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Structure-based design of inhibitors for dopamine receptors

Bachelor's thesis

done at

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under the supervision of

dr. hab. Rajmund Kaźmierkiewicz

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ABSTRACT

Molecular modeling allows for *in silico* analysis of molecules, which in the future may be a useful tool in drug design. In the case of dopamine receptors, inhibitors are sought that, after specific binding to a given receptor, could effectively block the production of dopamine to reduce the effects of diseases related to the dopaminergic regions such as bipolar disorder, drug addiction and schizophrenia. Thanks to *de novo* molecules design provided by LigBuilder program, after choosing the best cavity model and selecting the most appropriate candidates, I managed to get one inhibitor for each of the five dopamine receptors that mostly passed toxicity tests and can bind at the dopamine binding site, block docking of it.

INTRODUCTION

Dopamine is one of the most important human's neurotransmitters. It belongs to catecholamine family and plays major role in behavioral biology of human body. Its expression in brain is responsible for locomotion activity, reward and reinforcement mechanisms, addiction, and cognition. Dopamine is also present in peripheral - kidney, adrenal gland, sympathetic ganglia, and blood vessels - where it acts mainly as a vasodilator and paracrine messenger.

Dopamine can exert its effects after binding to the cell surface dopamine receptors which belongs to the class A of seven transmembrane domain G protein-coupled receptors family. There are five types of dopamine receptors in human that we know of: D_1 – D_5 receptors. They differ in amino acids sequences, their function and quantity in a cell concerned. We can classify them into two families: D_1-like and D_2-like. D_1-like consists of D_1 and D_5 receptors and D_2-like of D_2, D_3 and D_4 receptors. Classification is based on increasing or decreasing levels of cAMP inside the cells. D_1-like is known of activation of adenylate cyclase which as a result increase intracellular cAMP level and D_2-like works as inhibitor of adenylate cyclase decreasing cAMP level inside the cells. Furthermore, another difference is that D_1-type have large C-terminal domain and D_2-type have large intracellular loop-3.

Dysfunctions of dopaminergic pathways may lead to various diseases. They are caused by underproducing (e.g., Parkinson Disease, Tourette Syndrome, Hypertension, Major Depression) or overproducing (Bipolar Disorder, Drug Addiction, Schizophrenia, Nausea and vomiting) dopamine in given system. There are already many drugs that can

inhibit or stimulate dopamine receptors that we know. The main problem for them are side effects and their undesirable interaction with other receptors. In my work, I will focus on specific inhibitors for each of dopamine receptors, which may also be called antipsychotics or neuroleptics, to reduce overproduction of dopamine that causes above-mentioned diseases.

1. Extraction and optimization

First step was to obtain the spatial structure of dopamine receptors and prepare them to further analysis. Each of the structures, except the D_5 receptor, were available in the PDB database as a component of the protein complexes. Extraction was possible by removing unnecessary chains and ligands. In D_5 receptor case, I had to perform structure prediction by homology using Protein HomologY/analogy Recognition Engine V 2.0 (Phyre2) program. Phyre2, using PSI-BLAST algorithm, scans databases for similar sequences to the given one. Then, the generated profile is processed by neural network structure prediction program (PsiPred) and protein disorder predictor program (Disopred). The preparation process consisted of energy minimalization and adding missing hydrogens and counterions (Cl^-) to neutralize the protein. For that, I used molecular dynamics package - GROMACS. The visualization of molecules was made possible by the PyMOL program.

1.1 Dopamine receptor D_1

Dopamine receptor D_1 on which my work was based belonged to the protein complex from PDB database with ID 7LJD and has a length of 446 amino acids. It is *Allosteric modulator LY3154207 binding to dopamine-bound dopamine receptor 1 in complex with miniGs protein*. The result of the optimization was the addition of 15 counterions and achieving neutral energy after 1449 ps of approximately equal -1,946e+06 kJ/mol.

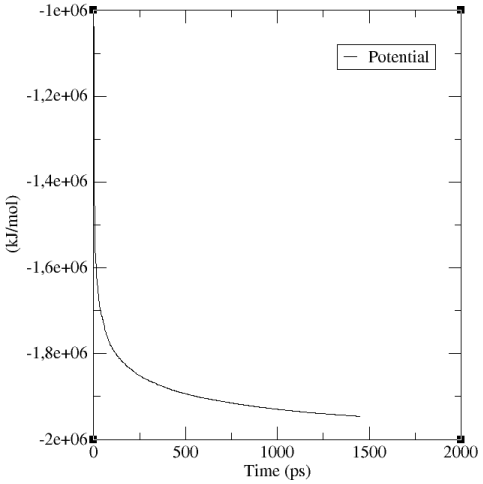


Fig. 1a Energy minimalization of D_1 receptor.

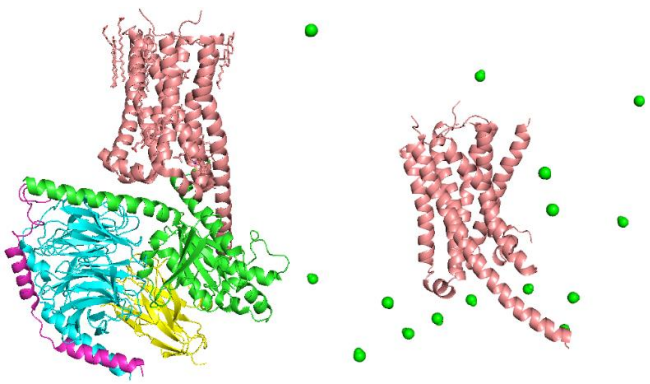


Fig. 1b 7LJD (left) and D_1 receptor after optimalization (right).

1.2 Dopamine receptor D_2

Structure for the first member of D_2-like family was obtained from 7JVR PDB ID with the title: *Cryo-EM structure of Bromocriptine-bound dopamine receptor 2 in complex with Gi protein* and is 443 amino acids long. Optimization was successful, adding to structure 10 counterions and lowering the potential system energy to approximately -1,2065e+06 kJ/mol after 1296 ps.

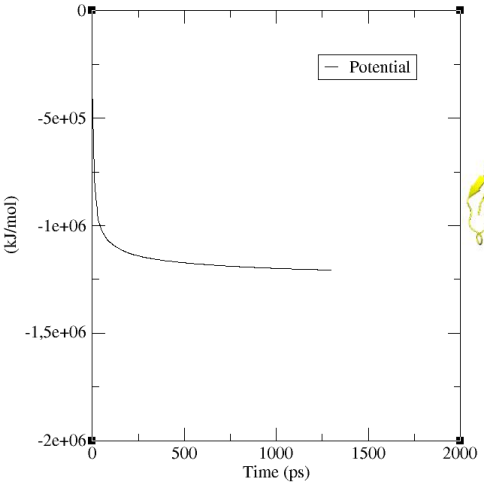


Fig. 2a Energy minimalization D_2 receptor.

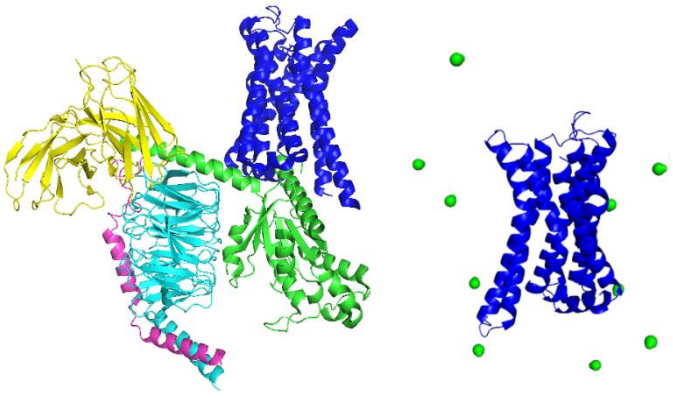


Fig. 2b 7JVR (left) and D_2 receptor after optimization (right).

1.3 Dopamine receptor D_3

In the case of D_3 receptor, complex from which I extracted the receptor, having a length of 400 amino acids, was *Dopamine Receptor D3R-Gi-PD128907 complex* (7CMV). Optimization has brought results, in form of 8 counterions and system with potential energy approximately equal to -1,8665e+06 kJ/mol after 1742 ps.

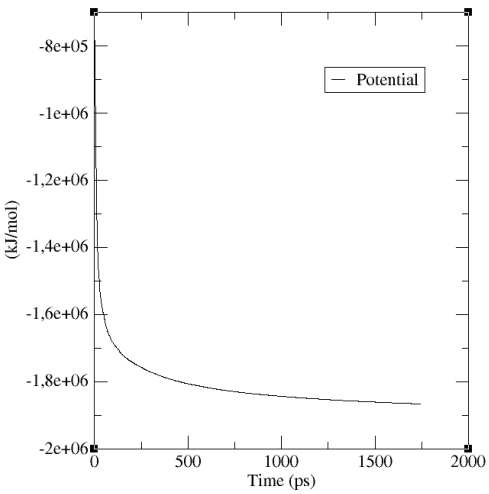


Fig. 3a Energy minimalization D_3 receptor.

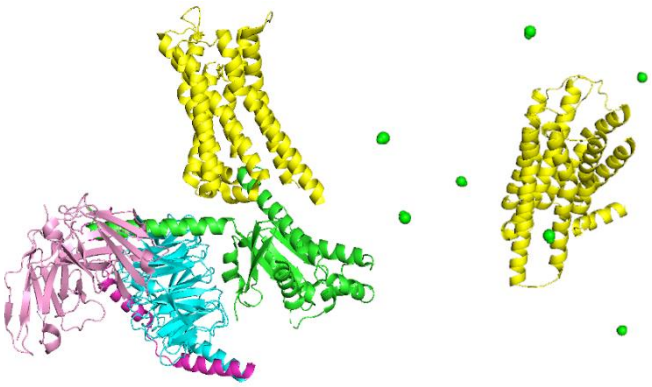


Fig. 3b 7JVR (left) and D_3 receptor after optimization (right).

1.4 Dopamine receptor D_4

D_4 receptor had its spatial structure in the PDB file titled: *Structure of the human D4 Dopamine receptor in complex with Nemonapride* with ID 5WIU. As in this case, the receptor sequence was mutated, so I had to restore the receptor's sequence to its original state, obtaining desired protein made up of 462 amino acids.

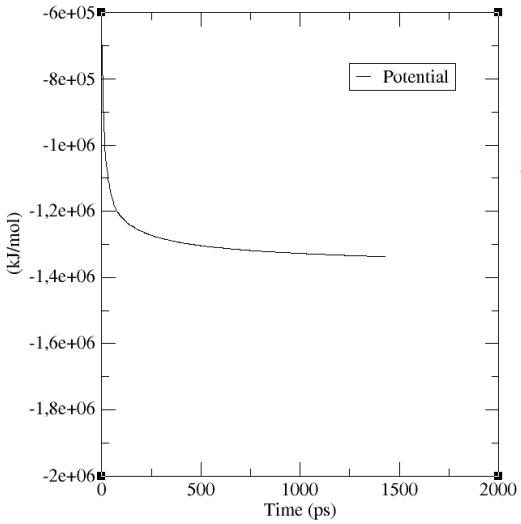


Fig. 4a Energy minimalization D_4 receptor.

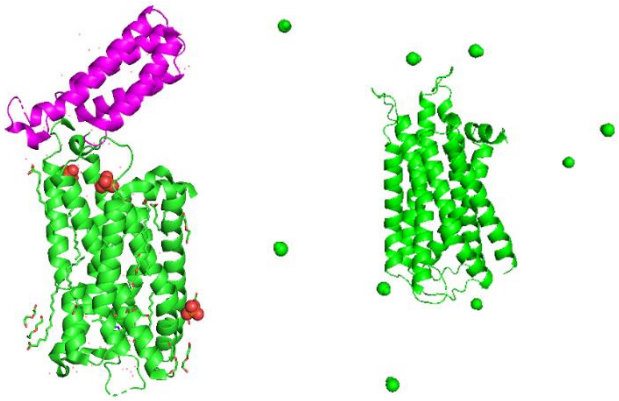


Fig. 4b 5WIU (left) and D_4 receptor after optimization (right).

1.5 Dopamine receptor D_5

Phyre2 program, based on dopamine receptor D_5 sequence chain with length of 477 amino acids, created sum of 120 templates, where 20 was modelled. The most accurate model was based on muscarinic acetylcholine receptor m4 (6KP6), belonging to G protein-coupled cell surface receptors, just like dopamine receptors. Program evaluated, that 64% of D_5 receptor sequence have been modelled with 100% confidence. Furthermore, after analyzing alignment with the related D_1 receptor structure, belonging to the same family of D_1-like receptors, it most closely resemble it. The difference is the location of the longer C-terminal, which in the case of predicted structure occurs instead

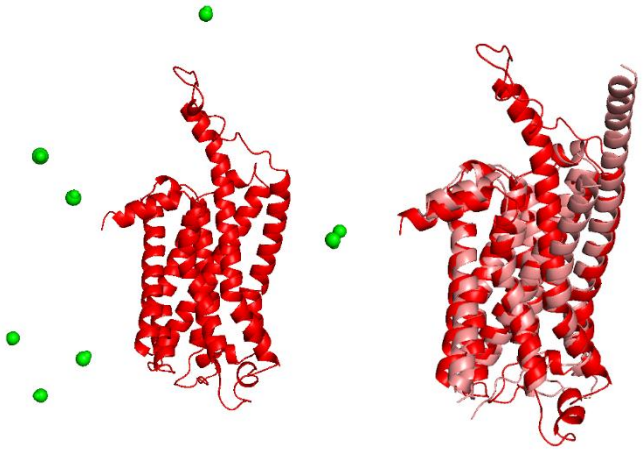


Fig. 5 D_5 receptor after optimization (left) and result of D_1 receptor and D_5 receptor alignment with visible similarity (right).

of on seventh transmembrane domain on the sixth one. Fortunately, it did not affect the next steps negatively, as dopamine binds at the opposite site (extracellular), which in predicted structure looks identical to the D_1 receptor. Optimization step consisted only of adding counterions in amount of 8, since the potential energy of the system was already minimized automatically during structure prediction and was in approximately equal to -2.2019e+06 kJ/mol.

2. Binding sites and ligands search

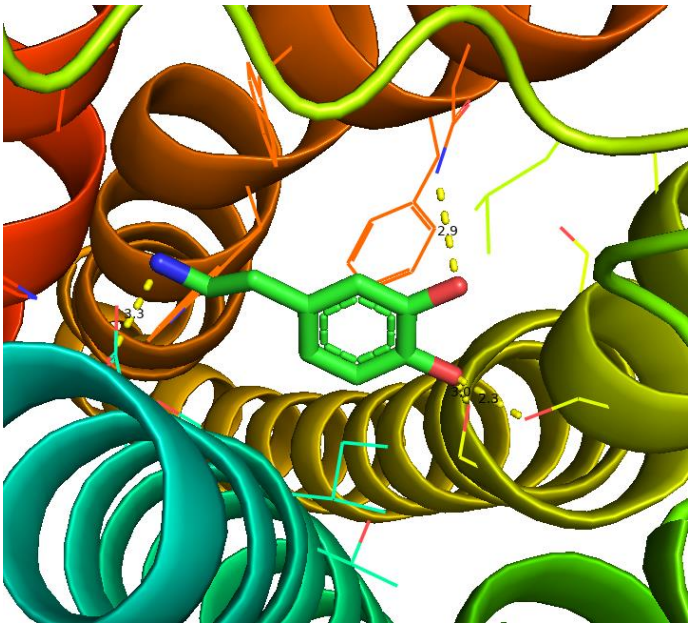


Fig. 6 Dopamine binding site in D_1 receptor

The basis for determining the site of the cavity where I built the ligands in each receptor was actual known binding site for dopamine in D_1 receptor from, mentioned earlier, 7LJD PDB structure. As shown on Fig. 6 Dopamine binds through 1 Pi Stacking with 289 phenylalanine and hydrogen bonds with 4 amino acids: 198 serine with length of 2.3Å and 202 serine with length of 3.0 Å – both present on TM domain 7. The other two are 292 asparagine with length of 2.9Å on TM domain 6 and 103 aspartic acid with length of 3.3Å on TM domain 5. Binding happening on the N-terminus site (extracellular space).

Once I had the information I needed, I was able to move on to the next step of searching pocket site for each receptor and choosing the correct one. For this, I use the program Cavity, which is part of the LigBuilder V3 program. LigBuilder V3 is a multi-purposed program developed for structure-based de novo drug design and optimization. Cavity, using structure-based geometrical method, identify ligand binding sites to then use geometrical structure and physical chemistry property information to locate and create pocket surface. Cavities were assessed by predicting maximal pK_d value (ligandability of the binding site). Also helpful were created along with cavities pharmacological models, which is ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with an active site. In addition, it shows the potential places where ligands can bind to.

Build module from LigBuilder V3 program enabled the key activity of constructing ligand molecules. Molecules creating by Build are developed and evolved with a Genetic Algorithm procedure, which is inspired by process of natural selection, and its purpose is to mimics the evolution of population under selection pressure. Furthermore, due to search for inhibitors that could be used as potential drugs, I used drug-like design option, which aim to focus on molecules, that will fit in Internal/External Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) filters.

Final operation was to cluster obtained ligands, also carried out by the LigBuilder, which was based at two levels. First, chemical clustering, were grouped molecules shared the same two-dimensional structures and thus could be synthesized via the same reactions. Second clustering method, conformation cluster, focused on orientations of hydrogen atoms, which different position could provide local perturbation. Even though hydrogen atoms do not have much of an influence on protein-ligand binding, they are responsible for growing site for connecting newly added fragments. Every cluster has its own representative molecule. In total, I received total of 4091 ligands, which were cluster to sum of 151 clusters.

2.1 Dopamine receptor D_1

Cavity I obtained have predicted maximal pK_d value equal 7.53 and is druggable. Total surface area is 892,5 $Å^2$. Pharmacological models showed that every bond created

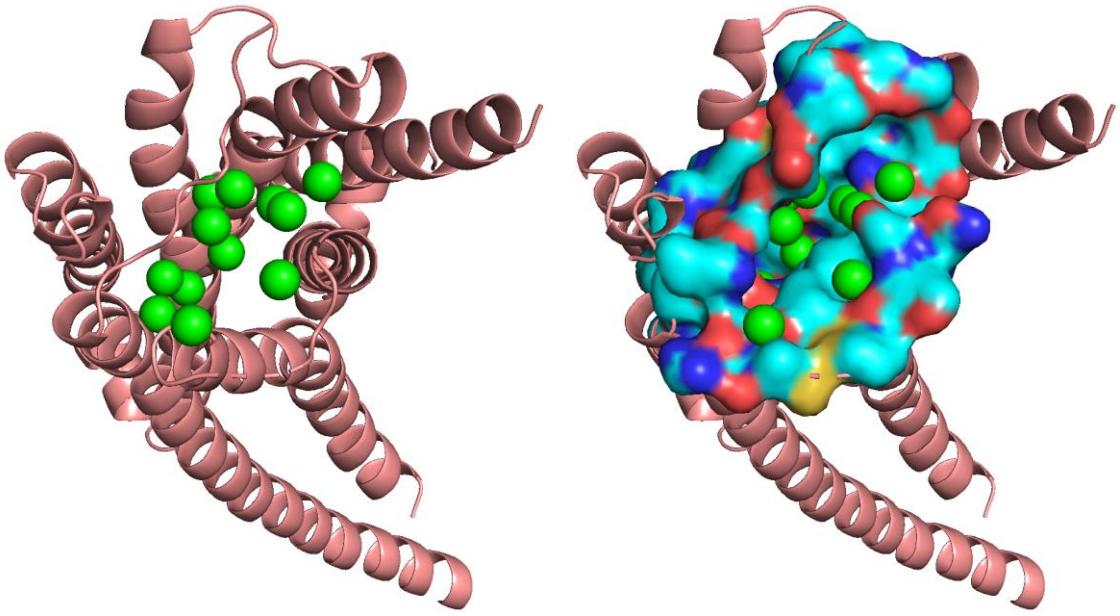


Fig. 7 D_1 receptor pharmacore model with no cavity shown (left) and with cavity shown (right)

in binding site are hydrogen. As a result, Build module, working with cavity shown on Fig. 7, created sum of 1248 ligands grouping them to 43 clusters.

2.2 Dopamine receptor D_2

In D_2 case, cavity I chose have predicted maximal pK_d value equal 3.34 and is druggable. Total surface area equal 1504,5 $Å^2$. Every bond created in cavity with Pharmacological models are hydrogen. The sum of created ligands equal 1237 and was clustered to 22 clusters.

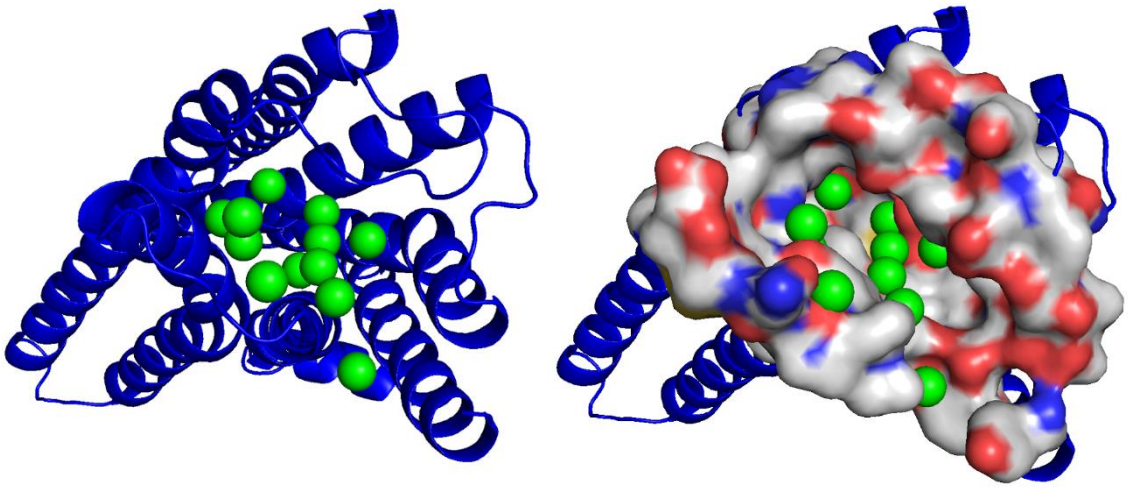


Fig. 8 D_2 receptor pharmacore model with no cavity shown (left) and with cavity shown (right)

2.3 Dopamine receptor D_3

Cavity have predicted maximal pK_d value equal 2.63 and is druggable. Total surface area is 1219 $Å^2$. Pharmacological models created only hydrogens bonds with protein. Program through the Build module created 1053 ligands and cluster them into 27 clusters.

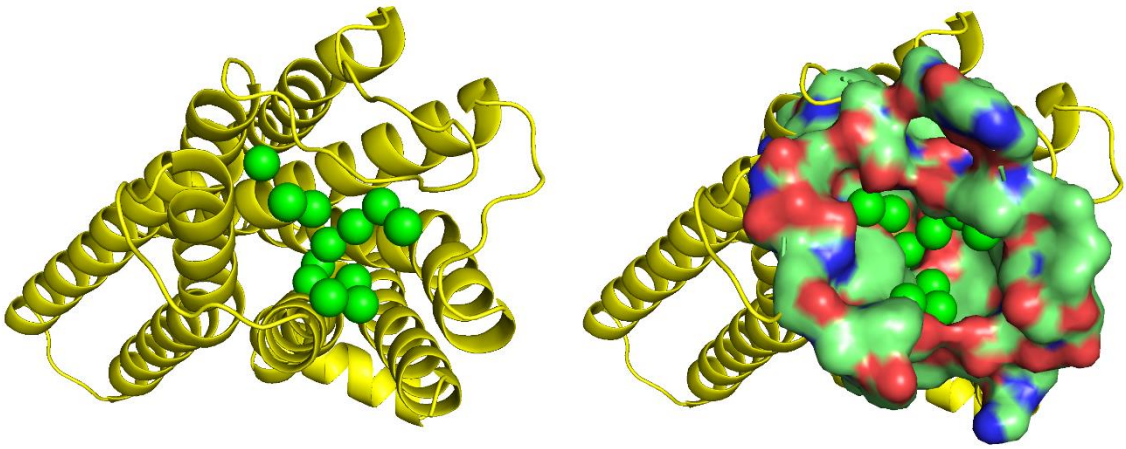


Fig. 9 D_3 receptor pharmacore model with no cavity shown (left) and with cavity shown (right)

2.4 Dopamine receptor D_4

Cavity present in the dopamine binding site have predicted maximal pK_d value equal 6.79 and is druggable. Total surface area is 1076,25 $Å^2$. Thanks to pharmacological models, I was able to determine that each bond is hydrogen. In D_4 case, I obtained sum of 530 molecules created by Build module, which ten was clustered into 33 clusters.

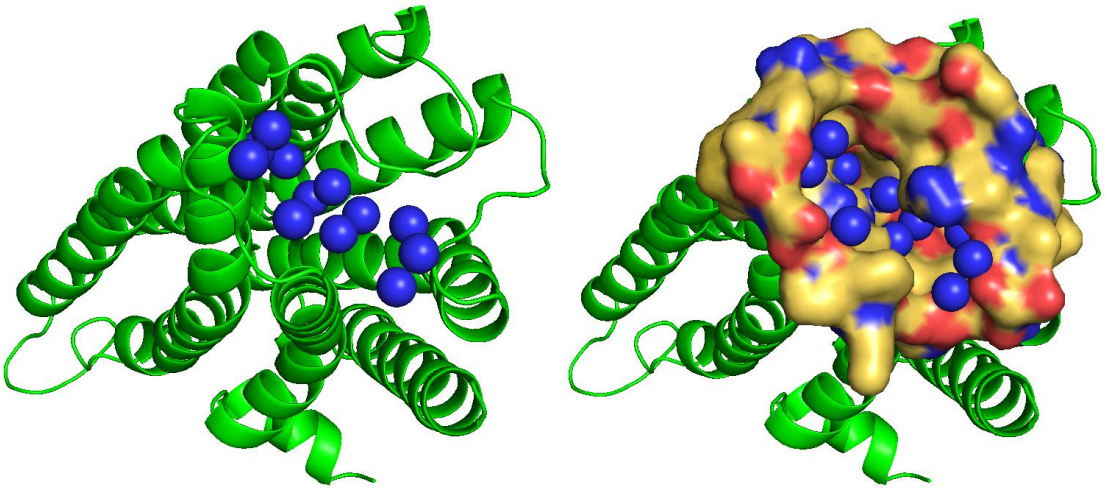


Fig. 10 D_4 receptor pharmacore model with no cavity shown (left) and with cavity shown (right)

2.5 Dopamine receptor D_5

PK_d defining function calculated that predicted maximal pK_d of the cavity is equal 4.54 and is druggable. Total surface area, that cavity occupy, equals 2184.25 $Å^2$. Cavity module also showed that every donor which pharmacological model uses are hydrogen.

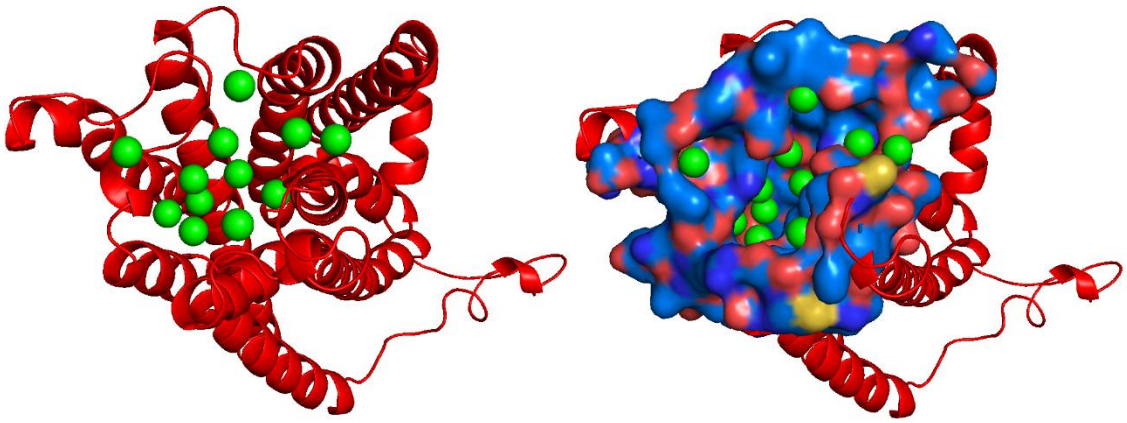


Fig. 11 D_5 receptor pharmacore model with no cavity shown (left) and with cavity shown (right)

3. Toxicity evaluations and docking interactions

As it comes to pharmacokinetics and bioavailability, there was couple rules for designed molecules that I had follow. In the case of dopamine receptors inhibitors, the

availability of the drug to the brain was important, because of their occurrence in dopaminergic brain regions. For this reason, molecules that I focused on had to have the ability to cross the blood-brain barrier. Another very important factor was human intestinal permeation and interaction with P-glycoprotein 1 (multidrug resistance protein 1) which role, in general, is based on passing or removing xenobiotics from the body. It means, that potential drug could not be P-glycoprotein 1 substrate. Defined druglikeness criterion, were based on Lipinski's rule (rule of 5), Ghose filter, Veber's rule, Egan rule and Muegge rule. Summary of druglikeness is Bioavailability score. Responsible for all previously defined assessments was swiss-ADME program. Schema of Human Intestinal Absorption, Blood-Brain barrier access and interaction with P-glycoprotein was presented as Brain Or IntestinaL EstimateD permeation method (BOILED-Egg). Points located in BOILED-Egg's yolk (yellow area) are molecules predicted to passively permeate through blood-brain barrier and passively been absorbed by the gastrointestinal tract. Points on BOILED-Egg's white (white area) are molecules which need active transport through barrier, but they been passively absorbed by the gastrointestinal tract. In addition, points colored in blue are molecules predicted to be effluated from the central nervous system by the P-glycoprotein, unlike red ones, which are predicted not to be effluated from the central nervous system. Chosen ligands were red dots colored green.

As a future human drug, another step in toxicity evaluation was to predict carcinogenicity, Ames mutagenesis and hepatotoxicity. For that purpose, I used admetSAR2 program, which also showed useful information about eco-toxicity such as honey bee toxicity, biodegradation, crustacea aquatic toxicity and fish aquatic toxicity. Analysis yielded two values: positive (toxic) and negative (non-toxic) with corresponding probability.

Blockage of the hERG K^+ channels could lead to lethal cardiac arrhythmia. The aim was to consider those ligands that will not block hERG potassium channels after binding to them, or those that will not bind at all. At this stage, the Pred-hERG program was helpful. Visualization of results is shown as green (positive contribution), pink (negative contribution to hERG blockage) and gray (neutral contribution) regions with proper intensity of the color depending on contribution power (the more intense color, the greater the contribution).

Thanks to the Autodock/Vina docking function used as plugin of PyMol, I was able to show the interaction of a selected ligand with binding site of target receptor.

3.1 Dopamine receptor D_1

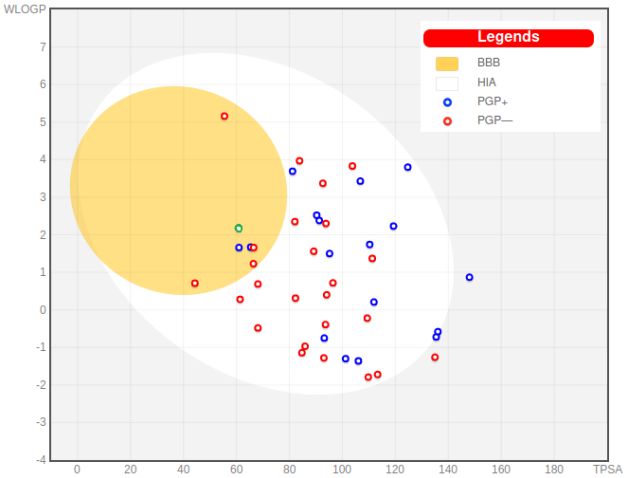


Fig. 12a D_1 receptor cluster's representatives presented on BOILED-Egg scheme in swiss-ADME.

BOILED-Egg method results presented on Fig 12a showed, that from sum of 43 potential inhibitors, 6 molecules made it passively through blood-barrier in which 4 of them were not effluated by P-glycoprotein. I decided to chose one of them (green) by their final bioavailability score and LigScore evaluted by binding affinity with D_1 receptor. The highest

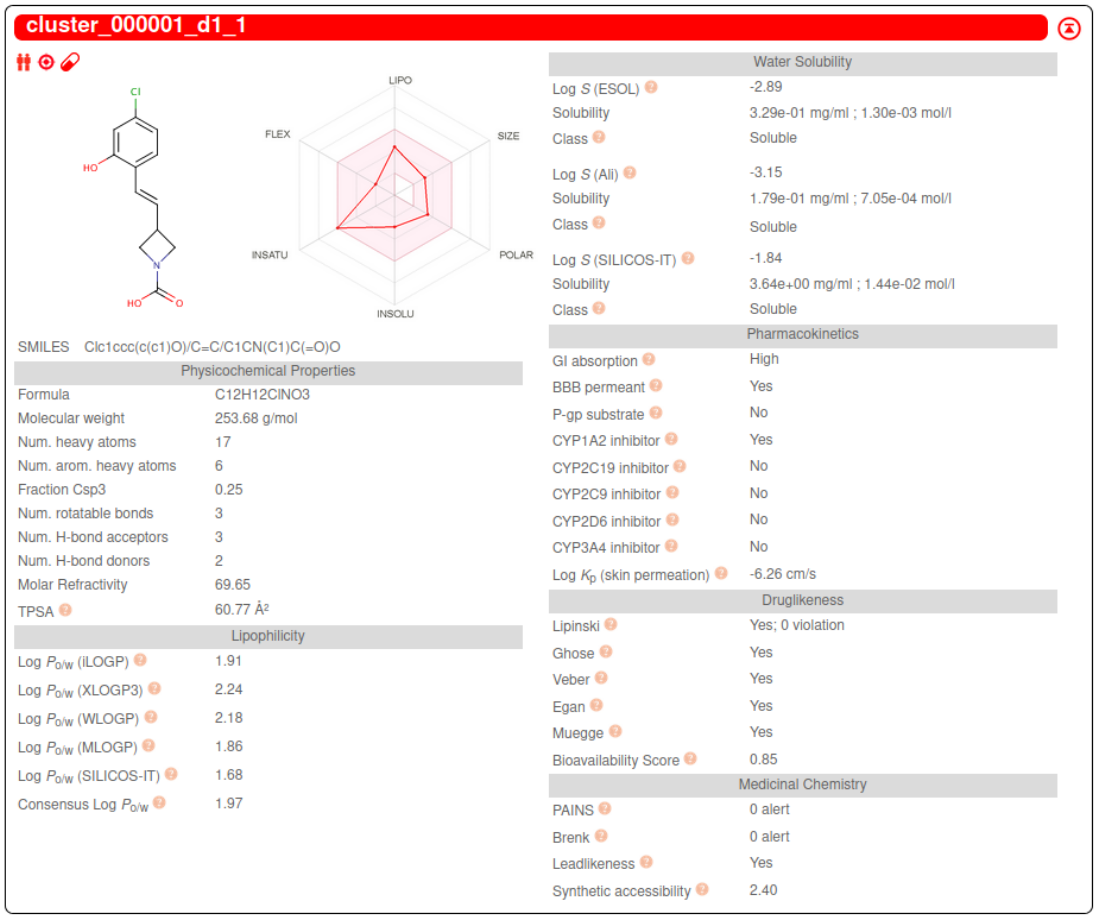


Fig 12b Results of swiss-ADME analysis of selected ligand (D_1 receptor ligand)

bioavailability score (0.85) and LigScore (1.67) belong to structure shown on Fig. 12b. Cluster from which the representative was selected was made up of 10 molecules. Swiss-ADME assessed that molecule met all criteria of druglikeness with 0 violations. It has weight of 253,68 g/mol and is soluble.

Molecule did well with the carcinogenicity (negative 0.71), Ames mutagenesis (negative 0.76) and little worse with hepatotoxicity (negative 0.65) tests. As for the rest of the tests it was non-toxic with neutral probability in honey bee toxicity (negative 0.53), biodegradation (negative 0.67), crustacea aquatic toxicity (negative 0.62) and failed with the fish aquatic toxicity (positive 0.95). In case of hERG blockage, program Pred-hERG evaluated that compund is non-cardiotoxic with 60% confidence. Even that there is a lot of potential positive contribution sites (green), carboxyl group and oxygen atom make molecule non-blockader of hERG K^+ channels.

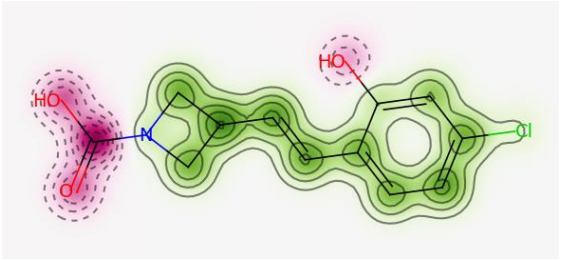


Fig 12c Probability map of Pred-hERG result

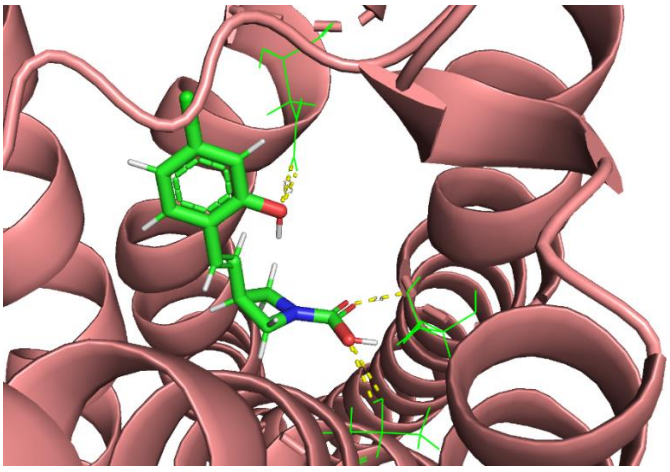


Fig 12d D_1 inhibitor interaction with binding site in D_1 dopamine receptor

Docking program showed, that molecule can bind to active site by hydrogen bonds of length equal 2.9Å or 2.9Å with 108 threonine, 2.6Å with 202 serine and 2.2Å or 3.1Å with 292 asparagine. It's worth mentioning that 202 serine and 292 asparagine are also amino acids which dopamine bind to.

3.2 Dopamine receptor D_2

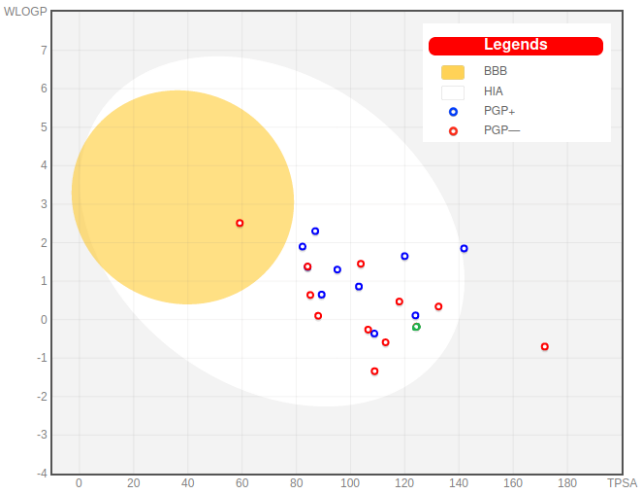


Fig 13a D_2 receptor cluster's representatives presented on BOILED-Egg scheme in swiss-ADME.

BOILED-Egg showed that only one molecule met both ability to passively pass the blood-brain barrier and not being effluated by P-glycoprotein critiera. Unfortunately, after careful analysis, it turned out that the molecule was not the best candidate, because of it toxicity and low LigScore. The best representative turned out to be molecule which belongs to the

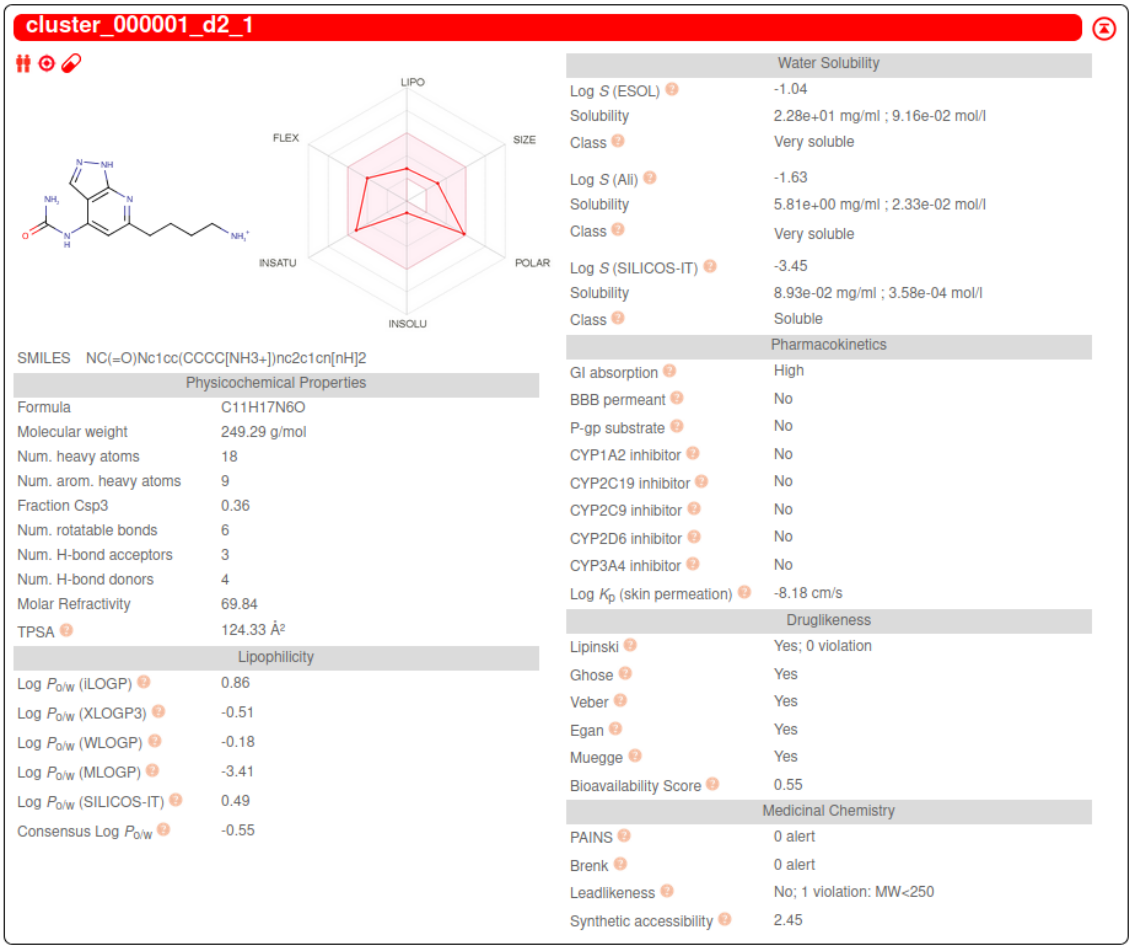


Fig 13b Results of swiss-ADME analysis of selected ligand (D_2 receptor ligand)

molecules not rejected by the glycoprotein and those which been passively absorbed by the gastrointestinal tract and is marked in green in Fig. 13a. It has LigScore equal 1.15, bioavailability score equal 0.55, meets all the rules of druglikeness and the cluster from which molecule came was made of 336 molecules. The lack of the ability to passively penetrate the blood-brain barrier is the only drawback so far. However, this is not a reason for the molecule to be rejected as it will be able to enter the dopaminergic brain regions via active transport, which will requiere more energy. Moreover, molecule is very soluble and weights 249,29 g/mol.

As successive toxicity analyzes have shown, molecule did almost perfectly at carcinogenicity tests (negative 0.97) but had failed in Ames mutagenesis (positive 0.51) and hepatotoxicity (positive 0.67) tests. The molecule performed well in subsequent toxicity test: honey bee toxicity (negative 0.59),

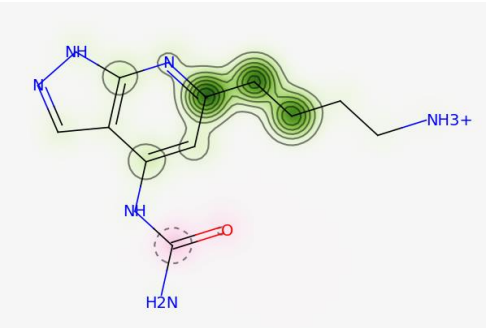


Fig 13c Probability map of Pred-hERG result

biodegradation (negative 0.77), crustacea aquatic toxicity (negative 0.59) and fish aquatic toxicity (negative 0.83). Pred-hERG results shown, that molecule isn't cardiotoxic with 50% confidence. As shown in Fig. 13c, area were carbon atom is connected with oxygen and amino group was main factor on negative contribution to the hERG blockage.

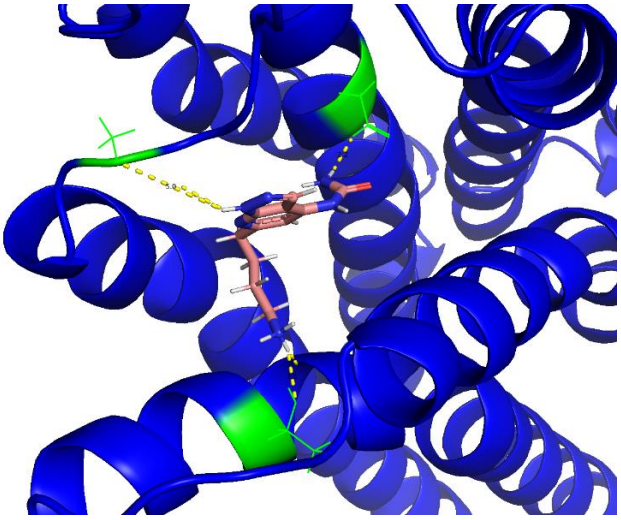


Fig 32c D_2 inhibitor interaction with binding site in D_2 dopamine receptor

Final docking, gave insight into the amino acids with which the molecule binds. It is 185 alaine with length of 5Å, 114 aspartic acid with 2.2Å and 392 threonine with 2.6Å. Any of amino acids aren't involved in dopamine binding, but the ligand binding site is similar a dopamine binding spatially.

3.3 Dopamine receptor D_3

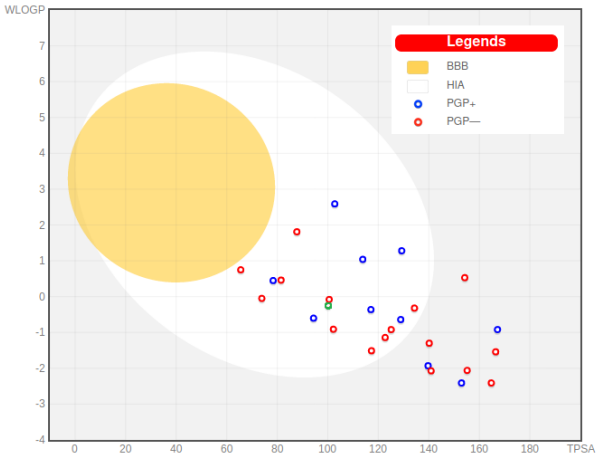


Fig 14a D_3 receptor cluster's representatives presented on BOILED-Egg scheme in swiss-ADME.

In D_3 inhibitor search case, there was no ligand that successfully passed through blood-brain barrier without active transport. I had to base my choice only on other BOILED-Egg's factors (P-glycoprotein rejection and absorption by gastrointestinal tract), toxicity check and LigScore. After analysis, the best canditate was ligand presented as green dot at Fig. 14a. Molecule, besided Muegge rule (1 violation caused by XLOGP3 equal -2.04), passed all toxicity rules. It has molecular weight equal 265.29 g/mol, LigScore equal 1.09, bioavailability score equal 0.55 and has a high level of solubility. Cluster from which molecule was chosen was created with 72 similar ligands.

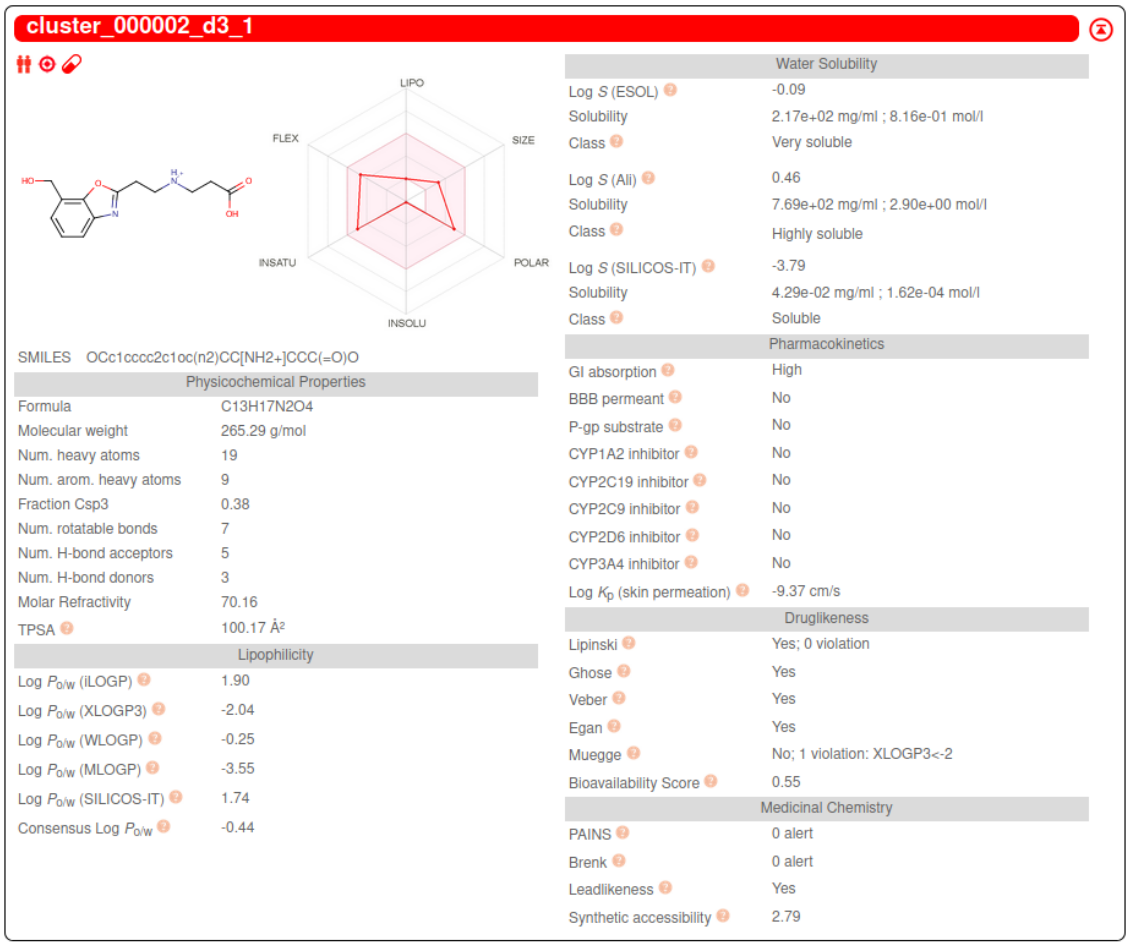


Fig 14b Results of swiss-ADME analysis of selected ligand (D_3 receptor ligand)

The ligand shows no sign of toxicity. As admetSAR2 shows, molecule passed in carcinogenicity tests (negative 0.92), Ames mutagenesis (negative 0.67) and hepatotoxicity (negative 0.5). Remaining tests were also carried out with satisfactory resulsts: honey bee toxicity (negative 0.57), biodegradation (negative 0.57), crustacea aquatic toxicity (negative 0.84) and fish aquatic toxiciy (negative 0.9). Pred-hERG results predicted, that molecule is non-cardiotoxic with 60% confidence. Regions that contributed to non-blocking hERG was hydroxide and carboxyl group.

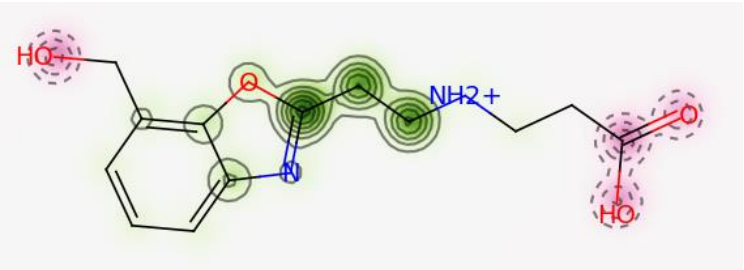
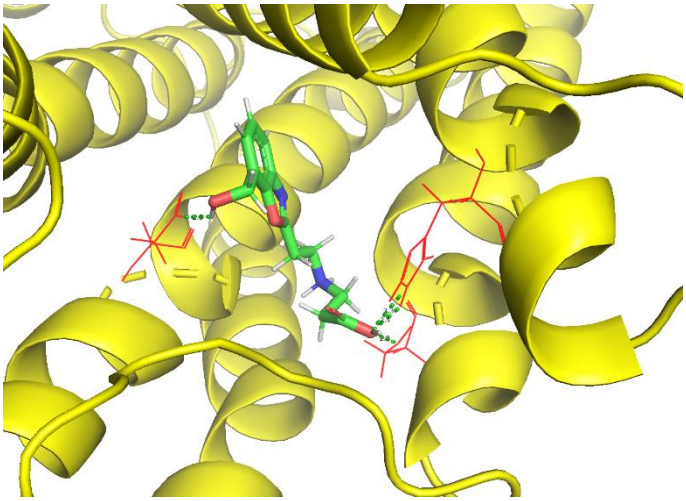


Fig 14c Probability map of Pred-hERG result



When it comes to docking the molecule in D_3 receptor, as shown on Fig. 14 it can bind by hydrogen bonds with 110 aspartic acid with 2.3Å, 192 serine with 2.8Å and 349 histidine with 3.3Å or 2.4Å bond. Docking site is like the one where dopamine binds, this is quite a satisfactory result.

Fig 14d D_3 inhibitor interaction with binding site in D_3 dopamine receptor

3.4 Dopamine receptor D_4

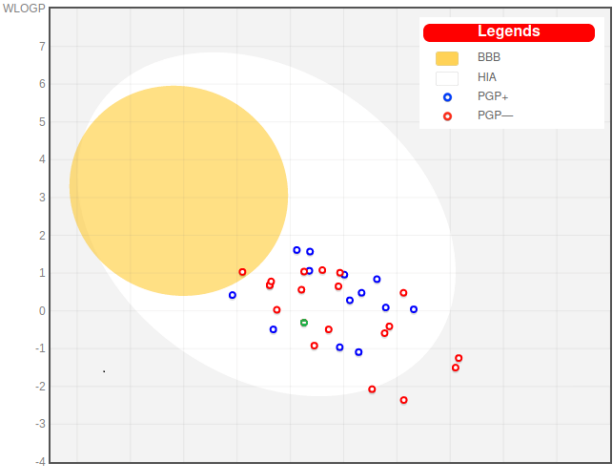


Fig 15a D_4 receptor cluster's representatives presented on BOILED-Egg scheme in swiss-ADME.

As shown in Fig. 14a, swiss-ADME predicted, that only one molecule was able to passively pass through blood-brain barrier. Although, it wasn't suitable enough for later analysis due to high toxicity and low LigScore. After careful analysis, I decided, that the best ligand was non-substrate of P-glycoprotein and passively absorbing by gastrointestinal tract ligand marked in green. It is very soluble, 171,20 g/mol weighing ligand, that has only one violation in druglikeness check (Muegge – molecular weight is lower than 200 g/mol).

Toxicity tests, evaluated by admetSAR2, showed, that molecule shown no sign of toxicity in carcinogenicity (negative 0.91), Ames mutagenesis (negative 0.73), and hepatotoxicity (negative 0.77) . When it comes to eco-toxicity, honey bee toxicity (negative 0.72), biodegradation (negative 0.57), crustacea aquatic toxicity (negative 0.77) and fish aquatic toxicity

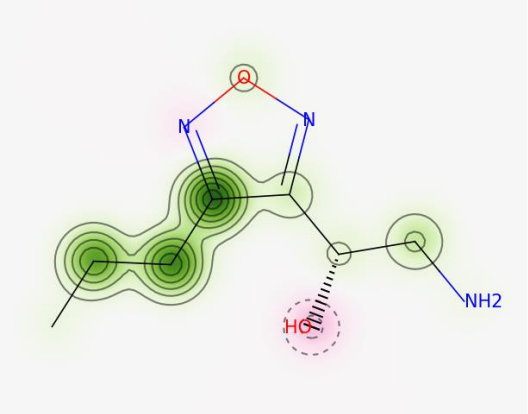


Fig 15c Probability map of Pred-hERG result

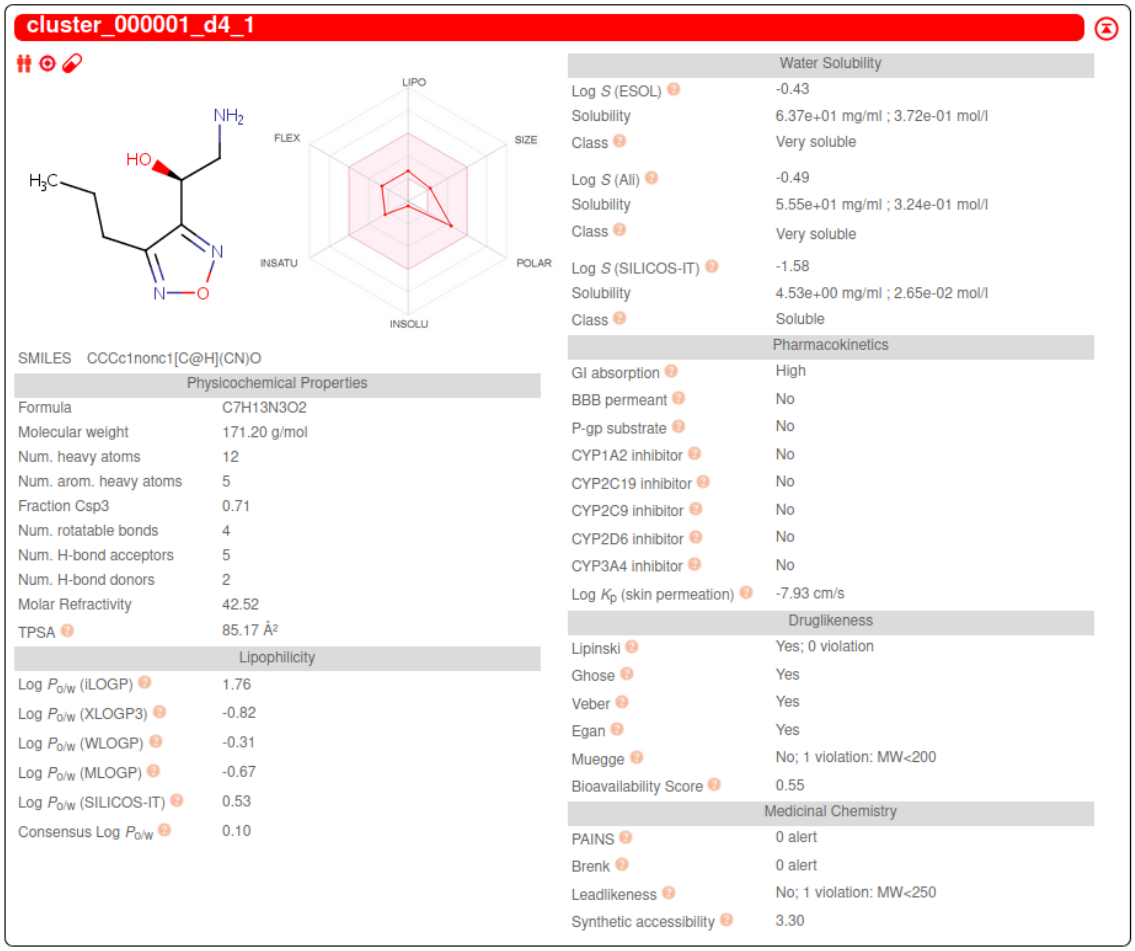


Fig 15b Results of swiss-ADME analysis of selected ligand (D_4 receptor ligand)

(negative 0.94) also show non-toxic result. Unfortunately, program Pred-hERG calculated with 60% confidence, that molecule is potential cardiotoxic (Fig 15c). This assumption was predicted to has weak or moderate potency with 50% confidence. Main reason is the carbon chain attached to the ring, which contributes to the hERG K^+ channels blockage.

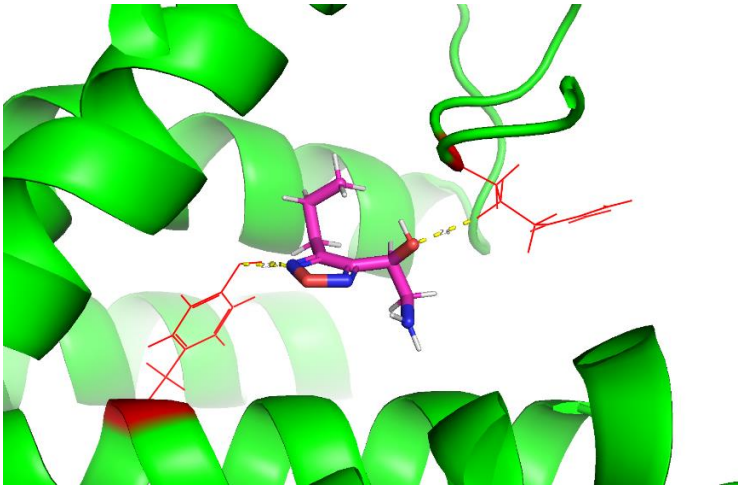


Fig 15d D_4 inhibitor interaction with binding site in D_4 dopamine receptor

Docking analysis showed, that molecule is connected by only two hydrogen bonds, which suggests that molecule is not the most stable. The connection with D_4 receptor is via 186 arginie with 2.3Å or 2.1Å and 438 tyrosine with 2.6Å.

3.5 Dopamine receptor D_5

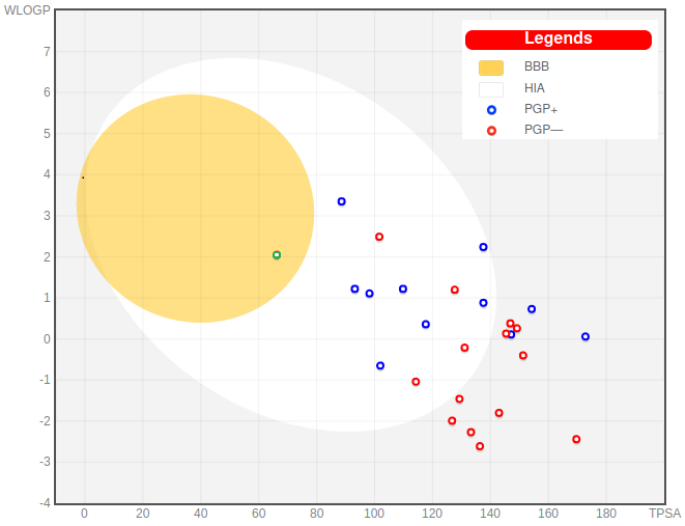


Fig 16a D_5 receptor cluster's representatives presented on BOILED-Egg scheme in swiss-ADME.

Last chosen inhibitor that has managed to passively penetrate the blood-brain barrier and the gastrointestinal system was not removed from the body by P-glycoprotein, so it fits perfectly in the BOILED-Egg algorithm. It is a soluble 255,27 g/mol molecule that complies with all the rules of druglikeness without any violations and having 0.55 bioavailability score.

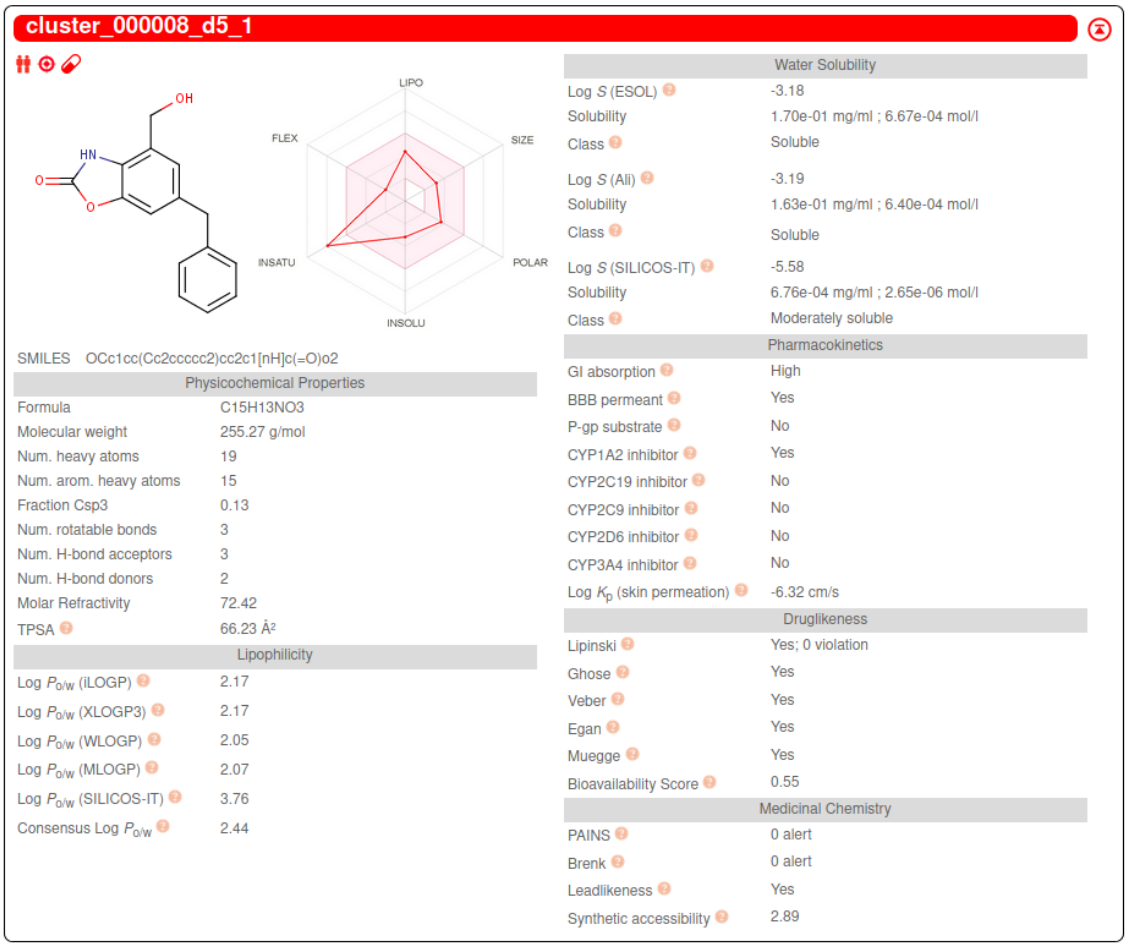


Fig 16b Results of swiss-ADME analysis of selected ligand (D_5 receptor ligand)

Molecule is hepatotoxic having positive value with 0.95 probability. Option would be to potentially administer her along with medications for liver immunity. Altough, it did well in carcinogenicity (negative 0.92 probability) and Ames mutagenesis (negative 0.64 probability) tests. When it comes to eco-toxicity, molecule showed toxic values with honey bee toxicity (positive 0.81) and fish aquatic toxicity (0.78) and non-toxic values with biodegradation (0.92) and crustacea aquatic toxicity (0.64) tests. It is, as Pred-hERG showed in Fig 16b, non-cardiotoxic with 60% confidence.

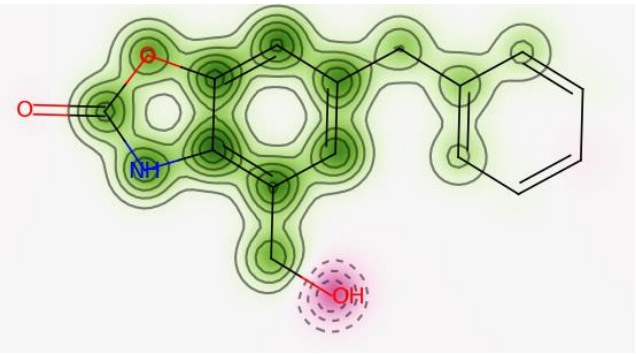


Fig 16c Probability map of Pred-hERG result

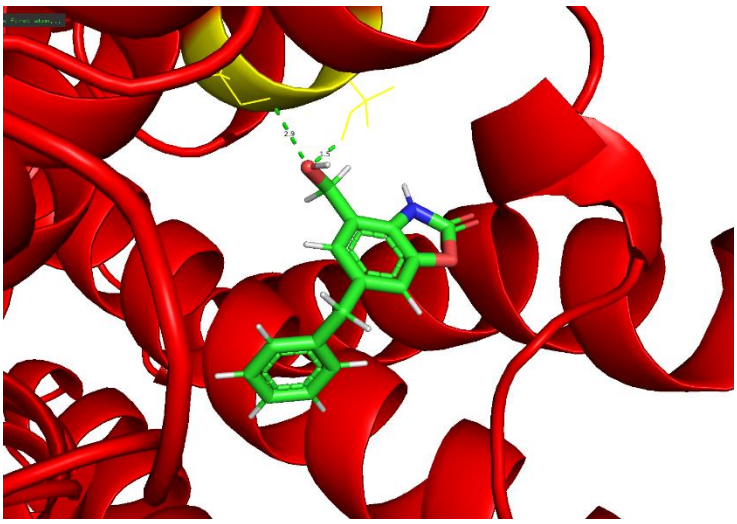


Fig 16d D_5 inhibitor interaction with binding site in D4 dopamine receptor

The binding of selected ligand is not the most stable. What brought me down to this assumption is that there are only two hydrogen bonds: 229 Serine 1.5 and 230 Serine with 2.9 located on only one TM domain (Fig 16d). Nevertheless, the binding site is similar to the dopamine binding site.

CONCLUSION

In parallel with the progression of advanced diseases related to the dopaminergic system, drug research is also progressing. In my work, I have managed to create one inhibitor for each dopamine receptor. For this purpose, I have prepared cavities that define the dopamine binding site, and then, using genetic algorithm, created ligands that fit into them creating blockage for dopamine. The choice of inhibitors was firstly based on interaction with blood-brain barrier, gastrointestinal tract and P-glycoprotein, to then run the ADMET toxicity tests, which included organ toxicity, genomic toxicity, eco-toxicity and cardiotoxicity. Finally, I analyzed the binding site of the inhibitors.

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STATEMENT

I, the undersigned, declare that the submitted thesis was done by me myself, it does not infringe the copyrights, legal and material interests of other people.

5.19.2021	Kamil Kowalewski
(date)	(signature)