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Molecular Docking and Simulation of Rhodanine and Rhodanine-3-Acetic Acid Derivatives as Potential Aldose Reductase Inhibitors

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Abstract
Aldose reductase (AR) is a crucial metabolic enzyme in the polyol pathway, responsible for converting glucose to sorbitol in tissues independently of insulin. This process is significantly accelerated in hyperglycemic conditions, leading to sorbitol accumulation in tissues and resulting in microvascular complications. To discover new aldose reductase inhibitors, a group of naturally occurring compounds: seven Rhodanine (RH-1 to RH-7) and seven Rhodanine-3-acetic acid (RA-1 to RA-7) derivatives were investigated through in silico analysis. Among all the compounds, RA-2 exhibited potent inhibitory activity against aldose reductase, with a binding affinity of -9.6 kcal/mol. Further molecular dynamics simulations and binding free energy calculations suggested that RA-2 showed greater stability, flexibility, and binding energy. These findings suggest that RA-2 could be promising for treating diabetic complications, pending further in vitro and in vivo investigations.
Keywords: Aldose reductase, Rhodanine, Rhodanine – 3 -acetic acid, molecular docking, molecular dynamics simulation

Introduction

About 8.8% of the adult population is in the risk of diabetes mellitus due to change in the lifestyle and food pattern. It is estimated that about 10.4% of adult population are expected to be diabetic by 2040 (Ogurstova et al., 2017, Ramu et al., 2014), and life-threatening diabetes will be in the seventh position worldwide. Diabetes is an amalgamation of insufficient insulin secretion and resistance to insulin (Sreepathi et al., 2022, Martiz et al., 2023). The prolonged repercussion of diabetes leads to secondary complication including kidney diseases or microalbuminuria, foot amputation, eyes dumbness, congestive heart failure, chest pain, coronary blocks, and stroke.

In body under normal circumstances, cellular-glucose is phosphorylated into glucose-6-phosphate, and enters the glycolytic pathway and approximately 3% of non-phosphorylated glucose enters the polyol pathway. Contrarily, under diabetic condition non-phosphorylated glucose entering the polyol pathway will be increased to 30% (Antony and Vijayan., 2017). Aldose reductase plays a vital role in this pathway, were enzyme catalyses nicotinamide adenosine dinucleotide phosphate-dependent reduction of glucose to sorbitol (Tang et al., 2007). Unfortunately, enzyme aldose reductase is found in all the target tissue that are prone to develop diabetic complication. Excessive sorbitol in the heart, eyes, vasculature, neurons, and kidney leads to reactive oxygen species accumulation and local hyperosmotic conditions causing retinopathy, nephropathy, and neuropathy in diabetic individuals (Kalita et al., 2018, Mallikarjunaswamy et al., 2020). Therefore, enzyme aldose reductase is emerged as promising drug target in management of diabetic complication.

So far various aldose reductase inhibitors including alrestatin, epalrestat, lidorestat, ponalrestat, sorbinil, zopolrestat and Zenerastat has been developed to treat secondary complication in diabetes, but many of them have been withdrawn due to secondary failures and other side effects. The most repeated adverse effect encountered in consumers are increased liver enzymes, thrombocytopenia, adult respiratory distress syndrome, splenomegaly, and lymphadenopathy (Quattrini et al., 2019). Thus, identifying potential aldose reductase inhibitors with untoward side effect is in focus.

In various scientific studies, the biological activities of synthetic rhodanine (RH) are been discussed, a five membered heterocyclic compound is found to exhibits antitubercular, antimicrobial, antiviral, anticancer, antimalarial, anti-inflammatory and antioxidant activities and emerged as a promising antidiabetic molecule (Patel et al., 2016). According to Day and Bailey (2016) & (Wang et al., 2017) Rhodanine derivatives also have ability to bind PPARγ (ciglitazon) and α-glucosidase respectively and inhibit their activity. Similarly, synthetically developed Rhodanine-3-acetic acid (RA) and its condensation products i.e. RA with different aldehydes has been studies for their antihypertensive, antimycobacterial, pesticidal, antifungal and antineoplastic activities (Dolezel et al., 2009, Kumar et al., 2021, Honnavar et al., 2020).

Virtual screening, a widely adopted method in computer-aided drug design, plays crucial role in identifying the new hit molecules via bioinformatic tools. The process involves multiple steps including identifying potential binding sites on the target protein, generation of candidate molecules, assessment of drug likeness, docking the ligand with respective targets and ranking them based on the binding affinity. Additionally, the tools of bioinformatic are also employed in analysing the stability of the designed drug, target protein and ligand-protein complex (Umamaheshwari et al., 2011). Therefore, in this study, given the multiple biological activities attributed with RA and RH, we investigated the inhibitory effects of naturally occurring RA-RH on aldose reductase utilizing bioinformatic tools.

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, Martiz 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), 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 50ns of MD trajectories were considered.

Results and Discussion

Molecular docking simulation

Molecular docking studies were conducted to identify the potentiality of the ligand to inhibit the target protein, evaluating non-bonded interactions, conventional hydrogen bonds and binding affinity. After docking procedure, a compound expressing highest negative binding affinity is considered to exhibit higher activity (Nadavapalli et al., 2023). In this study, to elucidate all possible interactions and complex formations between protein and ligand, 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 aldose reductase target protein, with quercetin serving as control. The docking results are tabulated in Table 1. Among the ligand set, compound RA-2 was chosen due its high binding affinity, number of non-bonded interactions and hydrogen bonds.

Table 1 Binding affinity and non-bonding interactions of ligand with

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	-7.9	14	2
2	**RA-2**	**-9.6**	**17**	**6**
3	RA-3	-8.1	14	5
4	RA-4	-7.6	11	4
5	RA-5	-9.1	13	6
6	RA-6	-7.6	12	6
7	RA-7	-7.1	10	2
8	RH-1	-9.3	9	1
9	RH-2	-9.6	14	4
10	RH-3	-7.6	12	3
11	RH-4	-8.5	13	5

12	RH-5	-9.5	9	1
13	RH-6	-8.6	7	1
14	RH-7	-7.0	8	2
15	**Quercetin**	**-6.9**	**11**	**3**

Compound RA-2 interacted with protein aldose reductase with the binding affinity of -9.6 kcal/mol. Compound RA-2 formed 17 non-bonded interactions, out of which 6 were hydrogen bonds with THR A:19 (2.51 Å), TRP A: 20 (2.26 Å), TRP A: 20 (2.50 Å), LYS A: 77 (2.86 Å), GLY A: 18 (2.68 Å), UNL A: 1 (2.78 Å) residue, and 8 hydrophilic bonds with TRP A: 20 (4.64 Å), TRP A: 20 (3.94 Å), TYR A: 209 (4.21 Å), TRP A: 20 (5.47 Å), PHE A: 122 (4.58 Å), TRP A: 20 (4.97 Å), CYS A: 298 (4.59 Å). The results of hydrogen bond interaction in the binding pocket of aldose reductase enzyme is in par with the studies by (Nadavapalli et al.,2023)&(Umamaheshwari et al.,2011). However, the binding affinity of RA-2 surpasses those two aldose reductase inhibitors mentioned in the aforementioned studies. The compound with lowest non-bonded interaction, hydrogen bond and binding affinity was considered as negative control and the graphical representation of RA2-aldose reductase is presented in **Figure 1**.

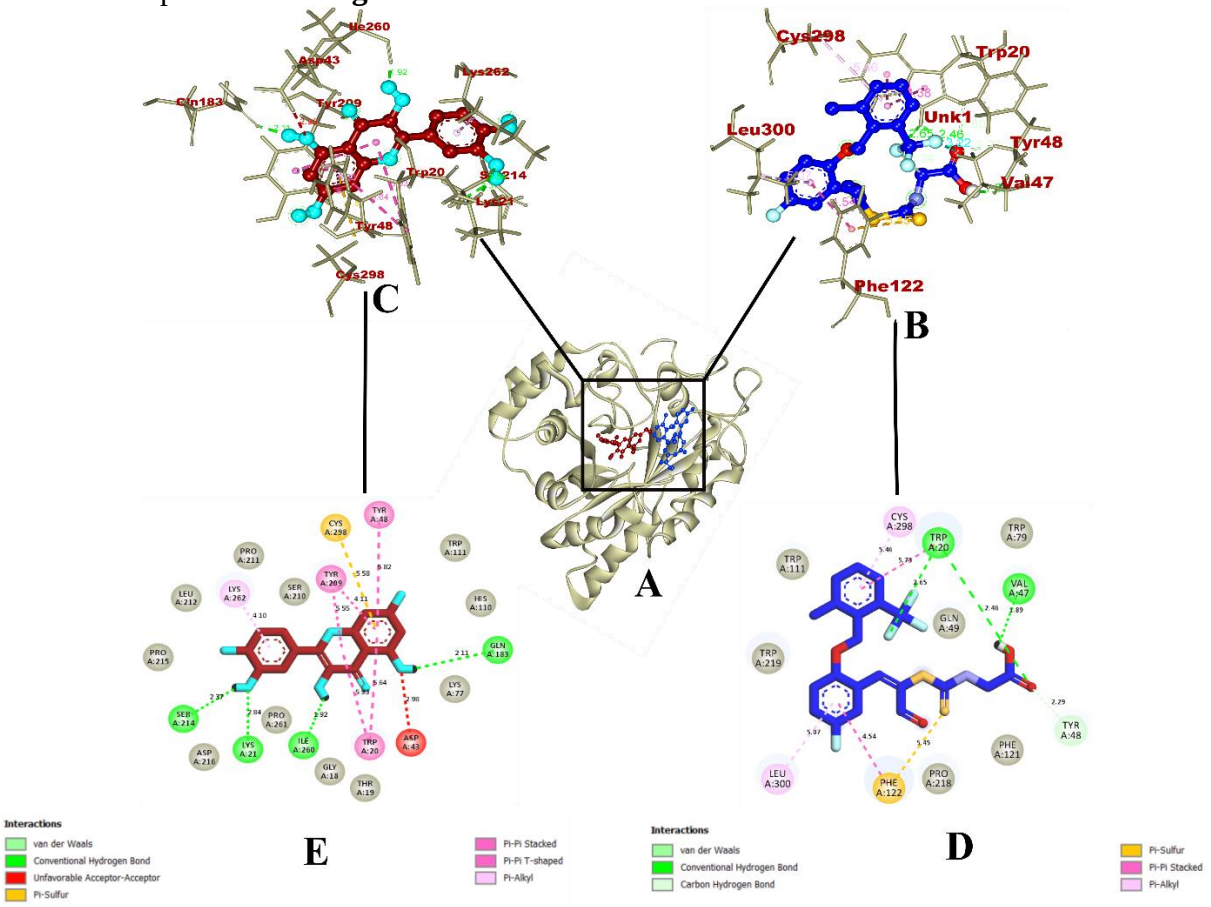

Figure 1 A) Interaction of compound RA2 and control Quercetin in binding site of protein aldose reductase, B) represents the 3D structure of RA2 interaction with protein, C) represents the 3D structure of Quercetin interaction with protein, D and E) is a 2D representation of compound RA2 and Quercetin with bonded and non-bonded residues along with respective distance.

Molecular dynamics simulation

The interaction between ligand and the target protein is well understood through molecular docking studies. However, the impact of these interaction on stability and flexibility of the

protein and ligand-protein complex remains unclear. To address this aspect molecular dynamic simulation is carried out (Patil et al.,2022, Pushpa et al., 2023). In this study, MD simulation of RA-2 was performed for the time scale of 100ns. All the MD simulation trajectories were plotted in terms of RMSD, RMSF, Rg, SASA and ligand hydrogen bond. The respective graph for each trajectory is provided in **Figure 2**.

The RMSD plot typically gives information on the stability of the complex formed (Fajar et al., 2021). In this study, the RMSD plot depicts that the RA2-aldose reductase complex is more stable compared to quercetin-aldose reductase complex. The stability of the RA2-aldose reductase complex is evident from 40ns-70ns with variations. The RMSD value of RA2-aldose reductase complex ranges from 1.1-3.0nm, while the complex quercetin-protein aldose reductase was found to be in the range of 1.5-3.5nm, with fluctuations in loop and terminal region.

RMSF analysis is performed to examine the binding efficacy of the ligand with target protein (Fajar et al., 2021). In this study, the RMSF value of both formed complexes were found to be almost similar. The possible changes that take place in protein structure during complex formation is analysed through Rg values (Kumar et al., 2022). Here, the Rg value of compound, protein and control drug has ranged between 1.87-1.90nm.

The SASA plot is evaluated to predict the conformational change in the binding region (Patil et al., 2023). The predicated SASA value of protein is between 130-145nm^2, and SASA value of both complexes fall within the same range i.e. 137-185nm^2. Ligand H-bond is analysed to understand the structural agreement after complex formation (Patil et al., 2023). Based on the hydrogen bond plot, it is predicated that the complex RA2-aldose reductase and quercetin-aldose reductase forms a maximum of 6 and 3 hydrogen bonds respectively. Although the RMSD, RMSF, Rg and SASA value of RA-2 are at par with quercetin drug, the RA-2 compound exhibit better ligand hydrogen bond counts, which is crucial in determining the orientation of inhibitor binding to receptor with high binding affinity (Khadri et al., 2023, Patil et al., 2022, Patil et al., 2023). This observation elucidates the enhanced stability and flexibility of RA-2 as a drug candidate.

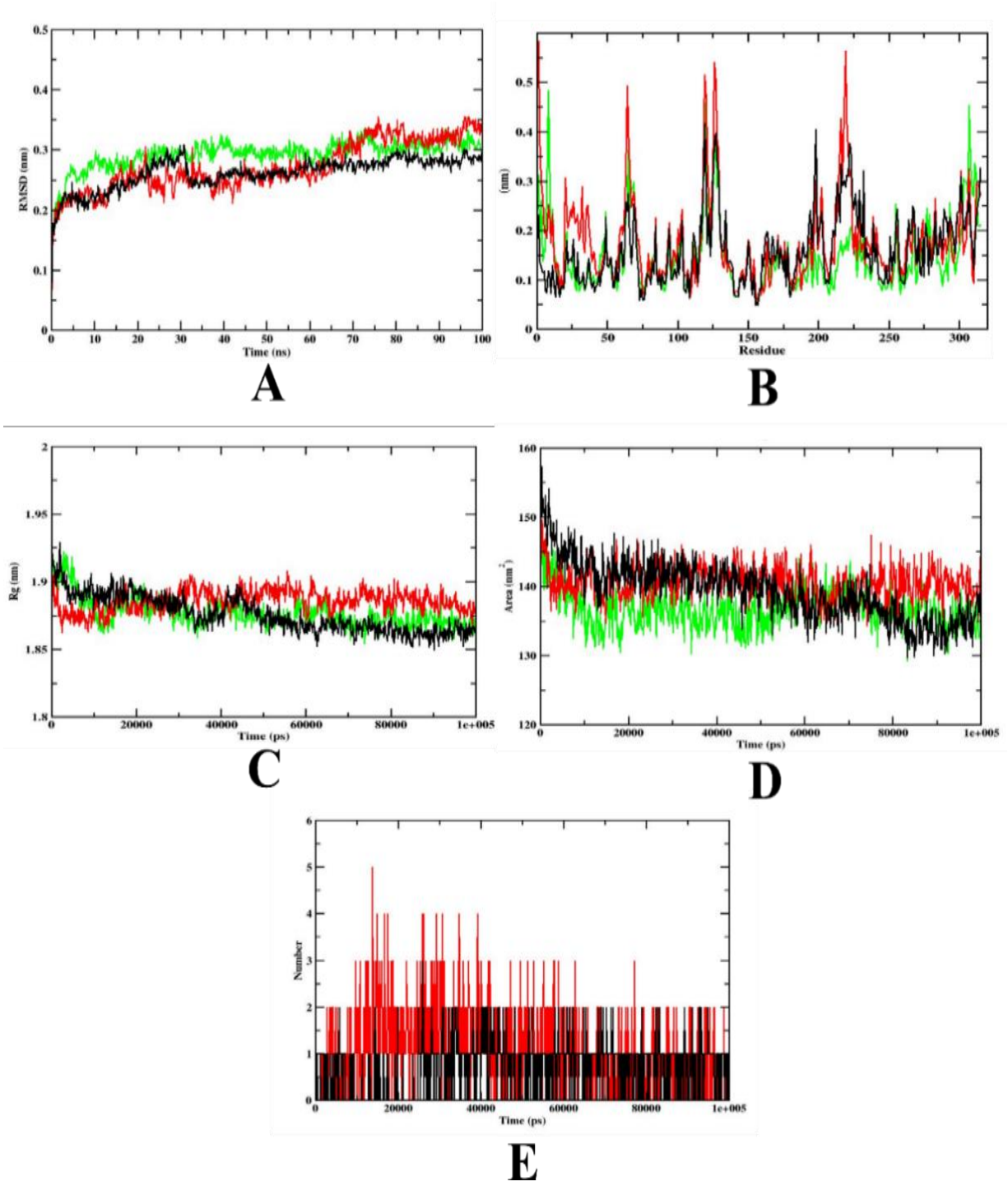

Figure 2 Plots of the molecular dynamics simulations trajectories obtained after 100 ns for RA2 and quercetin bound with aldose reductase protein. (A) RMSD (B) RMSF (C) Rg (D) SASA, and (E) ligand hydrogen bonds; Green: protein backbone atoms, black: protein-RA2 complex, red: protein-quercetin complex (negative control).

Binding free energy calculations

To evaluate the energy formed during the process of complex formation, the binding free energy was calculated by considering all the obtained MD trajectories using MMPBSA method. The outline of binding free energy is depicted in **Table 2**, including Van der Waal energy, electrostatic energy, SASA energy, polar solvation energy and binding energy. This analysis suggests that the RA2-aldose reductase complex exhibits better polar solvation

energy and binding energy, while the values of other energy terms are comparable between the two complexes. This indicates the higher stability of the RA2-aldose reductase complex.

Table 2 Binding free energy calculations of RA2 and their controls with Aldose reductase proteins.

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 - Aldose reductase complex	−148.669	−22.911	89.945	−12.899	−105.615
Quercetin - Aldose reductase complex	-159.669	-30.870	82.920	-13.796	-100.299

Conclusion

This study aimed to identify a potential compound which effectively inhibited aldose reductase activity. Molecular docking of the compounds revealed inhibition of the target enzyme, with binding affinities ranging from -6.9 to -9.6 kcal/mol. RA-2 emerged as the most promising compound due to its highest binding affinity, non-bonded interactions, and numerous hydrogen bonds. Analysis of molecular dynamics simulation trajectories and binding free energy calculations indicated that RA-2 exhibits stability comparable to the effective drug quercetin. These findings provide valuable data for future in vitro and in vivo experimental studies evaluating RA-2 as an aldose reductase inhibitor for treating diabetes mellitus and its complications.

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CONFLICT OF INTEREST

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

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