Original Research Article

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

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 in Humans which is difficult to treat. Geninthiocin is a macrocyclic thiopeptide with a 35-membered core moiety, which was isolated from marine streptomyces spp ICN19 , which has proven potent activity against MRSA. Five target proteins PDB ID 4YMX, 3ZDS, 3QLB, 4IEN, 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 H-bond interactions, two possible proteins 4YMX, 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 homogentisate 1,2-dioxygenase for exhibiting an effective antimicrobial activity.

***Keywords*:** Geninthiocin, target proteins, Docking studies, MRSA

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the *Staphylococcus aureus* isolate which is resistant to all currently available β-lactam antibiotics, namely, penicillins, cephalosporins, and carbapenems. The emergence of MRSA is associated with significantly poor clinical outcomes, high morbidity, mortality, and treatment costs.[1] It is becoming increasingly difficult to combat MRSA because of emerging resistance to other antibiotic classes severely limiting the available treatment options[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 the antimicrobial resistance. To overcome the situation, different combinations of antimicrobial drugs are being prescribed to treat serious MRSA infections [2].

Geninthiocin is a thiopeptide with 35-membered macrocyclic core moiety. It has potent anti-Gram-positive (G^+) bacteria activity [3].

Thiopeptide antibiotics are a prominent class of antimicrobials with potent activity against Gram-positive bacteria, produced primarily by *Streptomyces* species. 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 through direct physical binding to one or more cellular proteins. The detection of drug-target interactions is therefore necessary for the characterization of compound mechanism of action[5].

Hence, target identification is considered necessary for the comprehensive inference of the action mechanisms of a compound. In this context, Molecular docking is a very efficient tool for novel drug discovery for targeting protein. Due to its vast application in medicine field, protein-ligand docking gains special interest, amongst different types of docking.

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

Current computational methods for drug target discovery are of three categories viz., 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. The ligand-based methods are based on using similarities between known ligands to speculate on unknown structures of receptor sites; thus, such methods are not appropriate for the analysis of proteins without known ligands [8].

Molecular dynamics (MD) simulation is a vital tool for studying macromolecules such as nucleosomes, ribosomes, membrane proteins, organic solids, proteins-ligand complexes and 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 in the analysis of the structure to function relationship of protein and protein-ligand complexes [9].

In the present work we did molecular docking studies for five possible target proteins for Geninthiocin isolated from MRSA. These five proteins targets were subjected to docking studies. Further, based on their respective docking scores and H-bond 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 were retrieved from protein databank (PDB). The three-dimensional structure of the selected ligand molecule Geninthiocin was downloaded from 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. The binding affinity between the selected protein structures and the ligand were analyzed using Autodock Vina (1) [10].

Prior to the docking the cocrystallized ligand and the water molecules attached the protein were 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) [11] .

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

proteins and the interaction between proteins and ligands were studied using Pymol visualization software Further, the prepared ligand was saved in the PDBQT format.

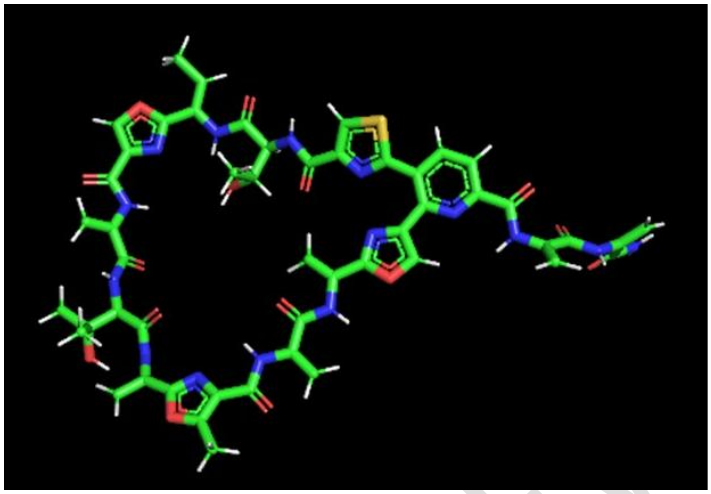


Fig 1: 3D structure of compound Geninthiocin

2.2 Molecular dynamics simulation:

The compound geninthiocin was docked with the three protein targets using Auto dock. The pose with higher binding affinity and maximum hydrogen bond interaction was selected to analyze the stability of the ligand protein complex using molecular dynamic simulation approach. Molecular dynamic simulations studies were performed using GROMACS 5.1. software [12].

The ligand parameters were analyzed using the PRODRG server in the GROMOS force-field 43a1 framework [13].

The ligand protein complex was then solvated using simple point charge water box under periodic boundary conditions from box faces to protein. Energy minimization was performed using steepest decent 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. Particle Mesh Ewald method was applied to treat the electrostatic interactions[14].

The potential of each trajectory generated after the Molecular dynamic simulations were 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

3.1 Molecular Docking Studies:

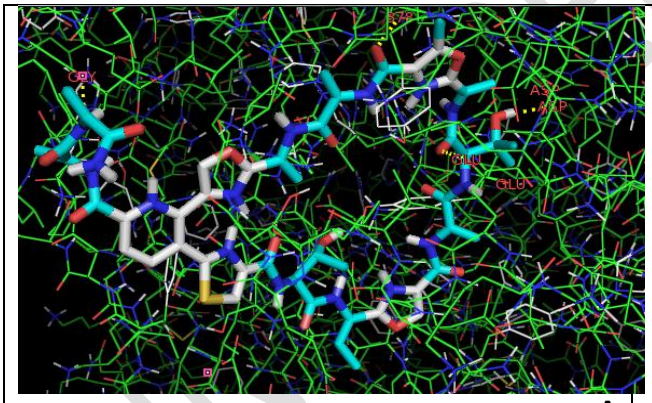
The selected protein targets were docked with Geninthiocin using Autodock. Docking scores, and intermolecular interactions are listed in Table 1. The inhibition susceptibility was evaluated using the Binding affinity value generated from Autodock. The compound Geninthiocin exhibited a significant

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 binding score of -12.1 Kcal/mol. The hydrogen bond interactions between the protein and ligand were vizualized using Pymol viewer.

Geninthiocin exhibited 7 hydrogen bond interactions with the protein 3ZDS at the aminoacid 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 -11.4Kcal/mol and exhibited five hydrogen bond interactions at the position ASN114, ARG364, GLN411, ASP482, 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 7 hydrogen bond interaction 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 respectively with good number of hydrogen bond interactions. The details of the hydrogen bond interactions are listed in Table.1 and the interactions are displayed in Fig 2.

Thus, it is evident that three 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.



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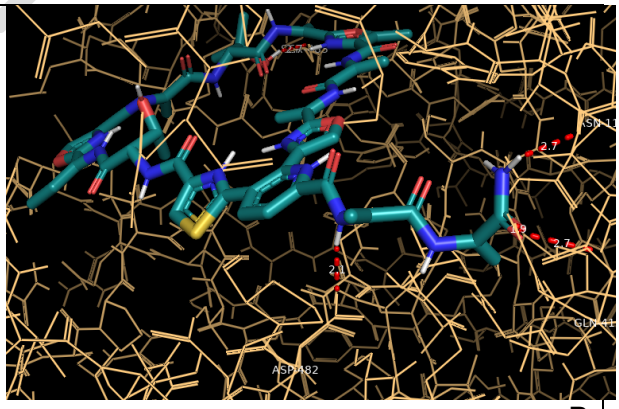

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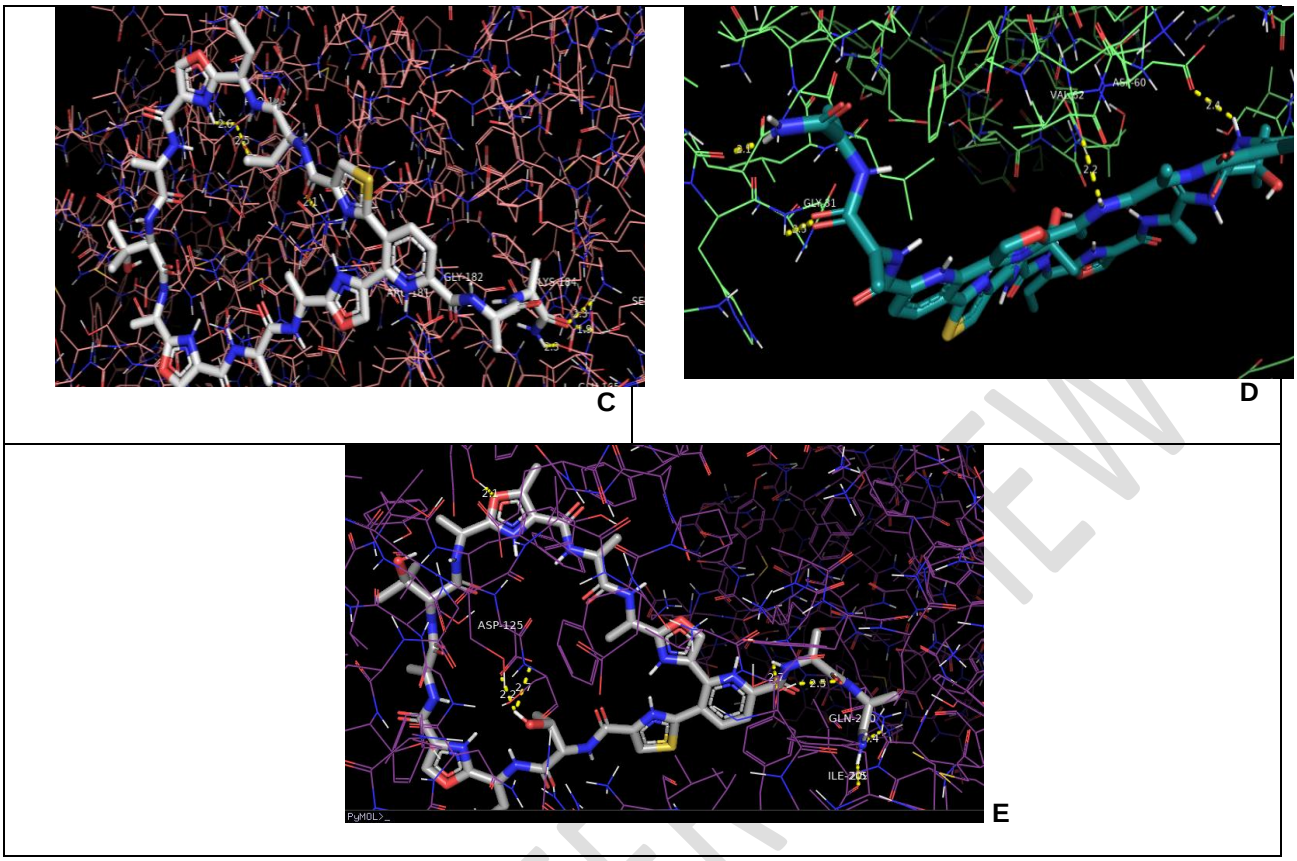

Fig 2: Docking poses of Geninthiocin with protein targets (A) Interaction of Geninthiocin with a Dihydrolipoamide dehydrogenase protein (1DXL), (B) Interactions between Geninthiocin and 3QLB, (C) Interactions between Geninthiocin and 3ZDS, (D) Interactions between Geninthiocin and 4IEN, (E) Interactions between Geninthiocin and 4YMX

Table 1. AutoDock score and hydrogen bond interaction of Geninthiocin against target proteins

PDB ID	Docking Score (Kcal/mol)	Number of H bond 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

3.2 Molecular dynamics Simulation Studies:

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 3).

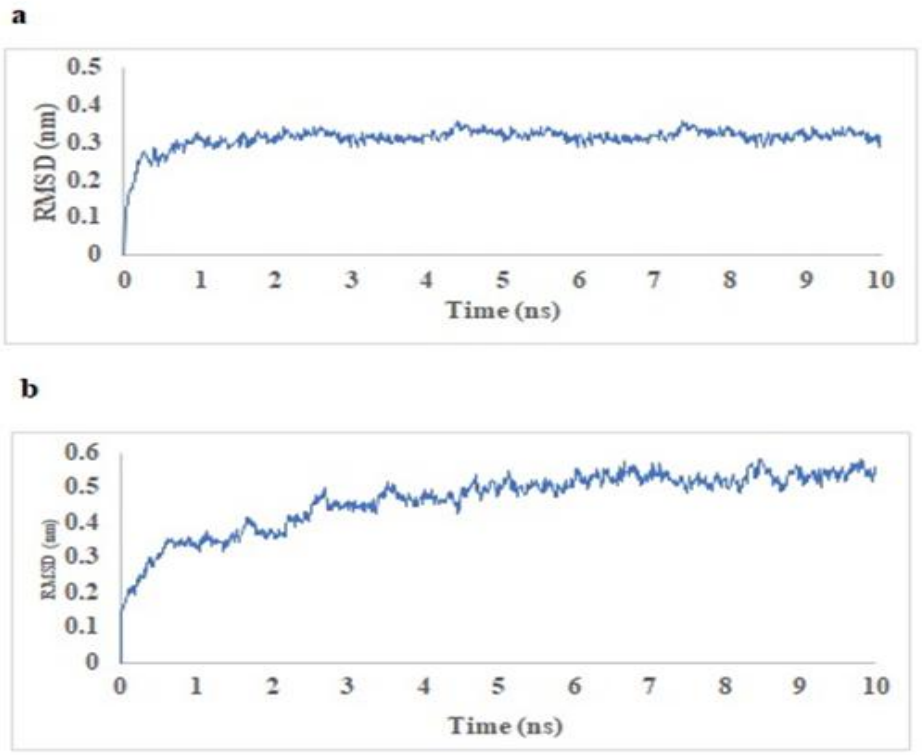

Fig 3. Root mean square deviation (RMSD) studies of ligand with two protein targets (a) PDB ID : 3ZDS ; (b) PDB ID : 4YMX

However, protein complexes 4YMX exhibited lesser stability respectively.
RMSD was calculated 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 [15].

Root Mean Square Fluctuation (RMSF):
The root mean square fluctuation (RMSF) was evaluated to identify the average fluctuation of all residues during simulation. To appreciate the continuation and advancement in dynamic stability of protein complex after binding of ligands, the RMSF of the residues is inspected and plotted as a

function of residue number. Higher the RMSF value, higher the flexibility of the protein ligand complex and vice versa. RMSF is given as:

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

Where, R_k refers to the position vector of atom k [16]. 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 4).

Radius of gyration:

The compactness of 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.

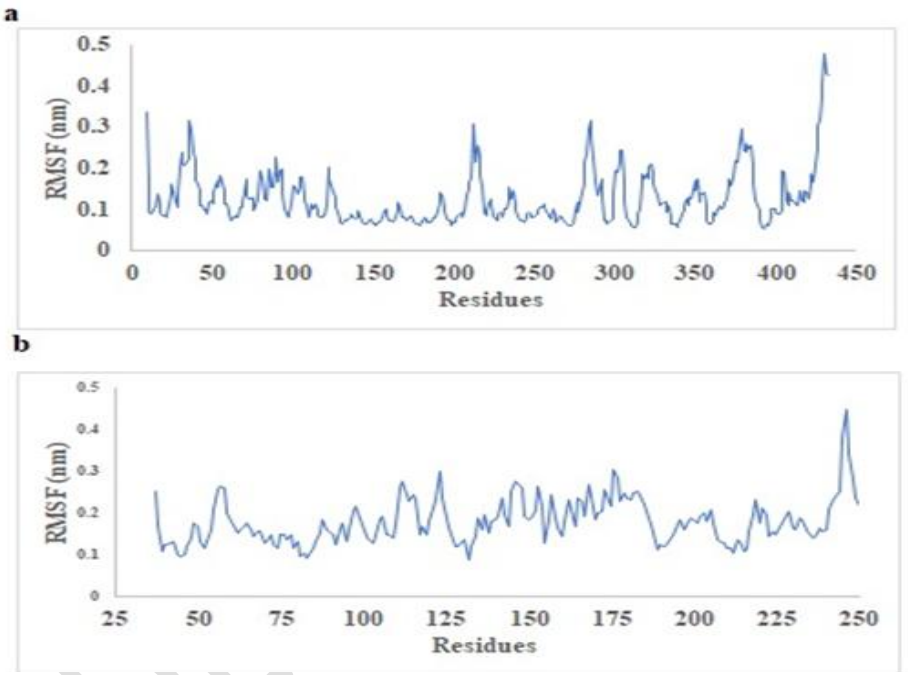

Fig 4. Root mean square fluctuation (RMSF) studies of ligand with three protein targets (a) PDB ID : 3ZDS ; (b) PDB ID : 4YMX

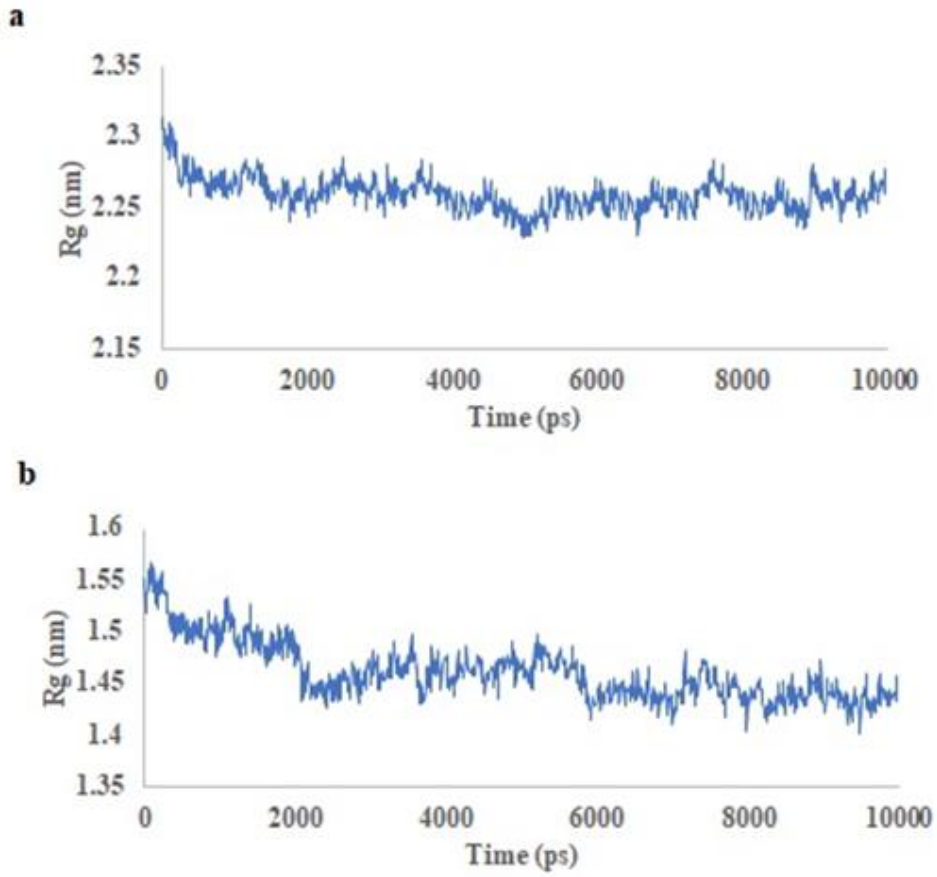

Fig.5. Radius of gyration (Rg) studies of ligand with three protein targets (a) PDB ID : 3ZDS ; (b) PDB ID : 4YMX

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 5). whereas the ligand with the protein 4YMX exhibited higher fluctuations

4. CONCLUSION:

Geninthiocin exhibited highest binding affinity score of -12.1 (kcal/mol) towards the homogentisate 1,2-dioxygenase and hydrogen bond interaction with seven aminoacid residues. Moreover the RMSD, RMSF and Rg analysis indicted that the ligand binding was more stable with the protein homogentisate 1,2-dioxygenase (PDB ID : 3ZDS) as 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research 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.

5. REFERENCES:

[1]. Mehta Y, Hegde A, Pande R, Zirpe KG, Gupta V, Ahdal J, Qamra A, Motlekar S, Jain R. Methicillin-resistant *Staphylococcus aureus* in Intensive Care Unit Setting of India: A Review of Clinical Burden, Patterns of Prevalence, Preventive Measures, and Future Strategies. Indian J Crit Care Med. 2020 Jan;24(1):55-62. doi: 10.5005/jp-journals-10071-23337. PMID: 32148350; PMCID: PMC7050173.

[2]. Kurosu M, Siricilla S, Mitachi K. Advances in MRSA drug discovery: where are we and where do we need to be? Expert Opin Drug Discov. 2013 Sep;8(9):1095-116. doi: 10.1517/17460441.2013.807246. Epub 2013 Jul 6. PMID: 23829425; PMCID: PMC3750092.

[3]. Li S, Hu X, Li L, Liu H, Yu L, You X, Jiang B, Wu L. Geninthiocins C and D from Streptomyces as 35-membered macrocyclic thiopeptides with modified tail moiety. J Antibiot (Tokyo). 2019 Feb;72(2):106-110. doi: 10.1038/s41429-018-0127-y. Epub 2018 Nov 27. PMID: 30479394.

[4]. Schneider O, Simic N, Aachmann FL, Rückert C, Kristiansen KA, Kalinowski J, Jiang Y, Wang L, Jiang CL, Lale R, Zotchev SB. Genome Mining of *Streptomyces* sp. YIM 130001 Isolated From Lichen Affords New Thiopeptide Antibiotic. Front Microbiol. 2018 Dec 19;9:3139. doi: 10.3389/fmicb.2018.03139. PMID: 30619207; PMCID: PMC6306032.

[5]. Chen, S., Jiang, H., Cao, Y. *et al.* Drug target identification using network analysis: Taking active components in *Sini* decoction as an example. *Sci Rep* **6,** 24245 (2016). https://doi.org/10.1038/srep24245

[6] Sun, H., Shen, Y., Luo, G. *et al.* An integrated strategy for identifying new targets and inferring the mechanism of action: taking rhein as an example. *BMC Bioinformatics* **19,** 315 (2018). https://doi.org/10.1186/s12859-018-2346-4

[7] Zhou W, Wang Y, Lu A, Zhang G. Systems Pharmacology in Small Molecular Drug Discovery. Int J Mol Sci. 2016 Feb 18;17(2):246. doi: 10.3390/ijms17020246. PMID: 26901192; PMCID: PMC4783977.

[8] Hu, S., Zhang, C., Chen, P. *et al.* Predicting drug-target interactions from drug structure and protein sequence using novel convolutional neural networks. *BMC Bioinformatics* **20,** 689 (2019). https://doi.org/10.1186/s12859-019-3263-x

[9] C S, S DK, Ragunathan V, Tiwari P, A S, P BD. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main-protease. J Biomol Struct Dyn. 2020 Sep 8:1-27. doi: 10.1080/07391102.2020.1815584. Epub ahead of print. PMID: 32897178; PMCID: PMC7573242.

[10]. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry 2010;31(2):455-61.

[11]. Pathak RK, Gupta A, Shukla R, Baunthiyal M. Identification of new drug-like compounds from millets as Xanthine oxidoreductase inhibitors for treatment of Hyperuricemia: A molecular docking and simulation study. Computational Biology and Chemistry 2018;76:32-41.

[12]. Berendsen, H. J., van der Spoel, D., & van Drunen, R. (1995). GROMACS: a message-passing parallel molecular dynamics implementation. Computer physics communications, 91(1-3), 43-56.

[13]. Van Aalten, D. M., Bywater, R., Findlay, J. B., Hendlich, M., Hooft, R. W., & Vriend, G. (1996). PRODRG, a program for generating molecular topologies and unique molecular descriptors from coordinates of small molecules. Journal of computer-aided molecular design, 10(3), 255-262.

[14]. Darden, T., York, D., & Pedersen, L. (1993). Particle mesh Ewald: An N· log (N) method for Ewald sums in large systems. The Journal of chemical physics, 98(12), 10089-10092.

[15] Sargsyan K, Grauffel Cd, Lim C. How molecular size impacts RMSD applications in molecular dynamics simulations. Journal of chemical theory and computation 2017;13(4):1518-24

[16]. Lugsanangarm K, Nueangaudom A, Pianwanit S, Kokpol S, Nunthaboot N, Tanaka F, et al. D ynamics of the protein structure of T169S pyranose 2-oxidase in solution: M olecular dynamics simulation. Proteins: Structure, Function, and Bioinformatics 2017;85(10):1913-24.