Electronic Supplementary Material (ESI) for Materials Advances.

Supporting Information

Ferrous Metallacage Combined with Oxaliplatin for Synergistic Chemodynamic/Chemo Therapy

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1. Materials and Reagents

Solvents and raw materials are available on the market without further processing and purification. NaOH, H_2O_2, ethanol, methanol was purchased from Sinopharm Chemical Reagent Co., Ltd. Tetrakis (triphenylphosphine) palladium, Pd/C were purchased from J&K Scientific Co.,Ltd. 4-nitrophenylboronic acid, pyridine-2-formaldehyde, potassium carbonate, 3-chloro-1,2-propanediol, DMSO-d_6, D_2O, 1,4-dioxane, $FeSO_4{\cdot}7H_2O$, 1,4-diiodine-2,5-dimethyl ether, Trypsin-EDTA, PBS, DMEM high glucose, fetal bovine serum (FBS) and RPMI-1640 medium, Oxaliplatin (OXA), terephthalic acid (TPA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Adamas. 4T1 and 3T3 cells were obtained from ATCC. Calcein AM, propidium iodide (PI) were purchased from Sigma Aldrich. 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA), Mitochondrial membrane potential assay kit with 5,5',6,6'-tetrachloro-1,1'-3,3'-tetraethyl-benzimidazolylcarbocyanine iodide (JC-1), Hydrogen Peroxide Assay Kit were purchased from Biyotime Biotechnology Co., Ltd. PEG-COOH 2000 were purchased from Xi'an ruixi Biological Technology Co.,Ltd.

2. Characterization

^{1}H NMR spectra were probed on 400 MHz Bruker spectrometer using D_2O as the solvent. The fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was recorded on Bruker Compact spectrometer using methanol as mobile phase. Zeta potential and dynamic light scattering (DLS) measurements were measured using ZS90 Malvern Instrument Ltd. UV-vis measurements were obtained on a Lambda 20 UV-vis spectrometer (Perkin Elmer, Inc., USA). Inductively coupled plasma (ICP, Vista MPX ICP) was used for quantitative determination of iron. X-ray photoelectron spectroscopy was performed on Thermo Scientific K-Alpha+. Scanning electron microscope (SEM) images were collected on a Zeiss EVO MA 25/LS 25. Transmission electron microscope (TEM) images and energy dispersive spectra (EDS) mapping were collected on a Talos 200S. Fluorescent measurements were obtained using Shimadzu F-4500 spectrofluorophotometer. Confocal laser scanning microscopy (CLSM) images were acquired by a SP5 II Leica confocal microscope. Hematoxylin and eosin (H&E) staining images were obtained with an ECLIPSE Ci-L microscope. Tunel staining images were obtained with a panoramic MIDI digital slice scanner. Analytical high performance liquid chromatography (HPLC) was performed on a Agilent 1260 with UV detection. Analytical Tc-C18 column (4.6 mm × 25 cm, 5 μm) from Agilent was used.

3. Methods

3.1 Synthesis of ligand

Synthesis of **1**.

A mixture of 1,4-diiodine-2,5-dimethyl ether (4 g, 10.2 mmol) and boron tribromide (10.7 g, 43.1 mmol) was stirred in nitrogen atmosphere for 16 h. At the end of reaction, the mixture was poured into ice-cold water and the white solid was filtered to afford compound **1** (3.31 g, yield: 89.2%). ^{1}H NMR (DMSO-d_6, 400 MHz): 9.78 (2 H, s, H^1), 7.13 (2H, s, H^2).

Synthesis of **2**.

1 (3.3 g, 9.1 mmol) and NaOH (0.84 g, 21 mmol) dissolve in a mixed solvent of ethanol and water (V/V= 10/1). The mixture was refluxed at 80 °C for 1 h. After 3-chloro-1,2-propanediol (2.18 g, 19.8 mmol) dissolve in ethanol (5 mL) was add in to it, refluxed at 80 °C for 24 h. The mixture solvent was removed by rotary evaporator. (2.53 g, yield: 54.3%).^{1}H NMR (DMSO-d_6, 400 MHz): 7.33 (2 H, s, H^1), 4.91 (2 H, d, H^2), 4.65 (2 H, t, H^3), 3.92 (2, dd, H^4), 3.88 (2 H, dd, H^5), 3.74 (2 H, m, H^6), 3.49 (4 H, m, H^7).

Synthesis of **3**.

2 (250 mg, 0.5 mmol), 4-nitrophenylboronic acid (625 mg, 3.7 mmol), tetrakis(triphenylphosphine)palladium (22.75 mg, 2.4 mmol) and potassium carbonate (421.5 mg, 3 mmol) were dissolved in 1,4-dioxane and water (V/V= 3/1). The mixture was stirred at 100 °C for 16 h under nitrogen atmosphere. The precipitate was separated and purified by column chromatography (dichloromethane: methanol = 30:1) to produce a yellow solid (965 mg, yield: 38.9%).^{1}H NMR (DMSO-d_6, 400 MHz):8.25 (4 H, d, H^1), 7.93 (4 H, d, H^2), 7.20 (2 H, s, H^3), 4.05 (2 H, dd, H^4), 3.96 (2 H, dd, H^5), 3.74 (2 H, m, H^6), 3.36 (4 H, dd, H^7).

Synthesis of **L**.

A mixture of **3** (40 mg, 0.08 mmol) and Pd/C (8.5 mg, 0.08 mmol) was stirred in methanol under hydrogen atmosphere for 3 h. After the reaction was over, the impurities were removed by filtration, and the filtrate was steamed in vacuum to obtain **L** (33.7 mg, yield: 95.7%).^{1}H NMR (DMSO-d_6, 400 MHz): 7.31 (4 H, d, H^1), 6.87 (2 H, s, H^2), 6.58 (4 H, d, H^3), 5.12 (4 H, s, H^4), 3.88 (2 H, dd, H^5), 3.82 (2 H, dd, H^6), 3.71 (2 H, m, H^7), 3.42 (4 H, m, H^8).

3.2 Synthesis of FM

A mixture of **L** (19 mg, 0.04 mmol), pyridine-2-formaldehyde (8.5 mg, 0.08 mmol), $FeSO_4 \cdot 7H_2O$ (17 mg, 0.06 mmol) were dissolved in D_2O (5 mL) and the mixture was stirred at R.T. for 12 h. ^1H NMR (D_2O, 400 MHz): 8.88 (12H, s, H^1), 8.43 (12H, d, H^2), 8.24 (12H, dd, H^3), 7.56 (12H, dd, H^4), 7.27 (12H, d, H^5), 7.04 (24H, s, H^6), 6.80 (12H, s, H^7), 5.41 (24H, s, H^8), 3.80 (12H, s, H^9), 3.63 (24H, s, H^{10}), 3.34 (24H, s, H^{11}).

3.3 Synthesis of FM@OXA-PEG

FM@OXA-PEG was synthesized through hydrophilic and hydrophobic interaction with PEG-COOH 2000 and oxaliplatin. PEG-COOH 2000 (30 mg) and oxaliplatin aqueous solution (100 µL, 1 mg/mL) was added dropwise into FM solution (1 mM, 100 mL) successively under ultrasonic condition. The free components were removed by centrifugation with an ultrafiltration tube (MWCO 8000) and the upper purple solution FM@OXA-PEG was concentrated for later use.

4. Figures and table.

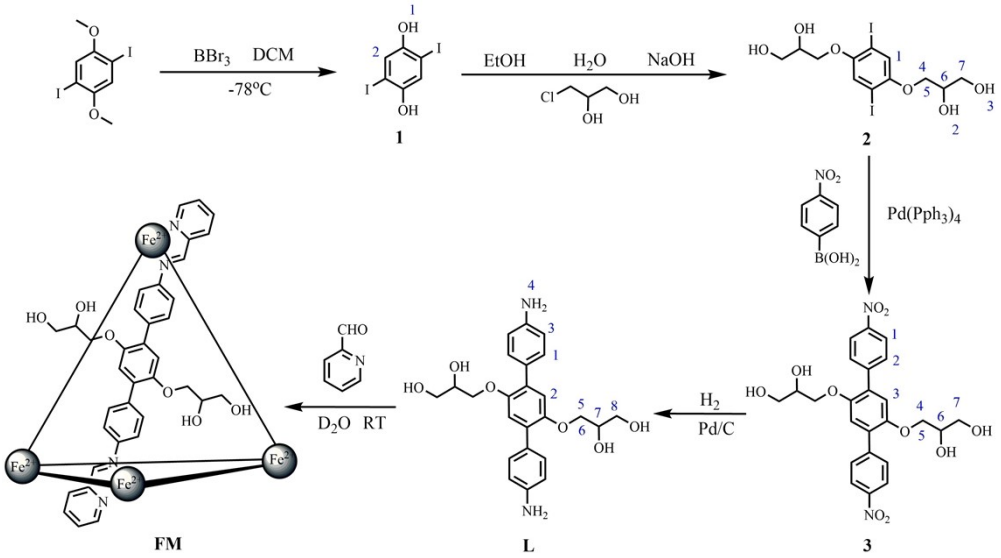

Fig. S1. Synthetic route of ligand and FM.

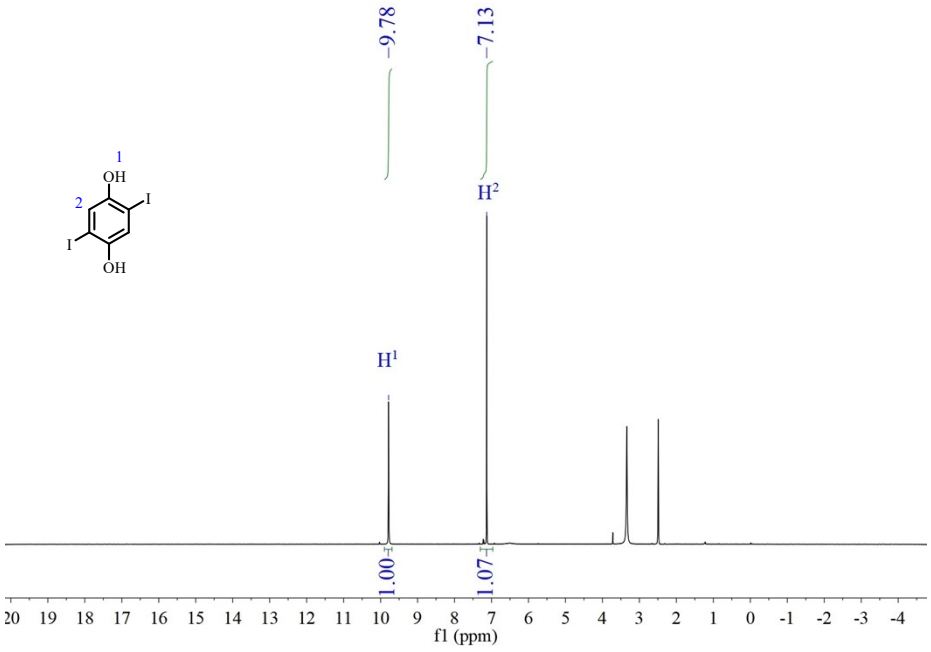

Fig. S2. ^1H NMR spectrum of **1** in DMSO-d_6.

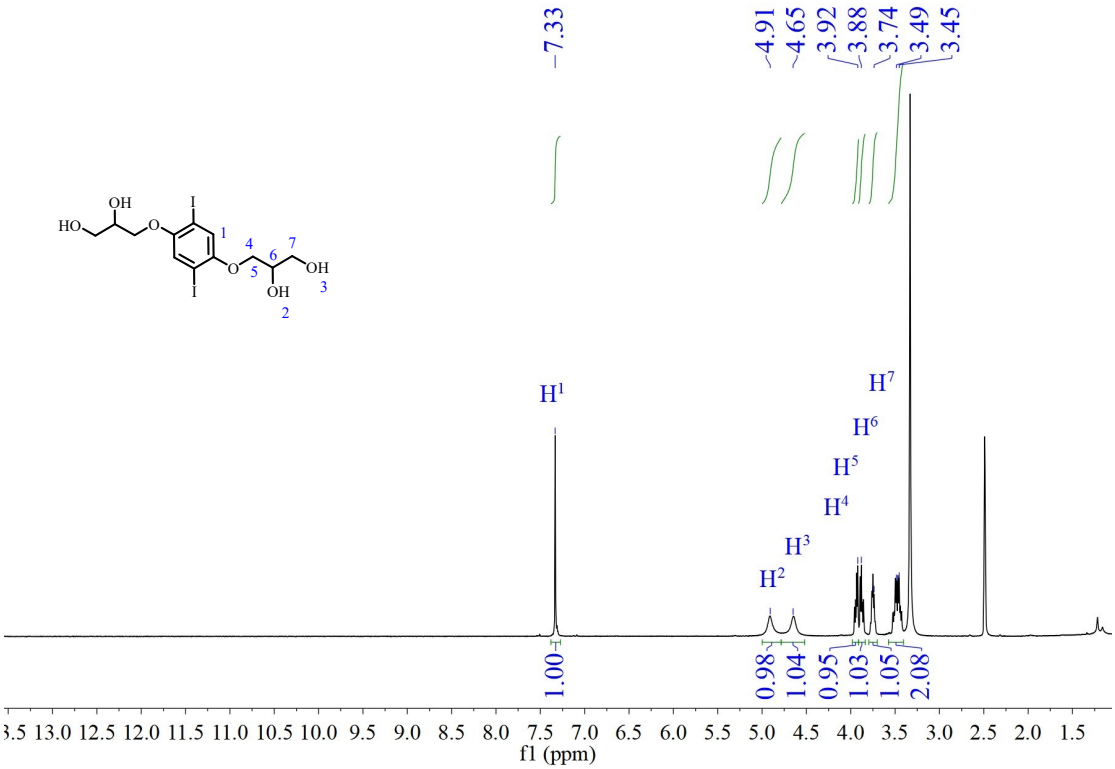

Fig. S3. ^{1}H NMR spectrum of **2** in DMSO-d_6.

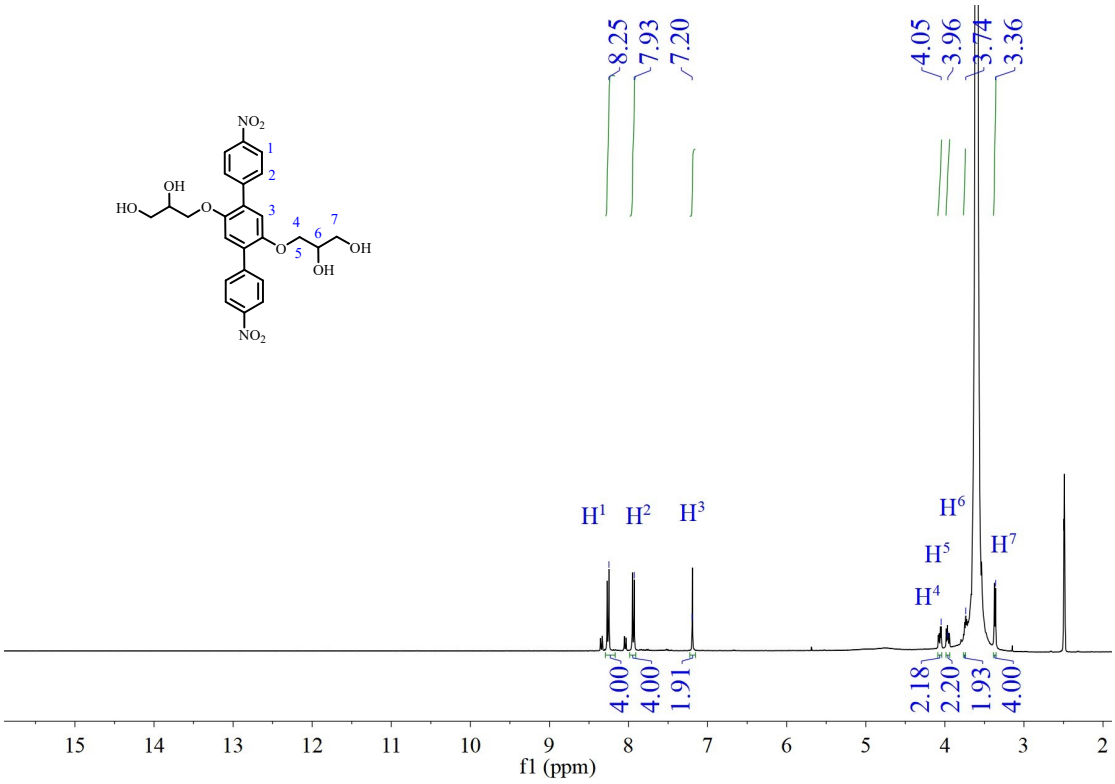

Fig. S4. ^{1}H NMR spectrum of **3** in DMSO-d_6.

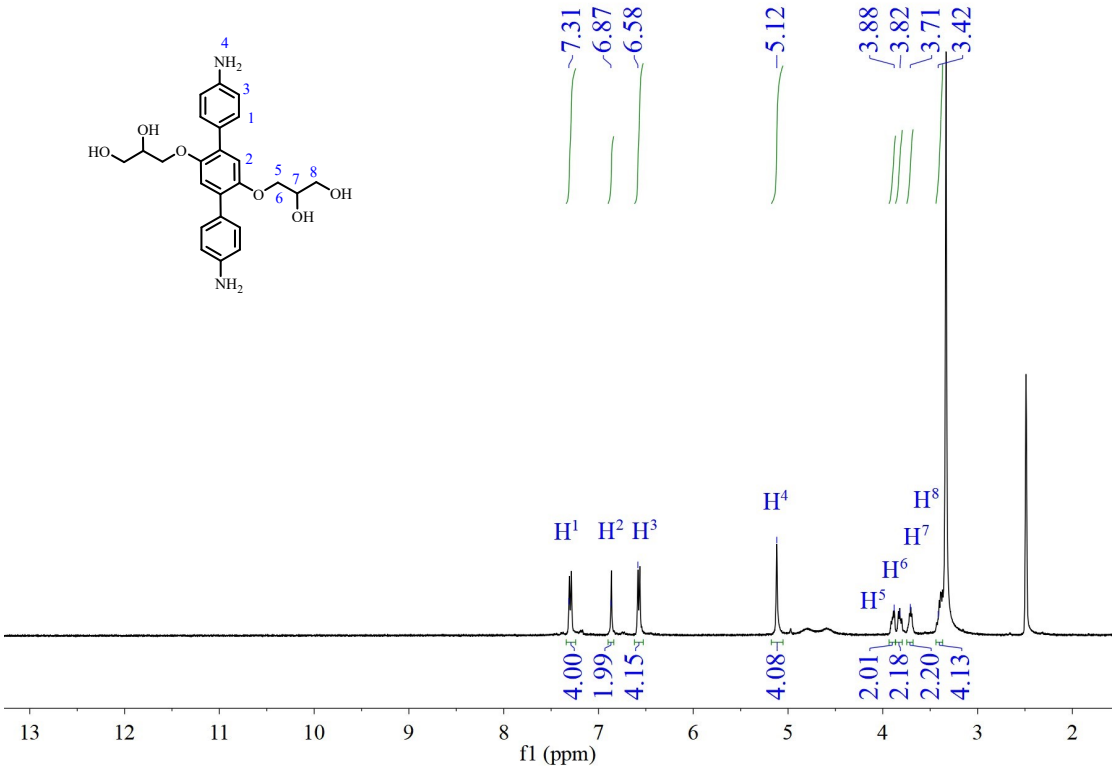

Fig. S5. ^{1}H NMR spectrum of **L** in DMSO-d_6.

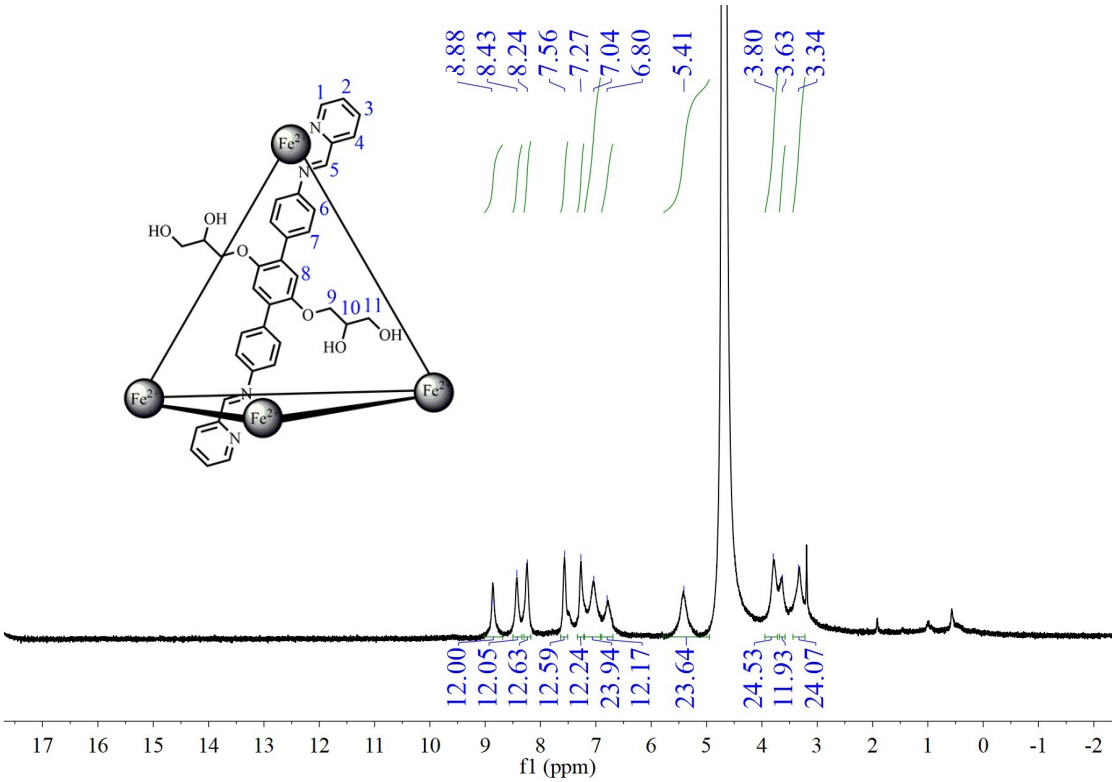

Fig. S6. ^{1}H NMR spectrum of FM in D_2O.

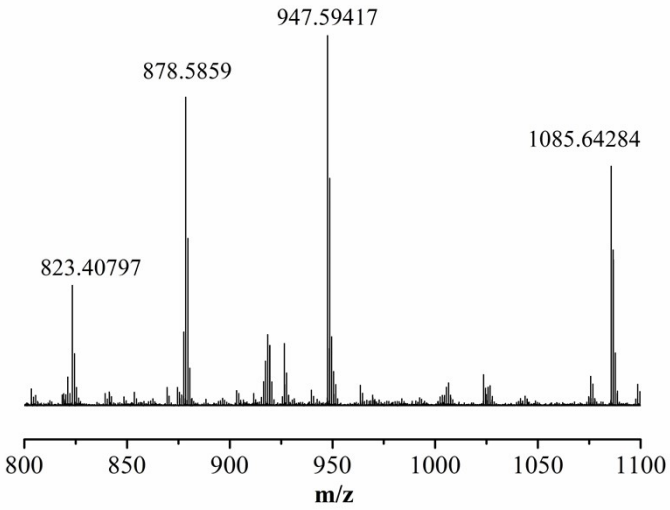

Fig. S7. Mass spectrum of FM.

Table S1 Mass fragment assignment for FM.

m/z	fragment
823.41	$[Fe_4L_6SO_4{\cdot}5H_2O\text{-}H^+]^{5+}$
878.59	$[Fe_4L_6SO_4{\cdot}20H_2O\text{-}H^+]^{5+}$
947.59	$[Fe_4L_6SO_4{\cdot}39H_2O\text{-}H^+]^{5+}$
1085.64	$[Fe_4L_6(SO_4)_2{\cdot}12H_2O]^{4+}$

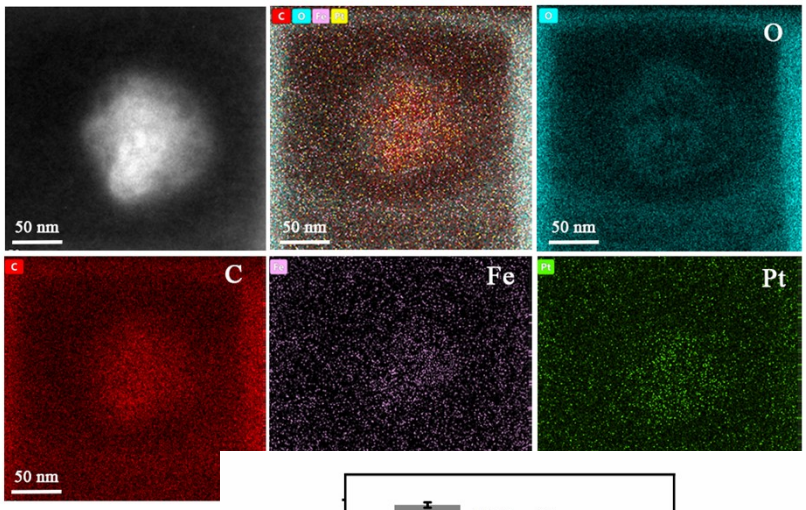

Fig. S8. EDS mapping of FM@OXA-PEG.

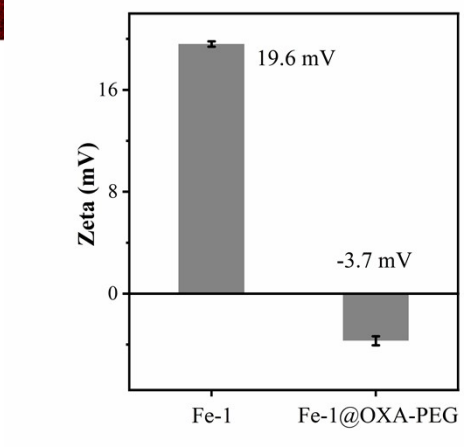

Fig. S9. The zeta potentials of FM and FM@OXA-PEG.

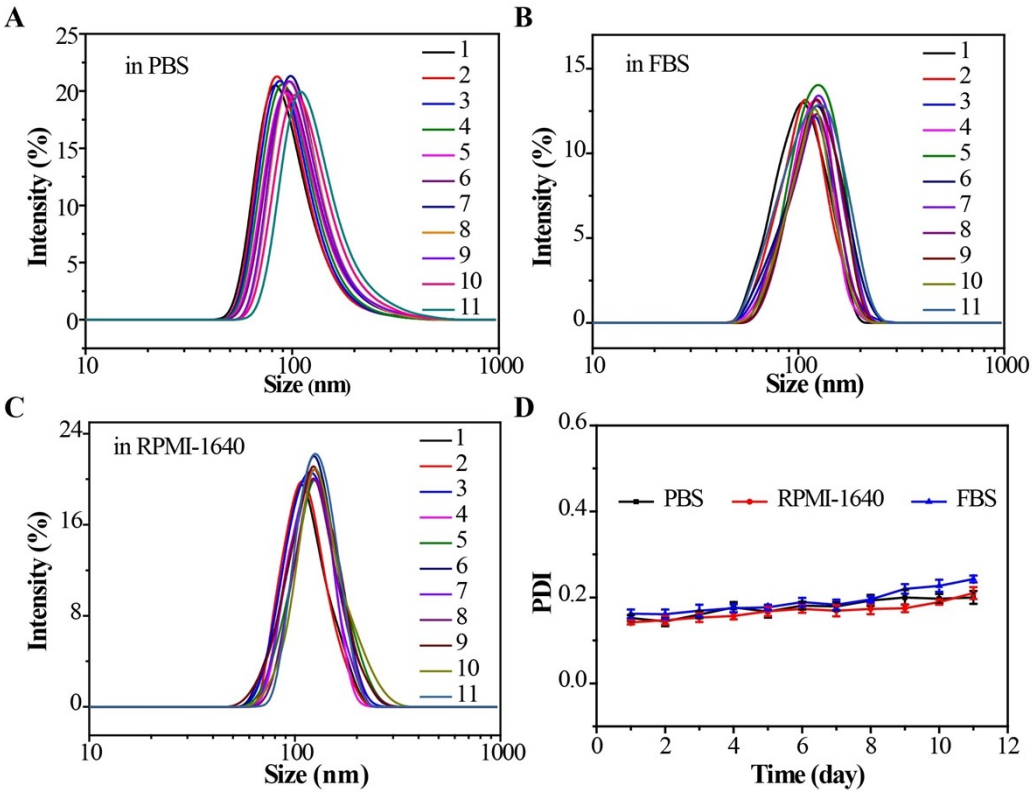

Fig. S10. The stability of FM@OXA-PEG in PBS (A), FBS (B) and RPMI-1640 culture medium (C) determined by DLS distributions and PDI changes (D) during 11 days.

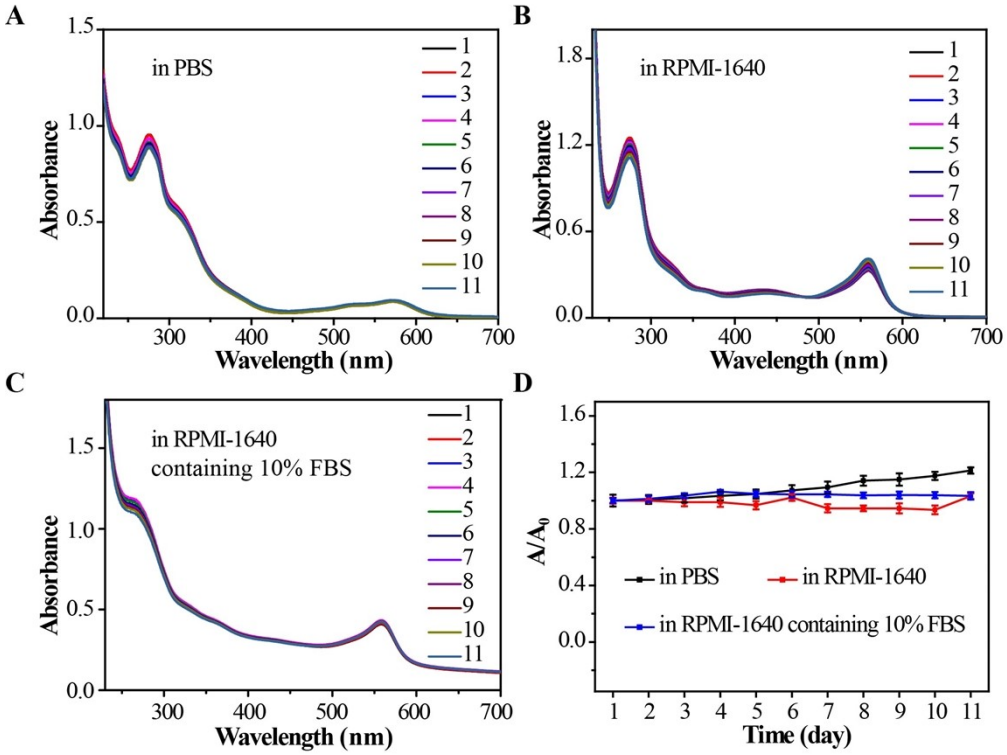

Fig. S11. UV-vis spectra of FM@OXA-PEG in PBS (A), RPMI-1640 (B) and RPMI-1640 containing 10% FBS culture medium (C) during 11 days. UV-vis absorbance ratio (A/A_0 at 572 nm) in different conditions (D), A_0 is initial adsorption, A is adsorption at different time.

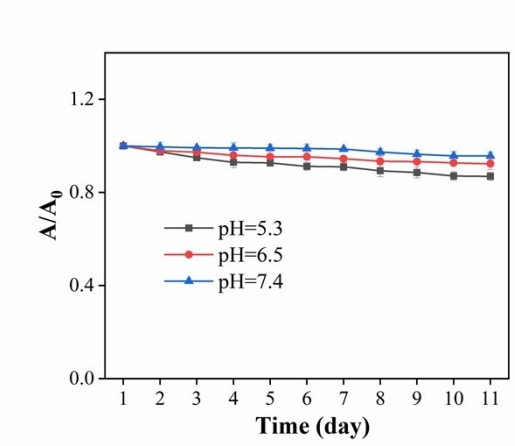

Fig. S12. Stability of FM@OXA-PEG at different pH (5.3, 6.5, 7.4) during 11 days by measuring UV-vis absorbance ratio A/A_0 at 572 nm.

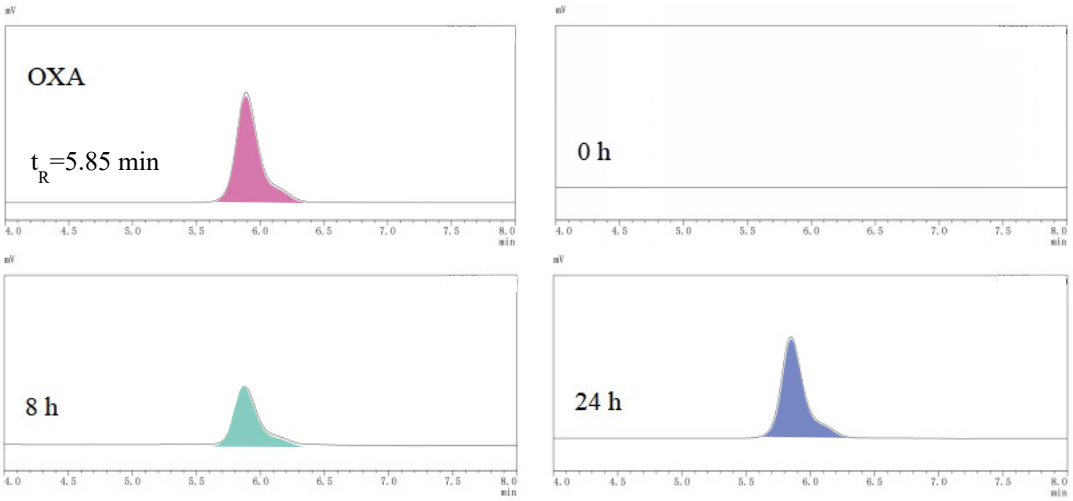

Fig. S13. Drug release: HPLC determination of OXA at different time (0, 8 and 24 h).

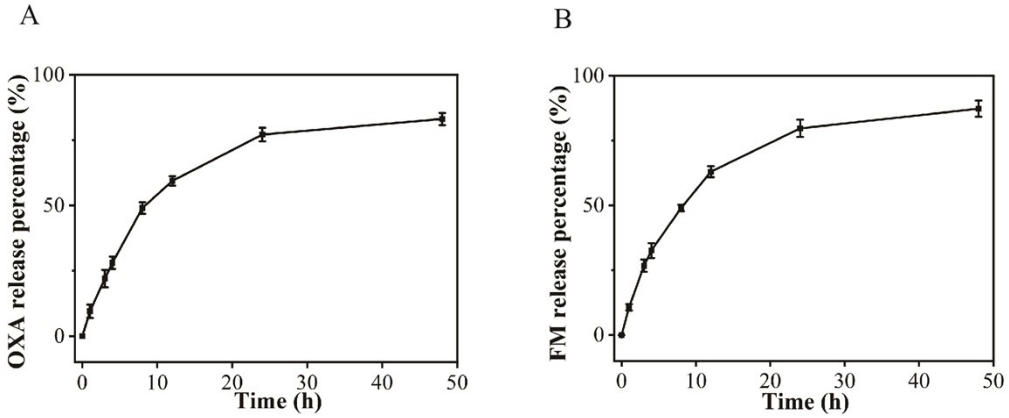

Fig. S14. (A) Release curve of OXA determined by HPLC. (B) Release curve of FM determined by UV-vis spectra.

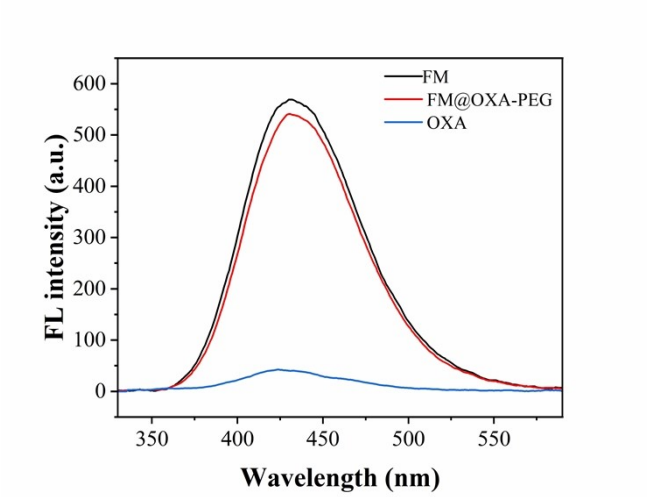

Fig. S15. Fluorescence intensity of TPA for FM, FM@OXA-PEG, and OXA groups.

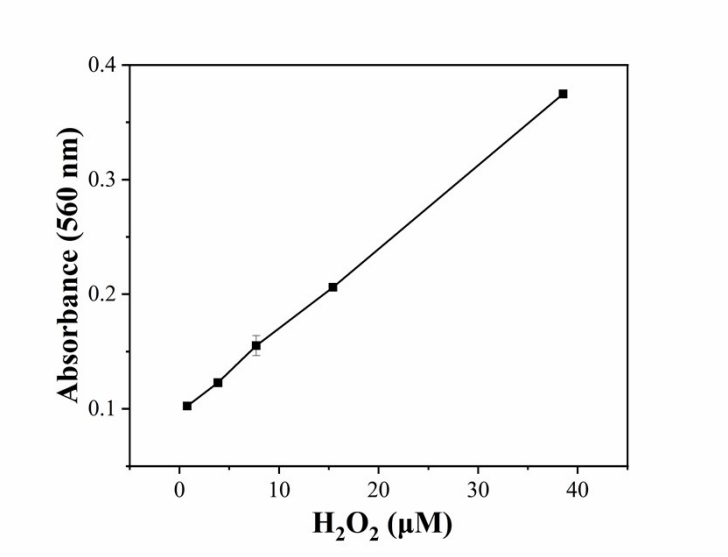

Fig. S16. Standard curve of H_2O_2 used to determine intracellular H_2O_2 concentration.

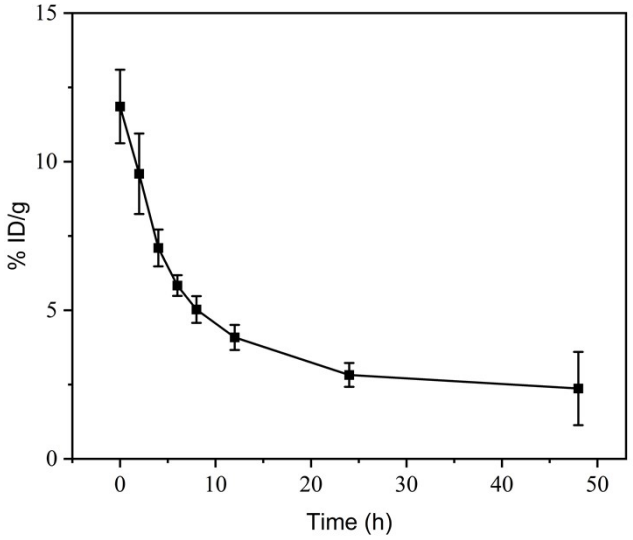

Fig. S17. The metabolism of iron ions within 48 h after intravenous injection in mice. Data are presented as means ± SD (n = 3)

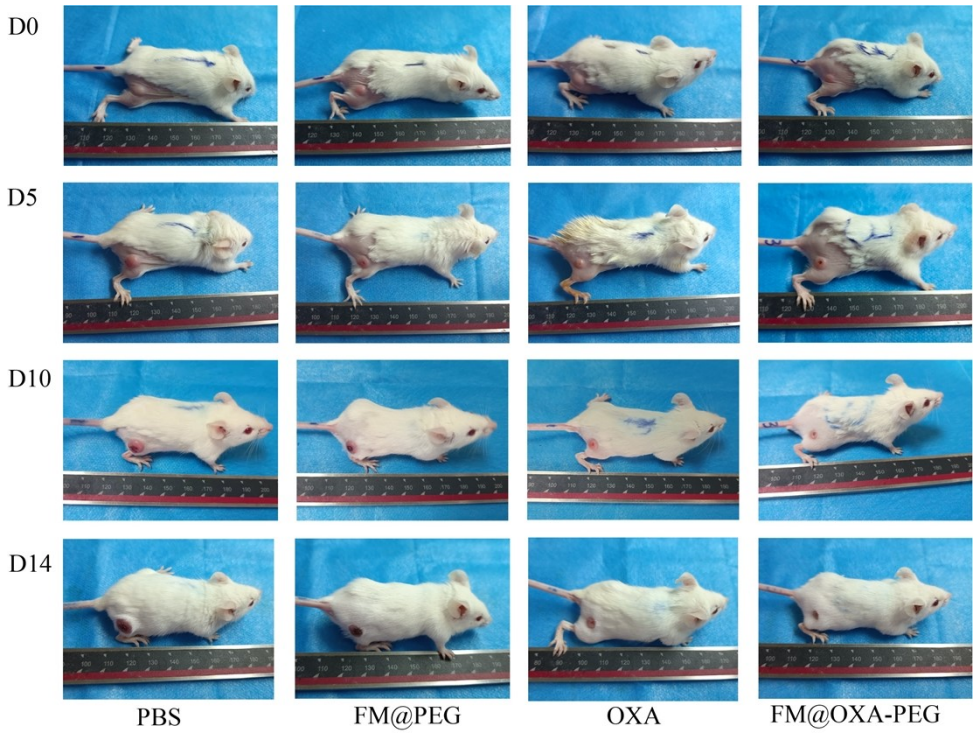

Fig. S18. Photographs of tumor-bearing mice on days 0, 5, 10 and 14 days of during follow-up treatment.

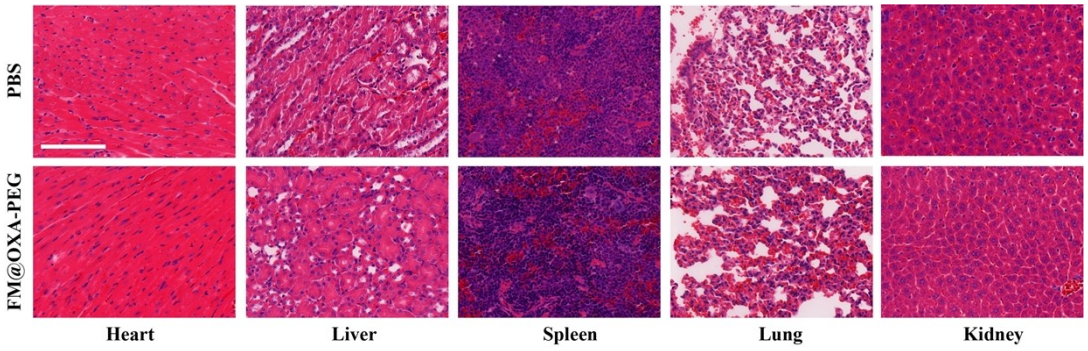

Fig. S19. H&E staining of mice heart, liver, spleen, lung, and kidney after treatment by PBS and FM@OXA-PEG. Scale bar: 100 μm.