

## Supporting Information

### A New Class of Ru(II) Polyazine Agents with Potential for Photodynamic Therapy

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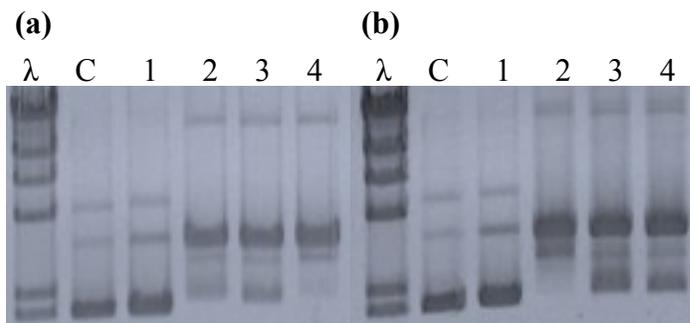
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## Effect of 5,5-dimethyl-1-pyrroline N-oxide (DMPO) on interaction of anthracene-[Ru]-dpp complexes with DNA



**Figure S1:** DNA gel shift assay for (a) [(AnthbpyMe)(bpy)Ru(dpp)](PF<sub>6</sub>)<sub>2</sub> and (b) [(AnthbpyMe)<sub>2</sub>Ru(dpp)](PF<sub>6</sub>)<sub>2</sub> using a 5:1 BP:MC ratio. λ: DNA weight marker, C = pUC19 DNA, 1 = MC + DNA, 2 = MC + DNA + hv, 3 = DMPO (10 mM) + MC + hv, 4 = DMPO (100 mM) + MC + hv. hv = 455 nm LED irradiation with <sup>3</sup>O<sub>2</sub>. C: DNA control, no compound

### Materials and methods

**Materials.** All solvents/chemicals were used as received unless otherwise noted. Circular and linear pUC19 plasmid DNA were purchased from Bayou Biolabs. Lambda DNA/*Hind*III molecular weight marker was obtained from Promega. Electrophoresis grade (boric acid, agarose), and molecular biology grade glycerol were purchased from Fisher Scientific. **Mini-PROTEAN TGX Stain-Free Precast Gels and running buffers were obtained from BioRad.**

**DNA gel shift assay.** DNA-metal complex (MC) solutions were prepared in a 5:1 BP (base pairs):MC ratio in 10 mM phosphate buffer (pH = 7.4) with 16.5 μM metal complex to ensure an absorbance of at least 0.1 at the excitation wavelength (455 nm) in all samples. The complexes were dissolved in 200 μL of DMF and the concentration was determined using known extinction coefficients at the λ<sub>max</sub> for each complex.<sup>1</sup> Anaerobic solutions were deoxygenated with argon for 15 min prior to photolysis. The former solutions were then blanketed with Ar<sub>(g)</sub> during photolysis, while aerobic solutions were exposed to atmospheric conditions. Solutions were photolyzed for 1 h with an LED array.<sup>2</sup> A 0.8% w/w gel was prepared and placed in a model B1A stage Owl Separation Systems with 300 ml of 1X TB buffer (90 mM tris base, 90 mM boric acid). Each sample was prepared for loading by adding 2 μl of loading dye to a 10 μl aliquot of sample and then loaded into their respective wells. A potential of 100 V was applied through the gel for 1 h. Following electrophoresis, gels were stained in 0.5 μg/ml ethidium bromide for 0.5 h and washed using double deionized water for 0.5 h. The gels were visualized on a Fisher Biotech UV-transilluminator and images were captured using an Olympus SP-320 camera fitted with an ethidium bromide filter.

**Scavenger assay.** Concentration of DNA, metal complex, and buffer in the solutions were the same as described above. However, individual ROS scavengers were added before photolysis. Sodium iodide, sodium benzoate, and DMSO were used as hydroxyl radical scavengers. Sodium chloride was utilized as an ionic control in order to determine if the change in the activity of the complexes was due to an ionic component instead of an ROS scavenger effect. Little or no

difference was observed between the NaCl ionic control and the sample photolyzed under standard conditions (82.5  $\mu\text{M}$  DNA, 16.5  $\mu\text{M}$  metal complex, 10 mM buffer).

**CT-DNA titration.** The concentration of CT DNA was calculated by using known extinction coefficient at 260 nm ( $6,600 \text{ M}^{-1} \text{ cm}^{-1}$  per base).<sup>3</sup> The ratio of absorbance for the DNA sample at 260 nm and 280 nm was taken and was always greater than 1.8, indicating that the DNA was substantially free of protein.<sup>4</sup> A solution of 30  $\mu\text{M}$  complex in 5 mM in Tris buffer (pH = 7.0), 50 mM NaCl, and was prepared. This solution was titrated with increasing amounts of DNA from  $10^{-6}$  to  $10^{-4}$  M. The solution was incubated in the dark for 5 min prior to each measurement to allow formation of non-covalent interaction and establishment of equilibrium. Binding constants were determined by triplicate utilizing the model proposed by Schmechel and Crothers (1971) and latter modified by Meehan *et al* (1987).<sup>5</sup>

**SDS-PAGE assay.** A bovine serum albumin (BSA) stock solution was prepared by dissolving 100 mg of BSA in 5 ml of 20 mM  $\text{NaH}_2\text{PO}_4$  to give a 20 mg/ml solution. The complexes were dissolved in 200  $\mu\text{L}$  of DMF and the concentration was determined by using known extinction coefficient at a  $\lambda_{\text{max}}$  for each complex.<sup>1</sup> Protein-metal complex solutions were prepared in a 10:1 P:MC ratio in 20 mM phosphate buffer (pH = 7.4) by diluting 50  $\mu\text{L}$  of 20 mg/ml BSA protein with the appropriate amount of complex to a total volume of 500  $\mu\text{L}$ . Anaerobic solutions were deoxygenated through 5 cycle of freeze pump thaw prior to photolysis for 1 h with an LED array.<sup>2</sup> Aerobic solutions were exposed to atmospheric conditions during photolysis for 1 h with an LED array.<sup>2</sup> A 10% precast polyacrylamide Mini-PROTEAN TGX Stain-Free Gels,  $8.6 \times 6.7 \text{ cm}$  ( $W \times L$ ), was used for the SDS-PAGE analysis. Prior to loading, each samples was combined with an equal volume of loading buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 0.1% bromophenyl blue, and 2%  $\beta$ -mercaptoethanol) and heated at  $95^\circ\text{C}$  for 5 min. A potential of 200 V was applied through the gel for 30 min followed by visualization on a Fisher Biotech UV-transilluminator. The images were captured using an Olympus SP-320 camera.

## References

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