Development of Selective GPCR Ligands – 5-HT$_{1B/2B}$ Case Study

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The development of compounds capable of selectively targeting particular GPCR receptors has always been a major hurdle in the process of computer-aided drug design. The miniscule differences between the sequences and structures of closely related proteins make it extremely difficult to find a chemical moiety which would be able to differentiate between them.

Fortunately, as observed by Michino et al. [1], many of the GPCRs show signs of containing a secondary binding pocket (SBP) within their structure (Fig. 1). This allows to create compounds with two major parts; one active towards the orthosteric binding pocket (OBP); the second binding selectively to the secondary binding pocket of a particular receptor (Fig. 2).

In this research, an approach using SBP-OBP type compounds was employed in order to find ligands selective towards 5-HT1B and 5-HT2B receptors from within the MCule database.

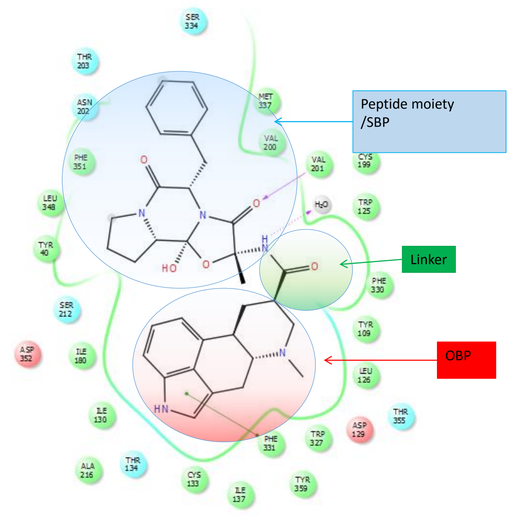

Fig. 2: An example of an SBP – selective compound; the OBP part is active towards both receptors, while the SBP part is responsible for selectivity

	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-HT1B and 5-HT2B receptors. The residues within orthosteric binding pocket are marked in blue and residues within secondary binding pockets are marked in yellow.

Fig. 3: Orthosteric binding pocket (A) and secondary binding pockets (B) present in 5-HT1B receptor structure.

In order to find compounds selective towards 5-HT1B and 5-HT2B receptors, three classifier sets were created, based on the compounds from the ChEMBL [2] database, and using the Substructural Connectivity Fingerprint (Fig. 4), as well as multiple machine learning methods, including SVM and EEM [3].

The first classifier set was a typical activity classifier based on known active and inactive compounds extracted from ChEMBL. Analysis of known ligands with these classifiers allowed to discard 1B compounds which were predicted to posess activity towards 2B and *vice versa*.

The second classifier set was based on selective compounds, that is reported to express activity towards one target and no activity towards the other. This step highlighted known ligands with possibility of being selective.

The final, third classifier set was a selectivity classifier created based only on the SBP parts of known selective ligands. To do so, all selective ligands for both targets were scanned in order to select those with the SBP-OBP type of structure. Next, the selected compounds were cut into the respective parts, and the SBP moieties were used as the input for the classifier. This step ensured, that the compounds selected for further testing would have the SBP-OBP type of structure, and that the SBP part would be responsible for eventual selectivity.

Finally, the three classifiers were used to scan the MCule database (4.9M compounds) for putatively selective compounds for both targets and a consensus set was selected for docking experiments.

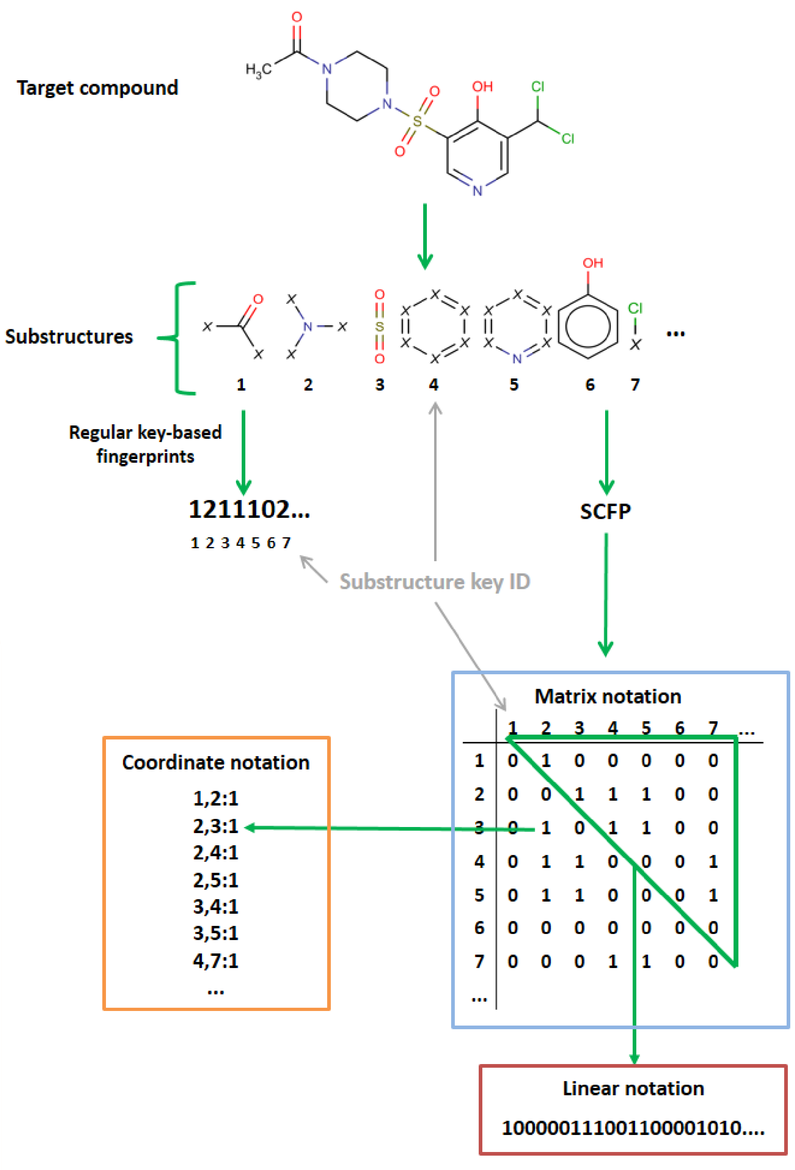

Fig.4: Visualization of the SCFP fingerprint

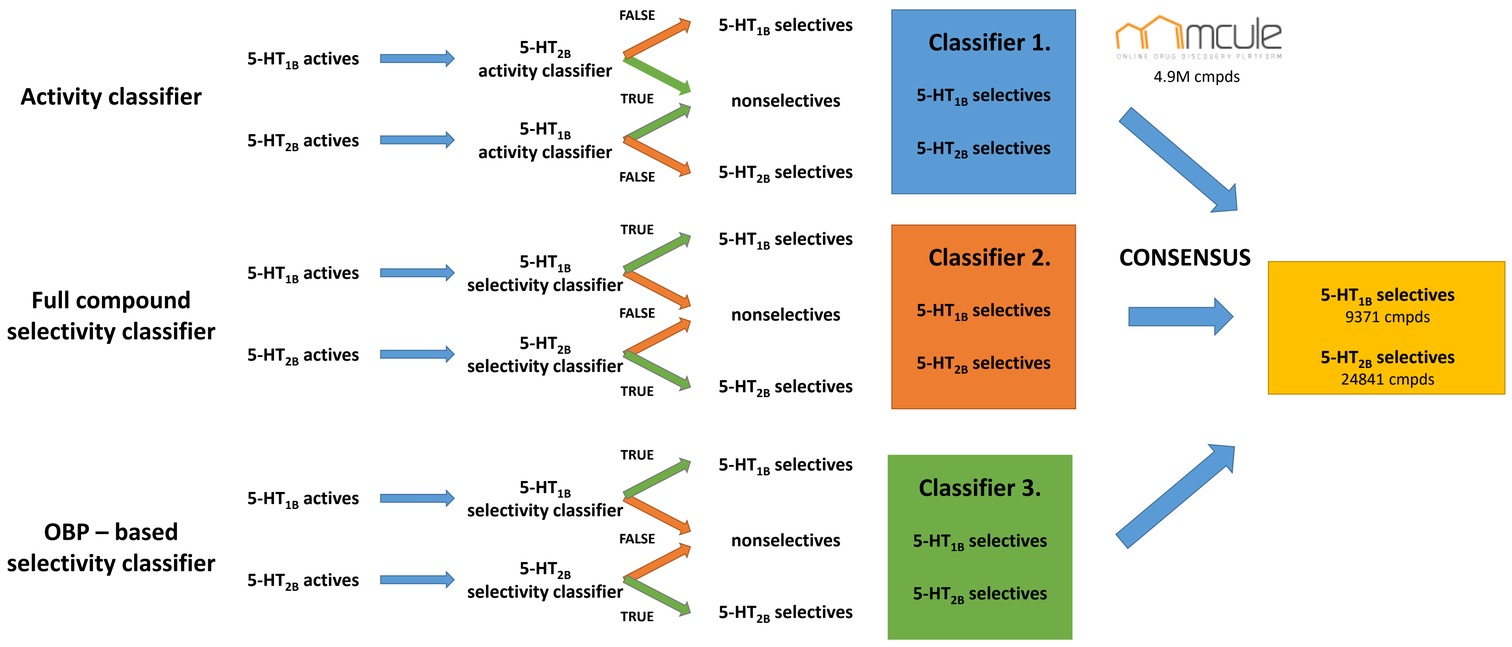

Fig.5: Workflow of classifier creation and usage

The compounds selected in the ML-based classification step were then used in series of docking experiments, in order to highlight the final set for *in vitro* screening. For that purpose 2 crystal structures of 5-HT1B and 5-HT2B were used, and 9371 putative 1B-selective and 24841 2B-selective compounds were docked into both structures. The docking created multiple ligand-receptor conformations which were then visually inspected for the proper interactions within the SBP.

In order to select the best compounds, a custom scoring function has been created including cross-scoring in case a compound was able to dock into both 1B and 2B crystal structures.

Finally, a set of 8 compounds was highlighted for each selectivity type, out of which 5 were chosen for *in vitro* screening.

Acknowledgments:

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