Supporting Information

Optochemical Control of Therapeutic Agents through Photocatalyzed Isomerization

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Supporting Information

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Materials and methods

All LED irradiations were conducted with a collimated LED light 11 cm above the plate (455 nm, 1W: Thorlabs, part numbers M455L2-C1, M00259452, COP-1A) (6.2 mWcm⁻² unless otherwise stated - measured with a PM100USB power meter and S-170c power sensor - Thorlabs). H NMR spectra were recorded at 300 or 400 MHz on AVANCE 3 HD using DMSO-d₆ or DMF-d₇ as solvent, with residual solvent peaks (δ = 2.50 ppm; δ = 8.03, 2.92, 2.75 ppm). LC-MS spectra were recorded by using a DIONEX Ultimate 3000 UHPLC coupled with a Thermo LCQ Fleet Mass Spectrometer System (electrospray ionization (ESI)) operated in positive mode (condition for elution gradient: 0 min, A:B = 100:0; 4 min, A:B = 10:90; solution A: 0.01% aqueous TFA solution; solution B, 0.1 % TFA in HPLC grade acetonitrile; flow rate: 0.750 mL/min). Absorption spectra were measured using a Molecular Devices Spectra Max M5. HPLC purification was performed with an Agilent Technologies 1260 infinity HPLC mode (condition for elution gradient: 0 min, A:B = 95:5; 0.5 min, A:B = 95:5: 0.6 min, A:B = 60:40; 3.5 min, A:B = 0:100; solution A: 0.1% aqueous TFA solution; solution B, 0.1 % TFA in HPLC grade acetonitrile; flow rate: 0.680 mL/min) using a Thermoscientific Hypersil GOLD C-18 column (2.1 x 50 mm, 1.9 µm, 175Å). Fluorescence imaging was carried out using a Leica SP8 inverted confocal microscope (objective 63x oil – laser 488 nm), a Leica SP8 dive falcon multiphoton confocal microscope (objective 63x oil – white light laser 488 nm – multiphoton laser 916 nm), a Nikon BioStation widefield microscope (objective 10x – irradiation light 488 nm), and a Molecular DevicesTM ImageXpress Micro (IXM) XL automated microscope (objective 60x dry and 20x dry – laser 358 and 488 nm). For EB3-GFP HeLa cells, a 488 nm light source was used for fluorophore excitation. For the laser isomerization of Res-3M, a 916 nm multiphoton laser was used to trigger the reaction. Nuclear counting was carried out using a Molecular DevicesTM ImageXpress Micro (IXM) XL automated microscope with DAPI settings. Excitation = 100 ms. Objective 20x. The cellular apoptotic state was investigated using CellEvent Caspase-3/7 Green Detection Reagent (ThermoFisher) in combination with a 488 nm widefield microscope. All fluorescence images were analysed with Image J. or MetaXpress® software. CA-4 was purchased from abcr as the trans isomer and converted to the cis isomer by literature procedure.[1] All other alkenes were obtained as the trans isomer from the following suppliers: MedChemExpress (GW4064, QNZ46, mubrutinib), abcr (CNB-001), Cayman (Palvarotene), Fluorochem (Res-3M) and Apollo (pterostilbene). Ru(bpy)₃Cl₂ hexahydrate was obtained from Sigma Aldrich.

Cell culture

Stably expressing (under continuous antibiotic selection) EB3-GFP HeLa cells were a kind gift from Prof. Patrick Meraldi (University of Geneva, Switzerland). Cells were grown in DMEM medium (Gibco) containing 10% FCS, 1% pen-strep, 0.3 µg ml⁻¹ puromycin, and 500 µg ml⁻¹ G418 at 37 °C under 5% CO₂ in a humidified incubator. Cells were regularly tested for mycoplasma contamination by qPCR using the MycoplasmaCheck test service (Eurofins). Colonies were kept in culture for no more than 15 passages. Wild type HeLa cells were obtained from the American Type Culture Collection (ATCC) and expanded following their instructions. Cells were grown in DMEM medium (Gibco) containing 10% FCS, 1% pen-strep at 37 °C under 5% CO₂ in a humidified incubator. Cells were regularly tested for mycoplasma contamination by qPCR using the MycoplasmaCheck test service (Eurofins). Colonies were kept in culture for no more than 20 passages.
Monitoring of photoisomerization by NMR

Compounds were dissolved to a final concentration of 3.2 mM in the relevant solvent system. Ru(bpy)$_3$Cl$_2$ was added as stock in either DMF-d$_7$ or DMSO-d$_6$ to a final concentration of 32 µM (1 mol %) and an initial NMR was obtained. The sample was then irradiated in the NMR tube for the prescribed time at 455 nm with a collimated LED light 11 cm above the plate. The experiments were protected from external light by performing experiments inside a protected enclosure (see Figure S-1A for experimental setup). After irradiation reaction progression was monitored by proton NMR.

**Figure S-1:** Diagram of photoirradiation setups using Thor labs LED. All experiments were conducted using set up A) except for *in cellulo* 96 well plate experiments, which used set up B). NMR and eppendorf tubes were laid horizontally across the elevated sample stage to maximize irradiation cross-section. A surface flux density of 6.2 mW/cm$^2$ and 0.82 mW/cm$^2$ was measured using a PM100USB power meter and S-170c power sensor (Thor labs) for setup A and B respectively. To validate the homogenous irradiation in absence of a collimator, the radiant exposure was measured for 52 seconds in 5 positions of the plate. As shown in Fig. S-1B the positional maximal flux variation < 2%
Figure S-2: Photocatalytic isomerisation of trans-CA-4. CA-4 was dissolved in DMF-d7 and the experiment conducted as described in the general procedure to compare treated sample at 60 min (middle) to cis (top) and untreated trans-CA-4 (bottom). Asterisks indicate peaks integrated to determine percentage completion.

Figure S-3: Photocatalytic isomerisation of trans-CA-4. CA-4 was dissolved in 9:1 D2O:DMSO-d6 and the experiment conducted as described in the general procedure to compare treated sample at 60 min (middle) to cis (bottom) and untreated trans-CA-4 (top). Asterisks indicate peaks integrated to determine percentage completion.
Figure S-4: Control NMR studies on trans-CA-4. CA-4 was dissolved in DMF-d$_7$ and the experiment conducted as described in the general procedure to compare treatment in the absence of light (bottom) or photocatalyst (middle) to the untreated trans-CA-4 (top) after 60 min.

Figure S-5: Control NMR studies on cis-CA-4. cis-CA-4 was dissolved in DMF-d$_7$ and the experiment conducted as described in the general procedure to compare treatment in the presence (aqua) or absence (green) of photocatalyst to the untreated cis-CA-4 (red) and trans-CA-4 (purple) after 60 min. Asterisks indicate peaks integrated to determine percentage completion.
Figure S-6: Control NMR studies on trans-CA-4. trans-CA-4 was dissolved in DMF-d$_7$ and the experiment conducted as described in the general procedure to compare treatment in the presence of 1% riboflavin (blue) to the untreated cis-CA-4 (red) and trans-CA-4 (green) after 60 min. Asterisks indicate peaks integrated to determine percentage completion.
Figure S-7: LCMS chromatogram and mass spectrum of CA4 before (top) and after 60 min of irradiation in the presence of 1 mol % Ru(bpy)$_2$Cl$_2$. Solvent: (middle) water + 2% DMSO or (bottom) Fluorobrite cell culture media without phenol red 2% DMSO.
Determination of kinetics of photoisomerization by reverse-phase HPLC

Samples were prepared from a 5 mM stock of trans-CA-4 in DMF or a 3.3 mM stock in DMSO depending on the solvent system tested. Appropriate concentration samples were prepared by dilution and addition of Ru(bpy)₃Cl₂ from a 25 µM stock in either DMF or water. Samples were irradiated for designated time periods as described for the NMR experiments in Eppendorf tubes, then diluted to a concentration of 100 µM and 20 µL injected onto the HPLC system for analysis. Samples were analyzed over 40-100% B gradient over 2.9 min at 0.68 mL/min, with chromatography monitored at 287 and 329 nm. The area under the curve for trans-CA-4 was determined at 329 nm between 3.0-3.1 min and expressed as a percentage of the initial area at T0 using the formula: P_{cis} = (A_{trans} - A) / (A_{trans} - A_{cis}) where P_{cis} is the percentage converted to cis, A_{trans} is the area under the curve for the trans isomer, A_{cis} is the area under the curve for the cis isomer and A is the area under the curve for the sample in question.

From this the concentration of the trans isomer remaining was determined. The slope of the relation between the natural log and irradiation time was determined to give the pseudo-first-order rate constant, with this value then divided by the catalyst concentration to give the second-order rate constant of the reaction. All time points were conducted in triplicate and were analyzed independently.

Figure S-8: Photoisomerisation kinetics of CA-4 in DMF. Experiments were conducted according to the general procedure with a final concentration of 0.5 mM trans-CA-4 and 5 µM Ru(bpy)₃Cl₂. A) The reaction progression was monitored at 329nm by triplicate injection on reversed-phase HPLC. B) Remaining concentration of the trans isomer was calculated as a function of the absorbance of the pure cis and trans samples at 329 nm, with the natural log taken to arrive at the pseudo-first order rate constant. The second order rate constant: 2.245 x10². Error bars represent standard deviation.
**Figure S-9:** NMR spectra of CA-4 before (upper) and after (lower) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$ +1% DMF. Asterisks indicate peaks integrated to determine percentage completion (corresponds to 4x OMe).

**Figure S-10:** LCMS chromatogram and mass spectrum of *trans*-CA-4 (top) and *cis*-CA-4 (bottom)
Figure S-11: NMR spectra of pterostilbene before (lower) and after (upper) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-$d_6$ +1% DMF. Asterisks indicate peaks integrated to determine percentage completion (corresponds to 2x OMe).

Figure S-12: LCMS chromatogram and mass spectrum of pterostilbene before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-$d_6$ +1% DMF.
Figure S-13: NMR spectra of Res-3M before (lower) and after (upper) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$ +1% DMF. Asterisks indicate peaks integrated to determine percentage completion (correspond to 3x OMe).

Figure S-14: LCMS chromatogram and mass spectrum of Res-3M before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$ +1% DMF.
Figure S-15: NMR spectra of CNB-001 before (lower) and after (upper) 60 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$+1% DMF. Asterisks indicate peaks integrated to determine percentage completion (2x OMe).

Figure S-16: LCMS chromatogram and mass spectrum of CNB-001 before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$+1% DMF.
Figure S-17: NMR spectra of GW4064 before (lower) and after (upper) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-$d_6$ +1% DMF. Asterisks indicate peaks integrated to determine percentage completion (benzyllic CH$_2$ indicated).

Figure S-18: LCMS chromatogram and mass spectrum of GW4604 before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-$d_6$ +1% DMF.
Figure S-19: NMR spectra of QNZ46 before (lower) and after (upper) 30 min of irradiation in the presence of 1 mol % Ru(bpy)_3Cl_2. Solvent: DMSO-d_6 +1% DMF. Asterisks indicate peaks integrated to determine percentage completion (alkene proton indicated).

Figure S-20: LCMS chromatogram and mass spectrum of QNZ46 before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)_3Cl_2. Solvent: DMSO-d_6 +1% DMF.
Figure S-21: NMR spectra of palvarotene before (lower) and after (upper) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-$d_6$ +1% DMF. Asterisks indicate peaks integrated to determine percentage completion (benzylic CH$_2$ indicated).

Figure S-22: LCMS chromatogram and mass spectrum of palvarotene before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-$d_6$ +1% DMF.
Figure S-23: NMR spectra of mubrutinib before (lower) and after (upper) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$ +1% DMF. Asterisks indicate peaks integrated to determine percentage completion.

Figure S-24: LCMS chromatogram and mass spectrum of mubrutinib before (top) and after (bottom) 30 min of irradiation in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$. Solvent: DMSO-d$_6$ +1% DMF.
Figure S-25: NMR spectra of the effect of light flux on the rate of isomerization of CA-4. Trans-CA-4 was irradiated for 7 min at either full power (middle) or 10% power (bottom) at 455nm in the presence of 1 mol % Ru(bpy)$_3$Cl$_2$ and compared to an unirradiated sample (top). Solvent: DMF-$d_7$. Asterisks indicate peaks integrated to determine percentage completion.
Estimation of IC$_{50}$ for CA-4 and ReS-3-M

As comets number is a well-known indicator of tubulin polymerization state, we decided to measure the IC$_{50}$ of isomerized Res-3M and CA-4 using comets number as a descriptor$^2$. To do so, HeLa cells expressing EB3-GFP$^3$ were seeded at a concentration of 100000 cells ml$^{-1}$ in a 96-wells black/clear bottom plate one day before the experiment. The in vitro isomerization was carried out by mixing 10 µL of a 100 mM Res-3M solution in DMSO and 10 µL of a 1 mM Ru(bpy)$_3$Cl$_2$ to a final concentration of 50 mM and 1% mol. of catalyst, or 10 µL of a 20 mM CA-4 solution in DMSO and 10 µL of a 0.2 mM Ru(bpy)$_3$Cl$_2$ to a final concentration of 10 mM and 1% mol. of catalyst. Then, the sample was irradiated in a microcentrifuge tube for 30 minutes at 455 nm with a collimated LED light 11 cm above the tube (flux density 6.2 mW/cm$^2$ – radiant exposure 11.16 J/cm$^2$). While the drugs were isomerizing, cells were treated with a 2 µg/mL solution of Hoechst 33342 (Chemodex) for 30 minutes. Then, cells were washed 3 times with warm media and treated with dilution series of the drugs for 30 minutes. Finally, cells were imaged using a Molecular Devices™ ImageXpress Micro (IXM) XL automated microscope equipped with a 60x dry objective. A total of 16 tiles and 3 z stacks were imaged using DAPI and FITC settings. Cells were kept at 37 °C and with 5% CO$_2$ for the whole duration of the experiment. The isomerization was carried out using the setup shown in figure S-1A.

Comet’s analysis protocol

Microscopy images were analyzed using the MetaXpress® software. For nuclear segmentation, DAPI was used with the following settings: minimum width = 8 µm, maximum width = 25 µm, intensity above background = 1000. FITC channel was used to segment the whole cell body using the following settings: minimum width = 8 µm, maximum width = 50 µm, intensity above background = 100. Cells touching the frame’s border were excluded from the analysis.

Figure S-26: Segmentation of nuclei and cell body (right) using the blue and green channels (left), respectively.
To reduce the background and facilitate the segmentation of EB3 comets, a top hat modification was applied. This modification was only used to ease the segmentation but not for the intensity quantification of the comets.

**Figure S-27:** Top up transformation (right) of the green channel image (left).

To remove artifacts like protein aggregates or condensates, the comets were segmented and filtered by area. In particular, all the objects with an intensity above 80 and a width between 0.3 µm and 3 µm were identified as a comet. All objects with a minimum width below 0.2 µm were removed from the mask. This threshold has been used to analyze images taken with a Leica SP8 dive falcon used during MP-Laser isomerization. For the pictures taken using IXM-C microscope the threshold is way higher due to the higher GFP signal.

**Figure S-28:** Comets segmentations (center) of the green channel image. Merge of the two (right).
Figure S-29: IC₅₀ curves for change in comet length for the parent trans and photoisomerized variants of A) CA-4 and B) Res-3M. Photoisomerization was conducted in vitro in DMSO at 10 mM (CA-4) or 50 mM (Res-3M) with 1 mol.% Ru(bpy)₂Cl₂ and irradiated at 455 nm for 30 min which provides trans:cis ratios of 18:82 and 10:90, respectively.
Figure S-30: Effect of CA-4 and Res-3M treatments on average cell size. From the experiments used to calculate IC₅₀ for CA-4 and Res-3M we extrapolated the average cell area (GFP signal). No significant change in size can be correlated with our treatments. Therefore, we speculated that the number of comets can be used as a valid descriptor for tubulin depolymerization.
Cell toxicity using in vitro isomerized trans-Res-3M

To assess whether isomerized Res-3M can induce cell death HeLa cells expressing EB3-GFP were seeded at a concentration of 100’000 cells ml⁻¹ in a 96-wells black/clear bottom plate one day before the experiment. The in vitro isomerization was carried out by mixing 10 µL of a 100 mM Res-3M solution in DMSO and 10 µL of a 1 mM Ru(bpy)₃Cl₂ to a final concentration of 50 mM and 1% mol. of catalyst. Then, the sample was irradiated in a microcentrifuge tube for 30 minutes at 455 nm with a collimated 455 nm LED light 11 cm above the tube (flux density 6.2 mW/cm² – radiant exposure 11.16 J/cm²). Finally, cells were treated with 50, 5, 0.5, and 0.05 µM solution of the isomerized compound, same concentrations of trans-Res-3M, 0.5 µM of Ru(bpy)₃Cl₂, or 0.1 % of DMSO. After 8 or 20 hours of incubation, the supernatant was gently decanted to minimize cells loss, and cells were fixed in 4 % PFA for 30 minutes. Before imaging, the cells were stained for 30 minutes with a 2 µg/mL solution of Hoechst 33342 (Chemodex). Cells were imaged using a Molecular DevicesTM ImageXpress Micro (IXM) XL automated microscope with DAPI settings. Excitation = 100 ms. Objective 20×. The number of healthy cells was quantified by using a nuclei count protocol where the particle mask size was width = 10 μm; height = 25 μm; fluorescence cutoff threshold = 1000. Each well was analyzed by the acquisition of a matrix of images covering the well (16 tiles). The isomerization was carried out using the setup shown in figure S-1A.

In cells cytotoxicity

HeLa cells were seeded at a concentration of 20’000 cells ml⁻¹ in a 96-wells black/clear bottom plate one day before the experiment. The next day, the cells were treated with trans-Res-3M (5 µM), Ru(bpy)₃Cl₂ (0.5 µM), or a combination of both. After 20 minutes, the plate was placed at 37 °C in an aluminum-coated box on a shaker and irradiated for 60 minutes (flux density 0.82 mW/cm² – radiant exposure 3.3 J/cm²). Once irradiated the cells were incubated with the compounds for 24 hours. Before imaging, Hoechst 33342 (Chemodex) was added to the cells to a final concentration of 2 µg/mL for 1 hour. No washing was performed to avoid any loss of dead or dying cells. After that, cells were imaged using a Molecular DevicesTM ImageXpress Micro (IXM) XL automated microscope with DAPI settings. Excitation = 100 ms. Objective 20×. The number of healthy cells was quantified by using a nuclei count protocol where the particle mask size was width = 10 μm; height = 25 μm; fluorescence cutoff threshold = 1000. Each well was analyzed by the acquisition of a matrix of images covering the well (6 tiles). Nuclei were counted using the MetaXpress® software and the statistical analysis was performed using GraphPad. The statistical significance was determined using Student’s t-test where **** is equal to p-value ≤ 0.0001, *** is equal to p-value < 0.001, ** is equal to p-value < 0.01, * is equal to p-value < 0.05 and ns is equal to p-value > 0.05 (Figure S-31). The isomerization was carried out using the setup shown in figure S-1B. Since the plate was irradiated without a collimator, we needed to be sure that no significant difference in radiant exposure could be measured by changing the position of the sensor inside the box. To this end, we recorded a brief light exposure by placing an S-170c power sensor connected to a PM100USB power meter in different positions for 52 seconds (Fig. S-1B). The maximal positional variation in radiant exposure between the various positions was measured to be < 2%. Therefore, we concluded that our setup was suitable for in cells isomerization.
**Figure S-31:** Cell death induced by combinations of Ru(bpy)\(_2\)Cl\(_2\), *trans*-Res-3M, and irradiation of cells (flux density 0.82 mW/cm\(^2\) – radiant exposure 3.3 J/cm\(^2\)). Each dot is representative of a technical replicate. Nuclei were detected and a mask was applied to discriminate between healthy and dying cells using the MetaXpress® software. \(p\) values ≥ 0.05 denoted as ns (no significance), \(p\) values < 0.001 denoted as ***, \(p\) values < 0.01 denoted as **.

**Apoptosis induction and quantification using in vitro isomerized *trans*-Res-3M**

To assess whether treatment with isomerized *trans*-Res-3M was able to induce apoptosis, cells were seeded at a concentration of 160'000 cells ml\(^{-1}\) in a µ-Slide 8 Well chamber. After one day, solutions containing Ru(bpy)\(_2\)Cl\(_2\) 0.5 µM, *trans*-Res-3M 50 mM, DMSO 0.1% or a combination of Ru(bpy)\(_2\)Cl\(_2\) (0.5 µM) and *trans*-Res-3M (50 mM) were either irradiated or not using setup S-1A for 30 minutes (flux density 6.2 mW/cm\(^2\) – radiant exposure 11.16 J/cm\(^2\)). After the isomerization, the cells were treated with one of the aforementioned solutions and 3 µM of CellEvent™ Caspase-3/7 Green Detection Reagent (Thermo - C10740). Finally, after 24 hours, cells were imaged using a Nikon BioStation widefield microscope (objective 10x – irradiation light 488 nm). A total of four tiles per condition were taken (Figure S-32). The isomerization was carried out using the setup shown in figure S-1A.
**Figure S-32:** Apoptosis induced by combinations of Ru(bpy)$_3$Cl$_2$, trans-Res-3M and irradiation (6.2 mW/cm$^2$ 455 nm for 30 minutes, isomerization performed prior to cell treatment, cells were not irradiated). Bright green spots are indicative of apoptotic cells with a fluorogenic substrate to measure caspase 3/7 activity. Images for GFP channel were acquired as follows: excitation = 488 nm – emission = 500 to 550 nm. Panels “trans-Res-3M + Ru(bpy)$_3$Cl$_2$” and Res-3M + Ru(bpy)$_3$Cl$_2$ – irradiated” also appear in the main text (Figure 4E).
Res-3M isomerization on cells using an LED light source

EB3-GFP HeLa cells were seeded at a concentration of 75’000 cells ml⁻¹ in a 3.5 cm glass-bottom dish with a 10 mm microwell (Mattek). After one day cells were treated with either 1% DMSO, 5 µM Res-3M, 0.05 µM Ru(bpy)₃Cl₂ or a combination of drug and catalyst. After 15 minutes, the cells were irradiated for 180 seconds with a collimated 455 nm LED light 11 cm above the plate in a thermal oven set at 37 °C (flux density 6.2 mW/cm² – radiant exposure 11.16 J/cm²). The cells were irradiated for no longer than 5 minutes and then left to equilibrate in a humidified incubator for at least 15 minutes before imaging. The experiments were protected from external light by performing the experiments inside a protected enclosure. Finally, the EB3-GFP comets were imaged using a Leica SP8 microscope equipped with a 488 nm laser and a 63x oil objective, taking stacks of the whole cell (Figure 5). Shown data are a representative selection of a biological triplicate. The isomerization was carried out using the setup shown in figure S-1A.

Res-3M isomerization on cells using a multiphoton laser

HeLa cells stably expressing EB3-GFP were seeded at a concentration of 75’000 cells ml⁻¹ in a 3.5 cm glass-bottom dish with a 10 mm microwell (Mattek). After one day cells were incubated with 10 µM Ru(bpy)₃Cl₂ for 3 hours. The spermatant containing the catalyst that was not taken up cells was then removed, and cells were extensively washed with warm media before the addition of 5 µM Res-3M. To trigger the isomerization, the dishes were irradiated using a multiphoton laser (InSight X3 – Spectra-Physics) at 916 nm and real-time images were acquired on the green channel. Cells were maintained at 37 °C for the full duration of the experiment (Figure 6A – Video 1/2). Shown data are a representative selection of a biological triplicate (Figure S-33).

Experimental imaging settings – Multiphoton laser power: 1%, 4 mW, 916 nm; single-photon laser power: 15%, 0.017 mW, 488 nm (used to visualize EB3-GFP); scan direction: bidirectional X; scanning speed: 200 Hz; objective: 63x/1.4 NA (numerical aperture), oil; zoom: 2.5; line accumulation: 2; resolution: 1024x1024. Note: FRAP interface was used to facilitate the acquisition of complex time series requiring the use of different lasers at different power.

Photophysical calculations – The Airy disk is defined as the area of the diffraction pattern containing 84% of the light intensity. Using a 916 nm wavelength and a 1.4 NA objective we can calculate that the Airy disk has a diameter \( d = \frac{\lambda}{2 NA} = 327 \text{ nm} \) which means an area \( A = 83981.84 \text{ nm}² \). The amount of time the laser irradiates each dot, referred to as laser dwell time \( (Ld) \) can be calculated as a function of the scanning speed and number of scanned dots (related to the 1024x1024 resolution). The laser dwell time is \( Ld = \frac{1}{\text{scanning speed} \times \text{number of points}} = \frac{1}{200 \text{ s}^{-1} \times 1024 \times 1024} = 4.88 \text{ ns} \). From that, the radiant exposure or fluence – defined as the amount of energy that irradiates a surface for a given irradiation time can be roughly estimated as \( H_e = \frac{4 \times 10^{-3} W \times 4.88 \times 10^{-9} s}{8.4 \times 10^{-10} \text{cm}²} \approx 0.024 \text{ J} \text{cm}^{-2} \).

Taking into account that each dot is scanned two times per frame and that there are 36 frames per experiment, an area scanned frame corresponding to an Airy disc is irradiated 72 times for 4.88 ns over the experiment the total energy provided is \( H_e = \frac{72 \times 4 \times 10^{-3} W \times 4.88 \times 10^{-9} s}{8.4 \times 10^{-10} \text{cm}²} \approx 1.7 \text{ J} \text{cm}^{-2} \).

Similarly, the fluence for the 488 nm light is \( H_e = \frac{0.017 \times 10^{-3} W \times 4.88 \times 10^{-9} s}{9.2 \times 10^{-10} \text{cm}²} \approx 9.03 \times 10^{-5} \text{ J} \text{cm}^{-2} \).

And the total energy provided is therefore \( H_e = \frac{72 \times 0.017 \times 10^{-3} W \times 4.88 \times 10^{-9} s}{9.2 \times 10^{-10} \text{cm}²} \approx 0.0065 \text{ J} \text{cm}^{-2} \).
Figure S-33: Isomerization induced disassembly of EB3-GFP from tubulin filaments. Bright spots are EB3-GFP agglomerates bound to the growing end of tubulin. Their disassembly indicates Res-3M induced tubulin depolymerization. See videos 1 and 2 for a time-lapse of shown conditions (trans- Res-3M + Ru(bpy)$_2$Cl$_2$ or trans- Res-3M alone respectively). Panels “Res-3M + Ru(bpy)$_2$Cl$_2$” and Res-3M” also appear in the main text (Figure 6A).
Figure S-34: Single cell two-photo irradiation shows localization dependent loss of comets. The experiment was performed as above using 50 uM Res-3M with the two photon irradiation limited to the area indicated in red. Comet signal recovers within 5 min post two photon irradiation indicating that the active isomer generated diffuses out of the irradiated cell. This diffusion to adjacent cells can also be observed across the 12.5 s to 75 s time point with a radial expansion of the loss of comets.
Figure S-35: Two photon irradiation in the extracellular space is not sufficient to cause comet loss. The experiment was performed as above using 50 uM Res-3M with the two photon irradiation limited to the area indicated in red.
Ru(bpy)$_3$Cl$_2$-induced singlet oxygen generation

As Ruthenium is a well know photosynthesizer used in photodynamic therapy,[4] we asked ourselves whether singlet oxygen production might be responsible for the observed phenotypes. To address this question, we leveraged a recently developed singlet oxygen sensor called BioTracker Si-DMA Singlet Oxygen Live Cell Dye (Sigma – SCT063 – Figure S-34)\(^5\). HeLa cells were seeded at a concentration of 75’000 cells ml$^{-1}$ in a 3.5 cm glass-bottom dish with a 10 mm microwell (Mattek). After one day, Ru(bpy)$_3$Cl$_2$ was added to a final concentration of 10 µM (0.1% DMSO). The catalyst was incubated for 24 hours and then washed three times with warm DPBS. A solution of Si-DMA (500 nM in Fluoro Brite media - Gibco) was added to the cells for 1 hour. Finally, cells were washed two times with warm PBS before imaging. Ruthenium was irradiated using a multi-photon laser at 916 nm (laser power 1% equal to 4 mW - measured with a PM100USB – Thorlabs) for 60 seconds. At the same time, the Si-DMA probe was irradiated with a 640 nm white light laser. For the whole duration of the experiment fluorescence between 665 and 675 nm was detected. As a positive control, HeLa cells were incubated with 5-aminolevulinic acid (5-ALA - Acros) for 4 hours to increase the endogenous production of protoporphyrin IX (PpIX). Differently, the positive control was irradiated with an Argon laser at 405 nm, where PpIX shows a maximum of absorption, for 60 seconds. Just before imaging, trans-Res-3M was added to a final concentration of 5 µM for 15 minutes. The detection of Si-DMA over time was carried out identically to the Ru(bipy)$_3$Cl$_2$ treated cells. For the quantification, cytoplasmatic regions of interest were defined at T$_0$, and fluorescence was quantified within these regions throughout the temporal scan (Figure S-34, S35-A, S35-B). Each experiment was carried out as a biological replicate, statistical analysis was carried out via a Student's t-test using the program GraphPad.

**Figure S-36:** Detection of single oxygen using the Si-DMA probe. Samples were irradiated for 60 seconds while the increase in fluorescence was recorded as a time series. The green corresponds to the lowest values of fluorescence while blue corresponds to fluorescence maximum in an 8-bit range. Images were acquired as follows: excitation = 405 nm (excitation of PpIX)/916 nm (multiphoton laser – excitation of Ru(bipy)$_3$Cl$_2$)/640 nm (excitation of Si-DMA) – emission = 665 to 675 nm.
Figure S-37: Quantification of singlet oxygen production. Multiple regions of interest were defined to calculate the Si-DMA fluorescence signal over time (A). The intensity of Si-DMA emission after 60 seconds of irradiation was normalized by the intensity at $T_0$ and plotted as single values. Each dot represents a different region of interest. $p_{\text{value}} \geq 0.05$ denoted as ns (no significance), $p_{\text{value}} \leq 0.0001$ denoted as ****.
1^H NMR of cis-CA-4 (400 MHz, DMF-\textit{d}_7) \delta 9.01 (s, 1H), 6.92 (dd, \textit{J} = 5.2, 3.1 Hz, 2H), 6.78 (ddd, \textit{J} = 8.2, 2.1, 0.6 Hz, 1H), 6.50 (d, \textit{J} = 12.3 Hz, 1H), 6.44 (d, \textit{J} = 12.2 Hz, 1H), 3.82 (s, 3H), 3.71 (d, \textit{J} = 10.2 Hz, 8H).

1^H NMR of trans-CA-4 treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 60 min (400 MHz, DMF-\textit{d}_7). Diagnostic peaks (4x OMe): trans $\delta$ 3.90, 3.85, 3.75 (1.21 H); cis 3.82, 3.72, 3.70 (11.48 H). Trans : cis $\approx$ 10:90
$^1$H NMR of trans-CA-4 (400 MHz, DMF-$d_7$) $\delta$ 9.01 (s, 1H), 7.25 – 7.12 (m, 2H), 7.08 – 6.95 (m, 5H), 3.90 (s, 6H), 3.86 (s, 3H), 3.75 (s, 3H).

$^1$H NMR of trans-CA-4 (400 MHz, Deuterium Oxide) $\delta$ 7.78 (s, 1H), 7.37 – 7.05 (m, 5H), 7.00 (s, 2H), 3.97 (s, 6H), 3.94 (s, 3H), 3.85 (s, 3H).
$^1$H NMR of cis-CA-4 treated with Ru(bpy)$_2$Cl$_2$ and 455 nm light for 60 min (400 MHz, Deuterium Oxide). Diagnostic peaks (4x OMe): trans $\delta$ 3.97, 3.94, 3.85 (11.49 H); cis 3.90, 3.82, 3.75 (11.88 H). Trans : cis = 49:51

$^1$H NMR of cis-CA-4 (400 MHz, Deuterium Oxide) $\delta$ 7.08 (d, $J = 8.1$ Hz, 1H), 6.96 (d, $J = 9.9$ Hz, 2H), 6.77 (s, 2H), 6.72 (d, $J = 12.2$ Hz, 1H), 6.63 (d, $J = 12.2$ Hz, 1H), 3.93 (s, 3H), 3.85 (s, 3H), 3.78 (s, 6H).
$^1$H NMR of trans-CA-4 treated with 455 nm light for 60 min (400 MHz, DMF-$d_7$) δ 9.01 (s, 1H), 7.25 – 7.12 (m, 2H), 7.08 – 6.95 (m, 5H), 3.90 (s, 6H), 3.86 (s, 3H), 3.75 (s, 3H).

$^1$H NMR of trans-CA-4 treated with Ru(bpy)$_3$Cl$_2$ for 60 min (400 MHz, DMF) δ 9.00 (s, 1H), 7.25 – 7.09 (m, 1H), 7.07 – 6.90 (m, 2H), 3.90 (s, 1H), 3.85 (s, 1H), 3.74 (s, 1H).
$^{1}H$ NMR of **trans-CA-4** (400 MHz, DMF-$d_7$) $\delta$ 9.00 (s, 1H), 7.24 – 7.11 (m, 2H), 7.07 – 6.94 (m, 5H), 3.90 (s, 6H), 3.85 (s, 3H), 3.74 (s, 3H).

$^{1}H$ NMR of **cis-CA-4** treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 60 min (400 MHz, DMF-$d_7$).

Diagnostic peaks (4x OMe): **trans** $\delta$ 3.90, 3.85, 3.74 (1.4 H); **cis** 3.82, 3.72, 3.69 (11.92 H). Trans : cis = 11:89
$^1$H NMR of cis-CA-4 treated with 455 nm light for 60 min (400 MHz, DMF-$d_7$) \( \delta \) 9.01 (s, 1H), 6.92 (dd, \( J = 5.2, 3.1 \) Hz, 2H), 6.78 (ddd, \( J = 8.3, 2.1, 0.7 \) Hz, 1H), 6.69 (s, 2H), 6.50 (d, \( J = 12.3 \) Hz, 1H), 6.44 (d, \( J = 12.3 \) Hz, 1H), 3.82 (s, 3H), 3.72 (s, 3H), 3.70 (s, 6H).

$^1$H NMR of cis-CA-4 (400 MHz, DMF-$d_7$) \( \delta \) 9.00 (s, 1H), 6.91 (dd, \( J = 5.2, 3.1 \) Hz, 1H), 6.68 (s, 1H), 6.49 (d, \( J = 12.2 \) Hz, 1H), 6.43 (d, \( J = 12.3 \) Hz, 1H), 3.81 (s, 2H), 3.72 (s, 2H), 3.69 (s, 3H).
$^1$H NMR of trans-CA-4 treated with riboflavin and 455 nm light (60 min) (400 MHz, DMF-$d_7$). Diagnostic peaks (4x OMe): trans $\delta$ 3.90, 3.86, 3.75 (11.84 H); cis 3.82, 3.72, 3.70 (2.23 H). $\text{Trans: cis} = 84:16$

$^1$H NMR of trans-CA-4 treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 30 min (400 MHz, DMSO-$d_6$). Diagnostic peaks (4x OMe): trans $\delta$ 3.82, 3.78, 3.66 (2.53 H); cis 3.73, 3.64, 3.61 (11.74 H). $\text{Trans: cis} = 18:82$
$^1$H NMR of trans-pterostilbene (400 MHz, DMSO-$d_6$) $\delta$ 7.41 (d, $J = 8.6$ Hz, 2H), 7.15 (d, $J = 16.4$ Hz, 1H), 6.93 (d, $J = 16.4$ Hz, 1H), 6.76 (d, $J = 8.6$ Hz, 2H), 6.71 (d, $J = 2.2$ Hz, 2H), 6.37 (t, $J = 2.2$ Hz, 1H), 3.76 (s, 6H).

$^1$H NMR of trans-pterostilbene treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 30 min (400 MHz, DMSO-$d_6$). Diagnostic peaks (2x OMe): trans $\delta$ 3.76 (1.05 H); cis 3.63 (6.00H). Trans : cis = 15:85
$^1$H NMR of trans-3,5,4'-trimethoxystilbene (400 MHz, DMSO-$d_6$) δ 7.53 (d, $J = 8.8$ Hz, 2H), 7.21 (d, $J = 16.3$ Hz, 1H), 7.02 (d, $J = 16.4$ Hz, 1H), 6.95 (d, $J = 8.8$ Hz, 2H), 6.74 (d, $J = 2.3$ Hz, 2H), 6.38 (t, $J = 2.2$ Hz, 1H), 3.78 (s, 3H), 3.77 (s, 6H).

$^1$H NMR of trans-Res-3M treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 30 min (400 MHz, DMSO-$d_6$).

Diagnostic peaks (3x OMe): trans δ 3.78, 3.77 (1.00 H); cis 3.73, 3.63 (9.05H). Trans : cis = 10:90
$^1$H NMR of trans, trans-CN001 (400 MHz, DMSO-\textit{d}_6) \delta 7.68 – 7.49 (m, 4H), 7.49 – 7.40 (m, 1H), 7.24 – 7.10 (m, 3H), 7.10 – 6.98 (m, 3H), 7.02 – 6.90 (m, 2H), 6.84 – 6.67 (m, 3H), 3.84 (s, 3H), 3.77 (s, 3H).
$^1$H NMR of trans, trans-CNB001 treated with Ru(bpy)$_2$Cl$_2$ & 455 nm light for 30 min (400 MHz, DMSO-$d_6$). Diagnostic peaks (2x OMe): trans, trans $\delta$ 3.84, 3.77 (2.64 H); trans, cis $\delta$ 3.81, 3.60 (5.69). Trans : cis = 38:62

$^1$H NMR of trans-GW4604 (300 MHz, DMSO-$d_6$) $\delta$ 8.09 (s, 1H), 7.83 (t, $J = 6.8$ Hz, 2H), 7.76 (d, $J = 8.8$ Hz, 1H), 7.65 (d, $J = 2.1$ Hz, 1H), 7.62 (s, 1H), 7.59 – 7.45 (m, 2H), 7.39 (d, $J = 16.3$ Hz, 1H), 7.25 (d, $J = 16.4$ Hz, 1H), 6.95 (d, $J = 2.5$ Hz, 1H), 6.79 (d, $J = 8.9$ Hz, 1H), 4.91 (s, 2H), 3.58 – 3.43 (m, 1H), 1.34 (d, $J = 7.0$ Hz, 6H).
$^1$H NMR of *trans*-GW4064 treated with Ru(bpy)$_2$Cl$_2$ and 455 nm light for 30 min (300 MHz, DMSO-$d_6$). Diagnostic peaks (1x CH$_2$): *trans* $\delta$ 4.91 (0.56 H); *cis* 4.86 (6.98 H). *Trans : cis = 7:93*
$^1$H NMR of trans-QNZ46 (400 MHz, DMSO-$d_6$) δ 8.24 (t, $J = 2.0$ Hz, 1H), 8.18 – 8.13 (m, 3H), 7.93 – 7.91 (m, 1H), 7.86 – 7.82 (m, 1H), 7.79 – 7.75 (m, 1H), 7.67 – 7.59 (m, 3H), 7.56 – 7.50 (m, 2H), 6.49 (d, $J = 15.6$ Hz, 1H), 3.90 (s, 3H).

$^1$H NMR of trans-QNZ46 treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 30 min (400 MHz, DMSO-$d_6$). Diagnostic peaks (alkene CH): trans δ 6.49 (0.36 H); cis 6.11 (1.00 H). Trans : cis = 26.74
$^1$H NMR of trans-palvarotene (400 MHz, DMSO-$d_6$) δ 7.95 (obs, 2H), 7.76 (dd, $J = 2.2, 0.8$ Hz, 1H), 7.70 (d, $J = 8.0$ Hz, 2H), 7.65 (d, $J = 15.9$ Hz, 2H), 7.46 (dd, $J = 1.9, 0.7$ Hz, 1H), 7.19 (d, $J = 16.2$ Hz, 1H), 7.04 (s, 1H), 6.25 (t, $J = 2.1$ Hz, 1H), 5.49 (s, 2H), 1.63 (s, 4H), 1.30 (s, 6H), 1.18 (s, 6H).

$^1$H NMR of trans-palvarotene treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 30 min (400 MHz, DMSO-$d_6$). Diagnostic peaks (1x CH$_2$): trans δ 5.49 (1.00 H); cis 5.31 (36.50 H). Trans : cis = 3:97
$^1$H NMR of trans-nubrutinib (300 MHz, DMSO-$d_6$) δ 8.25 (s, 1H), 8.11 (s, 1H), 7.98-7.94 (d, 2H, hidden), 7.76 (d, $J = 8.2$ Hz, 2H), 7.70 (s, 1H), 7.62 (d, $J = 16.5$ Hz, 1H), 7.34 (d, $J = 16.5$ Hz, 1H), 7.10 (d, $J = 8.4$ Hz, 2H), 6.94 (d, $J = 8.4$ Hz, 2H), 4.98 (s, 2H), 4.39 (t, $J = 7.1$ Hz, 2H), 2.57-2.48 (t, 2H, hidden), 1.81 (p, $J = 7.2$ Hz, 2H), 1.49 (q, $J = 7.8$ Hz, 2H).

$^1$H NMR of trans-nubrutinib treated with Ru(bpy)$_3$Cl$_2$ and 455 nm light for 30 min (300 MHz, DMSO-$d_6$). Diagnostic peaks (alkene CH): trans δ 7.34 (0.41 H); cis 6.62 (1.00 H). Trans : cis = 29:71
H NMR of trans-CA-4 treated with Ru(bpy)\(_2\)Cl\(_2\) and 455 nm light (7 min, 100% power) (400 MHz, DMF-
\(d_7\)). Diagnostic peaks (4x OMe): trans \(\delta\) 3.85, 3.81, 3.70 (12.04 H); cis 3.77, 3.67, 3.65 (7.95 H). Trans:cis = 60:40

H NMR of trans-CA-4 treated with Ru(bpy)\(_2\)Cl\(_2\) and 455 nm light (7 min, 10% power) (400 MHz, DMF-
\(d_7\)). Diagnostic peaks (4x OMe): trans \(\delta\) 3.85, 3.81, 3.70 (11.90 H); cis 3.77, 3.67, 3.65 (0.37 H). Trans:cis = 97:3
$^1$H NMR of cis-CA-4 treated with 254 nm light for 2 h (400 MHz, DMF-$d_7$). Diagnostic peaks (4x OMe): 
trans $\delta$ 3.82, 3.78, 3.67 (4.92 H); cis 3.74, 3.64, 3.62 (12.05 H). Trans:cis = 29:71
References

[1] K. Gaukroger, J. A. Hadfield, L. A. Hepworth, N. J. Lawrence, A. T. McGown, *J. Org. Chem.* **2001**, *66*, 8135-8138.

[2] M. Borowiak, W. Nahaboo, M. Reynders, K. Nekolla, P. Jalinot, J. Hasserodt, M. Rehberg, M. Delattre, S. Zahler, A. Vollmar, D. Trauner, O. Thorn-Seshold, *Cell* **2015**, *162*, 403-411.

[3] J. W. Armond, E. Vladimirou, M. Erent, A. D. McAinsh, N. J. Burroughs, *J. Cell Sci.* **2015**, *128*, 1991-2001.

[4] J. Karges, S. Kuang, F. Maschietto, O. Blacque, I. Ciofini, H. Chao, G. Gasser, *Nat. Commun.* **2020**, *11*, 3262.

[5] S. Kim, T. Tachikawa, M. Fujitsuka, T. Majima, *J. Am. Chem. Soc.* **2014**, *136*, 11707-11715.