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Molecular Docking: Virtual Screening of Phytoconstitutents for Drug Discovery

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Abstract—*Molecular docking is an approach to predict receptor-ligand binding modes. The use of high-throughput screening in the pharmaceutical and biotechnology industries has led to the investigation of large number of compounds for their biological activity. In-vitro screening of number of compounds is an expensive and time-consuming process. Docking is a clear computational strategy of choice to augment and accelerate structure-based drug discovery. Xanthine oxidase (XO) is the metabolizing enzyme that is physiologically involved in the conversion of xanthine to uric acid; which in high concentration is responsible for inflammatory conditions. In-silico screening of the phytoconstituents with the enzyme XO will identify the most suitable drug candidate which can be further evaluated using in-vitro experimentation. This focused approach will reduce cost and time. Here, we docked a library of phytoconstituents using 'Maestro Schrodinger' software with XO; forming the basis of rational drug discovery. Phloretin showed potential interaction with the enzyme in this study.*

1. INTRODUCTION

The xanthine oxidase (XO) family comprises of molybdenum-dependent enzymes that usually form homodimers or dimers of heterodimers/trimers organized in three domains. N-terminal domain has two [2Fe-2S] clusters, one FAD molecule at intermediate domain, and a Molybdopterin (Mo-Co) cofactor in the C-terminal domain. The Mo-Co has two Mb atoms. Mb atoms are contained as molybdopterin cofactor and the active sites of the enzyme[1,16]. Xanthine oxidase is a form of Xanthine oxidoreductase (XOR) which is a highly conserved member of the molybdoenzyme family, which also includes aldehyde oxidase (AO) and sulphite oxidase (SO)[3]. In 1902, Schardinger identified xanthine oxidase in milk. In 1921, Frederick Gowland Hopkins discovered, Xanthine oxidase, an enzyme that is widely distributed in tissue and milk [4,5].

Xanthine oxidase (XO) is a metabolizing enzyme that plays an important role in the catabolism of purines in some species including human. In the last two reactions of purine catabolism, it converts hypoxanthine to xanthine and then xanthine to uric acid. It also generates reactive oxygen species (ROS) during this conversion. The oxidation of xanthine takes place at the molybdenum center, and the electrons thus introduced are rapidly transferred to FAD via the Fe–SI and Fe–SII centers [2]. The reoxidation of the reduced enzyme by the oxidant substrate, NAD+ or molecular oxygen, occurs through FAD. The reaction is as follows [18]:

$$\text{Hypoxanthine} + O_2 + H_2O \rightarrow \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + 2O_2 + H_2O \rightarrow \text{Uric Acid} + 2O_2^- + 2H^+$$

$$\text{Xanthine} + O_2 + H_2O \rightarrow \text{Uric Acid} + H_2O_2$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

High concentration of uric acid and ROS damages tissue, resulting in the release of lysosomal enzymes that induce an inflammatory response [8]. Inflammation is the cause of the various disorders such as complications in diabetes, neurodegenerative diseases which include Parkinson disease, epilepsy, Alzheimer's disease. Therefore, the inhibition of XO with potent inhibitors could be one of the strategies to prevent the inflammation and consequent disorders.[9,10,11,17,22].

There are two classes of XO inhibitors, one is purine analogues such as allopurinol, oxypurinol etc., and other is non-purine analogues such as quercetin, febuxostat etc.

In-vitro and in-vivo drug discovery takes approximately 12-14 years and costs up to 76 billion INR. Therefore, *in-silico* drug discovery is most suitable to screen and predict the active molecules which could be further evaluated for their proposed activity in order to save time as well as money [6, 17].

Molecular Docking is an approach to predict the most suitable and stable orientation of ligand- receptor complex. It is a structure based virtual screening approach to predict most suitable and stable receptor- ligand binding modes. The ideal structure is to identifying a better key for a given lock. The process of docking a ligand to a binding site mimics the natural course of interaction of the ligand and its receptor via lowest energy pathway. It computationally predicts the structures of protein-ligand complexes from their

conformations and orientation. The orientation that maximizes the interaction reveals the most accurate structure of the complex. The first approximation is to allow the substrate to do a random walk in the space around the protein to find the lowest energy. The success of a docking program depends on two components: the search algorithm and the scoring function [13,14,17,19,21].

In the present study, we docked a library of phytoconstituents with the enzyme XO using Schrodinger (maestro, Glide)[12,20,21]. Allopurinol and Oxypurinol were taken as standard XO inhibitors [7, 15]. Therefore, the objective of this study was to screen the phytoconstitutents for its interaction with enzyme Xanthine Oxidase, with a possible anti-inflammatory activity after doing the actual enzyme interaction *in-vitro*.

2. MATERIALS AND METHODS

2.1 Molecular docking

The docking studies were performed using Glide module (version 6.3, Schrödinger, LLC, NY) installed on redhat Linux workstation. Docking consists of five steps ligand preparation, protein preparation, receptor grid generation, actual docking procedure and finally viewing the docking results using the pose-viewer [12].

2.2 Ligand preparation

The following molecules were selected from SS Laboratory (Pharmacology lab II) (Institute of Chemical Technology, Mumbai) based on their reported activity:

Table 1: Non-purine analogues:

Serial No.	Names	Sources
1	Apigenin	*Matricaria recutita*
2	Apocynin	*Apocynum cannabinum*
3	Arecoline	*Areca catechu*
4	Daidzein	*Pueraria mirifica*
5	Diosgenin	*Costus speciosus*
6	Ellagic acid	*Quercus alba*
7	Ethyl ferulate	*Phaseolus vulgaris*
8	Gentesic acid	*Alchornea cordifolia*
9	Lutein	*Spinacia oleracea*
10	Luteolin	*Lamiaceae species*
11	Naringenin	*Citrus sinensis*
12	Phloretin	*Prunus mandshurica*
13	Psoralen	*Ficus carica*
14	Quercetin	*Capparis spinosa*
15	Thymol	*Thymus vulgaris*
16	Thymoquinone	*Nigella sativa*
17	Ursolic Acid	*Mirabilis jalapa*

Table 2: Purine analogues:

Serial No.	Names	
1	Acetyl xanthine	Derivative of xanthine
2	Allopurinol	Standard
3	Oxypurinol	Standard

Activator of XO: Histamine.

Ligand preparation was accomplished on all the phytoconstitutents using Ligprep (maestro, Schrödinger) module to clean the structure [12].

2.3 Protein preparation

Protein preparation was performed by using Protein Preparation Wizard of maestro (Schrodinger) software. XO with oxypurinol (PDB ID- 3BDJ at resolution 2.0 Å) and XO with quercetin (PDB ID- 3NVY at resolution 2.0 Å) were taken and treated to add missing hydrogen, assign proper bond orders, treat metal (i.e., breaking bonds to metal and correct the formal charge on metal and neighboring atoms), and to delete water molecules that are more than 5 Å from the heterogeneous groups. The H bonds were optimized using sample orientations. All the polar hydrogens were displayed. Finally, the protein structures were minimized to the default root mean square deviation value of 0.30 Å. The inhibitor structure was minimized using OPLS-2005 force field [12].

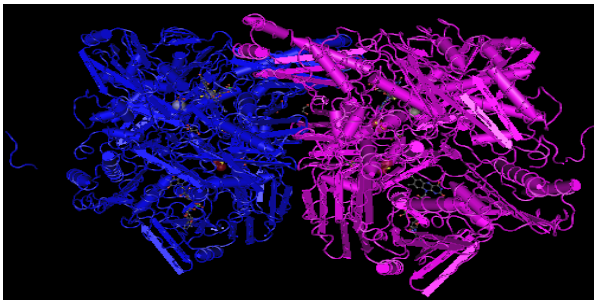

Figure 1. PDB ID- 3BDJ (Crystal structure of bovine XO with inhibitor oxypurinol) [24]

Figure 2. PDB ID- 3NVY (Crystal structure of bovine XO with inhibitor Quercetin) [24]

2.4 Receptor grid generation

Receptor was defined and the co-crystallized ligand was differentiated from the active site of receptor chain B for 3BDJ (XO with oxypurinol) and chain L for 3NVY (XO with quercetin). The atoms were scaled by van der Waals radii of 1.0 Å with the partial atomic charge less than 0.25 default. The active site for both the protein was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder. The ligands similar in size to the workspace ligand were allowed to dock into the active site. No constraints either positional, H bonding or hydrophobic were defined [12].

2.5 Ligand docking:

Ligand docking was performed using OPLS-2005 force field. The receptor grid defined in the receptor grid generation folder was selected for the docking of ligands prepared using Ligprep. Flexible docking was performed using the Extra Precision (XP) feature of Glide module. The van der Waals radii was scaled using a default scaling factor of 0.80 and default partial cutoff charge of 0.15 to decrease the penalties for close contacts. The core pattern comparison and similarity mode were not used since our aim was to study the binding of ligands to the active site. The constraints to defined ligand–receptor interactions were not set. The structure output form was set to pose viewer file so as to view the output of the resulting docking studies from pose-viewer [12].

2.6 Viewing docking results:

It was done using pose-viewer. The H bonds and bad and ugly van der Waals contacts to the receptor were visualized using default settings to analyze the binding modes of the ligands to receptor. The constraints to define ligand–receptor interactions were not set. The final ligand binding poses were ranked according to a computed model score that encompasses the grid score, proprietary GLIDE score, and the internal energy strain. The structure output format was set to pose-viewer file so as to view the output of the resulting docking studies from pose-viewer. The score function of Glide, or Glide score, a modified and expanded version of ChemScore, was used for binding affinity prediction and ligand ranking. The docking can be on the level of either standard (SP) or XP. The improvement of XP over SP includes the addition of large desolvation penalties to both ligand and protein, assignment of specific structural motifs that contribute significantly to binding affinity, and expanded sampling algorithms required by scoring function improvement (Glide 2014). Therefore, XP mode was used for molecular docking of XO (PDB ID- 3BDJ & 3NVY) with library of phytoconstitutents.

3. RESULTS AND DISCUSSION:

3.1 Molecular docking

The docking studies were carried out to explore the interaction mechanism between inhibitors and the receptor. The score function of Glide, gives highest binding affinity prediction as well as molecule ranking. The molecules along with their Glide docking score have been listed in Table 3.

Table 3. Docking score of XO with phytoconstitutents

Title	Docking Score	Title	Docking Score
3NVY		**3BDJ**	
Quercetin	-11.2	**Phloretin**	**-9.692**
Luteolin	-9.895	Quercetin	-9.578
Ellagic Acid	-8.787	Luteolin	-8.884
Naringenin	-8.497	Naringenin	-8.011
Phloretin	-8.068	Apigenin	-7.891
Apigenin	-7.88	Gentisic Acid	-7.558
Ethyl Ferulate	-7.165	Acetyl Xanthine	-5.65
Diadzein	-7.153	**Allopurinol**	**-5.525**
Acetyl Xanthine	-6.203	Ethyl Ferulate	-5.442
Psoralen	-6.1	Thymol	-4.941
Thymol	-5.97	**Oxypurinol**	**-4.155**
Oxypurinol	-5.702	Thymoquin-one	-4.144
Allopurinol	-5.687	Diadzein	-3.972
Histamine	-5.539	Psoralen	-3.513
Gentisic Acid	-5.318	Apocynin	-3.286
Apocynin	-5.155		
Thymoquinone	-4.743		
Diosgenin	-3.936		
Arecolin	-3.62		
Ursolic acid	-1.806		

In this study, we docked a library of phytoconstitutents with XO. Since there are two classes of inhibitors, we selected two XO-inhibitor crystal complexes, one was XO with inhibitor oxypurinol which comes under the class purine analogue and other was XO with inhibitor quercetin which is non-purine analogue.

In 3BDJ docking, phloretin (-9.692) showed maximum dock score which is much higher than the standard inhibitors (allopurinol: -5.525, oxypurinol: -4.155).

Many molecules have higher dock score than the standard inhibitors i.e., allopurinol with a dock score of -5.687 and oxypurinol with a score of -5.702 for 3NVY. In 3NVY, quercetin showed maximum interaction with the enzyme XO. Phloretin showed potential interaction with the enzyme. We have thus identified phloretin as a molecule for further experimentation on XO *in-vitro*.

According to literature, histamine is an activator of XO which is majorly responsible for inflammation [23].It would be worthwhile to look into the interaction of this docking site with many drugs to understand about their potential inflammatory activity/allergenicity. This will also help in identifying the adverse drug effects of certain drugs.

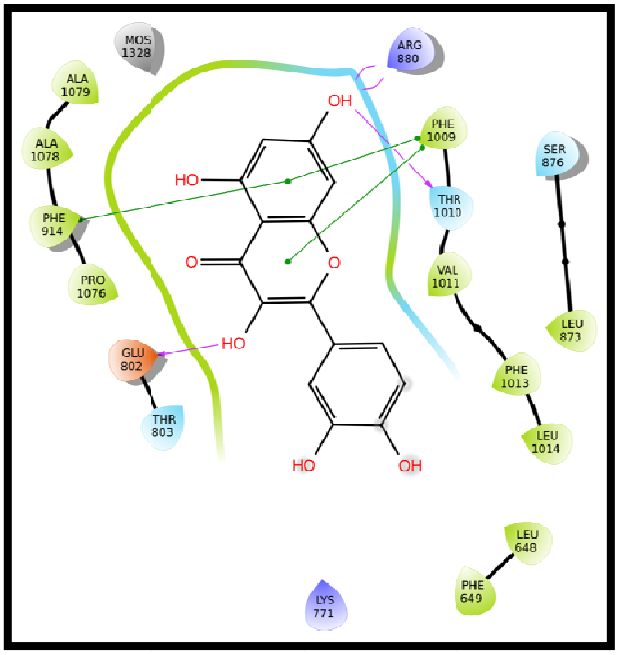

Figure 3. 2D interaction of 3NVY-QUERCETIN (most docked)

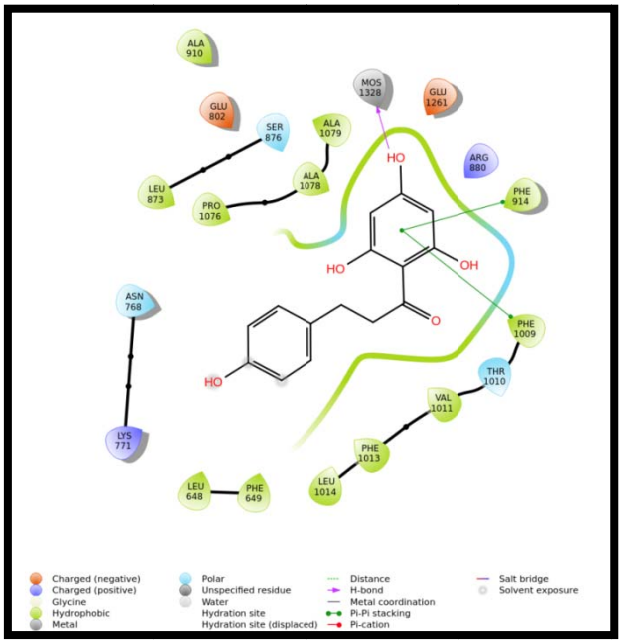

Figure 4. 2D interaction of 3NVY-PHLORETIN

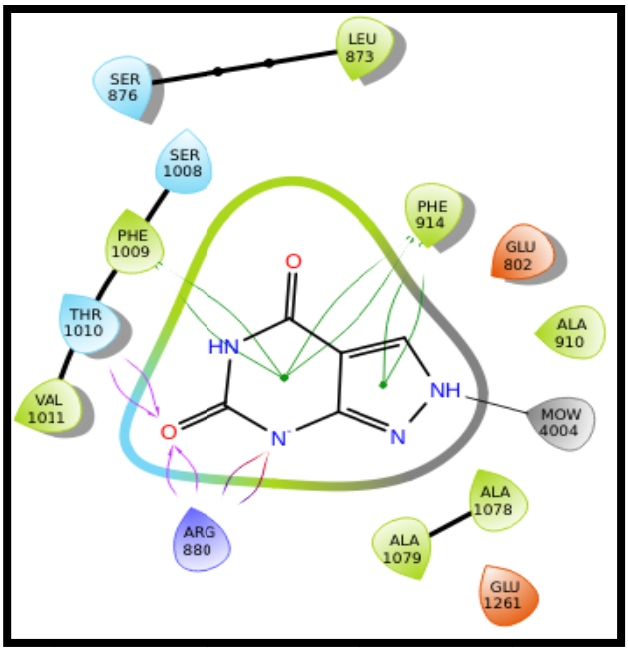

Figure 5. 2D interaction of 3BDJ-OXYPURINOL (Standard)

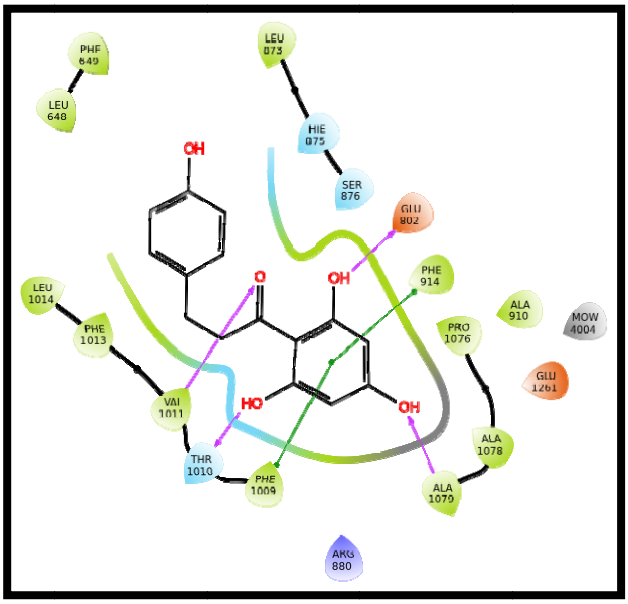

Figure 6. 2D interaction of 3BDJ-PHLORETIN (most docked)

4. CONCLUSION

The library of phytoconstituents have been docked with 3BDJ (XO with Oxypurinol) and 3NVY (XO with Quercetin). Phloretin showed potential interaction with the enzyme XO. Although certain molecules have more docking score than phloretin, they have already been studied. *In-silico* Naringenin showed good interaction with XO but according to literature it failed to show activity *in-vitro*. Hence, *in-vitro* experimentation is necessary for confirmation. It is important

to study the enzyme interaction *in- vitro* to comment on its stimulatory or inhibitory interaction with the enzyme.

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REFERENCES

[1] Cristine E. Berry and Joshua M. Hare; Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications; *J Physiol* 555.3 (2004), pp 589–606.

[2] Cristofer Enroth, Bryan T. Eger, Ken Okamoto, Tomoko Nishino, Takeshi Nishino and Emil F. Pai; Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: Structure-based mechanism of conversion; *www.pnas.org* 2000.

[3] Mohammad-Reza Rashidi and Roghiyeh Pashaei-Asl; Role of Aldehyde Oxidase and Xanthine Oxidase in the Metabolism of Purine-Related Drugs; *World's largest Science, Technology & Medicine*;published by intech.

[4] Hellsten-Westing Y; Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle; 1993 Sep; 100(3):215-22.

[5] Ichikawa M, Nishino T, Nishino T, Ichikawa A.; Sub cellular localization of xanthine oxidase in rat hepatocytes: high-resolution immunoelectron microscopic study combined with biochemical analysis; *J Histochem Cytochem*. 1992 Aug; 40(8):1097-103.

[6] L. Geris, Y. Guyot, J. Schrooten and I. Papantoniou; *In silico* regenerative medicine: how computational tools allow regulatory and financial challenges to be addressed in a volatile market; *Interface Focus*. 2016 Apr 6; 6(2): 20150105.

[7] Arumugam Madeswaran*, Muthuswamy Umamaheswari, Kuppusamy Asokkumar, Thirumalaisamy Sivashanmugam, Varadharajan Subhadradevi, Puliyath Jagannath; Computational drug discovery of potential phosphodiesterase inhibitors using in silico studies; *Asian Pacific Journal of Tropical Disease* 2012, S822-S826.

[8] Bruce CN, Terkeltaub R. The inflammatory process of gout and its treatment Res Ther. 2006:8(S3).

[9] Fridovich I . Quantitative Aspects of the Production of Superoxide Anion Radical by Milk Xanthine Oxidase*. J Biol Chem*. 1970;245:4053-4057.

[10] Borges F, Fernandes E, Roleira F. Progress towards the discovery of xanthine oxidase inhibitors. *Cur Med Chem.* 2002;9:195-217.

[11] Mehta SK, Nayeem N. Natural xanthine oxidase inhibitors for management of gout: a review. Research and Reviews: *J Med. Health Scie.* 2014;3:1-3.

[12] (http://www.schrodinger.com/kb/1635, Schrödinger Prime User Manual)

[13] Sahi C, Singh A, Kumar K, Blumwald E, Grover A. Salt stress response in rice: genetics, molecular biology, and comparative genomics. *Functional & integrative genomics.* 2006 Oct 1;6(4): 263-84.

[14] Nishtha Singh, Sonal Upadhyay, Ankur Jaiswar and Nidhi Mishra*.In silico Analysis of Protein.*JSM Bioinformatics, Genomics and Proteomics.* 2016 September 07;1(2);1-7.

[15] Okamoto K, Eger BT, Nishino T, Pai EF, Nishino T. Mechanism of inhibition of xanthine oxidoreductase by allopurinol: crystal structure of reduced bovine milk xanthine oxidoreductase bound with oxypurinol. *Nucleosides, Nucleotides, and Nucleic Acids*. 2008 Jul 23;27(6-7):888-93.

[16] Enroth C, Eger BT, Okamoto K, Nishino T, Nishino T, Pai EF. Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. *Proceedings of the National Academy of Sciences.* 2000 Sep 26;97(20):10723-8.

[17] Lin S, Zhang G, Liao Y, Pan J, Gong D. Dietary Flavonoids as Xanthine Oxidase Inhibitors: Structure–Affinity and Structure–Activity Relationships. *Journal of agricultural and food chemistry.* 2015 Aug 21;63(35):7784-94.

[18] Okamoto K, Kusano T, Nishino T. Chemical nature and reaction mechanisms of the molybdenum cofactor of xanthine oxidoreductase. *Current pharmaceutical design*. 2013 Apr 1;19(14):2606-14.

[19] Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. *Molecules.* 2015 Jul 22;20(7):13384-421.

[20] Xie H, Qiu K, Xie X. 3D QSAR studies, pharmacophore modeling and virtual screening on a series of steroidal aromatase inhibitors. *International journal of molecular sciences*. 2014 Nov 14;15(11):20927-47.

[21] Eweas AF, Maghrabi IA, Namarneh AI. Advances in molecular modeling and docking as a tool for modern drug discovery. *Der Pharma Chemica*. 2014;6(6):211-28.

[22] Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *The Journal of physiology.* 2004 Mar 16;555(3):589-606.

[23] Activation of xanthine oxidase by histamine, Preliminary notes, *Biochem biophys Acta*, 60 (1962) 445-447.

[24] https://www.rcsb.org/pdb/home/home.do