Electronic Supporting Information

A novel chiral oxazoline copper(II)-based complex inhibits ovarian cancer growth in vitro and vivo by regulating VEGF/VEGFR2 downstream signaling pathways and apoptosis factors

Rong Fan [#, a], Jing-chen Wei [#, a], Bing-Bing Xu [a], Nan Jin [a], Xiao-Yi Gong [a], Xiu-Ying Qin* [, a, b]

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. *L*-Methioninol was purchased from Sequoia precision Chemical Co., Ltd. 4-Hydroxyisophthalaldehyde was purchased from SAAN Chemical Technology Co., Ltd. Potassium hydroxide (A.R.), anhydrous ethanol (A.R.) and copper nitrate trihydrate (A.R.) were purchased from Xilong Science Co., Ltd. MTT, penicillin/streptomycin, and dimethylsulfoxide were purchased from Sigma-Aldrich, USA. Dulbecco's modified eagle medium (DMEM, Gibco), Fetal bovine serum (FBS, GEMINI), pancreatic enzyme (Gibco), cell culture plates (Corning) were used. Annexin V/PI apoptosis kit was purchased from BD Bioscience. The JC-1 mitochondrial membrane potential detection kit was from Beyetime (Shanghai, China). HUVECs and SKOV3 cell lines were purchased from Shanghai oulu biological technology Co, ltd. Cell culture: HUVECs were cultured in DMEM medium supplemented with FBS (10%), penicillin (100 μg/mL), and streptomycin (100 μg/mL); SKOV3 cells were cultured in DMEM 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. BALB/c nude mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. Chin.

Instruments.

IR spectras were taken on a IRAffinity-1 FT-IR spectrometer with KBr pallets in the range of 4000～400 cm^{-1}. The crystal structures were determined by a four-circle CCD diffractometer (XtaLAB Synergy, Dualflex, HyPix). Apoptosis assays, reactive oxygen species and mitochondrial membrane potential detections were determined by BD FACSCanto. The animal experiments were approved by the Experimental Animal Ethics Committee of Guilin Medical University (Giulin, China). Cells were cultured in a CO_2 incubator (170S, Galaxy, New Brunswick). Cells were observed with an inverted microscope (OLYMPUS CKX35, Japan).

Supporting Tables

Table S1. Cystal data and structure refinement parameters for $[Cu(C_{13}H_{14}NO_3S)_2]_2$.

Parameters	**$[Cu(C_{13}H_{14}NO_3S)_2]_2$**
Empirical formula	$C_{52}\ H_{56}\ Cu_2\ N_4\ O_{12}\ S_4$
Formula moiety	$2(C_{26}\ H_{28}\ Cu\ N_2\ O_6\ S_2)$
Formula weight	1184.32
Temperature (K)	273(2)
Radiation type	Cu K\a
Wavelength (Å)	1.54184
Crystal system, Space group	Triclinic, *P*1
a, b, c (Å)	10.68048(15), 11.38830(10), 12.62996(13)
α, β, γ (°)	72.3194(8), 67.0804(11), 84.0598(9)
V (Å^3)	1347.91(3)
Z, D_{Calcd} (Mg.m^{-3})	1, 1.459
Abs. coefficient (mm^{-1})	2.958
F (000)	614
θ range for data collection / refinement (°)	3.9470~77.2020 / 3.968 ~74.437
	$-13 \leq h \leq 11$
Limiting indices	$-14 \leq k \leq 13$
	$-15 \leq l \leq 15$
Reflections collected	42630
Independent reflections (*R*int = 0.035)	9946
Observed data ($I > 2\sigma(I)$)	9745
Refinement method	Full-matrix least-squares on F^2
Nref / Npar / Nres	9946 /690/ 34
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.0878, 0.2431, 1.003
	$w = 1/[\sigma^2(F_o^2) + (0.2440P)^2 + 0.0250P]$
	where $P = (F_o^2 + 2F_c^2)/3$
H-atom treatment	H-atom parameters constrained
Shift max / mean	0.000 / 0.000
Completeness to theta	0.999
Absolute structure parameter*	-0.03(4)

* Classical Flack method preferred over Parsons because s.u. lower.

Table S2. Selected bond distances (Å) and angles (°) for $[Cu(C_{13}H_{14}NO_3S)_2]_2$.

Bond	**Dist. (Å)**	**Bond**	**Dist. (Å)**
Cu1 — O1	1.902(6)	Cu2 — O10	1.908(6)
Cu1 — O4	1.912(6)	Cu2— O7	1.911(6)
Cu1 — N2	1.965(6)	Cu2 — N3	1.971(6)
Cu1 — N1	1.969(6)	Cu2 — N4	1.968(6)
S1 — C12	1.811(10)	S1 — C13	1.754(17)
S2 — C25	1.808(13)	S2 — C26	1.80(3)
S3 — C38	1.814(13)	S3 — C39	1.80(2)
S4A — C51	2.23(2)	S4A — C52A	1.98(4)
S4B — C51	1.815(18)	S4B — C52B	1.79(6)
C8 — O3	1.345(8)	O2— C5	1.186(14)
N1 — C8	1.289(9)	C21 — N2	1.284(10)
N1 — C10	1.475(8)	C21 — O6	1.337(10)
C9 — O3	1.443(10)	C22 — O6	1.482(12)
C9 — C10	1.520(10)	C23 — N2	1.491(11)
C34 —O9	1.348(9)	C47 —O12	1.347(9)
Angle	(°)	**Angle**	(°)
O1 —Cu1— O4	174.3(3)	O10— Cu2— O7	167.4(3)
O1 —Cu1— N2	88.4(3)	O10—Cu2 —N3	90.3(2)
O1— Cu1 —N1	91.5(2)	O10— Cu2—N4	91.4(2)
O4 —Cu1 —N2	91.8(3)	O7— Cu2— N3	91.8(2)
O4— Cu1 —N1	89.2(2)	O7 —Cu2 —N4	90.3(3)
N2 —Cu1—N1	171.2(3)	N4— Cu2— N3	162.8(2)
C13 —S1—C12	100.1(7)	C26—S2—C25	100.2(12)
C39 —S3—C38	99.9(9)	C52A —S4A—C51	84.3(12)
C52B —S4B—C51	111.8(19)	O2— C5—C4	126.8(10)
N1 —C8—C7	127.4(6)	N2 —C21—C20	126.9(7)
N1 —C8—O3	115.6(6)	N2 —C21—O6	116.3(7)
O3 —C8—C7	116.9(6)	O6 —C21—C20	116.7(7)
N3 —C34—C33	127.2(7)	N4 —C47—C46	127.3(6)
N3 —C34—O9	115.8(6)	N4 —C47—O12	116.6(6)
C8—N1— C10	108.8(6)	C21— N2— C23	108.1(6)
C8 —O3 —C9	106.8(6)	C21— O6 —C22	106.6(6)
O9 —C34—C33	117.0(6)	O12 —C47—C46	116.1(6)

Table S3. Hydrogen bond lengths (Å), angles (°) for $[Cu(C_{13}H_{14}NO_3S)_2]_2$.

D*—H••• *A	***D*—H**	**H ••• *A***	***D* ••• *A***	**<*D*H*A***
C9—H9B••• O5 [i]	0.97	2.59	3.441 (11)	146.6
C5—H5 ••• O9 [i]	0.93	2.63	3.497 (12)	156.2
C37—H37B ••• S1 [ii]	0.97	2.97	3.821(9)	146.9

C24—H24A ••• O1	0.97	2.55	3.136 (12)	118.5
C51—H51D ••• S1^{ii}	0.97	2.87	3.788(18)	158.5

Symmetry codes: (i) x, y, z-1; (ii) x, y+1, z.

Supplementary Figures

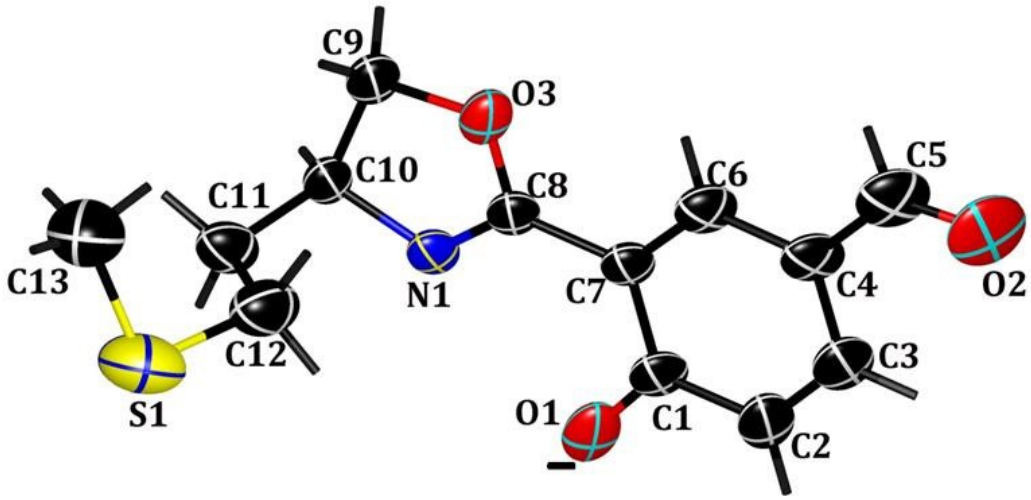

Figure S1 The structure of anion ligand $(C_{13}H_{14}NO_3S)^-$ in the complex $[Cu(C_{13}H_{14}NO_3S)_2]_2$.

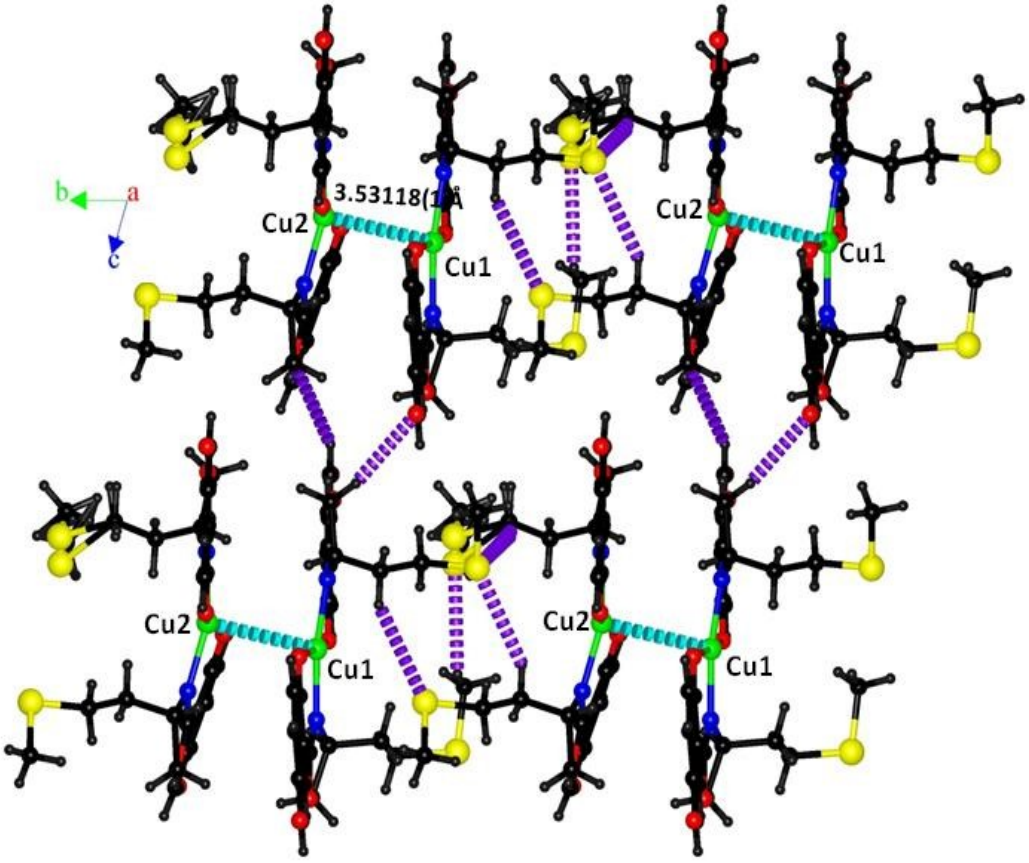

Figure S2 Stacking diagram of complexes in *bc* plane. The distance between Cu1 and Cu2 is 3.53118(1) **Å**, and the purple dotted line represents hydrogen bonding.

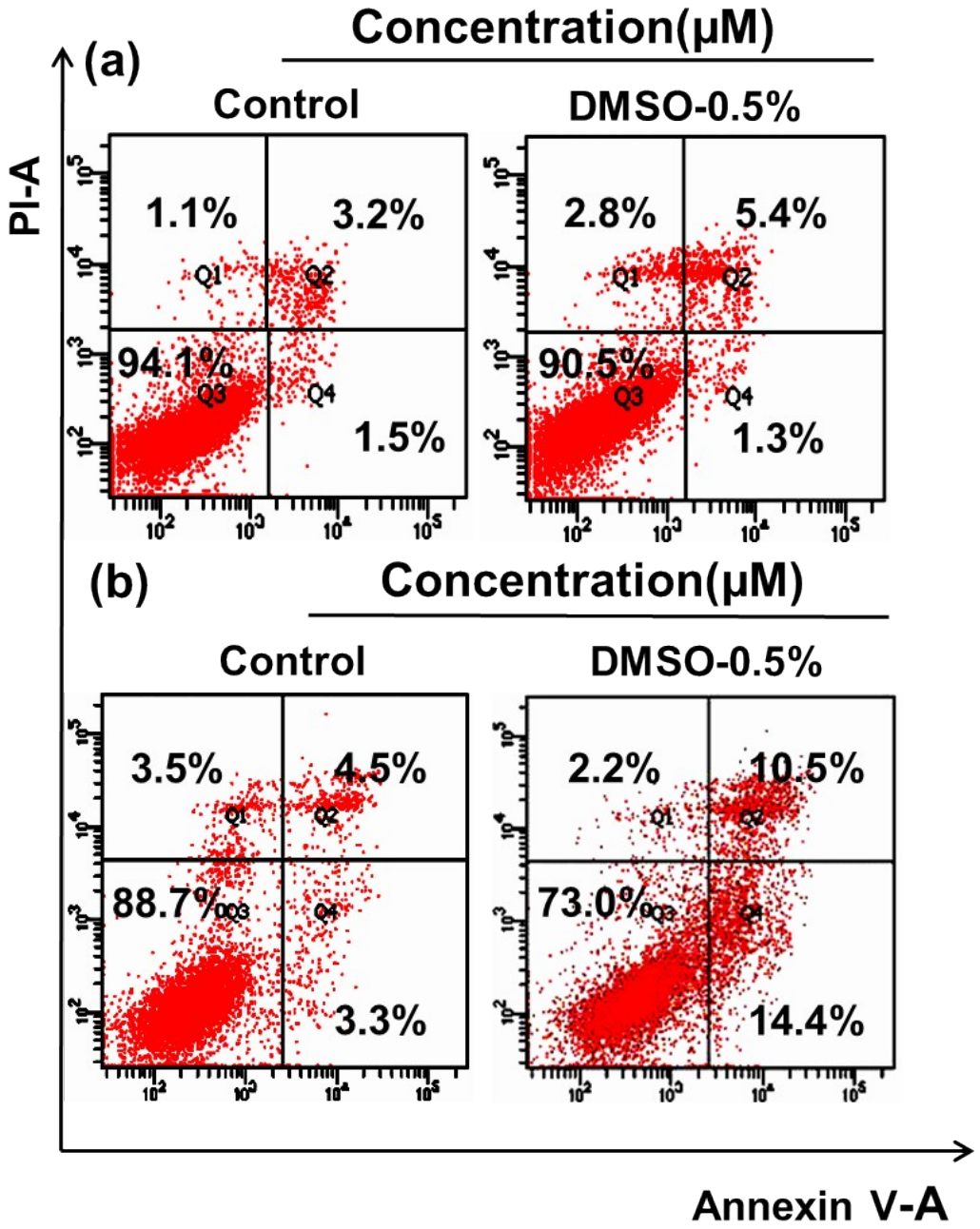

Figure S3 SKOV3 cells (a) and HUVECs (b) were treated with the solvent used at the maximum concentration of $[Cu(C_{13}H_{14}NO_3S)_2]_2$ (DMSO-0.5%) for 27h, respectively, cells were stained with annexin V/PI and were analyzed by flow cytometry for detecting apoptosis and injury.

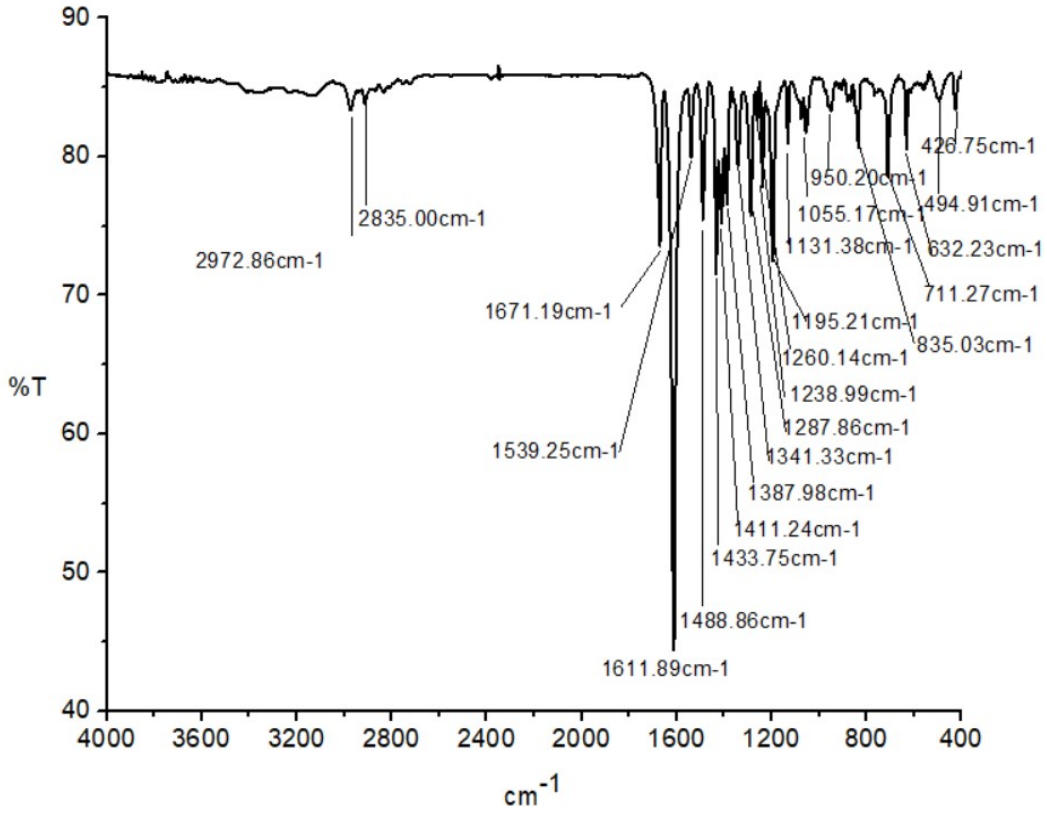

Figure S4 FT-IR of $[Cu(C_{13}H_{14}NO_3S)_2]_2$.