Electronic Supplementary Information

Facile Template Synthesis of Water-Soluble Triazine-Based Schiff Base Ligand Bridged-Coordination Polymers of Co(II), Ni(II), and Cu(II): Structure, Biomolecular Interactions, and Cytotoxic Activity

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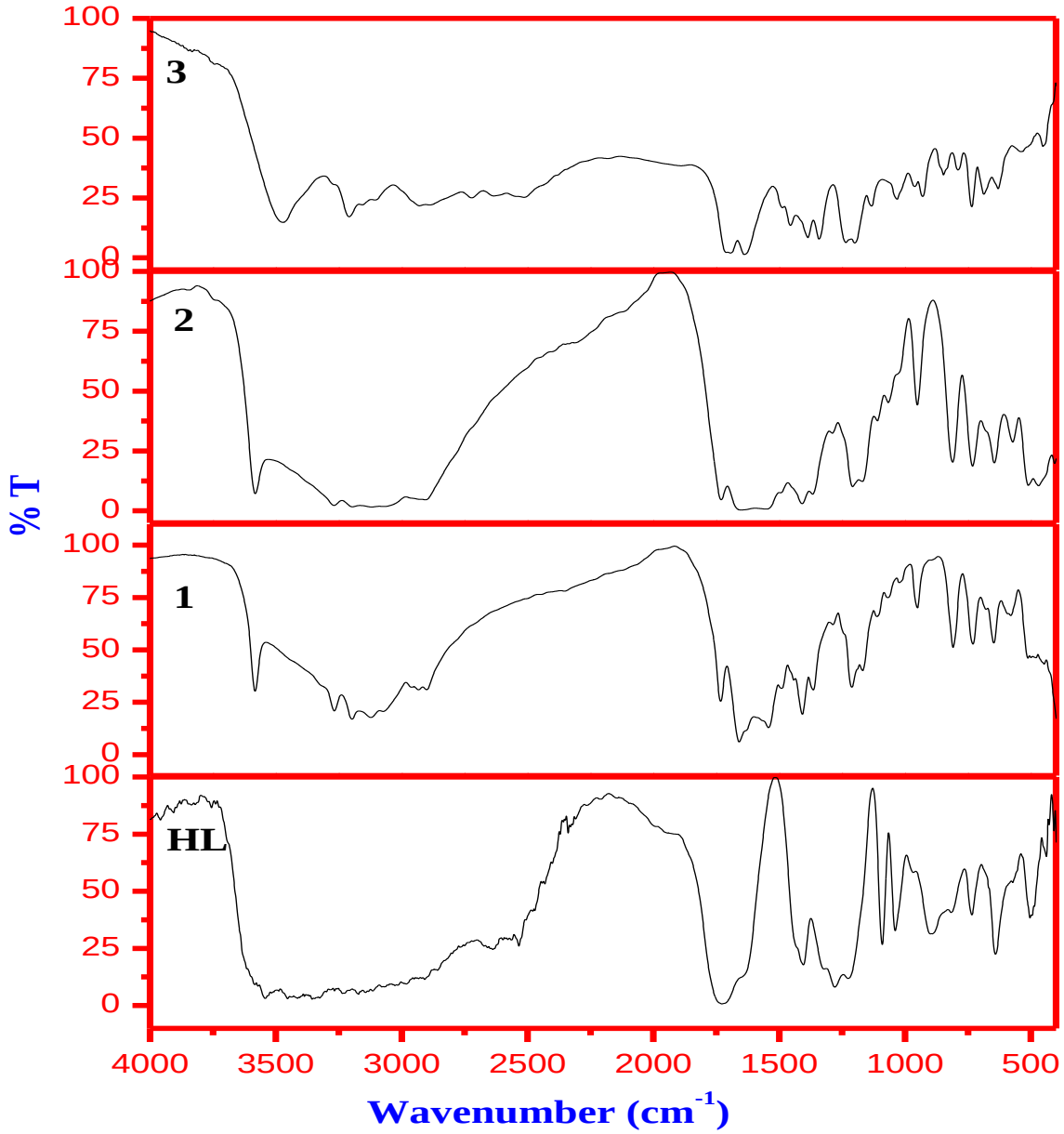

Fig. S1. FT-IR spectra of **HL** and complexes **1-3**.

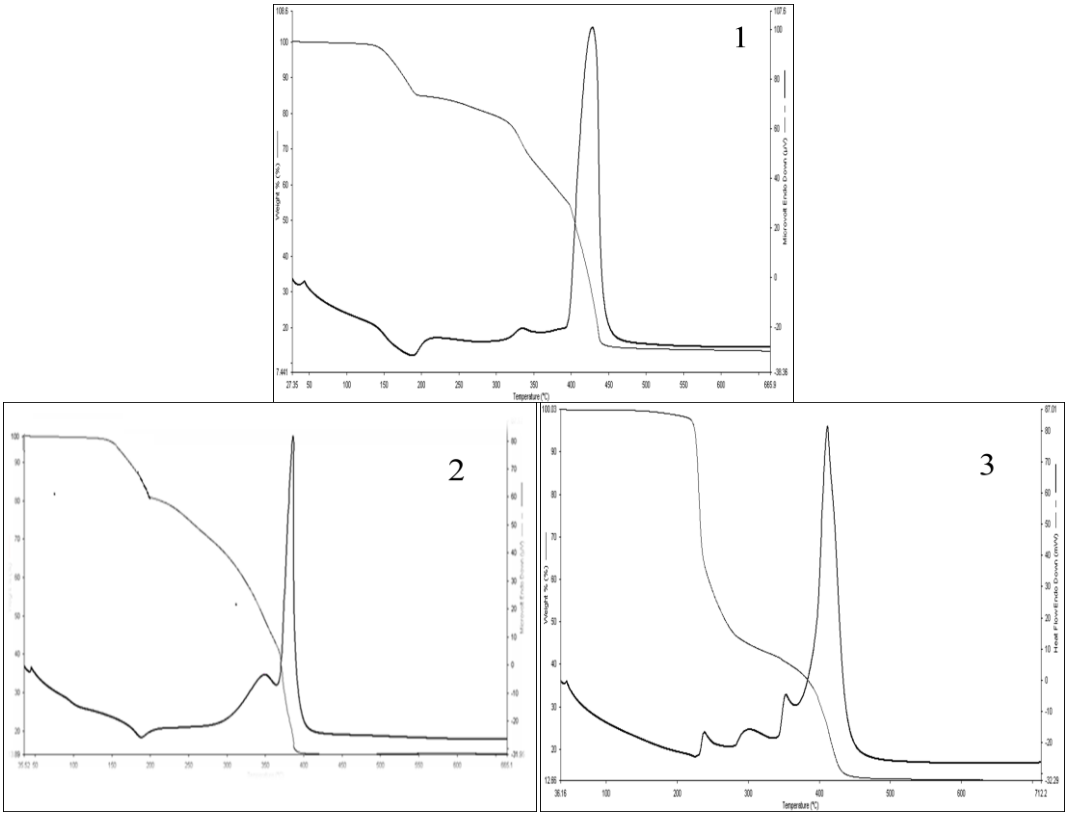

Fig. S2. Simultaneous TG-DTA of complexes **1-3.**

Table S1. Thermal data of complexes **1-3**

Compounds	DTA peak temp (^{o}C)	Thermogravimetry			Intermediates/ End products
		Temp.range (^{o}C)	Mass loss %		
			Obsd.	Calcd.	
1	(+)190	120-230	14.2	13.6	Dehydration
	(-)336, (-)423	230-600	85.00	84.89	Formation of Co_3O_4
2	(+)186	120-230	20.00	19.65	Dehydration, deamination
	(-)350, (-)390	230-500	86.11	86.02	Formation of NiO
3	(-)236, (-)300	200-300	62.00	62.50	Formation of copper acetate complex
	(-)354, (-)418	300-600	83.5	82.8	Formation of CuO

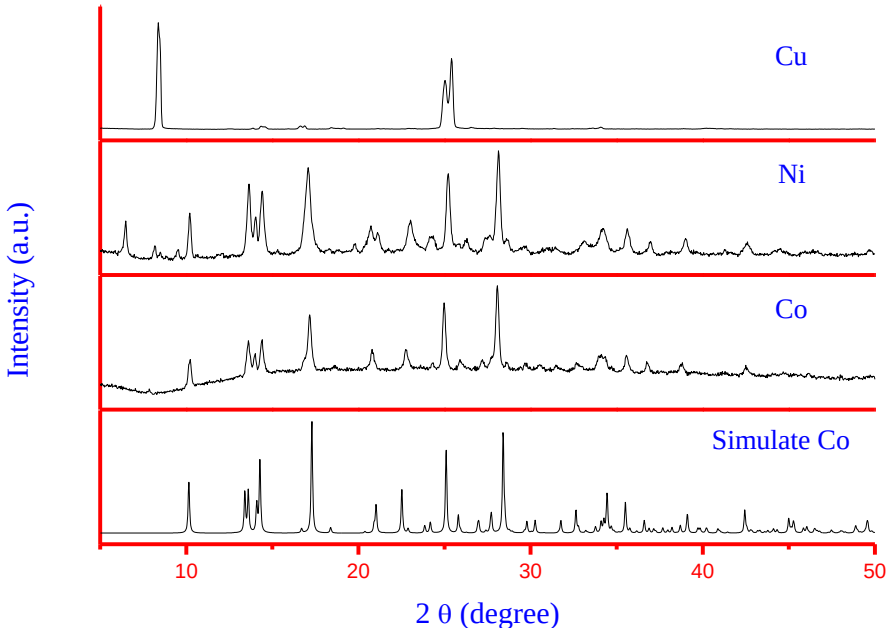

Fig. S3. PXRD patterns of the synthesized Co(II), Ni(II), and Cu(II) complexes and PXRD pattern simulated from the single-crystal crystallography data of **1**.

Table S2. Bond lengths (Å) and bond angles (º) of **1.**

1						
Bond length/Å				Bond angle/º		
Co1	$N4^{1}$	2.2117(16)	$N4^{1}$	Co1	$N4^{2}$	180.0
Co1	$N4^{2}$	2.2117(16)	$O8^{3}$	Co1	$N4^{2}$	96.67(5)
Co1	O8	2.0750(13)	O8	Co1	$N4^{2}$	83.33(6)
Co1	$O8^{3}$	2.0749(13)	$O8^{3}$	Co1	$N4^{1}$	83.33(6)
Co1	O10	2.0607(14)	O8	Co1	$N4^{1}$	96.67(5)
Co1	$O10^{3}$	2.0607(14)	$O8^{3}$	Co1	O8	180.0
C7	O8	1.280(2)	$O10^{3}$	Co1	$N4^{2}$	88.42(6)
C7	O9	1.251(2)	$O10^{3}$	Co1	$N4^{1}$	91.58(6)
N1	C4	1.288(2)	O10	Co1	$O8^{3}$	91.06(5)
N3	C3	1.376(2)	O10	Co1	$O10^{3}$	180.00(9)

Table S3. Hydrogen bonding data for **1.**

1				
D-H...A	d(D-H)/Å	d(A-H)/ Å	d(D-A)/ Å	D-H-A/º
N4-H4A...$O2^{i}$	0.910	2.508	3.165	129.46
N4-H4B...$O3^{ii}$	0.910	2.119	2.971	155.36
O11-H11B...$O2^{iii}$	0.870	2.380	3.044	142.39
N2-H2...$O9^{iv}$	0.880	1.890	2.702	152.74
O10-H10B...$O11^{v}$	0.874	1.907	2.656	143.17
O11-H11B...$O3^{vi}$	0.870	2.492	3.053	122.88
O11-H11A...$O8^{vi}$	0.870	1.886	2.741	167.18

i. 2-x, 1-y, -z iv. 1+x, y,zvi. 2-x, 2-y, 2-z
ii. 1-x, 1-y, -z v. 1-x,2-y,2-z
iii. 2-x,1-y,1-z vi. 1-x,1-y,1-z

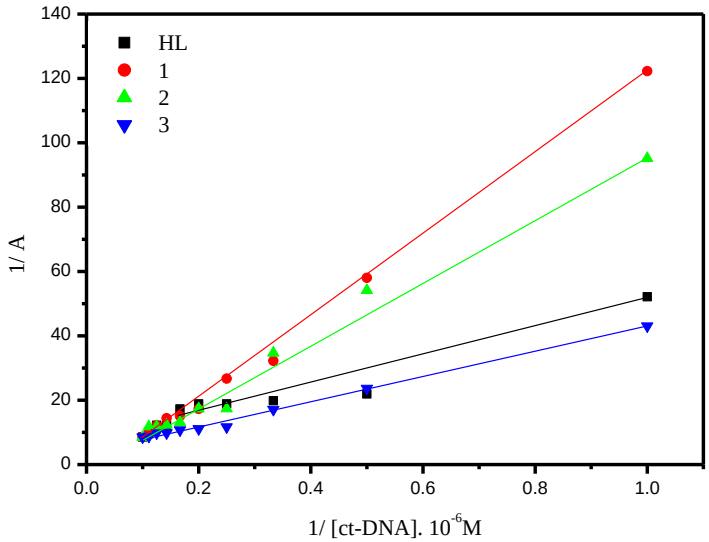

Fig. S4. Benesi-Hildebrand plots of absorption titrations of **HL**, **1**, **2**, and **3** with CT-DNA.

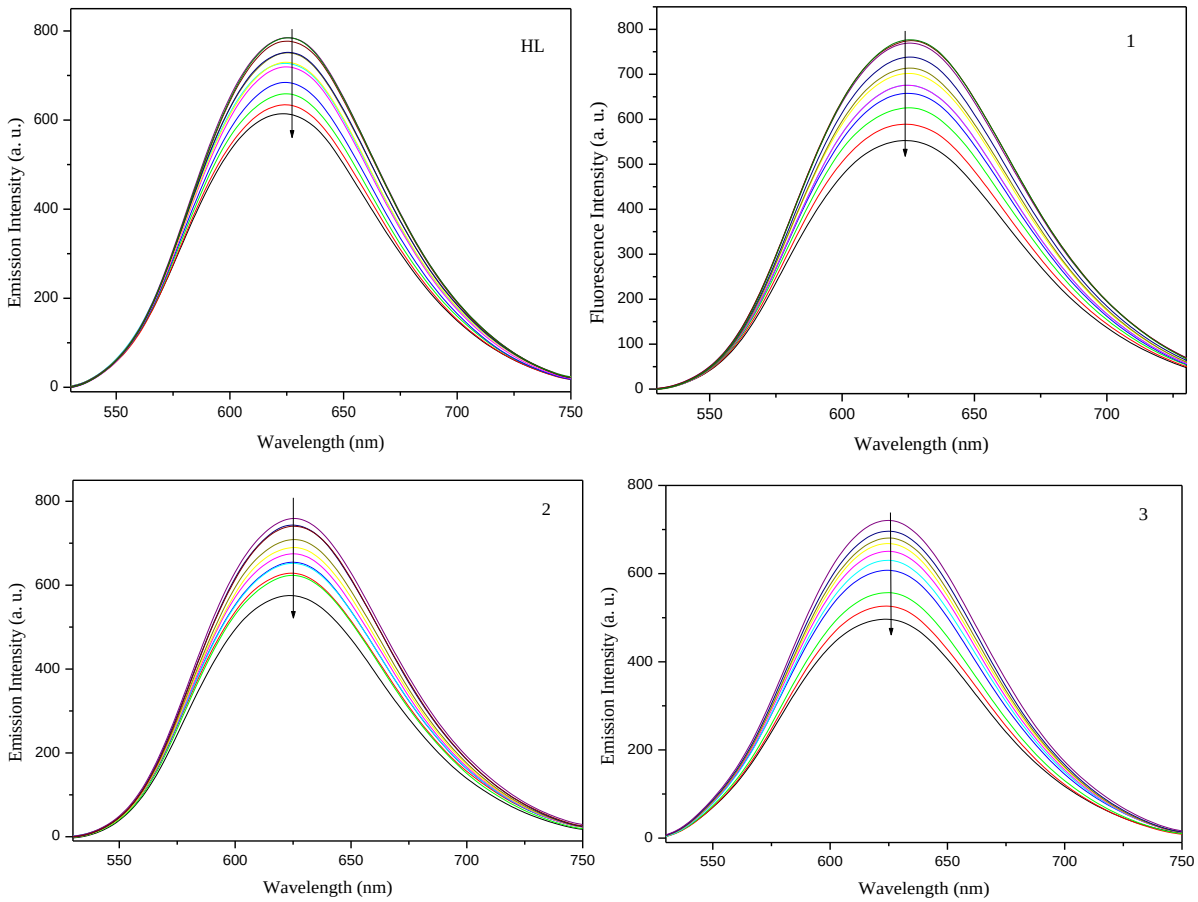

Fig. S5. Fluorescence titrations of **HL**, **1**, **2**, and **3** (0-40 μM) with EB (2 μM)-bound CT-DNA (4 μM).

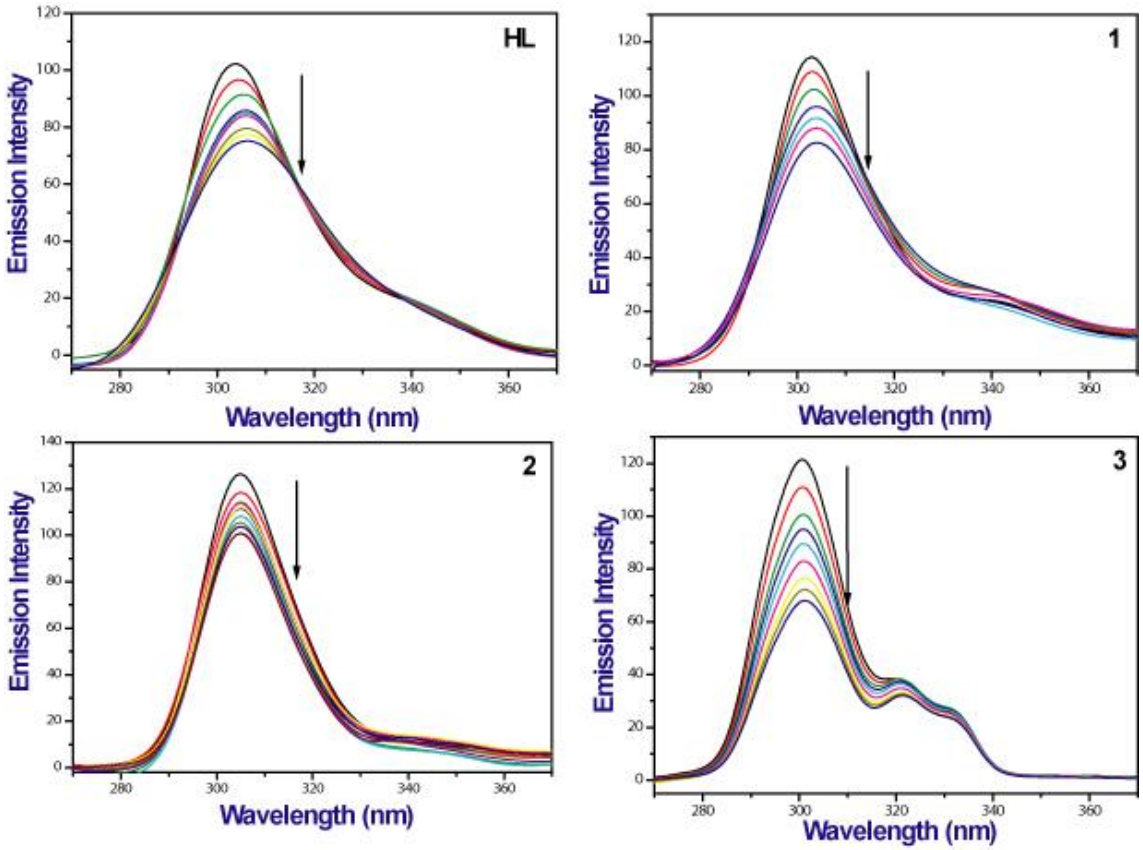

Fig. S6. Synchronous fluorescence spectra of **HL**, **1**, **2**, and **3** at $\Delta\lambda$ = 15 nm.

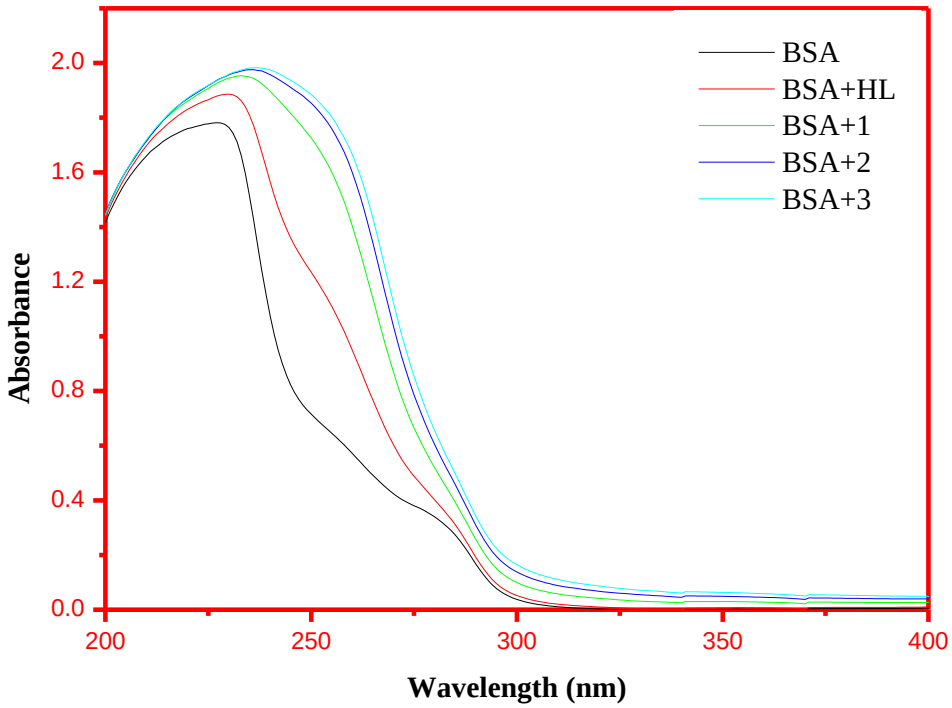

Fig.S7. Absorption spectra of BSA (10 μM) in the absence and presence of **1-3** (10 μM).

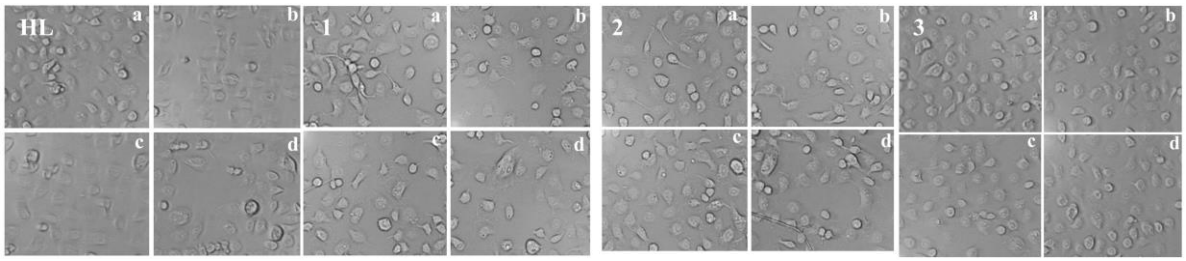

Fig. S8. Microscopic images of MCF-7 cancer cells treated with compounds at 0 (a), 10 (b), 20 (c), or 40 μM (d).

Table S4. Primers used

S. No	Genes	bp size	T[a]	Primer sequence 5'-3'
	BAD	123	61.6	F.5'-GGTTCTGAGGGGAGACTGAGG
1				R.5'-CTTCCTCTCCCACCGTAGCG
2	Bcl2	152	60.25	F.5'-CTTTGAGTTCGGTGGGGTCA
				R.5'-CACCAAGTGCACCTACCCAG
3	Cytochrome c	121	59.7	F.5'-CCCAAGAAGTACATCCCTGGAA
				R.5'-AGGCAGTGGCCAATTATTACTCA
4	Casp-3	102	60.2	F.5'- CAGTGGAGGCCGACTTCTTG
				R. 5'- TGGCACAAAGCGACTGGAT
5	Bax	149	59.6	F.5'- GGACGAACTGGACAGTAACATGG
				R.5'- CAAAGTAGAAAAGGGCGACAAC
				F:5'- CCCCTCCATCAACTTCTTCA-3'
9	PI3K	155	57.4	R:5'-CGGTTGCCTACTGGTTCAAT-3'
				F:5'-GGGTTTCTCCCAGGAGGTTT-3'
10	AKT	179	59.1	R:5'-GTCCATGGTGTTCCTACCCA-3'
				F:5'-TCCCAGACATGACAGCCATC-3'
12	PTEN	187	58.8	R:5'-TGTCTTTCAGCACAACTTACTACA-3'
				F:5'- GTCATTCCAAATATGAGATGCGT-3'
14	β-Actin	124	57.2	R:5'- AATGCTATCACCTCCCCTGT-3'

***Materials and instrumentation*:**

All the chemicals and reagents were of analytical reagent grade and used without purification. Calf thymus-DNA, ethidium bromide (EB), tris(hydroxymethyl)aminomethane hydrochloride, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma. Stock solution of CT-DNA was prepared in 5 mM Tris HCl /50 mM NaCl (pH 7.2), and stock solutions of BSA were made with 50 mM phosphate buffer (pH 7.4). All stock solutions were stored at 5 ˚C and used within 2 days. Metal analyses were carried out using complexometric titration with a standard 0.01 M EDTA solution. Normal breast epithelial and human breast cancer (MCF-7) cells were purchased from the National Centre for Cell Sciences (NCCS), Pune, India. FT-IR spectra were recorded on a JASCO-4100 spectrophotometer with KBr pellets in the range of 4000-400 cm^{-1}. Elemental analysis for C, H, and N was performed on a Vario-EL III elemental analyzer. $^{1}H/^{13}C$ NMR spectra were recorded on a Bruker Avance III 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal reference. UV-Vis spectra were measured on a Shimadzu UV-2600 spectrophotometer operating in the range of 200–900 nm. The luminescence spectra were analysed with a PerkinElmer LS-55 spectrophotometer with whole excitation and emission slit widths of 10 and 5 nm, respectively. Simultaneous TG-DTA analyses were performed on a PerkinElmer SII thermal analyser using platinum cups to hold 5-10 mg of the samples, which were heated at a rate of 10 ˚C/min in air. The PXRD patterns were recorded using a Bruker D2 PHASER powder diffraction instrument with Kα radiation of a Cu target as the light source (λ = 0.154056 nm) and a Si plate as the sample holder.

Antioxidant assays: The total antioxidant ability of the compounds was determined using the ABTS radical cation decolorization assay.[1] The DPPH free radical scavenging capacity was determined using the approach described by Shih-Chuan Liu et al.[2] Nitric radical scavenging ability was measured according to the method of Olabinri et al.[3] The superoxide radical scavenging capacity was measured using the nitroblue tetrazolium reduction technique described by Wei Fu et al.[4] All measurements were performed in triplicate at each concentration to generate consistent data with statistical errors. The % inhibition was determined using the following equation:

$$\%\ \text{scavenging capacity} = \frac{A_0 - A_c}{A_0} \times 100$$

Where$A_c$and A_0 are absorbance in the presence and absence of the tested compounds, respectively. The IC_{50} (50% activity) was determined using percentage activity.

DNA binding studies: The interactions of the ligand and complexes 1-3 with CT-DNA were analysed using UV-Vis spectroscopy to examine the possible modes of binding and to calculate the binding constants. The concentration of DNA was measured using UV absorbance at 260 nm with 6600 $M^{-1}cm^{-1}$ as the molar absorption coefficient. The ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}) was approximately 1.8, indicating that the DNA solution contained an adequately small amount of protein.[5] Absorption titrations were conducted at a constant complex concentration (10 μM) and changing the CT-DNA concentration (0-40 μM). Titration was continued until the saturation point. Fluorescence binding studies were performed in a similar way by varying CT-DNA concentration. Competitive binding studies were performed at fixed EB (2 μM) and CT-DNA (4 μM) concentrations and increasing concentration of complexes (0-40 μM) and then measuring the fluorescence spectra at an excitation wavelength at 520 nm.

BSA binding studies: The UV absorption spectra of BSA (5 μM) were measured in phosphate buffer at pH 7.4 in the presence of varying concentration of complexes (0-40 μM). Fluorescence quenching studies were performed in phosphate buffer at pH 7.4. A 2.5-mL portion of BSA (5 μM) was titrated with varying complex concentration (0-40 μM). Each complex solution was shaken and then allowed to stand for 10 min, and the fluorescence spectra were recorded at an excitation wavelength at 280 nm. Synchronous fluorescence spectra of the solutions in the range of 270-400 nm were recorded at $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm for the Tyr and Trp residues, respectively

MTT assay: The IC_{50} value was assessed using MTT assay. Cancer cells (1×10^4 cells/well) were grown in a 96-well plate for 48 h until 75% confluence, and selected MCF-7 cells were treated with varying concentrations of HL and compounds 1-3 dissolved in dimethyl sulfoxide (DMSO). The culture medium was removed, and 100 μL of MTT solution was added to each well and incubated at 37 ^{o}C for 4 h. After removal of the supernatant, 50 μL of DMSO was added to each of the wells and incubated for 10 min to solubilize the

formazan crystals. The optical density (OD) was determined at 620 nm in an ELISA multiwell plate reader (ThermoMultiskan EX, USA). The OD value was used to determine the percentage of viability using the following formula:

$$\%\ \text{viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

Cell morphological analysis: MCF-7 human cancer cells were grown on cover slips to 1×10^5 cells/cover slip, incubated for 6-24 h with compounds at the IC_{50} concentration, and fixed in acetic acid:ethanol solution (1:3 v:v). The cover slips were then carefully mounted on glass slides for morphometric analysis. Three monolayers per experimental group were photomicrographed using a Nikon brightfield inverted light microscope at 40x magnification. Morphological analysis using AO and EB staining was performed by adding 1 µL of dye mixture (100 mg/mL each of EB and AO in distilled water) to 9 mL of cell suspension on a clean microscope cover slip. The selected MCF-7 cells were isolated, washed with phosphate buffered saline (pH 7.2), and stained with 1 mL of AO/EB. After incubation for 2 min, the cells were washed twice with phosphate buffered saline (5 min each) and visualized using a fluorescence microscope at 400x magnification with an excitation filter at 480 nm. Similarly, the cells were grown on glass coverslips in a 24-well plate and treated with complex for 24 h. The fixed cells were permeabilized with 0.2% Triton X-100 (50 µL) for 10 min at room temperature and incubated for 3 min with 10 µL of DAPI by placing a coverslip over the cells to allow uniform spreading of the stain.

References

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