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Multi-Target Molecular Docking Study of a Chloromethylated Chalcone Against Bacterial Enzymes

Dr. Kishor G. Huge*[1], Satish Y. Mane[2]

*[1]Assistant Professor, Department of Chemistry K.K.M College Manwat, Dist.: Parbhani, Maharashtra, India.
[2]Assistant Professor, Department of Chemistry, Shivneri Mahavidyalaya, Shirur (A), Dist.: Latur, 413544, MS India.

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ABSTRACT

The emergence of multidrug-resistant bacterial strains necessitates the development of new antibacterial agents targeting multiple essential pathways. In the present study, a chloromethylated chalcone, (2E)-1-[3-(chloromethyl) phenyl]-3-(4-methylphenyl) prop-2-en-1-one, was evaluated through a multi-target molecular docking approach using the Schrödinger Glide platform. The three essential bacterial enzymes -DNA Gyrase B (PDB ID: 1KZN), Dihydrofolate Reductase (PDB ID: 3SRW), and Penicillin-Binding Protein 3 (PDB ID: 6I1E) were selected to represent DNA replication, folate metabolism, and cell wall biosynthesis pathways, respectively. The docking studies were performed using Glide Extra Precision (XP) modes. The ligand exhibited favorable Glide scores and stable interactions within the possible active sites of all three proteins, forming hydrogen bonds and hydrophobic interactions comparable to standard antibiotics. The results suggest that the investigated chalcone scaffold possesses promising multi-pathway antibacterial potential and warrants further in vitro and in vivo validation.

Keywords: Chalcone, Molecular docking, Antibacterial, Bacterial enzymes, Proteins.

INTRODUCTION:

The rapid spread of antimicrobial resistance has become a global health concern [1]. The conventional antibiotics typically act on a single molecular target, which facilitates resistance development. Hence, the multi-target inhibition strategies are gaining increasing importance in antibacterial drug discovery. Chalcone derivatives, characterized by an α, β-unsaturated carbonyl system, have demonstrated diverse biological activities including antibacterial, anti-inflammatory, and anticancer properties [2]. Their planar aromatic scaffold enables hydrophobic and π–π stacking interactions within enzyme active sites. Structural modification through halogen and alkyl substitution further enhances membrane permeability and target affinity [3]. Chalcones exhibited a great potential of biological activities, specially chalcone having a large number of antibacterial activities [4]. The presence of dual functionality in the chalcone it provides a good platform for the different kinds of reactions. The hydrophobic characters of chalcone also increase antibacterial activity of drug molecule. The cyclisation reaction of chalcone enhance the synthetic and medicinal utility by forming the heterocyclic compounds like pyrazole, isoxazole, pyrimidine Thiopyrimidines, quinazoilin-2,4-diones, azepines etc. [5]. The substitution on the phenyl ring of chalcone interestingly enhance the antibacterial activities [6]. The unsubstituted planar chalcones itself act as biological target [7].

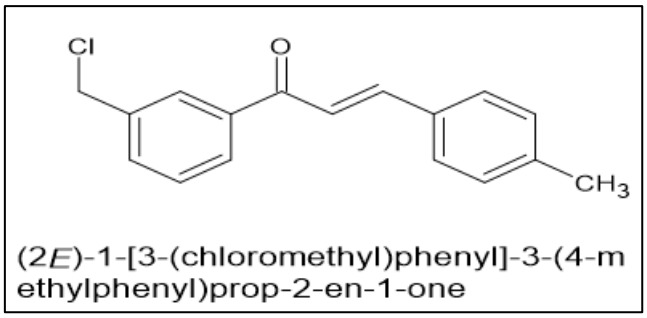

Fig 1: Structure of Experimental Ligand Chalcone

In this study, a substituted chalcone derivative containing chloromethyl and methyl functionalities was evaluated using a structure-based multi-target molecular docking strategy against three essential bacterial enzymes representing distinct biochemical pathways.

Materials and Methods:

All the required protein structure was taken from PDB repository [8]. The structure of ligand chalcone (2E)-1-[3-(chloromethyl) phenyl]-3-(4-methylphenyl) prop-2-en-1-one was prepared in the ACD Lab software and further converted in the sdf form in the BIOVIA Discovery studio 2024 Software. The PDB protein binding analysis including solvent Accessibility, hydrophobicity, Hydrogen bond interaction, Ionizability was studied in discovery studio Visualizer [9].

The following stepwise protocol is followed for the experiment.

A) Ligand Preparation:

The 2D structure of (2E)-1-[3-(chloromethyl) phenyl]-3-(4-methylphenyl) prop-2-en-1-one was constructed using Maestro 12.5 (Schrödinger Release 2024-1). Lig Prep was employed to generate optimized 3D structures using the OPLS4 force field. Ionization states generated at pH 7.0 ± 2.0 using Epik. Energy minimization was performed with default parameters.

B) Protein Selection and Preparation

a) The crystal structures of different resolutions of proteins for experimental purpose were downloaded from the Protein Data Bank.

- DNA Gyrase B (PDB ID: 1KZN; resolution 2.30 Å)
- Dihydrofolate Reductase (PDB ID: 3SRW; resolution 1.70 Å)
- Penicillin-Binding Protein 3 (PDB ID: 6I1E; resolution 1.64 Å)

b) Protein Preparation Wizard was used for:

- Assigning bond orders
- Adding hydrogens
- Removing the crystallographic water molecules beyond 5 Å
- Optimizing the hydrogen-bonding networks
- Perform restrained minimization (RMSD cutoff 0.3 Å)

C) Receptor Grid Generation

Receptor grids were generated at the centroid of co-crystallized ligands.

Table 1: Selected protein PDB code and Ligand information

Sr. No	Protein	Selected experimental ligand	Reference Ligand
1	1KZN	substituted chalcone (2E)-1-[3-(chloromethyl) phenyl]-3-(4-methylphenyl) prop-2-en-1-one	Clorobiocin
2	3SRW		7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline-2,4-diamine
3	6I1E		Amoxicillin

D) Docking Protocol:

The docking was performed using Glide in both Standard Precision (SP) and Extra Precision (XP) modes. Flexible ligand sampling was enabled ,10 poses per ligand were generated, and the best-ranked pose based on Glide Score was selected for interaction analysis. Docking validation was carried out by redocking the native co-crystallized ligand, ensuring RMSD values ≤ 2.0 Å.

Result:

The output of the experiment is given below.

Table 2: Docking Score of selected ligands with different protein target

Sr No	Target	Glide XP Score (kcal/mol)	Docking Score	Characteristics Interactions
1	DNA Gyrase B	−5.518	-5.518	Strong π–alkyl interactions Val42, Val167 and Pro79, water-mediated hydrogen bond through Asp 73
2	DHFR	−7.674	-7.674	H-Bond with Thr47, π–alkyl, π– π with Phe93
3	PBP3	−4.733	-4.733	Thr487Conventional H-bond, Val333 Alkyl, π-alkyl, Lys348 Alkyl contact

Table 3: 1KZN Residual Interaction with ligand

Sr No	Residue	Van der Waal interaction	Coulombic interaction	Nature	Distance	Type of interaction
1.	Val 167	-----	------	Hydrophobic	--------	Alkyl
2.	THR 165	-4.100	-0.160	Polar	3.115	Van der Waals
3.	PRO 79	-0.309	0.071	Hydrophobic	7.461	π-alkyl
4.	llE 78	-----	-----	Hydrophobic	-------	Alkyl
5.	ASP 73	-0.011	0.872	Acidic	11.138	Water mediated H-bond
6.	VAL 71	-0.011	0.027	Hydrophobic	9.956	π-alkyl
7.	ALA 47	-2.85	0.022	Hydrophobic	6.826	Van der Waals
8.	VAL 43	----------	--------	Hydrophobic	-------	Alkyl / π-alkyl

Table 4: 3SRW Residual Interaction with ligand

Sr No	Residue	Key interactions	Van der Waal Interaction	Coulombic Interaction	Hydrogen Bond	Distance
1	ALA 8	Conventional H-bond	-2.137	-0.303	-1	2.022
2	ILE 15	Hydrophobic	--------	--------	0	--------
3	LEU 21	π-alkyl	-3.205	-0.045	0	2.24
4	THR 47	H-bond			0	
5	PHE 93	π-alkyl / π–π	-3.19	-0.623	0	2.281

Table 5: 6I1E Residual Interaction with ligand

Sr No	Residue	Key Interactions	Van der Waal Interaction	Coulombic Interaction	Hydrogen Bond	Distance
1	VAL 333	Alkyl / π-alkyl	-3.707	-1.061	0	2.939
2	LYS 348	Alkyl contact	-2.354	-0.015	0	3.366
3	THR 487	Conventional H-bond	-4.819	-0.72	-0.247	2.401
4	PHE 533	π–π T-shaped	-3.707	-1.061	0	2.939

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Interaction Analysis: The ligand demonstrated favorable binding affinity across all three targets.

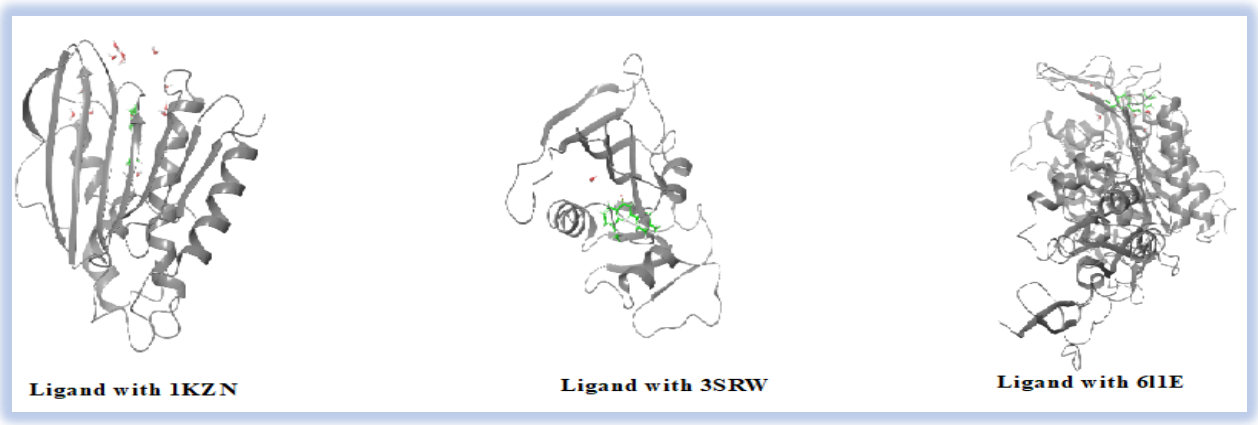

Fig 2: Cartoon model of proteins with docked ligand

Pocket Analysis of Chalcone Binding with DNA Gyrase 3D & 2D Interaction:

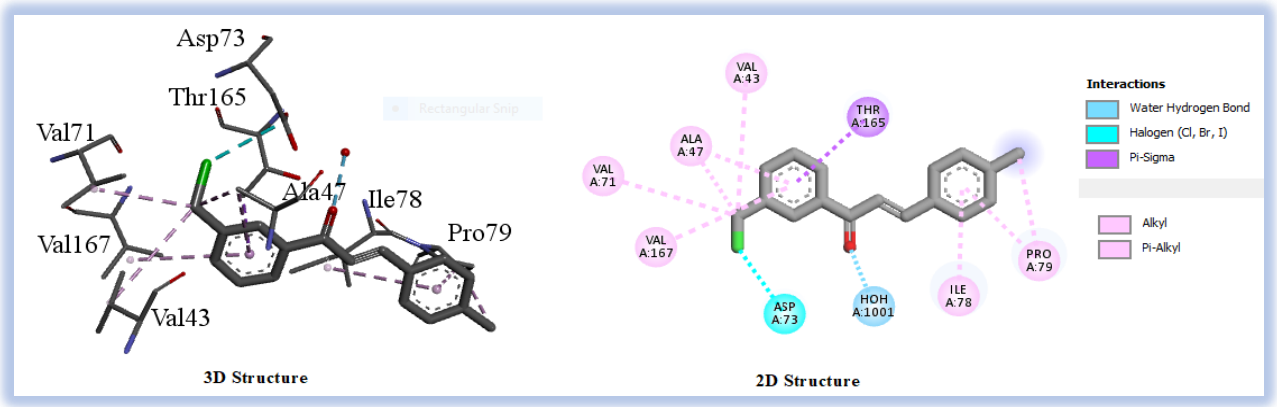

Fig 3: Binding pockets of protein 1KZN with Ligand

The docking figure shows ligand chalcone occupying a well-defined hydrophobic pocket of DNA gyrase. The binding cavity appears compact and primarily nonpolar, with limited but significant polar stabilization. The ligand is positioned inside a narrow cleft surrounded mainly by hydrophobic amino acid residues. The pocket is characteristic of the ATP-binding or adjacent allosteric region of DNA gyrase, commonly targeted by antibacterial agents.

Hydrophobic interaction shows Strong π–alkyl interactions between chalcone aromatic rings and Val 42, Val 167 and Pro 79 in which Alkyl interactions stabilize the phenyl rings within the lipophilic pocket, these interactions are crucial for antibacterial DNA gyrase inhibitors and This suggests hydrophobic stabilization is the primary driving force.

A water-mediated hydrogen bond is observed through Asp 73 with carbonyl oxygen of chalcone which helps the ligand orientation [10]. The π-sigma interaction observes between aromatic ring and nearby hydrophobic residue Val 42 enhance binding stability through orbital overlaps. The planar structure of chalcone fits well into elongated hydrophobic channel which indicates good steric complementarity [11].

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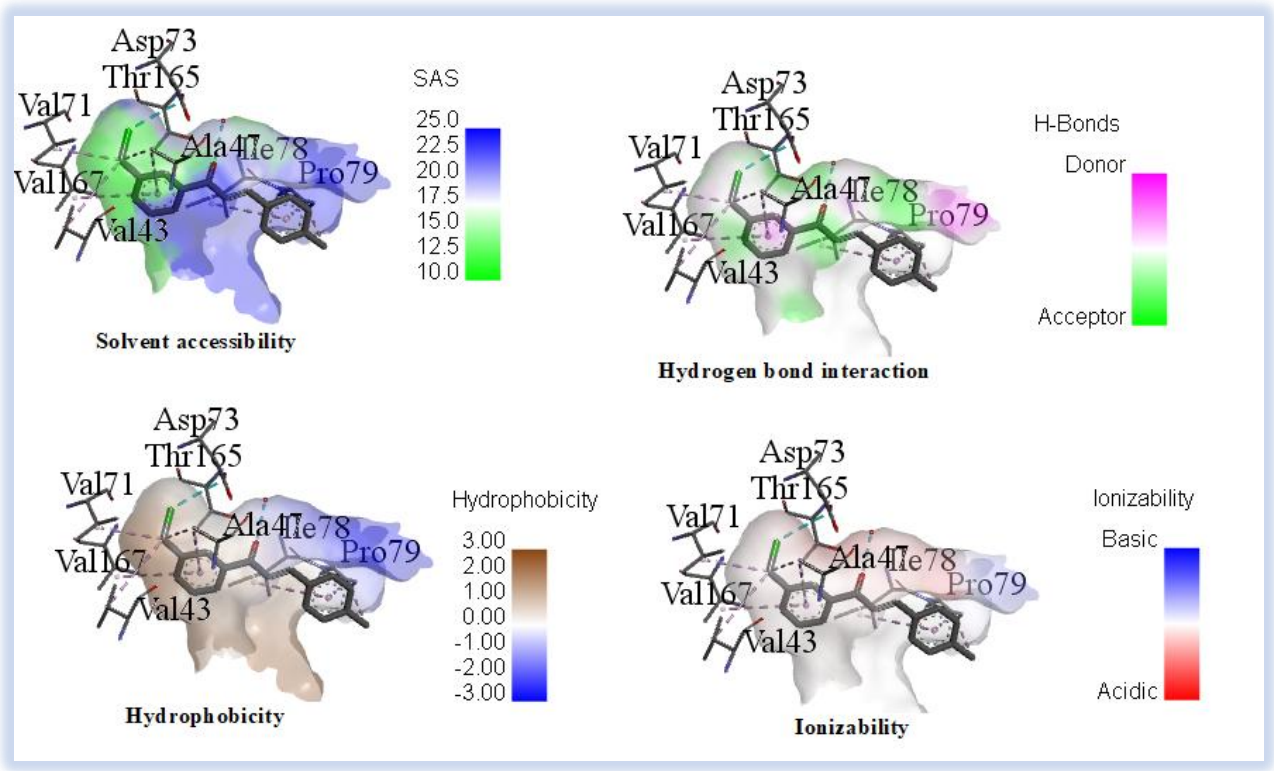

Fig 4: Various interaction of Ligand with protein1KZN

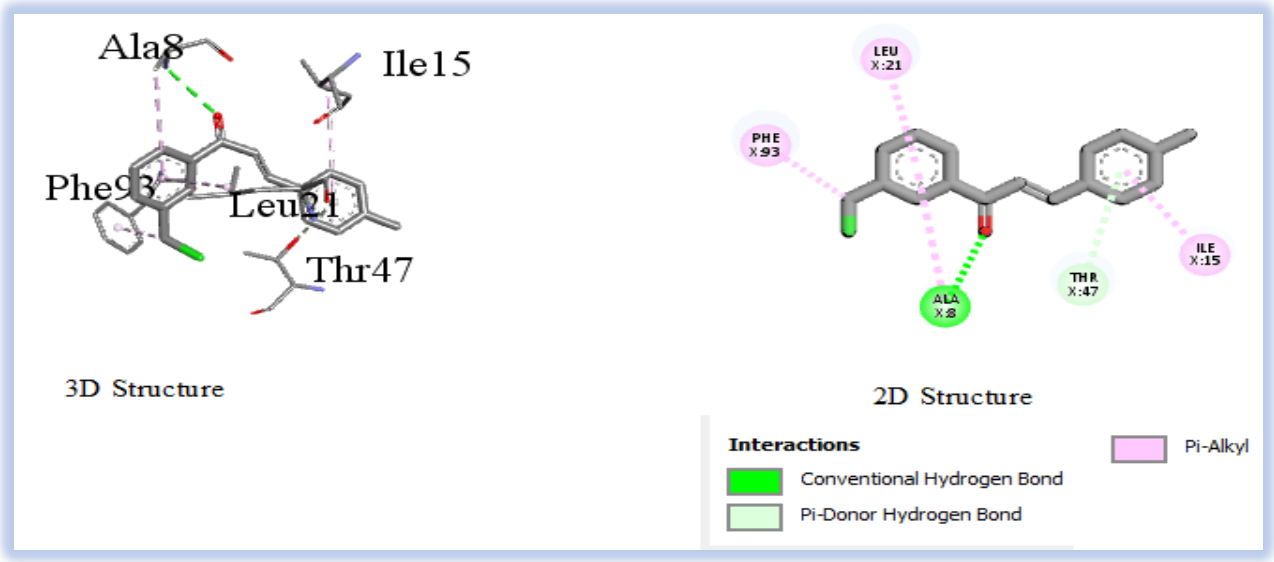

Fig 5: Binding pockets of protein 3SRW with Ligand

The pocket is dominated by residue Leucine, Isoleucine and Phenylalanine which represent the preference for aromatic rings, Suitability for lipophilic substituents and good compatibility with chalcone and aryl systems. The ligand chalcone is located within a hydrophobic cleft formed primarily by aliphatic and aromatic residues. The pocket appears Moderately deep, predominantly hydrophobic, stabilized by both hydrogen bonding and π-interactions and hence suitable for aromatic planar scaffolds chalcone derivatives [12]. The residue Ala8 makes a conventional hydrogen bond showing green dashed line in 2D structure which Contributes to ligand anchoring at one terminus. The Thr47 Participates in hydrogen bonding, The hydroxyl (-OH) group of Thr47 acts as H-bond donor or acceptor contribute for directional stabilization. Hydrophobic interaction of Phe93 forms π–π or π–alkyl interactions

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with aromatic ring which Enhances hydrophobic stabilization and suggests aromatic stacking compatibility. Leu 21 Engages in π-alkyl interaction to contributes to hydrophobic enclosure. The residue lle15 provides additional hydrophobic contacts to stabilizes non-polar region of ligand. Therefore, ligand chalcone demonstrates a stable and energetically favorable binding mode within the ATP-binding site of DNA gyrase B. The combination of hinge-region hydrogen bonding and hydrophobic pocket occupancy supports its potential as a promising ATP-competitive inhibitor.

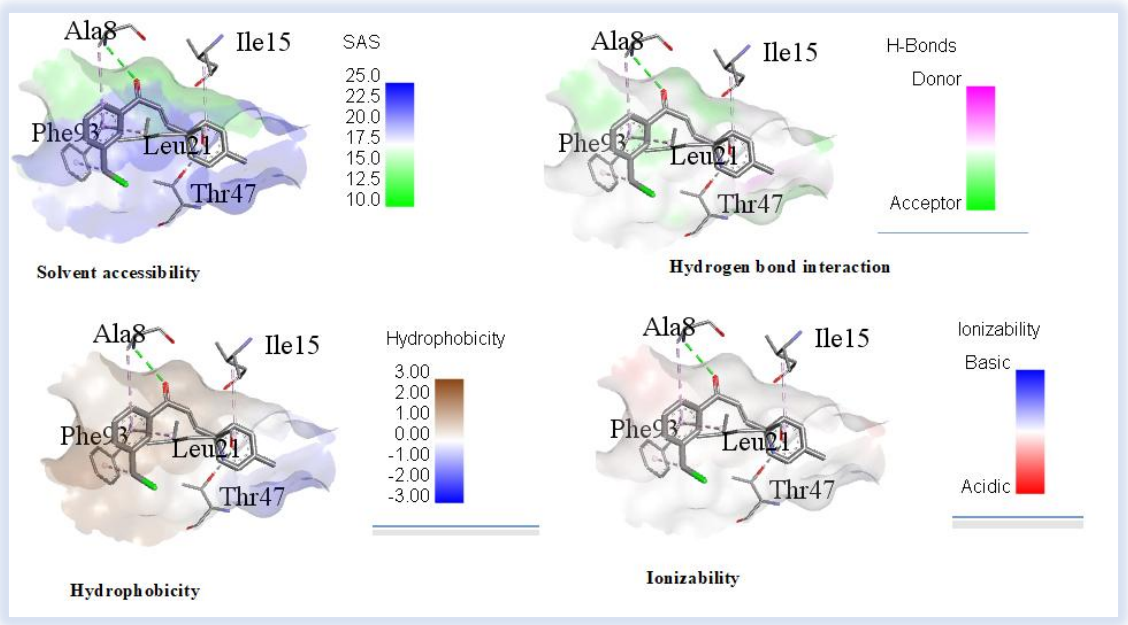

Fig 6: Various interaction of Ligand with protein 3SRW

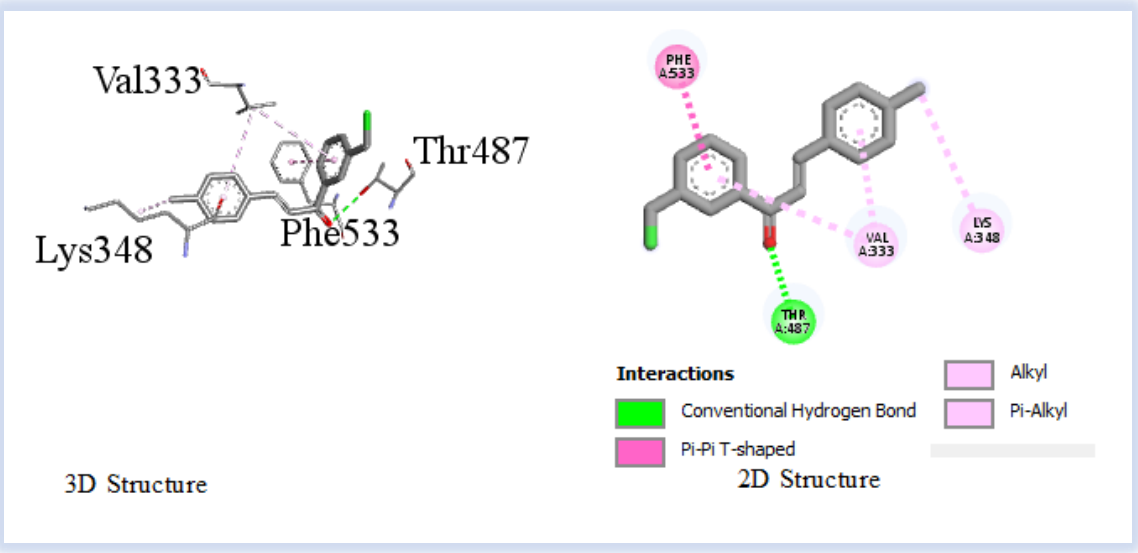

Fig 7: Binding pockets of protein 6I1E with Ligand

The residue Thr487 shows hydrogen bonding by forming conventional hydrogen bond in fig showing by green dashed line. The Thr487 Serves as a critical anchoring residue and Controls ligand orientation within the cavity provides binding specificity and directional stabilization. Phe533 shows aromatic and hydrophobic interactions by engaging in π–π T-shaped interaction with the ligand aromatic ring which Provides strong aromatic stacking stabilization and plays a major role in binding affinity. Val333 Contributes hydrophobic alkyl, π-alkyl Stabilizes non-polar region of ligand. Lys348 participates in alkyl or hydrophobic contact it May provide electrostatic environment modulation although positively charged, interaction appears predominantly hydrophobic.

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Interaction in the binding pocket of 6I1E appears to consist of an aromatic-rich hydrophobic core supported by a strategically positioned polar residue that provides directional hydrogen bonding. Such an arrangement favors ligands possessing rigid aromatic frameworks with appropriately placed hydrogen bond acceptor or donor functionalities [13]. The dominance of π-interactions suggests that electronic properties of the aromatic rings may significantly influence binding affinity.

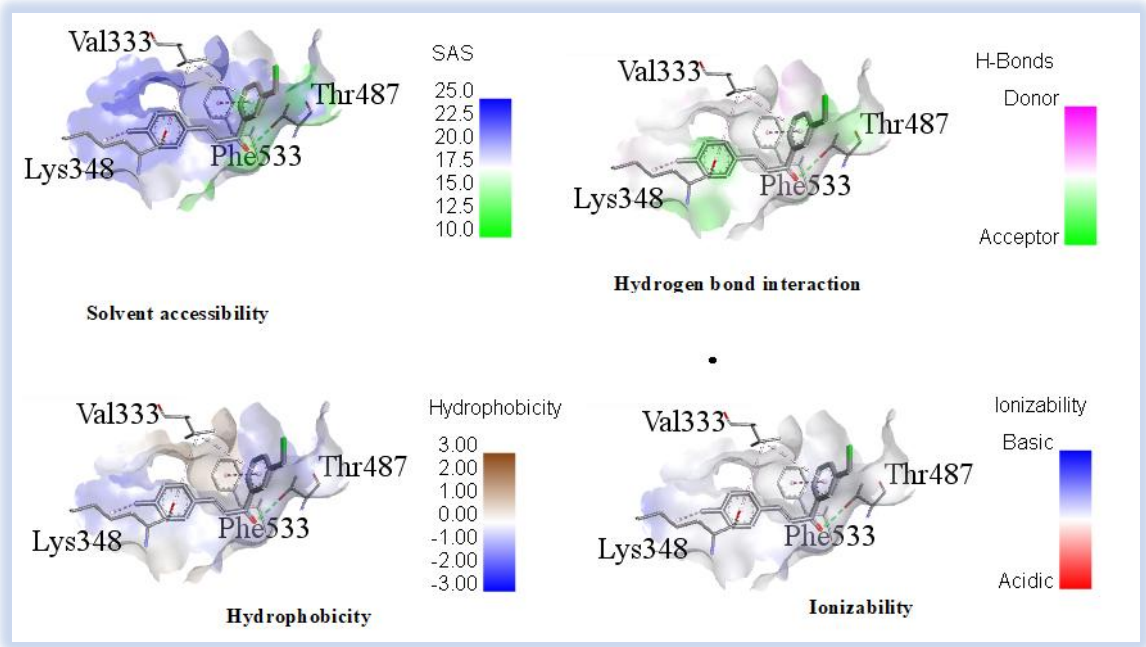

Fig 8: Various interaction of Ligand with protein 6I1E

The docking results suggest that the investigated chalcone derivative can interact favorably with three independent antibacterial targets. The α, β-unsaturated carbonyl moiety facilitates hydrogen bonding, while substituted aromatic rings promote hydrophobic stabilization.

The multi-target binding profile indicates potential broad-spectrum antibacterial activity. Notably, the strongest affinity toward DHFR suggests possible inhibition of folate metabolism [14]. The chalcone my serv as a potential antimicrobial target in the future [15]. Compared with standard antibiotics, the binding scores were within an acceptable range, supporting further experimental validation.

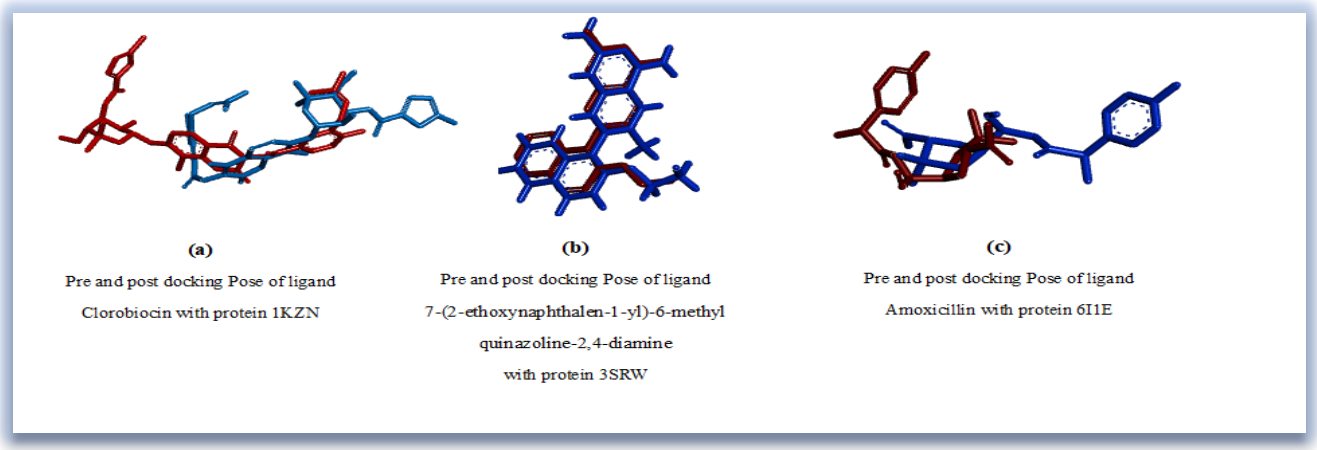

Fig 9: Pre and post docking poses of reference ligand with proteins

Discussion:

The docking results suggest that the investigated chalcone derivative can interact favorably with three independent antibacterial targets. The α, β-unsaturated carbonyl moiety facilitates hydrogen bonding, while substituted aromatic rings promote hydrophobic stabilization. The multi-target binding profile indicates potential broad-spectrum antibacterial activity. Notably, the strongest affinity toward DHFR suggests possible inhibition of folate metabolism. Compared with standard antibiotics, the binding scores were within an acceptable range, supporting further experimental validation.

Conclusion:

The present multi-target molecular docking study demonstrates that the substituted chalcone derivative exhibits promising binding affinity toward essential bacterial enzymes involved in DNA replication, folate metabolism, and cell wall biosynthesis. These findings support the potential of chalcone scaffolds as multi-pathway antibacterial agents and provide a basis for further in vitro antibacterial screening and lead optimization.

Conflict of Interest: Author has no any conflict of interest.

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