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Potent Lead Identification against Xanthine oxidase for the Treatment of Gout: a Computational Approach

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ABSTRACT

Gout is a frequent inflammatory arthritis characterized by urate crystal accumulation within joints. Xanthine oxidase (XO) is an enzyme that converts hypoxanthine and xanthine to uric acid and, when overproduced, causes gout. As a result, blocking XO activity is critical for reducing urate production. Here, we present the result of In silico screening using ligand-based computational method to identify most suitable compounds as xanthine XO inhibitor. We used the known 11 xanthine inhibitors in our Pharmacophore modelling using pharmagist webserver to uncover the novel entities. Through the ZINCPharmer webserver, 1000 most comparable pharmacophoric ligands that are feasible were identified. By the application of Datawarrior tool, compounds were further filtered and used for molecular docinkg using Autodock vina. Based on binding energy and amino acid interaction top 10 ligands are subsequently evaluated for ADMET properties using PKCSM webserver. The ligand ZINC27683167, ZINC89510657 and ZINC91046011 showed more binding energy values (-9.6, -9 and -9Kcal/mol) when compared with binding energies of standard drug allopurinol (-6.6kcal/mol.). Filtered ZINC compounds to be more potent than Allopurinol. These findings highlight the discovery of a novel class of XO inhibitors that have the potential to be more effective than allopurinol in the treatment of gout.

KEYWORDS: *Gout, Allopurinol, Xanthine oxidase, Docking, Pharmacophore modelling, ADMET.*

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INTRODUCTION

Gout is the most frequent kind of inflammatory arthritis. Hyperuricaemia, or increased serum uric acid (SUA) levels, is a key factor in the development of gout. As SUA levels rise and the physiological saturation threshold for uric acid in body fluids is exceeded, monosodium urate crystals form and deposit in joints and soft tissues, causing inflammation and degeneration in addition to crystal formation [1].Males over the age of 40 are more likely to acquire it than women, making it the most common type of inflammatory arthritis in this age group [2,3]. Gout is growing more common and more common for a variety of causes. Increasing longevity, rising comorbidity rates, the use of specific prescription medicines, and changing dietary and lifestyle patterns have all been linked to an increased risk of acquiring gout [4-6]. Furthermore, certain populations, such as transplant patients who use cyclosporine, are considered high risk. Furthermore, certain populations, such as transplant patients who use cyclosporine, are considered high risk [7,8]. Gout has a substantial economic and social impact on society. Patients with acute gouty flares or chronic gout have a reduced health-related quality of life due to discomfort, restricted activity, and disability. Productivity and work-related activity are both much lower in this cohort. Furthermore, the small number of people with chronic gout who are resistant to traditional therapy bear an exceptionally high burden of the illness [9-11]. Side effects associated with Anti gout drugs are ulcers, bleeding, stomach pain, Nausea, vomiting, diarrhea, Mood swings, raised blood sugar, high blood pressure, fever, rash, hepatitis, and kidney issues. Even a brief course of therapy can have negative effects because glucocorticoid toxicity is more prevalent with prolonged and repeated treatments [12]. The application of computer-aided drug discovery/design (CADD) techniques at various stages of the drug development process helps to reduce total costs. As a result, CADD approaches

are becoming increasingly widespread and have played a key part in the discovery of therapeutically relevant compounds during the previous few decades. These facts highlight the need to adopt more effective treatment strategies for gout treatment[13].
Hence the present study aims to identify novel lead moieties to inhibit xanthine oxidase for the treatment of gout through the application of computational tools.

MATERIAL AND METHODS

Pharmacophore modeling: A pharmacophore is a molecular framework that outlines the critical elements responsible for a molecule's biological activity [14]. Pharmacophore models are created to improve knowledge of ligand-protein interactions. They can be used to find novel compounds that meet the pharmacophore requirements and are thus predicted to be active [15].If the target structure is not available, pharmacophore models can be constructed using structural information from the active ligands that bind to the target. This is referred to as the ligand-based pharmacophore modelling technique [16]. Pharmacophore models can be developed utilizing the structural attributes of the target when the target's structure is accessible. This method is known as structure-based pharmacophore modelling. Pharmacophore modelling has been used at many stages of the drug discovery process using pharmagist software. In the drug development process, there are two main techniques to pharmacophore modelling: Pharmacophore modelling based on ligands and structure-based pharmacophore modelling. Novel ligands are designed utilizing a set of active ligands available in the ligand-based pharmacophore modelling approach [17]. If the target structure is not available, this strategy is used. Similarly, when the structure of the target protein is known, the structure-based pharmacophore method is used [18]. In the ligand-based pharmacophore modelling, first active ligands are identified by using the literature available or database search. The data set is split into a training set and test set. The training set ligands' features are then analyzed. The alignment of the active ligands reveals the similar properties. The next step is to generate pharmacophore models and rank the models that are generated. Finally, the pharmacophore model is validated, and the best pharmacophore model is chosen based on the results [19].
Molecular docking: Docking is a method in molecular modelling that predicts the preferred orientation of one molecule to another when a ligand and a target are coupled together to form a stable complex. Using scoring functions, for example, knowledge of the preferred orientation can be used to predict the strength of connection or binding affinity between two molecules. Because of its capacity to anticipate the binding-conformation of small molecule ligands to the proper target binding site, molecular docking is one of the most commonly utilised strategies in structure-based drug design. Characterization of binding behavior is vital in both rational drug design and elucidating fundamental biological processes [20].The initial condition for docking is the protein structure (xanthine oxidase). Typically, a biophysical approach such as x-ray crystallography or NMR spectroscopy was used to determine the structure. A docking tool uses this protein structure and a database of possible ligands as inputs. A docking program's success is determined by two factors: the search algorithm and the scoring mechanism. Then once a target is ready the ligands. Then the ligands are made cluster by using discovery studio. Then the cluster and target are uploaded in pyrx and then are autodocked by using this software. Then the obtained result is taken and verified. Then the interaction between ligand and the target is found by using pymol. Then the 2D and 3D interaction image are taken with the help of discovery studio [21].
ADMET:ADMET profiles were analyzed using PKCSM web tool. All the ligands' Simplified Molecular Input Line Entry System (SMILES) formats were obtained from the PubChem database for this analysis. Lipinski's rule of 5 was applied to all the ligands' drug-likeness to see if all the properties were within the acceptable range. The PubChem database was used to get all the ligands' Simplified Molecular Input Line Entry System (SMILES) forms for this investigation. Lipinski's rule of five was used to establish whether each ligand's drug-likeness is within the allowed range. The atom-based logarithm of the partition coefficient was used to calculate lipophilicity levels (ALogP). The blood-brain barrier (BBB) was investigated in terms of drug distribution. For substrate or inhibition, drug metabolism was estimated. In addition to these, drug toxicity was examined, with a focus on hepatotoxicity, AMES toxicity [22].

RESULT

Ten diverse compounds ZINC07988619, ZINC08323999, **ZINC27683167,** ZINC31314416, ZINC38600479, ZINC67246986, ZINC72510810, ZINC87026910, ZINC89510657, ZINC91046011 After optimization, the training set with the highest activity and diversity of chemical structure was chosen. The Feature Mapping tool in Discovery Studio was used to extract and cluster essential characteristics in this protocol. Allopurinol, Oxypurinol, Benzbromarone, Celecoxib, Fenofibrate, losartan, Probencid, Colchicine, Febuxostat, Topiroxostat, Lensinurad, are used to inhibit xanthine oxidase. Table 1 displays the docking score and binding interaction. Celecoxib was discovered among the inhibitors with a

docking score of -9.1 kcal/mol and conventional hydrogen bonding with ARG313, GLN100, GLN415, THR416. Pi - alkyl interaction with PHE198, Alkyl interaction with ALA151, Interaction of halogen with GLN372. These ligands attach to the protein by hydrogen bonding, Alkyl interactions, Halogen interactions, and Pi-alkyl interactions. The residue tacking part in interaction were ARG313, GLN100, GLN415, THR416, ALA151, PHE198, GLN374, PHE372. As a result, the foregoing interaction could be crucial and explain why molecules act as Xanthine oxidase inhibitors. The compound was docked after extracting the molecule from the ZINC database using ZINC Pharma based on the pharmacophore. In this case, ten chemicals were chosen for molecular docking with the target protein PDB ID: 7MSD. The docking was examined to obtain compounds with the highest docking score (most negative) to compare with the binding interactions of compounds. This comparison aids in identifying the hit molecule as a Xanthine oxidase inhibitor. The chemicals are identified based on their binding interaction. A docking score of between -8.2 and -9.6 kcal/mol is possible. A molecule named ZINC27683167 was discovered through molecular docking to have the best docking score of -9.6 kcal/mol and to interact with TRP103 in the Pi-Pi stacking and Pi-Pi T shapes. pi-alkyl interaction with PRO200, as well as alkyl interaction with PRO200. Hence, these 10 compounds can block xanthine oxidase.

Table 1: Standard and test compounds binding affinity

Ligands	Binding affinity	ZINC ID	Binding affinity
Febuxostat	-8.4	ZINC07988619	-8.2
Oxypurinol	-6.8	ZINC08323999	-8.3
Allopurinol	-6.6	**ZINC27683167**	**-9.6**
Benzbromarone	-8.3	ZINC31314416	-8.5
Celecoxib	**-9.1**	ZINC38600479	-8.8
Fenofibrate	-7.7	ZINC67246986	-8.2
Losartan	-8.9	ZINC72510810	-8.7
Probencid	-7.1	ZINC87026910	-8.3
Topiroxostat	-8.6	ZINC89510657	-9
Lensinurad	-8.5	ZINC91046011	-9
Colchicine	-8.8		

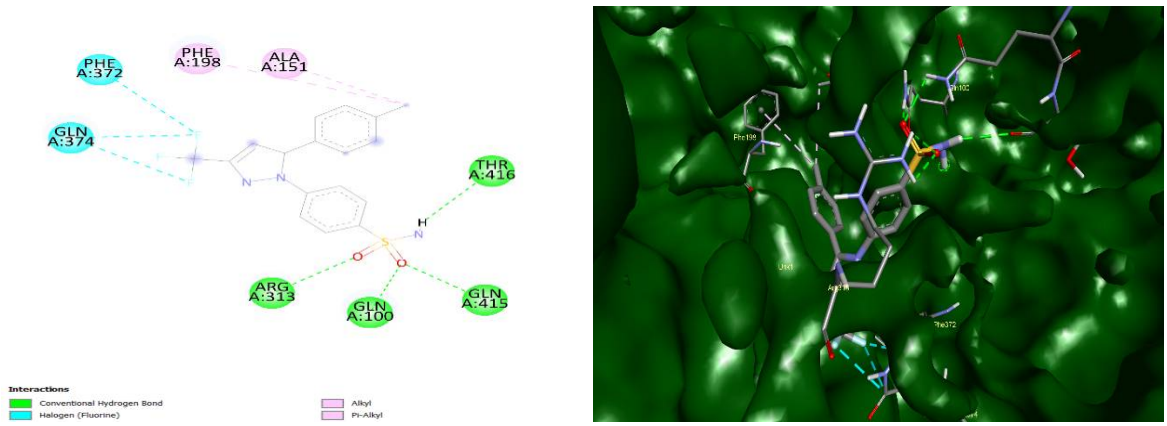

Fig 1: 2D view of molecular interaction of amino acid residues of xanthine oxidase with celecoxib

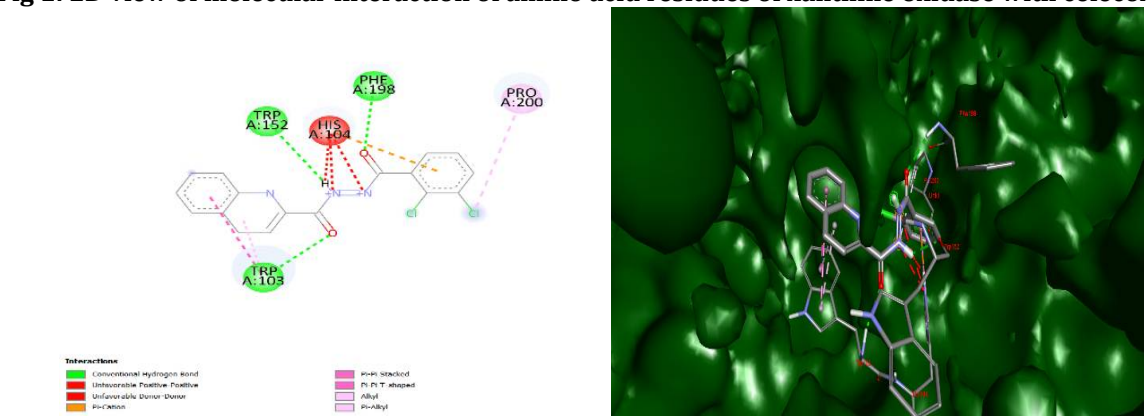

Fig 2: 2D(left) and 3D(right) view of molecular interaction of amino acid residues of xanthine oxidase with ZINC27683167.

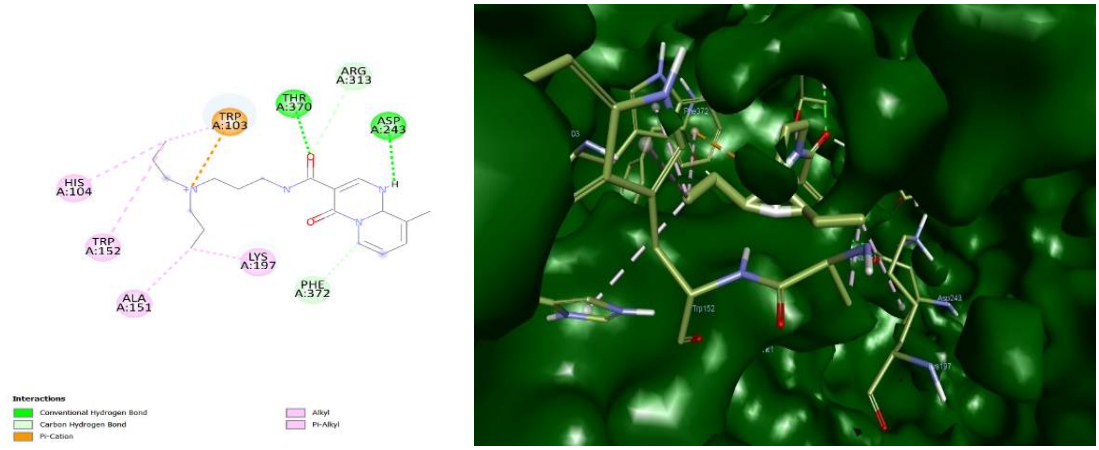

Fig3: 2D(left) and 3D(right) view of molecular interaction of amino acid residues of xanthine oxidase with ZINC91046011

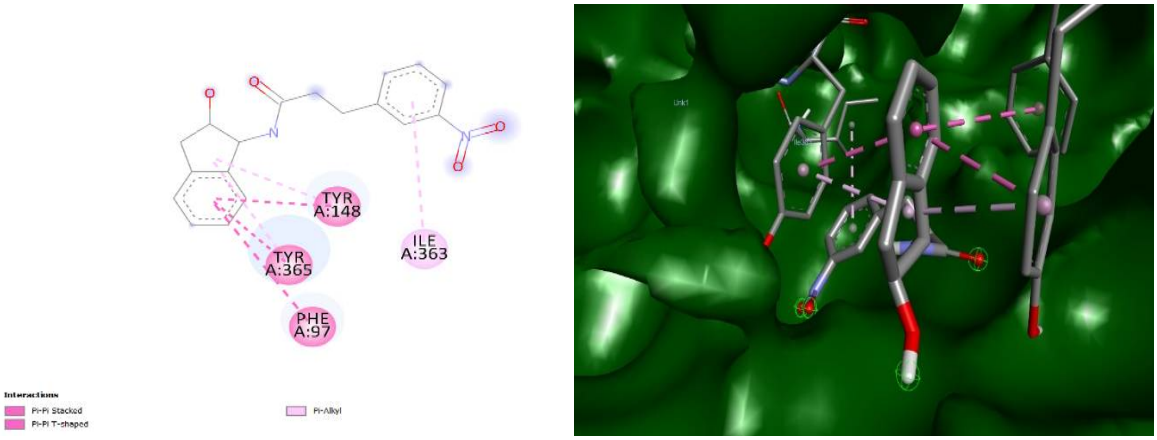

Fig 4: 2D(left) and 3D(right) view of molecular interaction of amino acid residues of xanthine oxidase with ZINC89510657

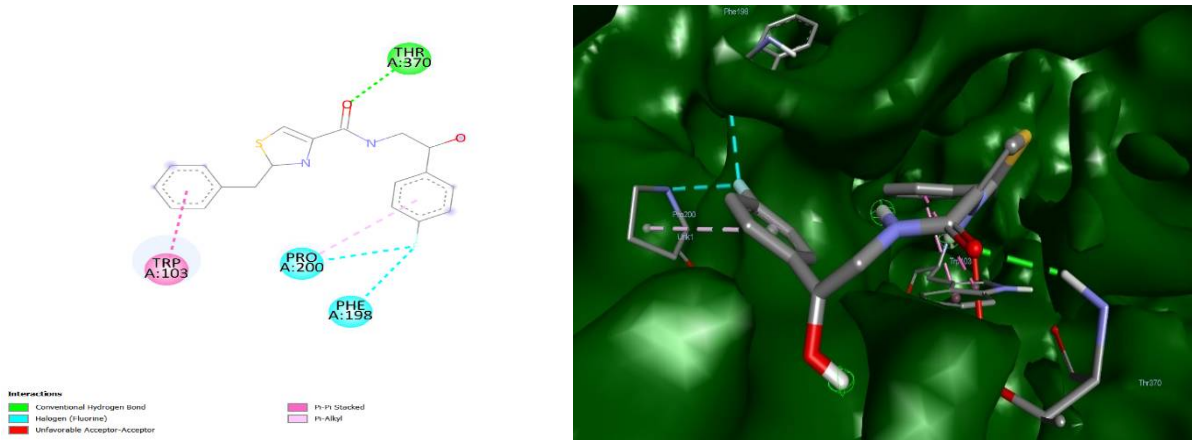

Fig 05: 2D(left) and 3D(right) view of molecular interaction of amino acid residues of xanthine oxidase with ZINC38600479

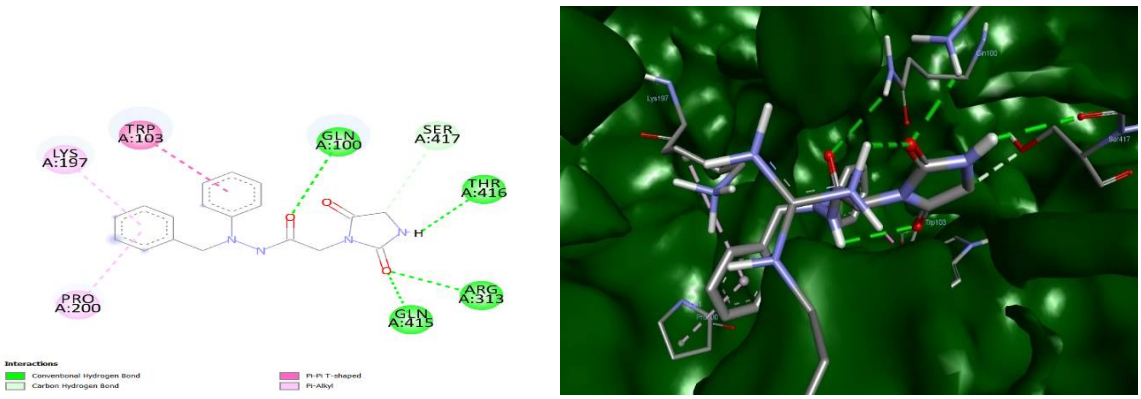

Fig 6: 2D(left) and 3D(right) view of molecular interaction of amino acid residues of xanthine oxidase with ZINC72510810

A molecule's fate inside the body is largely determined by the ADMET characteristics. When a molecule is administered orally, a good absorption is sought. The distribution and absorption of a molecule throughout the system will be governed by the balance of lipophilic and hydrophilic groups in the structure. From a therapeutic standpoint, effectiveness and toxicity are important. The liver contains a variety of cytochrome enzymes that are responsible for the molecule's metabolism. As a result, after metabolization, the activity of the metabolite should be known and removed from the body when the intended therapeutic impact has been achieved using the PKCSM software to determine whether a molecule cluster has likeliness and to forecast the ADMET attribute. Lipinski rule of 5 for ZINC compounds as shown in the table 2

Table 2: Lipinski rule of 5 for ZINC compounds

Zinc Compounds	**PHYSICAL PROPERTY**				
	MOL_WEIGHT	LOGP	#ROTATABLE_BONDS	#ACCEPTORS	#DONORS
ZINC27683167	359.426	2.9439	8	5	2
ZINC38600479	359.426	2.9439	8	5	2
ZINC31314416	356.422	3.3364	6	4	2
ZINC91046011	345.467	0.82772	9	4	2
ZINC72510810	338.367	1.2762	6	4	2
ZINC07988619	333.432	2.2478	6	3	2
ZINC08323999	333.413	1.4986	3	4	2
ZINC89510657	326.352	2.3019	5	4	2
ZINC67246986	316.361	1.1222	7	5	2

CONCLUSION

Some possible structures were examined as XO inhibitors using a pharmacophore modelling and molecular docking combination. discovery of a new class of XO inhibitors that may be more effective at treating gout. Additionally, ZINC27683167 can be tested in vitro and in vivo to find a powerful lead for the treatment of gout.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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