An analysis of molecular interactions between the 5-HT$_6$ receptor and non-basic ligands

Dawid Warszycki, Krzysztof Rataj, Andrzej J. Bojarski

Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Kraków, Poland

e-mail:warszyc@if-pan.krakow.pl

Serotonin receptors play an importatnt role in many physiological and behavioral functions in humans and animals. One of the recently identified members of the 5-HTR family is the 5-HT$_6$ subtype. It was found that this receptor is distributed almost exclusively in areas of central nervous system (essential for cognitive process) which minimalizes the chance of occuring undesirable peripheral side effects. All typical serotonin receptor ligands possess basic nitrogen atom in their structure. The charge assisted hydrogen bond between the protonated nitrogen atom and aspartic acid D3.32 was considered to be crucial for anchoring to the receptor. Recently, high affinity non-basic ligands of serotonin receptors have been reported. The newest version of the ChEMBL database (version 20, January 2015), the largest collection of information about the biological activity of chemical compounds, contains 1626 compounds acting on the 5-HT$_6$ receptor (K_i or equivalent equal or less than 100 nM)[1]. Within this collection nearly 15% of actives (234 compounds) has low basicity (basic pKa less than 6) [2,3].

To examine binding modes of non-basic 5-HT$_6$R ligands, class-specific homology models were generated utilizing previously applied methodology[4].

The homology models were created based on eight class-A GPCR templates (A_{2A}, β_1-adrenergic, β_2-adrenergic, chemokine, D_3, H_1, M_2 and M_3). For each of those templates 200 models were constructed using Modeller 9.13 software, resulting in 1600 models built in total. This ensured that a wide range of possible protein conformations is covered, through the randomness factor incorporated in the modelling algorithm. All models were evaluated by docking of set consisting of all non-basic 5-HT$_6$R ligands and 426 compounds with confirmed inactivity towards 5-HT$_6$R (K_i or equivalent > 1000 nM; fetched from ChEMBL database) by using Schrodinger's Glide software. Models characterized by the highest values of the AUROC parameter in virtual screening experiments were selected for binding mode evaluation (Table 1, Figure 1).

All active, non-basic 5-HT$_6$R ligands were clustered by Hierarchical Clustering tool implemented in Canvas under the default settings. Finally 23 clusters were created using the Kelly criterion. Binding modes of the the compounds across all top scoring receptors were carefully evaluated.

For the two the most populated clusters (thieno[2,3-e][1,2,3]triazolo[1,5-a]pyrimidynes and pyrazolo[1,5-a]pyrimidines, Figure 2, panel A and B) binding modes are similar. Compounds are close to the TM5 and posses van der Waals interaction with this helix. Structures are located shallowly due to hydrophobic interactions with ECL2. Terminal aromatic systems posses face-to-edge and face-to-face interactions with aromatic cluster from TM6, however interactions with F6.52 are rarely seen. The most important diffence in comparison to classical 5-HT$_6$R ligands is lack of close contacts with TM3.

Binding modes of compounds from the next two the most populated clusters (6-(phenyl)sulfonyltetralines and N-benzenesulfonylindoles, Figure 2, panel C and D) are also similar. Contrary to previously disscussed complexes H-bond interaction with D3.32 is formed here, however without any significant charge-assisted contribution. This contact is formed by non-basic amine groups. Deeper placement of ligands in binding pockets neglects hydrophobic interactions with ECL2. Stacking interactions with aromatic cluster were also weakened. Both groups are still close to TM5, and some N-benzenesulfonylindoles interact with T5.46 by H-bond. Nevertheless, 6-(phenyl)sulfonyltetralines firstly described by Harris, did not show this interaction in binding mode proposed in original work[5].

The analysis of remaining clusters indicated that the non-basic ligands also bind to the receptor through hydrogen bonding with the D3.32 although the charge assisted contribution is missing; hydrogen bonds with T5.46 and T7.39 are also widespread. The most common way of non-basic ligands binding are hydrophobic interactions, among which stackings with aromatic cluster (W6.48, F6,51 and F6.52) are crucial.

Table 1. AUROC values for five the best models in active/inactive discrimation tests. Results for the best model developed on other templates (one per template) are also shown.

Model number	Template	AUROC
12	H_1	0.666
113	H_1	0.665
97	Chemokine	0.654
181	H_1	0.654
123	H_1	0.654
6	M_3	0.639
28	M_2	0.632
149	β_2-adrenergic	0.568
183	D_3	0.561
114	β_1-adrenergic	0.508
161	A_{2A}	0.182

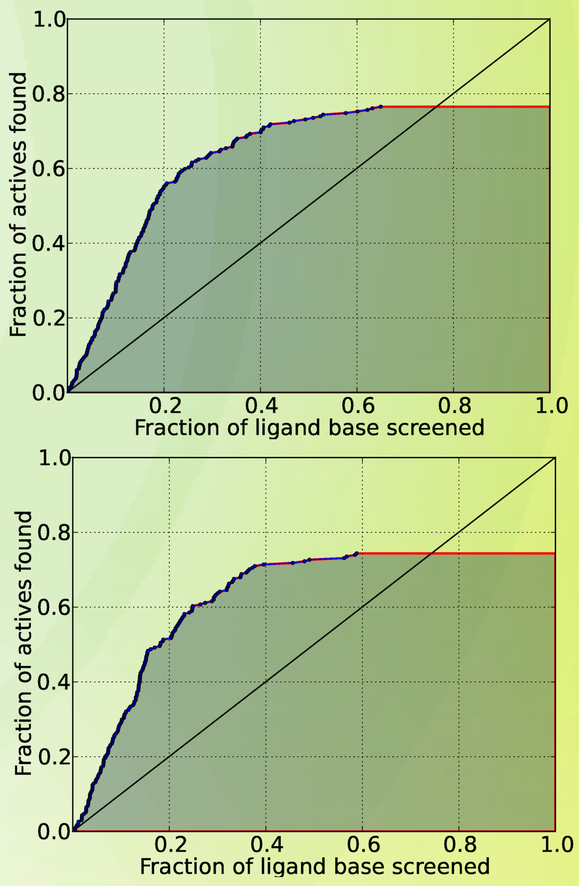

Figure 1. ROC curves for the two best performing homology models.

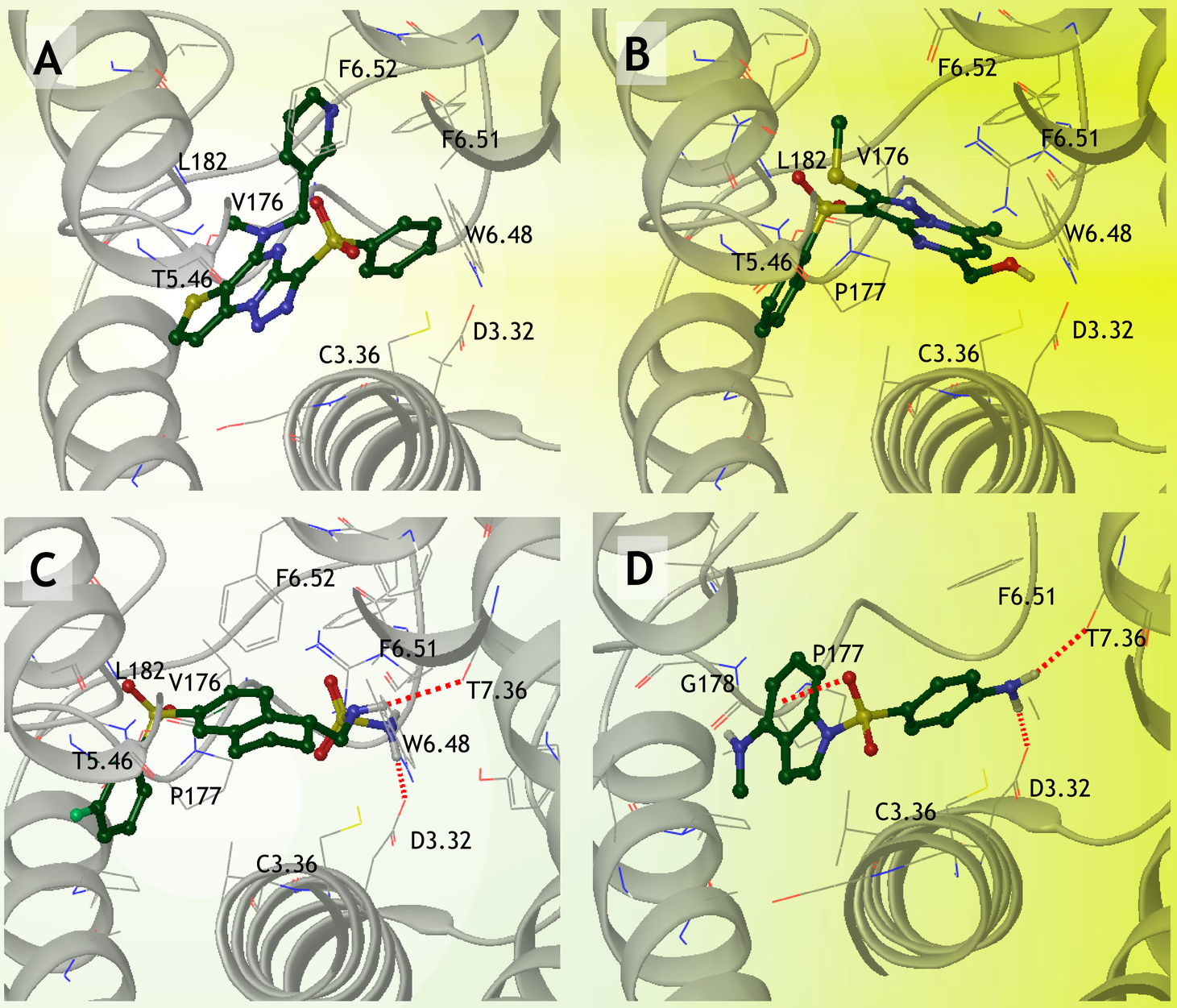

Figure 2. Binding modes for representative compounds from four the most populated structural groups of non-basic 5-HT$_6$R ligands (A – thieno[2,3-e][1,2,3]triazolo[1,5-a] pyrimidynes, B - pyrazolo[1,5-a]pyrimidines, C - 6-(phenyl)sulfonyltetralines and D - N-benzenesulfonylindoles). Compounds are rendered as a ball and stick representation. Only residues situated less than 3Å from the docked compounds have been shown. Hydrogen bonds between 5-HT$_6$R and ligands are labelled as red dashed line.

References

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