Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 138 (2015) 780–788

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Synthesis, DNA interactions and antibacterial PDT of Cu(II) complexes of phenanthroline based photosensitizers via singlet oxygen generation

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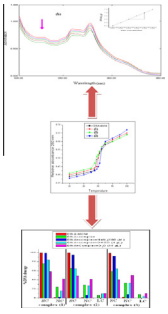
HIGHLIGHTS

- Synthesis of biologically active Cu(II) mixed ligand metal complexes and their characterization.
- All the complexes exhibited efficient DNA groove-binding propensity.
- The complexes were able to cleave pUC19 DNA effectively via singlet oxygen generation.
- Screening of newly synthesized complexes as an antibacterial photosensitizer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 July 2014
Received in revised form 8 November 2014
Accepted 20 November 2014
Available online 11 December 2014

Keywords:
Groove-binding
Metal complexes
Cleavage
Photonuclease activity
PACT assay

ABSTRACT

Cu(II) complexes [Cu(mqt)(B)H_2O]ClO_4(**1**–**3**) of 2-thiol 4-methylquinoline and phenanthroline bases (B), viz 1,10-phenanthroline (phen in **1**), Dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq in **2**) and Dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz in **3**) have been prepared and characterized by elemental analysis, IR, UV–Vis, magnetic moment values, EPR spectra and conductivity measurements. The spectral data reveal that all the complexes exhibit square-pyramidal geometry. The DNA-binding behaviors of the three complexes were investigated by absorption spectra, viscosity measurements and thermal denaturation studies. The DNA binding constants for complexes (**1**), (**2**) and (**3**) were determined to 2.2×10^3, 1.3×10^4 and 8.6×10^4 M^{-1} respectively. The experimental results suggest that these complexes interact with DNA through groove-binding mode. The photo induced cleavage studies shows that the complexes possess photonuclease property against pUC19 DNA under UV–Visible irradiation via a mechanistic pathway involving formation of singlet oxygen as the reactive species. Antimicrobial photodynamic therapy was studied using photodynamic antimicrobial chemotherapy (PACT) assay against *Escherichia coli* and all complexes exhibited significant reduction in bacterial growth on photoirradiation.

Introduction

Exploration of the interaction between transition metal complexes and DNA has fascinated many benefits due to their significance in cancer therapy and molecular biology [1–4]. In recent years, there is a growing importance in the design of metal complexes that DNA on photoirradiation for their potential use in PDT because of the noninvasive nature of this therapeutic process requiring photoactivation of the drug at the tumor cells, leaving other healthy cells unexposed to light [5–7]. Hence, to a great extent attention has been targeted on the design of novel metal-based complexes which can bind and cleave DNA.

The universal rise in the rates of antibiotic resistance of bacteria emphasizes the need for alternate antibacterial agents. A promising approach to destroy antibiotic-resistant bacteria uses light in

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http://dx.doi.org/10.1016/j.saa.2014.11.085
1386-1425/

combination with a photosensitizer to persuade a phototoxic reaction. This photodynamic approach could be a possible alternative to general medications in treating localized infections, thus avoiding the development of microbial resistance to general drugs. Easily reachable oral or skin infections could be good candidates for PACT treatment [8–12].

Photosensitizing uniqueness has become predominantly relevant due to their potential applications in photodynamic antimicrobial chemotherapy (PACT), which takes up photosensitizers and visible or ultraviolet light. The basic principles are the interaction between light and photoactive drugs, forms reactive oxygen species (ROS) produced through either electron transfer (type I) or energy transfer (type II) reactions [13]. These ROS will react with many cellular components that will persuade oxidative processes leading to cell death [14–16]. Copper is a bioessential element in all living systems and has been found to be involved in mixed ligand complex formation in a number of biological processes [17]. Copper complexes containing polypyridine ligands and their derivatives are of great significance since they exhibit various biological activities such as antitumor [18], anti-candida [19], antibacterial [20] and antimicrobial [21,22] activities, etc. The presence of both the copper(II) center and photoactive ligand has been found to be essential for light induced DNA cleavage activity [23,24]. Complexes with ligands containing nitrogenated aromatic rings have deserved an immense importance since the complex with 1,10-phenanthroline proved its ability to break DNA chains [25–28]. Dhar et al. reported [29,30] that the sulfur-containing ligands act as photosensitizers and the photosensitizing effect is greater when the sulfur is bound to the metal ion. The literature reveals to thio or thione moieties are known to show efficient intersystem crossing to the triplet state on photo-irradiation [31–33].

So, we aimed to synthesis engineered bichromophoric photosensitizers (photoactive drugs) competent to mediate intramolecular energy and/or charge transfer through DNA double helix which results in improved efficiency toward bacterial cells.

Herein, we present the synthesis, DNA binding and photoinduced DNA cleavage activity of [Cu(mqt)(B)H_2O] (**1**)–(**3**), where 2-thiol 4-methylquinoline (mqt) acts as a photosensitizer and planar phenanthroline bases (B), *viz*. 1,10-phenanthroline (phen in **1**), dipyridoquinoxaline (dpq in **2**) and dipyridophenazine (dppz in **3**) are DNA binders. In this study, [Cu(mqt)$(B)_2H_2O$] has emerged to be a promising molecule as a photoactive component for PACT.

Material and methods

Chemicals and instrumentations

All chemicals used for the synthesis were of analytical grade and procured from HiMedia Laboratories Pvt., Ltd. All the solvents were purified by distillation and used. The $Cu(ClO_4)_2{\cdot}6H_2O$ was purchased from Merck (India) and used as received without further purification. D_2O purchased by Sigma Aldrich. Calf thymus (ds)DNA and Super coiled (SC) pUC19 DNA were purchased from Bangalore Genie (India), Agarose (molecular biology grade) ethidium bromide were purchased from Himedia (India). Tris–HCl buffer solution used for binding was prepared using deionised double distilled water. Tris–borate ethylenediaminetetraacetate (TBE) electrolyte buffer used for DNA cleavage study. Elemental analysis was done on Perkin-Elmer Model 240-C CHN analyzer. Conductivity measurements were determined in DMF (10^{-3} M) using an Equip-Tronic Digital conductivity meter model No. EQ-660A. Magnetic measurements were carried out by the Gouy method at room temperature (28 ± 2 °C) using Hg[$Co(SCN)_4$] as calibrant. The electronic spectra of the complexes were measured using Shimadzu spectrometer model UV-1650 PC double beam spectrophotometer. IR spectra were recorded in 4000–250 cm^{-1} region using KBr pellets on Shimadzu (Kyoto, Japan) FTIR-8400S spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker FT NMR spectrometer (300 MHz) at 25 °C in DMSO-d_6 with TMS as the internal reference. Electron paramagnetic resonance (EPR) spectra was measured using Bruker BioSpin GmbH.

Viscosity measurements were carried out on semimicro dilution capillary viscometer. Thermal denaturation studies were carried out with a Perkin–Elmer model 554 with a Shimazdu UV–Vis recording spectrophotometer coupled to a temperature controller (Model TCC-240A) using quartz cuvettes of 10 mm light-path. A monochromatic 12 W UV lamp of 365 nm was used as light source for photonuclease activity.

Synthesis and characterization

Synthesis of ligand (methylquinoline-2-thiol)

The starting compounds 2-hydroxy-4-methylquinoline and 2-chloro-4-methylquinoline were synthesized according to the method reported earlier [34]. Ethanolic solution of above synthesized 2-chloro-4-methyl quinoline was mixed with sodium sulfide in 1:3 ratios and refluxed for about 4 h in the presence of catalytic amount of HCl. The completion of the reaction was monitored by TLC eluting the phase ethyl acetate: carbon tetrachloride (8:2). The reaction mixture was poured into ice cold water and neutralized; finally product was filtered and recrystallized.

Anal. Calc. for $C_{10}H_9NS$: C, 68.51; H, 5.20; N, 7.99. Found: C, 68.57; H, 5.12; N, 8.01; UV–Visible. λ_{max} (nm): 281, 380, IR (KBr) cm^{-1}: 1653 ν(C=N), 2480 ν(C–SH); ^{1}H NMR (δ ppm): 7.1–8.09 (m, ArH, 5H), 2.4 (s, 3H, CH_3), 13.4 (s, SH), M.W. 175.26.

Synthesis of ligands (dpq and dppz)

The dpq ligand (dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline) and dppz ligand dipyrido[3,2-*a*:2′,3′-*c*] phenazine were prepared by reported procedure [35,36].

*Synthesis of complexes [Cu(mqt)(B)H_2O]ClO_4 (**1**)–(**3**)*

mqt: 2-thiol 4-methylquinoline and B = phen, **1**; dpq, **2**, dppz,**3**.

All the present complexes were synthesized by following the same general procedure. Mixture of methanolic solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (1 mmol, 0.37 g) and solution of the mqt (1 mmol, 0.175 g) was stirred for 30 min. To the above reaction mixture, 1 mmol of methanolic solution (10 ml) of B was added and the stirring was continued for 30 min more. Then obtained precipitate was separated by filtration.

*[Cu(mqt)(phen)H_2O]ClO_4 (**1**).* Yield 76%, C, Anal. Calc. for $C_{22}H_{18}ClCuN_3O_5S$ (%): 49.35; H, 3.39; N, 7.85. Found (%): C, 49.37; H, 3.40; N, 7.83. IR (KBr) cm^{-1}: 3456 br, 3050m, 2925s, 1600s, 1091 vs (ClO_4), 1633 vs 993m, 755s, 485w, 386m (br, broad; m, medium; s, strong; vs very strong; w, weak). UV–Visible. λ_{max} (nm): 276, 320, 400, 520. μ_{eff} = 1.81 B.M. Ω_M = 70 mhos cm^2 mol^{-1}

*[Cu(mqt)(dpq)H_2O]ClO_4 (**2**).* Yield 72%, Anal. Calc. for $C_{24}H_{18}ClCuN_5O_5S$ (%): C, 49.07; H, 3.09; N, 11.92. Found (%): C, 49.02; H, 3.13; N, 11.90. IR (KBr) cm^{-1}: 3489 br, 3052m, 2924s, 1606s, 1084 vs (ClO_4), 1626 vs 989m, 776s, 478m, 392m. UV–Visible. λ_{max} (nm): 265, 370, 452, 602. μ_{eff} = 1.76 B.M. Ω_M = 58 mhos cm^2 mol^{-1}

*[Cu(mqt)(dppz)H_2O]ClO_4 (**3**).* Yield 67%, Anal. Calc. for $C_{28}H_{20}ClCuN_5O_5S$ (%): C, 52.75; H, 3.16; N, 10.98. Found (%): C, 52.69; H, 3.21; N, 10.89. IR (KBr) cm^{-1}: 3471 br, 3052m, 2925s, 1602s, 1087 vs (ClO_4), 1618 vs 989w,738s, 470 w, 398w. UV–Visible. λ_{max} (nm): 279, 356, 450, 628, μ_{eff} = 1.81 B.M. Ω_M = 67 mhos cm^2 mol^{-1}

Solubility and stability

The complexes are soluble in dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and buffer solution. The complexes are sufficiently stable in the solid and solution phases.

Caution! Perchlorate salts are potentially explosive and only small quantity was handled with care.

DNA binding experiments

Absorption spectroscopic studies

DNA binding experiments were executed in Tris–HCl/NaCl buffer (5 mM tris (hydroxymethyl) aminomethane, pH 7.2, 50 mM NaCl) solution of the complexes. The concentration of CT-DNA (in the base pairs) was determined using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}) [37]. The absorbance at 260 nm and 280 nm for CT-DNA was measured in order to check the purity level. The ratio A260/A280 was found to be 1.8–1.9, signifying that CT-DNA was adequately free from protein [38,39].

UV–Visible absorption titration experiments were performed by varying the DNA concentration (0–100 μM) and maintaining the metal-complex concentration constant (0.5 μM). Absorption spectra were recorded after each successive addition of DNA and equilibration for 5 min to allowing the complexes to bind to the CT DNA. For Cu(II) complexes, the observed data were then interpreted by using Eq. (1), to obtain the intrinsic binding constant, K_b:

$$\frac{\text{DNA}}{(\varepsilon_a - \varepsilon_f)} = \frac{\text{DNA}}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad (1)$$

where ε_a, ε_f, and ε_b are the apparent, free and bound metal complex extinction coefficients respectively. A plot of [DNA]/$(\varepsilon_b - \varepsilon_f)$ vs [DNA] gave a slope of $1/(\varepsilon_b - \varepsilon_f)$ and a y intercept equal to $1/K_b$ $(\varepsilon_b - \varepsilon_f)$, where K_b is the ratio of the slope to the y intercept [23].

Viscosity measurements

Viscosity measurements were performed using semi-micro dilution capillary viscometer at room temperature and flow time was measured with a digital stopwatch. Each experiment was carrying out three times and an average flow time was calculated. The data was presented as (η/η_o) vs binding ratio, where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone in 5 mM Tris–HCl buffer medium [40].

Thermal denaturation

Melting studies were carried out by monitoring the absorption intensity of CT DNA (50 μM) at 260 nm various temperatures in the presence (5–10 μM) and absence of each complex. As such, the melting temperature (T_m), at which 50% of double-stranded DNA becomes single-stranded (absorption increase was noticed in the curve width (σ_T) and temperature range between 10% and 90%) occurred and was calculated as reported [41].

DNA photocleavage experiments

The extent of photoinduced cleavage of supercoiled (SC) pUC19 DNA by the complexes was studied by agarose gel electrophoresis. Each pUC19 DNA sample (0.5 μg) in 50 mM Tris–HCl buffer (pH, 7.2; ionic strength, 25 mM) containing 50 mM NaCl in the presence of the complex (50 μM) followed by dilution with Tris–HCl buffer to a total volume of 20 μL. For DNA photo-neuclease activity, the reactions were carried out under illuminated conditions using UV light at 365 nm (12 W). After photoexposure of samples under UV light, these samples were incubated for 1 h at 37 °C followed by addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol and the solution was finally loaded on 0.8% agarose gel containing 1.0 μg/ml ethidium bromide. Electrophoresis was carried out in a dark chamber for 3 h at 50 V in Tris–borate buffer (45 μmol/L Tris–borate, 1 μmol/L EDTA) TBE buffer. Bands were visualized using UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled (SC) DNA to nicked circular (NC) form and linear (LC) form.

The inhibition reactions were carried out in the presence of different additives (NaN_3, 100 μM; DMSO, 4 μL; D_2O 14 μL) at 365 nm for mechanistic insights of the photo-induced DNA cleavage reactions. These reactions were done by adding reagents to SC DNA prior to addition of the complex before photoirradiation [42,43].

Bacterial strains and growth conditions

Escherichia coli ATCC- 25922, was grown aerobically overnight in LB broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl w/v) at 37 °C.

PACT assay

PACT assay was carried out according to the reported procedure elsewhere [44]. Bacterial cultures were diluted in 15 ml of fresh LB broth to obtain a cell density of 10^8 cfu/ml and after the addition of the 25 μM and 50 μM concentrations of the copper complexes under investigation, incubated in foil-covered tubes to avoid accidental exposure to the light. DMSO in low concentrations (the range from 1% to 8%), was necessary for the preparation of aqueous solution of the copper complexes. After incubation in the dark, cells were harvested by centrifugation, washed with 0.1 M phosphate buffer saline (pH 7.4) and resuspended in the same volume of PBS. The flasks were placed under a 500 W halogen–tungsten lamp at a distance of 20 cm. During light exposure, cell suspensions were maintained under gentle magnetic stirring. Control cultures, with no copper complexes added, were exposed to the light for the same period of PS treated cultures. Aliquots of undiluted and serially diluted treated and control cultures were plated on LB agar medium. The percentage of survivors was calculated according to the equation $N_1/N_0 \times 100$, where N_0 represents the number of cfu/ml in samples before the irradiation and N_1 the number of cfu/ml after light exposure.

Results and discussion

Characterization of metal complexes

The elemental analysis data, UV–Visible, IR and magnetic susceptibility of the new complexes are summarized in the experimental section. The formula of the complexes $[Cu(mqt)(phen)(H_2O)]ClO_4$ (**1**), $[Cu(mqt)(dpq)(H_2O)]ClO_4$ (**2**) and $[Cu(mqt)(dppz)(H_2O)]ClO_4$ (**3**), respectively. These new complexes are soluble in DMF, DMSO and in buffer (pH 7.2) solution and stable in the solid as well as in a solution phase. The molar conductance values of the complexes in DMF solutions fall in the range 58–70 ohm^{-1} cm^2 mol^{-1}, demonstrating their electrolytic nature. The complexes are one-electron paramagnetic at room temperature corresponding to d^9 electronic configuration for copper(II) center.

The complexes exhibit a prominent broad *d–d* band in the range of 520–628 nm for complexes (**1**)–(**3**) in DMF. In addition, strong bands in the UV region assigned to ligand to metal charge transfer (LMCT). The electronic spectra of the complexes are in very good agreement with the previously reported square pyramidal geometry of the complexes Cu(II) complexes [45–47]. The dpq and dppz complexes display a moderately strong band near 440 nm possibly due to n–π^* transition involving the quinoxaline or phenazine moieties as this band is not observed in the phen complexes

In IR spectra of the complexes, the characteristic frequencies exhibit significant changes as compared with those of the parent ligand. The spectrum of the ligand mqt shows a strong band at 2480 cm^{-1} assignable to –SH group. The absence of this band in the spectra of the complexes indicating the deprotonation of the –SH group on complexation. The complexes (**1**), (**2**) and (**3**) clearly exhibited strong band ν(C=N) in the range of 1618–1633 cm^{-1} which shows a shifting to the lower frequencies by 20–35 cm^{-1} in compared with ligand and metal chelates also show some new bands in the region 485–479 cm^{-1} and 386–402 cm^{-1} which are due to formation of M–N and M–S bands respectively. All the complexes exhibit a board band at 3400–3500 cm^{-1} attributable to water molecules followed by appearance of rocking ρr (HOH) frequency at 723–790 cm^{-1}, assignable to coordinated water molecules. Besides, complexes are display characteristic infrared band for the perchlorate anion at 1090 cm^{-1}.

Complexes (**1**)–(**3**) are one-electron paramagnetic having μ_{eff} value of ~1.8 BM. The complexes display axial EPR spectra giving $g_{\|}$ and $g_{\perp}$ values of ~2.2 ($A_{\|}$ = 162 G) and ~2.0, respectively. The EPR spectral features are characteristic of an essentially square-pyramidal geometry [48,49].

Based on the analytical data and physicochemical properties, the following structure is proposed for the complexes (See Fig. 1).

DNA binding studies

Absorption spectral features of DNA binding

The mode and propensity of binding of the complexes to calf thymus (CT) DNA are studied by different techniques like absorption spectral technique, DNA thermal denaturation and viscosity measurements.

The absorption spectral technique is used to evaluate the intrinsic binding constants (K_b) of the complexes to CT DNA by monitoring the absorption intensity of the charge transfer spectral band of the complexes near 300 nm attributed to the LMCT transition involving the heterocyclic base and the metal ion with increasing concentration of CT DNA keeping the complex concentration as constant. The extent of the hypochromism gives the strength of the interaction of complex to DNA. On increasing the CT-DNA concentration, the hypochromism is found to increase with a red shift in the UV band of the complexes. All complexes show minor bathochromic shift of the spectral band of ~2–3 nm with significant hypochromisity of 12%, 19.8% and 28.6% for complexes (**1**)–(**3**) respectively (Fig. 2a and b), suggesting mainly groove-binding propensity of the complexes to the double-stranded DNA. The intrinsic binding constant (K_b) values are in the range of 2.2×10^3, 1.3×10^4 and 8.6×10^4 M^{-1} follows the order (**3**) > (**2**) > (**1**). The dpq and dppz complexes prove significantly higher DNA binding propensity in comparison to their phen analogue possibly due to the presence of quinoxaline and phenazine rings of extended planar structure which could undergo π-stacking interactions with the DNA bases [50].

The complex (**3**) having a dppz ligand with its extended fused aromatic rings show significant binding propensity whereas the complex (**1**) which show less binding affinity to the double-stranded CT-DNA. The evaluated K_b values and the results are summarized in the Table 1.

Viscosity measurements

The hydrodynamic measurements that are sensitive to length change (viscosity and sedimentation) are regarded as the least ambiguous and the most critical test of a binding in solution, in the absence of crystallographic structural data [51]. This method is based on the well-known fact that, unwinding and elongation of the DNA double helix occur upon intercalation leads to the significant increase in the viscosity of DNA [52]. In contrast, complexes binding to DNA grooves result in minor variation or no variation in the viscosity of the DNA solution.

The viscosity measurements were carried out on CT-DNA by varying the concentration of added complex. The effect of Cu(II) complexes on the viscosity of DNA is depicted in Fig. 3. A marginal increase of the relative viscosity is observed on the addition of the present complexes to the DNA solution, suggesting the groove-binding nature of the complexes.

Thermal denaturation study

Thermal behavior of DNA in the presence of metal complexes can give insight into their conformational changes when

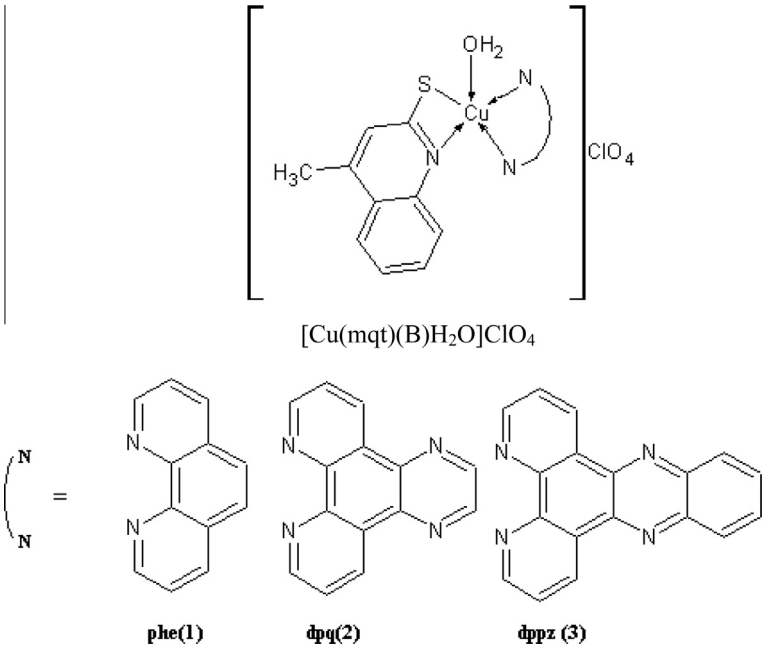


Fig. 1. Structures of Cu(II) complexes.

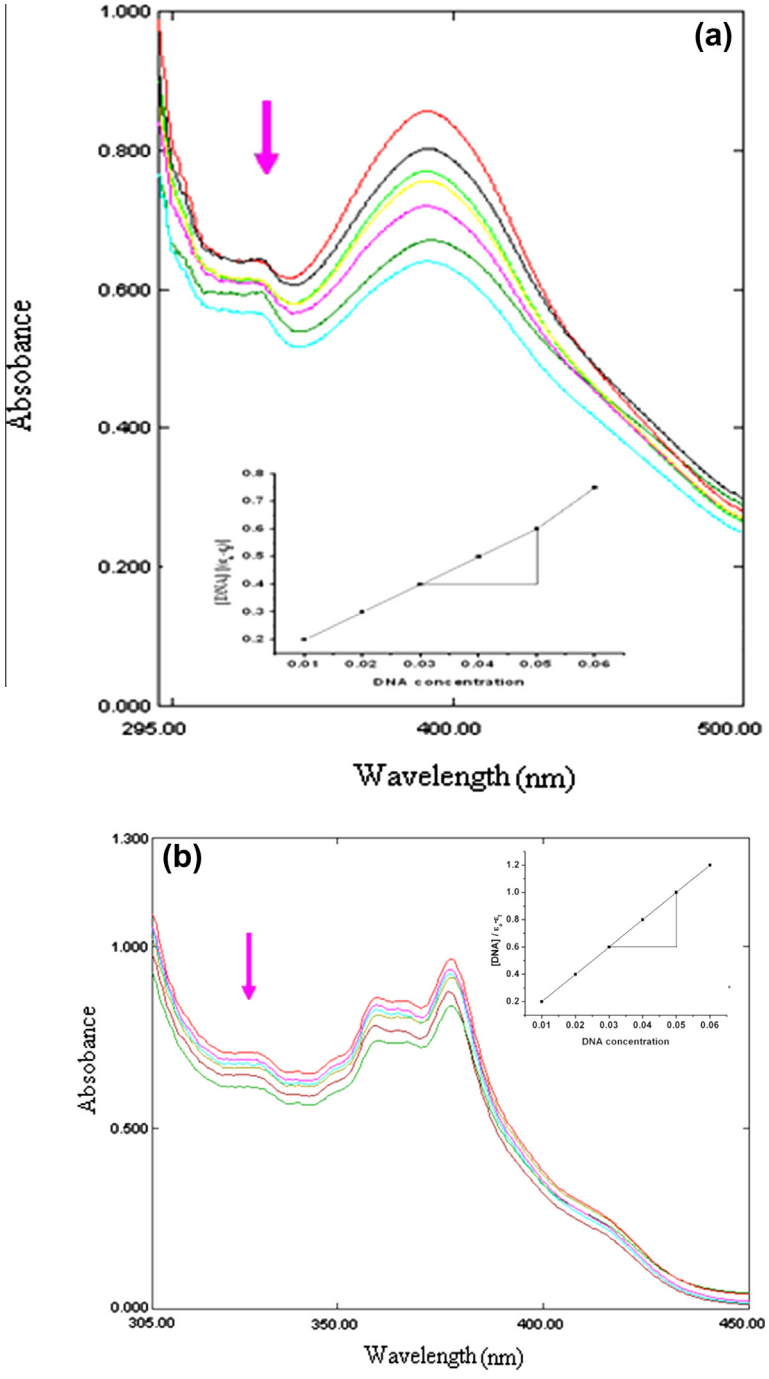


Fig. 2. Absorption spectra of copper complex in Tris–HCl buffer upon addition of DNA. [Complex] = 0.5 μM, [DNA] = (0–100 μM). Arrow shows the absorbance changing upon increase of DNA concentration. The inner plot of $[DNA]/\varepsilon_a - \varepsilon_f$ vs [DNA] for the titration of DNA with complex. (a) Complex (**1**) and (b) complex (**2**).

Table 1
Effect of CT-DNA on absorbance and binding constant of the Cu(II) complexes.

Complex	$\Delta\lambda$ (nm)	H (%)	K_b (M^{-1})
(**1**)	2	12.0	2.2×10^3
(**2**)	2	19.8	1.3×10^4
(**3**)	3	28.6	8.6×10^4

temperature is raised and information about the interaction strength of the complexes with DNA. The double- stranded DNA tends to gradually dissociate to single strands on increase in the solution temperature and generates a hyperchromic effect on the absorption spectra of DNA bases (λ_{max} = 260 nm). In order to identify this transition process, the melting temperature T_m, which is defined as the temperature where half of the total base pairs is bounded, is usually introduced [53].

A moderate positive shift in the DNA melting temperature (ΔT_m) ranging from 1.4 to 3.6 is observed on the addition of the complexes (**1**)–(**3**) to DNA (Fig. 4). The low value of ΔT_m primarily suggest the groove-binding nature of the complexes to DNA in preference over an intercalative mode of binding to DNA that normally gives significantly high positive ΔT_m values [54].

Photo-induced DNA cleavage activity

The cleavage of supercoiled pUC 19 DNA was monitored by gel electrophoresis to examine the capability of the present complexes

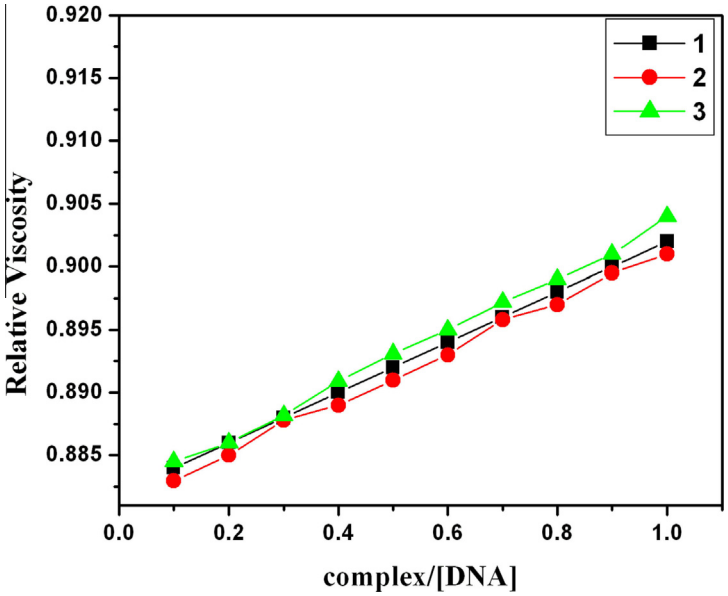


Fig. 3. Plot of relative viscosity vs [Complex]/[DNA]. Effect of Cu(II) complexes on the viscosity of CT-DNA at 25 °C. Complex = 0–100 μM, [DNA] = 50 μM.

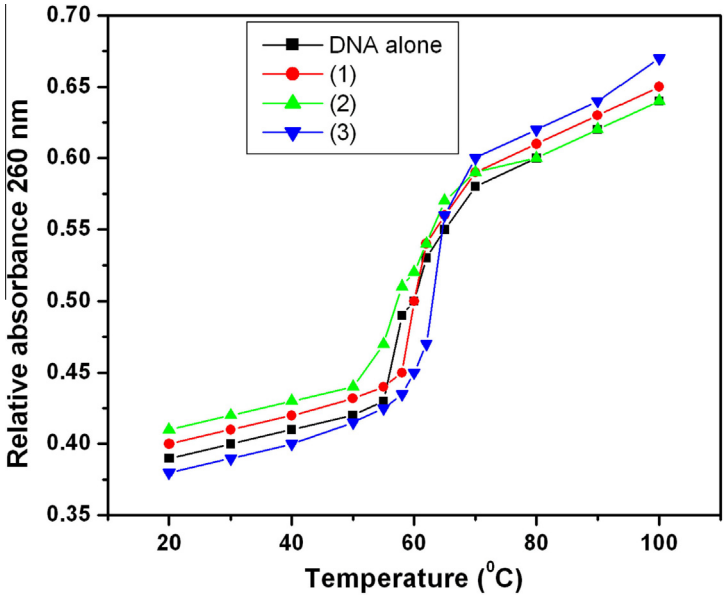


Fig. 4. Thermal denaturation of CT-DNA in the absence and presence of Cu(II) complexes. [DNA] = 50 μM, [complex] = 10 μM, buffer: phosphate.

(**1**)–(**3**) to serve as metallonucleases. The photo-induced DNA cleavage experiments have been carried out in UV (365 nm, 12 W) using 50 μM concentration of the complexes and SC pUC19 DNA (0.5 μg) reagent. The dpq and dppz complexes show more significant cleavage activity with the formation of both nicked-circular and linear DNA in comparison to their phen analogue showing formation of only NC form under similar experimental conditions (Fig. 5). The DNA binder heterocyclic bases dpq and dppz have photoactivatable quinoxaline and phenazine moieties with conjugated C=N bonds that could generate photoexcited $3(n–\pi^*)$ or $3(\pi–\pi^*)$ state(s) effecting DNA cleavage following an oxidative pathway in addition to the photoactive sulfur of the mqtH ligand show a significant photosensitization effect, enhancing the cleavage activity of complexes (**2**) and (**3**) to a greater extent along with the formation of linear DNA by attacking two complementary DNA strands at close proximity [55,56]. The cleavage efficiency follows the order (**3**) > (**2**) > (**1**). The reason for difference in the cleavage activity also depends on the DNA binding capabilities.

Mechanistic aspects of the photoinduced DNA cleavage reactions are investigated in the presence of various additives in UV light (Figs. 5 and 6). Addition of singlet oxygen quencher like sodium azide to complexes (**1**)–(**3**) inhibits the cleavage reaction, that signifying $_1O^2$ generation plays a key role in photo cleavage of pUC 19 DNA. While the hydroxyl radical scavenger DMSO has no apparent effect on the cleavage process, thus excluding the possibility of a type-I pathway forming hydroxyl radical (·OH). The formation of singlet oxygen is further supported by the presence of D_2O. An enhancement of photocleavage of DNA is observed in D_2O solvent in which $_1O^2$ has longer lifetime so induce more strand scissions [57,58]. Data from control experiments unequivocally suggest the involvement of singlet oxygen in a type II process in

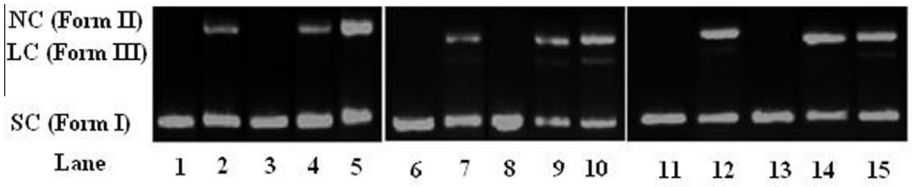


Fig. 5. Gel electrophoresis diagram showing the photocleavage of SC pUC19 DNA (0.5 μg) by complex (**1**), (**2**) and (**3**) (50 μM) and other additives at 365 nm for an exposure time of 1 h. lane 1, DNA control; lane 2, DNA+(**1**); lane 3, DNA+(**1**)+NaN_3 (100 μM); lane 4, DNA+(**1**)+DMSO (4 μL); lane 5, DNA+(**1**)+D_2O (14 μL);. lane 6, DNA control; lane 7, DNA+(**2**); lane 8, DNA+(**2**)+NaN_3 (100 μM); lane 9, DNA+(**2**)+DMSO (4 μL); lane 10, DNA+(**2**)+D_2O (14 μL); lane 11, DNA control; lane 12, DNA+(**3**); lane 13, DNA+(**3**)+NaN_3 (100 μM); lane 14, DNA+(**3**)+DMSO (4 μL); lane 15, DNA+(**3**)+D_2O (14 μL).

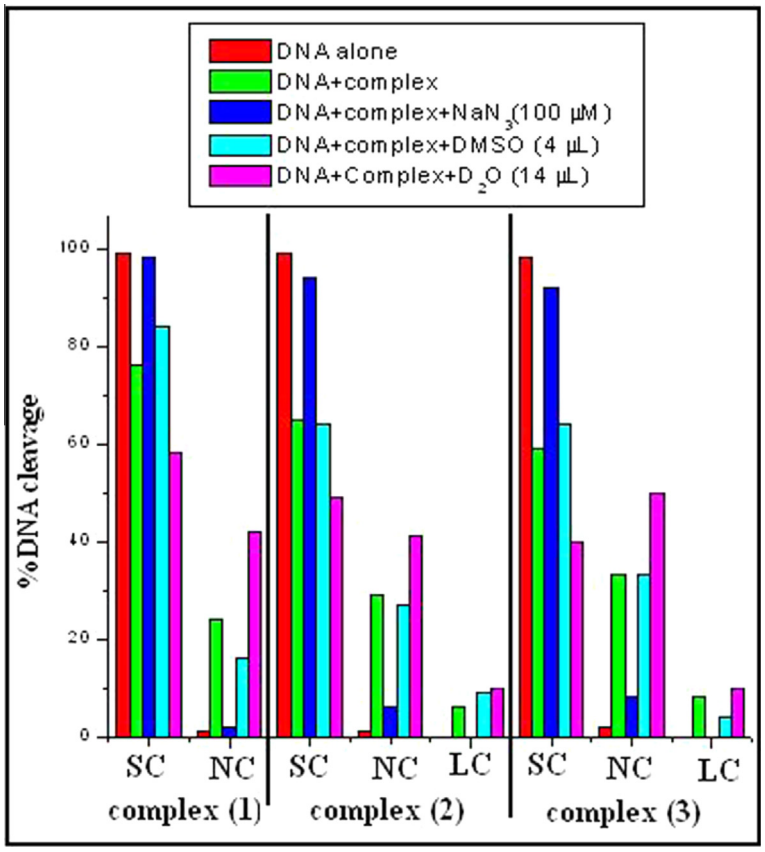
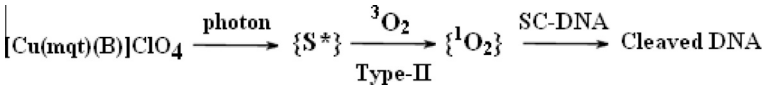


Fig. 6. Cleavage of SC pUC19 DNA (50 μM) by complexes (**1**), (**2**) and (**3**) in the presence of various additives upon photo irradiation at 365 nm in 50 mM Tris–HCl/NaCl buffer (pH 7.2).



Scheme 1. Mechanistic pathways for the cleavage of DNA on photoirradiation.

the metal assisted photo-excitation process involving ligand n–π^* and π–π^* transitions (Scheme 1).

PACT assay

The novel copper complexes (**1**), (**2**) and (**3**) were tested for their ability to kill *E. coli* on photoirradiation by a tungsten–halogen 500 W lamp. The effect of cell viability on irradiation of visible light emitted by a 500 W halogen–tungsten lamp was investigated to obtain dependable data about the efficiency of the photodynamic treatment on *E. coli*. When *E. coli* cultures (108 cfu/ml) alone, in absence of complexes, were illuminated for 60 min, no substantial decrease in the number of viable cells was observed. Thus, irradiation time was maintained for 60 min in all PACT assays. To standardise the time of incubation with the complexes before light exposure, suspensions (10^8 cfu/ml) of the bacteria was incubated with the complexes in the dark for 30, 60 and 90 min, washed once in PBS and then irradiated with light for the period previously determined. The percentage of survivors for *E. coli* after the light treatment were found to be 0.043%, 0.041%, 0.044% for complex (**1**), 0.068%, 0.067%, 0.065% for complex (**2**) and 0.043%, 0.042%, 0.044% for complex (**3**). Consequently results suggested that, increasing the time of the contact between the complexes and the bacterial cells did not improve the performance of the

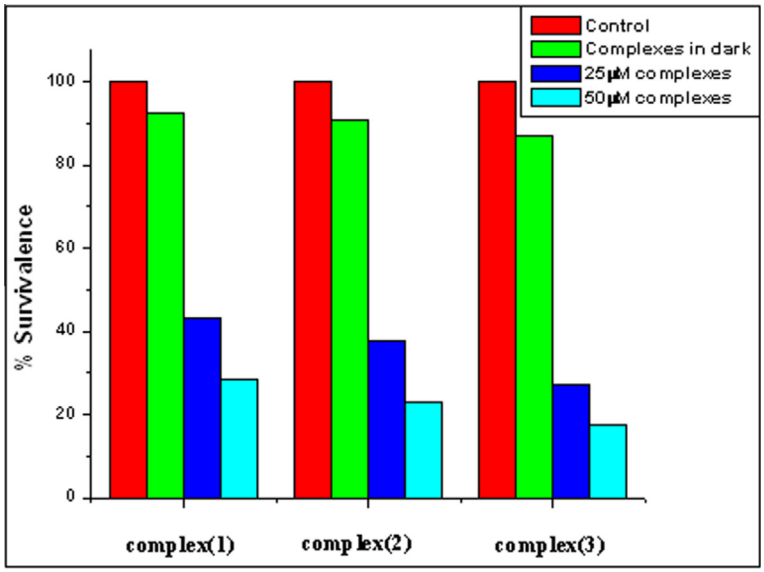


Fig. 7. CFU reduction (%) in *E. coli* after treatments.

complexes against the above mentioned bacteria. So, for all photoinactivation experiments of copper complexes under study 30 min incubation period was maintained.

The protocol for photodynamic treatment of *E. coli* tested was then further standardized evaluating the effects of complexes up to 50 μg/ml. *E. coli* showed a apparent dose-dependent lethality when irradiated after the exposure to the different complexes at 25 μM and 50 μM concentrations. The DMSO used in low concentrations for the preparation of aqueous solution of the copper complexes also did not alter cell viability. By counting colony forming unit (CFU) of *E. coli* remaining in the control, complexes (**1**), (**2**) and (**3**) alone and photoirradiated complexes at 25 μM and 50 μM concentrations was made to determine effectiveness of PDT. The results of PDT on *E. coli* in terms of CFU counts (%) after treatment can be seen in Fig. 7. The% survival statistical analysis for *E. coli.* revealed a nonsignificant reduction of CFU counts between the control and Complexes 7.6%, 9.3%, 12.8% of complexes (**1**), (**2**) and (**3**) respectively. However, a significant reduction of CFU counts by 56.8%, 62.3%, 72.4% for 25 μM concentration and 71.3%, 76.8%, 82.3% for 50 μM concentration was observed for the photoirradiated complexes (**1**), (**2**) and (**3**) respectively when compared with the control group.

In the Control group a significantly higher ($p < 0.06$) bacterial growth (10^8 CFU/mL) was exhibited, while the complexes alone presented no statistically significant ($p > 0.06$) bacteria reduction, indicating that the complexes alone seems not to significantly reduce bacterial viability. However, all the photo irradiated complexes were sensitive to *E. coli* for the PACT assay, presenting a significantly low bacterial growth. There was a significant reduction in the CFU counts ($p < 0.06$) compared with the initial numbers recorded in both Control and complexes.

Conclusions

Three novel copper(II) complexes having N,S donor of mqtH and N,N-donor heterocyclic base (B) are prepared and structurally characterized. The planarity and extended conjugation of the phenanthroline bases have a profound effect on the DNA binding and cleavage activity of the complexes. Binding study shows a minor groove-binding nature of the copper(II) complexes. The significant results include our observation of biologically important photoinduced DNA double-strand breaks leading to linear DNA formation due to the photosensitizing effect of both dpq and dppz. Mechanistic studies reveal direct involvement of $_1O^2$ in the photo-cleavage process. Pathways involving singlet oxygen in the DNA photocleavage reactions are proposed from the observation of the complete inhibition of the cleavage in presence of NaN_3 and enhancement of cleavage in D_2O. Sulfur containing ligands with their longer triplet state lifetimes are suitable for activation of molecular oxygen. We are making further efforts to understand the DNA clevage ability of copper complexes in longer wavelength (PDT window). These results may through light toward the design and development of copper-based complexes with sulfur containing ligands for PDT applications and as alternatives to the PDT anticancer drug.

Acknowledgment

This work has been supported by providing Post Doctoral Fellowship for women (2009–10 & 2010–11) from the University Grants Commission, New Delhi.

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