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Original Article

In vitro Antibacterial Evaluation and *in silico* Docking of Schiff Base as a Potential Inhibitor of Nucleoside Diphosphate Kinases

Archana Tomer[1], Vishnu Vats[2], Smriti Bansal[1], Pravita Kumar[3], Yogendra Kumar[3,*], Anjana Sarkar[1,*]

[1]Department of Chemistry, Netaji Subhas University of Technology (N.S.U.T), Erstwhile N.S.I.T, Azad Hind Fauj Marg, Dwarka, Delhi, INDIA.
[2]Department of Applied Science and Humanities, G.B Pant DSEU Okhla-1 Campus, Delhi, INDIA.
[3]Department of Chemistry, Sri Aurobindo College Delhi (Delhi University), Delhi, INDIA.

ABSTRACT

Aim: Schiff bases are vital Compounds of organic chemistry; they have exhibited more potential biological activities namely antiviral, antibacterial, and antifungal characteristics. Molecular docking studies suggested that strong binding energies, as well as favorable hydrogen bonds and hydrophobic interactions, could be beneficial for the pharmacological application of the compounds. Moreover, the aid of the ADMET score may support the therapeutic action of potential pharmaceutical compounds. **Materials and Methods:** In the present work, *In vitro* studies were conducted on five Schiff bases named BIP, CIP, PT, NIP, and DPIP against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *In silico* studies were also performed on the same five Schiff bases, evaluating their interaction with the target proteins of *Staphylococcus aureus* and *Escherichia coli*. **Results:** *In vitro* results of BIP and NIP showed mild MIC (minimum inhibition concentration) against *Staphylococcus aureus* and *Escherichia Coli* respectively, while PT showed good MIC against pathogenic bacteria *Staphylococcus aureus* which was upheld by *in silico* study. **Conclusion:** We hope the findings of the present study should contribute to developing a good anti-bacterial drug.

Keywords: Antibacterial Study, Schiff base ligand, *Escherichia coli*, *Staphylococcus aureus*, Molecular Docking.

Correspondence:
Anjana Sarkar
Department of Chemistry, Netaji Subhas University of Technology (N.S.U.T), Erstwhile N.S.I.T, Azad Hind Fauj Marg, Dwarka, Delhi-110017, INDIA.
Email: anjisarkar@gmail.com

Yogendra Kumar
Sri Aurobindo College Delhi (Delhi University), Delhi-110062, INDIA.
Email: dr_yogendrak@yahoo.com

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INTRODUCTION

Health-care-Associated Infections (HAIs) and nosocomial infections have spread comprehensively by the common gram-positive bacteria, *Staphylococcus aureus*,[1] and gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae*,[2] *Pseudomonas aeruginosa*,[3] *Acinetobacter baumannii*,[4] *E. coli* is typically present in the intestines of humans and animals.[5] Most of the strains of *E. coli* are beneficial, playing a crucial role in maintaining the gut microbiome. However, some strains of *E. coli* can cause illness, ranging from mild diarrhea to severe diseases like urinary tract infections, meningitis, and sepsis. Understanding the biology of *E. coli* and developing new treatments and preventative measures is an active area of research in the scientific community. *Klebsiella pneumoniae*, a gram-negative bacterium that can originate a range of diseases, for instance urinary tract infections, pneumonia, and bloodstream infections is commonly found in the intestines and feces of humans, as well as in soil and water. *K. pneumoniae* is a significant healthcare-associated pathogen, particularly in critical care units and skilled nursing facilities, where hospitalized persons have weakened immune systems. The bacterium is known to develop resistance to multiple antibiotics, including carbapenems, which are often used as a last-line defense for curing multiple exposures caused by superbugs. As a result, *K. pneumoniae* infections can be challenging to treat and can be associated with high mortality rates. Ongoing research is focused on understanding the biology of *K. pneumonia* and developing new treatments and preventative measures to combat its spread.[6] *Pseudomonas aeruginosa*, gram-negative bacterium that is widespread in clay and moisture is an adaptable disease-causing bacterium that can trigger a range of infections, notably in hospitalized patients, including pneumonia, sepsis, pyelonephritis, and surgical wound infestation. *P. aeruginosa* is known to develop resistance to multiple antibiotics, making treatment challenging. It is also capable of forming biofilms, which can protect it from antibiotics and the host's immune response. *P. aeruginosa* infections can be particularly dangerous for patients with weakened immune systems, such as those with cystic fibrosis. Efforts to understand the biology of *P.*

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aeruginosa and to develop new treatments are ongoing in the scientific community.[7] *Acinetobacter baumannii*, a gram-negative bacterium that is present in fluid and dirt is known to trigger some infestation, along with pneumonia, bloodstream infections, and surgical site infection, particularly in hospitalized patients and those with weakened immune systems. *A. baumannii* has become a growing concern in healthcare settings due to its ability to create resistance to antibiotics, making treatment challenging. As a result, *A. baumannii* infections can be life-threatening, especially in critically ill patients. Research shows that effort are being made to better understand this pathogen and to develop new treatments.[8] *S. aureus* is a spherical-shaped, gram-positive-bacterium which belongs to the *Bacillota phylum* of bacteria. Almost 20-30% worldwide human population is a long-term carrier of a pathogen. *Staphylococcus aureus* is ubiquitously found on the cutis and in the nasal canal of humans. It is a considerable disease-causing agent that can produce a spectrum of diseases, ranging from minor epidermis infections to survival-threatening conditions such as pulmonary inflammation, sepsis, and bacterial endocarditis. *S. aureus* is a leading cause of healthcare-associated infections, particularly in hospitals, where it can be transmitted between patients and healthcare workers. It is known to develop resistance to multiple antibiotics, including methicillin, which has directed to the emanation of Methicillin-Resistant *S. aureus* (MRSA). MRSA infections are particularly challenging to heal and can be related to high fatality rates. *S. aureus*, gram-positive bacteria has a smooth mono-layered outer wall of a cell consisting of murein, and teichoic acids and is wall-cojoined with cross-linkage of Peptidoglycan.[9] *S. aureus* and *E. coli* scale up various lethal diseases such as pneumonia, meningitis, and osteomyelitis, etc.[10,11] Based on reported data to EARS-Net, 2021 more than 670000 infections in EU/EEA happen due to multi-drug resistant bacteria and this infection is get affected lethal on approximately 33000 people. As of May 2021, the Global Antimicrobial Surveillance System (GLASS) was conducted by World Health Organization (WHO).[11] The Centre for Disease Control and Prevention (CDC) reported that each year in the United States STEC (Shiga Toxin *E. coli*) leads to 3,600 individuals requiring hospital care and results in 30 causalities.[12] The discovery of antibacterial drugs gave a huge relief to overcome this problem. Antibiotics saved the human race worldwide, however, the use of antibiotics increased some bacteria naturally and some through mutations, causing resistance against these drugs. Therefore, a novel drug is needed to combat bacterial infections,[13-15] and the selection of appropriate antimicrobial agents for the treatment of bacterial infections is also crucial.[16] MIC knowledge of antimicrobial agents against a specific bacterial strain is essential for selecting the appropriate antimicrobial agent and dosing regimen for the treatment of bacterial disease. Molecular docking is a beneficial tool for predicting the potential drug candidate against bacterial strains of *E. coli*, and *S. aureus*.[17-21] Schiff bases have been found to exhibit a diverse array of biological screening in the field of biological chemistry. These activities include analgesic, antimalarial, growth-inhibiting, inflammation-reducing, antiviral, antifebrile, fungicidal, and antibacterial properties.[22-24] It is believed that the imine or azomethine group (>C=N-) is important for the bio screening of Schiff bases.[25-27] The present study mainly deals with the *in vitro* and *in silico* studies of five Schiff bases against these hazardous bacteria.

MATERIALS AND METHODS

Synthesis of Schiff Bases

The Schiff bases used in the present study were synthesized using the methods previously reported in the literature.[28]

MIC Determination

The broth microdilution method is a widely used technique in microbiology research to examine the Minimum Inhibitory Concentration (MIC) of potential drug agents versus a specific bacterial strain. In this study, we intended to examine the MIC of various potential drug candidates against a clinical isolate of *S. aureus* using the broth microdilution technique. We followed the standard procedure described in Andrews (2001) with some modifications. The bacterial inoculum was prepared by growing the bacterial strain in Mueller-Hinton broth for 18-24 hr at a specified temperature of 37°C with shaking. The bacterial inoculum was injected into each well, and the plate was incubated at specific conditions. The MIC was analyzed as the lowest concentration of potential drugs that prevented the growth of bacterial colonies. Our results showed the MIC value of Levofloxacin and Schiff bases. In conclusion, the broth microdilution method is a reliable and accurate technique for determining the MIC and provides important information for the selection of appropriate antimicrobial potential compounds for the treatment of *S. aureus* disease.

In this work, we focused to examine the MIC of various potential pharmaceutical agents against a clinical isolate of *S. aureus* by the broth microdilution method. The bacterial strain was obtained from a clinical specimen and identified by standard biochemical tests. The bacterial inoculum was prepared by growing the bacterial strain in Mueller-Hinton broth for 18-24 hr at 37°C with shaking. The bacterial concentration was made up to similar the turbidity of a 0.5 McFarland standard which corresponds to approximately 1-2x10^8 CFU/mL. The antimicrobial agents (vancomycin, ciprofloxacin, and erythromycin) were prepared at appropriate concentrations and added to the microtiter plate in twofold dilutions. The bacterial inoculum was injected into each well and the plate was incubated for about 18-24 hr at a specified temperature of 37°C. The experiment was recurred three times to ensure reproducibility and accuracy.[29,30]

Molecular docking study

3D structure of target protein in Staphylococcus aureus

3Q8U (PDB ID) with 2.22 Å resolution was retrieved from the RCSB PDB database from the X-ray crystallography structure. It is a heteromer containing chains A B C D E and F and the protein was cleaned by BIOVIA Discovery Studio (DS). 149 Amino Acid (AA) residue chain A (MET1 to GLU149) is part of the cleaned protein structure.[31]

3D structure of target protein in *Escherichia coli*

Data of 3D X-ray crystal structure of the bacterium *E. coli* was searched and 3T88 (PDB ID) with 2.00 Å resolution was recovered from the RCSB PDB database. Protein was loaded in BIOVIA Discovery Studio (DS) and underwent the removal of water, hetero atoms. The cleaned protein structure contained chain A, spanning from amino acid residue ASP5 to PRO285 (total 281 residues).[32]

Preparation of Schiff base ligands

The 2D and 3D cleaning of reported structures occurred via Marvin's application. Target protein was used to assess the potential biological activity of previously reported Schiff base ligands (Figure 1a-e).

Prediction of binding site

The LIGPLOT represents the binding site via both hydrogen and non-hydrogen bond between the target and ligand. The binding pocket of target 3Q8U with ADP and 3T88 with S0N was found in the LIGPLOT figure obtained from the PDB sum database (Figure 2a, 2b). Target protein 3Q8U with ligand ADP illustrates hydrogen bonds with LYS9, HIS52, THR91, ARG102, and ASN112. 3T88 protein with ligand S0N shows hydrogen bonds with ARG 45, SER 84, GLY 86, GLN88, LYS89, TYR97, GLY133, THR 155, TYR258, and LYS273.

Protein preparation

In Discovery Studio Visualizer, the water molecules, heteroatoms, and ligand groups of the PDB structures 3Q8U and 3T88 were cleaned. The final cleaned structure was depicted in Figure 3a, 3b. The hydrogen atoms (polar only) and Kollman charges were added to the cleaned protein using AutoDock Vina. The grid box for the cleaned 3Q8U and 3T88 macromolecule was defined with the XYZ coordinates of the active site and were slide to 30, 30 and 30 dimensions and 36,36,36 dimensions respectively in AutoDock Vina.[33]

ADME Studies of Potential Drug Ligand

ADME stands for Absorption, Distribution, Metabolism, and Excretion are important parameters that depict the ligand's drug-likeness properties. Swiss ADME tool is used to predict physico chemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness rule (Lipinski rule, Ghose, Veber, Egan, Mugge, Bioavailability score), and medicinal chemistry.[34] Most cited Lipinski rule states if ligand satisfied the following parameters that it can be a good potential drug candidate.

- Less than 500 molecular weight.
- Less than 5 Octanol-water coefficient logP.
- Not more than 5 hydrogen bonds elements.
- Not more than hydrogen bond acceptors.
- Molar refractivity value should lie between 40-130.

Reliant on Lipinski's rule of 5, Schiff bases moved towards the step of the pre-filtration of a potentially life-saving drug candidate. Such pre-filtration of drug candidates secures the pharmacological interest.[35,36]

RESULTS AND DISCUSSION

In vitro antibacterial study

The broth microdilution MIC method is a laboratory technique used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent against a particular bacterial isolate. The method involves taking a sterile multi-well plastic tray with various concentrations of the antimicrobial agent, which are injected with a standardized number of test bacteria strains. After being left to incubate under specific conditions overnight.[37] A total of five Schiff-bases compounds were examined for potential antibacterial activity versus the *S. aureus ATCC 29213, E. coli ATCC 25922, K. pneumoniae BAA 1705, A. baumannii BAA1605, P. aeruginosa ATCC 27853*. These compounds were then examined further for the MIC value by using a broth micro-dilution assay. The test showed that, PT was most active versus *S. aureus ATCC 29213* (Table 1) strains having a16 µg/mL value of MIC and BIP also showed mild value of MIC as per Table 1.

Druglikness Property of Schiff bases

Schiff bases were put on the first screening of a potentially life-saving drug candidate by virtue of Lipinski's rule of 5.[38] According to rule parameters such as molecular weight (m.wt), HBA (hydrogen bond acceptor), HBD (hydrogen bond donor), log P (Octanol-water partition coefficient), and molar refractivity index. All five Schiff bases were found to fit on the first scrutiny conditions and outcomes of druglikeness condition are depicted in Table 2. These Schiff bases also ensured Veber's law which put forward the bioavailability score of drug candidates. The bioavailability score of these Schiff bases is 0.55 and the synthetic accessibility (SA) of Schiff bases is greater than one and less than 10 to represent the easy productivity of chemicals in the laboratory.[39] Synthetic accessibility is a critical role in drug design

selection. Incorporating SA parameters into CAD workflows can improve the efficiency and success rates of drug designing Above all; it reflects more potential drug candidate designs ahead.

Molecular docking

The molecular docking approach is an inevitable technique nowadays to design a potential drug, it accelerates the process of selection of drugs by obtaining the binding energy of the molecule and the interaction of molecule and protein through virtual portrayal.[40] Hydrogen bond interaction is a key role to figure out the compatibility of protein and ligand complexes, it assists to understand the anti-bacterial mechanism of a potential drug. On account of this, *in silico* studies of Schiff bases are formed by Autodock Vina. These Schiff bases generated good binding energy with the active pocket of *E. coli* (PDB ID 3T88) *S. aureus* (PDB ID 3Q8U). It is cited in the literature, a greater score of the negative value of binding energy is good for the binding affinity between a target protein and ligand.[41]

Docking studies of Schiff bases with 3Q8U

The docked structures of Schiff bases with target protein 3Q8U are represented in Table 3 and 2D structures of docked Schiff bases are shown in Figure 4 (a-e). As mentioned in Table 3, All Schiff bases have the same core structures. However, containing different substituents, these Schiff bases BIP, CIP, PT, NIP, and DPIP have shown different and well binding scores -7.3 kcal/mol, -7.2 kcal/mol, -7.5 kcal/mol, -7.6 kcal/mol, and -7.3 kcal/mol sequentially against the target protein. In addition, Schiff bases have more than 7 kcal /mol binding energy, which is a good sign for protein complex affinity. From Table 3, NIP showed the highest binding affinity -7.6 Kcal/mole, and methyl-containing Schiff bases also have binding energy almost the same as the NIP value. NIP is surrounded by 2 hydrogen bonds in active site pocket amino acid residues ARG45 and ARG102. BIP, PT is surrounded with 3 hydrogen bonds in active site pocket amino acid residues LYS9, TYR49, HIS115, and CIP interact with one hydrogen bond with LYS9 AA residue. Inside the active pocket of 3Q8U, protein amino acid residue ARG45 is aligned along with the oxygen atom of a nitro group of Schiff base by one hydrogen bond interaction, another hydrogen bond interaction is found with ARG102 and the other oxygen atom of a nitro Schiff base (NIP). PT Schiff bases have been shown to have good bonding energy -7.5 kcal/mol, against the target protein and followed by three promising hydrogen bonds with amino acids LYS9, TYR49, and HIS115. These hydrogen bonds are created by the oxygen atom near to (-C=N) imino group and amino acid residue LYS9, TYR49, HIS115. CIP and DPIP Schiff bases form only one hydrogen bond with LYS9 and GLY110 AA residue respectively.

Apart from conventional hydrogen bonds, other interactions like Van der Waals, Pi cation Pi alkyl and Pi-Pi stacked are created with amino acids LYS9, TYR49, HIS52, PHE57, ARG85, ILE88, ARG102, GLY110, HIS115, GLY116, ASP118, THR9, VAL109 and Schiff base NIP. PT Schiff bases generated other hydrophobic interactions with target protein amino acid residue LYS9, TYR49, GLU51, HIS52, PHE57, LEU61 THR91, ARG102, GLY110, ASN112, VAL109 HIS115, GLY116, and ASP118. BIP show non-conventional interaction with AA residue LYS9, TYR49,GLU51,HIS52,PHE57,LEU61,THR91,ARG102,VAL109,GLY110, ASN112,HIS115,GLY116,ASP118,and CIP Schiff base with AA residue LYS9,,TYR49, HIS52, PHE57, LEU61,THR91, ARG102, VAL109, GLY110, ASN112, HIS115, GLY116, ASP118. DPIP Schiff base also show interaction with AA residue LYS9, TYR49, GLU51, HIS52, PHE57, LEU 61, THR91, VAL109, GLY110, HIS 115, SER122.

Based on the good binding energy and interaction of NIP, PT, BIP, CIP, and DPIP Schiff bases with target protein 3Q8U is revealed that the NIP, PT, CIP, BIP, and DPIP Schiff bases may be effectively growth inhibitory compounds against bacteria as per Table 3 having 2D interactions.

Docking studies of Schiff bases with 3T88

The docked Schiff bases with target protein 3T88 are represented in Table 4 and 2D Structures of docked Schiff bases are shown in Figure 5 (a-e). As mentioned in Table 4, These Schiff bases have been obtained to have binding energy -6.9 kcal/mol, -6.9 kcal/mol, -7.1 kcal/mol, -6.9 kcal/mol, and -6.8 kcal/mol against target protein 3T88. From Table 4, Nitro-containing Schiff base (NIP) showed the highest hydrogen bond with binding affinity -6.9 Kcal/mole. NIP Schiff bases show connection between the target protein with 4 hydrogen bonds in active site pocket amino acid residue (O: GLN154, O: THR155, O: GLY156). In the active

Table 1: MIC values.

Compound	Solubility	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*K. pneumoniae* BAA 1705	*A. baumannii* BAA 1605	*P. aeruginosa* ATCC 27853
BIP	DMSO	>64	64	>64	>64	>64
CIP	DMSO	>64	>64	>64	>64	>64
PT	DMSO	>64	16	>64	>64	>64
NIP	DMSO	64	>64	>64	>64	>64
DPIP	DMSO	>64	>64	>64	>64	>64
Levofloxacin	DMSO	0.0156	0.125	64	4	1

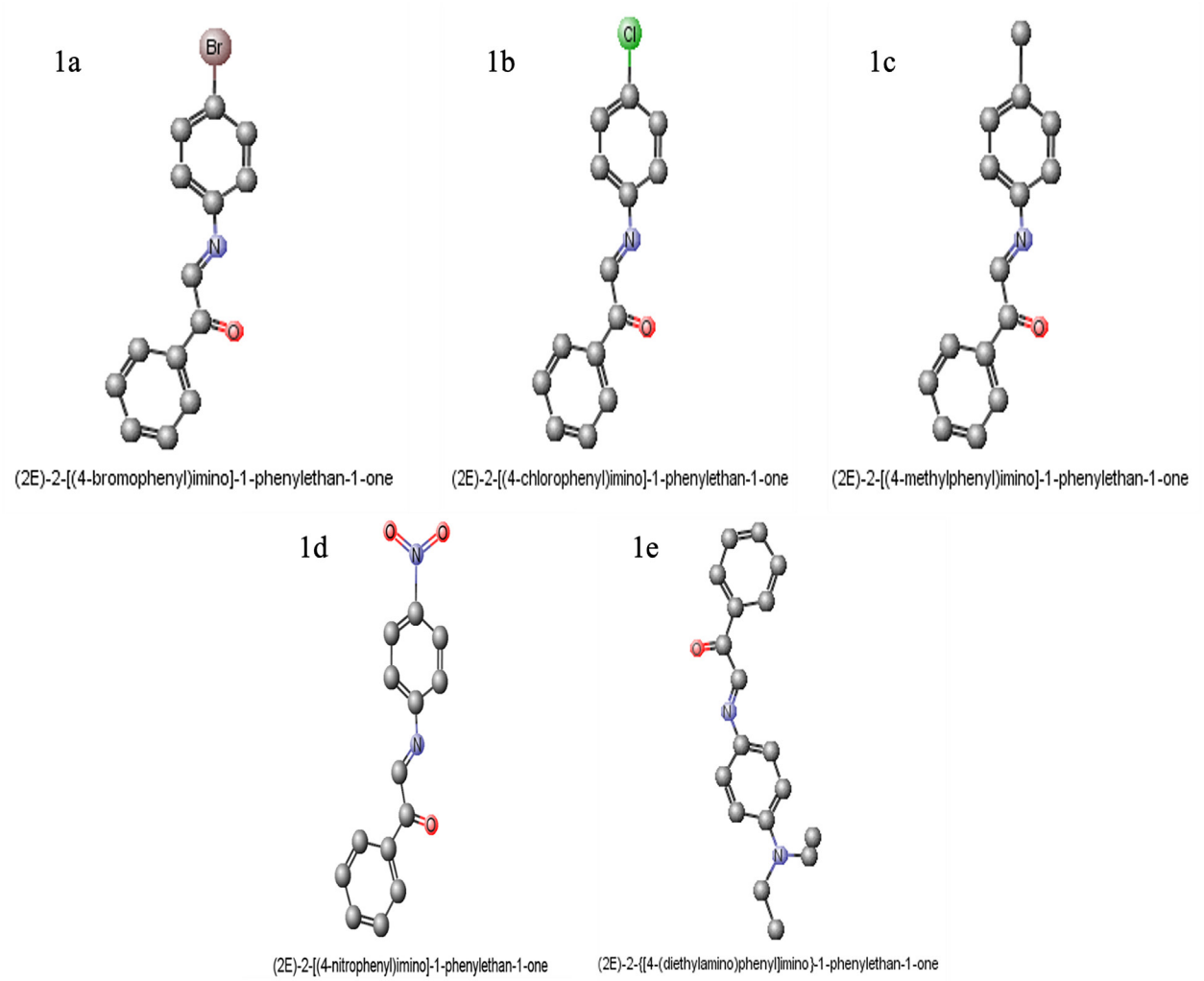

Figure 1(a-e): 3D structure of Schiff base ligand.

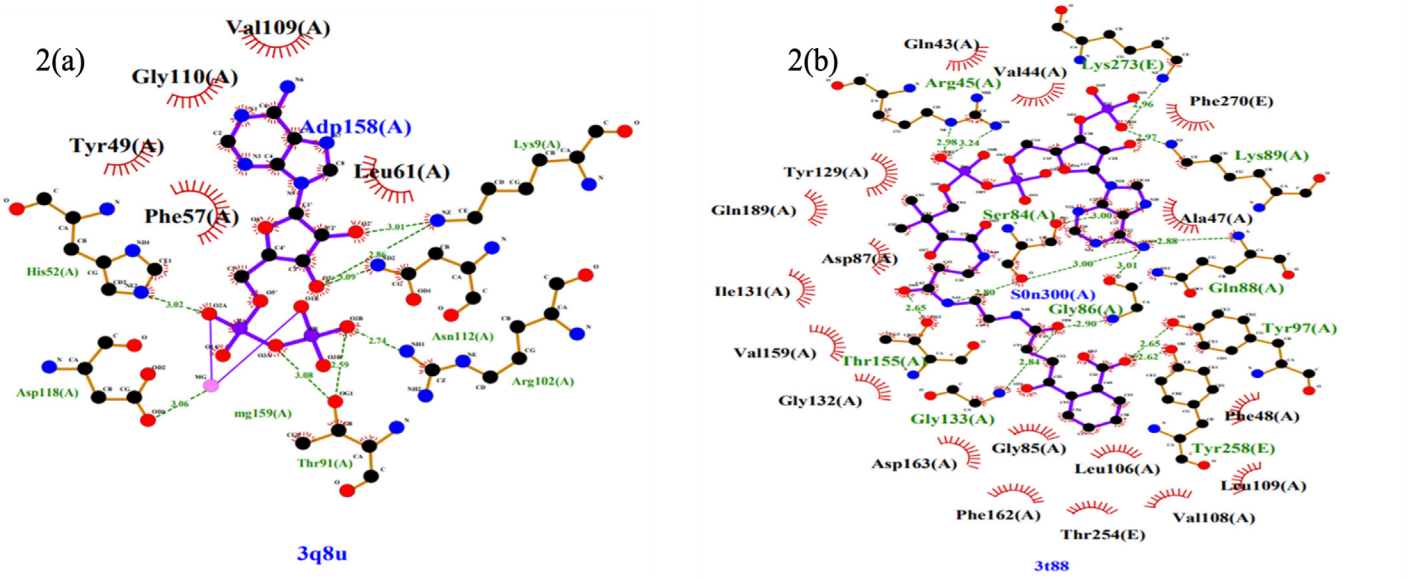

Figure 2(a-b): Ligplot of 3Q8U and 3T88.

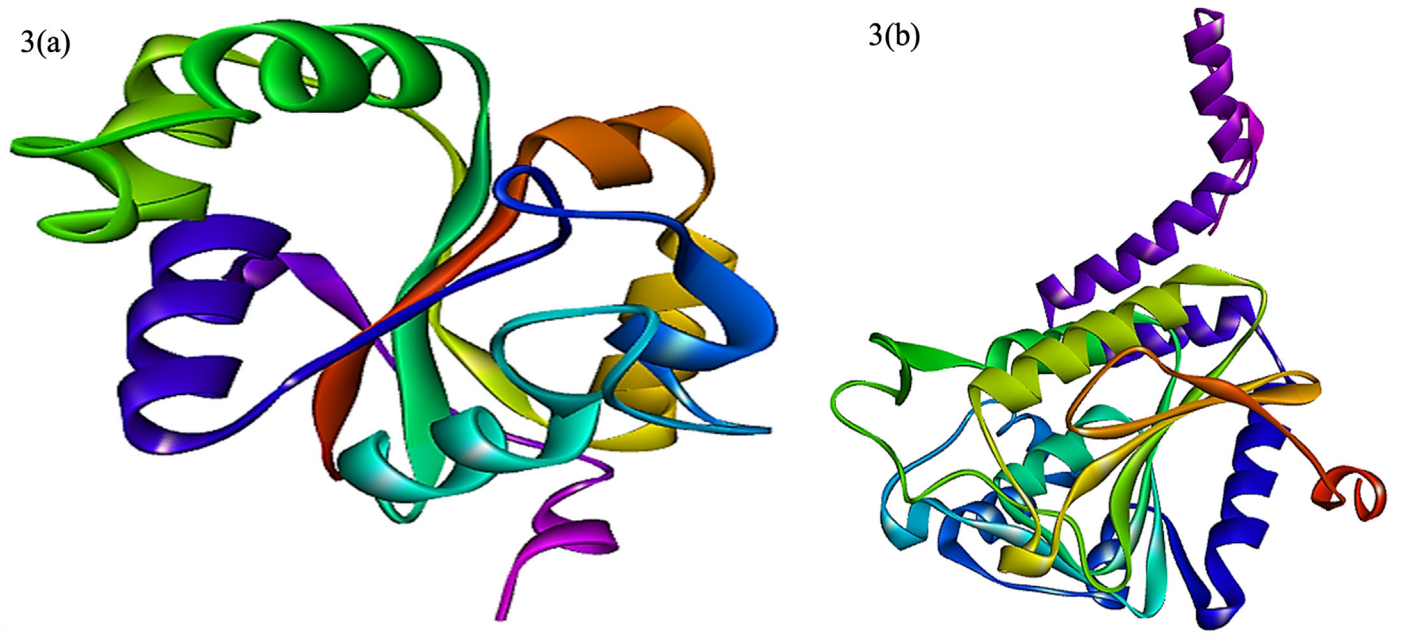

Figure 3(a-b): 3D Structure of 3Q8U and 3T88.

Table 2: **Lipinski Rule of Five.**

Drug likeness property Parameter	Schiff bases				
	BIP	CIP	PT	NIP	DPIP
M.Wt<500	288	243	223	254	280
HBA<5	2	2	2	4	2
HBD<10	0	0	0	0	0
logP<5	3.55	3.48	3.26	2.29	3.64
Molar refractivity 40-130	73.07	70.38	70.33	75.25	89.19
Lipinski Rule	Yes	Yes	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55
Veber's Rule	Yes	Yes	Yes	Yes	Yes
Synthetic accessibility	2.29	2.21	2.31	2.42	2.56

pocket, one of the 4 hydrogen bonds is originated with oxygen atom of a nitro moiety. Schiff base and THR155 amino acid residue, and the other three hydrogen bonds are created among the other oxygen atom of a nitro Schiff base, and GLN154, THR155, GLY15 amino acid residue. PT Schiff bases also interacted against the target protein with good bonding energy -7.1 kcal/mol, and noticeable two hydrogen bonds with GLY86, and SER161 amino acids. One of the two hydrogen bonds is created by nitrogen element of Schiff bases (-C=N) and amino acid residue SER161, another hydrogen atom is followed between an oxygen atom and amino acid AA residue GLY86.BIP and CIP have same binding energy -6.9 kcal/mol and show 3(O: GLY86, N: GLN88, O: GLN88) and 22(O: GLY86, N: GLN88) hydrogen bonds respectively. 1(THR155) hydrogen bond is noticeable in DPIP with binding energy -6.8 kcal/mol. Apart from the conventional hydrogen bond, other interactions like Pi Sigma, Pi Alkyl, and the rest of Van der Waals are created with amino acids LEU106, LEU109 and PHE48, GLY86, TYR97, LEU106VAL108, LEU109, GLY132, GLY133, GLN154, THR155, GLY156, VAL159, SER161, PHE162, ASP163, TRP184, and Schiff base NIP respectively. PT Schiff bases generated other hydrophobic interactions with target protein amino acid residue PHE48, GLY86, ARG91, GLY95, GLY96, TYR97, GLY132, GLY133, GLN154, THR155, THR155, GLY156, PHE162, ASP163, TRP184(van der Waals interaction) and LEU106, (Pi sigma interaction). SER 84,GLY85, GLY86, GLN88, TYR97,GLY133, GLN154, THR155, GLY156, SER161, TRP184, ASP163, PHE162, VAL159 AA residue show non-conventional bond with BIP Schiff bases, and GLY86, ARG91, GLY95, GLY96, TYR97, LEU106, GLY132, GLY133, GLN154, THR155, GLY156, SER161, PHE162, ASP163, TRP184 AA residue show non-conventional interaction with CIP. DPIP also give interaction apart from conventional bond with ALA47,

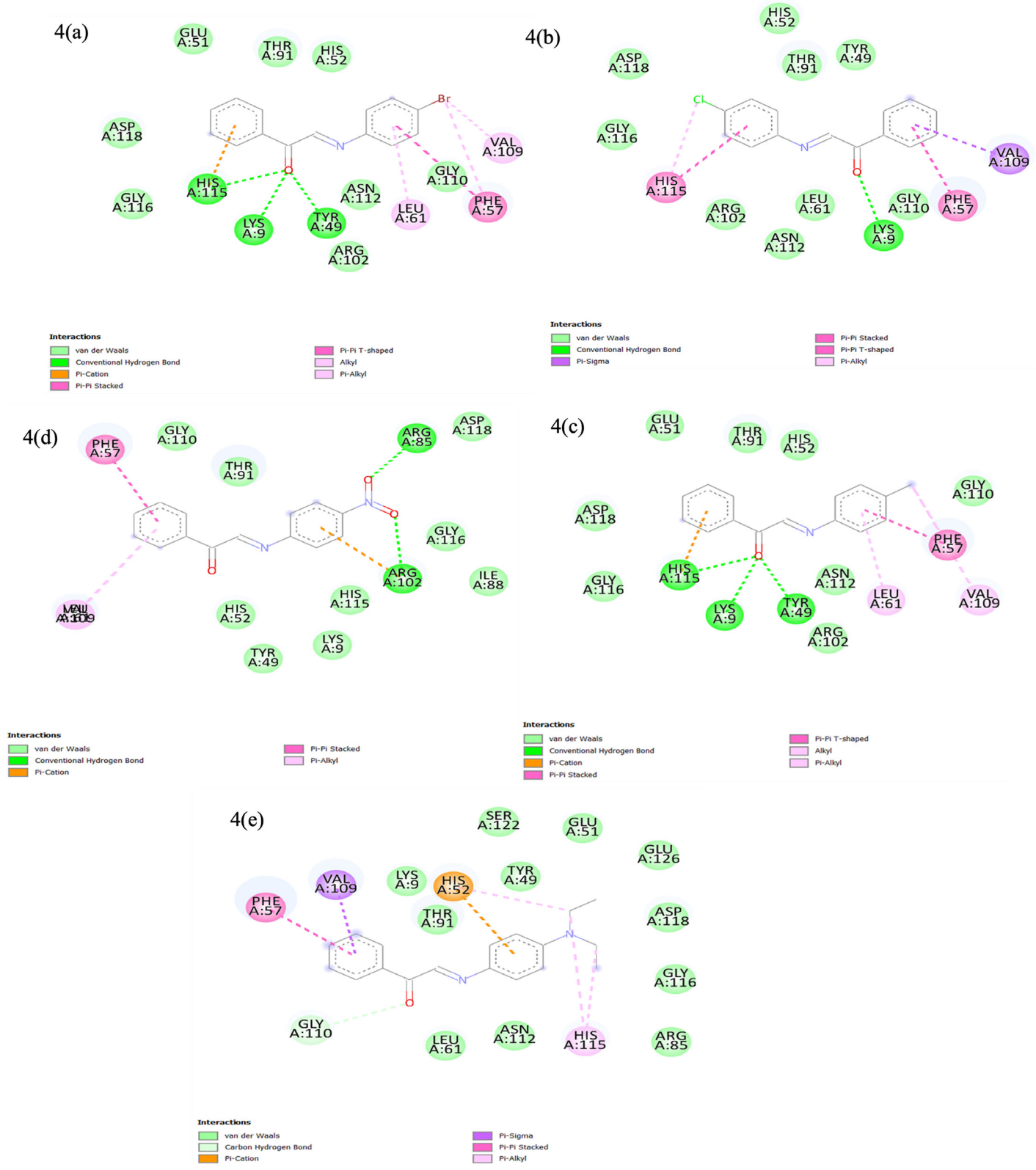

Figure 4(a-e): 2D Interaction between *Staphylococcus aureus* (protein PDB ID 3Q8U) with Schiff bases BIP, CIP, PT, NIP, and DPIP.

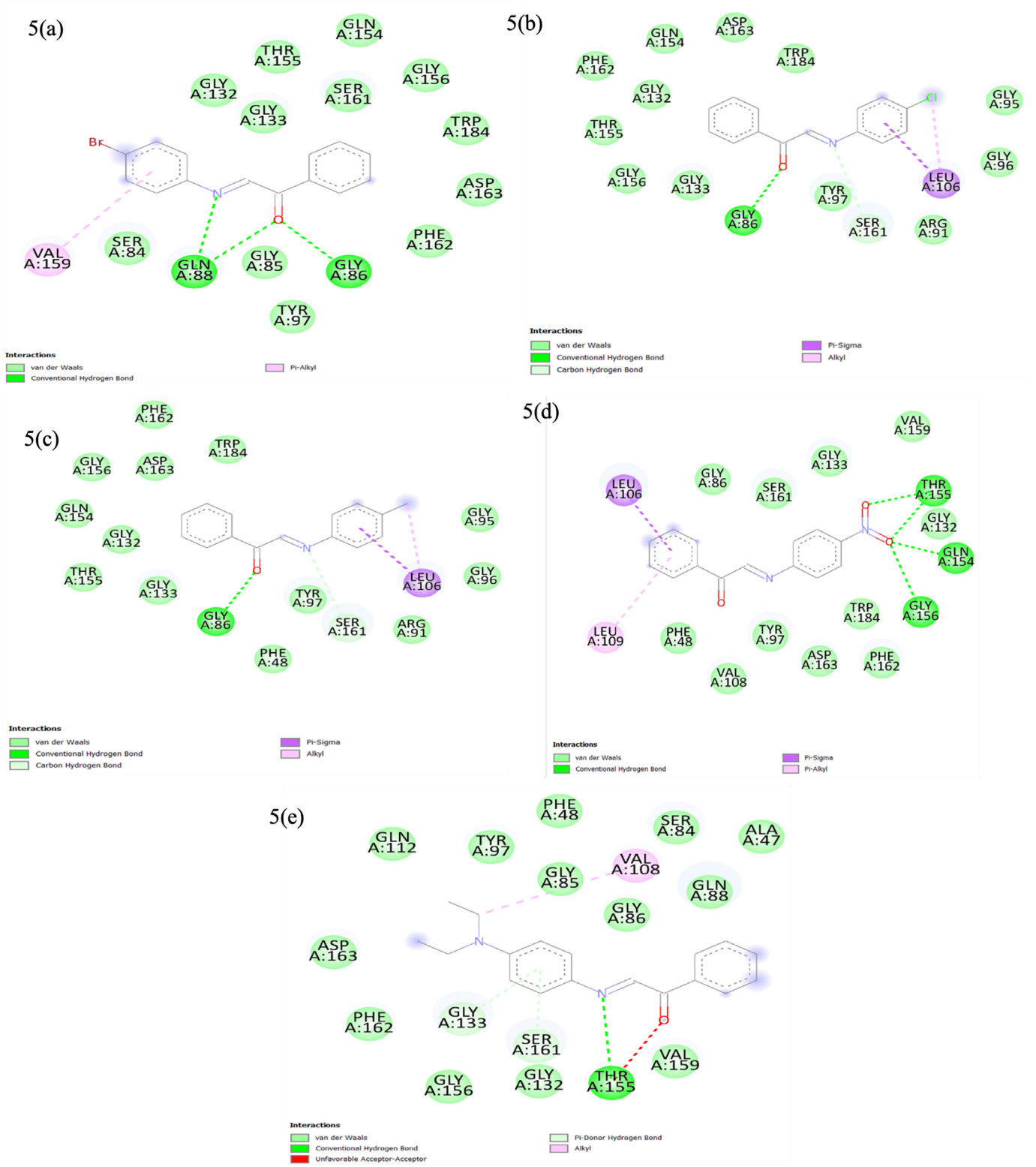

Figure 5 (a-e): 2D Interaction between *Escherichia coli* (protein PDB ID 3T88) with Schiff bases BIP, CIP, PT, NIP, and DPIP.

Table 3: 2D interaction of 3Q8Uand Schiff base.

Target Protein (PDB ID)	Ligand	Interacting amino acid at the active site	Interaction type	H-bond (LA:AA)	Binding Energy (BE) (Kcal/mol)
3Q8U	BIP	LYS9,TYR49,GLU51,HIS52 PHE57,LEU61,THR91,ARG102,VAL109,GLY110,ASN112,HIS115,GLY116,ASP118.	H-bond Van der Waals Pi-alkyl	3(O:LYS9,O:TYR49, O: HIS115)	-7.3
	CIP	LYS9,TYR49,HIS52,PHE57,LEU61 ,THR91,ARG102,VAL109,GLY110, ASN112, HIS115,GLY116,ASP118.	H-bond Van der Waals Pi-alkyl	1(O:LYS9)	-7.2
	PT	LYS9,TYR49,GLU51,HIS52,PHE57,LEU61,THR91,ARG102,GLY110,ASN112,VAL109 HIS115, GLY116, ASP118.	H-bond Van der Waals Pi-alkyl	3(O: LYS9, O: TYR49. O: HIS115}	-7.5
	NIP	LYS9,TYR49,HIS52,PHE57,ARG85,ILE88,VAL109,ARG102,GLY110,HIS115,GLY116, ASP118,THR91.	H-bond Van der Waals Pi-alkyl	2(O:ARG45.O:ARG102)	-7.6
	DPIP	LYS9,TYR49,GLU51,HIS52,PHE57,LEU61,THR91,VAL109,GLY110,HIS115,SER122.	H-bond Van der Waals Pi-alkyl	1(O:GLY110)	-7.3

Table 4: 2D interaction of 3T88 and Schiff base.

Target Protein (PDB ID)	Ligand	Interacting amino acid at the active site	Interaction type	H-bond (LA:AA)	Binding Energy (BE) (Kcal/mol)
3T88	BIP	SER84,GLY85,GLY86,GLN88,TYR97,GLY133,GLN154,THR155,GLY156,SER161,TRP184,ASP163,PHE162,VAL159.	H-bond Van der Waals Pi-alkyl	3(O:GLY86, N:GLN88, O:GLN88)	-6.9
	CIP	GLY86,ARG91,GLY95,GLY96,TYR97,LEU106,GLY132,GLY133, GLN154, THR155, GLY156,SER161, PHE162,ASP163,TRP184.	H-bond Van der Waals Pi-alkyl	2(O:GLY86,N: GLN88)	-6.9
	PT	PHE48,GLY86,ARG91,GLY95,GLY96,TYR97,LEU106,GLY132,GLY133, GLN154,THR155,THR155,GLY156, PHE162,ASP163,TRP184.	H-bond Van der Waals Pi-alkyl	2(O:GLY86,N: GLN88)	-7.1
	NIP	PHE48, GLY86,TYR97, LEU106 VAL108,LEU109, GLY132,GLY133,GLN154,THR155,GLY156,VAL159,SER161,PHE162,ASP163,TRP184.	H-bond Van der Waals Pi-alkyl	4(O:GLN154,O: THR155, O:GLY156)	-6.9
	DPIP	ALA47,PHE48,SER84,GLY85,GLY86, GLN88,TYR97,VAL108,GLN112,GLY132,GLY133,THR155,GLY156,VAL159, SER161, PHE162, ASP163. ,	H-bond Van der Waals Pi-alkyl	1(THR155)	-6.8

PHE48, SER84, GLY85, GLY86, GLN88, TYR97, VAL108, GLN112, GLY132, GLY133, THR155, GLY156, VAL159, SER161, PHE162, and ASP163.

Given the good binding energy and interaction of NIP and PT, CIP, BIP, and DPIP Schiff bases, the NIP and PT, CIP, BIP, and DPIP Schiff bases have good affinity with target protein 3T88. Therefore, NIP and PT, CIP, BIP, and DPIP Schiff bases may lead to effective growth inhibitory compounds against bacteria as per Table 4 showing 2D interactions.

CONCLUSION

On the basis of *in vitro* studies, NIP has mild Minimum Inhibitory Concentration (MIC) against *E. coli.* BIP also has mild minimum inhibitory concentration against *S. aurens*, but PT has good Minimum Inhibitory Concentration (MIC) against *S. aureus ATCC 29213*, with a MIC value of 16 µg/mL. *In silico* approach with the help of Auto dock Vina and Swiss ADME shows that PT has good binding energy against *S. aureus* (PDB ID 3Q8U) and promising conventional, non-conventional hydrogen bonds, and obeyed Lipinski rule of 5 and drug-likeness parameters. *In vitro* descriptors also support the virtual screening approach. Therefore, PT Schiff base can be used as a potential drug candidate against pathogenic bacteria *S. aureus.*

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NDKs: Nucleoside Diphosphate Kinases; **ADME:** Absorption, Distribution, Metabolism And Excretion, **AA:** Amino Acids; **BE:** Binding Energy; **CDC:** Centre for Disease Control and Prevention; **FDA:** Food and Drug Administration; **GISRS:** Global Influenza Surveillance and Response System; **PDB:** Protein Data Bank; **WHO:** World Health Organization; ***S. aureus*:** *Staphylococcus aureus*; ***P. aeruginosa*:** *Pseudomonas aeruginosa*; ***E. coli*:** *Escherichia coli*; ***A. baumannii:*** *Acinetobacter baumannii*; ***K. pneumoniae*:** *Klebsiella pneumoniae;* **BIP:** (2E)-2-[(4-bromophenyl)imino]-1-phenylethan-1-one; **CIP:** (2E)-2-[(4-chlorophenyl)imino)-1-phenylethan-1-one; **PT:** (2E)-2-[(4-methylphenyl)imino]-1-phenylethan-1-one; **NIP:** (2E)-2-[(4-nitrophenyl)imino]-1-phenylethan-1-one; **DPIP:** (2E)-2-[(4-(diethylamino)phenyl)imino]-1-phenylethan-1-one; **S0N-C:** Succinylbenzoyl-N-Coenzyme; **ADP:** Adenosine-5'-Diphosphate**. SER:** Serine; **GLY:** Glycine; **GLN:** Glutamine; **TYR:** Tyrosine; **THR:** Threonine; **TRP:** Tryptophan; **ASP:** Aspartic acid; **PHE:** Phenylalanine; **VAL:** Valine; **HIS:** Histidine; **LEU:** Leucine.

SUMMARY

Schiff bases have great importance in organic chemistry, having demonstrated significant biological activities including antiviral, antibacterial, and antifungal properties. *In silico* studies utilizing AutoDock Vina and Swiss ADME tools have further revealed that PT Schiff base exhibits favorable binding energy against the crystal structure of *S. aureus* (PDB ID 3Q8U), Additionally, PT adheres to the Lipinski rule of 5 and possesses drug-like properties, suggesting its potential as a suitable drug. *In vitro* results of the PT Schiff base against the pathogenic bacteria *S. aureus* aligned with the findings from the *in silico* studies. These outcomes have the aptitude to contribute to the development of a potential inhibitor.

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