REGULAR ARTICLE

DNA interactions and biocidal activity of metal complexes of benzothiazole Schiff bases: synthesis, characterization and validation

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Abstract. Binary complexes of Cu(II), Ni(II) and Co(II) were synthesized using two novel Schiff bases L_1 = 2-(benzothiazol-6-ylimino)methyl-4-chlorophenol (BTEMCP), L_2 = 2-(benzothiazol-6-ylimino)methyl-4-nitrophenol. The Schiff bases and metal complexes were characterized by analytical and spectral methods like elemental analysis, Mass, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, UV-Vis, IR, ESR, SEM, EDX, XRD and magnetic susceptibility measurements. From the analytical data, square planar geometry has been proposed for all the metal complexes. The binding interaction between the metal complexes and DNA was investigated by means of electronic absorption, fluorescence spectroscopy and viscosity measurements. The DNA cleavage ability of the metal complexes was also evaluated by agarose gel electrophoresis method. These studies revealed that the complexes showed an intercalative mode of binding to CT DNA and also effectively cleaved the supercoiled pBR DNA. The synthesised compounds were evaluated for *in vitro* antibacterial activity against Gram positive and Gram negative bacteria, and found that the metal complexes showed more potent biocidal activity than the Schiff bases.

Keywords. Schiff base; binary complex; DNA binding cleavage; fluorescence; viscosity.

1. Introduction

In recent years, the study of interactions between transition metal complexes of Schiff base and DNA have gained much attention due to their possible applications in cancer therapy¹⁻³ and molecular biology.⁴⁻⁶ DNA is the primary target molecule of many anti-cancer agents, and the binding between DNA and metal complexes were used in understanding the interaction between the drugs and DNA. In general, the tumour cells can be smashed by stopping the replication of the unnatural DNA. Using Schiff base transition metal complex in particular, affected DNA may be dented by either following cleavage or binding approach. Transition metal complexes can bind to DNA through the noncovalent way on the groove, by intercalation and electrostatic binding.⁷ From the literature survey, we find that a vast number of metal complexes have been used as cleavage agents for DNA. Metal complexes can cleave the DNA through three types of mechanisms, which are hydrolytic, oxidative and photolytic cleavages.⁸⁻¹⁰ Cisplatin and its second generation com-

pounds are the most widely used metal-based drugs for cancer treatment. However cisplatin, oxaliplatin and carboplatin possess inherent limitations such as resistance, toxicity and other side effects.^{11,12} To overcome these short comings of cisplatin derivatives, and to design less toxic, cheaper and non-covalently bound new chemotherapeutic drugs, the focus has been shifted to the Schiff bases containing transition metal complexes. Schiff bases derived from benzothiazole compounds have attracted a great deal of interest due to their biological and pharmacological properties such as anti-HIV, antitubercular, anti-inflammatory, anticonvulsant, antileishmanial, antimalarial, analgesic, antimicrobial, lipid peroxidation inhibitor and anticancer.¹³⁻¹⁹ Cu(II) complexes act as best alternatives to platinum based antitumor agents.^{20,21} Cytotoxicity of Cu(II) complexes is mainly due to their ability to bind and cleave DNA which leads to cell cycle prevention and apoptosis.²²⁻²⁴ The numerous reports have also narrated the reactivity of DNA with mononuclear Ni(II) complexes.²⁵ The chemistry of Ni(II) complexes with Schiff base ligands may provide the origin for models of the active sites of biological systems or to act as catalysts.²⁶ Cobalt complexes have gained importance for their

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applicability in the biological field.^{27,28} Some Co(II) complexes such as hexamine cobalt induced DNA condensation.²⁹ DNA interactions and antimicrobial studies of binary metal complexes of isoxazole and benzothiazole Schiff bases were reported earlier from our laboratory.^{30–32}

In view of the above facts, herein, we report the synthesis, characterization, DNA binding, cleavage and antibacterial activity of Cu(II), Ni(II) and Co(II) complexes containing benzothiazole Schiff bases.

2. Experimental

2.1 Materials

The ligand precursors 6-amino benzothiazole, 5-chloro salicylaldehyde/5-nitro salicylaldehyde and all metal salts used for the preparation of metal complexes were purchased from Sigma-Aldrich Bangalore, India. The solvents methanol, petroleum ether, chloroform, DMF and DMSO were used after purification by the standard methods described in the literature. The CT-DNA (Product number of CT-DNA is 615100780041730) and supercoiled pBR322 DNA were procured from Genei, Bangalore and stored at 4°C. Tris-HCl/NaCl buffer, ethidium bromide were obtained from Merck, Hyderabad, India.

2.2 Instrumentation

The elemental analysis (C, H, N, S) of the compounds was carried out on a Perkin Elmer 240C (USA) elemental analyzer. Melting points of the compounds were determined with open glass capillary tubes on a Polmon instrument (Model No. MP-96). Metal content of the complexes was estimated by atomic absorption spectroscopy using GBC Avanta 1.0 AAS. NMR spectra of all the synthesized ligands were recorded in CDCl₃/Deuterated DMSO using TMS as an internal standard reference on a Bruker 400 MHz spectrometer. Scanning electron micrography (on Zeiss scanning electron microscope) with associated energy dispersive spectrometry (on INCA EDX instrument) were used for morphological evaluation. ESI mass spectra were obtained on a Vergleichbare Gerate (VG) micromass 7070-H instrument. Infrared spectra of the ligand and the metal complexes were recorded in the range of 4000–400 cm⁻¹ using a Perkin-Elmer Infrared Model 337 spectrophotometer using KBr discs. Electronic spectra of the complexes were recorded on a Shimadzu Model 1601 UV-Vis Spectrophotometer in the wavelength range of 200–800 nm using DMSO as solvent. Shimadzu RF-5301PC Spectrofluorometer was used to record the fluorescence spectra of the compounds. Effective magnetic moments of the complexes were determined on a Guoy balance Model 7550 using Hg[Co(NCS)₄] as standard. The thermogravimetric analysis was carried out in dynamic nitrogen atmosphere with a

heating rate of 10°C min⁻¹ using Mettler Toledo Star system in the temperature range of 30–1000°C. EPR spectra of the Cu(II) complexes were recorded using JES-FA 200 ESR spectrometer (JEOL-Japan) at liquid nitrogen temperature (77K).

2.3 Synthesis of Schiff bases

Hot methanolic solutions of 6-aminobenzothiazole (10 mM) and 5-chlorosalicylaldehyde (10 mM)/5-nitrosalicylaldehyde (10 mM) were mixed and refluxed on an oil bath for 4 h with constant stirring. The resulting solid product was isolated by filtration and recrystallized from hot methanol.

2.3a Ligand L₁: M.p.:180°C; C₁₄H₉ClN₂OS: Anal. Found: C, 58.03; H, 3.18; N, 9.70; S, 11.15%. Calc.: C, 58.23; H, 3.14; N, 9.70; S, 11.10%. IR (KBr, ν/cm⁻¹): (ν_{O-H}) 3434, (ν_{CH=N}) 1620, (ν_{C-O}) 1163. UV-Vis (DMSO) λ_{max}/nm (ε/M⁻¹cm⁻¹): 258 (38760), 300 (33333). ESI-MS (m/z): Calc.: 288; Found: 289. ¹H-NMR (400 MHz, CDCl₃, TMS): δ 13.0 (s, 1H); 9.01 (s, 1H); 8.60 (s, 1H); 8.16 (d, 1H); 7.83 (s, 1H); 7.46–7.18 (m, 3H); 6.98 (d, 1H) (Figure S1 in Supplementary Information). ¹³C NMR (100 MHz, CDCl₃ TMSO): δ 161.7 159.6, 154.5, 152.4, 145.7, 135.0, 133.3, 131.3, 124.3, 123.8, 120.1, 119.8, 118.9, 114.4.

2.3b Ligand L₂: M.p.:195°C; C₁₄H₉N₃O₃S: Anal. Found: C, 56.14; H, 3.06; N, 14.08; S, 10.74%. Calc.: C, 56.18; H, 3.03; N, 14.04; S, 10.71%. IR (KBr, ν/cm⁻¹): (ν_{O-H}) 3430, (ν_{CH=N}) 1625, (ν_{C-O}) 1160. UV-Vis (DMSO) λ_{max}/nm (ε/M⁻¹cm⁻¹): 256 (39062), 308 (32467). ESI-MS (m/z): Calc.: 299; Found: 300 (M+1). ¹H-NMR (400 MHz, CDCl₃, TMS): δ 14.17 (s, 1H); 9.433 (s, 1H); 9.238 (s, 1H); 8.296–8.016 (m, 3H) 7.726 (s, 1H); 7.168 (s, 1H). ¹³C-NMR (100 MHz, CDCl₃ TMSO): δ 166.1, 161.6, 157.0, 152.2, 144.5, 139.3, 134.8, 128.0, 127.9, 123.6, 120.2, 118.9, 118.1, 115.3.

2.4 Synthesis of metal complexes

To a hot methanolic solution of the Schiff base [2-(benzo[d]thiazol-6-ylimino)methyl]-4-chlorophenol/2-(benzo[d]thiazol-6-ylimino)methyl)-4-nitrophenol] (10 mmol), a solution of metal(II) acetate of copper, nickel or cobalt (10 mmol) in hot methanol was added drop wise and the resulting mixture was refluxed for 2–4 h, kept reaction in basic condition. The solid product obtained was separated, washed thoroughly with methanol and dried in vacuum.

2.4a [Cu(BTEMCP)₂] (1): M.p.:265°C; C₂₈H₁₆Cl₂N₄O₂S₂Cu: Anal. Found: C, 52.65; H, 2.50; N, 8.80; S, 10.06; Cu; 9.92%. Calc.: C, 52.63; H, 2.52; N, 8.77; S, 10.04; Cu, 9.94%. IR (KBr, ν/cm⁻¹): ν_(CH=N) 1602, ν_(C-O) 1176, ν_(M-O) 530, ν_(M-N) 450. UV-Vis (DMSO) λ_{max}/nm (ε/M⁻¹cm⁻¹): 265 (37735), 400 (25000), 570 (17547). ESR: g_{||} = 2.15, g_⊥ = 2.09, G = 1.53. μ_{eff}(BM):1.82. ESI-MS (m/z): Calc.: 636. Found: 659 [M + Na]⁺.

2.4b $[Ni(BTEMCP)_2]$ (**2**): M.p.:310°C; $C_{28}H_{16}Cl_2N_4O_2S_2Ni$: Anal. Found: C, 53.05; H, 2.50; N, 8.85; S, 10.12; Ni, 9.21%. Calc.: C, 53.03; H, 2.54; N, 8.83; S, 10.11; Ni, 9.25%. IR (KBr, ν/cm^{-1}): $\nu_{(CH=N)}$ 1605, $\nu_{(C-O)}$ 1180, $\nu_{(M-O)}$ 520, $\nu_{(M-N)}$ 437. UV-Vis (DMSO) λ_{max}/nm ($\epsilon/M^{-1}cm^{-1}$): 280 (35714), 411 (24330), 564 (17667). ESI-MS (m/z): Calc.: 631; Found: 670 $[M + K]^+$.

2.4c $[Co(BTEMCP)_2]$ (**3**): M.p.:280°C; $C_{28}H_{16}Cl_2N_4O_2S_2Co$: Anal. Found: C, 53.05; H, 2.56; N, 8.85; S, 10.12; Co 9.25% Calc: C, 53.01; H, 2.54; N, 8.83; S, 10.11; Co, 9.29%. IR (KBr): $\nu_{(CH=N)}$ 1600, $\nu_{(C-O)}$ 1179, $\nu_{(M-O)}$ 532, $\nu_{(M-N)}$ 429. UV-Vis (DMSO) λ_{max}/nm ($\epsilon/M^{-1}cm^{-1}$): 259 (38610), 410 (24390), 550 (19011). $\mu_{eff}(BM)$: 2.14. ESI-MS (m/z): Calc.: 632. Found: 655 $[M + Na]^+$.

2.4d $[Cu(BTEMNP)_2]$ (**4**): M.p.:290°C; $C_{28}H_{16}N_6O_6S_2Cu$: Anal. Found: C, 50.92; H, 2.42; N, 12.69; S, 9.68; Cu, 9.60%. Calc.: C, 50.94; H, 2.44; N, 12.73; S, 9.71; Cu, 9.63%. IR (KBr, ν/cm^{-1}): $\nu_{(CH=N)}$ 1609, $\nu_{(C-O)}$ 1182, $\nu_{(M-O)}$ 515, $\nu_{(M-N)}$ 426. ESR: $g_{||} = 2.12$, $g_{\perp} = 2.07$, $G = 1.61$. UV-Vis (DMSO) λ_{max}/nm ($\epsilon/M^{-1}cm^{-1}$): 260 (38462), 442 (22624), 538 (18587). $\mu_{eff}(BM)$:1.72. ESI-MS (m/z): Calc.: 659. Found: 698 $[M + K]^+$.

2.4e $[Ni(BTEMNP)_2]$ (**5**): M.p.:315°C; $C_{28}H_{16}N_6O_6S_2Ni$: Anal. Found: C, 51.30; H, 2.44; N, 12.79; S, 9.76; Ni 8.93%. Calc.: C, 51.32; H, 2.46; N, 12.82; S, 9.79; Ni, 8.96%. IR (KBr, ν/cm^{-1}): $\nu_{(CH=N)}$ 1608, $\nu_{(C-O)}$ 1173, $\nu_{(M-O)}$ 545, $\nu_{(M-N)}$ 430. UV-Vis (DMSO) λ_{max}/nm ($\epsilon/M^{-1}cm^{-1}$): 260 (38461), 410 (24390), 526 (19011). ESI-MS (m/z): Calc.: 653. Found: 653 $[M]^+$.

2.4f $[Co(BTEMNP)_2]$ (**6**): M.p.:300°C; $C_{28}H_{16}N_6O_6S_2Co$: Anal. Found: C, 51.32; H, 2.48; N, 12.80; S, 9.80; Co, 8.9%. Calc.: C, 51.30; H, 2.46; N, 12.82; S, 9.78; Co, 8.99%. IR (KBr, ν/cm^{-1}) $\nu_{(CH=N)}$ 1598, $\nu_{(C-O)}$ 1170, $\nu_{(M-O)}$ 503, $\nu_{(M-N)}$ 415; UV-Vis (DMSO) λ_{max}/nm ($\epsilon/M^{-1}cm^{-1}$): 262 (38168), 411(24330), 556 (17985). $\mu_{eff}(BM)$: 2.16. ESI-MS (m/z): Calc.: 654; Found: 693 $[M + K]^+$.

2.5 DNA binding studies

2.5a Electronic absorption spectroscopic studies:

The UV-Vis titration experiments were performed by maintaining a constant concentration of the complexes at 10 μM throughout experiment (5 mM Tris-HCl/50 mM NaCl buffer at pH 7.4). The ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm was given by CT-DNA in tris HCl-NaCl buffer solution, indicating that the DNA was sufficiently free of protein.³³ The concentration of the source DNA is 1 mg/mL. We determined the concentration of CT-DNA stock solution by employing a molar absorptivity ($6600 M^{-1}cm^{-1}$) at $A_{260} nm$,³⁴ after 1:30 dilution of source DNA with 5 mM Tris-HCl/50 mM NaCl buffer at pH = 7.2. Thus, the concentration of the source DNA (1 mg/mL) is estimated to

be 5700 μM . In experiments, the concentration of CT-DNA was varied between 0–10 μM keeping the total volume of the reaction mixture constant (3 mL). After each addition of CT-DNA to the complex, the resulting solution was allowed to equilibrate at 25°C for 5 min followed by recording of absorption spectrum. The binding constants (K_b) were calculated from the spectroscopic titration data by the plot between $[DNA]/(\epsilon_a - \epsilon_f)$ and $[DNA]$.³⁵

2.5b Fluorescence study: Further support for the binding of complexes to DNA was given through the fluorescence quenching study. A mixture of the CT-DNA (125 μM) and ethidium bromide (EB) (12.5 μM) was subjected to competitive binding with increasing amount of complex (0–60 μM). The DNA bound EB/complexes was excited at 350 nm. The binding constants K_b ($=K_{SV}$) were calculated using following equation and observed the changes of fluorescence intensity with increasing concentration of quencher (complex).

$$I_0/I = 1 + K_{SV} r \quad (1)$$

Where, I_0 and I are the fluorescence intensities in the absence and presence of complexes respectively, K_{SV} is a well-known linear Stern–Volmer constant, and r is the concentration of the quencher (complex).

2.5c Viscosity study: Viscosity experiments were carried out on an Ostwald's viscometer, immersed in a thermostatic water bath at constant temperature ($30 \pm 1^\circ C$). Concentration of metal complexes was varied (0–100 μM) and concentration of DNA (100 μM) was kept constant. Flow time was recorded with a digital stopwatch for three times of each sample and an average flow time was calculated. Data are presented as plot of $(\eta/\eta_0)^{1/3}$ versus $[complexes]/[DNA]$, where η_0 is the viscosity of DNA alone and η is the viscosity of DNA in the presence of complex.

2.6 DNA cleavage

Interaction between pBR322 plasmid DNA and complexes were examined by gel electrophoresis experiments. The complexes were moving on agarose gel under the influence of electric field. The stock solutions of complexes in DMSO were prepared and used freshly. The experiment involves incubating the samples (20 μM) with pBR322 plasmid DNA (0.2 $\mu g/\mu L$) in tris-HCl/NaCl buffer (pH 7.2) at 37°C for 2 h. The gel was then bleached using ethidium bromide (EB) and photographed under UV light. All the experiments were performed at room temperature.

2.7 Antibacterial assay

All the ligands and complexes were screened against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* for antibacterial activity. The tests were performed using the disc diffusion method. All the stock solutions were prepared by dissolving compounds in DMSO. The test microorganisms were grown on nutrient agar medium in

Scheme 1. Synthesis of ligands and their metal complexes.

Petri plates. The compounds were soaked in a filter paper disk of 1 mm thickness and 5 mm diameter. The discs were placed on Petri plates and incubated at 37°C. The diameter of inhibition zone around each disc was measured after 24 h.

3. Results and Discussion

3.1 FT-IR spectroscopy

The IR spectra of ligands L_1 and L_2 showed sharp peaks at 1620 and 1625 cm^{-1} , respectively (Figure S2 in Supplementary Information), which corresponds to

azomethine ($-\text{C}=\text{N}$) group. These peaks are shifted to lower frequency by 18 to 20 cm^{-1} due to coordination of nitrogen of azomethine to the metal ion.^{36,37} The broad band at 3434 and 3430 cm^{-1} due to $-\text{OH}$ group of in metal-free ligands (L_1 and L_2) disappeared in the metal complexes (**1–6**). A medium intensity band which showed at 1160 and 1163 cm^{-1} due to phenolic $\text{C}-\text{O}$ group of the ligands L_1 and L_2 are shifted towards positive side by 15–25 cm^{-1} ³⁸ upon complexation. The formation of complexes is further confirmed by the new bands observed at lower frequency region 415–450 and 515–545 cm^{-1} ,^{39,40} respectively due to $\text{M}-\text{N}$

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

Compound	$\nu(\text{O-H})$	$\nu(\text{CH=N})$	$\nu(\text{C-O})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
BTEMCP (L_1)	3434	1620	1163	–	–
[Cu(BTEMCP) $_2$] (1)	–	1602	1176	530	450
[Ni(BTEMCP) $_2$] (2)	–	1605	1180	520	437
[Co(BTEMCP) $_2$] (3)	–	1600	1179	532	429
BTEMNP (L_2)	3430	1625	1160	–	–
[Cu(BTEMNP) $_2$] (4)	–	1609	1182	515	426
[Ni(BTEMNP) $_2$] (5)	–	1608	1173	545	430
[Co(BTEMNP) $_2$] (6)	–	1598	1170	503	415

and M–O. Some of the important bands are shown in Table 1.

3.2 Electronic spectra and magnetic susceptibility

The UV-Vis spectra of all the compounds were measured in DMSO at room temperature. The ligands showed two absorption peaks between the range 256–258 and 300–308 nm and these are assigned to $\pi - \pi^*$ and $n - \pi^*$ transition, respectively. The metal complexes showed d-d band in the range of 526–570 nm and CT bands at lower wavelengths. The Cu(II) complexes showed a band due to $^2\text{B}_{1g} - ^2\text{E}_g$ transition⁴¹ with magnetic moment of 1.82 (**1**) and 1.76 (**4**) BM for single unpaired electron. The nickel complexes are diamagnetic with one band corresponding to $^1\text{A}_{1g} - ^1\text{B}_{1g}$ transition.⁴² The Co(II) complexes showed a band at lower region attributable to $^1\text{A}_{1g} - ^1\text{B}_{1g}$ transition⁴³ with magnetic moment of 2.14 (**3**) and 2.16 (**6**) BM. Based on the electronic spectral data and magnetic moment, a square planar geometry is assigned to all the complexes.

3.3 SEM and EDX

The surface morphology of ligands and metal complexes was determined by SEM in order to know the change of surface morphology and particle size upon coordination. The micrographs of Schiff base L_1 and its metal complexes are shown in Figure 1. Ligand L_1 depicts non-uniform platelet structures, particle size is 10 μm ; on the other hand, micrographs of complex **1** indicated the rough surface containing irregular particles with 2 μm size. The magnification of image of complex **2** showed cloud-like appearance with particle size of 2 μm . The complex **3** displayed irregular small spherical shape particles of size 2 μm . The Figure S3 (in SI) depicts the L_2 and its metal complexes. Bundle of elongated needles are observed with particle size of 2 μm in micrograph of L_2 , irregular flakes-like particles are observed in micrograph of **4**, rough fungi-like

surface is observed in complex **5**, and agglomeration of smaller and larger spherical particles faceted on surface of complex **6**. The observed sizes of complexes **4**, **5** and **6** are 2, 10 and 10 μm , respectively. Further, it is observed that upon the change of metal ion in the complex, surface morphology and particle size are also changed.⁴⁴ The chemical composition of Schiff base complexes was determined using energy dispersive X-ray diffraction (EDX). EDX profiles of L_1 and its complexes **1**, **2** and **3** are shown in Figure 1. The peaks for elements like C, N, O, S, Cl and respective metal ion Cu(II), Ni(II) and Co(II) which constitute the ligand molecule ($\text{C}_{14}\text{H}_9\text{ClN}_2\text{OS}$) and complexes such as ($\text{C}_{28}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_2\text{S}_2\text{Cu}$), ($\text{C}_{28}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_2\text{S}_2\text{Ni}$) and ($\text{C}_{28}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_2\text{S}_2\text{Co}$) are clearly identified, supporting the proposed structures.⁴⁵

3.4 XRD

The X-ray powder diffraction analysis of the compounds has been performed in order to determine whether the nature of the sample is crystalline or amorphous. The powder diffraction analysis of ligands L_1 and L_2 shows sharp peaks due to their crystalline nature while the complexes do not exhibit well-defined sharp peaks due to their amorphous nature. The Figure 2 shows powder XRD patterns of L_1 , **1**, **2** and **3**. The crystallite sizes were calculated by using the following Debye Scherrer's equation:

$$D = 0.9 \lambda / \beta \cos \theta$$

where, constant 0.9 is the shape factor, λ is the X-ray wavelength (1.5406 Å), θ is the Bragg diffraction angle and β is the full width at half maximum (FWHM). The experimental average grain sizes of ligands and their metal complexes were found to be 64.32 (L_1), 23.45 (**1**), 53.52 (**2**), 43.21 (**3**), 44.82 (L_2), 35.42 (**4**), 23.23 (**5**) and 24.32 nm (**6**).

SEM photograph of L_1 EDX graph of L_1

SEM photograph of 1

EDX graph of 1

SEM photograph of 2

EDX graph of 2

SEM photograph of 3

EDX graph of 3

Figure 1. SEM Morphology of L₁, 1, 2 and 3.

Figure 2. Powder XRD patterns of **L₁**, **1**, **2** and **3**.

3.5 Mass spectra

The mass spectra of ligands **L₁** and **L₂** showed their molecular ion peaks at $m/z = 289$ ($M+1$)⁺ and $m/z = 300$ ($M+1$)⁺, respectively. The copper complexes showed their molecular ion peaks at m/z 659 ($M+Na$)⁺ and 698 ($M+K$)⁺ which confirmed their stoichiometry as Cu(**L₁**)₂ and Cu(**L₂**)₂, respectively. The peaks for the Ni(II), Co(II) complexes were observed at m/z 670 [(**L₁**)₂Ni + K]⁺, 653 [(**L₂**)₂Ni]⁺, 655 [(**L₁**)₂Co + Na]⁺, 693 [(**L₂**)₂Co + K]⁺ (Shown in Figure S4 in SI). Thus, the mass spectral results along with elemental analysis agreed with the formation of (M(**L₂**)) complexes of 1:2 stoichiometry.

3.6 Thermal analysis

The thermal stability of all the synthesized complexes was determined by the TG-technique. The experiment was carried out under a dynamic nitrogen atmosphere in the temperature range 30–1000°C at heating rate of 10°C min⁻¹. The thermal behaviour of the complexes

were found to be similar for the six complexes. Two-steps were involved in the weight loss process. The first step corresponds to the collapse of the starting material. The second step involves the removal of total ligand moiety and above this temperature metal oxide (MO) was left as residue. Representative thermograms of **1–3** complexes are shown in Figure 3. In complexes **1**, **2** and **3**, the first step degradation in the range of 250–390°C corresponds to removal of partial ligand moiety. Second step corresponds to departure of total ligand moiety in the range of 332–753°C, and above this temperature, horizontal curve was obtained suggesting formation of metal oxide.

3.7 ESR spectra

The electron spin resonance spectra of Cu(II) complexes were recorded in DMSO at liquid nitrogen temperature (LNT). The ESR studies give information about the sharing of the single unpaired electron of Cu(II) complexes with ligand and hence the nature of the bonding between the metal ion and its ligands can be

Figure 3. Thermograms of complexes **1**, **2** and **3**.

Figure 4. Electron spin resonance spectra of complexes **1** and **4**.

known. Figure 4 depicts the ESR spectra of **1** and **4**. The trend $g_{\parallel} > g_{\perp} > g_e(2.0023)$ observed in these complexes indicate that the unpaired electron residing in the $d_{x^2-y^2}$ orbital, and this evidence is in favour for Cu(II) ion having square planar geometry. The $g_{\parallel}$ value of these complexes lies below 2.3 ($g_{\parallel} < 2.3$) which indicate covalent environment around the Cu(II) ion.⁴⁶ In Cu(II) complexes, the calculated G [$G = (g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)$] value is 1.539 for complex **1** and 1.618 for **4**. The G values of Cu (II) complexes less than 4 give a hint of considerable exchange interaction in the complexes.⁴⁷

3.8 DNA binding studies

3.8a Electronic absorption spectroscopic studies:

Electronic absorption study is one of the most reliable technique to examine the binding affinity and binding mode of metal complexes with CT-DNA.^{48,49} In this study, absorption titration concentration of CT-DNA is

varied while the concentration of metal complex is kept constant. In general, the absorption spectrum of a complex shows a red shift and hypochromism, indicating intercalation mode of binding between complexes and DNA. The binding tendency was determined by monitoring the change of the absorbance of the complex when the concentration of CT-DNA was increased. Absorption spectra of **1**, **3**, **4** and **6** are shown in Figure 5. Upon increasing concentration of CT-DNA to the metal complex, absorbance decreased (hypochromism) and wavelength shifted towards long wavelength (red shift) due to a strong stacking interaction between the aromatic chromophore of the complex and the adjacent base pairs of DNA.⁵⁰ The extent of the hypochromism commonly parallels with the intercalative binding strength.⁵¹ The electronic absorption spectra of **1** (400 nm), **2** (401 nm), **3** (403 nm), **4** (377 nm), **5** (391 nm) and **6** (390 nm) show intense absorption bands. In order to compare the DNA binding propensity of the compounds quantitatively, the intrinsic binding constant K_b of the complexes with CT-DNA was determined according to the eq. 2.

$$[\text{DNA}] / (\epsilon_a - \epsilon_f) = [\text{DNA}] / (\epsilon_b - \epsilon_f) + 1 / K_b (\epsilon_b - \epsilon_f) \quad (2)$$

Here, K_b is the binding constant, $[\text{DNA}]$ is the concentration of DNA in the base pairs, ϵ_a is apparent coefficient equal to $A_{\text{obsd}} / [\text{complex}]$, 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 constants K_b were calculated and found to be, $1.57 \pm 0.16 \times 10^5 \text{ M}^{-1}$ for complex (**1**), $1.36 \pm 0.15 \times 10^5 \text{ M}^{-1}$ (**4**), $3.14 \pm 0.13 \times 10^4 \text{ M}^{-1}$ (**2**), $1.14 \pm 0.16 \times 10^4 \text{ M}^{-1}$ (**5**), $2.32 \pm 0.2 \times 10^4 \text{ M}^{-1}$ (**3**) and $2.1 \pm 0.18 \times 10^4 \text{ M}^{-1}$ (**6**). This absorption spectral result reveals that the Cu(II) complexes have greater binding affinity than the Ni(II) and Co(II) complexes.

3.8b Fluorescence studies: The EB fluorescence displacement experiments were carried out to explore further the interaction mode between CT-DNA and the complexes. EB in buffer solution has low fluorescence intensity due to fluorescence quenching of the free EB by the solvent molecule, and its emission intensity dramatically increases when (incubate 15 min) it intercalatively binds with DNA (Figure S5 in Supplementary Information). The intensity saturates for $[\text{EB}] \sim 12.5 \mu\text{M}$ for $[\text{DNA}] = 125 \mu\text{M}$. This high intensity is reduced by successive addition of DNA binding agents (complexes) to EB-DNA system.^{31,52,53} In the present work, on increasing the concentration of the Cu(II), Ni(II) and Co(II) complexes, the fluorescence emission intensity of the DNA-EB complex decreased slowly (Figure 6). The flu-

Figure 5. Absorption spectra of complexes **1**, **3**, **4** and **6** (In Tris-HCl/NaCl buffer) on addition of increasing concentration of CT-DNA. Conditions: [complex] = 10 μ M, [DNA] = 0–10 μ M. Arrow shows changes in absorbance upon increasing amounts of CT DNA. Inset: Plot of $[DNA]/(\epsilon_a - \epsilon_f)$ versus $[DNA]$ for the titration of DNA with metal complexes.

orescence emission intensities at 592 nm (excitation at 350 nm) diminished with the increase of complex concentration, which suggested that the complexes could displace DNA-bound EB and bind to CT-DNA at the intercalation site with almost the same affinity.⁵⁴ The K_{sv} is calculated from the slope of the plot I_0/I versus r . The apparent binding constant was calculated to be $1.57 \pm 0.16 \times 10^5 \text{ M}^{-1}$ (**1**), $1.36 \pm 0.15 \times 10^5 \text{ M}^{-1}$ (**4**), $7.4 \pm 0.13 \times 10^4 \text{ M}^{-1}$ (**2**), $6.1 \pm 0.17 \times 10^4 \text{ M}^{-1}$ (**5**), $1.9 \pm 0.13 \times 10^4 \text{ M}^{-1}$ (**3**), and $1.3 \pm 0.19 \times 10^4 \text{ M}^{-1}$ (**6**). These results indicate Cu(II) complex binds to the DNA by intercalative mode more efficiently than Ni(II) and Co(II) complexes, which is consistent with electronic absorption spectral results described in Section 3.8a.

3.8c Viscosity studies: The universally acceptable tool to find the binding mode of complexes to CT-DNA further is viscometric measurement and it is one of the most definite methods in illustrating the binding mode of complexes to DNA in solution in the absence of crys-

tallographic structural data. In classical intercalation, length of DNA helix increases due to complexes binding between the base pairs of the DNA leading to increase in viscosity of the DNA solution. In dissimilarity, a partial intercalation mode can kink or bend the DNA helix, resulting in a decrease in the effective length and also viscosity.⁵⁵ Whereas, non-intercalation binding, such as electrostatic or groove binding, has a smaller effect on viscosity. The effect of all the metal complexes on the viscosity of DNA solution at $30 \pm 1^\circ\text{C}$ is shown in Figure 7 and Figure S5 (in SI). The results on viscosity clearly showed that all the complexes can bind intercalatively. The increase is in the following order: **1** > **4** > **2** > **5** > **3** > **6**. The affinity complexes to DNA are comparable with groove binding (GB) agents like copper(II) or nickel(II) mesalamine complexes⁵⁶ (shown in Figure 7 and Figure S6). These results confirmed that binding is intercalative. The obtained results of viscometric measurements are consistent with the results of the absorption and emission studies.

Figure 6. Quenched Fluorescence spectra of DNA (125 μM) bound to EB (12.5 μM) system with the addition of **1**, **3**, **4** and **6**. Arrow shows the decreasing emission intensity upon increasing concentration of the complexes (0–60 μM). Inset: I_0/I versus r .

Figure 7. Viscosity of the solutions of EB, complexes **1**, **2**, **3** and Nickel (II) mesalamine complex (GB).

3.9 DNA cleavage

The chemical nuclease efficacy of complexes **1-6** has been studied by gel electrophoresis by using supercoiled pBR322 DNA as the substrate in the medium of 5 mM Tris-HCl/50 mM NaCl buffer (pH = 7.4) in the presence of hydrogen peroxide and UV-light. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the super coiled (SC) form-I. If one strand is cleaved, the SC form will relax to produce a slow-moving open circular (OC) form-II or nicked form. If both strands are cleaved, a linear form-III will be generated that migrates at a rate in between the form-I and form-II.^{57,58}

Figure 8 exhibits the cleavage patterns of synthesized Cu(II), Ni(II) and Co(II) metal complexes. In the pho-

Figure 8. **a** Photoactivated cleavage of supercoiled pBR322 DNA (0.2 μ g, 33.3 μ M) by the complexes at 37°C in 5 mM Tris HCl/50 mM NaCl buffer by UV irradiation at 345 nm. Lane 1, DNA control; Lane 2, DNA + L_1 (20 μ M); Lane 3, DNA + **1** (20 μ M); Lane 4, DNA + **2** (20 μ M); Lane 5, DNA + **3** (20 μ M). **b** Oxidative cleavage of supercoiled pBR322 DNA (0.2 μ g/ μ L, 33.3 μ M) at 37°C in 5 mM Tris HCl/50 mM NaCl buffer. Lane 1, DNA control; Lane 2, DNA + H₂O₂ (1 mM); Lane 3, DNA + H₂O₂ (1 mM) + L_1 ; Lane 4, DNA + H₂O₂ (1 mM) + **1** (20 μ M); Lane 5, DNA + H₂O₂ (1 mM) + **2** (20 μ M); Lane 6, DNA + H₂O₂ (1 mM) + **3** (20 μ M).

Table 2. Minimum inhibition zone (mm) of complexes (1mg/mL)

Compound	Bacterial inhibition zone(mm)			
	Gram-negative bacteria			Gram-positive bacteria
	<i>E. coli</i>	<i>P. putida</i>	<i>K. pneumoniae</i>	<i>B. subtilis</i>
BTEMCP (L_1)	11	8	9	10
[Cu (BTEMCP) ₂] (1)	26	23	21	24
[Ni (BTEMCP) ₂] (2)	18	15	16	17
[Co (BTEMCP) ₂] (3)	20	16	17	19
BTEMNP (L_2)	9	7	10	8
[Cu (BTEMNP) ₂] (4)	22	19	17	21
[Ni (BTEMNP) ₂] (5)	15	12	13	15
[Co (BTEMNP) ₂] (6)	19	17	14	17
[Cu(Aco) ₂ H ₂ O]	2	–	–	3
[Ni(Aco) ₂ 4H ₂ O]	2	–	–	1
[Co(Aco) ₂ 4H ₂ O]	1	–	–	2
Ampicillin	30	29	25	28

tolytic method, control (lane 1) and L_1 (lane 2) do not show any cleavage of plasmid DNA whereas complexes **1** (lane 3), **2** (lane 4) and **3** (lane 5) effectively cleaved the plasmid DNA into nicked form, and, especially, complex **1** cleaved DNA into linear form. In the oxidative method, control (lane 1), DNA+H₂O₂ (lane 2) and L_1 (lane 3) do not show any significant cleavage of plasmid DNA whereas complexes **1** (lane 4), **2** (lane 5) and **3** (lane 6) cleaved DNA into nicked form. These results showed that complex **1** (Cu) has a good cleavage activity than complexes **2** (Ni) and **3** (Co). Further, it is observed that the complexes **1**, **2** and **3** showed better cleavage activity than complexes **4**, **5** and **6** (Figure S7 in SI)

3.10 Antibacterial activity

The *in vitro* biological effects of the two ligands and six complexes were tested against various gram positive, gram negative bacteria, namely, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putida*, and *Klebsiella pneumonia* and the results are presented in Table 2 and Figure 9. All the metal complexes (1 mg/mL in DMSO) showed better activity compared to free ligands. The results are compared with Ampicillin. Increased activity of the complexes is attributed to increase in the lipophilic nature of the complexes arising from chelation, which reduces the polarity of the metal ion mainly because

Figure 9. Antibacterial activity of synthesized compounds with *E. coli*, *P. putida*, *K. pneumonia* (Gram Negative) and *B. subtilis* (Gram Positive) and Ampicillin as a standard.

of possible π electron delocalization within the intact chelate ring and partial sharing of its positive charge with the donor groups. The lipophilic nature of the metal ion is also increased by chelation which subsequently favors the permeation through the lipid layer of the cell membrane. The mode of action of the complexes involves the formation of hydrogen bonds with the imino group by the active sites leading to interference with the cell wall synthesis. This hydrogen bond formation damages the cytoplasmic membrane, and the cell permeability may also be distorted leading to cell fatality.⁵⁹ Further, it is observed that the Cu(II) complexes showed larger inhibition zone than Ni(II) and Co(II) complexes.

4. Conclusions

In the present investigation, we have synthesized mononuclear Cu(II), Ni(II) and Co(II) complexes using novel Schiff base ligands L_1 and L_2 . The Schiff bases and metal complexes have been characterized by spectral and analytical techniques. Spectral and magnetic susceptibility measurements revealed that all the complexes exhibit square planar geometry. The binding of the complexes with CT-DNA were studied by UV-Vis absorption, fluorescence spectroscopy and viscosity measurements. It was found that binding with CT-DNA is by intercalative mode. The cleaving tendency of the complexes was tested with pBR322 DNA in the presence of H_2O_2 and UV light, which revealed that the complexes effectively cleave the DNA. Among all the complexes, Cu(II) complex (1) showed better cleavage

activity. Further, antibacterial activity of the synthesized compounds were studied and the metal complexes have shown promising more activity than the corresponding Schiff bases.

Supplementary Information (SI)

Spectral (1H -NMR, IR, SEM and Mass), Fluorescence spectra of EB bound to CT DNA (Figure S5), viscosity (Figure S6 for 4, 5 and 6) and DNA cleavage (Figure S7 for complexes 4, 5 and 6) are given as Supplementary information, which is available at www.ias.ac.in/chemsci.

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