Development of selective GPCR ligands – 5-HT$_{1B/2B}$ case study

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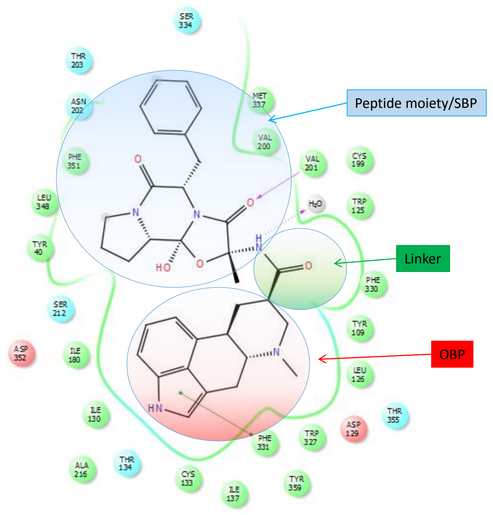


Fig. 2: An example of an SBP – selective compound; the OBP part is active towards both receptors, while the SBP part is responsible for selectivity

Selectivity of ligands targeting GPCRs has always been an issue troubling the drug design community. Due to high structural similarities between the receptors in that family, compounds suggested as new drugs are usually highly promiscuous. This comes with some advantages, as multiple targets can be addressed by a single molecule, however it is also the main cause of most adverse side effects of said drugs.

Since the sequences of orthosteric binding pockets (OBPs) of class A GPCRs are often highly conserved, it is very hard to find compounds capable of distinguishing between closely related proteins. Nevertheless, Michino et al. [1] found that there are some crucial differences in the secondary binding pockets (SBPs) (Fig. 1) – putative allosteric binding sites – in multiple GPCRs. This led to the idea of screening for selective compounds for two closely related targets whose structures had been recently resolved: the 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors, using the differences in their SBPs (Fig. 2).

	2.61	2.64	2.65	EL 1.50	3.28	3.29	3.32	3.33	3.36	3.37	3.40	4.57	EL2.48	EL2.49	EL2.50	EL2.51	EL2.52	EL2.53	EL2.54	EL2.55	5.38	5.39	5.42	5.43	5.46	5.47	6.44	6.48	6.51	6.52	6.55	6.56	6.58	6.59	7.32	7.35	7.36	7.39	7.40	7.42	7.43
5-HT1B	S	Y	T	W	W	L	D	I	C	T	I	S	S	E	C	V	V	N	T	D	Y	T	S	T	A	F	F	W	F	F	S	L	M	P	L	F	D	T	W	G	Y
5-HT2B	A	T	I	W	W	L	D	V	S	T	I	A	I	T	C	V	L	T	K	E	F	M	G	S	A	F	F	W	F	F	N	I	L	V	Q	L	E	V	W	G	Y

Fig. 1: Alignment of ligand-interacting residues of 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors. The residues within orthosteric binding pocket are marked in blue and residues within secondary binding pockets are marked in yellow. The residues crucial for 1B/2B selectivity are marked with green circles.

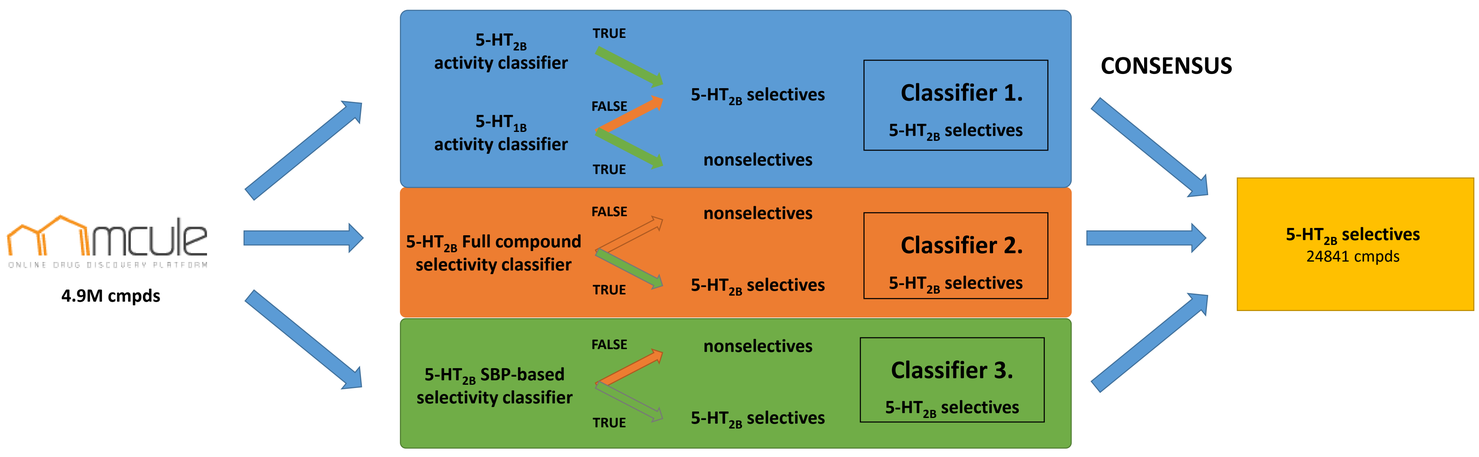
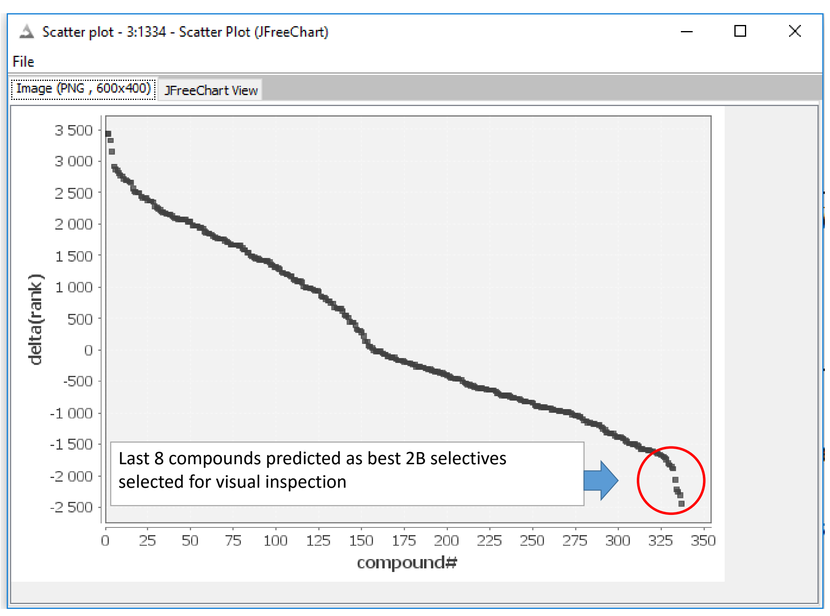


Fig. 3: Workflow of classifier creation and usage

Compounds were prepared using Ligprep software resulting in ~74k compounds with various conformational and protonation states. The docking was carried out using Glide software and 4 crystal structures: 2 of 5-HT$_{1B}$ (4IAQ i 4IAR) and 2 of 5-HT$_{2B}$ (4IB4 i 4NC3). Each of these structures have been crystallized with ergotamine as their ligand, which ensures that the only differences in the receptor-ligand complexes will be within the SBP part. To each of the structures all 74k compounds were docked, in order to determine their putative selectivity (good docking pose and glide scores when docking to 5-HT$_{2B}$ crystals and weak scores when docking to 5-HT$_{1B}$).

The docking results were first filtered by the existence of desired interactions within the compound (as shown in Fig. 4), and then scored based on the ranks achieved by each compound for each crystal (Fig. 5). Finally, 8 compounds were selected for visual inspection out of which 5 were chosen for *in vitro* studies.



$$\Delta rank = rank_{2B,4IB4} + rank_{2B,4NC3} - (rank_{1B,4IAQ} + rank_{1B,4IAR})$$

$$\Delta rank < 0, if\ the\ compound\ is\ 2B\ selective$$

Fig. 5: The ranking equation and the distribution of ranks between all tested compounds.

The 5 selected putative 5-HT$_{2B}$ compounds were screened *in vitro*, and 2 of them had a tested activity towards 5-HT$_{2B}$ receptor. Out of those 2 compounds, 1 expressed extremely high activity towards the receptor and a selectivity factor of 10000 (Fig. 6). This result proves that the methodology employed in this research is of **high reliability in producing selective GPCR compounds**.

The first step in this research was to filter out compounds from the Mcule database which had low probability of being active.

To do so, a set of 3 activity and selectivity machine learning classifiers have been developed, using known active and inactive compounds for 5-HT$_{1B}$ and 5-HT$_{2B}$ extracted from the ChEMBL [2] database and Substructural Connectivity Fingerprints, a new method of compound representation.

The database used for screening was the Mcule database, which totals 35M compounds [3]. We chose a subset of „In stock compounds", which contained 4.9M compounds at the time of conducting the research. This set was classified using the 3 built classifier sets, and each classifier highlighted a set of compounds with putative selectivity. The final set was constructed by performing a consensus scoring, that is a compound needed to have been classified as selective by all three classifiers. As a result, the set used further in docking contained ~25k compounds.

5-HT$_{1B}$	5-HT$_{2B}$
OBP:	OBP:
Asp H-bond with Asp129$^{3.32}$	Asp H-bond with Asp135$^{3.32}$
Atom-distance from Thr134$^{3.37}$	Atom-distance from Ser139$^{3.36}$
SBP:	SBP:
Atom-distance from SBP Ser212$^{5.42}$	Atom-distance from SBP Glu359$^{7.32}$
Atom-distance from SBP Met337$^{6.58}$	Atom-distance from SBP Met218$^{5.39}$
H-bond with SBP Val201$^{ECL2.52}$	H-bond with SBP Leu209$^{ECL2.52}$

Table 1: Pose filtering parameters for docking to 5-HT$_{1B}$ and 5-HT$_{2B}$ crystal structures

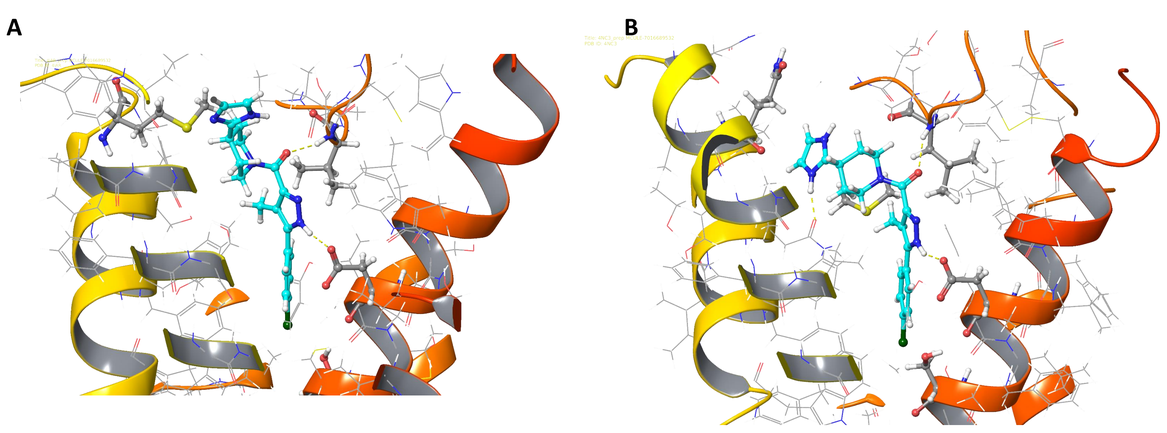
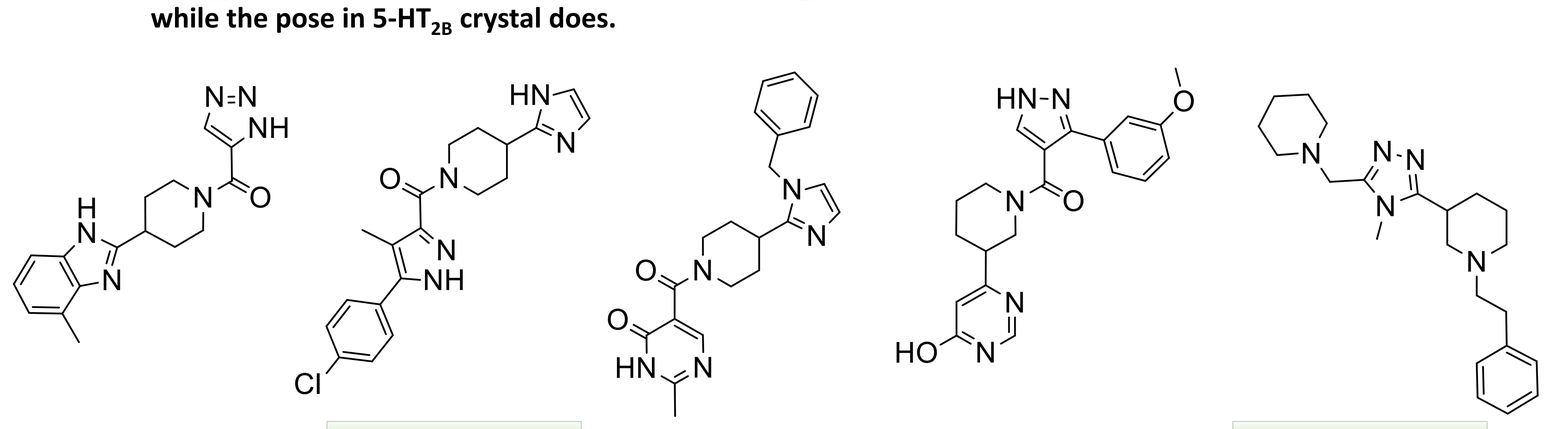


Fig. 4: Docking poses of MCULE-7016689532 to 5-HT$_{1B}$ crystal (A) and to 5-HT$_{2B}$ crystal (B). The pose present within the 5-HT$_{1B}$ crystal does not fulfill all preset constraints, while the pose in 5-HT$_{2B}$ crystal does.



MCULE-9820045710	MCULE-7016689532	MCULE-3664870072	MCULE-3991561829	MCULE-9933184416
5HT$_{1B}$ % (10uM): 12%	5HT$_{1B}$ IC50 (nM): 2934,7 nM	5HT$_{1B}$ % (10uM): 19%	5HT$_{1B}$ % (10uM): 7%	5HT$_{1B}$ % (10uM): 7%
5HT$_{2B}$ % (10uM): 40%	5HT$_{2B}$ IC50 (nM): 0,3 nM	5HT$_{2B}$ % (10uM): 34%	5HT$_{2B}$ % (10uM): 36%	5HT$_{2B}$ IC50 (nM): 2612,9 nM

Fig. 6: The 5 compounds selected for screening together with screening results.

Acknowledgments:

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