Supporting Information

Exploring the Activity of a Polyazine Bridged Ru(II)–Pt(II) Supramolecule in F98 Rat Malignant Glioma Cells

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1. Synthesis and characterization of $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$

The bimetallic complex, $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$ was synthesized by the previously reported method using a building block strategy (Figure S1).[1] $[(Ph_2phen)_2Ru(dpp)]Cl_2$ (0.50 g, 0.47 mmol) and $[PtCl_2(DMSO)_2]$ (1.0 g, 2.4 mmol) were heated at reflux in 25 mL of ethanol for ca. 2 h in the dark. The reaction mixture was cooled to room temperature and the solid was collected by vacuum filtration. The solid was dissolved in a minimal amount of 2:1 C_2H_5OH/CH_3CN and purified by LH-20 size exclusion chromatography using 2:1 C_2H_5OH/CH_3CN eluent. The first orange-red band was collected, solvent was removed under vacuum, and the complex was flash precipitated from dry acetone into diethyl ether, with a yield of 75%.

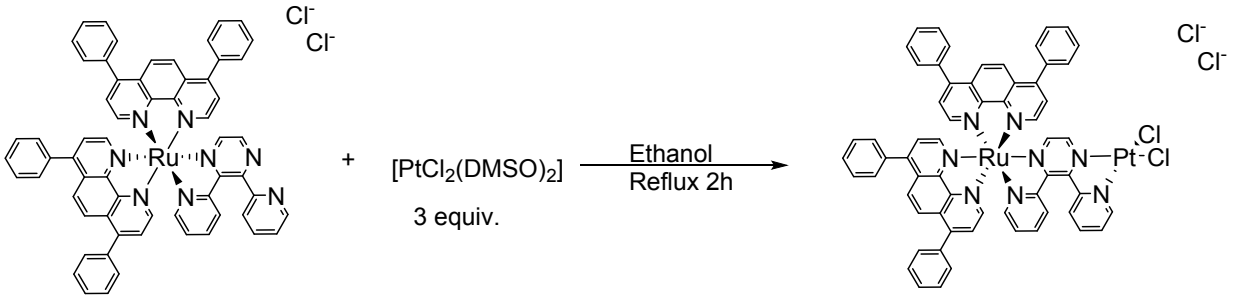


Figure S1. Synthetic scheme of $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$.

The complex was metathesized to the PF_6^- salt by mixing an aqueous solution of $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$ into a saturated solution of NH_4PF_6. $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)$ was recovered by filtration and flash precipitated from acetonitrile into diethyl ether, with a yield of 70%.

The electrochemistry of the $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$ complex is shown in Figure S2. The oxidative electrochemistry shows a reversible $Ru^{II/III}$ couple at +1.57V *vs.* Ag/AgCl and an irreversible oxidation of $Pt^{II/IV}$ at +1.47V *vs.* Ag/AgCl, respectively, while the $dpp^{0/-}$ is reduced at -0.50V *vs.* Ag/AgCl and $Ph_2phen^{0/-}$ is reduced at -1.05V *vs.* Ag/AgCl.[2]

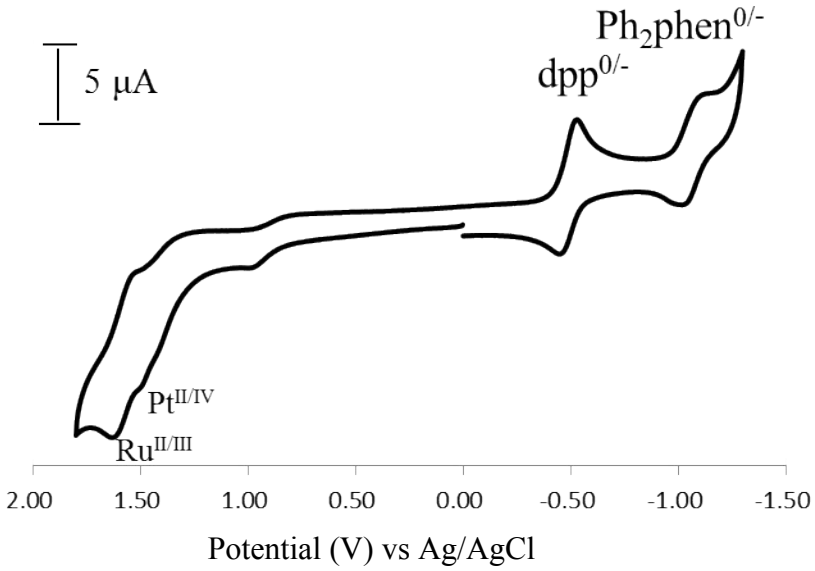


Figure S2. Cyclic voltammogram of $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$ in 0.1 M $TBAPF_6$ acetonitrile, E vs. Ag/AgCl, ν = 100 mV/s.

The electronic absorption spectrum exhibits a broad low energy Ru(dπ)→dpp(π*) metal–to–ligand charge transfer (^{1}MLCT) transition at 525 nm with ε = 12,100 $M^{-1}cm^{-1}$, which extends throughout the visible region; while the UV region is dominated by characteristic ligand-based π→π* transitions (Figure S3). Emission of this bimetallic complex occurs at 740 nm upon excitation at 520 nm.[2]

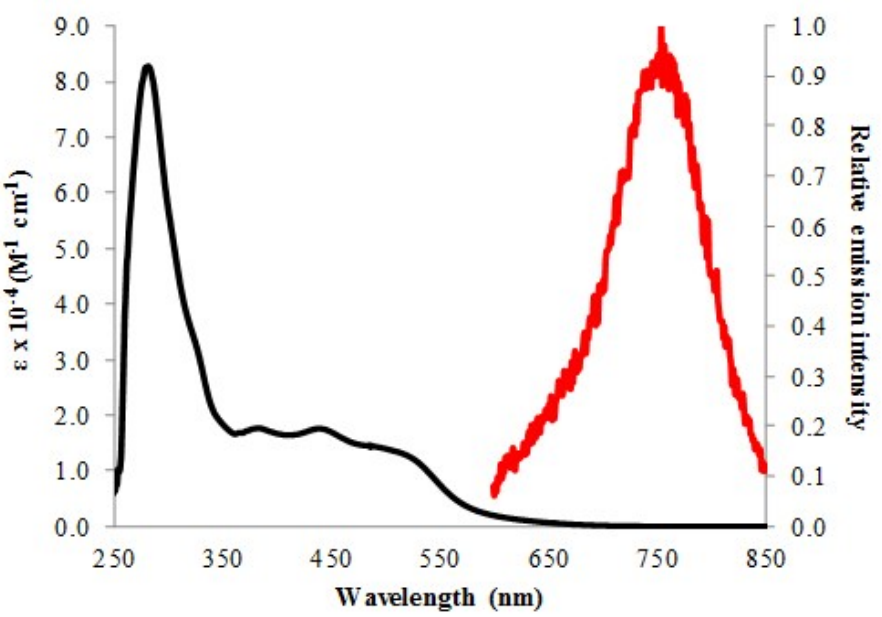


Figure S3. Electronic absorption (black) and emission (red) spectra upon excitation at 520 nm of $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$ in CH_3CN at room temperature.

2. Partition coefficient

The partition coefficient of the complex relative to three well-known anticancer agents was determined as an approximation of the ability of these complexes to cross the cell membrane. The partition coefficient measures the relative concentration of a molecule in equilibrated immiscible solvents, in this case equilibrated phosphate buffer (pH 7.4) and *n*-octanol. The buffer and octanol solvents were equilibrated and separated prior to use in the experiments. The complexes were dissolved in 30 mL of buffer and 30 mL of octanol was added. The samples were stirred at 1000 rpm for 30 min and added to a separatory funnel. After 12 h of equilibration the layers were drained and the concentrations of the complexes determined based on their molar absorptivity using an electronic absorption spectrophotometer. The log*P* or partition coefficients were determined using equation 1,

$$\mathrm{Log}P_{o/w} = \log\left(\frac{[\text{octanol}]}{[\text{buffer}]}\right) \qquad \text{(Eq. 1)}$$

where [octanol] and [buffer] are the concentrations of the compound in octanol and buffer after separation.

Partition coefficients using octanol and water are reported as Log$P_{O/W}$, where a value of 0 represents equal concentrations of the molecule in the octanol and water phases. A Log$P_{O/W}$ value greater than 0 indicates a larger concentration of the molecule in the octanol phase and therefore a more lipophilic molecule.

3. DNA Gel Shift Assay

DNA-complex solutions were prepared in aqueous 10 mM phosphate buffer (pH 7.4) with 20 μM $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$ and 100 μM pUC19 DNA (1:5 complex:base pair molar ratio). Dark (D) and thermal (T) controls were incubated in a dark compartment at room temperature or 37 °C, respectively, while photolysis was being conducted. Photolysis was performed for 1 h with a blue (455 nm, B and B-Ar) or red (625 nm, R and R-Ar) LED array. Oxygen depleted samples (B-Ar and R-Ar) were bubbled with argon for 20 min prior to photolysis and closed under an argon atmosphere. After 1 h, 5 μL of each sample were diluted with 5 μL of 10 mM phosphate buffer and 2 μL of Type 3 loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol in water). Samples were analyzed using a 0.8 % w/w agarose gel with Tris-boric acid buffer (90 mM Tris base, 90 mM boric acid), placed in an Owl Separation Systems model B1A stage, where electrophoresis was conducted for 90 min at 100 V. Gels staining was performed using 0.5 μg/mL ethidium bromide (Biotium Inc.) for 30 min, followed by washing with deionized water for 15 min and visualization on a Fisher Biotech UV-transilluminator. Images were captured with an Olympus SP-320 camera fitted with an ethidium bromide filter.[3]

4. Protein Gel Shift Assay

Bovine serum albumin (BSA)-complex solutions were prepared in aqueous 10 mM phosphate buffer (pH 7.4) with BSA: metal complex molar ratios of 1:1 or 1:10, with a fixed metal complex concentration of 20 μM. Photolysis was performed for 1 or 3 h using a blue (455 nm, B) or red (625 nm, R) LED array. Dark (D) and thermal (T) controls were incubated in a dark compartment while photolysis was being conducted. Fractionation was performed using 1 mm 4-12% Bis-Tris gels (Invitrogen). Gels were loaded with samples treated with Nupage LDS buffer and Nupage reducing agent according to the manufacturer's instructions, followed by electrophoresis in MES buffer at 200 V for 35 min. Gels were washed three times with water for 5 min before being treated

with Simplyblue Safestain (Invitrogen) for 1 h. Gels were then washed overnight in water and imaged using a Bio-Rad ChemiDoc XLS system.[3] (Figure S4)

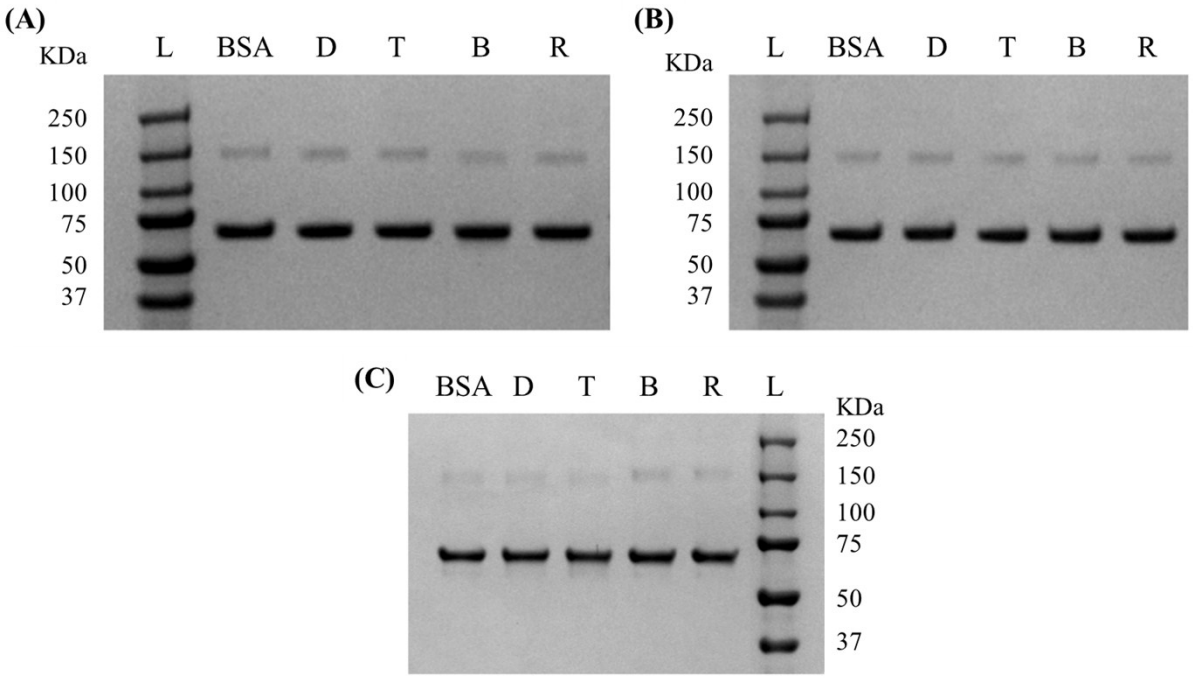


Figure S4. Protein gel shift assay. L = molecular weight ladder; BSA = untreated BSA control; D = samples incubated in the dark. T = samples incubated at 37 °C, B = samples photolyzed with blue light; R = samples photolyzed with red light. **(A)** Samples treated for 1 h at 1:1 complex:BSA molar ratio. **(B)** Samples treated for 1 h at 10:1 complex:BSA molar ratio. **(C)** Samples treated for 3 h at 10:1 complex:BSA molar ratio.

5. Cell culture

F98 rat malignant glioma cells (ATCC) were maintained in DMEM supplemented with 10% fetal bovine serum (FBS, ATCC) and 1% penicillin-streptomycin and incubated at 37 ˚C in a humidified incubator at 5% CO_2.

6. Cell uptake of Ru(II)-Pt(II) by F98 MG cells

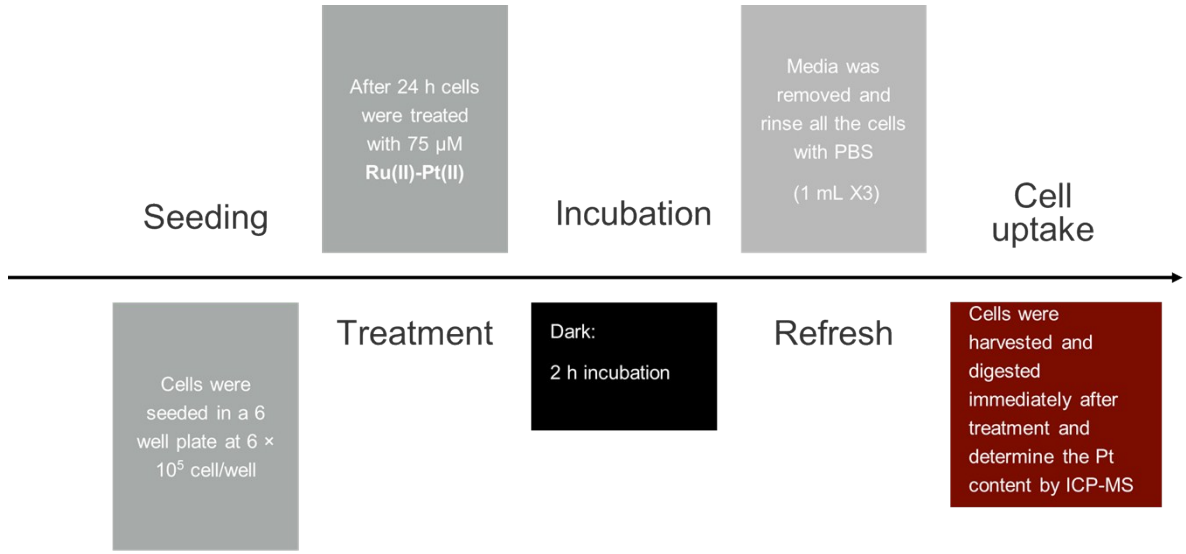


Figure S5. Protocol for **Ru(II)-Pt(II)** uptake in F98 MG cell line.

Cell uptake was quantified by inductively coupled plasma mass spectrometry (ICP-MS) analysis of intracellular platinum contents. F98 cells were grown in 6-well plates for 24 h at 37˚C and 5% CO_2 before the treatment. **Ru(II)-Pt(II)** or cisplatin were dissolved in DMSO and added to cells to give a final concentration of 75 µM (final concentration of DMSO 3% v/v). Cells were incubated with either compound for 2 h at 37 ˚C in a 5% CO_2 humidified incubator. The culture medium was discarded, and the cells were washed three times with PBS. Cells were harvested, suspended in PBS, and centrifuged at 3000 rpm for 15 min. After discarding the buffer, cell pellets were digested in 70% nitric acid at 90 °C for 1 h. Samples were diluted further to a final concentration of 7% nitric acid with D.I. water containing 0.1% Triton X100. The Pt content of the cells was estimated using a Thermo Electron X-Series inductively coupled plasma mass spectrometer (ICP-MS) in accordance with Standard Method 3125-B.

7. Cytotoxicity and photocytotoxicity

The cytotoxicity of $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$ was evaluated both under dark conditions and following photolysis to evaluate the photodynamic activity of the compound. F98 rat malignant glioma cells were plated in triplicates in the 6-well plates at a density of approximately 6×10^5 cells/well in DMEM. The cells were incubated at 37 ˚C in a 5% CO_2 humidified atmosphere for

24 h before treatment. Medium was removed from each well and replaced with solution containing different concentrations of the complex, ranging from 25 to 75 μM, dissolved in 29% v/v phosphate buffered saline (PBS), 4% v/v dimethyl sulfoxide (DMSO), and 67% v/v RPMI 1640 (without L-glutamine and phenol red). A control sample was prepared using the same ratio of PBS, DMSO, and RPMI 1640 but without $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$. For cytotoxicity assay, the plates were incubated in the dark for 1 h. For photocytotoxicity, the cells were incubated in the dark for 15 min and then were irradiated for 30 min using a lab-built blue light LED array (470 nm, 3.75 mW). A final 15 min dark incubation was performed after photolysis. The solutions were then aspirated and the cells were washed with fresh medium. Half of the plates were supplemented with DMEM and incubated for 48 h prior to counting cells, while cells in the rest of the wells were harvested immediately after incubation.

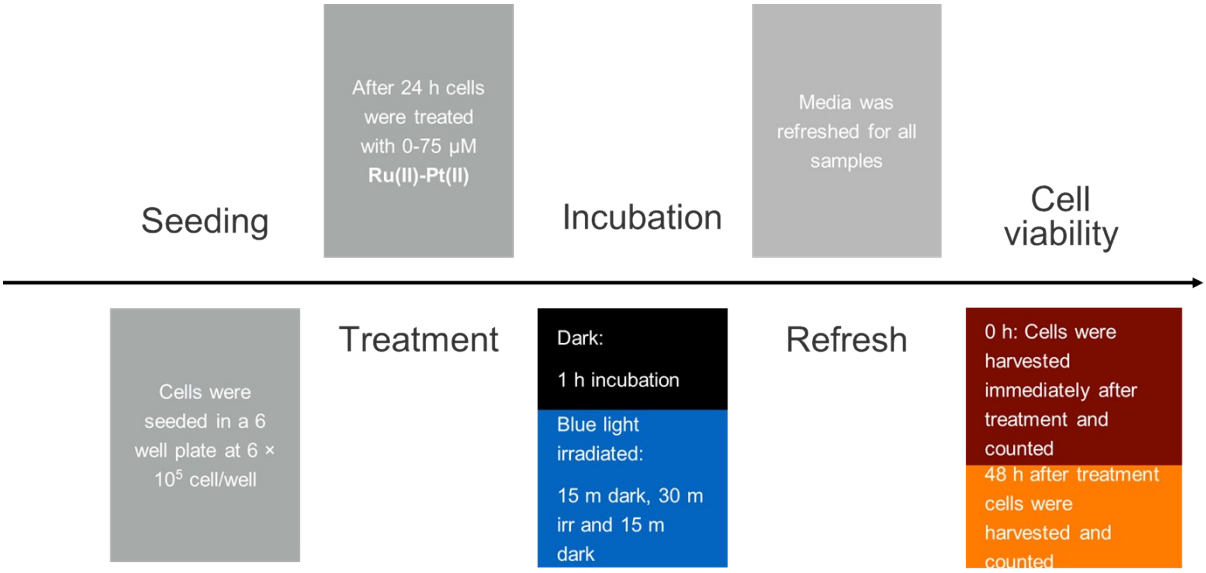


Figure S6. Protocol for F98 MG cell line photocytotoxic response to $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$.

Cells were harvested using 0.05% trypsin in PBS/EDTA. An equivalent amount of DMEM was added to the trypsinized cells once they were visibly detached from the well. Trypan blue was used as a dye exclusion assay and cells were counted using an automated Vi-CELL® Cell Viability Analyzer.

Each experiment was performed in triplicate and the average values were used to calculate the final cell viability for each concentration of $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$. The results are shown in Figure S7.

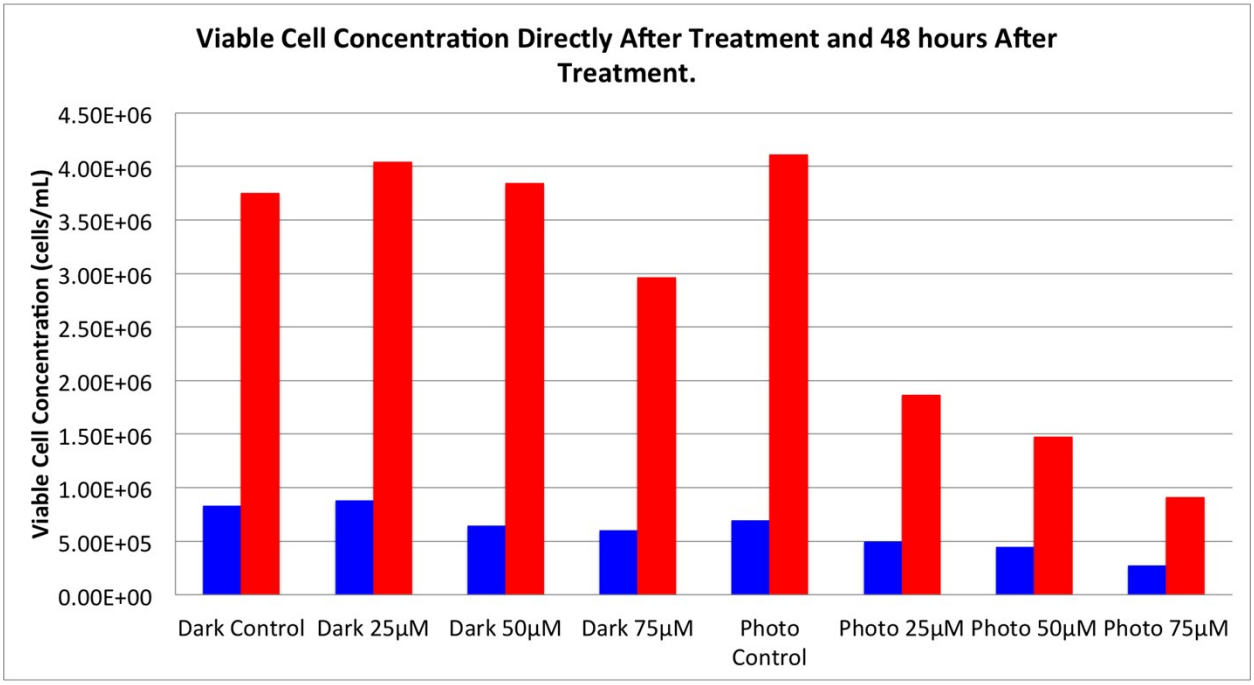
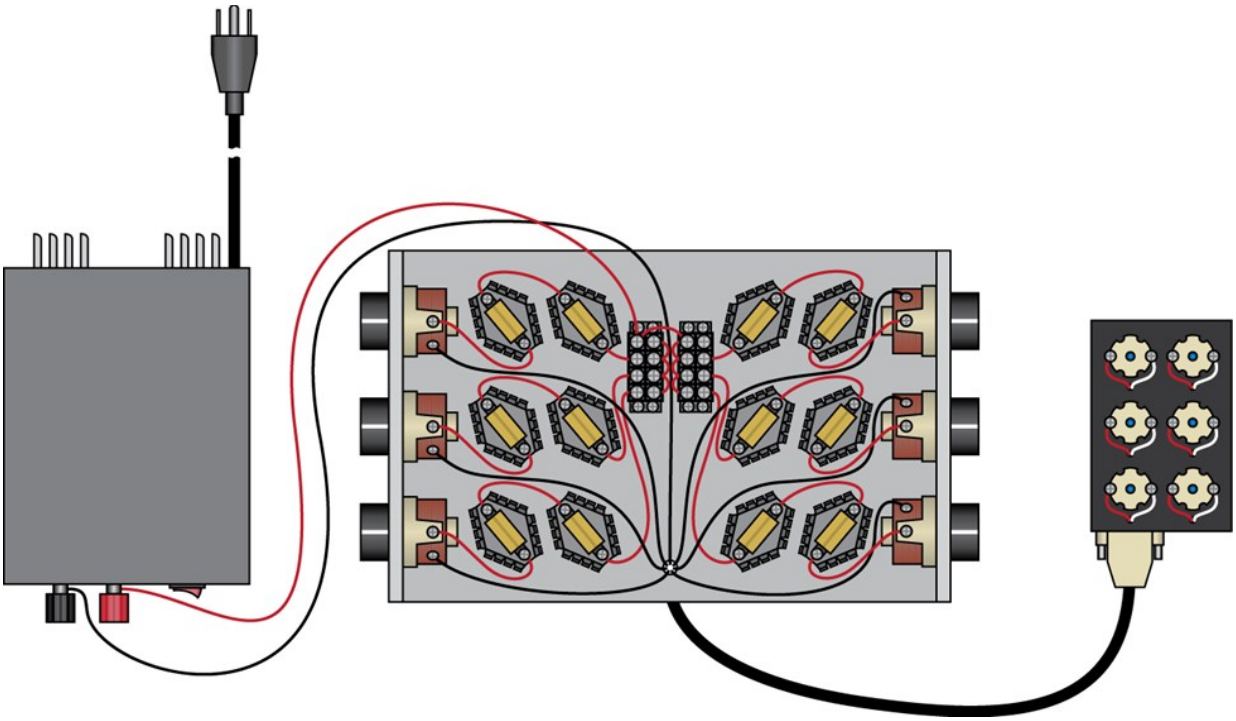


Figure S7. Viability assay of F98 MG cells after treatment with **Ru(II)-Pt(II)** for 0 h (blue) or 48 h (red). Results represent the mean value for two experiments. Photo = irradiated samples.

8. Design of LED Array:

A LED array was designed to insure homogenous and controlled light delivery to the cells during treatment. The power source input was 120 V AC with an output of 13.5 V DC. A circuitry box was used to calibrate the output of each LED separately prior to the treatment. The circuitry diagram is displayed in Figure S8. Two 5 Ω resistors, wiring, a voltage controlling knob, and a LED were placed together for six LED channels, with one light source per well in the treatment containers. The six LEDs were placed on a separate board from the circuitry to avoid excessive heating of the plates during treatment. The LED head board was placed on a mount designed to fit the dimensions of the well plates used in treatment and not let light either enter or escape to interrupt the photolysis. Illumination of the cells was performed through the bottom of the plate in order to insure effective delivery of light.



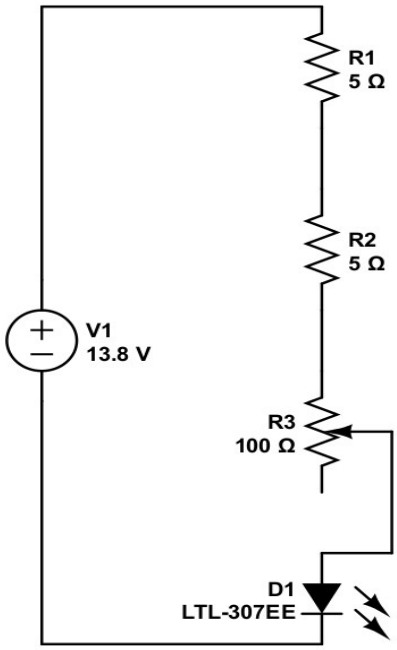


Figure S8. Circuitry diagram for calibration box

For calibration of each LED prior to the treatment, a ThorLabs sensor was used to detect the light output at the center of each well. Adjustments were made accordingly with the voltage

controlling knob and the average power delivered to those sites was 3.75 mW. This output was checked each time a plate was removed to confirm that the power remained constant throughout the treatment.

References:

1. S. L. H. Higgins, A. J. Tucker, B. S. J. Winkel and K. J. Brewer, *Chem. Commun.*, 2012, **48**, 67-69.
2. S. L. H. Higgins, T. A. White, B. S. J. Winkel and K. J. Brewer, *Inorg. Chem.*, 2011, **50**, 463-470.
3. J. Zhu, A. Dominijanni, J. Á. Rodríguez-Corrales, R. Prussin, Z. Zhao, T. Li, J. L. Robertson and K. J. Brewer, *Inorg. Chim. Acta*, 2016.