THE MODELING OF THE 5-HT$_{1A}$ RECEPTOR, BASED ON AN INTERACTION WITH ARYLPIPERAZINE DERIVATIVES WITH RESTRICTED CONFORMATIONAL FLEXIBILITY

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INTRODUCTION

Complex arylpiperazine derivatives constitute one of the most important classes of serotonin 5-HT$_{1A}$ receptor ligands, being a valuable source of potential therapeutic agents in the treatment of psychiatric disorders. Of the several published models of the serotonin 5-HT$_{1A}$ receptor, only two explored arylpiperazine interaction mode [1, 2]. Computationally derived models of ligand-receptor complexes remain ambiguous, mainly due to the flexibility of the investigated ligands.

Fig. 1.

Recently, a set of novel, rigid arylpiperazine derivatives was synthesized (Fig. 1), in which a flexible aliphatic chain connecting the arylpiperazine fragment with the second terminal pharmacophoric group was replaced by the cyclohexane ring [3]. The present study describes the application of those highly active arylpiperazine derivatives for the optimization of the 5-HT$_{1A}$ receptor model. Our approach is based on the assumption that the rigidity of a compound encodes significant information about the binding site geometry.

METHODOLOGY

Methodology presented here is based on reverse virtual screening of large number of “raw" model structures of receptor on several high affinity antagonists in order to select the receptor conformation able to accommodate the rigid ligand and reproduce the putative binding mode. The choice of solely antagonists is based on the assumption, that the unique antagonist binding mode stabilizes inactive conformation. In the first step 400 homology models of 5-HT$_{1A}$ receptor were produced with the use of Modeller 6.1 [4]. X-ray structure of inactivated bovine rhodopsin (PDB code 1F88 [5]) was used as a template. The models differ mainly in side chain conformation, but also in TM helices arrangement (Fig. 2). We used sequence alignment based on recurring, the most conserved residue patterns in TM regions of GPCR family. All models were energy optimized by Modeller in Charm forcefield. Subsequently, docking experiments with 3 ligands (mp245, mp252, mp349) were conducted for all 400 tested receptor models with the use of Autodock 3.0 [6]. 200 docked conformations were stored for each run resulting in 320000 ligand-receptor pairs. The docked conformations were primarily scored with respect to distance between basic nitrogen of a ligand and carboxy- group of Asp116 in TM3 of receptor (N--O distance): only complexes in which that distance was shorter than 3.5 A were taken into account. This interaction is considered crucial for binding of both agonists and antagonists by monoamine receptors. Five models with highest number of ligand-receptor pairs filling N--O distance criterion were submitted to further step: docking of other 11 high affinity (nanomolar K_i) ligands.

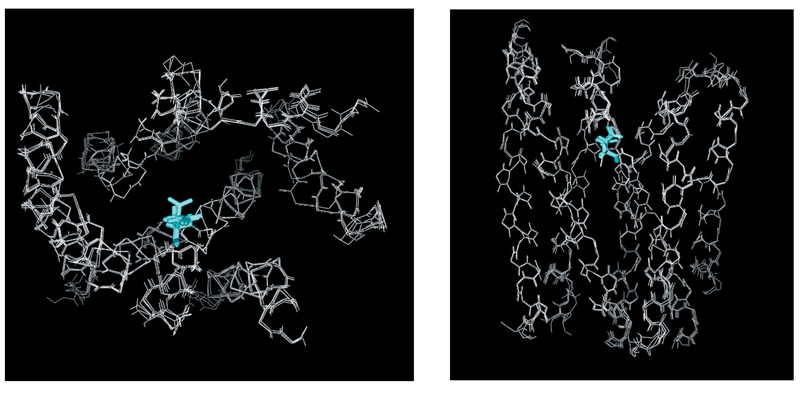
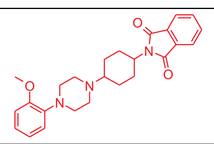
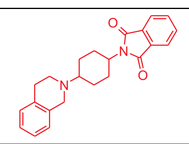
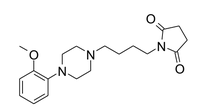
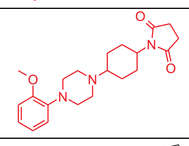
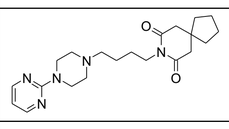
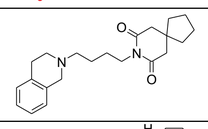
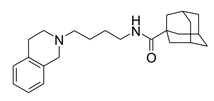
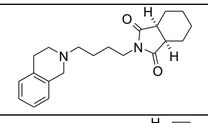
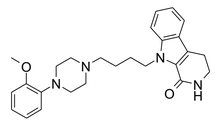
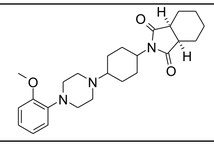
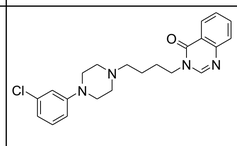
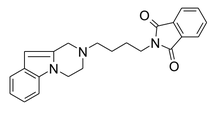
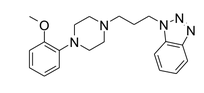
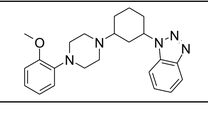


Fig. 2. Five best receptor models chosen on the basis of NO criterion. Superimposition of the backbone atoms, Asp 116 colored cyan.

Table. Structures and affinity constants for 5-HT$_{1A}$ receptors of the compounds used in receptor models selection (in red) and others used in subsecquent docking studies.

Compd.	Structure	*K*i	Compd.	Structure	*K*i
mp245		8	mp252		34
mm77		6.4	mp349		15
buspiron		12	mm199		5
mm223		0.95	mm233		50
b758		0.3	mp273		72
pk47		50	id138		74
mp3022		15	mp346		47

RESULTS

Docking of complex arylpiperazine derivatives to all five chosen models enabled us to establish the putative binding mode for this class of compounds (see Fig. 4). In all the cases the aryl substituent is pointing slightly towards the outside of receptor, whereas terminal fragment is placed in the cavity formed by transmembrane helices 3, 4, 5 and 6.

Current models studied here were not able to predict single binding mode for all tetrahydroisoquinoline derivatives. THIQ fragment was situated either between helices 1, 2 and 7 or between helices 4, 5 and 6.

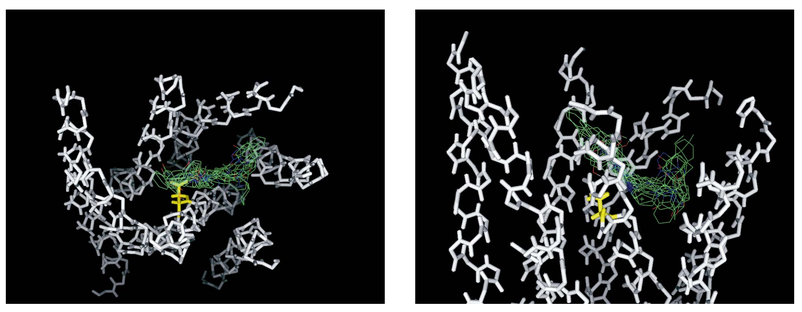


Fig. 3. Lowest energy dockings of all studied compounds in the binding site of model 255. Asp116 is shown yellow.

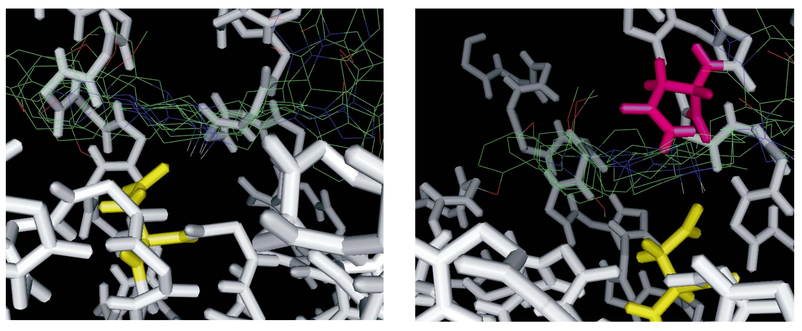


Fig. 4. Lowest energy dockings of all arylpiperazine derivatives. On the left: in all cases interaction between Asp116 (yellow) and basic nitrogen is visible (hydrogen atoms of ligands pointing towards carboxy oxygen of Asp). On the right: possible interaction of *ortho*-methoxyphenylpiperazines with Asn386 (magenta) is visible for 3 compounds (mp3022, mp349 and mm77). The contribution of Asn386 to the antagonist binding site has been confirmed by mutagenesis experiments and other modeling work [2, 7].

The quality of models for prediction of binding mode was assessed by checking the correlation between experimental K_i values and estimated free energy of binding of the best conformation from each docking. The most significant correlation is present in case of model 255 (Fig. 5).

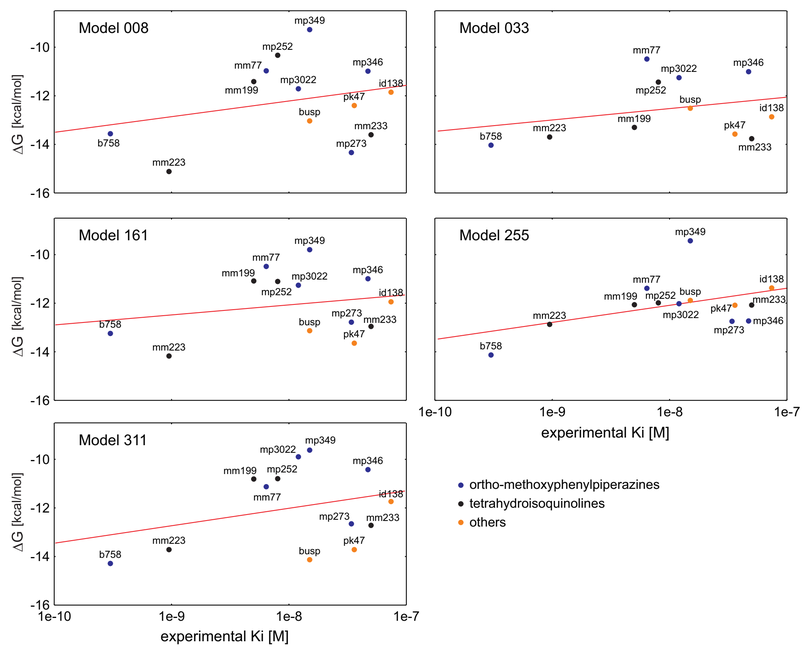


Fig. 5. Correlation between experimental Ki (M, logarithmic scale) and the lowest estimated free energy of binding (kcal/mol) in all docking experiments for 5 best receptor models. Only ligand conformations filling N--O distance criterion were taken into account.

CONCLUSIONS

We have shown the usefulness of approach based on reverse virtual screening of large number of receptor models with high affinity ligands in the process of optimization of the receptor model. Model 255, showing the best correlation between docking energies and experimental K_i, is currently optimized with the use of molecular dynamics simulation methods.

Additionally the putative binding mode for 5-HT$_{1A}$ containing arylpiperazine fragment was proposed. It is generally in agreement with models of antagonists binding site published by Kuipers [7] and Jacoby [2].

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