SUPPORTING INFORMATION

Synthesis and spectral characterizations of water soluble Cu(II) complexes containing *N*-heterocyclic chelates: cell-proliferation, antioxidant and nucleic acid/serum albumin interactions

Materials and methods

All the reagents and starting ancestors used were analytically and chemically pure grade and the solvents were dried according to the literature procedure.[1] 8-methyl-2-oxo-1,2-dihydro-quinoline-3carbaldehyde was synthesized according to the literature procedure.[2] Melting points were determined on Lab India apparatus. Elemental analysis of carbon, hydrogen, nitrogen and sulphur was determined by using Vario EL III CHNS at the department of chemistry, Bharathiar University, Coimbatore-641046. Infrared spectra were recorded in the range of 400-4000 cm^{-1} (KBr pellets) on a Jasco FT-IR spectrometer. Electronic absorption spectra of the compounds were recorded using JASCO 600 spectrophotometer and emission measurements were carried out by using a JASCO FP-6600 spectrofluorometer.^{1}H NMR spectra were measured on Brucker DRX-500 Avance spectrometer at 500 MHz, using TMS as the internal reference in DMSO-d_6. The EPR spectra were recorded on a Brucker spectrometer operating at the X-band using 100 kHz magnetic field modulation. Molar conductivity measurements have been carried out on a conductivity bridge with a dip-type cell, using 1 mM solution of complexes in 5% aqueous DMSO.[2] Single crystal data collections and corrections for the ligand $\mathbf{H_2L^2}$ and complexes **2**, **3** and **4** were done at 293 K with CCD Kappa Diffractometer using graphite monochromated Mo Ka (k = 0.71073 A) radiation.[3] The structural solution were done by using SHELXS-97[4] and refined by full matrix least square on *F2* using SHELXL-2014.[5]CT-DNA, BSA and ethidium bromide (EB) were obtained from HiMedia.

Experimental section

Preparation of ligands[H_2-8MOQtsc-R](H_2L^{1-4}) (R= H, CH_3, C_2H_5 and C_6H_5)

Ligand **[H_2-8MOQtsc-H]** was prepared by refluxing the stoichiometric solution of 4*N*-thiosemicarbazide (0.242 g, 2.6 mmol) and 8-methyl-2-oxo-1,2-dihydroquinoline-3-carboxaldehyde (0.5 g, 2.6 mmol) in 20 cm^3 of methanol and refluxed for about 30 minutes. During which a yellow precipitate was formed, then the reaction mixture was cooled to room temperature and the product formed was filtered, washed several times with cold methanol,

and dried under vacuum. Other ligands (**H_2L^{2-4}**) were also prepared by adopting the comparable procedure as in the case of ligand **[H_2-8MOQtsc-H]**, using corresponding thiosemicarbazide i.e., methylthiosemicarbazide (0.2794 g, 2.6 mmol), ethyl thiosemicarbazide (0.3167 g, 2.6 mmol) and phenylthiosemicarbazide (0.444 g, 2.6 mmol) as summarized in **scheme** 1.

[H_2-8MOQtsc-H] (H_2L^1): Yield: 85 %, Mp: >263 °C. Selected IR bands ('ν' in cm^{-1}): 1651 (C=O); 1563 (C=N); 839 (C=S). UV-vis (DMSO), λ_{max}: 254 (87,934) (dm^3 $mol^{-1}cm^{-1}$) nm (intra-ligand transition); 353 (14,146) ($dm^3mol^{-1}cm^{-1}$) nm (n→л* transition); ^{1}H NMR (500 MHz, DMSO-d^6): (δ, ppm): 11.62 (s, 1H, N(1)H); 10.26 (s, 1H, N(3)H); 8.49 (s, 1H, -CH=N); 8.07 (s, 1H, C(4)H); 7.75-7.77 (d, 1H, *J=8 Hz* C(5)H); 7.50-7.52 (t, 1H, *J=8 Hz*, C(6)H); 7.12-7.19 (m, 1H, C(7)H); 7.36-7.38 (d, 2H, terminal $–NH_2$), 2.43-2.45 (d, 3H, *J=8 Hz*, C(8)H, $-CH_3$).

[H_2-8MOQtsc-Me] (H_2L^2): Yield: 87 %, Mp: >267 °C. Selected IR bands ('ν' in cm^{-1}): 1654 (C=O); 1552 (C=N); 839 (C=S). UV-vis (DMSO λ_{max}: 254 (10,793) ($dm^3mol^{-1}cm^{-1}$) nm (intra-ligand transition); 358 (17,355) ($dm^3mol^{-1}cm^{-1}$) nm (n→л* transition); ^{1}H NMR (500 MHz, DMSO-d^6): (δ, ppm): 11.67 (s, 1H, N(1)H); 11.16 (s, 1H, N(3)H); 8.67 (s, 1H, N(4)H), 8.59-8.60 (d, 1H, *J=4 Hz*, -CH=N, C(1)H), 8.30 (s, 1H, C(4)H), 7.52-7.54 (d, 1H, *J=8 Hz*, C(5)H), 7.38-7.39 (d, 1H, *J=8 Hz*, C(6)H), 7.14-7.17 (t, 1H, *J=12 Hz*, C(7)H) 3.05-3.06 (d, 3H, *J=4 Hz*, terminal $–CH_3$). 2.44 (S, 3H, C(8)H, $-CH_3$).

[H_2-8MOQtsc-Et] (H_2L^3): Yield: 81 %, Mp: >265 °C. Selected IR bands ('ν' in cm^{-1}): 1644 (C=O); 1529 (C=N); 858 (C=S). UV-vis (DMSO), λ_{max}: 252 (13,549) (dm^3 $mol^{-1}cm^{-1}$) nm 316 (45,781) (dm^3 $mol^{-1}cm^{-1}$) nm (intra-ligand transition); 359 (16,430) ($dm^3mol^{-1}cm^{-1}$) nm (n→л* transition); ^{1}H NMR (500 MHz, DMSO-d^6): (δ, ppm): 11.61 (s, 1H, N(1)H), 11.15 (s, 1H, N(3)H), 8.67-8.61 (q, 2H, *J=24Hz*, N(4)H & -CH=N), 8.31(s, 1H, C(4)H), 7.56-7.54 (d, 1H, *J=8 Hz*, C(5)H), 7.38-7.39 (d, 1H, *J=4 Hz*, C(6)H), 7.13-7.17 (t, 1H, *J=16 Hz*, C(7)H), 3.60-3.66 (p, 2H, *J=24 Hz*, terminal $–CH_2$), 1.17-1.20 (t, 3H, *J=12 Hz*, terminal $–CH_3$), 2.44 (s, 3H, $-CH_3$, C(8)).

[H_2-8MOQtsc-Ph] (H_2L^4): Yield: 89 %, Mp: > 267 °C. Selected IR bands ('ν' in cm^{-1}): 1653 (C=O); 1526 (C=N); 826 (C=S). UV-vis (DMSO), λ_{max}: 261 (13,549) (dm^3 $mol^{-1}cm^{-1}$) nm (intra-ligand transition); 363 (16,430) (dm^3 $mol^{-1}cm^{-1}$) nm (n→л* transition); ^{1}H NMR (500 MHz, DMSO-d^6): (δ, ppm): 12.01 (s, 1H, N(1)H), 11.18 (s, 1H, N(3)H), 8.86

(s, 1H, N(4)H), 8.42 (s, 1H, -CH=N, C(1)H), 7.13-7.59 (m, aromatic protons), 2.45 (s, 3H, $-CH_3$, C(8)).

Deoxy-Ribo Nucleic acid interaction

CT-DNA binding experiment was performed according to the previously reported literature procedure.[6]

Ethidium-bromide to CT-DNA displacement studies

According to the literature procedure,[6] EB-DNA displacement experiment was carried out.

Viscosity studies

For viscosity measurements, the Ubberhold viscometer (1 Ml capacity) was thermostated in a water bath maintained at 25 ˚C. The flow time for each sample was measured thrice using digital stopwatch and an average flow time was calculated. The flow rate for buffer (10 mM Tris), DNA (100 μM) and DNA with the copper(II) complexes at various concentrations (5-50 μM) was measured. The relative specific viscosity was calculated using the equation, $\eta = (t - t_o)/ t_o$, where t_o is the flow time for the buffer and t is the observed flow time for DNA in the absence and presence of the complex. Data are presented as $(\eta/\eta_o)^{1/3}$ versus 1/R {R = [complex]/ [DNA]}, where h is the viscosity of DNA in the presence of the complex and η_o is the viscosity of DNA alone.[7,8]

Protein binding

According to the literature procedure,[8,9] the serum albumin (BSA/HSA) bindings and three dimensional fluorescence spectra were carried out for all the ligands/complexes.

Radical scavenging assays

DPPH and OH radical scavenging assays were done according to the literature methods.[10,11]

MTT cell viability assay

According to the literature procedure,[12] the cytotoxic effects of *N*-heterocyclic ligands $\mathbf{H_2L^{1-4}}$, copper(II) complexes **(1-4)** and standard drugon MCF-7 and HaCaT cells were determined by MTT reduction assay. Further, AO/EB dual staining and DAPI staining assay were carried out by the previously reported literature.[13]

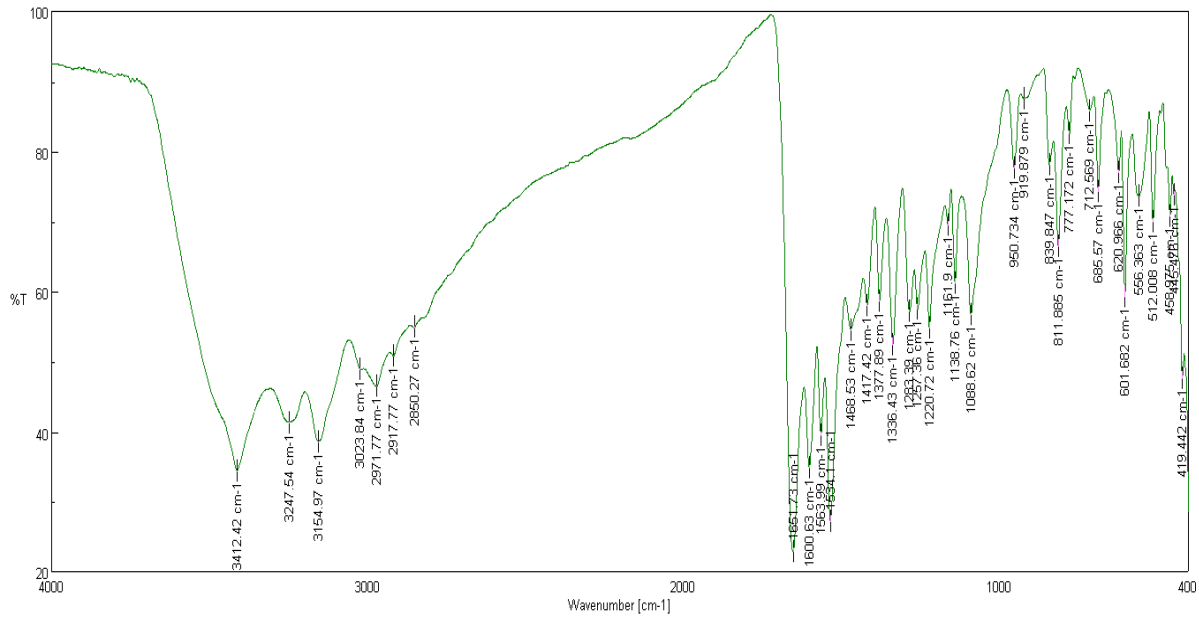


Fig. S1 IR spectra of ligand $\mathbf{H_2L^1}$.

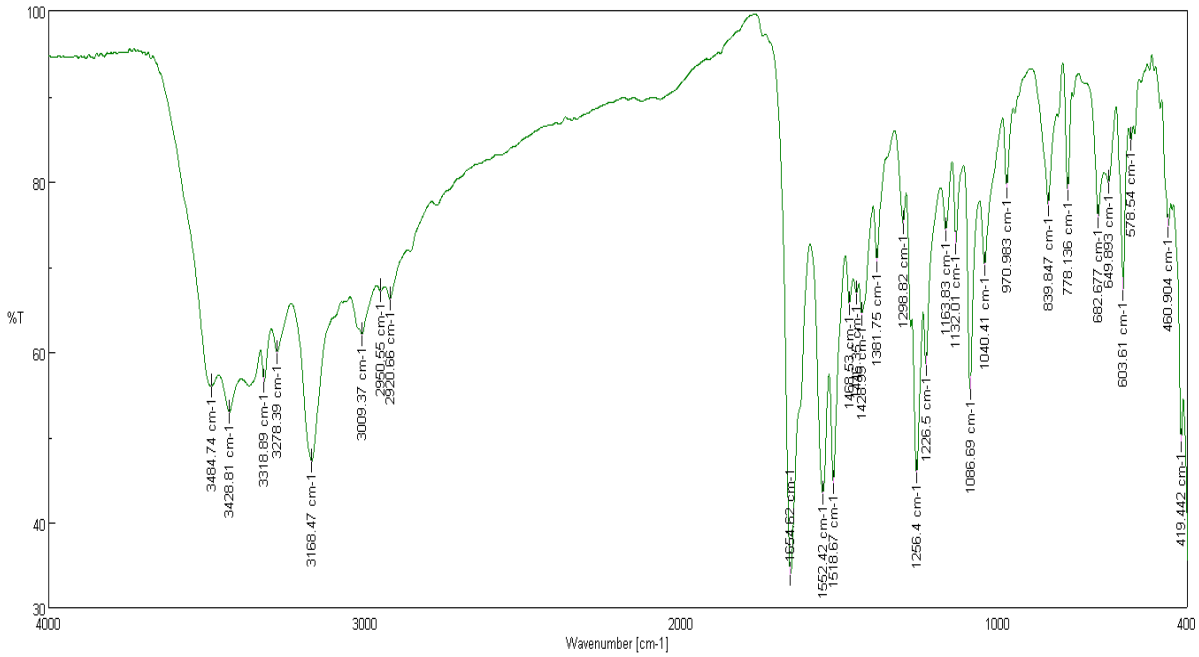


Fig. S2 FT-IR spectra of ligand $\mathbf{H_2L^2}$.

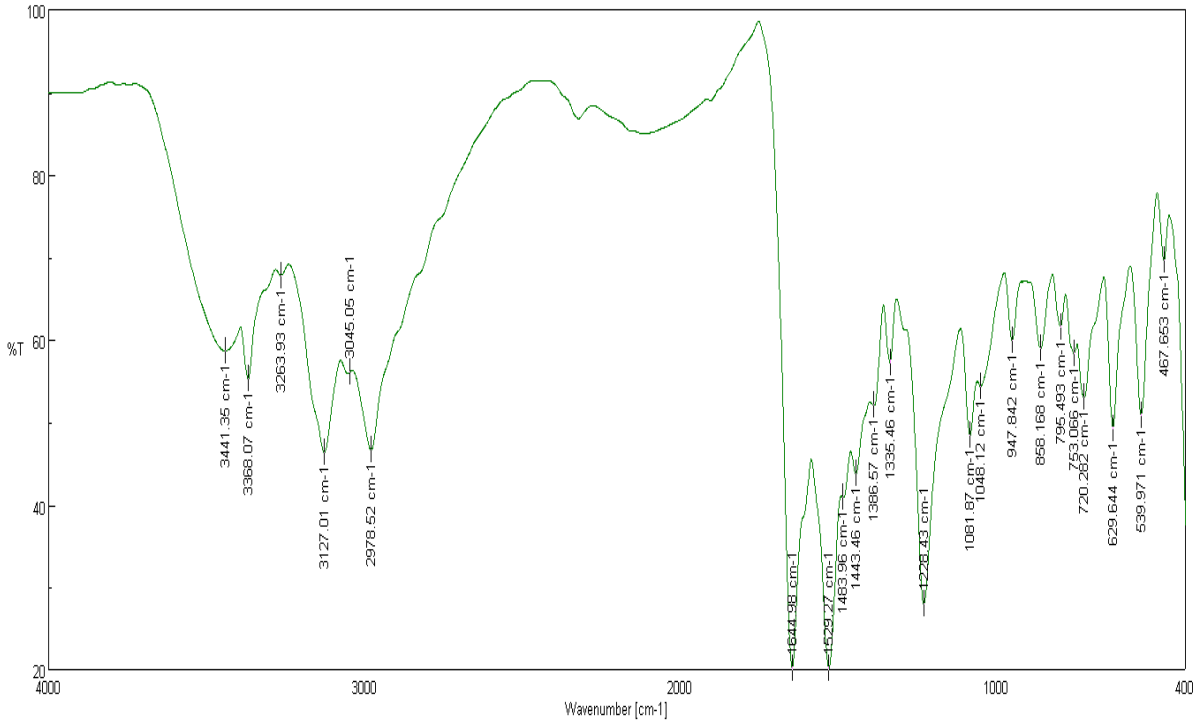


Fig. S3 FT-IR spectra of ligand $\mathbf{H_2L^3}$.

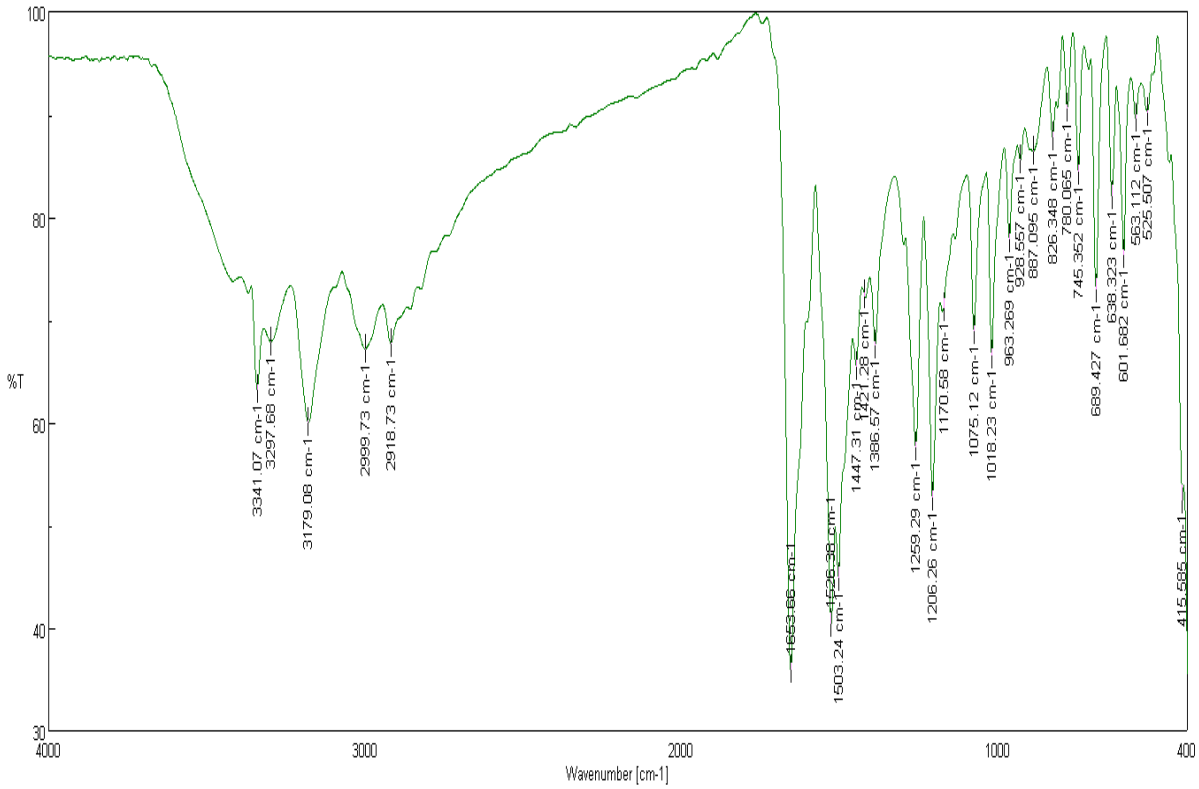


Fig. S4 FT-IR spectra of ligand $\mathbf{H_2L^4}$.

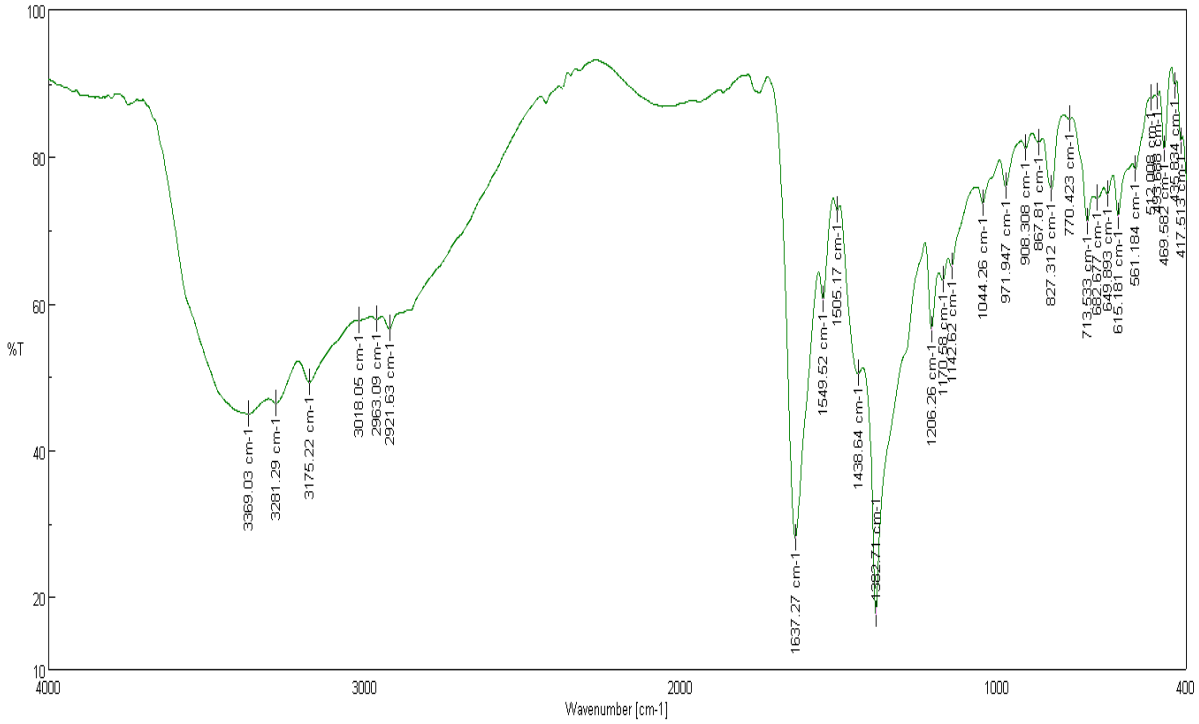


Fig. S5 FT-IR spectra of Complex **1.**

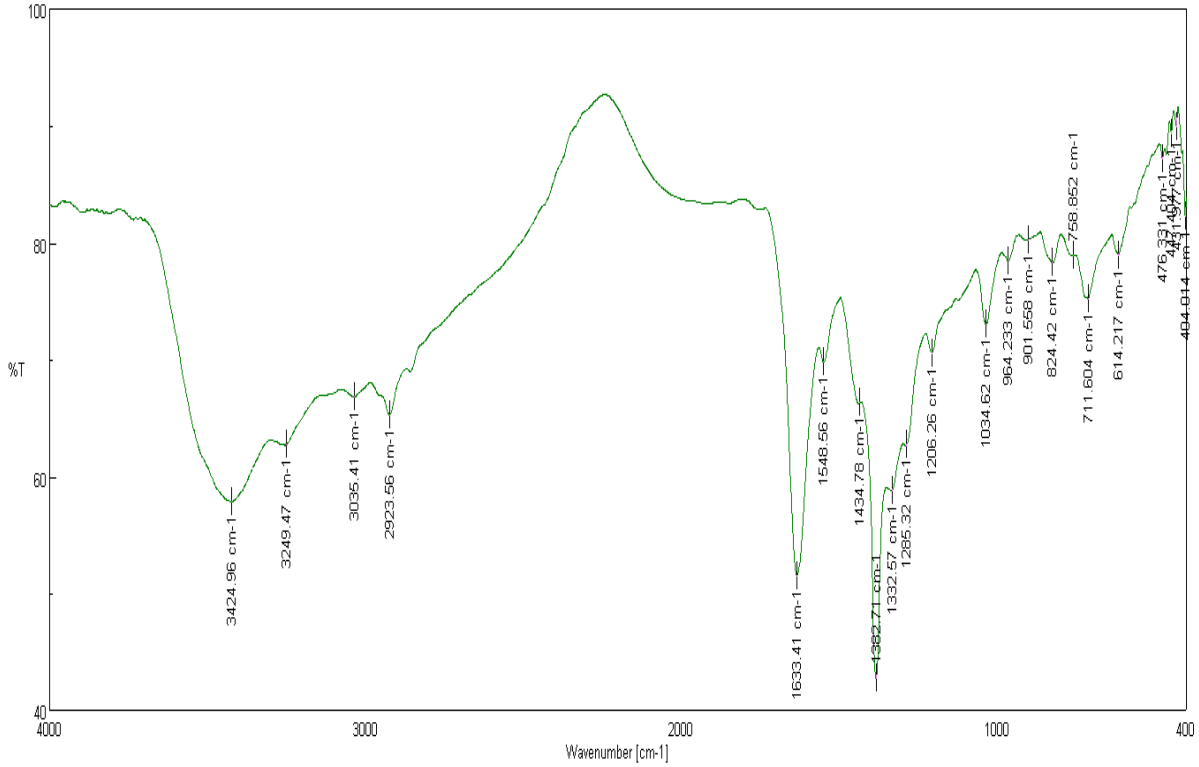


Fig. S6 FT-IR spectra of Complex **2.**

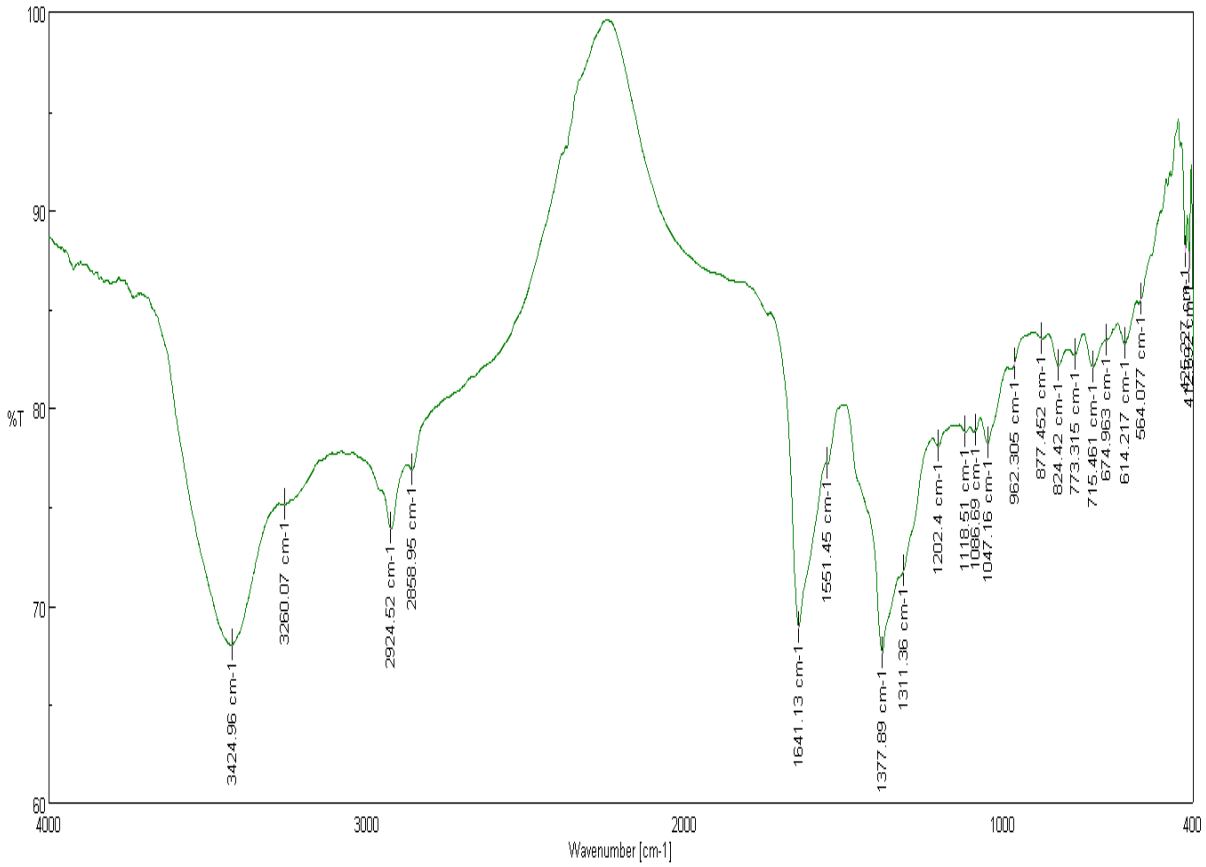


Fig. S7 FT-IR spectra of Complex **3.**

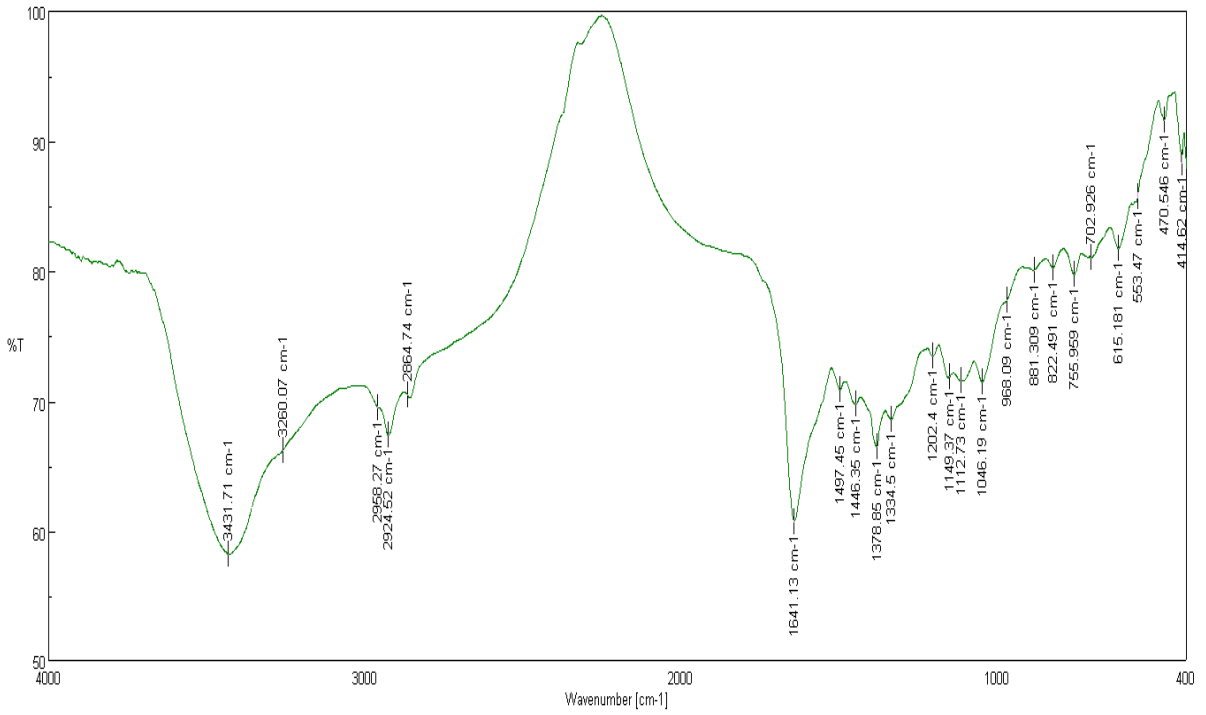


Fig. S8 FT-IR spectra of Complex **4.**

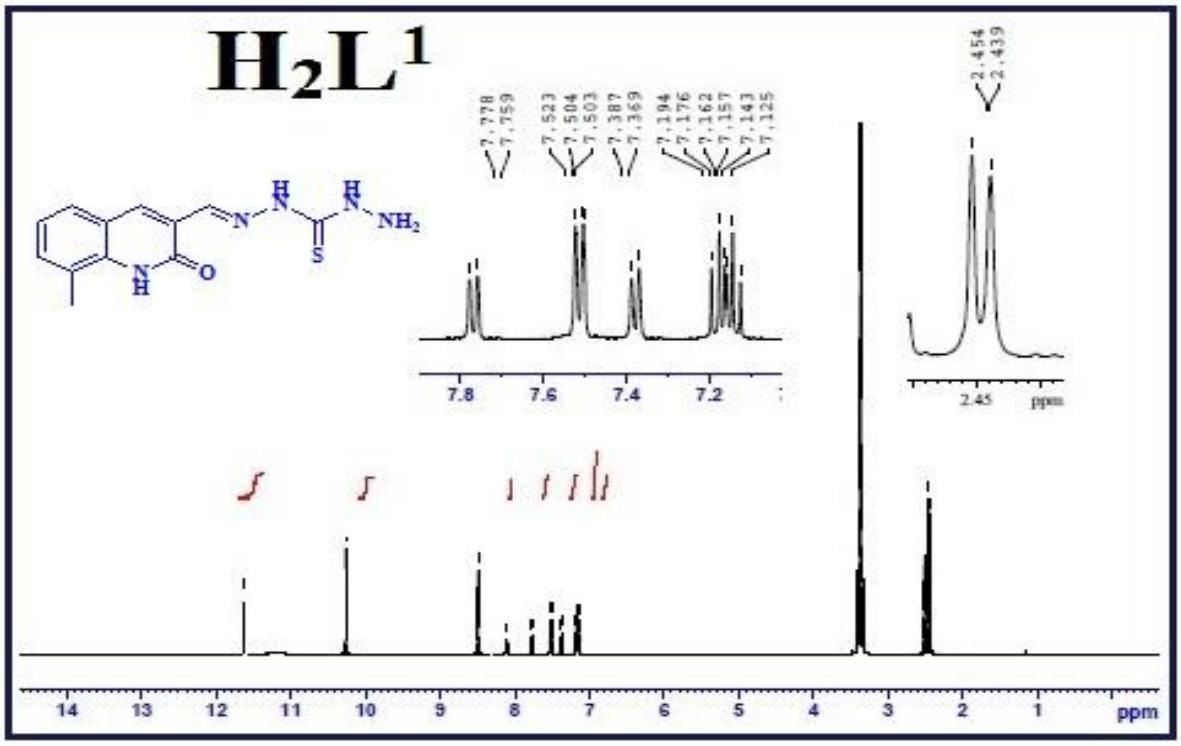


Fig. S9[1]H-NMR spectrum of $\mathbf{H_2L^1}$

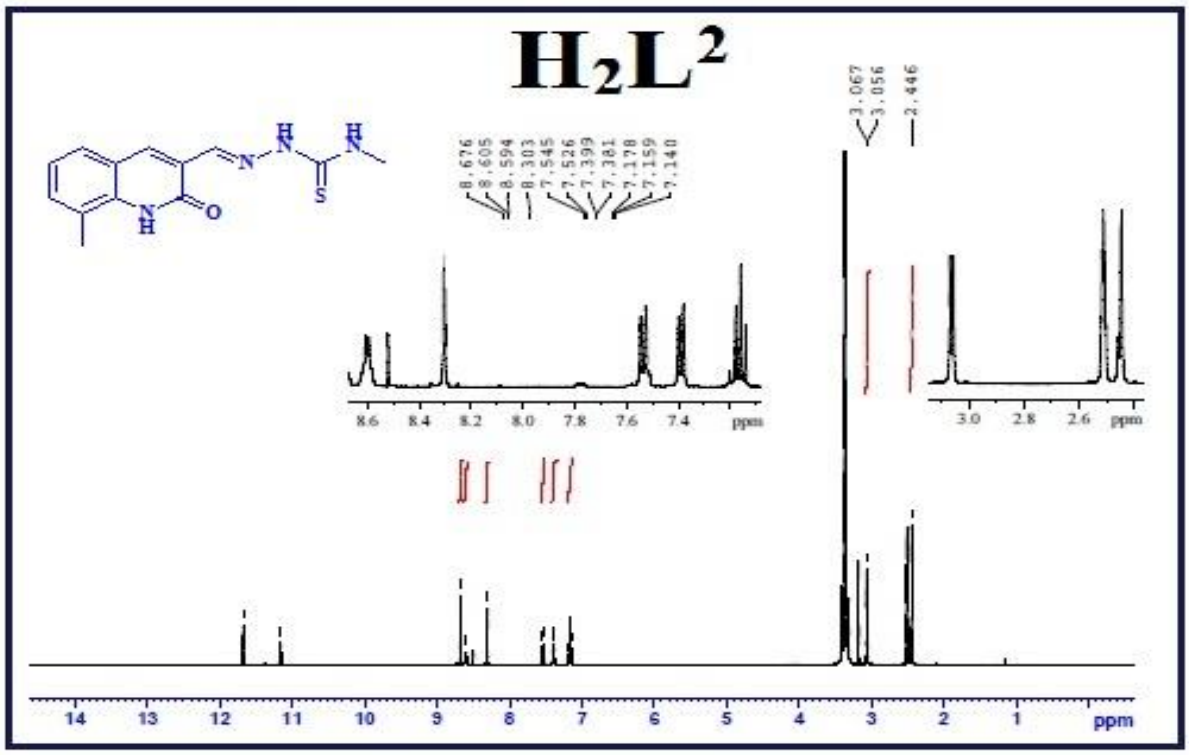


Fig. S10 [1]H-NMR spectrum of $\mathbf{H_2L^2}$

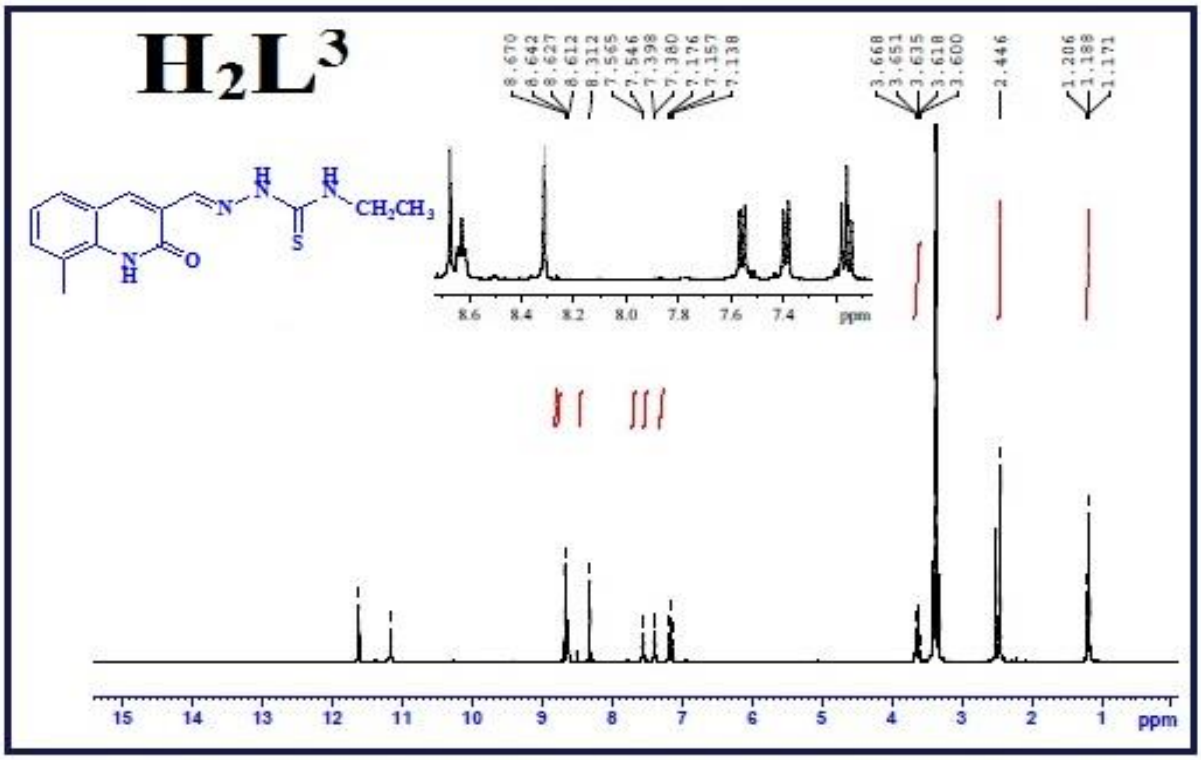


Fig. S11 ^{1}H-NMR spectrum of $\mathbf{H_2L^3}$

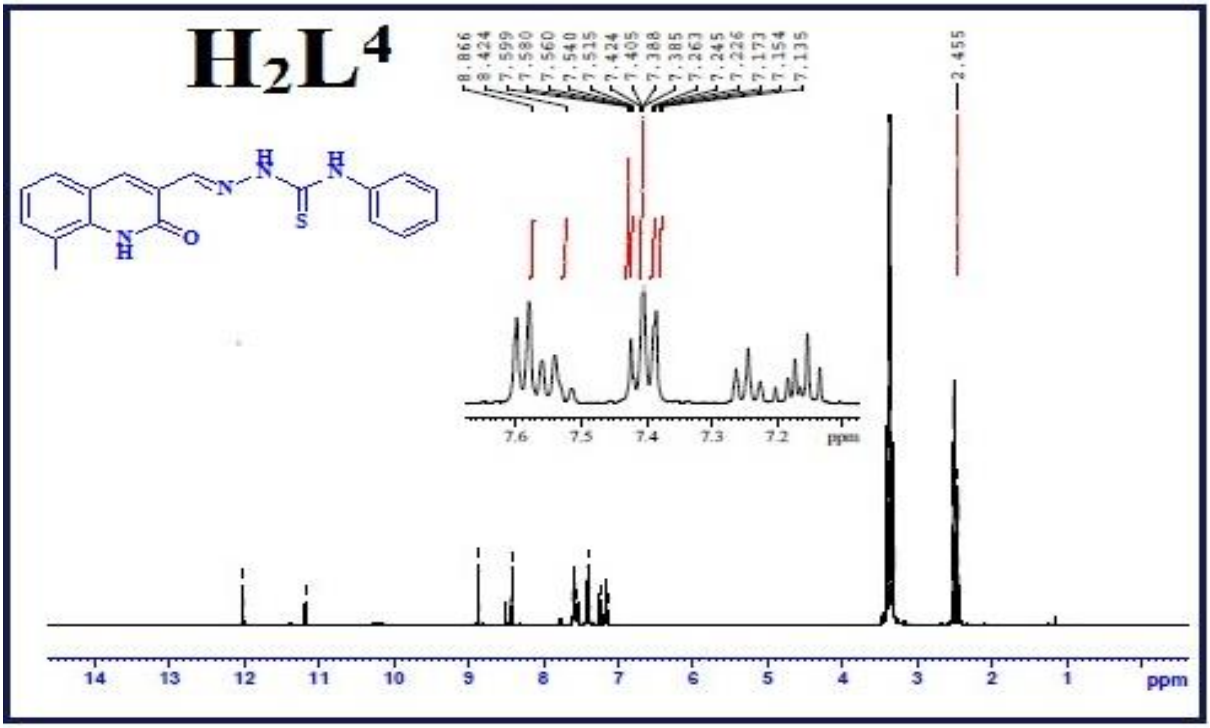


Fig. S12 ^{1}H-NMR spectrum of $\mathbf{H_2L^4}$

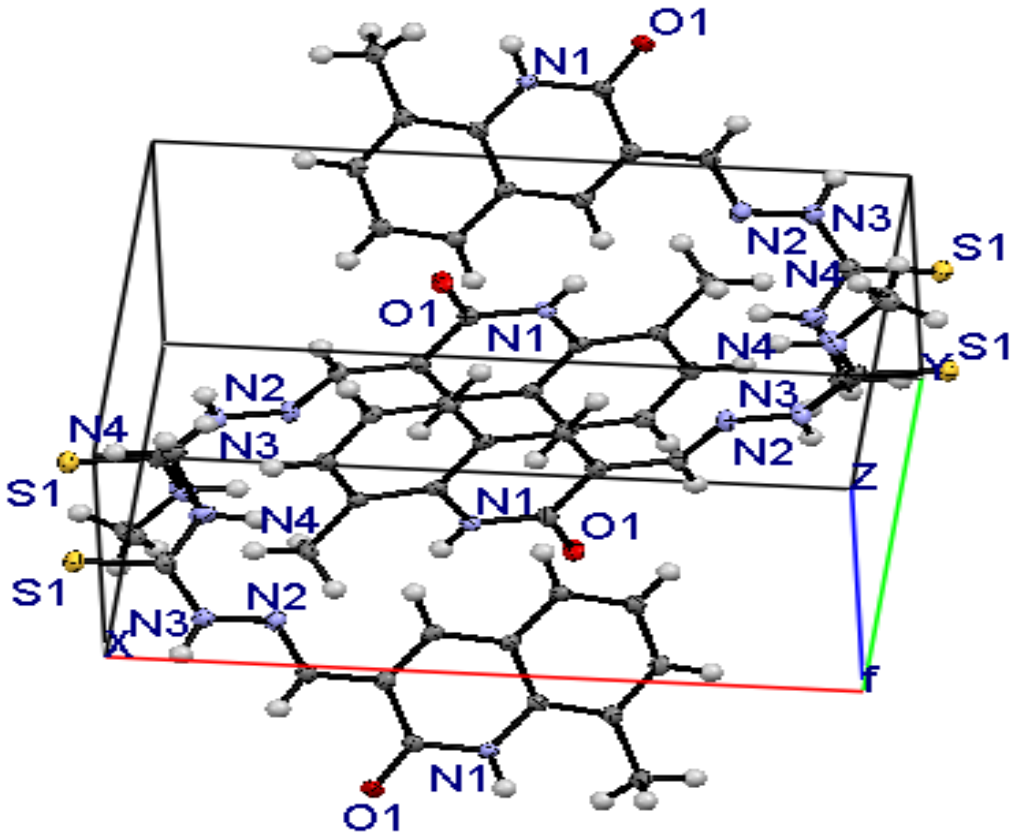


Fig. S13 Molecular packing diagram of H_2L^2

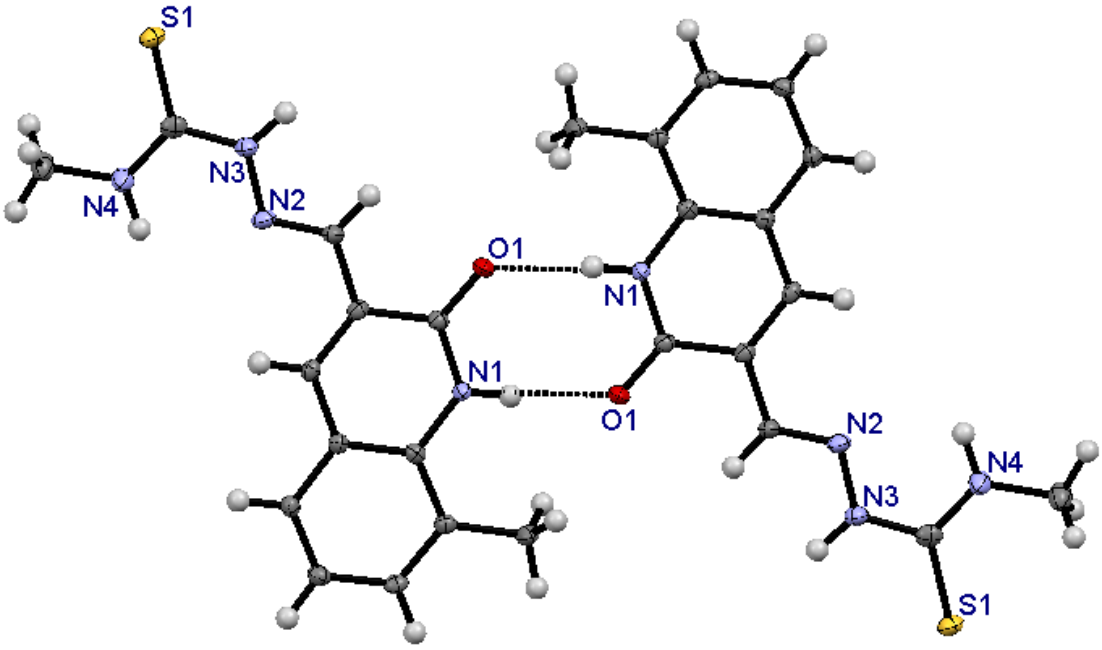


Fig. S14 Hydrogen bonding diagram of H_2L^2

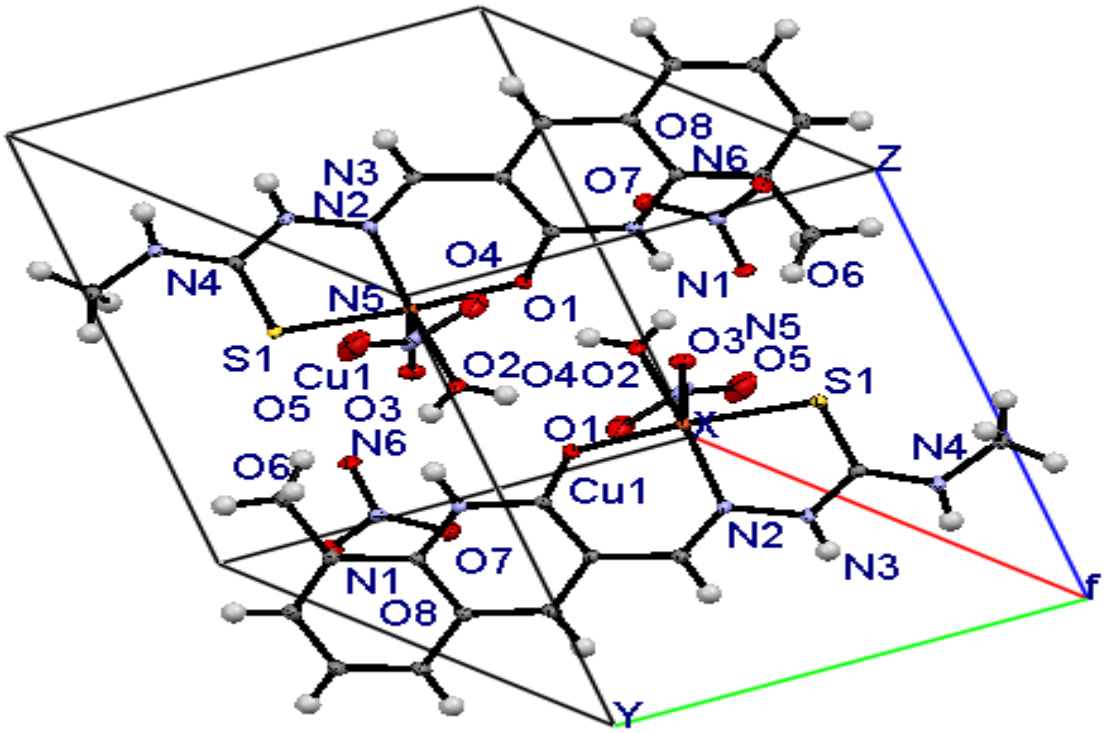


Fig. S15 Molecular packing diagram of **[Cu(H$_2$-8MOQtsc-Me)(NO$_3$)(H$_2$O)]·NO$_3$ (2).**

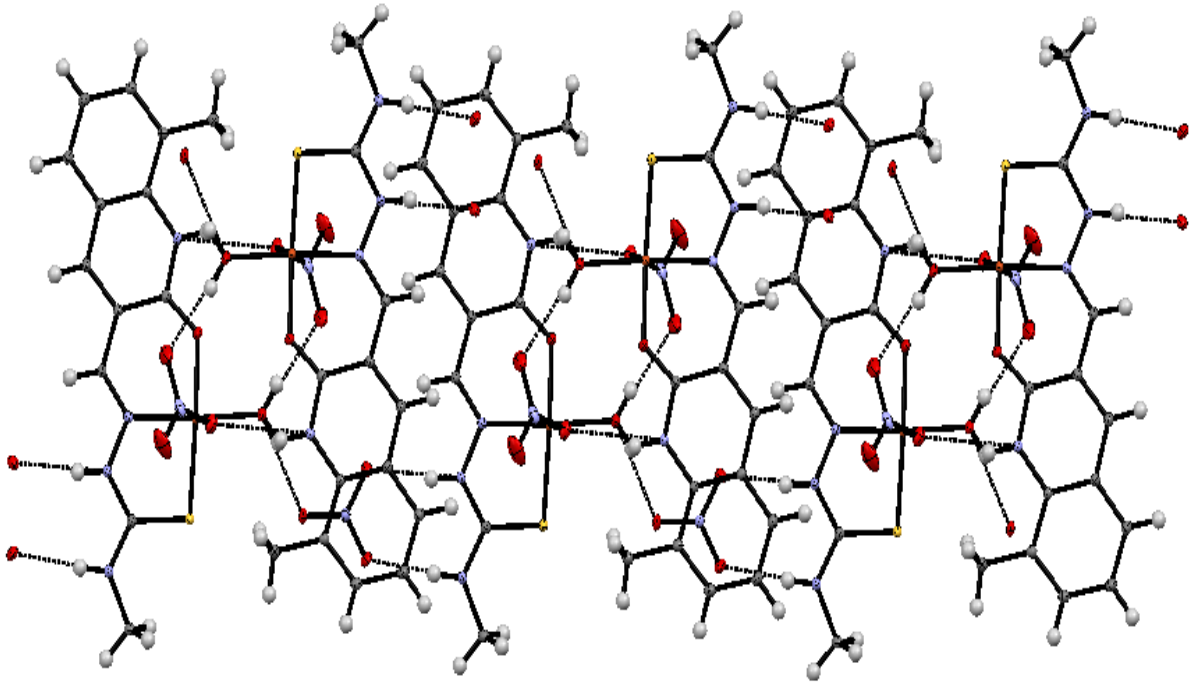


Fig. S16 Hydrogen bonding diagram of **[Cu(H$_2$-8MOQtsc-Me)(NO$_3$)(H$_2$O)]·NO$_3$ (2)**

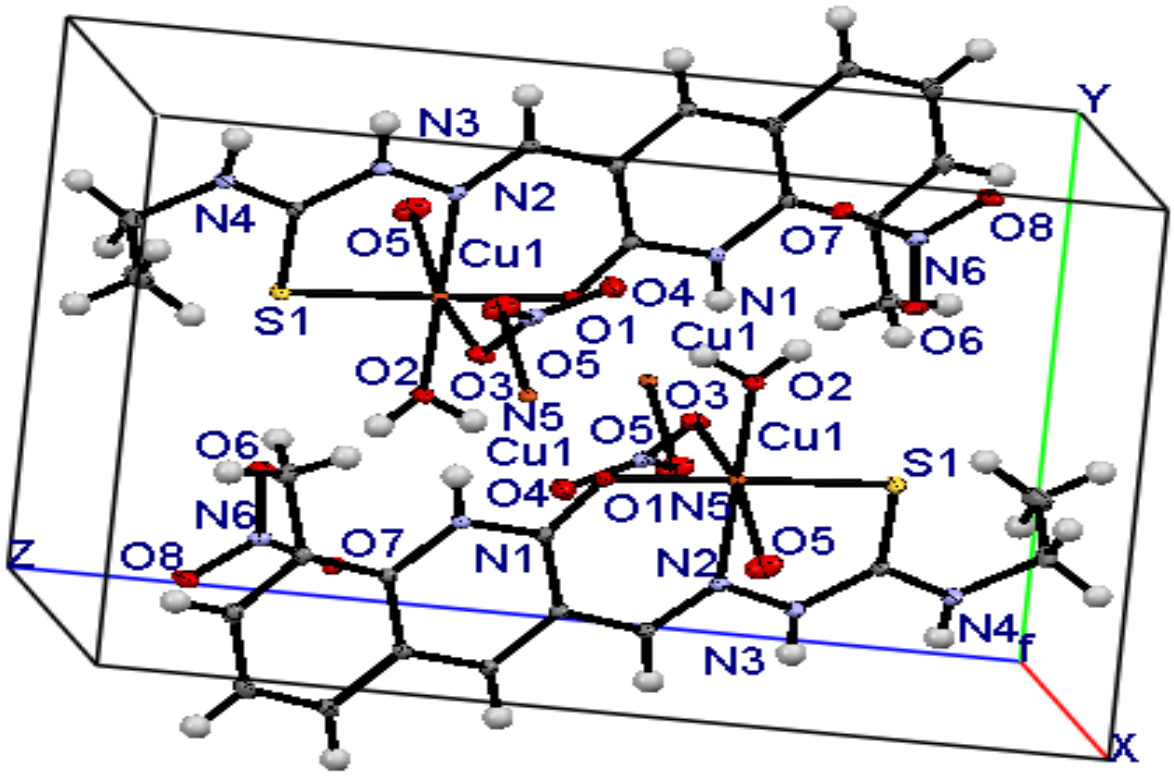


Fig. S17 Molecular packing diagram of [**Cu(H_2-8MOQtsc-Et)((H_2O)(NO_3)]n.NO_3 (3)**.**

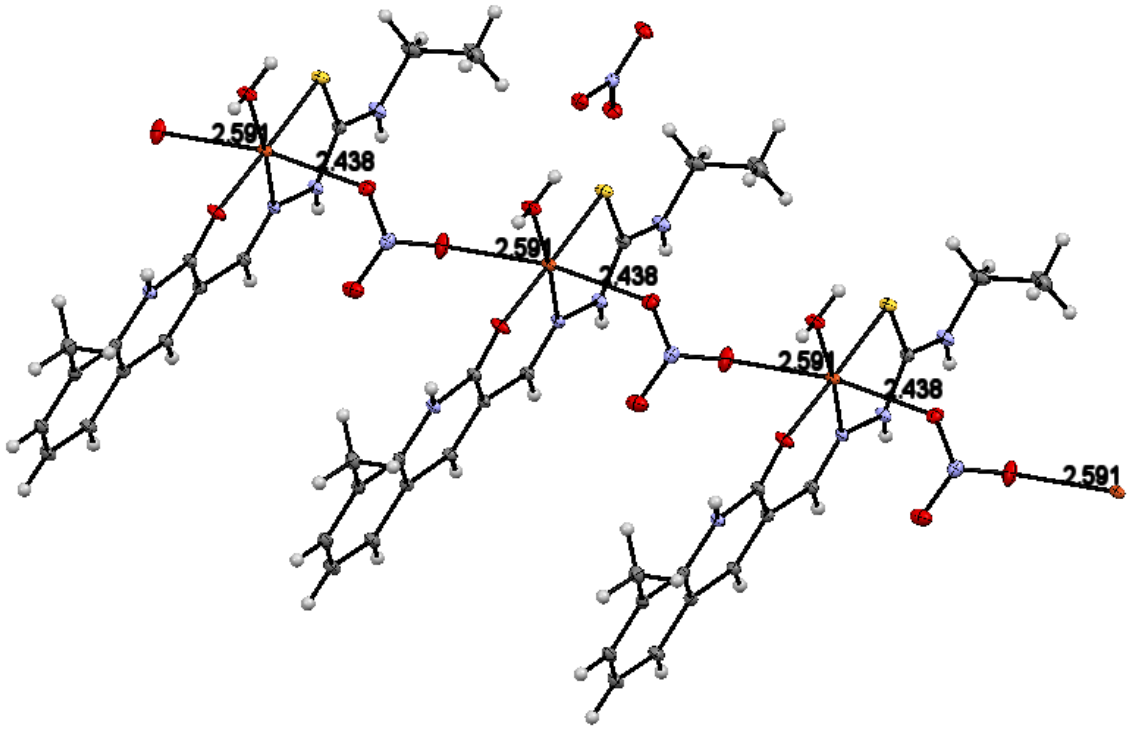


Fig. S18 Polymeric structure of [**Cu(H_2-8MOQtsc-Et)((H_2O)(NO_3)]n.NO_3 (3)**.**

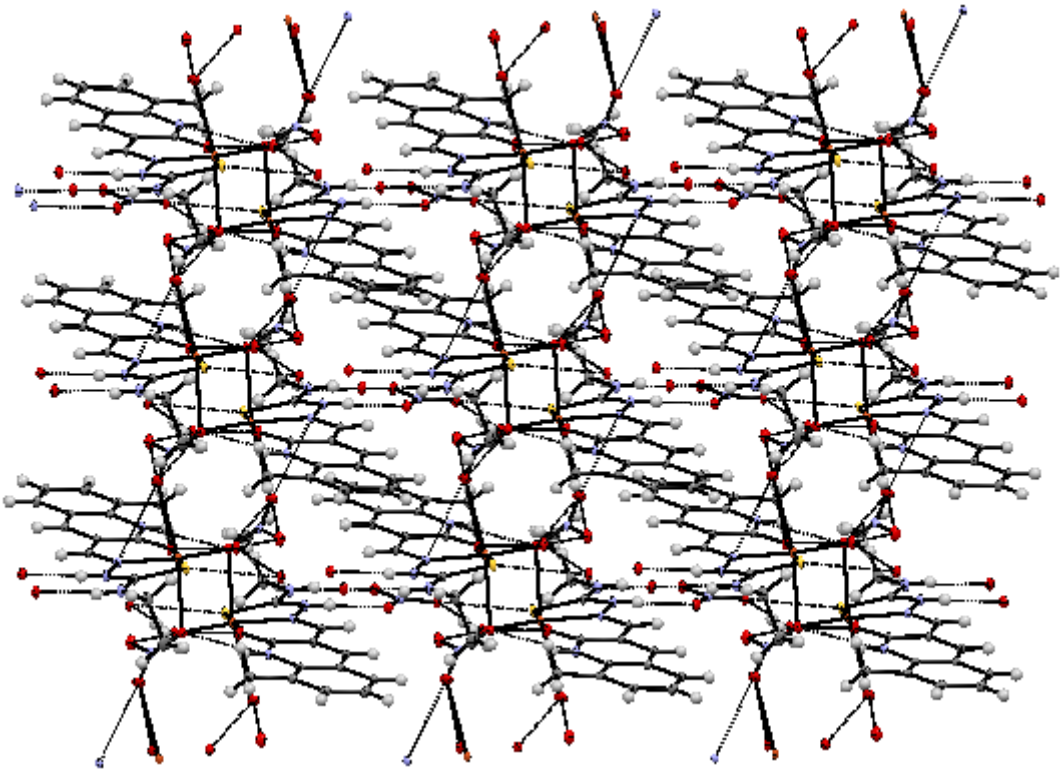


Fig. S19 Hydrogen bonding diagram of [**Cu(H_2-8MOQtsc-Et)((H_2O)(NO_3)]n.NO_3 (3)**.

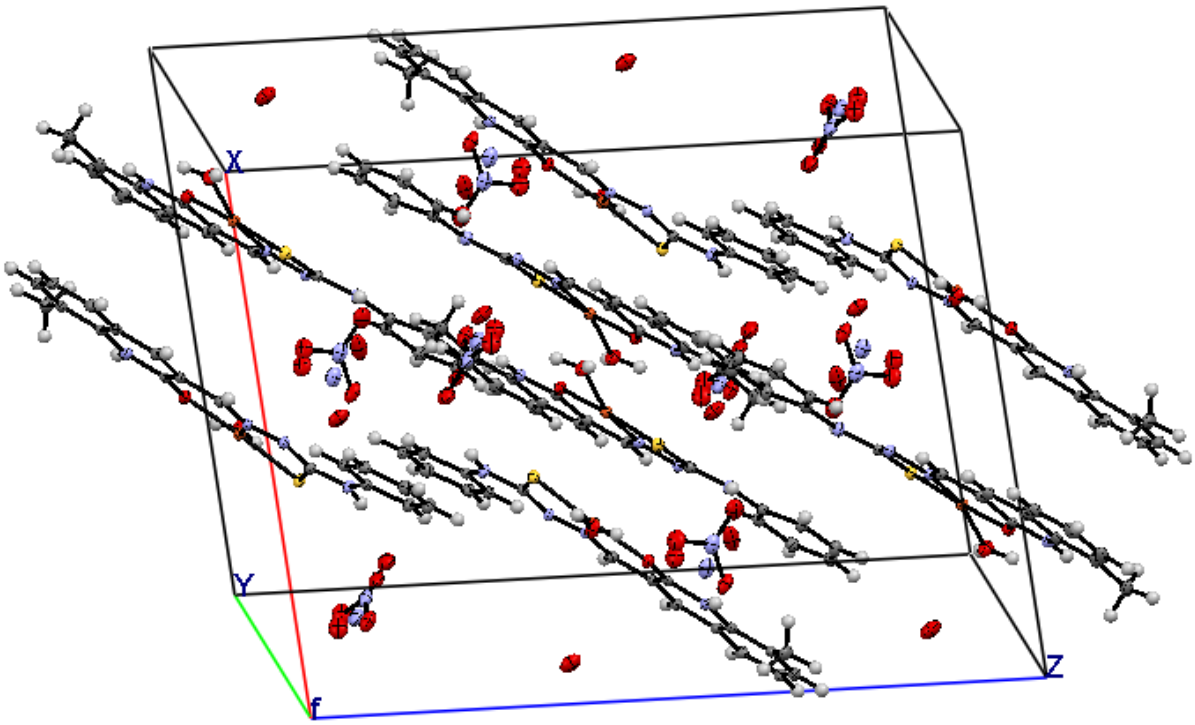


Fig. S20 Molecular packing diagram of**[Cu(H-8MOQtsc-Ph)(H_2O)].(NO_3) (4)**

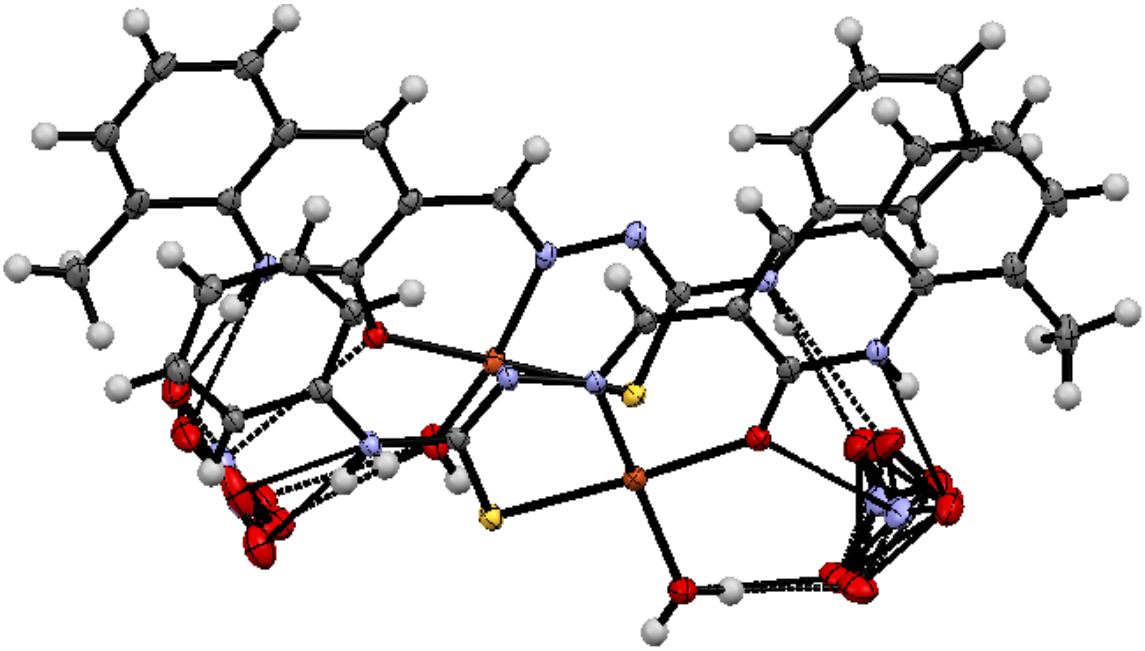
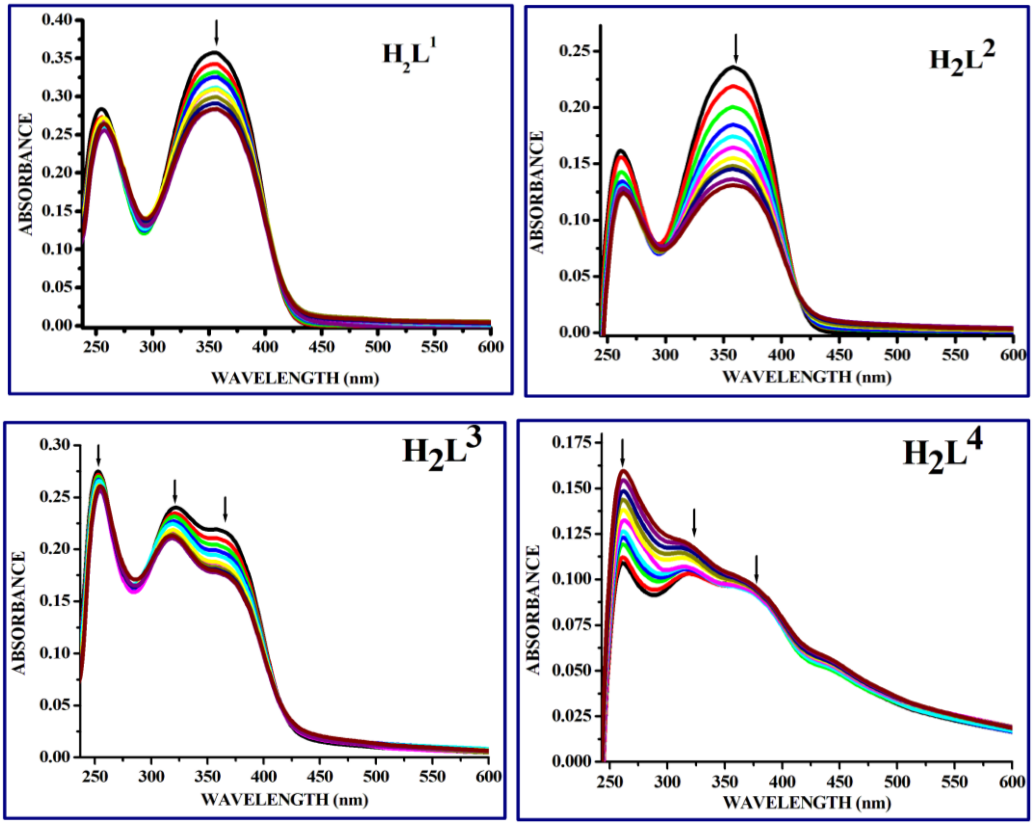


Fig. S21 Hydrogen bonding diagram of**[Cu(H-8MOQtsc-Ph)(H_2O)].(NO_3) (4)**



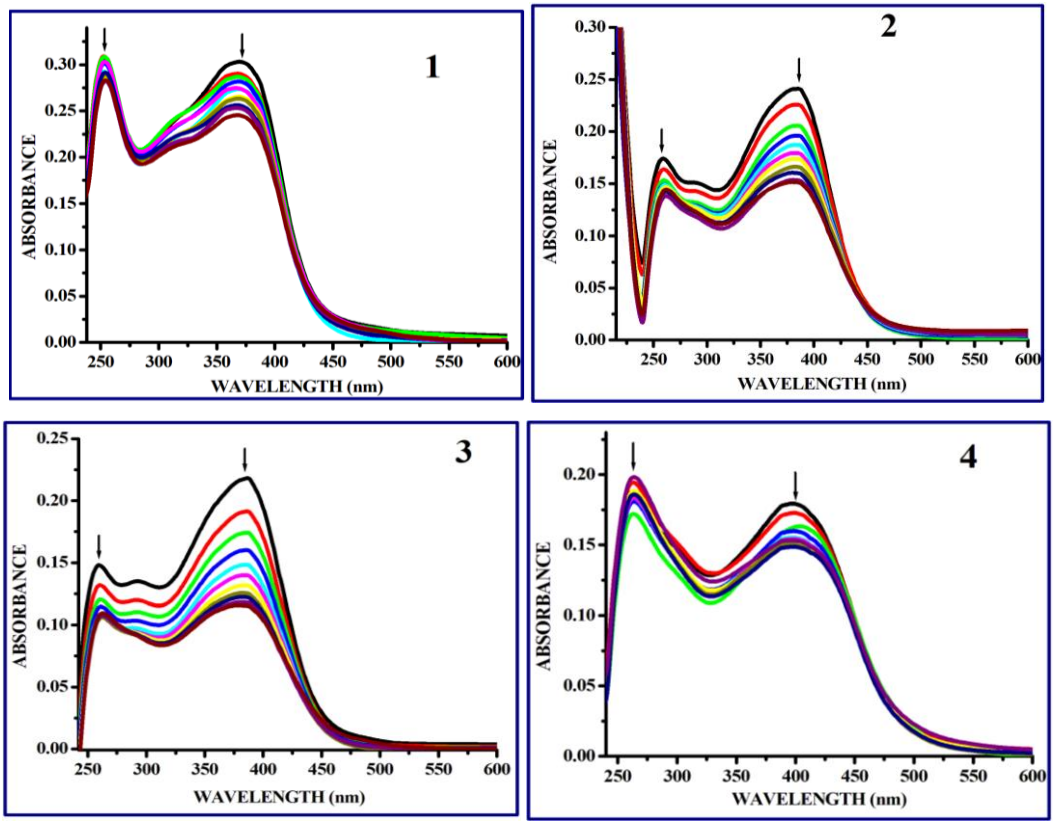


Fig. S22 Absorption spectra of ligands and complexes (10 μM) in the absence and presence of increasing the concentration of CT-DNA (0-100 μM) at room temperature in 5 mM Tris-HCl/50 mM NaCl buffer(pH = 7.2).

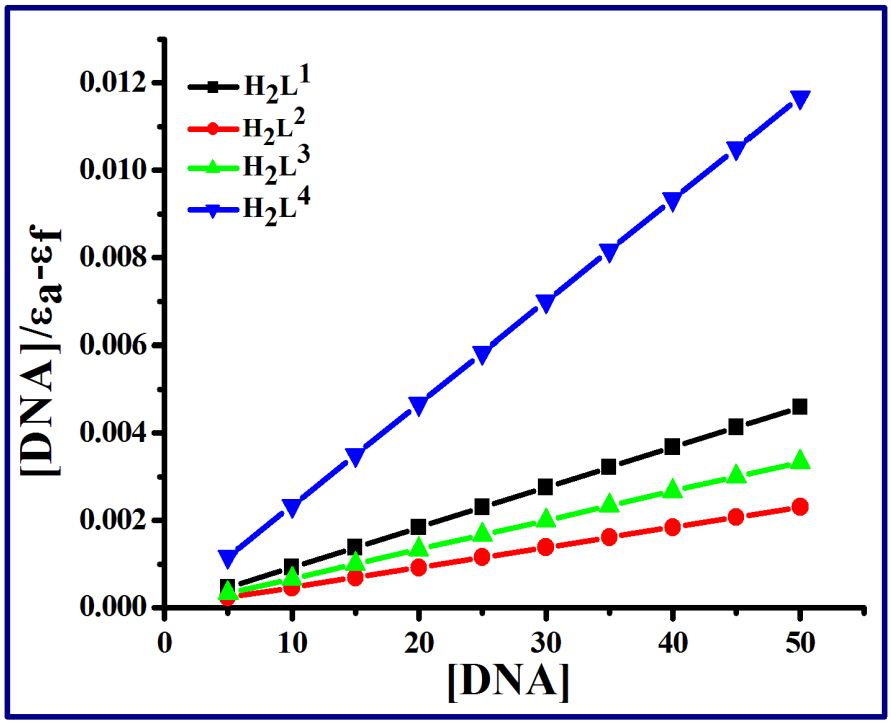


Fig. S23 Plots of [DNA] *versus* [DNA]/ε_a- ε_f for ligands (**H_2L^{1-4}**).

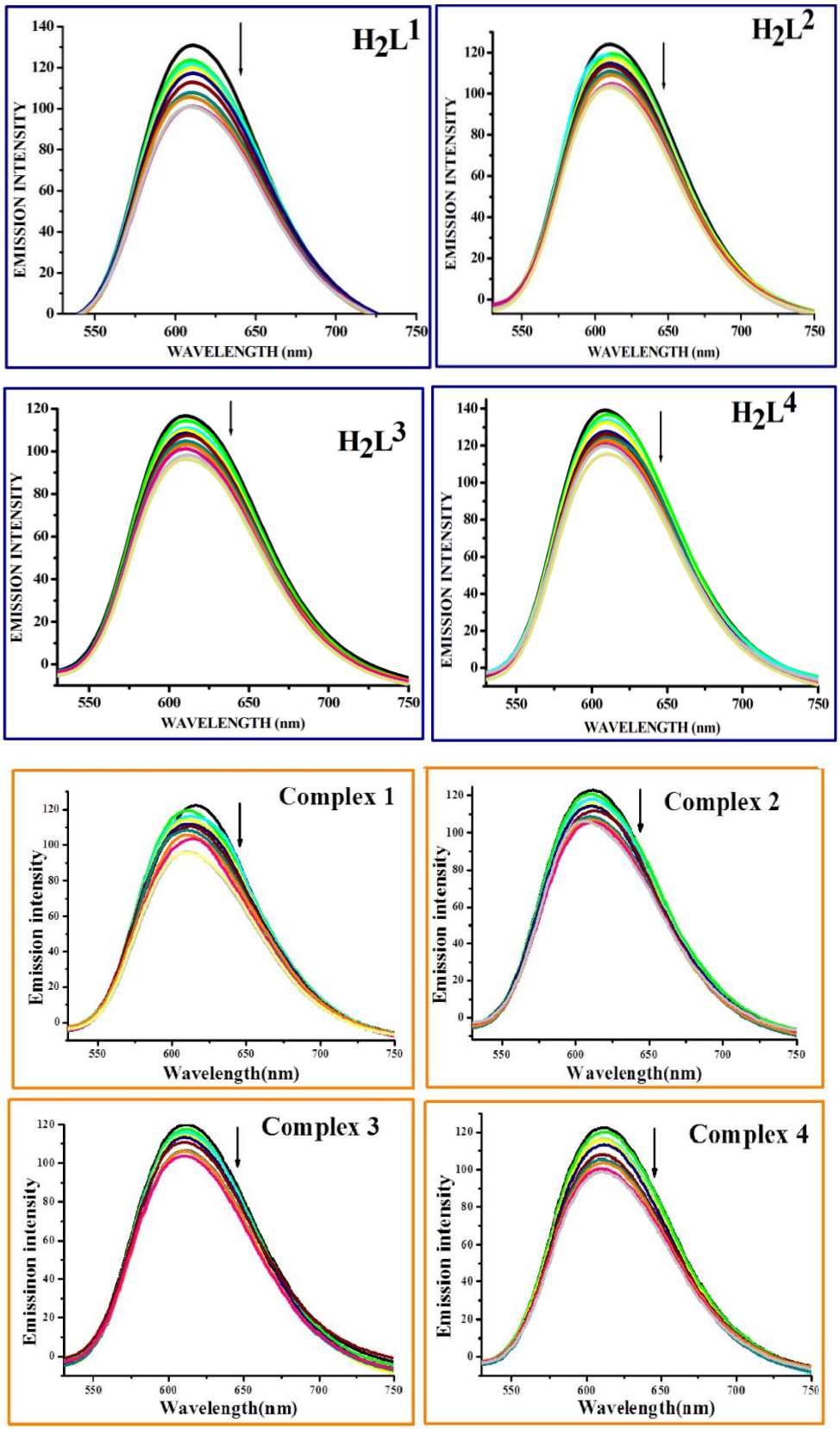


Fig. S24 Emission spectra of EB (5 μM) bound to DNA (5 μM) in absence and presence of ligands and complexes (0-100 μM) using Tris-HCl/50 mM NaCl buffer (pH = 7.2).

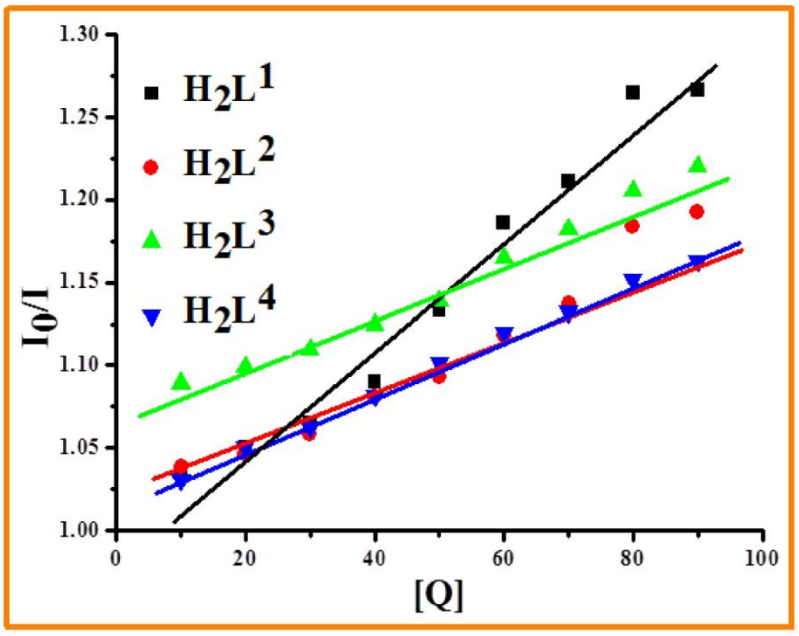


Fig. S25 Stern volmer plots of EB + DNA of the fluorescent titration of the ligands (**H_2L^{1-4}**).

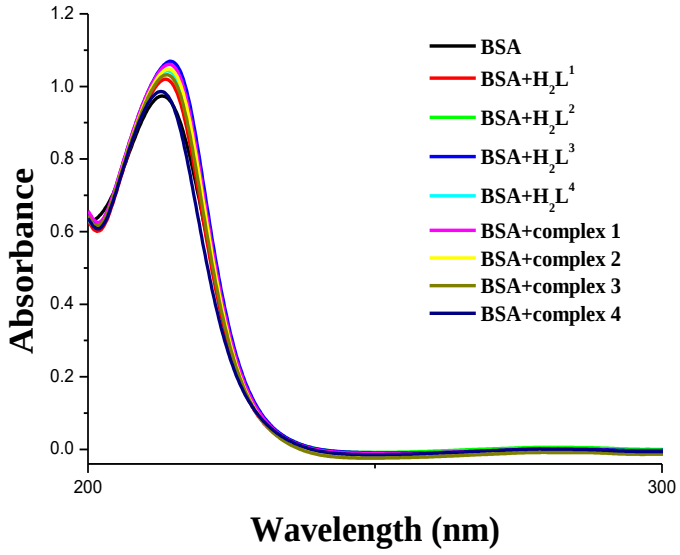


Fig. S26 absorption spectra of BSA (10 μM) in presence of ligands and complexes (10 μM).

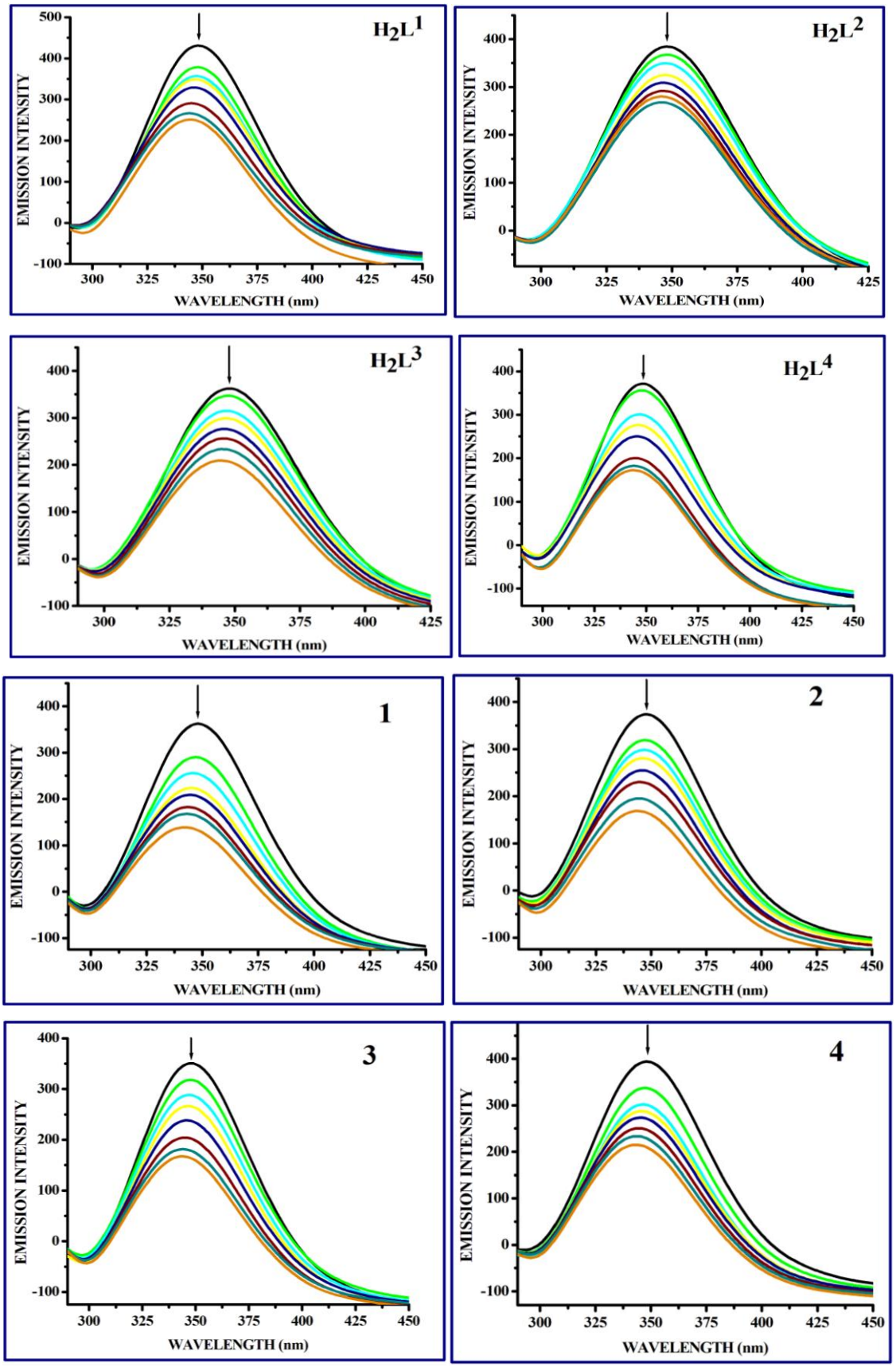
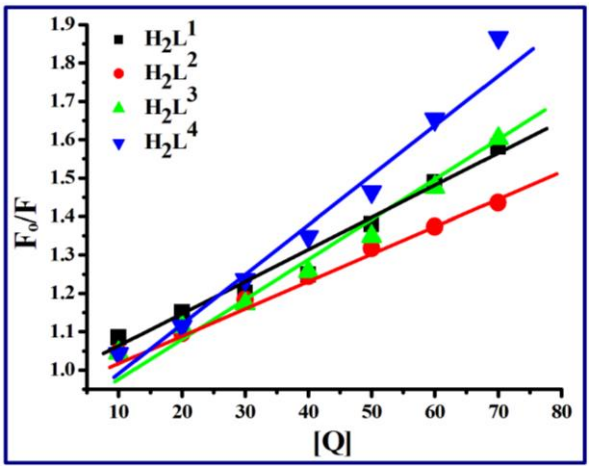


Fig. S27 Florescence spectra for titration of BSA (10 μM) with increasing amounts of ligands and complexes (10-100 μM), at pH = 7.2.



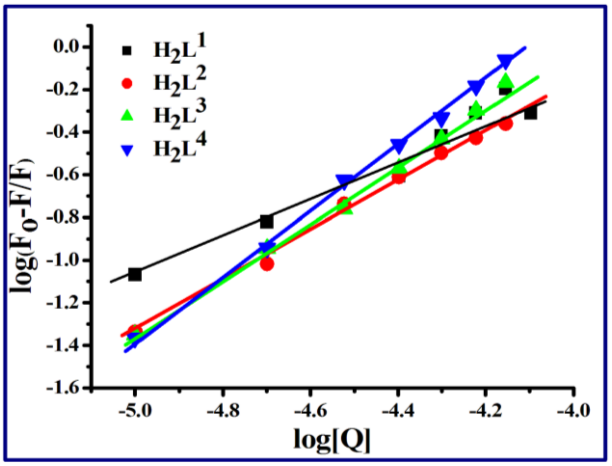
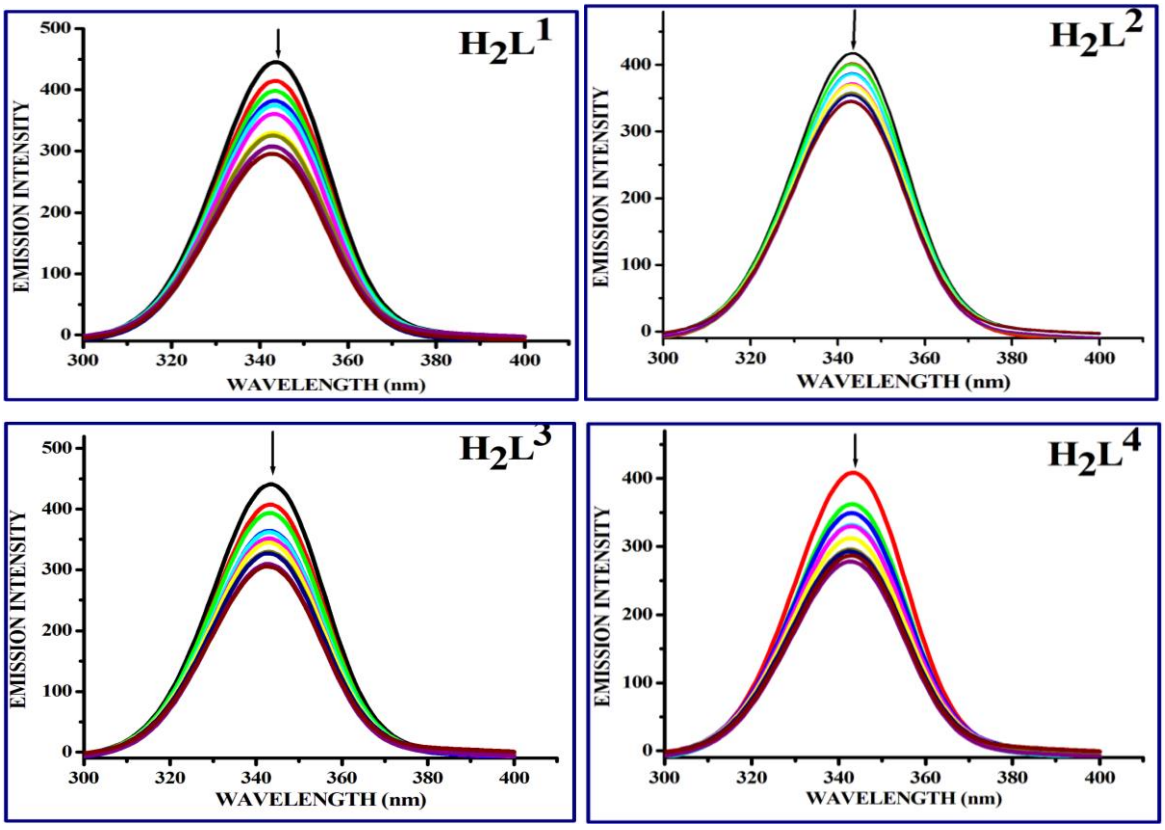


Fig. S28 Stern-Volmer plot for ligands **Fig. S29** Scatchard plot for ligands with BSA.With BSA.



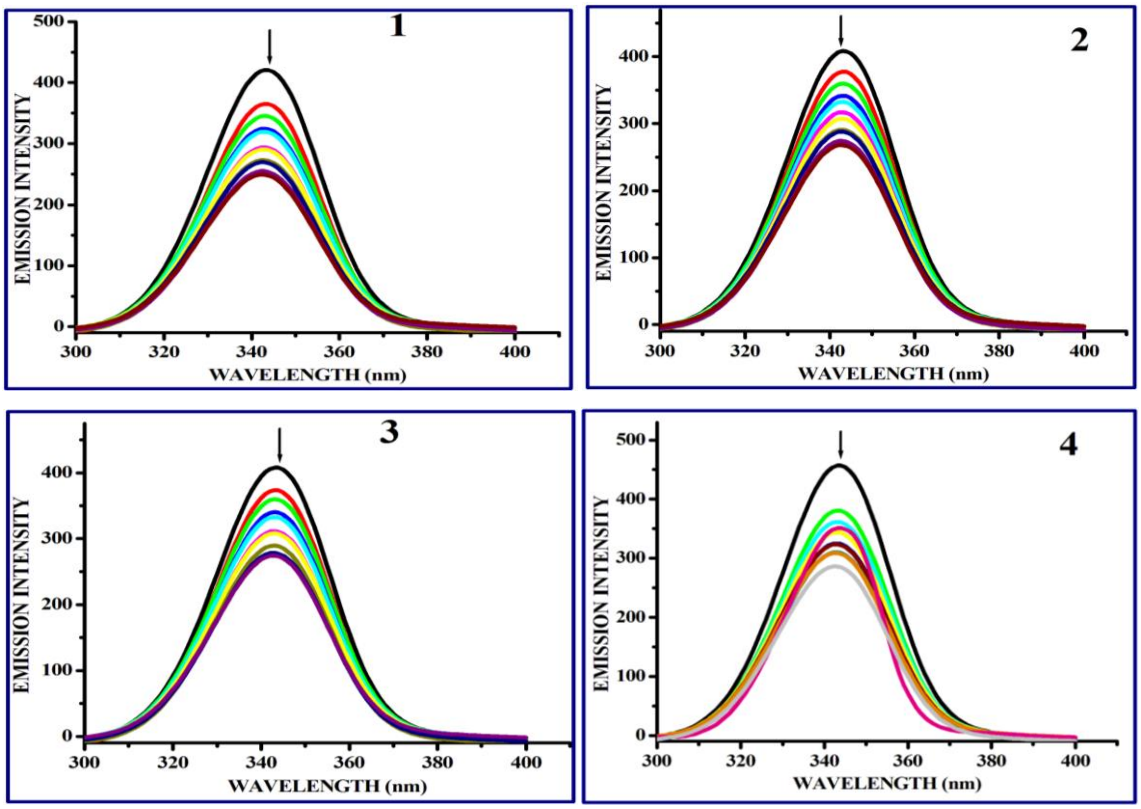
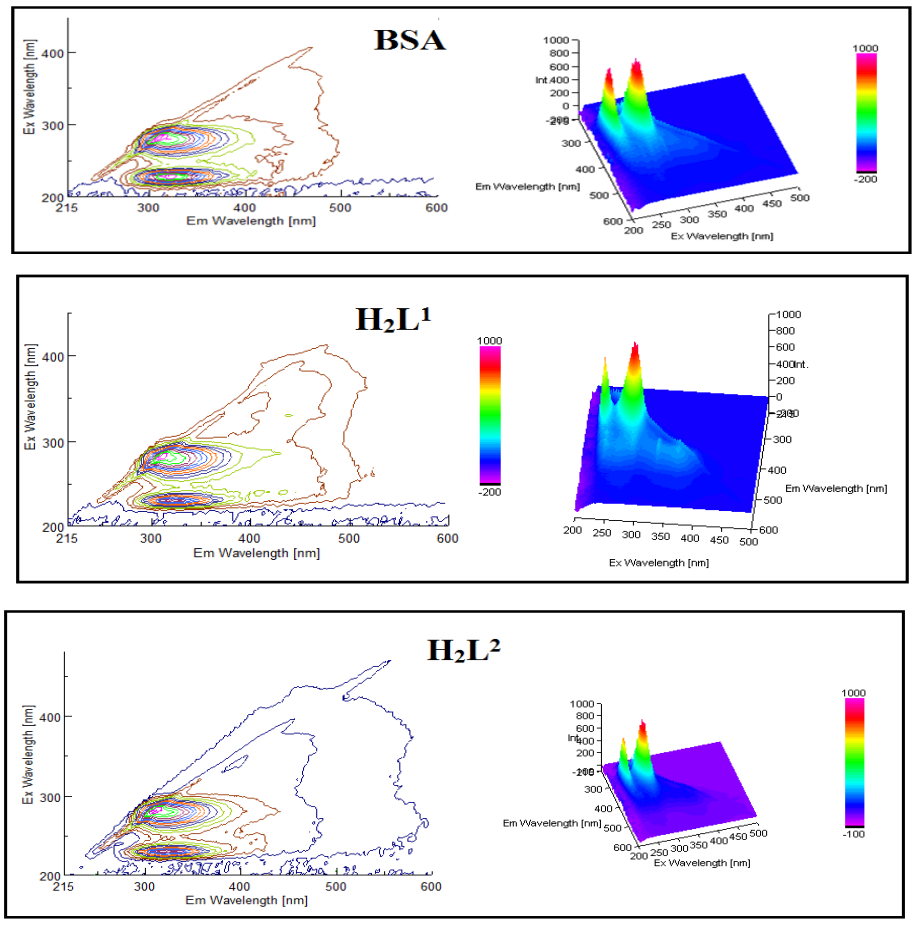
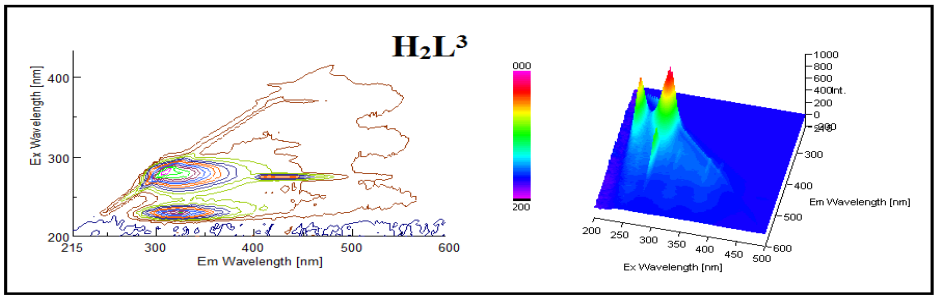
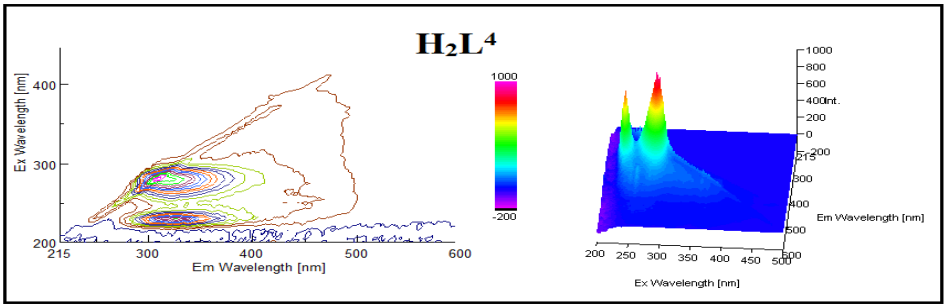
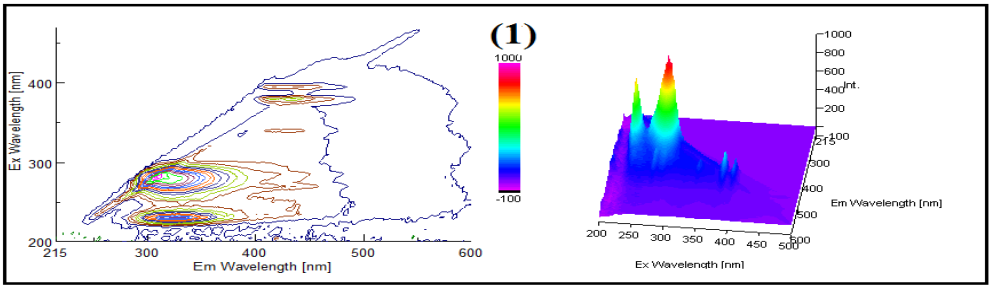


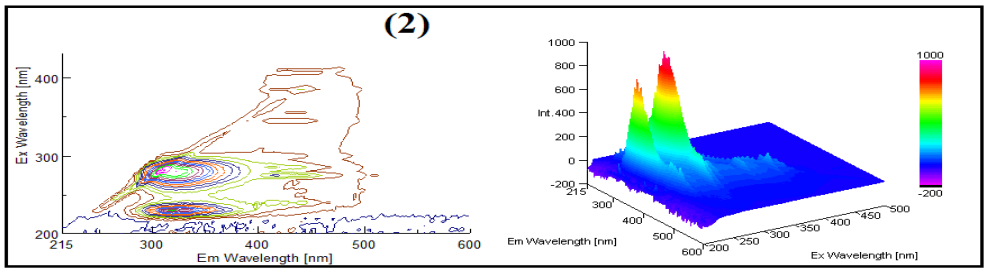
Fig. S30 Synchronous spectra of BSA (10 μM) as a function of concentration of ligands/complexes (10-100 μM) with $\Delta\lambda$ = 60 nm.




H2L3
Ex Wavelength [nm]
Em Wavelength [nm]
Int.
Em Wavelength [nm]
Ex Wavelength [nm]


H2L4
Ex Wavelength [nm]
Em Wavelength [nm]
Int.
Em Wavelength [nm]
Ex Wavelength [nm]


(1)
Ex Wavelength [nm]
Em Wavelength [nm]
Int.
Em Wavelength [nm]
Ex Wavelength [nm]


(2)
Ex Wavelength [nm]
Em Wavelength [nm]
Int.
Em Wavelength [nm]
Ex Wavelength [nm]

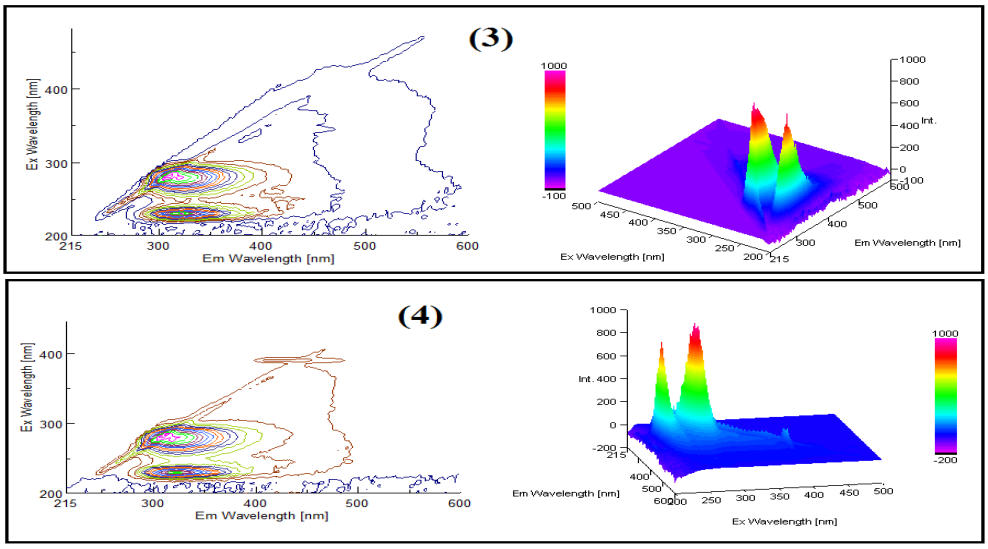


Fig. S31 Three-dimensional spectra of BSA (10 μM) in the (BSA) absence and presence of the test ligands and complexes (10 μM). The contour plots of the corresponding three-dimensional diagrams are provided in the same row of each.

Table. S1. EPR spin Hamiltonian parameters as a consequence of changing N(4) terminal substitutions in copper(II) complexes

Complexes	g_{iso}	$g_{\parallel}$	$g_{\perp}$	g_{av}
1	**-**	**2.2082**	**2.1381**	**2.1617**
2	**2.0447**	**-**	**-**	-
3	**-**	**2.2271**	**2.1772**	**2.1938**
4	**2.0273**	**-**	**-**	-

Table. S2. Selected bond lengths (Å) and bond angles [°] for $\mathbf{H_2L^2}$

Bond lengths		**Bond angles**	
S1-C11	1.686 (2)	C1-N1-C9	124.9 (2)
O1-C1	1.245 (3)	C10-N2-N3	116.21(19)
N1-C1	1.366 (3)	C11-N3-N2	119.6 (2)
N2-C10	1.281 (3)	C11-N4-C13	123.3 (2)
N2-N3	1.373 (3)	O1-C1-N1	120.8 (2)
N3-C11	1.358 (3)		
N4-C11	1.325 (3)		
N4-C13	1.455 (3)		

Table. S3. Hydrogen bonds for ligand **H_2L^1** (Å and °)

D-H···A	*D*-H	H···*A*	*D*···*A*	*D*-H···*A*
N1-H1*N*···O1^{i}	0.87 (3)	1.98 (3)	2.833 (3)	166 (3)
N3-H3*N*···S1^{iii}	0.95 (3)	2.43 (3)	3.373 (2)	174 (2)

Symmetry codes; (i) –x+1,-y,-z+1; (ii) –x,-y+1,-z+1; (iii) –x,-y+2,-z+1.

Table. S4. Selected bond lengths (Å) and bond angles [°] for complex **2**

Bond lengths		**Bond angles**	
Cu1-O1	1.9217 (8)	O1- Cu1-O2	85.31 (4)
Cu1-O2	1.9569 (8)	Cu1-O1-N2	91.07 (4)
Cu1-N2	1.9794 (9)	O1-Cu1-S1	171.55 (3)
Cu1-S1	2.2858 (3)	O2- Cu1-N2	173.58 (4)
Cu1-O3	2.2984 (9)	O2- Cu1-S1	96.06 (3)
		N2- Cu1-S1	86.76 (3)
		O1- Cu1-O3	94.03 (4)
		O2- Cu1-O3	86.98 (4)
		N2- Cu1-O3	98.57 (3)
		S1- Cu1-O3	94.37 (3)

Table. S5. Hydrogen bonds for Complex **2** (Å and °)

D-H···A	*D*-H	H···A	*D*···A	*D*-H···A
O2-H21···O6	0.80 (2)	1.87 (2)	2.6526(12)	167.0 (19)
O2-H22···O4^{i}	0.81 (2)	1.99 (2)	2.7958 (14)	170.7 (19)
N1-H1*N*···O3^{i}	0.870 (17)	2.113 (17)	2.9339 (13)	157.2 (15)
N3-H3*N*···O7^{ii}	0.866 (17)	1.914 (17)	2.7742 (12)	171.9 (16)
N4-H4*N*···O8^{ii}	0.842 (18)	2.019 (18)	2.8588 (13)	174.6 (16)

Symmetry codes; (i) –x+1, -y+1, -z+1; (ii) x,y,z+1; (iii) –x+2,-y+1,-z+1 (iv) x+1,y,z.

Table. S6. Selected bond lengths (Å) and bond angles [°] for complex **3.**

Bond lengths		**Bond angles**	
Cu1-O1	1.9087 (5)	S1-Cu1-O1	178.03(2)
Cu1-O2	1.9537 (5)	S1-Cu1-O2	94.83(2)
Cu1-N2	1.9797 (5)	S1-Cu1-O3	87.14(2)
Cu1-S1	2.2622 (2)	S1-Cu1-N2	87.11(2)
Cu1-O3	2.4383 (5)	S1-Cu1-O5	96.06(2)
Cu1-O5	2.5912(6)	O1-Cu1-O2	85.97(2)
		O1-Cu1-O3	94.58(2)
		O1-Cu1-N2	91.92(2)
		O1-Cu1-O5	82.07(2)
		O2-Cu1-O3	95.59(2)
		O2-Cu1-N2	174.15(2)
		O2-Cu1-O5	95.48(2)
		O3-Cu1-N2	90.01(2)
		O3-Cu1-O5	168.17(2)
		N2-Cu1-O5	78.81(2)

Table. S7. Hydrogen bonds for Complex **3** (Å and °).

D-H···A	*D*-H	H···A	*D*···A	*D*-H···A
O2-H21···O6	0.833 (14)	1.873 (14)	2.6785 (17)	162.2 (14)
O2-H22···O4^{i}	0.852 (14)	1.960 (14)	2.7902 (7)	164.3 (13)
N1-H1···O3^{i}	0.83 (1)	2.12 (1)	2.9008 (7)	157 (1)
N3-H3*N*···O7^{ii}	0.82 (1)	1.95 (1)	2.7670 (7)	177 (1)
N4-H4*N*···O8^{ii}	0.81 (1)	2.00 (1)	2.7917 (8)	168 (1)

Symmetry codes; (i) –x+1, -y+1, -z+1; (ii) x,y,z+1; (iii) –x+1,-y+1,-z+2.

Table. S8. Selected bond lengths (Å) and bond angles [°] for complex **4.**

Bond lengths		**Bond angles**	
Cu1-O1	1.9507 (13)	O1- Cu1-O2	85.19 (6)
Cu1-O2	1.9533 (14)	O1- Cu1-N2	93.06 (6)
Cu1-N2	1.9614 (15)	O1-Cu1-S1	176.30 (4)
Cu1-S1	2.2454 (5)	O2- Cu1-N2	165.03 (6)
		O2- Cu1-S1	96.92 (4)

		N2- Cu1-S1	85.68 (4)

Table. S9.Hydrogen bonds for Complex **4** (Å and °).

D-H···A	*D*-H	H···A	*D*···A	*D*-H···A
O1-H21···O3*A*	0.83 (2)	1.98 (2)	2.807 (8)	173 (3)
N1-H1*N*···O4*A*	0.83 (2)	2.15 (2)	2.951 (3)	162 (2)
N4-H4*N*···O5*A*^{i}	0.81 (2)	2.17 (2)	2.963 (3)	165 (2)

Symmetry codes; (i) –x+3/2,y,-z+1.

Table. S10. The K_b (binding constant), K_{sv} (Quenching constant) and K_{app}(apparent with EB) values for the interactions of the ligands (**H_2L^{1-4}**) with CT-DNA.

Compounds	K_b/M^{-1}	K_{sv}/M^{-1}
H_2L^1	3.45 × 10^4±0.22	1.71 × 10^4±0.20
H_2L^2	2.75 × 10^4±0.19	1.68 × 10^4±0.09
H_2L^3	5.01 × 10^4±0.13	2.09 × 10^4±0.18
H_2L^4	7.01 × 10^4±0.16	2.34 × 10^4±0.07

Table. S11. Quenching constant (**K_{sv}**), binding constant (**K_{bin}**) and number of binding sites (**n**) for the interactions of ligands (**H_2L^{1-4}**) with BSA

Compounds	K_{sv}/M^{-1}	K_b/M^{-1}	**n**
H_2L^1	1.61 × 10^3±0.14	8.26 × 10^3±0.15	1.01
H_2L^2	1.34 × 10^3±0.11	3.68 × 10^3±0.22	1.18
H_2L^3	1.89 × 10^3±0.17	7.96 × 10^3±0.19	1.91
H_2L^4	8.35× 10^3±0.29	8.91 × 10^3±0.14	1.21

Table. S12. Three-dimensional fluorescence spectral characteristics of bovine serum albumins and BSA-ligand systems.

Compounds	**Peak 'a'**			**Peak 'b'**		
	Peak position λex/λem (nm/nm)	**Stokes Δλ(nm)**	**Intensity (F)**	**Peak position λex/λem (nm/nm)**	**Stokes Δλ(nm)**	**Intensity (F)**
BSA	280/335	55	972.26	230/326	96	897.74
BSA+H_2L^1	280/326	46	875.45	230/322	92	747.51
BSA+ H_2L^2	280/328	48	760.65	230/319	89	715.16
BSA+ H_2L^3	280/330	50	591.74	230/325	95	765.91
BSA+ H_2L^4	280/334	54	766.52	230/320	90	625.98

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