Supporting Information

Taurine Modified Ru(II)-Complex Targets Brain Cancer Cell for Photodynamic Therapy

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Content

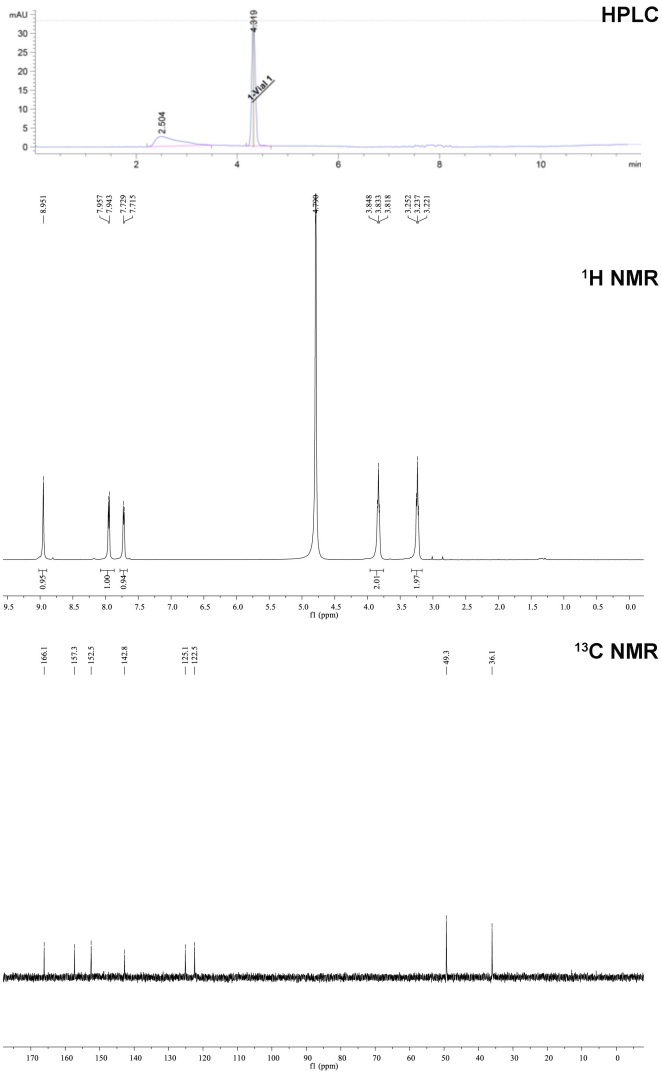
Experimental Section

Materials and Methods:

Synthesis and Characterization: All chemicals were obtained from commercial sources (Sigma, Nacalai or Wako) and were used without further purification. HPLC was performed on a Agilent 1260 Infinity Preparative Pump using a Agilent 1260 Infinity Diode Array Detector VL as the detector. A 19 mm×150 mm XBridge® Peptide BEH C18 column (Waters) was used for semi-preparative HPLC applications. Mass spectra were recorded using a Thermo LTQ-ETD mass spectrometer (ESI-MS). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend 400 (400 MHz and 100MHz, respectively) spectrometer.

Complex 1': This compound was prepared by a published procedure. [1] ^{1}H NMR (400 MHz, D_2O/NaOD) δ 8.87 (s, 1H), 7.87 (d, J = 5.6 Hz, 1H), 7.67 (m, 1H); ^{13}C NMR (100 MHz, D_2O/NaOD) δ 171.0, 157.4, 151.9, 145.7, 126.2, 123.2.

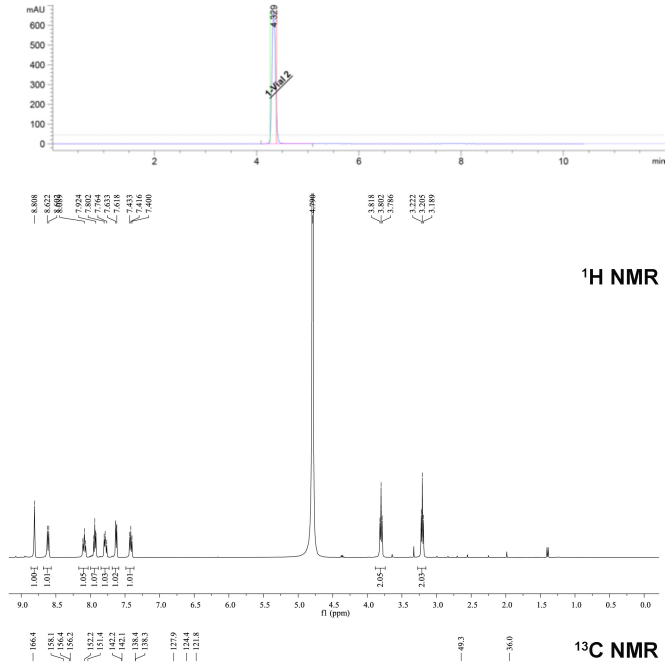
Complex 1: Complex **1'** (1.00 g, 1.20 mmol), HBTU (3.65 g, 9.59 mmol) and DIEA (1.69 mL, 9.59 mmol) were added to anhydrous DMF (25 mL) and anhydrous DMSO (1 mL) successively. After stirring for 30 min, Taurine (1.20 g, 9.59 mmol) was added into the mixture solution and kept stirring at 60°C for 48 h. Then it was concentrated under reduced pressure and the residue was treated with acetone. The precipitate was filtered and separated on Sephadex LH-20 column chromatography with MeOH as eluent. Then the raw product was further purified by semi-preparative HPLC to afford the pure complex **1** (140 mg, 7.9%). MS (ESI), *m/z*: 1477.00 ([M+H]$^{+}$); Found: C 37.14; H 4.05; N 10.76% (Calculated for $C_{48}H_{54}Cl_2N_{12}O_{24}RuS_6 \cdot 0.5H_2O$: C 37.04; H 3.56; N 10.80%); ^{1}H NMR (400 MHz, D_2O) δ 8.95 (s, 1H), 7.95 (d, J = 5.6 Hz, 1H), 7.72 (d, J = 5.6 Hz, 1H), 3.83 (t, J = 6.0 Hz, 2H), 3.24 (t, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, D_2O) δ 166.1, 157.3, 152.5, 142.8, 125.1, 122.5, 49.3, 36.1. Elemental analysis of **1**: Found: C 37.14; H 4.05; N 10.76% (Calculated for $C_{48}H_{54}Cl_2N_{12}O_{24}RuS_6 \cdot 0.5H_2O$: C 37.04; H 3.56; N 10.80%).



Complex 2': This compound was prepared by a published procedure. [2] ^{1}H NMR (400 MHz, D_2O) δ 8.82 (s, 1H), 8.60 (d, J = 8.0 Hz, 1H), 8.08 (m, 1H), 7.89 (m, 1H), 7.81 (m, 1H), 7.65 (m, 1H), 7.39 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 171.0 (2C), 157.6, 157.5 (2C), 157.4, 156.8, 156.7 (3C), 151.9, 151.8, 151.7, 151.4, 151.3, 151.2, 145.2, 145.1, 137.8 (2C), 127.3, 125.8, 124.2, 122.8.

Complex 2: DIC (0.42 mL, 2.68 mmol) was added to a solution of complex **2'** (570 mg, 0.81 mmol) and N-hydroxy succinimide (308 mg, 2.68 mmol) in anhydrous DMF (8 mL) and DMSO (0.5 mL) at 0°C, and the mixture was stirred for 0.5 h at the same temperature, then for an additional 1.5 h at room temperature. To the mixture was added Taurine (335 mg, 2.68 mmol) at room temperature. The reaction mixture was stirred at 50^{o}C for 24 h, and then adding 30 mL of acetone followed by filtration. The precipitate was separated on Sephadex LH-20 column chromatography with MeOH as eluent. Then the raw product was further purified by semi-preparative HPLC to afford the pure complex **2** (200 mg, 24.6%). MS (ESI), m/z: 1022.02 ([M-H]$^-$); ^{1}H NMR (400 MHz, D_2O) δ 8.81 (s,

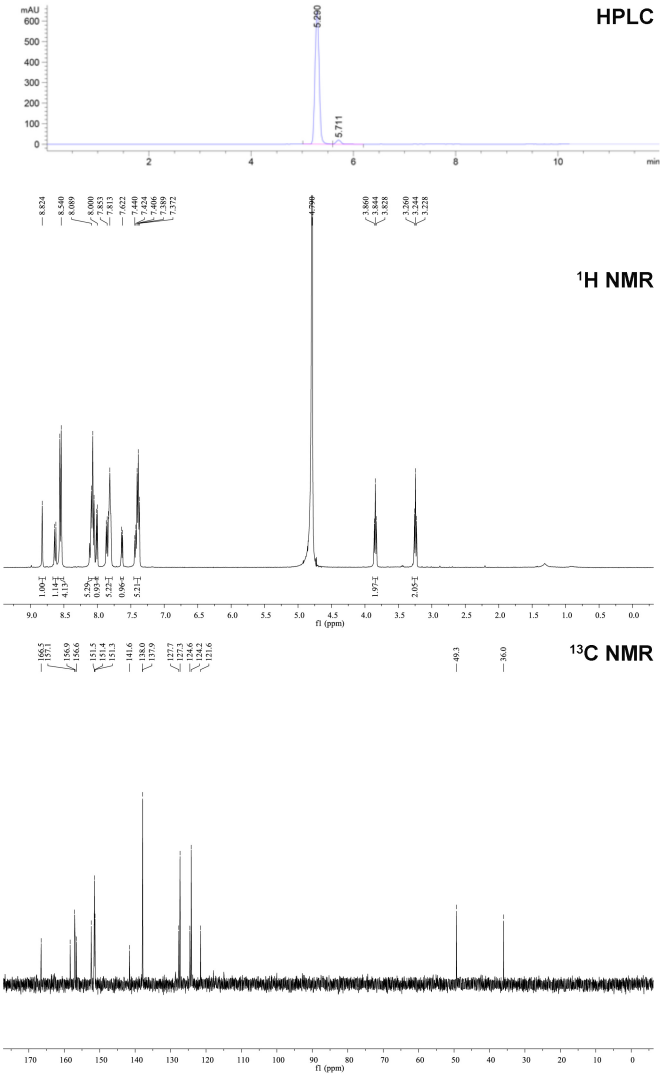
1H), 8.61 (d, J = 8.0 Hz, 1H), 8.09 (m, 1H), 7.94 (m, 1H), 7.79 (m, 1H), 7.63 (dd, J = 6.0, 1.6 Hz, 1H), 7.42 (t, J = 6.4 Hz, 1H), 3.80 (t, J = 6.4 Hz, 2H), 3.21 (t, J = 6.4 Hz, 2H); ^{13}C NMR (100 MHz, D_2O) δ 166.4, 158.2, 158.1, 158.0, 156.4, 156.3, 156.2, 152.5, 152.3, 152.2, 151.6 (2C), 151.4, 142.2, 142.1, 138.4, 138.3, 127.9, 124.8, 124.4, 121.8 (2C), 121.7, 49.3, 36.0.



Complex 3': This compound was prepared by a published procedure .[3] ^{1}H NMR (400 MHz, D_2O) δ 8.99 (s, 1H), 8.65 (d, J = 8.0 Hz, 1H), 8.55 (d, J = 8.0 Hz, 4H), 8.07 (m, 6H), 7.86 (d, J = 5.6 Hz, 1H), 7.81 (m, 5H), 7.39 (m, 5H); ^{13}C NMR (100 MHz, D_2O) δ 167.3, 158.5, 157.1, 157.0 (2C), 156.9, 156.6, 152.5, 151.5 (2C), 151.4, 151.3, 138.7, 138.0, 137.9 (4C), 127.7, 127.3 (4C), 126.0, 124.5, 124.2 (3C), 123.2, 117.9, 115.0.

Complex 3: Ru-complex **3'** (1.00 g, 1.63 mmol), HBTU (0.92 g, 2.43 mmol) and DIEA (0.43 mL, 2.47 mmol) were added to anhydrous DMF (25 mL) and anhydrous DMSO (1 mL) successively. After stirring for 30 min, Taurine (0.30 g, 2.40 mmol) was added into the mixture solution and kept stirring at 60°C for 48 h. Then it was concentrated

under reduced pressure and the residue separated on Sephadex LH-20 column chromatography with MeOH as eluent. The raw product was further purified by semi-preparative HPLC to afford the pure complex **3** (950 mg, 80.8%). MS (ESI), *m/z*: 722.22 ($[M+H]^+$); ^{1}H NMR (400 MHz, D_2O) δ 8.82 (s, 1H), 8.63 (d, J = 8.0 Hz, 1H), 8.55 (d, J = 8.0 Hz, 4H), 8.07 (m, 5H), 8.00 (d, J = 5.6 Hz, 1H), 7.86 (d, J = 5.6 Hz, 1H), 7.81 (m, 4H), 7.63 (d, J = 6.0 Hz, 1H), 7.40 (m, 5H), 3.84 (t, J = 6.4 Hz, 1H), 3.24 (t, J = 6.4 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 166.5, 158.4, 157.1 (2C), 157.0, 156.9, 156.6, 152.4, 151.5 (2C), 151.4, 151.3, 141.6, 138.0, 137.9 (4C), 127.7, 127.3 (4C), 124.6, 124.2 (4C), 121.6, 117.9, 115.0, 49.3, 36.0.



Absorption and emission spectrascopy: UV-vis spectra were performed with a Thermo Nanodrop 2000C spectrophotometer. The path length of the cuvette was 1 cm. The detection range was set to 250-700 nm and the spectral resolution to 1.0 nm. All emission spectra were measured using a Hitachi F-7000 fluorescence spectrometer. The excitation wavelength was set to 460 nm and emission collection from 500 to 800 nm.

Emission quantum yields: The quantum yields (Φ) were calculated according to the equation.[4]

$$\Phi_R = \Phi_{ref} \frac{I}{A} \frac{A_{ref}}{I_{ref}} \frac{n^2}{n_{ref}^2}$$

where A is the absorbance at the excitation wavelength, I is the integrated fluorescence intensity calculated from the area under the emission spectrum from 500 to 800 nm, n is the refractive index of the solvents (for buffer solutions, no refractive index correction was made) and the subscripts R and ref stand for the samples and reference, respectively. An aqueous solution of $[Ru(bpy)_3]^{2+}$ was used as a standard (0.063 in deaerated H_2O).[5]

Lifetime measurement: The fluorescence lifetimes (τ) were measured with a Hamamatsu Streakscope Time-resolved spectrometer. Emission for all lifetime measurements was excited at 460 nm. Samples used for quantum yield and lifetime determinations were carried out in deaerated buffer solutions (bubbling with solvent-saturated argon for 30 min).

Cell culture assay: PC12, F98 cell lines were purchased from American-type Culture Collection (ATCC, USA) and cultured in ATCC recommended media. F98 cell line was cultured in DMEM containing 10% FBS, whereas PC12 cell line was in RPMI-1640 medium supplemented with 10% FBS and 5% horse serum. Incubation was carried out at 37°C with a humidified atmosphere of 5% CO_2. The cells were maintained at 80% confluency and used for the bioassays.

Intracellular uptakes: Imaging flow cytometer (ImageStream X Mark, Germany) is applied for the cell uptake experiments. Excitation wavelength at 488 nm with power of 50 mW, and emission range 595-640nm are selected for all the experiments. The integrated fluorescence intensities (595-640 nm) of the complexes in citrate buffer (pH 4.5) were applied for the calibration of the cellular uptakes. Eventually, we obtained the relative cellular uptakes of the complexes in different cell lines.

Cell viability assay: Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 1×10^4 cells/well for F98 cell, and 5×10^4 cells/well for PC12 cell. The cells were allowed to attach to the wells for 12 h at 37ºC, 5% CO_2. The culture medium was removed followed by addition of 100 µL culture medium containing different concentrations (20, 50, 100, 200 and 500 µM) of Ru-complexes (immediately diluted from 10 mM stock solution in PBS). After the desired time of exposure, 10 µL MTT solution (5 mg/mL) was added to each well and incubated at 37°C for another 4 h, and then 100 µL of SDS solution (10% in Milli-Q water) was added to stop the reduction reaction and dissolve the purple formazan. The absorbance of each well at 570 nm was measured by a Tecan microplate reader. All experiments were conducted triplicate. The results were calculated as means, which are expressed as cell viability (%).

Measurement of ROS generation in buffer: The ROS levels under irradiation in citrate buffer solution were also measured with DHE assay. To the buffer solution of Ru-complexes (20 μM), DHE was added at a final concentration of 30 μM. The solution was irradiated with laser (450 nm, 0.45±0.01 mW) for desired time. After 5 min, the ROS level was examined by detecting fluorescence intensity of DHE conducted by Hitachi F-7000 fluorescence spectrometer. The excitation wavelength was set to 514 nm and emission collection from 590 to 610 nm.

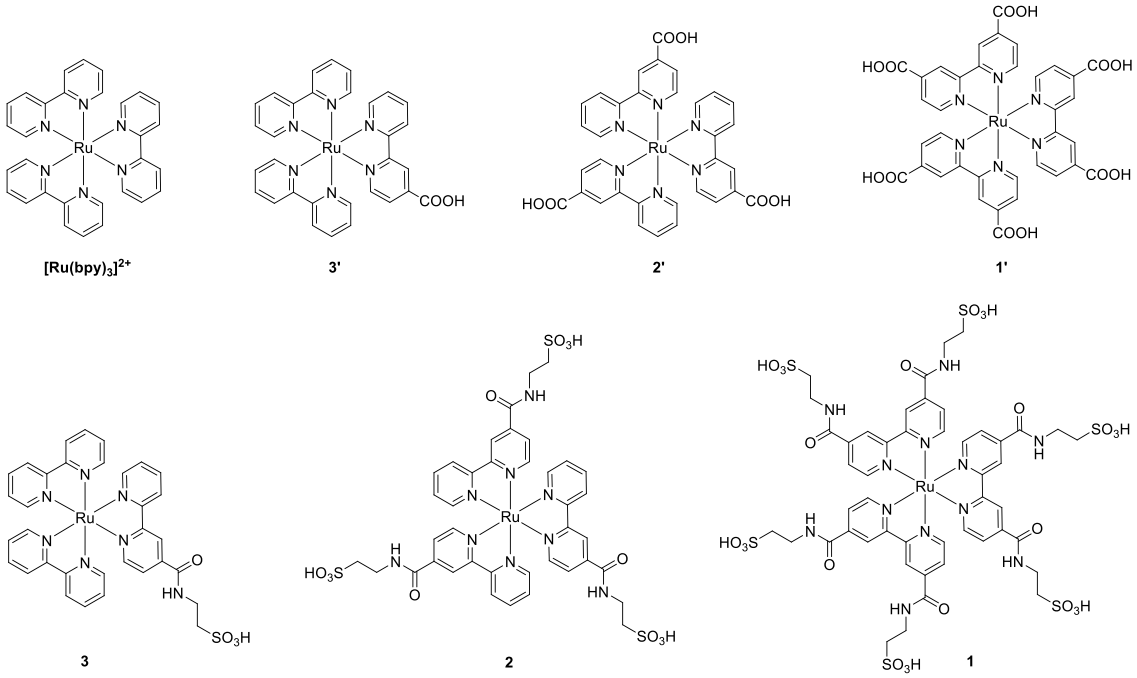
Measurement of intracellular ROS generation: The intracellular ROS under irradiation was measured using the fluorescent probe dihydroethidium (DHE). Briefly, the cultured cells were treated with 100 μM of complex **1** in the dark. After 9 h, the cells were co-incubated with 30 μM of DHE at 37^{o}C for 7 min. The cells, washed with PBS twice and resuspended in fresh live-cell imaging solution were subjected to irradiation (Zeiss LSM 780 with 60X objective, λ_{ex}: 458 nm, Laser powder: 3%). The intracellular ROS level was examined by detecting fluorescence intensity of DHE detivative.

Photo-cytotoxicity Assay: Cells were seeded on 35 mm cell culture dishes and incubated (37 °C, 5% CO2) overnight to allow the cells to attach to the wells. The culture medium was refreshed and 100 μM of **1** was added, and cultured for 6 hr. After washing with PBS, 1 mL fresh culture medium with 5 μL Alexa Fluor® 488 (invitrogen) was added immediately before placing the dishes in a Nikon BioStation CT. Cells were irradiated with the internal LED illuminator for 10 min. Images of cells in 8 different fields for each treatment condition were taken with a 10 × objective every 60min for 24hrs. At least three independent experiments were carried out for each experimental condition. Image analysis for the living and dead cell counting was done with ImageJ. By skipping the cell culture step with Ru-complex **1**, same procedure was applied for the control experiments.

Cell Viability Imaging Assay: ReadyProbes Cell Viability Imaging Kit (Blue/Green) from Life Technologies, ThermoFisher Scientific was applied for the experiments. Both PC12 cells and F98 cells are seeded in 35 mm glass-bottom dish (2×10^4 cells) and incubated with **1** (100 μM) for 9 hr. Remove the culture medium and change to fresh medium without phenol red. Monitor the cell viability by Zeiss LSM780 confocal microscope with stage-top incubator. And 60x objective is applied for both observation and photo-irradiation. Cells are irradiated by 458 nm laser with 3% laser power for 3 min. After that, time-lapse images are recorded very 20 min. After 3 hr, we observe cell death.

Supporting Figures and Tables:

Scheme S1: Chemical structures of Ru-complexes.



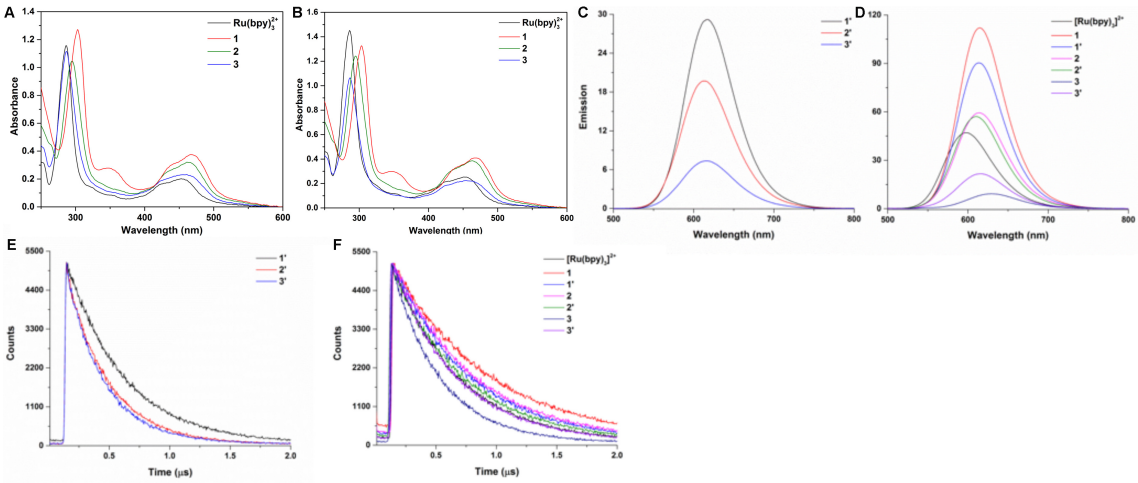


Fig. S1 Absorption spectra of Ru-complexes (20 μM) in citrate buffer (pH=4.5) **(A)**, and PBS buffer (pH = 7.4) **(B)**. Emission spectra of Ru-complexes (2 μM) in citrate buffer solution **(C)** and PBS buffer solution **(D)** (except complex **1** at 1.5 μM), λ_{ex} = 460 nm, under argon atmosphere. Time-resolved emission decay of Ru-complexes (50 μM) in citrate buffer solution **(E)** and PBS buffer solution **(F)**, λ_{ex} = 460 nm, under argon atmosphere.

Table S1. Physical properties in citrate buffer (pH = 4.5) and PBS (pH = 7.4) solutions under argon atmosphere.

	λmax (absorption, nm)[a]			λmax (emission, nm)		Φ_R (%)		τ (ns)	
Complex	LC (π-π*)	MC (d-d)	MLCT (d-π*)	pH 4.5	pH 7.4	pH 4.5	pH 7.4	pH 4.5	pH 7.4
$[Ru(bpy)_3]^{2+}$	286		453	597	598	8.7	13.8	607	583
1	303	347	467	615	615	15.3	12.9	866	859
1'	302	344	466	618	614	4.3	13.1	504	681
2	295		462	613	614	5.6	8.5	740	713
2'	295		461	613	610	2.4	9.0	364	643
3	287		458	630	629	3.3	2.2	440	482
3'	287		455	617	616	2.4	8.6	340	568

[a] This group absorbance data was measured under air condation, and the data was nearly the same in two different buffer solutions.

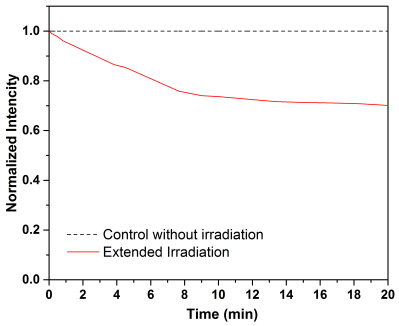


Fig. S2 Normalized emission intensity with and without extended laser irradiation.

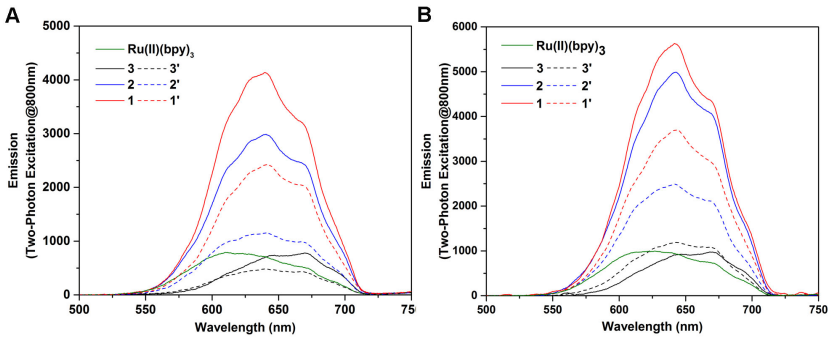


Fig. S3 Two-photon emission spectra of Ru(II)-complexes (λ_{ex} = 800 nm) in citrate buffer (pH = 4.5) (A), and PBS buffer (pH = 7.4) (B).

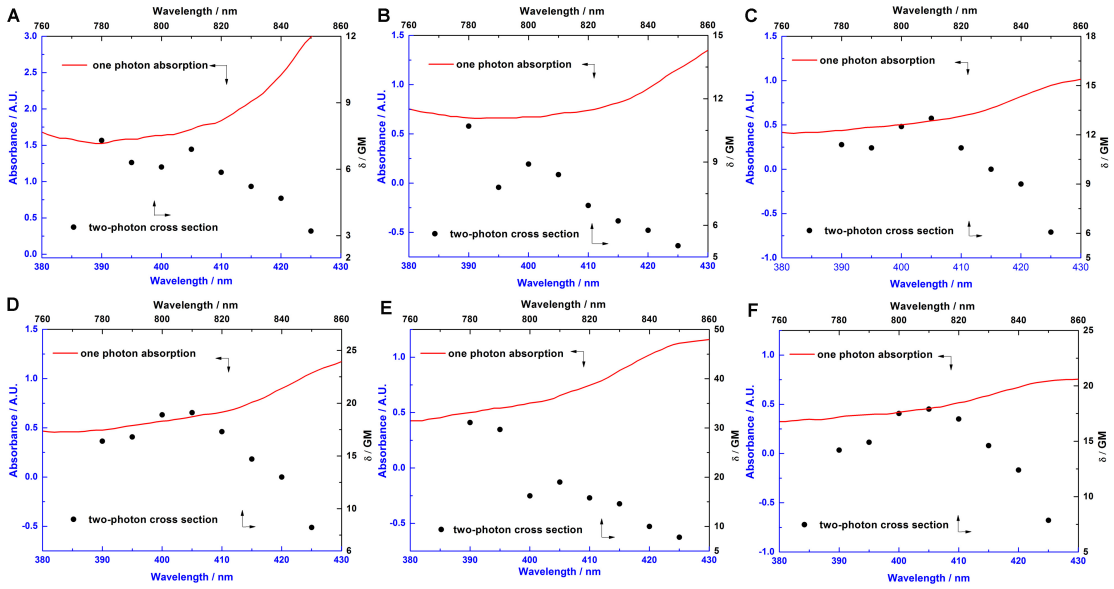


Figure S4. Two-photon cross sections of Ru-complexes **3'** (A), **3** (B), **2'** (C), **2** (D), **1'** (E), and **1** (F) with corresponding one photon absorption spectra. 1GM = 10^{-5} $cm^4 s$ $photon^{-1} molecule^{-1}$.

Table S2. The two-photon absorption crossing sections (δ) of the Ru-complexes at various excitation wavelength (λ_{ex}) in citrate buffer (pH = 4.5).

δ (GM) / **λ_{ex} (nm)**	**3**	**3'**	**2**	**2'**	**1**	**1'**	**RhB** [1]
780	10.7	7.3	16.4	11.4	14.2	31.1	115
790	7.8	6.3	16.8	11.2	14.9	29.7	133
800	8.9	6.1	18.9	12.5	17.5	16.2	151
810	8.4	6.9	19.1	13	17.9	19	170
820	6.94	5.9	17.3	11.2	17	15.8	180
830	6.2	5.2	14.7	9.9	14.6	14.6	200
840	5.8	4.7	13	9	12.4	10	210
850	5.0	3.2	8.2	6.1	7.9	7.8	126

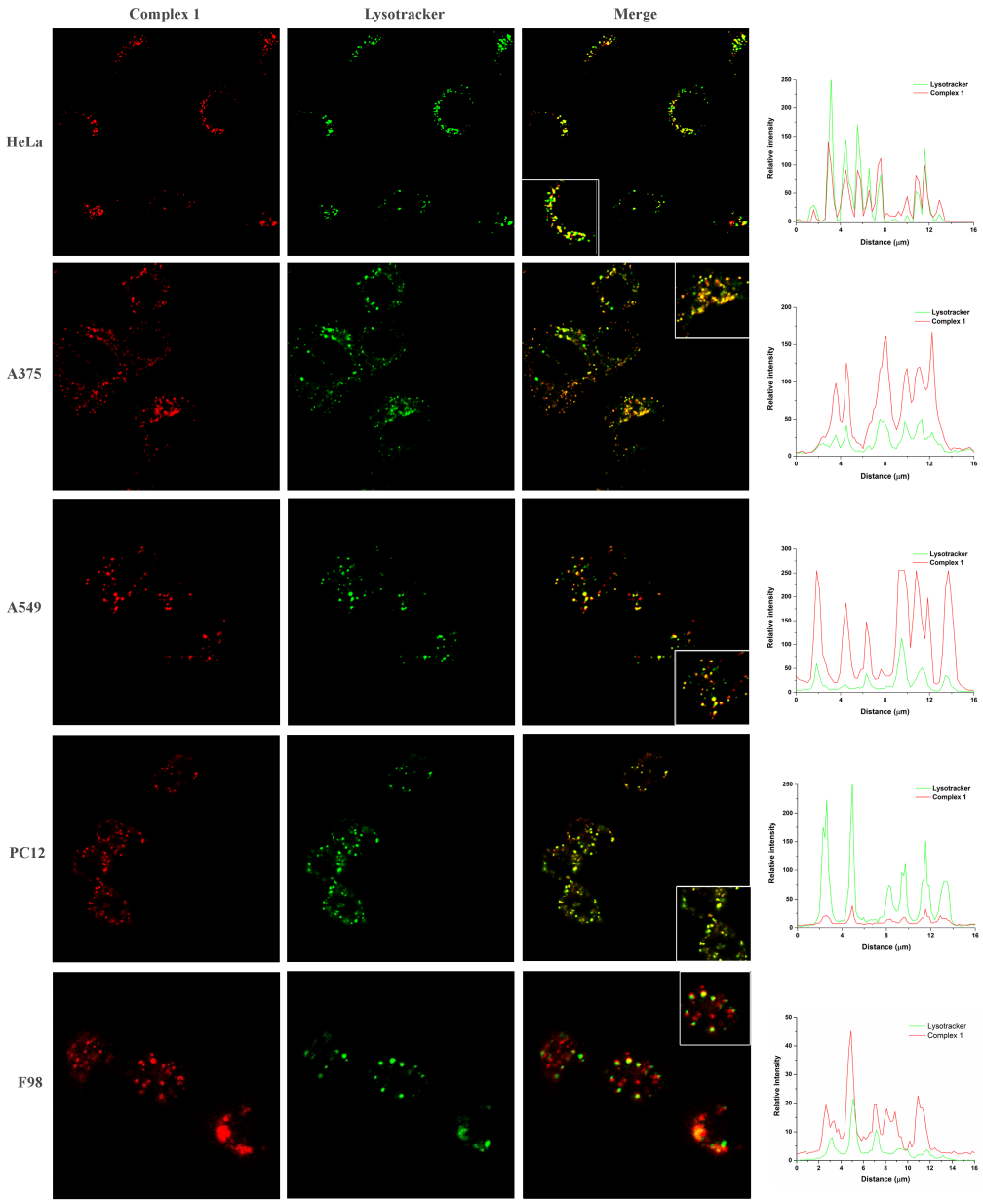


Fig. S5 Fluorescent images of HeLa, A375, A549, PC12 and F98 cells cultured with Ru-complex (100 μM) stained with LysoTracker Green. Red color represents the Ru-complex **1**, green color represents lysosomes, and the third column images are the merged fluorescent images. The last column spectra are the plot profiles of high-lightened cell images with white frames.

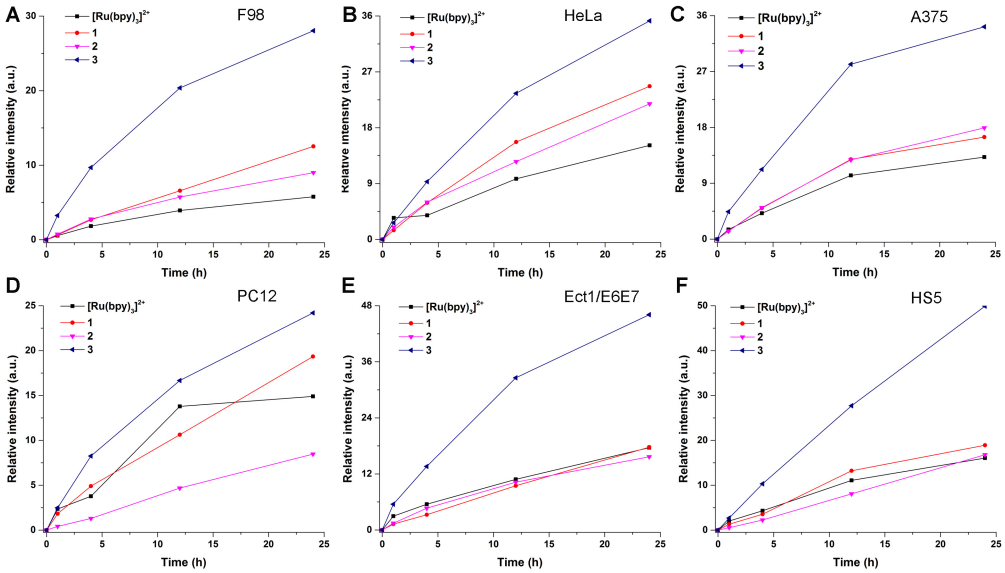


Fig. S6. The time-dependent cell uptakes of Ru-complexes in F98 (A), HeLa (B), A375 (C), PC12 (D), Ect1/E6E7 (E), and HS5 cells (F).

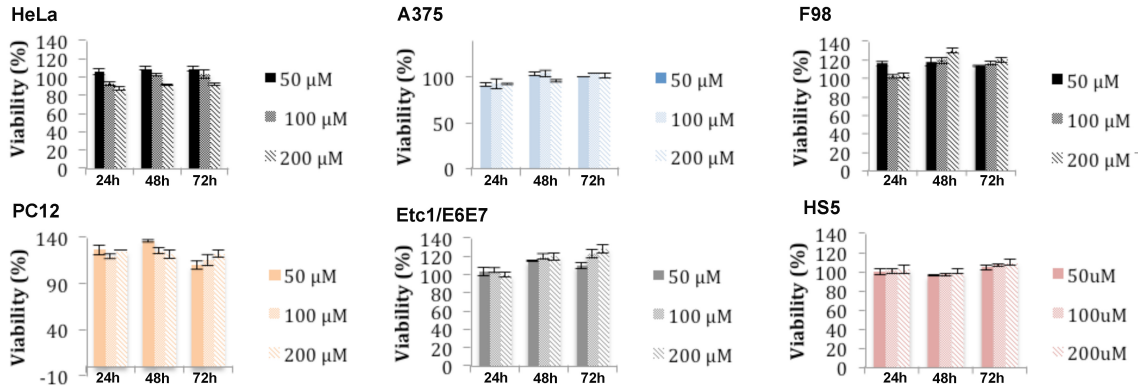


Fig. S7 HeLa, A375, F98, PC12, Etc1/E6E7 and HS5 cell viabilities incubated with Ru-complex **1** in darkness.

Reference:

1. P. G. Hoertz, Y. I. Kim, W. J. Youngblood and T. E. Mallouk, *J Phys Chem B*, 2007, **111**, 6845-6856.
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