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CHAPTER 10
MATERIALS AND METHODS

The methods and steps used for *in vitro* antibacterial and *in silico* molecular docking studies are discussed in this chapter. Details about the protein targets adopted for docking studies are also included.

***In vitro* antibacterial studies**

In vitro antibacterial analysis of the Schiff base compounds 2,2'-(5,5-dimethylcyclohexane-1,3-diylidene)bis(azanylylidene))diphenol (DMCHDP), N,N'-(5,5-dimethylcyclohexane-1,3-diylidene)dianiline (DMCHDA), 2,2'-(5,5-dimethyl cyclo hexane-1,3-diylidene)bis(hydrazinecarboxamide) (DMCHHC), 2-((2-hydroxy benzylidene)amino)phenol (2HBAP), 2-(cyclohexylideneamino)phenol (2CHAP), 3-(1-(2-phenylhydrazono)ethyl)pyridine (3PHEP), 2-(1-(pyridine-3-yl)ethylidene)hydrazine carboxamide (2PEHC), 2-(1-(pyridine-3-yl)ethylidene) hydrazine carbothioamide (2PEHCT), 3-((thiophen-2-ylmethylene)amino)benzoic acid (3TMAB) and 2-(1-(2-phenylhydrazono)ethyl)pyridine (2PHEP) were conducted against *Staphylococcus aureus* and *Escherichia coli*. Three Schiff bases 2PEHC, 2PEHCT, 3TMAB and their La(III), Nd(III) and Sm(III) complexes were also subjected to antibacterial studies against the pathogens such as *Enterococcus faecalis***,** *Enterococcus casseliflavus*, *Pseudomonas aeruginosa* and *Enterobacter hormaechei* apart from the former two bacteria. Disk diffusion method is employed for this purpose. Mueller- Hinton agar was used for preparing the medium. Ampicillin is the standard antibiotic used for comparison.

Disc diffusion method

Also called Kirby–Bauer test or agar diffusion test [48]. This method is used to determine efficient antibiotic against pathogenic microorganisms. First step is the preparation of agar plate followed by spreading bacteria on it [49]. Then added the discs containing sample and incubated at 37^0C for 24 h. If the sample is effective against the bacteria, there will be a region around the bacteria where there is no bacterial growth. This region is called zone of inhibition. Diameter of the zone of inhibition was measured and compared with zones produced by reference compounds. Representation of disc diffusion method is shown in Fig. 10.1.

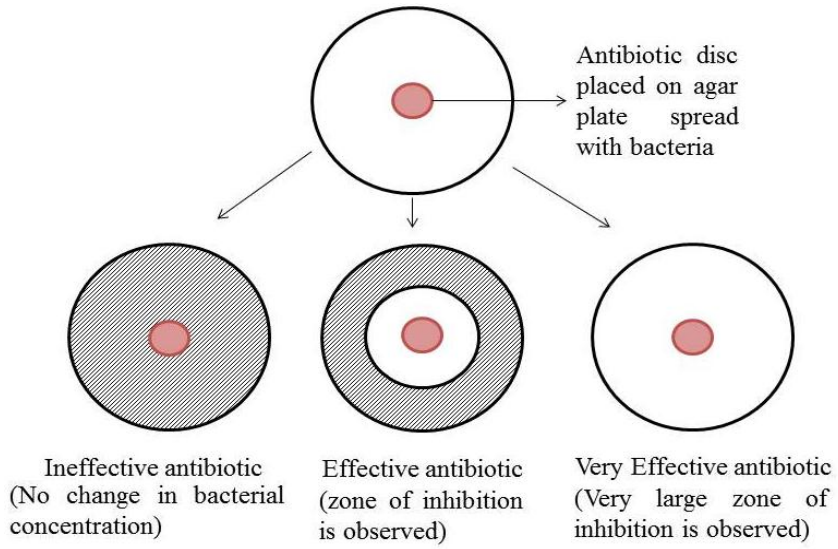

Fig. 10.1 Representation of disc diffusion method

Steps involved in the in vitro antibacterial studies

Steps involved in the *in vitro* antibacterial studies are described as follows

Mueller-Hinton agar (MHA) plate preparation: All the ingredients (Beef-300 g, Starch-1.5 g, Casein acid hydrolysate-17.5 g, agar-17 g, pH -7.3, Distilled water-1000 ml) were dissolved in distilled water and autoclaved after closing it with a sterilized cotton ball at 121^0C for 15 minutes. Then cooled for half an hour and poured into a sterilized petri dish (diameter-90 mm and depth-4 mm) and placed in laminar flow hood

chamber. Allowed the plate to solidify and examined each set of plates for sterility by incubating at 30-35^{0}C for 24 h.

Nutrient broth for inoculum preparation: All the ingredients (Peptone -5 g, NaCl-5 g, Beef extract-3 g, Yeast extract-3 g, Agar- 20 g, Distilled water-1000 ml, pH-7.4) were dissolved in distilled water by heating it and take 5-7 ml of the medium in separate test tubes. The test tubes were closed using cotton wool and autoclaved for 15 minutes at 121^{0}C.

Inoculum preparation: For the preparation of inoculums growth method is used. From an agar plate culture, four to six isolated bacterial colonies having similar morphology was selected and then the growth is transferred to a tube consisting of nutrient broth (5 ml). Then incubation of the broth culture at 35^{0}C was done until it gains the turbidity of 0.5 McFarland standards. Normally 2-6 hours will take to produce turbidity.

Disc preparation: Three Schiff base ligands, its La(III), Nd(III) and Sm(III) complexes and standard antibiotic ampicillin were dissolved in DMSO to prepare the stock solutions. It is then diluted to obtain various ranges of concentrations from 100-500 µgdisc^{-1}. Disc diffusion method was utilized to inoculate drug molecules. Using a micropipette samples were inoculated to the discs. Disc containing DMSO (control) was also tested. Discs are then incubated at 37^{0}C for 24 h after placing it in a petri dish.

Test plate inoculation: A cotton swab is immersed into the inoculum suspension after controlling its turbidity using a sterile saline solution or broth. Rotate the cotton swab several times in suspension and strongly press it on the walls of the tube above the liquid level in order to remove excess inoculums. Then the inoculum was uniformly swabbed over the surface of Mueller-Hinton agar plate. This process was repeated by streaking and rotating the plate for uniformly distributing the inoculums and then dried for 20 minutes.

Allocation of disc containing samples on inoculated agar plates: Discs containing samples are allocated on the surface of the agar plate by means of forceps and then gently pressed down to attain uniform contact with agar plate. A minimum distance of 30 mm was preserved between the discs. Then the petri dishes were inverted and incubated in air ambience at 35^0C for 24 h. If the sample (drug) is effective against the bacteria, there will be a region around the bacteria where there is no bacterial growth. This region is called zone of inhibition. Diameter of the zone is measured and compared with zones produced by reference compounds.

***In silico* molecular docking studies**

As a preliminary step Lipinski rule of five was evaluated in all Schiff base compounds to predict the drug like properties and then conducted docking studies. Molecular docking studies were conducted using AutoDock 4.2 program in order to establish the mechanism by which the Schiff base molecules inhibit the growth of bacteria. For this purpose 6 different targets (PDB ID: 1T2P, 3U2D, 2W9S, 1N67, 2ZCO and 4H8E) of one gram-positive bacteria (*S. aureus*) and 7 targets (PDB ID: 1HNJ, 1G2A, 2VF5, 2MBR, 2GT1, 2X5O and 2W6O) of one-gram negative bacteria (*E. coli*) were selected. Ten Schiff bases 2,2'-(5,5-dimethylcyclohexane-1,3-diylidene) bis(azanylylidene))diphenol (DMCHDP), N,N'-(5,5-dimethylcyclohexane-1,3-diylidene) dianiline (DMCHDA), 2,2'-(5,5-dimethylcyclohexane-1,3-diylidene)bis(hydrazine carboxamide) (DMCHHC), 2-((2-hydroxybenzylidene)amino)phenol (2HBAP), 2-(cyclohexylideneamino)phenol (2CHAP), 3-(1-(2-phenylhydrazono)ethyl)pyridine (3PHEP), 2-(1-(pyridine-3-yl)ethylidene)hydrazine carboxamide (2PEHC), 2-(1-(pyridine-3-yl)ethylidene)hydrazine carbothioamide (2PEHCT), 3-((thiophen-2-yl methylene)amino)benzoic acid (3TMAB) and 2-(1-(2-phenylhydrazono)ethyl)pyridine

(2PHEP) were docked with these 13 target proteins. Binding affinities of these molecules in the 4 binding sites of all bacteria (except in 4H8E) were also evaluated.

Lipinski rule of five

Also called Pfizer's rule of five or rule of five. The rule was developed in 1997 by Christopher A. Lipinski It is a theoretical method to evaluate the drug ability of a chemical compound [50]. This rule is based on the view that an orally active drug will be small and slightly lipophilic. It depicts molecular properties and not predicts pharmacological activity. According to this rule a drug has good oral activity if it satisfies the following conditions

1. Molecular weight < 500
2. Octanol-water partition coefficient logP < 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

The origin of the name arises from the observation that all are multiples of five.

Softwares used for docking studies

Softwares such as Openbabel, Pymol, AutoDock Tools (MGL Tools), vina.exe and BIOVIA Discovery Studio were used to carry out the docking study.

The software Openbabel is used to interconvert different chemical file formats. Major features are chemical expert system, inter conversion of many chemical file formats, substructure search, based on simplified molecular-input line-entry system (SMILES), fingerprint calculation, 3D coordinate generation, wrappers for Python, Perl, Java, Ruby, C#.

Pymol software is used for chemical visualization and preparation of protein.pdb files for docking. MGL tools were used for preparation of protein.pdb files and

ligand.pdb files for docking. AutoDock [51] has applications in X-ray crystallography, structure-based drug design, lead optimization, virtual screening (HTS), combinatorial library design, protein-protein docking and chemical mechanism studies. Vina.exe is the docking software to execute docking analysis and give binding energy of ligand with protein. In order to derive active sites of protein and docking result analysis, the software Discovery Studio is employed.

Steps involved in docking process

The docking procedure involves the following steps.

Preparation of ligand*:* Structures of the ligands were drawn using ChemSketch software and saved in MOL format. This is then converted to PDB format using Open babel software. Ten Schiff base compounds were used for docking studies such as DMCHDP, DMCHDA, DMCHHC, 2HBAP, 2CHAP, 3PHEP, 2PEHC, 2PEHCT, 3TMAB and 2PHEP

Preparation of protein*:* Structures of the proteins were downloaded in PDB format from RCSB PDB web server. Then 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 from *Staphylococcus aureus* and seven from *Escherichia coli* were utilized to check the interaction with synthesized ligands. Structures of the target proteins in *S. aureus* and *E. coli* are shown in Fig. 10.2 and 10.3 respectively.

Prediction of active site: Prediction of active sites is important in structure based drug design. Coordinates 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-ligand complexes was obtained. ***Visualization of protein-ligand complexes***: The protein-ligand complexes were

visualized using the software BIOVIA Discovery Studio. 3D and 2D interaction diagrams of the protein-ligand complexes were obtained. Hydrogen bond interactions such as conventional H bond 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, unfavorable donor-donor and acceptor-acceptor interactions are commonly seen between protein and ligands. Binding affinity of the compound with the target protein is the resultant of all the interactions and binding energy existing between them.

Target proteins in Staphylococcus aureus

Sortase-A, DNA gyrase, dihydrofolate reductase, clumping factor A, dehydrosqualene synthase, undecaprenyl diphosphate synthase are the main target proteins in *Staphylococcus aureus*, which were selected for the present studies.

Staphylococcus aureus sortase-A: Sortases are extracellular transpeptidases of Gram-positive bacteria [52-53]. The function of the enzyme is sorting proteins into the cell wall compartment of gram-positive bacteria, hence named sortases. A C-terminal sorting signal with a canned LP*X*TG motif (*X* denotes any amino acid) is involved in the sorting mechanism. Sortases are of two types, sortase A (SrtA) and sortase B (SrtB). Former one was first separated from *S. aureus* in 1999 and it is also called housekeeping sortases as they are substantial for surface protein sorting and visible in almost all gram-positive bacteria. Transpeptidase Sortase A, with cysteine as the active site will break the sorting signal between threonine and glycine of the LP*X*TG motif. Sortases have a great role in the cell wall envelope assembly and in bacterial pathogenicity. Thus they are good targets for therapeutic agents that may reduce infections in human because of gram-positive bacteria.

PDB ID	:	1T2P
Classification	:	Hydrolase
Expression System	:	*Escherichia coli*
Chains present	:	A, B and C
Sequence length	:	146
Ligands present	:	No ligands

DNA gyrase: Topoisomerase is an isomerase enzyme that provokes dramatic change in topology of DNA structure [54-55]. Topoisomerase are categorized as topoisomerase I and topoisomerase II, based on 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 on DNA replication. Presence of a metal ion and ATP is required for the functioning of topoisomerase II in all the stages. It is a tetrameric enzyme consisting of two Gyr A and two Gyr B subunits, which form an A_2B_2 complex in the active enzyme. Studies on DNA gyrase showed that it has catalytic activity and the shape is like a pair of scissors, with A subunits and B subunits as cutting blades (the breaking–resealing component) and handles (the ATP-driven energy transduction component) respectively. Gyrase cleaves the helix in both strands of closed circular DNA and passes another segment of DNA through the break and at last reseal the broken ends.

PDB ID	:	3U2D
Classification	:	Isomerase/Isomerase inhibitor
Expression System	:	*Escherichia coli* BL21 (DE3)
Chains present	:	A, B
Sequence length	:	198
Ligands present	:	4-bromo-5-methyl-N-[1-(3-nitropyridin-2-yl)

piperidin-4-yl]-1H-pyrrole-2-carboxamide(08B), Magnesium (ii) ion (MG)

Dihydrofolate reductase (DHFR): 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) [56-57]. Also it has a great role in the synthesis of thymidylate, purines, methionine and some other important metabolites. These enzymes are required for the cell proliferation. Thus inhibition of dihydrofolate reductase will results in destruction of intracellular tetrahydrofolate pool and hence prevents biosynthesis of RNA, DNA, thymidine and protein. Due to the wide range of cellular functions they are targets for anticancer and antimicrobial agents.

PDB ID	:	2W9S
Classification	:	Oxidoreductase
Expression System	:	*Escherichia coli* BL21 (DE3)
Chains present	:	A, B, C, D, E and F
Sequence length	:	161
Ligands present	:	Glycerol (GOL), Trimethoprim (TOP), NADPH, dihydro-nicotinamide-adenine-dinucleotide phosphate (NDP)

Clumping factor A (ClfA): In blood plasma there is a glycoprotein called fibrinogen (Fg) which is present at ~3 mg/ml concentration and had a significant role in coagulation and hemostasis. Six polypeptide chains are present in fibrinogen such as 2 Aa, 2 Bb and 2 γ-chains, that 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) [58-59] was the first Fg γ -chain-binding *S. aureus* adhesin identified.

PDB ID	:	1N67
Classification	:	Cell adhesion
Expression System	:	*Escherichia coli*
Chains present	:	A
Sequence length	:	359
Ligands present	:	Magnesium (ii) ion (MG)

Dehydrosqualene synthase (CrtM): The golden carotenoid pigment staphyloxanthin is a virulence factor for *S. aureus*. Dehydrosqualene synthase [60-61] is involved in the synthesis of this pigment. In the synthesis pathway of the pigment two farnesyl diphosphate molecules undergo head to head condensation to synthesize the C30 hydrocarbon dehydrosqualene by the dehydrosqualene synthase (CrtM). Main responsibility of the pigment is that it preserves *S. aureus* against oxidative stress as a result of host immune defense by reactive oxygen species and neutrophils by acting as an antioxidant.

PDB ID	:	2ZCO
Classification	:	Trasferase
Expression System	:	*Escherichia coli*
Chains present	:	A
Sequence length	:	293
Ligands present	:	Nil

Undecaprenyl diphosphate synthase (UPPS): The role of undecaprenyl diphosphate synthase (UPPS) in the biosynthesis of cell wall of *Staphylococcus aureus* is very

significant [62-63]. There are many essential steps during cell wall biosynthesis. Some of them are formation of farnesyl diphosphate (FPP), reaction between two molecules of isopentyl diphosphate (IPP) and dimethylallyldiphosphate (DMAPP) in the presence of enzyme named farnesyl diphosphate synthase (FPPS), and after that eight isopentyl diphosphate molecules are added to generate undecaprenyl diphosphate (UPP). Undecaprenyl diphosphate synthase catalyse the formation of UPP. Then UPP is hydrolyzed to form monophosphate, it is then converted into lipid I and II resulting in the formation of the cell wall polymer peptidoglycan. Among the proteins mentioned above UPPS is important since it is vital for the formation of peptidoglycan. Also UPPS is not present in human and is an additional merit for the development of good antibacterial agents.

PDB ID	:	4H8E
Classification	:	Transferase/transferase inhibitor
Expression System	:	*Escherichia coli*
Chains present	:	A
Sequence length	:	256
Ligands present	:	Sulfate ion (SO4), Magnesium ion (MG), Farnesyl diphosphate (FPP)

Target proteins in Escherichia coli

The major target proteins in *Escherichia coli* include β-ketoacyl-acyl carrier protein synthase, peptide deformylase, L-glutamine: D-fructose-6-phosphate amido-transferase, murB, heptosyltransferase WaaC, murD and biotin carboxylase.

β-ketoacyl-acyl carrier protein synthase III (ecKAS III): In gram-negative bacteria, type II fatty acid (FAS II) has proved to be a good target. β-ketoacyl-acyl carrier protein synthase (KAS) is a type II fatty acid which is acting as an essential target in the field of

antibacterial drug discovery [64-65]. Three KAS enzymes such as KAS I, KAS II, and KAS III are present in bacteria. The KAS III initiate the starting steps of fatty acid biosynthesis and thus act as most appropriate target for antibacterial drug design.

PDB ID	:	1HNJ
Classification	:	Transferase
Expression System	:	*Escherichia coli*
Chains present	:	A
Sequence length	:	317
Ligands present	:	Phosphate ion (PO4), Malonyl-Coenzyme A (MLC)

Peptide deformylase (PDF): It is a prokaryotic metalloenzyme that is vital for the growth of bacteria [66-67]. This enzyme is not seen in mammalian cells and hence acts as a promising target for the development of potential antibacterial agents. The method of protein synthesis is similar in both mammalian and bacterial cells. The main difference is that *N*-formylmethionine initiates the protein synthesis in bacteria whereas in mammalian cells methionine takes the role of initiator. *N*-formylmethionine is formed through enzymatic transformylation of methionyl-tRNA in the presence of formylmethionine tRNA transferase. The methionine amino peptidase and peptide deformylase consequently remove *N*-formylmethionine of nascent bacterial protiens in successive steps. This cycle of formylation and deformylation is important for the growth of bacteria. Thus it will be a promising target for the development of chemotherapeutic agents.

PDB ID	:	1G2A
Classification	:	Hydrolase
Expression System	:	*Escherichia coli* BL21 (DE3)

Chains present	:	A, B, C
Sequence length	:	168
Ligands present	:	Actinonin (BB2), Nickel (II) ion (NI)

***L-glutamine: D-fructose-6-phosphate amido*-transferase**: It is also named as glucosamine-6-phosphate synthase (GlcN-6-P synthase). The hexosamine metabolism involves conversion of fructose 6- phosphate into glucosamine 6-phosphate by utilizing glutamine [68-69]. This reaction catalyzed by glucosamine-6-phosphate synthase is irreversible, hence considered as an important step. The vital building unit of bacterial cell wall N-acetyl glucosamine is the final product formed in this pathway. Inactivation of GlcN-6-P synthase even for a short duration is harmful to bacterial cells.

PDB ID	:	2VF5
Classification	:	Transferase
Expression System	:	*Escherichia coli*
Chains present	:	X
Sequence length	:	608
Ligands present	:	Glucosamine-6-phosphate (GLP)

MurB: This enzyme is important for the survival of bacterial cells [70-71]. They are good targets for antibacterial agents. Enzymes involved in the biosynthesis of peptidoglycan are essential for the survival of bacterial cell wall. The osmotic integrity of the cell is maintained by the peptidoglycan and the lactyl ether connecting units link both peptide and glycan strands. UDP-N-acetylglucosamine is the stating component of peptidoglycan biosynthesis and proceeds through the formation of UDP-N-acetylmuramic acid, which is catalysed by the mur enzymes murA and murB. In the first step enolpyruvyl moiety present in the phosphoenolpyruvate is added to the 3$^{'}$ hydroxyl exist in UDP-*N*-acetylglucosamine with the help of murA enzyme. In the second step

enol ether is reduced to lactyl ether by using 1 equivalent of NADPH and a proton derived from solvent with the help of murB enzyme.

PDB ID	:	2MBR
Classification	:	Oxidoreductase
Expression System	:	-
Chains present	:	A
Sequence length	:	340
Ligands present	:	Uridine-diphosphate-2(N-acetylglucosaminyl butyric acid (EPU), Flavin-adenine dinucleotide (FAD)

Heptosyltransferase WaaC: The cell membrane prevents contact of cell with external environment. The outer leaflet of the membrane present in gram negative bacteria is created by lipopolysaccharides and hence essential for the growth of cells. The glycosyltransferace named heptosyltransferase WaaC is engaged in the synthesis of inner core region of lipopolysaccharides [72-73]. It catalyzes the addition of the first l-glycero-d-manno-heptose molecule to one 3-deoxy-d-manno-oct-2-ulosonic acid (Kdo) residue of the Kdo2-lipid A molecule. These heptose is an important component of the lipopolysaccharides core domain and its absence will cause truncated lipopolysaccharide associated with the deep-rough phenotype causing a greater susceptibility to antibiotic.

PDB ID	:	2GT1
Classification	:	Transferase
Expression System	:	*Escherichia coli*
Chains present	:	A, B
Sequence length	:	326
Ligands present	:	Nil

Mur D: Enzymes having great role in the peptidoglycan (a vital cell wall polymer of bacteria) biosynthesis are wildly known targets for developing selective and efficient antibacterial agents. Cytoplasm is the place where the initial stage of peptidoglycan biosynthesis takes place and a monomeric building block is generated. This is then translocated through the cellular membrane to its outer part, and by glycosyl transfer and transpeptidation reactions incorporated into the growing peptidoglycan macromolecule. UDP-N-acetylmuramoyl-pentapeptide (UDP MurNAc-pentapeptide) is the building block of peptidoglycan, which is synthesized through four consecutive reactions catalysed by the cytoplasmic enzymes named mur ligases (murC, murD, murE and murF) [74-75]. Mur ligases catalyses the attachment of a pentapeptide chain to the terminal carboxyl group present in UDP-N-acetylmuramic acid (UDP-MurNAc). This is taking place through addition of L-Ala, D-Glu, meso-diaminopimelic acid or L-Lys, D-Ala- D-Ala dipeptide catalyzed by murC, murD, murE and murF respectively. Mechanism of operation of all Mur ligases is same. In order to activate the carboxyl group of UDP-N-acetylmuramic acid, one ATP molecule is required. An acyl phosphate intermediate is thus formed and the amino group present in the incoming dipeptide or aminoacid attacks this intermediate to produce a new peptide or aminoacid after eliminating the inorganic phosphate from the tetrahedral intermediate.

PDB ID	:	2X5O
Classification	:	Ligase
Expression System	:	*Escherichia* DH5[alpha]
Chains present	:	A
Sequence length	:	439
Ligands present	:	Sulfate ion (SO4), Sulfite ion (SO3), Chloride ion (CL), Azide ion (AZI**),** N-

({3-[({4-[(z)-(2,4-dioxo-1,3thiazolidin-5-ylidene)methyl]phenyl}amino)methyl] phenyl}carbonyl)-d-glutamic acid (VSV)

Biotin carboxylase (BC): In the field of drug discovery for antibacterial activity the biosynthetic pathway of type II fatty acid has considerable importance. The multifunctional enzyme acetyl-CoA carboxylase (ACCase) is biotin-dependent and comprised of three proteins such as biotin carboxylase (BC), biotin carboxylase carrier protein (BCCP) and carboxyltransferase (CT). During the first half of the reaction a carboxyl group is transferred from bicarbonate to biotin of biotin carboxylase carrier protein in the presence of ATP molecule. This is catalyzed by BC. In the second half transfer of carboxyl group from carboxybiotinylated BCCP to acetyl-CoA will take place to form malonyl-CoA. Since biotin carboxylase catalyzes the most important initial step in fatty acid biosynthesis, it is a promising target for antibacterial agents [76-77].

PDB ID	:	2W6O
Classification	:	Ligase
Expression System	:	*Escherichia coli*
Chains present	:	A, C
Sequence length	:	449
Ligands present	:	4-amino-7,7-dimethyl-7,8- dihydroquinazolin-5(6H)-one (OA3), Chloride ion (CL)

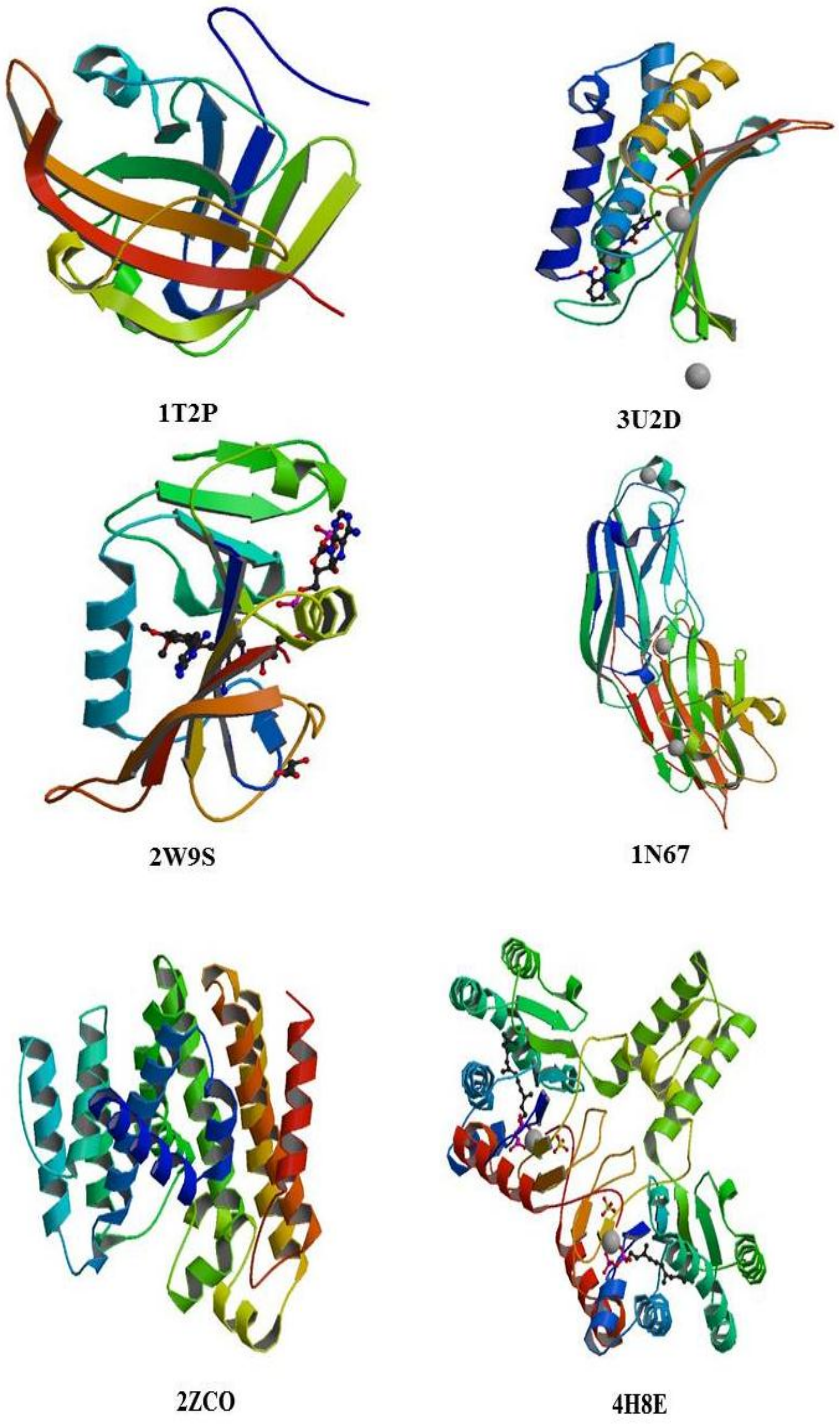

Fig. 10.2 Structures of the target proteins in *S.aureus*

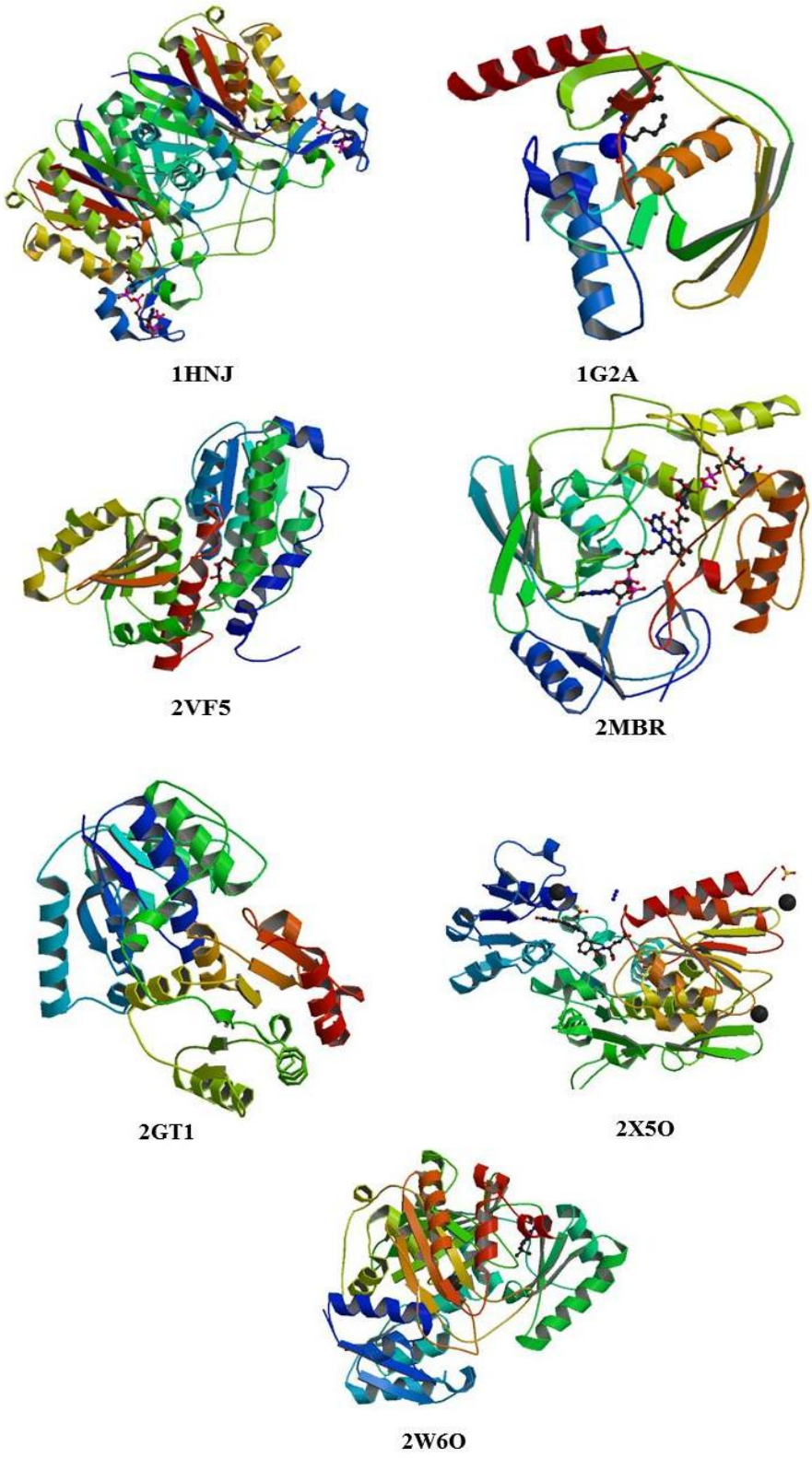

Fig. 10.3 Structures of the target proteins in *E. coli*