Discovery of Novel Chemotypes to a G-Protein-Coupled Receptor through Ligand-Steered Homology Modeling and Structure-Based Virtual Screening

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Melanin-concentrating hormone receptor 1 (MCH-R1) is a G-protein-coupled receptor (GPCR) and a target for the development of therapeutics for obesity. The structure-based development of MCH-R1 and other GPCR antagonists is hampered by the lack of an available experimentally determined atomic structure. A ligand-steered homology modeling approach has been developed (where information about existing ligands is used explicitly to shape and optimize the binding site) followed by docking-based virtual screening. Top scoring compounds identified virtually were tested experimentally in an MCH-R1 competitive binding assay, and six novel chemotypes as low micromolar affinity antagonist "hits" were identified. This success rate is more than a 10-fold improvement over random high-throughput screening, which supports our ligand-steered method. Clearly, the ligand-steered homology modeling method reduces the uncertainty of structure modeling for difficult targets like GPCRs.

Introduction

Melanin-concentrating hormone (MCH[a])is a disulfide linked cyclic 19-residue neuropeptide[1] that has been found to selectively bind and activate two receptors: MCH-R1, which is expressed predominantly in the hypothalamus in humans and rodents, and MCH-R2, which is found only in humans.[2–8] MCH-Rs belong to the class A family of G-protein-coupled receptors (GPCRs), which are integral membrane proteins containing seven transmembrane helices. Experimental studies show that knockout mice lacking either the MCH peptide gene or the MCH-R1 gene have high metabolic rates and are generally lean and hypophagic whereas mice injected with MCH have a higher food consumption than normal.[9–13] This indicates that the MCH system is intrinsically linked with feeding behavior and energy balance.

Obesity is a medical condition that is on the rise in the Western world.[14,15] Obesity places people at a higher than normal risk of contracting chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension, stroke, and certain forms of cancer. Given the role of MCH and its receptor(s) in feeding and energy balance, there has been considerable interest in developing MCH-R1 receptor antagonists for antiobesity therapy.[16]

A number of MCH-R1 antagonists have been reported that reduce the amount of food intake in rats including **1**, *N*-[6-(dimethylamino)methyl]-5,6,7,8-tetrahydro-2-naphthalenyl]-4′-fluoro[1,1′-biphenyl]-4-carboxamide (T-226296),[17] aryltetrazole derivatives, and quinoline derivatives.[18,19] Weight loss in obese mouse models has been observed with several MCH-R1 antagonists[20–22] including GW-803430.[23]

Recently, bicycloalkylurea antagonists have been identified that exhibit in vivo efficacy in rat models and a novel series of bis-aminopyrrolidine ureas have been identified as potent functional MCH-R1 antagonists.[24,25] Studies have also been undertaken to improve the properties of a series of phenylamine subunit antagonists by replacing the middle phenyl ring of the biphenylamine moiety with bicyclo[4.1.0]heptanes.[26] In 2004, Clark et al.[27] used published structures of MCH-R1 antagonists to perform a ligand-based screening that led to the discovery of a structurally distinct series of MCH-R1 antagonist chemotypes.

A structure-based approach to discover new scaffolds of MCH-R1 ligands is hampered by the lack of atomic structural data pertaining to GPCRs that is mainly due to the challenges associated with expression and crystallization of this family of proteins.[28] This makes homology modeling an attractive tool to generate high-quality structures of binding pockets to be later used in the drug discovery process.

Most comparative modeling methods rely on sequence similarity to a structural template, from which the backbone structure is inherited. There are three key factors that need to be considered: (i) the percentage of sequence identity between target and template, (ii) the accuracy of the alignment, and (iii)

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[a] Abbreviations: MCH, melanin concentrating hormone; MCH-R1, melanin concentrating hormone receptor type 1; GPCR, G-protein-coupled receptor; bRho, bovine rhodopsin; MD, molecular dynamics; RED, receptor ensemble docking; HTS, high-throughput screening.

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structural conservation. Once a model has been generated, it can be improved by an efficient energy sampling procedure for backbone and side chain atoms, although this is challenging especially when all-atom approaches are undertaken (see recent reviews[29–31]).

In the case of GPCR modeling the only structural template available is bovine rhodopsin (bRho).[32–36] For a model based on 20–30% sequence identity it is common to find that structural conservation is also very low. Fortunately for GPCR modelers there is structural conservation between GPCRs in the form of seven hydrophobic transmembrane helices. However, if sequence similarity within functional regions such as the ligand binding pocket is not very high, which is the most common scenario in GPCR modeling, there is uncertainty not only regarding backbone positioning but also about side chain conformations. One approach to solve this is to optimize the side chains in an empty pocket, but this may be considered inadequate because of the possibility of collapse. The limitations described here are highly critical in structure-based drug discovery, where most of the docking-based virtual screening methods rely on a rigid-receptor approach. Another approach is to use molecular dynamics (MD) and ligand data as a restraints;[37] however, a recent MD validation exercise reveals that even during a long simulation (100 ns) the sampling quality is poor, which presents a major hurdle for using this technique in homology modeling.[38]

It is natural to try to incorporate information about ligand binding poses in the process of modeling binding pockets, if known ligands are available. However, most of the approaches that attempt this are limited to an a posteriori optimization with the ligand, after the model has been built, either by manual placing or local energy minimization. There are exceptions though. Using knowledge-based potentials, Klebe and coworkers developed and benchmarked a homology modeling method where the information about ligand binding is considered in the modeling process by user-defined ligand−protein restraints derived from manual placement or rigid-ligand−rigid-receptor docking.[39] Preliminary models are generated with MODELLER,[40] and once the ligand(s) is placed, it is kept static during the modeling step and its information included by restraints. Further refinement through local energy minimization using a common force field is also possible. Models are ranked using DrugScore scoring function.[41] From these results, it is clear that more accurate binding sites result from considering ligand information in the modeling process. In a later application of this method to the NK1 receptor, the best model was considered the one that better satisfied the postulated ligand−protein interaction model.[42]

An alternative approach using ligand binding information was developed by Cavasotto et al., where flexible-ligand−flexible-receptor docking of known inhibitors was used to generate diverse binding sites in protein kinases.[43] These alternative binding sites spanning protein flexibility were successfully used in a small-scale structure-based virtual screening under the receptor ensemble docking (RED) approach (see refs 44–46 for review). A similar approach but using a normal mode-based methodology to mimic backbone flexibility has been reported,[47,48] and characterization of ligand-induced changes through full flexible docking has been also performed on nuclear receptors.[49]

In an expansion of our above-mentioned previous work, we present and validate here a ligand-steered homology modeling method, where existing ligands are explicitly used to shape and optimize the binding site through a docking-based stochastic global energy minimization procedure. Structural models were used as templates in a structure-based virtual screening project on class A GPCR MCH-R1. Top-scoring compounds were tested experimentally in a competitive binding assay, and six compounds were identified to be active with K_i values in the low-micromolar range (<20 μM). This relates to a hit enrichment rate of more than 10-fold compared to traditional random high-throughput screening (HTS). The docked orientation of the most potent identified agent was used to generate a pharmacophore model describing MCH-R1 binding determinants that greatly enhanced identification of active agents. It is thus clear that the ligand-steered homology modeling method reduces the uncertainty in binding site modeling, as has been suggested by others.[39,42]

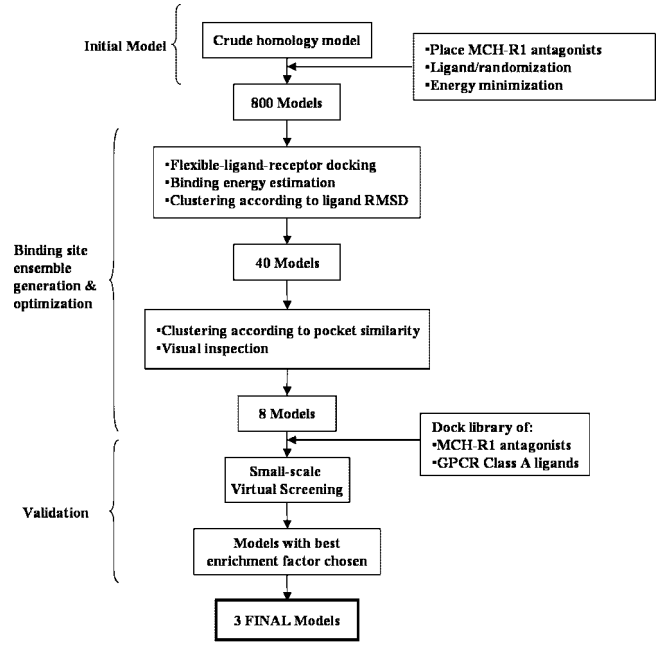
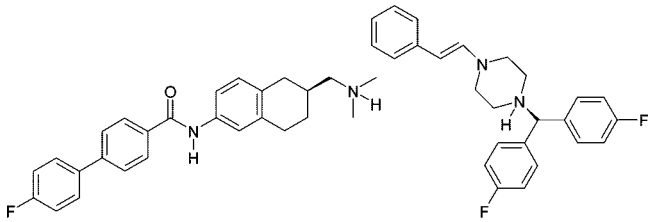


Figure 1. Ligand-steered homology modeling method: description and application to class A GPCR MCH-R1.

Results and Discussion

Ligand-Steered Homology Modeling Method: Overview and Application to the Structural Modeling of MCH-R1. The ligand-steered method for structural modeling of MCH-R1 is summarized in Figure 1. From an initial crude homology model, a large collection of receptor−antagonist complexes were built by placing one or more known ligands into the binding site. Ligands were placed in different orientations and conformations. Each member of the ensemble was subjected to flexible-ligand−flexible-receptor Monte Carlo based docking and further filtered down through a crude estimation of ligand−receptor interaction energy, binding pocket clustering, and visual inspection. Because of the lack of accurate binding energy calculations, limitations involved in the modeling of the system (e.g., lack of phospholipids and water molecules, lack of abundant mutagenesis data, absence of a wealth of solid experimental evidence to be used as structural constraints), and lack of and uncertainties in ligand−protein interaction patterns in many systems, especially in GPCRs (cf. ref 50 where models are selected according to docking score and quality of binding pose), we felt it was better to validate the final set of models through the performance of the modeled binding sites in a small-scale virtual screen. In this way, we test models in the demanding experiment of discriminating between known MCH-R1 antagonists described previously[27] and GPCR class A



1 2

Figure 2. Two chemically diverse MCH-R1 antagonists, **1** and **2**, as well as their two stereoisomers were used to generate a collection of structurally diverse MCH-R1 antagonist binding sites.

binders. This is a more stringent test than using a random library where compounds that do not share the physicochemical profile of known binders may facilitate binder/nonbinder separation. If a large pool of ligands were available, a QSAR-homology modeling approach to validate the models could also be attempted, as has been suggested.[39] Our method is especially useful when scarce ligand−protein interaction patterns are available, since both ligand and receptor are regarded as flexible throughout the modeling process. However, whenever experimental information about ligand−protein interactions are available, it could be included in the form of distance restraints.

Building of a Homology Model from Bovine Rhodopsin. A homology model of MCH-R1 based on the available crystal structure of bovine rhodopsin (bRho, PDB code 1L9H) was built, followed by a restraint-minimization (see Experimental Procedures and Supporting Information for details on protein sequence alignment). The objective of the energy minimization step was to relieve the structural strain stemming from the replacement of nonconserved residues in the homology modeling process while not allowing the pocket to collapse. Because of the small number of insertions/deletions of MCH-R1 compared to bRho, we found it meaningful to include both helices and loops in the model. Loops in the extracellular region might also be important constraints in helical positioning. The disulfide bond between Cys in the E2-loop and H3 was also included in the homology model.

Generation of an Ensemble of Ligand-Binding Sites through Ligand Docking. It is emphasized that there is not a great deal of experimental evidence regarding the ligand pose in MCH-R1. The only evidence of ligand–receptor interaction is the salt bridge established between D123 in transmembrane helix 3 (D3.32) and the charged amine in ligands.[51] Taking into account that MCH-R1 is observed to bind ligands of different chemotypes and by use of the minimized crude model from the previous step, two chemically diverse compounds (see Figure 2) were selected to generate a collection of structurally diverse binding sites, in a similar way as described for the generation of alternative structures for protein kinases.[43] Both chiralities of compounds **1** and **2** (compound **5** in ref 27) were also considered, so four ligands in total were used in this ligand-docking-based homology model. The four ligands were seeded into the pocket, and an ensemble of 200 structures for each ligand was generated by randomizing the position and orientation of the ligand followed by a multistep energy minimization, where the van der Waals interaction is gradually switched from soft to full interaction, in a similar fashion as performed in other cases.[43,47,53] The ligand and receptor were held flexible in this stage. A distance restraint between 1.5 and 2.0 Å was imposed between the hydrogen of the charged amine of the ligand and one of the oxygens of D3.32 in MCH-R1 (the functional form of the distance restraint is given in ref 54). (If further knowledge of ligand−protein interaction patterns are known, they may be taken into account at this stage.) The structures in the ensemble were then ranked using a crude binding energy estimation (see Experimental Procedures) and clustered according to ligand root-mean-squared deviation (rmsd), after superposition of the backbone atoms. A total of 20 models were generated in this step.

Generation of Refined MCH-R1 Models through Optimization of Side Chains in Binding Sites. The 20 structures obtained in the previous step were subjected to a full flexible-ligand−flexible-side chain Monte Carlo based global energy optimization as has been performed before in GPCRs and other proteins.[43,47–49,55,56] Side chains within 6 Å of the ligand were considered free, while the backbone was kept fixed. In this process, the restraint between the charged amine and D3.32 was removed. The top 20 best-energy complexes were selected and merged with the original 20 structures.

Analysis and Inspection of the MCH-R1 Models. The backbone atoms of the 40 models were superimposed, and the shape and residues surrounding the ligand binding pocket were determined. The rmsd values of the backbone atoms of residues within 6 Å of the ligand binding pocket were calculated. A matrix of rmsd values for each of the 40 complexes was built, and pockets were clustered according to rmsd similarity (a 0.4 Å rmsd cutoff was used). From each cluster, a representative structure was chosen on the basis of the binding affinity estimation, which resulted in the elimination of 20 models. The binding sites of each of the models were then visually inspected, and structures were retained if they met the following criteria: (1) the side chain of the critical binding pocket residue D3.32 was orientated directly into the pocket; (2) the pocket was orientated involving helices 5 and 6. These criteria were based on what has been reported on biogenic amine receptors and their ligand–receptor interactions.[57,58] Following this visual inspection, eight models remained; five of these models were based on compound **2** (models A−E), and three models were based on **1** (models F−H).

To verify the structure-based virtual screening approach on our models, compounds **1** and **2** used in the ligand-steered modeling were docked using a rigid receptor approach. The eight models were able to "redock" the ligands within 2 Å rmsd, making the key hydrogen bond with D3.32. In some cases, the hydrogen bond distance was larger than the optimal one, mainly because of the approximations in the rigid receptor docking approach. Those compounds were also docked to discarded model structures during the previous steps, failing to dock in all of the cases examined.

Model Validation through Small-Scale Virtual Screening. In the absence of solid experimental evidence to validate our models, we evaluated the accuracy of the models by their ability to discriminate binders and nonbinders in a virtual screening of known MCH-R1 antagonists seeded within a GPCR class A ligand library (see Experimental Procedures). The fraction of ligands recovered as a function of the ranked database is shown in Figure 3. Only MCH-R1 antagonists exhibiting the key hydrogen bond with D3.32 were taken into account to compute the fraction of ligands recovered, since a robust use of structure-based virtual screening should be based on correct docking poses[59] (although the correct pose is not known in advance, on the basis of ref 51, we assumed that the absence of a D3.32-ligand hydrogen bond relates to an incorrect pose). This is the reason that in Figure 3 the curves do not reach a 100% of ligands recovered when considering the whole chemical library. Only models recovering more than 50% of the known binders with the expected hydrogen bond between the charged amine and

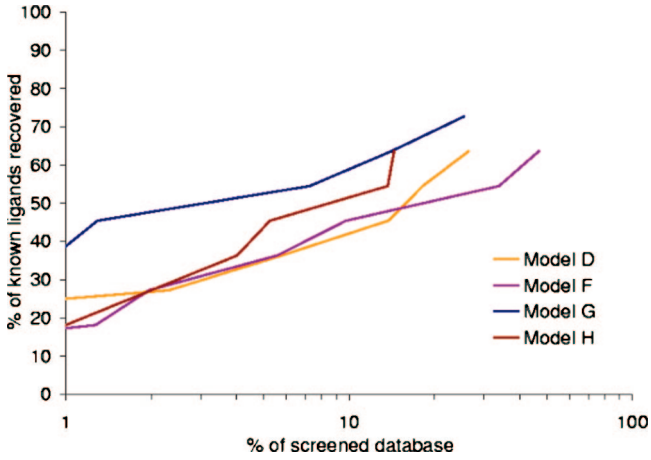


Figure 3. Fraction of known MCH-R1 antagonists recovered as a function of the ranked database. Only models recovering more than 50% of the ligands with the expected hydrogen bond between the charged amine and D3.32 are shown.

D3.32 are included. Although model G performs best, model H was selected for screening stage because of higher chemotype diversity in the top-ranking compounds. Our methodology, however, allows protein flexibility to be accounted for in two ways: (i) through using an ensemble of the receptor conformations obtained by modeling (RED approach) and (ii) through using smoothed potential maps to represent the receptor.[60] The RED (use of several structurally distinct receptors in virtual screening) has been reported as one of the best ways to incorporate receptor flexibility into virtual screening.[44–46] It is mentioned here that virtual screening of known antagonists against models generated without considering explicitly ligand information failed to dock any of them (data not shown).

Identification of Novel MCH-R1 Antagonist Scaffolds. Structure-Based Virtual Screening on the MCH-R1 Receptor. A filtered database (see Experimental Procedures for description) containing 187 084 compounds was then docked to the MCH-R1 model H using the ICM virtual screening module.[61] After screening, the results from each of the three docking runs were merged and the best score per compound was kept. A postscreening set of filters was imposed, requiring (i) absence of docked-ligand−receptor clashes and (ii) presence of a hydrogen bond between the ligand and the carboxylic oxygens of D3.32. The remaining compounds (about 7000) were clustered according to chemical similarity, and the highest scoring compounds per cluster were chosen. Compounds with a total charge of +1 were given priority for biological evaluation, resulting in a pool of 281 compounds for testing.

Bioevaluation of MCH-R1 Antagonist Candidates. Only 129 compounds (out of 281) were tested experimentally because of availability. From the MCH-R1 competitive binding assay (see Experimental Procedures for details), six compounds (see Table 1) were active with K_i values ranging within 7–20 μM, a hit enrichment rate of 11.8-fold compared to random screening of a corporate file collection (data not shown).

Analysis of Proposed Binding Modes. All six hits (Table 1) make a strong H-bond between the charged amine and D3.32 [D192] in helix 3 (Figure 4). Other common features in binding modes between the lead compounds include high contact areas with residues Q3.36 [Q196] (helix 3), W6.48 [W338] (helix 6), I7.39 [I366] (helix 7), G7.42 [G369] (helix 7), and Y7.43 [Y370] (helix 7). Compound **8**, **6**, and **4** all make a hydrogen bond with Q3.36 via a secondary amine hydrogen. The methylbenzyl rings of compounds **3** and **4** make a $\pi-\pi$

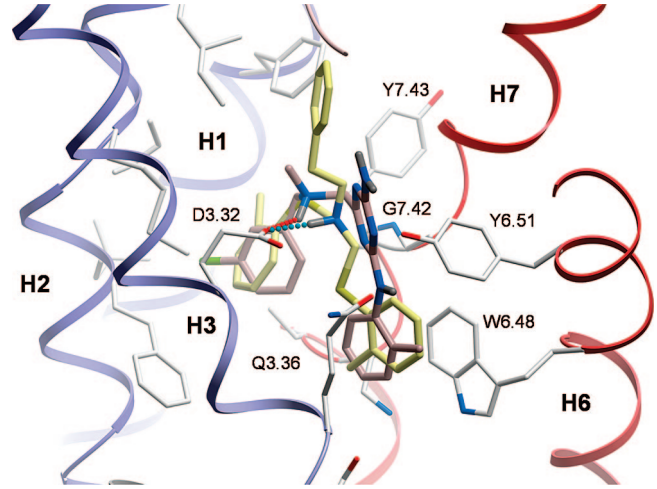


Figure 4. MCH-R1 complexed with compound **3** (yellow carbon atoms) and compound **4** (brown carbon atoms). The hydrogen bond for each ligand with D3.32 [D192] is represented as blue and red spheres. The $\pi-\pi$ stacking can be seen with W6.48 [W338]. Helices are colored blue (N-terminal) to red (C-terminal), and helices 4 and 5 are cut away in the front plane for clarity.

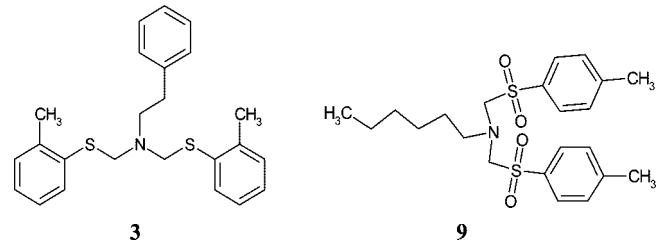


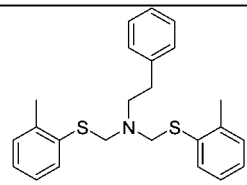
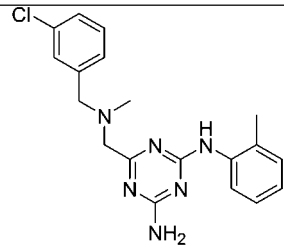
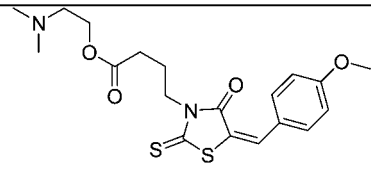
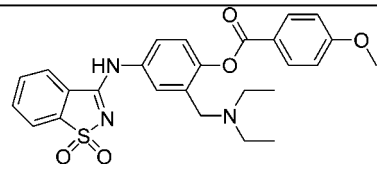
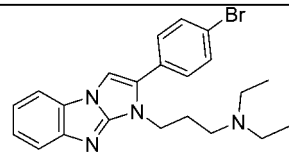
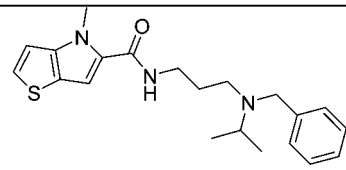
Figure 5. Structures of hit compound **3** discovered through structure-based virtual screening (K_i = 7.5 μM) and analogue **9** discovered by similarity search (K_i = 1.7 μM).

interaction with W6.48. The methoxybenzyl of compound **5** protrudes between helices 5 and 6, and the methylbenzyl ring of compound **A1** is not involved in $\pi-\pi$ stacking. The methoxybenzyl of compound **6**, the benzyl ring of compound **9**, and, the chlorobenzyl of compound **4** extend outward toward helices 1 and 2.

Ligand-Based and Pharmacophore-Based Virtual Screening. Available analogues of the six assay hits were clustered on the basis of similarity using Pipeline Pilot (Accelrys/SciTegic, San Diego, CA), and representatives of each cluster were tested to expand structure–activity for each chemotype (see Tables 1–5 in Supporting Information). All tested analogues of compound **5** were determined to be inactive (data not shown). An analogue of compound **3** was identified with a K_i of 1.7 μM, compound **9** (see Figure 5).

Generation of a Pharmacophore Model. From the docked pose of compound **3**, a Catalyst (Accelrys Inc., San Diego, CA) pharmacophore model was generated using excluded volume features to represent the MCH-1R receptor excluded regions. The pharmacophore model is shown in Figure 6. The model included two aromatic hydrophobic, one general hydrophobic, and one tertiary amine feature, as suggested by the structure–activity relationship of tested compound **A1** analogues (data not shown). A search with this pharmacophore query of the corporate file yielded 535 hits. For 147 of the hits, MCH-R1 percent inhibition data were available and 48% were known active MCH-R1 ligands (30% or better inhibition at 2 μg/mL as mixtures of 8). In addition, K_i values for 28 of the 535 hits were measured, and all but compound **3**, which was also

Table 1. Chemical Structures of the Lead Antagonists and Their Respective K_i Values Identified by Ligand-Docking-Based Modeling of MCH-R1, Followed by Structure-Based Virtual Screening and Bioevaluation[a]

Compound ID	Structure	K_i(μM)	Available Analogs	Tested Cluster Representatives
3		7.5	9	4
4		7.8	4	4
5		8.1	223	4
6		11.3	117	3
7		11.9	557	3
8		20.9	28	3

[a] Pharmacophore analysis was undertaken on each "hit", resulting in a set of available analogues from which a sample of representative compounds were further tested experimentally.

retrieved by the query, had a K_i value of 5 μM or better. Among the hits were eight previously reported biarylureas (compare to compound **4** shown in ref 62). The excellent retrieval rate of known MCH-R1 ligands by the pharmacophore query based on the GPCR model is a further validation of the model and demonstrates that additional potential MCH-R1 inhibitors could be obtained by a pharmacophore search.

Conclusions

These data illustrate that explicit ligand information incorporated in the ligand-steered homology modeling method can be applied to shape and optimize the binding site, thus reducing the uncertainty in its structural characterization by homology modeling. This approach appears especially suited to model (i) proteins with flexible binding sites, (ii) targets that are difficult to characterize experimentally, like GPCRs, and (iii) pockets with low homology to a template structure. Model validation was performed through a small-scale virtual screening, namely, the same type of experiment for which the models are constructed. Thus, uncertainties in accurate energy prediction and lack of knowledge of ligand−protein interaction patterns are avoided. This methodology appears to be attractive when little structural information about ligand−protein interaction is available, because of the fact that both ligand and receptor are considered flexible throughout the modeling process by a stochastic global energy minimization procedure, which ensures a thorough coverage of the energy landscape. However, if solid experimental knowledge about ligand−protein interactions is available, it could be included as additional distance restraints. This ligand-steered homology modeling method could be merged with the receptor ensemble docking[44,45] to account for flexibility in the virtual screening stage. The six novel antagonists found in this work are clearly not reducible to any existing scaffold, accomplishing one of our goals to search for chemotype

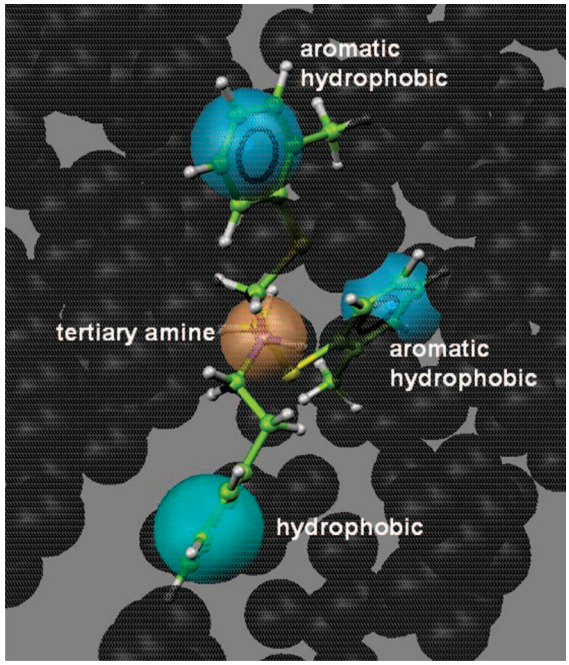


Figure 6. Pharmacophore model developed with Catalyst for compound **3** docked within the MCH-R1 binding site.

novelty and diversity. Moreover, the structure-based virtual screening approach to drug discovery is shown again to be a complementary tool to the more traditional HTS, exemplified by the more than 10-fold better performance, which can then be improved when subsequent pharmacophore-based screening is included. It is stressed here that 40–50% of the marketed drugs involve an interaction with a GPCR and this method can clearly be applied to improve current and discover new therapeutics.

Experimental Procedures

Biomolecular Representation and Energy Calculation. The molecular system was described in terms of internal coordinates variables[54] using modified ECEPP/3 potentials,[63] as implemented in the ICM program (MolSoft LLC, La Jolla, CA).[61] Charges for ligands were taken from the MMFF force field.[64] The stochastic global energy minimization was performed according to a Monte Carlo procedure with local minimization.[65] Ligand–receptor binding energy was estimated as the ligand–receptor interaction energy, where the van der Waals, electrostatic, hydrogen bonding, and torsional energy terms were considered.

Sequence Alignment between MCH-R1 and Bovine Rhodopsin. The alignment between bRho and MCH-R1 was initially generated using ICM and then refined according to published GPCR sequence residue conservation tables[66] (see Supporting Information). Fold similarity and conservation of key residues in each of the helices ensure a reliable alignment despite the low overall sequence identity between bRho and MCH-R1 (19%). The nomenclature of Ballesteros and Weinstein[67] is used, whereby the most conserved residue in helix X is labeled as X.50.

Preparation of Virtual Chemical Libraries Used in Ligand-Docking-Based Modeling. A MCH-R1 antagonist library was built by taking the 2-D structures of 11 known antagonists from ref 27 (compounds **1**−**11** in that reference). Whenever the stereoisomer was not clearly specified, both chiralities were considered (per chiral center). The decoy library of GPCR class A ligands consisted of 5497 compounds collected from an in-house database.

Large Compound Database for Virtual Screening. An initial raw database of compounds was compiled from 37 different vendors, resulting in 7 379 648 small-molecule structures. Refinement showed that 3 349 510 nonredundant compounds were present. The nonredundant database was then filtered according to a set of parameters based on those used by Clark et al.[27] in their ligand-based screening for MCH-R1 antagonists. Maximum allowed molecular weight was increased to 600 from 550, and compounds that did not have an amine nitrogen (single bonded nitrogen not bonded to any sp^2 heavy atom) were removed from the database, resulting in 187 084 compounds.

Structure-Based Virtual Screening. The ICM structure-based virtual screening method was used.[60] The receptor is represented by six grid potential maps accounting for hydrophobicity, van der Waals interactions (three), hydrogen-bonding, and electrostatic potential. The ligand is considered fully flexible in the field of the receptor. To ensure convergence of the Monte Carlo based global energy minimization, each ligand was docked three times into the receptor, and the best scoring pose per compound was kept.

MCH Receptor Binding Assay. Membranes from CHO cells expressing the human MCH-R1 receptor were prepared by lysing cells with 5 mM HEPES for 15 min at 4 °C. Cell lysates were centrifuged (12500*g*, 15 min), and the pellet was resuspended in 5 mM HEPES. For each 96-well plate (Microlite, Dynex Technologies), 1 mg of cell membranes was incubated with 10 mg of wheatgerm aggluttinin SPA beads (Amersham) for 5 min at 4 °C in a volume of 10 mL of binding buffer (25 mM HEPES, 10 mM $MgCl_2$, 10 mM NaCl, 5 mM $MnCl_2$, 0.1% BSA). The membrane/bead mixture was centrifuged (1500*g*, 3.5 min), the supernatant was aspirated, and the pellet was resuspended in 10 mL of binding buffer. The centrifugation, aspiration, and resuspension were then repeated. The membrane/bead mixture (100 μL) was then added to 96-well plates containing 50 μL of 500 pM [^{125}I]MCH (NEN) and 50 mL of the appropriate concentration of compound (4× the desired final concentration). Nonspecific binding was determined by including 1 μM MCH in the binding reaction. The binding reaction mixture was incubated at room temperature for 2 h. Plates were then analyzed in a TOPCOUNT microplate scintillation counter (Packard). Data were analyzed, and K_i values were determined using Graphpad Prism software.

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Supporting Information Available: Sequence alignment of bRho and MCH-R1; tables of structure-based hits, tested analogues, and binding data. This material is available free of charge via the Internet at http://pubs.acs.org.

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