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# Synthesis And Biological Evaluation of Fluoroluminescent Ruthenium Complexes as Potential Cancer Therapeutics

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**Abstract:** Ruthenium (II) polypyridyl complexes are one of the most extensively studied and developed systems in the family of luminescent transition-metal complexes. There has been a large amount of interest in the biological applications of these luminescent ruthenium (II) complexes because of their rich photo physical and photochemical properties with a focus on their use as phototherapeutic agents. A new ligand BMPIP (2-(3-methoxy-4 phenoxy phenyl)-1-H-imidazo[4,5-f][1,10]phenantroline) and its four luminescent ruthenium complexes were synthesized and characterized DNA binding studies, photo cleavage studies, anti microbial studies were performed and the cytotoxic activity of the complexes against cancer HeLa cells was evaluated by MTT method. The IC50 values range from 41 ± 0.5 to 62 ± 0.5 μM. Photodynamic therapy (PDT) has recently emerged as a potential valuable alternative to treat microbial infections. In PDT, singlet oxygen is generated in the presence of photo sensitizers and oxygen under light irradiation of a specific wavelength, causing cytotoxic damage to bacteria. However, both complexes exhibit lower cytotoxicity than cisplatin toward HeLa cell lines under identical conditions.

***Key words:*** *pBR322DNA, calf thymus DNA, cytotoxic, BMPIP, polypyridyl complexes, Absorption, emission, viscosity, photocleavage, anciliary, Intercalating.*

## INTRODUCTION

During the past decade, cancer incidence rates are increasing alarmingly due to many reasons [1-2]. Now designing effective anti-cancer drugs are an active research area in the field of pharmaceutical chemistry. Although cisplatin has been in clinical use for over 30 years, due to its dose-limiting nephrotoxicity and the development of drug resistance prevents its potential efficacy [3-5]. While some second generation platinum complexes are less toxic than cisplatin, and others can partially overcome acquired resistance, there has been little success in developing drugs that are active in cancer cell lines resistant to cisplatin. Consequently, there has been considerable interest in the development of ‘‘non-classical’’ platinum complexes that can bind DNA in a different manner than cisplatin and its analogues [6–11]. This provides the impetus to search for alternative metal based drugs..

Ruthenium complexes are considered promising alternatives to platinum complexes. These complexes show promising antiproliferative activity and lower toxicity than platinum-based drugs developed in the last two eras; Clinical studies have been carried out in particular on NAMI-A and KP1019 [12], whose effect is based on activation by reduction of Ru(III) to Ru(II). Furthermore, synthesized ru(II)arene complexes such as RM 175 [13] (or) RAPTA-T [14] have shown antitumor activity both in vitro and in vivo. Research on Ru(II) polypyridyl complexes is particularly extensive due to their ease of synthesis, their interesting chemical, physical and photophysical properties, and their high affinity for nucleic acids. Previous studies have focused on characterizing the in vitro interactions of these complexes with DNA [15-18] and quantifying the effectiveness of the compound as both traditional

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cytotoxic agents and light-activated agents, in photodynamic therapy (PDT) or phototherapy and as sensors or biocatalysts. . An attractive feature of Ru(II) polypyridyl complexes that makes them particularly useful for applications such as biological probes and effectors is the diversity of chemical structures that are readily accessible via coordinated ligand modifications ( 28 ).

In recent years, many authors have published their findings on the DNA binding and anticancer activity of Ru(II) polypyridyl complexes containing various intercalating ligands [29–31]. Some reports suggest that accessory ligands play an important role in biomolecular interactions and recognition processes, so the variability of accessory ligands of Ru(II) complexes may lead to differences in biological activity [32–34]. Our research group has also published several reports on DNA binding studies and anticancer studies on polypyridylruthenium(II) complexes with various intercalating and accessory ligands [35–40]. In this article, we report the synthesis and characterization of the four complexes 1 to 4. DNA binding, photocleavage ability, antimicrobial assays, light on/off properties, and antitumor activity were investigated.

## 2.1 SYNTHESIS

### 2.1.1 Synthesis of ligand 2-(3-methoxy-4phenoxyphenyl)-1-H-imidazo[4,5f][1,10] phenanthroline (BMPIP)

A mixture of phendione (1 mmol) and 4(benzyloxy)3-methoxybenzaldehyde (1.5 mmol), ammonium acetate and glacial acetic acid was heated to reflux with stirring for 2 hours. The solution was cooled and diluted with 25 ml of water. It was neutralized by adding concentrated ammonia dropwise. The precipitate was collected and purified by silica gel column chromatography (60–100 mesh) with ethanol as eluent to produce golden yellow BMPIP needles, which were aspirated, filtered, and recrystallized.(Fig. 1)

**Analytical Data**: $C_{26}H_{18}N_4O_2$Calcd.(%) : C:74.63; H: 4.34; N: 13.39; Found(%): C: 73.98; H: 4.28; N: 12.99; ESI-MS(m/z): 418. $^1$H NMR (DMSO-$d_6$, 400M Hz; TMS, δ-ppm): 8.92681(d, 2H), 7.85505 (d, 2H), 7.49900 (d, 1H), 7.43150 (t, 2H), 7.39470 (t, 2H), 7.34893 (d,1H), 7.21134 (d, 2H), 7.19002 (d, 1H), 5.59111(s,1H).

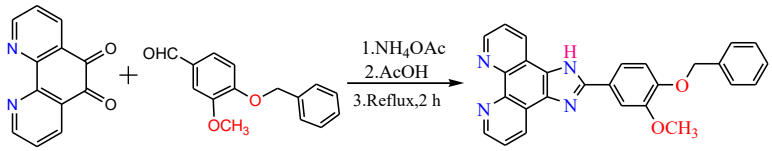



**FIGURE. 1**.Synthesis of BMPIP

### 2.1.2 Synthesis of [Ru(phen)$_2$ BMPIP] $(ClO_4)_2$ $2H_2O$(1)

A mixture of phendione (1 mmol) and 4(benzyloxy)3-methoxybenzaldehyde (1.5 mmol), ammonium acetate and glacial acetic acid was heated to reflux with stirring for 2 hours. The solution was cooled and diluted with 25 ml of water. It was neutralized by adding concentrated ammonia dropwise. The precipitate was collected and purified by silica gel column chromatography (60–100 mesh) with ethanol as eluent to produce golden yellow BMPIP needles, which were aspirated, filtered, and recrystallized.(Fig. 2.1.)

This complex [Ru(phen)$_2$ BMPIP] $(ClO_4)_2$ $2H_2O$ is prepared by the similar procedure described for the above complex with a mixture of cis-[Ru(phen)$_2$Cl$_2$].$2H_2O$ (0.5mmol) and BMPIP (0.5mmol).

Analytical Data: $RuC_{50}H_{38}Cl_2N_8O_{12}$Calcd.(%) C: 53.87; H: 3.44; Cl, 6.36; N: 10.05; Found(%):C: 53.87; H: 3.44; N: 10.05; ESI-MS(m/z): 1114.9. $^1$H NMR (DMSO-$d_6$, 400MHz; TMS, δ-ppm): 9.01327, 8.69094, 8.03417, 7.87807, 7.64645, 7.4075, 7.15914, 5.16826, 3.8678, 3.50643, 2.49590.$^{13}$C-NMR (DMSO-$d_6$, 400MHz;δ-ppm): 125, 40.489, 40.279, 40.072, 39.864, 39.655, 39.448, 39.240, 21.174, 20.

### 2.1.3 Synthesis of [Ru(bpy)$_2$BMPIP] $(ClO_4)_2$ $2H_2O$ (2)

This complex [Ru(bpy)$_2$BMPIP] $(ClO_4)_2$ $2H_2O$ is prepared by the similar procedure described for the above complex with a mixture of cis-[Ru(bpy)$_2$Cl$_2$].$2H_2O$ (0.5m mol) and BMPIP (0.5m mol).

Analytical Data: $RuC_{46}H_{38}Cl_2N_8O_{12}$ Calcd.(%) C, 51.79; H, 3.59; N, 10.50;. Found(%):C, 50.63;

H, 2.83; N, 9.68;. ESI-MS(m/z): 1066. $^1$H NMR (DMSO-$d_6$, 400MHz; TMS, δ-ppm): 8.886(d,2H), 8.8508(d,4H), 8.5306(d,4H), 8.0521(d,2H), 7.8840(t,4H),), 7.6157(t,4H), 7.593(t,2H), 7.4241(d,2H), 7.4053(t,1H), 7.3725(d,1H), 7.3576(d,1H), 7.3406(d,1H). $^{13}$C-NMR (DMSO-$d_6$, 400MHz;δ-ppm): 157, 152, 150, 139, 138, 129, 71, 70.424, 56.336, 40.568, 40.360, 40.151, 39.943, 39.734, 39.526, 39.315.

**2.1.4 Synthesis of [Ru(dmb)$_2$BMPIP] (ClO$_4$)$_2$ 2H$_2$O (3)**

This complex [Ru(dmb)$_2$BMPIP] (ClO$_4$)$_2$ 2H$_2$O is prepared by the similar procedure described for the above complex with a mixture of cis-[Ru(dmb)$_2$Cl$_2$].2H$_2$O (0.5mmol) and BMPIP (0.5mmol).

Analytical Data: $RuC_{50}H_{46}Cl_2N_8O_{12}$Calcd.(%) C, 53.48; H, 4.13; N, 9.98; Found(%):C, 52.63; H, 3.78; N, 8.54; ESI-MS(m/z): 1122.8. $^1$H NMR (DMSO-$d_6$, 400MHz; TMS, δ-ppm): 9.04058, 8.71482, 8.4, 8.04936, 7.89818, 7.4136, 7.16193. $^{13}$C-NMR (DMSO-$d_6$, 400MHz;δ-ppm): 9.04058, 8.71482, 8.4, 8.04936, 7.89818, 7.4136, 7.16193.

**2.1.5 Synthesis of [Ru(Hdpa)$_2$BMPIP] (ClO$_4$)$_2$ 2H$_2$O (4)**

A mixture of cis- [Ru(Hdpa)$_2$Cl$_2$]Cl (0.26 mmol) and BMPIP (0.26 mmol) was heated to reflux in water (30 mL) for 15 min under $N_2$ atmosphere, after which the solution was cooled then the reducing agent 30% $H_3PO_3$ neutralized with NaOH was added to the reaction mixture and the reflux were continued to 3 h and treated with an excess of $NaClO_4$. The precipitated complex cis-[Ru(Hdpa)$_2$BMPIP] (ClO$_4$)$_2$ 2H$_2$O (Fig. 2.5.3.) was dried, dissolved in a small amount of acetonitrile, and purified by chromatography over alumina using acetonitrile – methanol (3:1, v/v) as an eluent. The red coloured compound was obtained after drying in vaccum.

Analytical Data: $RuC_{46}H_{36}Cl_2N_{10}O_{12}$ Calcd.(%) C, 50.56; H, 3.32; N, 12.8; Found (%):C: 49.68; H: 2.89; N, 11.6;. ESI-MS (m/z): 1092.8.$^1$H NMR (DMSO-$d_6$, 400MHz; TMS, δ-ppm): 9.0094 (d, 2H), 8.59 (d, 4H) 8.0 (d, 2H), 7.7612 (d, 4H) 7.3842 (t, 4H), 7.33 (d, 1H), 7.3 (t, 2H), 7.22 (d, 2H),6.98 (t, 1H), 6.8456 (d, 1H), 6.54 (d, 1H), 5.1654 (d, 1H). $^{13}$C-NMR (DMSO-$d_6$, 400MHz;δ-ppm):158,157,154,152.9,152, 149.8, 137,131.1,127, 123.6, 122, 121.5, 116, 110.3, 104.1.

**2.2 Physical Measurements**

All synthesized compounds were characterized by elemental analysis, mass analysis, infrared, electron absorption and nuclear magnetic resonance spectroscopy. Electron absorption spectroscopy in the visible and visible range recorded with an Elico BL 198 spectrophotometer. Fluorescence emission spectroscopy was performed with an Elico SL 174 spectrofluorometer, FTIR spectra were recorded on KBr disks with a Perkin Elmer FT-IR-1605 spectrometer. Microanalyses (C, H, and N) were performed using a Perkin_Elmer 240elemental analyzer. ESI-MS in DMSO was recorded in the LQC system (Finnigan MAT, USA) using CH3CN as mobile phase. Nuclear magnetic resonance spectra (1HNMR and 13C[1H]NMR) were recorded on a 400 MHz Bruker spectrometer with DMSO-d6 as a solvent at room temperature and tetramethylsilane (TMS) as an internal standard. Viscosity experiments were carried out using an Ostwald viscometer. Gel electrophoresis was imaged on a Gel Doc system (Alpha InfoTech Corporation). The Thermo Scientific Multi Skan EX Elisareader was used for the MTT test.

**2.3 DNA Binding Experiments**

**Electronic Absorption AndFluorescence Titrations**

The electronic absorption titrations and emission titrations of the four complexes were monitored in both the absence and presence of DNA-CT. Uptake titrations of the complex in Tris buffer were performed by titrating a fixed concentration of the complex to which successive portions of the DNA stock solution were added. The assembled DNA solutions were incubated for 5 min before recording absorption or emission spectra.To evaluate the binding strength of the complex, the intrinsic binding constant Kb to CT-DNA was determined by monitoring the absorbance change in the metal–ligand charge transfer (MLCT) band with increasing DNA concentration. The internal binding constant Kb of DNA-bound Ru(II) complexes was calculated according to equation [41].

$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/Kb\ (\varepsilon_b - \varepsilon_f)$

where [DNA] is the concentration of DNA. The apparent extinction coefficient ($\varepsilon_a$) was obtained by calculating $A_{obs}$/ [Ru]. The terms $\varepsilon_f$ and $\varepsilon_b$ correspond to the extinction coefficients of free (unbound) and the fully bound complex

respectively. From plot of [DNA]/ ($\varepsilon_a - \varepsilon_f$) against [DNA] will give a slope 1/ ($\varepsilon_a - \varepsilon_f$) and an intercept $1/K_b(\varepsilon_b - \varepsilon_f)$. $K_b$ is the ratio of the slope to the intercept.

In fluoroscence titrations the fraction of the ligand bound was calculated from the relation [42]

$C_b = C_t [(F - F_0)/(F_{max} - F_0)]$,

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 $F_{max}$ is when complex is fully bound to DNA. Binding constant ($K_b$) was obtained from a Scatchard plot of $r/C_f$ against r, where r is the $C_b$/[DNA] and $C_f$ is the concentration of free complex.

**2.4. Light Switch On-Off Effect**

Molecular studies of light switching in the DNA of Ru(II) complexes were carried out in Tris buffer solution at room temperature. A constant complex excitation wavelength was assumed and the emission range was adjusted before measurements. These titrations were performed by sequential addition of Co2+ and EDTA to the DNA-bound complex.

**2.5. Viscometric Titrations**

Viscosity experiments were performed using an Ostwald viscometer placed in a thermostatic water bath to maintain a constant temperature of 30.0 ± 0.1 °C. Calf thymus DNA samples of approximately 200 base pairs were prepared by sonication to minimize complexity due to DNA flexibility. The flow time was measured with a digital stopwatch, each sample was measured three times and the average flow time was calculated. The data are presented as $(\eta/\eta 0)1/3$ versus[complex]/[DNA], where η is the viscosity of the DNA in the presence of the complex and η0 is the viscosity of the DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t>100 s), corrected for the flow time of the buffer itself (t0) [43, 44].

**2.6. DNA Photocleavage And Agarose Gel Electrophoresis**

For the gel electrophoresis experiments pH 8.0 buffer of 40 mM Tris base, 20 mM acetic acid, and 1 mM EDTA were used. A buffer of 10 mM Tris–HCland 1 mM $Na_2EDTA$ was used for dilution of pBR322 DNA. Supercoiled pBR322 DNA (0.1 g/μL) was treated with ruthenium (II) complexes with concentrations of 20–60 μM, and the mixtures were irradiated at room temperature with a UV lamp (365 nm, 10 W) for 60 min. A loading buffer containing 25 % bromophenol blue, 0.25 % xylene cyanol, and 30 % glycerol (2 μL) was added. The samples were then analyzed by 0.8 % agarose gel electrophoresis at 50 V for 2 h. The gel was stained with 2 μL (from 1 mg/100 μL) ethidium bromide [45] and photographed under UV light. The gels were viewed with a gel documentation system and photographed using a CCD

**2.7 *Biological* Activity**

The in vitro antifungal and antibacterial activities of the four complexes were tested using the agar well diffusion method. Agar bioassay was used to test antifungal activity. The prepared potato dextrose agar (PDA) medium (Hi medium, 39 g) was suspended in distilled water (1000 ml) and heated to boiling until completely dissolved. The medium and Petri dishes were autoclaved for 20 minutes at 15 lbs/boost. Once the plating medium had solidified, 0.5 ml (1 week) of theculture of the test organism was inoculated and spread evenly on the agar surface using a sterile L-shaped swab. The solutions were prepared by dissolving the compound in DMSO at different concentrations. were received. After inoculation, wells were removed with a sterile 6 mm corkscrew and lids were placed on the plates. Different concentrations of the test solutions were added to each well. The controls were maintained. Processed samples and controls were stored at 27 °C for 48 h.inhibition zones were measured and the diameter was calculated in millimeters. Three to four replicates were performed for each treatment.

**2.8. Cytotoxicity Assay**

Standard MTT testing procedures were used [46]. Cells were added to 96-well microassay culture plates (8 × 103 per well) in 200 μL and cultured overnight at 37 °C in a 5% CO2 incubator. Complexes in the concentration range 1–100 μM, dissolved in DMSO, were added to the wells. Control wells were prepared by adding culture medium (200 μl). The plates were incubated for 48 hours at 37 °C in a 5% CO2 incubator. After 48 hours of incubation, approximately 20 μL of MTT dye stock solution was added to eachwell, the plates were wrapped in aluminum foil and incubated at 37 °C for 4 hours. The purple formazan product was dissolved by adding 100 μL of 100% DMSO to each well. Absorbance was monitored at 620 nm using a 96-well plate reader. Wells with cell-free culture medium were used as a negative control and cisplatin was used as a

positive control. DMSO was used as a vehicle control. A cisplatin stock solution (10 mM in DMSO) was prepared for eachexperiments. Stock solutions of metal complexes were prepared in DMSO and the DMSO percentage was kept between 0.1 and 2% in all experiments. DMSO itself was found to be non-toxic to cells up to a concentration of 2%. The data were collected in triplicate to obtain average values. IC50 values were determined by plotting percent viability versus concentration on a logarithmic graph and reading the concentration at which 50% of cells remained viable compared to the control. This study used HeLa (a human cervical cancer cell line).

**2.9. Molecular Docking:**

The DNA crystal structure was downloaded from the protein database (PDB ID: 5J3G) into the Molecular Operating Environment (MOE) software ( 47 ). All water molecules were removed and all polar hydrogen atoms were added to the DNA structure, preserving its standard geometry. Energy minimization was then applied. The 3D structure of the Ru(II) complexes was drawn using Chemsketch software and saved in Mol2 file format, and the energy was minimized in Discovery Studio. All complexes were docked to DNAusing MOE. It contains a list of conformations and the best fit to the active site was found based on the interaction energy between the DNA and the complex. The MOE-S score is used to estimate the binding energy of the highest scoring conformations.

## 3. CHARECTERIZATION

### 3.1 Elemental Analysis

The percentage of elements such as carbon, nitrogen and hydrogen (C, N and H analysis) in the complexes is determined by elemental analysis. As a result of this analysis, the empirical formulas of transition metal complexes are determined. As expected, this elemental analysis agrees well with the calculated values except for experimental errors.

### 3.2. Viscosity Measurement

Viscosity measurements explain the interactions of Ru(II) complexes with DNA. How the complexes bind can be determined by viscosity measurements. Photophysical and optical probes provide essential assistance in changing DNA length after binding. In the absence of crystallographic studies, viscosity is considered the least ambiguous and most critical test of the binding pattern in solution (48, 49). In classical intercalation, the DNA helix elongates as the base pairs moveapart to accommodate the bound ligand, resulting in an increase in the viscosity of the DNA solution ( 42 ). In Fig. 2, the relative viscosities of complexes 1 to 4 are compared with the relative viscosity of the known intercalator ethidium bromide. For all four complexes, the relative viscosity increased steadily with the intercalation binding mode, as expected [50].The order of relative viscosity of the 1-4 complexes are as follows

EtBr > $[Ru(phen)_2BMPIP]^{2+}$>$[Ru(Hdpa)_2BMPIP]^{2+}$>$[Ru(bpy)_2BMPIP]^{2+}$>$[Ru(dmb)_2BMPIP]^{2+}$

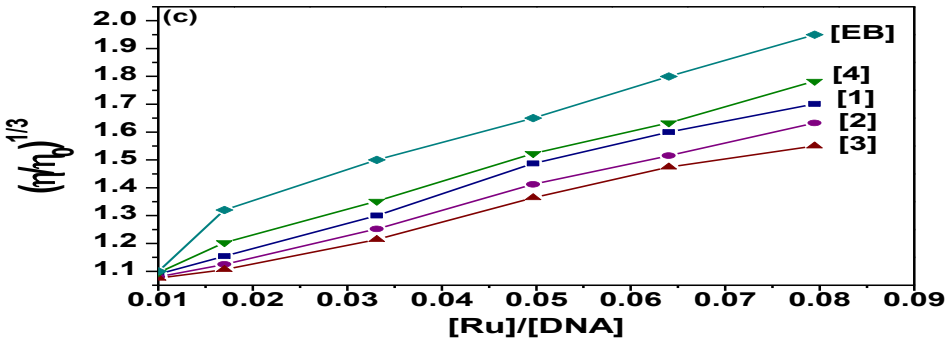



**FIGURE2.** shows effect of increasing amount of ethidium bromide and complexes 1-4 on relative viscosity of CT-DNA at 30 ± 0.1°C.

### 3.3. Absorption spectral studies (UV-Vis spectra):

The absorption spectrum of the BMPIP ligand shows a peak at 330 nm, which corresponds to the transition of the ligands. The absorption spectrum of complexes 1 to 4 showed a peak at 360 nm, corresponding to the transition of the ligands, and another peak at around 460 nm due to the

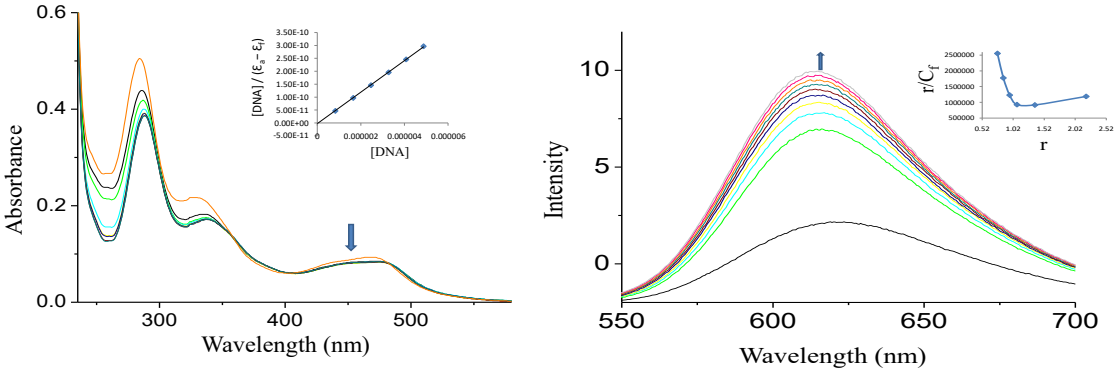
absorption peak for charge transfer from the MLCT metal to the ligand, indicating the formation of complexes. It was also observed that in the complexes the peak shifted from 330 nm to 360 nm, which corresponds to a longer wavelength. In the visible region, the lowest energy bands at 438 nm,for complex 1, 460 nm for complex 2, 472 nm for complex 3 and 460 nm for complex 4, are attributed to the metal-ligand charge transfer (MLCT) Ru (dπ) → Attributed to ligand (π*) transitions. In the case of metal intercalators, DNA binding is associated with hypochromism and red shift in the MLCT band. These complexes showed hypochromia of 28.2%, 25.6%, and 23.7% and 21.1% in the MLCT band, respectively. To further elucidate the binding strength of the complexes, internal binding constants ofKb were determined by monitoring the absorbance changes in the MLCT band. These spectral features obviously suggest that complexes 1, 2, 3, and 4 interact strongly with DNA in a manner that involves a stacking interaction between the aromatic chromophore and the DNA base pairs. The electronic absorption spectrum of the [Ru(phen)2BMPIP] $^{2+}$ complex is shown in Fig 3(A). The values of the binding constants are listed in Table 1.

**3. 4. Fluorescence spectroscopic studies:**

Fluorescence titrations were performed to explore drug-DNA interaction, because it is one of the most common and sensitive methods in DNA-binding studies. The emission titration of complexes 1-4 were carried out in tris buffer at room temperature. the fluorescence spectra were recorded in the presence of varying concentrations of the complexes[51, 52]. The complex emits prominent luminescence in Tris-buffer with a maximum emission wavelength of about 650 nm. The well-behaved titration of the complex with CT-DNA is displayed in Fig 3.(B) shows fluorescence spectrum of complexes 1 with addition of CT–DNA, in Tris buffer with increasing concentration of CT-DNA. The arrow shows the fluorescence intensity change upon increase of DNA concentration. Inset: Scatchard plot of $r/C_f$ vs r.

On addition of CT-DNA to the complex, enhancement in fluorescence without wavelength shift was observed in the region of 340-360 nm

The complexes $[Ru(phen)_2BMPIP]^{2+}$ (1), $[Ru(bpy)_2BMPIP]^{2+}$ (2) $[Ru(dmb)_2BMPIP]^{2+}$(3) and $[Ru(Hdpa)_2BMPIP]^{2+}$(4) exhibits fluorescence in the absence and presence of CT–DNA, in tris buffer at ambient temperature with $\lambda_{max}$ at 616, 612, 608 and 605 nm, respectively. The ratio of [DNA]/[Ru] for complexes 1,2,3 and 4 increased to about 1.72 times (1), 1.65 times (2), 1.59 times (3) and 1.53 times (4) more than their respective complex alone 3.3.(c).




(A) (B)

**FIGURE. 3.(A)**Absorption spectrum of $[Ru(phen)_2(BMPIP)]^{2+}$ (1) in tris-buffer upon addition of CT–DNA in absence and presence of CT–DNA, the [complex] = 10–15 µM; [DNA] = 0–126 µM. Insert plots of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA] for the titration of DNA with complex. Arrow shows change in absorption with increasing DNA concentration.

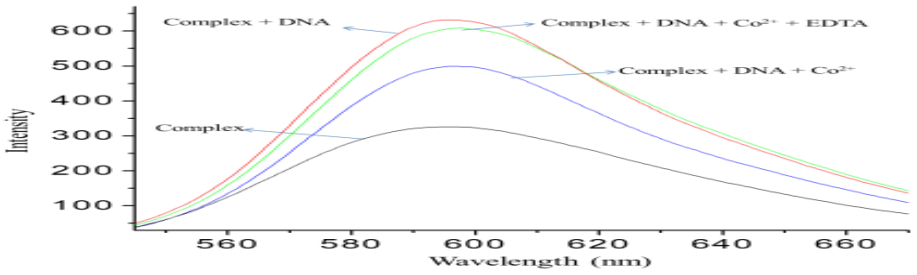
**FIGURE. 3.(B)**The fluorescence spectrum of $[Ru(phen)_2BMPIP]^{2+}$(9) with addition of CT–DNA, in Tris buffer with increasing concentration of CT-DNA. The arrow shows the fluorescence intensity change upon increase of DNA concentration. Inset: Scatchard plot of $r/C_f$ vs r.

**Table 1: $K_b$ values of complexes 1-4**

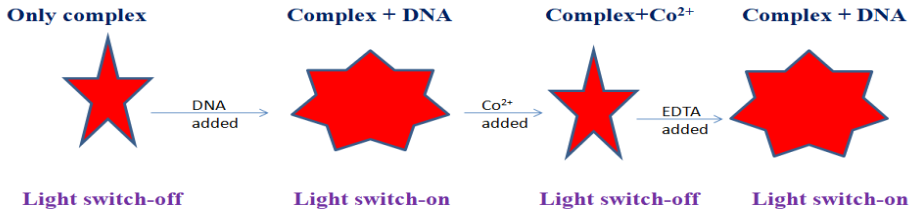
| Complex | UV/Vis $K_b$ | Fluorescence $K_b$ |
|---|---|---|
| 1 | $6.38x10^5$ | $8.50x10^5$ |
| 2 | $3.17x10^5$ | $3.88x10^5$ |
| 3 | $3.00x10^5$ | $3.63x10^5$ |
| 4 | $4.79x10^5$ | $8.01x10^5$ |

### 3.5 Light Switch On-Off Effect

Cyclic switching on and off of Ru(II) complexes studied in the presence of CT-DNA. Transition metals are generally believed to quench light to confirm DNA intercalation. Molecular "light switch" research has primarily consisted of the search for a new "light switch" complex and DNA. When the complex binds to DNA, the emission intensity is maximum (turn on), when Co2+ solution is added to the DNA-bound complex, the emission intensity is, which is due to the formation of a low emission species, more likely. the formation of Co2+-[Ru(phen)2BMPIP]2+, resulting in electron transfer or change in electronic structure. After further addition of EDTA to the Co2+-[Ru(phen)2BMPIP]2+ system, the emission intensity of the complex is restored (light on), as shown in Fig 4.(A) and Fig 4 (B). This means that the heterometallic complex is released after the formation of a strong Co2+-EDTA complex. A similar observation was made for other complexes. The change in luminescence of the DNA-bound complex indicates its use in modulating drug therapy.




(A)




(B)

**FIGURE4(A)**DNA light switch on and off experiments showing the luminescence changes upon addition of $Co^{2+}$,EDTA to $[Ru(phen)_2BMPIP]^{2+}$ + DNA.

**FIGURE 4(B)** Pictorial representation: Luminescence modulation routes of complexes

### 3.6 Photoactivated Cleavage Studies

When circular plasmid DNA is subjected to electrophoresis, relatively rapid migration to the intact supercoiled form (Form I) is observed; When a cleavage (cut) occurs on a filament, the supercoil relaxes and forms a slower moving open circular shape (Form II) [53]. Figure.5 shows the separation of pBR322 DNA by gel electrophoresis after incubation with four complexes 1 to 4 at different concentrations and irradiation at 365 nm for 60 minutes. No obvious DNA cleavage was observed in the control containing no complex. As the concentration of the four complexes increased, the amount of Form I gradually decreased while the amount of Form II increased. Furthermore, the cleavage effect was found to be concentration

dependent. Under comparable experimental conditions, all complexes 1–4 exhibit efficient DNA photocleavage activity, confirming their DNA binding affinity.

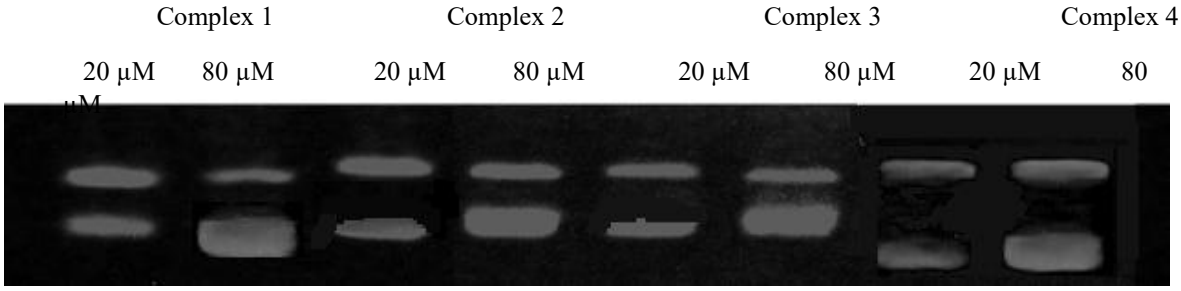



**FIGURE 5** shows the separation of pBR322 DNA by gel electrophoresis after incubation with four complexes 1 to 4 at different concentrations and irradiation at 365 nm for 60 minutes

### 3.7 In Vitro Cytotoxicity Assay

The cytotoxicity of all complexes against HeLa cell lines was evaluated by MTT assay. Cisplatin and DMSO used as positive and negative controls, respectively. After treatment of HeLa cell line for 48 h with complexes 1-4 in the range of concentrations (4-100µM). The inhibitory percentage against growth of cancer cells was determined. The cytotoxicity of complexes was found to be concentration-dependent. Cell viability decreased with increasing concentration of complexes 1 to 4 (Fig. 6). The $IC_{50}$ values for complexes 1 to 4 and cisplatin are shown in Table 2.

**Table 2. $IC_{50}$ values of complexes 1-4**

| COMPLEX | $IC_{50}$ µM |
|---|---|
| 1 | 41.34±0.5 |
| 2 | 48.32±0.5 |
| 3 | 62.53±0.5 |
| 4 | 57.99±0.5 |
| CISPLATIN | 4.8±0.5 |

**FIGURE6**.HeLa cells were treated with Cisplatin and complexes 1-4 for 48 hrs incubation.

### 3.9. Biological Activity OfComplexes

The experimental results of antimicrobial activity indicated a variable degree of efficacy of the compounds against different strains of bacteria.

The in vitro antifungal activity of four complexes against fungal strains such as Candida albicans (MTCC 227), Saccharomyces cerevisiae (MTCC 36), Rhizopus oryzae (MTCC 262), and Aspergillus niger (MTCC 282) was tested using the well diffusion method of agar. Amphotericin was used as a standard. The antibacterial activity of complexes 1 to 4 was tested against three representative gram-positive

organisms, namely, Bacillus subtilis (MTCC 441), Staphylococcus aureus (MTCC 96), Staphylococcus epidermidis andgram-negative organisms, viz. Escherichia coli (MTCC 443), Pseudomonaseruginosa (MTCC 741), and Klebsiella pneumoniae (MTCC 618) using the broth dilution method recommended by the National Committee for Clinical Laboratory (NCCL). Penicillin and streptomycin will be used as standards under conditions identical to the purpose of comparison. . The minimum inhibitory concentrations (MIC) of complexes 1 to 4 are listed in Table 3.3.3. Experimental results of antimicrobial activity showed different levels of effectiveness ofcompounds against different bacterial strains. The compounds did not show significant antifungal activity, but did show antibacterial activity.

**Table 3The MIC values of the complexes 1-4.**

| MIC (µg/ml ) | | | | | | |
|---|---|---|---|---|---|---|
| **Complex** | ***B.Subtilis*** | ***S.aureus*** | ***S.epidermidis*** | ***E.coli*** | ***P.aeroginosa*** | ***K.pneumoniae*** |
| 9 | 25 | 31 | 35.7 | 34.2 | 42 | 61.5 |
| 10 | 75 | >150 | 75 | >150 | 150 | 150 |
| 11 | 58 | 35 | 27 | 51 | 39 | 81 |
| 12 | 41 | 46 | 40 | 79 | 52 | 64 |
| Penicillin | 1.562 | 1.562 | 3.125 | 12.5 | 12.5 | 6.25 |
| Streptomycin | 6.25 | 6.25 | 3.125 | 6.25 | 1.562 | 3.125 |

### 3.10 Docking Studies

Molecular docking [54] was performed to further investigate the theoretical binding affinity of the complex to DNA. The best pose was selected and used for further study. The docking results showed that Ru(II) complexes bind to the DNA molecule via hydrogen bonds and vander Waals interactions, which can be evaluated very quickly during the docking process. Hydrogen bonds play an important role in the interaction of the complex with DNA. It is evident that all complexes bound to theDNA binding cavity and formed base pair interactions. Receptor interacting residues, S-score and hydrogen bond distance for all complexes are listed in Table 4. The interactions between the complex and the DNA are shown in the fig. 7

**Table.4. Interacting residues, S-Score and H- bond distance (Å) of complexes 1-4**

| **Complex** | **S-Score** | **Receptor Interacting residues** | **Hydrogen Bond residues** | **Hydrogen Bond distance** |
|---|---|---|---|---|
| 1 | -9.6887 | DG4, DC5 | Compound N1 – DG4;<br>Compound N4 – DC5 | 2.91<br>2.65 |
| 2 | -9.7852 | DG6, DG3 | Compound N2 – DG6;<br>Compound N3 – DG3 | 2.78<br>2.53 |
| 3 | -8.8743 | DG6, DG4 | Compound N2 – DG6;<br>Compound N2 – DG4 | 2.16<br>4.02 |
| 4 | -9.9048 | DG4 | Compound N2 – DG4;<br>Compound N1 – DG4 | 2.64<br>2.94 |

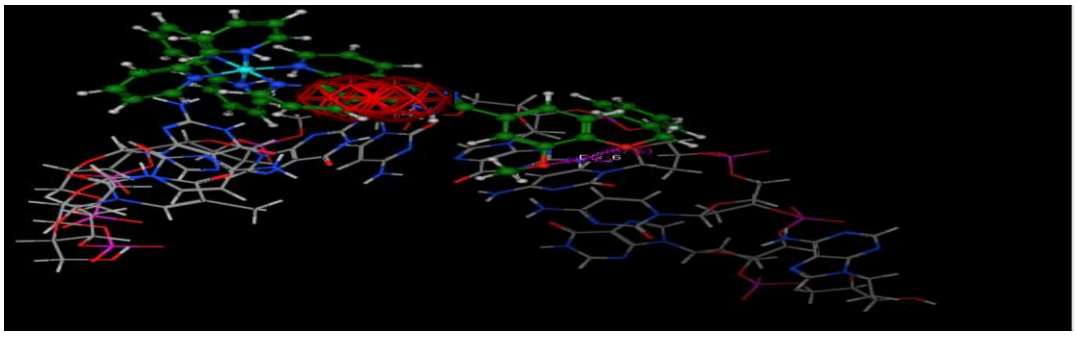


**FIGURE7** Binding interactions between complex 1 DNA (PDB ID: 5J3G)

## 4. CONCLUSION

The focus of this work is to investigate DNA binding and cleavage studies of metallopolypyridyl complexes that have photoactive intercalating ligands and various accessory ligands. Taken together, these results provide new insights into understanding the fundamental biological interactions and molecular mechanisms of Ru(II) polypyridyl complexes as well as the development of Ru(II)-specific neutral anticancer drugs. The four Ru(II) complexes $[Ru(phen)_2(BMPIP)]^{2+}$(1), $[Ru(bpy)_2(BMPIP)]^{2+}$(2),$[Ru(dmb)_2(BMPIP)]^{2+}$ $[Ru(hdpa)_2(BMPIP)]^{2+}$(4) weresynthesized and characterized. In vitro cytotoxicity tests showed that four complexes have antitumor activity, but it is weaker than cisplatin. Complex (1) exhibits stronger antitumor activity than the other three complexes. The antiproliferative effect of 1–4 on tumor cells is consistent with their binding strength, which is significantly improved by increased lipophilicity. Using fluorescence studies, light on/off studies, andphotodivision studies, we can confirm that these complexes can serve as PDT probes. The results of binding and anticancer studies of all of these complexes are comparable to those of their parent intercalating ligands pip, ppip, with minor differences in results. The information in the article is useful for the design and synthesis of Ru(II) complexes with potent cytotoxic activity with various intercalated ligands and auxiliary ligands.

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