Supplementary Information

Rational Construction of a Triphenylphosphine-Modified Tetra-nuclear Cu(I) Coordinated Cluster for Enhanced Chemodynamic Therapy†

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Experiments

X-ray crystallography data

Single-crystal data of the metal complexes were collected on an Agilent Supernova diffractometer (Cu, λ= 1.54184 Å) at room temperature (293 K). The crystal structures were solved using direct methods with the SHELXL program[1,2] and refined with a full-matrix least-squares technique within the ShelXL and OLEX2.[3] 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 MASKS command in OLEX.2. Selected bond lengths and angles for **1** is collected in Table S2. Selected bond lengths and angles for **2** is collected in Table S3. The CCDC reference numbers for **1** is 2121711. The CCDC reference numbers for **2** is 2121712.

MASKS results for 2 are as follows:

loop_

_smtbx_masks_void_nr

_smtbx_masks_void_average_x

_smtbx_masks_void_average_y

_smtbx_masks_void_average_z

_smtbx_masks_void_volume

_smtbx_masks_void_count_electrons

_smtbx_masks_void_content

1 0.000 0.000 -0.828 278.8 66.2 ?

2 0.333 0.667 -0.501 278.8 66.2 ?

3 0.667 0.333 -0.834 278.8 66.2 ?

_smtbx_masks_special_details ?

That is, MASKS gives 198 electrons/unit cell for the voids. If these electrons are all from H_2O (10 e^-), each unit cell has 198/10 =19.8 H_2O molecules, and each formula unit has 1 H_2O molecules (since Z = 18). So the suitable formula for this compound should be $[Cu(Ph_3P)_2L^2_2]\cdot NO_3\cdot MeOH\cdot H_2O$.

Detection of •OH using MB and DMPO

Comparative analysis of the influence of different incubation times, different concentrations of H_2O_2 and different concentrations of complex on the colorimetric indicator of MB based on single variable method. The mixing system was incubated at 37 °C, then absorbance was measured by UV-vis spectroscopy.

The EPR experiment refer to the previously published reports. 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.

In vitro experiments

The in vitro experiments are done adopting the published experimental method in the paper.[4-6]

Cellular uptake: T24 cells were seeded in a 10 cm cell culture dish at a density of 10^6 cells and incubated for 12 h. Then the medium was replaced by a fresh one containing 3 μM **1** and **2** separately, the cells were incubated for different times (3, 6, 12, 24, 36 and 48 h) at 37 °C in an incubator with 5% CO_2. The cells were hydrolysis by pancreatic enzyme and washed with PBS and then harvested. The content of copper in the whole cells was determined by ICP-MS.

Cytotoxicity assay: T24 cells were seeded in 96-well culture plate for 12 h at incubator (37 °C, 5 % CO_2), and different concentrations of **1** and **2** were added to the cell medium separately. After 24 h of incubation, 10 μL of MTT (5 mg·mL^{-1}) was added into each well and incubated for another 4-6 h. The purple formazan crystals obtained were dissolved in 100 μL of DMSO, and then measure the absorbance and calculate the cell viability.

In Vitro cytotoxicity: T24 cells were seeded into 6-well plates at a density of 10^5 cells/well for 12 h at incubator (37 °C, 5 % CO_2), **1** and **2** solutions at various concentrations (0, 1, 2 and 3 μM) were added into each well separately for 24 h at incubator, and then 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. 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.

Determination of intracellular ROS: T24 cells were seeded into 6-well plates at a density of 10^5

cells/well for 12 h at incubator (37 °C, 5 % CO_2), **1** and **2** solutions at various concentrations (0, 1, 2 and 3 μM) were added into each well separately for 8 h at incubator, and then washing with serum-free medium, cells were incubated with 500 μL 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. Fluorescence imaging was observed by using a laser scanning confocal microscope.

Cell cycle: T24 cells were seeded in a 70 mm cell culture dish at a density of 10^5 cells/well for 12 h at incubator (37 °C, 5 % CO_2), **1** and **2** solutions at various concentrations (0, 1, 2 and 3 μM) were added into each well separately for 24 h at incubator, then cells were washed with PBS and then harvested. and then fixed with 70% ethanol overnight. The cells were stained with cell cycle kit according to the manufacture's protocol and then analyzed by flow cytometry.

In vivo antitumor efficiency

This study was performed in strict accordance with the guidelines of Animal Care and Use Committee of Guangxi Normal University (Animal Care and Ethical Examination Certificate No. 202107-001). Animal handling procedures were approved by the Animal Ethics Committee of Guangxi Normal University. BalB/C mice were used to construct tumor-bearing mouse xenograft model. 5×10^6 T24 cells were injected into the BalB/C mice at the right armpit. When the volume of the tumor reached 85 mm^3, the mice were randomly divided into 4 groups (n = 6) for antitumoral studies. The T24 tumor-bearing mice were treated orally with saline (control group), 0.02mmol/kg **1** or 0.02mmol/kg **2** (A total of 4.788 mg of the **1** was precisely weighed and ultrasonically dissolved in 120 ul DMF + 1080 ul normal saline, with the solution concentration of 3.99 mg / ml, the intragastric administration volume was 0.2 mL / 20 g; A total of 2.34 mg of the **2** was precisely weighed and ultrasonically dissolved in 120 ul DMF + 1080 ul normal saline, with the solution concentration of 1.95 mg / ml, the intragastric administration volume was 0.2 mL / 20 g.).The administration was repeated every day, and a total of fourteen orally were performed. Meanwhile, the positive contrast group were intra-peritoneally injected 2 mg/kg cisplatin, the administration was repeated every 2 days, and a total of seven injections were performed. The body weight and tumor volume were recorded every 2 days. 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 were excised for further characterization at the 15th day after the first administration.

References:

[1] G. M. Sheldrick. A short history of SHELX. *Acta Cryst*. **2008**, *64*, 112-122.

[2] G. M. Sheldrick. Crystal structure refinement with SHELXL. *Acta Cryst*. **2015**, *71*, 3-8.

[3] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann. OLEX2: a complete structure solution, refinement and analysis program. *J. Appl. Cryst.* **2009***, 42,* 339-341.

[4] Z. Wang, B. Liu, Q. Sun, S. Dong, Y. Kuang, Y. Dong, F. He, S. Gai and P. Yang, *ACS Appl Mater Interfaces*, 2020, **12**, 17254-17267.

[5] M. Wang, M. Chang, Q. Chen, D. Wang, C. Li, Z. Hou, J. Lin, D. Jin and B. Xing, *Biomaterials*, 2020, **252**, 120093.

[6] S. Chen, X. Liu, X. Ge, Q. Wang, Y. Xie, Y. Hao, Y. Zhang, L. Zhang, W. Shang and Z. Liu, *Inorganic Chemistry Frontiers*, 2020, **7**, 91-100.

Table S1 Crystal data for **1** and **2**

	1	**2**
Compound	$[Cu_2(Ph_3P)_2L^1_2]$	$[Cu(Ph_3P)_2L^2_2]\cdot NO_3\cdot MeOH\cdot H_2O$
Formula	$C_{44}H_{40}Cu_2N_8O_4P_2S_2$	$C_{43}H_{46}CuN_7O_5P_2S_4$
Formula Weight	997.98	994.57
Crystal System	monoclinic	trigonal
Space Group	*P*2/n	*R*-3
a (Å)	29.17370(17)	46.0372(3)
b (Å)	11.66423(5)	46.0372(3)
c (Å)	29.95363(18)	11.65150(7)
a (°)	90	90
β (°)	115.6403	90
g (°)	90	120
V/ (Å ^{3})	9189.17(10)	21386.0(3)
Z	8	18
D_x (Mg·m^{-3})	1.443	1.365
F (000)	4096	9108.0
T(K)	293 K	293 K
λ(Å)	1.54184	1.54184
Mu (mm^{-1})	3.066	3.319
GOF on F^2	1.055	1.078
R_1, wR_2[I>=2σ(I)]	R_1 = 0.0345, wR_2 = 0.0929	R_1 =0.0465，wR_2 = 0.1528
R1, wR2 (all data)	R_1 = 0.0405, wR_2 = 0.0965	R_1 = 0.0482, wR_2 = 0.1543

Table S2 Selected bond lengths (Å) and angles (°) for **1.**

Bond Lengths (Å)	
Cu1—S1A	2.3646 (5)
Cu1—S2	2.3448 (5)
Cu1—P1	2.2525 (5)
Cu1—N1	2.0311 (16)
Cu2—S1A	2.3490 (5)
Cu2—S2A	2.3569 (5)
Cu2—P2	2.2632 (6)
Cu2—N5	2.0440 (16)
Cu3—S3B	2.3717 (6)
Cu3—S4	2.3328 (5)
Cu3—P4	2.2655 (6)
Cu3—N9	2.0472 (16)
Cu4—S3B	2.3536 (5)
Cu4—S4B	2.3713 (6)
Cu4—P3	2.2657 (6)
Cu4—N13	2.0363 (17)
Angles (°)	
S2—Cu1—S1A	112.156 (19)
P1—Cu1—S1A	106.54 (2)
P1—Cu1—S2	109.29 (2)
N1—Cu1—S1A	99.30 (5)
N1—Cu1—S2	107.58 (5)
N1—Cu1—P1	121.61 (5)
S1A—Cu2—S2A	110.828 (19)
P2—Cu2—S1A	107.92 (2)

P2—Cu2—S2A	109.02 (2)
N5—Cu2—S1A	108.09 (5)
N5—Cu2—S2A	100.80 (5)
N5—Cu2—P2	119.94 (5)
S4—Cu3—S3B	112.147 (19)
P4—Cu3—S3B	108.50 (2)
P4—Cu3—S4	109.20 (2)
N9—Cu3—S3B	104.09 (5)
N9—Cu3—S4	107.66 (5)
N9—Cu3—P4	115.24 (5)
S3B—Cu4—S4B	112.711 (19)
P3—Cu4—S3B	112.11 (2)
P3—Cu4—S4B	104.05 (2)
N13—Cu4—S3B	105.31 (5)
N13—Cu4—S4B	103.32 (5)
N13—Cu4—P3	119.17 (5)

(A) -*x*+3/2, *y*, -*z*+1/2; (B) -*x*+1/2, *y*, -*z*+1/2.

Table S3 Selected bond lengths (Å) and angles (°) for **2**.

Bond Lengths (Å)	
Cu1—P2	2.3073 (5)
Cu1—P1	2.2867 (5)
Cu1—S3	2.3912 (5)
Cu1—S1	2.3735 (5)
Angles (°)	
P2—Cu1—S3	99.820 (18)
P2—Cu1—S1	108.554 (19)
P1—Cu1—P2	122.781 (19)
P1—Cu1—S3	112.829 (19)
P1—Cu1—S1	104.126 (18)

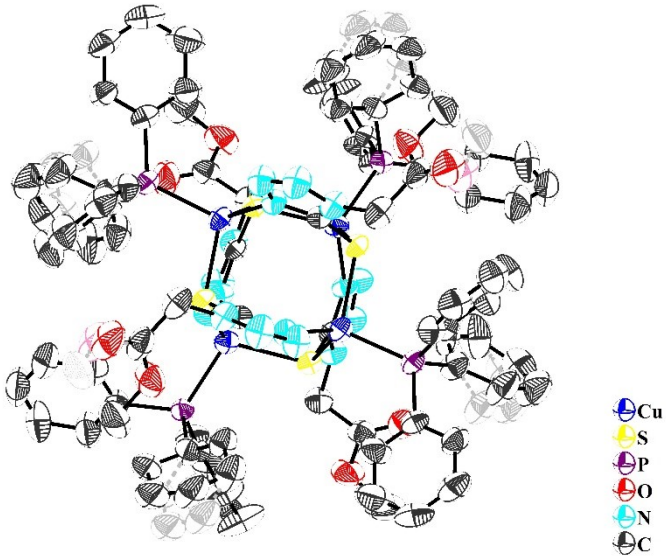

Figure S1 View of the coordination environment of the Cu(I) center in **1**. Thermal ellipsoids are drawn at 50% probability level. H anions are omitted for clarity.

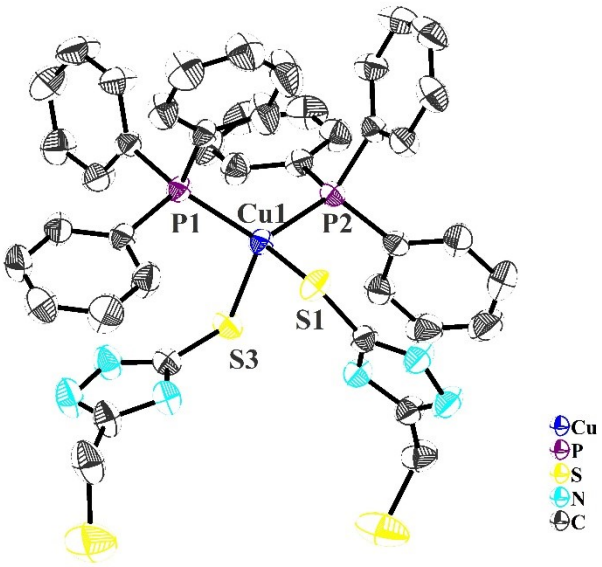

Figure S2 Crystal structures of **2**. Thermal ellipsoids are drawn at 50% probability level. Some anions and solvent molecules are omitted for clarity.

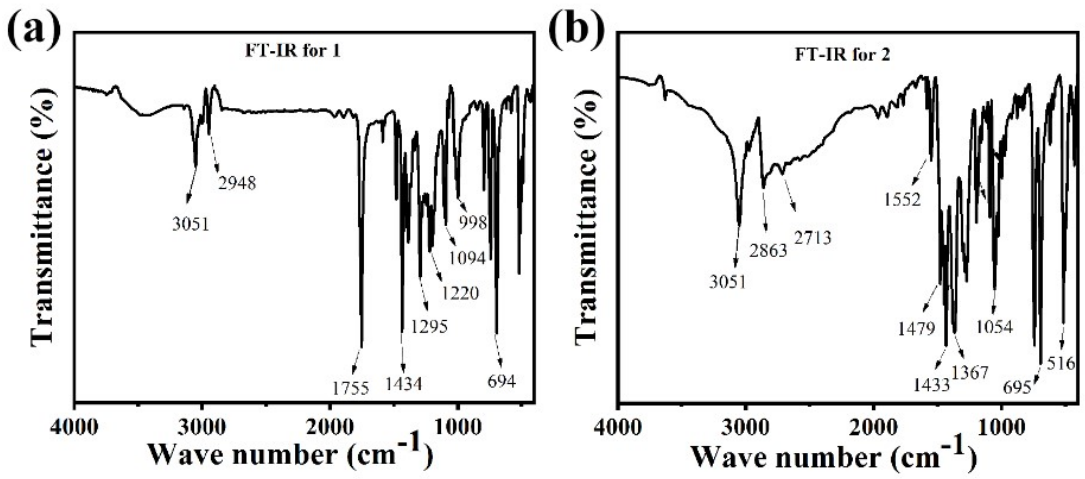

Figure S3 The FT-IR spectra of (a) **1** and (b) **2** recorded in KBr palates.

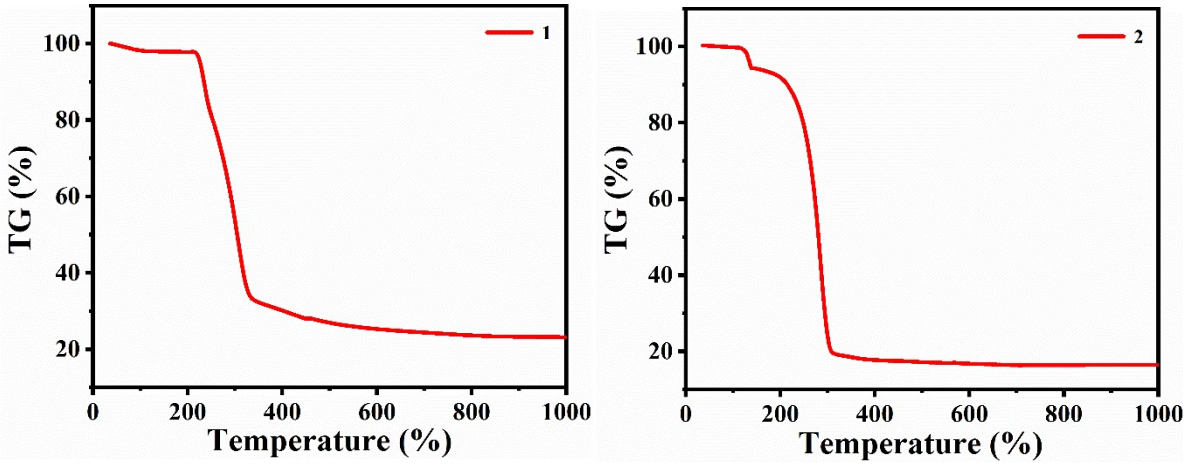

Figure S4 The TG curve of **1** and **2** under heating rate of 10 °C /min over the temperature range of 35-1000 °C in flowing N_2.

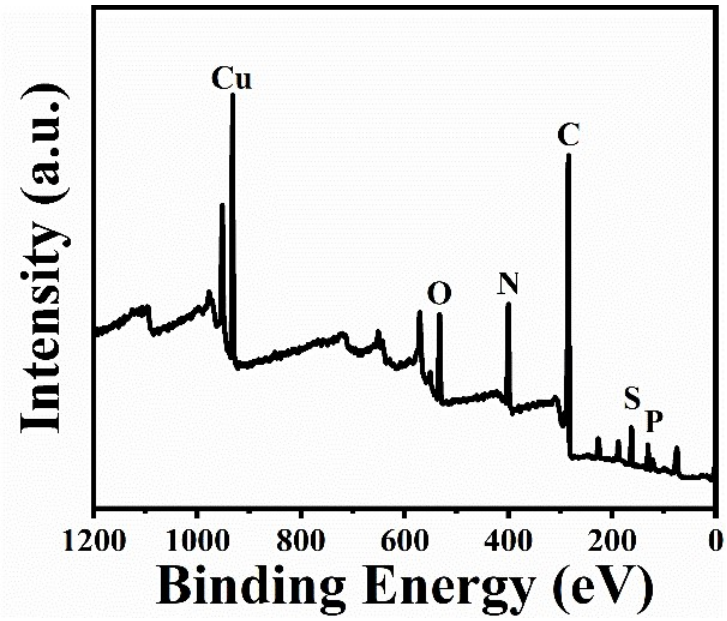

Figure S5 XPS spectra of **1**.

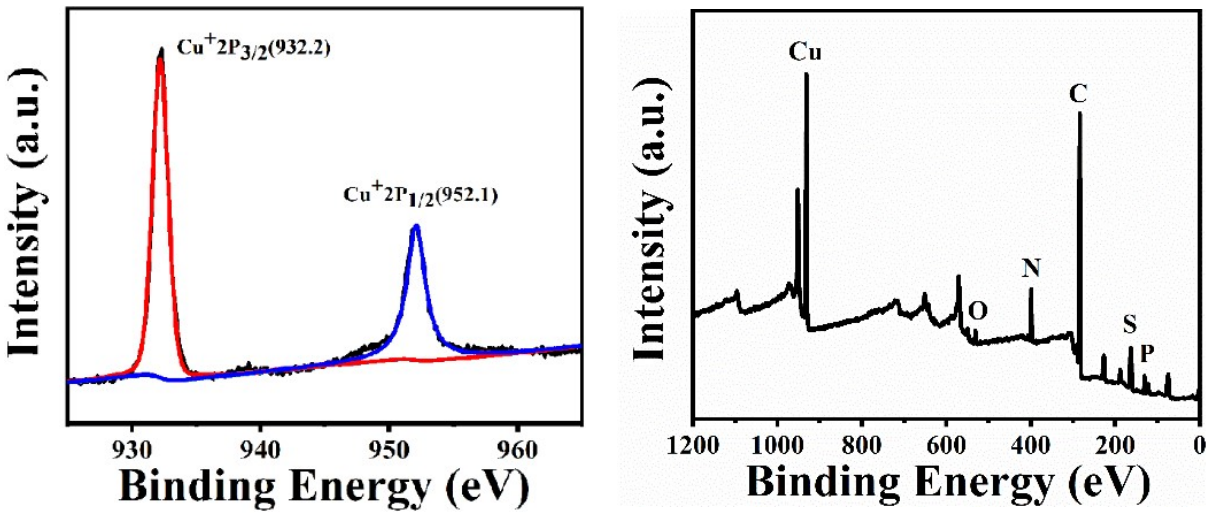

Figure S6 XPS spectra of **2**.

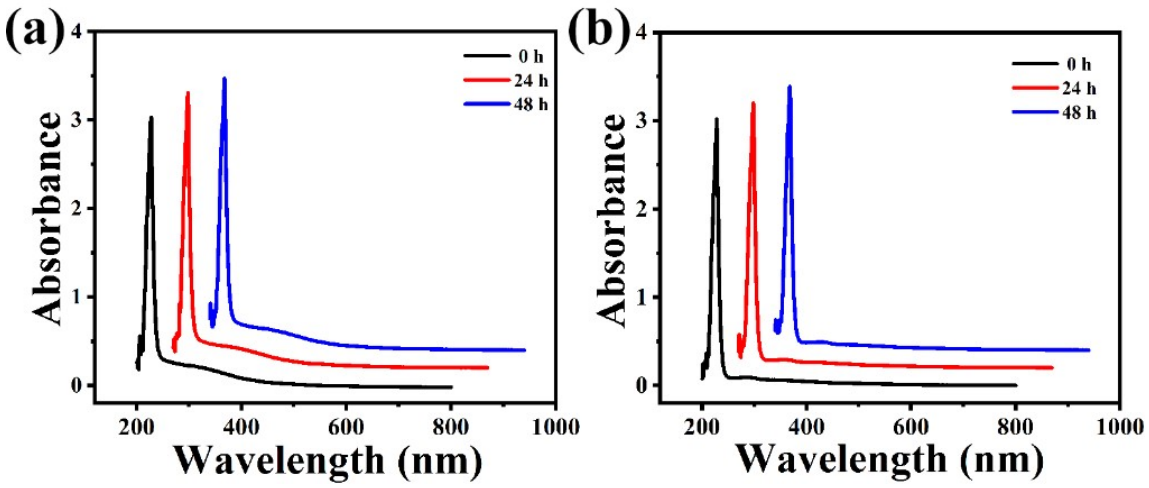

Figure S7 UV-Vis spectra of (a) **1** and (b) **2** in PBS solution at different incubation time.

Table S4 Some fitting fragment of different peaks of **1** in Figure 2.

m/z	Fragment
1118.89	$[Cu_4L^1{}_4(CH3O)(DMF)(H2O)_2H]^-$
1352.96	$[Cu_4L^1{}_4\ (Ph_3P)(CH3O)(DMF)(CH3CN)]^-$
1592.93	$[Cu_4L^1{}_4\ (Ph_3P)_2(CH3O)(HCl)_2(H2O)]^-$
1827.11	$[Cu_4L^1{}_4\ (Ph_3P)_3(CH3O)(CH3OH)_2]^-$
2068.13	$[Cu_4L^1{}_4\ (Ph_3P)_4(CH3O)(CH3CN)]^-$

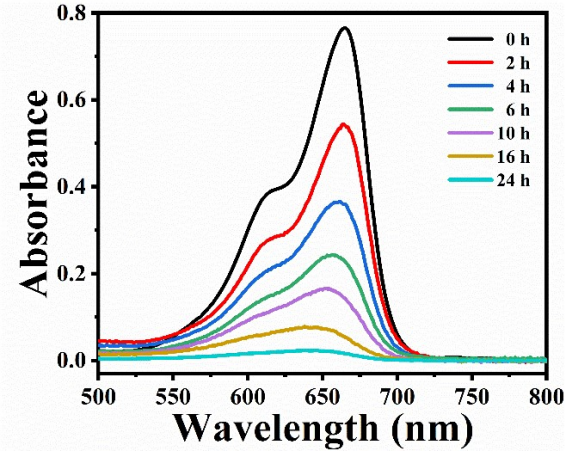

Figure S8 The degradation process of MB containing 5 mM H_2O_2 and 30 μM **2** at different intervals (pH 6.5).

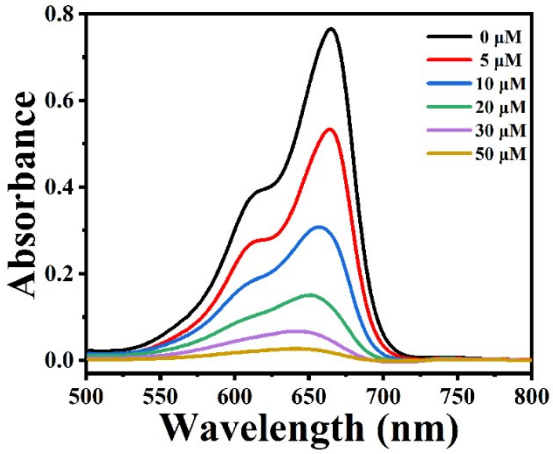

Figure S9 The degradation process of MB treated with different concentration of **1** containing 5 mM H_2O_2 (6 h of incubation time, pH 6.5).

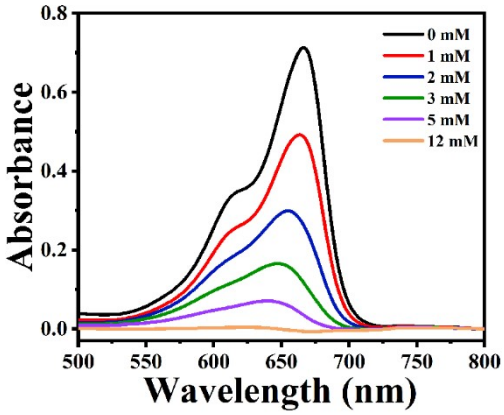

Figure S10 The degradation process of MB treated with different concentration of H_2O_2 containing 30 μM **1** (6 h of incubation time, pH 6.5).

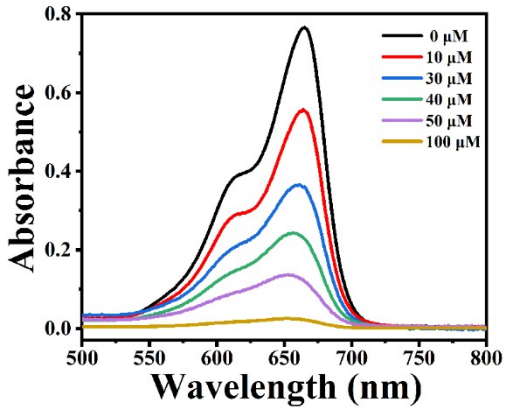

Figure S11 The degradation process of MB treated with different concentration of **2** containing 5 mM H_2O_2. (6 h of incubation time, pH 6.5).

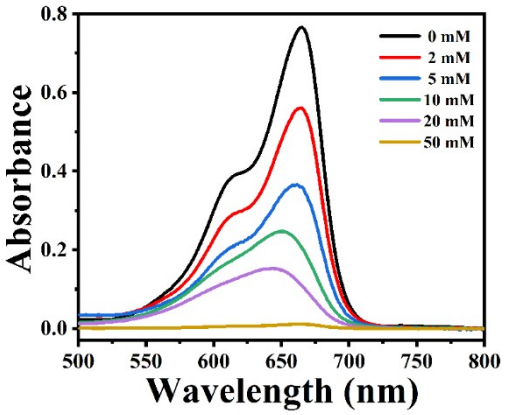

Figure S12 The degradation process of MB treated with different concentration of H_2O_2 containing 30 μM **2**. (6 h of incubation time, pH 6.5).

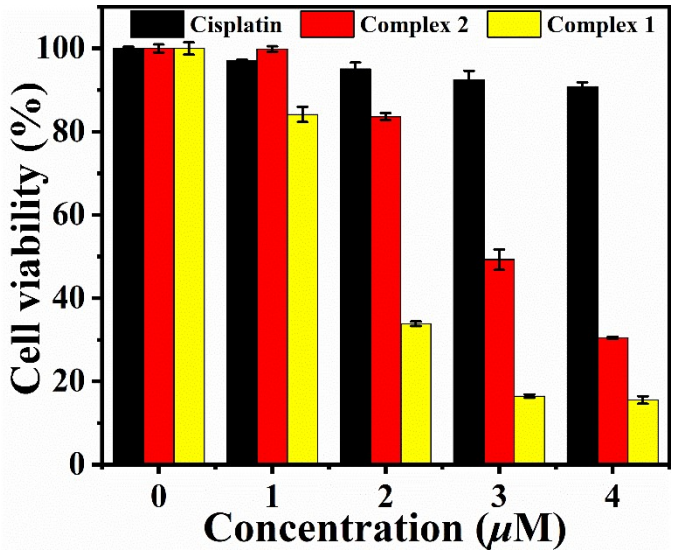

Figure S13 Cell viability of T24 cells treated with **1**, **2** and cisplatin for 24 h.

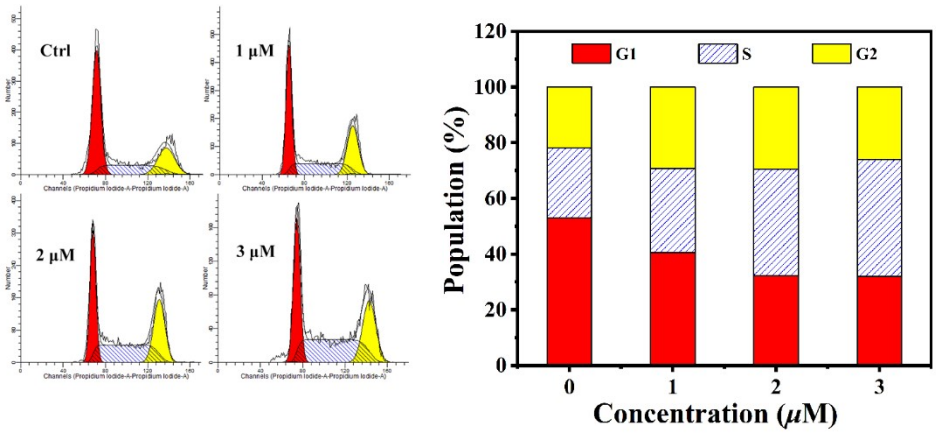

Figure 14 Cell cycle of T24 treated by **1** for 24 h using flow cytometry**.**

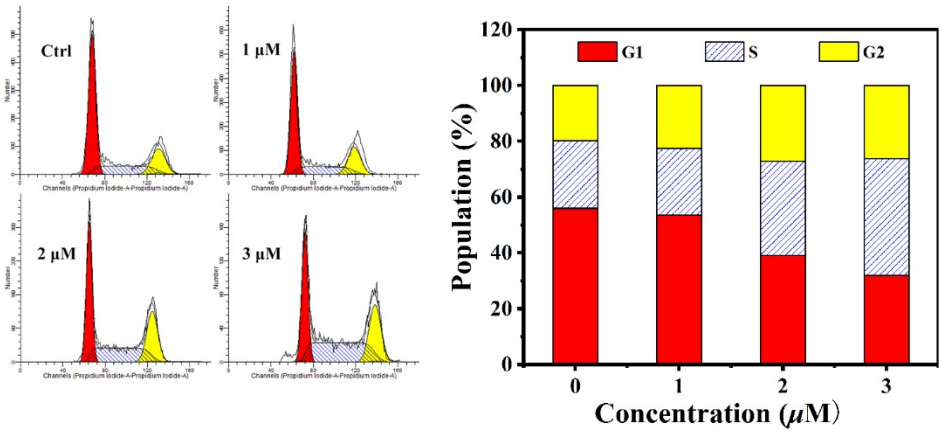

Figure 15 Cell cycle of T24 treated by **2** for 24 h using flow cytometry.

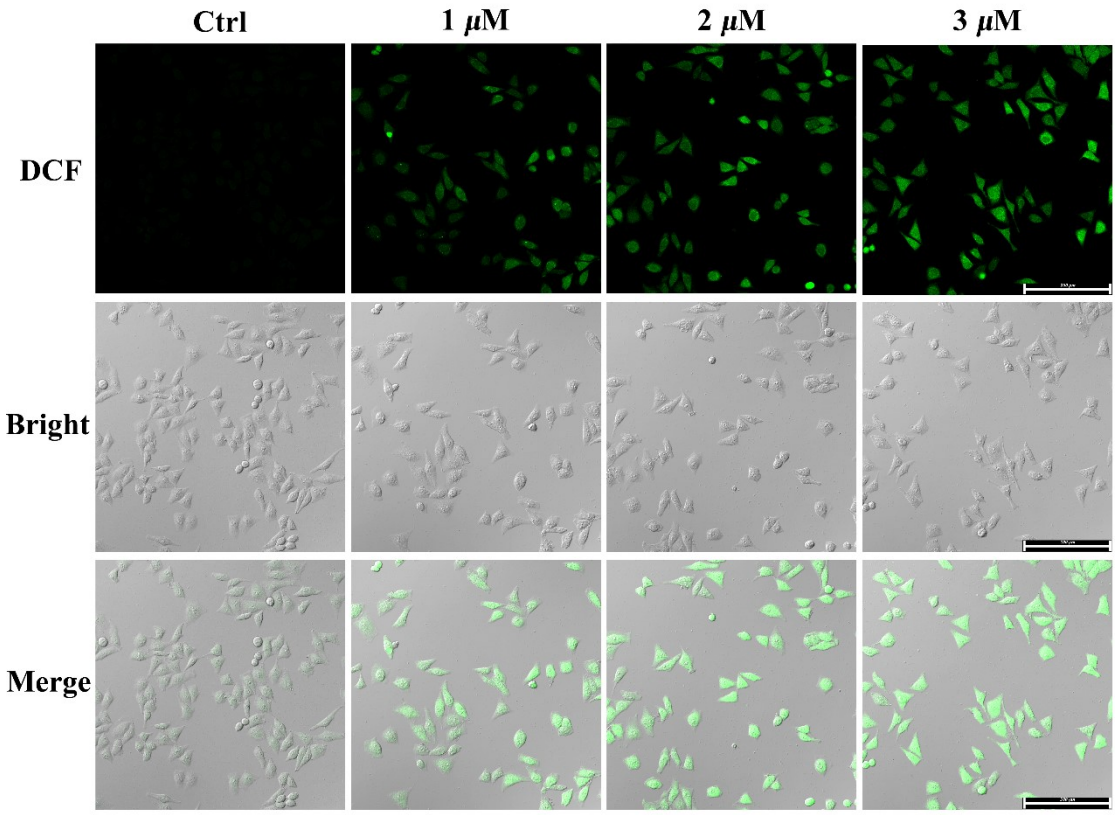

Figure S16 The intracellular ROS detection in T24 cells by DCFH-DA after treatment with **1** using a laser scanning confocal microscope. Scale bar: 200 μm.

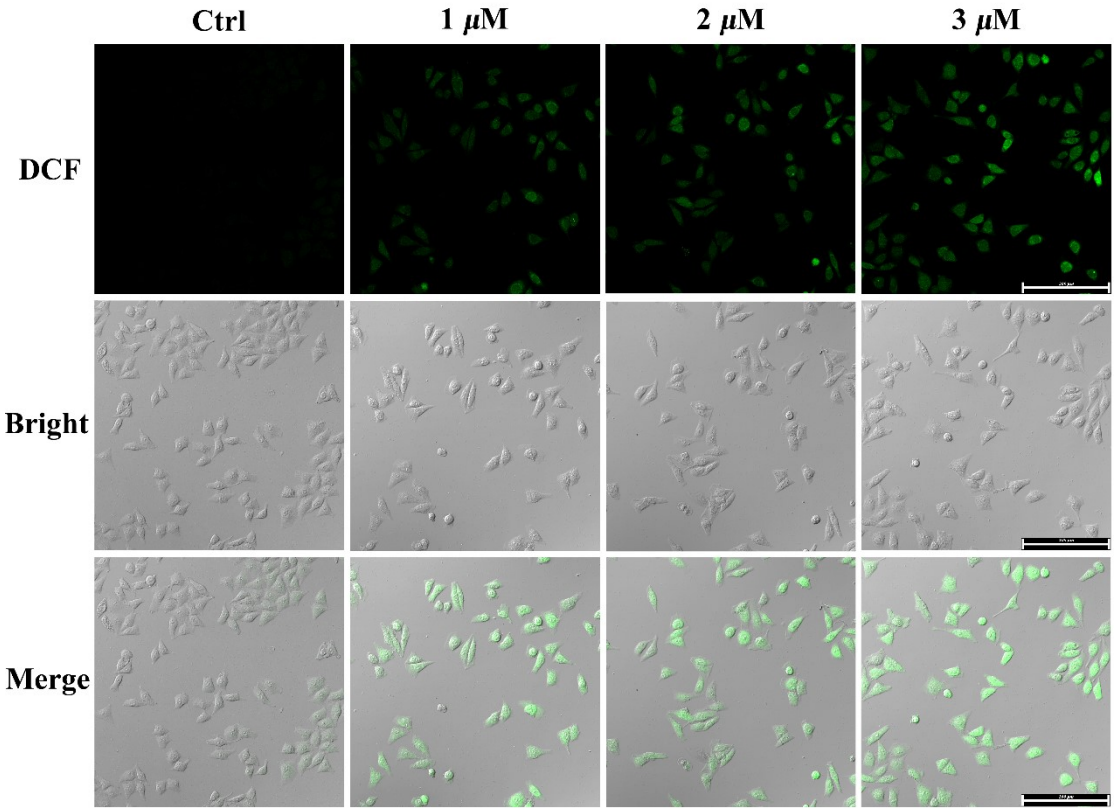

Figure S17 The intracellular ROS detection in T24 cells by DCFH-DA after treatment with **2** using a laser scanning confocal microscope. Scale bar: 200 μm.

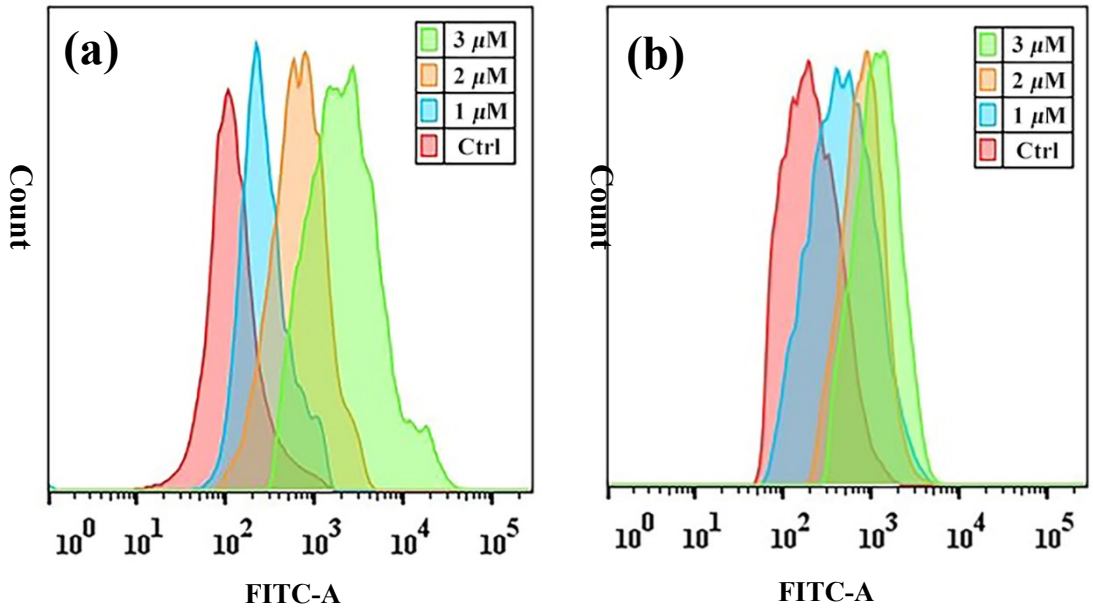

Figure S18 Analysis of ROS in T24 cells after treating with **1** (a) and **2** (b) using flow cytometry.