Electronic Supporting Information for

Phototoxicity of Strained Ru(II) Complexes: Is it the Metal Complex or the Dissociating Ligand?

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Experimental Section

Instrumentation. Absorption data were acquired on a Shimadzu UV-1650PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on an AC500 Bruker spectrometer operating at 500 MHz and 62 MHz, respectively. Chemical shifts are reported in delta (δ) units, expressed in parts per million (ppm) using the residual protonated solvent as an internal standard. HR-ESI MS measurements were performed at MSU Mass Spectrometry Core Facility. Mass spectra (Figures S8-S9) were acquired using an LC/MSMS system (Ultimate 3000 RSLC/TSQ Endura from ThermoScientific). 50 µM solutions of aqueous Ru(II) samples were prepared and directly infused (2 µL injection, 100 µL/min flow rate of LC-MS grade water containing 0.05% formic acid) into the heated ESI probe [sheath gas (2.71 L/min), aux gas (5.78 L/min), sweep gas (1.5 L/min), ion transfer tube temperature (325 °C), vaporizer temperature (200 °C), positive ion spray voltage (3700 V)]. The chromatogram in Figure S3 was acquired using a Shimadzu analytical HPLC equipped with a quaternary pump, a C18-Shim-pack column (250 mm length x 4.6 mm I.D. x 5 µm particle size) and a PDA detector. Data presented in absorbance spectra in Figure S4 were acquired using an ocean optic spectrometer (HR2000+) equipped with a deuterium/halogen light sources (DT-MINI-2-GS, Ocean Optics) while irradiating at a right angle with He/Cd laser excitation (λ_{exc} = 442 nm; $P_{incident}$ = 33 mW).
White LED specifications: Philips LEDspot LV D 10-50W 4000K MR16 36D, broadband spectral output (Fig. S10), operated at full-power (325 lm) driven by OSRAM HTM 105/230-240 transformer and Clipsal 32E450TM dimmer.
Blue LED specifications: LED Engin LZ4-40B208-000, cooled by a Star LED heat sink, 460 nm output (Fig. S11), operated at 50% of its full-power (130 lm), coupled with a focusing lens (LLNS-1T06-H) and a home-built LED dimmer driven by a Philips Xitanium LED driver LEDINTA0700C210FO.

Materials. 2,9-dimethyl-1,10-phenanthroline hydrate (neocuproine), cisplatin, *cis*-bis(2,2'-bipyridine)dichlororuthenium(II) hydrate, Dowex 22 chloride form, and all other chemicals, solvents and stationary phase materials were obtained from Aldrich and used without any further purification. LC-MS grade water and formic acid were purchased from Fisher Chemical.

Proliferation Inhibition Assay (cytotoxicity). Cytotoxicity assays were carried out as described previously.[1, 2] Cells were plated in flat-bottom 96-well plates at a density of 2-3×10^4 cells/well in 100 µL of their respective growth medium. Four-fold serial dilutions of the different compounds were made directly in the cell culture medium in round-bottom, 96-well plates to yield a total of 9 or 10 concentration points, in triplicates, ranging from 50 to 0.003 µM (200 to 0.003 µM in the case of cisplatin). Dilutions were then directly transferred to the cell plates containing the cells. The wells at all edges were left free of cells in order to prevent edge effect. An additional row with only the compounds was added as a control for their effect. All compounds were freshly prepared as stock solutions in DMSO and the volumes of DMSO contained in the compound dilutions were also tested in duplicates as a control. Typically the percentage of DMSO was maintained below 2% in all experiments ensuring no cytotoxicity due to DMSO. In the case of light activation, plates were exposed to blue LED light for 30 minutes after 6 hrs of cell incubation with the compounds. Excitation occurred from the top at a fixed distance (4.5 cm) from the plate. Note that the compounds that were not taken up by the cells were not removed from the medium before irradiation especially that ML2 cells are non-adherent. The dark and light data were generated from two separate plates and these types of cytotoxicity assays regularly show a plate to plate variability of 2 to 3-fold. Plates were then incubated for 72 hrs at 37 °C, 5% CO_2. Detection of cell viability was performed using the XTT Cell Proliferation Assay – Roche, as per manufacturer's instructions. Briefly, 50 uL of the XTT mixture was added to each well and the plates were incubated for 4 hrs at 37 °C. Absorbance was then read at 450 nm using an ELISA Microplate Reader. All graphs were plotted and analyzed using GraphPad Prism 5 software.

Synthesis and characterization. The synthesis of $Ru(bpy)_2dmphen(PF_6)_2$ was handled under low ambient light and the product was isolated as PF_6 salt. For biological testing the PF_6 salt was converted to chloride salts using Dowex chloride ion exchange resins in order to promote the solubility of the complex in water.

$Ru(bpy)_2dmphen(PF_6)_2$. This compound was synthesized using a modified literature procedure.[3, 4] cis-$Ru(bpy)_2Cl_2.2H_2O$ (200 mg, 0.384 mmol) and 2,9-dimethyl-1,10-phenanthroline hydrate (neocuproine) (88.1 mg, 0.423 mmol) were added to 8 mL of degassed ethylene glycol in a round bottom flask. The mixture was heated in the dark for 6 hours, allowed to cool at room temperature then saturated aq. KPF_6 solution was then added producing a dark orange precipitate that was collected by vacuum filtration. The crude product was dissolved in a minimum amount of acetonitrile and purified by chromatography (aluminum oxide, 2:1 benzene:acetonitrile ramped to 1:1 benzene:acetonitrile) to give a red-orange fraction that was dried using rotavap to form an orange solid. Yield: 283 mg; 82%.^{1}H NMR (CD_3CN, 500 MHz): δ 8.50 (d, *J* =8.50 Hz, 2H), 8.49 (d, *J*=8.50 Hz, 2H), 8.45 (d, *J* =8.50 Hz, 2H), 8.10 (s, 2H), 8.02 (t, *J*=8.50 Hz, 2H), 7.98 (t, *J*=8.50 Hz, 2H), 7.71 (d, *J*=5.50 Hz, 2H), 7.64 (d, *J*=5.50, 2H), 7.61 (d, *J*=8.50 Hz, 2H), 7.28-7.24 (m, 4H), 1.91 (s, 6H). ^{13}C NMR (CD_3CN, 500 MHz): δ 168.03, 158.57, 158.44, 153.88, 153.76, 152.94, 152.81, 149.55, 138.72, 138.61, 138.56, 130.45, 128.63, 128.53, 128.31, 128.13, 128.03, 125.37, 26.16. HR-ESI MS m/z for $[M\text{-}2(PF_6)]^{2+}$ calcd. 311.0714; found 311.0722. UV/Vis (MeCN): MLCT λ_{max} nm (ε $M^{-1}cm^{-1}$) 452 (19,500).
Chloride-ion exchange: 10 mg of $Ru(bpy)_2dmphen(PF_6)_2$ was added to pre-washed Dowex beads in a 50 mL round bottom flask. 10 mL of water was added and the suspension was

stirred gently in the dark for 24 h. The ion exchange promoted the solubility of the complex in water and the resulting $Ru(bpy)_2dmphenCl_2$ was isolated by filtration. The solution was concentrated to about 2 mL volume under vacuum, the compound was then purified on a sephadex LH-20 column with water as eluent and finally dried under rotovap. After chloride counter-ion exchange: HPLC purity by area >98% (Fig. S3) and ESI-MS mz for $[M-2(Cl)]^{2+}$ calcd. 311.07; found 311.13 (Fig S12).

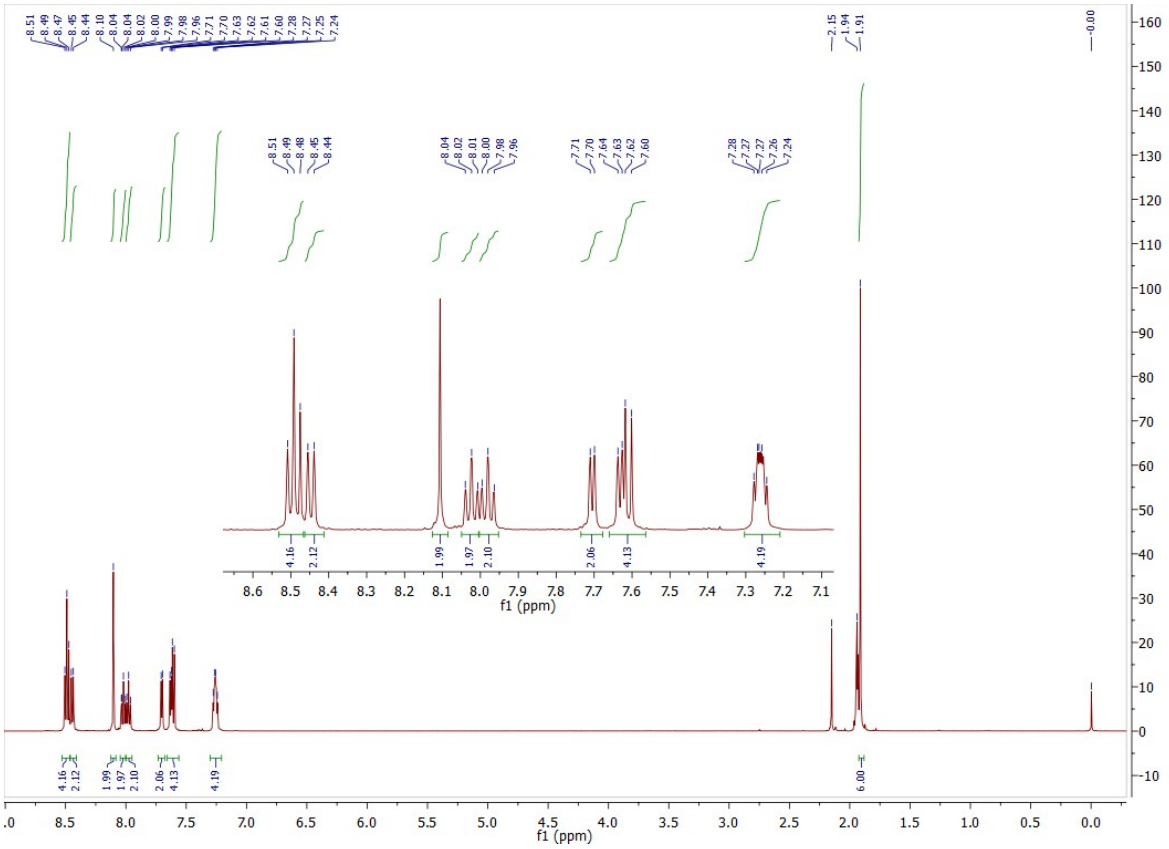


Fig. S1 ^1H NMR (500 MHz) spectrum of $Ru(bpy)_2dmphen(PF_6)_2$ in CD_3CN.

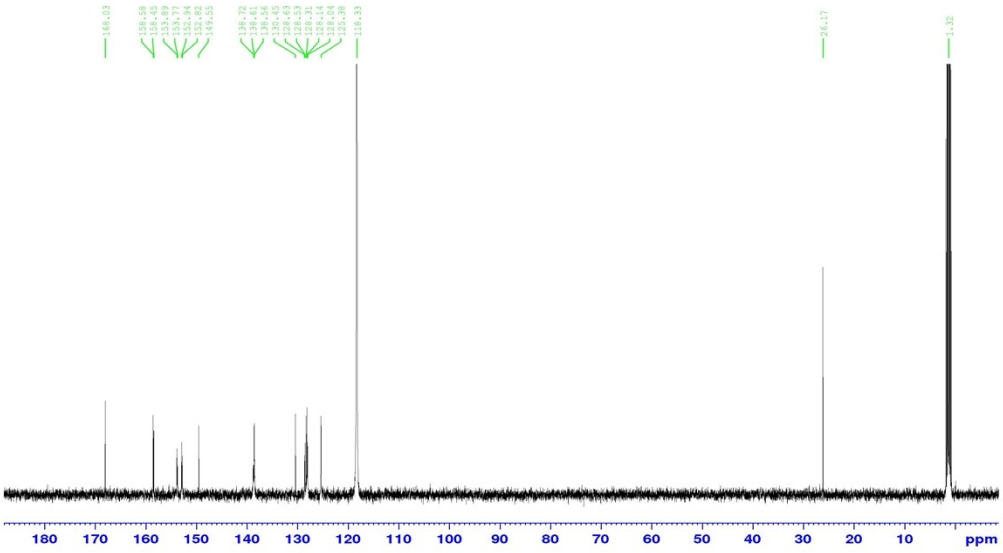


Fig. S2 ^{13}C NMR (500 MHz) spectrum of $Ru(bpy)_2dmphen(PF_6)_2$ in CD_3CN.

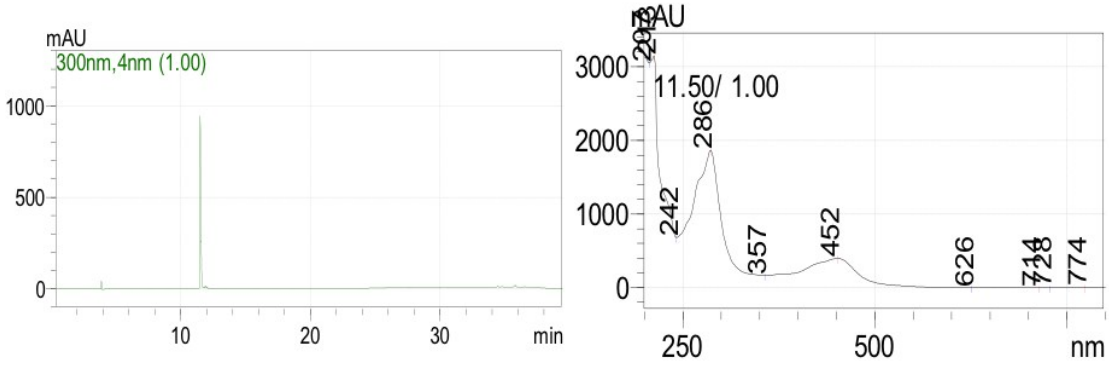


Fig. S3 (**left**) HPLC chromatogram of $Ru(bpy)_2dmphenCl_2$ (purity >98% by area). (**right**) UV-vis spectrum extracted from the peak at 11.5 min retention time using a previously published gradient method.[5]

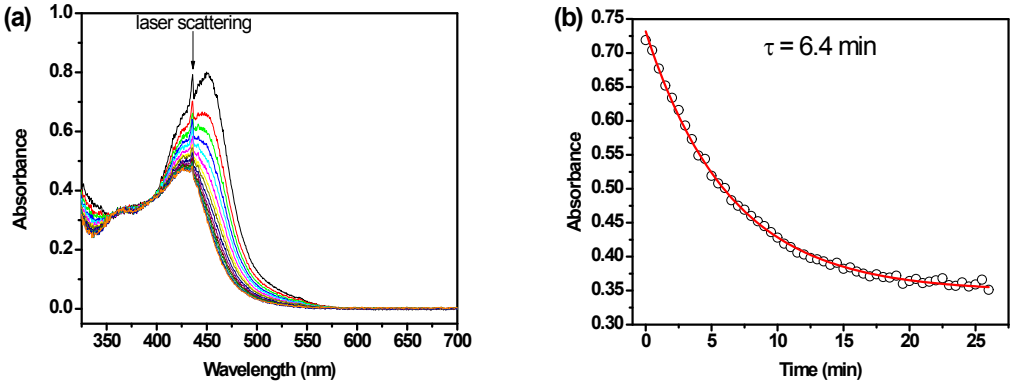


Fig. S4 (a) Absorption spectra of $Ru(bpy)_2dmphen(PF_6)_2$ in acetonitrile (V = 3×10^{-3} L, pathlength = 1 cm) as a function of irradiation time. The time interval used was 1 min and the excitation source was 442 nm He/Cd laser excitation at P = 33 mW excitation (ε_{442nm} = 18,600 $M^{-1}cm^{-1}$). The arrow indicates laser scattering. **(b)** Absorbance at 442 nm as a function of time recorded at 30 seconds interval and fitted to a single exponential decay.

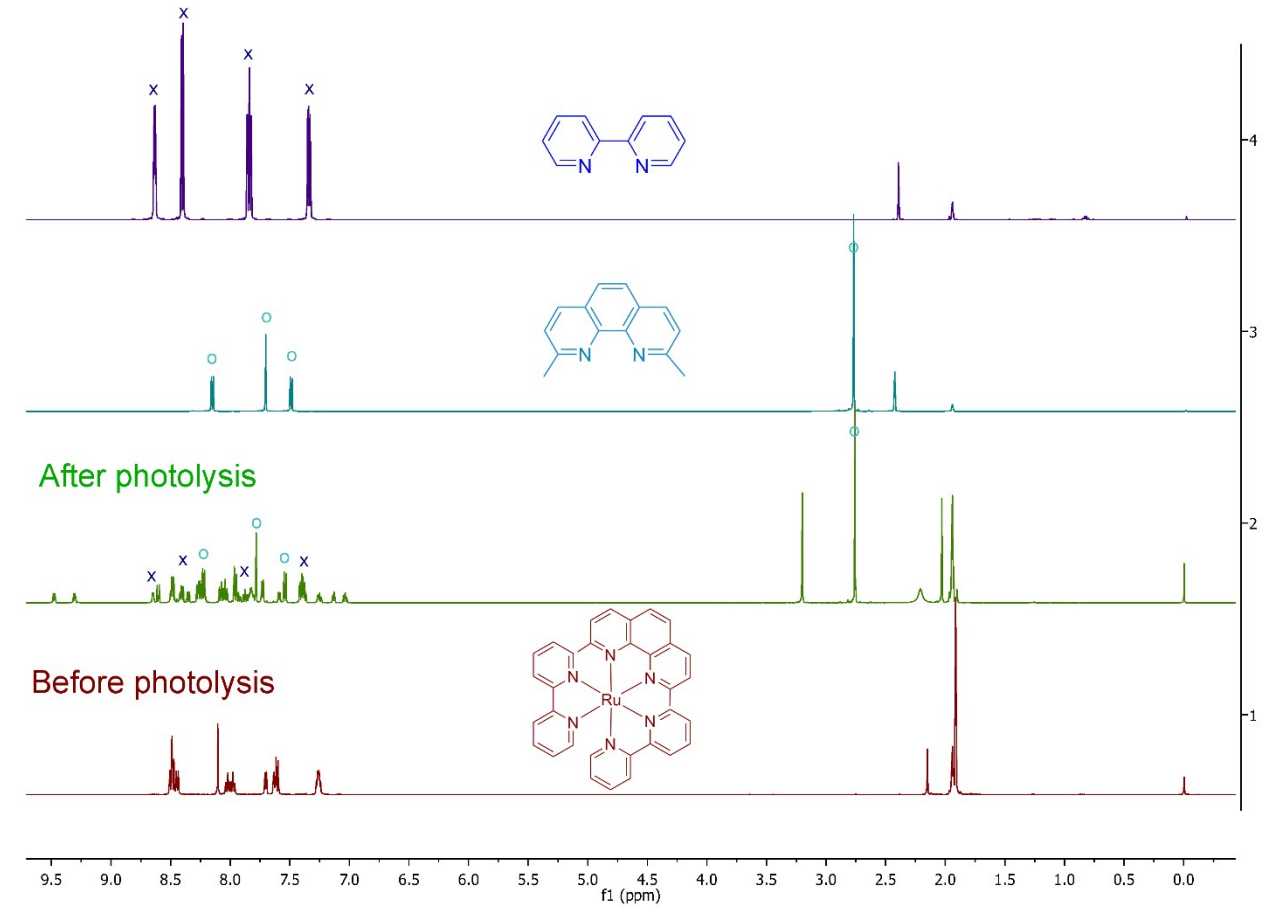
The quantum yield was calculated using a slightly modified literature method.[6] The initial rate of photons absorption and the initial rate of photo-dissociation were calculated.

- initial rate of photodissociation $= k_{obs}[A]_0 = \frac{[A]_0}{\tau} =$ 1.07×10^{-7} M s^{-1}
- initial rate of photons absorption $= \frac{P \times (1 - e^{-\ln(10)O.D.})}{E_{photons} \times V} =$ 3.33×10^{-5} M s^{-1}

where $[A]_0 =$ 4.1×10^{-5} M, τ = 384 s, the initial O.D. = 0.755 at 442 nm and $E_{photons}$ = 2.7065×10^{5} J/mol.

The quantum efficiency for photodissociation (η) is:

$$\eta = \frac{initial\ rate\ of\ photodissociation}{initial\ rate\ of\ photons\ absorption} = 0.0032$$


After photolysis
Before photolysis
Ru
f1 (ppm)

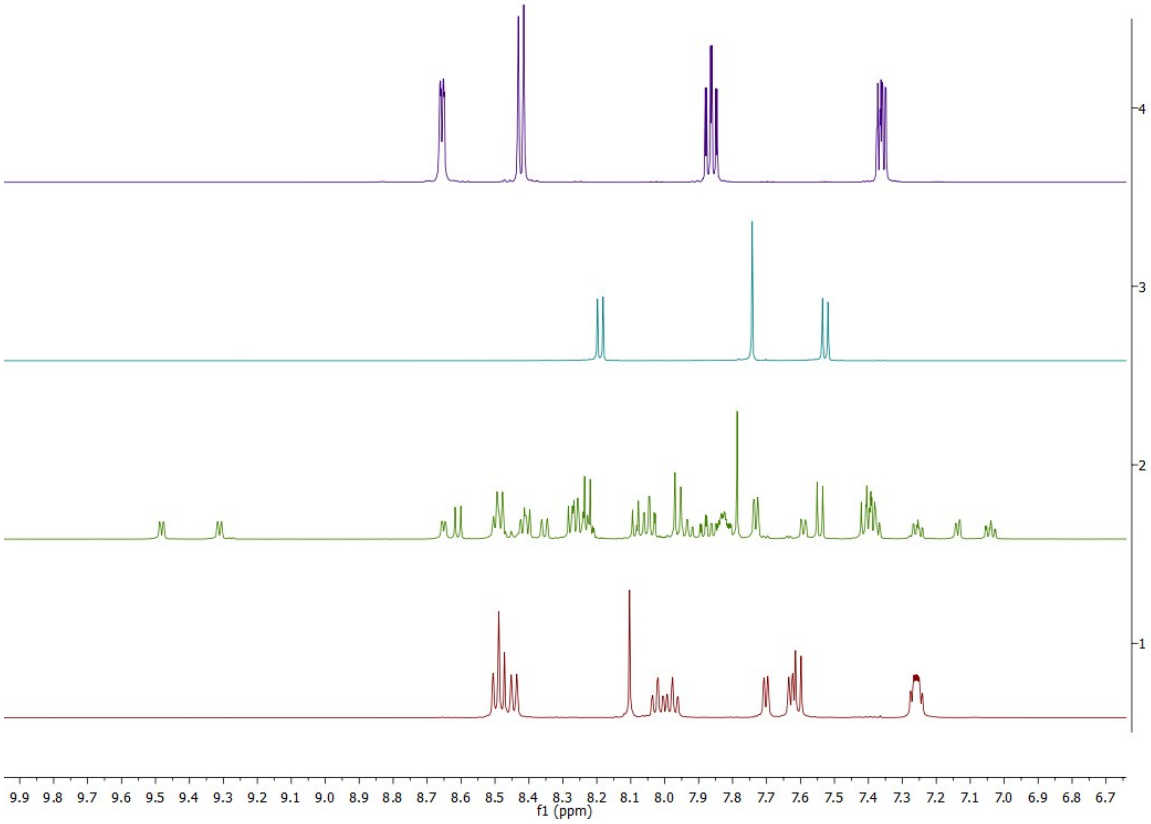

f1 (ppm)

Fig. S5 (top) ^{1}H NMR spectra (500 MHz) in CD_3CN of $Ru(bpy)_2dmphen(PF_6)_2$ before photolysis (red), after photolysis (green) as well as the free ligands 2,2'-bipyridine (purple) and 2,9-dimethyl-1,10-phenanthroline (light blue); **(bottom)** magnification of the aromatic region. The white LED light source was placed 2 cm away from the NMR tube. The reaction progression was monitored by NMR until the starting material completely disappears (~6 hours). Since the aromatic region was crowded we relied on the integration of the protons of the methyl groups of the free dmphen ligand (which results from dmphen ejection) at 2.76 ppm versus the methyl groups of the presumed $Ru(bpy)(dmphen)(CD_3CN)_x^{2+}$ [x = 1 or 2] (which results from bpy ejection) at 3.20 ppm and 2.03 ppm. Peaks marked with a cross are for 2,2'-bipyridine and the ones marked with circles are for 2,9-dimethyl-1,10-phenanthroline.

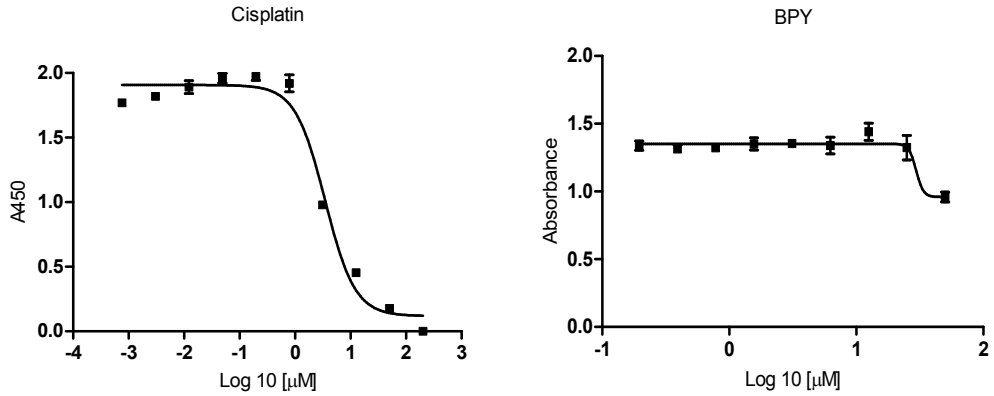


Fig. S6 Cytotoxicity data acquired on ML2 cell line of cisplatin plotted as absorbance at 450 nm vs log of the concentration (μM) of cisplatin (**left**) and 2,2'-bipyridine (bpy) (**right**).

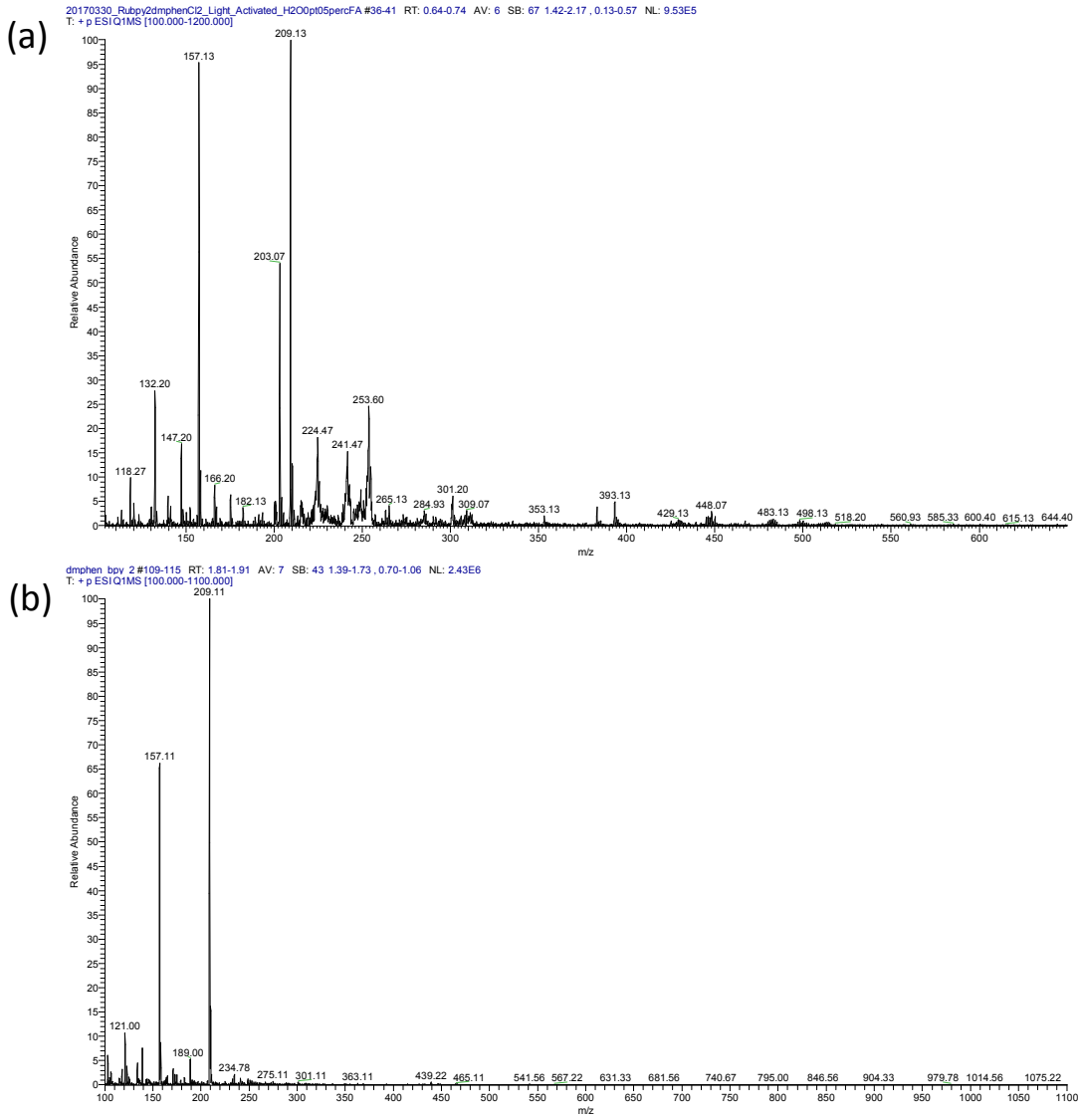


Fig. S7 ESI-MS (full scan, positive and profile mode at 0.4 Da resolution) of **(a)** $Ru(bpy)_2(dmphen)Cl_2$ in water subjected to 30 min irradiation in the same setup used for biological testing and **(b)** an equimolar mixture of bpy and dmphen ligands. The peaks at m/z of 157.1 were assigned to bpy, at 209.1 to dmphen, at 224.5 to $Ru(bpy)_2(H_2O)_2^{2+}$, at 241.5 and 253.6 to $Ru(bpy)(dmphen)(H_2O)^{2+}$ and its sodiated form respectively. Based on the ratio of the peaks at m/z 157.1 and 209.1 in spectrum (b), it was estimated that both bpy and dmphen dissociate in a ratio of 3 to 2 respectively (a).

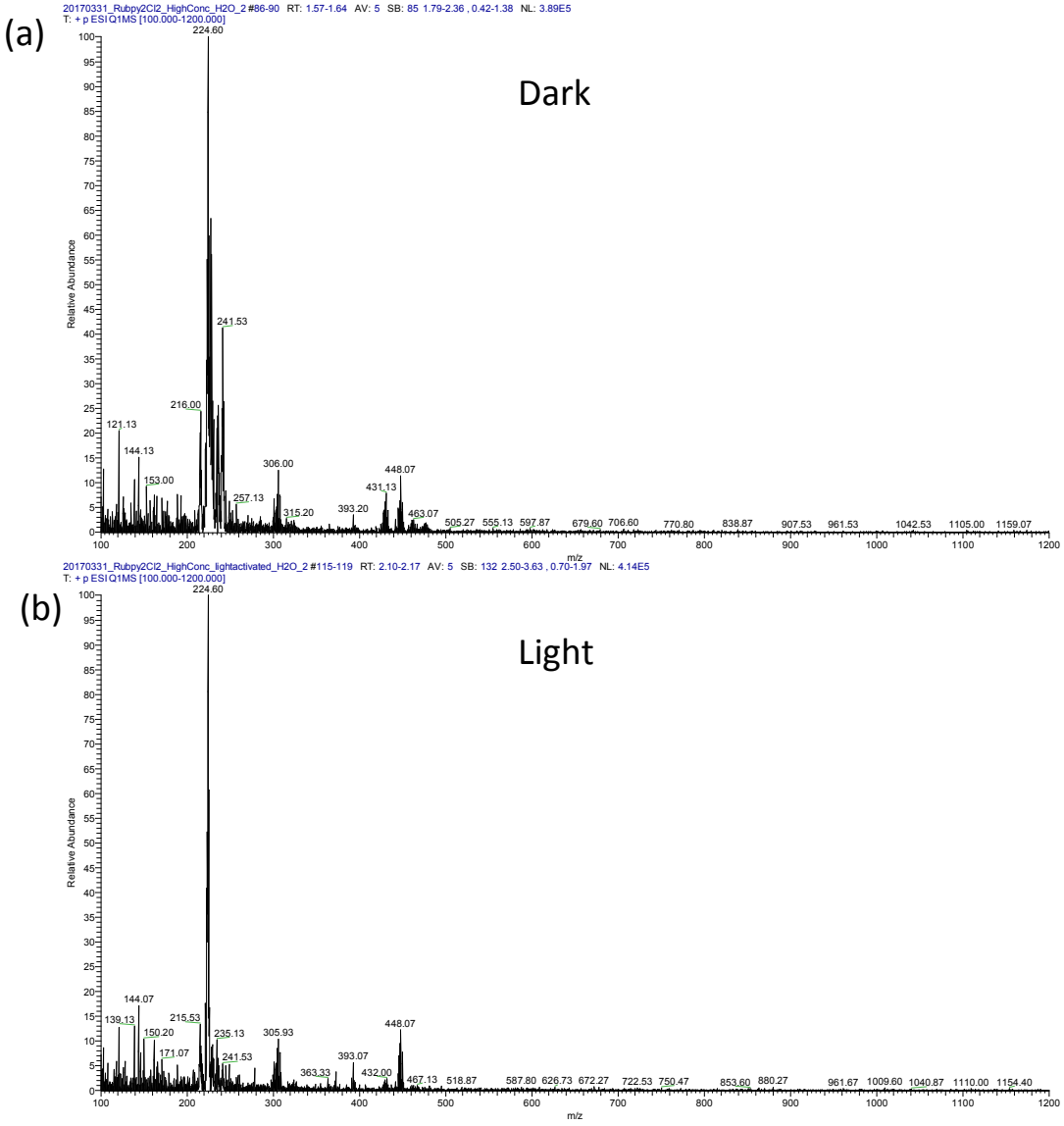


Fig. S8 ESI-MS (full scan, positive and profile mode at 0.4 Da resolution) of $Ru(bpy)_2Cl_2$ in water in the dark for 6 h **(a)** and then subjected to 30 min blue LED irradiation **(b)** in the same setup used for biological testing. Note that the same base peak (m/z 224.6) exists in (a) and (b) which was attributed to $Ru(bpy)_2(H_2O)_2^{2+}$.

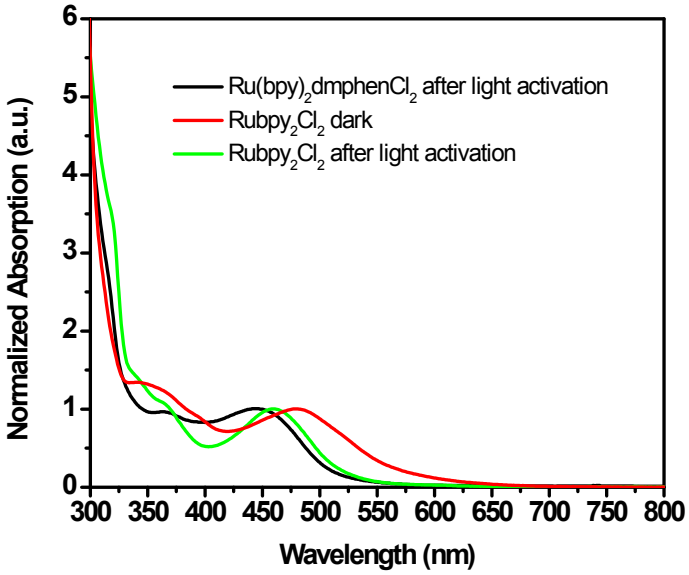


Fig. S9 Normalized absorption spectra of $Ru(bpy)_2dmphenCl_2$ after light activation in water, $Ru(bpy)_2Cl_2$ in the dark (in water for 6 hrs, forming $Ru(bpy)_2(H_2O)_2^{2+}$, Fig. S8a) and $Ru(bpy)_2Cl_2$ after light activation (in water for 6 hrs then activated using blue LED light for 30 min). The hypsochromic shift of the MLCT absorption peak is attributed to cis-trans photoisomerization.[7] It is important to note that the exact peak position of the MLCT absorption depends on the medium (buffer composition and pH) inducing a red-shift when coordinating anions such as acetate and phosphate are used.[5, 8]

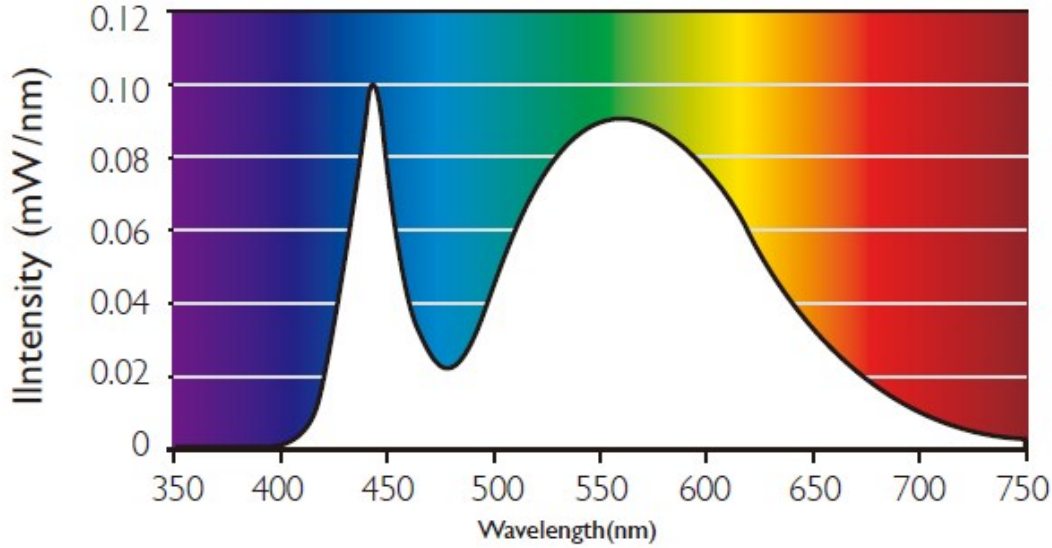


Fig. S10 Spectral output of the white LED used for irradiation as extracted from manufacturer's specification sheet.

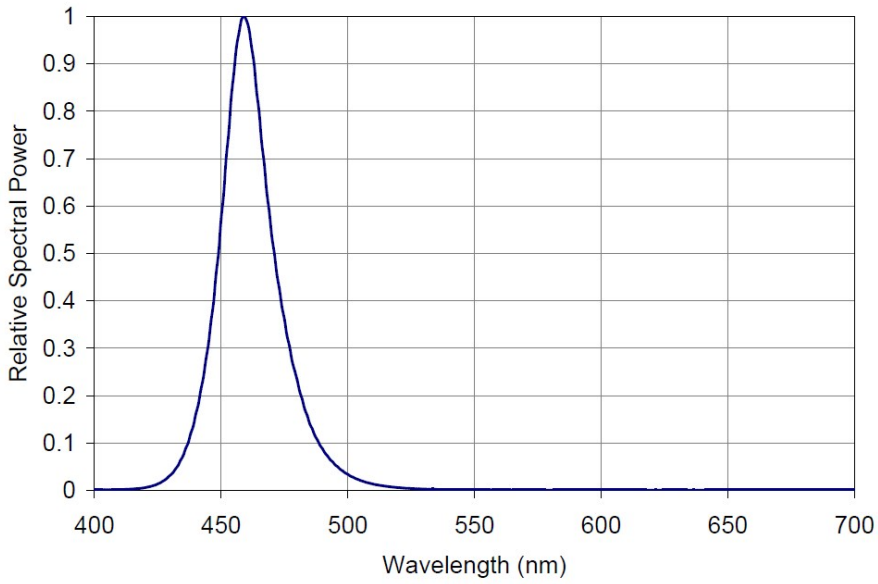


Fig. S11 Spectral output of the blue LED used for irradiation as extracted from manufacturer's specification sheet.

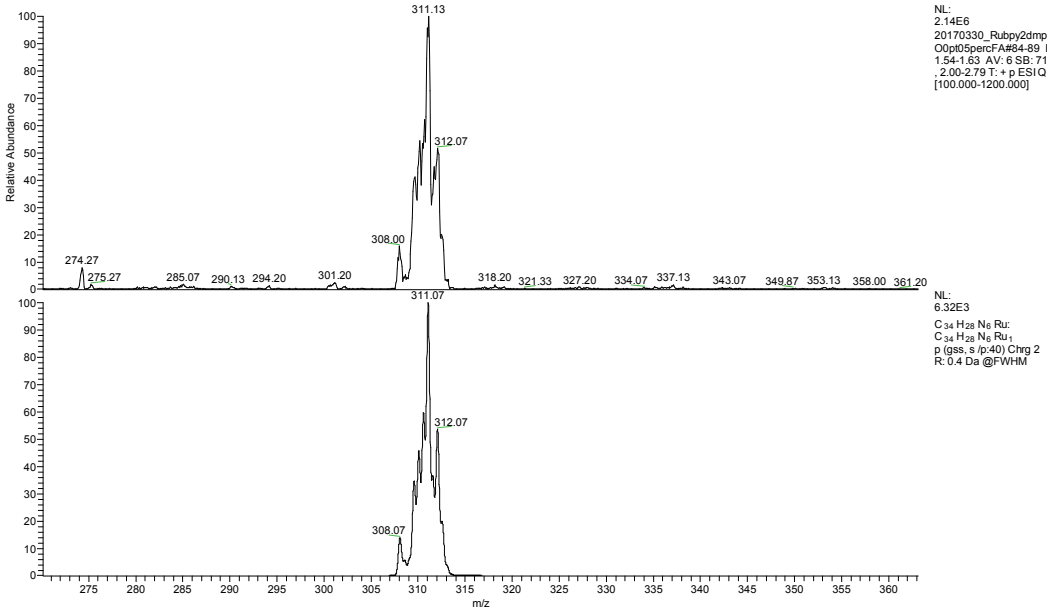


Fig. S12 ESI-MS (full scan, positive and profile mode at 0.4 Da resolution) of $Ru(bpy)_2(dmphen)Cl_2$ in water (top) and simulated at the same resolution based on the molecular formula of $[M\text{-}2Cl]^{2+}$ (bottom).

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