Green light-induced apoptosis in cancer cells by a tetrapyridyl ruthenium prodrug offering two *trans* coordination sites

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Supporting Information

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1 Synthesis and crystallography

All ^{1}H NMR spectra were recorded on a Bruker DMX-400 or a Bruker AV-600 spectrometer. Chemical shifts are indicated in ppm relative to tetramethylsilane. Mass spectra were recorded by using a Thermoquest Finnagen AQA Spectrometer and a MSQ Plus Spectrometer positive ionization mode. See Figure S1 for the specific NMR-assignments of $[1a]^{2+}$ and $[2]^{+}$. For NMR experiments under argon, J. Young NMR tubes with PTFE stopper were used. UV-vis experiments were performed on a Cary 50 Varian spectrometer equipped with a Cary Single Cell Peltier for temperature control. he elemental ultra-trace analyses were performed with an FAST (Elemental Scientific, Ohama, Nebraska, USA), i-CAP-Q ICP-MS (Thermo Scientific, Walthem, Massachusetts, USA) MP2 peristaltic pump controlled flow 110 µl/min standardized setup.
The ligand 6,6'-bis[N-(isoquinolyl)-1-amino]-2,2'-bipyridine (biqbpy), $[Ru(dmso)_4Cl_2]$, and $[Ru(bpy)_3]Cl_2$ were synthesized according to literature procedures.[1] N-acetyl-L-methionine (HAmet) was purchased from Alfa Aesar. The complexes were synthesized in dim light and stored in the dark in the freezer.

1.1 [Ru(biqbpy)(dmso)Cl]Cl ([1]Cl)

In a 2-necked round-bottom flask $[Ru(dmso)_4Cl_2]$ (86 mg, 0.34 mmol) and biqbpy (71 mg, 0.34 mmol) were added in degassed ethanol (10 mL). The solution was stirred overnight at 80 °C under argon resulting in a dark red suspension. Then, the flask was cooled in an ice bath and the red suspension was filtered over a membrane filter, washed with cold ethanol (3 × 10 mL), diethylether (3 × 10 mL), and hexanes (3 × 10 mL). The complex was obtained as a red brown powder. Yield: 48 mg (43%). *^{1}H NMR (600 MHz, 300 K, δ in D_2O in ppm):* δ 8.66 (dd, *J* = 8.6, 0.9 Hz, 2H, qi-4), 8.37 (d, *J* = 7.9 Hz, 1.0 Hz, 2H, H^3), 8.36 (d, *J* = 7.3 Hz, 2H, qi-10), 8.25 (dd, *J* = 8.4, 7.7 Hz, 2H, H^4), 8.05 (d, *J* = 8.0 Hz, 2H, qi-7), 7.96 (ddd, *J* = 8.1, 7.0, 1.0 Hz, 2H, qi-6), 7.89 (ddd, *J* = 8.4, 7.0, 1.3 Hz, 2H, qi-5), 7.86 (dd, *J* = 8.4, 0.9 Hz, 2H, H^5), 7.60 (d, *J* = 6.7 Hz, 2H, qi-9), 2.41 (s, 6H, H^{α}) ppm; *^{13}C NMR (150 MHz, 300 K, δ in D_2O in ppm):* δ 156.3 (C2), 151.9 (qi-2), 151.7 (C6), 144.3 (qi-10), 140.0 (C4), 137.0 (qi-3), 133.4 (qi-6), 129.8 (qi-5), 128.3 (qi-7), 123.3 (qi-4), 120.6 (qi-8), 119.5 (C3), 118.0 (qi-9), 117.2 (C5), 44.6 (C^{α}) ppm; *UV-vis:* λ_{max} (ε in $M^{-1}.cm^{-1}$) in H_2O: 308 nm (2.3×10^4); *High resolution ES MS m/z (calc):* 310.0465 (310.0464, $[M - Cl]^{2+}$). *Elem. Anal. Calcd. For $C_{30}H_{26}Cl_2N_6O_2RuS,H_2O$:* C, 50.85; H, 3.98; N, 11.86. *Found:* C, 50.85; H, 4.23; N, 11.76.

1.2 [Ru(biqbpy)(HAmet)(Amet)]PF_6 ([2]PF_6)

In a 2-necked round-bottom flask [**1**]Cl (30 mg, 0.043 mmol) and N-acetyl-L-methionine (160 mg, 0.87 mmol) were added in degassed demineralized water (15 mL) under argon. The solution was stirred overnight at 80 °C under argon resulting in a red solution. Solid KPF_6 (2.0 g) was added and the complex was extracted using ethyl acetate. After rotary evaporation at 30 °C the compound was purified using size exclusion chromatography (Sephadex, LH-20 in MeOH). After rotary evaporation at 30 °C the compound was obtained as a red powder. Yield: 20 mg (43%). *^{1}H NMR (400 MHz, 300 K, δ in CD_3OD in ppm):* 8.75 (d, *J* = 7.8 Hz, 1H, qi-4), 8.53 (d, *J* = 8.0 Hz, 1H, H^3), 8.47 (d, *J* = 6.7 Hz, 1H, qi-10), 8.28 (dd, *J* = 8.1, 8.1 Hz, 1H, H^4), 8.07 (dd, *J* = 8.1, 1.6 Hz, 1H, qi-7), 8.02 – 7.85 (m, 3H, qi-6 + H^5 + qi-5), 7.66 (d, *J* = 6.8 Hz, 1H, qi-9), 3.99 (*m*, *J* = 7.8, 3.0 Hz, H^{δ}), 1.76-1.65 (m, 6H, $H^{\gamma b}$ + H^{β}), 1.58 (s, 6H, H^{ζ}), 1.47 -1.41 (*m*, 2H, $H^{\gamma a}$), 1.40 (s, 6H, H^{α}). *^{13}C NMR (100 MHz, 300 K, δ in CD_3OD in ppm):* 173.8 (COOH), 173.0 (CON), 156.9 ($C^{2/6}$), 152.8 ($C^{2/6}$), 152.0 (qi-2), 146.1 (qi-10), 139.4 (C4), 137.7 (qi-3), 133.7 (qi-5/6), 130.5 (qi-5/6), 129.0 (qi-7), 124.1 (qi-4), 121.3 (qi-8), 120.6 (H^3), 118.8 (qi-9), 117.8 (H^5), 51.7 (C^{δ}), 33.2 (C^{β}), 29.9 (C^{γ}), 22.2 (C^{α}), 16.1 (C^{ζ}). *UV-vis:* λ_{max} (ε in $M^{-1}.cm^{-1}$) in H_2O: 363 nm (8.9×10^3). *High resolution ES MS m/z (calc):* 923.1954 (923.1951,

[M]$^{+}$), 462.1008 (462.1012, [M+H]$^{2+}$). *Elem. Anal. Calcd. for* $C_{42}H_{45}F_6N_8O_6PRuS_2$*,3MeOH:* C, 46.43; H, 4.94; N, 9.63 *Found:* C, 46.20; H, 5.05; N, 9.30.

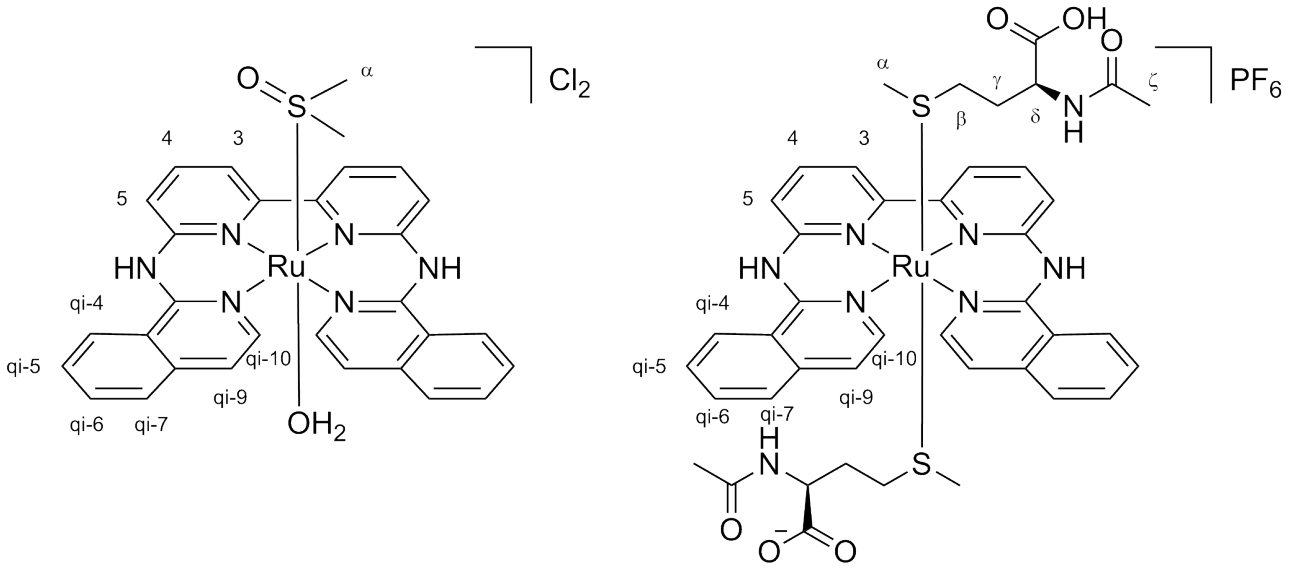


Figure S1. Schematic representation of [Ru(biqbpy)(dmso)(OH_2)]Cl_2 and of [Ru(biqbpy)(HAmet)(Amet)]PF_6 with atom numbering used in NMR attribution.

1.3 Crystal growing.

Single crystals of [**1**]Cl were obtained by recrystallization through liquid-vapor diffusion using MeOH as solvent and EtOAc as counter-solvent. In short, 1 mg of [**1**]Cl was dissolved in 1 mL of MeOH and placed in a small vial. This vial was placed in a larger vial containing 2.8 mL EtOAc. The large vial was closed and vapor diffusion occurred within a few days to afford X-ray quality crystals.

1.3.1 ***Crystal structure determination***

For X-ray diffraction all reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Cu *K*α radiation (λ= 1.54178 Å) under the program CrysAlisPro (Version 1.171.36.32 Agilent Technologies, 2013). The program CrysAlisPro (Version 1.171.36.32 Agilent Technologies, 2013) was used to refine the cell dimensions. Data reduction was done using the program CrysAlisPro (Version 1.171.36.32 Agilent Technologies, 2013). The structure was solved with the program SHELXS-2013 (Sheldrick, 2013) and was refined on F^2 with SHELXL-2013 (Sheldrick, 2013). Analytical numeric absorption corrections based on a multifaceted crystal model were applied using CrysAlisPro (Version 1.171.36.32 Agilent Technologies, 2013). The temperature of the data collection was controlled using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions using the instructions AFIX 43 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 times *U*eq of the attached C atoms. The H atoms attached to N2, N5 and O1S were found from difference Fourier maps, and their coordinates and isotropic temperature factors were refined freely.

1.3.2 ***Details of the crystal structure.***

The X-ray crystal structure is ordered. [**1**]Cl**:** Fw = 722.64, orange plate, 0.23 × 0.15 × 0.04 mm^3, triclinic, *P*-1 (no. 2), a = 9.5443(3), b = 12.2675(3), c = 13.2983(4) Å, α = 68.395(3), β = 89.878(2), γ = 84.475(2)°, V = 1439.98(8) Å^3, Z = 2, D_x = 1.667 g cm^{-3}, μ = 7.139 mm^{-1}, abs. corr. Range: 0.309–0.765. 22804 Reflections were measured up to a resolution of (sin $\theta/\lambda)_{max}$ = 0.62 Å^{-1}. 5599

Reflections were unique (R_{int} = 0.0365), of which 5286 were observed [$I > 2\sigma(I)$]. 403 Parameters were refined using 3 restraints. $R1/wR2$ [$I > 2\sigma(I)$]: 0.0283/0.0743. $R1/wR2$ [all refl.]: 0.0301/0.0760. S= 1.043. Residual electron density found between −0.60 and 0.98 e $Å^{-3}$.

2 (Photo)Stability of [1]Cl and [2]PF_6

2.1 Overview

The (photo)stability studies were used to determine the impact of various ionic strengths on the complexes in the light and dark. In Figure S2 an outline and references are given.

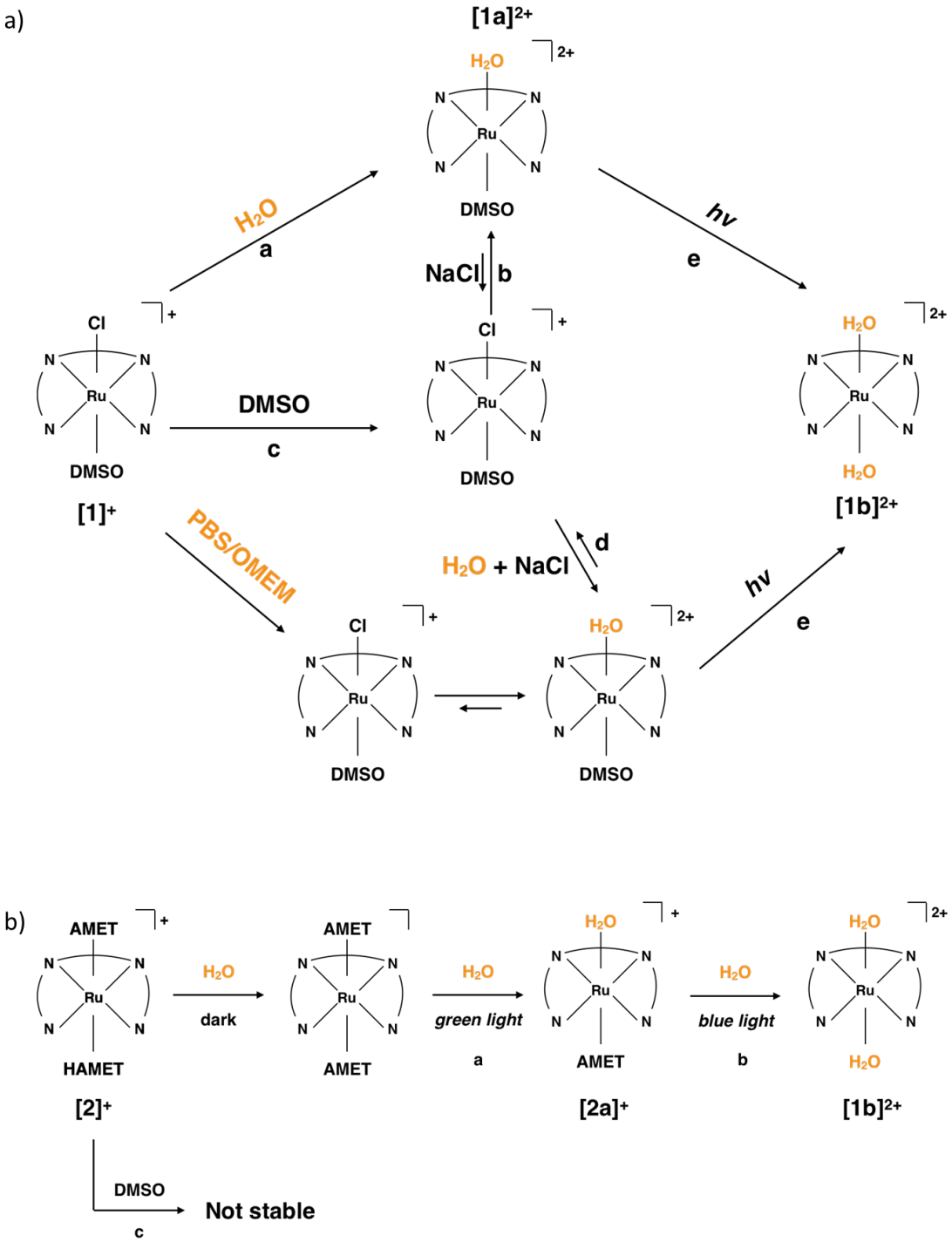


Figure S2. An overview of the stability for complex [**1**]Cl (a) and complex [**2**]PF_6 (b) in various solvent systems. The letters in each figure reference the experiment. For complex [**1**]Cl: a) Dissolving [**1**]Cl in H_2O yields in immediate ligand exchange (Figure S3). b) Concentration dependent back coordination to $[\mathbf{1}]^+$ can be observed under addition of Cl ions (Figure S3). c) The Ru-Cl bond in $[\mathbf{1}]^+$ is stable in DMSO-d_6 (Figure S5). d) Adding water or brine to a DMSO-d_6 solution (Figure S7) of $[\mathbf{1}]^+$ yields a mixture of the mono aqua complex $[\mathbf{1a}]^{2+}$ and $[\mathbf{1}]^+$. e) Photoinduced excitation results in ligand expulsion, and thus in the formation of $[\mathbf{1b}]^{2+}$ (Figure 2 and S8). For complex [**2**]PF_6: a) Green-light induced excitation results in ligand expulsion of one $Amet^-$ ligand (Figure S17). b) Extensive blue-light irradiation of [**2**]PF_6 leads to the formation of $[\mathbf{1b}]^{2+}$ (Figure S12). c) Dissolving [**2**]PF_6 in DMSO-d_6 leads to thermal decomposition (Figure S6).

2.2 Behavior of [1]Cl and [2]PF_6 in aqueous solution in the dark

2.2.1 ***In D_2O under increasing chloride concentrations: Indirect proof of immediate hydrolysis***

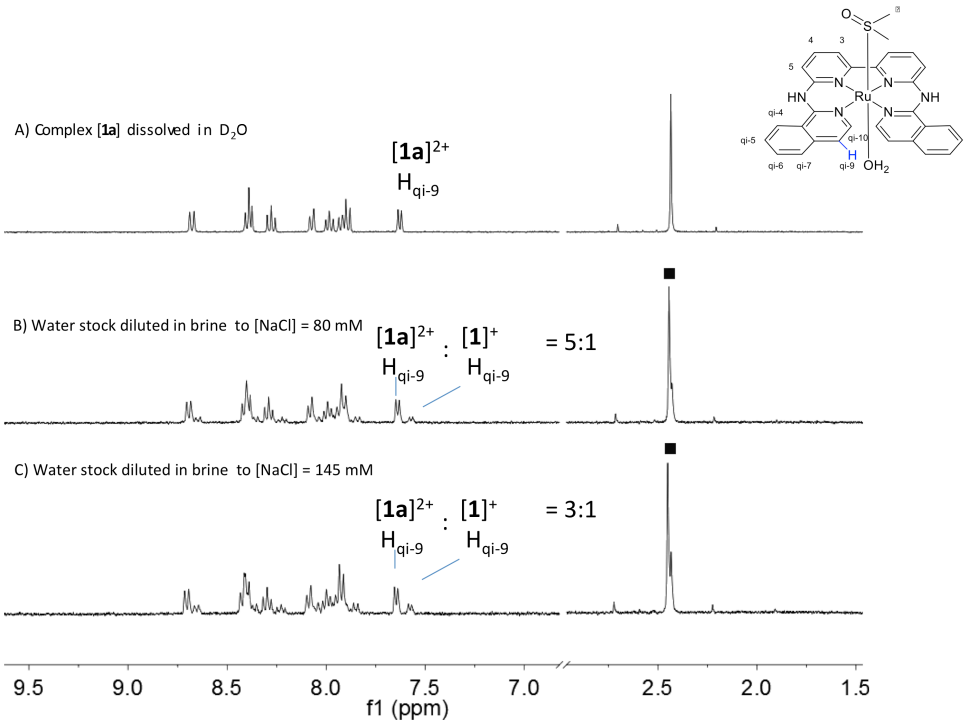


Figure S3. Evolution of the ^{1}H NMR spectra (400 MHz, D_2O, region 9.5-7.0 and 3.5-1.5 ppm) of [**1a**]$^{2+}$ under addition of NaCl at 298 K in D_2O. The initial spectrum (at [NaCl] = 0 M) is that of [**1a**]$^{2+}$, the spectrum at [NaCl] = 0.08 M and [NaCl] = 0.15 M a mixture of [**1a**]$^{2+}$ and [**1**]$^{+}$. Notations: methyl group of dmso in [Ru(biqbpy)(dmso)(D_2O)]$^{2+}$ (■), splitting of ■ indicates partial formation of [Ru(biqbpy)(dmso)Cl]$^{+}$. $[Ru]_0$ = 0.5 mM.

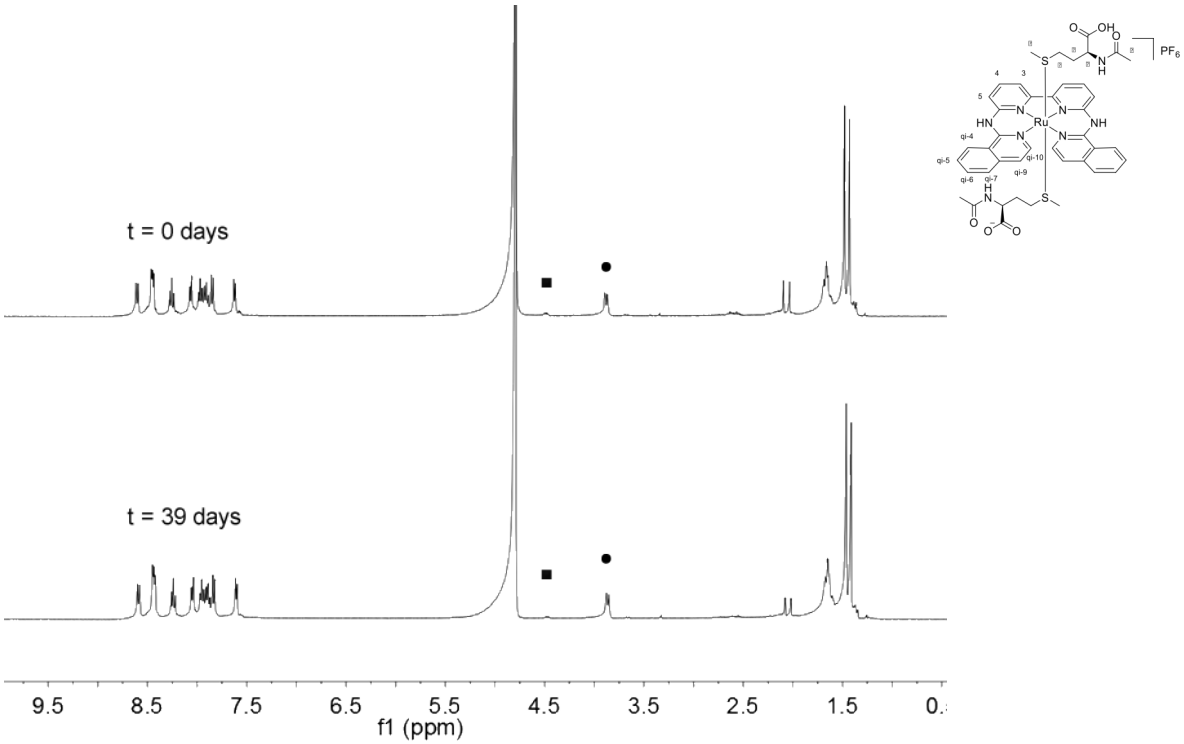


Figure S4. Stability of the ^{1}H NMR spectra (400 MHz, D_2O, region 9.0 – 1.0 ppm) of a solution of [**2**]PF_6 in D_2O under argon. Notations: (■) characteristic chiral proton (δ) of free Amet$^-$, and (●) characteristic chiral proton (δ) of ruthenium-coordinated Amet$^-$ in [**2a**] (see Scheme 2). $[Ru]_0$= 0.38 mM

2.2.2 *In pure DMSO for storage of stock solutions (-20 °C, dark)*

For biological experiments diluted solutions of [**1**]Cl were prepared from a DMSO stock solution by adding aqueous buffers (i.e. phosphate buffer saline, phosphate buffer, or cell culture media). The stability of DMSO-d6 solution of [**1**]Cl is shown in Figure S5. Stock-solutions of this complex could be stored at −20 °C and in the dark for up to 9 month. [**2**]PF6 was unstable in DMSO-d6 over the course of 16 h and DMSO stock solutions of this compound must be prepared freshly.

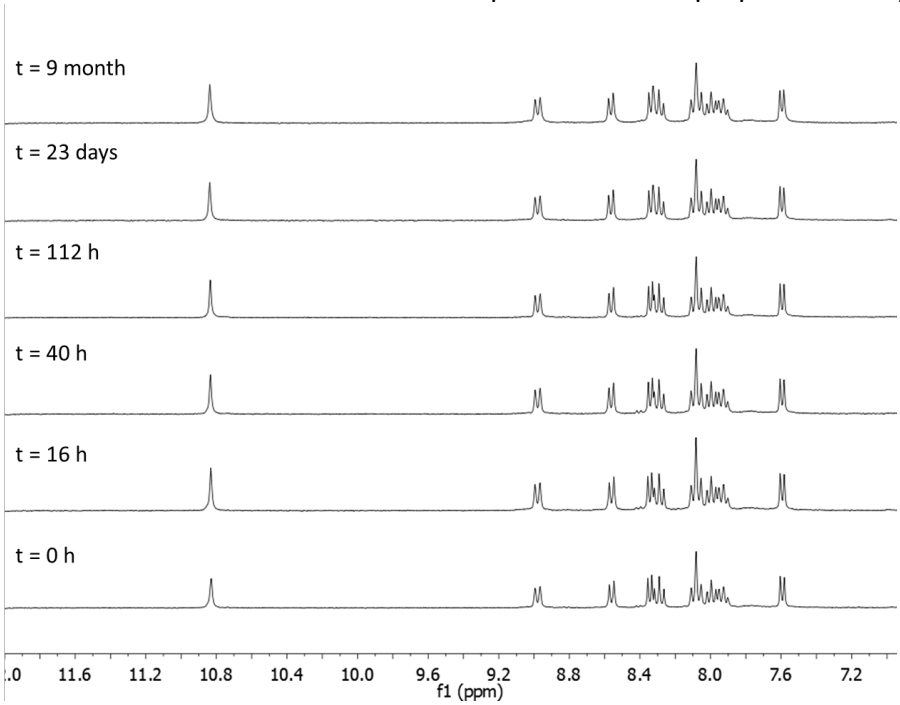


Figure S5. Time evolution of the ^{1}H NMR spectra (300 MHz, region 12 - 7 ppm) of [**1**]Cl (2.5 mM) in DMSO-d6.

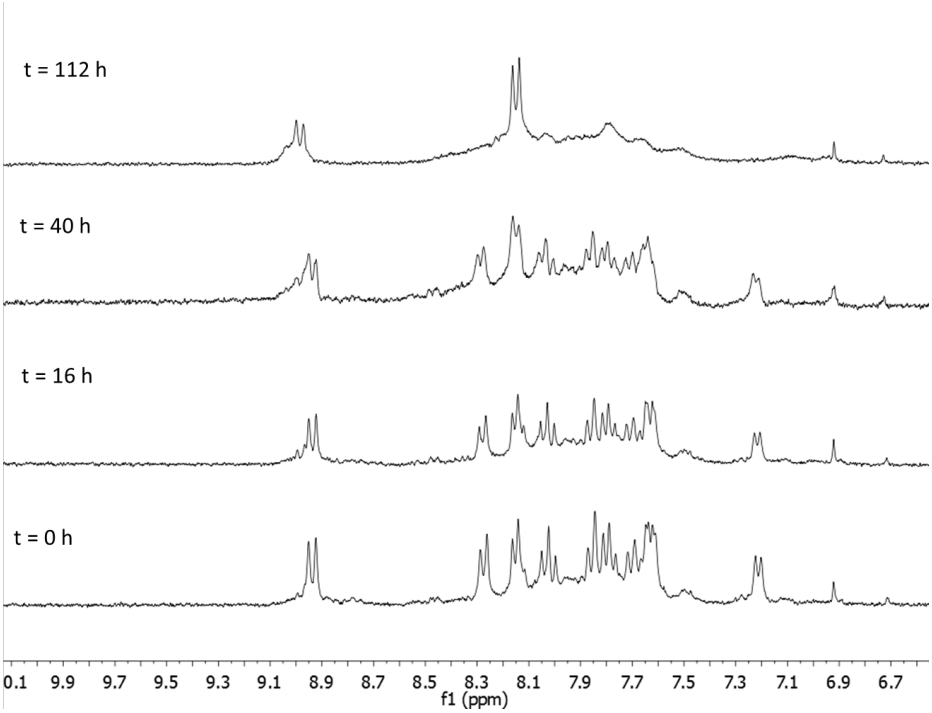


Figure S6. Time evolution of the ^{1}H NMR spectra (300 MHz, region 10.1 – 6.5 ppm) of [**2**]PF_6 (1.5 mM) in DMSO-d6.

2.2.3 *Stability of [1]Cl in D_2O and deuterated brine ([NaCl] = 110 mM).*

^{1}H NMR studies starting from DMSO stock solution of [**1**]Cl in D_2O and D_2O-based brine were done to predict the relevant ionic species of [**1**]Cl in cell culture media ([NaCl] = 110 mM) and inside the cells [NaCl] = 10 mM. As shown in Figure S7 the cells are actually treated with a mixture of $[\mathbf{1}]^+$ and $[\mathbf{1a}]^{2+}$. The active species inside the cells will be most likely $[\mathbf{1a}]^{2+}$ due to the lower chloride concentration.

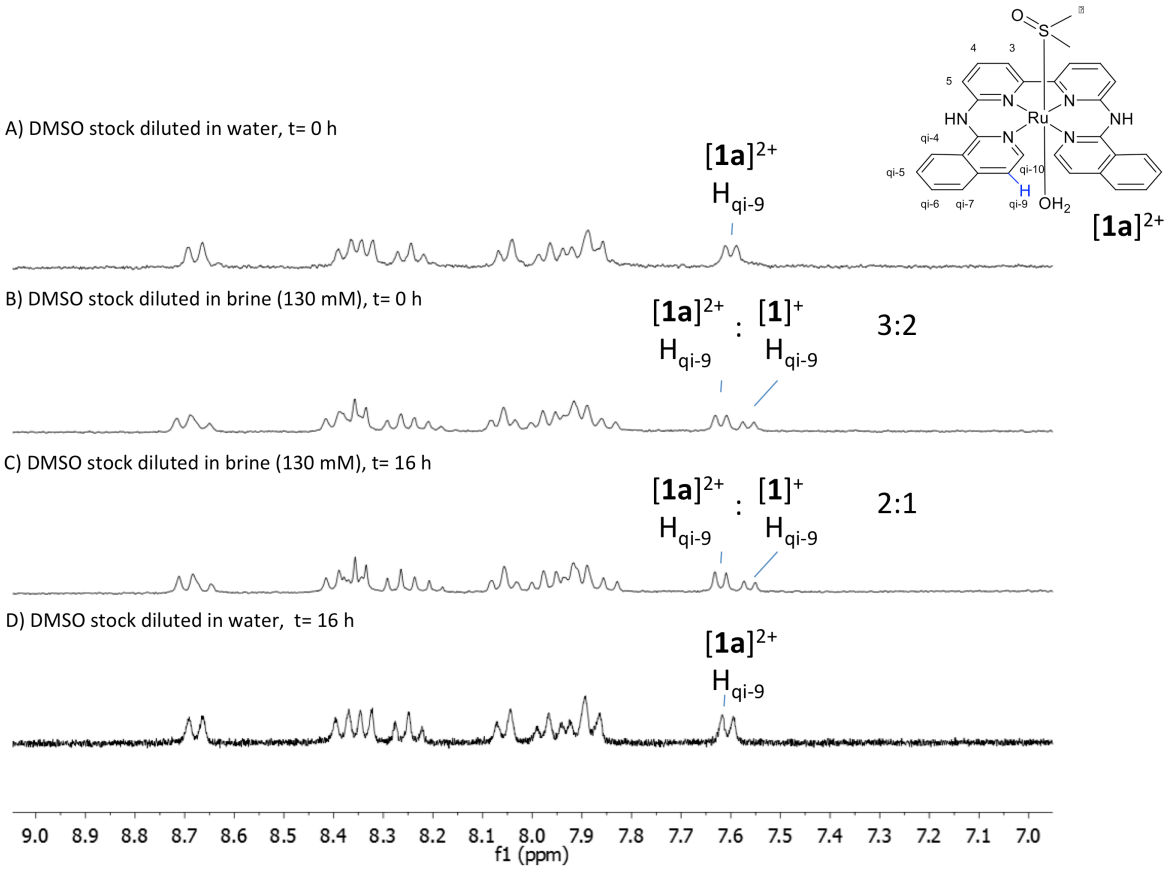


Figure S7. Evolution of the ^{1}H NMR spectra (300 MHz, D_2O, region 9.0 – 7.0 ppm) of $[\mathbf{1}]^+$ (130 µM) disolved in DMSO and diluted with D_2O and D_2O-based brine vs. time.

2.3 Photoreaction of [1]Cl and [2]PF_6 in aqueous solutions

2.3.1 ***Irradiation setup***

The UV-vis spectroscopy study of Figure 2 was performed using a UV-vis spectrometer equipped with temperature control set to 298 K and a magnetic stirrer. The measurements were performed in a quartz cuvette, containing 3 mL of solution (10^{-5} M). The stirred sample was degassed by argon, irradiated perpendicularly to the axis of the spectrometer with the beam of a green (λ = 530 nm, $\Delta\lambda_{1/2}$ = 25 nm, 3.02 mW, 2.1×10^{-8} Einstein.s^{-1}) or blue LED (450 nm, $\Delta\lambda_{1/2}$= 25 nm, 0.653 mW, 8.53×10^{-9} Einstein.s^{-1}) fitted to the top of the cuvette, and an absorption spectrum was measured at regular time intervals and analyzed using Microsoft Excel. Mass spectrometry was performed at the beginning and at the end of the irradiation to confirm the nature of the reagent and products.

2.3.2 ***Evolution of absorbance spectra of [1]$^+$ under blue vs. green light irradiation***

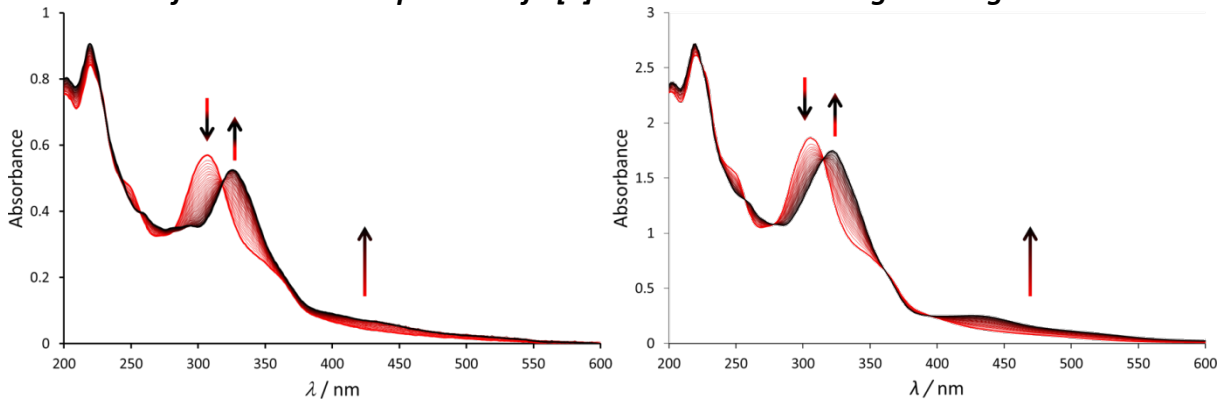


Figure S8. Evolution of the UV-vis spectrum of a solution of [**1**]Cl in demi water, thus of [**1a**]$^+$, upon blue (left) or green light (right) irradiation under argon. Conditions $[Ru]_0 = 2.5 \times 10^{-5}$ (left), 7.5×10^{-5} (right), irradiated volume 3.0 mL, 298 K. Light source: λ = 450 nm, $\Delta\lambda_{1/2}$ = 25 nm, 0.65 mW, 8.53×10^{-9} Einstein.s^{-1} for blue light, λ = 530 nm, $\Delta\lambda_{1/2}$= 25 nm, 3.02 mW, 2.1×10^{-8} Einstein.s^{-1} for green light.

2.3.3 ***Evolution of absorbance spectra of [2]PF_6 under blue vs. green light irradiation***

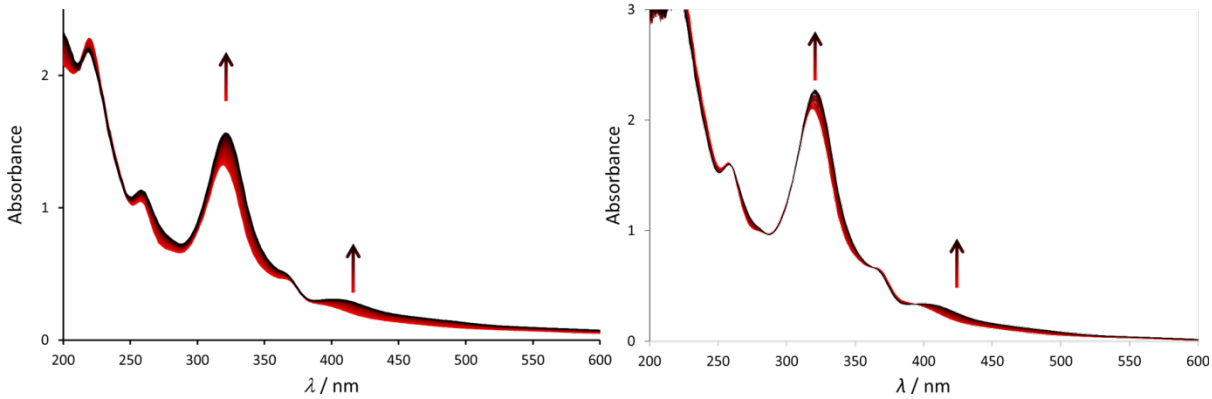


Figure S9. Evolution of the UV-vis spectrum of a solution of [**2**]PF_6 in demi water upon blue (left) or green light (right) irradiation under argon. Conditions $[Ru]_{0=}$ 4.8×10^{-5} (left), 7.8×10^{-5} (right) irradiated volume 3.0 mL, 298 K. Light source: λ= 450 nm, $\Delta\lambda_{1/2}$ = 25 nm, 0.65 mW, 8.53×10^{-9} Einstein.s^{-1} for blue light, λ=530 nm, $\Delta\lambda_{1/2}$= 25 nm, 3.02 mW, 2.1×10^{-8} Einstein.s^{-1} for green light.

2.3.4 *Evaluation by HPLC traces of a solution of [1]Cl irradiated with blue light and dark control*

Experimental condition as given in 2.3.1 were used.

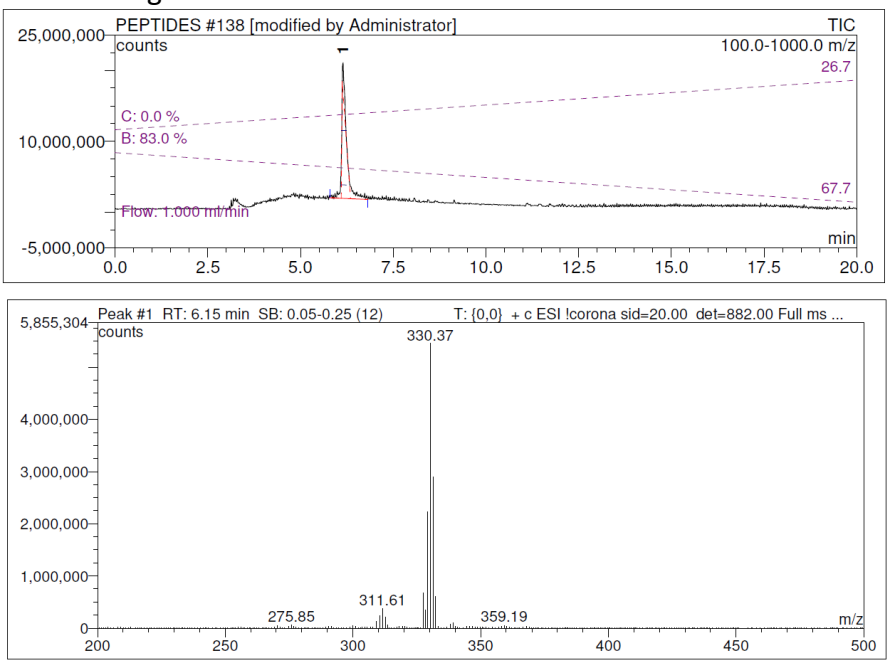


Figure S10. HPLC trace of $[Ru(biqbpy)(dmso)(MeCN)]^{2+}$ with m/z = 330.4 (calc. m/z = 330.6) at 6.15 min after placement in the dark under the same conditions as Figure S8.(MeCN was used as eluent on the HPLC column).

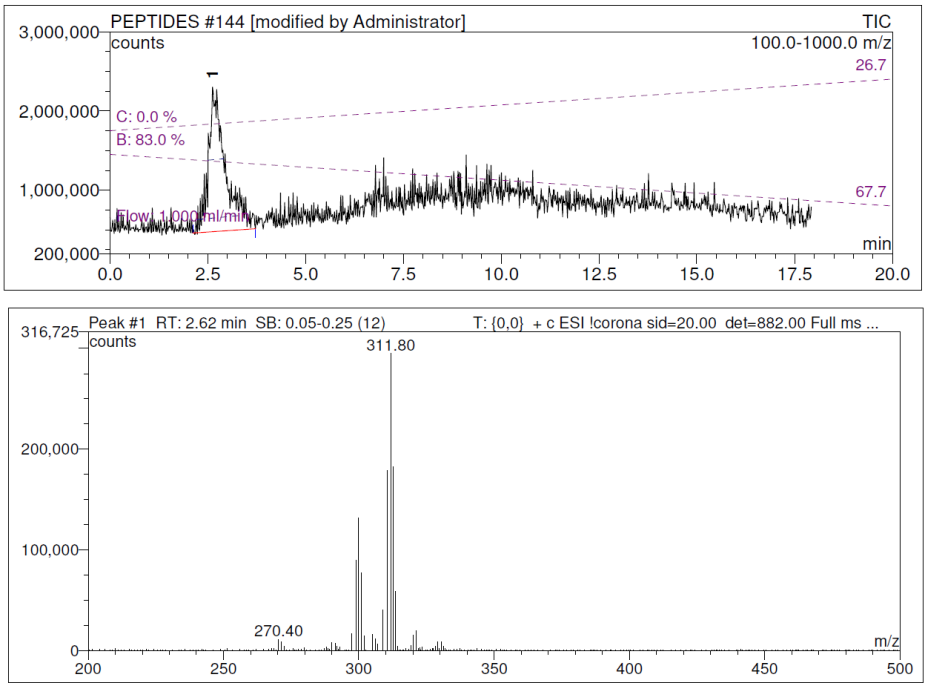


Figure S11. HPLC trace of $[Ru(biqbpy)(MeCN)_2]^{2+}$ with m/z = 311.8 (calc. m/z = 312.1) at 2.62 min after blue light irradiation under the same conditions as Figure S8. MeCN was used as eluent on the HPLC column. Absence of other traces and dmso-bound complexes in this spectrum demonstrates that the photosubstitution of $[\mathbf{1a}]^{2+}$ to $[\mathbf{1b}]^{2+}$ has gone to full conversion in this experiment.

2.3.5 *Evaluation by HPLC traces of a solution of [2]PF_6 irradiated with blue light and dark control*

Experimental condition as given under 2.3.1 were used.

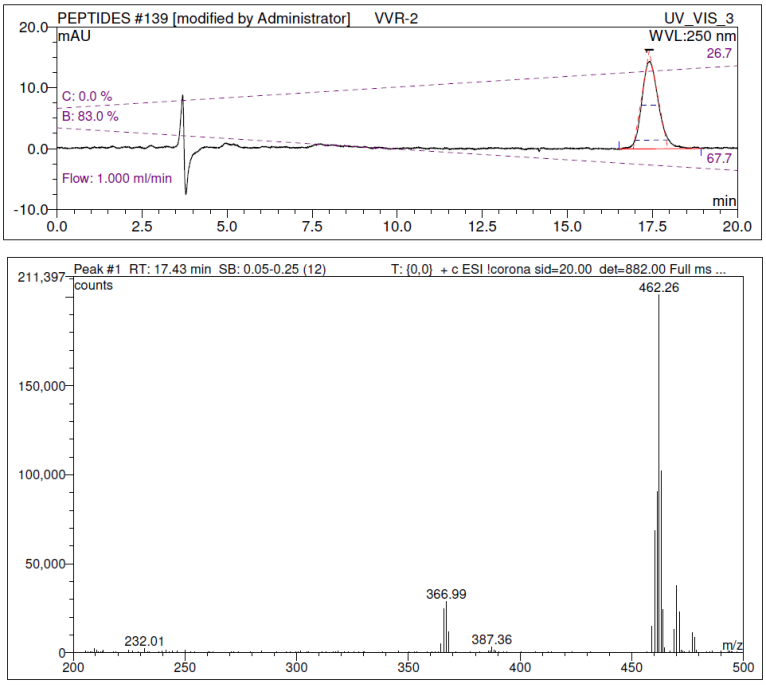


Figure S12. HPLC trace of [**2**]PF_6 kept in the dark under the same conditions as Figure S9. The species at 17.53 min gives a peak at m/z = 462.0 corresponding to $[Ru(biqbpy)(HAmet)_2]^{2+}$ (calc. m/z = 462.1)

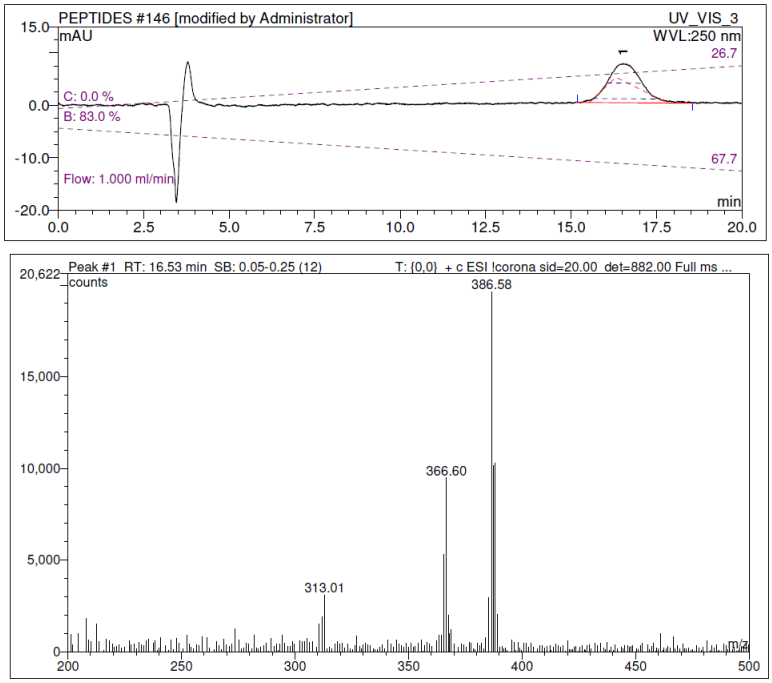


Figure S13. HPLC trace of [**2**]PF_6 after blue light irradiation under the same conditions as Figure S8. The peak at 2.62 min is characterized by m/z = 386.6 corresponding to $[Ru(biqbpy)(MeCN)_2]^{2+}$ (calc. m/z = 387.11). MeCN was used as eluent on the HPLC column). Absence of other peaks on the chromatogram, and of any peak for $[Ru(biqbpy)(HAmet)_2]^{2+}$ at m/z = 462.0 in the mass spectrum (as shown in Figure S12) demonstrates that the photosubstitution of $[\mathbf{2a}]^0$ to $[\mathbf{1b}]^{2+}$ (Scheme 2) as gone to full conversion in such conditions.

2.3.6 *Green light irradiation in the cell irradiation setup*

2.3.6.1 Followed by UV-vis

The photochemical reactivity of [**1**]Cl (38 µM) and [**2**]PF_6 (88 µM) in 96-well plates was measured using UV-vis spectroscopy by dissolving each compound in Opti-MEM complete and by irradiating the plate with green light (520 nm, 75 $J.cm^{-2}$) using the green LED source described in details in Hopkins et al[2] to mimic the conditions used in the photocytotoxicity assay. Figure S14-S15 show that the absorbance change at 315 nm for [**1**]Cl and 320 nm for [**2**]PF_6 after 60 min irradiation with green light are plateauing for [**1**]Cl, *i.e.*, that no further activation occurs after 1 h irradiation, and is significantly activated for [**2**]PF_6. Since further activation for [**2**]PF_6 would become experimentally impractical, 60 minutes was chosen as the green light irradiation time in the photocytotoxity assay.

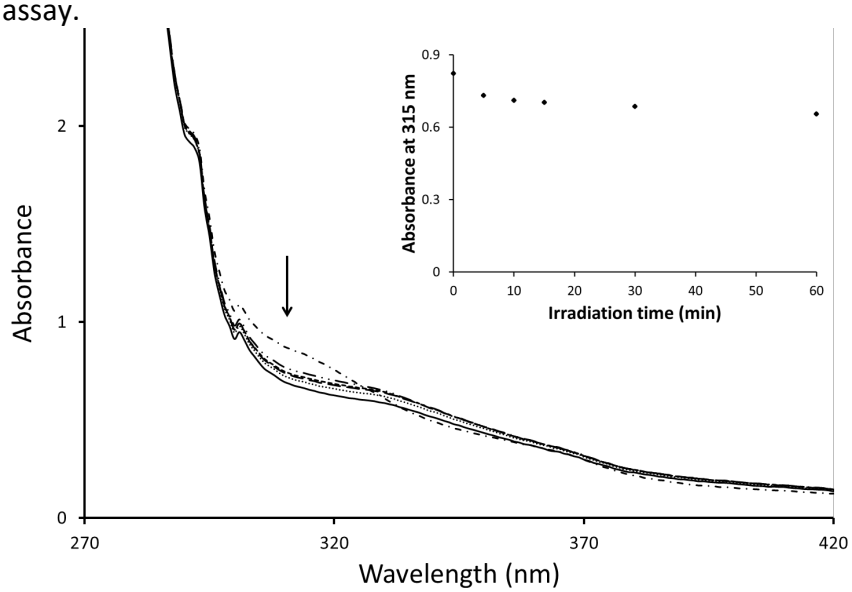


Figure S14. Evolution of the UV-vis spectrum of a well in a 96-well plate containing compound [**1**]Cl (38 µM) in the medium Opti-MEM complete under green light irradiation (37 °C) at 0 min (- · -), 5 min (—··), 10 min (— —), 15 min (- - -), 30 min (···), and 60 min (—).

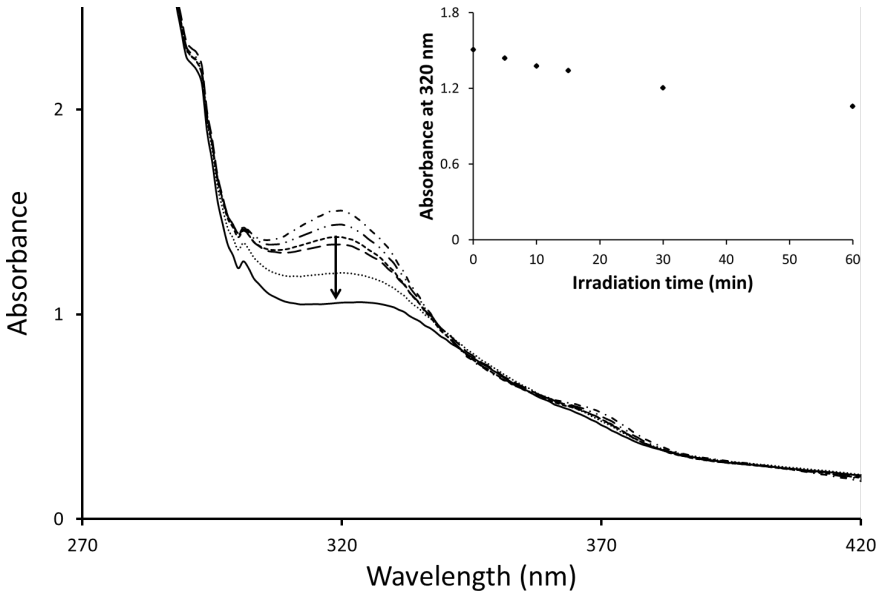


Figure S15. Evolution of the UV-vis spectrum of a well of a 96-well plate containing compound [**2**]PF_6 (88 µM) in the medium Opti-MEM complete under green light irradiation (37 °C) at 0 min (- · -), 5 min (—··), 10 min (— —), 15 min (- - -), 30 min (···), and 60 min (—).

2.3.6.2 Followed by ^{1}H NMR

Green light irradiation experiments in D_2O were performed in a 96-well plate with the LED source described in details in Hopkins et al[2] for the photocytotoxicity assay. Three adjacent wells per compound were loaded with 200 µL of a 38 µM solution of [**1**]Cl or [**2**]PF_6, and irradiated for 60 min (520 ±20 nm, 75 J.cm^{-2}) at 37 °C, while control wells were kept in the dark at the same temperature. For each condition the content of the 3 wells was mixed, and ^{1}H NMR spectroscopy was performed on the 4 samples (2 compounds, dark or light). As shown in Figure S16, for [**1**]Cl clear release of free dmso is observed (new peak at 2.71 ppm) and a complete reorganization of the aromatic peaks indicate that full conversion to [**1b**]$^{2+}$ is achieved in such conditions. For [**2**]PF_6 (Figure S17) the photosubstitution is demonstrated by the formation of the characteristic peak of the chiral proton of free HAmet at 4.5 ppm (square). The remaining peak at 3.7 ppm indicated by a circle represents the chiral proton of the coordinated HAmet. Photosubstitution under the irradiation conditions used for cell testing (1 h green light) does not lead to photosubstitution of the second Amet$^-$ ligand, *i.e.*, the photoproduct is [**2b**]$^+$ (Scheme 2), which is in line with our other photochemical experimental data under green light (only ~1 eq. HAmet is photosubstituted).

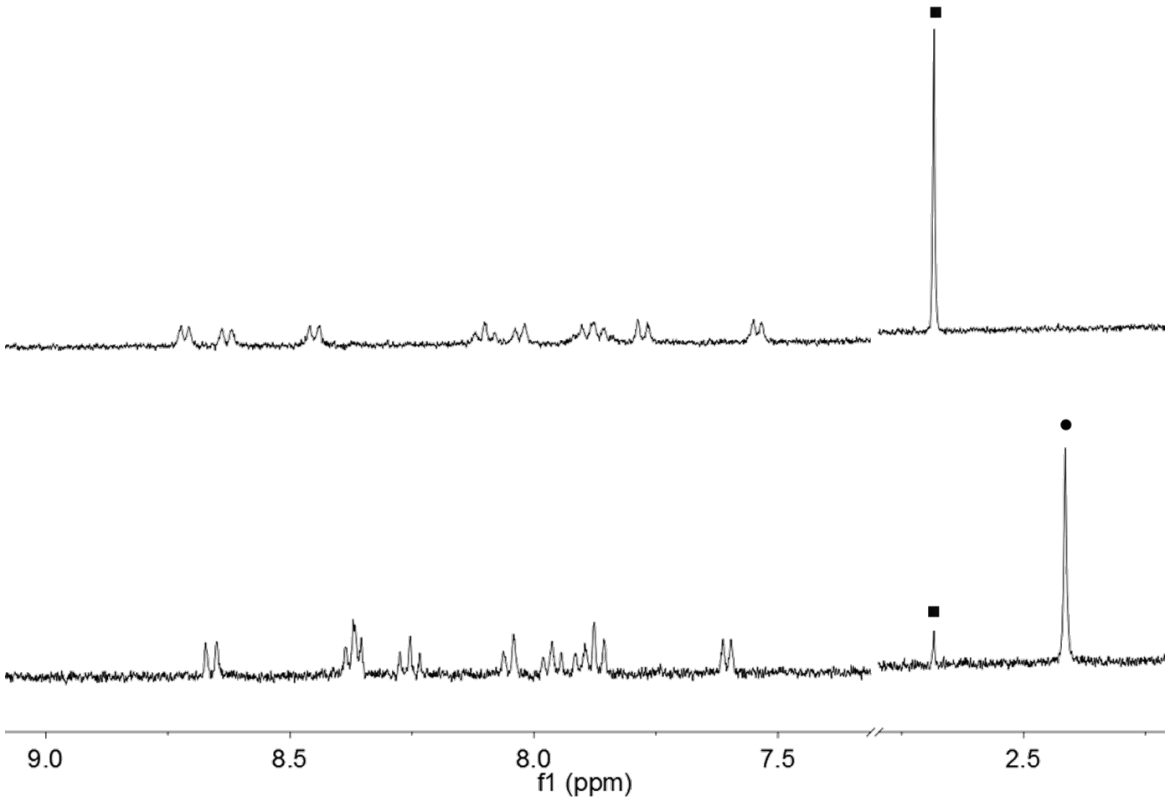


Figure S16. ^{1}H NMR spectra (400 MHz, region 9.0–7.5 and 3.0–2.0 ppm) of a solution of [**1**]Cl in D_2O that has been irradiated by green light (top) and stored in the dark (bottom) at 310 K in a 96-well plate. Notations: free dmso (CH_3) (■), sulfur-coordinated dmso (CH_3) in [**1**]Cl (●). $[Ru]_0$ = 38 µM. Light source: green LED array, 520 nm, 75 J.cm^{-2}.

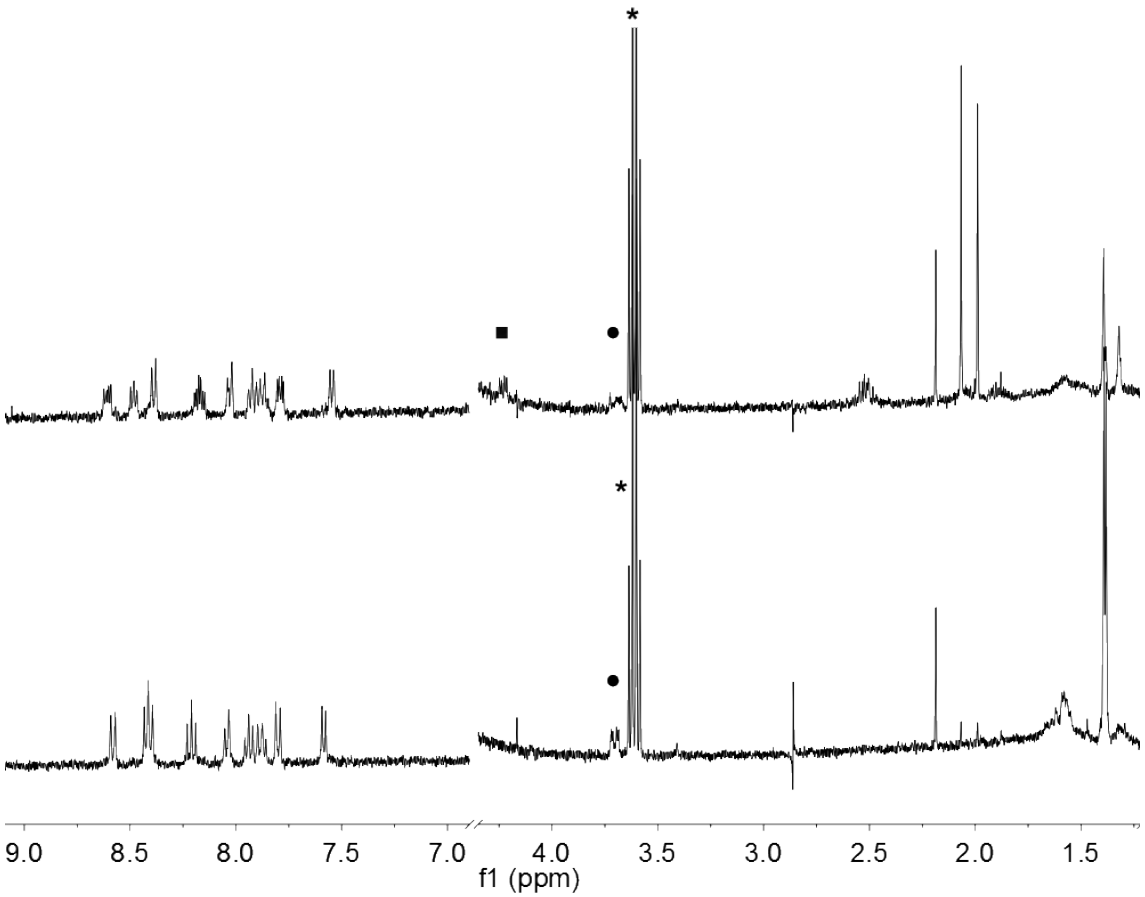


Figure S17. ^{1}H NMR spectra (400 MHz, D_2O, region 9.0–7.0 and 4.5–1.0 ppm) of a solution of [**2**]PF_6 in D_2O that has been irradiated by green light (top) or stored in the dark (bottom) at 310 K in a 96-well plate. Notations: (■) characteristic chiral proton (δ) of free Amet$^-$, (●) characteristic chiral proton (δ) of ruthenium-coordinated Amet$^-$, and ethanol originating from working in ethanol sterilized environment for cell experiments (*). $[Ru]_0$ = 87 µM. Light source: green LED array, 520 nm, 75 J.cm^{-2}.

3 Cell culturing and EC_{50} (photo)cytotoxicity assay

3.1 Cell Culturing

Cells were thawed and at least passaged twice before starting photocytotoxicity experiments. Each cell line was cultured in Dulbecco's Modified Eagle Medium with phenol red, supplemented with 8.0% v/v fetal calf serum (FCS), 0.2% v/v penicillin/streptomycin (P/S), and 0.9% v/v Glutamine-S (GM). Cells were cultured in either 25 cm^2 or 75 cm^2 flasks and split at 70-80% confluence (three times per week for 25 cm^2 flasks, once per week for 75 cm^2 flasks). The flasks were incubated at 37 °C at 7.0% CO_2. The medium was refreshed three times a week. Cells used in all biological experiments were cultured for a maximum of 8 weeks.

3.2 Cell irradiation setup

The cell irradiation system consists of a Ditabis thermostat (980923001) fitted with two flat-bottom microplate thermoblocks (800010600) and a 96-LED array fitted to a standard 96-well plate. The 520 nm LED (OVL-3324), fans (40 mm, 24 V DC, 9714839), and power supply (EA-PS 2042-06B) were ordered from Farnell. Full description of the cell irradiation setup is given in Hopkins et al.[2] Human cancer cell lines (A431, human epidermoid carcinoma; A549, human lung carcinoma; MRC-5, fetal lung fibroblasts) were distributed by the European Collection of Cell Cultures (ECACC), and purchased through Sigma Aldrich. Dulbecco's Minimal Essential Medium (DMEM, with and without phenol red, without glutamine), 200 mM Glutamine-S (GM), trichloroacetic acid (TCA), glacial acetic acid, sulforhodamine B (SRB), tris(hydroxylmethyl)aminomethane (tris base), 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein disodium salt (rose bengal), and *cis*-diamineplatinum(II) dichloride (cisplatin), were purchased from Sigma Aldrich. (2*R*,3*R*,4*R*,5*R*)-hexan-1,2,3,4,5,6-hexol (D-mannitol) was purchased from Santa Cruz Biotechnology via Bio-Connect. Fetal calf serum (FCS) was purchased from Hyclone. Penicillin and streptomycin were purchased from Duchefa and were diluted to a 100 mg/mL penicillin/streptomycin solution (P/S). Trypsin and Opti-MEM® (without phenol red) were purchased from Gibco® Life Technologies. Trypan blue (0.4% in 0.81% sodium chloride and 0.06% potassium phosphate dibasic solution) was purchased from BioRad. Plastic disposable flasks and 96-well plates were from Sarstedt. Cells were counted using a BioRad TC10 automated cell counter with BioRad Cell Counting Slides. UV-vis measurements for analysis of 96-well plates were performed on a M1000 Tecan Reader. Cells were inspected with an Olympus IX81 microscope. Cells were stained for fluorescence-assisted-cell-sorting (FACS) using Annexin V-FITC purchased from Bioconnect and propidium iodide purchased from Sigma Aldrich and analyzed by a Cell Lab Quanta SC flow cytometer from Beckman Coulter.

3.3 Optimized assay for the determination of photocytotoxicity.

The photocytotoxicity of A431 (human epidermoid carcinoma) and A549 (human lung carcinoma) cell lines was assessed using an assay adapted from Hopkins et al. and shortly described above.[2] Cell cultures with a maximum confluence of 70-80% were trypsinized and centrifuged (1.5 min, 1.2 relative centrifugal force), trypsin and DMEM complete were removed, and the cells were re-suspended using Opti-MEM supplemented with 2.4% v/v FCS, 0.2% v/v P/S, and 1.0% v/v GM, (hereafter called Opti-MEM complete). 10 µL of cell suspension and 10 µL of trypan blue were mixed and pipetted into a cell counting slide, and cells were counted using a BioRad TC10 automated cell counter. The cell suspension was diluted to the appropriate seeding density (A431, 8×10^3 cells/well; A549, 5×10^3; MRC-5, 6×10^3 cells/well) and seeded in the wells of a 96-well plate. Cisplatin positive control solution was prepared from a stock solution based on clinical formulation (3.3 mM cisplatin, 55 mM mannitol, 154 mM NaCl).[3] Sterilized dimethylsulfoxyde

(DMSO) was used to dissolve [**1**]Cl and [**2**]PF_6 in such amounts that the maximum v/v% of DMSO per well did not exceed 0.5% v/v%.

The complete photocytotoxicity experiment lasted 96 h: cells were seeded at t = 0 h, treated at t = 24 h, irradiated at t = 30 h or t = 48 h, and fixed at t = 96 h. For every irradiated plate a parallel control plate was prepared and treated identically to the irradiated plate, but without irradiation. 96-well plates were seeded with the correct amount of cells in 100 µL Opti-MEM complete per well. To prevent border effects cells were only seeded in the inner 60 wells as shown in Figure S18 (unseeded cells indicated by blue). Border wells B12-F12 (Figure S18, grey, pink) were seeded with cells as qualitative positive controls. In the remaining outer wells 200 µL of Opti-MEM complete was pipetted.

The cells were incubated in the dark for 24 h at 37 °C at 7.0% CO_2. At t = 24 h the cells were treated with freshly prepared solutions of [**1**]Cl and [**2**]PF_6 dissolved in Opti-MEM complete. Different concentrations of [**1**]Cl (*dark conditions*: 0.4, 1.9, 4.0, 7.5, 19, and 38 µM; *light conditions*: 0.13, 0.63, 1.25, 2.5, 6.25, and 12.5 µM) and [**2**]PF_6 (*light and dark conditions*: 0.6, 3.1, 6.3, 13, 31, 63 µM; concentrations were increased after first assay to ensure 0% cell death: 0.9, 4.4, 8.8, 18, 44, 88 µM) were prepared and of each concentration 100 µL was added to three wells (B3:G11, Figure S18, red). Six wells (B2:G2, Figure S18 green) were treated with Opti-MEM complete as cell growth control without ruthenium compound, three wells (B12:D12, Figure S18, grey) were treated with the positive dark control compound cisplatin (3.3 µM), and three wells (E12:G12, Figure S18, pink) were treated with positive green light control compound rose bengal (5.0 µM). At t = 30 h or t = 48 h the 96-well plates were taken out of the incubator and were irradiated using the 96-well LED 520 nm array system for 1 h (dose: 75 $J.cm^{-2}$). During irradiation the temperature of the wells was controlled at 35-37 °C by the thermoblock preheated to 39 °C coupled to LED cooling with a fan.[2] After irradiation all 96-well plates were placed back into a standard (dark) incubator for the remainder of the experiment, until the cells were fixated by addition of 100 µL trichloroacetic acid per well (TCA, 10% w/v).

The plates were stored at 4 °C for at least 4 h as part of the sulforhodamine B (SRB) assay that was adapted from Vichai at al.[4] In short, after fixation the TCA medium mixture was removed, rinsed with demineralized water three times, and air dried. Then, each well was stained with 100 µL SRB solution (0.6% w/v SRB in 1% v/v acetic acid) for 30 min, the SRB solution was removed, and washed with acetic acid (1% v/v) until no SRB came off, normally requiring 3-5 times. Once air dry, 200 µL of tris base (tromethamine, 10 mM) was pipetted to each well. To determine the cell viability the absorbance at 510 nm was measured using a M1000 Tecan Reader. To make sure all the SRB was dissolved, this measurement was performed at least 30 minutes after addition of tris base. The SRB absorbance data per compound per concentration were averaged over three identical wells (technical replicates, $n_t = 3$) in Excel and made suitable for use in GraphPad Prism. Relative cell populations were derived from the average of the untreated controls ($n_t = 6$). In any case it was checked that the cell viability of the control cells of the samples irradiated without compound were similar (maximum difference of 10%) to the unirradiated samples without ruthenium compound to make sure no harm was done by the light (Figure S19). The data from three independent biological replications were used to obtain the dose-response curves shown in Figure S20-24 and the EC_{50} table (Table 1) using non-linear regression of hills-slope equation with a fixed Y maximum (100%) and minimum (0%) relative cell population values.[2] Photo indices (PI) reported in Table 1 were calculated, for each cell type, incubation time, and light dose, by dividing the EC_{50} value obtained in the dark by the EC_{50} value determined under light irradiation.

3.4 96-well plate layout used in photocytotoxicity assay

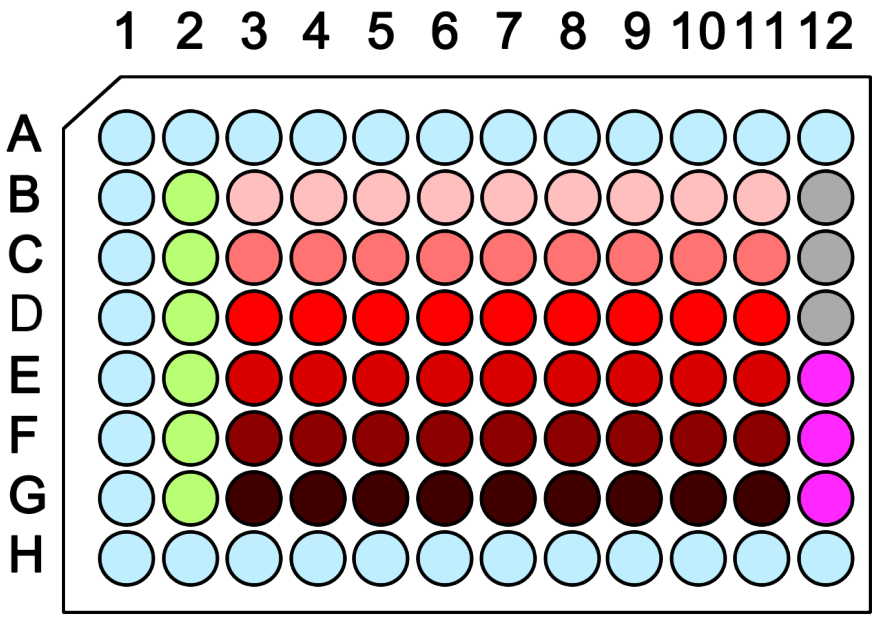


Figure S18. Layout of a 96-well plate used in the photocytotoxicity assay. All the wells are seeded with cells apart from the outer 36 blue wells that are filled with 200 µL Opti-MEM complete. The wells in green (B2:G2) are the untreated control cells, the wells colored by a different intensity of red (B3:B11), are cells treated with various concentration of the compound. The grey wells (B12:D12) are wells treated with the positive dark control cisplatin (3.3 µM). The pink wells (E12:G12) are wells treated with the positive light control Rose Bengal (5.0 µM).

3.5 Effect of green light irradiation on control cells

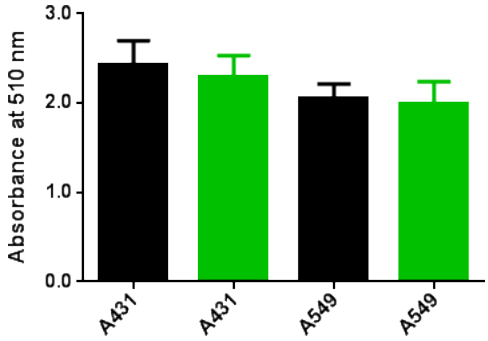


Figure S19. Average absorbance at 510 nm (with standard deviation) of control cells used in photocytotoxicity assay for A431 and A549 cells kept in the dark (black bar) or irradiated with green light (520 nm, 60 min, 75 $J.cm^{-2}$) indicated with a green bar. Phototoxicity assay outline: cells seeded at 8×10^3 (A431) or 5×10^3 (A549) cells/well at t = 0 h, treated with Opti-MEM complete at t = 24 h, irradiated at t =30 h or 48 h, and SRB assay performed at t = 96 h. Incubation conditions: 37 °C and 7% CO_2.

3.6 *Photocytotoxicity results for cisplatin for A431 cells or A549 cells irradiated 6 h after treatment*

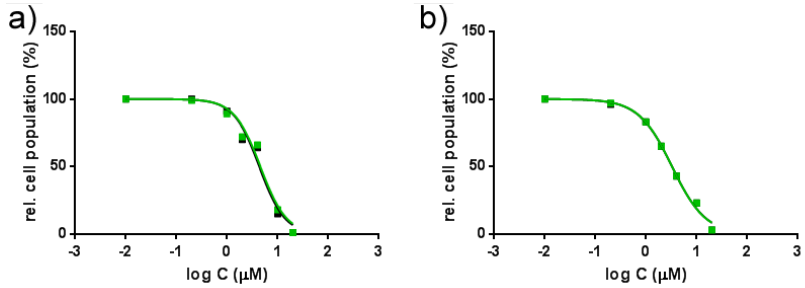


Figure S20. Dose-response curves for A431 (a) and A549 (b) cells in presence of cisplatin irradiated 1 h with green light 6 h after treatment (green data points) or left in the dark (black data points). Phototoxicity assay outline: cells were seeded at 8 × 10^3 (A431) or 5 × 10^3 (A549) cells/well at t = 0 h, treated with cisplatin at t = 24 h, irradiated at t = 30 h, and the SRB assay was performed at t = 96 h. Conditions: cells were incubated at 37 °C and 7% CO_2. Data points represent the average of technical triplicates.

3.7 *Photocytotoxicity results for cisplatin for A431 cells or A549 cells irradiated 24 h after treatment*

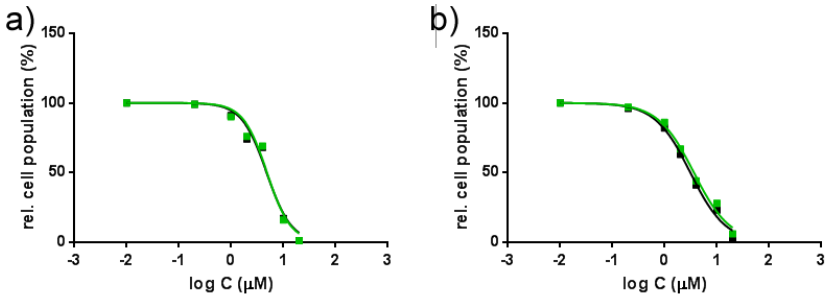


Figure S21. Dose-response curves for A431 (a) and A549 (b) cells in presence of cisplatin irradiated 1 h with green light 24 h after treatment (green data points) or left in the dark (black data points). Phototoxicity assay outline: cells were seeded at 8 × 10^3 (A431) or 5 × 10^3 (A549) cells/well at t = 0 h, treated with cisplatin at t = 24 h, irradiated at t = 48 h, and the SRB assay was performed at t = 96 h. Conditions: cells were incubated at 37 °C and 7% CO_2. Data points represent the average of technical triplicates.

3.8 *Photocytotoxicity results of [1]Cl and [2]PF_6 for A431 cells irradiated 24 h after treatment*

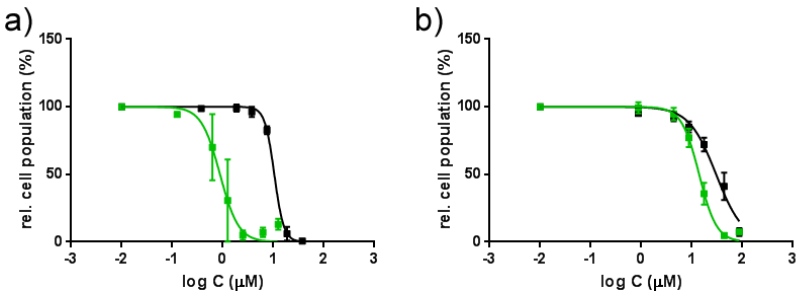


Figure S 22. Dose-response curves for A431 cells in presence of [**1**]Cl (a) or [**2**]PF_6 (b) irradiated 1 h with green light 24 h after treatment (green data points) or left in the dark (black data points). Phototoxicity assay outline: cells were seeded at 8 × 10^3 cells/well at t = 0 h, treated with [**1**]Cl or [**2**]PF_6 at t = 24 h, irradiated at t = 48 h, and the SRB assay was performed at t = 96 h. Conditions: Cells were incubated at 37 °C and 7% CO_2.

3.9 *Photocytotoxicity assay results of [1]Cl and [2]PF_6 for A549 cells irradiated at 6 h and at 24 h after treatment*

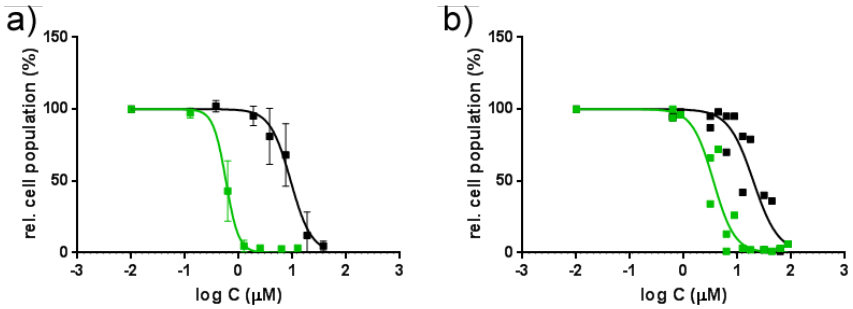


Figure S23. Dose-response curves for A549 cells incubated with [**1**]Cl (a) or [**2**]PF_6 (b) and irradiated 1 h with green light 6 h after treatment (green data points), or left in the dark (black data points). Phototoxicity assay outline: cells seeded at 5 × 10^3 cells/well at t = 0 h, treated with [**1**]Cl or [**2**]PF_6 at t =24 h, irradiated at t = 30 h, and SRB assay performed at t = 96 h. Incubation conditions: 37 °C and 7% CO_2.

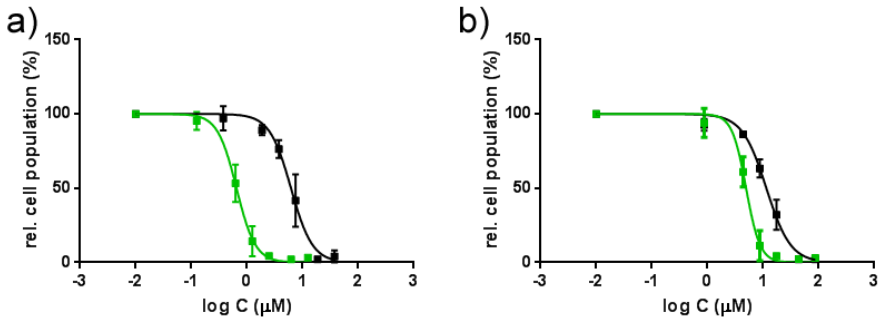


Figure S24. Dose-response curves for A549 cells incubated with [**1**]Cl (a) or [**2**]PF_6 (b) and irradiated 1 h with green light 24 h after treatment (green data points), or left in the dark (black data points). Phototoxicity assay outline: cells seeded at 5 × 10^3 cells/well at t = 0 h, treated with [**1**]Cl or [**2**]PF_6 at t = 24 h, irradiated at t = 48 h, and SRB assay performed at t = 96 h. Incubation conditions: 37 °C and 7% CO_2.

3.10 *Photocytotoxicity assay results of [1]Cl and [2]PF_6 for MRC-5 cells irradiated at 6 h and at 24 h after treatment*

Table S1. Cytotoxicity (EC_{50} with confidence interval in μM) of [**1**]Cl and [**2**]PF_6 on lung (MRC-5) non-cancerous cell lines. n.d. not determined.

	6 h (dark)	6 h (light)	24 h (dark)	24 h (light)
[**1**]Cl	13 ± 1.3	1.6 ± 2.4	8.3 ± 1.0	1.7 ± 2.3
[**2**]PF_6	8.5 ± 3.5	< 1	18.3 ± 1.4	< 1
CDDP	3.8 ± 1.5	n.d.	6.9 ± 1.2	n.d

4 Singlet oxygen quantum yield measurements

The quantum yield of singlet oxygen generation was determined in a custom-built setup (Figure S25), in which both UV-vis absorption and UV-vis and NIR emission spectroscopy could be performed. All optical parts were connected with optical fibers from Avantes (Apeldoorn, The Netherlands), with a diameter of 200-600 µm. 2 mL of sample, consisting of the compound in methanol-d4, was placed in a stirred 111-OS macro fluorescence cuvette from Hellma in a CUV-UV/VIS-TC temperature-controlled cuvette holder from Avantes. The sample was allowed to equilibrate for 5 minutes. Emission spectroscopy was performed with a 450 nm fiber-coupled laser (Laser system LRD-0450 from Laserglow, Toronto, Canada), which was set to 101 mW at the cuvette (4 mm beam diameter; 0.4 W.cm^{-2}) at a 90° angle with respect to the spectrometers. The excitation power was measured using a S310C thermal sensor connected to a PM100USB power meter (Thorlabs). Two spectrometers that were coupled to the cuvette holder with a bifurcated optical fiber, visualized the emission spectrum from 300 nm to 900 nm (Avantes 2048L StarLine spectrometer) and from 1000 nm to 1700 nm (Avantes NIR256-1.7TEC spectrometer). The infrared emission spectrum was acquired in ≤50 seconds, after which the laser was turned off directly. UV-vis absorption spectra before and after emission spectroscopy were measured using an Avalight-DHc halogen-deuterium lamp (Avantes) as light source (turned off during emission spectroscopy) and the Avantes UV-vis spectrometer (2048L StarLine spectrometer) as detector, both connected to the cuvette holder at a 180° angle. All spectra were recorded with Avasoft software from Avantes and further processed with Microsoft Office Excel 2010 and Origin Pro software.

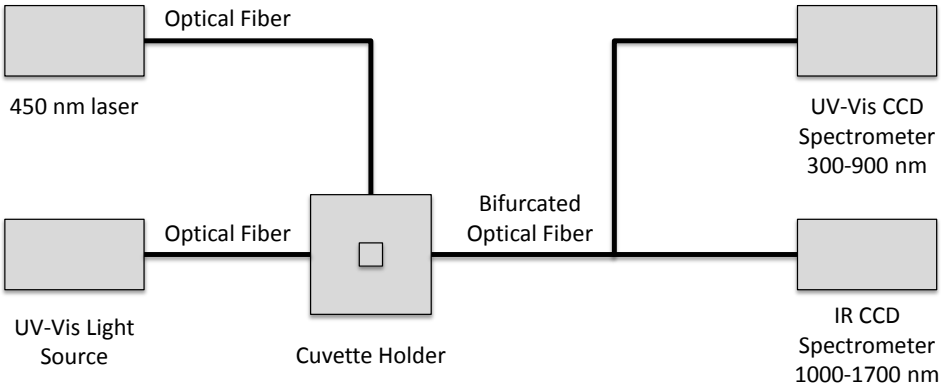


Figure S25. Schematic overview of the custom-built setup to determine singlet oxygen quantum yield.

The quantum yield of singlet oxygen production was calculated using the relative method with $[Ru(bpy)_3]Cl_2$ as the standard ($\Phi^{\Delta}_{std} = 0.73$ in CD_3OD), according to Equation 1:

$$\Phi^{\Delta}_{sam} = \Phi^{\Delta}_{std} \times \frac{A^{450}_{std}}{A^{450}_{sam}} \times \frac{E_{sam}}{E_{std}} \quad \text{Equation 1}$$

where Φ^{Δ} is the quantum yield of singlet oxygen generation, A^{450} is the absorbance at 450 nm (always kept below 0.1 for a 1 cm path length cuvette), E is the integrated emission peak of singlet oxygen at 1270 nm, and *sam* and *std* indexes denote the "sample" and "standard", respectively.

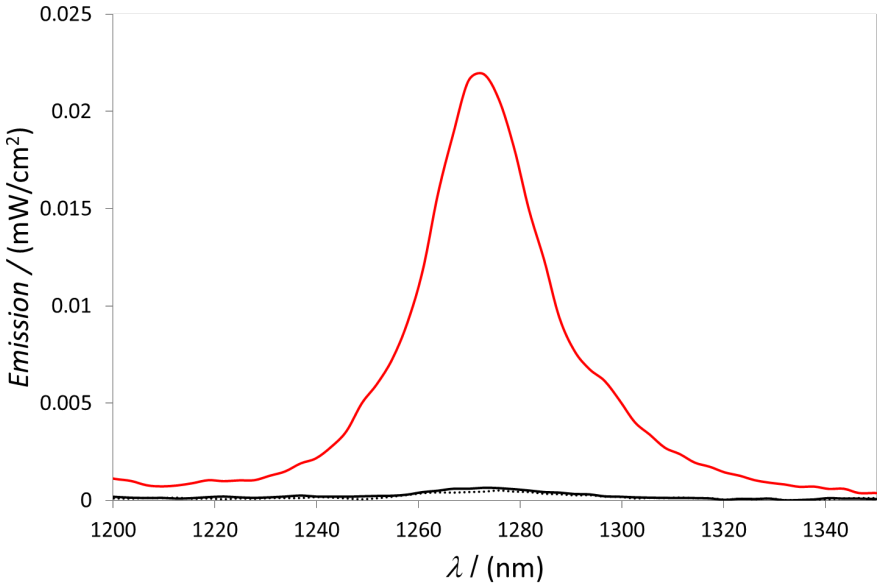


Figure S26. Time-integrated emission spectra of $[Ru(bpy)_3]Cl_2$ (—), [**1**]Cl (···), [**2**]PF_6 (—) irradiated with blue light (450 nm, 101 mW, photon flux= 0.53 µEinstein.s^{-1}), stirred under air in CD_3OD at 298 K. Emission was measured and averaged over 4 × 40 s.

Table S2. Time-integrated emission data for $[Ru(bpy)_3]Cl_2$, [**1**]Cl, and [**2**]PF_6 measured in CD_3OD stirred under air at 298 K. Emission was averaged 4 × 40 s.

Compound	**Concentration (µM)**	**Absorbance at 450 nm**	**Integrated Emission ($mW.cm^{-2}$) at 1273 nm**	**Quantum yield (%)**
$[Ru(bpy)_3]Cl_2$	5.3	0.11	0.02192	73 [a]
[1]Cl	23	0.14	0.00045	1.3
[2]PF_6	8.9	0.084	0.00065	2.3

[a] Value taken from reference [5].

5 Flow cytometry and cellular morphology

5.1 Annexin V-FITC/PI assay

200 × 10^3 A549 cells were seeded in 35 mm petri dishes in Opti-MEM complete media (2 mL). After 24 h, the media was replaced with drug loaded media (2 ml) using the following concentrations: Rose Bengal 10 µM, Staurosporine 0.3 µM, [**1**]Cl 1.5 µM, [**2**]PF_6 10 µM. The non-light sensitive protein kinase inhibitor Staurosporine, and Rose Bengal, a green light absorbing PDT reagent, were used as positive controls.[6] As control also compound-free Opti-MEM complete was added to one dish. Cells were irradiated (520 nm, 75 J.cm^{-2}) using the same irradiation setup as for the EC_{50} determination, leading to a set of light irradiated cells and a set of dark controls. 24 h after irradiation, all cells were collected, washed twice with PBS, and suspended in Annexin V binding buffer (1 × 10^6 cells/mL). The cell suspension (100 µL) was stained with propidium iodide (5 µL, 10 µg/ml) and the Annexin V-FITC conjugate (3 µL, Bio connect) in the dark for 15 minutes. After addition of Annexin V binding buffer (200 µL) the cells were submitted to FACS measurement.[7] Quantification of the induced cell death was performed with FlowJo, using a standard protocol (Figure 4 and Figure S28).[8]

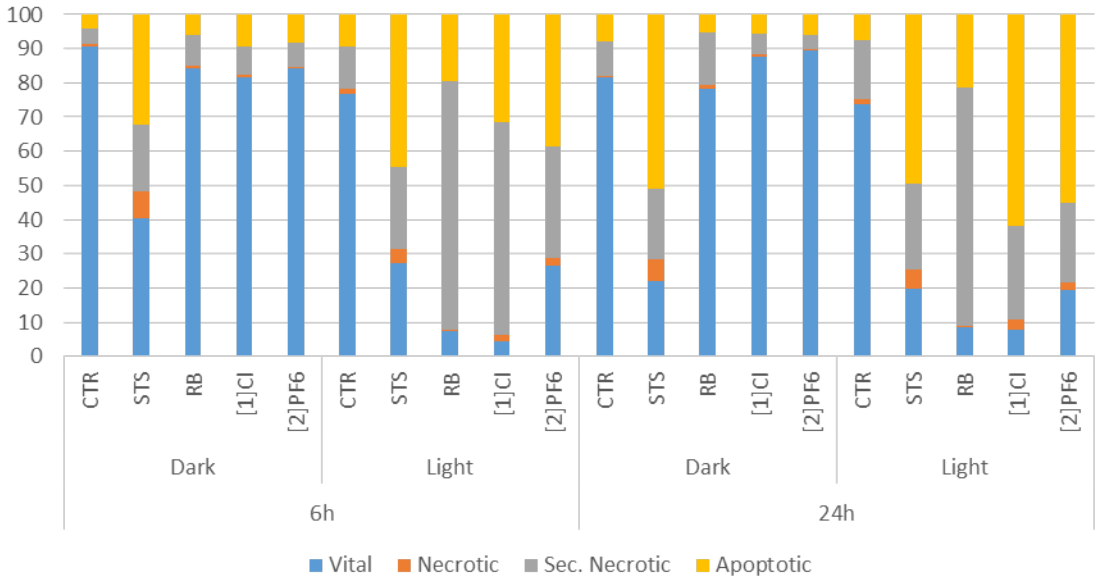


Figure S27. Annexin V FITC/ propidium iodide FACS analysis of cells treated with Staurosporine (STS, 0.3 µM), Rose Bengal (10 µM), [**1**]Cl (1.5 µM), or [**2**]PF_6 (10 µM). Cells were seeded at t = 0, grown for 24 h, treated with the appropriate compound for 6 h or 24 h, then irradiated with green light (520 nm, 60 min, 75 J.cm^{-2}, "Light"), then incubated for a further 24 h before flow cytometric analysis. In parallel, a second set of cells were treated with the same protocol, except that they were kept in the dark instead of irradiated ("Dark"). The diagram shows the relative distribution (cell count) of vital, necrotic, apoptotic, and secondary apoptotic cells in the different samples.

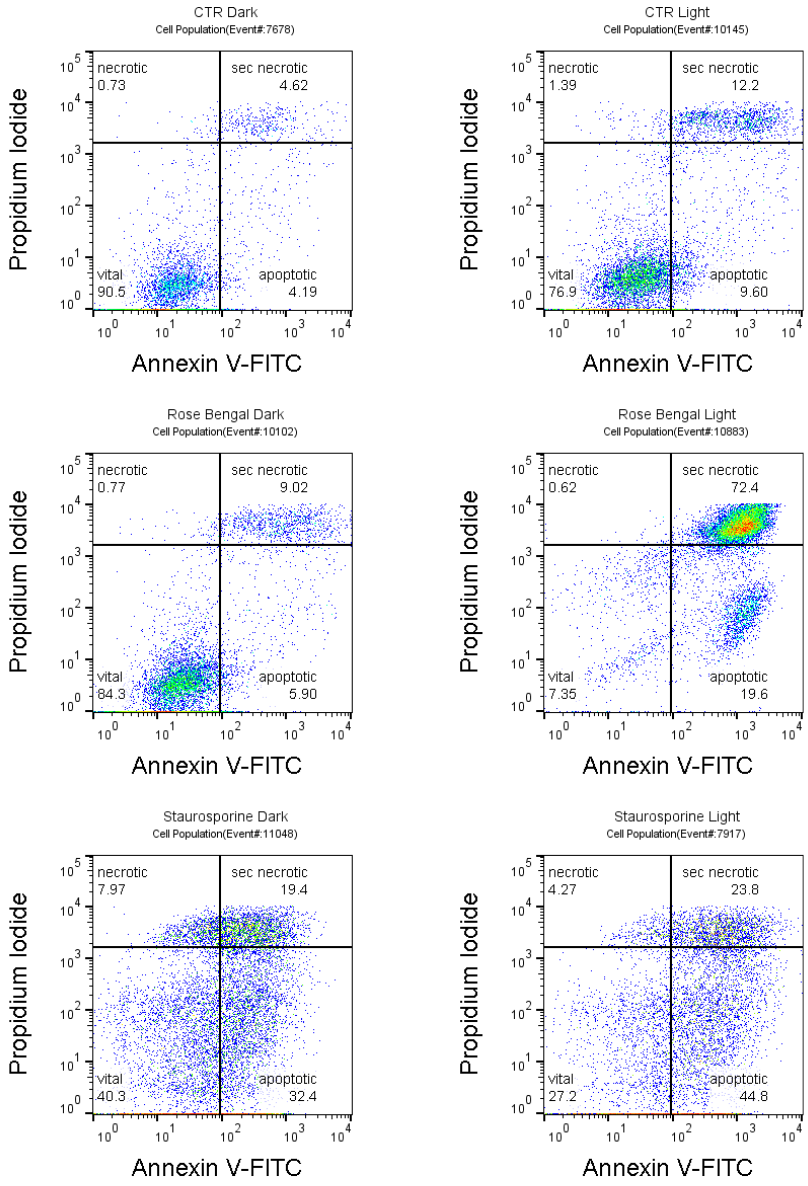


Figure S28. Representative flow cytometric density plots (Annexin V - FITC (525 nm) / propidium iodide (670 nm) of A549 cells treated with Staurosporine (STS, 0.3 µM), Rose Bengal (10 µM) and left in the dark (left column) or irradiated with green light 6 h after treatment (right column). Analysis was performed 24 h after light irradiation. Irradiation conditions: 520 nm, 60 min, 75 J.cm^{-2}.

5.2 Phase Contrast microscopy images

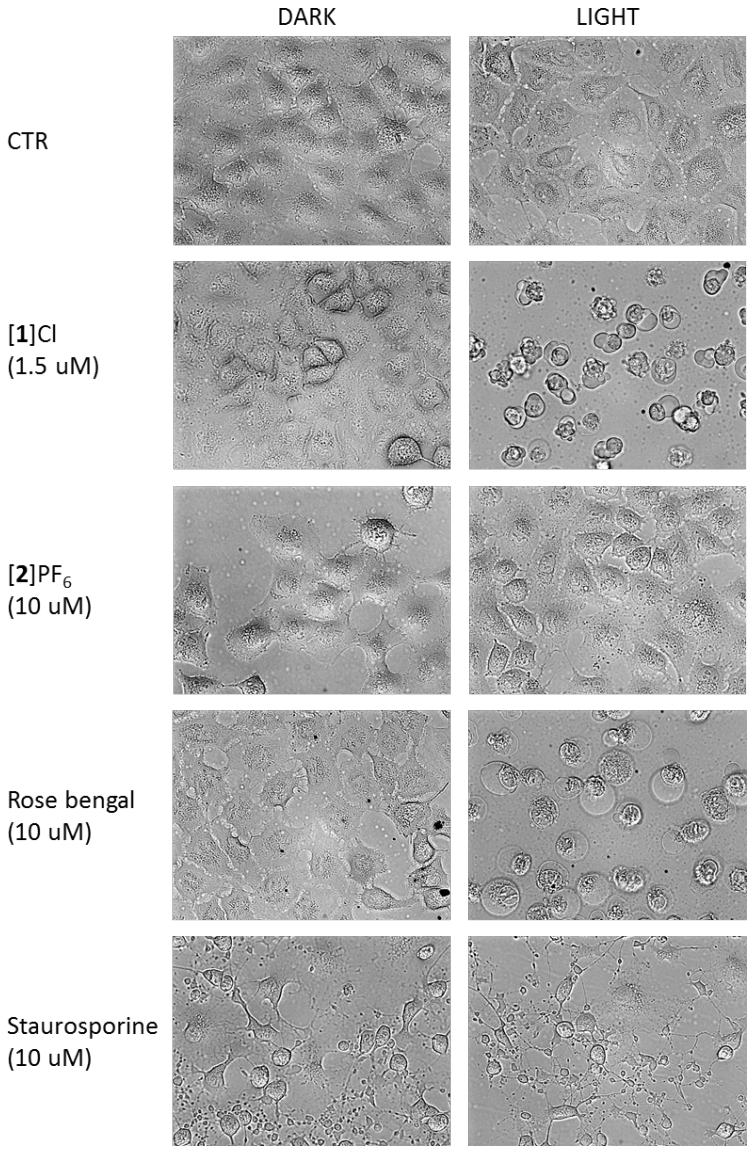


Figure S29. Micrographs (40×) of A549 cells directly after green light irradiation (520 nm, 60 min, 75 J.cm^{-2}). Cells seeded at t = 0 (200.000 cells per 35 mm petri dish), incubated in the dark for 24 h (7% CO_2, 37 °C), treated with the indicated drug at t = 24 h, either irradiated at t = 6 h (LIGHT) or left in the dark (DARK), and then directly imaged. Cells treated with positive control compounds Rose Bengal and Staurosporine are also depicted.

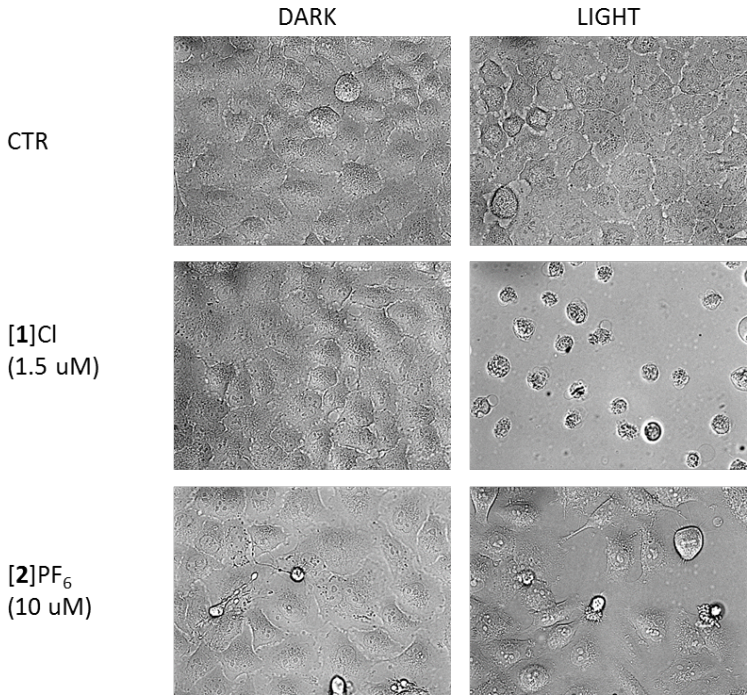


Figure S30. Micrographs (40×) of A549 cells 24 h after green light irradiation (520 nm, 60 min, 75 J.cm^{-2}). Cells seeded at t = 0 (200.000 cells per 35 mm petri dish), incubated in the dark for 24 h (7% CO_2, 37 °C), treated with [**1**]Cl or [**2**]PF_6 or with no drug (CTR) at t = 24 h, either irradiated at t = 6 h (LIGHT) or left in the dark (DARK), further incubated for 24 h in the dark, and then imaged.

6 Investigation of the mitochondrial potential

A549 cells were seeded at 10.000 cell/well in a eight chamber (ibidi, µ-Slide 8 well). After 24 h, the media was replaced with drug loaded media (300 µL, OMEM complete) using the following concentrations: Rose Bengal 10 µM, Staurosporine 0.3 µM, cisplatin 5 µM, [**1**]Cl 1.5 µM, [**2**]PF_6 10 µM. The non-light sensitive protein kinase inhibitor Staurosporine, and the green light absorbing PDT agent Rose Bengal were used as positive controls. Further, compound-free Opti-MEM complete was added to one chamber as control. Cells were irradiated (520 nm, 75 $J.cm^{-2}$) using the same irradiation setup as for the EC_{50} determination, leading to a set of light irradiated cells and a set of dark controls. Directly after irradiation the media was replaced by fluorescence-dyes loaded media. Tetramethylrhodamine ethyl (600 µM), a mitochondrial sensitive dye, and Draq5 (1 µM), a DNA-staining fluorophore were used. After 30 min of incubation the media was removed and replaced with TMRE loaded media (150 µM). Subsequently, the microscope slides were transferred to a Leica SPE confocal microscope.

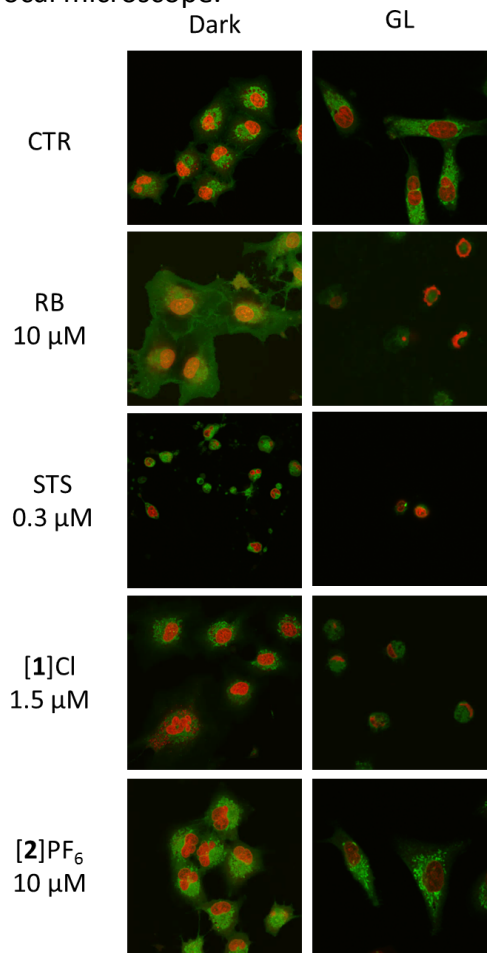


Figure S31. Confocal micrographs of the emission of the mitochondrial dye tetramethylrhodamine ethyl ester (Ex/Em = 551/593 nm) and the emission of the DNA intercalator Draq5 (Ex/Em = 646/681 nm) in A549 cells treated with Rose Bengal (10 µM) Staurosporine (STS, 0.3 µM), [**1**]Cl (1.5 µM), and [**2**]PF_6 (10 µM) left in the dark (left column) or irradiated with green light 24 h after treatment (right column). Analysis was performed 24 h after light irradiation. Irradiation conditions: 520 nm, 60 min, 75 $J.cm^{-2}$.

7 Cell fractionation

Cell fractionation for intracellular distribution studies for complexes [**1**]Cl and [**2**]PF_6 were conducted on A549 lung cancer cells. 3×10^6 cells were seeded at t = 0 h in Opti-MEM complete in 175 cm^2 flasks. At t = 24 h cells were treated with complexes to give a final concentration corresponding to the EC_{50} values in the dark after 6 h in a total volume of 24 mL. After 6 h of drug incubation at 37 °C, the medium was aspirated, the cells were washed with PBS-buffer, trypsinized, counted and pelleted by centrifugation 700 × g for 5 min. Then, the pellets were fractionated using to FractionPREP cell fractionation kit from BioVision according to the suppliers's instructions. Samples were digested overnight in concentrated nitric acid (>65%) and diluted with MilliQ water to obtain a final concentration of 5% HNO_3. For ICP-MS measurements, the system was optimized with a ruthenium-platinum solution. The calibration range was from 0 to 25 µg/l, and obtained detection limit for all isotopes was 0.01 µg/l. Silver and Indium were used for internal standard, to correct for sample dependent matrix effects. No reference sample was available; therefore several samples were spiked with a known concentration. The recoveries of the spiked concentrations were all within a 10% deviation. The data from three independent biological replications were used to obtain the uptake bars shown in Figure S32.

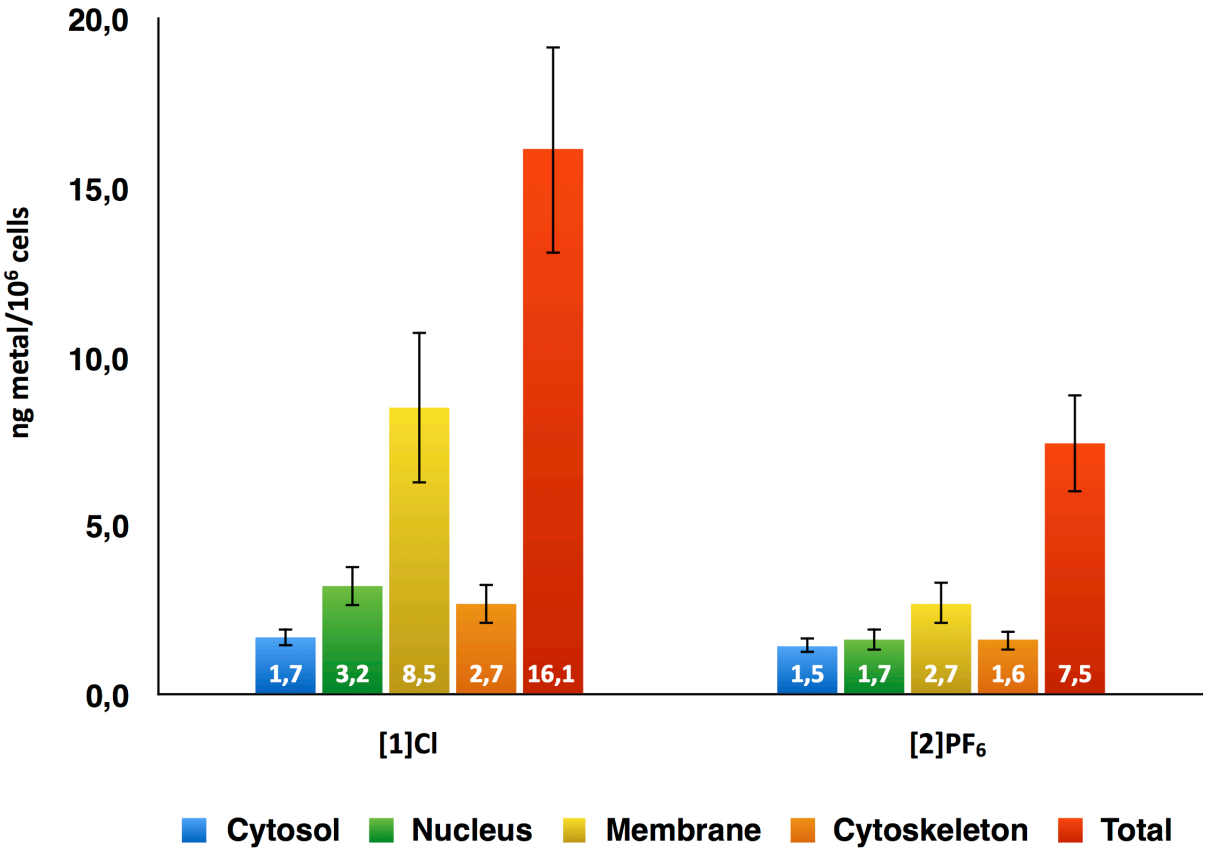


Figure S32. Relative distribution of ruthenium in the different cellular fractions of A549 cells (expressed in ng Ru/millions cells) after 6 h treatment with EC_{50} concentration of [**1**]Cl or [**2**]PF_6.

8 DNA (photo)binding studies

8.1 Principle

The pUC19 plasmid used for this study (2686 bp) exists in three forms: supercoiled (SC), single-nicked open circular (OC) and linear dimer (LD). Of particular interest are the SC and OC forms. Although these two forms have the same number of bp, the SC form migrates faster through the agarose gel compared to the OC form due to the condensed SC form. However, as positively charged metal complexes associate with the SC form, the shape may become larger and it will be less negatively charged, ultimately resulting in slower migration. Alternatively, if metal complexes coordinatively modify the OC form, it may induce coiling or a condensed structure causing an increase in migration and thus coalescence of the SC and OC form on increasing metal complex concentration. However, if there is no condensation of the OC plasmid structure, but the positively charged metal complexes associate with the OC form then the observed migration would be retarded. Finally, if the metal complexes generate enough singlet oxygen, then upon irradiation the SC form is converted via a nick in one of the DNA strands to the relaxed OC form. However, due to the low Φ_{1O2}, DNA photocleaveage was unexpected.

8.2 General DNA agarose gel information

Agarose gel electrophoresis was used to assay the thermal and photoinduced binding of [**1**]Cl and [**2**]PF_6 to pUC19 plasmid DNA. Two buffers were used for the experiments: 5X tris-boric acid buffer (TBA) and phosphate buffer (PB). The 5X tris-boric acid (TBA) buffer (45 mM tris(hydroxymethyl)amino methane and 45 mM boric acid, pH = 7.4) was used in the gel and running buffer. Phosphate buffer (PB, 100 mM NaH_2PO_4, pH = 7.0) was used for DNA-MC interactions. The agarose gels were 0.8% w/w agarose gel (0.24 g agarose, 24 g DI H_2O, and 6 mL TBA) and were cast in the OWL B1A Easycast system.

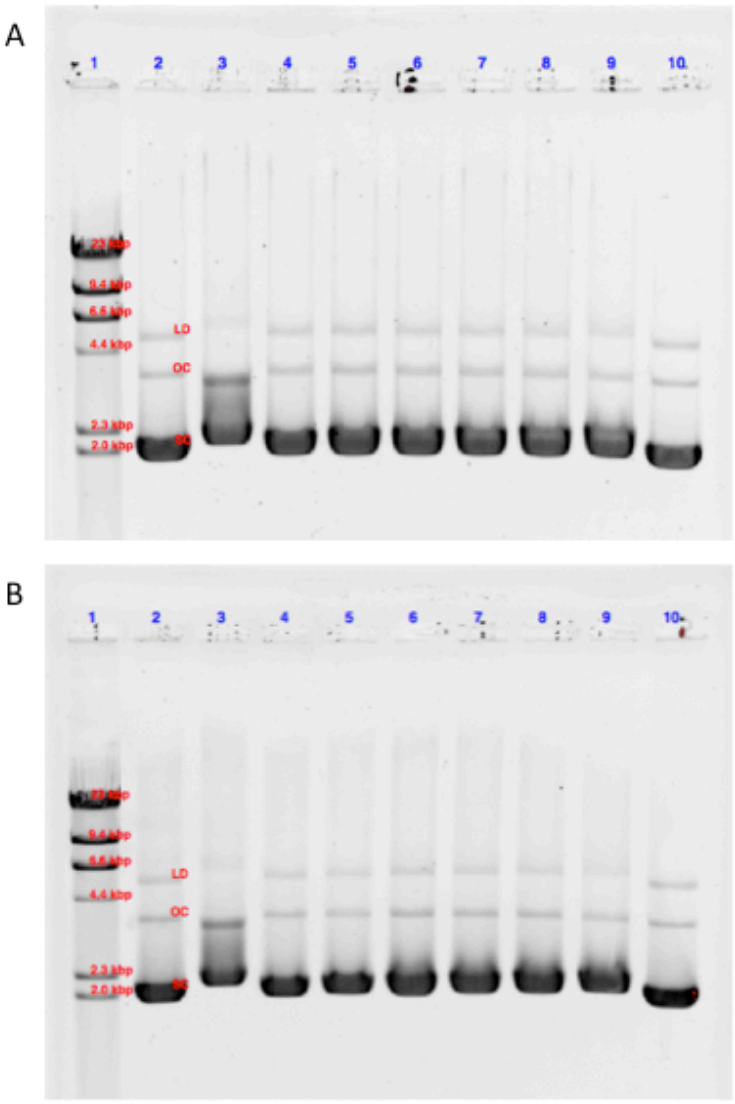
The molar concentration of the pUC19 plasmid DNA base pairs (BP) was determined using the extinction coefficient ($\varepsilon_{260\ nm}$ = 13,200 $M^{-1}cm^{-1}$).[9] All aliquots were prepared with a final volume of 20 µL and prior to loading 4 µL of 6X loading dye was added. The λ DNA-*Hind*III digest molecular weight (MW) marker was prepared by adding 2 µL (1 µg) of the DNA MW marker, 18 µL PB, and 4 µL 6X loading dye. The MW marker was heated for 3 min at 60 °C prior to loading. In each well, 12 µL (1 µg of pUC19 DNA or 0.5 µg of MW marker) of each sample was loaded.

For each gel, the electrophoresis chamber was filled with 50 mL TBA and 210 mL DI H_2O. Each gel was run at a constant voltage of 105 V for 90 min. All gels were stained using 10 µL (10 mg/mL) ethidium bromide in 200 mL DI H_2O for 30 min with slight shaking and then destained in 200 mL DI H_2O for 20 min. Immediately following destaining, the gel was imaged using a BioRad ChemiDoc imaging system (ethidium bromide setting). Image Lab software was used to process the images.

*8.3 Thermal binding of [**1**]Cl and [**2**]PF_6 to pUC19 plasmid DNA at 37 °C for 24 h*

The samples for thermal binding were prepared under ambient light in amber centrifuge tubes. For each sample 2 µL of pUC19 plasmid (2 µg, $[BP]_f = 1.95 \times 10^{-3}$ M) was used and the amount of metal complex and PB were adjusted to a final volume of 20 µL. Several ratios of base pairs of the plasmid (BP) to metal complex (MC) (100:1, 50:1, 25:1, 15:1, 10:1, and 5:1 BP:MC) and incubated at 37 °C for 24 h. Additionally, the negative control (DNA without metal complex) and a positive control (cisplatin, 5:1 BP:MC ratio) were incubated under the same conditions. Lanes were loaded

as follows: **(1)** λ MW marker, **(2)** DNA only control, **(3)** cisplatin (5:1 BP:MC, $[cisplatin]_f$ = 390 µM) control, **(4)** Ru complex (100:1 BP:MC, $[Ru]_f$ = 20 µM), **(5)** Ru complex (50:1 BP:MC, $[Ru]_f$ = 40 µM), **(6)** Ru complex (25:1 BP:MC, $[Ru]_f$ = 80 µM), **(7)** Ru complex (15:1 BP:MC, $[Ru]_f$ = 130 µM), **(8)** Ru complex (10:1 BP:MC, $[Ru]_f$ = 195 µM), **(9)** Ru complex (5:1 BP:MC, $[Ru]_f$ = 390 µM), and **(10)** DNA only control. The gels were run, stained, and processed as described above (Figure S33).

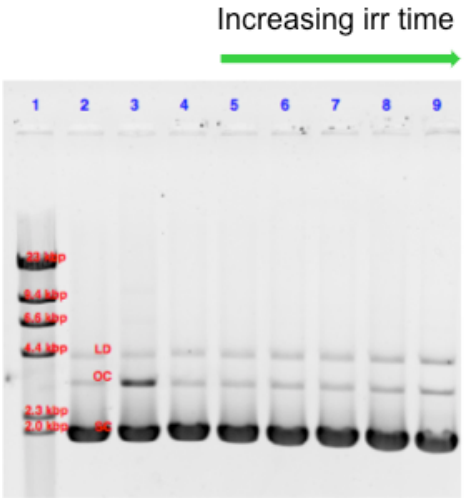


Lane	BP:MC	$[DNA\ bp]_f$ (M)	$[MC]_f$ (M)
1	λ MW marker		
2	37 °C control	1.95E-03	0
3	Cisplatin 5:1 BP:MC	1.95E-03	1.95E-05
4	100:1 BP:MC	1.95E-03	3.91E-04
5	50:1 BP:MC	1.95E-03	1.95E-04
6	25:1 BP:MC	1.95E-03	1.30E-04
7	15:1 BP:MC	1.95E-03	7.82E-05
8	10:1 BP:MC	1.95E-03	3.91E-05
9	5:1 BP:MC	1.95E-03	1.95E-05
10	λ MW marker		

Figure S33. Agarose gels showing the thermal binding of metal complexes $[\mathbf{1a}]^{2+}$ (A) and $[\mathbf{2a}]^{0}$ (B) to pUC19 plasmid DNA. The table below shows the lane assignment, description (DNA base pair (BP) to metal complex (MC) ratios) and the final concentrations. The λ MW marker bands correlate to 23, 9.4, 6.6, 4.4, 2.3, and 2.0 kbp. The control DNA band consists of three bands correlating to linear dimer (LD), open circular (OC), and supercoiled (SC) forms.

8.4 Photoinduced binding of [1]Cl, [2]PF_6, and cisplatin to pUC19 plasmid DNA following 5, 15, 30, 45, and 60 min irradiation (λ_{irr} = 520 nm)

The samples for photoinduced binding were prepared under ambient light and then irradiated in a well of a 96-well plate using the LED array system described for cell culturing (λ_{irr} = 520 nm). A 50:1 BP:MC ratio ($[BP]_f = 1.95 \times 10^{-3}$ M and $[\text{Metal complex}]_f$ = 40 µM) was used for all metal complexes. For each gel, a dark DNA only control, irradiated DNA only control, dark 50:1 BP:MC control ($[\text{Metal complex}]_f$ = 40 µM), and irradiated 50:1 BP:MC sample were prepared. The DNA only controls consisted of 8 µL pUC19 plasmid DNA and 72 µL PB. The dark 50:1 BP:MC controls were composed of 4 µL pUC19 plasmid DNA, 33 µL PB, and 3 µL of the metal complex in PB (0.5 mM). A total volume of 200 µL (20 µL pUC19 plasmid DNA, 164 µL PB, and 16 µL metal complex in PB (0.5 mM) was prepared for the irradiated 50:1 BP:MC samples and 20 µL aliquots were removed for each time point. The time points were 5, 15, 30, 45, and 60 min correlating to light doses of 6.3, 18.8, 37.6, 56.4, and 75.2 Jcm^{-2}, respectively. At the end of the experiment, 20 µL of the dark DNA control, irradiated DNA control, and dark DNA-metal complex control (t = 0 min irr) were removed. Loading dye was added to each of the aliquots and the gel was loaded. The lanes were as follows: **(1)** λ MW marker, **(2)** dark DNA control, **(3)** irradiated DNA control (75.2 Jcm^{-2}), **(4)** dark 50:1 BP:MC control, **(5)** irradiated 50:1 BP:MC (5 min, 6.3 Jcm^{-2}), **(6)** irradiated 50:1 BP:MC (15 min, 18.8 Jcm^{-2}), **(7)** irradiated 50:1 BP:MC (30 min, 37.6 Jcm^{-2}), **(8)** irradiated 50:1 BP:MC (45 min, 56.4 Jcm^{-2}), and **(9)** irradiated 50:1 BP:MC (60 min, 75.2 Jcm^{-2}. The gels were run, stained and processed as specified above.



Lane	BP:MC	Time of irradiation (min)	Jcm^{-2}
1	λ MW marker		
2	Dark DNA control		
3	Irr DNA control	60	75.2
4	50:1 BP:MC	0	0.0
5	50:1 BP:MC	5	6.3
6	50:1 BP:MC	15	18.8
7	50:1 BP:MC	30	37.6
8	50:1 BP:MC	45	56.4
9	50:1 BP:MC	60	75.2

Figure S34. Agarose gels showing the photoinduced binding of cisplatin to pUC19 plasmid DNA. The table shows the lane assignment, description (DNA base pair (BP) to metal complex (MC) ratios), time of irradiation (min) and calculated light doses ($J.cm^{-2}$). The λ MW marker bands correlate to 23, 9.4, 6.6, 4.4, 2.3, and 2.0 kbp. The control DNA band consists of three bands correlating to linear dimer (LD), open circular (OC), and supercoiled (SC) forms.

9 References

(1) a) Z. Arcis-Castíllo, S. Zheng, M. A. Siegler, O. Roubeau, S. Bedoui and S. Bonnet, *Chem. Eur. J.*, 2011, **17**, 14826-14836; b) E. Dulière, M. Devillers and J. Marchand-Brynaert, *Organometallics*, 2003, **22**, 804-811.

(2) S. L. Hopkins, B. Siewert, S. H. C. Askes, P. Veldhuizen, R. Zwier, M. Heger and S. Bonnet, *Photochem. Photobiol. Sci.*, 2016, DOI: 10.1039/C1035PP00424A.

(3) M. D. Hall, K. A. Telma, K.-E. Chang, T. D. Lee, J. P. Madigan, J. R. Lloyd, I. S. Goldlust, J. D. Hoeschele and M. M. Gottesman, *Cancer Res.*, 2014, **74**, 3913-3922.

(4) V. Vichai and K. Kirtikara, *Nat. Protoc.*, 2006, **1**, 1112-1116.

(5) M. C. DeRosa and R. J. Crutchley, *Coord. Chem. Rev.*, 2002, **233–234**, 351-371.

(6) a) R. Bertrand, E. Solary, P. O'Connor, K. W. Kohn and Y. Pommier, *Exp. Cell Res.*, 1994, **211**, 314-321; b) E. Panzarini, V. Inguscio and L. Dini, *Int. J. Photoener.*, 2011, **2011**.

(7) G. Koopman, C. P. Reutelingsperger, G. A. Kuijten, R. M. Keehnen, S. T. Pals and M. H. van Oers, *Blood*, 1994, **84**, 1415-1420.

(8) O. Williams, in *Apoptosis Methods and Protocols*, ed. H. M. Brady, Humana Press, 2004, vol. 282, ch. 3, pp. 31-42.

(9) S. M. Zeman and D. M. Crothers, in *Drug-Nucleic Acid Interactions*, Elsevier, 2001, vol. 340, pp. 51-68.