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Supporting Information

Rational design of copper(I)-doped metal-organic frameworks as dual-functional nanocarriers for combined chemo-chemodynamic therapy

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1. Experimental section

1.1 Chemicals and Materials

Anhydrous copper acetate ($Cu(CH_3COO)_2$, ≥98%) was purchased from Aladdin; Zinc acetate ($Zn(CH_3COO)_2$, ≥99.0%) and imidazole-2-formaldehyde (ICA, ≥98%) were purchased from TCI; Doxorubicin·HCl was purchased from meilunbio. 3-(4,5-dimethylthiazol-2-yl)-2-diphenyltetrazolium bromide (MTT) assay was purchased from KeyGEN BioTECH Corp., Ltd. N, N-dimethylformamide (DMF, AR). and anhydrous methanol (MeOH, AR) was purchased from Sinopharm. All reagents are commercially available and used without further purification.

1.2 Apparatus

The morphology of the samples was characterized by a field emission scanning electron microscope (SEM, Hitachi S-4800) and transmission electron microscopy (TEM, HT-7700). Powder X-ray diffraction (PXRD) patterns were collected on a Shimadzu XRD7000 powder X-ray diffractometer with a recording rate of $2°\cdot min^{-1}$ in the range of 2θ = 3°-50° at room temperature. UV-vis absorption spectroscopy was obtained on a UV-2600 UV-vis spectrophotometer (Shimadzu, Japan). Dynamic light scattering (DLS) was carried out on a Zetasizer Nano-ZS instrument (Malvern, England). The released DOX was detected by an F4600 fluorescence spectrophotometer. The concentration of ions was measured by an inductively coupled plasma emission spectrometer (ICP, Varian-730ES). CLSM study was performed on Olympus FV-1000 confocal laser scanning microscope. FT-IR spectra were collected on a Spectrum One in the spectral range of 400-4000 cm^{-1} (Thermo Scientific Nicolet iS20). N_2 sorption isotherms were measured by Micromeritics ASAP 2460 surface area analyzer. N_2 sorption measurement was maintained at 77 K with liquid nitrogen. ESR spectra were obtained from Bruker EMXplus equipment. Thermal gravimetric analysis (TGA) was performed on a Netzsch TGA 209 F3 analysis with a heating rate of 10 °C min^{-1} under a nitrogen flow of 20 mL min^{-1} h. Differential scanning calorimetry (DSC) was performed using a TA DSC Q100 with a heating rate of 10 °C min^{-1}.

1.3 Synthesis of CuZn-ZIF and CuZn-ZIF@DOX.

Synthesis of CuZn-ZIF: CuZn-ZIF nanoparticles were synthesized according to the literature with some modifications. [1] First, 10 mM $Zn(CH_3COO)_2$ DMF solution (solution A), 10 mM $Cu(CH_3COO)_2$ DMF solution (solution B), and 20 mM ICA DMF solution (solution C) were prepared. Solution D was prepared by mixing solution A with B at different molar ratios (8.5:1.5, 9:1, 9.5:0.5, 1:0). Later, the specific synthesis steps are as follows: 10 mL solution C and 10 mL solution D were injected simultaneously into the round-bottomed flask which contained 10 mL solution C, at different rates under stirring. The products were collected by centrifugation, rinsed by DMF and methanol twice, respectively, and dried in a vacuum oven at 60 °C for 12 hours. Finally, the powder was stored at room temperature for further use.

Synthesis of CuZn-ZIF@DOX: CuZn-ZIF and DOX with different mass ratios were stirred in methanol at room temperature for two days. Centrifuge and rinse the products three times with methanol. The products were dried in a vacuum oven, and stored in the refrigerator at 4 °C.

1.4 Detection of •OH produced by CuZn-ZIF

The •OH produced by CuZn-ZIF was detected by DMPO through electron spin resonance spectroscopy. In detail, 2 mL sodium acetate buffer solution containing DMPO (25 mM) and H_2O_2 (100 μM) was prepared at first. Then, 40 μL 1 mg/mL CuZn-ZIF aqueous dispersion system was added. After incubating for 5 minutes, the ESR spectra were collected.

1.5 Drug release experiment

The CuZn-ZIF@DOX aqueous dispersion system (5 mg/mL) was added into dialysis bags (interception MWCO = 3500 Da), and then soaked into phosphate buffered saline with different pH values (pH 7.4, pH 6.5, pH 5.5). Drug release experiments were performed at 37 °C under stirring. 1 mL solution was collected for further testing to determine the amount of released DOX. Meanwhile, 1 mL of fresh solution was added.

1.6 Cellular experiment

Cell culture: HeLa cells were cultured in high-glucose DMEM containing 10% fetal bovine serum (FBS) and 1% (penicillin and streptomycin) under a humidified

atmosphere with 5% CO_2 at 37 °C. HL-7702 (L02, human normal liver cells) were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS) and 1% (penicillin and streptomycin) under the same condition.

Cellular uptake: HeLa cells were incubated in a confocal dish for 4 h. After that, the medium was replaced with a fresh medium containing 100 μg mL^{-1} CuZn-ZIF@DOX. After incubation for 4 h and 8 h, the cells were washed three times with PBS. Then the cells were fixed with 4% paraformaldehyde for 30 minutes. Finally, the HeLa cells were observed by confocal laser scanning microscope (CLSM) to observe cell uptake.

Intracellular ROS detection: Intracellular ROS were detected by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). HeLa cells were inoculated into confocal dished and incubated for 4 h. After that, the media were replaced with fresh media containing 100 μg mL^{-1} ZIF-90, CuZn-ZIF, and CuZn-ZIF@DOX, respectively. After incubation for 8 h, the cells were rinsed with PBS and then incubated with DCFH-DA (10 μM) for another 20 minutes. Finally, cells were rinsed with PBS and the fluorescence imaging was conducted on CLSM.

Cytotoxicity of CuZn-ZIF and CuZn-ZIF@DOX: Cell compatibility was assessed by normal cell line. Briefly, cells were seeded into 96-well plates for 4 h. Then the medium was removed and replaced with fresh medium containing different concentrations of CuZn-ZIF (0-150 μg mL^{-1}). After 24 h incubation, 50 μL 1×MTT solution was added to each well and incubated for another 4 h. The absorbance was measured by the microplate reader at 492 nm. For the antitumor cells ability of CuZn-ZIF and CuZn-ZIF@DOX, HeLa cells were co-cultured with fresh medium containing different concentrations of CuZn-ZIF and CuZn-ZIF@DOX (Cu, 0-1 μg mL^{-1}; DOX, 0-1.6 μg mL^{-1}) for 24 h or 48 h. The cell viability was measured with the microplate reader at 492 nm.

Live/dead cells staining: The Live/Dead cells staining was carried out using calcein-AM/PI staining. Briefly, HeLa cells were seeded in confocal dishes at 1×10^5 cells per dish. After 4 h, HeLa cells were treated with ZIF-90, CuZn-ZIF, and CuZn-ZIF@DOX and incubated for another 24 h, followed by dying with calcein AM and PI and observation at 480 nm.

1.7 In vivo antitumor study

All animal experiments were performed based on the relevant laws and institutional guidelines, and the experiments were approved by the institutional ethics committee. Healthy nude mice were purchased from the Laboratory Animal Company of Shanghai Slac. HeLa cells were digested and suspended in DMEM at a concentration of 1.5×10^7 cells, and 100 μL aliquots were subcutaneously injected under the arm. Mice were used for further experiments when the tumor size grew to ~100 mm^3, and the mice were assigned to three groups (n = 5/group) including saline, CuZn-ZIF, and CuZn-ZIF@DOX group. Mice-bearing HeLa cells were intratumorally injected with 100 μL of 4 mg/mL CuZn-ZIF and CuZn-ZIF@DOX. Tumor volume and body weight were measured every two days. The tumor volume (V) can be calculated according to the following formula: $V = (L \times W^2)/2$, where L and W correspond to the longest and shortest diameter of the tumor, respectively. After 14 days of treatment, all mice were killed, and the major organs (heart, liver, spleen, lung, and kidney) and tumors were sliced and stained with hematoxylin and eosin (H&E). The tumor slices were stained with TUNEL and Ki67. In addition, the obtained tumors were photographed and weighed.

2. Supporting Figures and Table

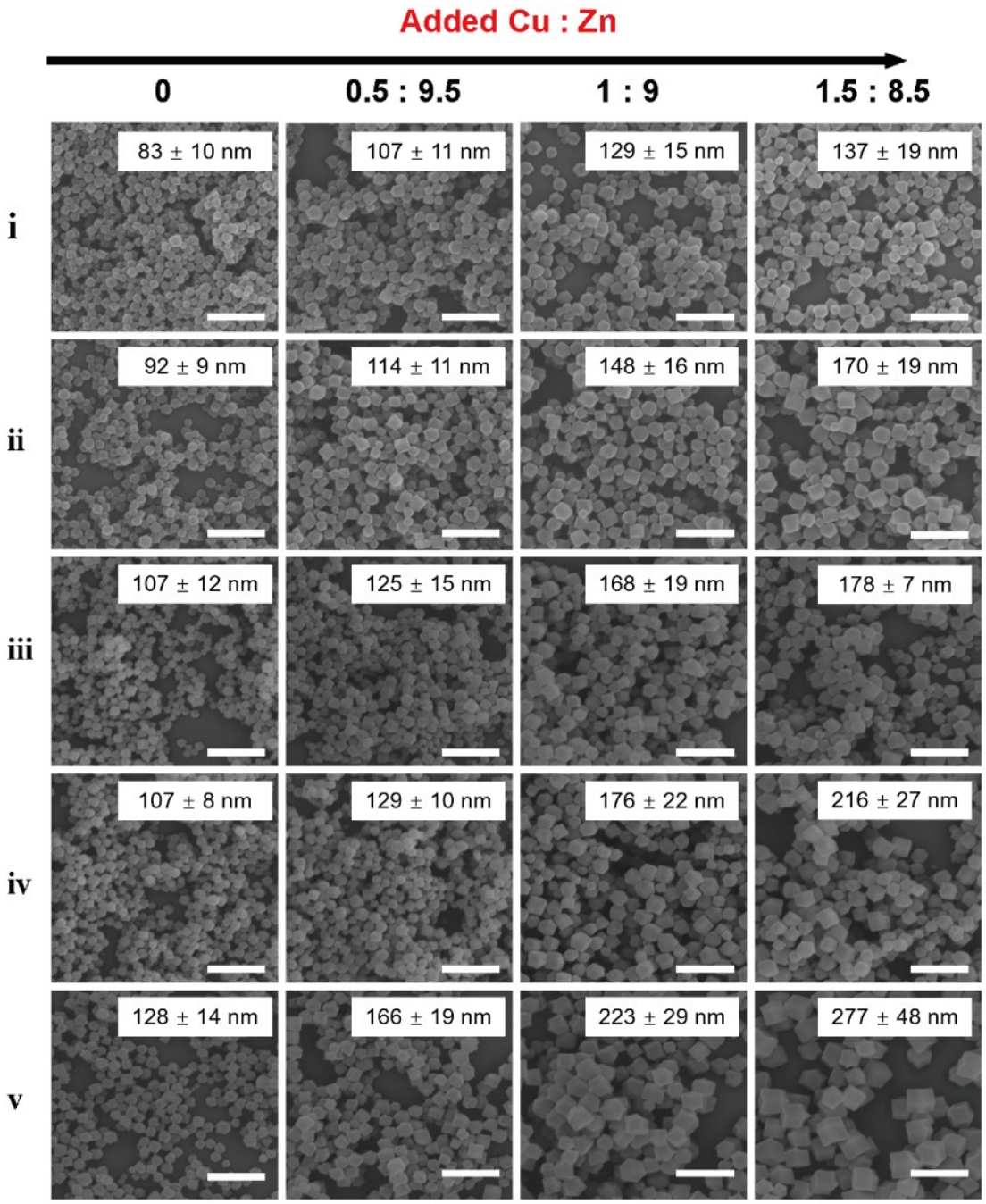

Figure S1. SEM images of nanoZIF-90 and CuZn-ZIF synthesized with different cooper content and a series of feed rates (i-v corresponds to 235, 160, 84, 54, and 24 mL/h, respectively). Scale bar: 500 nm.

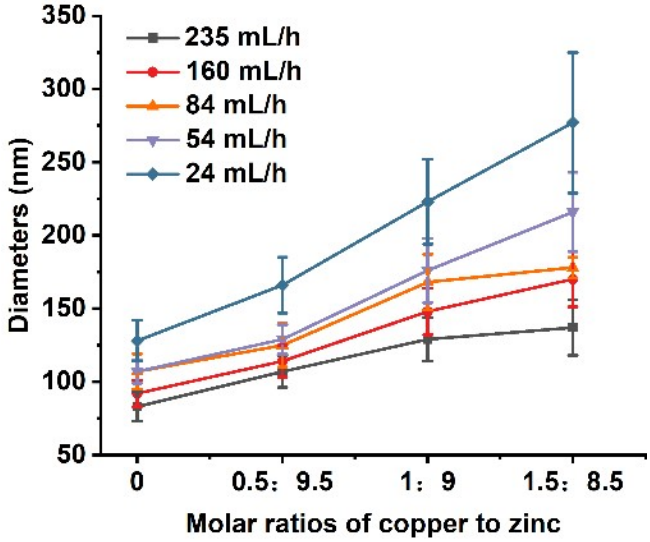

Figure S2. Nanoparticle sizes versus the molar ratios of copper to zinc under different feed rates.

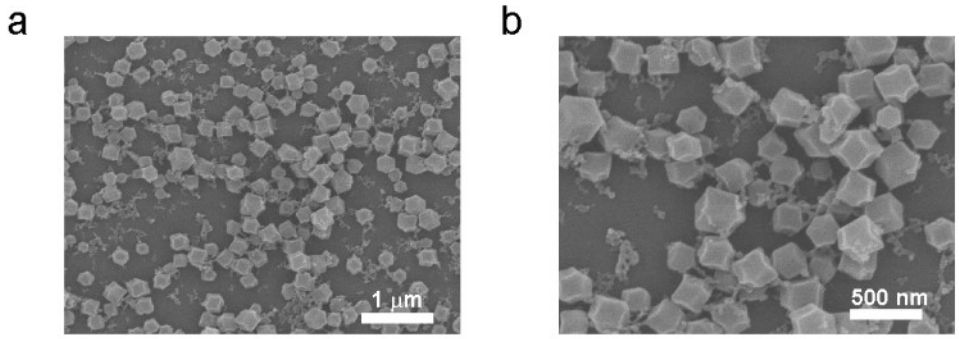

Figure S3. (a) SEM image and (b) the corresponding enlarged image of CuZn-ZIF (Cu/Zn = 0.2/0.8).

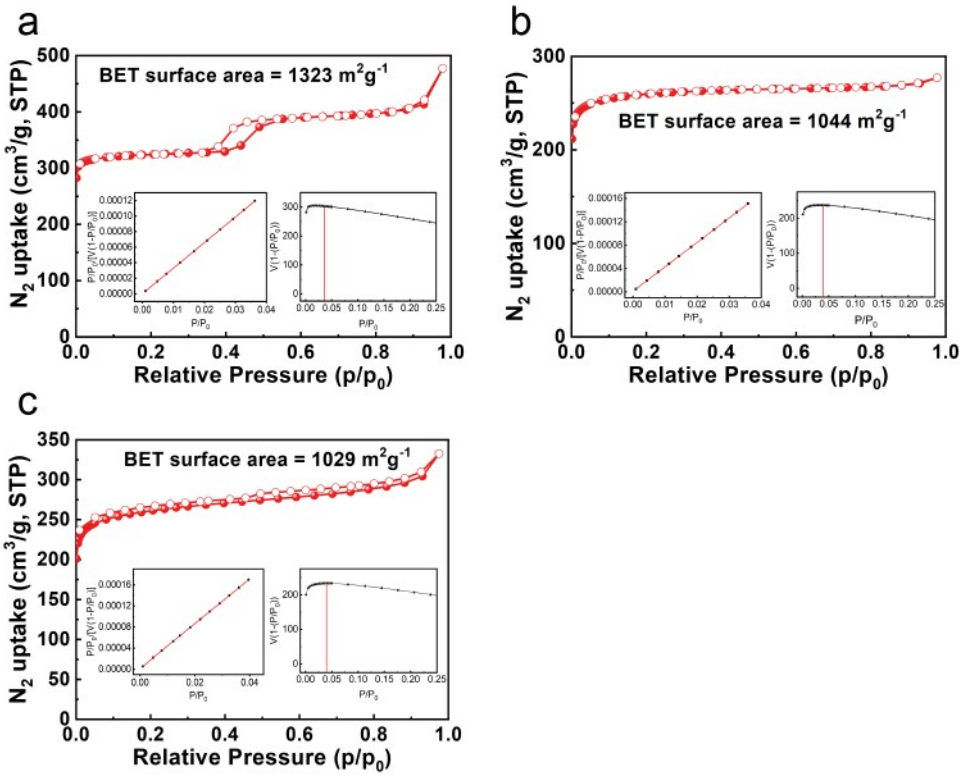

Figure S4. Nitrogen isotherms of ZIF-90 (a), CuZn-ZIF (b), and CuZn-ZIF@DOX (c) at 77K.

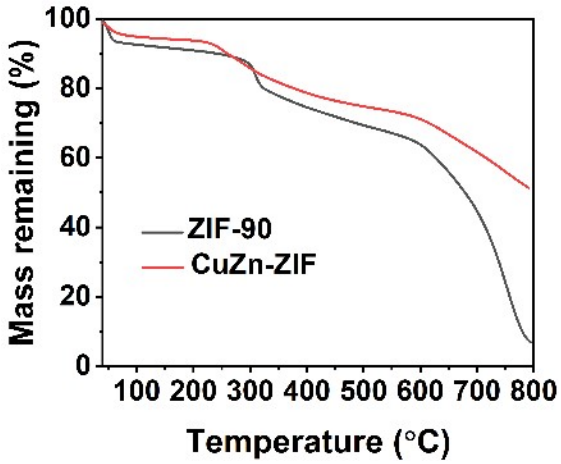

Figure S5. Thermogravimetric analysis (TGA) showing the weight loss and decomposition for ZIF-90 and CuZn-ZIF samples.

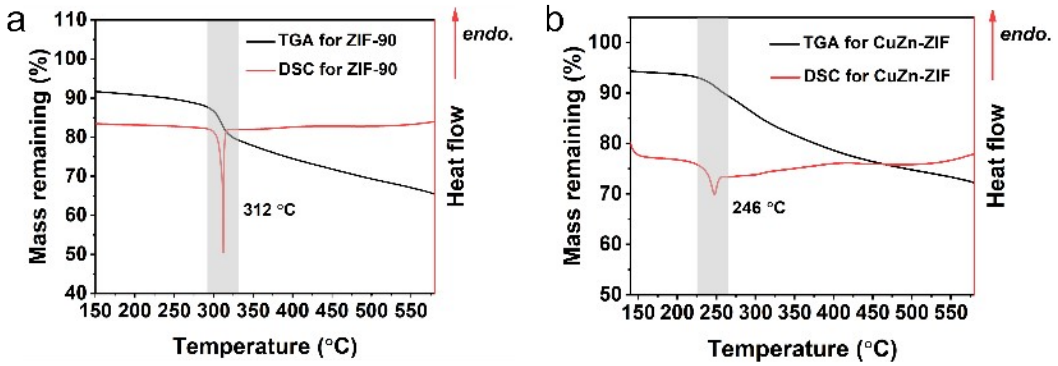

Figure S6. Thermogravimetric (black) and enthalpic response (red) of ZIF-90 (a) and CuZn-ZIF (b) on DSC heating upscans at 10 °C min^{-1}.

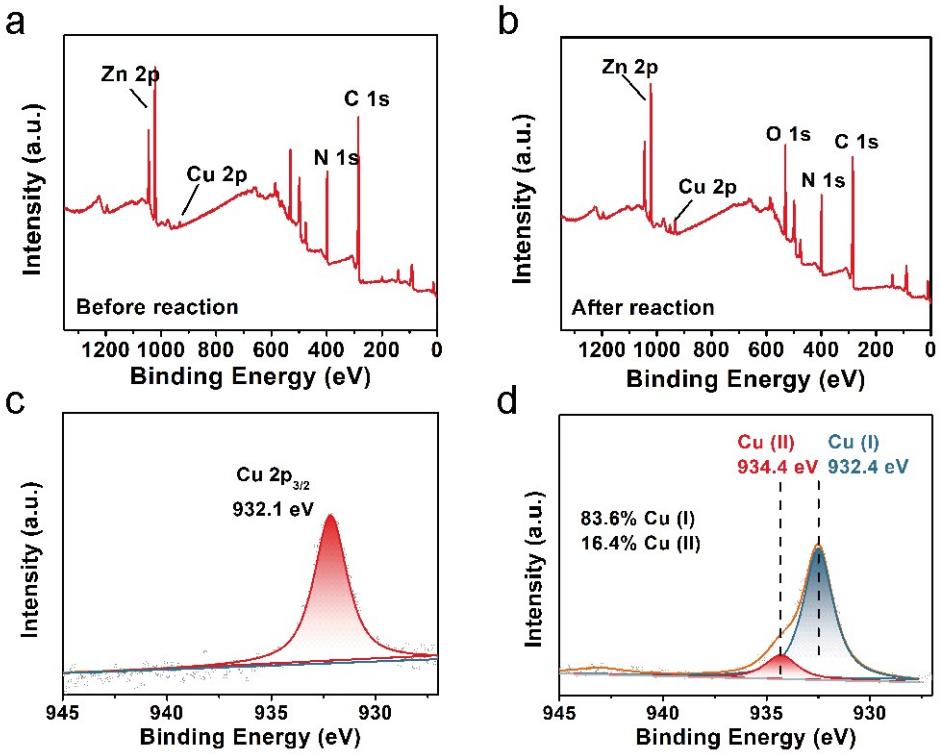

Figure S7. (a, b) XPS spectra and (c, d) typical elements Cu 2p spectra of CuZn-ZIF before and after reaction with H_2O_2.

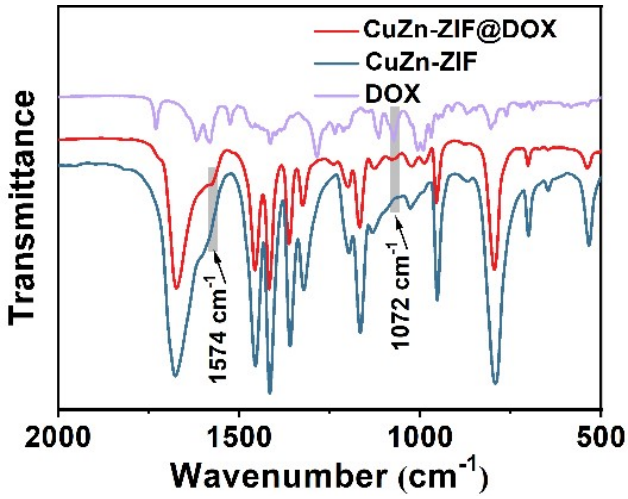

Figure S8. FT-IR spectra of DOX, CuZn-ZIF and CuZn-ZIF@DOX.

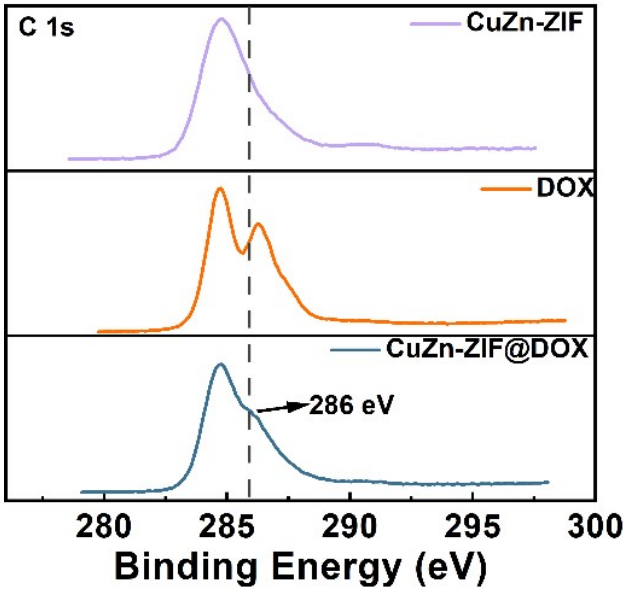

Figure S9. High-resolution XPS spectra of C 1s from CuZn-ZIF, DOX, and CuZn-ZIF@DOX.

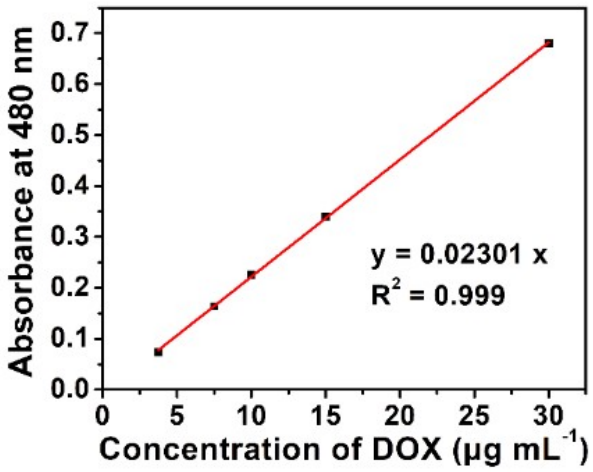

Figure S10. Standard curve of DOX from UV-vis spectra.

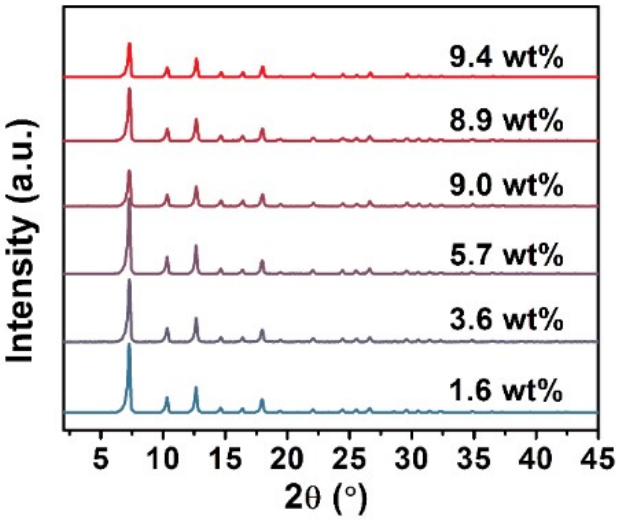

Figure S11. PXRD patterns of CuZn-ZIF@DOX with different content of DOX.

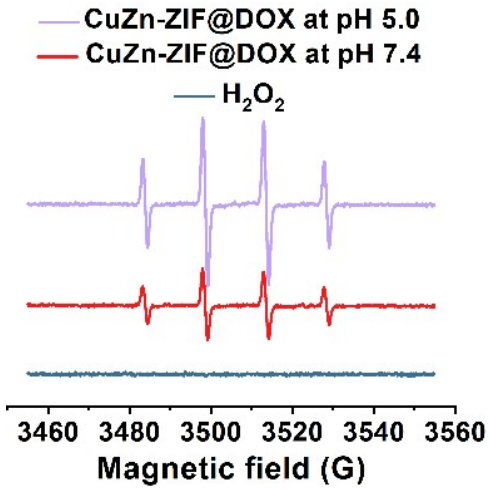

Figure S12. ESR spectra of CuZn-ZIF at different pH values.

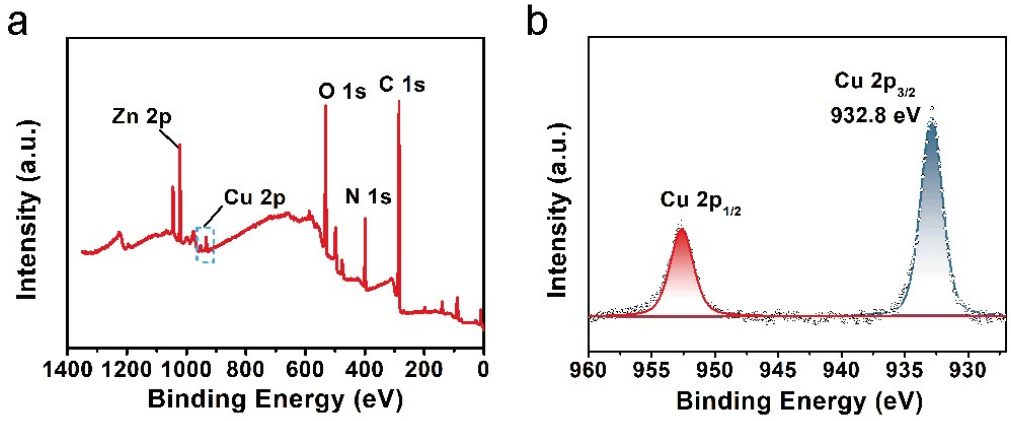

Figure S13. (a) XPS survey spectrum of CuZn-ZIF@DOX and (b) high-resolution XPS spectrum of Cu 2p in CuZn-ZIF@DOX.

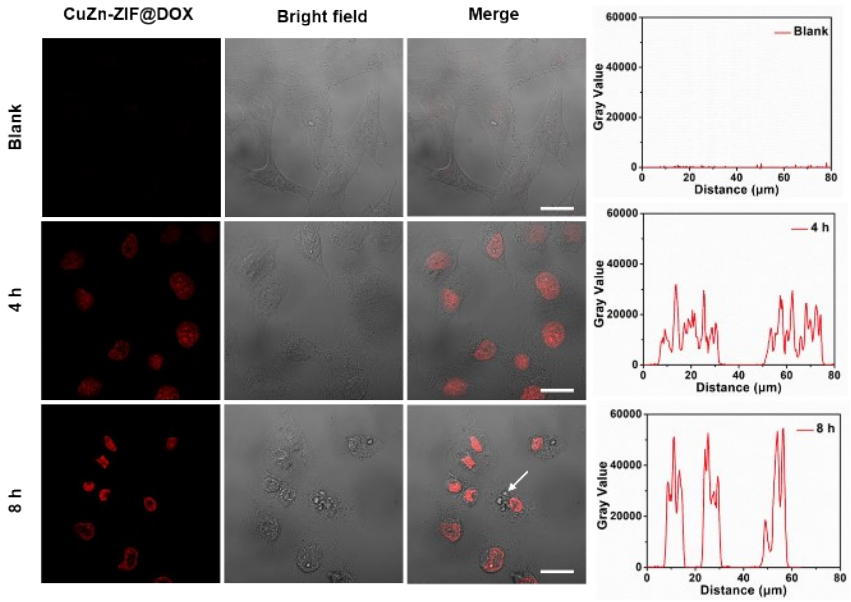

Figure S14. (a) CLSM images of HeLa cells incubated with CuZn-ZIF@DOX for different time. (b) The fluorescence intensity profile of ROI analysis of HeLa cells with CuZn-ZIF@DOX in the red channel. Scale bar: 30 μm.

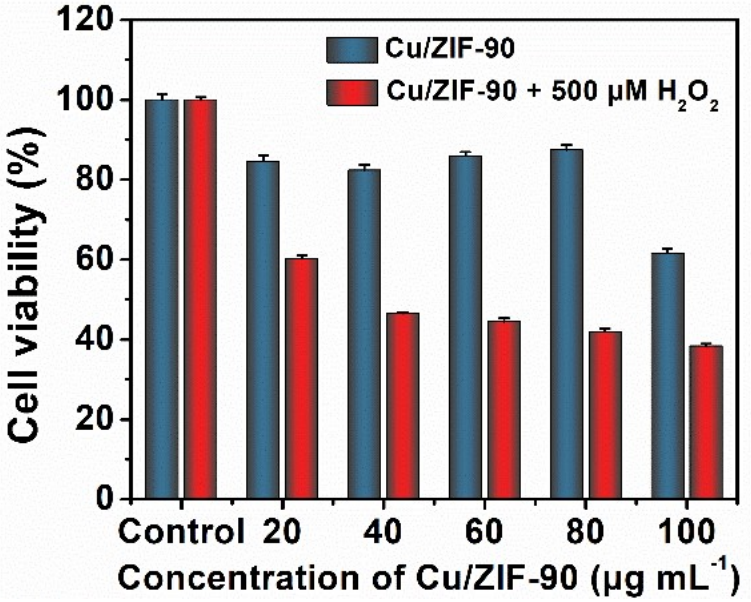

Figure S15. Cell viability of HeLa cells after incubating with CuZn-ZIF and CuZn-ZIF + H_2O_2 for 24 h.

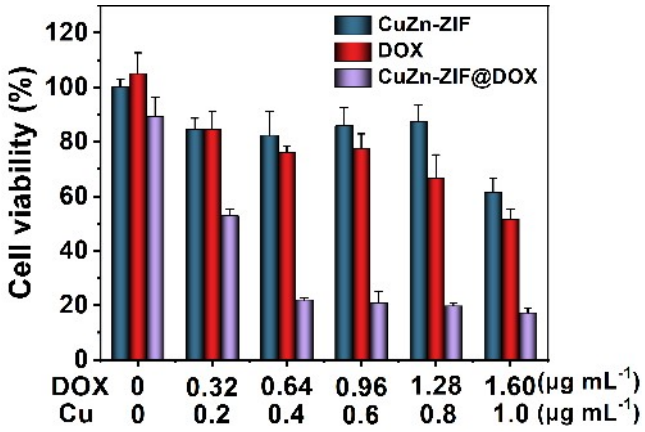

Figure S16. Cell viability of HeLa cells after incubating with DOX, CuZn-ZIF, or CuZn-ZIF@DOX for 48 h.

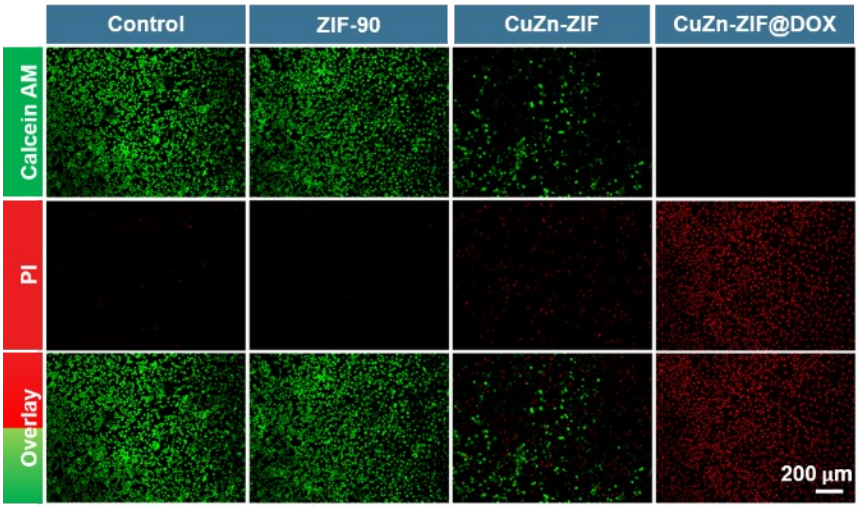

Figure S17. CLSM images of HeLa cells co-stained with Calcein-AM (green, for live cells) and propidium iodide (red, for dead cells) after different treatments. Scale bar: 200 μm.

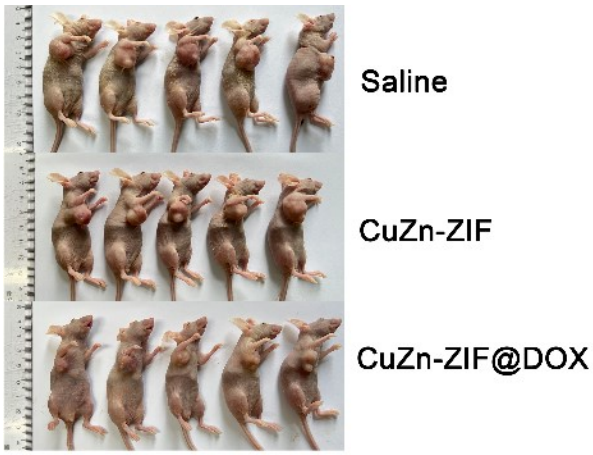

Figure S18. Representative photographs of mice with different treatments after 14 days.

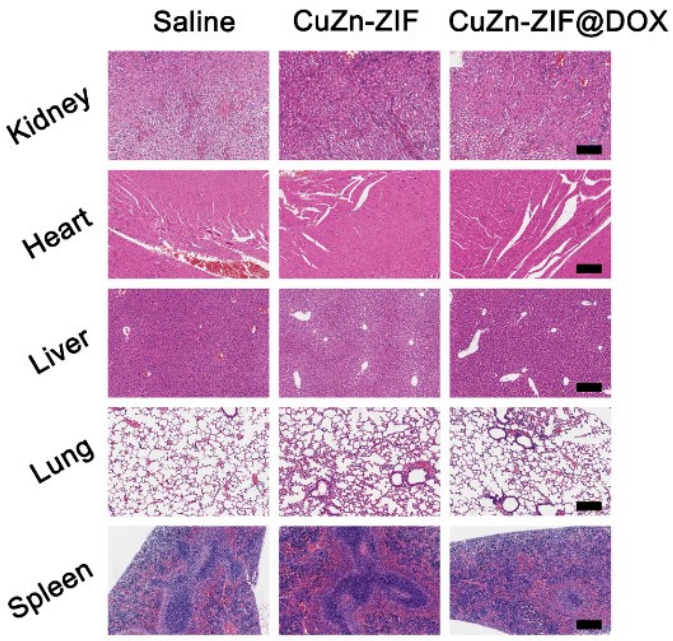

Figure S19. H&E-stained images of major organs after different treatments. Scale bar: 200 μm.

Table S1 The atomic ratios between copper and zinc of CuZn-ZIF determined by ICP-MS.

Cu: Zn atomic ratio (%)				
Experimental	0	0.5:9.5 (5%)	1:9 (10%)	1.5:8.5 (15%)
Observed (ICP)	0	1:18.9 (5%)	1:9.64 (9%)	1:7.09 (12.4%)

References

[1] X. Wang, Q. Cheng, Y. Yu and X. Zhang, *Angew. Chem. Int. Ed.*, 2018, **57**, 7836-7840.