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CHAPTER 11
IN VITRO ANTIBACTERIAL AND *IN SILICO* MOLECULAR DOCKING STUDIES ON SCHIFF BASES AND THEIR INNER TRANSITION METAL COMPLEXES

This chapter deals with screening of growth inhibitory power of the 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-dimethyl cyclohexane-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) against *Staphylococcus aureus* and *Escherichia coli.* The *in silico* molecular docking studies were also carried out to understand the mechanism by which the Schiff base compounds inhibit the growth of these two bacteria by selecting suitable targets present in them. Detailed procedures for the synthesis and characterization of Schiff bases such as DMCHDP, DMCHDA, DMCHHC, 2HBAP, 2CHAP, 3PHEP, 2PEHCT and 3TMAB are discussed in the chapters 3 and 8, and reported procedures were adopted for 2PEHC and 2PHEP [78-79]. The *in vitro* antibacterial analysis of the three Schiff base ligands 3PHEP, 2PEHCT, 3TMAB and their La(III), Nd(III) and Sm(III) complexes were also carried out against the pathogens such as *Enterococcus faecalis*, *Enterococcus casseliflavus*, *Pseudomonas aeruginosa* and *Enterobacter hormaechei* apart from the former two bacteria. Disc diffusion method was employed for the *in vitro* antibacterial analysis. Antibacterial

activity of all the ligands and complexes were compared with the activity of the standard drug ampicillin.

***In vitro* antibacterial studies of the Schiff bases**

In *vitro* antibacterial studies of the Schiff base compounds DMCHDP, DMCHDA, DMCHHC, 2HBAP, 2CHAP, 3PHEP, 2PEHC, 2PEHCT, 3TMAB and 2PHEP against *Staphylococcus aureus* and *Escherichia coli* were carried out at different concentrations such as 50, 100, 250 and 500 µgdisc^{-1} in DMSO. Ampicillin was used as standard antibiotic to compare the activity of synthesized ligands. Antibacterial activity of the Schiff base compounds are shown in Table 11.1.

Table 11.1 Antibacterial activity of the Schiff base compounds

Schiff base	Diameter of zone of inhibition (mm) at different concentrations (µgdisc^{-1})							
	S. aureus				*E. coli*			
	50	100	250	500	50	100	250	500
DMCHDP	12	16	22	26	12	15	19	22
DMCHDA	10	16	22	25	12	19	20	24
DMCHHC	10	16	20	25	10	19	21	22
2HBAP	9	17	21	22	8	10	14	16
2CHAP	11	15	19	25	9	9	12	15
3PHEP	10	17	20	25	8	12	13	16
2PEHC	7	10	14	17	2	3	10	13
2PEHCT	4	8	8	11	0	1	5	9
3TMAB	10	12	20	24	7	11	13	15
2PHEP	9	16	19	23	8	12	16	18
Ampicillin	15	21	28	30	12	19	21	25

Even though all Schiff bases have less activity than the standard antibiotic, all of them have appreciable growth inhibitory power. Diameter of zone of inhibition exhibited by ampicillin in *S. aureus* and *E. coli* are 30 and 25 mm respectively. Zone of inhibition was found to be increasing with concentration of the ligands. In *S. aureus* maximum zone of inhibition of about 26 mm was shown by DMCHDP and in *E. coli* maximum zone of inhibition of about 24 mm was shown by DMCHDA. Antibacterial activity against both *S. aureus* and *E. coli* was low for the ligands 2PEHC and 2PEHCT.

Fig. 11.1 represents the antibacterial activity of DMCHDP at different concentrations against *S. aureus*.

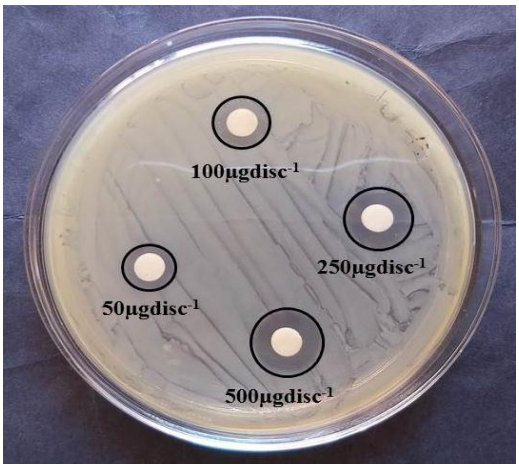

Fig. 11.1 Antibacterial activity of DMCHDP at different concentrations against *S. aureus*

***In silico* molecular docking studies**

In silico approach was used to predict the mechanism by which the Schiff base compounds inhibit bacterial growth. Compounds were first pre- filtered using Lipinski rule of five to check the drug like properties. Parameters such as mass, number of hydrogen bond donors, number of hydrogen bond acceptors, log P (octanol-water partition coefficient), molar refractivity of all compounds were evaluated by Lipinski rule of five and is given in Table 11.2. According to this rule an orally active drug will have fewer than two violations. Results showed that the ligands DMCHDP, DMCHDA, DMCHHC have only one violation and all other ligands have no violation of Lipinski rule. This suggests that these compounds have the potential of acting as an orally active drug.

In molecular docking studies the 3D structures of the compounds were docked with four active sites of the target proteins, PDB ID 1T2P, 3U2D, 2W9S, 1N67, 2ZCO and two active sites of the target proteins, PDB ID 4H8E of *Staphylococcus aureus* and with four active sites of the target proteins, PDB ID 1HNJ, 1G2A, 2VF5, 2MBR, 2GT1,

2X5O, 2W6O of *Escherichia coli* with the aid of AutoDock 4.2. Parameters such as binding energy, number of conventional hydrogen bond (HB) interactions and other interactions were used to determine the best binding mode.

Table 11.2 Lipinski rule of five

Parameters		Mass	Hydrogen bond donor	Hydrogen bond acceptor	log P	Molar Refractivity
Limiting value for drug like property		<500	<5	<10	<5	40-130
Schiff base	DMCHDP	322	2	4	5.15	98.03
	DMCHDA	290	0	2	5.74	94.7
	DMCHHC	254	6	8	0.24	67.94
	2HBAP	213	2	3	2.84	63.46
	2CHAP	189	1	2	3.42	58.28
	2PEHC	164	3	5	0.08	44.55
	3PHEP	211	1	3	2.91	66.51
	2PEHCT	180	3	4	0.24	51.75
	3TMAB	231	1	3	3.19	64.97
	2PHEP	211	1	3	2.91	66.51

Docking studies of Schiff base compounds with targets in Staphylococcus aureus

Binding affinity of the ten Schiff bases in the four sites (except in 4H8E, two sites) of 6 target proteins present in *Staphylococcus aureus* such as sortase-A (PDB ID: 1T2P), DNA gyrase (PDB ID: 3U2D), dihydrofolate reductase (DHFR) (PDB ID: 2W9S), clumping factor A (ClfA) (PDB ID: 1N67), dehydrosqualene synthase (CrtM) (PDB ID: 2ZCO), undecaprenyl diphosphate synthase (UPPS) (PDB ID: 4H8E) were studied. Stability of final protein-ligand complex was evaluated on the basis of two essential criteria: (1) the highest binding energy and (2) number of interactions of the ligand with the active site residues. Highest binding energy and number of interactions of the Schiff base compounds with protein models under study were enlisted in Table 11.3 and 11.4. A ligand can mainly undergo interactions such as Van der Waals, 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 in predicting the best binding mode.

Table 11.3 Binding energy and number of interactions of Schiff bases, DMCHDP, DMCHDA, DMCHHC, 2HBAP and 2CHAP, docked with target proteins in *S.aureus*

Schiff base	Binding energy and interactions	Target proteins and active sites																					
		1T2P				3U2D				2W9S			1N67				2ZCO				4H8E		
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
DMCHDP	-BE	7.6	7.5	6.3	6.2	6.7	6.8	6.8	6.9	9.3	9.9	10.3	7	9	9	9	9	8.3	7.8	7.6	8.4	7.8	6.2
	HB	2	2	1	1	1	1	1	0	1	2	1	1	2	2	1	2	2	2	1	3	3	3
	Other	7	5	5	10	8	5	3	6	8	8	14	5	9	7	11	7	6	6	6	6	8	4
DMCHDA	-BE	8	7.3	5.9	6.1	7.2	7.1	7.2	7.1	9.1	10.2	9.5	6.9	8.6	6.6	8.5	8.4	8	5.9	7.1	8.1	7.5	6.2
	HB	0	0	1	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	1	0	0
	Other	7	8	6	6	6	9	7	10	14	9	8	7	8	4	9	8	7	2	6	4	8	8
DMCHHC	-BE	7.1	6.6	5.4	6	6.2	7.2	6.2	7.2	7.8	8.5	7.8	6.2	8.1	8.1	8	8.1	7.5	7	6.2	7.5	7.8	6.2
	HB	4	5	2	3	7	5	7	6	1	3	4	3	5	5	3	5	6	7	2	4	6	2
	Other	7	3	4	7	3	3	3	3	6	6	4	6	8	6	6	8	2	5	4	2	5	9
2HBAP	-BE	7.4	7.4	5.2	5.3	5.8	6.7	7	6.7	7.8	7.7	7.7	7.7	7.3	7.3	7.3	7.2	7.6	6.5	6.5	7	7.8	7.2
	HB	2	2	1	1	0	2	1	0	0	0	0	1	3	2	4	2	2	1	0	1	0	1
	Other	6	7	5	6	2	5	4	4	5	5	5	6	8	3	4	3	7	3	5	5	9	9
2CHAP	-BE	6.8	6.8	5.1	5.7	6.4	6.8	7	6.9	7.4	7.4	7.4	6.5	7	6.9	6.9	6.9	7.7	5.8	7.8	6.5	8.2	8.2
	HB	1	1	1	3	1	1	0	0	0	1	1	1	2	2	1	1	1	3	1	2	1	0
	Other	4	4	3	5	3	6	6	5	6	6	5	5	6	4	4	7	6	4	7	2	7	5

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

Table 11.4 Binding energy and number of interactions of Schiff bases, 3PHEP, 2PEHC, 2PEHCT, 3TMAB and 2PHEP, docked with target proteins in *S.aureus*

Schiff base	Binding energy and interactions	Target proteins and active sites																					
		1T2P				3U2D				2W9S				1N67				2ZCO				4H8E	
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2
3PHEP	-BE	6.8	6.7	5.5	5.4	6.8	7	7.3	6.6	7.9	7.8	8	6.6	7.2	7.6	7.5	7.5	7.9	6.3	7.9	6.6	8.2	8.2
	HB	2	2	1	0	0	1	1	1	1	1	1	0	1	0	0	0	0	1	0	2	1	1
	Other	6	5	4	7	4	4	8	6	6	1	6	6	7	7	6	8	8	5	8	3	7	7
2PEHC	-BE	5.8	5.8	4.3	4.8	5.1	5.5	6.1	5.1	6.1	6.3	6.1	6.1	6.3	6.9	6.2	6.9	6.1	5.6	5.7	6.1	6.2	6.3
	HB	6	5	2	3	4	5	2	4	1	3	1	4	4	5	4	5	2	4	1	2	0	0
	Other	4	5	4	4	2	2	1	3	4	2	7	2	3	2	3	2	9	0	4	9	6	5
2PEHCT	-BE	5.1	5	4.2	4.1	4.7	4.9	5.2	5	6.5	6.3	6.4	5.8	5.6	5.6	5.5	5.6	5.7	5	5.3	5.9	6.1	6.1
	HB	3	4	3	2	3	2	2	3	3	3	3	2	3	3	3	3	3	3	1	3	2	2
	Other	3	5	8	8	2	4	5	4	7	7	7	2	4	5	5	7	5	4	5	5	5	4
3TMAB	-BE	6.3	6	4.8	5	5.8	6.1	6.1	6.1	6.7	6.8	7	5.7	7	7	7	7	7.3	5.5	6.6	6.7	6.8	7
	HB	1	0	2	1	3	3	4	2	0	1	2	1	2	3	2	2	1	3	0	2	2	0
	Other	6	3	3	6	4	1	2	4	7	7	8	4	4	7	5	4	6	2	7	4	7	8
2PHEP	-BE	6.8	6.7	5.2	5.3	5.9	7	7.1	7	7.7	7.5	7.9	6.9	7.1	7	6.9	7.1	7.6	6.6	7.5	6.8	7.9	7.8
	HB	2	0	2	1	1	0	0	0	2	1	1	1	1	0	1	1	0	0	0	2	0	0
	Other	4	4	4	5	2	3	6	4	5	6	7	4	7	5	5	6	5	3	9	4	6	7

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

Among the interactions conventional hydrogen bond (HB) (more prominent) and hydrophobic interactions are more effective than the others [80-81]. Coordinate values of the four binding sites selected for docking are shown in Table 11.5. Considering the binding energy and number of interactions, the compounds DMCHDP, DMCHDA, 2PEHCT and 2PHEP have high binding affinity towards the target dihydrofolate reductase (DHFR) (PDB ID: 2W9S). Binding affinity of DMCHHC, 2PEHC and 3TMAB was high towards the target clumping factor A (ClfA) (PDB ID: 1N67) whereas 2CHAP and 3PHEP have high affinity towards the target undecaprenyl diphosphate synthase (UPPS) (PDB ID: 4H8E). Dehydrosqualene synthase (CrtM) (PDB ID: 2ZCO) was found to be active target for 2HBAP. Details of binding energy and interactions of the site having highest binding affinity between ligand and target are mainly considered for discussion.

Table 11.5 Coordinate values of active sites in target proteins of *S.aureus*

Active site and coordinates		PDB ID of target proteins of *S.aureus*					
		1T2P	3U2D	2W9S	1N67	2ZCO	4H8E
1	x	-17.38	17.99	-0.48	27.16	59.12	27.46
	y	-9.54	-2.66	5.99	42.54	11.62	3.94
	z	-7.59	11.95	33.4	71.29	52.34	8.95
2	x	-10.63	5.99	23.51	17.91	66.37	38.21
	y	-19.54	8.83	-22.25	52.54	-2.12	5.94
	z	-12.59	20.45	30.20	61.04	47.09	4.70
3	x	-13.63	28.99	2.769	25.91	51.62	-
	y	-20.54	-9.16	-23.50	55.54	27.62	-
	z	9.40	9.45	22.45	76.79	62.09	-
4	x	0.37	9.99	-26.23	20.41	59.62	-
	y	-6.04	16.58	9.24	33.29	1.12	-
	z	-28.84	27.20	68.20	70.79	41.34	-

Docking studies of DMCHDP with dihydrofolate reductase (DHFR): From Table 11.3 it is clear that the ligand 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. 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 the active site 2, first hydrogen bond is formed between nitrogen atom of the Schiff base (-C=N-) and H of LEU20. 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 H atom of ALA7 (2.24 Å). 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 the site 3 of 2W9S interacted by means of pi-pi T shaped and amide-pi stacked interactions respectively. Amino acid residues LEU20 and ILE50 formed two alkyl interactions each and the residues ALA7, ILE31, ILE5 and PHE92 forms pi-alkyl interactions. Considering binding energy and interactions we assume that DMCHDP has more binding affinity towards the site 3 of the target protein 2W9S. Thus the inhibition mechanism of S.aureus by DMCHDP may involve deactivation of the function of dihydrofolate reductase enzyme.

Docking studies of DMCHDA with dihydrofolate reductase (DHFR): 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 site 2 and 3 respectively. In addition to these interactions there is an amide pi-stacked interaction with ASN18 in site 2. Considering binding energy and interactions we assume that DMCHDA has more binding affinity towards the site 2 of the target 2W9S.

Thus DMCHDA also deactivate dihydrofolate reductase enzyme preferentially than the other five enzymes.

Docking studies of DMCHHC with clumping factor A (ClfA): In the case of DMCHHC high binding energy of about -8.5 kcal/mol is observed in the active site 2 of 2W9S and -8.1 kcal/mol in the active sites 1, 2 and 4 of 1N67. In the target 2W9S it forms three conventional H bond between H of terminal NH_2 with ASN18 (2.29 Å) and carbonyl oxygen with TYR98 (2.87 Å) and GLN95 (2.35 Å). In addition to this 2 Van der Waals and 4 hydrophobic interactions are also present. In the target 1N67 it makes 5 conventional hydrogen bond interactions with VAL450, HIS252, PRO25, ASP385 residues of site 1, 2 and 4. The H bond is formed between the N atom of imine group with H of HIS252 (2.74 Å), H atom of terminal NH_2 group with carbonyl O of carboxyl group in PRO251 (2.74 Å) and ASP385 (2.43 Å), H atom of terminal NH_2 group with carbonyl O in VAL450 (2.28 Å), H atom of NH group with carbonyl O of carboxyl group in ASP385 (2.08 Å). Number of interactions other than H bond was eight (five alkyl and 3 Van der Waals) for sites 1and 4 while it is six for second site. The binding affinity was comparable in 2W9S and 1N67. Considering all the factors DMCHHC is slightly more effective against the enzyme Clumping factor A (1N67).

Docking studies of 2HBAP with dehydrosqualene synthase (CrtM): Considering the three factors to select the good binding site, site 1 of 2ZCO may be active for 2HBAP. Binding energy of -7.6 kcal/mol, two H bond between phenolic H of the ligand with carbonyl O in VAL133 (2.94 Å) and GLN165 (2.83 Å), three Van der Waals, six hydrophobic and one electrostatic interaction were observed. Binding affinity in site 4 of 2W9S is also comparable with a binding energy of -7.7 kcal/mol. But only one hydrogen bond interaction and six other interactions are present. Hence 2HBAP is more powerful to deactivate dehydrosqualene synthase (2ZCO).

Docking studies of 2CHAP and 3PHEP with undecaprenyl diphosphate synthase (UPPS): Schiff bases such as 2CHAP and 3PHEP are very effective against the enzyme undecaprenyl diphosphate synthase. Observed binding energy for site 1 of 2CHAP is -8.2 kcal/mol and forms an H bond with ILE92. In addition to this there are 7 other interactions. 3PHEP molecule also has same binding energy value and number of interactions in both sites of 4H8E. In the case of 2CHAP the H bond interaction is between phenolic H with carbonyl O in ILE92 (2.72 Å) whereas in the case of 3PHEP it is between H atom of NH group present in ligand with carbonyl O in ILE92 (2.96 Å). The number of hydrophobic interactions present in 2CHAP and 3PHEP are 5 and 6 respectively. A pi-sulfur interaction is observed between pyridine ring of ligand with S atom of MET32 residue in the case of 3PHEP.

Docking studies of 2PEHC with clumping factor A (ClfA): Highest binding energy of -6.9 kcal/mol was observed in site 2 and 4 of 1N67 upon docking of 3PEHC with this target protein. There are five H bond interactions. H atom of terminal NH_2 group forms three H bond interactions. 1)With O atom of carbonyl group in TYR448 (2.10 Å), 2) with O atom of carbonyl group in SER447 (2.77 Å), 3) with O atom of OH group in TYR399 (2.25 Å). Carbonyl oxygen forms H bond interaction with TYR399 (2.75 Å). N atom in the pyridine moiety forms an H bond with ARG395 (2.76 Å). Also present two pi-alkyl interactions in site 2 and 4 of 1N67.

Docking studies of 2PEHCT with dihydrofolate reductase (DHFR): When 2PEHCT is docked with target protein it is found that the first three sites of 2W9S have highest binding energy of -6.5, -6.3 and -6.4 kcal/mol respectively. Thus it deactivates dihydrofolate reductase. There are 3 conventional H bond and 7 other interactions in all cases. Considering the interactions along with binding energy site 1 is found to be slightly more active than other two sites. The H bond interaction is between pyridine N

with H in ALA7 (2.83 Å), NH hydrogen with phenolic oxygen in TYR98 (2.51 Å) and NH hydrogen with carbonyl oxygen in PHE92 (2.05 Å). All hydrophobic interactions are between pyridine ring of 2PEHCT and amino acid residues of 2W9S. The S atom of the ligand interacts with phenyl ring in TYR98.

Docking studies of 3TMAB with clumping factor A (ClfA): The best binding mode of 2TMAB was observed in site 2 of 1N67 with a binding energy -7 kcal/mol. Three conventional H bond interactions with TYR399, PRO251, ASP385 (between N atom of imine group with H of TYR399 (2.71 Å), H of COOH group with carbonyl O in PRO25 (2.41 Å) and ASP385 (2.68 Å)) and seven other interactions were observed in this site. On comparing with site 2 of 1N67, the binding energy of site 1 of 2ZCO (-7.3 kcal/mol) and site 3 of 2W9S (-7 kcal/mol) is comparable but less interactions.

Docking studies of 2PHEP with dihydrofolate reductase (DHFR): Same binding energy value of about -7.9 kcal/mol is observed in site 3 of 2W9S and site 1 of 4H8E when 2PHEP is docked with different sites of various target protein in *S. aureus*. Also slightly comparable affinity in site 1 of 2W9S with a binging energy -7.7 kcal/mol is observed. More probability of best binding is in 2W9S due to the presence of conventional hydrogen bond interaction between NH hydrogen with carbonyl O in PHE92 (1.90 Å) and 8 other interactions such as Van der Waals, non-conventional H bond, pi-sigma, pi-alkyl and pi-pi T shaped interaction.

In brief out of 6 target protein the Schiff base compounds are active against 2W9S, 1N67, 4H8E, 2ZCO than IT2P and 3U2D. Maximum binding energy of the ligands varies between -6.5 to -10.3 kcal/mol. Maximum binding energy of -10.3 kcal/mol is observed when DMCHDP was docked with 2W9S. It is observed that DMCHDP and DMCHDA have high binding energy compared to the other Schiff bases. This is attributed to the fact that in the active pockets of the target having large size the

bulky ligands will bind strongly. The ligands 2PEHC and 2PEHCT have lowest binding energy compared to other ligands. This is supported by *in vitro* antibacterial analysis of the ligand molecules. Diameter of zone of inhibition exhibited by DMCHDP and DMCHDA are 26 mm and 25 mm respectively at 500 μgdisc^{-1}. In the case of 2PEHC and 2PEHCT it is only 17 mm and 11 mm respectively at 500 μgdisc^{-1}. Total number of interactions is high in the case of DMCHHC and is supportive to the results of Lipinski rule of five. In the case of ligands having high molecular mass the effect of hydrophobic interactions will slightly predominate than conventional hydrogen bond [79]. Table 11.6 and 11.7 indicate the amino acid residues interacted with Schiff base compounds. 3D and 2D interaction diagrams of Schiff bases are shown in Fig. 11.2(a-j) and Fig. 11.3(a-j) respectively. Schiff base compounds derived from 5,5-dimethylcyclohexanone such as DMCHDP, DMCHDA and DMCHHC are found to be active against the target 2W9S. The ligands DMCHDP and 2PHEP are found to occupy in the same active pocket of the target protein 2W9S whereas 2CHAP and 3PHEP will occupy in the same active pocket of the target 4H8E.

Docking studies of Schiff base compounds with targets in Escherichia coli

The binding affinity of the ten Schiff bases in the four sites of 7 targets in *Escherichia coli* such as β-ketoacyl-acyl carrier protein synthase III (ecKAS III) (PDB ID: 1HNJ), Peptide deformylase (PDF) (PDB ID: 1G2A), L-glutamine: D-fructose-6-phosphate amido-transferase (PDB ID: 2VF5), murB (PDB ID: 2MBR), heptosyltransferase WaaC (PDB ID: 2GT1), mur D (PDB ID: 2X5O) and biotin carboxylase (BC) (PDB ID: 2W6O) were studied. Highest binding energies and number of interactions of all Schiff base compounds with protein models under study are enlisted in Table 11.8 and 11.9. Here also the active site and efficiency are predicted on

Table 11.6 Interactions of Schiff bases, DMCHDP, DMCHDA, DMCHHC, 2HBAP and 2CHAP, with amino acid residues present in the binding pockets of various target proteins of *S.aureus*

Schiff base	Active target	Active site	Binding energy (kcal/mol)	Interactions	Amino acid residue
DMCHDP	2W9S	3	-10.3	Van der Waals	ILE14, GLY15, VAL6, LYS45
				Hydrogen bond	ALA7 (HB-2.24 Å), THR46 (NCHB-3.28 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)
DMCHDA	2W9S	2	-10.2	Van der Waals	ILE14
				Hydrogen bond	SER49 (HB - 2.06 Å)
				Hydrophobic	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)
DMCHHC	1N67	1	-8.1	Van der Waals	ILE339, PHE449, ARG395
				Hydrogen bond	VAL450 (HB - 2.28 Å), HIS252(HB - 2.74 Å), ASP385(HB - 2.08 Å), ASP385(HB - 2.43 Å), PRO251(HB - 2.74 Å)
				Hydrophobic	PRO341(R- 4.43 Å), PRO341(R- 4.68 Å), VAL288(R- 3.43 Å), VAL288(R- 4.08 Å), VAL288(R- 5.13 Å)
2HBAP	2ZCO	1	-7.6	Van der Waals	ASN168, ASP48, TYR41
				Hydrogen bond	VAL133(HB - 2.94 Å), GLN165 (HB - 2.83 Å)
				Hydrophobic	PHE22 (π-T - 4.90 Å), LEU164 (π-σ - 3.98 Å), VAL137 (π-σ - 3.81 Å), ALA134 (π-R - 5.31 Å), CYS44 (π-R - 5.39 Å), val137 (π-R - 4.80 Å)
				Electrostatic	ARG45(π+ - 4.42 Å)
2CHAP	4H8E	1	-8.2	Van der Waals	HIS50, MET32
				Hydrogen bond	ILE92(HB - 2.72 Å)
				Hydrophobic	ALA76(R- 4.95 Å), PRO96 (R- 4.06 Å), PHE148(π-R - 4.42 Å), PHE99(π-R - 5.29 Å), ILE92(π-R - 5.15 Å)

HB - Conventional hydrogen bond, NCHB – Non-conventional hydrogen bond, π-T - π-π T shaped, amide-π stack- amide-π stacking, R – alkyl, π-R- pi-alkyl, π-σ- pi-sigma, π+ - pi-cation, π-stack – pi-pi stacking, π-S – pi-sulfur

Table 11.7 Interactions of Schiff bases, 3PHEP, 2PEHC, 2PEHCT, 3TMAB and 2PHEP, with amino acid residues present in the binding pockets of various target proteins of *S.aureus*

Schiff base	Active target	Active site	Binding energy (kcal/mol)	Interactions	Amino acid residue
3PHEP	4H8E	1	-8.2	Hydrogen bond	ILE92(HB - 2.96 Å)
				Hydrophobic	HIS50(π-stack - 4.83 Å), PHE99(π-T - 5.56 Å), LEU95(amide-π stack - 4.59 Å), PRO96(π-R - 3.86 Å), ALA76(π-R - 4.95 Å), ILE92(π-R - 4.98 Å)
				Pi-sulfur	MET32(π-S - 5.35 Å)
2PEHC	1N67	2	-6.9	Hydrogen bond	ARG395 (HB - 2.76 Å), TYR399 (HB - 2.75 Å), TYR399 (HB - 2.25 Å), TYR448 (HB - 2.10 Å), SER447(HB - 2.77 Å)
				Hydrophobic	VAL288(π-R - 3.71 Å), PRO341(π-R - 5 Å)
2PEHCT	2W9S	1	-6.5	Van der Waals	VAL6
				Hydrogen bond	ALA7(HB - 2.83 Å), TYR98(HB - 2.51 Å), PHE92(HB - 2.05 Å)
				Hydrophobic	PHE92(π-T - 4.95 Å), ILE5(amide-π stack - 4.39 Å), ILE31(π-σ - 3.78 Å), ALA7(π-R - 5.20 Å), ILE5(π-R - 5.21 Å)
				Pi-sulfur	TYR98(π-S - 5.40 Å)
3TMAB	1N67	2	-7.0	Van der Waals	SER447, TYR369, VAL288
				Hydrogen bond	PRO251(HB - 2.41 Å), ASP385(HB - 2.68 Å), TYR399(HB - 2.71 Å)
				Hydrophobic	HIS252(π-T - 4.74 Å), PRO341(π-R - 4.25 Å), PRO341(π-R - 4.49 Å)
				Electrostatic	HIS252(π+ - 3.79 Å)
2PHEP	2W9S	3	-7.9	Van der Waals	THR46
				Hydrogen bond	PHE92(HB - 1.90 Å), ASP27(NCHB - 3.64 Å)
				Hydrophobic	PHE92(π-T - 4.88 Å), LEU20(π-σ - 3.86 Å), ILE31(π-σ - 3.59 Å), ILE14(π-R - 4.90 Å), ILE5(π-R - 5.32 Å), ALA7(π-R - 5.25 Å)

HB - Conventional hydrogen bond, NCHB – Non-conventional hydrogen bond, π-T - π-π T shaped, amide-π stack- amide-π stacking, R – alkyl, π-R- pi-alkyl, π-σ- pi-sigma, π+ - pi-cation, π-stack – pi-pi stacking, π-S – pi-sulfur

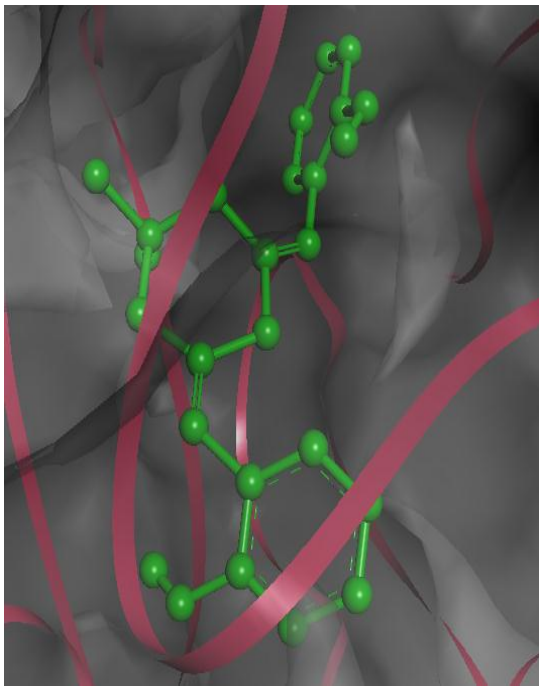

Fig. 11.2a 3D interaction diagram of DMCHDP with active site 3 of 2W9S

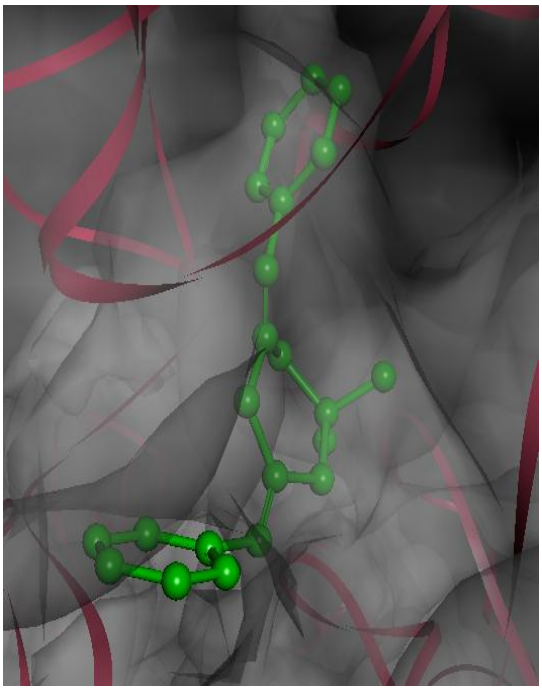

Fig. 11.2b 3D interaction diagram of DMCHDA with active site 2 of 2W9S

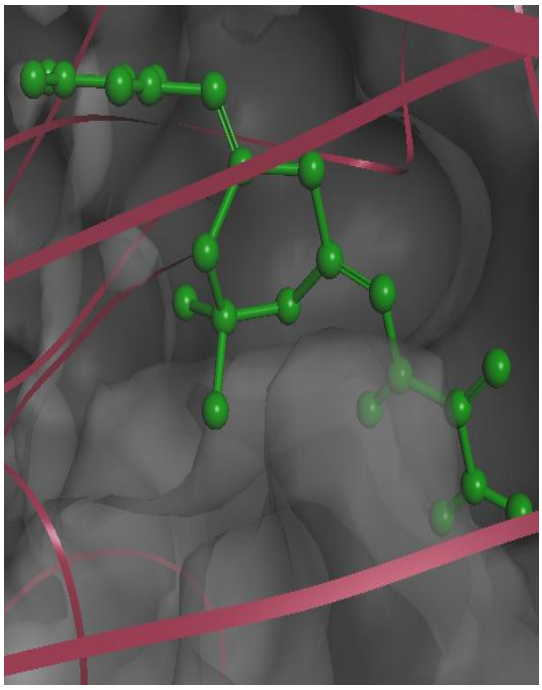

Fig. 11.2c 3D interaction diagram of DMCHHC with active site 1 of 1N67

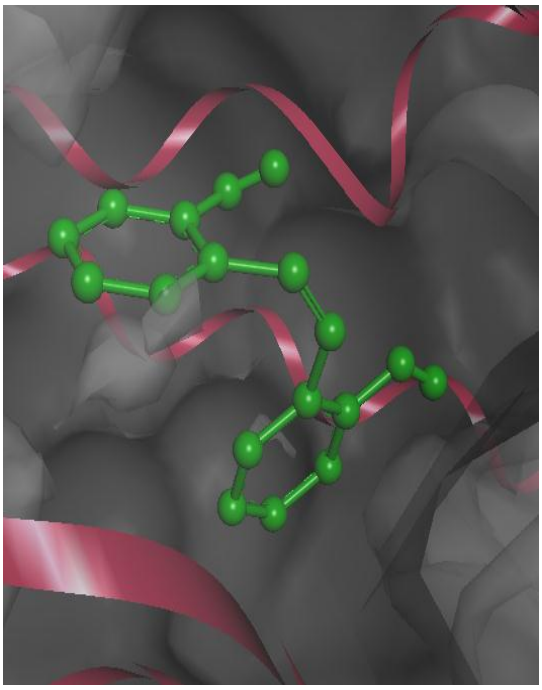

Fig. 11.2d 3D interaction diagram of 2HBAP with active site 1 of 2ZCO

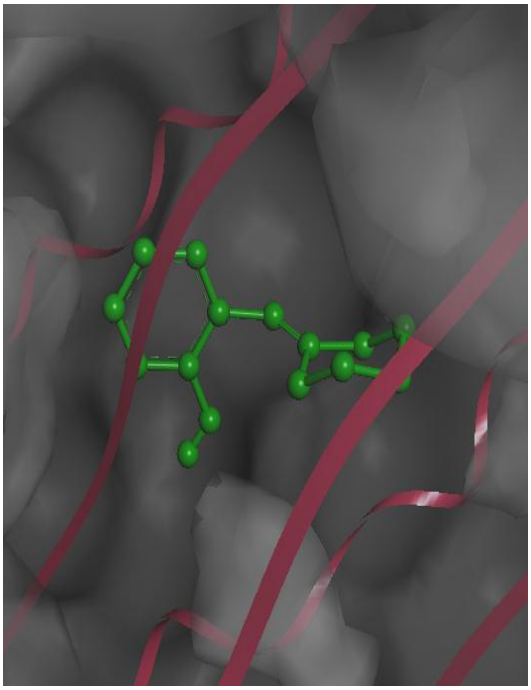

Fig. 11.2e 3D interaction diagram of 2CHAP with active site 1 of 4H8E

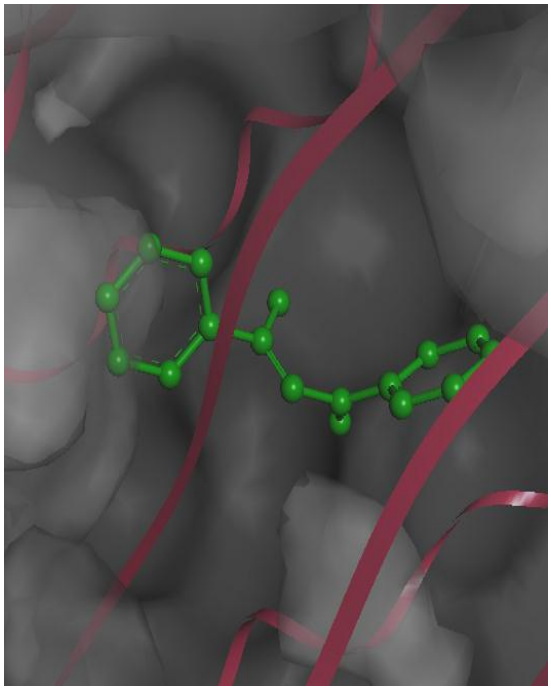

Fig. 11.2f 3D interaction diagram of 3PHEP with active site 1 of 4H8E

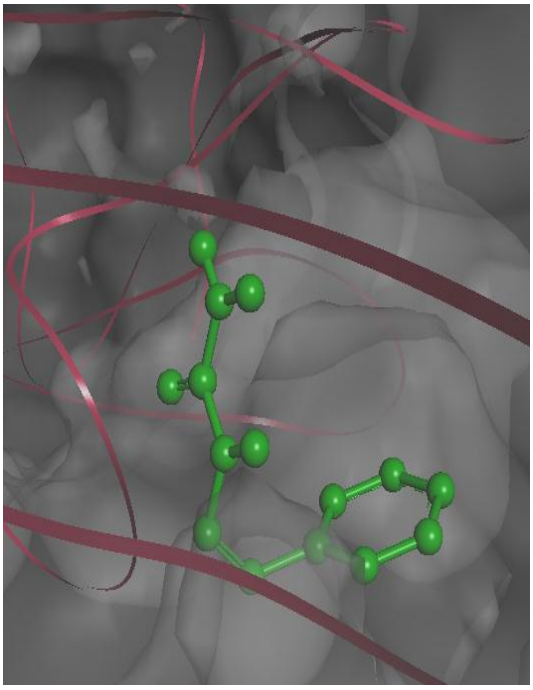

Fig. 11.2g 3D interaction diagram of 2PEHC with active site 2 of 1N67

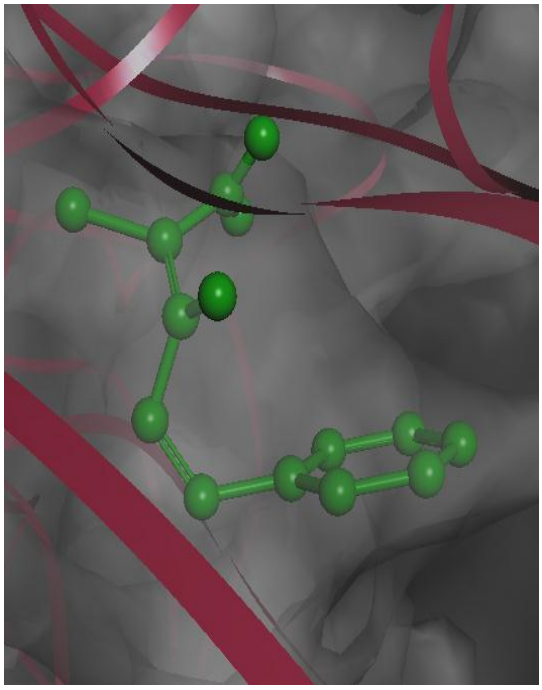

Fig. 11.2h 3D interaction diagram of 2PEHCT with active site 1 of 2W9S

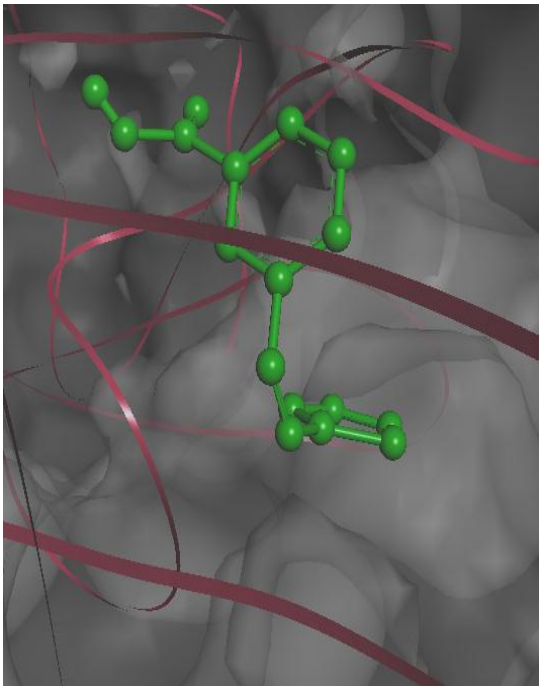

Fig. 11.2i 3D interaction diagram of 3TMAB with active site 2 of 1N67

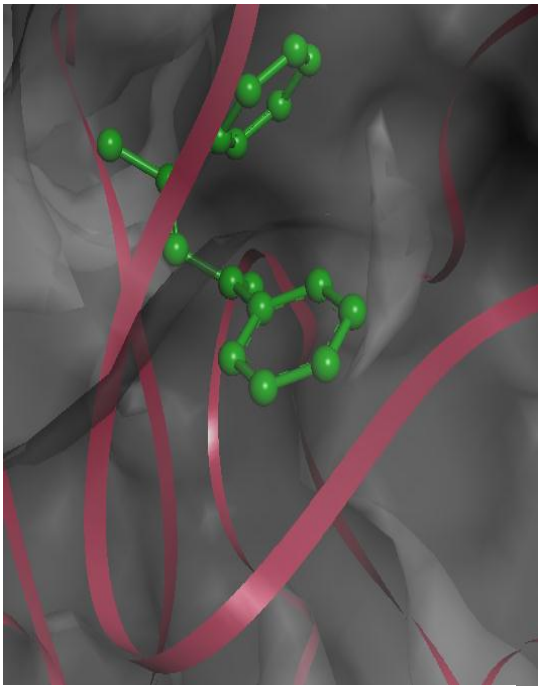

Fig. 11.2j 3D interaction diagram of 2PHEP with active site 3 of 2W9S

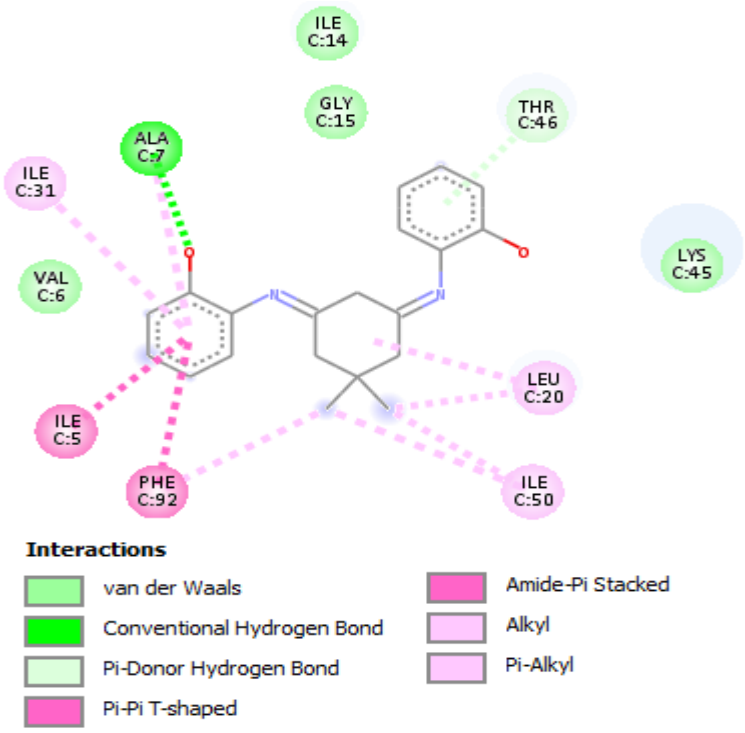

Fig. 11.3a 2D interaction diagram of DMCHDP with active site 3 of 2W9S

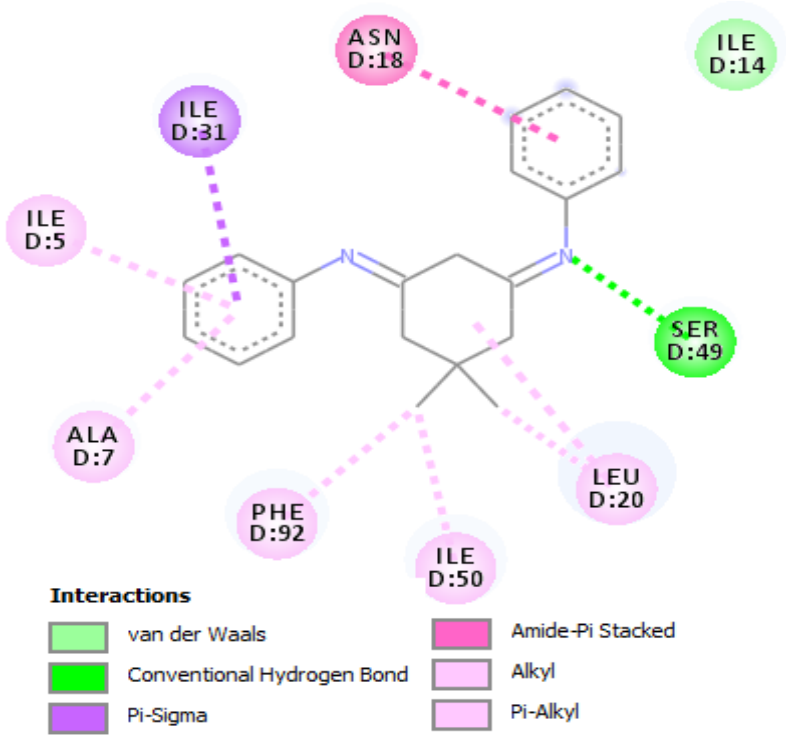

Fig. 11.3b 2D interaction diagram of DMCHDA with active site 2 of 2W9S

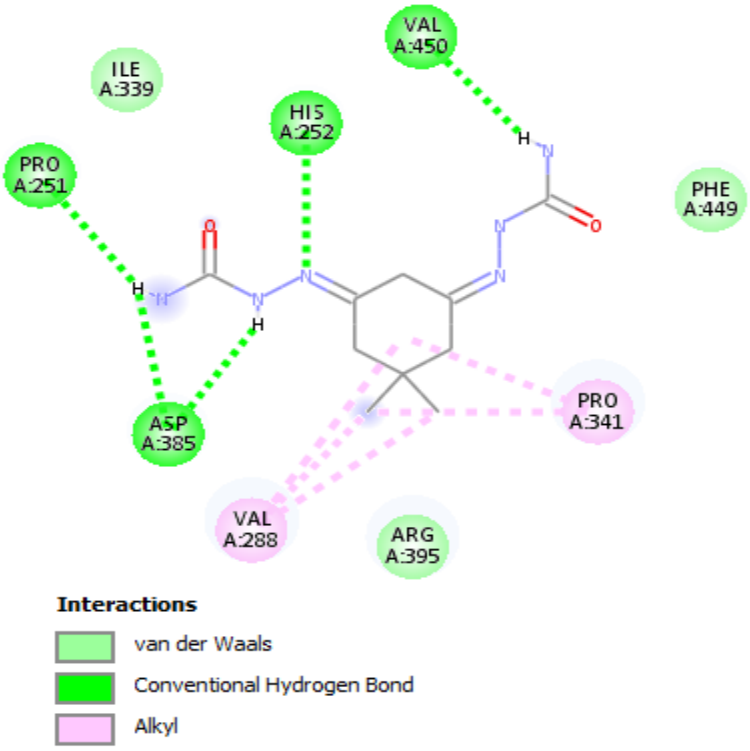

Fig. 11.3c 2D interaction diagram of DMCHHC with active site 1 of 1N67

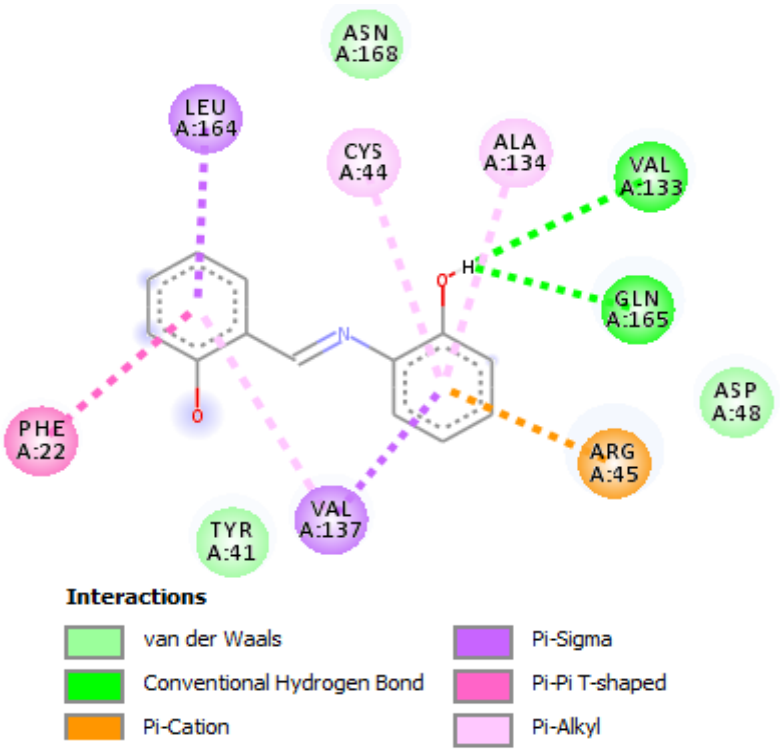

Fig. 11.3d 2D interaction diagram of 2HBAP with active site 1 of 2ZCO

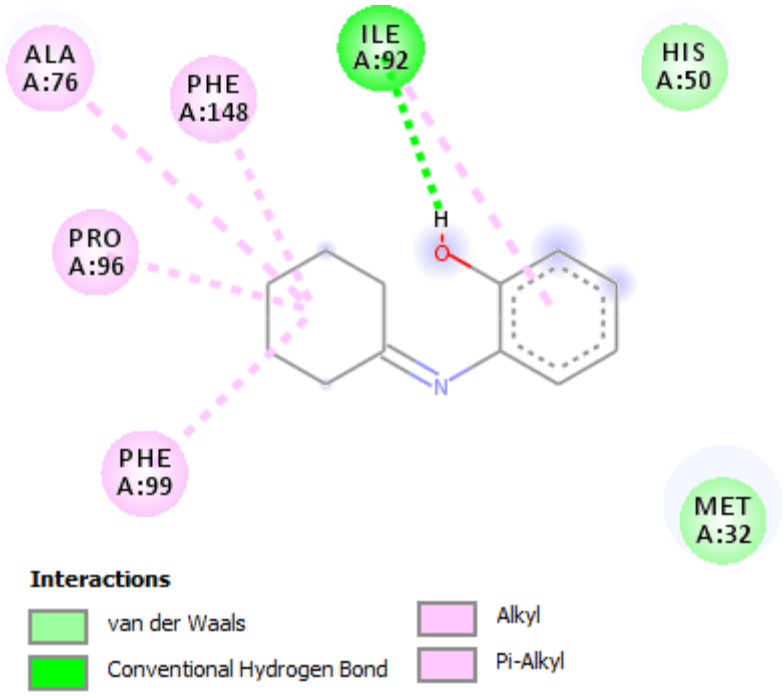

Fig. 11.3e 2D interaction diagram of 2CHAP with active site 1 of 4H8E

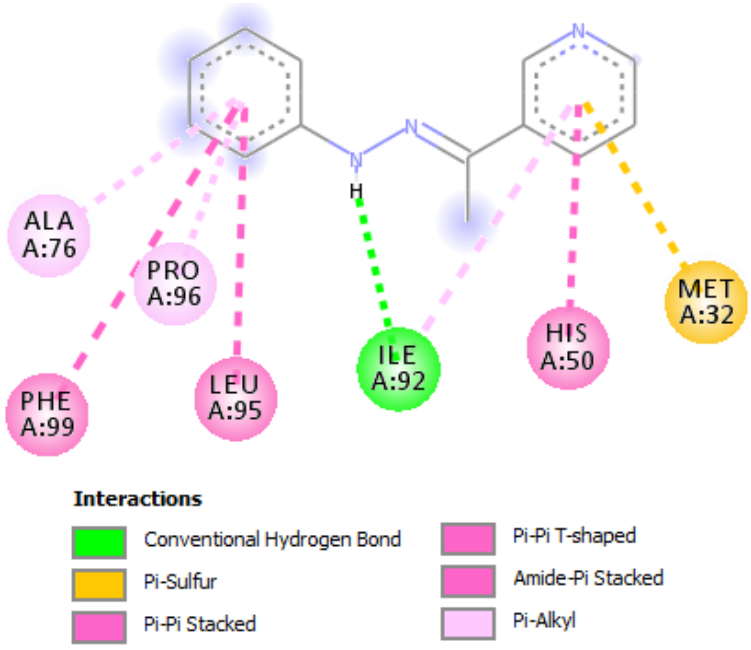

Fig. 11.3f 2D interaction diagram of 3PHEP with active site 1 of 4H8E

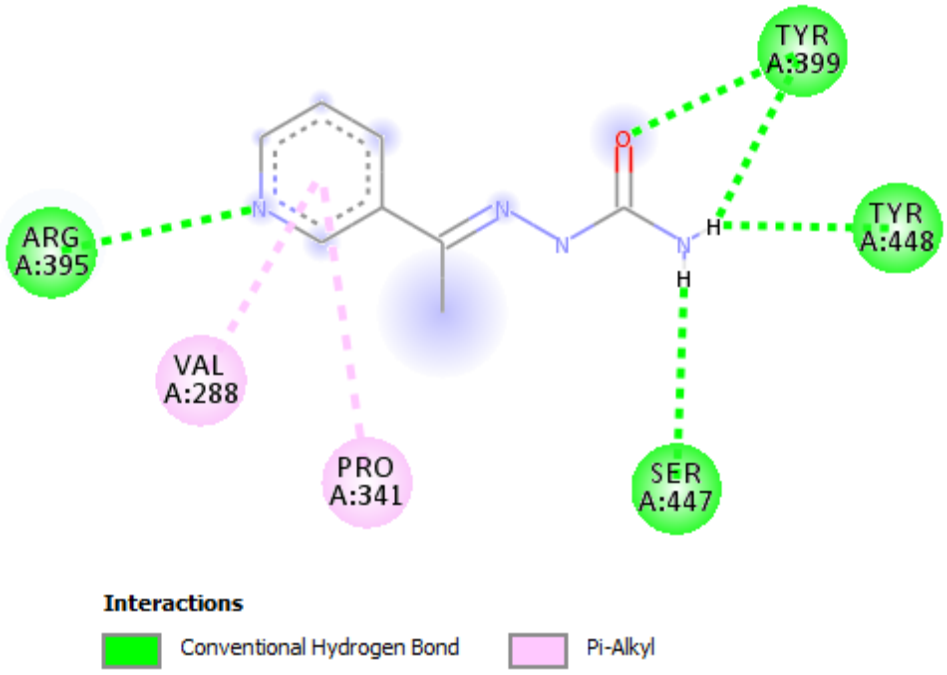

Fig. 11.3g 2D interaction diagram of 2PEHC with active site 2 of 1N67

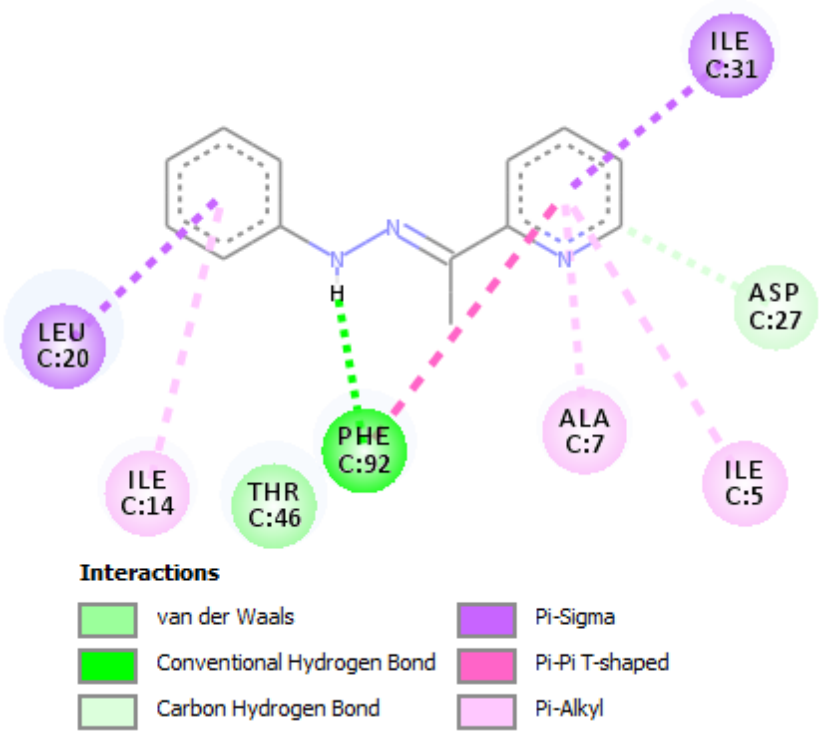

Fig. 11.3h 2D interaction diagram of 2PEHCT with active site 1 of 2W9S

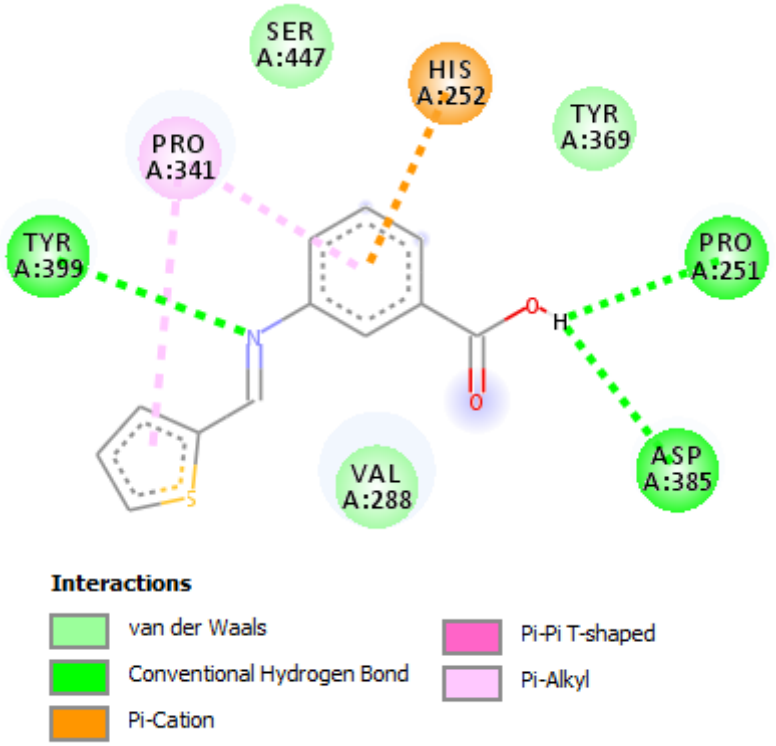

Fig. 11.3i 2D interaction diagram of 3TMAB with active site 2 of 1N67

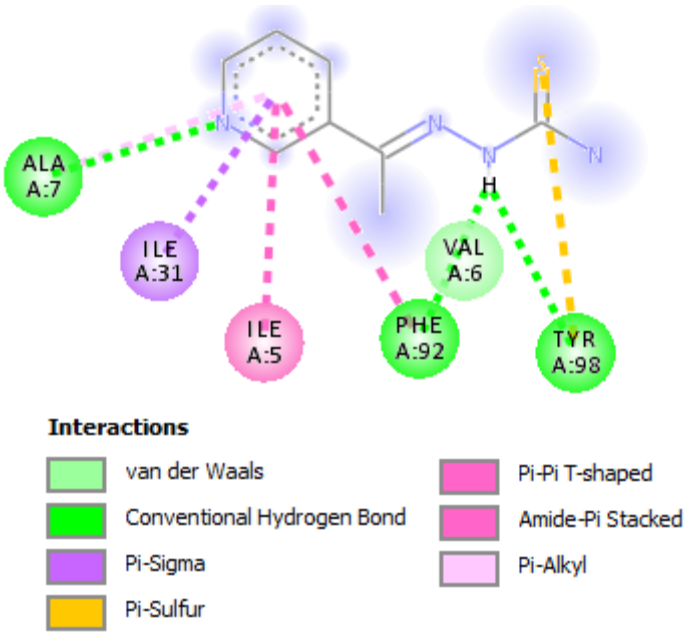

Fig. 11.3j 2D interaction diagram of 2PHEP with active site 3 of 2W9S

the basis of binding energy and interactions. The coordinate values of the four binding sites selected for docking are shown in Table 11.10.

Docking studies of DMCHDP with biotin carboxylase (BC): Highest binding energy of -8.8 and -8.9 kcal/mol is observed in the site 2 and 4 of 2W6O respectively for the ligand DMCHDP. In site 2 there are 2 H bond interactions between N of imine with H of GLY364 (3.08 Å) and phenolic O with GLY364 (2.74 Å) whereas in site 4 only one H bond between phenolic H with ASN340 (1.86 Å). In addition to this 13 other interactions are present in site 2. Hydrophobic interactions such as pi-sigma and pi-alkyl, Van der Waals, pi-donor H bond and unfavourable acceptor-acceptor interaction are present. In site 4 there is only 8 other interactions. So DMCHDP is slightly more active in site 2 than site 4 of 2W6O and deactivate the function of biotin carboxylase.

Table 11.8 Binding energy and number of interactions of Schiff bases, DMCHDP, DMCHDA, DMCHHC, 2HBAP and 2CHAP, docked with target proteins in *E.coli*

Schiff base	Binding energy and interactions	Target proteins and active sites																											
		1HNJ				1G2A				2VF5				2MBR				2GT1				2X5O				2W6O			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
DMCHDP	-BE	8.1	8	6.5	5.8	8.3	7.8	6.8	8	7.8	6.2	7.4	6.8	8.7	8.3	7.3	6.6	7.8	7.6	7.1	7.4	7.5	7.5	7.5	6.9	7.2	8.8	8.7	8.9
	HB	1	3	1	2	0	1	1	4	3	1	0	2	1	1	2	0	1	1	1	2	2	2	2	1	1	2	1	1
	Other	5	9	5	8	5	8	8	5	6	2	6	5	6	7	2	4	6	8	4	9	4	7	4	6	7	13	5	8
DMCHDA	-BE	7.6	7.4	6.5	5.3	8	7.1	6.5	7.1	7.1	6.1	7.1	6.5	9.1	7.8	6.9	6.3	7	7.3	7.3	7.3	7	7	7	6.7	6.7	8.1	8.3	8.3
	HB	1	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
	Other	4	10	8	7	5	6	6	7	9	5	6	7	7	8	3	4	7	5	5	7	4	4	5	6	4	9	5	5
DMCHHC	-BE	6.7	6.7	6.1	5.7	7.3	7.8	7.8	8	7.7	7.2	6.7	7.8	8.2	7.6	6.4	6.1	7.7	7.1	6.6	5.9	7.2	6.7	6.5	6.4	6.6	8.7	7.1	7.1
	HB	2	3	5	6	7	6	5	8	4	4	3	5	4	2	3	5	5	3	4	6	6	4	5	4	2	3	3	2
	Other	6	3	5	3	6	5	8	6	4	2	6	5	5	5	4	2	5	6	2	2	1	1	4	1	6	9	1	1
2HBAP	-BE	6.9	6	5.6	5.1	6.4	7.1	7.1	6.2	7.2	7.2	6	7.2	7.2	7.2	5.8	6	6.7	5.8	5.8	5.5	6.4	5.9	6	5.9	6.2	7.1	6.2	6.1
	HB	1	2	0	3	1	2	3	1	3	4	1	2	2	2	4	1	1	1	2	1	2	1	1	2	3	0	3	2
	Other	4	7	4	5	8	6	5	6	4	2	4	4	1	2	0	6	5	4	1	7	3	6	7	3	5	3	1	2
2CHAP	-BE	6.7	6.1	5.1	4.9	6.8	6.4	6.4	6.9	6	6	5.8	6	7.5	7.1	5.5	5.8	6.1	6	5.7	5.1	5.7	5	5.5	5.7	5.7	7	6.5	6.5
	HB	2	0	0	0	0	0	1	1	2	1	0	1	1	1	0	0	0	2	0	1	2	0	2	1	0	0	1	1
	Other	3	4	3	2	6	6	4	6	3	2	3	3	5	3	1	6	4	3	3	5	1	3	6	5	3	4	3	4

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

Table 11.9 Binding energy and number of interactions of Schiff bases, 3PHEP, 2PEHC, 2PEHCT, 3TMAB and 2PHEP, docked with target proteins in *E.coli*

Schiff base	Binding energy and interactions	Target proteins and active sites																											
		1HNJ				1G2A				2VF5				2MBR				2GT1				2X5O				2W6O			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
3PHEP	-BE	6.8	6.3	5.4	5	6.6	6.8	6.8	6.8	6.6	6.6	6.3	6.6	7.9	7	6.1	5.7	6.1	6.6	5.7	5.8	6.8	5.9	6	6.2	6.4	7.2	3	6.8
	HB	0	1	0	2	1	1	1	1	2	2	1	2	1	1	3	1	2	0	1	1	3	1	1	0	0	1	1	1
	Other	6	4	8	4	7	4	3	6	4	4	3	5	9	7	2	4	7	4	6	4	4	3	3	8	4	2	5	5
2PEHC	-BE	5.9	5.4	5.1	4.5	6.8	6.6	6.7	6.7	6.5	6.6	5.8	6.5	6.4	5.9	4.8	5	5.7	5.7	5.3	5	5.9	6.1	5.5	5.5	5.3	6.6	6.1	6.1
	HB	3	3	0	1	4	3	4	5	4	3	4	4	3	3	1	1	2	2	4	2	3	4	4	4	1	5	3	4
	Other	6	4	2	2	3	5	5	7	3	1	5	3	1	2	1	4	3	4	1	3	1	4	3	1	4	2	4	3
2PEHCT	-BE	5.3	5	4.8	4.3	5.6	5.7	5.7	5.7	5.8	5.6	5.2	5.8	6	5.6	4.8	5	5.4	4.9	4.8	4.8	5.9	5.1	5.2	4.9	5.2	5.8	5.7	5.6
	HB	1	3	2	2	4	4	4	2	3	5	3	4	4	3	5	1	1	2	3	2	2	2	2	2	2	3	2	3
	Other	6	2	2	4	5	6	6	3	2	0	5	1	1	1	2	4	5	3	2	2	0	4	4	2	2	2	4	3
3TMAB	-BE	5.9	6.1	5.5	5.1	6.5	6.1	6.5	6.4	6.7	6.5	6	6.7	7.2	6.6	5.5	5.6	6.1	6.1	5.4	5.4	6	5.4	6.1	5.4	6.3	6.8	6.2	6.3
	HB	1	2	2	1	1	1	2	2	3	3	2	2	3	1	3	0	2	2	2	1	5	1	2	1	3	2	0	0
	Other	6	4	3	3	9	8	7	7	3	1	5	1	5	6	2	4	5	2	3	2	1	7	3	4	5	3	2	1
2PHEP	-BE	6.7	6.5	5.7	5.2	7.1	6.8	6.8	7.1	6.4	6.4	6.4	6.2	7.6	5.9	5.3	6	6.3	6.4	5.7	5.8	6.6	5.7	5.7	6.2	6.3	6.9	6.8	6.8
	HB	0	1	0	2	2	2	2	2	1	2	1	0	1	1	1	0	0	0	1	1	1	1	1	0	0	1	0	1
	Other	5	7	4	4	7	7	8	7	3	4	5	3	10	4	2	5	8	4	6	7	1	3	7	5	3	4	5	5

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

Table 11.10 Coordinate values of active sites in target proteins of *E. coli*

Active site and coordinates		PDB ID of target proteins of *E. coli*						
		1HNJ	1G2A	2VF5	2MBR	2GT1	2X5O	2W6O
1	x	26.31	11.47	29.58	2.24	20.56	-3.93	11.86
	y	15.79	-9.03	17.24	11.38	33.89	23.60	1.57
	z	31.30	10.24	-0.64	15.58	56.00	16.79	5.54
2	x	44.56	-9.53	39.08	-7.26	37.81	-20.68	-8.13
	y	21.29	-6.78	6.74	23.63	43.14	23.85	9.32
	z	26.05	33.74	4.35	6.58	60.50	16.79	20.29
3	x	22.06	-2.28	10.83	15.24	-2.43	-19.18	-35.13
	y	31.79	-15.03	29.24	-5.12	50.14	34.85	16.82
	z	17.30	27.24	11.60	15.33	42.50	16.79	37.29
4	x	31.06	45.72	23.58	-6.76	-17.43	-18.68	-20.63
	y	34.54	4.713	15.74	-13.12	41.89	18.35	8.07
	z	4.05	14.74	9.10	20.33	35.25	5.29	38.79

Docking studies of DMCHDA with murB: DMCHDA may inhibit the growth of *E. coli* by deactivating murB enzyme (2MBR). Binding energy, number of conventional hydrogen bond interactions, number of hydrophobic interactions are respectively -9.1 kcal/mol, 1 (N of imine with H of ARG214 (2.67 Å) and 7. Out of seven other interactions, four are alkyl interactions and three are pi-alkyl interactions. Alkyl interaction is between cyclohexanone part of the ligand and the amino acid residues PRO111, ILE110, ILE122 whereas pi-alkyl interaction is between phenyl ring and amino acid residues PRO221, LEU218 and PRO111.

Docking studies of DMCHHC with biotin carboxylase (BC): DMCHHC is more active in site 2 of 2W6O with a binding energy of -8.7 kcal/mol. Three H bond is formed between H atom of terminal NH_2 group with VAL365 (2.74Å) and carbonyl O with ARG33 (2.87Å) and GLY364 (2.11Å). Six hydrophobic interactions arise from the cyclohexanone part of the ligand and also there are three Van der Waals interactions. Thus it deactivates the enzyme biotin carboxylase.

Docking studies of 2HBAP with L-glutamine: D-fructose-6-phosphate amido-transferase: Even though the binding energy of sites 1 and 2 of both 2VF5 and

2MBR are same (-7.2 kcal/mol) when 2HBAP is docked with these target proteins the activity is high against 2VF5 due to large number of interactions. In site 2 of 2VF5 there are 4 H bond interactions (phenolic O with THR302 (2.63 Å), N of imine with SER401 (2.74 Å), phenolic H with ALA602 (2.27 Å) and LYS603 (2.46 Å) and 2 other interactions. Thus it deactivates the function of L-glutamine: D-fructose-6-phosphate amido-transferase in preference to murB enzyme.

Docking studies of 2CHAP and 3PHEP with murB: The ligands 2CHAP and 3PHEP are active in site 1 of 2MBR with a binding energy of -7.5 and -7.9 kcal/mol respectively. Number of H bond interactions is same in both cases whereas other interactions are 5 and 9 respectively for 2CHAP and 3PHEP. H bond is formed with same amino acid residue SER116 by imine N of 2CHAP (2.29 Å) and N in pyridine moiety of 3PHEP (2.26 Å). Both the ligands thus deactivate the function of the enzyme murB.

Docking studies of 2PEHC with peptide deformylase (PDF): Binding energies of -6.6, -6.7 and -6.8 kcal/mol were observed in different sites of the target protein 1G2A when 2PEHC is docked into it. But considering the interactions and applying hypothetical equation it is found that 2PEHC is more active in site 4 of 1G2A where binding energy is -6.7 kcal/mol. Here 5 H bonds are formed between O atom of carbonyl group with LEU91 (1.89 Å) and GLN50 (2.55 Å), NH hydrogen with O of COO group in GLU133 (2.28 Å) and H atoms of terminal NH_2 with same O atom of COO group in GLU133 (1.96 Å) and with O atom of carbonyl group in GLN50 (2.31 Å). In addition there are 7 other interactions present in this site. Here the pi-pi stacking interaction is between pyridine ring and imidazole ring in HIS132 residue. So the deactivating ability of 2PEHC is more prominent against peptide deformylase.

Docking studies of 2PEHCT with peptide deformylase (PDF): It is found that 2PEHCT has comparable activity against both 1G2A and 2VF5. The sites 2 and 3 of 1G2A are found to be equally active with a binding energy of -5.7 kcal/mol and same number of interactions. In 2VF5 sites 1 and 4 are more active. Considering the three factors to select the active site, 2PEHCT has more binding affinity in sites 2 and 3 of 1G2A. In site 2 there are 4 conventional hydrogen bond interactions (H atom of NH group with carbonyl oxygen of GLY89 (2.12 Å), H atom of terminal NH_2 group with carbonyl oxygen of GLY45 (2.30 Å), S atom with H of LEU9 (2.69 Å) and HIS132 (2.98 Å)) and 6 other interaction. As in the case of 2PEHC the pi-pi stacking interaction is between pyridine ring and imidazole ring in HIS132 residue. The sulphur atom present in the ligand undergoes pi-sulfur interaction with imidazole ring in HIS136 residue.

Docking studies of 3TMAB and 2PHEP with murB: Both 3TMAB and 2PHEP are found to be active in site 1 of 2MBR with a binding energy of -7.2 and 7.6 kcal/mol. When 3TMAB is docked into site 1 of 2MBR three H bond interaction were observed between carbonyl oxygen of COOH group and H of GLY47 (1.87 Å), GLY49 (2.01 Å), and GLU48 (2.68 Å), whereas only one H bond (N of pyridine moiety with ASP169(2.29 Å)) is observed in the case of 2PHEP. Number of interaction other than H bond is 5 and 10 respectively for 3TMAB and 2PHEP. Thus they deactivate murB enzyme.

In brief out of 7 target proteins the Schiff bases are more active against the targets 2MBR, 2W6O, 2VF5 and 1G2A than 1HNJ, 2GT1 and 2X5O. Majority of the compounds are more active against murB enzyme (2MBR) and site 1 of the enzyme is most active. Maximum binding energy of the compounds varies between -5.7 to -9.1 kcal/mol. Highest binding energy is observed for DMAN

(-9.1 kcal/mol). The binding energy of DMCHDP, DMCHDA and DMCHHC are high compared to the other Schiff bases. This is also attributed to the high molecular mass. Here also 2PEHC and 2PEHCT have the lowest activity against target proteins compared to other ligands which is supported by experimental data. The diameter of zone of inhibition exhibited by DMCHDP, DMCHDA and DMCHHC are 22 mm, 24 mm and 22 mm respectively at 500 µgdisc^{-1}. But in the case of 2PEHC and 2PEHCT it is only 13 mm and 9 mm respectively at 500 µgdisc^{-1}. The Table 11.11 and 11.12 indicates the amino acid residues interacted with ligands. The 3D and 2D interaction diagram of ligands are shown in Fig. 11.4(a-j) and Fig. 11.5(a-j) respectively. It is observed that the Schiff bases DMCHDA, 2CHAP, 3PHEP, 3TMBA and 2PHEP occupy in the same active pocket of the target 2MBR.

In general, the stability of a protein-ligand complex is proportional to the binding energy. But some complexes which display moderate binding score may have increased number of H bond interactions and hydrophobic interactions. In order to consider the overall affinity of a ligand towards a protein receptor, factors like binding energy, H bond and other interactions have to be taken into account. By analysing the docking result we suggest a hypothetical equation 80%X+15%Y+5%Z for predicting the overall affinity of a ligand towards a receptor. Here X=binding energy, Y= number of conventional hydrogen bond interactions and Z= number of interactions other than conventional hydrogen bond.

Table 11.11 Interactions of Schiff bases, DMCHDP, DMCHDA, DMCHHC, 2HBAP and 2CHAP, with amino acid residues present in the binding pockets of various target proteins of *E.coli*

Schiff base	Active target	Active site	Binding energy (kcal/mol)	Interactions	Amino acid residue
DMCHDP	2W6O	2	-8.8	Van der Waals	PHE363, ARG331, TYR391
				Hydrogen bond	GLY364(HB - 2.74 Å), GYY364(HB - 3.08 Å), TYR391(HB - 2.78 Å)
				Hydrophobic	MET302(π-σ - 3.67 Å), ARG366(π-R - 4.71 Å), ARG331(π-R - 5.44 Å), TYR391(π-R - 4.74 Å), PHE363(π-R - 5.14 Å), PHE363(π-R - 4.10 Å), PHE363(π-R - 4.80 Å), TYR391(π-R - 4.63 Å)
				Unfavourable acceptor-acceptor	MET302(2.89 Å)
DMCHDA	2MBR	1	-9.1	Hydrogen bond	ARG214(HB - 2.67 Å)
				Hydrophobic	ILE110(R - 4.54 Å), PRO111(R - 5.35 Å), ILE110(R - 3.70 Å), ILE122(R -4.74 Å), PRO221(π-R - 5.49 Å), LEU218(π-R - 5.22 Å), PRO111(π-R - 3.82 Å)
DMCHHC	2W6O	2	-8.7	Van der Waals	VAL365, MET302, PHE363
				Hydrogen bond	VAL365 (HB - 2.74 Å), GLY364(HB - 2.11 Å), ARG331(HB - 2.87 Å)
				Hydrophobic	MET302 (R - 5.40 Å), MET302 (R- 4.28 Å), TYR391(π-R - 4.69 Å), PHE363(π-R - 5.03 Å), TYR391(π-R - 5.21 Å), PHE363(π-R - 5.07 Å)
2HBAP	2VF5	2	-7.2	Hydrogen bond	THR302(HB - 2.63 Å), SER401(HB - 2.74 Å), ALA602(HB - 2.27 Å), LYS603(HB - 2.46 Å)
				Electrostatic	GLU488(π- - 4.35 Å)
				Unfavourable donor- donor	SER303(1.26 Å)
2CHAP	2MBR	1	-7.5	Van der Waals	ASN51
				Hydrogen bond	SER116(HB - 2.29 Å)
				Hydrophobic	ILE119 (R - 5.26 Å), ILE45(π-σ - 3.99 Å), ILE173(π-R - 5.18 Å), ILE119(π-R - 5.24 Å)

HB - Conventional hydrogen bond, NCHB – Non-conventional hydrogen bond, π-T - π-π T shaped, amide-π stack- amide-π stacking, R – alkyl, π-R- pi-alkyl, π-σ- pi-sigma, π+ - pi-cation, π-stack – pi-pi stacking, π-S – pi-sulfur

Table 11.12 Interactions of Schiff bases, 3PHEP, 2PEHC, 2PEHCT, 3TMAB and 2PHEP, with amino acid residues present in the binding pockets of various target proteins of *E.coli*

Schiff base	Active target	Active site	Binding energy (kcal/mol)	Interactions	Amino acid residue
3PHEP	2MBR	1	-7.9	Van der Waals	GLY115, GLU334
				Hydrogen bond	SER116 (HB - 2.26 Å), ILE45(NCHB - 3.42 Å)
				Hydrophobic	ILE45(π-σ - 3.81 Å), LEU44 (π-R - 5.33 Å), ARG327(π-R - 4.81 Å), ILE173(π-R - 5.40 Å)
				Electrostatic	ARG327(π+ -4.06 Å), ARG327(π+ -3.88 Å)
2PEHC	1G2A	4	-6.2	Van der Waals	GLY89, GLY45
				Hydrogen bond	LEU91(HB - 1.89 Å), GLN50(HB - 2.55 Å), GLY50(HB - 2.31 Å), GLU133(HB - 1.96 Å), GLU133(HB - 2.28 Å), GLU133(NC - 3.49 Å)
				Hydrophobic	HIS132(π-stack - 4.03 Å), ILE44(π-R - 4.87 Å), CYS129(π-R - 4.71 Å)
				Electrostatic	HIS132(π+ - 4.33 Å)
2PEHCT	1G2A	2	-5.7	Hydrogen bond	GLY45(HB - 2.30 Å), LUE91(HB - 2.69 Å), HIS132(HB - 2.98 Å), GLU89(HB - 2.12 Å)
				Hydrophobic	HIS132(π-stack - 4.34 Å), CYS129(π-R - 4.87 Å), ILE44(π-R - 5.45 Å)
				Electrostatic	HIS132(π+ - 4.83 Å)
				Pi-sulfur	HIS136(π-S - 5.69 Å)
				Unfavourable donor- donor	GLY45(1.92 Å)
3TMAB	2MBR	1	-7.2	Van der Waals	ASN51
				Hydrogen bond	GLY47(HB - 1.87 Å), GLU48(HB - 2.68 Å), GLY49 (HB - 2.01 Å)
				Hydrophobic	LEU44(π-σ - 3.85 Å), ILE119(π-R - 4.89 Å), ARG327(π-R - 4.67 Å)
				Electrostatic	ARG327(π+ - 4.25 Å)
2PHEP	2MBR	1	-7.6	Van der Waals	ILE45, ILE73
				Hydrogen bond	ASP169 (HB - 2.29 Å), PHE163(NCHB - 3.59 Å), TYR167(NCHB - 3.56 Å), GLN168(NCHB - 3.55 Å)
				Hydrophobic	ILE119(π-R - 5.22 Å), LEU44(π-R - 5.47 Å), ARG327(π-R - 4.63 Å)
				Electrostatic	ARG327(π+ - 3.89 Å), ARG327(π+ - 3.73 Å)

HB - Conventional hydrogen bond, NCHB – Non-conventional hydrogen bond, π-T - π-π T shaped, amide-π stack- amide-π stacking, R – alkyl, π-R- pi-alkyl, π-σ- pi-sigma, π+ - pi-cation, π-stack – pi-pi stacking, π-S – pi-sulfur

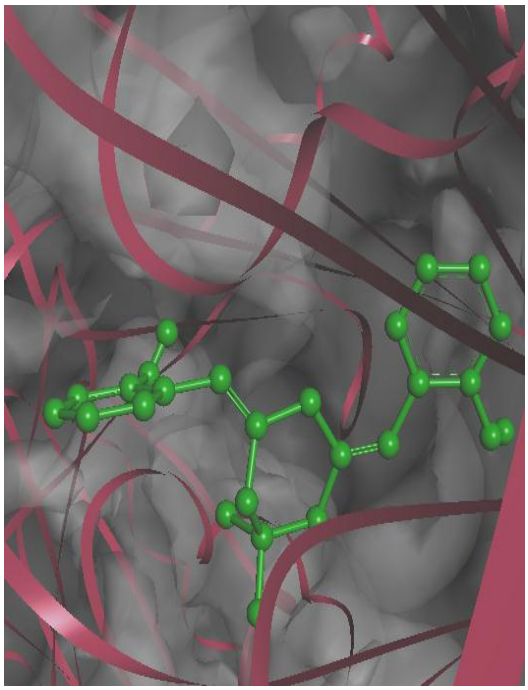

Fig. 11.4a 3D interaction diagram of DMCHDP with active site 2 of 2W6O

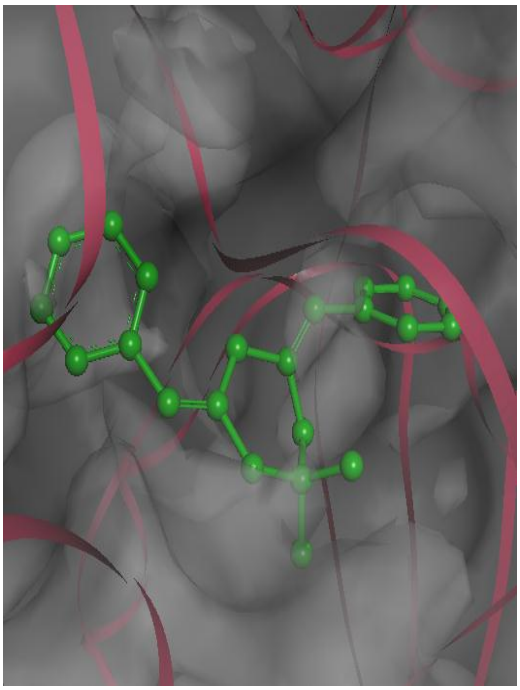

Fig. 11.4b 3D interaction diagram DMCHDA with active site 1 of 2MBF

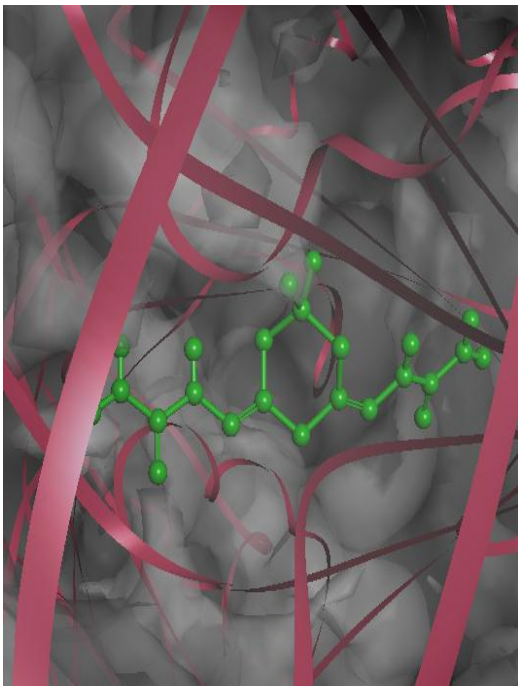

Fig. 11.4c 3D interaction diagram of DMCHHC with active site 2 of 2W6O

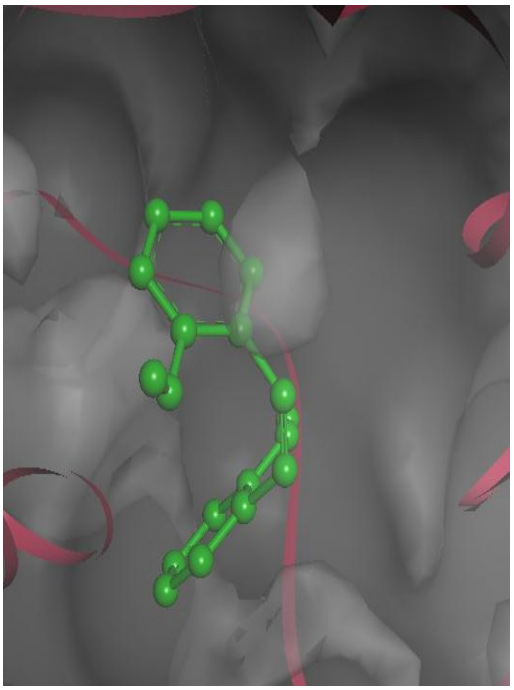

Fig. 11.4d 3D interaction diagram of 2HBAP with active site 2 of 2VF5

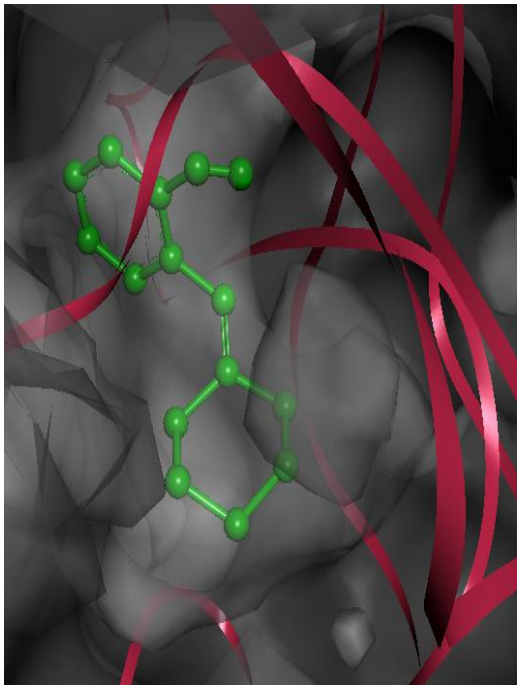

Fig. 11.4e 3D interaction diagram of 2CHAP with active site 1 of 2MBR

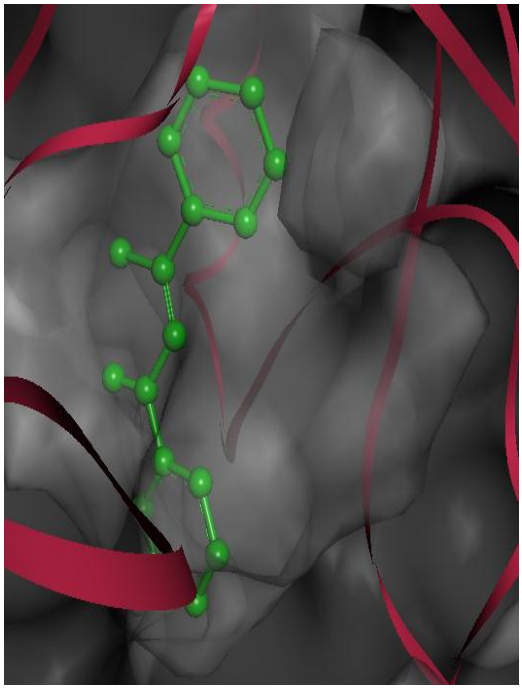

Fig. 11.4f 3D interaction diagram of 3PHEP with active site 1 of 2MBR

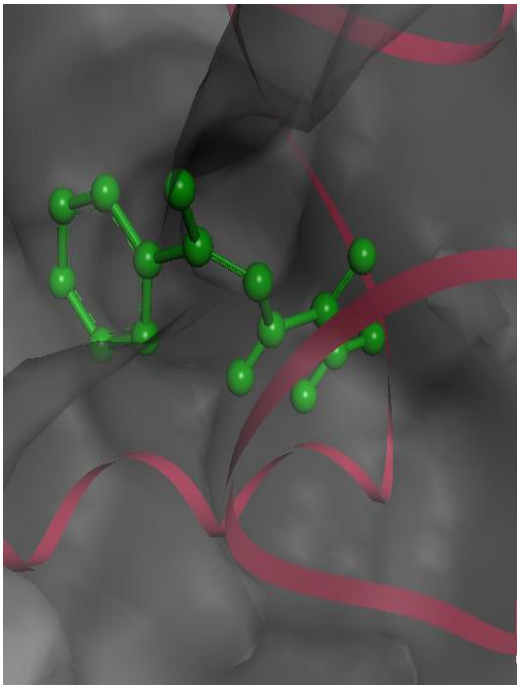

Fig. 11.4g 3D interaction diagram of 2PEHC with active site 4 of 1G2A

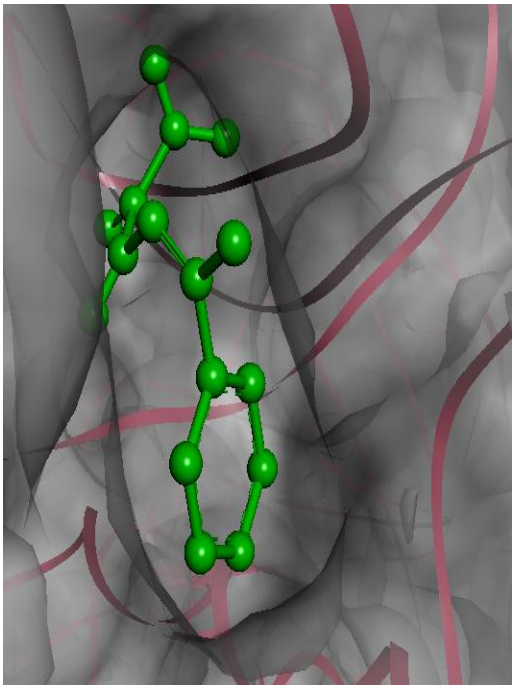

Fig. 11.4h 3D interaction diagram of 2PEHCT with active site 2 of 1G2A

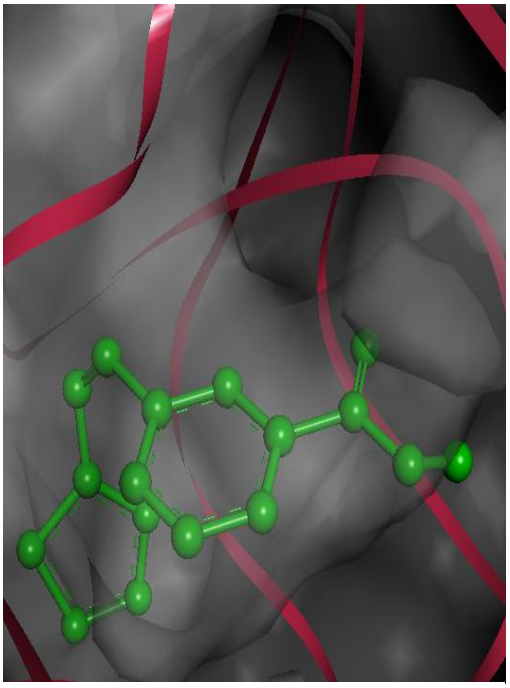

Fig. 11.4i 3D interaction diagram of 3TMAB with active site 1 of 2MBR

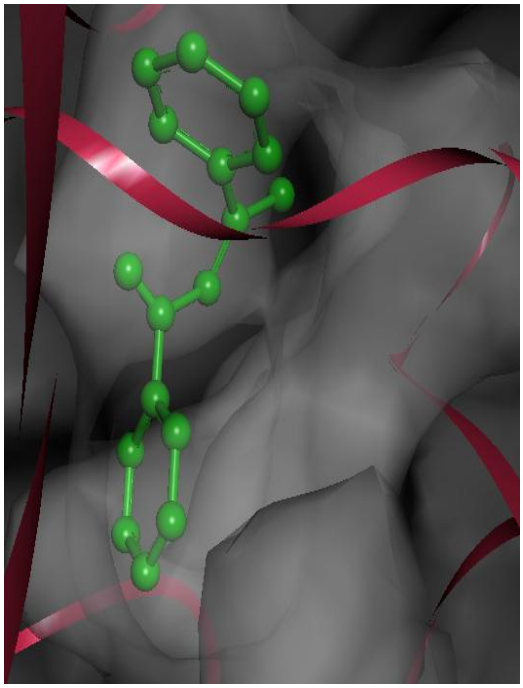

Fig. 11.4j 3D interaction diagram of 2PHEP with active site 1 of 2MBR

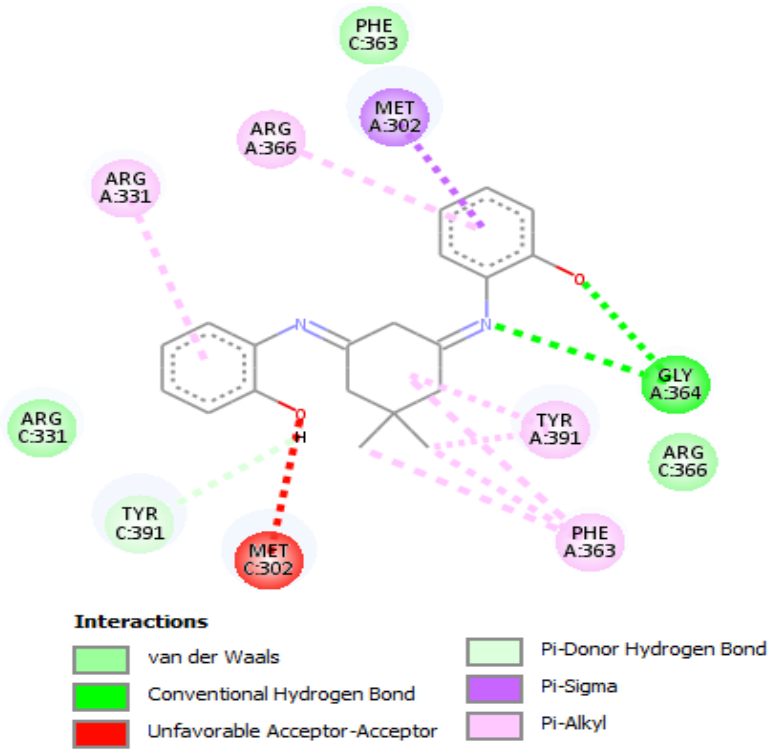

Fig. 11.5a 2D interaction diagram of DMCHDP with active site 2 of 2W6O

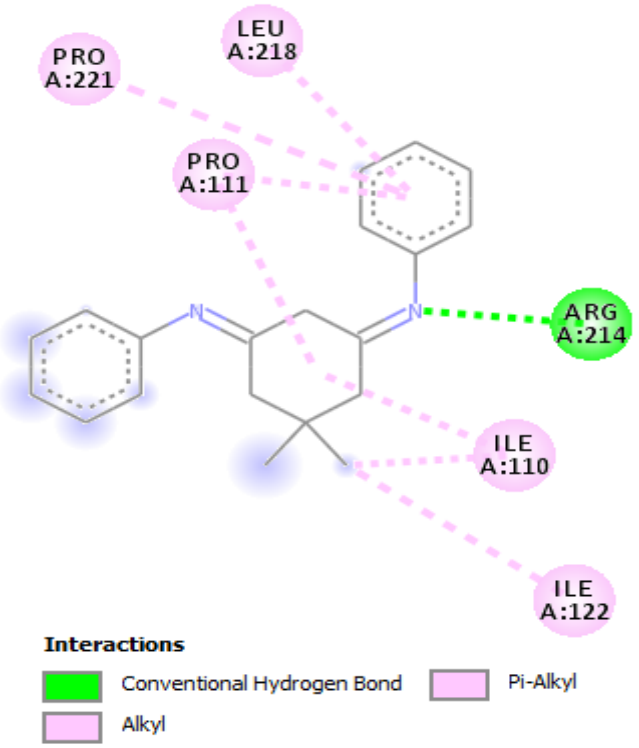

Fig. 11.5b 2D interaction diagram of DMCHDA with active site 1 of 2MBR

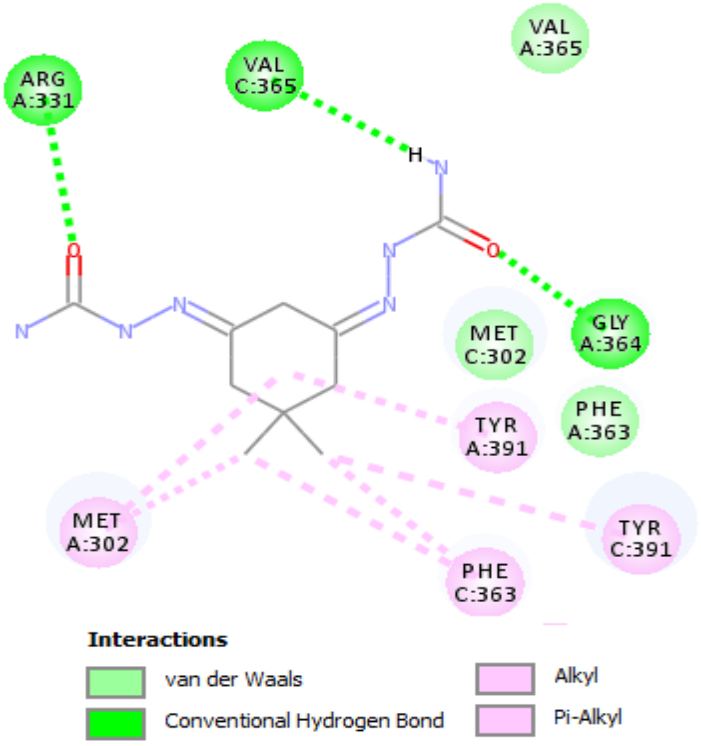

Fig. 11.5c 2D interaction diagram of DMCHHC with active site 2 of 2W6O

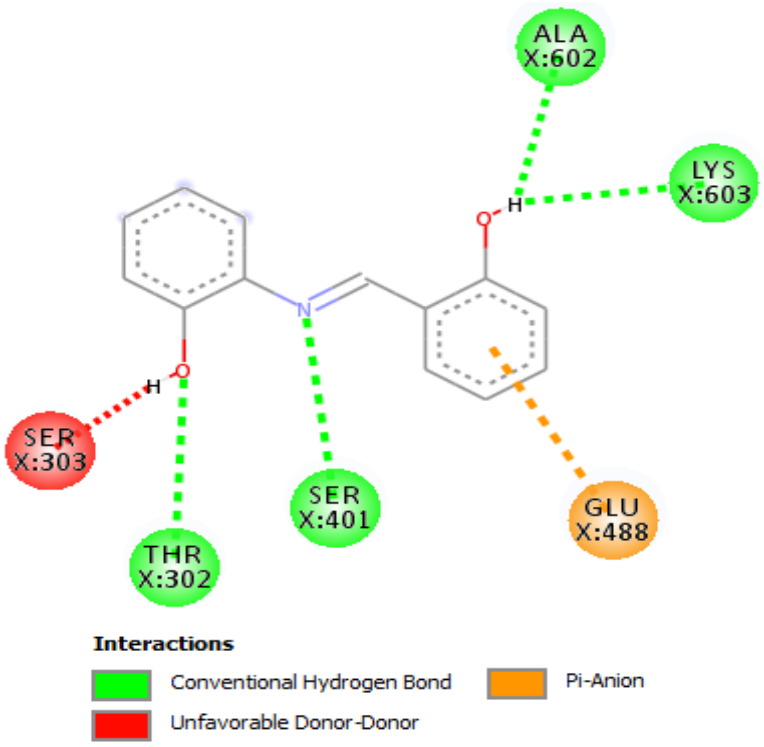

Fig. 11.5d 2D interaction diagram of 2HBAP with active site 2 of 2VF5

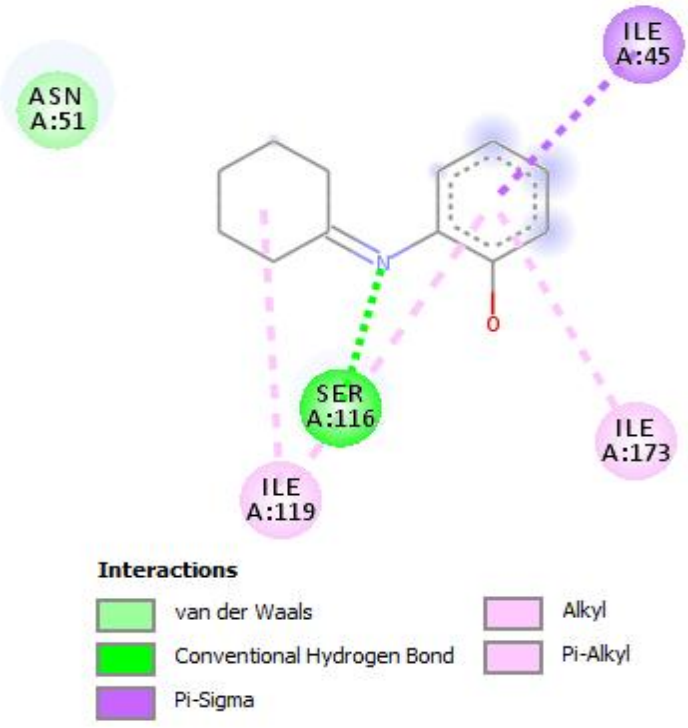

Fig. 11.5e 2D interaction diagram of 2CHAP with active site 1 of 2MBR

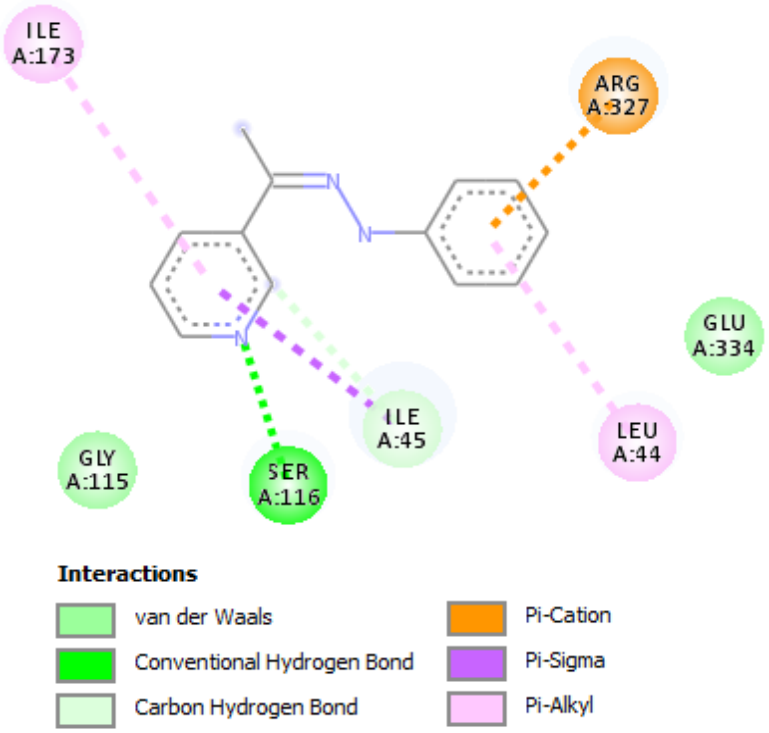

Fig. 11.5f 2D interaction diagram of 3PHEP with active site 1 of 2MBR

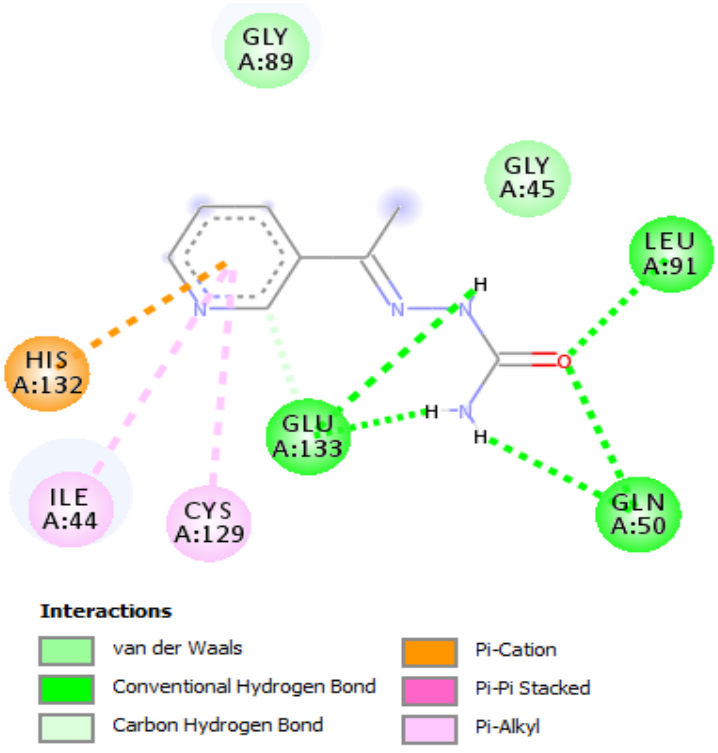

Fig. 11.5g 2D interaction diagram of 2PEHC with active site 4 of 1G2A

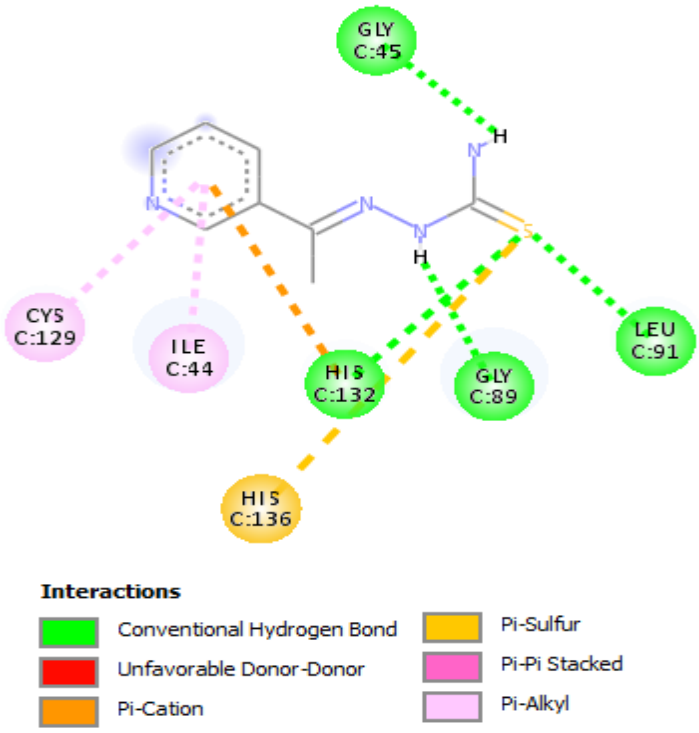

Fig. 11.5h 2D interaction diagram of 2PEHCT with active site 2 of 1G2A

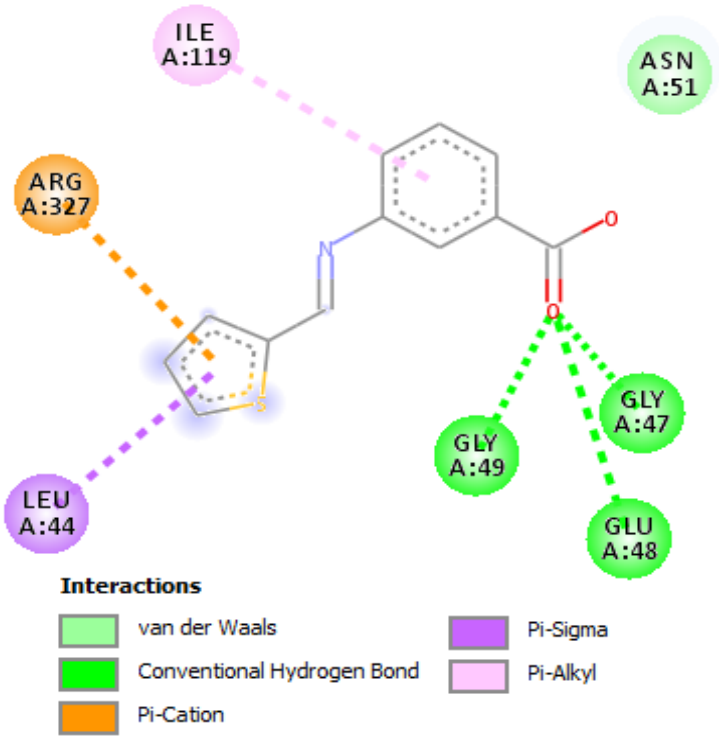

Fig. 11.5i 2D interaction diagram of 3TMAB with active site 1of 2MBR

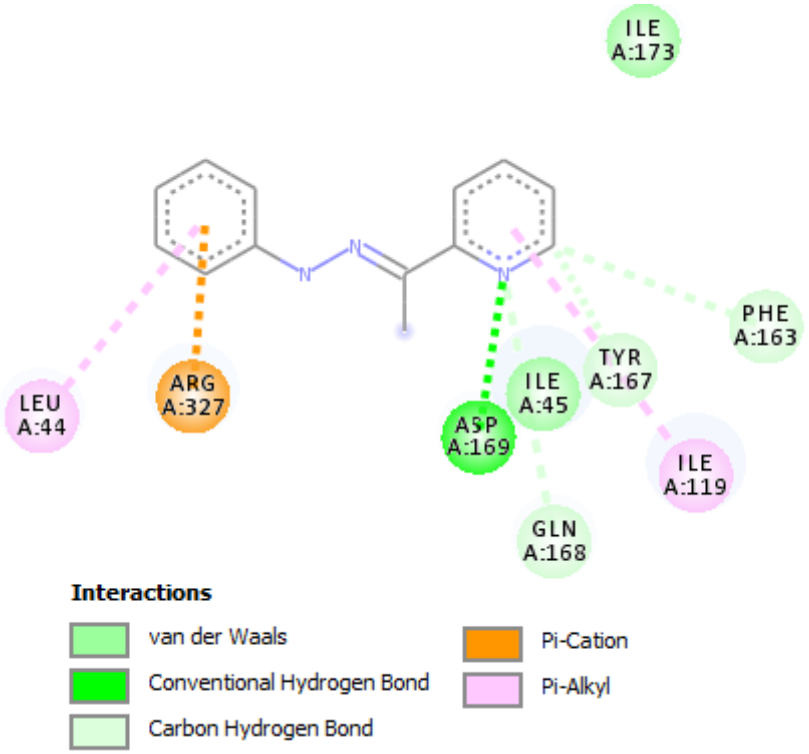

Fig. 11.5j 2D interaction diagram of 2PHEP with active site 1 of 2MBR

***In vitro* antibacterial studies of the heterocyclic Schiff bases and their inner transition metal complexes**

Schiff bases ligands 3-(1-(2-phenylhydrazono)ethyl)pyridine (3PHEP), 2-(1-pyridine-3-yl)ethylidene)hydrazinecarbothioamide (2PEHCT), 3-((thiophen-2-yl methylene)amino)benzoic acid (3TMAB) and their inner transition metal complexes of La(III), Nd(III) and Sm(III) were subjected to antibacterial studies against three gram-positive and three gram-negative bacterial strains using disc diffusion method. *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus casseliflavus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter hormaechei* are the bacterial strains used for the screening. In order to conduct the antibacterial studies the compounds were dissolved in the solvent DMSO to prepare solutions having concentrations 50 $\mu gdisc^{-1}$, 100 $\mu gdisc^{-1}$, 250 $\mu gdisc^{-1}$ and 500 $\mu gdisc^{-1}$. Ampicillin was used as the standard

antibiotic to compare the antibacterial activity of the compounds. The solvent DMSO was also subjected to antibacterial screening.

Schiff base 3PHEP and its inner transition metal complexes

Antibacterial activity of the Schiff bases and their inner transition metal complexes are given in Table 11.13. Schiff base ligand 3PHEP and its La(III), Nd(III) and Sm(III) complexes were found to be less active than the standard antibiotic. 3PHEP is more active against the bacterial strains *P. aeruginosa* and *E. hormaechei* and less active against *E. casseflavis*. Standard antibiotic ampicillin exhibited appreciable activity against all the bacterial strains. La(III), Nd(III) and Sm(III) complexes also exhibits high activity against *P. aeruginosa* and *E. hormaechei*, but less active than the ligand 3PHEP. La(III), Nd(III) complexes are more active than Sm(III) against *E. faecalis* and *E. coli* whereas Sm(III) complex is more active than La(III), Nd(III) complex in *S. aureus* and *E. casseflavis.* Also at high concentration of about 500 µgdisc^{-1} the activity of La(III) and Nd(III) complexes are slightly greater than that of the ligand. Same activity is observed for 3PHEP and its complexes against *E. hormaechei*. The solvent DMSO was found to be inactive towards all bacterial strains under study.

Schiff base 2PEHCT and its inner transition metal complexes

In the case of 2PEHCT and its complexes the activity is high against the bacterial strains *P. aeruginosa* and *E. hormaechei*. Activity of the standard antibiotic is higher than the ligand and complexes. 2PEHCT exhibited low activity against *E. coli.* Nd(III) and Sm(III) complexes are more active than ligand against *E. faecalis* and *P. aeruginosa*. Diameter of zone of inhibition of Nd(III) and Sm(III) complexes are 15 mm and 11 mm respectively at 500 µgdisc^{-1}. The enhancement of growth inhibitory power of Schiff base ligands upon coordination can be explained on the basis of Tweedy's chelation theory [82].

Table 11.13 Diameter of zone of inhibition (mm) at different concentration (µgdisc^{-1}) of the Schiff bases and their inner transition metal complexes

Bacteria	Conc. (µgdisc^{-1})	3PHEP (L)				2PEHCT (L$'$H)				3TMBA (L$''$H)				Ampicillin
		L	La	Nd	Sm	L$'$	La	Nd	Sm	L$''$	La	Nd	Sm	
S. aureus	50	10	6	6	7	4	6	5	6	10	6	5	8	15
	100	17	7	7	8	8	8	6	7	12	8	6	10	21
	250	20	8	9	10	8	9	9	8	20	9	9	12	28
	500	25	10	10	12	11	10	10	10	24	10	10	15	30
E. casseflavis	50	3	5	5	7	4	5	7	3	4	5	4	2	9
	100	5	7	7	9	7	6	7	5	6	6	6	4	14
	250	7	8	9	11	8	7	9	6	9	9	8	6	16
	500	10	10	10	12	10	10	10	8	10	10	10	8	20
E. faecalis	50	8	6	7	1	5	5	8	7	5	5	6	3	10
	100	8	9	9	2	6	7	10	8	5	7	7	4	15
	250	10	10	11	4	8	8	12	10	7	8	9	6	18
	500	11	12	13	6	10	10	15	11	10	10	10	8	20
E. coli	50	8	10	9	5	0	6	9	3	7	7	6	5	12
	100	12	11	10	5	1	7	10	5	11	9	7	7	19
	250	13	13	11	8	5	9	13	7	13	11	9	8	21
	500	16	15	12	10	9	10	15	10	15	12	10	11	25
P. aeruginosa	50	19	12	11	10	14	14	18	14	16	13	16	9	20
	100	21	13	13	15	18	18	21	19	21	15	18	12	26
	250	23	17	16	18	21	20	23	21	23	17	20	16	28
	500	25	20	20	20	24	22	25	25	25	20	25	20	30
E. hormaechei	50	7	4	6	5	4	7	7	6	5	5	4	5	7
	100	7	6	7	5	5	9	7	8	5	6	5	8	9
	250	9	8	9	8	8	10	8	9	6	7	8	9	11
	500	10	10	10	10	10	12	10	10	10	10	10	10	15

When coordinate with metal, ligand will reduce the positive charge on the metal by partial sharing of delocalized electrons. As a result metal polarity gets reduced and lipophilicity of the molecule increases compared to the free ligand. This enables the

complexes to cross the lipid membrane and to enter into the cytoplasm of the cell. As a result the cytotoxicity of the metal chelates will enhance. In the bacterial strain *E. hormaechei* all the complexes are slightly more active than the ligand. Compared to other two complexes and ligand, Nd(III) complex is more active against *E. coli* and exhibits a zone of inhibition of about 15 mm at 500 µgdisc^{-1}. Antibacterial activity of 2PEHCT, Nd(III)-2PEHCT and Sm(III)-2PEHCT at 500 µgdisc^{-1} against *E. faecalis* is shown in Fig. 11.6.

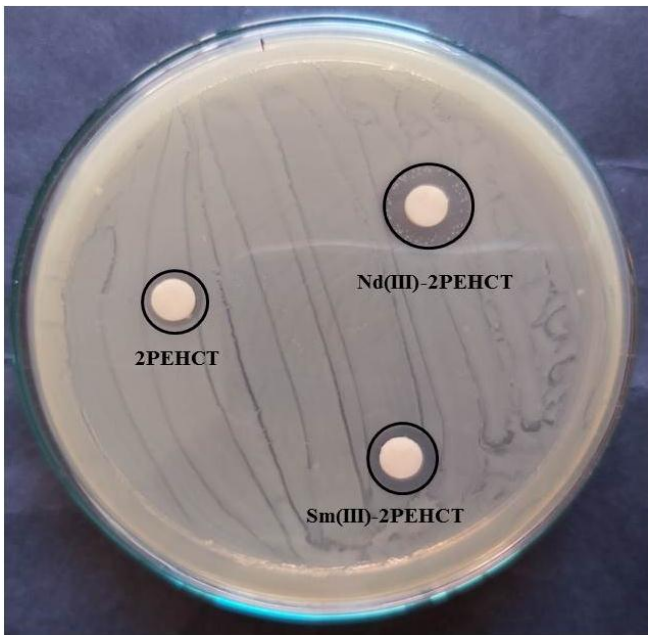

Fig. 11.6 Antibacterial activity of 2PEHCT, Nd(III)-2PEHCT and Sm(III)-2PEHCT at 500 µgdisc^{-1} against *E. faecalis*

Schiff base 3TMBA and its inner transition metal complexes

The activity is also high in the case of 3TMBA and its complexes against the bacterial strains *P. aeruginosa* and *E. hormaechei*, but less than that of standard antibiotic. Maximum zone of inhibition of about 25 mm is shown by the Nd(III) complex and ligand in *P. aeruginosa.* In the case of *E. hormaechei* the activity of ligand and complexes are almost same and the diameter of zone of inhibition exhibited at a maximum concentration of 500 µgdisc^{-1} is 10 mm. La(III) and Nd(III) complexes exhibits slightly higher activity than 3TMBA against *E. faecalis*

SUMMARY

In vitro antibacterial studies of the 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-dimethylcyclo 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 determined using disc diffusion method. *Staphylococcus aureus* and *Escherichia coli* are the pathogens taken for antibacterial screening. Different concentration of the compounds in DMSO such as 50, 100, 250 and 500 µgdisc^{-1} were employed for antibacterial investigations. Ampicillin was taken as the standard antibiotic to compare the activity of the Schiff base compounds.

Even though all Schiff bases have less activity than the standard antibiotic, all of them have appreciable growth inhibitory power against *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition was found to be increased with concentration of Schiff bases. Maximum inhibition was exhibited by DMCHDP and DMCHDA in *S. aureus* and *E. coli* respectively. The compounds 2PEHC and 2PEHCT have less activity in both strains.

In order to understand the mechanism by which the Schiff base compounds inhibit the growth of these two bacteria, *in silico* molecular docking studies were also carried out by selecting suitable targets present in them. All Schiff bases obey Lipinski rule of five and hence possess drug like properties. Molecular docking studies were

conducted to evaluate the binding affinity of the compounds with target proteins present in *S. aureus* and *E. coli*. The compounds were docked with four active sites of the targets such as Sortase-A (PDB ID: 1T2P), DNA gyrase (PDB ID: 3U2D), dihydrofolate reductase (DHFR) (PDB ID: 2W9S), clumping factor A (ClfA) (PDB ID: 1N67), dehydrosqualene synthase (CrtM) (PDB ID: 2ZCO) and two sites of the target undecaprenyl diphosphate synthase (UPPS) (PDB ID: 4H8E) of *S. aureus* and with four active sites of the targets such as β-ketoacyl-acyl carrier protein synthase III (ecKAS III) (PDB ID: 1HNJ), peptide deformylase (PDF) (PDB ID: 1G2A), L-glutamine: D-fructose-6-phosphate amido-transferase (PDB ID: 2VF5), murB (PDB ID: 2MBR), heptosyltransferase WaaC (PDB ID: 2GT1), mur D (PDB ID: 2X5O) and biotin carboxylase (BC) (PDB ID: 2W6O) of *E. coli* by using the software AutoDock 4.2.

In *S. aureus* the Schiff base compounds are found to be more active against the targets 2W9S, 1N67, 4H8E and 2ZCO whereas in *E. coli* the activity was high against the targets 2MBR, 2W60, 2VF5 and IG2A. Thus the compounds inhibit the growth of these pathogens by deactivating their target proteins. The binding energy of DMCHDP and DMCHDA are high compared to other Schiff bases when docked with target proteins of both pathogens. This can be due to high molecular mass. Maximum binding energy of the compounds varies between -6.5 to -10.3 kcal/mol and -5.7 to -9.1 kcal/mol in *S. aureus* and *E. coli* respectively. In *S. aureus* maximum binding energy of -10.3 kcal/mol was observed when DMCHDP was docked with 2W9S whereas in *E. coli* maximum binding energy of -9.1 kcal/mol was observed when DMCHDA was docked with 2MBR. The Schiff bases 2PEHC and 2PEHCT exhibits low binding affinity to all target proteins and is supported by the results of *in vitro* antibacterial screening. In *S. aureus* the Schiff bases DMCHDP and 2PHEP occupy the active site 3 of the target 2W9S whereas 2CHAP and 3PHEP occupy site 1 of the target 4H8E. In *E. coli* site 1 of

target 2MBR was found to be the most active pocket for the compounds. The ligands DMCHDP, 2CHAP, 3PHEP, 3TMBA and 2PHEP were found to occupy in this active pocket.

Screening of *in vitro* antibacterial activity of the three heterocyclic Schiff bases 3PHEP, 2PEHCT, 3TMBA and their La(III), Nd(III) and Sm(III) complexes were also conducted against three gram positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus casseliflavus* and three gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter hormaechei* using disc diffusion method. Ampicillin was taken as the standard antibiotic and 50-500 $\mu g disc^{-1}$ in DMSO was the concentration range employed for the study.

Standard antibiotic ampicillin exhibits appreciable activity in all bacterial strains. The activity of all the ligands and complexes are found to be less than the standard antibiotic. All the ligands and complexes are more active against the bacterial strains *P. aeruginosa* and *E. hormaechei*. The Nd(III) and Sm(III) complexes of 2PEHCT are more active than the ligand against *E. faecalis* and *P. aeruginosa.* In *E. hormaechei* all the complexes of 2PEHCT are slightly more active than the ligand whereas the ligands 3PHEP, 3TMBA and their complexes have same activity. The La(III) and Nd(III) complexes of 3PHEP are more active than its Sm(III) complex against *E. coli* and *E. faecalis* whereas Sm(III) complex of 3PHEP is more active against *S. aureus* and *E. casseliflavus* than its La(III) and Nd(III) complexes.

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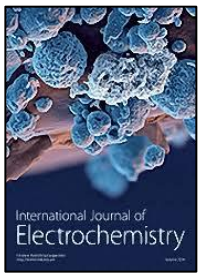

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