

Pharmacophore Modeling and Molecular Docking-Based Virtual Screening of Adenosine A2A Inhibitor as Antiparkinson

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Abstract:

Background: Parkinson's disease is caused by the lack of dopamine in basal ganglia due to nigrostriatal system degeneration. Inhibition of the Adenosine A2A receptor can improve dopamine release for the treatment of Parkinson's disease. Thus, by inhibiting the Adenosine A2A receptor, Parkinson's disease can be overcome. This study aims to find new drug candidates that have the potential to inhibit the adenosine A2A receptor as a drug for Parkinson's disease.

Materials and Methods: In this research, we used a 3-dimensional structure file for adenosine A2A receptors with a PDB ID 5IU4 obtained from the RCSB protein data bank (www.rcsb.org) and 12 ZINC Natural Product databases consisting of 151,837 compounds as data sets. Pharmacophore-based virtual screening using LigandScout 4.3 software, and molecular docking-based virtual screening using Pyrx Screening Tools software, previously validated, were carried out to screen drug candidates from the databases. The validation of the pharmacophore-based virtual screening method showed that model 8, which has four pharmacophore features consisting of 2 aromatic ring interactions, one hydrogen bond acceptor, and one hydrogen bond donor, is the best pharmacophore model with the values of AUC 0.61; EF 5.5; Se 0.22; Sp 0.99; ACC 0.99; Yes 0.085; and GH 0.12. Docking validation using Autodock tools 4.2 and VinaWizard showed RMSD values of 1.32 Å and 1.75 Å, respectively. The validation of the pharmacophore-based virtual screening method showed the AUC and EF values of 0.514 and 662.52, respectively.

Results: The pharmacophore-based virtual screening gave 321 drug candidates, and further screening using molecular docking simulations gave 50 potential Adenosine A2A inhibitors with ΔG value lower than the native ligand (-9.8 kcal/mol).

Conclusion: The ZINC38932599 and ZINC98365141 originated from Princeton Natural Products, and IBScreen Natural Products gave the lowest ΔG value of -11.9 Kcal/mol and were concluded as the best candidates as antiparkinson.

Key Words: Virtual Screening, Adenosine A2A, antiParkinson

I. INTRODUCTION

Parkinson's disease, or simply Parkinson's, is a neurodegenerative disorder that affects dopaminergic neurons in substantia nigra¹. It belongs to a group of conditions called motor system disorders, which cause unintended or uncontrollable movements of the body. In Parkinson's, brain cells are damaged or die in the part of the brain that produces dopamine².

Adenosine is an organic compound that is widely found in nature in the form of various derivatives. The molecule consists of adenine which is bound to ribose via β -N9-glycosidic bonds. Adenosine is one of the four nucleoside building blocks for DNA and RNA, which are essential for all life³. Adenosine decreases the neuronal firing rate and inhibits both synaptic transmission and the release of most neurotransmitters⁴. When given in very high amounts, adenosine can affect intracellular nucleotide pools and even provide a source of metabolizable energy⁵.

There are four different adenosine receptors, denoted A1, A2A, A2B, and A3. Later pharmacological studies revealed that the A2 receptors, coupled to adenylyl cyclase, were heterogeneous, necessitating subdivision into A2A and A2B⁵. The A1 receptors are linked negatively to adenylyl cyclase, whereas the A2a receptors are linked positively to this enzyme. Adenosine A1 receptors are found in almost all parts of the brain, with high levels in the

hippocampus, cerebral and cerebellar cortex, and the thalamus. On the contrary, the A2a receptors are located almost exclusively in the striatum, nucleus accumbens, and olfactory tubercle⁴.

In the latter regions, A2a receptors are coexpressed with enkephalin and dopamine D2 receptors in striatal neurons. Administering adenosine A2a receptor agonists will decrease dopamine affinity for D2 receptors on the striatal membrane. Antagonizing the negative modulation effect of adenosine receptors on dopamine receptors will lead to inhibition and blockade of adenosine A2 receptors, thereby, leading to potentiation of dopaminergic neurotransmission⁴.

In new drug discovery, drug testing is not only carried out in vivo or in vitro but also in silico or by computer simulations^{6,7,8}. Virtual screening is a high-performance computational method for analyzing a database set of chemical compounds to identify drug candidate compounds. This computational method can reduce research costs as well as streamline research time compared to pharmacological screening⁹. The current study aims to find new drug candidates that have the potential to inhibit the adenosine A2A receptor as a drug for Parkinson's disease using pharmacophore modeling and molecular docking-based virtual screening.

II. MATERIAL AND METHODS

Hardware: This study was carried out using a computer unit with the specification of Windows 8.1 Pro 64-bit operating system, Intel® Core™ i7-4790 CPU @ 3.60 GHz 8 (CPUs) processor, 32 GB of RAM DDR3 memory, and 6 GB 128-bit dedicated VGA.

Protein structure preparation: The 3-dimensional structure of the A2A receptor (PDB ID: 5IU4) was obtained from the RCSB protein data bank website (www.rcsb.org). The crystal structure of human (*Homo sapiens*) A2A receptor in complex with ZM241385 was determined using the X-ray diffraction method at 1.7 Å resolution¹⁰.

Ligand preparation: The database of the testing compounds was 12 ZINC Natural product consisting of 151,837 compounds, which was obtained from <http://zinc.docking.org/>, including AfroDB Natural Products, AnalytiCon Discovery Natural Products, Herbal Ingredients In-Vivo Metabolism, Herbal Ingredients Targets, IBScreen Natural Products, Indofine Natural Products, NPACT Database, Nubbe Natural Products, Princeton Natural Product, Specs Natural Products, TCM Database Taiwan, and UEFS Natural Products.

Active and decoy set compounds: 50 active compounds as a positive control in the validation of the virtual screening were obtained from the website <https://www.ebi.ac.uk/chembl/> with the range of IC₅₀ value of 0 - 4000 nM. On the other hand, 31,977 decoy compounds as negative control were obtained from the website <https://dude.docking.org>.

Pharmacophore-based virtual screening: The pharmacophore modeling was carried out using the LigandScout 4.3¹¹ software. Before doing pharmacophore modeling, the native ligand was optimized first using the MMFF94 method. The pharmacophore of the optimized native ligand was created and also validated. The validation was carried out by applying the overall pharmacophore features of the native ligand against the active and decoy compounds. The validation was performed by observing the values of the hit compounds and the ROC curves, which contained the values of AUC of more than 0.5 and of EF of over 1.0¹² and also by calculating other classic enrichment validation parameters such as Se values^{13,14}, Sp values¹⁴, ACC values^{15,16,17}, Ya values¹⁸, and GH values¹⁹. The validated pharmacophore models were used for the virtual screening of compounds in the ZINC Natural Product Database.

Molecular docking-based virtual screening: The molecular docking was carried out using the PyRx Screening Tool 0.8²⁰, which includes the Vina Wizard and the AutoDock Wizard. The active site of the A2A receptor was made following the native ligand binding site to the receptor. The grid box width was set to 40x40x40 on the XYZ axis with a grid point spacing of 0.375 Å. The redocking process of the native ligand to the receptor was carried out with the number of GA Run of 100. The results of the validation of the docking method are declared valid if the RMSD value is less than 2 Å²¹. The validation process of virtual screening was carried out using all the docking parameters above to the active and the decoy set compounds to the receptor. The ROC curve was created with the requirements that AUC is more than 0.5 and EF is more than 1.0. The validated model was then employed for the virtual screening of compounds of the ZINC natural product database from the previous screening.

III. RESULT AND DISCUSSION

Pharmacophore-based virtual screening

The validation of pharmacophore features was employed before the virtual screening was carried out. The native ligand bound to the A2A receptor (PDB ID: 5IU4), 4-{2-[(7-amino-2-furan-2-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)amino]ethyl}phenol, was optimized first using the MMFF94 method. The pharmacophore features were generated from the ligand, and 12 features were obtained, consisting of four hydrogen bond donors, four hydrogen bond acceptors, two aromatic ring interactions, and two hydrophobic interactions (Figure 1a). These pharmacophore features were then validated with active and decoy set compounds (Table 1). The AUC and EF values of all pharmacophore models met the requirements, with model 8 gave the largest AUC value of 0.61. According to this data, model 8 was created and used as a virtual pharmacophore model for screening the natural product databases. This model had four pharmacophore features consisting of two aromatic ring interactions, one hydrogen bond donor, and one hydrogen acceptor (Figure 1b).

Table 1: Validation of pharmacophore features

| Model | Hits | AUC | EF | Se | Sp | ACC | Ya | GH | Feature(s) |
|-------|------|------|-------|------|------|------|-------|------|------------|
| 1 | 1 | 0.5 | 640.5 | 0.02 | 0.99 | 0.99 | 1 | 0.75 | 11 |
| 2 | 1 | 0.5 | 640.5 | 0.02 | 0.99 | 0.99 | 1 | 0.75 | 10 |
| 3 | 3 | 0.52 | 427 | 0.04 | 0.99 | 0.99 | 0.67 | 0.51 | 9 |
| 4 | 4 | 0.52 | 320.3 | 0.04 | 0.99 | 0.99 | 0.5 | 0.38 | 8 |
| 5 | 11 | 0.52 | 116.5 | 0.04 | 0.99 | 0.99 | 0.18 | 0.15 | 7 |
| 6 | 38 | 0.52 | 33.7 | 0.04 | 0.99 | 0.99 | 0.52 | 0.04 | 6 |
| 7 | 67 | 0.54 | 38.2 | 0.08 | 0.99 | 0.99 | 0.59 | 0.06 | 5 |
| 8 | 128 | 0.61 | 55 | 0.22 | 0.99 | 0.99 | 0.085 | 0.12 | 4 |
| 9 | 816 | 0.58 | 7.1 | 0.18 | 0.99 | 0.99 | 0.01 | 0.05 | 3 |

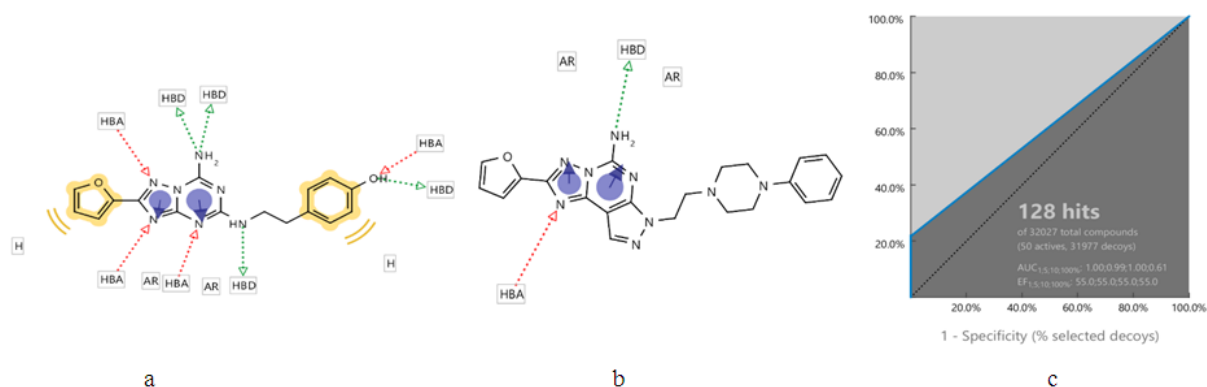


Figure 1. Pharmacophore features of native ligand (a), validated pharmacophore features (b), and ROC curves for model 8 (c).

The virtual screening was carried out using model 8 as the validated model to the 12 databases of natural products with a total of 151,837 compounds resulting in 321 candidate compounds (Table 2). However, the outputs were still considered too many candidates; thus, further screening was needed to obtain fewer candidate compounds.

Table 2: Pharmacophore modeling-based virtual screening result.

| No | ZINC Database | Hits |
|-------|---------------------------------------|------|
| 1 | Afropan Natural Product | 1 |
| 2 | AnalytiCon Discovery Natural Products | 2 |
| 3 | Herbal Ingredients In-Vivo Metabolism | - |
| 4 | Herbal Ingredients Targets | 2 |
| 5 | IBScreen Natural Product | 284 |
| 6 | Indofine Natural Product | - |
| 7 | NPACT Database | - |
| 8 | NuBBe Natural Product | 1 |
| 9 | Princeton Natural Product | 18 |
| 10 | Specs Natural Product | 1 |
| 11 | TCM Database | 12 |
| 12 | UEFS Natural Product | - |
| TOTAL | | 321 |

Molecular Docking-based Virtual Screening

The 321 candidate compounds obtained from the previous screening proceeded to the molecular docking-based screening stage. Validation of the docking method was applied by re-docking the native ligand to the A2A target receptor using Vina Wizard in Pyrx 8.0 software. Grid Box width of 10.08x17.36x13.44 on the XYZ axis, whereas Grid Spacing of 1.0 Å with Grid Center X: -20.05, Y: 9.02, and Z: 16.03 were set for the Vina Wizard and gave the RMSD value of 1.75 Å. Thus, the docking method used was declared valid. These docking parameters then validated for virtual screening using active and decoy set compounds. The ROC curve of Vina Wizard showed that the AUC and EF values of 0.514 dan 662.5 (Figure 2), respectively, and met the requirements. Thus, this docking model was valid, and therefore, screening could be carried out for the test compounds.

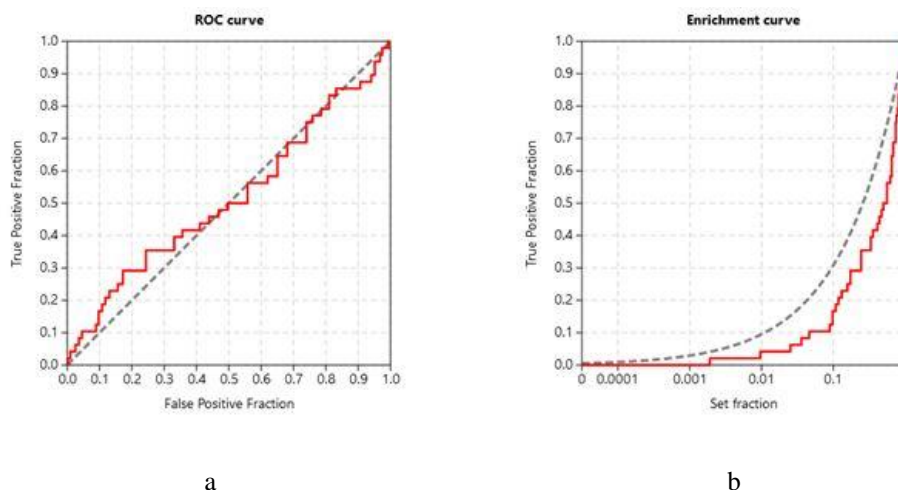


Figure 2. ROC curve (a) and Enrichment curve (b) of Vina Wizard validation results of the virtual screening method

An AUC value that is more than 0.5 indicates that the binding site area that has been covered with a gridbox is able to provide good selectivity to be used as a molecular docking place for the test compounds so that candidates for new drug compounds that have the best bonds and interactions can be obtained as a result of virtual screening²².

The screening results obtained 50 candidate compounds, whose free binding energy were smaller than the native ligand (Table 3).

Table 3: Summary of the screening results of zinc natural product compounds.

| No | ZINC Database | Hits | |
|-------|---------------------------------------|------------------------|------------------|
| | | Pharmacophore-based VS | Docking-based VS |
| 1 | Afropan Natural Product | 1 | - |
| 2 | AnalytiCon Discovery Natural Products | 2 | - |
| 3 | Herbal Ingredients In-Vivo Metabolism | - | - |
| 4 | Herbal Ingredients Targets | 2 | - |
| 5 | IBScreen Natural Product | 284 | 40 |
| 6 | Indofine Natural Product | - | - |
| 7 | NPACT Database | - | - |
| 8 | NuBBE Natural Product | 1 | - |
| 9 | Princeton Natural Product | 18 | 10 |
| 10 | Specs Natural Product | 1 | - |
| 11 | TCM Database | 12 | - |
| 12 | UEFS Natural Product | - | - |
| TOTAL | | 321 | 50 |

Identification of the best candidate compounds

The 50 candidate compounds obtained from the virtual screening were further analyzed in order to discover the best candidate compounds. The selection of the best compound can be seen from the free binding energy (ΔG). The smaller the free binding energy, the better the compound is. The value of free binding energy of the 50 candidate compounds, which were lower than the native ligand (-9.9 kcal/mol), indicated that the binding strength between the ligands from the screening results and the receptor was better. Ligands with the code ZINC38932599 from the Princeton Natural Product database and ZINC98365141 from IBScreen Natural Product database had the smallest free binding energy of -11.9 kcal/mol.

IV. CONCLUSION

The pharmacophore modeling-based virtual screening of 12 ZINC Natural Product databases using model 8, involving aromatic ring interactions and hydrogen bonds gave 321 candidate compounds and eliminated approximately 99.79% of 151.837 compounds. Further, the molecular docking-based virtual screening gave 50 candidate compounds whose binding strength was better than the native ligand to the A2A receptor. The ZINC38932599 and ZINC98365141 originated from Princeton Natural Products, and IBScreen Natural Products gave the lowest ΔG value of -11.9 Kcal/mol and were concluded as the best candidates as antiParkinson's.

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