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Research Article

DNA AND BSA INTERACTIONS OF TETRA-DENTATEMETAL (II) COMPLEXES: SYNTHESIS AND CHARACTERIZATON

Malathy M, Manikandan P and Rajavel R*

Department of Chemistry, Periyar University, Periyar Palkalai Nagar, Salem-636011, Tamilnadu, India.

ABSTRACT

Synthesis of two new series of tetra-coordinatedCu(II), Co(II), Ni(II) and Zn(II) chelates with the Schiff base ligands derived from Urea/Thiourea, 5-bromo-2-hydroxyacetophenone and 1-(2,4-dihydroxy-phenyl)-ethanone are described. These complexes have been characterized by elemental analyses, molar conductance, IR, electronic spectral studies, ¹H NMR, magnetic measurements and ESR spectra. The 3D molecular modelling and analysis for bond lengths and bond angles have also been carried out for the two representative Copper complexes ([CuL¹]& [CuL²] to substantiate the proposed structures. Antibacterial studies against Gram +ve and Gram -ve bacterias reveals high antibacterial activity of the synthesized complexes. The interactions of the compounds with DNA and bovine serum albumin (BSA) were also investigated. The result shows that all the complexes exhibited a strong cleavage activity to DNA and BSA.

Keywords: 3D molecular modelling, Antibacterial activity, DNA cleavage, BSA cleavage.

INTRODUCTION

A great attention has been devoted to the design and the synthesis of unsymmetrical Schiff base ligands¹. Also their transition metal complexes are a captivating area of research, owing to their importance in basic and applied chemistry. In particular, Schiff bases derived from urea have proposed to comprise of an excellent systems with their well-known ability to form stable complexes with different metal cations. Detailed investigations of their stereo chemical, electronic, magnetic, and catalytic properties have allowed the proposal of new, highly efficient molecular devices or probes for a number of specific applications². The literature contains numerous examples of the use of hydroxyl radical-generating systems for the modification and cleavage of DNA and related chemistry has been applied to eject protein cleavage. Serum albumin is the most abundant protein in animals including human circulatory system that is in-charge for the transport, distribution and deposition of a

of endogenous and exogenous variety substances in body. Knowledge of interaction mechanisms between drugs and plasma proteins is very important to understand the pharmacodynamics and pharmacokinetics of a drug. Drug binding influences their distribution, excretion, metabolism and interaction with the target tissues. Bovine serum albumin (BSA) has been proven to have a high homology and a similarity to human serum albumin (HSA) in sequence and conformation. Therefore, it is always selected as a particularly relevant protein and has become the best-studied model of general drug-protein interactions⁴. Proteins the most abundant are macromolecules in cells and are crucial to maintaining normal cell functions. Among bio macromolecules, the serum albumins are the major soluble protein constituent of the they circulatory system; have many physiological functions. Bovine serum albumin (BSA) has been one of the most extensively studied proteins, especially of its structural homology with human serum albumin⁵.

MATERIALS AND METHODS

All the chemicals and Solvents were reagent grade and were used as received. Elemental analyses were performed using Perkin-Elmer 2400-II elemental analyzer. Infrared spectra were recorded as KBr disks using a Thermo Nicolet, Avatar 370 model spectrometer. Molar conductance was measured in DMF using a conductance bridge of the type Elico model conductivity meter calibrated with KCI solution. ¹H NMR spectra were measured at room temperature on Bruker Avance III, 400MHz model spectrometer. The chemical resonances (δ) were reported in parts per million (ppm) with the ¹H NMR shifts referenced to the residual proton signal of deutrated solvent (DMSO-d⁶). UV-Vis spectral measurements were carried out in DMF within the wavelength range of 200-800 nm using a Perkin-Elmer Lambda 40(UV-Vis) spectrometer. Magnetic susceptibilities of the complexes were determined by Gouy's method using magnetic susceptibility instrument. The Redox nature of the complex in DMF was measured using CHI -760 Electrochemical Analyzer by employing a platinum electrode as working electrode, Ag/AgCl as a reference electrode and platinum wire as auxiliary electrode. The working media consist of DMF containing 0.1M tetrabutylammonium perchloride (TBAP) as supporting electrolyte. X-band ESR spectra of the complexes were measured on E-112 ESR Spectrometer using powdered samples at room temperature.

SYNTHESIS OF SCHIFF BASE LIGANDS AND ITS METAL COMPLEXES Scheme-I

Synthesis of Schiff Base Ligand (L¹)

The tetradentate Schiff base ligands were prepared by the reported method⁶ by condensation in an ethanolic solution of 5-bromo-2- hydroxybenzaldehyde (1 mM), 1-(2,4-dihydroxy-phenyl)-ethanone (1 mM) and Urea/ thiourea (1 mM) was mixed and refluxed for 2 hrs on a water bath(Figure 1). The isolated brown colour solid product was separated and dried. Also the purity of the ligand was checked by TLC.

Synthesis of Schiff Base Metal Complexes

The Schiff base metal complexes were prepared by literature procedures⁷ (Figure 2).

Synthesis of Cu (II) Schiff base complexes

An absolute ethanolic solution containing copper acetate monohydrate (0.1997 g, 1mM) was added drop-wise to a solution of Schiff base ligands (L^1 and L^2)(1mM) in 20mL of absolute ethanol with constant stirring condition. After refluxing for 3 hrs, the solution was allowed to cool at room temperature and the solid product obtained is dried in air.

Synthesis of Co (II) Schiff base complexes

The clear solution containing 1mM of Schiff base ligands (L^1 and L^2)was added to a solution of cobalt acetate tetrahydrate (0.2491g) in ethanol (20ml). The stirred mixture was refluxed for 3hrs. A colored product appeared on standing and cooling the above solution. The precipitated solid complexes were filtered, washed with ethanol to remove any traces of the unreacted starting materials. Finally, the complexes were dried under vacuum.

Synthesis of Ni(II) Schiff base complexes

An ethanolic (20ml) solution of Schiff base ligands (L^1 and L^2) (1mM) was mixed with Nickel acetate tetrahydrate (0.2488g) in ethanolic (20ml) solution. The mixture was then refluxed for 3hrs on a water bath. The resulting solution was then allowed to cool at room temperature and the precipitate obtained was dried and washed with ethanol.

Synthesis of Zn (II) Schiff base complexes

To an ethanolic solution (20ml) of zinc acetate tetrahydrate (0.253g), an ethanolic solution (20ml) Schiff base ligands(L^1 and L^2)(1mM) was added drop wise with continuous stirring and gently refluxed for about 3hrs. The solution is allowed to cool at room temperature and solid product obtained is dried and purified

Molecular Modelling Studies

The 3D molecular modelling of the complexes was carried out on Chem3D Pro 12.0 Molecular Modelling and Analysis Program.

Antibacterial Assay

The *in vitro* antibacterial activity of the tetradentate Schiff base ligands(L^1 and L^2) and its metal complexes were tested against the Gram +ve bacterial species such as *Staphyloccus aureus* and *Bacillus subtilis* and Gram –ve bacterial species such as *Escherichia coli* and *Klebsiella pneumoniae*. The screening was carried out at room temperature by the disc diffusion method using the standard procedure⁸. *Streptomycin* was used as the standard drug for bacterial screening. Nutrient agar medium was employed as culture media for bacterial screening. The disc of whatmann No.1 filter

paper having the diameter 5mm were soaked into the solution of the complexes dissolved in DMSO at different concentrations ($50\mu g \text{ mL}^{-1}$ and 75 $\mu g \text{ mL}^{-1}$). After drying at room temperature, the discs were then placed on the inoculated plates containing respective medium. The plates were then incubated at 37° C for 24-48 hrs. After the period of incubation, the antibacterial activities were recorded by measuring the diameter (mm) of the inhibitory zone. The antibacterial activities shown by the ligands and their metal complexes were compared with the standard drugs.

DNA Cleavage Studies

CT DNA cleavage study was carried out by using gel electrophoresis. The reaction mixture containing supercoiled 40μ M CT DNA, 50μ M metal complexes and 50μ M H₂O₂ in Tris- HCI buffer (pH 7.2) was incubated at 37°C for 2hrs. After incubation, the samples were electrophoresed for 2hrs at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH-7.2). After electrophoresis, the gel was stained using 1µg cm-³ethidium bromide (EB) and photograpged under UV light at 360nm⁹.

BSA cleavage studies

The protein cleavage experiment was done in a dark room at 25°Cusing BSA in 50mM Tris-HCI buffer and the complex. In this experiment, BSA (5 mg) was treated with Schiff base complexes in Tris buffer containing (5 mM Tris/50 mM NaCl, pH 7.5) and the reaction mixture was incubated for 6 h at 37°C. After incubation, the samples were electrophoresed for 2hrs at 50 V on 1% agarose gel (SDS-PAGE) using Tris-HCI (62.5mM, pH 6.8) buffer solution. After electrophoresis, the gel was stained with silver stain and photographed under UV light at 360nm.

RESULTS AND DISCUSSION

All the synthesized ligands and its metal complexes are stable and non-hygroscopic in nature. The complexes are insoluble in water but soluble in DMF and DMSO.

Elemental Analysis and Molar Conductance

The elemental analyses shows that, the Cu(II), Co(II), Ni(II) and Zn(II) complexes have 1:1 stoichiometry of the type ML wherein L acts as a tetradentate ligand and agrees well with the proposed structure(Table 1). The molar conductance of the metal complexes in DMF at room temperature was observed in the range 3.6-5.9scm²mol⁻¹. The observed valves are tabulated in Table 2. This data shows that the complexes are non-electrolytes and thus confirming the neutral structure proposed for the synthesized complexes ¹⁰.

Infrared Spectra

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom¹¹. The important IR frequencies exhibited by the Schiff base ligand and their corresponding metal complexes are tabulated in Table 3. The band in the range 1611-1616cm⁻¹ are observed due to v(C=N)which has been shifted towards lower region at around 1545-1603cm⁻¹ in the complexes indicating the participation of the azomethine group in the complexes formation¹², this shift is also due to the reduction of double bond character of carbon-nitrogen bond of azomethine group¹³. The Schiff base ligands shows a medium intensity band at around 1259 & 1275cm⁻¹ due to phenolic v(C-O) which is shifted towards higher region in metal complexes indicating the coordination through the phenolic oxygen atom and also due to the conversion of hydrogen bonded structure into a covalent metal bonded structure. The ligand shows broad band at around 3310-3377cm⁻¹ due to two phenolic hydroxyl groups in free ligand¹⁴. The appearance of this peak in the spectra of complex between 3320-3396cm⁻¹ indicates that one of the -OH group is free from complexation The coordination through nitrogen of azomethine and oxygen of v(C-O) of ligand are further evidenced by the appearance of non-ligand bands around 432-491cm⁻¹and 531-566cm⁻¹ are due to M-N and M-O bonds respectively¹⁵⁻¹⁶.Thus the IR spectral results provide strong evidences for the complexation of Schiff base with metal ions in tetradentate mode.

Electronic Spectra and Magnetic Moment

The UV-Vis spectra of Schiff base ligands and their complexes were recorded in DMF solution between 200-800nm. The Schiff base ligands exhibit two bands between 265-270nm and 336-340nm(Table 4). In both ligands, the inner ligand transitions such as $\pi \rightarrow \pi^*$ and n $\rightarrow \pi^*$ are common due to the presence of C=N, C=C and C-O groups in the ligand structures. The electronic spectrum of Cu(II) complexes showed a band between 601-620nm assignable to ${}^{2}B_{1}g \rightarrow {}^{2}A_{1}g$ transitions, respectively which corresponds to distorted square planar geometry around Cu(II) ion¹⁷. The measured value of the magnetic moment for Cu(II) complex was 1.81B.M., which confirm the square planar structure. The electronic spectrum of Ni(II) complexes exhibited a d-d transition between 542-555nm which may be assigned to three spin allowed transitions, ${}^{1}A_{1}g \rightarrow {}^{1}A_{2}g$ characteristic of distorted square planar geometry around Ni(II) ion¹⁸. The diamagnetic nature revealed by magnetic moment studies further confirm the square planar environment around the Ni(II) ion. However, the electronic spectrum of Co(II) complexes showed a d-d transition between 547-588nm assignable to ${}^{4}A_{2q} \rightarrow {}^{4}T_{1q}(P)$ transitions, respectively. However, a band at 255 and 265nm may be assigned to $\pi-\pi^*$ transition supporting a distorted square planar geometry ¹⁷. The observed magnetic moment of 3.12B.M.further authenticates the electronic spectral finding. The Zn(II) complexes show an absorption band at 415 and 440nm attributed to the $L \rightarrow M$ charge transfer transition, which is compatible with this complex having a planar geometry distorted square electronic configuration¹⁹.

¹H-NMR Studies

In the ¹H NMR spectrum of the Schiff base, the aromatic OH proton exhibited signal at 10.21ppm (s, 2H) and the characteristic signal observed at 8.2ppm (s, H) was due to azomethine proton. The multiplet signals around 6.24-7.77ppm are ascribed to aromatic protons. The signals observed at 2.54ppm are due to methyl protons²⁰. These ¹H-NMR results further support the IR inferences.

Electrochemical Study

The redox properties of the complexes have been investigated in DMF solution at glassy carbon electrode using tetrabutylammonium perchlorate as the supporting electrolyte and Standard Calomel Electode as reference electrode. The electrochemical data such as cathodic peak (Epc) and anodic peak (Epa) are given in Table 5&6. The electron transfer behaviour of transition metal Schiff base complexes in a subject of considerable interest from the point of view of understanding the redox function of the complexes. The cyclic voltammogram of Cu(II) complexes displays a reduction peak at Epc = -1.4V and -1.6V with an associated oxidation peak at Epa = -0.85V and -1.8V. The peak separation of this couple (ΔEp) is 200mV and 550mV. Thus, the analyses of cyclic voltametric responses give the evidence for guasi-reversible one electron reduction. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple²¹. The Schiff base Co(II) complexes exhibit one electron quasi reversible transfer process with a reduction peak at Epc = -1.2V and -1.7V and a corresponding oxidation peak at Epa = -1.2V and -1.7V. The peak separation (AEp) of this couple is 400mV and 150mV. This further evidence for the guasi-reversible Co(II)/Co(I) couple. The difference between forward and backward peak potentials can provide a rough

evaluation of the degree of the reversibility²². The redox property of Ni(II) complex displayed an reversible peak between 1.2 to -1.15V, corresponding to the reduction of Ni(II)/Ni(I). The irreversibility of the Ni(II)/Ni(I) was checked by varying the scan rates with peak potentials. The Schiff base Zinc(II) complexes exhibits one-electron reduction peak at 1.1V and the corresponding oxidation occurs at 0.8V, which can be assigned to the formation of zinc(I) complex species. The investigated zinc(II) complex that undergoes the following redox reaction.

Zn (II)≒ Zn(I)

This finding suggests that the one–electron transfer process is easily reversible²³⁻²⁵ and suggests that Zn(II)/Zn(I) redox couple is quasi-reversible behaviour with a slow electron transfer²⁶.

3D Molecular Modelling

All the synthesized mononuclear complexes were soluble in solvents like DMSO and DMF, crystals that are suitable for single-crystal studies were not obtained. The optimized structure of the complexes was obtained by the 3D molecular modelling method(Figure 3 & 4). The actual bond lengths and bong angles were found to be close to the optimal values, which supports the proposed square planar geometry for the complexes(Table 7&8).

ESR spectrum

The ESR spectrum of Cu(II) complexes were recorded on X-band at frequency 9.1GHz at room temperature. The spectra exhibited a single anisotropic intense broad signal at room temperature with no hyperfine splitting This suggests that copper ions are in mononuclear environment. From the observed values, it is clear that gll =2.19> g_1=2.02>2, which suggest that the complex has a square-planar geometry²⁷.

Antimicrobial activity

The in vitro anti-microbial activity of the investigated compounds was tested against Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia and Escherichia coli by the well diffusion method. The minimum inhibitory concentration values of the compounds against the growth of the microorganisms are summarized in Table 9. A comparative study of the ligands (L^1 and L^2) and its copper(II), cobalt(II), Nickel(II) and Zinc(II) complexes indicated that all the complexesexhibited either higher or lower antimicrobial activity than the free ligand²⁸. These observations showed that the majority of the complexes were more active than their respective Schiff base ligands. In some cases, Schiff bases are more active than their metal complexes against bacteria and fungai. The chelation either enhances or reduces the antimicrobial activity: sometimes it remains neutral. Thus, metal chelation may increase or decrease the therapeutic value of synthesized compounds. It may maintain the property intact by further stabilizing the drug and/ or reducing the biodegradability/ metabolic decay of the synthesized Schiff base ligands through chelation²⁹. In general, the antimicrobial activity of metal complexes is concerned with the following five principal factors such as,

- The chelate effect, i.e. multidentate Schiff base ligands, show higher antimicrobial efficiency towards complexes with monodentate ligands.
- The nature of the ligands.
- The total charge of the complex; generally the antimicrobial efficiency decreases in the order cationic > neutral > anionic complex.
- The nature of the counter ion in the case of the ionic complexes.

Hence the synthesized ligands with its NNOO donar atoms act moderately against bacteria and fungai, whereas the copper complexes show higher activity than other complexes due to its smaller size and high liphophilic nature formed after complexation with the Schiff base ligands³⁰.

DNA Cleavage Study

DNA Cleavage study was done by Gel electrophoresis method using H_2O_2 as an oxidant. From the result it is clear that at low concentration the complexes were able to cleave the CT-DNA, as compared to the control DNA. The different DNA cleavage efficiency of the complexes was due to the different binding affinity of the metal complexes to DNA. Cu(II) complex was found

to be highly active in cleaving CT DNA in present of H_2O_2 , due to the increased production of hydroxyl radical (OH) by cuprous ions³⁰⁻³¹.

BSA Protein Cleavage

The protein photo cleavage activity of all the metal complexes were investigated at 365nm and analyzed on a SDS-PAGE in tris-HCI. The SDS PAGE gels are shown in Figure 7 &8. From the results it is clear that all the complexes cleave the BSA into smaller fragments³². The photo-excitation generates reactive metal species from a photo-redox mechanism with subsequent activation of molecular oxygen to form superoxide anion radical, which is then converted into hydroxyl radicals in the reaction:

$$3O_2^{-} + 2H + \rightarrow OH + HO^{-} + 2O_2$$

Moreover the copper (II) complex shows higher cleavage activity than the other complexes, due to the increased production of hydroxyl radical (OH⁻) by cuprous ions.

CONCLUSION

In this study, the complexes of Co(II), Ni(II), Cu(II) and Zn(II) complexes with Schiff base derived from 5-bromo-salicylaldehyde, 2,4hydroxyacetophenone with urea/ thiourea were synthesized and characterized. The Schiff bases act as versatile tetradentate ligand. On the basis of spectral techniques, a distorted square planar structure was proposed for the complexes, which was supported by the 3D molecular modelling. The electrochemical properties of the metal complexes revealed the quasi-reversible one electron transfer redox process for all the synthesized complexes. The biological assay indicates that the complexes show higher activity than free ligand. DNA and BSA cleavage studies of the complexes reveal the higher activity of the [CuL1]& [CuL2] complexes.

.OH



Fig. 2: Synthesis of Schiff base metal complexes



Lane 1: DNA alone, Lane 2: DNA $+H_2O_2$, Lane 3: DNA $+H_2O_2 + C$ opper complex1, Lane 4: DNA $+H_2O_2 + C$ obalt complex1, Lane 5: DNA $+H_2O_2 + N$ ickel complex1, Lane 6: DNA $+H_2O_2 + Z$ inc complex1



Lane 1: DNA alone, Lane 2: DNA $+H_2O_2$, Lane 3: DNA $+H_2O_2$ + Copper complex2, Lane 4: DNA $+H_2O_2$ + Cobalt complex2, Lane 5: DNA $+H_2O_2$ + Nickel complex2, Lane 6: DNA $+H_2O_2$ + Zinc complex2





Lane -1BSA Control; Lane-2 BSA+ L1; Lane-3 BSA + Copper Complex1; Lane -4 BSA +Nickel Complex1; Lane-5 BSA + Cobalt complex1; Lane-6 BSA + Zinc Complex1; Fig. 7: SDS PAGE gel diagram for the photocleavage

of BSA by the L^1 and its corresponding metal complexes



Lane -1BSA Control; Lane-2 BSA+ L2; Lane-3 BSA + Copper Complex2;

Lane -4 BSA +Nickel Complex2; Lane-5 BSA + Cobalt complex2;

Lane-6 BSA + Zinc Complex2;

Fig. 8: SDS PAGE gel diagram for the photocleavage of BSA by the L^2 and its corresponding metal complexes

Compound	Molecular Colour		M. Pt	% Nitro	% of Nitrogen		of tal
Compound	formula	ooloui	(°)	Cal	Exp	Cal	Exp
L ¹	C ₁₆ H ₁₃ BrN ₂ O ₃ S	Pale brown	155	7.43	7.40	-	-
L ²	C ₁₆ H ₁₃ BrN ₂ O ₄	Dark brown	168	7.12	7.09	-	-
[CuL ¹]	C ₁₆ H ₁₁ BrCuN ₂ O ₃ S	Green	246	6.39	6.33	14.48	14.41
[CoL ¹]	C ₁₆ H ₁₁ BrCoN ₂ O ₃ S	Dark Brown	252	6.45	6.41	14.74	14.70
[NiL ¹]	$C_{16}H_{11}BrNiN_2O_3S$	Light green	243	6.46	6.42	13.53	13.49
[ZnL ¹]	$C_{16}H_{11}BrZnN_2O_3S$	Pale Yellow	239	6.36	6.32	14.84	14.80
[CuL ²]	$C_{16}H_{11}BrCuN_2O_4$	Green	258	6.16	6.11	13.97	13.94
[CoL ²]	C ₁₆ H ₁₁ BrCoN ₂ O ₄	Dark Brown	265	6.22	6.19	13.09	13.01
[NiL ²]	$C_{16}H_{11}BrNiN_2O_4$	Dark green	231	6.23	6.19	13.04	13.00
[ZnL ²]	$C_{16}H_{11}BrZnN_2O_4$	Pale brown	242	6.13	6.10	14.32	14.29

Table 1: Analytical data of the Schiff base ligands and its complexes

Table 2: Molar conductance data of the Schiff base complexes

Compounds	Molar conductance scm ² mol ⁻¹	Type of electrolyte			
[CuL ¹]	5.9				
[CoL ¹]	4.8				
[NiL ¹]	4.1				
[ZnL ¹]	3.6	Non			
[CuL ²]	5.0	electrolyte			
[CoL ²]	5.5				
[NiL ²]	4.6				
[ZnL ²]	3.8				

Table 3: Infrared spectroscopic data of the Schiff base ligands and its complexes

Compound	Free-OH	-CH=N	C-0	M-O	M-N
L	3310	1611	1259	-	-
L ²	3377	1616	1275	-	-
[CuL ¹]	3396	1620	1301	566	442
[CoL ¹]	3308	1545	1274	533	455
[NiL ¹]	3378	1620	1274	548	473
[ZnL ¹]	3310	1608	1275	566	458
[CuL ²]	3343	1615	1317	543	464
[CoL ²]	3354	1570	1274	531	432
[NiL ²]	3355	1580	1265	533	454
[ZnL ²]	3366	1603	1275	533	491

Table 4: Electronic Spectral data of Schiff base ligands	
and its metal complexes	

Compound	Ele	ctronic sp	ectra (nn	Coomstry of the complex	
Compound	$\pi \rightarrow \pi^*$	n→π*	L→M	d-d	Geometry of the complex
L	270	340	438	-	-
L ²	265	336	-	-	-
[CuL ¹]	226	348	415	620	
[CoL ¹]	265	356	432	588	
[NiL ¹]	254	360	430	542	
[ZnL ¹]	242	346	415	-	
[CuL ²]	290	350	445	601	Distorted
[CoL ²]	255	346	412	547	Square planar
[NiL ²]	225	354	432	555	
[ZnL ²]	230	345	440	-	

In Divir meanum (oxidation at anodic potential)										
Complex	Epc(V)	Epa(V)	E _{1/2} (V)	∆Ep(mV)						
[CuL ¹]	0.65	0.9	0.78	250						
[CoL ¹]	0.9	0.7	0.8	200						
[NiL ¹]	1.2	0.8	1.0	400						
[ZnL ¹]	0.7	0.85	0.78	150						
[CuL ²]	0.9	1.1	1.0	200						
[CoL ²]	0.95	0.8	0.88	150						
[NiL ²]	1.1	0.8	0.95	300						
[ZnL ²]	1.1	0.8	0.95	300						

Table 5: Electrochemical data of the Schiff base metal complexes in DMF medium (oxidation at anodic potential)

Table 6: Electrochemical data of the Schiff base metal complexes in DMF medium (reduction at cathodic potential)

Complex	Epc(V)	Epa(V)	E _{1/2} (V)	∆Ep(mV)
[CuL ¹]	-1.4	-0.85	-1.13	550
[CoL ¹]	-1.2	-0.8	-1.0	400
[NiL ¹]	-1.15	-0.7	-0.93	450
[ZnL ¹]	-1.5	-1.6	-1.55	100
[CuL ²]	-1.6	-1.8	-1.7	200
[CoL ²]	-1.7	-1.85	-1.78	150
[NiL ²]	-1.15	-0.65	-0.9	500
[ZnL ²]	-0.95	-0.8	-0.88	150

Table 7: Various bond lengths of [CuL¹] complex

S No. Atomo		Actual Bond	Optimal			
3.NU.	Atoms	length	Bond length			
1	C(22)-H(35)	1.114	1.113			
2	C(22)-H(34)	1.1139	1.113			
3	C(22)-H(33)	1.1139	1.113			
4	O(20)-H(32)	0.9709	0.972			
5	C(17)-H(31)	1.1036	1.1			
6	C(15)-H(30)	1.1033	1.1			
7	C(14)-H(29)	1.1013	1.1			
8	C(8)-H(28)	1.1054	1.1			
9	C(6)-H(27)	1.1035	1.1			
10	C(3)-H(26)	1.1031	1.1			
11	C(1)-H(25)	1.1031	1.1			
12	N(21)-Cu(24)	1.3342	1.303			
13	N(9)-Cu(24)	1.3341	1.303			
14	O(19)-Cu(24)	1.8076				
15	O(7)-Cu(24)	1.8088				
16	C(11)-S(23)	1.5829	1.576			
17	C(11)-N(21)	1.2857	1.426			
18	C(18)-C(22)	1.5268	1.497			
19	C(18)-N(21)	1.2788	1.26			
20	C(16)-O(20)	1.3601	1.355			
21	C(12)-O(19)	1.3654	1.355			
22	C(13)-C(18)	1.3577	1.503			
23	C(17)-C(12)	1.3467	1.42			
24	C(16)-C(17)	1.3413	1.42			
25	C(15)-C(16)	1.3402	1.42			
26	C(14)-C(15)	1.3407	1.42			
27	C(13)-C(14)	1.3479	1.42			
28	C(12)-C(13)	1.3521	1.42			
29	N(9)-C(11)	1.261	1.426			
30	C(2)-Br(10)	1.8927	1.881			
31	C(8)-N(9)	1.2659	1.26			
32	C(4)-C(8)	1.3563	1.503			
33	C(5)-O(7)	1.3866	1.355			
34	C(6)-C(1)	1.3413	1.42			
35	C(5)-C(6)	1.3466	1.42			
36	C(4)-C(5)	1.3607	1.42			
37	C(3)-C(4)	1.3448	1.42			
38	C(2)-C(3)	1.3419	1.42			
39	C(1)-C(2)	1.3411	1.42			

S.No.	Atoms	Actual Bond	Optimal Bond	S.No.	Atoms	Actual Bond	Optimal Bond
		angle	angle			angle	angle
1	N(21)-Cu(24)-N(9)	87.2347	_	34	C(18)-C(13)-C(12)	120.2911	120
2	N(21)-Cu(24)-O(19)	111.6941	-	35	C(14)-C(13)-C(12)	118.0118	120
3	N(21)-Cu(24)-O(7)	129.8319	-	36	O(19)-C(12)-C(17)	118.1536	124.3
4	N(9)-Cu(24)-O(19)	109.4067	-	37	O(19)-C(12)-C(13)	123.0725	124.3
5	N(9)-Cu(24)-O(7)	95.4914	-	38	C(17)-C(12)-C(13)	118.766	120
6	O(19)-Cu(24)-O(7)	114.3141	-	39	S(23)-C(11)-N(21)	135.1325	-
7	H(35)-C(22)-H(34)	106.0864	109	40	S(23)-C(11)-N(9)	132.1515	-
8	H(35)-C(22)-H(33)	105.5332	109	41	N(21)-C(11)-N(9)	92.5644	-
9	H(35)-C(22)-C(18)	113.599	110	42	Cu(24)-N(9)-C(11)	88.9702	-
10	H(34)-C(22)-H(33)	109.5792	109	43	Cu(24)-N(9)-C(8)	118.1359	-
11	H(34)-C(22)-C(18)	111.0094	110	44	C(11)-N(9)-C(8)	137.8374	-
12	H(33)-C(22)-C(18)	110.7707	110	45	H(28)-C(8)-N(9)	118.5605	116.5
13	Cu(24)-N(21)-C(11)	87.9334	-	46	H(28)-C(8)-C(4)	122.0536	120
14	Cu(24)-N(21)-C(18)	121.9168	-	47	N(9)-C(8)-C(4)	119.2559	123.5
15	C(11)-N(21)-C(18)	113.2461	-	48	Cu(24)-O(7)-C(5)	106.7752	-
16	H(32)-O(20)-C(16)	109.0702	108	49	H(27)-C(6)-C(1)	118.9172	120
17	Cu(24)-O(19)-C(12)	112.152	-	50	H(27)-C(6)-C(5)	118.8337	120
18	C(22)-C(18)-N(21)	111.1447	115.1	51	C(1)-C(6)-C(5)	122.2478	-
19	C(22)-C(18)-C(13)	120.7468	121.4	52	O(7)-C(5)-C(6)	119.6765	124.3
20	N(21)-C(18)-C(13)	128.0609	120	53	O(7)-C(5)-C(4)	123.2167	124.3
21	H(31)-C(17)-C(12)	118.3945	120	54	C(6)-C(5)-C(4)	117.096	120
22	H(31)-C(17)-C(16)	118.0039	120	55	C(8)-C(4)-C(5)	117.7586	120
23	C(12)-C(17)-C(16)	123.6005	-	56	C(8)-C(4)-C(3)	121.1527	120
24	O(20)-C(16)-C(17)	121.2838	124.3	57	C(5)-C(4)-C(3)	121.0817	120
25	O(20)-C(16)-C(15)	121.8646	14.3	58	H(26)-C(3)-C(4)	119.9311	120
26	C(17)-C(16)-C(15)	116.8476	120	59	H(26)-C(3)-C(2)	119.829	120
27	H(30)-C(15)-C(16)	119.2107	120	60	C(4)-C(3)-C(2)	120.2369	-
28	H(30)-C(15)-C(14)	119.9686	120	61	Br(10)-C(2)-C(3)	120.2095	118.1
29	C(16)-C(15)-C(14)	120.8206	-	62	Br(10)-C(2)-C(1)	120.1097	118.1
30	H(29)-C(14)-C(15)	115.9727	120	63	C(3)-C(2)-C(1)	119.6808	120
31	H(29)-C(14)-C(13)	122.0806	120	64	H(25)-C(1)-C(6)	119.268	120
32	C(15)-C(14)-C(13)	121.9454	-	65	H(25)-C(1)-C(2)	121.0879	120
33	C(18)-C(13)-C(14)	121.6872	120	66	C(6)-C(1)-C(2)	119.6407	-

Table 8: Various bond angles of [CuL¹] complex

Table 9: Antibacterial activity of Schiff base ligands and its metal complexes

		Diameter of infibition zone (infi)														
Samples	Staphylococcus aureus			reus	В	Bacillus subtilis			Klebsiella pneumonia			Escherichia coli				
Samples	10µl	20µl	30µl	40µl	10µl	20µl	30µl	40µl	10µl	20µl	30µl	40µl	10µl	20µl	30µl	40µl
L	6	8	9	12	5	7	8	11	6	7	10	13	4	7	10	11
L ²	5	7	9	11	4	5	8	10	5	9	10	12	4	6	10	11
[CuL ¹]	19	22	25	28	16	23	24	26	18	21	24	27	14	16	19	22
[CoL ¹]	17	21	23	26	15	20	21	25	18	20	23	25	16	19	22	24
[NiL ¹]	16	20	23	24	14	15	18	22	15	19	20	23	12	18	19	22
[ZnL ¹]	14	15	20	23	14	16	19	21	13	17	20	22	11	15	16	19
[CuL ²]	20	24	26	30	17	20	23	27	19	22	24	27	15	16	19	22
[CoL ²]	18	21	23	27	16	20	22	25	18	20	23	25	14	17	18	21
[NiL ²]	18	20	24	28	14	17	20	24	16	22	25	26	13	16	20	22
[ZnL ²]	13	15	18	20	10	13	14	17	13	16	18	19	9	12	15	17

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