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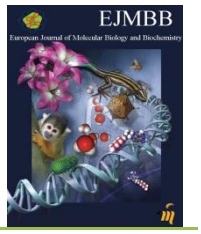

MOLECULAR DOCKING STUDIES OF XANTHINE OXIDASE INHIBITORS IDENTIFIED FROM *PSEUDARTHRIA VISCIDA*

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ABSTRACT

The enzyme xanthine oxidase catalyses the oxidation of hypoxanthine and xanthine to uric acid, which plays a crucial role in gout. During the reoxidation of xanthine oxidase, molecular oxygen acts as electron acceptor, producing superoxide radical and hydrogen peroxide. Gouty arthritis is an acute rheumatoid disorder that occurs in connection with the deposit of monosodium urate crystals in the joints. Monosodium urate crystal-induced inflammation is triggered by infiltration of neutrophils, and subsequent production of damage-causing superoxide. Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found brought to clinical trials and eventually released to the marketplace. Computer Aided Drug Design (CADD) is a specialised discipline that uses computational methods to stimulate drug-protein interaction. Discovery studio 2.1 provides a set of protocols for predicting and analysing the interaction between protein and ligands. Molecular docking experiments were carried out for compounds identified from *Pseudarthria viscida* root extract with Xanthine oxidase using Accelry's Discovery Studio 2.1. Out of thirteen compounds characterized from *Pseudarthria viscida* root, six compounds docked with Xanthine oxidase. Of that, n-Hexadecanoic acid and Tetradecanoic acid can be considered as a lead compound to treat hyperuricemia.

INTRODUCTION

Xanthine oxidase is the enzyme responsible for catalyzing the hydroxylation of hypoxanthine to xanthine and of xanthine to urate. Gout and hyperuricemia are the common metabolic disorders in human, associated with an elevated uric acid level in the blood, leading to deposition of urate crystals in the joints leading to gouty arthritis [1, 2]. The treatment for gout is either increasing the excretion of uric acid or reducing the uric acid production. Xanthine oxidase inhibitors are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents. Allopurinol is the only clinically used xanthine oxidase inhibitor, which also suffers from many side effects such as hypersensitivity syndrome [3]. Thus there is a need to develop compounds with xanthine oxidase inhibitory activity which is devoid of undesirable side effects of allopurinol. A potential source of such compounds can be obtained from medicinal plants [4, 5]. Flavonoids and polyphenolic crude extracts have been reported to posses' xanthine oxidase inhibitory activity [6, 7].

Secondary metabolites are the substances, which are produced by plants as defense chemicals. It includes alkaloids, flavonoids, essential oils, phenols, terpenes etc. These metabolites are sought after because they are known to exhibit number of biological activities that promotes health effects [8]. Plants have played a remarkable role in healthcare since the ancient times. Traditional plant based medicines still exert a great deal of importance to people living in developing countries and also leads to the discovery of new drug candidates [9].The plants were initially used in unmodified form, later as extracts, and in

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the 19th century, advances in chemistry made it possible to isolate the active compounds of some medicinal plants. A large number of the pharmaceutical agents used today contain natural compounds, including those with various modification of the original molecule [10]. In addition, bioactive plant compounds have served as templates for several synthetic drugs, and as precursors used in the production of semi-synthetic drugs [11, 12, 13].

Pseudarthria viscida (L) wight & Arn (Fabaceae) is a semi-erect diffuse under shrub, distributed throughout South India. The extract from the leaf, root, stem and callus of *Pseudarthria viscida* showed anti-fungal property [14]. A potential anti-oxidant activity has been reported from the stem and root extracts [15]. Literature survey revealed, the extract of this plant has been showed to exert anti-diabetic [16], anti-diarrhoeal [17] and anti-cancer effect [18].

In the field molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [19]. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Molecular docking is a study of how two or more molecular structures, for example drug and enzyme or receptor of protein, fit together. In other words, the problem is like solving a 3-dimensional puzzle. For example, the action of a harmful protein in human body may be prohibited by finding an inhibitor, which binds to that particular protein. Molecular docking softwares are mainly used in drug research industry. The most important application of docking software is virtual screening. In virtual screening the most interesting and promising molecules are selected from an existing database for further research. This places demands on the used computational method; it must be fast and reliable [20].

MATERIALS AND METHODS

Compounds identified from *Pseudarthria viscida* root

The presence of compounds like 3-O-Methyl-d-glucose, Butane-1, 1 Diethoxy-3-methyl, d-Mannitol-1-decyl sulfonyl, n-Hexadecanoicacid, Oleic acid, Oxirane tetra decyl, Tetradecanoic acid, Undecanoic acid was identified by GC-MS study. By HPLC analysis, the existence of phenolic compounds such as Rutin, Quercetin, Gallic acid, Ferulic acid and Caffeic acid was characterized. So in total 13 compounds identified in the root of *Pseudarthria viscida* was taken for binding analysis with Xanthine oxidase.

Ligand preparation

The three dimensional structures of compounds taken for binding analysis were downloaded in .sdf format from PubChem database. Hydrogen bonds were added and the energy was minimized using CHARMm force field. Lipinski properties such as Molecular weight, XLog P, number of hydrogen bond donors and acceptors for the compounds were obtained from PubChem (Table 1)

Protein preparation

The PDB is a key resource in areas of structural biology, is a key repository for 3D structure data of large molecules. The molecule which taken is Xanthine Oxidase for our consideration. The PDB ID is 3NVW and a resolution factor is 1.60A° and the method of incorporation is X-ray diffraction method. The ligand and crystallographic water molecules were removed from the protein; and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were connected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMm Force field.

Docking studies

The docking method used in this study is LigandFit. To perform docking process the modeled protein, a protocol called "Dockligands" (LigandFit) is selected among those listed under receptor-ligand interaction protocol cluster. Each ligand compoundis given as input in the parameter meant for "input ligands" and the protocol was run for each of the inhibitors selected for the study. The various conformations for ligand in this docking procedure were generated by Monte Carlo trials. The final energy refinement of the ligand pose (or) pose optimization in ligandfit occurs by Broyden-Flecher Gold Farbshanno (BFGS) method. The Dockscore of the best poses docked in to the enzyme for all the 13 compounds is calculated.

RESULTS AND DISCUSSION

The crystal structure of Xanthine Oxidase with PDB ID 3NVW having structural weight 280751.2 is retrieved from PDB. Resolution for 3NVW is 1.60 A° and found to be incorporated by X-ray diffraction method. In total this protein has six chains A, B, C, J, K and L. Out of these, 3 are sequence unique. Each chain has 164 amino acid residues. The structure of xanthine oxidase has 9 helices and 7 stands. 32% of the structure comprising 56 amino acid residues belongs to helical part and 18% of the structure comprising of 31 amino acid residues belong to strands.

Out of different compounds (ligands) taken for docking analysis only one compound docked with the protein Xanthine oxidase. Docked pose of the compound with protein (Xanthine Oxidase) is presented in Figure1.The dockscore values includes Ligscore 1&2 [21]; Piecewise Linear Potential (PLP) - PLP1 [22, 23] and PLP 2, Jain [24]; Potential of Mean Force- PMF [25]; PMF04 [26], Ligand internal energy and dockscore obtained using LigandFit protocol of Discovery studio 2.1.

The dock score value for the various compounds (ligands) identified from *Pseudarthria viscida* root with

xanthine oxidase enzyme were shown in Table 2. Ligands n-Hexadecanoic acid and Tetradecanoic acid showed highest dockscore with enzyme xanthine oxidase with comparison to other ligands. Ligands d-mannitol 1-decyl sulfonyl, Rutin and oelic acid form four hydrogen bonds, while n-hexadecanoic acid and tetra decanoic acid forms three hydrogen bonds with the enzyme. The detailed information about the atoms involved in forming the hydrogen bond and the number of hydrogen bonds formed between the ligands and enzyme were provided in Table 3

To ensure that the ligand orientation obtained from the docking studies was likely to represent valid and reasonable binding modes of the inhibitors, the ligand Fit program docking parameters had to be first validated for the crystal structure's active site. Protein utilities and health protocol of Discovery's studio was used to find out the active site contains amino acids such as Ser 9**,** Lys 11, Ala 156, Leu 157 etc. Results of docking showed that the Ligand Fit determined the optimal of the docking inhibitor, exactly to these active sites. Here top ranked ligand is taken for binding affinity studies. The validation process consisted of two parts;

1. Hydrogen bond details of the top-ranked docked pose.
2. Prediction of binding energy between the docked ligand and the enzyme using various score calculated using Discovery studio.

Table 1. Physiochemical Properties of Compounds Identified In *PseudarthriaViscida*

S.No.	Compound name	Mol. wt	X logp	H- Bond donor	H- Bond acceptor
1	3-O-Methyld- glucose	194.18246	-2.9	4	6
2	Butane -1,1- diethoxy-3-	160.2539	2.5	0	2
3	d-Mannitol-1- decyl sulfonyl	370.50	0.9	5	7
4	n- Hexadecanoic acid	256.42	6.4	1	2
5	Oleic acid	282.46	6.5	1	2
6	Oxirane tetra decyl	240.42	7.3	0	1
7	Tetradecanoic acid	228.37	5.3	1	2
8	Undecanoic acid	186.29	3.7	1	2
9	Rutin	610.5175	-1.3	10	16
10	Quercetin	302.2357	1.5	5	7
11	Gallic acid	610.5175	-1.3	10	16
12	Ferrulic acid	194.18	1.5	2	4
13	Caffeic acid	180.15	1.2	3	4

Table 2. Results for protein-ligand interaction

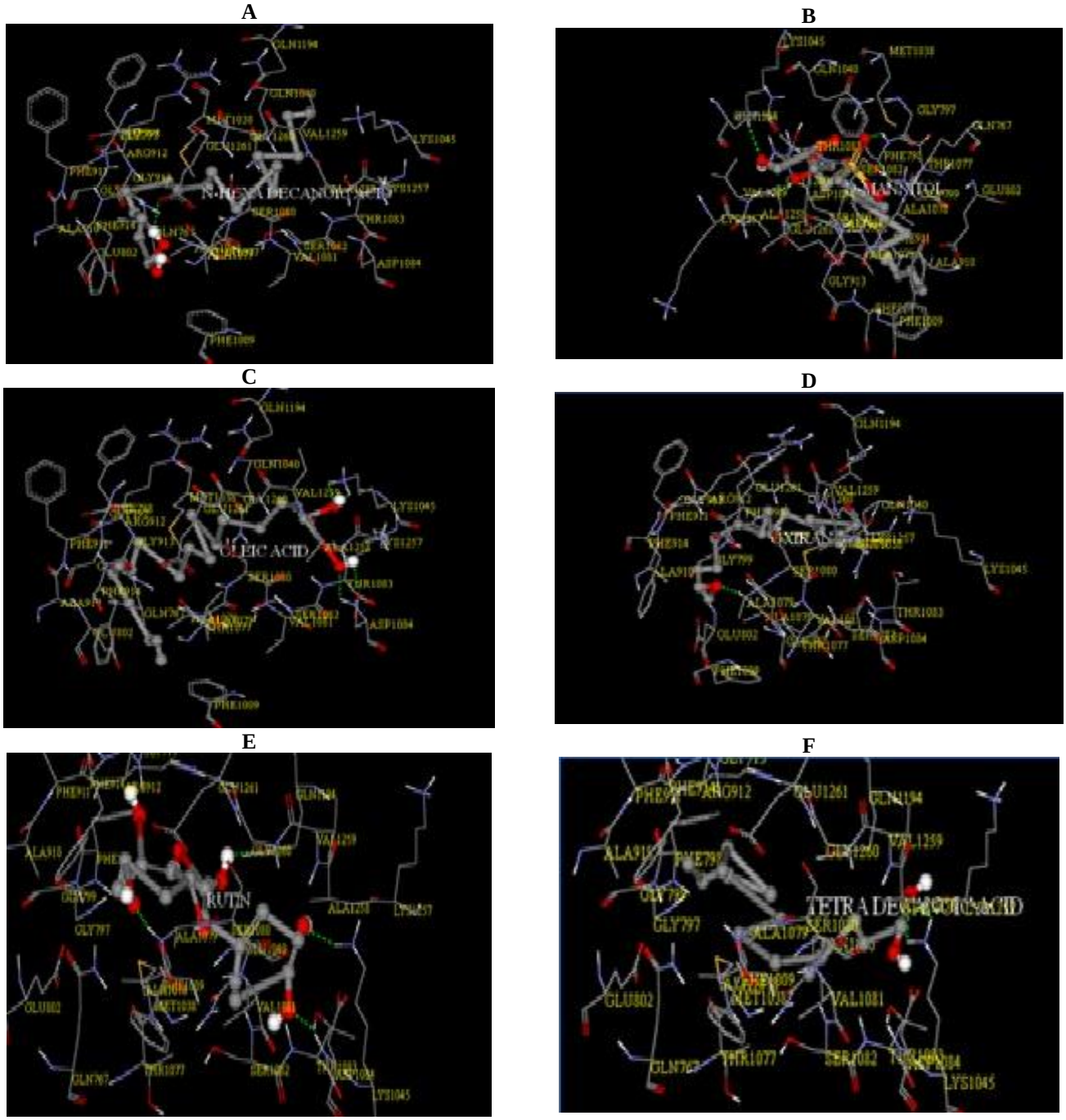
S.No.	Compound name	Lig Score 1	Lig Score 2	PLP 1	PLP 2	Jain	PMF	Dock Score
1	n Hexadecanoic acid	4.66	5.29	74.8	86.07	-0.38	9.91	62.81
2	Tetradecanoic acid	3.85	4.38	63.75	70.36	-1.24	-1.45	59.597
3	Rutin	5.17	3.53	44.79	70.77	1.23	9.54	39.705
4	Oleic acid	4.44	3.35	59.94	79.21	0.27	-5.93	37.976
5	d-Mannitol 1-decyl sulfonyl	4.21	3.89	76.13	92.05	-.032	15.77	33.901
6	Oxirane tetradecyl	2.96	3.08	46.44	55.66	-1.41	3.28	30.803

Table 3. **Hydrogen bond interaction details between xanthine oxidase and docked ligands**

S.No.	Name of the Ligand	Protein			Atom in ligand	No. of Interaction
		Chain	Amino acid	Atom in Amino acid		
1	D-Mannitol, 1-decylsulfonyl	C C C C	Phe 798 Lys 1045 Thr 1083 Gly 1260	HN HZ HN HN	012 020 022 023	4
2	Oleic acid	C C C C	Lys 1045 Lys 0145 Asp 1084 ASP 1084	HZ HZ HN OD1	020 020 19 H21	4
3	Rutin	C C C C	Lys 1045 Ala 1079 Thr 1083 Gln 1194	HZ HQ HG OE	021 018 022 H26	4

4	n-Hexadecanoic acid	C C C	Arg 880 Arg 880 Glu 1261	HE HH OE	018 018 H19	3
5	Tetradecanoic acid	C C C	Lys 1045 Lys 1045 Asp 1191	HZ HZ OD	018 015 H17	3
6.	Oxirane Tetradecyl	C	Ala 1079	HN	017	1

Fig 1. Summary of docked pose of compounds with Xanthine oxidase



Docked model of (A) n-Hexadecanoic acid (B) D-Mannitol, 1-decylsulfonyl (C) Oleic Acid (D) Oxirane (E) Rutin (F) Tetradecanoic Acid with Xanthine Oxidase

CONCLUSION

Bioinformatics approaches contribute supportive evidences for the promising action of a drug molecule under research and also help in saving time and minimizing the number of pre-clinical trials. It is imperative that bioinformatics and pharmaceutics complement each other and play an equal role in drug research which will prove effective in developing novel, specific and safe drugs to achive sustained response.

The present study indicates that *Pseudarthria viscida* can be used in the treatment of hyperuricemia, which shows a strong binding affinity towards Xanthine oxidase. This brings a strong focus towards this plant that, when administered during the treatment of hyperuricemia may block Xanthine oxidase. N-Hexadecanoic acid and Tetradecanoic acid showed the highest affinity towards Xanthine oxidase compared to other compounds. This creates a strong hypothesis that the effects of complex formation by Xanthine oxidase with this compounds contribute towards combating against hyperuricemia. Hence, Xanthine oxidase may become a prospective target for inhibition of hyperuricemia and may unlock a strong initiative in developing novel ligand which is specified towards it.

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