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Molecular Modeling of the Dopamine D_2 and Serotonin 5-HT_{1A} Receptor Binding Modes of the Enantiomers of 5-OMe-BPAT

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Abstract—Molecular modeling studies were undertaken in order to elucidate the possible dopamine D_2 and serotonin 5-HT_{1A} receptor binding modes of the enantiomers of 5-methoxy-2-[*N*-(2-benzamidoethyl)-*N*-*n*-propylamino]tetralin (5-OMe-BPAT, **1**). For this purpose, a combination of indirect molecular modeling and direct construction of the seven transmembrane (7TM) domains of the receptors was employed in a stepwise, objective manner. Pharmacophore models and corresponding receptor maps were identified by superimposing selected sets of receptor agonists in their presumed pharmacologically active conformations, while taking the conformational freedom of the ligands into account. The 7TM models were then constructed around the agonist pharmacophore models, by adding the TM domains one-by-one. Initially, the relative positions of TM3, TM4, and TM5 were determined using the three-dimensional structure of bacteriorhodopsin, but subsequently the orientations of all TM domains were adjusted in order to mimic the topology of the TM domains of rhodopsin. The presumed dopamine D_2 receptor binding conformations of (*S*)- and (*R*)-**1** were determined by using the semirigid dopamine D_2 receptor antagonist *N*-benzylpiquindone as a template for superposition. Similarly, the selective serotonin 5-HT_{1A} receptor agonist flesinoxan was employed for identifying the serotonin 5-HT_{1A} receptor binding conformations of the enantiomers of **1**. After docking of the presumed pharmacologically active conformations in the 7TM models and subsequent optimization of the binding sites, specific interactions between the ligands and the surrounding amino acid residues, consistent with the structure–activity relationships, were observed. Thus, both enantiomers of **1** bound to the dopamine D_2 receptor model in a similar fashion: a reinforced electrostatic interaction was present between the protonated nitrogen atoms and Asp114 in TM3; their carbonyl groups accepted a H-bond from Ser121 in TM3; their amide NH groups acted as H-bond donor to Tyr416 in TM7; and their benzamide phenyl rings were involved in a hydrophobic edge-to-face interaction with Trp386 in TM6. Differences were observed in the orientations of the 2-aminotetralin moieties, which occupied the agonist binding site. Whereas the (*S*)-enantiomer could form a H-bond between its 5-methoxy substituent and Ser193 in TM5, the (*R*)-enantiomer could not, which may account for the differences in their intrinsic efficacies at the dopamine D_2 receptor. In the serotonin 5-HT_{1A} receptor model, the benzamide phenyl rings of both enantiomers were involved in hydrophobic face-to-face interactions with Phe112 in TM3, while their protonated nitrogen atoms formed a reinforced electrostatic interaction with Asp116 in TM3. Consistent with the structure-affinity relationships of **1**, the amide moieties were not involved in specific interactions. Both enantiomers of **1** could form a hydrogen bond between their 5-methoxy substituent and Thr200 in TM5, which may account for their full serotonin 5-HT_{1A} receptor agonist properties.

Introduction

Dopamine and serotonin exert their physiological effects by interacting with specific receptors. With the exception of the serotonin 5-HT_3 receptor, all dopaminergic and serotonergic receptor subtypes currently identified belong to the superfamily of G-protein-coupled receptors (GPCRs). Although several hundred different members of this family have been identified to date,[1] little is known about their molecular properties. Since it has not been possible to unequivocally establish the exact molecular structure of any of these receptors by techniques such as X-ray crystallography thus far, structural information has only been obtained from indirect methods, such as multiple sequence alignments,[2] identification of evolutionary conserved amino acid residues,[3] and hydropathy analyses.[4] A common picture emerging from these indirect methods is that GPCRs are embedded in the cell membranes in a specific manner. Presumably they traverse the lipid bilayers seven times, with the amino-terminal and carboxy-terminal regions located extra- and intracellularly, respectively. The seven transmembrane (TM) domains,

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corresponding to relatively hydrophobic regions in the amino acid sequence, are believed to comprise α-helices, and to be interconnected via intracellular and extracellular loops of relatively hydrophilic nature. A similar pattern has been observed for bacteriorhodopsin (bR), from which a three-dimensional (3-D) structure at a resolution of 7 Å has been obtained by electron cryomicroscopy (Fig. 1).[5,6] This structure has recently been confirmed to a large extent by an X-ray structure, which was resolved at 2.5 Å.[7] bR constitutes a light-driven proton pump embedded in the cell membranes of *Halobacterium salinarium*, which responds to light by isomerization of a retinal molecule, that is covalently bound in the core of the protein. The net result of the subsequent conformational changes is the translocation of a proton from the inside to the outside of the bacterium.[8] The apparent structural similarities between bR and GPCRs have prompted a number of researchers to use the molecular structure of bR as a structural template for the construction of molecular models of GPCRs (e.g. see refs 9–13), a technique which is referred to as homology modeling.[14–16] However, unlike GPCRs, bR is not coupled to a G-protein. Mammalian opsins do belong to the family of GPCRs, but nevertheless they also share some structural and functional features with bR. Thus, similar to bR, they function by photoisomerization of a covalently bound retinal molecule, but instead of proton extrusion, this results in an intracellular signal transduction mediated by the associated G-protein termed transducin.[17] Therefore, the mammalian opsins have been suggested to form the evolutionary link between bR and ligand-binding GPCRs, which provides a rationale for using the structure of bR as a structural template for the construction of molecular models of GPCRs.[16,18,19] Supportive of this hypothesis is a two-dimensional (2-D) density projection map derived from bovine rhodopsin by electron cryo-microscopy, which showed a pattern that could be readily interpreted when assuming that this receptor forms a bundle of seven TM domains.[20] However, the suitability of the structure of bR for homology-based modeling of GPCRs may be questioned, since no significant (< 20%) sequence homology exists between bR and any of the GPCRs, including the opsins.[21] Furthermore, theoretical[22–24] as well as experimental[5,20,25] data suggest that, despite similarities in the overall membrane topology, the arrangement of the individual TM domains of bR and rhodopsin may be considerably different (Fig. 2).

Alternatively, when the structure of the receptor is not available, indirect molecular modeling techniques, such as pharmacophore identification or receptor mapping, may be undertaken in order to gain insight into the structural properties of the molecular target.[26,27] A pharmacophore may be defined as an ensemble of structural features which are required for a ligand to be recognized by the receptor. The spatial arrangement of these structural features is referred to as a pharmacophoric pattern, while the spatial arrangement of structural features of the target molecule, complementary to those of the pharmacophore, is referred to as the receptor map. In order to develop a pharmacophore or a receptor map, capable of accommodating a set of ligands, the active analogue approach may be employed. Two stages can be recognized in this approach. First, the relative importance of the different functional groups present or absent in a set of known active or inactive ligands is determined, taking the SAR of the compounds into consideration. This may reveal information about the nature of the complementary functional groups in the receptor, involved in the binding of the ligands. Secondly, a hypothesis is proposed about the spatial arrangement between either the functional groups of the ligands, resulting in a pharmacophoric pattern, or the recognition points postulated to belong to the receptor, resulting in a receptor map. The hypothesis is evaluated by searching for a spatial arrangement of the functional groups *c.q.* interaction points which is common to all of the ligands. Since the global minimum energy conformation of a ligand does not necessarily need to be the pharmacologically active one, the conformational

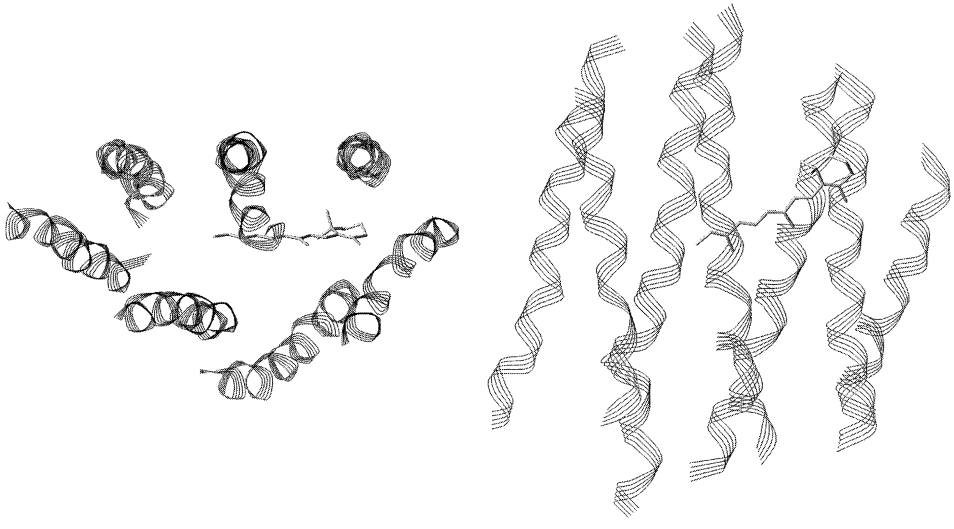

Figure 1. Inside-to-outside (left) and othogonal view (right) of the 3-D electron cryomicroscopy-derived structure of bR, showing the relative arrangements of the 7TM domains, visualized as line ribbons, and the position of the retinal chromophore.

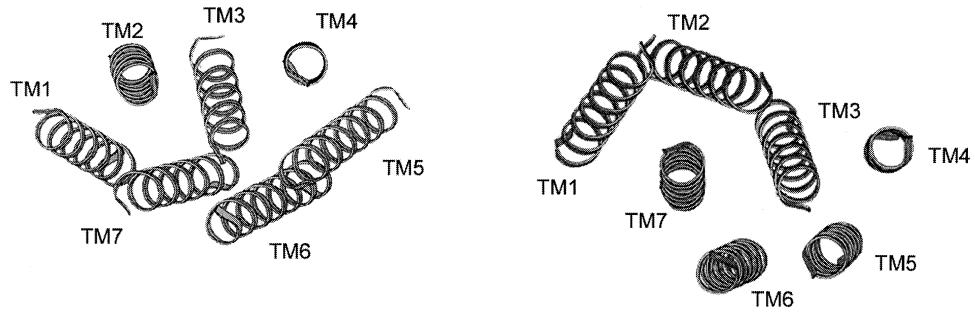

Figure 2. Schematic representation of the topology of the TM domains of bR (left) and rhodopsin (right) based on helix assignment calculations by Herzyck and Hubbard. The view is from the intracellular side. Adapted from ref 23.

freedom of the ligands under investigation has to be taken into consideration during the processes of pharmacophore identification and receptor mapping. For flexible molecules, this poses a potential problem, since their presumed pharmacologically active conformations have to be deduced from a large number of minimum energy conformations. However, this problem may be tackled by including an active ligand with restricted conformational freedom in the data set, which can then be used as a template for matching the optimal conformations of the flexible ligands.

In order to improve the quality of a GPCR model, the techniques of homology modeling and indirect molecular modeling may be combined. The active site of the receptor can be determined by probing the homology model with a pharmacophore *c.q.* receptor map obtained from indirect modeling approaches. The combination of these two approaches seems attractive, since it includes more experimental data than the homology-based model alone. Furthermore, a more realistic binding site, capable of accommodating structurally diverse ligands and rationalizing their affinities and activities, may be obtained. Several successful applications of this approach have appeared in literature.[9,11,28,29]

The enantiomers of the potential atypical antipsychotic agent 5-methoxy-2-[*N*-(2-benzamidoethyl)-*N*-*n*-propylamino]tetralin (5-OMe-BPAT, **1**, Chart 1) were shown to possess different intrinsic efficacies at dopamine D_2 and D_3 receptors both in vitro and in vivo.[30] Particularly the in vivo results suggested that (*S*)-5-OMe-BPAT [(*S*)-**1**] acts as a dopamine D_2 receptor agonist, while (*R*)-5-OMe-BPAT [(*R*)-**1**] behaves as a dopamine D_2 receptor antagonist. Furthermore, both enantiomers were shown to act as full serotonin 5-HT_{1A} receptor agonists in vitro and in vivo. The interactions with the receptors in vitro were stereospecific, since (*S*)-**1** had the highest serotonergic affinity, while (*R*)-**1** preferred the

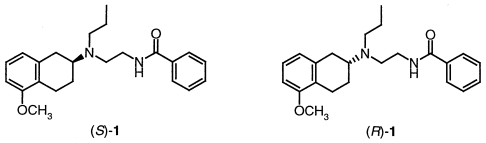

Chart 1. Chemical structures of the enantiomers of 5-methoxy-2-[*N*-(2-benzamidoethyl)-*N*-*n*-propylamino]-tetralin (5-OMe-BPAT, **1**).

dopaminergic receptors. Earlier investigations on the SAFIR of structural analogues and derivatives of racemic **1** revealed several important clues about the possible receptor binding modes of this compound.[31–33] First, the 2-aminotetralin moiety of **1** is likely to occupy the same binding sites as the 2-aminotetralin moiety of the *N,N*-di-*n*-propylaminotetralins (DPATs) in both the dopaminergic and serotonergic receptors. Furthermore, the benzamidoethyl side chain seemed to enhance the affinities of **1** for both the dopaminergic and serotonergic receptors, but not in an identical way. Thus, the SAFIR suggested that the benzamide moiety of the side chain may occupy the same binding sites in the dopaminergic receptors as the benzamide moieties of the 2-pyrrolidinylmethyl-derived substituted benzamides, presumably by forming specific interactions between the amide functionality and certain amino acid residues in the receptors. At the serotonin 5-HT_{1A} receptor, the benzamidoethyl side chain as a whole seemed to act merely as a 'spacer', which enhanced the affinity due to interactions not involving the amide functionality. The 5-methoxy substituent of **1** appeared to be important for the dopaminergic affinities, but not for the serotonergic affinity.

In the present investigation, molecular modeling studies were undertaken in order to rationalize the modes of binding of (*S*)- and (*R*)-**1** to the dopamine D_2 and serotonin 5-HT_{1A} receptor. A combination of indirect molecular modeling, that is, pharmacophore identification and receptor mapping, using the active analogue approach, and homology-based modeling of the 7TM domains of the receptors was used for this purpose, while taking the SAFIRs as described above into account.

Molecular Modeling

Ligand selection

Sets of well-known dopamine D_2 and serotonin 5-HT_{1A} receptor agonists of various chemical classes were selected, in order to be able to identify the agonist binding sites in the two receptor subtypes. The following dopamine D_2 receptor agonists were taken into consideration: dopamine (**2**, Chart 2), (*S*)-5-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin [(*S*)-5-OH-DPAT, **3**], (*R*)-7-hydroxy-

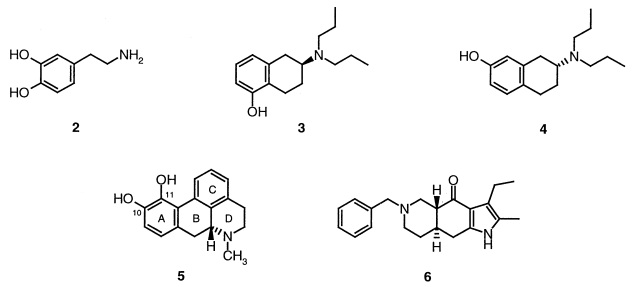

Chart 2. Chemical structures of the dopamine D_2 receptor agonists dopamine (**2**), (*S*)-5-OH-DPAT (**3**), (*R*)-7-OH-DPAT (**4**), and (*R*)-apomorphine (**5**), and the dopamine D_2 receptor antagonist *N*-benzylpiquindone (**6**).

2-(*N,N*-di-*n*-propylamino)tetralin [(*R*)-7-OH-DPAT, **4**], and (*R*)-apomorphine (**5**). In addition, the pyrroloisoquinoline derivative *N*-benzylpiquindone[34] (**6**) was used as a semirigid template for identifying the dopamine D_2 receptor binding conformations of (*S*)- and (*R*)-**1**. Similarly, for the identification of a serotonin 5-HT_{1A} receptor agonist receptor map, the following serotonin 5-HT_{1A} receptor agonists were selected: serotonin (**7**, Chart 3), (*R*)-8-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin [(*R*)-8-OH-DPAT, **8**], the partial ergoline LY 197,206[35] (**9**), and (*R*)-10-methyl-11-hydroxyaporphine[36] (**10**). The enantiomer of compound **9**, which is homochiral to **8**, was considered. In addition, the selective serotonin 5-HT_{1A} receptor agonist flesinoxan[37] (**11**) was chosen as a template molecule for determining the serotonin 5-HT_{1A} receptor agonist conformations of (*S*)- and (*R*)-**1**.

Conformational analyses

Prior to the pharmacophore identification, all ligands considered in this investigation were submitted to extensive conformational analyses in MacroModel.[38] Only conformations with conformational energies within 3 kcal/mol above the global minimum energy conformation were considered to be relevant.[39] All ligands were considered in their protonated, positively charged forms. Water was simulated as the solvent during the analyses. *N*-*n*-Propyl groups were truncated to *N*-methyl groups, and benzamide aromatic rings were maintained in a coplanar fashion with the amide moieties during all minimizations. The arylpiperazine moiety of **11** was fixed in the conformation as described by Kuipers et al.[37] during all minimizations. For ligands containing a protonated nitrogen atom with three different substituents (i.e. **1**, **5**, **6**, and **10**), the possibility of inversion of this nitrogen atom was taken into account by calculating two subpopulations of minimum energy conformations for these ligands, which only differed in the orientation of the proton at the basic nitrogen.[40] These subpopulations were then combined, and conformations with energies more than 3 kcal/mol above the global energy minimum were discarded.

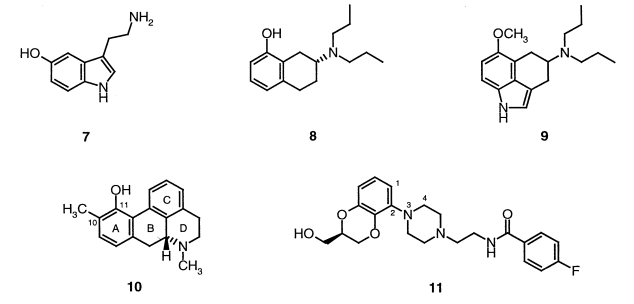

Chart 3. Chemical structures of the serotonin 5-HT_{1A} receptor agonists serotonin (**7**), (*R*)-8-OH-DPAT (**8**), LY 197,206 (**9**), (*R*)-10-methyl-11-hydroxyaporphine (**10**), and flesinoxan (**11**).

Pharmacophore identification

The program APOLLO (Automated PharmacOphore Location through Ligand Overlap)[41,42] was used for establishing the dopamine D_2 and serotonin 5-HT_{1A} receptor binding conformations of the ligands under investigation, and for the identification of different pharmacophoric patterns and receptor maps for binding at these receptor subtypes. Although APOLLO also allows to explicitly use specific functional groups *c.q.* atoms as pharmacophoric elements, the main purpose of the program is to identify from a set of ligands their mutual interaction points belonging to the receptor site (i.e. a receptor map). In this way additional flexibility is introduced, since different ligands are allowed to interact with the same receptor point from different directions. Using APOLLO, vector points emanating from essential functional groups are defined in all minimum energy conformations of the ligands under investigation. Subsequently the appropriate vector points of the different ligands are superimposed, while considering all possible combinations of the minimum energy conformations of the ligands. Different weights may be assigned to different fitting points, and in cases where functional groups may form interactions from different sides, APOLLO can choose which vector results in the best superposition. Specific matches are scored using root mean square (RMS) deviations, and conformational energies may be included in the scoring procedure. Finally, APOLLO can reproduce the superimposed ligands of all matches, and places water molecules at the mutual interaction points, mimicking H-bond donating or accepting amino acid residues belonging to the receptor, and thus representing a simple receptor map.

Receptor construction

Construction of the 7TM models of the dopamine D_2 and serotonin 5-HT_{1A} receptors was performed in SYBYL.[43] The amino acid sequences of the human dopamine D_{2A} and the human serotonin 5-HT_{1A} receptor were obtained from the literature[44,45] and were manually aligned with the sequences of other dopaminergic and serotonergic receptor subtypes. The alignment was guided by identifying evolutionary conserved amino acids. The amino acids to be included in the TM domains were visually selected, based on their relatively hydrophobic nature. The TM domains thus selected are shown in Figure 3. The 7TM models were constructed in a stepwise manner, with the aid of the receptor maps as identified by APOLLO, and the 3-D structure of bR,[5] which was retrieved from the Brookhaven Protein

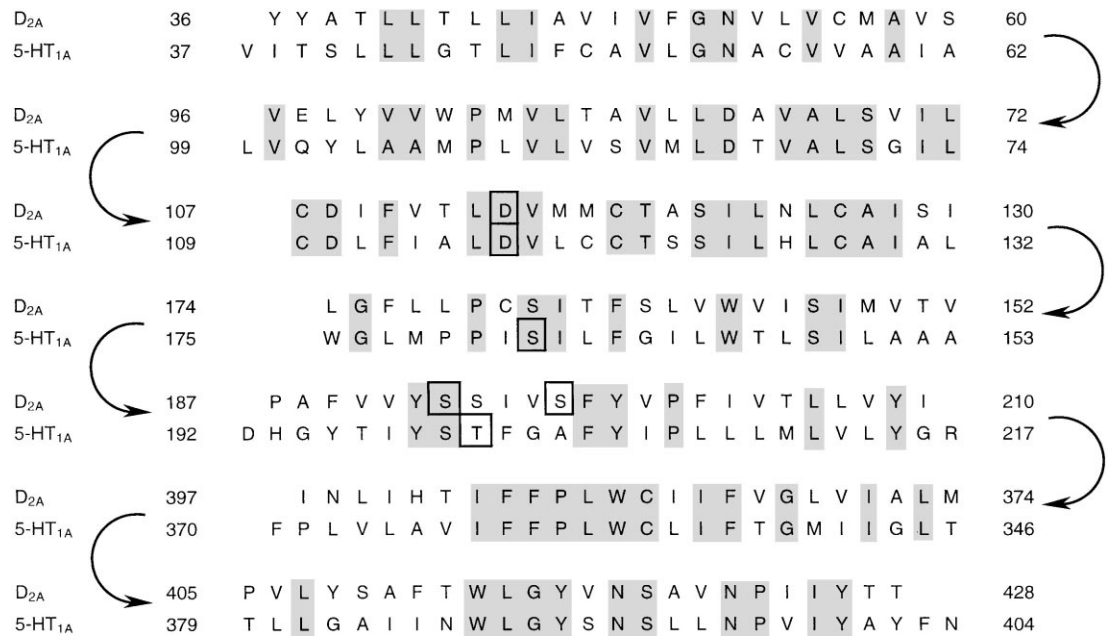

Figure 3. Alignment of the seven putative TM domains of the human dopamine D_{2A} and serotonin 5-HT_{1A} receptors. The domains are displayed back and forth, starting from left to right with TM1 at the top. Numbers correspond to the amino acids at the start and end of the rows. Evolutionary conserved amino acids are shaded, and amino acids presumed to be important for binding of the endogenous ligands are boxed.

Databank (PDB code: 1BRD; see Fig. 1). Construction of the models was started by positioning TM3 and TM5 at opposite sides of the superimposed agonists, while superimposing specific amino acid residues, presumably involved in the primary interactions with the ligands, with their side chains replacing the water molecules included in the receptor maps. The other TM domains were then added one by one, in the following order: TM4, TM6, TM2, TM7, and TM1. The 3-D structure of bR was used for their initial crude positioning, but subsequently their relative arrangements were manually adjusted in order to obtain 7TM models which more closely resemble the topology of the TM domains of rhodopsin, according to the model calculated by Herzyk and Hubbard[23] (see Fig. 2).

Ligand docking

All ligands considered in this investigation were individually and manually docked into the active sites of the final 7TM models in their presumed pharmacologically active conformations, as determined with APOLLO. The ligands were initially positioned in such a way that the interactions between their functional groups and the appropriate amino acid side chains, presumably primarily involved in the binding process, could be visualized as H-bonds. The interaction energies between the receptors and the ligands were then minimized by manually adjusting the positions of the ligands in the binding sites, while attempting to maintain the primary interactions displayed as H-bonds, using the Docking procedure as implemented in SYBYL.

Active site optimization

The interactions between the amino acids in the binding sites and the individually docked ligands were finally optimized using the pseudoreceptor-generating program PrGen. This program was originally designed for the construction of mini- and pseudoreceptors around a set of ligands, superimposed in their presumed pharmacologically active conformations.[46,47] For this purpose, PrGen relies on the Yeti force field, in which the directionalities of H-bonds and electrostatic interactions have been strongly implemented.[48] Translational, rotational, and torsional degrees of freedom for both ligands and amino acid residues can be specified to the accuracy of single bonds. This makes the program very suitable for optimizing binding sites derived from homology-based models, since it allows the backbone atoms to be fixed, and hence to maintain the overall shape of the binding site.

Results and Discussion

The purpose of the present investigation was to gain insight into how the enantiomers of 5-OMe-BPAT exert their effects at the molecular level, using molecular modeling techniques. Although both enantiomers have high affinities for dopamine D_2, D_3, and serotonin 5-HT_{1A} receptors, only their binding modes at the dopamine D_2 and the serotonin 5-HT_{1A} receptor were investigated. The results from the in vivo studies suggested that their effects are predominantly mediated via these two receptor subtypes.[30] Furthermore, since all dopamine receptor agonists, previously designated to be selective for the dopamine D_2 receptor, also have comparable or even higher affinities for the dopamine D_3 receptor, and since the dopamine D_2 and D_3 receptors share a sequence homology of about 80% in the TM domains, the modeling approach employed here would in all probability result in virtually identical binding sites for the two receptor subtypes. Dopamine D_2 versus

D_3 receptor selectivities would then have to be explained based on very subtle differences in receptor conformations (e.g. see ref 28). Therefore, the dopamine D_3 receptor binding modes of (*S*)- and (*R*)-**1** were not considered in this study.

The process of receptor construction was started by mapping the agonist binding sites in the two receptor subtypes, using the active analogue approach. It is very likely that receptor agonists share the same binding site, since they also share the ability to stimulate the receptor during the binding process. Furthermore, sequence alignments and site-directed mutagenesis studies (see below) have identified several amino acid residues which are presumed to be important for agonist binding. Definition of the agonist binding sites was therefore considered to be a good starting point for the construction of the 7TM models. For the identification of the dopamine D_2 receptor agonist pharmacophore, several structural features of the selected agonists were considered to be important for their activity.[49–52] First, all dopamine receptor ligands known to date contain a basic nitrogen atom, which will to a large extent be protonated under physiological conditions. It is generally believed that the protonated nitrogen atoms are involved in a reinforced electrostatic interaction with a highly conserved aspartic acid residue in TM3 (Asp114 in D_{2A}). This interaction was simulated by placing vector points emanating from the protonated nitrogen atoms of **2**–**5**. Second, similar to dopamine, all dopamine receptor agonists contain an aromatic ring (or a bioisostere) at a certain distance from the basic nitrogen atom, capable of forming hydrophobic interactions. In order to include them for superposition, centroids and normals were defined in these rings (i.e. the A-ring of **5**). Third, the *meta*-hydroxy substituent of dopamine is important for binding to the dopamine D_2 receptor, but the *para*-hydroxy substituent is not. SARs suggest that the *meta*-hydroxy substituent functions both as H-bond donor and acceptor. Thus, the *meta*-hydroxy substituent of dopamine, the hydroxy substituents of **3** and **4**, and the 11-hydroxy substituent of **5** were defined as both H-bond donor and acceptor. The best matching superposition, resulting from fitting the appropriate vector points emanating from the hydroxy substituents, the centroids and normals defined through the aromatic rings, and the vector points emanating from the protonated nitrogen atoms, is shown in Figure 4.[53] In the best match, dopamine adopts a folded conformation, while the 2-aminoteralin moieties of **3** and **4** are in a half-chair conformation with a pseudo-equatorial amino group.

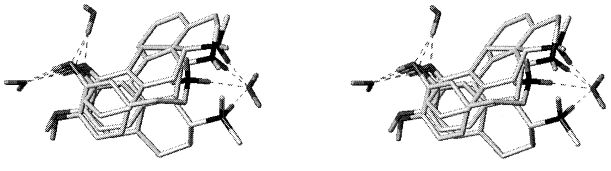

Figure 4. Stereo representation of the superposition of the dopamine D_2 receptor agonists **2**–**5** in their presumed dopamine D_2 receptor binding conformations. The water molecules mimic putative acid residues belonging to the receptor, capable of forming interactions (dotted lines) with the ligands.

The *N*-methyl substituent of **5** is also oriented in a pseudo-equatorial fashion. The aromatic rings and the hydroxy substituents, corresponding to the meta-hydroxy substituent of dopamine, are oriented in a similar fashion and show good overlap. However, the fact that all ligands form an interaction with their protonated nitrogens and a mutual putative receptor point, although they approach this point from different directions, clearly shows that explicit overlap of functional groups belonging to the ligands (in this case the protonated nitrogen atoms) is not required for a good interaction with the receptor. In this respect it is noteworthy that the superposition of **3** and **4**, which possess opposite absolute configurations in their active enantiomers, strongly resembles the pharmacophore models proposed by Grol et al.[54] and Johansson et al.,[55] and hence differs substantially from the well-known McDermed model,[56] in which the hydroxy substituents, the aromatic nuclei and the protonated nitrogen atoms of **3** and **4** explicitly overlap.

Similar to dopamine receptor agonists, formation of a reinforced electrostatic interaction via a protonated basic nitrogen atom presumably is essential for binding of serotonin 5-HT_{1A} receptor ligands to the receptors. Furthermore, the 5-hydroxy substituent of serotonin may act as a H-bond acceptor, since the methoxy analogues of serotonin and hydroxy-containing synthetic serotonin 5-HT_{1A} receptor agonists are usually equipotent. The indole NH of serotonin is also important for binding, since *N*-alkylation of this group decreases its affinity for the serotonin 5-HT_{1A} receptor.[57] Taking these observations into account, the appropriate vector points, centroids and normals were defined in **7**–**10** and superimposed. The A-ring of **10** was used for superposition of the aromatic rings. Despite its importance, the indole NH group could not be included in the fitting procedure, since not all ligands contain this functionality. However, the best matching superposition (Fig. 5) shows that the indole NH groups of **7** and **9** coincide

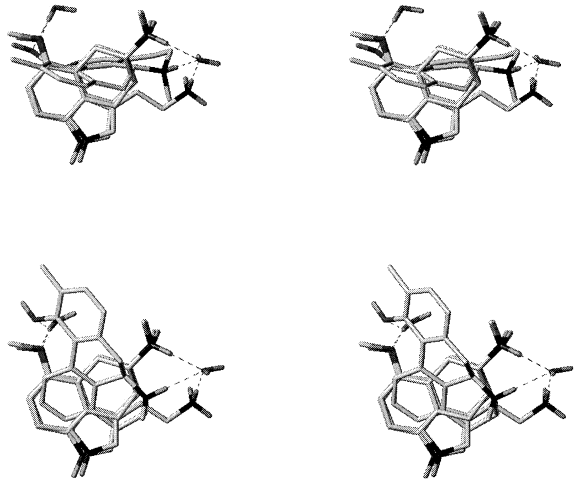

Figure 5. Stereo representation of the two superposition modes of the serotonin 5-HT_{1A} receptor agonists **7**–**10** in their presumed receptor binding conformations, using the A-ring (top) and C-ring (bottom) of **10** for superposition. The water molecules mimic putative amino acid residues belonging to the receptor, capable of forming interactions (dotted lines) with the ligands.

and point in the same direction, indicating that they may interact with a mutual H-bond accepting amino acid residue. The side chain of serotonin is oriented in an all-*trans* conformation, lying in the plane of the indole nucleus. The cyclohexyl rings of **8** and **9** have adopted a half-chair conformation, bearing pseudo-equatorial amino substituents. The hydroxy groups of the ligands coincide well and have a mutual H-bond-donating interaction point. However, because the 11-hydroxy group of **10** can only be approached from one side due to steric hindrance caused by the C-ring, and as a consequence of using the A-ring for aromatic superposition, **10** is oriented almost perpendicular to the plane through **7**–**9**. Nevertheless, it is still capable of forming the essential interactions with the receptor map. It is also noteworthy that **10** has an axial *N*-methyl substituent in the best match. During the process of receptor construction (see below) it soon became clear that **10** would be difficult to accommodate in the orientation as revealed by APOLLO, using the A-ring for superposition. Difficulties resulted from the 10-methyl group causing steric hindrance with TM5. Furthermore, due to the perpendicular orientation compared to the other agonists, the C-ring of **10** protruded in the direction of TM4, thus preventing this helix to be tightly packed with TM3 and TM5. Therefore, an alternative fitting mode of **7**–**10** was considered, using the C-ring of **10** for aromatic superposition. The same conformations of all ligands were identified, and the superposition of **7**–**9** was identical to the first approach (Fig. 5). Furthermore, the mutual interaction points were located at almost identical positions in the two fitting modes. However, in this alternative orientation, **10** could be well accommodated by the binding site, since it is lying in the plane of the other agonists, while the 10-methyl group points more away from TM5, in the direction of the binding site crevice. Particularly the alternative superposition mode of **7**–**10** stresses the conclusion drawn for the dopaminergic pharmacophore that corresponding functional groups of the different ligands may approach mutual interaction points belonging to the receptor from different angles.

All G-protein-coupled monoaminergic receptors contain a highly conserved aspartic acid residue at similar positions in TM3, corresponding to Asp114 and Asp116 in the dopamine D_{2A} and serotonin 5-HT_{1A} receptor, respectively. Site-directed mutagenesis studies have revealed that mutation of Asp114 to an uncharged residue completely abolishes the binding of both dopamine D_2 receptor agonists and antagonists,[58] strongly suggesting that the negatively charged carboxy terminus of Asp114 is involved in forming a reinforced electrostatic interaction with the protonated, positively charged nitrogen atom of dopamine and synthetic dopamine receptor ligands. In addition, a highly conserved aspartic acid residue in TM2, corresponding to Asp80 and Asp82 in the dopamine D_{2A} and serotonin 5-HT_{1A} receptor, respectively, is present in all monoaminergic GPCRs. Site-directed mutagenesis of this residue in the dopamine D_2 and α_2-adrenergic receptor revealed its importance for the pH and sodium regulations of agonist affinities, suggesting that it is involved in stabilizing the receptor in a certain conformation required for signal transduction, by complexation of cations.[59,60] Furthermore, catecholamine receptors contain a cluster of serine residues in TM5, corresponding to Ser193, Ser194, and Ser197 in the dopamine D_2 receptor, capable of forming H-bonds with a catecholamine moiety. Since the intracellular loop connecting TM5 and TM6 presumably is involved in the coupling of the receptor to its associated G-protein, the ability of a ligand to interact with one or more of these serine residues seems to be crucial for agonistic properties. However, the specific contributions of these serine residues in the binding process of dopamine and synthetic receptor agonists are not completely clear, since site-directed mutagenesis experiments on these residues resulted in different, sometimes contradicting effects for different ligands.[58,61–63] Nevertheless, Ser193 and Ser197 seem to be of importance for agonist binding, while Ser194 may be involved in mediating the ability of dopamine to inhibit cAMP production. Finally, mutation studies have shown that Phe389 and Phe390 in TM6 are crucial for ligand binding.[64] Possible interactions with the amino acids discussed above were taken into consideration when the 7TM model of the dopamine D_2 receptor was constructed around the superimposed ligands **2**–**5**. Thus, TM3 was positioned in such a way that the terminal carboxy terminus of Asp114 replaced the water molecule in the receptor map, which interacted with the protonated nitrogen atoms of **2**–**5**. Similarly, the terminal hydroxy groups of Ser193 and Ser197 replaced the water molecules interacting with the hydroxy groups of **2**–**5**. Ser193 was defined as H-bond donor and Ser197 as H-bond acceptor (Fig. 6). TM4 was the placed on the correct side of TM3 and TM5 with the aid of the 3-D structure of bR, resulting in a counterclockwise orientation of the TM domains when viewed from the outside. The positioning of TM4 could also be guided by the possibility to form a disulfide bridge between Cys118 in TM3 and Cys168 in TM4 (Fig. 6), as proposed by Moereels and Leysen.[65] Cys118 is a conserved amino acid, present in the sequences of a number of GPCRs. Cys168, however, is only found in the dopamine D_2 and D_3 receptors.[66] The possibility to form this disulfide bridge may therefore be important for maintaining the relative orientation of TM3 to TM4, as well as the overall shape of the dopamine D_2 and D_3 receptors. Javitch et al., however, claimed that Cys118 is exposed in the binding site, based on studies with sulfhydryl-specific methanethiosulfonate reagents that were allowed to react with cysteine residues, suggesting that Cys118 is not involved in disulfide bridge formation.[67] Therefore, as suggested by Moereels and Leysen,[65] Cys118 should be an interesting candidate for site-directed mutagenesis studies.

Site-directed mutagenesis studies have confirmed that Asp116 in TM3 of the serotonin 5-HT_{1A} receptor probably is involved in the formation of a reinforced electrostatic interaction with the protonated nitrogen atoms of ligands binding to this receptor subtype. TM5 of the serotonin 5-HT_{1A} receptor contains a serine (Ser199) and a threonine (Thr200) residue, both capable of forming H-bonds with the 5-hydroxy group of

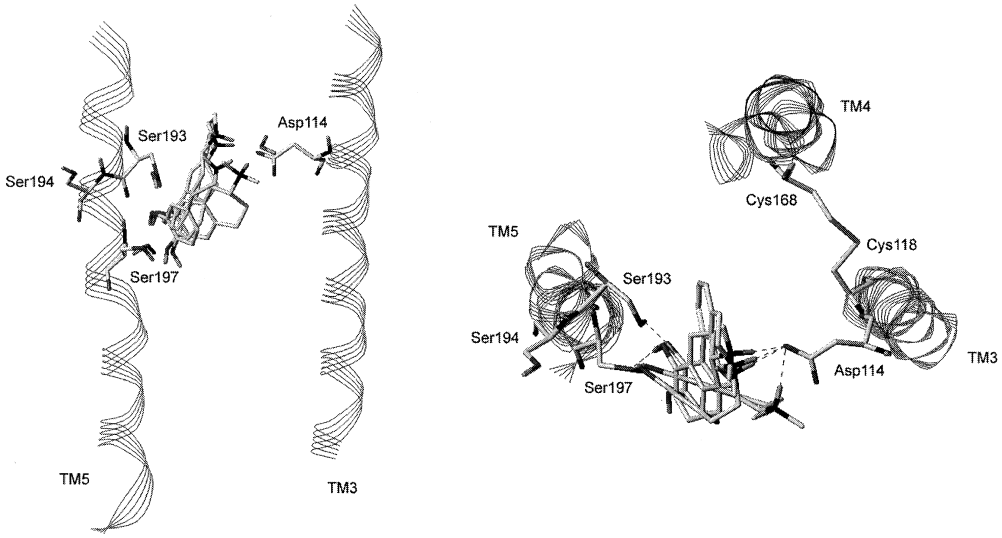

Figure 6. Pictures illustrating the stepwise construction process of the dopamine D_2 receptor model. The left picture shows the positioning of TM3 and TM5 with the aid of the indirectly obtained receptor map. The right picture gives a top-to-bottom view of the relative positions of TM3, TM4 and TM5. The positioning of TM4 could be guided by the possibility to form a disulfide bridge between Cys118 in TM3 and Cys168 in TM4, which is clearly visible. The backbones of the TM domains are displayed as line ribbons.

serotonin. Thr200 is conserved in all 5-HT_1 subclass receptors. Since these receptor subtypes also require the presence of the 5-hydroxy substituent for serotonin to bind, Thr200 appears to be important for the affinity of serotonin, and presumably acts as a H-bond donor to the 5-hydroxy substituent of serotonin. The importance of Thr200 for serotonin binding was supported by site-directed mutagenesis studies.[68] However, Ser199 also seems to be involved in serotonin binding, since mutation of this residue was found to decrease the affinity of serotonin as well.[68] Kuipers et al. suggested that Ser199 may be involved in binding the indole NH of serotonin.[10] This assumption was based on the observation that Ser199 is conserved in serotonin 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors, while in serotonin 5-HT_{2A} and 5-HT_{2C} receptors, this residue is replaced by a glycine. This appears to be consistent with the observations that an unsubstituted indole nitrogen of serotonin is required for high affinity at the 5-HT_1 but not for the 5-HT_2 receptor subtypes. In analogy with the dopaminergic 7TM model, the construction process was started by bringing TM3 and TM5 in close contact with the superimposed agonists **7**–**10**. Asp116 in TM3 was directed at the protonated nitrogen atoms, thus merely replacing the water molecule in the receptor map, while the terminal hydroxy group of Thr200 was defined as H-bond donor towards the 5-hydroxy substituent of serotonin. It soon became evident that a simultaneous interaction between Ser199 and the indole NH groups of serotonin and **9** was unlikely, since the orientation of the cluster of agonists required for this to occur caused serious steric hindrance between the ligands and the backbone of TM5. During the construction process however, an interaction between the indole NH groups of serotonin and **9** and Ser168 in TM4 became possible, which was also useful for determining the position of TM4 relative to TM3 and TM5. The 7TM models of the dopamine D_2 and serotonin 5-HT_{1A} receptor finally obtained are shown in Figure 7. The overall topological arrangement of the 7TM domains in both models is clearly dissimilar from bR (Figs 1 and 2), and show much more resemblance with rhodopsin (Fig. 2). Nevertheless, differences in the relative positions of the TM domains are also clear. Particularly the relative arrangement of TM3, TM4, and TM5 in the serotonin 5-HT_{1A} receptor model differs considerably from that of rhodopsin and the dopamine D_2 receptor model. Deviations in these arrangements probably arise from

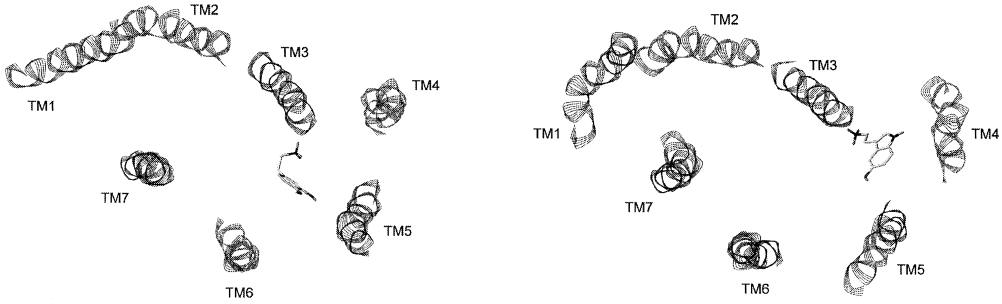

Figure 7. Pictures showing the topological arrangements of the TM domains of the final 7TM models of the dopamine D_2 (left) and serotonin 5-HT_{1A} receptor (right) and the locations of the endogenous ligands in the active sites. The backbones of the TM domains are displayed as line ribbons. The view is from the intracellular side.

differences in packing of the helices, due to differences in amino acid sequences and in the receptor maps used for the construction of the agonist binding sites. Since most published 7TM models of the dopamine D_2 and serotonin 5-HT_{1A} receptor were constructed either by mutating the amino acids in the 3-D structure of bR,[10,12,37] or by using the 3-D structure of bR as an explicit template for positioning all TM domains,[13,14,28,69,70] it will be difficult to compare those models with the results obtained in the present investigation.

The interactions of the agonists with the receptors, revealed after individual docking and optimization of their binding sites, are exemplified by dopamine (Fig. 8) and serotonin (Fig. 9). Dopamine interacts with the receptor in a folded conformation. The protonated nitrogen atom forms a reinforced electrostatic interaction with the carboxylate group of Asp114 on TM3. The *meta*-hydroxy substituent is involved in two H-bonds: it acts as a H-bond donor to the oxygen of Ser197, while at the same time accepting a H-bond from Ser193. Furthermore, Ser197 donates a H-bond to the *para*-hydroxy substituent. Both hydroxy substituents of dopamine are rotated slightly out of the plane of the aromatic ring. Ser194 is pointing towards TM6 and not involved in the binding of dopamine. In a model reported by Moereels and Leysen[65] dopamine was also found to interact with Ser193 and Ser197. However in their model, the *meta*-hydroxy substituent acts as H-bond donor to Ser193, while the *para*-hydroxy substituent acts as H-bond donor to Ser197. Furthermore, Phe390 in TM6 is in close contact with the aromatic ring of dopamine, presumably contributing to its affinity by hydrophobic interactions.

Compounds **3**–**5** occupied the agonist binding site in a similar fashion as dopamine (not shown). Thus, the hydroxy substituents of **3** and **4**, and the 11-hydroxy substituent of **5** acted as H-bond donor and acceptor to Ser197 and Ser193, respectively. In addition, similar to the *para*-hydroxy substituent of dopamine, the 10-hydroxy substituent of **5** accepted a H-bond form Ser197. All synthetic agonists formed a reinforced electrostatic interaction with their protonated nitrogens atoms and Asp114. It should be noted, however, that the relative positions of **2**–**5** after individual docking and optimization of the binding sites had changed considerably compared to the relative positions in the pharmacophore model (Fig. 4). The *N*-*n*-propyl groups of **3** and **4** could easily be accommodated by the binding site models. The so-called 'propyl group phenomenon' of the DPATs and structurally closely related compounds, which involves the observation that one of the N-substituents should be an unbranched group not larger than *N*-*n*-propyl, while the structural requirements for the other N-substituent are less demanding for optimal affinity, has been rationalized by the presence of a small lipophilic pocket in the dopamine D_2 receptor, capable of accommodating the *N*-*n*-propyl substituent.[71–73] Malmberg et al. were able to identify and visualize this 'propyl pocket' in the dopamine D_{2A} and D_3 receptor, using homology-based molecular modeling studies.[28] Our model did not clearly reveal the presence of such a pocket. Differences with the Malmberg model presumably result from differences in the arrangements of the TM domains, since their model was explicitly based on the 3-D structure of bR.

The extended conformation of serotonin, as revealed by APOLLO, was maintained after optimization of its position in the binding site (Fig. 9). Thr200 acts as H-bond donor to the 5-hydroxy substituent of serotonin, while Asp116 in TM3 forms a salt bridge with the protonated nitrogen atom. The indole NH acts as H-bond donor to Ser168 in TM4. Other neighbouring amino acid residues are Met172 in TM4, and Thr196, Ala203 and Phe204 in TM5. Similarly, the hydroxy substituents of **8** and **10**, and the methoxy substituent of **9** accepted a H-bond from Thr200, while their protonated nitrogens atoms formed a reinforced electrostatic interaction with Asp116 (not shown). In addition, like serotonin, the indole NH of **9** donated a H-bond to Ser168. Based on homology-based modeling studies, this serine residue was proposed by Hedberg et al. to function as H-bond donor to the 11-hydroxy substituent of **10**,[29] although in an earlier report they proposed a similar interaction with Ser199 in TM5.[69] In our model, Ser168 is not involved in the binding of **10**, in all probability due to a completely different orientation of

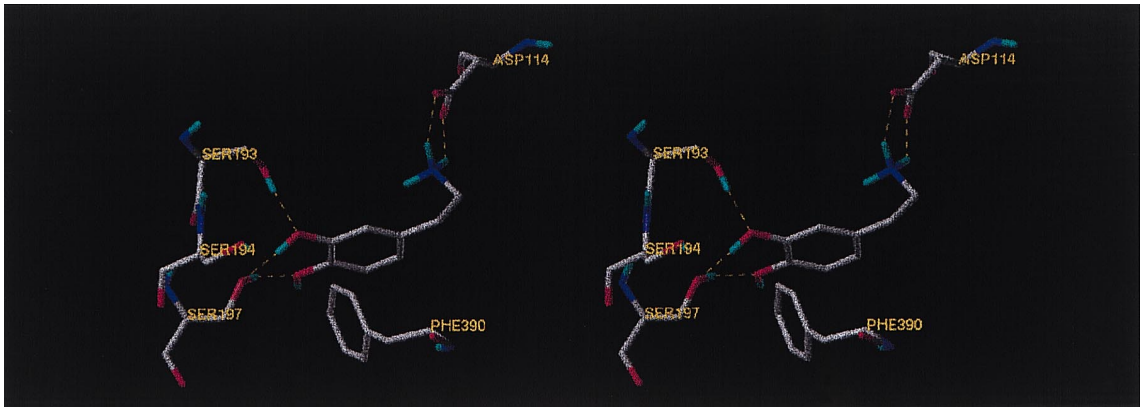

Figure 8. Stereo representation of dopamine in its optimized binding site model.

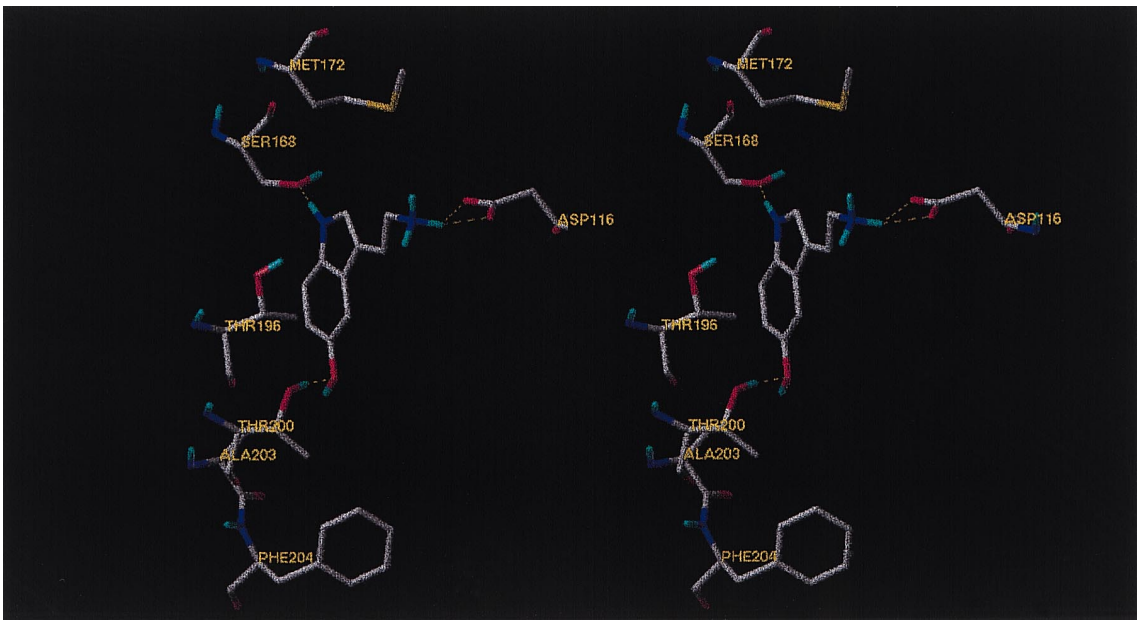

Figure 9. Stereo representation of serotonin in its optimized binding site model.

the ligand in the binding site and to differences in the overall TM domain topology as argued above. In view of the agonist properties of **10**, an interaction with either Thr200 or Ser199 seems the most reasonable.

SAFIRs of the 2-aminotetralin-derived benzamides suggested that the 2-aminotetralin moieties of the compounds may occupy the same binding site as the 2-aminotetralin moieties of the DPATs (i.e. the agonist binding site), while the 2-benzamidoethyl side chains may share the same binding site as the 2-pyrrolidinylmethyl-derived substituted benzamides in the dopamine D_2 receptor. Obviously, the dopamine D_2 receptor binding conformations of (*S*)- and (*R*)-**1** cannot be identified by including their 2-aminotetralin moieties in the fitting procedure as described for **2**–**5**, since this would not reveal the relative position of the benzamidoethyl side chains. Therefore, a suitable template molecule, containing pharmacophoric elements corresponding to both the benzamidoethyl side chains and the 2-aminotetralin moieties of (*S*)- and (*R*)-**1** needed to be considered. *N*-Benzylpiquindone (**6**) was selected for this purpose. This semirigid compound is a member of a series of pyrrolo[2,3-*g*]isoquinoline derivatives, which are highly stereoselective dopamine D_2 receptor antagonists.[34] (−)-Piquindone, the *N*-methyl analogue of **6**, and lead compound of the series,[74] has been frequently used as a rigid template for identifying the dopamine D_2 receptor binding conformations of 2-pyrrolidinylmethyl-derived substituted benzamides.[40,75–77] Although belonging to different chemical classes, (−)-piquindone and the 2-pyrrolidinylmethyl-derived substituted benzamides have several structural and pharmacological features in common, which is essential when employing the active analogue approach. Thus, both classes of compounds contain an aromatic ring capable of forming hydrophobic interactions, a carbonyl group, and a basic nitrogen atom at a certain distance from the aromatic ring. In addition, the binding of both classes of compounds is highly stereoselective and sodium-dependent, suggesting that they may share the same binding site and possess similar binding modes. Using homology-based receptor modeling, the dopamine D_2 receptor binding mode of several derivatives of (−)-piquindone has been elucidated by Teeter et al.[34] An interesting observation from their study was that **6** had a 55-times higher affinity for the dopamine D_2 receptor than the lead compound, suggesting that the binding of **6** was strongly enhanced by the presence of its *N*-benzyl group. In the receptor model, the *N*-benzyl group was located in the agonist binding site, forming favourable interactions with Phe389 and Phe390 in TM6. Therefore, **6** was considered to be a suitable template for identifying the dopamine D_2 receptor binding conformations of (*S*)- and (*R*)-**1**: the pyrrolo[2,3-*g*]isoquinoline skeleton could be used for fitting the benzamidoethyl side chains, while the *N*-benzyl group should identify the orientation of the 2-aminotetralin moieties. Thus, the pyrrole ring of **6**, corresponding to the benzamide nuclei of (*S*)- and (*R*)-**1**, the carbonyl groups, the protonated nitrogen atoms, and the *N*-benzyl group of **6**, corresponding to the 2-aminotetralin aromatic rings, were defined as pharmacophoric elements. The superpositions of the best matching conformations of (*S*)- and (*R*)-**1** and **6** are shown in Figure 10. Whereas the conformations of the benzamidoethyl side chains of (*S*)- and (*R*)-**1** are identical, obviously as a result of the identical fitting procedure, differences in the overall conformations occur in the orientations of the 2-aminotetralin moieties, as a consequence of the opposite chiralities of the C2-carbon.

Flesinoxan (**11**) is a selective serotonin 5-HT_{1A} receptor agonist, which shares the presence of a benzamidoethyl

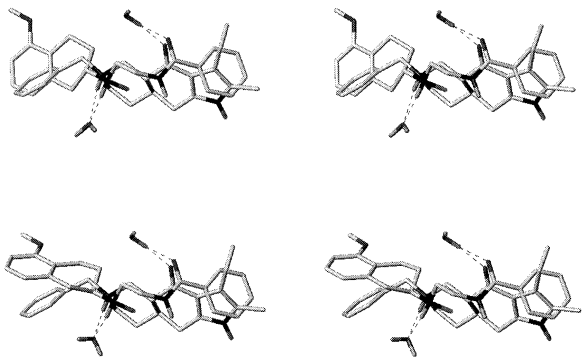

Figure 10. Stereo representations of (*S*)-**1** and **6** (top), and (*R*)-**1** and **6** (bottom), superimposed in their presumed dopamine D_2 receptor binding conformations. The water molecules mimic putative amino acid residues belonging to the receptor, capable of forming interactions (dotted lines) with the ligands.

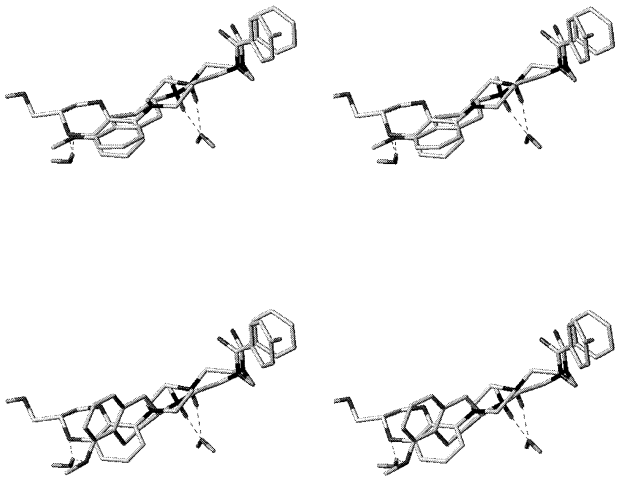

Figure 11. Stereo representations of (*S*)-**1** and **11** (top), and (*R*)-**1** and **11** (bottom), superimposed in their presumed serotonin 5-HT_{1A} receptor binding conformations. The water molecules mimic putative amino acid residues belonging to the receptors, capable of forming interactions (dotted lines) with the ligands.

side chain with **1**. Modeling studies performed by Kuipers et al. suggested that the arylpiperazine moiety of **11** occupies the same binding site in the serotonin 5-HT_{1A} receptor as serotonin and other agonists, including **8** and **10**,[10,37] supporting the observation that the arylpiperazine moiety, when appropriately substituted, can function as a bioisostere of the 2-aminotetralin moiety.[57] According to Kuipers et al., the plane angle between the benzene and piperazine rings of **11** in its receptor binding conformation is approximately 30°, corresponding to a torsion angle $\tau_{1-2-3-4}$ (see Chart 3) of about −11°. In the active site, the protonated arylpiperazine N4-atom interacted with Asp116, while the most distant oxygen atom of the dioxane ring accepted a H-bond from Thr200. The amide functionality of **11** was not involved in the formation of specific interactions. The lack of dopamine D_2 receptor affinity of **11** was explained by the inability of this receptor subtype to accommodate the hydroxymethyl substituent at the dioxane ring. In view of the structural and pharmacological similarities with **1**, **11** was considered to be a suitable template for identifying the serotonin 5-HT_{1A} receptor binding conformations of (*R*)-and (*S*)-**1**. Thus, vector points emanating from the protonated N4-atom and the most distant dioxane oxygen atom, and centroids and normals through both aromatic rings were defined in **11**. These points were matched with the appropriate points defined in the enantiomers of **1**, that is, vector points emanating from the protonated nitrogens and the 5-methoxy oxygen atoms, and centroids and normals through both aromatic rings. The best-matching conformations thus identified are shown in Figure 11. Similar to the presumed dopamine D_2 receptor binding conformations (Fig. 10), the benzamidoethyl side chains of (*S*)- and (*R*)-**1** have adopted identical conformations, whereas the orientations of the 2-aminotetralin moieties are different. However, the overall conformations of both enantiomers found here are completely different from their presumed dopamine D_2 receptor binding conformations.

The presumed dopamine D_2 receptor binding conformations of (*S*)- and (*R*)-**1** were docked into the binding site of the 7TM model with their 2-aminotetralin moieties positioned in the agonist binding site, and their protonated nitrogen atoms interacting with Asp114. Consequently, the benzamidoethyl side chains were directed downwards (i.e. towards the intracellular side, in a more or less parallel fashion with TM3). Inspection of the surrounding amino acid residues revealed possible interactions between the amide carbonyl oxygen atoms and Ser121 in TM3, which is situated almost two turns below Asp114. In addition, a H-bond could be formed between the amide hydrogen atoms of the ligands and the *para*-hydroxy group of Tyr416 in TM7, which was directed towards TM3. These observations support the SAFIRs revealing the importance of the carbonyl moiety and the presence of an unsubstituted amide nitrogen necessary for high affinity of **1** at the dopamine D_2 receptor.[32] Furthermore, edge-to-face interactions between the indole NH of Trp386 in TM6 and the benzamide moieties were present. The *N*-*n*-propyl groups of both enantiomers adopted an all-*trans* conformation and were directed towards TM7. Moreover, the 5-methoxy substituents of both enantiomers were located close to Ser193 in TM5, but after optimization of the complexes, only the 5-methoxy substituent of (*S*)-**1** formed a H-bond with this amino acid residue (Fig. 12). The aminotetralin moiety of the (*R*)-enantiomer was directed more towards TM6, which prevented its 5-methoxy substituent to interact with Ser193. Since stimulation of the dopamine receptors by agonists probably requires binding to one of the conserved serine residues in TM5, this subtle but important difference may explain why (*S*)-**1** behaves as a dopamine D_2 receptor agonist, while (*R*)-**1** behaves as an antagonist.[30] It is noteworthy that the 5-methoxy substituent of (*S*)-**1** was rotated 49° out of the plane of the aromatic ring of the 2-aminotetralin nucleus, while the 5-methoxy substituent of (*R*)-**1** was oriented in a coplanar fashion with this ring. Semi-empirical AM1 calculations[78] revealed that this unfavourable orientation of the 5-methoxy substituent of (*S*)-**1** can be adopted at the cost of 2.5 kcal/mol. This may explain the lower affinity of (*S*)-**1** for the dopamine D_2 receptor

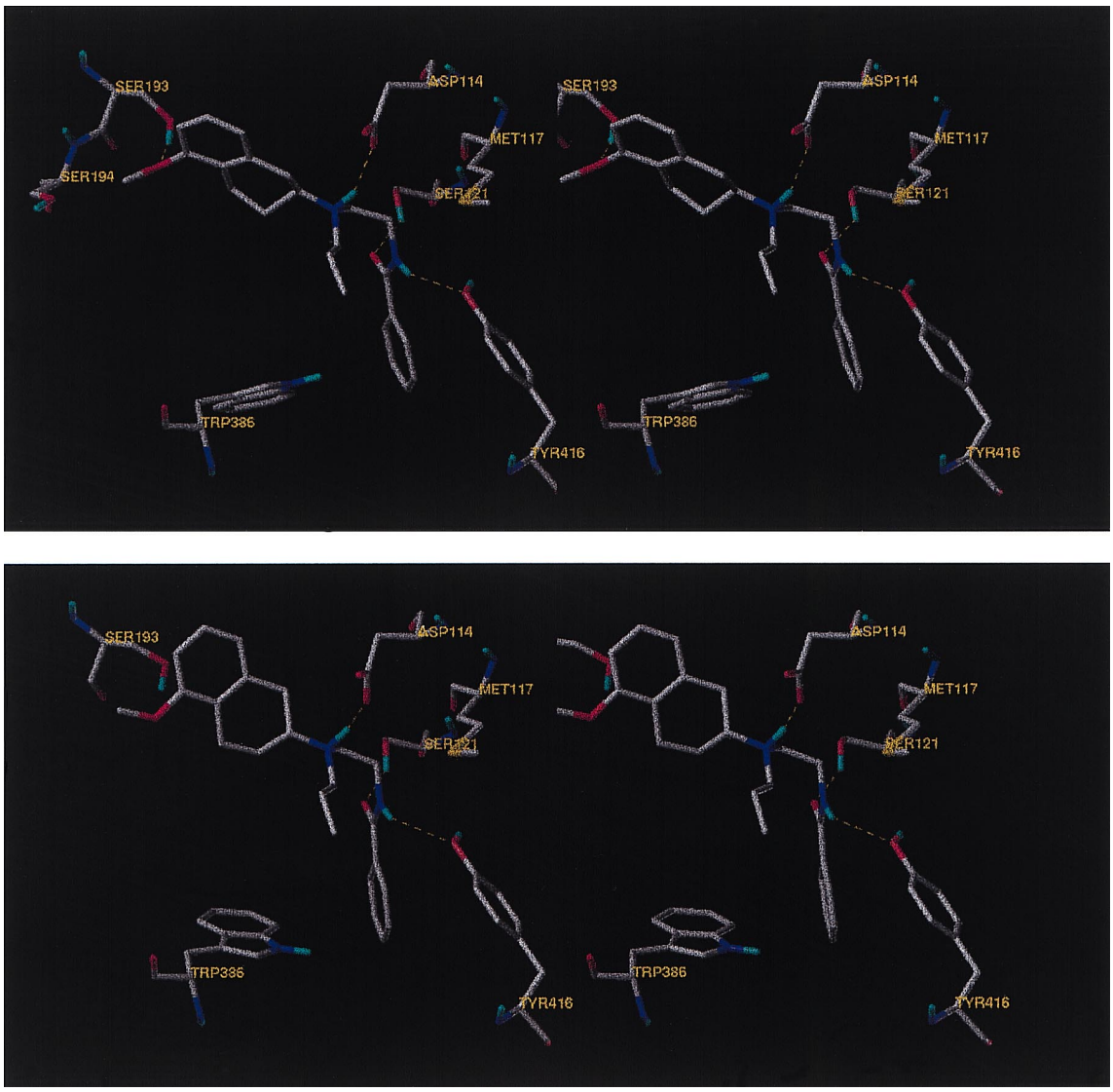

Figure 12. Stereo representations of (*S*)-**1** (top) and (*R*)-**1** (bottom) located in the optimized dopamine D_2 receptor binding site models.

compared to (*R*)-**1**, despite its apparent ability to form a H-bond with its 5-methoxy substituent.

In contrast to their dopaminergic receptor binding modes, the benzamidoethyl side chains of (*S*)- and (*R*)-**1** were directed upwards along TM3 (i.e. towards the extracellular space) in the serotonin 5-HT_{1A} receptor model. No amino acid residues capable of H-bond formation were surrounding the benzamide moieties, but specific face-to-face interactions between the benzamide aromatic rings and the aromatic ring of Phe112 in TM3 were possible. The *N*-*n*-propyl groups of both compounds were pointing towards TM4, both having adopted an all-*trans* conformation. In addition, the 5-methoxy substituents of both enantiomers were located close to Thr200 in TM5. Although their overall orientations within the binding site were not identical, both enantiomers formed a reinforced electrostatic interaction with their protonated nitrogens and Asp116, and a H-bond between their 5-methoxy substituent and Thr200 after optimization of the individual complexes. In addition, the benzamide moieties of both enantiomers were in close contact with Phe112, forming face-to-face interactions with their aromatic rings (Fig. 13). The lack of specific interactions with the benzamide moieties is consistent with the findings from SAFIRs that this functionality does not contribute to the affinity of **1** for the serotonin 5-HT_{1A} receptor.[32] Due to steric hindrance of their 5-methoxy terminal CH_3 groups with Thr200, the 5-methoxy substituents of (*S*)- and (*R*)-**1** were rotated 56 and 64° out of plane, requiring energy penalties of 2.1 and 3.2 kcal/mol, respectively. The energy difference in favour of the (*S*)-enantiomer may explain its higher affinity for the serotonin 5-HT_{1A} receptor. Out-of-plane orientation of the 5-methoxy substituents abolishes the steric hindrance and simultaneously favours the formation of an H-bond with Thr200. Although previous SAFIR studies on the role of the 5-methoxy substituent of **1** revealed that H-bond formation of the C5-substituent does not contribute to

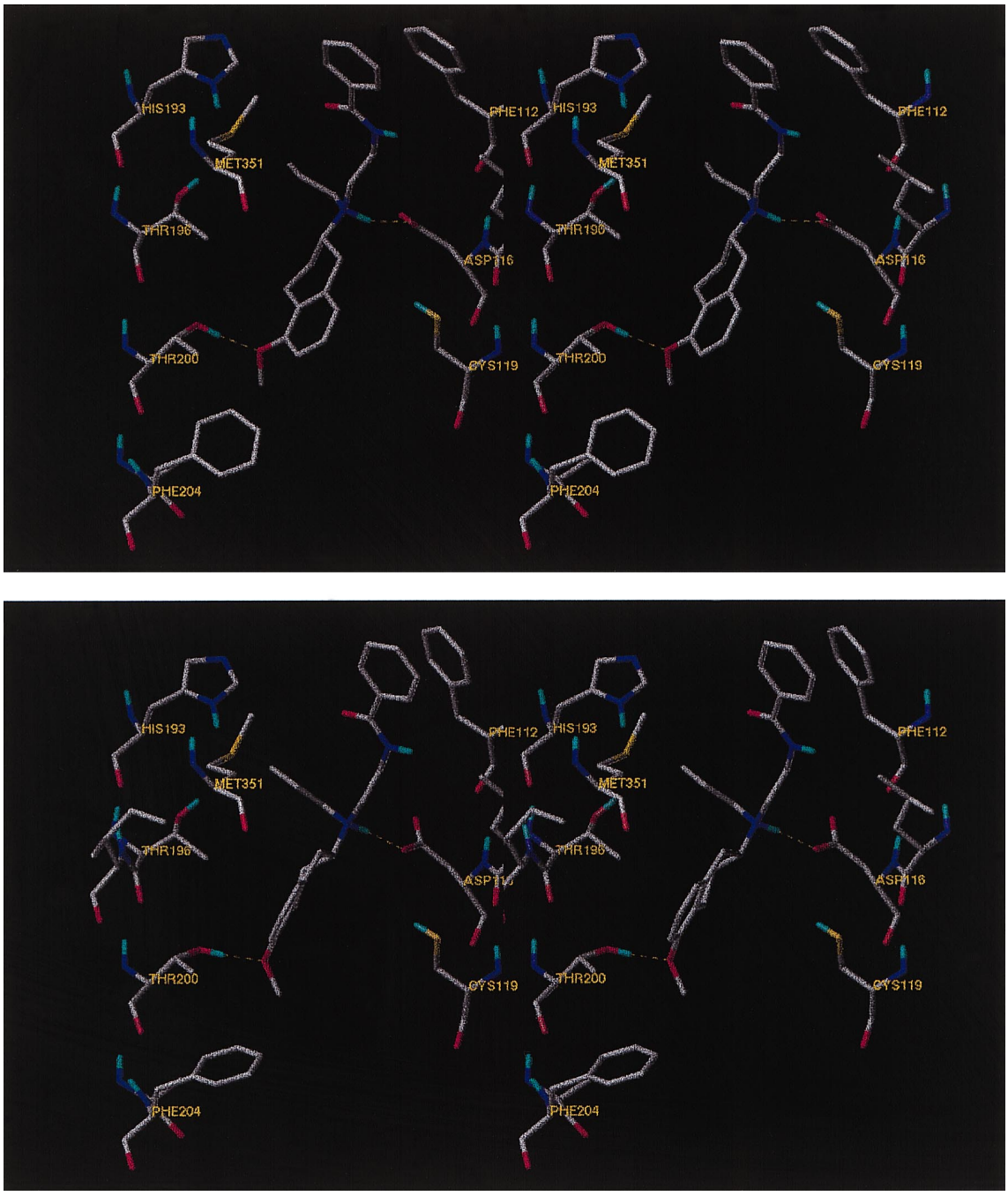

Figure 13. Stereo representations of (*S*)-**1** (top) and (*R*)-**1** (bottom) located in the optimized serotonin 5-HT_{1A} receptor binding site models.

the serotonin 5-HT_{1A} receptor affinity,[33] it may prove to be essential for stimulation of the receptor (i.e. the intrinsic efficacy). Therefore, the ability of both enantiomers to interact with Thr200 may explain why they both behave as full serotonin 5-HT_{1A} receptor agonists in vitro and in vivo.

The unbiased nature of the 7TM receptor models presented here, resulting from the combination of indirect and direct modeling during the objective, stepwise construction process should be emphasized. A distinct advantage of this procedure seems to be the ability of the resulting receptor models to rationalize the binding modes and support the SARs of ligands (i.e. the enantiomers of **1**) which have not been used during the construction process.

Conclusions

Models of the dopamine D_2 and serotonin 5-HT_{1A} receptor, which resemble the projection structure of rhodopsin in their TM domain topology, were developed by using a combination of indirect molecular modeling (i.e. the active analogue approach) and direct

construction of the TM domains of the receptors, in a stepwise, objective manner. The models could be used to rationalize the dopamine D_2 and serotonin 5-HT_{1A} receptor binding modes of the enantiomers of 5-OMe-BPAT. (*S*)- and (*R*)-5-OMe-BPAT bound to the dopamine D_2 receptor model in a similar but not identical fashion, the most important difference being the ability of (*S*)-5-OMe-BPAT to interact with Ser193 in TM5, which may explain its dopamine D_2 receptor agonist properties. The ability of the amide moieties to form specific interactions were consistent with the SAFIRs earlier observed. Similarly, the observed binding modes of (*S*)- and (*R*)-5-OMe-BPAT in the serotonin 5-HT_{1A} receptor model were consistent with the SAFIR of 5-OMe-BPAT, and could explain their full agonist properties at this receptor subtype.

Experimental

Conformational analyses

All calculations were performed on a Silicon Graphics IRIS Indigo XS/4000 Workstation or a Silicon Graphics Indy Workstation, running IRIX 5.3. Conformational analyses were performed in MacroModel version 4.5,[38] using the Monte Carlo Multiple Minimum (MCMM) search protocol.[79] Ligands with the correct stereochemistry were built from standard fragments. All ligands were considered in their protonated, positively charged forms. *N*-*n*-Propyl groups were truncated to *N*-methyl groups during the conformational analyses in order to reduce the number of torsion angles. All minimizations were performed within the MM3* force field,[80–82] while simulating a distance-dependent GB/SA water continuum,[83] as implemented in MacroModel. Benzamide moieties were fixed in a coplanar fashion during all minimizations. Prior to submitting them to the MCMM protocol, all ligands were minimized with default options. In order to mimic the possibility of inversion of the protonated nitrogen atom, a second starting conformation was generated for ligands containing a protonated nitrogen atom bearing four different substituents, by inverting its 'chirality'. The two starting conformations thus obtained for these ligands were independently submitted to the MCMM protocol. To search conformational space 1000–5000 MC steps were performed on each starting conformation, dependent on the number of torsion angles. Starting conformations for each step were systematically generated using the SUMM option.[84] The number of torsion angles to be varied in each MC step was set between 2 and *n*-1, *n* being the total number of variable torsion angles. Ring closure bonds were defined in 6-membered non-aromatic rings in order to allow torsion angles within these rings to be varied as well. Ring closure distances were limited to 0.5–2.0 Å. The randomly generated structures were minimized using the Truncated Newton Conjugate Gradient (TNCG) minimizer, allowing for 250 iterations per structure, until an initial gradient of 0.01 kcal/Å mol was reached. Least squares superposition of all non-hydrogen atoms was used to eliminate duplicate conformations. For non-chiral ligands, rejection of mirror images was prevented by specifying the NANT option. The minimum energy conformations thus obtained were submitted to a final minimization, using the Full Matrix Newton Raphson (FMNR) minimizer, allowing for 1000 iterations per structure, until a final gradient of 0.002 kcal/Å mol was reached. An energy cut-off of 3.0 kcal/mol was applied to the search results. For ligands containing a 'chiral' protonated nitrogen atom, the search results of the independent analyses performed on the starting conformations with inverted nitrogen atoms were combined and subsequently filtered on energy ($\Delta E \leq 3.0$ kcal/mol) using the Filter module.

Pharmacophore identification

The sets of minimum energy conformations of the ligands, as obtained from the conformational analyses, served as input for the VECADD module of the pharmacophore-identifying program APOLLO.[41,42] This module was used to add extension vectors pointing from H-bond donating or accepting groups towards putative complementary receptor points, and to define centroids and normals through the planes of aromatic rings in each conformation of each ligand. In all cases a minimum density of vectors was specified, representing the ideal position for H-bond formation. The RMSFIT module of APOLLO was then used to identify the conformation of the different ligands that exhibited the best overall least squares fit with respect to the specified fitting points. All fitting points were weighed equally. Extension vectors emanating from oxygen atoms, centroids, and the extremes of normals were defined as choices. Conformational energies and root mean square deviations were used to rank the matches. The best match was then extracted with the MMDFIT module of APOLLO.

Receptor construction

The 7TM models of the dopamine D_2 and serotonin 5-HT_{1A} receptors were constructed using the Biopolymer module of the integrated molecular modeling package SYBYL version 6.4.[43] Right-handed α-helices (2 turns, $\phi = -57°$, $\psi = -47°$, $\Omega = 180°$) were built from the appropriate L-amino acids. Proline residues were fixed, hydrogen atoms were added, and side chain torsion angles were scanned. Gasteiger–Hückel charges were defined on all atoms, and the helices were independently minimized within the Tripos force field. A distance-dependent dielectric constant of 5 and nonbonded cut-off value of 8 Å were specified. Minimizations were performed using the Conjugate Gradient minimizer. An RMS energy gradient of 0.1 kcal/Å mol was set as the final convergence criterion. The complete 7TM models were constructed in a stepwise manner, starting with positioning TM3 and TM5, guided by the receptor maps as obtained from the pharmacophore identification procedure. The structure of bR was then used to determine the relative position of TM4. Subsequently, TM3, TM4, and TM5 were packed as tightly as possible, by slightly adjusting (i.e. tilting, rotating, or shifting) their relative positions, without disturbing the primary

interactions with the superimposed agonists. After each adjustment the TM domains were inspected for overlap of the van der Waals volumes of their side chains, using the Z-clipping option of SYBYL. This feature allows to cut slices of any desired width at any desired position through a molecule, and hence is very suitable for visualizing overlap of van der Waals volumes between adjacent TM domains. In a similar fashion, the other TM domains were added one by one, in the following order: TM6, TM2, TM7, and TM1. The more hydrophilic sides of the helices were directed towards the binding site crevice, while the more hydrophobic sides faced the hypothetical lipid bilayer. The TM domains were positioned according to the topological arrangement of rhodopsin. Each time a new TM domain had been added and appropriately positioned, the energy of the entire 7TM model was minimized.

Ligand docking

The presumed pharmacologically active conformations of the ligands, as identified by APOLLO, were individually docked into the active sites of the receptors. Where appropriate, *N*-*n*-propyl groups were introduced and extended in an all-*trans* conformation. Atomic charges were calculated semi-empirically on all ligands, using the AM1[78] method as implemented in MOPAC 5.0 and accessed through SYBYL. In all cases the additional keyword 1SCF was supplied, while for benzamides the keyword MMOK(0) was supplied as well. Attractive interactions between the ligands and the receptors were then optimized and repulsive interactions minimized by manually rotating and translating the ligands in the binding sites, using the Docking procedure as implemented in SYBYL. H-bonds between the ligands and the receptors were dynamically displayed during this process. When necessary, side chain torsion angles were manually adjusted in order to minimize overlap or to optimize attractive interactions between amino acid residues in the binding sites and the ligands.

Active site optimization

The pseudoreceptor-generating program PrGen version 2.0[46] was used for optimizing the interactions between the individual ligands and the amino acid residues surrounding them. For this purpose, all amino acid residues having one or more atoms located within a sphere of 4 Å surrounding a ligand, were extracted from the receptors in SYBYL, and transferred to PrGen. Force field atom types were assigned to the ligands, and their solvation energies, entropy corrections, and reference energies were calculated. Minimizations in PrGen were performed within the Yeti force field,[48] using the Conjugate Gradient type 2 minimizer. During the minimizations, only torsion angles in the side chains, conjugated bonds, and terminal CH_3 groups of the amino acid residues were allowed to be varied. The ligands were allowed to be translated and rotated, and all freely rotatable torsion angles, conjugated bonds, and terminal CH_3 groups were allowed to be varied, except for the coplanar benzamide moieties. A sufficient number of iterations was specified in order to reach a convergence criterion of 0.001 kJ/Å mol. All other convergence criteria were set at default values.

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