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NEW RUTHENIUM (II) POLYPYRIDYL COMPLEXES BEARING 2-(4-METHYLTHIO) PHENYL-1H-IMIDAZO [4, 5-F][1,10] PHENANTHROLINE LIGAND: SYNTHESIS, CHARACTERIZATION, BIOPHYSICAL STUDY AND BIOLOGICAL ACTIVITY.

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Abstract:

Two mononuclear Ru(II) complexes of the type $[Ru(A)_2(MTPIP)]^{+2}$ in which A = bpy (1) 2,2'-bipyridine and dmb (2) 4,4'-dimethyl-2,2'-bipyryridine and an intercalative ligand, MTPIP = 2-(4-methylthio)phenyl)-1H-imidazo [4,5-f][1,10] phenanthroline have been synthesized and characterized by spectroscopic techniques (Electronic, Infrared, ^{1}H NMR as well as ^{13}C NMR, ESI-Mass spectroscopy). The interaction of the synthesized complex with DNA was studied by electronic absorption titration, luminescence titration and viscosity measurements; as well as these complexes effectively cleaved pBR 322 DNA.

The study reveals that the Ru(II) polypyridyl complex bind to DNA preponderantly via intercalation. The binding constant K_b was $1.5x10^5$ and $1.1x10^5 M^{-1}$ (from UV-Vis absorption studies) and $4.0x10^4$ and $3.8x10^4$ (from fluorescence emission studies) and Stern-Volmer quenching constant (K_{sv}) was $5.3x10^3$ and $3.4x10^3$ for complexes 1 and 2 respectively. Finally viscosity measurements revealed that the binding of the complexes with CT-DNA could be an intercalative mode of binding. The antimicrobial activity of Ru(II) complexes was done against Gram positive and Gram negative microorganisms and found to have profound activity. The anti cancer activities of the complexes were investigated by MTT assay against MCF-7 cell line with IC_{50}= 20.96±0.57 µg/ml and 21.11±0.08µg/ml. The result indicates that the synthesized Ru(II) complex 1 having slightly stronger DNA binding affinity and anticancer activity than complex 2, suggesting that anti cancer activity of the Ru(II) complexes could be related to its interaction with DNA.

Keywords: Polypyridyl ligand, DNA binding, Photo cleavage, Ru(II) complexes, Cytotoxicity, Antimicrobial activity, Biophysical Studies.

1. INTRODUCTION

Over the past decades, the DNA binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron transfer probes, DNA foot printing and sequence-specific cleaving agents and potential anti cancer drugs [1-6].Earlier studies have deals with the in vitro interactions of these complexes with DNA [7-13]. Since DNA is the material of inherence and controls the structure and function of cells, the binding of metal complexes with DNA has been extensively studied [14-15].Metal chelates which bind to DNA-Strand as a reactive models for protein-nucleic acid interaction provided routes towards rational drug design to develop sensitive probes for DNA structure [16] and to get information about drug design tool of molecular biology [17-18]

Bacterial infections are a significant global health concern due to the enhanced multidrug resistant pathogens [19-20]. Significant efforts are dedicated to design new antibiotics as well as improving the existing ones. Infection diseases and resistance to antimicrobial drugs is an increasing threat to humans [21-23]. IDSA (Infectious Disease Society of America) has classified bacteria of particular concern. *Entero Coccus faecium, staphylococcus aures,klebsilla pneumonia, Acinoto bacter baumannii, pseudomonas aeruginosa*, and *Enterobacter Spp*. as the ESKAPE pathogens causing a wide range of hospital- acquired infection [24-25]

Metal chelates which bind to DNA-strands as reactive models for protein- nucleic acid interaction provide routes towards rational drug design to develop sensitive probes for DNA structure [26] and to get information about drug design tools of molecular biology [27-28].Polypyridyl metal Complexes can bind to DNA in a non-covalent interaction mode, such as groove-binding for ancillary ligands,electrostatic binding for cations[29].The DNA lengthening and the double helix Stabilization happen when an intercalator binds owing to the increased hydrophobic stacking of the molecule with the base pairs. The method of DNA stretching has also been used successfully studied by binding simple intercalator and groove binders. Ruthenium based compounds are known for their significance as drug nominees, though they have very little in common with the existing platinum-based drugs.

Antitumor Potential of these compounds was established over two decades ago, but interest to explore their cytotoxic profile was very low, possibly because they do not mimic cisplatin in their mode of action[30].There are several metal-based drugs in literature, especially ruthenium-based drugs to be used[31a-d].Three major important properties of ruthenium complexes make it suitable for medicinal application: (i) the range of accessible oxidation state,(ii) the rate of ligand-exchange and (iii) the ability of ruthenium to mimic iron in binding to certain biological molecules. Ruthenium-based complexes show very good antimicrobial activity [31e-g]

Ruthenium the most attractive metal owing to its chemical and air stability; structural variety, low toxicity, and capability to resemble iron binding in a biological system[32-34].The distinct photophysical properties of ruthenium complexes compel as DNA probes, cellular imaging, protein monitoring, and anticancer activity[35-42]. Presently, a Ruthenium complex [NKP-1339] (trans-[tetrachloride bis(1H-indazole) Ruthenate(III)] has efficiently entered into the clinical trials [43-44].

Ruthenium compounds possess light switch behavior and enhance the emission intensity upon intercalation into base pairs of DNA[45-46].The photocleavage studies of these complexes with pBR 322 DNA, cytotoxic studies against to the specific selected cell lines shows that all the complexes demonstrated effective activity in a dose-dependent manner. Kinetically Ru(III) complexes are less reactive than Ru(II) complexes[47].

4-(Methylthio)benzaldehyde is a essential moiety for the synthesis of various pharmaceutical and biological active compounds. It is the intermediate for the synthesis of a pyrrole derivatives showing anti-inflammatory activity [48-49]. Imidazoles are heterocycles with a wide range of applications and are receiving growing attention[50].The imidazole ring systems is of particular interest because it is a component of histidine and its decarboxylation metabolite histamine[51].The potency and wide applicability of the hydrogen bond donar-acceptor capability as well as high affinity for metals, which are present in many protein active site[52] (e.g.Zn,Fe,Mg). Ruthenium polypyridyl complexes have been intensively investigated due to their excellent chemical stability, facile electron transfer, strong luminescent emission and relatively long-lived excited states as well as their extensive applications in the field of photochemistry, photophysics and biochemistry[53-56].

In this current study, the ligand MTPIP and two of its Ru(II) polypyridyl complexes have been synthesized, characterized and investigated for their DNA binding, photo cleavage, anti-microbial action and cytotoxicity. Studies on absorption, emission and viscosity, and photo cleavage indicate that the complexes interact with DNA in an intercalating manner.

2. EXPERIMENTAL SECTION

2.1. Materials and physical measurements

The required Reagents were of analytical quality and used as received unless otherwise specified.1,10-Phenanthroline monohydrate (phen), 2,2'-bipyridine (bpy), and 4,4'-dimethyl-2,2'-bipyridine (dmb), $RuCl_3.3H_2O$, dimethyl sulfoxide, CT-DNA were acquired Sigma-Aldrich. Supercoiled pBR 322 plasmid DNA (stored at 20^0C) obtained from Ferments Life Sciences. Agarose gel was purchased from Genei. Remaining all other chemicals and solvents were obtained from local available sources. The other materials were obtained from commercial sources and used without further purification.All solvents were purified before use as per standard procedures. Ultrapure Milli-Q water (18.2 mX) was used in all experiments.Tris buffer(PH=7.2) was prepared in double distilled water using 5 mmol Tris-HCl and 50 mmol NaCl to carried out the study of the binding affinity of the complexes with CT-DNA. Elemental analyses were performed on PerkinElmer 240 analyzer for the elements of C, H and N. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400/DRX spectrometer with DMSO-d6 as solvent. Infrared spectra were recorded on a PerkinElmer 1605 FTIR spectrometer as KBr disks. UV–Vis spectra were obtained on a Shimadzu UV-2600 UV-visible spectrometer. The Cary Eclipse instrument serial number (MY 12400004) Spectroflurometer was used to record the luminescence spectral data needed to calculate the binding constant. Ostwald Viscometer was used for viscosity measurements. A Quattro LC triple quadrupole mass spectrometer equipped with the Mass Lynx software (Micro mass, Manchester, UK) 3 was used to record the mass spectra. Calf thymus DNA (CT-DNA) was obtained from Sigma Chemicals and used as received. The concentration of CT-DNA solutions was determined spectrophotometrically using the reported molar absorptivity of $\varepsilon 260nm = 0.66 \times 10^4\ M^{-1}\ cm^{-1}$. A solution of CT-DNA in Tris–HCl buffer gave a ratio of UV absorbance at 260 and 280 nm, $A260/A280 \geq 1.9$, indicating that the CT-DNA was sufficiently free from protein.

Stock solutions of CT-DNA were prepared in the buffer (U.V. absorbance at 260 nm after 1:20 dilutions) and were kept at 40°C were used for not more than four days. A Stock solution of metal complexes was made by dissolving computed amounts of metal complexes in DMSO and diluting to the concentrations required for all experiments with the appropriate buffer.

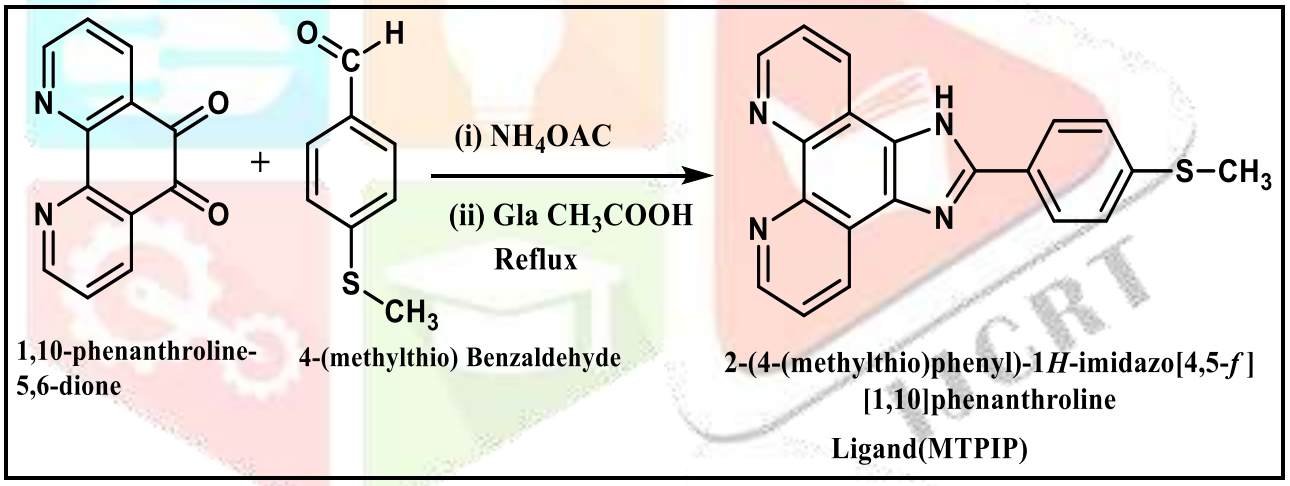
2.2. Synthesis and Characterization of MTPIP Ligand and its Ru(II) Complexes

The starting materials 1,10-Phenanthroline-5,6-dione (Phen-dione), cis- $[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (bpy = 2,2' -bipyridine) and $[Ru(dmb)_2Cl_2].2H_2O$ (dmb=4,4'-dimethyl-2,2'-bipyridine) were synthesized according to the literature methods.

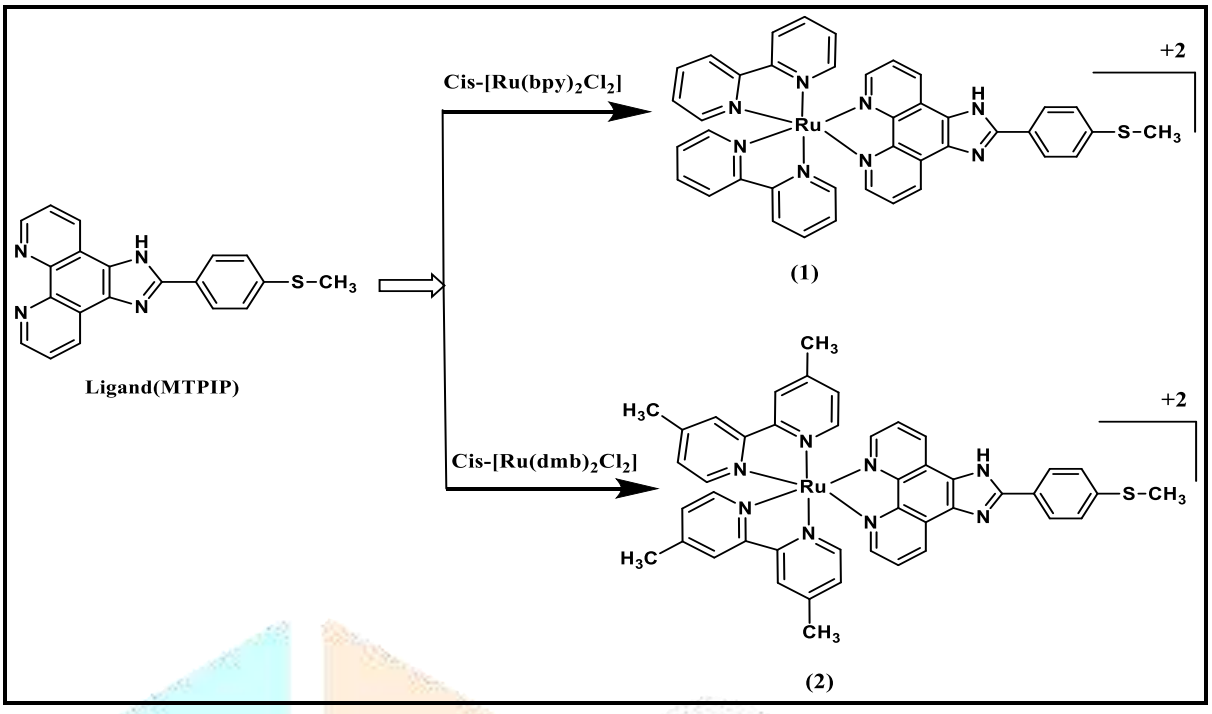
2.3. Synthesis of MTPIP Ligand

As per the literature method, the MTPIP ligand was synthesized using 1,10-Phenanthroline-5,6-dione (0.25gm, 1.18 mmol), 4-(Methylthio) Benzaldehyde (0.18 gm, 1.18 mmol) and ammonium acetate (2.334 gm,30 mmol) were dissolved in glacial acetic acid (15ml) and then refluxed for 5 hour .The result was a distinct brick-red colour solution, cooled to room temperature and transferred to distilled water, where the con NH_3 was added drop wise to produce an orange-yellow precipitate that was collected. Washed with H_2O and dried. The crude product recrystallized with $C_2H_5N.H_2O$ and dried. Yield: 68.4%, Scheme 1 illustrates.

Analytical data for $C_{20}H_{14}N_4S$: Calc (%): C,70.1; H,4.09; N,16.36; Found (%):C,70.3; H,4.12; N,16.29; ESI-MS (m/z):Calc 342; Found:343.4. ^{1}H NMR (DMSO-d6, 400 MHz and δ ppm): 9.04(d,2H), 8.94(d,2H), 8.24 (d,2H), ,7.85(t,2H),7.51(d,2H),2.50 (s,3H,CH_3).^{13}C [^{1}H] NMR (100, MHz, DMSO-d6, ppm):150.35,147.73, 143.35,140.43,129.75, 126.61 ,126.29, 125.85, 123.35, 14.32; IR (KBr,cm^{-1}): 3454 (*ϑ*,N-H), 2924 (*ϑ*,C-H), 1741 (*ϑ*,C=N), 1368 (*ϑ*,C-N),1213(*ϑ*,C-S)



Scheme.1. Synthetic route for the preparation of MTPIP ligand



Scheme 2 Synthetic route for the preparation of Ruthenium (II) Polypyridyl Complexes

2.4. Synthesis of Ru(II) complexes 1&2

2.4.1. Synthesis of $[Ru(bpy)_2(MTPIP)](ClO_4)_2.2H2O$

A mixture of cis-$[Ru(bpy)_2Cl_2].2H_2O$ (0.5mM,0.172g) and MTPIP ligand (0.5 mM,0.171 g) in ethanol (15ml) refluxed for 14 h at 120^0C under a purge of N_2 gas. The resulting light purple colour solution was cooled to room temperature before adding an equivalent amount of saturated aqueous $NaClO_4$ solution under vigorous stirring. The red solution under was collected and cleaned with modest amount of diethyl ether, ethanol and water, before being vacuum dried (Yield 62.3), Scheme 2 illustrates.

Analytical date for $C_{40}H_{30}$ N_8 Ru S; Calc (%): C, 63.56; H,4.0; N,14.82; Found (%):C,63.24;H,3.92;N,13.97;ESI–MS(m/z):$[M\text{-}2ClO_4\text{-}H^+]^+$:755,

$[M^{+2}\text{-}2(ClO_4)]$:378.3. ^{1}H NMR data(DMSO-d_6,400MHz and δ ppm): 9.09(d,2H), 8.89(d,4H),8.86(d,2H),8.26(t,4H),8.13(t,2H),8.07(d,4H),7.91(d,2H),7.85(d,2H),7.34(t,4H), 2.51(s,3H,CH_3). ^{13}C [^{1}H] NMR (100 MHz,DMSO,D_6,ppm): 156.78,156.57 , 151.51,151.42,137.98,127.90,126.9,125.9,124.4,14.27.

IR (KBr, CM^{-1}):3599(ϑ, N-H), 2924 (ϑ, C-H), 1741 (ϑ, C=N), 1088 (ϑ, C-N), 623 (ϑ, Ru-N)

2.4.2. Synthesis of $[Ru(dmb)_2(MTPIP)](ClO_4)_2.2H2O$

A mixture of cis-$[Ru(dmb)_2Cl_2]2H_2O$ (0.5mM,0.260g) and MTPIP ligand (0.5 mM,0.171 g) in ethanol (15ml) refluxed for 14 h at 120^0C under a purge of N_2 gas. The resulting light purple colour solution was cooled to room temperature before adding an equivalent amount of saturated aqueous $NaClO_4$ solution under vigorous stirring. The red solution under was collected and cleaned with modest amount of diethyl ether, ethanol and water, before being vacuum dried (Yield 62.3), Scheme 2 illustrates.

Analytical date for $C_{44}H_{38}$ N_8 Ru S; Calc (%): C, 65.09; H,4.72; N,13.80; Found (%): C,65.24; H.7.58; N,13.74; ESI–MS (m/z):$[M\text{-}2ClO_4\text{-}H^+]^+$: 811,

$[M^{+2}\text{-}2(ClO_4)]$:406.2. ^1H NMR data (DMSO-d_6, 400MHz and δ ppm): 9.20(S,4H), 9.08(d,2H),8.73(d,4H),8.26(t,2H),7.54(d,4H),7.42(d,2H),716(d,2H),3.38(S,CH_3),2.50(S,3H).$^{13}C[^1H]$NMR(100 MHz,DMSOd_6,ppm):158.1,149.9,148.6,139.2,139.4,136.4,129.2,127.6,125.2,121.5,14.8.IR(KBr,CM^{-1}) :3589(ϑ,N-H),2971(ϑ,C-H),1741 (ϑ,C=N),1369 (ϑ,C-S),1088 (ϑ,C-N),623 (ϑ, Ru-N)

2.5. Biophysical Study of DNA Binding Affinity of Ru(II) Polypyridyl Complexes

The DNA binding studies of Ru(II) complexes were studied using various Biophysical methods, such as Absorption, Fluorescence, Quenching, DNA cleavage and viscosity methods.

2.6. DNA - binding by electronic Absorption Studies

The DNA binding affinities were measured via absorption titrations by increasing the concentration of DNA to a fixed metal complex quantity (20 µl) at room temperature. Ru-DNA solutions were incubated (for 5 min) before recording the absorption spectra. Equation 1 was used to derive the intrinsic binding constants, K_b [38].

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_a - \varepsilon_f) + 1/K_b(\varepsilon_a - \varepsilon_f) \quad (1)$$

Where, ε_a, ε_b, and ε_f represent the noticeable absorption extinction coefficient (A_{obsd} / [complex]), the extinction coefficient of the complex in the fully bound form, and the extinction coefficient of the free complex respectively and [DNA] is the concentration of DNA. The plots of [DNA]/ (ε_a-ε_f) versus [DNA],the binding constant K_b is given in the ratio between slope and the intercept.

2.7. Florescence (Luminescence) Studies

The luminescence titrations were carried out similar to absorption titrations using the Tris–HCl buffer. To a fixed metal concentration (10 µl), different concentrations (10- 200 µl) of DNA were added. The fraction of the compound bound was calculated from the equation

$$C_b = C_t\,[(F - F_0)/(F_{max} - F_0)] \quad (2)$$

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 Fmax is when the complex is maximum bound to DNA. The fluorescence binding constant (K_b) of complexes 1 & 2 was obtained From Scatchard equation and data were cast into the scatchard plot of r/C_f against r. Where r is the C_b / [DNA], and C_f is the concentration of the free complex.

2.8. Quenching studies

The Tris-HCl buffer solution (p^H=7.5) was employed in the florescence experiments. Different concentrations of the complexes (1 and 2) were added to an ethidium bromide and CT-DNA solution to allow for reaction .The concentrations of the complexes was kept between 10 and 100 µM , whereas ethidium bromide and CT-DNA were kept at 130 and 40 µm, respectively. Ethidium bromide's emission range was kept between 560 and 760 nm, and its emission spectra was observed at 520 nm. By using the Stern-Volmer equation: $I_0/I=1+K_{sv}$ r, where I and I_0 Stand for flurorescence intensities in the presence and absence of complexes, respectively, and K_{SV} linear Stern-Volmer quenching constant based on the ratio of r_{EB} (the ratio of the bound concentration of EB to the concentration of DNA) and total concentration of the complex to that of DNA, is r thus the spectra were examined (Zhang, 2016; Mariappan, 2018).

2.9. Viscosity experiment

Ostwald viscometer was used for the viscosity studies (maintained at a constant temperature of 29 ± 0.1°C in a thermostat water bath). Each sample's flow time was measured three times using a digital stopwatch, and the average flow time was computed. Data are displayed as $(\eta/\eta_0)^{1/3}$ vs binding ratio (Tan, 2008), where η is the DNA's viscosity when the complex is present and η_0 is the DNA's viscosity when CT-DNA is used alone. The measured flow time of DNA-containing solutions (t) was used to determine the viscosity values, which were then adjusted for the observed flow time of the buffer alone (t_0)

2.10. DNA cleavage experiment

By using the agarose gel electrophoresis method, the metal complexes capacity to cleve DNA through photolytic investigation was calculated. In this test, metal complexes were applied to super coiled pBR322 DNA at several quantities, and then DNA was diluted with Tris-Hcl buffer at P^H 7.2.The pretreatment DNA-sample system was mixed with bromophenol blue (2L) and then incubated for a further two hours at 37 °C. The samples were then loaded onto the wells of a 1% agarose gel that was set in a tray containing TAE buffer (p^H 8.0) and electrophoresis for 45 minutes at 70 V. Before electrophoresis, the gel was treated with ethidium bromide.With the use of a BIO-RAD Gel documentation system, bands were seen under an ultraviolet (UV) Tranisilluminator and the gel that resulted was photographed (Aveli,2021).

2.11. Antimicrobial activity

Antimicrobial studies were performed by using pour plate method. The complex was tested for their antimicrobial activity against staphylococcus, Bacillus, E.coli ,Klebsiella, Candida and Aspergillus. Four different concentrations (25µL, 50µL, 75µL &100µL) in DMSO were used for testing spore germination of each fungus. In this method 1% of active bacterial and antibiotic (Streptomycin/Chloramphenicol) cultures were mixed into autoclaved agar media just before solidifyling temperature and poured into the petric plates. After the plates were solidified, wells were made using steril well borer and samples were loaded 100µL each into the wells respectively. Plates were incubated at 37°C for 18-24 hours in a bacterial incubator and at 25°C for 96 hours in fungal incubator. They were the observed and diameters of the inhibition zone (in mm) were measured and tabulated. These results were compared with standard antibacterial drug streptomycin (5µg/ml) and antifungal agents Fluconazole(5µg/ml) for candida, Mancozeb Wp75(5µg/ml) for aspergillus at same concentration.

2.12. Cytotoxicity

A standard literature 3-(4,5-dimethylthiazole)-2,5-diphenyltetrazolium bromide (MTT) assay procedure was used to evaluated in vitro cytotoxicity of synthesized compounds.The cells were seeded in a 96-well flat - bottom micro plate and maintained at 37° C in 95% humidity and 5% CO_2 overnight. Different concentration (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) of sample were treated. The cells were incubated for another 48 hours .The wells were washed twice with PBS and 20 µl of the MTT staining solution was added to each well and the plate was incubated at 37°C .After 4h, 100 µL of DMSO was added dissolve to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using micro plate reader (Ravi Kumar 2020).

Formula: Surviving cells (%) =Mean OD of test compound /Mean OD of negative control x 100

Using graph pad Prism version 5.1, we calculate the IC_{50} of compound

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization

The MTPIP ligand and its corresponding Ru(II) complexes 1 and 2 were synthesized and characterized according to literature procedures. The synthetic route for the ligand and its complexes are shown in figure 1.The structures of ligand and its complexes were confirmed by UV-Vis, IR, mass, elemental analysis, and ^{1}H and $^{13}C[^{1}H]$-NMR spectral studies. In the UV-Vis absorption spectra, the two complexes 1 and 2 showed MLCT bands at ~465 nm, which were not seen with the free ligand, indicating the complex formation. The IR bands of metal complexes were shifted to lower frequencies when compared to the ligand. A new IR band was observed at 623 cm^{-1} for the complex 1 and at 624 cm^{-1} for the complex 2 which was assigned to the formation of a metal nitrogen bond and indicating formation of complexes. The mass spectral studies were also suggested the formation of complexes. In the ^{1}H-NMR spectra shows the ligand characteristic aliphatic peaks at δ 2.50 ppm, these protons were shifted to around δ2.51ppm of complexes 1 and 2 formation, due to the coordinated ligands and all peaks were shifted downfield compared to the free ligand. In the ^{13}C $[^{1}H]$ NMR spectra, the peaks are shifted to downfield when compared to ligand suggesting formation of complex. The imidazole ring nitrogen proton was not observed in ^{1}H-NMR, due to the fast proton exchange between the two imidazole nitrogen atoms. [54,55]

3.2. Electronic absorption spectra

The UV–Vis absorption titration is one of the most useful basic techniques to make out the binding affinity of complexes with DNA. The change in the absorbance value and wavelength shift in the UV–Vis region while adding DNA to the solution of Ru(II) complexes indicate the binding affinity of complex with DNA. When the complex binds to DNA, it usually results in hypochromism and bathochromism due to the intercalative mode of binding, which involves a strong stacking interaction between an aromatic chromophore and the DNA base pairs.

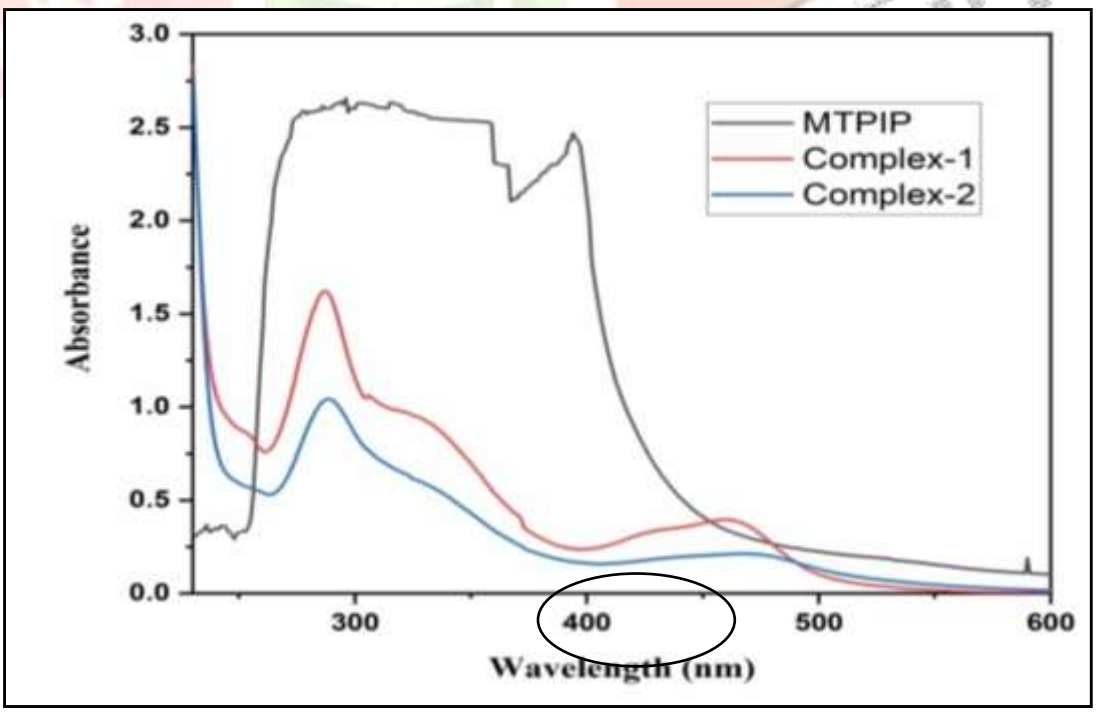


Fig.1. Electronic absorption spectra of Ligand and its complexes, circle shows MLCT peaks of complexes but no MLCT peak for ligand.

Table.1. Electronic spectral data of ligand and its two complexes

Ligand/Ru(II) Complexes	Absorption region ,λ_{max}	Band assigned
MTPIP ligand	**265**	**π → π** ∗(INCT)
[Ru(bpy)$_2$(MTPIP)]$^{+2}$	**280** **462**	**π → π** ∗(INCT) MLCT
[Ru(dmb)$_2$(MTPIP)]$^{+2}$	**282** **465**	**π → π** ∗(INCT) MLCT

Absorption studies of synthesized Ru(II) complexes with DNA are assessed and are shown in Fig.2.The spectral changes of Ru(II) polypyridyl complexes are indicative of intercalation binding. The bands below 300 nm are attributed to intraligand (IL) p^{π}-p^{π} transitions, whereas the bands in the visible region are attributed to MLCT (metal to ligand charge transfer) transitions. The intrinsic binding constants (K_b) of Ru(II) complexes 1 and 2 were determined from the decrease of the absorbance monitored at the MLCT band upon addition of DNA to complex, and binding constants (K_b) of Ru(II) complexes were calculated and tabulated in Table 3. The hypochromism (H% = 100% ($A_{free} - A_{bound}$)/A_{free}) and bathochromism of the MLCT bands for complexes 1 and 2 are tabulated in Table 3. From the results obtained, it has been found that all complexes show a good binding affinity to DNA. The binding constants of the complexes 1 is more than complex 2, which indicates that complex 1 has a stronger ability to bind with DNA, due to in dmb, methyl substitutions in the 4,4'- positions of the dmb ancillary ligand, which may cause steric hindrance (steric hindrance: bpy < dmb), which in turn causes a decrease in the binding constant.

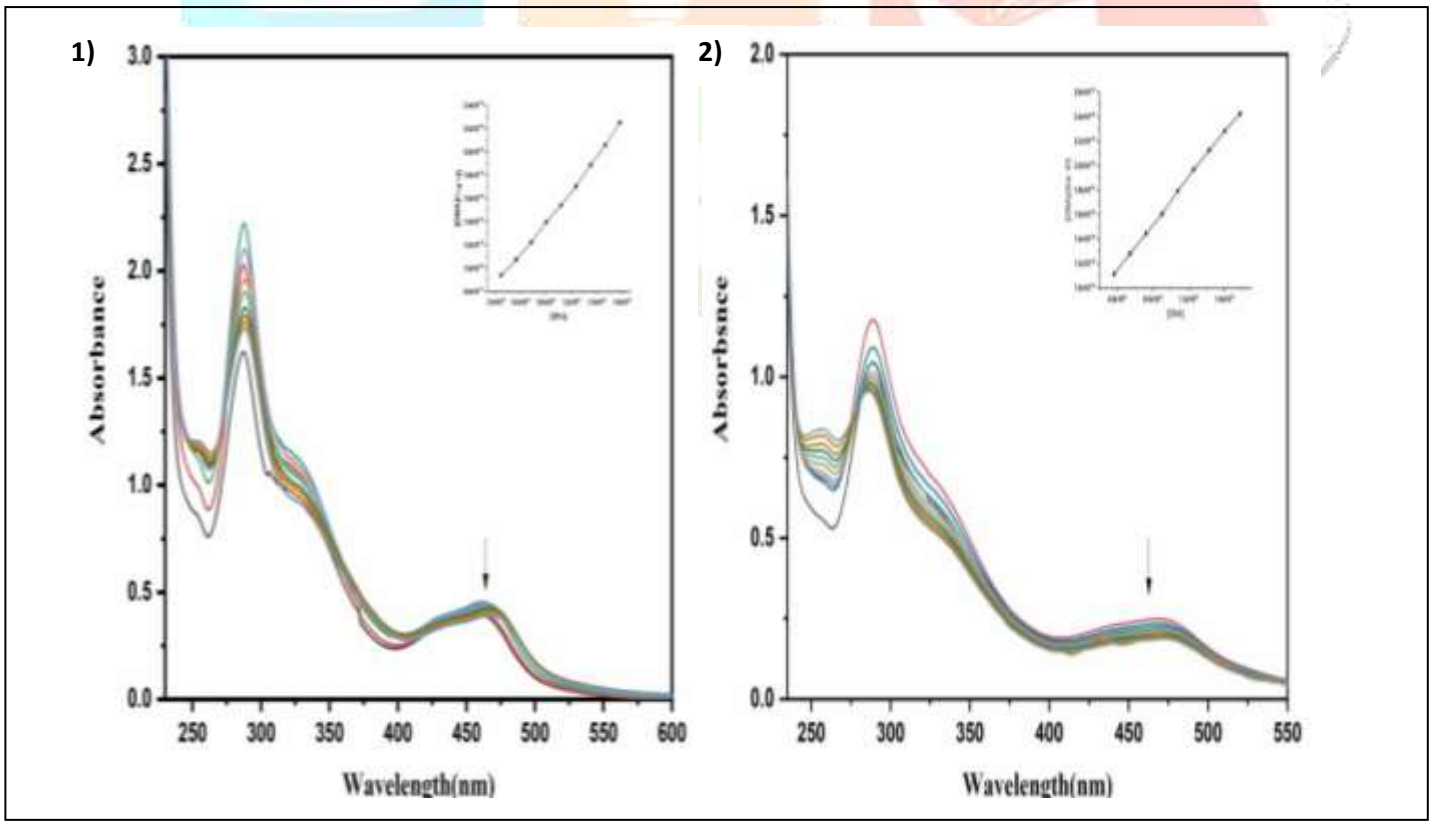


Fig.2 Changes upon increase of DNA concentration is represented with the arrows, inserted a Plot by taking [DNA]/ ($\varepsilon_a - \varepsilon_f$) Vs [DNA] for the titration of DNA with Ru(II) polypyridyl Complex which gives intrinsic binding constant (K_b) further. Where [Ru(bpy)$_2$(MTPIP)]$^{+2}$ and [Ru(dmb)$_2$(MTPIP)]$^{+2}$ are complex 1 and 2.

3.3. Fluorescence emission studies

Luminescence spectroscopy is one of the most sensitive and useful way to analyze drug interaction with bimolecular such as DNA .The complexes excited at 462 nm and 465 nm then strong emission observed at 607 nm and 608 nm band region respectively. While increasing the concentration of CT- DNA change in the emission intensity of the complexes are shown in the figure.3.The complexes emission intensities gradually raise with increasing DNA concentration until they reach a steady level. The binding constant was calculated using modified scatchard equation used for the calculation of intrinsic binding constant (K_b) from emission data. Binding constants (K_b) were obtained from scatchard plots where r/c_f Vs r is plotted. Where C_f is free ligand concentration,r is the binding ratio C_b / [DNA].The K_b values for complex 1 and 2 are $4.0x10^4$ and. $3.8x10^4$ respectively. The difference in K_b value is due to change in ancillary ligand.The complex 2 has lower value than complex 1 due to steric hindrance present methyl groups in dmb. The values obtained from absorption and emission titration were in accordance with each other.

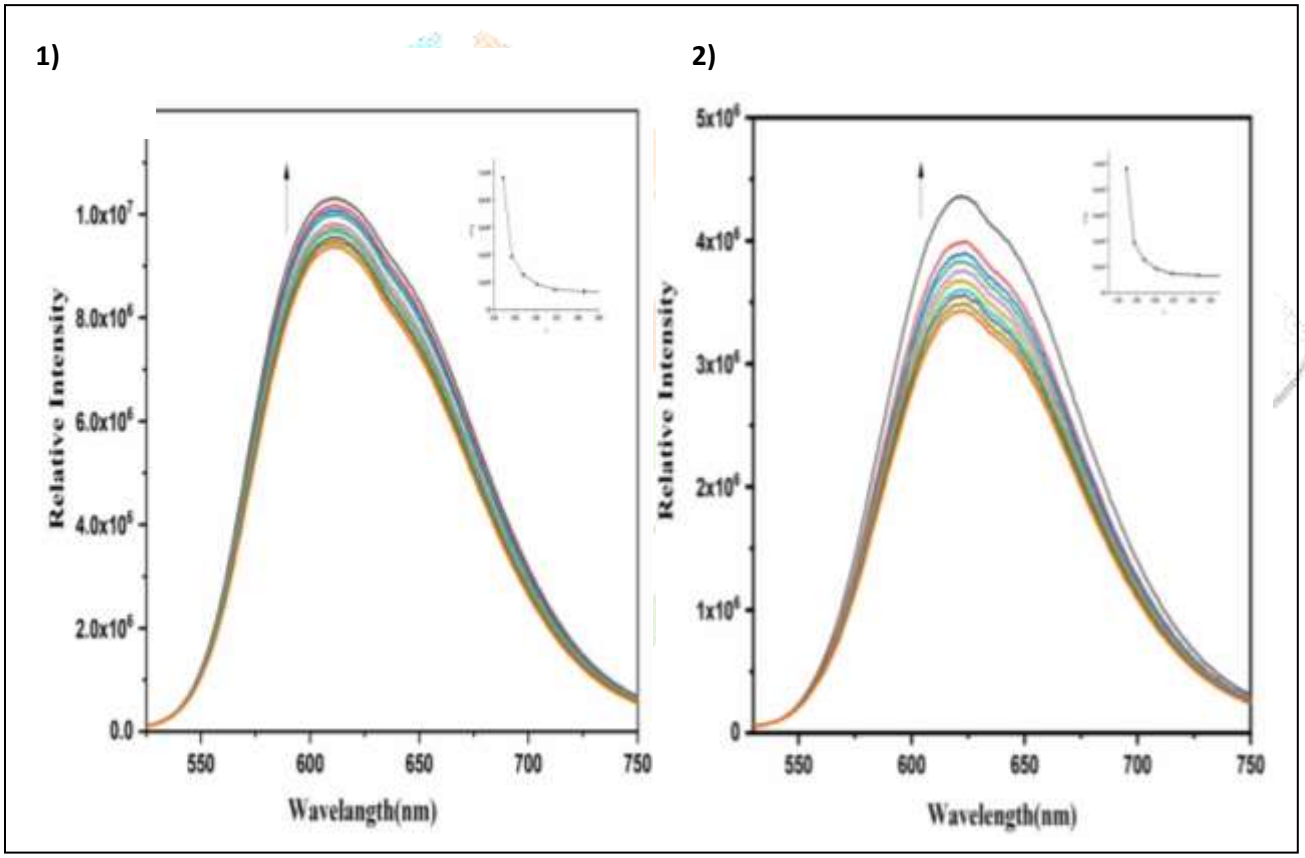


Fig.3. The Ruthenium (II) complex emission spectra in Tris-HCl buffer. Upon the addition of CT-DNA; the arrow shows the intensity change by increasing DNA concentration. insent: Scatchard plot of the complexes, from which binding constant(K_b) calculated.

3.4. Quenching studies

It has been shown that DNA groove binders and intercalator can both diminish the fluorescence intensity. However, the reduction caused by groove binders is only minor, whereas the replacement of ethidium bromide (EB) by intercalator can result in a considerable reduction in intensity. Further fluorescence investigations using the quenching method were used to determine the intercalative mode of complex binding to CT-DNA.This exeperiment was performed by taking a well-know intercalating agent ethidium bromide. When EB intercalate with DNA, there is a dramatic increase in emission intensity. This represents the way that EB interacts with

DNA base pair units. In the current study, the solution of ethidium bromide [40 µM] and CT-DNA [130 µM] increased amount of complex [10-100 µM] added. As a result,the intensity of the fluorescence emission was reduced which was shown figure.4.The outcome was approximated using the Stern-Volmer equation and indicates that complexes may bind to CT-DNA and replace EB from DNA .This indicates the intercalation of the complexes to CT-DNA. Stern-Volmer equation used to calculate the results. K_{sv} value is obtained from the linear fit plot of I_0/I versus [complex]/ [DNA] are $5.3x10^3$ and $3.4x10^4$ for complex 1 and 2 respectively.

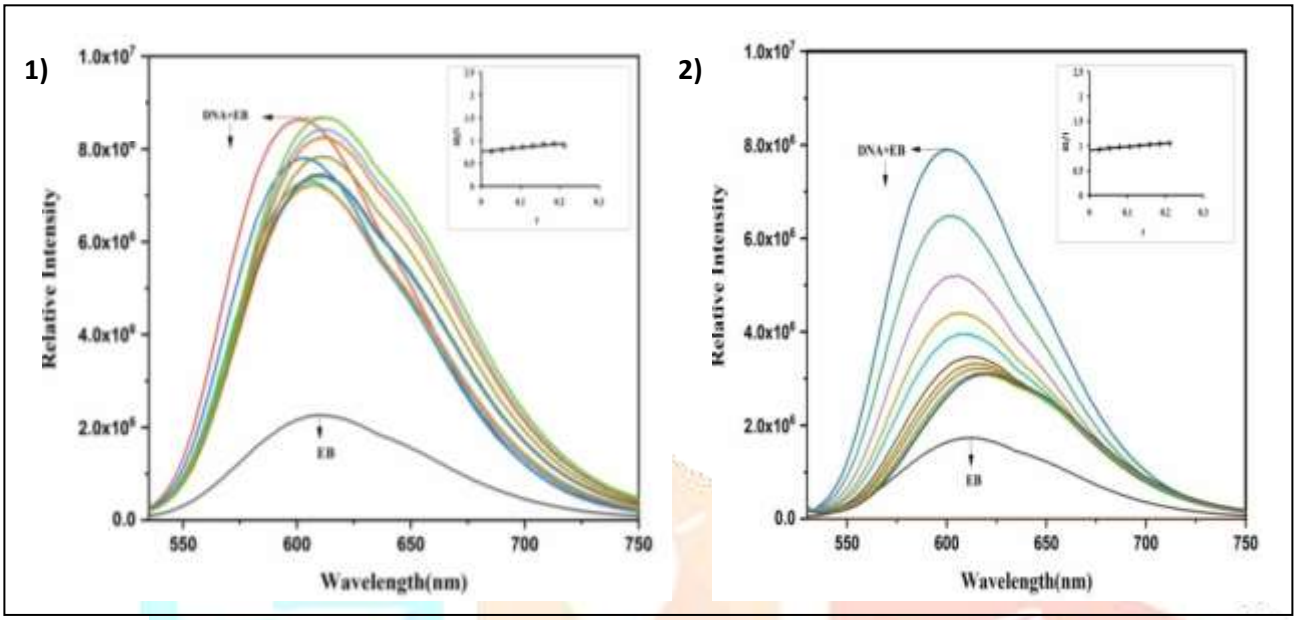


Fig.4. Fluorescence quenching studies of DNA–EB complex (DNA[130mM] & EB[40mM]) system, with the addition of complex arrow shows the decreasing emission intensity by increasing the concentration of complex[10 – 100 mM], Inset :I_0/I versus r. Where $[Ru(bpy)_2(MTPIP)]^{+2}$ and $[Ru(dmb)_2(MTPIP)]^{+2}$ are complexes 1 and 2.

Table.2. Absorption, emission, and quenching binding constants of Ruthenium (II) complexes with CT-DNA.

Complex	**$K_b(M^{-1})$ (Absorption)**	**$K_b(M^{-1})$ (Emission)**	**K_{sv} value**
$[Ru(bpy)_2(MTPIP)]^{+2}$	$1.5x10^5$	$4.0x10^4$	$5.3x10^3$
$[Ru(dmb)_2(MTPIP)]^{+2}$	$1.1x10^5$	$3.8x10^4$	$3.4x10^3$

3.5. Viscosity studies

The binding modes of complexes with CT-DNA were further investigated by Viscosity studies.In the absence of crystallographic data, hydrodynamic experiments are very important to understand the length change and stiffening of the rod like DNA by insertion of ligand between neighboring base pairs of DNA in solution. Intercalating ligands, intercalates in between the base pairs of DNA which lengthens the double helix and by increases the viscosity of DNA solution. Classical intercalation binding model shows increased viscosity of DNA by lengthening of DNA helix due to the separation of double helix to accommodate the ligand in between the base pairs (chaires, 1997).The best example for classical intercalation binding mode was observed in EtBr. In this study, increased concentrations of metal complexes were added to the CT-DNA, an extension in the

DNA length there by increased viscosity observed.Further on comparison with EtBr an intercalating ligand as shown in the figure 5 the results of the complex binding in between the base pairs of DNA which support intercalation binding mode.The results are showing two complexes bind in between the base pairs of DNA.The viscosity order of complexes follows EtBr>1>2.The results from this study strongly supported by observation from electronic spectroscopy and fluorescence emission.

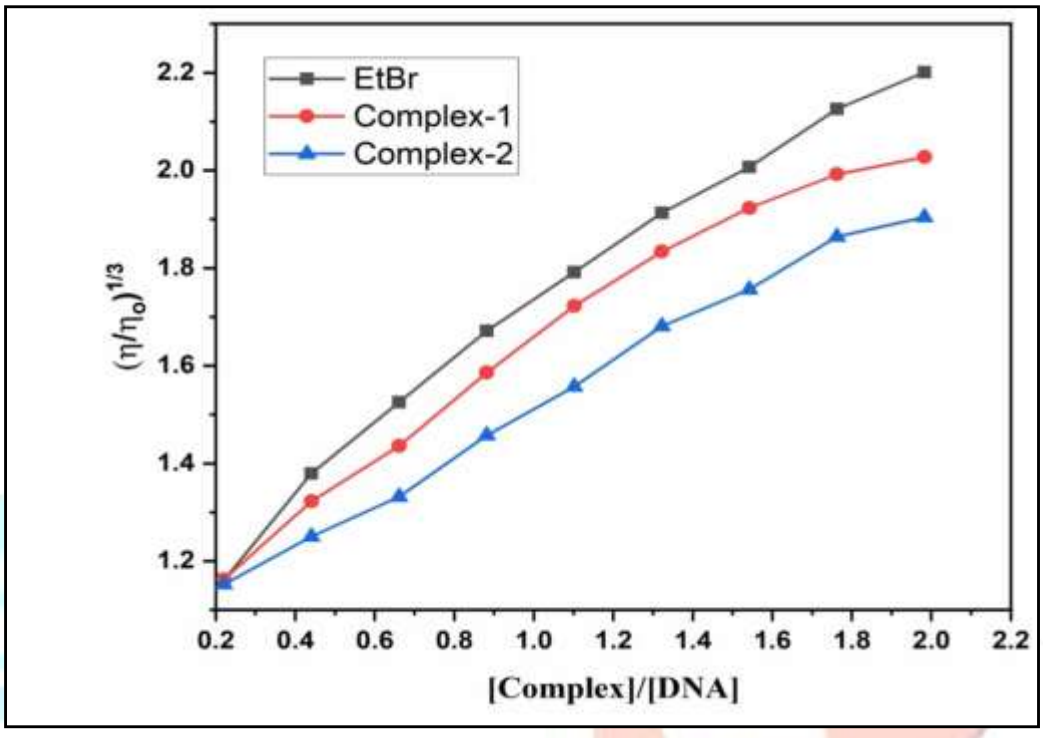


Fig.5. Viscosity studies of the complexes in Tris-HCl buffer with increasing amounts of complexes 1- 2 and Ethidiumbromide(EtBr) on the relative viscosity of CT-DNA at room temperature.

3.6. Photo cleavage

Using pBR322 DNA, nuclear activity of the Ru (II) Complex was measured using agarose gel electrophoresis. Plasmid DNA becomes electrophoresis, a reasonably quick process. When the super coiled shape is intact, migration is seen in form I. if nicking, or scission on a single strand, happens, then a slower-moving, open circular form (form II) will result from the super coiled form relaxing. When both threads are split, a linear form known as form III moves. This from is migrates in between I and II. Photo cleavage experiment was performed by taking pBr322 which was incubated along with the complex (20 µM) and irradiated for 60 min at 365 nm under UV light continuously. When the metal complex was absent no signs of DNA cleavage shown in lane. 1. Once the amount of Ru(II) complex was added, the equivalent of form I progressively dropped, while the quantity of type II enhanced, as Fig 6 illustrates.

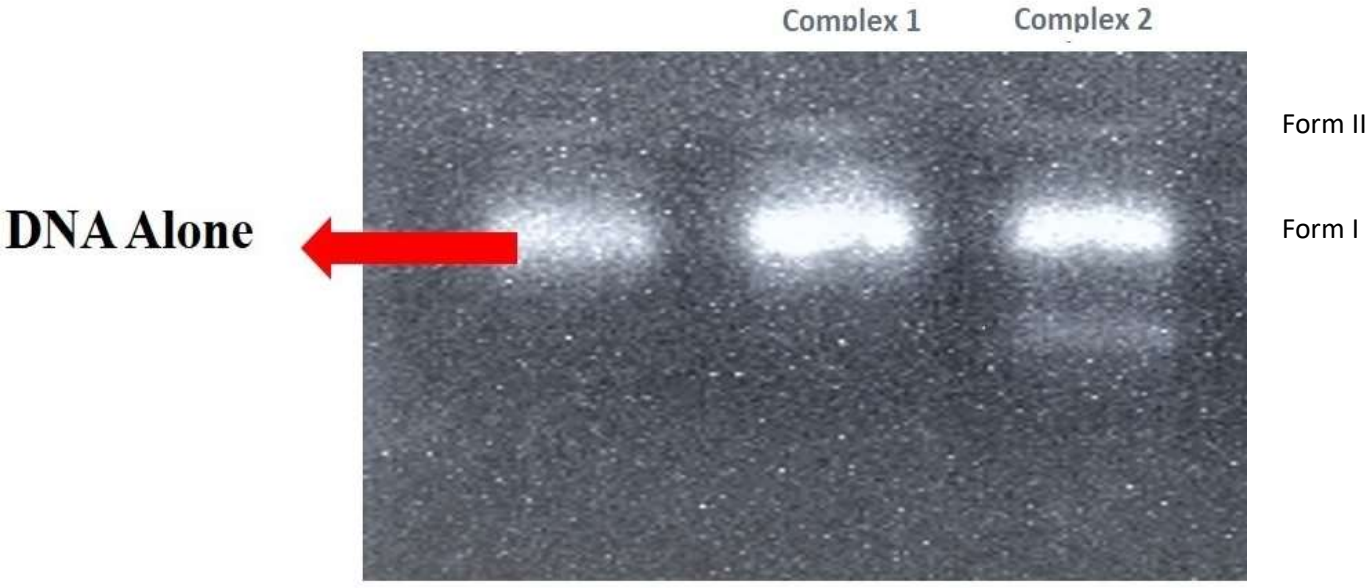


Fig.6. Photo activated cleavage $[Ru(bpy)_2MTPIP]^{+2}$ (1) and $[Ru(dmb)_2MTPIP]^{+2}$ (2) complexes with the concentration range of 20 µM

3.7. Antimicrobial Activity

The antibacterial activity data (table 3) indicates that the complexes 1 and 2 shows a high activity against Bacillus (gram positive bacteria) at 75 µL and 25 µL of sample concentration respectively and complexes 1 and 2 shows appreciable activity against Klebsiella (gram negative bacteria) at 50µL of sample concentration (Fig. 7). The antifungal activity data (table 4) indicate that the complexes 1 and 2 show an appreciable activity against Aspergillus at 50 µL and 75 µL of sample concentration and are expressed as inhibition zone diameter (in mm) versus standard (Fig. 8).

Table.3. Anti - Bacterial activity of $[Ru(bpy)_2(MTPIP)]^{+2}$(1) and $[Ru(dmb)_2(MTPIP)]^{+2}$ (2) Complexes

Minimum Inhibitory Concentration-MIC (mm)												
Gram positive bacteria							**Gram negative bacteria**					
Staphylococcus				**Bacillus**			**E.Coli**			**Klebsiella**		
	75 µL	100 µL	MIC of sample (µL)	75 µL	100 µL	MIC of sample (µL)	75 µL	100 µL	MIC of sample (µL)	75 µL	100 µL	MIC of sample (µL)
Complex -1	08 mm	08 mm	75 µL	10 mm	10 mm	75 µL	08 mm	12 mm	50 µL	10 mm	12 mm	50 µL
Complex-2	08 mm	10 mm	75 µL	10 mm	12 mm	25 µL	08 mm	10 mm	75 µL	08 mm	12 mm	50 µL
Standard	16mm			14mm			12mm			12mm		

*The zone of inhibition was measured in mm

Antibacterial activity Images

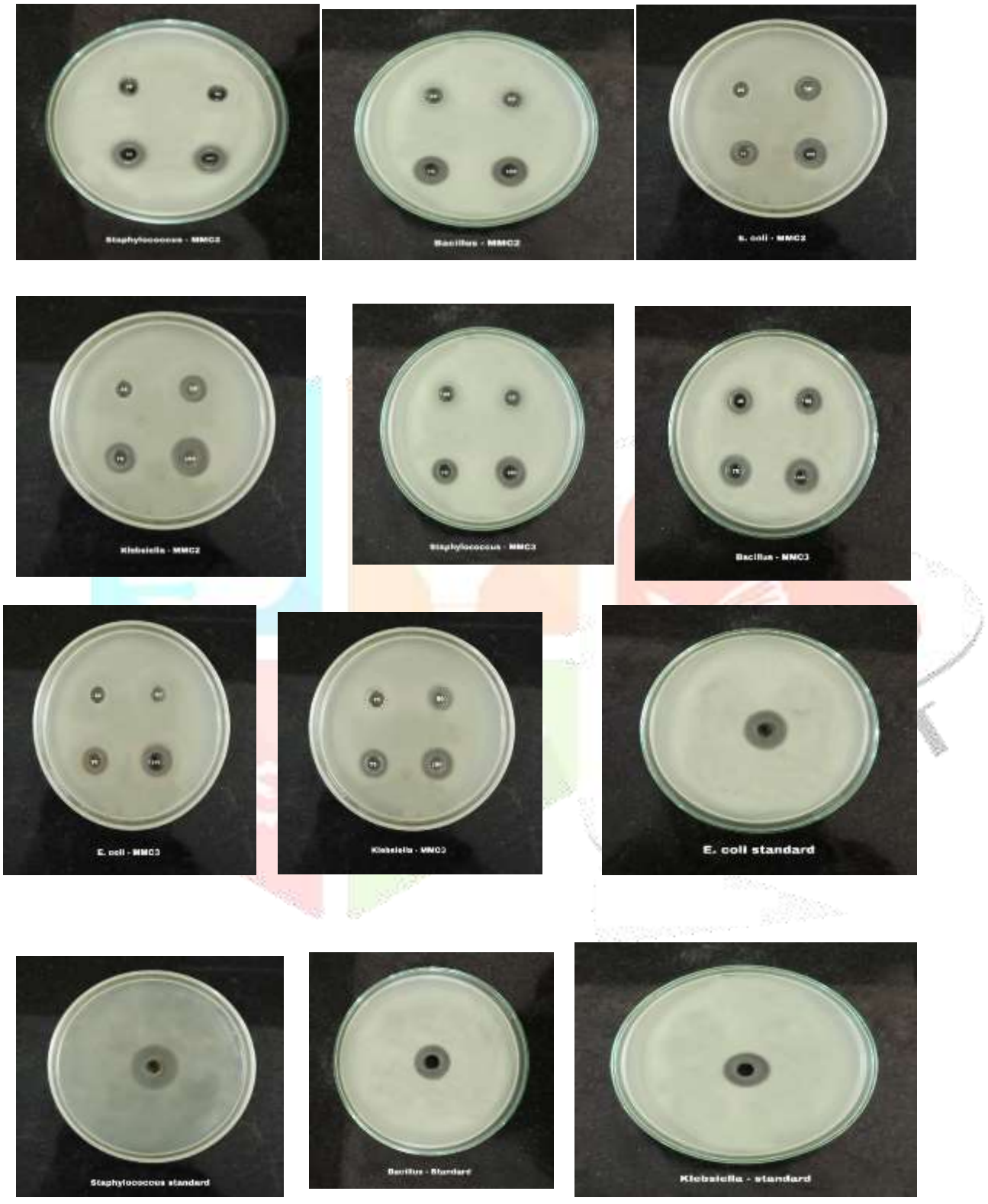


Fig.7. Anti Bacterial activity of $[Ru(bpy)_2(MTPIP)]^{+2}$(1) and $[Ru(dmb)_2(MTPIP)]^{+2}$ (2) Complexes

Table.4. Antifungal activity of $[Ru(bpy)_2(MTPIP)]^{+2}$(1) and $[Ru(dmb)_2(MTPIP)]^{+2}$ (2) Complexes

Minimum Inhibitory Concentration-MIC (mm)						
	Aspergillus			Candida		
	75 µL	75 µL	MIC of Sample (µL)	75 µL	75 µL	MIC of Sample (µL)
Complex-1	12 mm	12 mm	50 µL	08 mm	10 mm	75 µL
Complex-2	12 mm	12 mm	75 µL	08 mm	12 mm	25 µL
Standard	12 mm			12 mm		

*The zone of inhibition was measured in mm

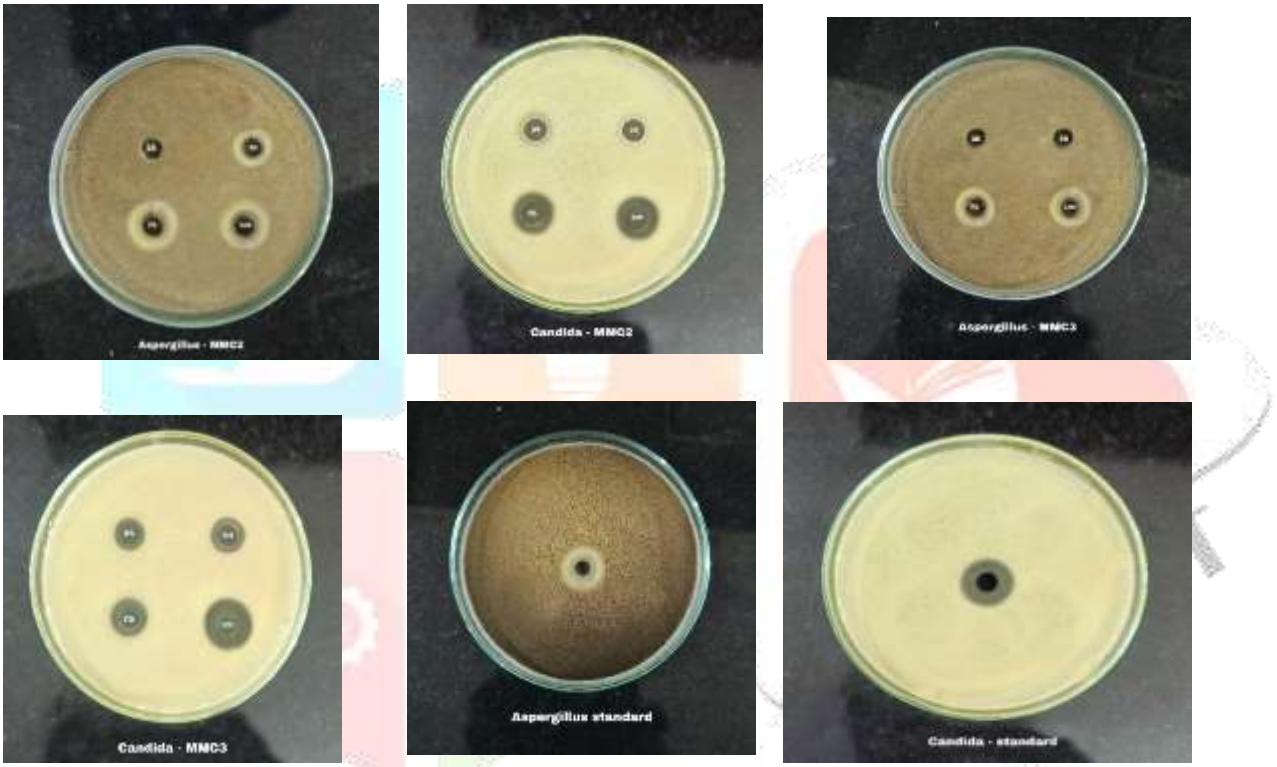


Fig. 8. Antifungal activity of $[Ru(bpy)_2(MTPIP)]^{+2}$(1) and $[Ru(dmb)_2(MTPIP)]^{+2}$ (2) Complexes

3.8. Cytotoxicity

The MTT method was used to evaluate the antiproliferative activities of the Ru(II) Complexes again MCF-7(Human breast cancer), comparison with-doxorubicin as positive control. The Ru (II) polypyridyl complex displays excellent antitumour activity towards the selected cancer cell line. DMSO was used as a positive control.After treatment of MCF-7 cell lines for 48 h with complexes 1 and 2 in a range of concentration(3.125-100 µg/ml),the the percentage inhibition of growth of the cancer cells was determind.The cytotoxicity of the complexes was found to be concentration dependent.The cell viability decreased with increasing concentrations of both complexes.Ru(II) complexes exhibited well activity against MCF-7 cells with IC_{50} = 20.96±0.57 µg/ml and 21.11±0.08 µg/ml standard doxorubicin IC_{50} = 3.66 ± 0.07 µg/ml. Cell viability of MCF-7 is more in case of complex 1 than complex 2, shown in the figure.9.and 10 Thus this complexes exhibits high inhibitory effect on the cell growth in MCF-7 cell line and may be used as a potent anticancer drugs against breast cancer.

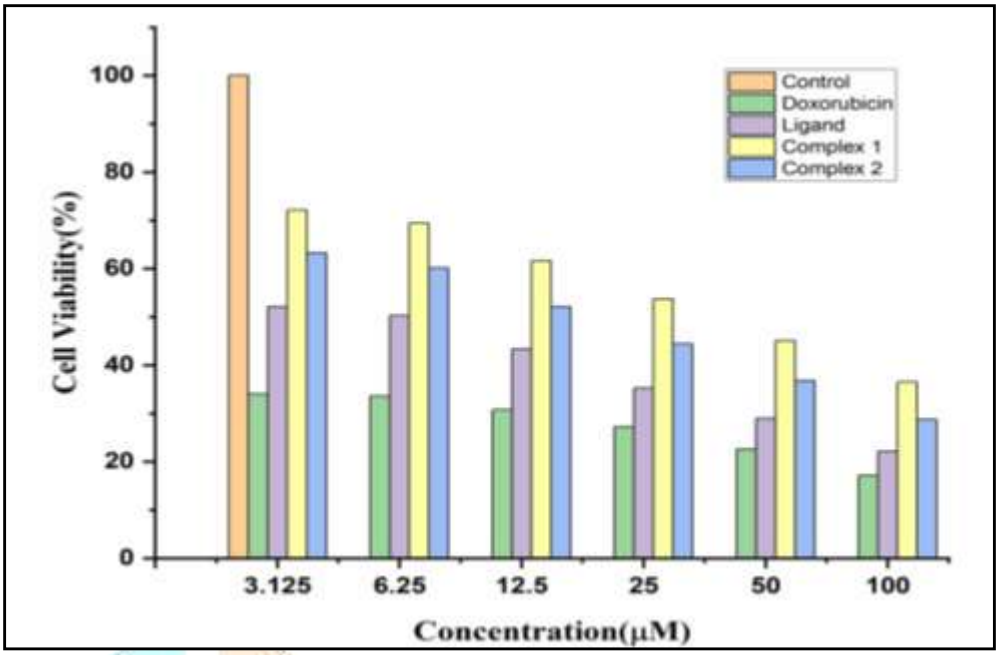


Fig.9. Graphical representation of anticancer activity of metal complexes 1&2

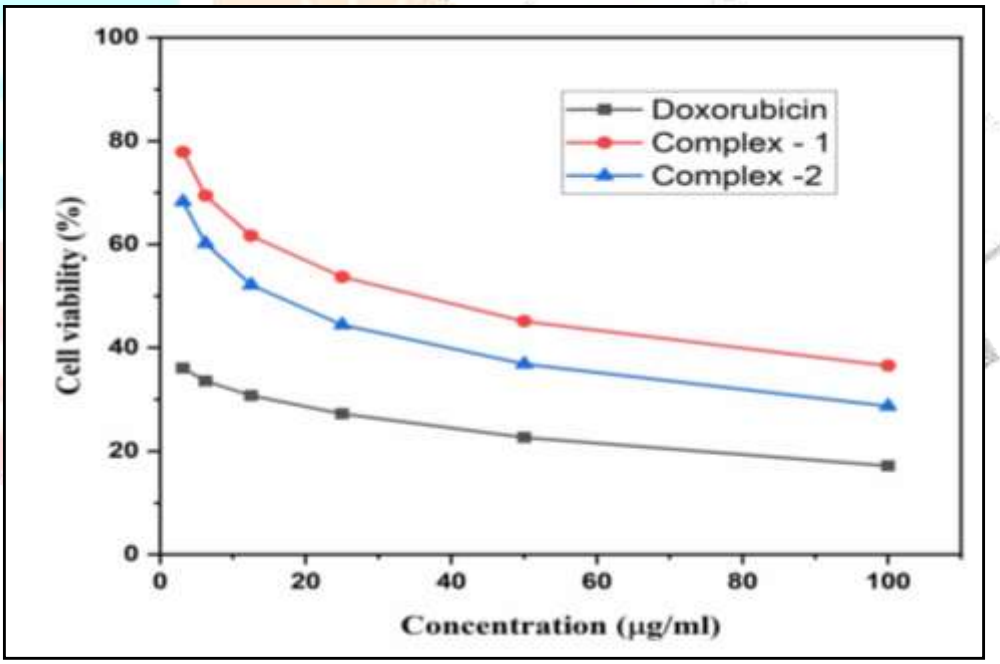


Fig.10. Cell viability of MCF-7 cell lines in vitro treatment with complexes $[Ru(bpy)_2 MTPIP]^{+2}$ (1) and $[Ru(dmb)_2 MTPIP]^{+2}$ (2).Each data point is the mean ± standard error obtained from of three independent experiments.

CONCLUSION

Ru(II) polypyridyl complexes are synthesized with novel intercalator MTPIP ligand, characterized and their interaction with CT-DNA was studied by UV-Vis absorption, fluorescence quenching titrations and viscosity measurement which concluded that binding mode of complex against DNA is an intercalative mode. For the complex 1 and complex 2 the binding constants K_b from UV-Vis absorption studies are 1.5×10^5 and $1.1x10^5$, Stern-Volmer quenching constant (K_{sv}) from fluorescence studies are 5.3×10^3 and $3.4x10^3$ and the binding constant (K_b) from emission studies are $4.0x10^4$ and $3.8x10^4$ respectively. The above data showed that the

complexes bind to DNA effectively. Upon irradiation, this complex can effectively cleave pBR 322 DNA. Antimicrobial activity indicated that complexes were active against the tested microorganisms. The invitro cytotoxicity of Ru(II) polypyridyl complexes show promising anticancer activity against cell line MCF-7. From the all above results suggesting as complex 1 is effective than complex 2.

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Conflict of Interest- On behalf of all authors, the corresponding author states that there is no conflict of interest.

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