Electronic Supplementary Information (ESI)

Biotinylated photoactive Pt(IV) anticancer complexes

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Experimental section

1. Materials and instruments

O-(Benzotriazol-1-yl)-*N*, *N*, *N*', *N*'-tetramethyluronium tetrafluoroborate (TBTU) was purchased from Merck, pyridine was from Fischer Scientific UK, biotin, avidin, 2-(4-hydroxyphenylazo)benzoic acid (HABA) and other chemicals were from Sigma Aldrich and used without further purification.

NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer and referenced to the residual signal of the solvent. ESI-HRMS spectra in positive mode were recorded on a Bruker microTOF instrument in the range of 200–3000 m/z. Electronic absorption spectra were recorded on a Varian Cary 300 UV-vis spectrophotometer in a quartz cuvette at 298 K using neat solvent as reference.

Analytical reversed-phase HPLC analyses were carried out on an Agilent 1100 HPLC equipped with an Agilent ZORBAX Eclipse XDB-C18 column (250×4.6 mm, 5 μm, flow rate: 1 mL/min), using 0.1% v/v TFA in H_2O (solvent A) and 0.1% v/v TFA in CH_3CN (solvent B) as mobile phase. A linear gradient from 10% to 80% B over 30 min was applied. LC-MS were carried out on Bruker Amazon X connected online with an Agilent 1260 HPLC.

The light source with λ_{max} = 420 nm used for photoactivation was a LZC-ICH2 photoreactor (Luzchem Research Inc.) equipped with a temperature controller and 8 Luzchem LZC-420 lamps without light filtration, and LED light source with λ_{max} = 465 nm (4.8 mW cm^{-2} per LED) was used in *in vitro* growth inhibition assay and cellular morphology.

Platinum content was analysed on ICP-OES 5300DV (Perkin Elmer) or ICP-MS 7500cx (Agilent) instruments. Samples were prepared in HNO_3 (3.6% v/v) solution.

2. Synthesis and characterisation

Caution! Due care and attention with appropriate precautions should be taken in the synthesis and handling of shock-sensitive heavy metal azides, even though no problem was encountered during the work reported here. All syntheses and purifications were carried out in the dark with minimal light exposure.

Trans, trans, trans-[$Pt(py)_2(N_3)_2$(biotin)(OH)] (**2a**). To a solution of complex **1** (40 mg, 85 μmol), biotin (21 mg, 86 μmol), and TBTU (27 mg, 85 μmol) in DMF (2 mL), *N*, *N*-diisopropylethylamine (DIPEA) (100 μL) was added. The reaction mixture was stirred for 24 h at 298 K under a nitrogen atmosphere. After evaporation to dryness, the oily residue was collected and purified by column chromatography on silica gel (8% methanol / 92% DCM, v/v). ^{1}H NMR (DMSO-d_6, 400 MHz): 8.82 (d with Pt satellites, J = 5.5 Hz, J ^{195}Pt-^{1}H = 26.8 Hz, 4H, H_{ortho} *py*), 8.28 (t, J = 7.6 Hz, 2H, H_{para} *py*), 7.85 (t, J = 6.9 Hz, 4H, H_{meta} *py*), 6.39 (s, 1H, *NHC(O)NH*), 6.35 (s, 1H, *NHC(O)NH*), 4.31-4.28 (m, 1H, *CH*), 4.11-4.08 (m, 1H, *CH*), 3.51 (s, 1H, *OH*), 3.06-3.01 (m, 1H, *CH*), 2.81 (dd, J = 12.4, 5.1 Hz, 1H, CH_2), 2.59 (s, 1H, CH_2), 2.23 (t, J = 7.3 Hz, 2H, CH_2), 1.62-1.24 (m, 6H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): 175.92 (*NHC(O)NH*), 163.18 (*COO*), 149.75 (C_{ortho} *py*), 142.45 (C_{para} *py*), 126.68 (C_{meta} *py*), 61.55 (*CH*), 59.64 (*CH*), 55.98 (*CH*), 55.38 (CH_2), 36.74 (CH_2), 28.72 (CH_2), 28.58 (CH_2), 26.34 (CH_2). ESI-HRMS: $[M + Na]^+$ (*m/z*) calcd 720.1405, found 720.1398. Anal. Calcd: $C_{20}H_{26}N_{10}O_4PtS\bullet(CH_2Cl_2)_{0.5}$: C 33.27, H 3.68, N 18.93. Found: C 33.67, H 3.70, N 18.59. $\varepsilon_{299\ nm}$ = 19330 M^{-1} cm^{-1}, $\varepsilon_{260\ nm}$ = 12188 M^{-1} cm^{-1} (5% DMSO / 95% RPMI-1640, v/v).

Trans, trans, trans-[$Pt(py)_2(N_3)_2$(biotin)(DCA)] (**2b**). Three mol. equiv. of dichloroacetic anhydride (DCA anhydride) was stirred with complex **2a** (35 mg, 50 μmol) in DMF solution at 298 K under a nitrogen atmosphere for 24 h. After evaporation to dryness, the oily residue was collected and purified by column chromatography on silica gel (3% methanol / 97% DCM, v/v). ^{1}H NMR (DMSO-d_6, 400 MHz): 8.77 (d with Pt satellites, J = 5.6 Hz, J ^{195}Pt-^{1}H = 26.2 Hz, 4H, H_{ortho} *py*), 8.34 (t, J = 7.6 Hz, 2H,

H$_{para}$ *py*), 7.90 (t, J = 7.0 Hz, 4H, *H*$_{meta}$ *py*), 6.61 (s, 1H, *CHCl*$_2$), 6.38 (s, 1H, *NHC(O)NH*), 6.35 (s, 1H, *NHC(O)NH*), 4.31-4.28 (m, 1H, *CH*), 4.10-4.09 (m, 1H, *CH*), 3.04-2.99 (m, 1H, *CH*), 2.81 (dd, J = 12.4, 5.1 Hz, 1H, *CH*$_2$), 2.58 (d, J = 12.5 Hz, 1H, *CH*$_2$), 2.30 (t, J = 4.9 Hz, 2H, *CH*$_2$), 1.59-1.23 (m, 6H, *CH*$_2$). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): 175.71 (*NHC(O)NH*), 163.16 (*COO*), 149.57 (*C*$_{ortho}$ *py*), 143.24 (*C*$_{para}$ *py*), 127.26 (*C*$_{meta}$ *py*), 66.32 (*CHCl*$_2$), 61.52 (*CH*), 59.64 (*CH*), 55.90 (*CH*), 55.36 (*CH*$_2$), 49.07 (*CH*$_2$), 35.52 (*CH*$_2$), 28.47 (*CH*$_2$), 26.12 (*CH*$_2$). *COO* for DCA was too weak to detect. ESI-HR MS: [M + Na]$^+$ (*m/z*) calcd 830.0731, found 830.0738. Anal. Calcd: $C_{22}H_{26}Cl_2N_{10}O_5PtS\bullet(CH_2Cl_2)_{0.8}$: C 31.24, H 3.17, N 15.98. Found: C 31.49, H 3.20, N 15.31. $\varepsilon_{308\,nm}$ = 20983 $M^{-1}\,cm^{-1}$, $\varepsilon_{260\,nm}$ = 17827 $M^{-1}\,cm^{-1}$ (5% DMSO / 95% RPMI-1640, v/v).

Adducts **2a**-avi and **2b**-avi were formed in the phenol red-free RPMI-1640 cell culture medium from **2a**/**2b** and 0.25 mol. equiv. of avidin, and were used in the biological tests directly.

3. Dark stability and photodecomposition in aqueous solution

Complexes **2a** and **2b** were dissolved in 5% DMSO / 95% phenol red-free RPMI-1640 (v/v) and their dark stability was monitored by UV-vis spectroscopy. The photodecomposition of complexes **2a** and **2b** in aqueous solution with 5% DMSO was monitored by UV-vis spectroscopy at different time intervals after irradiation with blue light (420 nm) at 298 K.

4. Electron paramagnetic resonance

The mixture of **2a** (2.5 mM) and DMPO (40 mM) was prepared in H_2O with 5% DMSO, while that of **2a** (2.5 mM) and TEMP (20 mM) was prepared in CH_3CN with 5% DMSO. Samples (*ca*. 100 μL) were transferred to a standard quality quartz tube with inner diameter of 1.0 mm and outer diameter of 2.0 mm (Wilmad LabGlass) using a plastic syringe with metal needle. The EPR spectra were recorded on a Bruker EMX (X-band) spectrometer at 298 K. Using the y-incremental sweep mode with an accumulation of 5 scans in the x dimension, with key EPR spectrometer settings (modulation amplitude 1.0 G, microwave power 6.32 mW, 1.0 $\times 10^5$ receiver gain, conversion time 10.24 ms, time constant 10.24 ms, sweep width 200 G). The LED light (463 nm) was mounted within the EPR magnet, supported by a foam sponge, to maintain the distance from the tip of the irradiation light bulb to the EPR cavity being *ca*. 3 cm. Data were processed by Matlab R2016b with easyspin 5.1.12 through a multicomponent fit.

Azidyl radicals were trapped by DMPO in H_2O after an accumulation of 75 scans with the key parameters for DMPO-N_3• : g = 2.006, $\boldsymbol{a}_{\mathbf{NO}}^{\mathbf{N}}$ = 1.47 mT, $\boldsymbol{a}_{\boldsymbol{\beta}}^{\mathbf{H}}$= 1.43 mT, $\boldsymbol{a}_{\mathbf{N}\boldsymbol{\alpha}}^{\mathbf{N}}$ = 0.31 mT; singlet oxygen was trapped by TEMP in CH_3CN after 10 min irradiation with the key parameters for TEMPO: g = 2.0049, $\boldsymbol{a}_{\mathbf{NO}}^{\mathbf{N}}$ = 1.57 mT.

5. Photoreaction with 5'-GMP

Guanosine 5′-monophosphate disodium salt hydrate (5′-GMP-Na_2, 2 mol. equiv.) was mixed with 30 μM complexes in aqueous solution (1% DMSO / 99% water, v/v). The solution was irradiated for 1 h with blue light (420 nm) after 1 h incubation at 310 K and analysed immediately. LC-MS analysis for Pt adducts was carried out on an Agilent ZORBAX Eclipse XDB-C18 column (250 × 4.6 mm, 5 μm, flow rate: 1 mL/min). The mobile phases for HPLC were A (0.1 % formic acid in H_2O, v/v) and B (0.1 % formic acid in acetonitrile, v/v, 10–80% in 30 min).

6. DNA melting temperatures

The melting curves for *ct*-DNAs were recorded by measuring the UV-vis absorbance at 260 nm. Phosphate buffer (1 mM, pH = 7.9) was used as medium. The nucleotide concentration of *ct*-DNA was determined using molar extinction coefficient of 6600 at 260 nm.[1] The concentration of DNA base pairs was 15 μM with the ratio of drug-to-base pairs 0.2. All solutions were prepared in duplicate, one was incubated at 310 K for 1 h and then irradiated for 1 h (420 nm), while the other one was incubated for 2 h in the dark. The DNA melting temperature (T_m) was determined as the temperature corresponding to the mid-point absorbance of the melting curves.

7. Interaction with avidin

To a mixture of avidin (8 μM) and HABA (160 μM) in phosphate-buffered saline (PBS) solution (500 μL), aliquots of a biotin or biotinylated complexes solution (1.6 mM) in DMSO were added. The concentration of avidin was calibrated by titration with biotin, since the concentration of avidin should be 0.25x as that of standard biotin solution at the end titration point. UV-vis spectra were recorded in a quartz cell (l = 1 cm) after 1 min equilibration.

8. Cell culture

A2780 ovarian, A549 lung and PC3 prostate cancer cells were obtained from the European Collection of Animal Cell Culture (ECACC), Salisbury, UK. All cell lines used in this work were grown in Roswell Park Memorial Institute media (RPMI-1640) supplemented with 10% v/v of foetal calf serum (FCS) and 1% v/v penicillin/streptomycin. The adherent monolayers of cells were grown at 310 K in a humidified atmosphere containing 5% CO_2 and passaged regularly at *ca*. 80% confluence.

9. Photo-dark cytotoxicity

Approximately 1.5×10^4 cells were seeded per well in 96-well plates. Two independent plates were used, one for dark and the other for irradiation experiments. Cell viability for each drug concentration was determined in triplicate. The cells were pre-incubated in drug-free medium with phenol red at 310 K for 24 h. Complexes were dissolved first in DMSO and then diluted in phenol red-free RPMI-1640 to make stock solution of the drug. These stock solutions were further diluted using phenol-red free cell culture medium until working concentrations were achieved. In these solutions the maximum DMSO concentration was less than 0.5% v/v. Cells were exposed to various concentrations of the drugs for 1 h. Then one plate was irradiated for 1 h using blue light (4.8 mW cm^{-2} per LED at 465 nm) while the dark plate was kept in the incubator. Then, supernatants of both plates were removed by suction and the plates washed with PBS. Photocytotoxicity was determined after 24 h recovery at 310 K in drug-free phenol red-containing medium in comparison to untreated controls which were only exposed to vehicle. Untreated controls were also compared between the irradiated and the non-irradiated plates to ensure that the observed differences in cell viability were not due to irradiation. The SRB assay was used to determine cell viability.[2] Absorbance measurements of the solubilised dye (on a Promega microplate reader) allowed the determination of viable treated cells compared to untreated controls. IC_{50} values (concentrations which caused 50% of cell death), were determined as the average of triplicates and their standard deviations were calculated. Stock concentrations for all metal complexes used in these biological assays were adjusted/verified after ICP-OES metal quantification.

10. Platinum accumulation in cancer cells

Approximately 5×10^6 A2780 cells were plated in 100 mm Petri dishes and allowed to attach for 24 h. The plates were exposed to 10 μM complexes. Additional plates were incubated with medium alone as a negative control. After 1 h of incubation at 310 K in the dark, cells were rinsed three times with cold PBS and harvested by trypsinisation. The number of cells in each sample was counted manually using a haemocytometer. Then the cells were centrifuged in Eppendorf vials to obtain the whole cell pellet, which was dissolved in concentrated 72% v/v nitric acid (200 μL), and heated in an oven at 343 K overnight. The vials were then allowed to cool, and each cellular sample solution was transferred into a falcon tube and diluted with Milli-Q water (3.8 mL), to give a final HNO_3 concentration of *ca*. 3.6% v/v for ICP-MS analysis. All experiments were conducted in triplicate.

11. Cellular ROS generation

A549 cells (1×10^4) were seeded per well in 96-well plates and allowed 24 h for attachment, then exposed to complexes (10 μM) for 1 h in the dark. One plate was kept in the dark, while the other one was irradiated by blue light (465 nm) for 1 h. Drugs were removed, and cells were stained by DCFH-DA (20 μM) for 40 min. Cells were washed by PBS. The fluorescence intensity was determined on a Promega microplate reader with comparison to negative control.

12. Cellular morphological change

Confocal fluorescence microscopy. A2780 cells (2×10^5) were seeded in two glass-bottom cell culture dishes (CELLview) and allowed 24 h for attachment, then exposed to complexes (1 or 2× photo IC_{50} concentration) for 1 h in the dark. One dish was irradiated by blue light (465 nm) for 1 h, while the other was kept in the dark for comparison. After 24 h recovery in the medium, supernatants of both plates were removed by suction. Cells were washed with PBS and stained by SYTO^{TM} 17 red fluorescent nucleic acid stain (2.5 μM) for 30 min. The fluorescence images were recorded on a confocal microscope (LSM 880, AxioObserver, $\lambda_{ex}/\lambda_{em}$ = 633/638–759 nm).

Flow cytometry. A2780 cells (5×10^6) were plated in 100 mm Petri dishes and allowed to attach for 24 h, then exposed to complexes (1 or 2× photo IC_{50} concentration) for 1 h in the dark. One dish was irradiated by blue light (465 nm) for 1 h, while the other was kept in the dark for comparison. After 24 h recovery in the medium, supernatants of both plates were removed by suction. Cells were washed with PBS, harvested by trypsinisation and analysed using a Becton Dickinson FACScan flow cytometer in the School of Life Sciences at Warwick University.

Table S1. Change in DNA melting temperature caused by 0.2 mol. equiv. of Pt(IV) complexes with or without irradiation (420 nm, 1 h). Experiments were performed in triplicate and a two-tail t-test with unequal variances used to compare each dataset to T_m of DNA alone. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

Complexes	ΔT_m/K	
	Dark	Irrad
2a	1.4±0.9	5.3±0. 4***
2b	1.5±0.7	4.3±0.7*
1	2.0±0.8	5.1±0.8***

Table S2. Relative fluorescence intensity of A549 cells treated with complex (10 μM, 2 h in dark or 1 h in dark and 1 h irradiation, 465 nm) then probed with DCFH-DA. The statistical significance between dark and irradiated samples were evaluated by a two-tail t-test with unequal variances. *p < 0.05, **p < 0.01.

Complexes	I/I_c	
	Dark	Irrad
2a	0.96±0.02	1.06±0.05
2b	0.94±0.04	1.23±0.07*
1	0.95±0.06	1.01±0.02

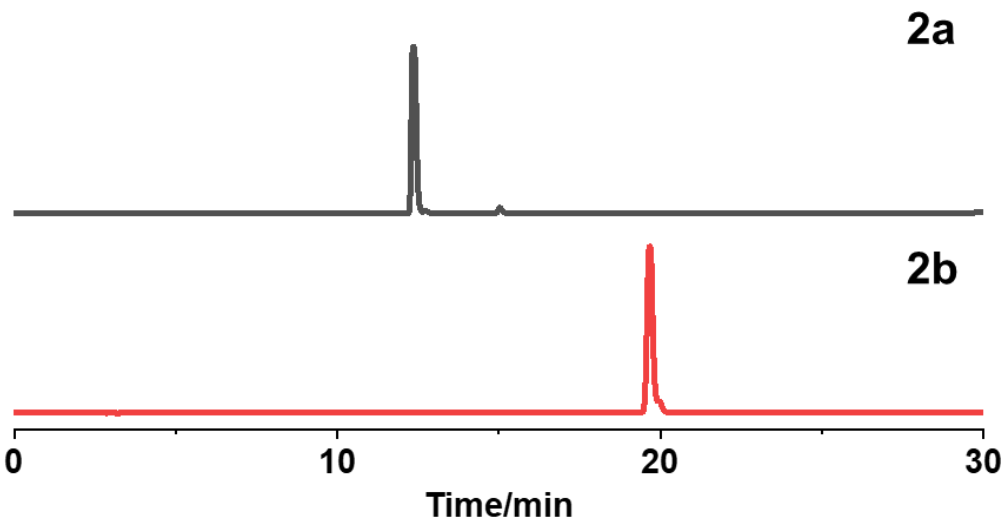

Figure S1. HPLC purity of complexes **2a** and **2b**. (10% to 80% CH_3CN in 30 min, detection wavelength 254 nm)

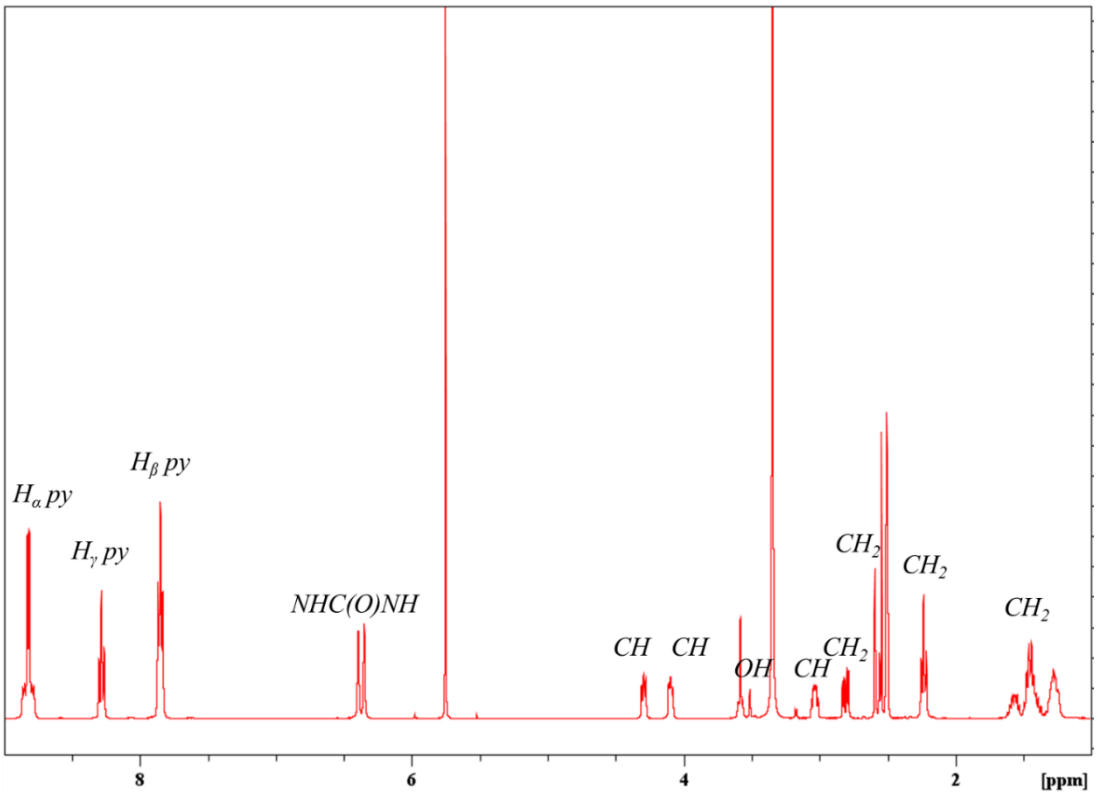

Figure S2. 400 MHz ^{1}H NMR spectrum of complex **2a** in DMSO-d_6 at 298 K.

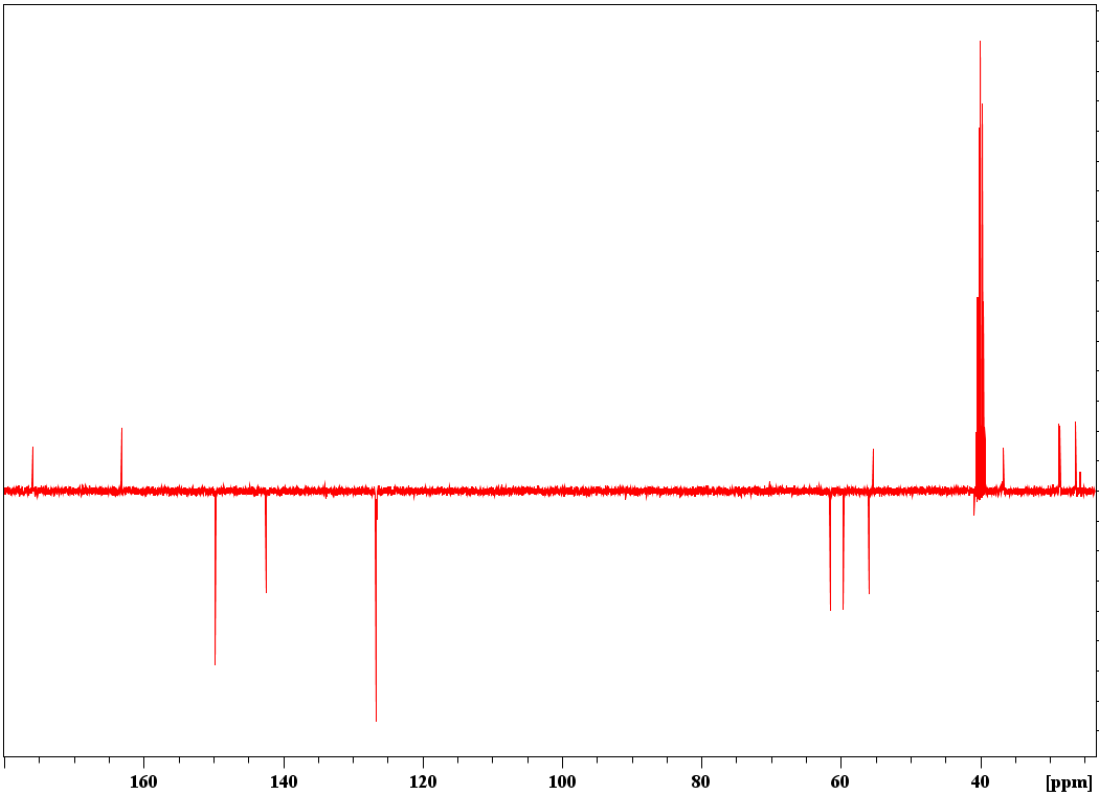

Figure S3. 100 MHz DEPT 135° ^{13}C NMR spectrum of complex **2a** in DMSO-d_6 at 298 K.

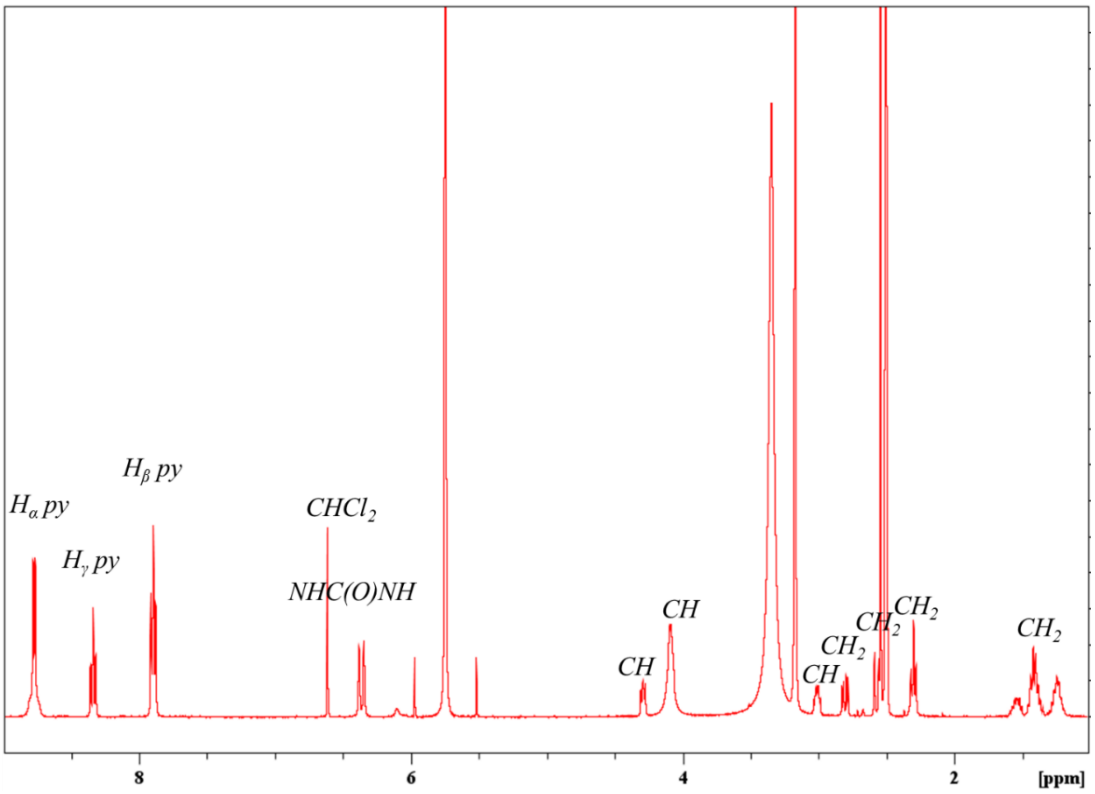

Figure S4. 400 MHz ^{1}H NMR spectrum of complex **2b** in DMSO-d_6 at 298 K.

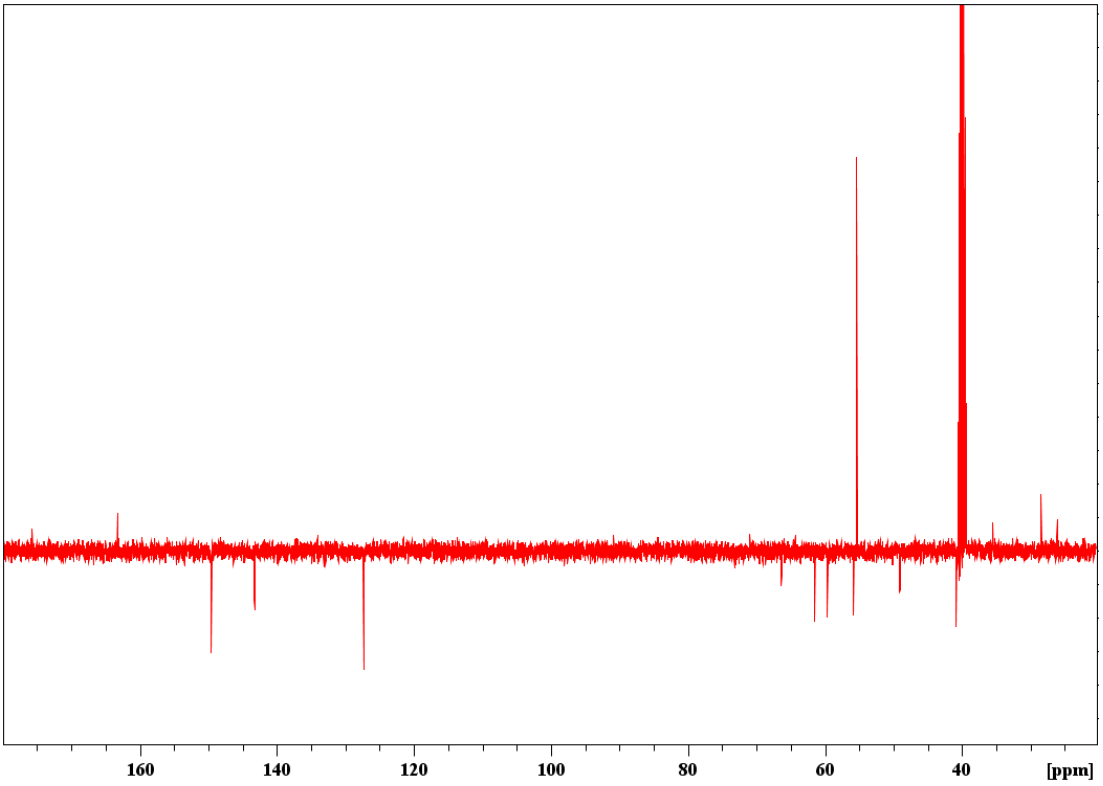

Figure S5. 100 MHz DEPT 135° ^{13}C NMR spectrum of complex **2b** in DMSO-d_6 at 298 K.

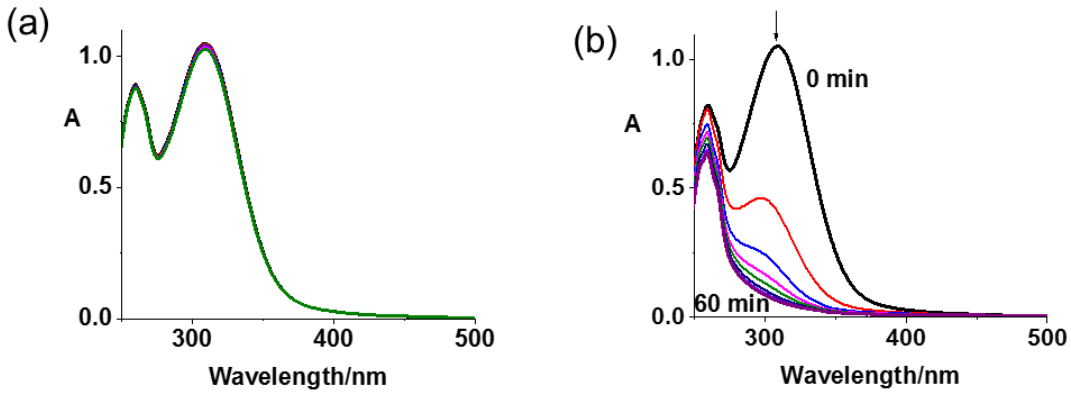

Figure S6. (a) UV-vis spectral change of **2b** (50 μM) in RPMI-1640 with 5% DMSO in the dark for 2 h, and (b) in H_2O with 5% DMSO upon 1 h irradiation with blue light (420 nm)

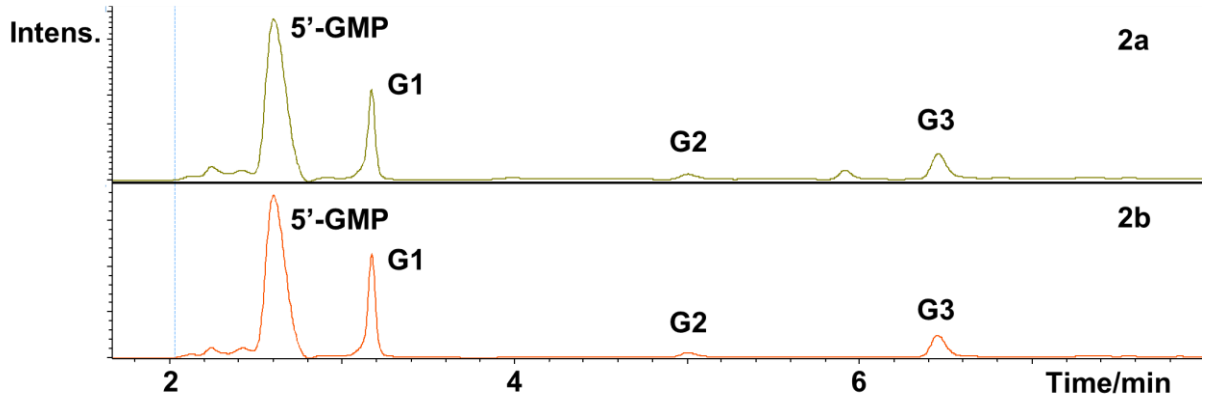

Figure S7. Photoreactions between Pt(IV) complexes and 5′-GMP monitored by LC-MS (detection at 254 nm). $[Pt^{II}(CH_3CN)(py)_2(GMP\text{-}H)]^+$ (G1, 756.48 m/z); $[Pt^{II}(HCOO)(py)_2(GMP)]^+$ (G2, 762.18 m/z); $[Pt^{II}(N_3)(py)_2(GMP)]^+$ (G3, 758.23 m/z).

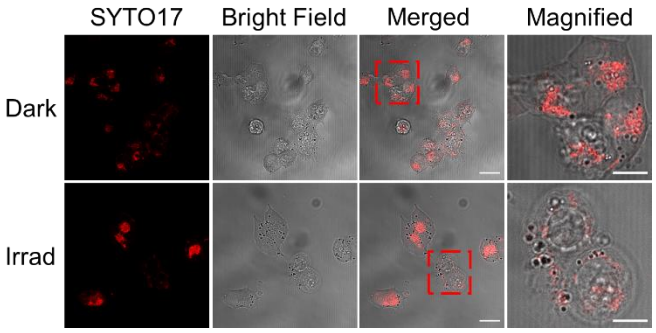

Figure S8. Fluorescence images of A2780 ovarian cancer cells treated with **2a** (photo IC_{50} concentration) in the dark and after irradiation with blue light (λ = 465 nm). Cells were stained by SYTO™ 17 (2.5 μM, $\lambda_{ex}/\lambda_{em}$ =633/638–759 nm) for 30 min. Scale bar = 20 μm (10 μm in magnification).

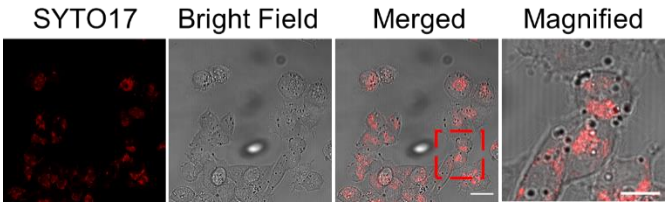

Figure S9. Fluorescence images of untreated A2780 ovarian cancer cells after irradiation with blue light (λ = 465 nm). Cells were stained by SYTO™ 17 (2.5 μM, $\lambda_{ex}/\lambda_{em}$ =633/638–759 nm) for 30 min. Scale bar = 20 μm (10 μm in magnification).

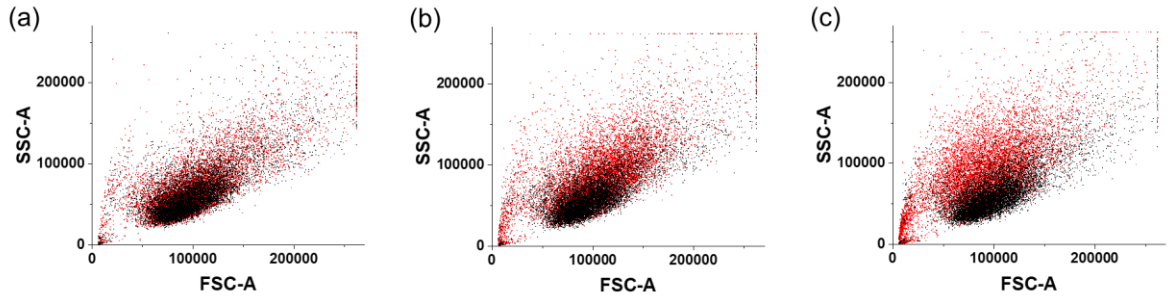

Figure S10. (a) FSC-SSC profiles of A2780 ovarian cancer cells with (red) and without (black) irradiation with blue light (λ = 465 nm) in absence of **2a**, in the presence of **2a** at (b) photo IC_{50} concentration and (c) 2× photo IC_{50} concentration.

References

1. M. F. Reichmann, S. A. Rice, C. A. Thomas, P. Doty, *J. Am. Chem. Soc.*, 1954, **76**, 3047–3053.
2. V. Vichai, K. Kirtikara, *Nat. Protoc.*, 2006, **1**, 1112−1116.