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Molecular Docking and Antimicrobial Screening of Alanine Derived Water-Soluble Cu(II) Complexes

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Abstract

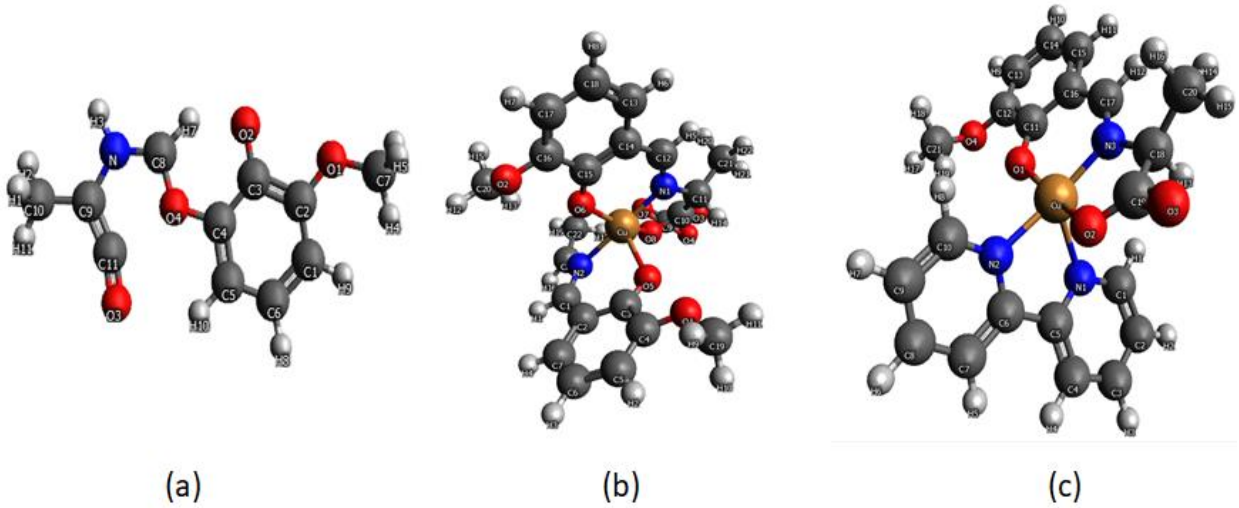
The rise of antibiotic-resistant bacteria reduces the effectiveness of existing antibiotics. This limits treatment options for common infections such as urinary tract infections, pneumonia, and skin infections. To combat antibiotic resistance, there's an urgent need to develop new antimicrobial agents that can effectively treat resistant infections. To address this problem and its possible solution, aniline and ortho vanillin derived biocompatible and water soluble compounds reported earlier [1] were examined for their potential to inhibit the growth of pathogenic bacteria and control the resulting infection. An *in-silico* binding investigation was conducted against the potential chemotherapeutic target bacterial DNA (PDB id:1BNA), and the compounds demonstrated significant binding. *In vitro* Antimicrobial activity of these compounds against pathogenic bacteria *Salmonella typhi (NCTC786)* and *S. aureus* (*NCIM 5345)* at different concentrations. Both the complexes have shown significant results compared to free ligand and with reference to the standard drugs Amoxicillin and erythromycin.

Keywords: Molecular docking, Bacterial DNA, MIC, Disc Diffusion, antibiotic

1. Introduction

The leading cause of human mortality across the globe is bacterial infection. A recent study reveals about 33 different species of bacteria responsible for 7.7 million deaths worldwide in 2019 alone. That amounts to 1 in 8 deaths worldwide [2]. The statistics for bacterial infections rank second only to ischemic heart disease in terms of cause of mortality. Common bacterial infections include strep throat, UTI, gonorrhea, syphilis, etc [2]. Over the years several antibiotic medicines have been successfully used against these pathogens. However, their overuse has given rise to antibiotic-resistant bacterial strains [3]. Hence, it is crucial to make new antibacterial agents from period to period to invade its resistance. It is still a big problem to develop new drugs, because of severe side effects, costs, and many-step processes. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the main microorganisms responsible for many bacterial diseases [4]. One of the most significant and effective advances in modern science and technology for the prevention and treatment of infectious illnesses is the discovery and development of new metal-based drugs [5]. Coordination chemistry has played an immense role as a therapeutic agent because of the coordination of metal complexes with cell membranes and some microorganisms. Interactions between transition metals and proteins are ubiquitous in biochemistry [6]. Metal complexes with Schiff base interact with bacterial DNA and Virus DNA to create new bioactive molecules used in biotechnology and medicine [7]. Transition metal complexes interact with DNA through various binding modes, with non-covalent intercalation being the most significant [8]. Virtual sorting and docking are effective techniques for discovering new compounds and increasing productivity. Docking can identify critical interactions between ligands to bind to enzymes or receptors, as well as predict

binding affinities between protein-ligand complexes [9]. In this paper, we propose to close these gaps by proposing a new class of antimicrobial drugs based on alanine amino acid-derived ligands and their metallic conjugates from our previous work [1]. The structure of ligand(a), complex1(b) and complex2(c) is given below.



(a) (b) (c)

The workflow includes in silico drug targeting studies, primarily against the bacterial DNA (pdb id:1BNA), an essential drug target. Using the disc diffusion method, antimicrobial activity against two bacterial strains, gram-positive and gram-negative *S. Aureus* (NCIM 5345) & *S. Typhi* (NCTC786), was assessed and results were analyzed. Minimum inhibitory constant (MIC) was also obtained.

2.0 Material and Methods

2.1 Disc diffusion method

The Ligand and its metal complexes were tested for antibacterial activity against pathogenic bacteria gram-positive and gram-negative *S. Aureus (NCIM 5345) & S. Typhi(NCTC786)* using control amoxicillin and Erythromycin which were obtained from Microbiology Laboratory of Sharda University, On Mueller-Hinton agar, suspensions of the bacteria with an optical density (OD) of 1.5 units were evenly swabbed for the disc well diffusion method [10]. A sterile cork-borer was used to form wells 6 mm in diameter, filling with a range of Compounds. 0.5 mL of 100,500 and 1000 ppm of the compounds were injected into the well and allowed to disperse evenly. The plates were incubated at 37 °C for 24 hours to check the antibacterial activity of synthesized compounds. The disc diffusion method [11] was used to measure the inhibition zone diameter, and the minimal inhibitory concentration (MIC) was determined using culture samples. Following 24 hours of incubation, the diameter of growth inhibition around the disc was measured. Three replications were used for each treatment, and the results were reported as means.

2.2 In silico Study-Target and lead identification

Molecular docking is an important technique to predict the possibility of binding between two biological molecules [12]. Here, to identify the affinity of our test molecules with bacterial DNA, Molecular docking study was conducted using Autodock4.0 software [13]. The bacterial DNA pdb file (PDB ID:1BNA) was downloaded from protein database with a resolution of 2.0. The energy minimization was conducted by SPDBV [14]. The receptor molecule's active site was defined using a 40X40X40 Å grid with X, Y, and Z coordinates of 20.837, 14.423, and 14.910, respectively. The structures of ligands and metal complexes 1 and 2 were drawn using the ACD/ChemSketch software. Docking experiments were performed on the energy-minimized DNA molecule and all test molecules using the Lamarckian search algorithm. To predict the best fit orientation of binding to the DNA helix, all rotatable bonds within the test molecules were allowed to rotate freely, while the receptor was considered rigid [15].

3.0 Result and Discussion

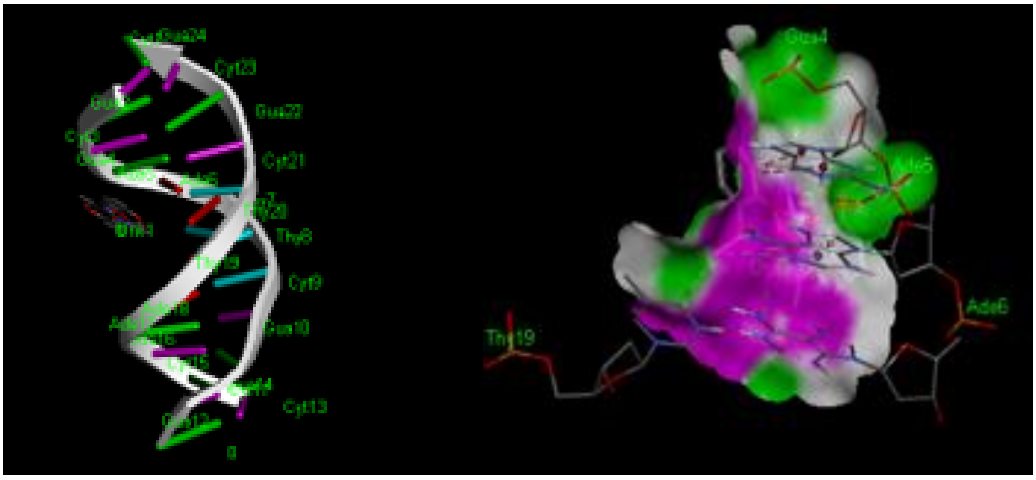
3.1Molecular docking with Bacterial DNA

The results obtained from Docking study were analysed by using discovery studio free version visualization tool. The ligand and both the complexes have showing binding with 1BNA among which complex 1 has shown better binding energy which is -6.93 Kcal/mole. It was expected from the structure of complex1, which is a perfect ensemble of hydrophilic and hydrophobic parts in the molecule which makes easy to non-covalent intermolecular interaction between test molecule and

DNA. Non-covalent interactions in the form of hydrogen bonding, hydrophobic, π-π stacking along with a few electrostatic interactions were observed. The detailed data on the docking study is presented in the table1 and figure 1&2

Table1: Summary of all binding interactions with residues, binding energy and inhibition constant of complexes with Bacterial DNA (1BNA)

Test molecule	Receptor	Binding energy (Kcal/mol)	Hydrogen bonding		Hydrophobic		II-Stacking		Inhibition constant
			Residue	Bond length(Å)	Residue	Bond length (Å)	Residue	Bond length (Å)	
L_1H	1BNA	-5.76	DA5, DA6		DA5, DG4		DA5, DG4		69.75nm
$L_1Cu(II)$ complex1		-6.42	DA5, DA6		DA5, DG4 DT19		DA5, DG4		34.87µM
$L_1Cu(II)$-Bpy complex2		-6.93	DA5, DA6		DA5, DG4		DA5, DG4		46.78µM



(a) (b)

Figure 1: Binding interactions of molecules in the active site with Bacterial DNA (pdb id:1BNA) of Complex 1 (C1)

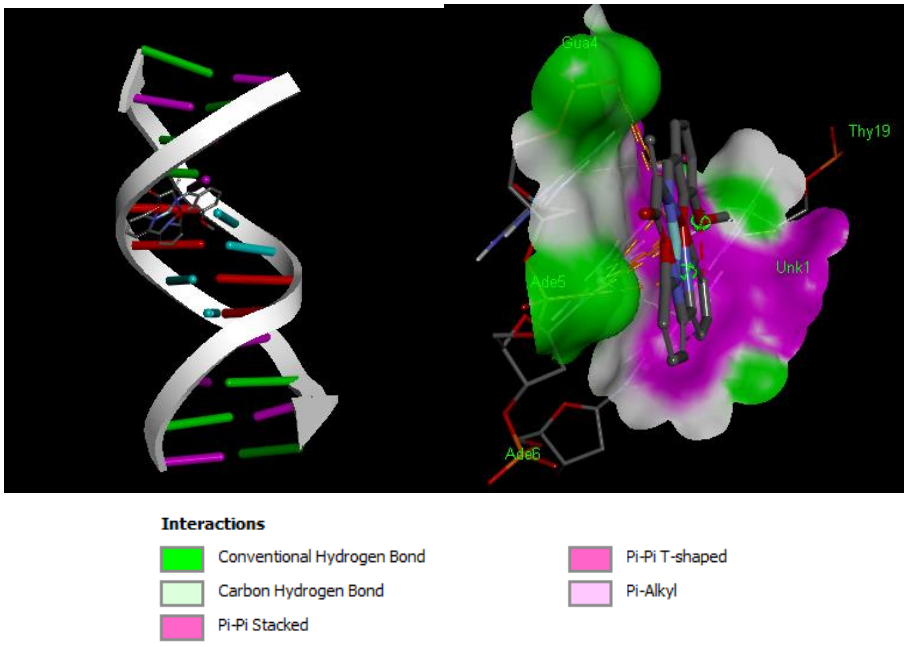


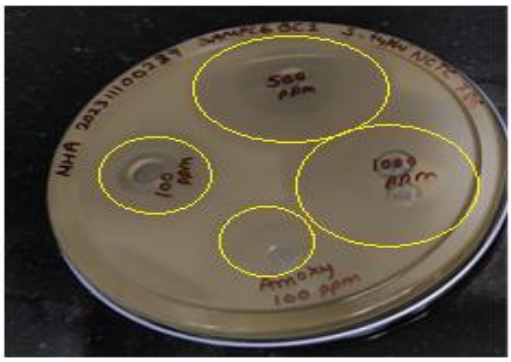
Figure 2: Binding interactions of molecules in the active site with Bacterial DNA (pdb id:1BNA) of Complex 2 (C2)

3.2 Antimicrobial activity

Antimicrobial activities at three concentrations (100 ppm, 500 ppm, and 1000 ppm) are shown using Erythromycin and Amoxicillin as reference drugs. Figure 3 shows the zones of inhibition created by ligand and complexes against the test organisms. The results of antimicrobial activity screening are shown in Table 2 for Ligand, Complex 1, and Complex 2. Compared to the parent ligands, the complexes demonstrated increased activity against Bacterial strains. There was a slight increase in activity with increased concentration. At 1000 ppm, both Complexes exhibit the highest inhibitory activity against *S. Aureus* and *S. Typhi.* Complexes had the highest inhibitory activity against *S. Aureus* but were slightly less active against the gram(-ve) bacteria *S. Typhi* compared to the *S.Aureus* gram(+ve). (Figure4 & 5)

Table 2: Zone of Inhibition in bacterial growth for Ligand, Complex 1 and Complex 2 with positive control

Complex	Bacterial Strains	Zone of inhibition(mm)in different Concentration(ppm)			Zone of control(mm)	
Ligand		**100**	**500**	**1000**	**Erythromycin**	**Amoxycillin**
	S.Aureus(NCIM 5345)	7.6±0.04	20.6±0.06	22.8±0.12	17.28±0.06	25.66±0.08
	S.Typhi(NCTC786)	8.6±0.05	14.6±0.09	19.8±0.10	9.25±0.07	22.46±0.07
Complex1	*S.Aureus(NCIM 5345)*	21.6±0.11	25.2±0.06	27.6±0.05	17.28±0.14	25.66±0.03
	S.Typhi(NCTC786)	12.35±0.02	17±0.11	24.6±0.07	9.25±0.09	22.46±0.04
Complex 2	*S.Aureus(NCIM 5345)*	21.6±0.09	26.7±0.04	29.6±0.04	17.28±0.12	25.66±0.07
	S.Typhi(NCTC786)	14.2±0.05	19.4±0.08	25.1±0.06	9.25±0.09	22.46±0.15



(a)

(b)

(c)

Figure3: Zone of inhibition (in mm) of the complexes tested against (*S. Aureus*) at three concentrations (100, 500 & 1000ppm)

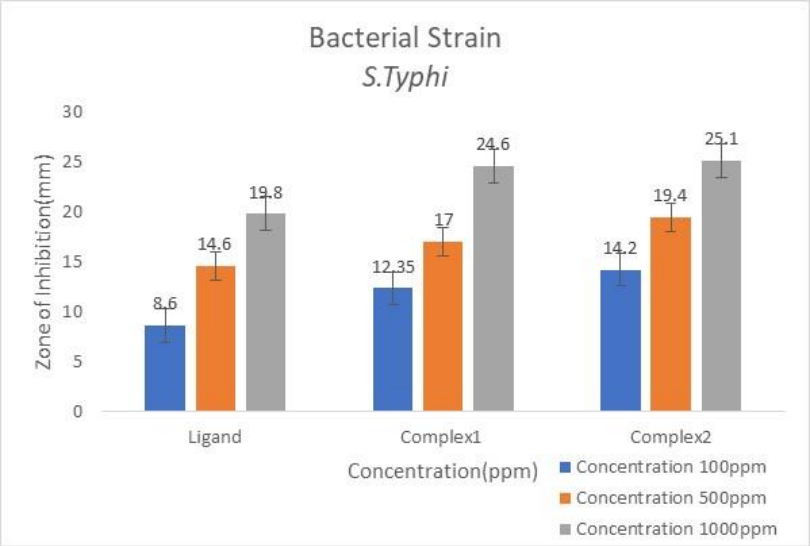


Figure4: Comparative analysis of antibacterial potential of the complexes tested against (*S. Typhi*) at different concentrations

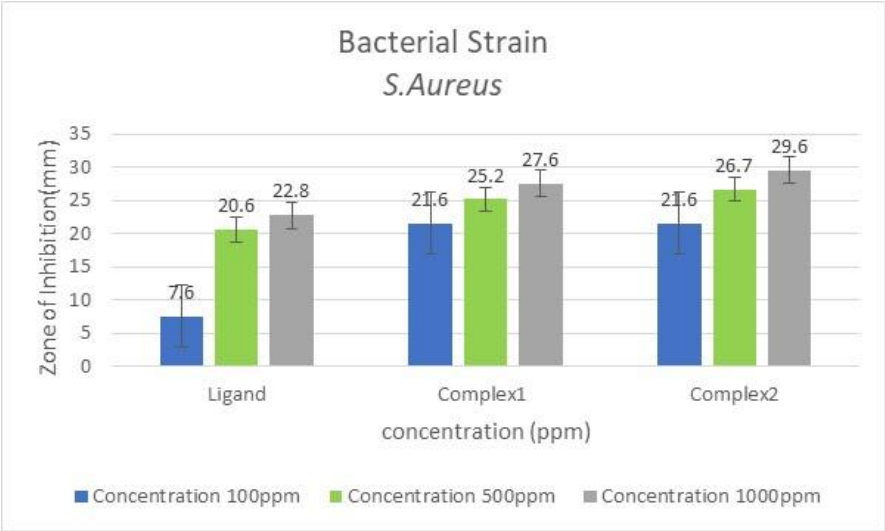


Figure 5: Comparative analysis of antibacterial potential of the complexes tested against (*S.Aureus*) at different concentrations

MICs were determined using the broth microdilution method. The nutrient broth, containing logarithmic serially two-fold diluted amounts of test compound and controls, was inoculated with active bacteria cells. Bacterial strains were cultured at 37°C for 24 hours, and growth was measured visually and spectrophotometrically [16]. The minimum inhibitory concentration (MIC) is the lowest concentration to inhibit bacterial growth. Erythromycin (standard antibiotic drug) and Amoxycillin (standard antifungal drug) were used as positive controls (table 3). Copper plays a significant role in biological systems. Copper is an essential trace element and plays a significant role in forming complexes that promote nucleic acid cleavage, making it useful as a metallodrug for DNA damage [17] . This is because Chelation reduces the polarity of metal ions, resulting in increased activity. Also, due to the partial sharing of positive charge with ligand donor groups and possible π-electron delocalization on aromatic rings [18] . This enhances the drug's lipophilicity and improves its effectiveness by increasing its permeability to the target site [19] . Different modes of mechanism are possible which include: disruption in of the cell wall, an injury that might change the permeability of the cell, or disarray of the lipoprotein that would result in cell death (figure6) [20]. Also, the formation of a hydrogen bond between the active center of the cell's components and the azomethine group, interferes with the regular cell process [21]. Complex2 has shown most effective MIC value with both the strains as mentioned in the table2

Table 3: MIC values for complexes with positive control Erythromycin and Amoxycillin

Complex	**Gram(+ve) Bacteria** ***S. Aureus*** **(MIC** in µg/ml**)**	**Gram(-ve) Bacteria** ***S. Typhi*** **(MIC)**
Ligand	>100	>100
Complex 1	19.26±0.06	18.25±0.05
Complex 2	23.5±0.05	22.67±0.075
Erythromycin	17.28±0.07	25.66±0.10
Amoxycillin	9.25±0.10	22.46±0.09

MIC (µg/ml) = minimum inhibitory concentration, i.e., the lowest concentration of the compound that completely inhibits bacterial growth.

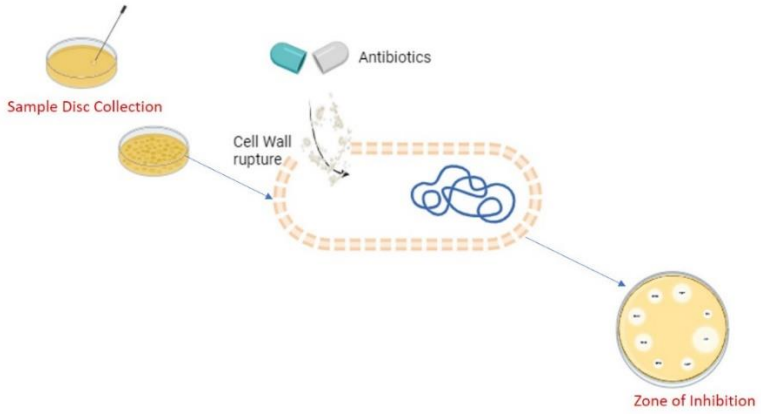


Figure 6: Mechanistic graphical representation of cell wall disruption using test drugs against bacterial cell

Metal complexes have the potential to release metal ions which harm the integrity of the bacterial cell wall. For instance, metal ions with antibacterial qualities, like copper (Cu,Ag) , can break down the structural strength of the cell wall by attaching to vital elements like lipopolysaccharides or peptidoglycan, which causes cell lysis.When certain metal complexes come into contact with bacterial cells[4], they can release reactive oxygen species (ROS). The cell wall is one of the components of the cell that can sustain oxidative damage from ROS. Cell death may result from this oxidative stress's disruption of the cell wall's structure and functionality.[5] Metal complexes can also disrupt bacterial cell membranes, leading to increased permeability. This disruption can compromise the integrity of the cell wall and facilitate the entry of the metal complex or other antimicrobial agents into the cell, causing further damage and ultimately cell death.

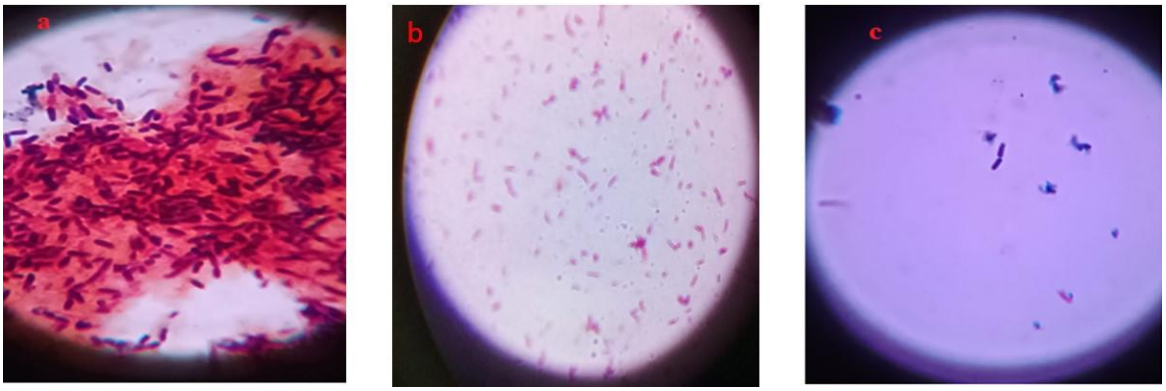


Figure 7: Microscopic images of *S.Aureus* (a) blank (b) Complex 1 (C) Amoxicillin at the lowest concentration of 100ppm.

Before examining the bacterial growth under a microscope, 10 µL of samples from the blank(culture), bacteria loaded with Complex 1, complex2, and amoxicillin were stained with gram stain on different glass slides. As shown in figure 7 when the slides were exposed to visible light, metal complexes undergo photocatalytic generation of electrons, hydroxyl radicals, and reactive oxygen species (ROS), which results in oxidative stress and ultimately leads to inactivation of bacterial cell [22]. Metal Complex 1has shown 79% inhibition of bacteria which is quite close to the results obtained for Amoxicillin (95%).

4. Conclusion

This paper shows N, O donor ligand and its water soluble and biocompatible Cu (II) complexes as potential antibacterial agents that significantly inhibit bacterial growth. Both the tested complexes show the higher anti-microbial activity than that of ligand and were comparable to Erythromycin and Amoxycillin reference drugs. Molecular docking results support these findings in terms of comparative binding energy with receptor bacterial DNA (1BNA). Complex 2 shows a higher binding energy of -6.93 Kcal/mol amongst all. These water soluble and biocompatible compounds offer significant anti-bacterial efficacy which can solve the problem of antibiotic resistance in bacteria thereby providing alternative antibiotic medicines.

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