Supporting Information

Benzophenone core based AIE-active photosensitizers for mitochondria-targeted photodynamic therapy

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General information

Unless otherwise noted, commercial reagents were purchased from Bidepharm, Adamas-beta (Titan-Tansoole), General-reagent (Titan-Tansoole), Energy Chemical or *J&K Scientific* and used without further purification. The dry solvents were dried and distilled before use. All reactions were performed under an inertia atmosphere unless otherwise specified. Flash chromatography was performed using silica gel (300–400 mesh). Analytical thin-layer chromatography was performed on 0.20 mm silica gel HSGF-254 plates (Huanghai, China) and visualized under 254 or 365 nm UV light. Column chromatography was performed using 200-300 mesh silica gel (Huanghai, China). Methylene blue (MB) and 9,10-anthracenediylbis(methylene) dimalonic acid (ABDA) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Fetal bovine serum (FBS) and basal medium were purchased form GIBCO. (USA). 2', 7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased form Dalian Meilun Biotechnology Co., Ltd (Dalian, China). ROS Brite^{TM} HPF (HPF) was purchased form AAT Bioquest Inc. (USA). Mitochondrial probe and Lysosomal probe were purchased form Jiangsu Kaiji Biotechnology Co., Ltd. (Jiangsu, China). CCK-8 was purchased form Dojindo (Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained at 25 °C on a Bruker Advance III 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) with reference to TMS or residual nuclei in deuterated solvents [^{1}H NMR (DMSO-d_6): δ =2.50 ppm; ^{13}C NMR (DMSO-d_6): δ = 39.5 ppm]. Coupling constants (*J*) are denoted in Hz. Multiplicities are denoted as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broad. MALDI-TOF-MS spectra

were recorded on an UltrafleXtreme MALDI-TOF/TOF instrument (Bruker Daltonics, Germany) equipped with a 355 nm Nd: YAG laser at the analytical center of Sun Yat-Sen University. The UV-vis absorption spectra was measured with a SHIMADZU UV-3600 UV-VIS-NIR spectrometer. Steady-state photoluminescence (PL) spectra were recorded on a SHIMADZU RF-5301PC or Edinburgh Instruments FLS980 spectrometer.

Measurement in aqueous media

General ROS detection by fluorescence analysis

The general ROS generation measurements were conducted using 2,7-dichlorodihydrofluorescein (DCFH) as the indicator, which was converted from 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, 0.5 mL, 1 mM in ethanol) reacting with an aqueous solution of NaOH (2 mL, 1.0 mM) for 30 min at room temperature. The hydrolysate was then neutralized with 7.5 mL PBS buffer solution to get the stock solution with a concentration of 50 μM. PBS buffer solution containing 25 μM DCFH was added to 10 μM **OMEPYH** (stock solution: 10 mM in dimethylsulfoxide (DMSO)), **OMEPY** (stock solution: 10 mM in DMSO), The fluorescence signal of the indicator was monitored in a range of 500-600 nm with the excitation wavelength at 490 nm after the solution was irradiated by white light irradiation of 20 mW/cm^2.

·OH detection by fluorescence analysis

The ·OH generation measurements were conducted using hydroxyphenyl fluorescein (HPF) as the indicator. PBS buffer solution containing 10 μM HPF (stock solution: 20 mM in DMF) was added to 10 μM **OMEPYH** (stock solution: 10 mM in DMSO), **OMEPY** (stock solution: 10 mM in DMSO), The fluorescence signal of the indicator was monitored in a range of 500-600 nm with the excitation wavelength at 490 nm after the solution was irradiated by white light irradiation of 20 mW/cm^2. The fluorescence intensity was recorded to indicate the OH· generation rate.

$^1O_2$ detection by fluorescence analysis

The $^1O_2$ generation measurements were conducted using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) as the indicator. PBS buffer solution containing 100 μM ABDA (stock solution: 10 mM in DMSO) was added 10 μM **OMEPYH** (stock solution: 10 mM in DMSO), **OMEPY** (stock solution: 10 mM in DMSO), MB (stock solution: 10 mM in water with additional DMSO), or pure DMSO. The UV Absorption of the indicator was monitored in a range of 350-430 nm after the solution was irradiated by white light irradiation of 20 mW/cm^2.

Cell culture: The 4T1 cells were cultured in a 1640 medium containing 10% FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a humidified incubator with 5% CO_2 at 37 ℃.

Biocompatibility measurement

4T1 cells were seeded in 96-well plates at a density of 1 × 10^5 cells per well. After 24 h of culture, different concentrations of **OMEPYH** or **OMEPYH** were added and incubated at 37 ℃ for 24 h in dark. The sample and control wells were washed twice with PBS buffer and added with cck8 solution. After incubation at 37 ℃ for 4 h. The absorbance of sample and control wells at 450 nm was then measured by a microplate reader. Cell viability was then calculated by the ratio of the absorbance of sample wells to control cells.

In vitro PDT evaluation

4T1 cells were seeded in 96-well plates at a density of 1 × 10^5 cells per well. After 24 h of culture, different concentrations of **OMEPYH** or **OMEPYH** were added and incubated at 37 ℃ for 4 h in dark. Without or with the exposure to white light irradiation of 20 mW/cm^2 for 30 min, the cells were further incubated at 37 ℃ to 24 h. CCK-98 assay was conducted as described in Biocompatibility measurement.

ROS generation measurement in vitro

4T1 cells were grown in a petri dish at 37 ℃. After incubation with medium a containing 30 μM **OMEPY** and **OMEPYH** (stock solution: 10 mM in DMSO) or pure DMSO for 4 h and treatment with a medium containing 10 μM DCFH-DA, HPF (stock solution: 10 mM in DMSO) for 30 min at 37 ℃, Without or with the exposure to white light irradiation of 20 mW/cm^2 for 10 min the cells were imaged by fluorescence microscope.

Confocal co-localization

4T1 cells were grown in a petri dish at 37 ℃. After incubation with a medium containing 30 μM **OMEPY** and **OMEPYH** (stock solution: 10 mM in DMSO) for 8 h and stain cells with lysosomal or mitochondrial probes, Cells were washed with PBS and observed with a confocal microscope

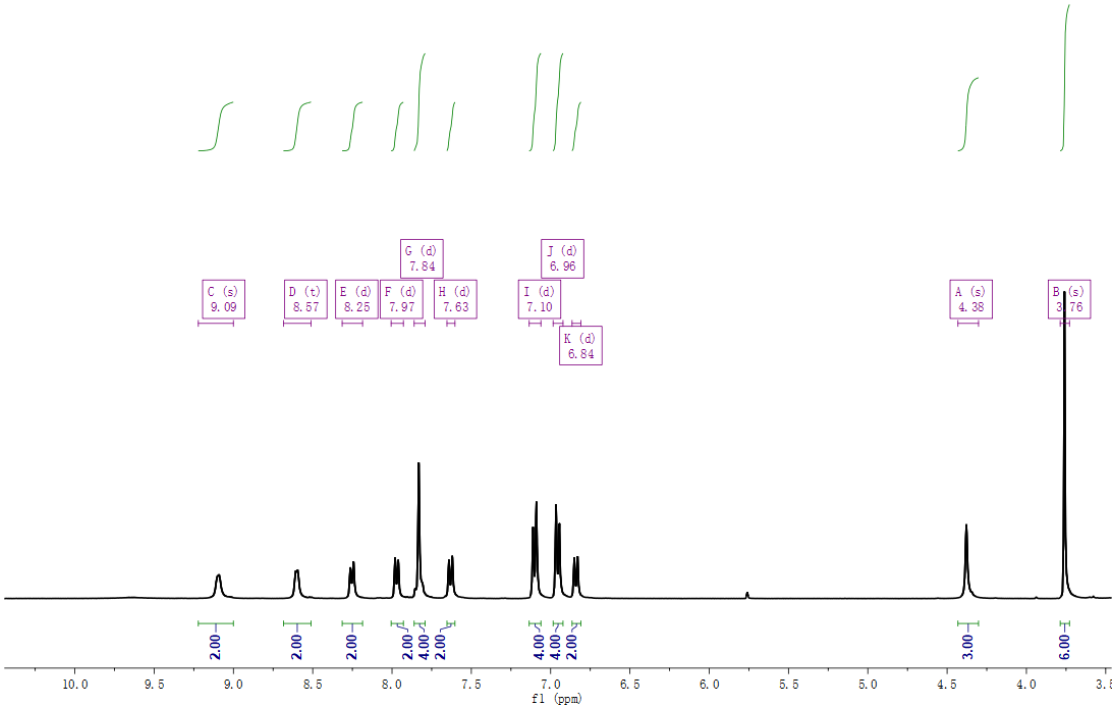

Fig. S1 ^{1}H NMR spectrum of **OMEPYH** (400 MHz, DMSO-d_6).

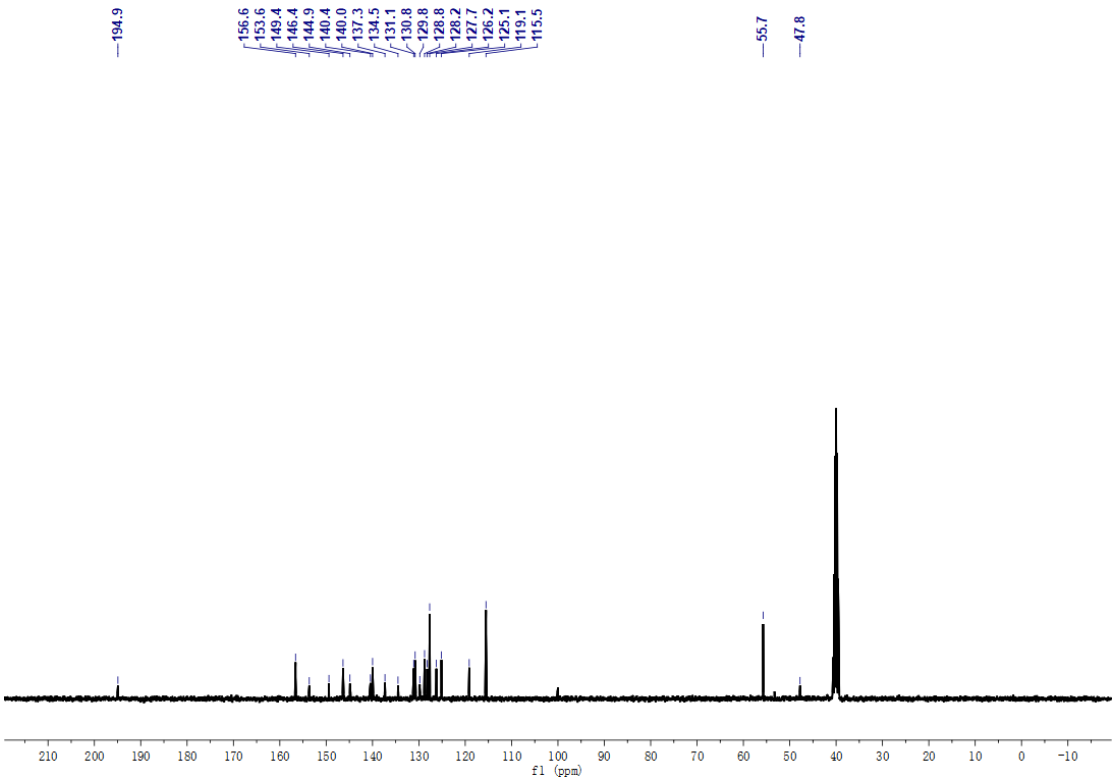

Fig. S2 ^{13}C NMR spectrum of **OMEPYH** (400 MHz, DMSO-d_6).

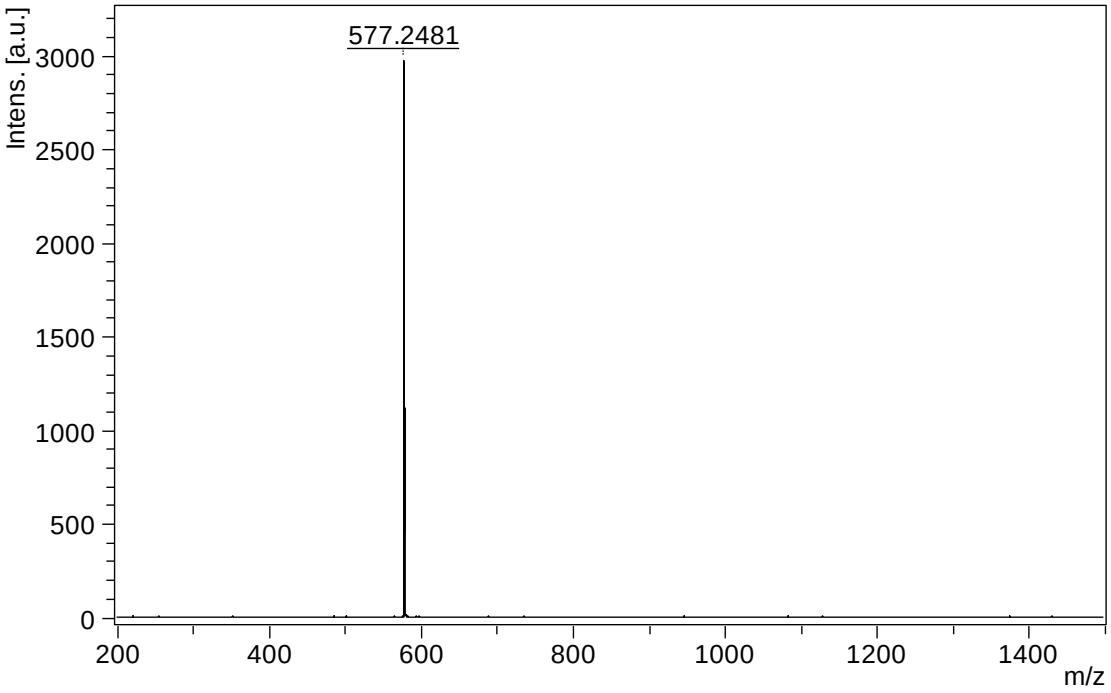

Fig. S3 MALDI-TOF-MS spectrum of **OMEPYH**.

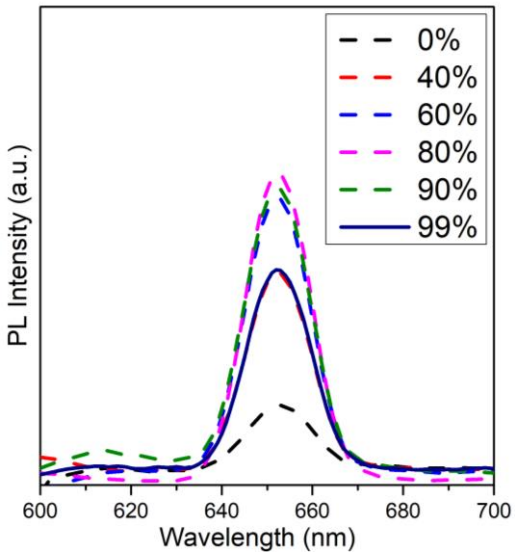

Fig. S4 Fluorescence spectra of **OMEPYH** in DMSO/toluene mixtures with different toluene fractions.

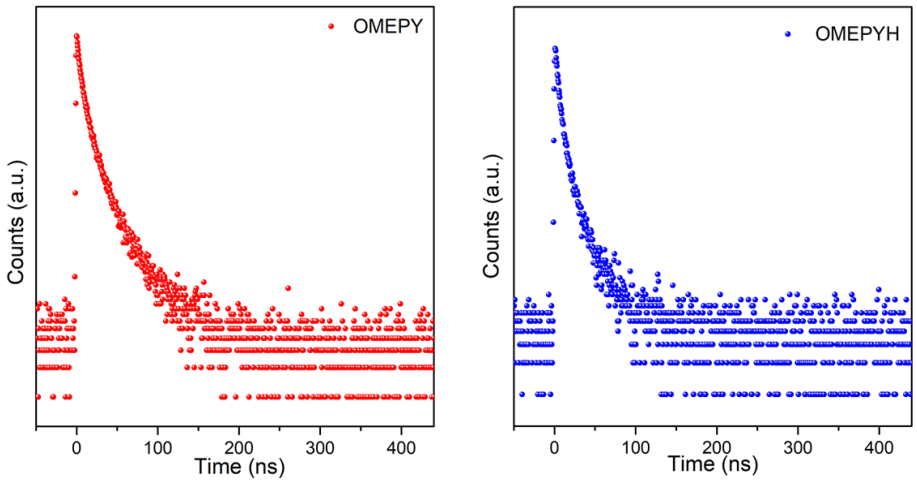

Fig. S5 The fluorescence intensity decay of **OMEPY and OMEPYH**.

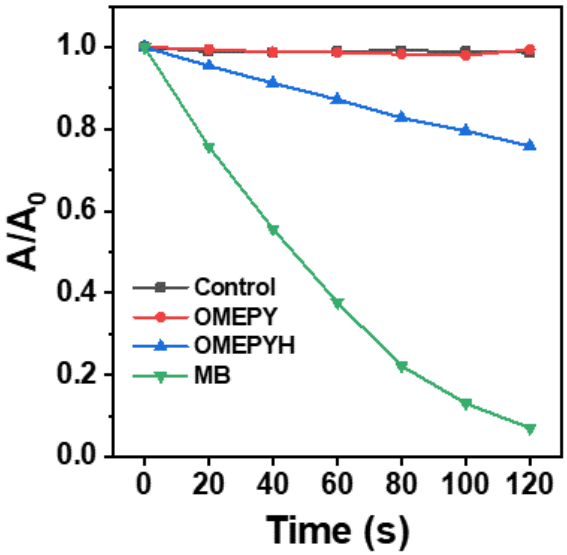

Fig. S6 Absorption changes of ABDA (for $^1O_2$ detection) with PSs under white light irradiation with different time. (20 mW/cm^2, 100 μM (ABDA), 10 μM (**OMEPY**, **OMEPYH** and MB))

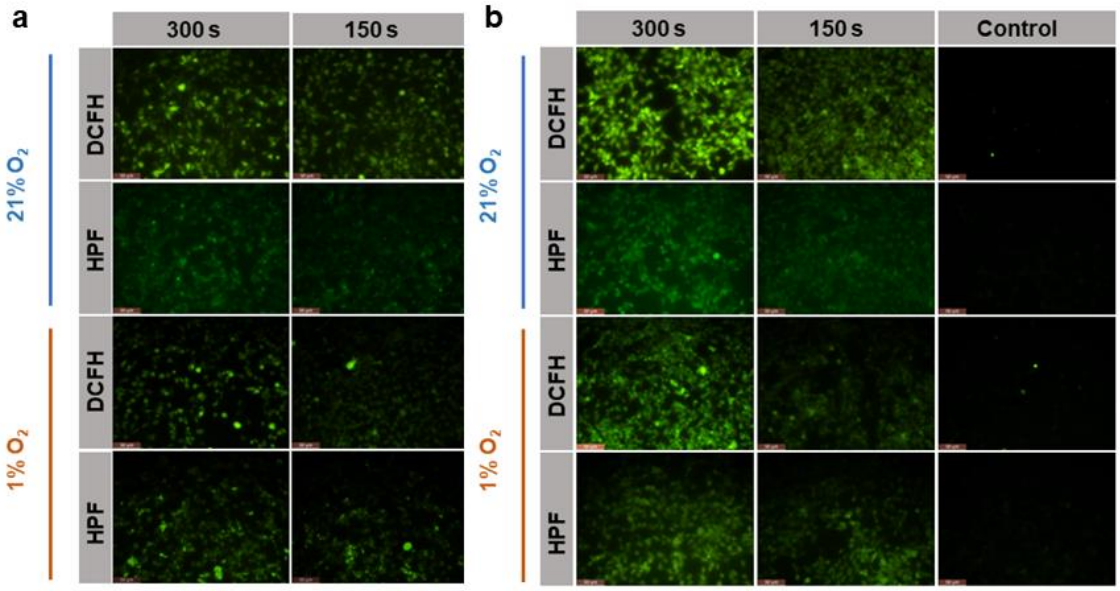

Fig. S7 Confocal images of 4T1 cells incubation with DCFH and HPF as fluorescence probes after treated with (a) **OMEPY** and (b) **OMEPYH**. (20 mW/cm^2, 10 μM (HPF), 10 μM (**OMEPY** or **OMEPYH**), scale bar: 50 μm).

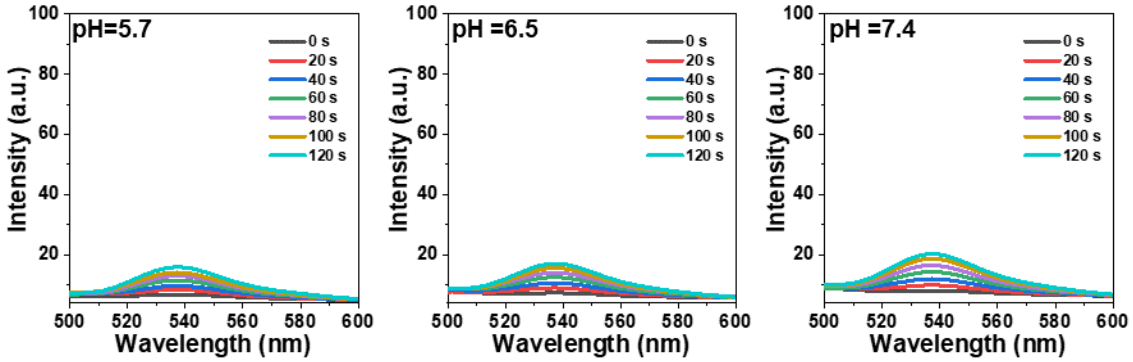

Fig. S8 The PL spectra of DCFH in absence of **OMEPY** in PBS buffers with different pH values.

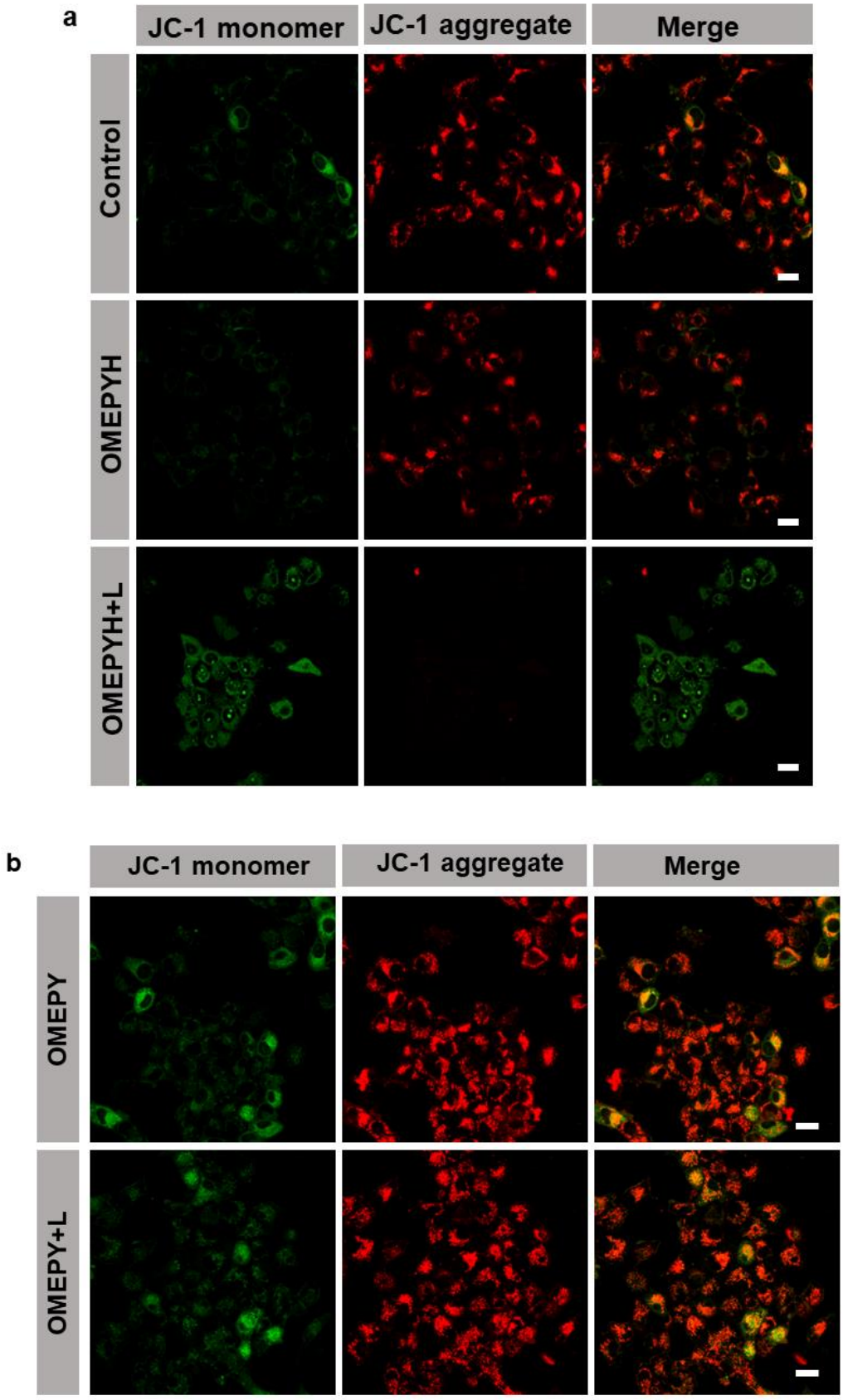

Fig. S9 (a) Confocal images of 4T1 cells co-stained with P16 and JC-1 kit in the absence or presence of light irradiation. (b) Confocal images of 4T1 cells co-stained with P15

and JC-1 kit in the absence or presence of light irradiation. (In the light irradiation group, after 30 minutes of white light (20 mW/cm^2) irradiation, the culture was continued for 4 hours, and then stained with JC-1 and photographed. JC-1 monomer Ex/Em 490/530 nm, JC-1 aggregate Ex/Em 525/590 nm, scale bar 20 μm).

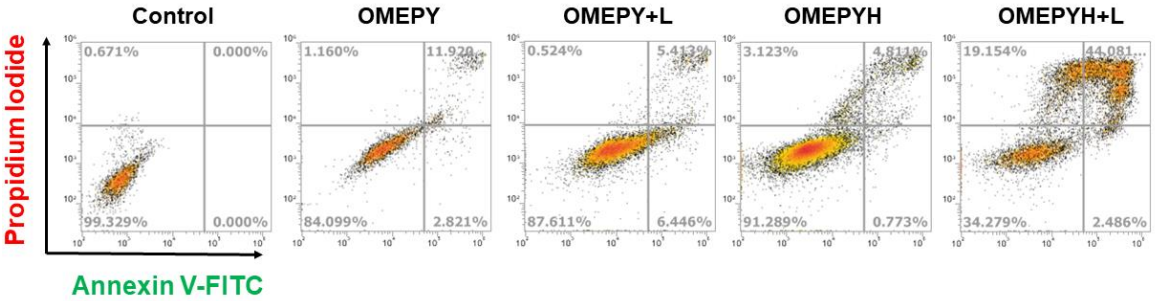

Fig. S10 Apoptosis analysis using flow cytometry toward 4T1 cells after different treatments in various groups.