Verification of virtual screening results for 5-HT$_6$ receptor in *in vitro* experiments

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INTRODUCTION: There are a lot of data indicating pro-cognitive properties and potential therapeutic activity of 5-HT$_6$ receptor ligands which can be beneficial, among others, in Alzheimer's disease [1], schizophrenia [2] and depression [3]. Virtual screening (VS) is a cheminformatic tool used in a search for new biologically active compounds. Depending on available structural data about biological target and number of know ligands, VS methods are divided on "ligand-based" and "structure-based". In the present work both approaches were used for VS of the in-house (Department of Medicinal Chemistry) library, to identify activity for serotonin 5-HT$_6$ receptor. The VS results were verified in *in vitro* experiments and Ki values for selected group of 33 structurally related compounds (in which VS indicated 8 active ligands) were measured.

In silico studies

The development of homology models of transmembrane domain of 5-HT$_6$ receptor.

Models of a 5-HT$_6$ receptor were generated using β_1-adrenergic template (PDB ID: 2VT4). Selection of models was based on docking results of a set 106 ligands (65 referenced ligands from publication [4] and 41 ligand-like compoumds with Ki > 1000 nM). The three models with the highest discrimination ratio were next used in VS of the whole set (about 1000) of our compounds library.

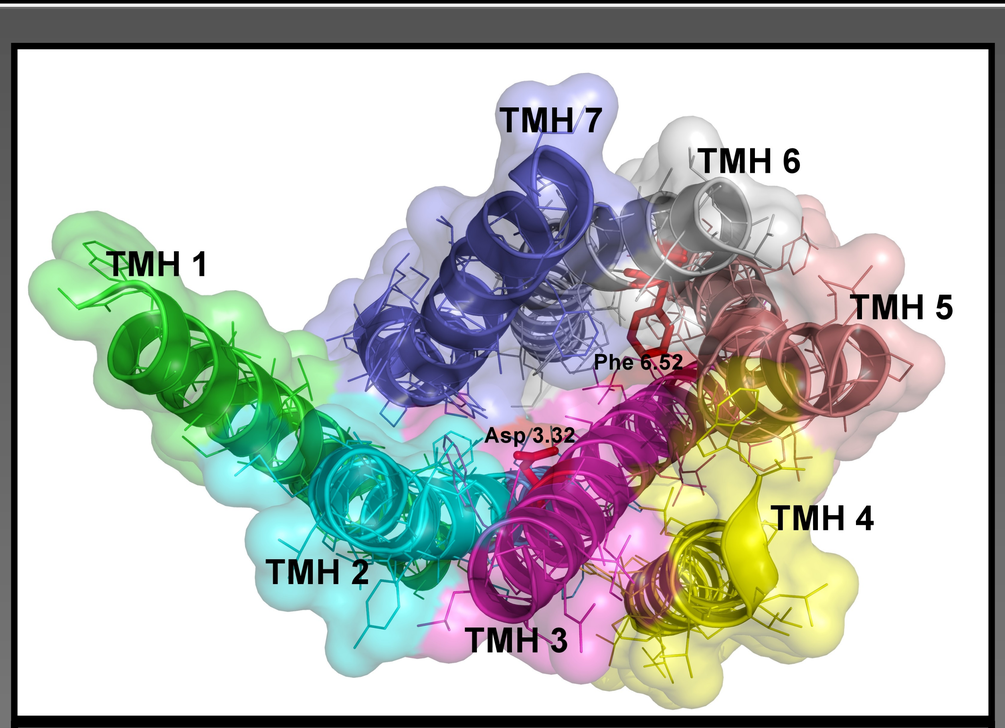


Figure 1. One of the three representative models of 5-HT$_6$ receptor.

The application of Support Vector Machine (SVM) methods to fingerprint representation of compounds library for preselection of derivatives.

Six kernel functions were used to learn SVM: linear, polynomial of the degree 2-5 and radial base function. If the compound was at least once positively rated by one of them, it was qualified to second stage of virtual screening, i.e. docking.

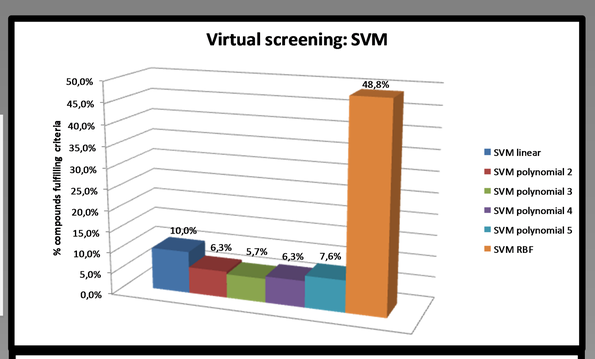


Figure 2. The graph showing % of compounds fulfilling the criteria of kernel functions.

Glide docking of 505 agents which passed SVM stage to the developed homology models at accuracy level: XP.

Hydrogen bond between Asp 3.32 and the protonated amine group of the ligand and interaction between the aromatic rings with Phe 6.52 were applied as constrains in all docking simulations.

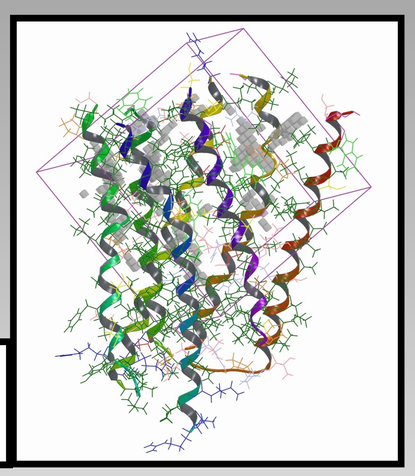


Figure 3. The grid size (big box), and hydrophobic regions of the receptor (grey cubes).

As a result of VS more than 200 compounds with potential 5-HT$_6$ receptor activity were identified.

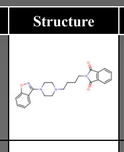
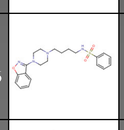
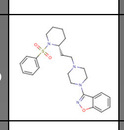
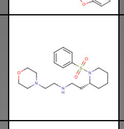
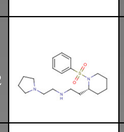
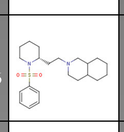
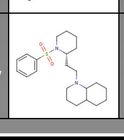
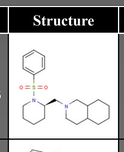
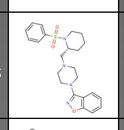
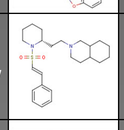
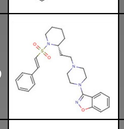
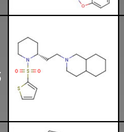
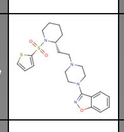
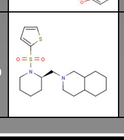
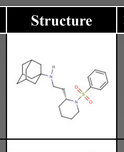
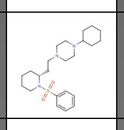
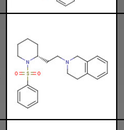
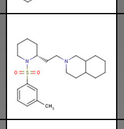
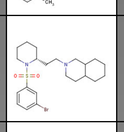
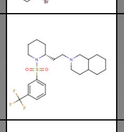
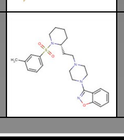
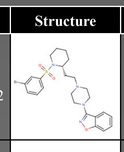
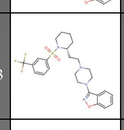
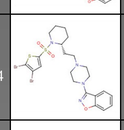
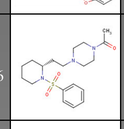
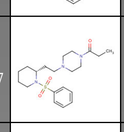
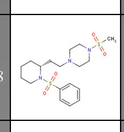
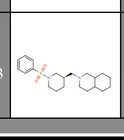
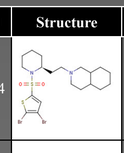
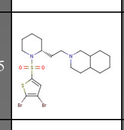
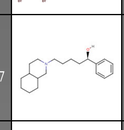
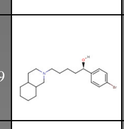
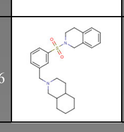
In vitro experiments

Next, the *in silico* results were verified in *in vitro* experiments. At first, radioligand methods were optimized to the format of 96-well plate and MultiProbe II HT EX – an automated liquid handling system.

The saturation and competition experiments with the use of [^{3}H]-LSD, HEK293 cell line with a stable expression of 5-HT$_6$ receptor, and reference agents (clozapine, olanzapine, ritanserin and dihydroergotamine) validated the applied procedure.

Figure 4. Automated liquid handling system – MultiProbe II HT EX.

In the successive stage, Ki values for a selected group of 33 structurally related compounds (in which VS indicated 8 active ligands) were measured.

Compd	Structure	Ki [nM] 5-HT6
1 RB-131		874
2 RB-156		341
3 RB-159		393
4 RB-161		228100
5 RB-162		60700
6 RB-166		25360
7 RB-167		27110
8 RB-173		25340
9 RB-175		5626
10 RB-177		7917
11 RB-179		91
12 RB-185		15540
13 RB-187		527
14 RB-189		30730
15 RB-198		7837
16 RB-201		5468
17 RB-202		15160
18 RB-207		11970
19 RB-208		2999
20 RB-209		9287
21 RB-211		123
22 RB-212		62
23 RB-213		198
24 RB-214		42
25 RB-226		54460
26 RB-227		796300
27 RB-228		262200
28 RB-233		4818
29 RB-234 (S)		2394
30 RB-235 (R)		397
31 RB-237		23690
32 RB-239		16710
33 RB-246		4869

In *in vitro* experiments significant affinity was found for 10 compounds, 5 of which were correctly predicted by VS protocols, therefore shownig good model predictability. Majority of active ligands were successfully docked to the best model (50%), which recognized also only 8% of inactive compounds.

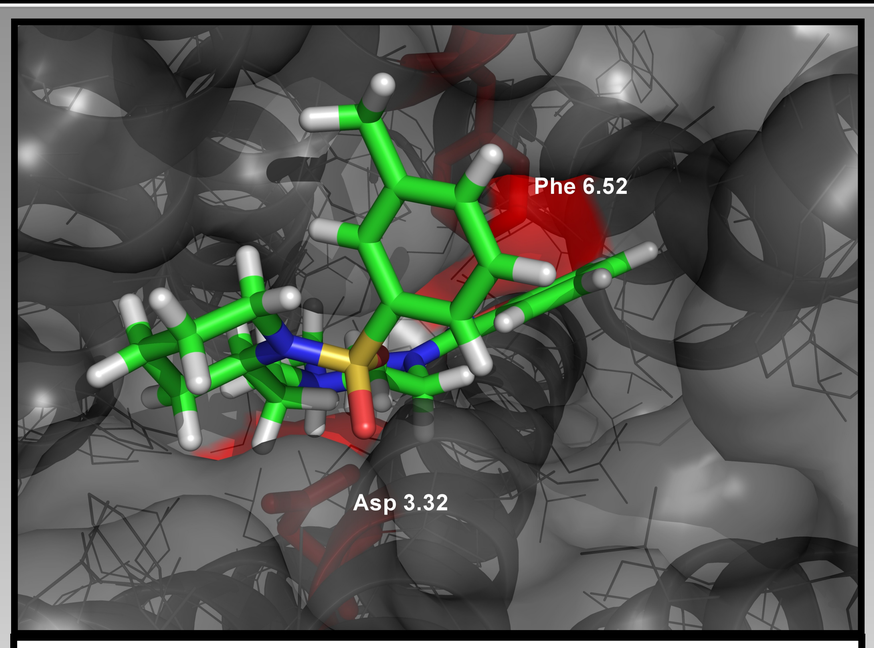


Figure 5. Representative binding mode: compound RB-211 within the 5-HT$_6$ receptor model.

CONCLUSION: The developed and validated 5-HT$_6$ models will be used in VS of commercially available compounds databases.

References:

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