Structure-based Drug Discovery Using GPCR Homology Modeling: Successful Virtual Screening for Antagonists of the Alpha1A Adrenergic Receptor

Andreas Evers* and Thomas Klabunde

Aventis Pharma Deutschland GmBH, Ein Unternehmen der Sanofi-Aventis Gruppe, DI&A Chemistry, Computational Chemistry, Industriepark Höchst, Building G838, D-65926 Frankfurt am Main, Germany

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In this paper, we describe homology modeling of the alpha1A receptor based on the X-ray structure of bovine rhodopsin. The protein model has been generated by applying ligand-supported homology modeling, using mutational and ligand SAR data to guide the protein modeling procedure. We performed a virtual screening of the company's compound collection to test how well this model is suited to identify alpha1A antagonists. We applied a hierarchical virtual screening procedure guided by 2D filters and three-dimensional pharmacophore models. The ca. 23 000 filtered compounds were docked into the alpha1A homology model with GOLD and scored with PMF. From the top-ranked compounds, 80 diverse compounds were tested in a radioligand displacement assay. 37 compounds revealed K_i values better than 10 μM; the most active compound binds with 1.4 nM to the alpha1A receptor. Our findings suggest that rhodopsin-based homology models may be used as the structural basis for GPCR lead finding and compound optimization.

Introduction

G-protein coupled receptors (GPCRs) form a large protein family that plays an important role in many physiological and pathophysiological processes. Historically, the discovery of drugs acting at GPCRs has been extremely successful with 50% of all recently launched drugs targeting against GPCRs.[1] Especially the subfamily of biogenic amine binding GPCRs has provided excellent targets (given in brackets) for the treatment of several CNS diseases such as schizophrenia (mixed D2/D1/5-HT2), psychosis (mixed D2/5-HT2A), depression (5-HT1), or migraine (5-HT1). This GPCR subfamily has also proven to provide drugable targets for other disease areas such as allergies (H1), asthma (beta2), ulcers (H2), or hypertension (alpha1 antagonist, beta1 antagonist).

The alpha1 adrenergic receptors are involved in blood pressure maintenance by modulating the vascular muscle tone. They are subdivided into the alpha1A, alpha1B and alpha1D adrenoreceptor subtypes.[2] Antagonists of the alpha1 adrenergic receptors such as indoramin and prazosin are employed as antihypertensitive agents. In addition, alpha1A antagonists such as alfuzosin and prazosin are thought to be effective in the management of benign prostatic hypertrophy.

Considering the high sequence (and probably structural) similarity of the ligand binding site of the biogenic amine binding receptors, this family represents a challenge for ligand design with respect to the problem of selectivity. Since GPCRs are membrane-bound proteins, experimental determination of their 3D structures is still an extremely difficult task. To gain understanding into the determinants of molecular recognition at biogenic amine binding receptors, we have started to generate pharmacophore and homology models for the alpha1A receptor.

Due to lack of crystal structures, traditionally, successful computer-aided drug design for GPCRs was mainly achieved by applying ligand-based modeling techniques.[3,4] Drug discovery based on virtual screening with rhodopsin-based GPCR models has recently been reported in the literature.[5−11] Although the generation of a lot of GPCR models is reported in the literature (e.g. refs 12−22), these models were only used to explain the binding of known GPCR agonists/antagonists retrospectively. The discovery of novel ligands derived from structure-based design was, so far, only reported in refs 6 and 9−11. In ref 9, ligand information was used for the selection and optimization of rhodopsin-based homology models of the neurokinin-1 (NK1) receptor. Applying pharmacophore modeling and docking, this model was successfully used for the discovery of a novel NK1 antagonist binding in the submicromolar range to the receptor. Varady et al. reported on an impressive discovery of novel potent D3 ligands using a hybrid pharmacophore- and structure-based database searching approach.[11] Applying in a stepwise fashion a "hybrid" protein- and ligand-based computational approach, virtual screening was performed. Out of 20 experimentally tested compounds, eight showed K_i values better than 1 μM.

Encouragingly, in these cases, virtual screening was successfully applied to rhodopsin-based homology models of GPCRs belonging to different subfamilies (biogenic amine and peptide binding GPCRs). Thus, the bovine rhodopsin crystal structure may be a template of general relevance for generating GPCR homology models for the purpose of structure-based drug design.

Here, we describe the generation and validation of a homology model for the alpha1A receptor. The protein model was generated using bovine rhodopsin as structural template. We applied a modified version of the

* To whom correspondence should be addressed. Phone +49(0)-69305-12636. Fax +49(0)69331399. E-mail: Andreas.Evers@sanofi-aventis.com.

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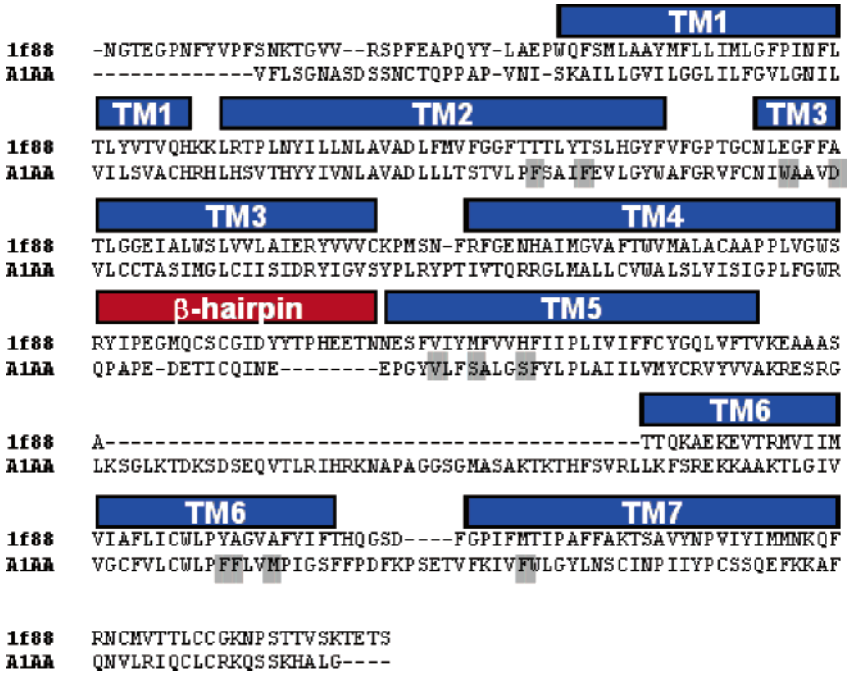


Figure 1. Sequence alignment of bovine rhodopsin (PDB code 1f88) and the alpha1A receptor (Swissprot code A1AA). All residues comprising the putative binding pocket as given in Figure 2 are marked in gray.

MOBILE approach (*mo*delling *b*inding sites *i*ncluding *l*igand information *e*xplicitly), which models proteins by homology including information about bound ligands as restraints, thus resulting in more relevant geometries of protein binding sites.[23] We furthermore considered mutational and ligand binding data reported in the literature to obtain only models which are in agreement with these experimental data. As an ultimate validation criterion for the relevance of the generated model, we tested its suitability to discover potent alpha1A antagonists. Therefore, we virtually screened the company's compound repository and tested the top-scored compounds in an experimental assay.

Results

Generation of a Ligand-Supported Homology Model of the Alpha1A Receptor. A homology model of the alpha1A receptor was generated using the bovine rhodopsin structure determined at 2.8 Å resolution as template based on the sequence alignment given in Figure 1. The global sequence identity between the alpha1A receptor and bovine rhodopsin amounts to only 21%, which is generally considered not to be sufficient for reliable homology modeling.[24,25] When only the transmembrane region is considered, the identity increases to 27%. However, regarding the proposed binding pocket as depicted in Figure 2, no sequence identity is given. Since none of the amino acids in the putative binding pocket are conserved, the orientations of the side chains cannot be derived by homology but have to be predicted de novo with the force field implemented in the homology modeling program. It was shown previously that, depending on the composition of the binding pocket to be modeled, considerable deviations from the native structure may be obtained.[23] We therefore applied a modified version of the MOBILE approach.[23] In a first step, a ligand is docked into an ensemble of crude homology models of the target protein

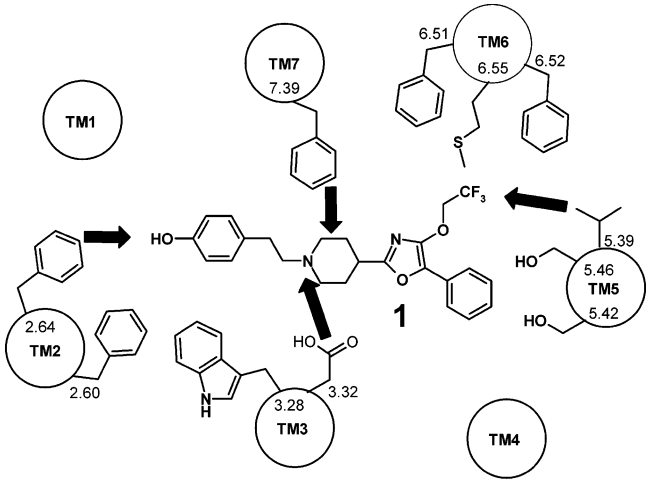


Figure 2. Schematic representation of the postulated interactions between the alpha1A receptor and compound **1** derived from refs 30 and 31 The arrows indicate proposed key interactions between the receptor and the ligand.

(paragraph B). In the next step, improved homology models are generated, explicitly considering the previously placed ligand. Subsequently, the most favorable models are selected by ranking the interactions between the ligand and the generated models. Final models are obtained by combining the best ranked side-chain conformers from a set of different models followed by an energy optimization of the entire complex using a common force-field (paragraph C).

(A) Generation of a Topographical Interaction Model for the Alpha1A Receptor. For the generation and validation of a ligand-supported homology model, details about the interaction between protein and ligand should be available. We generated a topographical interaction model to guide the subsequent homology modeling process. Through mutational studies and comparative affinity determinations based on ligand binding,[26–33] essential amino acids involved in antago-

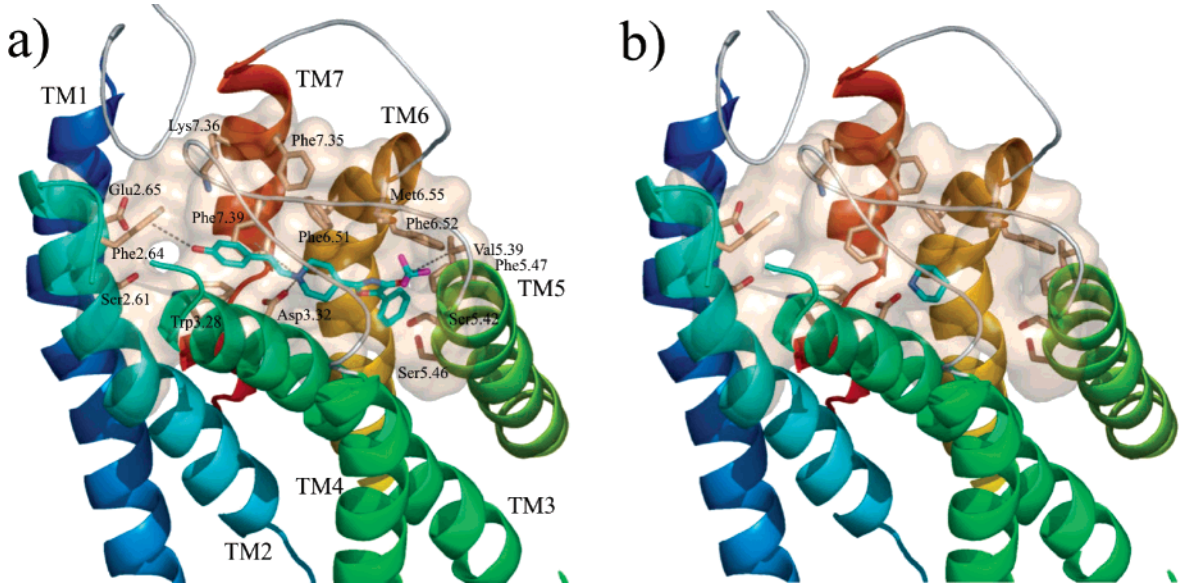


Figure 3. (a) Homology model of the alpha1A receptor complexed with compound **1**. The dashed lines indicate the key interactions as depicted in Figure 2. (b) The piperidine moiety that was used as "template similarity constraint" during the docking procedure of the virtual screening.

nist recognition could be identified. Considering additional mutational studies performed on close relatives of the biogenic amine binding receptor family,[27,34−48] we derived a crude topographical interaction model (see Figure 2). According to our homology model and these mutational data, the antagonist binding pocket of the alpha1A receptor overlaps with the binding site of retinal in bovine rhodopsin. It is generally accepted for all biogenic amine binding GPCRs that Asp3.32 (according to the Ballesteros−Weinstein nomenclature[49]) is involved in binding the biogenic amine group. The studies of Hamaguchi et al.[30,31] furthermore reveal that Phe2.64, Phe7.39, and Val5.39 are involved in binding compound **1** as depicted in Figure 2. Ser5.42 and Ser5.46, which are supposed to be involved in binding the catechole moieties of adrenaline and epinephrine,[28] do not seem to be involved in binding this particular antagonist. Since not all residues in the alpha1A receptor have been mutated to test their role in ligand binding and/or receptor activation, a completely validated 2D picture of the antagonist binding pocket cannot be provided here. In fact, our homology model indicates that further residues may be involved in antagonist binding.

(B) Generation of Initial Alpha1A Homology Models and Ligand Docking. A set of initial protein models of the alpha1A receptor was generated by using MOE (Chemical Computing Group, Montreal, Canada). In total, 100 different models are obtained reflecting to some extent the conformational side-chain variability. In the next step, compound **1** was docked into each single alpha1A model using GOLD2.0[50−52] with "standard default" parameter settings. Since the alpha1A model was generated by homology based on the rhodopsin structure, the modeled E2 loop linking helices 4 and 5 reaches deeply into the putative binding pocket. It was removed from the models for this preliminary docking procedure.

To reduce the search space of the docking procedure and to obtain only docking poses that are in agreement with the experimental (mutational) data, distance constraints were defined according to the interactions indicated as arrows in the topographical interaction model given in Figure 2. All resulting complexes were scored with DrugScore,[53] and the best scored ligand pose was selected after visual inspection.

(C) Generation of Refined Alpha1A Models Including Ligand Information and Optimization of the Modeled Protein−Ligand Complexes. In the following step, 100 new homology models were generated using MOE. Compound **1** was now considered in its docked orientation as an additional restraint in this step of the homology modeling procedure using the "Environment" option within MOE. The generated protein−ligand complexes were further refined (for details, see also ref 23). First, a DrugScore value was assigned to each binding-site-exposed amino acid (i.e., all amino acids within 6 Å distance to the ligand) to describe the interaction of this amino acid with compound **1**. Subsequently, the best scored individual amino acids from the different models were assembled in a combinatorial fashion. Finally, the best composite complex model was identified in the following way: Avoiding any intramolecular clashes between individual amino acid side chains extracted from the different models, we selected the model that yielded the best total DrugScore value to the ligand. To relax the composed model, the entire binding pocket (i.e., all amino acids within 6 Å distance to the ligand) was minimized with the MMFF94 force field[54] available in MOE (using default parameters), keeping ligand and binding-site residues flexible. The E2 loop was added to the model in the orientation adopted from bovine rhodopsin. As clashes with the docked ligand were observed, all loop atoms were minimized while the ligand and the rest of the protein were kept fixed. This procedure moved the loop away from the ligand and expanded the putative ligand binding site.

(D) Description of the Alpha1A Receptor Model. The complex of the alpha1A receptor model is depicted in Figure 3a. The proposed interactions (as suggested by the interaction model, Figure 2) are displayed as

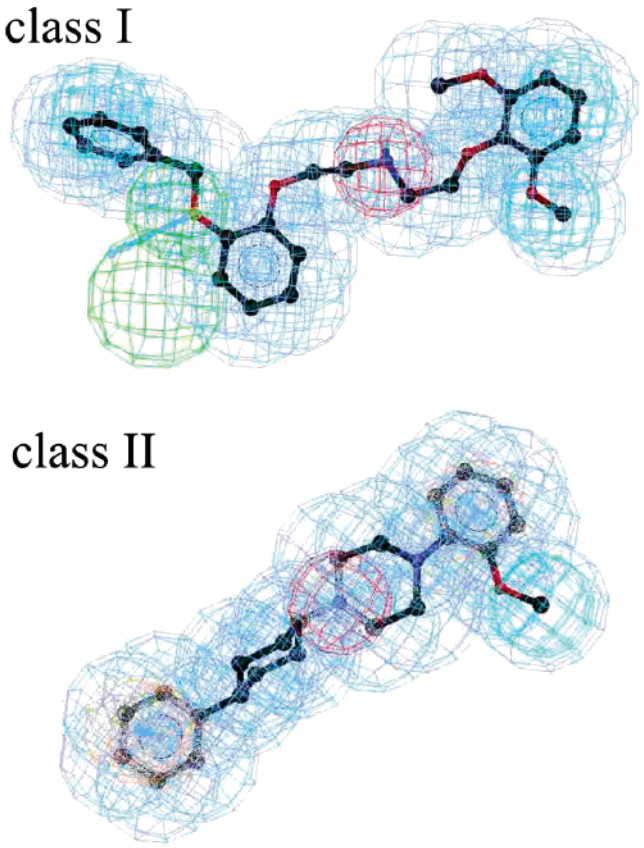


Figure 4. Catalyst pharmacophore models used for virtual screening of the Aventis compound repository. Shown are the two pharmacophore models (class I and class II) mapping two different classes of high-affinity ligands of the alpha1A receptor.

dashed lines. As mentioned above, there is no sequence identity between the alpha1A receptor and bovine rhodopsin in the assumed antagonist binding pocket. Since we applied homology modeling, the backbone trace of the alpha1A model is kept with similar conformation to the rhodopsin template, whereas the arrangement of the side chains of the binding-site residues is predominantly determined by the docked binding mode of compound **1**. The final spatial arrangement of the binding-site residues agrees well with the topographic interaction model depicted in Figure 2. Analysis of the modeled complex reveals that Asp3.32 constitutes a central anchoring point for ligands and divides the binding site into two different subpockets. The subpocket defined by helices 4−7 consists of amino acids offering hydrophobic side chains (Val5.39, Phe6.51, Phe6.52, Met6.55, Phe7.35, and Phe7.39). Similarly, the second subpocket is formed by mainly aromatic residues contributed by helices 1−3, and 7 (Phe2.60, Phe2.64, Trp3.28, Phe7.35, and Phe7.39).

These characteristics of the binding pocket are implicitly reflected by two ligand-based pharmacophore models that were generated on the basis of a diverse set of known alpha1A receptor antagonists (see Figure 4).[55] These models describe and predict alpha1A affinity across several different chemotypes. Pharmacophore model "class II" (Figure 4b) contains a central positively ionizable pharmacophoric element (interacting with Asp3.32). The hydrophobic subpockets of the antagonist binding site are addressed by the hydrophobic and

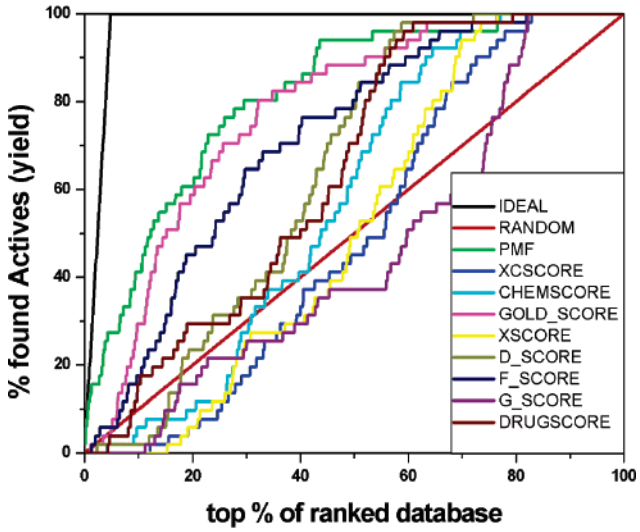


Figure 5. Enrichment of alpha1A antagonists obtained from nine different scoring functions.

aromatic pharmacophoric features. Some alpha1A antagonists contain an additional hydrogen-bond acceptor, which is reflected by pharmacophore model "class I". A possible interaction partner for this acceptor is (according to our model) Lys7.36. However, this assumption is only speculative, since no mutational data are available and analysis of known ligand SAR does not conclusively suggest an essential role of this pharmacophoric element.

Evaluation of Different Scoring Functions. Following a similar approach as described by Bissantz et al.,[7] we evaluated different scoring functions using an MDDR data set that is composed of 990 drug-like molecules and 50 embedded alpha1A antagonists (see Methods). All compounds of the test set were docked into the homology model with GOLD (applying "7−8 times speed-up" parameter setting) and scored with nine different scoring functions [DrugScore,[53] GoldScore,[51] Xscore,[56] XCScore[57] and D_Score,[58] G_Score,[51] ChemScore,[59] PMF,[60] and F_Score[61] as implemented in the Cscore module of Sybyl6.92 (Tripos Associates, Inc., St. Louis, MO, 2001)]. Again, to include experimentally derived knowledge about the interaction of piperidines with the receptor into the docking procedure, the piperidine moiety of docked compound **1** has been defined as a "template similarity constraint" as depicted in Figure 3b. This guides the docking of ligands that contain piperidines or similar groups toward binding poses found for compound **1**. Furthermore, we defined a "protein hydrogen-bond constraint" with one of the carboxylate oxygen atoms of Asp3.32. In Figure 5, enrichments plots obtained with different scoring functions are depicted. Table 1 lists the enrichment factors obtained when analyzing the top 1%, 5%, and 10% of the scored data set. For the applied data set, the best results are obtained when PMF[60] is used as scoring function, providing an up to 13-fold hit rate than a random selection. An overview over the compounds retrieved as the top 10 is given in Table 2. Among these, six are alpha1A antagonists. From the remaining four compounds, two are registered as antidepressants binding to the 5HT1A receptor, a biogenic amine receptor closely related to the alpha1A receptor. The affinity of these compounds on the alpha1A receptor is not known.

Table 1. Enrichments of Active Compounds Embedded into a Database of 50 Alpha1A Antagonists and 990 Further Druglike Molecules Extracted from the MDDR[a,b]

scoring function	1%	5%	10%	scoring function	1%	5%	10%
PMF_Score	13.1	5.4	4.1	D_Score	0.0	0.4	0.2
XScore	0.0	0.0	0.0	F_Score	0.0	1.2	1.8
ChemScore	0.0	0.4	0.6	G_Score	0.0	0.0	0.0
GoldScore	0.0	1.2	2.9	DrugScore	0.0	0.8	1.6
XScore	0.0	0.0	0.0	Ideal	20.6	19.8	10.0

[a] For details, see Materials and Methods. [b] Enrichments of the alpha1A antagonists are given at 1%, 5%, and 10% of the screened test database.

Virtual Screening for Novel Alpha1A Antagonists. For the validation of our alpha1A receptor model, we tested how well it is suited for the discovery of novel alpha1A antagonists. Therefore, the company's compound repository was virtually screened to retrieve putative alpha1A receptor antagonists. Similarly to other previous virtual screening experiments described in the literature (i.e. refs 11, 62, 63), the search has been performed in a stepwise fashion using hierarchical filters of increasing complexity with respect to their computational requirements. As an initial step, a rather unspecific and target-independent filter was applied: Only compounds with up to nine rotatable bonds and a molecular mass of less than 600 Da have been considered. This avoids highly flexible ligands that could possibly (1) experience reduced binding affinity due to entropic considerations and (2) increase the complexity and thus reduce the success rate of the attempted 3D searches. In the second step, compounds fulfilling the pharmacophoric requirements of known alpha1A receptor antagonists (Figure 4)[55] were identified with Catalyst. 22 950 compounds were selected and docked into the binding site of our alpha1A homology model using GOLD with the same settings as applied for the MDDR data set. All docking solutions were rescored with PMF.

The top-scoring 300 compounds were clustered according to their UNITY fingerprint similarity (Tripos Associates, Inc., St. Louis, MO, 2001), and a diverse set of 80 compounds was submitted for experimental testing using a radioligand displacement assay. From these, 38 compounds showed percent inhibition values >50% at 10 μM. These compounds were submitted for K_i value determination. Figure 6 and Table 4 give an overview of the number of compounds binding at different affinity ranges to the alpha1A receptor. Thirty-seven compounds showed affinity below 10 μM. Twenty-four compounds exhibit binding in the submicromolar range, 10 of these under 100 nM and three compounds even below 10 nM (see Table 3). Table 4 shows the 2D structures of four representative strong binders retrieved by virtual screening. Docking modes of these compounds are depicted in Figure 7. The hit list from the virtual screening contains compounds with scaffolds of known alpha1A antagonists, for example, compound **12**, which is an analogue of the known alpha1A antagonist 5-methylurapidil. It binds with K_i = 3.6 nM at the alpha1A receptor. According to our docking mode (see Figure 7a), the methoxyphenyl moiety addresses the hydrophobic subpocket defined by amino acids from helices 3−6. Here, the 2,6-dichlorophenyl moiety establishes aromatic−aromatic stacking interactions with the side chains of Trp3.28 and Phe2.64. As mentioned before, Phe2.64 was shown to be involved in binding several alpha1A antagonists by Hamaguchi et al.[30,31]

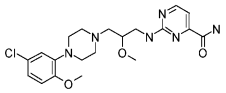
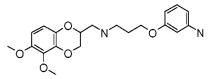
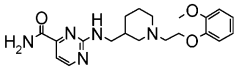
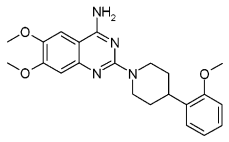
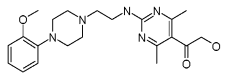
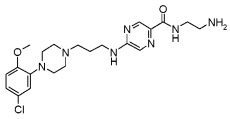
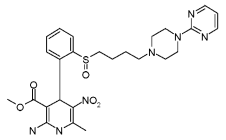
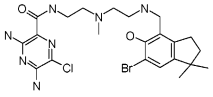
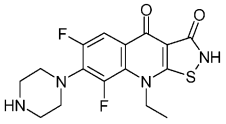
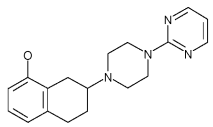
The docking mode of the most active compound identified by virtual screening, compound **13**, is given in Figure 7b. Interestingly, this class of compounds has been developed as antagonist of the dopaminergic D3 receptor.[64] Again, aromatic interactions of the pyrimidine ring with Trp3.28 and Phe2.64 are observed in our complex model. In addition, the N3 atom of the pyrimidine ring is in close distance to the hydroxyl group of Ser2.61, allowing for the formation of a hydrogen bond. A further hydrogen bond is possibly established between the amide oxygen atom of the ligand and Lys7.36 of the receptor. Interestingly, as mentioned before, this hydrogen-bond-acceptor functionality is also found as a pharmacophoric feature in the ligand-based alpha1A pharmacophore model "class I" (Figure 4).[55] Since this functionality is observed in compounds **13** and **15**, it may indeed be a feature that is not absolutely required but beneficial for strong binding to the alpha1A receptor. Ligands similar to compound **15** (Figure 7d) have already been reported to bind against the 5HT1A receptor, a further biogenic amine receptor. Like compound **13**, compound **15** could establish a hydrogen bond to Ser2.61 via its terminal amino group, suggesting an important role of this amino acid for binding these compounds. Mutational data addressing the role of Ser2.61 in ligand binding have not been reported in the literature. However, it was shown by mutational studies that the amino acid in the corresponding position of the dopamine D4 receptor (Phe2.61) is important for the binding of several antagonists.[48] In addition to series with known activity on the alpha1A receptor or other biogenic amine receptors, we also identified novel alpha1A scaffolds by our virtual screening approach, for example, compound **14** (Figure 7c). Whereas the ethylbenzene "tail" which is pointing toward helix 2 is often observed in alpha1A structures, the dioxolan "head" directed toward helices 5 and 6 represents a scaffold known from the fungicide field. Interestingly, according to our docking mode, the piperazine group of compound **14** assumes a different orientation in the alpha1A binding pocket from that of compounds **1**, **12**, **13**, and **15**.

Discussion

In this contribution, we presented the generation of a 3D homology model for the alpha1A receptor based on the rhodopsin X-ray structure and successful virtual screening of the company's compound repository. From the 80 top-scored hits, 37 revealed affinity below 10 μM, with 24 compounds binding in the submicromolar range. The most potent hit revealed an affinity of 1.4 nM. Among the virtual hits, several ligands represent structural classes that have not been reported as alpha1A antagonists before.

Our approach, which can in principle be applied to any member of the GPCR family with known ligand information and site-directed mutagenesis data, is based on Catalyst pharmacophore models and a homology model generated from the crystal structure of bovine rhodopsin as structural template. It is widely discussed whether bovine rhodopsin is a proper template for homology models of GPCRs.[65] It is clear that it is the

Table 2. Compounds Retrieved among the Ten Best Scored Compounds of the MDDR Test Data Set[a]

No	Compound	Activity	No	Compound	Activity
2		**alpha1 Blocker**	**7**		5HT1A Agonist
3		**alpha1 Blocker**	**8**		5HT Antagonist **alpha1 Blocker**
4		**alpha1 Blocker**	**9**		**alpha1 Blocker**
5		**alpha1 Blocker**	**10**		Calcium Channel Blocker
6		Topoisomerase Inhibitor	**11**		5HT1A Agonist

[a] Shown are the structures and the biological activity as stated in the MDDR.

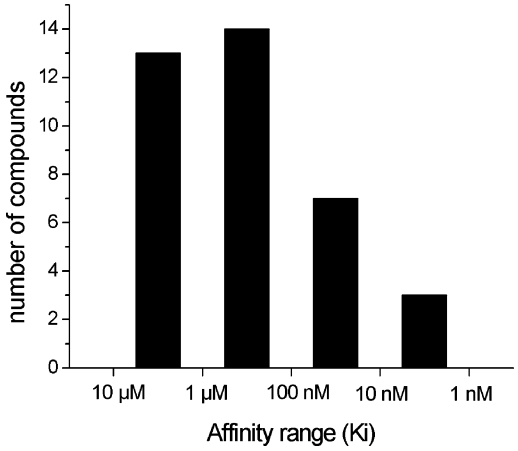


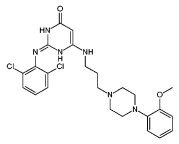
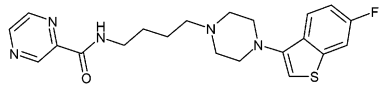
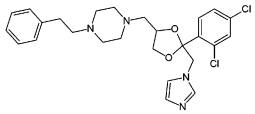
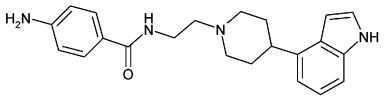
Figure 6. Overview of affinity ranges of alpha1A antagonists identified by virtual screening. From 37 hits discovered by virtual screening, 13 compounds were binding in the range between 10 and 1 μM, 14 compounds showed affinity between 1 μM and 100 nM, seven compounds were binding between 100 and 10 nM, and three compounds showed affinity between 1 and 10 nM.

Table 3. Overview of Affinity Ranges of Alpha1A Antagonists Identified by Virtual Screening

	no. of compds	hit rate, %
K_i < 10 μM	37	46.8
K_i < 1 μM	24	30
K_i < 100 nM	10	12.5
K_i < 10 nM	3	3.8

only reasonable template since it is the only GPCR resolved to sufficient resolution, but it remains to be proven whether its use results in homology models reliable enough for structure-based drug design. Regarding only the putative antagonist binding site of the alpha1A receptor, no sequence identity to bovine rhodopsin is given. This fact points to uncertainties in modeling GPCR binding pockets on the basis of the bovine rhodopsin structure. Usually, if sequence identity falls beyond 35%, the accuracy of any produced homology model is considered insufficient to allow for virtual screening and docking of small ligands.[24,25] Whereas among related globular proteins, the ligand binding site is generally more conserved for related proteins than their entire fold, the opposite holds for GPCRs.[66] Furthermore, the E2-loop, which links helix 4 with helix 5, is an integral part of the retinal binding site and it was shown by mutational studies (i.e. refs 27, 47, 67) to possibly be involved in ligand binding for the biogenic amine receptors. The loop is probably "fixed" by a highly conserved disulfide bridge. But since this loop is variable in length and amino acid composition, it must be assumed that it adopts conformations different from that observed in the rhosopsin crystal structure.[65] For

Table 4. List of Four Selected Hits Discovered by Virtual Screening

No	Compound	K_i [nM]
12		3.6
13		1.4
14		300
15		9.5

the virtual screening, we decided to keep the loop in the model, since preliminary docking studies of the MDDR data set (data not shown) revealed that better enrichments of active compounds among the top scorers are obtained when the E2-loop is included. Visual analysis of docking modes revealed that neglect of the loop provokes docking solutions of large, highly flexible "inactive" compounds that are artificially oriented into the unoccupied region. These ligands establish additional artificial interactions with amino acids beyond the E2-loop, resulting in better scores and, thus, higher enrichment rates of false positives among the top scorers.

Of course, these uncertainties justify the debate whether homology modeling is the appropriate method for generating GPCR models of an accuracy sufficient for drug design. We cannot state how close the putative antagonist binding pocket of the presented alpha1A homology model is to reality. However, we have shown that our model is in agreement with experimental (mutational and ligand SAR) data and it is of suited quality for finding novel ligands by structure-based drug design. The fact that further successful applications of GPCR homology models for virtual screening have recently been reported in the literature (e.g. refs 5−9, 11, 68) supports the assumption that bovine rhodopsin provides a useful template structure. However, at this point it should be mentioned that the success rate of a virtual screening approach not only depends on the quality of the protein structure but also on the applied screening method and the composition of the screened compound collection. If a compound collection does not contain any active compounds for the actual target or an unsuited combination of docking program and scoring function is used, the resulting hit rate will be low, even if a crystal structure of the target protein is available. Still, we are convinced that a large contribution to the success rate of the presented virtual screening must be attributed to the quality of the homology model and the fact that it has been refined and validated using ligand (SAR) and mutational data. Without experimental validation data, these computational GPCR homology models possibly lack the accuracy needed to apply them within the GPCR drug discovery process.

Furthermore, it must be noted that precise affinity predictions are not feasible on the basis of GPCR homology models. Limitations may surely arise from inaccuracies of the model. In addition, the currently available scoring functions are still considered to be inapplicable for accurate affinity prediction, even if the molecular protein−ligand interactions are available from crystal structures.[56,69] On the contrary, it was also shown that docking programs and scoring functions are well-suited for generating near-native ligand binding poses in protein binding sites. Thus, our homology model could be used as structural basis to generate relevant binding poses and ligand alignments in the alpha1A binding pocket useful for the subsequent generation of 3D-QSAR models as described in refs 12, 70, and 71. Indeed, we were able to generate significant 3D-QSAR models from docking modes of alpha1A antagonists covering different chemotypes. Such models allow for a reliable affinity prediction within these chemical series. In addition, the good agreement between contour maps obtained from such 3D-QSAR models and the protein model confirms the relevance of both models and allows for an easy interpretation of the features that are important for binding.

Besides serving as a structural platform for structure-based drug design, a further potential application of our generated alpha1A homology model is to serve as a structural template for homology modeling of further members of the biogenic amine receptor family. Since significant sequence conservation is observed in the putative antagonist binding pocket within this subfamily of GPCRs, our validated alpha1A homology model presents an appropriate structural template. Combined with docking modes of selective ligands, the homology models of closely related biogenic amine binding GPCRs could ideally be used to determine which amino acids are potentially important for selectivity.

Indeed, the central role that many of these biogenic amine binding GPCRs play in cell signaling also poses a risk for new drug candidates that reveal side affinities toward these receptor sites: These candidates have the potential to interfere with the physiological signaling process and to cause undesired effects in preclinical or clinical studies. As the alpha1A receptor is involved in blood pressure maintenance by modulating the vascular muscle tone, it is not only a molecular target for antihypertensitives (e.g. prazosine). It has also been suggested as an "antitarget" that mediates cardiovascular side effects of many GPCR drug candidates, causing orthostatic hypotension, dizziness, and fainting spells.[72] In our company, we have established biogenic amine receptor binding assays to monitor affinities of new GPCR drug candidates and to predict negative side effects of compounds during lead optimization. Surprisingly, many of the hit and lead compounds developed for chemokine or peptide binding GPCRs reveal affini-

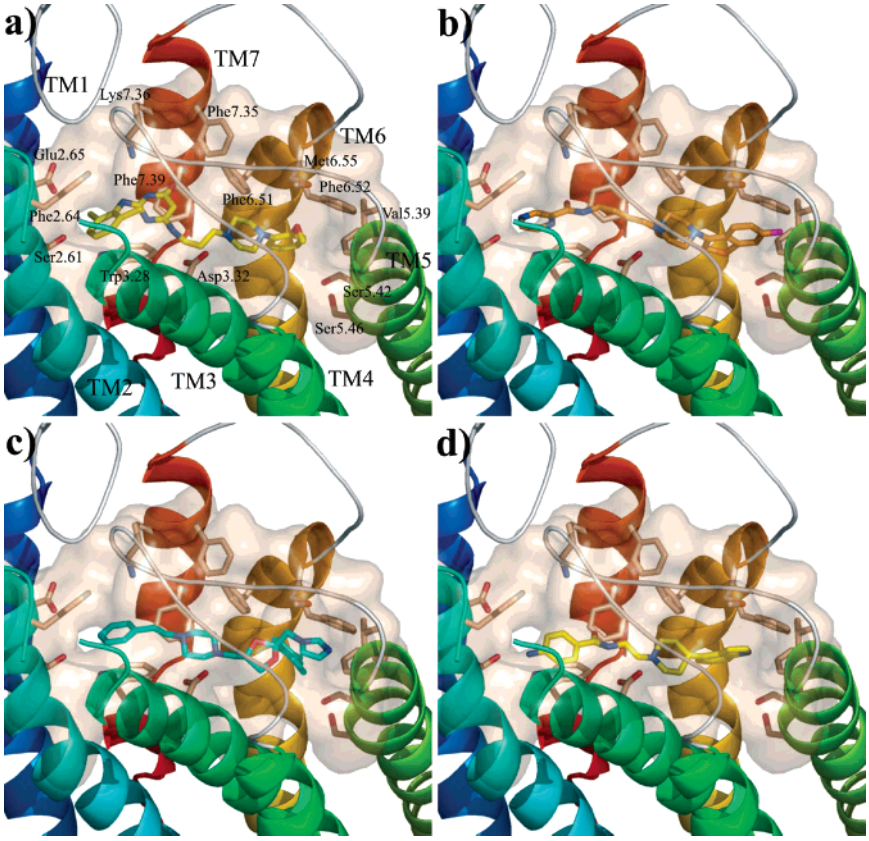


Figure 7. Docking mode of four alpha1A antagonists discovered by virtual screening (see also Table 4): (a) compound **12** (binding with 3.6 nM to the alpha1A receptor), (b) compound **13** (1.4 nM), (c) compound **14** (300 nM), (d) compound **15** (9.5 nM).

ties toward several members of the biogenic amine "antitarget" panel. To eliminate side affinities toward these antitargets, a general understanding of the features important for binding of ligands to these GPCRs is necessary. This study demonstrates that the presented alpha1A receptor homology model provides a relevant structural basis for rationalizing alpha1A receptor binding. Combined with ligand-based pharmacophore models and 3D-QSAR modeling, the homology model will guide the compound optimization within projects toward development candidates with an improved safety profile.

Materials and Methods

Test Data Set. To establish the best protocol for the virtual screening procedure, we compiled a test set extracted from the MDL Drug Data Report (MDDR). The MDDR is an annotated database covering the patent literature, journals, meetings, and congresses that contains over 141 000 biologically relevant compounds and well-defined derivatives such as drugs launched or in development phase. We extracted a test set consisting of 50 alpha1A antagonists and 990 further compounds that were not stated as alpha1A antagonists. The performance of different virtual screening protocols evaluating different scoring functions was assessed by calculating the enrichment of alpha1A antagonists among the top-scored compounds. It cannot be excluded that some of the randomly selected 990 decoy compounds that were not (explicitly) marked as alpha1A blockers actually reveal (side) affinity against the alpha1A receptor. Nevertheless, the enrichment values appear to be useful criteria to benchmark different scoring functions for a particular protein to select the optimal protocol for a subsequent virtual screening.

The effectiveness of the scoring functions ability to assign high ranks to alpha1A antagonists is reported in terms of enrichment factors in graphical and tabular form. The enrichment factor is represented by

$$\mathrm{EF} = \frac{\mathrm{Hits}_{\mathrm{sampled}}/N_{\mathrm{sampled}}}{\mathrm{Hits}_{\mathrm{total}}/N_{\mathrm{total}}}$$

where EF is the enrichment factor,

$\mathrm{Hits}_{\mathrm{sampled}}$ is the number of true hits in the hit list,
N_{sampled} is the number of compounds in the hit list,
$\mathrm{Hits}_{\mathrm{total}}$ is the number of hits in the full database, and
N_{total} is the number of compounds in the full database.

Testing for Binding. Alpha1A receptor binding assay was performed as described.[73] Briefly, the binding of [^{3}H]prazosine (0.5 nM, supplier NEN) to human recombinant alpha1A receptor in CHO-K1 cell membranes (30.4 μg/well) was measured after incubation for 40 min at 37 °C in 200 μL of 50 mM Tris/HCl (pH 7.7). Binding reactions were terminated by filtration through Millipore GF/B filter plates, and radioactivity was determined with a liquid scintillation counter (Perkin-Elmer). For all 80 hits from the virtual screening, double determinations were done at 10 μM. For those compounds that showed more than 50% inhibition, IC_{50} values were calculated from the averages of double determinations at eight different concentrations. K_i values were determined from the IC_{50} and K_d values of the radioligand by using the Cheng−Prusoff equation.

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