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ORIGINAL PAPER

Insights into binding modes of 5-HT2c receptor antagonists with ligand-based and receptor-based methods

Chunhua Lu · Fangfang Jin · Cui Li · Weihua Li · Guixia Liu · Yun Tang

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Abstract 5-hydroxytryptamine-2c (5-HT2c) receptor antagonists have clinical utility in the management of nervous system. In this work, ligand-based and receptor-based methods were used to investigate the binding mode of h5-HT2c receptor antagonists. First, the pharmacophore modeling of the h5-HT2c receptor antagonists was carried out by CATALYST. Then, the h5-HT2c antagonists were docked to the h5-HT2c receptor model. Subsequently, the comprehensive analysis of the pharmacophore and docking results revealed the structure-activity relationship of 5-HT2c receptor antagonists and the key residues involved in the interactions. For example, three hydrophobic points in the ligands corresponded to the region surrounded by Val135, Val208, Phe214, Ala222, Phe327, Phe328 and Val354 of the h5-HT2c receptor. The carbonyl group of compound **1** formed a hydrogen bond with Asn331. The nitrogen atom in the piperidine of compound **1** corresponding to the positive ionizable position of the best pharmacophore formed the electrostatic interactions with the carbonyl of Asp134, Asn331 and Val354, and with the hydroxyl group of Ser334. In addition, a predictive CoMFA model was developed based on the 24 compounds that were used as the training set in the pharmacophore modeling. Our results were not only useful to explore the detailed mechanism of the interactions between the h5-HT2c receptor and antagonists, but also provided suggestions in the discovery of novel 5-HT2c receptor antagonists.

Keywords Docking · Homology modeling · 5-HT2c receptor · 5-HT2c receptor antagonist · Pharmacophore

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5-Hydroxytryptamine (5-HT) is a serotonergic neurotransmitter widely distributed in various tissues of human and animals, especially in synapses. As an endogenous medium, it plays diverse roles by acting on 5-HT receptor which belongs to the seven transmembrane G protein-coupled receptor superfamily. Recent studies showed that 5-HT receptor family can be divided into seven subtypes namely 5-HT1 ~ 7 receptor individually, in which h5-HT2cR was comprised in 5-HT2R class. Several evidences suggested that h5-HT2cR was related to the pathological mechanism of nervous system and the antagonists of h5-HT2cR can be used to treat depression, anxiety and schizophrenia [1]. The h5-HT2c receptor antagonists may also be used to treat the tardive dyskinesia (TD) [2]. With the increasing emphasis of the second-generation antipsychotic, attempts are now being made to explore the therapeutic potential of h5-HT2c receptor antagonists and to investigate the mechanisms of antagonism of the 5-HT2c receptor antagonists [3].

Since the first selective, high binding-affinity h5-HT2c receptor antagonist RS102221 [4] was published in 1997, there have been a large number of 5-HT2cR antagonists such as bisaryl cyclic ureas [5, 6], heterotricycle [7], diaryl substituted pyrrolidines and pyrrolones [8], N-substitued-pyridoindolines [9], oxepin derivatives [10, 11], 1H-indole-3-carboxylic acid pyridine-3-ylamides [12] with good activity. Most of the earlier studies were focused on the

ligand-based study like pharmacophore modeling or receptor-based study like the homology modeling of the 5-HT2c receptor alone. For example, Micheli F et al. generated a 5-HT2cR antagonist pharmacophore model by HipHop using compounds with binding assay pK_i>8.5 [8], and Zuo ZL et al. built the h5-HT2c receptor model using 3D structure of bovine rhodopsin at 2.8 Å resolution as template [13]. However, relatively little research was implemented in comprehensive consideration on both ligand and receptor. There are some inevitable limitations in explaining the target only by the ligand-based pharmacophore method [14]. It would be better to simultaneously consider the structure of the receptor when designing innovative ligands. Although there has been no crystal structure of the 5-HT2c receptor, its homology model may play important roles in interpreting the ligand-receptor interaction and guiding the design of novel ligands. So, it is required to build a reasonable structure of the receptor and gain the key features to fully master the mechanism of h5-HT2c receptor antagonists bound to the h5-HT2cR in order to find new chemical entities.

In the present study, the pharmacophore model was developed employing HypoGen module in CATALYST software. Meanwhile, a reliable 3D structure of the h5-HT2c receptor was constructed using the crystal structure of the human β2-adrenergic receptor as the template. After that, the compounds for developing the pharmacophore model were docked into the modeled protein structure. With the combination of the docking mode and the pharmacophore, the key residues participating in the ligand binding were analyzed. In addition, a CoMFA model was developed using SYBYL 7.0. Our results obtained by both ligand-based and receptor-based methods may be helpful for further understanding the interacting mechanism between the 5-HT2cR and its antagonists, even for finding new h5-HT2cR antagonists.

Materials and methods

Pharmacophore modeling

Pharmacophore generation

All workflow for this procedure was performed with CATALYST 4.11 integrated in Discovery Studio 2.1 (DS 2.1) [15]. According to the training set selection rule [16] in CATALYST, the number of compounds used in HypoGen should be more than 15 with active value spanning four orders of magnitude and the skeletons be as various as possible. Following these rules, a series of h5-HT2cR antagonists with different activity levels and structures from the published literatures were collected. 24 diverse compounds [4–7, 9–12, 17–21] of the above collection were manually selected as training set with binding affinity (K_i) ranging from 0.63 nM to 7244 nM to generate models. A set of 164 chemicals were chosen to validate the developed model.

All the structures were drawn in the ISIS/Draw and saved in sdf format. The uncertainty parameter, which is a ratio of the reported activity value to the minimum and the maximum values, was set as 3 and activity data was also input in the file. The subsequent operations were carried out in HypoGen module. Because the compound pose contributing to biological effect relies on the specific conformer, the first process was to generate conformation using poling algorithm in order to consider the flexibility of ligand. The option in conformation generation was set as BEST to ensure the best coverage of conformational space although it required much CPU time. In the default assumption of discarding existing conformation, we allocated the maximum conformations 255 and assigned energy threshold 20.0 kcal mol^{-1} to search the exact binding orientation. Several trials were carried out for exploring the feature selection. Taking previous h5-HT2c receptor antagonist pharmacophore and the structures of the published h5-HT2cR antagonists into account, five kinds of features in the feature dictionary were selected. They were hydrogen-bond acceptor (HBA), hydrogen-bond donor (HBD), hydrophobic (HY), positive ionizable position (PI) and ring aromatic (RA). Ten ranking cost pharmacophores were yielded by automatic running in the DS 2.1 program.

Validation of pharmacophore model

Considering delta cost, correlation, RMSD, configuration all together, hypothesis 1 was thought to be the best pharmacophore. And the pharmacophore was then validated using a test set including 164 various compounds. Besides, 2000 compounds randomly chosen from the Specs database and other 95 known h5-HT2cR antagonists were put together as a new data set. The reunited data set was used for virtual screening to get the enrichment factor. The enrichment factor (E) was calculated as follows Eq. 1:

$$E = \frac{Ha}{Ht} \div \frac{A}{D} \quad (1)$$

where Ha is the number of active molecules in the hit list, Ht is the number of hits retrieved, A is the number of active molecules in the combined data set and D is the number of total molecules in the subjected database.

Homology modeling

The sequence of human 5-HT2c receptor (entry: P28335) with 458 amino acids was downloaded from Swiss-Prot

(UniProtKB/Swiss-Prot) [22] in fasta format. Human β2-adrenergic G protein-coupled receptor has a certain high identity with the sequence of h5-HT2cR. They both belong to the same species of GPCR family. The high resolution crystal structure from Protein Data Bank (PDB identity: 2RH1) at 2.4 Å resolution was selected as the template. Then, both of the sequences were aligned in the running of aligning multiple sequences protocol included in DS 2.1. The aligning result showed their identity was 26.1%. The parameters used in this process were as follows: BLOSUM when multiple alignment scoring matrix and true for use secondary structure. After that, according to the forming disulfide residues Cys127, Cys207 and the experimental key residue Asp134 [23, 24], the resultant aligned sequences were adjusted for the best fit alignment manually. Based on the above manual alignment, five h5-HT2cR models were obtained at high optimization level and medium loops optimization level refinement with DOPE method in the protein modeling module of DS 2.1. The best one was chosen to run the side chain refinement for all the residues. The final model was detected by PROCHECK and WHAT-IF programs [25] after minimization for residues 128–138 and the second extracellular loop.

Molecular docking

The following work was done by Glide module in Maestro 9.0 [26]. First, the most active compound, compound **1**, was docked into the constructed protein model using the Glide module in the Maestro. Second, residues spanning from 128 to 138 and the second extracellular loop of the docked complex were optimized using molecular mechanics methods with the following parameters: a distance-dependent dielectric constant of 1.0; extended cutoff; OPLS2005 force field; frozen atoms of the invert residues; maximum iterations of 500; conjugate gradient algorithm; 0.05 convergence threshold in the Macromodel. Third, the former minimized h5-HT2cR structure was run through the process of protein preparation including preprocess, optimization as well as minimization for the following receptor grid generation. Residue Asp134 was specified as the centroid to define the enclosing box. Finally, all 24 compounds that were used to develop the pharmacophore model were docked into the constructed receptor model using the Glide module in Maestro program. All 24 compounds were prepared using force field OPLS2005 at target pH 7.0±2.0 with output at most 32 per ligand in the application of LigPrep. The foregone conformations of the compounds were then docked into the obtained grid by standard precision with 5 poses per docked ligand. To analyze the individual residue contribution on the binding, per residue interaction scores of 10 Å were written.

Mapping of pharmacophore onto the homology model

The best pharmacophore model was mapped onto the docked h5-HT2cR model using the pharmacophore protocol integrated in Discovery Studio 2.1 package. Consequently, the detailed interaction information of h5-HT2cR antagonists with h5-HT2c receptor was analyzed and compound **1** was used in the diagram to facilitate the understanding.

CoMFA study

The 24 compounds for developing the pharmacophore model were used to generate a CoMFA model. Atomic charges were computed with the Gasteiger-Huckel method. Steric and electrostatic potentials were created using Tripos Standard field. A probe atom with vdW properties of sp^3 carbon and a charge of +1 were served to generate steric and electrostatic field energies. The CoMFA calculations were performed with a distance-dependent dielectric constant of $1/r^2$, and a truncation of 30 kcal·mol^{-1}. The regress analysis was executed using leave-one-out cross-validation partial least squares (PLS) method with column filtering 2.0 firstly to determine the optimal number of components. Then the final CoMFA model (noncross-validated conventional analysis) was developed with the components determined before. All the computation of CoMFA was conducted with SYBYL 7.0.

Results and discussion

Pharmacophore modeling

Construction of pharmacophore model

The pharmacophore models were generated on the basis of the training set (Table 1) including 24 diverse structures. Ten hypotheses (Table 2) were obtained with the Null cost 158.2. Fixed cost is the cost of a perfect hypothesis with no deviation between predicted and experimental activities, and the value of the generation was 95.2. The best pharmacophore model (Fig. 1) with the total cost 105.8 was chosen. Null cost minus total cost was delta cost and the value of our pharmacophore was 52.4. Delta cost reflects the diversity of the selected training compounds. The value of the pharmacophore generation stands for the confidence of 40%~60%. On the whole, the cost values met the following rules of CATALYST. The closer the fixed cost and the total cost is, the better the hypothesis is. And the greater the difference between the fixed and null cost is, the higher the probability for finding useful hypotheses is. Also, the configuration value was 13.36 which was lower

Table 1 The structures of 24 5-HT2c receptor antagonists with the binding value (K_i, nM) in the training set for HypoGen running

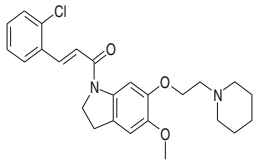
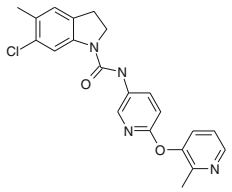
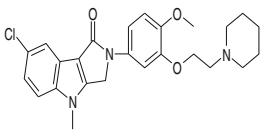
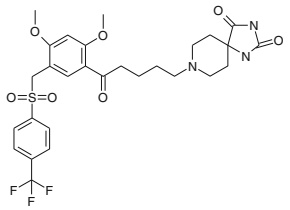
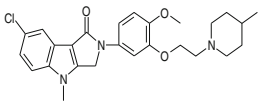
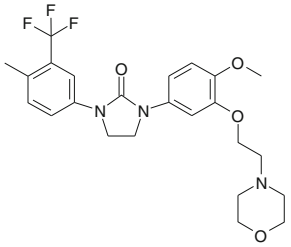
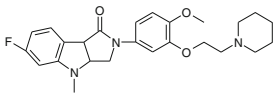
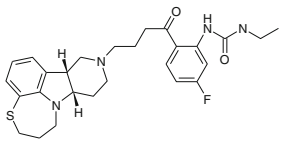
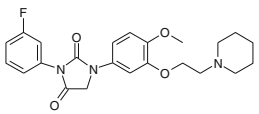
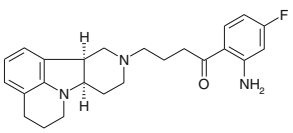
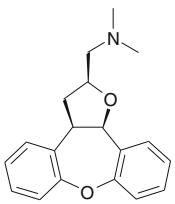
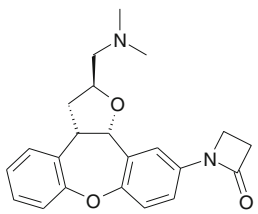
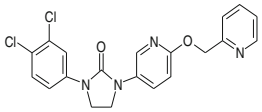
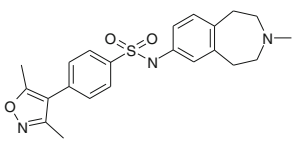
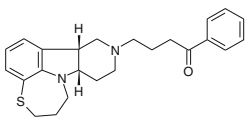
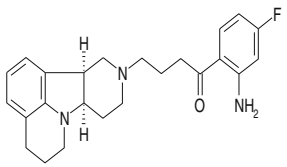
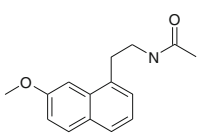
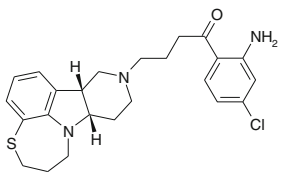
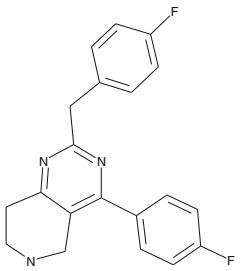
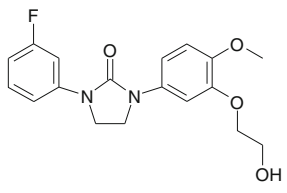
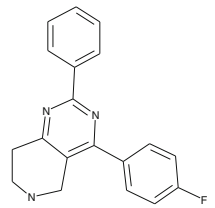
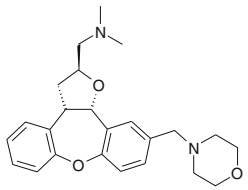
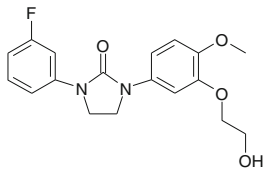
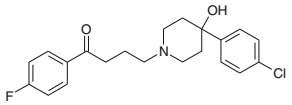
 **1** (K_i = 0.63 nM) [17]	 **2** (K_i = 1.0 nM) [5]	 **3** (K_i = 2.5 nM) [6]	 **4** (K_i = 3.2 nM) [4]
 **5** (K_i = 3.2 nM) [12]	 **6** (K_i = 7.9 nM) [7]	 **7** (K_i = 12.6 nM) [6]	 **8** (K_i = 48 nM) [9]
 **9** (K_i = 50 nM) [7]	 **10** (K_i = 71 nM) [9]	 **11** (K_i = 182 nM) [11]	 **12** (K_i = 356 nM) [10]
 **13** (K_i = 398.1 nM) [6]	 **14** (K_i = 398 nM) [18]	 **15** (K_i = 417 nM) [9]	 **16** (K_i = 664 nM) [9]
 **17** (K_i = 708 nM) [20]	 **18** (K_i = 754 nM) [9]	 **19** (K_i = 1000 nM) [9]	 **20** (K_i = 1259 nM) [7]

Table 1 (continued)

			
21 (K_i = 4000 nM) [9]	**22** (K_i = 4105 nM) [10]	**23** (K_i = 5000 nM) [21]	**24** (K_i = 7244) nM) [19]

than 17. It indicated that the flexibility of the molecules in the training set was suitable for HypoGen to analyze the complexity of the hypothesis space. The RMSD and correlation coefficient values were 0.936 and 0.930 (Fig. 2), respectively. It meant that the estimated activities by the model correlated well with the actual activities, and the pharmacophore model had the high predictive reliability. Most of the h5-HT2cR antagonists have a nitrogen atom. It was not strange that all ten models had one PI just as Table 2 showed. It can be seen from Fig. 1 that there were five features in the best pharmacophore model: three hydrophobic positions (3HY), one positive ionizable position (1PI), and one hydrogen acceptor (1HBA). The spatial arrangement of these pharmacophore features (Fig. 1) was generally consistent with previous models [8, 27] whose features were 1HBA, 1PI, 1RA, 3HY [8] and 1HBA, 1PI, 3HY [27] in succession. Our pharmacophore model was more identical with the latter [27]. The small difference between our pharmacophore model and Micheli's [8] may be caused by the discrepancies in both the methods and the structures of the training set compounds.

If mapping the pharmacophore model to the compounds, we could find that the nitrogen in the piperidine was in the positive ionizable position. The carbonyl group has been considered critical in the skeleton during compound reconstruction experiment. It was mapped to the hydrogen bond acceptor here. And the benzene ring, indole ring and methyl group were hydrophobic located in three regions. The most active compound, compound **1**, could map all the five features of the pharmacophore very well.

From Table 3, we could find that the pharmacophore hypothesis was able to discriminate the active and the inactive molecules. That was to say, it could be used to discover the h5-HT2cR antagonists from the unknown database. For compounds with high binding affinity, the accuracy of the prediction was higher than those with low binding affinity. Sometimes when estimating compounds with low activity, the predicted activity may be higher or

Table 2 Results of top ten pharmacophore hypotheses for h5-HT2c receptor antagonists by means of CATALYST/HypoGen

Hypo no	Total cost[a]	RMSD[b]	Rtr-set correlation[c]	Hypo features	▵cost[d]
1	105.8	0.936	0.930	1HBA 3HP PI	52.4
2	109.6	1.094	0.903	1HBA 3HP 1PI	48.6
3	112.6	1.180	0.886	1HBA 3HP 1PI	45.6
4	115.1	1.289	0.862	1HBA 2HP 1PI	43.0
5	116.2	1.323	0.854	1HBA 3HP 1PI	41.9
6	116.3	1.322	0.854	1HBA 1HP 1PI 1RA	41.9
7	117.0	1.347	0.848	1HBA 3HP 1PI	41.1
8	117.4	1.357	0.845	1HBA 2HP 1PI 1RA	40.7
9	118.0	1.364	0.844	1HBA 3HP 1PI	40.1
10	118.0	1.377	0.840	1HBA 3HP 1PI	40.1

[a] Total cost=Error cost+Weight cost+Configuration

[b] RMSD: deviation of the log (estimated activities) from the log (experimental activities) of this generation

[c] Correlation is derived from the fit index (simple linear regression)

Hypothesis 1 had the highest Δ cost and correlation while the lowest RMSD, so it was selected for further study

[d] ΔCost=(Null cost−Total cost); All cost units are given in bits. Here, Null cost was 158.2

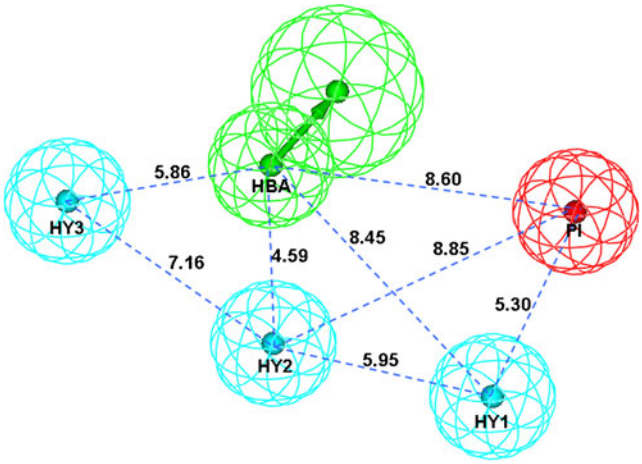


Fig. 1 The best pharmacophore model with distance constraints (Å) between features. PI (red) is positive ionizable point, HY1, HY2, HY3 (blue) are hydrophobic points, and HBA (green) is hydrogen bond acceptor

lower than the actual value. Although this may happen, it can predict correctly for most of the compounds with medium and high activity. In all, it can predict more accurately for high-affinity h5-HT2cR antagonists than the lower ones.

Validation of the pharmacophore

To validate the rationality of the pharmacophore, two methods were applied. One was the regression in a test set containing 164 diverse h5-HT2c receptor antagonists. The other method was the virtual screening based on a combined data set including 2000 randomly selected compounds from Specs database and 95 active 5-HT2c receptor antagonists. Just as the plot shown in Fig. 2, the correlation coefficient between the predicted activity and the experimental value was 0.79, which implied that the model could be used to estimate the unknown molecules precisely. From 2095 compounds with 95 reported 5-HT2c receptor antagonists, 95 compounds were retrieved as hits in which 90 were known antagonists for h5-HT2c receptor by our pharmacophore model. Therefore, the enrichment factor (Eq. 1) was up to 20.89, showing that the pharmacophore had more than 20 times greater probability in detecting active compounds than inactive compounds from virtual database.

Homology modeling of the h5-HT2c receptor

For the absence of the crystal structure of the h5-HT2c receptor, it becomes an obstacle to study the exact mechanism about how the antagonists interact with the receptor to trigger the corresponding biological effects. It has been verified that h5-HT2cR belongs to the GPCR A family and the common feature of this family is the conserved seven transmembrane helix bundles. On this basis, the unknown h5-HT2cR can be modeled by the template of the known human β2-adrenergic receptor with the automatic sequence alignment and the subsequent manual accommodation (Fig. 3).

To validate the constructed 3D model of the h5-HT2c receptor, the Ramachandran plot analysis (Fig. S1) was carried out, which indicated that 88.7% residues in the model were in the most favored regions, 9.0% residues in the additional allowed regions, 1.3% residues in the generously allowed regions and 1.0% regions in the disallowed regions. The analysis of the PROCHECK showed that the modeled structure was reliable. At the same time, the WHAT-IF packing scores per residue for the homology model were calculated (Fig. S2). Just as depicted in Fig. S2, all the packing scores were higher than −5.0. RMS Z-Scores for bond angles and bond lengths were 0.705 and 1.310 which were both close to 1. Thus, the WHAT-IF evaluation also revealed that the model was reasonable [28].

The modeled 3D structure of h5-HT2cR with seven transmembrane helix bundles (TM1–7) and the extracellular loop (ECL1–3) close to the membrane was shown in Fig. 4. The conserved residues, Cys127 at the beginning of TM3 and Cys207 in ECL2 among the 5-HTR subfamily are helpful to stabilize the three dimensional structure. And the pose of the docked compound **1** was shown in CPK style.

Analysis from the docking complex together with the pharmacophore model

There has existed a bitarget market drug playing the role in both melatonergic and serotonergic system, agomelatine (i.e., compound **17** in Table 1) [20, 29]. Its binding affinity

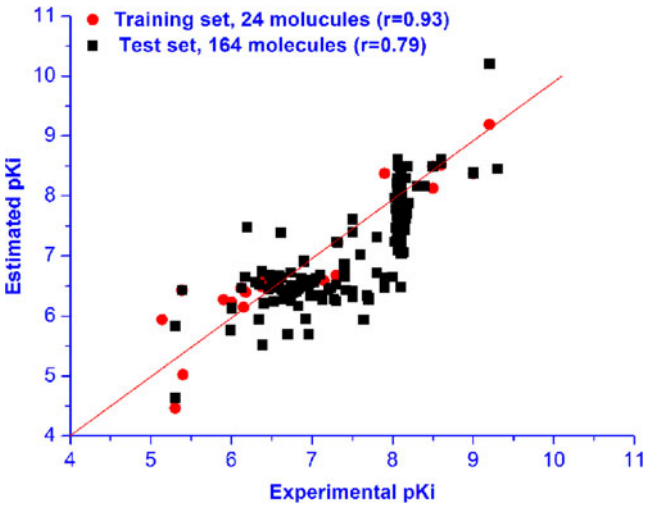


Fig. 2 Regression analysis of the best pharmacophore applied to the training set and the test set showing the correlation (r) between the estimated and the experimental activities

Table 3 Output of the score hypothesis process on the training set using CATALYST/HypoGen

Compound no	K_i (nM)		Fit value[a]	Error cost[b]	Activity scale			Mapped features			
	Estimated	Experimental			Estimated[c]	Actual[d]	HA	PI	HY1	HY2	HY3
1	0.63	0.63	8.72	−1	+++	+++	+	+	+	+	+
2	4.2	1	7.90	+4.2	+++	+++	+	–	+	+	+
3	3.1	2.5	8.03	+1.2	+++	+++	+	–	+	+	+
4	7.8	3.2	7.63	+2.5	+++	+++	–	+	+	+	+
5	3.3	3.2	8.00	+1.1	+++	+++	+	–	+	+	+
6	24	7.9	7.15	+3	++	+++	+	+	–	+	+
7	4.1	13	7.91	−3.1	+++	++	+	–	+	+	+
8	61	48	6.74	+1.3	++	++	+	–	+	+	+
9	180	50	6.27	+3.6	++	++	+	–	+	–	+
10	260	71	6.10	+3.7	++	++	+	–	–	+	+
11	310	180	6.03	+1.7	++	++	–	+	+	+	–
12	230	360	6.16	−1.6	++	++	+	–	+	+	–
13	210	400	6.20	−1.9	++	++	–	–	+	+	+
14	290	400	6.06	−1.4	++	++	–	+	+	+	–
15	330	420	6.01	−1.3	++	++		–	–	+	+
16	390	660	5.93	−1.7	++	++	+	–	+	+	–
17	690	710	5.68	−1	++	++	+	–	–	+	+
18	340	750	5.99	−2.2	+	++	+	–	+	+	–
19	630	1000	5.72	−1.6	++	++	+	–	–	+	+
20	480	1300	5.84	−2.6	++	+	+	–	+	+	+
21	9200	4000	4.56	+2.3	+	+	+	–	–	+	+
22	370	4100	5.95	−11	++	+	–	+	+	+	–
23	35000	5000	3.98	+7	+	+	+	–	–	–	+
24	1200	7200	5.46	−6.3	+	+	–	–	+	–	+

[a] Fit value indicates how well the features in the pharmacophore overlap the chemical features in the molecule

[b] When Est. activity>Act. activity, then Error=Est./ Act.; when Est. activity<Act.activity, then Error = −Act. / Est

[c, d] Activity scale: +++, K_i<10 nM (highly active); ++, 10≤K_i<1000 nM (moderately active); +, K_i≥1000 nM (low active)

(K_i=708 nM) with the 5-HT2c receptor was not too high. To further study how the h5-HT2c receptor antagonists interacted with the receptor, the 24 compounds for developing the pharmacophore model were docked into the binding site of h5-HT2cR model. All 24 compounds were bound in the active binding site of h5-HT2cR homology model in a similar conformation, indicating that they shared some common binding features for each other.

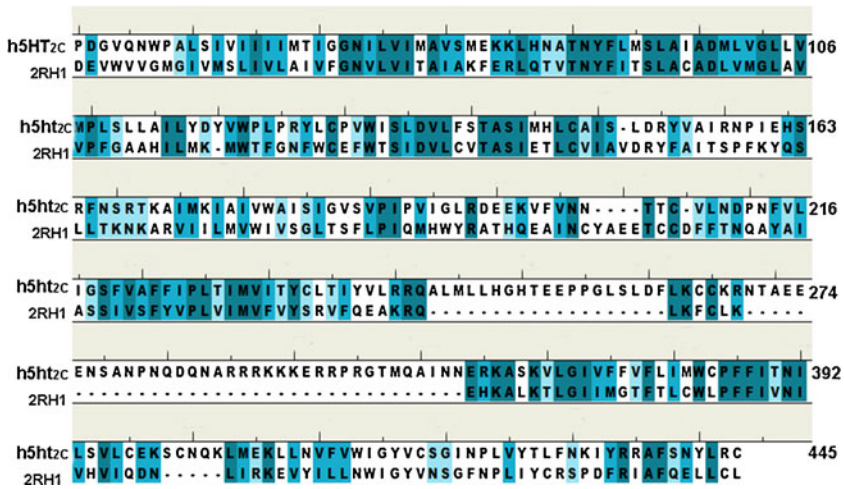


Fig. 3 Sequence alignment between the h5-HT2c receptor (p28335) and the β2-adrenergic receptor (2RH1) using ClustalW algorithm as well as manual adjustment

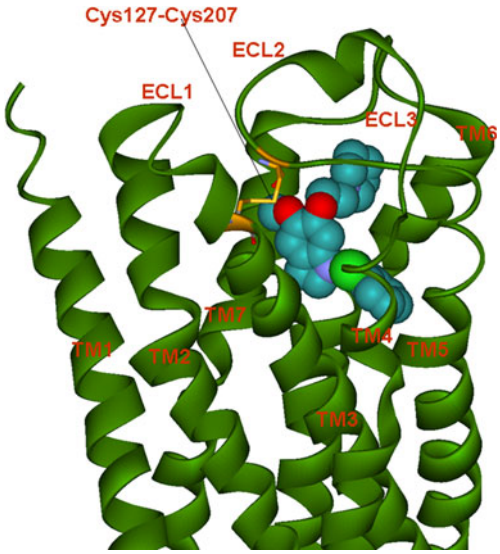


Fig. 4 The homology modeled 3D structure of the 5-HT2c receptor. Seven transmembrane helix bundles were marked from TM1 to TM7 for clarity. Three extracellular loops were labled ECL1, ECL2, ECL3. The conserved disulfide bridge Cys127-Cys207 between TM3 and ECL2 was shown in stick. The docked compound 1 was in CPK style

To illustrate the interaction mechanism, compound **1,** the most active molecule among the 24 compounds, was selected for more detailed analysis. The knowledge of the ligand interaction mode is useful for us to understand the reason why compound **1** is of high activity while some other compounds are not so active. The study may provide a guide on how to modify other low-affinity compounds and improve their binding affinity.

There were kinds of binding poses from the docking result. The docking mode was chosen in the consideration of both the Glide gscore and the ligands' binding pose. As shown in Fig. 4, the disulfide bridge formed between Cys127 and Cys207 was highlighted in stick. Through the docking result of the most active h5-HT2cR antagonist, it was identified that the possible binding site was located at TMs including TM3, TM5, TM6 and TM7, partly at the extracellular loop of the h5-HT2c receptor (Fig. 4). According to the polarity, the hydrophilic subdomain covered Asp134, Asn331 and Ser334, while the hydrophobic cavity was composed of Val135, Val208, Phe214, Ala222, Phe327, Phe328 and Val354. The key residues involved in the antagonist-receptor interactions were somewhat similar to those (Val135, Val208, Phe327, Phe328 and Asn331) responsible for the binding of agonists to the h5-HT2cR [13]. The comparison in the binding mode of agonists and antagonists implied that the h5-HT2c receptor antagonists and agonists may share the same pocket while different orientation. This similarity was essentially coincident with the idea, namely, synthetic antagonists bound to residues in the agonist-binding pocket. [30]. Nitrogen in the piperidine of compound **1** corresponding to the positive ionizable position of the best pharmacophore formed electrostatic interactions with the carbonyl of Asn331 and Val354 as well as with hydroxyl group of Ser334 with distance 4.05 Å, 4.97 Å and 5.21 Å. Carbonyl serving as a hydrogen bond acceptor was H-bonded with nitrogen in the Asn331 with the bond length of 2.92 Å. It contributed greatly to the binding affinity. The HY1 occupied the region mainly constituted by Val208 and Val354, HY2 by Phe327 and Phe328, and HY3 by Val135, Phe214, Ala222 and Phe328 (Fig. 5a, b).

Asp134 is a conserved residue in the 5-HT2c receptor and plays the key role for ligand binding. Eint is the abbreviation of the interaction energy. It is the total interaction energy between residues and the ligand composed of Coulombic, van der Waals and hydrogen bonding terms. And the interaction energy of Asp134 was as low as -19 kcal mol^{-1}, which was the lowest energy of all the interacting residues. It was not surprising for the importance of Asp134. The Asp134 Coulomb potential, which is the electrostatic interaction between the residue and the ligand, was -18.00 kcal mol^{-1}. When formal charge of nitrogen of piperidine was zero, the Asp134 Coulomb potential increased up to -1.85 kcal mol^{-1}. The comparison indicated that the electric charge of the nitrogen of the piperidine had enormous influence on the electrostatic interaction of Asp134. In consequence, Asp134 made the electrostatic attraction with the nitrogen in the piperidine. Aromatic residues help to anchor the phenyl ring via stacking or π-π type interaction. Phe214 and Phe328 formed T-stacking interactions with the phenyl group, while Phe327 had a π-π type interaction with the indole of compound **1,** individually. The conserved Phe327 and Phe328 play another pivotal role in 5-HT2c receptor. Choudhary MS et al. found the binding affinity of some 5-HT2c receptor antagonists didn't diminish when Phe327 was mutated, while some other compounds also had no change in binding affinity if Phe328 was mutated employing site-directed mutation [31]. Therefore, in order to further study these two residues, the interaction energies of Phe327 and Phe328 with 5-HT2c receptor were analyzed. For Phe327 and Phe328, the Eint values were -1.00 kcal mol^{-1} and -2.42 kcal mol^{-1}. And the distance between the two residues and the ligand were 1.86 and 2.74 Å, respectively. Why Phe327 was closer to the ligand, but the contribution to the interaction was less? The main reason for the different energy may be that the distance between Phe327 and ligand was so close that they resulted in repulsion. So Phe328 made more positive influence on the interaction for compound **1** binding to 5-HT2c receptor. Early in 2006, the h5-HT2cR model was built to study the interaction of the 5-HT2c receptor with agomelatine and lisuride [32]. In their work 5-HT2c receptor antagonist was docked into the modeled 3D structure to identify the

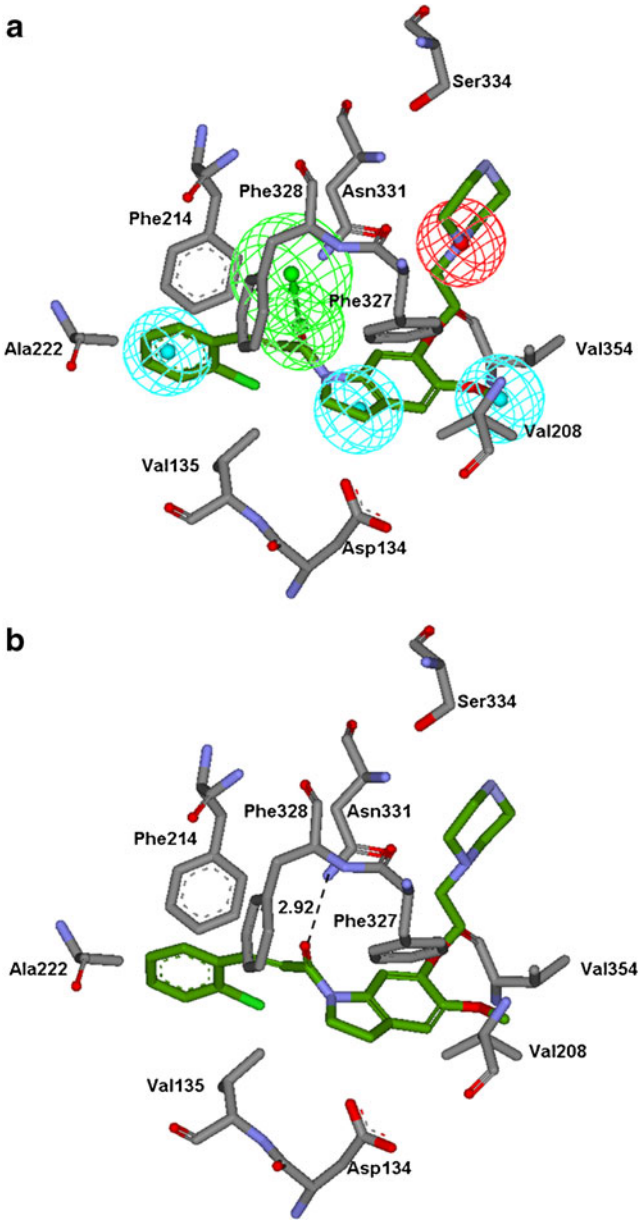


Fig. 5 (**a**) The best pharmacophore model was mapped onto the binding site of h5-HT2cR homology model. Nitrogen atoms were colored light blue; oxygen atoms were colored red. Carbon atoms of the ligand and receptor were colored green and gray. The pharmacophore features were color-coded as follows: green, hydrogen bond acceptor (HBA); red, positive ionizable position (PI); blue, hydrophobic position (HY). The residues were labeled in three letters. (**b**) The detailed interactions of compound **1** with h5-HT2cR. The hydrogen bond was shown in black line with distance (Å). All hydrogen atoms were not displayed. The color of each atom was the same as **a**

binding site information. Though our docked compound **1** was different from lisuride, the resulted key residues Asp134, Ala222, Phe327 and Phe328 were identical. It is definitely no accident that different ligands interact with the same key residues. It is another confirmation for the correctness of our binding modes.

CoMFA contour map

A CoMFA model of the h5-HT2cR antagonists was developed on the basis of the binding conformations of the 24 compounds to h5-HT2cR. The resulting q^2 and r^2 were 0.94 and 0.54, respectively.

The CoMFA contour maps were shown in Fig. 6. Detrimental and beneficial steric interactions were each displayed in yellow and green contours (Fig. 6a), while blue and red contours illustrated the regions of desirable positive and negative electrostatic interactions (Fig. 6b). The steric contour of the model indicated that bulky substituents in the sites of methyl and phenyl group would enhance the h5-HT2cR antagonism. And the substitution with bulky groups in the region close to indole was unsuitable. Positive-charge-favored areas were near Asp134 and Asn331. It can be indicated that positively charged substituents may increase the activity through reinforcing the electrostatic interaction with Asp134 and Asn331. The red polyhedron indicated that high electron density near amid may play a favorable role in antagonistic potencies.

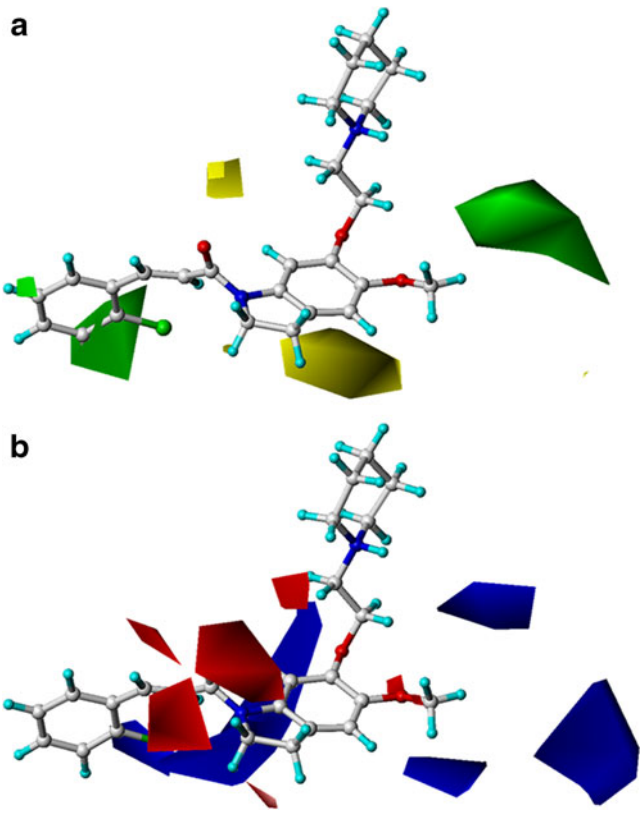


Fig. 6 Views of the contour plots of the CoMFA model. Steric (**a**) and electrostatic (**b**) maps were shown. Sterically favored regions were in green; sterically disfavored regions were in yellow. Areas favoring positive potential field were indicated by blue polyhedron while favoring negative potential field by red polyhedron

Conclusions

In this study, in terms of ligands, an h5-HT2c receptor antagonist pharmacophore model was developed on the basis of a training set including 24 molecules on the platform of HypoGen module of CATALYST. All the features possessed in the pharmacophore were: one PI, one HBA and three HP. These findings may be a guide for the discovery of new structures with better binding affinity. The enrichment 20.89 through screening true 5-HT2c receptor antagonists from virtual database indicated that the model was reliable and could be used to detect novel structure possessing the property of h5-HT2c receptor antagonism.

Moreover, an h5-HT2c receptor model was also constructed based on the crystal structure of β2-adrenergic receptor, and was then validated reasonable and reliable.

In addition, the compounds for developing the pharmacophore model were docked into the modeled three dimensional structure of the h5-HT2c receptor. And the key residues participating in the interaction between the h5-HT2c receptor antagonists and the receptor were analyzed in detail.

Finally, a CoMFA model based on the 24 compounds was constructed to further understand the binding mechanism and provide some useful information for ligand optimization.

Taken together, the combined ligand-based and receptor-based studies were systematically made for the 5-HT2c receptor system, which is useful for the design and development of novel 5-HT2c receptor antagonists for the treatment of neuropsychiatric disorders related to a hypofunction of central dopamine.

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