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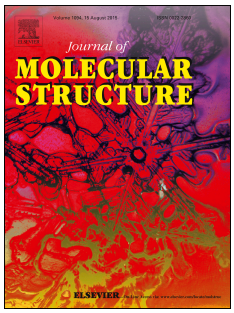

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Research Article

Preparation of Octahedral Cu(II), Co(II), Ni(II) and Zn(II) Complexes Derived from 8-formyl-7-hydroxy-4-methylcoumarin: Synthesis, Characterization and Biological study

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ABSTRACT

A series of octahedral Cu(II), Co(II), Ni(II) and Zn(II) complexes has been synthesized with ONO donor Schiff base ligand **(L)** derived from the reaction of 8-formyl-7-hydroxy-4-methyl coumarin and *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide. The chemical structures of the compounds were elucidated by elemental analysis and various physico-chemical techniques. Thermal analyses studies indicates the presence of coordinated water molecules in Cu(II) and Zn(II) complexes. The compounds were screened for their antibacterial and antifungal activities by MICs method and DNA cleavage activity AGE method. Also, brine shrimp bioassay was also carried out to study the *in vitro* cytotoxic properties, the compounds reveals the significant activity.

Key words: Transition metal complexes; Schiff base; *in vitro* cytotoxic; DNA cleavage; coumarin; antimicrobial.

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1. Introduction

It has long been known that many of the metal ions are actively involved in the biological processes of life and have been a topic of interest. The mode of action of these metal ions are often complex, but are believed to involve in bonding through the various hetero atoms (ONO/S) of the heterocyclic residues of biological molecules, i.e., proteins, enzymes, nucleic acids, etc. [1-3]. Some metal complexes have been used as the anticancer drug in clinic. Currently Lanthanide (III) complexes are being investigated extensively for a variety of functionalities and applications, such as medicine, optical probes, microelectronics, and others [4-7]. Schiff base ligands with N and O donor atoms continue to occupy an important role in metal coordination chemistry, resulting in an enormous number of publications, ranging from pure synthetic work to modern physicochemical and biochemically relevant studies of metal complexes [8-10].

Sulfa drugs have attracted the attention of the chemist because their therapeutic significance as they were used against a wide spectrum of bacterial ailments. Also, some sulfa drugs were used in the treatment of tuberculosis, malaria, cancer and leprosy [11,12]. The core molecule thiazole is an important class of heterocycles contains two hetero atoms (S and N) placed in the heterocyclic ring at 1 and 3 positions respectively. Compounds containing this moiety have wide range of applications in pharmaceutical, phytosanitary, analytical industrial aspects and have diverse biological activities [13,14].

Coumarins are also an important class of compounds obtained from nature and synthetic origin that possess diverse array of pharmacological and biological relevance such as antitumor, anti-inflammatory, anticoagulant, anti-HIV, herbicidal and fungicidal activities [15-17]. Some of the coumarin derivatives are used as DNA intercalators used in cancer treatment because of its planar chromophore [18]. Also, it has been proved that the naturally occurring 7-hydroxy-4-methylcoumarin is potential lead molecule for cancer drug development [19]. Also, the compounds containing this moiety form very stable complexes with various metal ions due to the presence of phenolic hydroxyl group at its *ortho*-position, which coordinates to the metals ion *via* deprotonation [20].

Herein, octahedral Cu(II), Co(II), Ni(II) and Zn(II) complexes has been synthesized with ONO donor Schiff base ligand **(L)** derived from the reaction of 8-formyl-7-hydroxy-4-methyl coumarin and *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide containing carbonyl (C=O), azomethine (C=N) and hydroxyl group (OH) as potential chelating sites. The structures of the complexes are elucidated using various physicochemical techniques. The

corresponding biological relevance of all the complexes have been compared with those of the free Schiff base ligand **(L)** and standard drug.

2. Experimental

2.1. Materials and methods

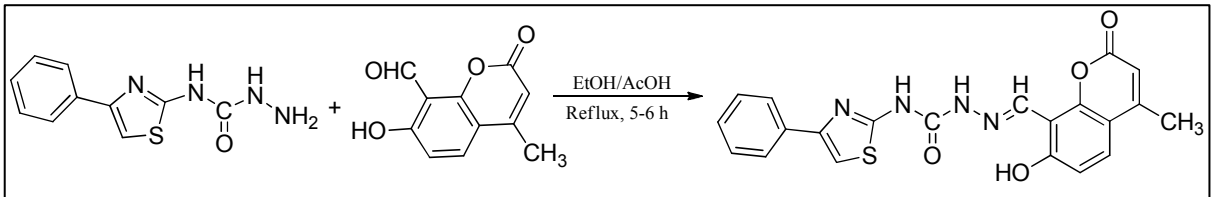
All solvents and starting materials were purchased from commercial chemical suppliers and used without further purification, unless otherwise stated. The precursor, 8-formyl-7-hydroxy-4-methyl coumarin and *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide are prepared as per literature methods [20-22].

2.2. Instrumentation

The metal and chloride contents of the complexes were estimated gravimetrically as per standard methods [23]. Carbon, Hydrogen and Nitrogen analysis was performed on Vario EL III CHNS analyser. IR spectra were recorded on Perkin Elmer Spectrum RX-I FTIR spectrophotometer. ^{1}H NMR spectra were recorded on Bruker, 400 MHz spectrometer using d_6-DMSO as solvent. ESI mass spectra were recorded on mass spectrometer equipped with ESI source having mass range of 4000 amu in quadruple and 20,000 amu in Tof. Electronic spectra were recorded on ELICO SL-164 double beam UV-visible spectrophotometer (ca. 10^{-3} M in DMF). Molar conductivity of metal complexes was measured on Elico-CM 180 Conductivity Bridge (ca. 10^{-3} M DMF). ESR measurements of solid $[Cu(L)(Cl)(H_2O)_2]$ complex was carried out on BRUKER Bio Spin spectrometer working at a microwave frequency of 8.75-9.65 GHz using DPPH as reference with field set at 3000 Gauss using tetracynoethylene as the 'g' marker (g = 2.00277). Thermo gravimetric analyses data were measured on Perkin Elmer thermal analyser in nitrogen atmosphere with a heating rate of 20 °C min^{-1}.

2.3. Synthesis of Schiff base ligand (L)

A solution of 8-formyl-7-hydroxy-4-methyl coumarin and *N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide in absolute ethyl alcohol (30 mL) was refluxed on water bath for about 5-6 with an addition of a catalytic amount of glacial acetic acid (2-3 drops). A light yellow precipitate was collected by filtration, washed with absolute ethyl alcohol, and dried in vacuo. The pathway for the synthesis of Schiff base ligand **(L)** is presented in **Scheme 1**.



Scheme 1- Synthesis of 2-((7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)methylene)-*N*-(4-phenylthiazol-2-yl)hydrazinecarboxamide **(L)**

2.4. Preparation of metal complexes

Mixture of Schiff base ligand **(L)** (0.001 mol) and respective metal chlorides (0.001 mol) in 20 mL of absolute ethyl alcohol was refluxed on water bath for about 6-7 h and pH of the reaction mixture was adjusted to ca.7.0-7.5 using sodium acetate (0.5 g) and reflux is continued for about an hour more. The reaction mixture was cooled to room temperature and poured in to distilled water the separated precipitates were filtered off, washed with distilled water, then with hot ethanol and finally dried in a vacuum over fused calcium chloride.

2.5. Biological Evaluation

2.5.1. Antibacterial and antifungal assay

The antimicrobial activity of **L** and its $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ 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 four bacteria, *S. aureus* (MTCC 3160), *B. subtilis* (MTCC 736), *E. coli* (MTCC 46) and *S. typhi* (MTCC 98) and antifungal activity against four fungi, *C. albicans* (MTCC 227), *C. oxysporum* (MTCC 1777), *A. flavus* (MTCC 1883) and *A. niger* (MTCC 1881) by MIC method [24].

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.

2.5.2. DNA cleavage studies

The DNA cleavage activity was performed using super coiled plasmid DNA pBR322 as a target molecule as per the literature method [25] and obtained results were compared with the standard DNA marker.

2.5.3. *In vitro* Cytotoxicity study

For the study, Brine shrimp nauplii (*Artemia salina*) were used for cytotoxicity assay and followed the protocol given by *Meyer et al*. [26] with some modifications. Brine shrimp nauplii eggs were hatched in a shallow rectangular dish (22 X 32 cm) filled with artificial sea water. An unequal partition was made in the dish with the help of punctured device. About 50

mg of eggs were sprinkled into the large compartment, which was darkened and the minor compartment was open to ordinary light. After two days nauplii were collected by a pipette from the lighter side.

The stock solutions of the each test compounds (1 mg mL^{-1}) were prepared by dissolving 10 mg of each test compound in 10 mL of DMSO, different concentrations of the test compounds were placed in separate vials and final volume is adjusted to 10 mL using artificial sea water. After two days, when shrimp larvae were ready, 10 nauplii were then placed in each vial, after 24 h incubation the vials were observed using a magnifying glass and the number of survivors in each vial was counted. Tests were performed in duplicate and the data were analysed by a Finney computer program to determine the LD_{50} values [27]. The results were compared with a positive control Bleomycin, an anti-cancer drug.

3. Results and discussion

3.1. Chemistry

The synthesized $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes are colored solids, stable at room temperature and infusible at high temperature. The elemental analysis data provided in supplementary material **Table S1** are agreeing well with the proposed composition of **L** and its metal complexes. These data supports the metal to ligand stoichiometric ratio of the complexes is 1:2 of the type $[M(L)_2]$ (M = Co and Ni) and 1:1 stoichiometry of the type $[M(L)(Cl)(H_2O)_2]$ (M = Cu and Zn). The observed molar conductance values for all the metal complexes are too low to account for any dissociation, indicating their non-electrolytic nature.

3.2. IR spectral studies

The IR spectrum of **L**, displayed a broad band at 3420 cm^{-1} due to phenolic OH and showed high intensity strong bands at 1710 cm^{-1}, 1692 cm^{-1}, 1663 cm^{-1} and 1270 cm^{-1} are due to lactone carbonyl, amide carbonyl, azomethine function and phenolic C-O respectively. Also, medium intensity bands are observed at 3232 cm^{-1} and 3048 cm^{-1} due to amide NH and NH attached to thiazole moiety respectively.

On comparing, the IR spectra of the **L** and its $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes, it was clearly observed that, the absence of absorption band due to phenolic OH at 3420 cm^{-1} of ligand in all the complexes indicates the formation of coordination bond between metal ion and phenolic oxygen atom of coumarin moiety *via* deprotonation. This is further confirmed by sharp increase in the absorption frequency about 21-45 cm^{-1} of phenolic ν(C-O) which appeared in the region 1291-1315 cm^{-1} in all the

complexes representing the involvement of oxygen atom of phenolic oxygen in coordination. The most prominent change is the shift of amide carbonyl ν(C=O) to lower frequency side about 05-66 cm^{-1} which appeared in the region 1687-1626 cm^{-1} in all the complexes confirms the involvement of oxygen atom of amide ν(C=O) in coordination as such without undergoing enolization. Also, the shift of azomethine function ν(C=N)to lower frequency side about 32-81 cm^{-1} and appeared in the region 1582-1631 cm^{-1} in all the metal complexes indicating the participation of nitrogen atom of azomethine function in coordination. The medium intensity weak bands appeared in all the metal complexes at 3201-3237 cm^{-1} and 3050-3063 cm^{-1} were due to amide NH and NH attached to thiazole moiety respectively, which appeared almost at about the same region as in case of ligand, thus confirming their non-involvement in coordination. Also, the absorption band due to lactone carbonyl function was appeared in all the complexes at 1700-1728 cm^{-1} i.e., at about the same region as in the case of ligand confirming its non-involvement in coordination.

Formation of metal complexes was further confirmed by the appearance of new weak intensity, non-ligand bands in the region 509-540 cm^{-1}, 449-495 cm^{-1} in all the complexes and peak at 366 cm^{-1} and 343 cm^{-1} (in case of Cu and Zn complexes) are assigned to frequencies of ν(M-O), ν(M-N) and ν(M-Cl) bonds respectively. In case of $[Cu(L)(Cl)(H_2O)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes, the appearance of broad band at 3430 cm^{-1} and 3372 cm^{-1} respectively are due to coordinated water molecule. The prominent IR spectral data of **L** and its metal complexes together with their assignments are presented in **Table 1.**

3.3. ^{1}H NMR spectra

The ^{1}H NMR spectrum of **L** displayed (Supplementary data, **Fig. S1**) three singlets each at 12.2, 11.4 and 11.1 ppm which are ascribed to protons of phenolic OH, amide NH and NH of thiazole moiety respectively. A characteristic singlet appeared at 8.1 ppm is assigned to azomethine proton (HC=N). In addition to this nine aromatic protons have resonated as multiplet in the region 6.9-7.9 ppm and a signal at 2.4 ppm is due to three protons of methyl group of coumarin moiety. The ^{1}H NMR spectrum of diamagnetic $[Zn(L)(Cl)(H_2O)_2]$ complex (Supplementary data, **Fig. S2**) displayed two singlets each at 11.1 and 10.5 ppm are ascribed to protons of amide NH and NH of thiazole respectively. A singlet proton signal at 8.3 ppm is assigned to azomethine proton (HC=N). In addition to this the nine aromatic protons have resonated as multiplet in the region 7.1-8.0 ppm and a signal at 2.5ppm is due to three protons of methyl group of coumarin moiety.

The phenolic OH signal of **L,** which was found at 12.2 ppm has completely disappeared in $[Zn(L)(Cl)(H_2O)_2]$ complex confirming the involvement and bonding of phenolic oxygen atom in chelation with metal ion *via* deprotonation. Also, appearance of a new signal at 3.6 ppm corresponds to four protons of two coordinated water molecules.

3.4. ESI mass spectral studies

The ESI mass spectrum (Supplementary data, **Fig. S3**) of **L** displayed a molecular ion peak recorded at M^+420 (100%) is equivalent to its molecular weight and which is also a base peak.

The ESI mass spectrum of $[Co(L)_2]$ complex (Supplementary data, **Fig. S4**) displayed M^++1 peak recorded at 897 (7%). The molecular ion peak recorded at M^+ 896 (5%) underwent fragmentation in two routes **(Scheme - S1)**. First, on loss of C_3H_3 radical and CO_2 molecule of coumarin moiety gave a fragment ion peak recorded at m/z 813 (6%). In another route, the molecular ion underwent fragmentation and gave a peak recorded at m/z 338 (100%) which is also a base peak due to the loss of a deprotonated ligand, CO_2 molecule, benzene molecule, CH_3 radical and two hydrogen radicals. The Schematic mass spectral fragmentation pattern of $[Co(L)_2]$ complex is in consistency with its proposed structure which is provided in the supplementary data, **Scheme-S1.**

The ESI mass spectrum of $[Zn(L)(Cl)(H_2O)_2]$ complex (Supplementary data, **Fig. S5**) displayed M^++1 peak recorded at m/z 556, 558 (9%, 3%). This molecular ion recorded at M^+ 555, 557 (3%, 1%) underwent a fragmentation in two routes **(Scheme - S2).** First, on loss of $C_6H_4O_2$ molecule, C_2H radical of coumarin moiety and two coordinated water molecule giving a fragment ion peak recorded at m/z 386, 388 (90%, 30%). Further this on simultaneous loss of C_3H_2Ospecies of coumarin, Chloride radical and two hydrogen radicals gave a fragment ion peak recorded at m/z 295 (80%). In another route, the molecular ion underwent fragmentation and gave a peak recorded at m/z 364 (100%) which is also a base peak, this is due to the loss of CO molecule, two coordinated water molecule and phenyl, CH_3 and Chloride radicals. This on further loss of C_9H_3O radical of coumarin moiety gave a peak recorded at m/z 321 (31%). The Schematic mass spectral fragmentation pattern of $[Zn(L)(Cl)(H_2O)_2]$ complex is in consistency with its proposed structure which is provided in the supplementary data, **Scheme - S2.**

3.5. Electronic spectra and magnetic susceptibility studies

The green coloured $[Cu(L)(Cl)(H_2O)_2]$ complex displayed a low intensity single broad asymmetric band in the region 15149-17986 cm^{-1}. The broadness of the band

designates the three transitions $^2B_{1g} \rightarrow {}^2A_{1g}$ (v_1), $^2B_{1g} \rightarrow {}^2B_{2g}$ (v_2) and $^2B_{1g} \rightarrow {}^2E_g$ (v_3) which are similar in energy and give rise to only one broad band and the broadness of the band may be due to dynamic Jahn-Teller distortion. The obtained data suggests the distorted octahedral geometry for $[Cu(L)(Cl)(H_2O)_2]$ complex [28]. The obtained μ_{eff} value of the $[Cu(L)(Cl)(H_2O)_2]$ complex is 1.84 BM, which is slightly higher than the spin-only value due to one unpaired electron 1.73 BM, indicates a octahedral geometry around the Cu (II) ion [29]. The electronic spectra of brown coloured $[Co(L)_2]$ complex displayed two absorption bands at 16887 cm^{-1} and 19695 cm^{-1}. These bands are assigned to be $^4T_{1g}$ (F) $\rightarrow {}^4A_{2g}$ (F) (v_2) and $^4T_{1g}$ (F) $\rightarrow {}^4T_{2g}$ (P) (v_3) transitions respectively, which are in good agreement with the reported values for octahedral geometry [30]. The lowest band, v_1 could not be observed due to the limited range of the instrument used, but it could be calculated using the band fitting procedure given by Underhill and Billing [31]. The transition values of v_1, v_2 and v_3 suggest the octahedral geometry arrangement for $[Co(L)_2]$ complex. The observed lower magnetic moment value for $[Co(L)_2]$ complex is μ_{eff} 5.02 BM, which is well within the range of μ_{eff} 4.70-5.20 BM for octahedral geometry [32]. The $[Ni(L)_2]$ complex under present investigation exhibited two absorption bands in the region 14946 cm^{-1} and 25168 cm^{-1}, which are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (v_2) and $^3A_{2g}$ (F) $\rightarrow {}^3T_{1g}$ (P) (v_3) transitions respectively, suggest an octahedral geometrical environment around Ni (II) ion [33]. The transition value of band v_1 was calculated by using a band fitting procedure [31]. The observed magnetic moment value for $[Ni(L)_2]$ complex is μ_{eff} 2.94 BM, which is also well within the expected range of μ_{eff}2.83-3.50 BM, suggesting the consistency with its octahedral geometry [34]. The $[Zn(L)(Cal)(H_2O)_2]$complex is found to be accordingly diamagnetic in nature and it is proposed to have a octahedral geometry.

The proposed geometry of $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$ and $[Ni(L)_2]$ complexes was further confirmed by the calculated values of ligand field parameters such as, nephelauxetic parameter (β), Racah inter electronic repulsion parameter (B'), ligand field splitting energy (10 Dq) and ligand field stabilization energy (LFSE) [35]. The calculated B' values for the $[Co(L)_2]$ and $[Ni(L)_2]$ complexes are lower than the free ion values, which is due to the orbital overlap and delocalization of d-orbitals. The β values are significant in determining the covalency for the metal-ligand (M-L) bond and they were found to be less than unity, suggesting a considerable amount of covalency for the M-L bonds. The β value for the $[Ni(L)_2]$ complexes was less than that of the $[Co(L)_2]$ complexes, indicating the greater

covalency of the metal-ligand bond. The position of absorption band maxima assignments are presented in **Table 2.**

3.6. Electronic Spin Resonance spectra

The ESR spectrum of $[Cu(L)(Cl)(H_2O)_2]$ complex **(Fig. 1)** at room temperature exhibits anisotropic signals with g value $g_{\|} = 2.139$ and $g_{\perp} = 2.034$ which is characteristic for axial symmetry [36]. Since the $g_{\|}$ and $g_{\perp}$ values are closer to 2 and $g_{\|} > g_{\perp}$ suggesting an octahedral geometry [37]. The trend $g_{\perp} > g_{\|} > 2.0023$ shows that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital in the ground state. In addition, the exchange coupling interaction between two Cu(II) ions is explained by Hathaway and Billing expression $G = g_{\|} - 2.0023/g_{\perp} - 2.0023$. When 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 study, that G value is 4.20, indicate the exchange coupling effects are not operative. The ESR spectra of $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes at room temperature do not show ESR signal because the rapid spin lattice relaxation of the $[Co(L)_2]$ and $[Ni(L)_2]$ broadness the lines at higher temperatures [36] and the diamagnetic nature of the $[Zn(L)(Cl)(H_2O)_2]$ complex. The ESR spectra show signals that may be accounted for the presence of free radicals that can result from the cleavage of any double bond and distribution of the charge on the two neighbour atoms. The presence of unpaired electrons from any source inside the molecule can be responsible for the appearance of these signals [38].

3.7. Thermal analyses

The thermogram of $[Cu(L)(Cl)(H_2O)_2]$ complex **(Fig. 2)** showed that the complex is decomposed in four successive stages. The first stage of degradation occurred at 165 °C, due to the loss of two coordinated water molecules (Obs.5.84%, Calc. 6.50%). The resultant complex on further degradation furnished a break at 310 °C by the loss of a coordinated Chlorine atom and CH_3 species of coumarin moiety (Obs. 10.27%, Calc. 9.66). Further, thecomplex showed third stage of decomposition at 340 °C due to loss of C_5HO_2 molecule of coumarin moiety (Obs. 20.68%, Calc. 19.89%). Further, the complex at 430 °C showed the break which was due to the loss of C_8H_6 species of thiazole moiety (Obs. 24.42%, Calc. 27.23%). Thereafter, the complex showed a gradual weight loss up to 731 °C, due to loss of remaining organic moiety. The final weight of the residue corresponds to cupric oxide.

The thermogram of $[Co(L)_2]$ complex indicated its degradation in three successive stages. The first stage of decomposition represents the weight loss of a $C_4H_4O_2$ species of coumarin moiety at 358 °C (Obs. 9.33%, Calc. 9.36%). The resultant complex further

underwent degradation in second stage and gave break at 304°C (Obs. 46.35%, Calc. 45.14%) which corresponds to the loss of two moles of 2-amino-4-phenylthiazole molecules. Further, the complex underwent third stage of decomposition and gave a break at 375 °C (Obs. 43.20%, Calc. 41.71%), which corresponds to the weight loss of $C_{11}H_6O_3$group of coumarin moiety. Thereafter, the complex showed a gradual weight loss up to 632 °C, due to remaining organic moiety. The weight of the residue corresponds to cobalt oxide.

Similarly, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes underwent a decomposition in three and four successive stages respectively and the final weight of the residue corresponds to the formation of respective metal oxides. The proposed stepwise degradation pattern of $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes at different stages are due to the loss of different organic moieties with respect to temperature, which is illustrated in **Table 3**.

3.8. Biological Evaluations

3.8.1. Antibacterial and antifungal assay

The MIC values of the tested compounds and standards against the respective strains are summarized in supplementary data **Table S2 and S3**. 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 ONO 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 [39, 40]. 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 [41] 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 [42].

3.8.2. DNA cleavage activity

From the DNA cleavage activity gel picture **(Fig. 3),** it is clearly evident that there was a difference in the migration of the lanes (Lane 1 to 5) of **L** and its $[Cu(L)(Cl)(H_2O)_2]$,

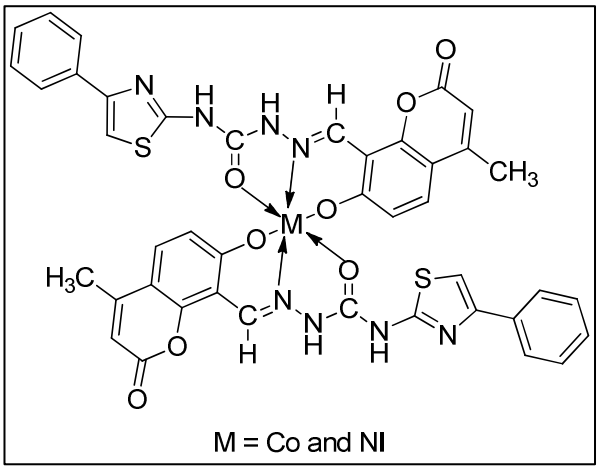
$[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes respectively. Control experiments clearly revealed that the untreated DNA does not show any cleavage (Lane C), whereas all the compounds have exhibited cleavage of pBR322 DNA. On the basis of these outcomes, we can infer that all the newly synthesized compounds under present study are good pathogenic microorganism inhibitors. These compounds inhibit the growth of pathogenic organism by cleaving the genome [43].

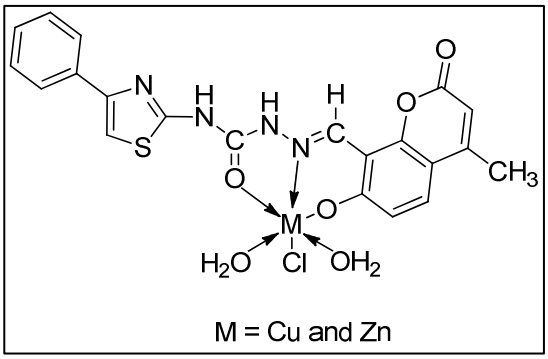
3.8.3. Cytotoxicity

It is an excellent tool for monitoring the preliminary assessment of toxicity. Brine shrimp lethality bioassay is a development in the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (*viz.*antiviral, anti-cancer, insecticidal, pesticidal, AIDS, etc.) of the compounds [44]. Results for the lethality were noted in terms of number of deaths of larvae. The summary of the cytotoxicity assay results were presented in **Table 4.** The standard anti-cancer drug Bleomycin showed the LD_{50} value of 0.410 X 10^{-4}. Among all the tested compounds $[Co(L)_2]$ complex showed the highest cytotoxicity with LD_{50} value 1.106 X 10^{-4}, followed by $[Zn(L)(Cl)(H_2O)_2]$ complex with LD_{50} value1.112 X 10^{-4} respectively. On the other hand, the **L** and its $[Cu(L)(Cl)(H_2O)_2]$, $[Ni(L)_2]$ complexes were showed moderate activity.

4. Conclusions

A new octahedral $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes were synthesized with ONO donor Schiff base ligand 2-((7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)methylene)-N-(4-phenylthiazol-2-yl)hydrazinecarboxamide**(L)**. The spectral data suggest the octahedral coordination geometrical arrangement for all the complexes having 1:2 stoichiometric ratio of the type $[M(L)_2]$ (M = Co and Ni) and $[M(L)(Cl)(H_2O)_2]$ (M = Cu and Zn). Thermal analyses data provides the information regarding the presence of coordinated water molecules in $[Cu(L)(Cl)(H_2O)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes. The antimicrobial activity of all the metal complexes was found to be enhanced on the formation of complex. The results of DNA cleavage studies infer that, all the newly synthesized compounds are able to carry out an efficient cleavage of pBR322 DNA. In addition to that, the $[Co(L)_2]$and $[Zn(L)(Cl)(H_2O)_2]$ complexes showed good cytotoxic property. Hence from these observations, it was concluded that **L** and its metal complexes gave remarkable, versatile and valuable information of coordination compounds and also they are found to be powerful bioactive compounds. Based on the spectral data following geometries have been proposed for the metal complexes:





Proposed structures of the $[Cu(L)(Cl)(H_2O)_2]$, $[Co(L)_2]$, $[Ni(L)_2]$ and $[Zn(L)(Cl)(H_2O)_2]$ complexes

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Supplementary Data

Supplementary data associated with this article i.e., Fig's., Tables and Schemes are provided in electronic form.

Declaration of interest

The authors declared no conflicts of interest.

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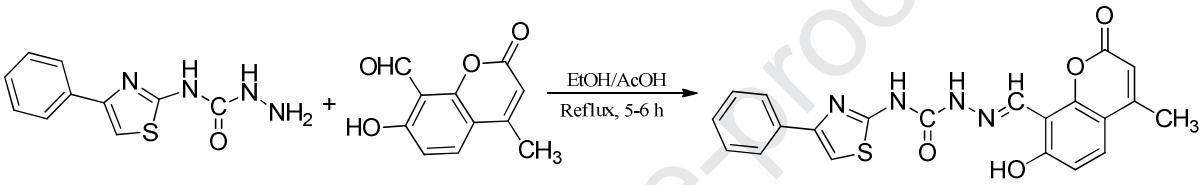
GRAPHICAL ABSTRACT



- Synthesis of new class of bioactive metal complexes containing thiazole core.
- All the newly prepared compounds showed complete cleavage of super-coiled DNA pBR322.

Table 1 - IR spectral data (cm^{-1}) of Schiff base ligand **(L)** and its metal complexes

Ligand/ Complexes	ν_{OH} (phenolic)	ν_{H2O}	ν_{NH} (amide)	ν_{NH} (thiazole)	$\nu_{C=O}$ (lactone)	$\nu_{C=O}$ (carbonyl)	$\nu_{C=N}$ (azomethine)	ν_{C-O} (phenolic)	ν_{M-O}	ν_{M-N}	ν_{M-Cl}
L	3420	--	3232	3048	1710	1692	1663	1270	--	--	--
$[Cu(L)(Cl)(H_2O)_2]$	--	3430	3226	3050	1728	1683	1614	1291	528	449	366
$[Co(L)_2]$	--	--	3237	3050	1709	1687	1631	1291	540	495	--
$[Ni(L)_2]$	--		3201	3050	1700	1626	1582	1295	520	465	--
$[Zn(L)(Cl)(H_2O)_2]$	--	3372	3225	3063	1705	1685	1619	1315	509	473	343

Table 2 - Electronic spectral data and ligand field parameter data

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						
$[Cu(L)(Cl)(H_2O)_2]$	15149-17986			--	--	--	--	--	28.40
$[Co(L)_2]$	7887	16887	19695	894	861	0.886	11.32	2.14	15.42
$[Ni(L)_2]$	9160	14946	25168	916	841	0.808	31.40	1.63	31.40

*Calculated values

Table 3 - Thermal degradation pattern of metal complexes with respect to temperature

Metal Complexes	Temperature (°C)	Weight loss (%)		Metallic residue (%)		Assignment
		Obs.	Calc.	Obs.	Calc.	
$[Cu(L)(Cl)(H_2O)_2]$	165	5.84	6.50	--	--	Loss due to two coordinated water molecules
	310	10.27	9.66	--	--	Loss due to a coordinated Chlorine atom and CH_3 species of coumarin moiety
	340	20.68	19.89	--	--	Loss due to C_5HO_2 molecule of coumarin moiety
	430	24.42	27.23	--	--	Loss due to C_8H_6 species of thiazole moiety
	Up to 731	--	--	11.22	12.90	Loss due to remaining organic moiety
$[Co(L)_2]$	258	9.33	9.36	--	--	Loss of a $C_4H_4O_2$ species of coumarin moiety
	304	46.35	45.14	--	--	Loss of two moles of 2-amino-4-phenylthiazole molecules
	375	43.20	41.71	--	--	loss of $C_{11}H_6O_3$ group of coumarin moiety
	Up to 632	--	--	12.11	13.97	Loss due to remaining organic moieties.
$[Ni(L)_2]$	154	11.59	11.04	--	--	Loss due to CH_3 group and $C_4H_4O_2$ species of coumarin moiety
	358	36.73	36.85	--	--	Loss due to $C_7H_2O_2$ molecule of coumarin and $C_9H_8N_2S$ molecule of thiazole moiety
	372	26.95	26.60	--	--	Loss due to C_8H_6S species
	Up to 380	--	--	10.14	12.13	Loss due to remaining organic moiety
$[Zn(L)(Cl)(H_2O)_2]$	160	7.02	6.48	--	--	Loss due to two coordinated water molecules
	220	9.78	9.62	--	--	Loss due to coordinated Chlorine atom and methyl species of coumarin moiety
	334	36.44	37.49	--	--	Loss due to a molecule of 2-amino-4-phenylthiazole
	490	26.34	23.51	--	--	Loss due to C_2H species and CO_2 molecule
	Up to 731	--	--	10.71	12.23	Loss due to remaining organic moiety.

Table 4 - Brine shrimp bioassay data

Compounds	LD_{50} (M/mL)
L	2.262×10^{-4}
$[Cu(L)(Cl)(H_2O)_2]$	2.142×10^{-4}
$[Co(L)_2]$	1.106×10^{-4}
$[Ni(L)_2]$	2.216×10^{-4}
$[Zn(L)(Cl)(H_2O)_2]$	1.112×10^{-4}
Bleomycin	0.410×10^{-4}

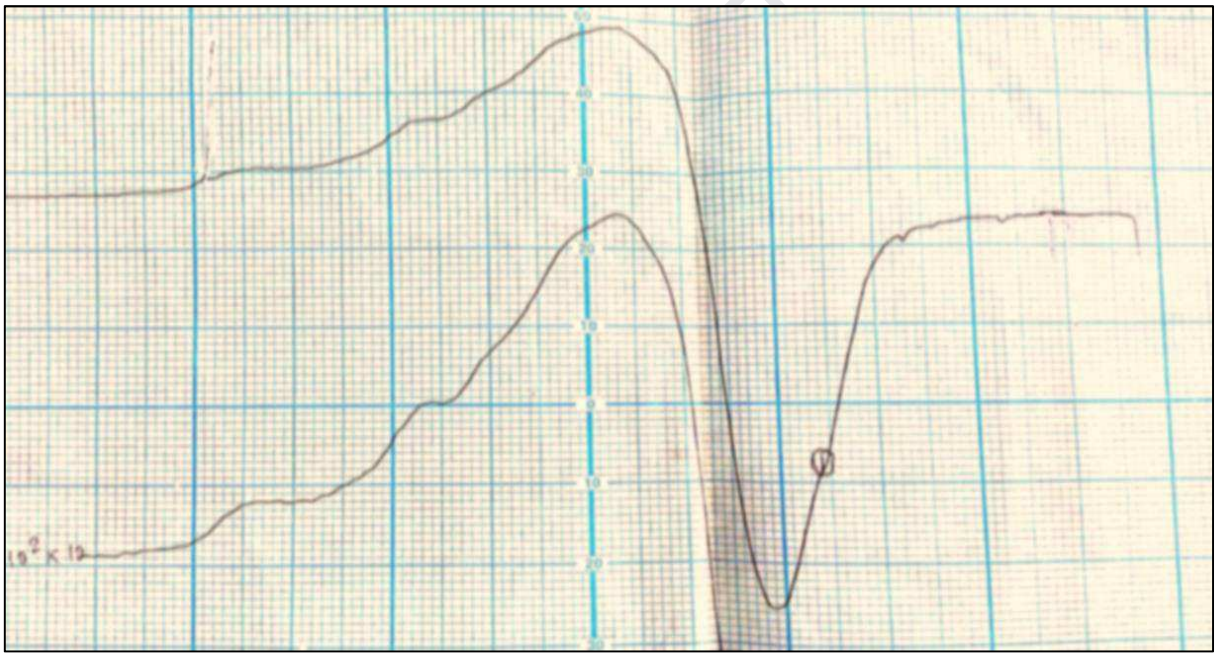


Fig. 1 - ESR spectrum of $[Cu(L)(Cl)(H_2O)_2]$ complex

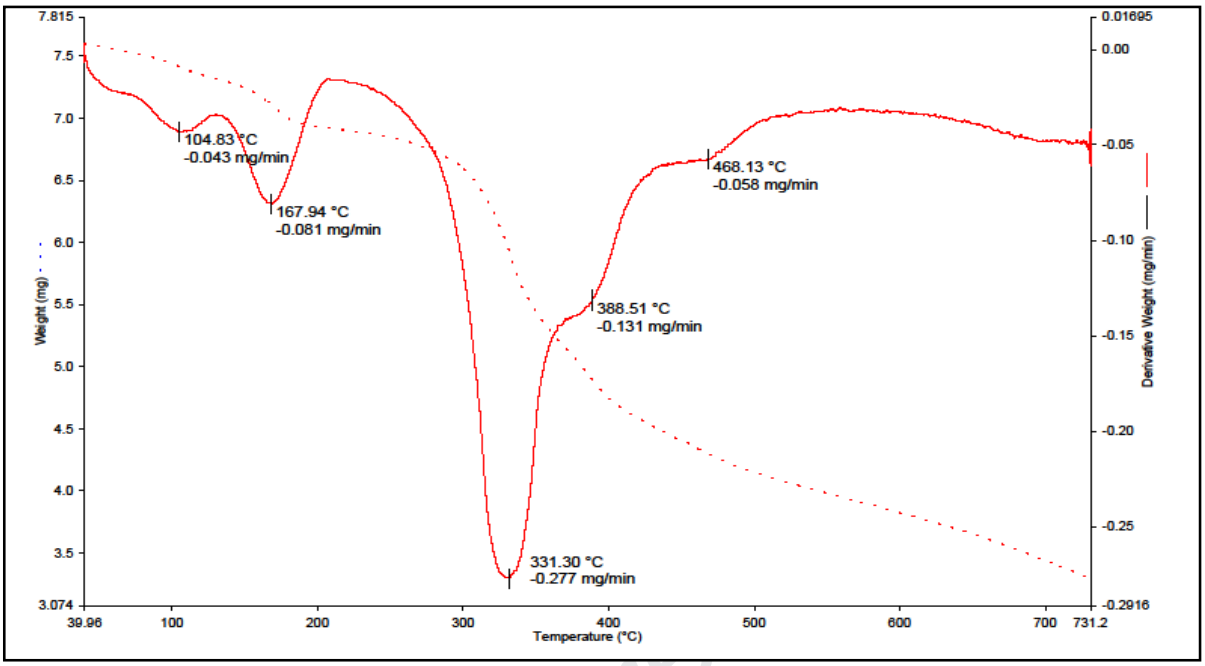


Fig. 2 - The Thermogram of $[Cu(L)(Cl)(H_2O)_2]$ complex

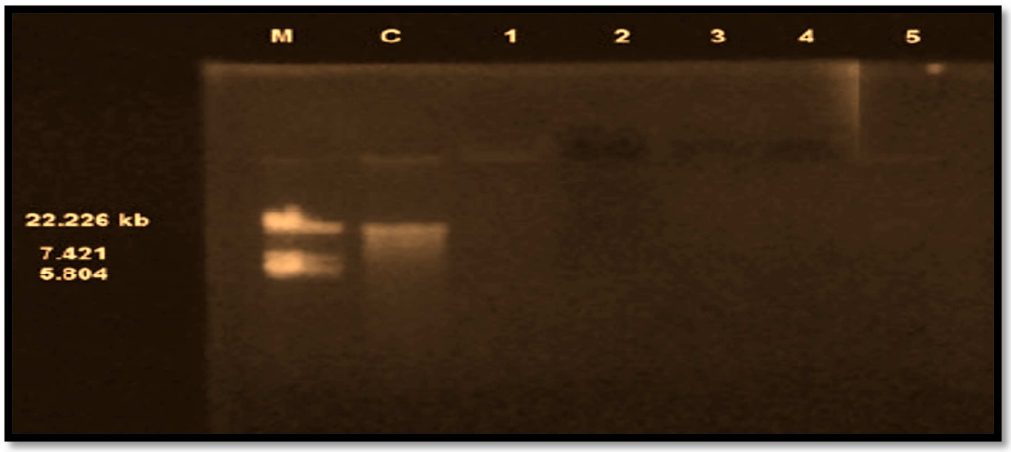


Fig.3 - DNA cleavage on plasmid pBR 322

M: Standard DNA, C: Control DNA (untreated pBR 322), **1**: **L**, **2:**$[Cu(L)(Cl)(H_2O)_2]$**3:**$[Co(L)_2]$ **4:**$[Ni(L)_2]$ **5:**$[Zn(L)(Cl)(H_2O)_2]$

Figure captions:

1. Fig. 1 - ESR spectrum of $[Cu(L)(Cl)(H_2O)_2]$ complex
2. Fig. 2 - The Thermogram of $[Cu(L)(Cl)(H_2O)_2]$ complex
3. Fig. 3 - DNA cleavage on plasmid pBR 322
   M: Standard DNA, C: Control DNA (untreated pBR 322), **1**: **L**, **2:**$[Cu(L)(Cl)(H_2O)_2$ **3:**$[Co(L)_2]$ **4:**$[Ni(L)_2]$ **5:**$[Zn(L)(Cl)(H_2O)_2]$

Research Highlights:

- Synthesis of Octahedral Metal (II) complextes.
- The synthesized complexes shows promising antimicrobial activities compared to ligands.
- Thermogravimetric studies for Cu(II) and Zn(II) complexes indicated the presence of coordinated water molecules.
- The prepared complexes have good ability to cleave the pBR322 DNA.

Research Article

Preparation of Octahedral Cu(II), Co(II), Ni(II) and Zn(II) Complexes Derived from 8-formyl-7-hydroxy-4-methylcoumarin: Synthesis, Characterization and Biological study

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Conflict of Interests:

The authors do not have any agreement, financial assistance or sponsorship from any institution except providing the data which are of purely academic interest and to encourage young researchers in India. The IR spectra of compounds were recorded on Perkin Elmer-Spectrum RX-I FTIR spectrophotometer using KBr disc technique, ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz NMR Spectrometer in *d*6-DMSO using TMS as an internal standard, ESI mass spectra were recorded by electrospray ionization (ESI) on a Waters Micromass Q-Tof Micro spectrometer, ESR spectrum was recorded on JES-FA200 ESR spectrometer, and so forth. These names are mentioned in the experimental protocol as these are the instrument models and it is mandatory for authors to mention the instrument models used to scan the spectra of unknown compounds. Otherwise, the corresponding author or coauthor has no-direct financial relationship with the commercial identity in any form of the institutes mentioned in our paper.