Electronic Supplementary Information for

A Smart Perylene Derived Photosensitizer for Lysosome-Targeted and Self-Assessed Photodynamic Therapy

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Materials

(1,3-diphenylisobenzofuran) DPBF and (5,5-dimethyl-1-pyrroline-1-oxide) DMPO were obtained from Energy Chemicals (China). (2,2,6,6-tetramethyl-4-oxopiperidine) TEMP and (methylthiazolyldiphenyl-tetrazolium bromide) MTT were bought from J&K Chemicals (China). Mito Tracker® Deep Red (MTDR), LysoTracker® Red (LTR), and Annexin V-Alexa Fluor 647/PI Apoptosis Detection Kit were obtained from Yeason (China). Hoechest 33342 and (propidium iodide) PI were purchased from Life Technology (China). Lipid Peroxidation MDA Assay Kit and BCA Protein Assay Kit were bought from Beyotime (China). A stock solution of PC4 (5mM) was prepared in sterile water, stored at room temperature, and covered to avoid light exposure. Other reagents were of high purity (≥ 95%) and used as received.

Physical measurements

UV-Vis absorption spectra were recorded with a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped with a xenon flash lamp. Fluorescence emission spectra were recorded using a Fluoromax-4spectrofluorometer (Horiba JobinYvon Inc., USA) at room temperature. CLSM images were obtained from Nikon ECLIPSE Ti(Japan).

ROS generation assay

The generation of ROS was detected using DPBF as the indicator.[S1] DPBF stock solution (250 μg/mL) was freshly prepared, and diluted to the final concentration of 17 μg/mL (solvent: CH_3OH). The stock solution of PC4 in CH_3OH was 350 μM and the final concentration was 8 μM. A 473 nm laser was used as the light source. The samples contained DPBF+PC4, DPBF, or PC4 were under continuous light irradiation

for 10 s intervals, and the changes in UV-vis absorption were recorded. The intensity decrease of DPBF at 412 nm was plotted against the irradiation time. As a comparison, a sample mixture of PC4 and DPBF was kept in the dark, and changes in DPBF UV-vis absorption were studied. The intensity changes of DPBF at 412 nm were calculated as:

$$Y = (A - A_p)/(A_o - A_P) \quad (1)$$

A: the absorption value of the sample mixture at 412 nm after light irradiation, *Ap*: the absorption value of PC4 at 412 nm, A_o*:* the absorption value of the sample mixture at 412 nm before light irradiation. Please note that the absorption value of PC4 was not affected by light irradiation.

Singlet oxygen quantumn yield of the probe was calculatedusing DPBF as indicator and the $Ru(bpy)_3^{2+}$ as the standard photosensitizer. The quantumn yield of the probe was calculated using the following formula:

$$\Phi_{pc4} = \Phi_{Ru} \cdot K_{pc4} \cdot \frac{A_{Ru}}{K_{Ru} \cdot A_{pc4}} \quad (2)$$

Where K_{pc4} and K_{Ru} were the decomposition rate constant of DPBF caused by PC4 and $Ru(bpy)_3^{2+}$, respectively. A_{pc4} and A_{Ru} represented the light absorption by PC4 and $Ru(bpy)_3^{2+}$ at 473 nm, respectively. Φ_{Ru} was the $^1O_2$ quantum yield of $Ru(bpy)_3^{2+}$ in methanol (0.73).[S2]

ESR assay

The ESR spectra were recorded with a Bruker A300 spectrometer (Japan) at 298 K.The capillary tubes were put into the ESR cavity, and the spectra were recorded under a UV lamp irradiation for 3 min. Conditions: 1 mW microwave power; 100 G scan range; and 1 G field modulation; 2,2,6,6-tetramethylpiperidine (TEMP, 40 mM, $^1O_2$ trapper); 5,5-dimethyl-1-pyrroline-1-oxide (DMPO, 100 mM, for free radical trapping); PC4, 50 μM.

NIR emission spectrum of $^1O_2$

The $^1O_2$ emission at 1270 nm was detected with a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA) using a NIR detector (cooled with liquid nitrogen). Conditions: 35 μM of PC4 in CH_3CN/D_2O (19/1, v:v) solution, excitation: 450 nm.

Lipophilicity/hydrophilicity assay

500 μL of octanol was added to equal volume of H_2O, cell culture PBS (150 mM, pH7.4), HAc-NaAc buffer (200 mM, pH 4.0), and phosphate buffer (200 mM, pH6.0). The samples were equilibrated for 12 h. 4 μL of the probe stock solution (5 mM) was added to the sample mixtures, and the samples were put on an oscillator overnight (18 h) at 60 rpm and 25ºC. The samples were centrifuged at 3000 rpm for 10 min, and the photographs were taken. The fluorescence emission of the PC4 in each phase was also recorded.

Cell culture

A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% 100 U/mL antibiotics penicillin/streptomycin at 37 ºC, in a 100% humidified atmosphere containing 5% CO_2. After the amount of cell reached 60-80%, the culture medium was removed and the cells were lysed by trypsin and harvested by centrifugation at 800 rpm for 5 min. The harvested cells were seeded onto the confocal dish and allowed to grow overnight.

Cell uptake and localization assay

The cells grown in the confocal dishes were incubated with PC4 (5 μM) at 37ºC for 30 min, 6 h, and 12 h, respectively. The cells were rinsed with PBS three times and the fluorescence images were taken with a confocal fluorescence microscope using a 100X oil-immersion objective lens. The excitation wavelength of PC4 was 488 nm. For co-localization assay, cells in confocal dishes were incubated with 5 μM of PC4

for 30 min in culture medium at 37 ºC, and rinsed with PBS three times. The samples were then incubated with 1 μL of Hoechst 33342 (1 mg/mL), 10 μL of Mito Tracker (MTR, 10 μM in DMSO) or 10 μL of Lyso Tracker (LTDR, 5 μM in DMSO) for 30 min, respectively. The final density of the trackers was 1 μg/mL, 100 nM and 50 nM, respectively. The cells were rinsed with PBS and the fluorescence images were taken. The excitation wavelength was 405 nm for Hoechst 33342, 488 nm for PC4, 561 nm for MTR, and 640 nm for LTDR.

Cytotoxicity assay

200 μL of the collected cells (10^4 cells/mL) were seeded in a 96-well plate and grown overnight in an incubator. Various amounts of PC4 (5 mM) were added, and the samples were incubated for an additional 6 h. The culture medium was removed and 200 μL of fresh culture medium was added. One set of the cell samples was irradiated with light for 5 min (14 mW/cm^2, 450 nm). Another set of the cell samples was not irradiated. All the samples were incubated for another 24 h, and 10 μL of MTT solution (5 mg/mL) was added and the samples were incubated for 4 h. After incubation, the cell culture was discarded, 150 μL of DMSO was added to completely dissolve the purple crystal, and the absorption of each well at 490 nm was recorded by a microplate reader.

Real-time monitoring therapy process

The harvested cells were incubated in confocal dishes overnight, incubated with 5 μM of PC4 for 30 min, washed with PBS for three times, and then continuous irradiated (488 nm laser, 50 mW, 6% laser intensity) and observed under CLSM. For control experiment, cells without PC4 were irradiated and observed. For Hoechst 33342-labeling experiment, cells were incubated with PC4 (5 μM, 30 min), and with Hoechst 33342 (1 μg/mL, 30 min), washed with PBS, and then continuous irradiated (488 nm laser, 50 mW, 6% laser intensity) and observed under CLSM.

Apoptotic/necrotic detection assay

Annexin V-Alexa Fluor 647/PI Apoptosis/Necrosis Detection Kit was used following the protocol described by the manufacturer. The cells were culture in a 10 cm dish medium and used when its density reached 10^7 cells/mL. The cells were incubated with the probe (8 μM) for 30 min, washed with PBS 3 times, and irradiated with blue LED light (450 nm, 4.2 J/cm^2). The cells with light irradiation but no PC4, and the cells with PC4 but not light irradiation were used as control. The cells werecollected,washed with PBS and re-suspended in 100 μL binding buffer. The cell suspension was stained with 5 μL Annexin V-Alexa Fluor 647 and 10 μL PI for 15 min at room temperature, and re-suspended in 400 μL PBS. The cells were then analyzed by fluorescence-assisted cell sorting.

MDA detection assay

An MDA detection kit and a BCA detection kit were used following given protocols. Cells were plated in 10 cm petri dish at a density of 10^7 cells/well. After incubated with PC4 (8 μM) and irradiated with blue LED light (450 nm, 4.2 J/cm^2), cells were washed and harvested by trypsinization. Cellular extracts were prepared by an ultrasonic disruptor and the lysed cells were centrifuged at 12,000 rpm for 5 min to remove the sediment. MDA level and protein amount of the cells were quantified in the supernatant. 100 μL cell supernatant were mixed with 200 μL TBA detection solution and incubated at 100 ºC for 15 min with a heat block. After cooling to room temperature, the mixture was centrifuged at 1,200 rpm for 10 min. Then 200 μL supernatant was added to the 96-well plate, and the absorption at 540 nm was obtained using a microplate reader. The protein detection kit was used to measure the protein concentration.

PI co-staining assay

The harvested cells were incubated in confocal dishes overnight, and then incubated

with PC4 (5 μM) for 30 min, washed with PBS three times. After light irradiation (450 nm, 4.2 J/cm^2), the cells were incubated with PI (1 μg/mL). The cells were washed with PBS three times and the fluorescence microscope images were obtained. The excitation wavelength was 488 nm for PC4 and 561 nm for PI.

Supplementary figures

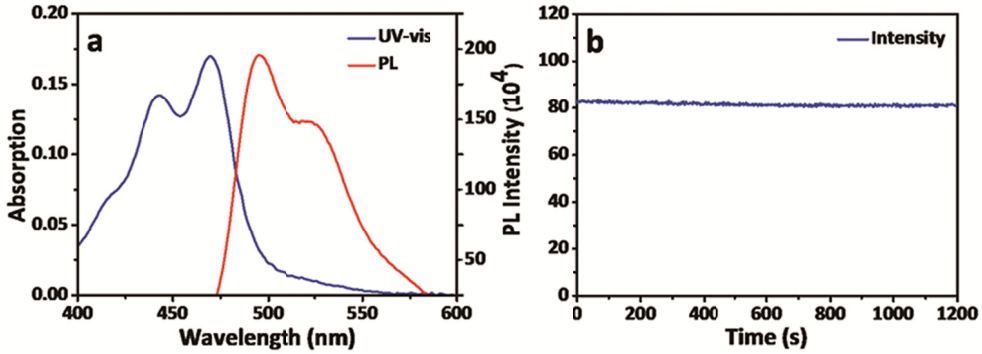

Fig. S1 (a) UV-vis absorption and PL spectra of PC4. (b) PL intensity changes of PC4 at 490 nm under continuous 470 nm light irradiation. PC4: 5 μM; Phosphate buffer (20 mM, pH 7.4).

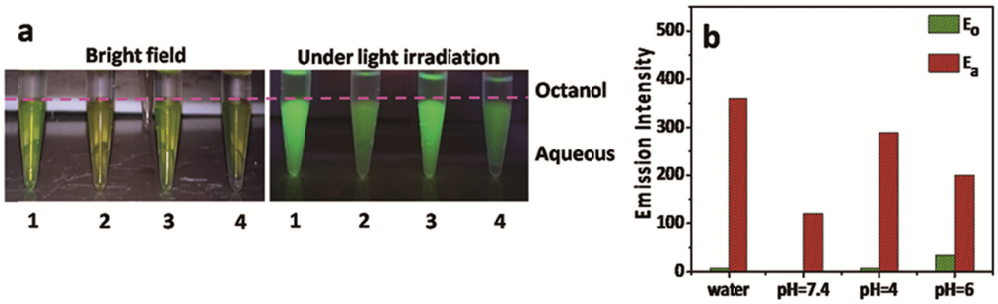

Fig. S2 (a) Partition of PC4 between the octanol and the aqueous phases. (1) Water; (2) Phosphate buffer saline for cell culture (150 mM, pH 7.4); (3) HAc-NaAc buffer (200 mM, pH 4.0); (4) Phosphate buffer (200 mM, pH 6.0). (b) Emission intensity of PC4 at 490 nm in various phases (E_o: the octanol phase, E_a: the aqueous phase), excitationat 450 nm.

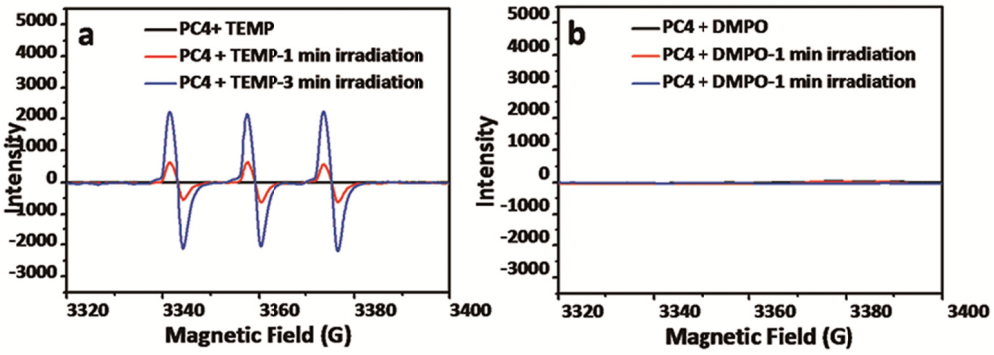

Fig. S3 (a) ESR experiments of $^1O_2$ trapped by TEMP. (b) ESR experiments of radicals trapped by DMPO. TEMP: 40 mM; DMPO: 100 mM; PC4: 50 μM.

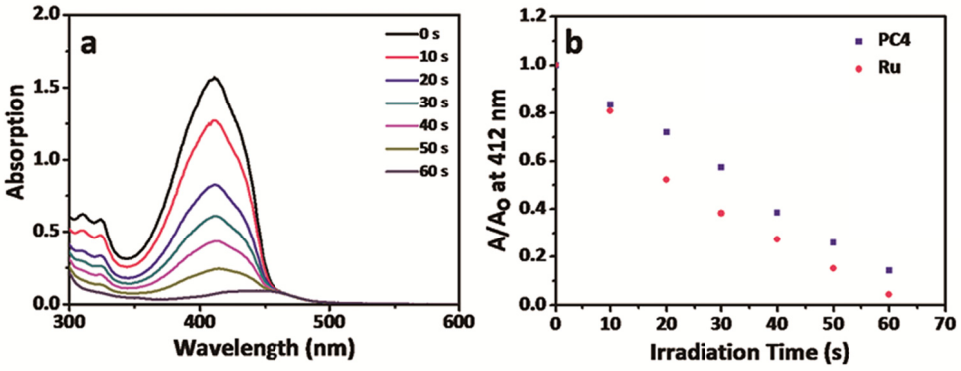

Fig. S4 (a) UV-vis absorption spectra of DPBF (17 μg/mL) in the presence of $[Ru(bpy)_3]^{2+}$ (4.6 μM) and under different duration of 473 nm light irradiation. (b) The corresponding A/A_o ratio changes of DPBF at 412 nm with irradiation time. $A=A_{mixture}- A_{pc4}$ or $A= A_{mixture}-A_{Ru}$; $A_0= A_{0mixture}-A_{Pc4}$ or $A_o= A_{0mixture}-A_{Ru}$. Solvent: methanol.

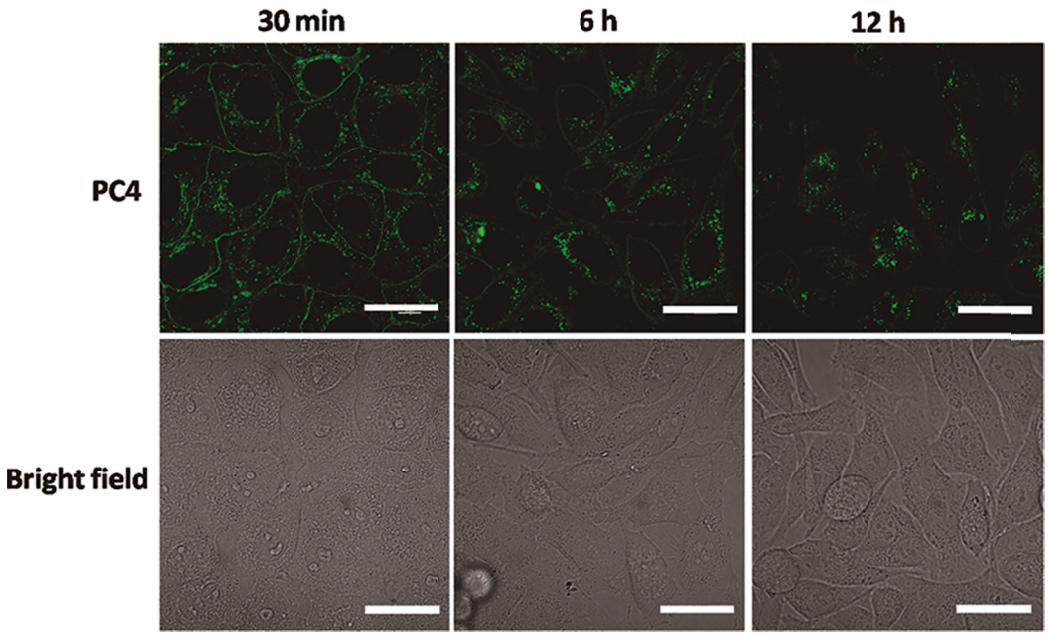

Fig. S5 CLSM images of A549 cells incubated with 5 μM of PC4 for different periods of time. Scale bar: 30 μm.

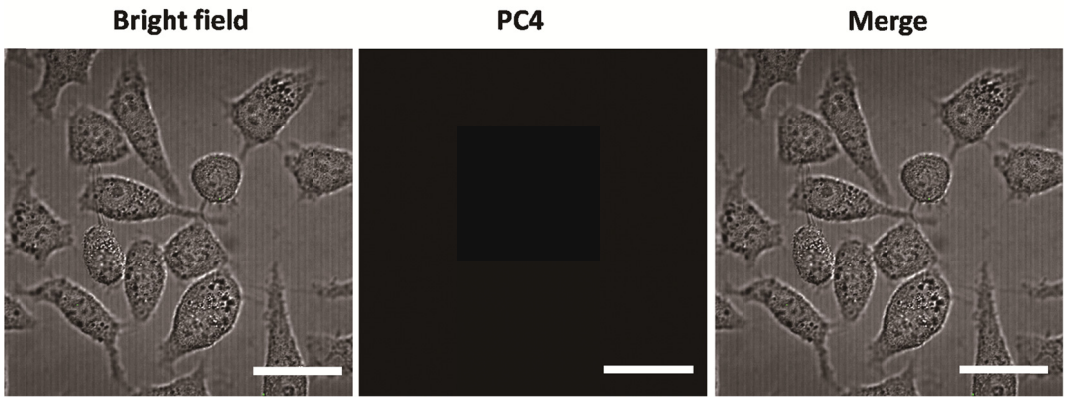

Fig. S6 CLSM images of A549 cells incubated with PC4 (5 μM) at 4 ºC. Scale bar: 30 μm.

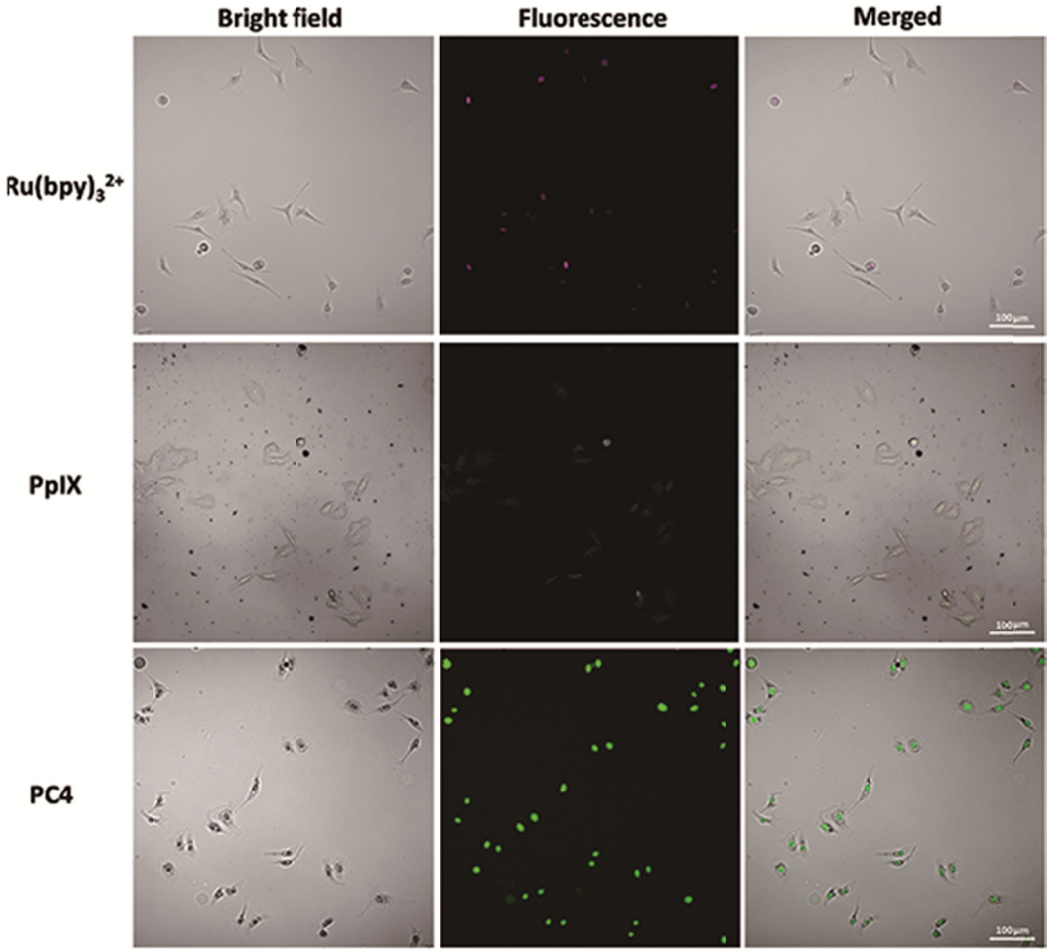

Fig. S7 CLSM images of cells after treated with different photosensitizers. Scale bar: 100 μm.

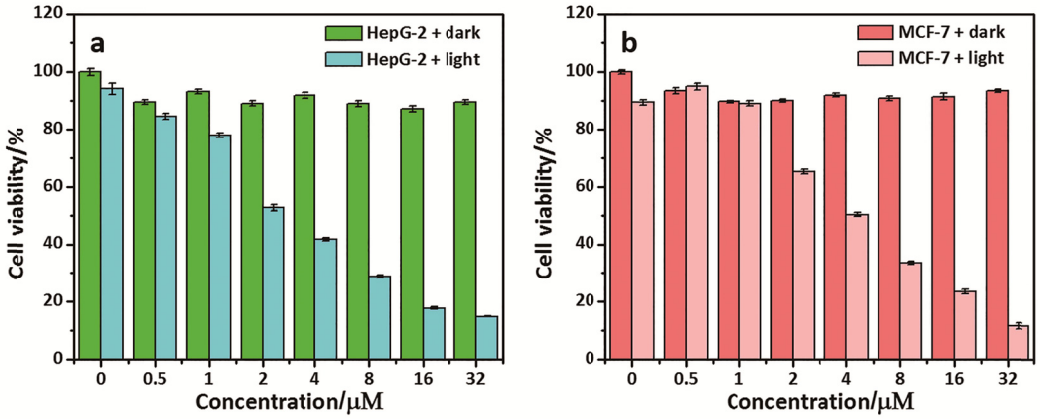

Fig. S8 Cytotoxicity of PC4 towards HepG-2 (a) and MCF-7 (b) cells in the dark or under light irradiation (450 nm, 4.2 J/cm^2).

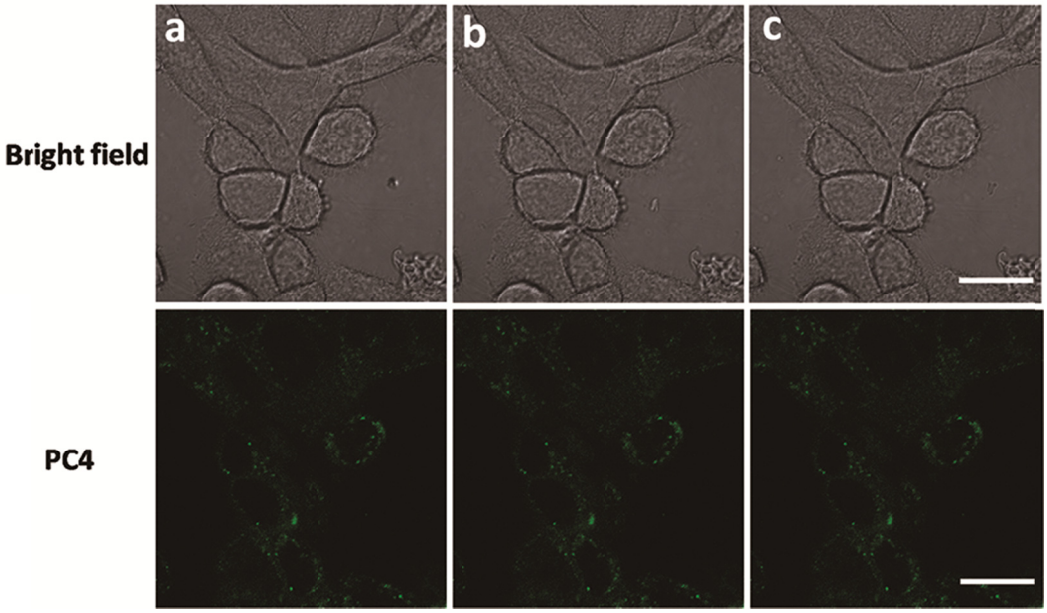

Fig. S9 Phototoxicity of PC4 in the presence of Vitamin C (50 μM) as ROS scavenger. Light irradiation time:(a) 0, (b) 2, and (c) 4 min. Scale bar: 30μm.

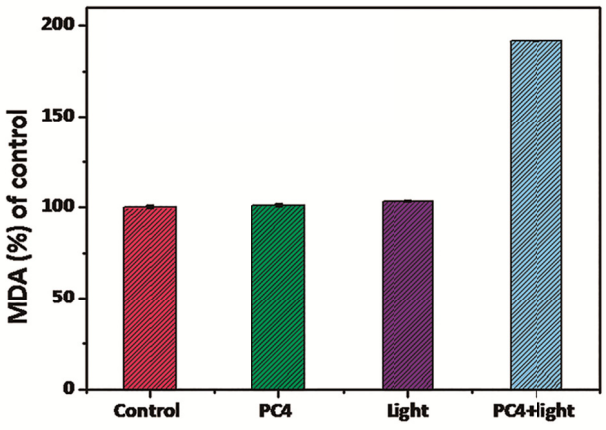

Fig. S10 MDA contents (%) in cells treated with PC4 (8 μM), light (4.2 J /cm^2), and PC4 + light (8 μM+4.2 J /cm^2).

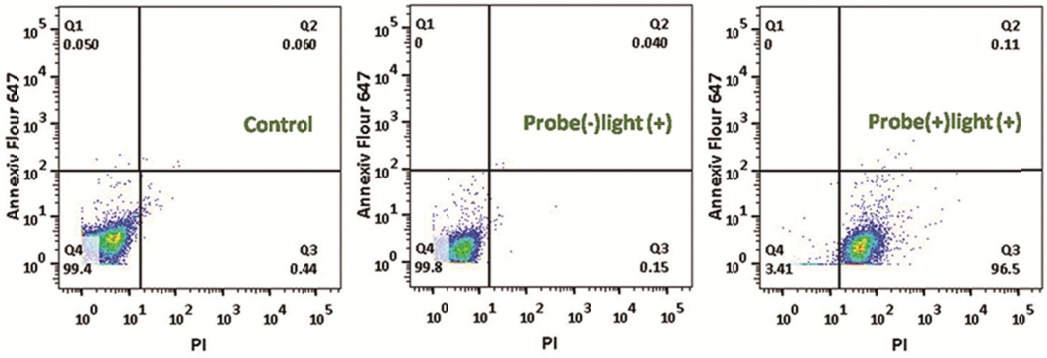

Fig. S11 Flow cytometry quantification of Annexin V-Alexa Fluor 647 and PI double-labeled A549 cells.

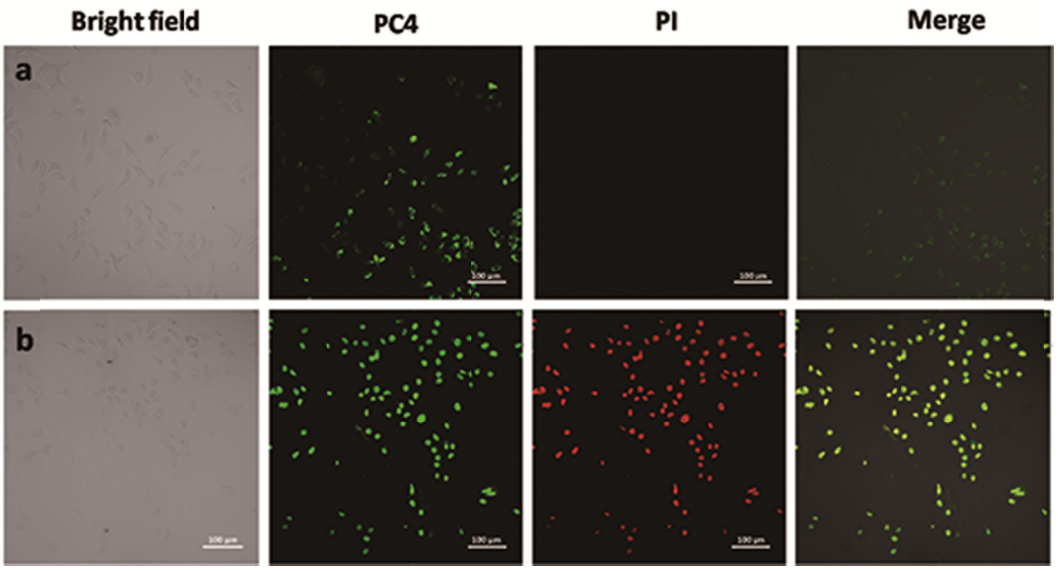

Fig. S12 CLSM images of A549 cells incubated with PC4 (8 μM), irradiated with light of (a) 0, (b) 4.2 J/cm^2, respectively, and stained with PI (1 μg/mL). Scale bar: 100 μm.

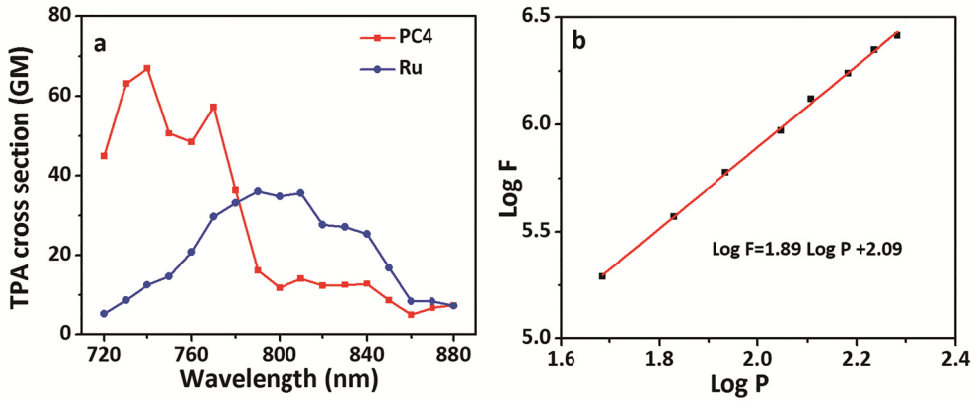

Fig. S13 (a) The two-photon absorption cross section of the probe and $Ru(bpy)_3^{2+}$ at excitation of 720 – 880 nm with rhodamine B as reference. (b) The relationship of the probe emission intensity with laser power (excitation at 750 nm).

Supporting movies

Movies S1-S2: A549 cells incubated with 5 μM of PC4 were monitoredcontinuously in the bright field channel and FITC green fluorescence channel by CLSM, respectively (excitation at 488 nm).

Movie S3: A549 cells withoutPC4 were monitored continuously in the bright field channel by

CLSM (excitation at 488 nm).

Supporting References

S1. X. Zheng, L. Wang, Q. Pei, S. He, S. Liu and Z. Xie, *Chem. Mater.*, 2017, 29, 2374-2381.

S2. D. GarciaFresnadillo, Y. Georgiadou, G. Orellana, A. M. Braun and E. Oliveros, *Helv. Chim. Acta*, 1996, 79, 1222-1238.