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Cobalt(II), Nickel(II) and Copper(II) complexes of a tetradentate Schiff base as photosensitizers: Quantum yield of $^1O_2$ generation and its promising role in anti-tumor activity

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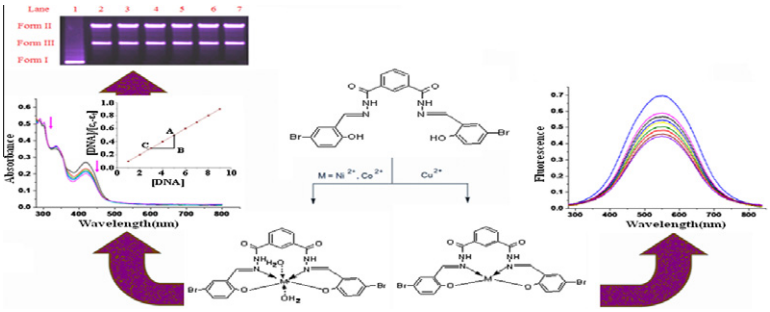
HIGHLIGHTS

- We have synthesized biologically active M(II) (where M=Co, Ni, Cu) complexes.
- The DNA-binding modes of M(II) complexes were studied by different techniques.
- The DNA-photocleavage abilities of M(II) complexes were studied on pUC 19 DNA.
- The quantum yields of $^1O_2$ generation of M(II) complexes were determined.
- The cytotoxicity of M(II) complexes were performed on A549 lung cancer cell lines.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present investigation, a Schiff base N'^1,N'^3-bis[(*E*)-(5-bromo-2-hydroxyphenyl)methylidene]benzene-1,3-dicarbohydrazide and its metal complexes have been synthesized and characterized. The DNA-binding studies were performed using absorption spectroscopy, emission spectra, viscosity measurements and thermal denatuaration studies. The experimental evidence indicated that, the Co(II), Ni(II) and Cu(II) complexes interact with calf thymus DNA through intercalation with an intrinsic binding constant K_b of $2.6 \times 10^4\ M^{-1}$, $5.7 \times 10^4\ M^{-1}$ and $4.5 \times 10^4\ M^{-1}$, respectively and they exhibited potent photodamage abilities on pUC19 DNA, through singlet oxygen generation with quantum yields of 0.32, 0.27 and 0.30 respectively. The cytotoxic activity of the complexes resulted that they act as a potent photosensitizers for photochemical reactions.

Introduction

Photosensitizers (PSs) are a group of compounds which performs an vital role in photodynamic therapy (PDT). The activation of a photosensitizer compound (PS) by radiation of appropriate wavelength has been applied in PDT to treat diseases characterized by uncontrolled cell growth, such as cancer [1]. A huge number of photosensitizers together with their generating mechanisms of singlet oxygen have been discussed in detail [2–4]. Among these studies, the types of photosensitizers generating singlet oxygen frequently concentrate on the organic molecules, such as organic dyes, porphy-

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rins, phthalocyanines, and some macrocyclic systems etc. However, many of the studied PSs are unstable against light, which alters their capability to absorb light during illumination, resulting in a decreased efficiency of production of excited molecules [5].

Success of photofrin as an efficient photosensitizer agent has drawn the concentration of many bioinorganic chemists towards developing other active non-porphyrin transition metal complexes with superior efficiency [6–10]. Under the irradiation of suitable light, these metal complexes will be excited and, further, produce reactive oxygen species in the presence of molecular oxygen, and display potential utility on PDT. Recently, many of transition metal complexes have been found to be more efficient as the singlet oxygen producers than the well studied organic systems. For example, some complexes of ruthenium(II) and platinum(II) can be the proficient photosensitizers to induce singlet oxygen [11,12]. The interaction of Schiff base metal complexes with DNA has been widely studied in the past decades. Due to the site specific binding properties and many fold applications in cancer therapy, these coordination compounds were suitable candidates as DNA secondary structure probes, photo cleavers, and antitumor drugs [13–15]. In addition to this, several metal complexes of Schiff bases derived from salicylaldehyde and amino acid [16–22] and reduced salicylidene amino acid [23–25] were reported and some of them have been confirmed to be efficient DNA cleavers [26–28] and as novel tumor chemotherapeutic and tumor radio imaging agents [29].

In view of the diversified roles of Schiff base metal complexes, we synthesized a Schiff base ligand N'^1,N'^3-bis[(*E*)-(5-bromo-2-hydroxyphenyl)methylidene]benzene-1,3-dicarbohydrazide (L_1) and its Co(II), Ni(II) and Cu(II) complexes and the structures were elucidated by physico-chemical methods. Their DNA binding, photosensitizing ability and antitumor activity were also described.

Experimental

Materials

All reagents and solvents required were of AR grade, purchased commercially. All the solvents were purified by distillation and $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, and Tris–HCl were purchased from Merck (India), calf thymus (ds)DNA and super coiled (SC) pUC19DNA were purchased from Bangalore Genie (India), Agarose (molecular biology grade) and ethidium bromide were purchased from Himedia. Tris–HCl buffer solution used for binding and cleavage studies was prepared using deionised double distilled water.

Physical measurements

The melting points are determined by open capillary methods and are uncorrected. The UV–Visible spectra are recorded on a Shimadzu model impact 1650 UV–Visible double beam spectrometer. The FT-IR spectra are recorded on a Shimadzu model impact 8400S FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution), ^1H, ^{13}C NMR spectra on a Bruker 400 MHz and mass spectra are recorded on LCMS Shimadzu, Japan 800 MHz spectrometer. Elemental analyses are done on Vario EL.CHNOS elemental analyser. Viscosity measurements are studied by semi micro dilution capillary viscometer (Viscomatic Fica MgW) with a thermostated bath D40S.

Synthesis of Schiff base ligand (L_1)

A solution of benzene-1,3-dicarbohydrazide (0.01 mol) in anhydrous EtOH (50 cm^3) were added drop wise to the ethanolic solution (50 cm^3) of 5-bromo-2-hydroxybenzaldehyde (0.02 mol) in the presence of acetic acid and the mixture was heated under reflux for 5–6 h. The resulting solution was concentrated to half of its initial volume and then cooled to room temperature. The yellow precipitate was separated by filtration, washed with EtOH and dried in a vacuum desiccator. The yield was 76% and mp = 180–182 °C. Anal. (%) Calc. for [$C_{22}H_{16}Br_2N_4O_4$]: C, 47.17; H, 2.88; N, 10.00. Found: C, 47.19; H, 2.87; N, 10.02. LC–MS (m/z) 559 [$C_{22}H_{16}$ $Br_2N_4O_4$—H]$^+$. IR (KBr, cm^{-1}): 3217 (—OH), 1658 (C=O), 1557 (C=N), 3058 (N—H), 1270 (N—N); ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 11.20 (s, O—H, 2H), 12.30 (s, N—H, 2H), 9.54 (s, —CH=N), 6.8–8.65 (m, Ar-10H). UV–Visible in DMF [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 360 (798), 310 (7522), 298 (7800).

Synthesis of Co(II), Ni(II) and Cu(II) complexes

A ethanolic solution of ligand (0.01 mol) and respective metal chloride salt (0.01 mol) was refluxed for 2–3 h under nitrogen. The complex was precipitated by adding distilled water. The complex separated was filtered, washed with water, then with hot alcohol and finally dried in vacuum desiccator over P_2O_5 (yield 60–70%).

Co(II) complex

Yield: 70%; M.P.: > 300 °C; Anal. (%) Calc. for [$C_{22}H_{18}Br_2CoN_4O_6$]: C, 40.46; H, 2.78; N, 8.58. Found: C, 40.44; H, 2.80; N, 8.55; LC–MS (m/z) 652 [$C_{22}H_{18}Br_2CoN_4O_6$—H]$^+$. IR (KBr, cm^{-1}): 3402 (OH lattice H_2O), 1658 (C=O), 1608 (C=N), 540 (M—O), 425 (M—N); UV–Visible in DMF [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 440 (2540), 334 (5614), 302 (7450), 288 (8350).

Ni(II) complex

Yield:68%; M.P.: > 300 °C; Anal. (%) Calc. for [$C_{22}H_{18}Br_2NiN_4O_6$]: C, 40.47; H, 2.78; N, 8.58. Found: C, 40.45; H, 2.79; N, 8.57; LC–MS (m/z) 651 [$C_{22}H_{18}Br_2NiN_4O_6$—H]$^+$. IR (KBr, cm^{-1}): 3425 (OH lattice H_2O), 1658 (C=O), 1609 (C=N), 545 (M—O), 437 (M—N); UV–Visible in DMF [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 430 (2690), 335 (4630), 302 (6266).

Cu(II) complex

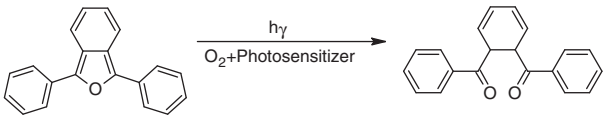
Yield: 65%; M.P.: > 300 °C; Anal. (%) Calc. for [$C_{22}H_{14}Br_2CuN_4O_4$]: C, 42.50; H, 2.27; N, 9.01. Found: C, 42.48; H, 2.25; N, 8.98; LC–MS (m/z) 620 [$C_{22}H_{14}Br_2CuN_4O_4$—H]$^+$. IR (KBr, cm^{-1}): 1658 (C=O), 1614 (C=N), 548 (M—O), 440 (M—N); UV–Visible in DMF [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 407 (6032), 336 (6560), 321 (6520), 303 (6380).

DNA-binding experiments

Electronic absorption spectroscopy has been widely employed to determine the binding characteristics of metal complexes with DNA. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [30]. Relative binding of the complex to CT DNA was studied by fluorescence spectrometry using ethidium bromide (EB) bound CT DNA solution in Tris–HCl/NaCl buffer (pH 7.2) at room temperature [31].

Viscosity measurements were carried out using a semi micro dilution capillary viscometer at room temperature. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Data were presented as (η/η_o) vs [complex]/[DNA], where η is the viscosity of DNA in the presence of the complex and η_0 is that of DNA alone [32,33].

Thermal denaturation experiments were carried out with a Shimadzu Model UV-160A spectrophotometer coupled to a temperature controller (Model TCC-240A) by monitoring the absorption at 260 nm of CT-DNA at various temperatures [34,35].



Scheme 1. The reaction of DPBF with singlet oxygen.

DNA cleavage experiments

For the gel electrophoresis experiments, supercoiled pUC19 DNA was treated with Co(II), Ni(II) and Cu(II) complexes in tris buffer (50 mM tris–acetate, 18 mM NaCl, pH 7.2), and the solution was irradiated at room temperature with a UV lamp (365 nm, 10 W) after being incubated at 37 °C for 1 h. Electrophoresis was carried out at 50 V for 2 h in tris–borate EDTA (TBE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using UVITEC Gel Documentation System [36,37].

Quantum yield of $^1O_2$ generation

The reaction of $^1O_2$ with 1,3-diphenylisobenzofuran (DPBF), (Scheme 1) was adopted to evaluate the quantum yield of $^1O_2$ generation by Co(II), Ni(II) and Cu(II) complexes. A sequence of 2 mL of air-saturated DMSO solutions containing DPBF (20 μM) and complexes, of which the absorbance at 440, 430 and 407 nm originating from the absorption of Co(II), Ni(II) and Cu(II) complexes was adjusted to the same (OD_{440} nm, OD_{430} nm and OD_{407} nm = 0.25), were separately charged into an opened 1 cm path quartz cuvette and illuminated with light of 440 nm, 430 nm and 407 nm (obtained from a Shimadzu model impact 1650 UV–Visible double beam spectrophotometer, 2.0 nm of slit width). The consumptions of DPBF were followed by monitoring its loss of absorbance at 417 nm (λ of irradiation = 440, 430 and 407 nm) at different irradiation time. $[Ru(bpy)_3]^{2+}$ was used as standard, whose $^1O_2$ generation quantum yield was determined to be 0.81 in air saturated methanol [38].

Antitumor activity

Human lung cancer cell line A549 was cultured in Dulbecco's modified Eagle's medium (Thermo Scientific HyClone, Logan, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) in 5% CO_2 atmosphere at 37 °C, respectively. For cell viability assay, the cancer cells were plated at a density of 104 cells/ well in 96-well plates and incubated for 24 h. The medium was removed, and complex solutions were added into a new medium without FBS to the required final concentrations between 5 μM and 200 μM. The untreated A549 cells were used as a control.

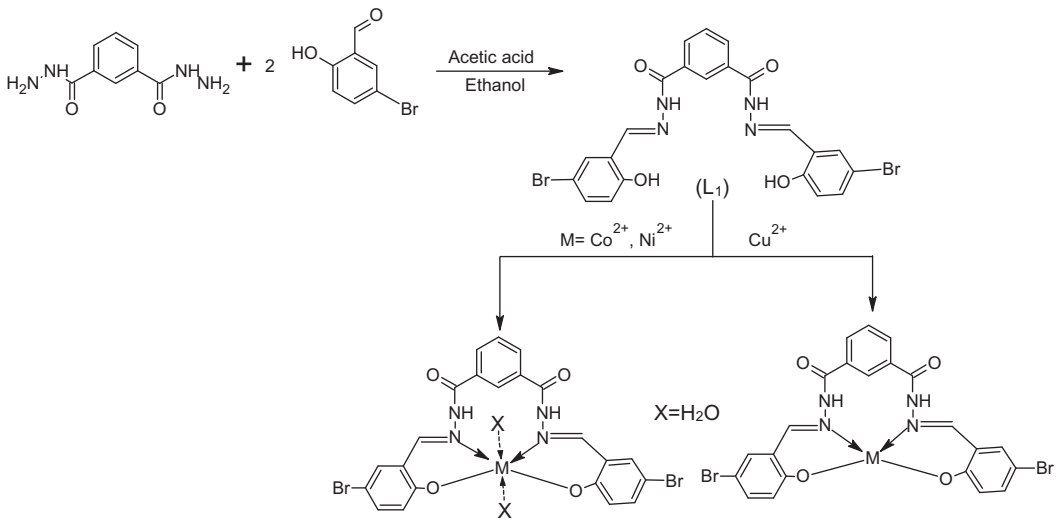
After 72 h of incubation, 10 μl of a stock MTT solution was added to give a final concentration of 0.5 mg/ml and incubated for a further 4 h. Then, the medium was replaced with 100 μl of pure dimethyl sulfoxide and the absorbance of the dark blue formazan was measured with an ELISA plate reader at 550 nm. Cell viability = $(A_{sample}/A_{control}) \times 100\%$. After 72 h of incubation with complexes, 30 μl of washed cell suspension was mixed thoroughly with 30 μl of trypan blue solution (0.4%) and allowed to stand for 3 min at room temperature. The total number of cells and the number of blue-stained cells (dead cells) were counted using a microscope [39].

Results and discussion

One pot synthesis of the Schiff base ligand N'^1,N'^3-bis[(*E*)-(5-bromo-2-hydroxyphenyl)methylidene]benzene-1,3-dicarbohydrazide (L_1) (Scheme 2) and its metal(II) complexes were prepared by direct reaction of metal chlorides and the ligand in 1:1 M ratio (metal to ligand), using methanol as the reaction medium. The complexes are stable in both air and light. They are soluble in DMF, DMSO and buffer solution. The conductivity measurement in DMF at 10^{-3} M concentration gave a Λ_o value in the range of 65–70 ohm^{-1} mol^{-1} cm^{-1} at 300 k, suggesting that the complexes are non-electrolytic in nature [40].

Spectroscopic studies

It is well established in the IR spectral studies that the Schiff base having o-hydroxy group either on aldehyde or on amine residue can form intramolecular hydrogen bond. This has direct impact on the υ(OH) vibration, and shifts to the lower frequency with broadening. The extent of shift depends on the strength of hydrogen bonding observed [41]. The observed bands at 3217 cm^{-1}, a strong band at 1557, and 1658 cm^{-1} in the IR spectrum of the Schiff base are assigned to H-bonded —OH stretching, υ(C=N) of azomethine and carbonyl υ(C=O) vibrations, respectively. An intense band at 3058 cm^{-1} is due to the



Scheme 2. Synthesis of Co(II), Ni(II) and Cu(II) complexes.

—NH— vibrations of the hydrazine group, the band at 1270 cm^{-1} is assigned to hydrazinic υ(N—N) of the free ligand.

For the Co(II), Ni(II) and Cu(II) complexes, the following changes were observed; the high intense band due to phenolic —OH appeared in the region at 3217 cm^{-1} in the Schiff base was disappeared in the complexes. These observations support the formation of M—O bonds via deprotonation. So, the H-bonded —OH groups have been replaced by the metal ion. The presence of broad stretching vibrations in the 3402–3452 cm^{-1} region can be attributed to coordinated or lattice water molecules in these complexes. The medium intense band at 1608 cm^{-1} due to υ(C=N) indicates that the C=N of the ligand coordinates to the metal through nitrogen. The unaltered position of the υ(C=O) (carbonyl) confirms non-involvement in coordination. The υ(M—O) and υ(M—N) bands have been assigned in the region 540–550 cm^{-1} and 425–450 cm^{-1}, respectively.

The electronic spectra of all the compounds were recorded in DMF solution in the range 200–800 nm. The electronic spectra of the free dihydrazone ligand exhibit characteristic bands at 298 nm (ε_{max}, 7800 M^{-1} cm^{-1}), 310 nm (ε_{max}, 7522 M^{-1} cm^{-1}) and 360 nm (ε_{max}, 798 M^{-1} cm^{-1}). The bands present in the region 298–310 nm are assigned to intraligand π–π^* transition while the band at 360 nm is assigned to the n–π^* transition which is characteristic of azomethine (C=N) function of the Schiff base. The electronic spectra for the complexes show two to four bands in the 300–450 nm region. The ligand bands at 298, 310 and 360 nm show red shift on complexation. The bands appearing in the region 302–340 nm, in the complexes are attributed to intraligand π–π^* transition. On the other hand, the appearance of a new bands with high molar extinction coefficient in the region 440–407 nm for Co(II), Ni(II) and Cu(II) complexes, may be assigned for n–π^* and ligand to metal charge transfer transitions. The red shift of the ligand bands provides good evidence for chelation by ligand L_1 to the metal center. The magnitude of shift of ligand bands on complexation indicates strong bonding between the ligand and the metal center [42,43].

^{1}H NMR spectra

The ^{1}H NMR spectra of the Schiff base ligand L_1 was recorded in DMSO-d_6. The down field shift of the OH proton in the ligand which resonates at 11.2 ppm in its ^{1}H NMR spectrum indicates that the OH proton in ligand is probably involved in the formation of strong intramolecular hydrogen bonding.

The ^{1}H NMR spectrum of the ligand L_1 exhibits NH proton at 12.3 ppm, aromatic ring protons at 6.8–8.65 ppm (each as a doublet). The ^{1}H NMR spectra of the complexes cannot be obtained due to interference in their paramagnetic properties.

DNA binding properties of the complexes

The interactions of Co(II), Ni(II) and Cu(II) complexes in the absence and presence of increasing amount CT-DNA (at a constant concentration of complexes) are given in Figs. 1–3, respectively.

The electronic absorption spectroscopy is one of the most common method to study the DNA-binding properties of M(II) complexes, since there are strong MLCT (metal-to-ligand), LMCT (ligand-to-metal) and IL (intraligand) charge transfer features were observed in the absorption spectra of these complexes. In general, Hypochromism is suggested to occur due to an intercalative mode of binding involving a tough stacking interaction between an aromatic chromophore and the base pairs of DNA [44–46]. Upon addition of CT-DNA, the observed absorption band of Co(II) complex at 288 nm exhibited hypochromism of about 20.8%, without showing any changes in the wavelength, while the LMCT band at 440 nm exhibited hypochromism of about 23.0%, with a slight

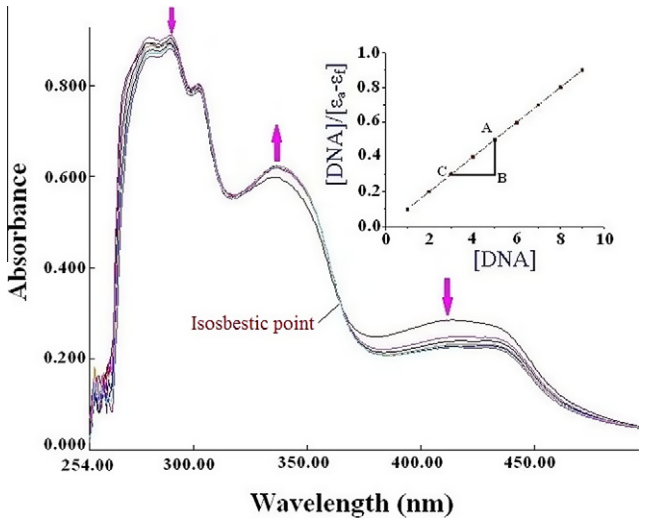


Fig. 1. Absorption spectral traces of $[Co(L_1)(H_2O)_2]$ complex in Tris–HCl buffer (0.01 M, pH 7.2) upon the addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; shows the absorbance changing upon an increase of DNA concentration. Arrow shows the absorbance changing upon the increase of DNA concentration. The inner plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ vs [DNA] for the titration of DNA with Co(II).

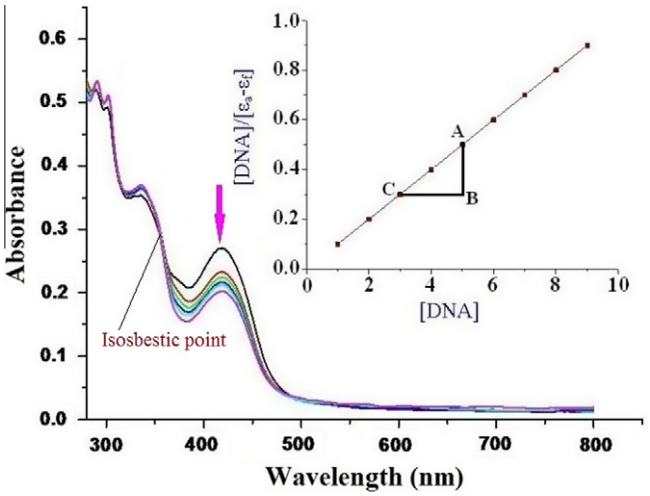


Fig. 2. Absorption spectral traces of $[Ni(L_1)(H_2O)_2]$ complex in Tris–HCl buffer (0.01 M, pH 7.2) upon the addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; shows the absorbance changing upon an increase of DNA concentration. Arrow shows the absorbance changing upon the increase of DNA concentration. The inner plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ vs [DNA] for the titration of DNA with Ni(II).

bathochromism of about 2 nm (Fig. 1). Similarly, Ni(II) and Cu(II) complexes exhibits an evident hypochromism of about 23.3% and 25.1% at 430 and 407 nm, respectively, without any apparent bathochromic shift (Figs. 2 and 3).

The spectral changes are characterized by an isosbestic point maintained throughout the set of experiments. Small changes in λ_{max} and hypochromicities have been observed in all the metal complexes upon interaction with CT-DNA. The isosbestic points was observed at 364 nm for Co(II) complex (Fig. 1), 356 nm for Ni(II) complex (Fig. 2), 451 and 341 nm for Cu(II) complex (Fig. 3), respectively. The appearance of strong hypochromism in the absorption spectra of the complexes is attributable to the interaction between the electronic states of the binding chromophore and that of CT-DNA. The intrinsic binding constant (K_b) for the association of Co(II), Ni(II) and Cu(II) complexes with CT-DNA (insets of resp. Fig.) were found to be 2.6×10^4 M^{-1},

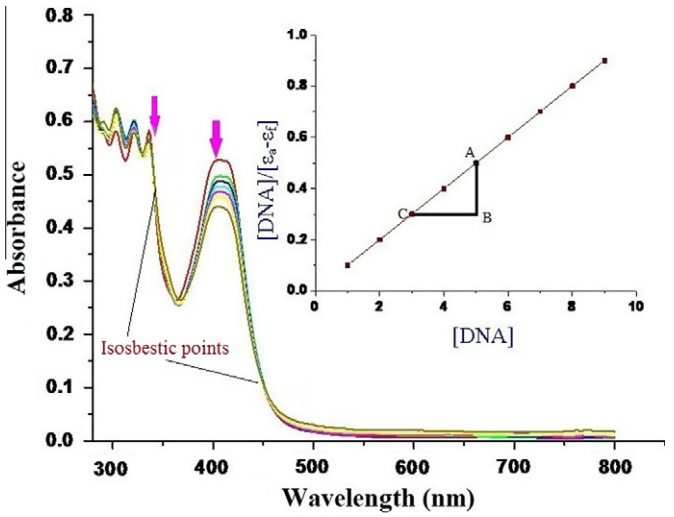


Fig. 3. Absorption spectral traces of [Cu(L_1)] complex in Tris–HCl buffer (0.01 M, pH 7.2) upon the addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; shows the absorbance changing upon an increase of DNA concentration. Arrow shows the absorbance changing upon the increase of DNA concentration. The inner plot of [DNA]/($\varepsilon_a-\varepsilon_f$) vs [DNA] for the titration of DNA with Cu(II).

$5.7 \times 10^4\ M^{-1}$, $4.5 \times 10^4\ M^{-1}$, respectively which are comparable to that observed for typical classical intercalators [44–46], it suggests a mode of intercalative binding that involves a stacking interaction between the complexes and the base pairs of DNA.

Viscosity measurements

Optical photophysical techniques are widely used to study the binding of ligands and their complexes to DNA, but do not give sufficient information to determine a binding model. Therefore, viscosity measurements were taken to further clarify the interaction between metal complexes and DNA. Hydrodynamic measurements that are sensitive to the length change of DNA (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data [47,48]. For the ligand and complexes, as the amount of compound is increased, the viscosity of DNA increases steadily. The values of (η/η_0) were plotted against [compound]/[DNA] (Fig. 4). In classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the ligand, leading to increased DNA viscosity, whereas a partial, non-classical ligand intercalation causes a bend (or kink) in the DNA helix, so reducing its effective length and thereby its viscosity [47,48].

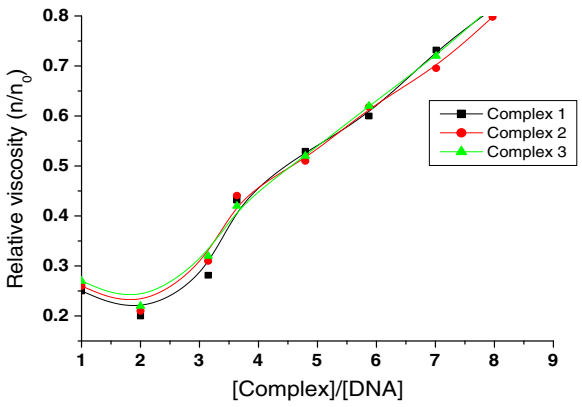


Fig. 4. Effects of increasing amount of Co(II) **1**, Ni(II) **2** and Cu(II) **3** complexes on the relative viscosity of CT-DNA at 25 ± 0.1 °C.

Fluorescence studies

The interactions of Co(II), Ni(II) and Cu(II) complexes with CT-DNA were also monitored via steady-state fluorescence emission spectra, and the results are shown in Fig. 5. It reveals that, upon the addition of CT-DNA, the emission strength of the complexes exhibits obvious reduction [49,50].

The addition of each of the complexes to DNA, causes obvious reduction in emission intensity, and the quenching constants K of all the complexes were calculated according to the classical Stern–Volmer equation [51]:

$$I_0/I = 1 + Kr$$

where I_0 and I are the fluorescence intensities in the absence and presence of the complex, and r is the ratio [M]/[DNA]. K is a linear Stern–Volmer quenching constant. The fluorescence quenching curves of complexes are shown in Fig. 6. The quenching plots illustrate that the binding of DNA by the complexes is in good agreement with the linear Stern–Volmer equation. In the linear fit plot of I_0/I vs [complex], the K values calculated for Co(II), Ni(II) and Cu(II) complexes are 3.4, 3.6 and 4.1, respectively. The above results showed that all the complexes could replace EB from the DNA–EB system, and a complex-DNA system was formed. The reduced emission of the DNA–EB system was caused by EB being expelled from the hydrophobic environment into the water solution [49,50]. Hence, the fluorescence studies indicated that Co(II), Ni(II) and Cu(II) complexes binds to DNA by intercalation.

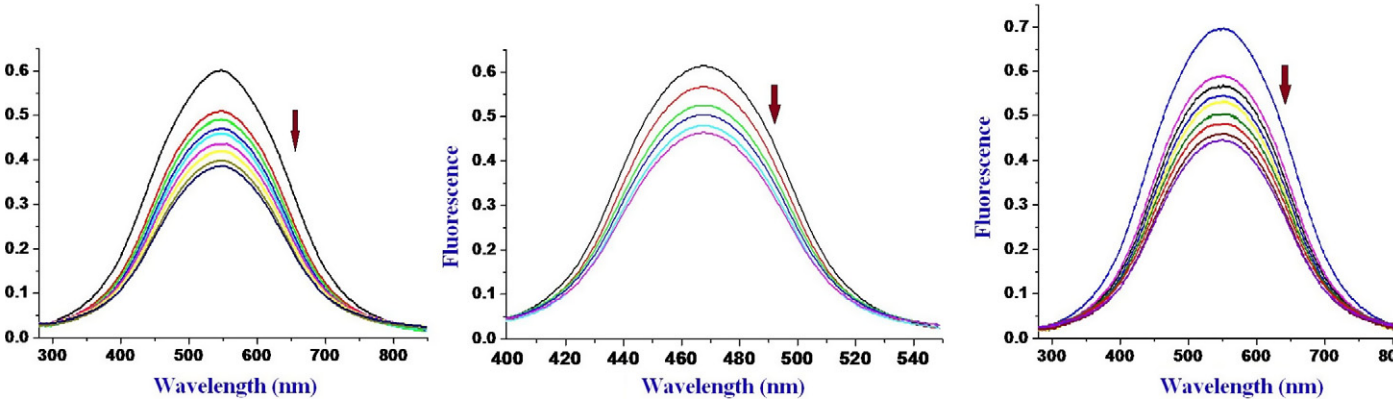


Fig. 5. The emission spectra for Co(II), Ni(II) and Cu(II) metal complexes in the presence and absence of CT-DNA.

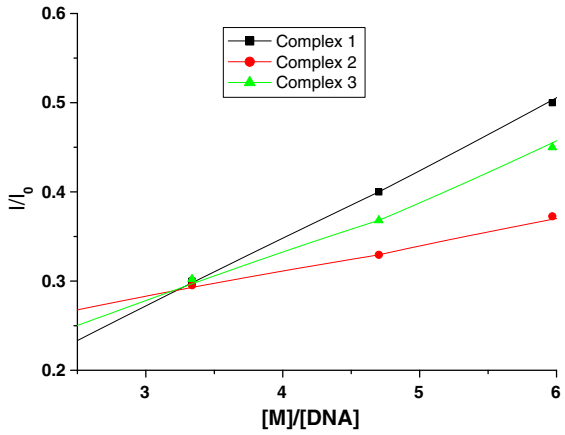


Fig. 6. The fluorescence quenching curves of Co(II) **1,** Ni(II) **2** and Cu(II) **3** complexes in the presence and absence of CT-DNA.

Thermal denaturation studies

The thermal behavior of DNA is a measure of the stability of DNA double helix temperature. An interaction between DNA and complexes were indicated by the increase in the thermal melting temperature (T_m) as shown in Fig. 7. Thermal denaturation experiments also revealed the intercalation of these complexes with DNA. The CT-DNA alone melt at 61 ± 1 °C (10 mmol NaCl, 1 mmol phosphate) in the absence of any added complex. The σ_T values of DNA were also increased by 4 ± 1 °C for these complexes. The increase T_m and σ_T of DNA could be interpreted in terms of the stabilization that results from the intercalation of these metal complexes with DNA. The observations made during the absorption titration, viscosity measurements and thermal denaturation experiments are reminiscent of those reported earlier for various metallointercalators, thus suggesting that the complexes bound to DNA by intercalations [52–55].

DNA photocleavage studies

The ability of the complexes to mediate DNA cleavage was assayed using agarose gel electrophoresis at physiological pH and temperature. When supercoiled circular pUC 19 DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand, the supercoiled form will relax to generate a slower-moving open nicked form (Form II), and if both strands are cleaved,

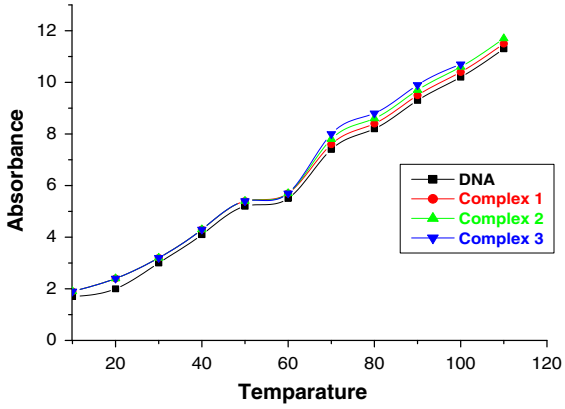


Fig. 7. Melting curves of CT-DNA in the presence and absence of Co(II) **1,** Ni(II) **2** and Cu(II) **3** complexes.

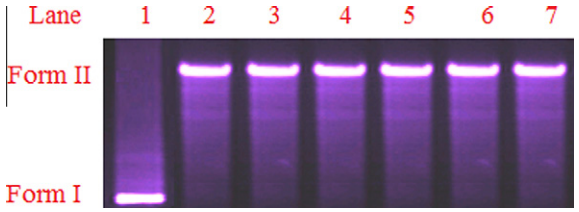


Fig. 8. Photo-induced DNA cleavage by the M(II) complexes. The complexes were irradiated with UV light at 365 nm, Lane; 1: control DNA (with out complexes), Lane; 2: 20 μM Co(II), Lane; 3: 40 μM Co(II), Lane; 4: 20 μM Ni(II), Lane; 5: 40 μM Ni(II), Lane; 6: 20 μM Cu(II), Lane; 7: 40 μM Cu(II).

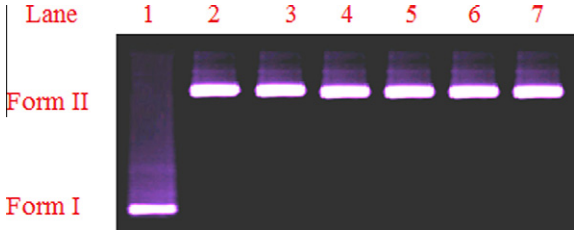


Fig. 9. DNA cleavage by the M(II) complexes. Lane; 1: control DNA (with out complexes), Lane; 1: control DNA (with out complexes), Lane; 2: 60 μM Co(II), Lane; 3: 80 μM Co(II), Lane; 4: 60 μM Ni(II), Lane; 5: 80 μM Ni(II), Lane; 6: 60 μM Cu(II), Lane; 7: 80 μM Cu(II).

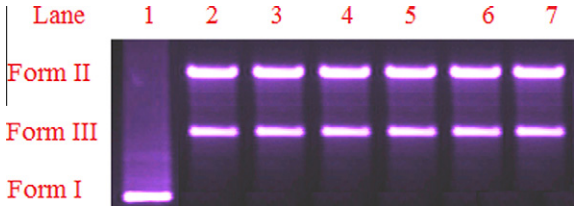


Fig. 10. Light-induced DNA cleavage by the M(II) complexes. Supercoiled DNA runs at position I (SC), nicked DNA at position II (NC) and Linear DNA at position III (LC). Lane; 1: control DNA (with out complexes), Lane; 2: 100 μM Co(II), Lane; 3: 120 μM Co(II), Lane; 4: 100 μM Ni(II), Lane; 5: 120 μM Ni(II), Lane; 6: 100 μM Cu(II), Lane; 7: 120 μM Cu(II).

a linear form (Form III) that migrates between Form I and Form II will be observed. Figs. 8–10 show the gel electrophoretic separations of pUC 19 DNA induced by Co(II), Ni(II) and Cu(II) complexes. All three complexes promote photocleavage of DNA under physiological conditions (pH 7.2, 37 °C), but with similar cleavage activities. As shown in Figs. 8–10 the DNA cleavage activities of the complexes are obviously concentration-dependent. With the increase of complex concentration, the supercoiled DNA decreases and nicked circular DNA gradually increases. It was found that Co(II), Ni(II) and Cu(II) complexes were the most potent nuclease mimic in terms of molecular structure. Chemical environment and their geometric structures may also affect the nucleolytic efficiency of these complexes. The different DNA-cleavage efficiency of the complexes may be considered due to the different binding affinity to DNA [36,37,56].

Quantum yield of $^1O_2$ generation

Numerous organic compounds can react with reactive oxygen species such as $^1O_2$, leading to the changes of absorbance and/or fluorescence intensity, and consequently can be utilized to

quantitatively evaluate the $^1O_2$ generation quantum yields of photosensitizers by simply monitoring the fluorescence or UV–Visible spectrum [57]. 1,3-Diphenylisobenzofuran (DPBF, Scheme 1) with a reported β value of about 10^{-4} is presently one of the most reactive with $^1O_2$. The consumptions of DPBF were followed by monitoring its loss of absorbance at 417 nm and be articulated as a function of photosensitizer's $^1O_2$ generation quantum yield (Φ_Δ) according to the literature method [58,59]. Using $[Ru(bpy)_3]^{2+}$ as standard ($\Phi_{\Delta s} = 0.81$), the Φ_Δ of Co(II), Ni(II) and Cu(II) complexes in DMSO were determined to be 0.32, 0.27 and 0.30 respectively (Fig. 11), implying their prospective application in $^1O_2$-involving processes, such as photocleavage of DNA.

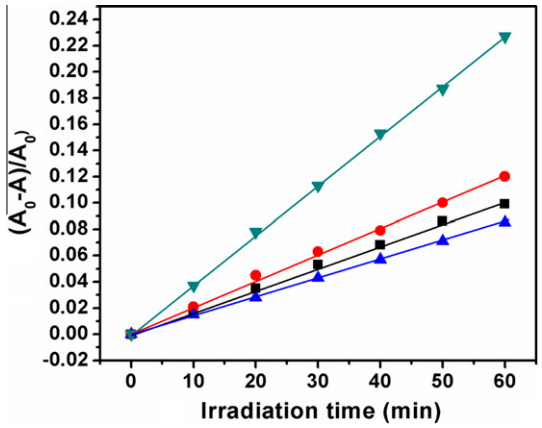


Fig. 11. The DPBF consumption percentage as a function of irradiation time in the air-equilibrated DMSO solution of Co(II) (●), Ni(II) (◄) and Cu(II) (■). $[Ru(bpy)_3]^{2+}$ (►) as standard ($\Phi_{\Delta s} = 0.81$) in air equilibrated CH_3OH.

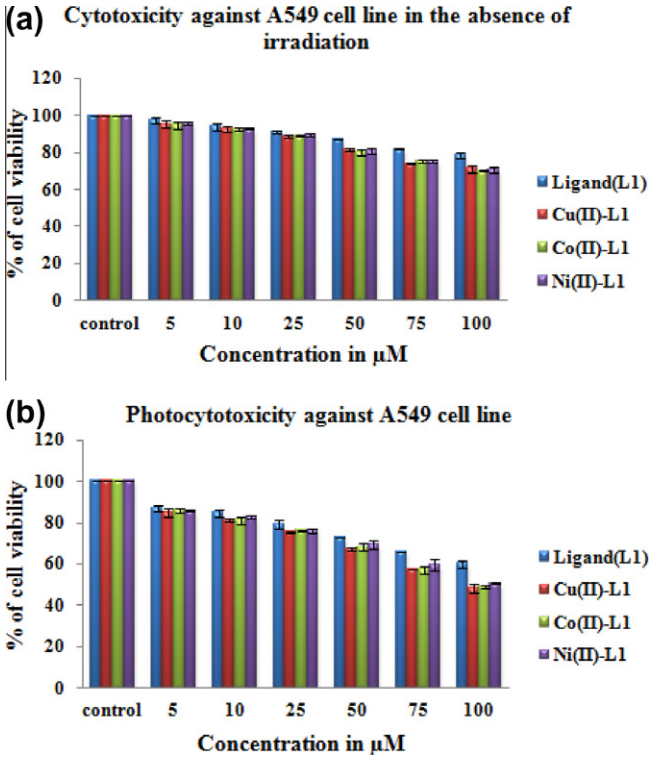


Fig. 12. (a) Cytotoxicity of Co(II), Ni(II) and Cu(II) complexes to A549 cells in the absence of irradiation. (b) Cytotoxicity of Co(II), Ni(II) and Cu(II) complexes to A549 cells in the presence of irradiation.

Anti-tumor study on A549 (lung carcinoma) cell

The cytotoxicity of Co(II), Ni(II) and Cu(II) complexes to human lung tumor cells of A549 was measured by MTT reduction assay [60]. The metabolic activity of the cells was assessed by their ability to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue water-insoluble formazan crystal. Fig. 12 shows the results of the cell viability experiments intended at determining the cytotoxicity of Co(II), Ni(II) and Cu(II) complexes. Fig. 12a showed dose dependent, dark cytotoxicity of all the three complexes to human lung carcinoma cells of A549 and Fig. 12b showed significant dose dependent, photocytotoxicity to human lung carcinoma cells of A549.

In the present investigation, we supplementary employed trypan blue exclusion assay to compute the cytotoxicity of Co(II), Ni(II) and Cu(II) complexes to human lung tumor cells of A549. Viable cells characterized by a structurally vital cell membrane do not uptake trypan blue dye. In contrast, dead cells (necrotic or late apoptotic cells) characterized by the failure of integrity of their membrane are stained blue by the dye. At all concentration, in the absence of irradiation the Co(II), Ni(II) and Cu(II) complexes moderately reduced the viable A549 cells after staining with trypan blue at 72 h (effects on cell proliferation), which was slightly more at higher concentrations (50–100 μM) (Fig. 12a). On the other hand, under similar experimental conditions but with irradiation, the complexes show enhanced cytotoxicity and reduced maximum number of viable A549 cells at both lower and higher concentrations (Fig. 12b). These results further sustaining the conclusion reached by MTT reduction assay that these complexes show considerable dose dependent cytotoxicity to A549 cells in presence of light irradiation through singlet oxygen generation.

Conclusion

In summary, three novel M(II) complexes of Schiff base ligand N'^1,N'^3-bis[(*E*)-(5-bromo-2-hydroxyphenyl)methylidene]benzene-1,3-dicarbohydrazide have been synthesized and structurally characterized. The DNA binding abilities resulted that the complexes bind to CT-DNA by an intercalative mode. When irradiated by UV–Visible light (at 365 nm) the complexes are found to be efficient photocleaving agents of DNA. The mechanism studies reveal that singlet oxygen ($^1O_2$) play an important role in the DNA photocleavage. Hence, these complexes may be useful as potential PDT agents in photochemical therapy.

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