Hydrolytic cleavage of DNA by ruthenium(II) polypyridyl complex

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Supplementary Information

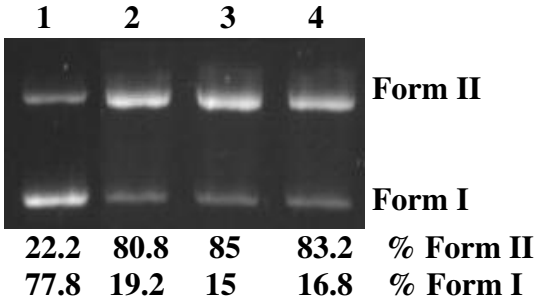

Figure S1. Cleavage of plasmid pBR322 DNA (60 μM, in base pairs) (incubation for 18 h); Form I - Supercoiled plasmid DNA; Form II - nicked circular plasmid DNA; Lane 1 – DNA control; Lane 2 - DNA + **1** in Ar atmosphere; Lane 3- DNA + **1** in air; Lane 4 - DNA + **1** (20 μM **1** in D_2O)

Rhodamine B Assay for Hydroxyl Radicals:

Hydroxyl radical formation was quantitated using rhodamine B [8c,26] (10 μM) as a reporter molecule in the presence of **1** (50 μM). Reactions were performed in a 3 ml volume in phosphate buffer (pH 7.2) under aerobic conditions. Degradation of dye was monitored at 552 nm. The change in concentration of rhodamine B at varying times is a direct measure of the concentration of hydroxyl radicals produced in the reaction mixture. Anaerobic reactions using $FeCl_3$ and H_2O_2 (Fenton conditions) with rhodamine B were used to verify the dye degrades in the presence of hydroxyl radicals.

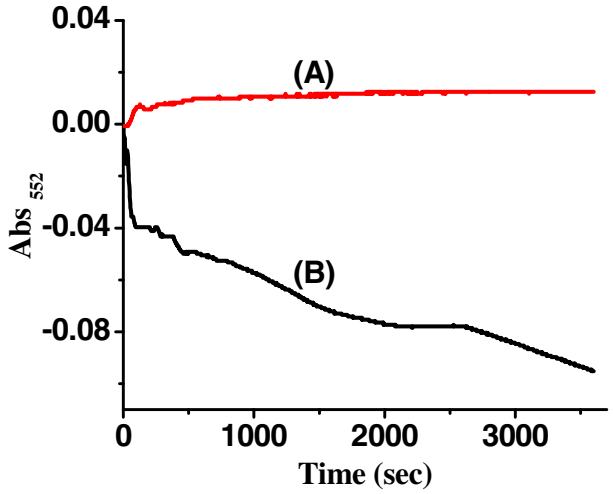

Figure S2. (A) Quantitation of hydroxyl radicals by following the degradation of rhodamine B at 552 nm in phosphate buffer, pH 7.2 under aerobic condition at 50 μM concentration of **1**; (B) Reaction of rhodamine B with $FeCl_3$ and H_2O_2 under anaerobic condition.

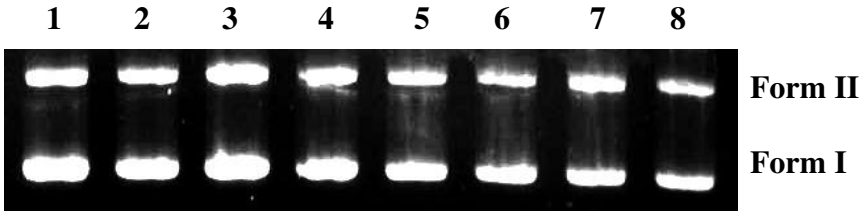

Figure S3. Cleavage of plasmid pBR322 DNA (90 μM, in base pairs) in the presence of 20 μM **1** and different inhibitors (incubation for 18 h); Form I - Supercoiled plasmid DNA; Form II - nicked circular plasmid DNA; Lanes 1 – DNA control; lane 2 – DNA + **1**; lane 3 - DNA + **1** + DMSO (1 mM); lane 4 - DNA + **1** + mannitol (50 mM); lane 5 - DNA + **1** + DABCO (10 mM); lane 6 - DNA + **1** + NaN_3 (20 mM); lane 7 - DNA + **1** + L-Histidine (20 mM); lane 8 - DNA + **1** + SOD (15 units).

Table S. Comparison of the pBR322 DNA cleavage efficiency of **1** for inhibition experiments.

Serial No.	Reaction Condition	Form (%)	
		I	II
1.	pBR322 DNA control	72.1	27.9
2.	DNA + **1**	66.3	33.7
3.	DNA + DMSO + **1**	65.5	34.5
4.	DNA + mannitol + **1**	63.9	36.1
5.	DNA + DABCO + **1**	64.4	35.6
6.	DNA + NaN_3 + **1**	66.1	33.9
7.	DNA + L-Histidine + **1**	54.5	45.5
8.	DNA + SOD + **1**	59.8	40.2

Experimental procedure for the T4 ligase-religation studies

An enzymatic assay was performed using T4 DNA ligase to determine whether the cleaved products were consistent with hydrolysis of the phosphodiester linkages in DNA. For the religation experiments, the solution was incubated for 18 h at 16 °C prior to gel electrophoresis. The NC DNA obtained from the hydrolytic cleavage reaction, was recovered from agarose gel by phenol extraction method and purified by ethanol precipitation. This was followed by addition of 10 X ligation buffer and T4 DNA ligase (4 units) to the purified NC DNA.

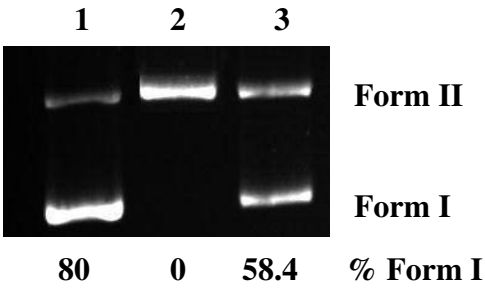

Figure S4. Analysis of the capacity of T4 DNA ligase to relegate DNA cleaved by complex **1**; Lane 1 – DNA control; Lane 2 – the products (nicked plasmid *p*BR322) obtained from the reaction with the complex **1** after an incubation period of 18 hours; Lane 3 – Lane 2 + T4 ligase.

Details of Kinetic Measurement:

For kinetic measurements, the decrease in the intensities of form I were plotted against complex concentrations and these were fitted well with a single-exponential decay curve (pseudo first order kinetics) by use of equation 1, where y_0 is the initial percentage of a form of DNA, y is the specific form of DNA at time t, const is the percentage of uncleaved DNA, k_{obs} is the hydrolysis rate or apparent rate constant and V_{max} is the maximal reaction velocity. Careful optimization of electrophoretic and densitometric techniques led to pseudo-first order kinetics and allowed the determination of true Michaelis - Menten kinetic parameters.

$$y = (y_0 - \text{const}) \exp(-k_{obs}\, x) + \text{const} \qquad [1]$$

k_{obs}' versus [Ru] was plotted and fitted using equation 2, which allows determination of both rate constants and Michaelis – Menten – type kinetic values. Similar experiments with constant complex **1** concentration and varying DNA (10-90 μM) concentrations were performed, and the intensities were plotted against substrate concentrations by use of equation 3. k_{cat} is calculated as $V_{max}/[E]_0$, where $[E]_0$ is the concentration of $[Ru(bpy)_2BPG]^{2+}$ = 20 μM.

$$k_{obs'} = V_{max'}\,[\text{catalyst}] / (K_M + [\text{catalyst}]) \qquad [2]$$

$$k_{obs} = V_{max}\,[\text{substrate}] / (K_M + [\text{substrate}]) \qquad [3]$$

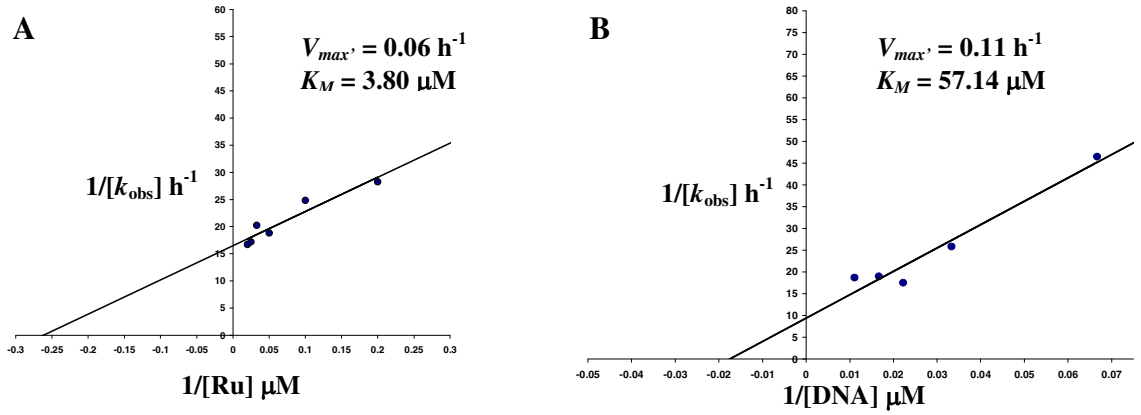

Figure S5. (A) Double reciprocal plot of pseudo-Michaelis-Menten kinetic experiment of **1** with different complex concentration; (B) Double reciprocal plot of true-Michaelis-Menten kinetic experiment of **1** with different DNA concentration.

Synthesis and characterization of $[Ru(bpy)_2(BPG)]Cl_2$ (1)

cis-$[Ru(bpy)_2Cl_2].2H_2O$ (0.100 g, 0.192 mmol) and BPG (0.056 g, 0.192 mmol) were refluxed in 50% methanol/50% water for 8 hours. The red solution was filtered hot and was cooled to room temperature. After evaporation of the solvent, the red solid was collected and washed with small amounts of methanol and diethyl ether and then dried under vacuum. The product was purified by column chromatography on active alumina using acetone and methanol as eluent. The red fraction was collected and concentrated in vacuum, a small amount of diethyl ether was added to the concentrated solution and red solid was obtained. Yield - 65 %.

Anal.Calc.for $C_{69.5}$ H_{75} N_{20} O_{14} Ru_2 Cl_4: %C 47.45; %H 4.30; %N 15.93; Found: %C 47.64; %H 4.25; %N 16.14; λ_{max} = 446 nm (ε = 1.53 x 10^4 $dm^3mol^{-1}cm^{-1}$), λ_{em} = 625 nm.; ^{1}H NMR (DMSO-d_6) room temperature, ppm) δ = 8.90 (3H), 7.78 (2H), 8.64 (2H), 8.52 (2H), 8.22(5H), 7.85 (2H), 7.72 (10H). I.R. (KBr) cm^{-1} - C=O (1693), N-H (3215), O-H (3404), C=N (1606), C=C (1444).

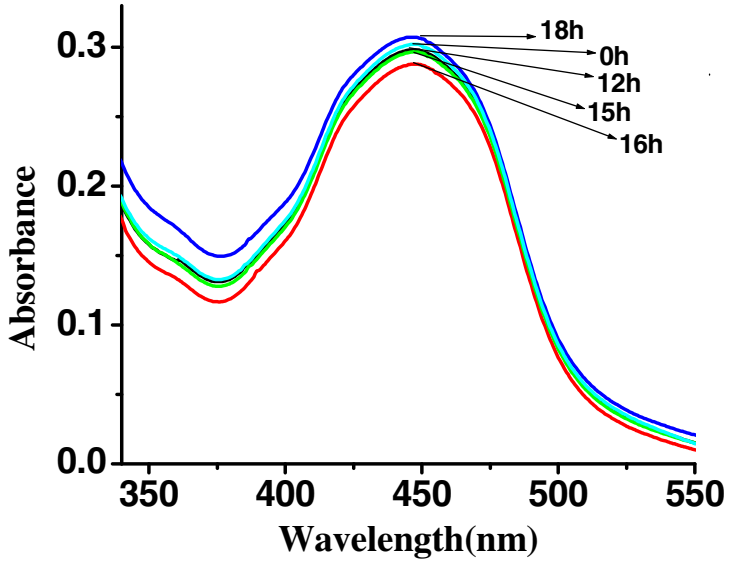

Figure S6. Electronic absorption spectra of **1** in phosphate buffer (pH-7) in the presence of CT-DNA at various time intervals; ([Ru] = 20 μM; [DNA] = 20 μM) incubation at 37°C.

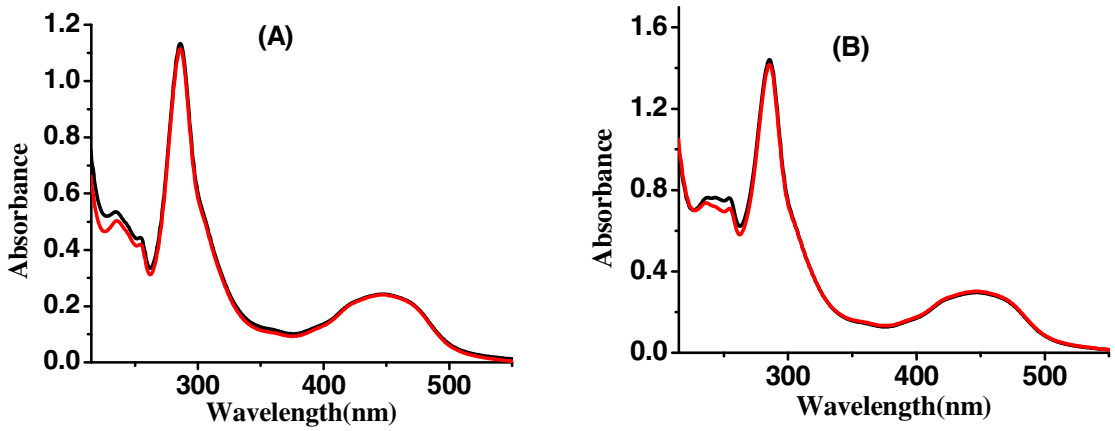

Figure S7. Electronic absorption spectra of **1** in phosphate buffer (pH-7) (A) **1** alone at zero time (red) and after 18 hours (black) incubation (B) in the presence of CT-DNA at zero time (red) and after 18 hours (black) incubation; ([Ru] = 20 μM; [DNA] = 20 μM at 37°C).

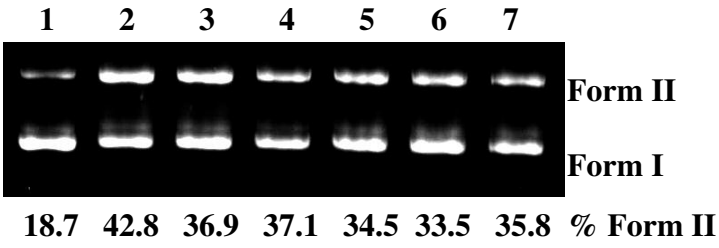

Figure S8. Cleavage of plasmid pBR322 DNA (60 μM, in base pairs) with varying concentration of **1** (incubation for 14 h at 37°C); Lane 1- DNA control, lane 2 - DNA + 80 μM 1, lane 3 - DNA + 100 μM 1, lane 4 - DNA + 200 μM 1, lane 5 - DNA + 400 μM 1, lane 6 - DNA + 600 μM 1, lane 7 - DNA + 800 μM 1; Form I - Supercoiled form; Form II - nicked circular form.