

# Flavones scaffold of *Chromolaena odorata* as a potential xanthine oxidase inhibitor: Induced Fit Docking and ADME studies

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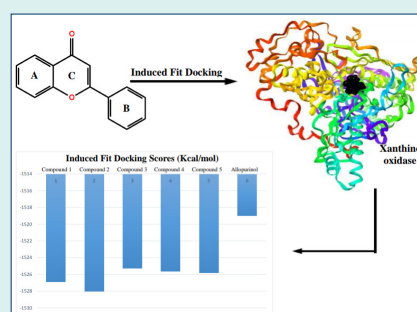
## Abstract

**Introduction:** Gout is a type of painful inflammation initiated by the interactions between monosodium urate crystals and connective tissue. Xanthine oxidase (XO) catalyzes the oxidation of hypoxanthine to xanthine, then to uric acid. The primary treatments for gout include XO inhibitors. At present, allopurinol is the most used XO inhibitor for the treatment of gout. However, it can cause adverse effects commonly known as allopurinol hypersensitivity syndrome, thereby limiting its usage. Consequently, it is necessary to develop potent and less toxic inhibitors of XO. *Chromolaena odorata* is one of such plants under investigation for its diverse health benefits.

**Methods:** Phytochemicals of *C. odorata* were screened against XO receptor, using molecular docking. The top five hit compounds of glide docking yield flavones scaffold which were subjected to induced fit docking (IFD) and absorption, distribution, metabolism, and excretion (ADME) studies.

**Results:** The result showed that flavones scaffold of *C. odorata* can bind with higher affinity and lower free energy values when compared to that of the standard, allopurinol. The IFD scores of the flavones scaffold range from -1525.25 to -1527.99 kcal/mol.

**Conclusion:** Our results have shown that flavones scaffold might have the potential to act as an effective drug candidate when compared to allopurinol in treating and/or preventing gout and some inflammatory condition.



## Introduction

Gout is a prevalent metabolic disease with high public distribution and continues to be a major health challenge. It affects circa 13% and 5% of male and female populations, respectively.<sup>1</sup> Gout is a type of painful inflammation initiated by the interactions between monosodium urate crystals and connective tissue<sup>2</sup> during purine catabolism by the enzyme of xanthine oxidase (XO).<sup>3</sup> XO is a very flexible enzyme that has a wide distribution among various species from bacteria to humans and within several tissues of mammals. It is a member of a class of enzymes called molybdenum iron-sulfur flavin hydroxylases.<sup>4</sup> XO plays an essential role in the breakdown of purines, it catalyzes

the oxidation of hypoxanthine to xanthine, then to uric acid (UA), the last step in the catabolism of purine bases.<sup>5</sup> The build-up of UA in the body is accountable for the formation of several disorders, therefore, it plays a crucial role in the development of hyperuricemia and gout.<sup>6</sup> Inborn xanthine oxidase reductase (XOR) deficiency is a genetical dysfunction of XOR, and it is characterized by a very low or undetectable concentration of UA in the blood and urine, leading to a high level of urinary xanthine called xanthinuria.<sup>7</sup> Previous studies have reported that gout causes some complications, such as tophi, kidney stones and, joint deformities.<sup>8</sup>

Drugs approved for the treatment of gout, include



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allopurinol, cyclooxygenase 2 inhibitors, steroids, and non-steroidal anti-inflammatory drugs. These drugs are effective but they are characterized by several side effects such as allergic skin conditions, skin rashes, fever, renal dysfunction, aseptic meningitis, renal and hepatic dysfunction.<sup>9,10</sup> Allopurinol is the most used XO inhibitor for the treatment of gout,<sup>11</sup> it inhibits XO and thereby increases re-utilization of hypoxanthine and xanthine for the formation of nucleotide and nucleic acid in a reaction catalyzed by hypoxanthine-guanine phosphoribosyltransferase, the consequent increase in nucleotide concentration results in feedback inhibition of *de novo* purine synthesis. Allopurinol thereby lowers UA concentrations in both urine and serum.<sup>12</sup> Allopurinol causes adverse effects commonly known as allopurinol hypersensitivity syndrome. Consequently, it is necessary to develop potent and less toxic inhibitors of XO. Recently, treating disease using medicinal plants is capturing new interest,<sup>13</sup> and research on medicinal plants has increased worldwide<sup>14,15</sup> due to fewer side effects and lower costs.<sup>16</sup> Ojeh et al.<sup>17</sup> reported that the main aim of sourcing for plants drug is to discover the active constituent in plants that can produce definite pharmacological effects since the results of such research would often serve as a lead compound for the treatment of diseases.

*Chromolaena odorata* is one of the common plants under investigation for its various health benefits. It is a tropical and subtropical species of flowering shrub in the family of sunflower, a scrambling and perennial plant belonging to *Asteraceae*, and has been classified as the weed. Previous studies showed that this plant possesses anti-inflammatory, anti-astringent, anti-diuretic and anti-hepatotropic activities,<sup>18</sup> and contains triterpenes, alkaloids and flavonoids.<sup>19</sup> The biological activities of flavonoids in the treatment of diseases have attracted high interest in drug research, they have been reported to possess numerous useful properties.<sup>20</sup> They interfere in carbohydrates metabolism and also have anti-allergic, anti-inflammatory, antiviral, and anticancer properties.<sup>21</sup> Flavonoids have a C6-C3-C6 parental skeleton, based on the structural difference on ring C, they are sub-classified into anthocyanidins, flavones, flavonols, flavanones, flavonols, and isoflavones. Thus, the present study uses *in silico* analysis via molecular docking, Induced fit docking (IFD), and pharmacological properties to evaluate the anti-gout phytoconstituents of *C. odorata*. Molecular docking is an *in-silico* approach that predicts the preferred orientation of small molecules in the active site of a receptor or target protein.<sup>22</sup> It predicts the best binding mode and bio affinities of ligands with their receptor,<sup>23</sup> at present it has been widely applied to virtual screening for the optimization of lead compounds. The basic tools of the molecular docking method are search algorithm and scoring functions for generating and analyzing different conformations of the ligand.

## Materials and Methods

### Protein preparation

Three-dimensional (3D) crystal structure of XO was retrieved from protein data bank with id: 1FIQ and prepared using the protein preparation wizard of Schrödinger Maestro 11.5.<sup>24</sup> Protein was prepared by assigning bond orders, adding hydrogens, filling missing loops and side-chain using prime, deleting water beyond 5.00 Å, generating tautomeric states of heteroatom groups at a pH of  $7.0 \pm 2$  using Epik and heteroatom state was generated for ligand, then optimized at neutral pH. Using OPLS3 forcefield, restrained minimization was performed setting heavy atom root mean square deviation (RMSD) to 0.30Å.

### Ligand preparation

Phytochemicals of *C. odorata* were obtained from reported literatures and their 2D structures were retrieved from the NCBI PubChem database. The 3D structures were built using LigPrep of Schrödinger Maestro 11.5 with an OPLS3 force field. Ligands ionization states were generated at a pH range of  $7.0 \pm 2.0$  (general pH of biological systems) using Epik.<sup>25</sup> Stereoisomers computation was left at retaining specified chiralities (vary other chiral centers), up to 4 possible stereochemical structures were generated per ligand.

### Receptor grid generation

Receptor grid generation allows defining the position and size of the active site for ligand docking. The scoring grid was defined based on the co-crystallized ligand (Salicylic acid) using the receptor grid generation tool in Schrödinger Maestro 11.5. The van der Waals (vdW) radius scaling factor of nonpolar receptor atoms were scaled at 1.0, with a partial charge cut-off of 0.25.

### Molecular docking procedure

On a defined receptor grid, the glide tool on Schrödinger Maestro 11.5 was used to carry out molecular docking studies. The prepared ligands were docked using standard precision (SP), setting ligand sampling to flexible, and then docked with extra precision (XP) with the ligand sampling set to none (refine only). The vdW radius scaling factor was scaled at 0.80 with a partial charge cut-off of 0.15 for ligand atoms.

### Induced fit docking

IFD protocol was carried out on the top 5 hit compounds using Schrödinger Maestro 11.5. IFD<sup>26</sup> aims to introduce flexibility for both ligand and its target receptor during molecular docking. This is achieved by joining different iterations of docking of flexible ligands into a rigid receptor followed by the protein active site refinement to adopt the best conformation for a ligand. The scoring grid was defined based on the co-crystallized ligand (Salicylic acid) in 1FIQ and no constraints were defined. Sample

ring conformation at an energy window of 2.5 kcal/mol were selected for ligand conformational sampling. In glide docking, the vdW radii of protein and ligand were at 0.5 and to generate a maximum of 20 poses per ligand. Refine residues within 5.0Å of ligand poses were refined with prime. Final structures within 30 kcal/mol of the best structure and the top 20 structures overall were re-docked with XP precision.

### Molecular docking validation

The employed docking protocol was validated with the blasting of the FASTA sequences XO (PDB id:1FIQ), obtained from protein data bank into the ChEMBL database server ([www.ebi.ac.uk/chembl/](http://www.ebi.ac.uk/chembl/)). From the search results, the bioactivity generated by the database with the IC<sub>50</sub> value of 476, and an inhibition value of 121, was downloaded with a canonical smile of the compounds. The bioactivity was sorted out; missing or misplaced data were removed. Only 55 of drug-like compounds were recovered. The complied compounds were converted to 2D (in the sdf format) by data warrior software (version 2). The ligands were imported into Schrödinger Maestro 11.5 and the 3D structures were built by using LigPrep with an OPLS3 force field. The ligands were dock using glide into the target protein receptor using SP precision. A correlation coefficient graph was mapped between the docking scores of 55 compounds generated, and

their corresponding PCHEMBL\_VALUE was plotted as shown in Fig. 1. Spearman rank correlation coefficient graph was plotted to obtain the correlation (R<sup>2</sup>) between the PCHEMBL\_VALUE and the docking scores of the compounds.

### Pharmacology parameters

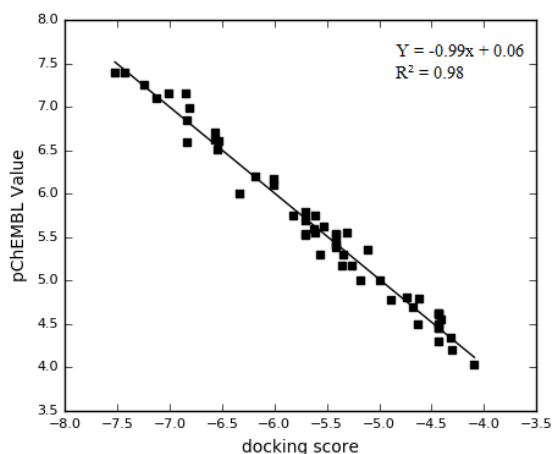
Schrödinger QikProp was employed to calculate the Absorption, Distribution, Metabolism and Excretion (ADME) properties of selected phytoconstituents along with the standard compound, for their pharmacological properties such as drug-likeness by checking for any violation of Lipinski's rule of five (ROF) and several pharmacological parameters including Human Oral Absorption, Predicted octanol/water partition coefficient and prediction of binding to human serum albumin.

### Results and Discussion

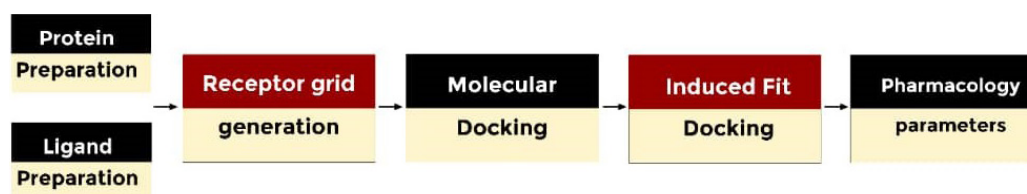
The present study features *in silico* experimental procedures described in Fig. 2 to evaluate the molecular interactions, binding mode, pharmacological profile, and inhibitory potential of novel XO inhibitor.

### Molecular docking

Protein docking is a significant tool in the study and provision of a proper understanding of protein-ligand interactions. Protein docking is regularly used in various stages to facilitate the design of potentially active leads.<sup>27</sup> The library of compounds generated was subjected to Glide and IFD to determine compounds with high binding mode and docking scores when compared with the small molecule inhibitor of XO from the protein data bank, Allopurinol. Glide docking scores make use of the basic assumption of a rigid receptor; its result may not be accurate if the ligands induce conformational changes in the target receptor. For this reason, IFD was used to generate flexible receptors (only for hit compounds).<sup>28</sup> The top five hit compounds of glide docking yield flavones scaffold (as shown in Table 1 and Fig. 3), which can also be called 'skeleton key' as it plays an important role in numerous compounds that act at different targets to produce pharmacological properties with various substitution pattern.<sup>29</sup> According to the study, flavones scaffold report an induced fit score of -1526.86 kcal/mol, -1527.99 kcal/mol, -1525.25 kcal/mol, -1525.65 kcal/mol and -1525.80 kcal/mol for compounds 1, 2, 3, 4 and 5, respectively. The Glide Gscore and IFD scores of flavones



**Fig. 1.** The correlation coefficient graph between the experimentally determined pIC<sub>50</sub> of 1FIQ and their docked score with R<sup>2</sup> of 0.98 indicating that docking experiment can replicate the experimentally determined values of the inhibitors.



**Fig. 2.** Flow chat for identifying potential inhibitors against xanthine oxidase.

**Table 1.** Flavones scaffold generated from scaffold analysis of *Chromolaena odorata*

Compound Name	
Compound 1	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
Compound 2	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one
Compound 3	2-(3,4-dihydroxyphenyl)-3,5,6,7-tetrahydroxychromen-4-one
Compound 4	3,5,7-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one
Compound 5	5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)chromen-4-one

scaffold are lower when compared to that of the standard, Allopurinol which recorded an IFD score of -1519.00 kcal/mol. Based on these results, it is obvious that the flavones scaffold has a relatively better inhibitory than allopurinol. These results are consistent with earlier reports gotten from both *in silico* and *in vitro* experiments.<sup>30,31</sup> The docking scores calculated by glide XP-scoring function and IFD are shown in Table 2.

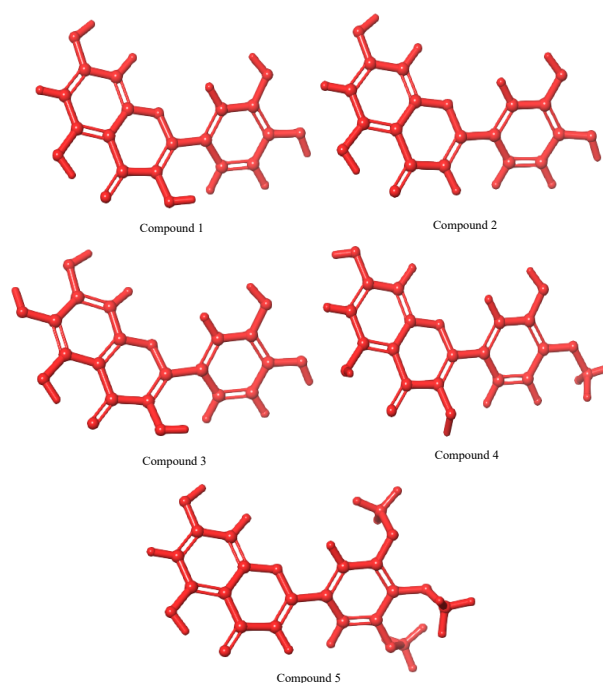
### Interaction profiling and scaffold analysis

The active site of XO is believed to contain several amino acid residues. GLU1216, ARG880 and GLU802 residues in the molybdenum center of the gorge play crucial roles in the Xanthine oxidation reaction. At the entrance of the cavity, LEU648, PHE649, PHE914, PHE1009, VAL1011, PHE1013 and LEU1014 regulate the entry of small molecules.<sup>32</sup> Interestingly, Figs. 4-9 shows that flavones scaffold interacted through the formation of pi-pi stacking, hydrogen bond and hydrophobic interaction with the molybdenum atomic domain and some other amino acid residues of XO, which are crucial for the enzymatic activity and hence blocked catalytic activities that is similar to the report of Yan et al.<sup>30</sup>

Flavones scaffold consist of phenyl rings A and B joined by a heterocyclic Ring C. The phenyl Ring A and heterocyclic Ring C of flavones scaffold, were buried deep in the hydrophobic side chains, therefore contributing to the stability of the compounds at the hydrophobic pocket by forming pi-pi staking with PHE 914 and PHE1009. The direct staking interactions between PHE 914 and PHE 1009 seem to be a general feature in the binding of

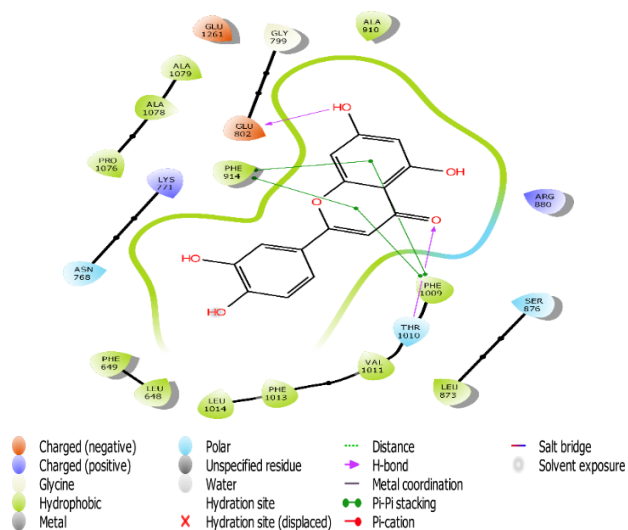
substrates in the active site of XO and constitute a good starting point for consideration of competitive inhibition of the enzyme.<sup>33</sup>

Hydrogen bond also has a key role in enzyme catalysis and structural stability of many biological molecules. Hydrogen bond interactions exist between flavones scaffold and XO as shown in Figs. 4-8. Compounds 1, 2, 3, and 5 form hydrogen bond interaction with the

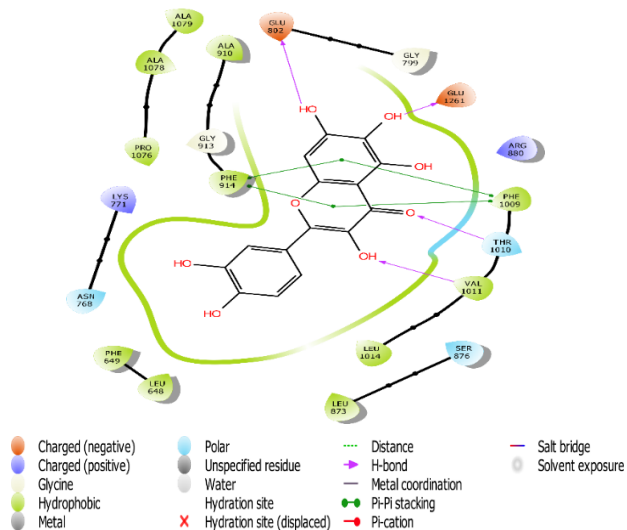
**Fig. 3.** 3D chemical structure of compounds 1-5**Table 2.** Docking score calculated by glide XP-scoring function and induced fit docking (IFD)

Phyto-constituents	Glide XP-Docking Score (kcal/mol)	Induced Fit Docking	
		Glide Gscore (kcal/mol)	IFD score (kcal/mol)
Compound 1	-11.339	-14.614	-1526.86
Compound 2	-10.872	-14.130	-1527.99
Compound 3	-11.804	-16.303	-1525.25
Compound 4	-11.185	-13.343	-1525.65
Compound 5	-10.081	-12.948	-1525.80
5281702	-9.999		
5280443	-9.717		
1794427	-9.298		
5281697	-8.834		
Allopurinol	-4.109	-9.003	-1519.00

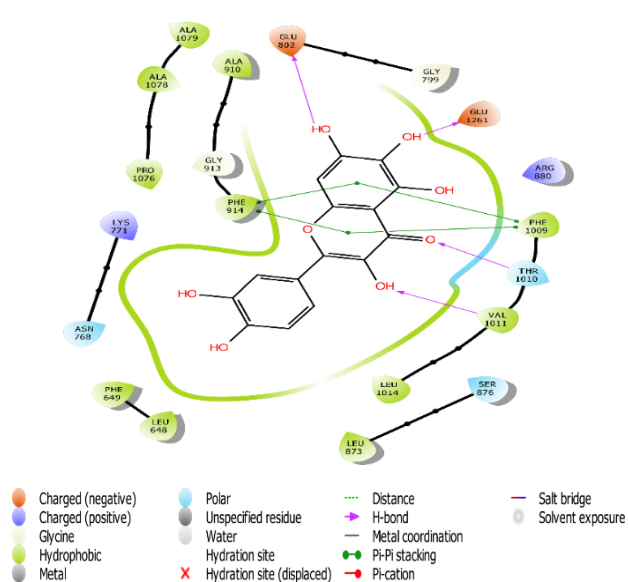
IFD was calculated for hit compounds and standard compound (allopurinol).



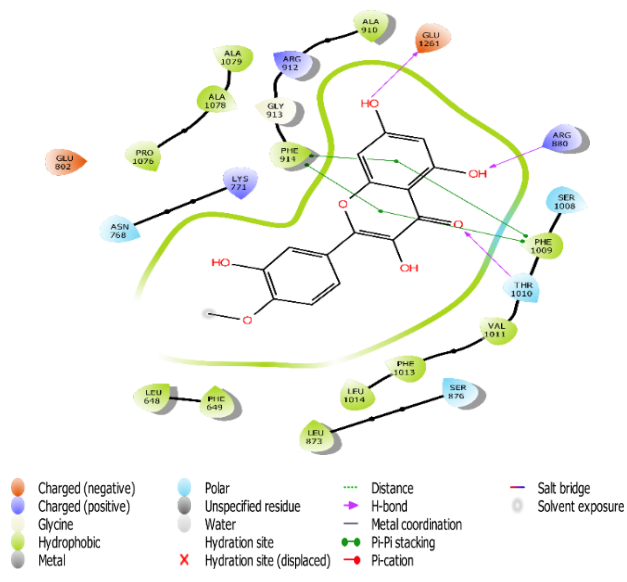
**Fig. 4.** The 2D interaction view of compound 1 with amino acid residues at the active site of XO.



**Fig. 6.** The 2D interaction view of compound 3 with amino acid residues at the active site of XO.



**Fig. 5.** The 2D interaction view of compound 2 with amino acid residues at the active site of XO.

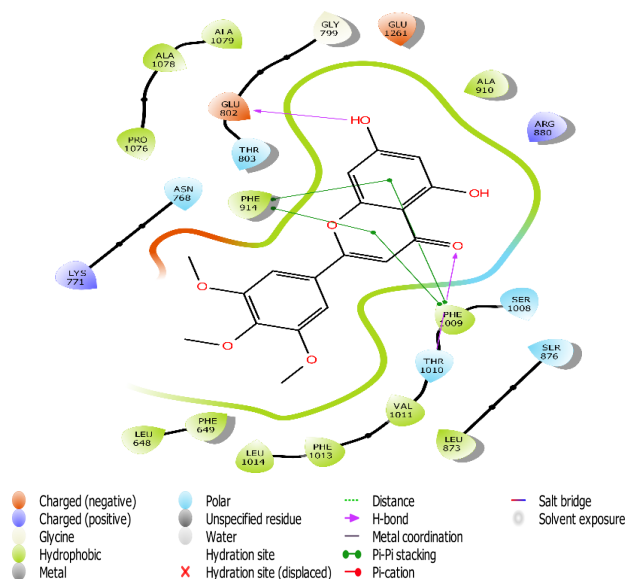


**Fig. 7.** The 2D interaction view of compound 4 with amino acid residues at the active site of XO.

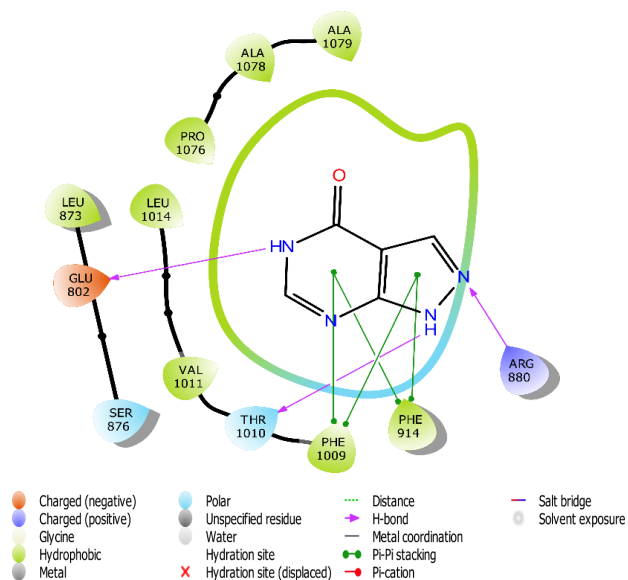
hydroxyl group at their phenyl ring A with GLU802 and, VAL1011 and GLU1261 in the case of compounds 1 and 3, respectively. Compound 5 also forms hydrogen bond interaction with the hydroxyl group at its phenyl ring A with ARG 880 and GLU1261. Compounds 1, 3, and 4 form hydrogen bond using the carbonyl group at their ring C with polar THR1010. Compounds 2 and 5 also form hydrogen bonds using the carbonyl group at ring C with hydrophobic PHE1009.

Scaffold and substituent analysis were carried out for the top five compounds, and the essentiality of the flavonoid 3-ring structure was revealed both spatially and chemically. Spatially, the 3-ring structure was essential for optimal occupancy of the active site, thus enabling the

observed binding pose within the active site. The number and positioning of hydroxyl groups on Ring A have a role to play in displacing the ALA910, a crucial residue for maintaining the hydrophobic shell surrounding it. Only compound 3 with three OH- substituents on Ring A was able to form two hydrogen bonds with the polar GLU802 and GLU 1261, thus displacing ALA910 effectively. Another important observation is the relationship between solvent (water) exposure and the nature of substituents on Ring B. Although they did not seem to engage in any interaction, they play an essential role in the reversible binding of the ligand by disrupting the strong hydrophobicity that surrounds them. Compounds 1, 2, and 3 share similar Ring B nature. Improved active site



**Fig. 8.** The 2D interaction view of compound 5 with amino acid residues at the active site of XO.



**Fig. 9.** The 2D interaction view of Allopurinol with amino acid residues at the active site of XO.

opening was observed with compound 5 where polar 3 carbonyl groups readily interact with water molecules. The reverse is seen in the case of allopurinol which enjoys a tight hydrophobic space, thus discouraging reversibility, a consequence of desolvation.<sup>34</sup> The result is a zero-order binding kinetic which may have been linked to allopurinol accumulation and toxicity.<sup>35</sup>

### ADME/Tox

ADME properties of molecules obtained by the Glide docking were checked, using Schrödinger QikProp. Qikprop helps in analyzing the pharmacokinetics properties of molecules by checking the drug-like properties. The predicted ADME properties include the human oral absorption, the number of rotatable bonds, the molecular weight of the molecule, the number of hydrogen bond acceptors, the prediction of binding to human serum albumin, the number of hydrogen bond donors, the predicted hexadecane/gas partition coefficient, and the number of violations of Lipinski's Ro5.<sup>36</sup> Lipinski's Ro5 helps to evaluate the drug-likeness, and determine the prospect of small molecules in becoming an orally active drug for human. The rule permits a molecular weight <500 Da, octanol-water partition coefficient < 5, hydrogen bond donor  $\leq 5$ , and hydrogen bond acceptor  $\leq 10$ . Prospective drug candidates that obey the Ro5 tend to have lower attrition rates at the stage of clinical trials and for this reason, it has an increased chance of becoming and staying marketable.<sup>36</sup> Compounds of *C. odorata* have shown excellent result and are in accordance with this rule. Therefore, they can be developed as a promising lead in the design of XO inhibitors. The results of the ADME properties are shown in Table 3.

### Conclusion

The docking study and pharmacology parameters revealed that flavones scaffold could be a useful drug candidate for preventing and treating gout, flavones scaffold has a higher binding affinity with XO, interacting through the formation of pi-pi stacking, hydrogen bond,

**Table 3.** QikProp results of selected compounds based on docking score with Standard compound

Phyto-constituents	#rotor	Mol_MW	donorHB	acceptHB	QPlogPo/w	QPlogKhsa	HOA	ROF
	A	B	C	D	E	F	G	H
Compound 1	5	302.240	4.000	5.250	0.315	-0.364	2	0
Compound 2	4	286.240	3.000	4.500	0.926	-0.200	3	0
Compound 3	6	318.239	5.000	6.000	-0.306	-0.501	2	1
Compound 4	5	316.267	3.000	5.250	1.152	-0.175	3	0
Compound 5	5	344.320	1.000	5.250	2.688	0.172	3	0
Allopurinol	0	136.113	2.000	4.500	-0.551	-0.741	3	0

A. Number of rotatable bonds (Range: 0 to 15) B. Molecular weight of the molecule (Range: 130.0 to 725.0) C. Number of hydrogen bond donors (Range: 0.0 to 6.0) D. Number of hydrogen bond acceptors (Range: 2.0 to 20.0) E. Predicted octanol/water partition coefficient. (Range: -2.0 to 6.5) F. Prediction of binding to human serum albumin (Range: -1.5 to 1.5) G. Human Oral Absorption (Range: 1, 2, or 3 for low, medium, or high) H. Number of violations of Lipinski's rule of five (Range: maximum is 4).

## Research Highlights

### What is the current knowledge?

✓ Flavones scaffold interacts with the molybdenum atomic domain and some other amino acid residues of XO that are essential for enzymatic action and hence blocked catalytic activities.

### What is new here?

✓ Flavones scaffold has relatively better inhibitory than allopurinol and may poses lesser side effects in the treatment of gout.

and hydrophobic interaction. Therefore, *in vivo* and or *in vitro* assays could be examined to further demonstrate its potential to inhibit XO.

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### Ethical statement

No ethical issue to be declared.

### Competing interests

Authors declare no conflicts of interest.

### Authors' contribution

KB brought the presented idea. KB, OAJ, IOK and ANS designed the computational flow chat. MDS and IOK provided the study equipment and materials. ANS, DTI and OOO retrieved ligands and protein from their respective database for molecular docking studies. KB and ITT wrote the manuscript with input from all the authors. OOI reviewed the article before submission.

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