ISSN 1672-9145 **Acta Biochimica et Biophysica Sinica** 2007, **39**(6): 413–422 **CN** 31-1940/Q

Pharmacophore-directed Homology Modeling and Molecular Dynamics Simulation of G Protein-coupled Receptor: Study of Possible Binding Modes of 5-HT_{2C} Receptor Agonists

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Abstract A new pharmacophore-based modeling procedure, including homology modeling, pharmacophore study, flexible molecular docking, and long-time molecular dynamics (MD) simulations, was employed to construct the structure of the human 5-HT_{2C} receptor and determine the characteristics of binding modes of 5-HT_{2C} receptor agonists. An agonist-receptor complex has been constructed based on homology modeling and a pharmacophore hypothesis model based on some high active compounds. Then MD simulations of the ligand-receptor complex in an explicit membrane environment were carried out. The conformation of the 5-HT_{2C} receptor during MD simulation was explored, and the stable binding modes of the studied agonist were determined. Flexible molecular docking of several structurally diverse agonists of the human 5-HT_{2C} receptor was carried out, and the general binding modes of these agonists were investigated. According to the models presented in this work and the results of Flexi-Dock, the involvement of the amino acid residues Asp134, Ser138, Asn210, Asn331, Tyr358, Ile131, Ser132, Val135, Thr139, Ile189, Val202, Val208, Leu209, Phe214, Val215, Gly218, Ser219, Phe223, Trp324, Phe327, and Phe328 in agonist recognition was studied. The obtained binding modes of the human 5-HT_{2C} receptor agonists have good agreement with the site-directed mutagenesis data and other studies.

Keywords 5-HT_{2C} receptor; Flexi-Dock; G protein-coupled receptor; homology modeling; molecular dynamics simulation

5-Hydroxytryptamine (5-HT; serotonin) is a major neurotransmitter that is thought to be involved in many central nervous system processes including feeding, anxiety, aggression, sexual behavior, mood and pain [1–3]. 5-HT exerts its physiological effects by an action on a diverse family of cell surface receptor proteins. The 5-HT_{2C} receptor is one of the serotonin receptors belonging to a large group of seven transmembrane (TM)-spanning G protein-coupled receptors (GPCRs). Direct and indirect evidence from clinical studies suggest that 5-HT_{2C} receptor agonists can effectively resist obesity without inducing any tolerance [4–7]. Although approximately half of all marketed drug targets are GPCRs [8], there are no selective 5-HT_{2C} receptor agonists developed and marketed for the treatment of obesity [4]. Because of the huge market in this area, tremendous interest has been attracted to research and development of anti-obesity drugs.

Generally, the structure of the human 5-HT_{2C} receptor could be used as a starting point for the receptor structure-based design of new agonists. However, like other GPCRs belonging to the rhodopsin family, the 3-D struc-

Received: February 5, 2007 Accepted: March 15, 2007
This work was supported by the grants from Shanghai Key R&D Program (No. 036505003), the National Key R&D Program (2005BA711A04), and the Major State Basic Research Development Program of China (2004CB518901)
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DOI: 10.1111/j.1745-7270.2007.00295.x

ture of the receptor is not easily amenable to determination by either X-ray crystallography or nuclear magnetic resonance spectroscopy. Therefore, the 3-D structure of bovine rhodopsin [9,10] serves as a template to construct 3-D models for other GPCRs [11]. But the low sequence similarity among GPCRs makes the homology modeling imperfect or questionable.

In this study, an integrated strategy, including homology modeling, pharmacophore study, long-time molecular dynamics (MD) simulations in an explicit membrane environment, and Flexi-Dock, was employed for constructing a reliable 3-D model of the 5-HT_{2C} receptor, and determining the features of the binding modes of 5-HT_{2C} receptor agonists. Under the guidance of a pharmacophore derived from published agonists, a complex of the receptor with an agonist was established based on a 3-D model of the 5-HT_{2C} receptor from homology modeling. Then long-time MD simulation was carried out on the complex that was placed in a phospholipid bilayer and solvated in solvent water, to find the most probable structure of the complex. Finally, a series of agonists were docked into the final structure of the 5-HT_{2C} receptor to explore the binding mode between the 5-HT_{2C} receptor and its agonist. The obtained binding mode is in good agreement with the site-directed mutagenesis data and other studies. Therefore, this binding mode should be helpful in determining the roles of the key residues involved in agonist binding, and useful in discovering potential agonists for the 5-HT_{2C} receptor.

Materials and Methods

Molecular modeling of 5-HT_{2C} receptor

Modeling of the α-helix bundle, extracellular and intracellular regions were carried out sequentially.

The sequence of the 458 amino acids of the human 5-HT_{2C} receptor was taken from the Swiss-Prot Database (entry P28335; http://us.expasy.org/sprot/). The crystal structure of bovine rhodopsin at 2.80 Å resolution (PDB entry 1F88) [9] from the Protein Data Bank [12] was used as a template, the Homology module of InsightII (version 2000; Molecular Simulation Inc., San Diego, USA) and the ClustalW algorithm [13] were applied in sequence alignment, and the Blosum scoring matrix [14] was employed to obtain the best-fit alignment. The best alignment was selected according to not only the value of the alignment score, but also the reciprocal positions of conserved residues.

The sequence alignment results carried out by the ClustalW algorithm [13] are depicted in **Fig. 1**. The sequence identity between rhodopsin and the 5-HT_{2C}

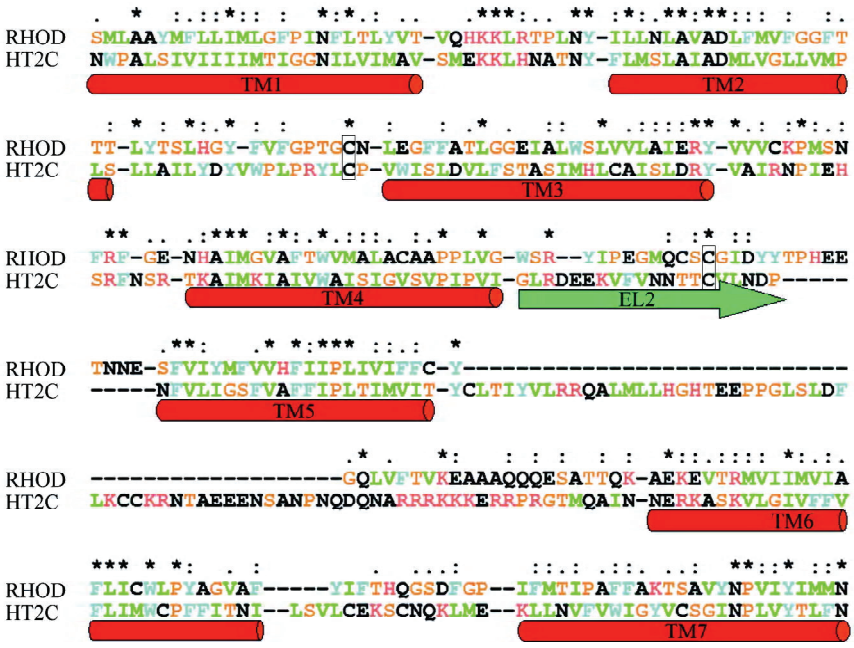


Fig. 1 Sequence alignment of 5-HT_{2C} (HT2C) with rhodopsin (RHOD; 1F88) generated by the ClustalW algorithm

In the sequences, an asterisk (*) indicates an identical or conserved residue, a colon (:) indicates a conserved substitution, a stop (.) indicates a semi-conserved substitution. EL, extracellular loop; TM, transmembrane.

receptor is 20.5% except at the N-terminus and C-terminus.

The conserved disulfide bond between residues Cys127 at the beginning of TM3 and Cys207 in the middle of extracellular loop 2 was also created and kept as a constraint in the geometric optimization. The N-terminus, C-terminus and intracellular loop CL-III were not modeled as they are not directly involved in the binding of agonists, and poor sequence conservation exists in this region within the GPCR family [15].

The resultant structure of the 5-HT_{2C} receptor was optimized using molecular mechanics methods with the following parameters: a distance-dependent dielectric constant of 1.0; non-bonded cutoff 8 Å, Amber force field [16] and Kollman all-atom charges; and conjugate gradient minimization until 0.05 kcal/(mol·Å). The minimized structure was validated using PROCHECK [17].

3-D pharmacophore study

A common set of distances among the hypothetical pharmacophoric points was constructed by the DISCO module of SYBYL 7.1 (Tripos Association, St. Louis, USA). The initial structures of these compounds were built and energetically minimized using the Tripos force field with Gasteiger-Hückel charges [18]. The classical procedure of DISCO was followed: (1) combining conformer databases to create a molecular spreadsheet; (2) assigning features to the rows automatically; (3) moving features to the conformers; (4) scanning the reference compound 6; (5) admitting between three and seven points of feature requirements; (6) computing the model; and (7) examining and analyzing the results spreadsheet.

MD simulation

Nowadays, the commonly accepted method of carrying out MD simulations of membrane proteins is the use of the phospholipid bilayer solvated by water, to provide the optimum environment [19–22].

A pre-equilibrated 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) bilayer with 128 lipid molecules was obtained from Dr. TIELEMAN (http://moose.bio.ucalgary.ca/; University of Calgary, Calgary, Canada), then the GROMACS 3.1.4 package [23] and an in-house program were used to generate and equilibrate the POPC bilayer with 272 lipid molecules. The protein was embedded in the pre-equilibrated POPC lipid bilayer using similar procedures to those used in our previous work [22]. A cylindrical hole was made in the center of the bilayer by removing lipids whose P atoms fell within 2.0 Å of the 5-HT_{2C} receptor. A short MD simulation with a radially acting repulsive force was used to drive any remaining atoms out of the cylinder and into the bilayer. As shown in **Fig. 2**, the protein was oriented with the main axis along the Z-axis. In the 5-HT_{2C} receptor, Arg and Lys concentrate at the cytoplasmic boundary of membrane proteins, where they interact with the lipid at the level of the phospholipids headgroups [24]. In our model, the positively charged side chains of the Arg and Lys residues in the 5-HT_{2C} receptor are positioned at the level of the phospholipid headgroups. Thus, the TM helical domains reside in a complex environment consisting of three distinct phases: a hydrophobic core of the membrane defined by the phospholipids

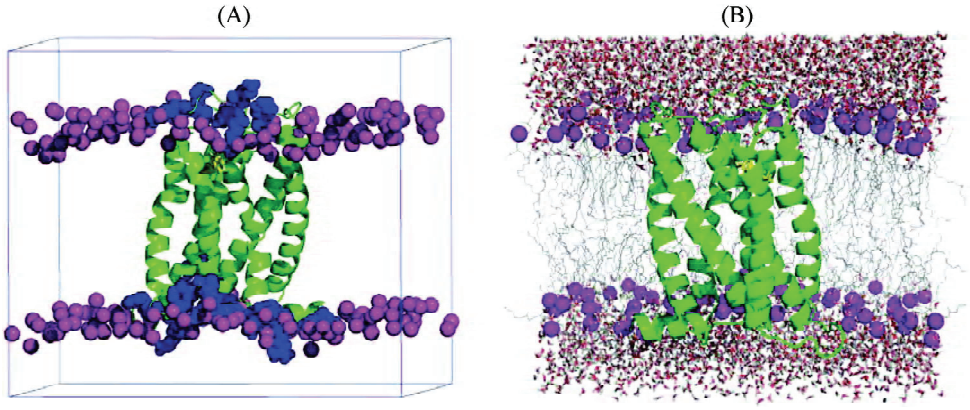


Fig. 2 3-D structure of molecular dynamics simulations model

(A) A 3-D molecular representation of the distribution of the charged residues in the 5-HT_{2C} receptor. The side chains of Arg and Lys are shown in magenta spheres, which are mainly located in the lipid headgroup layer near the intracellular region; the side chains of Asp and Glu are shown in blue spheres, which are mainly located in the lipid headgroup layer near the extracelluar region. These residues interact with the lipid at the level of the phospholipids headgroup. To represent the membrane bilayer, P atoms in lipid are shown in salmon spheres. (B) Overview of the 5-HT_{2C}/POPC/water simulation systems, with the dimensions and direction of the applied electrical field. The protein is shown in cartoon format, and the P atoms are shown in magenta spheres. Compound 6 is colored yellow in the cluster of helices.

chains; a mixed hydrophobic-hydrophilic region comprised of the phospholipids headgroups; and the aqueous cytoplasm.

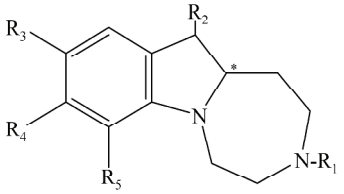
Before starting MD simulations, the pKa was calculated to determine if any of the residues in the receptors were likely to adopt non-standard ionization states. The surface-accessibility-modified Tanford-Kirkwood method of Matthew [25,26] encoded in MacroDox version 3.2.2 [27] was employed to determine the protonation status of each titratable residue in the receptor at body pH value. The MD on 5-HT_{2C}-agonist complex in a POPC/water environment was carried out with the parallel version of the MD program GROMACS 3.1.4 [23], using constant number of particles, pressure and temperature (NPT) and periodic boundary conditions. The LINCS method [28] was used to constrain bond lengths, allowing an integration step of 2 fs. Electrostatic interactions were calculated with the particle mesh Ewald algorithm [29,30]. A constant pressure of 1 bar was applied independently in X, Y and Z directions of the whole system with a coupling constant of 1.0 ps. Water, lipids and protein were coupled separately to a temperature bath at 300 K with a coupling time of 0.1 ps using a Berendsen thermostat [31]. The values of the semi-isothermal compressibility were set to 4.5×10^{-5} bar^{-1} for water simulations. The molecular topology for agonist 6 was generated by PRODRG (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/prodrg.html) [32]. The atomic charges of agonist 6 were assigned using the CHELPG procedure at the Hartree-Fock level with a 6-311G* basis set [33].

MD simulations were carried out using the software package GROMACS 3.1.4 [23]. The GROMACS force field was applied for protein and the lipid parameters adopted were those used in previous MD studies of lipid bilayers [22,34].

Molecular docking

To further validate the 3-D model of the activated conformation of the 5-HT_{2C} receptor, all agonists in **Table 1** were docked into the binding pocket after MD simulation. As the ligands might interact with the receptor in different and diverse ways, even among homologous ligands acting

Table 1 Structure and activity of 5-HT_{2C} receptor agonists



	R_1	R_2	R_3	R_4	R_5	Ki (nm)
1(+)–	H	H	H	H	2-EtOPh	103
2(–)–	H	H	H	H	2-EtOPh	196
3	H	H	H	H	2-CLPh	23
4(+)–	H	H	H	H	2-MePh	30
5(–)–	H	H	H	H	2-MePh	39
6(+)–	H	H	H	H	2-CF_3Ph	14
7(–)–	H	H	H	H	2-CF_3Ph	59
8(+)–	H	H	CL	H	2-EtOPh	32
9(–)–	H	H	CL	H	2-EtOPh	178
10	H	H	CL	H	3-EtOPh	472
11	H	H	CL	H	4-EtOPh	265
12	H	H	CL	H	2-MePh	43
13	H	H	CL	H	2-CLPh	57
14	H	H	CL	H	2-FPh	223
15	H	H	CL	H	4-FPh	310
16	H	H	CL	H	2,4-diCLPh	386
17	H	H	CL	H	2,5-diCLPh	160
18	H	H	CL	H	5-CL-2MeOPh	793

at homologous receptors [35,36], the flexible docking was carried out using the Flexi-Dock unity in the Biopolymer module of SYBYL 7.1 to determine the most energetically favorable binding conformation and orientation. After the hydrogen atoms were added to the receptor, atomic charges were recalculated using Kollman all-atom for the protein and Gasteiger-Hückel for ligands. H-bonding sites were marked for all residues in the binding pocket and for ligands that were able to act as H-bond donors or acceptors. Ligands were manually pre-positioned in the putative binding pocket as a starting point for Flexi-Dock. During the flexible docking, not only the ligands were defined with rotatable bonds, but the bonds in the binding pocket of the 5-HT_{2C} receptor were also defined as rotatable. Genetic algorithms were set at 30,000 generations. Finally, the energy minimization of the obtained protein-ligand complexes was carried out by Amber force field [16] with a distance-dependent dielectric constant of 5.0, until the conjugate gradient reached 0.05 kcal/(mol·Å).

Results and Discussion

Homology modeling

The molecular mechanism by which agonists bind to the human 5-HT_{2C} receptor remains a central unresolved problem for biochemists and pharmacologists, because of the absence of clear structural data. For this reason, the homology modeling of the human 5-HT_{2C} receptor was carried out using bovine rhodopsin as the template [9]. In order to describe the structure clearly, the TM segments from all seven subunits were designed as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. It is well known that all rhodopsin-like receptors consist of seven TM α-helices with the same arrangement connected by hydrophilic loops. So the hydrophobic α-helices of the 5-HT_{2C} receptor were constructed by homology modeling based on the crystal structure of rhodopsin. As some fragments of the intracellular loops of the rhodopsin are missing in the crystal structure, the configuration of the hydrophilic loops of the 5-HT_{2C} receptor was determined using the LOOP-SEARCH utility in the Homology module of InsightII [22], taking into account both the root mean square values of the top 10 candidates from the default loop database and geometrical compatibility with other parts of the receptor.

In the procedure of modeling the hydrophilic loops, a key point is the formation of the highly conserved disulfide bond between the Cys127 at the beginning of TM3 and the Cys207 in extracellular loop 2. The modeled 3-D structure of the 5-HT_{2C} receptor is shown in **Fig. 3**.

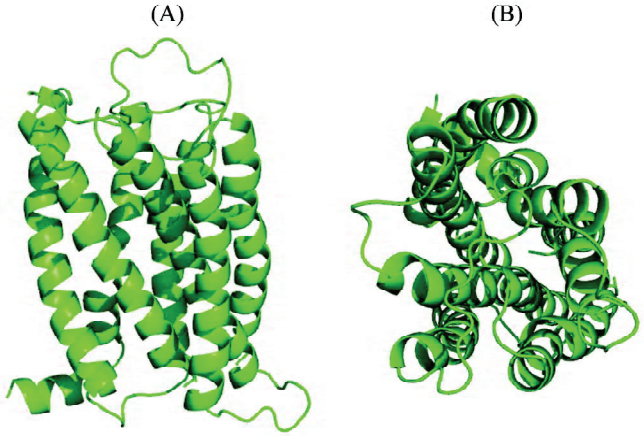


Fig. 3 Molecular model of the human 5-HT_{2C} receptor
(A) Side view. (B) Top view.

The Ramachandran plot by PROCHECK [17] analysis indicated that approximately 94% of residues in the 5-HT_{2C} receptor model are in either the most favored or in the additionally allowed regions, suggesting that the constructed 3-D model of the 5-HT_{2C} receptor should be reliable and could be used for further studies.

Pharmacophore hypothesis and binding pocket

The template (bovine rhodopsin) used in the homology modeling was crystallized with a ground conformation different from that of the active state [9]. It is suitable for the binding of antagonists, but not accurate enough for retrieving known agonists [37]. Therefore, the model of the 5-HT_{2C} receptor based on this crystal structure would be the inactive form rather than the agonist bound form. To build a topographical model of the agonist binding conformation, 18 agonists of the 5-HT_{2C} receptor from published reports [38] with high activities and common chemical features were used to generate an agonist-based pharmacophore model (**Table 1**) by the DISCO module of SYBYL 7.1. Four hypothetical pharmacophore elements, including three aromatic points and one hydrogen bond point, were obtained, as shown in **Fig. 4(A)** (annotated with a, b, c and d). This pharmacophore model was used to calibrate the 3-D structure of the constructed structure of the 5-HT_{2C} receptor.

The possible binding site of the receptor for the agonist was identified on the extracellular side of the TM domain and partly covered by the second extracellular loop, through docking of the pharmacophoric elements and taking into account some published data about the position of the agonist binding site [37,39–48]. The cavity is mainly com-

posed of the conserved residues of Asp134, Phe223, Trp324, Phe327, Phe328, Val354, and Tyr358 on the helix TM3, TM5, TM6 and TM7. The site can be divided into two parts with different properties: the area near helix of TM3 and TM7 that is hydrophilic; and the other near TM5 and TM6 that is hydrophobic. In the binding pocket, the agonists interact with these areas by hydrogen-bonding and hydrophobic interactions, respectively. To give a clear representation of these interactions, a schematic picture is given in **Fig. 4(B)**.

MD simulation

With the identified binding site, the most active agonist (compound 6; **Table 1**) was manually docked into the receptor. In the binding pocket, the aromatic end of agonist 6 was put into the hydrophobic cave, and the head of the agonist with a nitrogen points to the hydrophilic cave. During this process, some residues of the 5-HT_{2C} receptor, especially the angles of the side chains of the residues lining the binding pocket, were modified manually to avoid unacceptably steric contacts with the ligand. This is understandable as the conformation of the preliminary 5-HT_{2C} receptor model was constructed based on the crystal structure of rhodopsin with an inactive form. This initial structure was then subjected to geometrical optimization and simulation by the MD method.

To generate a correct model of an activated state, the complex of the 5-HT_{2C} receptor and compound 6 was minimized using molecular mechanics followed by long-time MD simulation in the real phospholipid bilayer/water environment. As shown in **Fig. 2**, the complex was placed into the water-solvated phospholipid bilayer. Single-point charged (SPC) was used as a water model [31], which has been shown to behave well in lipid bilayer/water simulations [49,50]. In order to provide a neutral simulation system, 7 Cl^- ions replaced water molecules at the positions of low Coulomb potential in 5-HT_{2C}/agonist/POPC/water systems. Lastly, the MD simulation system contains 241 POPC lipids, 8007 water molecules, and 7 Cl^-, giving a total of 39,317 atoms in a box of dimensions 8.8×8.8×7.7 nm^3. The whole MD simulation system was set up as described above.

The potential energy of the whole system and heavy atom root mean square deviation (RMSD) from the starting structure are important criteria for the convergence of the free MD simulations. As is shown in **Fig. 5**, the potential energy of the whole system under study became stable at approximately -6.365×10^5 KJ/mol after approximately 2.0 ns of the MD simulation, and the RMSD with respect to the starting structure reached stability, with an RMSD value of 0.25 nm, also after approximately 2.0 ns. These results suggested that a relatively stable conformation of

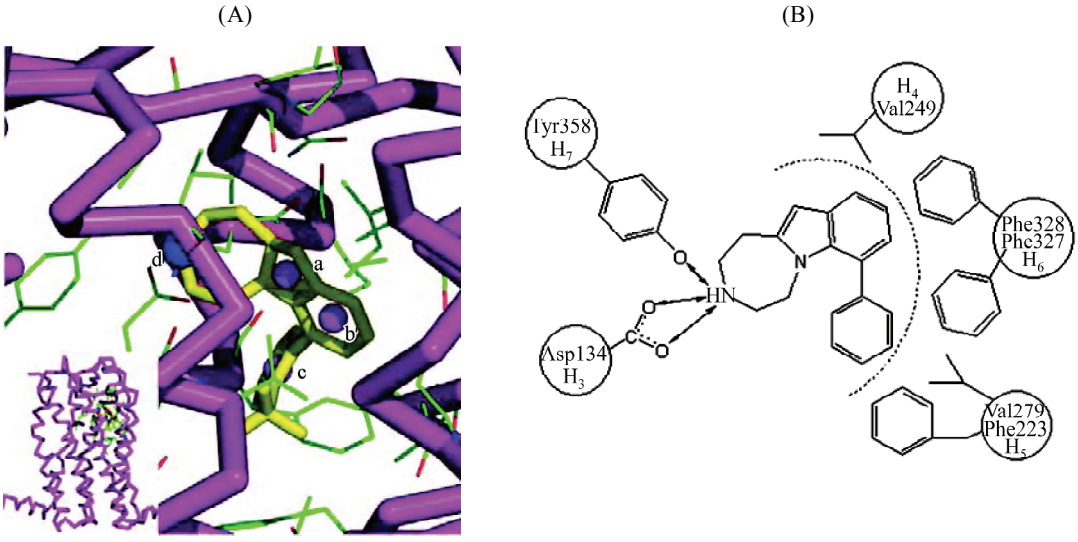


Fig. 4 5-HT_{2C} receptor and its agonist

(A) An agonist with pharmacophore hypotheses from DISCO is docked to the possible binding pocket of the receptor. The binding position of the agonist in the receptor is indicated by the inset picture (bottom left). Two major kinds of interaction features are revealed from the model: aromatic (three magenta points, a–c); and hydrogen-bonded (blue point, D). (B) Schematic representation of postulated interactions of the 5-HT_{2C} receptor with its agonist skeleton. The possible hydrogen bond interactions between receptor and agonist are indicated as arrows, and hydrophobic interactions are depicted as a dashed arc line.

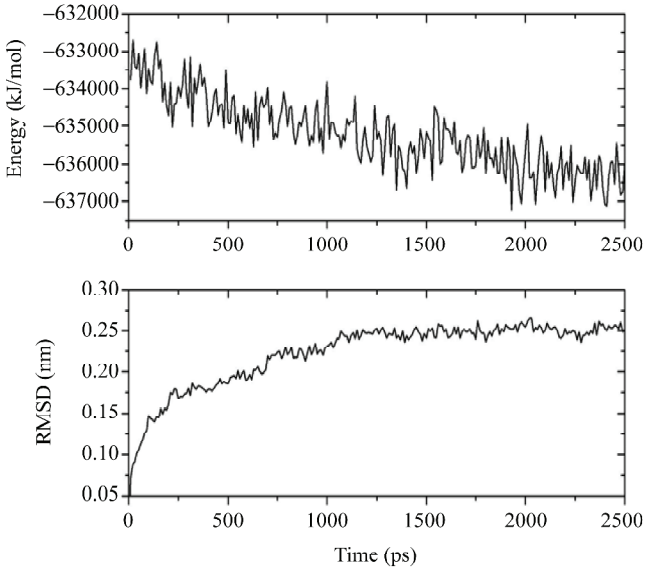


Fig. 5 Time-dependences of potential energy of the 5-HT$_{2C}$ receptor and Cα root mean square deviation (RMSD) with respect to the starting structure along the molecular dynamics simulation

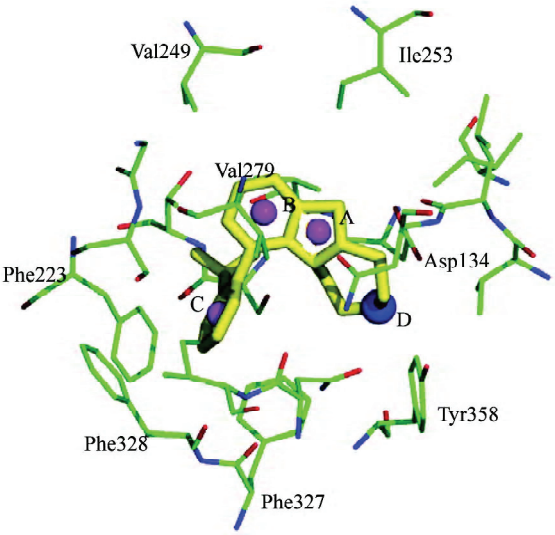


Fig. 6 3-D view of the binding mode of the 5-HT$_{2C}$ receptor and the agonist compound 6 with pharmacophore elements from the DISCO study

the 5-HT$_{2C}$ receptor was discovered through the MD simulation.

In order to clearly compare the binding mode with the pharmacophore hypothesis, the four pharmacophore elements, including three aromatic elements (A, B and C) and one hydrogen-bonding element (D), with agonist 6 and some key residues within the distance of 5 Å of the agonist were depicted (**Fig. 6**), which were extracted from last snapshot of the MD simulation. It can be seen from **Fig. 6**, after the long-time MD simulation, the hydrogen-bonding element (D) was positioned to the residues of Asp134 and Tyr358, and the aromatic elements (A, B and C) point to hydrophobic parts near TM5 and TM6. The characteristic of the interaction between the agonist and the 5-HT$_{2C}$ receptor agrees well with our pharmacophore hypothesis established by the above DISCO study. A comparison between the model after MD simulation, which was built based on the pharmacophore hypothesis, and the model from conventional homology modeling was also made. We found that the binding site of pharmacore-directed modeling was larger than the conventional one and the conformation of some residues was different.

Molecular docking

To further explore the common characteristics of agonist binding, all the agonists listed above were flexi-docked into the binding pocket of the 5-HT$_{2C}$ receptor with the active conformation after MD simulation. Geometrical optimization with molecular mechanics was carried out for the docked complexes. Then the interaction between the docked agonists and the 5-HT$_{2C}$ receptor in each complex was studied by LIGPLOT [51]. The 2-D representatives of the interaction models of all complexes were studied by LIGPLOT. Four representative pictures of the 2-D interaction models are shown in **Fig. 7**. We found that: agonist 2 and 9 might have hydrogen-bond interactions with the residue of Ser138; agonist 7 interacted with Asp134 by hydrogen bond; agonist 11 might interact with Asn210, Asn331 and Tyr358; and the rest of the agonists might have hydrogen-bond interactions with the residue of Tyr358. The obtained results of the molecular docking suggested that five amino acid residues of the receptor, that is, Asp134, Ser138, Asn210, Asn331, and Tyr358, might be hydrogen-bonded with the agonists. The residue of Tyr358 is the most popular because it provides an important hydrogen-bond interaction to the agonists. The amino acid residues of Ile131, Ser132, Val135, Thr139, Ile189, Val202, Val208, Leu209, Phe214, Val215, Gly218, Ser219, Phe223, Trp324, Phe327, and Phe328 form a suitable hydrophobic pocket between TM3, TM5, TM6, and TM7 to host the hydrophobic moiety of the agonists.

The experiments of Choudhary *et al.* [48] found that the mutation of phenylalanines to leucine in TM6 impact markedly on the binding of ligands. The involvement of the highly conserved aspartic acids of TM3 in the binding

of some ligands was shown by Wang *et al.* [47] using an aspartic acid to asparagines mutant. The work of Roth *et al.* [44] has shown that mutation of the completely conserved tyrosine in TM7 had significant effects on the binding of some ligands to the 5-HT_{2A} receptor. As depicted in the homology modeling study by Bissantz *et al.* [37], the agonist binding pockets of different D3 receptors and β2 receptors are mainly composed of aspartic acid in TM3, two residues of phenylalanine in TM6, and some residues of serine in TM5. Our binding modes established by ligand-based homology modeling and MD simulations were found to be in close agreement with the results from these studies.

Conclusions

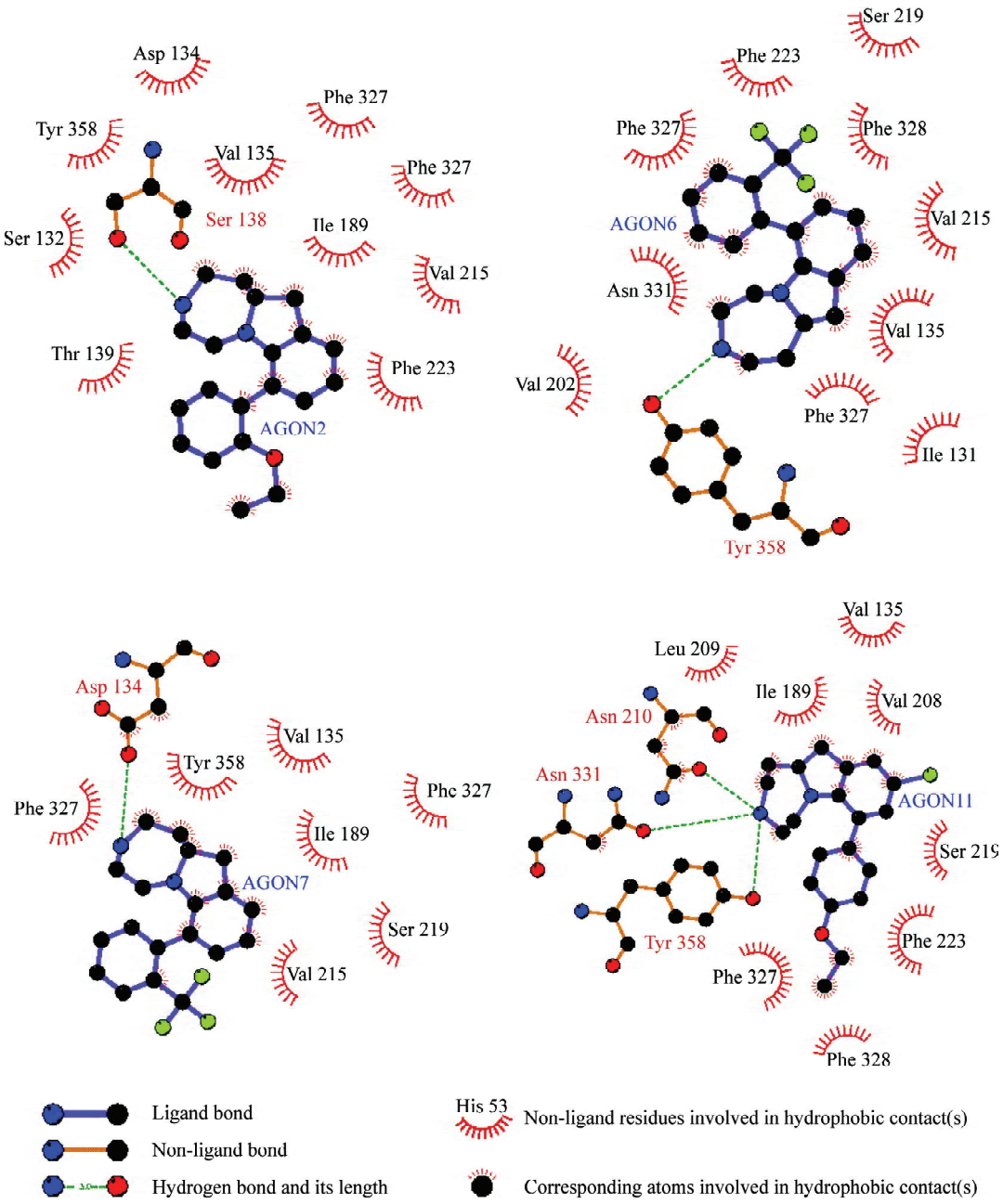


Fig. 7 2-D representative illustrations of the interaction model of agonists (AGON) with the 5-HT_{2C} receptor

The distance between the donor and acceptor of less than 3.4 Å is considered as a hydrogen bond, and a 4.1 Å distance between two hydrophobic atoms is considered to be a hydrophobic interaction. Illustrations generated by the LIGPLOT program[51].

Although the availability of high-resolution structural information for GPCRs has opened new vistas for molecular modeling of membrane proteins, homology modeling can not give a perfect structure alone for agonist binding because the conformation of the template is an inactive one. Therefore, other methods are needed to explore the binding mode of 5-HT_{2C} receptor agonists.

The molecular modeling and MD simulations presented in this work provide the first detailed study of the human 5-HT_{2C} receptor structure inserted into the phospholipid bilayer. A molecular model of the human 5-HT_{2C} receptor was created by homology modeling in this work. A reasonable and valuable pharmacophore hypothesis model was constructed based on some high active agonists by DISCO. MD simulations of the complex of the 5-HT_{2C} receptor with an agonist in an explicit membrane environment were carried out. The flexible molecular docking of the agonists of the 5-HT_{2C} receptor was carried out. The results of MD simulations and molecular docking allowed us to explore the binding mode of these agonists and to determine the amino acid residues involved in the recognition of 5-HT_{2C} receptor agonists. The amino acid residues of Asp134, Ser138, Asn210, Asn331, and Tyr358 are mainly in TM3 and TM7 hydrogen-bonded with agonists. Some aromatic residues and hydrophobic residues of Ile131, Ser132, Val135, Thr139, Ile189, Val202, Val208, Leu209, Phe214, Val215, Gly218, Ser219, Phe223, Trp324, Phe327, and Phe328 are involved in ligand recognition by the means of hydrophobic interaction or π-π interaction. The obtained binding modes of the 5-HT_{2C} receptor agonists are in agreement with studies by others [37,44,47, 48].

References

1 Roth BL. Multiple serotonin receptors: Clinical and experimental aspects. Ann Clin Psychiatry 1994, 6: 67–78

2 Roth BL, Shapiro DA. Insights into the structure and function of 5-HT_2 family serotonin receptors reveal novel strategies for therapeutic target development. Expert Opin Ther Targets 2001, 5: 685–695

3 Tierney AJ. Structure and function of invertebrate 5-HT receptors: A review. Comp Biochem Physiol A Mol Integr Physiol 2001, 128: 791–804

4 Bickerdike MJ. 5-HT_{2C} receptor agonists as potential drugs for the treatment of obesity. Curr Top Med Chem 2003, 3: 885–897

5 Carlton J, Rowland N. Effects of initial body weight on anorexia and tolerance to fenfluramine in rats. Pharmacol Biochem Behav 1985, 23: 551–554

6 Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D. Eating disorder and epilepsy in mice lacking 5-HT^{2C} serotonin receptors. Nature 1995, 374: 542–546

7 Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT_{2C} receptor gene polymorphism. Lancet 2002, 359: 2086–2087

8 Klabunde T, Hessler G. Drug design strategies for targeting G-protein-coupled receptors. Chembiochem 2002, 3: 928–944

9 Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I *et al*. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 2000, 289: 739–745

10 Teller DC, Okada T, Behnke CA, Palczewski K, Stenkamp RE. Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-protein-coupled receptors (GPCRs). Biochemistry 2001, 40: 7761–7772

11 Baker D, Sali A. Protein structure prediction and structural genomics. Science 2001, 294: 93–96

12 Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN *et al*. The protein data bank. Nucleic Acids Res 2000, 28: 235–242

13 Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994, 22: 4673–4680

14 Henikoff S, Henikoff JG. Amino acid substitution matrices from protein blocks. Proc Natl Acad Sci USA 1992, 89: 10915–10919

15 Strader CD, Dixon RA, Cheung AH, Candelore MR, Blake AD, Sigal IS. Mutations that uncouple the β-adrenergic receptor from Gs and increase agonist affinity. J Biol Chem 1987, 262: 16439–16443

16 Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM, Ferguson DM, Spellmeyer DC *et al*. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. J Am Chem Soc 1995, 117: 5179–5197

17 Laskowski RA, MacArthur MW, Moss DS, Thomton JM. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993, 26: 283–291

18 Gasteiger J, Marsili M. Iterative partial equalization of orbital electronegativity: A rapid access to atomic charges. Tetrahedron 1980, 36: 3219–3228

19 Ivanov AA, Baskin II, Palyulin VA, Piccagli L, Baraldi PG, Zefirov NS. Molecular modeling and molecular dynamics simulation of the human A2B adenosine receptor. The study of the possible binding modes of the A2B receptor antagonists. J Med Chem 2005, 48: 6813–6820

20 Elmore DE, Dougherty DA. Molecular dynamics simulations of wild-type and mutant forms of the *Mycobacterium tuberculosis* MscL channel. Biophys J 2001, 81: 1345–1359

21 Xu Y, Shen J, Luo X, Zhu W, Chen K, Ma J, Jiang H. Conformational transition of amyloid β-peptide. Proc Natl Acad Sci USA 2005, 102: 5403–5407

22 Xu Y, Barrantes FJ, Luo X, Chen K, Shen J, Jiang H. Conformational dynamics of the nicotinic acetylcholine receptor channel: A 35-ns molecular dynamics simulation study. J Am Chem Soc 2005, 127: 1291–1299

23 Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: A message-passing parallel molecular dynamics implementation. Comp Phys Commun 1995, 91: 43–56

24 Ballesteros JA, Weinstein H. Analysis and refinement of criteria for predicting the structure and relative orientations of transmembranal helical domains. Biophys J 1992, 62: 107–109

25 Matthew JB. Electrostatic effects in proteins. Annu Rev Biophys Biophys Chem 1985, 14: 387–417

26 Matthew JB, Gurd FR. Calculation of electrostatic interactions in proteins. Methods Enzymol 1986, 130: 413–436

27 MacroDox Macromolecular Simulation Program (Software). Version 3.2.1. Tennessee Technological University, 1999. Available from URL: http://pirn.chem.tntech.edu/macrodox.html

28 Hess B, Bekker H, Berendsen HJC, Fraaije JGEM. LINCS: A linear constraint solver for molecular simulations. J Comp Chem 1997, 18: 1463–1472

29 Darden T, York D, Pedersen L. Particle mesh Ewald: An N-log(N) method for Ewald sums in large systems. J Chem Phys 1993, 98: 10089–10092

30 Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle mesh Ewald potential. J Chem Phys 1995, 103: 8577–8592
31 Berendsen HJC, Postma JPM, Gunsteren WF, DiNola A, Haak JR. Molecular dynamics with coupling to an external bath. J Chem Phys 1984, 81: 3684–3690
32 van Aalten DM, Bywater R, Findlay JB, Hendlich M, Hooft RW, Vriend G. PRODRG, a program for generating molecular topologies and unique molecular descriptors from coordinates of small molecules. J Comput Aided Mol Des 1996, 10: 255–262
33 Breneman CM, Wiberg KB. Determining atom-centered monopoles from molecular electrostatic potentials. The need for high sampling density in formamide conformational analysis. J Comp Chem 1990, 11: 361–373
34 Stachel SJ, Coburn CA, Steele TG, Jones KG, Loutzenhiser EF, Gregro AR, Rajapakse HA *et al*. Structure-based design of potent and selective cell-permeable inhibitors of human beta-secretase (BACE-1). J Med Chem 2004, 47: 6447–6450
35 Shacham S, Topf M, Avisar N, Glaser F, Marantz Y, Bar-Haim S, Noiman S *et al*. Modeling the 3D structure of GPCRs from sequence. Med Res Rev 2001, 21: 472–483
36 Gether U. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr Rev 2000, 21: 90–113
37 Bissantz C, Bernard P, Hibert M, Rognan D. Protein-based virtual screening of chemical databases. II. Are homology models of G-Protein Coupled Receptors suitable targets? Proteins 2003, 50: 5–25
38 Ennis MD, Hoffman RL, Ghazal NB, Olson RM, Knauer CS, Chio CL, Hyslop DK *et al*. 2,3,4,5-tetrahydro- and 2,3,4,5,11,11a-hexahydro-1H-[1,4] diazepino[1,7-α]indoles: New templates for 5-HT_{2C} agonists. Bioorg Med Chem Lett 2003, 13: 2369–2372
39 Kroeze WK, Kristiansen K, Roth BL. Molecular biology of serotonin receptors structure and function at the molecular level. Curr Top Med Chem 2002, 2: 507–528
40 Kristiansen K, Dahl SG. Molecular modeling of serotonin, ketanserin, ritanserin and their 5-HT2C receptor interactions. Eur J Pharmacol 1996, 306: 195–210
41 Quirk K, Lawrence A, Jones J, Misra A, Harvey V, Lamb H, Revell D *et al*. Characterisation of agonist binding on human 5-HT2C receptor isoforms. Eur J Pharmacol 2001, 419: 107–112
42 Roth BL, Willins DL, Kristiansen K, Kroeze WK. 5-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine2C): Where structure meets function. Pharmacol Ther 1998, 79: 231–257
43 Rashid M, Manivet P, Nishio H, Pratuangdejkul J, Rajab M, Ishiguro M, Launay JM *et al*. Identification of the binding sites and selectivity of sarpogrelate, a novel 5-HT_2 antagonist, to human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes by molecular modeling. Life Sci 2003, 73: 193–207
44 Roth BL, Shoham M, Choudhary MS, Khan N. Identification of conserved aromatic residues essential for agonist binding and second messenger production at 5-hydroxytryptamine2A receptors. Mol Pharmacol 1997, 52: 259–266
45 Bywater RP. Location and nature of the residues important for ligand recognition in G-protein coupled receptors. J Mol Recognit 2005, 18: 60–72
46 Shi L, Javitch JA. The binding site of aminergic G protein-coupled receptors: The transmembrane segments and second extracellular loop. Annu Rev Pharmacol Toxicol 2002, 42: 437–467
47 Wang CD, Gallaher TK, Shih JC. Site-directed mutagenesis of the serotonin 5-hydroxytrypamine2 receptor: Identification of amino acids necessary for ligand binding and receptor activation. Mol Pharmacol 1993, 43: 931–940
48 Choudhary MS, Craigo S, Roth BL. A single point mutation (Phe340→Leu340) of a conserved phenylalanine abolishes 4-[125I]iodo-(2,5-dimethoxy)phenylisopropylamine and [3H]mesulergine but not [3H]ketanserin binding to 5-hydroxytryptamine2 receptors. Mol Pharmacol 1993, 43: 755–761
49 Tieleman DP, Berendsen HJ, Sansom MS. An alamethicin channel in a lipid bilayer: Molecular dynamics simulations. Biophys J 1999, 76: 1757–1769
50 Tieleman DP, Berendsen HJ. A molecular dynamics study of the pores formed by *Escherichia coli* OmpF porin in a fully hydrated palmitoyloleoylphosphatidylcholine bilayer. Biophys J 1998, 74: 2786–2801
51 Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. Protein Eng 1995, 8: 127–134

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