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### AND
## RESEARCH

# SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF RUTHENIUM (II) COMPLEXES OF SUBSTITUTED 2-{4, 5-BIS[(E)-2-PHENYLETHENYL]-1H-IMIDAZOL-2-YL}-1H-BENZIMIDAZOLE

M. Kiruthika [* 1], R. Elayaperumal [2] and C. Hariharan [3]

Department of Chemistry [1], Arignar Anna Government Arts College, Musiri, Trichy - 621211, Tamil Nadu, India.
Department of Chemistry [2], Paavai Engineering College, Namakkal - 637018, Tamil Nadu, India.
Department of Physics [3,] Jamal Mohamed College, Tiruchirappalli - 620020, Tamil Nadu, India.

**ABSTRACT:** Ruthenium(II) complexes of the type [Ru(bpy) 2(L)](PF6)2, where L= 2-{4,5-bis[(E)-2-phenylethenyl]-1H-imidazol-2-yl}-1H-benzimidazole (1), 2-{4,5-bis[(E)-2-(4-chlorophenyl)ethenyl]-1H-imidazol-2-yl}-1H-benzimidazole (2), 2-{4,5-bis [(E)-2-(4-fluorophenyl) ethenyl]-1H-imidazol-2-yl} -1H-benzimidazole (3), 2-{4 ,5-bis[(E)-2- (4-methylphenyl) ethenyl] -1H-imidazol-2-yl }-1H-benzimidazole (4), 2-{4, 5-bis[(E)-2-(4-methoxyphenyl) ethenyl]-1H-imidazol-2-yl}-1H-benzimidazole (5) have been synthesized and characterized by elemental analyses and spectral (IR, UV-vis, NMR, ESI-MS) techniques. The redox behavior of the complexes has been studied by cyclic and differential pulse voltammetry. In acetonitrile solution, all the complexes exhibit characteristic metal to ligand charge transfer (MLCT) absorptions and ligand-based transitions. The complexes showed efficient DNA cleavage activity in the presence of light at the wavelength of 480 nm. The complexes are also able to cleave supercoiled pUC19 plasmid DNA *via* guanine base oxidation in a concentration-dependent manner. *In-vitro* cytotoxic activity of the complexes shows that complex 1 has better anticancer activity against MCF7 human breast cancer cells with an $IC_{50}$ value of 7.9 μM. The antimicrobial activities of the ligand and their metal complexes were screened by agar diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

**INTRODUCTION:** Throughout the previous three decades, anticancer chemotherapy has focused on cisplatin derivates. Regardless of their predictable reactions, cisplatin derivates have a focal part in most anticancer treatments [1]. A large number of platinum compounds have been combined, trying to defeat the issues of cisplatin.

Incredibly none of these has possessed the capacity to supplant cisplatin in routine chemotherapy medicines. In the look for medications with cutting edge clinical viability, decreased harmfulness, and a more extensive range of action, metals other than platinum, for example, rhodium and ruthenium, have been considered.

Non-platinum active compounds are having different mechanisms of action and bio-distribution so that they are found to be active against human malignancies. Because of a less toxic nature, ruthenium complexes are found to be superior to cisplatin. Ruthenium has found its way into the clinic, where its properties are exploited for very

miscellaneous uses. The radiophysical properties of 97Ru can be connected to radiodiagnostic imaging [2, 3]. Other ruthenium compounds have potential as immunosuppressants (cis-[Ru (III) (NH$_3$)$_4$ (HIm) 2]$^{3+}$), antimicrobials ([Ru (II) Cl$_2$ (chloroquine)2]), antibiotics, antioxidants, vasodilator / vaso-constrictor agents and, as previously mentioned, as drugs for cancer chemotherapy [4]. The ligand-exchange kinetics of ruthenium (II) and ruthenium (III) complexes have been observed to be like those of platinum (II) complexes. This property makes them the first choice in the search for compounds that exhibit similar biological effects to platinum (II) drugs [5].

The scope of available oxidation states of ruthenium under physiological conditions makes this metal novel among the platinum compounds. The ruthenium compounds, prevalently octahedral, can be Ru (II), Ru (III), or Ru (IV). Ru (III) compounds have a tendency to be more biologically inert compared to Ru (II) and Ru (IV) complexes [6]. The redox capability of ruthenium compounds can be exploited to enhance the viability of Ru-based medications in the clinic [5, 6]. The passage of two Ru(III) based drugs, NAMI-A ([H2Im]{trans-[Ru (III) Cl$_4$ (dmso) (imidazole)]})[7] and KP1019 (trans-[RuCl$_4$ (indazole)2]-)8 into clinical trials for the treatment of metastatic tumors expanded the enthusiasm for this metal.

Additionally, more number of Ru (II) compounds are being synthesized to show the cytotoxic and antitumor properties [9, 10] and are presently under dynamic examination. Benzimidazoles are important group of heterocyclic compounds that are naturally active and of noteworthy significance in therapeutic chemistry. The derivatives of benzi-midazoles have been of huge enthusiasm to medicinal chemistry since its substituents had different biological activities, for example, antioxidant [11], antimicrobial [12], anticancer [13,] and anti-inflammatory [14] activities. All benzimidazole subordinates with their two-ring frameworks bear distinctive useful substituents, and this prompts basic alteration of the physico-chemical, pharmaco-kinetic and metabolic properties of these drugs. In the previous couple of decades, benzimidazole and its derivatives have received much consideration because of their chemotherapeutic properties. Since, the metal ion, its oxidation state, and

chelating ligand circle assume imperative parts in deciding chemical and biological properties, a series of Ru (II) compounds encasing benzi-midazole based ligands have been synthesized and structurally characterized. All the complexes have been subjected to biological studies such as DNA photocleavage, antioxidant, antimicrobial, and cyto-toxic activity testing as DNA photocleavage, anti-microbial and cytotoxic activity testing.

## EXPERIMENTAL:

**MATERIALS AND METHODS:** Ruthenium chloride trihydrate, ammonium hexafluoro phos-phate, 4-chlorobenzaldehyde, 4-fluoro-benzal-dehyde, 4-methyl benzaldehyde, 4-methoxy benzal-dehyde and benzaldehyde were purchased from Sigma-Aldrich. Benzimidazole- 2-carbo-xaldehyde was prepared by following a reported procedure [15.] Acetic acid, ammonium acetate, methanol, acetonitrile, and ethanol were purchased from SD Fine chemicals.

Absorption spectra were recorded on Shimadzu UV-160A UV-Visible spectrophotometer. Cyclic (CV) and differential pulse voltammetry (DPV) were performed by using CH instrument (USA) model CH-620 B electrochemical analyzer. A conventional three-electrode system consisting of the platinum disc as a working electrode, platinum wire as an auxiliary electrode, and saturated calomel (SCE) as a reference electrode was used for the electrochemical measurements. 0.1 M tetrabutylammonium perchlorate (TBAP) was used as the supporting electrolyte for all the experiments. Positive ion electrospray ionization mass spectra of the complexes were obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer. Elemental analyses were performed using Carlo Erba 1108 analyzer at Cochin University. IR spectra were recorded as KBr pellets in the 400-4000 cm$^{-1}$ region using a Shimadzu FT–IR 8000 spectrophotometer. All the DNA gel images were taken using UVITEC gel documentation system, and fragments were analyzed using UVI chem and UVI-band software.

**Synthesis of Ligands:** The ligands (1E, 5E)-1, 6-diphenylhexa-1, 5-diene-3, 4-dione, (1E, 5E)-1, 6-bis (4-chlorophenyl) hexa-1, 5-diene-3, 4-dione, (1E, 5E)-1, 6-bis (4-fluorophenyl) hexa-1, 5-diene-3, 4-dione, (1E, 5E)-1, 6-bis(4-methylphenyl)hexa-

1, 5-diene-3, 4-dione and (1E, 5E)-1, 6-bis(4-metthoxyphenyl) hexa-1, 5-diene-3, 4-dione were prepared as reported elsewhere [16].

**Synthesis of 2-{4, 5-bis[(E)-2-phenylethenyl]-1H-imidazol-2-yl}-1H-benzimidazole (L1):** (1E, 5E)-1,6-diphenylhexa-1, 5-diene-3, 4-dione (0.5 g, 1.91 mmol), benzimidazole-2- carboxaldehyde (0.29 g, 2.00 mmol) and ammonium acetate (4 g, 50 mmol) were dissolved in 30 mL acetic acid and heated to reflux for 3 h. After cooling, cold water (20 mL) was added to the solution, during which reddish brown precipitate appeared. It was filtered and purified by column chromatography on silica gel using ethyl acetate:hexane (1:4) as an eluent (Yield: 0.416 g, 56%). ESI-MS: m/z (relative intensity): 389.9 $(M+1)^+$. $\delta_H$ (400 MHz; DMSO-d6; Me4Si) 7.29(4H, t, J=2.8 Hz), 7.21(4H, t, J=3.2 Hz), 7.18(4H, d, J=4.0 Hz), 7.31(4H, d, J=5.2 Hz), 7.71 (2H, d, J=2.8 Hz), 7.26(2H, t, J=7.6 Hz), 12.22(2H, s); Anal. Calc. for $C_{26}H_{20}N_4$: C, 80.91; H, 5.19; N, 14.42. Found: C, 80.88; H, 5.17; N, 14.38; IR, $cm^{-1}$ (KBr pellet) 3423, 2313, 1545, 1271, 745, 696; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 313(16000), 282(49314).

**Synthesis of 2-{4, 5-bis[(E)-2-(4-chlorophenyl) ethenyl] -1H-imidazol-2-yl} -1H benzimidazole (L2):** An analogous synthetic procedure using (1E, 5E)-1, 6-bis(4-chlorophenyl)hexa-1, 5-diene-3, 4-dione (0.5 g, 1.51 mmol) instead of (1E, 5E)-1, 6-diphenylhexa-1, 5-diene-3,4-dione was used to prepare 2-{4,5-bis[(E)-2-(4-chlorophenyl)ethenyl]-1H-imidazol-2-yl}-1H-benzimidazole (Yield: 0.438 g, 61%). ESI-MS: m/z (relative intensity): 458.1 $(M+1)^+$. $\delta_H$ (400 MHz; DMSO-d6; Me4Si) 7.32 (4H, d, J=2.8 Hz), 7.34(4H, d, J=3.2 Hz), 7.29 (4H, d, J=2.8 Hz), 7.71(2H, d, J=2.4 Hz), 7.30 (2H, t, J=1.6 Hz), 12.42(2H, s); Anal. Calc. for $C_{26}H_{18}Cl_2N_4$: C, 68.28; H, 3.97; N, 12.25. Found: C, 68.25; H, 3.95; N, 12.22; IR, $cm^{-1}$ (KBr pellet) 3417, 2312, 1606, 1489, 743, 419; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 339(15145), 285 (49430).

**Synthesis of 2-{4,5-bis[(E)-2-(4-fluorophenyl) ethenyl ]-1H-imidazol -2-yl}-1H benzimidazole (L3):** The same method was adopted using (1E, 5E)-1, 6-bis(4-fluorophenyl)hexa-1, 5-diene-3, 4-dione (0.5 g, 1.68 mmol) instead of (1E, 5E)-1, 6-bis (4-chlorophenylhexa-1, 5-diene-3, 4-dione was used to prepare -{4, 5-bis[(E)-2- (4-fluorophenyl)

ethenyl]-1H- imidazol-2-yl]- 1H-benzimidazole (Yield: 0.371 g, 52%). ESI-MS: m/z (relative intensity): 425.2 $(M+1)^+$. $\delta_H$ (400 MHz; DMSO-d6; Me4Si) 7.18(4H, d, J=5.2 Hz), 7.24(4H, d, J=6.0 Hz), 7.11(4H, d, J=6.8 Hz), 7.31(4H, d, J=5.2 Hz), 7.71(2H, d, J=7.2 Hz), 7.32(2H, t, J=2.8 Hz), 12.72(2H, s); Anal. Calc. for $C_{26}H_{18}F_2N_4$: C, 73.57; H, 4.27; N, 13.20. Found: C, 73.55; H, 4.26; N, 13.16; IR, $cm^{-1}$ (KBr pellet) 3398, 2924, 1601, 1506, 1230, 839, 428; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 326(17265), 282(46590).

**Synthesis of 2-{4,5-bis[(E)-2-(4-methylphenyl) ethenyl]- 1H-imidazol-2-yl} -1H-benzimidazole (L4):** While preparing 2-{4, 5-bis[(E)-2-(4-methyl-phenyl) ethenyl]- 1H-imidazol- 2-yl}-1H-benzi-midazole, (1E, 5E)-1, 6-bis(4- methylphenyl) hexa-1, 5-diene-3, 4-dione (0.5 g, 1.72 mmol) was used in place of (1E,5E)-1, 6-bis(4-fluorophenyl) hexa-1, 5-diene-3, 4-dione. A brown precipitate was appeared. It was filtered and purified by column chromatography on silica using ethyl acetate: hexane (1:4) as an eluent. (Yield 0.52 g, 72%). ESI-MS: m/z (relative intensity): 417.1 $(M+1)^+$. $\delta_H$ (400 MHz; DMSO-d6; Me4Si) 7.08 (4H, d, J=3.2 Hz), 7.10(4H, d, J=3.2 Hz), 6.97 (4H, d, J=6.8 Hz), 7.59(2H, d, J=2.0 Hz), 7.10 (2H, t, J=3.2 Hz), 2.24(6H, s), 12.42(2H, s); Anal. Calc. for $C_{28}H_{24}N_4$: C, 80.74; H, 5.81; N, 13.45. Found: C, 80.72; H, 5.78; N, 13.43; IR, $cm^{-1}$ (KBr pellet) 3417, 2922, 1607, 1513, 1272, 742, 424; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 323(17475), 283 (46090).

**Synthesis of 2-{4,5-bis[(E)-2-(4-methoxyphenyl) ethenyl ]-1H-imidazol-2-yl} -1H-benzimidazole (L5):** An analogous synthetic procedure using (1E, 5E)-1, 6-bis(4-metthoxyphenyl)hexa-1,5-diene-3,4-dione (0.5 g, 1.55 mmol) instead of (1E,5E)-1, 6-bis (4-methylphenyl) hexa-1, 5-diene-3, 4-dione was used to prepare 2-{4, 5-bis[(E)-2 -(4-methoxyphenyl) ethenyl]-1H-imidazol-2-yl}-1H-benzimidazole. (Yield 0.33 g, 47%). ESI-MS: m/z (relative intensity): 449.1 (M)$^+$. $\delta_H$ (400 MHz; DMSO-d6; Me4Si) 6.77(4H, d, J=3.2 Hz), 7.29 (4H, d, J=3.2 Hz), 7.01(4H, d, J=2.0 Hz), 7.71 (2H, d, J=2.8 Hz), 7.28(2H, t, J=2.8 Hz), 3.78 (6H, s), 12.41(2H, s); Anal. Calc. for $C_{28}H_{24}O_2N_4$: C, 74.98; H, 5.39; N, 12.49. Found: C, 74.95; H, 5.37; N, 12.44; IR, $cm^{-1}$ (KBr pellet) 3451, 2312, 1607,

1508, 1249, 833, 586; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 315(16495), 284 (46805).

## Synthesis of Ruthenium (Ii) Complexes:

**Synthesis of [Ru(bpy)$_2$(L1)] (PF6)$_2$ (1):** A mixture of [cis-Ru(bpy)$_2$Cl$_2$]•2H$_2$O$_{17}$ (0.2 g, 0.38 mmol) and L1 (0.149 g, 0.38 mmol) was suspended in an ethanol/water solvent mixture (3/1, v/v). The mixture was refluxed under an inert atmosphere for 4 h while vigorous stirring was maintained. The reaction mixture was cooled to room temperature; the solvent was reduced under vacuum to one-third of its initial volume. A saturated aqueous solution of NH4PF6 was added to precipitate [Ru (bpy) 2(L1)]$^{2+}$ as its hexafluorophosphate salt. The product was filtered and washed with water (3 × 10 mL) and dried. Yield: 0.34 g, 81%. Anal. Calc. for $C_{46}H_{36}F_{12}N_8P_2Ru$: C, 50.60; H, 3.32; N, 10.26. Found: C, 50.58; H, 3.28; N, 10.25. ESI-MS: m/z 946.0 (M − PF6)$^+$ ; IR, $cm^{-1}$ (KBr pellet) 3716, 2363, 1516, 845; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 464 (8700), 343 (20950), 290 (53750), 242 (43450).

**Synthesis of [Ru(bpy)$_2$(L2)](PF6)$_2$ (2):** The synthesis and purification of compound 2 were similar to those of 1 using [Ru(bpy)$_2$Cl$_2$]•2H$_2$O (0.2 g, 0.38 mmol) and L2 (0.17 g, 0.38 mmol). Yield: 0.366 g, 83%, Anal. Calc. for $C_{46}H_{34}Cl_2F_{12}N_8P_2Ru$: C, 47.60; H, 2.95; N, 9.65. Found: C, 47.57; H, 2.93; N, 9.62; ESI-MS: m/z 435.4 (M − 2PF6)$^{2+}$; IR, $cm^{-1}$ (KBr pellet) 3717, 3047, 2363, 1516, 844; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 462(7400), 346(19350), 292(52350), 244 (43400).

**Synthesis of [Ru(bpy)$_2$(L3)](PF6)$_2$ (3):** This compound was synthesized and purified by a procedure similar to that described for compound 1, except L3 (0.16 g, 0.38 mmol) was used instead of L2. Yield: 0.368 g, 86%, Anal. Calc. for $C_{46}H_{34}F_{14}N_8P_2Ru$: C, 48.99; H, 3.04; N, 9.94. Found: C, 48.95; H, 3.01; N, 9.92; ESI-MS: m/z 419.1 (M − PF6)$^{2+}$; IR, $cm^{-1}$ (KBr pellet) 3726, 3608, 1602, 1510, 1454, 1230, 763; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 463(7500), 338(19350), 291 (47850), 242(40200).

**Synthesis of [Ru(bpy)$_2$(L4)](PF6)$_2$ (4):** This complex was prepared by adopting the procedure used for the isolation of 3 but by using L4 (0.16 g, 0.38 mmol) instead of L3. Yield: 0.349 g, 82%.

Anal. Calc. for $C_{48}H_{40}F_{12}N_8P_2Ru$: C, 51.48; H, 3.60; N, 10.01. Found: C, 51.45; H, 3.58; N, 9.97. ESI-MS: m/z 487.3 (M − 2PF6)$^+$ ; IR, $cm^{-1}$ (KBr pellet) 3728, 2920, 2308, 1514, 843, 416; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 466(8550), 344 (21450), 290 (53900), 244 (43250).

**Synthesis of [Ru (bpy)$_2$(L5)](PF6)$_2$ (5):** The synthesis and purification of compound 5 were similar to those of 4 using [Ru(bpy)$_2$Cl$_2$]•2H$_2$O (0.2 g, 0.38 mmol) and L5 (0.17 g, 0.38 mmol). Yield: 0.372 g, 85%, Anal. Calc. for $C_{48}H_{40}O_2F_{12}N_8P_2Ru$: C, 50.05; H, 3.50; N, 9.73. Found: C, 50.02; H, 3.47; N, 9.71; ESI-MS: m/z 430.2 (M − 2PF6)$^{2+}$; IR, $cm^{-1}$ (KBr pellet) 3701, 2362, 1695, 1516, 1250, 842; UV-Visible $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) 470(8850), 332(24900), 289(59600), 245(41300).

**DNA Cleavage Activity:** Photonuclease activity of the complexes was monitored using gel electrophoresis of plasmid DNA (pUC19). The solutions were prepared for the photolysis experiment containing 3 μL of 100 μg mL$^{-1}$ plasmid DNA in Tris buffer and varying amounts of complexes 1- 5 (0–48 μM). Each solution was incubated for 1 hour and then irradiated at 450 nm for various time intervals varying from 10 min to 60 min.

The samples were then subjected to electrophoresis in 0.8% agarose gel (tris-boric acid-EDTA buffer, pH 8.0) at 50 V for 2 h. The gel was stained with 0.5 μg mL$^{-1}$ of ethidium bromide. The stained gel was illuminated under a UV lamp and gel documented. In a separate experiment, the DNA was incubated with 48 μM of the metal complex and 10 mM of histidine and irradiated at 440 nm. The photolyzed solution was subsequently subjected to electrophoresis.

***In-vitro* Anticancer Assay:** 3-[4, 5-dimethylthiazol -2-yl] 2, 5-diphenyltetrazolium bromide (MTT) is a yellow water-soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-de-hydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan.

Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37 ºC, 5% $CO_2$, 95% air and 100% relative humidity. For screening experiments, the cells were seeded into 96-well plates in 100 mL of the respective medium containing 10% FBS, at a plating density of 10 000 cells / well and incubated at 37 ºC, 5% $CO_2$, 95% air and 100% relative humidity for 24 h prior to the addition of compounds. The compounds were dissolved in DMSO and diluted in the respective medium containing 1% FBS. After 24 h, the medium was replaced with the respective medium with 1% FBS containing the compounds at various concentrations and incubated at 37 ˚C, 5% $CO_2$, 95% air and 100 % relative humidity for 48 h. Experiments were performed in triplicate and the medium without the compounds served as control. After 48 h, 15 µL of MTT (5 mg mL$^{-1}$) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ˚C for 4 h. The medium with MTT was then removed, and the formed formazan crystals were dissolved in 100 mL of DMSO, and the absorbance measured at 570 nm using a micro plate reader. The % cell inhibition was determined using the following formula, and a graph was plotted between % of cell inhibition and concentration. From this plot, the $IC_{50}$ value was calculated. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) × 100

A nonlinear regression graph was plotted between % Cell inhibition and Log concentration, and $IC_{50}$ was determined using GraphPad Prism software.

**Antimicrobial Assay:** Test Microorganisms: A gram-positive bacteria (*Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli*), and one Yeast *Candida albicans* were used in the present study for evaluation of antimicrobial activity of the synthesized compounds. The medium used for the antimicrobial testing was Muller Hilton agar media and autoclaved at 15 lbs/in 2 for 15 min.

Antimicrobial Activity: Agar disc diffusion method was used to study the antimicrobial activity of the newly synthesized compounds [18, 19].

For the evaluation of antimicrobial activity, the size of inoculums was adjusted to approximately 108 colony-forming units (cfu/mL) by suspending the culture in sterile distilled water. Petri dishes containing 20 mL of Muller Hilton agar medium were swabbed with a culture of the respective microbial strains and kept for 15 min for the absorption of culture. Sterile borer is used to create the wells (6 mm in diameter), and we added 25, 50, and 100 µL solution of each compound of 25, 50, and 100 µg/mL concentration respectively re-constituted in the DMSO on the preinoculated plates. All the plates were incubated at 37 ºC for 24 hrs. Antimicrobial activity of all the synthesized compounds was determined by measuring the zone of inhibition around the wells. DMSO was used as a negative control, whereas Gentamycin was used as a positive control. This procedure was performed in three replicate plates for each organism.
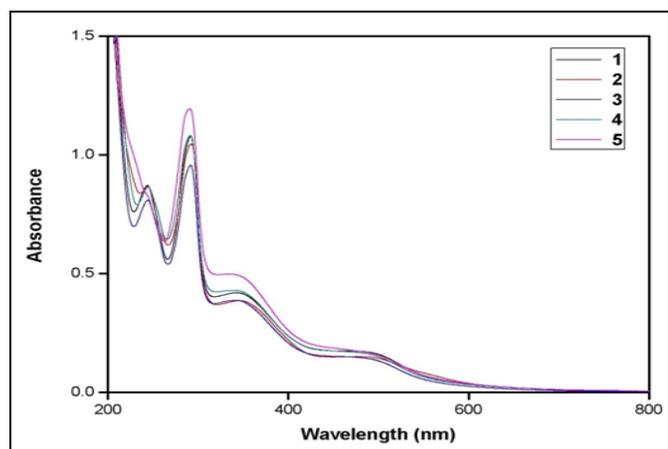
**RESULTS AND DISCUSSION:**
**Synthesis and Characterization:** The complexes are prepared in high yield by following a general procedure in which $[Ru(bpy)_2Cl_2] \cdot 2H_2O$ was reacted with the newly synthesized ligands (L1-L5) in an aqueous ethanolic medium. The synthesized complexes **Fig. 1** have been isolated as their hexa-fluorophosphate salts and characterized by various techniques such as FT-IR, UV-Visible, ESI-MS, elemental analyses, cyclic and differential pulse voltammetries. The synthesized ligands and complexes are stable at room temperature and non-hygroscopic. The analytical data **Table 1** obtained for these complexes are in good agreement with the proposed molecular formulae. The mass spectra of the complexes $[Ru(bpy)_2(L1)](PF6)_2$, $[Ru(bpy)_2(L2)](PF6)_2$, $[Ru(bpy)_2(L3)](PF6)_2$, $[Ru(bpy)_2(L4)(PF6)_2$ and $[Ru(bpy)_2(L5)](PF6)_2$ displayed base peaks at 946.0, 435.39, 419.07, 487.33 and 430.17 respectively. These peaks are reliable with the proposed molecular formulae of the corresponding ruthenium (II) complexes. The electronic spectra of all the complexes were recorded in acetonitrile, and the results are listed in **Table 1**. Representative spectra of the complexes (1-5) are shown in **Fig. 2**. If the field around $Ru^{2+}$ is assumed as octahedral, the ground state of ruthenium (II) (t2g6 configuration) should be 1A1g. The excited states corresponding to t2g5eg1 configuration are 3T1g, 3T2g, 1T1g, and 1T2g in increasing order of energy.

Thus, four bands corresponding to the transitions from 1A1g to 3T1g, 3T2g, 1T1g, and 1T2g should appear in the electronic spectra of the complexes.

In the low spin d6 system, no band due to ligand to metal charge transfer transitions is possible in the visible region [20].



**FIG. 1: SYNTHESIS OF RUTHENIUM (II) COMPLEXES**
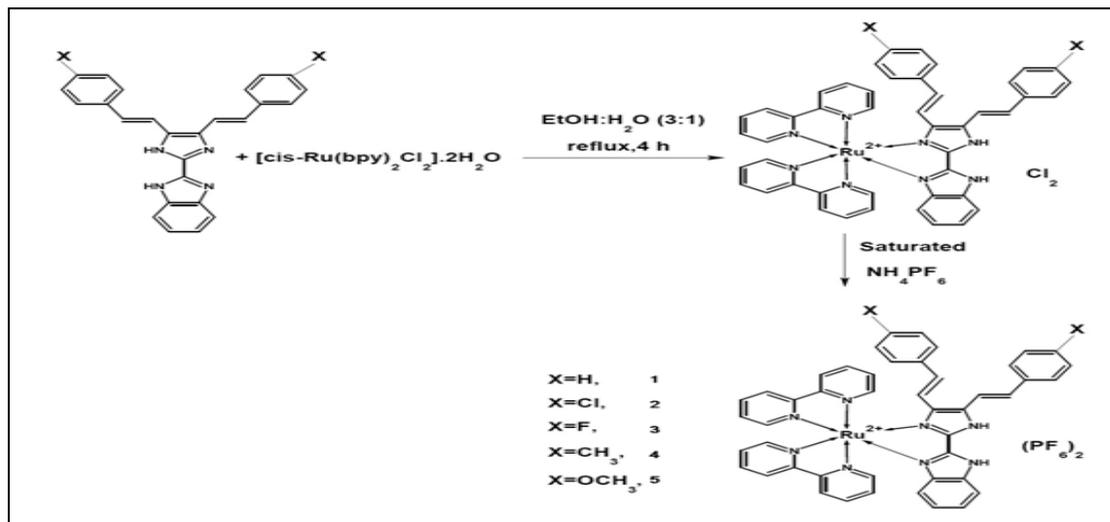
Each complex shows broadband in the region of 462-470 nm. Based on the values of molar extinction coefficient (7400-8850 $M^{-1}$ $cm^{-1}$), this band has been assigned to charge transfer transitions occurring from excitation of an electron from metal t2g level to unfilled molecular orbitals derived from the ∏* level of the ligands [21]. The $\lambda_{max}$ value for the well-defined lowest energy band illustrates a clear trend of increasing as the co-ligand (L1–L5) becomes more electron-donating, in the order L5 > L4 > L1 > L3 > L2. The nature of the observed electronic spectra and the position of absorption bands are reliable with those of other similar octahedral ruthenium (II) complexes [22]. The higher energy bands observed in the UV region (242-292 nm) are of intraligand ∏-∏* type.

The IR spectra of the complexes (1-5) contain many sharp bands of different intensities due to the vibrations arising from the coligands (L1-L5) and 2, 2'-bipyridine. The vC=N stretching frequency of the free ligands (L1-L5) appears near 1607 $cm^{-1}$ has been shifted to 1602 $cm^{-1}$ in the present complexes in accordance with the coordination of the azomethine function to the metal ion [23, 24]. In the IR spectra of all the ligands, a sharp band observed in the region of 1506-1544 $cm^{-1}$ has been assigned to vC=C stretching frequency. Upon coordination, these bands have been shifted around 1516 $cm^{-1}$, thereby indicating the coordination of the ligands with the ruthenium metal. The presence of a medium intensity band at 744-844 $cm^{-1}$ in the ligands is characteristic of CH out-of-plane bending mode [25].

The electrochemical features of the mononuclear ruthenium (II) complexes 1-5 were investigated in acetonitrile by employing cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on a glassy carbon working electrode. Typical cyclic voltammograms of the present complexes is depicted in **Fig. 3**. The redox parameters of these complexes are listed in **Table 1**. The values of anodic peak potentials (Epa) indicate the energy required for oxidation of the central metal ion in the complex species, while the values of cathodic peak potential (Epc) indicate the energy required for reduction of the metal ion.



**FIG. 2 ELECTRONIC ABSORBANCE SPECTRA OF RUTHENIUM COMPLEXES IN ACETONITRILE**

**TABLE 1: ELECTRONIC AND ELECTROCHEMICAL DATA OF MONONUCLEAR RUTHENIUM (II) COMPLEXES IN ACETONITRILE SOLUTION AT 25 ± 0.2 °C**

| Complex | $\lambda_{max}$, nm ($\varepsilon$, $M^{-1}cm^{-1}$) | $E_{p,a}$(V) | $E_{p,c}$(V) | $\Delta E_p$(mV) | $E_{1/2}$ $Ru^{II}/Ru^{III}$ *vs* SCE | |
|---|---|---|---|---|---|---|
| | | | | | CV (V) | DPV (V) |
| $[Ru(bpy)_2(L1)](PF_6)_2$ | 464 (8700) 343(20950) 290(53750) 242(43450) | 1.0834 | 0.9749 | 108 | 1.0291 | 1.0198 |
| $[Ru(bpy)_2(L2)](PF_6)_2$ | 462 (7400) 346(19350) 292(52350) 244(43400) | 1.1092 | 0.9964 | 112 | 1.0528 | 1.0489 |
| $[Ru(bpy)_2(L3)](PF_6)_2$ | 463 (7500) 338(19350) 291(47850) 242(40200) | 1.0691 | 0.9931 | 76 | 1.0311 | 1.0342 |
| $[Ru(bpy)_2(L4)](PF_6)_2$ | 466 (8550) 344(21450) 290(53900) 244(43250) | 1.1194 | 1.0246 | 94 | 1.0720 | 1.0677 |
| $[Ru(bpy)_2(L5)](PF_6)_2$ | 470 (8850) 332(24900) 289(59600) 245(41300) | 1.1507 | 1.0074 | 143 | 1.0791 | 1.0731 |
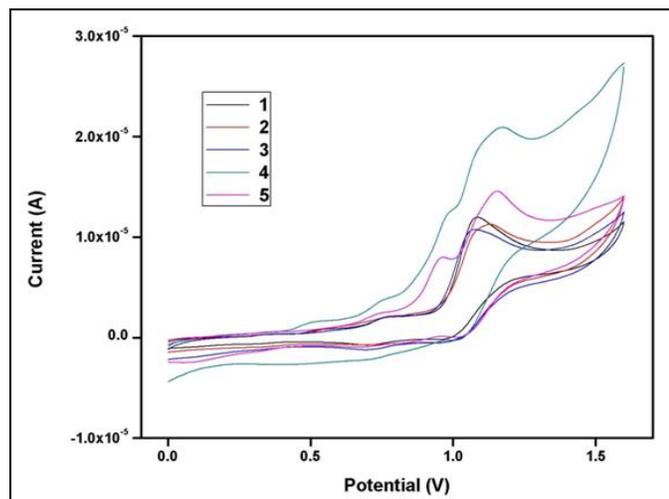


**FIG. 3: CYCLICVOLTAMMOGRAMS OF RUTHENIUM COMPLEXES IN ACETONITRILE AGAINST SCE AT A SCAN RATE OF 100 MVS⁻¹**

All the complexes exhibit a quasi-reversible metal-based oxidative response with E1/2 values falling in the range of +1.0291 - +1.0791 V. The peak potential separations $\Delta E_p$ (76-143 mV) are generally larger than the ideal Nernstian value of 59 mV for a one-electron transfer, but commonly observed for complexes of this type apparently due to uncompensated solution resistance 26. The Ru (II) / Ru (III) potentials follow the trend 5 > 4 > 2 > 3 > 1, which reflects the changes to the electronic environment around Ru (II) along with this series

upon varying the substituent in the ligand. The high Ru (II) / Ru (III) redox potentials of the present complexes may be due to the strong π-delocalization of benzimidazole based ligands. The redox potential of 1 has been observed at +1.0291 V with a peak separation of 108 mV. Whereas complexes 2 and 3 exhibited the redox peaks at +1.0528 and +1.0311 V, respectively.

Complex 5 showing a greater stabilization for the Ru (II) species could be related to the presence of methoxy group enhancing the π –acidity of the ligand. Further, the E1/2 values of the present complexes are lower than the analogous imidazole based complexes, which may be attributed to the electron withdrawing benzimidazole moiety present in the synthesized complexes [22].

**DNA Cleavage Activity of Ruthenium (II) Complexes:** The agarose gel electrophoresis method was employed to monitor the degree of DNA cleavage by the newly synthesized compounds. No cleavage activity was observed in the absence of light **Fig. 4**. On the other hand, on irradiating the compounds in the presence of light at the wavelength of 480 nm, cleavage of supercoiled plasmid DNA has been observed even at a concentration of 24 μM.
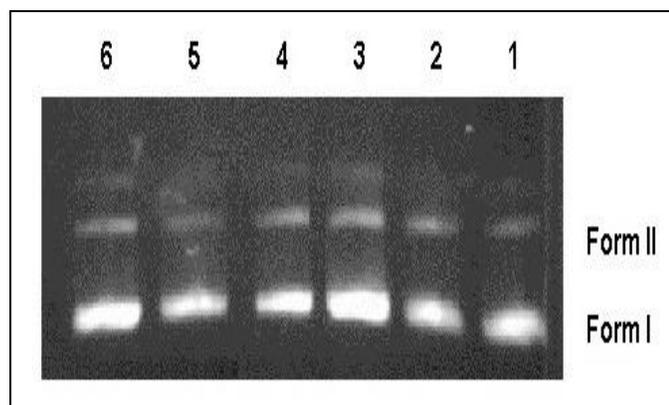
**FIG. 4: CLEAVAGE OF SUPERCOILED PUC19DNA BY THE COMPLEXES (1-5) IN THE ABSENCE OF LIGHT (LANE 2-6).** Lane 1: DNA alone; Lane 2: DNA+24 μM complex 1; Lane 3: DNA+24 μM complex 2; Lane 4: DNA+24 μM complex 3; Lane 5: DNA+24 μM complex 4; Lane 6: DNA+24 μM complex 5

On increasing the concentration of the compounds to 48 μM, complete cleavage of DNA has been observed **Fig. 5**. In order to find out the reactive species involved in the cleavage mechanism, a control experiment with DMSO (hydroxyl radical scavenger) and histidine (singlet oxygen quencher) has been carried out. In a reaction with DMSO (10 mM) no DNA cleavage has been observed, ruling out the possibility of DNA cleavage via OH-based depurination pathway and also a possible oxidative cleavage [27, 28]. While carrying out the reaction with histidine (10 mM), it has not been shown to exhibit any inhibitory effect in the DNA cleaving ability of the complexes **Fig. 6**. This clearly shows that the complexes cleave the DNA by direct guanine oxidation rather than through singlet oxygen.
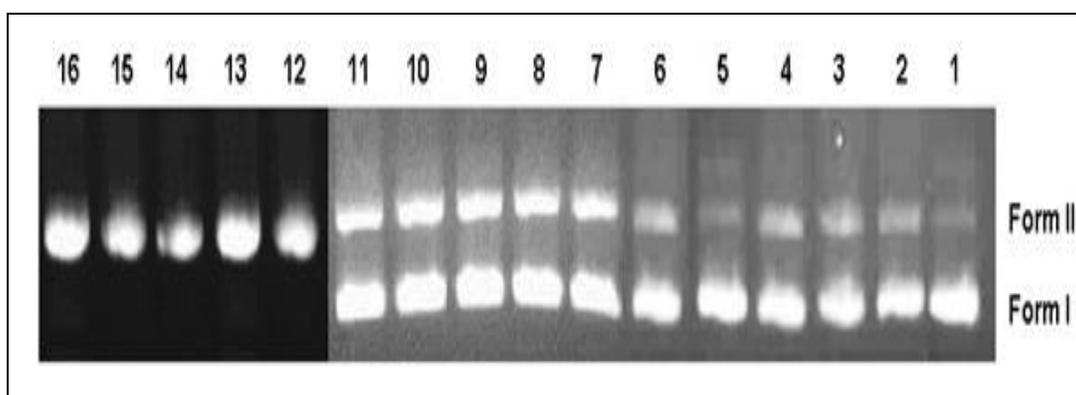


**FIG. 5: CLEAVAGE OF PUC19 DNA BY COMPLEXES (1-5) AT VARIOUS CONCENTRATIONS WHEN EXPOSED TO LIGHT AT 480 NM. DNA WAS INCUBATED WITH COMPLEX FOR 60 MIN IN TRIS BUFFER (PH 7.2) AT 37 °C.** Lane 1, DNA control Lane 2: DNA+12 μM complex 1; Lane 3: DNA+12 μM complex 2; Lane 4: DNA+12 μM complex 3; Lane 5: DNA+12 μM complex 4; Lane 6: DNA+12 μM complex 5; Lane 7: DNA+24 μM complex 1; Lane 8: DNA+24 μM complex 2; Lane 9: DNA+24 μM complex 3; Lane 10: DNA+24 μM complex 4; Lane 11: DNA+24 μM complex 5; Lane 12: DNA+48 μM complex 1; Lane 13: DNA+48 μM complex 2; Lane 14: DNA+48 μM complex 3; Lane 15: DNA+48 μM complex 4; Lane 16: DNA+48 μM complex 5
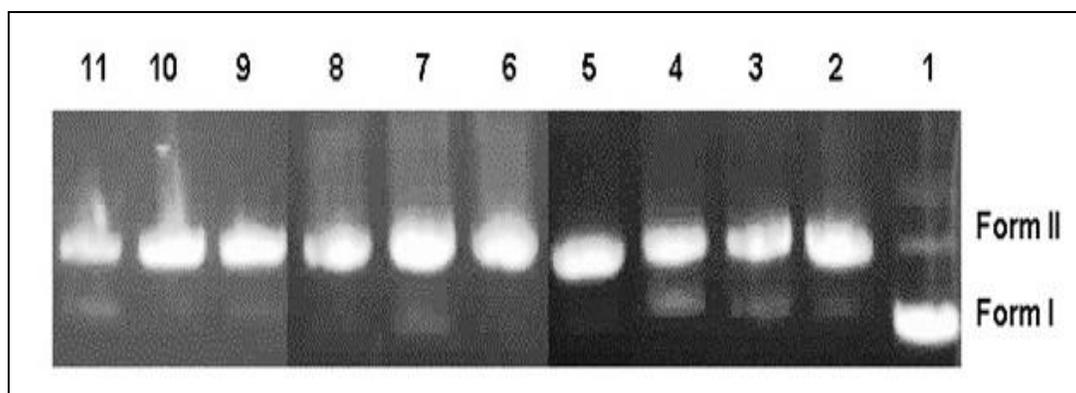


**FIG. 6: CLEAVAGE OF PUC19 DNA BY COMPLEXES (1-5) TO LIGHT AT 480 NM IN THE PRESENCE OF HISTIDINE AND DMSO. DNA WAS INCUBATED WITH COMPLEX FOR 60 MIN IN TRIS BUFFER (PH 7.2) AT 37°C.** Lane 1, DNA control; Lane 2, DNA + 1 (48 μM) + Histidine (10 mM); Lane 3, DNA + 2 (48 μM) + Histidine (10 mM); Lane 4, DNA + 3 (48 μM) + Histidine (10 mM); Lane 5, DNA + 4 (48 μM) + Histidine (10 mM); Lane 6, DNA + 5 (48 μM) + Histidine (10 mM); Lane 7, DNA + 1 (48 μM) + DMSO (10 mM); Lane 8, DNA + 2 (48 μM) + DMSO (10 mM); Lane 9, DNA + 3 (48 μM) + DMSO (10 mM); Lane 10, DNA + 4 (48 μM) + DMSO (10 mM); Lane 11, DNA + 5 (48 μM) + DMSO (10 mM)

**Antiproliferative Activity of Ruthenium (II) Complexes:** The present study examined the efficacy of ruthenium (II) complexes to inhibit the human breast cancer cell line (MCF-7) as determined by MTT assay. The cells were treated with five different concentrations ranging from 0.1 to 100 µM. The complexes suppressed the growth of breast cancer cell lines in a dose-dependent manner. The maximum cell inhibitions of 100, 99.13, 98.72, 98.63 and 92.87% at 100 µM concentration were determined for 1, 2, 4, 5 and 3 respectively and minimum inhibitions (-1.09, -0.82, 0.54, 1.1 and 2.60%) at 0.1 µM concentration were observed for 3, 2, 4, 1 and 5 respectively **Fig. 7**.
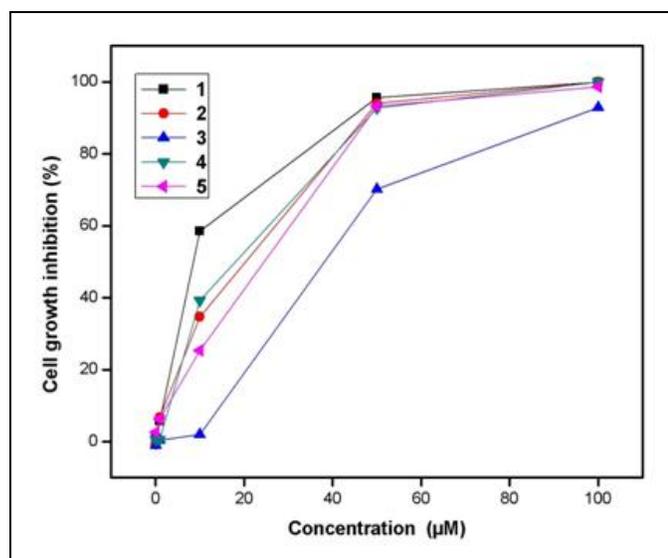


**FIG. 7: EFFECTS OF RUTHENIUM (II) COMPLEXES ON THE CELL GROWTH INHIBITION OF MCF-7 CELLS (HUMAN BREAST CANCER), FOLLOWING CONTINUOUS INCUBATION FOR 48 h, WITH INCREASING DRUG CONCENTRATION (0.1–100 MM). RESULTS ARE REPRESENTATIVE OF THREE INDEPENDENT EXPERIMENTS (N = 3)**

From the results presented in **Table 2**, it is clear that several ruthenium complexes exhibited a marked inhibitory effect on the proliferation of MCF-7 cancer cells. The $IC_{50}$ values for complexes treated with MCF-7 cells were obtained at 7.9, 13.49, 36.78, 12.57, and 16.0 µM for 1, 2, 3, 4, and 5, respectively.

Out of the five complexes tested, $IC_{50}$ value for 1 is found to be less, suggesting that 1 could exert a very strong anti-proliferative effect when compared to other complexes tested with breast cancer cell lines at low doses. The complexes exhibited higher cytotoxic effects on breast cancer cells with lower

$IC_{50}$ values indicating their efficiency in killing the cancer cells even at low concentrations. There are reports in the literature on the cytotoxic effects of the complexes with longer incubation time periods [29-31]. The longer incubation period may result in the development of cellular resistance for that particular complex.

**TABLE 2: *IN-VITRO* CYTOTOXIC ACTIVITY OF SYNTHESIZED RUTHENIUM (II) COMPLEXES IN HUMAN BREAST CANCER CELL LINE (MCF7)**

| S. no. | Compound | $IC_{50}$ value (µM) |
|--------|----------|----------------------|
| 1 | [Ru(bpy)$_2$(L1)](PF$_6$)$_2$ | 7.9 |
| 2 | [Ru(bpy)$_2$(L2)](PF$_6$)$_2$ | 13.49 |
| 3 | [Ru(bpy)$_2$(L3)](PF$_6$)$_2$ | 16.78 |
| 4 | [Ru(bpy)$_2$(L4)](PF$_6$)$_2$ | 12.57 |
| 5 | [Ru(bpy)$_2$(L5)](PF$_6$)$_2$ | 16.0 |

**Biocidal Studies of Ruthenium (II) Complexes:** *In-vitro* antimicrobial activity of a test drug is measured in terms of zone of inhibition produced. The higher the diameter of the zone higher is the microbial growth inhibition.

The *in-vitro* antimicrobial screening of the ligands and complexes were carried out against *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans* by disc diffusion method [32]. From the results in **Table 3**, it has been observed that ruthenium complexes showed better activity than the free ligands.

This can be attributed to Tweedy's chelation theory [33-35], according to which chelation reduces the polarity of the central metal atom mainly because of partial sharing of its positive charge with a donor group and possible ∏-electron delocalization taking place over the whole ring. This increases the lipophilic character of the metal chelate, which favors the permeation of the complexes through the lipid layer of the cell membrane.

The complexes possess activity, which is greater than the effectiveness of the standard drug, Gentamycin. It has been observed that the inhibition activity of the compounds increases with an increase in the concentration of the solution. The activity of the complexes against the microbes follows the order: *E. coli > S. aureus > C. albicans*. Moreover, the complexes are having pronounced antibacterial activity compared to antifungal activity.

**TABLE 3: ANTIMICROBIAL ACTIVITY OF LIGANDS AND THEIR RUTHENIUM (II) COMPLEXES**

| S. no. | Test Drug | Zone of Inhibition (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *S. aureus* | | | *E. coli* | | | *C. albicans* | | |
| | | 25 µg/mL | 50 µg/mL | 100 µg/mL | 25 µg/mL | 50 µg/mL | 100 µg/mL | 25 µg/mL | 50 µg/mL | 100 µg/mL |
| 1 | L1 | 9 | 10 | 13 | 15 | 17 | 20 | 12 | 14 | 20 |
| 2 | L2 | 10 | 13 | 15 | 12 | 15 | 19 | 11 | 12 | 15 |
| 3 | L3 | 10 | 12 | 15 | 12 | 16 | 18 | 09 | 11 | 15 |
| 4 | L4 | 9 | 11 | 14 | 13 | 16 | 19 | 11 | 13 | 16 |
| 5 | L5 | 9 | 11 | 15 | 12 | 14 | 17 | 10 | 12 | 16 |
| 6 | [Ru(bpy)$_2$(L1)](PF$_6$)$_2$ | 21 | 24 | 31 | 17 | 22 | 30 | 15 | 18 | 26 |
| 7 | [Ru(bpy)$_2$(L2)](PF$_6$)$_2$ | 12 | 16 | 26 | 17 | 20 | 28 | 14 | 16 | 25 |
| 8 | [Ru(bpy)$_2$(L3)](PF$_6$)$_2$ | 15 | 20 | 30 | 16 | 20 | 25 | 14 | 16 | 22 |
| 9 | [Ru(bpy)$_2$(L4)](PF$_6$)$_2$ | 10 | 15 | 20 | 17 | 20 | 28 | 13 | 16 | 25 |
| 10 | [Ru(bpy)$_2$(L5)](PF$_6$)$_2$ | 11 | 16 | 27 | 16 | 18 | 30 | 18 | 20 | 26 |
| 11 | Gentamycin Standard | - | - | 16 | - | - | 16 | - | - | - |

Note: Zone size less than 15 mm – Least active; 16 – 20 mm– moderatively active; Above 20 mm – highly active

**CONCLUSION:** The search for new molecular structures that exhibit effective antitumor activities is one of the most important goals of pharmacological research. This has driven inorganic chemists to look for new metal compounds with good activities, preferably against tumors that are responsible for high cancer mortality.

In this study, a new series of benzimidazole-based ruthenium (II) complexes were synthesized and characterized using various spectral techniques. The complexes are able to cleave supercoiled pUC19 plasmid DNA via guanine base oxidation in a concentration-dependent manner.

The cytotoxic studies showed that the complexes exhibit good cytotoxic activity against MCF-7 cancer cell lines. Furthermore, these complexes have potential practical applications to formulate into an efficient drug against cancer.

**CONFLICTS OF INTEREST:** The authors have no potential conflict of interest.

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