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In vitro antibacterial and in silico docking studies of two Schiff bases on *Staphylococcus aureus* and its target proteins

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Abstract

Background: Schiff base compounds have extensive applications in various fields such as analytical, inorganic, organic, and biological fields. They have excellent pharmacology application prospects in the modern era and are widely used in the pharmaceutical industry. In the present work in vitro antibacterial and in silico docking studies of two Schiff base compounds 2,2′-(5,5-dimethylcyclohexane-1,3-diylidene)bis(azan-1-yl-1-ylidene)diphenol (DmChDp) and N,N′-(5,5-dimethylcyclohexane-1,3-diylidene)dianiline (DmChDa) were carried out against the bacterial strain *Staphylococcus aureus* and its target proteins.

Results: The tests proved that the ligands have potential antibacterial activity. In the computational analysis, the drug-like properties of the compounds were first pre-filtered using the Lipinski rule of five. Then, molecular docking study was conducted using the AutoDock 4.2 program, to establish the mechanism by which the molecules inhibit the growth of *S. aureus*. For this purpose, 6 different target proteins (PDB ID: 1T2P, 3U2D, 2W9S, 1N67, 2ZCO, and 4H8E) of *S. aureus* were selected. Both the Schiff bases showed a good binding affinity with the target protein dihydrofolate reductase enzyme (PDB ID: 2W9S) but in different sites. Maximum binding energies of about − 10.3 and − 10.2 kcal/mol were observed when DmChDp and DmChDa were docked with 2W9S.

Conclusion: Schiff base compounds DmChDp and DmChDa have appreciable growth-inhibitory power against *S. aureus*, which can be attributed to the deactivation of the enzyme, dihydrofolate reductase.

Keywords: Schiff bases, Antibacterial study, Molecular docking

Background

The research in the field of therapeutics is of great importance for the improvement of the quality of human life and for reducing human diseases. A vast number of diseases is due to pathogenic organisms. Pathogens are microorganisms that are harmful to the human body. Bacteria, viruses, fungus, prion, protozoan, viroid, etc. are the different types of pathogens. Microbial infections are drastically increased in living beings due to multi-drug-resistant microorganisms even though the human body can defend against potential pathogens [1–3].

S. aureus is one of such multi-drug-resistant microorganisms. It is a round-shaped bacterium that is a member of Firmicutes [4]. The cell wall of this species is amorphous and tough. The major component of the cell wall is the peptidoglycan (50% of cell wall mass). The other component that contributes 40% of cell wall mass is teichoic acids and the remaining 10% consist of exoproteins, surface proteins, and peptidoglycan hydrolases. Naturally, this bacterium is found in the nasopharynx of the human body and on the skin. *S. aureus* can cause infections of the nose, skin, vagina, urethra, and gastrointestinal tract [5, 6]. They

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are non-sporing, non-motile, and few strains are encapsulated. Nearly 50% of the human population are carriers of *S. aureus*. Generally, beta (β)-lactum antibiotics such as Penicillin, Carbapenems, Monobactams, and Cephalosporins are used to heal infections caused by *Staphylococcus aureus*. These drugs bind with proteins in the bacteria and thereby inhibit the bacterial cell wall synthesis [7]. But several studies showed that beta (β)-lactum antibiotics have a low binding affinity with Penicillin-binding proteins and are a major cause for less antibiotic activity [8–10]. Even though a large number of antibiotics and chemotherapeutic agents such as linezolid and dalfopristin are available to resist such microorganisms, they are costly and are used only for patients having highly resistant strains [11]. Thus, the development of efficient and novel chemotherapeutic agents in the medical field is of great importance.

Schiff bases are generally prepared by the condensation of carbonyl compounds (aldehyde/ketones) with aromatic or aliphatic primary amines [12]. It is regarded as a nitrogen analogue of an aldehyde or ketone where the imine group substitutes the C=O group. Therefore, it is also known as imine or azomethine. The lone pair of electrons in the sp^2 hybrid orbital of N atom in –C=NH- linkage present in Schiff bases enhances their biological and chemical importance [13, 14]. They have widespread use as therapeutic agents and antibacterial agents [15–17]. The azomethine group can be seen in drugs like Thiacetazone 5 and Nifuroxazide (INN) 4 available in the market [18].

In the present study, the antibacterial activity of the Schiff bases 2,2'-(5,5-dimethylcyclohexane-1,3-diylidene)bis(azan-1-yl-1-ylidene)diphenol (DmChDp) and N, N'-(5,5-dimethylcyclohexane-1,3-diylidene)dianiline (DmChDa) were analysed using disc diffusion method against the pathogenic bacteria *S. aureus* (Fig. 1). Apart from this, a molecular docking study was also carried out using the AutoDock 4.2 program to predict the best-fit orientation of the drug molecule that binds to a specified protein target of interest to find out the activity and affinity of the drug molecule. This will enable to establish the mechanism by which the Schiff base prevents bacterial growth. Sortase-A (1T2P), DNA gyrase (3U2D), dihydrofolate reductase (DHFR) (2W9S), clumping factor A (ClfA) (1N67) dehydrosqualene synthase (CrtM) (2ZCO), and undecaprenyl diphosphate synthase (UPPS) (4H8E) are the target proteins of *S. aureus* considered for the study. The two sites of the target 4H8E and four sites of all other targets were selected for docking.

Methods

Analar grade samples were used for the synthesis of Schiff bases. 5, 5-dimethylcyclohexanone, 2-aminophenol, and aniline, were purchased from E. Merck. The percentage of elements such as carbon, hydrogen, and nitrogen were analysed by microanalysis using Elementar make Vario EL III model CHNS analyser. KBr disc technique on a Shimadzu model FT-IR Spectrometer (Model IR affinity) were used for recording IR spectra in the region 4000–400 cm^{-1}. Shimadzu UV-Visible-1800 Spectrophotometer was used for recording electronic spectra in DMSO. BRUKER AVANCE III HD was used for ^1H NMR and ^{13}C NMR studies in dmso-d_6. Mass spectra were recorded using QP 2010 model Shimadzu GCMS.

Synthesis and characterization of Schiff bases

2,2'-(5,5-dimethylcyclohexane-1,3-diylidene)bis(azan-1-yl-1-ylidene)diphenol (DmChDp)

To a stirred ethanolic solution of 2-aminophenol (0 .02 mol), 5,5-dimethyl-1,3-cyclohexanedione (0.01 mol) dissolved in hot ethanol was added, refluxed for 20 min and cooled. Brown-coloured precipitate formed was filtered, washed with ethanol, and recrystallized. Yield was 80%, and M.P. 180 °C [19].

Anal.calcd for $C_{20}H_{22}N_2O_2$ was C, 74.53; H, 6.83; and N, 8.69%. Found. was C, 73.91; H, 6.74; and N, 8.52%; IR (KBr) was 3240 cm^{-1} (OH), 1600 cm^{-1} (C=N), 1238 cm^{-1} (C-O), 3080 cm^{-1} (aromatic C-H), and 2960 and 2877 cm^{-1} (aliphatic C-H); UV was 22936 cm^{-1} ($n \rightarrow \pi^*$), and 33784 and 39682 cm^{-1} ($\pi \rightarrow \pi^*$); ^1H NMR was δ 0.99 (CH_3), δ 2.35 (CH_2 between two azomethine group), δ 2.02 (CH_2 adjacent to > $C(CH_3)_2$), and δ 6.81–7.07 (aromatic H); ^{13}C NMR was 95.88ppm (C=N), 27.84ppm (CH_3), 41.52ppm (C containing CH_3), 49.78ppm (C between azomethine groups), 32.32ppm (C adjacent to > $C(CH_3)_2$), and 116.28–151.55 ppm (aromatic C); mass was M^+ peak absent, m/z 216 (base peak) $[C_{14}H_{18}NO]^+$, m/z 231 $[C_{14}H_{19}N_2O]^+$, and m/z 178 $[C_{11}H_{16}NO]^+$.

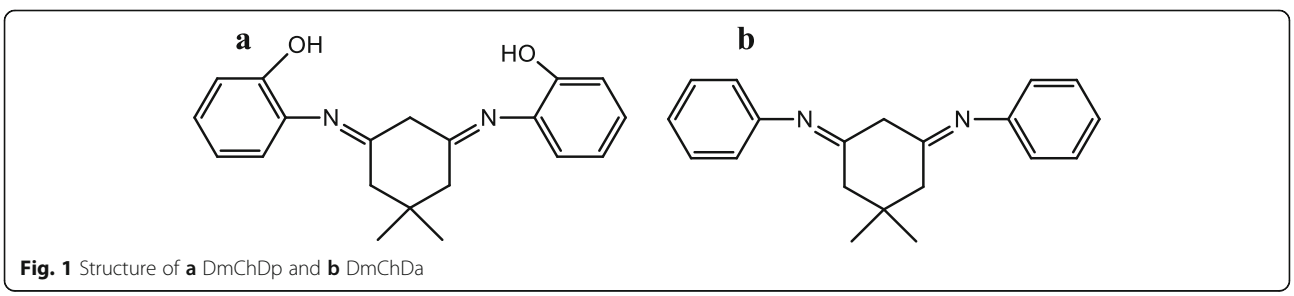

Fig. 1 Structure of **a** DmChDp and **b** DmChDa

N, N′-(5,5-dimethylcyclohexane-1,3-diylidene)dianiline (DmChDa)

0.01 mol of 5,5-dimethyl-1,3-cyclohexanedione was dissolved in hot ethanol and added to a stirred ethanolic solution of 0.02 mol of aniline. Refluxed for 3h and cooled. Yellow precipitate separated was filtered, washed with ethanol, and recrystallized. Yield was 78%, and M.P. 152 °C [20].

Anal.calcd for $C_{20}H_{22}N_2$ was C, 82.7; H, 7.5; and N, 9.6%. Found. was C, 81.3; H, 6.9; and N, 8.9%; IR (KBr) was 1564 cm^{-1} (C=N); 1249 and 3234 cm^{-1} (N-H); 2949, 2879, and 2810 cm^{-1} (CH_3 and CH_2); and 3055 cm^{-1} (aromatic H); UV was 32020 cm^{-1} ($n \rightarrow \pi^*$), and 39463 cm^{-1} ($\pi \rightarrow \pi^*$); ^1H NMR was δ 1.04 (CH_3), δ 1.51 (CH_2 between two azomethine group), and δ 7.26,7.09 (aromatic H); ^{13}C NMR was 28.35 ppm (CH_3); 98.94 ppm (C=N); 32.88, 43.71, and 50.35 ppm (two CH_2); and 123.39–138.14 ppm (aromatic C); mass was M^+ peak absent, m/z 159 (base peak-$[C_{11}H_{13}N]^+$), m/z 215 $[C_{14}H_{20}N_2]^+$, and m/z 198 $[C_{14}H_{18}N]^+$.

In vitro antibacterial studies

Mueller-Hinton agar was used for preparing the medium [21]. Schiff base compounds and the standard antibiotic ampicillin were dissolved in DMSO to prepare the stock solutions. Then, it is diluted to obtain various ranges of concentrations from 100 to 500 μg $disc^{-1}$. Disc diffusion method was adopted for the drug [22]. The petri dishes were incubated in an air ambiance at 35 °C for 24 h. The diameter of the zone of inhibition was measured and compared with zones produced by the reference antibiotic, ampicillin.

Target proteins in *Staphylococcus aureus*

Staphylococcus aureus sortase-A (PDB ID: 1T2P)

Sortases are extracellular transpeptidases of Gram-positive bacteria [23, 24]. The function of the enzyme is to sort proteins into the cell wall compartment of Gram-positive bacteria, hence named Sortases. Sortases have a great role in the cell wall envelope assembly and bacterial pathogenicity.

DNA gyrase (PDB ID: 3U2D)

Topoisomerase is an isomerase enzyme that provokes dramatic change in the topology of DNA structure [25, 26]. Topoisomerase is categorized as topoisomerase I and topoisomerase II, based on the number of strands cut in one phase of action. New topoisomerases, type III and IV have also been discovered recently. DNA gyrase subclass of type II topoisomerase is responsible primarily for DNA replication.

Dihydrofolate reductase (DHFR) (PDB ID: 2W9S)

Dihydrofolate reductase (DHFR) is an enzyme that catalyses the formation of tetrahydrofolate (THF) by the reduction of Dihydrofolate (DHF) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) [27, 28]. Also, it has a great role in the synthesis of thymidylate, purines, methionine, and some other important metabolites. These enzymes are required for cell proliferation. Thus, inhibition of dihydrofolate reductase will results in the destruction of the intracellular tetrahydrofolate pool thereby preventing biosynthesis of RNA, DNA, thymidine, and protein. Due to the wide range of cellular functions, they are targets for anticancer and antimicrobial agents.

Clumping factor A (ClfA) (PDB ID: 1N67)

In blood plasma, there is a glycoprotein called fibrinogen (Fg) which is present at ~ 3 mg/ml concentration and has a significant role in coagulation and haemostasis. Six polypeptide chains are present in fibrinogen such as 2 Aa, 2 Bb, and 2 nd 2 Bb, which are dimeric and symmetrical. The γ-chain has C-terminal residues which are biologically important. In the process of fibrinogen-dependent platelet adherence and aggregation, they interact with platelet integrin aIIb3. This C-terminal residue of γ-chain is also targeted by the pathogenic bacterium *Staphylococcus aureus*, resulting in fibrinogen-dependent cell clumping and tissue adherence. Clumping factor A (ClfA) [29, 30] was the first Fg γ-chain-binding *S. aureus* adhesin identified.

Dehydrosqualene synthase (CrtM) (PDB ID: 2ZCO)

The golden carotenoid pigment staphyloxanthin is a virulence factor for *S. aureus*. Dehydrosqualene synthase [31, 32] is involved in the synthesis of this pigment. The main responsibility of the pigment is to preserve *S. aureus* against oxidative stress as a result of host immune defence by reactive oxygen species and neutrophils by acting as an antioxidant.

Undecaprenyl diphosphate synthase (UPPS) (PDB ID: 4H8E)

The role of undecaprenyl diphosphate synthase (UPPS) in the biosynthesis of the cell wall of *Staphylococcus aureus* is very significant [33, 34]. UPPS is important since it is vital for the formation of peptidoglycan. Also, UPPS is not present in humans and is additional merit for the development of good antibacterial agents.

In silico molecular docking studies

Lipinski rule of five

Lipinski rule envisages that an orally active drug will be small and slightly lipophilic. This rule depicts molecular properties rather than pharmacological activity and

Table 1 Antibacterial activity of the Schiff bases and ampicillin

Compounds	Diameter of zone of inhibition (mm) at different concentrations (μg disc^{-1})			
	50	100	250	500
DmChDp	12	16	22	26
DmChDa	10	16	22	25
Ampicillin	15	21	28	30

states that a drug has good oral activity if it satisfies the five conditions such as the following [35]:

1. Molecular weight < 500
2. Octanol-water partition coefficient log*P* < 5
3. Less than 5 hydrogen bond donors (total number of NH and OH bonds)
4. Less than 10 hydrogen bond acceptors (total number of N and O atoms)
5. Molar refractivity between 40 and 130

Molecular docking

Docking studies were carried out to establish the mechanism by which the Schiff base compounds prevent bacterial growth. Binding affinity and interactions of the Schiff bases with different target proteins in *S. aureus* were derived from docking studies [36–38]. The steps involved in the docking process are as follows.

Preparation of ligands and proteins The structure of the Schiff bases in MOL format was derived using ChemSketch software and converted to PDB format using open babel software. The structures of the proteins were downloaded in PDB format from RCSB PDB. Using Pymol software, water molecules and ligands already present in the proteins were removed; hydrogen atoms were added and saved in PDB format. Six target proteins of *Staphylococcus aureus* were utilized to check the interaction with synthesized Schiff bases.

Prediction of active site Prediction of the active site is important in structure-based drug design. Co-ordinates of binding sites of the proteins were identified using the software BIOVIA Discovery Studio.

Docking Molecular docking calculations were carried out with the aid of the software AutoDock 4.2 and binding energy of the protein—Schiff base adducts were obtained [39].

Visualization of protein-ligand complexes The protein-ligand complexes were visualized using the software BIOVIA Discovery Studio and their 3D and 2D interaction plots were derived. Hydrogen bond interactions such as conventional and non-conventional H bonds, hydrophobic interactions such as amide-pi stacked, pi-pi stacked, pi-sigma, pi-pi T-shaped, alkyl and pi-alkyl interactions, electrostatic interactions such as pi-anion and pi-cation interactions, van der Waals interaction, and unfavourable donor-donor and acceptor-acceptor interactions are commonly seen between protein and ligand. The binding affinity of the compound with the target protein is the resultant of all the interactions and binding energy existing between them.

Results

The antibacterial activity of the Schiff base compounds is shown in Table 1. Results showed that they have appreciable growth-inhibitory power. The compounds were first pre-filtered using Lipinski rule of five to check the drug-like properties. The parameters such as mass, number of hydrogen bond donors, number of hydrogen bond acceptors, log *P* (octanol-water partition coefficient), and molar refractivity of both Schiff bases were evaluated by the rule and are given in Table 2.

In vitro antibacterial study showed that the ligands DmChDp and DmChDa have the potential to act as good antibacterial agents. Now, it is necessary to understand the mechanism by which the compounds inhibit the growth of bacteria. For that, molecular docking studies were conducted, by the aid of which we get an idea about the protein target in bacteria with which the ligands have more binding affinity. The stability of the protein-ligand complex was evaluated on the basis of two essential criteria: (1) the highest binding energy and (2) the number of interactions of the ligand with the active site residues. The highest binding energies (BE) and the number of interactions of the ligands with protein models under study were enlisted in Table 3. A ligand can mainly undergo interactions such as van der Waals,

Table 2 Lipinski rule of five

Parameters	Schiff bases		Condition for drug-like property
	DmChDp	DmChDa	
Mass	322	290	< 500
Hydrogen bond donor	2	0	< 5
Hydrogen bond acceptor	4	2	< 10
log *P*	5.15	5.74	< 5
Molar Refractivity	98.03	94.7	40–130

Table 3 Binding energy and number of interactions of Schiff bases docked with target proteins in *S. aureus*

	Schiff bases		DmChDp			DmChDa		
	Binding energy (BE) (kcal/mol) and other interactions		-BE	H bond	Other	-BE	H bond	Other
Target proteins and active site	1T2P	1	7.6	2	7	8	0	7
		2	7.5	2	5	7.3	0	8
		3	6.3	1	5	5.9	1	6
		4	6.2	1	10	6.1	0	6
	3U2D	1	6.7	1	8	7.2	0	6
		2	6.8	1	5	7.1	0	9
		3	6.8	1	3	7.2	0	7
		4	6.9	0	6	7.1	0	10
	2W9S	1	9.3	1	8	9.1	0	14
		2	**9.9**	**2**	**8**	**10.2**	**1**	**9**
		3	**10.3**	**1**	**14**	**9.5**	**0**	**8**
		4	7	1	7	6.9	0	7
	1N67	1	9	2	9	8.6	1	8
		2	9	2	7	6.6	1	4
		3	9	1	11	8.5	1	9
		4	9	2	7	8.4	1	8
	2ZCO	1	8.3	2	6	8	1	7
		2	7.8	2	6	5.9	0	2
		3	7.6	1	6	7.1	0	6
		4	8.4	3	6	8.1	1	4
	4H8E	1	7.8	3	8	7.5	0	8
		2	6.2	3	4	6.2	0	8

BE- Binding energy in kcal/mol, HB- Conventional hydrogen bond, Other- Other interactions

hydrogen bonding, hydrophobic, and electrostatic while docking into the active site. From literature, it is clear that binding energy has a great role than the number of interactions to predict the best binding mode. Among the interactions, conventional H bond (HB) (more prominent) and hydrophobic interactions are more effective than the others. Table 4 indicates the amino acid residues of different binding pockets interacted with the Schiff base molecules.

Binding energy and number of interactions clearly establish that both the Schiff bases have more binding affinity towards the enzyme dihydrofolate reductase (2W9S). The structure of the protein target dihydrofolate reductase (2W9S) is shown in Fig. 2.

Table 4 Interactions of Schiff bases with amino acid residues of target proteins in *S. aureus*

Schiff base		DmChDp	DmChDa
Active target		2W9S	2W9S
Active site		3	2
Binding energy (kcal/mol)		− 10.3	− 10.2
Interactions with amino acid residue	Van der Waals	ILE14, GLY15, VAL6, LYS45	ILE14
	Hydrogen bond	ALA7 (HB, 2.24 Å), THR46 (NCHB, 3.28 Å)	SER49 (HB, 2.06 Å)
	Hydrophobic	PHE92 (π-T, 5.02 Å), ILE5 (amide-π stack, 3.92 Å), LEU20 (R, 4.36 Å), LEU20 (R, 4.31 Å), ILE50 (R, 4.43 Å), ILE50 (R, 4.51 Å), ALA7 (π-R, 5.17 Å), ILE31 (π-R, 5.18 Å), ILE5 (π-R, 4.95 Å), PHE92 (π-R, 4.27 Å)	ASN18 (amide-π stack, 4.09 Å), LEU20 (R, 4.43 Å), LEU20 (R, 4.48 Å), ILE50 (R, 4.57 Å), ILE31(π-σ, 3.99 Å), ILE5 (π-R, 4.93 Å), ALA7 (π-R, 4.63 Å), PHE92 (π-R, 4.56 Å)

HB conventional H bond, *NCHB* non-conventional H bond, *π-T* π-π T-shaped, *amide-π stack* amide-π stacking, *R* alkyl, *π-R* pi-alkyl, *π-σ* pi-sigma

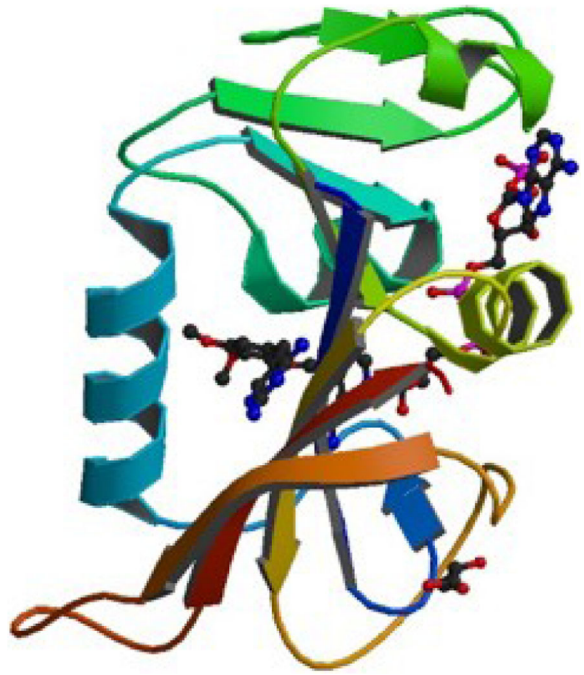

Fig. 2 Structure of the target protein dihydrofolate reductase (PDB ID: 2W9S)

Discussion

In vitro antibacterial studies

Even though both compounds (DmChDp and DmChDa) have less activity than the standard antibiotic ampicillin, they showed appreciable growth-inhibitory power. The diameter of the zone of inhibition exhibited by ampicillin in *S. aureus* was 30 mm, while the Schiff bases DmChDp and DmChDa exhibited 26 mm and 25 mm respectively, at 500 μg $disc^{-1}$ concentration. The zone of inhibition was found to be increasing with the concentration of the compounds. Thus, they can be considered good antibacterial agents for *S. aureus*.

Lipinski rule of five

According to this rule, an orally active drug must have less than two violations [35]. Results showed that the Schiff bases DmChDp and DmChDa have only one violation and hence they obey the Lipinski rule, suggesting that these compounds have the potential to behave as an orally active drug.

Docking studies of Schiff bases with targets in *Staphylococcus aureus*

Docking studies of DmChDp with 2W9S

From Table 3, it is clear that the Schiff base is more effective in sites 2 and 3 of the target 2W9S with a maximum binding energy of − 9.9 and − 10.3 kcal/mol, respectively. The interaction pattern showed that DmChDp interacted with the target 2W9S through 2 hydrogen bonds in active site 2 (LEU20, SER49 residues) and 1 hydrogen bond in active site 3 (ALA7 residue). In active site 2, the first hydrogen bond is formed between the nitrogen atom of the Schiff base (-C=N-) and H of LEU20. The second was originated from phenolic H to the N of SER49. In site 3, the hydrogen bond is formed between phenolic oxygen of the ligand and the H atom of ALA7 (2.24 Å).

The other interactions present in site 2 were carbon H bond (GLN19), alkyl interaction (LEU20, ILE50), pi-alkyl interaction (LYS29), and unfavourable acceptor-acceptor interaction (ILE14). In site 3, apart from conventional H bond, non-classical H bond, van der Waals, and hydrophobic interactions were also observed.

PHE92 and ILE5 residues present in site 3 of 2W9S interacted by means of pi-pi T-shaped and amide-pi stacked interactions, respectively. The amino acid

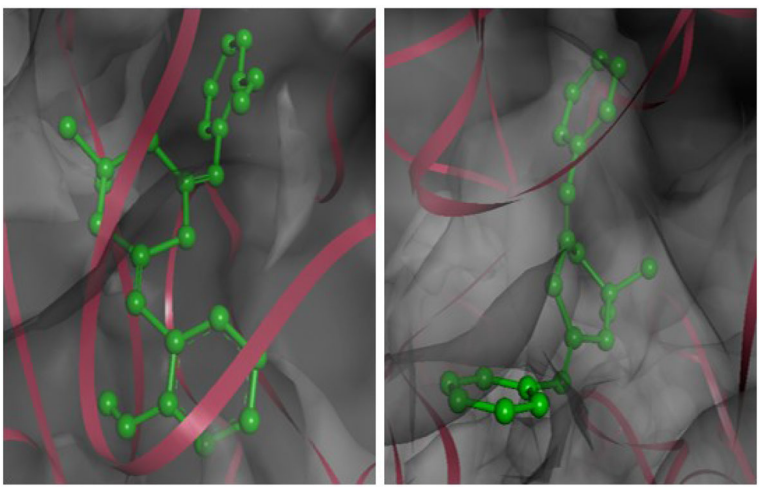

Fig. 3 3D interaction diagrams of **a** DmChDp in site 3 of 2W9S and **b** DmChDa in site 2 of 2W9S

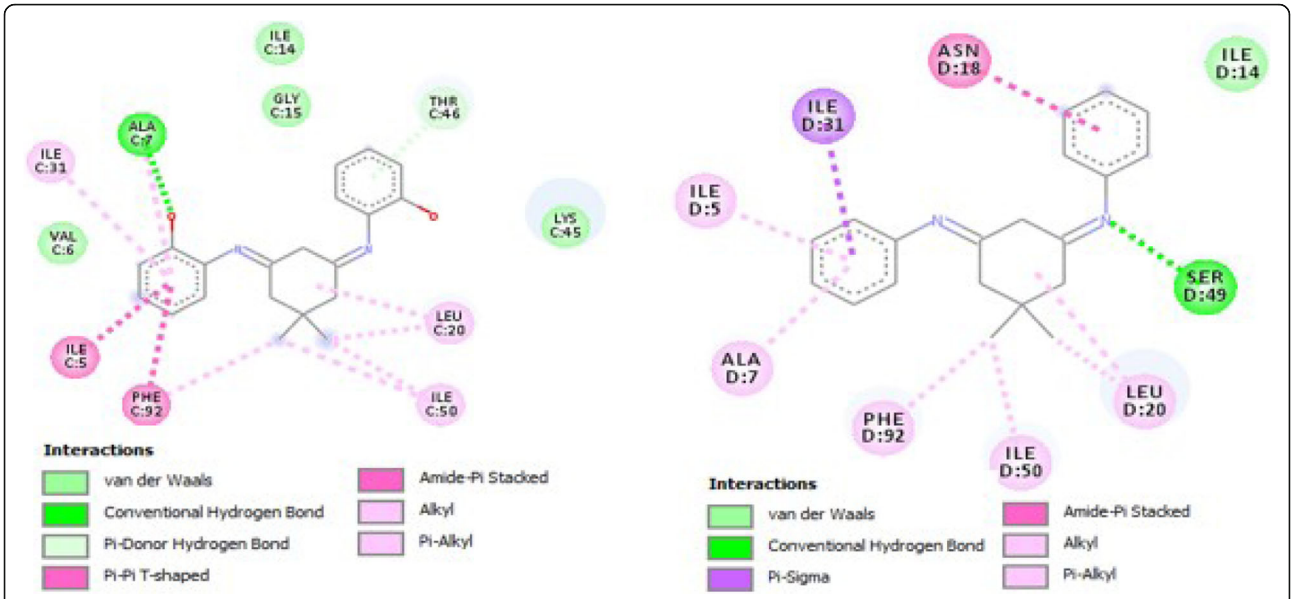

Fig. 4 2D interaction diagrams of **a** DmChDp in site 3 of 2W9S and **b** DmChDa in site 2 of 2W9S

residues LEU20 and ILE50 formed two alkyl interactions each and the residues ALA7, ILE31, ILE5, and PHE92 form pi-alkyl interactions. Considering binding energy and interactions, it is derived that DmChDp has more binding affinity towards site 3 of the protein target 2W9S. Thus, the inhibition mechanism of *S. aureus* by DmChDp may involve deactivation of the function of the dihydrofolate reductase enzyme.

Docking studies of DmChDa with 2W9S

DmChDa is also more effective in sites 2 and 3 of the target 2W9S with a maximum binding energy of − 10.5 and − 9.5 kcal/mol, respectively. In site 2, there is a conventional hydrogen bond interaction with SER49 residue (imine N with H of SER49, 2.06 Å), whereas in site 3, hydrogen bond interaction was absent. In both sites, there are three alkyl interactions (ILE50, LEU20), three pi-alkyl interactions (ILE14, ALA7, ILE5) and a pi-sigma interaction (ILE31). The van der Waals interaction is with ILE14 and PHE92 residue in sites 2 and 3, respectively. In addition to these interactions, there is an amide-pi stacked interaction with ASN18 in site 2. Based on the values of binding energy and interactions, it can be stated that DmChDa has more binding affinity towards site 2 of the target 2W9S. Thus, DmChDa also deactivates the dihydrofolate reductase enzyme preferentially than the other five enzymes.

In brief, out of the 6 target proteins selected for docking study, the Schiff base compounds are more active against dihydrofolate reductase enzyme and the growth-inhibitory power against *S. aureus* is attributed to the high binding affinity towards this enzyme. Also, the amino acid residues LEU20, ILE50, and PHE92 interacted with the cyclohexanone ring when both the compounds docked with the target 2W9S. 3D and 2D interaction diagrams of Schiff bases are shown in Figs. 3 and 4, respectively.

Conclusion

Schiff base compounds DmChDp and DmChDa have appreciable growth-inhibitory power on comparing with the inhibitory power of the standard antibiotic ampicillin against the pathogenic bacteria *S. aureus*. Maximum zone of inhibition of about 26 mm and 25 mm were shown by DmChDp and DmChDa, respectively, at a concentration of 500 μg $disc^{-1}$. Both of them obeyed Lipinski's rule of five and possess drug-like property. Among the target proteins selected for study, both the Schiff bases showed good binding affinity in sites 2 and 3 of the target protein dihydrofolate reductase enzyme (PDB ID: 2W9S). Maximum binding energies of − 10.3 and − 10.2 kcal/mol were observed for DmChDp and DmChDa docked with 2W9S, respectively, which clearly establish that the appreciable growth-inhibitory power of these Schiff bases against the pathogenic bacteria *S. aureus* is mainly due to deactivation of the enzyme, dihydrofolate reductase.

Abbreviations

DmChDp: 2,2′-(5,5-dimethylcyclohexane-1,3-diylidene)bis(azan-1-yl-1-ylidene)diphenol; DmChDa: N,N′-(5,5-dimethylcyclohexane-1,3-diylidene)dianiline

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Authors' contributions
RK carried out in vitro and in silico studies and drafted the manuscript. The author JTK and VPR designed the study and edited the manuscript. RJ and VTK helped to carry out the work. All authors read and approved the final manuscript.

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Availability of data and materials
Data and material are available upon request.

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare no competing interests.

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