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Research Article

IN SILICO ANALYSIS AND MOLECULAR INTERACTIONS STUDIES OF SELECTED PHYTOCONSTITUENTS FROM *ANDROGRAPHIS PANICULATA* AS POTENTIAL INHIBITORS OF MONOAMINE OXIDASE B

SIVA SANKARI[1], KANNAN RAMARAJ[2], SHYAM KUMAR RAJARAM[3], RONALDO ANUF ALEXANDER[3]*

[1]Department of Biotechnology, Mepco Schlenk Engineering College, Sivakasi, Virudhunagar, Tamil Nadu, India. [2]Bioinformatics Division, AU-KBC Research Centre, Madras Institute of Technology, Chennai, Tamil Nadu, India. [3]Department of Biotechnology, Kamaraj College of Engineering and Technology, Virudhunagar, Tamil Nadu, India. Email: ronaldoanuf@gmail.com

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ABSTRACT

Objective: The objective of the present study is to explore novel drug lead constituents from *Andrographis paniculata* for the treatment of Parkinson's disease.

Methods: Phytoconstituents from *A. paniculata* were screened, and their activity against the monoamine oxidase B (MAO-B) protein was analyzed using Molegro Virtual Docker software. The binding energy and interaction of the phytoconstituents with the protein were analyzed. The phytoconstituents were also analyzed for their compliance toward Lipinski's rule of five.

Results: Molecular docking studies were performed using Molegro Docking software. The compound neoandrographolide exhibited more potent inhibitory activity with a MolDock score of −126.78 Kcal/mol compared to that of the standard drug Zelapar which exhibited a MolDock score of −49.95 Kcal/mol. The docked pose of the compound neoandrographolide fits exactly at the active site with a maximum number of H-bond interactions.

Conclusion: The present study suggests that neoandrographolide could be used as a potent inhibitor of MAO-B protein. However, it has to be validated using *in-vivo* and *in-vitro* studies to suggest the potency of neoandrographolide to inhibit the target protein, which could make neoandrographolide as an effective drug lead for the treatment of Alzhemier's disease.

Keywords: *Andrographis paniculata,* Monoamine oxidase B protein, MolDock, Alzhemier's disease.

INTRODUCTION

Parkinson's disease is a neurodegenerative disease of the central nervous system which mainly affects the motor system. In industrialized countries, 0.3% of the population is being affected by Parkinson's disease [1]. The Parkinson's disease is associated with the degradation of nigrostriatal dopamine neurons, exhibiting symptoms such as bradykinesia, resting tremor, postural instability and dementia occurring in the advanced stages of the disease [2]. The symptoms of main motor systems are collectively called as Parkinsonism. The Parkinsonism was sub-classified into four types. They are primary (idiopathic), secondary (acquired), hereditary Parkinsonism, and multiple system degeneration [3]. Idiopathic, i.e., primary parkinsonism is common. Mutation in one of the several genes is found to be evident in more than 10% of Parkinson's cases. It is stated that the inhibition of key metabolic enzyme monoamine oxidase B (MAO-B) suppress the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's disease [4,5].

MAO-B is an enzyme, which was coded by the gene *MAO-B. MAO-B* presents throughout the body but have higher expression level in liver and brain. It is located in the outer mitochondrial layer and primarily present in non-neuronal cells such as radial glial and astrocytes [6]. It has bipartite hydrophobic cavities with the volume nearly 700 Å^3. The first cavity (~290 Å^3) is act as the entrance cavity and the second cavity (~390 Å^3) is the active site of the MAO-B [7].

The major function of MAO-B is generating hydrogen peroxide, which leads to the mitochondrial dysfunction and induces oxidative stress [8]. It degrades several chemicals such as benzylamine and phenylethylamine, which also including dopamine. The reduce in neurotransmission of dopamine leads to the cognitive decline [9]. In addition to that, during aging the dopamine levels decrease and the MAO-B expression is increased by three-folds [10]. These discoveries propose that the inhibition of MAO-B and maintenance of dopamine are the strategy to recover the cognitive function. The inhibition of MAO-B at the earlier stages of Parkinson's disease was proficient of stumbling or adjourning the subsequent mechanism involved [11]. So, there has been an emergence of novel drug discovery to target the MAO-B for Parkinson's disease.

Plant-derived phytochemicals have been traditionally used as a natural remedy in the treatment of diverse ailments. They are the backbone of traditional medicine. Usage of medicinal plants demonstrates promising and potential effect in treatment against many human diseases, due to the recognition that the natural products are non-toxic. In this content, there is an interest in developing novel lead molecules from plant sources because of their higher biological activity, safety, and lower cost as compared to the synthetic drugs.

Andrographis paniculata is a herbaceous plant, belongs to the family of *Acanthaceae*, whose roots and leaves are used as remedies over countries [12]. *A. paniculata* is reported to possess potent biological properties such as antimicrobial, anti-snake venom, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and cardiovascular activity [13]. So, the phytoconstituents from *A. paniculata* could therefore act as the good source of medicinal beneficial over the synthetic drugs.

In this study, we aim at exploring novel phytoconstituents from *A. panicualta* as potential lead molecules against the target protein MAO-B using Molegro Virtual Docker.

METHODS

Ligand preparation

The structure of phytoconstituents from *A. paniculata* was retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Chemspider database (http://www.chemspider.com/). The compounds were converted into a comfortable format (.sdf) using Open Babel tool. The energy-minimized structure was used for docking studies.

Protein preparation

The three dimensional crystal structure of the MAO-B was taken from Protein DataBank (http://www.rcsb.org/pdb/). The PDB ID of the target protein is 4CRT. The bonds, bond orders, explicit hydrogen, charges (calculated by Molegro Virtual Docker), flexible torsion, and Tripos atom types were assigned if they were missing using "Protein Preparation" module of Molegro Virtual Docker for the protein MAO-B (Fig. 1).

Molecular docking

Molegro Virtual Docker works on the basis of MolDock SE search algorithm. The docking algorithm was set at a maximum iteration of 1500 with a simple evolution size of 50 and minimum of 5 runs. The population size was set at 50 with energy threshold of 100 at each step. The least minute was set as 10 minutes, the torsions/translations/rotations of the ligand-protein interaction were tested and the one giving lower energies is chosen for further studies. The bond flexibility of the ligands was fixed, and the side chain flexibility of the amino acids in the binding cavity was set with a tolerance of 1.10 and strength of 0.90 for docking simulations. Root-mean-square deviation threshold for multiple cluster poses was set at <2.00.

The re-ranking score function is estimated more expensive than the scoring function used during the docking simulation, but it is commonly better than the docking score function at analyzing the best pose among several poses originating from the same ligand [14]. Binding affinities were estimated using Molegro data Modeler The scoring function used by MolDock is derived from the piecewise linear potential scoring functions which further improves these score with a new H-bonding term and new charge schemes [15].

Lipinski's rule of five

For any molecule to be used as a drug, it has to follow Lipinski's rule of five [12] which states that poor absorption or permeation are more likely when, (i) There are more than 5 H-bond donors, (ii) the molecular weight is over 500, (iii) the Log P (cLog P) is over 5, and (iv) there are more than 10 H-bond acceptors. Compound classes that are substrates for biological transporters are exceptions to the rule.

The above-listed phytochemicals which showed an affinity toward MAO-B were analyzed for their compliance to the Lipinski's rule of five using OECD QSAR toolbox [16]. The QSAR toolbox is an open source software to analyze the phytochemical properties of compounds.

RESULTS

Natural products derived from medicinal plants and their synthetic derivatives have been used as a good lead molecule [17]. A. *paniculata* has a number of active constituents which exhibits significant biological activities such as anti-inflammatory, immune stimulating, and liver protection activity [18]. Docking analysis was carried out for the phytoconstituents selected from A. *paniculata* against the target protein MAO-B using Molegro Docking Software so as to evaluate the efficacy of these compounds in suppressing the activity of target protein. The MolDock score of the phytoconstituents of A. *paniculata* was displayed in Table 1.

H-bond interaction

The strength of interaction between the compound and the protein is dependent on the number of H-bonds existing between them. The number of H-bonds interactions were calculated and tabulated in Table 2.

From the results, it was evident that four phytochemicals namely 14-deoxy-11,12-didehydroandrographolide, andrographoside, neo-andrographolide, and stigmasterin showed interaction with the target enzyme, whereas the rest of the phytochemicals did not show up any significant interactions with the protein of interest. Among these, compounds such as 14-deoxy-11, 12-didehydroandrographolide, andrographoside, and neoandrographolide showed a greater affinity

Table 1: MolDock score for phytoconstituents of *A. paniculata* against target protein (MAO-B)

S. No	Compound	MolDock score (Kcal/mol)	Re-rank score (Kcal/mol)	H-bond (Kcal/mol)
1	Neoandrographolide	−126.78	−51.9371	−14.3599
2	Stigmasterin	−118.141	−89.62	−2.5
3	14-deoxy-11,12-didehydroandrographolide	−116.945	−63.8776	−15.0094
4	Andrographoside	−113.875	−72.9157	−9.44802
5	Andrographolide	−95.4598	−84.2812	−5.27447
6	Isoandrographolide	−89.8945	−72.8517	−7.25545
7	Deoxyandrographolide	−87.8172	−73.6136	−2.7837
8	Zelapar (standard drug)	−49.9563	−10.6736	−0.12296
9	Andrographan	202	1553.43	0
10	14-deoxyandrographolide	8118.18	241.916	2.06533

A. paniculata: Andrographis paniculata, MAO-B: Monoamine oxidase B

Table 2: H-bond interaction of the phytoconstituents of *A. paniculata* with the target protein (MAO-B)

S. No	Compound	Number of H-bonds	H-bond interaction site
1	14-deoxy-11,12-didehydroandrographolide	11	Thr 408 A, Gln 409A, Asn 170 B, Glu 176 B, Thr 177 B, His 178 B
2	Andrographan	-	-
3	Andrographolide	4	Thr 408 A, Gln 409 A, Thr 177 B
4	Andrographoside	8	Lys 148 B, Asn 170 B, Gly 319 B, Glu 320 B, Leu 345 B, His 347 B
5	14-deoxyandrographolide	11	Thr 408 A, Gln 409 A
6	Deoxyandrographolide	3	Thr 408 A, Lys 149 B, Thr 177 B
7	Isoandrographolide	7	Asn 145 A, Gly 405 A, Thr 408 A, Gln 409 A, Thr 177 B, His 178 B
8	Neoandrographolide	12	Trp 135A, Glu 141A, Thr 408 A, Gln 409 A, Arg 412 A, Lys 149 B, Thr 177 B, His 178 B
9	Stigmasterin	2	Glu 159 B
10.	Zelapar (standard drug)	1	Gln 409 B

A. paniculata: Andrographis paniculata, MAO-B: Monoamine oxidase B

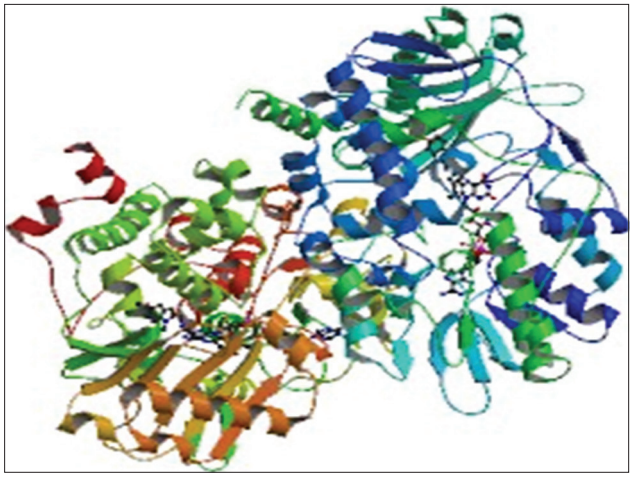

Fig. 1: Three-dimensional crystal structure of the target protein monoamine oxidase B (4CRT)

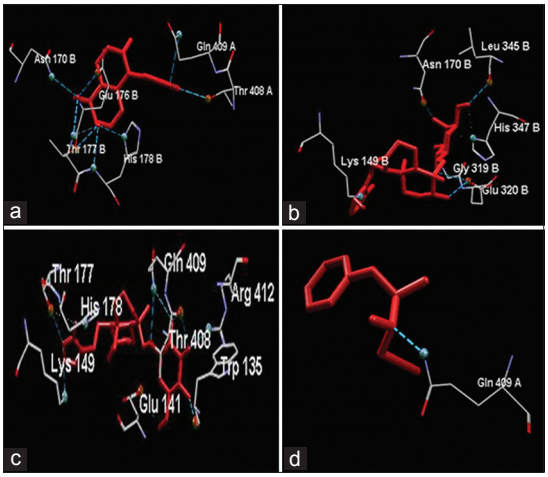

Fig. 2: (a) Interaction of 14-deoxy-11, 12-didehydroandrographolide with monoamine oxidase B (MAO-B) protein, (b) interaction of andrographoside with MAO-B protein, (c) interaction of neoandrographolide with MAO-B protein, (d) interaction of Zelapar (standard drug) with MAO-B protein visualized using Molegro viewer

with more number of H-bond interactions with 6, 6 and 8 aminoacid residues, respectively, present in the binding pocket of the target enzyme as shown in Table 1. 14-deoxy-11, 12-didehydroandrographolide, and neoandrographolide interact with the target protein in a similar pattern. Their interaction with the target majorly happens through the similar binding pattern with the same amino acids and at the same positions namely Thr 408 A, Gln 409A, Thr 177 B, and His 178 B (Fig. 2).

Lipinski's rule of five

The bioavailability of the phytoconstituents and standard drug were calculated based on the Lipinski's rule of five, and the results were shown in Table 3.

DISCUSSION

In recent scenario, computational techniques such as molecular docking tools are used to design novel lead molecules by exploring the interaction between the protein and the ligand molecules [19]. The docking results predict that the phytoconstituents from *A. paniculata* have effective interaction with the target protein. The compound neoandrographolide showed more potent inhibitory activity against MAO-B protein. Neoandrographolide is a naturally occurring compound present in *A. paniculata* nees and possesses a wide range of biological application because of its anti-inflammatory activity. The interaction of the compound with the target protein was analyzed using Molegro viewer module.

Table 3: Bioavailability of the phytoconstituents based on Lipinski's rule of five

Compound name	Status
14-deoxy-11,12-didehydroandrographolide	Bioavailable
Andrographan	Not bioavailable
Andrographolide	Bioavailable
Andrographoside	Not bioavailable
14-deoxyandrographolide	Bioavailable
Deoxyandrographolide	Bioavailable
Isoandrographolide	Bioavailable
Neoandrographolide	Bioavailable
Stigmasterin	Not bioavailable
Zelapar (standard drug)	Bioavailable

As evident from the Table 2, the MolDock score, Re-rank score, and H-bond score for the compounds, such as 14-deoxy-11,12-didehydroandrographolide, andrographoside, neoandrographolide, and stigmasterin, were higher. Neoandrographolide had the maximum score (126.78 Kcal/mol) with the highest value of H-bond interaction (−14.3599 Kcal/mol) and showed a greater affinity for the target protein. The activity of the compound neoandrographolide was greater compared to that of the standard drug Zelapar, which is used commercially for the treatment. Compounds such as androgrpahan and 14-deoxyandrographolide showed a very less MolDock score and hence have a very less affinity for the target. Development of neandrographlide as a potential inhibitor would assist in effective medication for the disease with minimal or lesser toxic side effects.

CONCLUSION

The present molecular docking studies provide insights into inhibition of MAO-B by phytoconstituents from *A. paniculata*. Docking study proposes that neoandrographolide has a high binding affinity for MAO-B protein. This study has led to the development of novel lead molecules which would help to develop enzymatic mechanisms allowing tumors to resist or escape immune rejection.

REFERENCES

1. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol 2006;5(6):525-35.
2. Dauer W, Przedborski S. Parkinson's disease: Mechanisms and models. Neuron 2003;39(6):889-909.
3. Jankovic J. Parkinson's disease: Clinical features and diagnosis. J Neurol Neurosurg Psychiatry 2008;79(4):368-76.
4. Thomas T. Monoamine oxidase-B inhibitors in the treatment of Alzheimer's disease. Neurobiol Aging 2000;21(2):343-8.
5. Stern G. Neuroprotection by selegiline and other MAO inhibitors. J Neural Transm Suppl 1998;52:99-107.
6. Levitt P, Pintar JE, Breakefield XO. Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. Proc Natl Acad Sci U S A 1982;79(20):6385-9.
7. Edmondson DE, Binda C, Mattevi A. Structural insights into the mechanism of amine oxidation by monoamine oxidases A and B. Arch Biochem Biophys 2007;464(2):269-76.
8. Shih JC. Cloning, after cloning, knock-out mice, and physiological functions of MAO A and B. Neurotoxicology 2004;25(1-2):21-30.
9. Sawamoto N, Piccini P, Hotton G, Pavese N, Thielemans K, Brooks DJ. Cognitive deficits and striato-frontal dopamine release in Parkinson's disease. Brain 2008;131:1294-302.
10. Kumar MJ, Nicholls DG, Andersen JK. Oxidative alpha-ketoglutarate dehydrogenase inhibition via subtle elevations in monoamine oxidase B levels results in loss of spare respiratory capacity: Implications for Parkinson's disease. J Biol Chem 2003;278(47):46432-9.
11. Siddiqui A, Mallajosyula JK, Rane A, Andersen JK. Ability to delay neuropathological events associated with astrocytic MAO-B increase in a Parkinsonian mouse model: Implications for early intervention on

disease progression. Neurobiol Dis 2011;43:527-32.
12. Kanokwan J, Nobuo N. Pharmacological aspects of *Andrographis paniculata* on health and itws major diterpenoid constitute andrographolide. J Health Sci 2008;54(4):370-81.
13. Mishra SK, Sangwan NS, Sangwan RS. *Andrographis paniculata* (Kalmegh): A review. Pharmacogn Rev 2007;1:283-9.
14. Ramaraj K, Ronaldo Anuf A. Inhibition of FGFR2 signal transduction in acne vulgaris using bioactive flavonoids: An *in silico* approach. Asian J Med Pharm Sci 2014;2(2):136-42.
15. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. J Med Chem 2006;49(11):3315-21.
16. Tebby C, Mombelli E, Pandard P, Péry AR. Exploring an ecotoxicity database with the OECD (Q)SAR Toolbox and DRAGON descriptors in order to prioritise testing on algae, daphnids, and fish. Sci Total Environ 2011;409(18):3334-43.
17. Ronaldo Anuf A, Breethi N, Shahila S, Ramaraj K. *In silico* analysis of plumbagin and its synthetic analogues as potential Bcl-2 inhibitors. Asian J Med Pharm Sci 2014;2(2):108-14.
18. Lo HW, Xia W, Wei Y, Ali-Seyed M, Huang SF, Hung MC. Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer. Cancer Res 2005;65(1):338-48.
19. Srivastava V, Gupta SP, Siddiqi MI, Mishra BN. Molecular docking studies on quinazoline antifolate derivatives as human thymidylate synthase inhibitors. Bioinformation 2010;4(8):357-65.