



# Evaluation of DNA Binding Antimicrobial and Cell Viability Activity of Furan-Based Cobalt (III) Polypyridyl Complexes

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## Abstract

This article describes the DNA binding properties of three new Cobalt (III) Polypyridyl complexes [Co(phen)<sub>2</sub>CMIP]<sup>3+</sup> (1), [Co(bpy)<sub>2</sub>CMIP]<sup>3+</sup> (2) and [Co(dmb)<sub>2</sub>CMIP]<sup>3+</sup> (3) where CMIP=2-(2-Chloro-8-methylquinolin-3-yl)w-1H-Imidazo[4,5-f][1,10]phenanthroline, phen = 1,10-phenanthroline, bpy = 2, 2' bipyridine and dmb = 4, 4 - dimethyl-2, 2' bipyridine. They have been synthesized and characterized by IR, <sup>1</sup>H & <sup>13</sup>C NMR and Mass spectra. The DNA – binding constant (K<sub>b</sub>) of the complexes was determined to be in the order of 10<sup>6</sup> with an intercalative mode of binding. Photoactivated cleavage and antimicrobial studies of three complexes were shown better results, Cytotoxicity (MTT assay) showed growth inhibition in dose dependent manner. Among three complexes, complex 1 was more active than other two complexes.

## Keywords

Co(III) polypyridyl complexes, Antimicrobial activity, Intercalation, Photoactivated cleavage, HeLa cells, MTT assay.

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## INTRODUCTION

The clinical drawbacks of cisplatin are apparent, including the limited applicability, the acquired resistance, and the serious side effects, such as neurotoxicity and nephrotoxicity<sup>1,2</sup>. These limitations of cisplatin have motivated extensive investigations into alternative metal-based cancer drugs. Over the years, complexes of Ru, Ir, Cu, Ni, Zn, Co, etc., have been reported to possess much better anticancer property than *cis*-platin<sup>3-6</sup>. Cobalt is an essential trace element present in the human body. It is involved in important biological functions such as

fatty acid and amino acid metabolism, haematopoiesis, and in the form of vitamin B<sub>12</sub> it is indirectly involved in synthesis of DNA. Interestingly, one cobalt complex containing Schiff base ligand (Doxovir) has recently passed phase II clinical trial for anti-viral treatment<sup>7</sup>. Several *in vitro* studies suggest that cobalt complexes possess promising anti-cancer activity<sup>8</sup>.

Cobalt is widely distributed in the biological systems such as cells and body, and thus the interaction of DNA with cobalt compound has attracted much attention<sup>9</sup>. The binding properties of cobalt with calf

thymus DNA were studied by several methods, and the experimental results showed that the size and shape of the intercalated ligand had an important effect on the binding affinity of the compounds with DNA<sup>10</sup>. Hisaeda and co-workers discovered a new water-soluble dicobalt compound having two cobalt-carbon bonds and reported that this dicobalt compound showed higher ability for DNA cleavage in comparison with the corresponding monocobalt compound<sup>11</sup>. The interaction of DNA with cobalt (II) tridentate compound, and the photocleavage studies showed that the cobalt (II) compound increased to nicking of DNA in the presence of plasmid DNA<sup>12</sup>. Our previous works have shown that Ru (II) polypyridyl complexes containing *N*, *N*-chelating ligands were DNA intercalative and antiproliferative agents<sup>13-20</sup>. This paper discussing about synthesis and various biological activities of newly synthesized furan derivatives of Co (III) polypyridyl complexes (**1-3**). Interactions of these compounds with CT-DNA were investigated by UV, visible spectroscopy, fluorescence spectroscopy and viscosity measurement.

## MATERIALS AND METHODS

### Materials

CoCl<sub>2</sub>·6H<sub>2</sub>O, 1, 10-Phenanthroline, 2, 2' bipyridine, 4, 4' dimethyl 2, 2' bipyridine and 2-Chloro-8-

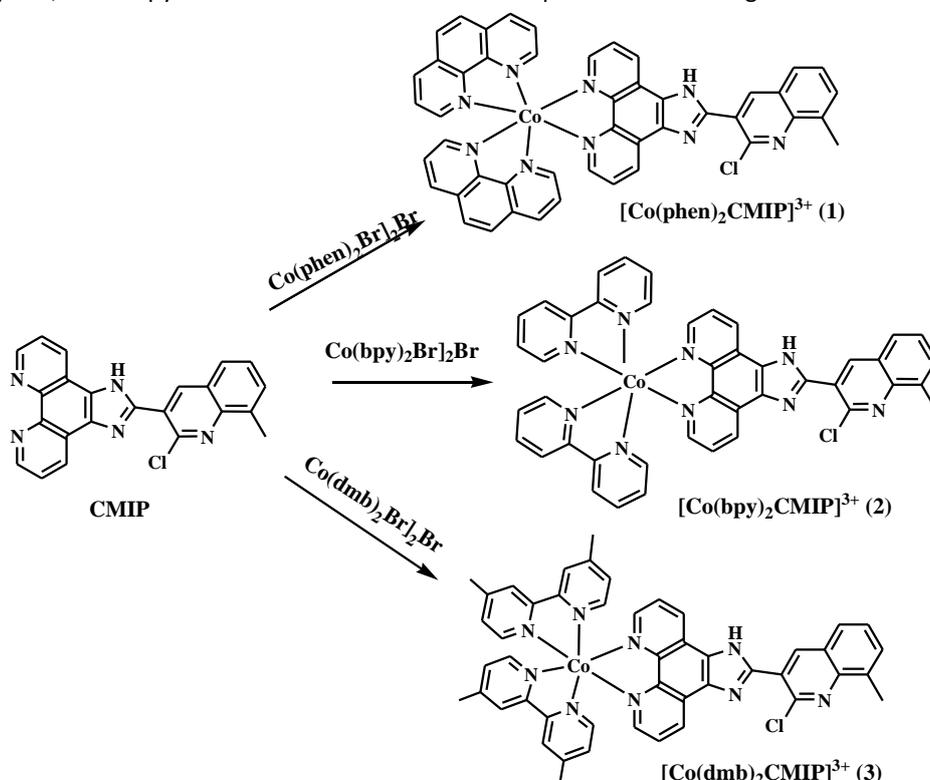
methylquinoline-3-carboxaldehydewere purchased from sigma. All the solvents were purified before use, as per standard procedures<sup>21</sup>. CT (Calf Thymus) DNA was purchased from Aldrich, its solution gives a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9, indicating that the DNA was sufficiently free of protein<sup>22</sup>, Supercoiled pBR 322 plasmid DNA (stored at -20 °C) was obtained from Fermentas life sciences, agarose (Genei) from Sigma. Double distilled water was used for preparing various buffers. Ampicillin for antimicrobial studies was purchased from local pharmaceuticals.

### Physical measurements

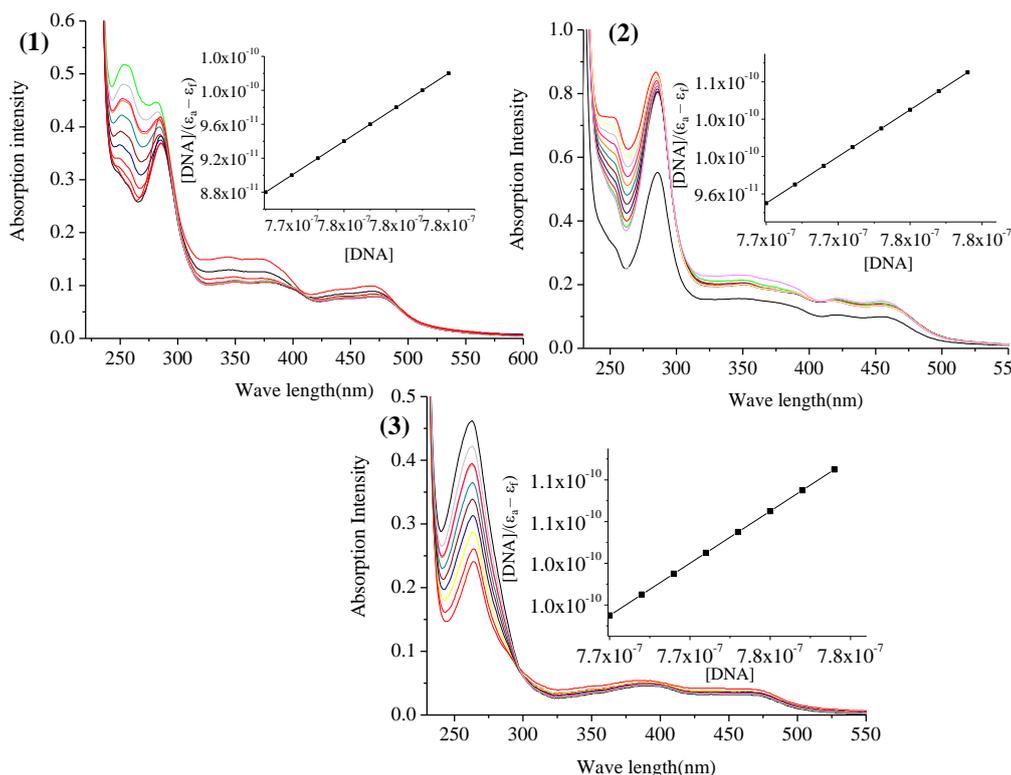
UV–Visible spectra recorded with an Elico BL 198 spectrophotometer. Fluorescence measurements performed on an Elico SL 174 spectrofluorometer. IR spectra recorded on KBr disks on a Perkin–Elmer FT-IR-1605 spectrometer. <sup>1</sup>H NMR spectra recorded on a Bruker 400 MHz spectrometer with DMSO-d<sub>6</sub> as solvent at RT and tetramethyl silane (TMS) as the internal standard. Microanalysis (C, H, and N) carried out with a Perkin-Elmer 240 elemental analyzers.

### Synthesis and characterization

Compounds 1, 10-phenanthroline-5, 6-dione<sup>23</sup>, cis-[Co(phen)<sub>2</sub>Cl<sub>2</sub>].2H<sub>2</sub>O, cis- Co(bpy)<sub>2</sub>Cl<sub>2</sub>·2H<sub>2</sub>O and cis-[Co(dmb)<sub>2</sub>Cl<sub>2</sub>].2H<sub>2</sub>O<sup>24</sup> were synthesized according to methods in the literature. Synthetic scheme of Co(III) complexes shown in Fig. 1.



**Fig. 1: Scheme;** Synthetic scheme for three complexes, [Co(phen)<sub>2</sub>CMIP]<sup>3+</sup> (**1**), [Co(bpy)<sub>2</sub>CMIP]<sup>3+</sup> (**2**) and [Co(dmb)<sub>2</sub>CMIP]<sup>3+</sup> (**3**).



**Fig. 2: Absorption spectra of three complexes 1, 2 and 3 in the absence (top red peak in each spectra) and in the presence of increasing concentration of CT-DNA. A plot of  $[DNA]/(\epsilon_a - \epsilon_f)$  vs  $[DNA]$  is shown in the inset.  $[DNA] = 2.5 \times 10^{-4} \text{ M}$ ,  $[Complex] = 10^{-3} \text{ M}$ .**

### Preparation of CMIP ligand

CMIP=2-(2-Chloro-8-methylquinolin-3-yl)-1H-Imidazo[4,5-f][1,10]phenanthroline was prepared by the addition of 1, 10-phenanthroline-5, 6-dione (0.53 g, 2.50 mM), 2-Chloro-8-methylquinoline-3-carboxaldehyde (0.7196 g, 3.5 mM), Ammonium acetate (3.88g, 50 mM) and glacial acetic acid (10 mL) were refluxed together for 4 h as per Steck and Day<sup>25</sup>, and then cooled to room temperature and diluted with water. Drop wise addition of ammonia gave a yellow precipitate which was collected, washed with water, dried, and purified by recrystallization from pyridine-H<sub>2</sub>O (9:1, v/v); Yield: 0.51 g (73%), Analytical data: Elemental Analysis for C<sub>21</sub>H<sub>12</sub>ClN<sub>5</sub>: Calc. (%): C: 68.20; H: 3.27; N: 18.94; Found: C: 68.18; H: 3.30; N: 18.97; ESI-MS (m/z): Calcd: 369, found: 370  $[M+H]^+$ . <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz):  $\delta$ : 8.9 (H-C=N), 7.2-8.2, m (H-C=C), 2.3 (CH<sub>3</sub>), <sup>13</sup>C[<sup>1</sup>H]-NMR (DMSO-d<sub>6</sub>, 100 MHz): 150 (-C=N), 122 (-C\*=C), 148 (-C(Cl)=N), 136(C-C\*(C)=C), 153 (N-C=C), 155 (N-C=C), 21 (1<sup>0</sup> C).

### Synthesis of [Co(phen)<sub>2</sub>(CMIP)] (ClO<sub>4</sub>)<sub>3</sub> · 2H<sub>2</sub>O (1)

This complex was prepared by mixing Cis-[Co(phen)<sub>2</sub>Br<sub>2</sub>] Br · 2H<sub>2</sub>O (0.143 g, 0.25 mM) and CMIP (0.5565, 1.5 mM) in 20.0 mL of ethanol and was refluxed for 6 hrs. After filtration it was then cooled to room temperature and added saturated

aq. solution of NaClO<sub>4</sub>. The obtained light yellowish solid was cooled and washed with small amount of water and then dried under vacuum. Yield: 0.45 g (71%). Elemental Analysis for C<sub>45</sub>H<sub>32</sub>Cl<sub>4</sub>N<sub>9</sub>O<sub>14</sub>Co, Calc. C: 48.11; H: 2.87; N: 11.22, Found: C: 48.13; H: 2.89; N: 11.25 %. ESI-MS (m/z): Calcd: 263, found: 264 for  $\{[Co(phen)_2(CMIP)]^{3+} - (ClO_4)_3 \cdot 2H_2O\}$ . <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz):  $\delta$ : 9.1 (H-C=N), 6.3-8.2, m (H-C=C), 4.8 (H-C-N), 2.3 (CH<sub>3</sub>), <sup>13</sup>C[<sup>1</sup>H]-NMR (DMSO-d<sub>6</sub>, 100 MHz): 153 (-C=N), 122 (-C\*=C), 118 (-C\*=C-Cl), 127 (3<sup>0</sup> C=), 123(=C-N), 138 (=C(H)-C=), 135 (=C(Cl)-C=), 21 (1<sup>0</sup> C).

### Synthesis of [Co(bpy)<sub>2</sub>(CMIP)] (ClO<sub>4</sub>)<sub>3</sub> · 2H<sub>2</sub>O (2)

This complex was synthesized with similar procedure of the above complex (1), with cis-[Co(bpy)<sub>2</sub>Br<sub>2</sub>] Br · 2H<sub>2</sub>O (0.578 g, 1 mM) in place of cis-[Co(phen)<sub>2</sub>Br<sub>2</sub>] Br · 2H<sub>2</sub>O Yield: 0.44 g (72%). Elemental Analysis for C<sub>41</sub>H<sub>32</sub>Cl<sub>4</sub>N<sub>9</sub>O<sub>14</sub>Co, Calc. C: 45.79; H: 3.00; N: 11.72. Found: C: 45.80; H: 3.03; N: 11.74 %. ESI-MS (m/z): Calcd: 246, found: 248 for  $\{[Co(bpy)_2(CMIP)]^{3+} - (ClO_4)_3 \cdot 2H_2O\}$ . <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz):  $\delta$ : 9.1 (H-C=N), 6.3-8.5, m (H-C=C), 4.8 (H-C-N), 5.4 (H-C=C-Cl), 2.3 (CH<sub>3</sub>), <sup>13</sup>C[<sup>1</sup>H]-NMR (DMSO-d<sub>6</sub>, 100 MHz): 153 (-C=N), 122 (-C\*=C), 118 (-C\*=C-Cl), 127 (3<sup>0</sup> C=), 123(=C-N), 138 (=C(H)-C=), 135 (=C(Cl)-C=), 21 (1<sup>0</sup> C).

### Synthesis of [Co(dmb)<sub>2</sub>(CMIP)] (ClO<sub>4</sub>)<sub>3</sub>. 2H<sub>2</sub>O (3)

This complex was synthesized with similar procedure of the above complex (1), with cis-[Co(dmb)<sub>2</sub>Br<sub>2</sub>] Br. 2H<sub>2</sub>O (0.721 g, 1 mM) in place of cis-[Co(phen)<sub>2</sub>Br<sub>2</sub>] Br. 2H<sub>2</sub>O. Yield: 0.39 g (69%). Elemental Analysis for C<sub>45</sub>H<sub>40</sub>Cl<sub>4</sub>N<sub>9</sub>O<sub>14</sub>Co, Calc. C: 47.76; H: 3.56; N: 11.14. Found: C: 47.77; H: 3.58; N: 11.17 %.). ESI-MS (m/z): Calcd: 265, found: 266 for {[Co(dmb)<sub>2</sub>(CMIP)]<sup>3+</sup> - (ClO<sub>4</sub>)<sub>3</sub>. 2H<sub>2</sub>O}. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz): δ: 9.1 (H-C=N), 6.3-8.4, m (H-C=C), 4.8 (H-C-N), 5.4 (H-C=C-Cl), 2.3 (-CH<sub>3</sub>), <sup>13</sup>C[<sup>1</sup>H]-NMR (DMSO-d<sub>6</sub>, 100 MHz): 153 (-C=N), 122 (-C\*=C), 118 (-C\*=C-Cl), 127 (3<sup>o</sup> C=), 123(=C-N), 138 (=C(H)-C=), 135 (=C(Cl)-C=), 36 (3<sup>o</sup> C), 21 (1<sup>o</sup> C), 24(-CH<sub>3</sub>).

### DNA binding and Photo activated Cleavage studies

The DNA-binding and photo activated cleavage experiments were performed at room temperature. Buffer A [5 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride, 50 mM NaCl, pH 7.0] was used for absorption titration, luminescence titration and viscosity measurements. Buffer B (50 mM Tris-HCl, 18 mM NaCl, pH 7.2) was used for DNA photocleavage experiments. Solutions of calf thymus DNA (CT DNA) in buffer A gave a ratio of UV-Vis absorbance of 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein<sup>26</sup>. The concentration of DNA was determined spectrophotometrically ( $\epsilon_{260} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$ ).

The absorption titrations in the buffer were performed using a fixed concentration (20  $\mu\text{M}$ ) complex to which increments of the DNA stock solution were added. The intrinsic binding constant K, based on the absorption titration, was measured by monitoring the changes in absorption at the MLCT band with increasing concentration of DNA using the following equation<sup>27</sup>.

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

where [DNA] is the concentration of DNA in base pairs,  $\epsilon_a$ ,  $\epsilon_b$  and  $\epsilon_f$  correspond to the apparent absorption coefficient  $A_{\text{obsd}} / [\text{Co}]$ , the extinction coefficient for the free Cobalt complex and the extinction coefficient for the ruthenium complex in the fully bound form, respectively. In plots of [DNA] / ( $\epsilon_a - \epsilon_f$ ) versus [DNA],  $K_b$  is given by the ratio of slope to the intercept.

In the emission studies fixed metal complex concentration (20  $\mu\text{M}$ ) was taken and to this DNA with varying concentrations was added. The excitation wavelength was fixed, and the emission range was adjusted before measurements. The fraction of the ligand bound was calculated from the relation  $C_b = C_t [(F - F_0) / F_{\text{max}} - F_0]$ , where  $C_t$  is the total complex concentration, F is the observed fluorescence emission intensity at a given DNA

concentration,  $F_0$  is the intensity in the absence of DNA and  $F_{\text{max}}$  is when the complex is fully bound to DNA. Binding constant ( $K_b$ ) was obtained from a modified Scatchard equation<sup>28</sup>. From a Scatchard plot of  $r/C_f$  vs  $r$ , where  $r$  is the  $C_b / [\text{DNA}]$  and  $C_f$  is the concentration of free complex.

Viscosity measurements were carried out on Ostwald Viscometer maintained at a constant temperature at 25.0 ( $\pm 0.1$ ) °C in a thermostatic bath. DNA samples approximately 200 base pairs in average length were prepared by sonication to minimize the complexities arising from DNA flexibility<sup>29</sup>. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Relative viscosities for DNA in the presence and absence of complex were calculated from the relation  $\eta = (t - t_0)/t_0$ , where  $t$  is the observed flow time of the DNA-containing solution and  $t_0$  is the flow time of buffer alone<sup>30,31</sup>. Data were presented as  $(\eta/\eta_0)^{1/3}$  versus binding ratio<sup>32</sup> where  $\eta$  is the viscosity of DNA in the presence of complexes and  $\eta_0$  is the viscosity of DNA alone.

For the gel electrophoresis experiment, supercoiled pBR 322DNA (0.1  $\mu\text{g}$ ) was treated with the Co (III) complex in buffer B, and the solution was then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 1.5 h at 80 V on a 0.8% agarose gel in TBE (89 mM Tris-borate acid, 2 mM EDTA, pH = 8.3). The gel was stained with 1  $\mu\text{g}/\text{ml}$  ethidium bromide and photographed on the Gel documentation system, UVDI - 312.

### Antimicrobial Studies

The antibacterial activity of the complexes was studied against Escherichia coli and Staphylococcus aureus. Each of the Cobalt (III) complex was dissolved in DMSO at different concentrations of 500  $\mu\text{M}$  and 1000  $\mu\text{M}$ . Paper disks of Whatman filter paper no. 1 were sterilized in an autoclave. The paper disks saturated with 10  $\mu\text{L}$  of the Cobalt (III) complex were placed aseptically in Petri dishes containing agar medium inoculated separately with E. coli and S. aureus. The Petri dishes were incubated at 37 °C and the inhibition zones were recorded after 24 h of incubation. The results were also compared with the results for the standard antibacterial drug ampicillin as a positive reference and DMSO as a negative reference at the same concentrations.

### Cell viability studies by MTT assay

The cell viability was evaluated by MTT assay and performed as per standard protocol. HeLa cells, (human cervical carcinoma cell line) were seeded 5,000/well in 96 well plates and after overnight incubation; cells were treated with complexes 1, 2

and 3 in varying concentrations for 48h. After incubation, MTT reagent was added (5 mg/ml) and incubated further for 4h at 37°C in dark. After then, media was aspirated and to dissolve the crystals DMSO was added to each well. The optical density was measured at 570 nm. Untreated cells were taken as controls. Experiment was repeated with three independent times, each time with triplicates. Data represented here as mean  $\pm$  SEM.

## RESULTS AND DISCUSSION

### DNA Binding studies

It has been reported that DNA damaging agents can activate the intrinsic pathway of apoptosis involving the release of cytochrome c and other mitochondrial apoptogenic factors<sup>33</sup> and trigger autophagy simultaneously as a self-defence mechanism<sup>34,35</sup>. DNA binding studies have been performed to determine whether Co(III)-induced apoptosis and autophagy are correlated with their abilities to cause DNA damage.

### Absorption spectroscopic studies

The MLCT bands ( $\approx$  450 nm) changes were monitored in the presence of increasing concentration of CT-DNA. The subsequent increase in the concentration of the DNA led to a hypochromic shift in the intensity

of the MLCT peak and it reached a saturation point for all three complexes as shown in the fig. 2. This decrease in intensity of the CT band was accompanied by considerable red shift in the spectral band. These observations clearly show that three complexes showed dual mode of DNA binding. It is possible that initially because of the  $\pi - \pi$  stacking within the metal complexes, bind DNA electrostatically and at higher concentration of DNA the complexes get destacked and get intercalated between DNA bases. Such dual mode of DNA binding by a Co (III) complex has previously been reported<sup>36</sup>. The DNA binding constant ( $K_b$ ) values of 1, 2 and 3 complexes were in the order of  $10^5$  as shown in the table 1. The difference in  $K_b$  values was due to their different ancillary ligands. Complex 3 shows the less binding strength to double-helical DNA. Due to the presence of methyl groups on the 4 and 4' positions of the ancillary ligand, dmb causes steric hindrance when the complex intercalates into the DNA base pairs. Electron deficient rings interact more strongly with polyanion (DNA) than electron rich rings, so methyl group present on dmb ring may enhance the electron density on complex moiety and make electron rich, hence the binding constant for complex 3 is less than other two complexes.

**Table 1:  $K_b$  values of three complexes**

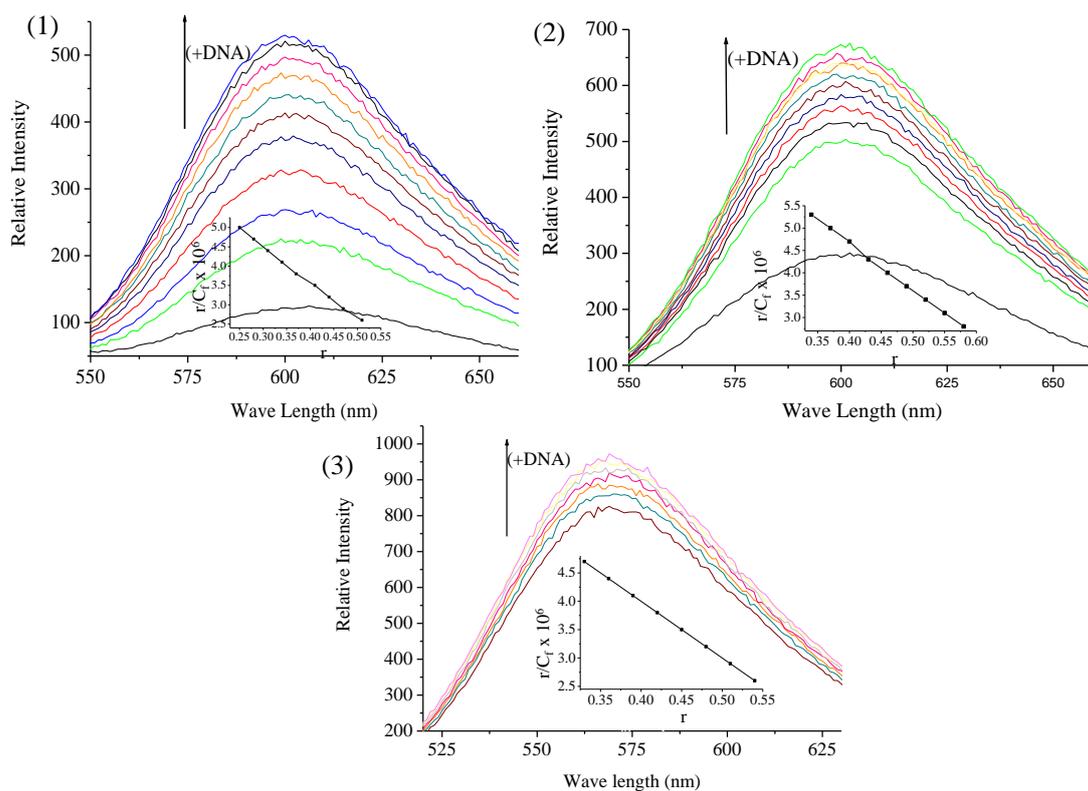
| Complex  | $K_b$ for absorption studies ( $M^{-1}$ ) | $K_b$ for Emission studies ( $M^{-1}$ ) |
|--|---|---|
| [Co(phen) <sub>2</sub> CMIP] <sup>3+</sup> (1) | 3.15 ( $\pm$ 0.01) $\times 10^5$          | 3.11 ( $\pm$ 0.02) $\times 10^5$        |
| [Co(bpy) <sub>2</sub> CMIP] <sup>3+</sup> (2)  | 2.73 ( $\pm$ 0.01) $\times 10^5$          | 2.75 ( $\pm$ 0.01) $\times 10^5$        |
| [Co(dmb) <sub>2</sub> CMIP] <sup>3+</sup> (3)  | 2.50 ( $\pm$ 0.02) $\times 10^5$          | 2.32 ( $\pm$ 0.01) $\times 10^5$        |

### Emission spectroscopic studies

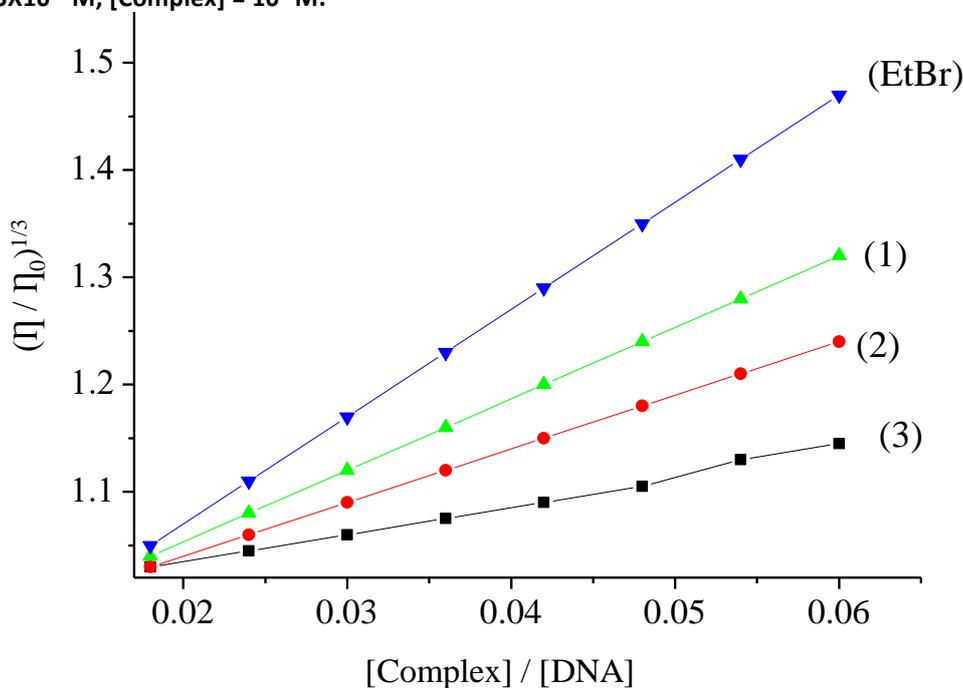
Emission intensity ( $\approx$  600 nm) of complexes from the MLCT excited states upon excitation at 460 nm is found to depend on DNA concentration. As shown in Fig. 3, upon the addition of DNA, the emission intensities increased from initial. The enhancement of emission intensity is indicative of binding of the complex to the hydrophobic pocket of DNA and is protected by DNA efficiently and the complexes mobility is restricted at the binding site, which leads to decrease in the vibrational modes of relaxation. The intrinsic binding constant from fluorescence data was obtained from a modified Scatchard equation<sup>37</sup> for all the complexes, and binding constants ( $K_b$ ) were in the order of  $10^5$  as shown in table 1.  $K_b$  values for both absorption and luminescence studies are almost comparable with small differences in numerical<sup>38</sup>.

### Viscosity experiment

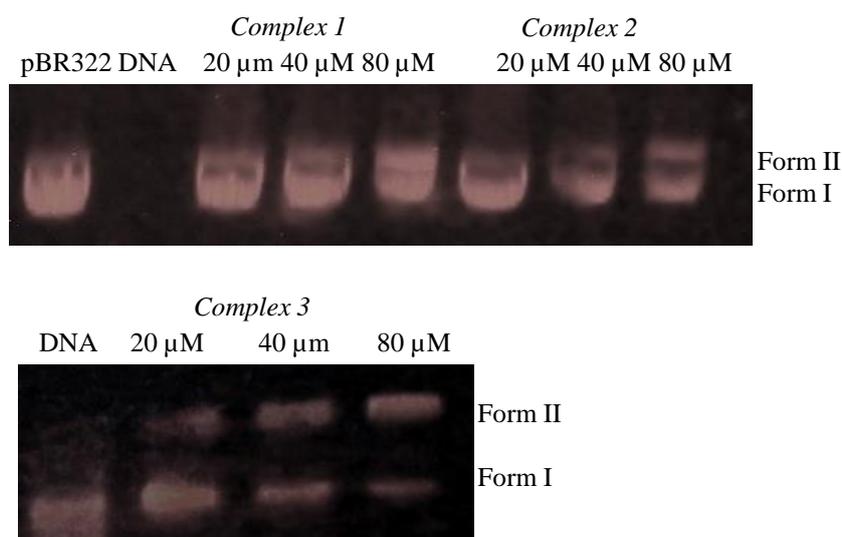
Viscosity experiments are sensitive to length changes to DNA and least ambiguous for binding mode in solution in the absence of crystallographic structural data<sup>39</sup>. The effects of the complex and ethidium bromide (EB) on the viscosities of CT-DNA are shown in Fig. 4. EB, being as a classical DNA intercalator, can strongly raise the relative viscosity by lengthening the DNA double helix through intercalation. As revealed in Fig. 4, upon increasing the amounts of the three complexes, the relative viscosity of DNA increases similar to the behaviour of EB. This observation suggests that the principal mode of DNA binding by the complex involves base-pair intercalation, in which, relative increased of viscosity is in agreement order with the results obtained by electronic absorption, fluorescence spectroscopy.



**Fig. 3:** Emission spectra of three complexes 1, 2 and 3 in the absence and in the presence of increasing concentration of CT-DNA. Inset: Scatchard plot of  $r/C_f$  above complexes, which gives binding constant ( $K_b$ ).  $[DNA] = 2.5 \times 10^{-4} M$ ,  $[Complex] = 10^{-3} M$ .



**Fig. 4:** Effect of increasing amounts of EtBr, complexes  $[Co(phen)_2CMIP]^{3+}$  (1),  $[Co(bpy)_2CMIP]^{3+}$  (2) and  $[Co(dmb)_2CMIP]^{3+}$  (3) on the relative viscosity of CT-DNA at  $25 (\pm 0.1)^\circ C$ .  $[DNA] = 2.5 \times 10^{-4} M$ .



**Fig. 5. Photo activated cleavage studies of  $[\text{Co}(\text{phen})_2\text{CMIP}]^{3+}$  (1),  $[\text{Co}(\text{bpy})_2\text{CMIP}]^{3+}$  (2) and  $[\text{Co}(\text{dmb})_2\text{CMIP}]^{3+}$  (3) with the concentration range of 20 to 80  $\mu\text{M}$ .**

#### Photo activated cleavage of pBR 322DNA

Nuclear activity of 1-3 Cobalt (III) complexes were performed on pBR322 DNA and monitored by agarose gel electrophoresis. When plasmid DNA is subjected to electrophoresis, relatively fast migration is observed in the intact supercoiled form (form I). If scission occurs on one strand (nicking), the supercoiled form will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (form III) that migrates between form I and form II will be generated<sup>40</sup>. Plasmid DNA incubated with three complexes with different concentrations (20  $\mu\text{M}$ , 40  $\mu\text{M}$  and 80  $\mu\text{M}$ ) pBR322DNA and irradiated at 365 nm for 60 min. We observed no DNA cleavage was in the control, in which the metal complex was absent (lane 1). When the concentration of 1-3 Cobalt (III) complexes was increased from 20  $\mu\text{M}$  to 80  $\mu\text{M}$ , the amount of form I gradually decreased, whereas the amount of form II increased as shown in fig. 5.

#### Antimicrobial activity

Three Cobalt (III) complexes were screened invitro for their antimicrobial activity against *E. coli* and *S. Aureus* with Ampicillin as positive control and DMSO as negative control. We observed variation in the inhibition zone (radius in mm) with two different concentrations (0.5 mg/ml and 1 mg/ml) 1-3 complexes. The antimicrobial activity increased as the concentration of the compounds increased. The antibacterial activity data for the complexes at various concentrations (Table 2) indicate that the complexes exhibited appreciable activity against *E. coli* and *S. aureus*. The complexes were more effective against *S. aureus* than against *E. coli* but were less effective than the standard drug ampicillin. As an increase in the lipophilic character of the complex favours its permeation through the lipid layer of the bacterial membrane, it shows more activity [41].

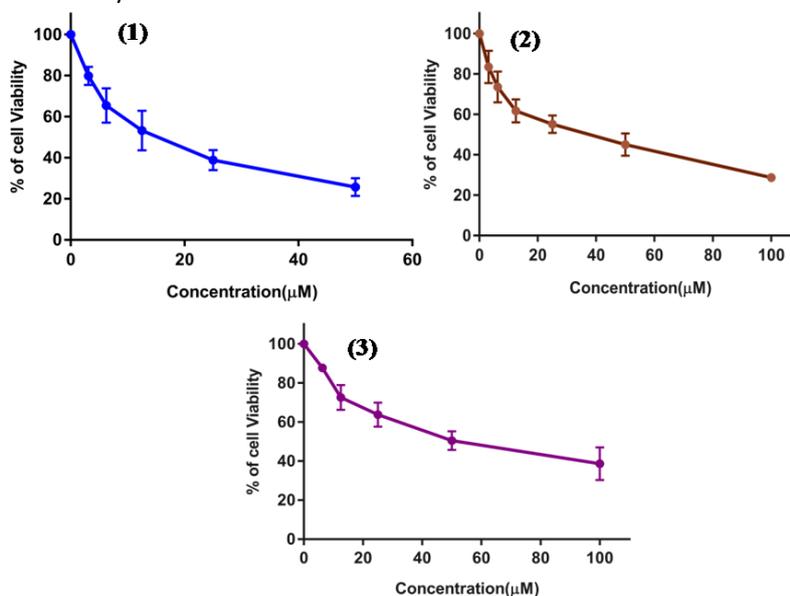
**Table 2: Antimicrobial studies of Complexes 1-3**

| Complex  | Bacterial Inhibition zone (mm) |        |                            |        |
|--|--------------------------------|--------|----------------------------|--------|
|  | Conc. (1000 $\mu\text{M}$ )    |        | Conc. (500 $\mu\text{M}$ ) |        |
|  | B.S                            | E.Coli | B.S                        | E.Coli |
| DMSO   | --                             | --     | --                         | --     |
| $[\text{Co}(\text{phen})_2\text{CMIP}]^{3+}$ (1) | 12.0                           | 10.2   | 6.9                        | 6.6    |
| $[\text{Co}(\text{bpy})_2\text{CMIP}]^{3+}$ (2)  | 10.1                           | 9.7    | 6.0                        | 5.5    |
| $[\text{Co}(\text{dmb})_2\text{CMIP}]^{3+}$ (3)  | 9.5                            | 9.5    | 5.7                        | 4.9    |
| Ampicilin  | 16.2                           | 14.3   | 10.0                       | 9.9    |

### Cell viability studies by MTT assay

All three complexes were tested against HeLa cells to assess the viability by using MTT assay. These three compounds show significant cell death in dose dependent manner. Viability of the cells decreased

upon increasing the concentration of the compound as shown in fig. 6. The IC<sub>50</sub> for complex **1** is 14.1 μM for 48h whereas for complex **2** and complex **3** were 36.2 μM and 52 μM.



**Fig. 6.** HeLa cells were treated with complexes **1**, **2** and **3** with different concentrations for 48h and the untreated cells were used as a control and then cell viability was evaluated by the MTT assay.

### CONCLUSION

Three new Cobalt (III) polypyridyl complexes [Co(phen)<sub>2</sub>CMIP]<sup>3+</sup> (**1**), [Co(bpy)<sub>2</sub>CMIP]<sup>3+</sup> (**2**) and [Co(dmb)<sub>2</sub>CMIP]<sup>3+</sup> (**3**) were synthesized and characterized. The DNA-binding behavior shows that the three Co (III) complexes were interacting with CT DNA through intercalative mode. Upon irradiation, these complexes can effectively cleave pBR322 DNA into three separate forms. The cytotoxicity assay indicates that complexes **1**, **2** and **3** can suppress the tumor cell proliferation in dose dependent manner. In DNA binding studies, complex **1** showed the greatest intrinsic binding constant due to π-π stacking with DNA base pairs may exert some additional interactions such as hydrogen bonding with functional groups present on the edge of the DNA.

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### Abbreviations

CT-DNA: calf thymus DNA  
 HEPES: [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]

DMSO: Dimethyl sulfoxide

TMS: Tetramethylsilane

UV-Vis: Ultra Violet – Visible Spectroscopy

NMR: <sup>1</sup>H & <sup>13</sup>C – Nuclear Magnetic Resonance Spectroscopy

IR: Infra-Red Spectroscopy

ESI-MS: Electrospray ionization mass spectrometry

MLCT: Metal-to-ligand charge transfer

MTT: 3-(4,5-Dimethylthiazol-2-Yl)-2,5-

Diphenyltetrazolium Bromide

Tris: Tris(hydroxymethyl)aminomethane

CMIP: 2-(2-Chloro-8-methylquinolin-3-yl) w-1H-

Imidazo[4,5-f] [1,10] phenanthroline

Phen: 1, 10- Phenanthroline

Bpy: 2, 2' – bipyridine

Dmb: 4, 4' – Methyl, 2, 2' - bipyridine

Ppt: Precipitation

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