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Supplementary material

SUPPLEMENTARY MATERIAL TO

Synthesis and efficacy of copper(II) complexes bearing *N*(4)-substituted thiosemicarbazide and diimine co-ligands on plasmid DNA and HeLa cell lines

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CHARACTERIZATION DATA

*1-Phenyl-2-((5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)ethanone* (***HL***). Yield: 68 %; Colorless; m.p.: 190–192 °C; Anal. calcd. for $C_{15}H_{12}N_4OS$: C, 60.79; H, 4.08; N, 18.19; S, 10.82 %. Found: C, 60.68; H, 4.02; N, 18.25; S, 10.77 %; ^{1}H-NMR (300 MHz, DMSO, δ / ppm): 9.15 (1H, *s*), 8.59 (1H, *s*), 8.26 (1H, *d*, J = 6.0 Hz), 8.06 (2H, *d*, J = 6.0 Hz), 7.66–7.61 (1H, *m*), 7.54–7.49 (2H, *m*), 7.39 (2H, *s*), 4.87 (2H, *s*); Λ_m (Ω^{-1} cm^2 mol^{-1}): 5.

*(*E*)-*N*-Methyl-2-(1-phenyl-2-((5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)-ethylidene) hydrazinecarbothioamide* (***H(L1)***). Yield: 74 %; Yellow; m.p.: 212–214 °C; Anal. calcd. for $C_{17}H_{17}N_7S_2$: C, 53.24; H, 4.47; N, 25.57; S, 16.72 %. Found: C, 53.36; H, 4.44; N, 25.61; S, 16.80 %; FT-IR (KBr, cm^{-1}): 3340 (s, N(4)H), 3199, (s, N(2)H), 1554 (s, C=N), 813 (s, C=S), 987 (s, N–N); ^{1}H-NMR (300 MHz, DMSO, δ / ppm): 10.88 (1H, *s*), 9.32 (1H, *s*), 9.23 (1H, *d*, J = 15 Hz), 8.66 (1H, *s*), 8.54 (1H, *d*, J = 9.0 Hz), 8.25 (1H, *d*, J = 3.0 Hz), 8.10 (1H, s), 7.85 (1H, *t*, J = 3.0 Hz), 7.56–7.52 (1H, *m*), 7.43 (3H, *d*, J = 3.0 Hz), 4.59 (2H, *s*), 3.19 (3H, *d*, J = 6.0 Hz); ^{13}C-NMR (75 MHz, DMSO, δ / ppm): 179.5, 162.9, 151.0, 148.2, 147.8, 145.5, 136.4, 134.9, 133.8, 130.0, 129.3, 128.9, 127.3, 124.1, 36.8, 31.7, 26.8; UV–Vis (λ_{max}, DMF, nm): 225, 295 355; Λ_m (Ω^{-1} cm^2 mol^{-1}): 4

*(*E*)-*N*-Ethyl-2-(1-phenyl-2-((5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)-ethylidene) hydrazinecarbothioamide* (***H(L2)***). Yield: 70 %; yellow; m.p.: 222–224 °C; Anal. calcd. for $C_{18}H_{19}N_7S_2$: C, 54.39; H, 4.82; N, 24.66; S, 16.13

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%. Found: C, 54.42; H, 4.91; N, 24.79; S, 16.09 %; FT-IR (KBr, cm^{-1}): 3240 (*m*, N(4)H), 2970 (*s*, N(2)H), 1518 (*s*, C=N), 808 (*s*, C=S), 977 (*s*, N–N); ^{1}H-NMR (300 MHz, DMSO, δ / ppm): 11.08 (1H, *s*), 9.51 (1H, s), 8.85 (2H, *d*, J = 30.0 Hz,), 8.53–8.51 (1H, *m*), 8.14–8.11 (1H, *m*), 7.96–7.94 (2H, *m*), 7.86 (1H, *s*), 7.62–7.60 (3H, *m*), 4.75 (2H*,* *s*), 3.95–3.93 (2H, *m*), 1.46 (3H, *t*, J = 6.0 Hz); ^{13}C-NMR (75 MHz, DMSO, δ / ppm): 179.7, 160.1, 157.8, 157.4, 155.3, 154.9, 148.0, 136.9, 134.2, 131.8, 128.9, 128.8, 128.4, 128.1, 124.3, 38.1, 39.9, 15.8; UV–Vis (λ_{max}, DMF, nm): 280, 360; Λ_m (Ω^{-1} cm^2 mol^{-1}): 7

*(*E*)-*N*-Phenyl-2-(1-phenyl-2-((5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)-ethylidene) hydrazinecarbothioamide (**H(L3)**)*. Yield: 61 %; Yellow; m.p.: 235–237 °C; Anal. calcd. for $C_{22}H_{19}N_7S_2$: C, 59.30; H, 4.30; N, 22.01; S, 14.39 %. Found: C, 59.26; H, 4.21; N, 22.11; S, 14.38 %; FT-IR (KBr, cm^{-1}): 3290 (*s*, N(4)H), 3147 (*s*, N(2)H), 1534 (*s*, C=N), 826 (*s*, C=S), 973 (*s*, N–N); ^{1}H-NMR (300 MHz, DMSO, δ / ppm): 11.60 (1H, *s*), 10.02 (1H, *s*), 9.74 (1H, *s*), 9.61 (2H, *s*), 8.99–8.96 (1H, *m*), 8.90 (1H, *d*, J = 6.0 Hz), 8.65 (1H, *d*, J = 6.0 Hz), 8.43 (1H, *d*, J = 9.0 Hz), 8.35 (2H, *s*), 8.03–7.95 (1H, *m*), 7.91–7.86 (1H, *m*), 7.81–7.72 (1H, *m*), 7.70–7.63 (1H, *m*), 7.59–7.57 (1H, *m*), 7.32–7.27 (1H, *m*), 5.17 (2H, *s*); ^{13}C-NMR (75 MHz, DMSO, δ / ppm): 184.7, 155.1, 157.9, 157.5, 155.4, 148.1, 138.7, 135.8, 135.8, 132.7, 133.1, 131.2, 129.2, 129.0, 128.9, 128.8, 128.5, 128.4, 128.2, 126.9, 124.1, 38.4; UV–Vis (λ_{max}, DMF, nm): 310, 345; Λ_m (Ω^{-1} cm^2 mol^{-1}): 11.

*[Cu(L1)$_2$] (**C1**)*. Yield: 69 %; green; Anal. calcd. for $C_{34}H_{32}CuN_{14}S_4$: C, 49.29; H, 3.89; N, 25.67; S, 15.48 %. Found: C, 49.17; H, 3.81; N, 23.62; S, 15.39 %; FT-IR (KBr, cm^{-1}): 3059 (*s*, N(4)H), 1562 (*s*, C=N), 738 (*s*, C–S), 1047 (*s*, N–N), 425 (*m*, Cu–N), 612 (*m*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 295, 360, 635 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 158×10^{-4}; $g_{||}$: 2.252; $g_{\perp}$: 2.057; g_{ll} / A_{ll} (cm): 140; G: 4.38; Λ_m (Ω^{-1} cm^2 mol^{-1}):16

*[Cu(L2)$_2$] (**C2**)*. Yield: 62 %; green; Anal. calcd. for $C_{36}H_{36}CuN_{14}S_4$: C, 50.48; H, 4.24; N, 22.89; S, 14.97 %. Found: C, 50.54; H, 4.29; N, 22.95; S, 14.89 %; FT-IR (KBr, cm^{-1}): 3061 (*s*, NH), 1556 (*s*, C=N), 738 (*s*, C–S), 1012 (*s*, N–N), 430 (*m*, Cu–N) and 621 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 290, 412, 645 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 162×10^{-4}; $g_{||}$: 2.249; $g_{\perp}$: 2.056; g_{ll} / A_{ll} (cm): 138; G: 4.59; Λ_m (Ω^{-1} cm^2 mol^{-1}): 17.

[Cu(L3)$_2$] (C3). Yield: 58 %; green; Anal. calcd. for: $C_{44}H_{36}CuN_{14}S_4$: C, 55.47; H, 3.81; N, 20.58; S, 13.46 %. Found: C, 55.56; H, 3.75; N, 20.51; S, 13.49 %; FT-R (KBr, cm^{-1}): 3091 (*s*, NH), 1572 (*s*, C=N), 745 (*s*, C–S), 1131 (*s*, N–N), 618 (*s*, Cu–N) and 428 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 318, 380, 715 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 160×10^{-4}; $g_{||}$: 2.258; $g_{\perp}$: 2.062; g_{ll} / A_{ll} (cm): 141; G: 4.28; Λ_m (Ω^{-1} cm^2 mol^{-1}): 18.

*[Cu(L1)(bpy)]Cl (**C4**)*. Yield: 61 %; green; Anal. calcd. for: $C_{27}H_{24}ClCuN_9S_2$: C, 50.86; H, 3.79; N, 19.77; S, 10.06 %. Found: C, 50.92; H,

3.71; N, 19.84; S, 50.88 %; FT-IR (KBr, cm^{-1}): 3098 (*s*, NH), 1553 (*s*, C=N), 761 (*s*, C–S), 1115 (*s*, N–N), 461 (*s*, Cu–N) , 641 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 310, 395, 745 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 163×10^{-4}; $g_{||}$: 2.257; $g_{\perp}$: 2.074; g_{ll} / A_{ll} (cm): 139; *G*: 4.55; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 83.

[Cu(L2)(bpy)]Cl ***(C5)***. Yield: 72 %; green; Anal. calcd. for: $C_{28}H_{26}ClCuN_9S_2$: C, 51.60; H, 4.02; N, 19.34; S, 9.84 %. Found: C, 51.70; H, 3.97; N, 19.34; S, 9.81 %; FT–IR (KBr, cm^{-1}): 3074 (*s*, NH), 1559 (*s*, C=N), 758 (*s*, C–S), 1109 (*s*, N–N), 456 (*s*, Cu–N), 643 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 295, 415, 725 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 160×10^{-4}; $g_{||}$: 2.268; $g_{\perp}$: 2.063; g_{ll} / A_{ll} (cm): 141; *G*: 4.38; Λ_m (Ω^{-1} cm^2 mol^{-1}): 89.

[Cu(L3)(bpy)]Cl ***(C6)*** Yield: 68 %; green; Anal. calcd. for: $C_{32}H_{26}ClCuN_9S_2$: C, 54.93; H, 3.75; N, 18.02; S, 9.16 %. Found: C, 54.89; H, 3.70; N, 18.07; S, 9.15 %; FT-IR (KBr, cm^{-1}): 3088 (*s*, NH), 1549 (*s*, C=N), 752 (*s*, C–S), 1008 (*s*, N–N), 441 (*s*, Cu–N), 628 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 335, 370, 758 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 162×10^{-4}; $g_{||}$: 2.254; $g_{\perp}$ = 2.071; g_{ll} /A_{ll} = 139 cm; *G* = 3.66. Λ_m (Ω^{-1} cm^2 mol^{-1}): 96

[Cu(L1)(phen)]Cl ***(C7)***. Yield: 75 %; green; Anal. calcd. for $C_{29}H_{24}ClCuN_9S_2$: C, 52.64; H, 3.66; N, 19.05; S, 9.69 %. Found: C, 52.70; H, 3.61; N, 19.11; S, 9.74 %; FT-IR (KBr, cm^{-1}): 3055 (*s*, NH), 1568 (*s*, C=N), 775 (*s*, C–S), 1045 (*s*, N–N), 424 (*s*, Cu–N), 642 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 290, 335, 685 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 163×10^{-4}; $g_{||}$: 2.257; $g_{\perp}$: 2.059; g_{ll} / A_{ll} (cm): 141; *G*: 4.49; Λ_m (Ω^{-1} cm^2 mol^{-1}): 93.

[Cu(L2)(phen)]Cl ***(C8)***. Yield: 68 %; green; Anal. calcd. for: $C_{30}H_{26}ClCuN_9S_2$: C, 53.32; H, 3.88; N, 18.66; S, 9.49 %; Found: C, 53.29; H, 3.81; N, 18.70; S, 9.43 %; FT-IR (KBr, cm^{-1}): 3055 (*s*, NH), 1516 (*s*, C=N), 777 (*s*, C–S), 998 (*s*, N–N), 426 (*s*, Cu–N), 653 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 295, 390, 725 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 162×10^{-4}; $g_{||}$: 2.258; $g_{\perp}$: 2.064; g_{ll} / A_{ll} (cm): 140; *G*: 4.14; Λ_m (Ω^{-1} cm^2 mol^{-1}): 98.

[Cu(L3)(phen)]Cl ***(C9)***. Yield: 60 %; green; Anal. calcd. for: $C_{34}H_{26}ClCuN_{14}S_4$: C, 56.42; H, 3.62; N, 17.42; S, 8.86 %. Found: C, 56.46; H, 3.59; N, 17.49; S, 8.89 %; FT–IR (KBr, cm^{-1}): 3059 (*s*, NH), 1543 (*s*, C=N), 771 (*s*, C–S), 1103 (*s*, N–N), 423 (*s*, Cu–N), 632 (*s*, Cu–S); UV–Vis (λ_{max}, DMF, nm): 335, 430, 735 (d–d transition); EPR: $A_{||}$ (cm^{-1}): 162×10^{-4}; $g_{||}$:= 2.260; $g_{\perp}$: 2.059; g_{ll} / A_{ll} (cm): 139; *G*: 4.54; HRMS: calcd. 768.12. Found: 765.17; Λ_m (Ω^{-1} cm^2 mol^{-1}): 103.

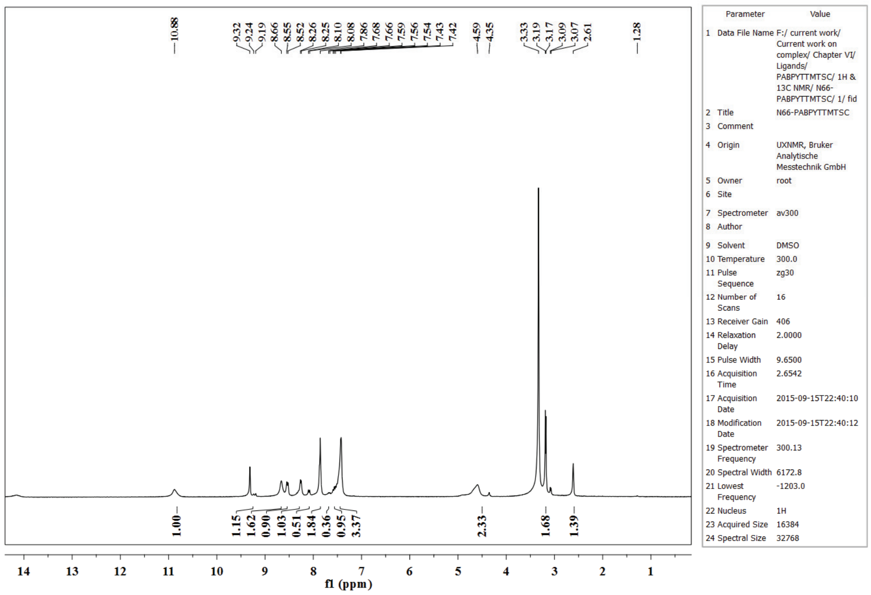

Fig. S-1. ^{1}H-NMR spectrum of H(L1) in DMSO-d_6.

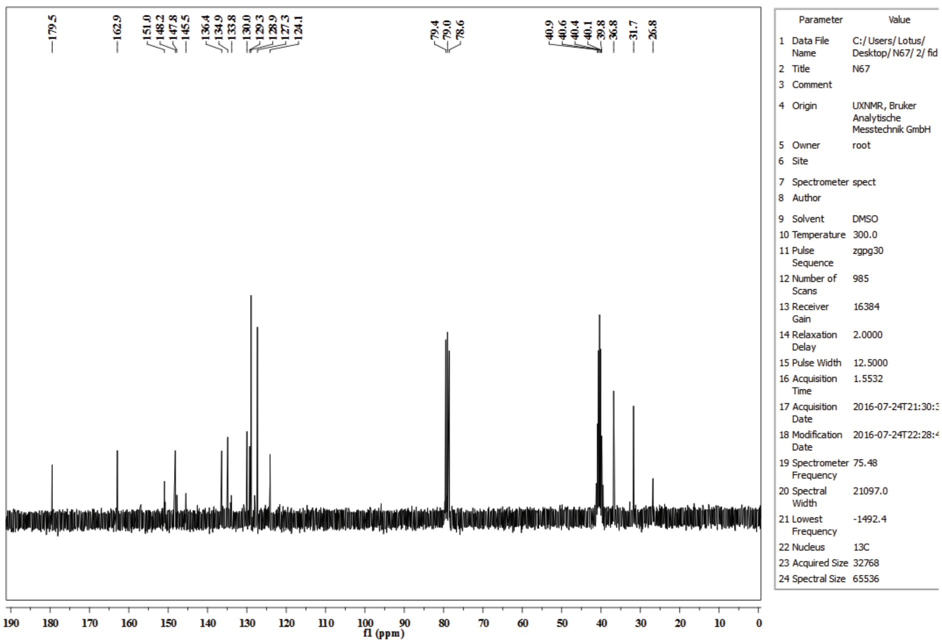

Fig. S-2. ^{13}C-NMR spectrum of H(L1) in DMSO-d_6.

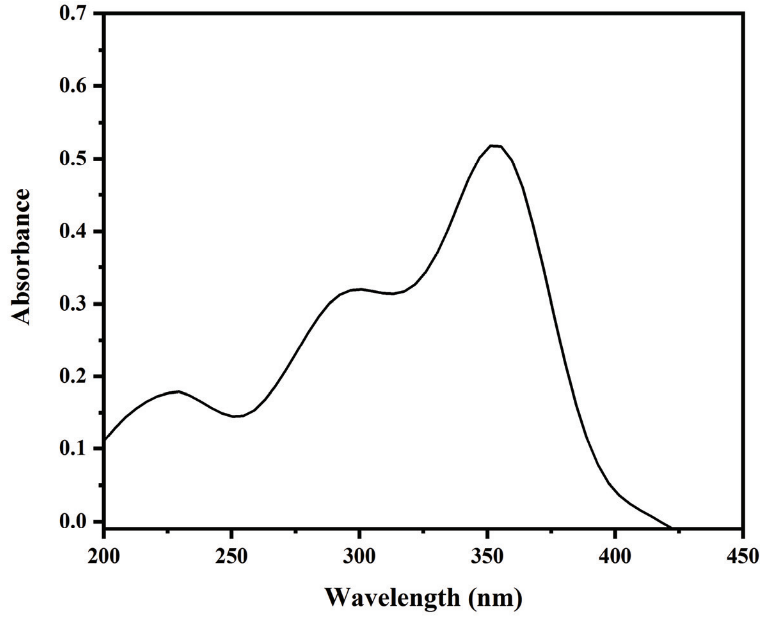

Fig. S-3. Electronic spectrum of ligand H(L1) in DMF.

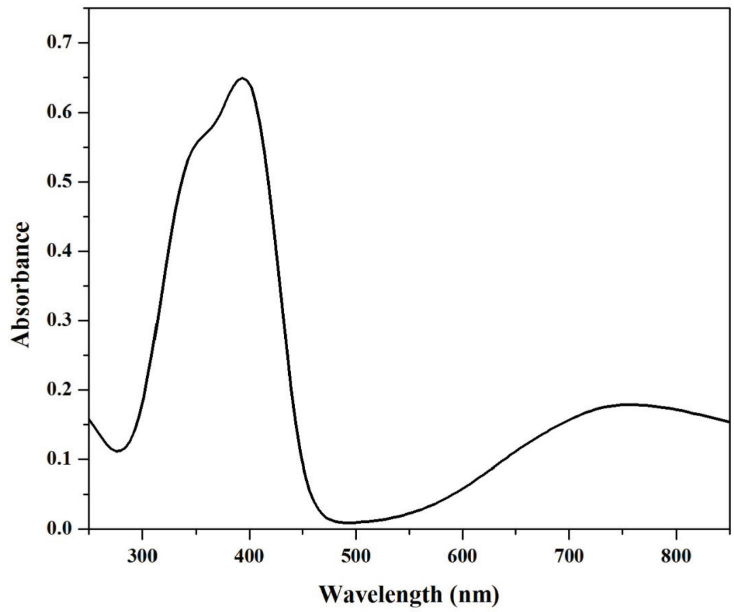

Fig. S-4. Electronic spectrum of complex **C5** in DMF.

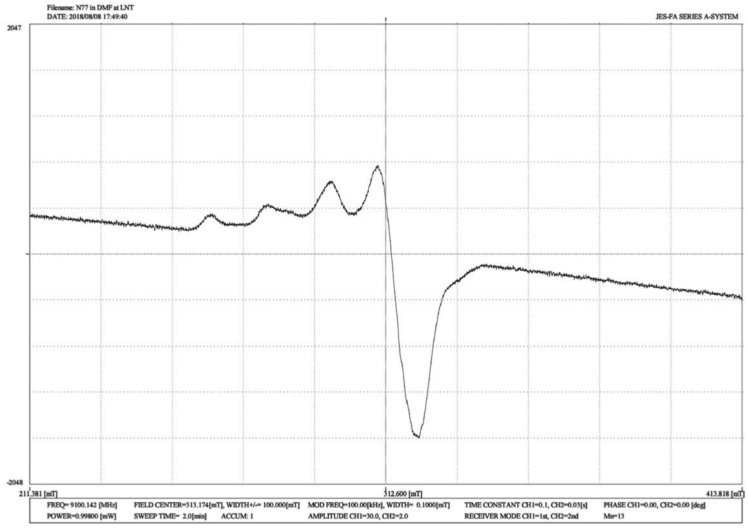

Fig. S-5. X-Band EPR spectrum of complex **C7** in frozen DMF solution.

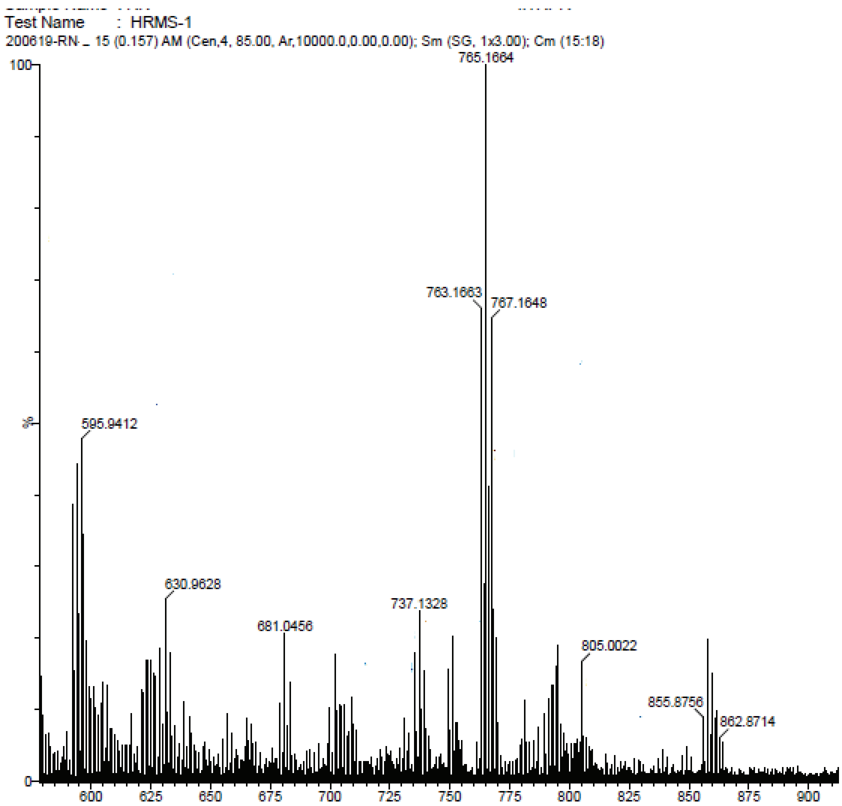

Fig. S-6. HRMS spectrum of complex **C9**.

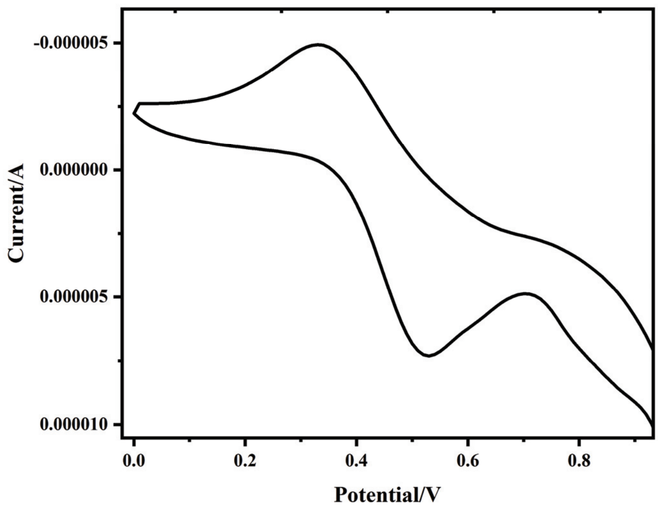

Fig. S-7. Cyclic voltammogram of complex **C2**.

TABLE S-I. Electrochemical data of thiosemicarbazone copper(II) complexes **C1**–**C9**

Complex	Cyclic voltammogram[a]			Differential pulse voltammogram[a]	Redox process
	E_{pa} / V	E_{pc} / V	$E_{1/2}$ / V	$E_{1/2}$ (DPV) / V	
$[Cu(L1)_2]$ (**C1**)	0.309	0.572	0.441	0.601	Cu(II) → Cu(I)
$[Cu(L2)_2]$ (**C2**)	0.324	0.542	0.433	0.529	Cu(II) → Cu(I)
$[Cu(L3)_2]$ (**C3**)	0.332	0.498	0.415	0.471	Cu(II) → Cu(I)
[Cu(L1)(bpy)]Cl (**C4**)	0.327	0.598	0.463	0.637	Cu(II) → Cu(I)
[Cu(L2)(bpy)]Cl (**C5**)	0.258	0.662	0.460	0.598	Cu(II) → Cu(I)
[Cu(L3)(bpy)]Cl (**C6**)	0.572	0.598	0.345	0.446	Cu(II) → Cu(I)
[Cu(L1)(phen)]Cl (**C7**)	0.327	0.559	0.659	0.643	Cu(II) → Cu(I)
[Cu(L2)(phen)]Cl (**C8**)	0.247	0.576	0.640	0.612	Cu(II) → Cu(I)
[Cu(L3)(phen)]Cl (**C9**)	0.022	0.551	0.638	0.587	Cu(II) → Cu(I)

[a]DMF (1×10^{-3} M), $E_{1/2} = 0.5$ ($E_{pa} + E_{pc}$), $\Delta E_p = E_{pa} - E_{pc}$, where E_{pa} and E_{pc} are the anodic and cathodic peak potentials, respectively (Scan rate 50 mV s^{-1})

TABLE S-II. Mean diameter of the zone of inhibition (mm) of the thiosemicarbazone ligands and the copper(II) complexes

Ligand or complex	Bacterium			
	E. coli		*Bacillus sp.*	
	c / µg ml^{-1}			
	5	50	5	50
H(L1)	1	2	0	1
H(L2)	1	4	0	1
H(L3)	1	2	0	1
$[Cu(L1)_2]$ (**C1**)	3	5	4	5
$[Cu(L2)_2]$ (**C2**)	3	6	2	4

TABLE S-II. Continued

Ligand or complex	Bacterium			
	E. coli		*Bacillus sp.*	
	c / µg ml^{-1}			
	5	50	5	50
[Cu(L3)$_2$] (**C3**)	2	5	1	3
[Cu(L1)(bpy)]Cl (**C4**)	2	4	4	6
[Cu(L2)(bpy)]Cl (**C5**)	2	5	5	7
[Cu(L3)(bpy)]Cl (**C6**)	3	6	6	8
[Cu(L1)(phen)]Cl (**C7**)	5	8	7	10
[Cu(L2)(phen)]Cl (**C8**)	7	9	6	11
[Cu(L3)(phen)]Cl (**C9**)	3	5	5	8
(Positive control)[a]	9	9	13	13
(Negative control)[b]	NA	NA	NA	NA

[a]Standard antibacterial agent used was chloramphenicol (positive control); [b]dimethyl formamide (negative control), NA = no activity

TABLE S-III. Cytotoxicity at 5 µM for 24 h with standard error values (%) of the thiosemicarbazone ligands and their copper(II) complexes against HeLa cervical cancer cell line

Compound	Value
H(L1)	56.38 ± 0.31
H(L2)	34.00 ± 0.10
H(L3)	46.00 ± 0.01
[Cu(L1)$_2$] (**C1**)	21.62 ± 0.10
[Cu(L2)$_2$] (**C2**)	66.00 ± 0.21
[Cu(L3)$_2$] (**C3**)	54.00 ± 0.09
[Cu(L1)(bpy)]Cl (**C4**)	60.84 ± 0.36
[Cu(L2)(bpy)]Cl (**C5**)	59.00 ± 0.05
[Cu(L3)(bpy)]Cl (**C6**)	60.00 ± 0.12
[Cu(L1)(phen)]Cl (**C7**)	81.79 ± 0.26
[Cu(L2)(phen)]Cl (**C8**)	84.00 ± 0.04
[Cu(L3)(phen)]Cl (**C9**)	73.00 ± 0.04
Cisplatin[a]	96.00 ± 0.01

[a]Positive control