Illuminating Cytochrome P450 Binding: Ru(II)-Caged Inhibitors of CYP17A1

Ao Li,[a] Rahul Yadav,[b] Jessica K. White,[c] Mackenzie K. Herroon,[d] Brian P. Callahan,[e] Izabela Podgorski,*[,d,f] Claudia Turro,*[,c] Emily Scott,*[,b] and Jeremy J. Kodanko*[,a,f]

[a]Department of Chemistry, Wayne State University, 5101 Cass Ave, Detroit, Michigan 48202, United States

[b]Department of Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States

[c]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio 43210, United States

[d]Department of Pharmacology, School of Medicine, Wayne State University, Detroit, Michigan 48201, United States

[e]Department of Chemistry, Binghamton University, Binghamton, New York 13902, United States

[f]Barbara Ann Karmanos Cancer Institute, Detroit, Michigan 48201, United States

Corresponding author:
jkodanko@chem.wayne.edu, scottee@med.umich.edu, turro.1@osu.edu, ipodgors@med.wayne.edu

Supporting Information

(22 Pages)

Part A. General Considerations

All reagents were purchased from commercial suppliers and used as received. NMR spectra were recorded on a Varian FT-NMR Agilent-400 MHz Spectrometer. IR spectra were recorded on a Nicolet FT-IR spectrophotometer (KBr pellet). UV-vis spectra were recorded on a Varian Cary 50 spectrophotometer. All reactions were performed under ambient atmosphere unless otherwise noted. Anaerobic reactions were performed by purging the reaction solutions with Argon.

Part B. Experimental Procedures

Synthesis of [Ru(tpy)(Me_2bpy)(ABI)]Cl_2 (1). [Ru(tpy)(Me_2bpy)Cl]Cl[1] (35.4 mg, 0.06 mmol) was added to a solution of abiraterone[2] (ABI) (42.0 mg, 0.12 mmol) in a 1: 1 mixture of EtOH and H_2O (15.0 mL) under inert atmosphere in a pressure flask. The solution was purged with Ar for 10 min at room temperature, then the pressure flask was sealed and the reaction mixture refluxed for 20 h under inert atmosphere. The color of the reaction mixture changed from purple to brown. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was purified over neutral alumina (5% MeOH/ DCM) in the dark to give the title complex as a brown solid (46.0 mg, 81.5%): ^{1}H NMR (400 MHz CD_3OD) δ 8.84–8.80 (m, 3H), 8.78–8.74 (m, 3H), 8.70 (d, 1H, *J* = 8.0 Hz), 8.66 (d, 1H, *J* = 8.0 Hz), 8.61 (d, 1H, *J* = 8.0 Hz), 8.55 (d, 1H, *J* = 8.0 Hz), 8.49 (d, 2H, *J* = 8.0 Hz), 8.42 (d, 1H, *J* = 5.2 Hz), 8.33–8.32 (m, 2H), 8.30–8.20 (m, 6H), 8.17–8.10 (m, 3H), 7.82–7.74 (m, 6H), 7.72–7.67 (m, 2H), 7.63–7.58 (m, 4H), 7.44 (d, 2H, *J* = 12.8 Hz), 7.11–7.09 (m, 2H), 7.03 (d, 2H, *J* = 7.6 Hz), 6.03 (s, 1H), 5.84 (s, 1H), 5.37 (s, 2H), 4.87–3.38 (m, 2H), 2.25–2.16 (m, 6H), 2.06–1.88 (m, 14H), 1.84–1.81 (m, 2H), 1.70–1.38 (m, 20H), 1.21–1.15 (m, 2H), 1.11-0.98 (m, 14H), 0.74 (s, 3H), 0.69 (s, 3H); IR (KBr) ν_{max} (cm^{-1}) 3751, 3649, 3386, 2972, 2928, 2896, 2844, 2380, 2350, 2315, 1600, 1560, 1542, 1445, 1386, 1282, 1238, 1156, 1120, 1062, 954, 910, 777, 735, 705, 673; EIMS calcd for $C_{51}H_{54}N_6ORu$ (M^{+2}) 434.17, found 434.02; UV-vis λ_{max} = 475 nm (ε = 7040 $M^{-1}cm^{-1}$); Anal. Calcd for $C_{51}H_{64}Cl_2N_6O_6Ru$: (**1**•5 H_2O) C, 59.53; H, 6.27; N, 8.17. Found: C, 59.51; H, 6.06; N, 8.08.

Synthesis of [Ru(tpy)(biq)(ABI)](Cl)$_2$ (2). To a solution of ABI (31.0 mg, 0.09 mmol) in a 1: 1 mixture of EtOH and H_2O (10 mL) under inert atmosphere in a pressure flask [Ru(tpy)(biq)(Cl)](PF$_6$)[3] (34.2 mg, 0.04 mmol) was added. The solution was then purged with Argon for 10 min at room temperature, which was followed by being refluxed for 20 h under inert atmosphere. Color of the reaction mixture changed from purple to pink. Reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was then purified over neutral alumina (1% MeOH/ DCM) in the dark to remove the excess [Ru(tpy)(biq)(Cl)](PF$_6$) (a purple band with $R_f = 0.7$). Then the column was flushed with 5% MeOH/ DCM and the fractions were concentrated under reduced pressure. Next, the residue was dissolved in H_2O (15 mL) and NH_4PF_6 (20 mg) was added to the solution, resulting in the formation of pink precipitate which was filtered, washed with ice cold water (200 mL) and dried under pressure. The residue was then dissolved in acetone (15 mL) and tetrabutylammonium chloride (20 mg) was added to the solution, resulting in the formation of pink precipitates which were also filtered, washed with acetone (200 mL) and dried under reduced pressure. The title complex was obtained as a pink solid after layering in MeOH with diethyl ether (40.0 mg, 89.1%): ^{1}H NMR (400MHz CD_3OD) δ 9.20(d, 2H, *J* = 8.8 Hz), 9.08(d, 2H, *J* = 8.8 Hz), 8.94-8.90(m, 3H), 8.84(t, 2H, *J* = 8.8 Hz), 8.79-8.75(m, 1H), 8.73-8.68(m, 2H), 8.63(d, 1H, *J* = 7.6 Hz), 8.57(d, 1H, *J* = 8.0 Hz), 8.48(d, 2H, *J* = 9.2 Hz), 8.40-8.35(m, 4H), 8.18(d, 1H, *J* = 5.6 Hz), 8.15-8.03(m, 6H), 7.93(d, 1H, *J* = 5.6 Hz), 7.88(d, 2H, *J* = 8.4 Hz), 7.79-7.75(m, 3H), 7.71(d, 1H, *J* = 7.6 Hz), 7.61(d, 1H, *J* = 5.6 Hz), 7.56-7.49(m, 8H), 7.43(s, 1H), 7.33-7.26(m, 6H), 7.09(t, 1H, *J* = 7.6 Hz), 7.03(t, 1H, *J* = 8.0 Hz), 6.79(d, 2H, *J* = 9.2 Hz), 5.84(s, 1H), 5.64(s, 1H), 5.35(s, 2H), 3.48-3.37(m, 2H), 2.24-2.19(m, 4H), 2.15-2.09(m, 3H), 1.98-1.81(m, 9H), 1.67-1.26(m, 15H), 1.09(s, 3H), 1.07(s, 3H), 0.95-0.89(m, 6H), 0.81-0.76(m, 3H), 0.65(s, 3H), 0.51(s, 3H); IR (KBr) ν_{max} (cm^{-1}) 3352, 3140, 3033, 2964, 2917, 2848, 2670, 2512, 2299, 2214, 1749, 1703, 1669, 1595, 1542, 1509, 1404, 1340, 1251, 1213, 1178, 1145, 1119, 1098, 1054, 1042, 1020, 954, 843, 774, 748, 700, 647, 634; EIMS calcd for $C_{57}H_{54}N_6ORu$ (M^{+2}) 470.17, found 469.99; UV-vis λ_{max} = 535 nm (ε = 9850 M^{-1}cm^{-1}); Anal. Calcd for $C_{57}H_{68}Cl_2N_6O_8Ru$: (2•7 H_2O) C, 60.20; H, 6.03; N, 7.39. Found: C, 60.09; H, 6.00; N, 7.41.

Stability of 1 and 2 in phosphate-based buffer (100 mM, pH 7.4) and cell growth media (pH 7.2 Dulbecco's Modified Eagle's Medium).

	1	**2**
Concentration (mM)	3.09	2.70

Make stock solutions of complexes **1** and **2** in phosphate-based buffer (100 mM, pH 7.4) as the concentrations shown above.

(1) Assays in 100% phosphate-based buffer (100 mM, pH 7.4).

Take 2.0 μl of stock solution **1** or **2**, and dilute with 200 μl phosphate-based buffer (100 mM, pH 7.4), respectively. The assays were monitored by UV-Vis spectroscopy for 24 h at 23.0°C. All the complexes were shown stable in the dark.

(2) Assays in cell growth media (< 5% phosphate-based buffer).

Take 2.0 μl of stock solution **1** or **2**, and dilute with 200 μl cell growth media, respectively. The assays were monitored by UV-Vis spectroscopy for 24 h at 37.0°C. All the complexes were shown stable in the dark.

Stability of the control complexes 3 and 4 in DMSO and cell growth media (pH 7.2 Dulbecco's Modified Eagle's Medium).

	3	**4**
Concentration (mM)	2.59	2.81

Make stock solutions of complexes **3** and **4** in DMSO as the concentrations shown above.

(1) Assays in 100% DMSO.

Take 2.0 μl of stock solution **3** or **4**, and dilute with 200 μl DMSO, respectively. The assays were monitored by UV-Vis spectroscopy for 24 h at 23.0°C. All the complexes were shown stable in the dark.

(2) Assays in cell growth media (< 5% DMSO).

Take 4 μl of stock solution **3** or **4**, and dilute with 200 μl cell growth media, respectively. The assays were monitored by UV-Vis spectroscopy for 24 h at 37.0°C. All the complexes were shown stable in the dark.

Cytotoxicity of complexes 1 and 2 and the control complexes 3 and 4 on prostate cancer DU145 cells. The viability of caged ABI complexes **1** and **2**, and pyridine control complexes **3** and **4**, were tested in DU145 cells via MTT (using clear 96-well plates; Invitrogen/Thermo Fisher Scientific, Waltham, MA) assays, according to manufacturer's instructions. Briefly, DU145 cells were plated in two 96-well plates (one plate for "light" condition, one plate for "dark" condition) at a concentration of $5x10^3$ cells per well in 100 µl DMEM containing 10% FBS. After 24 h, cells were treated with the complexes by adding 100 µl of serum-free media with the appropriate concentration of each compound or vehicle (DMSO) control (0.5%). Four biological replicates for each condition were included in the plate. The plates were loosely wrapped with aluminum foil and incubated at 37°C for 30 min, followed by 10 min incubation in the dark at room temperature ("dark") or irradiated with a 250 W tungsten halogen lamp (Osram Xenophot HLX) powered by a 24 V power supply, with bandpass and water filters ("light"), as described previously.[4] Following photolysis, cells were placed back in the incubator and kept in the dark under a 5% CO_2 atmosphere at 37°C for another 48 h prior to performing viability assays.

For MTT assays, the media was removed, and 100 µL of fresh media plus 10 µL of a 12 mM MTT stock solution (5 mg of MTT dissolved in 1 ml of sterile PBS) were added to each well. Empty (no cells) wells containing 100 µL of media plus 10 µL of MTT solution were used as negative controls. Plates were incubated in the dark for 4 h, after which 85 µL of media was removed from each well and 50 µL of DMSO was added to lyse the cells. Plates were incubated at 37°C for another 10 min before taking absorbance measurements at 540 nm on a Tecan Infinite 200 Pro microplate reader.

Cell viability was expressed as percent control, with 100% equal to the measurement shown in the dark and in the absence of the complex.

Protein expression and purification. The human CYP17A1 gene was previously modified to result in protein omitting the N-terminal transmembrane helix and adding a 4-residue C-terminal histidine tag and inserted into the pCWori$^+$ plasmid. The resulting

pCW17A1Δ19H construct was transformed into *E. coli* DH5α cells already containing the pGro7 plasmid for the co-expression of chaperone groES-groEL. Expression and purification of CYP17A1 protein was carried out as described[5] with modifications to accommodate coexpression of groES-groEL. Purified CYP17A1 protein was stored in 50 mM Tris-Cl, pH 7.4, 20% (v/v) glycerol, 100 mM glycine at -80°C until use.

Ligand binding assay. Stocks of 100 μM complex **1** and the reference [Ru(tpy)(Me_2bpy)Cl]Cl were prepared in water. Complex **1** was either kept in the dark to avoid photo-activated cleavage or deliberately exposed to light irradiation for 30 min to release ABI. Release of ABI is characterized by the disappearance of shoulder peak at 420 nm. Binding to CYP17A1 was measured by observing shifts in the heme Soret peak in difference mode as described.[6] Briefly, with 100 nM purified CYP17A1 in its storage buffer in both sample and reference cuvettes (5 cm path length) a baseline was collected from 350-550 nm (UV-2101; Shimadzu Scientific Instruments, Columbia, MD). Then dark or light-exposed complex **1** (0-280 nM) was added to the sample cuvette. An equal concentration of the reference compound [Ru(tpy)(Me_2bpy)Cl]Cl was added to the reference cuvette to compensate for background absorbance from the Ru(II) species itself. The difference between the absorbance peak at ~424.5 nm (indicating formation of a bond between the CYP17A1 heme iron and the ligand nitrogen) and the broader trough at ~388-396 nm (indicating disappearance of the water-bound heme) was plotted vs. ligand concentration. CYP17A1 titration with light-exposed complex **1** titration was repeated three times and the data fit to the Morrison tight-binding equation[6] using nonlinear regression in GraphPad Prism v5.

Photolysis and quantum yield measurement. Photochemical experiments were performed using a 150 W Xe arc lamp (USHIO) in a MilliArc lamp housing unit powered by an LPS-220 power supply and an LPS-221 igniter (PTI). A 395 nm long pass filter (CVI Melles Griot) was used for the photolysis experiments, while a 500 nm bandpass filter (Thorlabs) was used for the ligand exchange quantum yield experiments. A solution of the sample in either CH_3CN or H_2O was placed in a 1 × 1 cm quartz cuvette, and the electronic absorption spectra were recorded at various time points during irradiation. The

photon flux of the lamp was determined using potassium tris(ferrioxalate) as a chemical actinometer (λ_{irr} = 500 nm, flux = 3.16×10^{-8} mol photons/min) The change in absorption at very early times was monitored in calculating the quantum yields.[6]

Part C. Spectral Data for 1 and 2

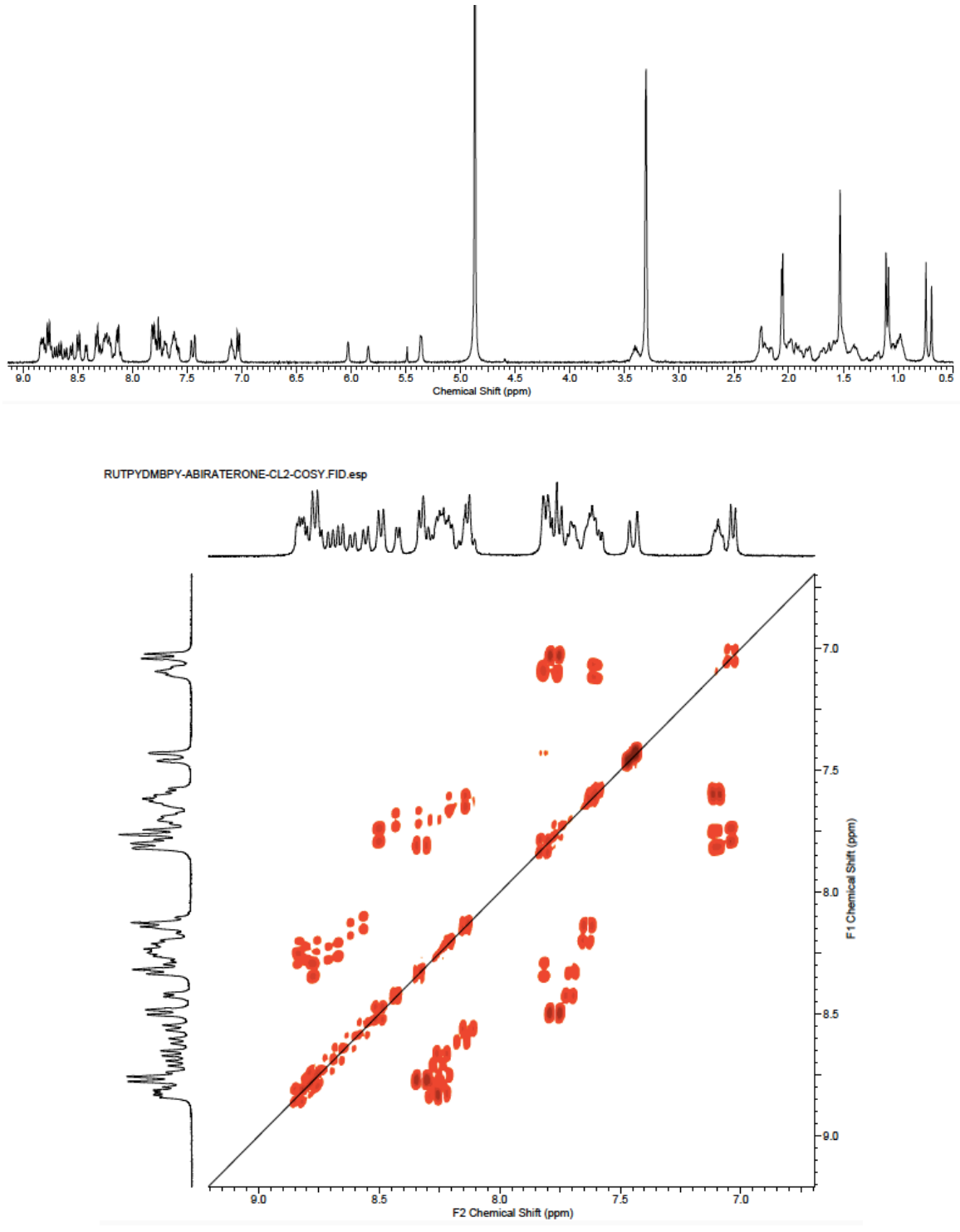
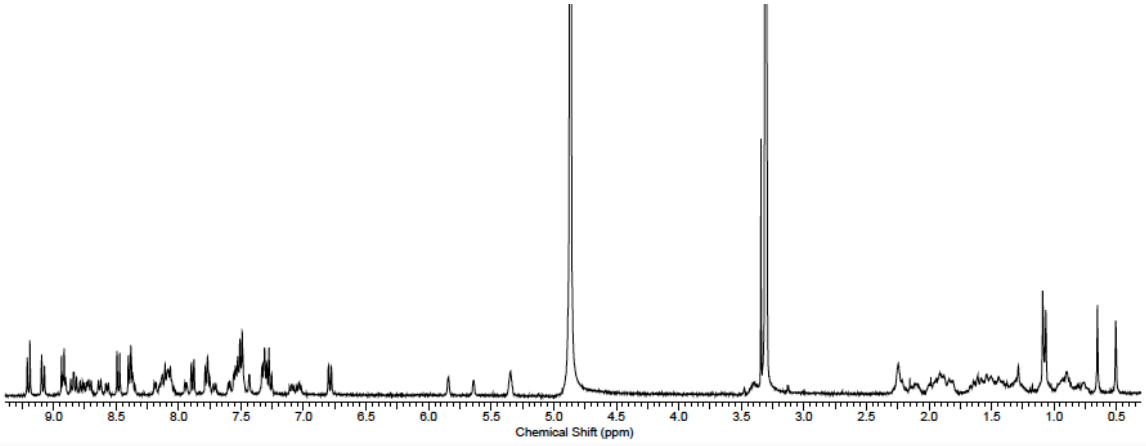


Fig. S1 ^{1}H NMR of **1** (top) and COSY (bottom) in CD_3OD.



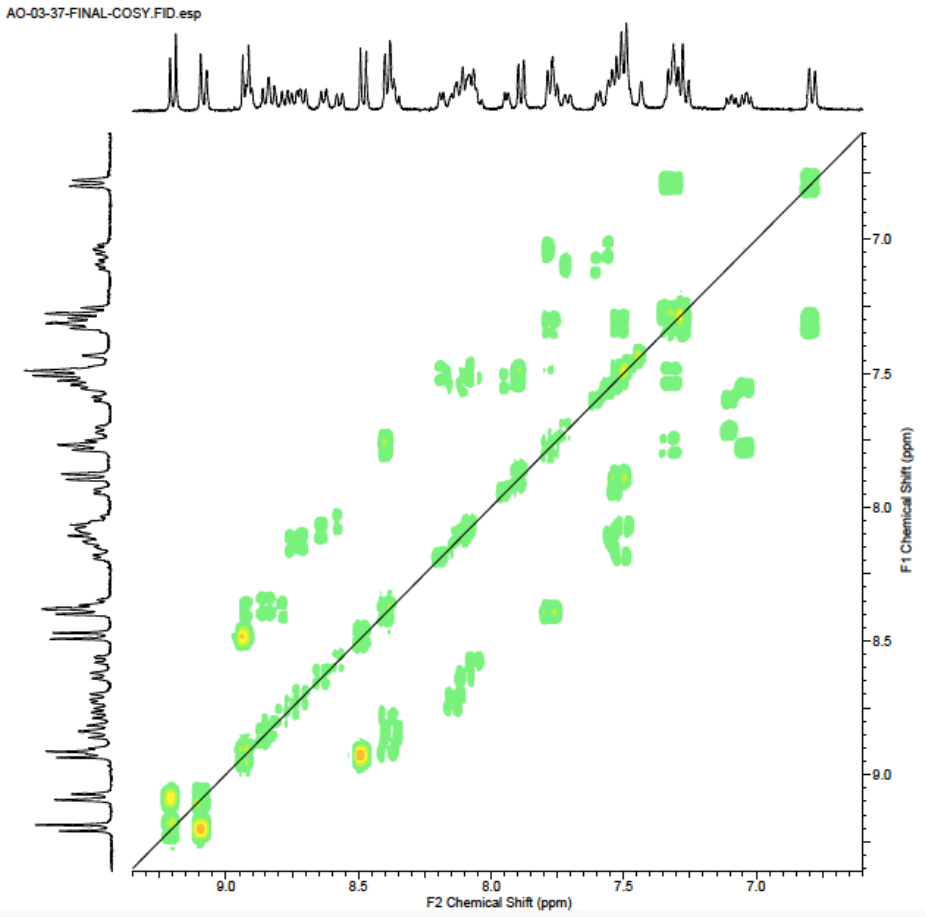


Fig. S2 ^{1}H NMR of **2** (top) COSY (bottom) in CD_3OD

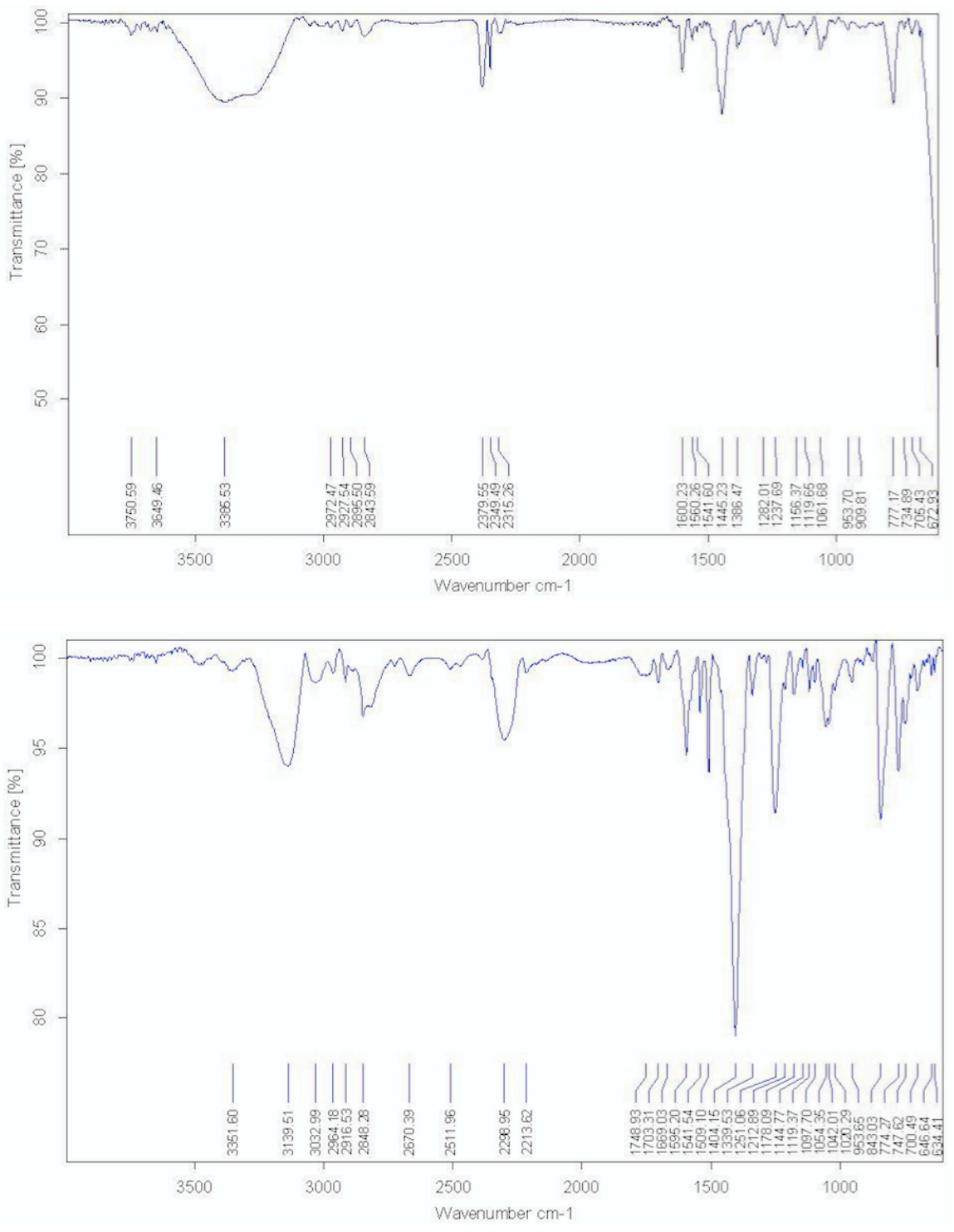


Fig. S3 IR (KBr) of **1** and **2**.

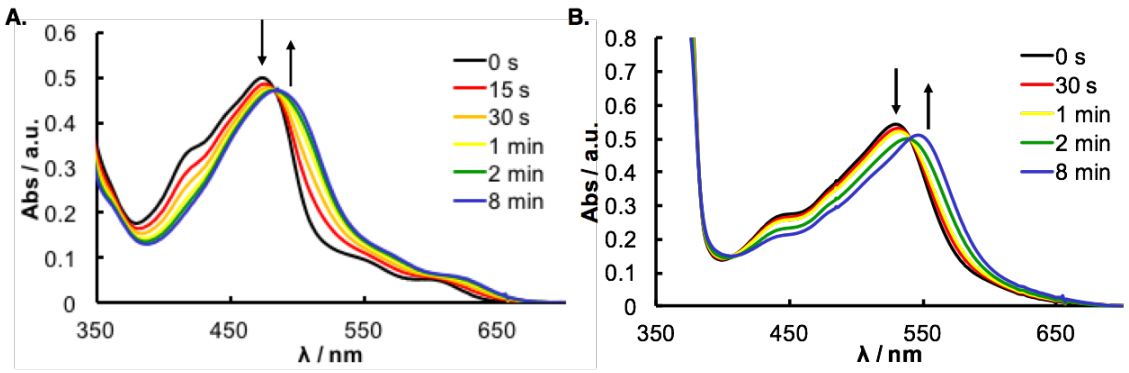


Fig. S4 Changes to the electronic absorption spectra of **1** (A) and **2** (B) as a function of irradiation time (λ_{irr} = 500 nm) in H_2O for 0–8 min.

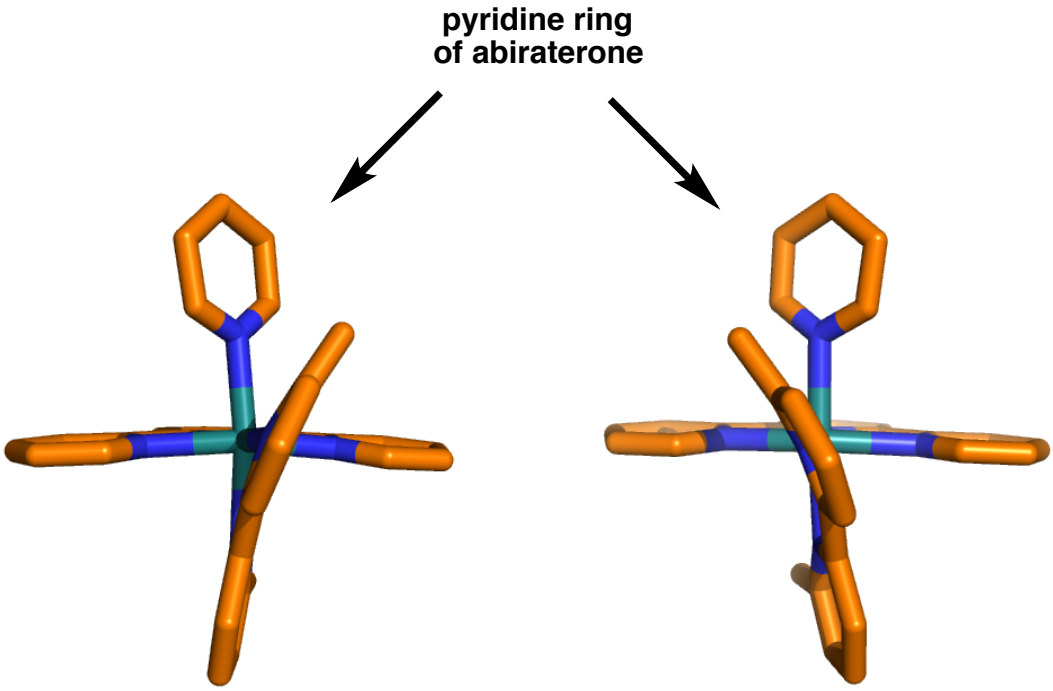
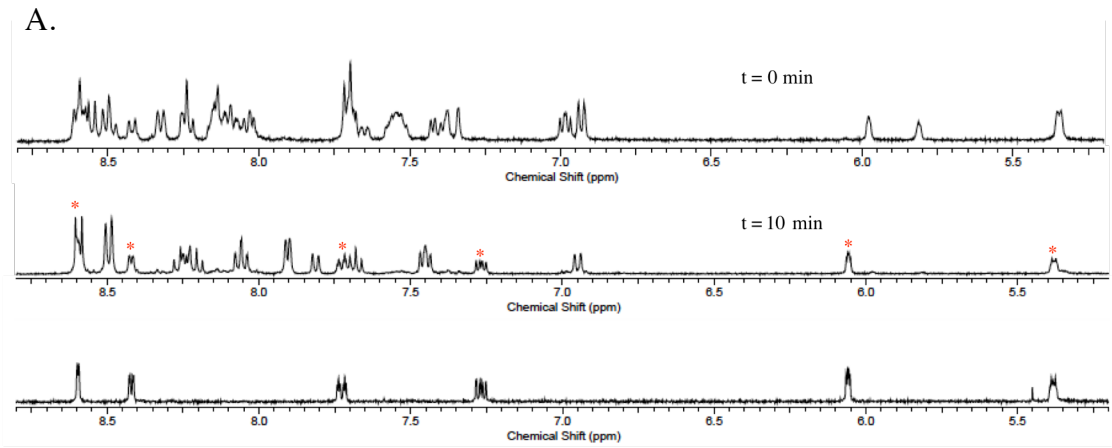


Fig. S5 Models of $[Ru(tpy)(Me_2bpy)(ABI)]^{2+}$ from complex **1** showing proposed diastereomeric atrope isomers generated from slow rotation of Me_2bpy ligand past the monodentate pyridine ring. Steroid skeleton of abiraterone is omitted for clarity. Models were generated using Spartan04.

Fig. S6 (A) ^{1}H NMR spectra of complex **1** in CD_3CN upon irradiation with visible light ($\lambda_{irr} \geq 395$ nm) at t = 0 (top) and 10 min (middle); ^{1}H NMR spectra of ABI in CD_3CN (bottom) (B) Schematic representation of ligand exchange of complex **1**.



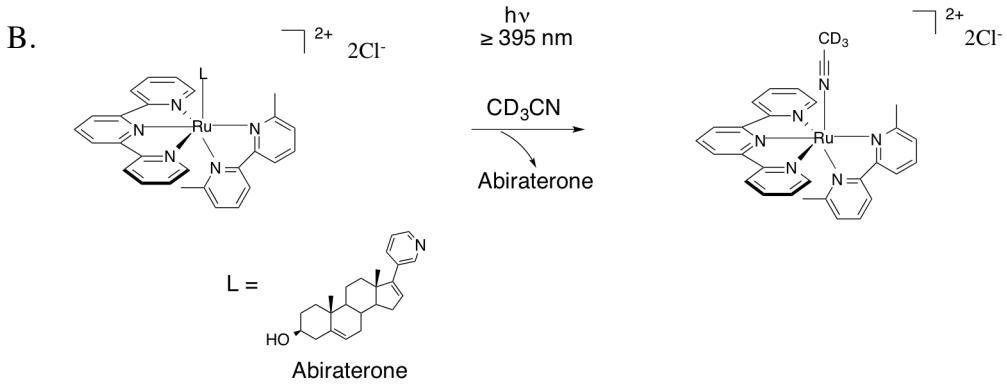
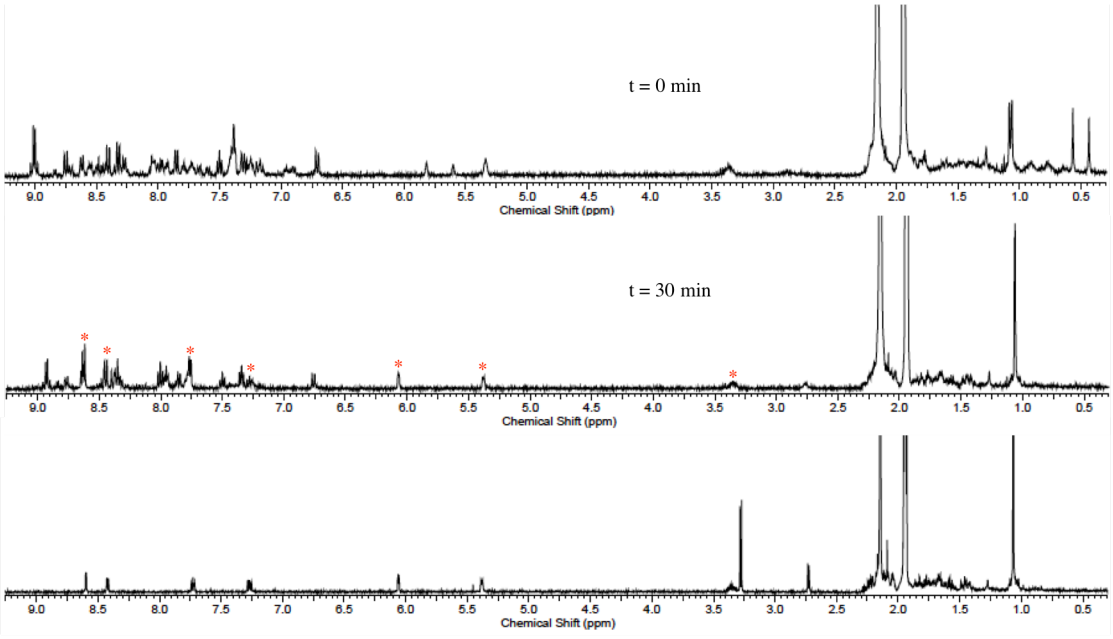
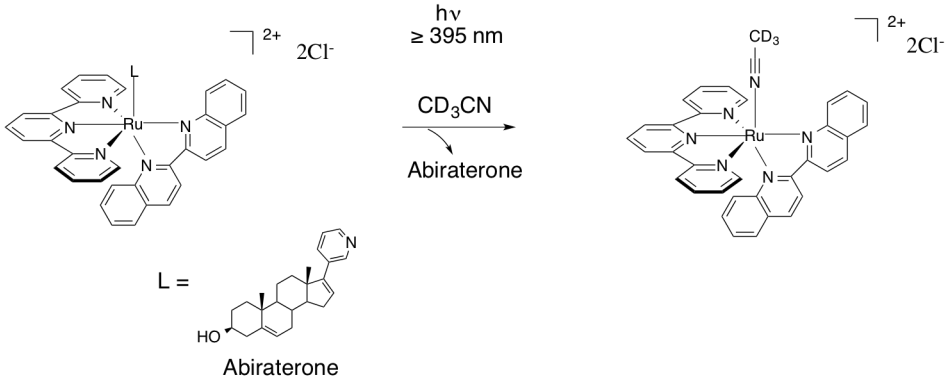


Fig. S7 (A) ^{1}H NMR spectra of complex **2** in CD_3CN upon visible light irradiation ($\lambda_{irr} \geq 395$ nm) at t = 0 (top) and 30 min (middle); ^{1}H NMR spectra of ABI in CD_3CN (bottom) (B) Schematic representation of ligand exchange of complex **2**





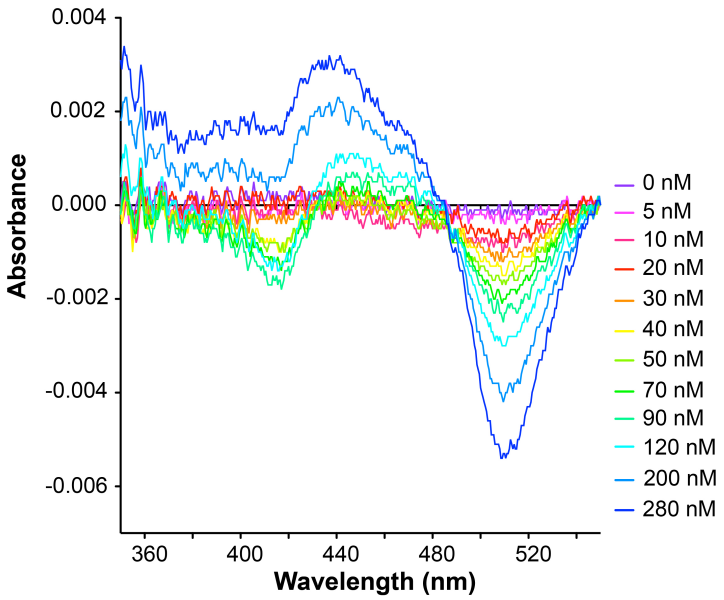
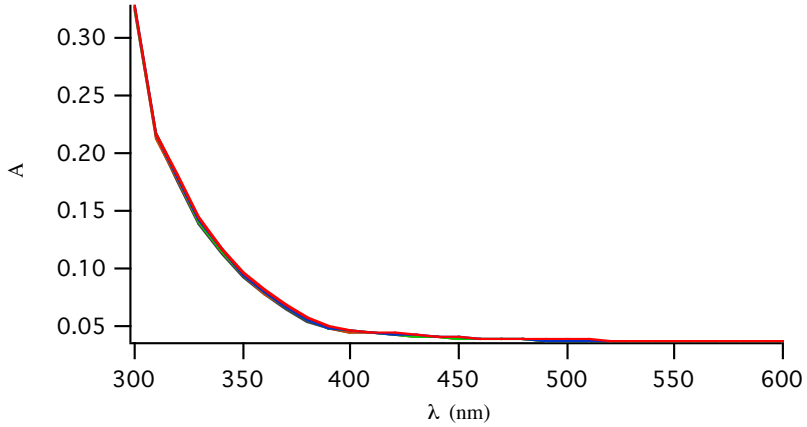
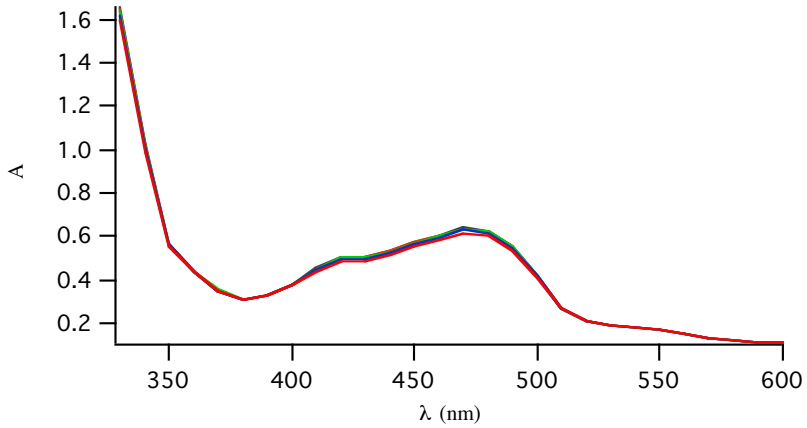


Fig. S8 Titration of CYP17A1 with increasing concentrations (0-280 nM) of complex **1** kept in the dark.

Phosphate-based buffer control at 23°C



Complex **1** in phosphate-based buffer at 23°C



Complex **2** in phosphate-based buffer at 23°C

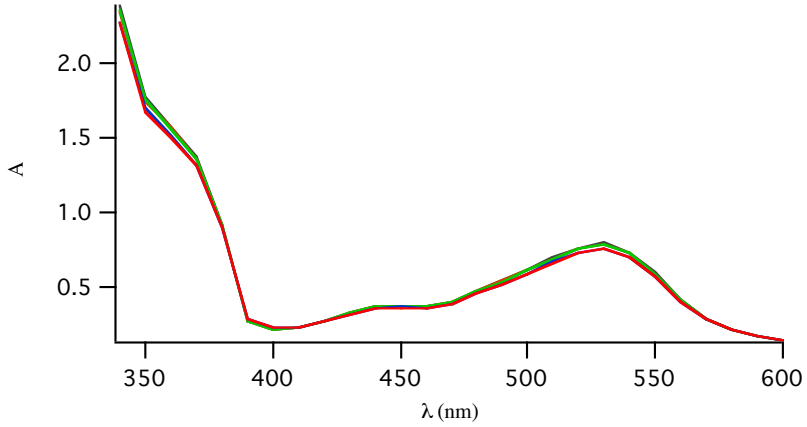
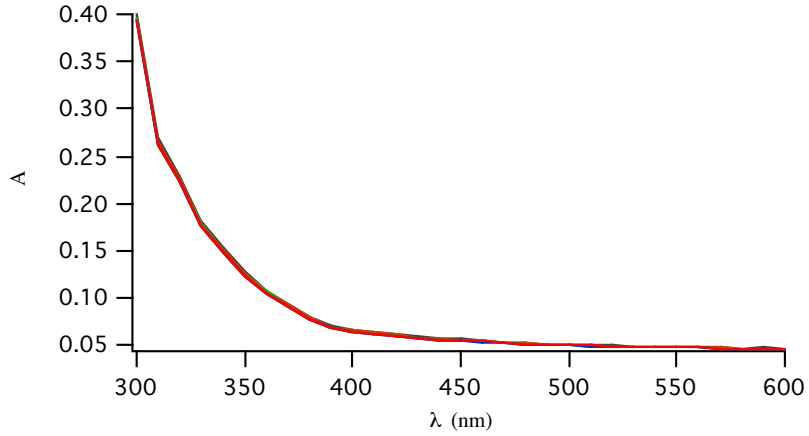
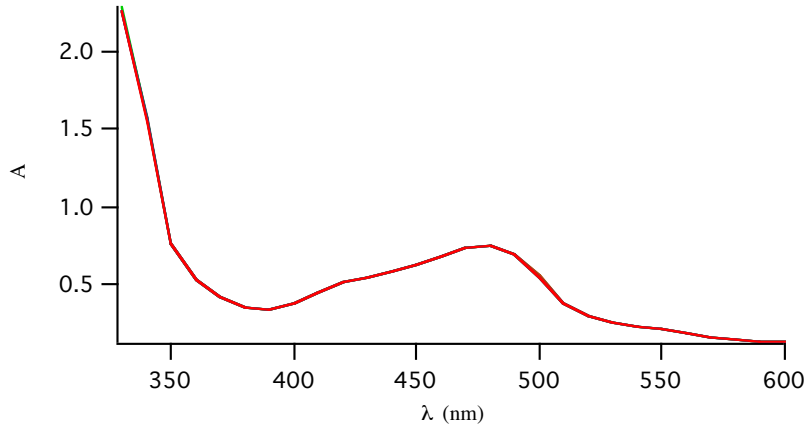


Fig. S9 Phosphate-based buffer control (top), complex **1** (middle) or **2** (bottom) in phosphate-based buffer (pH = 7.4) at 23°C, respectively. The spectra shown were taken at t = 0 (black), 4 (orange), 8 (green), 12 (blue) and 24 (red) h after baseline correction.

DMSO control at 23°C



Complex **3** in DMSO at 23°C



Complex **4** in DMSO at 23°C

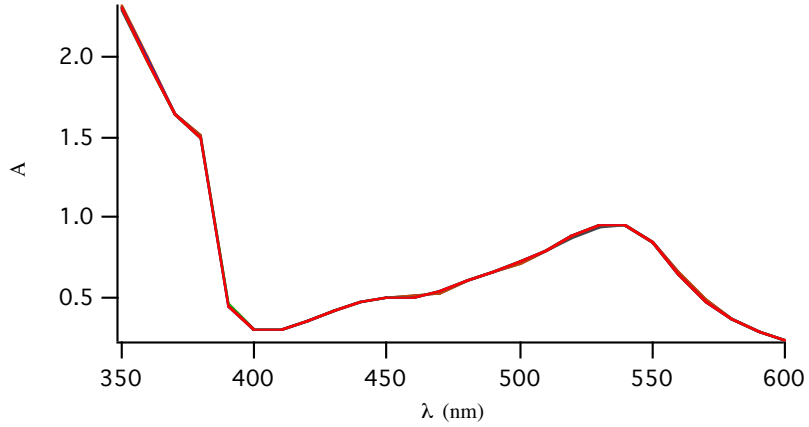
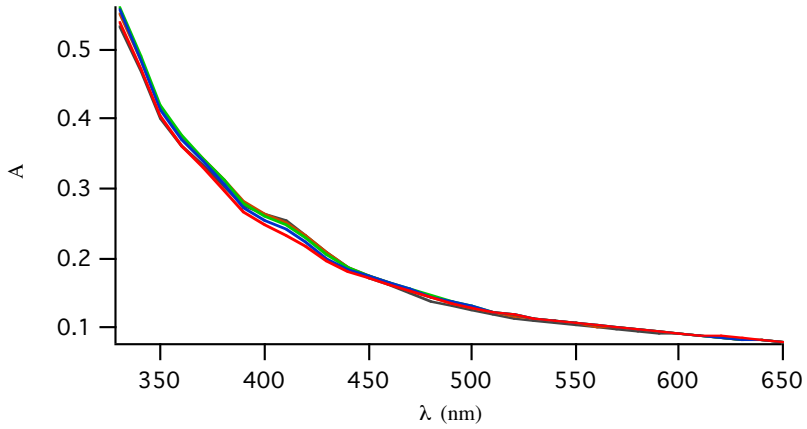
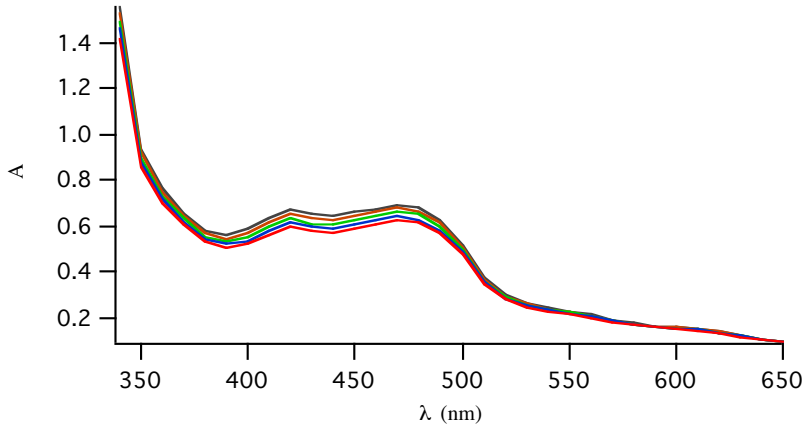


Fig. S10 DMSO control (top), complex **3** (middle) or **4** (bottom) in DMSO at 23°C, respectively. The spectra shown were taken at t = 0 (black), 4 (orange), 8 (green), 12 (blue) and 24 (red) h after baseline correction.

Cell growth media control at 37°C



Complex **1** in cell growth media at 37°C



Complex **2** in cell growth media at 37°C

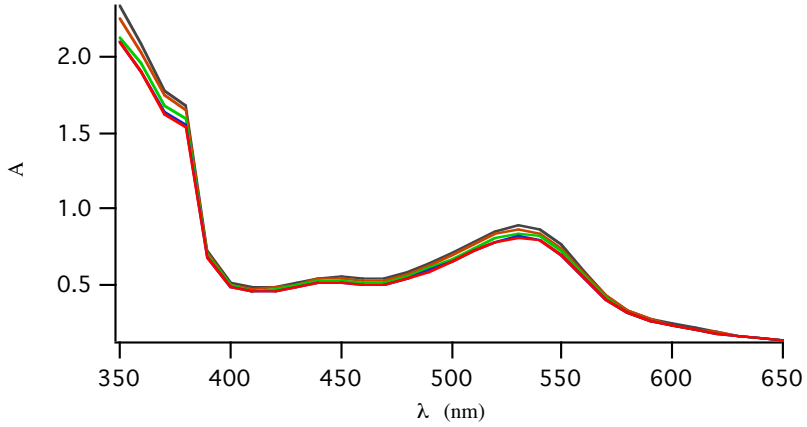
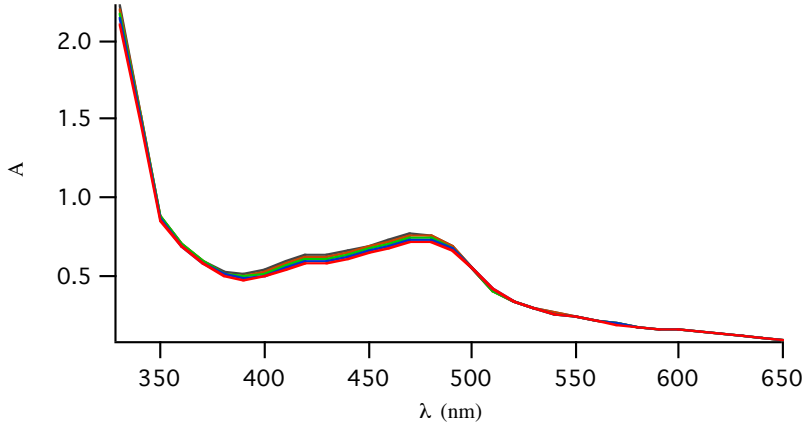


Fig. S11 Cell growth media control (top), complex **1** (middle) or **2** (bottom) in cell growth media at 37°C, respectively. The spectra shown were taken at t = 0 (black), 4 (orange), 8 (green), 12 (blue) and 24 (red) h after baseline correction. *Note:* The changes in absorbance of complex **1** and **2** were due to the background changes in cell growth media, and no isosbestic points were observed to indicate that the complexes were unstable.

Complex **3** in cell growth media at 37°C



Complex **4** in cell growth media at 37°C

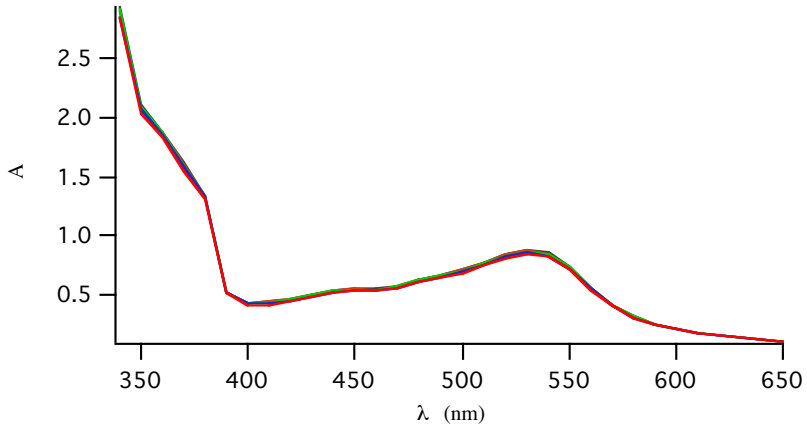


Fig. S12 Complex **3** (top) or **4** (bottom) in cell growth media at 37°C, respectively. The spectra shown were taken at t = 0 (black), 4 (orange), 8 (green), 12 (blue) and 24 (red) h after baseline correction.

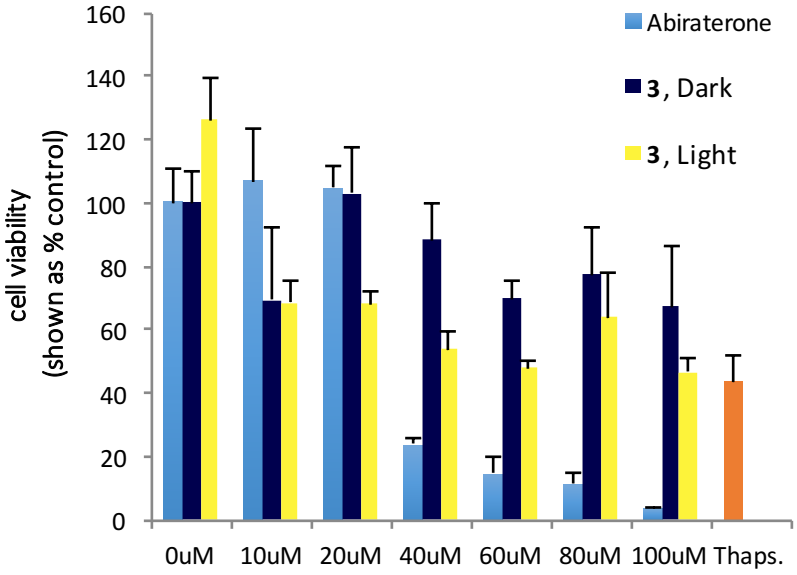


Fig. S13 Cell viability of **3** against DU145 cell line. The cell viability was determined by MTT assay after 48 h and is reported relative to control with only the vehicle (0.5% DMSO) added. Cells were incubated in the presence of **3** (10 μM to 100 μM) at 37°C for 30 min, followed by 10 min incubation in the dark at room temperature or irradiated with a 250 W tungsten halogen lamp ($\lambda_{irr} \geq 395$ nm). The cytotoxic compound thapsigargin (TPG; 10 μM) was used as a positive control. Error bars represent the standard error of the mean of quadruple wells, and data are representative of four independent experiments.

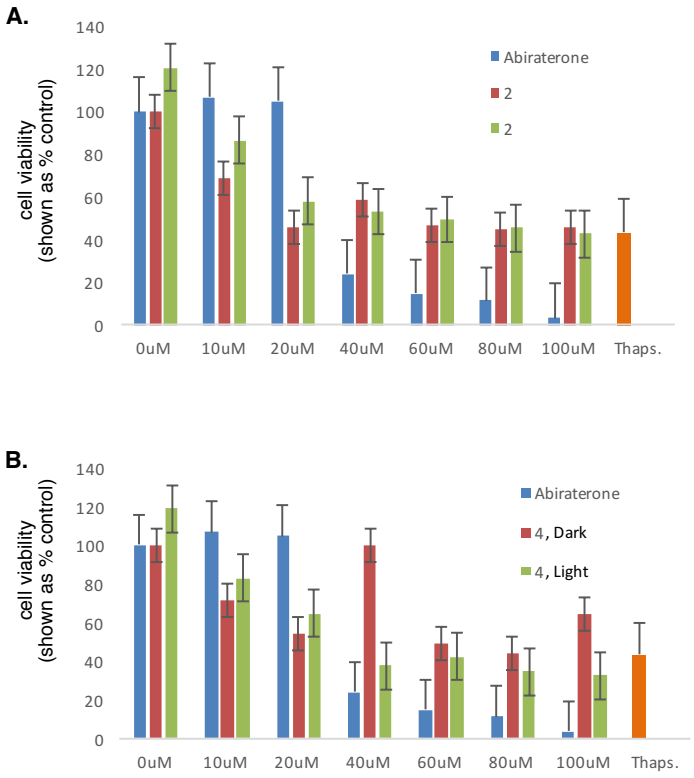


Fig. S14 Cell viability of **2** (A) and control complex **4** (B) against DU145 cell line. The cell viability was determined by MTT assay after 48 h and is reported relative to control with only the vehicle (0.5% DMSO) added. Cells were incubated in the presence of **2** or **4** (10 μM to 100 μM) at 37°C for 30 min, followed by 10 min incubation in the dark at room temperature or irradiated with a 250 W tungsten halogen lamp ($\lambda_{irr} \geq 395$ nm). The cytotoxic compound thapsigargin (TPG; 10 μM) was used as a positive control. Error bars represent the standard error of the mean of quadruple wells, and data are representative of four independent experiments.

Part D. References

1. Bahreman, A.; Limburg, B.; Siegler, M. A.; Bouwman, E.; Bonnet, S. *Inorg. Chem.* **2013,** *52* (16), 9456-9469.

2. Potter, G. A.; Hardcastle, I. R.; Jarman, M. *Org. Prep. Proc. Int.* **1997,** *29* (1), 123-128.

3. Bessel, C. A.; Margarucci, J. A.; Acquaye, J. H.; Rubino, R. S.; Crandall, J.; Jircitano, A. J.; Takeuchi, K. J. *Inorg. Chem.* **1993,** *32* (25), 5779-5784.

4. Respondek, T.; Sharma, R.; Herroon, M. K.; Garner, R. N.; Knoll, J. D.; Cueny, E.; Turro, C.; Podgorski, I.; Kodanko, J. J. *ChemMedChem* **2014,** *9* (6), 1306-1315.

5. Petrunak, E. M.; DeVore, N. M.; Porubsky, P. R.; Scott, E. E. *J. Biol. Chem.* **2014,** *289* (47), 32952-32964.

6. DeVore, N. M.; Smith, B. D.; Wang, J. L.; Lushington, G. H.; Scott, E. E. *Drug Metab. Dispos.* **2009,** *37* (6), 1319-1327.

7. M. Monalti, A. Credi, L. Prodi and M. T. Gandolfi, *Handbook of Photochemistry-Third Edition*, CRC Press LLC, 2006.