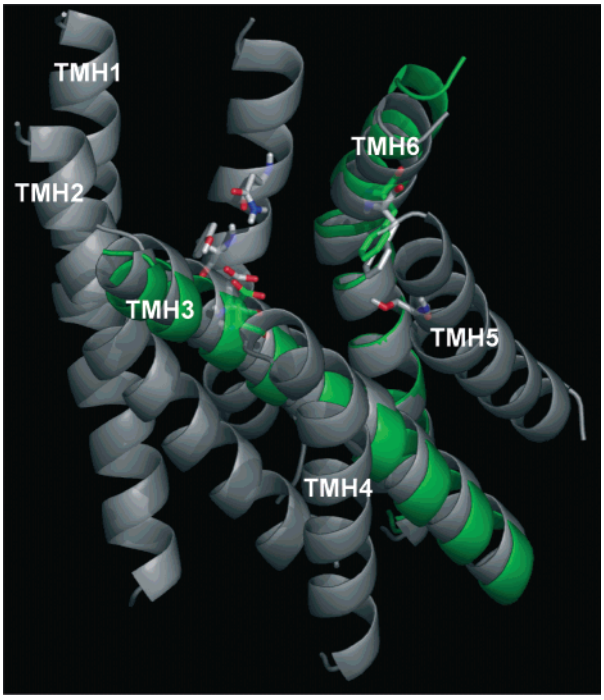


Figure 3. Spatial rearrangements in the heptahelical bundle, introduced to improve the ligand binding: intact helices, gray; modified helices, green. TMH3 was translated 1.5 Å toward the cytoplasmic side of the receptor, and a −5° rotation of TMH6 on the φ angle of Thr6.43 (64.1° → 59.4°) was introduced. Amino acids forming specific interactions with arylpiperazines are shown as sticks.

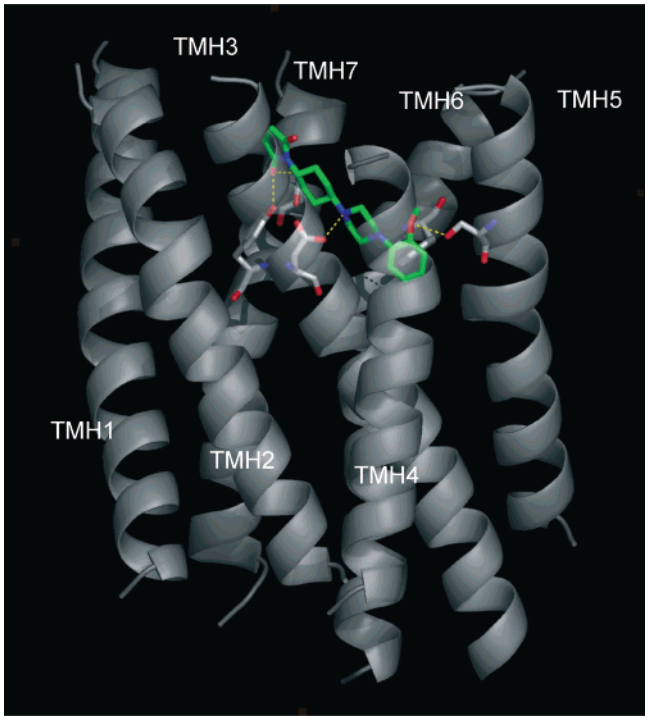


Figure 4. The binding mode of complex arylpiperazines in the 5-HT_{1A} receptor. H-bonds between MP349 (compound **1**) and amino acids are represented by a dotted yellow line. The linear ligand is situated in the extracellular part of a heptahelical bundle, along TMH3, and is anchored by a salt bridge formed with Asp3.32. The aryl moiety penetrates the binding cavity between TMHs 4−6, while the amide group is located between TMHs 3 and 7.

in some cases quite large volume. In contrast, possible aryl moieties consist of one- or two-ring systems only, and their substituents are rather small, if any. It is apparent that on the assumption that all the arylpiperazine derivatives have a consistent binding mode, the portion of the binding site that accommodates the imide part must have a substantially larger volume than the site responsible for the recognition of the aryl ring. Therefore, the part of the binding site located between TMHs 1, 2, and 7 seems much more likely to accommodate terminal imide moieties than the considerably more spatially limited cavity between TMHs 4, 5, and 6. This was proved by the fact that flexible compounds with the bulkiest terminal imide moieties (Table 1, compounds **25**−**31**) docked to the receptor only in orientations determined by the rigid ligands (**1**−**13**). The ligand binding mode was also additionally confirmed by docking simple N4-unsubstituted arylpiperazines (see Table 2 in Supporting Information: compounds **1**−**8**), as well as some other rigid compounds, which share common structural features with the arylpiperazines (see Table 2 in Supporting Information: compounds **9**, **10**). A vast majority of top-scored poses were found between TMHs 4, 5, and 6, which is in line with the results obtained for complex cyclohexylarylpiperazines. The same ligand positions were observed upon docking of all the ligands (**1**−**31**) to tuned receptor models; however in that case, alternative orientations were hardly ever observed.

2. Specific Interactions. According to the known SAR results,[49] specific interactions between ligands and the 5-HT_{1A} receptor may be classified as essential, determining the activity of the compound, and additional, enhancing its affinity or providing selectivity over other biological targets. The main essential interaction, which was confirmed experimentally (as has been mentioned in the Introduction), is the ionic bond between the carboxylic oxygen of the Asp3.32 side chain and the protonated N4-nitrogen of the piperazine moiety. This interaction was observed in the majority of low-energy ligand−receptor complexes obtained during the initial docking to the tested models; in consequence an essential constraint was applied to that interaction in all subsequent docking experiments. Our docking results suggested that the second essential interaction was the CH−π interaction between the aromatic rings of the arylpiperazine moiety and Phe6.52. The latter residue, present in all the receptors that are biological targets for arylpiperazines, was one of the residues proposed earlier as a potential counterpart for the aromatic ring, a crucial pharmacophoric feature of all 5-HT_{1A} receptor ligands.[10,14,50] An essential constraint was also applied to that interaction in docking experiments using tuned receptor models. The additional interactions observed in our models were hydrogen bonds between the hydrogen bond acceptors of the ligand and the hydrogen bond donors of the receptor. *O*-Methoxyphenyl and *m*-methoxyphenyl moieties were found to form a hydrogen bond with the hydroxyl group of Ser5.42 upon docking to tuned receptor models. The latter residue is present in all receptors for which *o*-methoxyphenylpiperazines show high affinity e.g. 5-HT_{1A}, 5-HT_7 serotonin, or α_{1A} adrenergic receptors. Moreover, the *o*-methoxy substitution in arylpiperazines significantly decreased the affinity for 5-HT_{2A} receptor, which has nonpolar glycine instead serine in the 5.42 position. Other hydrogen bond acceptors, connected with the phenyl ring of arylpiperazine, such as *m*-Cl or *m*-CF_3, interacted with Thr3.37 and Cys3.36, while the carbonyl groups of the terminal imide fragment were found to form hydrogen bonds with Tyr7.43 and Asn7.39 in our 5-HT_{1A} receptor model (Figures 5 and 6).

3. Bioactive Conformation of Flexible Ligands. Flexible (alkyl spacer) arylpiperazines in top-scored ligand−receptor complexes were identified in either (1) a linear conformation similar to that frozen in their rigid, cyclohexane analogues (Figure 7A), or (2) a fully extended conformation (Figure 7B).

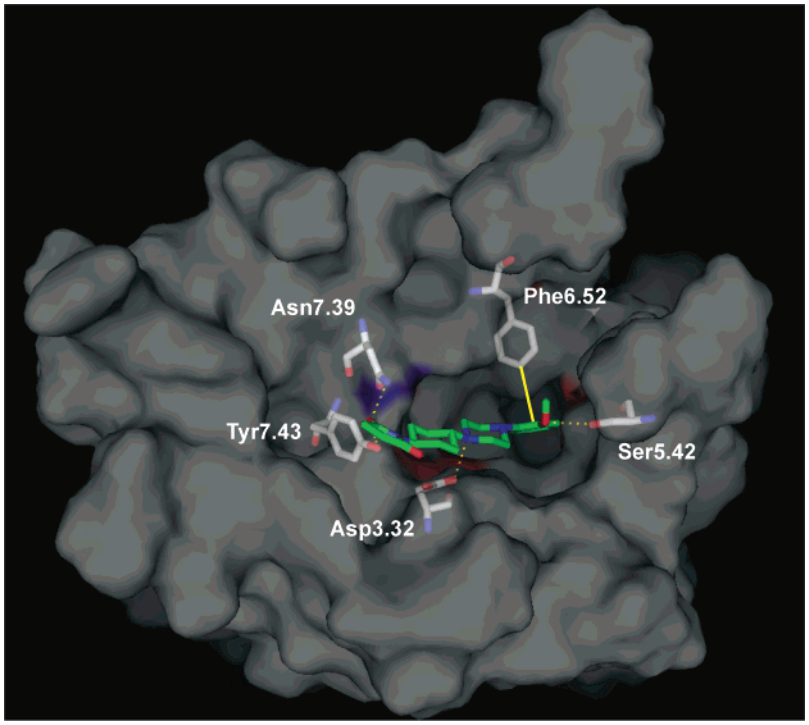


Figure 5. Ligand−receptor interactions responsible for the recognition of complex arylpiperazines (compound **1**). Dotted yellow lines represent H-bonds with Asn7.39, Tyr7.43, and Ser5.43, and a salt bridge with Asp3.32. A solid yellow line shows a CH−π interaction with Phe6.52.

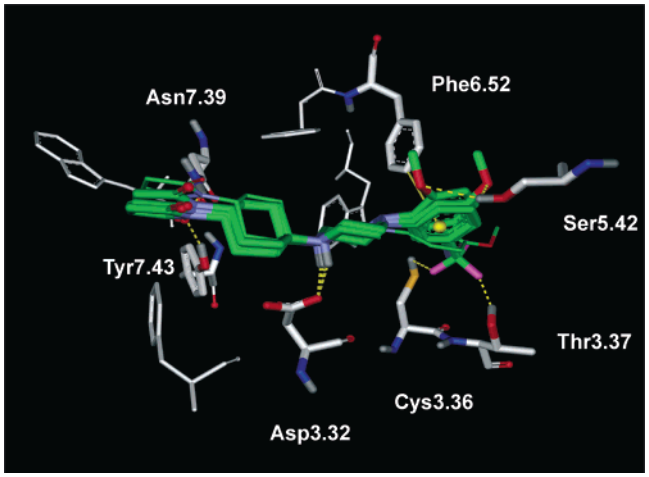


Figure 6. Interactions between complex arylpiperazines substituted in aryl ring and specific residues (Table 1, compounds **1**−**6**). Dotted yellow lines represent H-bonds with Asn7.39, Tyr7.43, Ser5.43, Thr3.37, and Cys3.36, and a salt bridge with Asp3.32. A solid yellow line shows a CH−π interaction with Phe6.52. *p*-OMe-substituted compound was shown as thin sticks.

Such conformations define the spatial arrangement of two pharmacophore fragments (arylpiperazine and terminal imide), which is optimal for their specific interaction with the above-mentioned important residues. The conformation (1) seems to be more suitable, as it lets the carbonyl group form a bifurcated hydrogen bond with both Tyr7.43 and Asn7.39 (Figure 7A). It is also noteworthy that such conformations correspond to minimum energy conformations determined by a theoretical conformational analysis with a simulated water environment or observed in NMR experiments (unpublished data).

Affinity Prediction. The process of model tuning and validation, which led to the detection of specific interactions responsible for ligand recognition, enabled the usage of the models in affinity prediction experiments.

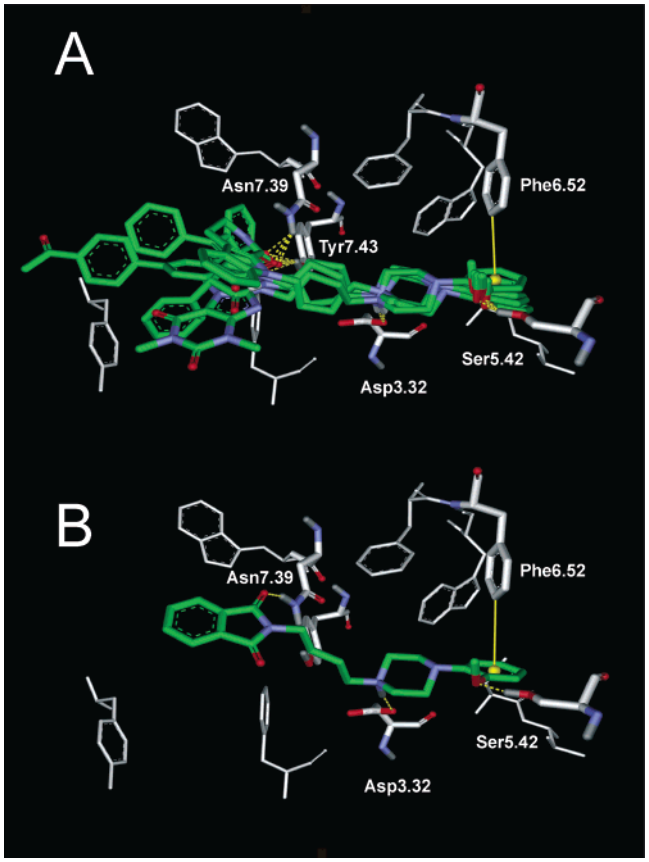


Figure 7. Flexible arylpiperazines with different terminal amides interacting with receptor binding site: (A) linear conformation (Table 1, compounds **25**−**27**, **29**, **30**); (B) fully extended conformation (Table 1, compound **21**). H-bonds are shown as dotted yellow lines. A solid yellow line shows a CH−π interaction with Phe6.52.

1. Recognition of a Substituent in the Arylpiperazine Fragment. Substitution at the phenyl ring, or introduction of

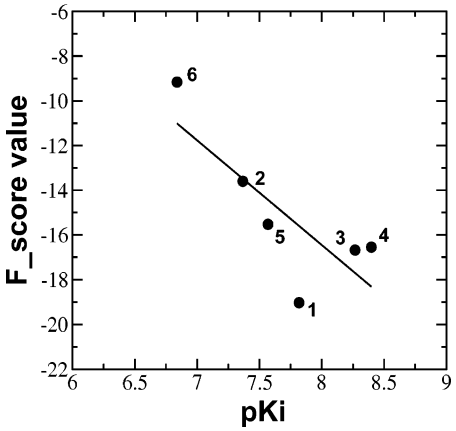


Figure 8. Correlation between F_score values and experimental pK_i obtained in docking of compounds **1**−**6** to one of the tuned models.

other aryl moieties to the arylpiperazine fragment, is a modification that most profoundly affects the affinity and selectivity of arylpiperazines. Considering this fact, we decided to select a receptor model that would optimally reproduce the experimentally derived structure−activity relationships of this crucial fragment. A set of six conformationaly restricted complex arylpiperazines which were recently developed in our laboratory (Table 1, compounds **1**−**6**)[35] was docked to all the tuned receptor models. All the compounds shared a common structure of cyclohexyl arylpiperazine, with the succinimide moiety as a terminal fragment, but were diversely substituted at the phenyl ring. The docking procedure was performed using a FlexX-Pharm module, with essential interaction constraints on the hydrogen bond between Asp3.32 and the protonated nitrogen of the ligand, as well as on the CH−π interaction between the aromatic rings of the arylpiperazine moiety and Phe6.52 (Figure 6). A consensus scoring with five scoring functions was applied, and only complexes with the highest ("5") CScore value were considered. A correlation ($r = 0.8$) between the predicted affinity, represented by the F_score function, and experimental pK_i values was obtained for one of the tuned receptor models (Figure 8). The correlation was also tested with the use of another scoring function (PMF_score), which provided worse correlation coefficients. The above-mentioned tuned model was used for further virtual screening experiment (see below).

2. "Virtual Screening-Like" Experiment. To evaluate the usefulness of the obtained receptor model in virtual screening procedures, a small-scale "virtual screening-like" experiment was performed. The test set used in the experiment consisted of 100 structurally diversified compounds with a known 5-HT_{1A} receptor affinity. At first, two groups of compounds, potent 5-HT_{1A} receptor ligands ($K_i < 10$ nM) and inactive compounds ($K_i > 1000$ nM), were extracted from our database containing over 2400 compounds (designed and tested as serotonin receptor ligands) published worldwide. Of the inactive compounds, only those fulfilling pharmacophore requirements for the 5-HT_{1A} receptor[4] were considered. Fifty compounds were randomly chosen from each group and were automatically docked to the selected tuned receptor model. Docking was held with essential interaction constraints and was followed by consensus scoring of all the results, as described above. Also in this experiment, only complexes with the highest ("5") CScore value were considered. The ranking of compounds was based on the PMF_score, since that scoring function was reported to provide the best enrichment factors in virtual screening experiments.[29] Among 50 top PMF-scored ligands, 34 active compounds were found, providing the enrichment over random selection. The same experiment performed on a crude receptor model led to identification of 31 active compounds.

Discussion and Conclusions

The modeling of GPCR can be focused on either studying the dynamical behavior of the receptor structure upon ligand binding by means of an MD simulation protocol or a thorough description of a possible ligand binding mode. Due to a high computational cost of MD simulations, only a few starting ligand−receptor complexes can be examined. In practice, a single trajectory is usually produced. Thus, in the first approach, the possible conclusions on the ligand binding mode may be biased by its arbitrary placement in a binding site or by a single starting receptor structure. Although an MD simulation produces a population of conformations of the ligand−receptor complex, this population explores only a part of a potential energy hypersurface (local minimum). The exploration of other minima is possible only provided that other optimal starting complexes were produced and investigated. In the second approach, a binding mode determination can either be conducted via manual docking using the knowledge from SAR and mutagenesis experiments, followed by energy optimization,[24] or be performed automatically, allowing for a vast number of ligand conformations to be probed. An additional incorporation of multiple receptor structures leads to an extensive sampling of possible ligand receptor complexes, thus yielding results with the highest confidence.[29] In our approach, the consideration of both receptor and ligand flexibility was combined with the incorporation of SAR and mutagenesis data in the process of receptor modeling. While the MD-based methodology can be useful as a source of information on ligand-induced conformational changes involved in GPCR activation, the strategy used in the present study seems more suitable if the binding mode determination or affinity prediction is a goal. Considering the main objectives of this study, to validate the model we used compounds with a decreased conformational flexibility, which is believed to encode the information on a shape of binding site and the spatial arrangement of specific interaction points within the binding pocket. As has already been mentioned in the "Model building" chapter, extra- and intracellular loops were excluded from our model. For the binding mode of arylpiperazines proposed here, mainly loop e2 may be in van der Waals contact with the ligand. Considering the length of this loop (18 residues), it may influence the shape of the remaining e1 and e3 loops. Inclusion of all the three loops (consisting of 31 residues in total) with large conformational freedom of both backbone and side chain dihedral angles would result in a huge number of possible receptor conformations, intractable by our approach. Nevertheless, with no loops included, we were able to obtain the correlation between specific ligand−receptor interactions and experimental binding affinity values. It turned out that all, or the vast majority, of residues responsible for specific interactions with the ligand were situated within the modeled, helical portion of the receptor.

The arylpiperazine binding mode described in this study is basically consistent with that proposed by Seeber et al.,[28] also obtained using automated docking. However, in our model, a larger number of specific interactions has been described, especially those responsible for the recognition of aromatic moiety. In the group of models proposed by Sylte et al., a ligand interacts with Ser7.46.[23] In our model, this residue is substantially buried and thus isolated from the ligand, mainly due to the distance between TMH3 and TMH7, which is comparable to the analogous distance in rhodopsin. Such an arrangement of helices causes the significant decrease in the conformational

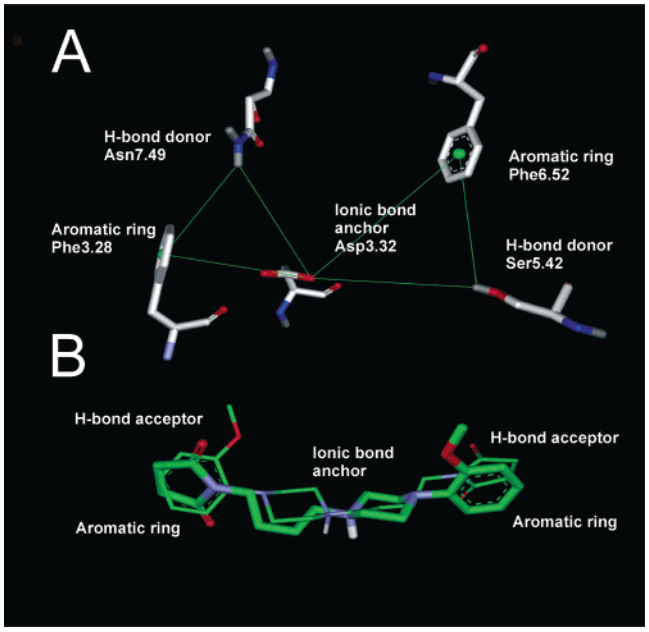


Figure 9. The symmetry of the binding site (A) and arylpiperazine ligand (B) interaction points. Anchoring points for all the pharmacophoric features of complex arylpiperazines are available, regardless of the ligand orientation within the binding pocket.

space of Phe3.28 and Tyr7.43, which obstruct the access to Ser7.46; hence such an interaction is impossible. The position of the ligand suggested by Lopez et al.[24] is similar to that observed in our model, whereas its orientation is opposite. This ambiguity is due to the symmetry of both 5-HT$_{1A}$ receptor binding site and complex arylpiperazines (Figure 9) and may constitute a serious obstacle to the determination of the binding mode. Summing up the arguments for the binding mode proposed in this study: (1) it has been established by means of automated docking to many conformations of the receptor model; (2) it is based on a group of rigid compounds; (3) flexible analogues adopt a similar binding pose and conformation; (4) the placement of arylpiperazine moiety has been additionally confirmed by docking of N4-unsubstituted phenylpiperazines and distant structural analogues of arylpiperazine (aporphine and ergoline derivatives); (5) the part of the binding site proposed here to accommodate the arylpiperazine moiety is very well conserved in all GPCRs to which the arylpiperazines display high affinity; (6) imide moieties (bulky in many cases) occupy more voluminous part of the binding site; (7) the majority of possible interaction points of the studied ligands have their specific anchoring points in our receptor model.

The model used in the virtual screening-like experiment was selected from the population on the basis of its ability to reproduce SAR data for a group of diversely substituted cyclohexyl arylpiperazines. This model accurately predicted the affinity of a relatively small, structurally homogeneous test set. The use of a single model for docking a larger and more diversified group of ligands cannot result in the affinity prediction with an equal confidence. However, it has been ascertained by the enrichment obtained in such an approach that this model can be a useful tool for virtual screening. Considering promising results of the virtual screening-like experiment described above, the use of our model for "real" virtual screening application is possible. The proposed binding mode and a detailed description of the possible specific interactions should also enable the use of the model in de novo ligand design approaches.

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Supporting Information Available: Sequence alignment (5-HT$_{1A}$ vs rhodopsin), a table of compounds used to confirm the complex arylpiperazine binding mode, and a table of compounds used in the virtual screening-like experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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