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Original article

Novel Copper (II) Complex Derived from Sulfadiazine Azo Resorcinol Ligand: Characterization, Antibacterial Evaluation, and Molecular Docking Study.

Hawraa S. Ibrahim[1*], Abeer A. Tuaimah [2], Zahraa Jalil Salman[3]

[1]Department of Medical Laboratory Techniques, College of Health and Medical Techniques / Kufa, University of Al-Furat Al-Awsat Techniques, Najaf 31003, Iraq

[2]Department of Pharmaceutical Chemistry, College of Pharmacy/ Kufa University, Najaf, Iraq

[3]Department of Dental Technology, College of Health and Medical Techniques / Kufa, University of Al-Furat Al-Awsat Techniques, Najaf 31003, Iraq

***Corresponding author** (Hawra.ibrahimckm@atu.edu.iq)

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Abstract

A novel copper complex was synthesized from a heterocyclic azo ligand, namely Sulfadiazine Azo Resorcinol (SAR). The first step involved preparing the azo ligand using a classical diazotization-coupling method. The copper complex was subsequently prepared with a 1:2 metal-to-ligand molar ratio. Following purification, the prepared ligand and its Cu(II) complex were characterized using FT-IR spectroscopy, UV-Vis spectroscopy, ^{1}H-NMR spectroscopy, CHN elemental analysis, molar conductivity, and magnetic susceptibility. The analytical results indicated that the ligand coordinated as a bidentate (N, O) donor, binding with two ligand molecules and two coordinated water molecules to the copper center, resulting in a neutral octahedral geometry. The antimicrobial activity of both the free ligand and its complex was evaluated against five pathogenic bacterial strains (*E. coli, P. aeruginosa, S. aureus, E. faecalis, and P. agglomerans*) using the agar well diffusion method. Biological activity evaluations demonstrated that both compounds exhibited moderate to high antimicrobial activity, with the copper (II) complex displaying significantly enhanced activity compared to the free ligand. Furthermore, a molecular docking simulation was conducted to evaluate the binding modes, affinities, and specific intermolecular interactions of the synthesized compounds within the active binding pockets of key bacterial target proteins. Two target proteins were selected for the docking study: dihydropteroate synthetase (PDB ID: 1AD4) and Dihydropteroate synthetase from *E. coli* (PDB ID: 3TYU). The molecular docking results were consistent with the in vitro antibacterial findings; the Cu(II) complex exhibited higher binding affinity toward both target proteins than the free ligand.

Keywords: Sulfadiazine, Azo ligand, copper, Biological activity, Resorcinol.

Introduction

Azo compounds are organic compounds widely used in coordination chemistry as ligands [1]. They are characterized by the presence of the azo group (-N=N-) in their structure, which acts as a donor site. This group can coordinate with the other donor groups attached to the azo moiety, enabling the formation of various metal complexes [2 -4] with various metal ions (Lewis acids). The conjugation between the azo group and the π- system of the aromatic rings leads to increased stability and enhanced color intensity. Therefore, azo compounds have numerous applications in analytical chemistry as reagents and in laser technology, in addition to

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various industrial applications [5]. Recently, considerable research interest has focused on azo ligands and their complexes due to their potential applications in medicine and pharmaceuticals as therapeutic agents [6]. These compounds exhibit significant antibacterial and antifungal activities through multiple mechanisms, including inhibition of cell wall or membrane synthesis, interaction with microbial DNA leading to cell death, and binding to specific enzymes. In addition, azo metal complexes are used as biological dyes owing to their lipophilic nature [7,8]. Furthermore, they have demonstrated notable anticancer [9] and antioxidant activities [10]. Sulfadiazine is a sulfonamide antibiotic widely used in clinical medicine due to its high activity against microorganisms by inhibiting dihydrofolate synthetase [11]. Many researchers have prepared azo compounds using Sulfadiazine as an amine source and have studied the therapeutic properties of the synthesized azo compounds, as well as their complexes, for potential antibiotic activity. Previous studies have reported that the azo derivatives exhibit enhanced or modified biological activities compared to the parent drug, depending on the type of substitution and the formation of metal complexes. These modifications may improve antimicrobial efficacy, broaden the spectrum of activity, and introduce new therapeutic properties [12]. To evaluate the underlying mechanisms of these interactions, molecular docking has emerged as a powerful computational tool to predict ligand-receptor binding profiles. It provides critical insight into the binding affinity and the specific interactions between ligands and target proteins, thereby enabling researchers to effectively predict the *in vitro* antibacterial potential of synthesized compounds against pathogenic strains [13,14].

A sulfadiazine azo resorcinol derivative was previously developed as a novel electrochemical sensor for the determination of paracetamol(PR), a corrosion inhibitor, and a forensic agent for latent fingerprint visualization [15]; however, its broader coordination chemistry and biological potential remain unexplored. To address this gap, the present study focuses on the synthesis and comprehensive characterization of this compound. Furthermore, we investigate its coordination behavior through complexation with copper(II), alongside evaluating the antibacterial activity and molecular docking profiles of the synthesized compounds.

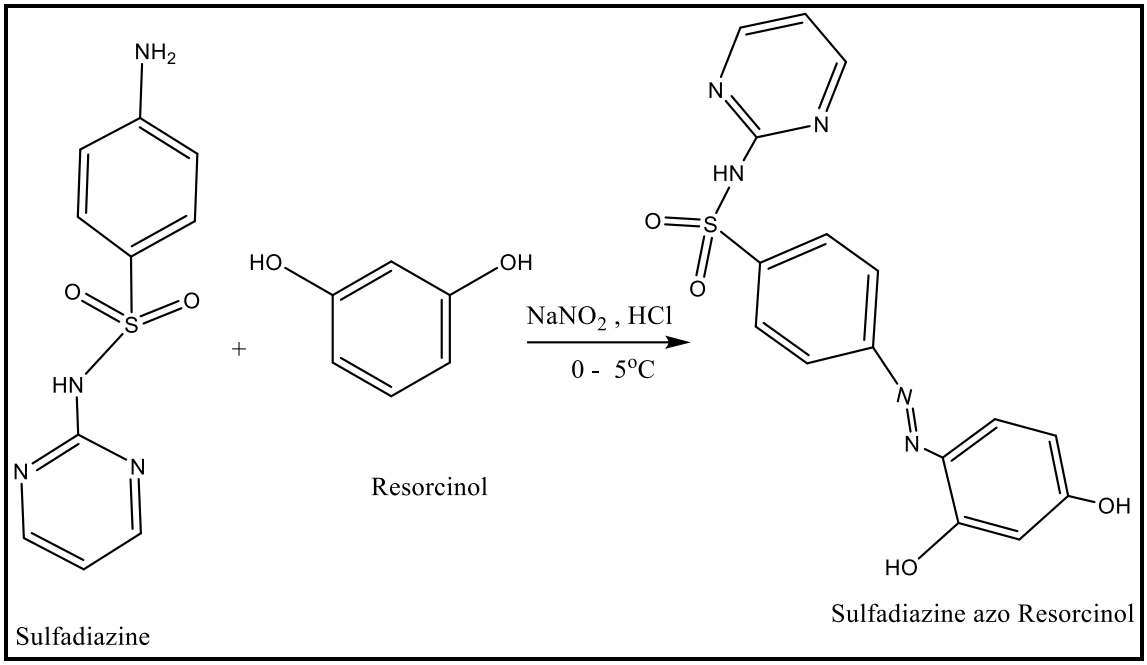
Materials

All chemicals used for the preparation of the ligand and copper complex were of analytical grade and obtained from commercial sources as follows: sulfadiazine 99% was obtained from Fluka; absolute ethanol 99.5% was obtained from G.C.C. company; hydrochloric acid 99% , Sodium nitrite 99%, Merck, Copper(II) chloride dihydrate 99% was obtained from Riedel –deHaën, dimethyl sulfoxide(DMSO) 98% was obtained from A.C.S. company.

Methods

Preparation of Sulfadiazine Azo Resorcinol (SAR)

Sulfadiazine Azo Resorcinol(SAR) ligand was synthesize by diazonium salt formation that involved dissolving (2.53g 0.01 mol) of 4-amino-N-(pyrimidin-2-yl)benzene sulfonamide sulfadiazine)in (10 mL) ethanol containing (1mL) of concentrated hydrochloric acid, the mixture was cooled in an ice bath to (0°C) then 10% Sodium nitrite solution was added gradually with continuous stirring, the diazonium solution was kept at (0°C) for 30 minutes with stirring after that this solution was added to the alkaline solution of(1.g, 0.01 mol) m-dihydroxy benzene (Resorcinol) with gradual and continuous stirring. An orange precipitate was formed. After leaving it in the refrigerator for 24 hours, the pH of the mixture was adjusted to (pH = 6). The precipitate was filtered and washed several times with distilled water, then with ethanol, to remove unreacted material and inorganic salts. The product was then dried in the oven at 70°C for several hours. After that, the melting point was measured.



Scheme1. SAR ligand preparation

Synthesis of Diaqua bis(sulfadiazine-azo-resorcinolato) copper (II)

The coppe(II) complex was prepared with mole ratio (1:2) (metal: ligand) by dissolve (0.17 g, 0.001 mol.) of copper chloride dehydrate $CuCl_2.2H_2O$ in 15 ml of distilled water as metal source, then this solution was added gradual with stirring to the solution of SAR ligand which prepared by dissolve (0.74g, 0.002 mol.) of sulfadiazine azo resorcinol in 15 ml of di methyl sulfoxide (DMSO), deep brown precipitate was formed then filtered and washed several times with distilled water, followed by ethanol and melting point was measured after purification and product ratio was calculated.

Antibacterial activity experiments

Antibacterial activity of the ligand and its copper complex was evaluated using the agar well diffusion method. Bacterial strains of *E. coli, P. aeruginosa, S. aureus, E. faecalis,* and *P. agglomerans were used*. Bacterial suspensions were adjusted to a 0.5 McFarland standard. Wells of 6 mm diameter were punched into the agar plates, and 50 µL of each compound solution at concentrations of 1000, 100, and 10 ppm in DMSO was added. DMSO alone served as the negative control; plates were incubated at 37 °C for 24 h, and the inhibition zone was measured in millimeters.

Molecular docking study

Molecular docking studies were performed using the Molecular Operating Environment (MOE 2015) software. The three-dimensional structures of the target proteins (PDB IDs: 3TYU and 1AD4) were obtained from the Protein Data Bank (PDB) https://www.rcsb.org/. Before docking, the protein structures were prepared by removing crystallographic water molecules, co-crystallized ligands, and cofactors. Hydrogen atoms were subsequently added to the protein structures, followed by energy minimization to obtain geometrically optimized protein structures. The potential ligand-binding sites were identified using the Site Finder module implemented in MOE 2015. The site identification was performed using the Atom and Backbone options, and dummy atoms were generated to represent the potential binding pocket. Among the predicted binding sites, the highest-ranked binding site was selected as the target binding site for the subsequent docking calculations.

The docking region was therefore defined by the Site Finder-generated dummy atoms rather than by applying a manually specified Cartesian docking-box dimension. The prepared ligands were subsequently docked into the selected binding pocket, and the resulting docking poses were evaluated according to their docking scores and the molecular interactions established with the amino acid residues within the binding site [16,17].

RESULTS AND DISCUSSION

Chemical characterization of ligand and copper complex

In this work, an azo heterocyclic ligand was prepared from sulfadiazine as the amine source and resorcinol through a diazotization-coupling reaction. Subsequently, the ligand's copper complex was synthesized. The prepared ligand and its complex exhibited deep orange and brown colors, respectively. Both compounds showed stability at room temperature and low solubility in distilled water, ethanol, and acetone, but were readily soluble in DMSO and DMF. The molar conductivity was determined for the copper complex in DMSO; the result was 2.49, indicating that the complex is a non-electrolyte**.** The physical properties of the SAR ligand and copper(II) complex and C.H.N. analysis data were listed in Table 1.

FT-IR spectrum characterization

FT-IR spectrum was taken to SAR ligand and its complex, in the sulfadiazine azo resorcinol (SAR), the results show percent of N=N band at 1494 cm^{-1}, this band was shifted to high frequency at 1502 cm^{-1}, when copper complex was formed, this resulted aggregated with literatures review [15,18,19] also, the spectrum shows a medium broad band at 3554 cm^{-1} belong to υ (O-H) for resorcinol, this band was disappeared in copper complex with shifting in the υ(C-O) bands from 1261 cm^{-1} to 1274 cm^{-1}, that indicate to sharing in coordination [20]. In addition, the appearance of new bands between 447 - 578 cm^{-1} belongs to υ(Cu-N) and υ (Cu-O) bonds. Also, in the copper complex, two bands were observed: coordinated water at 3525 cm-1 and 849 cm-1 [21]. Table 2 shows the important bands observed in the two ligand and copper complexes.

Table.1 The physical properties and C.H, N analysis data of the prepared compounds

						Found. (cal.)%			
Chemical formula of compounds	M.wt	m.p.°c	Color	Yield	Molar conductivity	C	H	N	**M**
$C_{16}H_{13}N_5O_4S$	371.38	259	orange	81%	---	50.88 (51.74)	3.36 (3.52)	18.51 (18.86)	---
$[Cu(SAR)_2(H_2O)_2]$	840.3	261	Brown	83%	2.49	45.29 (45.73)	3.64 (3.35)	16.58 (16.67)	**7.47 (7.56)**

Table2. FT-IR frequencies for the synthesis ligand and the copper complex

Compound	N=N	C=N (sulfa.)	S=O(sulfa.)	NH_2 (sulfa.)	C-O phenolic	O-H	O-H coordinated	Cu-O	Cu-N
SAR ligand	1494 **m**	**1653**	**1325 vs**	**3351 m**	**1261**	**3554 m,v**	---	-----	---
$[Cu(SAR)_2(H_2O)_2]$	1502 m	**1631**	**1313 vs**	**3451 w**	**1274**	---	**3525 sh,m, 849**	**578**	**447**

Sulfa. = sulfadiazine., s= strong, m= medium, w= weak, b= broad, sh=sharp, v= very

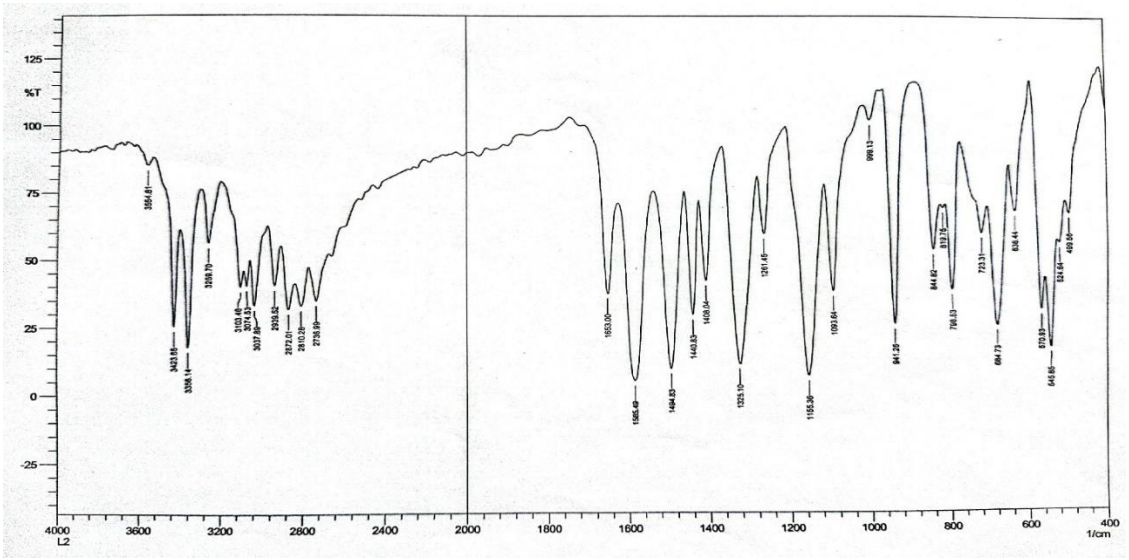


Figure1. FT-IR spectrum of sulfadiazine azo resorcinol

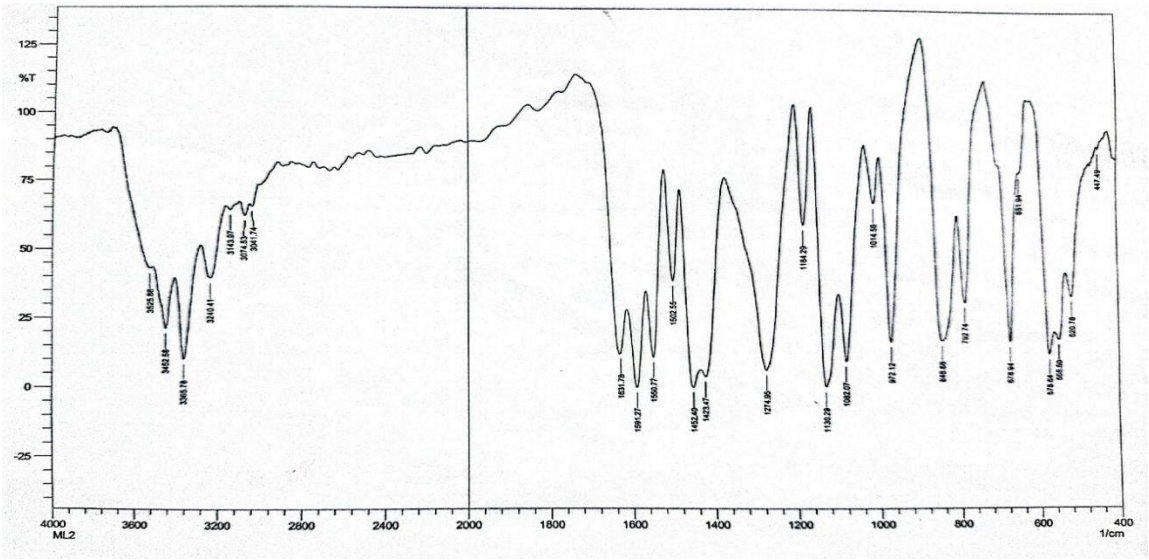


Figure2. FT-IR spectrum of Cu(II) complex

Electronic characterization.

Electronic absorption spectra of the synthesis ligand(SAR)and its copper complex were recorded at 300 K, 200 - 900 nm,and dimethyl sulfo xide (DMSO) as the solvent. Absorption peaks, in the presence of a strong SAR ligand, show strong peaks at 292 nm and 433 nm, belonging to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, respectively, for the azo group and aromatic system of the ligand. These assignments are in proximity to those reported in the research [22,23]. In the copper complex, these peaks shifted to higher wavelengths, at 268 nm and 371 nm, due to coordination. The weak peak observed between 620 and 750 nm corresponds to the d-d transition of the Cu(II) ion in a distorted octahedral geometry. The effective magnetic moment (μeff) of the synthesized complex was calculated from the molar magnetic susceptibility data, which were corrected for core diamagnetism using Pascal's constants. The measured magnetic moment was found to be 1.86 B.M.; this value clearly indicates the presence of a single unpaired electron in the d^9 orbital, which confirms the +2 oxidation state of the copper ion. This value supports the distorted octahedral geometry for the copper(II) complex. Assignments and magnetic moment for the ligand and copper complex were listed in Table 3.

Table 3. Assignments and magnetic susceptibility for ligand and copper complex

Compound	λ max (nm)	Assessment	μeff (B.M)	Geometry
SAR ligand	**292**	$\pi \rightarrow \pi^*$	**----**	
	433	$n \rightarrow \pi^*$		
$[Cu(SAR)_2(H_2O)_2]$	268	$\pi \rightarrow \pi^*$	**1.86**	**octahedral**
	371	$n \rightarrow \pi^*$		
	620	d-d transition		

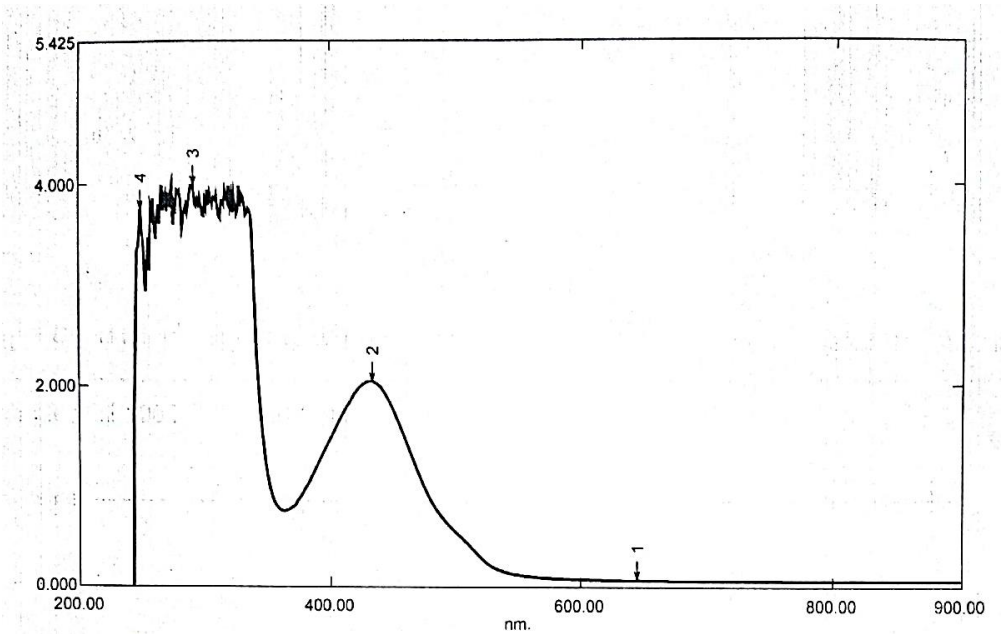


figure.3 UV-Visible spectrum of sulfadiazine azo resorcinol(SAR)

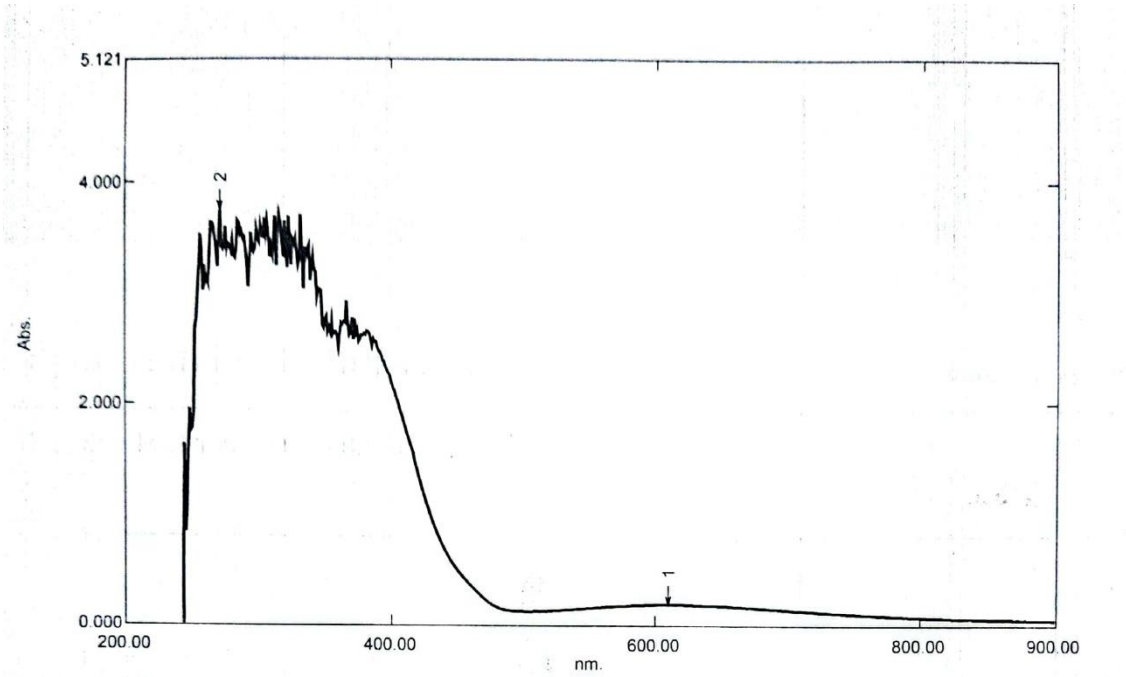


figure4. UV-Visible spectrum of Cu(II) complex

[1]H-NMR spectrum

[1]H-NMR spectrum of the sulfadiazine azo resorcinol (SAR) was recorded in DMSO-d6. A characteristic singlet observed at δ 11.292 ppm was assigned to the NH proton of the sulfonamide group. The aromatic protons of the pyrimidine, phenyl, and resorcinol rings appeared as a series of signals in the region δ 6.5 – 8.5 ppm. The two groups (OH) protons of resorcinol signals were not observed, probably due to oxidation or interaction with the DMSO-d6 [18,24]. [1]H-NMR data characterization for SAR ligands were listed in Table 4.

Table 4. [1]H-NMR data characterization for SAR ligand

Suggested Assignment	Integration	Multiplicity	Chemical Shift (δ, ppm)
Sulfonamide group NH	1H	s (singlet)	11.29
Aromatic protons (AR-H)	2H	m (multiplet)	8.51-8.48
Aromatic protons (AR-H	2H	m (multiplet)	7.63 -7.60
Aromatic protons (AR-H	2H	m (multiplet)	7.02 – 6.98
Aromatic protons CH		m (multiplet)	6.58 – 6.03

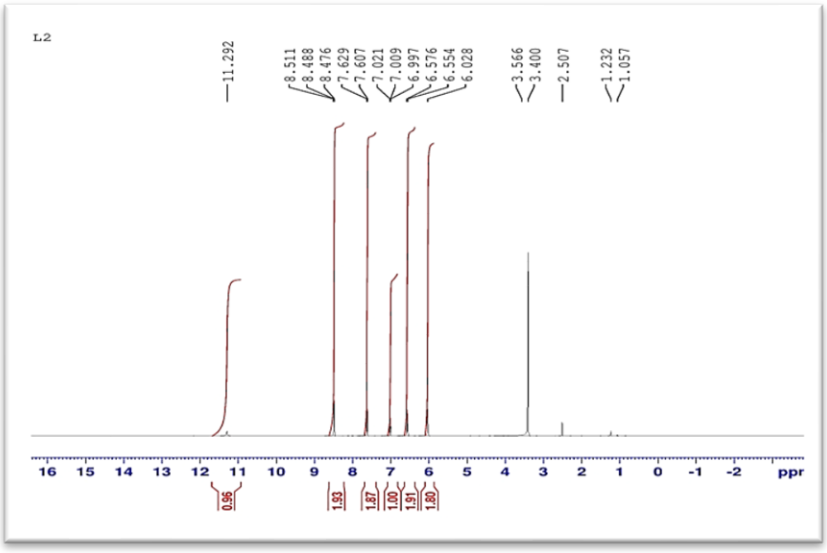


Fig.5: H-NMR spectrum of SAR ligand

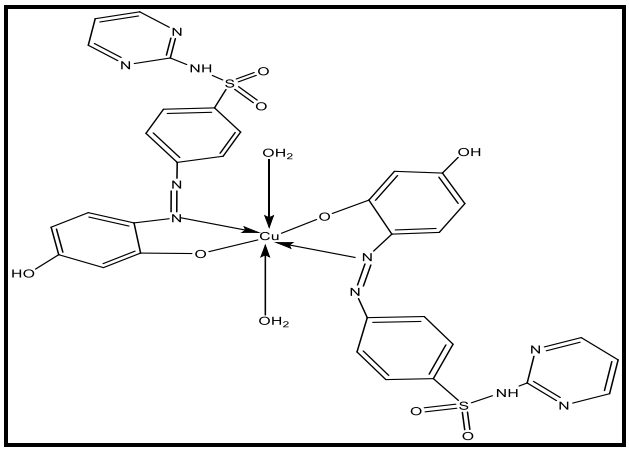


Figure 6. Suggested geometry of the copper complex.

Antibacterial activity study

The biological activity of SAR, the ligand, and its copper complex was evaluated against pathogenic bacterial isolates using the agar well diffusion method. The test bacterial strains included *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *P. agglomerans*. In general, the results demonstrated a concentration-dependent antibacterial activity for both the SAR ligand and the copper complex, with inhibition zone values increasing with concentration (1000 ppm > 100 ppm > 10 ppm). The results also showed no significant difference in antibacterial activity between Gram-positive and Gram-negative bacteria, indicating that both compounds were effective against the tested bacterial strains regardless of cell wall type.

In comparison, the copper complex exhibited larger inhibition zones than the SAR ligand against most bacterial isolates. This effect was particularly noticeable against *E. faecalis*, where the SAR ligand showed no inhibition activity, while the copper complex produced inhibition zones ranging from 24 to 30 mm. The enhanced antibacterial activity of the copper complex may be attributed to the chelation effect, which increases the complex's lipophilicity and stability relative to the free ligand. This increased lipophilicity facilitates the penetration of the complex through the bacterial cell membrane, according to Tweedy's theory of chelation and Overtone's concept [25-27]. All inhibition zone values for the SAR ligand and its copper complex are listed in Table 5 and Figure 7.

Table 5: antibacterial activity data of SAR ligand and copper(II) complex

organisms	compounds	inhibition zone (mm)		
		1000 ppm	100 ppm	10 ppm
P. aeruginosa	SAR ligand	26	22	19
	$[Cu(SAR)_2(H_2O)_2]$	28	24	20
E.coli	SAR ligand	26	20	10
	$[Cu(SAR)_2(H_2O)_2]$	28	20	16
S. aureus	SAR ligand	20	14	12
	$[Cu(SAR)_2(H_2O)_2]$	22	18	14
E. faecalis	SAR ligand	0	0	0
	$[Cu(SAR)_2(H_2O)_2]$	30	28	24
P. agglomerans	SAR ligand	28	24	20
	$[Cu(SAR)_2(H_2O)_2]$	26	27	20

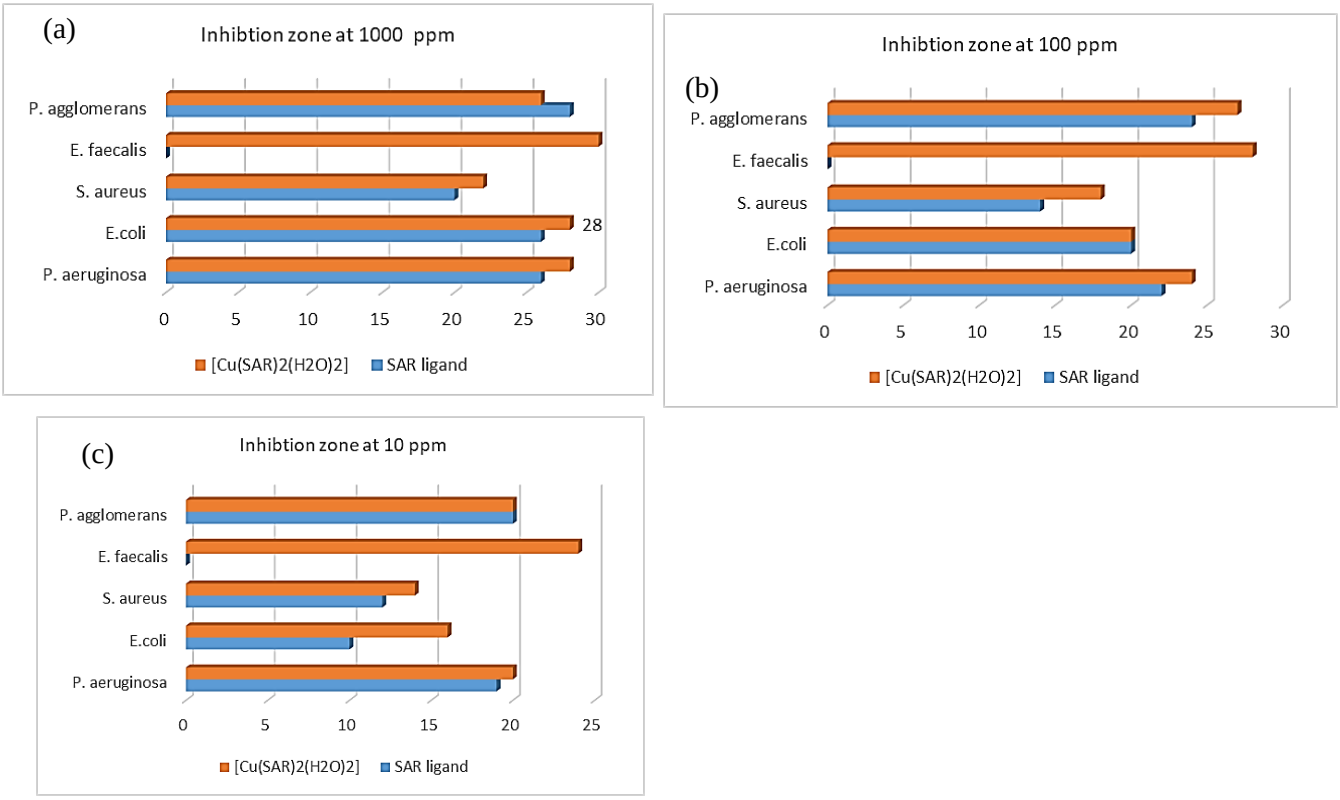


Figure7. Antibacterial activity of SAR ligand and copper(II) complex; a inhibition zone at 1000 ppm; b inhibition zone at 100 ppm; inhibition zone at 10 ppm.

Molecular docking study

To gain a deeper understanding of the mechanism of action and provide a structural rationale for the observed in vitro antibacterial efficacy, a molecular docking simulation was conducted for both the free SAR ligand and its copper(II) complex. This computational study aims to evaluate the binding modes, affinities, and specific intermolecular interactions of the synthesized compounds within the active binding pockets of key bacterial target proteins and compare these values with sulfadiazine. Two types of target proteins were selected for the molecular docking study, including Dihydropteroate synthetase from *Staphylococcus aureus* (PDB ID: 1AD4) and Dihydropteroate synthetase from *E. coli* (PDB ID: 3TYU), which were obtained from the Protein Data Bank; these proteins represent antibacterial drug design targets. For the 3TYU protein, the Cu(II) complex exhibited a high binding affinity of approximately -7.81(kcal/ mol), indicating greater thermodynamic stability. This interaction was enhanced by the formation of multiple hydrogen bonds with the amino acid residues, specifically LYS – 221, ARG-63, ARG-225, ARG- 63, ARG- 235, and HIS-257. In contrast, the binding affinity of the free ligand was -6.54 kcal/ mol, interacting with key amino acid residues including LYS -221, ARG-63, and ARG -255. These interactions formed three hydrogen bonds between the oxygen atoms of the sulphonamide group and the nitrogen of the pyrimidine ring. Compared to sulfadiazine, the molecular docking revealed a lower binding affinity of approximately -5.48 kcal/ mol. For sulfadiazine, three hydrogen bonds were formed between the oxygen atoms of the sulphonamide group and the nitrogen of the pyrimidine ring with LYS–221, ARG-63, and ASN-22; in addition, a π-H

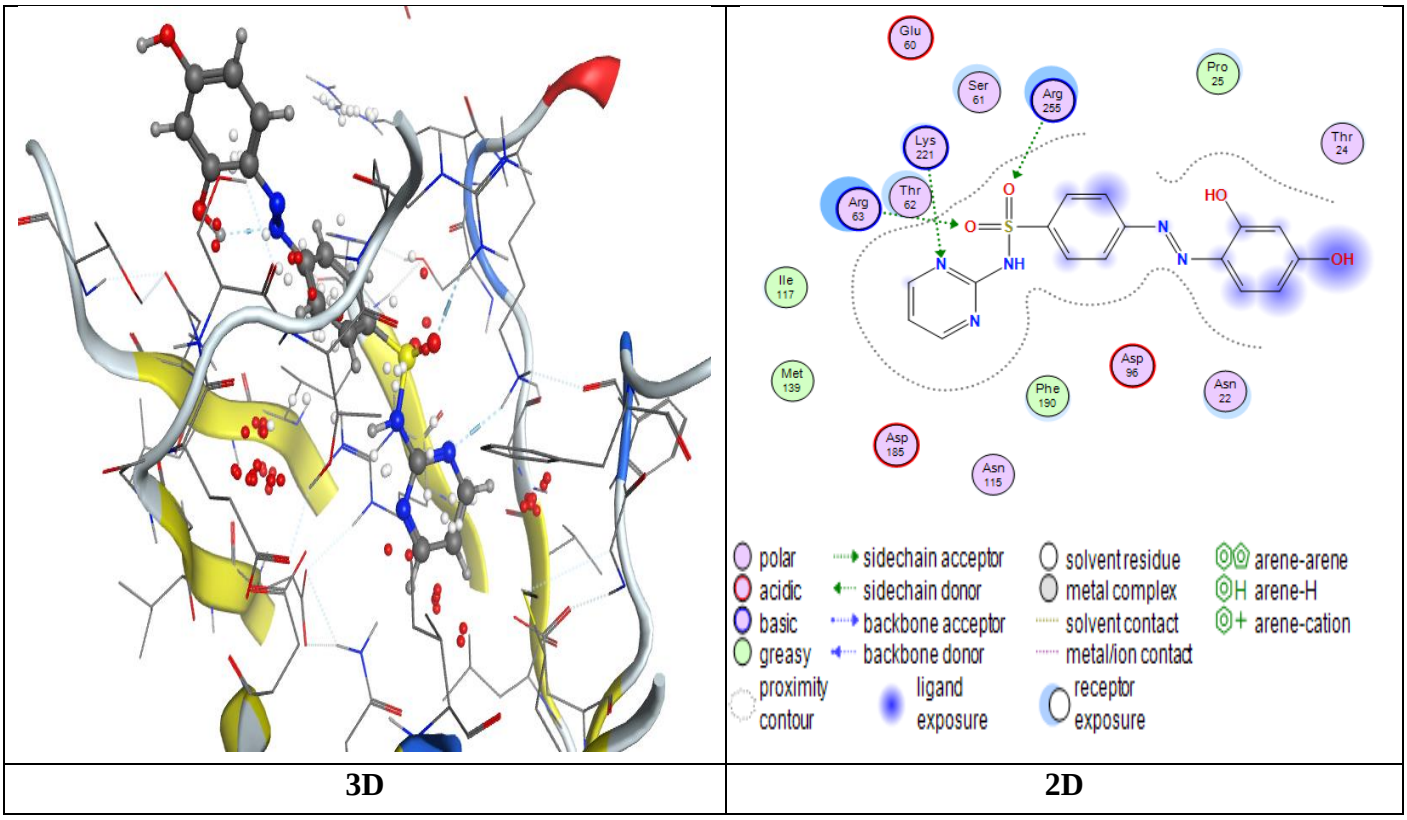
interaction was formed between the pyrimidine ring and the PHE-190 residue.

Notably, the binding affinity of the Cu(II) complex was significantly higher than that of the free SAR ligand and sulfadiazine. This superior affinity may be attributed to the larger number of hydrogen bonds formed compared to those of the free ligand and sulfadiazine. These molecular docking findings are strongly consistent with the observed in *vitro* results.

Regarding the Dihydropteroate synthetase (PDB ID:1AD4) protein, the molecular docking results revealed a binding affinity of -7.79 Kcal/ mol for the Cu(II) complex. This stability was driven by hydrogen bonding interactions involving ASN-162, ASN-162, andLYS-257. Conversely, the binding affinity for the free ligand was -6.21Kcal/ mol, where the Oxygen and nitrogen of the sulphonamide group and the hydroxyl group of the resorcinol moiety interacted with THR-2, ASN-162, and LYS-257 via hydrogen bonding. In contrast, the results demonstrated weak binding between sulfadiazine and the dihydropteroate synthetase (PDB ID: 1AD4), with a binding affinity of -5.48 Kcal/ mol. The oxygen and nitrogen atoms of sulfadiazine interacted with the LYS-257 and THR-2 residues within the active pocket through hydrogen bond formation. All docking interaction data and visual representations are summarized in Table 6 and Figures 7 - 8.

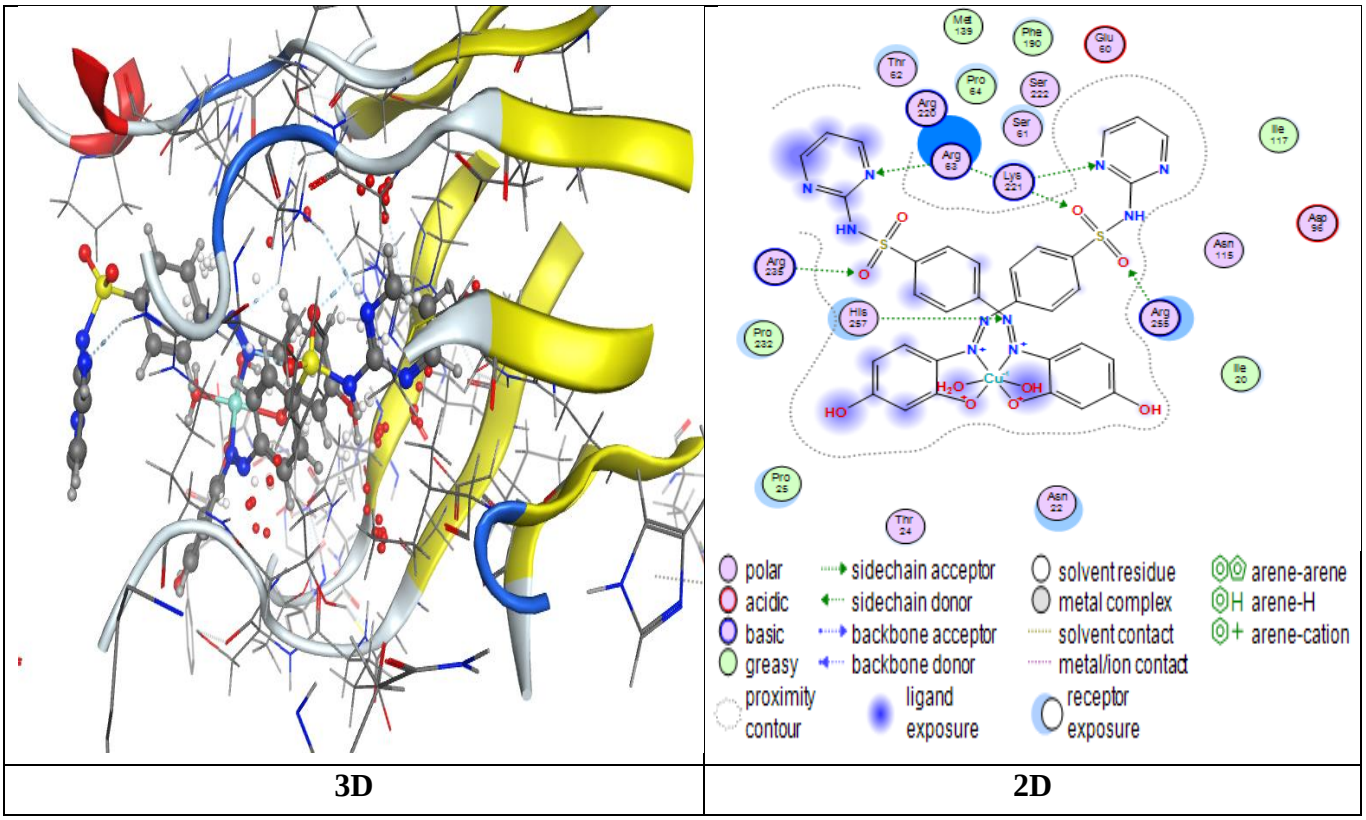
Table 6. The binding affinity values and the interacting amino acid residues in the SAR ligand and Cu(II) complex

Target protein	Compound	Binding Affinity (kcal/ mol)	RMSD (Å)	Hydrogen Bonding Residues (Amino Acids)
(PDB ID: 3TYU)	SAR ligand	-6.54	1.7614	LYS -221 ARG-63 ARG -255
	$[Cu(SAR)_2(H2O)_2]$	-7.81	2.7738	LYS – 221 ARG-63 ARG- 225 ARG- 63 ARG- 235 HIS-257
	sulfadiazine	-5.63	0.94	LYS – 221 ARG-63 ASN-22 PHE-190
(PDB ID: 1AD4)	SAR ligand	-6.21	1.97	THR-2 ASN-162 LYS-257
	$[Cu(SAR)_2(H2O)_2]$	-7.79	2.09	ASN-162 ASN-162 LYS-257
	sulfadiazine	-5.48	**2.44**	**THR-2** **LYS-257**



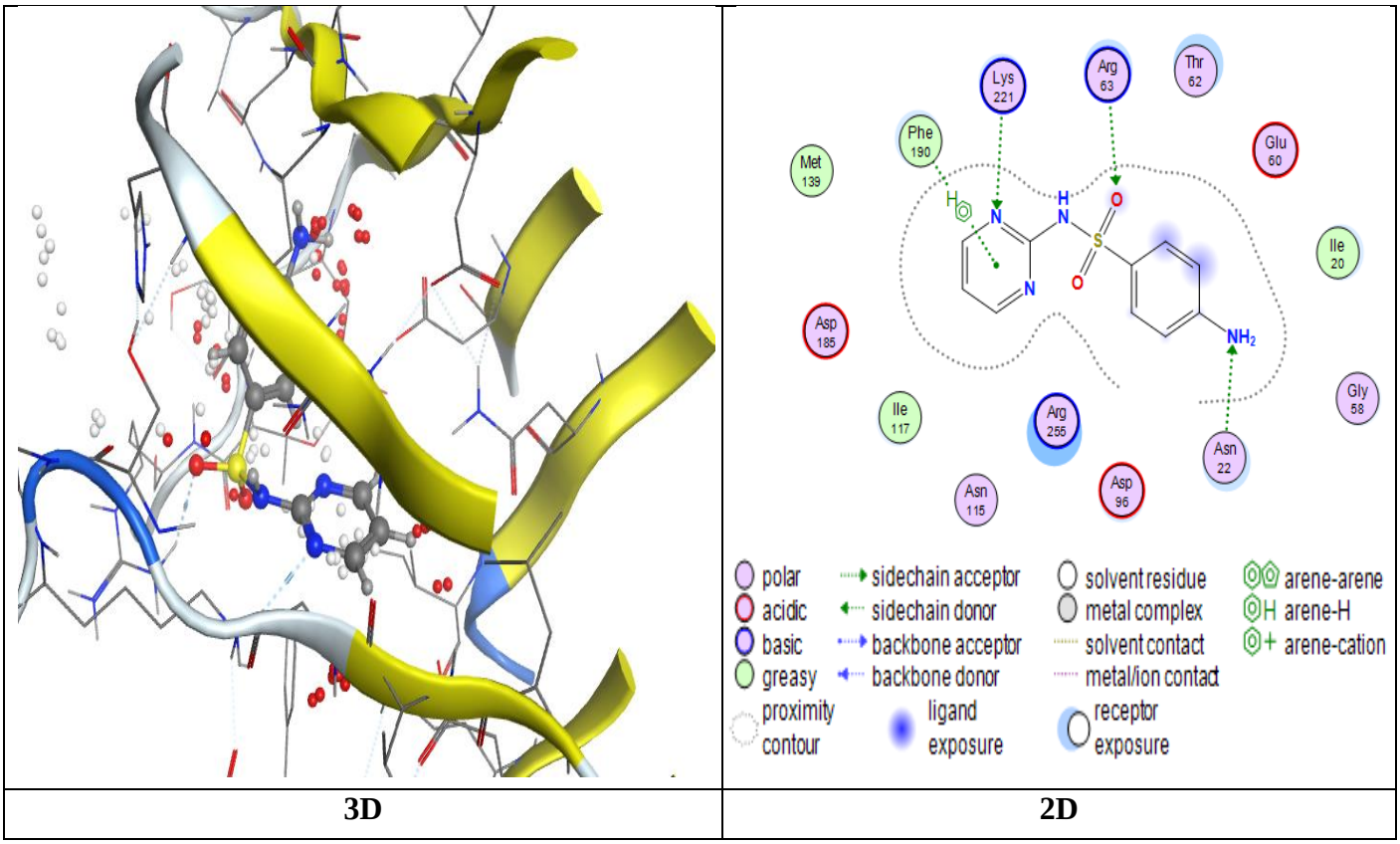
3D	2D

Fig. 7. 2D and 3D docking interactions of the SAR ligand with the **3TYU** protein.



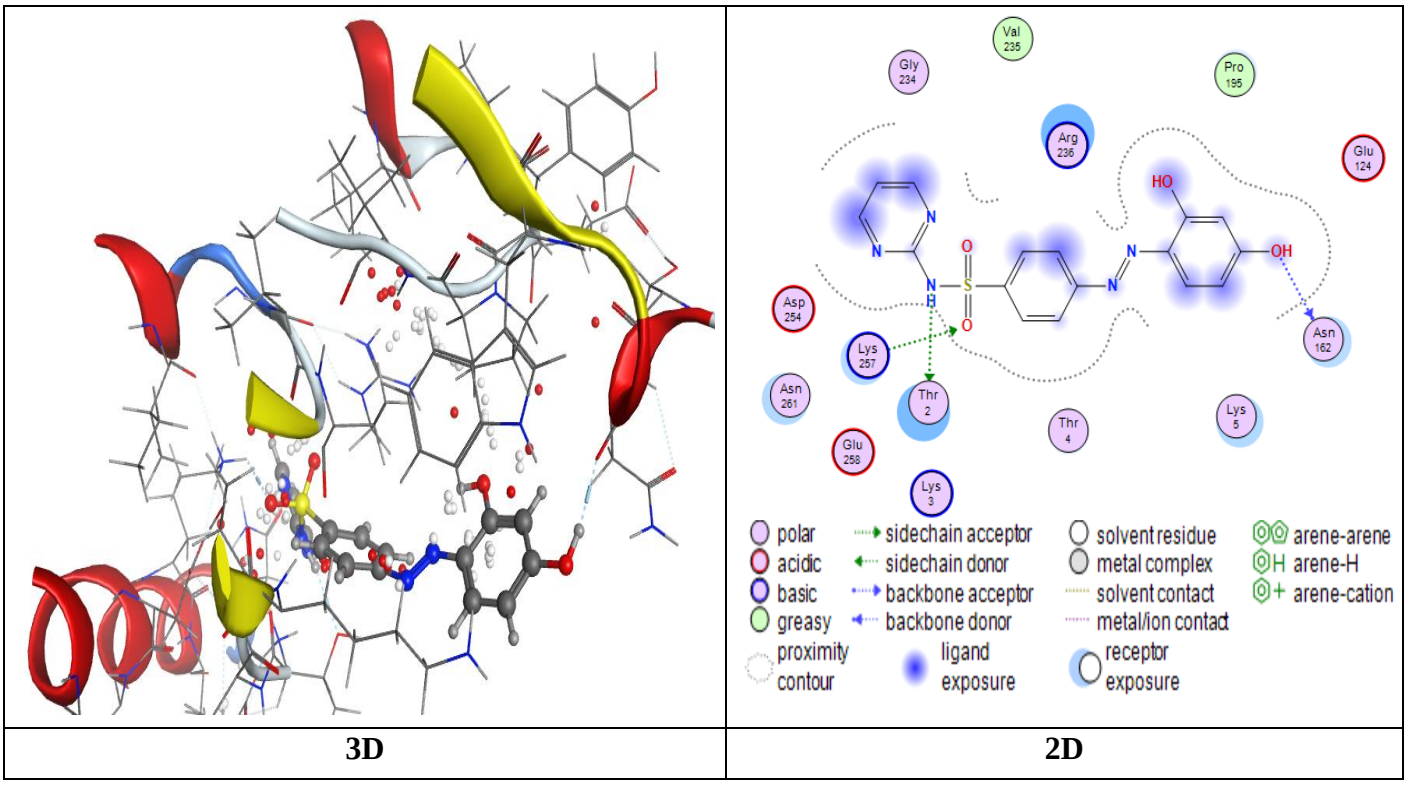
3D	2D

Fig. 8. 2D and 3D docking interactions of the Cu(II) complex with the **3TYU** protein.



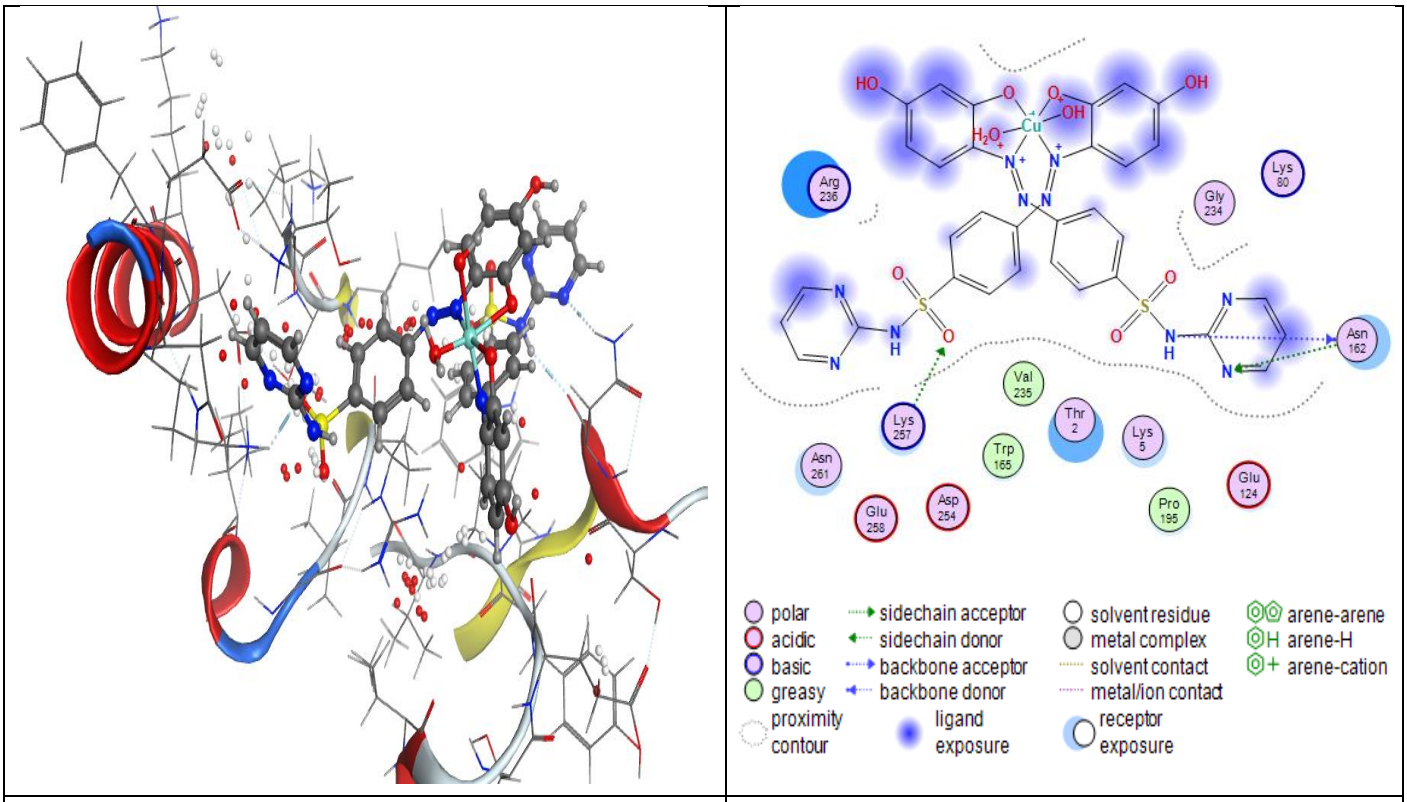
3D	2D

Fig. 9. 2D and 3D docking interactions of Sulfadiazine with the **3TYU** protein prostate.



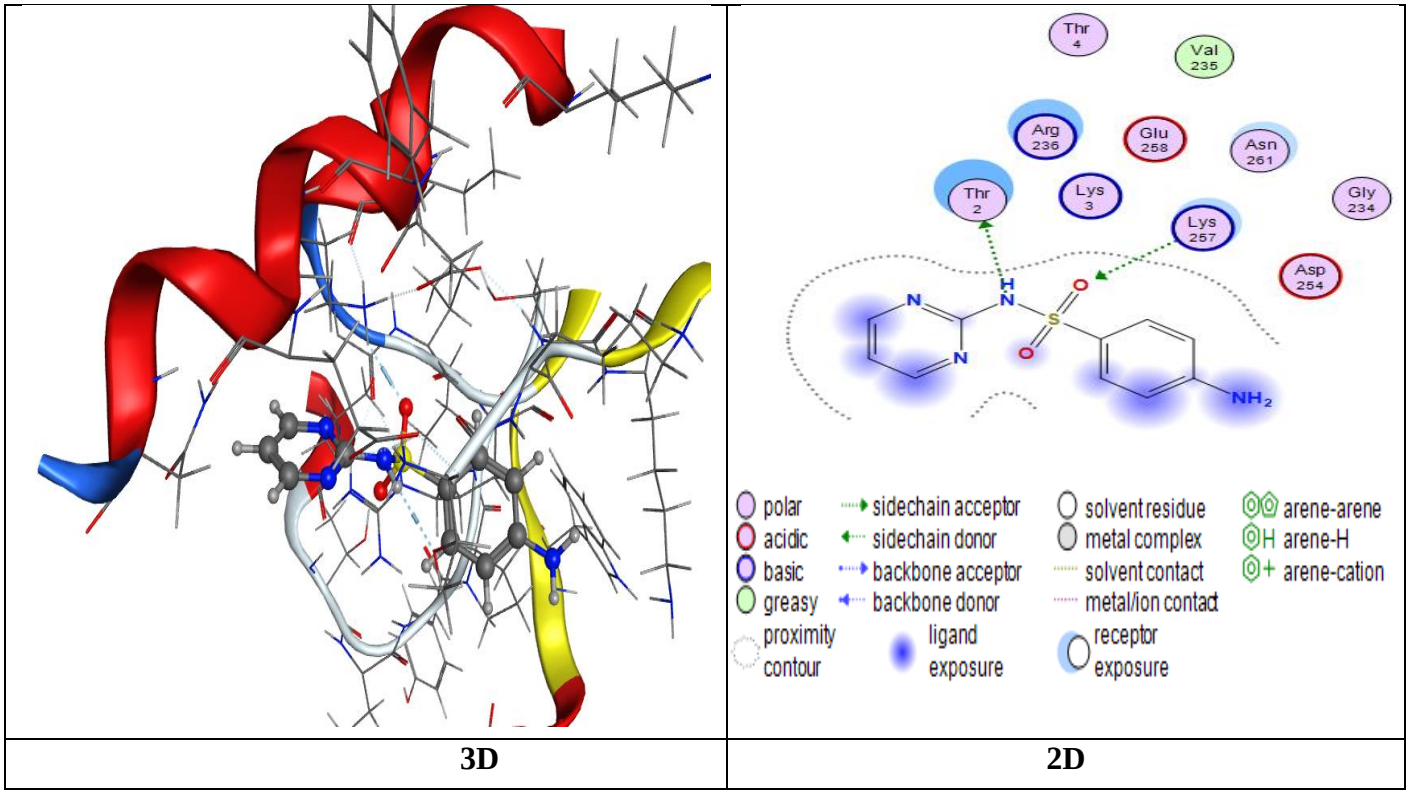
3D	2D

Fig. 10. 2D and 3D docking interactions of the SAR ligand with the **1AD4** protein.



3D	2D

Fig. 11. 2D and 3D docking interactions of the Cu(II) complex with the **1AD4** protein.



3D	2D

Fig. 12. 122D and 3D docking interactions of the sulfadiazine with the **1AD4** protein.

CONCLUSION

The azo ligand derived from sulfadiazine and resorcinol (SAR) was synthesized and characterized using various spectroscopic physicochemical techniques. The copper(II) complex of SAR was also successfully prepared and characterized. The results revealed that the ligand acts as a bidentate (N, O) donor, coordinated to the metal ion at a metal-to-ligand ratio of 1:2. The molar conductivity results indicate that it is a typical copper(II) complex. The data obtained were consistent with the proposed octahedral geometry of the copper(II) complex. Furthermore, biological activity studies showed that both the ligand and its copper complex exhibited moderate to high antimicrobial activity, with the copper(II) complex showing greater activity than the free ligand. The molecular docking study results showed higher binding affinity for the Cu(II) complex than the free ligand to all target proteins.

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