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ORIGINAL ARTICLE

DNA Binding, Cleavage and Antibacterial Activity of Mononuclear Cu(II), Ni(II) and Co(II) Complexes Derived from Novel Benzothiazole Schiff Bases

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Abstract A series of novel bivalent metal complexes $M(L_1)_2$ and $M(L_2)_2$ where M = Cu(II), Ni(II), Co(II) and L_1 = 2-((benzo [d] thiazol-6-ylimino)methyl)-4-bromophenol [BTEMBP], L_2 = 1-((benzo [d] thiazol-6-ylimino)methyl) naphthalen-2-ol [BTEMNAPP] were synthesized. All the compounds have been characterized by elemental analysis, SEM, Mass, ^{1}H NMR, ^{13}C NMR, UV–Vis, IR, ESR, spectral data and magnetic susceptibility measurements. Based on the analytical and spectral data four-coordinated square planar geometry is assigned to all the complexes. DNA binding properties of these complexes have been investigated by electronic absorption spectroscopy, fluorescence and viscosity measurements. It is observed that these binary complexes strongly bind to calf thymus DNA by an intercalation mode. DNA cleavage efficacy of these complexes was tested in presence of H_2O_2 and UV light by gel electrophoresis and found that all the complexes showed better nuclease activity. Finally the compounds were screened for antibacterial activity against few pathogens and found that the complexes have potent biocidal activity than their free ligands.

Keywords Schiff base · Binary complex · Viscosity measurements · Fluorescence · Antibacterial activity · DNA cleavage

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Introduction

Benzothiazoles have scaffold importance in medicine as it constitutes bioactive pharmacophore in many drugs due to their broad range of biological activity such as anticancer, anti-HIV activity, anti-inflammatory, antimicrobial, antimalarial, analgesic, anticonvulsant, antileishmanial, antitubercular, anticancer lipid peroxidation inhibitor activities [1–11]. A large number of benzothiazole derivatives possess potent anticancer activity and were considered as mechanistically distinct class of clinically proven chemotherapeutic agents [12–14]. To design effective chemotherapeutic agents and better anticancer drugs, the choice of metal ion and planarity of ligand are the most important factors [15]. A plenty of biological experiments were performed so far to suggest that DNA is the primary intracellular target of many anticancer drugs, because the interaction between complexes (small molecules) and DNA can induce DNA damages in cancer cells, blocking the division of aggressive growing cells leading cell death [16–21]. For DNA activity, the most important step is DNA binding interactions with the ligands as well as metal complexes [22, 23]. Metal complexes can bind to DNA through non-covalent modes, such as groove binding, electrostatic binding, intercalative binding [24–27]. Palaniandavar et al. pointed out that Cu(II) complexes are the best alternatives to cis platin. Nickel complexes play an important role in bioinorganic chemistry and redox enzyme systems, and may provide the basis for models of the active sites of biological systems [28]. Cobalt play an important role in biological system (constituent of coenzyme B12) and its complexes exhibit various biological properties viz., antifungal, antitumor, antiproliferative, antiviral, antimicrobial, antioxidant and anticancer [29–31]. DNA cleavage, cytotoxic and antimicrobial studies of binary and ternary Cu(II) complexes of isoxazole Schiff bases and heterocyclic compounds were reported earlier from our laboratory [32, 33].

Keeping in view the above facts, we herein report the synthesis, structural characterization, DNA binding, cleavage and biological studies of Cu(II), Ni(II) and Co(II) complexes of benzothiazole Schiff bases.

Experimental

Materials

All starting moieties, metal salts used for the preparation of ligands and metal complexes were procured from Sigma-Aldrich Bangalore, India. The solvents used for physical measurements were purified according to literature methods [34]. The CT-DNA and supercoiled pBR322 DNA were purchased from Genei, Bangalore and stored at 4 °C. Tris–HCl/NaCl buffer, ethidium bromide obtained from Merck, Hyderabad, India.

Instrumentation

The elemental analysis of the compounds was performed on a Perkin Elmer 240C (USA) elemental analyzer. Metal content of the complexes was estimated by atomic absorption spectroscopy using GBC Avanta 1.0 AAS. Melting points of the compounds were determined on a Polmon instrument (model No. MP-96). ^{1}H and ^{13}C NMR spectra of the ligands were recorded on a Bruker 400 MHz NMR instrument using TMS as internal standard. Morphology and particle-size of the compounds were determined on Zeiss scanning electron microscope. Surface elements of compounds were analyzed by the INCA EDX instrument. ESI mass spectra were recorded on a Vergleichbare Gerate (VG) micro mass 7070–H instrument, IR spectra of the compounds were carried out using KBr discs in the range of 4000–400 cm^{-1}on a Perkin-Elmer Infrared model 337. Electronic spectra were recorded on a Shimadzu UV-Vis 1601 spectrophotometer using DMSO as solvent. Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorometer. Magnetic susceptibilities of the complexes were determined on a Gouy balance model 7550 using $Hg[Co(NCS)_4]$ as standard. Thermograms of complexes were carried out on a Mettler Toledo Star system in the temperature range of 30–1000 °C. EPR spectra of the copper complexes were recorded using JES-FA200 ESR spectrometer (JEOL-Japan) at liquid nitrogen temperature (77 K).

Synthesis of Schiff Bases and Binary Metal Complexes

Synthesis of Schiff Bases L_1 and L_2

To a hot methanolic solution (50 ml) of 6-Amino benzothiazole (10 mM), hot methanolic solution (50 ml) of 5-bromo salicylaldehyde (10 mM)/2-hydroxy naphthaldehyde (10 mM) was added drop wise with constant stirring and refluxed on an oil bath for 4 h. The resulting solid product was isolated by filtration and recrystallized from hot methanol.

L_1; ($C_{14}H_9BrN_2OS$) Anal. Calc (%): C, 50.46; H, 2.72; N, 8.41; S, 9.62. found: C, 50.68; H, 2.92; N, 8.62; S, 9.92. IR (KBr): (ν_{O-H}) 3438, ($\nu_{CH=N}$) 1616, (ν_{C-O}) 1167. UV-Vis; λ_{max}/nm (cm^{-1}): 270 (37,037), 360 (27,777). ^{1}H-NMR ($CDCl_3$) (δ): 13.12 (s, 1H); 9.01 (s, 1H); 8.63 (s, 1H); 8.16 (s, 1H); 7.86 (d, 1H); 7.54–7.46 (m, 3H); 6.95(d, H) (Shown in Fig. S1). C^{13} -NMR ($CDCl_3$) (δ): 161.8, 160.1, 154.0, 152.4, 145.7, 136.0, 135.0, 134.3, 124.3, 120.5, 120.0, 119.3, 114.4, 110.6. ESI-MS (m/z): Calc: 333. Found: 335 (M + 2). MP-170 °C.

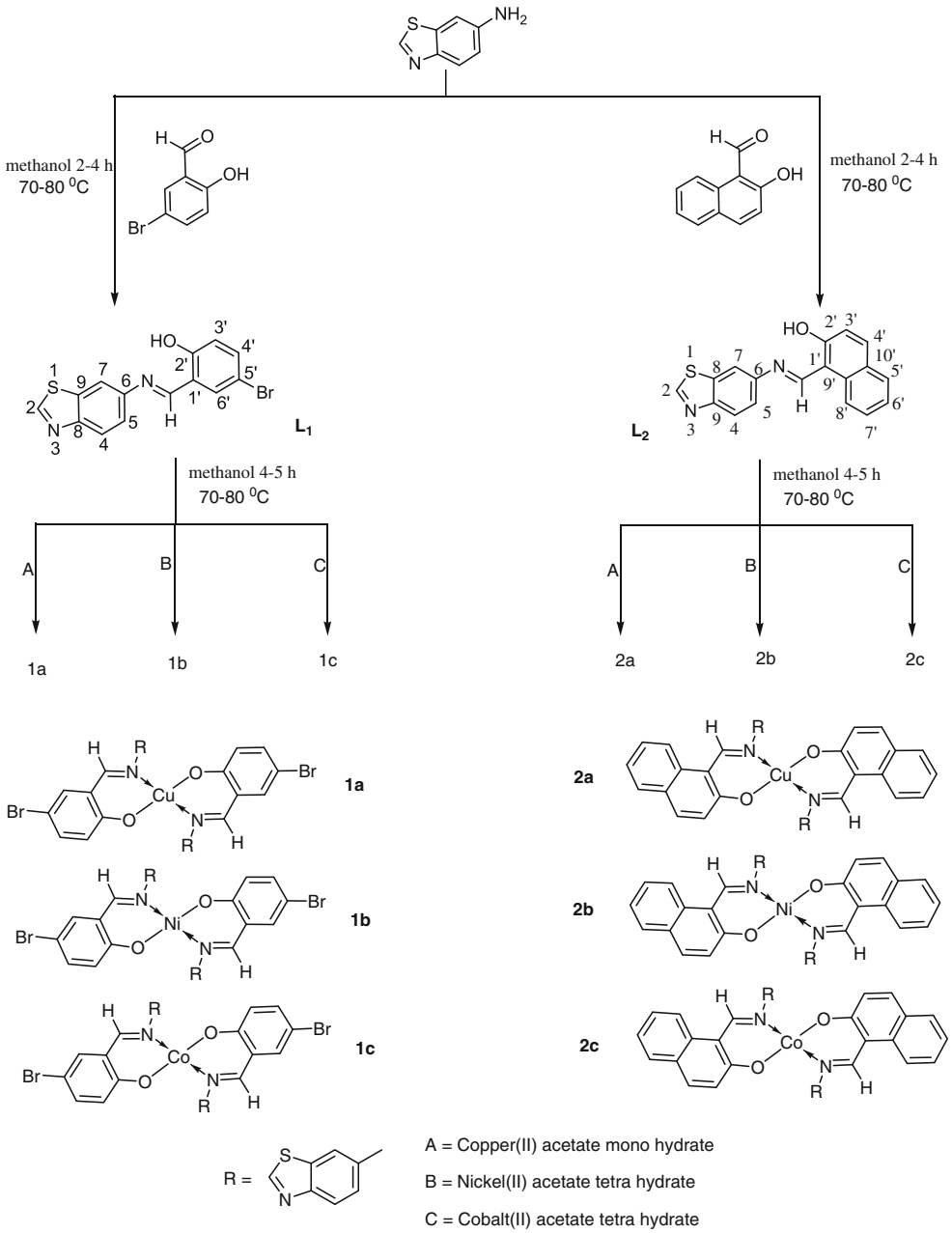
L_2; ($C_{18}H_{12}N_2OS$) Anal. Calc (%): C, 71.03; H, 3.97; N, 9.20; S, 10.54. found: C, 71.32; H, 4.23; N, 9.42; S, 10.84. IR (KBr): (ν_{O-H}) 3432, ($\nu_{CH=N}$) 1619, (ν_{C-O}) 1154. UV-Vis; λ_{max}/nm (cm^{-1}):, 243 (41,152),320 (31, 250), 360 (25,974). ^{1}H-NMR ($CDCl_3$) (δ): 15.33 (s, 1H); 9.40 (s, 1H); 8.973 (s, 1H); 8.16–7.09 (m, 9H). C^{13}-NMR ($CDCl_3$) (δ): 167.9, 156.4, 154.0, 151.9, 144.3, 136.4, 135.2, 133.0, 129.4, 128.16, 127.5, 124.4, 123.7, 121.3, 119.8, 119.0, 113.3, 109.2. ESI-MS (m/z): Calc: 304. Found: 305(M + 1). MP-190 °C.

Synthesis of Binary Metal Complexes

A hot methanolic solution of Copper acetate mono hydrate (10 mM) was added to a hot methanolic solution of Schiff base ligands (L_1/L_2) (20 mM) and the resulting mixture was refluxed for 2–4 h. The solid product obtained was separated, washed thoroughly with methanol and dried in vacuum. Similar experimental protocol was used for Ni(II) and Co(II) complexes using Nickel acetate tetra hydrate and Cobalt acetate tetra hydrate. The synthetic procedure of ligands and respective complexes was shown in Scheme 1.

1) $[Cu(BTEMBP)_2]$ (**1a**); ($C_{28}H_{16}Br_2N_4O_2S_2Cu$) Anal. Calc(%): C, 46.20; H, 2.22; N, 7.70; S, 8.81; Cu, 8.73. Found: C, 46.40; H, 2.44; N, 7.50; S, 8.6; Cu, 8.75. IR (KBr): $\nu_{(CH=N)}$ 1596, $\nu_{(C-O)}$ 1175, $\nu_{(M-O)}$ 504, $\nu_{(M-N)}$ 435. UV-Vis; λ_{max}/nm (cm^{-1})(DMSO): 258 (38,759), 357 (28,011), 573 (17,452). μ_{eff} (BM): 1.72. ESI-MS (m/z): Clac: 727. Found: 727 $[M]^{+}$. MP-255 °C.
2) $[Ni(BTEMBP)_2]$ (**1b**); ($C_{28}H_{16}Br_2N_4O_2S_2Ni$) Anal. Calc(%): C, 46.51; H, 2.23; N, 7.75; S, 8.87; Ni, 8.12. Found: C, 46.42; H, 2.52; N, 7.80; S, 8.66; Ni, 8.16. IR (KBr): $\nu_{(CH=N)}$ 1592, $\nu_{(C-O)}$ 1180, $\nu_{(M-O)}$ 537, $\nu_{(M-N)}$ 423. UV-Vis; λ_{max}/nm (cm^{-1})(DMSO): 258 (38,759), 300 (33, 333), 525 (19,047), 542 (18,450). μ_{eff} (BM): dia. ESI-MS (m/z): Calc: 719. Found: 737 $[M + NH_4]^{+}$. MP-300 °C.

Scheme 1 Synthesis of ligands and their metal complexes



3) [Co(BTEMBP)$_2$] (**1c**); ($C_{28}H_{16}Br_2N_4O_2S_2Co$) Anal. Calc(%): C, 46.49; H, 2.23; N, 7.75; S, 8.87; Co, 8.15. Found: C, 46.45; H, 2.45; N, 7.85; S, 8.57; Co, 8.17. IR (KBr): $\nu_{(CH=N)}$ 1593, $\nu_{(C-O)}$ 1176, $\nu_{(M-O)}$ 500, $\nu_{(M-N)}$ 432. UV-Vis; λ_{max}/nm (cm^{-1})(DMSO): 260 (38,461), 359 (27,855), 543 (18,416). μ_{eff} (BM): 2.11. ESI-MS (m/z): Calc: 720. Found: 721 [M + 1]. MP-270 °C.
4) [Cu(BTEMNAPP)$_2$] (**2a**); ($C_{36}H_{22}N_4O_2S_2Cu$) Anal. Calc.(%): C, 64.51; H, 3.31; N, 8.36; S, 9.57. Cu, 9.48. Found: C, 64.62; H, 3.12; N, 8.15; S, 9.87; Cu, 9.46. IR (KBr): $\nu_{(CH=N)}$ 1600, $\nu_{(C-O)}$ 1190, $\nu_{(M-O)}$ 564, $\nu_{(M-N)}$ 451. UV- UV-Vis; λ_{max}/nm (cm^{-1})(DMSO): Vis; λ_{max}/nm (cm^{-1}): 264 (37,878), 326 (30,674), 400 (25, 510), 563 (17,761). μ_{eff} (BM): 1.8. ESI-MS (m/z): Calc: 669. Found: 692 $[M + Na]^+$. MP-280 °C.
5) [Ni(BTEMNAPP)$_2$] (**2b**); ($C_{36}H_{22}N_4O_2S_2Ni$) Anal. Calc.(%): C, 64.98; H, 3.33; N, 8.42; S, 9.64; Ni, 8.82. Found: C, 64.76; H, 3.68; N, 8.24; S, 9.84; Ni, 8.79. IR (KBr): $\nu_{(CH=N)}$ 1602, $\nu_{(C-O)}$ 1180, $\nu_{(M-O)}$ 537, $\nu_{(M-N)}$ 423. UV-Vis; λ_{max}/nm (cm^{-1})(DMSO): 263 (38,022), 325 (30,769), 407 (25,000), 555 (18,018), 572 (17,482). μ_{eff} (BM): dia. ESI-MS (m/z): Calc: 664. Found: 664 $[M]^+$. MP-320 °C.

6) [Co(BTEMNAPP)$_2$] (**2c**); ($C_{36}H_{22}N_4O_2S_2Co$) Anal. Calc.(%): C, 64.98; H, 3.33; N, 8.42; S, 9.64; Co, 8.85. Found: C, 64.75; H, 3.12; N, 8.57; S, 9.92; Co, 8.87. IR (KBr) $\nu_{(CH=N)}$ 1606, $\nu_{(C-O)}$ 1188, $\nu_{(M-O)}$ 483, $\nu_{(M-N)}$ 425. UV-Vis; λ_{max}/nm (cm^{-1})(DMSO): 277 (36,101), 326 (35, 971), 435 (22,988), 567 (17,636). μ_{eff} (BM): 2.18. ESI-MS (m/z) Calc: 665. Found: 665 $[M]^+$. MP-290 °C.

DNA Binding Studies

Electronic Absorption Study

The UV–Visible absorption experiments were carried out by maintaining the constant concentration of metal complexes and varying the concentration of the CT-DNA. A Tris-buffer solution of CT-DNA gave a ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm, indicating that the DNA was suitably free of protein [35]. The DNA concentration per nucleotide was measured spectrophotometrically by using known molar extinction coefficient value 6600 M^{-1} cm^{-1} at 260 nm [36]. The stock solution of CT-DNA was prepared by diluting DNA in Tris–HCl/NaCl buffer (pH = 7.2, 50 mM NaCl 5 Mm Tris–HCl,). To measure the absorbance of complex and to eliminate the absorbance of CT-DNA itself, equal quantity of CT-DNA was added to both the complex solution and the reference solution. From the absorption data, the intrinsic binding constant (K_b) was calculated by a plot made between [DNA]/(ε_a-ε_f) and [DNA] [37].

Fluorescence Quenching Study

Competitive CT-DNA in ethidium bromide (EB) displacement study was carried out by pretreating the DNA (15 μM) containing EB (3 μM) in Tris–HCl buffer. To this metal complexes were added in succssive additions. Then samples were excited at 320 nm. The relative binding of the complexes to CT-DNA was determined by Stern–Volmer equation, $I_0/I = 1 + K_{SV}$ r, where I_0 and I are the fluorescence intensities in the absence and presence of complexes respectively, K_{SV} is a linear Stern–Volmer constant, and r is the concentration of complex to that of DNA.

Viscosity Study

Viscosity measurements were performed on Ostwald capillary viscometer immersed in a thermostatic waterbath at constant temperature (30 ± 1 °C). Metal complexes concentration was varied (0-100 μM) and DNA (100 μM) concentration was kept constant. The relative viscosities were calculated using formula $(\eta/\eta_0)^{1/3}$ where $\eta = (t\text{-}t_0)$ where t_0 and t represent the flow time of DNA solution in the absence and presence of complex and η_0 is the viscosity of CT-DNA alone. Average flow time was recorded after triplicates of sample with a digital stopwatch.

DNA Cleavage

Agarose gel electrophoresis was universally accepted method to delineate the interaction between synthesized metal complexes and the supercoiled (SC) pBR322 plasmid DNA. The experiment involves incubating the samples (20 μM) with pBR 322 plasmid DNA (0.2 μg/μL) in Tris-HCl/NaCl buffer (pH 7.2) at 37 °C for 2 h. After incubation bromophenol blue dye was added and then electrophoresed at 50 V for 1 h in Tris buffer using 1 % agarose gel. The resulting bands were blemished with ethidium bromide and images were taken under UV light.

Antibacterial Assay

All synthesized compounds were screened against different bacterial strains by the disc diffusion method using nutrient agar as the medium [38]. In typical procedure, standard and stock solutions of compounds were prepared by dissolving the compounds in DMSO. The plates were inoculated with microorganisms and filled with test compounds for 24 h at 30 °C. During the incubation period, the test solution diffused and the growth of the inoculated microorganisms was affected. The activity was measured in terms of inhibition zone.

Results and Discussion

FT-IR Spectroscopy

IR spectra of compounds provide information about nature of binding mode and functional group attached to metal ion. The binding modes were evaluated by comparing some important IR spectral bands of free ligands with their corresponding metal complexes (Table 1) and (Shown in Fig. S2). The azomethine stretching frequencies of $\mathbf{L_1}$ and $\mathbf{L_2}$ at 1616 and 1619 cm^{-1} are shifted to higher frequency region to the extent of 10–15 cm^{-1} in the complexes indicating the nitrogen of azomethine is coordinated to the metal ion [39–42]. The broad bands at 3438 and 3432 cm^{-1} due to free $\nu_{(O-H)}$ stretching frequencies of $\mathbf{L_1}$ and $\mathbf{L_2}$ are disappeared upon complexation indicating coordination through phenolic hydroxyl groups. A medium intensity band around 1167 cm^{-1} due to phenolic $\nu_{(C-O)}$ group of the ligand is shifted towards positive side by 15–20 cm^{-1} [43]. A lower frequency region new bands are

Table 1 Some important IR absorption frequencies (cm^{-1}) of Schiff bases and their Cu(II), Ni(II) and Co(II) metal complexes

Compound	$\nu_{(O-H)}$	$\nu_{(CH=N)}$	$\nu_{(C-O)}$	$\nu_{(M-O)}$	$\nu_{(M-N)}$
BTEMBP (**L_1**)	3438	1616	1167	-	-
$[Cu(BTEMBP)_2]$ (**1a**)	-	1596	1175	504	435
$[Ni(BTEMBP)_2]$ (**1b**)	-	1592	1180	537	423
$[Co(BTEMBP)_2]$ (**1c**)	-	1593	1176	500	432
BTEMNAPP (**L_2**)	3432	1619	1154	-	-
$[Cu(BTEMNAPP)_2]$ (**2a**)	-	1600	1190	564	451
$[Ni(BTEMNAPP)_2]$ (**2b**)	-	1602	1180	537	423
$[Co(BTEMNAPP)_2]$ (**2c**)	-	1606	1188	483	425

observed at 430 and 530 cm^{-1} due to $\nu_{(M-N)}$ and $\nu_{(M-O)}$ [44, 45] respectively.

Electronic Spectra and Magnetic Susceptibility

The geometry of metal complexes can be deduced from absorption spectra and magnetic data. Electronic spectra of the ligands and their metal complexes were measured in DMSO solution. The electronic spectra of ligands **L_1** and **L_2** shows two bands (270 and 360 nm) and three bands (243, 320 and 360 nm) respectively assigned to intraligand π-π* and π-π* transitions. The metal complexes Cu(II), Ni(II) and Co(II) show d-d bands at the range of 400–600 nm (Shown in Fig. S3). Due to $^2B_{1g}$-^2E_g for Cu(II) complexes [46], $^1A_{1g}$-$^1A_{2g}$ and $^1A_{1g}$-$^1B_{1g}$ for Ni(II) complexes [47] and $^1A_{1g}$-$^1B_{1g}$ transition for Co(II) complexes [48, 49]. The magnetic moments of Cu(II) and Co(II) are found to be 1.72 **(1a)**, 1.8 **(2a)**, 2.11 **(1c)** and 2.18 **(2c)** respectively whereas Ni (II) complexes are diamagnetic [50]. The electronic transitions and magnetic moments of these complexes are the characteristic features of square planar geometry.

SEM

The SEM analysis was carried out to check the surface morphology of Schiff bases and their metal complexes. All the compounds were analyzed by EDX analysis to find the elements present on surface. Figure 1 depicts the SEM photographs of the synthesized ligand **L_1**, and its metal complexes. The broken ice block like particles are observed in the ligand **L_1**, the grass like surface is observed in **1a** complex, irregular small rock circles like surface is observed in **1b** complex and the complex **1c** faceted with agglomeration of smaller and larger spherical particles. Figure S4 depicts the SEM photographs of the synthesized ligand **L_2**, and its metal complexes. An elongated flake are observed in ligand **L_2**, needle like particles are observed in **2a** complex**,** the bundle of irregularly broken ice piece shape particles are observed in **2b** complex and the spherical rock like particles are observed in **2c.** The SEM micrographs revealed that the surface morphology of metal complexes differ from ligand and each other due to the complexation and change of metal ion.

Mass Spectra

Mass spectra provide a preliminary clue for structure elucidation of compounds. The mass spectra of ligands L_1 and L_2 gave the molecular ion peak at m/z = 335, m/z = 305 respectively. The molecular ion peaks at m/z 727(M^+) and 692 (M + Na) of copper complexes are confirmed its stoichiometry as $[Cu(L_1)_2$ and $Cu(L_2)_2]$. For the Ni(II), Co(II) complexes peaks are observed at m/z 737 $[(L_1)_2Ni + NH_4]$, 721 $[(L_1)_2Co + H]$, 664 $[(L_2)_2Ni]$ and 665 $[(L_2)_2Co]$ (Shown in Fig. S5). The mass spectral results and elemental analysis are in good agreement with 1:2 stoichiometry for the formation of complexes.

Thermogravimetric Analysis

Thermal behaviors of all the complexes are studied by thermogravimetric analysis (TG) under nitrogen atmosphere in the temperature range 30–1000 °C. The TG curves indicated that Cu(II), Ni(II) and Co(II) metal complexes undergo pyrolysis in two stages. The thermograms of complexes (**1a, 1b, 1c, 2a, 2b** and **2c**) show similar decomposition pattern in two steps. The first step corresponds to the removal of starting material. The second step corresponds to removal of total ligand moiety and above this temperature left out was metal oxide (MO) residue. Representative thermograms of **1a** and **2a** complexes are shown in Fig. 2. In complexes **1a, 2a** the first step corresponds to departure of partial ligand moiety in the range of 266–390 °C. Second step degradation within the temperature range of 321–774 °C corresponds to removal of total ligand moiety and remaining left out was due to metal oxide (MO) residue.

ESR Spectra

The ESR spectra of Cu(II) complexes provide very useful information in studying the environment of Cu (II) ion. ESR spectra of **1a** and **2a** complexes are given in Fig. 3. The g values of the Cu(II) complexes could be used to obtain the ground state [51]. $g_\parallel$, $g_\perp$, Δg, g_{av} and G of the copper complexes have been calculated and presented in Table 2. The trend in the observed g values of copper(II) complexes at liquid nitrogen temperature (77 K) is $g_\parallel > g_\perp > g_e(2.0023)$. This trend provides an evidence of localization of the unpaired electron in $d_{x^2-y^2}$ orbital indicating square planar geometry. For Cu(II) complexes the G [$G = (g_\parallel\text{-}2.0023)/(g_\perp\text{-}2.0023)$] value found to be 2.442 (**1a**) and 2.296 (**2a**) suggesting considerable exchange interactions in Cu(II) complexes [52]. As $g_\parallel < 2.3$ these complexes are covalent in nature [53].

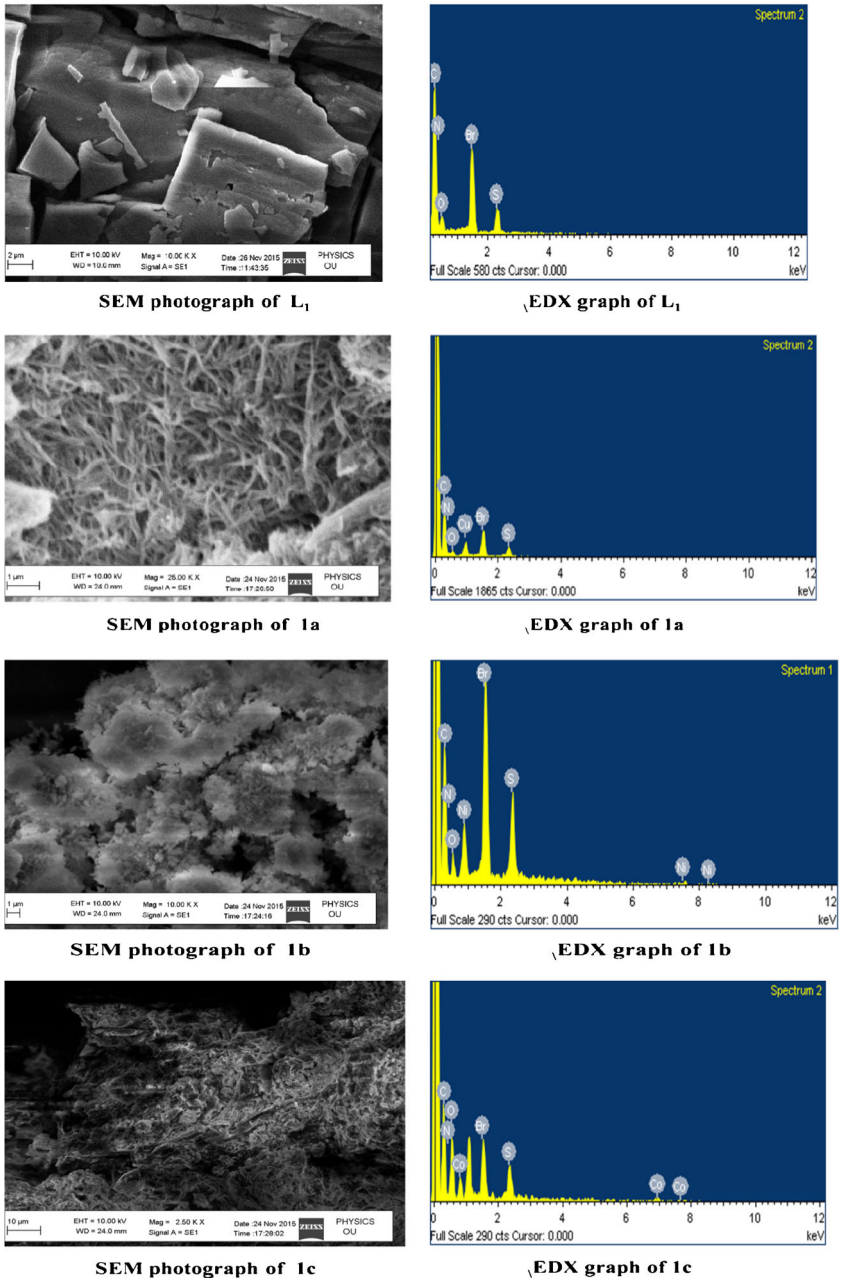


Fig. 1 **SEM** and **EDX** graphs of **L_1,** 1a, 1b and 1c

DNA Binding Studies

Electronic Absorption Study

UV-Vis spectroscopy has proven to be one of the most useful methods to determine the binding characteristics of metal complexes with CT-DNA [54, 55]. Absorption spectra of **1a, 1b** and **2c** are given in Fig. 4. The changes in absorption spectra of metal complexes (10 μM) were recorded in the absence and presence of CT-DNA. Upon increasing the concentration of CT-DNA, (0–10 μM) change in absorbance is effected, resulting in hypochromism (17–36 %) with slight red-shift (2-4 nm) (bathochromism) in all metal complexes indicating intercalative way of binding between DNA duplex and complexes [56, 57]. It was marked that the complexes bind to the base pair of DNA (intercalative), where the π* orbital of the intercalated ligand on the complexes can couple with π orbital of the base pairs, thus decreasing the π–π* transition energies. On the other hand, the coupled π* orbital is partially filled by electrons, thus decreasing the transition probabilities [58]. The extent of the hypochromism depends upon strength of intercalative binding [59]. The electronic absorption spectra of **1a** (401 nm), **1b** (406 nm), **1c** (408 nm), **2a** (444 nm), **2b** (440 nm) and **2c** (435 nm) shows intensive absorption bands. The intrinsic binding constant K_b of the complexes with CT-DNA is determined according to

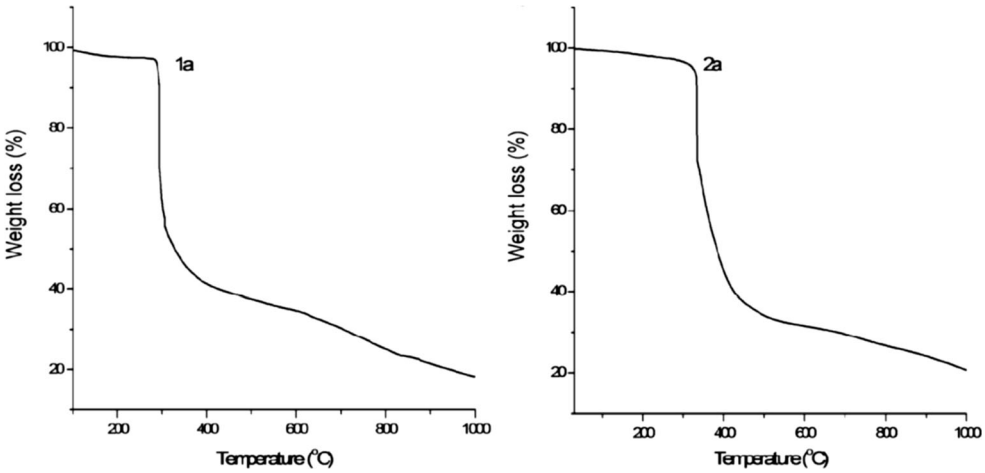


Fig. 2 TG curves of complexes 1a and 2a

the following equation,

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

Where [DNA] is the concentration of DNA in the base pairs, K_b is the intrinsic binding constant, ε_a is apparent coefficient of A_{obsd}/[complex], ε_f and ε_b correspond to the extinction coefficients of the free and fully bound forms of the complex, respectively. Using this formula, the binding constant are found to be $9.6 \pm 0.14 \times 10^4$ M^{-1} (**1a**), $9.39 \pm 0.17 \times 10^4$ M^{-1} (**2a**), $2.3 \pm 0.13 \times 10^4$ M^{-1} (**1b**), $2.12 \pm 0.18 \times 10^4$ M^{-1} (**2b**), $5.73 \pm 0.2 \times 10^4$ M^{-1} (**1c**) and $4.88 \pm 0.18 \times 10^4$ M^{-1} (**2c**). From the above values it is clear that, the copper complexes are strongly bind to DNA than the nickel and cobalt complexes.

Fluorescence Quenching Study

The evidences in favour of intercalative binding mode were also obtained by fluorescence titration studies. EB is a sensitive fluorescence probe, its fluorescence intensity is enhanced in the presence of DNA due to strong intercalation between the DNA base pairs [60, 61]. The experiment involves pretreating EB with DNA then followed by successive addition of complexes to the EB-DNA system. The fluorescence intensity is decreased due to complex competitive binding to EB-bound DNA system due to displacement of bound EB from DNA [62, 63]. As shown in Fig. 5, the fluorescence emission intensities at 619 nm (330 nm excitation) decreased with the increasing complex concentrations, which suggested that complexes could displace DNA-bound EB and bind to CT-DNA at the intercalation sites with almost the same affinity [64]. The K_{sv} is calculated from the slope of plot I_0/I versus r [65]. The apparent binding constant is found to be 8.4–3.32×10^3 M^{-1}. It may be due to the complexes interacting with DNA through intercalation binding, so releasing some free EB from the EB-DNA complex, which is consistent with the above absorption spectral results.

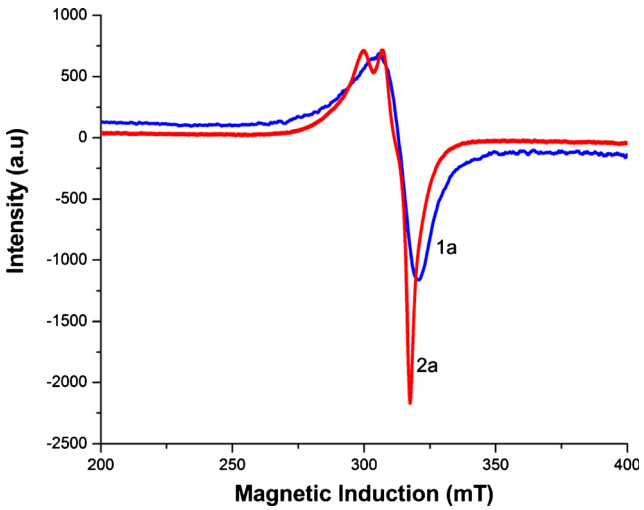


Fig. 3 ESR spectra of complexes 1a and 2a

Viscosity Studies

In order to get further confirmation of the intercalative binding nature of the complexes with CT-DNA, viscosity measurements have been performed. Viscosity studies give valuable information regarding binding mode of metal complexes with DNA in the absence of crystallographic structural data [66]. However, a partial and non-classical intercalation of ligand may bend the DNA helix, resulting in the decrease of its effective length and concomitantly its viscosity decrease [66]. The effects of investigated metal complexes on the viscosity

Table 2 ESR data of 1a and 2a complexes

Complex	$g_\parallel$	$g_\perp$	Δg	g_{av}	G
$[Cu(BTEMBP)_2]$ (**1a**)	2.213	2.0893	0.1242	2.1307	2.442
$[Cu(BTEMNAPP)_2]$ (**2a**)	2.155	2.0693	0.0857	2.098	2.296

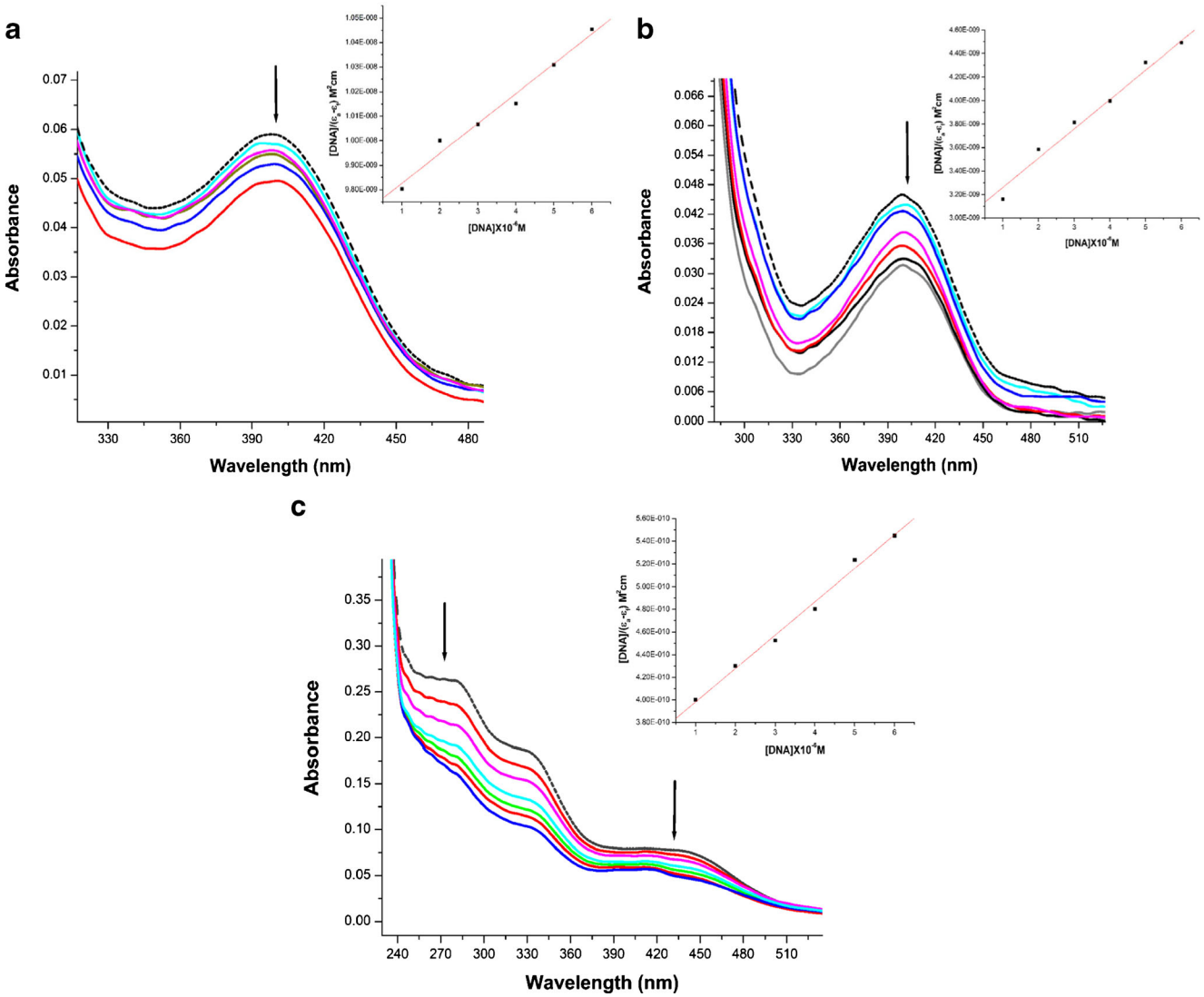


Fig. 4 UV-Vis absorption spectra of complexes **a** 1a, **b** 1b and **c** 2c DNA in Tris–HCl/NaCl buffer by addition of CT-DNA. Arrow shows the hypochromic and bathochromic shift upon increase of the DNA concentration. Plots of [DNA]/(ε_a–ε_f) Vs [DNA] for the titration of DNA with metal complexes

of DNA at 30 ± 1 °C are shown in Fig. 6. The experimental results showed that the relative viscosity of CT-DNA increased steadily on successive addition of increasing concentration of complexes. The Cu(II) complexes intercalate stronger than the Ni(II) and Co(II) complexes leading to the greater increase in viscosity of the CT-DNA. All the complexes intercalated between DNA duplex causing an increase in the viscosity of DNA solution. In conclusion, it is observed from experiment that, the complexes (**1a-2c**) may bind with DNA through intercalative mode.

DNA Cleavage Activities

The DNA cleavage activities of metal complexes with super coiled pBR322 plasmid DNA are carried out by oxidative and photolytic method [67–72]. The cleavage affinity of complexes are confirmed by comparing the band patterns obtained from untreated and treated (without and with complexes) plasmid DNA by oxidative and photolytic methods. When circular plasmid DNA is subjected to electrophoresis, the fast migration will be obtained for the covalently closed circular form. If one strand is nicked, the supercoil will relax to generate a slower moving open circular form (circular, open circular forms are known as Form I and Form II respectively). If both strands are nicked, a linear form that migrates between Form I and Form II will be generated [73].

The cleavage patterns of synthesised Cu(II), Ni(II) and Co(II) metal complexes are shown in Fig. 7. In oxidative method, no DNA cleavage was observed in lane 1 (**control**), lane 2 (**DNA + H_2O_2**) and lane 3 (**L_1**) but lane 4 (**1a**), lane 5 (**1b**) and lane 6 (**1c**) are efficiently cleaved supercoiled DNA

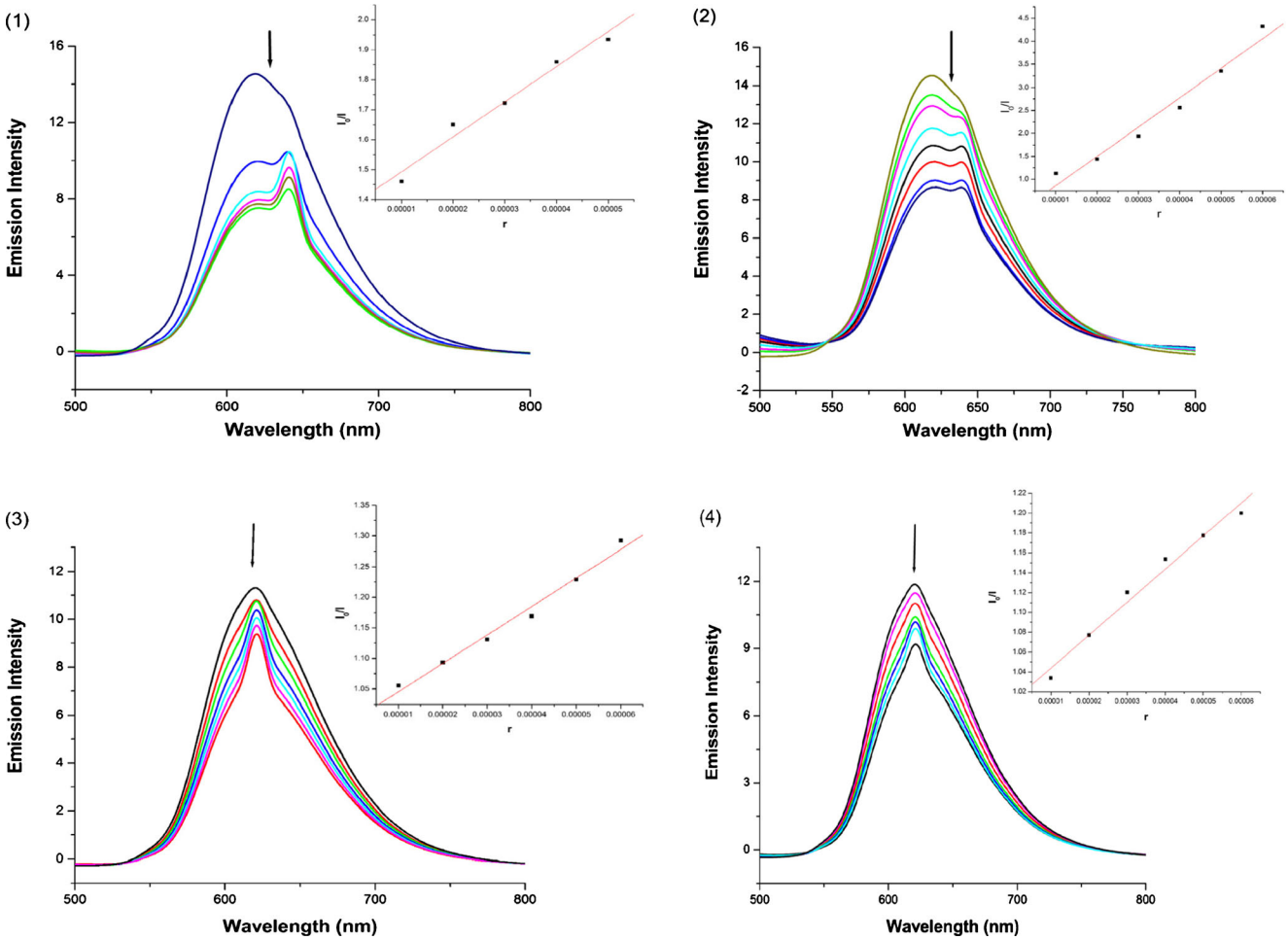


Fig. 5 Emission spectra of DNA–EB system **1** 1a**, 2** 1c**, 3** 2a and **4** 2b**.** Arrows shows the emission intensity changes upon increasing concentration of the complexes. Insert: I_0/I versus r

into nicked form. In photolytic method, no DNA cleavage is observed in lane 1, lane 2 (**L_1**) but lane 3 (**1a**), lane 4 (**1b**) and lane 5 (**1c**) efficiently cleaved into nicked form. In oxidative method DNA cleavage was more compared to photolytic

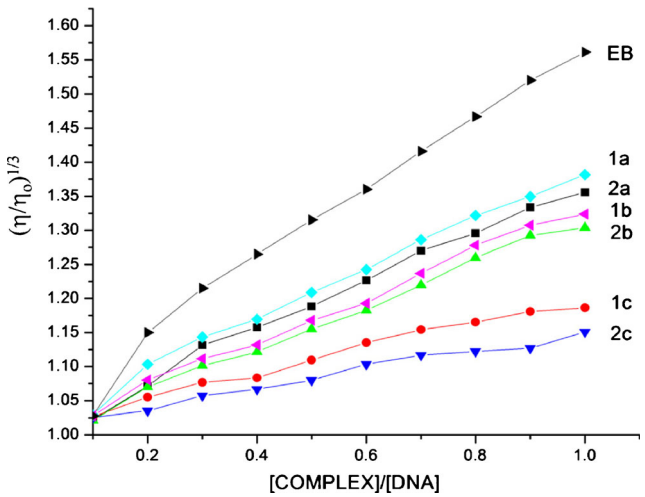


Fig. 6 Viscosity of the EB, complexes 1a, 2a, 1b, 2b, 1c and 2c

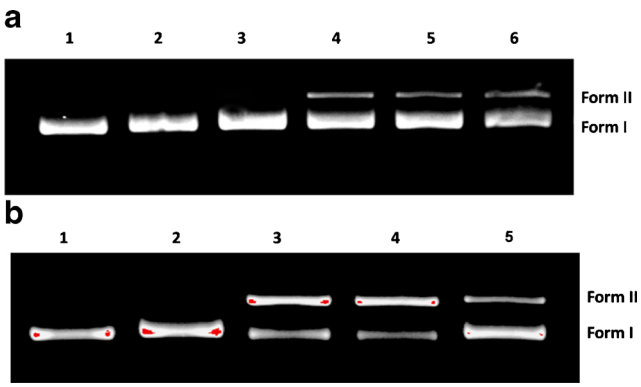


Fig. 7 **a** Oxidative cleavage of supercoiled pBR322 DNA(0.2 μg, 33.3 μM) at 37 °C in 5 mM Tris HCl/5 mM NaCl buffer by the metal complexes. Lane 1, DNA control; Lane 2, DNA + H_2O_2 (1 mM); Lane 3, DNA + H_2O_2 (1 mM) + L_1; Lane 4, DNA + H_2O_2 (1 mM) + 1a (20 μM); Lane 5, DNA + H_2O_2 (1 mM) + 1b (20 μM); Lane 6, DNA + H_2O_2 (1 mM) +1c (20 μM). **b** Photoactivated cleavage of supercoiled pBR322 DNA (0.2 μg, 33.3 μM) at 37 °C in 5 mM Tris HCl/5 mM NaCl buffer by the complexes UV irradiation of wavelength 345 nm. Lane 1, DNA control; Lane 2 DNA + L_1 (20 μM); Lane 3, DNA + 1a (20 μM); Lane 4, DNA + 1b (20 μM); Lane 5, DNA + 1c (20 μM)

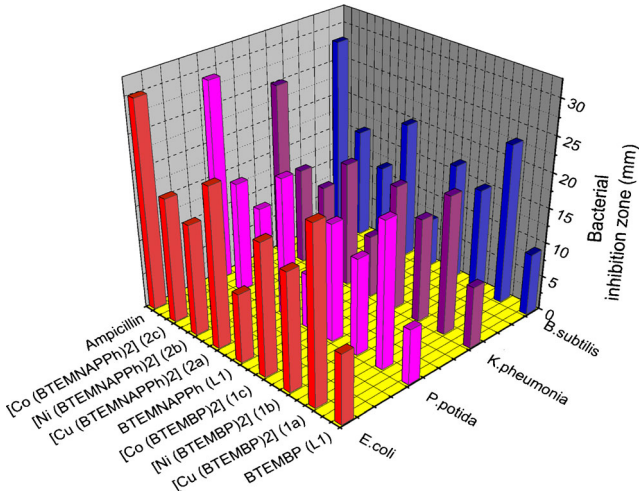


Fig. 8 Antimicrobial activity of Schiff bases and their Cu(II), Ni(II) and Co(II) metal complexes with *E. coli*, *P. putida*, *K. pneumoniae* (Gram Negative) and *B. subtilis* (Gram Positive) ampicillin as a standard

method. Further it is observed that copper complexes promote the cleavage of supercoiled pBR322 DNA more efficiently than Nickel and Cobalt complexes. In all complexes **1a**, **1b** and **1c** are shown greater cleavage property than **2a**, **2b** and **2c** complexes.

Antibacterial Activity

In vitro antibacterial activities of all the ligands and their complexes were tested against Gram-positive (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas putida*, *Klebsiella pneumonia.*), shown in Fig. 8 and Table 3. All the metal complexes (20 mg/mL in DMSO) showed significant activity compared to free ligand. Here the standard drug employed was Ampicillin**.** The higher activity of the complexes is explained through Overtone's concept [74] and chelation theory. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials. Antimicrobial activity depends on liposolubility. On chelation, the polarity of the metal ion is reduced to a greater extent owing to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donor groups. Further, it increases the delocalization of π -electrons over the whole chelating ring and augments the penetration of the complexes into lipid membranes and enzymes of microorganisms will be blocked by the metal binding sites. According to the microbial studies of the synthesized compounds the copper complexes are more active than nickel and cobalt complexes. The higher activity of copper complexes is deduced from the fact that increases in the size of the metal ion, decreases the polarization and further explained on the basis of chelation theory [75].

Conclusion

In the present investigation, two novel Schiff bases and their (Cu(II), Ni(II) and Co(II)) complexes have been synthesized and thoroughly characterized by analytical and spectral techniques. From the spectral data it is observed that all complexes tentatively adopted a square planar geometry. The binding mode of complexes with CT-DNA was investigated by UV-Vis spectroscopy, fluorescence studies and viscosity measurements**.** Binding studies reveal that all complexes are binding with CT-DNA through an intercalative mode. The DNA cleavage studies of Cu(II), Ni(II) and Co(II) complexes revealed that these complexes effectively cleaved supercoiled pBR322 DNA both in the presence of H_2O_2 and UV light. Further the antibacterial activity of all the synthesized ligands and their metal complexes revealed that all complexes showed high antibacterial activity compared to ligands.

Table 3 Inhibition zone (mm) of ligands and respective complexes (10 μL)

Compound	Bacterial inhibition zone(mm)			
	Gram-negative bacteria			Gram-positive bacteria
	E. coli	*P. putida*	*K. pneumoniae*	*B. subtilis*
BTEMBP (**L_1**)	10	8	9	9
[Cu (BTEMBP)$_2$] (**1a**)	25	21	20	23
[Ni (BTEMBP)$_2$] (**1b**)	17	14	15	15
[Co (BTEMBP)$_2$] (**1c**)	19	17	18	17
BTEMNAPP (**L_2**)	10	8	9	7
[Cu (BTEMNAPP)$_2$] (**2a**)	23	20	18	20
[Ni (BTEMNAPP)$_2$] (**2b**)	16	13	12	14
[Co (BTEMNAPP)$_2$] (**2c**)	18	16	14	16
Ampicillin	30	29	25	28

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