



Mononuclear Metal (II) Schiff Base Complexes Derived from Thiazole and *O*-Vanillin Moieties: Synthesis, Characterization, Thermal Behaviour and Biological Evaluation

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ABSTRACT

The novel Schiff base ligand 2-(2-hydroxy-3-methoxybenzylidene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide (**L**) obtained by the condensation of *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide with *o*-vanillin and its Cu(II), Co(II), Ni(II) and Zn(II) complexes were synthesized and characterized by elemental analysis and various physico-chemical techniques like, FT-IR, ¹H NMR, ESI mass, UV-Visible, ESR, TGA/DTA, magnetic measurements and molar conductance. The spectral results confirmed tridentate ONO donor binding of the ligand involving oxygen atom of amide carbonyl, azomethine nitrogen and oxygen of hydroxyl *via* deprotonation. Spectral analysis indicates octahedral geometry for all the complexes. Co(II) complex has 1:1 stoichiometry ratio of the type [M(L)(H₂O)₂Cl], whereas Cu(II), Ni(II) and Zn(II) complexes have 1:2 stoichiometric ratio of the type [M(L)₂]. In order to evaluate the effect of antimicrobial activity of metal ions upon chelation, all the newly synthesized compounds were screened for their antibacterial and antifungal activities by minimum inhibitory concentration (MIC) method. The DNA cleavage activities were also studied using plasmid DNA pBR322 as a target molecule.

Keywords: Thiazole; Schiff base; *o*-Vanillin, Antimicrobial; DNA-cleavage; Powder XRD

INTRODUCTION

Biologically active transition metal complexes have attracted a great deal of interest, largely due to their ability to interact with DNA molecule.¹ There has been significant interest in complexes that can bind or cleave DNA molecule at specific sites, because they play an important role in genomic investigation and in photodynamic therapy against cancer.² It is well known that some coordination compounds can inhibit the growth of cancer cells by binding to and damaging DNA.³

Thiazoles are heterocyclic organic compounds which have a five membered ring containing three carbons, one sulphur and one nitrogen atom. Many thiazole derivatives such as sulfathiazole, ritonavir, abafungin, bleomycine and tiazofurin are well known potent biologically active compounds.⁴ Also, thiazoles are the starting materials for several compounds including biocides, fungicides, sulphur drugs, dyes and chemical reaction accelerators which exhibit several biological activities such as anti-hypertensive, anti-inflammatory, anti-microbial, anti-HIV, antitumor and cytotoxic activity that can be well illustrated by the large number of drugs in the market containing this moiety.⁵ *o*-Vanillin is also the most prominent principal flavour and aroma compound in vanilla which is used as a food flavouring agent in foods, beverages and pharmaceuticals.⁶ Because of its numerous biological activities such as anti-inflammatory, analgesic, antiviral activities, it is extensively studied in medicinal field.⁷⁻¹⁰ In addition, it also can be used as efficient herbicide, pesticide bactericides¹¹ and Schiff bases containing *o*-vanillin moiety form stable complexes with various metal ions.^{12,13} Hence, *o*-Vanillin is an optimal

candidate for synthesizing various aromatic Schiff bases with significant bioactivities.

We have recently reported the synthesis, characterization, thermal study and biological evaluation of some metal (II) complexes derived from Schiff bases containing benzo[*b*]thiophene, thiazole and quinoline moieties.¹⁴⁻¹⁶ In continuation of our earlier work on metal complexes, we have synthesized the novel Schiff base ligand 2-(2-hydroxy-3-methoxybenzylidene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide (**L**) containing carbonyl, azomethines and hydroxyl groups as potential chelating sites and explored its ligational behaviour by preparing its Cu(II), Co(II), Ni(II) and Zn(II) complexes and studying their antimicrobial and DNA cleavage activities.

MATERIALS AND METHODS

Analysis and Physical Measurement

Microanalysis (C, H and N) were performed on a Vario EL III CHNS analyser. FT-IR spectral data were recorded on a Perkin Elmer Spectrum RX-I FTIR spectrophotometer. ¹H NMR spectra were recorded on the FT-NMR spectrometer model Bruker Avance II, 400 MHz using *d*₆-DMSO as solvent. ESI-MS were recorded on a mass spectrometer equipped with electrospray ionization (ESI) source having mass range of 4000 amu in quadruple and 20,000 amu in ToF. The UV-Visible spectra were recorded on a ELICO SL-164 double beam UV-Visible spectrophotometer. Electron spin resonance (ESR) measurements of solid [Cu(L)₂] complex was recorded at room temperature on BRUKER Bio Spin spectrometer at a microwave frequency 8.75-9.65 GHz. Thermal measurements (TGA/DTA) were carried out on Perkin Elmer Thermal Analyzer in nitrogen

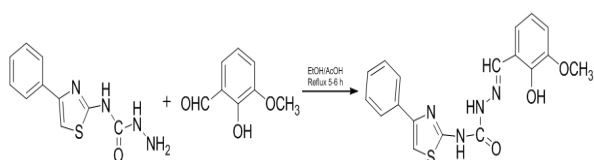


atmosphere with a heating rate of $20\text{ }^{\circ}\text{C min}^{-1}$. Powder X-ray diffraction (XRD) spectra of the metal complexes were recorded on Bruker AXS D8 Advance diffractometer (Cu, Wavelength 1.5406 \AA source).

All the reagents used for the synthesis of Schiff base ligand (**L**) were obtained from Sigma Aldrich chemical company, India. Metal salts were purchased from Loba Chemie. The metal and chloride contents of the complexes were determined as per standard procedures.¹⁷ The precursor *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide was prepared by the literature method.¹⁸

Synthesis of Schiff base Ligand (**L**)

An equimolar mixture of *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide (0.001 mol) and *o*-vanillin (0.001 mol) in ethanol (25 mL) was refluxed with a catalytic amount of glacial acetic acid (1-2 drops) for about 5-6 h on a water bath. The pale yellow coloured product which separated in hot was filtered off, washed with hot ethanol, dried and recrystallized from 1, 4-dioxane (**Scheme 1**).



Scheme 1: Synthesis of Schiff base ligand (**L**)

Synthesis of Metal (II) Complexes

To the hot solution of Schiff base ligand (**L**) (0.001 mol) in ethanol (20 mL) was added a hot ethanolic solution (20 mL) of respective metal chlorides (0.001 mol). The reaction mixture was then refluxed on a water bath for about 6-7 h, the pH of the reaction mixture adjusted ca.7.0-7.5 by adding sodium acetate (0.5 g) and refluxing continued for about an hour more. The reaction mixture was cooled to room temperature and poured into 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 desiccator.

Biological Evaluation

Antimicrobial Assay

The newly synthesized compounds were screened for their antibacterial and antifungal activities by the Muller-Hinton agar and potato dextrose media respectively by agar well dilution method. The *in vitro* antibacterial activity of the test compounds was tested against two Gram-positive [*Staphylococcus aureus* and *Bacillus subtilis*] bacteria and two Gram-negative [*Escherichia coli* and *Salmonella typhi*] bacteria. The *in vitro* antifungal activity was carried out against *Candida albicans*, *Cladosporiumoxysporum*, *AspergillusFlavus* and *Aspergillusniger* fungi. The activity was performed in accordance with the international recommendation

provided by the CLSI. The stock solutions of the each test compound and their respective metal chlorides (1 mg mL^{-1}) were prepared by dissolving 10 mg of the each test compound in 10 mL of freshly distilled DMSO. Further, the various concentrations of the test compounds (100 , 75 , 50 , 25 and $12.5\text{ }\mu\text{g mL}^{-1}$), were prepared by diluting the stock solutions with the required volume of freshly distilled DMSO.

The MIC of each test compounds was recorded as the lowest concentration of compound with no visible growth. The experiment done in triplicate and the average values were calculated. The obtained results were compared with that of gentamycin, a broad spectrum antibiotic for bacterial strains and fluconazole for fungal strains as positive control.

DNA Cleavage Activity

The DNA cleavage ability of the newly synthesized compounds was examined by using supercoiled plasmid pBR322 DNA (Bangal re Genei, Bengaluru, Cat. No 105850) as a target molecule in accordance to the literature method.¹⁹ Agarose gel electrophoresis method was used to study the efficiency of cleavage by the newly synthesized compounds.

RESULTS AND DISCUSSION

Chemistry

The newly synthesized metal (II) complexes are colored solids, stable at room temperature and possess high melting point ($>290^{\circ}\text{C}$).

The complexes are insoluble in water and common organic solvents; however these complexes are soluble to a large extent in DMF and DMSO.

Elemental analysis data (**Table 1**) agree well with the suggested composition of Schiff base ligand and its metal complexes. The results of conductivity measurements are too low to account for any dissociation of the complex in DMF ($17\text{-}22\text{ ohm}^{-1}\text{ cm}^2\text{ mole}^{-1}$). Hence, the complexes may be regarded as non-electrolytes.

IR Spectral Studies

The important IR bands of Schiff base ligand (**L**) were compared with those of the $[\text{Cu}(\text{L})_2]$, $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$, $[\text{Ni}(\text{L})_2]$ and $[\text{Zn}(\text{L})_2]$ complexes in order to ascertain the bonding mode of the ligand to the metal ion in the complex. The important IR bands for the ligand and its metal complexes together with their assignments are listed in **Table 2**.

The IR spectrum of Schiff base ligand (**L**), showed a broad band at 3475 cm^{-1} due to phenolic OH and medium intensity weak bands at 3355 cm^{-1} and 3114 cm^{-1} due to amide NH and NH attached to the thiazole moiety respectively. The high intensity strong bands observed at 1689 cm^{-1} , 1576 cm^{-1} and 1260 cm^{-1} are due to carbonyl function $\nu(\text{C}=\text{O})$, azomethine function $\nu(\text{C}=\text{N})$ and phenolic C-O respectively.

Table 1: Physical, Analytical data of Schiff base ligand (L) and its metal (II) complexes

Compound	M.W.	M.P. (°C)	Elemental Analysis, found (Calc.) [%]					λ_m	μ_{eff} (BM)
			C	H	N	M	Cl		
C ₁₈ H ₁₆ N ₄ O ₃ S (L)	368	278	58.72 (58.69)	4.28 (4.34)	15.24 (15.21)	--	--	--	--
[Cu(C ₁₈ H ₁₅ N ₄ O ₃ S) ₂] [Cu(L) ₂]	797.54	293	54.20 (54.16)	3.67 (3.76)	14.12 (14.04)	7.87 (7.96)	--	19	1.82
[Co(C ₁₈ H ₁₅ N ₄ O ₃ S)(H ₂ O) ₂ Cl] [Co(L)(H ₂ O) ₂ Cl]	496.93	297	43.42 (43.46)	3.88 (3.82)	11.34 (11.26)	11.88 (11.85)	7.14 (7.04)	22	4.72
[Ni(C ₁₈ H ₁₅ N ₄ O ₃ S) ₂] [Ni(L) ₂]	792.69	291	54.69 (54.49)	3.66 (3.78)	14.21 (14.12)	7.48 (7.40)	--	19	2.95
[Zn(C ₁₈ H ₁₅ N ₄ O ₃ S) ₂] [Zn(L) ₂]	799.40	296	54.09 (54.03)	3.70 (3.75)	14.08 (14.01)	8.15 (8.18)	--	17	Dia.

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

Compounds	ν_{OH}/ν_{H_2O}	Amide $\nu_{(NH)}$	Thiazole $\nu_{(NH)}$	$\nu_{(C=O)}$	$\nu_{(C=N)}$	Phenolic $\nu_{(C-O)}$	$\nu_{(M-O)}$	$\nu_{(M-N)}$	$\nu_{(M-Cl)}$
L	3475	3355	3114	1689	1576	1260	--	--	--
[Cu(L) ₂]	--	3362	3104	1682	1553	1321	468	413	--
[Co(L)(H ₂ O) ₂ Cl]	3425	3372	3107	1685	1532	1315	538	480	333
[Ni(L) ₂]	--	3355	3109	1683	1520	1280	569	454	--
[Zn(L) ₂]	--	3285	3110	1655	1536	1317	585	467	--

Table 3: Electronic spectral data and ligand field parameters

Complexes	Transitions in cm ⁻¹			Dq (cm ⁻¹)	B' (cm ⁻¹)	β	$\beta\%$	ν_2/ν_1	LFSE (k cal.)
	ν_1^*	ν_2	ν_3						
[Cu(L) ₂]	15228-17596			--	--	--	--	--	28.13
[Co(L)(H ₂ O) ₂ Cl]	7660	16390	19176	873	840	0.865	13.49	2.13	14.96
[Ni(L) ₂]	9550	15365	24912	955	791	0.760	23.94	1.60	32.74

*Calculated values

Table 4: Thermal data of Metal (II) Complexes

Complexes	Decomposition temp. (°C)	Weight loss (%)		Metal oxide (%)		Inference
		Obs.	Calc.	Obs.	Calc.	
[Cu(L) ₂]	243	11.11	9.65	--	--	Loss due to phenyl group of thiazole moiety.
[Co(L)(H ₂ O) ₂ Cl]	325	55.00	55.23	--	--	Loss due to a molecule of ligand and an OCH ₃ group of vanillin moiety.
	425	62.96	63.93	--	--	Loss due to C ₃ H ₃ N ₂ S species of thiazole moiety and C ₇ H ₄ O molecule of vanillin moiety.
	Up to 715	--	--	11.95	12.45	Loss due to remaining organic moiety
	124	6.66	7.24	--	--	Loss due to two coordinated water molecules.
[Ni(L) ₂]	338	7.93	7.59	--	--	Loss due to a coordinated chlorine atom.
	412	72.41	70.19	--	--	Loss due to C ₉ H ₈ N ₂ S molecule of 4-phenylthiazole moiety and C ₇ H ₇ O ₂ group of vanillin moiety.
	Up to 715	--	--	12.34	13.97	Loss due to remaining organic moieties.
	199	8.14	7.82	--	--	Loss due to two OCH ₃ groups of vanillin moieties.
[Zn(L) ₂]	315	25.80	26.20	--	--	Loss due to C ₉ H ₇ N ₂ S molecule of 4-phenylthiazole moiety.
	471	74.99	73.87	--	--	Loss due to C ₁₇ H ₁₂ N ₄ O ₂ S species of a ligand molecule.
	Up to 715	--	--	10.67	12.23	Loss due to remaining organic moiety.
	265	8.14	7.75	--	--	Loss due to two OCH ₃ groups of vanillin moieties.
[Zn(L) ₂]	440	83.87	84.21	--	--	Loss due to C ₁₇ H ₁₃ N ₄ O ₂ S molecule of a ligand and C ₁₁ H ₉ N ₄ OS of 4-phenylthiazole and C ₃ H ₃ molecules of vanillin moiety.
	Up to 715	--	--	13.47	14.29	Loss due to remaining organic moiety.

The IR spectra of the metal complexes exhibited ligand bands with the appropriate shifts due to complex formation. In the IR spectra of all the metal complexes it was observed that, the absence of absorption band due to phenolic OH at 3475 cm^{-1} of ligand indicates the formation of coordination bond between the metal ion and phenolic oxygen atom *via* deprotonation. This is further confirmed by the increase in absorption frequency about $20\text{-}61\text{ cm}^{-1}$ of phenolic $\nu(\text{C-O})$ which appeared in the region $1280\text{-}1321\text{ cm}^{-1}$ in all the complexes indicating the participation of oxygen atom of phenolic OH in the coordination. In the IR spectra of the metal complexes, medium intensity weak bands at $3285\text{-}3372\text{ cm}^{-1}$ and $3104\text{-}3110\text{ cm}^{-1}$ were due to amide NH and NH attached to thiazole moiety respectively, which appeared almost at about the same position as in the case of ligand, thus confirming their non-involvement in coordination. The shift of amide carbonyl $\nu(\text{C=O})$ to lower frequency side about $4\text{-}34\text{ cm}^{-1}$ and which appeared in the region $1655\text{-}1685\text{ cm}^{-1}$ in all the complexes confirms the coordination of oxygen atom of amide $\nu(\text{C=O})$ with the metal ions as such without undergoing enolization.²⁰ The absorption frequency due to azomethine $\nu(\text{C=N})$ function also shifted to lower frequency side about $23\text{-}56\text{ cm}^{-1}$ and appeared in the region $1520\text{-}1553\text{ cm}^{-1}$ suggesting the involvement of nitrogen atom of azomethine function in complexation with metal ions.²¹ Furthermore, the appearance of broad band at 3425 cm^{-1} in $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex is assigned to $\nu(\text{OH})$ of water molecules attached to the metal centre.

The coordination of metal ions with ligand was further confirmed by the appearance of new weak intensity, non-ligand bands in the region $468\text{-}585\text{ cm}^{-1}$ and $413\text{-}480\text{ cm}^{-1}$ in the IR spectra of the complexes are assigned to frequencies of $\nu(\text{M-O})$ and $\nu(\text{M-N})$ stretching vibration respectively. Also the appearance of new band at 333 cm^{-1} in $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex is due to $\nu(\text{M-Cl})$ stretching vibration.

¹H NMR Spectral Studies

The ¹H NMR spectrum of Schiff base ligand (**L**) displayed three singlets each at 11.459, 10.755 and 10.752 ppm are due to the proton of phenolic OH, amide NH and NH attached to thiazole moiety respectively. The signal due to azomethine proton (CH=N) resonated at 8.975 ppm. The signals due to nine aromatic protons (ArH) have resonated as multiplets in the region 7.227-7.754 ppm and signal at 3.180 ppm is due to three protons of methoxy group of *o*-vanillin moiety respectively. The Schiff base ligand (**L**) upon complexation with Zn(II) ion showed the disappearance of signal due to proton of phenolic OH confirms the involvement of bonding of phenolic oxygen to metal ion *via* deprotonation. The signals due to amide NH and NH attached to thiazole are appeared in the region 10.916 ppm and 10.450 ppm respectively. The signal due to azomethine proton (CH=N) resonated at 9.05 ppm. The signals due to nine aromatic protons (ArH) have resonated as multiplets in the region

7.322-7.921 ppm and signal at 3.60 ppm is due to three protons of methoxy group of *o*-vanillin moiety respectively.

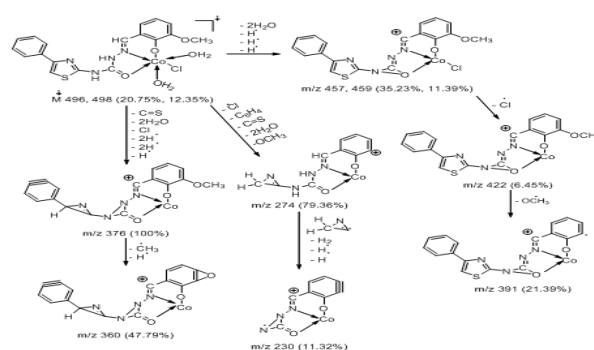
When compared to the ¹H NMR spectral data of Schiff base ligand (**L**) and its $[\text{Zn}(\text{L})_2]$ complex, all the signals due to protons have been shifted towards down field strength confirming the complexation of Zn(II) ion with the ligand. Thus, the ¹H NMR spectral results further supports the IR spectral inferences and complexation of Zn(II) ion with ligand.

ESI-Mass Spectral Studies

The mass spectra of $[\text{Cu}(\text{L})_2]$, $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ and $[\text{Zn}(\text{L})_2]$ complexes showed a molecular ion peaks recorded at m/z 797 (4.26%), m/z 496, 498 (20.75%, 12.35%) and m/z 799 (10.8%) respectively which are equivalent to their molecular weights.

Mass Fragmentation Pattern study of Co(II) Complex

The mass spectrum of $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex (**Figure 1**) showed a molecular ion peak recorded at m/z 496, 498 (20.75%, 12.35%), which corresponds to its molecular weight. The molecular ion underwent fragmentation by three routes. First, on simultaneous loss of C=S, two coordinated water molecules, chloride species and five hydrogen radicals gave a fragment ion peak recorded at m/z 376 (100%), which is also a base peak. This fragment ion on loss of a methyl and hydrogen radicals gave a fragment ion peak recorded at m/z 360 (47.79%). In second route, molecular ion on loss of two coordinated water molecules and three hydrogen radicals gave a fragment ion peak recorded at m/z 457, 459 (35.23%, 11.39%). This fragment ion on loss of a chloride radical gave a fragment ion peak recorded at m/z 422 (6.45%), which on loss of OCH_3 radical gave a fragment ion peak recorded at m/z 391 (21.39%). In another route, molecular ion underwent fragmentation and gave a peak recorded at m/z 274 (79.36%) which is due to the loss of chloride radical, C_6H_4 species, C=S, OCH_3 radical and two coordinated water molecules. Further, this on loss of $\text{C}_2\text{H}_2\text{N}$ radical and four hydrogen radicals gave a fragment ion peak recorded at m/z 230 (11.32%). The schematic mass spectral fragmentation pattern of $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ is in consistency with its structure which is illustrated in **Scheme 2**.



Scheme 2: Mass fragmentation pattern of $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex

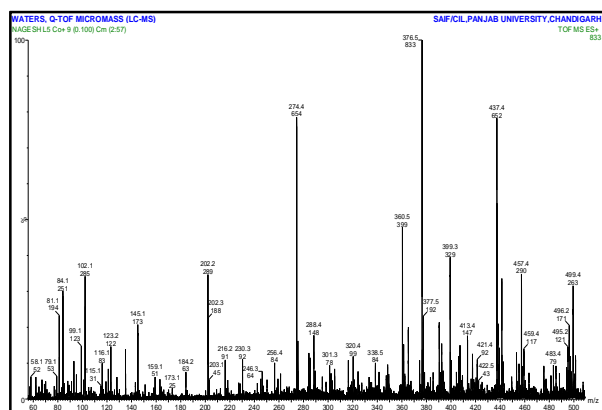


Figure 1: ESI mass spectrum of $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex

Electronic Spectral Studies

The green coloured $[\text{Cu}(\text{L})_2]$ complex showed a low intensity single broad asymmetric band in the region $15228\text{--}17596\text{ cm}^{-1}$. The broadness of the band designates the three transitions ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}(\nu_1)$, ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}(\nu_2)$ and ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g(\nu_3)$, 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 of these data suggested a distorted octahedral geometry around the $\text{Cu}(\text{II})$ ion.²²

The electronic spectra of brown coloured $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex displayed two absorption bands at 16390 cm^{-1} and 19176 cm^{-1} . These bands are assigned to be ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})(\nu_2)$ and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{P})(\nu_3)$ transitions respectively, which are in good agreement with the reported values.²³ 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.²⁴ The calculated ν_1 value is presented in **Table 3**. The obtained transition values of ν_1 , ν_2 and ν_3 suggest the octahedral geometry of the $\text{Co}(\text{II})$ complex.

The brown colored $[\text{Ni}(\text{L})_2]$ complex under present investigation exhibited two absorption bands in the region 15365 cm^{-1} and 24912 cm^{-1} , which are assigned to ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})(\nu_2)$ and ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})(\nu_3)$ transitions respectively in an octahedral environment. The transition value of band ν_1 , was calculated by using a band fitting procedure.²⁴ The band position of absorption band maxima assignments are presented in **Table 3**.

The proposed octahedral geometry of $[\text{Cu}(\text{L})_2]$, $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ and $[\text{Ni}(\text{L})_2]$ 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).²⁵ The calculated B' values for the $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ and $[\text{Ni}(\text{L})_2]$ complexes are lower than the free ion values, which is due to the orbital overlap and delocalization of d-orbitals. 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 $[\text{Ni}(\text{L})_2]$ complexes was less than that of the $[\text{Co}(\text{L})_2]$ complexes, indicating the greater covalency of the M-L bond.

Magnetic Susceptibility Studies

For $[\text{Cu}(\text{L})_2]$, $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ and $[\text{Ni}(\text{L})_2]$ complexes, the magnetic susceptibility measurements were carried out and they were found to be paramagnetic in nature. The observed magnetic moment value for $[\text{Cu}(\text{L})_2]$ complex is 1.82 BM. The observed value is slightly higher than the spin-only value due to one unpaired electron 1.73 BM, suggesting the octahedral geometry.²⁶ Thus, in the present $[\text{Cu}(\text{L})_2]$ complex is devoid of any spin interaction with distorted octahedral geometry. In octahedral $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex the ground state is ${}^4\text{T}_{1g}$. A large orbital contribution to the singlet state lowers the magnetic moment values for the various $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complexes which are in the range 4.12-4.70 for tetrahedral and 4.70-5.20 BM for octahedral geometry of the complexes respectively.²⁷ In the current study the observed magnetic moment value for $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex is 4.72 BM which suggests the octahedral geometry for the $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex. The observed magnetic moment value for $[\text{Ni}(\text{L})_2]$ complex is 2.95 BM, which is well within the expected range of 2.83 - 3.50 BM, suggesting the consistency with its octahedral environment of $[\text{Ni}(\text{L})_2]$ complex.²⁸

ESR Spectral Study of $[\text{Cu}(\text{L})_2]$ Complex

In the present study the observed measurements for $[\text{Cu}(\text{L})_2]$ complex is $g_{\parallel} (2.1467) > g_{\perp} (2.0283) > 2.0023$ indicate that the complex is axially symmetric and copper site has a $d_{x^2-y^2}$ ground state characteristic of octahedral geometry.²⁹ 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.³⁰ In the present study the g_{\parallel} value of $[\text{Cu}(\text{L})_2]$ 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 Billing³¹, 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 and the exchange interaction is noticed. In present observations the calculated G value for the $[\text{Cu}(\text{L})_2]$ complex is 5.55, indicate the exchange coupling effects are not operative in $[\text{Cu}(\text{L})_2]$ complexes.

Thermal Studies

The thermogram of $[\text{Cu}(\text{L})_2]$, $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$, $[\text{Ni}(\text{L})_2]$ and $[\text{Zn}(\text{L})_2]$ complexes (**Figure 2, 3**) showed decomposition in two/three stages. The percentage of metal content in all the complexes as done by elemental analysis agrees well with the thermal studies. The proposed stepwise thermal degradation pattern of all the complexes with respect to temperature and formation of respective metal oxides

are depicted in **Table 4**. The demonstrated results are in good agreement with the formulae suggested from the analytical data.

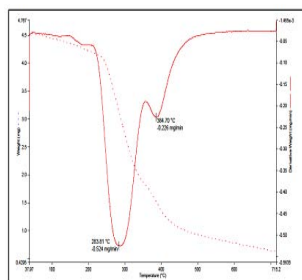


Figure 2: TGA-DTA curve of $[\text{Cu}(\text{L})_2]$ complex

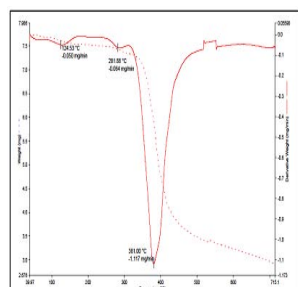


Figure 3: TGA-DTA curve of $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complex

Powder X-ray Diffraction Studies (Powder-XRD)

Though the newly synthesized metal complexes were soluble in some polar organic solvents like DMSO and DMF, crystals that are suitable for single crystal studies are not obtained. The powder X-ray diffraction pattern of $[\text{Cu}(\text{L})_2]$ complex is scanned in the range $3\text{--}60^\circ$ (θ) at wave length 1.54 \AA . The diffractogram and associated data depict the 2θ value for each peak, relative intensity and inter-planar spacing (d -values). Powder X-ray diffraction pattern of $[\text{Cu}(\text{L})_2]$ complex displayed a eleven reflections with maxima at $2\theta = 34.381^\circ$ corresponding to d value 2.606 \AA . The inter-planar spacing (d) has been calculated by using Bragg's equation, $n\lambda = 2d \sin\theta$. In the above complex, the trend of the curves decreases from maximum to minimum intensity indicating the amorphous nature of the complexes. The unit cell calculations have been calculated for $[\text{Cu}(\text{L})_2]$ complex from the entire important highly intense peaks and $h^2 + k^2 + l^2$ values were determined. The observed inter-planar d -spacing values have been compared with the calculated ones and it was found to be in good agreement with observed values. The $h^2 + k^2 + l^2$ values of $[\text{Cu}(\text{L})_2]$ complex are 1, 1, 3, 4, 6, 7, 8, 8, 13, 15 and 22. The calculated lattice parameter for $[\text{Cu}(\text{L})_2]$ complex is $a=b=c=12.174$. It was observed that the presence of forbidden number 7, 15 indicates that the $[\text{Cu}(\text{L})_2]$ complex may belong to hexagonal or tetragonal systems.

Biological Evaluations

In vitro Antimicrobial Results

The antimicrobial screening results in most of the cases, the newly synthesized metal complexes exhibited promising results than the free ligand. This activity found to be enhanced on coordination with metal ions. This enhancement in the antimicrobial activity of the complexes over the free ligand can be explained on the basis of chelation theory.^{32,33} The enhancement in the anti-microbial activity may be rationalized on the basis that ligands mainly possess azomethine ($\text{C}=\text{N}$) bond. More over in metal complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (N and O) present in the ligand and there may be π -

electron delocalization over the whole chelating system.³⁴ Hence the increase in the lipophilic character of the metal chelates favour its permeation through the lipid layer of the bacterial 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.³⁵ The minimum inhibitory concentration (MIC) values of the compounds against the respective bacterial and fungal strains are summarized in **Table 5**.

DNA Cleavage Activity

The electrophoresis analysis clearly revealed that the ligand and its metal complexes have acted on DNA because of a difference in molecular weight between the control and the treated DNA samples. In the present case, gel electrophoresis clearly revealed that (**Figure 4**) there was a difference in the migration of the lanes of Schiff base ligand (**L**) and its metal (II) complexes as compared to the control plasmid DNA pBR322 (**C**). It is clearly evident that the lane of Schiff base ligand (**L**) and its $[\text{Cu}(\text{L})_2]$ and $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$ complexes showed complete cleavage of supercoiled DNA whereas $[\text{Ni}(\text{L})_2]$ and $[\text{Zn}(\text{L})_2]$ complex showed partial cleavage of supercoiled DNA. This shows that the control DNA alone does not show any apparent cleavage, whereas the ligand and its metal complexes do show. On the basis of these findings, it can be concluded that all the newly synthesized compounds under present study are good pathogenic microorganism inhibitor as evident from the observation on the DNA cleavage of pBR322.

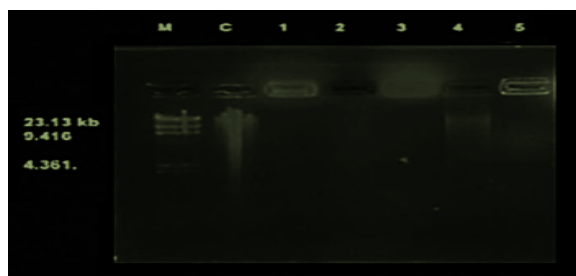


Figure 4: DNA cleavage on plasmid pBR 322M: Standard DNA, C: Control DNA (untreated pBR 322), 1: L, 2: $[\text{Cu}(\text{L})_2]$, 3: $[\text{Co}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$, 4: $[\text{Ni}(\text{L})_2]$, 5: $[\text{Zn}(\text{L})_2]$.

CONCLUSION

A series of $\text{Cu}(\text{II})$, $\text{Co}(\text{II})$, $\text{Ni}(\text{II})$ and $\text{Zn}(\text{II})$ complexes were prepared with tridentate ONO donor Schiff base ligand (**L**) derived from N -(4-phenylthiazol-2-yl)hydrazinecarboxamide and o -vanillin and characterized them by various spectral techniques. Spectral analysis indicates octahedral geometry for all the complexes. $\text{Cu}(\text{II})$, $\text{Ni}(\text{II})$ and $\text{Zn}(\text{II})$ complexes have 1:2 stoichiometric ratio of the type $[\text{M}(\text{L})_2]$ and $\text{Co}(\text{II})$ complex have 1:1 stoichiometry ratio of the type $[\text{M}(\text{L})(\text{H}_2\text{O})_2\text{Cl}]$. The antimicrobial activity results showed that all the newly synthesized metal (II) complexes have exhibited higher activity when compared to the free ligand. Also, DNA

cleavage studies revealed that all the compounds showed good efficiency towards DNA cleavage. Hence from all these extensive observations, it was concluded that the Schiff base ligand (**L**) and its metal complexes gave the remarkable, versatile and valuable information of coordination compounds about the study of bonding modes and elucidating the various structures of the metal complexes. Based on physicochemical evidences, we proposed the geometry/structure (**Figure 5**) of the metal complexes.

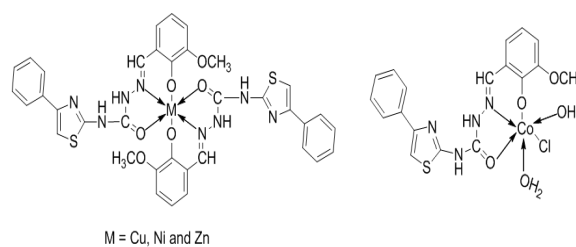


Figure 5: Proposed structures of Metal (II) Complexes

Table 5: MIC values of Schiff base ligand (L) and its metal (II) complexes

Compounds	Bacteria				Fungi			
	<i>S. aureus</i>	<i>B. Subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. oxysporum</i>	<i>A. Flavus</i>	<i>A. niger</i>
L	75	75	75	50	75	50	75	75
[Cu(L) ₂]	50	50	25	25	50	50	50	50
[Co(L)(H ₂ O) ₂ Cl]	25	25	25	25	50	25	25	50
[Ni(L) ₂]	50	50	50	50	50	50	50	25
[Zn(L) ₂]	50	50	50	50	50	25	25	50
Gentamicin	12.50	12.50	12.50	12.50	--	--	--	--
Fluconazole	--	--	--	--	12.50	12.50	12.50	12.50

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