Original Article

Biologically Active Metal Complexes Containing Thiazole core: Synthesis and Spectral Characterization

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ABSTRACT

The present study deals with the synthesis of new ligand *N*-(4-phenylthiazol-2-yl)-2-(pyridin-2-yl-methylene) hydrazinecarboxamide and it's Cu (II), Co (II), Ni (II) and Zn (II) complexes and study their antimicrobial activity. The newly prepared compounds are characterized by elemental analysis, FT-IR, ^{1}H NMR, mass, UV-visible and ESR spectral techniques. The elemental analysis data and spectral study indicates octahedral geometry for Cu (II), Co (II) and Ni (II) complexes and tetrahedral geometry for Zn (II) complex. All the compounds were tested for antimicrobial activity, the Cu (II) and Co (II) complexes showed good antimicrobial activity.

Key words: Schiff base, Thiazole, Pyridine-2-carboxaldehyde, Antimicrobial.

INTRODUCTION

Thiazoles are an important group of heterocyclic compounds possessing various applications in medicine. Thiazole moiety is found in thiamine (vitamin B_1). Also, some important drugs such as penicillin, sulfathiazole, ritonavir, abafungin, bleomycineand tiazofurin have thiazole moiety as their integral part. Thiazoles have attracted considerable attention due to their wide range of biological activities such as anti-inflammatoryactivity, antihypertensive activity, anti-allergic activity.[1,2] antibacterial activity, anti-schizophrenia activity, anti-HIV activity.[3] analgesic activity and anti-viral.[4] (Figure 1).

The activity of the compounds is strongly dependent upon the nature of the hetero atomic ring and the position of attachment to the ring.[5] These are studied extensively due to their flexibility, their selectivity and sensitivity towards the central metal atom, structural and similarities with natural biological substances, due to the presence of imine group (−N=CH−) which imparts the enhanced biological activity.[6]

Thus, this available literature encouraged us to synthesize the biologically active metal (II) complexes with new Schiff base ligand **(L)** derived from the reaction of pyridine-2-carboxaldehyde with *N*-(4-phenylthiazol-2-yl) hydrazinecarboxamide containing carbonyl (C=O) and azomethine (C=N) group as a potential chelating sites.

MATERIALS AND METHODS

Materials and instruments

The precursor *N*-(4-phenylthiazol-2-yl) hydrazinecarboxamide are prepared as per literature methods.[7,8] All the reagents, biological cultures and solvents were obtained from commercial suppliers Fluka, Merck and Sigma and were used without further purification. All reactions were monitored by TLC performed on silica gel plates (60F254 Merck).

IR spectra were recorded as KBr pellets on a Perkin Elmer-Spectrum RX-I FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a 400 MHz Bruker spectrometer

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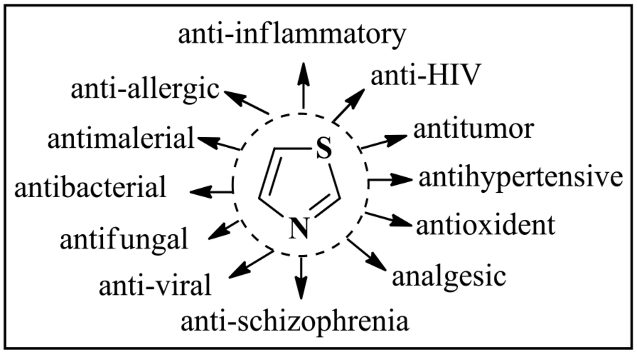


Figure 1: Biological activities of thiazole

and chemical shifts are expressed relative to TMS.ESI-MS were recorded on a mass spectrometer equipped with electro spray ionization source having mass range of 4000 amu in quadruple and 20,000 amu in Tof. The UV-visible spectra were recorded on an ELICO SL-164 double beam UV visible spectrophotometer. C, H and N analysis was performed on a Vario EL III CHN analyser. ESR spectrum of the Cu (II) complex in the polycrystalline state was recorded on a BRUKER BioSpin Gmbh spectrometer. Molar conductivity measurements were recorded on ELICO (CM-180) conductivity bridge in dry DMF ca. 10^{-3}M solution and magnetic susceptibility measurement were made at room temperature on a Gouy balance using $Hg[Co(NCS)_4]$ as the calibrant.

Synthesis of ligand (L)

An equimolar mixture of *N*-(4-phenylthiazol-2-yl) hydrazinecarboxamide and pyridine-2-carboxaldehyde in ethanol (15 mL) was refluxed with a catalytic amount of glacial acetic acid (1-2 drops) for about 3-4 h on a water bath. The pale yellow coloured product **(L)** which separated in hot was filtered off, washed with hot ethanol, dried and recrystallized from 1, 4-dioxane. The pathway for the synthesis of ligand **(L)** is presented in **Scheme 1.**

Preparation of metal complexes

To the 20 mL of hot ethanolic solution of ligand *N*-(4-phenylthiazol-2-yl)-2-(pyridin-2-yl-methylene) hydrazinecarboxamide (0.001 mol) add 10 mL of respective metal chlorides (0.002 mol). The reaction mixture was then refluxed on a water bath for about 5-6 h. The reaction mixture was cooled to room temperature and poured in to distilled water. The colored solids separated were collected by filtration, washed with distilled water, then with hot ethanol and finally dried in a vacuum over anhydrous calcium chloride in a desiccators.

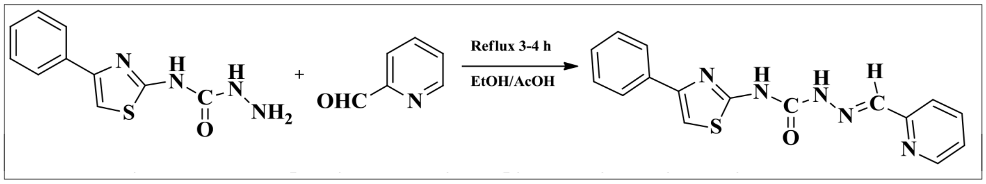
Antimicrobial activity

The antimicrobial activity of **L** and its metal (II) complexes have been studied for their antibacterial and antifungal activities using Muller-Hinton agar and potato dextrose agar (PDA) by disc and well diffusion methods respectively. The activities were carried out in four different concentrations (100, 50, 25 and 12.5 µg mL^{-1} in DMSO solvent). The antibacterial activity was tested against two bacteria's, *E. coli* (MTCC 46) and *S. aureus* (MTCC 3160) and antifungal activity against two fungi, *A. flavus* (MTCC 1883) and *A. niger* (MTCC 1881) by MIC method.[9] The activity was carried out in accordance with the international recommendation given by the CLSI. The minimum concentration of test compound with no visible growth of bacteria/fungi is reported as MIC for their respective strains. Blank tests have shown that DMSO in the preparation of the test solution does not affect the test organisms. The obtained results were compared under similar conditions using Gentamycin and Fluconazole, a broad-spectrum antibiotic for bacterial and fungal strains respectively.

RESULTS AND DISCUSSIONS

Chemistry

The newly synthesized metal complexes are colored solids, stable at room temperature and possess high melting point (> 290 °C). The metal complexes are insoluble in water and common organic solvents but completely soluble in DMF and DMSO. Elemental analysis and analytical data agree well with the proposed composition of Schiff base ligand **(L)** and its metal complexes (Table 1). These data of metal complexes suggest that the metal to ligand ratio of the complexes is 1:2 stoichiometry of the type $[M(L)_2]$ for Cu(II), Co(II) and Ni(II) complexes and [(M)(L)(Cl)] for Zn(II) complex. The measured molar conductance values of the metal complexes were too low to account for any dissociation of



Scheme 1: Synthesis of N-(4-phenylthiazol-2-yl)-2-(pyridin-2-ylmethylene) hydrazinecarboxamide (L)

Table 1: Physical and analytical data of ligand (L) and its metal complexes

Compounds	M.W.	M.P. (°C)	Color (Yield, %)	Elemental Analysis, found (Calc.) [%]					λ_m ($cm^2\,\Omega^{-1}$ mol^{-1})
				C	H	N	M	Cl	
$C_{16}H_{13}N_5OS$ **(L)**	323	248	Yellow (74)	59.44 (59.48)	4.02 (4.06)	21.67 (21.62)	--	--	--
$[Cu(C_{32}H_{26}N_{10}O_2S_2]$ $[Cu(L)_2]$	709.5	297	Green (62)	54.11 (54.07)	3.66 (3.59)	19.73 (19.77)	8.95 (8.89)	--	29
$[Co(C_{32}H_{26}N_{10}O_2S_2]$ $[Co(L)_2]$	704.9	294	Brown (53)	54.47 (54.51)	3.68 (3.66)	19.86 (19.90)	8.35 (8.40)	--	20
$[Ni(C_{32}H_{26}N_{10}O_2S_2]$ $[Cu(L)_2]$	704.6	294	Brown (51)	54.49 (54.55)	3.68 (3.67)	19.86 (19.92)	8.32 (8.28)	--	17
$[Zn(C_{16}H_{13}N_5OS)(Cl)]$ $[Zn(L)(Cl)]$	423.9	295	Orange (75)	42.46 (42.39)	2.83 (2.86)	16.51 (16.57)	15.42 (15.38)	8.25 (8.21)	30

Table 2: IR spectral data of Schiff base ligand (L) and its metal complexes.

Compounds	Amide $\nu_{(NH)}$	NH $\nu_{(NH)}$ thiazole	$\nu_{(C=O)}$	$\nu_{(C=N)}$	$\nu_{(M-O)}$	$\nu_{(M-N)}$	$\nu_{(M-Cl)}$
L	3312	3057	1684	1586	--	--	--
$[Cu(L)_2]$	3307	3053	1659	1574	595	468	--
$[Co(L)_2]$	3334	3108	1650	1541	541	421	--
$[Ni(L)_2]$	3290	3109	1632	1506	557	459	--
$[Zn(L)(Cl)]$	3336	3033	1616	1578	588	451	330

the complexes in DMF (17-30 ohm^{-1} cm^2 $mole^{-1}$) indicating their non-electrolytic behaviour.[10]

IR spectral studies

In the IR spectrum of the Schiff base ligand **(L),** Figure 2 shows the absorption band due to amide NH and NH attached to the thiazole moiety at 3312 cm^{-1} and 3057 cm^{-1} respectively. A high intensity band observed at 1684 cm^{-1} is due to carbonyl function ν(C=O). Absorption band due to azomethine function ν(C=N) has appeared at 1586 cm^{-1}(Table 2).

In the IR spectra of the metal complexes Figure 3, medium intensity weak bands at 3290 cm^{-1} – 3336 cm^{-1} and 3033 cm^{-1} – 3109 cm^{-1} were due to amide NH and NH attached to thiazole moiety respectively, which appeared almost at the same region as in the case of ligand, thus confirming their non-involvement in coordination. The shift of amide carbonyl ν(C=O) to lower frequency side about 25 cm^{-1} - 68 cm^{-1} which appeared in the region 1659 cm^{-1} - 1616 cm^{-1} in all the metal complexes confirms the coordination of oxygen atom of amide ν (C=O) with the metal ions as such without undergoing enolization.[11] The absorption frequency of azomethine ν(C=N) function shifted to lower frequency side about 06 cm^{-1} - 80 cm^{-1} and appeared in the region 1578 cm^{-1} - 1506 cm^{-1} sug-

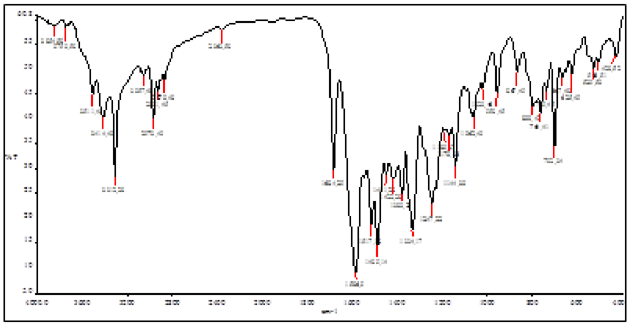


Figure 2: IR spectrum of Schiff base ligand (L).

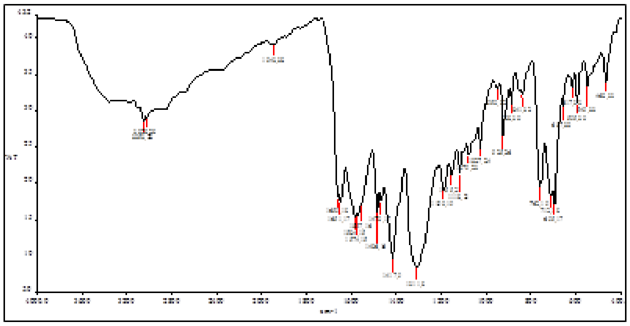


Figure 3: IR spectrum of $[Cu(L)_2]$ complex.

Table 3: ^{1}H NMR data of ligand (L).

Compound	^{1}H NMR data (ppm)
Ligand (L)	11.2 (s, 1H, CONH),10.5 (s, 1H, NH of thiazole),8.3 (s, 1H, HC=N), 7.1 - 7.9 (m, 11H, ArH)

gesting the involvement of nitrogen atom of azomethine function in complexation.[12]

The complexation of metal ions with ligand was further confirmed by the appearance of new weak intensity, non-ligand bands in the region 541 cm^{-1} - 595 cm^{-1} and 421 cm^{-1} – 468 cm^{-1} in the spectra of all the complexes which are assigned to frequencies of ν(M-O) and ν(M-N) stretching vibrations respectively. The appearance of new band at 330 cm^{-1} in Zn (II) complex is due to ν(M-Cl) band.[13]

H NMR spectral studies

The ^{1}H NMR spectrum (Figure 4) of Schiff base ligand **(L)** was recorded in *d6*-DMSO and data is presented in Table 3. The ^{1}H NMR spectrum of ligand **(L)** displayed two singlets each at 11.2 and 10.5 ppm are due to the proton of amide NH and NH attached to thiazole moiety respectively. The signal due to azomethine proton resonated at 8.3 ppm (s, 1H, CH=N). The signals due to eleven aromatic protons (m, 11H, ArH) have resonated as multiplets in the region 7.1 - 7.9 ppm.

ESI-mass spectral studies

The ESI mass spectrum of ligand **(L)** showed a molecular ion peak recorded at m/z 323 (5 %) which is equivalent to its molecular weight. Similarly, the ESI mass spectrum of Cu (II) complex showed a molecular ion peak recorded at m/z 709 (7%) which is equivalent to its molecular weight, thus confirming the structure of ligand and its Cu (II) complex respectively.

Electronic spectral studies

The green coloured Cu (II) complex displayed low intensity single broad asymmetric band in the region 15562-17742 cm^{-1}. The broadness of the band indicates the three transitions $^2B_{1g} \rightarrow {}^2A_{1g}$ (ν_1), $^2B_{1g} \rightarrow {}^2B_{2g}$ (ν_2) and $^2B_{1g} \rightarrow {}^2E_g$($\nu_3$), which are similar in energy and give rise to only one broad absorption band and the broadness of the band may be due to dynamic Jahn-Teller distortion. All these data suggested a distorted octahedral geometry around the Cu (II) ion.[14]

The electronic spectra of brown coloured Co (II) complex displayed two absorption bands at 16672 and 19726 cm^{-1}. These bands are assigned to be $^4T_{1g}$ (F) $\rightarrow {}^4A_{2g}$ (F) (ν_2) and $^4T_{1g}$ (F) $\rightarrow {}^4T_{2g}$ (P) (ν_3) transitions respectively. These data are in good agreement with the reported values.[15] The lowest band, ν_1 could not be observed due to the limited range of the instrument used, but it could be calculated using the band fitting procedure suggested by Underhill and Billing.[16] The above transitions suggest the octahedral geometry of the Co (II) complex.

The brown colored Ni(II) complex under present investigation exhibited two absorption bands in the region 15967 cm^{-1} and 25962 cm^{-1} which are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (ν_2) and $^3A_{2g}$ (F) $\rightarrow {}^3T_{1g}$ (P) (ν_3) transitions respectively in an octahedral environment. The band ν_1, was calculated by using a band fitting procedure.[17] The octahedral geometry of Cu(II), Co(II) and Ni(II) complexes was further supported by the calculated values of ligand field parameters, such as Racah inter electronic repulsion parameter (B'), nephelauxetic parameter (β), ligand field splitting energy (10 Dq) and ligand field stabilization energy (LFSE).[18]

The calculated B' values for the Co (II) and Ni (II) complexes are lower than the free ion values, which is due to the orbital overlap. The β values are important in determining the covalency for the metal-ligand bond and they were found to be less than unity suggesting a considerable amount of covalency for the metal-ligand bonds. The β value for the Ni (II) complexes was less than that of the Co (II) complex indicating the greater covalency of the M-L bond. The data were given in Table 4.

ESR spectral studies

The ESR spectrum of Cu (II) complex provides information about the environment of the metal ion within

Table 4: Electronic spectral data and ligand field parameters.

Complexes	Transitions in cm^{-1}			Dq (cm^{-1})	B′ (cm^{-1})	β	β%	ν_2/ν_1	LFSE (k cal.)
	ν_1^*	ν_2	ν_3						
[Cu(L)$_2$]	15562 – 17742			–	–	–	–	–	28.54
[Co(L)$_2$]	7782	16672	19726	889	870	0.895	10.40	2.53	15.24
[Ni(L)$_2$]	9970	15967	25962	997	800	0.769	23.07	1.60	34.18

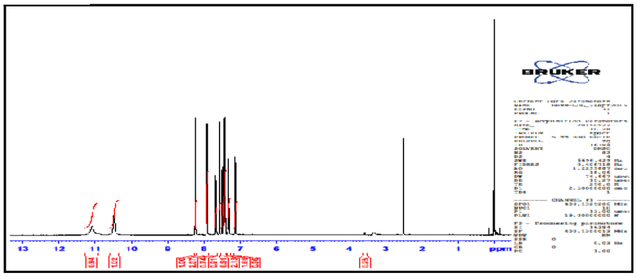
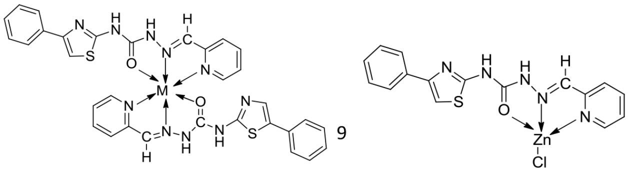


Figure 4: ^{1}H NMR spectrum of ligand (L).

the complex, i.e., the geometry and nature of the ligating sites of the Schiff base and metal. The X-band ESR spectrum of Cu(II) complex has been recorded in polycrystalline state at room temperature at a frequency of 9.1 GHz with a field set of 3000 G.

The spin Hamiltonian parameters for the Cu (II) complex is used to derive the ground state. In octahedral geometry with the g-tensor parameter $g_{\perp} > g_{\parallel} > 2.0023$, the unpaired electron lies in the d_{z^2} orbital and $g_{\parallel} > g_{\perp} > 2.0023$, the unpaired electron lies in the $d_{x^2-y^2}$ orbital in the ground state.[18] In the present case the observed measurements for Cu (II) complex is $g_{\parallel}$ (2.126) > $g_{\perp}$ (2.021) > 2.0023 indicate that the complex is axially symmetric and copper site has a $d_{x^2-y^2}$ ground state characteristic of octahedral geometry.[19] The $g_{\parallel}$ value is an important function for indicating the metal-ligand bond character, for covalent character $g_{\parallel} < 2.3$ and for ionic character $g_{\parallel} > 2.3$ respectively.[20] In the present study the $g_{\parallel}$ value of Cu (II) complex is less than 2.3, indicating an appreciable covalent character for the metal–ligand bond. The geometric parameter (G) is the measure of extent of exchange interactions and is calculated by using g-tensor values by the expression $G = g_{\parallel} - 2.0023/g_{\perp} - 2.0023$. According to Hathaway and Billing.[21] if the G value is greater than 4, the exchange interaction between the copper centres is negligible, whereas if its value is less than 4, the exchange interaction is noticed.



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

Figure 5: Proposed structures of metal (II) complexes.

Antibacterial and antifungal activity

The MIC values of the tested compounds and standards against the respective strains are summarized in Table 5. It is important to note that in most of the cases, metal complexes exhibited a more inhibitory effect than the **L**. This activity was found to be enhanced on coordination of the ONN donor atoms of the **L** with metal ions. This enhancement in the activity of the complexes over the ligand can be explained on the basis of chelation theory.[22] It is also known that chelation enhances the ligand to act as more powerful bactericidal/fungicidal agents by inhibiting the growth of bacteria/fungi, thus zone of inhibition of metal complexes was found to be higher compared to the ligand. The enhancement in the antimicrobial activity may be rationalized on the basis that ligands mainly possess azomethine (C=N) bond. More over in metal complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (N and O) of the ligand and there may be π-electron delocalization over the whole chelating system.[23] as a result the lipophilic character of the metal chelates was increased and favour their permeation through the lipoid layer of the bacterial cell membranes and blocking of the metal binding sites in the enzymes of microorganisms. In general, metal complexes are more active than the ligands because metal complexes may serve as a vehicle for activation of ligands as the principal cytotoxic species.[24]

Table 5: Antibacterial and antifungal activity results

Ligand/ Complexes	Bacteria		Fungi	
	E. coli	*S. aureus*	*A. Flavus*	*A. niger*
L	75	50	75	50
[Cu(L)$_2$]	25	25	50	25
[Co(L)$_2$]	25	25	25	50
[Ni(L)$_2$]	25	50	50	25
[Zn(L)(Cl)]	50	25	50	50
Gentamicin	12.50	12.50	12.50	12.50
Fluconazole	12.50	12.50	12.50	12.50

CONCLUSION

A series of Cu (II), Co (II), Ni (II) and Zn (II) complexes were prepared with tridentate ONN donor novel Schiff base ligand **(L)** derived from N-(4-phenylthiazol-2-yl) hydrazinecarboxamide and pyridine-2-carboxaldehyde and characterized by various physicochemical techniques. The spectral data demonstrate that Cu (II), Co (II) and Ni (II) complexes have an octahedral geometry and Zn (II) complex has a tetrahedral geometry. Based on these evidence, the following structures were proposed for the complexes (Figure 5). The non-electrolytic nature of the complexes was confirmed on the basis of their molar conductance values. The antimicrobial activity of all the newly prepared metal complexes was found to be enhanced on complexation. Hence, from all these extensive observations, it was concluded that the Schiff base ligand **(L)** and its newly synthesized metal complexes give the remarkable, versatile and valuable information of coordination compounds.

ABBREVIATIONS USED

FT-IR: Fourier Trans- form Infrared; **^{1}H NMR:** Proton Nuclear Magnetic Resonance; **ESI mass:** Electrospray Ionization Mass Spectrometry; **TLC:** Thin-layer chromatography; **DMSO:** Dimethyl sulfoxide; **TMS:** Tetramethylsilane; **DMF:** Dimethylformamide; **MTCC:** Microbial Type Culture Collection and Gene Bank; **PDA:** Potato dextrose agar; **EDTA:** Ethylene diamine tetraacetic acid; **MIC:** Minimum inhibitory concentration.

CONFLICT OF INTEREST

The authors declare that there are no competing interests regarding the publication of this paper.

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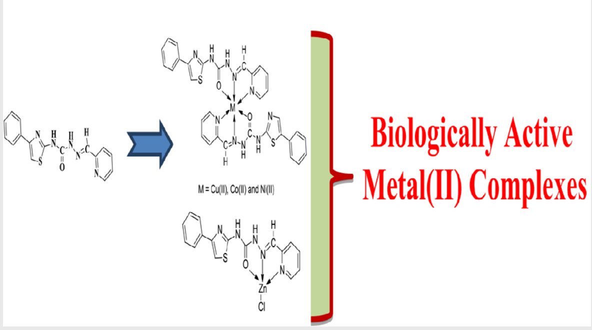
PICTORIAL ABSTRACT



SUMMARY

The newly prepared tridentate ONN donor novel Schiff base ligandN-(4-phenylthiazol-2-yl)-2-(pyridin-2-yl-methylene) hydrazinecarboxamide was derived from the condensation of N-(4-phenylthiazol-2-yl) hydrazinecarboxamide and pyridine-2-carboxaldehyde. The Cu (II), Co (II), Ni (II) and Zn (II) complexes of a ligand L are characterized by various physicochemical techniques. The spectral data demonstrate that Cu (II), Co (II) and Ni (II) complexes have an octahedral geometry and Zn (II) complex has a tetrahedral geometry. The antimicrobial activity of newly prepared metal complexes was found to be enhanced on complexation.

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