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Molecular Docking and Validation of Methicillin Resistant *Staphylococcus aureus* Targets against Geninthiocin

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Antibiotic resistance has been a serious public health concern in recent years. Methicillin resistant "*Staphylococcus aureus"* (MRSA) is a superbug that causes life threatening infections of Humanity which is difficult to treat. Geninthiocin is a macrocyclic thiopeptide with a 35-membered core moiety, which was isolated from marine *streptomyces sp.* ICN19, which has proven potent activity against MRSA. Five target proteins PDB ID: 4YMX, 3ZDS, 3QLB, 4IEN and 1DXL were identified from MRSA for their presumptive action for Geninthiocin. In this study, we used molecular docking and molecular dynamic simulation, in order to validate Geninthiocin's potential target protein. Target proteins were subjected to ligand-protein docking studies. Based on their docking scores and Hydrogen bonding interactions, two possible proteins 4YMX and 3ZDS were further subjected to simulation strategies to validate the protein-drug interaction. Out of which, homogentisate1,2 dioxygenase turned out to be a possible drug target for Geninthiocin. The compound Geninthiocin could be developed as a potential inhibitor against the target protein homogentisate1,2-dioxygenase for exhibiting an effective antimicrobial activity.

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Keywords: Geninthiocin; target proteins; docking studies; MRSA.

1. INTRODUCTION

Methicillin-resistant "Staphylococcus aureus" (MRSA) is resistant to all currently available β-lactam antibiotics, namely, penicillins, cephalosporins, and carbapenems. Due to the emerging resistance to MRSA, the options for treatment using available antibiotics are challenging nowadays [1].

Worldwide treatment methodology for MRSA infections continues to be a challenge for healthcare professionals as they struggle with treatment decisions. Moreover, the choice of antibiotic treatment for MRSA is increasingly becoming complex with antimicrobial resistance. To overcome the situation, different combinations of antimicrobial drugs are being prescribed to treat severe MRSA infections [2].

Geninthiocin is a thiopeptide having a macrocyclic core moiety of 35 members. It is effective against Gram-positive (G+) bacteria [3].

Thiopeptide antibiotics are a popular class of antimicrobials produced predominantly by Streptomyces species and have high antibacterial action against Gram-positive bacteria. Since many members of this class demonstrate activity against numerous drug-resistant bacteria, including methicillin-resistant *"Staphylococcus aureus"* (MRSA) [4], interest in this family of antibiotics has lately resurfaced.

Bioactive compounds exert their biological activities by directly binding to one or more cellular proteins. Therefore, the detection of drug-target interactions is necessary to characterize the compound mechanism of action [5].

As a result, target identification is vital for a complete understanding of a compound's action mechanisms. In this context, Molecular docking is a very efficient tool for novel drug discovery for targeting protein. Due to its vast application in the medical field, protein-ligand docking gains particular interest amongst different types of docking.

In addition, the application of *in silico* computational methods to predict targets of bioactive compounds has become more critical in recent years. However, wet lab experiments are found to be convincing [6].

Current computational methods for drug target discovery are of three categories: structure-based, ligand-based, and phenotype-based virtual screening [7].

The scoring function is used to estimate the likelihood of the ligand binding to a protein in structure-based approaches, which entail molecular docking between a ligand and a target. Because ligand-based methods rely on similarities between known ligands to guess on unknown receptor site configurations, they are ineffective for analyzing proteins that lack known ligands [8].

Molecular dynamics (MD) simulation is a vital tool for studying macromolecules such as nucleosomes, ribosomes, membrane proteins, organic solids, and proteins-ligand complexes. It has advanced rapidly over the last four decades thanks to advances in force fields made possible by quantum physics and computational chemistry. The simulation is widely used to analyze the structure to function relationship of protein and protein-ligand complexes [9].

We did molecular docking studies for five possible target proteins for Geninthiocin isolated from MRSA in the present work. These five protein targets were subjected to docking studies. Further, based on their respective docking scores and Hydrogen RG bonding interaction, two potential targets were shortlisted and molecular dynamic studies were carried out to validate their potentiality. Accordingly, successful protein target with excellent docking scores and validation values was identified.

2. MATERIALS AND METHODS

2.1 Molecular Docking

The crystal structure of the selected protein targets was retrieved from the protein databank (PDB). The three-dimensional structure of the selected ligand molecule Geninthiocin was downloaded from the PubChem database in sdf format. The structure of Geninthiocin is displayed below, Fig.1. The ligand molecule is converted into pdb file format using open Babel converter tool [10]. The binding affinity between the selected protein structures and the ligand was analyzed using Autodock Vina (1) [11].

Prior to the docking, the cocrystallized ligand and the water molecules attached to the protein was removed and the receptor proteins were

prepared using the Autodock tool, and Gasteiger charges were assigned. The prepared protein was saved in PDBQT format (2) [12].

The docking calculations were done using Lamarkian Genetic Algorithm method. After molecular docking, the pose with the minimum binding energy was selected as the best confirmation with respect to each protein. The interaction between proteins and ligands was studied using Pymol visualization software. Further, the prepared ligand was saved in the PDBQT format.

2.2 Molecular Dynamics Simulation Studies

The compound geninthiocin was docked with the two protein targets using Autodock. The pose with higher binding affinity and maximum hydrogen bonding interaction was selected to analyze the stability of the ligand protein complex using the molecular dynamic simulation approach. Molecular dynamic simulations studies were performed using GROMACS 5.1. software [13].

The ligand parameters were analyzed using the PRODRG server in the GROMOS force-field 43a1 framework [14].

The ligand protein complex was then solvated using a simple point charge water box under periodic boundary conditions from box faces to protein. Energy minimization was performed using the steepest descent method for 50,000 steps. The protein ligand complex was equilibrated at constant volume, temperature and number of particles at 300K for 100ps. The covalent bond and the hydrogen atoms were constrained using Linear constraint solver algorithm. The particle Mesh Ewald method was applied to treat the electrostatic interactions [15].

The potential of each trajectory generated after the Molecular dynamic simulations was analyzed using g_rms, g_rmsf, and g_gyration of GROMACS utilities to obtain the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (RG) formed between the protein and ligand.

3. RESULTS AND DISCUSSION

3.1 Molecular Docking Studies

The selected protein targets were docked with Geninthiocin using Autodock. Docking scores and intermolecular interactions are listed in Table 1. The inhibition susceptibility was evaluated using the Binding affinity value generated from Autodock. The compound Geninthiocin exhibited significant inhibitory activity in homogentisate 1,2-dioxygenase protein. Further analysis of the binding modes revealed that the ligand Geninthiocin displayed a significant binding affinity with a -12.1 Kcal/mol binding score. The hydrogen bonding interactions between the protein and ligand were vizualized using a Pymol viewer.

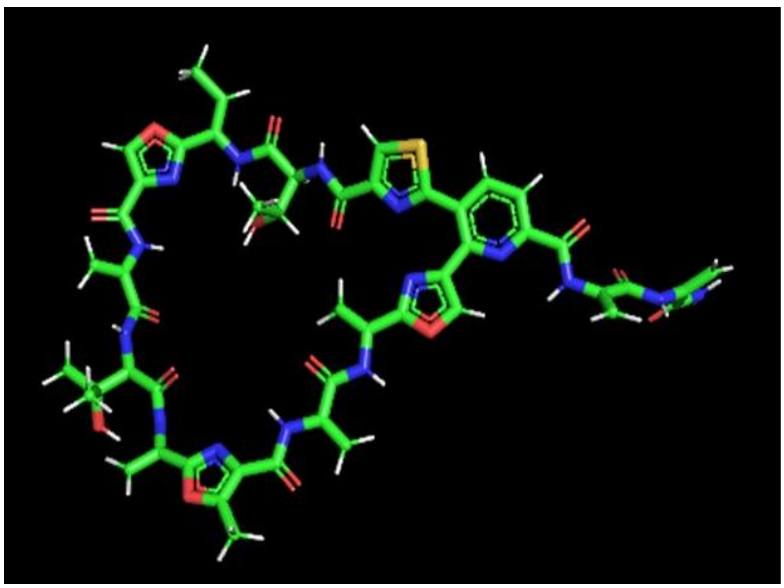


Fig. 1. 3D structure of compound Geninthiocin

Geninthiocin exhibited seven hydrogen bonding interactions with the protein 3ZDS at the amino acid positions GLU 165, LYS 184, GLY 182, SER 70, ARG 181, PRO 126, and PRO 126. Similarly, the compound also exhibited effective interaction with the target protein Ton B (PDB ID: 3QLB) with a binding affinity of -11.4 Kcal/mol and exhibited five hydrogen bonding interactions at the positions ASN114, ARG364, GLN411, ASP482, and SER683.

The protein target amino acid ABC transporter substrate-binding protein (PDB ID:4YMX) displayed a binding affinity of -10.4 Kcal/ mol and displayed seven hydrogen bonding interactions at the residue positions TYR 250, ASP 125, ASP 125, TYR 211, TYR 211, GLN 260, and ILE 205. Other protein targets Acetyl-CoA Hydrolase 4IEN, dihydrolipoamide dehydrogenase 1DXL exhibited a binding score of -9.3 & -11.8 Kcal/mol respectively, with good number of hydrogen bonding interactions.

The active site of five different proteins viz.,4YMX, 3ZDS, 4IEN,1DXL & 3QLB are shown in Fig.2.

Results of amino acids involved in forming active site for proteins viz.,4YMX, 3ZDS, 4IEN,1DXL & 3QLB are shown in Fig 3.

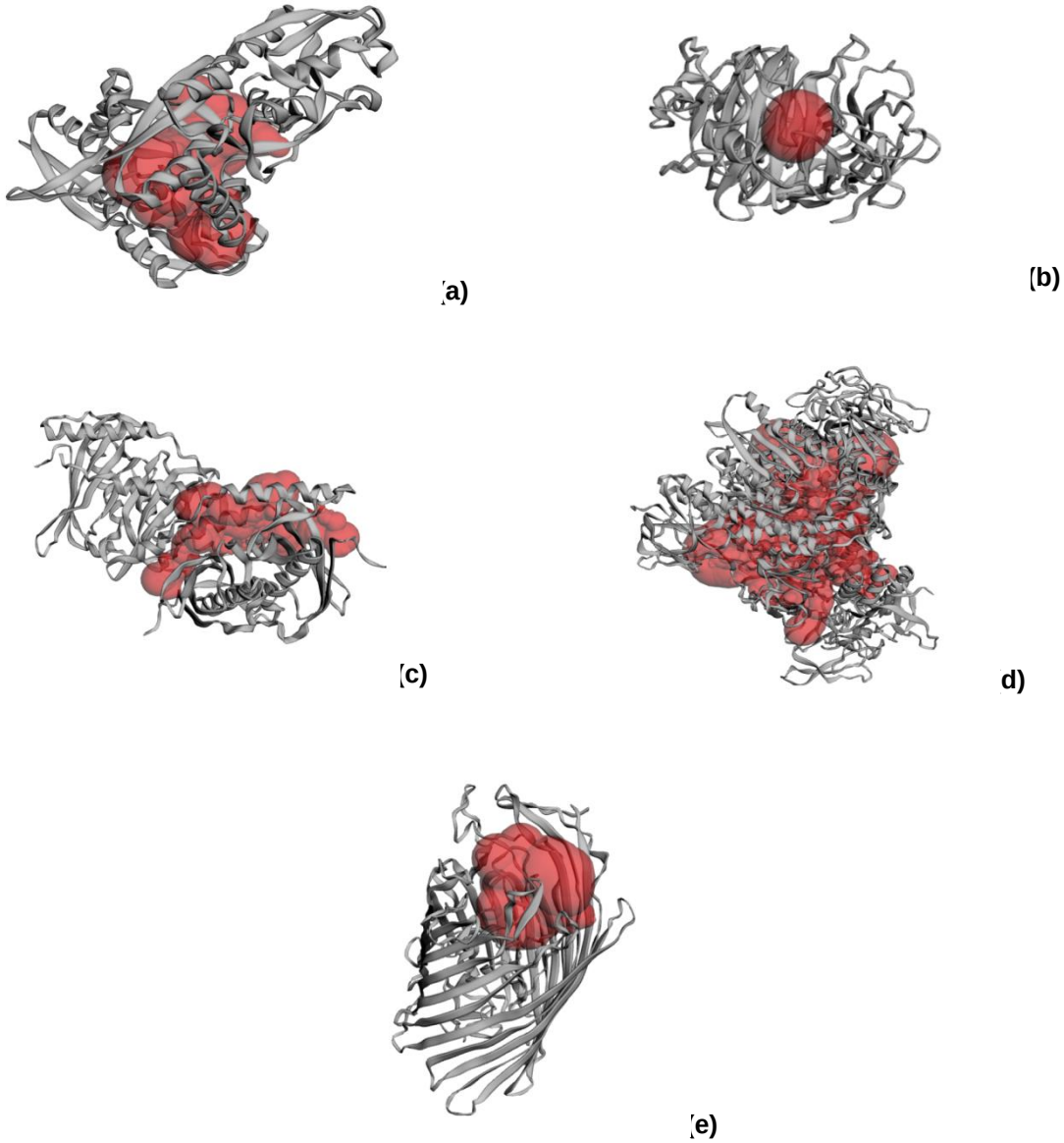
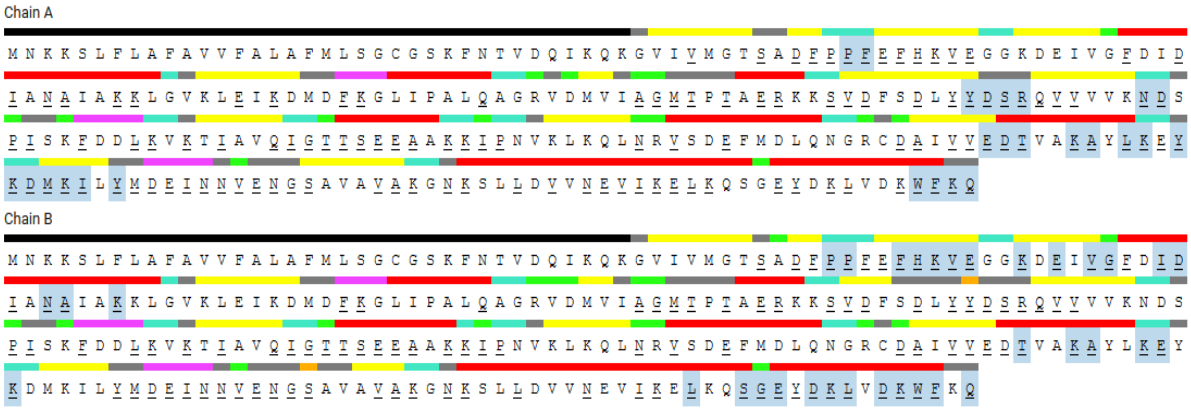


Fig. 2. Active site of five different proteins (a) 4YMX (b) 3ZDS (c) 4IEN (d) 1DXL(e) 3QLB

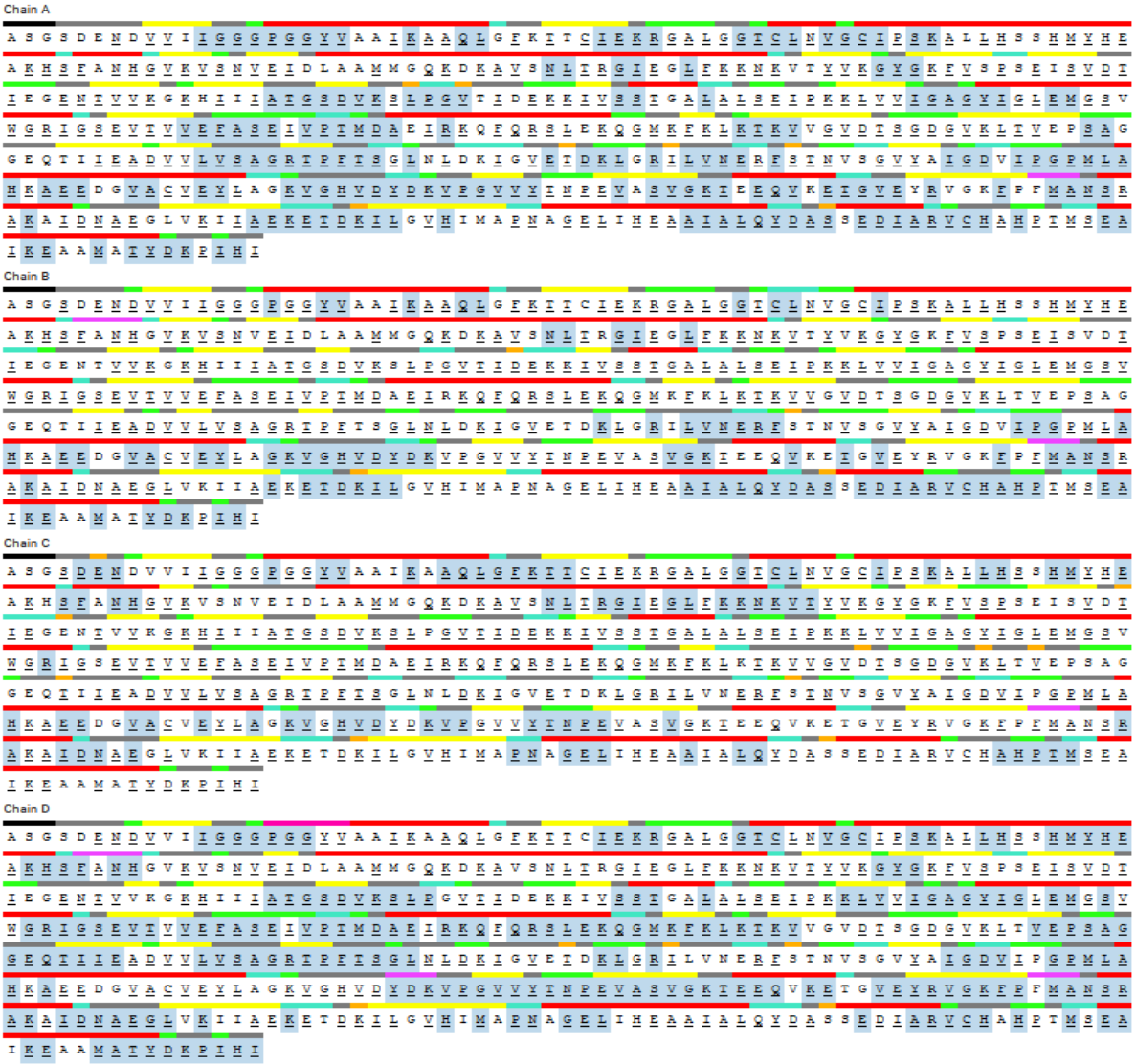
(a) 4YMX



(b) 3ZDS

(c) 4IEN

(d) 1DXL



(e) 3QLB

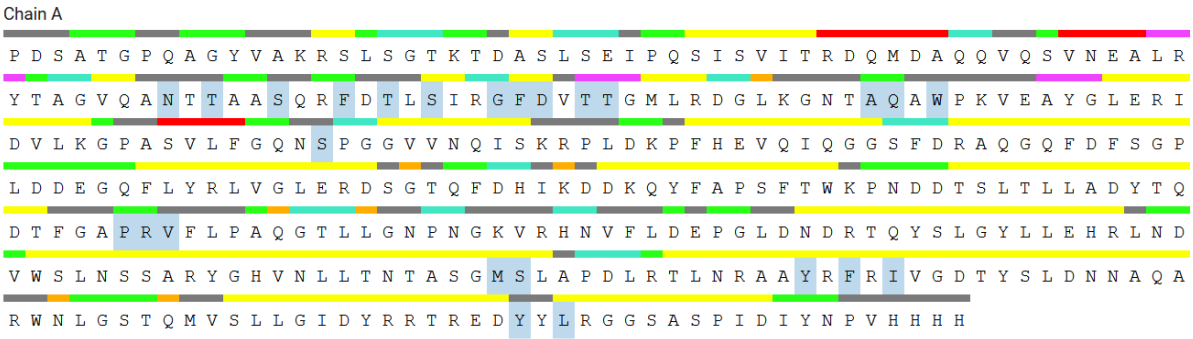


Fig. 3. Results of amino acids involved in forming active site for 05 proteins. (a) 4YMX (b) 3ZDS (c) 4IEN (d) 1DXL(e) 3QLB. Letters highlighted in blue indicate active site residues

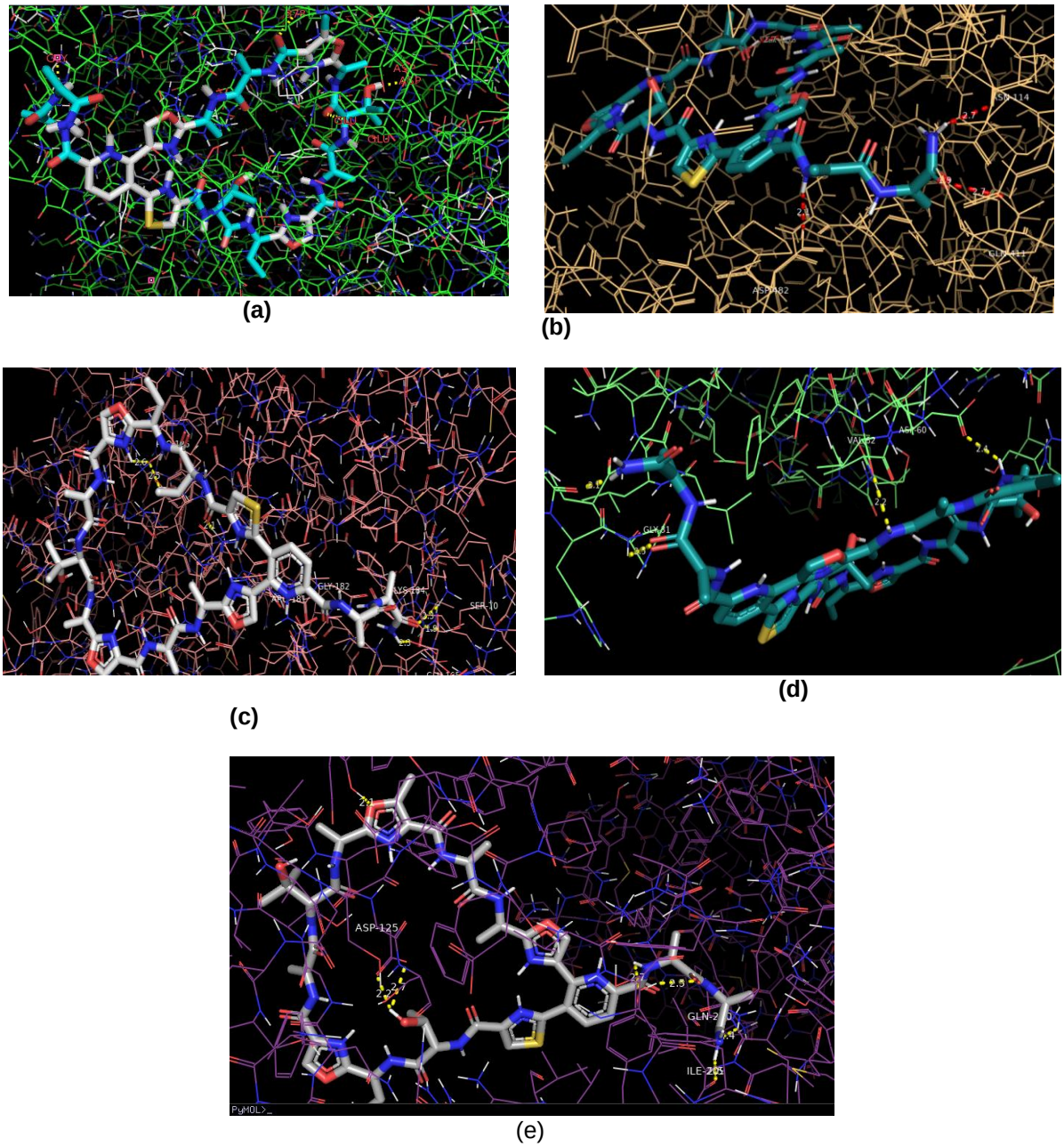


Fig. 4. Docking poses of Geninthiocin with protein targets (a) Interaction of Geninthiocin with a Dihydrolipoamide dehydrogenase protein (1DXL), (b) Interactions between Geninthiocin and 3QLB, (c) Interactions between Geninthiocin and 3ZDS, (d) Interactions between Geninthiocin and 4IEN, (e) Interactions between Geninthiocin and 4YMX

The details of the hydrogen bonding interactions are listed in Table 1 and the interactions are displayed in Fig. 4.

Thus, it is evident that two protein targets exhibited good interactions and further evaluations of their stability would assist in elucidating the potent role of Geninthiocin against the selected protein targets.

3.2 Molecular Dynamics Simulation Studies

3.2.1 Root mean square Deviation

Root mean square variation is an important parameter in analysis of equilibration of MD trajectories, which is estimated for backbone atoms of the enzyme-ligand complexes. Here,

the analysis of the deviation in backbone RMSD for the two enzyme ligand complexes revealed insights into the conformational stability of the complex. The RMSD trajectory of protein 3ZDS shows higher stability, Fig. 5.

However, protein complexes 4YMX exhibited lesser stability, respectively. RMSD was calculated as in Eq. 1, by

$$RMSD = \sqrt{\frac{\sum_{k=1}^{N} d_k^2}{n}}$$

Where, d is the distance of atom, k present in both structures, N is total number of equivalent atoms [16].

3.2.2 Root Mean Square Fluctuation (RMSF)

The root mean square fluctuation (RMSF) was evaluated to identify the average fluctuation of all residues during simulation. The RMSF of the residues is inspected and plotted as a function of residue number to appreciate the continuation and advancement in dynamic stability of the protein complex following ligand binding. Higher the RMSF value, higher the flexibility of the protein ligand complex and vice versa. RMSF is given in Eq. 2 as:

$$RMSF(k) = \sqrt{((R_k - (R_k)^2)}$$

Where, R_k refers to the position vector of atom k [17]. The RMSF of different protein complex with ligands were obtained after MD simulation, to infer the complete information on the position fluctuations. The ligand in complex with protein 3ZDS shows lower fluctuations. Whereas, the other two complex interactions with protein 4YMX exhibited a fall in stability over the binding of ligand, Fig. 6.

3.2.3 Radius of Gyration RG

The compactness of the tertiary structure of the protein was understood by the analysis of Radius of Gyration. RG value is inversely proportional to the packing of the proteins, Higher RG values indicate the lose packing of the system.

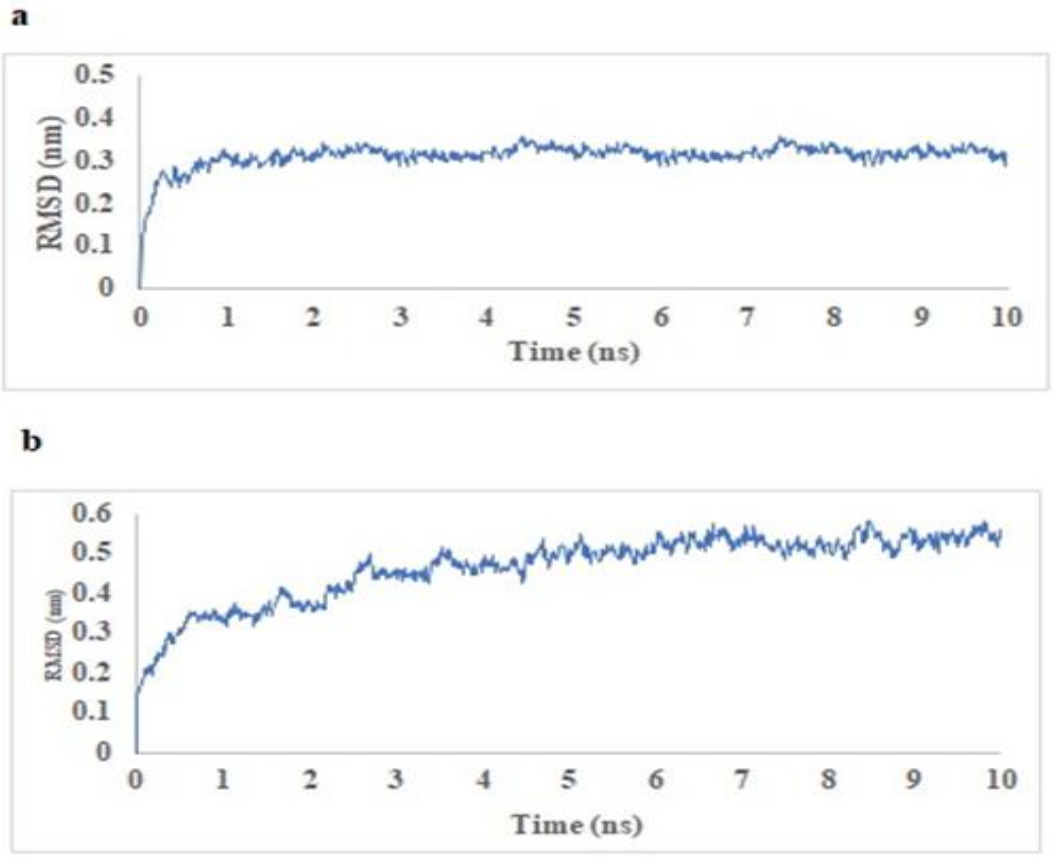


Fig. 5. Root mean square deviation (RMSD) studies of ligand with two protein targets (a) PDB ID: 3ZDS; (b) PDB ID: 4YMX

Table 1. Autodock score and hydrogen bonding interaction of Geninthiocin against target proteins

PDB ID	Docking Score (Kcal/mol)	Number of H bonding Interactions	Interacting Residues
4YMX	-10.2	7	TYR 250, ASP 125, ASP 125, TYR 211, TYR 211, GLN 260, ILE 205
3ZDS	-12.1	7	GLU 165, LYS 184, GLY 182, SER 70, ARG 181, PRO 126, PRO 126
4IEN	-9.3	4	VAL 29, GLY 31, VAL 62, ASP 60
1DXL	-11.8	4	ASP 436, GLU 368, THR 378, GLY 341
3QLB	-11.4	5	ASN 114, ARG 364, GLN 411, ASP 482, SER 683

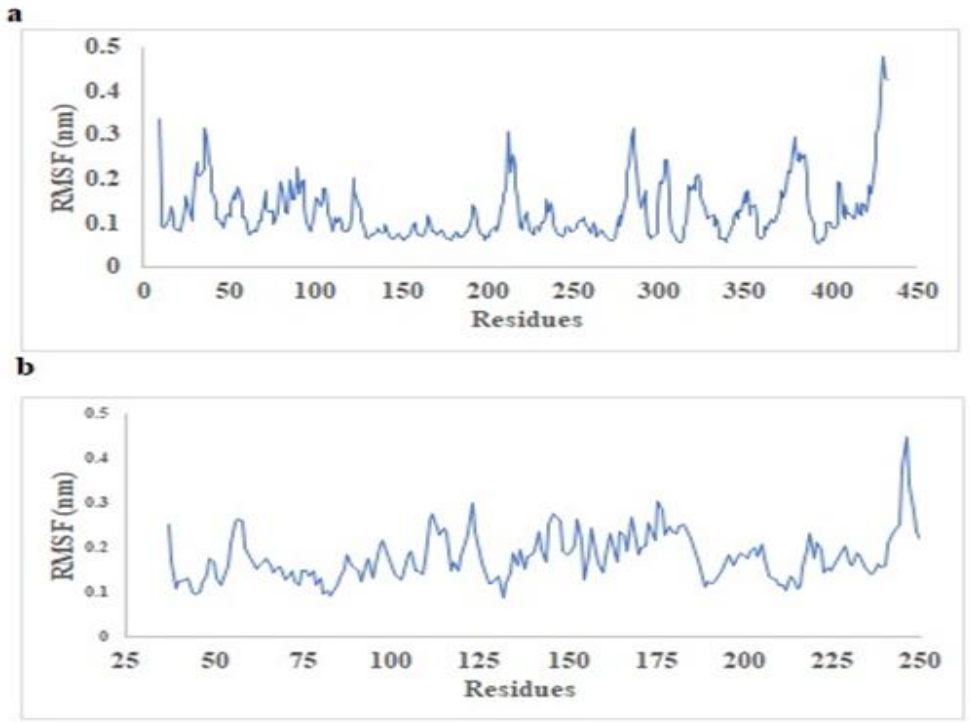


Fig. 6. Root mean square fluctuation (RMSF) studies of ligand with two protein targets PDB ID: (a) 3ZDS, and (b) 4YMX

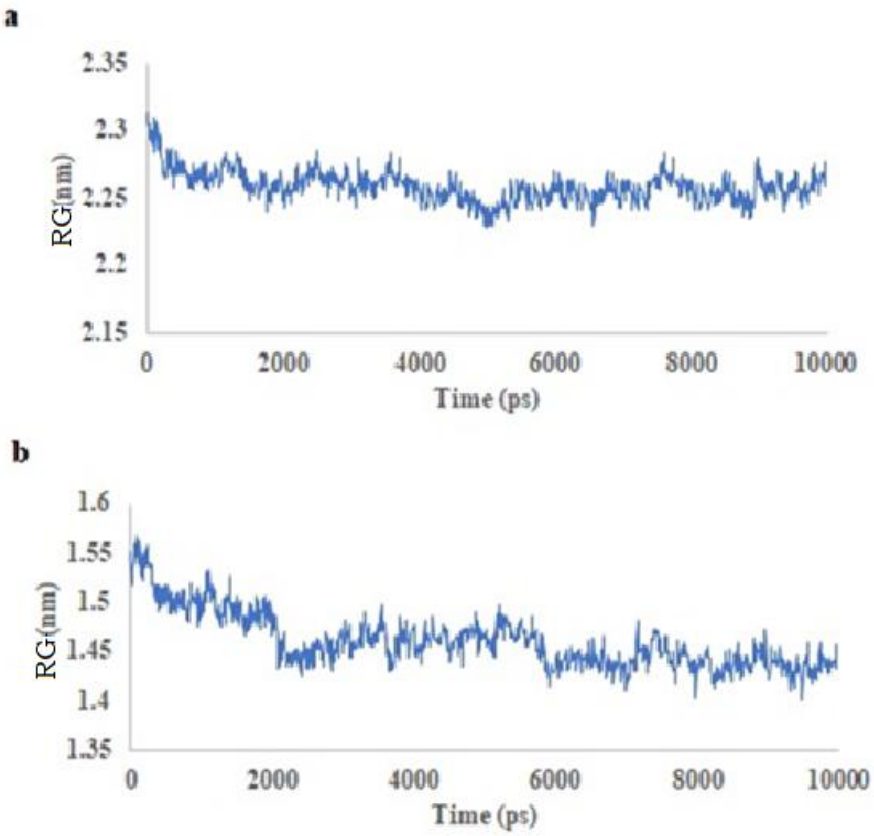


Fig. 7. Radius of Gyration (RG) studies of ligand with two protein targets PDB ID: (a) 3ZDS, and 4YMX

From the graph, it was clearly understood that the RG values of all the ligand complex were low indicating the compactness of complex protein. The ligand in the complex with protein 3ZDS shows lower fluctuations indicating the compactness of the protein complex, Fig. 7. Whereas, the ligand with the protein 4YMX exhibited higher fluctuations.

4. CONCLUSION

Geninthiocin exhibited the highest binding affinity score of -12.1 (kcal/mol) towards the homogentisate 1,2-dioxygenase and hydrogen bonding interaction with seven amino acid residues. Moreover, each RMSD, RMSF and RG analysis indicated that the ligand binding was more stable with the protein homogentisate 1,2-dioxygenase (PDB ID: 3ZDS) compared with the other targets. Therefore, the compound Geninthiocin could be developed as a potential inhibitor against the target protein homogentisate 1,2-dioxygenase for exhibiting an effective antimicrobial activity.

DISCLAIMER

The products used for this research are commonly and predominantly used in our research area and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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