Electronic Supporting Information Materials

Tryptanthrin derivatives copper(II) complexes with high antitumor activity by inhibiting telomerase activity, and inducing mitochondria-mediated apoptosis and S-phase arrest in BEL-7402

Qi-Pin Qin [a,c,1], Bi-Qun Zou [b,c,1], Ming-Xiong Tan [a,*], Shu-Long Wang [a,c], Yan-Cheng Liu [c] and Hong Liang [c,*]

Table S1. Crystal data and structure refinement details for **Try-Cu**.

Empirical formula	$C_{30}H_{16}Cl_2CuN_4O_4$
Formula weight	630.91
Temperature/K	293(2)
Crystal system	triclinic
Space group	P-1
a/Å	7.8422(3)
b/Å	8.7783(3)
c/Å	9.4903(3)
α/°	90.286(2)
β/°	104.150(2)
γ/°	97.796(2)
Volume/Å^3	627.15(4)
Z	1
ρ_{calc}mg/mm^3	1.670
m/mm^{-1}	1.132
F(000)	319.0
Crystal size/mm^3	0.41 × 0.23 × 0.12
2Θ range for data collection	4.42 to 52.72°
Index ranges	$-9 \le h \le 9$, $-10 \le k \le 10$, $-11 \le l \le 11$
Reflections collected	9770
Independent reflections	2554[R(int) = 0.0234]
Data/restraints/parameters	2554/0/187
Goodness-of-fit on F^2	1.067
Final R indexes [I>=2σ (I)]	R_1 = 0.0300, wR_2 = 0.0780
Final R indexes [all data]	R_1 = 0.0377, wR_2 = 0.0824
Largest diff. peak/hole / e Å^{-3}	0.64/-0.32

[a] $R_1 = \Sigma\, ||F_o| - |F_c||/\Sigma|F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{½}$.

Table S2. Selected bond lengths (Å) for **Try-Cu**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1	C2	1.384(3)	C9	C10	1.458(3)
C1	C6	1.387(3)	C9	O2	1.213(3)
C2	C3	1.389(3)	C10	C11	1.399(3)
C3	C4	1.373(4)	C10	C15	1.385(3)
C4	C5	1.397(3)	C11	C12	1.380(3)
C5	C6	1.409(3)	C11	N2	1.435(3)
C5	C7	1.467(3)	C12	C13	1.392(3)
C6	N1	1.408(3)	Cu1	O1[1]	2.7070(16)
C7	N2	1.399(3)	Cu1	O1	2.7070(16)
C7	O1	1.213(3)	Cl1	Cu1	2.2420(6)
C8	C9	1.509(3)	Cu1	Cl1[1]	2.2420(6)
C8	N1	1.284(3)	Cu1	N1	2.0790(17)
C8	N2	1.377(2)	Cu1	N1[1]	2.0790(17)

Table S3. Selected bond angles (°) for **Try-Cu**.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C2	C1	C6	119.7(2)	C15	C10	C11	120.84(19)
C1	C2	C3	120.6(2)	C10	C11	N2	109.44(17)
C4	C3	C2	120.3(2)	C12	C11	C10	121.8(2)
C3	C4	C5	120.1(2)	C12	C11	N2	128.7(2)
C4	C5	C6	119.5(2)	C11	C12	C13	116.6(2)
C4	C5	C7	118.98(19)	C14	C13	C12	122.3(2)
C6	C5	C7	121.49(19)	C13	C14	C15	120.7(2)
C1	C6	C5	119.9(2)	C10	C15	C14	117.8(2)
C1	C6	N1	119.51(18)	Cl1	Cu1	Cl1[1]	180.00(3)
N1	C6	C5	120.59(19)	N1[1]	Cu1	Cl1	90.38(5)
N2	C7	C5	112.26(17)	N1	Cu1	Cl1	89.62(5)
O1	C7	C5	126.3(2)	N1	Cu1	Cl1[1]	90.38(5)
O1	C7	N2	121.4(2)	N1[1]	Cu1	Cl1[1]	89.62(5)
N1	C8	C9	125.62(18)	N1[1]	Cu1	N1	180.0
N1	C8	N2	126.2(2)	C6	N1	Cu1	127.15(14)
N2	C8	C9	108.09(17)	C8	N1	C6	116.69(17)
C10	C9	C8	104.88(17)	C8	N1	Cu1	115.95(14)
O2	C9	C8	123.29(19)	C7	N2	C11	128.21(17)
O2	C9	C10	131.8(2)	C8	N2	C7	122.60(18)
C11	C10	C9	108.41(19)	C8	N2	C11	109.14(17)
C15	C10	C9	130.7(2)				

Table S4. Crystal data and structure refinement details for **BrTry-Cu**.

Empirical formula	$C_{30}H_{14}Br_2Cl_2CuN_4O_4$
Formula weight	788.71
Temperature/K	296.15
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	10.8609(12)
b/Å	13.7631(15)
c/Å	18.269(2)
α/°	90.00
β/°	97.096(5)
γ/°	90.00
Volume/Å^3	2709.9(5)
Z	4
ρ_{calc}mg/mm^3	1.933
m/mm^{-1}	4.000
F(000)	1548.0
Crystal size/mm^3	0.21 × 0.13 × 0.12
2Θ range for data collection	3.72 to 52.74°
Index ranges	$-13 \le h \le 13$, $-15 \le k \le 17$, $-22 \le l \le 22$
Reflections collected	19518
Independent reflections	5501[R(int) = 0.0921]
Data/restraints/parameters	5501/0/388
Goodness-of-fit on F^2	1.041
Final R indexes [I>=2σ (I)]	R_1 = 0.0944, wR_2 = 0.2623
Final R indexes [all data]	R_1 = 0.1780, wR_2 = 0.3087
Largest diff. peak/hole / e Å^{-3}	3.47/-0.77

[a] $R_1 = \Sigma\, ||F_o| - |F_c||/\Sigma|F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{½}$.

Table S5. Selected bond lengths (Å) for **BrTry-Cu**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Br1	C1	1.889(12)	C16	C21	1.407(16)
Br2	C16	1.905(13)	C17	C18	1.336(17)
C1	C2	1.356(16)	C18	C19	1.418(17)
C1	C6	1.415(16)	C19	C20	1.403(17)
C2	C3	1.395(17)	C19	N1	1.389(15)
C3	C4	1.390(16)	C20	C21	1.388(16)
C4	C5	1.373(17)	C20	C23	1.430(16)
C4	N3	1.432(16)	C22	C23	1.518(16)
C5	C6	1.375(16)	C22	N1	1.392(14)
C5	C7	1.457(17)	C22	N2	1.262(15)
C7	C8	1.449(17)	C23	O1	1.218(13)
C7	O4	1.220(13)	C24	C25	1.398(17)
C8	N3	1.379(14)	C24	N1	1.420(15)
C8	N4	1.326(15)	C24	O2	1.264(14)
C9	C10	1.425(18)	C25	C26	1.420(16)
C9	N3	1.397(15)	C25	C30	1.427(16)
C9	O3	1.215(14)	C26	C27	1.265(16)
C10	C11	1.432(17)	C27	C28	1.392(17)
C10	C15	1.430(18)	C28	C29	1.394(16)
C11	C12	1.305(17)	O1	Cu1	2.8945(91)
C11	N4	1.443(16)	O2	Cu1	2.7384(74)
C12	C13	1.390(17)	Cl1	Cu1	2.220(3)
C13	C14	1.398(18)	Cl2	Cu1	2.225(3)
C14	C15	1.380(18)	Cu1	N2	2.069(9)
C16	C17	1.374(17)	Cu1	N4	2.068(10)

Table S6. Selected bond angles (°) for **BrTry-Cu**.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C2	C1	Br1	120.5(9)	C21	C20	C19	122.7(11)
C2	C1	C6	120.4(12)	C21	C20	C23	129.5(12)
C6	C1	Br1	118.9(10)	C20	C21	C16	115.4(11)
C1	C2	C3	122.9(12)	N1	C22	C23	105.5(10)
C4	C3	C2	115.6(12)	N2	C22	C23	126.9(10)
C3	C4	N3	127.7(12)	N2	C22	N1	127.5(11)
C5	C4	C3	122.6(13)	C20	C23	C22	106.2(11)
C5	C4	N3	109.5(10)	O1	C23	C20	132.5(12)
C4	C5	C6	121.0(11)	O1	C23	C22	121.3(11)
C4	C5	C7	108.5(11)	C25	C24	N1	114.1(10)
C6	C5	C7	130.5(11)	O2	C24	C25	128.6(12)
C5	C6	C1	117.4(11)	O2	C24	N1	117.3(12)
C8	C7	C5	104.7(11)	C24	C25	C26	122.1(11)
O4	C7	C5	130.1(12)	C24	C25	C30	123.4(11)
O4	C7	C8	125.2(12)	C26	C25	C30	114.5(12)
N3	C8	C7	109.9(11)	C27	C26	C25	124.8(12)
N4	C8	C7	127.3(11)	C26	C27	C28	119.9(12)
N4	C8	N3	122.8(11)	C27	C28	C29	120.5(12)
N3	C9	C10	113.8(11)	C30	C29	C28	118.5(11)
O3	C9	C10	124.7(12)	C29	C30	C25	121.8(11)
O3	C9	N3	121.5(12)	C29	C30	N2	120.1(11)
C9	C10	C11	123.0(12)	N2	C30	C25	118.1(11)
C9	C10	C15	121.6(12)	Cl1	Cu1	Cl2	178.12(13)
C15	C10	C11	115.2(12)	N2	Cu1	Cl1	92.2(3)
C10	C11	N4	117.4(12)	N2	Cu1	Cl2	86.5(3)
C12	C11	C10	123.2(12)	N4	Cu1	Cl1	90.9(3)
C12	C11	N4	119.4(11)	N4	Cu1	Cl2	90.4(3)
C11	C12	C13	119.5(12)	N4	Cu1	N2	176.8(4)
C12	C13	C14	122.9(14)	C19	N1	C22	110.3(10)
C15	C14	C13	116.1(13)	C19	N1	C24	130.2(10)
C14	C15	C10	123.0(13)	C22	N1	C24	119.1(10)
C17	C16	Br2	121.2(10)	C22	N2	C30	117.5(10)
C17	C16	C21	122.2(12)	C22	N2	Cu1	116.1(8)
C21	C16	Br2	116.5(10)	C30	N2	Cu1	124.4(8)
C18	C17	C16	122.3(12)	C8	N3	C4	107.2(10)
C17	C18	C19	118.6(12)	C8	N3	C9	123.8(11)
C20	C19	C18	118.9(13)	C9	N3	C4	128.8(10)
N1	C19	C18	130.7(12)	C8	N4	C11	118.7(10)

N1	C19	C20	110.3(10)	C8	N4	Cu1	118.2(8)
C19	C20	C23	107.7(11)	C11	N4	Cu1	122.5(8)

Table S7. Inhibitory rates (%) of $CuCl_2 \cdot 2H_2O$, cisplatin, Try, BrTry and the corresponding complexes **Try-Cu** and **BrTry-Cu** toward on the selected five human cells for 48 h.

Compounds	T-24	BEL-7402	HepG2	MGC80-3	HL-7702
Try [a]	20.19±1.78	40.23±0.26	30.25±0.59	38.67±1.46	34.03±1.27
Try-Cu [a]	80.00±0.62	85.16±0.58	49.73±1.07	71.46±1.19	40.25±2.11
BrTry [a]	60.26±0.91	48.87±0.68	35.81±0.57	41.27±0.34	39.54±0.55
BrTry-Cu [a]	37.70±2.01	46.93±1.60	39.69±1.28	41.91±1.10	37.46±0.63
$CuCl_2 \cdot 2H_2O$ [b]	30.12±1.16	35.06±2.18	25.49±0.54	29.47±0.23	30.74±1.85
Cisplatin [a,c]	60.12±1.19	56.03±1.02	57.45±0.61	61.52±0.58	58.11±1.39

Results represent mean ± SD of at least six independent experiments. SD represents the standard deviation. [a] The concentration is 20 μM. [b] The concentration is 100 μM. [c] Cisplatin was dissolved at a concentration of 1.0 mM in 0.154 M NaCl.

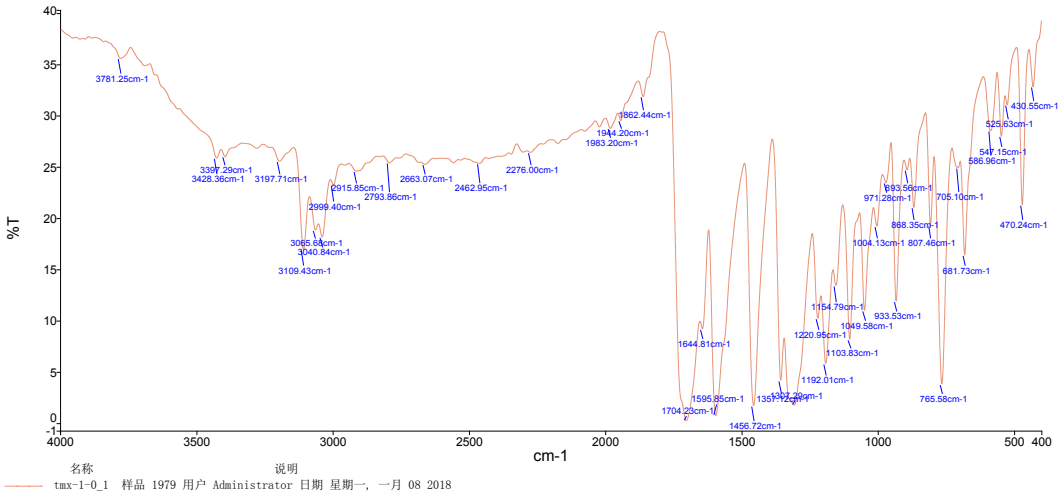

Fig. S1. IR (KBr) spectra of **Try-Cu**

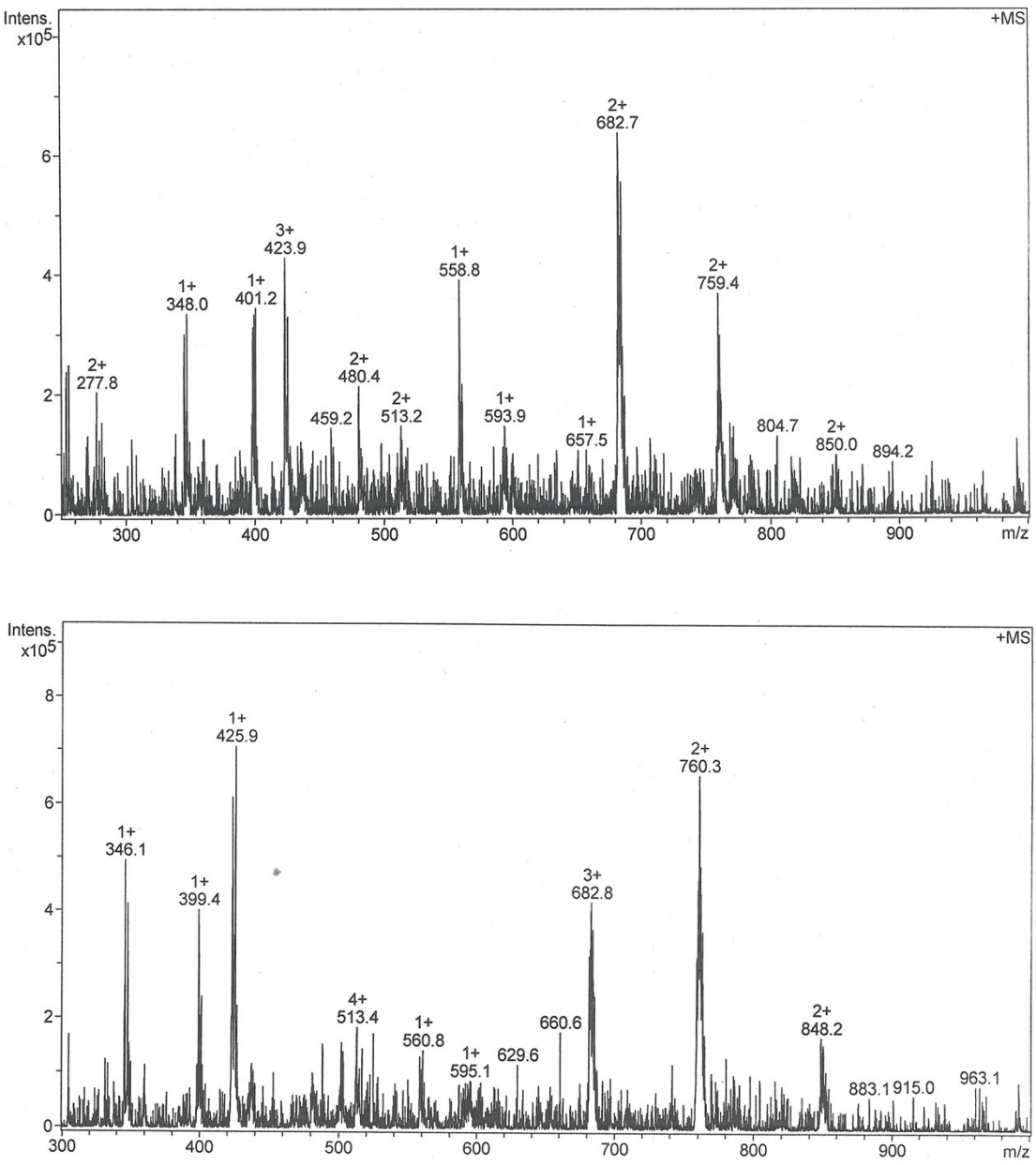

Fig. S2. The mass spectra of **Try-Cu** in Tris-HCl buffer solution (containing 5% DMSO) for 0 h (top) and 48 h (down), respectively.

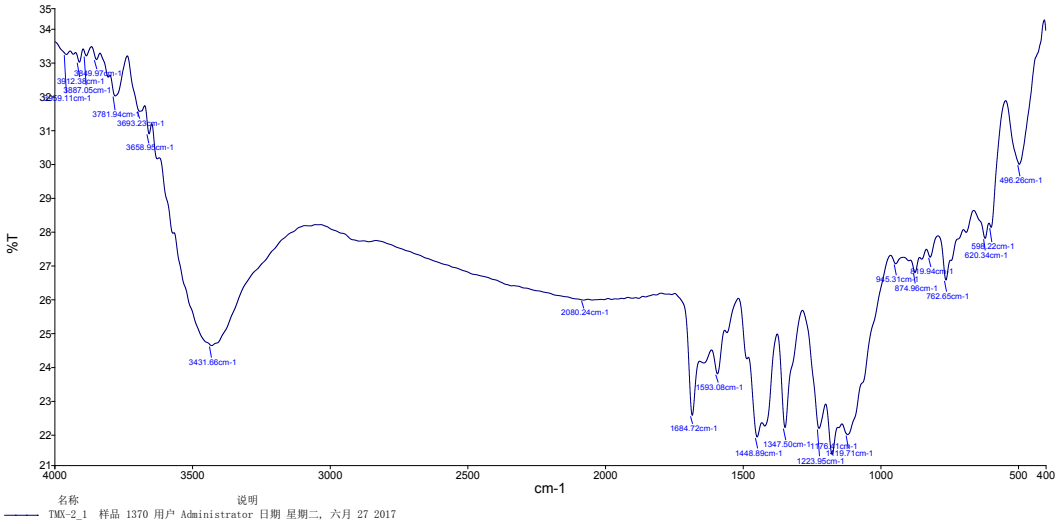

Fig. S3. IR (KBr) spectra of **BrTry-Cu**.

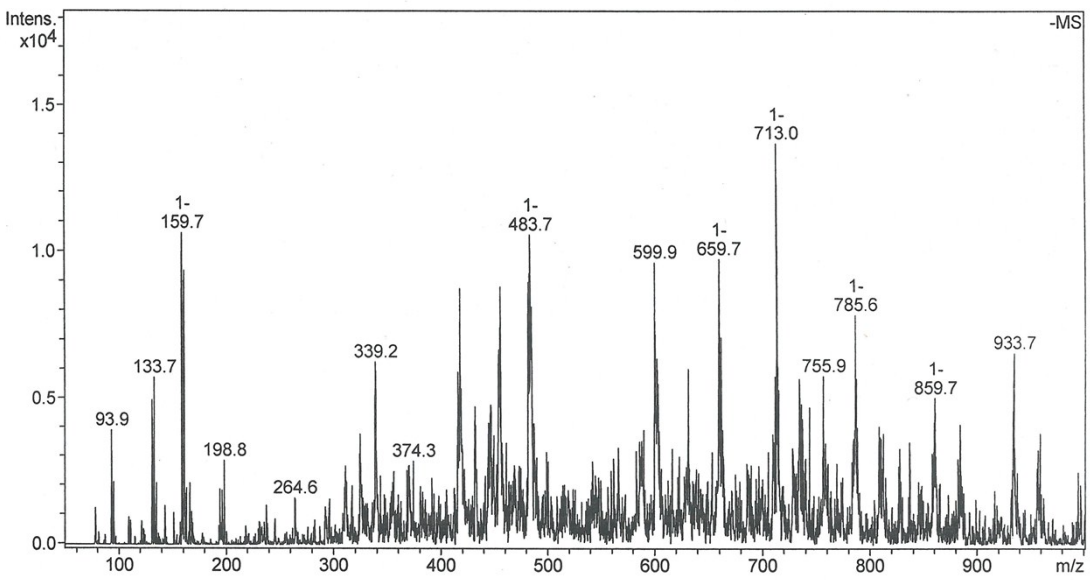

Fig. S4. The mass spectra of **BrTry-Cu** in Tris-HCl buffer solution (containing 5% DMSO) for 0 h.

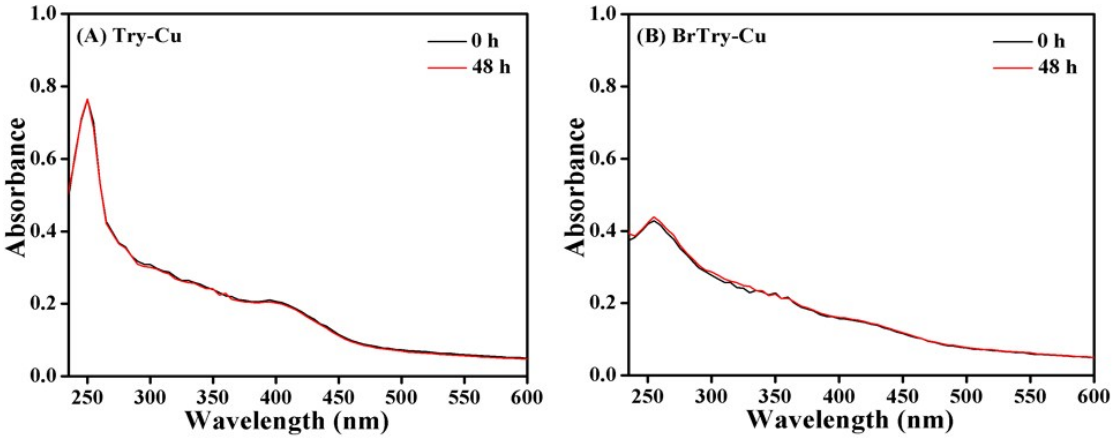

Fig. S5. UV-Vis absorption spectra of **Try-Cu** and **BrTry-Cu** (2.0×10^{-5} M) in TBS (Tris-HCl buffer solution, 10 mM, pH 7.35) solution in the time course 0 and 48 h, respectively.

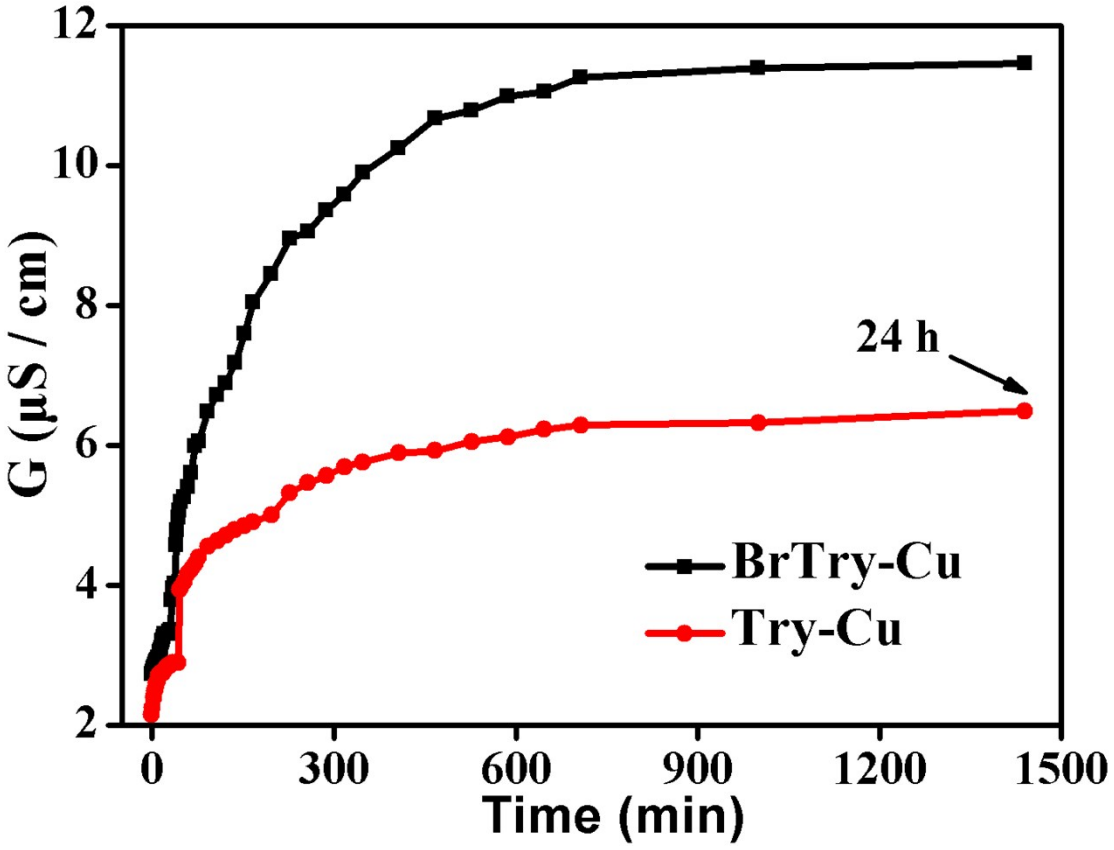

Fig. S6. The variations on the conductivity of **Try-Cu** (4.0 μM) and **BrTry-Cu** (30.9 μM) in cellular lysate (BEL-7402 cells) at 37 ºC, shown as the average value in a

triplicate experiment, which indicated that it would dissociated in TBS to give the species of **Try-Cu** (4.0 μM) and **BrTry-Cu** (30.9 μM), as suggested by the stability results of UV-Vis and ESI-MS spectroscopy.

Abbreviations

TBS	Tris-HCl buffer
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ROS	reactive oxygen species
IC_{50}	half maximal inhibitory concentration
T-24 cells	human bladder cancer cells
BEL-7402 cells	human hepatoma cells
HepG2 cells	human hepatocellular carcinoma cells
MGC80-3 cells	human gastric cancer cells
HL-7702 cells	human normal hepatocytes cells
PI	propidium iodide
Δψ	mitochondrial membrane potential
JC-1	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine