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

NEELAVENI RAJENDRAN[1], ABIRAMI PERIYASAMY [2], NITHYA KAMATCHI [3] and VASANTHA SOLOMON [1]*

[1]PG and Research Department of Chemistry, Lady Doak College, Madurai − 625002, Tamil Nadu, India [2] Department of Biotechnology, Lady Doak College, Madurai − 625002, Tamil Nadu, India and [3]PG and Research Department of Zoology, Lady Doak College, Madurai − 625002, Tamil Nadu, India

SUPPLEMENTARY DATA

Synthesis of thiosemicarbazone ligands

Preparation of 1-phenyl-2-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)ethanone HL

Color: Colorless; Yield: 68%; 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%; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 5; ^{1}H NMR (300 MHz, DMSO): δ_H 9.15 (s, 1H), 8.59 (s, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 8.06 (d, *J* = 6.0 Hz, 2H), 7.66 − 7.61 (m, 1H), 7.54−7.49 (m, 2H), 7.39 (s, 2H), 4.87 (s, 2H).

General method of preparation of H(L1) − H(L3)

(E)-N-methyl-2-(1-phenyl-2-((5-(pyridin-3-yl)-4H-1,2,4-triazol-3-yl)thio)ethylidene) hydrazinecarbothioamide H(L1)

Color: Yellow; Yield: 74%; 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 %; Λ_m ($\Omega^{-1}cm^2$ mol^{-1}): 4; ^{1}H NMR (300 MHz, DMSO): δ_H 10.88 (s, 1H), 9.32 (s, 1H), 9.23 (d, *J* = 15 Hz, 1H), 8.66 (s, 1H), 8.54 (d, *J* = 9.0 Hz, 1H), 8.25 (d, *J* = 3.0 Hz, 1H), 8.10 (s, 1H), 7.85 (t, *J* = 3.0 Hz, 1H), 7.56 (m, 1H), 7.43 (d, *J* = 3.0 Hz, 3H), 4.59 (s, 2H), 3.19 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO): 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)

[1] * Corresponding author E-mail: vasantha@ldc.edu.in (Tel:+919976366463)

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 (m, 1H), 8.14 (m, 1H), 7.96 (m, 2H), 7.86 (s, 1H), 7.62 (m, 3H), 4.75 (s, 2H), 3.95 (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 – 8.88 (d, *J* = 6.0 Hz, 1H), 8.65 – 8.63 (d, *J* = 6.0 Hz, 1H), 8.43 – 8.40 (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: $A_{\parallel}$ = 158 x 10^{-4} cm^{-1}; $g_{\parallel}$ = 2.252; $g_{\perp}$ = 2.057; $g_{\parallel}/A_{\parallel}$ = 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: $A_{\|}$ = 163 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.257; $g_{\perp}$ = 2.059; $g_{\|}/A_{\|}$ = 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: $A_{\|}$ = 162 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.258; $g_{\perp}$ = 2.064; $g_{\|}/A_{\|}$ = 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: $A_{\|}$ = 162 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.260; $g_{\perp}$ = 2.059; $g_{\|}/A_{\|}$ = 139 cm; G = 4.54.

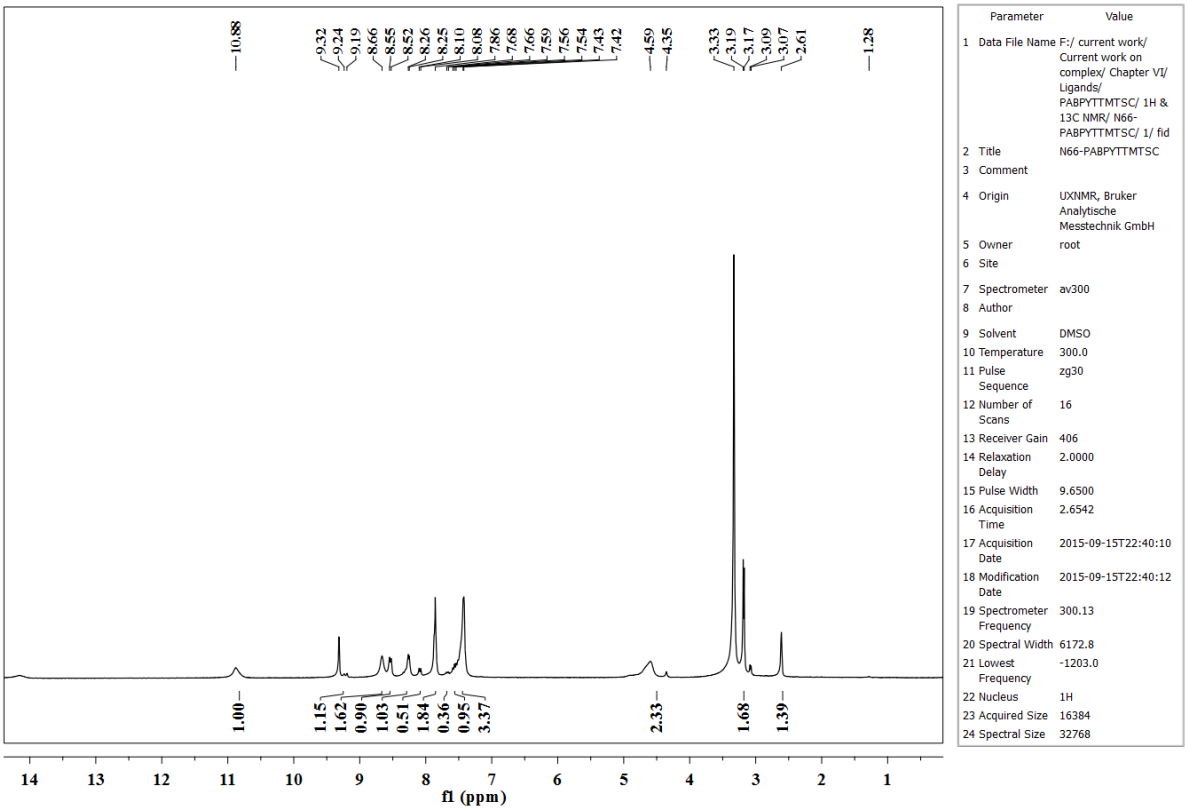

Fig. 1. ^{1}H NMR spectrum of H(L1) in DMSO-d$_6$

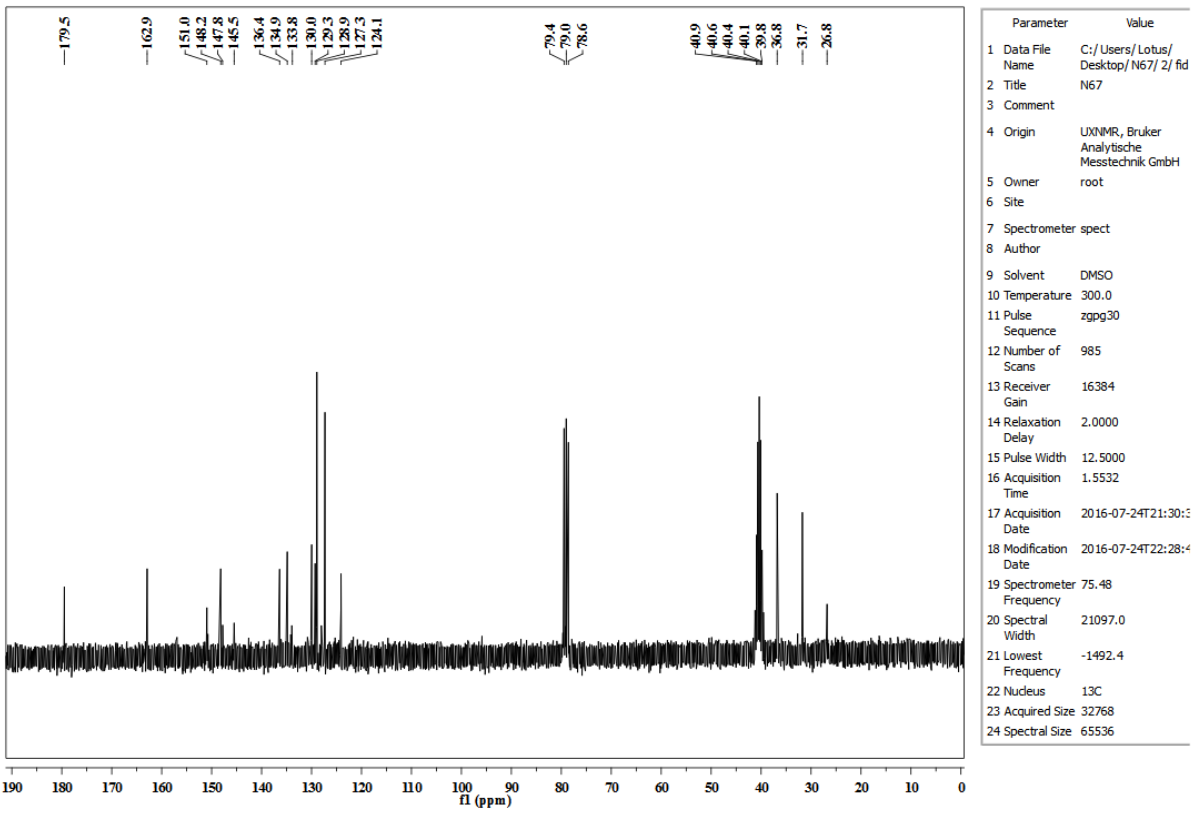

Fig. 2. ^{13}C NMR spectrum of H(L1) in DMSO-d$_6$

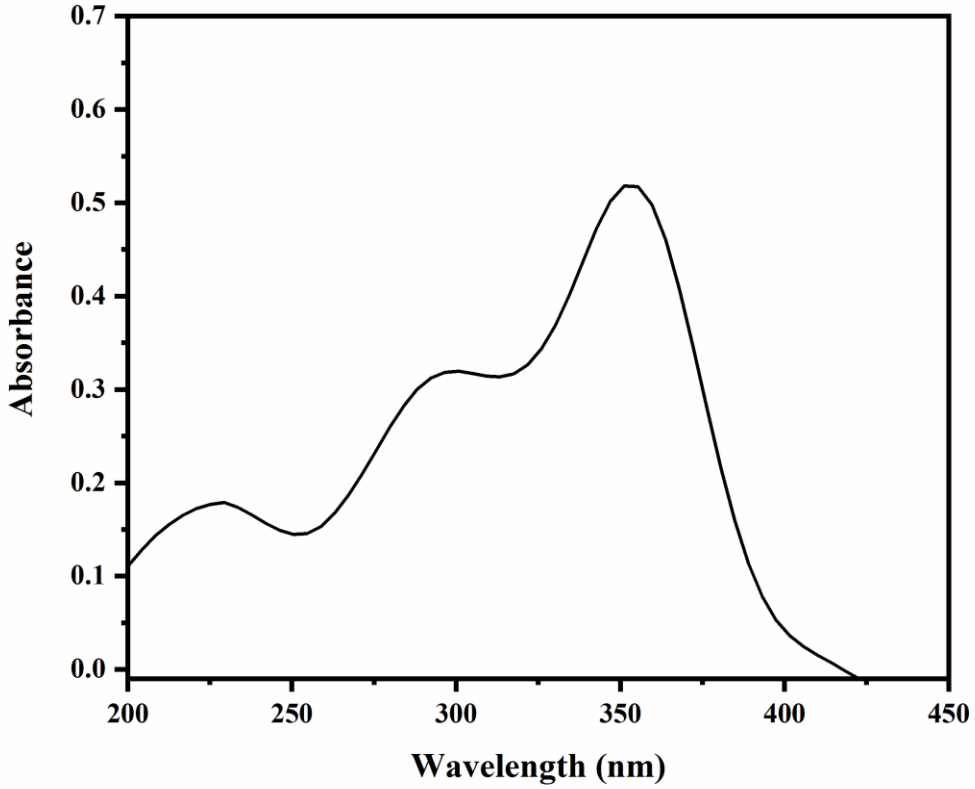

Fig. 3. Electronic spectra of ligand H(L1) in DMF

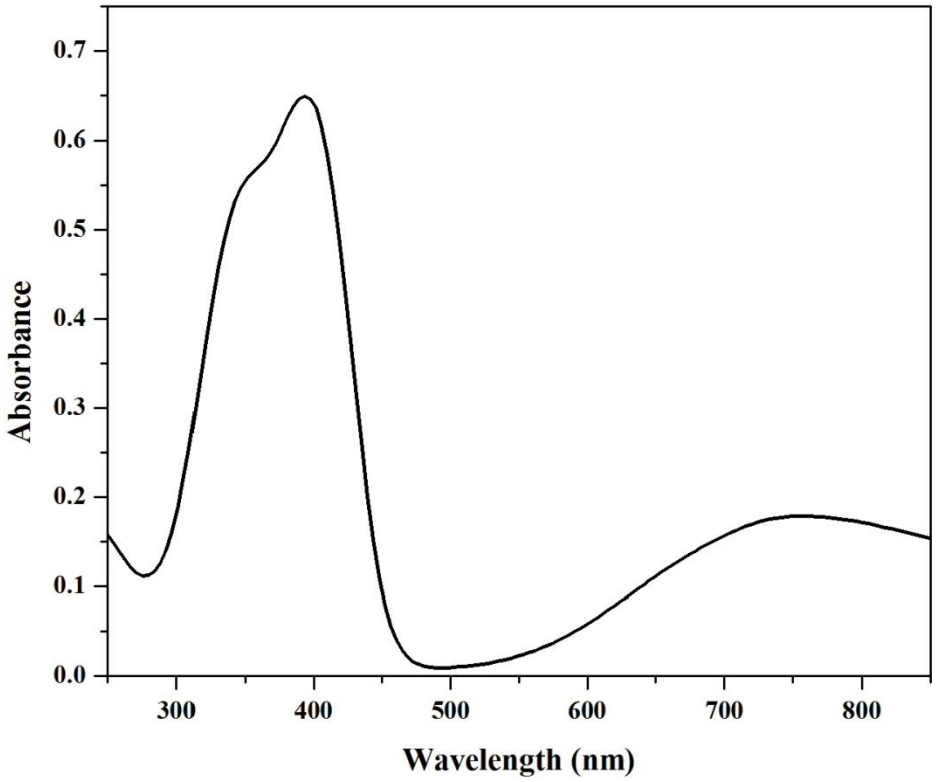

Fig. 4. Electronic spectra of complex C5 in DMF

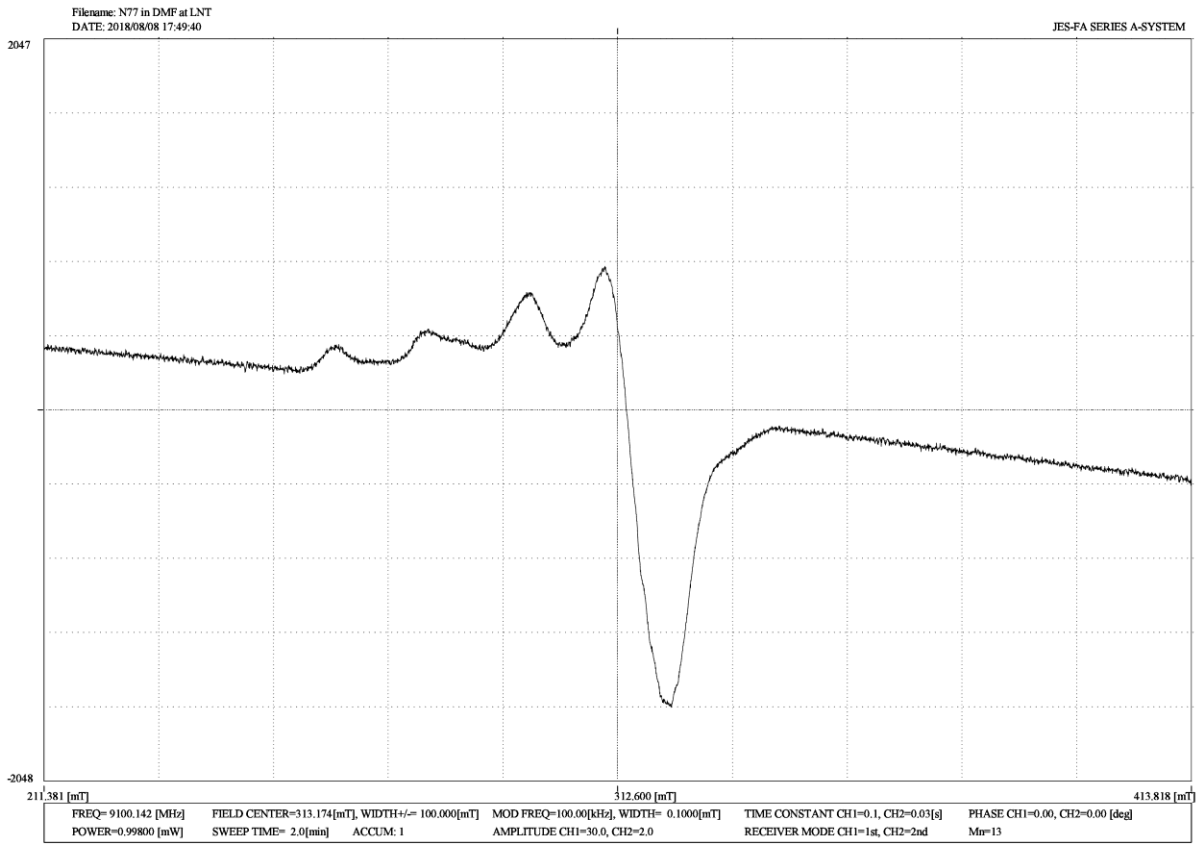

Fig. 5. X-band EPR spectrum of complex C7 in frozen DMF solution

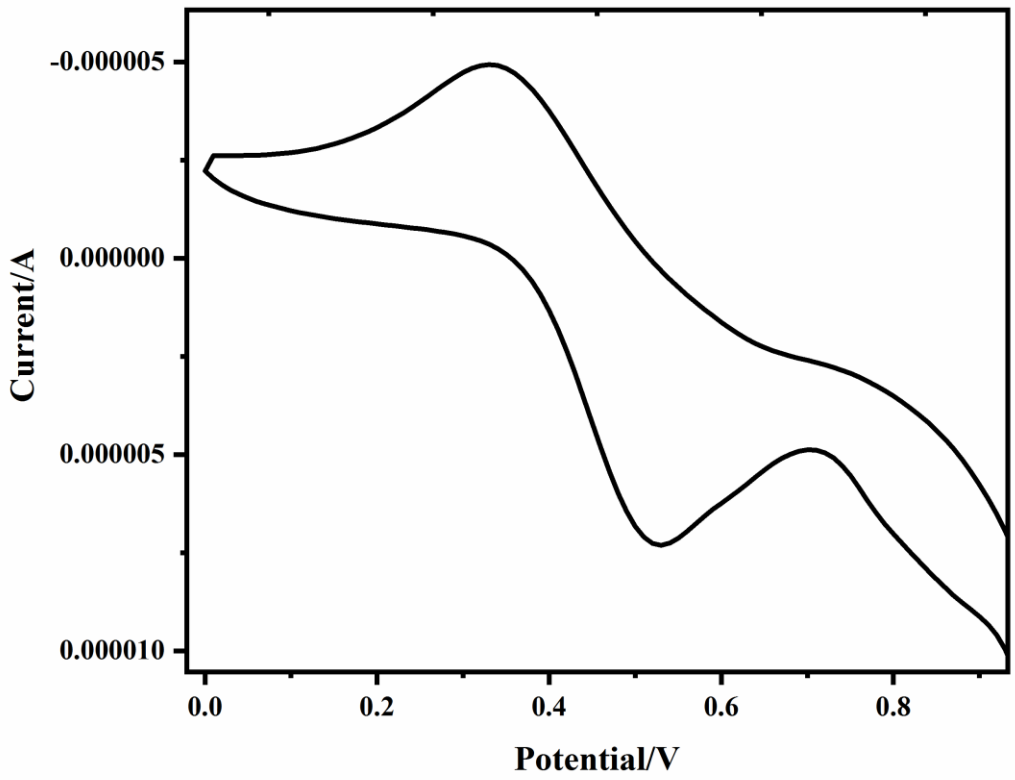

Fig. 6. Cyclic voltammogram of complex C2

Table I. 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 II. 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.*	
	5 (µg / ml)	50 (µg / ml)	5 (µg / ml)	50 (µg / ml)
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 III. 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