Supporting Information

Construction of a hydrogen-bonded organic framework-based therapeutic platform by one-pot method†

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Experimental Section

Chemicals and Materials. N, N-Dimethylformamide (C_3H_7NO, AR, 99.5%, Shanghai Macklin Biochemical Co. Ltd), N-Methyl Pyrrolidone (C_5H_9NO, 99%, Shanghai Macklin Biochemical Co. Ltd), Copper(II) tetra-(4-carboxyphenyl) porphyrin ($C_{48}H_{24}CuN_4O_8$, 95%, Jilin Chinese Academy of Sciences-Yanshen Technology Co. Ltd), Folic Acid ($C_{19}H_{19}N_7O_6$, 97%, Shanghai Macklin Biochemical Co. Ltd), Ethanol (99.8%, Shanghai Macklin Biochemical Co. Ltd), Tween 85 (Shanghai Macklin Biochemical Co. Ltd), Citric Acid ($C_6H_8O_7$, 99.5%, Shanghai Macklin Biochemical Co. Ltd).

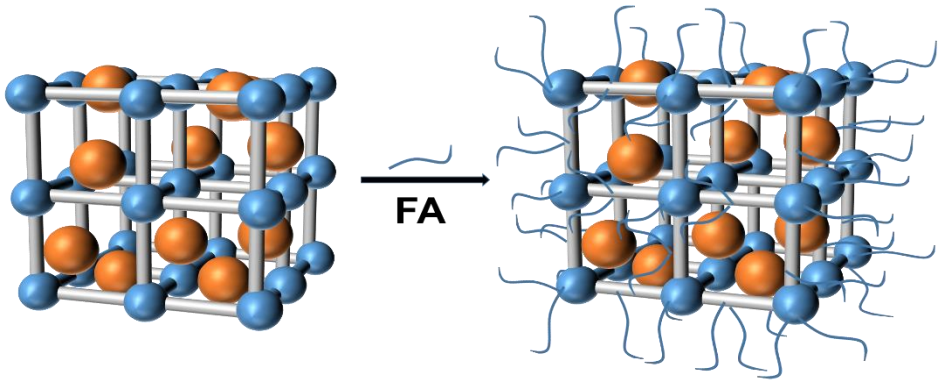
Characterization. Powder X-ray diffraction (PXRD) patterns were obtained on a Rigaku MiniFlex 600 diffractometer with graphite monochromatized CuKα radiation (λ = 0.15405 nm). The sample was scanned at a scanning rate of 5/min in the 2θ range from 3 to 20° at room temperature. A field emission scanning electron microscope (Thermo Fisher, Apero 2S) was used to characterize the morphology of the sample. The UV-Vis adsorption spectral values were collected on a UV-2600 spectrophotometer (Shimadzu). Dynamic light scattering (DLS) experiment was performed on Malvern Zeta Sizer-Nano ZS instrument at 25°C. MTT experiments were carried out using a microplate reader (Cytation5). The flow cytometry data was obtained by BD FACSymphony A1 Cell Analyzer.

Synthesis of HOF. Cu-TCPP (1mg, 0.0011 mmol) was dissolved in 0.3 mL of N,N-dimethylformamide (DMF) and 0.2 mL of N-methyl-2-pyrrolidone (NMP), and 0.042 mL of Tween 85 was then added. 2 mL of H_2O was introduced and stirred at room temperature for 5 min. 1.6 mL of ethanol and 0.03 mL of citric acid (in 5 mg/mL of EtOH) were finally added and stirred for another 25 min. The precipitate was collected by centrifugation and washed three times with ethanol.

Synthesis of CPT@HOF. Cu-TCPP (1 mg, 0.0011 mmol) and CPT (1mg, 0.0028 mmol) were dissolved in 0.3 mL of N,N-dimethylformamide (DMF) and 0.2 mL of N-methyl-2-pyrrolidone (NMP), and 0.042 mL of Tween 85 was then added. 2 mL of H_2O was introduced and stirred at room temperature for 5 min. 1.6 mL of ethanol and 0.03 mL of citric acid (in 5 mg/mL of EtOH) were finally added and stirred for another 25 min. The precipitate was collected by centrifugation and washed three times with ethanol.

Synthesis of HOF@FA. 2 mg of HOF was dispersed in 4 mL of deionized water and then 2 mg of FA was added. After stirring for 24 h, the product was collected by centrifugation and washed with deionized water three times

Synthesis of CPT@HOF@FA. 2 mg of CPT@HOF was dispersed in 4 mL of deionized water and then 2 mg of FA was added. After stirring for 24 h, the product was collected by centrifugation and washed with deionized water three times.



Drug release study. An equal amount (2 mg) of CPT@HOF@FA was dispersed in 3 mL of PBS solution with pH=5.0, and then shaken in a constant temperature oscillator. The samples were taken out after 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 h, 5 d, 6 d and 7 d respectively, and then centrifuged. The supernatant was sucked out and replaced by the fresh PBS solution. The content of CPT in the supernatant was measured by an ultraviolet-visible spectrophotometer with the help of a calibration curve.

In vitro cytotoxicity of HOF@FA. To study the in vitro biocompatibility of HOF@FA, L929 cells were seeded into 96-well plates with a density of 6000 cells per well and cultured in Dulbecco's modified eagle medium (DMEM) for 24 h. The original medium was then sucked out and the new medium containing different concentrations of HOF@FA was added again. After the cells were incubated for another 24 h, the medium inside was again discarded and re-added with fresh DMEM containing 20 μL of MTT. After placing the plates in the dark for 4 h, remove the culture medium and retain the Formazan crystals at the bottom of the wells, 150 μL of DMSO was added to each well, then gently shake the plate to completely dissolve the crystals and the absorption value of the medium was measured by a microplate reader at the wavelength of 630 nm.

In vitro cytotoxicity of CPT and CPT@HOF@FA. For the in vitro cytotoxicity test, 4T1 cells were seeded into 96-well plates with a density of 6000 cells per well and cultured in Advanced RPMI-1640 for 24 h. The original medium was then sucked out and the new medium containing different concentrations of CPT and CPT@HOF@FA was added again, respectively. Due the low stability of CPT@HOF@FA, some particles aggregated to form big size bulk crystals. After the cells were incubated for another 24 h, the medium as well as the insoluble CPT@HOF@FA were discarded, followed by the addition of fresh DMEM containing 20 μL of MTT. After placing the plates in the dark for 4 h, remove the culture medium and retain the Formazan crystals at the bottom of the wells, and 150 μL of DMSO was added to each well, then gently shake the plate to completely dissolve the crystals. The surviving rate of 4T1 cells was figured out by a microplate reader.

Cellular Internalization of RhB@HOF@FA and RhB@FA. Cu-TCPP (1 mg, 0.0011 mmol) and rhodamine B (RhB)-loaded (1 mg, 0.00209 mmol) were dissolved in 0.3 mL N,N-dimethylformamide (DMF) and 0.2 mL of N-methyl-2-pyrrolidone (NMP), and 0.042 mL of Tween 85 was then added. 2 mL of H_2O was introduced and stirred at room temperature for 5 min. 1.6 mL of ethanol and 0.03 mL of citric acid (in 5 mg/mL of EtOH) were finally added and stirred for another 25 min. The precipitate was collected by centrifugation and washed three times with ethanol.

1 mg of FA was then added. After stirring for 24 h, the product was collected by centrifugation and washed with deionized water three times. 4T1 cells were incubated with rhodamine B (RhB)-loaded HOF@FA (20 μg/mL) and HOF (20 μg/mL) for 1 h, respectively. It was then washed with PBS for three times. The nuclei were labeled with 4,6-diamino-2-phenylindole (DAPI) for 10 min and then observed under confocal laser scanning microscopy (CLSM) images.

Cellular Internalization of RhB@HOF@FA. To study the cellular uptake of CPT@HOF@FA, rhodamine B (RhB)-loaded HOF@FA was prepared first, and then 4T1 cells were incubated with rhodamine B (RhB)-loaded HOF@FA (50 μg/mL) for 1, 4, 6 h, respectively. It was then washed with PBS for three times. The nuclei were labeled with 4,6-diamino-2-phenylindole (DAPI) for 10 min and then observed under confocal laser scanning microscopy (CLSM) images.

Cellular Internalization of CPT@HOF and CPT@HOF@FA. To study the cellular uptake of CPT@HOF and CPT@HOF@FA, 4T1 cells were incubated with (20 μg/mL) for 1, 2, 6 h, respectively. It was then washed with PBS for three times. The mitochondria were labeled with MitoTracker Red for 15 min and then observed under confocal laser scanning microscopy (CLSM) images.

Mitochondrial Membrane Potential Assay. To investigate the effect of nanoparticles on mitochondrial membrane potential, a mitochondrial membrane potential assay kit containing JC-1 was used (Sparkjade). After co-incubating for 24 h, the cell samples washed by PBS, and prepared according to the instructions of the kit. Finally, the changes in mitochondrial membrane potential after various treatments were detected by Cell imaging.

Cell Apoptosis of CPT and CPT@HOF@FA Nanoparticles. To study the cell apoptosis process, Annexin V-FITC/PI Apoptosis Detection Kit was used. 4T1 cells were treated with PBS, HOF@FA (10 μg mL^{-1}), CPT (10 μg mL^{-1}), and CPT@HOF@FA (10 μg mL^{-1}), respectively, and then the cells were incubated in 6-well plates overnight. After that, the cells were harvested and washed with PBS, and then resuspended with binding buffer (400 μL). At last, 5 μL of Annexin V-FITC and 5 μL of PI were utilized to stain the samples for 15 min and 5 min in the dark, respectively. The cell apoptosis process was monitored via a flow cytometer.

In vivo antitumor efficacy of CPT@HOF@FA. Female Balb/C mice (about 18 g) were purchased from the Hunan Silaikejingda Experimental Animal Co, Ltd. The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Guangxi Normal University (No. 202410–006), and all procedures were performed in accordance with internationally accepted principles for the use and care of laboratory animals. The development of tumor model was achieved by subcutaneous injection of 4T1 cells into the right side of Balb/C mice. When the tumor volume reached up to 80-120 mm^3, 24 cancer-bearing mice were randomly divided into four groups, and treated with PBS, HOF@FA, CPT (5 mg Kg^{-1}) and CPT@HOF@FA, respectively. All groups of mice received twice drug injections on day 1 and day 7, respectively. The relative tumor volume was V/V_0 and the tumor volume was calculated by $V=4/3 \times \text{length} \times \text{width}^2/8$, where V_0 was the tumor volume before treatment.

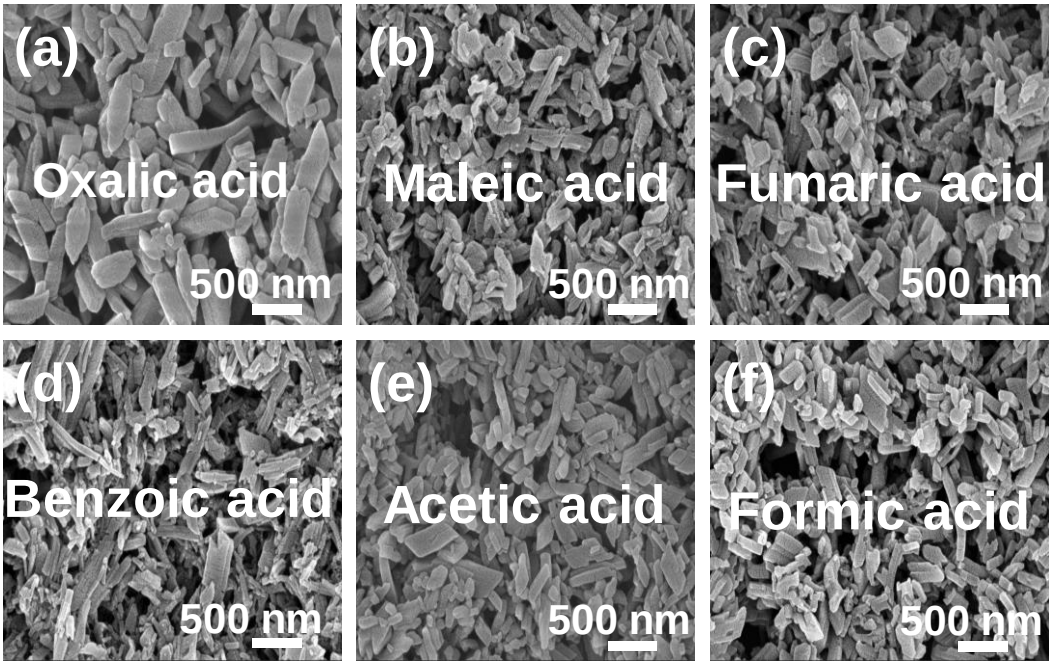


Fig. S1 SEM images of products under different acid conditions.

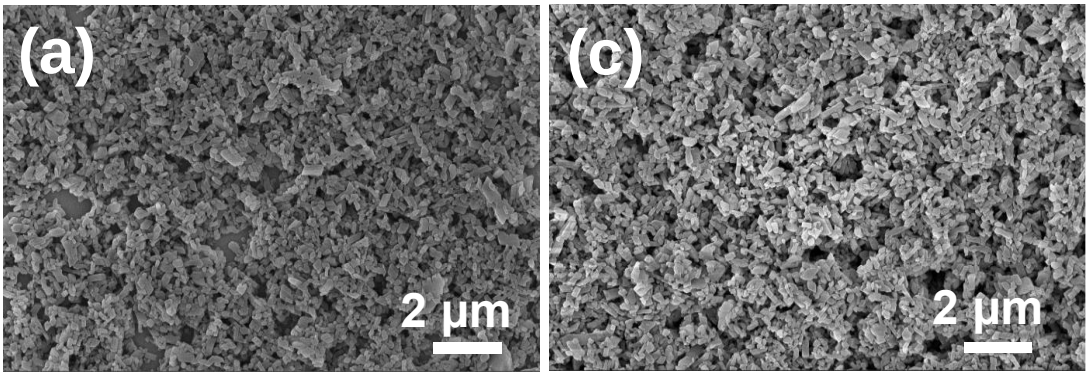


Fig. S2 SEM images of (a) HOF and (b) CPT@HOF.

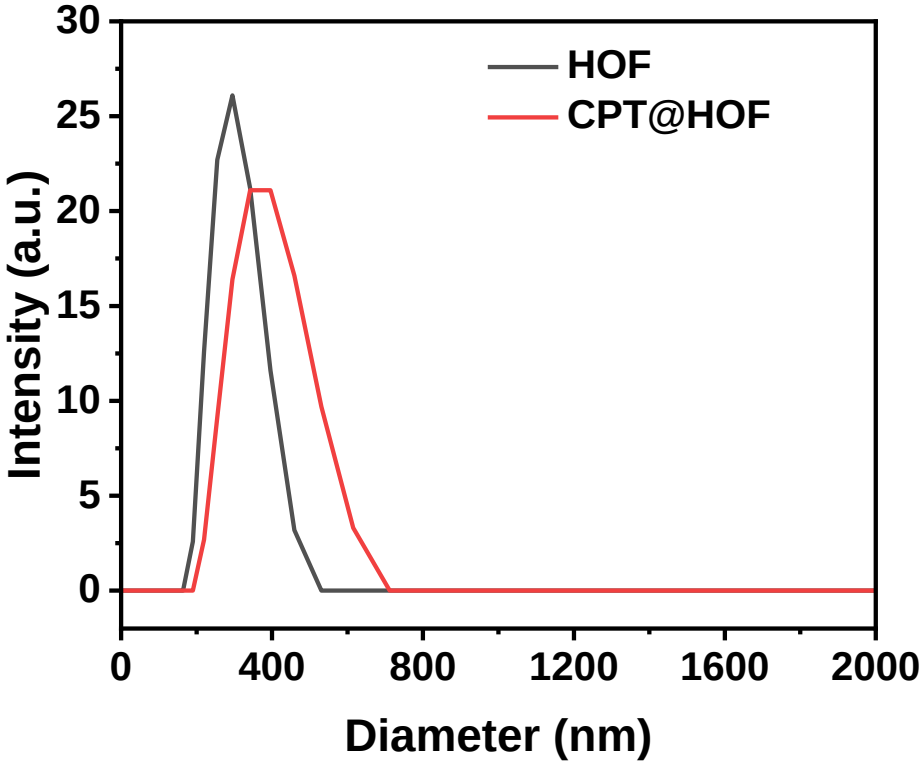


Fig. S3 The DLS measurement results for HOF and CPT@HOF.

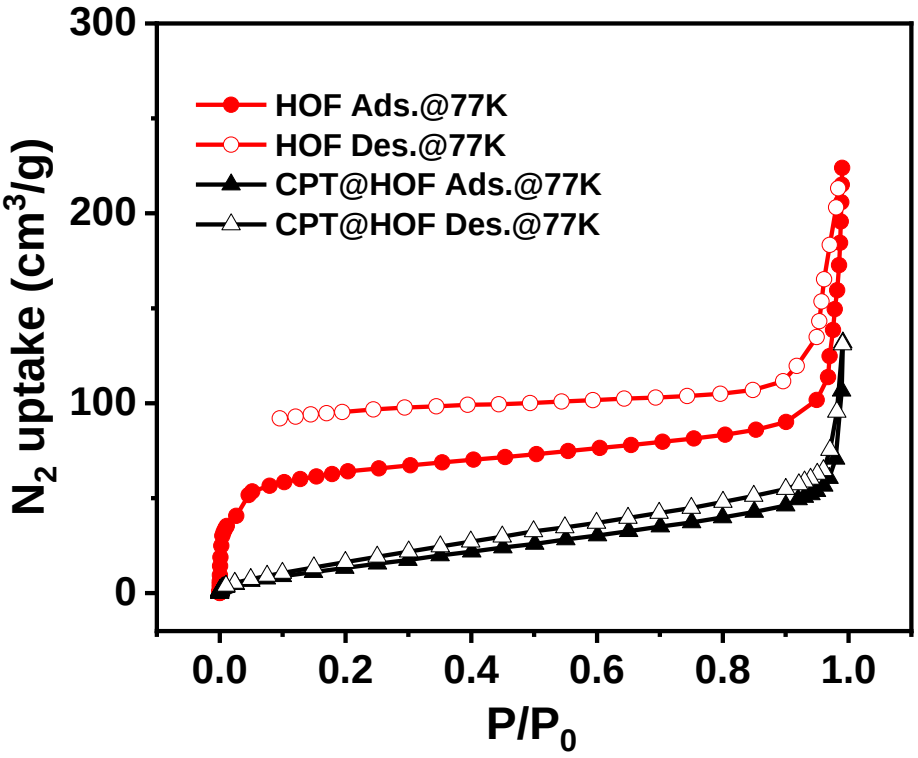


Fig. S4 The N_2 sorption isotherms and pore size distribution for HOF and CPT@HOF.

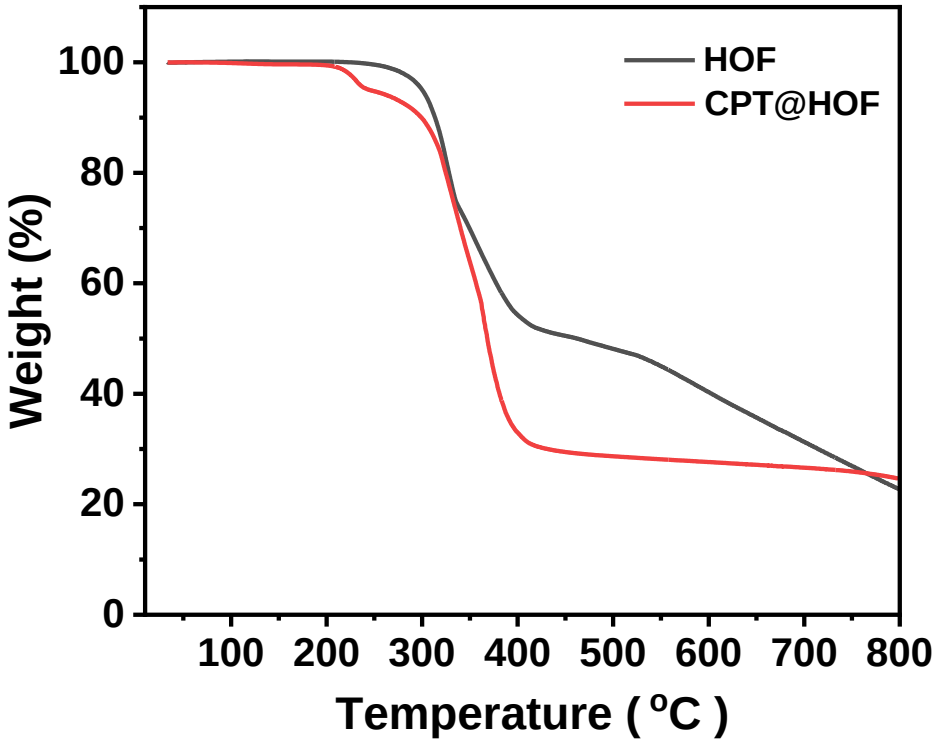


Fig. S5 The TGA curves for HOF and CPT@HOF, respectively.

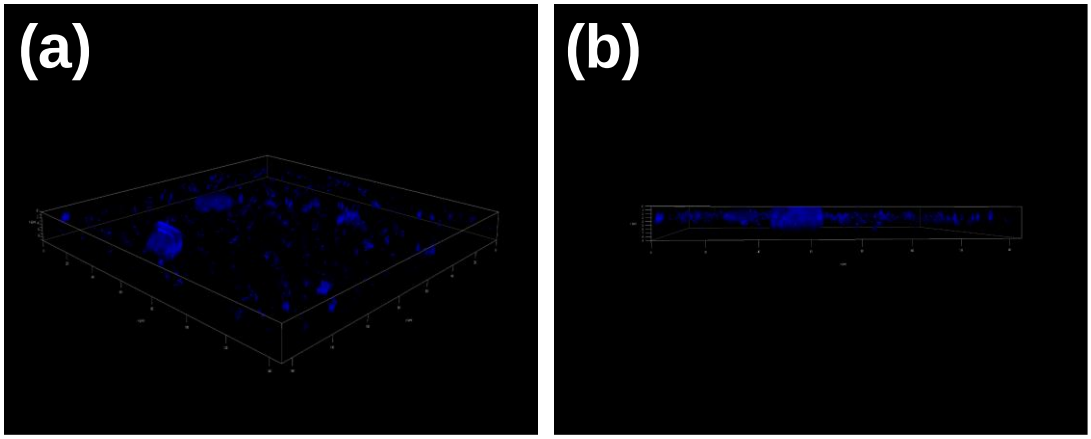


Fig. S6 3D-CLSM profile of the distribution of CPT in HOF

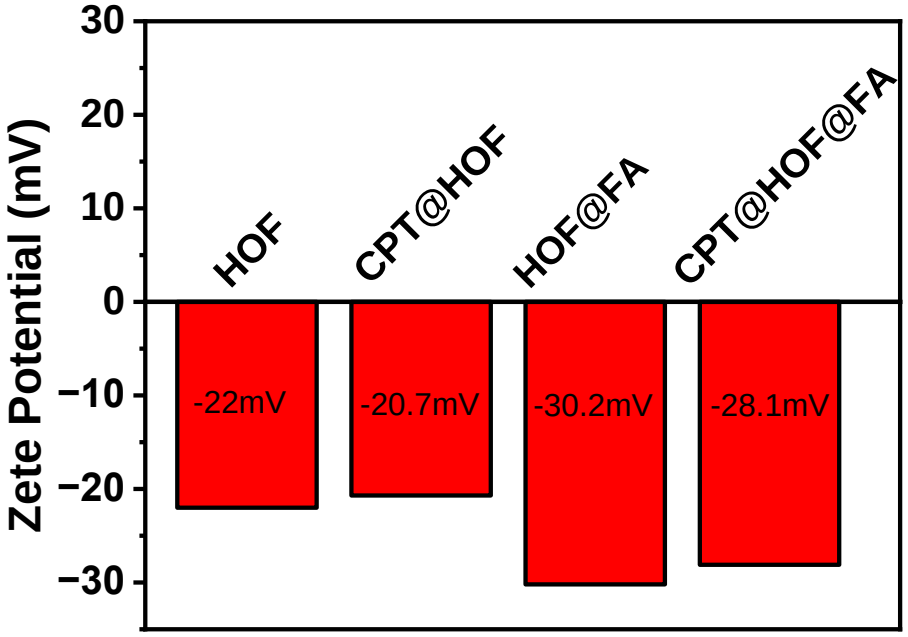


Fig. S7 Zeta potential of HOF, CPT@HOF, HOF@FA and CPT@HOF@FA.

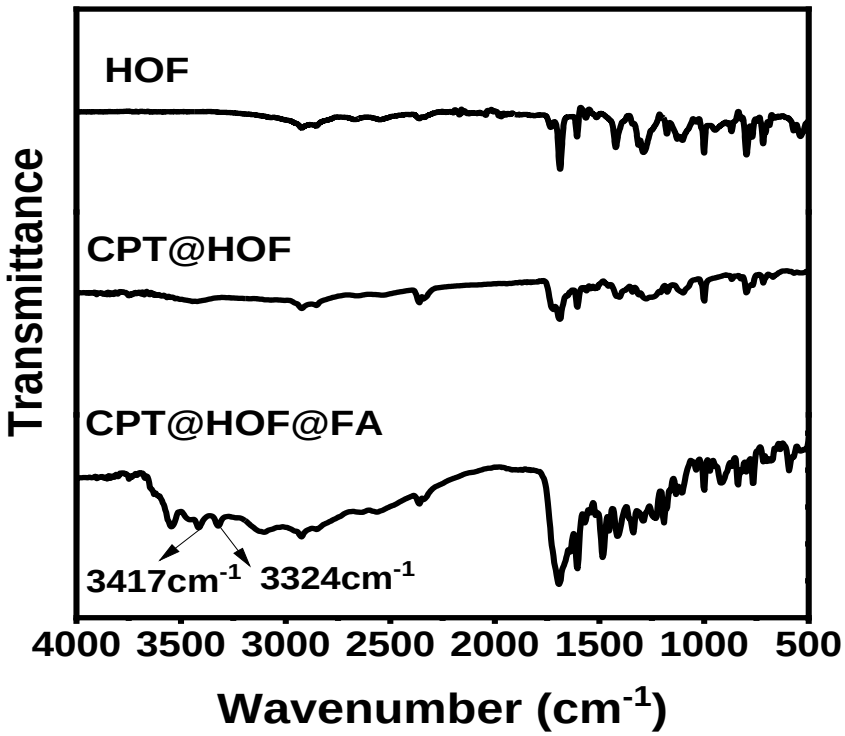


Fig. S8 The FT-IR spectra of HOF, CPT@HOF and CPT@HOF@FA.

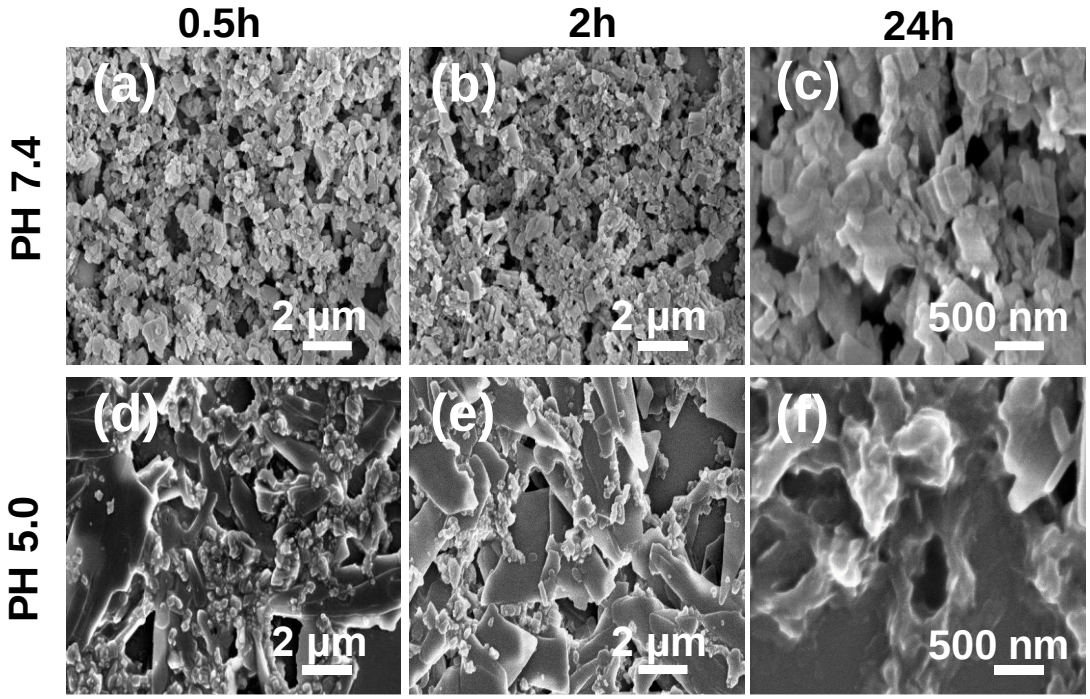


Fig. S9 (a)-(f) SEM image of CPT@HOF@FA after incubating with PBS at (a-c) pH=5.0, and (d-f) pH=7.4 for 0.5, 2 and 24 h, respectively.

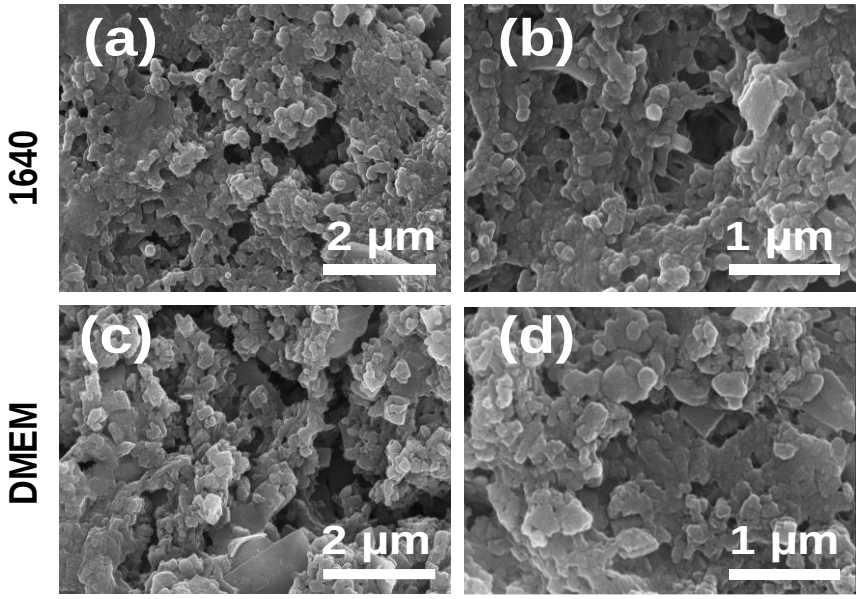


Fig. S10 SEM images of CPT@HOF@FA dispersed in (a), (b)1640 and (c), (d) DMEM for 24 h, respectively.

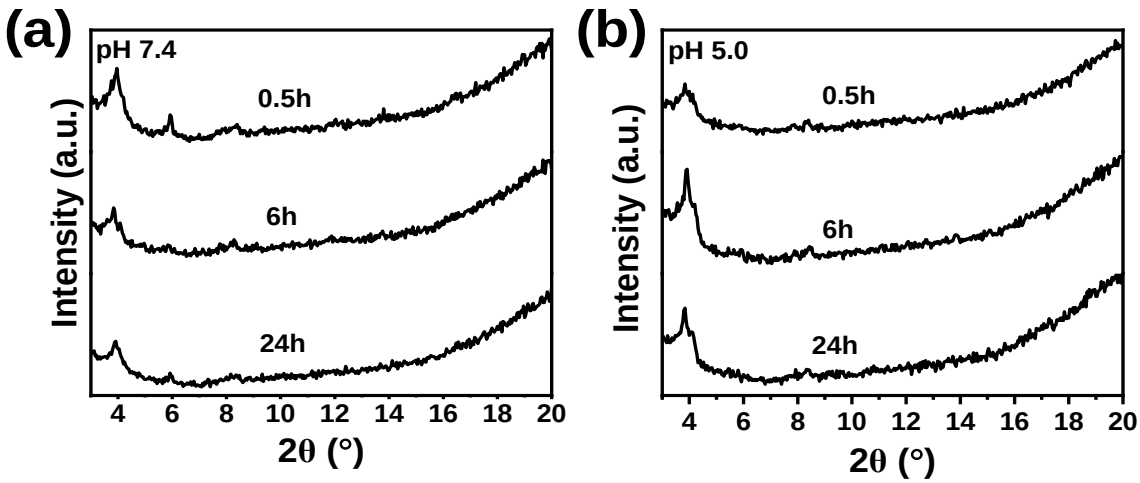


Fig. S11 The PXRD patterns of CPT@HOF@FA dispersed in PBS, at (a) pH=7.4, and (b) pH=5.0 for 0.5, 6 and 24 h, respectively.

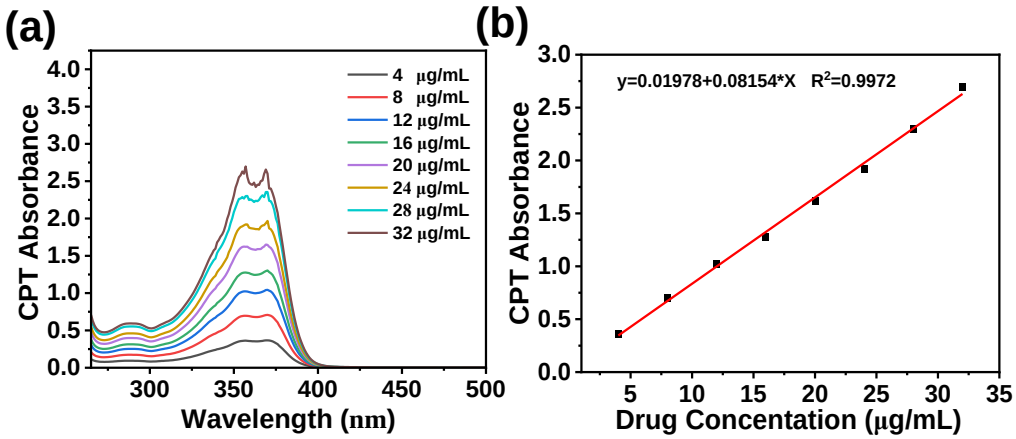


Fig. S12 (a) UV-vis absorbance-concentration standard line of CPT. (b) UV-vis absorption curve of CPT with different concentrations.

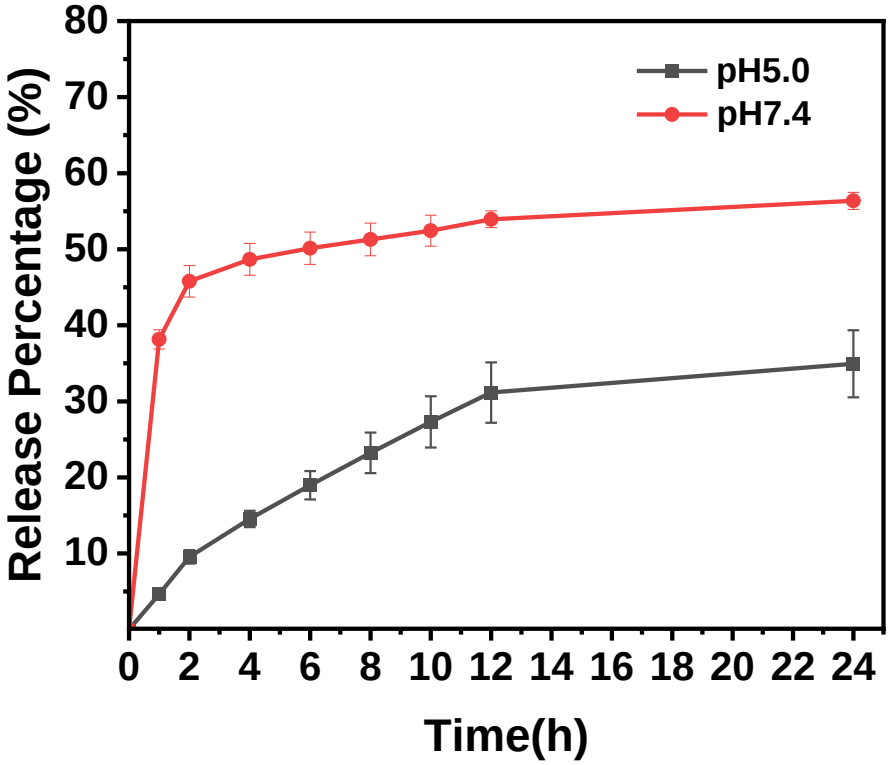


Fig. S13 Drug release curve of CPT@HOF@FA at pH=5.0 and pH=7.4

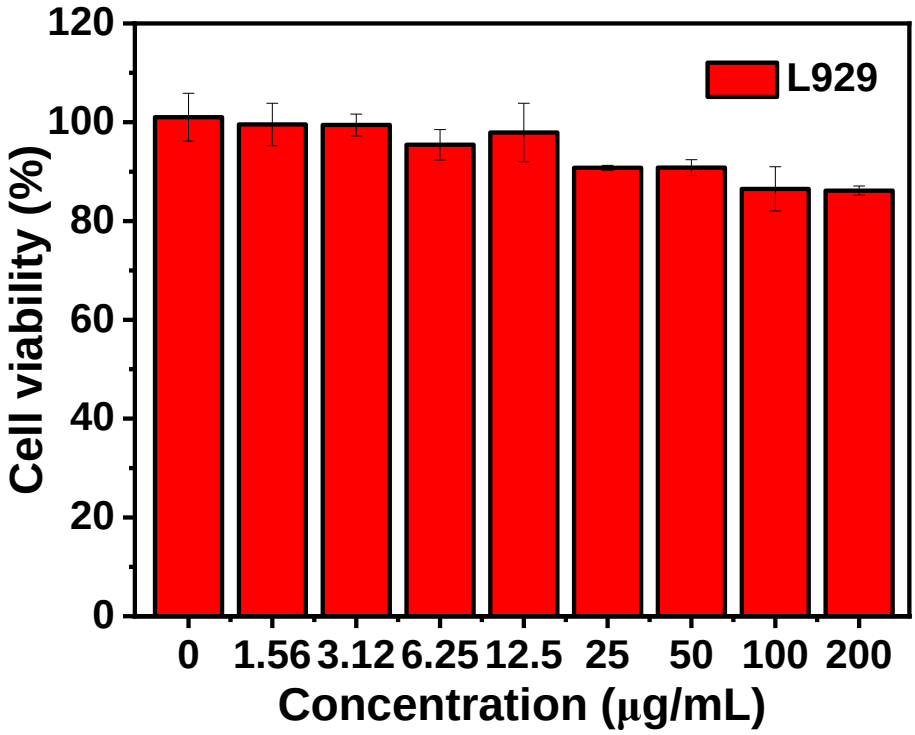


Fig. S14 In vitro cell viability of HOF@FA against L929 cells.

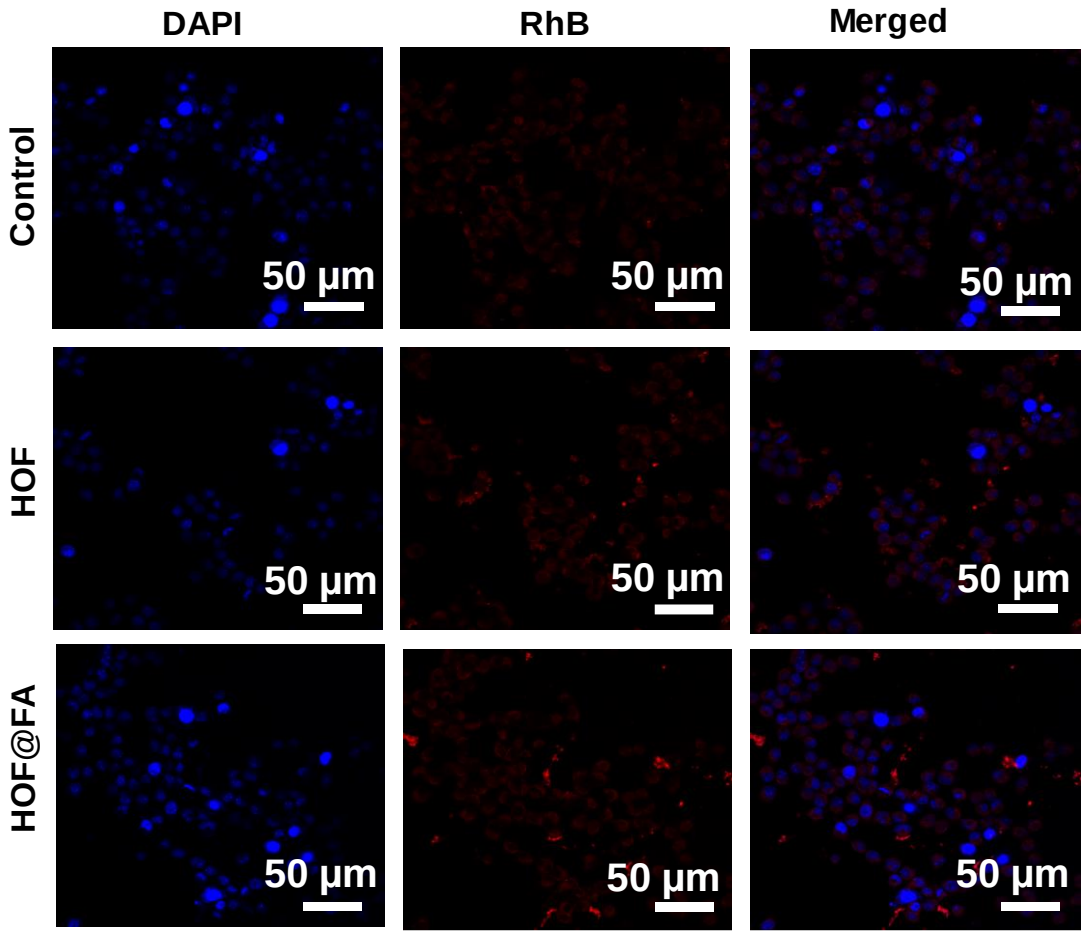


Fig. S15 CLSM images of 4T1 cellular uptakes of RhB-absorbed HOF and HOF@FA.

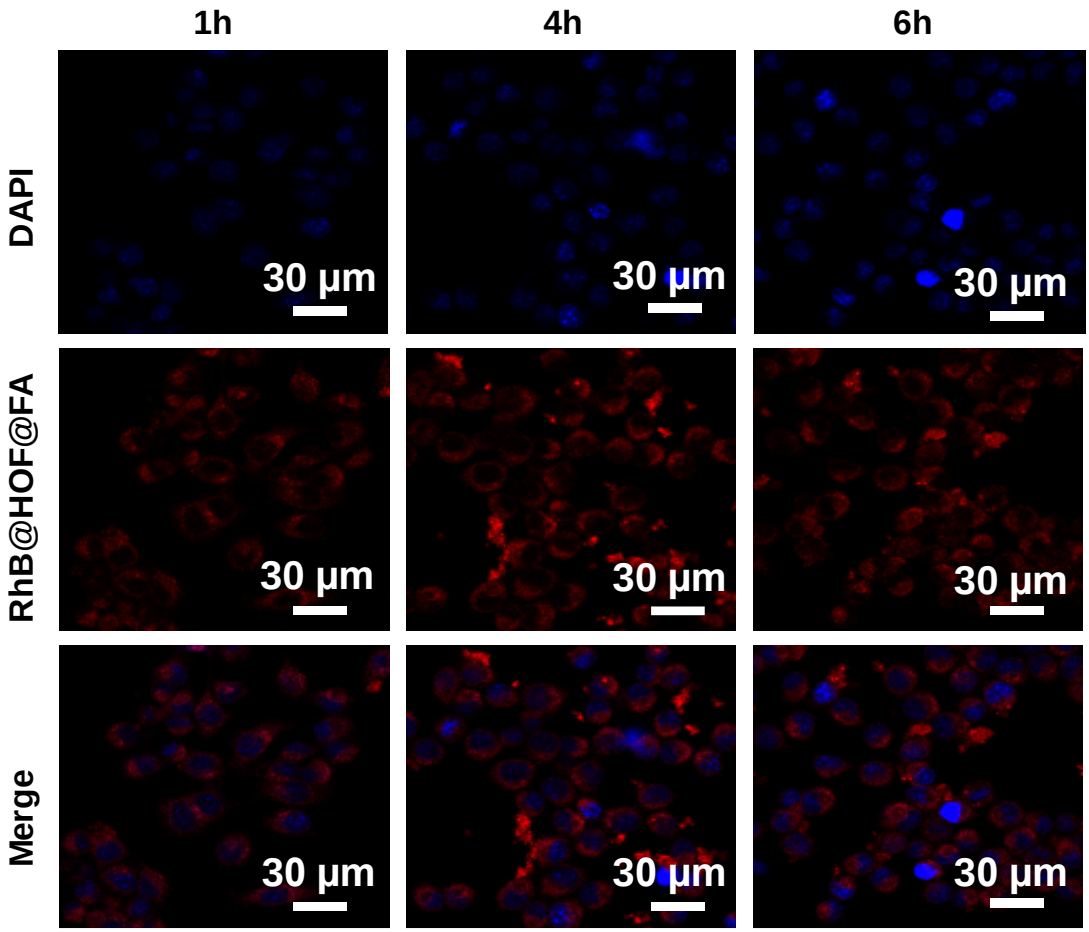


Fig. S16 CLSM images of 4T1 cells incubated with RhB-modified HOF for different times**.**

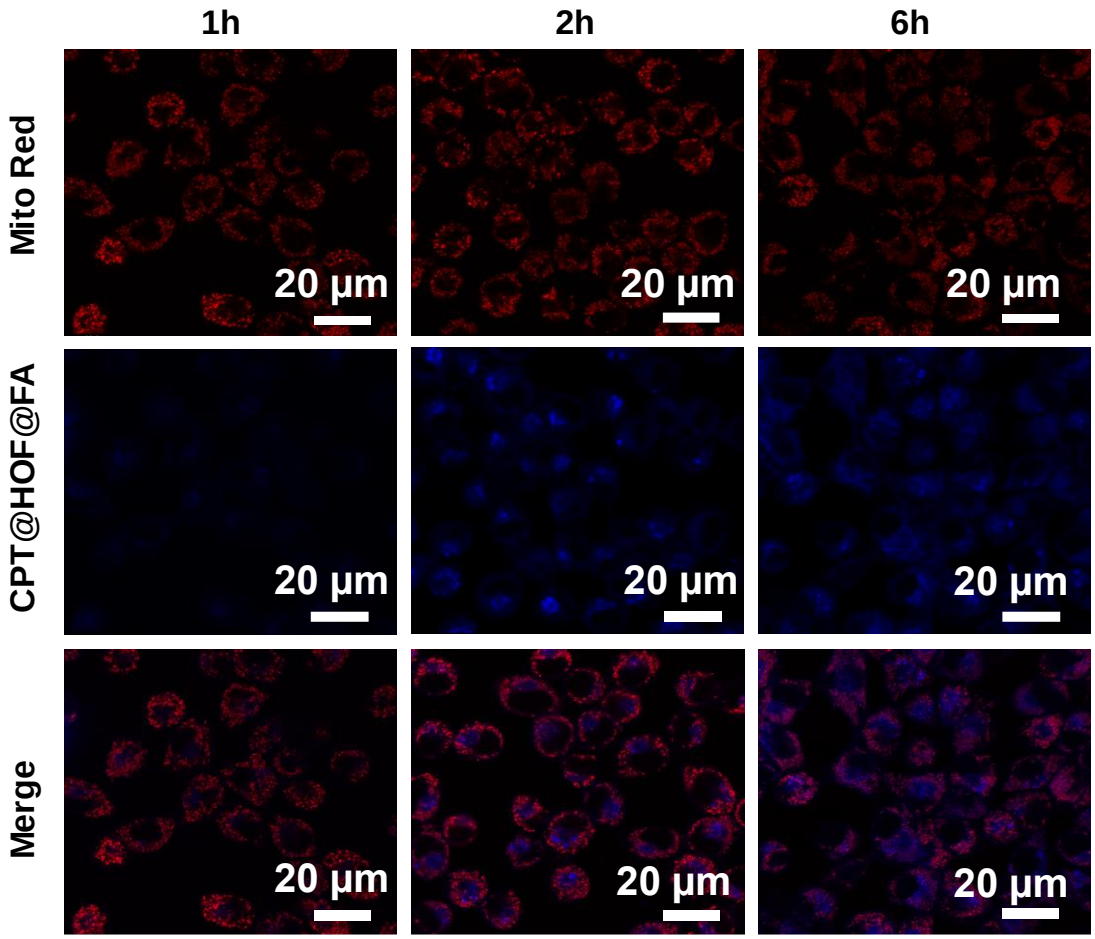


Fig. S17 CLSM images of 4T1 cells incubated with CPT@ HOF@FA for different times**.**

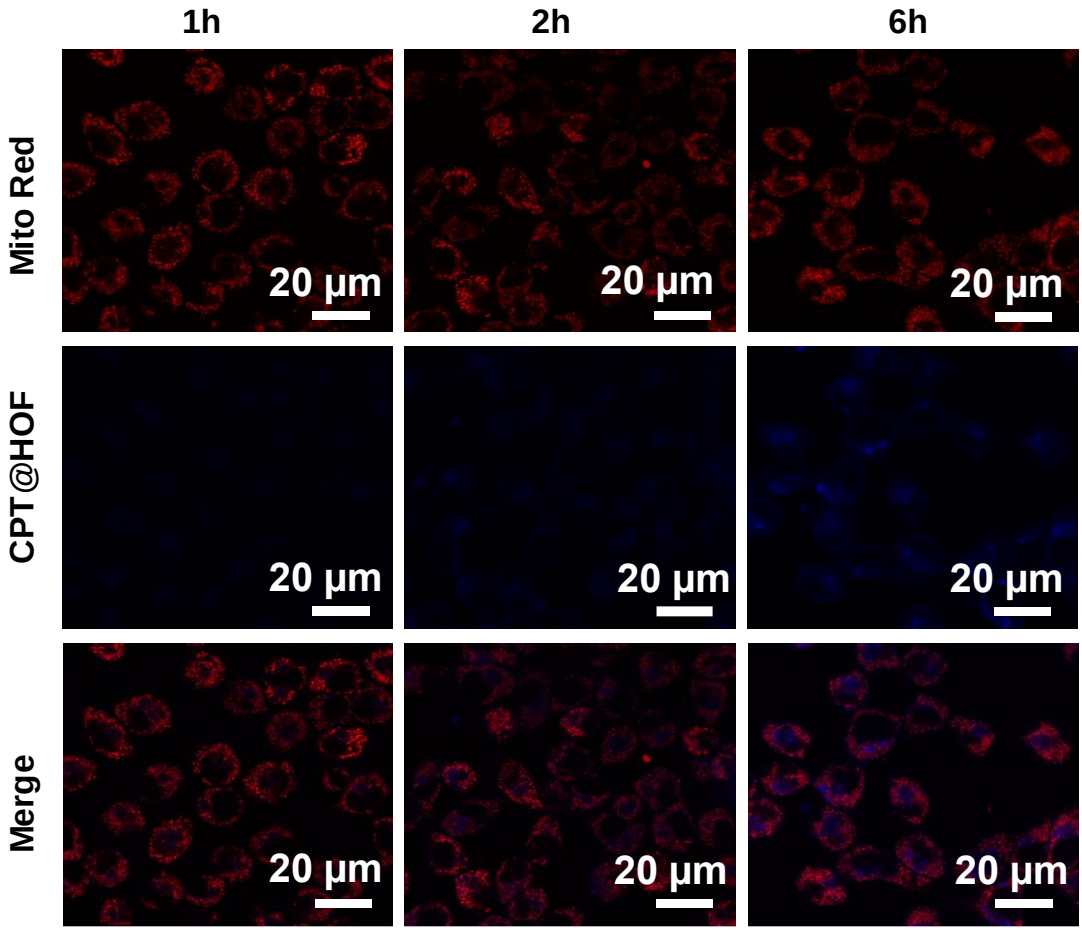


Fig. S18 CLSM images of 4T1 cells incubated with CPT@HOF for different times**.**