

Journal of Chemical, Biological and Physical Sciences



An International Peer Review E-3 Journal of Sciences

Available online at www.jcbpsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT

Research Article

Synthesis, Characterization, Thermal, Fluorescence, *In Vitro* Anti-Tuberculosis, Anthelmintic and Dna Cleavage Studies of Mononuclear Metal Complexes of 2h-Chromeme-2-One Schiff Base

Shivakumar S. Toragalmath, Sangamesh A. Patil* Shivashankar M. Kinnal and Shailaja S. Jawoor

P.G. Department of Studies in Chemistry, Karnatak University, Dharwad-580 003. Karnataka, India

Received: 09 April 2016; Revised: 16 May 2016; Accepted: 28 May 2016

Abstract: The mononuclear Co(II), Ni(II) and Cu(II) complexes have been synthesized with Schiff base, derived from 8-formyl-7-hydroxy-4-methylcoumarin and 2-hydrazino-4 (coumarin-3-yl)thiazole. The synthesized Schiff base and its mononuclear metal complexes have been successfully characterized in the light of FTIR, ^1H NMR, ^{13}C NMR, UV- Visible, ESR, magnetic, thermal, fluorescence, mass and ESI-MS studies. The synthesized metal complexes completely soluble in DMF and DMSO. The molar conductance values indicate that, all the synthesized complexes are non-electrolytic in nature. Elemental analysis study reveals stoichiometry of the type $[\text{ML}_2]$, where M= Co(II), Ni(II) and Cu(II), L= singly deprotonated ligand. The bonding between Schiff base and metal ion was supported by FTIR data, through deprotonation of phenolic oxygen of coumarin and imine nitrogen atoms. The octahedral geometry of the Co(II) and Ni(II) metal complexes and square planar nature of Cu(II) complex has been confirmed by solution electronic spectral study. The thermal stability of all the newly synthesized mononuclear metal complexes have been studied by TG/DTA. The newly synthesized Schiff base and its mononuclear metal complexes have been screened for their anti-tuberculosis and anthelmintic activity against *mycobacterium tubercuolsis* and *Pheretima posthuma* respectively. The CT-DNA cleavage activity has been studied employing the gel electrophoresis method.

Keywords: Coumarin Schiff base; Mononuclear Complexes; in-vitro Anti-tuberculosis, Anthelmintic; DNA Cleavage Study.

INTRODUCTION

Coumarin is a well-known oxygen heterocyclic white crystalline compound having typical benzopyrone framework, having fluorescence emission properties and occurs naturally in plants. Many coumarin derivatives present throughout the plant kingdom, it can be separated using various extraction procedures¹. Coumarin was first time isolated from Tonka bean in 1820 by Vogel² and chemically synthesized by Perkin in 1868³. Pechmann condensation is the simple and common method used for synthesis of coumarin, in which different phenols are used. 7-Hydroxycoumarin (*Umbelliferone*), on its structural and biogenetic sense is regarded as the parent coumarin among the structurally more complex coumarins. In plants, higher concentration of coumarin derivatives found in fruits, stems and leaves. Few coumarin derivatives have also been reported in microorganisms e.g. coumermycin and novobiocin from streptomyces and aflatoxins from *Aspergillus* species^{4, 5}. Since last couple of decades organic chemists have been carrying out investigation on coumarin derivatives, due the vital pharmacological applications such as antioxidants⁶, cytotoxic activity⁷, hypotensive⁸, anthelmintic⁹, inhibition of platelets aggregation¹⁰, antimicrobial¹¹, optical brighteners¹², fluorescent probes¹³. Since, last several years, inorganic chemists have paid their considerable attention towards the synthesis of coumarin compounds because of their promising biological activity upon forming complexes with metal ions. Many coumarin Schiff base metal complexes, were thoroughly characterized using various spectro-analytical techniques and screened for their different biological potency, have been reported from our laboratory¹⁴⁻¹⁷. Generally, biological activity of various coumarin derivatives depends on the presence and position of hydroxyl groups¹⁸. A recent study reveals that 7-hydroxycoumarin inhibits the release of Cyclin D1, which is over expressed in many types of cancer. Literature survey showed that no work has been done on Schiff base metal complexes of aldehydo hydroxy coumarin with 2-hydrazino-4 (coumarin-3-yl)thiazole.

Hence, in view of their wide applications, synthesis of new coumarin Schiff base metal complexes is thought to be worthwhile. All these improvements in the synthesis of Schiff base with various metal ions are most fascinating. Here, in this present investigation we have synthesized the mononuclear Co(II), Ni(II) and Cu(II) complexes with newly synthesized Schiff base derived from 8-formyl-7-hydroxy-4-methylcoumarin and 2-hydrazino-4(coumarin-3-yl)thiazole, which have been characterized by spectral (IR, NMR, UV-Vis, Mass, ESI-MS, ESR etc;), fluorescence, thermal and molar conductivity studies. The synthesized, Schiff base and its metal complexes were evaluated for their anti-tuberculosis, anthelmintic and DNA cleavage activities.

EXPERIMENTAL

Reagents: All chemical and solvents used for the synthesis of Schiff base and its mononuclear metal complexes were of reagent grade and used without further purification. The starting materials 7-hydroxy-4-methylcoumarin and 3-bromoacetylcoumarin were prepared according to previously reported methods with slight modification.^{19, 20}.

Analysis and Physical Measurements: Carbon, hydrogen and nitrogen were estimated by using Elemental Analyzer Carlo Erba EA1108 analyzer. The IR spectra of the Schiff base and its Co(II), Ni(II) and Cu(II) metal complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000-400 cm⁻¹ region in KBr pellet. The electronic spectra of all the complexes were recorded in HPLC grade DMSO on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1000

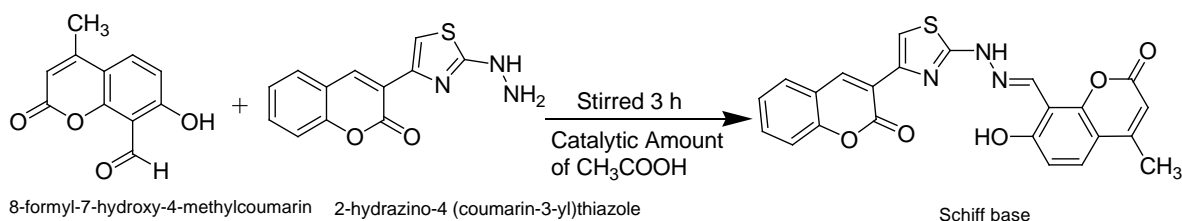
nm. The ^1H & ^{13}C -NMR spectra of the Schiff base were recorded in d_6 -DMSO on a BRUKER 400 MHz spectrometer at room temperature using TMS as an internal reference. The mass of Schiff base and its mononuclear metal complexes were recorded in GCMS and ESI-Mass respectively. The ESR spectrum of Cu(II) complex was recorded under at room temperature (RT) on Varian-E-4X-band EPR spectrometer. Thermogravimetric analyses data were measured from 25 to 1000 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C}/\text{min}$. The data were obtained by using a PERKIN-ELMER DIAMOND TG/ DTA instrument. Molar conductivity measurements were recorded for all the complexes on ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51, and magnetic moment measurement was carried out using Faraday balance.

Synthesis of 8-formyl-7-hydroxy-4-methylcoumarin: A mixture of 0.03 mol of 7-hydroxy-4-methylcoumarin and 0.07 mol of hexamine in 50 ml of glacial acetic acid were heated for 4-5 h and then 75 mL of 20% hydrochloric acid was added and heating was continued for another 20 minutes. The resulting mixture was cooled and extracted with ether. Pale yellow solid of 8-formyl-7-hydroxy-4-methylcoumarin was obtained after evaporating the ether. Yield: 19.01%; M.P.: 176-178 $^{\circ}\text{C}$.

Synthesis of 2-hydrazino-4 (coumarin-3-yl)thiazole: Potassium acetate 12.5 mmol was dissolved in 40 ml. of aqueous ethanol mixture (1:1) With constant stirring, thiosemicarbazide 12.5 mmole and 3-bromoacetylcoumarin 12.5 mmole were introduced. After 3 hours of stirring at room temperature, solid separated out. The separated solid was filtered, washed with cold ethanol and dried. The compound was recrystallized from alcohol. Yield: 78%; M.P.: 168-170 $^{\circ}\text{C}$.

Synthesis of Schiff base: Schiff base has been synthesized by condensing 1mmol of 8-formyl-7-hydroxy-4-methylcoumarin and 1mmol of 2-hydrazino-4 (coumarin-3-yl)thiazole in methanol medium. The progress of the reaction was monitored by TLC and light brown color product formed was set aside for 3 hours, then filtered and washed with water, cold methanol and dried over anhydrous CaCl_2 (Scheme-1).

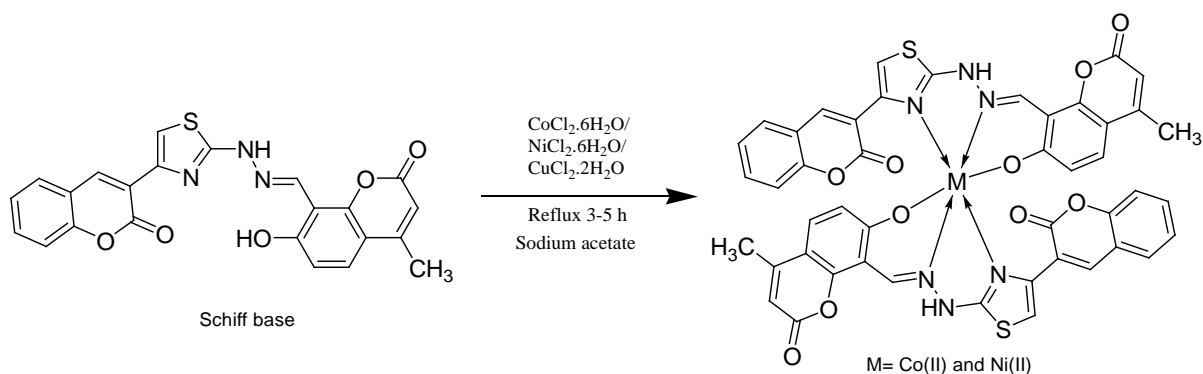
Yield: 75%; M.P.: 192-194 $^{\circ}\text{C}$; color: light brown. mass: m/z at 445, ^1H NMR (d_6 -dmso) δ : 12.28 (s, 1H, -OH), 11.31 (s, 1H, -NH), 8.79 (s, 1H, -CH=N), 8.60-5.96 (m, aromatic protons), 2.35 (s, H, -CH₃). Anal. Calc. for HL(%): C, 62.02; H, 3.39; N, 9.43. Found: C, 61.53; H, 3.16; 9.22. FTIR (cm^{-1}): ν (-OH) 3430; hydrazine, ν (-NH) 3140; ν (C=O) 1718; ν (-CH=N) 1589. λ_{max} (in nm): 279 π - π^* and 321 n - π^* .



Scheme-1: Synthesis of Schiff base

Synthesis of Co(II), Ni(II) and Cu(II) complexes [1-3]: A general method has been employed to synthesize the complexes using reaction of metal salts and Schiff base in molar ratio (M:L= 1:2). An ethanolic solution (30 ml) of Schiff base (1 mmol) was refluxed with ethanolic solution (30 ml) of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (1 mmol) on water bath for half an hour.

Then, to the reaction mixture 1 mmol of sodium acetate was added and refluxed for another 3-5 hours. The separated solid complexes were filtered, washed with water, alcohol and dry ether, finally dried in vacuum over fused anhydrous CaCl_2 (Scheme-2).



Scheme-2: Synthesis of metal complexes (1-3)

Bioassay Studies:

DNA Cleavage Study: The DNA cleavage study was carried out for the synthesised mononuclear metal complexes of coumarin Schiff base using Calf-thymus DNA (CT-DNA, Purchased from Bangalore Genei, Bengaluru, Cat. No. 105,850). The synthesized compounds were treated separately with CT-DNA, sample mixtures were then incubated at 37 °C for 2h. The cleavage products were analysed by agarose gel electrophoresis method²¹ and observed under UV transilluminator for DNA cleavage.

Agarose Gel Electrophoresis: In brief, 250mg of agarose was dissolved in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) by boiling. The warmed agarose was poured into the gel cassette fitted with comb. Later comb was removed carefully after the gel solidified and then gel placed in electrophoresis chamber flooded with TAE buffer. 20µl of 2h incubated sample mixture (mixed with bromophenol blue dye @ 1:1 ratio), were loaded carefully into individual wells, along with standard DNA marker and gel was run in electrophoresis chamber by passing constant 50 V of electricity for 45 min. Gel was then immersed in 10µg/ml ethidium bromide solution for 10-15 minutes to stain and bands observed under UV transilluminator and gel band pictures were recorded to determine the extent of DNA cleavage. The cleavage ability of treated samples were compared with standard DNA marker.

Anthelmintic Assay: The anthelmintic assay was carried out on adult *Pheretima posthuma*, an Indian earthworm because of its anatomical and physiological similarity with intestinal roundworm parasite of human beings²². The said assay was studied as per the method given in literature²³ with slight modifications. The *P. Posthuma* earthworms were collected from the moist soil, washed with the normal saline solution (5%) for about 20-30 seconds to remove the all faecal matter, were used for the present investigation. Earthworms of the 3-5cm in length were used to carry out this experiment. The newly synthesized Schiff base and its mononuclear metal complexes were treated to study the anthelmintic activity against earthworms at 2 and 10 µg/mL concentrations. DMSO in distilled water was used as a control and Albendazole as standard. The paralyzing and death times were recorded and their mean was calculated for triplicate sets. Death time was noted by placing earthworms in warm water (50 °C) and observed for stimulated movement, if the worm was alive.

Anti-tuberculosis Activity Study: The antituberculosis activity of the newly synthesized Schiff base and its mononuclear metal complexes were assessed against *M. tuberculosis* ATTC 27294 using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 mL of sterile distilled water was added to all outer perimeter wells of sterile 96 wells plate to minimize the evaporation of medium in the test wells at the time of incubation. The 96 wells plate

received 100 mL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 mg/mL. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 mL of freshly prepared 1:1 mixture of Almar Blue reagent and 10 % tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULTS AND DISCUSSION

The synthesis of Schiff base was schematically presented in **Scheme-1**. All the synthesized Co(II), Ni(II) and Cu(II) mononuclear metal complexes are stable at room temperature, non-hygroscopic, insoluble in water and many common organic solvents but soluble in DMF and DMSO. The analytical data showed that, the Co(II) and Ni(II) metal complexes having the stoichiometry of the type $[ML_2]$, where, 'L' stands for a deprotonated ligand and Cu(II) complex have the stoichiometry of the type $[Cu(L)Cl]$.

The molar conductivity of all the synthesized mononuclear metal complexes were studied at 10^{-3} M concentrations at room temperature. The results obtained are given in the **Table-1**, which indicate that all the synthesized mononuclear metal complexes have conductivity values in the range agreeable for the non-electrolytic nature suggesting that these complexes are non-electrolytic^{24, 25} in nature.

Table 1: Elemental analyses of Schiff Base and its Co(II), Ni(II) and Cu(II) metal complexes along with their molar conductance and magnetic moment data.

Compound No.	Empirical Formula	Color/Yield in %	M%		C%		H%		N%		Molar Cond. ($\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$)	Magnetic Moments μ_{eff} (BM)
			Obsd.	Cald.	Obsd.	Cald.	Obsd.	Cald.	Obsd.	Cald.		
Schiff base	$\text{C}_{23}\text{H}_{15}\text{N}_3\text{O}_5\text{S}$	Light Brown 75	-	-	61.04	62.02	3.30	3.39	9.35	9.43	-	-
1	$\text{Co}(\text{C}_{46}\text{H}_{28}\text{N}_6\text{O}_{10}\text{S}_2)$	Brown 67	6.11	6.22	58.21	58.29	2.87	2.98	8.79	8.87	12.01	4.89
2	$\text{Ni}(\text{C}_{46}\text{H}_{28}\text{N}_6\text{O}_{10}\text{S}_2)$	Green 71	6.09	6.19	58.26	58.31	2.91	2.98	8.81	8.87	13.28	3.27
3	$\text{Cu}(\text{C}_{23}\text{H}_{15}\text{N}_3\text{O}_5\text{S Cl})$	Dark Brown 77	6.61	6.67	50.10	50.83	2.05	2.60	7.20	7.73	16.15	1.52

IR spectral studies: The most prominent IR bands of the prepared Schiff base and its Co(II), Ni(II) and Cu(II) complexes are represented in **Table 2**. The IR spectra of Schiff base exhibited a broad band at 3430 cm^{-1} , strong band at 1718 , and 1589 cm^{-1} assigned to -OH stretching, $\nu(\text{C}=\text{O})$ lactonic carbonyl and $\nu(\text{C}=\text{N})$ vibrations respectively. A broad band at 3140 cm^{-1} is ascribed to the stretching vibration of -NH. In comparison with the spectra of the Schiff base, all the metal complexes exhibit downward shift $10\text{-}19 \text{ cm}^{-1}$ of $\nu(\text{C}=\text{N})$ indicating the participation of azomethine nitrogen in the coordination to the metal ion.

The high intensity band due to phenolic $\nu(\text{C}-\text{O})$ appeared in the region $1225\text{-}1295 \text{ cm}^{-1}$ in the Schiff base appeared as a medium to high intensity band in the $1322\text{-}1329 \text{ cm}^{-1}$ region in the complexes

indicating that, the ligand coordinated through deprotonated form and formation of metal-oxygen bonds. The characteristic band at 1444 cm^{-1} was attributed to $\text{C}=\text{N}$ of the thiazole moiety of the Schiff base. After complexation with the metal ions, the change in this band was observed in all the synthesized complexes²⁶ in the region $1483\text{--}1498\text{ cm}^{-1}$.

The most notable change in the Schiff base spectral features when coordinated to metal ion is the New bands at $547\text{--}551$ and $421\text{--}459\text{ cm}^{-1}$ are tentatively assigned to $\nu(\text{M-O})$ and $\nu(\text{M-N})$ stretching bands^{27, 28}. Thus, the IR spectral data results provide strong evidences for the complexation of the tridentate Schiff base.

Table 2: Prominent IR Frequencies (cm^{-1}) of Schiff Base and its Metal Complexes

Compound	$\nu(\text{-OH})$	$\nu(\text{-NH})$	$\nu(\text{HC}=\text{N})$	$\nu(\text{C}=\text{N})$ Thiazole	$\nu(\text{M-O})$	$\nu(\text{M-N})$
Schiff base	3430	3140	1589	1444	-	-
Co	---	3163	1570	1490	547	459
Ni	---	3163	1574	1483	550	458
Cu	---	3151	1580	1488	580	476

NMR studies of Schiff base: The spectral data of ^1H NMR and ^{13}C NMR of the Schiff base is given in synthesis of Schiff base. In the ^1H NMR spectrum of the Schiff base (**Figure-1**), the singlet at 12.28 and 11.31 ppm which are ascribed to -OH and -NH protons respectively. A singlet at 8.79 ppm is assigned to $\text{-CH}=\text{N}$ group. The aromatic ring protons are observed as multiplet, in the range $8.60\text{--}5.98$ ppm.

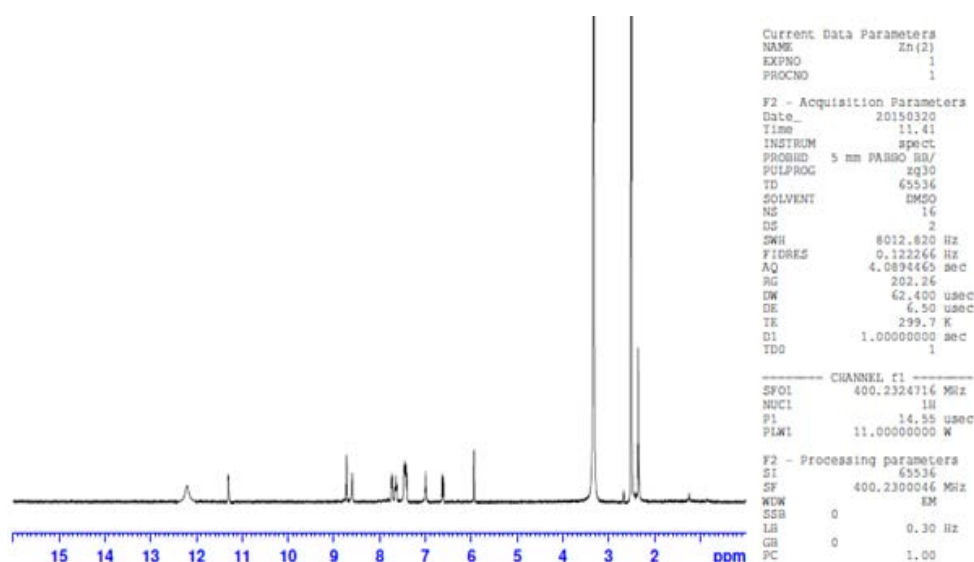


Figure 1: ^1H NMR Spectrum of Schiff base

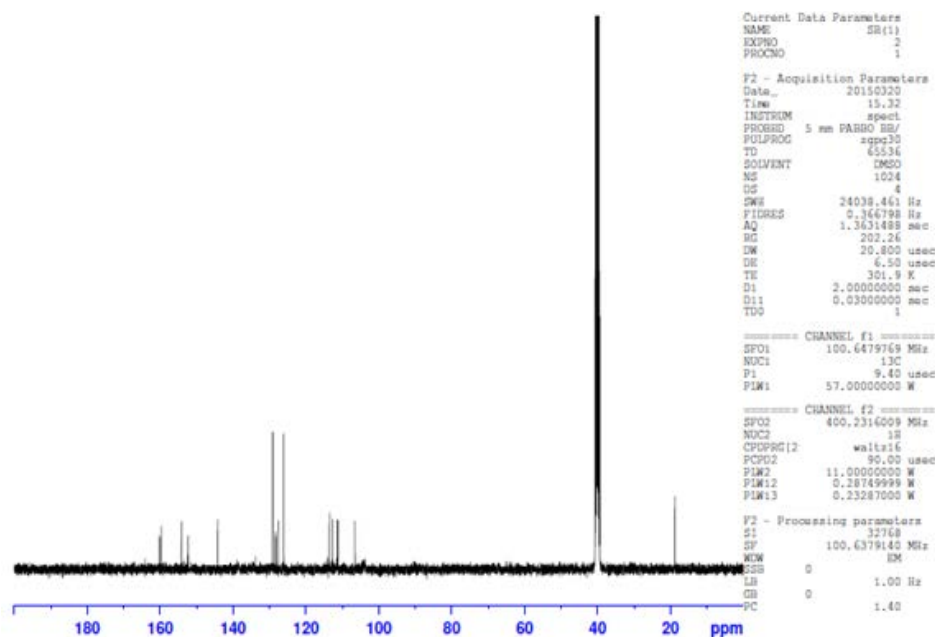


Figure 2: ^{13}C NMR Spectrum of Schiff base

In the ^{13}C NMR spectra of the Schiff base shown in **Figure-2** the signals appeared in the range 110.2-156.7 ppm are assigned to aromatic carbons. The signals at 144.1- 143.0 and 160-162.2 are due to C=N and C=O respectively.

Electronic spectral Studies: The electronic absorption spectra of the complexes were studied in DMSO were recorded at room temperature and the band positions of the absorption band maxima assignments and the proposed geometry is discussed in the present context. The free ligand exhibited strong absorptions in the range 260-280 nm due to intra-ligand $\pi\text{-}\pi^*$ transitions, these bands remain almost unchanged in the spectra of coordination compounds. Absorptions in the range 295-302 nm are attributed to the $n\text{-}\pi^*$ transition associated with azomethine, the bathochromic shift of this absorption upon complexation is due to the donation of a lone pair of electrons to the metal ion, indicating the coordination of azomethine nitrogen.

In the present study, the Co(II) complex showed the absorption bands at 10715 -11025 and 20985-24650 cm^{-1} are assigned to $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$ and $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{1g}(\text{P})$. These bands are the characteristic of high spin octahedral geometry of Co(II) complexes. The yellowish green Ni(II) exhibited three bands around 10989, 13495 and 24630 cm^{-1} attributed to $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{2g}(\text{F})$ (ν_1), $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{F})$ (ν_2) and $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{P})$ (ν_3) transitions respectively, which indicate octahedral geometry around Ni(II) ion.

The electronic spectrum of the Cu(II) complex shows two bands in the region 12685-14150 cm^{-1} and 13888- 16650 cm^{-1} assigned to $^2\text{B}_{1g} \rightarrow ^2\text{A}_{1g}$ and $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$ transitions respectively and these are assigned to square planar geometry for Cu(II) complex. Beside the above bands, the band observed $\sim 27000 \text{ cm}^{-1}$ may be assigned due to charge transfer.

Elemental Analysis and Magnetic Studies: The elemental analysis data and magnetic moment obtained at room temperature (**Table-1**). The experimental values are in good agreement with the calculated values for elemental analysis of all the synthesized compounds. The magnetic data obtained for Co(II) and Ni(II) are found to be well within the range of octahedral geometries of the

complexes. The magnetic moment value obtained for Cu(II) metal complex fit for the square planar nature of the complex.

ESR Studies: The ESR spectrum of the Cu(II) complex (**Figure-3**) was studied at room temperature show the isotropic character and did not contain any hyperfine splitting, it has given only one signal for which g^{\parallel} and g^{\perp} have been calculated. The calculated g^{\parallel} and g^{\perp} values are found to be 2.12 and 1.95 respectively, which supports the presence of unpaired electron in the $d_{x^2-y^2}$ orbital of Cu(II) ion with $3d^9$ configuration²⁹.

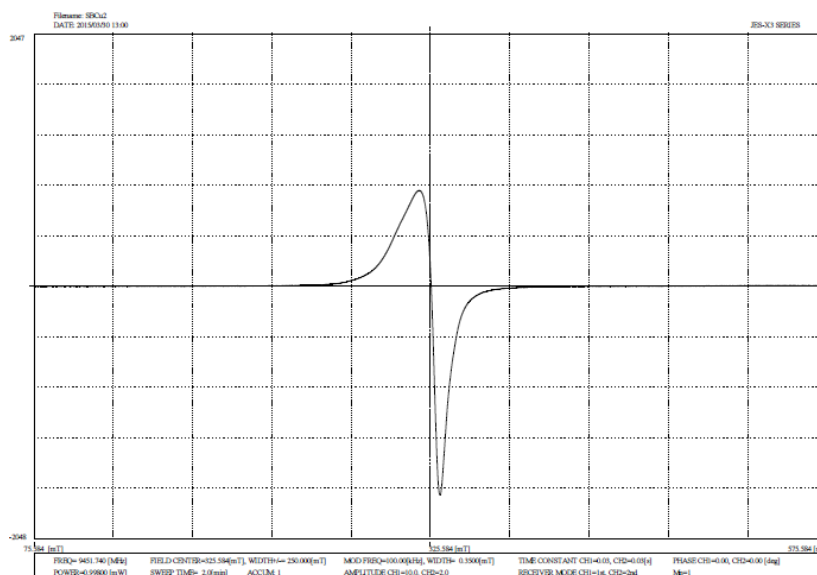


Figure 3: ESR Spectrum of Cu(II) Complex

Mass Spectral Studies of Schiff base and its Metal Complexes: The mass spectrum of the Schiff base is shown in **Figure-4**, the m/z peak found at 445 which is equivalent to its molecular weight. The ESI-Mass spectral studies of Co(II) and Ni(II) complexes exhibited $[M+H]^+$ peak at 948, 947 for Co(II) and Ni(II) complexes. The Cu(II) complex showed $[M-L]^+$ peak at 540 which is equivalent to its molecular weight. The representative Co(II) ESI-MS spectrum is showed in **Figure-5**.

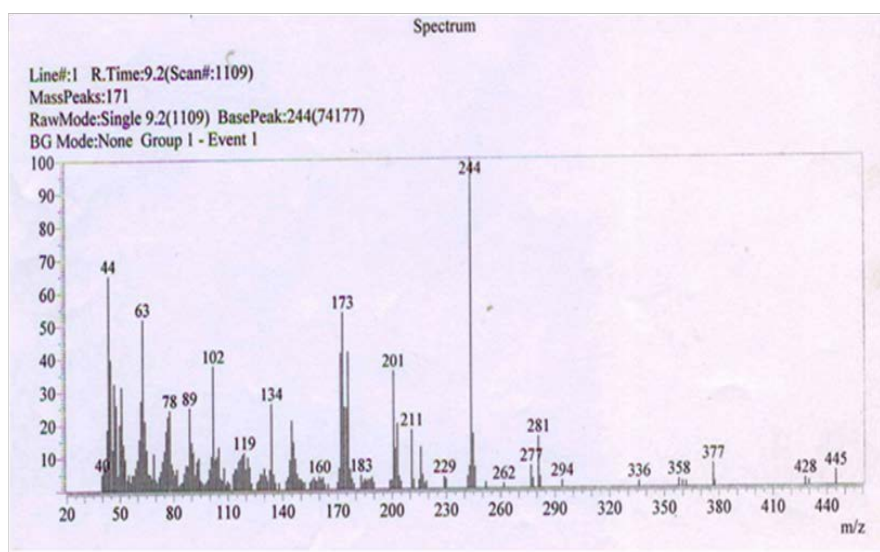


Figure 4: Mass Spectrum of Schiff base

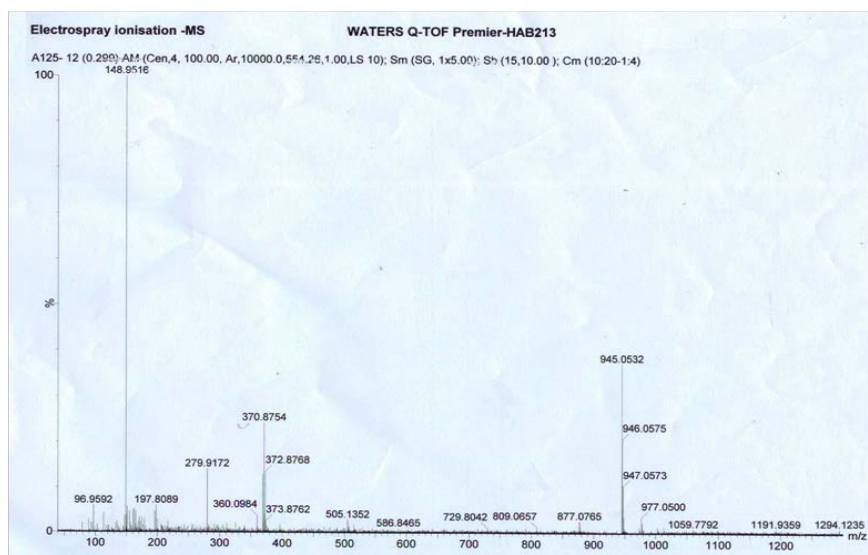


Figure 5: ESI-Mass Spectrum of Co(II) Complex

Thermal Studies: Thermal analyses carried out for all the synthesized metal complexes and it is almost similar for both Co(II) and Ni(II) complexes. The representative TG/DTA thermogram of Co(II) complex (**Figure-6**) and the decomposition takes place in two steps as indicated by DTG peak around 430 °C weight corresponds to loss of ligand and above 800 °C formation of metal oxide. The Cu(II) complex decomposition takes place in three steps, first steps loss at ~195 °C corresponds to loss of coordinated chloride. Second steps corresponds to loss of organic moiety at 350 °C. Finally, above 800 °C formation of metal oxide.

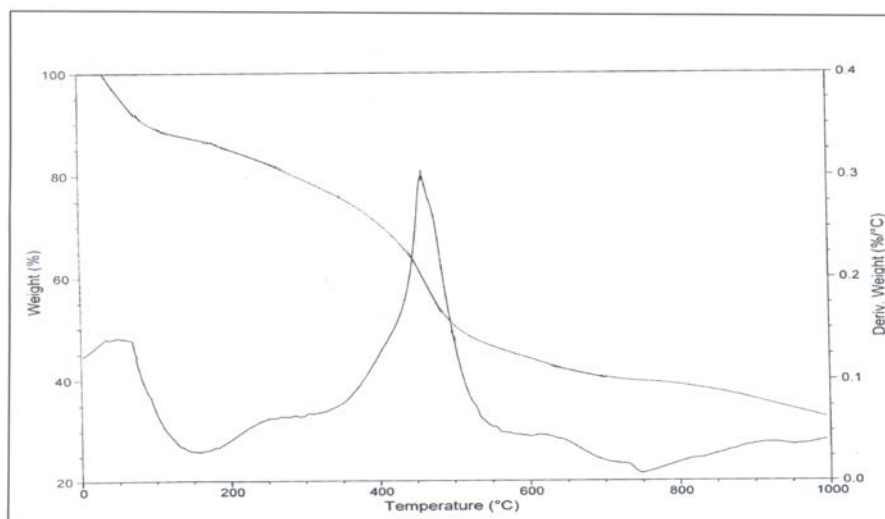


Figure 6: TG/DTA Curve of Co(II) Complex

Antituberculosis Activity: All the synthesized compound were screened for their antituberculosis activity against mycobacterium tuberculosis. The result reveals that, the metal complexes exhibited better activity compared to Schiff base.

Anthelmintic Activity: The anthelmintic activity studies results are shown in **Table-3**. From the observations it is clear that, the Schiff base and its metal complexes showed potent anthelmintic activity when compared to the standard drug (Albendazole). Results reveal that, paralytic and lethal

time for complexes is less than that of the Schiff base. The standard drug Albendazole at 4% concentration, the paralysis and death occur at 3 min and 7 min respectively. In case of Cu(II) complex at 4% concentration, the paralysis and death occur at 4 min and 9 min respectively.

Table 3: Anthelmintic studies of Schiff Base and its Metal Complexes (1-3)

Compound	Concentration ($\mu\text{g/mL}$)	Time of Paralysis (Min)	Lethal Time (Min)
Albendazole	4%	03	07
DMSO*		-	-
Schiff base		08	14
1		06	12
2		05	11
3		04	09

* DMSO used as Control

Fluorescence Study: The emission spectra of Co(II), Ni(II) and Cu(II) complexes have been studied in DMSO solvent at 10^{-5} concentration exhibited the emission bands at 525, 530 and 512 nm respectively. In **Figure-7** it is observed that, the emission band of Schiff base around 550 nm disappeared in complexes because of the interaction of the phenolic oxygen with the metal ion. The decrease in intensity of fluorescence of Co(II), Ni(II) and Cu(II) complexes was observed. Previous reports support that, the transition metal ions decrease the fluorescence quite effectively³⁰. The decrease in emission maxima were in the order of Co(II)>Ni(II)>Cu(II).

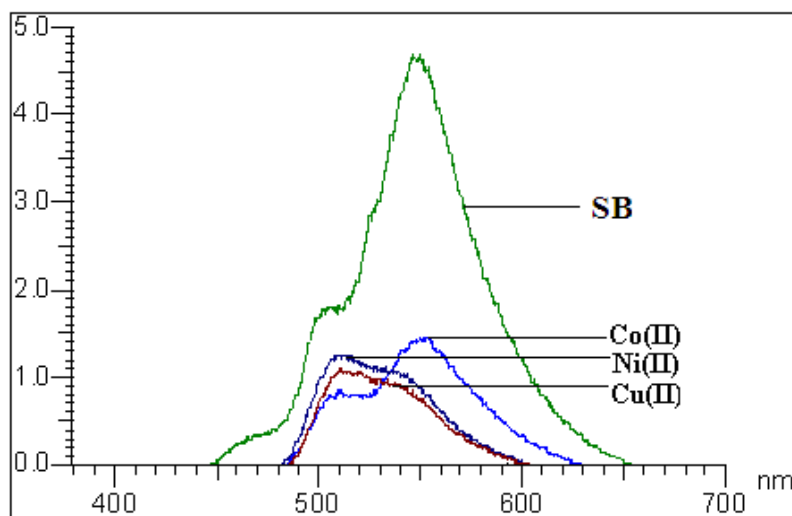


Figure 7: Emission Spectra of Schiff base and its Metal Complexes

DNA Cleavage Activity: The synthesized Schiff base and its Co(II), Ni(II) and Cu(II) complexes were studied for DNA cleavage activity by agarose gel electrophoresis method. The gel electrophoresis works on, migration of DNA under the influence of electric potential. **Figure-8** represents the DNA cleavage activity of Schiff base and its metal complexes, in which M- is standard DNA molecular weight marker, C-control CT-DNA i.e. untreated CT-DNA and lane 1-4 are CT-DNA treated with Schiff base, Co(II), Ni(II) and Cu(II) complexes respectively. From the photograph, It is clear that, copper complex shown complete cleavage of DNA whereas other complexes and Schiff base showed partial cleavage. As Cu(II) complex cleaved CT-DNA completely, this implies that

Cu(II) complex is more effective than the Co(II) and Ni(II) metal complexes and Schiff base. The cleavage efficiency of metal complexes compared with control is due to their efficient DNA-binding ability³¹. K. Mahendra Raj et al.³² have obtained similar results against CT-DNA using gel electrophoresis method.

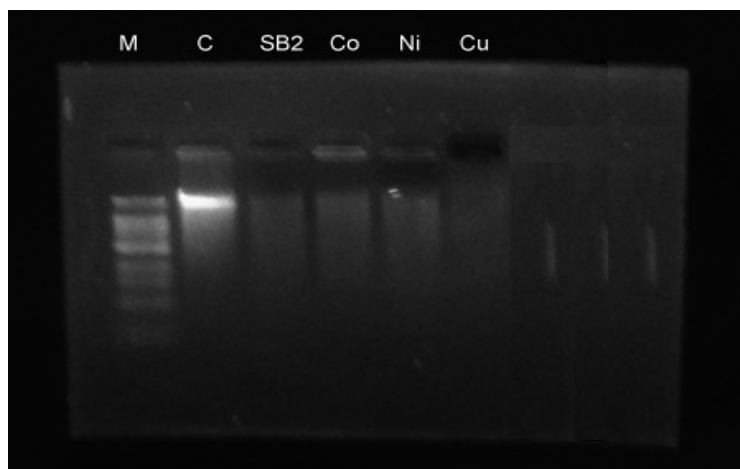


Figure 8: DNA Cleavage Activity of Schiff base and its Metal Complexes(1-3)

M-is standard DNA molecular weight marker, C-control CT-DNA i.e. untreated CT-DNA and lane 1-4 are CT-DNA treated with Schiff base, Co(II), Ni(II) and Cu(II) complexes respectively.

CONCLUSION

The newly synthesized Schiff act as tridentate ligand. The ligand is coordinated through the azomethine nitrogen, phenolic oxygen and thiazole nitrogen. The bonding of ligand to metal ion confirmed by spectral, analytical, magnetic, thermal and molar conductivity studies. The Schiff base and its metal complexes screened for their antituberculosis, anthelmintic and DNA cleavage activity and result reveals that, the metal complexes have prominent activity as compared to Schiff base, this means activity significantly increased on coordination. All these observations put together lead us to propose the following tentative structures (**Figure-9**) in which, the complexes having the stoichiometry of the type ML_2 [M= Co(II) and Ni(II) and L= deprotonated ligand]. The Cu(II) having the stoichiometry of the type $[Cu(L)Cl]$ **Figure-10**.

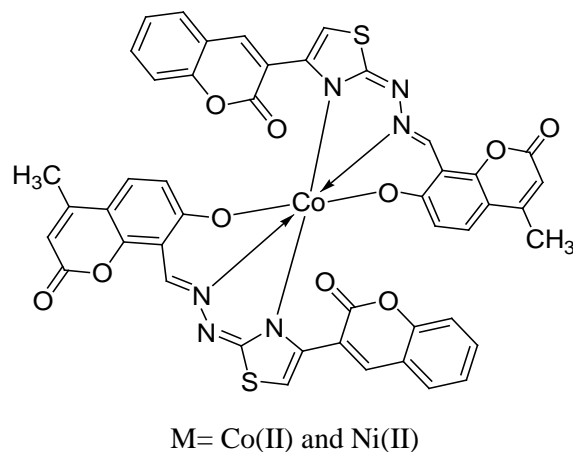


Figure 9: Proposed Structure of Metal Complexes

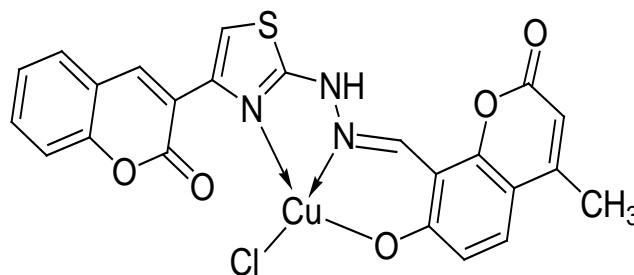


Figure 10: Proposed Structure of Cu(II) Complex

ACKNOWLEDGEMENT

Authors are grateful to the Chairman, Department of Chemistry, Karnatak University, Dharwad for providing necessary facilities. Authors are also thankful to USIC, Karnatak University, Dharwad, IISc Bengaluru, IIT Bombay, IIT Khanpur, IIT Madras and Biogenics Hubballi, India for providing spectral data and biological activity results. One of the authors, Shivakumar S. Toragalmath is thankful to UGC, New Delhi for awarding the BSR (RFSMS) fellowship.

REFERENCES

1. R.M.S. Celeghini, J.H.Y. Vilegas and F.M. Lanças, *Braz. Chem. Soc.*, 2001, 12 (6) 706.
2. A. Vogel, Darstellung von benzoessäure aus der Tonka-Bohne und aus den meliloten- oder steinklee-bäumen, *Ann. der Phys.*, 1820, 64 (2) 161.
3. W.H. Perkin, On the artificial production of coumarin and formation of its homologues, *J. Chem. Soc.*, 1868, 21, 53.
4. D. Cooke, B. Fitzpatrick, R. O' Kennedy, T. Mc Cormack, D. Egan, *Coumarin Biochemical Profile and Recent Developments*, third ed., John Wiley & Sons, 1997.
5. D. Cooke, *Recent Advances on Coumarin and its Synthetic Derivatives*, Dublin City University, Dublin, 1999.
6. N. Vukovic, S. Sukdolak, S. Solujic, N. Niciforovic, *Arch. Pharm. Res.*, 2010, 335.
7. S. Stanchev, G. Momekov, F. Jensen, I. Manolov, *Eur. J. Med. Chem.*, 2008, 43(4) 694.
8. A.H. Gilani, F. Shaheen, S.A. Saeed, S. Bibi, Irfanullah, M. Sadiq, S. Faizi, *Phytomedicine.*, 2000, 7, 423.
9. B. Sandhya, D. Giles, M. Vinod, G. Basavarajaswamy, A. Rekha, *Eur. J. Med. Chem.*, 2011, 46, 4696.
10. G. Cavettos, G.M. Nano, G. Palmisano, S. Tagliapietra, *Tetrahedron: Asymmetry*, 2001, 12 (5) 707.
11. M. Mladenovic, N. Vukovic, S. Sukdolak, S. Solujic, *Molecules.*, 2010, 15(6) 4294.
12. M. Zabradnik, *The Production and Application of Fluorescent Brightening Agents*, John Wiley and Sons, New York, 1992.
13. B.D. Wagner, The use of coumarins as environmentally-sensitive fluorescent probes of heterogeneous inclusion systems, *Molecules.*, 2009, 14 (1) 210.
14. S.A. Patil, V.H. Naik, A.D. Kulkarni, P.S. Badami, *Spectrochim. Acta, Part A.*, 2010, 75, 347.
15. S.A. Patil, S.N. Unki, A.D. Kulkarni, V.H. Naik, U. Kamble, P.S. Badami, *J. Coord. Chem.*, 2011, 64 (2) 323.

16. Gangadhar B. Bagihalli, Prakash Gouda Avaji, Sangamesh A. Patil, Prema S. Badami, Euro. J. of Med. Chem., 2008, 43, 2639.
17. A.D. Kulkarni, P.G. Avaji, G.B. Bagihalli, S.A. Patil, P.S. Badami, J. Coord. Chem., 2009, 62 (3) 481.
18. H. Kolodziej, O. Kayser, H.J. Woerdenbag, W.V. Uden, N.Z. Pras, Naturforsch., 1997, 52, 240.
19. V.K. Ahluwalia, P. Bhagat, R. Aggarwal, R. Chandra, Intermediates for Organic Synthesis, I.K. International Pvt. Ltd., Delhi, 2005.
20. P.P. Bhosale, R. S. Chavan and A. V. Bhosale, Ind. J. Chem., 2012, 51B 1649.
21. J. Sambrook, E. F. Fritsch, T. Maniatis, Molecular cloning, A laboratory Manual. Second Edn. Cold Spring Harbor Laboratory, New York. 1989.
22. R.D. Vidyarthi, A Text Book of Zoology, Fourteenth ed., S. Chand and Co., New Delhi, 1967.
23. L.C. Garg, C.K. Atal, Anthelmintic activity of *Myrsine africana*, Indian J. Pharm. Sci., 1963, 59, 240.
24. M.C.S. Lourenço, M.V.N. de Souza, A.C. Pinheiro, M.L. Ferreira, R.S.B. Gonçalves, T.C.M. Nogueira, M.A. Peraltab, Arkivoc., 2007, 15, 181.
25. A. Golcu, M. Tumer, H. Demirelli, R. A. Wheatley, Inorg. Chim. Acta., 2005, 358 (6) 1785.
26. M. Sobiesiak, T. Muzioł, M. Rozalski, U. Krajewska, E. Budzisz, New J. Chem., 2014, 38, 5349.
27. P.P. Dholakiya, M.N. Patel, Synth. React. Inorg. Metal-Org. and Nano-Met. Chem., 2002, 32 (4), 819.
28. K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds, Wiley-Interscience, New York, 1970.
29. M.V. Angelusiu, S.F. Barbuceanu, C. Draghici, G.L. Almajan, Eur. J. Med. Chem., 2010, 45, 2055.
30. A.W. Czarnik, Chem. Biol., 2 (1995) 423, A.W. Varnes, R.B. Dodson, E.L. Wehry, J. Am. Chem. Soc., 1972, 94, 946.
31. K. Puja, F. Nighat, R.V. Singh, Spectrochim. Acta, Part A., 2011, 83 (1), 74.
32. Mahendra Ra et al., J. Mol. Struct., 2014, 1074, 572.

Corresponding author: Sangamesh A. Patil

P.G. Department of Studies in Chemistry, Karnatak University, Dharwad-580
003. Karnataka, India