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In Silico Analysis of Rhodanine and Rhodanine Acetic Acid Derivatives as Inhibitors of Xanthine Oxidase

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Abstract
Hyperuricemia is a metabolic disorder associated with an increased concentration of uric acid in body fluid.Xanthine oxidase, a homodimer-metallo-flavoprotein, is significant target for treating hyper-uricemia as this enzyme leads a metabolic cascade responsible for uric acid synthesis. By leveraging natural molecules that tend to produce few adverse effects, naturally derived rhodanine (RH) and rhodanine acetic acid (RA) with various therapeutic properties has been investigated for their anti-hyperuricemia potency using *in silico* approach. The screening of potent xanthine oxidase inhibitor is performed using molecular docking, considering binding affinity (RH-5 = -8.2 kcal/mol), non-bonding interactions (RA-5 = 21) and hydrogen bonds (RA-5 = 11) parameters. The lead compound (RA-5) is further subjected to molecular dynamic simulation and binding free energy calculation. Based on these findings, the study concludes that compound RA-5 has higher binding affinity and more stability than the control allopurinol, suggesting its potential as a promising therapeutic agent for hyperuricemia.
Keywords: Hyperuricemia; rhodanine; xanthine oxidase; molecular docking

Introduction

The prevalence of hyperuricemia has increased in recent years, it is a metabolic disorder which gives raise to major illnesses such as diabetes, gout, cardiovascular problems, renal failure, and hypertension(Andruiser et al.,2008). This disease also acts as key characteristics of tumour-lysis syndrome. The over production or reduced excretion of uric acid is the major cause of this metabolic disorder(Vijeesh et al., 2022). Within the body, uric acid is produced by the activity of the xanthine oxidase enzyme, it is a homodimer-metallo-flavoprotein with molecular weight (MW) of 290 kDa, this enzyme catalysis the metabolic cascade i.e. hydroxylation of hypoxanthine to xanthine, and xanthine to uric acid at molybdenum center, which will get excreted by kidney (Chen et al., 2021).According to Pea (2005), Uric acid in the body fluids being the major cause, the disorder is treated either by reducing uric acid production by inhibiting the key enzyme or through increasing the clearance of uric acid by inhibiting the renal tubular reabsorption.Presently, most of thepharmacotherapies available for hyperuricemia work on the inhibition of key enzyme responsible for its production, and it is highly dependent on the type of secretion (more secretion or under secretion) (Gliozzi et al., 2016). Mostly overproducers are treated with allopurinol.

Allopurinol is a structural analog of natural purine base hypoxanthine, which interferes with the catabolism of purines by inhibiting the activity of xanthine oxidase. Current scientific studies support the statements on the substantial adverse of this synthetic medication (Zhang et al., 2022). The patients under this urate-lowering therapy often experience nausea, diarrhea, gastrointestinal complication and maculopapular pruritic rashes(Quire et al., 2018).Thus, the situation demands the identification of novel medication with untoward side effects and anti-hyperuricemia activity.

Rhodanine (RH) and rhodanine acetic acid (RA) are included in the study with the aim of discovering a natural molecule that can suppress the activity of xanthine oxidase enzyme. Rhodanine is a unique heterocyclic molecule that which possess various biological and agrochemical properties including anti-inflammatory (Panico et al., 2015), anti-tubercular(Takasu et al., 2002) antimalarial**,** anticonvulsant, antifungal (Sortino et al., 2007)pesticidal (Inamori et al., 1998),anti-neoplastic and anti-hypertensive (Singh et al., 2014)activities. RH also under go cAMP formation which is mediated by a parathyroid hormone that might be valuable in the treatment of osteoarthritis, degenerative arthrosis, and rheumatoid arthritis(Maddila et al., 2019) andit has also emerged as a promising antidiabetic drug (Kumar et al., 2021). Rhodanine acetic acid is a heterocyclic compound, that serves medical chemistry as it has a broad spectrum of pharmacological properties and is particularly noteworthy for its antidiabetic efficacy. RA has expressed inhibitory abilities against carbonic anhydraseacetylcholinesterase(Kratky et al., 2016),15-lipoxygenase(Shafii et al., 2015),cyclooxygenase (El -Miligy et al., 2017), cholesterol esterase(Sridhar et al., 2020), pancreatic lipase(Chauhan et al., 2019), α-amylase (Kumar et al., 2021, Kumari et al., 2022) and aldose reductase(Bacha et al., 2021) enzymes. Considering all the above-mentioned potentialities of RA-RH, the compounds were investigated for xanthine oxidase inhibitory activity.

Identifying new molecules with medicative capabilities against particular targetsconstitute a broad spectrum of research, where studies mustprovide data on specificity, interaction profile and drug stability. This, in turn reduce the chance of establishing unstable drugs. While the traditional approach of understanding the interaction profile and stability of a new therapeutic drug through series of *in vitro* and *in vivo*experiments yields most reliable outcome, it is a time-consuming as well as costly affair. At this juncture, *in silico* methodologies serveresearchers in structure-based drug design (Seeliger et al., 2010, Patil et al., 2022, Khadri et al., 2023&Simha et al., 2023) enabling early and fast prediction of drug capabilitiesbefore synthesis (Mouchilis et al., 2021). Understanding the interaction of

smallmoleculeswiththe receptor binding site of the target, and to estimate the binding affinity and stability of the formed complex, is a vital part of drug design**.** Various software tools includingAutoDock, DOCK, GOLD, FlexX and ICM facilitate molecular docking simulation and predict the binding libraries of the new ligand with targets(Azam et al., 2013, Patil et al., 2023,). In this study, considering the various medicative properties of RA-RH compounds and employing insilico methodologies, the effectiveness of RA-RH against the xanthine oxidase enzyme has been investigated.

Materials and methods

Molecular docking simulations

The crystal structure of aldose reductase was obtained from RCSB PDB database. The preparation of ligands, protein targets, binding site prediction and molecular docking studies were performed according to the previous studies by (Maradesha et al., 2021) & (Martiz et al., 2022).

Molecular Dynamics (MD) simulation

The compound's best docked pose underwent a 100 ns molecular dynamics (MD) simulation using GROMACS-2018.1. The CHARMM36 force field was used for protein parameters, while ligand parameters and topology were generated via the SwissParam server (Patil et al., 2021). A TIP3P water model with a 10 Å cutoff was employed, and Na^+ and Cl^- ions were included to maintain a 0.15 M salt concentration and system neutrality. Initial energy minimization utilized the steepest descent algorithm for 5000 steps (Shivanna et al., 2022). The system was equilibrated at 1 bar pressure and 310 K temperature using NTP and NVT ensemble classes with a 1 ps relaxation time. Analysis of the resulting trajectories included root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen bonds. Trajectory data were visualized using XMGRACE software (Gurupadaswamy et al., 2022; Martiz et al., 2022).

Binding free energy calculations

To calculate binding free energy by MM-PBSA approach, g mmpbsa program was used which is a plugin for GROMACS. As described by (Martiz et al., 2022, Martiz et al., 2023), the binding free energy of the complex (protein-ligand) was calculated using all the obtained trajectories. For the calculation of binding free energy, the last 50 ns of MD trajectories were considered.

Results

Molecular docking simulation

To understand the all-possible protein-ligand complex formations, the compounds (RA-1, RA-2, RA-3, RA-4, RA-5, RA-6, RA-7, RH-1, RH-2, RH-3, RH-4, RH-5, RH-6, RH-7) were docked with target proteins xanthine oxidase with allopurinol as control. The results of docking studies are tabulated**Table 1.** Of all the docked compounds RA-5 had the best binding affinity, non-bonded interactions, and total count of hydrogen bond.

Table 1: Binding affinity and non-bonding interactions of ligands.

Sl. No.	**Name of the compound**	**Binding affinity (kcal/mol)**	**Total no. of non-bonding interactions**	**Total no. of conventional hydrogen bonds**
1	RA-1	-7.4	14	4
2	RA-2	-7.5	17	4
3	RA-3	-6.7	20	8
4	RA-4	-6.3	15	3
5	**RA-5**	**-8.2**	**21**	**11**
6	RA-6	-6.6	15	5
7	RA-7	-6.6	17	7

8	RH-1	-7.7	10	3
9	RH-2	-7.5	13	4
10	RH-3	-8.1	10	3
11	RH-4	-7.4	16	7
12	RH-5	-7.7	12	6
13	RH-6	-7.2	17	6
14	RH-7	-6.6	7	4
15	**Allopurinol**	**-7.0**	**11**	**3**

Compound RA-5 compounds interacted with xanthine oxidase with the binding affinity of -8.2 kcal/mol. Compound RA-5 formed 21 non-bonded interactions, out of which 11 hydrogen bonds with ASN A: 650(3.00 Å), ASN A: 768(2.23 Å), LYS A: 771(2.84 Å), SER A: 876(2.41 Å), SER A: 876(2.40 Å), LEU A: 648(3.26 Å), ASN A: 650(1.81 Å), LEU A: 873(2.41 Å), SER A: 876(2.74 Å), SER A: 876(2.89 Å), PRO A: 1076(2.87 Å) was formed with hydrogen bonds. 7 hydrophilic bonds were formed of which PHE A: 649(5.03 Å), LEU A: 648(4.11 Å), LEU A: 1014(5.31 Å), VAL A: 1011(5.27 Å) were Pi-alkyl, PHE A: 649(4.89 Å) was Pi-Pi T shaped and LEU A: 648(5.38 Å), LEU A: 873(4.86 Å) are alkyl. The compound with the lowest binding affinity, least hydrogen bond and non-bonded interactions was considered as negative control. The graphical representation of compound interaction with xanthine oxidase is given in **Figure 1**.

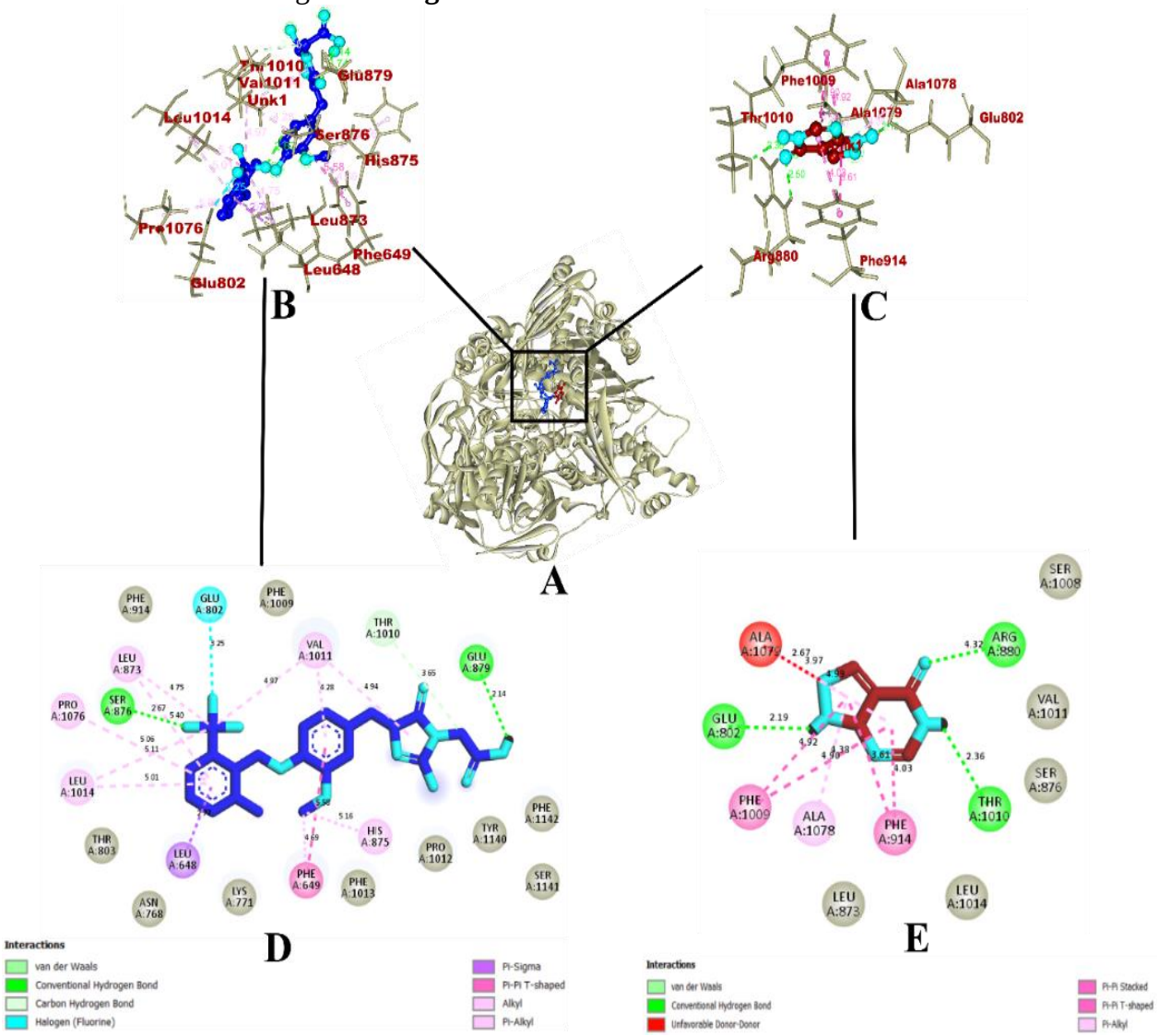

Figure 1: A) Interaction of compound RA-5 and control allopurinol in the binding site of protein xanthine oxidase, B) represents the 3D structure of RA5 interaction with protein, C) represents the 3D structure of allopurinol interaction with protein, D and E) is a 2D representation of compound RA5 and allopurinol with bonded and non-bonded residues along with respective distance.

Molecular dynamics simulation

To confirm the steadiness of the docked proteinMD simulation was performed for RA5-xanthine oxidase and allopurinol-xanthine oxidase complex. To explicate the pliability and conformational stability during the process of binding the MD simulation was performed to the time scale of 100ns. The obtained trajectories of MD simulation were plotted in terms of RMSD, RMSF, Rg, SASA and ligand hydrogen bond. The diagrammatic representation of MD simulation is given in **Figure 2.**

The plot of RMSD indicates that the complex RA5-xanthine oxidase is more stable in compression with theallopurinol-xanthine oxidase complex during simulation for 100ns. The predicted RMSD value of both complex and protein backbone was found to be stable from 10ns and 65ns respectively with imperceptible variations. The RMSD values of RA5, protein and control allopurinol are 0.25-0.31nm, 0.2-0.3nm and 0.25-0.5nm respectively.

The RMSF value of the complex with protein backbone was found to be almost similar with fluctuation throughout. The Rg value of the compound RA5, control drug and protein are found to be in the range between 3.12-3.20nm.

Future, the predicted SASA value of compound RA5, control and protein backbone were in the range of 425 nm^2-460 nm^2. Finally based on the plot of hydrogen bonds, it is predicted that the complexRA5-xanthine oxidase and allopurinol-xanthine oxidase formed maximum of 9 and 4 hydrogen bonds respectively. Considering all the plots of MD simulation it can be predicted that RA5-xanthine oxidase has better flexibility and stability.

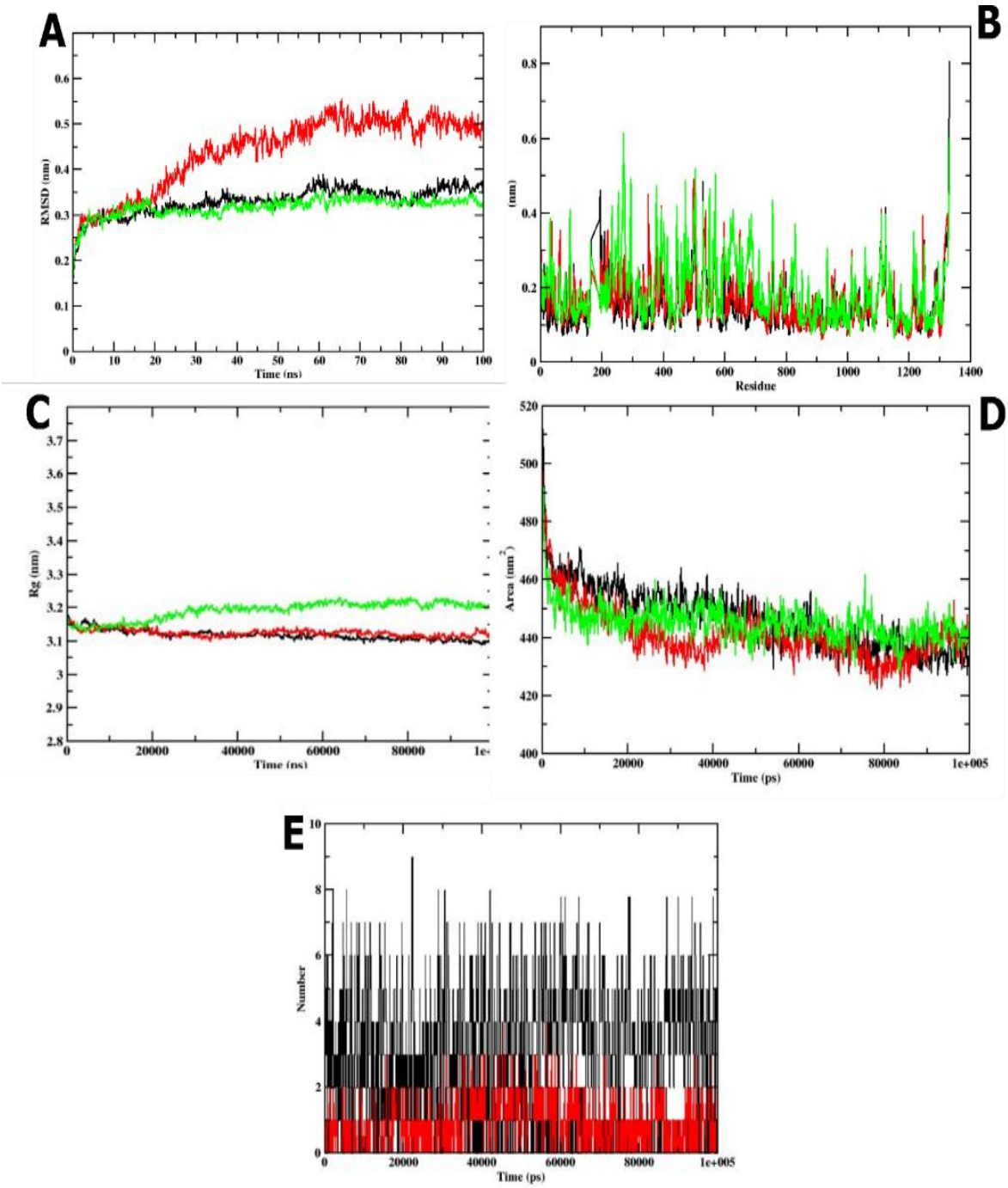

Figure 2: Plot of the molecular dynamics simulations trajectories obtained after 100 ns for RA5 and allopurinol bound with xanthine oxidase protein. (A) RMSD (B) RMSF (C) Rg (D) SASA, and (E) ligand hydrogen bonds; Green: protein backbone atoms, black: protein-RA5 complex, red: protein- allopurinol complex (negative control).

Binding free energy calculations

By using the MMPBSA method, the energy formed during the process of complex formation is evaluated and binding free energy is calculated by considering all the obtained MD trajectories. The values for various energy terms including Van der Waal energy**,** electrostatic energy, SASA energy, polar energy and binding energy are tabulated **Table 2,** with these values it is predicated that the Van der Waal energy and binding free energy of the complex RA5-xanthine oxidase is better in comparison with allopurinol-xanthine oxidase complex.

Table 2: Binding free energy calculations of RA5 and controls with xanthine oxidase

Types of binding free energies / Protein – ligand complexes	Van der Waal's energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
RA2 - Xanthine oxidase complex	-170.644	-20.421	83.161	-18.922	-158.441
Allopurinol – Xanthine oxidase complex	-169.669	-23.992	125.581	-19.988	-149.555

Discussion

Xanthine oxidase is responsible for the synthesis of uric acid, the abnormal levels of this acid in the body fluid effectively lead to hyperuricemia. Allopurinol, a xanthine oxidase inhibitor is the mainstay as an anti-hyperuricemia drug, over the past few decades scientific studies have reported on the detrimental side effects of this synthetic drug(Jordan and Gresser, 2018) (Liu et al., 2018). To address such complications, there is a need for novel medication with strong anti-hyperuricemia activity and possess negligible side effects. Active natural compounds have created a new era in the field of medicine and most of the compounds are involved as therapeutic agents. Rhodanine and rhodanine acetic acid are two natural heterocyclic molecules that exhibitvarious therapeutic properties as anti-inflammatory, anti-tubercula, antimalarial**,** anticonvulsant, antifungal, pesticidal**,** anti-neoplastic, anti-hypertensive agents. Considering all these medicative abilities of these two compounds,their ability as anti-hyperuricemia has been investigated in this study using an*in silico* approach. Molecular docking holds good promise in the field of structure-based computational drug discovery. The ligand with the most negative score represents a high binding ability. Hydrogen bond and hydrophobic bond play an important role in determining the ability and stability of the compound-protein complex (Martinez, 2015). In this study the binding affinity of the lead compound RA-5 is determined as -8.2kcal/mol, which is higher than the binding affinity score of allopurinol i.e. -7.0kcal/mol. In this case, the observed inhibition of the target enzyme is probably due to the loading of RA-5 into the active site of xanthine oxidase via hydrogen bond with Glu 879 and Ser 876, similar pattern of residual interaction is observed instudies by Martínez (2015)&(Marahatha et al., 2021).By this interaction, the catalytic center of the target enzyme would have undergone conformational changes and thus reduce the enzyme activity.Further, molecular dynamic simulation has been carried out to obtain trajectories including RMSD, RMSF, Rg, SASA values and ligand hydrogen bond counts. RMSD analysis usually explains the time-dependent motion of structure and its stability(Patil et al., 2022). Similarly, RMSF is another numerical measurement like RMSD used to predict individual residue flexibility(Patil et al., 2023). In this study, the RMSD value of RA5and control allopurinol are found to be in the range of 0.25-0.31nmand 0.25-0.5nm respectively and RMSF value of the complex with protein backbone is almost similar, illustrating that the stability of the RA-5 compoundis comparable to that of allopurinol. The Rg value of the compound RA5 and control drug was found in the range between 3.12-3.14nm, which indicates the consistency of the RA-5-xanthine oxidase complex similar to the allopurinol complex. Similarly, the SASA value of RA5 and thecontrol drug was found in the range 425 nm^2-450 nm^2, indicating the equal ability of RA-5 with allopurinol. The ligand hydrogen bond of RA5-xanthine oxidase and allopurinol-xanthine oxidase complex is found to be 9 and 4 respectively. The values of all the trajectories of MD simulation illustrate the potency of the

RA-5 compound. Even though few values of RA-5 are at par with allopurinol, the naturally derived RA-5 has expressed good count of ligand hydrogen bond, which is most important in dictating the orientation of an inhibitor binding in the receptor with great binding affinity (Yunta, 2017, Kumari et al., 2023, Huligere et al., 2023). Therefore,through MD simulation it is clear that compound RA5 has better stability and can extensively interacts with the protein at specific binding pocket. However, few variations observed in the trajectories plot can be considered exceptional in comparison with the stability of the control. Further, on investigating the binding free energy of both complex it is observed that the Van der Waal energy (-170.664 kJ/mol), and Binding energy (-158.441kJ/mol) of RA5-xanthine oxidase complex is higher thanthecontrol-target complex. Whereas values of other energy terms including electrostatic energy, SASA energy and polar solvationenergy of both the complex are at par with each other. Thus, this study intends to investigate the anti-hyperuricaemia activity of naturally derived RA and RH compounds using *in silico* approach resultedRA-5 compound as a drug for anti-hyperuricemia, the data obtained out of this study further helps to process *in vitro* and *in vivo* analysis against hyperuricemia.

Conclusion

The importance of naturally derived compounds in drug development is widely recognized, owing to their potential to offer therapeutic benefits with minimal side effects and drawbacks. In this study, our objective was to identify a natural inhibitor of xanthine oxidase. Through molecular docking, molecular dynamics simulations, and binding free energy calculations, RA-5 emerged as a highly effective inhibitor among the fourteen RH and RA compounds tested. Although some properties of RA-5 were comparable to those of the control drug, RA-5 offered the advantage of being a natural molecule with a lower likelihood of causing adverse effects. These findings suggest that RA-5 has the potential to be developed into a novel drug for hyperuricemia, pending further validation through in vitro and in vivo studies.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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