

Dalton Transactions

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Interaction of Ru(II) polypyridyl complexes with DNA mismatches and abasic sites**T. Nandhini[†], K.R.Anju[†], V.Manikandamathavan[¶], V.G. Vaidyanathan^{§,†,*} and B.U. Nair[¶]**

[†]*Biophysics Laboratory*, [§]*Academy of Scientific and Innovative Research (AcSIR)* and [¶]*Chemical Laboratory, CSIR-Central Leather Research Institute, Adyar, Chennai 600 020, India*

[†]Authors contributed equally

*Corresponding Author:

V.G. Vaidyanathan

Biophysics Laboratory

CSIR-Central Leather Research Institute

Adyar, Chennai 600020, India

Email: vaidyanathan@clri.res.in

Tel: +91-44-24437142

Abstract

Polypyridyl based ruthenium(II) complexes, [Ru(bpy)₂(furphen)](PF₆)₂ (**1**) and [Ru(bpy)₂(imiphen)](PF₆)₂ (**2**) { furphen: 2-(furan-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline and imiphen: 2-(1*H*-imidazol-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline} were synthesized and characterized by ESI-MS, NMR, UV-Visible and fluorescence spectroscopic techniques. Interaction of Ru(II) complexes with calf-thymus DNA (CT DNA) as well as oligonucleotides containing mismatches and abasic sites was studied along with unmodified control DNA. Based on absorption titration studies, binding constants (K_b) for the interaction of complexes **1** and **2** with DNA were found to be $6.7 \pm 0.2 \times 10^3$ and $4.9 \pm 0.2 \times 10^4 \text{ M}^{-1}$, respectively. Hydrodynamic studies revealed weak interaction between the two complexes and CT-DNA. Luminescence studies revealed that both the complexes exhibit five-fold increase in emission upon addition of CT-DNA. The integrated emission intensity of complex **1** and **2** with CC mismatch oligonucleotide was 1.5 and 1.2 fold higher than that of control GC match oligonucleotide, respectively. Both the complexes did not show any specificity towards abasic or other mismatch sites except for CC mismatch. The results from this study provide an insight on the requirement of ligand shape in recognising DNA mutations such as mismatch and selectivity between DNA mismatches.

Introduction

Interaction of the genome with chemicals or ionizing radiations leads to DNA damage like formation of abasic site, cyclobutane pyrimidine dimers (CPDs) or covalent-DNA adducts. Although human cells are armed with various repair enzymes to rectify the errors, DNA damage may escape from the repair process and enter into DNA synthesis pathway. During DNA synthesis, these adducts either introduce frameshift mutation or DNA mismatch.^{1, 2} In order to recognize DNA damage, different biological and chemical approaches have been applied. One of the approaches designed and reported is the application of transition metal complexes to study DNA mediated charge transport. Since the π -orbitals of the neighboring DNA bases overlap, DNA serves as bridge in long-range electron transfer reactions.

Barton et al. have demonstrated the electron transfer process using oxidative probes which can inject electrons through the DNA bases and cause damage at guanine sites at a distance of 200 Å away from the bound oxidant³. Recently, similar kind of propagation of redox signals has been demonstrated even at a distance of 100 base pairs or 340Å in DNA monolayers coated on gold electrodes.⁴ In particular, the application of rhodium(III) intercalator, $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ (phi: 9,10-phenanthrene quinone diamine, L: bipyridine(bpy) or 1,10-phenanthroline (phen)) complexes (strong photooxidant) have been studied extensively as they bind with DNA through intercalative mode of the phi ligand and propagate the charge.^{3, 5} Similar studies have also been performed with photoexcited iridium complex, $[\text{Ir}(\text{ppy})_2(\text{dppz})]^+$ (ppy: 2-phenylpyridine, dppz: dipyridophenazine) that acts as both reductant as well as oxidant and promotes reduction and oxidation of DNA. The redox property of iridium complex permits the study of electron transfer and hole injection process in the same DNA sequence.⁶ The above studies show that DNA mediated electron transfer is independent of distance between the

acceptor and donor. DNA damage such as presence of bulge sites or single mismatch either inhibits long range photochemical guanine oxidation or reduces the photoinduced electron transfer yield.^{5, 7-9} These studies suggest that the DNA mediated electron transfer process is highly sensitive to base stacking. The presence of mismatch/damage may induce perturbations in the electronic structure of the base pair.

Abasic site that arises from depurination or depyrimidination of nucleobases during excision repair process leads to destabilization of duplex DNA by about 3-11 kcal/mol. Moreover, the presence of abasic site affects the overall processivity of DNA polymerases. Similarly, DNA base mismatch occurs during the translesion DNA synthesis either by bypass or mutant polymerases.^{10, 11} Therefore, it is essential to develop probes to identify this kind of DNA damage. In this context, different organic molecules and inorganic transition metal complexes have been studied as probes for abasic sites, DNA and RNA mismatches.¹² $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ as well as $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and their derivatives have been studied as probes for abasic site as well as mismatches in DNA/RNA structures.^{10, 12-15} In the present study, we have attempted to understand the structural requirement of metal complex for recognition of DNA mutations. In this regard, we have synthesized Ru(II) complexes containing polypyridyl derivatives and studied their interaction with calf thymus DNA, oligonucleotides containing abasic site and base mismatches.

Results and discussion

Synthesis and characterization of ligands and Ru(II) complexes

The ligands, furphen (**L1**) and imiphen (**L2**) were synthesized by following the literature reported previously.¹⁶ The ligands were characterized using ESI-MS, ¹H and ¹³C NMR spectroscopic techniques. The mass spectrum of ligands **L1** and **L2** exhibited a $[\text{M}+\text{H}]^+$ ion peak

at 286.3 and 286.5, respectively. The formation of the ligands was further confirmed by ^1H NMR (Fig. S1). The synthesis of Ru(II) complexes was carried out by reacting the corresponding ligands with *cis*-[Ru(bpy) $_2$ Cl $_2$] solution in nitrogen atmosphere and their formation was confirmed using elemental analysis, ESI-MS and NMR spectroscopy. The electronic spectra of complexes was dominated by presence of two strong bands in 200-400 nm wavelength region that has been assigned as π - π^* transitions from the ligand, while metal-to-ligand charge transfer (MLCT) was observed between 450-470 nm.

Interaction of Ru(II) complexes with DNA

Absorption titration

Electronic absorption spectroscopy has been used to probe metal complex-DNA interactions by monitoring the spectral changes upon addition of DNA solution to metal complex solution. Generally, base binding perturbs the ligand field transition of the metal complex, while intercalative mode of binding usually results in hypochromism and bathochromism with strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of hypochromism is directly linked to the intercalative binding strength. On the other hand, metal complexes that bind either by partial intercalative/non-intercalative or electrostatic mode with DNA may result in either hyperchromism or hypochromism. In the present case, the change in absorption maxima of Ru(II) complexes upon addition of increasing amounts of CT-DNA was monitored at charge transfer band i.e. between 450-460 nm (Fig. 1). The absorption titration data reveal that both complexes **1** and **2** results in hypochromism with no blue or red shift on addition of DNA. From the titration, binding constants (K_b) values were calculated to be $6.7 \pm 0.3 \times 10^3$ and $4.9 \pm 0.2 \times 10^4 \text{ M}^{-1}$, respectively. The K_b values obtained here are relatively similar to other Ru(II)

bipyridyl complexes but less than that of the classical intercalators such as $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{bpy})_2(\text{chrysi})]^{2+}$, which exhibit higher binding strength in the order of 10^6 M^{-1} .¹⁷⁻²¹

Thermal denaturation studies

To understand the DNA binding mode of the two synthesized complexes thermal denaturation studies of DNA were performed in the presence and absence of the Ru(II) complexes. In general, in the absence of any added complex, the melting transition of DNA is sharp. However, intercalation of organic dyes or metallointercalators stabilizes the DNA duplex through pi-pi stacking resulting in large increase in melting temperature (T_m) of DNA (Fig. 2). The melting temperature (T_m) of DNA alone was found to be $68 \pm 0.2 \text{ }^\circ\text{C}$. On addition of complexes **1** and **2**, the change in DNA melting temperature (ΔT_m) was determined as 4 ± 0.2 and $6 \pm 0.2 \text{ }^\circ\text{C}$, respectively. The increase in T_m reported in this study is similar to other groove binders as well as partial intercalators but less than that of other intercalators like $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ that exhibits ΔT_m of around $7\text{-}14 \text{ }^\circ\text{C}$.¹⁸

Fluorescence studies

The aqueous solution of complexes **1** and **2** exhibit strong room temperature ³MLCT emission bands at 615 nm and 618 nm, respectively when excited between 450 and 480 nm. Both the complexes exhibit five-fold increase in luminescence when titrated with CT DNA by varying the ratio $[\text{DNA}]:[\text{complex}]$ from 0:1 to 10:1. The quantum yield of complexes **1** and **2** in aerated aqueous solution was measured using $[\text{Ru}(\text{bpy})_3]^{2+}$ as standard reference and found to be 0.01 and 0.02, respectively. As Ru(II) complexes exhibit strong luminescence profile, we tested these complexes for their application in detection of different DNA mutations such as DNA mismatches as well as abasic site (a base excision repair product) and compared with control Watson-Crick base paired G:C match duplex DNA. As seen from Figure 3, complex **1** with GC

match duplex DNA exhibit similar luminescence profile as with CT DNA i.e. enhancement in emission intensity. Interestingly, after addition of DNA mismatches, in particular, with CC mismatch, the integrated emission intensity of complex **1** and **2** was enhanced 1.5 and 1.2-fold respectively, than those of GC match control (Fig. 4). However, in the case of DNA mismatches such as GA or CA, there was no change in integral emission intensity of the metal complexes compared to GC match control (Fig. S2).

In the case of $[\text{Ru}(\text{bpy})_2(\text{tactp})]^{2+}$, a 12-fold increase in emission intensity was observed in the presence of match or mismatch oligonucleotides but with no discrimination.²² However, differential luminescence quenching studies have shown that these two complexes bind with mismatch DNA preferentially.²² In the present case the differential luminescence intensity of Ru(II) complexes between mismatch and match DNA was only 1.2-1.5 fold. This could be explained in terms of either (i) non-planar structure of the ligand and (ii) length of oligonucleotide sequence used. Interestingly in the case of $[\text{Ru}(\text{bpy})_2(\text{tpqp})]^{2+}$ instead of increase in emission, reduction was observed in the presence of DNA mismatch. Furthermore, while Ru(II) complex containing an extended aromatic system such as dppz exhibit a 1.5-fold higher differential luminescence intensity in 27-mer oligonucleotide, a 3-fold increase was observed with hairpin oligonucleotide containing mismatch site.²³ The increase in differential luminescence intensity between hairpin vs. 27-mer oligonucleotide has been explained in terms of higher binding affinity of Ru(II) complex towards 27-mer that results in reduction in the probability of binding of the complex to the destabilized CC mismatch site compared to that of shortened hairpin structure.²³

In addition to mismatch sequences, the functioning of these complexes as probes for detection of abasic site was tested with duplex DNA containing different complementary bases

against abasic site. Instead of enhancement, a decrease in emission intensity of these two complexes was observed (Fig. S2). Moreover, both complexes did not exhibit any specificity towards duplex DNA containing abasic sites with different complementary base sequences.

To further examine the specificity of Ru(II) complexes towards DNA mismatches, we compared the results from the interaction of ethidium bromide (an organic fluorophore) and oligonucleotides. No distinguishable difference in emission intensity was observed either with GC control or mismatch DNA or abasic site (Fig. S3). This study indicates that the classical intercalator did not show any specificity towards any DNA defect while the synthesized Ru(II) complexes did.

In order to determine the proximity of metal complex with match and mismatch DNA, luminescence studies were performed with sodium iodide, a well-known anionic fluorescent quencher. To maintain the ionic strength, KCl (100 mM) was added to the solution. Even in the absence of quencher, KCl itself quenched luminescence intensity of the complexes in control as well as in mismatch/abasic site DNA suggesting inhibition of electrostatic binding of metal complex with DNA in the presence of KCl (Fig. 5). Upon addition of sodium iodide, the luminescence intensity of the complexes was further quenched. In contrast to previous studies,²³ the synthesized complexes **1** and **2** did not show any increase in the luminescence ratio between mismatched and matched DNA upon addition of KCl or NaI. Based on the results, it is apparent that the synthesized Ru(II) complexes **1** and **2** interact with control and DNA mismatch/abasic sites with weak binding capacity, which is consistent with data from absorption titration as well as hydrodynamic studies data (Fig. S4).

Excited state lifetime measurement studies of the Ru(II) complexes

The excited state lifetime studies were carried out to examine the luminescent behavior of the Ru(II) complexes with match and mismatch oligonucleotide. The excited state decay profiles for the complexes with DNA or oligonucleotides were fitted to biexponential decay curves while single exponential decay fit was applied for the complexes alone. In the presence of CT DNA, the lifetime of complex **1** was increased from 517 ns to 853 ns, while with GC match control and CC mismatch, the lifetime increased to 1107 and 1198 ns, respectively (Table 1). Similarly, the lifetime of complex **2** with CT DNA was increased from 483 ns to 814 ns, while with GC match control and CC mismatch, the lifetime increased to 1026 and 1121 ns, respectively. The two lifetimes observed for the complexes with oligonucleotides/DNA could be due to orientation of the complexes leading to placement of the ligand moiety either in perpendicular or side-on mode with DNA. In perpendicular mode, the ligands lie along the DNA dyad axis yielding a longer lifetime compared to side-on approach in which ligand might be exposed to the solvent resulting in shorter lifetime. The observed results indicate that the complexes bind with DNA through intercalation thereby preventing the metal center from the exposure to water. The results are also in agreement with that of Δ -[Ru(bpy)₂(dppz)]²⁺, in which the complex exhibit similar enhancement in excited state lifetime with CC mismatch.²³ In spite of shortened 17-mer oligonucleotide used in this study compared to 27-mer oligonucleotide, it is hypothesized that the lower differential luminescence intensity witnessed in this case could be due to the topology of the ligand present in the complexes.

In summary, our data reveals that (i) both the complexes **1** and **2** bind with DNA weakly through partial intercalation mode based on absorption titration, thermal denaturation and hydrodynamic experiments (ii) a fivefold enhancement in luminescence intensity of these

complexes was observed with addition of DNA, (iii) integrated emission intensity of the complexes was 1.2-1.5 fold higher with CC mismatch as compared to GC control oligonucleotides, and (iv) absence of specificity between duplex DNA containing abasic site. The results from the present study suggest that both DNA sequence length and structure and nature of the ligand coordinated to the metal ion play an important role in the selectivity and specificity of the metal complex towards detection of DNA mutations.

Experimental section

Materials. Ruthenium(III) chloride hydrate, Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and calf thymus DNA (CT DNA) were purchased from SRL Chemicals. All the DNA related experiments were carried out at pH 7.2 at 25 °C. The solvents were purchased from Sigma-Aldrich Chemicals and used as received unless otherwise specified. Oligonucleotides were purchased from Eurofins India. The purity of the oligonucleotides was tested with RP-HPLC using 150 x 4.6 mm, 5 µm pore size Clarity column from Phenomenex India. The solvent gradient consisted of 10 mM ammonium acetate (solvent A) and acetonitrile (solvent B) with increase in solvent B percentage from 3% in 5 min to 16% in 15 min. Elemental analysis was performed using a EURO EA 3000–Single CHNS analyzer in order to check the composition of the element present in the proposed structure. Electrospray ionization (ESI) mass spectra of the ligands and complexes were recorded with a Thermofinnigan LCQ-6000 Advantage Max ion trap mass spectrometer equipped with an electron spray source.

Synthesis and characterization of ligands and Ru(II) complexes. Ru(II) complexes and ligands were synthesized using previously reported literature.¹⁶ A mixture of 1,10-phenanthroline-5,6-dione (1.5 mmol), furfuraldehyde or imidazole 2-aldehyde (1.5 mmol), ammonium acetate (30 mmol) and acetic acid (20 mL) was refluxed for 2 h, then cooled to room

temperature and diluted with water (c.a. 60 mL). Dropwise addition of concentrated aq. ammonia gave a yellow precipitate, which was collected and washed with water. The crude product in ethanol was purified by filtration through silica gel (60-100 mesh, ethanol). The principal yellow band was collected. On slow evaporation of the solution, a yellow solid was obtained, which was dried *in vacuo*.

2-(furan-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (Furphen, **L1**): Yield (0.37 g, 86 %) ESI-MS: *m/z*, 286.3 (M+H⁺); Elemental analysis: Anal. Calcd for C₁₇H₁₂N₄O: C, 71.32; H, 3.52; N, 19.57 %; Found C, 71.26; H, 3.42; N, 19.82 %.

2-(1*H*-imidazol-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (imiphen, **L2**): Yield (0.35 g, 81 %) ESI-MS: *m/z*, 286.5 (M+H⁺); Elemental analysis: Anal. Calc. for C₁₆H₁₀N₆: C, 67.12; H, 3.52; N, 29.35. Found: C, 67.18; H, 3.60; N, 29.31%.

[Ru(bpy)₂(furphen)](PF₆)₂ (**1**): A mixture of *cis*-[Ru(bpy)₂Cl₂].2H₂O (0.26 g, 0.5 mmol), Furphen (**L1**) (0.143 g, 0.5 mmol) were heated to reflux in 10mL ethanol-water (3:1) mixture for 5h under N₂ atmosphere. The solution was cooled to room temperature and concentrated to two third of its original volume. A saturated solution of ammonium hexafluorophosphate was added to precipitate the compound as PF₆ salt. The precipitate was washed with water and diethyl ether. The compound was purified by column chromatography on neutral alumina column using CH₃CN-CH₃OH (9:1) as eluent. For DNA related studies, chloride salts were prepared by following the previous literature.²³ Yield 0.28 g (56 %, 0.28 mmol), ESI-MS: *m/z* 350 [M-2PF₆]²⁺; ¹H NMR (400 MHz, δ, ppm) 7.07 (2H, m), 7.32 (1H, d), 7.45 (3H, m), 7.5 (2H, t), 7.6 (1H, d) 7.71 (1H, d), 7.93 (1H, s), 8.07 (3H, m), 8.16 (3H, m), 8.25 (1H, t), 8.52 (1H, s), 8.65 (3H, m), 8.71 (1H, t), 8.98 (1H, t), 9.16 (1H, t), 13.85 (1H, bs). Anal. Calcd for C₃₇H₂₆F₁₂N₈OP₂Ru: C, 44.90; H, 2.65; N, 11.32 %. Found: C, 44.50; H, 2.67; N, 11.40 %.

UV titration of Ru(II) complexes with DNA. A solution of calf thymus DNA was prepared by dissolving it in the tris buffer (10 mM, pH 7.2). The purity of DNA was confirmed from UV absorbance ratio at 260 and 280 nm of about 1.8–1.9:1, indicating that the DNA was sufficiently free from protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($6600 \text{ M}^{-1} \text{ cm}^{-1}$) at 260 nm.²⁴ Absorption titration experiments involving the interaction of the complex with DNA were performed in tris buffer (10 mM Tris, pH 7.2). Titration experiments were performed by maintaining the metal complex concentration constant (10 μM) while varying the concentration of nucleic acid from 0 to 180 μM . The corrected absorption spectra were obtained by adding an equal amount of DNA to both the complex solution and the reference solution to eliminate the absorbance arising from DNA. From the absorption data, the intrinsic binding constant K_b was determined using the following eq. (1) and by constructing a plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs. $[\text{DNA}]$

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

where $[\text{DNA}]$ is the concentration of DNA, the apparent absorption coefficient ϵ_a , ϵ_f and ϵ_b correspond to $A_{\text{obsd}}/[\text{Ru}]$, the extinction coefficient for free Ru(II) complex, and extinction coefficient for ruthenium complex in the fully bound form, respectively. The binding constant (K_b) was determined from slope/intercept of the linear plot.

Melting temperature

Thermal denaturation studies were carried out with CT DNA (100 μM) in 10 mM Tris buffer, pH 7.2 in the absence and presence of complexes **1** and **2** (50 μM). The change in absorbance of CT DNA at 260 nm was recorded in the temperature range 20–90° C with ramp rate of 2 °C/min using Peltier temperature controller system. The melting temperature of control

DNA and Ru(II) complex treated DNA were calculated by plotting temperature versus absorption. From the point of inflection of the curve, the melting temperature (T_m) was obtained. All the experiments were carried out in duplicates.

Fluorescence studies

(i) With Calf thymus DNA

Luminescence experiments were carried out using Cary 100 fluorescence spectrometer by keeping the excitation and emission slit width each as 20 and 20 nm, respectively. The luminescence studies of Ru(II) complexes with DNA were performed in 100 μ L quartz cuvette containing 10 μ M metal complex solution. The CT DNA concentration was varied from 20 to 200 μ M at an interval of 20 μ M concentration. The measurements were recorded from 500 nm to 800 nm by exciting the samples between 450-480 nm.

The quantum yield measurements for the complexes were determined using the equation $\Phi = \Phi_{\text{ref}} \times (\nabla / \nabla_{\text{ref}}) \times (\eta^2 / \eta_{\text{ref}}^2)$, where Φ and Φ_{ref} are the quantum yields of the sample and reference, ∇ and ∇_{ref} are the slopes of linear plots of the absorbance at the excitation wavelength vs. Integrated emission intensity, and η and η_{ref} are the refractive indices of the solvents used. Absorbance values were kept below 0.1 AU at the excitation wavelength. Both the complexes and reference were excited at 450 nm. The reference used for this study was $[\text{Ru}(\text{bpy})_3]^{2+}$ in aerated aqueous solution ($\Phi:0.042$)

(ii) With Oligonucleotides

Duplex DNA containing either GC match control or mismatch or abasic site were prepared by heating the respective primer and complementary sequences in Tris (10 mM, pH 7.2)/NaCl (50 mM) to 90 $^{\circ}$ C for 5 min followed by slow cooling to room temperature over a period of 3 h. For abasic site, tetrahydrofuran moiety was used. The samples were incubated at

4°C for overnight before carrying out the experiments. Fluorescence experiments were carried out by incubating Ru(II) complexes (1 μ M) with different concentrations of oligonucleotides (0-2 μ M) in Tris buffer (10 mM/NaCl 50 mM, pH 7.2). The sample solutions were excited between 450-480 nm, and the emission intensity was integrated over the region of 500 to 800 nm. In addition, emission experiments were performed by incubating organic fluorophore ethidium bromide (1 μ M) with varying concentrations of oligonucleotides containing match or mismatch DNA(0-2 μ M). The quenching experiments were performed using KCl (100 mM) and NaI (100 mM) by keeping the ratio of duplex DNA (oligonucleotides) and Ru(II) complexes as 2:1.

(iii) Lifetime measurements

Time resolved fluorescence measurements for the Ru(II) complexes in the presence and absence of DNA were performed using a picoseconds laser excited SPT (single-photon timing) spectrometer. The excitation source was a tunable Ti-Sapphire laser (Tsunami, Spectrophysics, USA) with a pulse width of <2 ps and a repetition rate of 82 MHz. The emission was monitored using a MCP-PMT Hamamatsu-C 4878) detector. Decay traces were deconvoluted by a non-linear least squares analysis using IBH software.

Acknowledgements

V.M. would like to thank CSIR for providing research fellowship. This research is supported by DST-SERB Ramanujan fellowship (V.G.V) (SR/S2/RJN- 07/2011) and Network Project STRAIT.

References

1. A. Luch, *Nature reviews. Cancer*, 2005, **5**, 113-125.
2. U. Hubscher and G. Maga, *Curr. Opin. Chem. Biol.*, 2011, **15**, 627-635.
3. M. E. Nunez, D. B. Hall and J. K. Barton, *Chem. Biol.*, 1999, **6**, 85-97.
4. J. D. Slinker, N. B. Muren, S. E. Renfrew and J. K. Barton, *Nat. Chem.*, 2011, **3**, 228-233.
5. D. B. Hall, R. E. Holmlin and J. K. Barton, *Nature*, 1996, **382**, 731-735.
6. F. Shao, B. Elias, W. Lu and J. K. Barton, *Inorg. Chem.*, 2007, **46**, 10187-10199.
7. S. O. Kelley, E. M. Boon, J. K. Barton, N. M. Jackson and M. G. Hill, *Nucleic Acids Res.*, 1999, **27**, 4830-4837.
8. D. B. Hall and J. K. Barton, *J. Am. Chem. Soc.*, 1997, **119**, 5045-5046.
9. S. O. Kelley, R. E. Holmlin, E. D. A. Stemp and J. K. Barton, *J. Am. Chem. Soc.*, 1997, **119**, 9861-9870.
10. H. A. Dahlmann, V. G. Vaidyanathan and S. J. Sturla, *Biochemistry*, 2009, **48**, 9347-9359.
11. K.-Y. Seo, S. A. Jelinsky and E. L. Loechler, *Mutat. Res.*, 2000, **463**, 215-246.
12. A. J. McConnell, H. Song and J. K. Barton, *Inorg. Chem.*, 2013, **52**, 10131-10136.
13. S. Xu, Y. Shao, K. Ma, Q. Cui, G. Liu, F. Wu and M. Li, *Analyst*, 2011, **136**, 4480-4485.
14. K. Ma, Q. Cui, G. Liu, F. Wu, S. Xu and Y. Shao, *Nanotechnology*, 2011, **22**, 305502.
15. F. Wu, Y. Sun, Y. Shao, S. Xu, G. Liu, J. Peng and L. Liu, *PloS one*, 2012, **7**, e48251.
16. S. Gopal and B. U. Nair, *Inorg. Chim. Acta*, 2013, **402**, 75-82.
17. A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 4960-4962.
18. V. G. Vaidyanathan and B. U. Nair, *J. Inorg. Biochem.*, 2002, **91**, 405-412.

19. V. G. Vaidyanathan and B. U. Nair, *Dalton Trans.*, 2005, 2842-2848.
20. G.-B. Jiang, J.-H. Yao, J. Wang, W. Li, B.-J. Han, Y.-Y. Xie, G.-J. Lin, H.-L. Huang and Y.-J. Liu, *New J. Chem.*, 2014, **38**, 2554-2563.
21. Y.-Y. Xie, H.-L. Huang, J.-H. Yao, G.-J. Lin, G.-B. Jiang and Y.-J. Liu, *Eur. J. Med. Chem.*, 2013, **63**, 603-610.
22. E. Rüba, J. R. Hart and J. K. Barton, *Inorg. Chem.*, 2004, **43**, 4570-4578.
23. M. H. Lim, H. Song, E. D. Olmon, E. E. Dervan and J. K. Barton, *Inorg. Chem.*, 2009, **48**, 5392-5397.
24. J. Marmur, *J. Mol. Biol.*, 1961, **3**, 208-IN201.

Table 1. Luminescence lifetime measurement parameters for complexes **1** and **2** with oligonucleotides and CT DNA

Complex	Oligonucleotide/CT DNA	τ_1 (ns)	τ_2 (ns)	$\tau_1:\tau_2$
[Ru(bpy) ₂ (Furphen)] ²⁺ (1)	CT DNA	350	853	70:30
	G:C match control	381	1107	64:36
	C:C mismatch	379	1198	49:51
	Free complex	517		
[Ru(bpy) ₂ (imiphen)] ²⁺ (2)	CT DNA	341	814	72:28
	G:C match control	361	1026	60:40
	C:C mismatch	378	1121	42:58
	Free complex	483		

Figure Legends

Scheme 1. Synthetic route of ligands and Ru(II) complexes

Scheme 2. Different oligonucleotide sequences used in this study

Fig. 1 Absorption titration of (a) complex **1** (10 μM) and (b) complex **2** (20 μM) upon addition of CT DNA(0-180 μM) in Tris buffer (10 mM, pH 7.2)

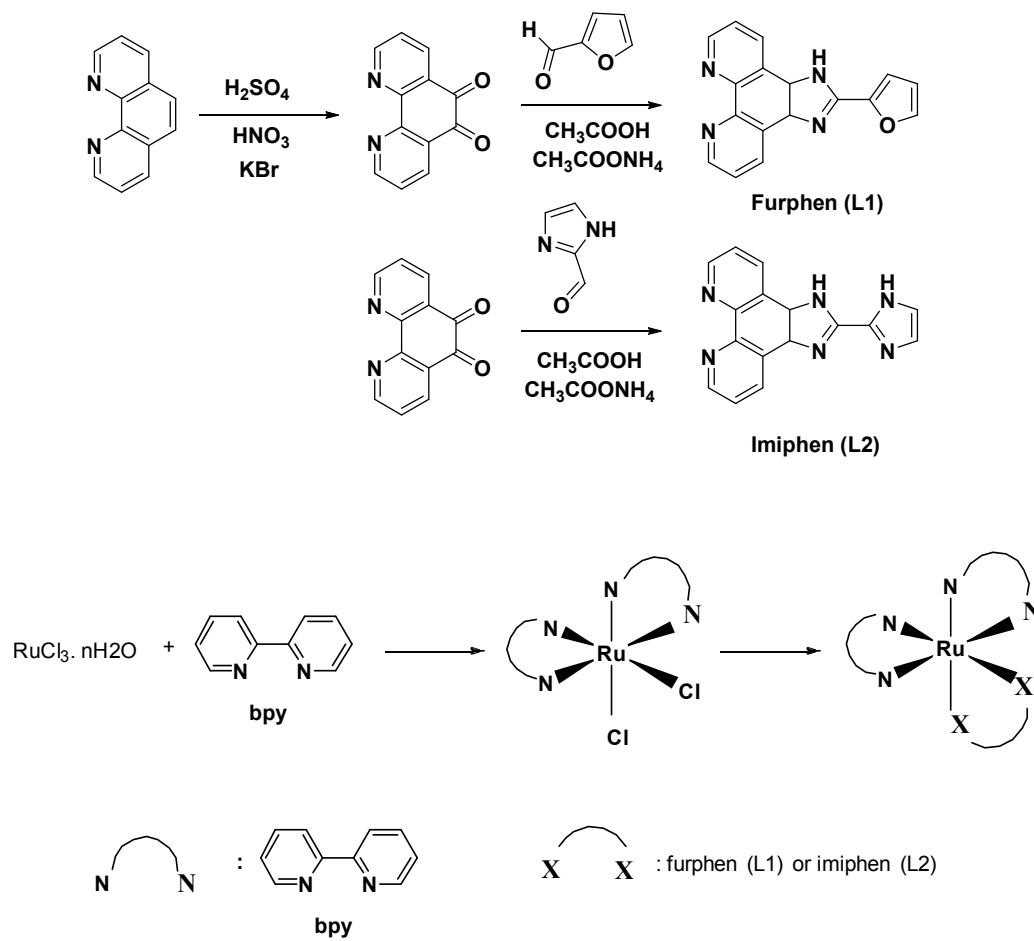
Fig. 2 Thermal denaturation curve of DNA in the absence (black) and presence of complexes **1** (red) and **2** (green) in Tris buffer. [DNA]: 100 μM ; [Ru]: 50 μM

Fig.3 Luminescence spectra of (a) complex **1** and (b) complex **2** in the absence and presence of varying concentrations of DNA. [Ru]: 10 μM ; [DNA]: 0-200 μM

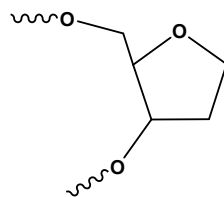
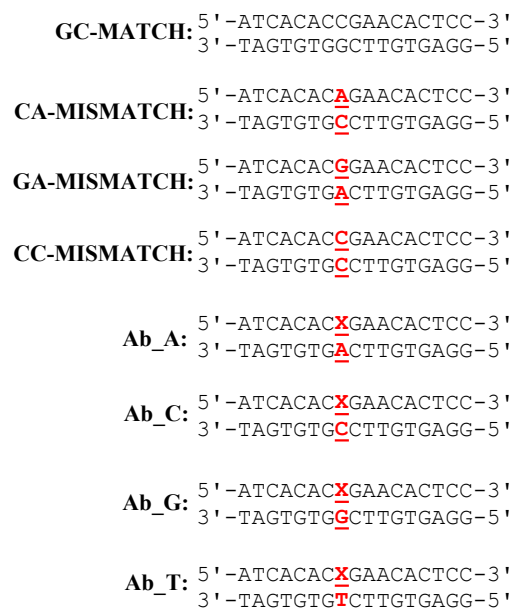
Fig. 4 Integrated emission intensities of Ru complexes (a) complex **1** and (b) complex **2** in the presence of GC, CC and abasic sites. [Ru]: 1 μM ; [Oligonucleotide]: 0-2 μM

Fig. 5 Effect of KCl (100 mM) and NaI (100 mM) on the luminescence intensity of Ru complexes (a) complex **1** and (b) complex **2** in the presence of oligonucleotides containing GC, CC and abasic sites. [Ru]:[oligonucleotide]::1:2

Scheme 1.



Scheme 2.



X: thf (abasic site model)

Figure 1.

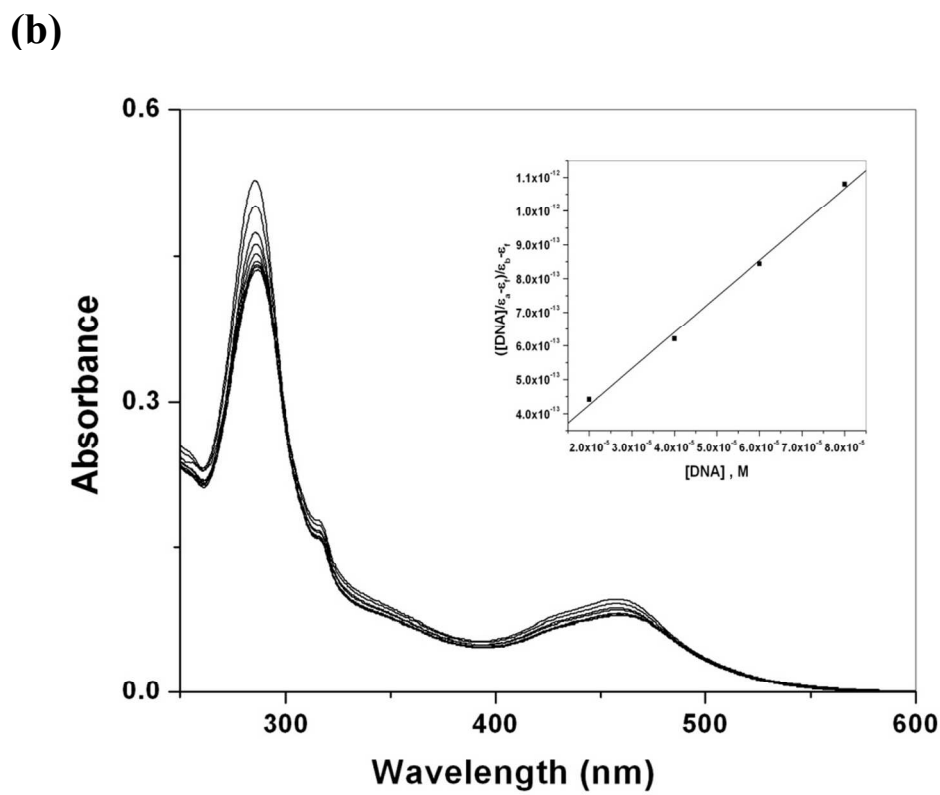
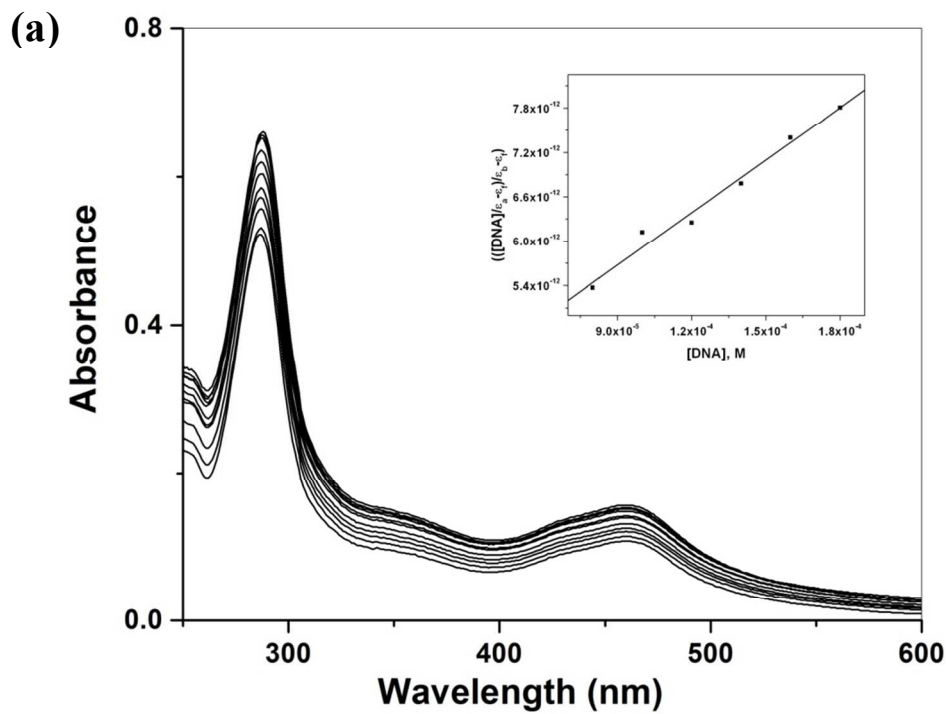


Figure 2.

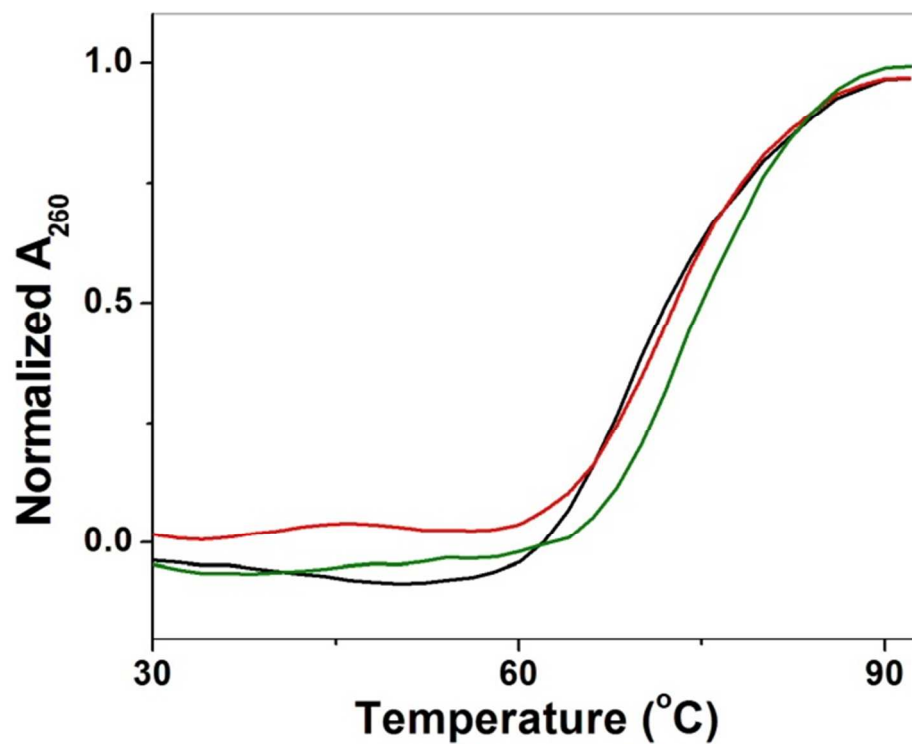
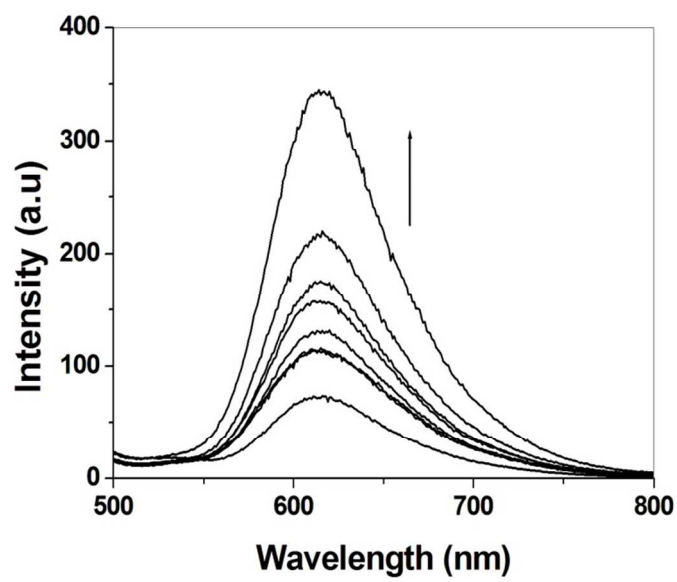


Figure 3.

(a)



(b)

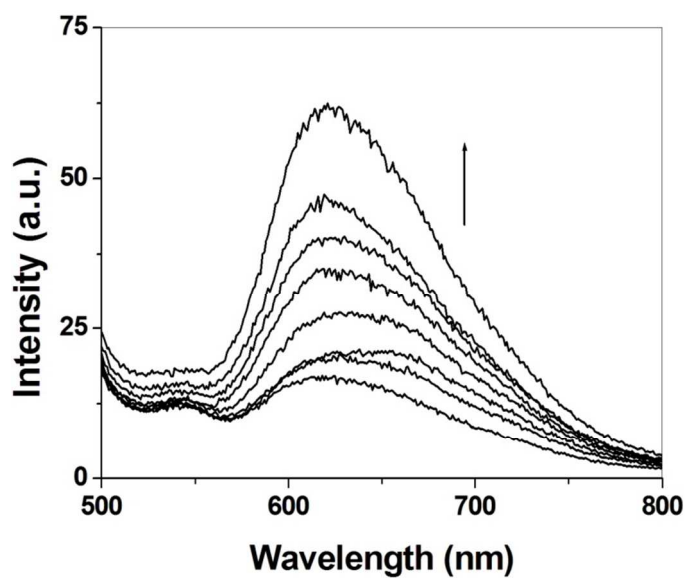
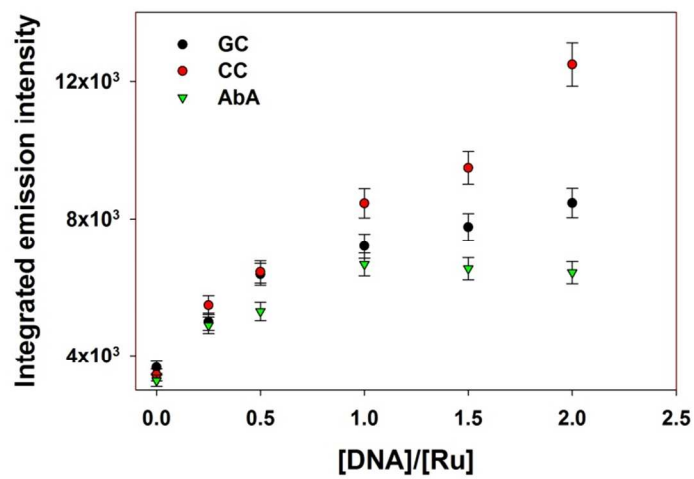


Figure 4.

(a)



(b)

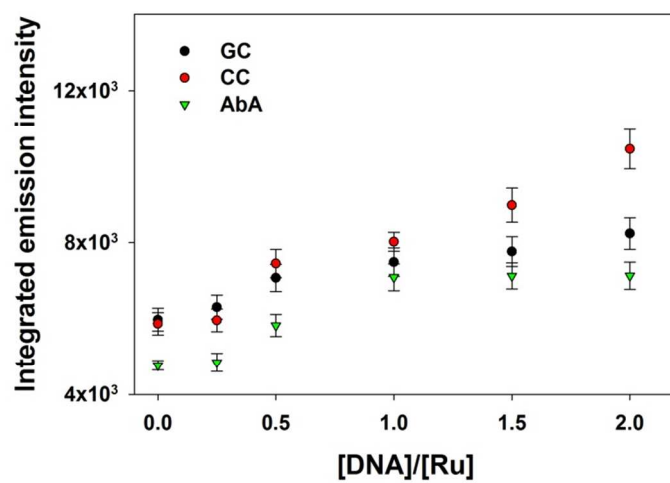
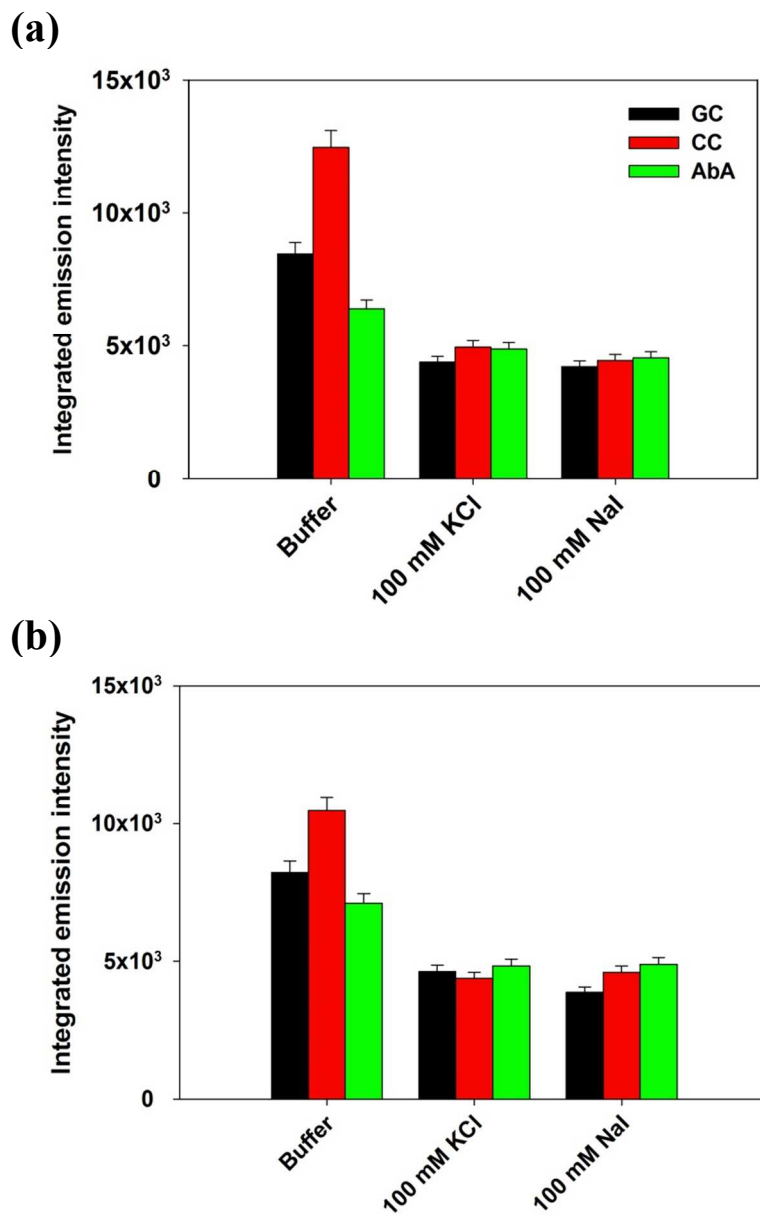
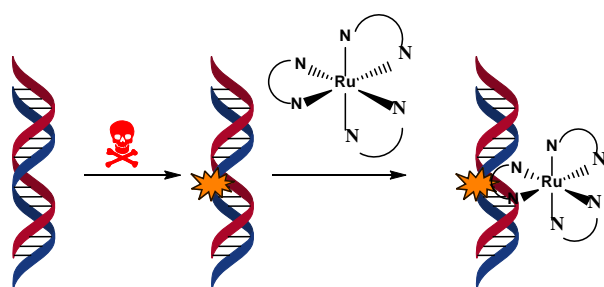


Figure 5.





Interaction of Ru(II) polypyridyl complexes with DNA mismatches and Abasic Sites

T.Nandhini, K.R.Anju,
V.M. Manikandamathavan,
V.G. Vaidyanathan and B.U. Nair

Ru(II) polypyridyl complexes binds to CC mismatch DNA with high selectivity