Supporting Information

Synthesis, characterization, and anticancer activity of two mixed ligand copper(Ⅱ) complexes by regulating VEGF/VEGFR2 signaling pathway

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Experimental Procedures

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. 1H NMR spectra were recorded on a Bruker 400MHZ NMR spectrometer with CD_3OD as solvent. X-ray powder diffractograms were recorded on a X-ray powder diffractometer(X' Pert PRO, Netherlands PANalytical company). Apoptosis assay were determined by BD FACSAriaⅢ. Tube formation assays of HUVECs were photographed with a bright field of inverted fluorescence phase contrast microscope(OLYMPUS IXTIFL, Japan). Cells were cultured in a CO_2 incubator(311, Thermo, USA). Cells were observed with a inverted microscope(OLYMPUS ckx31, Japan). Ultrasonic cell fragmentation apparatus (VCX-130, Sonics, USA), 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(DYCZ-24DN, Beijing LiuYi, China), rockers(TS-1000, Lin bell, jiangsu province, China), and gel imaging system UV projector(ZF-4, Shanhhai, China), were used in western blot assays.

Materials

Solvents and chemicals obtained from commercial sources were of reagent grade and were used without further purification unless specially noted. All the reagents used for syntheses of target complexes posses ⩾ 98% purity. 5-Bromo-3-methoxy-salicylaldehyde reagent was purchased from Alfa Aesar. 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(Corning), Anti-FAK, Anti-phospho-FAK, Anti-Akt, Anti-phospho-Akt, Anti-Erk1/2, and Anti-phospho-Erk1/2(Cell Signaling Technology) were used. GAPDH antibodies were procured from Thermo Scientific. Annexin V/PI apoptosis kit was purchased from BD Bioscience. HUVECs, HeLa, and C33A 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); C33A cells were cultured in DMEM medium supplemented with FBS(15%), penicillin (100 μg/mL), and streptomycin (100 μg/mL); HeLa cells were cultured in RPMI-1640 medium supplemented with FBS(10%), 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. The animal experiments were carried out in experiment animal center, SPF experimental animal lab, Guilin Medical University.

X-ray crystallography

A suitable dimension single crystal of Cu-1 and Cu-2, respectively, was selected for the measurement. The data were collected on Bruker APEX-II CCD diffractometer equipped with graphite Mo K\a radiation (λ = 0.71073 Å) and used aomega scans mode. Computing data collection: Bruker APEX2; computing cell refinement and data reduction: Bruker SAINT. The structures of Cu-1 and Cu-2 were solved by direct methods using SHELXS-97 (Sheldrick, 2008) and refined by full matrix least-squares on F^2 using the SHELXL 97 (Sheldrick, 2008)[1] program. The non-hydrogen atoms were assigned by anisotropic displacement parameters in the refinement. Hydroxyls were geometrically positioned and refined using a mixed model. All H atoms bonded to C atoms and bonded to hydroxyls were calculated hydrogens, and other H atoms bonded to O atoms from water molecules were geometrically positioned and refined using a riding model, with C–H = 0.9300 Å for aromatic [Uiso (H) = 1.2 Ueq (C)], C–H = 0.9700 Å for secondary methylene [Uiso (H) = 1.2 Ueq (C)], C–H = 0.9800 Å for tertiary methylene [Uiso (H) = 1.2 Ueq (C)], C–H = 0.9600 Å for methyl [Uiso(H) = 1.5 Ueq(C)], O–H = 0.8200 Å for hydroxyls [Uiso(H) = 1.5 Ueq(O)], and O–H = 0.8497 ~ 0.8504 Å for water [Uiso(H) = 1.5 Ueq(O)]. The crystal data were given in Table S1, and selected bond lengths and bond angles were listed in Table S2, and hydrogen bond lengths and hydrogen bond angles were listed in Table S3. The molecular structure of Cu-1 with the atom numbering scheme was illustrated in Fig. S1(a), and the single crystal morphology of Cu - 1 was illustrated in Fig. S1(b), and a 2-D sheet structure of Cu-1 in ac plane was illustrated in Fig. S1(c). The single crystal morphology of Cu - 2 was illustrated in Fig. S2(a), and the molecular structure of Cu-2 with the atom numbering scheme was illustrated in Fig. S2(b), and a 2-D sheet structure of Cu-2 in *bc* plane was illustrated in Fig. S2(c). The atomic coordinates and other parameters of the structure of Cu-1and Cu-2 had been deposited in the Cambridge Crystallographic Data Center(no. 1571872 and 1571873; deposit@ccdc.cam.ac.uk). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, CAMBRIDGE CB2 1EZ, UK ; Email:deposit@ccdc.cam.ac.uk. Moreover, X-ray powder diffractograms of Cu-1 and Cu-2 were recorded on a X-ray powder diffractometer, and were shown in Figure S3**.**

Cell viability assay

Cell viability was determined by a MTT (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide) method. Cells at the exponential growth phase were diluted to (8×10^4 cells per mL with the appropriate medium, respectively, and were seeded in 96-well plates at a volume of 100 μL per well with six duplicates. The other empty wells were filled with 1 × PBS. Cells were incubated at 37℃ in 5% CO_2 for 12 h, and were treated with various concentrations of complex (100 μL per well) diluted with the appropriate medium without FBS. The medium and drug-free control samples were prepared simultaneously. After incubation of the cells for up to 48 h, MTT (20 μL, 5 mg/mL) solution was added into each well. After a further incubation for 4 h at 37℃ in 5% CO_2, the medium was

sucked out by the microsyringe. DMSO (200 μL) was added to each well. The plates were oscillated for 10 minutes, and the values of OD were analyzed by an Microplate Reader at a wavelength of 490 nm. The percentage growth inhibitory rate of treated cells was calculated by (ODtested - ODmedia control) / (OD drug-free control - ODmedia control) × 100 %, where OD is the mean value calculated by using the data from six replicate tests. The IC_{50} values were determined by plotting the percentage viability versus concentration on a logarithmic graph and by reading the concentration at which 50 % of cells were viable, relative to the control. Cu-1, Cu-2, cisplatinum, and suramin were dissolved in DMSO, and diluted with water. The final concentration of DMSO is less than 0.1%.

Supplementary Table

Table S1. Cystal data and structure refinement parameters for complexes Cu-1 and Cu-2.

Parameters	Cu-1	Cu-2
Empirical formula	C_{60} H_{54} Br_2 Cu_2 N_6 O_{11}	C_{29} H_{30} Br Cu N_3 O_6
Formula moiety	$[Cu(C_{17}H_{14}NO_4Br)(C_{12}H_8N_2)]_2$•2($CH_3OH$) • H_2O	$[Cu(C_{17}H_{14}NO_4Br)(C_{10}H_8N_2)]$•2$CH_3OH$
Formula weight	1321.99	660.01
Temperature (K)	296(2)	296(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Triclinic	Triclinic
Space group	P-1	P-1
Unit cell dimensions		
a (Å)	11.2337(18)	10.2415(14)
b (Å)	11.6733(18)	11.8918(17)
c (Å)	22.478(4)	13.6063(19)
α (°)	98.836(3)	88.632(2)
β (°)	102.274(3)	70.613(2)
γ (°)	93.903(3)	66.244(2)
V (Å^3)	2830.6(8)	1419.1(3)
Z, D_{Calcd} (Mg.m^{-3})	2, 1.551	2，1.545
Abs. coefficient (mm^{-1})	2.230	2.225
F(000)	1344	674
Crystal size (mm^3)	0.34 × 0.27 × 0.25	0.34 × 0.24 × 0.22
θ range for data collection / refinement (°)	2.3 ~ 26.47 / 1.78 ~ 25.35	2.30~ 26.41 / 1.89 ~ 25.35
Limiting indices	-13 ≤ *h* ≤ 13 -14 ≤ *k* ≤ 14 -27 ≤ *l* ≤ 27	-12 ≤ *h* ≤ 12 -14 ≤ *k* ≤ 14 -16 ≤ *l* ≤ 16
Reflections collected	29493	11349
Independent reflections	10347 (Rint = 0.0594)	5159（Rint = 0.024）
Observed data	7287 (I > 2σ(I))	4223（I > 2σ(I)）
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Nref / Npar / Nres	10347 / 739 / 0	5159/365/1
Final R_1, wR_2, S [I > 2σ(I)]	R =0.0546, wR =0.1433, S =1.024	R =0.0350, wR =0.0956, S =1.049
	w = 1/[σ^2(F_o^2) + (0.0789P)^2 +4.0026P]	w = 1/[σ^2(F_o^2) + (0.0536P)^2 +0.6495P]
	where P = (F_o^2 + 2F_c^2)/3	where P = (F_o^2 + 2F_c^2)/3
Final R_1, wR_2, S (all data)	R =0.0843, wR =0.1593, S=1.024	R =0.0456, wR = 0.1017 S=1.049
Shift max / mean	0.001 / 0.000	0.000 / 0.000
Completeness to theta	0.998	0.991
CCDC	1571872	1571873

Table S2. Selected bond distances (Å) and angles (°) for complexes Cu-1 and Cu-2.

Cu-1

Bond	Dist. (Å)	Bond	Dist. (Å)
Cu(1)—O(2)	1.925 (3)	Cu(2)—O(6)	1.928 (3)
Cu(1)—N(1)	1.940 (3)	Cu(2)—N(4)	1.948 (4)
Cu(1)—O(3)	1.975 (3)	Cu(2)—O(7)	1.970 (3)
Cu(1)—N(3)	2.028 (4)	Cu(2)—N(6)	2.054 (4)
Cu(1)—N(2)	2.310 (4)	Cu(2)—N(5)	2.261 (4)
Angle	**(°)**	**Angle**	**(°)**
O(2)—Cu(1)—N(1)	93.75 (14)	O(6)—Cu(2)—N(4)	92.99 (14)
O(2)—Cu(1)—O(3)	167.74 (15)	O(6)—Cu(2)—O(7)	172.62 (14)
N(1)—Cu(1)—O(3)	82.48 (14)	N(4)—Cu(2)—O(7)	83.44 (15)
O(2)—Cu(1)—N(3)	91.34 (14)	O(6)—Cu(2)—N(6)	92.93 (14)
N(1)—Cu(1)—N(3)	169.23 (16)	N(4)—Cu(2)—N(6)	165.55 (16)
O(3)—Cu(1)—N(3)	90.57 (14)	O(7)—Cu(2)—N(6)	89.11 (14)
O(2)—Cu(1)—N(2)	92.83 (15)	O(6)—Cu(2)—N(5)	97.14 (14)
N(1)—Cu(1)—N(2)	111.32 (15)	N(4)—Cu(2)—N(5)	115.00 (16)
O(3)—Cu(1)—N(2)	99.41 (15)	O(7)—Cu(2)—N(5)	90.23 (14)
N(3)—Cu(1)—N(2)	77.85 (16)	N(6)—Cu(2)—N(5)	77.27 (16)
Cu-2			
Bond	**Dist. (Å)**	**Bond**	**Dist. (Å)**
Cu(1)—O(2)	1.937 (2)	Cu(1)—N(2)	2.027 (2)
Cu(1)—N(1)	1.940 (2)	Cu(1)—N(3)	2.251 (2)
Cu(1)—O(3)	1.967 (2)		
Angle	**(°)**	**Angle**	**(°)**
O(2)—Cu(1)—N(1)	93.03 (8)	O(3)—Cu(1)—N(2)	90.29 (9)
O(2)—Cu(1)—O(3)	166.64 (9)	O(2)—Cu(1)—N(3)	95.19 (9)
N(1)—Cu(1)—O(3)	83.55 (9)	N(1)—Cu(1)—N(3)	113.92 (9)
O(2)—Cu(1)—N(2)	90.73 (9)	O(3)—Cu(1)—N(3)	98.01 (8)
N(1)—Cu(1)—N(2)	168.38 (9)	N(2)—Cu(1)—N(3)	76.64 (9)

Table S3. Hydrogen bond lengths (Å) and angles (°)for complexes Cu-1 and Cu-2.

Cu-1				
D—H···*A*	*D*—H	H···*A*	*D*···*A*	<*D*H*A*
O(10)—H(10)···Br(1)$^{\#a}$	0.82	3.14	3.870 (6)	149.6
O(1W)—H(1WB)···O(10)	0.85	2.03	2.870 (9)	168.9
O(1W)—H(1WA)···O(9)	0.85	2.04	2.881 (8)	172.0
O(9)—H(9A)···O(8)	0.82	1.91	2.710 (6)	166.5
C(29)—H(29)···O5)	0.93	2.39	3.241	152.08
C(30)—H(30A)···O(3)	0.96	2.66	3.385	132.43
C(58)—H(58)···O(1)	0.93	2.47	3.224	138.13
Cu-2				
D—H···A	D—H	H···A	D···A	D—H···A
O6—H6···O5	0.82	1.97	2.765 (8)	161.7
O5—H5A···O4	0.82	1.89	2.695 (5)	165.5

Symmetry codes: For complex Cu-1 :($^{\#a}$) x+1, y, z.

Supplementary Figures

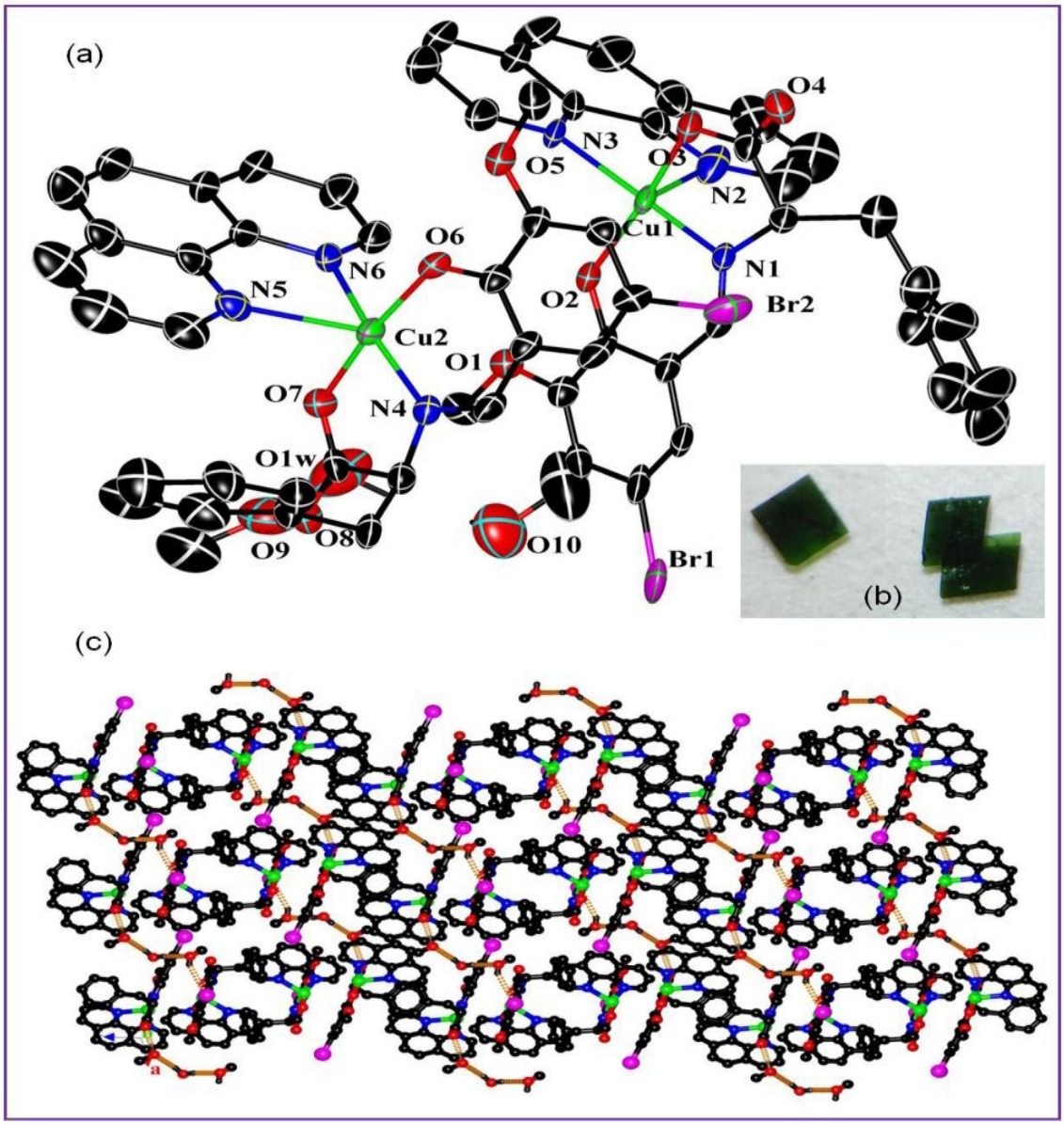

Figure S1. (a) Molecular view of Cu-1 with the atom labeling scheme, and ellipsoids were drawn at the 50% probability level; (b) The single crystal morphology of Cu - 1; (c) A view of 2-D stratified structure of Cu-1 in *ac* plane. Some H atoms were omitted for clarity. Dashed lines are hydrogen bonds in (c).

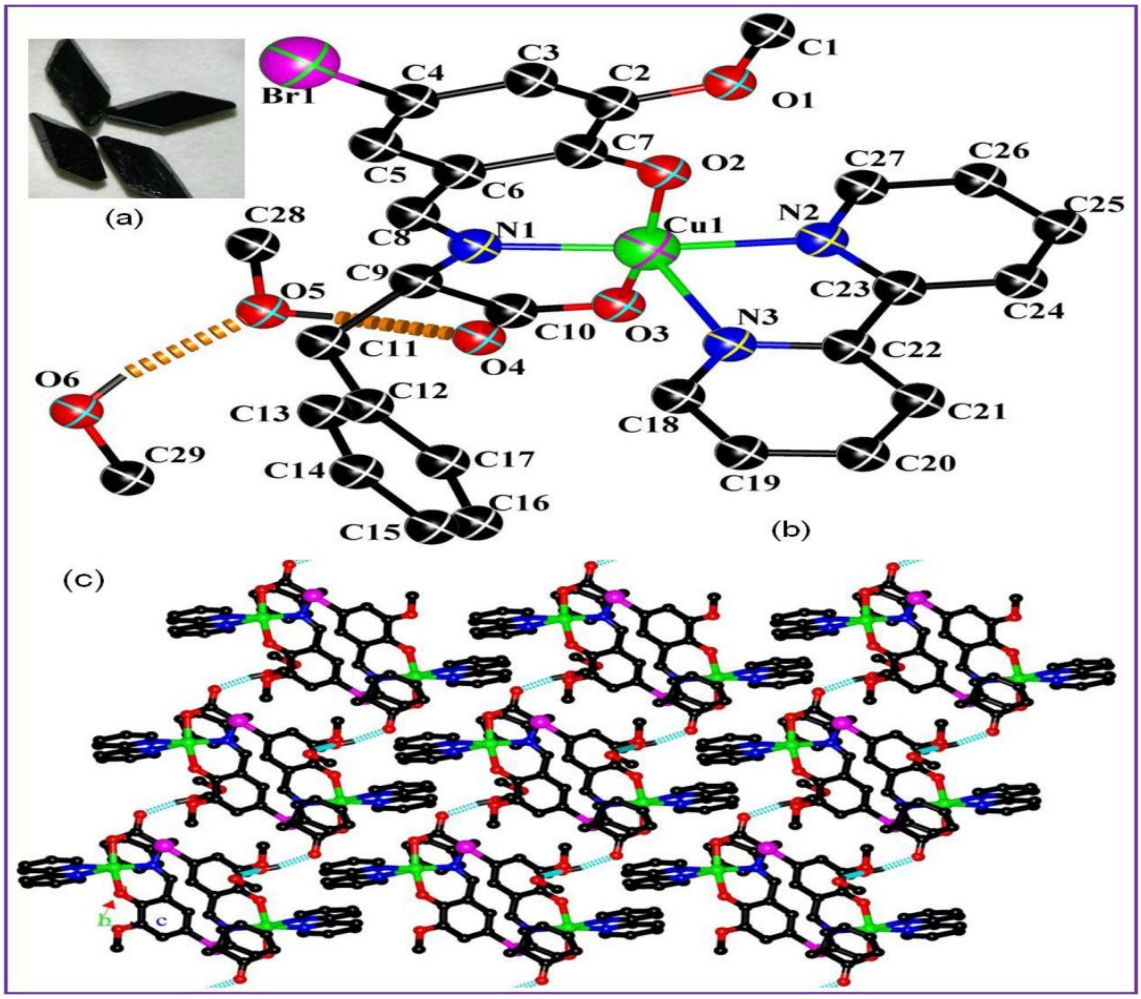

Figure S2. (a) The single crystal morphology of Cu - 2; (b) Molecular view of Cu-2 with the atom labeling scheme, and ellipsoids were drawn at the 50% probability level; (c) A view of 2-D stratified structure of Cu-2 in *bc* plane. Some H atoms were omitted for clarity. Dashed lines are hydrogen bonds in (c).

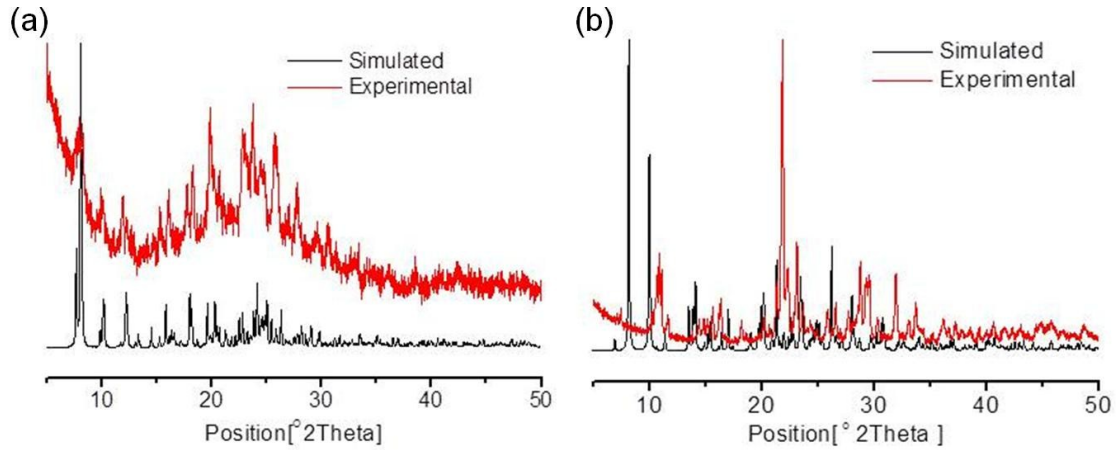

Figure S3. The powder diffractograms and their simulated diffractograms generated from single crystal X-ray diffraction data. (a) Cu-1; (b) Cu-2.

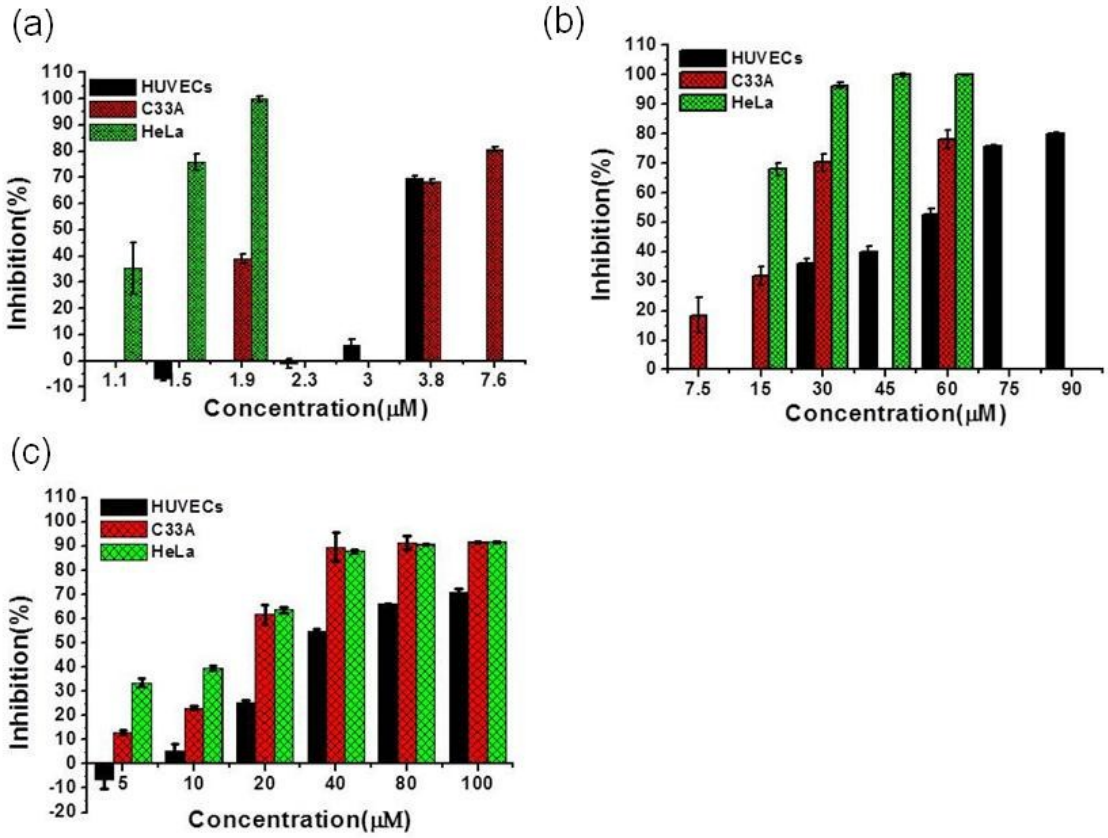

Figure S4. Cell inhibition rates assays of HUVECs, C33A, and HeLa cell lines treated with the various concentration of Cu-1, Cu-2, and cisplatinum for 48h using a MTT method, respectively. (a) Cu-1; (b) Cu-2; (c) Cisplatinum.

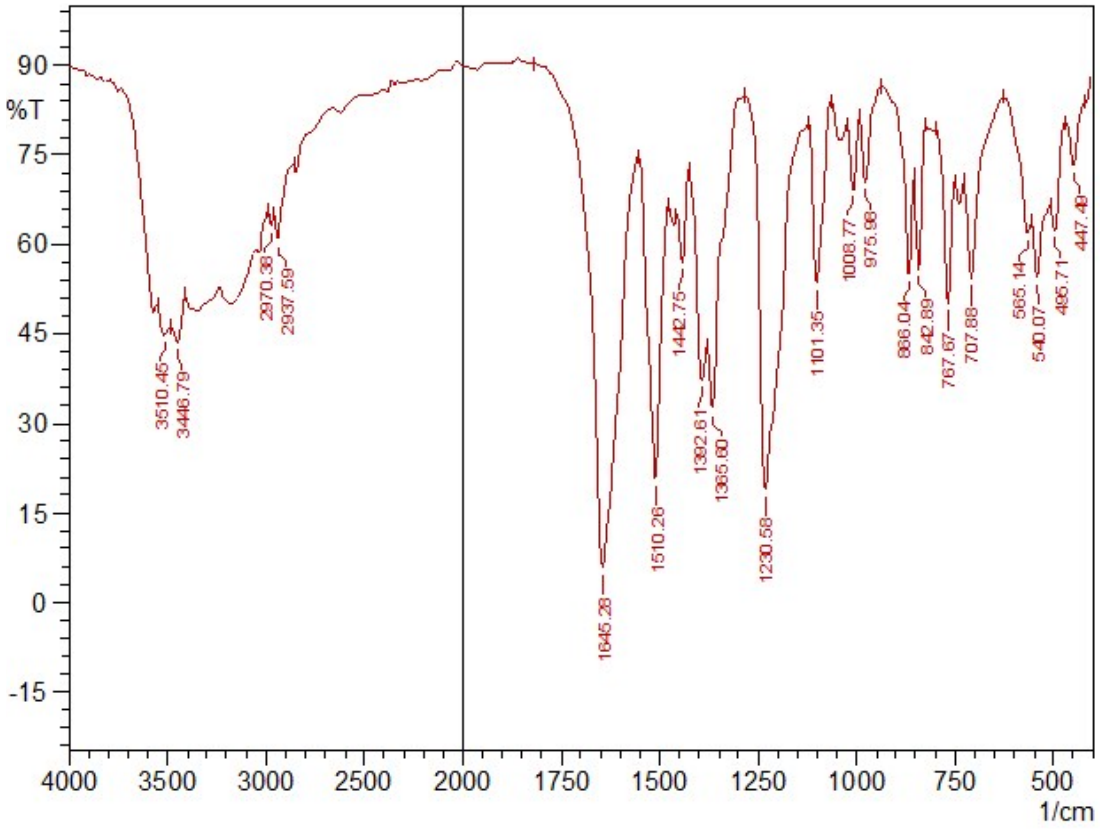

Figure S5. FT-IR of Schiff base ligand [$K_2(C_{17}H_{14}NO_4Br)$].

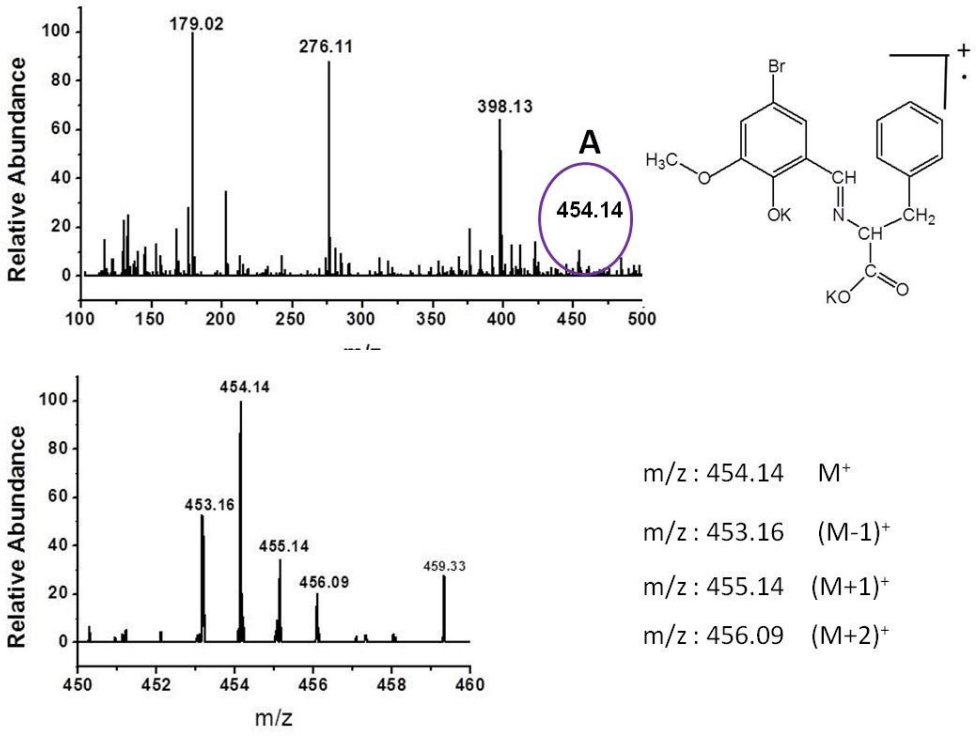

Figure S6. Liquid Chromatography Mass Spectrometry of positive ion of Shiff base ligand [$K_2(C_{17}H_{14}NO_4Br)$].

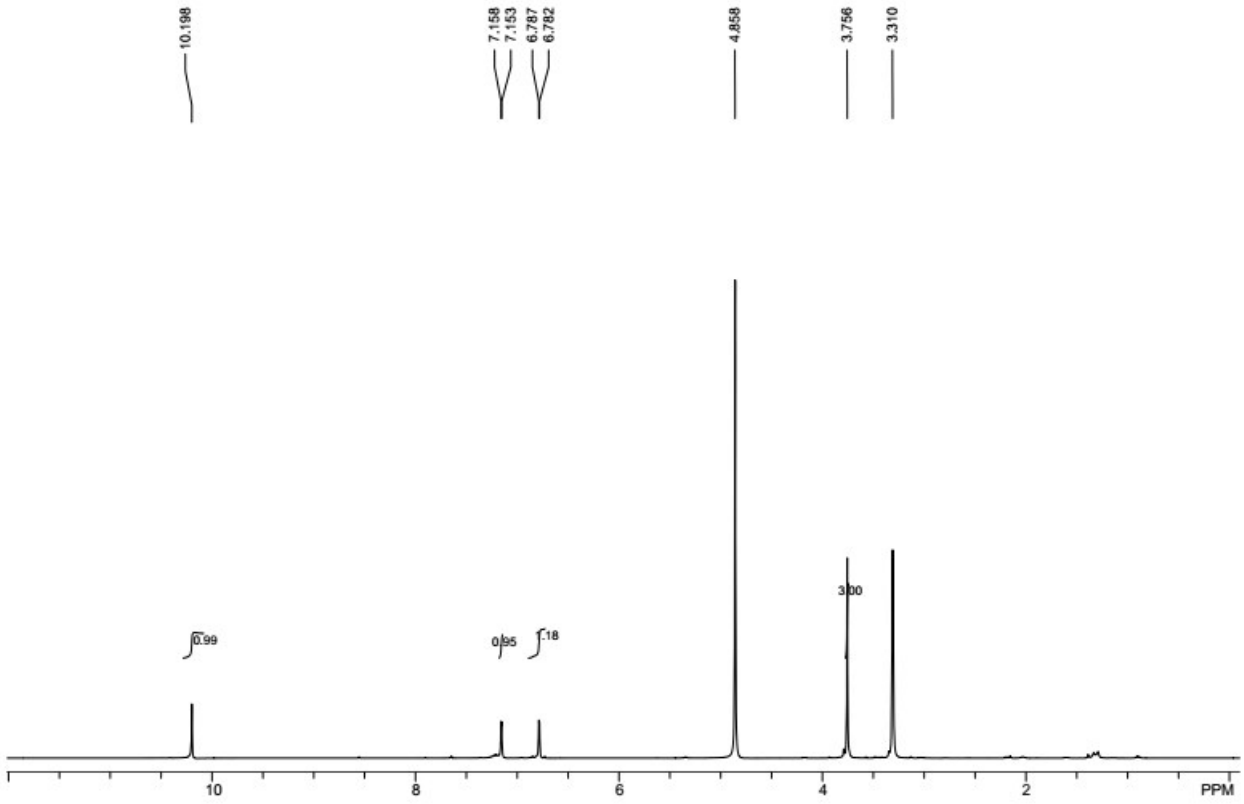

Figure S7. 1H NMR spectra [CD_3OD) of Shiff base ligand [$K_2(C_{17}H_{14}NO_4Br)$].

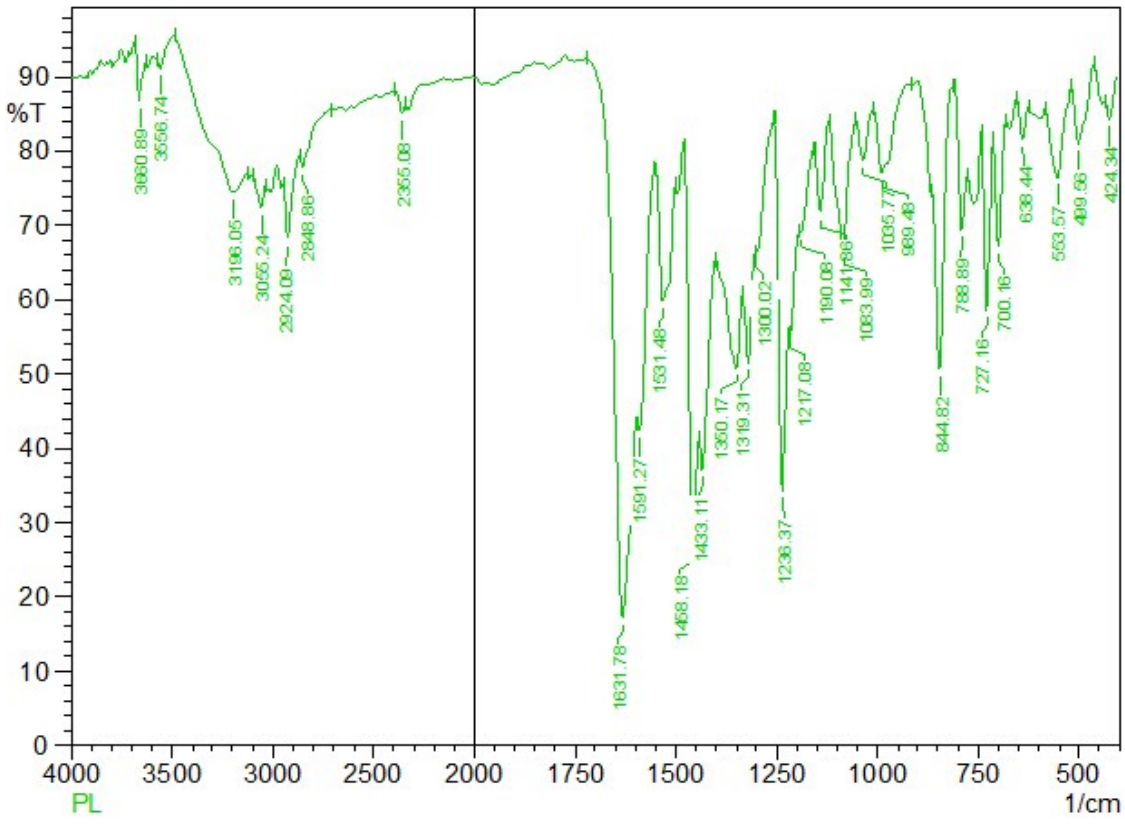

Figure S8. FT-IR of Cu-1.

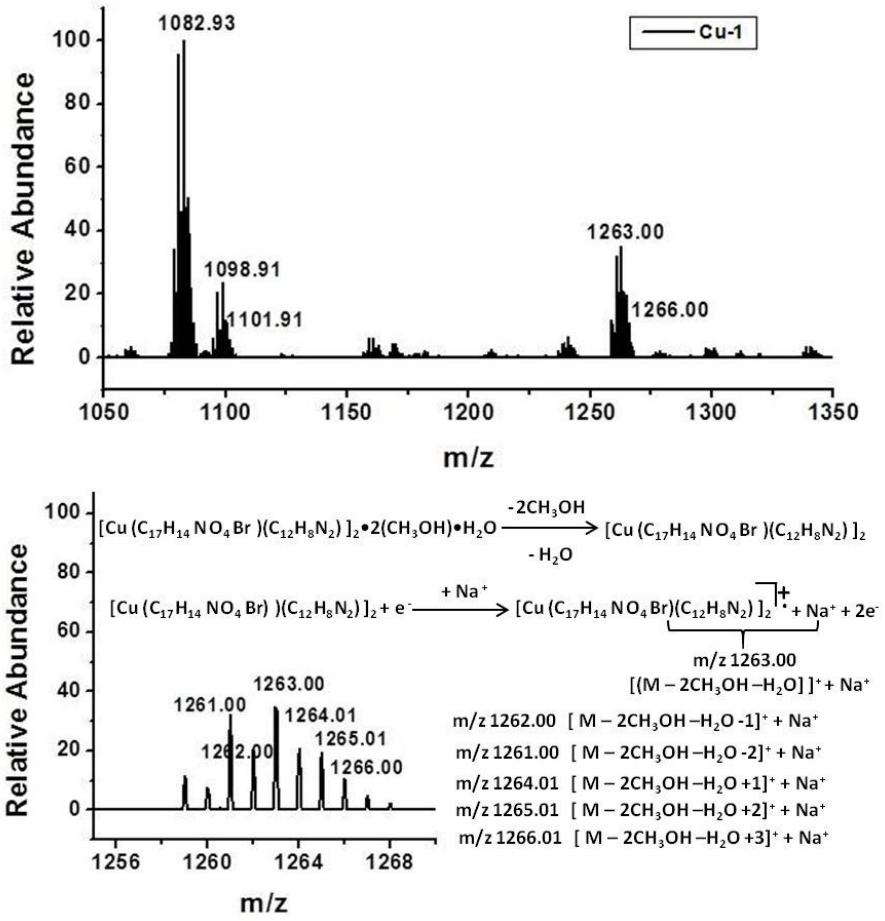

Figure S9. Liquid Chromatography Mass Spectrometry of positive ion of Cu-1.

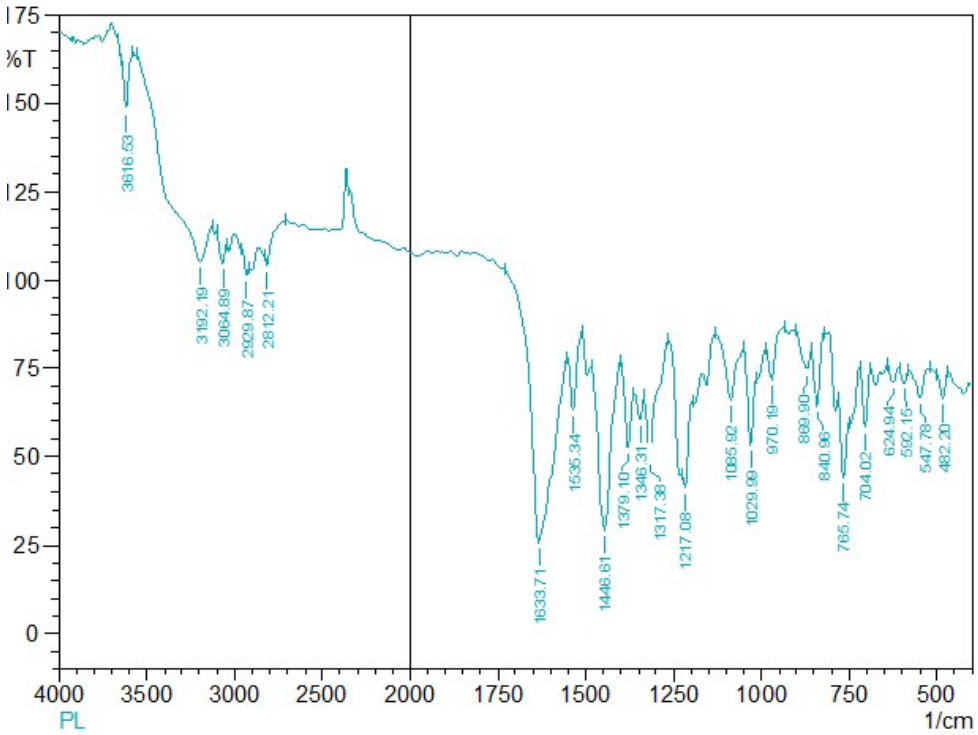

Figure S10. FT-IR of Cu-2.

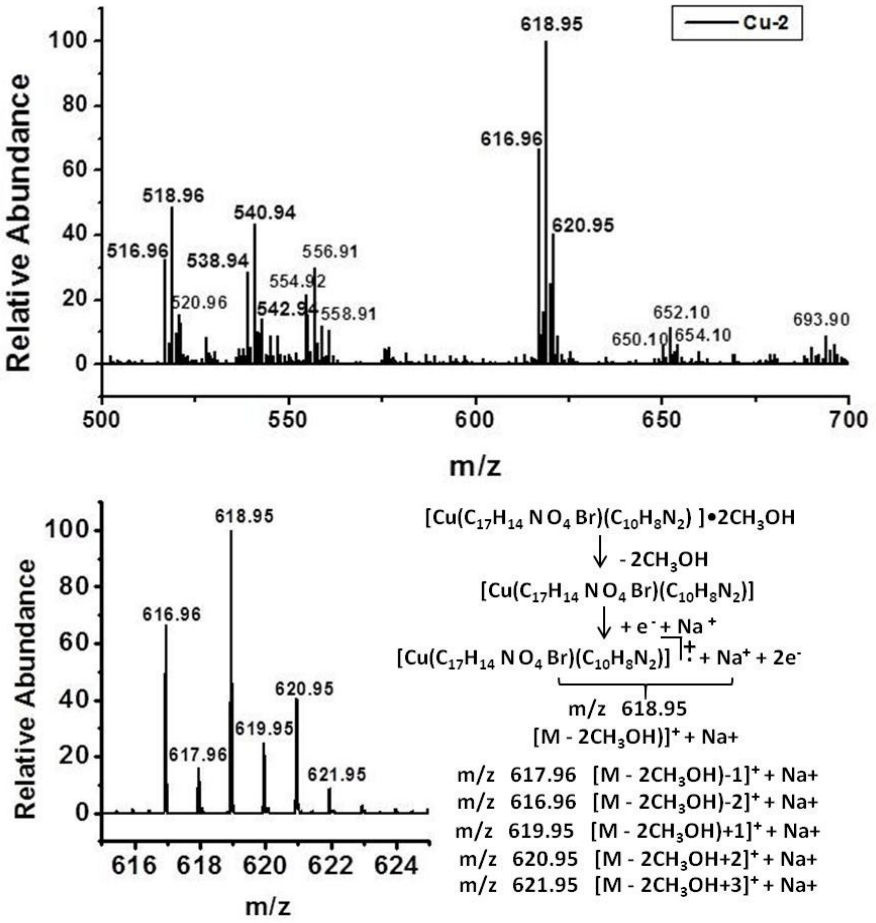

Figure S11. Liquid Chromatography Mass Spectrometry of positive ion of Cu-2.

References

1. G. M Sheldrick, *Acta Cryst. A.* 2008, **64**, 112.