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Electronic Supplementary Information

A Trinuclear Cobalt Coordination Complex Constructed using Novel Triazine Ligands via One-pot Green Synthesis: Structural Properties and Biological Evaluation

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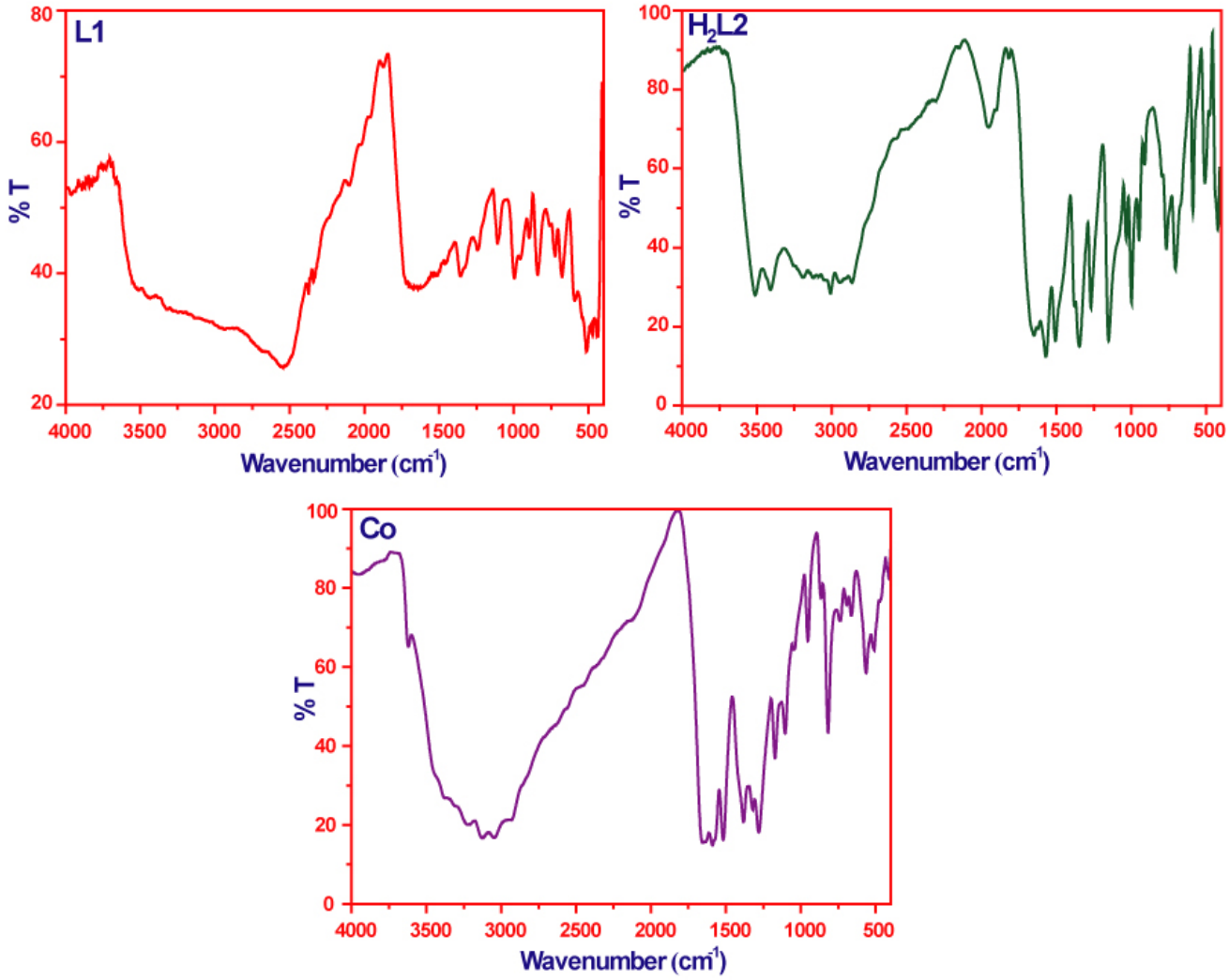

Fig. S1. FT-IR spectra of the ligands and complex.

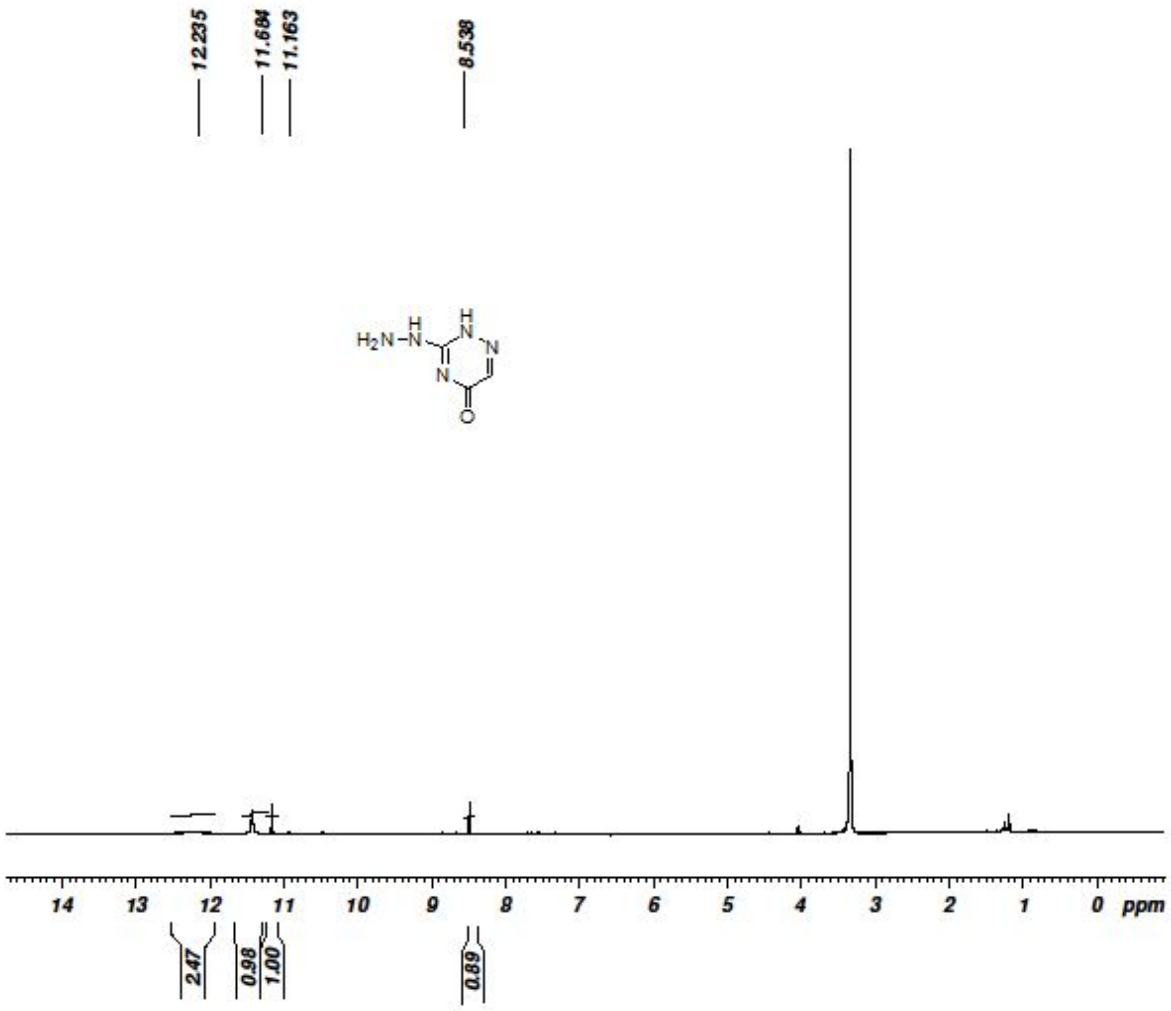

Fig. S2. ^{1}H-NMR spectrum of **L1** in DMSO-d_6.

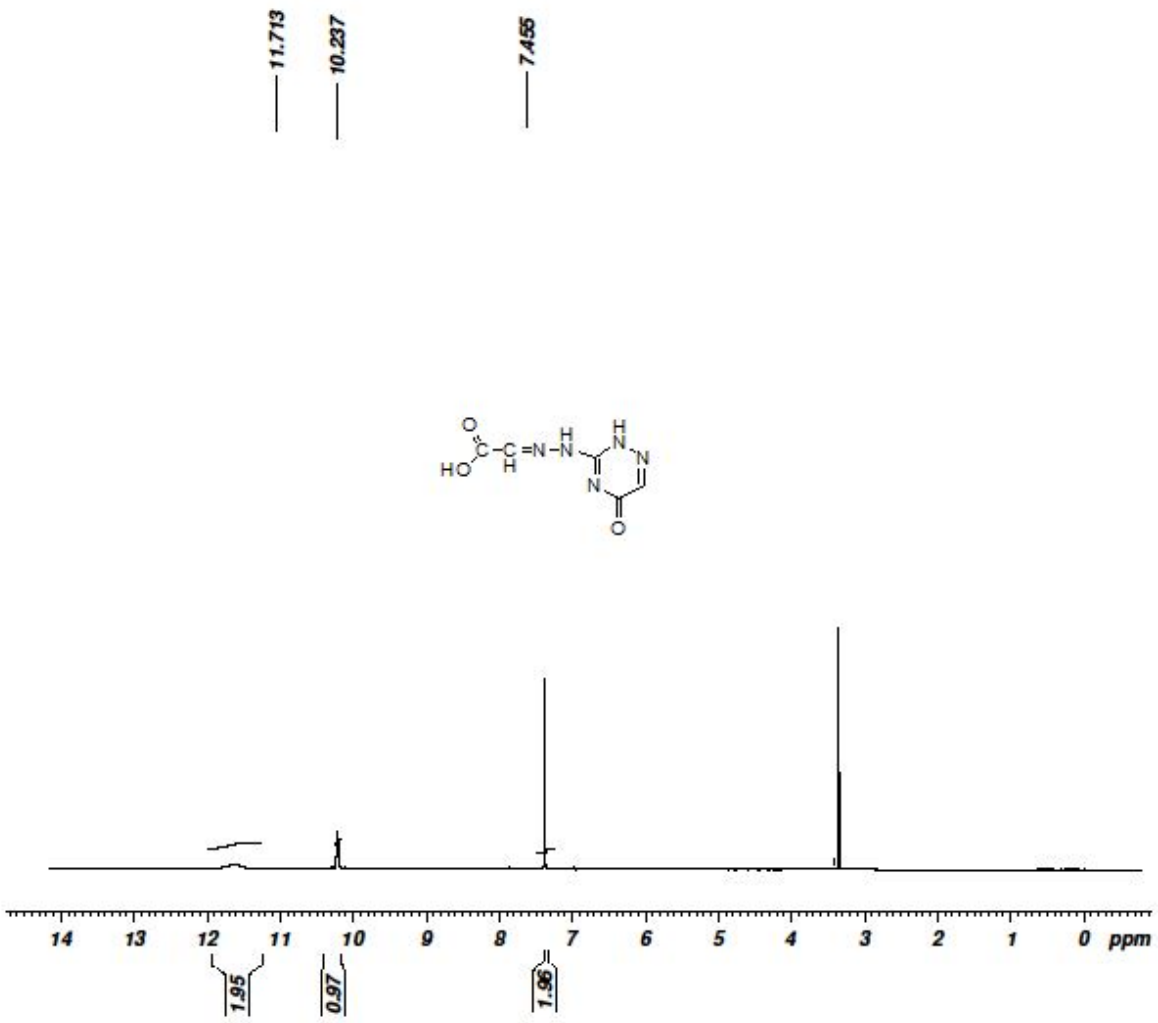

Fig. S3. ^{1}H-NMR spectrum of **H_2L2** in DMSO-d_6**.**

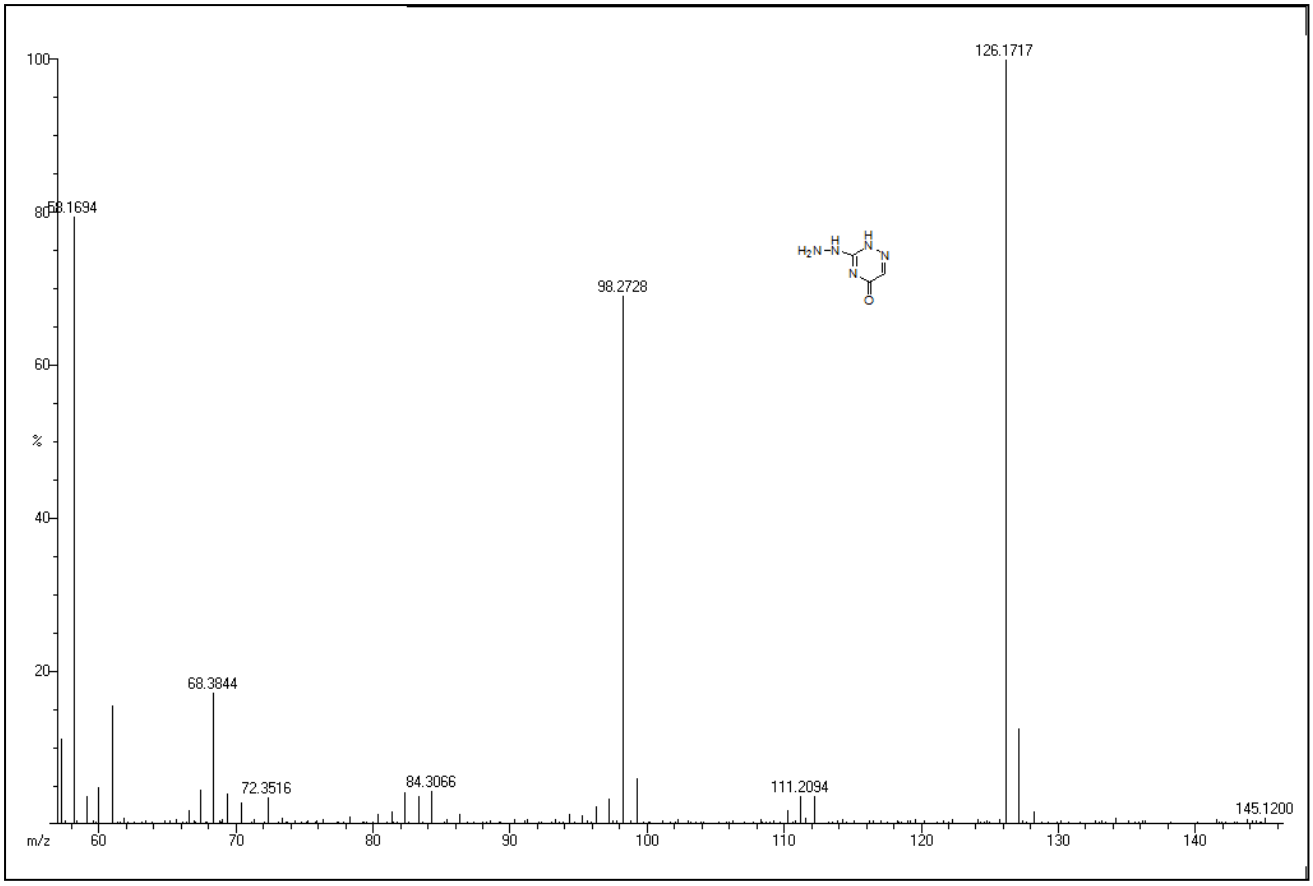

Fig. S4. Mass spectrum of **L1.**

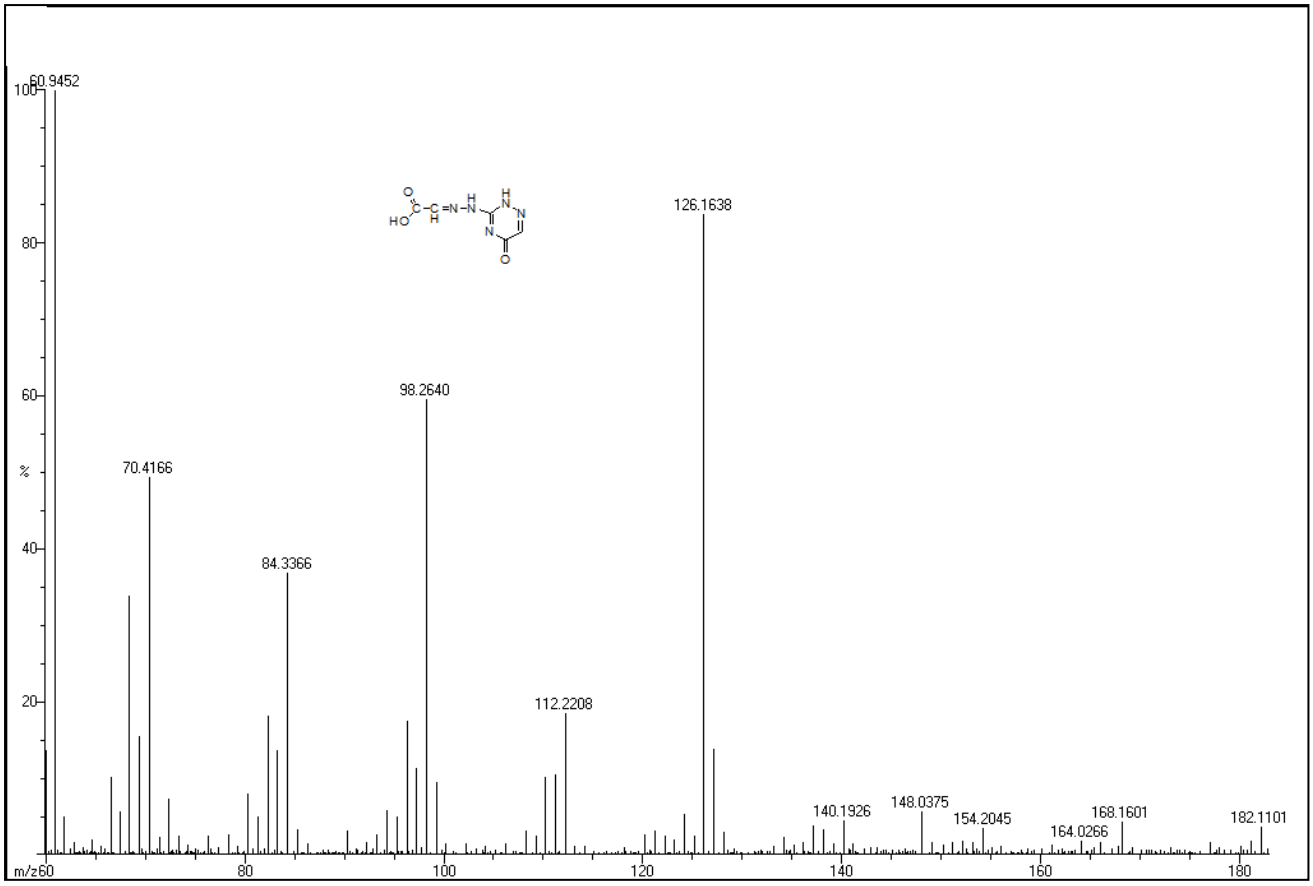

Fig. S5. Mass spectrum of $\mathbf{H_2L2}$.

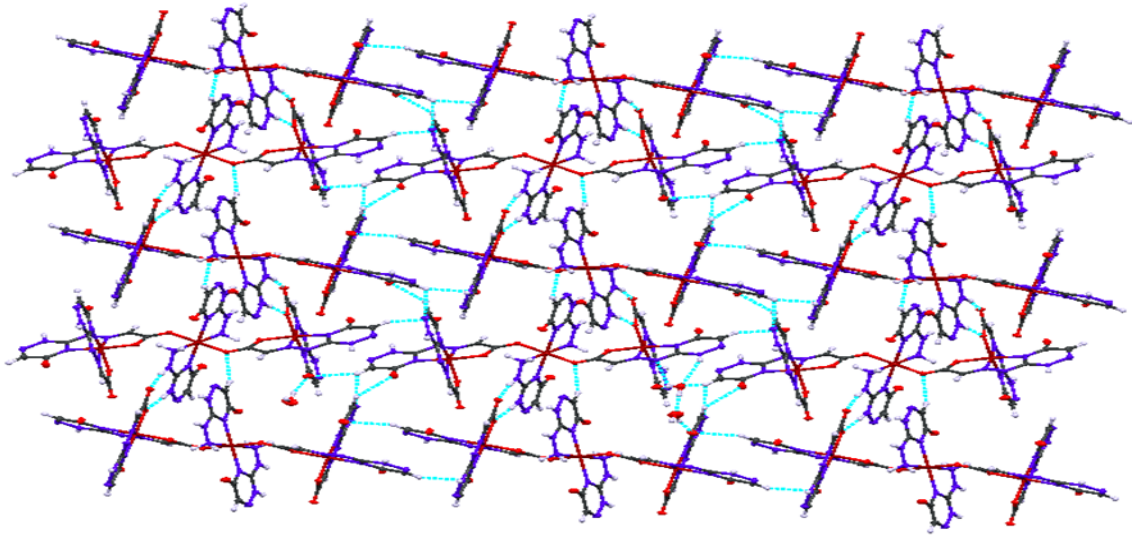

Fig. S6. Hydrogen bonding interactions.

Table S1. Thermal data

Compound	DTA peak temp (^oC)	Thermogravimetry			Decomposition phenomenon
		Temp (^oC)	Mass loss (%)		
			Obsd.	Calcd.	
L1	(+)100; (+)159	80-220	12.00	12.41	Dehydration
	(-)209	220-400	54.00	53.79	Dehydrazination, Decarboxylation
	(+)268 (+)381 (-)531	400-650	100	100	Complete decomposition
H_2L2	(+)122	80-180	17.00	17.52	Dehydrazination
	(+)227; (-)245	180-264	42.10	41.78	Decarboxylation
	(-)263 (+)305; (-)565	264-650	100	100	Complete decomposition
Co	(+)78	80-250	6.0	5.7	Dehydration,
	(-)288; (-)405	250-650	19.20	19.64	decarboxylation Formation of Co_3O_4

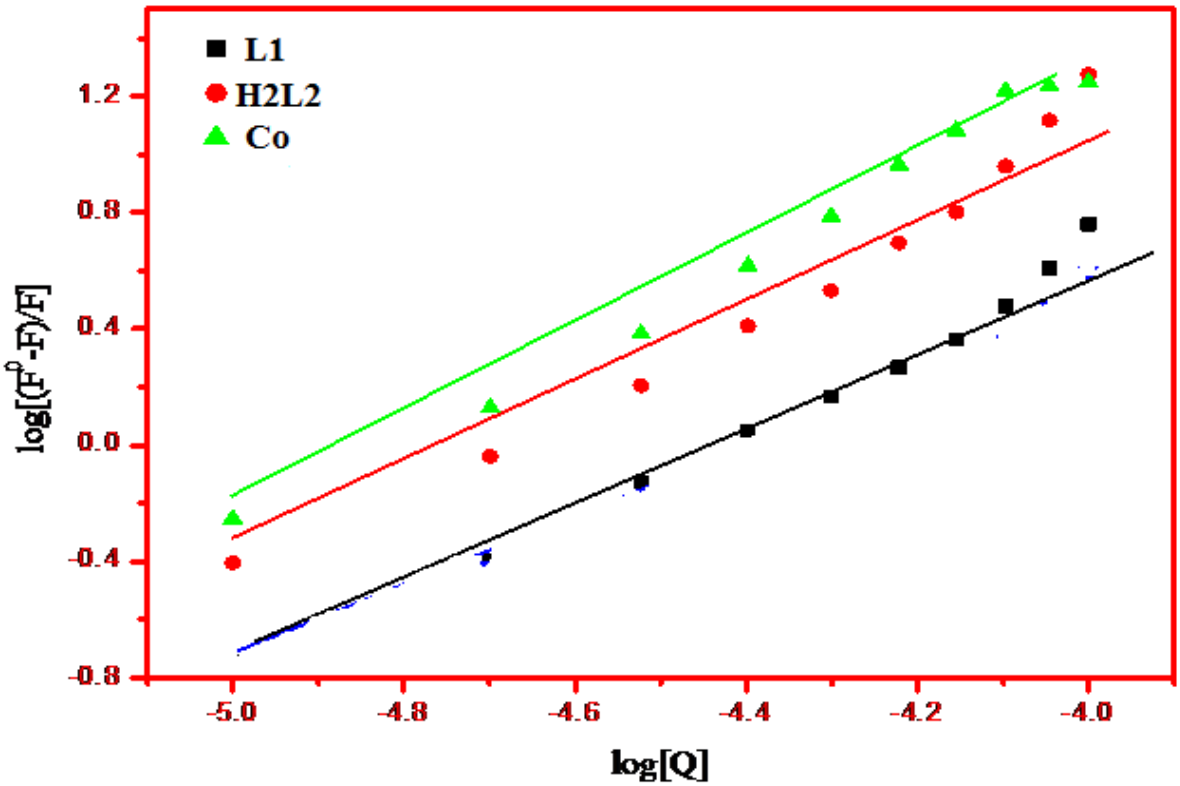

Fig. S7. Plot of log $[(F^0 - F]$ *vs.* log $[Q]$.

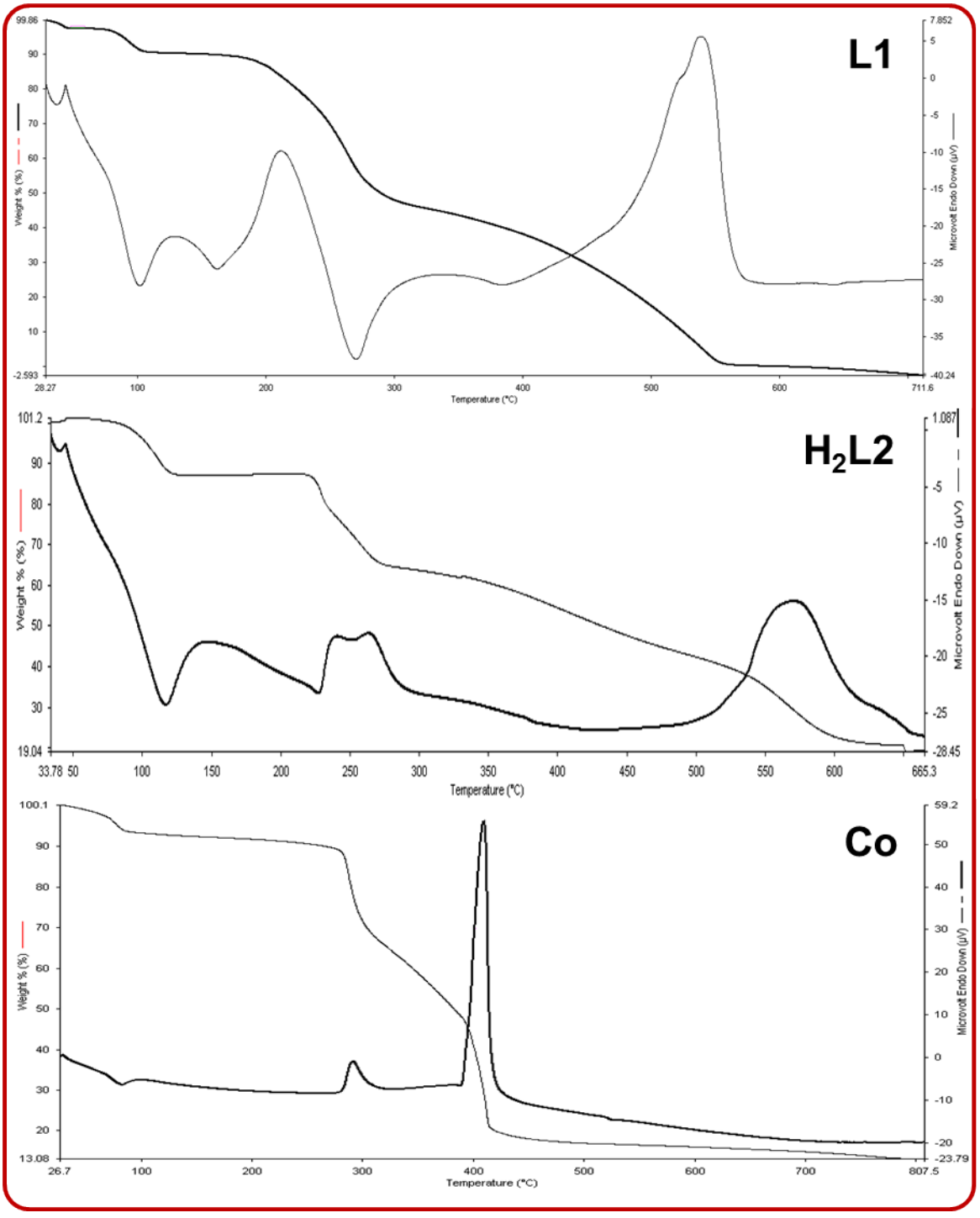

Fig. S8. Simultaneous TG-DTA thermograms of the ligands and complex.

Materials and Methods

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma. Nitroblue tetrazolium, nicotinamide adenine dinucleotide, 5-methyl phenazinium methyl sulfate, and sodium nitroprusside were procured from HiMedia. The stock solution of bovine serum albumin (BSA) was produced by dissolving BSA in a 50-mM phosphate buffer at pH 7.4. All solutions were preserved at 4°C and were used within 2 days. Elemental analyses for C, H, and N data were collected using a Vario-ELIII elemental analyzer. Fourier-transform infrared (FT-IR) spectra of the ligands and metal complex were obtained in KBr pellets in the region of 4,000–400 cm^{-1} using a JASCO-4100 spectrophotometer. Ultraviolet-visible (UV–Vis) absorption spectra in an aqueous medium were produced using a JASCO-N530 UV–Vis spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III spectrometer operating at 500MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts are reported in ppm and tetramethylsilane, which was used as an internal reference. Mass spectra for the ligands were performed on a FINNIGAN MAT 8280 mass spectrometer using an electrospray ionization (ESI) source with a resolution of 48000 at 10% valley in low-resolution mode. The mass pattern of the complex was recorded using a XEVO G2-XS QTOF mass spectrometer. Magnetic characteristics were examined using a vibrating sample magnetometer (Lakshore 7407, USA; system with a 7-inch electromagnet) with a maximum field of 1.5 T at room temperature. X-ray diffraction (XRD) data were obtained using a Bruker APEX-II CCD diffractometer with a graphite monochromated Mo-Kα (λ = 0.71073) radiation source at a temperature of 298.0 K. The structure was determined by dual space methods (SHELXT) using Olex2 and was subsequently refined using the ShelXL refinement package with least squares minimization.[1-3] Simultaneous thermogravimetry and differential thermal analysis

(TG-DTA) was performed using a Perkin–Elmer SII thermal analyzer, with curves obtained in an air atmosphere using platinum cups as holders with 3–5 mg of the samples while maintaining a final temperature at 700°C and a heating rate of 10°C/min.

Human breast adenocarcinoma cancer and normal breast epithelial cells (HBL-100) were procured from the National Center for Cell Sciences, Pune, India. The cells were preserved in a Dulbecco's modified eagles medium supplemented with a 2-mM l-glutamine and balanced salt solution adjusted to contain 1 mM of sodium pyruvate, 0.1 mM of nonessential amino acids, 1.5 g/L of Na_2CO_3, 1.5 g/L of glucose, 2 mM of l-glutamine, 10% fetal bovine serum (GIBCO, USA), and 10 mM of (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES). The concentration of penicillin and streptomycin (100 IU/100µg) was adjusted to 1 mL/L. Cells were preserved at 37°C with 5% CO_2 in a humidified CO_2 incubator.

Evaluation of cytotoxicity

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Here, cancer cells were grown (1×10^4 cells/well) in a 96-well plate for 48 h at a 75% confluence. The medium was replaced with fresh medium containing the serially diluted synthesized compounds and the cells were further incubated for 48 h. Following this, the culture medium was removed and 100 µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (HiMedia) solution was added to each well before being incubated at 37 for 4 h. Following the removal of the supernatant, 50 µL of DMSO was added to each of the wells and subsequently incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm using an ELISA multi-well plate reader (Thermo Multiskan EX,

USA). The optical density (OD) value was used to calculate the percentage of viability using the following formula:

% of viability = OD value of experimental sample/OD value of experimental control ×100

Morphological study

The MCF-7 cells that were grown on cover slips (1×10^5 cells/cover slip) were incubated for 6–24 h with the compounds at the IC_{50} concentration before being fixed in an ethanol:acetic acid solution (3:1; v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Three monolayers per experimental group were photo micrographed. The morphological changes of the MCF-7 selected cells were analyzed using a Nikon (Japan) bright-field inverted light microscope at a 40× magnification.

Fluorescence microscopic analysis of apoptotic cell death

Approximately 1 µL of a dye mixture (100 mg/mL acridine orange [AO] and 100 mg/mL ethidium bromide [EtBr] in distilled water) was mixed with 9 mL of the cell suspension (1×10^5 cells/mL) on clean microscope cover slips. The selected cancer cells were collected, washed with phosphate buffered saline (PBS) (pH 7.2), and stained with 1 mL of AO/EtBr. Following incubation for 2 min, the cells were washed twice with PBS (5 min each) and examined under a fluorescence microscope (Nikon Eclipse, Inc, Japan) at a 400× magnification with an excitation filter at 480 nm. Likewise, the cells were plated on glass coverslips in a 24-well plate and treated with the complex for 24h. The fixed cells were permeabilized with 0.2% triton X-100 (50 µL) for 10min at room temperature and then incubated for 3min with 10µL of DAPI by placing a coverslip over the cells to ensure uniform

spreading of the stain. The cells were observed under a fluorescent microscope(Nikon Eclipse, Inc, Japan).

References

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3 G. M. Sheldrick, ActaCrystallogr. C Struct. Chem., 2015, 71, 3.