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SYNTHESIS, CHARACTERIZATION, DNA BINDING/CLEAVAGE STUDIES AND BIOLOGICAL AND ANTICANCER ACTIVITY OF COPPER (II) COMPLEXES

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Abstract:

Two copper (II) complexes, $[Cu(B)(L\text{-}Ser)TU]ClO_4$ (B=1,10- phenanthroline (phen) in complex 1, and 2,2'-bypyridine (bpy) in complex 2, L-Ser =L-Serine,TU= thiourea), have been synthesized and characterized by Infra-red, EPR spectral and elemental analysis methods. The one-electron paramagnetic complexes display a d–d band near 600 nm in water. Binding modes of the complexes with calf-thymus DNA have been studied by electronic absorption spectroscopy, emission spectroscopy, viscosity and cyclic voltammetry. We continued to study the cytotoxicity activity of complex 1 by using HepG2 cells. Further complex 1 & 2 were tested for their antimicrobial activities and it was found to have good antimicrobial activities.

Key Words: Copper (II) Complexes, L-Serine, Thiourea, DNA Binding & Cytotoxicity,

1. Introduction:

Transition metals complexes interact with DNA under physiological conditions are important for their biological activity. Metal chelates have been used to investigate DNA structure in solution, as agents for mediation of strand scission of DNA and as anticancer agents [1–10]. Molecules able to irreversibly modify nucleic acids have received considerable interest because of their potential applications as chemotherapeutic agents. Copper complexes of 1,10-phenanthroline in the presence of secondary ligands and their derivatives are mainly used chemical nucleases employed as foot printing reagents to find ligand binding sites and recently also for chemotherapy [11-12]. Amino acids are the fundamental units of proteins. Copper ions and various amino acids are present in biological systems in cells and body fluids, and copper complexes of amino acids were reported to exhibit good antitumor and nuclease activity [13]. Copper (II) complexes have been found to use in the treatment of many diseases including cancer [14]. Among the various copper complexes which are containing phenanthroline and its derivatives have attracted much attention for their vital role in biological activities such as antimycobacterial, anticandida and antimicrobial activities [15-17]. Investigations of the interaction of transition metal complex with DNA are basic work to synthesize the new types of the molecules in pharmaceutical industry, to reveal the mechanisms involved in the specific binding site of DNA [18, 19].

Over the past several years, transition metal complexes with DNA have been continuous interest in determining the mode of binding, as such information are important for understanding the cleavage properties of metal complexes in order to develop cleaving agents for probing nucleic acid structures for other applications. Metal complexes are bound to DNA via both covalent and non-covalent interactions. In covalent binding a nitrogen base of DNA such as guanine replaces the labile ligand of the complexes. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove binding of cationic metal complexes along outside of DNA helix, in the minor and major groove, hydrogen bonding and Vander Waals interactions of functionalities bound along the groove of the DNA helix, on the outside of the helix (external electrostatic), the coordination anion and the nucleic acid cation interact through electrostatic interaction. Intercalation involves the partial insertion of aromatic heterocyclic rings of ligands between the DNA base pairs [20-23]. In this paper, the synthesis and characterisation of complexes 1 & 2 by elemental analysis, IR and EPR spectra have been reported. The binding properties of these complexes to calf thymus DNA have been studied using different physico-chemical methods and the binding modes are discussed. The experimental results show that the Copper (II) complex has potential cytotoxicity and anti-microbial agents.

2. Experimental:

Materials and Methods: The reagents such as $CuCl_2.2H_2O$, NaOH, $NaClO_4.H_2O$, 2,2-bipyridine, L-serine, Thiourea, 1,10-Phenanthroline, CT DNA, Tris HCl, NaCl and ethidium bromide were purchased from Aldrich. The spectroscopic titration was carried out in the buffer (50 mM NaCl–5 mM Tris–HCl, pH 7.1) at room temperature. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance 1.8 – 1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. Milli-Q water was used to prepare the solutions. Absorption spectra were recorded on a UV/VIS Shimadzu 2450 Spectrophotometer using cuvettes of 1-cm path length and emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. FT-IR spectra were recorded on a FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. EPR spectra

were recorded on Varian E-112 EPR spectrometer at room temperature, the field being calibrated with DPPH = 1, 10-diphenyl-2-picrylhydrazyl (g =2.0037). Cyclic voltammetry experiments were recorded on CHI 602D (CH Instruments Co., USA) electrochemical analyzer under oxygen free conditions using a three-electrode cell in DMF solution with TBAP (0.1 M) as the supporting electrolyte. A pt wire, glassy carbon, and the Ag/AgCl (in saturated KCl solution) electrodes were used as counter, working and reference.

Synthesis of [Cu(L-Ser)(phen)(TU)](ClO$_4$) (1): The complex [Cu(L-Ser)(phen)(H_2O)](ClO_4) was synthesized according to a published method [18]. To the aqueous solution of [Cu(L-Ser)(phen)(H_2O)](ClO_4) (1 mmol) was added thiourea (1 mmol) and stirred for 4 hrs. Finally the colour of the solution changed from blue to bluish green, the resulting solution was filtered and the filtrate was kept for slow evaporation, after two weeks bluish green colored complex was separated out. Yield 78%. Anal. Calcd. for $CuC_{16}N_5H_{18}ClO_7S$: C, 36.72; H, 3.47 ; N, 13.38%. Found: C, 35.82; H, 3.54; N, 13.61%. IR (KBr, Pellet, cm^{-1}): 3332, 3236, 3140, 2937, 1622, 1598, 1323, 1261, 1149, 1068, 858, 719, 650.

Synthesis of [Cu(L-Ser)(bpy)(TU)](ClO$_4$) (2): Synthesis was described above (in complex 1) using [Cu(L-Ser)(bpy)(H_2O)](ClO_4) (1 mmol) and thiourea (1 mmol). Yield: 66%. Anal. Calcd. for $CuC_{14}N_5H_{18}ClO_7S$: C, 33.67; H, 3.63 ; N, 14.02%. Found: C, 33.14; H, 3.21; N, 14.68%. IR (KBr, Pellet, cm^{-1}): 3338, 3284, 3232, 2196, 2171, 1741, 1622, 1602, 1344, 1315, 1228, 1068, 858, 719, 549. The detailed experimental setup of DNA binding, MTT assay and antimicrobial activity described in [24, 25].

3. Results and Discussion:

General Aspects: The ternary copper (II) complexes are synthesized with good yield from the reaction of $CuCl_2.2H_2O$ with L-serine, heterocyclic bases (phen, bpy). The complex formulated as [Cu(L-ser)(B)(U)]NO_3 where X is the heterocyclic bases (phen and bipy) and U is urea. The complexes (1 & 2) are stable and soluble in water. The complexes are characterized by physico chemical methods; analytical data infers that the experimental values agree with the calculated values. A d-d band near 610 nm in water due to d-d transition and band near 270 nm is assignable due to intraligand transitions (Fig. 1). EPR spectra of mononuclear complexes copper (II) species with S=1/2, those with two signals ($g_\perp$ and $g_\parallel$), on comparing these two signals $g_\perp(x,y) > g_\parallel(z)$ ($B_\perp(x,y) < B_\parallel(z)$) representing elongated axial symmetry of the spin tensor (Fig. 2).

DNA Binding Properties:

Absorption Spectral Studies: The UV/Vis absorption spectra of the complexes (1 & 2) in the presence and absence of calf-thymus DNA are shown in Fig. 3. In the presence of CT-DNA, an increase in absorption intensity (hyperchromism) was observed, with slight decrease wavelength for complex 1 and a decrease in absorption intensity (hypochromism) was observed, with slight decrease in wavelength for complex 2. These shifts are indicated that interaction between the complex and those of the DNA bases. The changes suggested that complex 1 was consistent with intercalative and complex 2 was electrostatic interaction into the DNA base pairs. The binding constants (k_b) of the two complexes (inset of Fig. 3) with CT-DNA were 9.49 X 10^3 and 5.62 X 10^3 M^{-1} for complex 1 and 2 respectively. The higher binding tendency of the phen complex 1 in comparison to its bpy complex 2 analogue could be due to the presence of planar aromatic ring in phen [26].

Fluorescent Spectral Studies: To further investigate the interaction between the complex and CT-DNA, the EB fluorescence displacement experiments were also employed. The fluorescence intensity of DNA is negligible; meanwhile, the EB in buffer solution is not significant due to quenching by the solvent molecules. However, if the complex can intercalate into DNA, the binding sites of the DNA available for EB will decrease, and hence the intensity of EB will be quenched. Therefore, EB can be used to explore the interaction of complex with DNA. It should be pointed out that the inner-filter effect (IFE), which refers to the absorbance of light at the excitation or emission wavelength by other compounds present in the solution, can also lead to the decrease in fluorescence intensity. The decrease in the fluorescence intensity of solution induced by the occurrence of IFE could lead to a nonlinear relationship between the observed fluorescence intensity and the concentration of the fluorophore, as well as result in the introduction of large errors in the data interpretation. Therefore, it is necessary to consider the IFE and eliminate its interference with the results. In our study, IFE was corrected with the following equation:

$$F_{corr} = F_{obs} \times 10^{\frac{A_{ex} \times d_{ex}}{2} + \frac{A_{em} \times d_{em}}{2}}$$

where F_{obs} and F_{corr} are the measured fluorescence and the correct fluorescence intensity, respectively; d_{ex} and d_{em} are the cuvette path length in the excitation and emission direction (in cm), and A_{ex} and A_{em} stand for the measured change in absorbance value at the excitation and emission wavelength, respectively. After the removal of IFE, the fluorescence intensities of EB bound to CT-DNA at 614 nm, as illustrated in Fig. 4, show remarkable decreasing trends with the increasing concentration of the complexes, indicating that some EB molecules were released into solution after the exchange with the two complexes, resulting in the fluorescence quenching of EB. These observations may be interpreted as the intercalation of the complexes between the base pairs of CT-DNA. The quenching of EB bound to the DNA by the complexes is in agreement with the linear Stern–Volmer equation [27-29]:

$$\frac{I_0}{I} = 1 + K_{sv}[Q]$$

where I_0 and I represent the fluorescence intensities in the absence and presence of complex, respectively. Ksv is a linear Stern–Volmer quenching constant, Q is the concentration of complex. In the quenching plot (insets in Fig. 4) of I_0/I vs [complex], the Ksv values for complexes **1** and **2** are 3.73 X 10^5 and 3.36 X 10^5 respectively. Thus, the evidences observed in the EB fluorescence displacement experiments are in agreement with the conclusion derived by the electronic absorption spectra measurements.

Viscosity Measurements: Mode of interaction between the metal complex and DNA was clarified by viscosity measurements. The intercalation mode demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity. In contrast, a partial non-classical intercalation of ligand could bend the DNA helix, reduce its effective length and concomitantly its viscosity. Effect of the complexes (1 & 2) on the viscosity of rod like DNA is shown in the (Fig. 5). The viscosity of DNA is slightly increased with the increase of the concentration of the complexes 1 and 2, in contrast to that of proven DNA intercalator EtBr (=ethidium bromide). Based on the viscosity results, it was observed that these complex 1 bind with DNA through intercalation and complex 2 through partial intercalation or groove binding [30].

Cyclic Voltammetry Studies: The cyclic voltammetric (CV) response for complexes 1 and 2 in Tris–HCl buffer in the presence and absence of CT DNA is shown in Fig. 6. In the cyclic voltammetry scan, a single cathodic and anodic peak were observed, which corresponds to the reduction as well as oxidation of complexes, which indicates that the process is reversible. When CT-DNA is added to a solution of complexes, marked decrease in the peak current and potential values were observed. The cyclic voltammetric behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complexes after the addition of DNA due to the binding of complexes to the DNA [31].

MTT Assay: The cytotoxicity of the complexes to be used as chemotherapeutic agents was studied using MTT assay. The ability of the complexes on HepG2 cells was tested with or without various concentrations (10–250 µg/ml) of complex 1. Cells incubated with different concentration of Doxorubicin served as positive control. After incubation period, MTT assay was carried out to calculate the cell death percentage. For each concentration, of the complexes cells were incubated in triplicate. The (Fig. 7) clearly illustrates that there is a clear decrease in the live cells number in the cells incubated with complex in a concentration dependent manner. Viability of cells incubated without any compound was considered as 100% and the percentage of live cells incubated with compound is given as relative to the control. The IC_{50} value of the complex 1 is 39.47 µg/ml.

Antibacterial and Antifungal Activity: The copper (II) complexes were screened in vitro for its microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. The complexes were found to exhibit considerable activity against bacteria and the fungus. Zoroddu et al [32] reported that copper complexes show considerable activity against the bacteria. Recently Patel et al [33] have indicated that the copper (II) complexes with L-phenylalanine has exhibited considerable activity against some human pathogens. In our biological experiments, using copper (II) complex, we have observed antibacterial activity antifungal activity. The complex 1 has shown high antibacterial activity (Table 1) against *Klebsiella pnemoniae* and *Escheria Coli* and complex 1 has shown high antifungal activity against *Rhizopus sps* and *Aspergillus niger*. It may be concluded that our complexes 1 inhibit the growth of bacteria and fungi to a greater extent.

4. Conclusion:

Ternary copper (II) complexes having heterocyclic bases and L-serine are prepared and characterized. The copper (II) complexes with heterocyclic base in CuN_4O coordination geometry shows DNA binding ability. The complex 1 and 2 show intercalative and electrostatic binding respectively. The DNA cleavage activity of complex 1 is more significant than complex 2. The MTT assay of complex 1 (higher IC_{50}) and antimicrobial activity of complexes (1 & 2) suggested that complexes have potential role in medicinal line.

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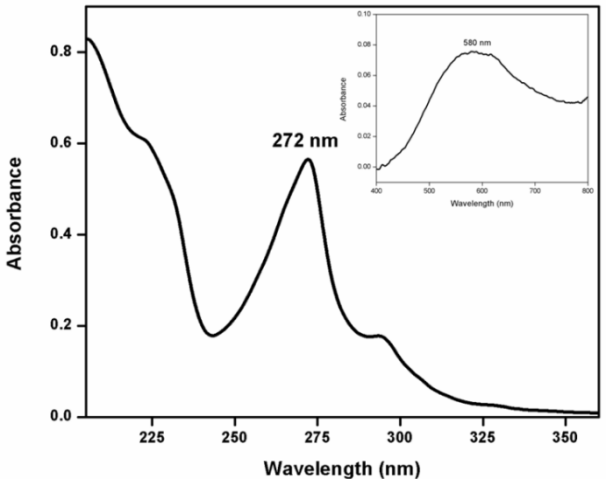
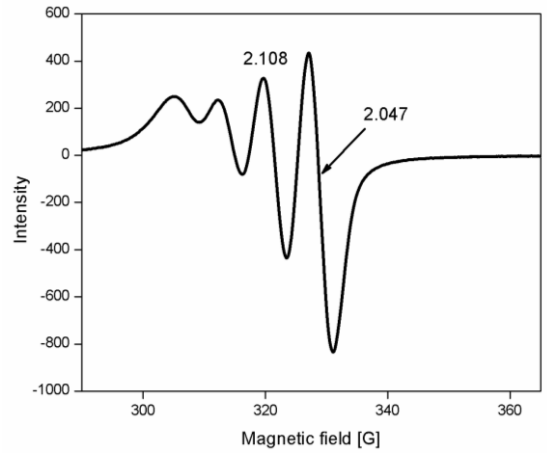


Figure 1



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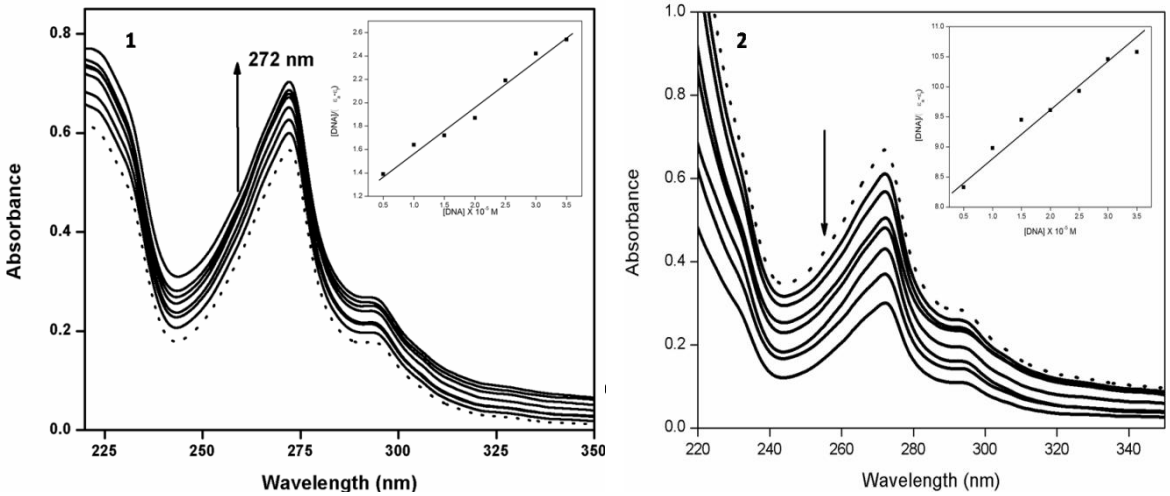

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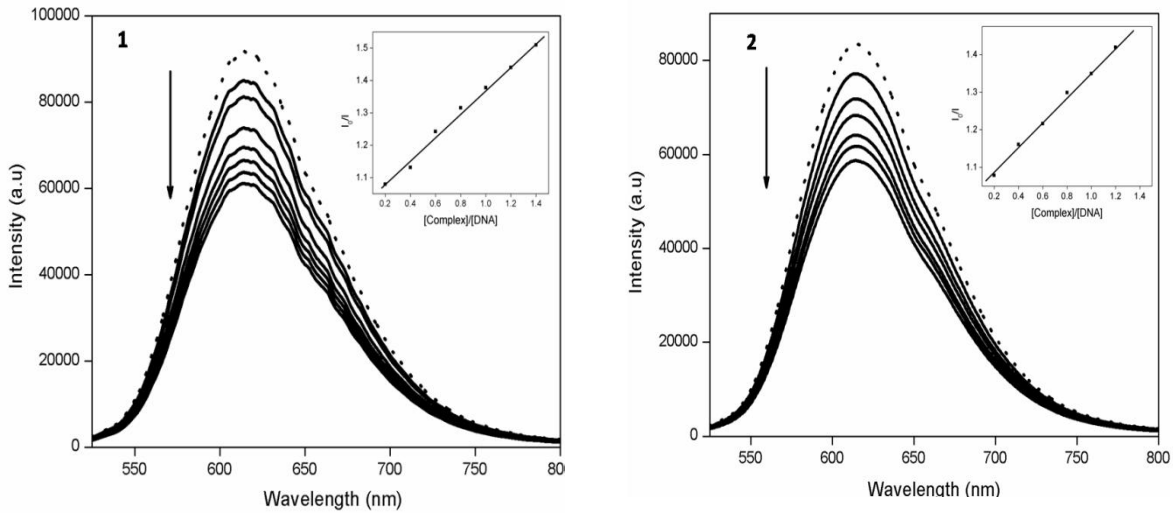

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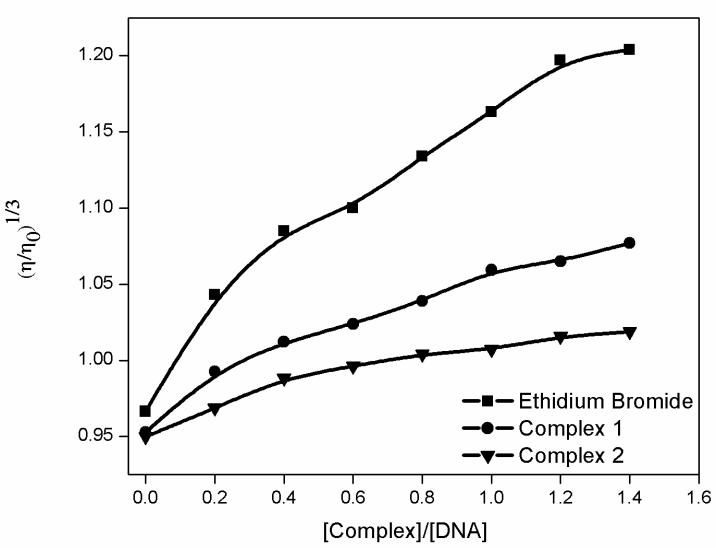

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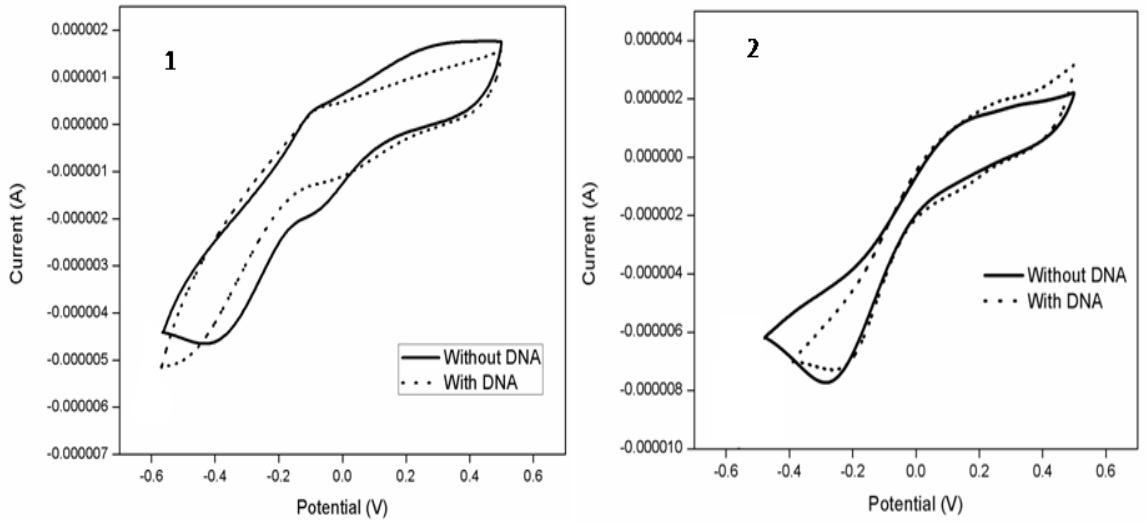

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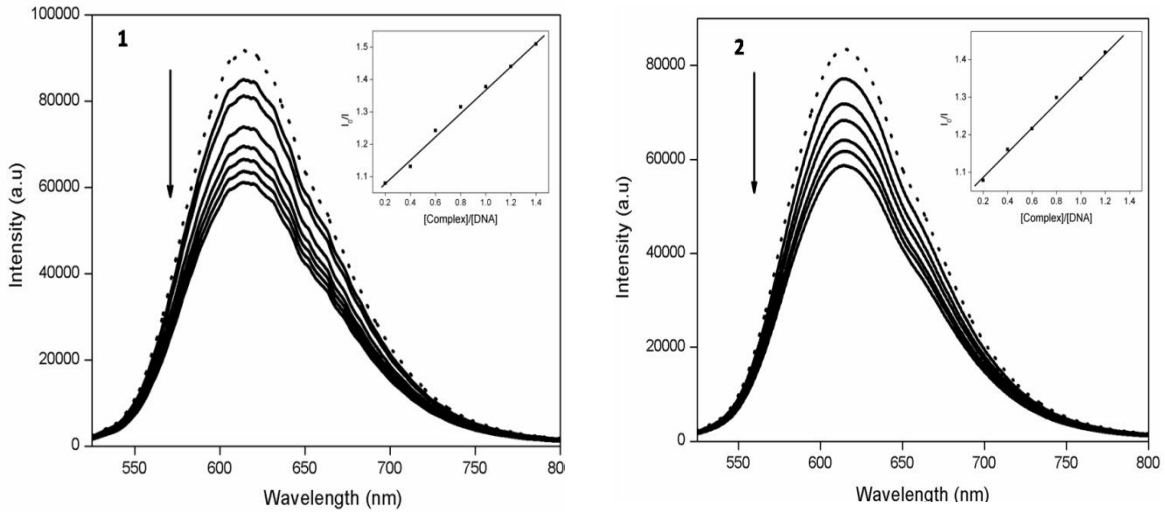

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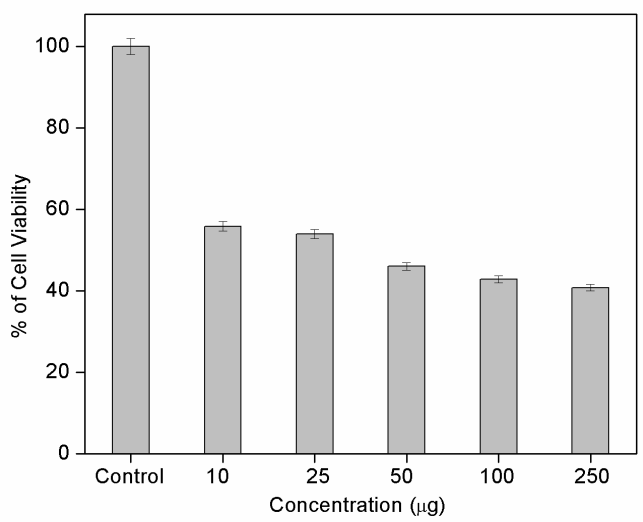

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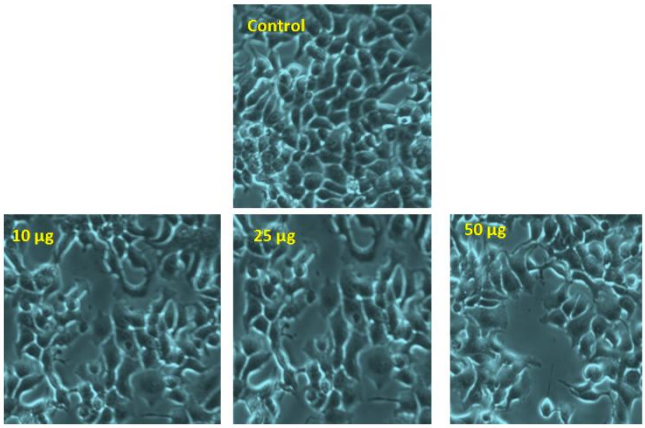
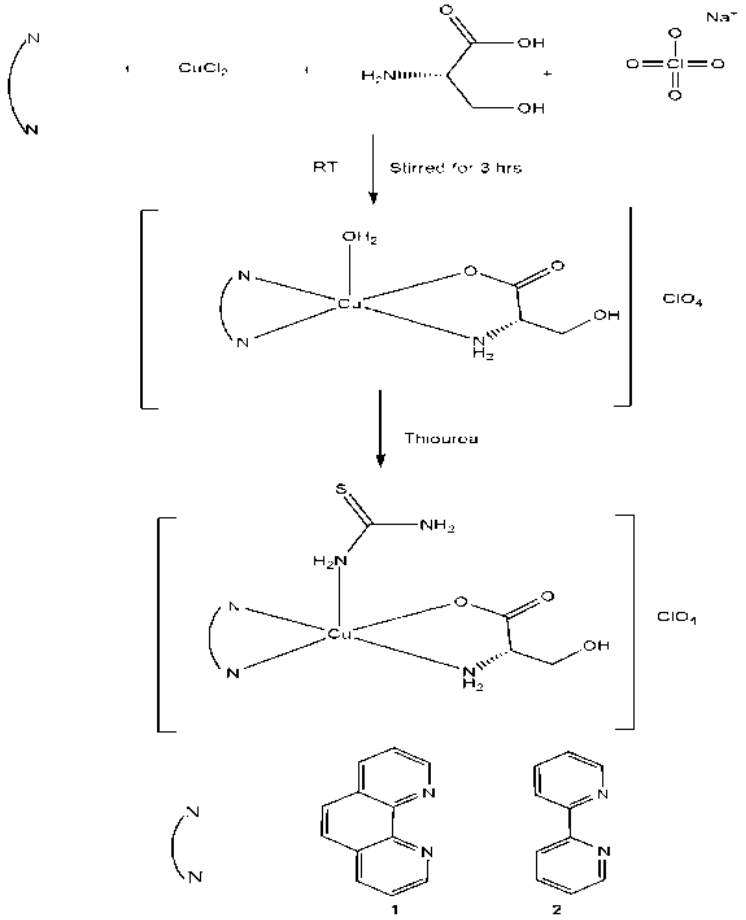


Figure 8

Figure 1: UV-Visible spectrum of complex 1
Figure 2: EPR spectrum of complex 1
Figure 3: Absorption spectral traces on addition of CT DNA to complexes 1 and 2 (shown by arrow). Inset plot of [DNA]/(εa-εf) vs [DNA] for absorption titration of CT DNA with complex at 271 nm.
Figure 4: Emission spectra of EB bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes **1** and **2**. Arrow (↓) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.
Figure 5: Effects of increasing amounts of complexes on the relative viscosities of CT DNA at 25^0C.
Figure 6: Cyclic voltammogram of complexes (**1** and **2)** in the absence (dashed line) and presence (dotted line) CT DNA.
Figure 7: Cell viability of HepG2 cells after treatment with complex **1** at different concentrations.
Figure 8: Morphology of HepG2 cells after treatment with complex **1** at different concentrations.

Table 1: Antibacterial and antifungal activity of complex 1

S.No	Micro Organisms	Control	Complex **1**	Ciproflaxacin/ Amphotericin-B
Bacteria				
1	*Staphylococcus aureus*	-	35	25
2	*Klebsiella pnemoniae*	-	40	37
3	*Escherichia coli*	-	40	25
Fungi				
4	*Aspergillus niger*	-	15	7
5	*Rhizopus sps*	-	15	15
6	*Penicillium sps*	-	10	20



Scheme 1: Synthesis of complex 1 and 2.