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Supporting Information

Small-Sized and Stable 2D Metal-Organic Framework: A Functional Nanoplatform with Effective Photodynamic Therapy

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Experimental Section

Materials

All chemicals, unless specified otherwise, were purchase from Sigma-Aldrich. All relevant reagents about cell culture were purchased from Gibco.

Synthesis and characterization of Sm-H_2TCPP nanosheets

$Sm(O_2C_2H_3)_3.6H_2O$ (0.043 g), benzoic acid (BA, 0.28 g), and 16 mg H_2TCPP were dissolved into 10 mL of DMF in hydrothermal reactor, which was heated to 120℃ and kept the reaction for 36 h. When the reactor has cooled down, the resulted purplish red Sm-H_2TCPP nanosheets were collected by centrifugation (12000 rpm, 10 min) and then washed three times with DMF and ethanol successively before being dispersed in ethanol as a stock suspension.

The morphology features of Sm-H_2TCPP nanosheets were observed using field-emission scanning electron microscope (FE-SEM), transmission electron microscopy (TEM, JEOL JEM-1010 microscope operated at 100 kV) and atomic force microscopy (AFM, Dimension ICON with Nanoscope V controller, Bruker, USA). The thickness of Sm-H_2TCPP nanosheets was measured by AFM.

The chemical structures of the Sm-H_2TCPP were analyzed using power X-ray diffraction pattern (XRD), which were recorded with a Shimadzu XRD-6000 power X-ray diffractometer, using Cu Kα radiation (λ=1.5406 Å). Meanwhile, the High-resolution TEM (HR-TEM) and the corresponding the fast fourier transform patterns (FFT) were used to further confirm the coordination mode between H_2TCPP and Sm^{3+}. The chemical compositions of Sm-H_2TCPP were accessed by X-ray photoelectron Spectroscopy (XPS, VG ESCALAB 220i-XL) equipped with a monochromatic Al Kα (1486.7 eV) X-ray source, and Fourier transform infrared spectrophotometry (FTIR, Nicolet 5700, USA). The ratio of the element of Sm-H_2TCPP was determined by Inductively coupled plasma mass spectrometry (ICP-MS), energy-dispersive X-ray spectroscope (EDS), and elemental mapping based on FE-SEM.

Thermogravimetric analysis on Sm-H_2TCPP was carried out on Shimadzu TGA-50 thermogravimetric analyzer. The heating speed was 5 ℃/min and the samples were heated to 800 ℃ under air atmosphere.

The photochemical properties of H_2TCPP and Sm-H_2TCPP nanosheets

The UV-Visible absorption spectra of H_2TCPP and Sm-H_2TCPP nanosheets were carried out on UV-vis spectrophotometer (UV-2600, Shimadzu, Japan). The absorption of H_2TCPP at the different concentration (0.2, 0.4, 0.8, 1.6, 3.2 μM), and Sm-H_2TCPP at different ligands concentration (0.94, 1.88, 3.75, 7.5, 15, 30 μM) were recorded. The standard curve of H_2TCPP and Sm-H_2TCPP nanosheets was plotted by linear fitting of the absorbance at 420 nm and 660 nm.

The fluorescence spectrum of H_2TCPP (Ex: 420 nm, Em, 650 nm) and Sm-H_2TCPP nanosheets were acquired using fluorescence spectrophotometer (RF-5301PC, Shimadzu, Japan).

The fluorescence lifetimes H_2TCPP and Sm-H_2TCPP nanosheets were determined by Time-Correlated Single Photon Counting (TCSPC) methods (Pluorolog 3-TCSPC).

Singlet oxygen generation of H_2TCPP and Sm-H_2TCPP nanosheets

2,7-dichlorofluorescein diacetate (DCFH-DA) was employed to measure the generation singlet oxygen according the reported literature. Firstly, the DCFH-DA was hydrolyzed into DCFH 2,7-Dichlorofluorescein, which could be oxidized to the highly fluorescent dichlorofluorescein (DCF) by ROS. Specifically, DCFH-DA was dissolved into DMSO followed by hydrolyzation with sodium hydroxide for 30 min. Then, the hydrolytic reaction was quenched by adding excess PBS buffer (PBS, 25 mM, pH 7.2) into the reaction system. The acquired DCFH solution was were fixed to 10 μM and kept on ice before use. For a typical measurement, H_2TCPP and Sm-H_2TCPP nanosheets at a concentration of 5 μM in PBS buffer were mixed with stocked DCFH solution followed by laser irradiation (660 nm, 100 mW/cm^2) for 0 s, 10 s, 20 s, 30 s, 40 s, 50 s, 1 min, 2 min, 3 min, 5 min, 8 min, 12 min. The fluorescence intensity (FI) at corresponding time was recorded. The relative fluorescence intensity (RFI) was defined with the ratio of

FI_t and FI_0 (FI_t / FI_0) in the peak intensity at 530 nm.

Cell culture

MCF-7 cells were cultured in RPMI-1640 completed medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were placed in a 37 °C and 5% carbon dioxide atmosphere.

In vitro cellular uptake

MCF-7 cells were seeded into confocal dish at a density of 1×10^5 cells. After 24 h of incubation, the cells were treated with Sm-H_2TCPP nanosheets at a concentration of 30 μM and continued to incubate for 6 h. Finally, the image of cellular uptake was observed using laser scanning confocal microscope (LSCM, Zeiss, Germany).

Qualitative and quantitative analysis of the singlet oxygen generation

For qualitatively detecting the intercellular singlet oxygen generation, the MCF-7 cells were seeded into confocal dish at a density of 1×10^5 cells. The cells were incubated for 24 h followed by treated with equal concentration of H_2TCPP and Sm-H_2TCPP nanosheets. After 6 h of incubation, the supernatant was removed and the dish was wished three times using PBS. Then, the cells were incubated with 10 μM of DCFH-DA for 25 min, and exposed under laser radiation (660 nm, 100 mW/cm^2, 5min). Finally, the cell images were observed by LSCM.

For quantatively detecting the intercellular singlet oxygen generation, MCF-7 cells were seeded into 6-well plate at a density of 1×10^5 cells per milliliter and incubated for 24 h. Then, cells were washed with PBS for twice and treated by completed medium containing different formation of therapeutic agents for another 24 h. After removing unconsumed drugs, the cells in the wells were digested and collected by centrifugation (1000 rpm, 5 min) followed by washing with PBS. Subsequently, the cells were incubated by DCFH-DA for 25 min and washed with PBS. Finally, the flow cytometer (FACS-Calibur, Becton Dickinson, USA) was used to quantitively detect the singlet oxygen generation.

MTT assay

The cytotoxicity of H_2TCPP and Sm-H_2TCPP nanosheets was evaluated by MTT assay. In brief, MCF-7 cells were seeded into 96-well at a density of 1×10^4 cells per well and cultured for 24 h. Then, the medium was replaced with 100 μL of fresh RPMI-1640 containing various ligand concentration of Sm-H_2TCPP and free H_2TCPP (6.25, 12.5, 25, 50, 100 μM). After 12 h of incubation, the cells were irradiated with LED light (660 nm, 15 min). Then, the cells were further cultured for 36 h. The cell viability was assayed by adding 20 μL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution to each well. The cell viability was calculated by Eq. (1).

$$\text{Cell viability (\%)} = A_T/A_C \times 100\% \quad (1)$$

Where A_T is the mean absorbance of treatment group, and A_C is the mean absorbance of control.

Cell apoptosis

MCF-7 cells from the exponential phase of the culture were harvested and seeded in 6-well plates (1×10^5 cells/well). After 24 h of incubation, cells were treated with equal concentration of H_2TCPP and Sm-H_2TCPP nanosheets (30 μM) and continued to incubate for 12 h, the cells were irradiated with LED light (660 nm 100 mW, 15 min). And the cells without irradiation treatment served as controls. After further 36 h of incubation, all cells were collected and dyed for 20 min using Alexa Fluor 488 Annexin V/dead cell apoptosis kit. The cells were analyzed by a flow cytometer (LSRII Orange, BD, USA).

In vivo efficacy

The vivo anticancer efficacy of Sm-H_2TCPP nanosheets was investigated by breast cancer (MCF-7) model. The tumor-bearing mice were established by subcutaneous inoculation of MCF-7 cells suspension (4×10^6 cells per mouse) into the right armpit of 6-week BALBc male mice. The mice were randomly assigned to three groups for comparison: a) PBS control; b) free H_2TCPP; c) Sm-H_2TCPP groups. When the tumor volume reached 80~100 mm^3, the mice were treated at a ligand dose of 10 μM/kg. 12 h post injection, the mice were anesthetized with 2% (v/v) isoflurane and tumors were irradiated with a 650 nm LED (100 mW/cm^2) for 30min. Noting that all mice only

receive single injection and irradiation.

To evaluate the anticancer efficacy, the tumor sizes and weight of mice were measured every 2 d. After 15 d of treatment period, all mice were sacrificed and the excised tumors were photographed and weighed. Tumor tissue and major organs including liver and kidney were collected for anatomy and histo-pathological analysis.

Ethical Statement

This study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985) and was approved by the Institutional Animal Care and Use Committee of National Tissue Engineering Center (Nanjing, Jiangsu, China)

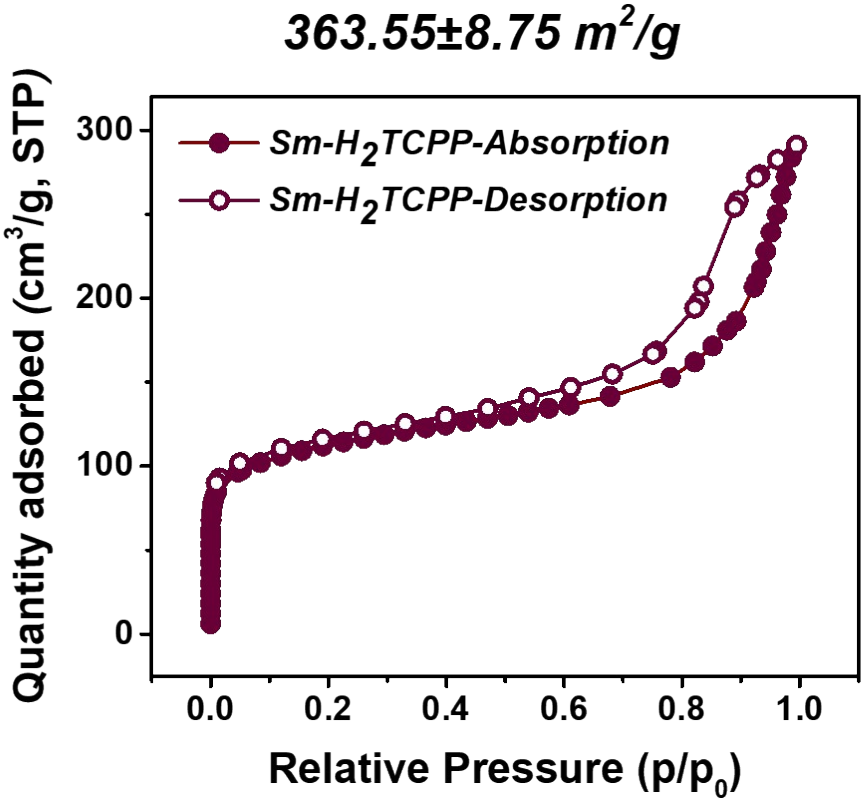

Figure S1. N_2 absorption isotherm of Sm-H_2TCPP nanosheets at 77K. The BET surface area is 363.55± 8.75 m^2/g.

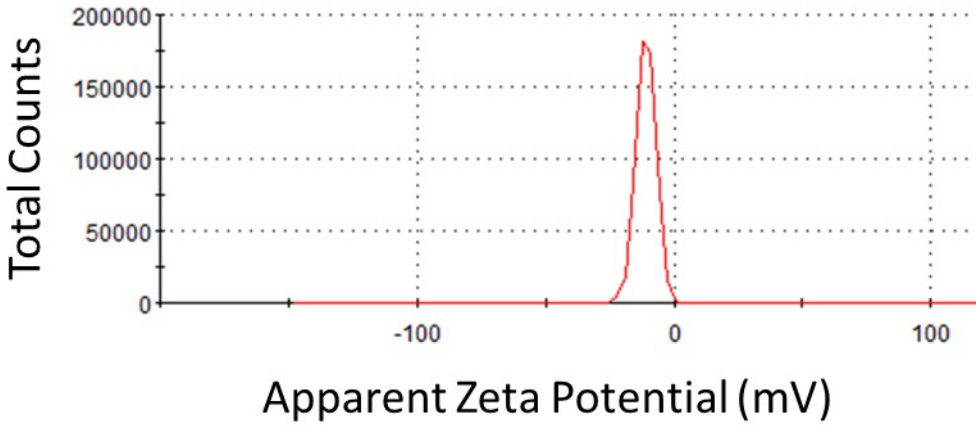

Figure S2. The Zeta potential of Sm-H_2TCPP nanosheets. The Zeta potential is -10.9 mV.

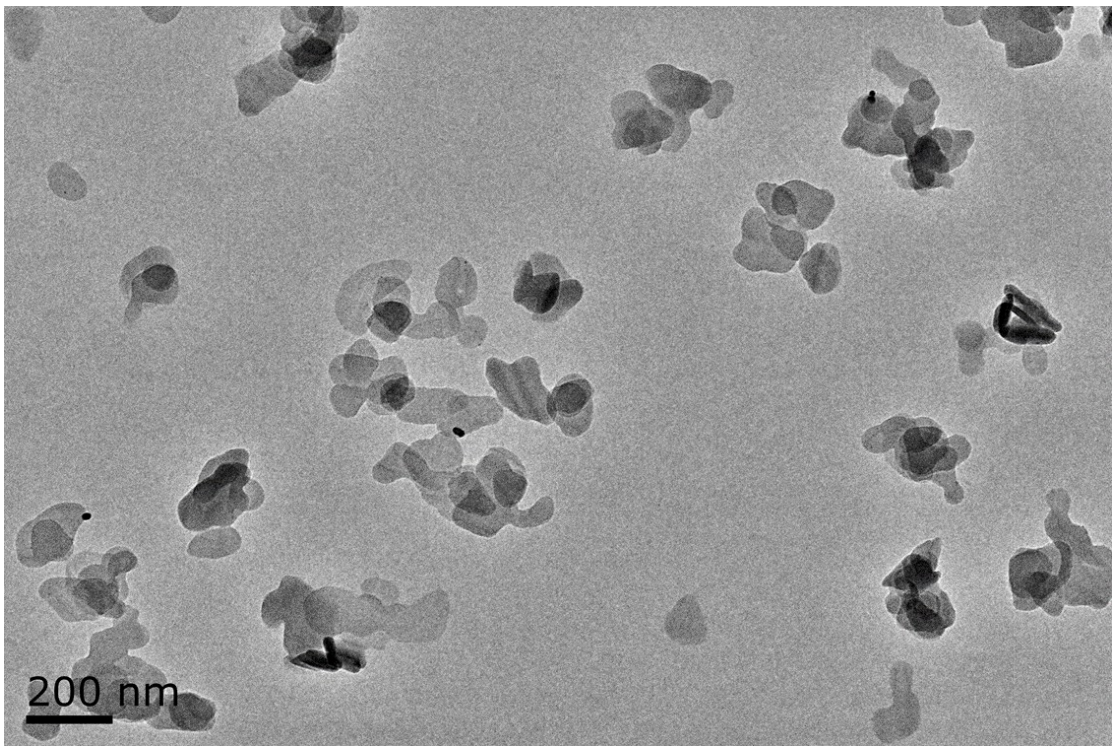

Figure S3. a) The TEM image of Sm-H_2TCPP nanosheets after 24 h of incubation of RPMI-1640 medium.

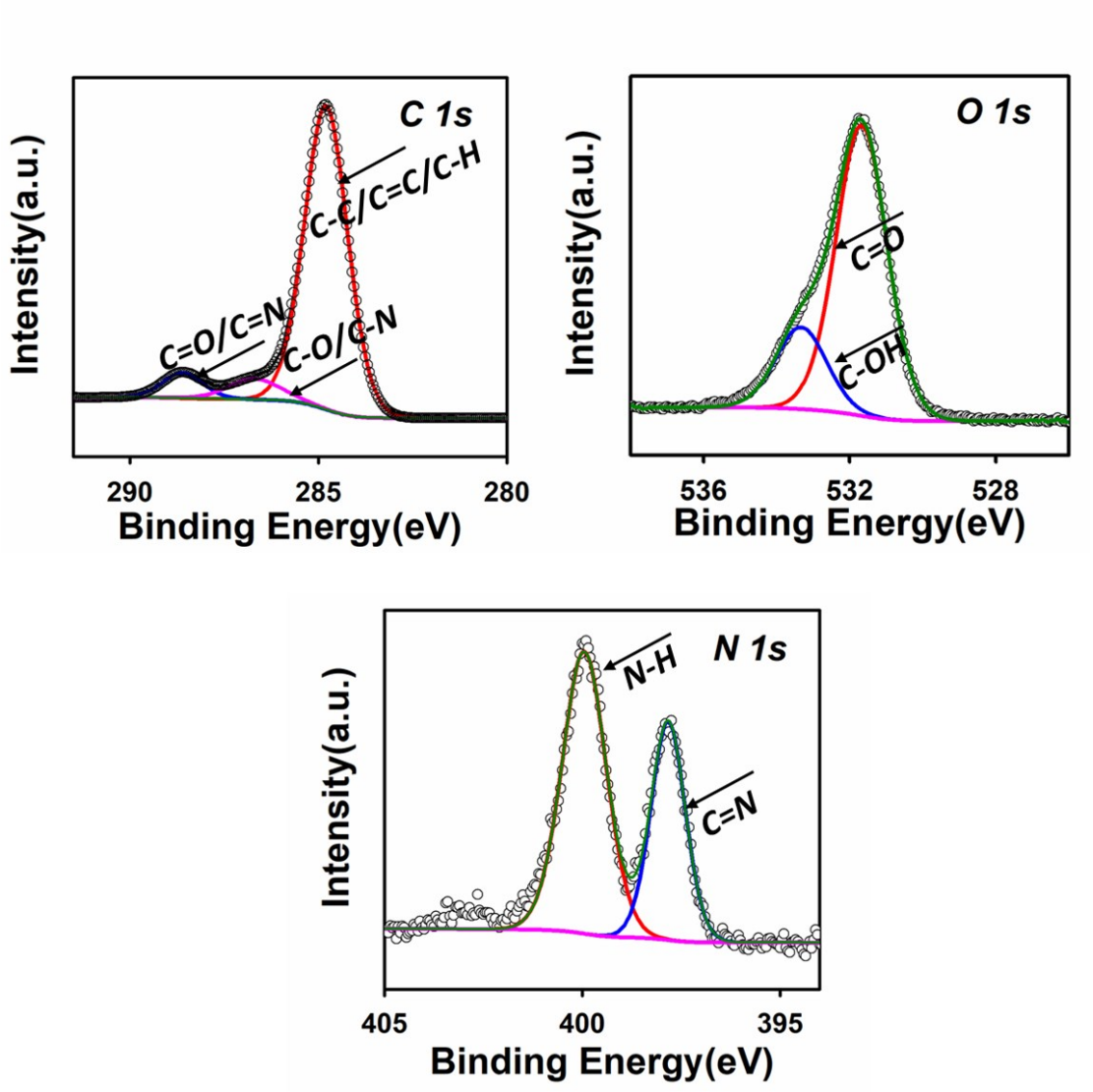

Figure S4. C 1S, O 1S, and N 1S high-resolution XPS pattern of Sm-H_2TCPP nanosheets.

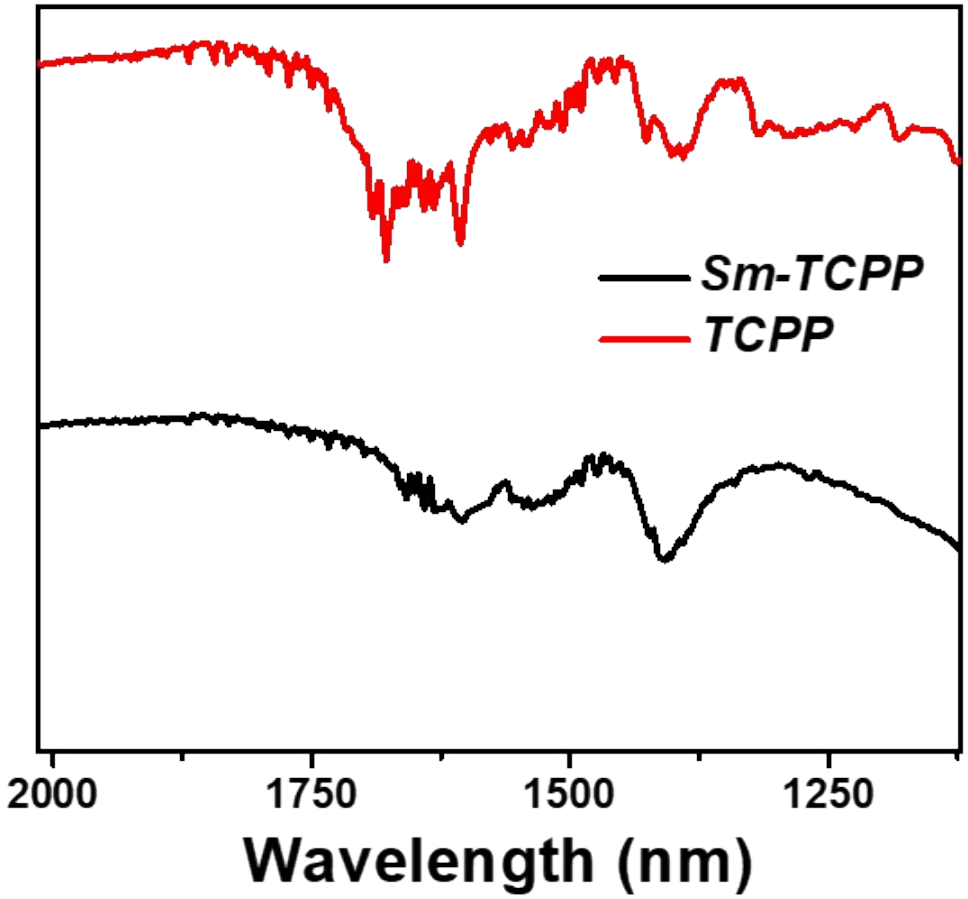

Figure S5. The FT-IR spectra of free H_2TCPP and Sm-H_2TCPP nanosheets.

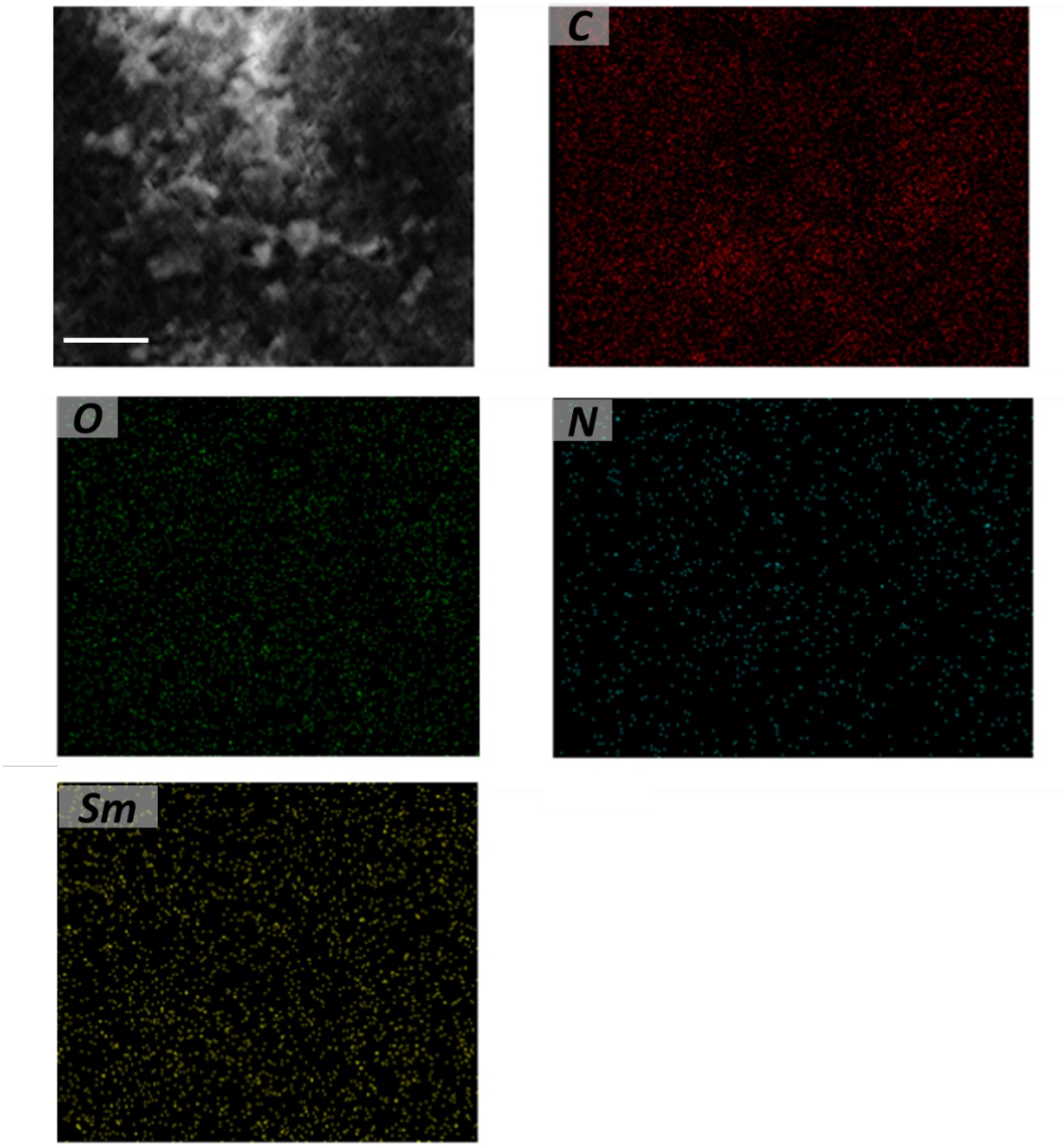

Figure S6. The elemental mapping of Sm-H_2TCPP nanosheets; Scale bar = 2 μm.

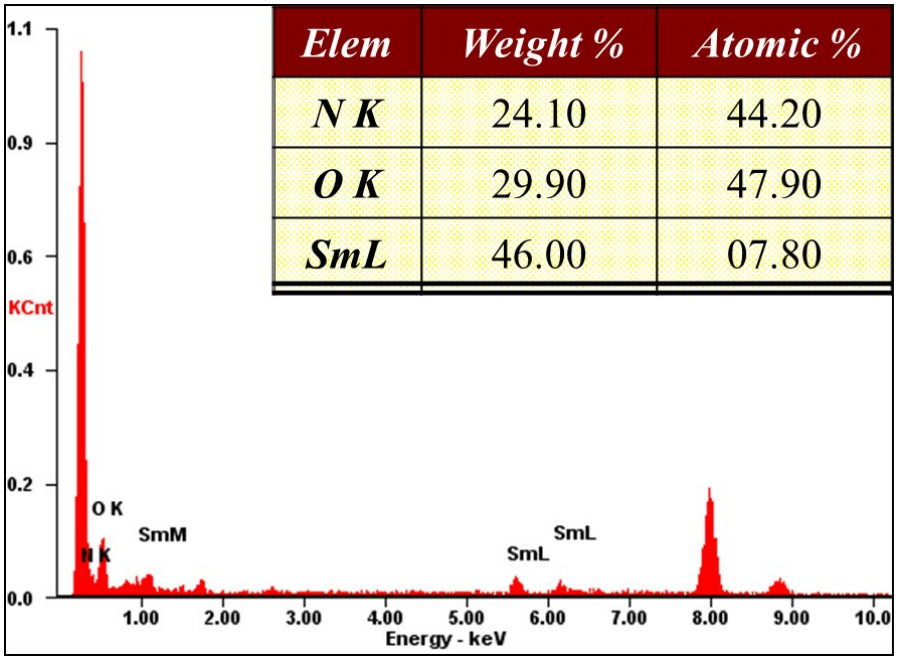

Figure S7. The EDX analysis of Sm-H_2TCPP nanosheets based on TEM.

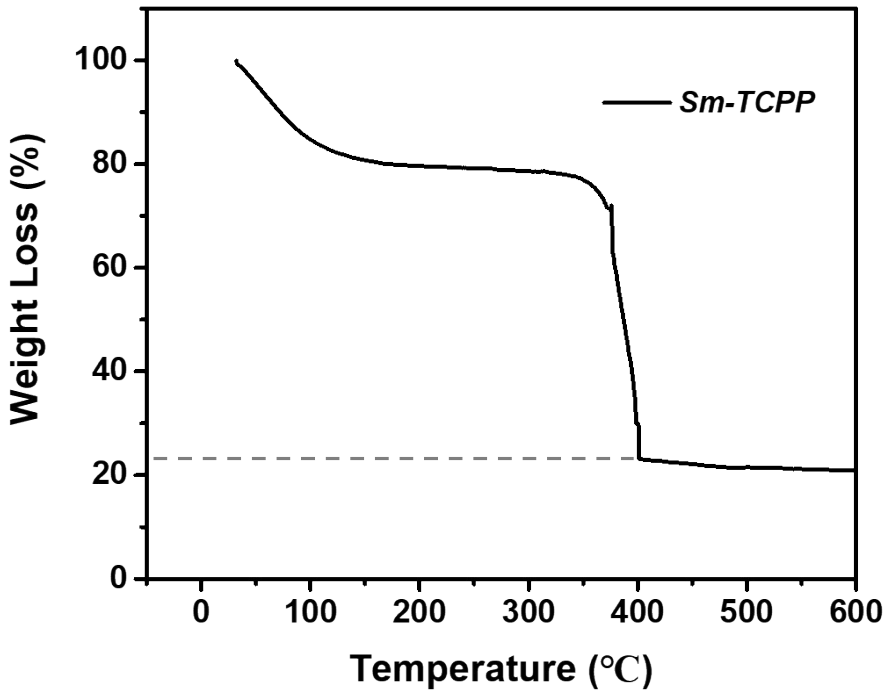

Figure S8. The thermogravimetric analysis of Sm-H_2TCPP nanosheets.

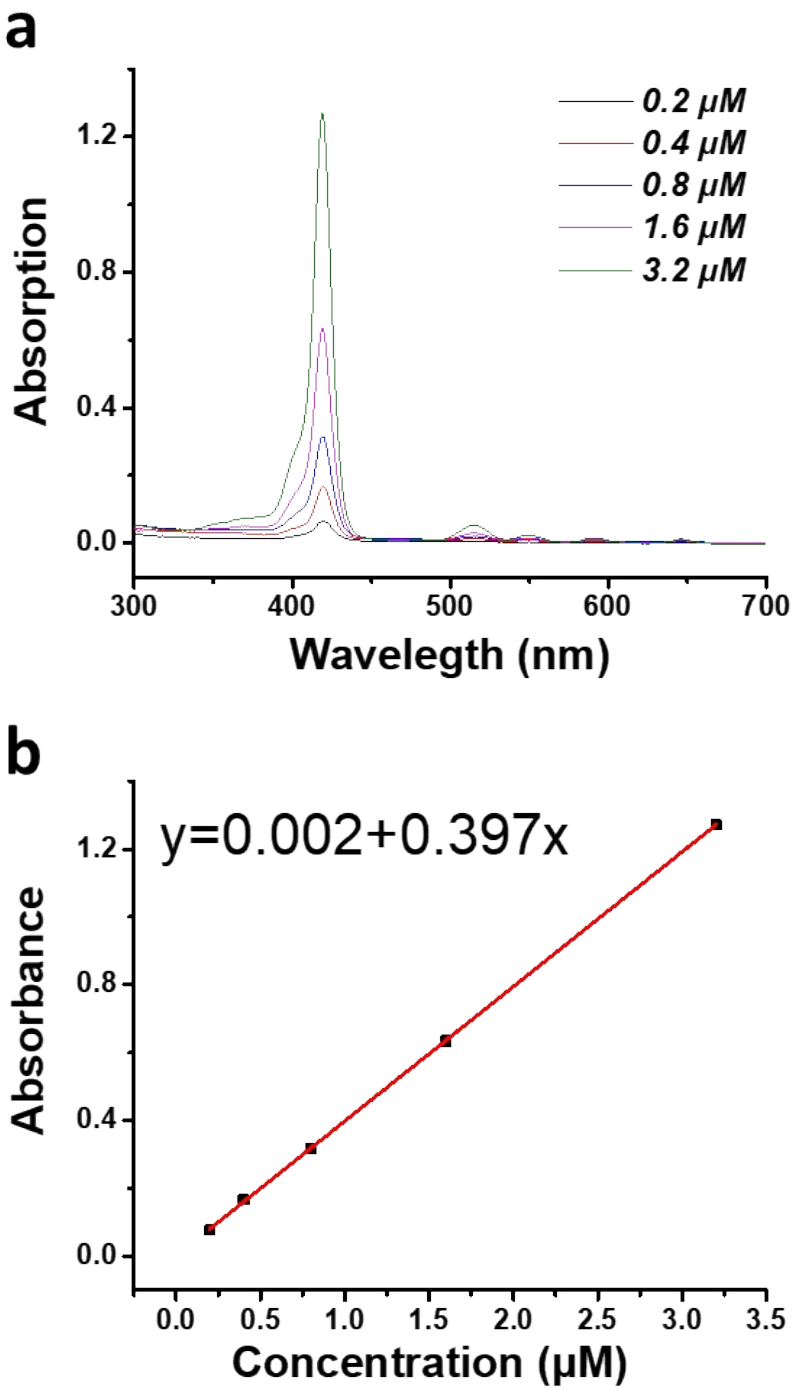

Figure S9. a) UV-vis absorption spectra of H_2TCPP at different concentration. b) Linear fit of H_2TCPP concentration at 420 nm.

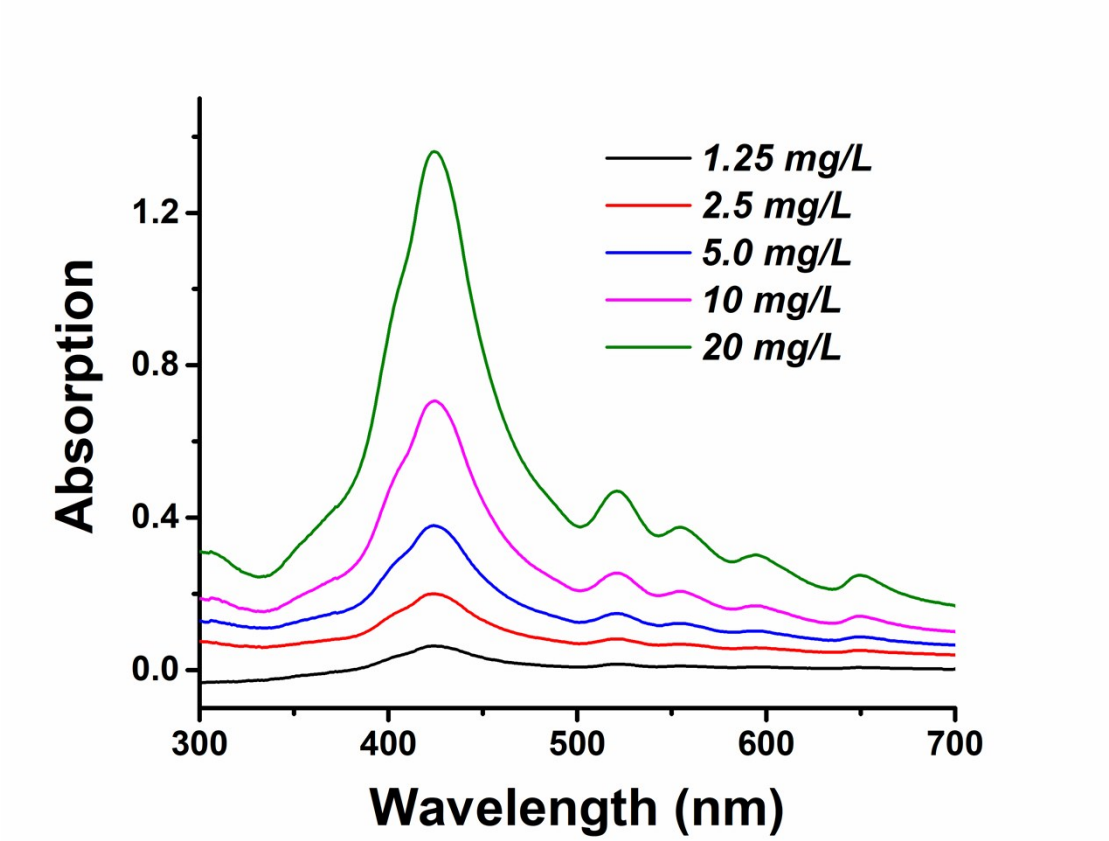

Figure S10. UV-vis absorption spectra of Sm-H_2TCPP at different concentration.

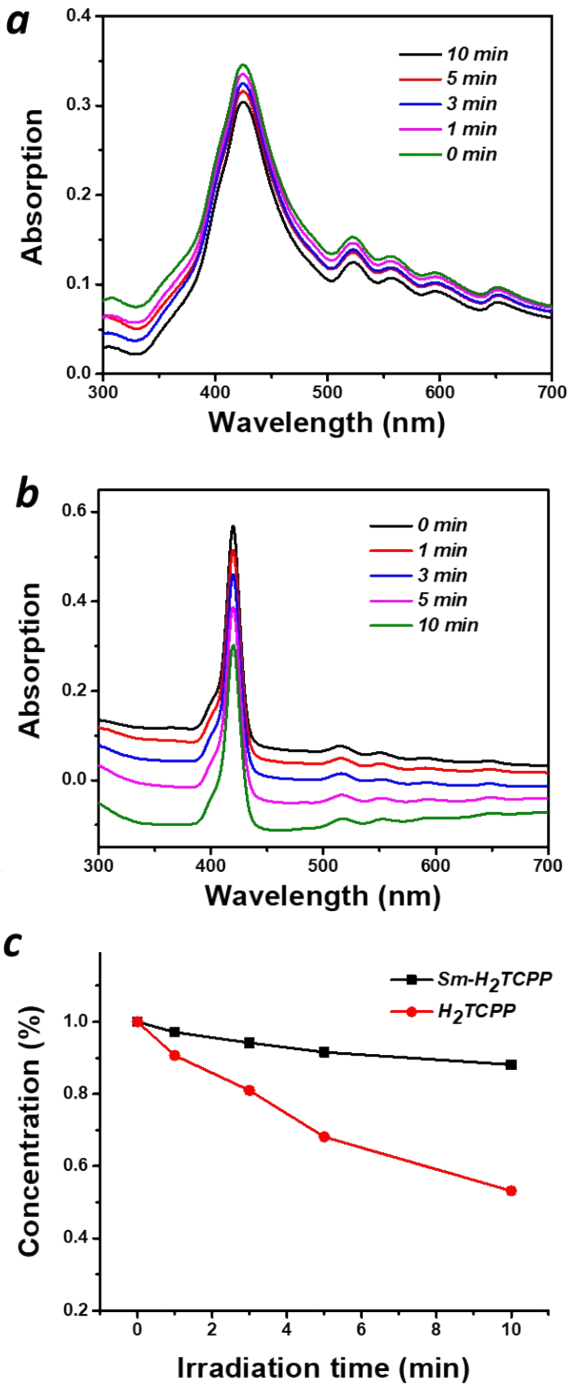

Figure S11 Photostability test of a) Sm-H_2TCPP nanosheets and b) H_2TCPP nanosheets. c) The corresponding dependence of initial concentration on the irradiation time.

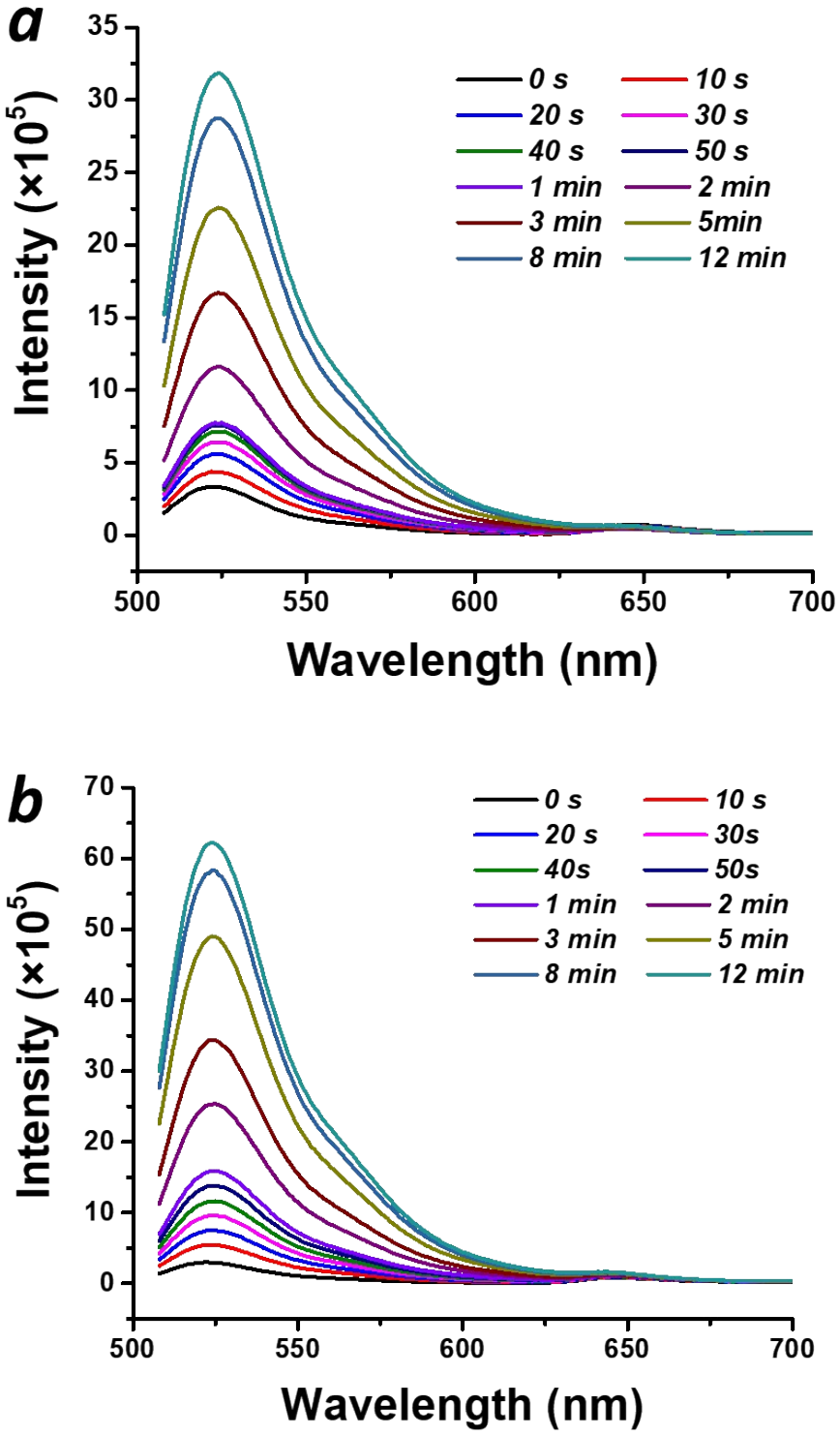

Figure S12. The fluorescence emission spectra of DCFH-DA indicating ROS generation from a) H_2TCPP b) Sm-H_2TCPP nanosheets under different irradiation time; (Ex: 485 nm, Em: 530 nm)

Table S1 Fitting parameters of ROS generation efficacy.

	A	*K*	r^2	*AK*
H_2TCPP	10.7	2.8×10^{-3}	0.998	0.03
$Sm\text{-}H_2TCPP$	23.0	3.9×10^{-3}	0.998	0.09
$H_2TCPP+Sm^{3+}$			0.902	0.003

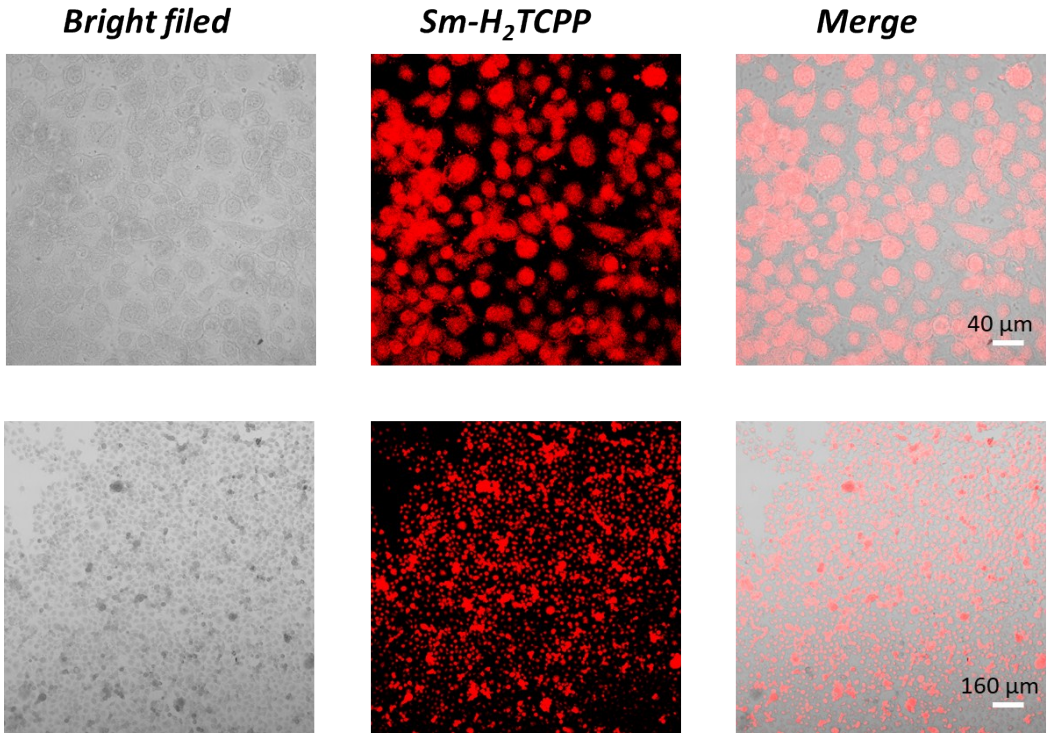

Figure S13. The cell uptake of Sm-H_2TCPP nanosheets in MCF-7 cells after 6 h of incubation with different magnifications by laser scanning confocal microscope (LSCM).

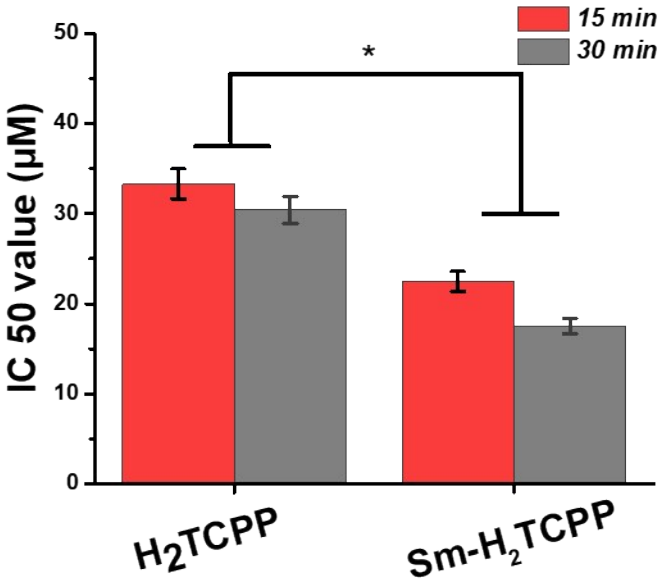

Figure S14. The IC50 value of H_2TCPP and Sm-H_2TCPP nanosheets under different irradiation time (660 nm, 100 mW/cm^2). Significance is defined such that a single asterisk indicates $P < 0.05$ and double asterisks indicate $P < 0.01$.

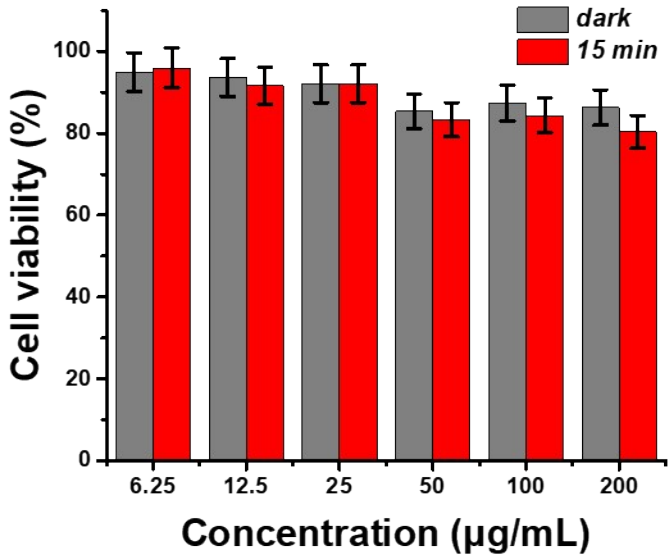

Figure S15. The cytotoxicity of Sm^{3+} to MCF-7 cells under dark and 15 min of irradiation condition.

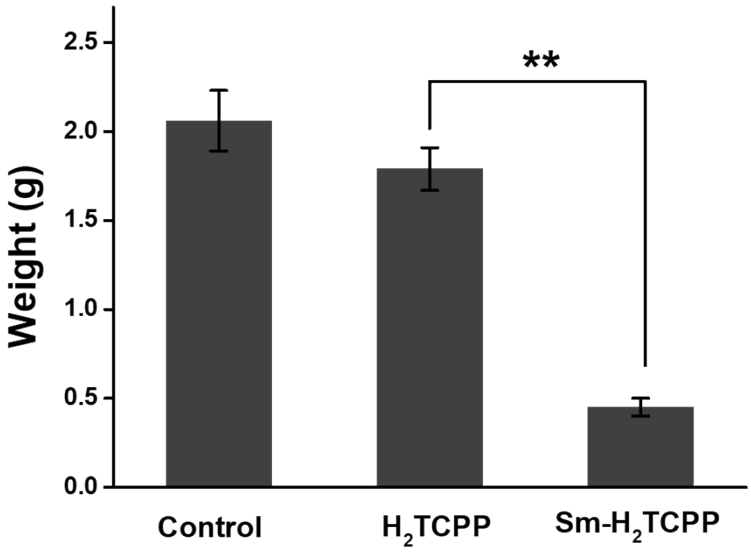

Figure S16. The tumor tissues weight of each groups after 14 days of treatment. Significance is defined such that a single asterisk indicates $P < 0.05$ and double asterisks indicate $P < 0.01$.

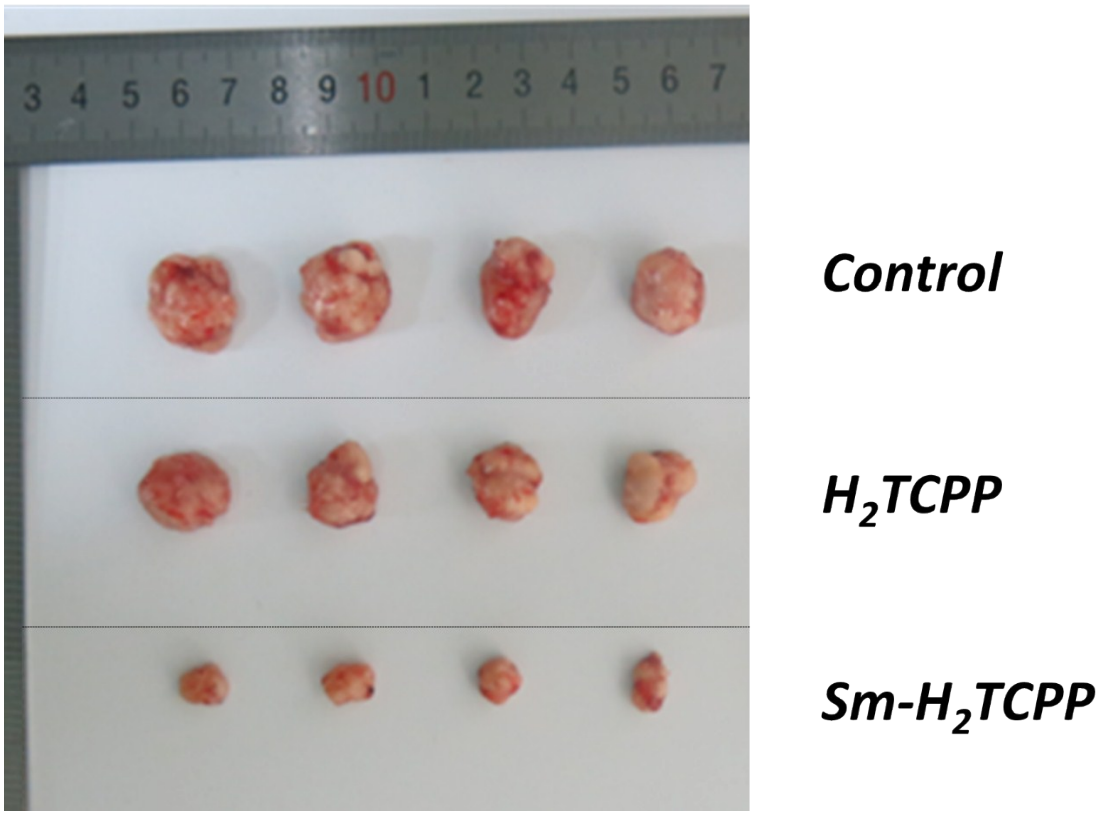

Figure S17. Tumor tissue images of each groups after PDT.