Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

A Triphenylphosphine Coordinated Cu(I) Fenton-like Agent with Ferrocene Moieties for Enhanced Chemodynamic Therapy

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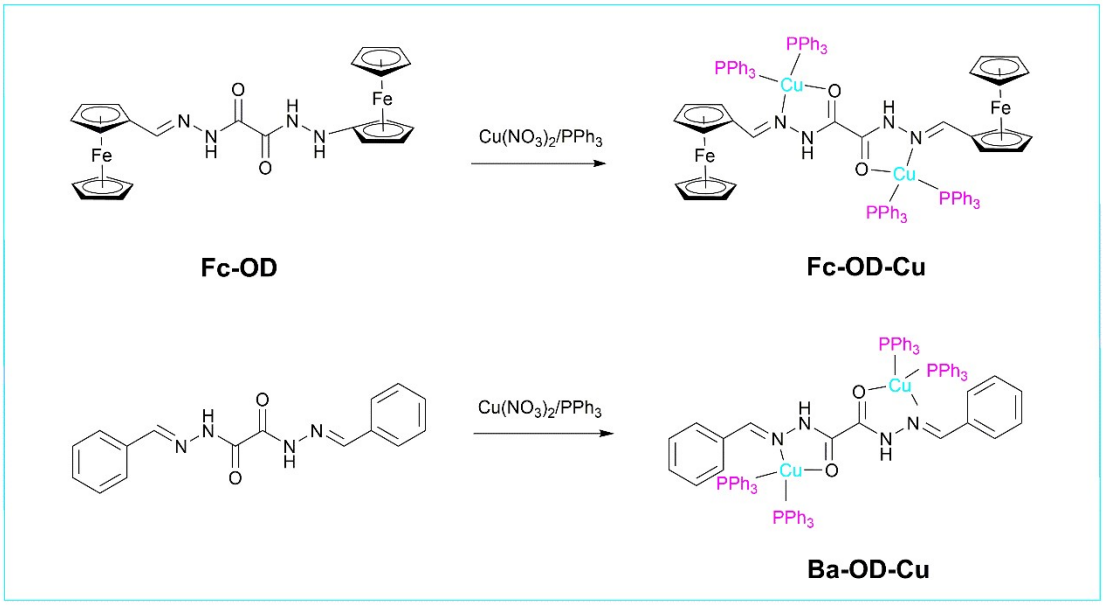

Scheme S1. The synthesis procedure of **Fc-OD-Cu**, **Ba-OD-Cu** and **Fc-OD**.

Table S2. Crystal data and structure refinement of **Fc-OD-Cu** and **Ba-OD-Cu**.

Complex	**Fc-OD-Cu**	**Ba-OD-Cu**
Empirical formula	$C_{96}H_{82}Cu_2Fe_2N_6O_8P_4$	$C_{90}H_{82}Cu_2N_6O_{10}P_4$
Formula weight	1810.33	1658.57
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/c$	P-1
a (Å)	13.7163 (2)	11.7360 (2)
b (Å)	20.0068 (3)	12.9128 (3)
c (Å)	16.7064 (2)	15.1245 (2)
α (°)	90.00	80.472 (1)
β (°)	109.234 (1)	82.941 (1)
γ (°)	90.00	65.549 (2)
Volume (Å^3)	4328.65 (11)	2053.92 (7)
Z	4	1
D_{calc} (g cm^{-3})	1.389	1.341
F(000)	1868	862
Reflections collected	38992	26739
Independent reflections	8544	8091
R_{int}	0.039	0.050
Goodness-of-fit	1.04	1.12
on F^2		
R_1, wR_2	0.0474, 0.1229	0.0491, 0.1430
[$I >= 2\sigma(I)$]		

R_1, wR_2[all data]	0.0571, 0.1286	0.0574, 0.1539

Table S3. Selected bond lengths (Å) and angles (°) for **Fc-OD-Cu**

Fc-OD-Cu			
Cu1—P1	2.2923 (8)	Cu1—O1	2.2194 (18)
Cu1—P2	2.2331 (7)	Cu1—N1	2.076 (2)
P2—Cu1—P1	124.04 (3)	N1—Cu1—P1	110.04 (7)
O1—Cu1—P1	91.32 (6)	N1—Cu1—P2	122.94 (7)
O1—Cu1—P2	115.61 (5)	N1—Cu1—O1	76.99 (8)

Table S4. Selected bond lengths (Å) and angles (°) for **Ba-OD-Cu.**

Ba-OD-Cu			
Cu1—P1	2.2949 (7)	Cu1—O1	2.2250 (15)
Cu1—P2	2.2450 (6)	Cu1—N1	2.1225 (19)
P2—Cu1—P1	119.56 (2)	N1—Cu1—P1	112.86 (6)
O1—Cu1—P1	93.10 (5)	N1—Cu1—P2	123.94 (6)
O1—Cu1—P2	117.38 (5)	N1—Cu1—O1	76.46 (7)

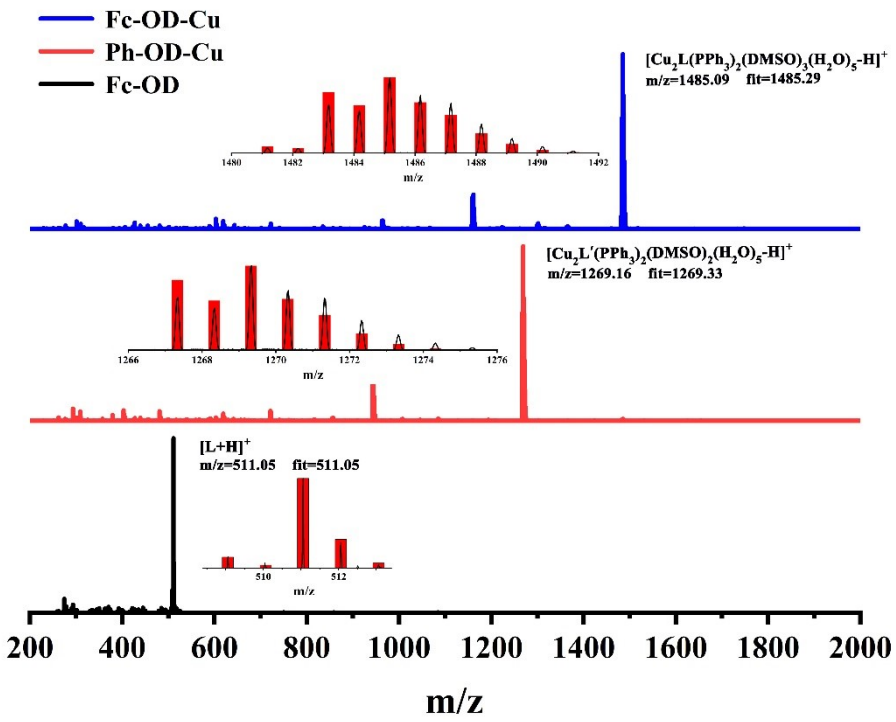

Fig. S1. Single crystal mass spectra and cation fragment fit diagrams of **Fc-OD-Cu**, **Ba-OD-Cu** and **Fc-OD** in DMF.

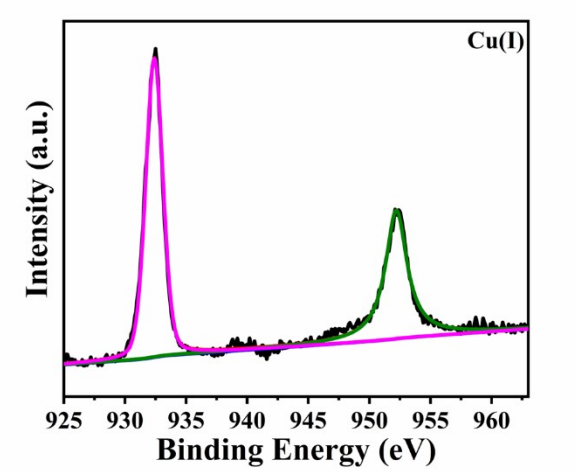

Fig. S2. XPS patterns of **Ba-OD-Cu.**

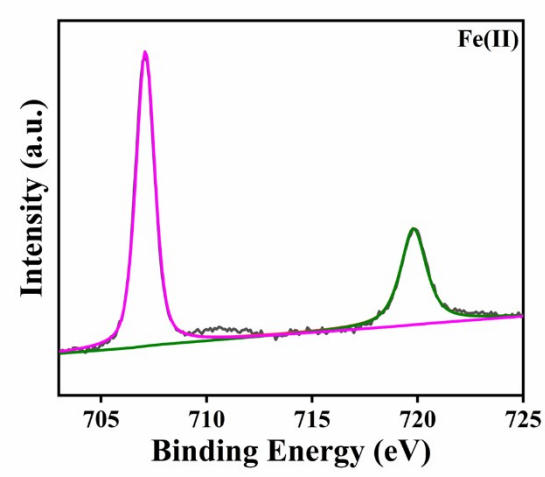

Fig. S3. XPS patterns of **Fc-OD.**

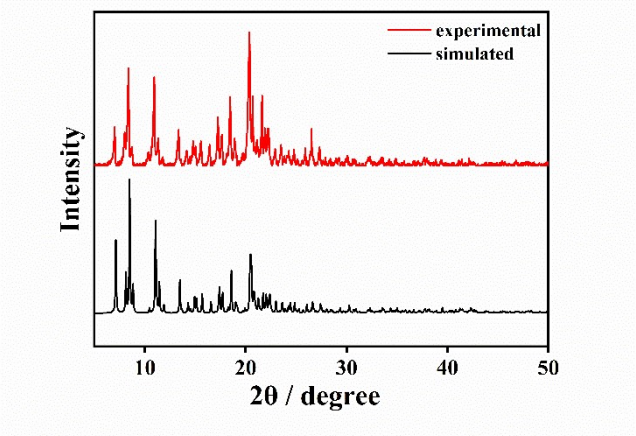

Fig. S4. XRD spectra of **Fc-OD-Cu.**

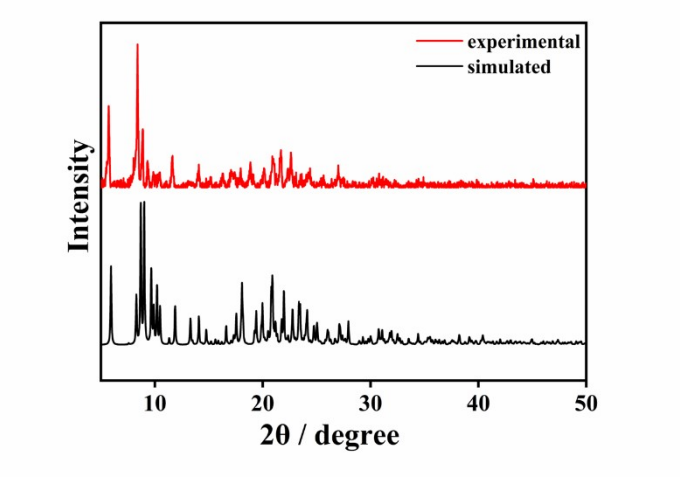

Fig. S5. XRD spectra of **Ba-OD-Cu.**

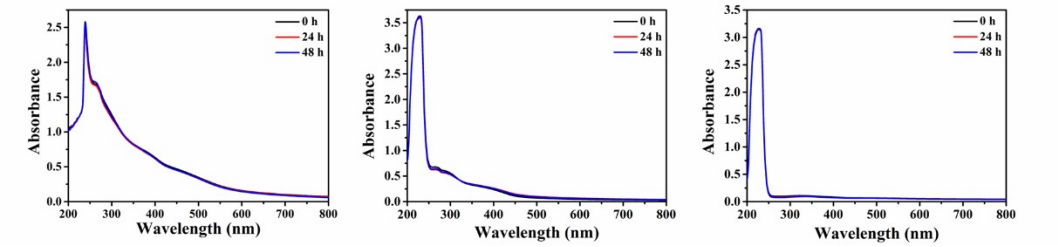

Fig. S6. Stability analysis of **Fc-OD-Cu**, **Ba-OD-Cu** and **Fc-OD** in Tris-HCl buffer (0.05 M, pH=7.4) at different times. (From left to right are **Fc-OD-Cu**, **Ba-OD-Cu** and **Fc-OD**).

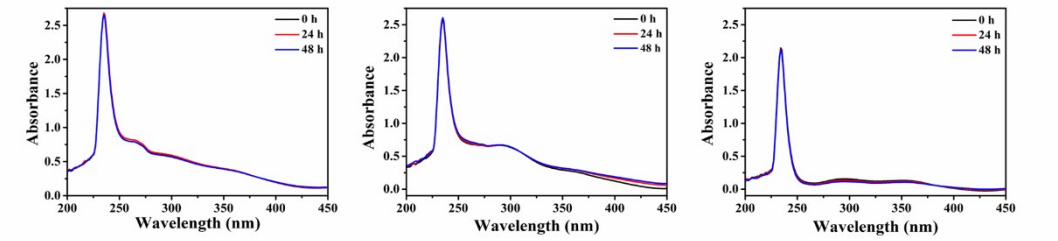

Fig. S7. Stability analysis of **Fc-OD-Cu**, **Ba-OD-Cu** and **Fc-OD** in cell culture medium (Dulbecco's modified Eagle's medium, DMEM) at different times. (From left to right are **Fc-OD-Cu**, **Ba-OD-Cu** and **Fc-OD**).

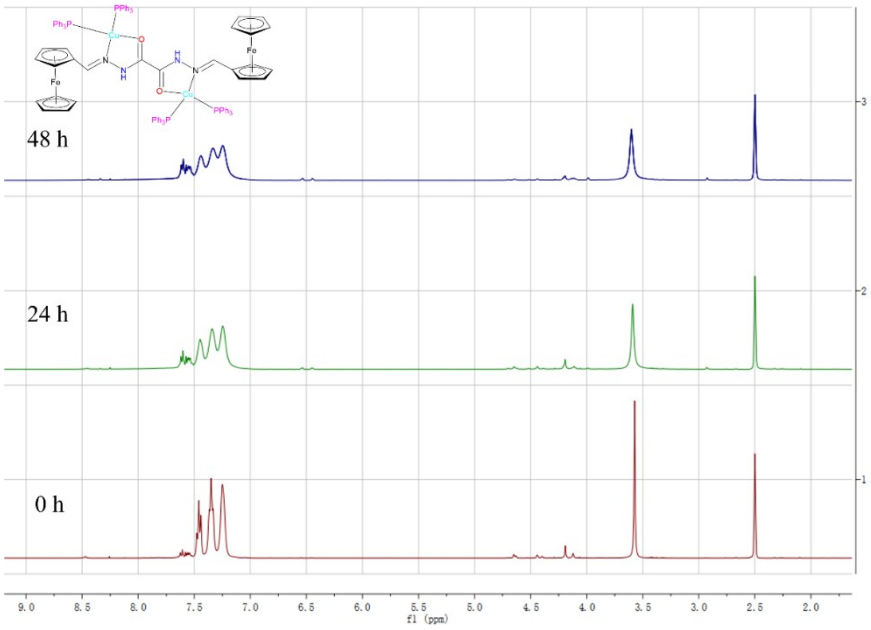

Fig. S8. ^{1}H NMR (400 MHz, DMSO-d_6) spectra of **Fc-OD-Cu** at different time.

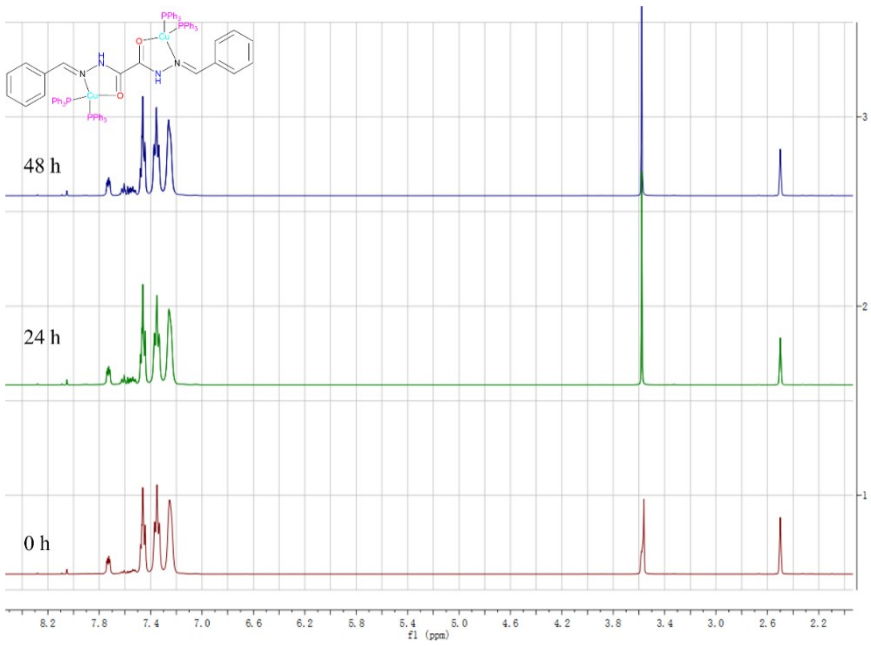

Fig. S9. ^{1}H NMR (400 MHz, DMSO-d_6) spectra of **Ba-OD-Cu** at different time.

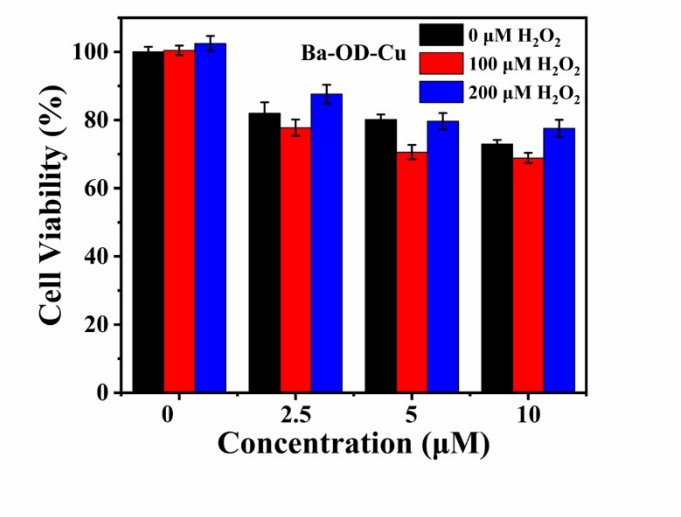

Fig. S10. Cell viability of Hep-G2 cells after incubation with **Ba-OD-Cu** in different concentrations of H_2O_2.

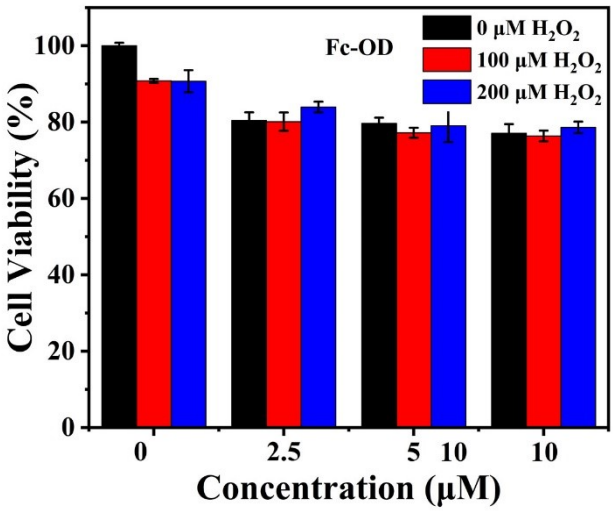

Fig. S11. Cell viability of Hep-G2 cells after incubation with **Fc-OD** in different concentrations of H_2O_2.

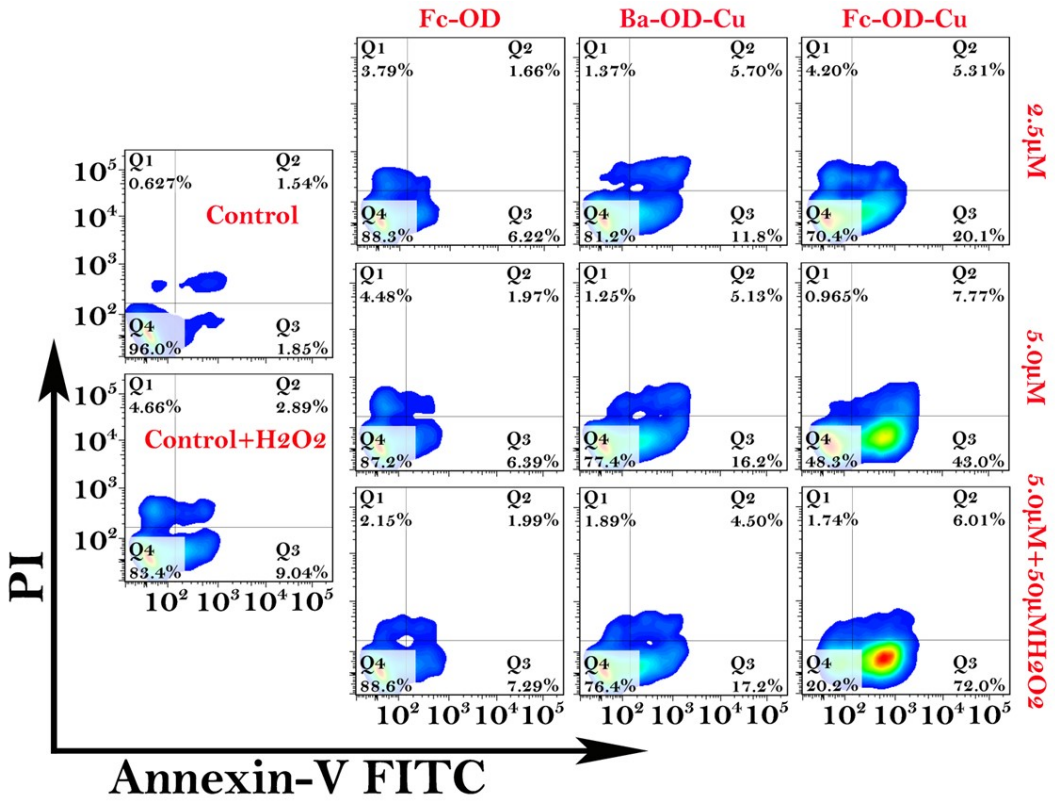

Fig. S12. Flow cytometry analysis of Hep-G2 cells apoptosis after treatment with **Fc-OD**/**Ba-OD-Cu**/**Fc-OD-Cu** at different concentration and **Fc-OD**/**Ba-OD-Cu**/**Fc-OD-Cu** in the presence of H_2O_2 (50 μM) for 48 h.

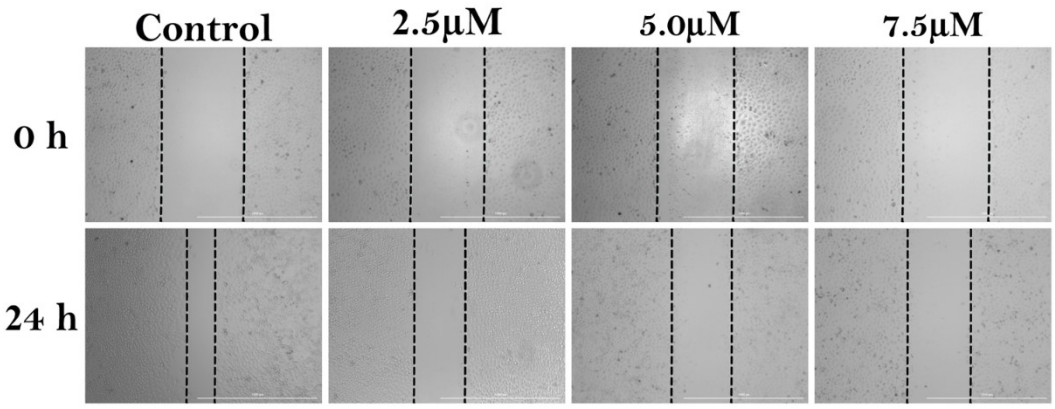

Fig. S13. Scratch assay for migration capability on Hep-G2 cells induced by different concentrations of **Fc-OD-Cu** for 24 h.

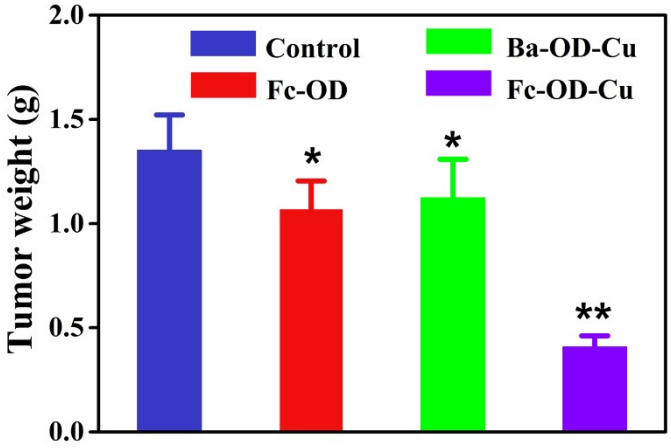

Fig. S14. Tumor weight change curves. *p < 0.05, **p < 0.01 determined by Student's t-test.

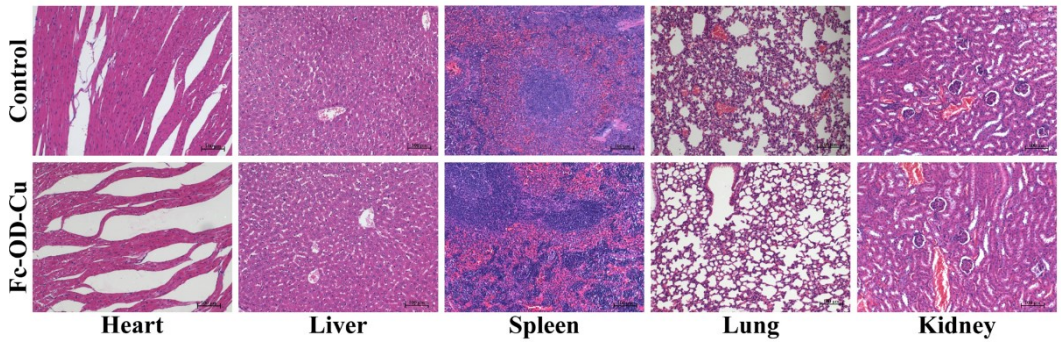

Fig. S15. Biosafety evaluation by H&E staining of major organs 15 days after treatments; scale bars: 100 μm.

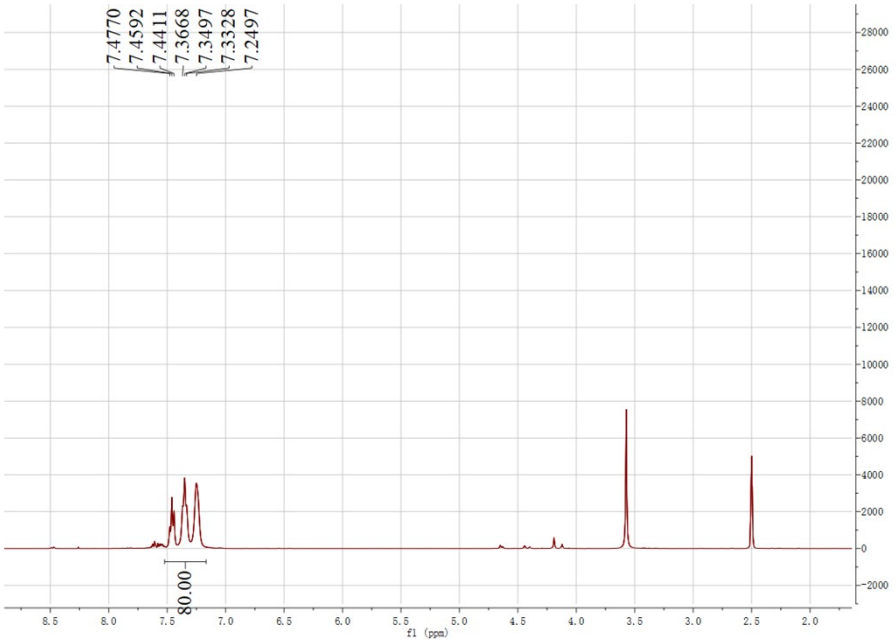

Fig. S16. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **Fc-OD-Cu**.

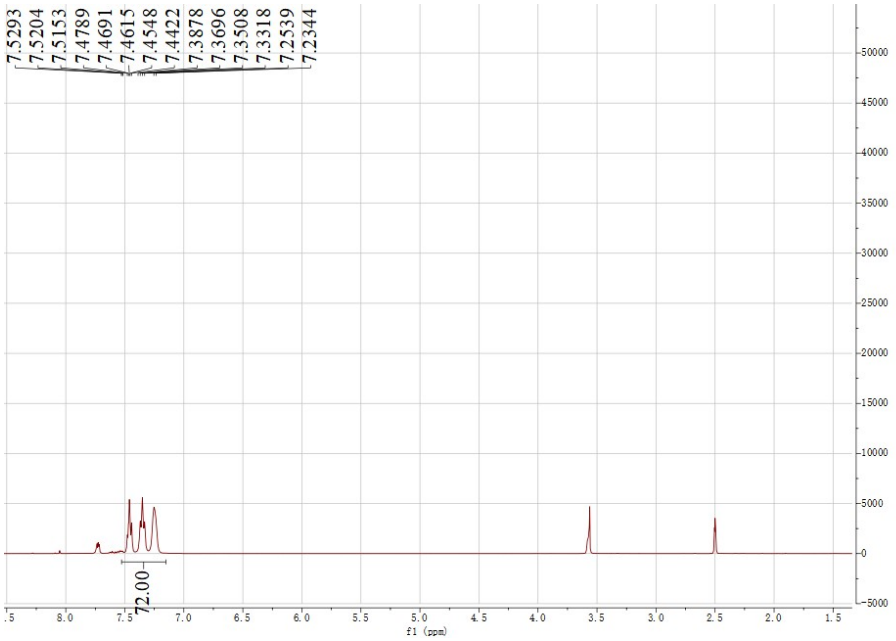

Fig. S17. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **Ph-OD-Cu**.

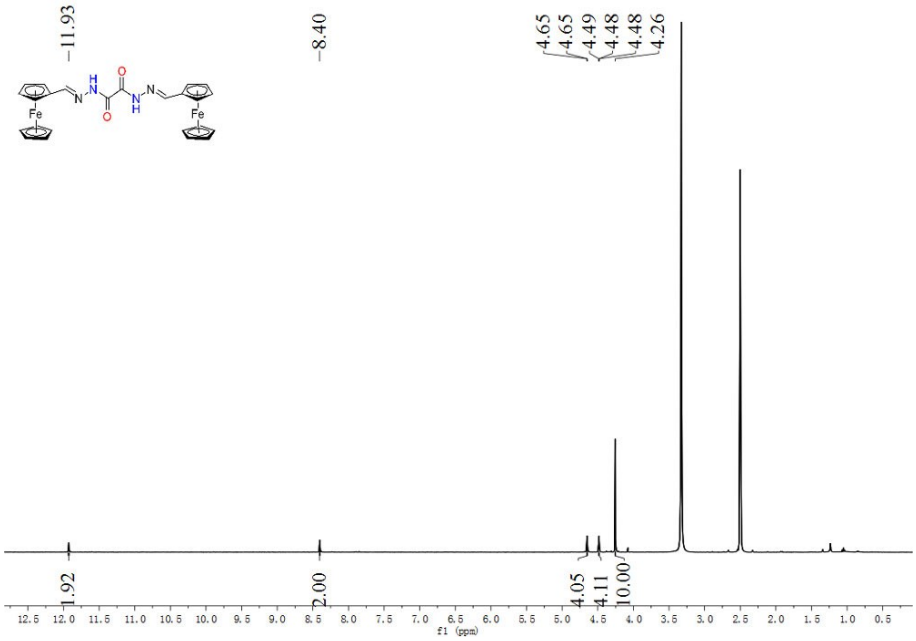

Fig. S18. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **Fc-OD**.

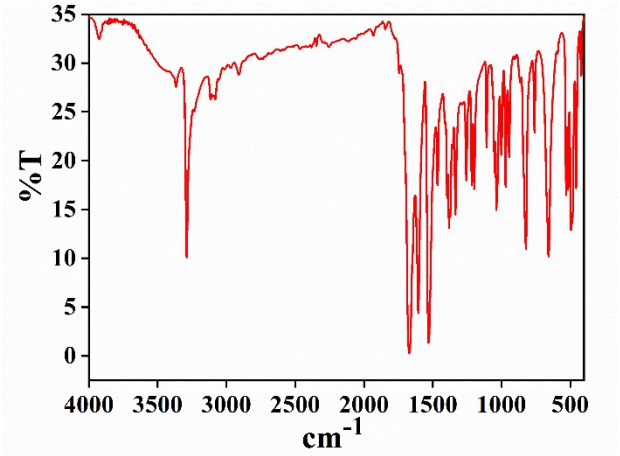

Fig. S19. FTIR spectrum of **Fc-OD**.

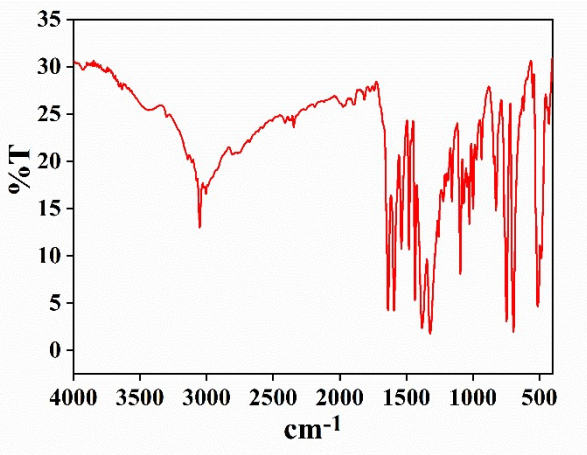

Fig. S20. FTIR spectrum of **Fc-OD-Cu**.

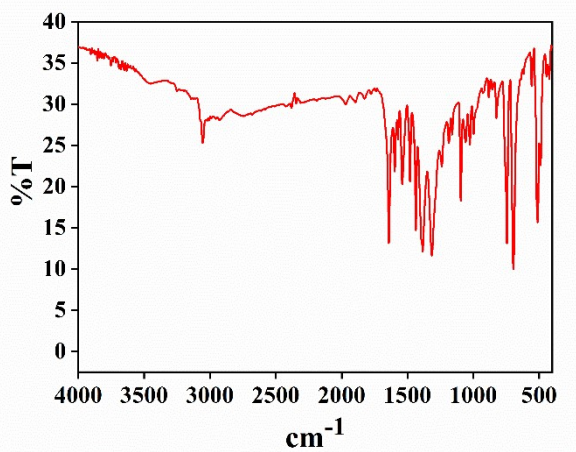

Fig. S21. FTIR spectrum of **Ba-OD-Cu**.

Experimental Section

Materials

All of the chemicals and reagents employed in this work were used without further purification. $CuNO_3 \cdot 3H_2O$, triphenylphosphine (TPP), oxalic dihydrazide and methylene blue (MB) were purchased from Bide Pharmaceutical Technology Co. Ltd. (Shanghai, China). Ferrocenecarboxaldehyde, Benzaldehyde and 5,5-dimethyl-1-pyrrolidine -N-oxide (DMPO) were purchased from Aladdin Holdings Group Co., Ltd. (Shanghai, China). Annexin V-FITC/propidium iodide, Calcein-AM/propidium iodide (CA/PI) staining agents

and 2′,7′-dichlorodihydrofluoresceinn diacetate (DCFH-DA) assay kit were purchased from Beyotime Biological Technology Co., Ltd. (Shanghai, China). Hep-G2 cells were obtained directly from the cell bank of Shanghai Institute of Life Sciences (China) and cultured in Roswell Park Memorial Institute (RPMI) DMEM Medium which containing 10 % FBS (Gibco) plus 1 % penicillin.

Instruments

Single crystal X-ray diffraction measurements were carried out using an XtaLAB Synergy-DS (Cu) X-ray Source (λ = 1.54184 Å). Absorbance was recorded on UV−Vis spectrophotometer (UV-2600i, Shmadzu). Powder X-ray diffraction was measured on a Rigaku D/max diffractometer equipped (Cu-Kα, λ = 1.54056 Å). XPS spectra were measured by Electron spectrometer ESCALAB 250Xi. EPR analysis was conducted on A300 equipment (Bruker, GER). High resolution mass spectrometric data were measured on a Q Exactive mass spectrometric (thermo scientific).

Synthesis of ligand Fc-OD

Ferrocenecarboxaldehyde (2.0 mmol, 0.428 g), oxalyldihydrazide (1.0 mmol, 0.118 g) were added in ethanol (50 mL). The mixture was refluxed in an oil bath at 85 °C and stirred for 2 hours. The precipitation was filtered, washed using ethanol, dried under vacuum overnight to obtain Fc-OD. Yield: 60 %. HRMS (ESI): m/z calculated for $[Fc\text{-}OD]^+$: 511.05; found 511.05. NMR: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 2H), 8.40 (s, 2H), 4.65 (d, J = 1.8 Hz, 4H), 4.49-4.48 (m, 4H), 4.26 (s, 10H). IR (KBr ν/cm^{-1}): 3289 m, 1670 w, 1605 w, 1529 w, 1381 vs, 1036 s, 970 s, 824 m, 656 vs, 493 m.

Synthesis of ligand Fc-OD-Cu

For **Fc-OD-Cu**, a mixture of **Fc-OD** (1.0 mmol, 0.51 g) and PPh_3 (5.0 mmol, 1.31g) in 5 mL methanol was added to an aqueous solution of 5 mL $Cu(NO_3)_2{\cdot}3H_2O$ (2.0 mmol, 0.38 g) with stirring at room temperature for 30 min, then, the solution was cooled and filtered. Several days later, red block crystals of **Fc-OD-Cu** suitable for X-ray structure analysis were crystallized from the solution after slow evaporation at room temperature, and collected by filtration, washed with ethanol and dried in air. Yield: 80%. Elemental analysis data: calculated (%) for $C_{96}H_{82}Cu_2Fe_2N_6O_8P_4$ (1810.33): C, 63.69; H, 4.75; N, 4.64. Found (%): C, 63.46; H, 4.51; N, 4.40. HRMS (ESI): m/z calculated for $[Cu_2(PPh_3)_2(Fc\text{-}OD)]^+$: 1485.09; found 1485.29. NMR: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35 (overlapped, 80H). IR (KBr ν/cm^{-1}): 3051 s, 1637 w, 1591 w, 1536 m, 1480 m, 1435 w, 1379 w, 1320 w, 1094 w, 825 s, 747 w, 694 w, 514 w.

Synthesis of ligand Ba-OD-Cu

Benzaldehyde (2.0 mmol, 0.212 g), oxalyldihydrazide (1.0 mmol, 0.118 g) were added in ethanol (50 mL). The mixture was refluxed in an oil bath at 85°C and stirred for 2 hours. The precipitation was filtered, washed using ethanol, dried under vacuum overnight to obtain Ligand L'. Subsequent synthesis reference **Fc-OD-Cu**. A light-yellow crystal was obtained. Yield: 55 %. Elemental analysis data: calculated (%) for $C_{90}H_{82}Cu_2N_6O_{10}P_4$ (1658.57): C, 68.85; H, 5.72; N, 4.27. Found (%): C, 68.64; H, 5.48; N, 4.02. HRMS (ESI): m/z calculated for $[Cu_2(PPh_3)_2L']^+$: 1269.16; found 1269.33. NMR: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (overlapped, 72H). IR (KBr ν/cm^{-1}): 3055 s, 1641 m, 1596 s, 1539 s, 1436 m, 1385 m, 1313 m, 1095 m, 743 m, 695 w, 510 m.

Crystallographic analysis

Single-crystal data of **Fc-OD-Cu** and **Ba-OD-Cu** were collected on a Rigaku SuperNova diffractometer equipped with Cu Kα radiation (1.54178 Å) at 100 K. The structure was solved by direct methods and refined with the full matrix least squares methods on F^2 with the SHELXT-2015[1] and SHELXL-2018[2] programs. All non-hydrogen atoms were refined with anisotropic displacement coefficients, and all hydrogen atoms were generated geometrically. The detailed crystallization data and structural refinement parameters are summarized in table S2. The selected bond lengths and angles of **Fc-OD-Cu** and **Ba-OD-Cu** were provided in tables S3 and S4. The crystal data of structural analysis have been saved in the Cambridge crystal data center (CCDC reference number of **Fc-OD-Cu**: 2205234, CCDC reference number of **Ba-OD-Cu**: 2205233).

Detection of extracellular ROS

Hydroxyl radicals were detected firstly through the UV-Vis absorption spectra of methylene blue (MB) at 665 nm. A certain concentration of MB solution was prepared in 0.05 M Tris/HCl buffer solution with 10 mM H_2O_2 and 2 mM **Fc-OD-Cu**. The mixing system was incubated at 37 °C, and then the absorbance of MB was measured by UV-vis spectroscopy. Additionally, the radical scavenger 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) was used to detect the production of •OH radicals in vitro with EPR spectroscopy. Take 5 μL

complex (2 mM) and mix of 90 μL ultrapure water and add DMPO (5 μL), then the mixture was transferred to a quartz capillary and measured on a Brooke A300 spectrometer.

Cytotoxicity of Fc-OD-Cu by MTT Assay

Hep-G2 cells were seeded in 96-well plates (1×10^4 cells per well) in 5 % CO_2 at 37 °C atmosphere for 24 h in dark, then, cells wereper-incubated with or without H_2O_2 (100 mM) for 2 h. After discarding the H_2O_2-containing medium, fresh cell culture medium containing different concentrations of **Fc-OD-Cu** (0, 2.5, 5.0, 10 and 20 μM) was added to each well for 48 h incubation. Lastly, 20 μL of standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added into each well and further incubated for 4 h. Afterwards, discard the medium and 100 μL DMSO was added into every well to dissolve formed formazan crystals. Absorbance at 570 nm of each well was measured on an enzyme-labeling instrument and the data was recorded using SPSS software. Experiments were performed 3 times as a parallel test.

Intracellular ROS generation

Intracellular total ROS generation in Hep-G2 cells was detected using ROS assay Kit DCFH-DA. Briefly, the Hep-G2 cells were planted into confocal dishes at a density of 1×10^5 cells/dish and incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2 in dark. Afterwards, the cells were pre-cultured for another 30 min in the present or absent of fresh DMEM medium containing 50 μM H_2O_2. Then, the cells were treated with the same concentration of **Fc-OD-Cu**, **Fc-OD** and **Ba-OD-Cu**, with DMEM as the control, and then incubated for another 24 hours. Subsequently, the cells were washed once with serum-free DMEM medium. Finally, the level of intracellular ROS was detected by Two-photon Confocal Scanning Laser Microscope photon (Leica, Leica TCS SP8 DIVE, GER) after 20 min incubation with DCFH-DA. For flow cytometry analysis, after incubated with DCFH-DA, the cells were washed with PBS and harvested by centrifugation and subsequently analyzed by FACS Aria II flow cytometry (BD Biosciences, San Jose, USA BD FACS).

Live/dead cytotoxicity assay

Cytotoxicity was detected by calcein AM and propidium iodide (PI) dual-color fluorescence staining. Hep-G2 cells were seeded in a cell culture dish and cultured overnight. The cells were treated with **Fc-OD-Cu** at different concentrations for 48 h. Then AM and PI were added to the cell culture dish according to the manufacturer's instruction manual. After multiple rinses with PBS, the dead and living cells were detected on Cell Imaging Multi-Mode Reader System (BioTek, citation 5, USA).

In vitro cell apoptosis study

The cell apoptosis assay was performed using Annexin V/PI Apoptosis Detection Kit. Hep-G2 cells were seeded in a six-well plate at a density of 2×10^5 and cultured overnight, and incubated for 30min in the presence or absence of H_2O_2 (50 μM). Then, the medium containing H_2O_2 was discarded and Hep-G2 cells were incubated in a medium containing different concentrations of (2.5 μM, 5.0 μM) **Fc-OD-Cu** for 48 hours. Cells incubated with fresh medium served as controls. Subsequently, cells were washed twice with PBS, the cells were washed twice with PBS, collected by trypsin solution and washed twice with ice-cold PBS. Finally, the gathered cells were stained with Annexin V-FITC/PI staining solution for 30 min under dark environment and then analyzed by flow cytometry. The data were processed and analyzed by FlowJo 7.6 software.

Animal Experiments

Animal experiments were consigned to Nanjing OG Science and Technology Service Co., Ltd. BALB/c nude mice (about 6 weeks aged) were purchased from Changzhou Cavens Laboratory Animal Co., Ltd. (Changzhou, China, approval No. SCXK 2016-0010). All animal experiment procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and all in vivo experiments were approved by Nanjing Lambda Pharmaceutical Co., Ltd. (Nanjing, China, Approval No. SYXK 2017-0017). Hep-G2 cells (5×10^6) were subcutaneous injected into the nude mice. When the tumor volume reached about 95 mm^3, the tumor-bearing mice were divided into four groups and each group was given saline (0.9 %), **Fc-Cu** (30 mg/kg), **Ba-OD-Cu** (30 mg/kg) and **Fc-OD-Cu** (30 mg/kg) respectively every day. The tumor volume and body weight of each mouse were carefully measured every other day. The tumor volume was calculated using the formula $V = LW^2/2$, where L and W stand for the maximum and minimum diameter of the tumors, respectively. On day 15, all mice were sacrificed, and the tumors were harvested and the main organs were collected for terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) or hematoxylin and eosin (H&E) staining assay.

1. Sheldrick, G. M. SHELXT–Integrated space-group and crystal-structure determination. Acta Crystallogr., Sect. A: Found. Adv. 2015, A71, 3-8.
2. Sheldrick, G. M. Crystal structure refinement with SHELXL. Acta Crystallogr., Sect. C: Struct. Chem. 2015, C71, 3-8.