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RESEARCH ARTICLE

SYNTHESIS, SPECTRAL STUDIES AND EVALUATION OF DNA BINDING, PHOTOCLEAVAGE, ANTICANCER ACTIVITY OF RU(II) POLYPYRIDYL COMPLEXES OF 2-(3,4,5-TRIMETHOXY-PHENYL)-H-IMIDAZOLE[4,5-F][1,10]PHENANTHROLINE

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Abstract

The two novel Ru(II) polypyridyl complexes of the type $[Ru(NN)_2L](ClO_4)_2.2H_2O$,where NN is dmp=4,4'-dimethyl-1,10 ortho phenanthroline and dmb=4,4'-dimethyl 2,2'-bipyridine and 'L' is Intercalator ligand, TMIP=2-(3,4,5-Trimethoxy-phenyl)-H-imidazole[4,5-f][1,10]phenanthroline were synthesized and characterized by different spectroscopic techniques. The binding ability of Ru (II) complexes with CT-DNA was investigated by Biophysical studies – UV-Visible absorption studies, Fluorescence studies (Emission & Quenching) and Viscosity studies. The binding constants of these complexes from absorption studies are $1.2x10^5$ $M^{-1}$and $8x10^4M^{-1}$respectively.These values are less as compared to the existing TMIP complex $[Ru(phen)_2TMPIP]^{+2}$, indicative of the effect of substitution over the ancillary ligand which reduce the binding capacity of metal complex to CT-DNA. The results from the viscosity studies suggest an intercalative binding mechanism. The DNA cleavage studies show that the Ru (II) complexes successfully cleaved the pBR322 DNA. Both the complexes exhibit not notable anti cancer acivity.

Introduction:-

The most important characteristic of the extremely diverse disease known as cancer is the unchecked, unrelenting development and spread of aberrant cells [1]. There are two basic groups into which the medications used to treat cancer fall. Drugs classified as cytotoxic (which kills cells) and cytostatic (which inhibits cell growth) are the first two types. Numerous scientists have discovered and studied a wide range of metal complexes, including rhodium, platinum, and ruthenium, as cytotoxic and cytostatic agents. The utilization of metal complexes as prospective anti-cancer options is one of the fast expanding fields of cancer therapy. In-depth research has been done on cytotoxic substances with more tolerable toxicity profiles since the coincidental discovery of Cisplatin's anticancer and anti-metastatic properties [2]. Metal coordinated complexes have been used as drugs to treat a variety of illnesses, including those with antimicrobial, anticancer, anti-diabetic, anti-arthritic, antiulcer, and anti-malarial properties. The development of new medications for cancer therapy is a crucial field of research. Because they can cleave DNA under physiological settings, metal complexes have drawn attention in recent decades for the development of metal-based anticancer medications [3-5].Recent progress in the field of medicinal (bio)inorganic chemistry has been

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provided by the discovery of cisplatin and its analogues for the treatment of cancer. These platinum drugs have a wide range of clinical uses, but they have serious limitations because of their high toxicity, range of side effects on normal cells, inactivity against many other cancer cell lines, and tendency to metastasize[6]. This motivated scientists to look for other possible metal-based anticancer drug options, and as a result, complexes of different transition metals were synthesized and tested for their anticancer activities [7]. Due to their rich photochemical reactions and photo physical characteristics, Ruthenium (II) polypyridyl complexes have drawn a lot of interest as substrates for DNA binding among the documented DNA binders [8-10]. Ruthenium complexes containing dipyridophenazine (dppz) ligand have been extensively researched due to their remarkable photophysical characteristics and robust DNA binding [11, 12]. By substituting on the dppz ligand, a number of derivatives of $[Ru(phen)_2(dppz)]^{+2}$ (phen is 1,10-phenanthroline) have been created [13]. It was discovered that altering the intercalating ligand's structure resulted in adjustments to the intercalated complex's orientation. A number of ruthenium complexes have been suggested as possible anticancer substances. Compared to platinum compounds, these complexes have exceptional anticancer efficacy and decreased overall toxicity; in certain situations, their anticancer activity surpasses that of cisplatin. $[Ru(bpy)_2(dppz)]^{+2}$ where bpy = 2,2'-bipyridine shows cytotoxicity at low half-maximum inhibitory doses (IC_{50}). Ru (II) complexes have been produced recently, possibly as a result of their structural composition, solubility, lipophilicity, charge, and photophysical properties, which may make them targets for cells [14–19]. According to more recent research, ruthenium (II) polypyridyl complexes can effectively cause apoptosis and exhibit high cytotoxicity in vitro [20–23].

Here, we report, the synthesis of novel Ruthenium(II) polypyridyl complexes derived from TMIP - [2-(3,4,5-Trimethoxy-phenyl)-H-imidazole [4,5-f][1,10]phenanthroline] ligand and Characterization of the complexes were accomplished by IR, UV,NMR (^{1}H and ^{13}C), Mass and Elemental analysis. The binding association of CT-DNA to these complexes was examined by using UV-Visible absorption titration, Fluorescence emission and viscosity experiments.

Methodology:-

Every reagent and solvent used was used precisely as supplied. The following materials were acquired from Merck: 4,4'-dimethyl-1,10 ortho phenanthroline (dmp) and 4,4'-dimethyl 2,2'-bipyridine (dmb).Calf thymus DNA (CT-DNA) and super coiled pBR322 plasmid DNA (which is kept at 20 °C) were purchased from Fermentas Life Sciences and Aldrich, respectively, and were utilised in their original forms. Agarose gel was bought from Genei. 18.2mX ultrapure Milli-Q water was utilized in all studies. The metal complex's binding affinity for CT-DNA was measured in tris (hydroxymethyl) amino methane, Tris-HCl buffer (5 mM TrisHCl, 50 mM NaCl, pH 7.2). Using spectrophotometry, protein-free DNA could be seen at absorbance of 260 and 280 nm and a concentration of DNA per nucleotide at a molar extinction of 6600 M^{-1} cm^{-1}. Metal complex stock solutions were made in DMSO solvent, maintained at 40 degrees Celsius, and consumed in fewer than five days. To achieve the necessary concentration in the buffer, all of the stock solutions were further diluted. The UV-Vis spectra were recorded using the Shimadzu UV-2600 spectrophotometer. The spectrofluorometer with the Cary Eclipse instrument serial number (MY12400004) was utilised to capture the fluorescence spectral data required to determine the binding constants. With KBr discs, IR spectra were recorded using a Perkin Elmer 1605 Fourier transform IR spectrometer.

Synthesis & Characterization of Ligand and its Ru(II) complexes:-

The starting compounds 1,10-phenanthroline-5,6- dione (Phendione) [24], cis-$[Ru(dmp)_2Cl_2]$ and cis- $[Ru(dmb)_2Cl_2]$ were synthesized according to reported literature procedures [25]. The ligand TMIP also synthesized according to our lab protocol [26].

Synthesis of $[Ru(dmp)_2(TMIP)](ClO_4)_2].2H_2O$ complex(1)

$Ru(dmp)_2Cl_2.2H_2O$ (0.206g, 0.5mM) was mixed TMIP (0.193g, 0.5mM) in a 15ml ethanol solution, stirred for 8 hours in a N_2 environment followed by the saturated sodium perchlorate solution-treated deep red-colored solution that was the end product. Solid orange-yellow separation occurs. This chemical was dried after being rinsed with distilled water, ethanol, and diethyl ether. (Yield: 70.24%)

Analytical data:

$C_{48}H_{42}N_8O_3$;cal.C,65.52;H,4.81;N,12.75;found:C,65.32;H,4.01;N,12.10.ESI-MS(m/z). 452.12$[M]^+$,^{1}HNMR(DMSO-d6,400MHz):δ1.74(s,5H), 1.94 (s,5H), 3.77 (s,4H), 3.93 (s,8H), 7.39 (t,4H), 7.51 (s,2H), 7.58 (s,2H), 7.99 (d,2H), 8.26 (d,2H), 8.44 (t,4H), 8.91 (d,3H), 9.0 (s,1H); ^{13}C [^{1}H] NMR (100MHz, $DMSOd_6$, ppm) : δ 153.81, 151.03,

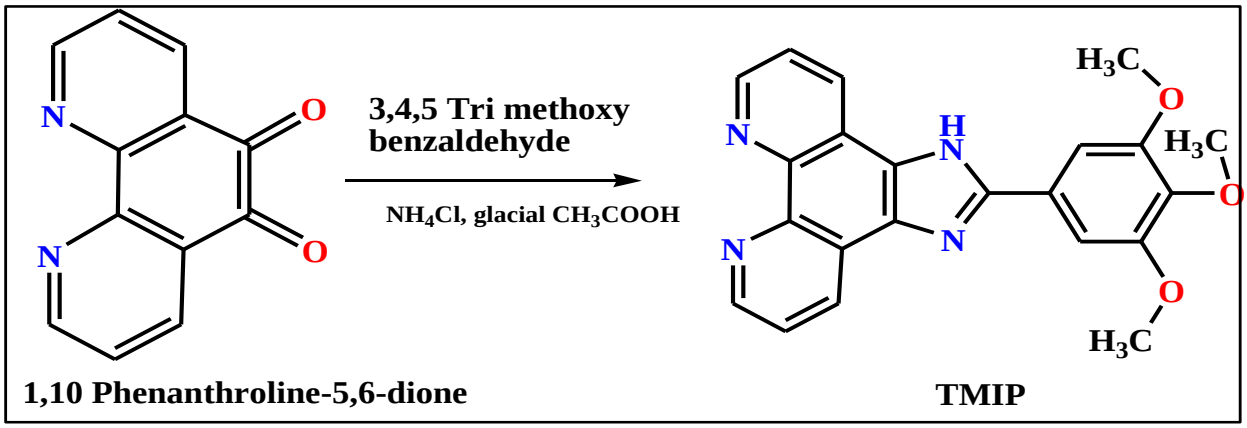
148.29, 144.02, 139.35, 130.14, 125.90, 123.71,104.29,60.69,56.67,40.59, 40.38. IR (KBr, cm^{-1}): 3556 (ν, N-H) ,1232(ν, C-N), 1092 (ν, C-O-C), 624(ν_{Ru-N}).

Synthesis of $[Ru(dmb)_2(TMIP)](ClO_4)_2.2H_2O$ complex(2)

In order to create this combination, $[Ru(dmp)_2Cl_2]2H_2O$ (0.260 g, 0.5 mM) and TMIP (0.193 g, 0.5 mM) were combined and refluxed for eight hours in a N_2 environment in a 15 ml ethanol solution. The saturated sodium perchlorate solution treatment produced a deep red coloured solution as a result. Solid orange-yellow separation occurs. This chemical was dried after being cleaned with ethanol, diethyl ether, and D.H_2O. (Yield: 71.84%)

Analytical data:

$C_{46}H_{42}N_8O_3$;cal.C,64.55;H,4.95;N,13.09;found:C,64.32;H,4.73;N,12.99.ESI-MS(m/z)=428.12,^{1}HNMR(DMSO-d6,400MHz):δ2.50(s,3H), 2.56 (s,4H), 3.79 (s,3H), 3.98 (s,5H), 7.91 (d,2H), 7.70 (s,1H), 7.67 (s,3H), 7.49 (d,3H), 7.37 (t,1H), 8.97 (s,1H), 8.93 (s,1H), 8.74 (m,2H), 8.16 (d,1H), 8.08 (m,1H), 9.21 (s,1H), 9.17 (s,1H), 9.08 (s,2H), 10.20 (s,1H), 10.10 (s,1H), ^{13}C [^{1}H] NMR (100MHz, $DMSOd_6$, ppm) : δ 168.41, 153.88, 149.33, 148.24, 138.60, 137.17, 131.29, 129.98, 128.00, 127.88, 127.59, 127.03, 104.70, 60.70, 56.76, 40.59, 40.38, 40.17, 39.96, 39.75, 39.55, 39.34, 24.99. IR (KBr, cm^{-1}): 3598 (ν, N-H),1239(ν, C-N), 1094(ν, C-O-C), 623(ν_{Ru-N}).



Scheme-1.Synthetic route for the preparation of ligand and structures of ruthenium (II) Complexes.

DNA Binding studies: UV-Visible Absorption Titrations

Our Lab procedures were followed in order to conduct these tests [26]. The DNA binding affinities were ascertained by increasing the DNA concentration to a fixed metal complex quantity (20 µl) at room temperature using absorbance titrations. The absorption spectra were acquired after five minutes of incubation of the Ru-DNA solutions. K_b values were calculated using the equation 1[27].The absorption extinction coefficient (Aobsd/[complex]), the complex's extinction coefficient in its fully bound form, and the extinction coefficient of the free complex are represented, respectively, by the values εa, εb, and εf in the equation. The symbol for DNA

concentration is [DNA]. Plotting [DNA] / (εa-εf) on the Y-axis and [DNA] on the x-axis resulted in the graph. K_b (intrinsic binding) is the intercept ratio from the slope.

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

Fluorescence emission titrations:

Fluorescence emission titration is also used to determine the strength of metal complex binding to CT-DNA, much as absorption titration. Prior to the experiment, the emission wavelengths were changed and the excitation wavelengths were maintained constant. 10µl of the metal complex was collected in Tris - HCl buffer for this experiment. Increased CT-DNA concentrations (10–100µl) were added to this. Equation 2, or the Scatchard equation, is utilized to determine the binding constant value [28].

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

Where F is the measured fluorescence emission intensity at a specific DNA concentration, F_0 is the level of emission in the absence of DNA, and F_{max} is the point at which the complex is maximum bound to DNA. Ct is the total complex concentration, denoted by C_t. from the r/C_f (equation 3) vs r Scatchard plot. The negative slope yields the K_b (intrinsic binding constant) of the complexes according to the equation, where r is the C_b/[DNA] and C_f is the amount of the free complex.

$$r/C_f = K_b(1 - nr) \quad \text{.....(3)}$$

Quenching & Light switch on-off experiment:

Ethidium bromide (EtBr) was used as a quencher in Tris–HCl buffer for room temperature fluorescence emission quenching research. The quenching constant K_{SV} is determined by the Stern–Volmer equation $I_0/I = 1 + K_{SV}[Q]$, where I_0 and I are the emission intensities in the presence and absence of the quencher EtBr, K_{SV} is the linear Stern–Volmer constant, and [Q] is the quencher concentration. K_{SV} values are obtained by drawing a linear fit graph between I_0/I and [complex]/[DNA]. Ru (II) polypyridyl complexes were investigated using a molecular light switch on and off in the presence of equimolar doses of EDTA and $CoCl_2$ [29, 30].

Viscosity Studies:

Ostwald's viscometer was used to investigate the viscosity of sample, and it was kept in a water bath with a thermostat set to maintain a constant temperature of 30 ±0.1ºC. Throughout the titration procedure, metal complexes concentration set to be in the range of 0 to 120 mM into account, DNA was maintained at a constant concentration of 20 mM. Three titrations of each were carried out, and the mean flow time value was calculated. a plot of η against complex concentration / CT-DNA concentration, where η represents DNA viscosities while the complex is present and η_0 represents DNA viscosities when the complex is absent; The formula for η is $\eta = t - t_0$ (which indicates the flow time of the buffer alone (t_0) and the flow time of DNA in the presence of complex (t), and $\eta_0 = t_1 - t_0$ ($t_1$which indicates the flow time of DNA alone) [31].

DNA-Cleavage study:

Oxidative and photolytic experiments were used to assess the metal complexes' capacity to break DNA employing the agarose gel electrophoresis technique. In this photolytic experiment, super coiled pBR322 DNA was diluted with Tris-HCl buffer at pH 7.2 after 20 µM of metal complexes were added. Following a two-hour incubation period at 37°C, 2µl of bromophenol blue was added to the preprocessing DNA sample system. Samples were then added to the 1% agarose gel wells, which were subsequently immersed in a tray filled with TAE buffer (pH 8.0), and electrophoresis was conducted for 45 minutes at 70V. The gel was stained with ethidium bromide (EtBr) before being electrophoresed. A UV transilluminator was used to see the bands, and photos of the finished gel were taken using the BIO-RAD Gel documentation equipment [32].

Cytotoxicity:

Using three separate studies and six concentrations (3.125-100 ug/ml) of complexes in triplicate, the MTT Assay was used to assess the viability of the cells. The cells were tallied using a hemocytometer, sown in 96-well plate culture medium at a density of 5.0 X 10^3 cells/well using 100 µl media, and incubated for a whole night at 37°C. Following incubation, remove the old media and replace it with 100 µl of fresh media containing various complex concentrations in the wells that are depicted on 96 plates. After 48 hours, discard the solution and replace it with fresh medium. Each well received 0.5 mg/mL^{-1} of MTT solution, and the plates were incubated for three hours at 37° C. When the incubation period comes to a conclusion, the cells with metabolically active mitochondria reduce the MTT salt to chromophore formazan crystals, which forms precipitates [33]. Using a micro plate reader, the

optical density of solubilized crystals in DMSO was determined at 570 nm. The algorithm below was used to compute the percentage growth inhibition.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The IC_{50} value was determined by using linear regression equation i.e. y =mx+c. Here, y = 50, m and c values were derived from the viability graph.

Results and Discussion:-

The intercalating ligand FT-IR spectrum depicts peaks at 1509 cm^{-1}(υC=N), 3592 cm^{-1} (υN-H), 1631 cm^{-1} (υC=C). Following the development of the complex, these peaks moved to the following locations: in complex 1, 1592 cm^{-1} (υC=N), 3556 cm^{-1} (υN-H), 1625 cm^{-1} (υC=C), and in complex 2, 1592 cm^{-1} (υC=N), 3598 cm^{-1} (υN-H), and 1619 cm^{-1} (υC=C). The existence of a new band at 624 cm^{-1} and 623 cm^{-1} in both complexes shows the formation of the complex.

Employing ^{1}H NMR spectra, the synthesized compounds were examined for resonance peak position and intensity. The ligand TMIP's ^{1}H NMR spectra revealed aromatic area peaks at δ9.04-7.63 ppm and an aliphatic peak of methyl protons at δ3.37-3.97 ppm [34]. Following the creation of the complex, some areas moved. The methyl protons peak changed to 3.3 ppm, while Complex 1 displayed peaks at the δ 8.93 - 7.38 ppm area. The peaks at δ 9.21-7.18 ppm in Complex-2 reveal that the CH_3 protons have relocated to 3.29 ppm. TMIP ^{13}C NMR ligand peaks were observed at δ 104.72 to 157.24 ppm. These peaks moved to δ 102.97 to 166.44 in the metal complexes, indicating complex formation.

The ligand TMIP ESI-MS spectra reveals a peak at m/z: 387.14(M+1), which is equivalent to the molecular weight (cal. m/z - 386.41). The complex $[Ru(dmp)_2TMIP]^{+2}$ ESI-MS spectra reveals a peak at m/z:428.12, which is equivalent to the complex's theoretical weight (cal. m/z: 428.12) and the complex $[Ru(dmb)_2TMIP]^{+2}$peak at m/z:452.12, which is equivalent to the complex theoretical weight (cal. m/z: 452.12). The $[M\text{-}2ClO_4\text{-}2H_2O]^{2+}$ signals have been observed in the metal complexes.

Excitation in the electronic spectra at 280 nm, which corresponds to the ligand's $\boldsymbol{\pi} \rightarrow \boldsymbol{\pi}^*$ transition. Metal complex absorption spectra revealed absorption bands at 275 and 285 nm, corresponding to $\boldsymbol{\pi} \rightarrow \boldsymbol{\pi}^*$ transitions, and at 472 and 468 nm, resulting from MLCT transition (metal to ligand charge transfer), which further suggests the creation of metal complexes [35]. The creation of metal complexes was verified by all the spectrum data.

DNA binding studies:

UV-Visible absorption titrations:

One helpful method of research to understand the interactions between DNA binding and metal complexes is UV-visible spectroscopy [36]. The MLCT band absorption region shifts when a metal compound bonds to DNA in the intercalation phase. Frequently, whenever a metal complex intercalates among the base pairs of CT-DNA, hypochromism and bathochromism are seen. The two complexes in Figure 1 exhibit bathochromic and hypochromic shifts as a result of mounting interactions among the aromatic chromophore and DNA base pairs.

Table 1:- Intrinsic Binding constant (K_b) of Ru (II) complexes with CT-DNA.

Complex	**K_b from absorption(M^{-1})**	**$K_b$from emission (M^{-1})**	**K_{sv} value(M^{-1})**
1.$[Ru(dmp)_2TMIP]^{+2}$	$1.2x10^5$ M^{-1}	$2.0x10^6$ M^{-1}	$2.41x10^4$ M^{-1}
2 $[Ru(dmb)_2TMIP]^{+2}$	$8.0x10^4$ M^{-1}	$1.8x10^6$ M^{-1}	$2.04x10^4$ M^{-1}

These alterations with 17.78% and 16.58% hypochromism were detected at MLCT band at 466 and 468 nm, respectively. For the two complexes, the intrinsic binding constants (K_b) were 1.20 $x10^5M^{-1}$ and $8.0x10^4$ M^{-1}, respectively. The difference between these values and the its corresponding unsubstituted metal complex $[Ru(phen)_2TMPIP]^{+2}$ K_b value = $1.5x10^5M^{-1}$, is caused by the auxiliary ligand's substitution, which results in steric hindrance at the binding center. As the substituted phenanthroline auxiliary ligand appears more planar than the substituted bipyridine, the binding ability order for the complexes is as follows: 1 > 2.

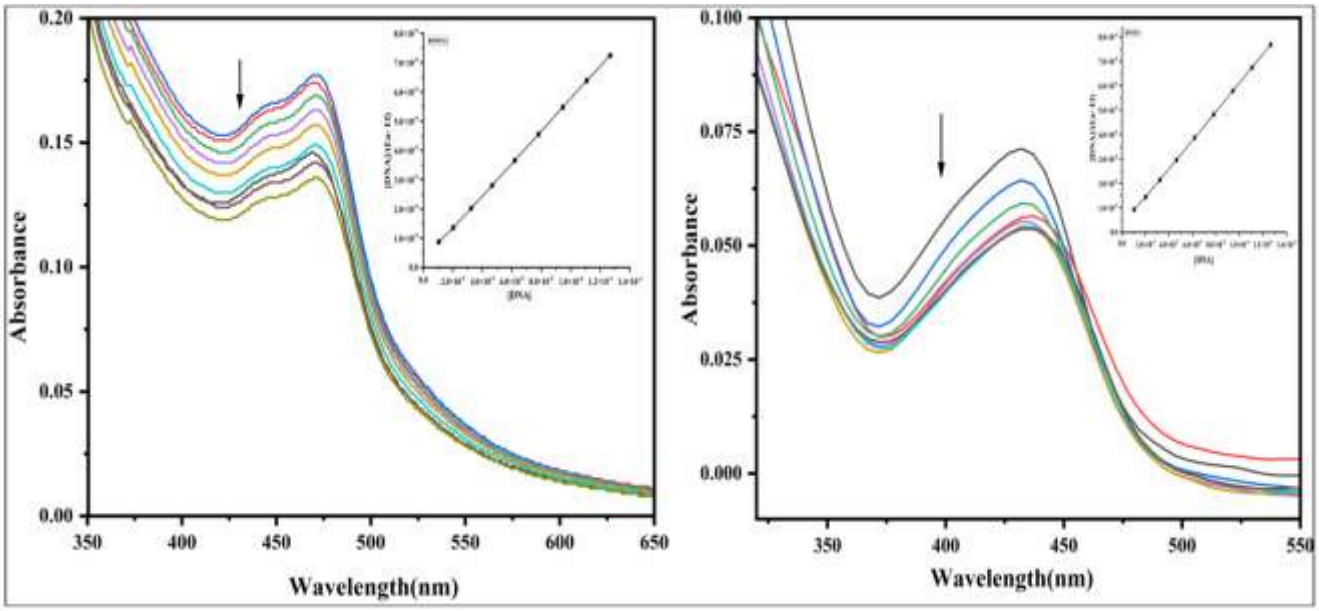


Fig 1:- UV-Visible Absorption spectra of Ru(II) complexes (1 to 2) in the presence (lower) and absence (top) of CT-DNA in Tris– HCl buffer. Changes upon increase of CT- DNA concentration is represented with the arrows, inserted a plot by taking [DNA]/(εa–εf) vs [DNA] gives intrinsic binding constant (K_b). 1) $[Ru(dmp)_2TMIP]^{+2}$, 2) $[Ru(dmb)_2TMIP]^{+2}$

Fluorescence emission titration:

Luminescence assays, performed at room temperature in Tris buffer (pH 7.2) with a constant concentration of 10 µl of metal complex, can explain the precise process of metal complex attachment to CT-DNA. When varying amounts of CT-DNA (10µl-100µl) exists, the emission intensity change is associated with the complex's degree of incorporation into the hydrophobic surroundings of the DNA. This result is shown in the figure 2. For complexes 1 and 2, luminescent emission titrations were carried out at 472, 468 nm excitation frequencies and 611 and 615 nm emission frequencies, respectively. The emission spectrum of metal complexes with and without DNA is explained in Figure 2.

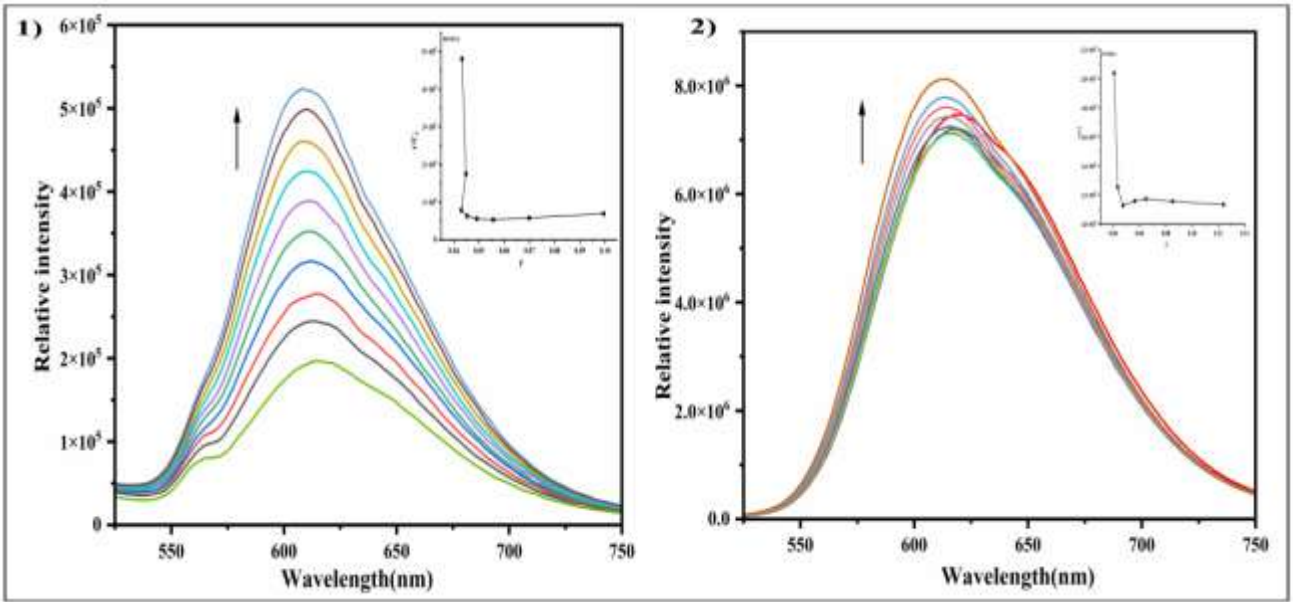


Fig 2:- Fluorescence emission spectra of Ru(II) complexes by addition of CT-DNA in Tris–HCl buffer. The arrow shows the change in the intensity by incremental addition of CT-DNA. Inset: Scatchard plot of the metal complexes 1) $[Ru(dmp)_2TMIP]^{+2}$, 2) $[Ru(dmb)_2TMIP]^{+2}$.

Fluorescence emission intensity rises as the amount of DNA added to the metal complex rises, supporting the intercalation mode of complexes 1 and 2 bonding. When a metal complex intercalates to CT-DNA, the emission intensity rises in comparison to the intensity when CT-DNA is absent [37].

The intrinsic binding constant (K_b) from the fluorescence data is obtained from the graph of r/C_f vs r from the Scatchard equation, where the binding ratio r is C_b/[DNA] and the concentration of free ligand is C_f. The Scatchard graphs for the two complexes constructed from fluorescence spectrum data showed that the K_b values for complexes 1 to 2 were 2.0 x10^6 M^{-1} and 1.8x10^6 M^{-1} respectively.

Luminescent quenching studies:

Figure 3 illustrates the quenching approach used to further investigate the strong intercalation attachment process of metal complexes to CT-DNA in presence of the strong intercalating ligand. In this experiment, EtBr was dissolved in Tris-HCl buffer. EtBr emits less light at first because of the influence of solvent molecules. However, the emission intensity of the identical solution increases abruptly when DNA is added. The intercalation of EtBr within DNA base pairs, which explains the intercalation mode of binding mechanism, is the source of this increased intensity.

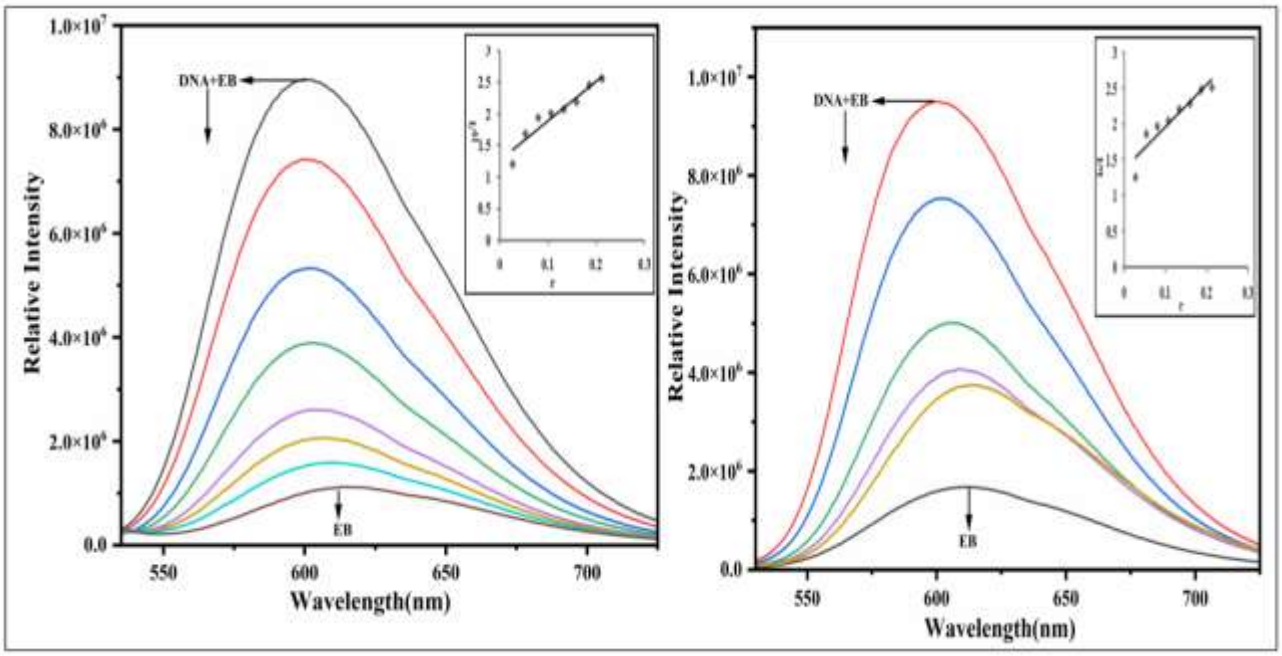


Fig 3:- Quenching studies of Ru(II) complexes(1-2) with EthBr in Tris-HCl in the absence (A) & and presence of DNA (B). 1) $[Ru(dmp)_2TMIP]^{+2}$, 2) $[Ru(dmb)_2TMIP]^{+2}$

The degree of emission appears to decrease when higher concentrations [10-100 mM] of metal complexes 1 and 2 are added to the solution containing EtBr [40 mM] and CT-DNA [130 mM] in Tris-HCl buffer. This implies that metal complexes bond with CT-DNA in between base pairs, potentially replacing the EtBr [38]. The Stern-Volmer formula and the I_0/I against [complex]/[DNA] linear fit graph used to derive the K_{SV} values. For complexes 1 and 2, the K_{sv} values were 2.41x10^4 M^{-1} and 2.04x10^4 M^{-1}, respectively. The K_b values from the absorption and fluorescence studies agree with the K_{sv} values. Table 1 included a list of K_b values.From absorption, emission, and quenching, the two complexes' binding orders are as follows: 1>2.

Light switch on & Switch off mechanism:

Using a DNA light switch test, the alteration in the luminosity behaviour of the metal complexes in the presence of exogenous reagents (Co^{+2}& EDTA) was examined. The results are displayed in Figure 4. The metal complex emission level was first lower in the tris-HCl buffer solution. The association of metal complex to DNA causes a quick increase in luminescence intensity (Switch on) upon the addition of DNA. When Co^{+2} (0.01 mM) is added to this solution, the emission intensity decreases as a result of the hetero metallic complex $[Ru(dmp)_2(TMIP)]^{2+}/Co^{2+}]$ (Complex1) forming [39].

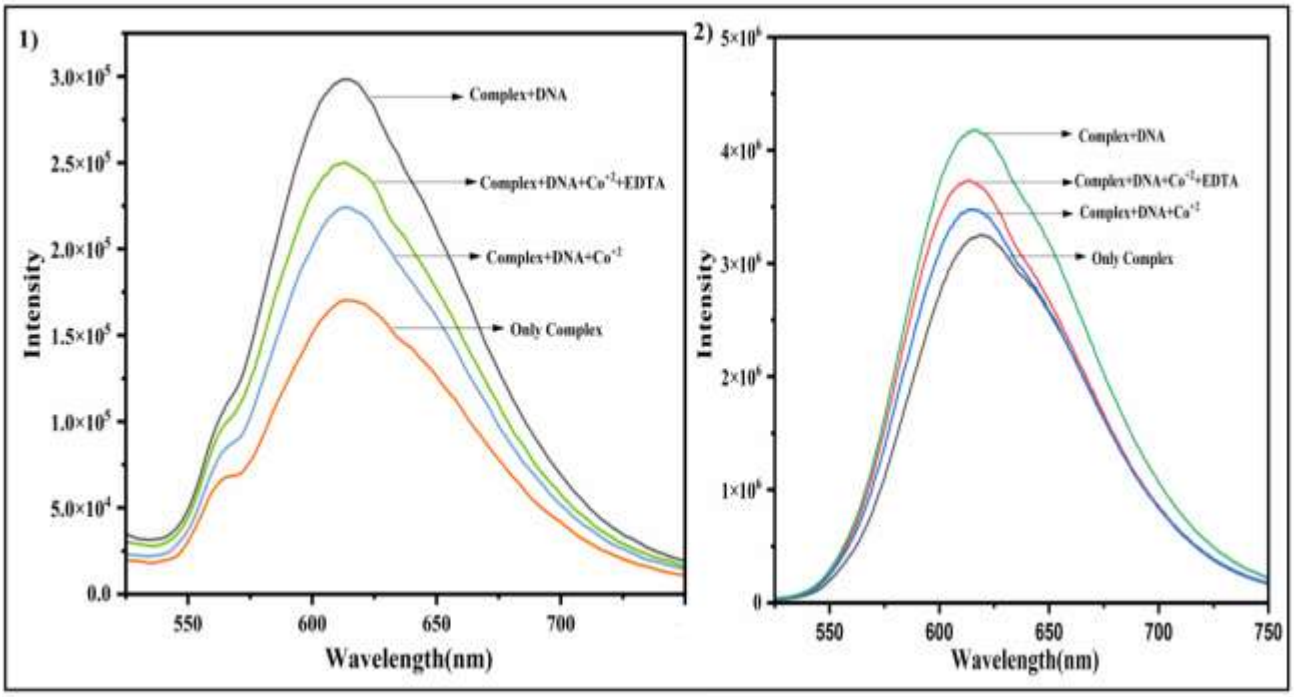


Fig 4:- DNA light switch on & off studies of Ru(II) complexes showing the emission intensity changes upon the addition of DNA (Switch on),Co^{2+} (Switch off) followed by EDTA (Switch on) to the complexes.1) $[Ru(dmp)_2TMIP]^{+2}$, 2) $[Ru(dmb)_2TMIP]^{+2}$

This effect is regarded as Light-switching off. This is known as "light switch on behaviour," where the addition of EDTA (0.01 mM) restores the emission strength. This is because the heterometallic complex is released by the complex formation between Co^{+2} and EDTA (Co^{+2}-EDTA) [40, 41]. This work validates the intercalation of metal complexes 1 and 2 to CT-DNA and indicates the distinctive feature of DNA-interclators [42].

Viscosity studies:

Although viscosity study is sensitive to changes in DNA length, it is probably the most significant and least ambiguous test of a binding model. The DNA helix lengthens and the viscosity of the DNA-containing fluid increases when a ligand/metal complex binds between DNA base pairs during the traditional intercalation binding mode. Ligands and metal complexes may bind to opposing ends of DNA base pairs during partial or non-classical intercalation, which shortens the DNA's length and decreases its viscosity [43]. Figure 5 illustrates how the incorporation of metal complex to CT-DNA causes variations in viscosity and length. DNA's relative viscosity increases when Ru(II) metal complex concentration grows (Complex 1and 2). In that sense, figure 5 is similar to $[Ru(phen)_2dppz]^{+2}$ [44]. Therefore, these complexes were identified as DNA intercalators. For instance, when the right circumstances are met, the intercalation of a dye such as ethidium bromide (EtBr) lengthens the DNA molecule overall. The viscosity order of the complexes comparable to the EtBr and the figure 5 order, which is as follows: EtBr>1>2. The additional data shows that the two Ru(II) complexes have an intercalative interaction process to CT-DNA, in accordance with the absorbance & emission titrations.

DNA cleavage study:

The complexes breaking reactions on plasmid DNA were examined using agarose gel electrophoresis. Circular plasmid DNA migrates swiftly in its intact supercoiled state (state I) when it is electrophoresed. When scission occurs on a single strand (nicking), the super coil relaxes to create Form II, a slower-moving open circular form.If both strands separate, a linear shape (form III) that transitions between Form I and II is created [45]. Figure 6 illustrates that lane-1 contains only DNA, while lane-2 contains DNA and a metal complex (20μM). In contrast, Form II of DNA rose significantly after 15 to 20 minutes of exposure to UV radiation at 365 nm, while Form I of DNA gradually dropped [46]. These results indicate that the complex possesses strong photocleaving ability for pBR322 DNA.

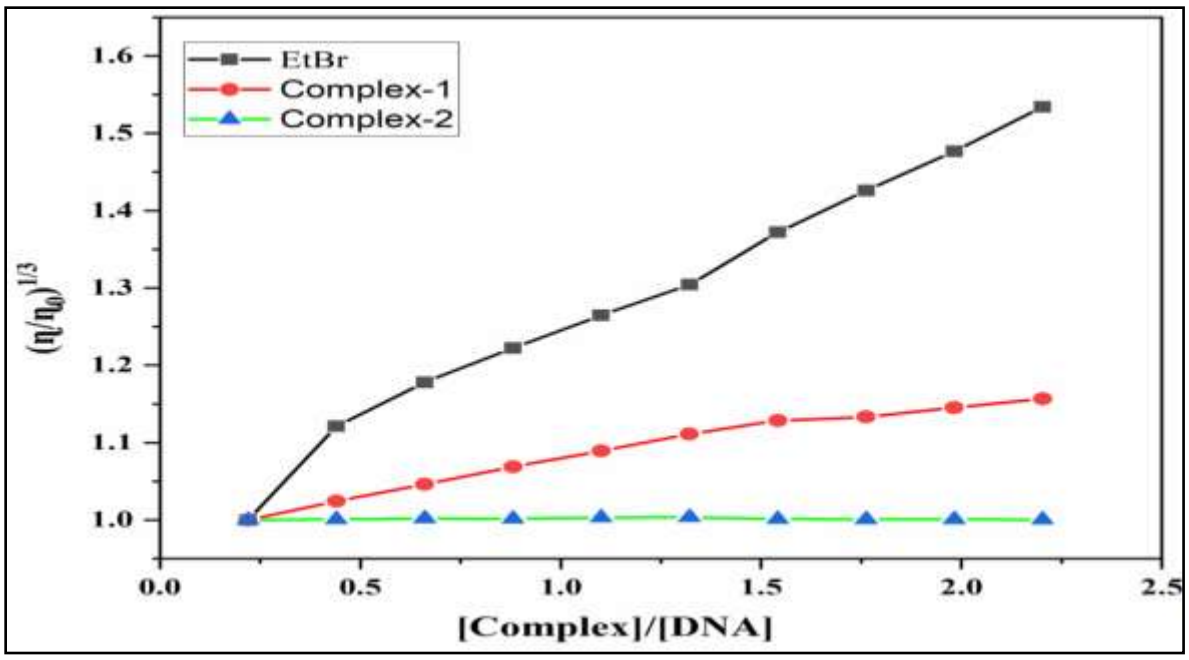


Fig 5:- Viscosity study of Ru (II) complexesin Tris-HCl buffer with increasing amounts of metal complexes and EtBr on the relative viscosity of CT-DNA at room temperature. 1) $[Ru(dmp)_2TMIP]^{+2}$, 2) $[Ru(dmb)_2TMIP]^{+2}$

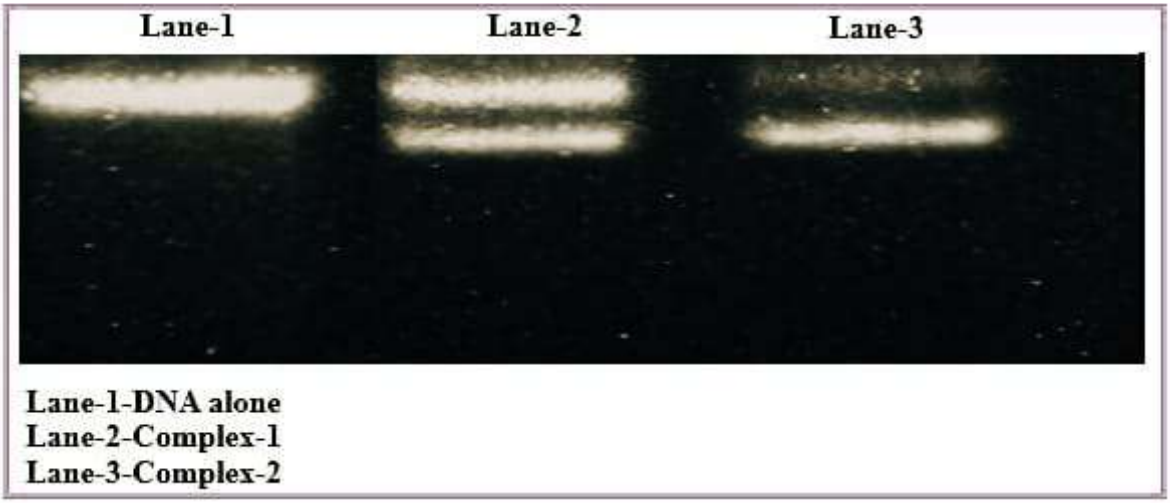


Fig 6:- Photo activated cleavage of pBR322 DNA in the presence of metal complexes(1&2) with the concentration range of 20 µM. 1) $[Ru(dmp)_2TMIP]^{+2}$, 2) $[Ru(dmb)_2TMIP]^{+2}$

Cytotoxicity:-

Many anticancer drugs employ DNA as their main intracellular target because the interaction of the metal complexes with DNA might damage DNA in cancer cells. This stops rapidly proliferating cells which eventually leads to cell death [47, 48].

Table 2:- Anti-cancer activity of Ru(II) Complexes.

S. No	Sample Name	IC_{50} (µg/ml)
		MCF 7
1	Complex 1	18.06±0.225
2	Complex 2	26.19±0.463
3	Doxorubicin	2.645±0.321

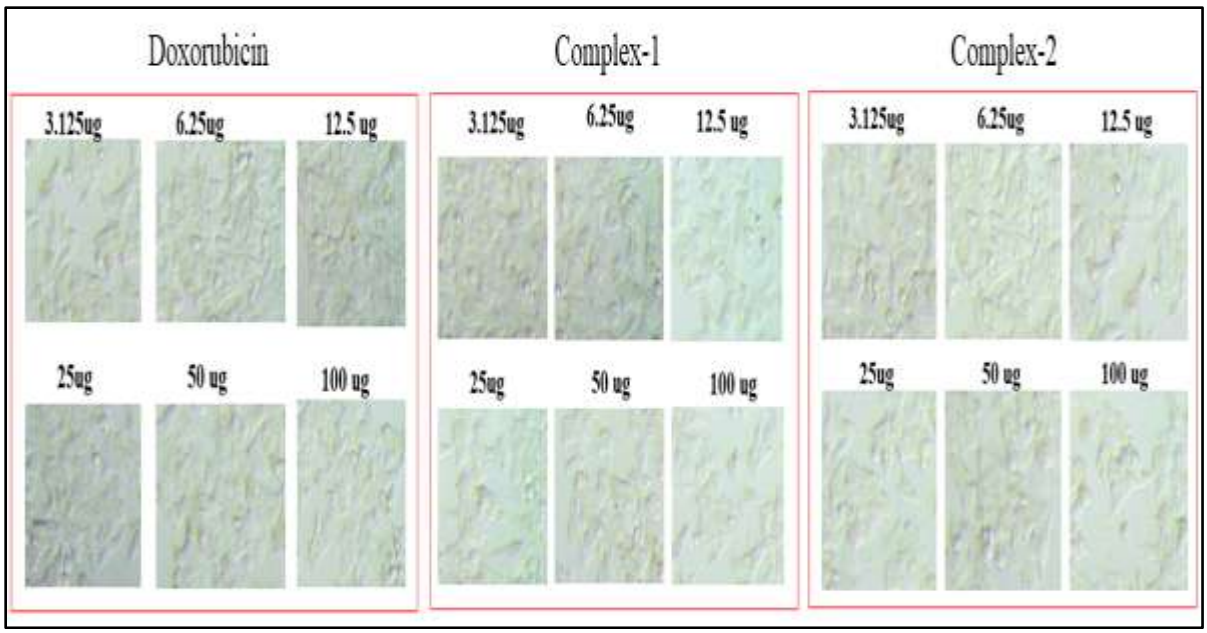


Fig 7:- Anticancer effect of complex 1&2 at different concentrations on MCF-7 Cell Line.

The cytotoxicity of metal complexes 1 and 2 has been investigated in relation to MCF-7 cancer cells (a kind of breast cancer cell) using the MTT assay. The IC_{50} values of metal complexes 1 and 2 using doxorubicin as the standard are shown in Figures 7 to 9 and Table 2. The IC_{50} results suggested that complex 1 was more active than complex 2.The activity determined follows the order doxorubicin > 1 > 2. This considerable increase in cytotoxicity implies that the type of auxiliary ligand that is affixed to the metal has a major impact on the anticancer activity of complexes. Increased activity was seen in the case of the phenanthroline ligand. Consequently, this combination exhibits a strong inhibitory impact on the cell growth of the MCF-7 cell line and holds great promise as an anticancer drug for breast cancer.

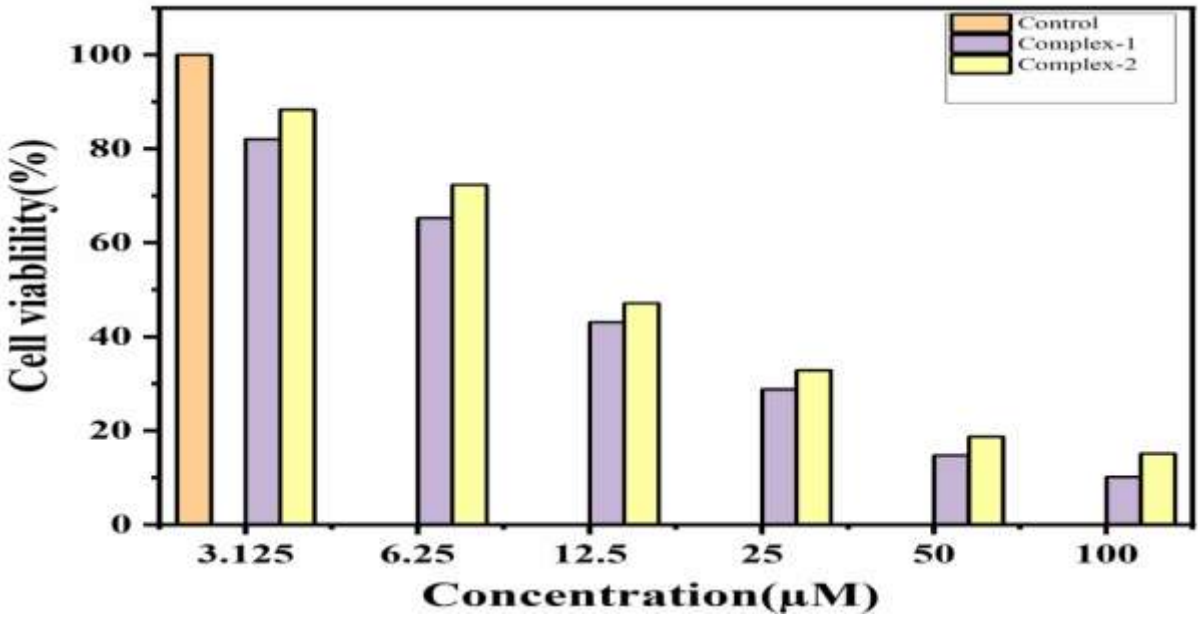


Fig 8:- Graphical representation of Anticancer activity of metal complexes 1&2.

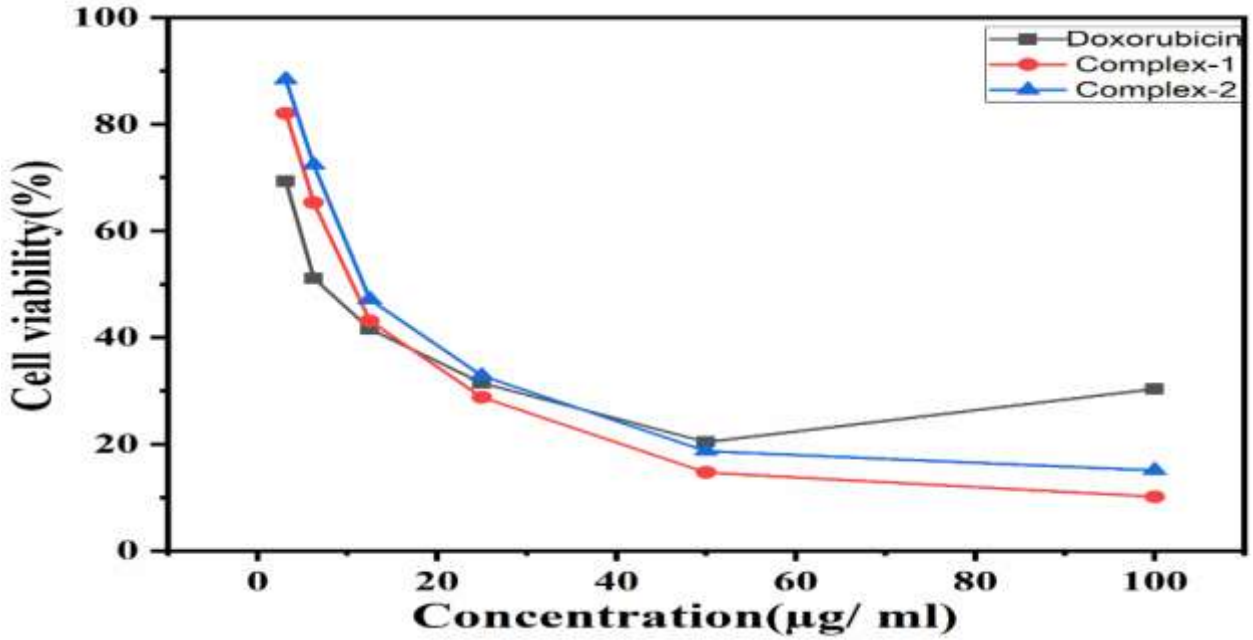


Fig 9:- Cell viability of MCF-7 cell line in vitro treatment with complexes $[Ru(dmp)_2(TMIP)]^{2+}$. Every data point is the average ± standard error derived from a minimum of three separate experiments.

Conclusion:-

The two novel Ru(II) Polypyridyl complexes were synthesized from the intercalator ligand (TMIP) and characterized by different spectroscopic techniques.These complexes interaction with DNA were studied by bio physical methods (UV Visible absorption, Fluorescence emission, and viscosity studies). The results indicating that planarity of ancillary ligand plays a crucial role in DNA binding. By comparing the K_b values with the existing phen complex of TMIP, these two complexes have shown less binding affinity this is due to the substitution over the ancillary ligand. Complex 1 binds to DNA more effectively than complexes 2, since complex 1 has substituted Phen as ancillary ligand.The binding affinity order is 1 > 2. Viscosity results revealed that Intercalative mode of binding observed in these metal complexes binding to DNA complimentary to the biophysical results. The findings suggest that an auxiliary ligand's persistence ensures its uniqueness for DNA binding. In the absence of DNA, the quencher (EtBr) successfully quenched the complexes. The complexes successfully cleaved the pBR322 DNA in photocleavage assays. The MTT assay results show the order of cytotoxicity is as follows: doxorubicin > 1 > 2. Cytotoxicity implies that the type of auxiliary ligand that is affixed to the metal has a major impact on the anticancer activity of complexes.

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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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