The Development and Validation of a Novel Virtual Screening Cascade Protocol to Identify Potential Serotonin 5-HT$_7$R Antagonists

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Introduction

The 5-HT$_7$ receptor (5-hydroxytryptamine$_7$) is a seven-transmembrane–domain G protein-coupled receptor positively linked to adenylyl cyclase and discovered through targeted cloning strategies about 16 years ago. It plays an important role in thermoregultion, circadian rhythm, learning and memory, hippocampal signaling and sleep. It is suggested that the 5-HT$_7$ receptor is also involved in other psychiatric and neurological disorders, such as schizophrenia, epilepsy and migraine.[1] Current research strongly indicate the development and investigation of 5-HT$_7$ antagonists determine new direction in the field of novel antidepressant agents research.[2]

The virtual screening is a new approach that allows discovery of novel ligand from large libraries of diverse and commercially available compounds by using information about the structure of protein binding site and/or known ligands. In literature it can be found many examples of successfully employment of this technique for the searching for new and potent ligands for a different targets.[3-5]

Using our rhodopsin-based homology model[6] and a set of 31 diversified 5-HT$_7$ receptor antagonists we have performed Virtual Screening Cascade Protocol by means of two-dimensional (2D) pharmacophore similarity, physico-chemical scalar descriptors, ADME/Tox filter, three-dimensional (3D) pharmacophore searches and docking protocol, to discover novel 5-HT$_7$ antagonists of new chemical scaffold. A set of about 730 000 commercially available drug-like compounds from the Enamine Screening Collection was served as the screening library.[7]

Computational Methods

The computational approach we developed for screening molecules acting as 5-HT$_7$ antagonists combines miscellaneous well-known methodologies and software ability within an integrated framework. A flowchart illustrating phases of the Virtual Screening Cascade Protocol implemented in our study is shown in figure below.

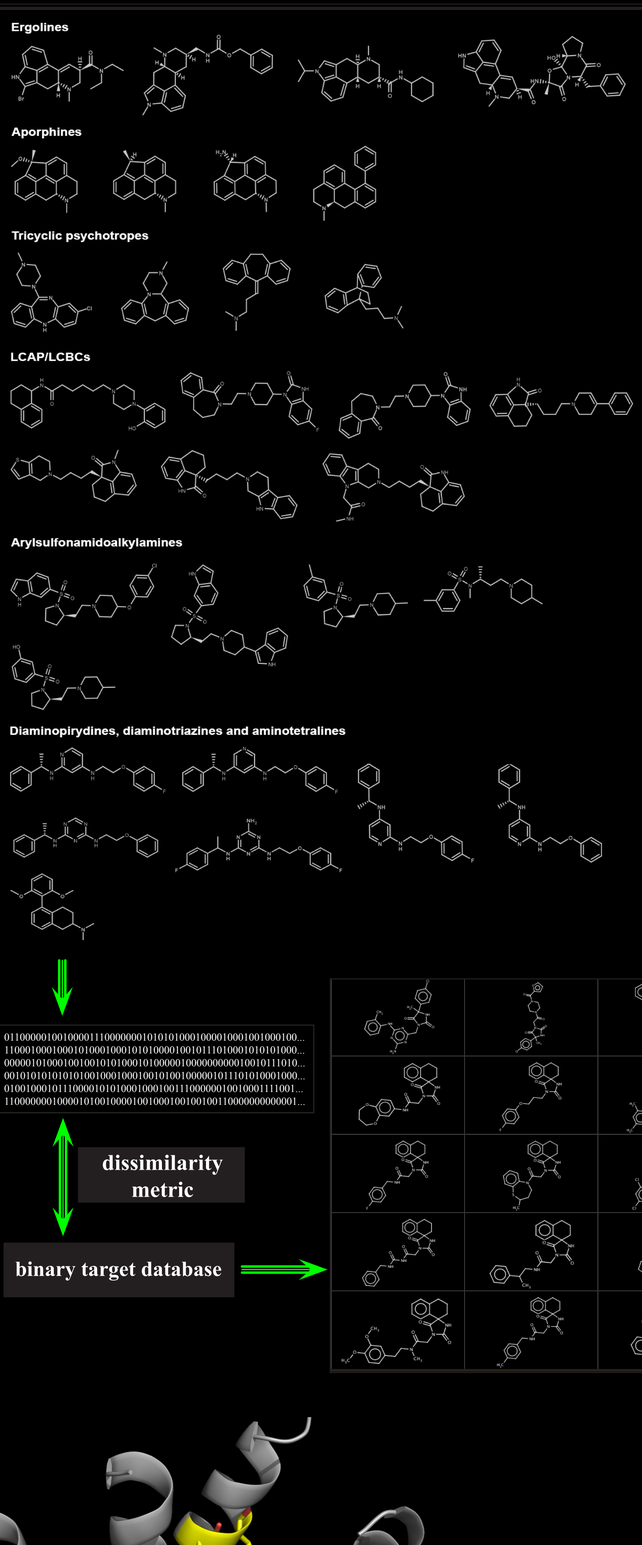
2D Pharmacophore-based Screening

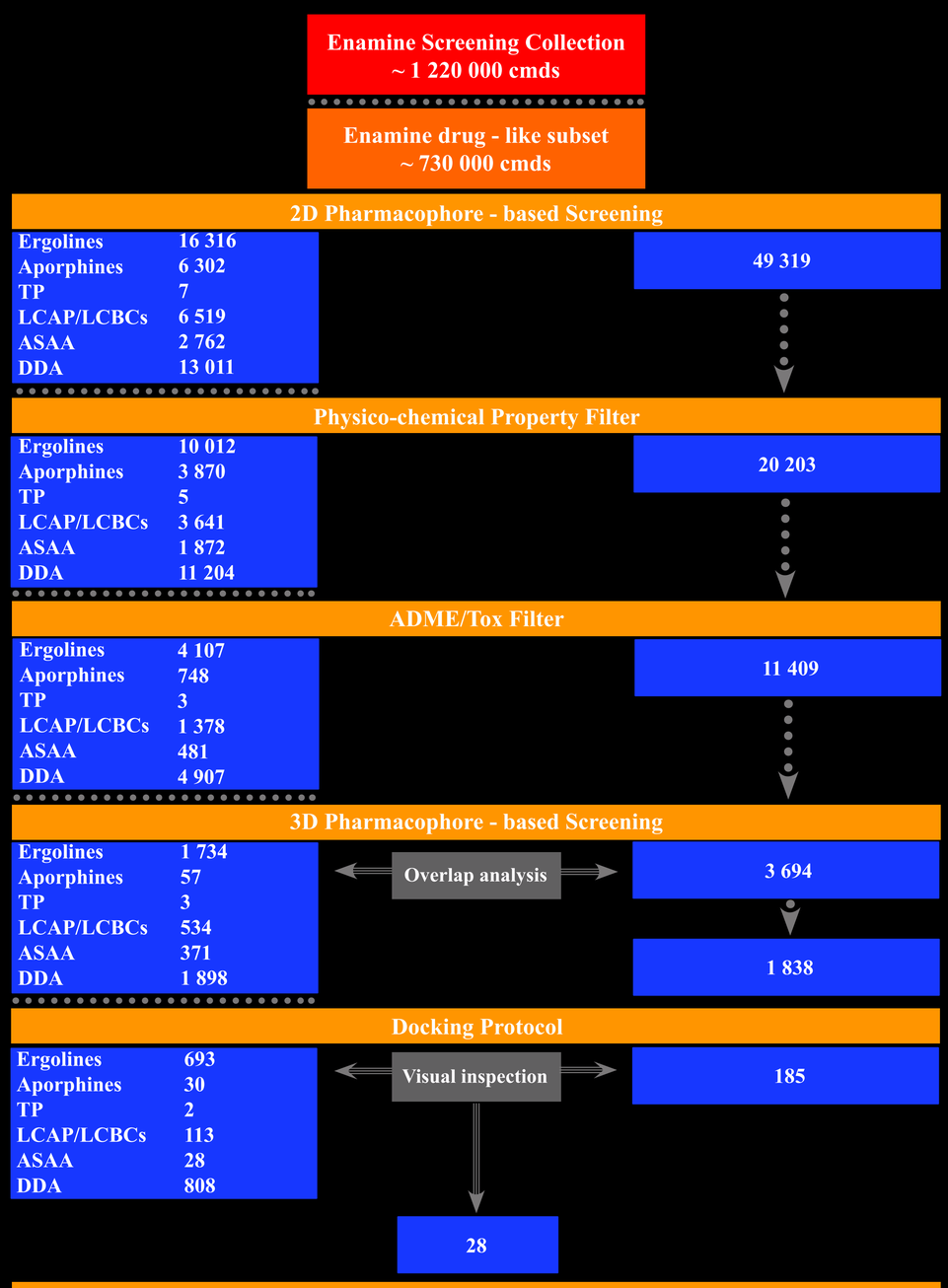
Molecular structures were encoded into easy to handled numerically molecular descriptors called as binary fingerprints. Query structures and target database are compared against each other by using similarity/dissimilarity metric. Only those virtual hits are accepted whose computed score is below predefined threshold.

A 31 diversified antagonists of 5-HT7 receptor were used as the set of query structures. Those the twofold approach of virtual hits generation was adopted. In the first path, query was built with respect to chemical classification of input structures whilst the pharmacophore hypothetical model generation for all input structures was implemented in the second path.

Enamine Screening Collection consists of more than 1 300 000 organic compounds that were produced by original synthetic techniques, developed by Enamine and its partners. Library of drug-like subset was created from Screening Collection by application of all filters and cutoffs proposed by Baurin, Baker and Richardson (see table below).[8]

Lipinsky's Rule of 5	Mol. weight	≤ 500
	QlogP	≤ 5
	H-bond acceptors	≤ 10
	H-bond donors	≤ 5
Vebr's Rule	Rotating bonds	≤ 10
	FISA	≤ 170 A^2



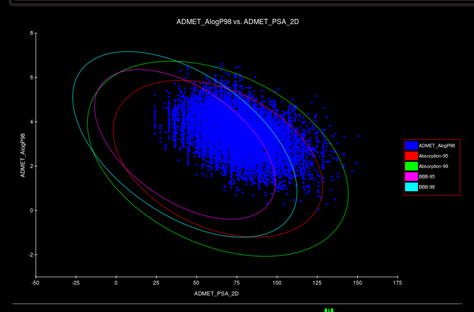


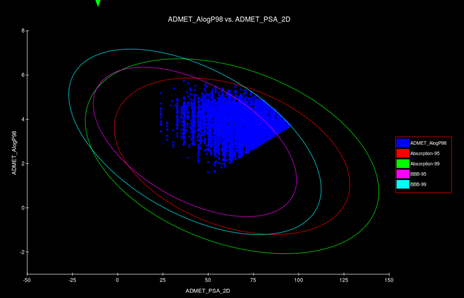
Physico-chemical Property Filter

Scalar descriptors are single values associated with an molecular structure and may involve the estimation of physico-chemical properties. The strongest basic pKa was taken into account to filter the initial set of compounds.

ADME/Tox Filter

Computing the ADMET descriptors allow in the fast and simple way to eliminate compounds having unfavorable ADMET profile. It tooks into account three descriptors predicted approximately: the Human Intestinal Absorption after oral administration, the solubility of each compound in water at 25ºC and the blood brain-barrier penetration by the molecule. Fulfilled the matching criteria lead to reduction of input set of molecules (see figures below).



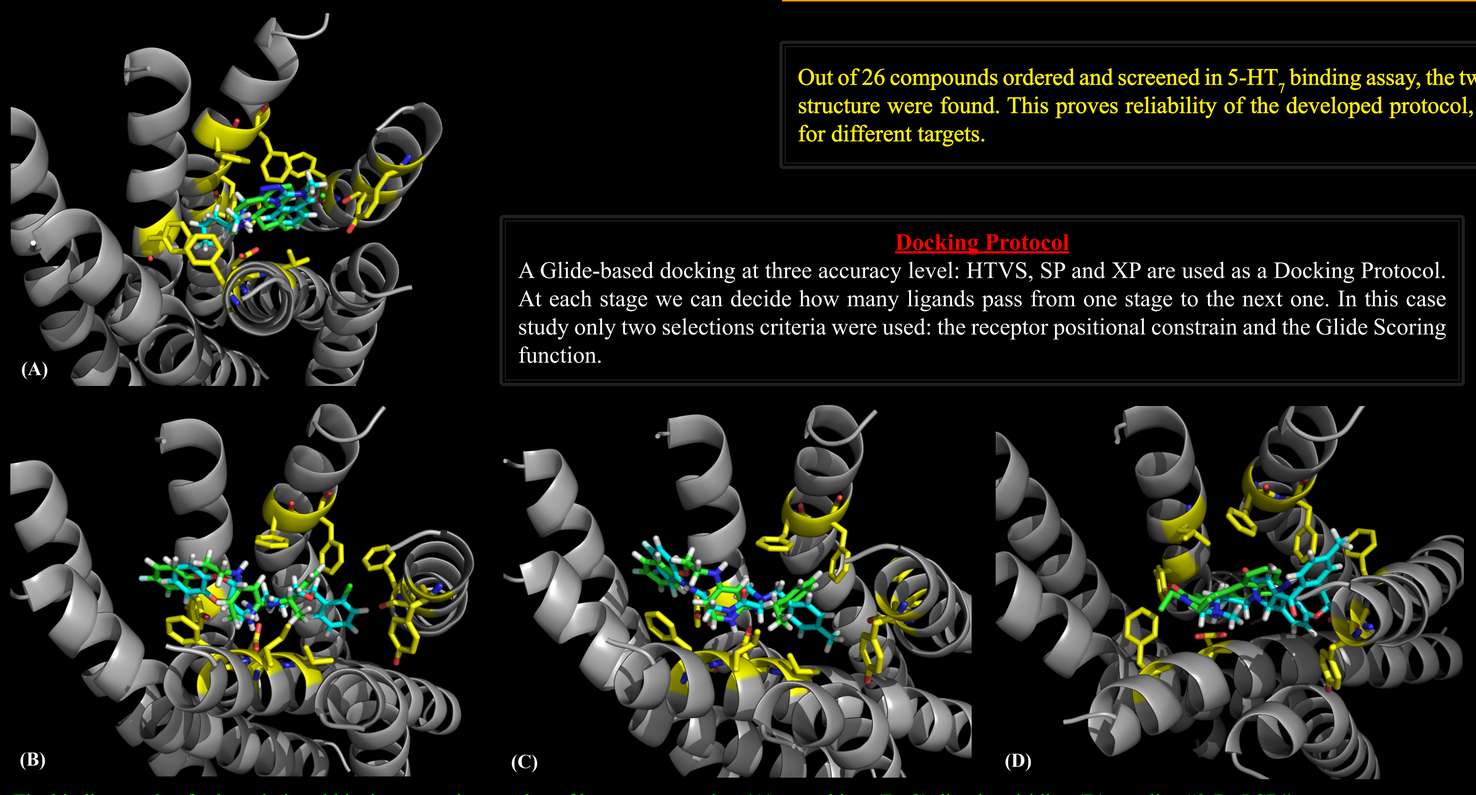


Plot of Polar Surface Area (PSA) vs. LogP for a sample compounds before and after the reduction, showing the 95% and 99% confidence limit ellipses corresponding to Blood-Brain Barrier and Intestinal Human Absorption models.

Out of 26 compounds ordered and screened in 5-HT$_7$ binding assay, the two new ligands of similar structure were found. This proves reliability of the developed protocol, which now can be used for different targets.

Docking Protocol

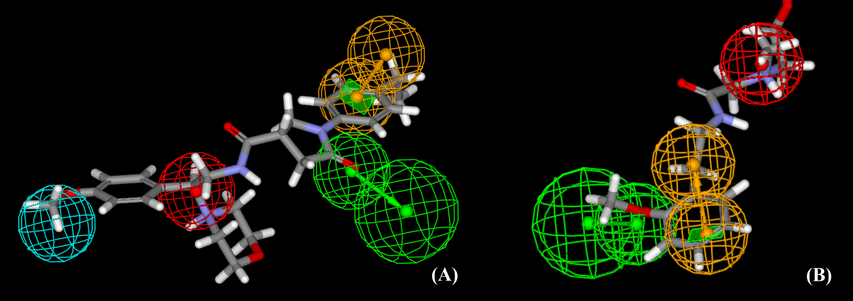
A Glide-based docking at three accuracy level: HTVS, SP and XP are used as a Docking Protocol. At each stage we can decide how many ligands pass from one stage to the next one. In this case study only two selections criteria were used: the receptor positional constrain and the Glide Scoring function.



The binding mode of selected virtual hits in comparison to that of known antagonists (A) aporphine, (B, C) diaminopiridine (D) ergoline "2-Br-LSD".

3D Pharmacophore-based Screening

Since molecules interacting at the same receptor site in the same way share a common pharmacophore model, 3D pharmacophore searching should be able



Two different compounds mapped on the pharmacophore models generated for (A) all antagonists (second path) and (B) LCAP/LCBCs family (first path).

Acknowledgment

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