Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Stepwise formation of a chemodynamic therapy agent of {Cu_8} macrocyclic complex recognized by iodide ions

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Table S1. Crystal data and structure refinement of **Cu_8I**.

Identification code	**Cu_8I**
Empirical formula	$C_{40}H_{84}ClCu_8I_3N_{40}O_4S_8$
Formula weight (M)	2370.42
Crystal system	Tetragonal
Space group	*P4/n*
a (Å)	19.3778 (19)
b (Å)	19.3778 (19)
c (Å)	11.9577 (13)
α (°)	90
β (°)	90
γ (°)	90
V/(Å ^{3})	4490.1 (8)
Z	2
D_c(Mg m^{-3})	1.753
F(000)	2320
Reflections	21736/ 3977
collected / unique	R(int)=0.090
Goodness-of-fit on F^2	1.024
Final *R* indices $I > 2\sigma(I)$	R_1 = 0.0826 wR_2 = 0.2305
R indices (all data)	R_1 = 0.1155 wR_2 = 0.2474

Table S2. Selected bond length and bond angle for **Cu_8I**.

Cu_8I. (A) *y*, −*x*+1/2, *z*; (B) −*y*+1/2, *x*, *z*; (C) *y*−1/2, −*x*+1, −*z*+1; (D) −*y*+1, *x*+1/2, −*z*+1; (E) −*x*+1/2, −*y*+3/2, *z*.			
Cu1—N9B	1.970 (8)	Cu1—S1	2.270 (3)
Cu1—S2	2.279 (3)	Cu1—Cu2	2.7461 (18)
Cu1—Cu2A	2.8083 (18)	Cu2A— S2	2.268 (3)
Cu2—S1	2.279 (3)	Cu2—N4C	1.995 (8)
N9—Cu1—S1	119.2 (3)	N9—Cu1-S2	116.2 (3)
S1—Cu1—S2	114.99 (10)	N9—Cu1—Cu2	87.9 (2)
S1—Cu1—Cu2	53.01 (7)	S2—Cu1—Cu2	154.25 (8)
N9B—Cu1—Cu2A	149.1 (3)	S1—Cu1—Cu2A	89.74 (8)
S2—Cu1—Cu2A	51.69 (7)	Cu2—Cu1—Cu2A	103.01 (7)
S2B—Cu2—Cu1	90.64 (7)	N4B—Cu2—Cu1	154.5 (2)
S1—Cu2—Cu1	52.73 (7)	N4B—Cu2—Cu1B	87.8 (2)
S2B—Cu2—Cu1B	52.03 (7)	S1—Cu2—Cu1B	155.22 (8)
Cu1—Cu2—Cu1B	102.69 (7)		

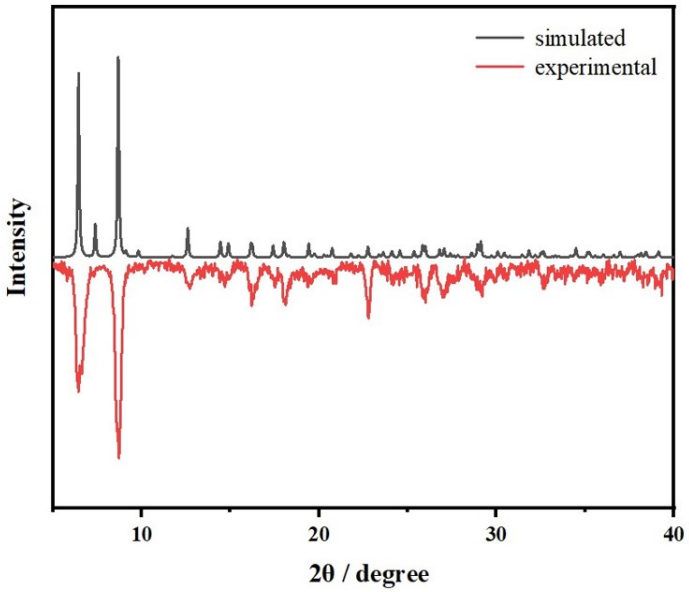

Figure S1. XRD spectra of **Cu_8I**.

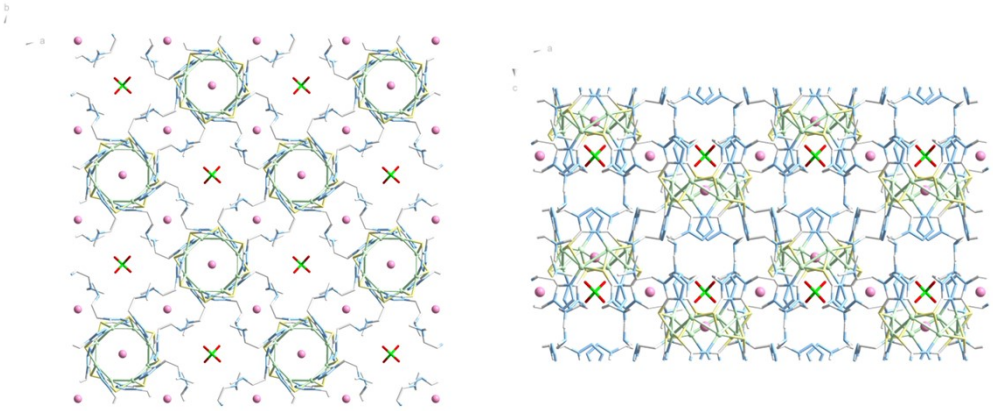

Figure S2. The packing arrangement of **Cu_8I** along the c-(left) and b-axis (right) directions, respectively.

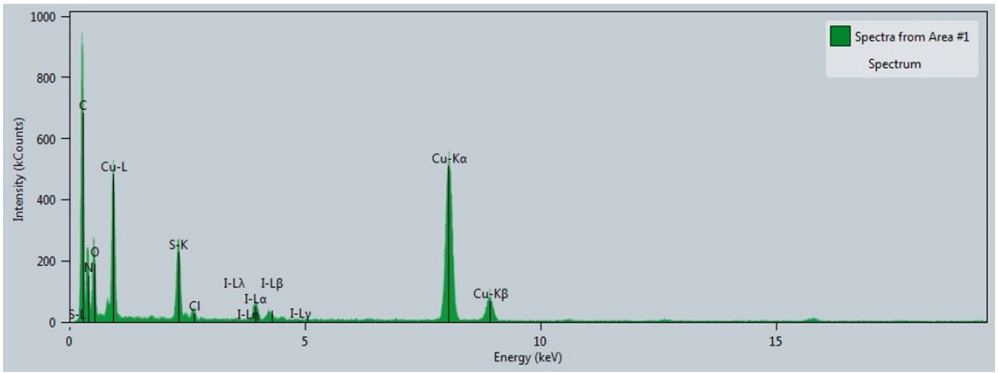

Figure S3. EDS spectra of **Cu_8I**.

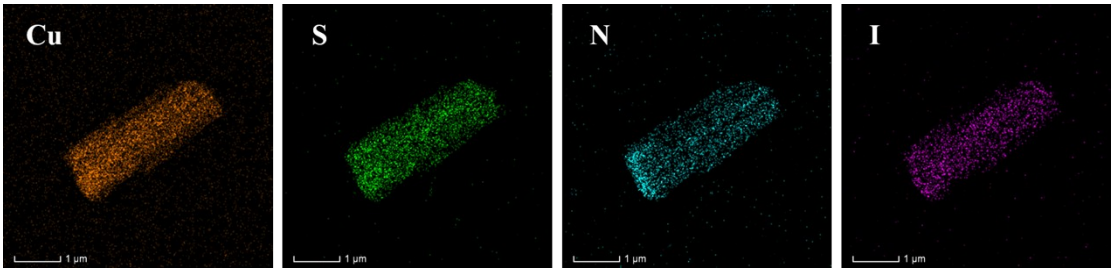

Figure S4. EDS elemental mapping images of Cu, S, N, and I in **Cu_8I**.

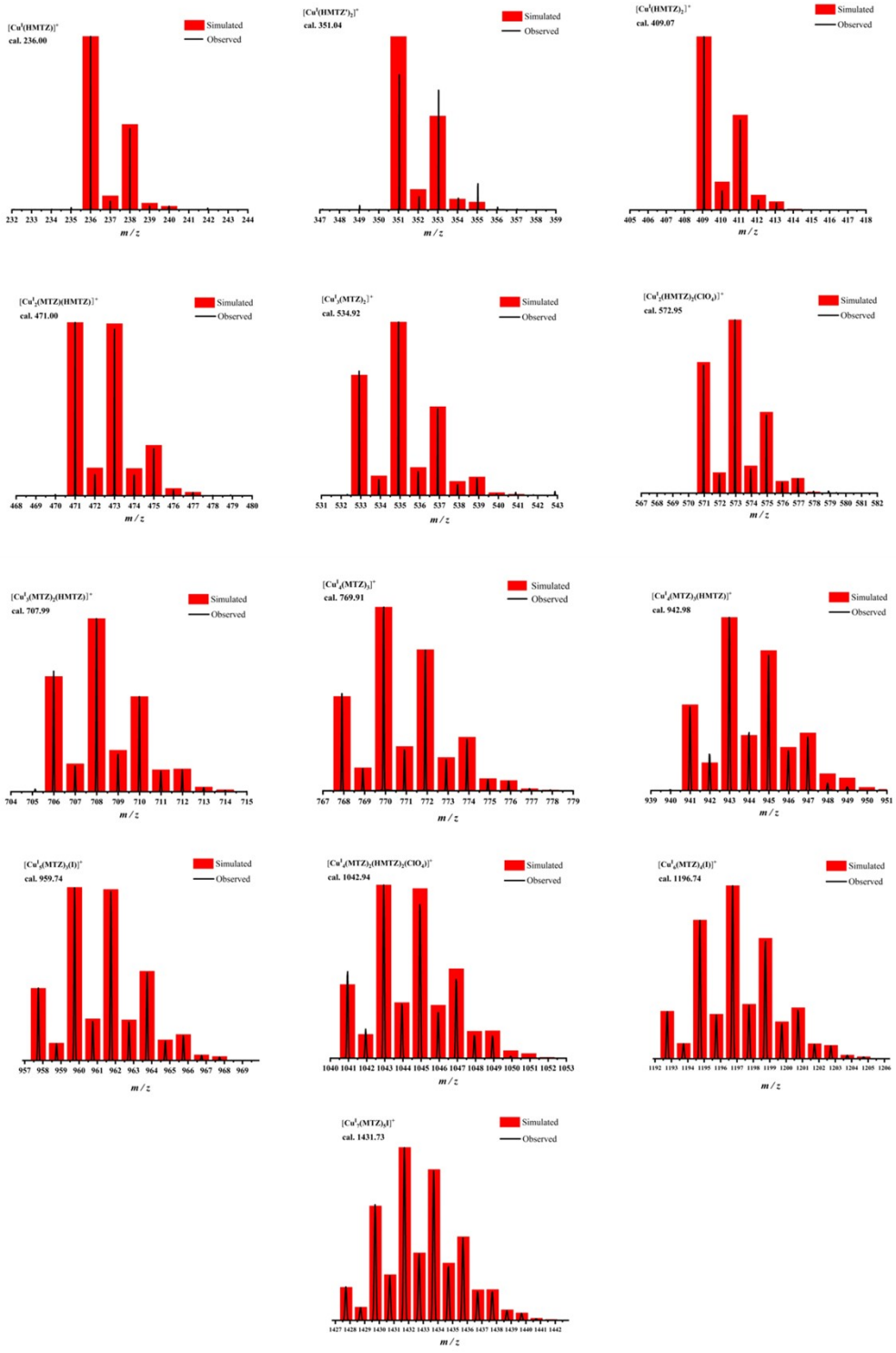

Figure S5. The superposed simulated and observed spectra of time-dependent ESI-MS species for **Cu_8I** in positive mode.

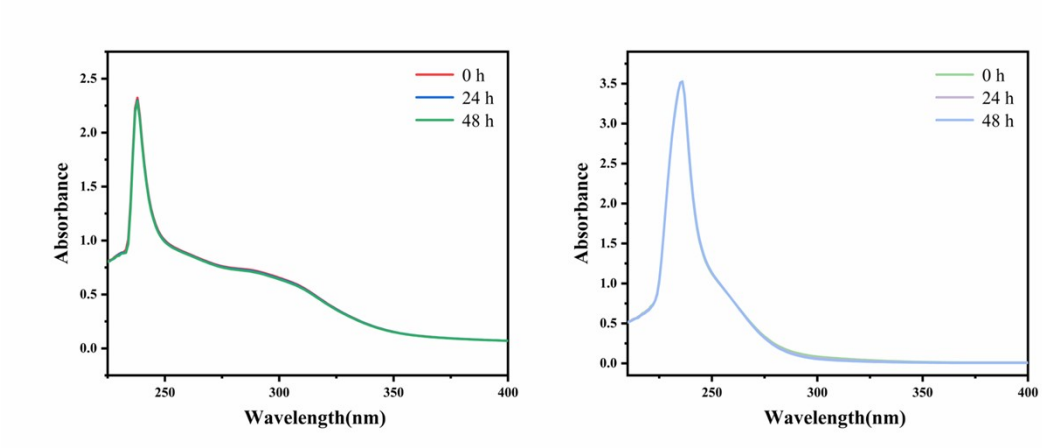

Figure S6. The absorption spectra of **Cu_8I** in PBS buffer solution (pH=7.4) and 1640 culture medium over 48 h.

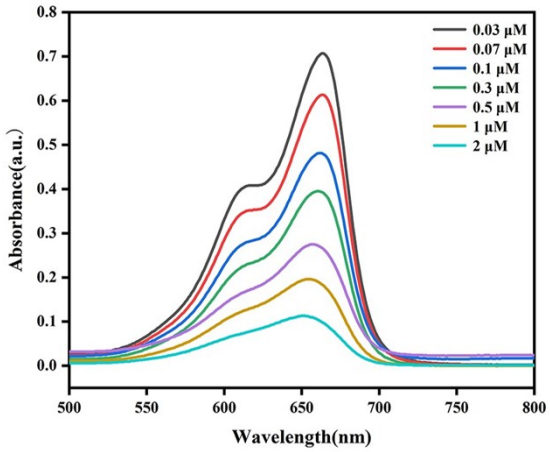

Figure S7. The degradation process of MB treated with different concentrations of **Cu_8I** containing 30 mM H_2O_2 (2.5 h of incubation time, pH 7.4).

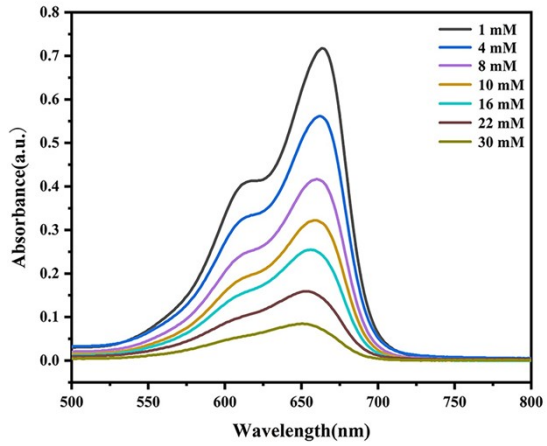

Figure S8. The degradation process of MB treated with different concentrations of H_2O_2 containing 2 μM **Cu_8I** (2.5 h of incubation time, pH 7.4).

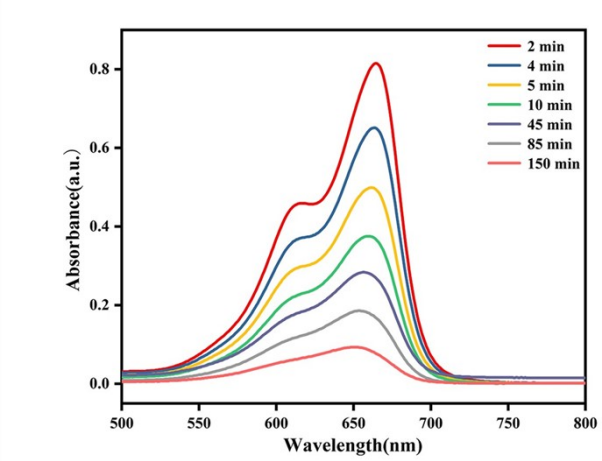

Figure S9. The degradation process of MB containing 30 mM H_2O_2 and 2 μM **Cu_8I** at different intervals (pH 7.4).

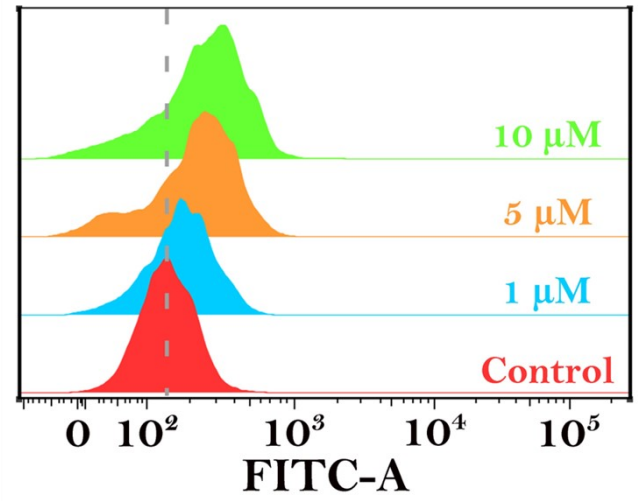

Figure S10. Intracellular ROS detection in HeLa cells using flow cytometry.

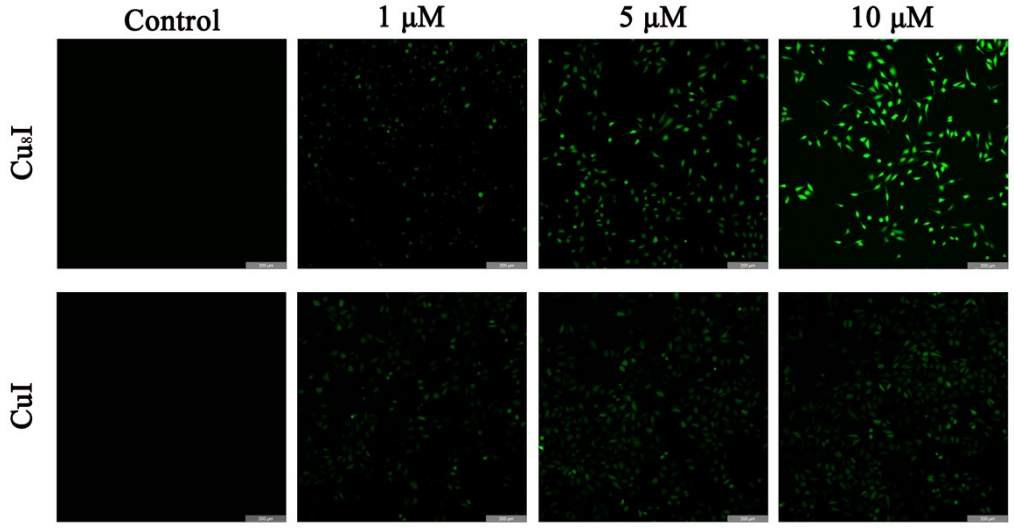

Figure S11. Intracellular ROS detection in HeLa cells after treatment with Cu_8I and CuI salt using a laser scanning confocal microscope. Scale bar: 200 μm.

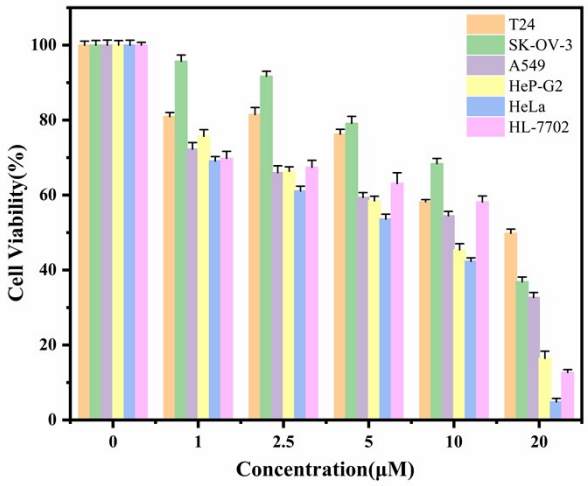

Figure S12. Effect of different concentrations of **Cu_8I** on the viability of different cells.

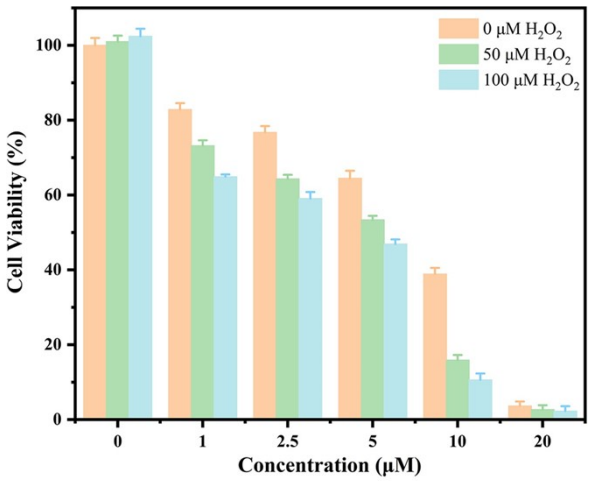

Figure S13. Cell viability of HeLa cells after incubation with **Cu_8I** in different concentrations of H_2O_2.

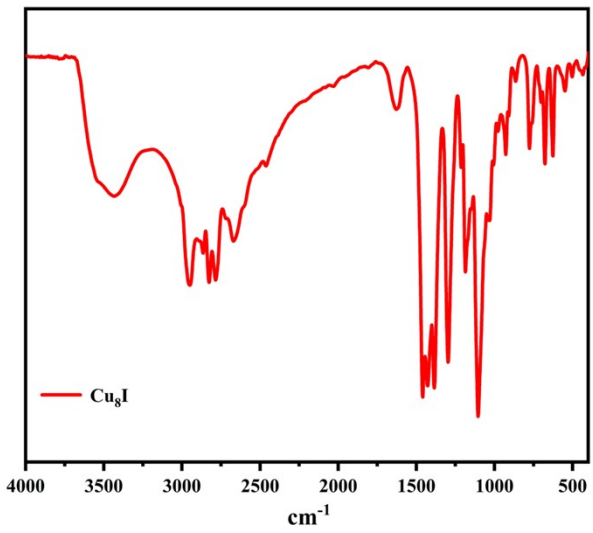

Figure S14. Fourier transform Infrared (FT-IR) spectrum of **Cu_8I**.

Experimental Section

Materials

All of the chemicals and reagents employed in this work were used without further purification. 1-[2-(N,N-dimethylamino)ethyl]-5-mercapto-1H-tetrazole and methylene blue (MB) were purchased from Bide Pharmaceutical Technology Co. Ltd. (Shanghai, China). $Cu(ClO_4)_2 \cdot 6H_2O$ 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). HeLa cells were obtained directly from the cell bank of Shanghai Institute of Life Sciences (China) and cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium which containing 10 % FBS (Gibco) plus 1 % penicillin.

Instruments

Absorbance was recorded on UV−Vis spectrophotometer (UV-2600i, Shimadzu). 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).

Synthesis of Cu_8I

A mixture of 1-[2-(N, N-dimethylamino)ethyl]-5-mercapto-1H-tetrazole (HMTZ) (69.3 mg, 0.400 mmol), $Cu(ClO_4)_2 \cdot 6H_2O$ (74.1 mg, 0.200 mmol) in H_2O (5 mL) and ethanol (5 mL) was stirred for 1 h in air, the mixed solution was adjusted to slightly turbid (pH = 7.0-8.0) by addition of 1 mol/L KOH solution, then added KI (66.4 mg, 0.400 mmol). The mixed solution was stirred under reflux for 4 h and then cooled and filtered. About two weeks later, colorless block crystals were obtained. Yield: 30%. IR (KBr ν/cm^{-1}): 3433 m, 2949 m, 2669 m, 1627 w, 1459 s, 1427 s, 1385 s, 1296 s, 1186 m, 1104 s, 677 w, 626 w.

Crystallographic analysis

Single-crystal X-ray diffraction data of **Cu_8I** were collected on a Bruker SMART CCD diffractometer by using graphite monochromatic Mo Kα radiation (k = 0.71073 Å) at room temperature. The structures were solved by direct methods using the program SHELXS-97 and refined by full-matrix least squares on F^2 using SHELXL-97. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were positioned geometrically and refined by a riding mode. The crystal data of structural analysis have been saved in the Cambridge crystal data center (CCDC reference number of **Cu_8I**: 1017362). The detailed structure and crystallographic data of **Cu_8I** are reported in our previous study[1].

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 PBS buffer solution with 30 mM H_2O_2 and 2 μM **Cu_8I**. 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. 100 μL aqueous solution containing DMPO (1 mM), 10 μL H_2O_2 (30%) and 100 μL complex solution (2mM). After that, the mixture was transferred into a quartz capillary and measured on an EPR spectrometer.

Cytotoxicity of Cu_8I by MTT Assay

HeLa 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 **Cu_8I** (0, 1, 2.5, 5.0, 10 and 20 μM) was added to each well for 24 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 HeLa cells was detected using ROS assay Kit DCFH-DA. Briefly, the HeLa 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 1640 medium containing 100 μM H_2O_2. Then, the cells were treated with different concentration of **Cu_8I**, with 1640 as the control, and then incubated for another 24 hours. Subsequently, the cells were washed once with serum-free 1640 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. HeLa cells were seeded in a cell culture dish and cultured overnight. The cells were treated with **Cu_8I** at different concentrations for 24 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, USA).

In vitro cell apoptosis study

The cell apoptosis assay was performed using Annexin V/PI Apoptosis Detection Kit. HeLa 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 (100 μM). Then, the medium containing H_2O_2 was discarded and HeLa cells were incubated in a medium containing different concentrations of (1 μM, 5.0 μM, 10.0 μM) **Cu_8I** for 24 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 2021-0013). 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 2021-0086). HeLa cells (5×10^6) were subcutaneous injected into the nude mice. When the tumor volume reached about 90 mm^3, the tumor-bearing mice were divided into two groups and each group was given saline (0.9 %), **Cu_8I** (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. All of the mice were sacrificed, and the tumors were excised for further characterization at the 15th day after the first administration.

References:

1. D. Y. Huang, H. M. Hao, P. F. Yao, X. H. Qin, F. P. Huang, Q. Yu, H. D. Bian, *Polyhedron*, 2015, **97**, 260–267.