

Homology Model and Docking-Based Virtual Screening for Ligands of the σ_1 Receptor

Erik Laurini,[†] Valentina Dal Col,[†] Maria Grazia Mamolo,[‡] Daniele Zampieri,[‡] Paola Posocco,[†] Maurizio Fermeglia,[†] Luciano Vio,[‡] and Sabrina Pricl^{*,†}

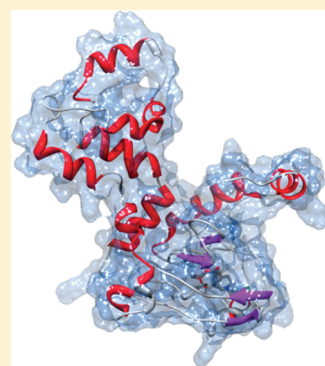
[†]Molecular Simulation Engineering (MOSE) Laboratory, Department of Industrial Engineering and Information Technology (DI3), University of Trieste, Via Valerio 10, 34127 Trieste, Italy

[‡]Department of Pharmaceutical Sciences, University of Trieste, Piazzale Europa 1, 34127 Trieste, Italy

 Supporting Information

ABSTRACT: This study presents for the first time the 3D model of the σ_1 receptor protein as obtained from homology modeling techniques, shows the applicability of this structure to docking-based virtual screening, defines a computational strategy to optimize the results based on a combination of 3D pharmacophore-based docking and MM/PBSA free energy of binding scoring, and provides evidence that these *in silico* models and recipes are powerful tools on which virtual screening of new σ_1 ligands can be based. In particular, the validation of the applicability of docking-based virtual screening to homology models is of utmost importance, since no crystal structure is available to date for the σ_1 receptor, and this missing information still constitutes a major hurdle for a rational ligand design for this important protein target.

KEYWORDS: σ_1 Receptor, homology modeling, 3D pharmacophore model, docking, free energy of binding, virtual screening



Since the findings that several neuroleptic drugs bind σ receptors,¹ a growing interest in σ ligands has been driven by the need of atypical antipsychotics devoid of motor side effects that are displayed by the classical neuroleptics. At the moment, σ receptors are distinguished in σ_1 and σ_2 subtypes,^{2,3} although only σ_1 receptor protein has been unequivocally identified to date. Both σ receptor subtypes are widely distributed in the central nervous system (CNS), liver, kidney, and lung and in endocrine, immune, and reproductive tissues,^{4,5} furthermore, they are overexpressed in several tumor cell lines.⁶ Direct functional assays are unknown. Nevertheless, deduced σ_1 receptor functions in CNS include modulatory roles on K^+ and Ca^{2+} channels and on dopaminergic, NMDA (*N*-methyl-D-aspartate), serotonergic, muscarinic neurotransmission, and opioid analgesia.⁷ The homology of mammalian cloned σ_1 receptor with a yeast sterol-isomerase (ERG2 protein) has suggested the σ_1 receptor intervention in steroid biosynthesis.⁸ Recent findings attribute EPS (extrapyramidal symptoms) to the activity at the σ_1 site.^{4,5} Although a real intrinsic activity cannot be ascribed to the σ ligands, putative σ_1 agonists have been proposed as antidepressants and anti-amnesics in learning and memory impairment.¹ Putative antagonists are thought to be useful agents for preventing neurodamage and treating cocaine abuse, schizophrenia, and other neurological disorders.⁹ Furthermore, both σ_1 and σ_2 ligands are required as cancer diagnostic tools for positron emission tomography (PET) analysis.⁶

The σ_1 gene is located on human chromosome 9, band p13, a region indeed known to be associated with different psychiatric

disorders.¹⁰ It encodes a unique protein consisting of 223 amino acids, which was first cloned and functionally expressed by Kekuda and colleagues.¹¹ From the structural standpoint, the only information available mostly concern the protein main structural motifs, which include an intracellular N-terminal end, two transmembrane spanning domains (residues 10–30 and 80–100) linked by an extracellular loop, and a partially arranged C-terminal end.¹² A typical arginine–arginine endoplasmic reticulum locating signal near the N-terminal end has also been identified. Importantly, σ_1 receptors contain two GXXXG motifs, a signature that occurs with high frequency in membrane proteins that favor helix–helix interactions.¹³ Because σ_1 is a membrane-bound protein, its expression, purification, crystallization, and structure determination are difficult processes. Thus, no information about the three-dimensional (3D) structure of the cloned σ_1 receptor has been released so far; this constitutes a serious hurdle in the development of new, potent ligands for this protein.

In this work, we present for the first time a 3D model for the σ_1 receptor based on a molecular modeling and simulation *ansatz* involving the following sequential steps: (i) generation and optimization of the complete 3D model of the receptor by homology modeling techniques;¹⁴ (ii) further 3D homology model refinement exploiting information derived from ligand

Received: June 21, 2011

Accepted: August 27, 2011

Published: August 27, 2011

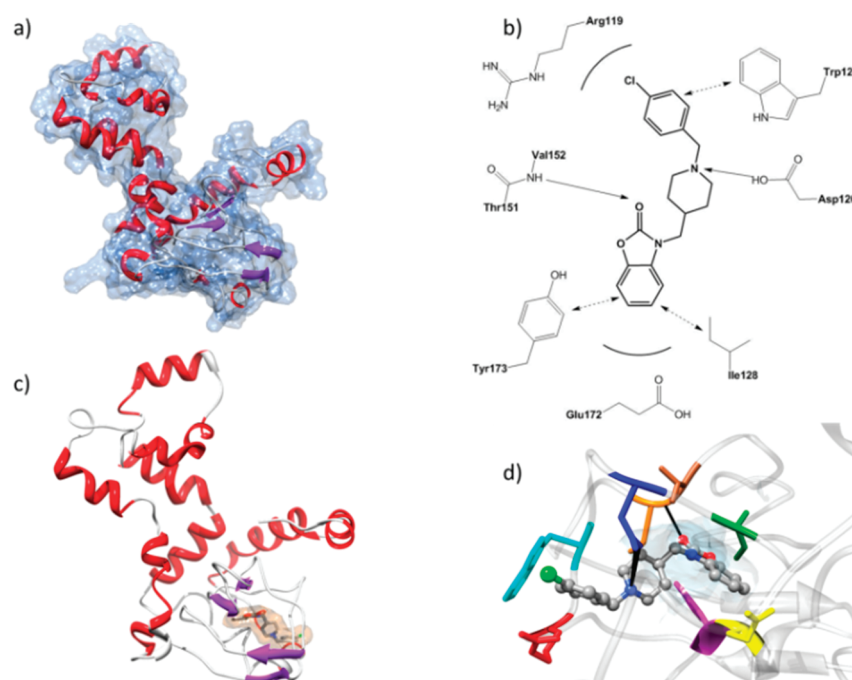


Figure 1. σ_1 Receptor and its ligand **1c**. (a) View of the σ_1 receptor MD-optimized homology model in complex with **1c**. (b) Two-dimensional schematic representation of postulated interactions between the σ_1 receptor and **1c**, established by analyzing published sequence–structure relationships, mutagenesis data, 3D pharmacophore model requirements, and direct affinity measurements. The lines/arrows indicate proposed key interaction between the receptor and its ligand. (c) View of the final σ_1 receptor homology model in complex with **1c**. (d) Modeled complex of the σ_1 receptor with **1c** showing the key interactions proposed in the topographical interaction model depicted in part b. The main protein residues involved in these interactions are Arg119 (red), Trp121 (cyan), Asp126 (blue), Ile128 (forest green), Thr151 (sienna), Val152 (orange), Glu172 (yellow), and Tyr173 (magenta).

docking,^{15–18} a 3D pharmacophore model,^{19–22} and mutagenesis experiments²³ as spatial restraints; (iii) docking of a series of bioactive ligands into the putative binding site, estimation of their binding affinity via the molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) methodology,²⁴ and comparison with the available experimental activities;^{19–22} and (iv) design of a set of new ligands with different degrees of affinity for the protein based on the derived receptor model to test the predictive capability of the σ_1 3D model. Detailed information about each single step mentioned above can be found in the Supporting Information.

The initial stage of step (i) consisted in the modeling of the transmembrane (TM) regions (three α -helices spanning residues 10–30, 80–100, and 180–200, respectively) and the prediction of the overall secondary structure of the protein, which included a few β -strands in the C-terminal half (residues 111–116, 133–135, 144–146, and 158–164), and some loops. The search for a suitable template to build the 3D receptor structure yielded four different protein sequences having both a considerable identity ($\geq 30\%$) with specific portions of the σ_1 sequence and an X-ray structure available in the Protein Data Bank (PDB entries 3CIA, 1I24, 2Z2Z, and 2Q8I) (see the Supporting Information, S3). Accordingly, each corresponding receptor/template sequence was aligned, and the Cartesian coordinates for σ_1 residues in structurally conserved regions were obtained from the corresponding sequence in the template PDB file. The first part of the N-terminal domain of the protein (residues 1–16) showed no homology with any other proteins in all queried databases and thus was built de novo. Finally, the overall receptor 3D structure was built, linking the different

template-based homology models and creating/optimizing the missing loop portions via several refinement processes. The structure was slightly adjusted to fully match the results of secondary structure predictions and optimized for backbone and side chain conformation. This initial 3D model was then subjected to extensive molecular dynamics (MD) refinement first in explicit solvent to attain an energetically stable and favorable conformation (see the Supporting Information, S4). The MD-refined homology model (Figure 1a), characterized by a very high stereochemical quality (more than 98% of residues in the favored or additional allowed regions of Ramachandran plot and only two residues in the disallowed region), was employed in the successive step.

Step (ii) required the initial identification of a putative binding site in the receptor model, based on preliminary information on sequence–structure relationships,²³ mutagenesis studies,²³ and some ligand-binding pharmacophore requirements (see Supporting Information, S5).^{19–22} In detail, a protein isoform missing residues 119–149 was found devoid of ligand binding capacity, and the conversion of residues D126 and E172 to glycine led to a several-fold reduction in ligand-binding function for the σ_1 receptor.²³ Moreover, a hydrophobicity analysis performed in the present study identified, aside for the TM regions, a third hydrophobic region matching the SBDLII (steroid binding domain-like II) region and centered on Asp188, a residue specifically photolabeled by [¹²⁵I]IACoc (3-iodo-4-azido cocaine).^{25,26}

Having localized this protein region as a possible zone for ligand binding, a thorough search for a set of complementary protein residues satisfying the chemical features imposed by our

Table 1. Binding Free Energies ΔG_{bind} (kcal/mol) for a Set of 12 Bioactive Ligands and the σ_1 Receptor Homology Model^a

compd	1c	1a	1j	2a
ΔG_{bind}	-12.05 (0.37)	-10.93 (0.44)	-8.38 (0.46)	-8.87 (0.48)
K_i (calcd)	1.48	9.80	730	315
K_i (exp)	0.098 (0.03)	3.58 (0.30)	1017 (117)	223 (47)
compd	2d	3a	3d	7a
ΔG_{bind}	-8.22 (0.42)	-10.53 (0.39)	-10.67 (0.45)	-10.24 (0.39)
K_i (calcd)	945	19.1	15.3	31.4
K_i (exp)	1147 (206)	2.60 (1.50)	7.10 (1.50)	22.5 (2.45)
compd	7b	FEN	PTZ	HALO
ΔG_{bind}	-10.99 (0.43)	-12.44 (0.43)	-10.02 (0.45)	-10.61 (0.42)
K_i (calcd)	8.70	0.77	45.5	16.8
K_i (exp)	12.9 (1.92)	0.011 (0.006)	15.0 (3.0)	5.70 (1.00)

^a Errors are given in parentheses as standard errors of the mean. The experimental and calculated K_i (nM), as estimated from the corresponding ΔG_{bind} values, are also reported for comparison.

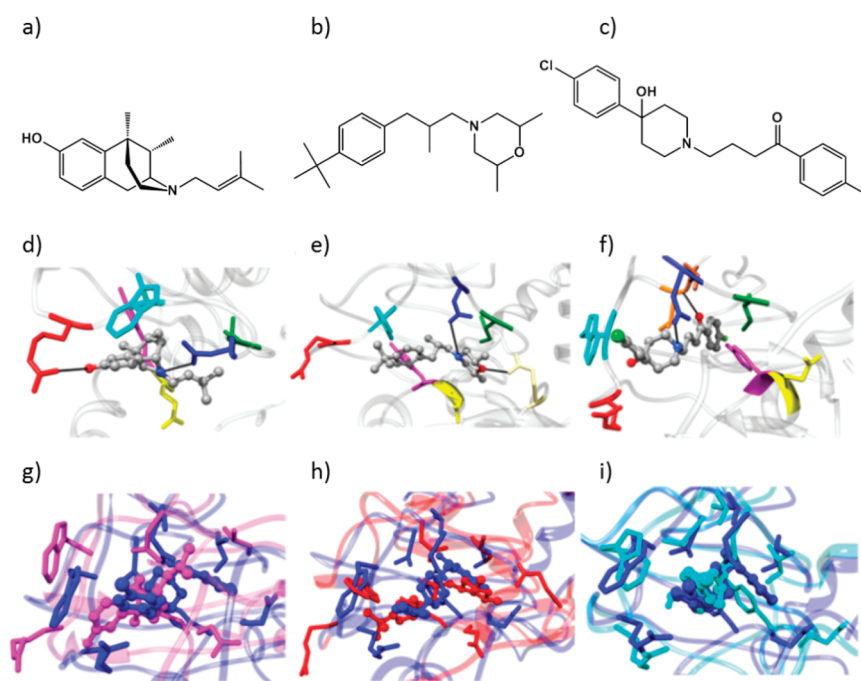


Figure 2. Structure of (a) pentazocine (PTZ), (b) fenpropimorph (FEN), and (c) (haloperidol (HALO)). Modeled complex of the σ_1 receptor with (d) PTZ, (e) FEN, and (f) HALO, showing the key interactions proposed in the topographical interaction model depicted in Figure 1b. The main protein residues involved in these interactions are Arg119 (red), Trp121 (cyan), Asp126 (blue), Ile128 (forest green), Thr151 (sienna), Val152 (orange), Glu172 (yellow), and Tyr173 (magenta). (c) Superposition of the σ_1 receptor binding site in complex with 1c (blue) and (g) PTZ (magenta), (h) FEN (red), and (i) HALO (cyan).

recently developed 3D pharmacophore model^{19–22} was performed and successfully retrieved (see Figure S7 in the Supporting Information). Then, from a previously synthesized set of σ_1 ligands,²² the lead compound 1c²² was docked into the σ_1 putative binding pocket. In the set of docked ligand conformations, a solution was found that best reproduced the key interactions from 3D pharmacophore model as depicted in the derived topographical model (Figure 1b). The resulting molecular complex was relaxed by energy minimization and MD simulations, and finally, a model without unfavorable van der Waals interactions was obtained (Figure 1c). Figure 1d shows the

ligand-binding site of the resulting σ_1 /1c complex, which retains the proposed interactions in the 2D topological model. Indeed, the interactions essential for the binding of ligand 1c are (1) a salt bridge between the 1c piperidine $-\text{NH}^+$ and the protein Asp126 $-\text{COO}^-$ (first 3D pharmacophore requirement); (2) the hydrogen bond (HB) between the backbone $-\text{NH}$ donor of the peptidic bond linking Thr151 and Val 152 on the receptor side and the acceptor oxygen atom of the benzoxazolone moiety of 1c (second 3D pharmacophore requirement); (3) T-stacking $\pi-\pi$ interaction between the protein residue Trp121 and the heteroaromatic condensed rings of 1c (third 3D pharmacophore

requirement); (4) parallel-stacking π - π interaction between the protein residue Tyr173 and the benzoxazolone phenyl ring of **1c** (fourth 3D pharmacophore requirement); (5) highly stabilizing van der Waals and electrostatic interactions between Arg119 and Glu172 with the *p*-Cl-phenyl and the benzoxazolonic heterocycle of **1c**, respectively; and (6) general hydrophobic favorable interactions between the side chains of Ile128, Thr151, and Val152 with both the aliphatic and the aromatic portions of the ligand.

In step (iii), the refined σ_1 homology model was used for the docking of a series of bioactive ligands (including **1c**) into the putative binding site, estimation of their binding affinity via the MM/PBSA methodology,²⁴ and comparison with the available experimental activities (see the Supporting Information, S6).^{19–22} Table 1 shows the results of the MM/PBSA ranking, from which the excellent agreement between computed and experimental affinities of these ligand series is apparent.

The quality of the model was further validated by probing its ability to accommodate other known σ_1 ligands belonging to structurally diverse compound classes. Thus, the strong σ_1 agonist pentazocine (PTZ)²⁷ and the two high-affinity σ_1 ligands fenpropimorph (FEN)²⁸ and haloperidol (HALO)¹⁹ were docked into the σ_1 binding site and scored according to the same protocol employed for **1c** and related compounds. Importantly, the adopted binding modes of these three compounds (Figure 2) were also in agreement with the proposed 2D interaction model (Figure 1b), and the MM/PBSA was able to

Table 2. Binding Free Energies ΔG_{bind} (kcal/mol) for the Set of Three New σ_1 Ligands Designed Exploiting the Developed 2D/3D Interaction Model^a

compd	EL-1	EL-2	EL-3
ΔG_{bind}	-11.31 (0.38)	-10.02 (0.35)	-7.75 (0.37)
K_i (calcd)	5.20	45.7	2100
K_i (exp)	1.87 (0.25)	30.3 (1.69)	1578 (135)

^aErrors are given in parentheses as standard errors of the mean. The experimental and calculated K_i (nM), as estimated from the corresponding ΔG_{bind} values, are also reported for comparison.

rank their affinity not only in the correct order but also with an outstanding agreement between computed and experimental K_i values^{19,29,30} ($R^2 = 0.93$, see Figure S9 in the Supporting Information).

Lastly, in step (iv), all information presented above and the new 3D homology model derived in this work were exploited for the design of a new, small series of σ_1 ligands starting from the structure of the lead compound **1c**. Subsequently, step (iii) of the multistep procedure outline above was applied to predict their affinity toward the protein. The rationale (see the Supporting Information, S7 and S8) behind this design was to check the predictive capabilities of the entire developed computational procedure and models in the design of novel σ_1 ligands. Under this perspective, three new compounds EL-1, EL-2, and EL-3 were conceived exploiting the developed 2D/3D interaction model (Figure 3) and ranked as highly affine, moderately affine, and poorly affine to the σ_1 protein by our *in silico* procedure according to the ΔG_{bind} values listed shown in Table 2. Importantly, these predicted affinities were confirmed by the corresponding experimental values of binding constant K_i , ultimately validating the predictive features of the present computational recipe. Of note, compound EL-1 exhibited a very promising K_i value of 1.87 nM, in utter agreement with the MM/PBSA predicted value of 5.20 nM. As shown in Figure 3, compound EL-1 adopted a binding mode within the receptor putative binding pocket, which satisfies all proposed 2D/3D interaction models.

In conclusion, the multistep homology modeling procedure adopted in this work produced a reliable 3D structure of the σ_1 receptor that can successfully be employed as a platform for structure-based drug design. Overall, this work illustrates that explicit ligand information can be successfully employed to optimize the binding site model. This approach can be especially helpful when modeling proteins with highly flexible binding sites, or targets that are difficult to characterize experimentally, and/or pockets with low homology to available template structures, as in the present case of σ_1 receptors. A thorough model validation was performed by a docking/MMPBSA-based small-scale virtual screening and by the receptor model-based design of three new

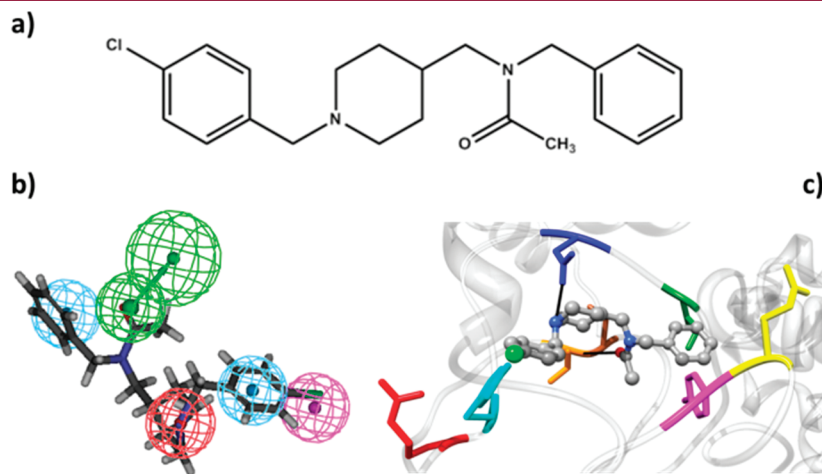


Figure 3. (a) Structure of compound EL-1 designed exploiting the 2D/3D interaction model and the 3D homology model of the σ_1 receptor developed in this work. (b) Mapping of EL-1 onto our 3D pharmacophore model developed for σ_1 ligands.^{19–22} (c) Modeled complex of the σ_1 receptor with EL-1 showing the key interactions proposed in the topographical interaction model depicted in Figures 1b and 3b. The main protein residues involved in these interactions are Arg119 (red), Trp121 (cyan), Asp126 (blue), Ile128 (forest green), Thr151 (sienna), Val152 (orange), Glu172 (yellow), and Tyr173 (magenta).

σ_1 ligands, featuring activity in a wide range. Although medicinal chemistry is still eager to obtain new X-ray structures of protein targets, this study with σ_1 receptor demonstrates that its derived 3D homology model is already an excellent substrate. Given the intense ongoing efforts in designing new compounds as potent σ_1 ligands, this work is rather an encouraging account.

■ ASSOCIATED CONTENT

S Supporting Information. Detailed experimental and computational procedures, 3D pharmacophore features, chemical formulas and structures of discussed compounds, syntheses and characterization of compounds, pharmacology, and more modeling-derived information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sabrina.pricl@dicamp.units.it

Notes

The coordinate file of the σ_1 homology model is available from the authors upon request.

Funding Sources

E.L., V.D.C., P.P., M.F., and S.P. acknowledge the financial support from ESTECO through the project DDOS.

■ ABBREVIATIONS

CNS, central nervous system; NMDA, N-methyl-D-aspartate; PET, positron emission tomography; EPS, extrapyramidal symptoms; TMtransmembranePTZ, pentazocine; FEN, fenpropionorph; HALO, haloperidol; MM/PBSA, molecular mechanics/Poisson–Boltzmann surface area

■ REFERENCES

- (1) Cobos, E. J.; Del Pozo, E.; Baeyens, J. M. Irreversible Blockade of Sigma-1 Receptors by Haloperidol and its Metabolites in Guinea Pig Brain and SH-SY5Y Human Neuroblastoma Cells. *J. Neurochem.* **2007**, *102*, 812–825.
- (2) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. A Proposal for the Classification of Sigma Binding Sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85–86.
- (3) Hellewell, S. B.; Bowen, W. D. A Sigma-like Binding Site in Rat Pheochromocytoma (PC12) Cells: Decreased Affinity for (+)-Benzomorphans and Lower Molecular Weight Suggest a Different Sigma Receptor Form From that in Guinea Pig Brain. *Brain Res.* **1990**, *527*, 244–253.
- (4) Fishback, J. A.; Robson, M. J.; Xu, Y.-T.; Matsumoto, R. R. Sigma receptors: Potential Targets for a New Class of Antidepressant. *Drug Pharmacol. Ther.* **2010**, *127*, 271–282.
- (5) Matsumoto, R. R.; Liu, Y.; Lerner, M.; Howard, E. W.; Brackett, D. J. Sigma Receptors: Potential Medications Development Target for Anti-cocaine Agents. *Eur. J. Pharmacol.* **2003**, *469*, 1–12.
- (6) Kashiwagi, H.; McDunn, J. E.; Simon, P. O., Jr.; Goedegebuure, P. S.; Xu, J.; Jones, L.; Chang, K.; Johnston, F.; Trinkaus, K.; Hotchkiss, R. S.; Mach, R. H.; Hawkins, W. G. Selective Sigma-2 Ligands Preferentially Bind to Pancreatic Adenocarcinomas: Applications in Diagnostic Imaging and Therapy. *Mol. Cancer* **2007**, *6*, 48.
- (7) Cobos, E. J.; Entrena, J. M.; Nieto, F. R.; Cendan, C. M.; Pozo, E. D. Pharmacology and Therapeutic Potential of Sigma(1) Receptor Ligands. *Curr. Neuropharmacol.* **2008**, *6*, 344–366.

(8) Moebius, M.; Striessnig, J.; Glossmann, H. The Mysteries of Sigma Receptors: New Family Members Reveal a Role in Cholesterol Synthesis. *Trends Pharmacol. Sci.* **1997**, *18*, 67–70.

(9) Maurice, T.; Su, T. P. The Pharmacology of Sigma-1 Receptors. *Pharmacol. Ther.* **2009**, *124*, 195–206.

(10) Prasad, P. D.; Li, H. W.; Fei, Y. J.; Ganapathy, M. E.; Fujita, T.; Plumley, L. H.; Yang-Feng, T. L.; Leibach, F. H.; Ganapathy, V. Exon–intron Structure, Analysis of Promoter Region, and Chromosomal Localization of the Human Type 1 Sigma Receptor Gene. *J. Neurochem.* **1998**, *70*, 443–451.

(11) Kekuda, R.; Prasad, P. D.; Fei, Y. J.; Leibach, F. H.; Ganapathy, V. Cloning and Functional Expression of the Human Type 1 Sigma Receptor (hSigmaR1). *Biochem. Biophys. Res. Commun.* **1996**, *229*, 553–558.

(12) Aydar, E.; Palmer, C. P.; Klyachko, V. A.; Jackson, M. B. The Sigma Receptor as a Ligand-Regulated Auxiliary Potassium Channel Subunit. *Neuron* **2002**, *34*, 399–410.

(13) Polgar, O.; Robey, R. W.; Morisaki, K.; Dean, M.; Michejda, C.; Sauna, Z. E.; Ambudkar, S. V.; Tarasova, N.; Bates, S. E. Mutational Analysis of ABCG2: Role of the GXXXG Motif. *Biochemistry* **2004**, *43*, 9448–9456.

(14) Webster, D. M., Ed. Protein Structure Prediction: Methods and Protocols. *Methods in Molecular Biology*; Humana Press: Totowa, NJ, 2000; Vol. 143.

(15) Evers, A.; Gohlke, H.; Klebe, G. Ligand-supported Homology Modelling of Protein Binding Sites Using Knowledge-based Potentials. *J. Mol. Biol.* **2003**, *334*, 327–345.

(16) Evers, A.; Klebe, G. Ligand-supported Homology Modeling of G-protein-coupled Receptor Sites: Models Sufficient for Successful Virtual Screening. *Angew. Chem., Int. Ed.* **2004**, *43*, 248–251.

(17) Evers, A.; Klebe, G. Successful Virtual Screening for a Submicromolar Antagonist of the Neurokinin-1 Receptor Based on a Ligand-Supported Homology Model. *J. Med. Chem.* **2004**, *47*, 5381–5392.

(18) Evers, A.; Klabunde, T. Structure-based Drug Discovery Using GPCR Homology Modeling: Successful Virtual Screening for Antagonists of the Alpha1A Adrenergic Receptor. *J. Med. Chem.* **2005**, *48*, 1088–1097.

(19) Glennon, R. A.; Ablordepey, S. Y.; Ismael, A. M.; El-Ashmawy, M. B.; Fischer, J. B.; Burke Howie, K. Structural Features Important for Sigma-1 (σ_1) Receptor Binding. *J. Med. Chem.* **1994**, *37*, 1214–1219.

(20) Zampieri, D.; Mamolo, M. G.; Laurini, E.; Florio, C.; Zanette, C.; Fermeglia, M.; Posocco, P.; Paneni, M. S.; Pricl, S.; Vio, L. Synthesis, Biological Evaluation, and Three-dimensional in silico Pharmacophore Model for Sigma(1) Receptor Ligands Based on a Series of Substituted Benzo[d]oxazol-2(3H)-one Derivatives. *J. Med. Chem.* **2009**, *52*, 5380–5393.

(21) Laurini, E.; Zampieri, D.; Mamolo, M. G.; Vio, L.; Zanette, C.; Florio, C.; Posocco, P.; Fermeglia, M.; Pricl, S. A 3D-pharmacophore Model for Sigma2 Receptors Based on a Series of Substituted Benzo[d]oxazol-2(3H)-one Derivatives. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2954–2957.

(22) Zampieri, D.; Mamolo, M. G.; Laurini, E.; Zanette, C.; Florio, C.; Collina, S.; Rossi, D.; Azzolina, O.; Vio, L. Substituted benzo[d]oxazol-2(3H)-one derivatives with preference for the sigma(1) binding site. *Eur. J. Med. Chem.* **2009**, *44*, 124–130.

(23) Seth, P.; Ganapathy, M. E.; Conway, S. J.; Bridges, C. D.; Smith, S. B.; Casellas, P.; Ganapathy, V. Expression Pattern of the Type 1 Sigma Receptor in the Brain and Identity of Critical Anionic Amino Acid Residues in the Ligand-binding Domain of the Receptor. *Biochim. Biophys. Acta* **2001**, *1540*, 59–67.

(24) Srinivasan, J.; Cheatham, T. E., III; Cieplak, P.; Kollman, P. A.; Case, D. A. Continuum solvent studies of the stability of DNA, RNA and phosphoramidate-DNA helices. *J. Am. Chem. Soc.* **1998**, *120*, 9401–9409.

(25) Pal, A.; Fontanilla, A. R.; Ramachandran, S.; Chu, U. B.; Mavlyutov, T.; Ruoho, A. E. Identification of regions of the sigma-1 receptor ligand binding site using a novel photoprobe. *Mol. Pharmacol.* **2007**, *72*, 921–933.

(26) Pal, A.; Chu, U. B.; Ramachandran, S.; Grawoig, D.; Guo, L. W.; Hajipour, A. R.; Ruoho, A. E. Juxtaposition of the steroid binding domain-like I and II regions constitutes a ligand binding site in the σ_1 receptor. *J. Biol. Chem.* **2008**, *283*, 19646–19656.

(27) De Costa, B. R.; Bowen, W. D.; Hellewell, S. B.; Walker, J. M.; Thurkauf, A.; Jacobson, A. E.; Rice, K. C. Synthesis and evaluation of optically pure [3H]-(+)-pentazocine, a highly potent and selective radioligand for sigma receptors. *FEBS Lett.* **1989**, *251*, 53–58.

(28) Moebius, F. F.; Reiter, R. J.; Hanner, M.; Glossmann, H. High affinity of sigma 1-binding sites for sterol isomerization inhibitors: Evidence for a pharmacological relationship with the yeast sterol C8-C7 isomerase. *Br. J. Pharmacol.* **1997**, *121*, 1–6.

(29) Laggner, C.; Schieferer, C.; Fiechtner, B.; Poles, G.; Hoffmann, R. D.; Glossmann, H.; Langer, T.; Moebius, F. F. Discovery of high-affinity ligands of sigma1 receptor, ERG2, and emopamil binding protein by pharmacophore modeling and virtual screening. *J. Med. Chem.* **2005**, *48*, 4754–4764.

(30) Zampieri, D.; Mamolo, M. G.; Laurini, E.; Zanette, C.; Florio, C.; Collina, S.; Urbano, M.; Azzolina, O.; Vio, L. Synthesis, biological evaluation, and three-dimensional in silico pharmacophore model for sigma(1) receptor ligands based on a series of substituted benzo-[d]oxazol-2(3H)-one derivatives. *Eur. J. Med. Chem.* **2009**, *44*, 124–130.