Supporting Information For

Boosting two-photon photodynamic therapy with mitochondria-targeting ruthenium-glucose conjugates

Jiangping Liu, a Xinxing Liao, a Kai Xiong, a Shi Kuang, a Chengzhi Jin, a Liangnian Ji, a and Hui Chaoab●

a MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry, Sun Yat-Sen University, Guangzhou 510275, China. E-mail: ceschh@mail.sysu.edu.cn, Fax: 86-20-84112245, Tel: 86-20-84110613
b MOE Key Laboratory of Theoretical Organic Chemistry and Functional Molecule, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan, 400201, P. R. China

03 Sep 2020

Note added after first publication: This supplementary information file replaces that originally published on 20 Apr 2020. Fig. S16 originally included incorrect images of the intestine sections for the saline+hv and Ru2 groups due to errors in image processing. This updated file contains the correct images. This does not affect the results and conclusions of the article.

Table of content

Experimental Section .................................................................................................................................................. 3

Materials and instrumentation ......................................................................................................................................... 3
Synthesis and characterization ....................................................................................................................................... 3
Luminescence quantum yield determination .................................................................................................................. 6
Two-photon absorption cross section determination ...................................................................................................... 6
1O2 quantum yield determination in solution ................................................................................................................ 7
LogP measurement ...................................................................................................................................................... 8
Cell line information, culture conditions, and cell pretreatment .................................................................................. 8
Cellular uptake level study in different cell lines .......................................................................................................... 8
Mixed cells co-incubation study .................................................................................................................................. 8
Cellular uptake mechanism study .................................................................................................................................. 9
Intracellular distribution studies ................................................................................................................................... 9
Mitochondrial membrane potential (MMP) assessment ..................................................................................................... 9
Caspase 3/7 detection ................................................................................................................................................... 10
MTT assay ................................................................................................................................................................. 10
Two-photon laser induced singlet oxygen generation in vitro ......................................................................................... 10
Annexin V/PI co-staining in the in vitro two-photon PDT .................................................................11
In vivo two-photon PDT experiments.................................................................................................11
Histological studies of tissue sections .................................................................................................12

Supporting Figures and Tables ...........................................................................................................13

Scheme S1. Synthetic routes of Ru2-Ru4 ............................................................................................4
Fig. S1. $^1$H NMR spectrum of 3a ........................................................................................................13
Fig. S2. $^1$H NMR spectrum of 3b ........................................................................................................13
Fig. S3. ESI-MS, and $^1$H NMR spectrum of Ru2 ...........................................................................14
Fig. S4. ESI-MS, and $^1$H NMR spectrum of Ru3 ...........................................................................15
Fig. S5. ESI-MS, and $^1$H NMR spectrum of Ru4 ...........................................................................16
Fig. S6. Absorption and emission spectra of Ru1-Ru4 in methanol .....................................................17
Fig. S7. Two-photon induced emission spectra of Ru2-Ru4 ..............................................................17
Fig. S8. DPBF consumption trajectory in the presence of Ru1-Ru4 ....................................................18
Fig. S9. Stability of Ru1-Ru4 in culture medium .................................................................................18
Fig. S10. Microscopic imaging of HeLa/L02 mixed cells co-incubation of Ru2 ...................................18
Fig. S11. Cellular uptake mechanism study by confocal luminescence imaging ...............................19
Fig. S12. Intracellular Ru distribution by ICP-MS quantification .......................................................20
Fig. S13. MMP measurement by JC-1 staining ..................................................................................20
Fig. S14. Caspase-3/7 activation detection in HeLa cells ...................................................................21
Fig. S15. Annexin V/PI co-staining of the HeLa cells before and after two-photon PDT ...............21
Fig. S16. Histological examination of primary organs and the HeLa xenografted tumor ...............22
Table S1. Photophysical properties of Ru1-Ru4 ..............................................................................23
Table S2. Estimation of TPA-induced ROS generating ability of Ru1-Ru4 ...........................................23
Table S3. Ru1-Ru4 cellular uptake level amongst different cell lines .................................................23
Table S4. Intracellular distribution of Ru1-Ru4 ..................................................................................24
Table S5. (Photo-)cytotoxicity of Ru1-Ru4 .......................................................................................24

References ...........................................................................................................................................25
Materials and instrumentation

Unless otherwise stated, all chemicals and reagents were commercially available and used without any further purifications. Double distilled (DD) water was used throughout all of the experiments. Deuterated solvents were purchased from Cambridge Isotope Laboratory (Andover, MA). RuCl₃·nH₂O, cisplatin, 4'-formylphenyl-β-D-glucopyranoside, Ru standard solution (1000 μg/mL), 1,3-diphenyliso-benzofuran (DPBF), PBS, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), H₂TPP, Hoechst 33342, and rhodamine B were purchased from Sigma-Aldrich. Dulbecco’s modified eagle medium (DMEM) with high/no glucose, fetal bovine serum (FBS), penicillin, streptomycin and MitoTracker Green were purchased from Thermo Fisher Scientific. JC-1 was purchased from Beyotime Biotechnology (China). Caspase-Glo 3/7 Assay Kit was purchased from Promega Corporation. Matrigel was obtained from Corning (BD Biocoat). Mitochondria isolation kit, nucleus and cytoplasm isolation kit were purchased from Thermo Pierce. The Ru(II) complexes were dissolved in DMSO prior to each experiment; the calculated quantities of the Ru(II) complexes solutions were added to appropriate volume of cultrue medium to yield a final solution with DMSO concentration of no more than 1%. Cisplatin stock solution (3333 μM) was prepared by using normal saline solution, stored at room temperature, protected from light, and used within a week.

The 1H NMR spectra were recorded on a Varian INOVA500NB Superconducting Fourier Transform Nuclear Magnetic Resonance Spectrometry. Electrospray ionization mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA). Microanalysis (C, H and N) was conducted by a Perkin-Elmer 240Q elemental analyzer. The UV-Vis spectra were recorded on a Varian Cary 300 spectrophotometer at room temperature. Emission spectra were recorded on a PerkinElmer LS 55 fluorescence spectrometer at room temperature. Two-photon absorption cross section measurements and two-photon in vivo PDT were performed in an open light pathway by the excitation of a modelocked Ti: Sapphire laser (pulse width 35 fs, Coherent Co., Ltd., USA). ¹O₂ phoshorescence were recorded on an Edinburgh FLSP-920 fluorescence spectroscopy (Edinburgh Instruments) equipped with a NIR detector using a picosecond pulse laser (450 nm) as the excitation source. The inductively coupled plasma mass spectrometry (ICP-MS) experiments were carried out on an Agilent’s 7700x instrument. The luminescence intensity in caspase detection assay was measured on a TECAN infinite M200 PRO multifunction microplate reader. Cell imaging and two-photon irradiation were conducted on an LSM 880 (Carl Zeiss, Germany) confocal laser scanning microscope (CLSM). Visible one-photon irradiation (λirr = 450 nm, 40 mW/cm², 100% power) in PDT was provided by a commercially available LED visible area light source (Height LED Instruments, China). MTT OD values were measured by a Molecular Deviceds absorbance reader (USA). All data were processed with the OriginPro 8.5 software package.

Synthesis and characterization

1,10-Phenanthroline-5,6-dione[1], cis-[Ru(DIP)₂Cl₂]·2H₂O, Ru₁[2] and compound 1[3] were synthesized according to published methods. The synthetic routes for Ru₂-Ru₄ were dipicted in Scheme S1. All of the final Ru(II) complexes were transferred into chloride salts for the biological tests by a reported method [2].

Synthesis of Ru₂

A mixture of comound 1 (91.2 mg, 19 mmol) and cis-[Ru(DIP)₂Cl₂]·2H₂O (159 mg, 0.19 mmol) was dissolved in 30 mL EtOH/H₂O (2:1, v/v) in a round bottom flask, purged with Ar, and refluxed at 85 °C for 8 h in the dark. Upon completion, the solvent was removed by a rotatory evaporator. The red crude product was
Scheme S1. Synthesis routes of Ru2-Ru4. (a) EtOH, CH$_3$COONH$_4$, reflux 5 h. (b) EtOH/H$_2$O (3:1, v/v), Ar, 85 °C, dark, 8 h. (c) Pyridine, RT, 18 h. (d) CH$_3$COOH, CH$_3$COONH$_4$, Ar, reflux overnight. (e) EtOH/H$_2$O (3:1, v/v), Ar, 85 °C, dark, 24 h. (f) MeONa/MeOH, 0 °C, 3 h.

purified by silica gel chromatography (100-200 meshes) with MeOH/H$_2$O/saturated NaNO$_3$ as the eluent, the red product was collected, evaporated to dryness, washed with ether. The obtained nitrate product was converted into the perchlorate salts by anion metathesis with aqueous NaClO$_4$; the precipitates were collected, and washed with water, and dried under vacuum to afford the red product (Yield = 50%). Anal. Calcd. for C$_{75}$H$_{58}$Cl$_2$N$_8$O$_{14}$Ru (%): C, 61.39; H, 3.98; N, 7.64; Found (%): C, 61.20; H, 4.09; N, 7.86. ES-MS: m/z = 620.10 [M-2ClO$_4$]$^{2+}$, 1239.2 [M-2ClO$_4$-H]$^{+}$.

$^1$H NMR (500 MHz, DMSO-d$_6$) δ 14.24 (s, 1H), 9.12 (dd, $^J$= 13.8, 8.2 Hz, 2H), 8.35 (d, $^J$= 5.5 Hz, 2H), 8.31 – 8.21 (m, 8H), 8.19 (d, $^J$= 5.2 Hz, 2H), 7.93 (ddd, $^J$= 22.5, 8.2, 5.2 Hz, 2H), 7.83 (d, $^J$= 5.5 Hz, 2H), 7.77 (t, $^J$= 5.0 Hz, 2H), 7.73 – 7.59 (m, 2H), 7.30 (d, $^J$= 8.4 Hz, 2H), 5.31 – 5.26 (m, 1H), 5.14 (d, $^J$= 6.7 Hz, 1H), 5.04 (d, $^J$= 3.7 Hz, 1H), 4.72 (d, $^J$= 7.3 Hz, 1H), 4.53 (t, $^J$= 5.7 Hz, 1H), 3.98 (d, $^J$= 3.2 Hz, 1H), 3.83 – 3.68 (m, 2H), 3.54 – 3.42 (m, 3H).

Synthesis of 2
A mixture of 4'-formylphenyl-β-D-glucopyranoside (1.0 g, 3.5 mmol) and acetic anhydride (2 mL, 21.1 mmol) was added to a 10 mL round-bottom flask and dissolved in 5 mL pyridine, stirred at ambient temperature for
18 h. Upon completion, the solution was swiftly neutralized and extracted with EtOAc. The organic layers were combined and washed by saturated Na$_2$CO$_3$ solution, and subsequently by saturated NaCl solution, and dried with Na$_2$SO$_4$. After removing the solvent by reduced pressure distillation, the obtained residue was purified with silica gel column chromatography (100-200 meshes) with an eluent of hexan/EtOAc (1:1, v/v) to give a white powder (Yield = 72%). Anal. Calcd. for C21H24O11 (%): C, 55.75; H, 5.35. Found (%): C, 55.25; H, 5.48 ES-MS: m/z = 453.1 [M+H]$^+$. 

Synthesis of 3a

A suspension of compound 2 (1.08 g, 2.38 mmol), 1,10-phenanthroline-5,6-dione (0.50 g, 2.38 mmol), ammonium acetate (2.20 g, 28.5 mmol), and aniline (0.22 g, 2.38 mmol) in 50 mL glacial acetic acid was stirred under Ar at 135 °C for overnight. After cooling down to room temperature, the resulting solution was poured into 200 mL DD water, neutralized with ammonium hydroxide, and extracted with chloroform. The organic layers were combined and washed with saturated NaCl solution, and dried with Na$_2$SO$_4$. The solvent was evaporated to give a crude residue which was subsequently purified by silica gel column chromatography with EtOH/DCM (1:50, v/v) as the eluent. The further recrystallization using toluene/DCM gave a white powder as the product (Yield = 72%). Anal. Calcd. for C39H34N4O10 (%): C, 65.17; H, 4.77; N, 7.80. Found (%): C, 64.93; H, 4.85; N, 7.94. ES-MS: m/z = 719.2 [M+H]$^+$, 359.0 [M+2H]$^{2+}$.

$^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.09 (dd, $J$ = 4.3, 1.8 Hz, 1H), 9.02 (dd, $J$ = 8.0, 1.8 Hz, 1H), 8.95 (dd, $J$ = 4.3, 1.6 Hz, 1H), 7.87 (dd, $J$ = 8.4, 4.3 Hz, 1H), 7.48 (dd, $J$ = 8.4, 4.3 Hz, 1H), 7.30 (dd, $J$ = 8.4, 1.7 Hz, 1H), 7.11 – 7.02 (m, 2H), 5.62 (t, $J$ = 3.1 Hz, 1H), 5.57 (d, $J$ = 8.2 Hz, 1H), 5.01 (dd, $J$ = 8.2, 3.1 Hz, 1H), 4.97 (dd, $J$ = 10.3, 3.0 Hz, 1H), 4.42 (ddd, $J$ = 10.3, 4.9, 3.3 Hz, 1H), 4.21 – 4.10 (m, 2H), 2.16 (s, 3H), 2.03 – 1.98 (m, 8H), 1.98 – 1.93 (m, 1H).

Synthesis of 3b

The compound was synthesized by a similar method to that of comound 3a except that aniline was replaced by equimolar p-phenoxyaniline to give a pale yellow powder (Yield = 25%). Anal. Calcd. for C45H38N4O11 (%): C, 66.66; H, 4.72; N, 6.91. Found (%): C, 66.38; H, 4.84; N, 7.04. ES-MS: m/z = 809.5 [M+H]$^+$.

$^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.09 (dd, $J$ = 4.3, 1.8 Hz, 1H), 9.02 (dd, $J$ = 8.0, 1.8 Hz, 1H), 8.95 (dd, $J$ = 4.3, 1.6 Hz, 1H), 7.87 (dd, $J$ = 8.4, 4.3 Hz, 1H), 7.30 (dd, $J$ = 8.4, 1.7 Hz, 1H), 7.11 – 7.02 (m, 2H), 5.62 (t, $J$ = 3.1 Hz, 1H), 5.57 (d, $J$ = 8.2 Hz, 1H), 5.01 (dd, $J$ = 8.2, 3.1 Hz, 1H), 4.97 (dd, $J$ = 10.3, 3.0 Hz, 1H), 4.42 (ddd, $J$ = 10.3, 4.9, 3.3 Hz, 1H), 4.21 – 4.10 (m, 2H), 2.16 (s, 3H), 2.03 – 1.98 (m, 8H), 1.98 – 1.93 (m, 1H).

Synthesis of 4a

A mixture of comound 3a (0.14 g, 0.20 mmol) and equimolar cis-[Ru(DIP)$_2$Cl$_2$]·2H$_2$O was dissolved in 60 mL EtOH/H$_2$O (3:1, v/v), protected from light, and refluxed under argon at 85 °C for 24 h. The solvent was then evaporated under reduced pressure and yielded a reddish black solid which was subsequently purified with flash column chromatography on alumina with MeCN/EtOH (20:1, v/v) to afford the reddish product. This product was used directly for the next step (Yield = 38%). ES-MS: m/z = 739.75 [M-2Cl]$^{2+}$.

Synthesis of 4b

This compound was synthesized by a similar method to that of comound 4a except that comound 3a was replaced by equivalent comound 3b, and a red powder was obtained (Yield = 33%). ES-MS: m/z = 786.15 [M-2Cl]$^{2+}$. 
Synthesis of Ru3

A solution of compound 4a (151 mg, 0.21 mmol) in 30 mL anhydrous MeOH was cooled to 0 °C, added with 28% NaOMe (0.04 mmol) solution, and stirred for 6 h at 0 °C. Upon completion, the mixture was neutralized with 1 M HCl solution to pH 7.0. The solution was concentrated, added with 200 mL DD water, and subjected to ultrasonicitation to promote dissolution. After filtration, the filtrate was added with saturated NaClO4 solution and the precipitates were collected, washed with cold water and ether, and dried under vacuo. The obtained solid were recrystalized with toluene/MeCN to afford the final reddish product (Yield = 69.2%). Anal. Calcd. for C79H58Cl2N8O14Ru (%): C, 62.62; H, 3.86; N, 7.39. Found (%): C, 62.32; H, 3.92; N, 7.48. ES-MS: m/z = 658.30 [M-2ClO4]2+.

1H NMR (500 MHz, DMSO-d6) δ 9.24 (dd, J = 8.3, 1.3 Hz, 1H), 8.35 (d, J = 5.5 Hz, 1H), 8.29 – 8.24 (m, 5H), 8.21 (dt, J = 6.0, 3.1 Hz, 2H), 8.17 – 8.12 (m, 1H), 7.96 (dd, J = 8.3, 5.3 Hz, 1H), 7.85 – 7.72 (m, 9H), 7.72 – 7.59 (m, 21H), 7.57 (dd, J = 8.8, 1.6 Hz, 2H), 7.50 – 7.43 (m, 1H), 7.08 – 7.00 (m, 2H), 5.14 (d, J = 5.5 Hz, 1H), 8.36 (dd, J = 5.5, 1.6 Hz, 1H), 8.33 (dd, J = 5.5, 1.6 Hz, 1H), 8.29 – 8.20 (m, 7H), 8.17 (d, J = 5.0 Hz, 1H), 8.01 – 7.92 (m, 1H), 7.86 – 7.75 (m, 6H), 7.73 – 7.58 (m, 24H), 7.49 (t, J = 7.8 Hz, 2H), 7.40 – 7.28 (m, 2H), 7.25 (t, J = 7.4 Hz, 1H), 7.20 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H), 5.19 (dd, J = 7.9, 1.6 Hz, 1H), 5.09 (s, 1H), 5.01 (s, 1H), 4.71 (s, 1H), 4.51 (s, 1H), 3.94 (d, J = 3.1 Hz, 1H), 3.73 – 3.62 (m, 2H), 3.48 – 3.36 (m, 3H).

Synthesis of Ru4

This compound is synthesized in a similar method to that of Ru3 except that compound 4a was replaced by equimolar compound 4b (Yield = 67.5%). Anal. Calcd. for C85H62Cl2N8O15Ru (%): C, 63.51; H, 3.89; N, 6.97. Found (%): C, 63.42; H, 3.94; N, 7.03. ES-MS: m/z = 704.25 [M-2ClO4]2+.

1H NMR (500 MHz, DMSO-d6) δ 9.25 (d, J = 8.2 Hz, 1H), 8.36 (dd, J = 5.5, 1.6 Hz, 1H), 8.33 (dd, J = 5.5, 1.6 Hz, 1H), 8.29 – 8.20 (m, 7H), 8.17 (d, J = 5.0 Hz, 1H), 8.01 – 7.92 (m, 1H), 7.86 – 7.75 (m, 6H), 7.73 – 7.58 (m, 24H), 7.49 (t, J = 7.8 Hz, 2H), 7.40 – 7.28 (m, 2H), 7.25 (t, J = 7.4 Hz, 1H), 7.20 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H), 5.19 (dd, J = 7.9, 1.6 Hz, 1H), 5.09 (s, 1H), 5.01 (s, 1H), 4.71 (s, 1H), 4.51 (s, 1H), 3.94 (d, J = 3.1 Hz, 1H), 3.73 – 3.62 (m, 2H), 3.48 – 3.36 (m, 3H).

Luminescence quantum yield determination

The luminescence quantum yield of the compounds were measured in methanol using [Ru(bpy)3]2+ as the reference compound of which the quantum yield is 0.042 in aerated H2O at 25 °C[4]. The luminescence quantum yield is calculated according to the following equation:

\[
\Phi_S = \Phi_R \times \frac{I_A A_R n_S^2}{I_R A_S n_R^2}
\]

where \(\Phi\) denotes the luminescence quantum yield, \(A\) denotes the absorbance intensity, \(I\) denotes the integrated emission intensity, and \(n\) denotes the refractive index of the solvents used. The subscript “S” stands for the tested sample, and “R” for the reference compound, i.e., [Ru(bpy)3]2+.

Two-photon absorption cross section determination

The two-photon cross sections were determined in the range of 800-880 nm by the well established TPEF method devised by Webb and Xu[5] using Rhodamine B as the standard compound.[6] The samples were excited by a modelocked Ti:Sapphire pulsed laser with a laser width of 35 fs combined with a femtosecond optical parametric amplifier, and the emission signals were captured at a 90 degree angle by a high numerical aperture lens and directed to a charge-coupled device (CCD). The quadratic dependence of two-photon absorption cross section is determined.
induced fluorescence intensity on the excitation power was validated at the optimal two-photon laser excitation wavelength. The two-photon cross section of the compounds were calculated by the following equation,

\[ \sigma_S = \sigma_R \times \frac{\Phi_R C_R I_S n_S}{\Phi_S C_S I_R n_R} \]  

where \( I \) denotes the stimulated integrated fluorescence intensity, \( \Phi \) stands for the fluorescence quantum yield, \( \sigma \) denotes the two-photon cross sections, \( C \) represents the concentration, and \( n \) stands for the refractive index of the solvent used. The subscript "s" stands for the tested sample, and "r" stands for the reference compound, i.e. Rhodamine B.

\(^1\text{O}_2\) quantum yield determination in solution

The singlet oxygen quantum yields were determined by both direct and indirect methods. The direct manner is to detect the 1273 nm\(^2\) \(^1\text{O}_2\) phosphorescence in the presence of the photosensitizer at various concentrations (of which the corresponding OD values at 450 nm were measured) upon excitation by 450 nm light source. The integrated \(^1\text{O}_2\) phosphorescence intensities were plotted against the corresponding optical densities (OD\(_{450\ \text{nm}}\)). The linear regression of the plots gives a slope to calculate the singlet oxygen quantum yield according to the following equation,

\[ \Phi_\Delta^s = \Phi_\Delta^r \times \frac{S^s}{S^r} \]  

where \( \Phi_\Delta \) is the singlet oxygen quantum yield, \( S \) is the slope, the superscription "s" denotes sample and "r" denotes reference compound, i.e., [Ru(bpy)\(_3\)\(^2+\)].

For the indirect method, DPBF was used as the singlet oxygen scavenger of which the emission intensity at 479 nm attenuates as the singlet oxygen generates. To be specific, a mixture of DPBF (30 \( \mu\)M) and the indicated compounds with adjusted concentration (total OD\(_{405\ \text{nm}} = 0.2\) were exposed to irradiation at 450 nm, and the emission intensity at 479 nm was recorded every 5 seconds. [Ru(bpy)\(_3\)\(^2+\)] was used as the standard (\( \Phi_\Delta = 0.81 \) in methanol\(^8\)). The slopes derived from a linear regression of the plots of \( \Delta[\text{DPBF}] \) versus time were calibrated by the control to eliminate the impact of bleaching, and were used to calculate the singlet oxygen quantum yields of Ru1-Ru4 according to the following equations\(^9\):

\[ -\frac{\Delta[\text{DPBF}]}{t} = \frac{I_0 - I_t}{I_0} = I_0 \Phi_{ab} \Phi_\Delta \Phi_r \]  

\[ \frac{S}{S^R} = \frac{\Phi_{ab} \Phi_\Delta}{\Phi_\Delta^R} \]

where \( I_0 \) denotes the incident monochromatic light intensity, \( \Phi_{ab} \) represents the light absorbing efficiency at 450 nm, \( \Phi_\Delta \) stands for the reaction quantum yield of DPBF, \( \Phi_\Delta \) is the singlet oxygen quantum yield, \( t \) is the cumulative radiation time, \( I_0/I_t \) is the fluorescence intensity before/after irradiation of the complexes, and \( S \) is the slope of plots. The superscript "R" indicates the reference compound, i.e., [Ru(bpy)\(_3\)\(^2+\)].

log P measurement
The log P value of each complex, expressed as
\[
\log P^0_{o/w} = \log \left( \frac{[solute]_{octanol}}{[solute]_{water}} \right)
\]  
was measured by a “shake-flask” method. To be specific, water and octanol were mixed at ambient temperature, fully shaken to equilibriant and the two phases were separated. The compound was then dissolved in 3 mL octanol-saturated water, and added with equal volume of water-saturated octanol. The mixture was shaken vigorously by shaking table for overnight. The concentrations of the compounds in water were determined by the measurement of UV absorbance intensity and the validation of standard curves in octanol-saturated water. The evaluation had two replicates and the results were expressed as “mean value ± standard deviation”.

**Cell line information, culture conditions, and cell pretreatment**

HeLa, L02, A549, A549R, HepG2 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cell lines were maintained in DMEM media supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37 °C in a CO₂ incubator (95% relative humidity, 5% CO₂).

Culture media without glucose (the ingredient of which contained 89% non-glucose DMEM, 10% FBS, 1% antibiotics, v/v) were utilized to refresh the culture medium and incubate the cells to be tested in half an hour before every cellular experiment.

**Cellular uptake level study in different cell lines**

This study covered several human cell lines including HeLa, L02, A549, A549R and HepG2. Particularly, a total number of over ca. 3 million exponentially grown cells were treated with 10 μM Ru1-Ru4 (with 1% DMSO, v%), respectively, in a non-glucose culture medium protected from light at 37 °C for 2 h. Upon completion, the cells were carefully washed with PBS for three times, trypsinized, collected and counted. The cells in the suspension were centrifuged, digested with 60% HNO₃ for over 24 h, and subjected to ICP-MS experiment. A series of Ru solutions with adjusted standard concentrations ranging from 0 to 100 ppb were prepared to plot the standard cuve using a Ru standard solution (1000 μg/mL). The samples were diluted with DD water to a volume of 10 mL (with an ultimate nitric acid content less than 2%). The cellular uptake level was calculated by the ICP-MS result associated with the cell numbers.

**Mixed cells co-incubation study**

L02 and HeLa single cell suspensions at a density of 1.5 × 10⁴ cells/mL were, respectively, charged into confocal dishes of which a mini covergalss was horizontally laid on the bottom in advance. The cells were incubated at 37 °C for 24 h to adhere to the glass. The two identical covergalsses with distinct adherent cells were then carefully placed against each other onto a Corning 35 mm cofocal dish and incubated at 37 °C in a non-glucose culture medium for half an hour. Subsequently, the cells were simultaneously incubated with 10 μM Ru2 at 37 °C for 2 h. Upon completion, the cells were carefully washed with PBS and subjected to microscopy for observation under a 20× objective.
Cellular uptake mechanism study

The cellular uptake mechanism study was conducted by a CLSM taking advantage of the pretreatment of several cellular uptake inhibitors.\textsuperscript{[10]} HeLa cells adherent to confocal dishes at a density of $1 \times 10^4$ cells/mL were refreshed with non-glucose culture media and treated with various inhibitors in different conditions before the incubation of complexes. Specifically, for the temperature dependent uptake study, HeLa cells were treated with 10 $\mu$M Ru1-Ru2 (1% DMSO) for 2 h at 4 °C and 37 °C, respectively. For bio-inhibiting uptake study, 2-deoxy-D-glucose (2-dG, 50 mM) and oligomycin (5 $\mu$M) were used as energy blockers to inhibit active transport. HeLa cells were pretreated with 2-dG and oligomycin in special culture medium without glucose at 37 °C for 45 min and further treated with 10 $\mu$M Ru1-Ru2 (1% DMSO) for 2 h in the dark. NH$_4$Cl (50 mM) and chloroquine (100 $\mu$M) were used to inhibit endocytotic uptake. HeLa cells pretreated with the indicated endocytotic inhibitors at 37 °C for 1 h were treated with 10 $\mu$M Ru1-Ru2 (1% DMSO) for 2 h in the dark. For GLUTs inhibition, Phlorizin (100 $\mu$M) was used to preincubate the cells for 1 h before the addition of the Ru(II) complexes under the same conditions. All of the HeLa cells were washed with PBS for three times, and subjected to the CLSM. The Ru channel was set to be identical to that of co-localization study.

Intracellular distribution studies

This study is comprised of two parts, co-localization study by a CLSM and isolation kit extraction study by ICP-MS. For the co-localization study, HeLa cells adherent to 35 mm Corning confocal dishes were incubated with 10 $\mu$M Ru1-Ru4 (with 1% DMSO, v%), respectively, in a non-glucose culture medium protected from light at 37 °C for 2 h. After wash with PBS, the cells were treated with MitoTracker Green (MTG) and Hoechst 33342 in sequence according to the manufacturer’s protocols. Upon completion, the cells were thoroughly washed with PBS and subjected to the CLSM for imaging study. For Ru(II) complexes, the excitation/emission wavelength was set as 458 nm/600-650 nm; For Hoechst, the excitation wavelength was 405 nm and the emission filter was between 410-450 nm; For MTG, it was excited at 488 nm and the emission signals centered at 525 nm were collected.

As for the cellular compartment isolation and ICP-MS study, ca. 5 million exponentially grown HeLa cells in a Corning culture dish were treated with the indicated complexes, respectively, under identical conditions. Upon completion, the cells were washed with PBS, trysinized, collected and counted. The cells were then divided into two equivalent parts which were subsequently treated with mitochondria isolation kit and nucleu and cytoplasm isolation kit, respectively, according to the manufacturer’s protocols. The asprepared samples were digested with 60% HNO$_3$ for at least 24 h and subjected to the ICP-MS experiment in an identical way to that in the uptake level study.

Mitochondrial membrane potential (MMP) assessment

MMP was assessed by JC-1 staining. HeLa cells were seeded onto 35 mm Corning confocal dishes at a density of $1 \times 10^4$ cells/mL. After adhering for overnight, the cells pretreated with non-glucose culture media as previously described were treated with non-glucose culture medium (control), 10 $\mu$M Ru1-Ru4 (1% DMSO), respectively, at 37 °C for 2 h in the dark. Then the cells were further incubated in normal culture media for
additional 22 h. Upon completion, the cells were treated with JC-1 (10 μg/mL) in PBS for 20 min in the dark. The JC-1 fluorescence were imaged by a CLSM. The excitation wavelength for JC-1 monomer (green channel) was 488 nm, and the emission filter was adjusted to around 529 nm for JC-1 monomer. While for the JC-1 aggregate (red channel), the excitation was set at 543 nm, and the emission signals were collected centered at 590 nm (red).

**Caspase 3/7 detection**

Caspase-3/7 activation level was detected on a TECAN multifunction microplated reader using a Caspase-Glo® Assay Kit (Promega) according to the manufacturer’s protocol. The HeLa single cell suspension was charged into white-walled nontransparent-bottomed 96 culture plates at a density of 1 × 10⁴ cells/well and incubated for one day before treatment. The culture media were refreshed with non-glucose culture media as previously described. Cells were then treated with culture medium, DMSO (1%, v%), cisplatin (10 μM), and the Ru complexes (10 μM), respectively, at 37 °C for 2 h in the dark. Then the cells were further incubated in normal culture media for additional 22 h. Upon completion, the cells were divided into two groups, one for PDT therapeutics at 450 nm (12 J/cm²), and the other in the dark for contrast. All cells were treated with Caspase-Glo 3/7 Assay Kit 1 h after the irradiation. The results were presented as relative caspase activation intensity using the negative control group without any PDT treatments as a standard.

**MTT assay**

(Photocytotoxicity of Ru1-Ru4 complexes were tested using MTT as the cell viability indicator, and H₂TPP was used as a positive control. Exponentially grown HeLa/L02/A549/A549R/HepG2 single cell suspensions were charged into 96-well culture plates at a density of 1 × 10⁴ cells/well, and incubated at 37 °C for 24 h to adhere. The culture medium were then refreshed non-glucose culture medium, and incubated for half and hour. The cells were then treated with the tested compounds (H₂TPP, cisplatin, Ru1-Ru4) at a set of increasing concentrations. Control wells were treated with equal volume of culture medium. Cells were incubated for 2 h, and then the culture media for all group but cisplatin were refreshed, and cells were incubated for another 22 h. Upon completion, cells of light group were subjected to irradiation (450 nm, 12 J cm⁻²), while cells of the dark group were kept in the dark. All groups were allowed to incubate for additional 48 h, subsequently treated with MTT solution (5 mg/mL) and incubated for 4 h. The generated formazan in each well was dissoved by DMSO and the optical density at 595 nm was measured by a Molecular Devices absorbance reader. The cell survival rate in control wells were considered to be 100%, and the optical density ratio of experiment wells to control wells were regarded as the survival rate. IC₅₀ values were determined by plotting the percentage of viability versus concentration on a logarithmic graph.

**Two-photon laser induced singlet oxygen generation in vitro**

The two-photon laser induced siglet oxygen in a PDT procedure was verified by using DCFH as the in vitro indicator,[¹¹] HeLa cells at a density of 1 × 10⁴ cells/mL were seeded onto Corning confocal dishes and allowed to adhere for overnignt. The cells pretreated with non-glucose culture media were incubated with 10 μM Ru2 at 37 °C for 2 h, and further incubated with 10 μM DCFH in PBS at room temperature for 20 min. Upon completion, the culture media were refreshed with fresh PBS, and the cells were exposed to the
irradiation of a Coherent femtosecond pulsed two-photon laser in a bleaching mode at 810 nm (25 mW, 120 s). The fluorescent images were captured by an LSM 880 Carl Zeiss CLSM. DCFH was excited at 488 nm and the emission signals centered at 525 nm were collected.

**Annexin V/PI co-staining in the in vitro two-photon PDT**

HeLa cell were seeded on a Corning confocal dishes at a concentration of $1 \times 10^4$ cells/mL and incubated for 24 h to adhere. The cells preincubated with non-glucose culture medium were treated with 10 μM Ru2 at 37 °C for 2 h. Cells treated with culture medium only were used as the control group. Upon completion of the incubation, the cells were washed with PBS for three times, treated with Annexin V/PI kit according to the manufacturer’s protocol, and subjected to two-photon laser irradiation at 810 nm (25 mW, 120 s) and the fluorescent and morphological changes before and after two-photon PDT were captured on a LSM 880 microscope. For the Annexin V channel, the dye was excited at 488 nm and the signals were collected between 510-535 nm. For the PI channel, the dye was excited at 536 nm and the fluorescence was collected in the range of 600-630 nm.

**In vivo PDT experiment**

BALB/c-(nu/nu) female nude mice of the age of eight weeks were purchased and bred following the protocols of the laboratory animal center. All experimental protocols were approved by the Sun Yat-sen University Animal Care and Use Committee. Each mouse was subcutaneously injected with 150 μL HeLa single-cell suspension (ca. $2.5 \times 10^6$ HeLa cells/mouse) in a mixture of Matrigel and saline (1:2, v/v), and the HeLa xenografted tumor models were established in 1-2 weeks. The tumor size was tracked by measuring the length and width of the tumor. When the volume of the tumor reached ca. 60 mm$^3$, the mice were randomly allocated into four groups (6 mice per group). The PDT process spanning 21 days was divided into three courses, and each course took seven days (i.e., the mice received PDT every seven days). The volume of the tumors and the weight of the mice were recorded every three days. The tumor volume was evaluated by the following formula,

$$Volume = \frac{1}{2} \times Width^2 \times Length$$

The four groups were divided according to the following treatment regimens: (i) Ru2+hv: 25 μL Ru2 saline solution was intratumorally injected into mice at a dosage of 5 mg/kg and the mice were irradiated with 810 nm laser (50 mW, 1 kHz, pulse width 35 fs, 120 s/mm along z axis) 2 h after the injection; (ii) Ru2: 25 μL Ru2 saline solution was intratumorally injected into mice at a dosage of 5 mg/kg; (iii) Saline+hv: 25 μL physiological saline was intratumorally injected into mice and the mice were irradiated with 810 nm laser (50 mW, 1 kHz, pulse width 35 fs, 120 s/mm along z axis) 2 h after injection; (iv) Saline: 25 μL physiological saline was intratumorally injected into mice. The mice were anesthetized by the intraperitoneal injection of 10% chloral hydrate aqueous solution (at a dosage of ca. 10 μL/g) prior to receiving two-photon PDT. Upon completion of the 21-day PDT therapeutics, all mice were sacrificed. One mouse from each group was randomly chosen and its tumor was carefully carved out and washed with 4% paraformaldehyde. A digital color camera was used to photograph the mice and tumors.
**Histological studies**

After the 21-day PDT treatment, the mice were sacrificed. The tumors and primary organs (including heart, liver, kidney, spleen, lung, brain, and intestine) were removed from a randomly chosen mouse of each group, washed with saline, and immersed in 4% paraformaldehyde for a week. The sections of the tumors and organs were obtained as paraffin-embedded samples and stained with hematoxylin and eosin (H&E). Deep blue-purple hematoxylin and pink eosin stains nucleic acids and proteins, respectively. A Carl Zeiss Imager microscope was used to observe the tissue structure and cell state of the sections.
Fig. S1. $^1$H NMR spectrum of 3a.

Fig. S2. $^1$H NMR spectrum of 3b.
Fig. S3. ESI-MS and $^1$H NMR spectrum of Ru2.
Fig. S4. ESI-MS and $^1$H NMR spectrum of Ru3.
Fig. S5. ESI-MS and $^1$H NMR spectrum of Ru4.
**Fig. S6.** UV-vis absorption spectra (a) and emission spectra (b) of Ru1-Ru4 (10 μM) in MeOH solutions at room temperature. Emission spectra were recorded under the excitation of 460 nm incident light.

**Fig. S7.** Two-photon induced emission spectra of Ru2 (a), Ru3 (b), and Ru4 (c). Inset: the logarithmic plots of the excitation power dependence of two-photon induced luminescence intensity of the complexes versus pump power at 810 nm. The solid red lines denote the best-fit straight lines with gradients around 2.
Fig. S8. Plots of the relative emission change of DPBF (30 μM) at 479 nm versus irradiation time in the presence of Ru1-Ru4 in aerated methanol at an adjusted concentration (OD$_{479 \text{nm}}$ = 0.08). The irradiation wavelength is 450 nm.

Fig. S9. The Luminescence emission spectra of Ru1-Ru4 with serum (10%) containing cell culture medium over a period of 24 hours
Fig. S10. The microscopic images of HeLa and L02 cells co-incubation of Ru2 (10 μM) at 37 °C for 2 h in non-glucose culture medium using a 20× objective. Cells of the two cell lines were seeded onto mini coverglasses at the same density, respectively, and allowed to adhere for one day. The coverglasses were put together into a confocal dish before the incubation. White chain line in Ru channel indicates the boundary of the two coverglasses.
Fig. S11. Confocal luminescence images and bright-field images of live HeLa cells preincubated with 10 μM Ru1 and Ru2, respectively, in non-glucose culture media for 2 hours under different conditions: (i) cells were incubated at 37 °C; (ii) cells were incubated at 4 °C; (iii) cells were pretreated with 50 mM NH₄Cl for 1 h before the incubation of the complexes; (iv) cells were treated with 50 μM chloroquine for 1 h in advance and then incubated with the complexes; (v) cells were incubated with 50 mM 2-deoxy-D-glucose and 5 μM oligomycin for 1 h prior to the incubation of the complexes; (vi) cells were incubated with 100 μM phlorizin for 1 h preceding the incubation of the complexes. Scale bars represent 20 μm.
Fig. S12. ICP-MS quantification of the intracellular Ru distribution of the HeLa cells. HeLa cells were treated with Ru1-Ru4 (10 μM) at 37 °C for 2 h in the dark. Nuclei (Nuc.), mitochondria (Mito.) and cytoplasm (without mitochondria, Cyto.) were isolated using mitochondrial and nuclear isolation kits.

Fig. S13. Effects of Ru1-Ru4 (10 μM) on mitochondrial membrane potentials of HeLa cells after 22 h incubation in the dark by JC-1 staining before PDT.
Fig. S14. Caspase-3/7 activation detection in HeLa cells. The cells were pretreated with culture medium (NC), vehicle cosolvent (1% DMSO), cisplatin (CDDP, 10 μM), and Ru1-Ru4 (10 μM), respectively, with/without PDT therapeutics 22 h after the incubation. This measurement was conducted in 1 h post-PDT.

Fig. S15. Annexin V/PI co-staining of the HeLa cells before and after two-photon PDT at 810 nm (25 mW, 120 s). Cells were pretreated with culture medium and stained with Annexin V/PI before the irradiation. Inset scale bars represent 20 μm.
**Fig. S16.** Histological examination of primary organs and the HeLa xenografted tumor of the mice allocated into four different treatment regimens: physiological saline injection (Saline); physiological saline injection and two-photon laser irradiation (Saline+\(hv\)); Ru2 saline solution injection (Ru2); Ru2 saline solution injection and two-photon laser irradiation (Ru2+\(hv\)). The organs examined include brain, heart, intestine, kidney, liver, lung, and spleen. All tissue sections were stained with H&E and imaged by light microscopy. No appreciable tumors were available for section preparation at the very end of the PDT treatment of the last group (i.e., Ru2+\(hv\)). The inset scale bars represent 100 μm.
Table S1. Photophysical properties of Ru1-Ru4

<table>
<thead>
<tr>
<th>Compd</th>
<th>λ_{abs} / nm (log \varepsilon) [a]</th>
<th>λ_{em} / nm [b]</th>
<th>Φ_{PL}</th>
<th>σ_2^{[c]}/GM</th>
<th>Φ_{PL} × σ_2^{[d]}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru1</td>
<td>467 (4.41)</td>
<td>617</td>
<td>0.031</td>
<td>124^{[d]}</td>
<td>3.8</td>
</tr>
<tr>
<td>Ru2</td>
<td>465 (4.42)</td>
<td>611</td>
<td>0.030</td>
<td>128</td>
<td>3.8</td>
</tr>
<tr>
<td>Ru3</td>
<td>466 (4.44)</td>
<td>607</td>
<td>0.035</td>
<td>164</td>
<td>5.7</td>
</tr>
<tr>
<td>Ru4</td>
<td>466 (4.56)</td>
<td>608</td>
<td>0.035</td>
<td>181</td>
<td>6.3</td>
</tr>
</tbody>
</table>

[a] Data were recorded in aerated MeOH. [b] The exitation wavelength was 460 nm. [c] Maximum two-photon absorption cross section in the range of 800-880nm. [d] Φ_{PL} × σ_2 higher than the 0.1 GM threshold indicates its capability for optical imaging application in live specimens. [d] Value was taken from reference[2].

Table S2. Estimation of TPA-induced ROS generating ability.[a]

<table>
<thead>
<tr>
<th>Compd</th>
<th>σ_2/GM</th>
<th>Φ_Δ (direct/indirect)[b]</th>
<th>Φ_Δ × σ_2(direct/indirect)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_2TPP</td>
<td>2.2^{[c]}</td>
<td>0.70^{[c]}</td>
<td>1.5</td>
</tr>
<tr>
<td>Ru1</td>
<td>124^{[d]}</td>
<td>0.74/0.74</td>
<td>92/92</td>
</tr>
<tr>
<td>Ru2</td>
<td>128</td>
<td>0.85/0.84</td>
<td>109/108</td>
</tr>
<tr>
<td>Ru3</td>
<td>164</td>
<td>0.75/0.73</td>
<td>123/120</td>
</tr>
<tr>
<td>Ru4</td>
<td>181</td>
<td>0.90/0.88</td>
<td>163/159</td>
</tr>
</tbody>
</table>

[a] Data of Ru1-Ru4 were collected in MeOH, and those of H_2TPP were in toluene. [b] Singlet oxygen quantum yield determined by direct and indirect method. [c] Value was taken from reference. [d] Value was taken from reference[1].

Table S3. Cellular uptake levels amongst different cell lines (ng/million cells)

<table>
<thead>
<tr>
<th>Compd</th>
<th>L02</th>
<th>A549R</th>
<th>A549</th>
<th>HepG2</th>
<th>HeLa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru1</td>
<td>12.9±1.1</td>
<td>19.7±2.2</td>
<td>17.6±3.1</td>
<td>16.9±5.5</td>
<td>22.9±5.1</td>
</tr>
<tr>
<td>Ru2</td>
<td>20.8±4.7</td>
<td>43.1±6.3</td>
<td>41.5±3.8</td>
<td>84.8±9.5</td>
<td>111.58±10.8</td>
</tr>
<tr>
<td>Ru3</td>
<td>13.8±2.2</td>
<td>27.4±1.6</td>
<td>30.1±2.7</td>
<td>40.1±7.5</td>
<td>68.0±8.8</td>
</tr>
<tr>
<td>Ru4</td>
<td>15.9±1.4</td>
<td>30.4±3.6</td>
<td>33.3±1.8</td>
<td>42.2±8.5</td>
<td>78.7±14.4</td>
</tr>
</tbody>
</table>
**Table S4.** The content of Ru in 10⁶ HeLa cells and their intracellular compartment distribution

<table>
<thead>
<tr>
<th>Compd</th>
<th>Ru content (ng)</th>
<th>whole cell</th>
<th>Mitochondria</th>
<th>Nuclei</th>
<th>Cytoplasm (Without mitochondria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru1</td>
<td>21.22 ± 2.15</td>
<td>14.83 ± 1.19</td>
<td>1.17 ± 0.25</td>
<td>5.21 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>Ru2</td>
<td>107.25 ± 11.15</td>
<td>94.24 ± 10.19</td>
<td>3.41 ± 0.85</td>
<td>9.60 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>Ru3</td>
<td>63.19 ± 7.24</td>
<td>51.52 ± 5.26</td>
<td>1.13 ± 0.15</td>
<td>10.54 ± 1.18</td>
<td></td>
</tr>
<tr>
<td>Ru4</td>
<td>76.23 ± 8.24</td>
<td>64.06 ± 7.26</td>
<td>3.98 ± 0.88</td>
<td>8.20 ± 0.98</td>
<td></td>
</tr>
</tbody>
</table>

**Table S5.** 72 h (photo-)cytotoxicity of Ru1-Ru4 towards different cell lines (IC₅₀ [μM]).[^a]

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>HeLa</th>
<th>Dark</th>
<th>Light</th>
<th>PI</th>
<th>A549R</th>
<th>Dark</th>
<th>Light</th>
<th>PI</th>
<th>A549</th>
<th>Dark</th>
<th>Light</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>dark</td>
<td>light</td>
<td></td>
<td>dark</td>
<td>light</td>
<td></td>
<td></td>
<td>dark</td>
<td>light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>dark</td>
<td>6.9±0.6</td>
<td>7.1±0.5</td>
<td>0.97</td>
<td></td>
<td>33.5±3.2</td>
<td>32.5±3.1</td>
<td>1.0</td>
<td></td>
<td>8.1±1.6</td>
<td>7.8±1.6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>H₂TPP</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>81.1±5.8</td>
<td>&gt;1.23</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>90.4±9.8</td>
<td>&gt;1.10</td>
<td>&gt;100</td>
<td>98.2±3.8</td>
<td>&gt;1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru1</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>14.2±2.8</td>
<td>&gt;7.0</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>16.1±0.8</td>
<td>&gt;6.2</td>
<td>&gt;100</td>
<td>19.5±1.4</td>
<td>&gt;5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru2</td>
<td>93.2±6.6</td>
<td>96.1±5.1</td>
<td>2.1±0.3</td>
<td>44</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>11.5±3.2</td>
<td>&gt;8.7</td>
<td>&gt;100</td>
<td>8.3±2.1</td>
<td>&gt;12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru3</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>5.5±0.2</td>
<td>&gt;18</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>14.2±2.1</td>
<td>&gt;7.0</td>
<td>&gt;100</td>
<td>9.8±1.1</td>
<td>&gt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru4</td>
<td>88.2±7.5</td>
<td>88.2±7.5</td>
<td>3.1±0.3</td>
<td>28</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>13.9±0.9</td>
<td>&gt;7.2</td>
<td>&gt;100</td>
<td>10.3±2.1</td>
<td>&gt;9.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>HepG2</th>
<th>Dark</th>
<th>light</th>
<th>PI</th>
<th>L02</th>
<th>Dark</th>
<th>light</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>dark</td>
<td>light</td>
<td></td>
<td>dark</td>
<td>light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>dark</td>
<td>6.1±0.4</td>
<td>5.7±0.5</td>
<td>1.1</td>
<td></td>
<td>5.6±0.8</td>
<td>5.9±0.8</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>H₂TPP</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>84.2±2.4</td>
<td>&gt;1.18</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>92.3±7.8</td>
<td>&gt;1.08</td>
<td></td>
</tr>
<tr>
<td>Ru1</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>13.3±2.4</td>
<td>&gt;7.5</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>24.0±4.5</td>
<td>&gt;4.2</td>
<td></td>
</tr>
<tr>
<td>Ru2</td>
<td>96.1±5.1</td>
<td>96.1±5.1</td>
<td>3.0±0.8</td>
<td>32</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>20.4±3.6</td>
<td>&gt;4.9</td>
<td></td>
</tr>
<tr>
<td>Ru3</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>6.8±0.6</td>
<td>&gt;14</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>25.2±3.4</td>
<td>&gt;4.0</td>
<td></td>
</tr>
<tr>
<td>Ru4</td>
<td>95.3±6.9</td>
<td>95.3±6.9</td>
<td>3.6±0.6</td>
<td>26</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>22.7±2.9</td>
<td>&gt;4.4</td>
<td></td>
</tr>
</tbody>
</table>

[^a]: Irradiated at 450 nm by an LED area light (450 nm, 12 J cm⁻²).