

SUPPORTING INFORMATION

Photoactivation of imatinib-antibody conjugate using low-energy visible light from Ru(II)-polypyridyl cages

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Materials. The precursors [Ru(tpy)(Me₂bpy)Cl](PF₆),^{S1} [Ru(tpy)(biq)Cl](PF₆),^{S2} [Ru(tpy)(Me₂dppn)Cl](PF₆),^{S3} and Ru(tpy-COOH)Cl₃,^{S4} were synthesized according to literature procedures. Ammonium hexafluorophosphate and deuterated acetone were purchased from Aldrich. Diethyl ether, acetone, and acetonitrile were obtained from Fisher Scientific, and 200 proof ethanol was purchased from Decon Laboratories. The c-kit/CD117 antibody was purchased from Santa Cruz Biotechnology and the Donkey anti-Rabbit antibody was obtained from LI-COR. 6,6'-dimethyl-2,2'-bipyridine (Me₂bpy), lithium chloride, sodium chloride, and potassium dihydrogen phosphate were purchased from Sigma-Aldrich. Potassium chloride, disodium hydrogen phosphate, *N*-hydroxysuccinimide (NHS), and *N,N'*-dicyclohexylcarbodiimide (DCC) were obtained from Aldrich. 2,2'-biquinoline (biq) was acquired from Acros, 4-ethylmorpholine was purchased from Fluka, and imatinib was obtained from Selleck Chemicals. EGF-Stimulated A431 cell lysate was purchased from EMD Millipore and the 10 to 180 kDa protein ladder was purchased from Thermofisher Scientific. The blotting grade Blockers (nonfat dry milk) was obtained from Bio-Rad.

Methods.

Synthesis of [Ru(tpy-COOH)(Me₂bpy)Cl]Cl. The compound was synthesized following a procedure adapted from a previously reported method.^{S4} To a 50 mL roundbottom flask were added Ru(tpy-COOH)Cl₃ (0.118 g, 0.243 mmol), Me₂bpy (0.060 g, 0.33 mmol), LiCl (0.100 g, 2.36 mmol), 4-ethylmorpholine (1 mL), and 24 mL of 5:1 ethanol/H₂O. The solution was heated at reflux for 4 h in the dark. After cooling to room temperature, the solvent was removed under vacuum. The product was purified by column chromatography using a silica stationary phase and a 3:1:1 acetone/methanol/LiCl(aq) mobile phase. The purple-red band was collected and the volume was reduced to about 20 mL. The product was precipitated following the addition of 1 mL of concentrated HCl and collected by vacuum filtration. Yield: 0.085 g, 55%.

Synthesis of [Ru(tpy-COOH)(biq)Cl]Cl. The compound was synthesized following the same procedure as described above using Ru(tpy-COOH)Cl₃ (0.102 g, 0.210 mmol), biq (0.080 g, 0.32 mmol), LiCl (0.100 g, 2.36 mmol), 4-ethylmorpholine (1 mL), and 24 mL of 5:1 ethanol/H₂O. Yield: 0.092 g, 62%.

Synthesis of 1. To a 50 mL round bottom flask were added 0.035 g (0.050 mmol) of [Ru(tpy)(Me₂bpy)Cl](PF₆), 0.052 g (0.11 mmol) of imatinib, and 15 mL of 2:1 ethanol/H₂O. The mixture was heated at reflux for 16 h while being protected from light with aluminum foil. The reaction was cooled to room temperature, and the resulting [Ru(tpy)(Me₂bpy)(imatinib)]Cl₂ was converted via

metathesis to [Ru(tpy)(Me₂bpy)(imatinib)](PF₆)₂ by the addition of the dark orange-brown solution to excess aqueous NH₄PF₆. After stirring while protected from light for 15 min, the resulting brown precipitate was collected by vacuum filtration and washed with 25 mL of H₂O and 25 mL of diethyl ether. The solid was dissolved in a minimal amount of acetone and filtered to remove undissolved impurities, and the solution was added dropwise to 100 mL of diethyl ether to induce precipitation. The product was collected by vacuum filtration and washed with 20 mL of diethyl ether. Yield: 0.046 g, 70%. ESI-MS: [M-2PF₆]²⁺, *m/z* = 506.2.

Synthesis of 2. The compound was synthesized following the same procedure as described above using [Ru(tpy)(biq)Cl](PF₆) (0.026 g, 0.034 mmol) and imatinib (0.050 g, 0.10 mmol). Yield: 0.035 g, 75%. ESI-MS: [M-2PF₆]²⁺, *m/z* = 542.2.

Synthesis of 3. The compound was synthesized following the same procedure as described above using [Ru(tpy)(Me₂dppn)Cl](PF₆) (0.030 g, 0.034 mmol) and imatinib (0.080 g, 0.16 mmol). Yield: 0.035 g, 70%. ESI-MS: [M-2PF₆]²⁺, *m/z* = 594.3.

Synthesis of 1-COOH. The compound was synthesized following the same procedure as described above using [Ru(tpy-COOH)(Me₂bpy)Cl](PF₆) (0.025 g, 0.039 mmol) and imatinib (0.040 g, 0.081 mmol). Yield: 0.034 g, 65%. ESI-MS: [M-2PF₆]²⁺, *m/z* = 528.1.

Synthesis of 2-COOH. The compound was synthesized following the same procedure as described above using [Ru(tpy-COOH)(biq)Cl](PF₆) (0.035 g, 0.050 mmol) and imatinib (0.052 g, 0.11 mmol). Yield: 0.051 g, 72%. ESI-MS: [M-2PF₆]²⁺, *m/z* = 564.1.

Synthesis of 1-Ab. The precursor **1-COOH** (0.005 g, 0.004 mmol), NHS (0.001 g, 0.0087 mmol), and DCC (0.002 g, 0.0097 mmol) were dissolved in 0.5 mL of dry DMF in an amber vial. The solution was stirred in the dark for 30 min at 0°C in an ice bath followed by 5 h at room temperature. The storage buffer of the antibody was exchanged for PBS buffer (pH 7.5) using 40K MWCO, 0.5 mL Zeba spin desalting columns. A 15 μL sample of the resulting solution was combined with 100 μL of the c-kit antibody in PBS buffer with a pH of 7.5 and allowed to incubate at room temperature in the dark for 1 h. The product was purified using spin desalting columns with a 40,000 g/mol molecular weight cut-off (MWCO). The **1-Ab** conjugate eluted upon centrifugation at 1500 × *g*, and the unreacted metal complex (MC) remained on the column. The MC:Ab ratio of the purified sample was determined to be 10:1 using the electronic absorption data for the individual components **1** and **Ab** compared to the product.

Synthesis of 2-Ab. The compound was synthesized following the same procedure as described above for **1-Ab** using **2-COOH** (0.005 g, 0.004 mmol). The MC:Ab ratio of the purified sample was determined to be 3:1 using the electronic absorption data for the individual components **2** and **Ab** compared to the product.

¹H NMR spectroscopy. The ¹H NMR spectra were collected with a Bruker 400 MHz DPX spectrometer. Samples were dissolved in acetone-*d*₆ and protected from light prior to analysis. Chemical shift values were referenced to the residual acetone signal.

Electrospray ionization mass spectrometry (ESI-MS). Electrospray ionization mass spectrometry was performed using a Bruker micrOTOF instrument with CH₃OH as the eluent.

Electronic absorption spectroscopy. Electronic absorption spectra were measured using a Hewlett-Packard 8453 diode array spectrometer. Samples were measured in a 1×1 cm quartz cuvette at room temperature.

Photochemistry. Solutions of complexes **1-3** in CH₃CN or H₂O and **1-Ab** and **2-Ab** in PBS buffer (pH 7.5) were irradiated in a 1×1 cm and 1×0.1 cm quartz cuvette, respectively, with red light using a 590 nm long-pass filter on a 150 W Xe arc lamp (USHIO) in a Milliarc lamp housing unit equipped with an LPS-220 power supply and an LPS-221 igniter (PTI). Electronic absorption spectra were recorded at time intervals until no more spectral changes were observed. The quantum yields for ligand dissociation with $\lambda_{\text{irr}} = 500 \text{ nm}$ (Φ_{500}) were determined by irradiating samples that were absorbance-matched at 500 nm using a 500 nm bandpass filter on the Xe arc lamp. The rate of moles of **1-3** reacted at early irradiation times was determined by monitoring the decrease in the absorbance of the MLCT maximum as a function of time. The lamp's photon flux was determined using the ferrioxalate chemical actinometer as previously described, resulting in a flux of $5.06 \pm 0.31 \text{ mol photons/min}$.^{S5}

¹H NMR Photolysis. ¹H NMR photolysis experiments were carried out in an NMR tube with acetonitrile-*d*₃ as the solvent for complex **1-3** and irradiated with a 395 nm long-pass filter on a 150 W Xe arc lamp (USHIO) in a Milliarc lamp housing unit equipped with an LPS-220 power supply and an LPS-221 igniter (PTI).

Singlet oxygen quantum yield. The quantum yields of ¹O₂ production (Φ_s) were measured in CH₃OH in a 1×1 cm quartz cuvette. [Ru(bpy)₃]²⁺ was used as a standard ($\Phi_s = 0.81$)^{S6} and 1,3-diphenylisobenzofuran (DPBF) was used as a ¹O₂ trap and fluorescent probe. The samples were absorbance matched at the irradiation wavelength ($A = 0.1$ at 460 nm) and irradiated in the presence of 1.0 μM DPBF. The decrease in emission of DPBF ($\lambda_{\text{exc}} = 405 \text{ nm}$ and $\lambda_{\text{em}} = 479 \text{ nm}$) was monitored as a function of time. The emission intensity plotted vs irradiation time produced a linear trend, the slope of which was compared to the slope of the [Ru(bpy)₃]²⁺ sample to determine the quantum yields.

Western Blot Analysis. The protein ladder and 12 micrograms of A431 cell lysate were electrophoresed on an 7.5% SDS-Polyacrylamide gel for 1.5 hours at 120V. The protein was transferred onto a PVDF membrane for 2.5 hours at room temp at 80V. After the transfer was complete, the membranes were incubated in 5% nonfat milk blocking solution (1X TBST buffer pH7.5) at room temperature for 1 hour. The membrane was then incubated with the primary antibody, c-Kit, **1-Ab**, or **2-Ab** (dilution 1:500) overnight at 4°C. After three washes with 1X TBST pH 7.5 buffer, the secondary antibody (donkey anti-Rabbit conjugated with an IR dye, dilution 1:5000) was incubated at room temperature for 1 hour. Lastly, the blots were imaged using a LI-COR Odyssey imaging system.

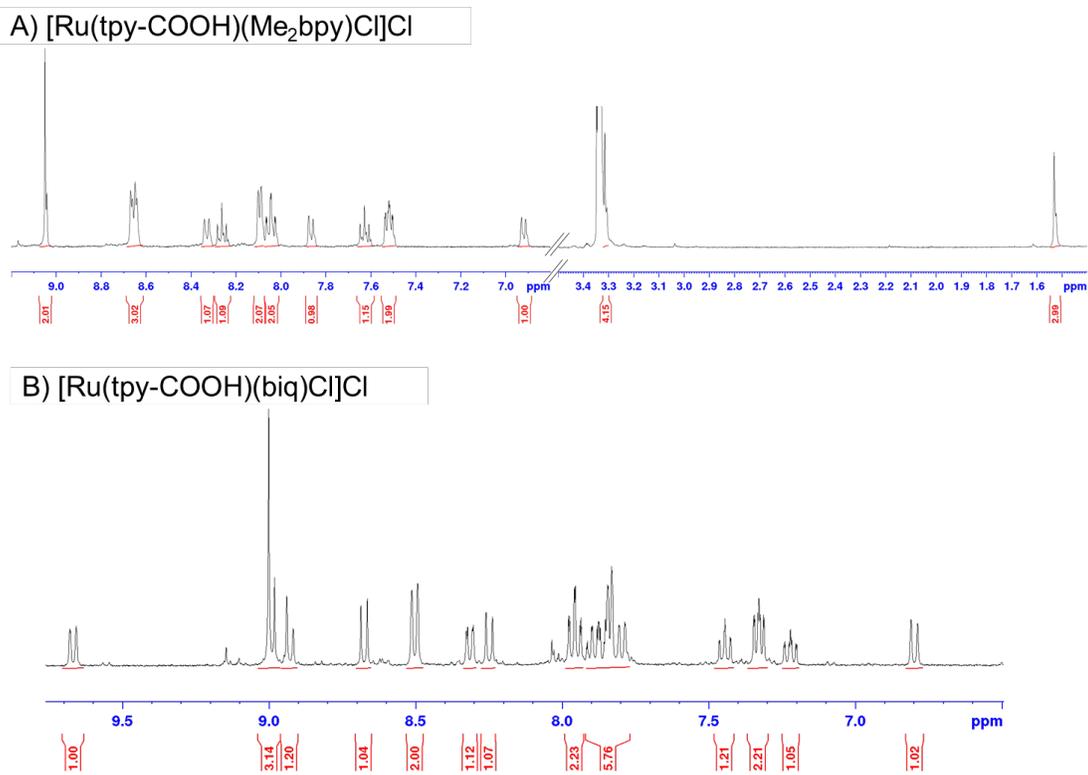


Figure S1. ^1H NMR spectra $[\text{Ru}(\text{tpy-COOH})(\text{Me}_2\text{bpy})\text{Cl}]\text{Cl}$ (A) and $[\text{Ru}(\text{tpy-COOH})(\text{biq})\text{Cl}]\text{Cl}$ (B) in CD_3OD .

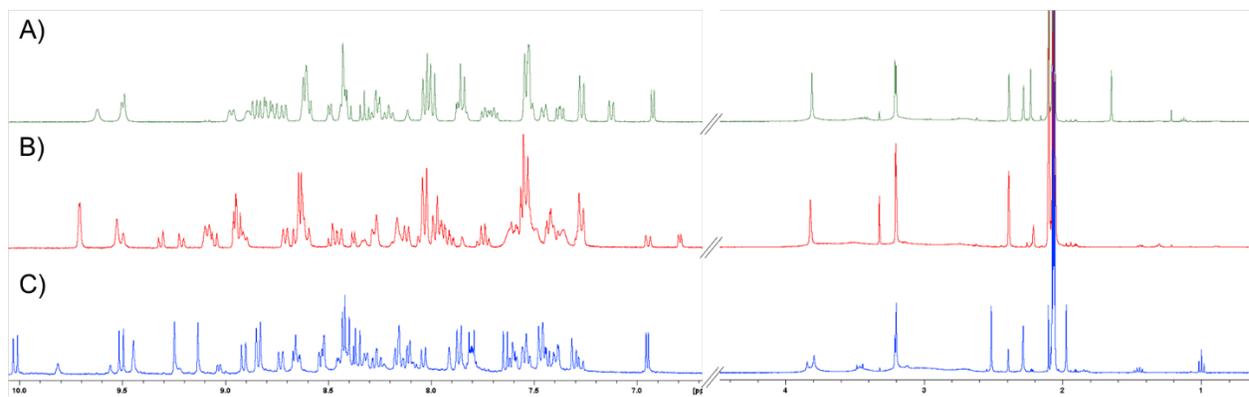


Figure S2. ^1H NMR spectra of **1** (A), **2** (B), and **3** (C) in $\text{acetone-}d_6$.

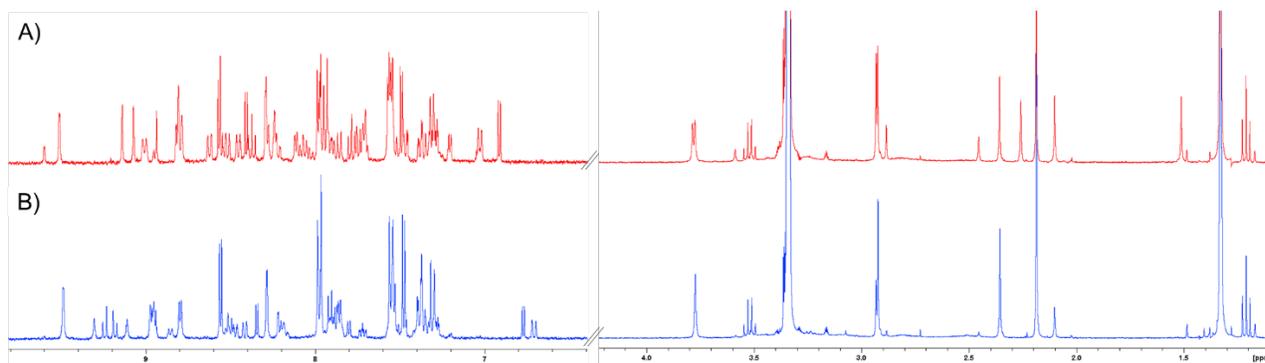


Figure S3. ^1H NMR spectra of **1-COOH** (A) and **2-COOH** (B) in acetone- d_6 .

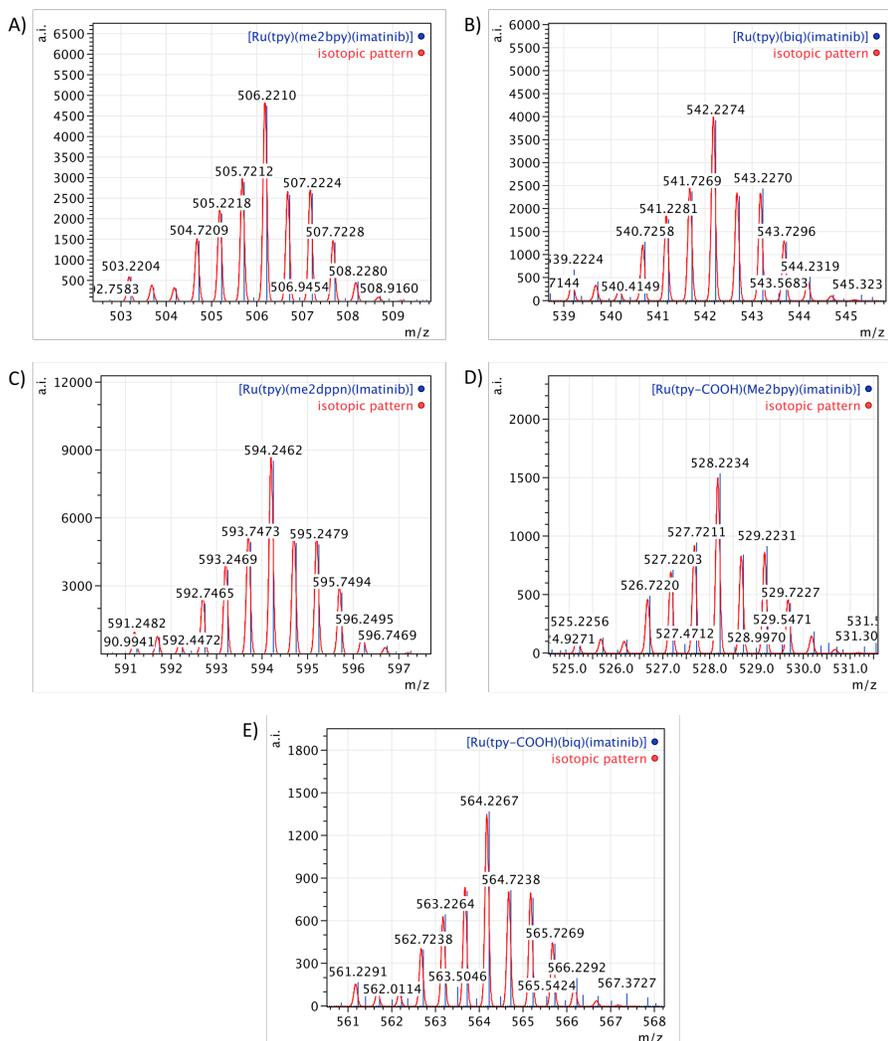


Figure S4. ESI-MS spectra of **1** (A), **2** (B), **3** (C), **1-COOH** (D), **2-COOH** (E) for the $[\text{M}-2\text{PF}_6]^{2+}$ peak with the experimental (—) and calculated isotopic pattern (—•—).

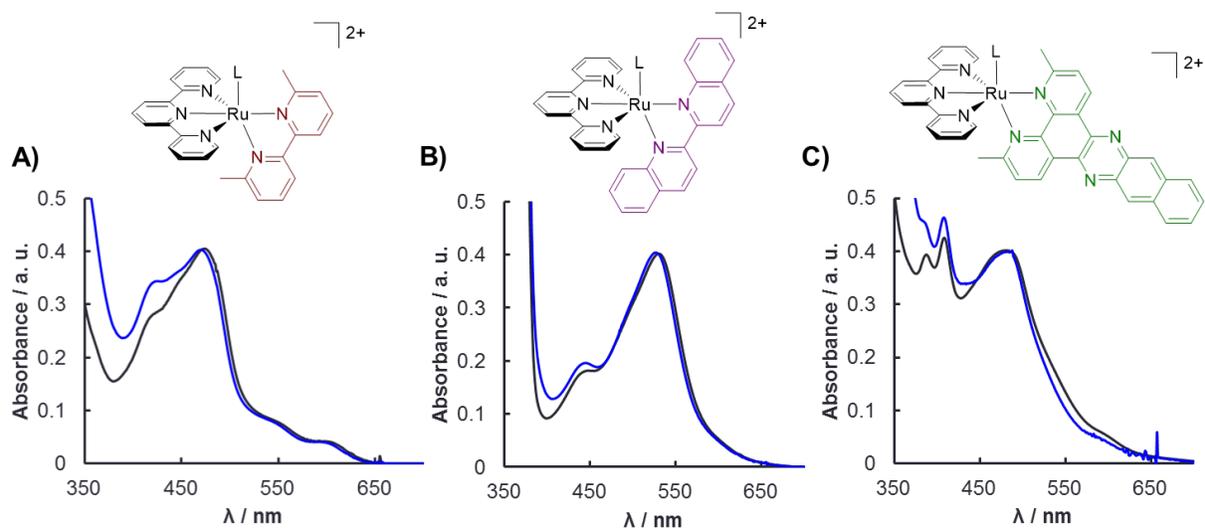


Figure S5. Overlaid electronic absorption spectra of $[\text{Ru}(\text{tpy})(\text{NN})(\text{py})](\text{PF}_6)_2$ (black) and $[\text{Ru}(\text{tpy})(\text{NN})(\text{imatinib})](\text{PF}_6)_2$ (blue) in CH_3CN when NN = Me₂bpy (A), biq (B), and Me₂dppn (C).

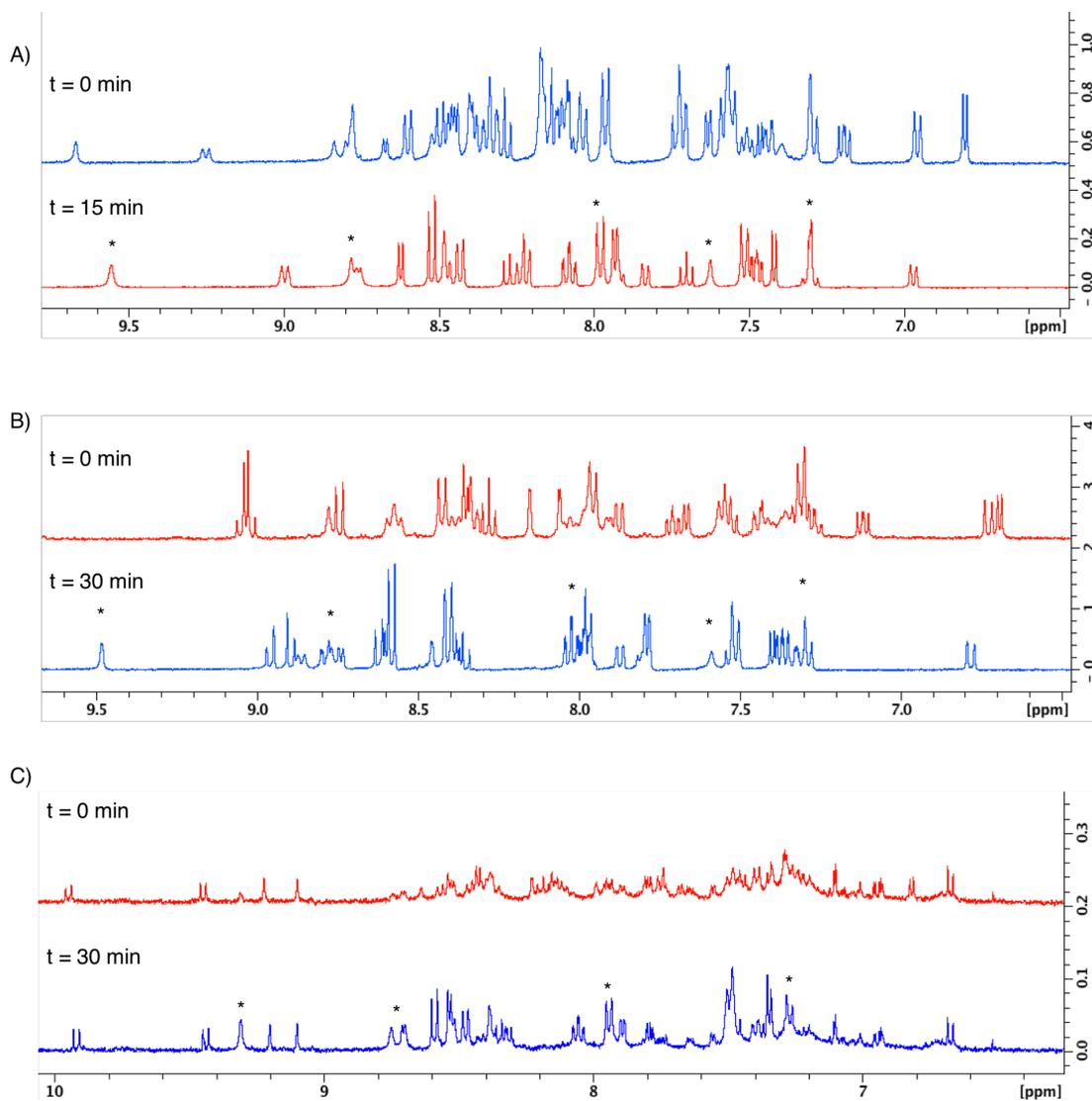


Figure S6. ¹H NMR spectra before and after photolysis with >395 nm light of (A) complex **1**, (B) complex **2**, and (C) complex **3** under a nitrogen atmosphere in CD₃CN, where the asterisks denote select NMR peaks assigned to the free imatinib ligand.

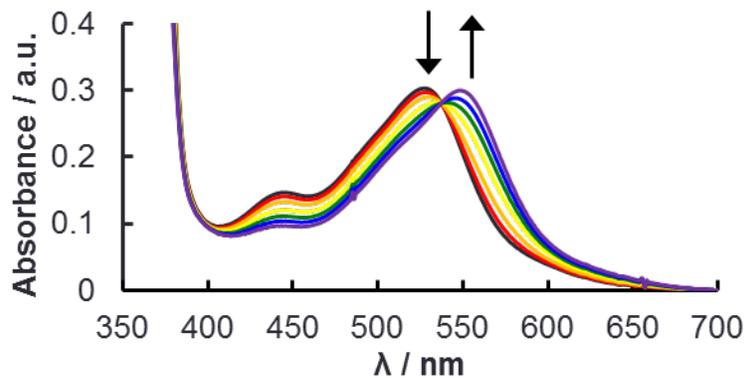


Figure S7. Changes in the electronic absorption spectroscopy of $[\text{Ru}(\text{tpy})(\text{biq})(\text{imatinib})](\text{PF}_6)_2$ in H_2O (< 5% acetone) upon irradiation with $\lambda_{\text{irr}} \geq 590$ nm for 0-60 min.

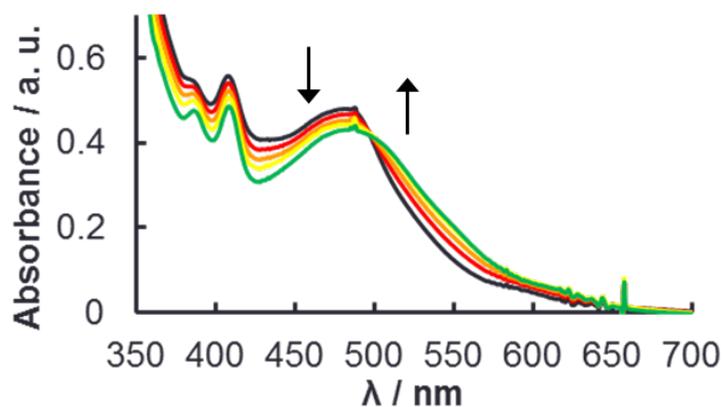


Figure S8. Changes in the electronic absorption spectroscopy of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{dppn})(\text{imatinib})](\text{PF}_6)_2$ in H_2O (< 5% acetone) upon irradiation with $\lambda_{\text{irr}} \geq 590$ nm for 0-4 h.

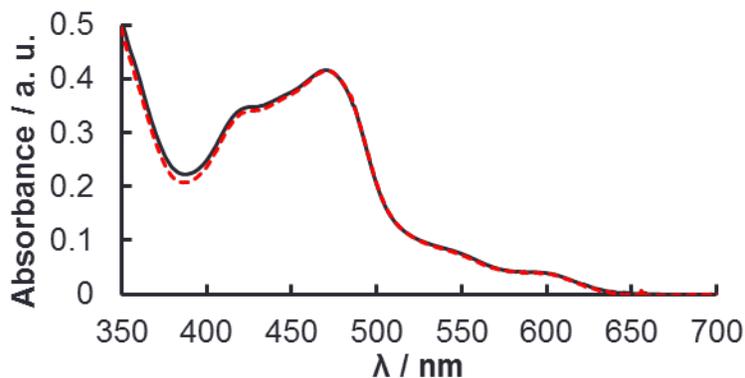


Figure S9. Electronic absorption spectra of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{imatinib})](\text{PF}_6)_2$ in H_2O (< 5% acetone) initially (black) and in the dark for 60 min (red).

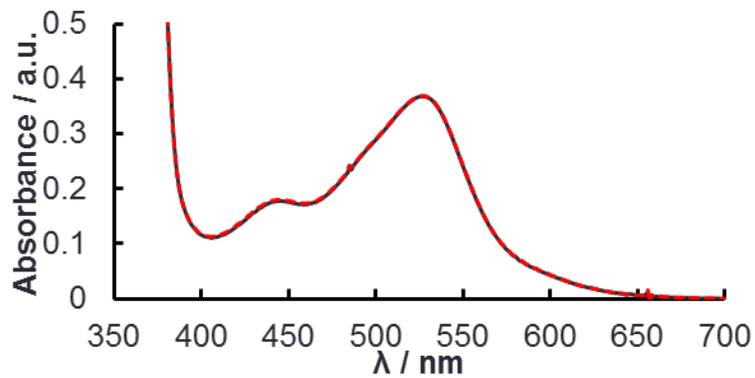


Figure S10. Electronic absorption spectra of $[\text{Ru}(\text{tpy})(\text{biq})(\text{imatinib})](\text{PF}_6)_2$ in H_2O (< 5% acetone) initially (black) and in the dark for 60 min (red).

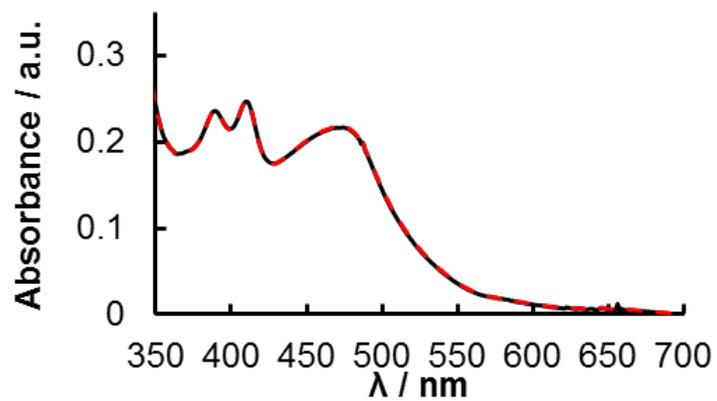


Figure S11. Electronic absorption spectra of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{dppn})(\text{imatinib})](\text{PF}_6)_2$ in H_2O (< 5% acetone) initially (black) and in the dark for 60 min (red).

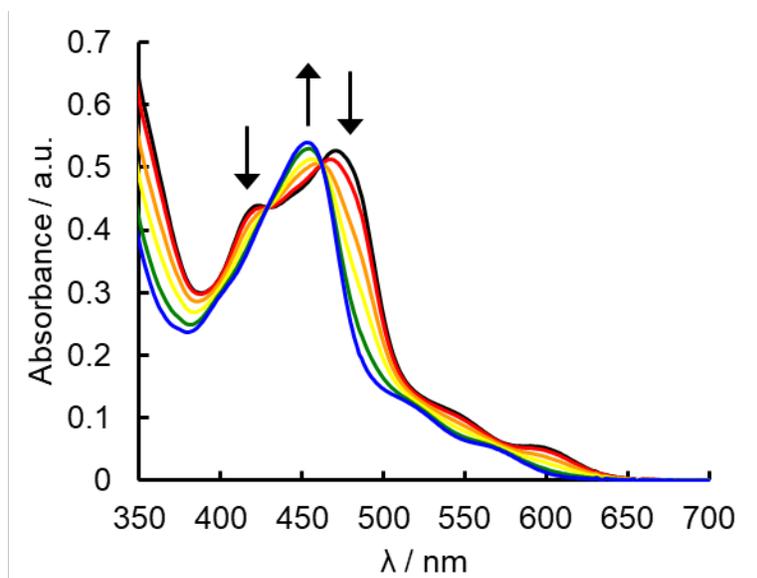


Figure S12. Changes in the electronic absorption spectroscopy of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{imatinib})](\text{PF}_6)_2$ in CH_3CN upon irradiation with $\lambda_{\text{irr}} \geq 590 \text{ nm}$ for 0-45 min.

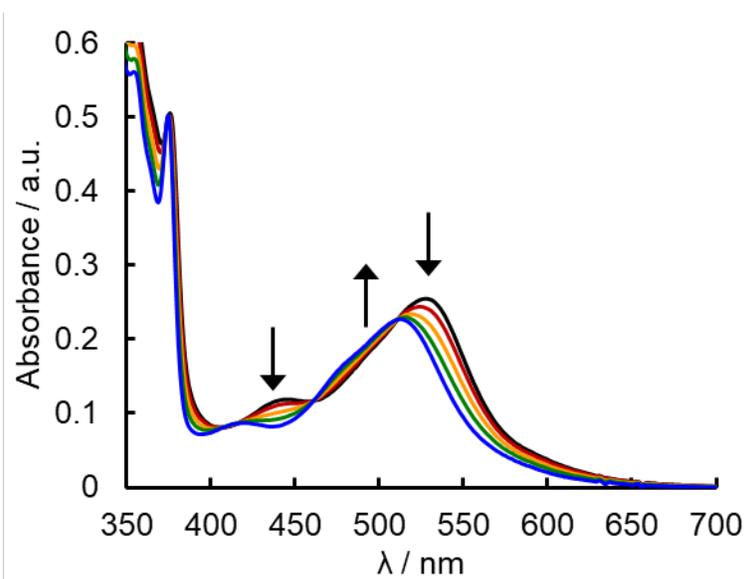


Figure S13. Changes in the electronic absorption spectroscopy of $[\text{Ru}(\text{tpy})(\text{biq})(\text{imatinib})](\text{PF}_6)_2$ in CH_3CN upon irradiation with $\lambda_{\text{irr}} \geq 590 \text{ nm}$ for 0-80 min.

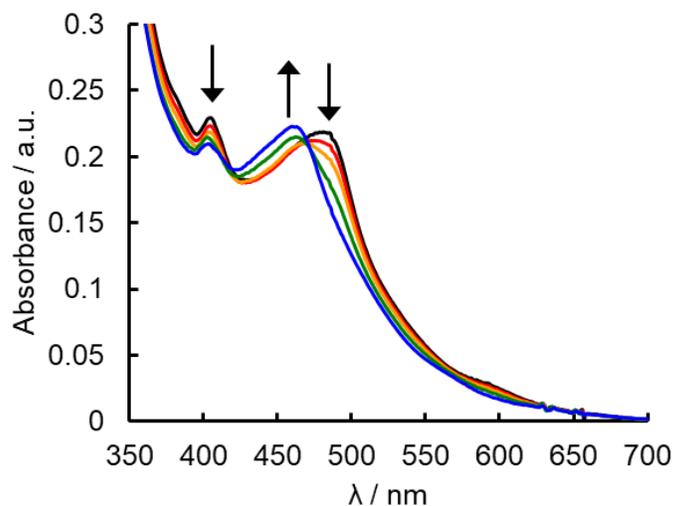


Figure S14. Changes in the electronic absorption spectroscopy of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{dppn})(\text{imatinib})](\text{PF}_6)_2$ in CH_3CN upon irradiation with $\lambda_{\text{irr}} \geq 590 \text{ nm}$ for 0-70 min.

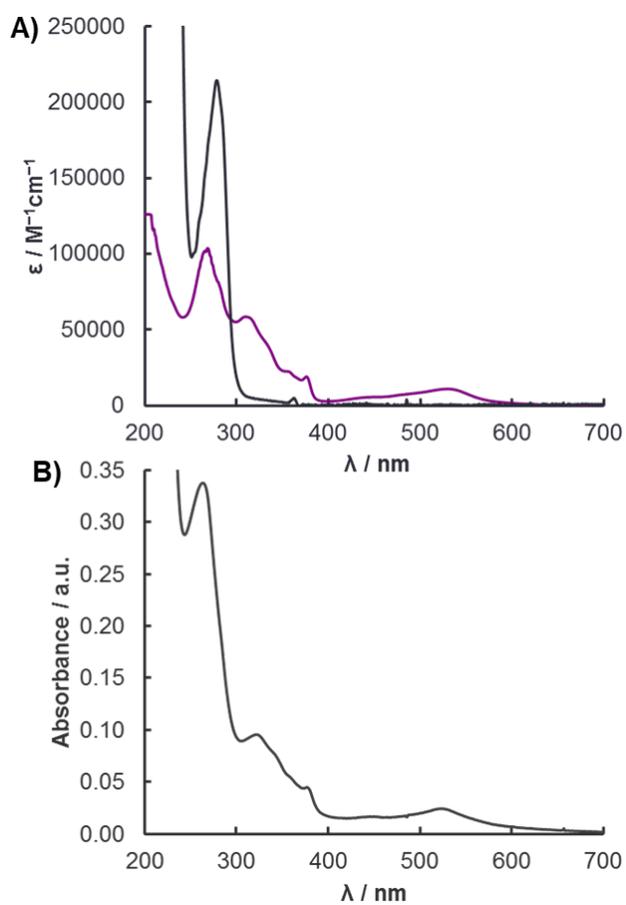


Figure S15. Electronic absorption spectra in PBS buffer for c-kit Ab (black) and **2** (purple) (A) and **2-Ab** (B).

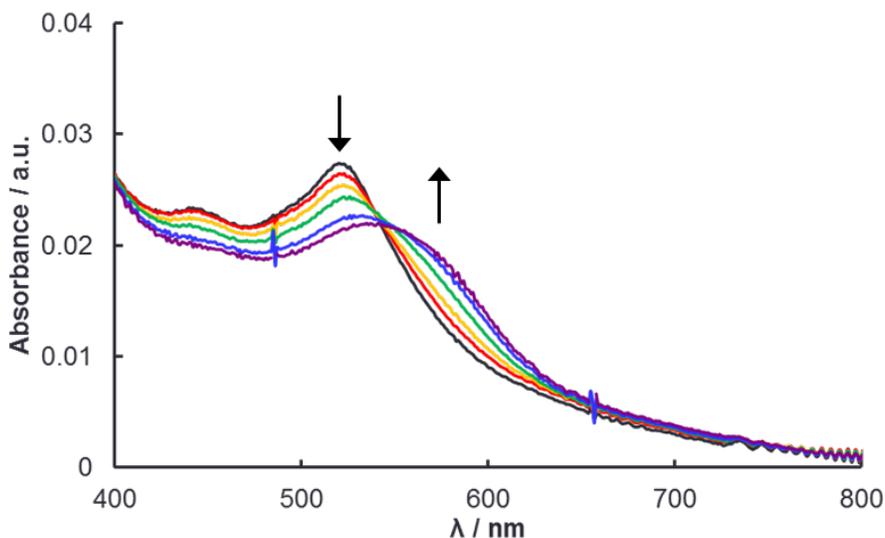


Figure S16. Changes in the electronic absorption spectroscopy of **2-Ab** in PBS buffer (pH = 7.5) upon irradiation with $\lambda_{\text{irr}} \geq 590$ nm for 0-90 min.

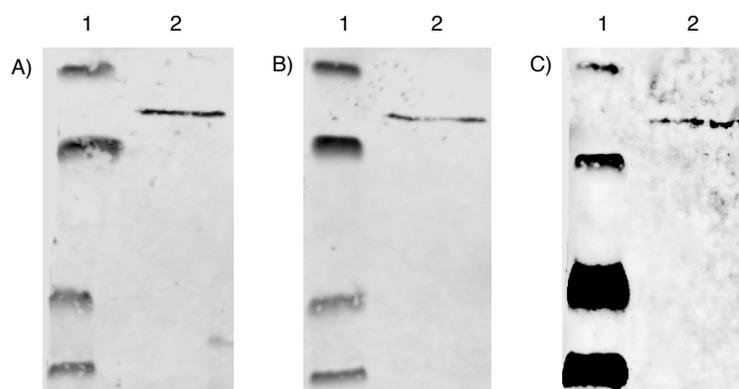


Figure S17. Western blot analysis of (A) unmodified c-Kit antibody, (B) **1-Ab**, and (C) **2-Ab** where lane 1 is a 10 to 180 kDa protein ladder and lane 2 is a EGF-Stimulated A431 cell lysate sample.

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