Effective Biopotencial properties of novel Schiff base metal(II) complexes derived from (1-Methyl-4-nitro-benzene(2-imino-1,2-dihydro-indol-3ylidene)-(4-nitro-phenyl)-amine)

T. Sujeshwari¹, E. Akila² & P. Maheswaran³

¹ Research Scholar, Department of Chemistry, P.G.P college of Arts and science, Namakkal.

² Department of Chemistry, Sri Sarada College for women (Autonomous), Salem-16.

³ Principal and Head, Department of Chemistry, P.G.P college of Arts and science, Namakkal.

ABSTRACT:

Transition metal complexes have been used widely by many bioinorganic chemists for developing active transition metal anticancer complexes with better efficiency. The interaction of transition metal complexes with DNA has been a subject of passionate research in the field of bioinorganic chemistry. To synthesize a new Schiff base ligand (1-Methyl-4-nitro-benzene(2-imino-1,2-dihydro-indol-3-ylidene)-(4-nitro-phenyl)-

amine) by the condensation reaction between isatin and p-nitroaniline and Its Co(II), Ni(II), Cu(II) and Mn(II) complexes were prepared by the interaction of the Schiff base ligand with metal(II) chloride. They were characterized by elemental analysis, IR, ¹H NMR, EPR, UV–Vis, magnetic susceptibility measurements and conductivity measurements. DNA cleavage activity by the complexes was performed by gel electrophoresis method. The Schiff base and its complexes have been screened for their antibacterial (Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Klebsila Pneumonia.). All the synthesized complexes have distorted octahedral geometry and the coordination of ligand to metal complexes confirmed by IR spectral studies . In all the biological studies, metal complexes show better activity than the ligands.

Keywords: Schiff base, Metal (II) complexes, DNA cleavage, Antimicrobial activity

INTRODUCTION:

The Schiff base ligands are well known due to their extensive range of applications in industrial and pharmaceutical fields [1]. Some Schiff base ligands derived from istain derivatives and their copper(II) complexes have also been shown to inhibit growth of tumor. These studies have exposed that complexation of metals to Schiff base ligands enhance the biopotentials of the ligands. Many reports are obtainable for the preparation and properties of above metal complexes as they mimic metalloproteins [2]. In adding up, the reports of Schiff base and their metal complexes have emerged more in recent times.

In recent years, the binding studies of transition metal complexes have turn into an significant field in the growth of DNA molecular probes and chemotherapeutics. Metal complexes such as Cobalt, Nickel, Copper and Manganese with N-containing ligands have shown outstanding

cleavage activities [3]. There is a considerable literature supporting the function of artificial DNA cleaving agents in biotechnology; structural studies of nucleic acids or development of new drugs. Transition metal complexes can interact with DNA during covalent binding, electrostatic interactions, groove binding or intercalation. Compounds showing the properties of effectual binding as well as cleaving double stranded DNA under physiological conditions are of immense importance since these could be used as diagnostic agents in medicinal and genomic research.

Although Schiff bases containing a N donor atoms have efficient biological activity, the studies based on 1-Methyl-4-nitro-benzene(2-imino-1,2-dihydro-indol-3-ylidene)-(4-nitro-phenyl)-amine Schiff base chemistry are less extensive. Our laboratory has been presently involved in exploring this chemistry [4]. Bearing all the above facts in mind, and as a part of our progressing research, herein the synthesis of Schiff base ligand (1-Methyl-4-nitro-benzene(2-imino-1,2-dihydro-indol-3-ylidene)-(4-nitro-phenyl)-amine) and its Co(II), Ni(II), Cu(II) and Mn(II) complexes has been reported. In order to investigate the coordination mode of ligand, the complexes of Co(II), Ni(II), Cu(II) and Mn(II) have been characterized by UV–Vis, IR, and ¹H NMR studies. The antimicrobial and cleavage properties of the Schiff base ligand (1-Methyl-4-nitro-benzene(2-imino-1,2-dihydro-indol-3-ylidene)-(4-nitro-phenyl)-amine) and its complexes have also been investigated.

MATERIALS AND METHODS:

The chemicals used in this work viz., isatin, p-nitroaniline, 2, 2' bipyridyl, acetic acid and Tris–HCl buffer were of Merck grade. The AR grade of metal salts CoCl₂.6H₂O, NiCl₂.6H₂O, CuCl₂.2H₂O and MnCl₂ .5H₂O were used. Commercial solvents were distilled and then used for the preparation of ligand and its complexes

Carbon, hydrogen and nitrogen analyses of the complexes were carried out on a CHN analyzer. Carlo Erba Model 1108. The infrared spectra (4000–200 cm⁻¹ KBr disks) of the samples were recorded on a Perkin–Elmer FT-IR-8300 spectrophotometer. The electronic absorbance spectra in the 200–800 nm were recorded on Double beam spectrometer Cyber lab between 200 - 800 nm. ¹H NMR spectra of ligand were recorded on Bruker Spectrometer at 400 MHZ in DMSO. The chemical shifts are given in PPM, using Tetramethylsilane (TMS) as the internal reference. Magnetic susceptibility measurements were carried out by employ the Gouy method at room temperature on powder sample of the complexes using CuSO₄.5H₂O as calibrant. The metal contents of the complexes were determined according to the literature method [5].

DNA cleavage activity Gel electrophoresis

The DNA cleavage of supercoiled pUC18DNA promoted by metal complex was proceeded by addition of reaction mixture (40µl). The reaction mixture (40µl) containing pUC18DNA, 50mM Tris-HCl, pH 7.4, 50 mm NaCl, 10 mM H₂O₂ added in a different volume, followed by adding Millipore water for final volume. Then the mixed solutions were incubated at 37° C for 1 hr. They checked by agarose electrophoresis methods [6].

Antibacterial activity

The antibacterial screening effects of the Schiff base ligand and their corresponding Co(II), Ni(II), Cu(II) and Mn(II) complexes were tested against four bacterial strains namely Bacillus subtilis, Staphylococcus aureus, Klebsilla pneumonia and Escherichia coli by disc diffusion method, using nutrient agar medium. The compounds were dissolved in DMF. The plates

were incubated aerobically at 37 °C for 24 hours and the antimicrobial activity was assess by measuring the inhibition of microbial development around the well. This method involves the experience of the zone inhibition toward the diffusion of micro-organism on agar plate [7].

Synthesis of Schiff base ligand (1-Methyl-4-nitro-benzene(2-imino-1,2-dihydro-indol-3-ylidene)-(4-nitro-phenyl)-amine)

The Schiff base ligand was synthesized by adding Isatin (1 mM) in 20 ml of ethanol, p- nitroaniline (2 mM) in 20 ml of ethanol. The mixture was refluxed for 2-3 hrs. Then solution of the ligand was kept for slow evaporation and yellow colored precipitate was collected and dried in air [8].

Synthesis of Schiff base metal complexes

The ethanolic solution of synthesized ligand (1 mM) was added dropwise stirring to an ethanolic solution of the metal chloride (1 mM) (Metal = Cu(II), Co(II), Ni(II) and Mn(II)) with constant stirring and followed by an ethanolic solution of 2, 2' bipyridyl is added and the mixture was boiled under reflux for 3-5 hrs. Then, the volume of the reaction mixture was reduced by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried in vacuuo [9].



Fig.1. Synthesis of Schiff base ligand



M=Cu(II), Ni(II), Co(II) and Mn(II)

Fig.2. Synthesis of Mononuclear Schiff base Metal(II) complexes

RESULTS AND DISCUSSION

Physicochemical properties of the synthesized compounds

The ligand was prepared by refluxing an appropriate amount of isatin and p-nitroaniline in 1:2 molar ratio and the complexes were formed with the metal salts and ligand in the ratio of 1:1:1. All the complexes show 1:1:1 metal– ligand stoichiometry. They are non-hygroscopic, decomposed above 200 °C and possess good keeping qualities. The molar conductance values in DMF (Table 1) reveal the non-electrolytic nature of the complexes [10].

Molecular Formula	color	Yield %	Melti ng	% of Nitrogen		% of Metal		Molar conductance		
			Point (°)	Cal	Exp	Cal	Exp	$\begin{array}{ c c c } \Lambda_m & (ohm^{-1} \\ {}^{1}cm^{2}mol^{-1} \end{array}) \end{array}$		
C ₂₀ H ₁₃ N ₄ O ₄	Pale yellow	85	136	12.90	12.89	-	-	-		
Cu(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	Dark green	75	>200	10.55	10.51	9.58	9.52	6.6		
Ni(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	Dark green	75	>200	10.63	10.61	8.91	8.90	5.7		
Co(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	Dark pink	80	>200	10.62	10.61	8.94	8.92	7.6		
Mn(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	Dark brown	80	>200	10.69	10.65	8.39	8.38	8.2		

 Table 1. Analytical data of the Schiff base ligand and its mononuclear metal complexes

Where L_1 = Ligand 1, L_2 = 2, 2' bipyridyl and X = Chloride ion.

Electronic spectra and magnetic properties of metal(II) complexes

The electronic spectral analysis was recorded in the solution of DMF and its data are depicted in the Table 2. The reported ligand which exhibits absorption bands in UV–Vis region of 340 and 364 nm. The very first band which is shown below 340 nm is attributed to $\pi \rightarrow \pi^*$ transition. An additional band observed at 364 nm is responsible to $n \rightarrow \pi^*$ transition which is originating from amide or imine function of the reported Schiff base ligand [11].

The magnetic moment value (1.90 B.M.) for the prepared $Cu(C_{30}H_{21}N_7O_4Cl_2)$ and also the broad band in its electronic spectrum centered at 505 and 604 nm, which areassigned to ${}^{2}B_{1g} \rightarrow {}^{2}E_{1g}$ and ${}^{2}B_{1g} \rightarrow {}^{2}A_{1g}$ transitions suggested distorted octahedral geometry around the Cu(II) metal ion. Though, the band exactly observed at 365 nm was almost certainly due to a ligand-metal charge transfer transition.

The Ni(C₃₀H₂₁N₇O₄Cl₂) complex reported which shows high spin with a room temperature of the magnetic moment value 3.12 B.M. is the normal range examined for octahedral Ni(C₃₀H₂₁N₇O₄Cl₂) complex. This result clearly indicates that, the synthesized Nickel(II) complex is probably octahedral. The electronic spectrum of Ni(C₃₀H₂₁N₇O₄Cl₂) complex were displayed three bands at 446, 578 and 625 nm, attributed to ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(F)$, ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)$ and ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(P)$ d–d transitions, correspondingly. The resulting spectrum also showed a band at 365 nm, which is exactly assigned to ligand to the metal charge transfer transition.

The magnetic moment value is measured at the range of (3.60 B.M.) due to $Co(C_{30}H_{21}N_7O_4Cl_2)$ complex was lower than spin-only value (3.87 B.M.) and as well lower than the value which is reported for complex shows octahedral geometry. The UV-Vis spectrum of $Co(C_{30}H_{21}N_7O_4Cl_2)$ resultant three broad bands of 464, 595 and 625 nm which are contributed to ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F)$, ${}^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(F)$ and ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(P)$ transitions,

correspondingly, in around the Co(II) ion. A quite intense peak that is observed at 365 nm is because of ligand to metal charge transfer transition.

The electronic spectrum of Mn(C₃₀H₂₁N₇O₄Cl₂) complex depict the absorption bands at the range of 694, 555 and 522 nm. These three absorption bands may be assigned to the ${}^{6}A_{1}g \rightarrow {}^{4}A_{1}g$, ${}^{6}A_{1}g \rightarrow {}^{4}A_{2}g$, and ${}^{6}A_{1}g \rightarrow {}^{4}Eg$, ${}^{4}A_{1}g$ transitions, respectively. These three bands show that the Mn(C₃₀H₂₁N₇O₄Cl₂) complex possess an octahedral geometry. The magnetic moment value of Mn(C₃₀H₂₁N₇O₄Cl₂) complex is 5.92 B.M. at room temperature equivalent to five unpaired electrons suggest octahedral geometry. In the spectra of reported Schiff base ligand, the absorption band which are observed between 281-294 nm contributed to intra-ligand $\pi \rightarrow \pi^*$ transition and the band exactly at 342-390 nm were resultant due to $n \rightarrow \pi^*$ transition that are associated with the azomethine chromophore (-C=N).

Complex	λmax	Band	Geometry	Magnetic			
	(nm)	assignments		moment			
				(B.M.)			
	340	INCT		1.90			
	365	LMCT					
$Cu(C_{30}H_{21}N_7O_4CI_2)$	505	$^{2}B_{1g} \rightarrow ^{2}E_{1g}$					
	604	$^{2}B_{1g} \rightarrow ^{2}A_{1g}$					
	352	INCT		3.12			
	365	LMCT	Distorted				
$Ni(C_{30}H_{21}N_7O_4Cl_2)$	446	$^{3}A_{2g}(F)$	octahedral				
	578	\rightarrow ³ T ₂ g(F),					
	625	$^{3}A_{2g}(F) \rightarrow ^{3}T_{1}g(F)$					
		${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(P)$					
	355	INCT		3.60			
$Co(C_{30}H_{21}N_7O_4Cl_2)$	365	LMCT					
	464	${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F),$					
	595	${}^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(F)$					
	625	${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(P)$					
	290	INCT		5.92			
Mn(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	365	LMCT					
	522	${}^{6}A_{1}g \rightarrow {}^{4}A_{1}g$,					
	555	$^{6}A_{1}g \rightarrow ^{4}A_{2}g$,					
	694	$^{6}A_{1}g \rightarrow {}^{4}Eg, {}^{4}A_{1}g$					

 Table .2. Electronic Absorption Spectral Data of Metal(II) complexes at 300 k.

Molar conductivity

With a view to study the electrolytic nature of the mononuclear metal complexes, their molar conductivities were measured in DMF at 10^{-3} M. The molar conductivity values of all the complexes are in the range 5.7–8.2 ohm⁻¹ cm² mol⁻¹ (shown in Table 1), which is in agreement with the non-electrolytic nature of the complexes.

Infrared spectra of the ligand and its metal(II) complexes

The infrared spectra of the prepared metal (II) complexes were measured in KBr disk and significant IR-spectral feature next to their tentative assignments are clearly shown in Table. 3. The IR spectra of the derived complexes were compared with the ligand $C_{20}H_{13}N_4O_4$ to settle on the change that may have taken place throughout the complexation process. A comparative study of synthesized IR spectra of ligand and metal complexes which disclose some peaks are general in nature and then, only important peaks, also have recently appeared or shifted, are discussed. Lowering the value of (C=N) in the complexes when compared to the ligand (1620 cm⁻¹) because of reduction of double bond nature of carbonnitrogen bond of azomethine group. Metal-ligand bond is furthermore confirmed by presence of a medium intensity band which is present at the range 444-468, cm⁻¹ in the spectra of reported complexes which are attributed to stretching frequencies of (M-N) bond formation respectively [12]. The band which are present in the region 310-317 cm⁻¹ may be assigned due to v(M-Cl) Vibrations.

Compounds	(C=N) (cm ⁻¹)	(M-N) (cm ⁻¹)	(M-Cl) (cm ⁻¹)
$C_{20}H_{13}N_4O_4$	1620		
Cu(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	1605	460	315
Ni(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	1609	454	317
Co(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	1595	468	310
Mn(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	1600	444	312

 Table 3. Infrared Spectroscopic Data of the Schiff Base Ligand and its mononuclear metal complex

¹H NMR spectra

The ¹H NMR spectra of reported Schiff base $C_{20}H_{13}N_4O_4$ ligand was recorded in DMSO d₆ at particular room temperature as given in Figure 4.3.15. The prepared ligand was prepared by dissolution in DMSO d₆ and the chemical shifts of the ligand recorded with respect to standard TMS. Two various type of protons were identified i) resonance due to azomethine proton in the $C_{20}H_{13}N_4O_4$ ligand appears at 8.23 ppm and ii) the other signals in the region 6.40–7.90 ppm clearly exhibits aromatic protons .



Fig. 3. ¹H NMR spectra of C₂₀H₁₃N₄O₄ ligand

EPR spectra

The EPR spectral analysis of complexes provides the information of significance in studying the metal ion environment. The ESR spectrum of $Cu(C_{30}H_{21}N_7O_4Cl_2)$ complexes recorded on X-band at frequency range of 9.1 GHz at particular room temperature. The ESR spectra showed a single anisotropic intense sharp signal at the room temperature with no hyperfine splitting as shown in Figure 4.3.22. This report suggests that Cu(II) ions are in mononuclear environment.

The EPR spectrum of the $Cu(C_{30}H_{21}N_7O_4Cl_2)$ complexes show a broad signal band with g_{iso} at 2.0001 which is consistent with that of distorted octahedral geometry [13]



Fig. 4. EPR spectra of Cu(II) Schiff base complex

Chemical nuclease activity

There has been substantial attention in DNA cleavage reactions that are initiated by transition metal complexes. The release of metal ion to the helix, in nearby generating oxygen or hydroxide radicals, yields a proficient DNA cleavage reaction. In order to consider the chemical nuclease activities of the Co(II), Ni(II), Cu(II) and Mn(II) complexes for DNA strand scission, pUC18DNA was incubated with all the above metal(II) complexes under the reaction conditions. The cleavage reaction can be monitored by gel electrophoresis. When circular pUC18DNA is subjected to electrophoresis, comparatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand (nicking), the supercoiled form will relax to produce a slower-moving nicked form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated.

The chemical nuclease activities of the four complexes have been studied using supercoiled pUC18DNA plasmid DNA as a substrate in a medium of 50 mM Tris–HCl/ NaCl buffer (pH = 7.2) in the presence of hydrogen peroxide under physiological conditions. There was no DNA-cleavage observed for the controls in which either in the absence of hydrogen peroxide with DNA or in the presence of hydrogen peroxide with DNA (Lanes 1 and 2). In Fig. 4, all the complexes were found to exhibit nuclease activity in the presence of hydrogen peroxide as reducing agent (Lanes 3–6). The dissimilar DNA-cleavage efficiency of the complexes may be due to the diverse binding affinity of the complexes to DNA. The nuclease effectiveness of the complexes is known to depend on the reducing agent used for initiating the DNA cleavage [14].



Fig. 5. Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H_2O_2 and metal complexes. Lane 1-DNA alone; Lane 2- DNA alone + H_2O_2 ; Lane 3-DNA + Cu complex + H_2O_2 ; Lane 4-DNA + Ni complex + H_2O_2 ; Lane5-DNA + Co complex Lane 6-DNA+ Mn complex H_2O_2 .

Antibacterial activity

Biological activity of the ligand and a series of its metal complexes [Cu(II), Ni(II), Co(II) and Mn(II)] were screened for anti-bacterial activity against *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis and Klebsilla pneumonia* by using disc-agar diffusion method (Figure 6). The results indicate that the complexes show more activity and the ligand 1919

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have less activity against same microorganisms under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favours its permeation through the lipid layer of the cell membrane. The copper(II) complex shows higher anti-fungal activity than other complexes. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells [15]

Table 4. Antibacterial activity for Schiff base ligands and its mononuclear metal complexes

	Zone of inhibition (mm)															
	Gram positive bacteria						Gram negative bacteria									
Compounds Stap auro			Staphylococcus xureus			Bacillus subtilis			Escherichia coli				Klebsiella pneumoniae			
	Concentration (µg/mL)															
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
$C_{20}H_{13}N_4O_4$	7	7	8	9	7	7	9	9	6	6	7	8	7	8	8	9
Cu(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	10	11	12	14	10	9	12	13	9	9	10	11	10	12	15	17
Ni(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	9	8	10	12	9	8	11	12	6	7	7	9	9	8	9	10
Co(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	10	9	12	13	10	11	12	14	8	9	9	11	9	10	11	12
Mn(C ₃₀ H ₂₁ N ₇ O ₄ Cl ₂)	8	9	9	10	8	9	9	10	7	8	9	9	7	8	9	10
Streptomycin	16	17	20	22	14	15	17	20	12	13	16	20	13	14	16	18



Fig. 6. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against Bacterial Strains

DPPH Radical Scavenging Activity

The antioxidant activity of the ligand $C_{20}H_{13}N_4O_4$ and reported complexes have attracted much more increasing interests and been considerably investigated. Figure 7. gives the plots of DPPH[•] free radical scavenging activity for the $C_{20}H_{13}N_4O_4$ and its complexes. It is very understandable that the scavenging activity increases with increasing the sample concentration in the range which is tested. As shown in Figure, the free ligand $C_{20}H_{13}N_4O_4$ has less scavenging activity compared to the complexes. As shown in Figure $Cu(C_{30}H_{21}N_7O_4Cl_2)$ complex possess high proficient activity in quenching DPPH• radical than the free ligand $C_{20}H_{13}N_4O_4$ consistence with previous to reported data for other elements. When the $C_{20}H_{13}N_4O_4$ interacts with the positively charged metal ions, and electron density which is drawn from the oxygen. Cu(II) complex which is displayed the highest scavenging activities than the other reported complexes might be due to the ionic size effect. This clearly indicates that the DPPH[•] scavenging activity of metal complexes depends on the central ion.



Figure 7. DPPH Scavenging Activity of C₂₀H₁₃N₄O₄ and Its Complexes.



Figure 8. DPPH Scavenging Capacities (IC₅₀) of Metal Complexes

CONCLUSION:

The present paper reports on the synthesis, spectral characterization and their electronic absorption spectra of Schiff base ligand and its metal complexes. The synthetic procedure in this work resulted in the formation of complexes in the molar ratio (1:1:1) (M:L₁:L₂), respectively. In these complexes, the bidentate Schiff base ligand is coordinated to metal ion through the azomethine-nitrogen atoms. The present study reveals that distorted octahedral geometry around Co(II), Ni(II), Cu(II) and Mn(II) complexes The conductance value show that all the metal complexes are non-electrolytes in nature. Noticeably, metal complexes have been found to promote cleavage of plasmid pUC18DNA from the supercoiled Form I to the open circular Form II or the linear Form III in the presence of H₂O₂. These findings clearly point out that transition metal based complexes have many possible practical applications, like the growth of nucleic acid molecular probes and new therapeutic reagents for diseases. The results of antimicrobial activity show that the metal complexes exhibit antimicrobial properties and it is significant to note that they show enhanced inhibitory activity compared to the parent ligand under identical experimental conditions. But the ligand and metal complexes have low activity as compared to standard drugs.

DECLARATIONS:

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Conflict of interests

The authors declare that they have no conflict of interest

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