A Novel Mn-Cu Bimetallic Complex for Enhanced Chemodynamic Therapy with Simultaneous Glutathione Depletion

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S1 Synthetic Procedures

S1.1 Materials and measurements

Amino-5-bromobenzoic acid (Alfa Aesar 98%), Ethyl chlorooxoacetate (Aladdin 98%), 3-(dimethylamino)-1-propylamine(Aladdin), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), calf thymus DNA (CT-DNA) and Ethidium bromide (EB) (Sigma-Aldrich), (5,5'-Dithiobis-(2-nitrobenzoic acid)) (DTNB) (Aladdin 98%), pUC19 DNA (thermo scientific). 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from Macklin (Shanghai). Annexin V-FITC/ Propidium Iodide double staining cell apoptosis kit, cell cycle kit and JC-1 apoptosis detection kit were purchased from KeyGEN BioTECH. All other chemicals were commercially available and used. All chemicals were used as received unless otherwise noted. ^{1}H and ^{13}C NMR spectra were determined using a Magnet System 400'54 Ascend NMR spectra (Bruker). High resolution mass spectrometric data were measured on a Q Exactive mass spectrometric (thermo scientific). The fluorescence imaging of cells was carried out on FV 3000 (OLYMPUS) confocal microscope with 60× objective lens. Single crystal X-ray diffraction measurement was carried out on Bruker D8 Venture diffractometer. Annexin V-FITC/PI double staining and cell cycle measurement were carried out on Attune NxT(Thermo Fisher Scientific) flow cytometry. FT-IR spectra were obtained as the KBr-pelleted samples on a 6700 spectrometer (Thermo Fisher Scientific). The inductively coupled plasma mass spectrometry (ICP-MS) data were determined on a NexION 300D ICP-MS (Perkin Elmer). UV-visible (UV-vis) spectra were measured on Cary 60 UV-Vis spectrophotometer. The fluorescence spectra were obtained on Cary Eclipse Fluorescence Spectrophotometer (Agilent techologies). Doubly purified water (from Milli-Q systems) was used to prepare buffers. A solution of CT-DNA in the buffer gave a ratio of UV absorbance at 260nm and 280nm, $A_{260}/A_{280}\approx1.9$, indicating that the protein content in DNA is zero[1]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($6600 M^{-1}cm^{-1}$) at 260nm[2].

S1.2 Synthesis of N-(2-carboxy-4-bromophenyl)-N'-[3-(dimethylamino)propyl]oxamide (H_3L).

The H_3L was prepared by modifying a reported procedure[3]. A solution of ethyl chlorooxoacetate

(1.638 g, 12 mmol) dissolved in tetrahydrofuran (8 ml) was added dropwise to a tetrahydrofuran solution (20ml) containing 2-amino-5-bromobenzoic acid (1.553 g, 10 mmol). The mixture was stirred for 1 h at 5℃. Then the solvents were evaporated by reduced pressure distillation. The resulting solid was washed by ethanol and dried under vacuum to afford the corresponding ethyl N-aryloxamate. Yield 90%.

A tetrahydrfuran solution (5ml) of 3-(dimethylamino)-1-propylamine (1.226 g, 12 mmol) was added dropwise to a solution of the above monoamide (2.7016 g, 10 mmol) in tetrahydrofuran (25 ml) with constant stirring for 12 h at 5℃. The resulting solid was filtered and washed by tetrhydrofuran and ethanol to afford the N-(2-carboxy-4-bromophenyl)- N'-[3-(dimethylamino) propyl] oxamide. Yield 86% IR (KBr ν/cm^{-1}):3333, ν(N-H); 1655, [V_{as}(COO) + V(C=O)]. ^{1}H NMR (DMSO-d_6): 14.78 (s, 1H, COOH), 8.97 (t, 1H, J = 6.0, 5.6 Hz, NH), 8.49 (d, 1H, J = 8.8 Hz,), 8.10 (d, 1H, J = 2.8 Hz,), 7.53 (dd, 1H, J = 2.8, 8.8 Hz,), 3.24 (m, 2H), 2.86 (t, 1H, J = 6.8 Hz), 2.55 (t, 1H, = 6.8 Hz), 2.38 (s, 6H), 2.18 (s, 3H), 1.72 (m, 2H). ^{13}C NMR (DMSO-d_6): 168.11 (COOH), 160.50, 159.01 (NHCOCONH), 138.58, 134.17, 133.03, 128.84, 121.23, 115.21 (Ph-H), 56.44, 56.38, 45.13, 44.42, 38.02, 37.72, 26.09, 24.89. HRESIMS m/z [M-H]$^{-}$ 370.0411 (calculated for $C_{14}H_{17}BrN_3O_4$, 370.0402).

S1.3 Synthesis of the monocopper(Ⅱ) complex (CuL)

A water solution (40 ml) of sodium hydroxide (1.2 g, 30 mmol) was added to a solution of above H_3L (10 mmol) in ethanol (50 ml) and water (10 ml) with stirring quickly for 15 min at ambient temperature. Then a water solution (10 ml) of cupric chloride (1.7 g, 10 mmol) added dropwise into the mixture in 30 min. The mixture was stirred for 3 h at ambient temperature. The resulting solid was filtered and washed by ethanol. Yield: (78%). IR (KBr ν/cm^{-1}): 1580.30 [V_{as}(COO) + V(C=O)].UV-Visible: λ_{max} (nm) [ε_{max} (M^{-1} • cm^{-1})] : 282 (11648)

S1.4 Synthesis of [Mn(phen)$_2$Cu(L)(CH$_3$OH)•ClO$_4$•2CH$_3$OH] (Complex 1)

A methanol solution (5 ml) of copper(II) perchloride hexahydrate (0.0371 g, 0.1 mmol) was added slowly into a methanol solution (10 ml) containing monocopper (Ⅱ) complex (0.04535 g, 0.1mmol). The mixture was stirred quickly for 30 min, then a methanol solution (5 ml) of 1,10-phenanthroline (0.03964 g, 0.2 mmol) was added dropwise into the mixture. The reaction solution

was heated at 60℃ with constant stirring for 5 h. The resulting blue solution was filtered and the filtrate was kept at room temperature. Dark blue crystals suitable for X-ray analysis were obtained from the filtrate by slow evaporation for seven days. Yield: 81%. IR (KBr ν/cm-1): 1610, [V_{as}(COO) + V(C=O)]; 1569, V(C=N). UV-Visible: λ_{max} (nm) [ε_{max} (M^{-1}•cm^{-1})] :270 (87619).

S1.5 Synthesis of [Ni(phen)$_2$Cu(L)(CH_3OH)•ClO_4•CH_3OH](Complex 2)

The synthesis method is similar to that of Complex 1, replacing copper perchlorate with nickel perchlorate. Yield: 79%. IR (KBr ν/cm^{-1}): 1610, [V_{as}(COO) + V(C=O)]; 1566, V(C=N). UV-Visible: λ_{max} (nm) [ε_{max} (M^{-1}•cm^{-1})] : 270 (92714).

S1.6 Synthesis of [Ni(bipyridine)$_2$Cu(L)(H_2O)•ClO_4](Complex 3)

The synthesis method is similar to that of Complex 2, replacing 1,10-phenanthroline ligand with 2,2-bipyridine ligand. Yield: 78%. IR (KBr ν/cm^{-1}): 1606, [V_{as}(COO) + V(C=O)]; 1565, V(C=N). UV-Visible: λ_{max}(nm)[ε_{max} (M^{-1}•cm^{-1})] :295 (41661), 307(40014).

S2 Characterize and analysis of complexes

S2.1 Crystal structure determination and refinement

The crystal structures of the heterobimetallic complexes were obtained by single crystal X-ray diffraction technique. Single crystal X-ray diffraction measurement was carried out on Bruker D8 Venture diffractometer at 123 k using graphite monochromatic microfocus MoKα radiation (λ = 0.71073 Å, 50 kV, 40 mA). Single crystal was selected and mounted on a nylon loop in Paratone-N cryoprotectant. Unit cell determination was performed in the Bruker SMART APEX III software suite. The data sets were reduced and a multi-scan spherical absorption correction was carried out in the SCALE interface. The structure of the heterobimetallic complexes were solved by the directed method and followed by full matrix least-squares procedures on F^2 using SHELXL-2018 Program package[4]. WinGX was employed for the final data presentation and structure plots[5]. Crystal data and refinement data are summarized in Table S1. Selected bond length and angles are given in Table S2, S3,S4.

S2.2 DNA interaction studies

Solutions of CT-DNA were prepared by dissolving CT-DNA in Tris-HCl/NaCl buffer at PH=7.4 (50 mM NaCl/5 mM Tris-HCl). The concentration of the prepared CT-DNA stock solution (PH 7.4) was determined by UV absorbance intensity at 260 nm with extinction coefficient of ε_{260}=6600 $M^{-1}cm^{-1}$. Stock solution of DNA was stored at 4℃ and used within 4 days.

The UV-Vis absorption titration experiments were carried out by keeping the constant concentration of the complexes with 10 μM, while varying the concentration of the CT-DNA. To eliminate the absorption of DNA itself, equal quantity of CT-DNA was added to both the complexes solution and the reference solution. Stock solutions of complexes and DNA were allowed to incubate for 5 min before recording.

The fluorescence spectral technique was performed to study the binding interaction between the heterobimetallic complexes and CT-DNA. In the ethidium bromide (EB) fluorescence quenching experiment, stock solution of the heterobimetallic complexes (1×10^{-2} M) in Tris-HCl/NaCl buffer was freshly prepared before use. Fluorescence intensities at 600 nm (525 nm excitation) were carried out by titrating complexes into EB-bound CT-DNA solution with [EB] =1 μM and [CT-DNA] =5 μM. The complexes were titrated into the solutions of DNA/EB and then obtained the solutions with the different mole ratio of the complexes to CT-DNA. Before measurements, the mixtures were well mixed and incubated for 10 min at room temperature. The fluorescence spectra of EB binding CT-DNA were obtained in the range of 530-750 nm.

The quenching of EB bound to DNA by complexes is in agreement with the linear Stern-Volmer equation[6]:

$I_0=I+K_{sv}[Q]$

Where I_0 and I represent the fluorescence intensities in the absence and presence of quencher, respectively. K_{sv} is a linear Stern-Volmer quenching constant, [Q] is the concentration of quencher. In the quenching plot (see Fig. 1(d)) of I_0/I versus [complex], K_{sv} is derived from the ratio of the slope to intercept. The K_{sv} values are 3.93×10^4 (R^2=0.99301), 1.78×10^4 (R^2=0.99013), 0.765×10^4 (R^2=0.99481) for Complex 1, Complex 2, Complex 3, respectively.

S2.3 DNA cleavage

Supercoiled DNA (pUC19) was purchased from thermo scientific. The solution of supercoiled pUC19 DNA (20 ng/ml) was incubated with the different concentration complexes for 12 h in Tris-HCl buffer(10 mM Tris-HCl, 1mM EDTA, PH 7.6, total 10 μl). Then 2 μl 6*loading buffer was added into above DNA and mixed evenly. Gel electrophoresis experiment was carried out with 1.5% (Tris-Acetate EDTA (TAE) buffer) agarose gel at 150 v 30 min in 1*TAE buffer. The agarose gel was stained 20 min with solution of Ethidium bromide (1 μg/ml) after electrophoresis, then analyzed with the FluorChem R (Proteinsample) gel imaging system.

S2.4 Detection the •OH using methylene blue (MB)

A certain concentration of MB solution was prepared in 25 mM $NaHCO_3$/ 5% CO_2 buffer solution with 8 mM H_2O_2 and 50 μM different complex. The mixing system was incubated 30 min at 37℃, then absorbance was measured by UV-vis spectroscopy. TEMPO was added to a sample containing MB, 50 μM Complex 1, 8 mM H_2O_2 and 25 mM $NaHCO_3$, followed by incubating for 30 min at 37℃. The absorption of MB was then measured.

S2.5 GSH depletion

GSH levels were evaluated by Ellman's reagent. Solutions with 10 mM GSH and 10 μM complexes were incubated at 37℃ for 0-12 h. The samples were centrifuged at 12000 rpm for 10 min, and the supernatants were collected. DTNB solution was added to the supernatant and co-incubated for 5 min at 25℃, then the absorbance was measured with a microplate reader.

S2.6 Cell incubation

Mouse breast cancer cells (4T1 cells), human liver cancer cells (HepG2 cells), human lung cancer cells (A549 cells), human breast cancer cells (MCF7 cells) and normal were purchased from Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences. All the cells were incubation in Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% Penicillin-Streptomycin Solution (Gibco) in 5% CO_2 incubator with 37℃.

S2.7 Cellular uptake

A549 cells were seeded into a six-well plate at a density of 107 cells and incubated for 12 h. Then the medium was replaced with fresh medium containing 4 μM Complex 1 and the cells were incubated for another 12 hours in a 37 ℃incubator with 5% CO_2. Discard the medium and wash the cells three times with PBS, then harvest the cells. Mitochondria and nucleus were isolated separately using the mitochondria/nuclear separation kit (KeyGEN BioTECH). The resulting mitochondria and nuclei were digested with nitric acid, 30% hydrogen peroxide, and concentrated hydrochloric acid. The content of copper in the nucleus, mitochondria and the whole cells was determined by ICP-MS.

S2.8 In vitro cytotoxicity assay

The in vitro cytotoxicity assay was carried out to evaluate the cytotoxicity of the complexes on 4T1, HepG2, A549 and MCF7 cells. The cell viability was determined based on the method of Mabley et al.[7] by the reduction of yellow MTT into purple formazan product by the enzymatic activity of mitochondrial dehydrogenase in the live cells [8]. In this assay, about 5000 cells of 4T1, HepG2, A549 and MCF7 cells were seeded in each well of a 96-well culture plate and incubated for 12 h in a 5% CO_2 incubator with 37℃. Then different concentrations of the complexes were added to the cells medium (200 μl). After 48 h incubate, 20μl MTT (5 mg ml^{-1}) in phosphate buffer saline (PBS) was added into each wells and incubated for another 4 h. The purple formazan crystals obtained were dissolved in 200 μl DMSO and the absorbance was measured at 490 nm using a Molecular Devices SpectraMax i3x plate reader and the cell viability was calculated by the following equation:

Cell viability (%)=(OD_{sample}-$OD_{blank\ control}$/$OD_{control}$-$OD_{blank\ control}$)×100%

The MTT assay was carried out in triplicates.

S2.9 Cell cycle

A549 cells were seeded into a six-well plate at a density of 10^6 cells and incubated for 12 h. Then the medium was replaced with fresh medium containing different concentration complexes (0, 1, 2,

4 μM) and the cells were incubated for another 12 h in a 37℃ incubator with 5% CO_2. The medium was discarded and the cells were washed with PBS, then the cells were harvested. The cells were washed again and fixed with 70% ethanol overnight. The cells were stained with PI according to the manufacture's protocol and then analyzed by flow cytometry.

S2.10 Determination of intracellular ROS

A549 cells were seeded in a six-well plate at a density of 10^6 cells and cultured overnight. The 4 μM different complexes were added to the cell culture dish and cultured for another 12 h at 37℃, 5% CO_2. The medium was discarded. The cells were washed three times with PBS then the cells were harvested and stained with 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) probe for a 30 min. Next, the cells was analyzed by flow cytometry.

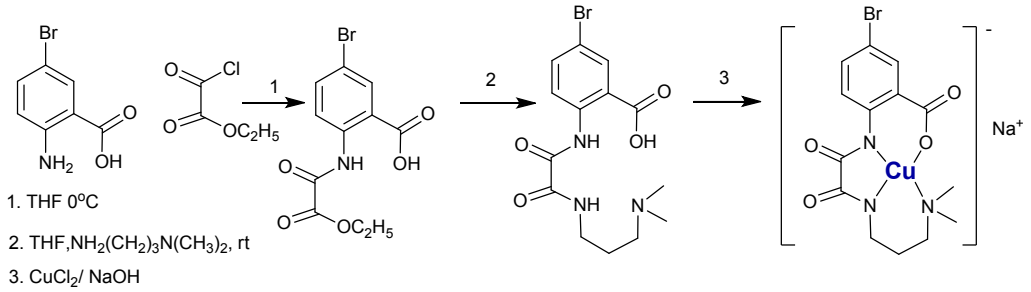
S2.11 Detection of mitochondrial membrane potential

The 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolo-carbocyanine Iodide (JC‐1) was used as an indicator for detecting the mitochondrial membrane potential. JC-1 apoptosis detection kit was purchased from KeyGEN BioTECH. The JC-1 solution was prepared according to manufacturer's instructions. A549 cells were seeded in 35 mm confocal dishes and incubated 12 h at 37℃, 5% CO_2, then the medium was replaced by fresh medium containing different concentration Complex 1 and incubated another 12 h. After treatment, cells were stained with JC-1 apoptosis detection kit according to the manufacture's instructions. Changes in mitochondrial membrane potential can be observed by single photon laser confocal microscopy with excitation wavelength of 488 nm and 561 nm, and emission wavelength was collected from 510-550 nm (green channel) and from 570-620 nm (red channel).

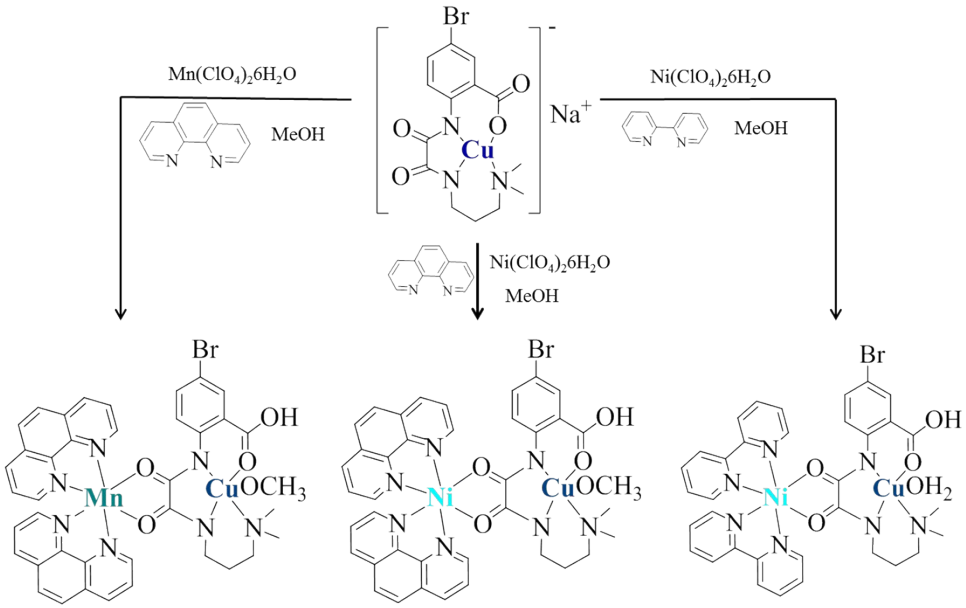
S2.12 Annexin V-FITC/PI double staining

A549 cells were seeded in a six-well plate at a density of 10^6 and cultured overnight. The different concentrations (4 μM, 8 μM and 16 μM) of Complex 1 were added to the six-well plate and cultured for another 12 hours. Cells were stained with Annexin V/PI according to the instructions and analysed by flow cytometry.

S3 Supplementary Figures



Scheme S1. The synthetic pathway of CuL.



Complex 1 Complex 2 Complex 3

Scheme S2. The synthetic pathway of Complexes 1-3.

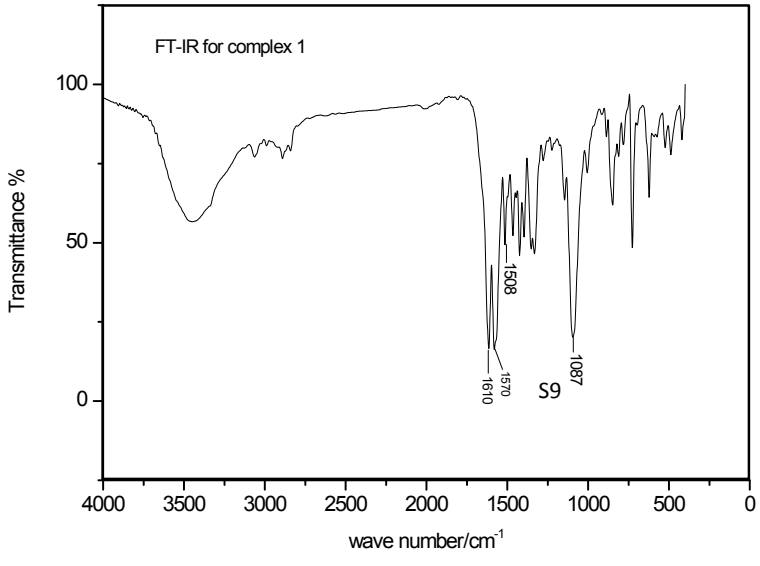


Fig. S1. FT-IR spetrum of Complex 1.

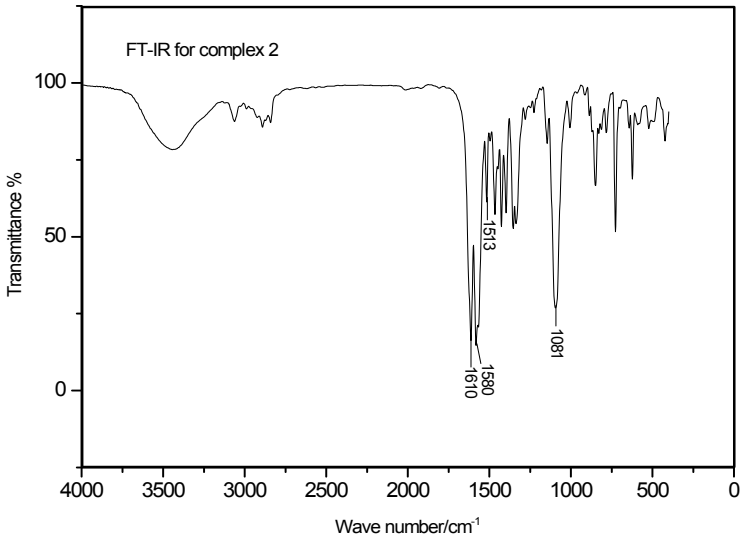


Fig. S2. FT-IR spetrum of Complex 2.

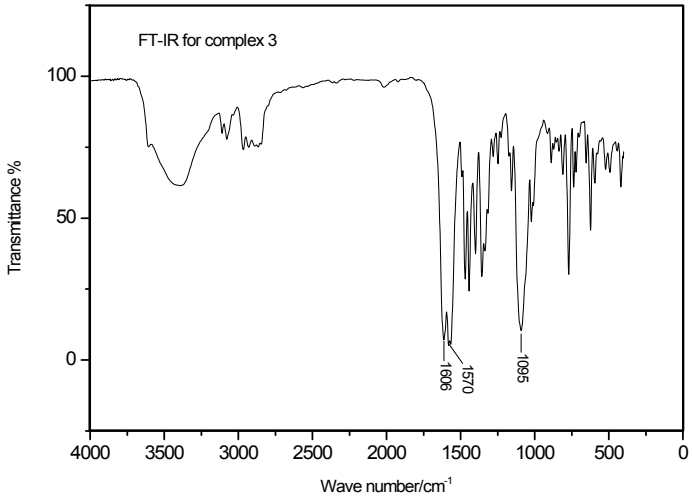


Fig. S3 FT-IR spectrum for Complex 3.

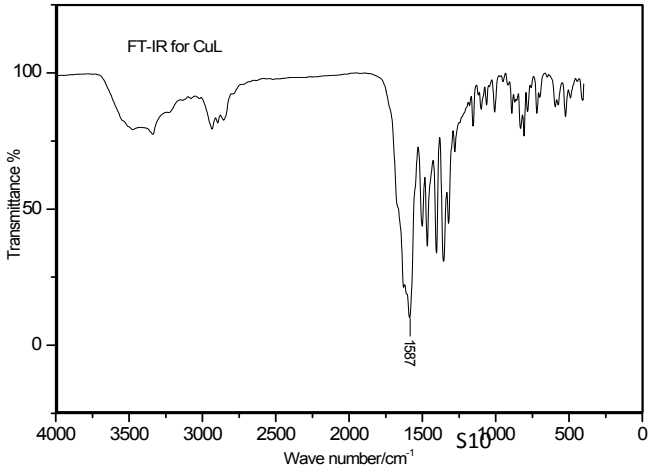


Fig.S4. FT-IR spectrum of CuL.

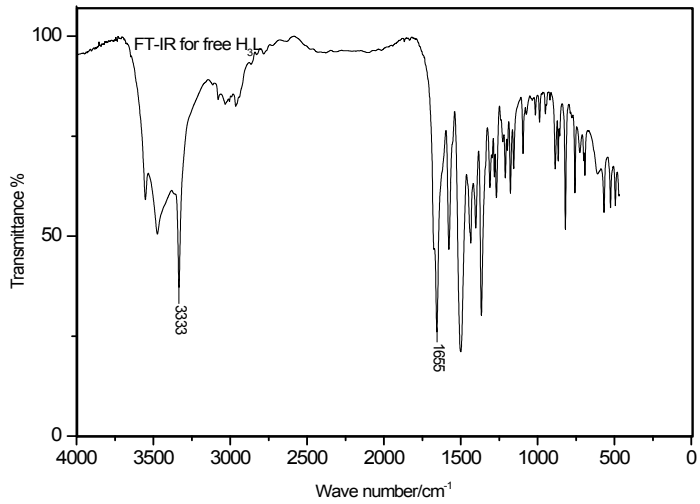


Fig. S5. FT-IR spectrum of H3L.

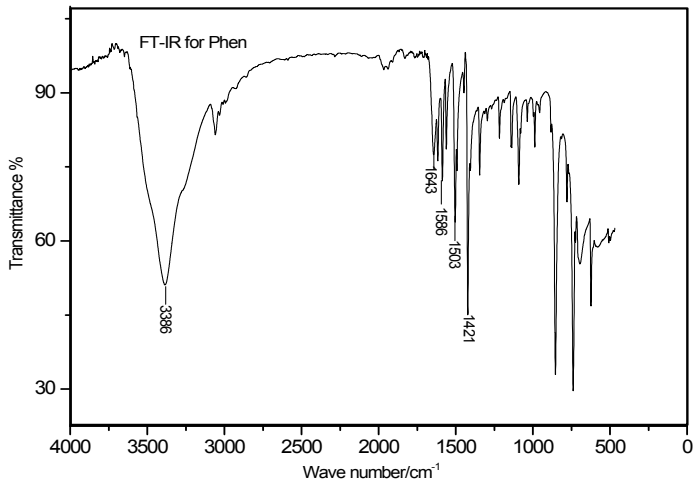


Fig. S6. FT-IR spectrum of 1,10-phenanthroline.

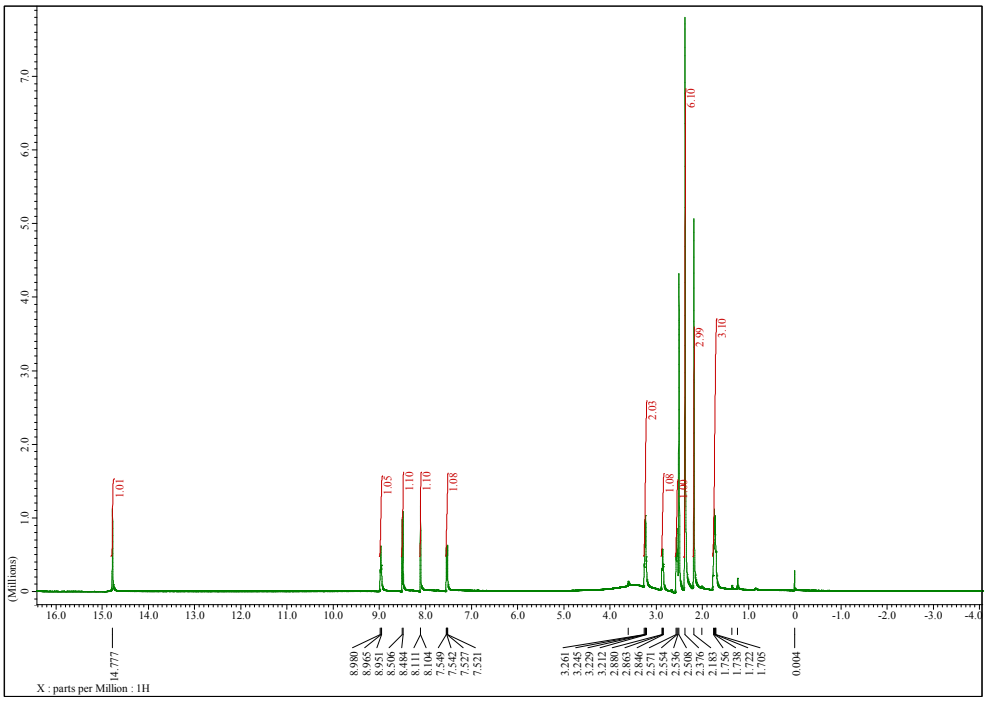


Fig. S7. ^{1}H NMR spectrum for H_3L.

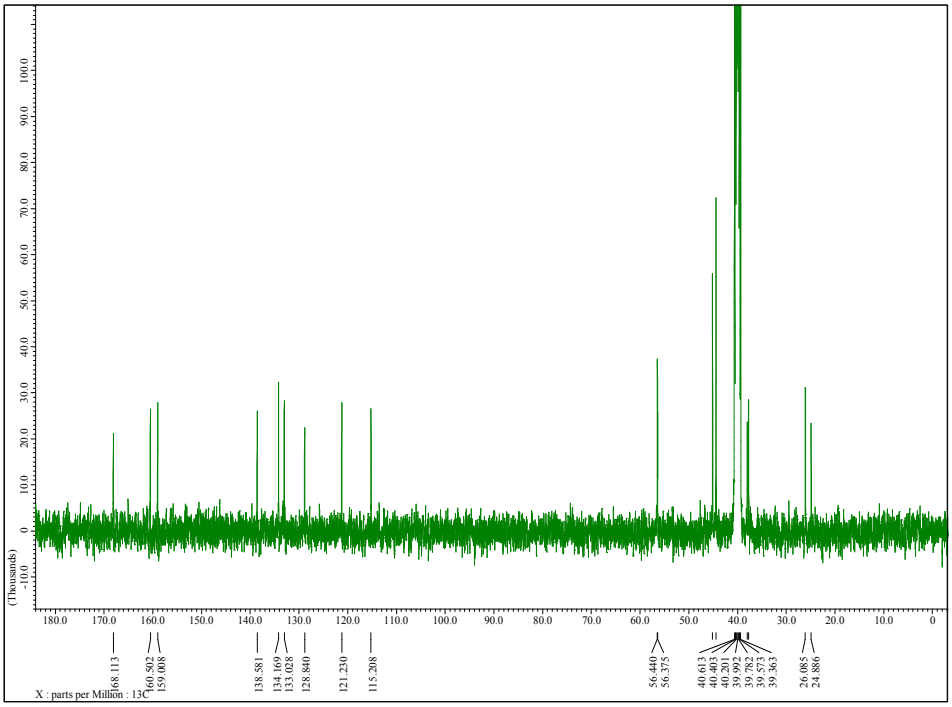


Fig. S8. ^{13}C NMR spectrum for H_3L.

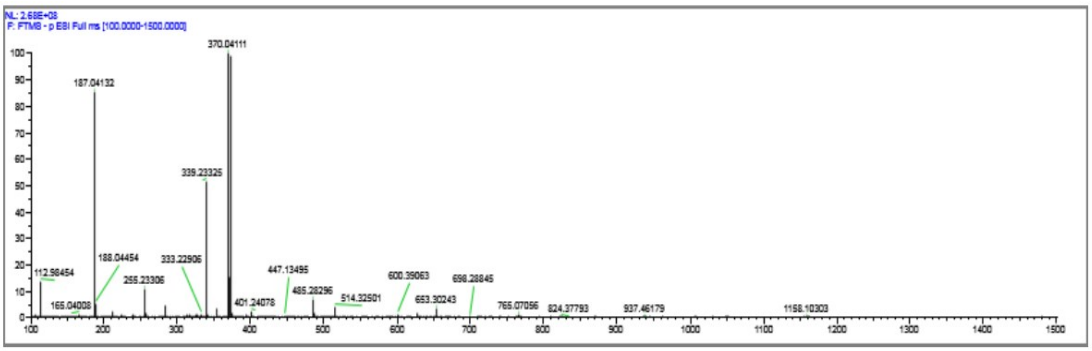


Fig. S9. HR-MS spectrum for H_3L.

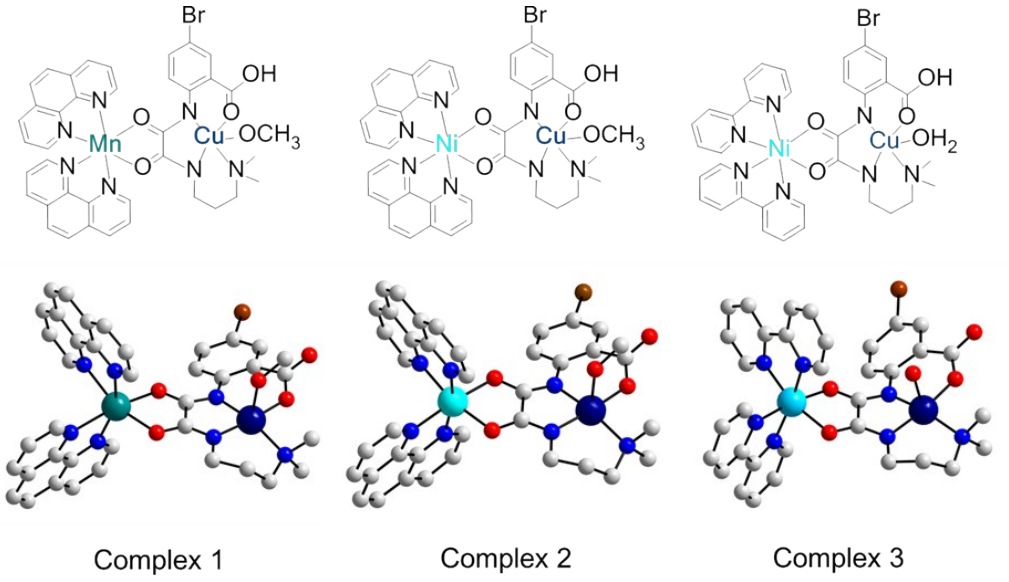


Fig. S10. Chemical and crystal structure of the complexes. C: gray; O: red; N: blue; Br: brown; Cu: dark blue; Ni: sky blue. The H atoms and solvent molecules are omitted for clarity.

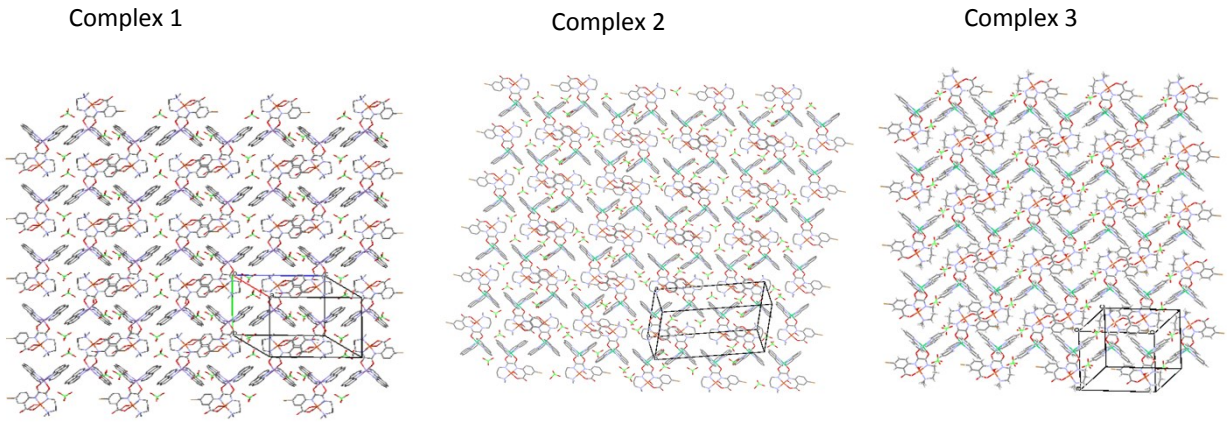


Fig. S11. Packing diagram of complexes in crystal of network structures.

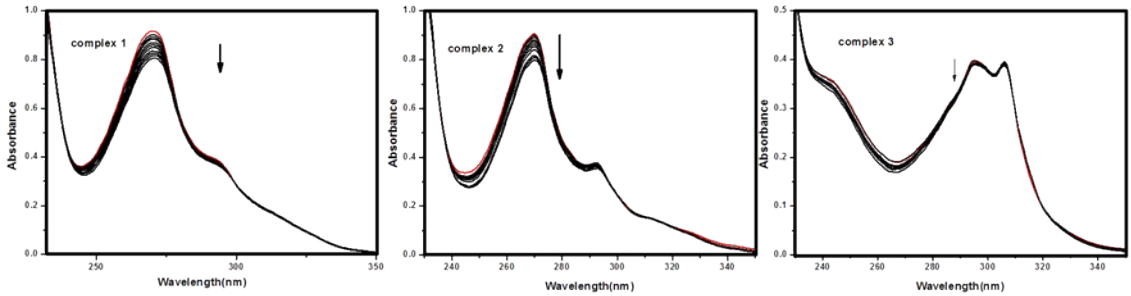


Fig. S12. UV-Vis spectra of the complexes in the absence and presence of CT- DNA (concentration increases downward). The absorption peaks of Complex 1 and Complex 2 both located at 270 nm, due to similar structures. While the complex 3 showed two intense absorption bands at about 295 and 307 nm, respectively.

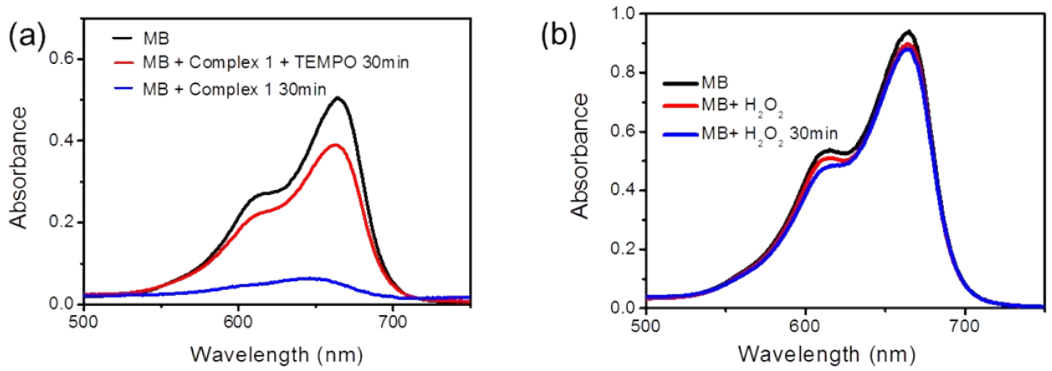


Fig. S13. The degradation of MB in (a) TEMPO, 50 μM Complex 1, 8 mM H_2O_2 and 25 mM $NaHCO_3$ and (b) in 1× PBS containing 100 μM H_2O_2.

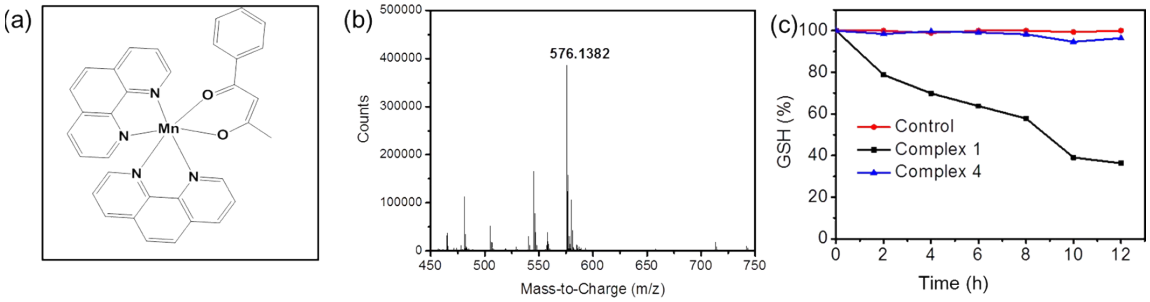


Fig. S14. (a) The structure of Complex 4; (b) The HR-MS spectrum of Complex 4, HR-MS m/z $[M]^+$ 576.1382 (calculated for $C_{36}H_{25}MnN_4O_2$, 576.1358).; (c) GSH depletion by Complex 4. Although the complex 2 or 3 contains Cu^{2+}, it can oxidize GSH to GSSG. However, Complex 2 or 3 contains Ni, and the reduction of Ni weakens the oxidation of Cu^{2+}. Compared to complex 1, GSH treated with complexes 2 and 3 decreased limited, indicating the redox process of Cu^{2+} and GSH to Cu^+ and GSSG was inhibited.

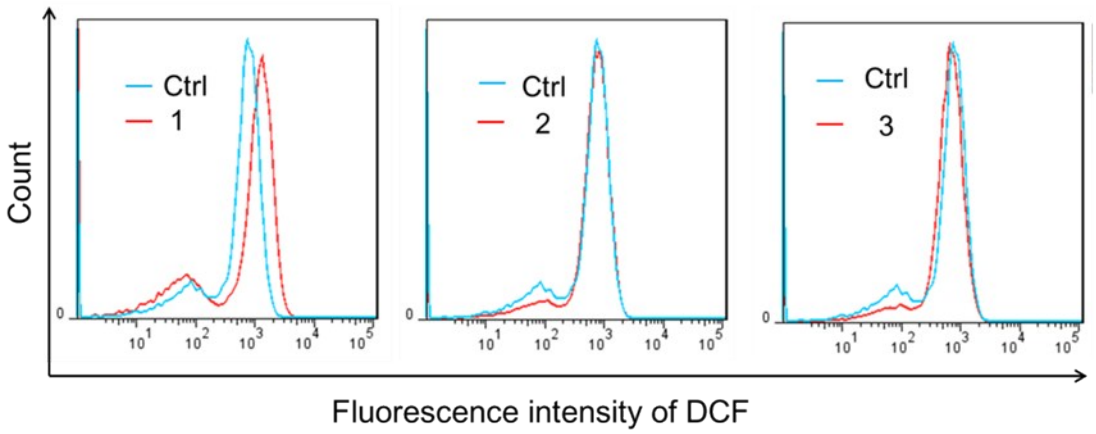


Fig. S15. The intracellular ROS detection by DCFH-DA using flow cytometry.

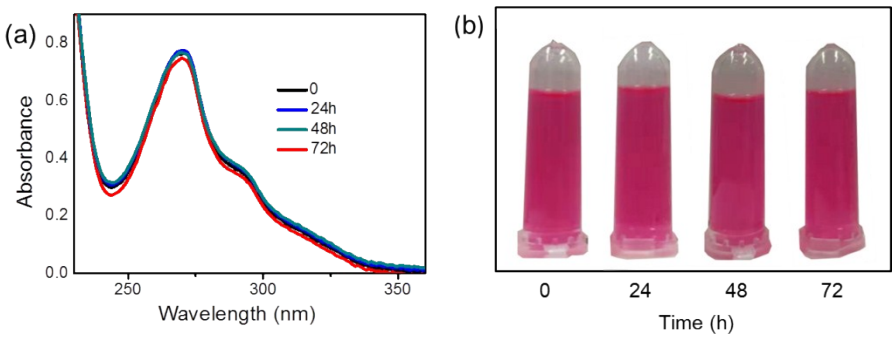


Fig. S16. The stability of the Complex 1 in (a) 1×PBS containing 25 mM NaHCO3 and (b) cell culture medium containing 10% FBS at different intervals.

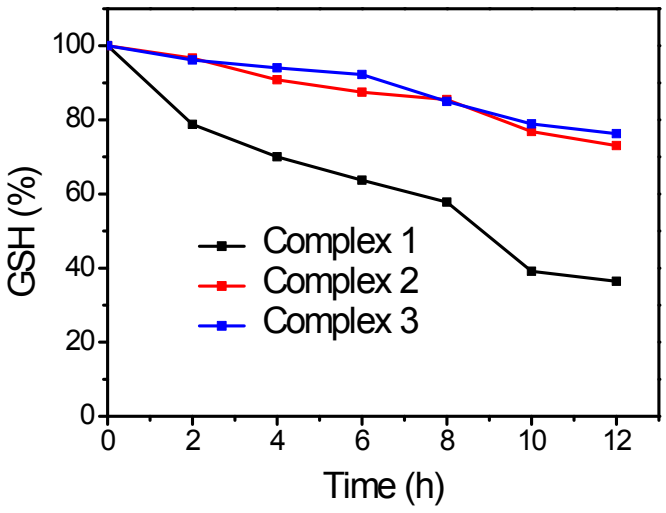


Fig. S17. GSH depletion by Complex 1-3.

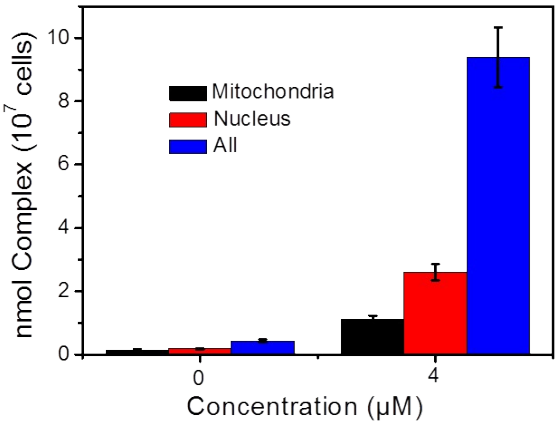


Fig. S18. Cell uptake and localization of Complex 1 in A549 cells after incubation for 12 h determined by analysis of Cu^{2+} content via using ICP-MS.

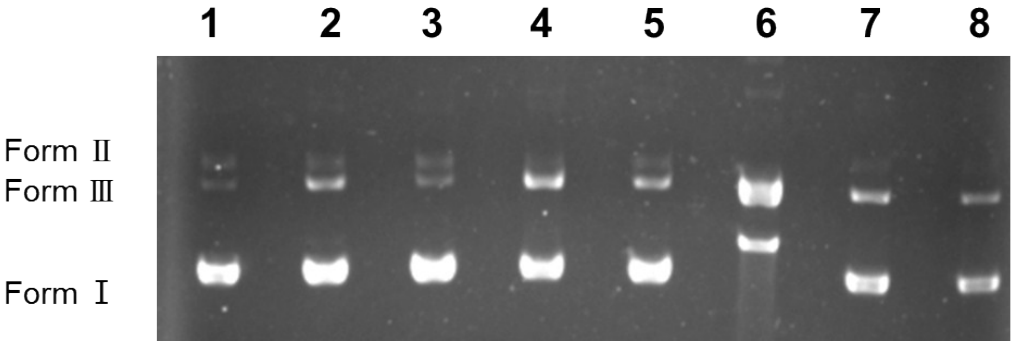


Fig. S19. Cleavage patterns of pUC19 DNA (20 ng/μl) by complex 1 in the agarose gel electrophoresis. Concentration of complex 1: Lane 1: 0; Lane 2: 10 μM; Lane 3: 20 μM; Lane 4: 30 μM; Lane 5: 40 μM; Lane 6: 50 μM; Lane 7: 60 μM; Lane 8:70 μM.

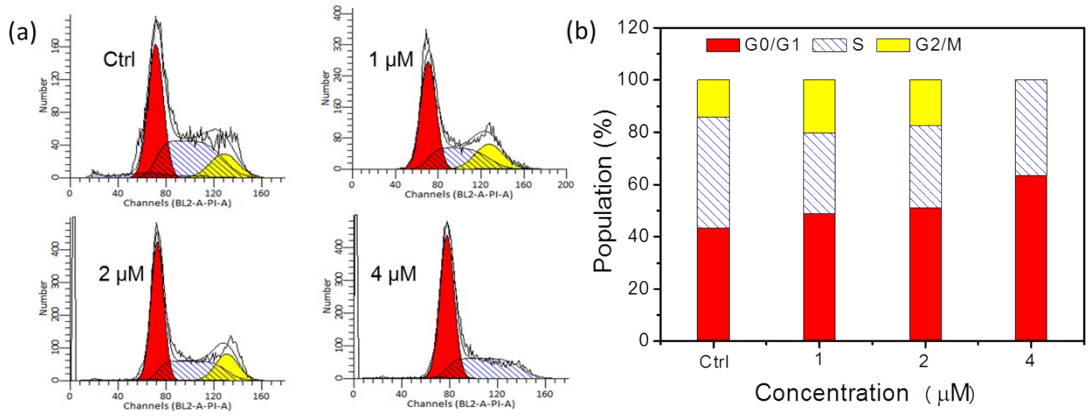


Fig. S20. Cell cycle of A549 treated by complex 1 for 12 h using flow cytometry.

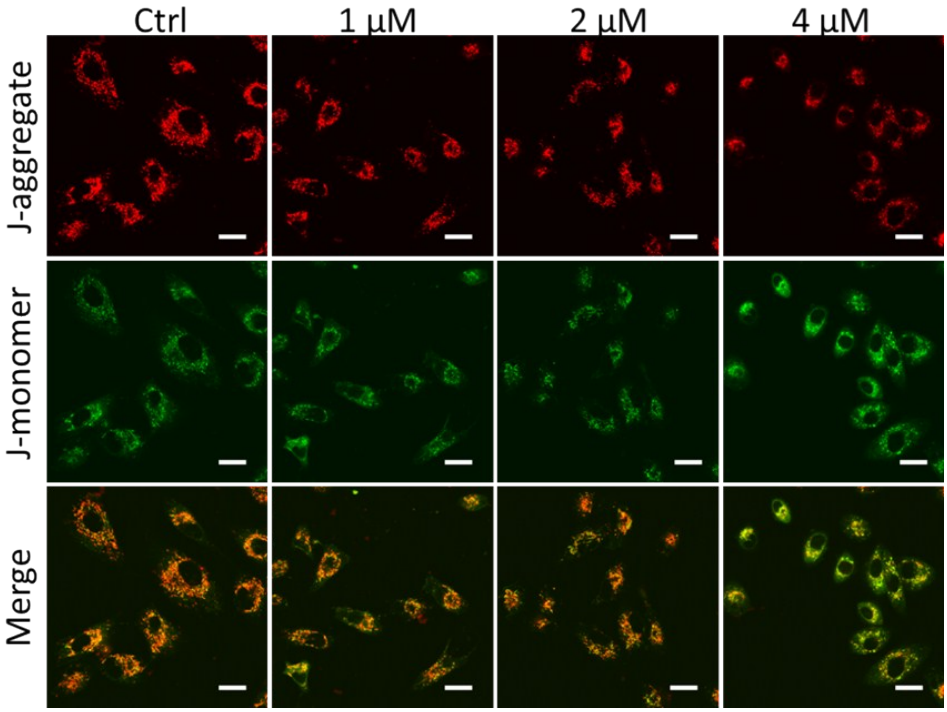


Fig. S21. Detection of mitochondrial membrane potential using JC-1. A549 cells treated with different concentration complex 1 for 12 h. Scale bar is 20 μm.

S4 Supplementary Tables

Table S1. Crystal data and structure refinement for complexes

	Complex 1	Complex 2	Complex 3
Formula	$C_{41}H43BrClCuMnN7O_{11}$	C39H35BrClCuN7NiO9	C34H33BrClCuN7NiO9
Formula weight	1065.87	983.35	921.28
Crystal system	Triclinic	Triclinic	monoclinic
Space group	P-1	P-1	$P2_1/n$
a(Å)	12.965(5)	13.1606(17)	12.7078(13)
b(Å)	13.304(5)	21.552(3)	15.6706(15)
c(Å)	15.631(5)	24.145(3)	18.6755(18)
α(°)	89.633(5)	79.506(2)	90
β(°)	66.535(5)	75.850(2)	94.217(3)
γ(°)	64.672(5)	89.599(3)	90
V(Å^3)	2191.3（14）	6524.5(15)	3708.9(6)
Z	2	6	4
ρ(Calcd)(g/cm^3)	1.61531	1.502	1.650
μ/mm^{-1}	1.877	1.961	2.294
F(000)	1054.00	2994.0	1868.0
Θrange for data collection(°)	2.46 to 25	2.168 to 25.5	2.067 to 24.999

GOF on F^2	1.033	1.012	1.100
Reflections collected	15806	43446	6436
Independent reflections [$R_{(int)}$]	7665[0.0492]	23918 [0.0923]	6436 [0]
Final R indexes [I>=2σ(I)]	R_1=0.0476,wR_2=0.1247	R1=0.0695, wR2=0.1383	R1=0.1165, wR2=0.2408
Final R indexes [all data]	R_1=0.0615,wR_2=0.1336	R1=0.1504,wR2=0.1584	R1=0.2100, wR2=0.2796
Largest diff. Peak/hole(eÅ-3)	1.55/-0.78	1.00/-1.00	2.44/-1.28

Table S2. Selected bond lengths and angles for Complex 1

Bonds/angles	(Å)/(°)	Bonds/angles	(Å)/(°)
Cu1-O3	1.925(3)	Mn1-O1	2.127(3)
Cu1-N2	1.949(3)	Mn1-O2	2.180(3)
Cu1-N3	2.010(3)	Mn1-N4	2.231(3)
Cu1-N1	2.081(3)	Mn1-N6	2.243(3)
Cu1-O5	2.366(3)	Mn1-N5	2.247(4)
		Mn1-N7	2.303(3)
O3-Cu1-N2	174.99(12)	O1-Mn1-O2	76.37(10)
O3-Cu1-N3	92.09(12)	O1-Mn1-N4	160.85(11)
N2-Cu1-N3	84.30(13)	O2-Mn1-N4	94.38(11)
O3-Cu1-N1	88.97(12)	O1-Mn1-N6	102.52(11)
N2-Cu1-N1	95.54(13)	O2-Mn1-N6	93.49(11)
N3-Cu1-N1	161.94(13)	N4-Mn1-N6	94.67(12)
O3-Cu1-O5	89.23(11)	N6-Mn1-N5	159.19(12)
N2-Cu1-O5	87.97(12)	O1-Mn1-N7	103.00(11)
N3-Cu1-O5	99.90(12)	O2-Mn1-N7	166.34(11)
N1-Cu1-O5	98.14(13)	N4-Mn1-N7	89.92(11)
		N6-Mn1-N7	73.21(11)
		N5-Mn1-N7	88.90(12)

Table S3. Selected bond lengths and angles for Complex 2

Bonds/angles	(Å)/(°)	Bonds/angles	(Å)/(°)
Cu1-O1	1.931(4)	Ni1-O4	2.047(4)
Cu1-N2	1.963(5)	Ni1-O3	2.057(4)
Cu1-N1	2.001(5)	Ni1-N7	2.070(6)
Cu1-N3	2.082(5)	Ni1-N6	2.074(5)
Cu1-O5	2.332(5)	Ni1-N4	2.097(5)
Cu2-O6	1.913(4)	Ni1-N5	2.102(6)
Cu2-N9	1.939(5)	Ni2-O9	2.047(4)
Cu2-N8	2.000(5)	Ni2-O8	2.062(4)
Cu2-N10	2.088(5)	Ni2-N13	2.064(5)
Cu2-O10	2.419(5)	Ni2-N14	2.082(6)
Cu3-O11	1.926(4)	Ni2-N12	2.084(6)
Cu3-N16	1.949(5)	Ni2-N11	2.098(5)

Cu3-N15	2.011(5)	Ni3-O14	2.058(4)
Cu3-N17	2.084(5)	Ni3-O13	2.076(4)
Cu3-O15	2.367(5)	Ni3-N18	2.084(5)
		Ni3-N21	2.096(6)
		Ni3-N19	2.100(6)
		Ni3-N20	2.118(5)
O1-Cu1-N2	176.6(2)	O4-Ni1-O3	80.31(17)
O1-Cu1-N1	93.0(2)	O4-Ni1-N7	91.0(2)
N2-Cu1-N1	83.6(2)	O3-Ni1-N7	97.21(19)
O1-Cu1-N3	88.1(2)	O4-Ni1-N6	167.0(2)
N2-Cu1-N3	95.3(2)	O3-Ni1-N6	90.74(18)
N1-Cu1-N3	160.5(2)	N7-Ni1-N6	80.7(2)
O1-Cu1-O5	89.51(19)	O4-Ni1-N4	98.30(18)
N2-Cu1-O5	90.7(2)	O3-Ni1-N4	171.78(19)
N1-Cu1-O5	100.17(19)	N7-Ni1-N4	90.9(2)
N3-Cu1-O5	99.3(2)	N6-Ni1-N4	91.9(2)
O6-Cu2-N9	175.1(2)	O4-Ni1-N5	96.05(19)
O6-Cu2-N8	92.3(2)	O3-Ni1-N5	91.89(19)
N9-Cu2-N8	83.6(2)	N7-Ni1-N5	169.3(2)
O6-Cu2-N10	88.5(2)	N6-Ni1-N5	93.7(2)
N9-Cu2-N10	96.1(2)	N4-Ni1-N5	80.2(2)
N8-Cu2-N10	165.4(2)	O9-Ni2-O8	80.28(17)
O6-Cu2-O10	89.8(2)	O9-Ni2-N13	168.8(2)
N9-Cu2-O10	87.8(2)	O8-Ni2-N13	93.57(19)
N8-Cu2-O10	93.5(2)	O9-Ni2-N14	91.2(2)
N10-Cu2-O10	101.0(2)	O8-Ni2-N14	93.0(2)
		N13-Ni2-N14	79.7(3)
		O9-Ni2-N12	93.96(19)
		O8-Ni2-N12	92.2(2)
		N13-Ni2-N12	95.7(2)
		N14-Ni2-N12	173.2(2)
		O9-Ni2-N11	95.76(18)
		O8-Ni2-N11	170.18(18)
		N13-Ni2-N11	91.7(2)
		N14-Ni2-N11	96.0(2)
		N12-Ni2-N11	79.0(2)
		O14-Ni3-O13	80.22(17)
		O14-Ni3-N18	167.3(2)
		O13-Ni3-N18	91.07(18)
		O14-Ni3-N21	95.52(19)
		O13-Ni3-N21	91.81(19)
		N18-Ni3-N21	94.0(2)
		O14-Ni3-N19	91.3(2)
		O13-Ni3-N19	97.69(19)

		N18-Ni3-N19	80.6(2)
		N21-Ni3-N19	169.2(2)
		O14-Ni3-N20	98.36(18)
		O13-Ni3-N20	171.38(19)
		N18-Ni3-N20	91.60(19)
		N21-Ni3-N20	79.8(2)
		N19-Ni3-N20	90.8(2)

Table S4. Selected bond lengths and angles for Complex 3

Bonds/angles	(Å)/(°)	Bonds/angles	(Å)/(°)
Cu1-O1	1.919(9)	Ni1-O3	2.064(8)
Cu1-N1	1.925(11)	Ni1-O4	2.069(8)
Cu1-N2	1.957(10)	Ni1-N4	2.071(10)
Cu1-N3	2.047(12)	Ni1-N7	2.076(10)
Cu1-O5	2.384(9)	Ni1-N5	2.085(11)
		Ni1-N6	2.089(11)
O1-Cu1-N1	91.7(4)	O3-Ni1-O4	80.3(3)
O1-Cu1-N2	172.8(4)	O3-Ni1-N4	93.2(4)
N1-Cu1-N2	84.2(4)	O4-Ni1-N4	92.1(4)
O1-Cu1-N3	89.1(4)	O3-Ni1-N7	95.6(4)
N1-Cu1-N3	162.6(4)	O4-Ni1-N7	90.1(4)
N2-Cu1-N3	96.7(4)	N4-Ni1-N7	171.2(4)
O1-Cu1-O5	90.7(3)	O3-Ni1-N5	88.4(4)
N1-Cu1-O5	98.7(4)	O4-Ni1-N5	165.7(4)
N2-Cu1-O5	84.1(4)	N4-Ni1-N5	79.7(4)
N3-Cu1-O5	98.7(4)	N7-Ni1-N5	99.8(4)
		O3-Ni1-N6	171.9(4)
		O4-Ni1-N6	93.1(4)
		N4-Ni1-N6	91.7(4)
		N7-Ni1-N6	79.6(4)
		N5-Ni1-N6	98.9(4)

Table S5. The cytotoxic activity of the complexes

	IC_{50}			
	A549	MCF7	4T1	HepG2
CuL	>40	>40	>40	>40
Complex 1	2.29±0.083	1.05±0.037	2.77±0.07	2.34±0.12
Complex 2	31.33±2.95	22.21±1.98	36.12±1.51	19.74±0.13
Complex 3	>40	>40	>40	>40

S5 References

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