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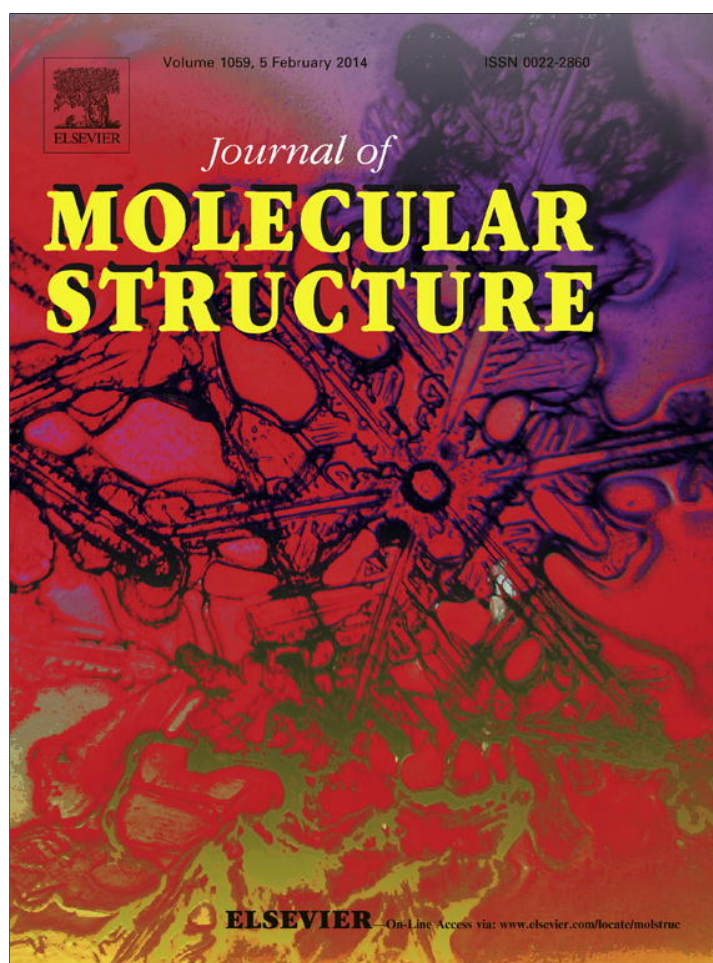


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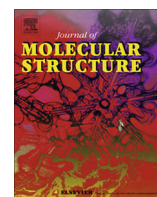
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Synthesis, spectroscopic characterization, electrochemistry and biological evaluation of some binuclear transition metal complexes of bicompartamental ONO donor ligands containing benzo[*b*]thiophene moiety



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HIGHLIGHTS

- Synthesis, characterization of bicompartamental ligands and their metal complexes.
- ESR spectral data proved the binuclear nature of the metal complexes.
- Cu(II) complexes showed the redox property.
- The synthesized complexes shows promising antimicrobial activities compared to ligands.
- The complexes have good ability to cleave the super coiled DNA.

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ABSTRACT

A series of new binucleating Cu(II), Co(II), Ni(II) and Zn(II) complexes of bicompartamental ligands with ONO donor were synthesized. The ligands were obtained by the condensation of 3-chloro-6-substituted benzo[*b*]thiophene-2-carbohydrazides and 4,6-diacetylresorcinol. The synthesized ligands and their complexes were characterized by elemental analysis and various spectroscopic techniques. Elemental analysis, IR, ¹H NMR, ESI-mass, UV–Visible, TG–DTA, magnetic measurements, molar conductance and powder-XRD data has been used to elucidate their structures. The bonding sites are the oxygen atom of amide carbonyl, azomethine nitrogen and phenolic oxygen for ligands **1** and **2**. The binuclear nature of the complexes was confirmed by ESR spectral data. TG–DTA studies for some complexes showed the presence of coordinated water molecules and the final product is the metal oxide. All the complexes were investigated for their electrochemical activity, only the Cu(II) complexes showed the redox property. Cu(II) complexes were square planar, whereas Co(II), Ni(II) and Zn(II) complexes were octahedral. Powder-XRD pattern have been studied in order to test the degree of crystallinity of the complexes and unit cell calculations were made. In order to evaluate the effect of antimicrobial activity of metal ions upon chelation, both the ligands and their metal complexes were screened for their antibacterial and antifungal activities by minimum inhibitory concentration (MIC) method. The results showed that the metal complexes were found to be more active than free ligands. The DNA cleaving capacities of all the complexes were analyzed by agarose gel electrophoresis method against supercoiled plasmid DNA. Among the compounds tested for antioxidant capacity, ligand **1** displayed excellent activity than its metal complexes.

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1. Introduction

In medicinal chemistry sulfur containing heterocycles have been very well known for their therapeutic applications. Compounds containing benzo[*b*]thiophene nucleus possess broad range of biological activities such as anti-inflammatory [1], antifungal

[2], analgesic [3], ocular hypotensive activities [4], alkaline phosphatase inhibitor [5] and antimicrobial activities [6]. Raloxifene drug containing benzo[*b*]thiophene nucleus has been approved by the U.S. Food and Drug Administration for the treatment of osteoporosis associated with woman postmenopausal [7,8].

On the otherhand 4,6-diacetylresorcinol, difunctional carbonyl compound acts as a precursor for the formation of various binucleating ligands [9–11] and also employed in the construction of ligands containing O, N, S donor atoms by its condensation with

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various thiosemicarbazides and thiocarbohydrazides [12]. Studies on binucleating ligands and binuclear metal complexes have stimulated interest owing to their unique physicochemical properties. Deoxyribonucleic acid (DNA) is the primary target molecule for most of the antiviral and anticancer therapies. Hence, the investigation on the interaction of DNA with small molecules is the basic work in the design of new type of pharmaceutical molecules. So, the interest in the interaction of small molecules with DNA, that are capable of binding and cleaving DNA constitute a significant area of research nowadays which has attracted the attention of both inorganic and biochemist which lead to the development of new drugs, synthetic restriction enzymes and DNA foot printing agents [13,14]. In spite of enormous scientific literature on Schiff bases derived from benzo[b]thiophene moiety, there is very little known about bicompartamental Schiff bases with the benzo[b]thiophene moiety and their metal complexes. Based on these finding and in continuation of our research work on coordination chemistry [15–18], we report herewith the synthesis, characterization and antimicrobial evaluation studies of (*N',N'',N',N''*)-*N',N''*-(1,1'-(4,6-dihydroxy-1,3-phenylene)bis(ethan-1-yl-1-ylidene))bis(3-chloro-6-substituted benzo[b]thiophene-2-carbohydrazide) Schiff bases obtained by the condensation of 3-chloro-6-substituted benzo[b]thiophene-2-carbohydrazides with difunctional 4,6-diacetylresorcinol and their Cu(II), Co(II), Ni(II) and Zn(II) complexes in order to obtain new class of biologically active compounds.

2. Experimental

2.1. Analysis and physical measurements

IR Spectra of the newly synthesized compounds were recorded as KBr pellets on a Perkin Elmer – Spectrum RX-IFTIR instrument in the region 4000–250 cm⁻¹. ¹H NMR spectra of ligands and their Zn(II) complexes were recorded in d₆-DMSO using a Avance III instrument. UV–Visible spectra of the Cu(II), Co(II) and Ni(II) complexes were recorded on Elico – SL 164 double beam spectrometer in the range 200–1000 nm in DMF solution (1 × 10⁻³ M). Elemental analysis was obtained from HERAEUS C, H, N–O rapid analyzer and metal analysis was carried out by following the standard methods. ESI-MS was recorded on Waters, model-Q-ToF micro mass spectrophotometer equipped with electrospray ionization (ESI) having mass range of 4000 amu in quadruple and 20,000 amu in ToF. ESR measurement of Cu(II) complexes in polycrystalline state were carried out on a BRUKER Bio Spin GmbH spectrometer at microwave frequency of 9.1 GHz. The experiment was carried out by using DPPH as reference with field set at 3000 gauss. Electrochemistry of all the complexes was recorded on a 600 D series model electrochemical analyzer in DMF using *n*-Bu₄NClO₄ as a supporting electrolyte. Powder-XRD of the complexes was recorded using Bruker AXS D8 Advance (Cu, Wavelength 1.5406 Å source). Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in dry DMF (10⁻³ M) solution using a dip-type conductivity cell fitted with a platinum electrode and the magnetic susceptibility measurements were made at room temperature on a Gouy balance using Hg[Co(NCS)₄] as the calibrant.

2.2. Methods

All the chemicals used were of reagent grade and procured from Hi-media. Solvents were dried and distilled before use. Melting points of the newly synthesized compounds were determined by electro-thermal apparatus using open capillary tubes. The metal and chloride contents of the metal complexes were determined

as per standard procedures [19]. 3-chloro-6-substituted benzo[b]thiophene-2-carbohydrazide was prepared by the literature method [20]. The 4,6-diacetylresorcinol was procured from Sigma Aldrich.

2.3. Synthesis of the ligands 1 and 2

A mixture of 3-chloro-6-substituted benzo[b]thiophene-2-carbohydrazide (0.02 mol) and 4,6-diacetylresorcinol (0.01 mol) with a catalytic amount of glacial acetic acid (1–2 drops) in ethanol (20 mL) was refluxed on a water bath for about 5–6 h. The reaction was monitored by TLC. The pale yellow colored solid separated was filtered, washed with little ethanol, dried and recrystallized from dioxane (Scheme 1).

Ligand 1: Yield = 71%. m.p. 297 °C; Mol. Formula = C₂₈H₂₀N₄O₄·S₂Cl₂ (Mol. Wt. = 611.52).

Ligand 2: Yield = 67%; m.p. 305 °C; Mol. Formula = C₃₀H₂₄N₄O₆·S₂Cl₂ (Mol. Wt. = 671.05).

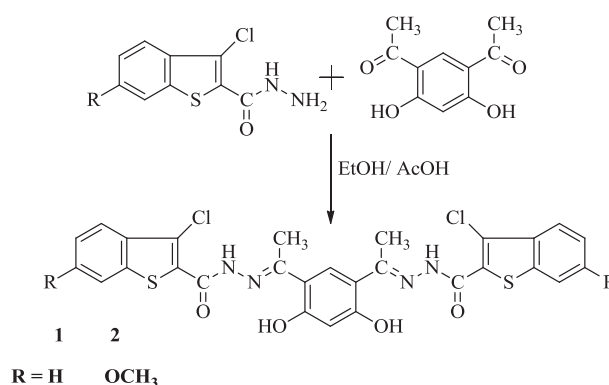
2.4. Synthesis of Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligands 1 and 2

To the hot solution of (*N',N'',N',N''*)-*N',N''*-(1,1'-(4,6-dihydroxy-1,3-phenylene)bis(ethan-1-yl-1-ylidene))bis(3-chloro-6-substituted benzo[b]thiophene-2-carbohydrazide) (1 and 2) (0.001 mol) in ethanol (30 mL) was added a hot ethanolic solution (15 mL) of respective metal chlorides (0.002 mol). The reaction mixture was then refluxed on a water bath for about 4–5 h, sodium acetate (0.5 g) was added to the reaction mixture and refluxing continued for about an hour more. The reaction mixture was then poured into distilled water. The separated solid complexes were collected by filtration, washed with sufficient quantity of distilled water, then with hot ethanol to apparent dryness and dried in a vacuum over anhydrous calcium chloride in a desiccator.

2.5. Pharmacological activity

2.5.1. Antimicrobial assays

The biological activities of the newly synthesized ligands 1 and 2 and their Cu(II), Co(II), Ni(II) and Zn(II) complexes were screened for their antibacterial and antifungal activities by disc and well diffusion method respectively [21]. The *in vitro* antibacterial activities of the compounds were tested against two Gram-negative [*Escherichia coli* (MTCC 46) and *Salmonella typhi* (MTCC 98)] and two Gram-positive [*Bacillus subtilis* (MTCC 736) and *Staphylococcus aureus* (MTCC 3160)] bacteria. The *in vitro* antifungal activities were carried out against *Candida albicans* (MTCC 227), *Cladosporium oxy-*



Scheme 1. Synthesis of ligands 1 and 2.

sporum (MTCC 1777) and *Aspergillus niger* (MTCC 1881) fungi. The stock solutions of the test chemicals (1 mg mL^{-1}) were prepared by dissolving 10 mg of the each test compound in 10 mL of freshly distilled DMSO. The different concentrations of the test compounds ($100, 50, 25$ and $12.5 \mu\text{g mL}^{-1}$), were prepared by diluting the stock solution with the required amount of distilled DMSO. The lowest concentration that inhibited microbial growth was called as the MIC value. Further the dilutions for the metal chlorides were prepared by using distilled water and control experiments were carried out by using DMSO solvent alone.

2.5.2. Antibacterial screening

Muller–Hinton agar media was used for the antibacterial studies. The pure dehydrated Muller–Hilton agar (38 g) was dissolved in 1000 mL distilled water. The pure cultures of the bacterial strains *E. coli*, *S. aureus*, *B. subtilis* and *S. typhi* were sub cultured by inoculating them in the nutrient broth and they were incubated at 37°C for about 18 h. The agar plates were prepared by using the above media. Each plate were inoculated with 18 h old bacterial culture ($100 \mu\text{L}$) using a micropipette and spread uniformly using bent glass rod. Wells were dug with the help of 6 mm sterile metallic cork borer. The drug gentamycin is used as standard. Different concentration of the test compounds and their respective metal chloride solutions were incorporated into the wells using micropipette and the plates were kept for incubation at 37°C for 24 h. Soon after the completion of incubation period, the diameter of the inhibition zone generated by each test compound against bacterial growth were measured using antibiogram zone measuring scale.

2.5.3. Antifungal screening

Potato dextrose agar (PDA) media was used for the antifungal studies. The following ingredients were used to prepare the media i.e. potatoes (sliced washed unpeeled) 200 g, dextrose 20 g, agar 20 g in 1000 mL distilled water. The pure cultures *C. albicans*, *C. oxysporum* and *A. niger* were inoculated on PDA slants. These slants were incubated at 32°C for 7 days. To these 7 days old slants of fungal strains, 10 mL of 0.1% tween-80 solution was added and the culture were scraped with sterile inoculating loop to get uniform spore suspension. The agar plates were prepared by using the above potato dextrose agar media. Each plate were inoculated with 7 days old spore suspension of each fungal culture ($100 \mu\text{L}$) using a micropipette and spread uniformly using bent glass rod. Wells were dug with the help of 6 mm sterile metallic cork borer. Each well was incorporated with the test compound solution of different concentrations and their respective metal chloride solution. The drug fluconazole is used as standard. All the inoculated plates were incubated at 32°C for about 48 h. Soon after the completion of incubation period the diameter of the inhibition zone generated by each test compound against fungal growth were measured using antibiogram zone measuring scale.

2.5.4. DNA cleavage experiment

The extent to which the newly synthesized ligands and their metal complexes could function as DNA cleavage agents was examined using supercoiled plasmid DNA pBR 322 (Bangal re Genei, Bengaluru, Cat. No. 105850) as a target molecule. The electrophoresis method was employed to study the efficiency of cleavage by the synthesized compounds. Each test compounds ($100 \mu\text{g}$) were added separately to the 225 ng pBR 322 DNA sample and these samples mixtures were incubated at 37°C for 2 h. The electrophoresis of the test compounds was done according to the literature method [22]. Agarose (600 mg) was dissolved in hot tris-acetate-EDTA (TAE) buffer (60 mL) (4.84 g Tris base, pH – 8.0, 0.5 M EDTA L^{-1}) and heated to boil for few minutes. When the gel attains approximately 55°C , it was then poured into the gas cassette fitted with comb. Slowly the gel was allowed to solidify

by cooling to room temperature and then carefully the comb was removed. The solidified gel was placed in the electrophoresis chamber containing TAE buffer. The DNA sample ($20 \mu\text{L}$) initially treated with the test compounds were mixed with bromophenol blue dye in equimolar ratio along with standard DNA marker containing TAE buffer was loaded carefully into the wells and the constant 50 V of electricity was supplied for about 30 min. Later, the gel was removed, stained with ethidium bromide solution ($10 \mu\text{g mL}^{-1}$) for 15–20 min and then the bands were observed and photographed under UV-illuminator.

2.5.5. Antioxidant assay (free radical scavenging activity)

The free radical scavenging activity of the test samples was determined with the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method [23]. Different concentrations of test compounds ($12.5 \mu\text{g}$, $25 \mu\text{g}$, $50 \mu\text{g}$ and $100 \mu\text{g}$) and standard butylated hydroxyanisole (BHA) were taken in different test tubes and the volume of each test tube was adjusted to $100 \mu\text{L}$ by adding distilled DMF. To the tubes containing sample solutions in DMF, 5 mL methanolic solution of DPPH (0.1 mM) was added to these tubes. The tubes were allowed to stand for 30 min. The control experiment was carried out as above without the test samples. The absorbance of test solutions was measured at 517 nm. The reduction of DPPH was calculated relative to the measured absorbance of the control. Radical scavenging activity was calculated using the following formula:

$$\% \text{ Radical scavenging activity} = \left[\frac{\text{Control optical density} - \text{Sample optical density}}{\text{Control optical density}} \right] \times 100$$

3. Results and discussion

All the synthesized metal complexes are colored solids, amorphous in nature and stable in air. Melting points of the newly synthesized metal complexes were above 320°C . The complexes are insoluble in water and common organic solvents but soluble in DMF and DMSO. Elemental analysis and analytical data of the complexes (Table 1) suggest that the metal to ligand ratio of the complexes is 2:1 stoichiometry of the type $[\text{M}_2(\text{L})(\text{Cl})_2]$ for Cu(II) complexes and $[\text{M}_2(\text{L})(\text{Cl})_2(\text{H}_2\text{O})_4]$ for Co(II), Ni(II) and Zn(II) complexes of both the ligands (**1** and **2**), where L stands for deprotonated ligand. The molar conductance values are too low to account for any dissociation of the complexes in DMF (14.76 – $31.21 \text{ ohm}^{-1} \text{ cm}^2 \text{ mole}^{-1}$), indicating non-electrolytic nature of the complexes in DMF.

3.1. IR spectral data

The important IR bands of the ligands and their metal complexes are represented in Table 2. In the IR spectra of ligands **1** and **2**, absorption due to phenolic OH exhibited bands at 3434 and 3385 cm^{-1} and absorption due to NH of CONH function displayed bands at 3354 and 3305 cm^{-1} respectively. The phenolic C–O function of ligands **1** and **2** displayed absorption bands at 1242 and 1260 cm^{-1} respectively. In both the ligands absorption bands due to carbonyl and azomethine functions have appeared at 1661 and 1622 cm^{-1} and 1671 and 1615 cm^{-1} respectively.

The absence of absorption bands due to phenolic OH groups at 3434 and 3385 cm^{-1} in the IR spectra of Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligands **1** and **2** indicates the formation of bonds between metal ion and phenolic oxygen atom via deprotonation. This is further confirmed by the increase in absorption frequency of phenolic C–O which appeared in the region 1278 – 1289 cm^{-1} and 1267 – 1271 cm^{-1} respectively in the metal complexes of both the ligands under the present study. The absorption

Table 1
Physical, analytical and magnetic susceptibility data of ligands **1** and **2** and their complexes.

Ligand/complexes	Molecular formula	Mol. Wt. (g)	Elemental analysis (%) Calcd. (Found)					Mag. moment		Color
			M	C	H	N	Cl	μ_{total}^a (BM)	μ_{eff}^b (BM)	
1 H ₂ L ₁	C ₂₈ H ₂₀ N ₄ O ₄ S ₂ Cl ₂	611.52	–	54.99 (55.19)	3.30 (3.35)	9.16 (9.29)	11.60 (11.59)	–	–	Yellow
2 H ₂ L ₂	C ₃₀ H ₂₄ N ₄ O ₆ S ₂ Cl ₂	671.05	–	53.65 (53.86)	3.60 (3.65)	8.34 (8.46)	10.56 (10.59)	–	–	Pale yellow
1a [Cu ₂ (L ₁)(Cl) ₂]	Cu ₂ [C ₂₈ H ₁₈ N ₄ O ₄ S ₂ Cl ₄]	807.50	15.74 (15.86)	41.65 (41.81)	2.25 (2.30)	6.94 (7.05)	17.56 (17.44)	2.72	1.49	Green
1b [Co ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	Co ₂ [C ₂₈ H ₂₆ N ₄ O ₈ S ₂ Cl ₄]	870.34	13.54 (13.65)	38.64 (38.82)	3.01 (3.04)	6.44 (6.52)	16.29 (16.19)	4.64	4.52	Brown
1c [Ni ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	Ni ₂ [C ₂₈ H ₂₆ N ₄ O ₈ S ₂ Cl ₄]	869.86	13.49 (13.63)	38.66 (38.87)	3.01 (3.08)	6.44 (6.53)	16.30 (16.21)	4.76	3.26	Green
1d [Zn ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	Zn ₂ [C ₂₈ H ₂₆ N ₄ O ₈ S ₂ Cl ₄]	883.23	14.80 (14.94)	38.08 (38.21)	2.97 (3.00)	6.34 (6.42)	16.06 (15.97)	Dia mag	Dia mag	Orange
2a [Cu ₂ (L ₂)(Cl) ₂]	Cu ₂ [C ₃₀ H ₂₂ N ₄ O ₆ S ₂ Cl ₄]	867.55	14.65 (14.77)	41.53 (41.73)	2.56 (2.63)	6.46 (6.55)	16.35 (16.27)	2.21	1.48	Green
2b [Co ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	Co ₂ [C ₃₀ H ₃₀ N ₄ O ₁₀ S ₂ Cl ₄]	930.39	12.67 (12.81)	38.73 (38.87)	3.25 (3.31)	6.02 (6.15)	15.24 (15.17)	4.50	4.39	Brown
2c [Ni ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	Ni ₂ [C ₃₀ H ₃₀ N ₄ O ₁₀ S ₂ Cl ₄]	929.91	12.62 (12.77)	38.75 (38.93)	3.25 (3.29)	6.02 (6.15)	15.25 (15.18)	4.90	3.34	Green
2d [Zn ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	Zn ₂ [C ₃₀ H ₃₀ N ₄ O ₁₀ S ₂ Cl ₄]	943.28	13.86 (13.99)	38.20 (38.35)	3.21 (3.28)	5.94 (6.01)	15.03 (14.94)	Dia mag	Dia mag	Orange

^a μ_{total} moment the total magnetic of the complex.

^b μ_{eff} where calculated for one metal ion in the complex.

Table 2
IR spectral data of ligands **1** and **2** and their complexes.

Ligands/complexes	$\nu_{\text{H}_2\text{O}}$	ν_{OH} (Phenolic)	ν_{NH} (amide)	$\nu_{\text{C=O}}$ (carbonyl)	$\nu_{\text{C=N}}$ (azomethine)	$\nu_{\text{C-S-C}}$	$\nu_{\text{C-O}}$ (phenolic)	$\nu_{\text{M-O}}$	$\nu_{\text{M-N}}$	$\nu_{\text{M-Cl}}$
1 H ₂ L ₁	–	3434	3354	1661	1622	1434	1242	–	–	–
2 H ₂ L ₂	–	3385	3305	1671	1615	1437	1260	–	–	–
1a [Cu ₂ (L ₁)(Cl) ₂]	–	–	3353	1636	1586	1435	1278	590	424	270
1b [Co ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	3414	–	3350	1633	1564	1434	1279	590	470	266
1c [Ni ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	3414	–	3353	1633	1557	1434	1289	590	493	293
1d [Zn ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	3400	–	3357	1626	1575	1434	1282	590	439	262
2a [Cu ₂ (L ₂)(Cl) ₂]	–	–	3305	1614	1599	1438	1271	559	428	266
2b [Co ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	3428	–	3306	1634	1586	1437	1267	540	439	273
2c [Ni ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	3400	–	3306	1634	1586	1437	1270	594	420	266
2d [Zn ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	3386	–	3305	1633	1585	1437	1271	590	439	285

band due to NH of CONH function of the above metal complexes of ligands **1** and **2** have displayed bands in the region 3357–3350 cm^{−1} and 3306–3305 cm^{−1} respectively, which have appeared at about the same region as in the case of the respective ligands thus confirming the non-involvement of NH of CONH function in coordination with the metal ions. The absorption frequency of carbonyl and azomethine functions in these metal complexes have been shifted to lower frequency by 35–25 and 57–37 cm^{−1} and 65–36 and 30–16 cm^{−1} and appeared in the region 1636–1626 and 1634–1614 cm^{−1} and 1586–1557 and 1599–1585 cm^{−1} respectively indicating the involvement of oxygen atom of carbonyl function as such without undergoing any enolization [24] and nitrogen atom of azomethine [25] function in complexation with the metal ions. This is further confirmed by the appearance of new bands in the region 594–540 and 493–420 cm^{−1} due to M–O and M–N stretching vibrations [26] in all the complexes of ligands **1** and **2** respectively. The appearance of new bands in the region 293–262 cm^{−1} in all the above synthesized complexes is due to M–Cl. The broad band due to co-ordinated water molecule in case of Co(II), Ni(II) and Zn(II) complexes of ligands **1** and **2** have appeared in the region 3414–3400 and 3428–3386 cm^{−1} respectively.

3.2. ¹H NMR spectral data

The ¹H NMR data of the ligands **1** and **2** and their Zn(II) complexes are depicted in Table 3. The ¹H NMR spectra of the ligands **1** and **2** displayed singlets each at 12.62 and 11.64 ppm and 12.62 two 12.04 ppm respectively, due to the two protons of amide NH and two OH protons of ligands **1** and **2** respectively. The aromatic protons of ligands **1** and **2** have resonated as multiplets in the region 7.65–7.97 ppm (m, 10H, ArH) and 7.34–7.60 ppm (m, 8H, ArH). Six protons of two methyl groups attached to azomethine carbon atoms in both the ligands respectively have resonated as distinct singlets at 2.66 ppm and 2.40 ppm respectively. Six protons of two methoxy groups attached to 6 -position of two benzo[b]thiophene moieties of ligand **2** have appeared as distinct singlet at 3.56 ppm.

In the case of Zn(II) complexes of ligand **1** and **2**, the absence of signal due to proton of two phenolic OH groups confirms the involvement of bonding of the phenolic oxygen atom to metal ion via deprotonation. The signals appeared at 12.63 and 12.63 ppm, 7.84–8.04 and 7.42–8.04 ppm and 2.72 and 2.55 ppm are due to two amide NH protons, aromatic protons and six protons of two methyl groups attached to two

Table 3¹H NMR data Zn(II) complexes of ligands **1** and **2**.

Ligand/Zn(II) complexes	¹ H NMR data (ppm)
1 /(H ₂ L ₁)	12.62 (s, 2H, two CONH), 11.64 (s, 2H, two phenolic OH), 7.65–7.97 (m, 10H, ArH), 2.66 (s, 6H, two CH ₃)
2 /(H ₂ L ₂)	12.62 (s, 2H, two CONH), 12.04 (s, 2H, two phenolic OH), 7.34–7.60 (m, 8H, ArH), 3.56 (s, 6H, two OCH ₃), 2.40 (s, 6H, two CH ₃)
1d /[Zn ₂ (L ₁)(Cl) ₂ (H ₂ O) ₄]	12.63 (s, 2H, two CONH), 7.84–8.04 (m, 10H, ArH), 5.92 (bs, 8H, 4H ₂ O), 2.72 (s, 6H, two CH ₃)
2d /[Zn ₂ (L ₂)(Cl) ₂ (H ₂ O) ₄]	12.63 (s, 2H, two CONH), 7.42–8.04 (m, 8H, ArH), 5.92 (bs, 8H, 4H ₂ O), 3.98 (s, 6H, two OCH ₃), 2.55 (s, 6H, two CH ₃)

azomethine carbon atoms in each of Zn(II) complexes of ligands **1** and **2** respectively. Singlet appeared at 3.98 ppm in case of Zn(II) complex of ligand **2** is due to six protons of two methoxy groups attached to 6-position of two benzo[*b*]thiophene moiety. A broad singlets at 5.92 ppm in both the Zn(II) complexes of ligands **1** and **2** is due to coordinated water molecules. A considerable degree of symmetry is present in these compounds so that the protons in the two halves of the molecules are magnetically equivalent. When compared to the ¹H NMR spectra of ligands **1** and **2** and their Zn(II) complexes, all the signals due to protons have been shifted towards down field strength confirming the complexation of Zn(II) ions with the ligands. Thus the ¹H NMR data supports the assigned structures.

3.3. ESI-mass spectral data

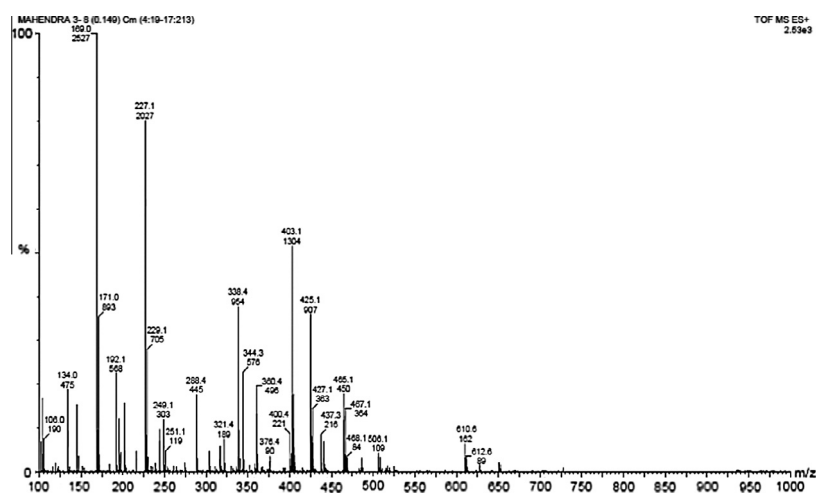
Ligands **1** and **2** and Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand **1** have been studied for their mass spectral studies. The ESI-mass spectra of all the above compounds exhibited molecular ion peaks equivalent of their molecular mass along with other fragment ion peaks. The representative mass spectra of ligands **1** (Fig. 1) and **2** showed molecular ion peak due to M⁺ at *m/z* 610, 612 (10%, 2.7%) and *m/z* 670, 672 (13%, 4%) which is equivalent of their molecular mass respectively. The fragmentation pattern of ligands **1** and **2** are depicted in Schemes 2 and 3 respectively, which are in consistency with their structures.

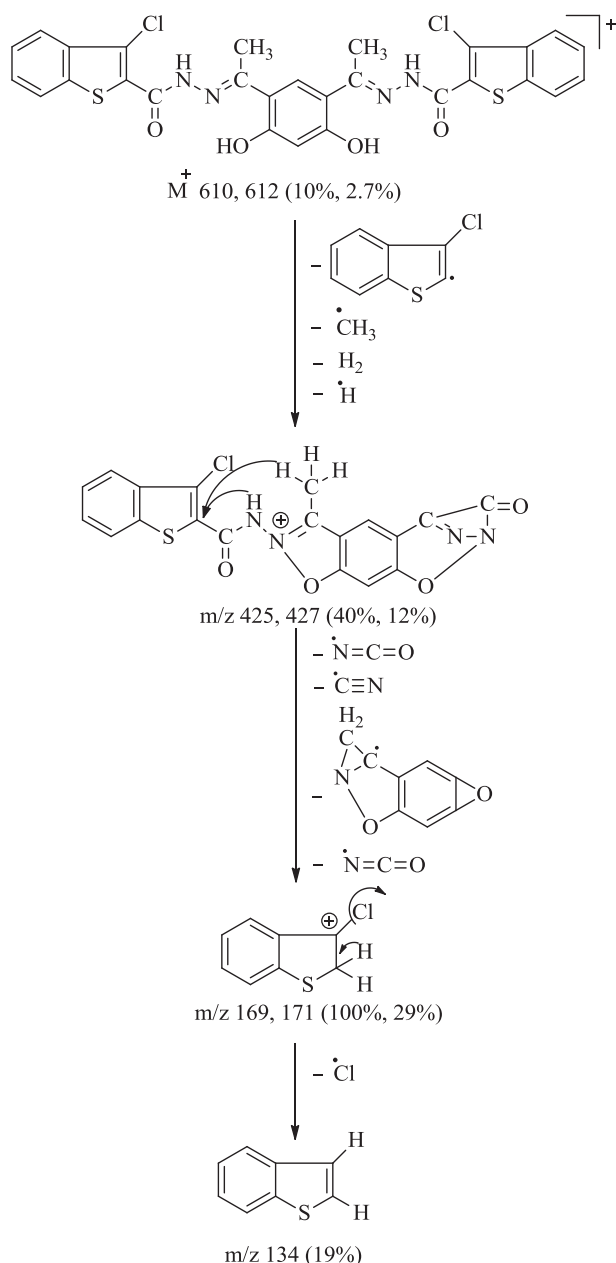
The mass spectra of Cu(II) (Fig. 2), Co(II), Ni(II) and Zn(II) complexes of ligand **1** exhibited the molecular ion peaks due to M⁺ at *m/z* 805, 807 (10%, 4.5%), *m/z* 867, 869 (25%, 9%), *m/z* 867, 869 (9%, 2.1) and *m/z* 880, 882 (7%, 2%) which are equivalent of their molecular mass of the respective complexes. The fragmentation pattern of the above complexes is presented in the Schemes 4–7 respectively. The mass spectral data of the above compounds are in conformity with their structures.

3.4. Electronic spectral and magnetic susceptibility data

The electronic absorption spectra of Cu(II), Co(II) and Ni(II) complexes of ligands **1** and **2** were recorded in distilled DMF (10^{−3} M) at room temperature. The band positions of absorption band maxima assignments are listed in Table 4. The electronic spectra of Cu(II) complexes (**1a** and **2a**) of the ligands **1** and **2** showed two absorption bands each at 576 (17,361 cm^{−1}) and 698 (14,326 cm^{−1}) nm and 569 (17,574 cm^{−1}) and 687 (14,556 cm^{−1}) nm respectively, which are assigned to ²E_g ← ²B_{1g} and ²A_{1g} ← ²B_{1g} transitions. Based on the electronic spectral data, the Cu(II) complexes are suggested to possess a square planar geometry [27]. This was further supported by their magnetic susceptibility measurements. The total magnetic moment value (μ_{total}) of Cu(II) complexes of ligands **1** and **2** are 2.72 and 2.21 BM respectively, at room temperature. The calculated μ_{eff} values of the magnetic moments for each Cu(II) complexes **1a** and **2a** (1.49 and 1.48 BM) may be due to antiferromagnetic interaction between the two electrons in the two adjacent Cu(II) ions [28], these values lie in the range reported for the square planar geometry.

The electronic spectra of Co(II) complexes (**1b** and **2b**) showed the two absorption bands each at 667 (14,992 cm^{−1}) and 554 (18,050 cm^{−1}) nm and 669 (14,947 cm^{−1}) and 549 (18,214 cm^{−1}) nm respectively, which assignable to ⁴A_{2g} (P) ← ⁴T_{1g} (F) (ν₂) and ⁴T_{1g} (P) ← ⁴T_{1g} (F) (ν₃) transitions in octahedral environment [29]. The lowest band ν₁ which is due to the limitations in the scanning range of the instrument used, it is calculated by using a band fitting procedure suggested by Underhill and Billing [30]. The magnetic moments of these complexes (μ_{total}) are 4.64 (**1b**) and 4.50 BM (**2b**), these values lies in the range reported for the octahedral geometry [31] for binuclear complexes. The octahedral geometry of Co(II) are further supported by the calculated values of ligand field parameters, such as Racah inter electronic repulsion parameter (B'), nephelauxetic parameter (β), ligand field splitting

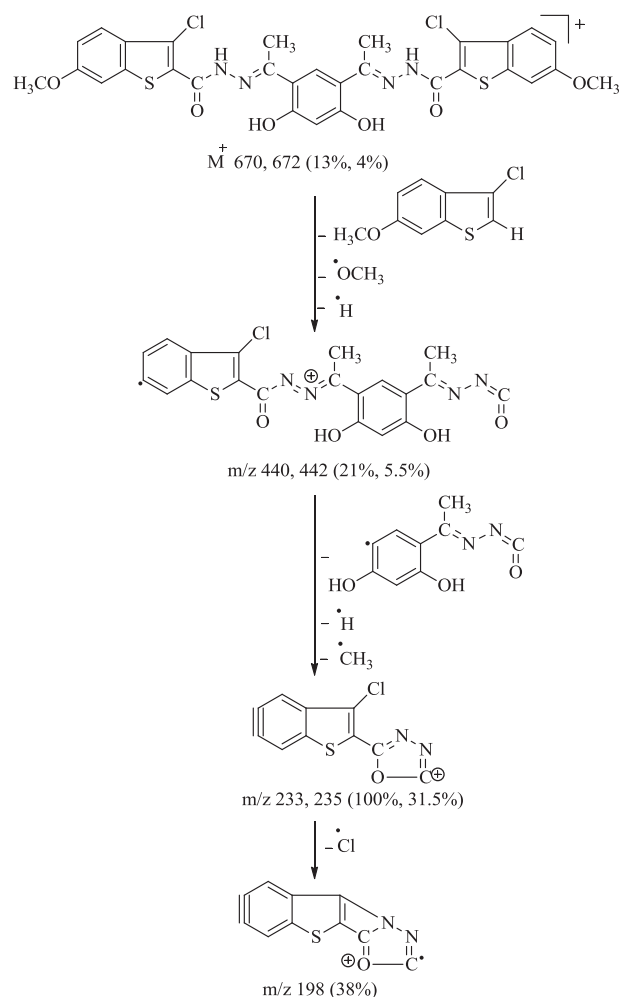
**Fig. 1.** ESI-mass spectrum of ligand **1**.



Scheme 2. Mass fragmentation of ligand 1.

energy (10Dq) and ligand stabilization energy (LFSE) [32]. The calculated B' value is less than the free ion value ($B'_{free\ ion} = 971\text{ cm}^{-1}$) for both the Co(II) complexes (**1b** and **2b**) which is an indication of orbital overlapping and delocalization of d-orbital. The nephelauxetic parameter which is calculated by using formula, $\beta = B_{complex}/B'_{free\ ion}$ for both the Co(II) complexes was found to be less than unity, suggesting a considerable cobalt-ligand bond character (Table 4).

The electronic spectra of green colored Ni(II) complexes (**1c** and **2c**) showed two bands each at 725 ($13,793\text{ cm}^{-1}$) and 398 ($25,125\text{ cm}^{-1}$) nm and 738 ($13,550\text{ cm}^{-1}$) and 390 ($25,641\text{ cm}^{-1}$) nm respectively, which are assignable to ${}^3T_{2rmg}(F) \leftarrow {}^3A_{2g}(F)(v_2)$ and ${}^3T_{1g}(F) \leftarrow {}^3A_{2g}(F)(v_3)$ transitions, in an octahedral environment [33], the band v_1 which is due to ${}^3T_{1g}(P) \leftarrow {}^3A_{2g}(F)$ was calculated by using a band fitting procedure [30]. The total magnetic moment of these complexes are 4.76 (**1c**) and 4.90 BM (**2c**) respectively, which are in agreement with Ni(II) ion in octahedral geometry. The octahedral geometry was further supported by the



Scheme 3. Mass fragmentation of ligand 2.

calculated values of ligand field parameters, such as Racah inter electronic repulsion parameter (B^1), nephelauxetic parameter (β), ligand field splitting energy (10Dq) and ligand stabilization energy (LFSE) [31] (Table 4).

Thus, based on electronic and magnetic susceptibility data, Co(II) and Ni(II) ions possess octahedral geometry by the addition of two water molecules each and this was confirmed by the thermal studies.

3.5. ESR Spectral Studies of the Cu(II) complexes (**1a** and **2a**)

To obtain the information about the hyperfine and superhyperfine structure in order to elucidate the geometry of the complex and the site of the metal–ligand bonding or environment around the metal ion, the X-band ESR spectra of Cu(II) complexes $[Cu_2(-L_1)(Cl)_2]$ (**1a**) and $[Cu_2(L_2)(Cl)_2]$ (**2a**) have been recorded in the polycrystalline state at room temperature at a frequency of 9.1 GHz with a field set of 3000 G. The spin Hamiltonian parameters for the Cu(II) complexes is used to derive the ground state. In square planar complexes, the g -tensor parameter with $g_{||} > g_{\perp} > 2.0023$, the unpaired electron lies in the $d_{x^2-y^2}$ orbital with ${}^2B_{1g}$ as ground state and with $g_{\perp} > g_{||} > 2.0023$, the unpaired electron lies in the d_z^2 orbital with ${}^2A_{1g}$ as ground state [34]. The observed measurements for Cu(II) complexes $[Cu_2(L_1)(Cl)_2]$ (**1a**), $g_{||}$ (2.117) $> g_{\perp}$ (2.031) > 2.0023 and $[Cu_2(L_2)(Cl)_2]$ (**2a**), $g_{||}$ (2.075) $> g_{\perp}$ (2.031) > 2.0023 indicating that the complexes are axially symmetric and copper site has a $d_{x^2-y^2}$ ground state character-

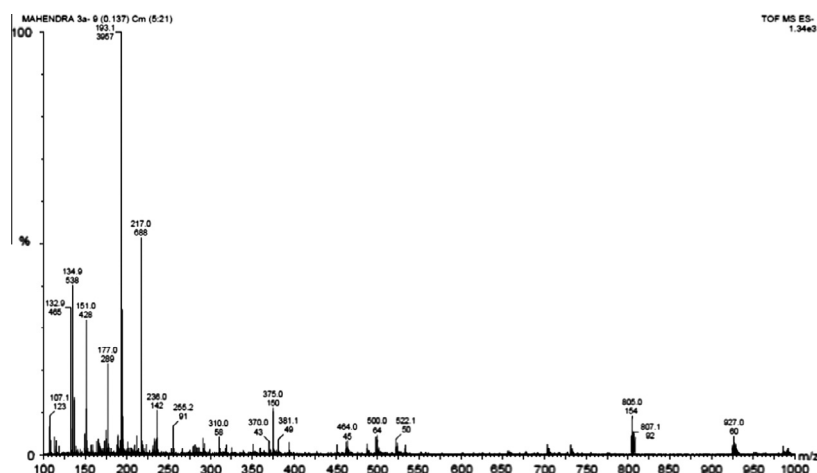
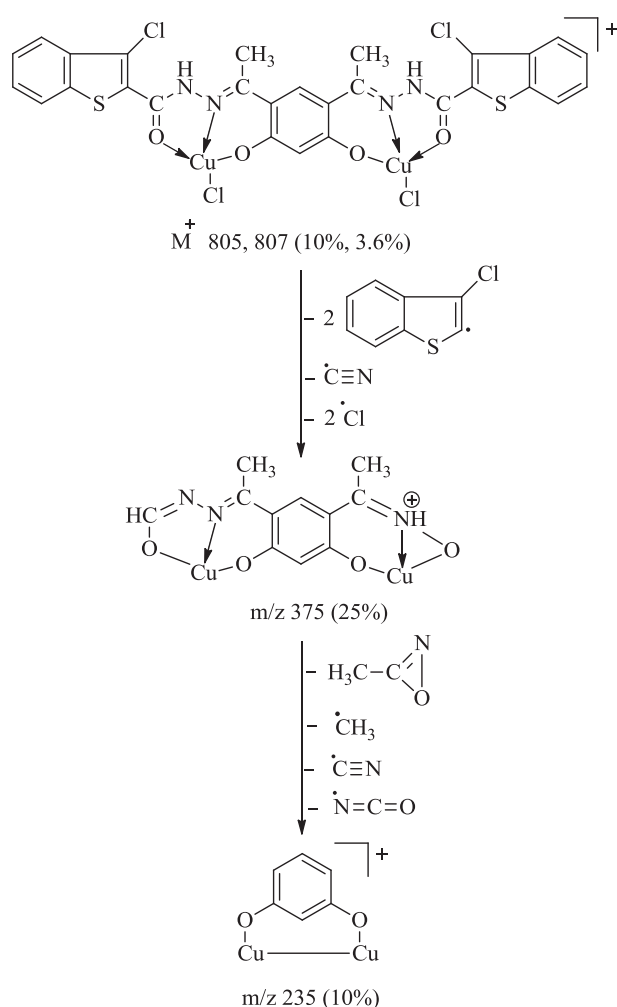


Fig. 2. ESI-mass spectrum of Cu(II) complex of ligand 1.



Scheme 4. Mass fragmentation of Cu(II) complex of ligand 1.

istic of square planar geometry for both the complexes [35]. The $g_{||}$ value is an important function for indicating the covalent character of metal–ligand bond, for ionic $g_{||} > 2.3$ and for covalent characters $g_{||} < 2.3$ respectively [36]. In the present Cu(II) complexes the $g_{||}$ values are less than 2.3, indicating an appreciable covalent character for the metal–ligand bond. The geometric parameter (G), which

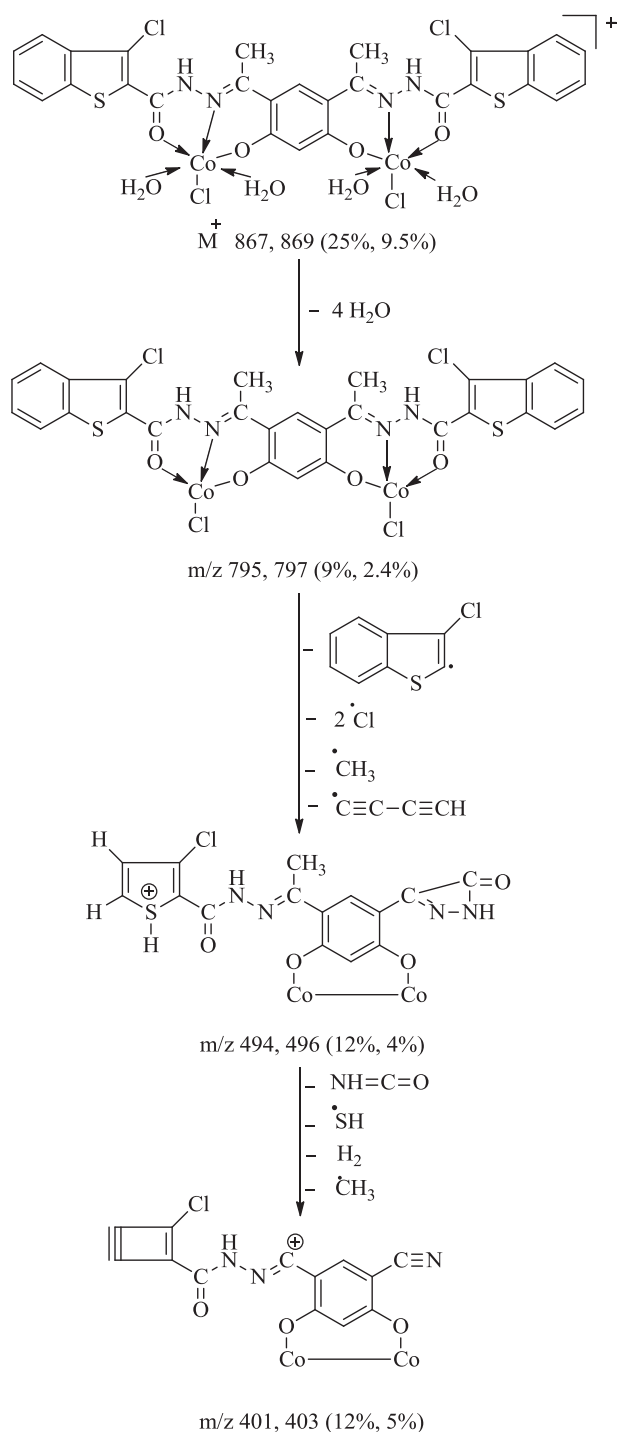
is the measure of extent of exchange interaction is calculated by using g -tensor values by the expression, $G = g_{||} - 2/g_{\perp} - 2$. According to Hathaway [37], if the G value is greater than 4, the exchange interaction between the copper centers is negligible whereas its value is less than 4 the exchange interaction is noticed. The calculated G -value for the present Cu(II) complexes are 3.70 (**1a**) and 2.39 (**1b**) indicating some interaction between Cu(II) centers in solid complex [38].

3.6. Thermal studies

The thermal stabilities were investigated for Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand **1** have been studied as a function of temperature. The proposed stepwise thermal degradation of the complexes with respect to temperature and the formation of respective metal oxides are depicted in Table 5. TG–DTA curve of Cu(II) complex (**1a**) (Fig. 3) shows that the complex is stable upto 330 °C and no weight loss occurs before this temperature. The first stage of decomposition represents weight loss of four chlorine atoms at 337 °C with practical weight loss of 15.70% (Cald. 17.38%). The resultant complex underwent second stage of degradation and gave break at 378 °C with a practical weight loss of 9.20% (Cald. 8.57%), which corresponds to the decomposition of NCO and methyl group. Further the complex underwent third stage of decomposition and gave a break at 390 °C with a weight loss of 27.71% (Cald. 28.77%) due to loss of one benzo[b]thiophene moiety with side chain attached to it (C_9H_4OS) and a methyl group. Thereafter, the compound showed a gradual decomposition in a gradual manner rather than with the sharp decomposition up to 715 °C and onwards due to the loss of the remaining organic moiety. The weight of the residue corresponds to two moles of cupric oxide.

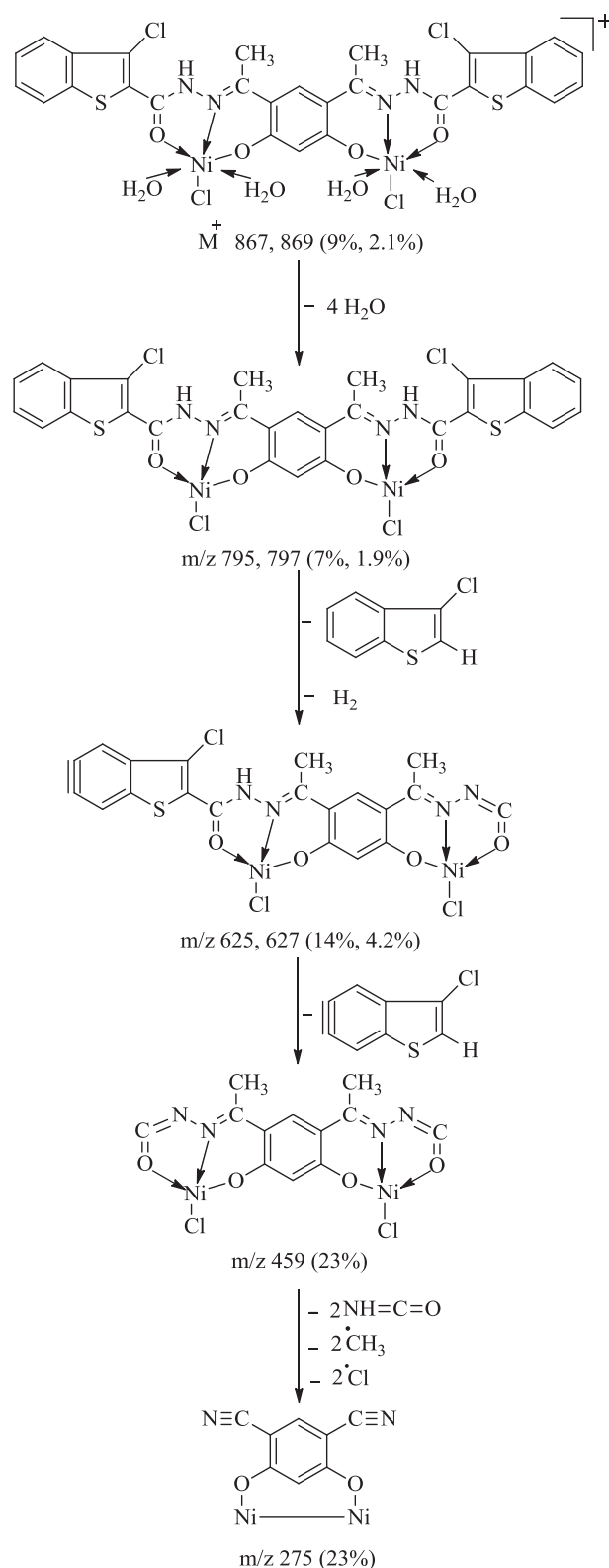
The thermogram of Co(II) complex (**1b**) shows the first stage of decomposition at 190 °C with practical weight loss of 8.03% (Cald. 8.29%), which corresponds to weight loss of four coordinated water molecules. Further the complex underwent decomposition and gave a break at 446 °C with a practical weight loss of 55.28% (Cald. 56.29%), corresponds to weight loss of two benzo[b]thiophene moieties with side chain attached to it ($2C_9H_5N_2OSCl$). Thereafter the complex showed gradual decomposition upto 750 °C with a weight loss of remaining organic moiety, the weight of the residue corresponds to two moles of cobalt oxide.

In the thermogram of the Ni(II) complex (**1c**) (Fig. 4), the first stage of decomposition represents the weight loss of four coordinated water molecules and two methyl groups at 272 °C with a practical weight loss of 11.43% (Cald. 11.75%). The resultant com-



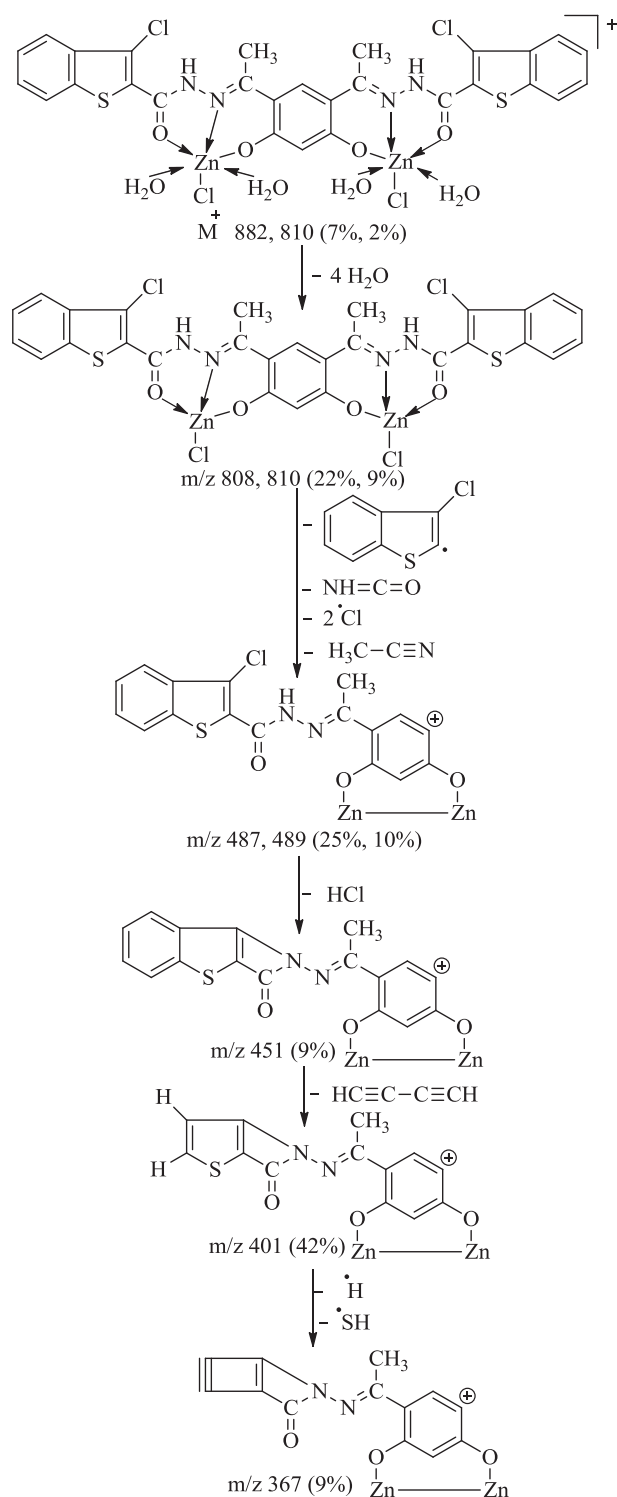
Scheme 5. Mass fragmentation of Co(II) complex of ligand **1**.

plex underwent further degradation and gave break at 444 °C with a practical weight loss of 16.51% (Cald. 17.60%), which corresponds to the decomposition of four chlorine atoms. Further the complex underwent decomposition and gave a break at 470 °C with a practical weight loss of 52.60% (Cald. 53.40), which corresponds to the weight loss of two benzo[b]thiophene moieties with side chain attached to it ($2C_9H_5OSN$). Thereafter, the compound showed a gradual decomposition up to 715 °C with a weight loss of remaining organic moiety. The weight of the residue corresponds to two moles of nickel oxide. In case of Zn(II) complex (**1d**) the first stage of



Scheme 6. Mass fragmentation of Ni(II) complex of ligand **1**.

decomposition occurs at 192 °C with practical weight loss of 7.06% (Cald. 8.17%), which represents the loss due to four coordinated water molecules. Further the complex underwent second stage of decomposition and gave a break at 452 °C with practical weight loss of 32.30% (Cald. 32.88%), due to loss of one benzo[b]thiophene



Scheme 7. Mass fragmentation of Zn(II) complex of ligand **1**.

moiety with side chain attached to it ($\text{C}_{10}\text{H}_5\text{N}_2\text{OSCl}$) and two methyl groups. The third stage of decomposition occurs at 470°C with practical weight loss of 51.14% (Cald. 51.58%), due to loss of one benzo[b]thiophene moiety with side chain attached to it ($\text{C}_9\text{H}_5\text{NOSCl}$) and two chlorine atoms. Thereafter, the compound showed a gradual decomposition up to 700°C with the weight loss of the remaining organic moiety. The weight of the residue corresponds to two moles of zinc oxide. The percentage metal content in all

the complexes as done by elemental analysis agrees well with the thermal studies.

3.7. Electrochemistry

The electrochemical behavior of ligands and their complexes were investigated in DMF (10^{-3} M) solution containing $0.05 \text{ M } n\text{-Bu}_4\text{NClO}_4$ as a supporting electrolyte in an oxygen free condition by cyclic voltammetry and it is the most versatile electro analytical technique for the study of electroactive species. Only the Cu(II) complexes of both the ligands exhibited redox properties. The voltammogram of two Cu(II) complexes are shown in Fig. 5. The cyclic voltammogram of Cu(II) complex (**1a**) in DMF at a scan rate of 0.1 V s^{-1} shows well defined redox process corresponding to the formation of Cu(II)/Cu(I) couple at (reduction peak) $E_{\text{pc}} = -0.281 \text{ V}$ and (oxidation peak) $E_{\text{pa}} = -0.170 \text{ V}$ versus Ag/AgCl. The peak separation of this couple is found to be quasi-reversible with $\Delta E_{\text{p}} = 0.111 \text{ V}$ and the ratio of anodic to cathodic peak height was less than one. The difference between forward and backward peak potential can provide a rough evaluation of the degree of the reversibility of one electron transfer reaction. Thus, the analysis of cyclic voltametric response to 0.1 V s^{-1} , 0.15 V s^{-1} and 0.2 V s^{-1} scan rates gives the evidence for quasi-reversible one electron redox process. The ratio of anodic to cathodic peak height was less than one and peak current increases with the increase of square root of the scan rates, establishing diffusion controlled electrode process [39]. From the peak separation value ΔE_{p} and peak potential increases with higher scan rates, therefore we can suggest that the electrode process are consistent with the quasi-reversibility of Cu(II)/Cu(I) couple [40].

The Cu(II) complex (**2a**) exhibits the reduction peak at $E_{\text{pc}} = -0.313 \text{ V}$ with the corresponding oxidation peak $E_{\text{pa}} = -0.147 \text{ V}$, which corresponds to the formation of Cu(II)/Cu(I) couple. The peak separation of this couple $\Delta E_{\text{p}} = 0.166 \text{ V}$ which increases with increasing scan rates. These characteristic are in consistency with the quasi-reversibility of Cu(II)/Cu(I) couple.

3.8. Powder X-ray diffraction studies (Powder-XRD)

Though the synthesized metal complexes were soluble in some polar organic solvents like DMSO and DMF, crystals that are suitable for single-crystal studies are not obtained. Hence powder-XRD pattern of Cu(II), Co(II), Ni(II) and Zn(II) complexes of the ligand **1** have been studied in order to test the degree of crystallinity of the complexes. Powder X-ray diffraction pattern for Cu(II) complex (**1a**) showed 4-reflections in the range of $3\text{--}30^\circ (2\theta)$, which are arisen from diffraction of X-ray by the planes of complex. The interplanar spacing (d) has been calculated by using Bragg's equation, $n\lambda = 2d \sin\theta$. The calculated interplanar d -spacing together with relative intensities with respect to most intense peak have been recorded and depicted in Table 6. The unit cell calculations have been calculated for cubic symmetry from the entire important peaks and $h^2 + k^2 + l^2$ values were determined. The observed interplanar d -spacing values have been compared with the calculated ones and it was found to be in good agreement. The $h^2 + k^2 + l^2$ values are 1, 1, 4 and 20. It was observed that the absence of forbidden numbers (7, 15, 23, etc.) indicate that the Cu(II) complex has cubic symmetry. The calculated lattice parameters were $a = b = c = 12.93 \text{ \AA}$.

Similar calculations were performed for Co(II), Ni(II) and Zn(II) complexes of ligand **1**. The Co(II) complex showed 5-reflections, whereas Ni(II) and Zn(II) complexes showed 3-reflections each in the range $3\text{--}30^\circ (2\theta)$ respectively, which are arisen from the diffraction of X-ray by the planes of these complexes. All the important peaks of Co(II), Ni(II) and Zn(II) complexes have been indexed and observed values of interplanar distances (d) have been

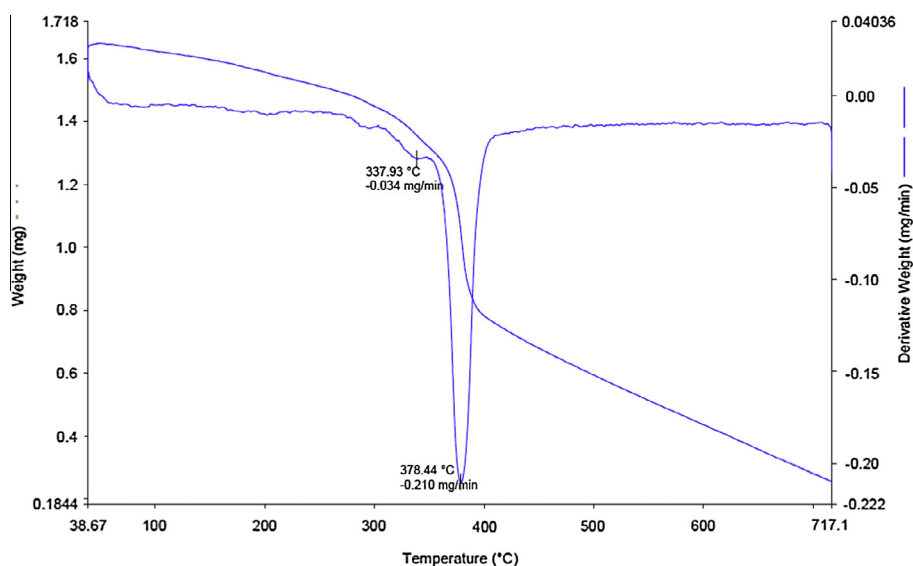
Table 4Electronic spectral data and ligand field parameters of the Cu(II), Co(II) and Ni(II) complexes in DMF (10^{-3} M) solution.

Complexes	Transitions in cm^{-1}			Dq (cm^{-1})	B' (cm^{-1})	β	$\beta\%$	ν_2/ν_1	LFSE (k cal.)
	ν_1^a	ν_2	ν_3						
(1a) $[\text{Cu}_2(\text{L}_1)(\text{Cl})_2]$	–	17,361	14,326	–	–	–	–	–	–
(1b) $[\text{Co}_2(\text{L}_1)(\text{Cl})_2(\text{H}_2\text{O})_4]$	6992	14,992	18,050	800	804	0.828	17.19	1.55	13.714
(1c) $[\text{Ni}_2(\text{L}_1)(\text{Cl})_2(\text{H}_2\text{O})_4]$	8250	13,793	25,125	825	944	0.793	9.23	1.67	28.285
(2a) $[\text{Cu}_2(\text{L}_2)(\text{Cl})_2]$	–	17,574	14,556	–	–	–	–	–	–
(2b) $[\text{Co}_2(\text{L}_2)(\text{Cl})_2(\text{H}_2\text{O})_4]$	6967	14,947	18,214	798	817	0.841	15.85	2.14	13.680
(2c) $[\text{Ni}_2(\text{L}_2)(\text{Cl})_2(\text{H}_2\text{O})_4]$	8040	13,550	25,641	804	1004	0.965	3.461	1.68	27.560

^a Calculated values.**Table 5**

Thermal data of the complexes of ligand 1.

Complex No.	Decomposition temp ($^{\circ}\text{C}$)	% Weight loss		% Metal oxide		Inference
		Obsd.	Cald.	Obsd.	Cald.	
1a	337	15.70	17.38	–	–	Loss due to four chlorine atoms
	378	9.20	8.57	–	–	Loss due to NCO and CH_3 group
	390	27.71	28.77	–	–	Loss due to one benzo[b]thiophene moiety with side chain attached to it ($\text{C}_9\text{H}_4\text{OS}$) and CH_3 group
1b	Upto 715	–	–	19.75	19.86	Loss due to remaining organic moiety
	190	8.03	8.29	–	–	Loss due to four coordinated water molecules
	446	55.28	56.29	–	–	Loss due to two benzo[b]thiophene moieties with side chain attached to it ($\text{C}_9\text{H}_5\text{N}_2\text{OSCl}$)
1c	Upto 750	–	–	17.26	17.39	Loss due to remaining organic moiety
	272	11.43	11.75	–	–	Loss due to four coordinated water molecules and two CH_3 groups
	444	16.51	17.60	–	–	Loss due to four chlorine atoms.
1d	470	52.60	53.40	–	–	Loss due to two benzo[b]thiophene moieties with side chain attached to it ($\text{C}_9\text{H}_5\text{N}_2\text{OSN}$)
	Upto 715	–	–	17.22	17.38	Loss due to remaining organic moiety
	192	7.06	8.17	–	–	Loss due to four coordinated water molecules
1d	452	32.30	32.88	–	–	Loss due to one benzo[b]thiophene moiety with side chain attached to it ($\text{C}_{10}\text{H}_5\text{N}_2\text{OSCl}$) and two CH_3 groups
	470	51.14	51.58	–	–	Loss due to one $\text{C}_9\text{H}_5\text{N}_2\text{OSCl}$ and two chlorine atoms
	Upto 700	–	–	18.48	18.60	Loss due to remaining organic moiety

**Fig. 3.** TG–DTA curve of Cu(II) complex of ligand 1.

compared with the calculated ones. The unit cell calculations were performed for cubic system and the $h^2 + k^2 + l^2$ values were determined for the above complexes. These complexes showed the absence of forbidden numbers (7, 15, 23, etc.) indicates that complexes have cubic symmetry. The calculated lattice parameters for Co(II) complex were $a = b = c = 10.82 \text{ \AA}$, for Ni(II) complex were $a = b = c = 10.83 \text{ \AA}$ and for Zn(II) complex were $a = b = c = 6.85 \text{ \AA}$.

3.9. Pharmacological activity results

3.9.1. In vitro antimicrobial activity

The synthesized ligands (**1** and **2**) and their metal complexes have been screened for their antimicrobial activity. The antibacterial activity were carried out against *E. coli*, *S. typhi*, *B. subtilis* and *S. aureus* bacterial strains and antifungal activity against *C. albi*.

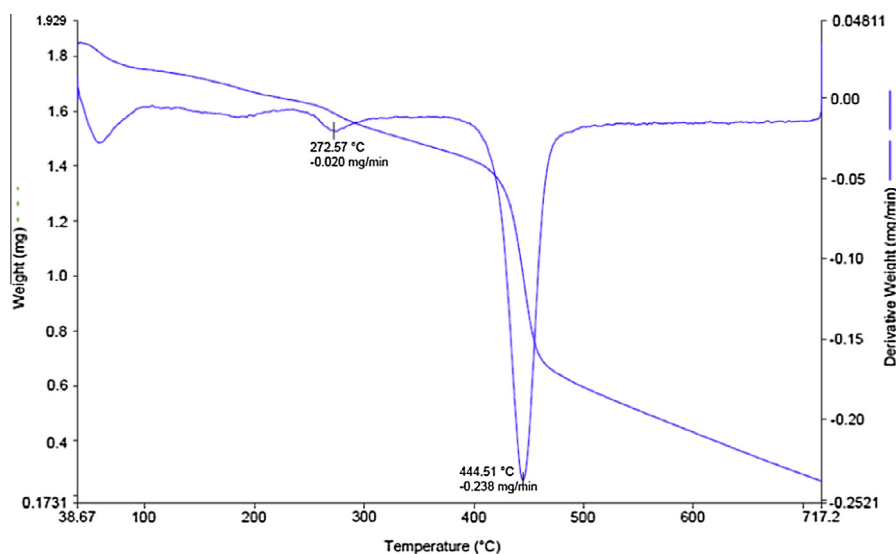


Fig. 4. TG-DTA curve of Ni(II) complex of ligand 1.

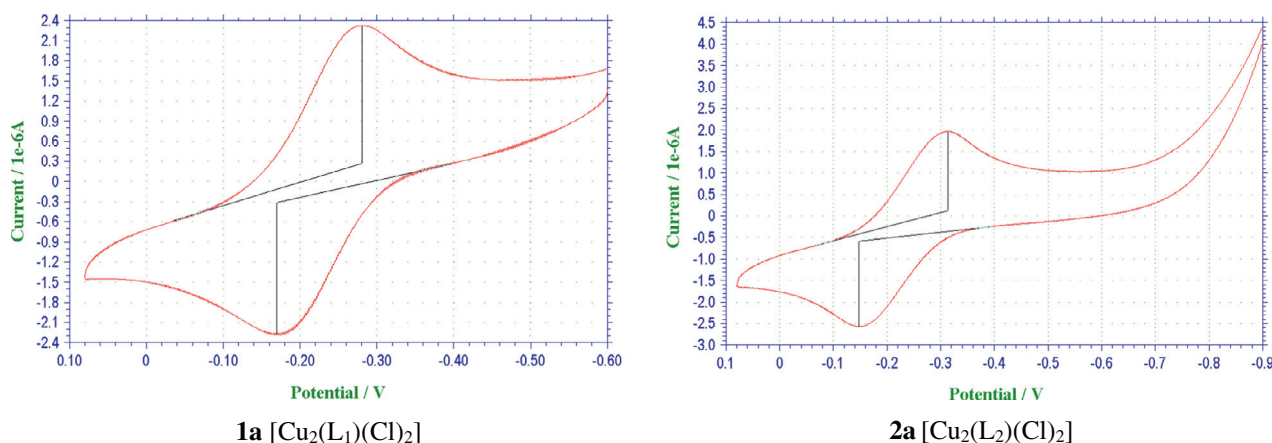


Fig. 5. Cyclic voltammogram of Cu(II) complexes at scan rate of 0.1 V s⁻¹.

Table 6

Powder X-ray diffraction data of Cu(II) complex of ligand 1.

Peak	2θ	θ	Sin θ	Sin ² θ	1000 Sin ² θ	1000Sin ² θ/CF (h ² + k ² + l ²)	h k l	d		a in Å
								Obsd.	Cald.	
1	5.812	2.906	0.0506	0.0025	2.56	1.00(1)	(100)	15.19	15.21	15.218
2	6.759	3.3795	0.0589	0.0034	3.46	1.351(1)	(100)	13.06	13.07	15.215
3	11.438	5.7190	0.0996	0.0099	9.92	3.875(4)	(110), (200)	7.730	7.730	15.218
4	25.879	12.939	0.2239	0.0501	50.13	19.58(20)	(420)	3.440	3.439	15.218

cans, *C. oxysporum* and *A. niger* fungal strains. The antimicrobial activity results and minimum inhibitory concentration (MIC) values of the compounds against the respective strains are summarized in Tables 7 and 8 respectively. The antimicrobial screening results of all the synthesized compounds exhibited antimicrobial properties and it is important to note that the metal complexes exhibited more inhibitory effect compared to their respective ligands and their metal chlorides. The enhanced antibacterial activity of the complexes over the ligands and their metal chlorides can be explained on the basis of chelation theory [41,42]. It is known that chelation enhances the ligand to act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand. The enhancement in the activity may be rationalized

on the basis that ligands mainly possess azomethine (C=N) bond. It has been suggested that the ligands with hetero donor atoms (nitrogen and oxygen) inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. It is observed that, in a complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (nitrogen and oxygen) present in the ligand, and there may be π -electron delocalization over the whole chelating system [43,44]. Thus the increase in the lipophilic character of the metal chelates and favors its permeation through the lipid layer of the bacterial membranes and blocking of the metal binding sites in the enzymes of microorganisms. The other factors namely solubility,

Table 7Antimicrobial activity of ligands (**1** and **2**) and their metal complexes.

Compound	Conc ⁿ ($\mu\text{g mL}^{-1}$)	Bacteria (Zone of inhibition in mm)				Fungi (Zone of inhibition in mm)		
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. oxysporum</i>	<i>A. niger</i>
1	100	++	++	++	+	++	++	++
	50	+	+	++	—	+	+	++
	25	—	—	—	—	—	—	—
	12.50	—	—	—	—	—	—	—
2	100	++	++	++	+	++	++	++
	50	+	+	+	—	++	+	++
	25	—	—	—	—	+	—	—
	12.50	—	—	—	—	—	—	—
1a	100	+++	++	++	+	++	++	++
	50	++	+	++	+	++	+	++
	25	+	+	+	—	++	+	+
	12.50	+	+	+	—	+	—	+
1b	100	++	++	+	+	++	++	++
	50	+	+	+	+	+	++	+
	25	—	+	+	—	+	+	+
	12.50	—	—	—	—	—	+	+
1c	100	++	++	++	+	++	++	++
	50	+	++	+	+	++	+	+
	25	+	+	+	—	++	+	+
	12.50	—	+	—	—	+	—	—
1d	100	++	++	++	+	++	++	++
	50	+	++	++	+	+	+	++
	25	+	+	+	—	+	+	+
	12.50	—	+	—	—	—	+	+
2a	100	++	+++	++	++	++	++	++
	50	+	++	+	++	++	+	++
	25	+	+	+	—	+	+	++
	12.50	+	+	—	—	+	+	+
2b	100	++	++	++	++	++	++	++
	50	+	+	++	+	+	+	++
	25	+	+	+	—	+	+	+
	12.50	+	—	+	—	+	—	—
2c	100	++	++	+	+	++	++	++
	50	+	++	+	+	+	++	+
	25	+	+	+	—	+	+	+
	12.50	—	—	—	—	+	+	—
2d	100	++	++	++	+	++	++	++
	50	+	++	+	+	+	+	+
	25	+	++	+	—	+	+	+
	12.50	+	—	—	—	+	+	—

— (inactive): less than 12 mm. Zone of inhibition of DMSO (solvent): 10 mm.

+ (weak active): 12–16 mm. Zone of inhibition of respective metal chlorides: 9–11 mm.

++ (moderately active): 17–25 mm. Zone of inhibition of standard drugs Gentamycin.

+++ (highly active): 26–35 mm. Fluconazole: 34 and 35 mm.

Table 8Minimum inhibitory concentration (MIC $\mu\text{g mL}^{-1}$) of ligands (**1** and **2**) and their metal complexes.

Compound	Bacteria				Fungi		
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. oxysporum</i>	<i>A. niger</i>
1	50	50	50	100	50	50	50
2	50	50	50	100	25	50	50
1a	12.50	12.50	12.50	25	12.50	25	12.50
1b	50	25	25	50	25	12.50	12.50
1c	25	12.50	25	50	12.50	25	25
1d	25	12.50	25	50	25	12.50	12.50
2a	12.50	12.50	25	50	12.50	12.50	12.50
2b	12.50	25	12.50	50	12.50	25	25
2c	25	25	25	50	12.50	12.50	25
2d	12.50	25	25	50	12.50	12.50	25
Gentamycin	12.50	12.50	12.50	12.50	—	—	—
Fluconazole	—	—	—	—	12.50	12.50	12.50

conductivity and bond length between the metal ion and the ligand also increase the activity. The increase in the activity of the metal complexes against the fungi is due to the formation of

a hydrogen bond between the azomethine nitrogen atom and active centers of the cell constituents, resulting in the interference with the normal cell process.

3.9.2. DNA cleavage activity

The ligand **1** and its Cu(II), Co(II), Ni(II) and Zn(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method against supercoiled plasmid DNA pBR 322 (Bangalore Genei, Bengaluru, Cat. No. 105850) as a target molecule and the gel picture showing cleavage is depicted in Fig. 6.

DNA-cleavage studies are used for the rational design and to construct new and more efficient drugs that are targeted to DNA [45]. The cleavage efficiency of all the compounds compared to the control is due to their efficient DNA-binding ability, which is observed by diminishing of the intensity of the lanes. The DNA-cleavage study by electrophoresis analysis clearly revealed that the lanes of ligand **1** and its Cu(II), Co(II) and Ni(II) complex showed complete cleavage of supercoiled DNA whereas Zn(II) complex showed partial cleavage of supercoiled DNA. The difference was observed in the bands of lanes of compounds compared with the control DNA. This shows that the control DNA alone did not show any apparent cleavage, whereas the ligands and metal complexes showed. These results indicate that the important role of coordination of nitrogen and oxygen to the metal ion in these isolated DNA cleavage reactions. On the basis of the cleavage of DNA observed in case of ligand **1** and its Cu(II), Co(II), Ni(II) and Zn(II) complexes, it can be concluded that all the compounds under present study inhibited the growth of pathogenic organism by DNA cleavage as has been observed on the DNA cleavage of supercoiled plasmid DNA pBR 322.

3.9.3. Antioxidant assay (DPPH free radical scavenging activity)

The newly synthesized ligand **1** and its metal complexes were screened for free radical scavenging activity by DPPH method. Antioxidant activity of these compounds was investigated by measuring radical scavenging effect of DPPH radicals. The results of the free radical scavenging activity of the compounds at different concentrations are shown in Fig. 7. It is evident from the results that the free radical scavenging activity of these compounds was concentration dependent. Among the examined compounds ligand **1** and its Cu(II), Co(II) and Ni(II) complexes have exhibited good scavenging activity. Whereas, Zn(II) complex has shown moderate activity. The marked antioxidant activity of the ligand **1** than its metal complexes is due to the presence of phenolic OH groups and moderate antioxidant activity of metal complexes is due to the coordination of metal with azomethine nitrogen, carbonyl oxygen of amide function attached to the 2-position of benzo[*b*]thiophene and phenolic oxygen of resorcinol moiety via deprotonation. In case of the ligand **1** hydrogen of OH groups donates to the DPPH and converts itself into the stable free radical and in metal complexes the hydrogen of amide NH is more acidic and could be easily

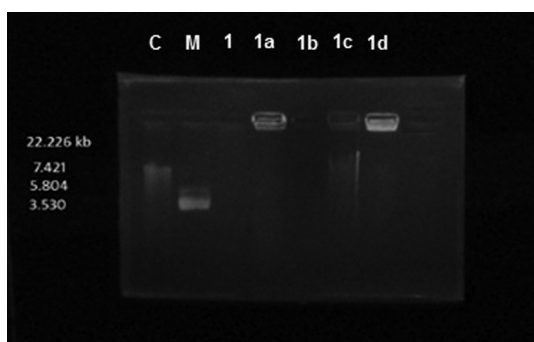


Fig. 6. DNA cleavage of supercoiled plasmid DNA pBR322. M, standard molecular weight marker; C, control. Lane 1, **1a**, **1b**, **1c**, and **1d** were treated plasmid DNA pBR322 with respective compounds.

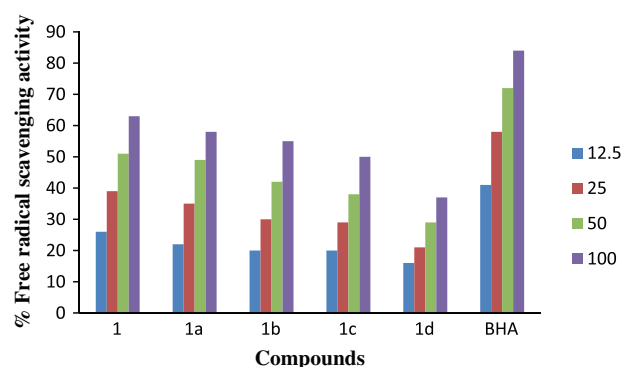


Fig. 7. Antioxidant activity results of ligand **1** and its metal complexes.

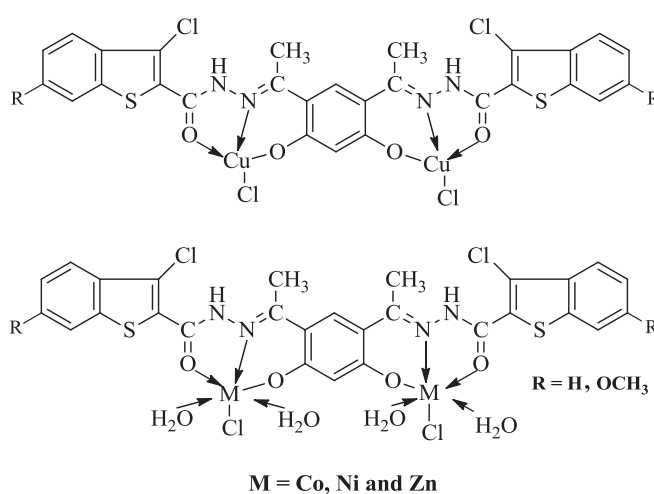


Fig. 8. Proposed structures of the complexes.

donated to the DPPH free radical and convert itself into the stable free radical. Moreover, the acidic nature of hydrogen atom attached to amide nitrogen increases on complexation with metal ions. Hence that hydrogen atom becomes more liable.

4. Conclusion

Based on the spectroscopic studies, the coordinating ability of the ligands have been proved in complexation reaction with Cu(II), Co(II), Ni(II) and Zn(II) ions. The newly synthesized ligands acts as a tridentate chelate around the metallic ion with two compartments and provides ONO donating sites to each metal ion in both compartments. Metal ion is coordinated through oxygen atom of carbonyl function, azomethine nitrogen and phenolic oxygen atom via deprotonation. ESR spectral data confirms the binuclear nature of the complexes. Cu(II) complexes of both the ligands have square planar geometry, whereas Co(II), Ni(II) and Zn(II) complexes of both the ligands possess octahedral geometries respectively. Cu(II) complexes of ligands **1** and **2** exhibit one electron transfer quasi-reversible redox activity in the applied potential range. The antimicrobial activity results showed that all the complexes have exhibited higher activity when compared to their respective ligand and metal chloride. The DNA cleavage studies revealed that the metal complexes showed good efficiency towards DNA cleavage. Ligand **1** and its Cu(II), Co(II) and Ni(II) complexes showed efficient antioxidant activity. Based on the analytical data and spectral studies, proposed structure of all the complexes are depicted in Fig. 8.

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