Supporting Information

Discovery of copper(II) complexes with plumbagin and bipyridine as chemodynamic therapy agents with enhanced antitumor activity by targeting mitochondria

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1. The characterization of Cu1–Cu4

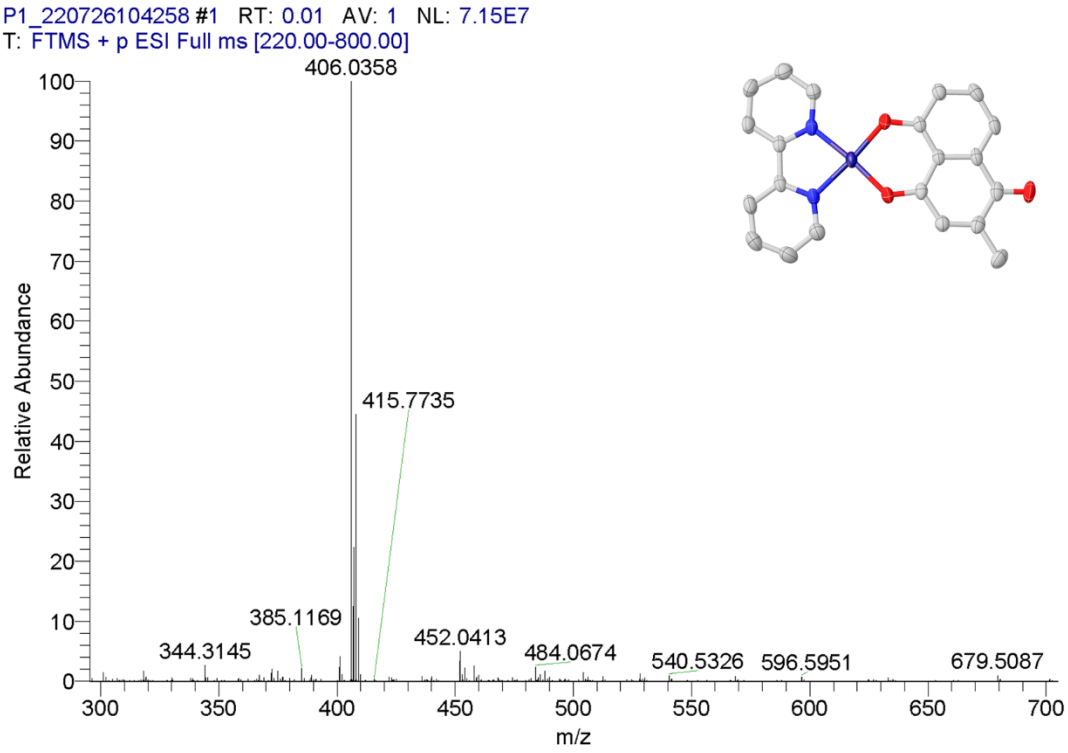

Figure S1. HRMS spectrum of **Cu1.**

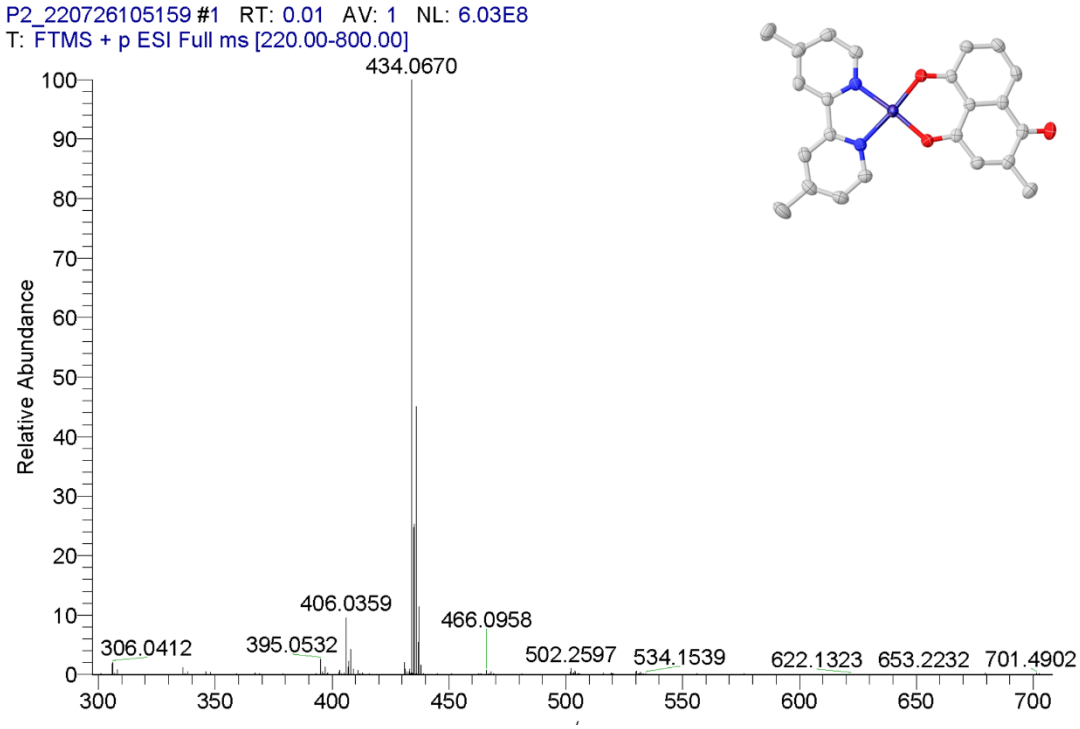

Figure S2. HRMS spectrum of **Cu2.**

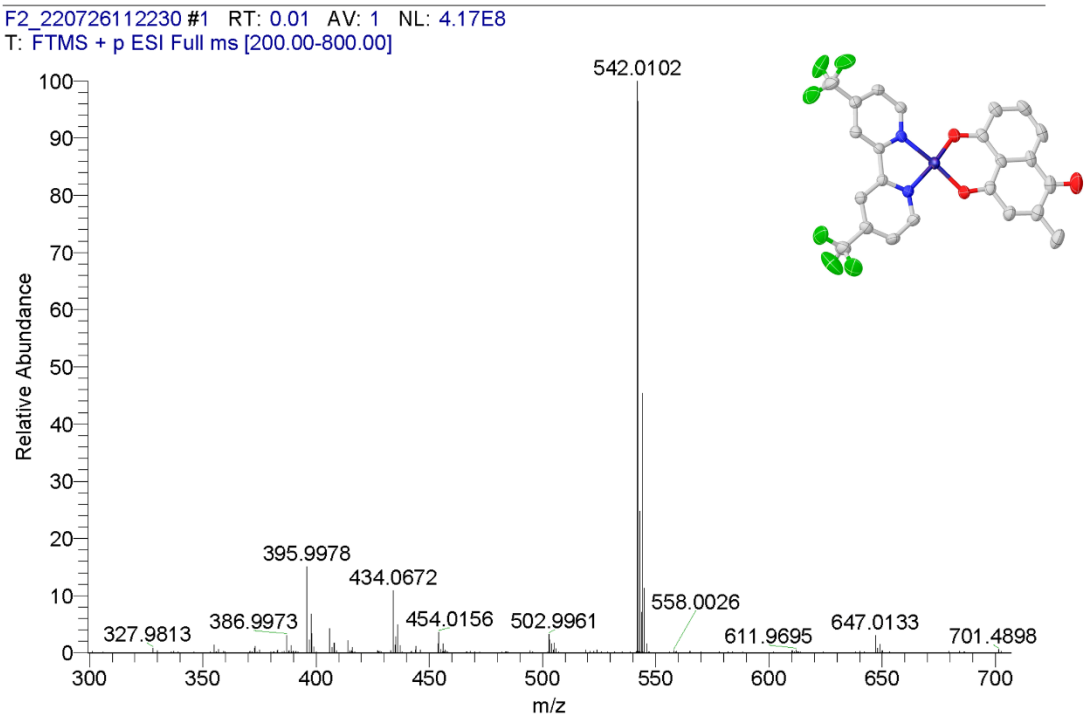

Figure S3. HRMS spectrum of **Cu3.**

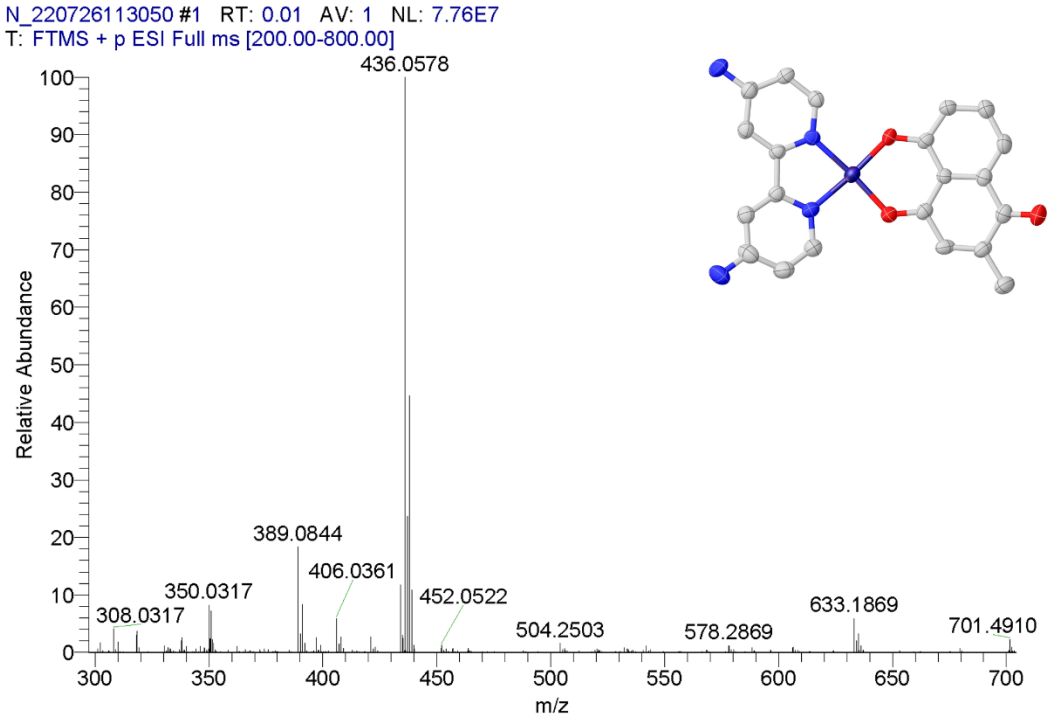

Figure S4. HRMS spectrum of **Cu4.**

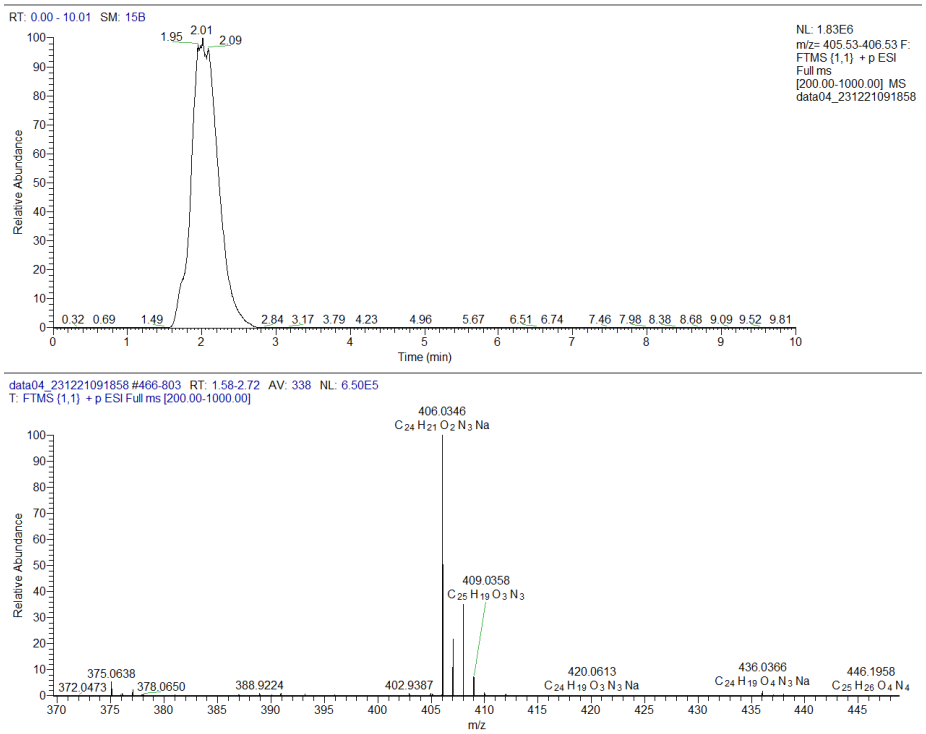

Figure S5. LC-MS spectrum of **Cu1**.

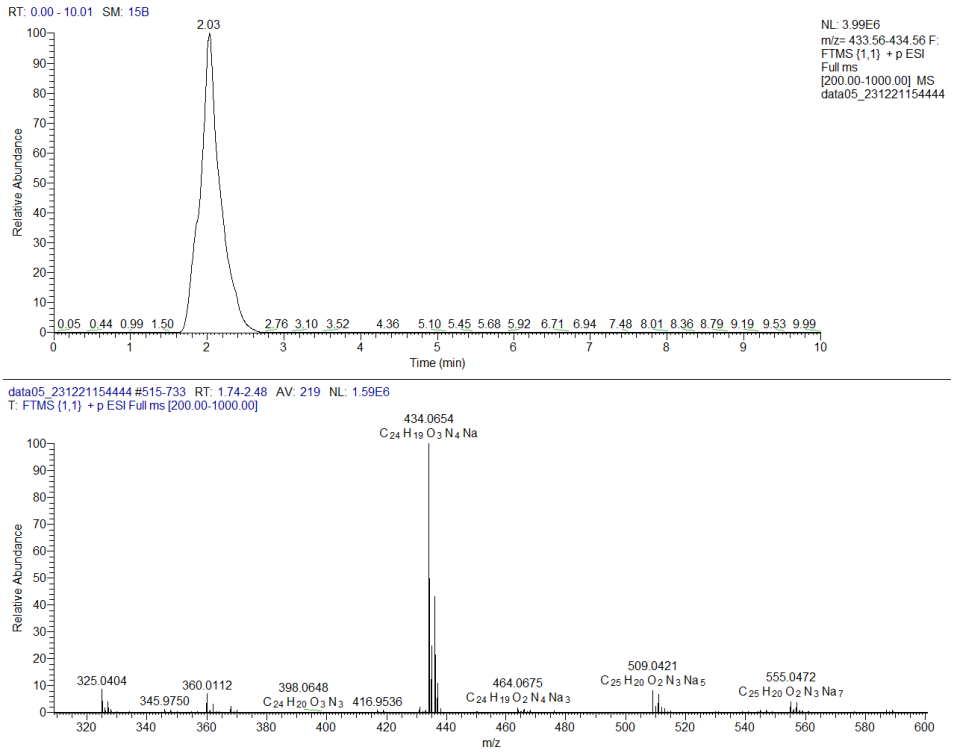

Figure S6. LC-MS spectrum of **Cu2**.

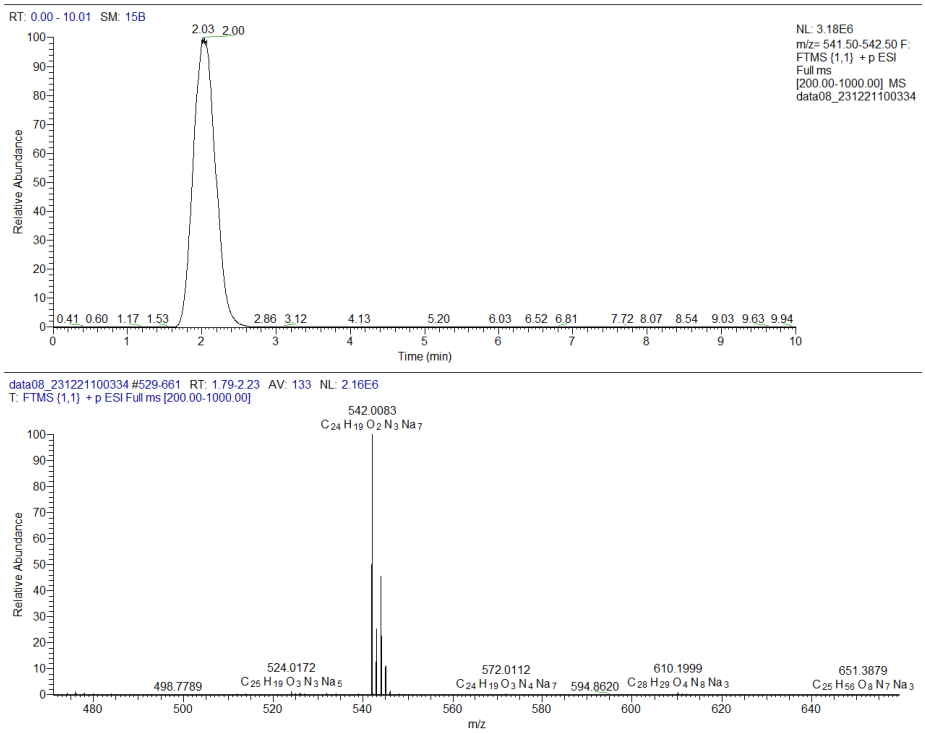

Figure S7. LC-MS spectrum of **Cu3**.

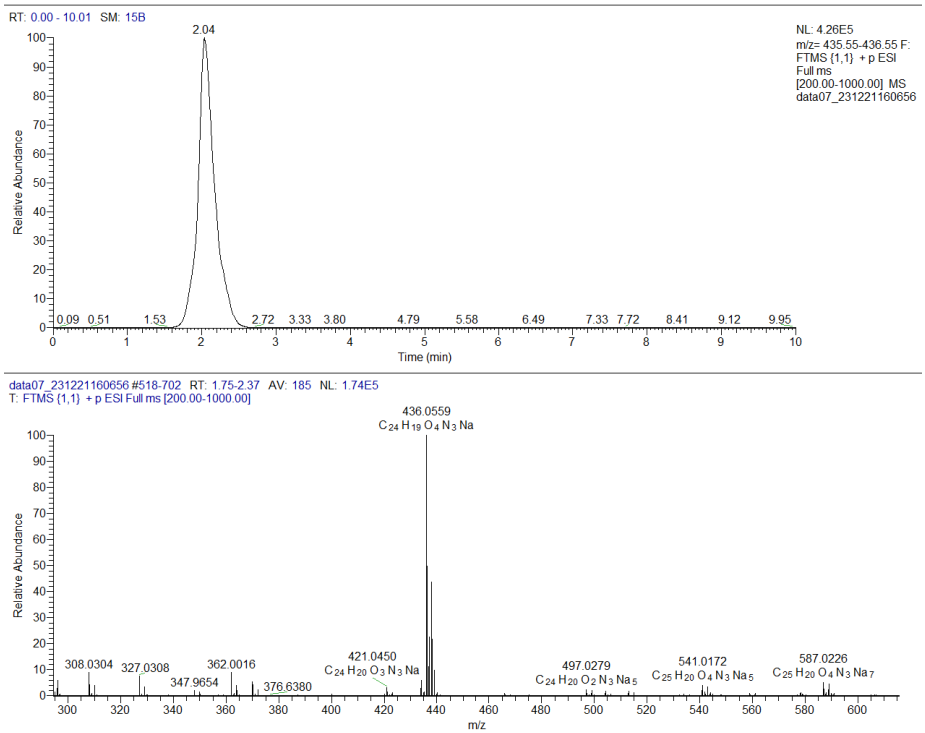

Figure S8. LC-MS spectrum of **Cu4**.

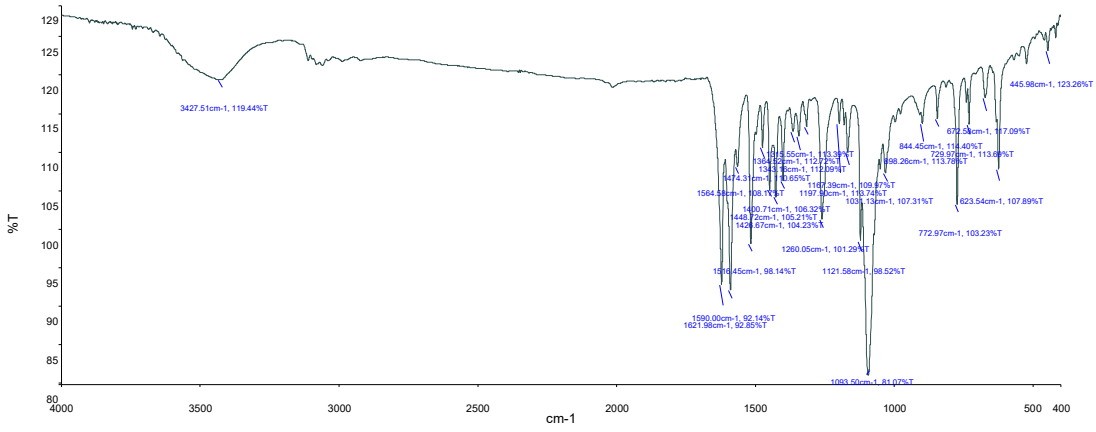

Figure S9. IR spectrum of **Cu1.**

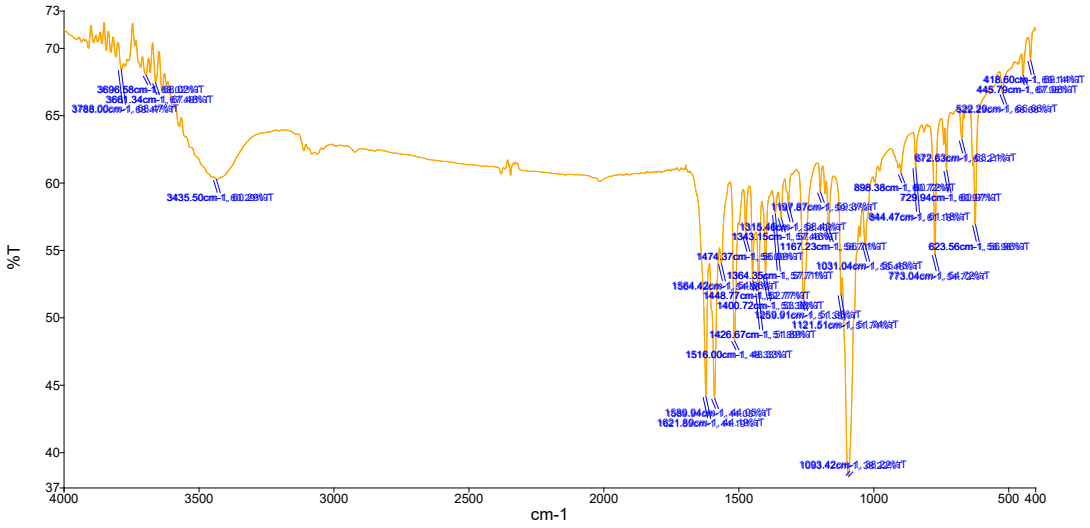

Figure S10. IR spectrum of **Cu2.**

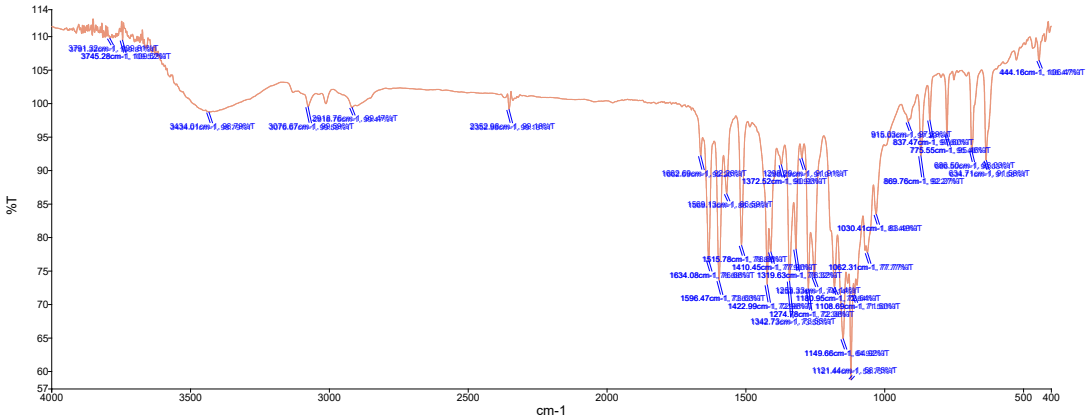

Figure S11. IR spectrum of **Cu3.**

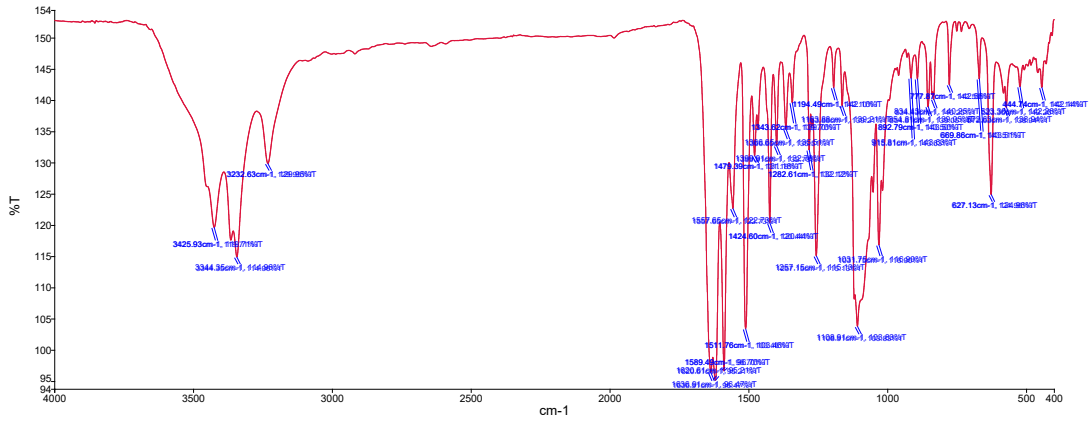

Figure S12. IR spectrum of **Cu4.**

2. Stability of Cu1–Cu4 in PBS

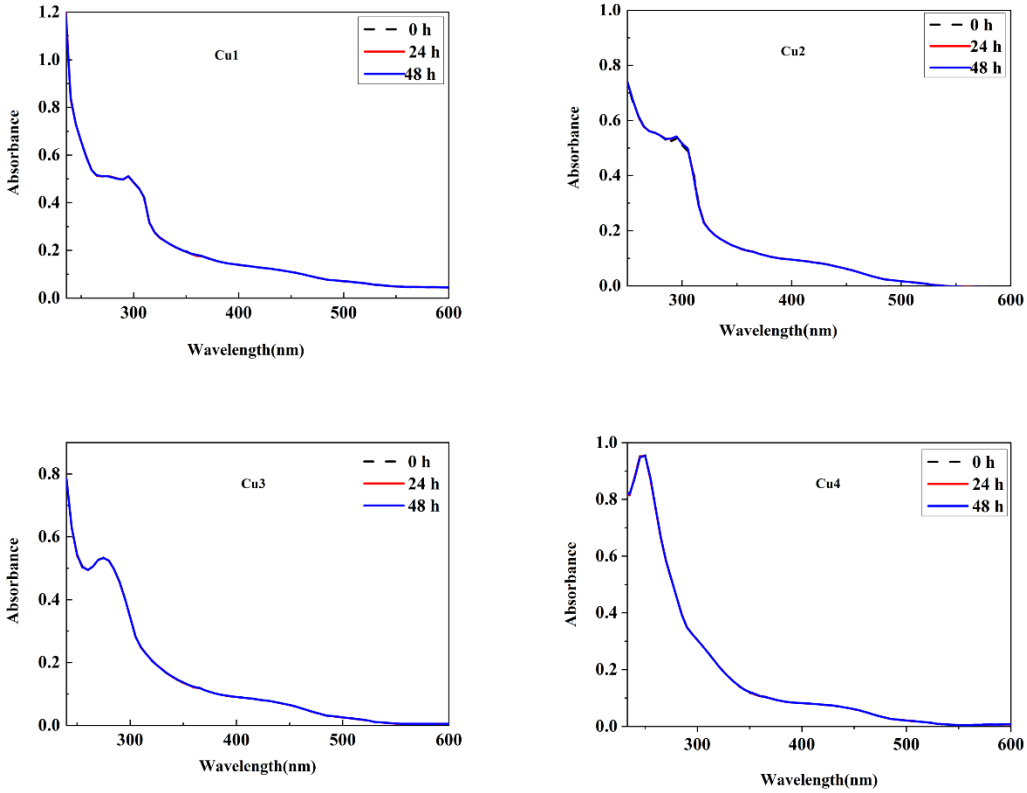

Figure S13. UV-Vis absorption spectra of **Cu1**–**Cu4** (2.0×10^{-3} M) in PBS buffer containing 1% DMSO solution in the time course 0 h, 24 h, and 48 h.

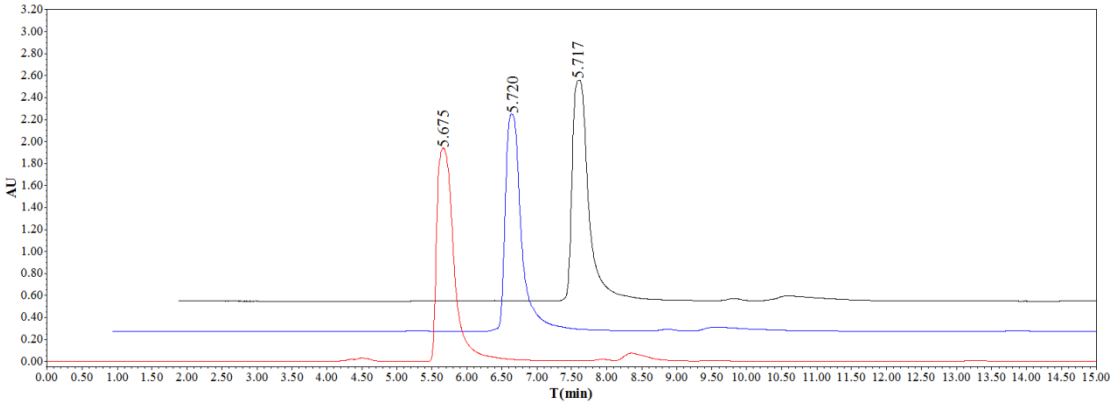

Figure S14. HPLC spectra of **Cu1** (2.0×10^{-3} M) at time 0 h, 24 h, and 48 h in DMSO/CH_3OH.

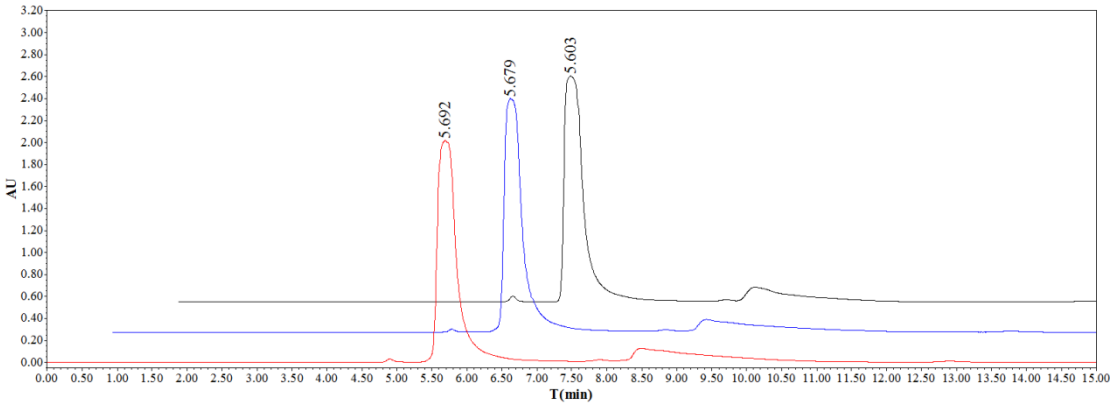

Figure S15. HPLC spectra of **Cu2** (2.0×10^{-3} M) at time 0 h, 24 h, and 48 h in DMSO/CH_3OH.

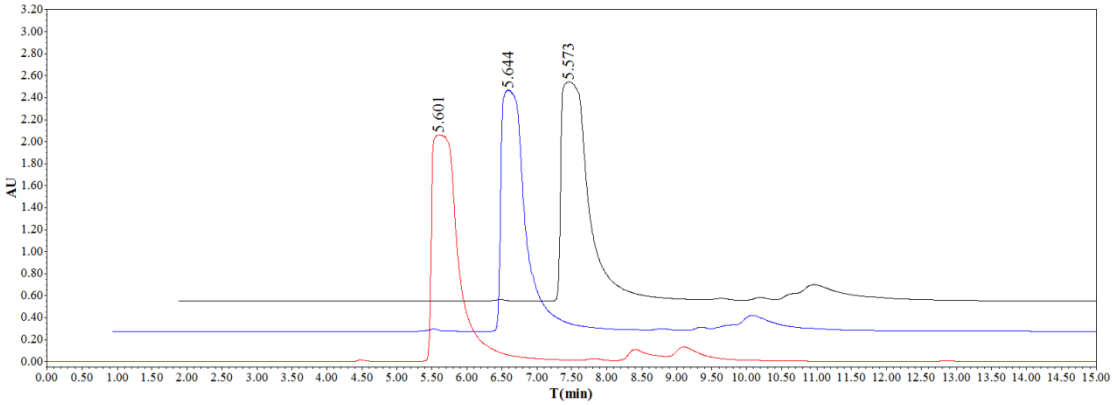

Figure S16. HPLC spectra of **Cu3** (2.0×10^{-3} M) at time 0 h, 24 h, and 48 h in DMSO/CH_3OH.

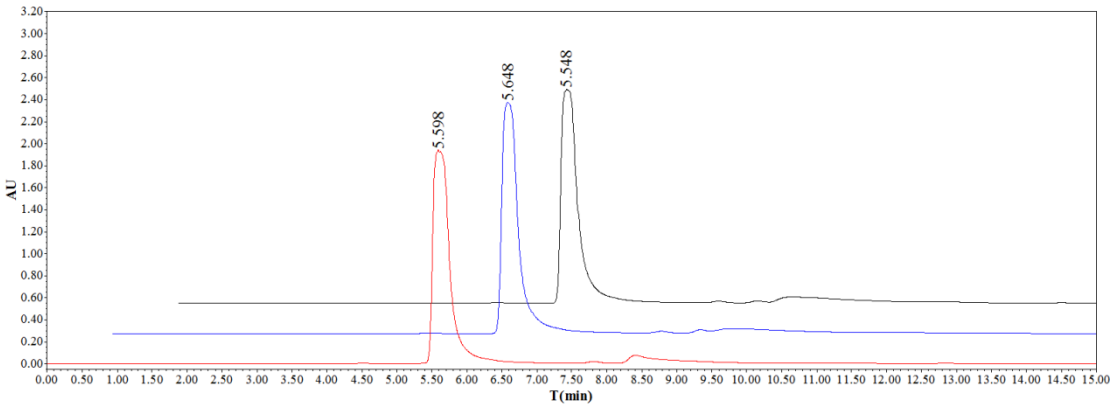

Figure S17. HPLC spectra of **Cu4** (2.0×10^{-3} M) at time 0, 24 h, and 48 h in DMSO/CH_3OH.

3. Solution behavior of Cu1–Cu4

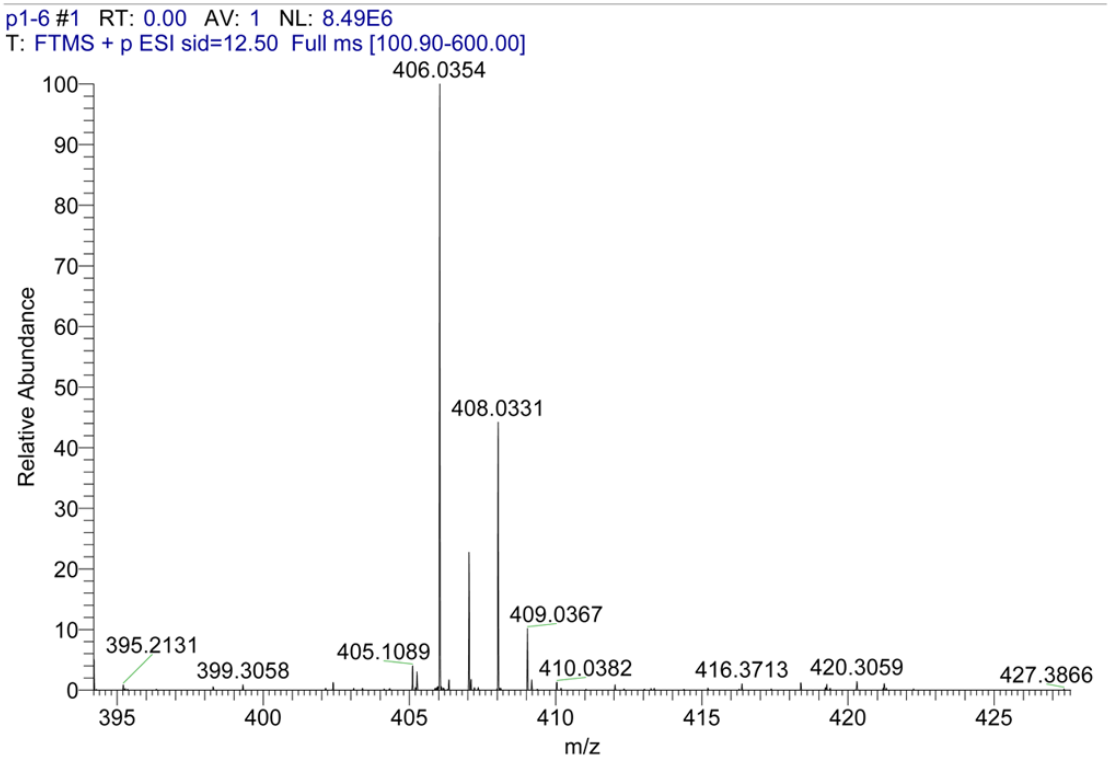

Figure S18. Mass spectrum of **Cu1** in solution.

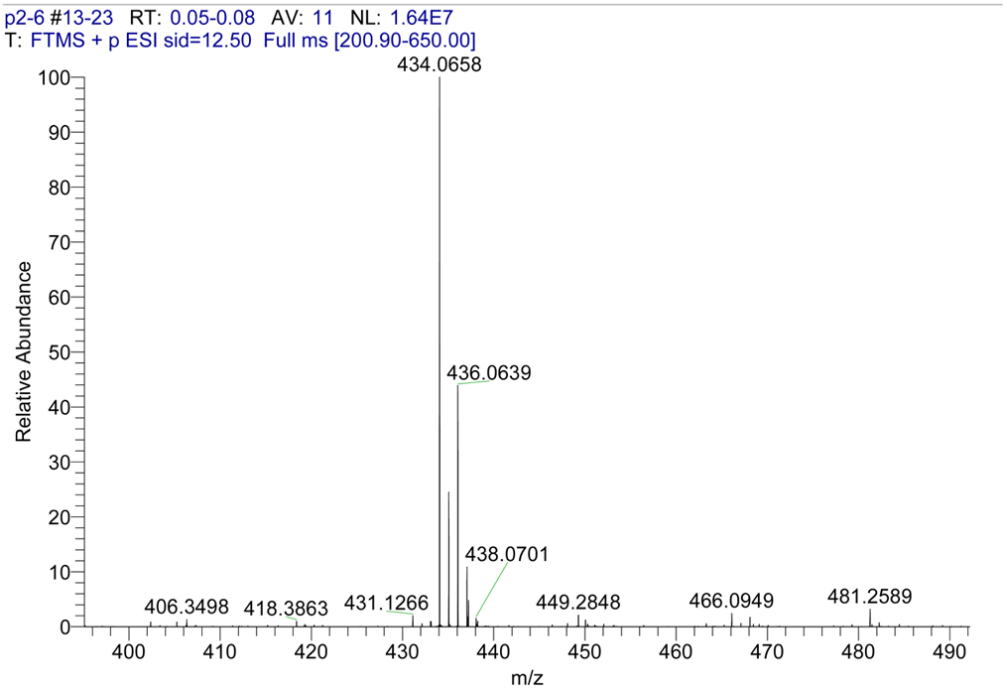

Figure S19. Mass spectrum of **Cu2** in solution.

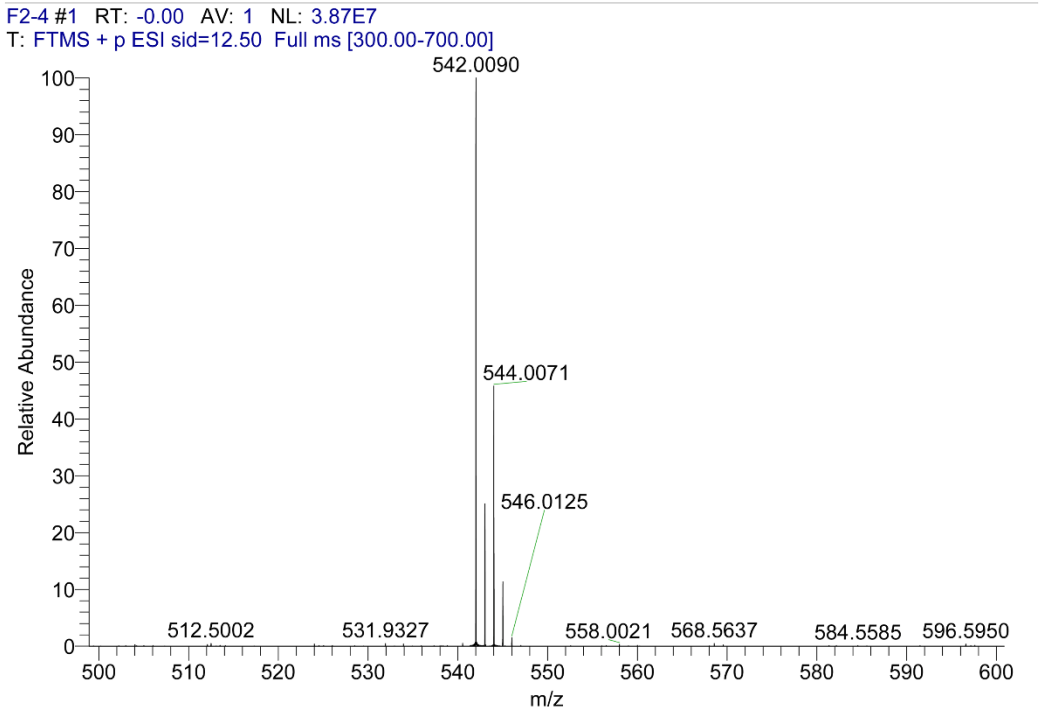

Figure S20. Mass spectrum of **Cu3** in solution.

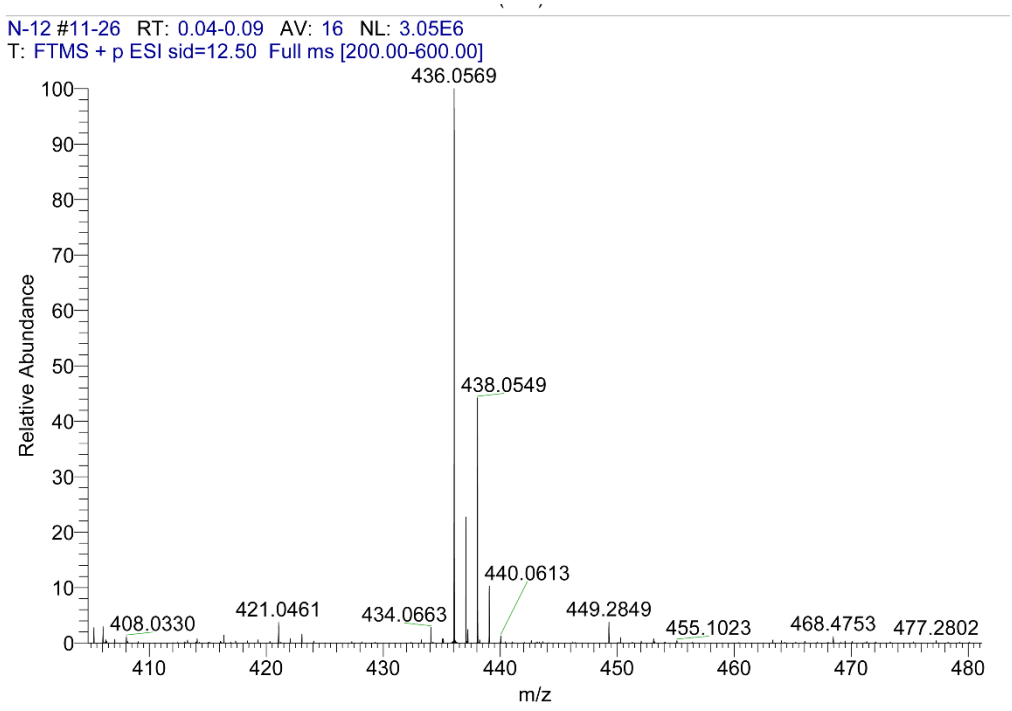

Figure S21. Mass spectrum of **Cu4** in solution.

4. Tables of crystal data of Cu1–Cu4

Table S1 Crystal data and structure refinement for **Cu1**

Complex	**Cu1**
Empirical formula	$C_{21}H_{15}ClCuN_2O_7$
Formula weight (M)	506.34
Crystal system	*Triclinic*
Space group	*P*-1
a (Å)	9.2985(5)
b (Å)	10.3543(7)
c (Å)	11.9616(7)
α (°)	112.775(6)
β (°)	107.363(5)
γ (°)	93.359(5)
V/(Å3)	993.44(11)
Z	2

Dc(Mg m^{-3})	1.693
F(000)	514.0
θ range for data collection (°)	3.4–26.3°
Reflections collected / unique	12106/4043 [$R_{(int)}$ = 0.0417]
Goodness-of-fit on F^2	1.049
R indices [$I > 2\sigma(I)$]	R_1 = 0.0527 ωR_2= 0.1248
R indices (all data)	R_1 = 0.0791 ωR_2= 0.1451

Table S2 Crystal data and structure refinement for **Cu2**

Complex	**Cu2**
Empirical formula	$C_{23}H_{19}ClCuN_2O_7$
Formula weight (M)	534.39
Crystal system	*Monoclinic*
Space group	$P2_1/n$
a (Å)	9.3791(2)
b (Å)	10.6291(2)
c (Å)	22.1108(4)
α (°)	90
β (°)	92.1043(17)
γ (°)	90
*V/(*Å3*)*	2202.78(8)
Z	4
Dc(Mg m^{-3})	1.611
F(000)	1092.0
θ range for data collection (°)	3.4–26.3°
Reflections collected / unique	13491/4498 [$R_{(int)}$ = 0.0257]
Goodness-of-fit on F^2	1.040
R indices [$I > 2\sigma(I)$]	R_1 = 0.0369 ωR_2= 0.0922
R indices (all data)	R_1 = 0.0453ωR_2= 0.0985

Table S3 Crystal data and structure refinement for **Cu3**

Complex	**Cu3**
Empirical formula	$C_{23}H_{13}ClCuF_6N_2O_7$
Formula weight (M)	642.34
Crystal system	*monoclinic*
Space group	$P2_1/c$
a (Å)	9.5260(4)
b (Å)	16.5988(5)
c (Å)	15.9965(5)
α (°)	90
β (°)	105.658(4)
γ (°)	90
V/(Å3)	2435.50(15)
Z	4
Dc(Mg m^{-3})	1.752
F(000)	1284.0
θ range for data collection (°)	3.3–28.8°
Reflections collected / unique	16165/5645 [$R_{(int)}$ = 0.0429]
Goodness-of-fit on F^2	1.060
R indices [$I > 2\sigma(I)$]	R_1 = 0.0612 ωR_2= 0.1314
R indices (all data)	R_1 = 0.1023 ωR_2= 0.1518

Table S4 Crystal data and structure refinement for **Cu4**

Complex	**Cu4**
Empirical formula	$C_{21}H_{17}ClCuN_4O_7$
Formula weight (M)	536.38
Crystal system	*triclinic*
Space group	*P*-1
a (Å)	9.4798(5)
b (Å)	10.4607(8)
c (Å)	12.3592(8)
α (°)	109.072(7)
β (°)	110.542(6)
γ (°)	90.238(5)
*V/(*Å3*)*	1074.86(14)
Z	2
Dc(Mg m^{-3})	1.657
F(000)	564.0
θ range for data collection (°)	3.2–28.8°
Reflections collected / unique	16062/5050 [$R_{(int)}$ = 0.0872]
Goodness-of-fit on F^2	1.001
R indices [$I > 2\sigma(I)$]	R_1 = 0.0708 ωR_2= 0.1518
R indices (all data)	R_1 = 0.1551 ωR_2= 0.1959

5. The HRMS spectra of Cu1–Cu4 reacted with GSH, separately

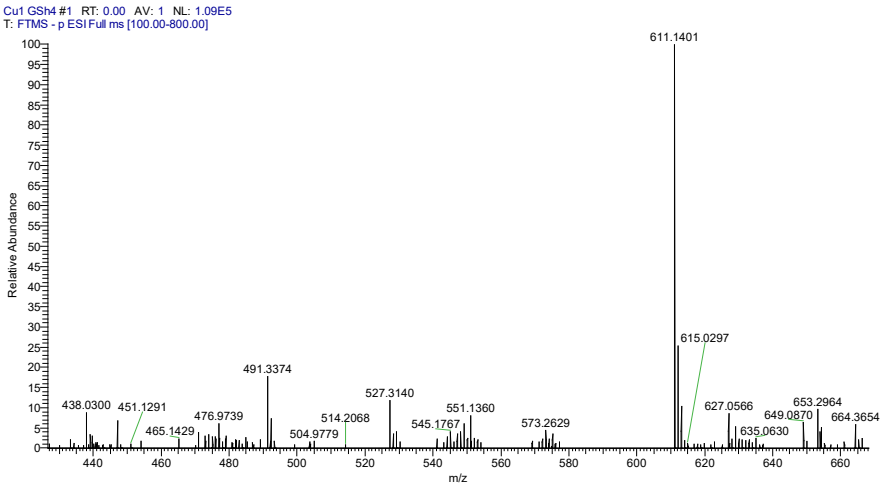

Figure S22. HRMS spectrum of **Cu1** with GSH.

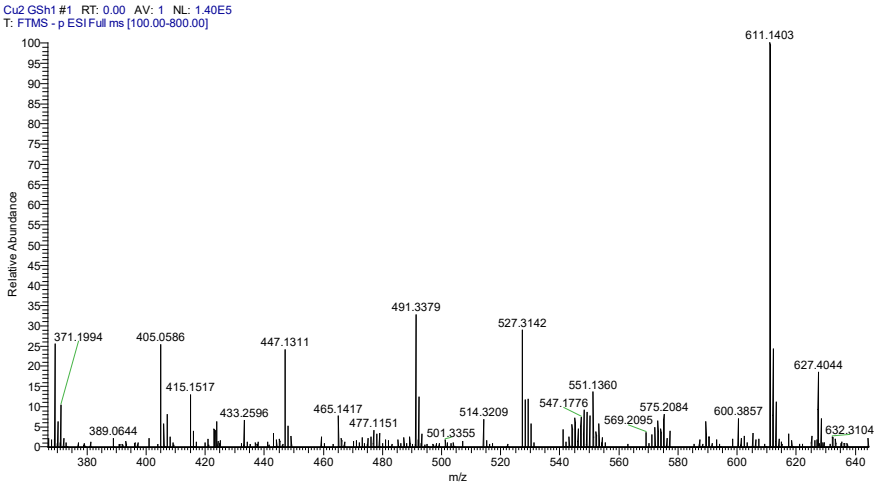

Figure S23. HRMS spectrum of **Cu2** with GSH.

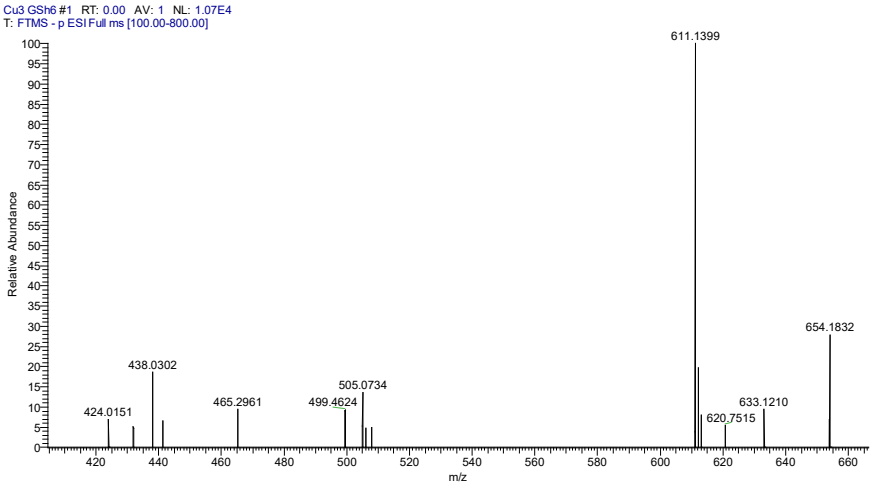

Figure S24. HRMS spectrum of **Cu3** with GSH.

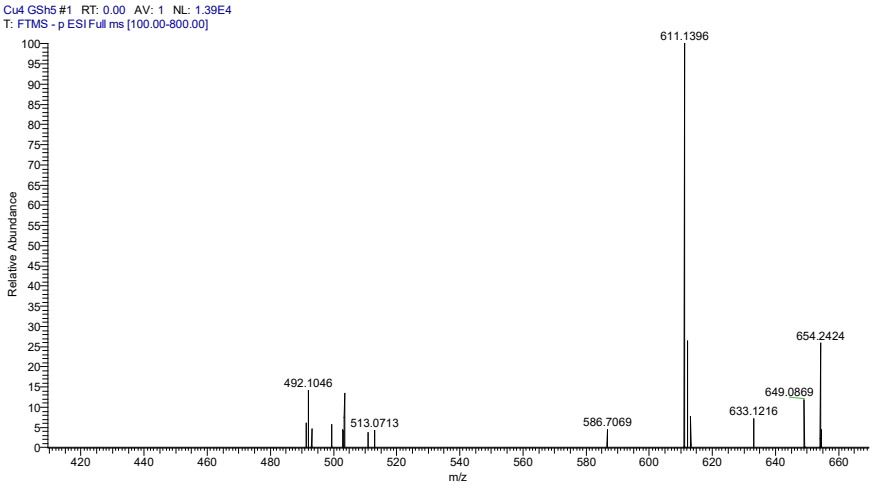

Figure S25. HRMS spectrum of **Cu4** with GSH.

6. Intracellular GSH / GSSG ratio

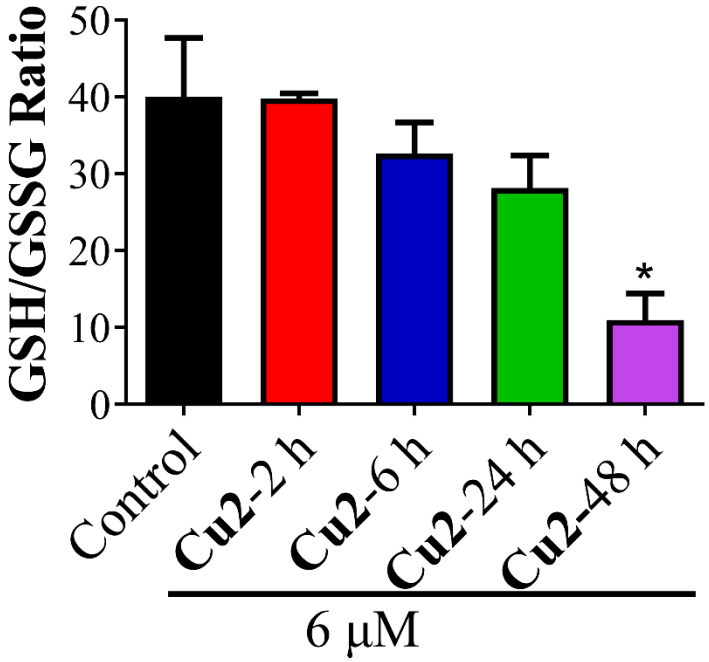

Figure S26. GSH/GSSG ratios in HeLa cells after 2 h, 6 h, 24 h and 48 h of exposure to **Cu2** (6 μM, *P < 0.05, ** P < 0.01, *** P < 0.001) .

7. Cell cycle arrest of Cu1–Cu4

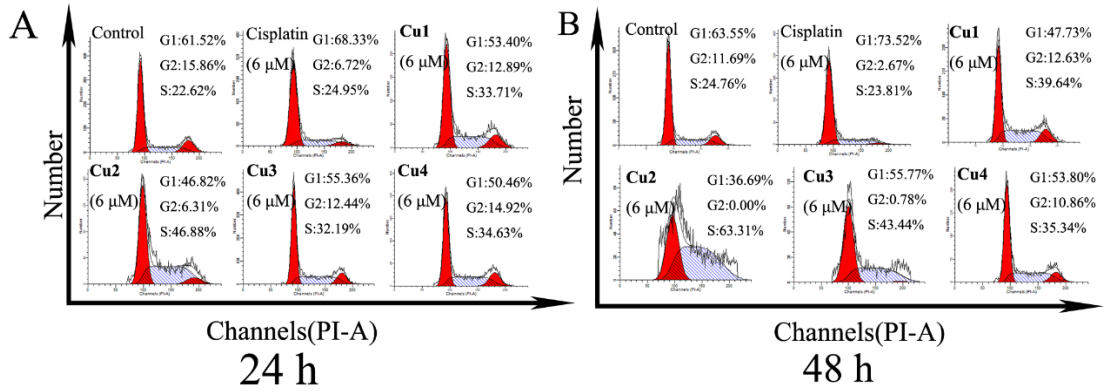

Figure S27. (A)(B) Cell cycle analysis of HeLa cells that treated with **Cu1**–**Cu4** (6 μM) and cisplatin (6 μM) for 24 and 48 h.

8. Expression of apoptosis-related proteins induced by Cu1-Cu4

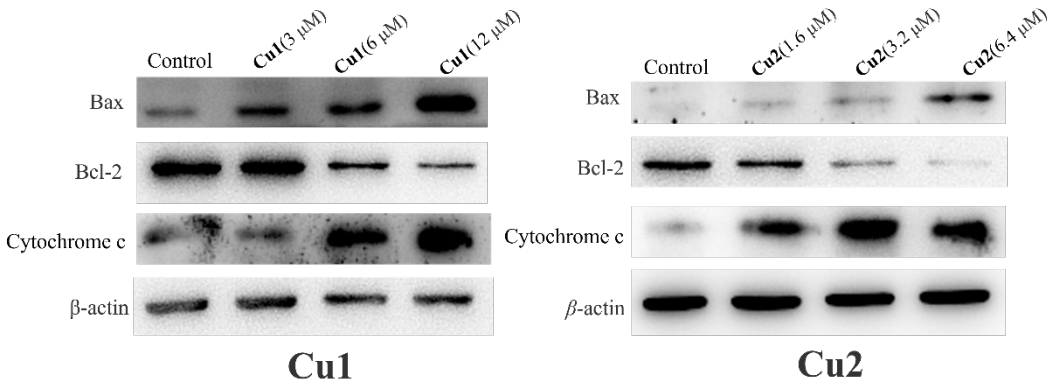

Figure S28**.** The expression level of apoptosis related proteins following **Cu1** and **Cu2** after exposure to 0.5x IC_{50}, 1x IC_{50}, and 2x IC_{50}.

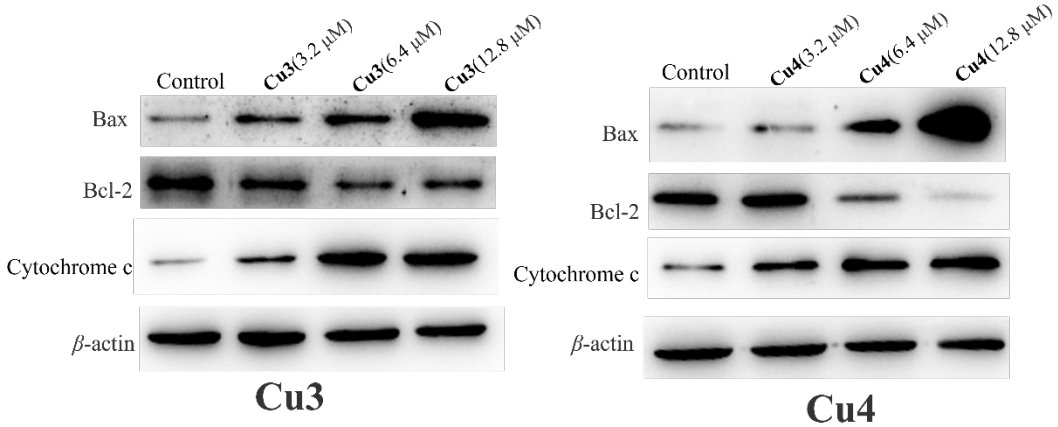

Figure S29**.** The expression level of apoptosis related proteins following **Cu3** and **Cu4** after exposure to 0.5x IC_{50}, 1x IC_{50}, and 2x IC_{50}.

9. pharmacokinetic evaluation of Cu2

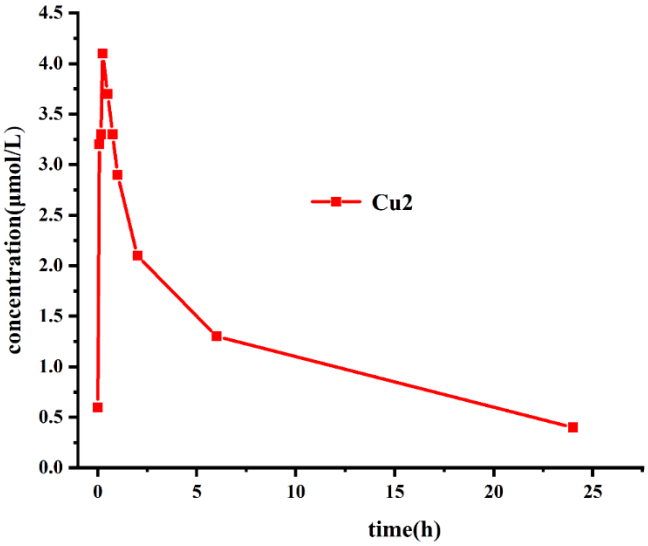

Figure S30. Pharmacokinetic evaluation in mice: concentration-time profile of **Cu2** in plasma following a single dose (20 mg/kg)

10. Acute toxicity test of Cu2

Table S5 acute toxicity of **Cu2** (alive/total)

	18.18 mg/kg	22.725 mg/kg	27.27 mg/kg
Cu2	6/6	6/6	4/6