Supporting Information

Two Novel Chiral Tetranucleate Copper-based Complexes: Crystal Structures, Nanoparticles, and Inhibiting Angiogenesis and the Growth of Human Breast Cancer by Regulating VEGF/VEGFR2 Signal Pathway in Vitro

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Instruments and materials

Materials. All materials and solvents were purchased commercially and used without further purification unless specifically noted. Ultrapure Milli-Q water was used in all experiments. 3-bromo-5- chlorosalicaldehyde and 5- nitrosalicylic aldehyde reagent were purchased from SAAN Chemical Technology Co., Ltd. *L*-Methioninol was purchased from Sequoia precision Chemical Co., Ltd. Potassium hydroxide(A.R.), Anhydrous methanol (A.R.)and Copper nitrate trihydrate (A.R.)were purchased from Xilong Science Co., Ltd. MTT, penicillin/streptomycin, and dimethylsulfoxide was purchased from Sigma-Aldrich, USA. Dulbecco's modified eagle medium (DMEM, Gibco), RPMI-1640 medium(Gibco), Fetal bovine serum (FBS, GEMINI), pancreatic enzyme(Gibco), cell culture plates(Eppendorf), Anti-VEGFR2, Anti-phospho-VEGFR2,Anti-FAK, Anti-phospho-FAK, Anti-AKT , Anti-phospho-AKT, Anti-Erk1/2, and Anti-phosphoErk1/2(Cell Signaling Technology) were used. GAPDH antibodies were procured from ZSGB-BIO. Annexin V/PI apoptosis kit was purchased from BD Bioscience. The JC-1 mitochondrial membrane potential detection kit and reactive oxygen species (ROS) assay kit were from Beyetime (Shanghai, China). HUVECs and MDA-MB-231 cell lines were purchased from Shanghai oulu biological technology Co, ltd. Vascular endothelial growth factor (VEGF) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Cell culture: HUVECs were cultured in DMEM medium supplemented with FBS(10%), penicillin(100 μg/mL), and streptomycin (100 μg/mL); MDA-MB-231 cells were cultured in DMEM medium supplemented with FBS(15%), penicillin (100 μg/mL), and streptomycin (100 μg/mL).They were incubated at 37℃ in a humidified incubator with 5 % CO_2 and 95 % air, and the medium was changed thrice weekly. Matrigel was purchased from Corning.

Instruments. IR spectras were taken on a IRAffinity-1 FT-IR spectrometer with KBr pallets in the range of 4000～400 cm^{-1}. The elemental analyses for C, H and N were performed on a Perkin-Elmer 2400C elemental analyzer. The crystal structures were determined by a four-circle CCD diffractometer (SuperNova, Single source at offset, Eos). Mass spectra were recorded on a Liquid Chromatography Mass Spectrometry (Exactive, Thermo Fisher Scientific) with DMSO as solvent

and CH_3OH diluent. X-ray powder diffractograms were recorded on a X-ray powder diffractometer (X' Pert PRO, Netherlands PANalytical company). Nanoparticle analysis was performed by transmission electron microscopy (HITACHI HT7700). ICP-MS were determined by NexION 300X. Apoptosis assay were determined by BD FACSCanto. Tube formation assays of HUVECs were photographed with a bright field of inverted fluorescence phase contrast microscope (DMi8 Leica, Germany). Cells were cultured in a CO_2 incubator (170S, Galaxy, New Brunswick). Cells were observed with a inverted microscope (OLYMPUS CKX35, Japan). High-speed refrigeration centrifuge (LegendRT-Plus, Thermo, USA), pure water ultra-pure water system (Elix3+30L+3YNERGY, Millipore, USA), high-speed centrifuge(Mini Spin, Eppendorf, Germany), protein transfer membrane system(TE22, GE, USA), protein electrophoresis tank(Bio-Rad Mini-PROTEAN Tetra Cell, USA), rockers(TS-1000, Lin bell, Jiangsu province, China), and gel imaging system UV projector(ZF-4, Shanghai, China), were used in western blot assays.

Supporting Tables

Table S1. Crystal data and structure refinement parameters for TNCu-1 and TNCu-2.

Parameters	**TNCu-1**	**TNCu-2**
Empirical formula	$C_{48}H_{56}Cu_4N_8O_{16}S_4$	$C_{96}H_{112}Br_8Cl_8Cu_8N_8O_{20}S_8$
Formula moiety	$[Cu_4(C_{12}H_{14}N_2O_4S)_4]$	$[Cu_4(C_{12}H_{13}NO_2SClBr)_4]_2$▪$4H_2O$
Formula weight	1383.40	3385.62
Temperature (K)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Tetragonal, P_{42}	Triclinic, P_1
a (Å)	14.2730 (3)	10.1515 (3)
b (Å)	14.2730 (3)	13.6106 (4)
c (Å)	13.9589 (3)	22.1971 (9)
α (°)	90	85.732 (3)
β (°)	90	84.872 (3)
γ (°)	90	85.986 (2)
V (Å ^{3})	2843.69(13)	3039.98 (18)
Z, D_{Calcd} (Mg.m $^{-3}$)	2, 1.616	1, 1.849
Abs. coefficient (mm $^{-1}$)	1.696	4.378
F (000)	1416	1680
Limiting indices	$-17 \le h \le 17$ $-17 \le k \le 17$ $-17 \le l \le 17$	$-12 \le h \le 12$ $-17 \le k \le 16$ $-27 \le l \le 27$
θ range for data refinement (°)	3.51~26.367	3.29~26.37
Reflections collected	24081	24959
Independent reflections	5803（*R*int = 0.027）	20039(*R*int = 0.027)
Observed data	4107（$I > 2\sigma(I)$	14762($I > 2\sigma(I)$)
Refinement method	Full-matrix least-squares on F2	Full-matrix least-squares on F2
Nref / Npar / Nres	5803/374/6	20039/1425/23
Flack parameter *	0.009(9)	-0.008(10)
Final R_1, R_2, S [$I > 2\sigma(I)$]	R_1= 0.0512, R_2 = 0.1435, S = 1.079 $=1/[\sigma^2(F_o^2)+(0.1P)^2]$ Where $P=(F_o^2+2F_c^2)/3$	R_1 = 0.0614, R_2= 0.1514, S = 1.042 $= 1/[\sigma^2(F_o^2) + (0.1000P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
Final R_1 , R_2 , S (all data)	R_1 = 0.0765, R_2 = 0.1676, S = 1.079	R_1 = 0.0939, R_2 = 0.1810, S = 1.043
hift max / mean	0.000/0.000	0.000/0.000
Completeness to theta	0.997	0.998

* For TNCu-1: Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259；For TNCu-2: Flack H D (1983), Acta Cryst. A39, 876-881.

Table S2. Selected bond distances (Å) and angles (°) for complexes TNCu-1.

Bond	Dist. (Å)	Bond	Dist. (Å)	Angle	Dist. (Å)
Cu(1)—O(4)	1.951 (6)	Cu(2)—O(4)^{i}	1.956(8)	O(5)—Cu(2)—O(4)^{i}	94.3(3)
Cu(1)—O(8)	1.930(7)	Cu(2)—O(5)	1.900(7)	O(8)—Cu(2)—O(4)^{i}	87.6(3)
Cu(1)—O(1	1.888(7)	Cu(2)—O(8)	1.934(6)	O(8)—Cu(2)—N(3)	83.7(3)
Cu(1)—N(2)	1.945(7)	Cu(2)—N(3)	1.943(8)	O(5)—Cu(2)—N(3)	94.5(3)
Angle	**(°)**	**Angle**	**(°)**	**Angle**	**(°)**
O(8)—Cu(1)—O(4)	87.4(3)	N(2)—Cu(1)—O(4)	83.9(3)	O(5)—Cu(2)—O(8)	177.9(3)
O(1)—Cu(1)—O(8)	94.4(3)	O(8)—Cu(1)—N(2)	165.6(3)	N(3)—Cu(2)—O(4)^{i}	169.0(3)
O(1)—Cu(1)—N(2)	94.4(3)	O(1)—Cu(1)—O(4)	178.1(3)		

Symmetry code: (i) −x, −y+1, −z.

Table S3. Selected bond distances (Å) and angles (°) for complexes TNCu-2.

Bond	Dist. (Å)	Bond	Dist. (Å)	Bond	Dist. (Å)
Cu(1)—O(2)	1.918 (10)	Cu(2)—O(8)	1.915 (7)	Cu(3)—O(2)	1.908 (8)
Cu(1)—O(4)	1.986 (8)	Cu(2)—O(4)	1.931 (8)	Cu(3)—O(5)	1.931 (9)
Cu(1)—O(1)	1.894 (10)	Cu(2)—N(2)	1.944 (9)	Cu(3)—O(6)	1.959 (8)
Cu(1)—N(1)	1.962 (9)	Cu(2)—O(3)	1.911 (9)	Cu(3)—N(3)	1.944 (9)
Cu(4)—N(4)	1.957 (9)	Cu(5)—O(12)	1.918 (7)	Cu(6)—O(12)	1.947 (8)
Cu(4)—O(7)	1.918 (10)	Cu(5)—O(9)	1.905 (7)	Cu(6)—O(16)	1.957 (7)
Cu(4)—O(8)	1.963 (8)	Cu(5)—O(10)	1.958 (7)	Cu(6)—O(11)	1.905 (8)
Cu(4)—O(6)	1.960 (8)	Cu(5)—N(5)	1.937 (8)	Cu(6)—N(6)	1.957 (9)
Cu(7)—O(14)	1.924 (7)	Cu(7)—O(10)	1.939 (7)	Cu(8)—O(16)	1.927 (7)
Cu(7)—O(13)	1.908 (8)	Cu(8)—O(15)	1.897 (8)	Cu(8)—O(14)	1.959 (7)
Cu(7)—N(7)	1.927(9)	Cu(8)—N(8)	1.943 (8)		
Angle	**(°)**	**Angle**	**(°)**	**Angle**	**(°)**
O(2)—Cu(1)—O(4)	87.6 (3)	O(4)—Cu(2)—N(2)	84.6(3)	O(2)—Cu(3)—O(5)	94.2 (4)
O(2)—Cu(1)—N(1)	83.9 (4)	O(3)—Cu(2)—O(8)	94.2 (3)	O(2)—Cu(3)—O(6)	89.8 (4)
O(1)—Cu(1)—O(4)	96.0 (3)	O(3)—Cu(2)—O(4)	177.4 (3)	O(2)—Cu(3)—N(3)	166.2 (4)
O(1)—Cu(1)—N(1)	93.3 (4)	O(3)—Cu(2)—N(2)	92.8 (4)	N(3)—Cu(3)—O(6)	83.2 (4)
N(1)—Cu(1)—O(4)	167.6 (4)	O(8)—Cu(2)—O(4)	88.2 (3)	O(5)—Cu(3)—N(3)	93.5 (4)
O(1)—Cu(1)—O(2)	174.1 (4)	O(8)—Cu(2)—N(2)	168.1 (4)	O(5)—Cu(3)—O(6)	175.3 (3)
O(6)—Cu(4)—O(8)	90.2 (3)	O(12)—Cu(5)—O(10)	89.3(3)	O(11)—Cu(6)—O(12)	172.9 (3)
O(7)—Cu(4)—N(4)	94.0 (4)	O(12)—Cu(5)—N(5)	169.3(3)	O(11)—Cu(6)—O(16)	96.5 (3)
N(4)—Cu(4)—O(6)	172.1 (4)	O(9)—Cu(5)—O(12)	93.7 (3)	O(11)—Cu(6)—N(6)	94.2 (4)
O(7)—Cu(4)—O(8)	176.2 (4)	O(9)—Cu(5)—O(10)	176.3 (3)	O(12)—Cu(6)—O(16)	87.7 (3)
O(7)—Cu(4)—O(6)	93.6 (4)	O(9)—Cu(5)—N(5)	93.1 (3)	O(12)—Cu(6)—N(6)	83.1 (3)
N(4)—Cu(4)—O(8)	82.3 (4)	N(5)—Cu(5)—O(10)	84.1 (3)	N(6)—Cu(6)—O(16)	163.3 (3)
N(7)—Cu(7)—O(10)	170.0 (4)	O(14)—Cu(7)—N(7)	82.3 (3)	O(15)—Cu(8)—O(16)	178.4 (3)
O(13)—Cu(7)—O(14)	176.1 (3)	O(14)—Cu(7)—O(10)	87.7 (3)	O(15)—Cu(8)—O(14)	94.0 (3)
O(13)—Cu(7)—O(10)	96.1 (3)	O(16)—Cu(8)—O(14)	87.5 (3)	O(15)—Cu(8)—N(8)	94.2 (3)
O(13)—Cu(7)—N(7)	93.8(4)	N(8)—Cu(8)—O(14)	167.0 (3)	O(16)—Cu(8)—N(8)	84.5 (3)

Table S4. Hydrogen bond lengths (Å) and angles (°) for complexes TNCu-1 and TNCu-2**.**

TNCu-1				
D—H•••A	*D*—H	H•••A	*D*•••A	<*D*HA
C(8)—H(8A)•••O(5) [i]	0.97	2.48	2.987(13)	112.5
C(8)—H(8A)•••O(7) [ii]	0.97	2.60	3.504 (16)	154.4
C(20)—H(20A)•••S(2)	0.97	2.99	3.581 (11)	120.4
C(24)—H(24C)•••S(1) [iii]	0.96	3.12	3.561 (17)	109.7
TNCu-2				
D—H•••A	*D*—H	H•••A	*D*•••A	<*D*HA
C(80)—H(80A)•••O(15)	0.97	2.49	3.038(14)	115.5
C(8)—H(8A)•••C(l5)	0.97	2.74	3.563(12)	143.6
C(8)—H(8A)•••S(1)	0.97	2.98	3.588(12)	121.5
C(92)—H(92A)•••O(2W)[i]	0.97	2.55	3.23(3)	126.9
C(90)—H(90)•••S(7)[ii]	0.93	3.00	3.816(12)	147.9
C(56)—H(56A)•••O(3W)	0.97	2.58	3.29(4)	129.5
C(94)—H(94B)•••Cl(2)[iii]	0.97	2.88	3.757(13)	150.9
C(64)—H(64)•••Br(1)[iv]	0.93	3.10	3.892(12)	144.6
C(93)—H(93)•••S(8)	0.98	2.65	3.176(11)	114.0
C(45)—H(45)•••S(4)	0.98	2.90	3.377(13)	110.9
C(20)—H(20B)•••O(1W)	0.97	2.61	3.22(3)	121.0
C(46)—H(46B)•••Br(6)v	0.97	3.09	3.732(12)	124.8
C(83)—H(83A)•••Br(6)	0.97	3.14	4.008(11)	149.9
C(10)—H(10A)•••S(2)[ii]	0.97	2.95	3.905(14)	168.4
C(71)—H(71A)•••S(8)[vi]	0.97	2.94	3.669(16)	133.1
C(42)—H(42)•••S(3)[ii]	0.93	2.78	3.661(17)	159.5
C(60)—H(60B)•••C(l6)[vii]	0.96	2.68	3.627(17)	170.2

Symmetry codes:

For complex TNCu-1：(i) −x, −y+1, z; (ii) y, −x+1, z−1/2; (iii) −x, −y+1, z+1

Symmetry codes:

For complex TNCu-2：(i) x+1, y, z; (ii) x, y−1, z; (iii) x, y, z−1; (iv) x+1, y+1, z−1; (v) x, y−1, z+1; (vi) x, y+1, z; (vii) x−1, y−1, z.

Supplementary Figures

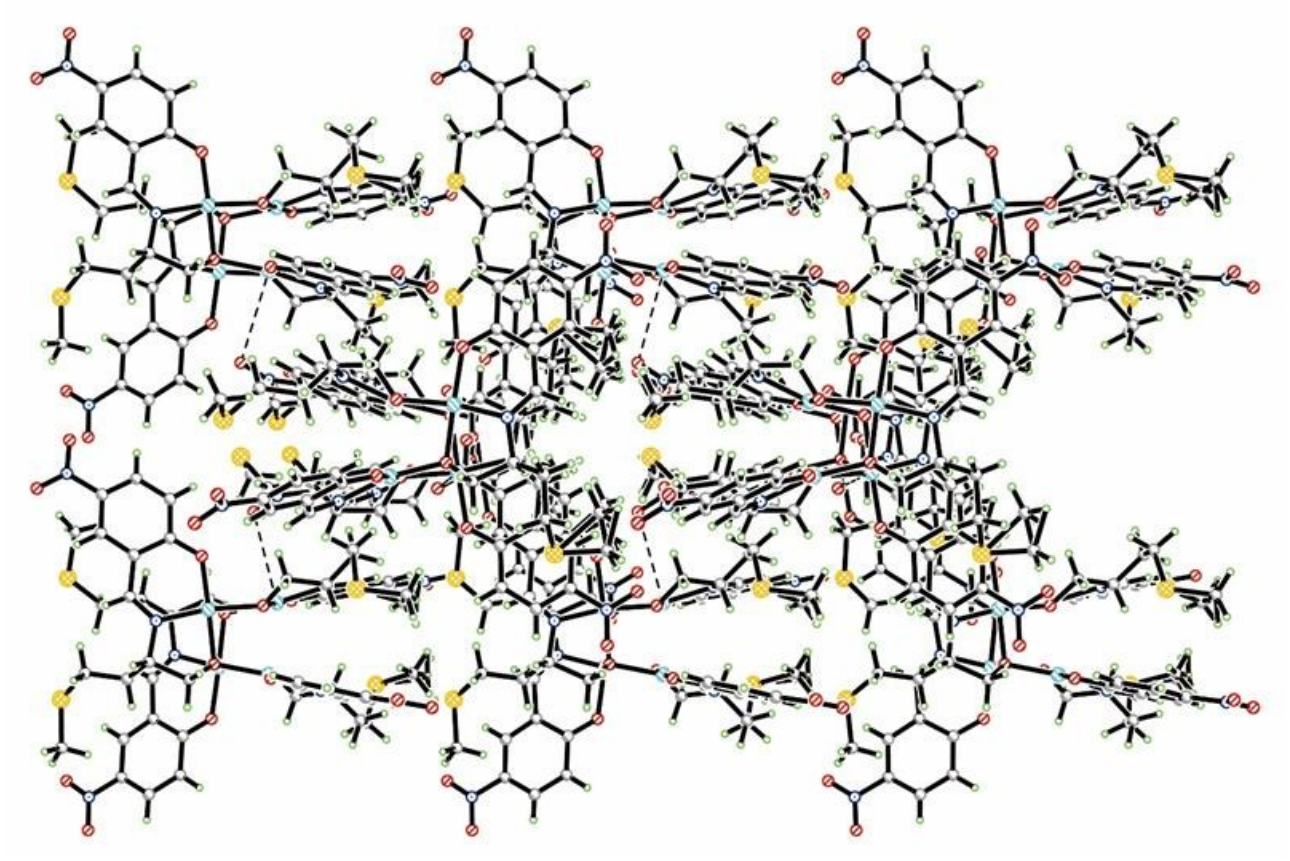

Figure S1. The two-dimensional network structure of TNCu-1 on the *ac* plane.

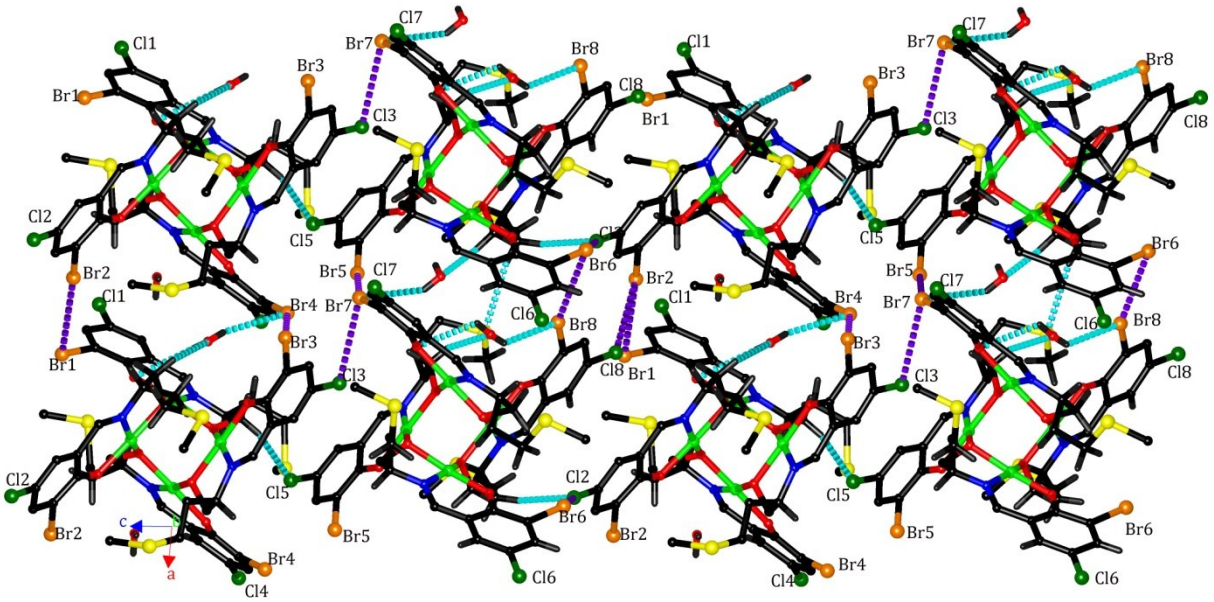

Figure S2. The two-dimensional network structure of TNCu-2 on the *ac* plane, the dotted lines in light blue indicate hydrogen bondings, and the dotted lines in purple indicate Halogen … Halogen interaction(the distance of Br … Br is 3.4844(1)~3.6032(1) **Å**, and the distance of Cl … Br is 3.7360(1)~3.7548(1) **Å).** For clarity, some of the hydrogens on the C atoms were omitted.

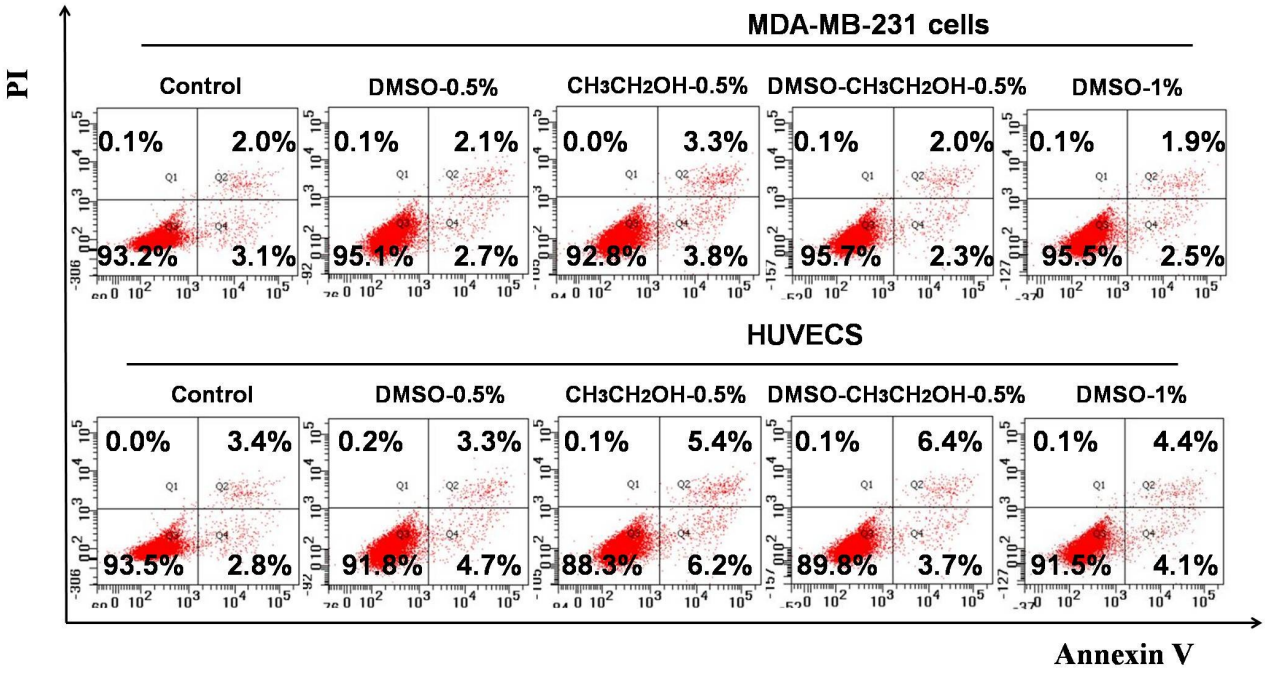

Figure S3. MDA-MB-231 cells and HUVECs were stained with annexin V/PI, respectively. Solvent induced apoptosis of MDA-MB-231 cells and HUVECs was analysed by flow cytometry after the cells were treated with different concentrations for 48 h.

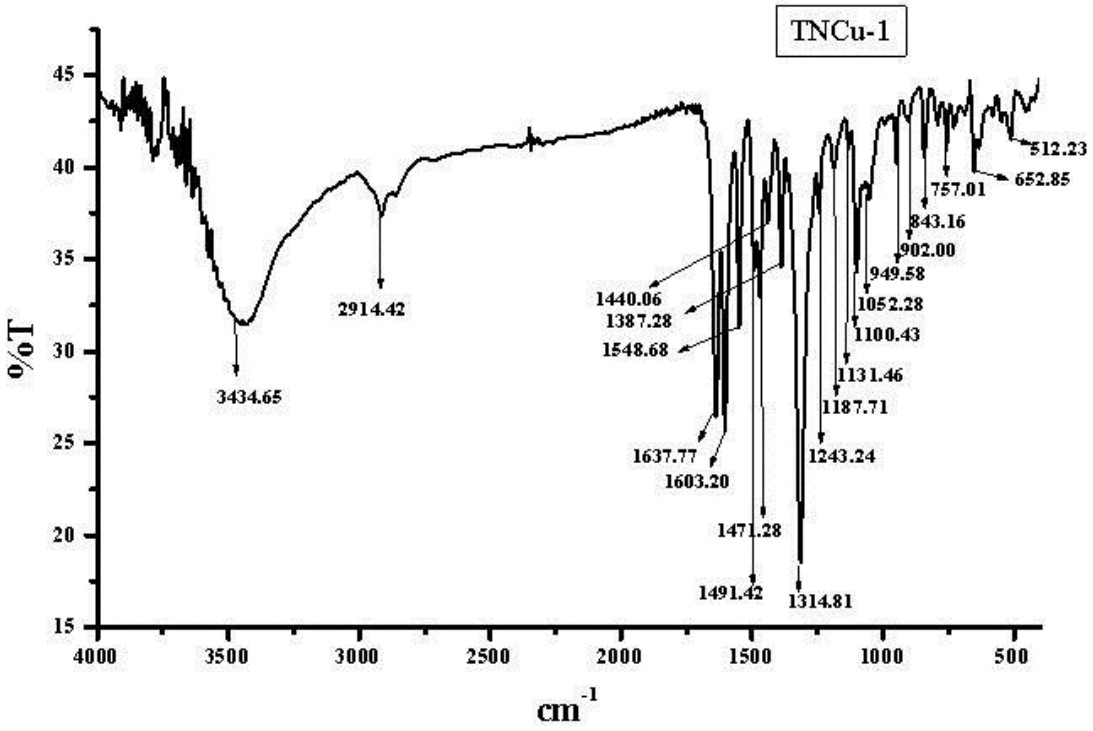

Figure S4. FT-IR of $[Cu_4(C_{12}H_{14}N_2O_4S)_4]$(abbreviated as TNCu-1), and $H_2(C_{12}H_{14}N_2O_4S)$= $C_6H_3(OH)(NO_2)CH{=}NCH(CH_2OH)(CH_2CH_2SCH_3)$. 3434.65(vs, O-H, Maybe the solvent didn't evaporate completely), 2914.42 (m, C-H), 1637.77 (vs,C=N), 1603.20 (vs, C=C for benzene), 1314.81(s, $-NO_2$), 1100.43(s, C-O), 652.85(w, C-S).

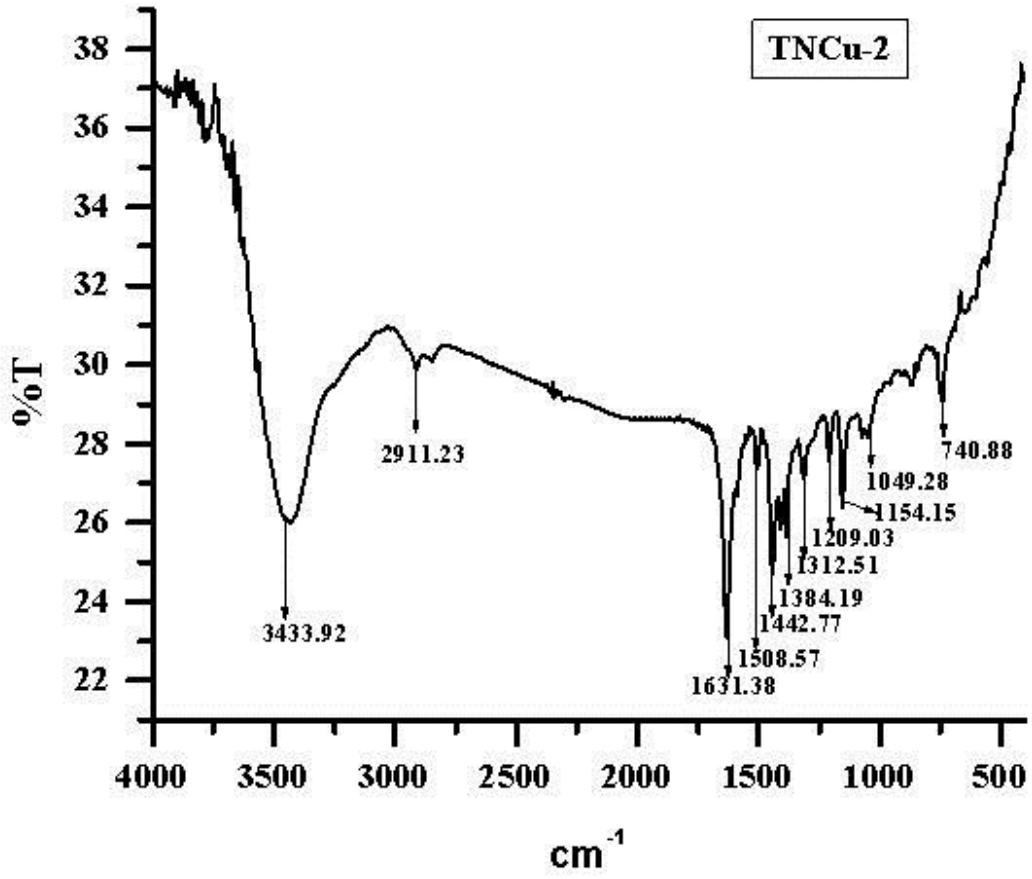

Figure S5. FT-IR of $[Cu_4(C_{12}H_{13}NO_2SClBr)_4]_2 \cdot 4H_2O$, (abbreviated as TNCu-2) and $H_2(C_{12}H_{13}NO_2SClBr)$ = $C_6H_2ClBr(OH)CH{=}NCH(CH_2OH)(CH_2CH_2SCH_3)$. 3433.92 (vs, O-H of H_2O), 2911.23(m, C-H),, 1631.38(vs,C=N), 1442.77(m, C-H), 1384.19(m, C-H), 1312.51(m, C-O), 740.88 cm^{-1}(m, C-Cl).

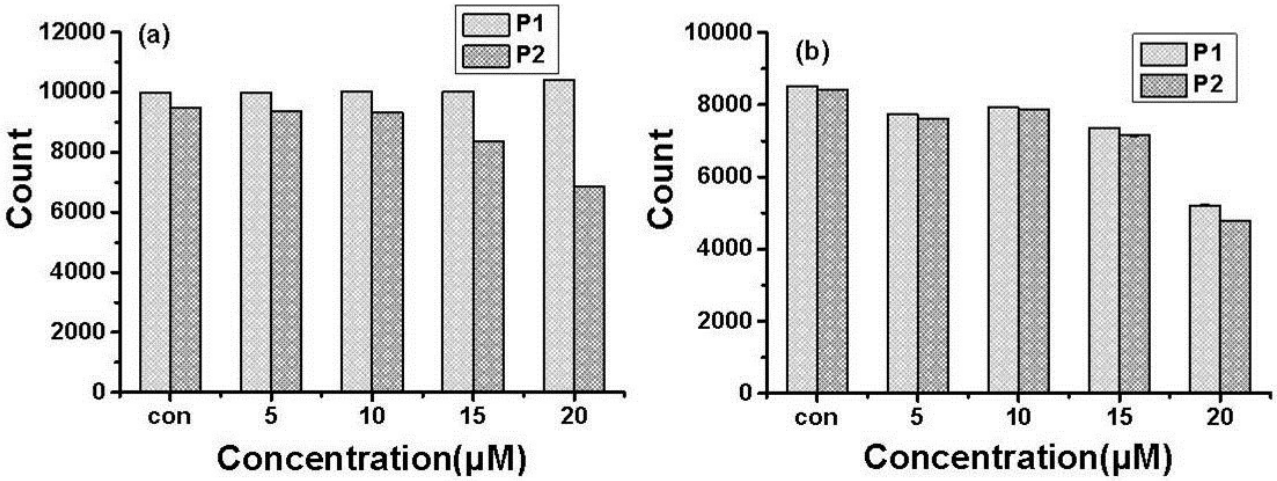

Figure S6. The number of cells counted in ROS detection. (a) The number of cells counted in the 23h N(TNCu-2) ROS experiment.;(b) The number of cells counted in the 30h N(TNCu-2) ROS experiment.

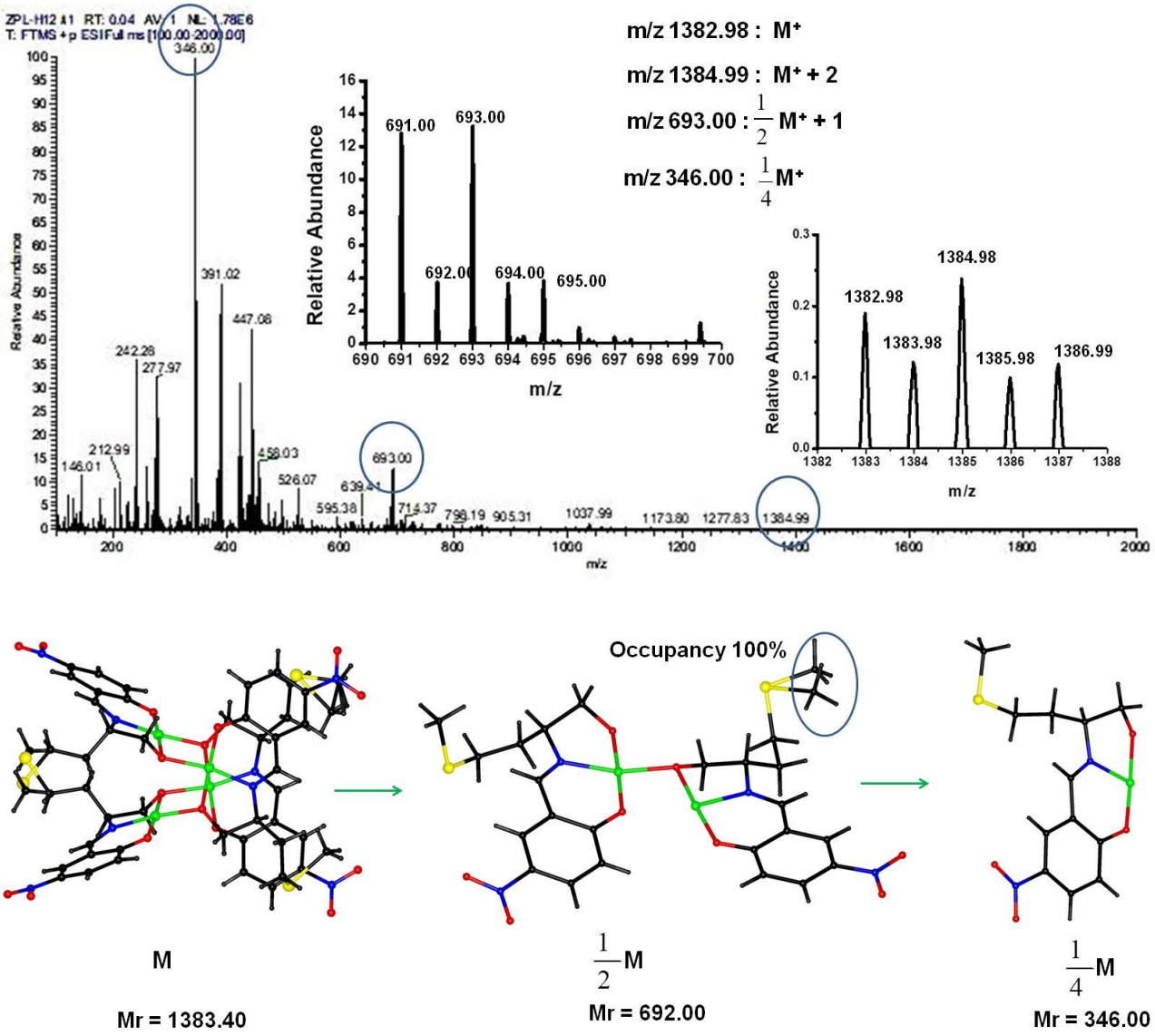

Figure S7. Liquid Chromatography Mass Spectrometry of positive ion of TNCu-1.

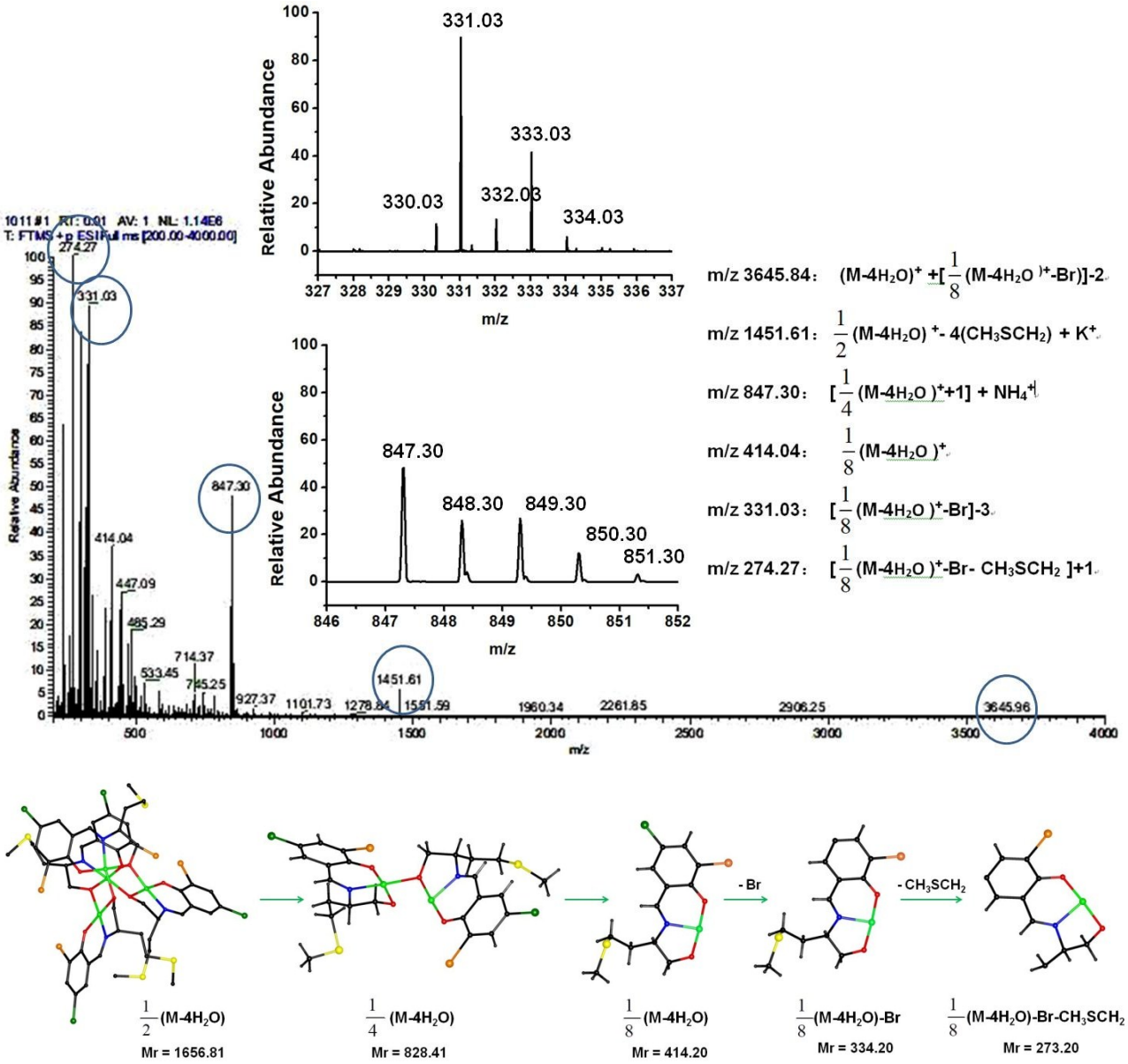

Figure S8. Liquid Chromatography Mass Spectrometry of positive ion of TNCu-2.