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The development and validation of a novel virtual screening cascade protocol to identify potential serotonin 5-HT$_7$R antagonists

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ABSTRACT

In an attempt to identify new ligands for the 5-HT$_7$ receptor (5-HT$_7$R), we developed and tested a hierarchical multi-step strategy of virtual screening (VS) based on two-dimensional (2D) pharmacophore similarity, physicochemical scalar descriptors, an ADME/Tox filter, three-dimensional (3D) pharmacophore searches and a docking protocol. Six chemical classes of 5-HT$_7$R antagonists were used as query structures in a double-path virtual screening scheme. The Enamine screening database, consisting of approximately 730,000 commercially available drug-like compounds, was adopted and used as a source of structures. A biological evaluation of 26 finally selected virtual hits resulted in finding two benzodioxane derivatives with significant affinity (K_i = 197 and 265 nM). The approach described in this case study can be easily used as a general rational drug design tool for other biological targets.

The 5-HT$_7$ receptor (5-HT$_7$R) is a seven-transmembrane–domain G protein-coupled receptor, positively linked to adenylyl cyclase and discovered through targeted cloning strategies about 16 years ago.[1] It is located in the central nervous system (thalamus, hypothalamus, hippocampus, cortex) and in peripheral tissues (pancreas, spleen, coronary artery, ileum).[2–4]

The 5-HT$_7$R plays an important role in thermoregulation,[5] circadian rhythm,[6] learning, and memory,[7] relaxation of vascular smooth muscles[8] and sleep.[9] The 5-HT$_7$R may also be involved in such psychiatric and neurological disorders and processes as schizophrenia,[10] epilepsy,[11] migraine,[12] and nociception.[13] Current research strongly indicates that the development and investigation of 5-HT$_7$R antagonists determine new direction in the field of novel agents for treating depression and anxiety.[14]

One way to discover novel ligands for a given target is application of virtual screening (VS) to large databases of diverse and commercially available compounds. This approach needs support by information about the structure of a protein binding site or/and chemical structures of known active ligands.[15]

The VS techniques were effectively employed to identify new ligands for different GPCRs[16–18] but, to the best of our knowledge, they were used only once for searching for new 5-HT$_7$R antagonists.[19]

The computational approach used in the present study combines miscellaneous well-known methodologies and different software within an integrated framework. The hierarchical multi-step virtual screening protocol is based on two-dimensional (2D) pharmacophore similarity, physicochemical scalar descriptors, ADME/Tox filter, three-dimensional (3D) pharmacophore searches and finally a docking protocol (Scheme 1). As an input, we used our modified rhodopsin-based homology models[20] and a set of 31 well-known 5-HT$_7$R antagonists. They were divided into several structural classes, as described previously,[20] namely ergolines (4 cmds), aporphines (4 cmds), tricyclic psychotropic agents (TP—4 cmds), long-chain arylpiperidines/piperazines or long-chain β-carbolines (LCAP/LCBCs—7 cmds), arylsulfonamidoalkylamines (ASAA—5 cmds), and, lastly, a class combining diaminopyridine, diaminopyrimidine, and diaminotriazine derivatives (DDA—7 cmds); (Fig. 1). A set of ca. 730,000 commercially available, drug-like compounds from the Enamine Screening Collection,[21] served as a screening library.

2D Pharmacophore-based similarity searches were performed with the use of Screen,[22] a command line tool of ChemAxon software, using two-dimensional pharmacophore-based fingerprints. All the fingerprints were generated from 2D molecular structures by defining the collection of all atom–atom pharmacophore feature pairs along with their topological distances, and were then handled numerically. To enhance the diversity of final results, we adopted two paths of similarity searching. In the first path, each chemical class of 5-HT$_7$R antagonists composed individual queries, while the intersection (understood as an average of query fingerprints) of all the 31 antagonist fingerprints was calculated and set up as the second path. The Enamine Screening Collection library was scanned, and only structures with similarity metrics values higher

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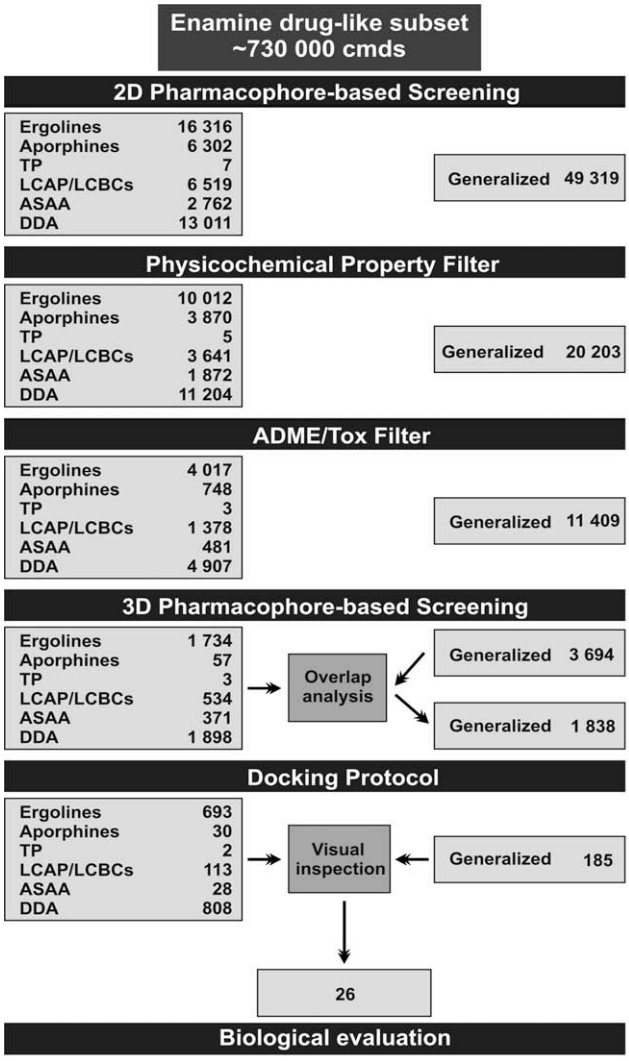

Scheme 1. A scheme of the multi-step virtual screening protocol designed for identification of 5-HT$_7$R antagonists. Numbers indicate the quantity of compounds at the output of each step. The left path shows a chemical-class-based search, where 2D and 3D ligand-based searches were performed separately for each class of 5-HT$_7$R antagonists. The right path explored the generalized hypothesis for all the classes of 5-HT$_7$R antagonists.

than assumed thresholds were regarded as virtual hits. The optimized Tanimoto or Euclidean metrics was used as a bit pairwise similarity measure, and threshold values differed for each optimized metrics for all the models.

Physicochemical property filter criteria were obtained from the physicochemical property distribution within a range of each chemical class of 5-HT$_7$R antagonists. We applied only the strongest basic pK_a as filter criteria (since the existence of a basic amine group is mandatory, due to the well-recognized ionic interaction with Asp3.32 of the receptor). Therefore only virtual hits for which the descriptor (calculated in Instant JChem[23]) covered the range of known antagonists (increased by ±20%) were maintained.

ADMET descriptors allow us to eliminate compounds with an unfavorable ADMET profile in a simplified but fast way. It is widely recognized that in silico prediction of these properties has a potential to significantly contribute to the streamlining and shortening of a drug design process, hence it should be considered as early as possible.[24] We took account of the three descriptors, predicted approximately by means of the ADMET Descriptor Protocol combined in Discovery Studio[25] software. Those descriptors were Human Intestinal Absorption after oral administration (HIA), the solubility of each compound in water at 25 °C, and the blood brain barrier penetration by a molecule (BBB). Only compounds predicted to have: moderate-to-good intestinal absorption (HIA Level < 2), optimal-to-good solubility in water (2 < Aqueous Solubility Level < 5), and medium-to-very high blood brain penetration index (BBB Level < 3) were selected for the next phase.

3D pharmacophore generation and screening of the remaining compounds were performed using the CATALYST module of Discovery Studio.[25] In the first screening path, separate pharmacophore models were generated for each chemical class of antagonists (see Supplementary data, Table S1). The arrangement of 3D features (PI—positive ion, HYD/AR—hydrophobic/aromatic, and/or HBA—H-bond acceptor) of each of those class-specific pharmacophore models mapped well on general, docking-based models published previously (separate pharmacophores for affinity and selectivity),[20] that is they constituted submodels for at least one hypothesis. For chiral compounds all possible stereoisomers were taken into account (FAST conformation generation algorithm).[25] The screened compounds were allowed to be maximally devoid of one feature during mapping to a given pharmacophore model to be considered as a hit. Partial mapping allows for a more diverse set of hits and reduces the risk of omitting compounds that can map incompletely due to their structural heterogeneity and/or the FAST algorithm[25] of conformation generation. To avoid multiple docking of the same compounds from both paths, a simple overlap analysis was carried out followed by the removal of duplicates from the second set.

The automated docking with interaction constraint (ionic interaction between the protonated amine group of the ligand and Asp3.32 side chain) was performed using Virtual Screening Workflow within Glide[26] software, consecutively at three accuracy levels: HTVS, SP, and XP. Stereoisomers were maintained by using a LigPrep tool to generate all the possible configurations that result from the combination of chiral centers. All the stereoisomers were treated separately. At each stage we decided on how many ligands were passed from one stage to the following one (HTVS: 50% compounds; SP: 70% compounds; XP: 80% compounds). We used as targets our previously published, modified rhodopsin-based homology models of 5-HT$_7$R receptor. Six different models (receptor binding site conformations) were used that the best accommodated ligands from a particular chemical group. In the first path, compounds detected by 2D and 3D ligand-based searches for a particular class were docked to 'their' receptor models. In the second, generalized path, all the compounds were docked to all the six receptor models.

At this final stage, all top results were evaluated independently by four team members. The main estimation criteria were the occurrence of particular interactions between ligands and the binding site, and diversity of chemotypes.

All evaluators scored 1 if a ligand fulfilled the selection rules, and 0 when it did not. The scores were gathered and added up. Only ligands with a final score 3 or 4 were accepted for biological investigation.

Finally, a set of 26 compounds, selected on the basis of structural diversity and visual inspection of their binding modes, was acquired from Enamine (see Supplementary data, Table S2) and tested for 5-HT$_7$R receptor activity at two compound concentrations (10^{-6} and 10^{-7} M).

Of the 26 compounds tested, only two showed a significant percentage of inhibition of the radioligand ([^{3}H]-5-CT) binding in cell lines with a stable expression of human 5-HT$_7$R receptors (67% and 75% at 10^{-6}, and 23% and 29% at 10^{-7} for T6053274 and T6068081, respectively). In full binding experiments, those ligands displayed significant affinity (Table 1). To the best of our knowledge, 5-HT$_7$R affinity has never been reported for such benzodioxanes, so our virtual screening protocol was able to identify novel hits from the library of