OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE www.BiointerfaceResearch.com ISSN 2069-5837

*Volume 1, Issue 4, 2011, 127-138*

**Received: 05.06.2011 / Accepted: 25.07.2011 / Published on-line: 15.08.2011**

# Synthesis, spectral characterization, DNA interaction studies, anthelmintic and antimicrobial activity of transition metal complexes with 3-(2-hydroxybenzylideneamino)-2-methylquinazolin-4(*3H*)-one and 1,10-phenanthroline

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## ABSTRACT

Mixed ligand complexes of cobalt(II) (1), copper(II) (2) and oxovanadium(IV) (3) with 3-(2-hydroxy benzylideneamino)-2-methylquinazolin-4(*3H*)-one and 1,10-phenanthroline have been synthesized and characterized by elemental analyses, IR, electronic, $^1$H-NMR, mass spectra, molar conductance and thermal studies. The synthesized compounds were screened for their *in vitro* antimicrobial activity. The results show a significant increase in antimicrobial activity of the complexes compared to ligand. Antihelmintic activity of the compounds has been tested on earthworms and the enhanced activity was observed upon complexation. In addition, DNA binding and DNA cleavage studies for the newly prepared compounds were also studied. These studies indicate that the DNA binding and cleavage efficacy were increased in the complexes relative to the parental ligand.

**Keywords:** *4(3H)-quinazolinone, transition metals, spectral studies, anthelmentic and antimicrobial activity, DNA interaction*

## 1. Introduction

Schiff bases and their metal complexes have attracted paramount importance due to their useful chemical, physical and medical applications. Some of them may be used as catalysts in various chemical processes [1], or as models for a better understanding of some biological systems [2]. There have been of considerable current interests to develop the chemistry of transition metal complexes those capable of cleaving DNA at specific sites, which play a major role in genomic research and photodynamic therapy of cancer [3, 4].

In recent years, there has been an increasing interest in the chemistry of 4(*3H*)-quinazolinones because of their biological significance. Many of them show antifungal, antibacterial, anticancer, anti-inflammatory, anticonvulsant and antiproliferative activities. On the other hand, heterocycles containing the pyridazine nucleus also exhibit various pharmacological activities; reduction of blood pressure, anticonvulsant activity and moreover there pyridazine derivatives that have shown an interesting affinity towards $A_1$-receptor and are potentially usable as

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cerebroprotective agents. Recently, a few researchers have been reported cobalt(II), copper(II), nickel(II) complexes with 1,2,4-triazole Schiff bases[5] and cobalt(II), copper(II), zinc(II), cadmium(II), nickel(II) and mercury(II) complexes with 3-[(2-hydroxy-quinolin-3-ylmethylene)-amino]-2-phenyl-*3H*-quinazolin-4-one [6]. The chemical literature survey reveals that metal complexes of quinazolinone Schiff base have not been reported so far or very scanty.

Further, transition metal complexes of 1,10-phenanthroline or their modified variants have been widely employed in DNA studies due to their applicability in several areas of research, including bioinorganic and biomedicinal chemistry [7-9]. The metal complexes can bind to DNA in non-covalent modes such as electrostatic, intercalative and groove binding. The above applications require that the complex can bind to DNA through an intercalative mode wherein the planar aromatic heterocyclic group insert and stacks between the base pairs of DNA [10-13]. Furthermore, upon irradiation these complexes can promote DNA cleavage, and they also exhibit enantioselectivity in DNA cleavage. These findings underscore the importance of an intimate association of the metal ion with the duplex DNA. The high level of recognition of DNA conformation by these chiral inorganic complexes suggests their utility in the design of stereospecific DNA binding drugs.

Thus, the chemical literature promotes us to synthesize a new Schiff base ligand and its metal complexes. Therefore, the present paper reports preparation and characterization of cobalt(II), copper(II) and oxovanadium(IV) complexes with a bidentate Schiff base ligand derived from 3-amino-2-methyl-4(3*H*)quinazolinone and salicylaldehyde forming the coordination units of a general formula [M(L)(X)] (where M= cobalt(II), copper(II), oxovanadium(IV); L= Schiff base ligand; X= 1,10-phenanthroline). As a part of extensive primary biological screening, the ligand and its complexes have also tested for *in vitro* antimicrobial activity, anthelmentic activity, DNA binding and DNA cleavage studies.

# 2. Experimental section

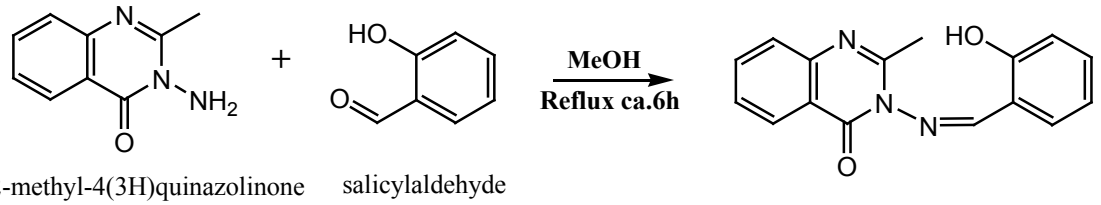
**2.1. Materials and methods.** All the chemicals and solvents were of AR grade. Solvents were used as supplied by commercial sources without any further purification. Elemental analyses (C, H, N) were determined using a Perkin-Elmer 240 elemental analyser. The IR spectra of the ligand and its cobalt(II), copper(II) and oxovanadium(IV) complexes were recorded on a Shimadzu FT-IR-8300 instrument using KBr discs in the range of 4000-200 $cm^{-1}$. Molar conductivity measurements were recorded on a CM-82T Elico conductivity bridge with a cell having cell constant 1. The $^{1}H$ NMR spectra of ligand and its metal complexes were recorded on Bruker DRX-300 spectrometer in DMSO-$d_6$ solution, with TMS as the internal standard. is recorded on VARIAN-2000 mass spectrometer by ESI-MS technique. Thermogravimetric analyses data were measured from room temperature to 700 °C at a heating rate of 20 °C/min. The data were recorded on a Shimadzu TG-50 thermobalance. Electronic spectra were recorded using an hp spectrophotometer (Agilent 8453).

**2.2. Chemistry**

**2.2.1. Synthesis of Schiff base ligand (L).** A methanolic solution of 3-amino-2-methyl-4(*3H*)quinazolinone (0.1752g, 1 mmol, 15 mL) was added to a solution of salicylaldehyde (1 mmol) in methanol (5 mL). The reaction mixture was refluxed for 6 h with continuous stirring. On cooling, a colorless product was obtained which was filtered, washed with $H_2O$ and recrystallized from methanol. (Yield: 71%; IR (KBr $cm^{-1}$): 3067.3, 2974.6, 2367.3 (C-H), 1656.0 (N-C=O), 1603 (C=N).; $^{1}H$-NMR (300 MHz, DMSO-$d_6$) δ: 2.7(s, $CH_3$, 3H, $C_8$), 9.1 (s, CH, 1H, -N=CH-), 10.6 (s, OH, 1H), 7.31-8.12 (m, Ar-H, 8H, Aromatic protons); Mass (m/Z): 280 [M+1, 79 %]; Anal: Calcd.

For $C_{16}H_{13}N_3O_2$: C 68.43, H 4.58, N 14.9, Found: C 68.5, H 4.61, N 15.02 %. Based on the above spectral data, the following structure has been assigned to the prepared ligand (Scheme 1).

**2.2.2. Synthesis of Cobalt(II), Copper(II) and Oxovanadium(IV) Schiff base complexes.** A methanolic solution of Schiff base ligand (0.279 g, 1 mmol) and 1,10-phenanthroline (0.198 g, 1 mmol) in methanol was added drop wise to a 20 mL hot methanolic solution of $CoCl_2.6H_2O$ (0.236 g, 1 mmol) or $CuCl_2.2H_2O$ (0.169 g, 1 mmol) and aqueous methanolic solution of $VOSO_4.3H_2O$ (0.163 g, 1 mmol). The reaction mixture was stirred for 30 min and refluxed for 4 h on a water bath. The volume of the solution was reduced to half of its original volume. The solid compound obtained was filtered off, washed with hot water, methanol, then with ether and dried in vacuum over $CaCl_2$.




3-amino-2-methyl-4(3H)quinazolinone salicylaldehyde

**(L)**

**Scheme 1:** Synthesis of 3-(2-hydroxybenzylideneamino)-2-ethylquinazolin-4(*3H*)-one ligand **(L)**

**2.3. Pharmacology**

**2.3.1. DNA binding experiments.** The experiments at room temperature involving interaction of the metal complex with calf thymus (CT) DNA were performed in Tris HCl buffer (5mM tris(hydroxyl methyl)aminomethane, tris, pH 7.2 and 50 mM NaCl). A solution of DNA in buffer gave a ratio of *ca* 1.84:1 at absorbance of 260 nm ($A_{260}$) and 280 nm ($A_{280}$), indicating that the calf thymus DNA was free from protein. The concentration of CT DNA per nucleotide was measured by using its known extinction coefficient at 260 nm (6000 $M^{-1}$ $cm^{-1}$). Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 min). From the observed data, the intrinsic binding constant, $K_b$ was calculated by using the following equation [14].

$$\mathbf{[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA]/ (\varepsilon_b - \varepsilon_f) + 1/ K_b (\varepsilon_a - \varepsilon_f)}$$

where $\varepsilon_a$, $\varepsilon_f$, $\varepsilon_b$ are the apparent, free and bound metal complex extinction coefficients. A plot of [DNA]/ ($\varepsilon_b$- $\varepsilon_f$) versus [DNA] gave a slope of 1/ ($\varepsilon_b$- $\varepsilon_f$) and a '*y*' intercept equal to $1/K_b(\varepsilon_b - \varepsilon_f)$, where $K_b$ is the ratio of the slope to the *y* intercept.

**2.3.2. Viscosity measurements.** In order to confirm the binding modes of the compounds in the interaction with CT-DNA, viscosity measurements were carried out using an Ubbelodhe viscometer at room temperature. Flow time was measured by hand with digital stopwatch, each sample was measured three times and the average flow time was calculated. The data were reported as $(\eta/\eta_o)^{1/3}$ versus the binding ratio, where $\eta$ is the viscosity of DNA in the presence of the compound and $\eta_o$ is the viscosity of DNA solution alone. Viscosity values were calculated from the observed flow time of DNA containing solution corrected for the flow time of the buffer alone.

**2.3.3. DNA cleavage experiment.** The cleavage of DNA was monitored using agarose gel electrophoresis. The experiments were performed using supercoiled pUC19 DNA. DMF solutions of both ligand **(L)** and vanadium complex **(3)** were placed in clean Eppendorf tubes and 1μg of pUC19 DNA was added. The contents were incubated for 30 min at 37 °C. The samples were electrophoresed at constant voltage (70 V) on a 1 % agarose gel in Tris-Boric acid-EDTA (TBE) buffer. The gel was stained with a 0.5 μg/mL ethidium bromide and visualized by UV light and photographed for analysis. The extent of cleavage was measured from the intensities of the bands using the Alpha Innotech Gel documentation gel system (AlphaImager 2200). For mechanistic

investigation, reactions were carried out by adding radical scavenging agents to supercoiled DNA prior to the addition of the complex before incubation [15].

**2.3.4. Anthelmintic activity.** Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Six groups of six earthworms each were released in to 10 mL of solutions of piperazine citrate and test solution in DMSO. Piperazine citrate was used as reference standard while DMSO as control.

**2.3.5. Antimicrobial activity.** The *in vitro* antimicrobial screening effects of the synthesized compounds were tested against five bacterial strains namely, *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Ralstonia solanacearum* and *Xanthomonas vesicatoria* by disk diffusion method, using nutrient agar medium [16]. The antifungal activities of the compounds were evaluated against *Aspregillus niger, Aspergilus flavus, Fusarium oxysporum* and *Alternaria solani* by disk diffusion technique using potato dextrose agar as medium. All the tests were performed in triplicate, and average is reported. The stock solution (1 mmol) was prepared by dissolving the compounds in DMSO and the solutions were serially diluted in order to find the MIC values [17]. The well was made on agar medium, inoculated with microorganisms. To this well, the test solution was added and the Petri dishes were incubated for 24 h for bacteria and 72 h for fungi at 35 °C. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted and was measured in mm. Results were compared with standards namely; Chloramphenicol against bacteria and Amphotericin B against fungi. The microbial cultures were obtained from department of Biotechnology, Manasagongotri, Mysore.

# 3. Results section

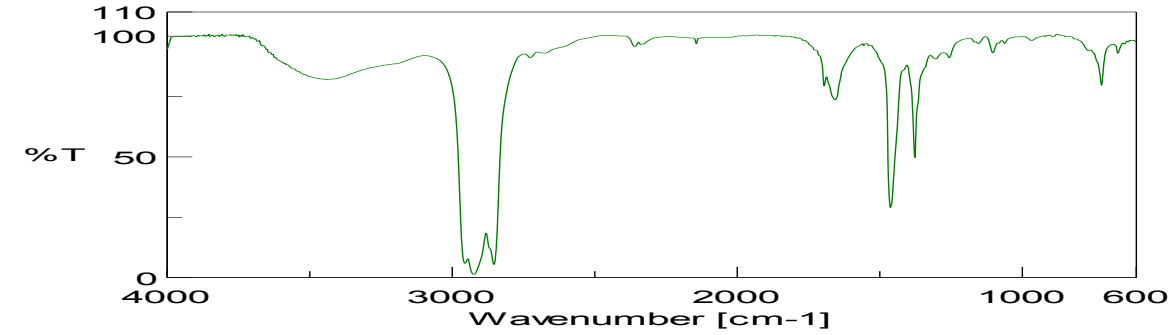
The elemental analyses agree well with the proposed formulae for the complexes. The cobalt(II), copper(II) and oxovanadium(IV) complexes are pinkish, green and brown, respectively. The complexes are air stable solids, soluble in DMSO and DMF and insoluble in water and ethanol. Their conductivity values were measured in DMSO at room temperature, fall within the usual range for non-electrolytes. Analytical data for the compounds are given in Table 1.

**Table 1:** Analytical data and some physical properties of the Schiff base and its metal complexes.

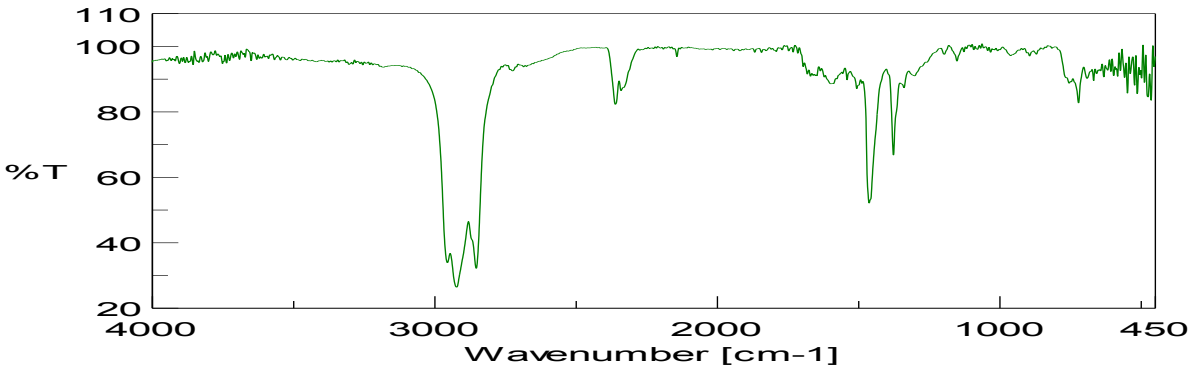
| Compd.no | Empirical formula | Molecular Mass | % | M % | | C % | | H % | | N % | | Molar Conductance S $cm^2Mole^{-1}$ | $\mu_{eff}$(BM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Obsd | Calcd | Obsd | Calcd | Obsd | Calcd | Obsd | Calcd | | |
| L | $C_{16}H_{13}N_3O_2$ | 279 | 87.3 | - | - | 68.5 | 68.7 | 4.62 | 4.76 | 15.11 | 15.23 | _ | _ |
| 1 | $C_{28}H_{21}N_5O_2Co.2H_2O$ | 554 | 67.2 | 10.26 | 10.34 | 58.62 | 58.78 | 3.35 | 3.43 | 12.24 | 12.39 | 21.7 | 4.7 |
| 2 | $C_{28}H_{21}N_5O_2Cu..H_2O$ | 541 | 66.4 | 11.71 | 11.89 | 62.0 | 62.14 | 3.52 | 3.63 | 12.91 | 13.12 | 21.9 | 2.1 |
| 3 | $C_{28}H_{21}N_5O_2VO$ | 526 | 66.1 | 12.35 | 12.51 | 61.62 | 61.79 | 3.44 | 3.57 | 12.82 | 12.9 | 23.5 | 1.9 |

**3.1. Infrared spectral studies**. The infrared spectra of the free ligand and its complexes were obtained in the range 4000-200 $cm^{-1}$. Figure 1 shows the IR spectra of ligand (L) and its cobalt complex. Appearance of weak to strong frequency difference in the region 2750-2739 $cm^{-1}$ can be attributed to the intramolecular H-bonding and this suggests the participation of OH group in the coordination with cobalt complex. The C=O stretching vibration appeared at 1656 $cm^{-1}$ in the Schiff base is shifted towards lower frequencies (10-14 $cm^{-1}$) in the complexes. This shift confirms the

participation of keto group in the coordination. A strong band appeared at 1603 $cm^{-1}$ in the ligand can be assigned to the ν(C=N) stretching vibration. In complexes of the prepared ligand, ν(C=N) bands are shifted by 10-21 $cm^{-1}$ to lower wave number. Thie indicated that the possibility of coordination of the imino nitrogen to metal ions. The ring skeletal vibrations ν(C=C) were consistent and were unaffected by complexation. All the IR data suggest that the metal was bonded to Schiff base through the oxygen of keto group and the imino nitrogen, but also with phenolic oxygen in cobalt complex. All these complexes exhibit a broad band in the region 3435-3453 $cm^{-1}$ indicating the presence of lattice or coordinated water molecule [18]. The IR spectra of 1,10-phenanthroline showed peak at 739 $cm^{-1}$ and in the complexes it was shifted in the range 717-721 $cm^{-1}$. This shift can be explained on the basis of the fact that the nitrogen atoms of phenanthroline donate a pair of electrons to the central metal ion, forming a coordinative covalent bond [19]. Besides, it is also confirmed by the shift of ν(C=N) of phenanthroline from 1665 $cm^{-1}$ in the free ligand to 1621-1626 $cm^{-1}$ upon coordination [20].




(a)




(b)

**Figure 1:** IR spectra of (a) L and (b) its Co(II) complex.

**3.2. Electronic spectra.** The UV-Visible spectra of the ligand and its complexes were recorded in DMF at room temperature. The electronic spectrum of ligand shows only one intense band at 292 nm, which is assigned to n→ π* transition of the C=N chromophore. Upon complexation, this is shifted to higher wavelength, suggesting the coordination of azomethine nitrogen (Figure 2).

The UV-Vis spectra of all the complexes showed intense band in the range 298-415 nm, which could be associated with metal to ligand charge transfer transition. The cobalt(II) complex exhibits three bands having $\lambda_{max}$ at 325, 397 and 415 nm. The first two bands were assigned to the π → π* transition of the >C=N and the ligand-to-metal ion charge-transfer band (L→ Co) as a result of complex formation. The last band can be assigned to $^4T_{1g}$ (F) → $^4A_{2g}$ (F) which reveals the octahedral geometry of the cobalt(II) complex. Similarly, copper(II) and oxovanadium(IV) complexes exhibits two bands each at 298, 349 and 300, 400 nm, respectively. These can be

assigned to the π→ π* transition and the ligand -to-metal-ion charge-transfer band, respectively for copper and oxovanadium complexes. This result suggest distorted octahedral geometry for copper(II) complex and square pyramidal geometry for oxovanadium(IV) complex.

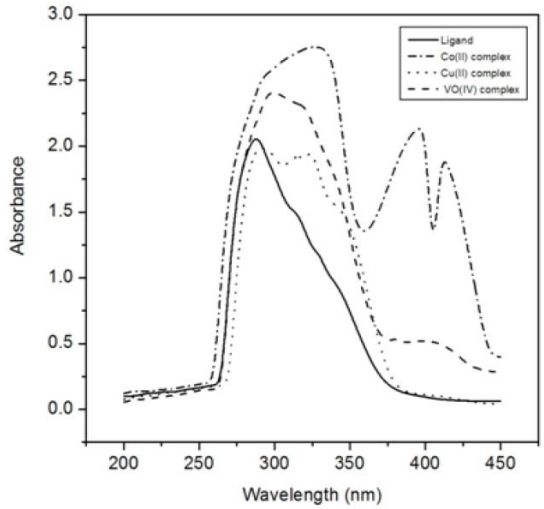



**Figure 2:** Electronic spectra of Schiff base ligand and its metal complexes.

**3.3. $^1$H-NMR spectra.** The $^1$H-NMR spectra of the compounds were obtained in DMSO-$d_6$ at room temperature using TMS as an internal standard. The proton NMR spectrum of ligand is shown in Figure 3. The chemical shift observed for the OH proton in the ligand (10.6 ppm) was also appeared in Cu(II) and VO(IV) complexes but disappeared in Co(II) complex. This confirms that the bonding of phenolic oxygen to the metal ions has taken place in Co(II) complex. The same result was confirmed by the IR spectra. The presence of a sharp singlet for the –(H)C=N proton in ligand (9.2 ppm) was shifted to 8.83 ppm (s) in cobalt(II) complex, 8.74 ppm (s) in copper(II) complex and 8.91 ppm (s) in the oxovanadium(IV) complex, confirming that the coordination of imino nitrogen. The multiplets of aromatic protons appeared within the range of 7.3-8.4 ppm and they are not affected by chelation.

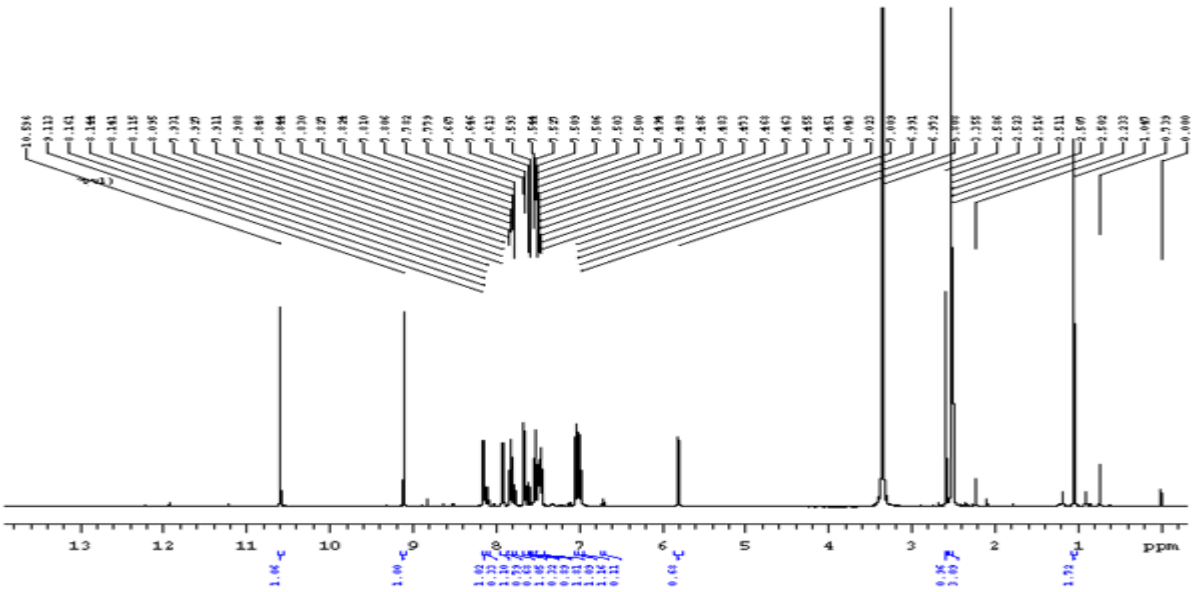



**Figure 3:** $^1$H-NMR spectrum of ligand (L).

**3.4. Mass spectra.** The mass spectrum of Schiff base **L** is recorded on VARIAN-2000 mass spectrometer by ESI technique and is shown in Figure 4. The spectrum showed molecular ion peak

at m/z = 280 corresponding the molecular weight confirming the molecular formula of the ligand **L**. The peaks at m/z 260, 176, 161, 147, 99 correspond to the fragmentation of the molecular ion.

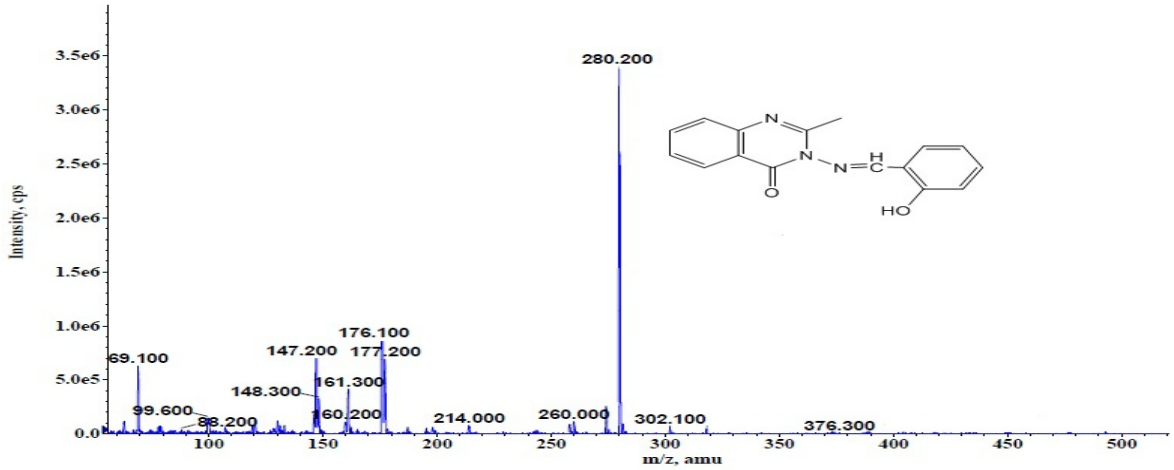
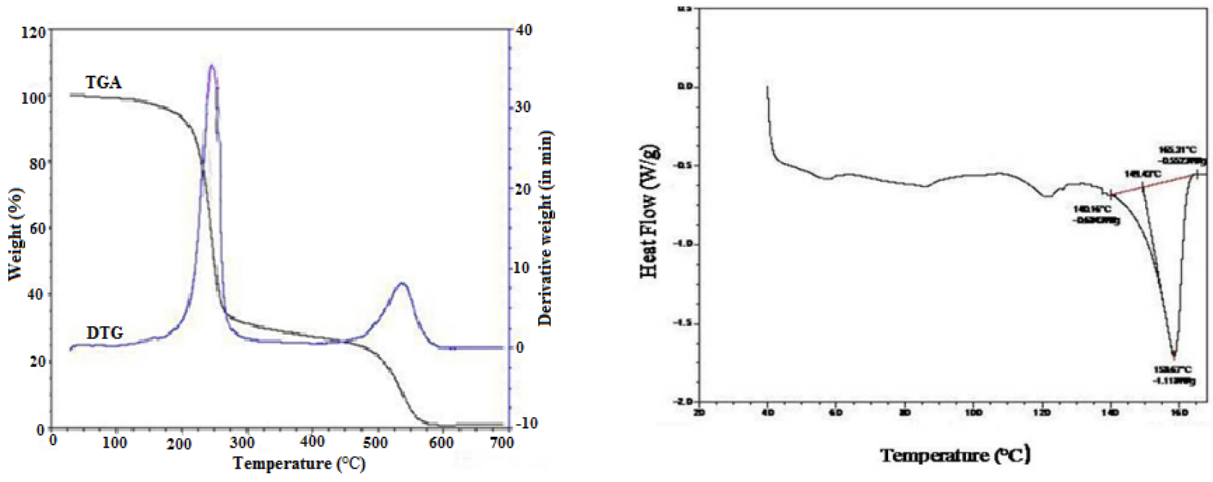



**Figure 4:** Mass spectrum of Schiff base ligand **(L)**.

**3.5. Thermal studies**. TGA/DTG curve of oxovanadium(IV) complex **(3)** and DSC curve of Schiff base ligand is represented in Figures 5(a) and 5(b), respectively. The decomposition of the complex undergoes in three stages. The first weight loss of 3.11 % (calcd. 2.98 %) in the temperature range 140-180 °C corresponds to the loss of coordinated water molecule in the complex. On pyrolysis, the dehydrated complex further decomposes in two steps. A weight loss of 71.35 % (calcd. 71.87 %) in the temperature range of 165-280 °C corresponds to the decomposition of the ligand. It is further supported by exothermic peak at 165.31 °C in DSC curve. Third stage occurs in 350-600 °C, corresponding to the decomposition of remaining part of the ligand, with the weight loss of 20.32 %. The maximum rate of weight loss is indicated by the DTG peak at 222.5 °C. The total weight loss (91.67 %) coincides with the theoretical value of 91.73 %. The final residue estimated as free vanadium oxide has the observed mass 8.21 % as against the calculated value of 8.27 %.




(a) (b)

**Figure 5:** a) Thermogravimetric (TGA and DTG) curve of oxovanadium(IV) complex**(3)** and (b) DSC curve of ligand **(L).**

### 3.6. Pharmacological results

**3.6.1. DNA binding experiments.** Absorption spectroscopy is one of the most useful techniques to study the binding of any drug to DNA [21]. The binding behavior of oxovanadium(IV) complex to DNA helix has been followed through absorption spectral titrations. The absorption spectra of the

vanadium complex, in the absence and in the presence of CT-DNA, are shown in Figure 6. With increasing concentration of calf thymus DNA, the absorption bands of the complexes were affected in the 310-390 nm region. The complexes can bind to the DNA in different binding modes on the basis of their structure, charge and type of ligands. Since, the oxovanadium(IV) complex contains Schiff base **L** and 1,10-phenanthroline ligand, it would provide an aromatic moiety extending from the metal center through which overlapping would occur with the base pairs of DNA by intercalation. The observed $K_b$ values for oxovanadium(IV) complex ($2.1 \times 10^6$ $M^{-1}$) is almost equal to classical intercalators [Ethidium-DNA, $K_b$, $1.8 \times 10^6$ $M^{-1}$] in 25 mM Tris-HCl/40 mM NaCl buffer, pH 7.9]. Thus, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

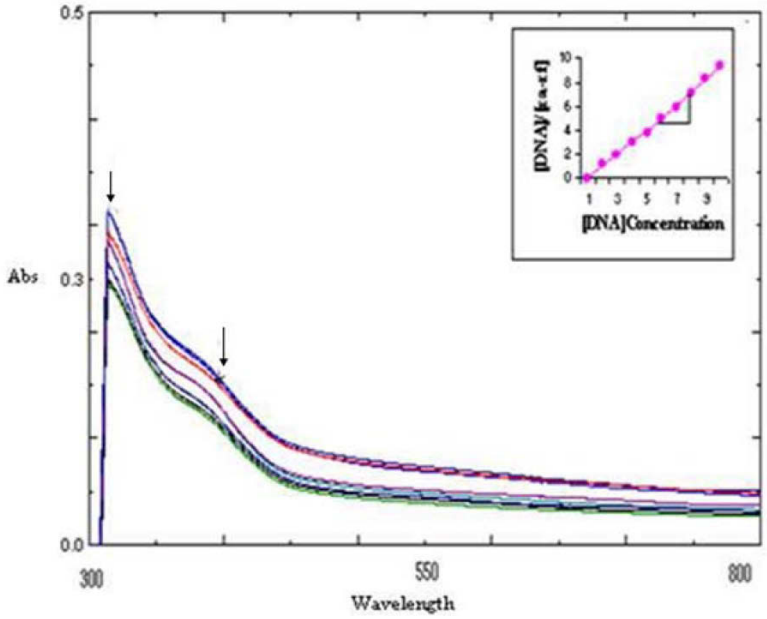



**Figure 6:** Absorption spectra of vanadium complex in Tris-HCl buffer upon addition of DNA = 0.5 μM, 0-100 μM. Arrow shows the absorbance changing upon increasing the concentration of DNA. The inner plot of[DNA]/ ($\varepsilon_a - \varepsilon_f$) versus [DNA] for the titration of DNA with oxovanadium(IV) complex.

**3.6.2. Viscosity measurements.** The binding modes of the oxovanadium(IV) complex were further investigated by viscosity measurements. In the absence of crystallographic structural data and hydrodynamic methods, which are sensitive to increase in DNA length, are regarded as the least ambiguous and the most critical tests of binding in solution. Under appropriate conditions, intercalation causes a significant increase in the viscosity of DNA solutions due to the separation of base pairs at intercalation sites and hence, increase the overall DNA contour length whereas ligands that bind exclusively in the DNA grooves, groove-face or electrostatic interactions typically cause a bend (or kink) in DNA helix reducing its effective length and thereby increase in its viscosity. The effect of the oxovanadium(IV) complex **(3)** and ligand (**L**) on the viscosity of DNA are shown in Figure 7.

**3.6.3. DNA cleavage**The DNA cleavage by the synthesized ligand and oxovanadium(IV) complex in the presence and absence of $H_2O_2$ as an oxidant was studied using pUC19 DNA under aerobic conditions. The results shown in Figure 8 indicate that the ligand does not exhibit significant activity in the absence and presence of $H_2O_2$ (lane 3, 4). It was observed that the cleaving activity of the complex was higher in the absence and presence of the oxidant (lane 5, 6). The significant cleaving activity of oxovanadium(IV) complex in the absence of oxidant may be due to the reaction of

hydroxyl radical with DNA. In the presence of $H_2O_2$, the cleavage may be due to hydrolytic cleavage of DNA catalyzed by oxovanadium(IV) complex.

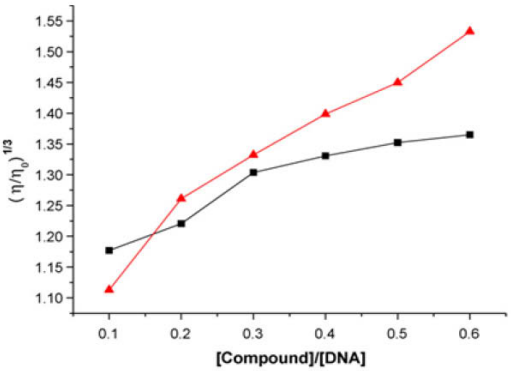



**Figure 7:** Effect of increasing amounts of oxovanadium(IV) complex (▲) and ligand (■) on the relative viscosities of CT-DNA at room temperature.

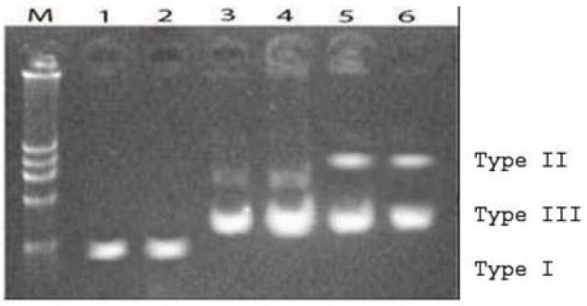



**Figure 8:** Cleavage of supercoiled pUC19 DNA (0.5 µg) by the ligand and oxovandium(IV) complex in a buffer containing 50 mM Tris-HCl at 37 $^0$C (30 min): lane M: marker; lane 1: DNA control; lane 2: Ligand ($10^{-3}$ M) + DNA; lane 3: DNA + $H_2O_2$; lane 4: Ligand + DNA + $H_2O_2$; lane 5: oxovanadium(IV) complex ($10^{-3}$ M) + DNA; lane 6: oxovanadium(IV) complex + DNA+ $H_2O_2$.

**3.6.4. Anthelmentic activity.** Antihelmentic activity was evaluated on earth worms (*Pheritima posthuma*) using the prepared ligand (**L**) and its metal complexes by reported method [22]. Ligand exhibited moderate activity and the antihelmentic activity is enhanced significantly upon complexation with cobalt(II), copper(II) and oxovanadium(IV) ions. The enhanced activity may be due to reduce in the excitability that leads to muscle relaxation and flaccid paralysis. The results of anthelminthic activity are shown in Table 2.

**Table 2:** Results of anthelmentic activity of Schiff base ligand (L) and its metal complexes.

| Compd. | Conc. (mg mL$^{-1}$) | Time taken for paralysis (min) | Time taken for death (min) |
|---|---|---|---|
| Normal saline[a] | 5 | no effect till ten hours | no effect till ten hours |
| Piperzine citrate[b] | 5 | 10 | 15 |
| L | 5 | 11 | 18 |
| 1 | 5 | 07 | 09 |
| 2 | 5 | 08 | 11 |
| 3 | 5 | 04 | 05 |

[a] blank and [b]standard.

**3.6.5. Antimicrobial results.** For *in vitro* antimicrobial activity, the newly synthesized Schiff base and its complexes were tested against the bacterial strains *B. subtilis, E.coli, S. aureus, R. solanacearum* and *X. vesicatoria* and fungi *A. niger, A. flavus, F. oxysporum* and *A. solani* by determining the zone of inhibition(Table 3 and 4). The free ligand **L** and its cobalt(II), copper(II) and oxovanadium(IV) complexes showed variable *in vitro* antimicrobial activities against tested

microbial strains (Table 5). The ligand **L** (128 µg $mL^{-1}$) showed the lowest MIC value against *R solanacearum*. The results were compared with that of Chloramphenicol (MIC= 4 µg $mL^{-1}$) [23], a standard antibiotic for bacterial strains and Amphotericin-B (4-8 µg $mL^{-1}$) for fungal strains. The stock solution of each test compound was prepared in DMSO. All the tested compounds were found to be moderate to better active against the tested strains. The metal complexes showed antimicrobial activities against all the tested bacterial strains with the MIC values in the range of 8-32 µg $mL^{-1}$ (Table 5). The complexes **1** and **3** was found to be most active against the tested bacterial strains at 8-16 µg $mL^{-1}$, whereas complex **2** inhibited the bacterial growth at a concentration of 32 µg $mL^{-1}$. The complexes exhibited effective antifungal activities with the MIC values in the range of 8-64 µg $mL^{-1}$. Particularly, complex **3** was effective against all the fungal strains at 8 µg $mL^{-1}$. The complexes **1** and **2** showed moderate to better effectiveness at variable concentrations (8-64 µg $mL^{-1}$). Metal salts were also evaluated for antimicrobial activity which showed very negligible activity compared to ligand and its metal complexes.

**Table 3:** *In vitro* antibacterial activity of the Schiff base ligand and its metal complexes.

| Compound | Conc.(µg $mL^{-1}$) | Antibacterial activity (Zone of inhibition in %)[a] | | | | |
|---|---|---|---|---|---|---|
| | | *B. subtilis* | *E.coli* | *S. aureus* | *R.solanacearum* | *X.vesicatoria* |
| L | 100 | 45 | 48 | 44 | 62 | 59 |
| 1 | 100 | 63 | 68 | 71 | 71 | 70 |
| 2 | 100 | 59 | 59 | 53 | 65 | 62 |
| 3 | 100 | 71 | 73 | 76 | 81 | 79 |
| Standard[b] | 100 | 100 | 100 | 100 | 100 | 100 |

[a]the values are the average of three replicates and [b]Chloramphenicol

**Table 4:** *In vitro* antifungal activity of the Schiff base ligand and its metal complexes.

| Compound | Conc.(µg $mL^{-1}$) | Antifungal activity (Zone of inhibition in %)[a] | | | |
|---|---|---|---|---|---|
| | | *A. niger* | *A. flavus* | *F. oxysporum* | *A. solani* |
| L | 100 | 72 | 78 | 65 | 67 |
| 1 | 100 | 74 | 75 | 67 | 71 |
| 2 | 100 | 75 | 77 | 72 | 75 |
| 3 | 100 | 81 | 81 | 77 | 82 |
| Standard[b] | 100 | 100 | 100 | 100 | 100 |

[a]the values are the average of three replicates and [b]Amphotericin B.

# 4. Conclusions

A novel Schiff base ligand (L) and its cobalt(II), copper(II) and oxovanadium(IV) complexes were synthesized and characterized. It was determined that the bidentate behavior of the ligand was accomplished *via* the keto group and the azomethine nitrogen atom. All the synthesized compounds showed moderate antimicrobial activity against selected bacteria and fungi. The anthelmentic activity of the Schiff base was enhanced upon complexation with studied metal ions. The binding behavior of the oxovanadium(IV) complex with calf thymus DNA was characterized by absorption titration and viscosity measurements. These studies indicate that the oxovanadium(IV) complex binds more strongly to DNA *via* intercalative binding. Further, the cleavage results show that the oxovanadium(IV) complex, has more nuclease activity compared to ligand. Based on the results of spectral and elemental analyses results, the following structures have been proposed for the prepared complexes (Figure 11).

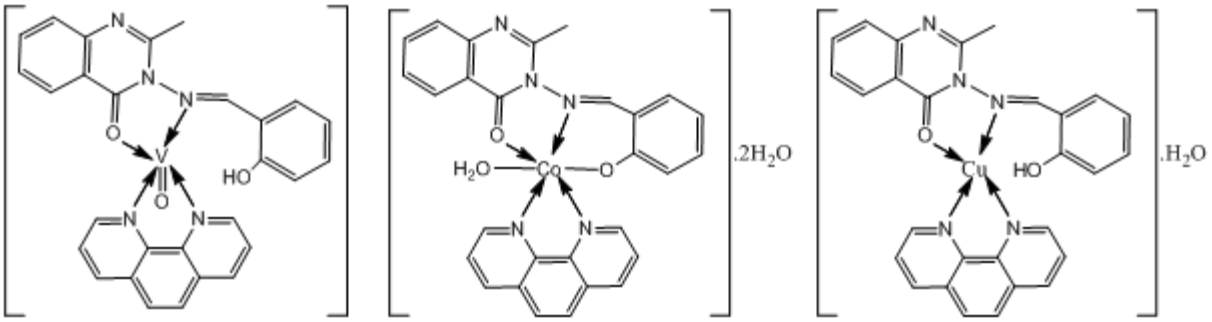



**Figure 11:** Proposed structures of the metal complexes.

# 5. References

[1] Sheldon R.A., Kochi J.K., *Metal catalyzed oxidation of organic compounds,* Academic Press, New York, 294, 162-171, **1999**

[2] Yan Z., Wang X., Ding L., Synthesis and DNA Binding Studies of Mg(II) Complex of Schiff Base Derived from Vanillin and L-Tryptophan, *Nucleosides, Nucleotides and Nucleic Acids*, 30, 49–62, **2011**

[3] Brenno A. D. N., Alexandre A. M. L., Recent Developments in the Chemistry of Deoxyribonucleic Acid (DNA) Intercalators: Principles, Design, Synthesis, Applications and Trends, *Molecules,* 14, 1725-1746, **2009**

[4] Tatiana D. R., Giampiero S., Maurizio P., Saison-Behmoaras T., Alexandre B., Cacciari B., Oligonucleotides and Oligonucleotide Conjugates: A New Approach for Cancer Treatment, *Current Medicinal Chemistry,* 12, 71-88, **2005**

[5] Gangadhar B. B., Prema S. B., Patil S. A., Synthesis, spectral characterization and in vitro biological studies of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases, *Journal of Enzyme Inhibition and Medicinal chemistry,* 24, 381-394, **2009**

[6] Siddappa K., Reddy T., Mallikarjun M., Reddy C. V., Synthesis, Characterization and Antimicrobial studies of 3-[(2-Hydroxy-quinolin-3-ylmethylene)-amino]2-phenyl-*3H*-quinazolin-4-one and its metal(II) complexes, *E-journal of chemistry*. 5, 155-162, **2008**

[7] Ji L. N., Zou X. H. Liu J. G., Shape-selective and enantioselective interaction of Ru(II)/Co(III) polypyridyl complexes with DNA, *Coordination Chemistry Reviews,*. 513, 216-217, **2001**

[8] Nagababu P., Naveena J., Latha L., Satyanarayana S., DNA-Binding Studies of Mixed-Ligand (Ethylenediamine)ruthenium(II) Complexes, *Chemistry & Biodiversity*, 3, 1219-1228, **2006**

[9] Kelly S. O., Barton J. K., In Sigel, A.; Sigel, H (Eds.); *Metal Ions in Biological Systems,* New York: Marcel Dekker, **1998**
[10] Archika C. B., Smita G., Anupa A. K., Avinash S. K., DNA-binding studies of mixed ligand cobalt(III) complexes, *Transition Metal Chemistry,* 30, 312–316, **2005**
[11] Qu X., Chaires J. B., Hydration Changes for DNA Intercalation Reactions, *Journal of American Chemical Society,* 123, 1-7, **2001**
[12] Nagababu P., Satyanarayana S., DNA binding and cleavage properties of certain ethylenediamine cobalt(III) complexes of modified 1,10-phenanthrolines, *Polyhedron,* 26, 1686-1692, **2007**
[13] Anbu S., Kandaswamy M., Electrochemical, magnetic, catalytic, DNA binding and cleavage studiesof new mono and binuclear copper(II) complexes, *Polyhedron*, 30, 123–131, **2011**
[14] Reichmann M. E., Rice S. A., Thomas C. A., Doty P., A further examination of the molecular weight and size of desoxypentose nucleic acid, *Journal of American Chemical Society,* 76, 3047-3053, **1954**
[15] Prabhakar M. C., Bhojya Naik H. S., Binding and oxidative cleavage studies of dna by mixed ligand co(iii) and ni(ii) complexes of quinolo [3,2-b]benzodiazapine and 1,10-phenanthroline, *Nucleosides, Nucleotides, and Nucleic Acids*, 26, 459–471, **2007**
[16] Cruickshank R., *Medicinal Microbiology, A Guide to Diagnosis and Control of Infection,* 11th ed, E & S Livingstone: Edinburgh and London, U.K, **1986**
[17] Vigar Z., *Atlas of Medical Parasitology*.: 2nd ed. P G Publishing House, Singapore, **1984**
[18] Koushik D., Jagnyeswar R., Mario M., Xin-Yi W., Song G., Pradyot B., Synthesis, crystal structure, magnetic property and oxidative DNA cleavage activity of an octanuclear copper(II) complex showing water–perchlorate helical network, *Journal of Inorganic Biochemistry,* 101(1), 95-103, **2007**
[19] Pansuriya P. B., Patel M. N., Synthesis, spectral, thermal, DNA interaction and antimicrobial properties of novel Cu(II) heterochelates. *Applied Organometallic Chemistry,* 21, 739-749, **2007**.
[20] Diaz G. F., Campos-Vallette M. M., Saavedra M. S., Clavijo R. E., Canales J. C., Costamagna J., Vargas J., Surface vibrational study of macrocycle complexes: Co(II), Ni(II), Cu(II) and Zn(II) bis(phenylhydrazine)-1, 10-phenanthroline, *Vibrational spectroscopy*, 28, 223-234, **2002**
[21] Farukh A., Mubashira A., Synthesis and characterization of dinuclear macrocyclic cobalt(II), copper(II) and zinc(II) complexes derived from 2,2,2′,2′-*S*,*S*[bis(bis-*N*,*N*-2-thiobenzimidazolyloxalato-1,2-ethane)]: DNA binding and cleavage studies, *European Journal of Medicinal Chemistry*, 44, 2, 834-844, **2009**
[22] Thorn G. W., Adams R. D., Braunwald E., Isselbacher K. J., Petersdrof R. G., *Harrison'sPrinciples of Internal Medicine*. In: Mcgraw Hill Co, New York, **1977**
[23] Dutta G. N., Gogoi J., Buragohain J., Inactivation of choramphenicol by *Staphylococcus aureus* biotype C from humans and animals. *Indian Journal of Medicinal Research,* 113, 11-13, **2001**

**Acknowledgement:** The authors are thankful to the University of Mysore, Mysore for financial support through minor research project. One of the authors, K. Shiva Prasad is thankful to the UGC-New Delhi for the award of meritorious fellowship. The authors are also thankful to the Head, Department of Biotechnology for providing facilities to do the nuclease activities.