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SUPPLEMENTARY MATERIAL TO

Synthesis and efficacy of copper(II) complexes bea[illegible]ng *N*(4)-substituted thiosemicarbazide and diimine co[illegible]ig[illegible] plasmid DNA and HeLa cell l[illegible]nes

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Synthesis of thiosemicarbazone ligands

Preparation of 1-phenyl-2-((5-(pyridin-3-yl)-4H[illegible]2[illegible]tria[illegible]l-3-yl)thio)ethanone HL

Color: Colorless; Yield: 68[illegible] m[illegible]19[illegible]– 192°C ; Anal.Calcd for $C_{15}H_{12}N_4OS$: C, 60.79; H, 4.08; N, 18.[illegible] S, 10.82 %; Found: C, 60.68; H, 4.02; N, 18.25; S, 10.77 %; Λ_m (Ω^{-1}[illegible]^2 mol[illegible]5; ^{1}H NMR (300 MHz, DMSO): δ_H 9.15 (s, 1H), 8.59 (s, 1H), 8[illegible] (d, [illegible]6.0 Hz, 1H), 8.06 (d, *J* = 6.0 Hz, 2H), 7.66 – 7.61 (m, 1H), 7.[illegible]54–7.49 (m, [illegible]), 7.[illegible]5 (s, 2H), 4.87 (s, 2H).

General method of prep[illegible]ation of H(L[illegible] H(L3)

(E)-N-methyl-2-(1-phenyl[illegible]((5-(p[illegible]idin-3-yl)-4H-1,2,4-triazol-3-yl)thio)ethylidene) hydrazinecarbothioamide H(L1)

Color[illegible]w; Yield: 74 %; m.p 212 – 214°C; Anal.Calcd for $C_{17}H_{17}N_7S_2$: C, 53.2[illegible] 4.4[illegible] 25.57; S, 16.72 %. Found: C, 53.36; H, 4.44; N, 25.61; S, [illegible]%; [illegible] (Ω^{-1}cm[illegible] mol^{-1}): 4; ^{1}H NMR (300 MHz, DMSO): δ_H 10.88 (s, 1H), 9.32 ([illegible]H), [illegible]3 (d, *J* = 15 Hz, 1H), 8.66 (s, 1H), 8.54 (d, *J* = 9.0 Hz, 1H), 8.25 (d, *J* = [illegible]Hz, 1H), 8.10 (s, 1H), 7.85 (t, *J* = 3.0 Hz, 1H), 7.56 – 7.52 (m, 1H), 7.43 (d, [illegible]= 3.0 Hz, 3H), 4.59 (s, 2H), 3.19 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 [illegible]MSO): δ_C 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; 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); UV–Vis: λ_{max} (DMF) nm: 225, 295 and 355.

(E)-N-ethyl-2-(1-phenyl-2-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)ethylidene) hydrazinecarbothioamide H(L2)

Color: Yellow; Yield: 70 %; 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 %. Found: C, 54.42; H, 4.91; N, 24.79; S,

16.09 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 7; ^{1}H NMR (300 MHz, DMSO): δ_H 11.08 (s, 1H), 9.51 (s, 1H), 8.85 (d, *J* = 30.0 Hz, 2H), 8.53 – 8.51 (m, 1H), 8.14 – 8.11 (m, 1H), 7.96 – 7.94 (m, 2H), 7.86 (s, 1H), 7.62 – 7.60 (m, 3H), 4.75 (s, 2H), 3.95 – 3.93 (m, 2H), 1.46 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO): δ_C 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; 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); UV−Vis: λ_{max} (DMF) nm: 280 and 360.

(E)-N-phenyl-2-(1-phenyl-2-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)ethylidene) hydrazinecarbothioamide H(L3)

Color: Yellow; Yield: 61 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 11;^{1}H NMR (300 MHz, DMSO): δ_H 11.60 (s, 1H), 10.02 (s, 1H), 9.74 (s, 1H), 9.61 (s, 2H), 8.99 – 8.96 (m, 1H), 8.90 (d, *J* = 6.0 Hz, 1H), 8.65 (d, *J* = 6.0 Hz, 1H), 8.43 (d, *J* = 9.0 Hz, 1H), 8.35 (s, 2H), 8.03 – 7.95 (m, 1H), 7.91 – 7.86 (m, 1H), 7.81 – 7.72 (m, 1H), 7.70 – 7.63 (m, 1H), 7.59 – 7.57 (m, 1H), 7.32 – 7.27 (m, 1H), 5.17 (s, 2H); ^{13}C NMR (75 MHz, DMSO): δ_C 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; 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); UV−Vis: λ_{max} (DMF) nm: 310 and 345.

Synthesis of copper(II) bis complexes (C1 − C3)

$[Cu(L1)_2]$ (C1)

Color: Green; Yield: 69 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}):16; 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) and 612 (m, Cu−S); UV−Vis: λ_{max} (DMF) nm: 295, 360 and 635 (d−d transition); EPR: $A_{||}$ = 158 x 10^{-4} cm^{-1}; $g_{||}$ = 2.252; $g_{\perp}$ = 2.057; $g_{||}/A_{||}$ = 140 cm; G = 4.38.The similar method was applied for the synthesis of complexes 2 and 3 using H(L2) and H(L3) instead of H(L1).

$[Cu(L2)_2]$ (C2)

Color: Green; Yield: 62 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 17; 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 and 645 (d−d transition); EPR: $A_{||}$ = 162 x 10^{-4} cm^{-1}; $g_{||}$ = 2.249; $g_{\perp}$ = 2.056; $g_{||}/A_{||}$ = 138 cm; G = 4.59.

$[Cu(L3)_2]$ (C3)

Color: Green; Yield: 58 %; 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 %; Λ_m

($\Omega^{-1}cm^2$ mol^{-1}): 18; FT−IR (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 and 715 (d−d transition); EPR: $A_{\|}$ = 160 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.258; $g_{\perp}$ = 2.062; $g_{\|}/A_{\|}$ = 141 cm; G = 4.28.

Synthesis of mixed ligand copper(II) complexes (C4−C9)

[Cu(L1)(bpy)]Cl (C4)

To the 2 mmol ethanolic solution of ligand H(L1), solution of 2,2'-bipyridyl (1 mmol in ethanol) was added by constant stirring for 1 h. To this solution, copper(II) chloride dihydrate was added dropwise and again stirred for 30 min. Subsequently, the green precipitate obtained was washed several times with cold ethanol and dried. Color: Green; Yield: 61 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 83; 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 and 745 (d−d transition); EPR: $A_{\|}$ = 163 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.257; $g_{\perp}$ = 2.074; $g_{\|}/A_{\|}$ = 139 cm; G = 4.55.

The similar method was applied for the synthesis of complexes 5 and 6 using H(L2) and H(L3) instead of H(L1).

[Cu(L2)(bpy)]Cl (C5)

Color: Green; Yield: 72 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 89; FT−IR (KBr; cm^{-1}): 3074 (s, NH), 1559 (s, C=N), 758 (s, C−S), 1109 (s, N−N), 456 (s, Cu−N) and 643 (s, Cu−S); UV−Vis: λ_{max} (DMF) nm: 295, 415 and 725 (d−d transition); EPR: $A_{\|}$ = 160 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.268; $g_{\perp}$ = 2.063; $g_{\|}/A_{\|}$ = 141 cm; G = 4.38.

[Cu(L3)(bpy)]Cl (C6)

Color: Green; Yield: 68 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 96; 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 and 758 (d−d transition); EPR: $A_{\|}$ = 162 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.254; $g_{\perp}$ = 2.071; $g_{\|}/A_{\|}$ = 139 cm; G = 3.66.

Complexes 7, 8 and 9 was prepared in a similar manner to complex 4, using 1,10-phenanthroline instead of 2,2'-bipyridyl**.**

[Cu(L1)(phen)]Cl (C7)

Color: Green; Yield: 75 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 93; 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 and 685 (d−d transition); EPR: $A_{\parallel}$ = 163 x 10^{-4} cm^{-1}; $g_{\parallel}$ = 2.257; $g_{\perp}$ = 2.059; $g_{\parallel}/A_{\parallel}$ = 141 cm; G = 4.49.

[Cu(L2)(phen)]Cl (C8)

Color: Green; Yield: 68 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 98; 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 and 725 (d−d transition); EPR: $A_{\parallel}$ = 162 x 10^{-4} cm^{-1}; $g_{\parallel}$ = 2.258; $g_{\perp}$ = 2.064; $g_{\parallel}/A_{\parallel}$ = 140 cm; G = 4.14.

[Cu(L3)(phen)]Cl (C9)

Color: Green; Yield: 60 %; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 103; 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 and 735 (d−d transition); EPR: $A_{\parallel}$ = 162 x 10^{-4} cm^{-1}; $g_{\parallel}$ = 2.260; $g_{\perp}$ = 2.059; $g_{\parallel}/A_{\parallel}$ = 139 cm; G = 4.54; HRMS Calculated for 768.12; Found 765.17.

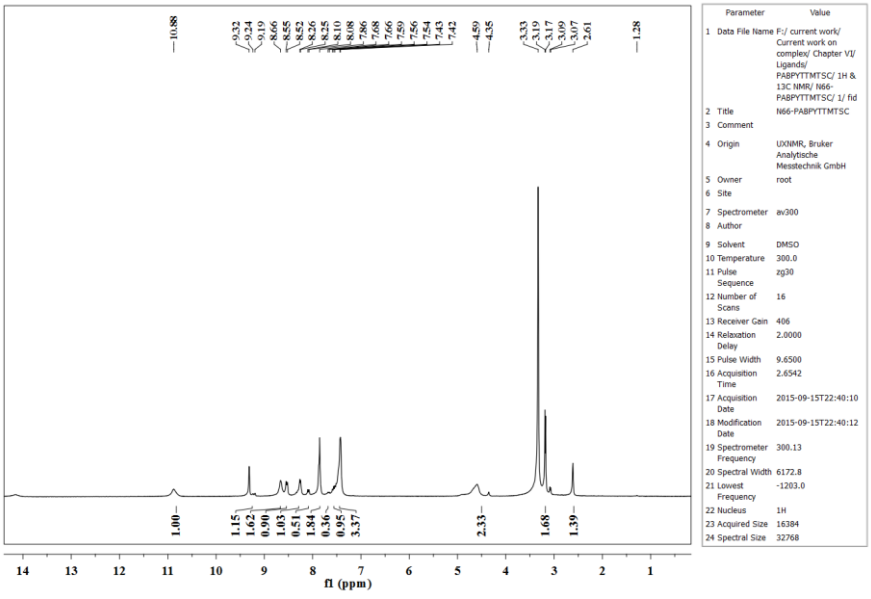

Fig. S1. ^{1}H NMR spectrum of H(L1) in DMSO-d_6

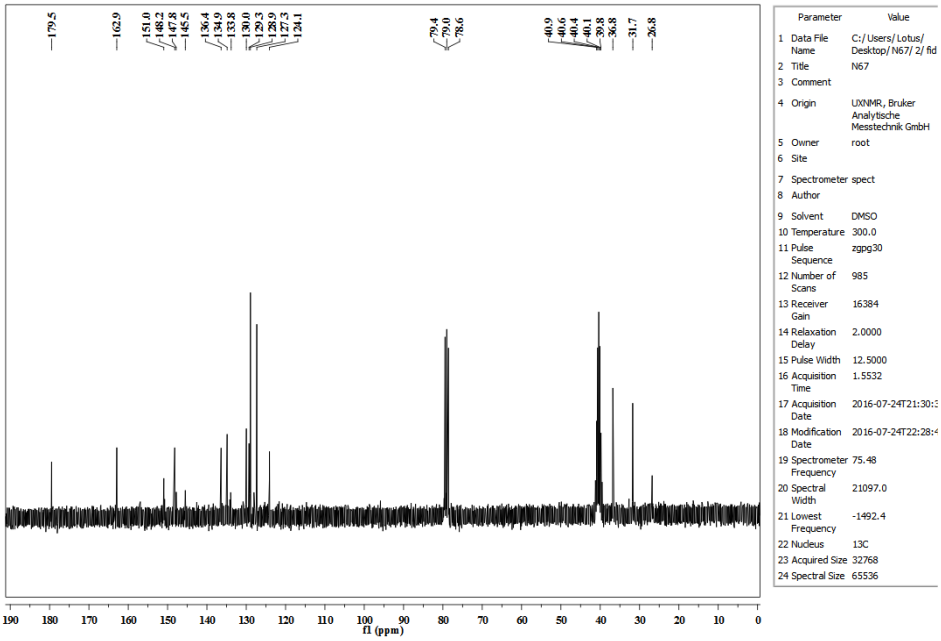

Fig. S2. ^{13}C NMR spectrum of H(L1) in DMSO-d_6

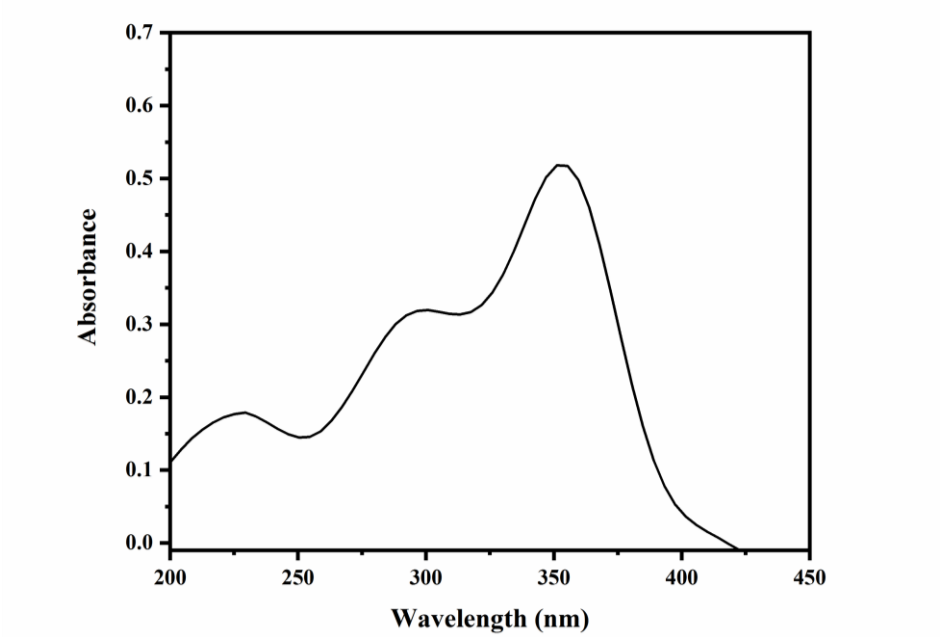

Fig. S3. Electronic spectra of ligand H(L1) in DMF

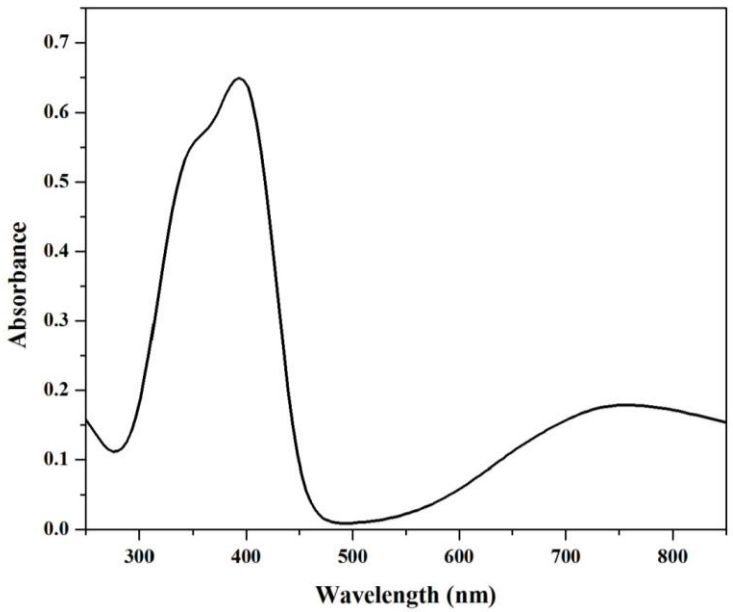

Fig. S4. Electronic spectra of complex C5 in DMF

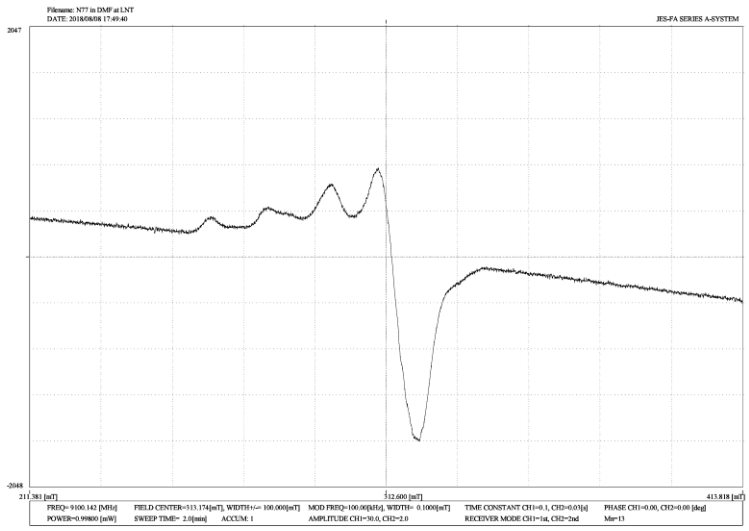

Fig. S5. X-band EPR spectrum of complex C7 in frozen DMF solution

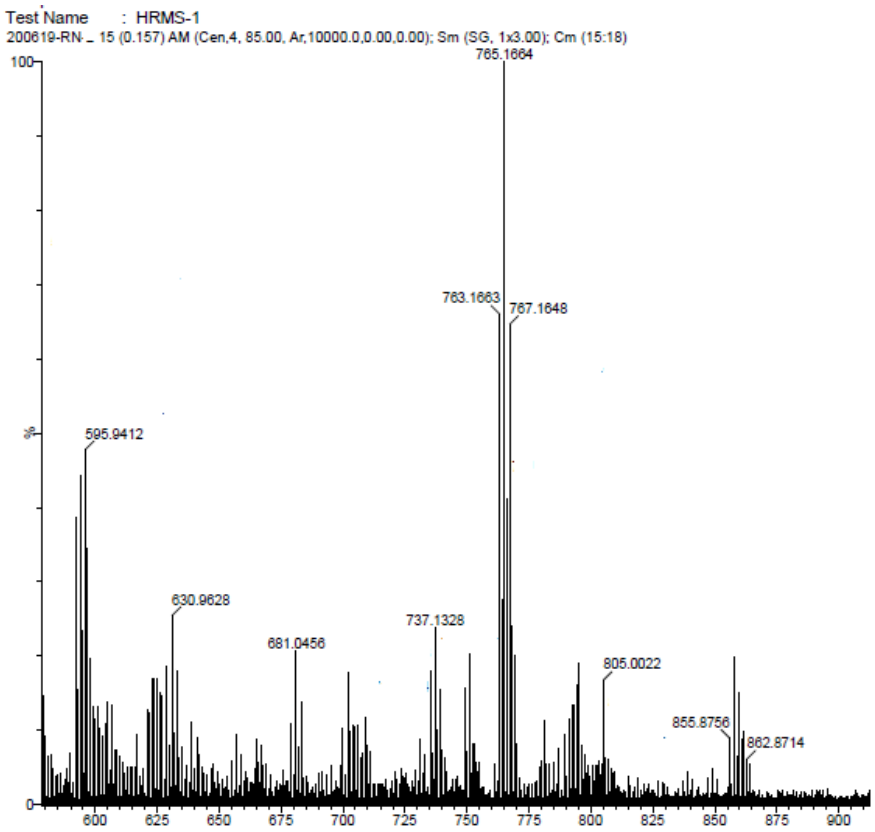

Fig. S6. HRMS spectrum of complex C9

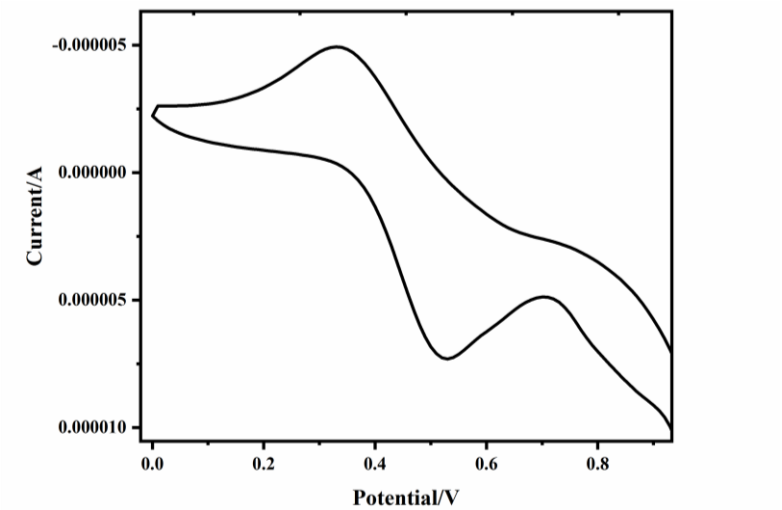

Fig. S7. Cyclic voltammogram of complex C2

Table SI. Electrochemical data of thiosemicarbazone copper(II) complexes 1 − 9

Complexes	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 x 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} were anodic and cathodic peak potentials respectively (Scan rate 50mVs^{-1})

Table SII. Diameter of zone of inhibition of thiosemicarbazone ligands and copper(II) complexes

Ligand and Complexes	Diameter of inhibition zone, mm[a]			
	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
[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)[b]	9	9	13	13
(Negative control)[c]	NA	NA	NA	NA

[a] Mean zone of inhibition in mm [b] standard antibacterial agent used was chloramphenicol (positive control) [c] Dimethyl formamide (negative control), NA = No activity

Table SIII. Percentage of cytotoxicity of the thiosemicarbazone ligands and its copper(II) complexes

Compounds	Cytotoxicity, %[a]
	HeLa [b]
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 [c]	96.00 ± 0.01

[a] Percentage of cytotoxicity at 5 μM for 24 h with standard error values [b] Cervical cancer cell lines [c] Positive control