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Experimental and Computational Insights of Schiff Base Copper(II) Complexes of Para-Substituted Benzaldehydes towards some Bacterial and Target Proteins

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Abstract: *The search for new antibacterials via in-vitro and computational techniques that support green and sustainable technologies for use in the future necessitated this study, as molecular docking has proved to be a promising lead in the development of target drugs. Herein, we report novel copper (II) complexes F1Cu-F3Cu with Schiff bases synthesized from para-substituted benzaldehydes and ortho-aminophenol. The compounds were characterized using elemental analysis, atomic absorption spectroscopy, infrared spectroscopy, ^{1}H NMR, and electronic absorption spectroscopy. In-vitro antibacterial study against 6 human pathogenic bacteria; Escheriachia coli (ATCC 8739), Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 19582), Bacillus cereus (10702), Enterococcus faecalis (ATCC 29212) and Kribsella pneumonia (ATCC 10031) revealed an increase in antibacterial activity with the copper complexes against the free ligands. This was compared with ampicillin as a reference. To further confirm the activity of the complexes, molecular docking was employed against some clinically important bacterial target proteins specific to each bacterium, which revealed a strong correlation with the experimental antibacterial activity (MIC values) for all the targets studied. The docking results show that the complexes exhibited a much stronger binding interaction with the proteins compared to the parent ligands, with the former having scores of −7.4 to −10.1 kcal/mol and the latter scoring −5.7 to −7.4 kcal/mol. Also, inhibition of the DHFR enzyme by the complexes prevents any substrate from accessing the enzyme, thus disrupting of the folate cycle that is important for the growth of bacteria*. The integration of experimental and computational findings in this study provides valuable insight into the structure–activity relationships of copper (II) Schiff base complexes and highlights their potential as promising lead compounds for the development of novel antibacterial agents capable of addressing multidrug-resistant bacterial infections.

Keywords: *Antibacterial, computational, protein targets, copper complexes, Schiff bases*

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1.0 Introduction

The increasing threat of antibiotic resistance worldwide, attributed to the development of multidrug-resistant strains like methicillin-resistant *Staphylococcus aureus*, trimethoprim-resistant *Klebsiella pneumoniae*, and virulent Gram-negative species such as *Pseudomonas aeruginosa* and *Escherichia coli*, requires an immediate search for new and chemically diverse antimicrobial drugs via computational techniques. Several Schiff base metal complex *in-vitro* studies have revealed promising results (Sankar & Sharmila 2023, Kumar *et al*., 2024; Nidhi *et al*., 2025), though many were never substantiated computationally.

Transition metal complexes, particularly those of copper(II), have attracted increasing attention due to their redox properties, structural versatility, and ability to interact with biological macromolecules, making them promising candidates in antimicrobial drug development**.** Presently, the use of molecular docking in the development of new drug candidates is drawing the attention of synthetic scientists, as this has led to the prediction of ligand-target interaction for the identification of novel compounds (Vaghela, 2026). The two primary components of molecular docking are the ligand and the protein. The protein acts as the binding site where the ligand attaches to trigger a specific biological response. This interaction offers valuable information about how effectively a ligand can bind to a protein, which helps to decode the molecular recognition between small ligands and larger biomolecules. These predictions help prioritize compounds likely to have strong biological effects for further experimental testing (Kitchen *et al*., 2004, Gschwend *et al*., 1996). Despite the reported antibacterial activities of Schiff base metal complexes, there is still a lack of integrated studies combining experimental antibacterial evaluation with detailed computational docking against multiple validated bacterial protein targets, particularly for para-substituted copper(II) Schiff base complexes. Furthermore, limited information exists on how para-substitution influences binding interactions across different bacterial enzymes and resistance-related proteins**.**

In this study, a combined experimental and computational approach was employed to investigate the antibacterial potential of newly synthesized copper(II) Schiff base complexes derived from para-substituted benzaldehydes. These selected protein targets—CapF, σ^s/Crl complex, HORMA domain proteins, and dihydrofolate reductase (DHFR)—are essential for bacterial virulence, stress response, DNA regulation, and folate biosynthesis, respectively, and represent key pathways associated with bacterial survival and antibiotic resistance**.**

The structure and function of CapF have been extensively studied in the literature (Nakano *et al*., 2015; Miyafusa *et al*., 2012; Miyafusa *et al*., 2008), with CapF being a very well-known bifunctional metalloenzyme characterized by a dumbbell structure, which consists of the cupin domain and the SDR domain containing clearly identifiable and approachable binding pockets, such as the zinc-containing active site of the cupin domain that makes this enzyme a promising candidate for structure-based computation studies of potential inhibitors (Nakano *et al*., 2015). CapF is crucial for the formation of the capsular polysaccharide precursor UDP-L-FucNAc in *Staphylococcus aureus*, a process which is well-conserved in many pathogenic bacteria; therefore, the inhibitors found by

computational methods against the active site of CapF would have great therapeutic value against many antibiotic-resistant pathogens, including both methicillin- and vancomycin-resistant *S. aureus* (Nakano *et al*., 2015). Moreover, the fact that CapF has already been shown to be inhibited via zinc coordination by 3-isopropenyl-tropolone can serve as a well-established pharmacophore and an inhibitor template for future molecular docking efforts (Nakano *et al*., 2015). These protein targets were selected based on their validated roles in bacterial survival mechanisms and their established structural availability for molecular docking studies, thereby enabling a multi-target inhibition strategy against resistant pathogens.

The docking studies aimed at targeting σ^s (Chain F) and Crl (Chain J) have great therapeutic value due to σ^s being the primary stress response regulator and transcription initiation factor in Gram-negative bacteria like *Salmonella enterica, Pseudomonas aeruginosa,* and *Escherichia coli*. The protein functions as a transcription initiation factor to initiate the process and is known to activate the expression of about 10% of genes present in *E. coli* and cause antibiotic tolerance, persistence, biofilm production, and virulence in the bacteria. As stated above, it activates the process under stress conditions like exposure to antibiotics (Battesti *et al*., 2011, Lange & Hengge-Aronis, 1991, Stewart *et al*., 2015). Since the high-resolution cryo-EM structure of the E. coli Crl-TAC complex at 3.80 Å resolution reveals the interaction interface between Crl and σ^s2 along with the RNAP core enzyme, one has the advantage of studying structurally defined and well-characterized binding sites for structure-based virtual screening and docking of small molecules (Xu *et al*., 2019; Feklistov *et al*., 2014; Liu *et al*., 2016). Moreover, the unique mode of activation of σ^s-dependent transcription by Crl, independent of DNA interaction, by which it stabilizes structural features in σ^s2 and facilitates holoenzyme and open complex formation, provides an unprecedented opportunity for computational drug targeting of the interface (Xu *et al*., 2019; Weber *et al*., 2005; Österberg *et al*., 2011).

The structural features of HORMA domain proteins are well-documented since they have well-established fold conformations and particular peptide-binding pockets, thus making them suitable subjects for studying by computational docking (Rosenberg & Corbett, 2015; Aravind & Koonin, 1998). The capability of HORMA domain proteins to interact with specific peptides and form complexes with other proteins (CD-NTase proteins) provides researchers with binding pockets where they can search for inhibitors using the docking technique (Burroughs *et al*., 2015, Whiteley *et al*., 2019). Additionally, considering that HORMA-containing CBASS systems provide protection against bacteriophages in important pathogenic bacteria, including *E. coli* and *Pseudomonas aeruginosa*, through the mechanism of abortive infection, the use of computational docking on HORMA domain proteins could help design new antibacterial drugs (Cohen *et al*., 2019).

Dihydrofolate reductase (DHFR) is an enzyme conserved throughout nature, which was proven to be a good target by the discovery of methotrexate and trimethoprim in the 1950s, and whose crystal structure, including the first crystallized complex of DHFR from *Klebsiella pneumoniae* in complex with propargyl-linked antifolates, is available at high resolution providing valuable information on ligand binding pockets allowing rational design of new inhibitors through computational docking (Lombardo *et al*., 2016, Schnell *et al*., 2004). Residues Leu28, Ile50, Ile94, and Leu54 play an important role in the active site of DHFR and determine inhibitor affinity, while the difference in DHFR from bacteria compared

to humans, namely the variations in residues N64E, P61G, F31L, and V115I as well as in the conformation of loop containing residues 49-53 make it possible to design selective antifolate derivatives targeting DHFR in resistant Gram-negative organisms based on computational docking (Lange *et al*., 2023, Lombardo *et al*., 2016, Hawser *et al*., 2006). Finally, because of the dramatic increase in trimethoprim resistance of *Entero - bacteriaceae* bacteria reaching up to 14-30% globally, along with very scarce treatment options against highly resistant *K. pneumoniae* bacteria, computational docking of inhibitors against both wild-type and resistant forms of DHFR is an urgent and effective way of designing potent antibiotics (Lombardo *et al*., 2016; Lange *et al*., 2023; Frey *et al*., 2010; Hawser *et al*., 2006). Considering the interesting mechanistic reports from molecular docking that support green and sustainable technologies for future use, our research focuses on the synthesis, characterization, in vitro evaluation, and molecular docking of para-substituted Schiff base copper complexes to establish lead compounds for drug discovery against antimicrobial resistance. This study aims to synthesize and characterize para-substituted Schiff base copper (II) complexes and evaluate their antibacterial activities both experimentally and through molecular docking studies against selected bacterial protein targets in order to establish their structure–activity relationships and potential as lead antimicrobial agents**.** Our previous report on ortho-substituted copper complexes with interesting antibacterial activity (Ejiah *et al*., 2024), necessitated further research into the para substituents and to establish mechanism of action via molecular docking.

2.0 Materials and Methods

2.1 Materials

All reagents and solvents were of analytical or spectroscopic grade and were used without further purification. Ethanol, chloroform, dimethylformamide (DMF), 2-aminophenol, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, and copper(II) chloride were purchased from Sigma-Aldrich and used as received.

2.2 Physical Measurements

Infrared (IR) spectra were recorded using a Bruker FT-IR (ATR) Tensor 27 spectrophotometer in the range of 400–4000 cm^{-1}. ^1H NMR spectra of the ligands were recorded in DMSO-d_6 using a Bruker Avance III 400 MHz spectrometer, with chemical shifts reported in ppm relative to tetramethyl-silane (TMS) as internal standard. Chemical shifts were reported in ppm relative to TMS as an internal standard. Electronic absorption spectra of the Schiff bases and metal complexes were recorded in chloroform ($CHCl_3$) and dimethylformamide (DMF) using a Cary 50 UV–Vis spectrophotometer over the range of 200–1100 nm. Metal analysis was determined using Analyst 200 atomic absorption spectrophotometer (AAS), Perkin Elmer. Melting points were determined on a Reichert Thermovar melting-point apparatus and are uncorrected. Magnetic susceptibility measurements were performed using a Sherwood Scientific magnetic susceptibility balance on powdered samples. $Hg[Co(SCN)_4]$ was used as the calibrant and corrections for diamagnetism calculated from Pascal's constants. Elemental (CHNS/O) analyses were performed using a PerkinElmer 2400 Series II analyzer.

2.3 Synthesis of Schiff Bases

Equimolar amounts (10 mmol each) of 2-aminophenol and para-substituted benzaldehydes were dissolved in ethanol (25 mL) and refluxed at 70 °C for 6 h under constant stirring. The resulting precipitate was filtered, recrystallized from ethanol, dried, and stored in a desiccator.

2.4 Synthesis of Schiff Base Copper(II) Complexes

An ethanolic solution (40 mL) of the Schiff base ligand (4 mmol) was mixed with a copper(II) chloride solution (2 mmol in 20 mL ethanol), maintaining a ligand-to-metal ratio of 2:1. The reaction mixture was made alkaline using triethylamine and refluxed for 4 h. The resulting solid was filtered, washed with ethanol, dried, and stored in a desiccator for further analysis.

2.5 Antibacterial Studies

The antibacterial activities of the ligands and metal complexes were evaluated against selected pathogenic microorganisms: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (ATCC 10702), *Enterococcus faecalis* (ATCC 29212), and *Klebsiella pneumoniae* (ATCC 10031) using the agar well diffusion method (Rivera et al., 2023).

The compounds were prepared in DMSO to obtain a final concentration of 10 mg/ml. Minimum inhibitory concentration (MIC) values were determined using a 96-well microplate dilution method (Barnes *et al*., 2023).

2.6 Molecular Docking

Construction of the structures of both the Schiff base compounds synthesized as well as their metal complexes was carried out using the software Avogadro (Ejiah *et al*., 2026). The structures of the Schiff base ligands and their copper(II) complexes were constructed using Avogadro software (Ejiah et al., 2026), and geometry optimization was performed using the PM3 semi-empirical method in Spartan'14 (Aina et al., 2024).

The crystal structures of the ATCC bacterial strain protein targets were acquired from the Protein Data Bank (PDB IDs for *Staphylococcus aureus*: 4YRD; *Escherichia coli*: 6KJ6 (ATCC 8739), chains F and J; *Pseudomonas aeruginosa*: 6P80 (ATCC 27853); and *Klebsiella pneumoniae*: 4OSG (ATCC 10031)). Protein preparation was done using BIOVIA Discovery Studio. All water molecules, as well as other co-crystal ligands and unnecessary heteroatoms, were removed during protein preparation. The polar hydrogen atoms were added, while their protonation states were correctly assigned according to physiological conditions.

The docking simulations were done on the PyRx software, which consists of AutoDock Vina with the Open Babel feature for converting the ligands from one file format to another. The ligands were subjected to energy minimization before docking in PDBQT format. The grid box was created to capture the entire region of activity of the target protein as defined in Table 1. All docking studies were conducted using AutoDock Vina on a computer running Windows 10, which had an Intel Core i5 CPU (2.71 GHz), 8 GB of memory, and a 500 GB solid-state drive. For each ligand-protein complex, various binding modes were created, and the most stable complex was chosen based on binding energy (kcal/mol). The docking results were used as a proxy for determining the strength of the binding interactions. Visualization and analysis after docking, such as hydrogen bonds, hydrophobic effects, and electrostatic effects, were carried out through PyMOL and BIOVIA Discovery Studio. Ligand-protein complexes with the highest stability were studied for their interactions with the important amino acids. The preferred binding modes with minimal binding energy were chosen for correlating with the antibacterial activity data for structure-activity relationships (SARs).

3.0 Results and Discussion

3.1 Synthesis

The Schiff base (SB) ligands were obtained in good yields through the condensation of 2-aminophenol with para-substituted benzaldehydes, namely 4-methoxybenzaldehyde (F1), 4-chlorobenzaldehyde (F2), and 4-nitrobenzaldehyde (F3), in a 1:1 molar ratio as shown in Scheme 1. The formation of the

desired ligands was confirmed by ^{1}H NMR and microanalysis Table 2. Treatment of the ligands with Cu(II) chloride afforded metal complexes corresponding to the general formula $[Cu_nF_2]$ where n = 1-2 Scheme 2.

Table 1: Grid Parameters of the Active Sites for the Targets

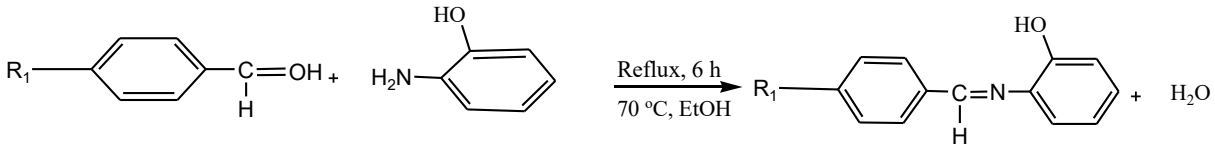
Target Organism	PDB ID	Chain	Grid Center (x, y, z) (Å)	Grid Size (x, y, z) (Å)	Spacing (Å)
Staphylococcus aureus	4YRD	A	(20.6665, 6.6397, −36.8372)	(26.3464, 25.2005, 26.5650)	1.0
Escherichia coli	6KJ6	F	(63.2925, 114.9958, 84.8150)	(28.2029, 30.6450, 42.0035)	1.0
Escherichia coli	6KJ6	J	(62.7653, 121.5927, 71.6813)	(26.6831, 47.5939, 37.3233)	1.0
Pseudomonas aeruginosa	6P80	A	(−13.4508, 2.6497, 0.0924)	(58.0868, 48.4820, 61.2048)	1.0
Klebsiella pneumoniae	4OSG	A	(19.9826, −53.7916, 7.7128)	(32.9235, 40.8029, 40.1650)	1.0

Microanalysis of the complexes supported the predicted structures of the complexes based on found and calculated Table 2. The mass of F1Cu $[M+NH_4]$ =681, F2Cu $[M+NH_4]$ =688 and F3Cu $[M^+]$ =545 supports the predicted structures of the complexes. The complexes were soluble in common organic solvents such as DMF and DMSO. Attempts to obtain single crystals suitable for X-ray diffraction analysis were unsuccessful.

The metal content analysis of the complexes revealed the percentage of metal ion per mole of complex, which corroborated the calculated. Elemental and AAS data suggest that the methoxy- and chloro-substituted complexes are binuclear species containing two copper ions per mole of complex, whereas the nitro-substituted complex appears to be mononuclear. Square planar complexes of the form $Cu_2Cl_2(L)_2$ containing 2 moles of copper per mole of complex have been reported from 2-acetylpyridine and s-benzyldithiocarbazate (Beshir *et al*., 2008) and bis(3-hydroxyquinoxaline-2-carboxalidene) 1,2-diaminobenzene) (Arun *et al*., 2021). To further confirm the presence of binuclear complexes, higher metal content via elemental and metal analysis has been reported (Prakash *et al*. 2020). This corroborates our results with higher metal content in F1Cu and F2Cu when compared with F3Cu with lower metal content.

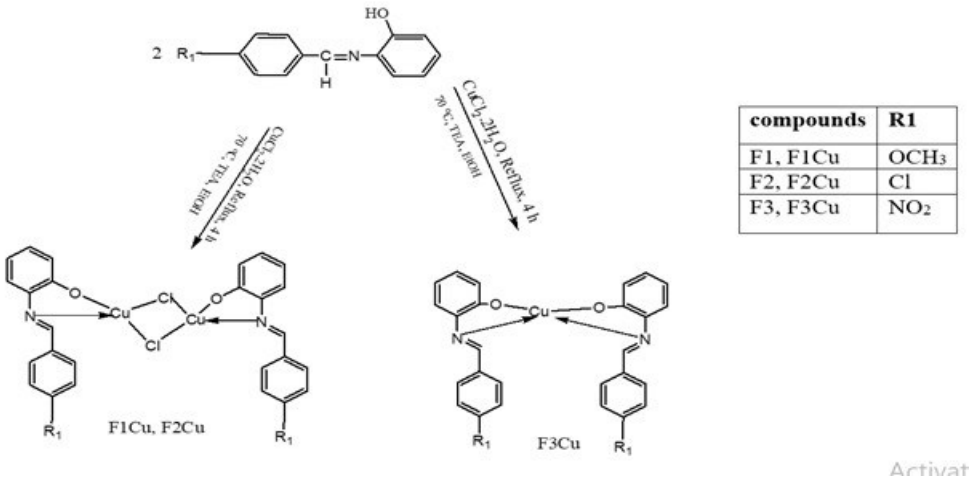
To study the binding modes of the ligands to the copper ion, the IR spectra of the free ligand were compared to the complex Table 3. The IR spectra of the ligands exhibited broad absorption bands in the range 3291–3333 cm^{-1} attributable to the phenolic O–H stretching vibration. The disappearance of these bands upon complexation indicates deprotonation and coordination of the phenolic oxygen atom to the copper(II) center.

The bands in the region 1601-1623 cm^{-1} due to C=N group shifted to a lower wavenumber 1585-1596 cm^{-1}. Furthermore, peaks at 350 cm^{-1} can be assigned to the Cu-Cl band.



P-Substituted benzaldehyde 2-aminophenol Schiff base

Scheme 1: Synthesis of Schiff bases



compounds	R1
F1, F1Cu	OCH_3
F2, F2Cu	Cl
F3, F3Cu	NO_2

Scheme 2: Synthesis of Schiff base copper (II) complexes

Table 2: Physical and analytical data of para substituted Schiff bases and metal complexes Infrared Spectroscopy

Compound	R_1	Empirical Formula (Formula Weight)	Yield (%)	Color	M.pt. (°C)	C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	Cu % Found (Calc.)
F1	OMe	$C_{14}H_{13}NO_2$ (227)	69	Yellow	56–57	74.08 (73.99)	5.67 (5.77)	6.13 (6.16)	–
F1Cu	OMe	$C_{29}H_{31}Cl_2Cu_2N_3O_4$ (681)	68	Brown	>250	50.78 (50.95)	4.85 (4.57)	6.01 (6.15)	20.98 (18.59)
F2	Cl	$C_{13}H_{10}NOCl$ (231)	63	Yellow	78–79	67.26 (67.39)	4.30 (4.35)	6.05 (6.05)	–
F2Cu	Cl	$C_{27}H_{25}Cl_4Cu_2N_3O_2$ (688)	52	Brown	>250	47.58 (46.83)	3.41 (3.64)	6.32 (6.07)	19.53 (18.36)
F3	NO_2	$C_{13}H_{10}N_2O_3$ (242)	77	Yellow	112	64.82 (64.46)	4.07 (4.16)	11.64 (11.56)	–
F3Cu	NO_2	$C_{26}H_{18}CuN_4O_6$ (545)	62	Brown	>250	57.13 (57.19)	3.09 (3.32)	10.82 (10.26)	10.36 (11.64)

The bands in the spectra of metal complexes 448-456 cm^{-1} and 577-594 cm^{-1} is assigned to stretching frequencies of ν(M-N) and ν(M-O) bond formation. These spectral changes confirm the coordination of the ligands through the azomethine nitrogen and phenolic oxygen donor atoms.

3.2 Spectroscopy

3.2.1 ^{1}H NMR

^{1}H NMR spectral data of the Schiff bases are

shown in Table 3. "The ^{1}H NMR spectra of all Schiff bases exhibited a singlet at δ 7.89–8.37 ppm attributable to the azomethine (–CH=N–) proton. A singlet at 8.63-8.77 ppm was observed due to the proton of the hydroxyl-OH group. Schiff bases with electron-donating groups appear upfield due to an increase in the electron density in the vicinity of the proton, which causes shielding from the magnetic field, while Schiff bases with electron-withdrawing groups appear downfield as a result of low electron density in the vicinity of the proton. These effects were similarly observed for the phenolic proton signals. The three protons of OCH_3 group resonated in the region 3.91 δ as expected.

Table 3: Spectroscopic data of para-substituted Schiff bases and metal complexes

Parameter	F1	F1Cu	F2	F2Cu	F3	F3Cu
R_1	**OMe**	**OMe**	**Cl**	**Cl**	**NO_2**	**NO_2**
ν(O–H) (cm^{-1})	3333	–	3291	–	3304	–
ν(C=N) (cm^{-1})	1618	1596	1601	1585	1623	1595
ν(C–O) (cm^{-1})	1245	1178	1238	1212	1299	1291
ν(Cu–Cl) (cm^{-1})	–	350	–	350	–	–
ν(M–O) (cm^{-1})	–	584	–	594	–	577
ν(M–N) (cm^{-1})	–	448	–	456	–	453
δ(HC=N) (s, 1H) (ppm)	7.89	–	7.98	–	8.37	–
δ(OH) (s, 1H) (ppm)	8.63	–	8.65	–	8.77	–
δ(OCH_3) (s, 3H) (ppm)	3.91	–	–	–	–	–

Note: ν = infrared stretching frequency; δ = chemical shift (ppm); s = singlet; M = Cu(II); OMe = methoxy; Cl = chloro; NO_2 = nitro.

3.2.2 Electronic Absorption Data and Magnetic Susceptibility Measurements

The UV-Visible absorption spectra for the binuclear complexes of transition copper complexes are shown in Table 4. The absorption bands are observed between 34,482-36,496 $cm^{-1,}$ indicating the formation of π-π* transition. The bands appearing between 27,567-28,169 cm^{-1} confirm the n-π* transition. The complexes showed d-d transition and charge transfer for Cu(II) complexes in the expected range of 22,883-22,988 and 30,088-30,959 $cm^{-1,}$ respectively (Jaffer & Orclin 1962). This could be attributed to the $^2B_{1g} \rightarrow {}^2A_{1g}$ transitions characterized Cu(II) ion in a square-planar geometry (Arun *et al*., 2021; Thirumavalavan *et al*., 2006; Cheng *et al*., 1996). These absorption bands of binuclear copper complexes also suggest square planar geometry (Kumar & Deo 2023).

The room temperature magnetic moment values of 1.56 and 1.52 B.M for the complexes, F1Cu and F2Cu, indicate that, as expected, magnetic exchange might have occurred between the two copper ions, which may have led to lower values (Lal *et al*., 2006) than expected (1.7-2.2 B.M). A subnormal magnetic moment value of 1.52 B.M/Cu atom at room temperature for a complex is an indication of the presence of two copper(II) ions in a molecule (Arun *et al*., 2021). On the basis of the magnetic data, the copper (II) complexes probably have a binuclear structure (Pardasani & Pardasani, 2021; Karabocek *et al*., 2007). The nitro-substituted copper(II) complex F3Cu exhibited a magnetic moment value of 1.87 in a tetrahedral field (Pardasani & Pardasani 2021).

3.3 Antibacterial Activity

The new compounds were subjected to an antibacterial study to evaluate their application in relation to the nature of substituent groups. Antibacterial activity of

all the compounds Table 5 was evaluated by applying minimum inhibition concentration test against six human pathogenic bacteria: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (10702), *Enterococcus faecalis* (ATCC 29212) and *Klebsiella pneumoniae* (ATCC 10031). Ampicillin was used as a reference compound.

Table 4: Electronic absorption data and magnetic susceptibility measurements of para-substituted Schiff bases and copper complexes

Parameter	F1	F1Cu	F2	F2Cu	F3	F3Cu
R_1	OMe	OMe	Cl	Cl	NO_2	NO_2
$CHCl_3$ Band 1 (cm^{-1})	34,482	31,152	36,496	22,573	36,496	–
(log ε)	(4.17)	(3.04)	(4.15)	(3.70)	(4.16)	
$CHCl_3$ Band 1 (cm^{-1}) Assignment	π→π*	CT	π→π*	d–d	π→π*	–
$CHCl_3$ Band 2 (cm^{-1})	27,567	23,255	28,169	19,762	27,839	–
(log ε)	(4.20)	(3.33)	(4.05)	(3.67)	(4.18)	
$CHCl_3$ Band 2 (cm^{-1}) Assignment	n→π*	d–d	n→π*	d–d	n→π*	–
DMF Band 1 (cm^{-1})	–	22,988	–	30,959	–	30,088
(log ε)		(3.80)		(4.08)		(4.56)
DMF Band 1 (cm^{-1}) Assignment	–	d–d	–	CT	–	CT
DMF Band 2 (cm^{-1})	–	–	–	22,883	–	22,988
(log ε)				(4.10)		(3.80)
DMF Band 2 (cm^{-1}) Assignment	–	–	–	d–d	–	d–d
μeff (B.M.)	–	1.56	–	1.52	–	1.87
Proposed Geometry	–	Square-planar	–	Square-planar	–	Tetrahedral

Note: CT = charge-transfer transition; d–d = transition between metal orbitals; μeff = effective magnetic moment; B.M. = Bohr Magneton; $CHCl_3$ = chloroform; DMF = dimethylformamide.

The complexes with methoxy and chloro substituents displayed higher antibacterial activity (MIC 0.62 mg/ml (F1Cu) and 0.15 – 1.25 mg/ml (F2Cu) respectively than free ligands (MIC= 1.25- >5.00 mg/ml. Table 5. This feature can be attributed to the chelation theory with copper(II) ions to produce a strong synergistic effect for efficient antibacterial activity. The FICu and F2Cu complexes form square planar, while F3Cu forms tetrahedral, which can also be attributed to the biological activity as reports have shown that some geometries can accommodate better interactions of enyzmes with compounds. Notably, analysis of the experimental data with computational methods also shows a synergy in the results as the results obtained have revealed anti-bacterial activity of complexes FICu and F2Cu.

3.4 Molecular Docking

Molecular docking of the Cu(II) complexes F1Cu-F3Cu against five clinically important bacterial target proteins (*Staphylococcus aureus* – 4YRD, *Escherichia coli* – 6KJ6 (ATCC 8739) chains F and J, *Pseudomonas aeruginosa* – 6P80 *(ATCC 27853)*, and *Klebsiella pneumoniae* – 4OSG (ATCC 10031)) suggests a positive relationship between

molecular docking affinity and the experimentally determined antibacterial activities (MIC values).

All of the investigated Cu(II) complexes, F1Cu-F3Cu display significantly increased binding abilities when compared to their un-complexed Schiff base ligands F1-F3, indicating that metal coordination plays a critical role in improving molecular recognition and binding interactions at the active sites of the target bacterial proteins. The docking results show that the Cu complexes have a much stronger interaction with the proteins compared to the parent ligands, with the former having scores of −7.4 to −10.1 kcal/mol and the latter scoring −5.7 to −7.4 kcal/mol. Table 6 below summarizes the docking studies.

Table 5: Minimum inhibitory concentration (MIC) of para-substituted Schiff bases and metal complexes

Microorganism	F1	F1Cu	F2	F2Cu	F3	F3Cu	Ampicillin
R_1	OMe	OMe	Cl	Cl	NO_2	NO_2	–
Staphylococcus aureus **(ATCC 6538)**	5.00	0.62	>5.00	0.15	>5.00	2.50	2.50
Enterococcus faecalis **(ATCC 29212)**	2.50	0.62	>5.00	0.31	>5.00	2.50	5.00
Bacillus cereus **(ATCC 10702)**	2.50	0.62	>5.00	1.25	>5.00	2.50	5.00
Escherichia coli **(ATCC 8739)**	1.25	0.62	>5.00	1.25	>5.00	2.50	1.25
Pseudomonas aeruginosa **(ATCC 19582)**	2.50	0.62	>5.00	0.31	>5.00	2.50	5.00

Note: MIC = Minimum Inhibitory Concentration. Values are expressed in mg mL^{-1}. Lower MIC values indicate higher antibacterial activity. Ampicillin was used as the reference antibacterial agent.

3.4.1 Staphylococcus aureus (4YRD)

Molecular docking studies for the PDB 4YRD target CapF of *Staphylococcus aureus* indicated that all designed ligands and their Cu(II) complexes docked into the active site of CapF enzyme with different levels of affinity, as shown in Fig. 1 below and supplementary material S1 -S7. It was found that the Cu complexes had higher docking energy (-8.7 to -9.3 kcal/mol) than their respective ligands (~ -7.0 to -7.4 kcal/mol), showing that metal complexation increased the binding energy of ligands. Notably, the conventional antibiotic Ampicillin displayed a docking energy value (-7.4 kcal/mol) which was lower than Cu complexes and specifically the values of F2Cu and F3Cu complexes, implying less affinity of Ampicillin towards CapF compared to those Cu complexes. CapF is a zinc-dependent cupin-domain enzyme involved in capsular polysaccharide biosynthesis and bacterial virulence.

In all the complexes studied, F2Cu exhibited the highest binding affinity (−9.3 kcal/mol) and the greatest antibacterial activity (MIC = 0.15 mg/mL), being superior to the two other complexes prepared as well as Ampicillin (MIC = 2.50 mg/mL). This binding is mediated through numerous interactions made by the ligand with essential residues such as Phe262, Lys285, Met351, Val353, and Phe357. These residues form the hydrophobic core of the CapF active site. The combined role of aromatic, hydrophobic, and polar groups suggests an optimal orientation of binding leading to efficient binding in the Zn^{2+}-binding site.

Favorable docking scores were also observed for F1Cu and F3Cu. (−8.7 and −9.0 kcal/mol, respectively), along with antibacterial activity superior to their parental ligands and Ampicillin. The compound F1Cu binds to the

receptor in an optimal manner and interacts with residues like Phe262, Glu264, Lys285, Met351, Val353, and Phe357, which leads to a moderate MIC value of 0.62 mg/mL. On the other hand, the compound F3Cu, despite exhibiting excellent docking scores, has shown lower antibacterial activity compared to its competitor (MIC = 2.50 mg/mL), which implies that, though it binds well within the cavity of the enzyme, the orientation of its interactions is inferior to F2Cu.

Table 6: Molecular interactions of compounds against bacterial targets

	Organism / Interaction	F1 (OMe)	F1Cu (OMe)	F2 (Cl)	F2Cu (Cl)	F3 (NO_2)	F3Cu (NO_2)	Ampicillin
S. aureus PDB: 4YRD	**Docking Score**	-7.0	-8.7	-7.4	-9.3	-7.3	-9.0	-7.4
	Hydrogen Interactions	His 288, Lys 293, Glu 295, Glu 353	—	—	—	Lys 293, Phe 357	Arg 259, Lys 285, Lys 293, Phe 357	His 288, Lys 293, Glu 355
	Hydrophobic Interactions	Lys 285, Met 351, Val 353	Phe 262, Glu 264, Lys 285, Met 351, Val 353, Phe 357	Lys 285, Lys 293, Met 351, Val 353	Phe 262, Lys 285, Met 351, Val 353, Phe 357	Lys 285, Met 351, Val 353	Arg 259, Phe 262, His 288, Met 351, Val 353	Phe 262, Lys 285
	Electrostatic Interaction	Glu 295	—	Glu 295	Glu 264, Phe 262	Glu 295	His 288, Glu 295	—
E. coli PDB: 6KJ6 (Chain F)	**Docking Score**	-6.0	-7.4	-6.2	-8.6	-6.3	-7.0	-6.3
	Hydrogen Interactions	Lys 133, Asp 135	—	—	—	Asp 135, Trp 145	Trp 149	Asp 135, Arg 138
	Hydrophobic Interactions	Arg 138, Phe 140, Tyr 145	Phe 140, Tyr 145, Trp 148, Trp 149	Phe 134, Phe 140, Trp 148	Lys 133, Phe 134, Asp 135, Arg 138, Phe 140, Trp 148, Trp 149	Arg 138, Phe 140	Phe 140, Tyr 145, Trp 148	Phe 140
	Electrostatic Interaction	—	—	—	—	—	—	—
E. coli PDB: 6KJ6 (Chain J)	**Docking Score**	-6.8	-8.4	-7.4	-8.8	-7.4	-8.4	-6.3
	Hydrogen Interactions	Leu 107, Leu 110	Leu 107	Leu 107	Leu 110	Arg 66, Phe 67, Leu 107	Phe 16, Arg 66, Phe 67	Arg 66, Phe 67
	Hydrophobic Interactions	Phe 16, Leu 19, Leu 60	Leu 12, Lys 15, Phe 16,	Phe 16, Leu 19,	Leu 12, Lys 15, Phe 16,	Phe 16, Leu 19,	Leu 12, Lys 15, Leu 19,	Phe 16, Leu 107, Arg 108

	Organism / Interaction	F1 (OMe)	F1Cu (OMe)	F2 (Cl)	F2Cu (Cl)	F3 (NO_2)	F3Cu (NO_2)	Ampicillin
P. aeruginosa PDB: 6P80			Leu 19, Phe 35, Leu 60, Leu 110, Leu 111, Leu 114	Leu 60, Leu 110	Leu 19, Leu 107, Arg 108, Leu 111	Leu 60, Leu 110	Leu 60, Leu 107, Leu 110, Leu 114	
	Electrostatic Interaction	—	—	—	—	—	—	—
	Docking Score	-5.7	-6.8	-5.7	-7.4	-5.8	-7.2	-5.5
	Hydrogen Interactions	Lys 25, Ala 39	Lys 60, Thr 75, Arg 76, Asp 78	Ala 39	Lys 72, Leu 73	Arg 41, Leu 117	Tyr 6, Trp 85	Lys 25, Ala 39
	Hydrophobic Interactions	Trp 42	Arg 76, Ser 23, Ala 102	Lys 25, Trp 42	Tyr 66, Leu 73, Ala 102, Ala 106, Lys 105, Lys 116	Trp 131, Lys 116	Val 4	Trp 42
	Electrostatic Interaction	—	Ser 23	—	—	—	—	—
K. pneumoniae PDB: 4OSG	**Docking Score**	-6.7	-8.8	-6.8	-10.1	-7.0	-9.9	-7.8
	Hydrogen Interactions	Ala 7, Ile 14	Gly 13, Phe 31	—	Ile 94	Ala 7, Ala 6	Ala 6, Gly 96, Arg 44	Thr 46, Gly 97, Arg 98
	Hydrophobic Interactions	Ala 6, Met 20, Phe 31	Ala 6, Ala 7, Asn 18, Met 20, Phe 31, Met 42, Leu 45, Ile 50, Leu 54, Ile 94, Gly 95	—	Ala 7, Met 20, Phe 31, Met 42, Leu 45, Trp 47, Ile 50, Leu 54, Ile 94, Gly 96, Gly 97, Val 99, Arg 98	Ile 14, Asn 18, Met 20, Leu 45	Met 20, Phe 31, Ile 50, Ile 94	Arg 44
	Electrostatic Interaction	—	—	—	—	—	—	—

On the other hand, the unbound ligands (F1, F2, and F3) presented a poor docking score (−7.0 to −7.4 kcal/mol) and low efficacy (MIC ≥ 5.00 mg/mL) against bacterial infection, revealing inadequate contact with the enzyme's binding site. These ligands were only able to bind partially through contact with amino acid residues such as His288, Lys293, and Met351, failing to occupy the whole catalytic site. Although Ampicillin displayed moderate docking affinity toward CapF, its antibacterial activity may involve additional biological targets beyond CapF inhibition. Overall, the combined docking and experimental data establish a clear structure–activity relationship where Cu(II)

complexation significantly enhances both CapF binding affinity and antibacterial potency. The consistent involvement of key residues such as Phe262, Lys285, His288, Met351, Val353, and Phe357 supports targeting of the Zn^{2+}-dependent cupin domain as the primary mechanism of inhibition. The superior performance of F2Cu over both other derivatives and Ampicillin highlights its potential as a lead scaffold. These findings suggest that metal-based Schiff base complexes act as more effective CapF inhibitors than the standard antibiotic in this system, likely by disrupting capsular polysaccharide biosynthesis and thereby reducing bacterial virulence.

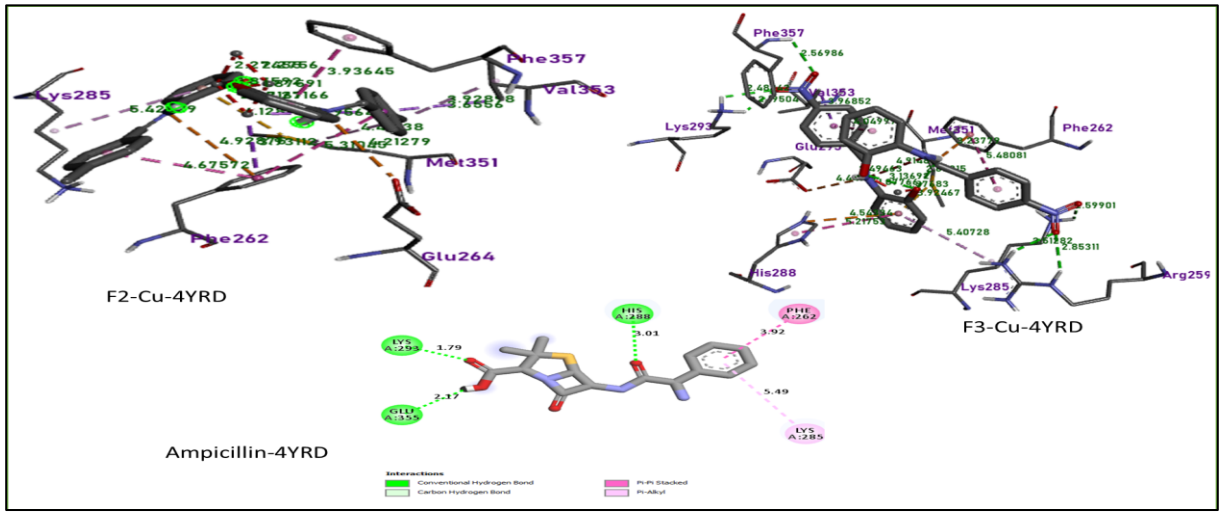


Fig. 1: Three-dimensional binding interaction diagrams of F2-Cu-4YRD (top left), F3-Cu-4YRD (top right), and Ampicillin-4YRD (bottom center) complexes with the 4YRD protein target, illustrating ligand–receptor interactions at the molecular level.

3.4.2 Escherichia coli (6KJ6 - ATCC 8739, chains F and J)

Furthermore, docking studies of synthesized Schiff bases (F1-F3) and their respective Cu(II) complexes (F1Cu-F3Cu) were extended to assess their interference with protein-protein interactions, particularly in the stress-responsive transcription machinery of ATCC 8739 strain of *E. coli*, 6KJ6, which is required for bacteria survival. In this study, it was established that all ligands docked in a specific interface zone that lies in-between $\sigma^{(s)}$ (Chain F) and Crl (Chain J) as shown in Figs. 2 and 3 below and supplementary material S8 - S21. The residues Asp135, Arg138, Phe140, Tyr145, Trp148, and Trp149 from Chain F, alongside the residues Phe16, Leu19, Leu60, and Leu110 from Chain J, ensured a favorable atmosphere where the ligands could form appropriate bonding through hydrogen bonding, electrostatics, and pi-pi interaction. The binding energies were in the range of −6.0 to −8.8 kcal/mol, where the complexes of metals had higher binding energy than the corresponding ligands alone. Out of all the compounds, the F2Cu metal complex bound more strongly (−8.6 and −8.8 kcal/mol) followed by F1Cu and F3Cu. The free ligands F1-F3 exhibited comparatively weak ligand binding energies. Superior binding of Cu(II) complexes could be due to the rigidity, square planar shape, and electronic properties.

Here as well, the docking results exhibit strong correlation with the experimentally acquired data on antimicrobial activities against *E. coli*. In comparison to other compounds that were tested, F1Cu is the most effective with a MIC value of 0.62 mg/mL,

while F2Cu is second (MIC=1.25 mg/mL). They are more active than their free counterparts (moderate F1 at 1.25 mg/mL and inactive F2, F3 above 5.00 mg/mL). Thus, it was shown that high binding affinity contributes directly to higher biological activity, because increased interaction with Crl–$\sigma^{(s)}$ complex correlates with increased bioactivity. It is important to mention that, while F1Cu shows higher activity than the common antibiotic Ampicillin (1.25 mg/mL), F2Cu demonstrates the same level of activity, indicating that the Cu(II) complexes possess promising antibacterial activity against the tested bacterial strain. On the contrary, Ampicillin exhibits weaker docking interactions and involves mainly residues Asp135 and Arg138. Therefore, Ampicillin displays lower activity. Increased biological activity of copper complexes can be explained by the higher lipophilicity of chelated metal ions.

In terms of a mechanism of action, it could be suggested that the key effect is exerted through interference with the Crl–$\sigma^{(s)}$ interaction, which is essential for transcription initiation under environmental stress conditions. If the $\sigma^{(s)}$ domain 2 gets stabilized through the complexation process, it may interfere with holoenzyme formation. This means that the DNA strand melting required for transcription cannot take place. This may impair the bacterial stress-response mechanism, leading to inhibition of growth. It seems like the square planarity of F1Cu and F2Cu is especially helpful for binding with the somewhat flattened surface of the protein–protein interface because it enables them to establish contacts both with charged (Asp135, Arg138) and aromatic (Phe140, Trp148) residues, while the tetrahedral configuration of F3Cu does not favor effective binding. Generally speaking, high consistency between docking scores, interacting residues, and MIC data emphasizes the importance of metal complexation in increasing the antimicrobial activity and makes a good argument for the use of transcriptional regulatory complexes as a novel target in antibiotic development.

3.4.3 Pseudomonas aeruginosa (6P80) HORMA

Docking studies of the synthesized Schiff bases (F1-F3) and their copper(II) complexes (F1Cu-F3Cu) with the HORMA domain protein of *Pseudomonas aeruginosa* (PDB ID: 6P8O) were performed to determine their binding capacity towards the receptor. As illustrated in Fig. 4 and supplementary material S22 - S28, docking studies showed that all the compounds are docked within a defined cavity containing several hydrophobic and polar amino acid residues such as Lys72, Leu73, Ala102, Val99, Ile94, Phe16, and Trp85. These residues together create a mixed interaction pattern that binds to the ligands via hydrogen bonding, hydrophobic, and π interactions. Binding scores varied between -5.7 and -7.4 kcal/mol, whereas all the copper complexes had higher affinities for the target receptor than their respective ligands.

Between all complexes, F2Cu proved to have the greatest binding affinity (−7.4 kcal/mol), closely followed by F3Cu (−7.2 kcal/mol), with both free ligands F1 and F2 having less binding affinity (−5.7 kcal/mol). The greater binding affinity for Cu(II) complexes could be due to their greater rigidity, squarish planarity, and electronic arrangement, leading to a greater affinity to interact with residues at the active sites. In the case of F2Cu, its interactions with Leu73, Ala102, and Val99 via hydrophobic interactions were complemented with polar interactions with Lys72 and Lys105, thereby facilitating the binding of the ligand into the pocket.

The correlation between the experimental antibacterial results (MICs) and docking analysis is evident. For *Pseudomonas aeruginosa*, the most effective compound in terms of antibacterial activity is F2Cu with an

MIC of 0.31 mg/mL, while F1Cu is ranked second (MIC of 0.62 mg/mL). On the contrary, there is no significant antibacterial activity for the two free ligands F2 and F3 (>5.00 mg/mL). These results are in line with the order of affinity calculated in the docking analysis, indicating that the higher the affinity to bind the 6P8O protein, the better its biological activity.

While the MIC of Ampicillin against *Pseudomonas aeruginosa* was 5.00 mg/mL, all the complexes tested had better activities, with the best being complex F2Cu, showing an activity that was over 15 times greater than that of Ampicillin. F1Cu (0.62 mg/mL) and F3Cu (2.50 mg/mL) had better activities than Ampicillin. The complexes exhibited superior activity compared with Ampicillin against the tested strain. These observations were supported by the docking experiments, with Ampicillin exhibiting poor interactions with the residues, with few hydrophobic interactions occurring. From a mechanistic perspective, the mode of action may be explained by the inhibition of the HORMA domain protein, which is involved in regulation and stress response in bacteria. The binding of copper (II) complexes in the active site disturbs interactions mediated by residues such as Lys72, Leu73, Ala102, and Trp85. The increased lipophilicity associated with metal complexation may contribute to improved cellular uptake, resulting in substantial inhibition of bacterial growth.

Notably, high correlation between docking score, interaction profile, and MIC values demonstrates the ability of metal chelation in improving the binding efficacy and anti-microbial activity, where F2Cu emerges as the lead compound.

3.4.4 Klebsiella pneumoniae (4OSG)

Molecular docking studies of Schiff bases F1-F3 and corresponding Cu(II) complexes F1Cu, F2Cu, and F3Cu were performed against the DHFR target protein (PDB ID: 4OSG) to study the interactions of these molecules in the enzyme active site. As represented in Fig. 5 and supplementary material S29 - S34, docking results showed that all the compounds dock into the DHFR active site constituted of critical residues such as Ile50, Leu54, Ile94, Phe31, Met42, Leu45, Gly96, and Arg98 that form the substrate binding pocket. From the results of the binding energy, it was observed that although the binding energies of F1-F3 were relatively weak (-6.7 to -7.0 kcal/mol), the copper complexes were better (-8.8 to -10.1 kcal/mol). Among all these, the most favorable binding energy was observed for F2Cu (-10.1 kcal/mol) and F3Cu (-9.9 kcal/mol).

There is also a clear correlation that is evident when the data is compared with that of the known antibiotic, Ampicillin. For instance, the MIC of Ampicillin against *K. pneumoniae* is 2.50 mg/mL. The synthesized copper complexes exhibited greater antibacterial activity than Ampicillin against the tested strain. The complex F2Cu was markedly more potent compared to Ampicillin, with a potency greater than one order of magnitude. From the structure of Ampicillin, it is evident that there are weak and non-specific interactions between the molecule and the DHFR active site. In addition, the molecule does not bind consistently with Ile50 and Ile94 amino acids present at the active site. This may explain why Ampicillin has inferior efficacy compared to the current complexes. In terms of mechanisms, the antimicrobial effects of the newly discovered copper complexes might be attributed to the inhibition of the DHFR enzyme by the compound. This is possible because the compound binds to the active site of the enzyme and, hence, may hinder substrate access to the active site inhibiting the enzyme, which results in disruption of the folate cycle that is important for the growth of bacteria.

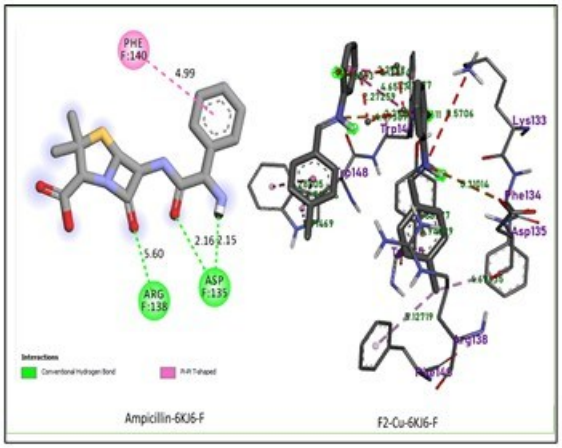


Fig. 2. Three-dimensional binding interaction diagrams of Ampicillin-6KJ6-F (left) and F2-Cu-6KJ6-F (right) complexes with the 6KJ6 protein target (chain F), depicting ligand–receptor interactions at the molecular level

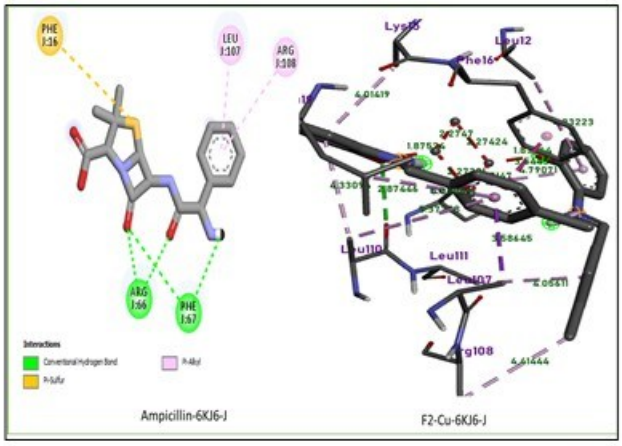


Fig. 3. Three-dimensional binding interaction models of Ampicillin-6KJ6-J (left) and F2-Cu-6KJ6-J (right) bound to their target protein 6KJ6, showing the interaction between the drug and its receptor at a molecular level.

Overall, molecular docking studies demonstrated that metal complexation significantly enhanced the binding affinity of the Schiff bases toward all investigated bacterial targets. The methoxy- and chloro-substituted complexes (F1Cu and F2Cu) consistently exhibited stronger protein–ligand interactions and lower MIC values than their corresponding free ligands, indicating that copper coordination plays an important role in improving antibacterial activity. The agreement between experimental and computational findings supports the potential of these copper(II) Schiff base complexes as promising antibacterial lead compounds.

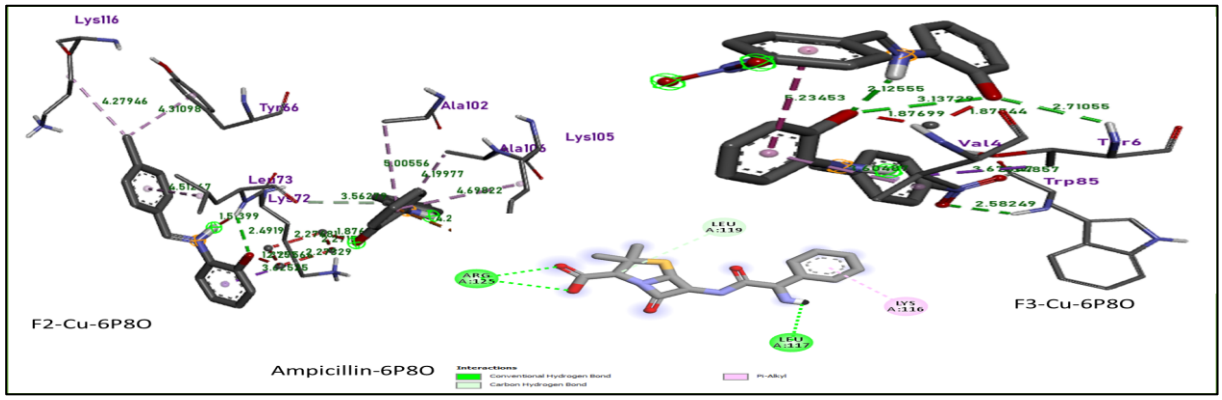


Fig. 4: Molecular docking interaction profiles of F2-Cu-6P8O, F3-Cu-6P8O, and Ampicillin-6P8O complexes illustrating key binding interactions within the active site.

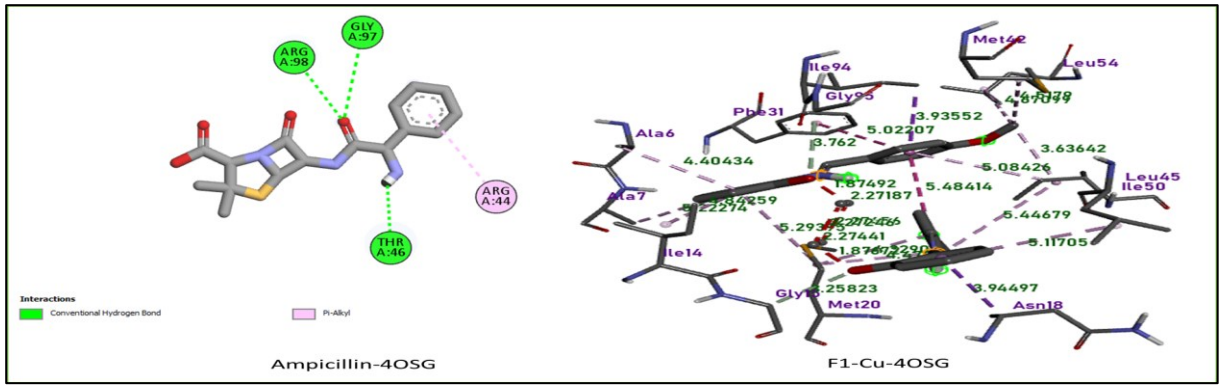


Fig. 5. Three-dimensional binding interaction diagrams of Ampicillin-4OSG (left) and F1-Cu-4OSG (right) complexes with the 4OSG protein target, showing ligand–receptor interactions at the molecular level.

4.0 Conclusion

In the present study, a series of novel Schiff base copper(II) complexes were successfully synthesized and characterized. The ligands were found to coordinate as bidentate donors through the imine nitrogen and phenolic oxygen atoms. Spectroscopic analyses, elemental microanalysis, metal-content determination, and magnetic susceptibility measurements revealed the formation of both binuclear and mononuclear copper complexes with square-planar and tetrahedral geometries. The synthesized compounds were evaluated using in vitro antibacterial assays and molecular docking studies against selected bacterial targets. The copper complexes exhibited significantly lower MIC values than their corresponding free ligands, indicating enhanced antibacterial activity upon metal coordination. Molecular docking studies further demonstrated stronger binding affinities for the copper complexes (−7.4 to −10.1 kcal/mol) compared with the free ligands (−5.7 to −7.4 kcal/mol), supporting the experimental observations. Among the synthesized compounds, F1Cu and F2Cu displayed the most promising antibacterial activities and binding interactions with the target proteins. The good agreement between the experimental and computational results suggests that copper coordination plays an important role in enhancing biological activity, highlighting these Schiff base copper(II) complexes as promising candidates for further antibacterial drug development studies.

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Declarations:

Conflict of interest

The authors declare that they have no conflict of interest

Data availability

All data used in this study will be readily available to the public.

Consent for publication

Not Applicable.

Ethical consideration

Not applicable

Competing interests

The authors declared no conflict of interest.

Authors' Contributions

Methodology, investigation, and writing original draft preparation were contributed by F. N. Ejiah, M. O. Rofiu; project administration, supervision, were done by F.N. Ejiah, T. M. Fasina, O. B. Familoni; writing and editing were performed by F. N. Ejiah, M. O. Rofiu.