Electronic Supplementary Information

A simple and feasible atom-precise biotinylated Cu(I) complex for tumor-targeted chemodynamic therapy

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Materials and methods

All chemicals were obtained from commercial sources and used as received. 5, 5-dimethyl-1-pyrrolidine -N-oxide (DMPO) and methylene blue (MB) were purchased from Aladdin. The Annexin V-FITC/PI apoptosis detection kit, Calcein-AM/PI assay kit, ROS assay kit, and JC-1 assay kits were obtained from BD Biosciences. All buffer solutions were prepared with double- distilled water.

The diffraction data of **Bio-CuCl** was obtained on an Agilent Technologies SuperNova diffractometer using Mo-Ka radiation (λ = 0.71073 A) at 293(2) K. The structures were solved by direct methods using ShelXS and refined using a full-matrix least-squares technique within ShelXL2015 and OLEX.2.[1,2] All non-hydrogen atoms were refined anisotropically thermal parameters, and hydrogen atoms were refined isotropically. The disordered solvent molecules were subtracted from the diffraction data by the SQUEEZE[3,4] command in PLATON. And SQUEEZE results which are approved by the elemental analysis. Further crystallographic data are provided in Table S1, and selected bond lengths and angles for **Bio-CuCl** is listed in Table S2. The CCDC reference numbers for the **Bio-CuCl** is 2005607.

Elemental analyses (C, H, and N) were attained on an Elementar Vario EL analyzer. UV-Vis absorption spectra were collected on a Cary 60 UV-Visible spectrophotometer. Cell apoptosis experiments were performed on FACS Aria II flow cytometry (BD Biosciences, San Jose, USA). A Bruker A300 electron paramagnetic resonance (EPR) spectrometer (Germany) was employed to collect the EPR signal.

References:

[1] Sheldrick, G. M. A short history of SHELXL. *Acta Crystallogr., Sect. A: Found. Crystallogr*. **2008**, 64, 112-122.

[2] Sheldrick, G. M. Crystal structure refinement with SHELXL. *Acta Crystallogr., Sect. C: Struct. Chem*. **2015**, 71, 3-8.

[3] Spek, A. L. Single-crystal structure validation with the program PLATON. *J. Appl. Cryst*. **2003**, 36, 7-13.

[4] Van der Sluis, P.; Spek, A. L. BYPASS: an effective method for the refinement of crystal structures containing disordered solvent regions. *Acta Cryst. Sect. A*. **1990**, 46, 194-201.

SQUEEZE results for Bio-CuCl are as follows:

$[CuCl(Bio)_2]\bullet 2H_2O$ (**Bio-CuCl)**

loop_

_platon_squeeze_void_nr

_platon_squeeze_void_average_x

_platon_squeeze_void_average_y

_platon_squeeze_void_average_z

_platon_squeeze_void_volume

_platon_squeeze_void_count_electrons

_platon_squeeze_void_content

1	0.250	0.750	0.836	526	83 ' '
2	0.250	0.250	0.261	345	80 ' '
3	0.750	0.250	0.336	526	83 ' '
4	0.750	0.750	0.761	345	80 ' '

_platon_squeeze_void_probe_radius 1.20

_platon_squeeze_details ?

That is, SQUEEZE gives 326 electrons/unit cell for the voids. If these electrons are all from H_2O (18 e^-), each unit cell has 326/18 =18 H_2O molecules, and each formula unit has 2 H_2O molecules (since Z = 8). So the suitable formula for this compound should be $[CuCl(Bio)_2]\bullet 2H_2O$. If other solvents (like cyclohexanone) are mixed in the structure, it would become more complicated and not easy to assign them.

Table S1 Crystallographic data and structure refinement parameters for the **Bio-CuCl**

Bio-CuCl (squeezed)	
Empirical formula	$C_{20}H_{32}ClCuN_4O_6S_2$
Formula weight (*M*)	587.60
Crystal system	tetragonal
Space group	$I4_1$
a (Å)	37.3667(5)
b (Å)	37.3667(5)
c (Å)	4.75497(13)
α (°)	90.00
β (°)	90.00
γ (°)	90.00
V/(Å ^{3})	6639.2(3)
Z	8
D_c(Mg.cm^{-3})	1.176
F(000)	2448.0
θ range for data collection (°)	6.542 to 52.734
Reflections collected / unique	40617
Goodness-of-fit on F^2	1.064
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0706, wR_2 = 0.1961
R indices (all data)	R_1 = 0.0970, wR_2 = 0.2220

Table S2 Selected band lengths [Å] and angles [°] for the **Bio-CuCl**

Bio-CuCl			
Cu1-Cl1A	2.749(7)	S1-Cu1-Cl1	106.1(2)
Cu1-Cl1	2.402(8)	S1-Cu1-Cl1A	94.7(2)
Cu1-S1	2.309(7)	S1-Cu1-S2	112.2(2)
Cu1-S2	2.365(7)	S2-Cu1-Cl1	111.5(2)
Cl1-Cu1-Cl1A	134.6(2)	S2-Cu1-Cl1A	96.3(2)

A: +X, +Y, 1+Z

Synthesis of Bio-CuCl

A mixture containing $CuCl_2 \cdot 2H_2O$ (2 mmol), biotin hydrazide (2 mmol) and cyclohexanone (10 mL) were sealed in a Teflon-lined stainless steel vessel (25 mL), which was heated at 80 ℃ for 48 h and then cooled to room temperature at a rate of 5 ℃ h^{-1}. Colorless needle crystals of **Bio-CuCl** were obtained and picked out, washed with distilled water several times, and dried in air. Then, the prepared **Bio-CuCl** stored at room temperature for the following experiments. Yield: 75 % [based on Cu]. IR data for **Bio-CuCl** (KBr, cm^{-1}): 3368(w), 3319(m), 2922(w), 1708(s), 1642(w), 1484(m), 1332(m), 1016(w), 645(m). Elemental analysis (%) for **Bio-CuCl** (Found/Calcd): C, 40.65/40.97; H, 6.46/6.19; N, 9.16/9.56; S, 5.56/5.47.

The ·OH generation by Cu^+-mediated Fenton-like reaction

MB was selected as a colorimetric indicator for ·OH generation. 0.2 mM MB, 10 mM H_2O_2, and 0.4 mM **Bio-CuCl** were mixed in PBS with different pH values (5.4, 6.5, and 7.4). The UV-Vis spectra of MB solution were then collected at different incubation times.

EPR spectrometer was employed to monitor the ·OH production by using DMPO as the spin trap agent. 100 μL aqueous solution containing DMPO (1 mM), 100 μL PBS with different pH values, 10 μL H_2O_2 and 100 μL **Bio-CuCl** solution. After that, the mixture was transferred into a quartz capillary and measured on an EPR spectrometer. The parameters were set as follows: modulation frequency = 100.0 kHz, microwave power = 0.998 mW, gain = 30 dB, microwave frequency = 9.85 GHz, and sweep time = 40 s.

Cell uptake study of Bio-CuCl

HeLa and WI38 cells were purchased from the cell bank of the Chinese Academy of Sciences. Cells were cultured in their corresponding media at 37 ℃ in a CO_2 incubator (5 %). The uptake of **Bio-CuCl** was studied by detecting intracellular Cu using inductively coupled plasma mass spectrometry (ICP-MS). HeLa and WI38 cells incubation with 12 μM **Bio-CuCl** for different times (6, 12, 24, 36, and 48 h). Cells were then washed with PBS and digested with trypsin. The collected cells were further digested by H_2O_2 and HNO_3 for testing the total intracellular Cu by ICP-MS. In the competitive experiment, 12 μg/mL free biotin was first incubated with HeLa cell for 6 h, and then 12 μM **Bio-CuCl** was added and incubated for 24 h. The following experimental procedure was the same as that of the above uptake study.

Tumor-cell targeted CDT induced by Bio-CuCl

HeLa cells and WI38 cells were seeded into 6-well plates at a density of 10^5 cells/well for 24 h at incubator (37 ℃, 5 % CO_2). Then, **Bio-CuCl** solutions at various concentrations (0, 12 and 24 μM) were added into each well, followed by the incubation for 48 h. The cells were harvested by

trypsinization and resuspended in 500 μL of PBS binding buffer and incubated with 5 μL Annexin V-FITC (100 ng/mL) for 20 min in the dark at 37 °C. And then 5 μL propidium iodide (2 μg/mL) were added and incubated in dark for another 20 min. The apoptosis results were detected by flow cytometry. Fluorescence imaging of live and dead cells using Calcein-AM/PI dual-staining kit was performed according to the manufacturer's instructions.

Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was detected by cytometry method using a JC-1 assay kit. HeLa cells were treated with **Bio-CuCl** at various concentrations (0, 12 and 24 μM). The cells were harvested by trypsinization after 48 h of treatment and resuspended in JC-1 staining solution (1×) at 37 °C for 20 min in the dark. Subsequently, the cells were washed three times by JC-1 staining buffer (1×). The cells were then measured by flow cytometry.

Intracellular ROS analysis

Cytometry method was used to detect ROS level in HeLa cells and WI38 cells with a ROS assay kit. HeLa cells and WI38 cells were treated with **Bio-CuCl** at different concentrations (0, 12 and 24 μM). After washing with serum-free medium, cells were incubated with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (100 μM) for 30 min in dark. After washing with serum-free medium for 3 times, the intracellular ROS were investigated by flow cytometry.

Tumor-targeted in vivo CDT

Animal handling procedures were approved by the Animal Ethics Committee of Guangxi Normal University. Female BalB/C mice (5 weeks) were used to construct tumor-bearing mouse xenograft model. 5×10^6 HeLa cells were injected into the BalB/C mice at the right mammary gland. When the volume of the tumor reached 100 mm^3, the mice were randomly divided into 2 groups (n = 6) for antitumoral studies. The HeLa tumor-bearing mice were treated with saline or 60 mg/kg **Bio-CuCl** via intravenous administration. The administration was repeated every 2 days, and a total of seven injections were performed over 15 days. The body weight and tumor volume were recorded every 2 dayst. The tumor volume was quantified by the formula $TV = 1/2\times a\times b^2$ (a, the length; b, the width). The relative tumor volume (RTV) was quantified by the formula $RTV=V_t/V_0$ (V_t, the tumor volume at each measurement; V_0, the tumor volume was measured when divided into cages). All of the mice were sacrificed, and the tumors and main organs were excised for further characterization at the 15th day after the first administration. For biosafety assessment, the major organs, including heart, liver, spleen, lung and kidney, were sliced (5 μm thickness) and analyzed using H&E staining test (n = 3).

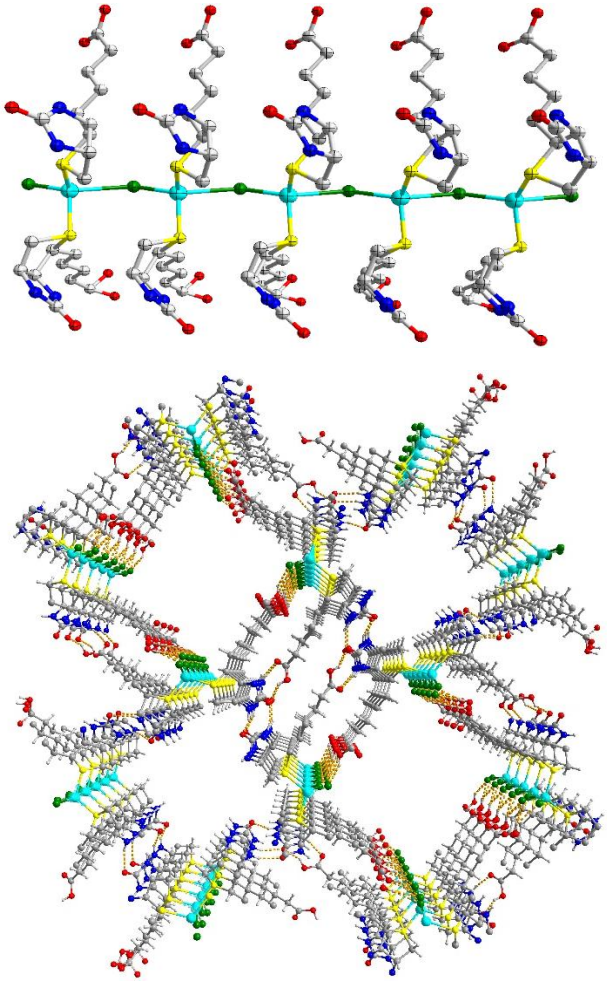

Fig. S1 View of the 1D chain and the 3D supramolecular structure in **Bio-CuCl**.

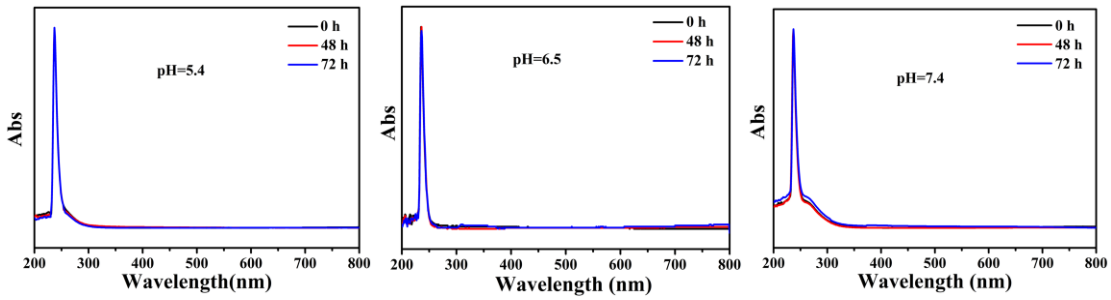

Fig. S2 UV-Vis spectra of **Bio-CuCl** in PBS solution with different pH at different incubation time.

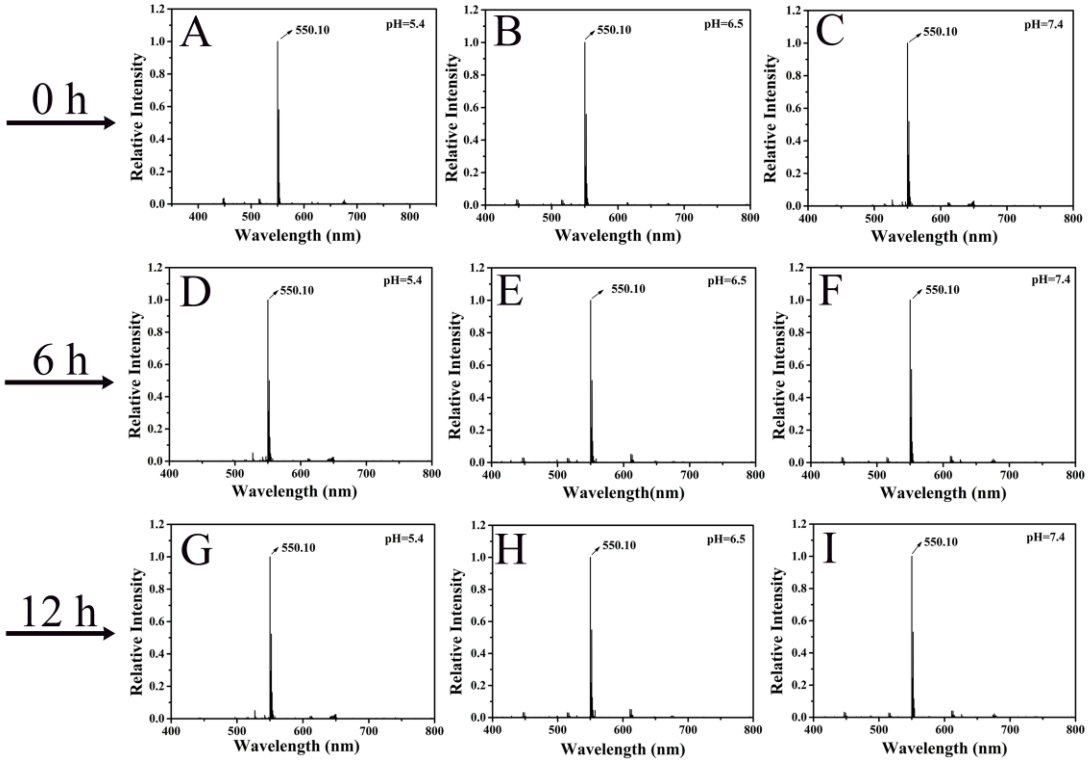

Fig. S3 MS spectra of **Bio-CuCl** after incubation with PBS (pH 5.4, 6.5, 7.4) at different incubation time. A-C: 0 h; D-F: 6 h; G-I: 12 h.

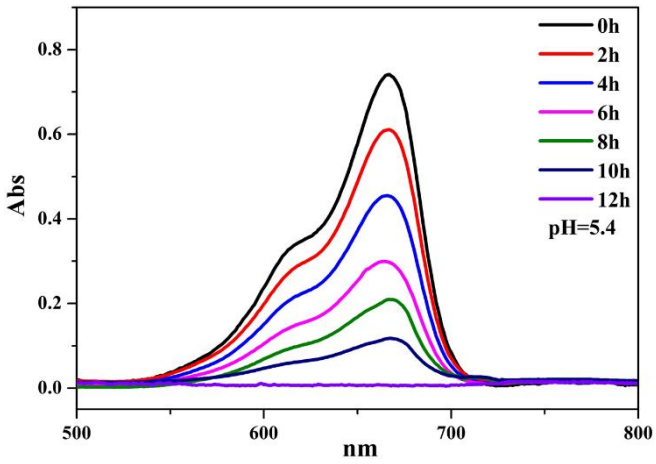

Fig. S4 MB degradation in PBS with pH 5.4.

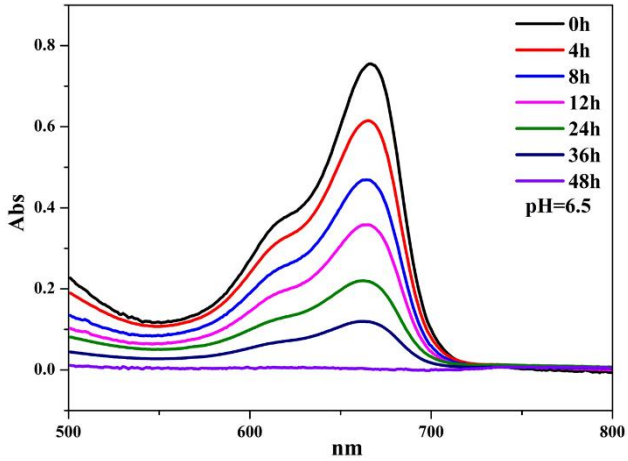

Fig. S5 MB degradation in PBS with pH 6.5.

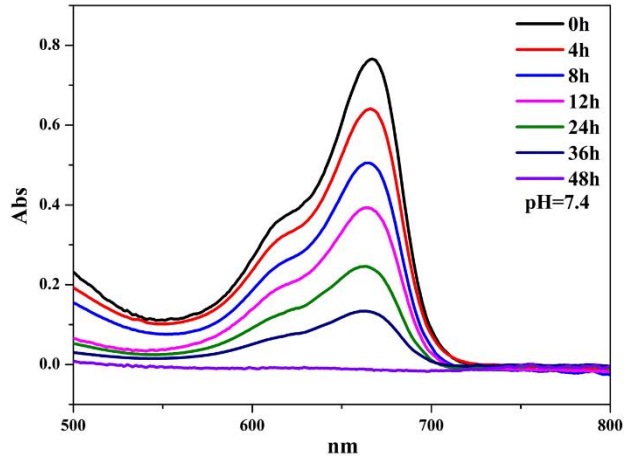

Fig. S6 MB degradation in PBS with pH 7.4.

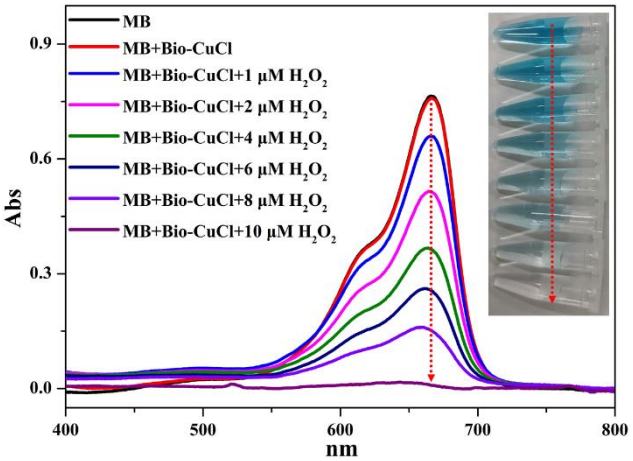

Fig. S7 H_2O_2-dependent MB degradation.

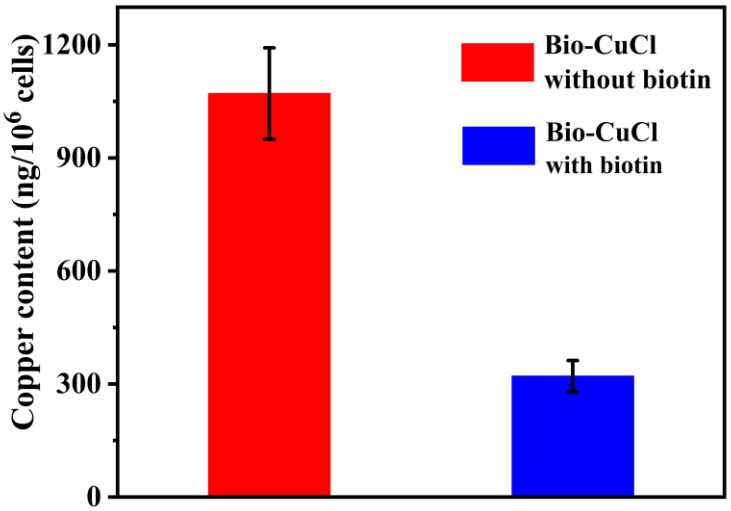

Fig. S8 Copper content in HeLa cells with or without competitive incubation of 12 μg/mL free biotin and 12 μM **Bio-CuCl**.

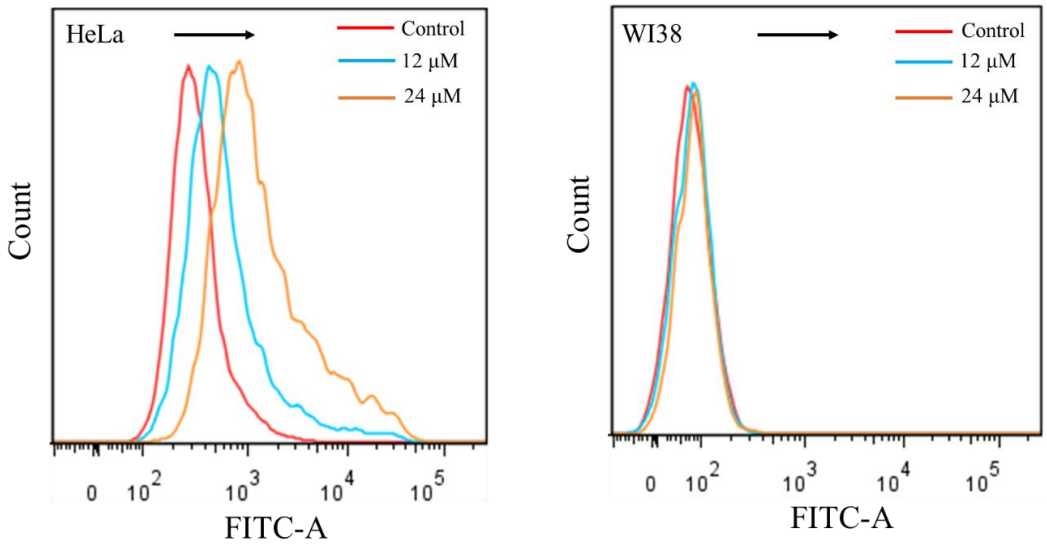

Fig. S9 Analysis of ROS by flow cytometry in HeLa and WI38 cells after 48 h of treatment with **Bio-CuCl**.

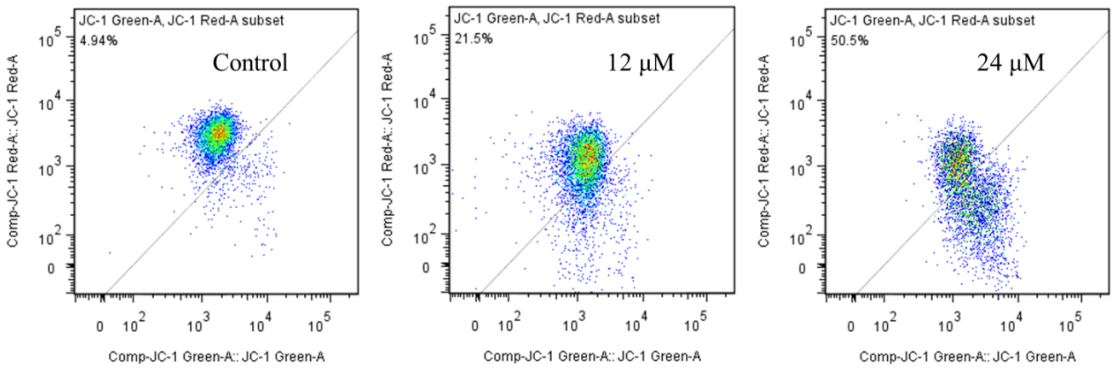

Fig. S10 Effect of **Bio-CuCl** on mitochondrial membrane potential of HeLa cells after 48 h treatment.

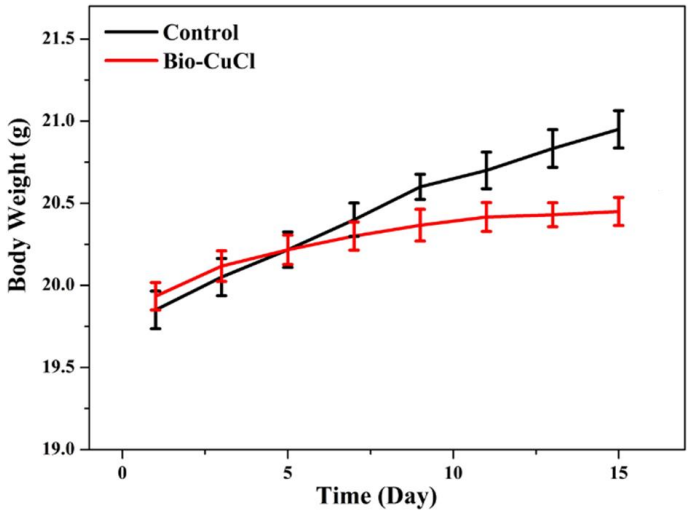

Fig. S11 The change of body weight of tumor-bearing mice during the therapy with injecting saline (control) or **Bio-CuCl**.

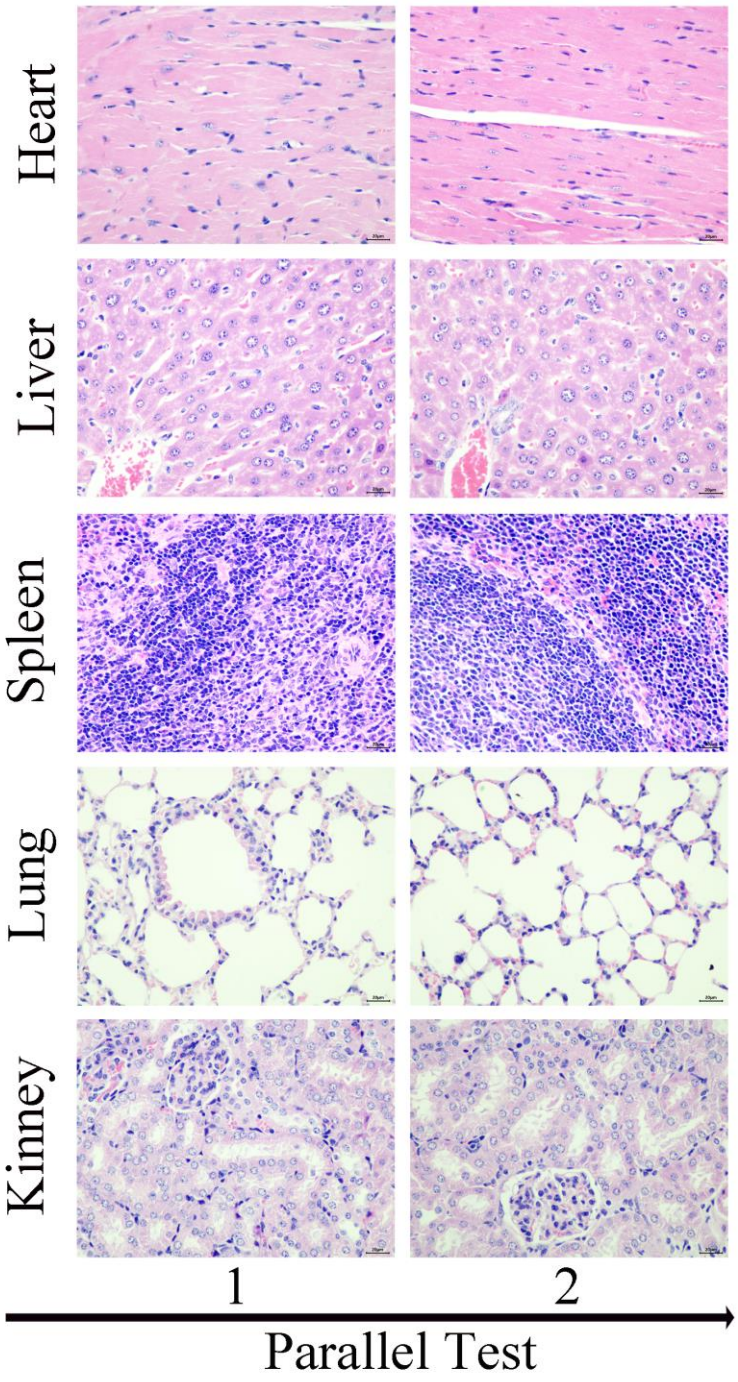

Fig. S12 H&E stained tissue sections from the major organs of HeLa-tumor-bearing mice after saline treatments (control group).

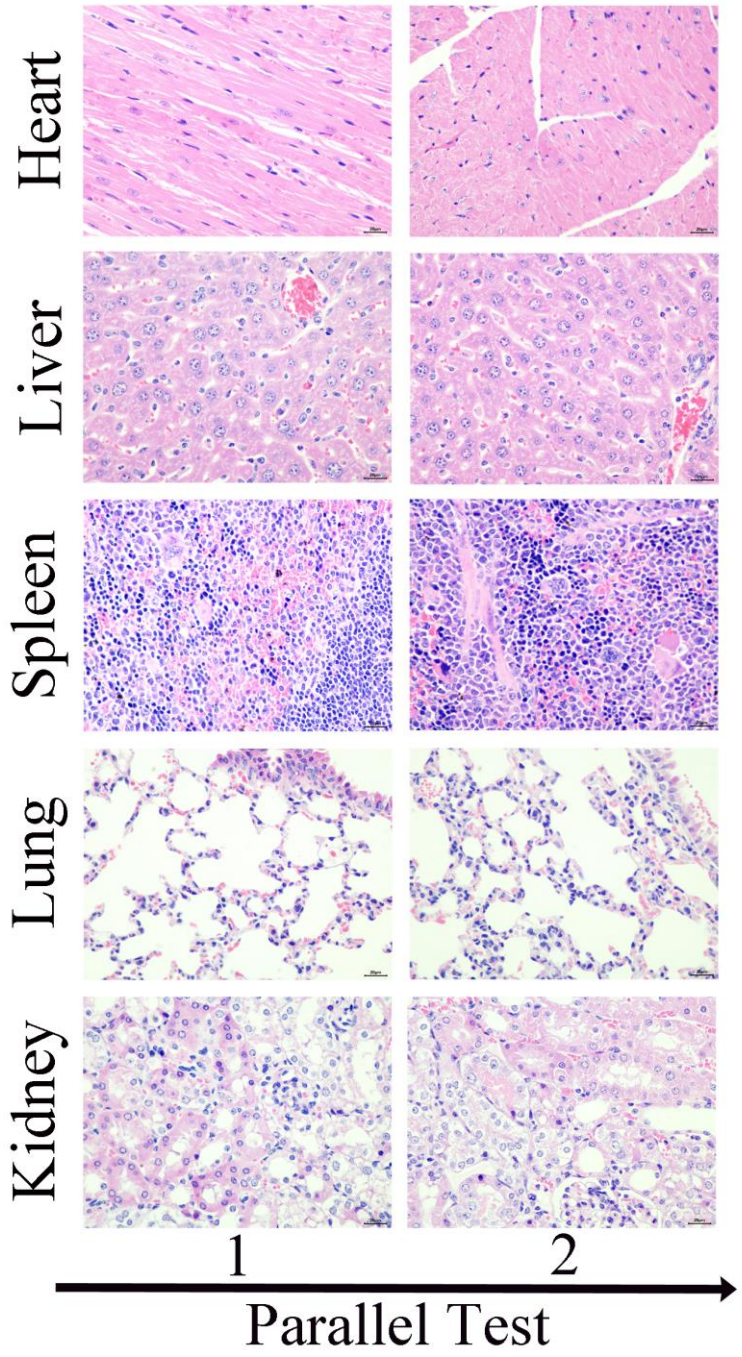

Fig. S13 H&E stained tissue sections from the major organs of HeLa-tumor-bearing mice after **Bio-CuCl** treatments.