Predictions of Binding for Dopamine D2 Receptor Antagonists by the SIE Method

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The control of tetralindiol derivative antagonists released through the inhibition of dopamine D2 receptors has been identified as a potential target for the treatment of schizophrenia. We employed molecular dynamics simulation techniques to identify the predicted D2 receptor structure. Homology models of the protein were developed on the basis of crystal structures of four receptor crystals. Compound docking revealed the possible binding mode. In addition, the docking analyses results indicate that five residues (Asp72, Val73, Cys76, Leu183, and Phe187) were responsible for the selectivity of the tetralindiol derivatives. Our molecular dynamics simulations were applied in combination with the solvated interaction energies (SIE) technique to predict the compounds' docking modes in the binding pocket of the D2 receptor. The simulations revealed satisfactory correlations between the calculated and experimental binding affinities of all seven tetralindiol derivative antagonists, as indicated by the obtained R^2 value of 0.815.

INTRODUCTION

The dopamine molecule has been associated with many physiological functions such as fine movement coordination, cognition, emotion, and memory by the pituitary reward system.[1] Alterations in dopaminergic function are involved in the pathogenesis of Parkinson's disease,[2] psychomotor diseases, and schizophrenia.[3] Dopamine receptors can be classified into two major subfamilies, D1 and D2 receptors, according to their pharmacological and domain functional characteristics.[4] Therefore, the dopamine receptor families have been targeted in the development of treatment medications for these disorders.

The dopamine D2 receptor is a G protein-coupled receptor located on postsynaptic dopaminergic neurons that is centrally involved in reward-mediating mesocorticolimbic pathways.[5] Signaling through D2 receptors governs physiologic functions related to locomotion, hormone production, and drug abuse. D2 receptors are also known targets of antipsychotic drugs used to treat psychomotor diseases such as schizophrenia, a debilitating mental illness that affects 0.5−1.5% of the worldwide population.[6] Although the biophysical and pharmacological properties of D2 receptors have been studied,[7] many problems remain unresolved due to the lack of three-dimensional structures and experimental limitations.

In recent years, a number of antagonists based on the cleavage of native substrates have been found, or designed, to inhibit D2 receptors.[1,4,8−18] For instance, stepholidine (SPD) isolated from the Chinese herb *Stephania* is a potential lead compound against D1 and D2 receptors. The pharmacophore of SPD molecules has been well studied in experiments[4] and molecular simulations.[19] We chose tetralindiol derivative antagonists for pharmacophore studies of D2 receptors as they possess the strong pharmacokinetic properties of D2 receptors.[12]

In the present study, tetralindiol derivative[8−18] compounds and their experimental biological binding affinities[8−18] (IC_{50}) were chosen to simulate D2 receptor pharmacological activities; these compounds are listed in Table 1. The transfer function[20] ($\Delta G_{bind} = -RT \ln(IC_{50})$) is used to transfer the IC_{50} values to the experimental ΔG_{bind} values; these experimental ΔG_{bind} values are listed in Table 5 in the Supporting Information. Considering that the crystal structures of D2 receptors and inhibitors are not available, homology modeling, molecular docking studies, molecular dynamics (MD) simulations, and binding free energy calculations were performed to gain further insight into the binding interactions between D2 receptors and a series of tetralindiol derivative inhibitors.

METHODS

Homology Modeling of Dopamine D2 Receptor. The dopamine D2 receptor sequence was collected from the National Center for Biotechnology Information (NCBI) protein database (ID: NP_000786).[21] A sequence similarity search for the D2 receptor against other protein databank sequences was performed using the NCBI BLAST server.[22] Four receptor structures were identified as homologous from the Protein Data Bank (PDB)[23−26] (Table 2). Automated sequence alignment (Figure 1) and template and target analyses were carried out using the Clustal X 2.09 program.[27] Meanwhile, the Modeler program package[28] was used to model a D2 receptor protein sequence. Four different template sequences were aligned with the D2 receptor sequence, and this alignment was supplied along with the 3D coordinates of the templates as an input to the program. The Modeler implements comparative protein structure

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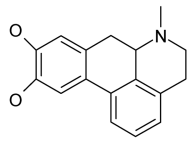
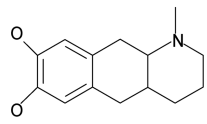
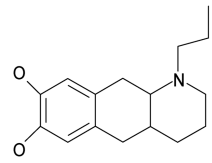
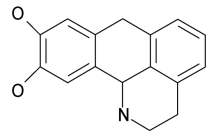
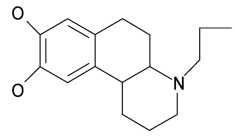
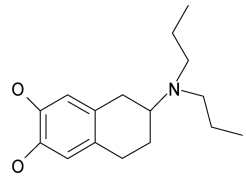
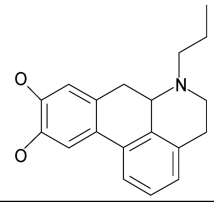
Table 1. Tetralindiol Derivative Antagonists

Compound	Structure	Molecular Weight	pIC_{50} (μM)
I		C17H17NO2, 267	4.73
II		C14H19NO2, 233	4.47
III		C16H23NO2, 261	4.77
IV		C16H15NO2, 253	6.00
V		C16H23NO2, 261	5.97
VI		C16H25NO2, 263	7.39
VII		C19H21NO2, 295	9.1

modeling by satisfying spatial restraints.[29,30] In order to obtain a relaxed conformation, the three models generated were initially subjected to an energy minimization process by using the conjugate gradient method for about 2000 iterations and to 2-ns isothermal, constant-volume MD simulation, with AMBER FF99 all-hydrogen amino acid parameters in the AMBER[31] program running on a LINUX server. To assess the quality of the minimized models, PROCHECK[32] analysis was also undertaken. Here, we considered two aspects in choosing the model: the "energy minima" and the optimum binding site to accommodate the D2 receptor inhibitors.

Molecular Docking. All tetralindiol derivative compounds were constructed and minimized using the SYBYL[33] modeling program. We aligned the model structure and the G protein-coupled receptor.[24] We then used the structure of the G protein-coupled receptor inhibitor (CUA: (2S)-1-(9H-carbazol-4-yloxy)-3-(isopropylamino) propan-2-ol) as a template to generate the receptor active site. The GOLD[34] program was used to dock all compounds into the active site of the model structure, which was defined as all residues within 10 Å of the aligned CUA inhibitor. This program, which makes use of hydrogen binding annealing parameters, GOLDscore, and a genetic algorithm, provides a fast and accurate means to dock small compounds into fixed protein binding sites. For every compound, 60 conformations were obtained from docking and then scored by the GOLDscore. The binding modes were identified after sorting the GOLDscore values, and the conformations of the best GOLDscores were selected for subsequent MD simulation.

Table 2. Templates Used for Dopamine D2 Receptor Homology Modeling

protein name	PDB ID	identity	resolution	reference
human beta2 adrenergic receptor	3D4S	28%	2.8 Å	11
human B2-adrenergic G protein-coupled receptor	2RH1	28%	2.4 Å	12
human B2-adrenergic G protein-coupled receptor	3EML	28%	2.6 Å	13
human beta2 adrenoceptor receptor	2R4S	33%	3.4 Å	14

Molecular Dynamics Simulation. Calculations were performed with the AMBER package using the AMBER FF99 all-hydrogen amino acid and general amber force field (GAFF) parameters. The GAFF partial atomic charges are often based on the RESP fitting procedure of the electrostatic potential obtained at the HF/6-31G(d) level of theory.[35,36] The B3LYP/6-31G(d,p) level of theory is also accurate and suitable for the electrostatic potential calculations of biomolecules and small organic molecules.[37−40] Those levels of theory overestimate the gas-phase partial atomic charges giving rise to effectively polarized force field. The geometries of the seven tetralindiol derivatives and dilauryl-phosphatidyl-ethanol-amine (DLPE) were fully optimized, and their electrostatic potentials were obtained using a single-point calculation; both operations were carried out at the B3LYP level with the 6-31G(d,p) basis set using the GAUSSIAN 03 program.[41] Subsequently, their partial charges were obtained by the restrained electrostatic potential (RESP) using Antechamber.[42] From the docking simulations, the complex structures were generated and then inserted into the solvents. The solvent molecules contain a DLPE lipid bilayer and water molecules. In addition, the initial compound II complex structures are shown in Figure 2. All MD simulations were performed in the canonical ensemble[43] (with a simulation temperature equal to 310 K), unless noted, using a leapfrog integrator, an integration time step of 0.002 ps, and SHAKE[44] data for all covalent bonds involving hydrogen atoms. In electrostatic interactions, atom-based truncation was undertaken using the PME method. In addition, the switch van der Waals functions were also used with a 1.8-nm cutoff for atom-pair lists. The complex structures were minimized for 50,000 conjugate gradient steps. The minimized complex structures were then subjected to a 5-ns isothermal, constant volume MD simulation. The final structures from these simulations were used to initiate the functionally important residues and the solvated interaction energies (SIE) calculations.

Functionally Important Residues. In a protein, every residue is not equally important. Some residues are essential

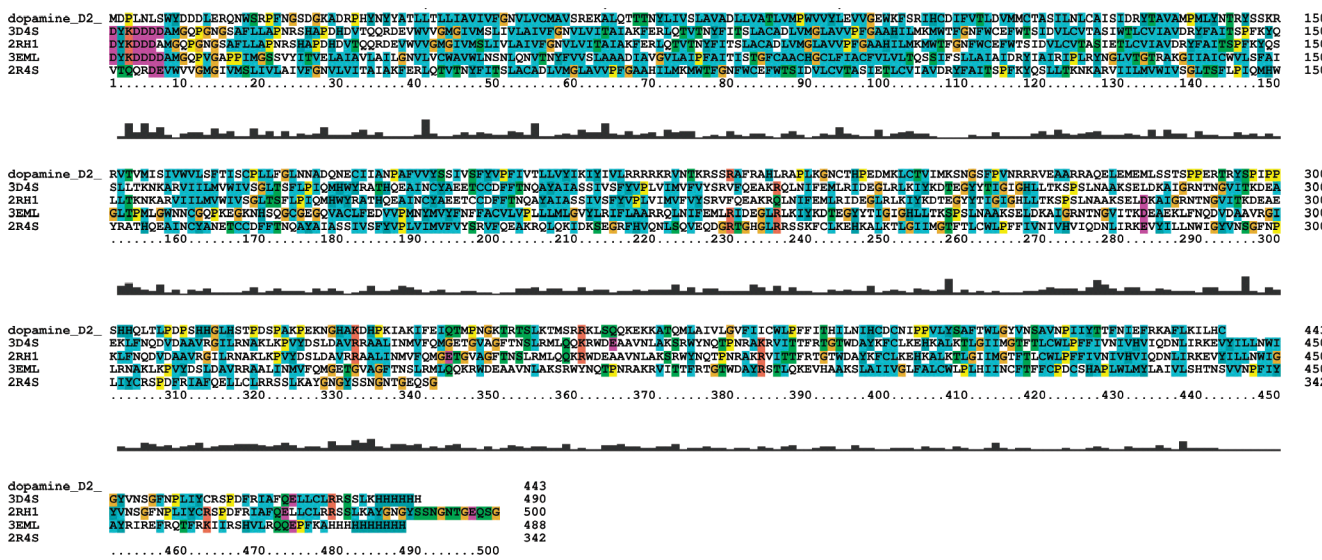


Figure 1. Dopamine D2 receptor (human) sequence aligned with four different template sequences: 3D4S, human beta2 adrenergic receptor; 2RH1, human B2-adrenergic G protein-coupled receptor; 3EML, human beta2 adrenergic receptor; 2R4S, human beta2 adrenergic receptor.

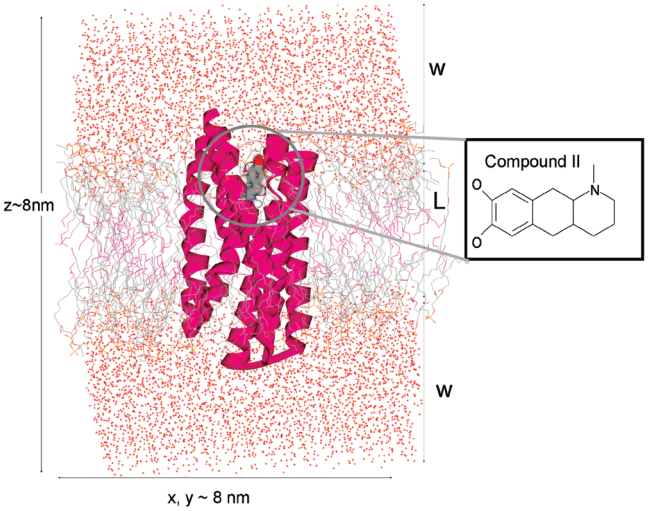


Figure 2. Overview of compound II—D2 receptor/DLPE/water system. The D2 receptor is shown in the ribbon; the hydrophobic chains of bilayer lipids are labeled L, and the regions containing water are labeled W. The 3-D size of the whole system is also shown. In the binding site, compound II is amplified and shown in a stick model.

Table 3. Data Reorient the GOLDscores by Docking the Seven Tetralindiol Derivatives Compounds into Two Homology Dopamine D2 Receptor Models

compound	model 1 (KJ/mol)	model 2 (KJ/mol)
I	NULL	–41.4052
II	NULL	–39.3230
III	NULL	–42.6992
IV	NULL	–41.6285
V	NULL	–37.3118
VI	NULL	–41.4983
VII	NULL	–44.8341

for important functions and structure stability and are known as conserved residues. However, others can be replaced by means of evolution. Identification of functionally important residues can provide a clear insight into the structural aspects of D2 receptors. In this work, the structure-based approach was applied to identify functionally important residues, while docking simulations were used to identify the residues involved in the binding pocket. From the earlier docking simulations, the important residues and pharmacophore regions were analyzed by the Ligandscout[45] program.

Solvated Interaction Energies (SIE) Method. The binding free energy calculations were performed by the SIE method. The SIE method is similar to the linear interaction energy (LIE) methods.[46−48] The binding free energies between receptor and inhibitors were calculated for snapshot structures taken from the MD trajectory of the system. From the 4000-ps complex-ligand MD trajectories of the D2 receptor and tetralindiol derivatives inhibitors, 50 snapshots were taken at regular intervals for the binding energy analyses. To provide error estimations for the free energies calculations, we calculated all the data based on the first 25 and last 25 snapshots. The average provided us with a result, and the difference between the two individual values and the average value yielded the estimated error value. The SIE[49] function to estimate protein−ligand free energy is written as

$$\Delta G_{bind}(\rho, D_{in}, \alpha, \gamma, C) = \alpha \times [E_c(D_{in}) + \Delta G^R_{bind}(\rho, D_{in}) + E_{vdw} + \gamma \Delta MSA(\rho)] + C \quad (1)$$

where E_C and E_{vdw} are the intermolecular Coulomb and van der Waals interaction energies in the bound state, respectively. These values were calculated using the AMBER molecular mechanics force field (FF99) with an optimized dielectric constant. ΔG^R_{bind} is the change in the reaction field energy between the bound and free states and is calculated by solving the Poisson equation with the boundary element method program, BRI BEM,[50,51] and using a molecular surface generated with a variable-radius solvent probe.[52] The ΔMSA term is the change in the molecular surface area upon binding. The following parameters are calibrated by fitting to the absolute binding free energies for a set of 99 protein−ligand complexes: AMBER van der Waals radii linear scaling coefficient (ρ), the solute interior dielectric constant (Din), the molecular surface area coefficient (γ), the global proportionality coefficient related to the loss of

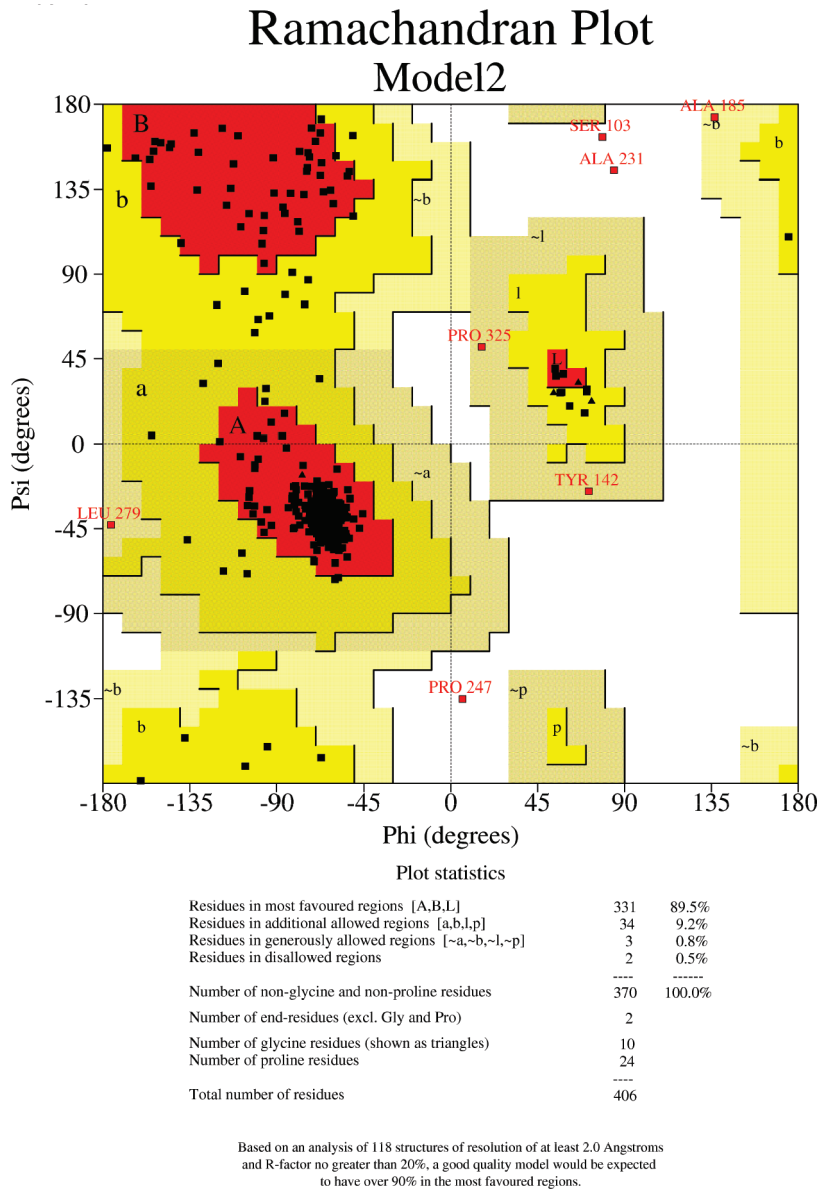


Figure 3. Ramachandran plot of D2 receptor model **2**.

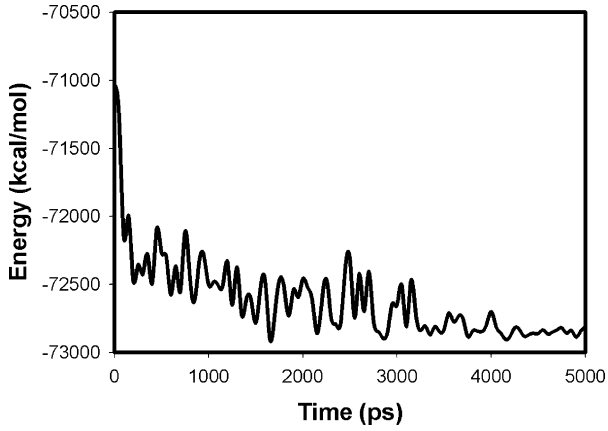


Figure 4. Potential energy plots for compound II—D2 receptor/DLPE/water system.

configurational entropy upon binding (α), and a constant (C).[46] The optimized values of these parameters are α = 0.1048, Din = 2.25, ρ = 1.1, γ = 0.0129 kcal/(mol Å^2), and C = −2.89 kcal/mol. The SIE calculations were carried out with the program sietraj.[53]

Table 4. Reoriented Data of the Important Residues of Model 2 (Dopamine D2 Receptor) from the Docking Simulations

compound	hydrogen bonding−related residues	nonbonding contact-related residues
I	Asp72	Val73
II	Trp60	Null
III	Thr188	Cys76, Met75, Tyr192
IV	Asp72	Val73, Cys76, Phe187
V	Cys76	His175, Leu183, Phe187
VI	Null	Val73, Cys76, Cys167, His175, Leu183, Phe187
VII	Asp72	Cys76, Leu122, Val130, Trp168, Phe171, His175, Leu183, Phe187

RESULTS AND DISCUSSION

Homology Model Selection, Molecular Docking, and MD Simulation. Initially, two protein models were generated—model 2 being considered the better model—on the basis of a least discrete optimized potential energy (DOPE) score of −23255; this score was found to be very close to that of the other model. The MD simulation (AMBER program) shows that the relaxed model has a DOPE score of −206.526 kcal/mol. Model 2 shows interactions with the seven compounds in the dopamine binding pocket without

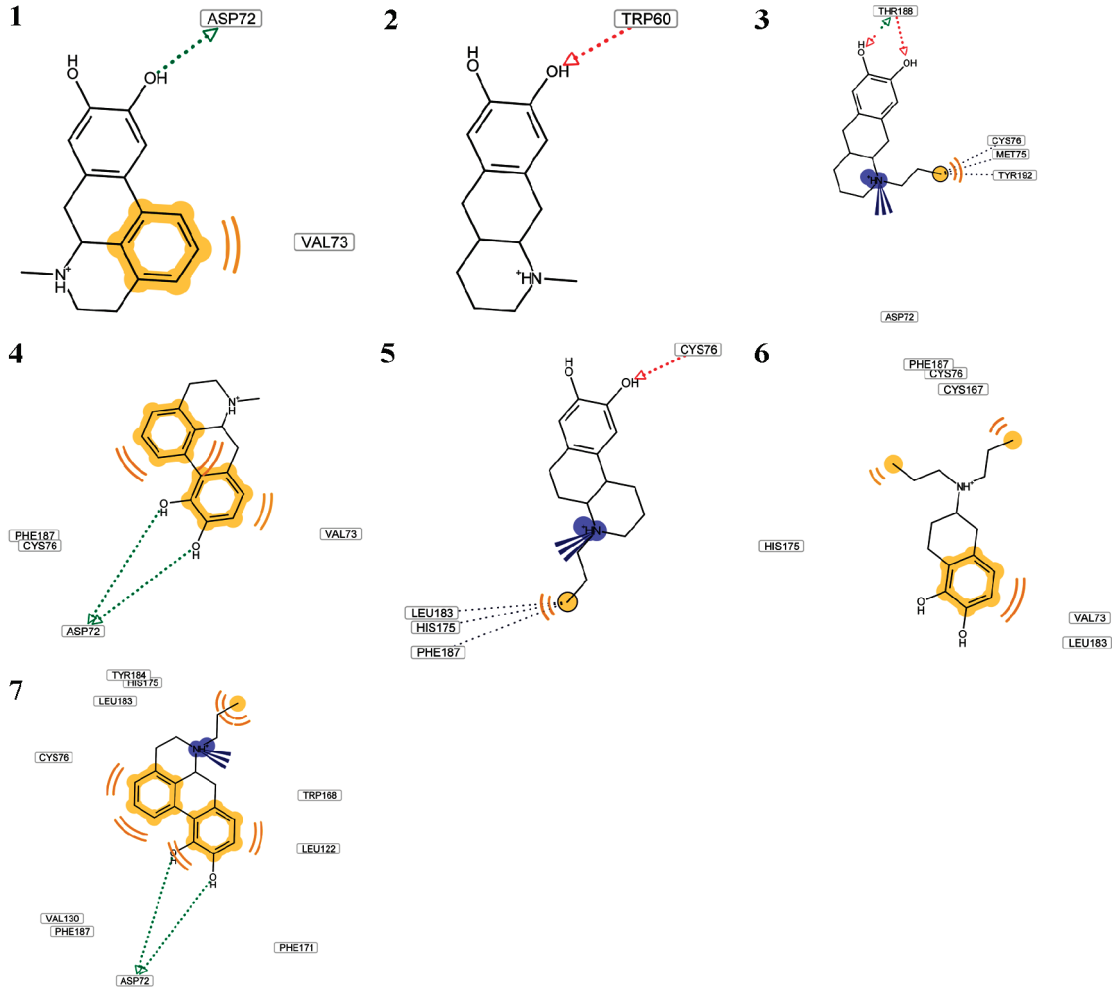


Figure 5. Analysis of the tetralindiol derivative binding modes. The green and red lines represent hydrogen binding modes. Yellow arcs represent nonbinding contacts.

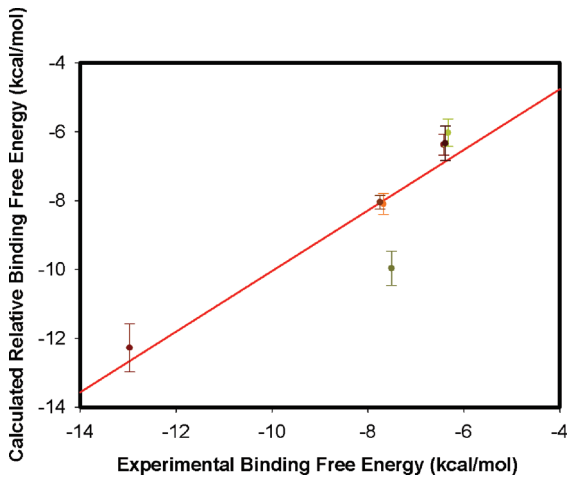


Figure 6. Calculated binding free energies (error bar) versus experimentally determined binding free energies of the series of seven compounds (each represented by a different colored symbol). The red line represents the regression line.

any docking constraints (Table 3), while model 1 does not show any interactions. Model 2 was also subjected to PROCHECK analysis to assess the stereochemical quality of the model. The Ramachandran plot (Figure 3) shows that 89.5% of residues were found in the most favored and additional allowed regions, 10.0% of the residues were in the generously allowed region, and 0.5% of residues in the disallowed region. The seven tetralindiol derivative compounds were then docked into model 2; Table 3 lists their best GOLD binding scores. Over the 5-ns MD trajectories of the D2 receptor with solvent molecules and tetralindiol derivatives, the overall structure of both complexes appeared to be equilibrated after 3500 ps. Here, we show the potential energy (Figure 4) of the complex system of compound I.

Key Residues of Dopamine D2 Receptors. Our studies of various known compounds have revealed that in the dopamine binding pocket residues can frequently interact with tetralindiol derivatives and that these residues are responsible for the selectivity of tetralindiol derivatives. The analysis results of our simulations are shown in Table 4 and Figure 5. For compound I, Asp72 forms a hydrogen bond with the compound, and Val73 has frequent nonbonding interactions with the compound. For compound II, Trp60 forms a hydrogen bond with the compound. For compound III, Thr188 forms a hydrogen bond with the compound, and the residues Cys76, Met75, and Tyr192 have frequent nonbonding interactions with the compound. For compound IV, Asp72 forms a hydrogen bond with the compound, and the residues Val73, Cys76, and Phe187 have frequent nonbonding interactions with the compound. For compound V, Cys76 forms a hydrogen bond with the compound, and the residues His175, Leu183, and Phe187 have frequent nonbonding interactions with the compound. For compound VI, the residues Val73, Cys76, Cys167, His175, Leu183, and

Phe187 have frequent nonbonding interactions with the compound. For compound VII, Asp72 forms a hydrogen bond with the compound, and the residues Cys76, Leu122, Val130, Trp168, Phe171, His175, Leu183, and Phe187 have frequent nonbonding interactions with the compound. The overall results of our simulations suggest that Trp60, Asp72, Cys76, and Thr188 play important roles in forming hydrogen bonds between D2 receptors and tetralindiol derivatives. Moreover, our simulations indicate that Val73, Met75, Cys76, Leu122, Val130, Cys167, Trp168, Phe171, Val173, His175, Leu183, Phe187, and Trp192 have frequent nonbonding interactions with the tetralindiol derivatives. While four residues (Trp60, Asp72, Cys76, and Thr188) were able to form hydrogen bonds between the receptors and the compounds, Asp72 formed the hydrogen bonds most frequently. In addition, Val73, Cys76, Leu183, and Phe187 often formed nonbonding interactions with the compounds. Therefore, our approach theoretically suggests that the five residues Asp72, Val73, Cys76, Leu183, and Phe187 are responsible for the selectivity of the tetralindiol derivatives.

Tetralindiol Derivatives Binding Free Energy. The complex structures of D2 receptors with the seven antagonists (compounds I−VII) and the solvent molecules (DLPE lipid bilayer and water molecules) were constructed using the GOLD docking program. The binding free energy of each complex was obtained from the MD simulation and the SIE method, with both processes using the same parameters. All the results are listed in Table 5 in the Supporting Information. In the simulations, the rank order of the predicted binding free energies of the seven compounds is in good agreement with the experimental results (Figure 6), with the correlation coefficient between the predicted and experimental energies being 0.815. This further verifies the predicted D2 receptor structure (model 2) and our docking simulations.

CONCLUSION

In this study, we used GOLD, solvent molecules (DLPE lipid bilayer and water molecules), MD simulations, and the SIE method to predict a dopamine D2 receptor structure and a docking model in which a series of tetralindiol derivative antagonists interact with the D2 receptor. In our simulation model, the correlation coefficient between the predicted binding free energies and experimental values of the seven antagonists is 0.815. The Asp72 residue frequently formed hydrogen bonds with the antagonists, and the four residues Val73, Cys76, Leu183, and Phe187 frequently formed nonbonding interactions with the compounds. Therefore, our approach theoretically suggests that the five residues Asp72, Val73, Cys76, Leu183, and Phe187 and the predicted D2 receptor structure can be used as a novel model for an antidopamine D2 receptor to further reconstruct and design new protease antagonists.

ACKNOWLEDGMENT

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Supporting Information Available: Actual versus predicted data for the tetralindiol derivative antagonists (Table 5). This material is available free of charge via the Internet at http://pubs.acs.org.

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