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Virtual Screening and Molecular Docking Studies of Compounds from *Phaseolus Vulgaris* against Nitric Oxide Synthase

Petchiammal C.
Research scholar, Department of Bioinformatics
School of Bioengineering, SRM University,
Kattankulathur, Tamilnadu, India
E-mail: petbiotech@gmail.com

Waheeta Hopper
Professor and Head, Department of Bioinformatics,
School of Bioengineering, SRM University,
Kattankulathur, Tamilnadu, India
E-mail:hod.bi@ktr.srmuniv.ac.in

Abstract - **Nitric-oxide synthase (NOS) is a therapeutic target to modulate pathologically high nitric oxide (NO) synthesis. Nitric oxide synthase could be a potential target for novel antioxidant strategies. *Phaseolus vulgaris* possesses many bioactive compounds. In this study the compounds from the plant were analyzed for their bioactivity against the nitric oxide synthase protein using virtual screening approach. The GLIDE docking result showed that allantoic acid from *Phaseolus vulgaris* possessed good Glide score and interacted well with the active site residues.**

Keywords-Antioxidant; Phaseolus vulgaris; molecular docking; allantoic acid

I. INTRODUCTION

Nitric oxide is a free radical widely produced in body in macrophages, endothelial cells, neutrophils, hepatocytes, neuron and many other cells [1, 2, 3&4] and can behave both as oxidant and antioxidant [3]. Its important functions are relaxation of smooth muscles (Vasodilation), neurotransmission, and inhibition of adhesion, activation and aggregation of platelets, cytoprotection, signal transduction and antioxidation. However, when produced in excess, it causes toxicity promoting diabetic complications, atherosclerosis, neurotoxicity, increased adhesion, stroke and neurodegenerative diseases, activation and aggregation of platelets and formation of another free radical nitrogen dioxide [5,8]. Nitric oxide is synthesized from arginine by three types of NOS: neuronal NOS (n-NOS) NOS I, inducible nitric oxide synthase (inos) NOS II and endothelial nitric oxide synthase (eNOS) NOS III. NOS could be a potential target for novel antioxidant strategies [6]. NOS have been developed as therapeutic agents to modulate pathologically high nitric oxide (NO) synthesis [7, 8]. The family of NOS catalyzes the oxidation of substrate L-arginine to L-citrulline and NO [9], or a related molecule [10-12], in an NADPH and O_2 dependent manner . Enzyme activity depends on multiple cofactors including Flavin adenine dinucleotide (FAD), Flavin mononucleotide (FMN), (6R)-5,6,7,8-tetrahydro-L-biopterin (H4BIP), and iron protoporphyrin IX (heme), which are localized within each by domain monomer consisting of an N-terminal oxygenase and a C-terminal reductase domain connected by a calmodulin (CaM) recognition sequences [13, 14]. Importantly, only homodimeric NOS is able to metabolize L-arginine and a single inter-subunit $ZnSO_4$ cluster appears to be important for stabilizing the dimer and H4Bip-binding site [14, 15].

NOS catalysis can be inhibited within the oxygenase domain by compounds binding to the heme prosthetic group such as indazoles and imidazoles for the heme binding site are questionable. [16, 17].

Phaseolus vulgaris, red kidney bean contains phytochemicals, like phenolics which have essential health benefits [18]. It contains many bioactive compounds of medical importance such as allantoic acid. Allantoic acid ($C_4H_8N_4O_4$) is the end product of allantoicase an enzyme involved in uric acid degradation (purine metabolism). Although it is commonly accepted that allantoicase is lost in mammals, it has been identified in mice and human.

In the present study the compounds from *Phaseolus vulgaris* were virually screened to identify inhibitors against the NOS protein target.

II. MATERIALS AND METHODS

A. Protein structure preparation

The crystal structure of NOS heme domain (Fig.1) (PDB ID: 3NLE) with resolution of 1.95 Å [19] was retrieved from the protein data bank (PDB) (http://www.rcsb.org). The protein has two polypeptides and is co-crystallized with the ligand and heme. The protein was optimized and energy minimized using the protein preparation wizard of the Schrodinger Suite v 9.0 (http://www.schrodinger.com).

B. Ligand preparation

28 natural compounds from *P.vulgaris* were retrieved from Dr. Duke's database (http://www.ars-grin.gov/duke/). The structure files were downloaded from the PubChem database (http://www.pubchem.ncbi.nlm. nih.gov). A database of all the compounds was created and energy minimized using ligprep module of Schrödinger Suite.

C. Receptor Grid generation

The receptor grid was generated around the active site residues identified using PDBSum database. The grid size was kept at default 20 Å.

D. *Virtual screening of compounds from Phaseolus vulgaris*

The natural compounds from *P. vulgaris* were virtually screened with the receptor grid file using Virtual screening workflow module. The screening was done with HTVS (High Throughput Virtual Screening), SP (Standard Precision) and XP (Extra Precision) mode.

III. RESULTS AND DISCUSSION

Of the total 28 compounds, allantoic acid showed best interaction with active site residues Arg367 and the heme subunit of NOS (Fig.2). The glide score of the compound was -5.822 Kcal/mol which was higher compared to other compounds. When compared to the co-crystallized ligand, allantoic acid showed better binding affinity towards the heme domain and active site residues.

IV. CONCLUSION

Compounds from *P.vulgaris* were screened against the NOS protein. Allantoic acid was showed good interaction based on the XP dock score. The *in silico* analysis of allantoic acid from *P.vulgaris* suggests that it could have a better possibility as an antioxidant drug by inhibiting NOS protein.

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TABLE I. XP OUTPUT OF ALLANTOIC ACID

Compound name	*D----H-----A*	*Distance (Å)*	*Glide score (Kcal/mol)*
Allantoic acid	(Arg 367)- N-H-O N-H-O (HEME) N-H-O(HEME) N-H-O(Trp 449)	1.703 1.833 1.991 1.911	-5.822
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Allantoic acid	(Arg 367)- N-H-O N-H-O (HEME) N-H-O(HEME) N-H-O(Trp 449)	1.703 1.833 1.991 1.911	-5.822

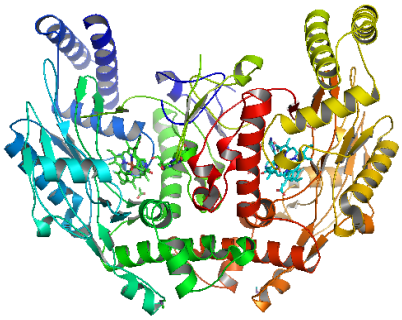

Figure 1. Structure of NOS heme domain

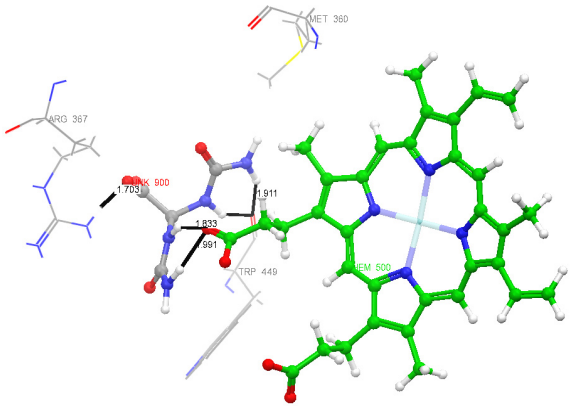

Figure 2. Interaction of allantoic acid with NOS