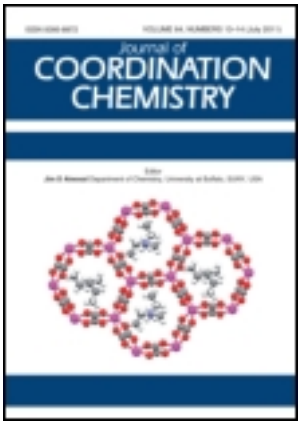

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DNA cleavage, antimicrobial, anti-inflammatory anthelmintic activities, and spectroscopic studies of Co(II), Ni(II), and Cu(II) complexes of biologically potential coumarin Schiff bases

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DNA cleavage, antimicrobial, anti-inflammatory anthelmintic activities, and spectroscopic studies of Co(II), Ni(II), and Cu(II) complexes of biologically potential coumarin Schiff bases

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A series of Co(II), Ni(II) and Cu(II) complexes, $[ML \cdot 2H_2O]$ of Schiff bases derived from 4, 4-diaminodiphenyl sulfone (dapsone) and 8-formyl-7-hydroxy-4-methylcoumarin/5-formyl-6-hydroxycoumarin have been synthesized. From analytical, spectral (IR, NMR, UV-Vis, ESR and FAB mass), and magnetic studies it has been concluded that the metal complexes possess octahedral geometry and are non-electrolytes. The redox behavior of the metal complexes is investigated by cyclic voltammetry. The Schiff bases and their metal complexes have been screened for antibacterial (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella*, *Salmonella*, *Streptococcus, Staphylococcus proteus*) and antifungal activities (*Fusarium*, *Candida*, *Rhizopus*, *Penicillium chrysogenum* and *Aspergillus niger*) by the minimum inhibitory concentration method. The anthelmintic activity of the ligands and their metal complexes against earthworms was investigated. The DNA cleavage study was done by agarose gel electrophoresis. Anti-inflammatory activity studies showed the test compounds are comparable to the standard drug diclofenac sodium.

Keywords: Antimicrobial; Anthelmintic activity; Anti-inflammatory; Schiff bases; Spectroscopy

1. Introduction

Coumarins contain the parent nucleus of benzo-a-pyrone and occur in plants of orchidaceae, leguminaceae [1], rutaceae, umbellliferae, and labiatae families. Coumarin and its derivatives exhibit biological properties such as anticoagulant, anti-inflammatory, anticancer drugs, in plants as growth regulating agents [2, 3] antithrombotic, vasodilatory [4], antimutagenic [5], and lipoxygenase, cyclooxygenase inhibition [6, 7]. 3-Acetyl/acetoacetoacetyl-4-hydroxy coumarin has been reported as anti-HIV agent [8]. Metal coordination to biologically active molecules can enhance biological activity and overcome resistance. Metal complexes derived from Schiff base coumarin derivatives

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are more potent and less toxic in many cases compared to the parent drug [9]. Some Schiff-base metal complexes have been tested successfully for antifungal [10], antibacterial [11], and antitumor [12] activities. Biologically active compounds like dapsone and its sulfonamide derivatives have been used to control dermatologic symptoms of dermatitis herpetiformis. We have reported spectroscopic, electrochemical, and biological studies of a number of metal complexes with various Schiff bases derived from coumarin [13–16]. Thus, in continuation of our work on Schiff bases and their metal complexes, we report here Schiff bases derived from dapsone and 8-formyl-7-hydroxy-4-methylcoumarin/5-formyl-6-hydroxycoumarin and their Co(II), Ni(II), and Cu(II) complexes.

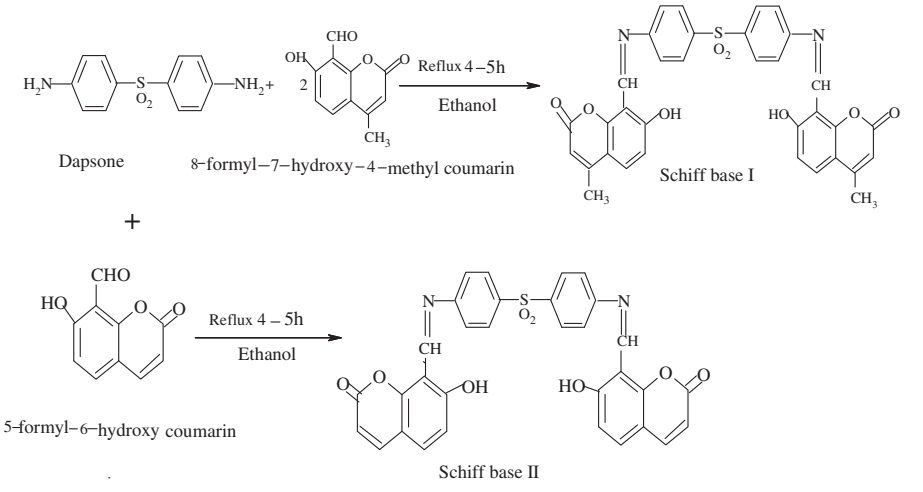
2. Chemistry

2.1. *Physical measurements*

Carbon, hydrogen, and nitrogen were estimated using a CHN Elemental Analyzer, Truespec LECO USA. IR spectra of the Schiff bases and their Co(II), Ni(II), and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer from 4000 to 250 cm^{-1} in KBr discs. Electronic spectra of the complexes were recorded in HPLC grade DMF on a VARIAN CARY 50-BIO UV-spectrophotometer from 200 to 1100 nm. ^{1}H-NMR spectra of ligands were recorded in DMSO-d_6 on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. FAB-mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10 Am) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature with *m*-nitrobenzyl alcohol as the matrix. The mass spectrometer was operated in the positive ion mode. Electrochemistry of the complexes was recorded on a CHI1110A-electrochemical analyzer (USA) in DMF containing 0.05 mol L^{-1} n-Bu_4NClO_4 as supporting electrolyte. EPR spectra were recorded on a Varian-E-4X-band EPR spectrometer at 3000 G and modulation frequency of 100 kHz at liquid nitrogen temperature using TCNE as g marker. Thermogravimetric analyses were measured from room temperature to 1000°C at a heating rate of 10°C min^{-1}. The data were obtained using a PERKIN-ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements were recorded on an ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51 and magnetic moment carried out by using a Faraday balance. The fluorescence studies of Schiff bases and their metal complexes were recorded on a HITACHI F-7000 Fluorescence Spectrophotometer (Japan). Solutions of 10^{-3} mol L^{-1} were prepared in HPLC grade DMF and DMSO solvents.

2.2. *Methods*

All chemicals were of reagent grade. 7-Hydroxy-4-methylcoumarin [17], 6-hydroxy coumarin [18], 8-formyl-7-hydroxy-4-methylcoumarin [19], and 5-formyl-6-hydroxy coumarin [19, 20] have been synthesized according to published procedures.



Scheme 1. Synthesis of Schiff bases.

2.2.1. Synthesis of Schiff bases (I and II). The Schiff bases have been synthesized as per scheme 1 by refluxing reaction mixture of hot ethanolic solution (30 mL) of dapsone (0.01 mol) and hot ethanolic solution (30 mL) of 8-formyl-7-hydroxy-4-methylcoumarin/5-formyl-6-hydroxycoumarin (0.02 mol) for 4–5 h with addition of 4–5 drops of conc. hydrochloric acid. Precipitate that formed during reflux was filtered, washed with cold EtOH, and recrystallized from hot EtOH, m.p. 189°C (Yield: 80%) and m.p. 173°C (Yield: 78%) of Schiff bases I and II, respectively.

2.2.2. Synthesis of Co(II), Ni(II), and Cu(II) complexes (1–6). A mixture of alcoholic solution (45 mL) of Schiff bases (1 mmol) and an alcoholic solution (5 mL) of 1 mmol of $CoCl_2 \cdot 6H_2O/NiCl_2 \cdot 6H_2O/CuCl_2 \cdot 2H_2O$ was refluxed on a water bath for 2 h. Then, to the reaction mixture sodium acetate of 1 mmol was added and reflux continued for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol, and ether and finally dried in vacuum over fused $CaCl_2$.

3. Pharmacology

3.1. *DNA cleavage experiment*

3.1.1. Preparation of culture media. Nutrient broth (peptone, 10; yeast extract, 5; NaCl, 10 in ($g\,L^{-1}$)) was used for culturing of *Escherichia coli*. The 50 mL medium was prepared and autoclaved for 15 min at 121°C under 15 lb pressure and then inoculated with the seed culture and incubated at 37°C for 24 h.

3.1.2. Isolation of DNA. Fresh bacterial culture (1.5 mL) is centrifuged to obtain the pellet, which is then dissolved in 0.5 mL of lysis buffer (100 mmol L^{-1} tris pH 8.0,

50 mmol L^{-1} EDTA, 10% SDS). To this, 0.5 mL of saturated phenol was added and incubated at 55°C for 10 min, then centrifuged at 10,000 rpm for 10 min. Equal volume of chloroform : isoamyl alcohol (24 : 1) and 1/20th volume of 3 mol L^{-1} sodium acetate (pH 4.8) was added to this supernatant and centrifuged at 10,000 rpm for 10 min, then 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugating. The dried pellet was dissolved in TE buffer (10 mmol L^{-1} tris pH 8.0, 1 mmol L^{-1} EDTA) and stored cold.

3.1.3. Agarose gel electrophoresis. Cleavage products were analyzed by agarose gel electrophoresis method [21]. Test samples (1 mg mL^{-1}) were prepared in DMF. The samples (100 μg) were added to isolated DNA of *E. coli*. The samples were incubated for 2 h at 37°C and then 20 μL of DNA sample (mixed with bromophenol blue dye at 1 : 1 ratio) were loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 mol L^{-1} EDTA/1 L) and finally loaded on agarose gel and 50 V of electricity passed through for 30 min. The gel was removed and stained with 10.0 μg mL^{-1} ethidium bromide for 10–15 min, the bands were observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with standard DNA marker.

3.1.4. *In vitro* antibacterial and antifungal assay. The biological activities of newly synthesized Schiff bases and their Co(II), Ni(II), and Cu(II) complexes have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion methods, respectively [22]. The antibacterial and antifungal activities were done at 200 and 500 μg mL^{-1} concentrations in DMF using six bacteria (*E. coli*, *Salmonella*, *Klebsiella*, *Streptococcus*, *Staphylococcus aureus*, and *S. proteus*) and five fungi (*Fusarium*, *Candida*, *Rhizopus*, *Penicillium chrysogenum,* and *Aspergillus niger*). The bacterial strains were incubated for 24 h at 37°C and fungal stains were incubated for 48 h at 37°C. Standard antibacterial (Gentamycin and Flucanozole) and antifungal drugs (Kanamycin) were used for comparison under similar conditions.

3.1.5. Anthelmintic activity (*in vitro*). Anthelmintic assay was done by the method given in the literature [23] with minor modifications. The assay was performed on adult Indian earthworms, *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [24]. Earthworms (*P. posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 3–4 cm in length and 0.1–0.2 cm in width were used for the experimental protocol.

The compounds were subjected to anthelmintic activity studies against earthworms at 2 and 10 mg mL^{-1} by using Albendazole as a standard drug. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50°C) which stimulated the movement, if the worm was alive. The results of anthelmintic studies are tabulated in Supplementary table 7.

3.1.6. Anti-inflammatory (paw edema induced by formalin). Anti-inflammatory studies were carried out by the reported method [25, 26]. Male Wistar Albino rats (weight 200 g) obtained from BIONEEDS Laboratory, Bangalore, Karnataka were used for this study. Animal experiments were carried out in the Department of Pharmacology, in the BIONEEDS Laboratory, Bangalore, Karnataka, India and were approved by the Institutional Animal Ethical Committee and Licensees (Reg No.: KTK37/20/2009). The animals were given 1 week time to get acclimated with laboratory conditions. The animals were housed in polypropylene cage (four per cage) with sterilized paper cuttings as bedding material under laboratory conditions with control environment of temperature $22 \pm 3^{\circ}C$, humidity ($60\% \pm 10\%$) and 12 h light/dark cycle. They were given commercial diet (Hindustan Lever Ltd., Bangalore) and tap water *ad libitum*. The animals were made to fast overnight before the experiment. The study protocol was approved by the institutional ethical committee.

3.1.6.1. *Drugs (acute toxicity test).* Dose selection of the test drugs, based on the acute toxicity test carried out over a dosage range varying from 50, 100, 200, 400, 600, and 800 mg kg^{-1} body weight (bw) orally and the LD_{50} was estimated as 400 mg kg^{-1} bw. In acute toxicity study, the test compounds did not show any toxicity and mortality up to a maximum dose of 400 mg kg^{-1} bw in rats. No gross change in behavior was observed at this dose. Weight of rats had a normal variation after 7 days of observations.

According to the results of acute toxicity test, doses of 20 mg kg^{-1} bw and 5 mg kg^{-1} of the standard drug were chosen for the experiment. The test drug in the form of suspension in normal saline was fed orally in a volume of 0.4 mL kg^{-1} bw. The standard drug was used as a 2% suspension in normal saline in a dose of 5 mg kg^{-1} bw.

3.1.6.2. *Acute anti-inflammatory model (paw edema induced by formalin).* The rats were divided into seven groups of four rats each. Group 1 received DMSO and served as a control. Groups 2 and 3 received doses of 20 mg kg^{-1} bw of ligand-1 and ligand-2 in DMSO, respectively. Groups 4–6 received 20 mg kg^{-1} bw of Co, Ni, and Cu complexes and Group 7 received 5 mg kg^{-1} bw standard drug, diclofenac sodium. Edema was induced by injecting 0.1 mL of 3.5% formalin in normal saline into the sub-plantar region of the left hind paw after 1 h of drug administration. The paw volume was measured with the help of mercury displacement technique with the help of a plethysmometer (Model 7140, UGO Basile, Italy) first at 0 h and then at 1, 2, 3, 4, and 5 h after administration of drugs. The dosage details are presented in Supplementary table 9; the percentage inhibition of edema compared with that of the control was taken as an anti-inflammatory activity. The percentage inhibition of edema was calculated by the formula,

$$\text{Percentage of inhibition} = \frac{(V_t - V_0)\ \text{control} - (V_t - V_0)\ \text{treated}}{(V_t - V_0)\ \text{control}} \times 100,$$

V_0 = Average volume of right paw before injection of formalin i.e., at 0th hour,
V_t = Average volume of right paw after injection of formalin.

4. Results and discussion

The Schiff bases (**I** and **II**) form octahedral complexes (**1–6**) with $CoCl_2 \cdot 6H_2O$/$NiCl_2 \cdot 6H_2O$/$CuCl_2 \cdot 2H_2O$ in ethanol. The complexes are colored, stable, non-hygroscopic, insoluble in common organic solvents but soluble in DMF and DMSO (table 1). The elemental analyses show 1 : 1 stoichiometry of the type $ML \cdot 2H_2O$, where "L" stands for a deprotonated ligand (scheme 1). The molar conductance values are too low to account for any dissociation of the complexes in DMSO, indicating non-electrolytes in DMSO [19].

4.1. *IR spectral studies*

IR spectral data of Schiff bases and their Co(II), Ni(II), and Cu(II) complexes are presented in Supplementary table 1. A strong band at 1620–1605 cm^{-1} in spectra of the Schiff bases is assigned to ν(CH=N) [27]. A strong band at 2658 cm^{-1} is assigned to ν(OH). High intensity bands at 1728–1722 cm^{-1} and 1231–1229 cm^{-1} in IR spectra of the Schiff bases are assigned to ν(C=O) (lactone carbonyl) and phenolic ν(C–O), respectively.

In comparison with the Schiff bases, all the Co(II), Ni(II), and Cu(II) complexes exhibited ν(CH=N) at 1595–1580 cm^{-1} shifted to lower wavenumber, indicating azomethine is coordinated [28]. A medium to high intensity band at 1308–1305 cm^{-1} due to phenolic ν(C–O) indicates coordination of phenolic oxygen *via* deprotonation. The increase in frequency of ν(C–O) in the complexes may be due to the expected high mesomeric interaction activated by the presence of metal. New bands at 465–445 cm^{-1} and 510–520 cm^{-1} for the metal complexes are assigned to stretching frequencies of (M–N) [29] and (M–O) [30], respectively. Coordinated water was confirmed by a broad band at 3436–3420 cm^{-1} and two weak bands at 750–800 and 700–720 cm^{-1} due to ν(−OH) rocking and wagging, respectively [31]. Thus, IR spectral data provide evidence for complexation of Schiff bases with metal.

4.2. *NMR spectral studies of Schiff base*

Both Schiff bases are characterized by ^{1}H NMR and ^{13}C NMR (Supplementary table 2). In the ^{1}H NMR spectrum of Schiff base I, proton signals at 9.14 ppm (s, 1H) and 10.45 ppm (s, 1H) are assigned to −CH=N and phenolic −OH, respectively. Signals at 6.31–7.93 ppm (m, 8H) and a sharp signal at 2.09 ppm (s, 3H) are due to aromatic and CH_3 protons, respectively.

In the ^{13}C NMR spectrum of Schiff base I, the signal at 160.86 ppm is ascribed to lactonyl carbon (C=O) and a signal at 154.35 ppm is assigned to azomethine carbon. Characteristic signals at 114.1–132.87 ppm are assigned to aromatic carbons and 22.10 ppm to methyl. Thus, the NMR results support the IR inferences.

4.3. *Electronic spectral and magnetic studies*

The brownish Co(II) complexes showed absorptions at 9578–9632 and 18,693–18,779 cm^{-1} corresponding to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1) and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3),

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

Complex No.	Empirical formula	Color/Yield (%)	M (%)		C (%)		H (%)		N (%)		Molar conductance $\Omega^{-1}\,cm^2\,mol^{-1}$	μ_{eff} (BM)
			Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd		
I	$C_{34}H_{24}N_2O_8S$	80	–	–	65.67	65.80	3.68	3.87	4.44	4.51	–	–
II	$C_{32}H_{20}N_2O_8S$	78	–	–	61.45	61.93	3.32	3.37	4.65	4.72	–	–
1	$Co(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$	Brown/70	8.21	8.27	57.12	57.22	3.34	3.08	3.56	3.92	28.11	4.83
2	$Co(C_{32}H_{18}N_2O_8S \cdot 2H_2O)$	Brown/69	8.45	8.61	56.23	56.05	2.13	2.63	4.14	4.08	18.87	4.62
3	$Ni(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$	Yellowish green/68	8.11	8.15	57.11	57.30	3.14	3.08	3.55	3.93	24.55	3.15
4	$Ni(C_{32}H_{18}N_2O_8S \cdot 2H_2O)$	Yellowish green/69	8.15	8.48	56.45	56.14	2.34	2.63	4.34	4.09	22.65	3.22
5	$Cu(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$	Dark green/66	8.12	8.79	56.56	56.90	3.56	3.06	3.67	3.90	25.13	1.74
6	$Cu(C_{32}H_{18}N_2O_8S \cdot 2H_2O)$	Dark green/69	9.11	9.14	55.50	55.73	2.43	2.61	4.23	4.06	29.56	1.79

respectively, characteristic of octahedral Co(II) [32]. However, ν_2 is not observed because of its proximity to the strong ν_3 transition. The Co(II) complexes exhibit magnetic moment values from 4.51 to 4.74 agreeing with octahedral range of 4.83–4.62 BM [33].

The greenish $Ni(C_{34}H_{22}O_5N_2S)\cdot 2H_2O$ complexes exhibit three bands at 10,250, 15,245, and 26,134 cm^{-1} attributed to the $^3A_{2g}\rightarrow {}^3T_{2g}$ (ν_1), $^3A_{2g}\rightarrow {}^3T_{1g}(F)$ (ν_2), and $^3A_{2g}\rightarrow {}^3T_{1g}(P)$ (ν_3) transitions, respectively, indicating an octahedral geometry around Ni(II) [34]. The ligand field parameters are given in Supplementary table 3. The ν_2/ν_1 ratio is 1.47. The values of the nephelauxetic parameter, β, indicate low covalent character of the metal–ligand σ bonds [35, 36]. Ni(II) complexes showed magnetic moment values of 3.15–3.22 within the range of 2.8–3.5 BM, suggesting [37] octahedral environment. Hence the ligand field parameters correlate the electronic spectral and magnetic properties.

Electronic spectra of Cu(II) complexes display two prominent bands. A low intensity broad band at 15382 cm^{-1} is assignable to $^2E_g\rightarrow {}^2T_{2g}$ transition and a high intensity band at 30789 cm^{-1} is due to symmetry forbidden ligand $\rightarrow$ metal charge transfer. On the basis of electronic spectra distorted octahedral geometry around Cu(II) ion is suggested [38]. The Cu(II) complexes showed magnetic moment in the range of 1.74–1.79 BM, slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [39].

4.4. *FAB-mass spectral studies*

A representative FAB-mass spectrum of Schiff base I is depicted in Supplementary figure 1. The spectrum showed a molecular ion peak at m/z 620 which is equivalent to its molecular weight. In addition to this, fragment peaks observed at m/z 432, 324, and 136 are due to cleavage of $C_{11}H_8O_3$, $C_{12}H_8O_2N_2S$, and $C_{11}H_8O_3$, respectively. For Schiff base II, the molecular ion peak is observed at m/z 268, which is ascribed to $C_{32}H_{20}N_2O_8S$.

FAB-mass spectra of all the complexes exhibited molecular ion peak equivalent to their molecular weight along with other fragmentation peaks. Only the representative $Co(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ (**1**), $Ni(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ (**3**), and $Cu(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ (**5**) are discussed here. The spectrum of $Co(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ showed a molecular ion peak M^+ at m/z 713, which is equivalent to its molecular weight. The fragmentation peaks at 677, 490, 382, and 195 correspond to the coordinated water molecule and $C_{11}H_8O_8$, $C_{12}H_8O_2N_2S$, and $C_{11}H_8O_8$ species, respectively. For $Ni(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ the molecular ion peak M^+ at m/z 712 corresponds to its molecular weight. The complex exhibited fragmentation peaks at 676, 489, 381, and 194 for cleavage of coordinated water, $C_{11}H_8O_8$, $C_{12}H_8O_2N_2S$, and $C_{11}H_8O_8$, respectively. The spectrum of $Cu(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ (Supplementary figure 2) exhibited a molecular ion peak M^+ at m/z 717 which is equivalent to its molecular weight, with other fragments observed at 681, 494, 386, and 199 from cleavage of coordinated water, $C_{11}H_8O_8$, $C_{12}H_8O_2N_2S$, and $C_{11}H_8O_8$, respectively.

4.5. *ESR spectrum of $Cu(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$*

The ESR spectrum of $Cu(C_{34}H_{22}N_2O_8S\cdot 2H_2O)$ was recorded in DMSO at LNT and room temperature. The spectrum at room temperature showed one intense absorption

in the high field region, that is isotropic due to tumbling of the molecules. However, this complex at LNT shows four well-resolved peaks in the low-field region. The $g_{\parallel}$ and $g_{\perp}$ values are 2.065 and 2.035, respectively. The trend $g_{\parallel} > g_{\perp} > 2.0023$ indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of Cu(II) and are characteristic for axial symmetry [40]. The g_{av} was calculated to be 2.043. The results are in accord with Cu(II) complexes possessing distorted octahedral geometry.

4.6. *Thermal studies of metal complexes*

The thermal behavior of the complexes has been studied as a function of temperature; thermal behaviors are all almost the same. $Co(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$, $Ni(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$, and $Cu(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$ complexes are chosen as representative complexes. Supplementary figure 3 represents the TG/DTG spectrum of $Co(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$.

The thermal decomposition of **1** takes place in four steps as indicated by DTG peaks. Loss of coordinated water took place at 135–145°C corresponding to mass loss of 5.11% (Calcd 5.04%). The two coumarin and one dapsone moieties were decomposed at 200–210°C, 250–265°C, and 530–550°C with mass losses of 26.23% (Calcd 26.36%), 26.12% (Calcd 26.36%) and 33.11% (Calcd 33.66%). Formation of metal oxide took place above 500°C.

Decomposition of $Ni(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$ occurred in four steps at 130–145°C, 205–220°C, 240–250°C, and 540–560°C corresponding to cleavage of coordinated water, two coumarin and one dapsone, respectively, with the mass losses of 5.02% (Calcd 5.05%), 26.35% (Calcd 26.40%), 26.29% (Calcd 26.40%), and 33.56% (Calcd 33.71%), respectively. The formation of metal oxide took place at 520°C. Decomposition of $Cu(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$ also took place in four steps: 130–145°C, 205–220°C, 240–250°C, and 540–560°C with mass loss of 5.05% (Calcd 5.02%), 26.34% (Calcd 26.22%), 26.43% (Calcd 26.22%), and 33.44% (Calcd 33.47%) corresponding to coordinated water, two coumarin and dapsone moieties, respectively. The formation of metal oxide took place at 500°C.

4.7. *Fluorescence spectral studies-emission spectroscopy*

Schiff base I has an emission band at 490 nm in DMF. Upon addition of 2–3 drops of aqueous alkali (2% NaOH) to Schiff base I, we observe λ_{max} at 391 nm due to formation of phenoxide [41]. The Co(II), Ni(II), and Cu(II) complexes of Schiff base I exhibited the emission band at 539, 535, and 530 nm, respectively, in DMF. The red shift of λ_{max} of the complexes compared with Schiff base I may be due to deprotonation of the hydroxyl (Supplementary figure 4). This indicates that Schiff base I and its complexes are fluorescent. The fluorescence emission intensity of Schiff base I decreases upon complex formation with metal ion in the order Co(II) < Ni(II) < Cu(II).

4.8. *Electrochemistry*

All the complexes were studied for their electrochemical behavior. Both Cu(II) complexes exhibited similar electrochemical properties. A cyclic voltammogram of

$Cu(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$ (Supplementary figure 5) displays a reduction peak at $E_{pc} = 0.63$ V with a corresponding oxidation peak at $E_{pa} = 0.82$ V. The peak separation of this couple (ΔE_p) is 0.19 V at 0.1 V and increases with increasing scan rate. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple. The difference between forward and back peak potentials provides a rough evaluation of the degree of reversibility of one electron transfer. Analyses of cyclic voltammetric responses with scan rate varying from 50 to 300 mV s^{-1} gives evidence for quasi-reversible, one-electron transfer. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with increasingsquare root of the scan rates, establishing the electrode process as diffusion controlled [42]. The separation in peak potentials increases at higher scan rates. These features are consistent with quasi-reversibility.

The cyclic voltammogram (Supplementary figure 6) of $Co(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$ exhibits a reduction peak at $E_{pc} = 0.631$ V with an oxidation peak at $E_{pa} = 0.822$ V corresponding to the Co(II)/Co(I) redox couple. The peak separation of this couple (ΔE_p) is 0.191 V. This Co(II) complex also has a quasi-reversible character. The peak currents rise with increasing square root of the scan rates.

4.9. *Pharmacological results*

4.9.1. *In vitro* antibacterial and antifungal activity. Schiff bases (I and II) were highly active against *Klebsiella* and *Salmonella* and moderately active against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa,* and *S. proteus*. The Co(II), Ni(II) and Cu(II) complexes showed more antibacterial activity against all the bacterial strains (Supplementary table 5) than the respective Schiff bases. For antifungal activity, the Schiff bases and their complexes were highly active with metal complexes, more active than the Schiff bases [43, 44]. Minimum inhibitory concentrations of some selected compounds, which showed significant activity against selected bacterial and fungi, indicated that these compounds were most active at 500 μg mL^{-1} concentration (Supplementary table 6).

4.9.2. DNA cleavage activity. Schiff base I and $Co(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$, $Ni(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$, and $Cu(C_{34}H_{22}N_2O_8S \cdot 2H_2O)$ have been studied for their DNA cleavage activity by agarose gel electrophoresis against isolated DNA of *E. coli* [45]. The electrophoresis clearly revealed that Schiff base I and its metal complexes act on DNA with molecular weight difference between the control and the treated DNA samples. The difference was observed in bands of Lanes 1–4 compared to the control (Supplementary figure 6) which does not show any apparent cleavage. However, the nature of reactive intermediates involved in DNA cleavage by the complexes is not clear.

4.9.3. Anthelmintic activity (*in vitro*). The anthelmintic activities of the Schiff bases and complexes were tested on earthworms (*P. posthuma*). The metal complexes exhibit higher anthelmintic activity than the Schiff bases with complexes of Co(II) and Cu(II) showing more activity. We conclude that the Schiff bases have enhanced anthelmintic activity on complexation with Co(II) and Cu(II) (Supplementary table 7).

4.9.4. Anti-inflammatory (paw edema induced by formalin). The anti-inflammatory effect of the test compounds on Formalin induced edema in right hind paws of rats are presented in Supplementary tables 8 and 9. There was gradual increase in edema paw volume of rats in the control (Formalin treated). However, in the test groups, the compounds showed significant reduction in the edema paw volume. As indicated in Supplementary table 9, significant anti-inflammatory effect appeared at 1–2 h, progressively increased and reached maxima of 71.4%, 70% and 65.5%, respectively, at 5 h; maximum anti-inflammatory effect of Schiff base I appeared at 1 h (71.4%). The anti-inflammatory effect induced by Diclofenac sodium progressively increased and reached a maximum (75%) at 2 h.

Inflammatory results showed that the Schiff bases possess significant role in inhibition of inflammation which increases after addition of metal ions. Further studies in humans should be undertaken to develop potent anti-inflammatory agents with low toxicity and better therapeutic index.

5. Conclusion

The newly synthesized Schiff bases are tetradentate, coordinating to metal through azomethine nitrogen atoms and phenolic oxygen atoms *via* deprotonation. Emission spectra reveal that the Schiff bases and their complexes are fluorescent. The Schiff bases and their complexes were active against some antibacterial and antifungal species. The activity is significantly increased on coordination. DNA cleavage studies revealed that the complexes show non-specific cleavage of DNA. The Schiff base metal complexes have potent anthelmintic activity when compared with the standard anthelmintic drug. Anti-inflammatory activity by the carrageenan induced paw edema method showed that the synthesized compounds exhibit good activities.

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