Molecular Docking for Predicting Alternative Ligands to Inhibit Xanthine Oxidase for Treatment of Uric acid Crystal Formation (gout)

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ABSTRACT— ***According to the world health organization (WHO), gout and hyperuricemia have been affecting over 41 million people worldwide, due to uric acid crystal accumulation in joints which causes severe pain and inflammation. Xanthine oxidase (XO) is the key enzyme in uric acid synthesis and a major therapeutic target. Although febuxostat effectively inhibits XO, its side effects and inconsistent efficacy necessitate safer alternatives. This study employed molecular docking using AutoDock 4.2.6, Chimera 1.17.2, and SwissADME to evaluate binding affinity and pharmacokinetic properties of potential ligands such as ulodesine, arhalofenate, and verinurad. Results revealed that ligands with lower binding energies and favorable pharmacokinetic profiles may serve as promising XO inhibitors. The findings highlight ulodesine and related compounds as potential safer and more effective alternatives to febuxostat for gout treatment.***

Keywords— Molecular docking, uric acid crystal formation, gout, febuxostat, ulodesine, allopurinol, topiroxostat, binding affinity, pharmacokinetic properties.

1. INTRODUCTION

Gout is a metabolic condition that has affected humans for ages, first noted in ancient Egypt and documented by the physician Hippocrates (around 460-370 BCE), who referred to it as "the unwalkable disease" due to the extreme pain in the joints [1]. Gout results from the buildup of uric acid crystals in the joints, causing severe pain, inflammation, and limited mobility [2].

In India, a vast number of individuals suffer from this distressing condition, with an uptick in cases attributed to lifestyle and dietary choices such as excessive purine consumption, obesity, and diabetes. Historically referenced in Indian literature like the Charaka Samhita and Sushruta Samhita, gout continues to pose a substantial health issue.

This condition arises when purines, which are present in foods such as red meat, seafood, and alcoholic beverages, are metabolised into uric acid. For those with gout, the kidneys struggle to effectively eliminate uric acid, resulting in its buildup in the synovial joints. The accumulation of these uric acid crystals triggers severe pain, especially in the big toe and other synovial joints, moving quite uncomfortable.

A primary treatment option for gout involves the use of xanthine oxidase (XO) inhibitors (Figure 1), which inhibit the enzyme that converts purines into uric acid. Febuxostat, a widely recognised XO inhibitor, is often prescribed to lower uric acid levels in individuals suffering from gout and hyperuricemia [3]. Nevertheless, febuxostat comes with certain drawbacks, including potential side effects and varying efficacy among different patients, which has created a demand for alternative inhibitors that might offer greater safety and effectiveness.

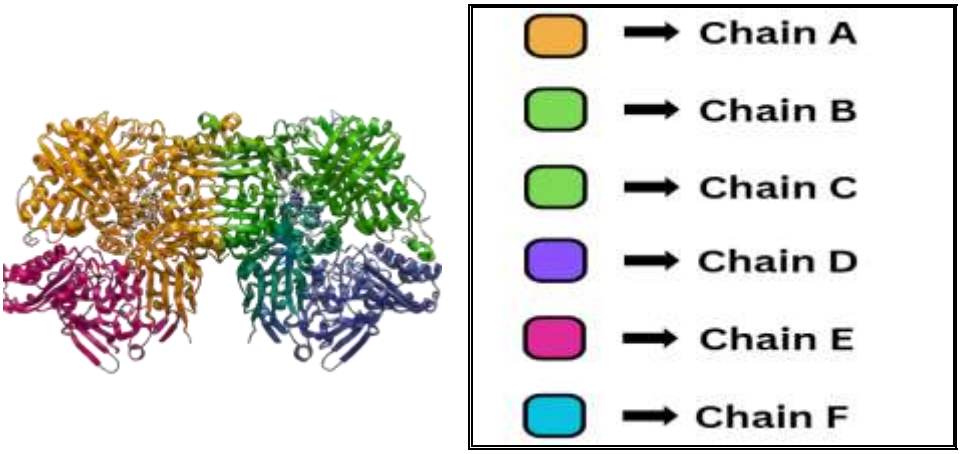

Figure 1: Structure of Xanthine Oxidase

This study aims to predict alternative ligands that may inhibit XO more effectively than febuxostat using molecular docking techniques and pharmacokinetic analysis. Ligands like ulodosine [4], topiroxostat [5], allopurinol [6], topiroxostat [7] , lesinurad [8] , Arhalofenate [9] , Benzbromarone [10], dotinurad [11] , Niraxostat [12], are considered in this experiment. The used ligands are shown below in Figure 2. In this, the Xanthine Oxidase structure illustrated in the figure consists of six unique chains identified as A through F, each shown in a different colour.

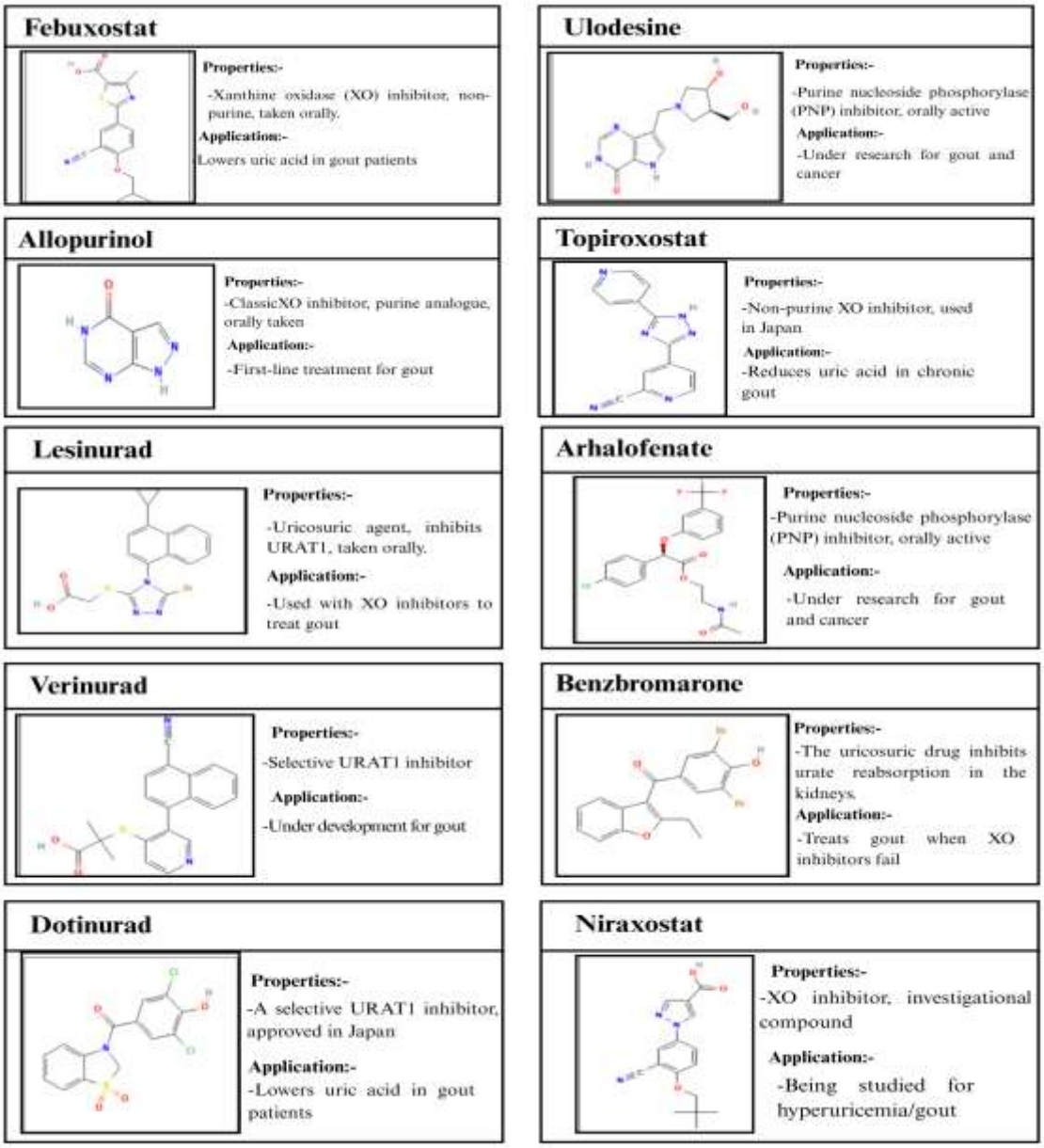

Figure 2: Structure, properties and applications of ligands used in this experiment.

Chain A (orange) serves as the main catalytic subunit and houses the molybdenum cofactor (MoCo) domain, where the key oxidation of xanthine to uric acid takes place. This chain is essential for the enzyme activity, facilitating substrate binding and redox processes. Chains B (light green) and C (green) are linked to iron-sulfur (Fe-S) clusters, which function as intermediate electron carriers that help transfer electrons from the catalytic site in Chain A to the ultimate electron acceptors. Their function is crucial for sustaining the enzyme's internal redox cycle.

Chain D (violet) participates in FAD (flavin adenine dinucleotide) binding and electron transport, playing a role in delivering the electrons obtained from the Fe-S clusters to molecular oxygen or other electron acceptors, which results in the production of reactive oxygen species. Chain E (magenta) contributes to the structural stability of the enzyme and may assist in stabilizing the MoCo and FAD domains by offering additional binding sites or aiding in the proper folding of the protein.

Lastly, Chain F (cyan) seems to help in the dimerisation or assembly of the enzyme into multimeric forms, ensuring the correct spatial arrangement and functional coordination of the other chains. Collectively, these six chains create an integrated system that allows xanthine oxidase to proficiently catalyse the oxidation of purine derivatives.

The primary docking software employed was Autodock 4.2.6 [13], which facilitated the assessment of binding affinities and the simulation of protein-ligand interactions. In this investigation, various bioinformatics tools, including ChemSketch, PyMOL, and Chimera [14], were utilised for ligand design, molecular visualisation, and interaction analysis. ChemSketch [15] was used to create and visualise the chemical structures of ligands, including Febuxostat and other potential candidates. PyMOL [16], a three-dimensional molecular visualisation tool, assisted in analysing the spatial interactions between the protein and ligands, enabling the examination of binding pockets in XO. The pharmacokinetic properties were predicted using SwissADME [17], allowing us to predict some compounds with potential medicinal applications.

The visual insights obtained facilitated the creation of new, potentially more effective inhibitors. The docking data from Autodock 4.2.6 were further examined using Chimera to visualize the binding configurations and confirm the docking forecasts. By employing these tools, the study concentrated on docking different ligands to XO and assessing their binding strengths in comparison to Febuxostat.

The objective is to discover novel ligands with superior inhibitory capabilities, resulting in more effective treatments for individuals afflicted by gout. This research aims to aid in the identification of enhanced therapeutic solutions, ultimately improving patient care by offering more powerful and safer xanthine oxidase inhibitors and also to predict more medically safer ligands.

2. METHODOLOGY

In this study, the approach involves utilizing ChemSketch for molecular design, AutoDock 4.2.6 for protein-ligand docking, Open Babel for file format conversions, UCSF Chimera and PyMOL for the 3D visualization of the protein-ligand complex, and SwissADME for evaluating the absorption, distribution, and pharmacokinetics of ligands. These tools facilitated the preparation and visualization of the protein and ligand structures, providing detailed simulations of their interactions. Free versions of all tools have been used.

2.1. Selection and retravel of data

The protein data for xanthine oxidase was obtained from the RCSB Protein Data Bank (PDB), while the ligand was created using ChemSketch, which involved several preparation steps such as eliminating water molecules, adding hydrogens, and applying Kollman charges to the protein. Ligands were crafted and processed with ChemSketch and OpenBabel (to convert the file format) to ensure they met the simulation requirements of AutoDock [18].

The docking procedure was then established with a grid box surrounding the active site of xanthine oxidase, concentrating on areas expected to show significant protein-ligand interactions. Subsequently, a SwissADME analysis was conducted to evaluate the medical safety of the ligand [17].

2.2. Preparation of protein and ligand

The docking process commenced with the acquisition and organization of the necessary software. ChemSketch was utilized to create ligand structures, which were saved in SDF format and then transformed into PDBQT format using OpenBabel. This conversion was vital for ensuring compatibility with AutoDock.

The xanthine oxidase protein was obtained in PDB format from the RCSB Protein Data Bank, after which the file was uploaded into the software. It was then processed by removing water molecules, adding hydrogen atoms, and assigning Kollman charges.

The PDBQT file for the ligand was then directly imported into the software. This preparation was crucial for accurately simulating the interactions between the protein and ligand in a biological setting.

2.3. Preparation of the grid box

A grid box was set up around the active site of xanthine oxidase and the ligand to define the docking region [19]. This step was critical for ensuring the accuracy of the docking simulations. The grid box was configured to encompass the entire ligand and the critical amino acids in the protein's active site.

Blind docking, which covers the entire protein structure, was considered but deemed less precise compared to targeted docking within the active site [20]. The grid parameters and coordinates were saved, and the grid box was validated to ensure proper alignment with the target region, by which this protein-ligand complex is formed. Then Autogrid was simulated, which had all the calculations inside the grid.

2.4. Autodock and AutodockVina simulation

In this, various parameters were set up. Initially, both the protein and ligand were selected, and any required adjustments were made to the rigid and genetic parameters, which were then confirmed. The crucial step in the docking process involved establishing the docking parameters, resulting in an output file that contains all calculations related to

the protein-ligand complex docking. Subsequently, the Autodock program was executed, initiating the process to obtain binding energies [21].

A configuration file was then generated, encompassing all coordinates and data associated with the complex, followed by the execution of AutodockVina to obtain the final docking outcomes, specifically the binding affinities [22]. This entire process was repeated for every ligand to predict the most effective inhibitor of xanthine oxidase.

2.5. Pharmacokinetics analysis

In this analysis, we conducted a pharmacokinetics evaluation of various ligands using the SwissADME software to determine which ligand is the safest for medical use. We gathered the SMILES formats of each ligand individually and subsequently processed them through the software, resulting in a pharmacokinetics report that included attributes such as absorbance, molecular weight, and the number of hydrogen bond acceptors. Ultimately, the report provided information on physicochemical characteristics, lipophilicity, water solubility, pharmacokinetics, and drug-likeness.

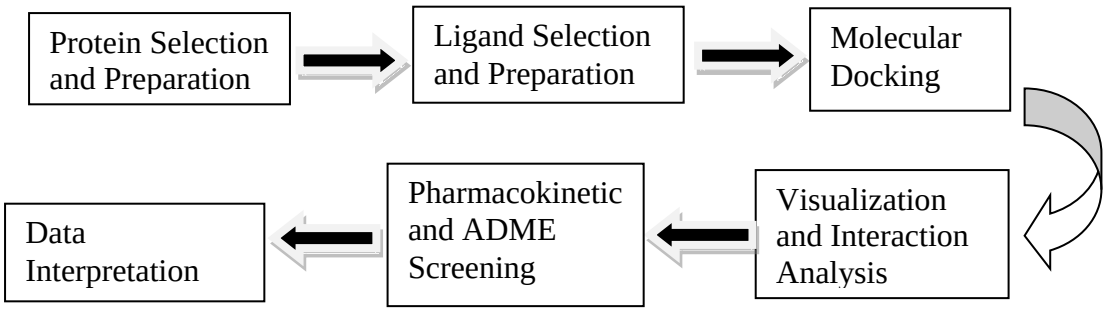

Figure 3: Flowchart of Methodology.

3. RESULTS AND DISCUSSION

3.1. Binding Affinities

Visualisation tools such as PyMOL and Chimera were utilised to analyse the docking outcomes. These tools offered comprehensive insights into the complexes formed between the protein and ligands, emphasising crucial interactions like hydrogen bonding, electrostatic interactions, and hydrophobic effects. The advanced visualisation features of Chimera facilitated the identification of essential residues, including ASN 988, ARG 996, and GLU 990, which were significant in stabilising the ligands in the binding site.

Based on these findings, it became evident that more effective alternatives to febuxostat were necessary; thus, molecular docking was performed, revealing several ligands with higher binding affinities compared to Febuxostat. The enhanced binding affinity of Lesinurad can be attributed to its capability of forming hydrogen bonds and electrostatic interactions with critical residues in the active site of xanthine oxidase. But Lesinurad is not a safe ligand to be used to treat gout or hyperuricemia; it may be dangerous.

To support this, we conducted SwissADME for all ligands to know the pharmacokinetics of all ligands and to predict a safer ligand that can treat gout with different medical therapies. By performing this, it was predicted that Ulodesine, Allopurinol and Topiroxostat are safer than Febuxostat. This study builds on the gout treatment as presented in the introduction, by which we can predict effective ligands that can be used in different therapies to reduce uric acid levels. This research contributes to the ongoing effort to improve patient outcomes and quality of life for those affected by gout and related conditions. Table 1 presents the binding affinity values for various inhibitors employed in managing gout and hyperuricemia, allowing for a comparative assessment of their effectiveness based on their binding strengths. Lesinurad, Arhalofenate, and Verinurad exhibit the strongest binding affinities at -9.5 kcal/mol, -9.4 kcal/mol, and -9.3 kcal/mol, respectively, suggesting that these compounds have the highest potential effectiveness among those listed.

Table 1: Binding affinity of different ligands

Ligand Name	Binding Affinity(kcal/mol)
Lesinurad	-9.5
Arhalofenate	-9.4
Verinurad	-9.3
Benzbromarone	-8.9
Topiroxostat	-8.5
Dotinurad	-8.4
Febuxostat	-8.3
Niraxostat	-8.2
Ulodesine	-7.4
Allopurinol	-5.9

Benzbromarone, with a binding affinity of -8.9 kcal/mol, also demonstrates noteworthy efficacy and is in close range to Topiroxostat (-8.5 kcal/mol) and Dotinurad (-8.4 kcal/mol). Febuxostat, a commonly prescribed treatment, displays a binding affinity of -8.3 kcal/mol, which, while slightly lower than its counterparts, still indicates significant binding strength.

Niraxostat, with a binding affinity of -8.2 kcal/mol, stands as another competitive alternative, whereas Ulodesine demonstrates a moderate binding affinity at -7.4 kcal/mol. Allopurinol, the conventional treatment for gout, shows the lowest binding affinity at -5.9 kcal/mol, implying it may be less effective compared to the more modern alternatives.

The results outline the binding affinities (in kcal/mol) for various binding modes, focusing on the top mode. The highest-ranked binding mode displays a binding affinity of -9.5 kcal/mol, suggesting the most robust interaction between the ligand and the protein. The other modes demonstrate increasingly weaker binding affinities, varying from -9.4 to -5.9 kcal/mol.

Overall, the docking simulation identified multiple potential binding modes, with the top-ranked mode demonstrating the highest affinity and no deviation from the ideal binding conformation. This information can guide further experimental validation and optimisation in the drug development process. But when it is compared with other ligands, it is slightly lower. Lesinurad, Arhalofenate, and Verinurad show the strongest binding affinities at -9.5 kcal/mol, -9.4 kcal/mol, and -9.3 kcal/mol, respectively, indicating that these compounds have the highest potential potency among the listed molecules. Benzbromarone, with a binding affinity of -8.9 kcal/mol, also shows significant effectiveness and is very close to Topiroxostat (-8.5 kcal/mol) and Dotinurad (-8.4 kcal/mol) and these two are also very good inhibitors of xanthine oxidase.

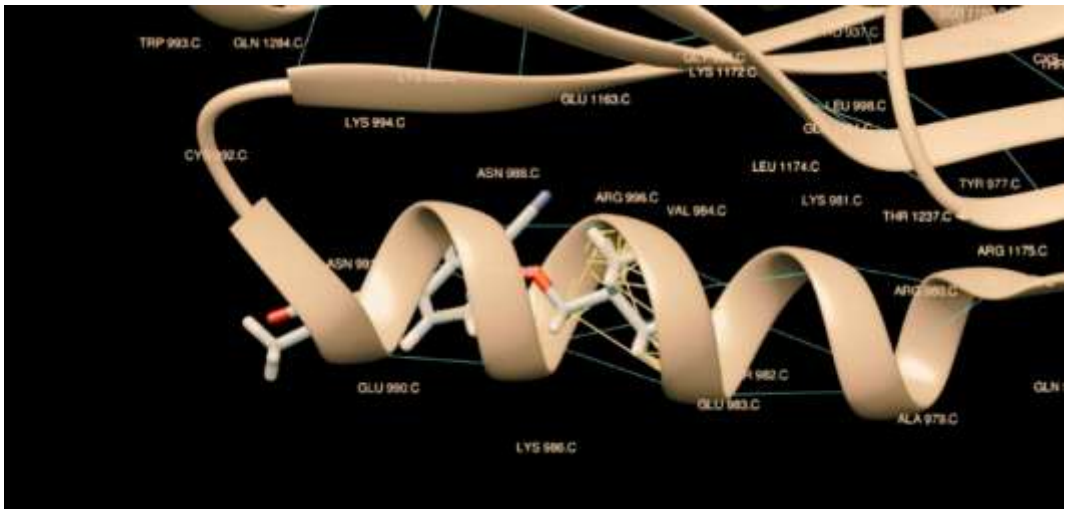

Figure 4: Protein-ligand interaction binding sites image

3.2. Visualisation of Protein-Ligand Interaction:

Figure 3 depicts a detailed visualisation of a protein-ligand interaction focusing on the ligand binding site, key residues involved in the interaction, and the nature of these interactions. The protein is depicted in a spiral for visualisation, which indicates alpha helices, which are common structural motifs in proteins.

It has some important amino acid residues, which are labelled with their respective positions in the protein sequence. These include residues such as LYS(Lysine), GLU (Glutamic acid), ASN(Asparagine), PHE(Phenylalanine), and ARG(Arginine), among others.

The binding pocket consists of several residues, including ASN 988. C, ARG 996. C, GLU 990. C, in which C represents the chain in which the amino acid is present. These results are critical for ligand binding and are very important in building new molecules. The yellow dashed lines indicate hydrogen bonds between the ligand and the protein. Important hydrogen bonding interactions are observed between: The ligand and ASN 988. C, The ligand and ARG 996. C

The presence of charged residues like GLU 990. C (negatively charged) and ARG 996. C (positively charged) shows the electrostatic interactions with the ligand and the potential hydrophobic interactions stabilising the ligand within the binding pocket. These interactions will affect the binding affinity values, so they are essential.

3.3. Pharmacokinetics

In Table 2, the pharmacokinetic analysis of ten xanthine oxidase inhibitor ligands using SwissADME is displayed. Several key properties were assessed to determine their potential medical safety. These included gastrointestinal (GI) absorption [23], blood-brain barrier (BBB) permeability [24], interaction with P-glycoprotein (P-gp), cytochrome P450 (CYP) enzyme inhibition [25], and skin permeability expressed as Log Kp [26].

Table 2: Pharmacokinetics results for all ligands

Ligand Name	***Gi absorption***	***BBB permeant***	***P-gp substrate***	***CYP Inhibitor***	***Log Kp (cm/s)***
Ulodesine	High	No	No	No	-8.66
Allopurinol	High	No	No	No	-7.61
Febuxostat	High	No	No	No	-4.95
Topiroxostat	High	No	No	CYP1A2, CYP2D6 & CYP3A4	-6.95
Lesinurad	High	No	No	CYP1A2, CYP2C19 &CYP2C9	-5.42
Arhalofenate	High	Yes	No	CYP1A2, CYP2C19 ,CYP2C9, CYP2D6 & CYP3A4	-5.75
Verinurad	High	No	No	CYP1A2, CYP2C19 ,CYP2C9& CYP3A4	-5.49
Benzbromarone	High	Yes	No	CYP1A2, CYP2C19 &CYP2C9	-4.87
Dotinurad	High	No	No	CYP2C19 &CYP2C9	-6.39
Niraxostat	High	No	No	CYP1A2, CYP2C19 &CYP2C9	-6.12

All ten ligands showed high GI absorption, which is favourable as it suggests good oral bioavailability. Most compounds, including ulodesine, allopurinol, dotinurad, and others, were not BBB-permeant. This is desirable for non-CNS-targeting drugs like xanthine oxidase inhibitors, as CNS penetration can lead to unnecessary side effects. Additionally, none of the ligands were identified as P-gp substrates, which indicates that they are less likely to be pumped out of cells and therefore can maintain intracellular concentration efficiently.

The most critical factor for drug safety assessment was CYP enzyme inhibition. Cytochrome P450 enzymes are responsible for the metabolism of many drugs; their inhibition can lead to drug–drug interactions or altered drug metabolism. Ulodesine and Allopurinol showed no inhibition of any CYP enzymes, making them pharmacokinetically safe and unlikely to interfere with other medications. Dotinurad showed limited inhibition of only CYP2C19 and CYP2C9, making it safer compared to other ligands like Arhalofenate and Verinurad, which inhibited four or more CYP isoforms.

In skin permeability, all ligands showed low Log Kp values (ranging from -4.87 to -8.66 cm/s), indicating poor transdermal absorption. This is expected and acceptable since these drugs are intended for oral administration, not for topical use.

Considering all these factors, the three most pharmacokinetically safe ligands—excluding Febuxostat—are Ulodesine, Allopurinol, and Dotinurad. Ulodesine stood out as the safest with high GI absorption, no BBB permeability, no P-gp substrate characteristics, no CYP inhibition, and optimal skin permeability.

Allopurinol also demonstrated an excellent safety profile with no interaction issues and a long history of clinical use. Dotinurad, though it inhibited two CYP enzymes, was safer than many other ligands that inhibited more and had potential for more complex interactions.

3.4. Drug likeness

According to Table 3, the drug-likeness criteria and bioavailability ratings, various ligands demonstrate significant potential for gout treatment. Ulodesine has a good drug likeness as it complies with most drug-likeness standards and has a bioavailability score of 0.55. Although it does not fulfil the Ghose criteria [27], it remains a viable candidate for lowering uric acid levels by inhibiting purine nucleoside phosphorylase.

Allopurinol, a well-established xanthine oxidase inhibitor, also presents a reliable profile, though it does not meet the Ghose and Muegge filters. Nevertheless, its clinical effectiveness underscores its ongoing importance in the management of gout.

Febuxostat, a non-purine xanthine oxidase inhibitor, satisfies all drug-likeness criteria with a notable bioavailability score of 0.56, establishing it as one of the most effective and dependable oral medications for managing chronic gout.

Similarly, Topiroxostat also adheres to all guidelines, earning a slightly lower bioavailability score of 0.55, which tells it has good oral activity. Lesinurad, which functions by inhibiting uric acid reabsorption through URAT1, meets all criteria and demonstrates a robust bioavailability score of 0.56, supporting its role as a uricosuric agent, particularly in combination therapies.

Arhalofenate, Verinurad, and Niraxostat each meet all drug-likeness standards and boast the highest bioavailability score of 0.56. These newer medications reduce uric acid levels and display anti-inflammatory effects, making them promising candidates for future development in gout treatment.

Although Benzbromarone and Dotinurad do not completely adhere to Muegge compliance, their bioavailability score of 0.55 and uricosuric properties still render them valuable options, especially for patients who do not respond to traditional treatments.

So overall, Febuxostat, Verinurad, Lesinurad, and Ulodesine show the greatest promise in terms of drug-likeness and pharmacokinetic properties for treating gout effectively. Their alignment with essential drug-likeness criteria and good bioavailability underscores their potential for safe and effective oral use.

Table 3: Drug likeness results for all ligands

Ligand Name	**Lipinski**	**Ghose**	**Veber**	**Egan**	**Muegge**	**Bioavailability Score**
Ulodesine	Yes	No	Yes	Yes	Yes	0.55
Allopurinol	Yes	No	Yes	Yes	No	0.55
Febuxostat	Yes	Yes	Yes	Yes	Yes	0.56
Topiroxostat	Yes	Yes	Yes	Yes	Yes	0.55
Lesinurad	Yes	Yes	Yes	Yes	Yes	0.56
Arhalofenate	Yes	Yes	Yes	Yes	Yes	0.55
Verinurad	Yes	Yes	Yes	Yes	Yes	0.56
Benzbromarone	Yes	Yes	Yes	Yes	No	0.55
Dotinurad	Yes	Yes	Yes	Yes	Yes	0.55
Niraxostat	Yes	Yes	Yes	Yes	Yes	0.56

3.5 Physicochemical Properties

In Table 4, the selected ligands exhibit favourable physicochemical characteristics that suggest good drug-likeness and pharmacokinetic suitability for oral gout treatment. Ulodesine presents a moderate molecular weight of 264.28 g/mol, with a reasonable number of hydrogen bond donors (4) and acceptors (5), along with a commendable molar refractivity of 72.74, indicating a strong potential for interaction and solubility.

Its well-balanced number of rotatable bonds and aromatic content further enhances its potential for good bioavailability. Allopurinol, possessing the lowest molecular weight at 136.11 g/mol and lacking any rotatable bonds, is characterised by limited flexibility but exhibits optimal size and simplicity, consistent with its established role as a classic xanthine oxidase inhibitor. Although its molar refractivity is comparatively low at 34.51, it remains adequate for its intended therapeutic action.

Febuxostat, a newer xanthine oxidase inhibitor, possesses a higher molecular weight (318.37 g/mol), a balanced profile of hydrogen bonding (5 acceptors, 1 donor), and an increased molar refractivity (83.40), which indicates strong polarizability and the ability to bind to receptors, thereby improving its efficacy.

In contrast, Topiroxostat has just 2 hydrogen bond acceptors and 1 donor, along with a low number of rotatable bonds and a minimal aromatic fraction, which leads to a lower molar refractivity (67.56) while maintaining acceptable oral bioavailability. Lesinurad and Arhalofenate have larger molecular weights (404.28 and 415.79 g/mol, respectively) and a greater potential for hydrogen bonding, with Arhalofenate exhibiting 10 acceptors and 7 donors, which implies strong interaction capabilities but may also result in reduced permeability. Nevertheless, their molar refractivity values of 97.33 and 95.75, respectively, suggest good lipophilicity.

Table 4: Physicochemical results for all ligands

Ligand Name	Formula	Molecular Weight	No. of Heavy atoms	No. of Aromatic Heavy Atoms	Fraction Csp3	No. of Rotatable Bonds	No. of H-bond acceptor	No. of H-bond donors	Molar Refractivity
Ulodesine	$C_{12}H_{16}N4O_3$	264.28	19	9	0.50	3	5	4	72.74
Allopurinol	$C_5H_4N_4O$	136.11	10	9	0.00	0	3	2	34.51
Febuxostat	$C_{15}H_{16}N_3O_3S$	318.37	22	11	0.33	5	5	1	83.40
Topiroxostat	$C_{13}H_8N_6$	248.24	19	17	0.00	2	5	1	67.56
Lesinurad	$C_{17}H_{14}BrN_3O_2S$	404.28	24	15	0.24	5	4	1	97.33
Arhalofenate	$C_{19}H_{17}CIF_3NO_4$	415.79	28	12	0.26	10	7	1	95.75
Verinurad	$C_{20}H_{16}N_2O_2S$	348.42	25	16	0.15	4	4	1	99.85
Benzbromarone	$C_{17}H_{12}Br_2O_3$	424.08	22	15	0.12	3	3	1	93.28
Dotinurad	$C_{14}H_9Cl_2NO_4S$	358.20	22	12	0.07	2	4	1	86.71
Niraxostat	$C_{16}H_{17}N_3O_3$	299.32	22	11	0.31	5	5	1	80.70

Verinurad features a well-balanced structure (MW 348.42 g/mol), notable flexibility (16 rotatable bonds), and impressive molar refractivity (99.85), indicating strong binding affinity and favourable absorption characteristics. Benzbromarone and Dotinurad also demonstrate positive drug-like attributes, with reasonable molecular weights, suitable hydrogen bonding capabilities, and respectable molar refractivity values (93.28 and 86.71, respectively).

Lastly, Niraxostat displays significant binding potential due to its high level of aromaticity (11 atoms), a well-balanced hydrogen bonding profile (5 acceptors, 1 donor), and substantial molar refractivity (80.70).

The ligands Febuxostat, Verinurad, Lesinurad, and Ulodesine show the most advantageous physicochemical characteristics for oral delivery and effective target interaction in the treatment of gout. Their well-balanced molecular weight, hydrogen bonding features, and molar refractivity suggest robust pharmacokinetic and pharmacodynamic performance.

3.6. Implications of CYP Inhibition in Clinical Settings

Cytochrome P450 (CYP) enzymes play a crucial role in drug metabolism, and inhibition of these enzymes can have significant clinical implications. Many XO inhibitors, including allopurinol and febuxostat, are known to interact with CYP isoforms, which can alter the metabolism of co-administered drugs. Strong CYP inhibition may lead to elevated plasma concentrations of other drugs, increasing the risk of adverse drug reactions or toxicity.

Therefore, newly identified XO inhibitors must be carefully evaluated for their potential to inhibit or induce key CYP isoforms (e.g., CYP3A4, CYP2C9, CYP2D6). This is particularly important in gout patients, who are often on polypharmacy regimens for comorbid conditions such as hypertension, diabetes, or renal impairment. A compound with strong XO inhibitory activity but minimal CYP interference would represent an ideal candidate, reducing the risk of drug–drug interactions and improving clinical safety.

3.7. Medicinal Chemistry

In Table 5, all the ligands assessed demonstrated no alerts for either PAINS (Pan Assay Interference Compounds) [28] or Brenk filters, indicating that they are likely free from significant structural liabilities or tendencies to produce false positives in bioassays. This increases their credibility as authentic therapeutic candidates.

Table 5: PAINS and Brenk assessment for all ligands

Ligand name	PAINS	Brenk	Leadlikeness	Synthetic accessibility
Ulodesine	0	0	Yes	3.08
Allopurinol	0	0	No	1.76
Febuxostat	0	0	No	3.10
Topiroxostat	0	0	No	2.45
Lesinurad	0	0	No	2.86
Arhalofenate	0	0	No	3.37
Verinurad	0	0	No	3.05
Benzbromarone	0	0	No	3.14
Dotinurad	0	0	No	2.89
Niraxostat	0	0	Yes	2.37

Regarding lead-likeness, only Ulodesine and Niraxostat were identified as lead-like compounds, positioning them as more appropriate for initial drug development due to their ideal balance of molecular weight, lipophilicity, and hydrogen bonding attributes. Although other compounds like Febuxostat, Verinurad, and Lesinurad were categorised as "No" in terms of lead-likeness, they still hold potential for development but may necessitate further optimisation for later stages.

In terms of synthetic accessibility, which assesses the simplicity of chemical synthesis (with lower scores indicating easier synthesis), Allopurinol achieved the most favourable score of 1.76, indicating that its production is relatively straightforward and cost-efficient, aligning with its established clinical usage. Niraxostat (2.37) and Topiroxostat (2.45) also demonstrate good feasibility for synthesis.

Conversely, Febuxostat (3.10), Ulodesine (3.08), Verinurad (3.05), and Benzbromarone (3.14) are moderately accessible, implying that the synthetic processes involved are manageable but somewhat more intricate. Arhalofenate, which has the highest synthetic accessibility score of 3.37, may present greater challenges in its synthesis, possibly affecting its scalability for therapeutic applications.

While molecular docking provides valuable insights into potential ligand receptor interactions, several limitations must be acknowledged. The ligand protonation states and tautomeric forms at physiological pH may differ from those modeled, influencing interaction patterns [29]. Thus, while docking serves as a powerful in silico screening tool, it must be complemented with molecular dynamics simulations, in vitro enzymatic assays to validate inhibitory potential and drug-likeness of the candidate ligands.

So at last the absence of PAINS/Brenk alerts for all compounds indicates their structural integrity, while Ulodesine and Niraxostat are identified as strong lead-like candidates with feasible synthetic profiles. Although Allopurinol is not considered lead-like, it is notable for its simplicity in synthesis. In general, this evaluation endorses the continued development of compounds such as Ulodesine, Niraxostat, and Febuxostat as promising drug candidates for gout treatment.

4. CONCLUSION

This study effectively employed molecular docking and pharmacokinetic assessment to predict alternative ligands capable of significantly inhibiting xanthine oxidase, a key enzyme involved in gout development. Using tools such as AutoDock 4.2.6, PyMOL, Chimera, and SwissADME, the study analysed binding affinities, drug-likeness, and medicinal chemistry profiles of ten potential ligands.

Docking results revealed that compounds like lesinurad, arhalofenate, and verinurad exhibited stronger binding affinities than the benchmark drug Febuxostat. However, pharmacokinetic evaluation indicated that some of these high-affinity ligands, particularly lesinurad, might not be safe for clinical use due to cytochrome P450 (CYP) enzyme inhibition, which can lead to adverse drug–drug interactions and metabolic interference in patients taking multiple medications.

Among the tested ligands, ulodesine, allopurinol, and dotinurad were identified as the most pharmacokinetically favourable candidates, demonstrating high gastrointestinal absorption, minimal CYP inhibition, and good oral bioavailability. Ulodesine, in particular, exhibited an excellent pharmacological profile, making it a promising and safer alternative to Febuxostat.

Nevertheless, this study is limited by the inherent constraints of molecular docking. Docking simulations predict theoretical binding affinities based on static protein–ligand interactions and may not fully replicate dynamic physiological conditions such as solvation effects, protein flexibility, or in vivo metabolism. Therefore, while docking provides valuable insights into potential inhibitors, experimental validation through in vitro enzyme assays and clinical studies is necessary to confirm efficacy and safety.

In summary, this study not only identifies ulodesine as a strong candidate for future therapeutic development but also highlights the critical importance of evaluating CYP inhibition in clinical settings to avoid metabolic complications. These findings contribute to the rational design of safer and more effective xanthine oxidase inhibitors for gout management and pave the way for further experimental research and optimization

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