Electronic Supplementary Information (ESI†)
for

Visible light-induced cytotoxicity studies on Co(II) complexes having an anthracene-based curcuminoid ligand

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light (hollow symbols, 400-700 nm, 10 J cm^{-2}).

Fig. S20: Uptake of the complexes **1** and **2** (10 μM) at 37 °C for 2 and 4 h incubation time in HeLa cells determined by flow cytometry. The shift in the band position for different incubation time indicates time dependent uptake of the complexes **1** and **2** in HeLa cells.

Fig. S21: Flow cytometric profiles of Annexin V-FITC-PI staining of HeLa cells undergoing apoptosis induced by complex **2** (10 μM) in dark and visible light (400-700 nm, 10 J cm^{-2}).

Fig. S22: Control flow cytometric profiles of Annexin V-FITC-PI staining of HeLa cells without addition of any cobalt complex.

Cell Viability Assay. HeLa (human cervical carcinoma), MCF-7 (human breast adenocarcinoma), MDA-MB-231 (metastatic mammary adenocarcinoma) and HPL1D (immortalized peripheral lung epithelial cells) cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 IU/mL of penicillin, 100 μg/mL of streptomycin and 2 mM Glutamax at 37 °C in a humidified 5% CO_2 incubator. The adherent cultures were grown as monolayer and were passaged once in 4-5 days by trypsinizing with 0.25% Trypsin-EDTA. The photocytotoxicity of the complexes was assessed using MTT assay based on the ability of mitochondrial dehydrogenases in the viable cells to cleave the tetrazolium rings of MTT and forming dark blue membrane impermeable crystals of formazan that were measured at 540 nm giving an estimate of the number of viable cells. Approximately, 8000 cells of HeLa, MCF-7, MDA-MB-231 and HPL1D were plated in a 96-well culture plate in DMEM supplemented with 10% fetal bovine serum and cultured overnight. Different concentrations of the complexes were added to the cells, and incubation was continued for 4 h in dark. After incubation, the medium was replaced with 50 mM phosphate buffer, pH 7.4, containing 150 mM NaCl (PBS) and photo-irradiation was done for 1 h in visible light of 400-700 nm using Luzchem Photoreactor (Model LZC-1, Ontario, Canada; light fluence rate: 2.4 mW cm^{-2}; light dose = 10 J cm^{-2}). PBS was replaced with 10% DMEM after irradiation. Incubation was continued for a further period of 19 h in dark for light exposed cells and for 20 h in dark for light unexposed cells followed by addition of 25 μL of 4 mg mL^{-1} of MTT to each well and incubated for an additional 4 h. The culture

medium was discarded, and a 100 μL volume of DMSO was added to dissolve the formazan crystals.[S1] The absorbance at 570 nm was determined using an ELISA microplate reader (BioRad, Hercules, CA, USA). The cytotoxicity of the test compounds was measured as the percentage ratio of the absorbance of the treated cells over the untreated controls. The IC_{50} values were determined by nonlinear regression analysis (GraphPad Prism, version 7).

Flow Cytometry for cellular uptake. Uptake of the fluorescent Co(II) complexes **1** and **2** in HeLa cells were evaluated by flow cytometric method.[S2,S3] HeLa cells (3.0×10^5), maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 IU/mL of penicillin, 100 μg/mL of streptomycin and 2 mM Glutamax at 37 °C in a humidified 5% CO_2 incubator, were incubated with the complexes **1** and **2** (10 μM) for 2 and 4 h respectively. The cells were harvested by trypsinization and a single cell suspension in PBS was made. The distribution of fluorescent complexes HeLa cells were determined by flow cytometry in the FL-1 channel and compared with auto-fluorescence of untreated control cells.

ICP-MS for cellular uptake. A dose-dependent uptake of the cobalt complexes **2** and **4** in terms of cobalt content in HeLa cells was studied by ICP-MS method.[S4] Approximately 10^6 HeLa cells were seeded in 60 mm culture dishes. The cells were treated with different concentrations (5 and 10 μM) of the complexes **2** and **4** for 4 h. The medium was aspired and the cells in both the plates were washed with PBS. The cells in one of the plates were treated with 0.5 mL hot conc. HNO_3 (~90 °C) for 2 h, diluted to 10 mL (2% HNO_3) and were analyzed by ICP-MS to estimate the amount of cobalt internalized. Cobalt content obtained in μg/L unit were then expressed as ng/10^6 cells. The cells in the other plate were trypsinized and live cells were counted by trypan blue method. The untreated control cells in the wells with only medium were taken as controls. The amount of internalized cobalt into HeLa cells were calculated by subtracting the average amount of cobalt found in the control cells from the average amount of cobalt found in the cells treated with the cobalt complexes **2** and **4** in dose dependent manner and normalizing to the average number of cells per well.

DCFDA Assay for detection of ROS. The DCFDA assay was used to detect the generation of cellular reactive oxygen species (ROS).[S5] For flow cytometric analysis for detection of ROS generation, 3.0 x 10^5 HeLa cells were incubated with the complex **2** (10 μM) for 4 h followed by photo-irradiation (400-700 nm) for 1 h in PBS. The cells were harvested by trypsinization and a single cell suspension in PBS was made. The cells were then treated with 10 μM DCFDA solution in dark for 5 min at room temperature. The distribution of DCFDA stained HeLa cells was determined by flow cytometry in the FL-1 channel.

Nuclear staining experiment (DAPI staining). 4',6-Diamidino-2-phenylindole (DAPI) nuclear staining assay was employed to monitor changes in chromatin organization, if any, after visible light irradiation in complex pre-treated HeLa cells.[S6] Approximately 2.0 x 10^4 cells were cultured on a cover slip placed in 12-well plates. The cells were treated with the complexes **1** and **2** (10 μM) for 4 h in the dark, followed by irradiation for 1 h with visible light (400-700 nm, 10 J cm^{-2}). The corresponding dark controls experiments were carried out. The cells were allowed to recover for 2 h in fresh media, fixed with 3.7% (v/v) paraformaldehyde in PBS for 10 min at room temperature followed by two washes with DPBS. The cells were permeabilized for 10 minutes using 50 μL of DPBS containing 0.1% Triton X-100, stained with DAPI (10 μM) for 15 min followed by two washed with DPBS and finally observed microscopically using confocal laser scanning microscope with 360/40 nm excitation and 460/50 nm emission filters (Zeiss LSM 510 apochromat).

Nuclear staining experiment (AO/EB dual staining). Insight into the mechanistic aspects of cell death in HeLa cells induced by Co(II) complexes under visible light irradiation was ascertained by the acridine orange/ethidium bromide (AO/EB) dual nuclear staining assay microscopically.[S7] Approximately 2 x 10^4 cells were cultured on a cover slip placed in 12-well plates. The cells were treated with the complexes **1** and **2** (10 μM) for 4 h in the dark, followed by irradiation with visible light (400-700 nm, 10 Jcm^{-2}). The corresponding dark controls experiments were carried out. The cells were allowed to recover for 20 h, washed three times with DPBS, stained with an AO/EB mixture (1 : 1, 10

μM) for 15 min, and observed microscopically using confocal laser scanning microscope (Zeiss LSM 510 apochromat).

Annexin-V/FITC/PI Assay. Annexin-V/FITC/PI assay was carried out for complex **2** (10 μM) in 1% DMSO/ DMEM.[S8] Approximately 3.0 x10^5 HeLa cells were seeded in six-well plates and cultured for 24 h. The cells were incubated with the complex for 4 h in the dark and then exposed to light (1 h, λ = 400-700 nm, light dose = 10 Jcm^{-2}) in phenol red free media or kept in dark. Cells were then kept for another 19 h and 20 h respectively for the light exposed and unexposed plates in DMEM/10% fetal bovine serum (FBS) buffer in the dark, after which the medium was discarded, and the cells were trypsinized and re-suspended in 140 μL Annexin V binding buffer (100 mM HEPES/NaOH, pH 7.4 containing 140 mM NaCl and 2.5 mM $CaCl_2$). Annexin-V/FITC (0.5 mL) and propidium iodide (PI; 1 mL) were added to the cell suspensions and incubated for 5 min. Readings were taken with the FACS instrument.

References:

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Table S1. Selected Crystallographic Data for the complex $[Co(dbm)(phen)_2](ClO_4)$ (**3a**)

Empirical formula	C39 H27 Cl Co N4 O6
Fw, g M^{-1}	742.02
Crystal system	monoclinic
Space group	$P2_1/n$
a, Å	15.1317(10)
b, Å	16.2342(12)
c, Å	16.7244(11)
α, °	90
β, °	112.679(2)
γ,°	90
V, Å^3	3790.7(5)
Z	4
T, K	296(2)
ρ_{calcd}, g cm^{-3}	1.300
λ, Å (Mo-Kα)	0.71073
μ, cm^{-1}	0.573
Data / restraints / parameters	7431 / 0 / 460
F(000)	1524
Goodness-of-fit	1.107
R (F_o)[a], I>2σ(I) [Rw (F_o)[b]]	0.0536 [0.1638]
R (all data) [Rw (all data)]	0.0643 [0.1716]
Largest diff. peak and hole (e Å^{-3})	0.540, -0.574

[a]$R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, [b]$Rw = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o)^2]\}^{½}$; $w = [\sigma^2(F_o)^2 + (AP)^2 + BP]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$, A = 0.0864; B = 3.3918

Table S2. Selected bond distances (Å) and bond angles (º) of $[Co(dbm)(phen)_2](ClO_4)$ (**3a**)

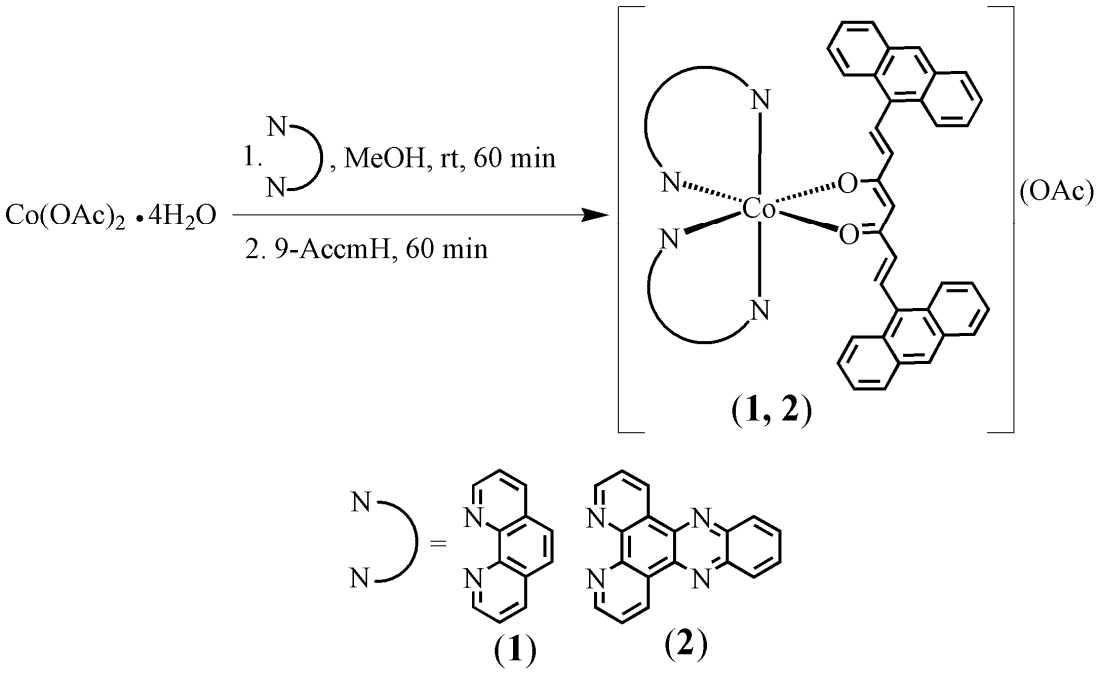
Co1-O1	2.022(2)	N3-Co1-N2	170.07(10)
Co1-O2	2.028(2)	O1-Co1-N4	92.32(9)
Co1-N1	2.164(3)	O2-Co1-N4	172.32(9)
Co1-N2	2.142(3)	N3-Co1-N4	77.69(10)
Co1-N3	2.123(2)	N2-Co1-N4	97.11(10)
Co1-N4	2.155(3)	O1-Co1-N1	173.72(9)
O1-Co1-N3	92.32(10)	O2-Co1-N1	93.10(10)
O1-Co1-N3	92.32(10)	N3-Co1-N1	93.50(10)
O2-Co1-N3	94.68(10)	N2-Co1-N1	77.64(10)
O1-Co1-N2	96.37(10)	N4-Co1-N1	86.61(10)
O2-Co1-N2	90.31(10)		

Table S3. DNA and human serum albumin (HSA) binding constants for the complexes [Co(9-accm)$(L)_2$](OAc) (L = phen in **1** and dppz in **2**) and [Co(dbm)$(L)_2$](OAc) (L = phen in **3** and dppz in **4**)

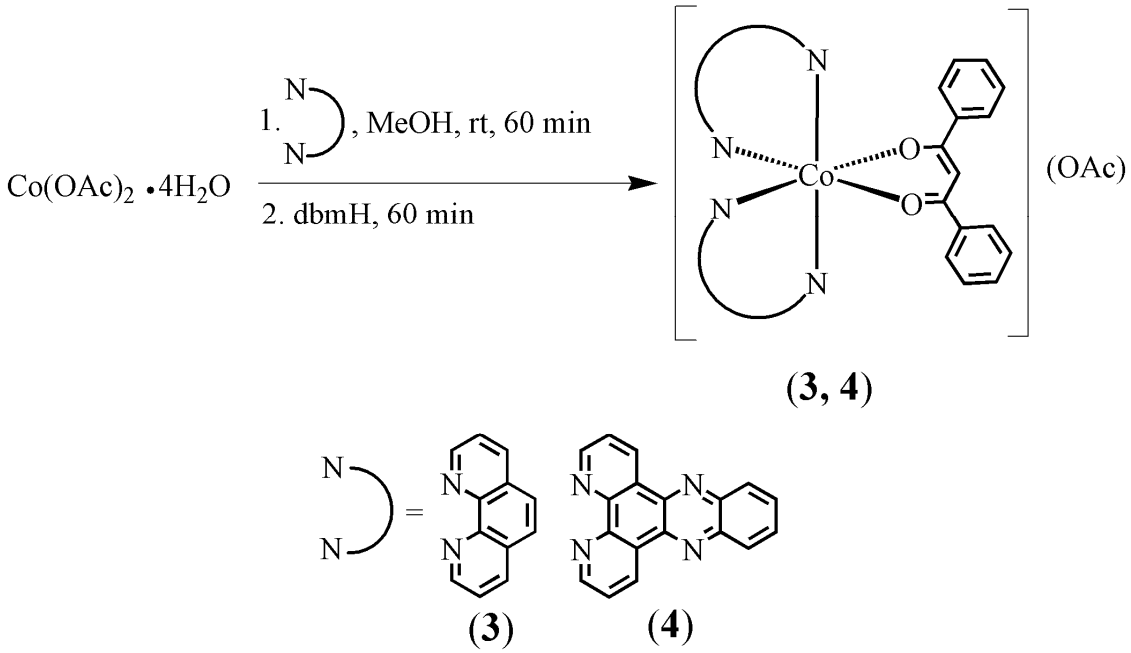
Complex No	Binding constant (M^{-1})	
	ct-DNA	HSA
1	2.42 (±0.10) x 10^5	7.70 (±0.12) x 10^4
2	1.51 (±0.78) x 10^6	2.80 (±0.04) x 10^5
3	3.05 (±0.13) x 10^5	6.97 (±0.08) x 10^4
4	3.24 (±0.13) x 10^6	2.40 (±0.03) x 10^5

Table S4: The content of cobalt (ng per 10^6 cells) in HeLa cells after incubation with different concentrations (5 and 10 μM) of the complexes **2** and **4** for 4 h.

[Complex]	Cobalt (ng per 10^6 cells)	
	Complex **2**	Complex **4**
5 μM	45.0 ± 0.6	12.0 ± 0.1
10 μM	113.0 ± 1.3	29.0 ± 0.3



Scheme S1 Synthetic scheme for the complexes **1** and **2**.



Scheme S2 Synthetic scheme for the complexes **3** and **4**.

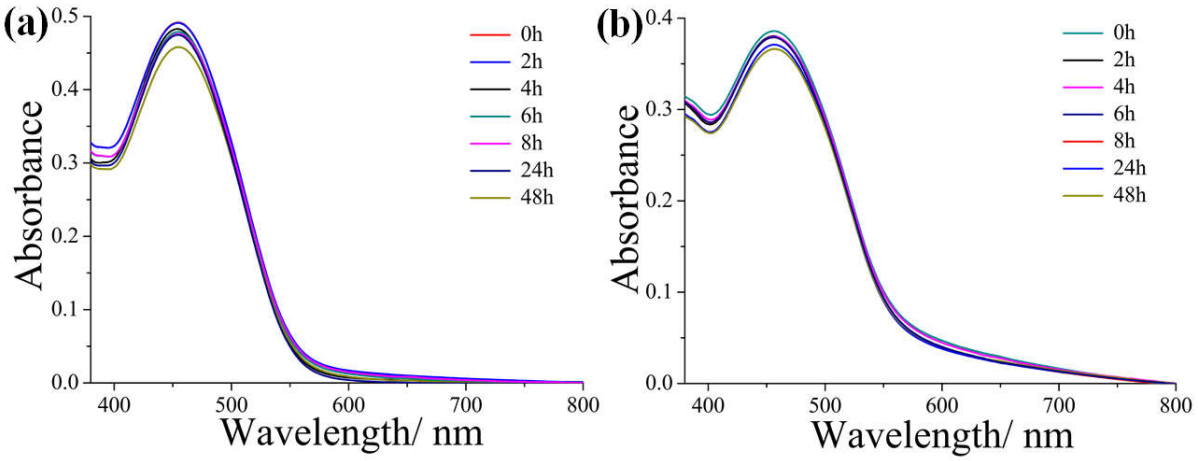


Fig. S1 Absorption spectral traces of the complexes **1** (a) and **2** (b) in DMSO-Tris-HCl buffer (pH 7.2) (1:9 v/v) at different time points showing the stability of the complexes.

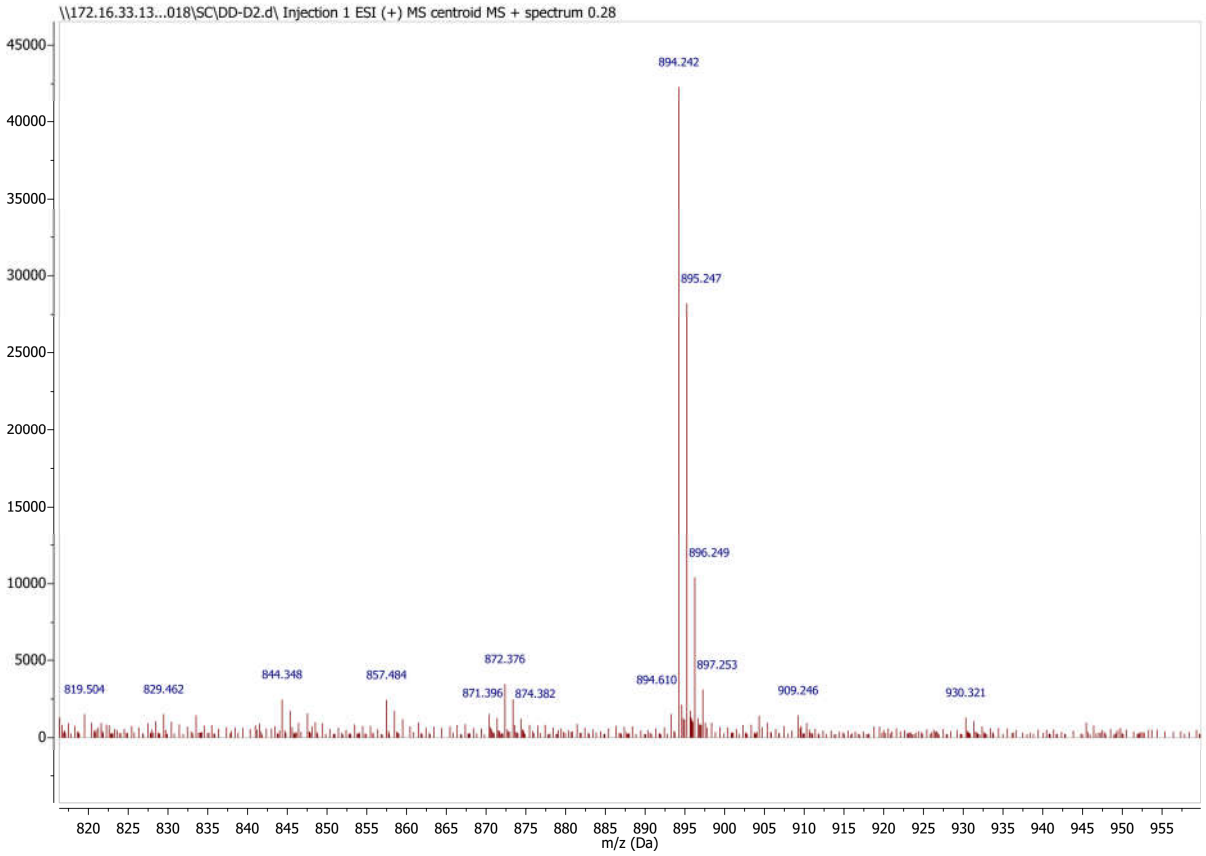


Fig. S2 Mass spectrum of complex **1** in methanol showing the $[M-(OAc)]^+$ peak at m/z = 894.242

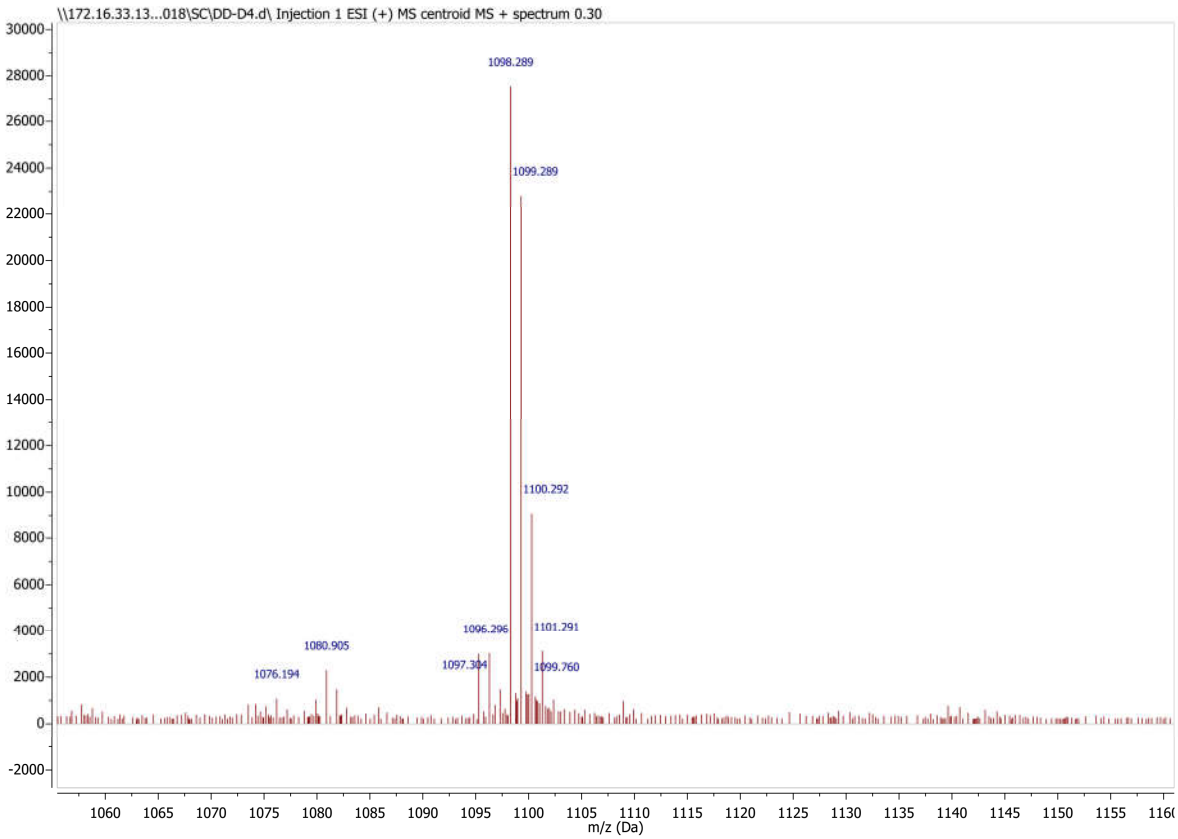


Fig. S3 Mass spectrum of complex **2** in methanol showing the $[M-(OAc)]^+$ peak at m/z = 1098.289

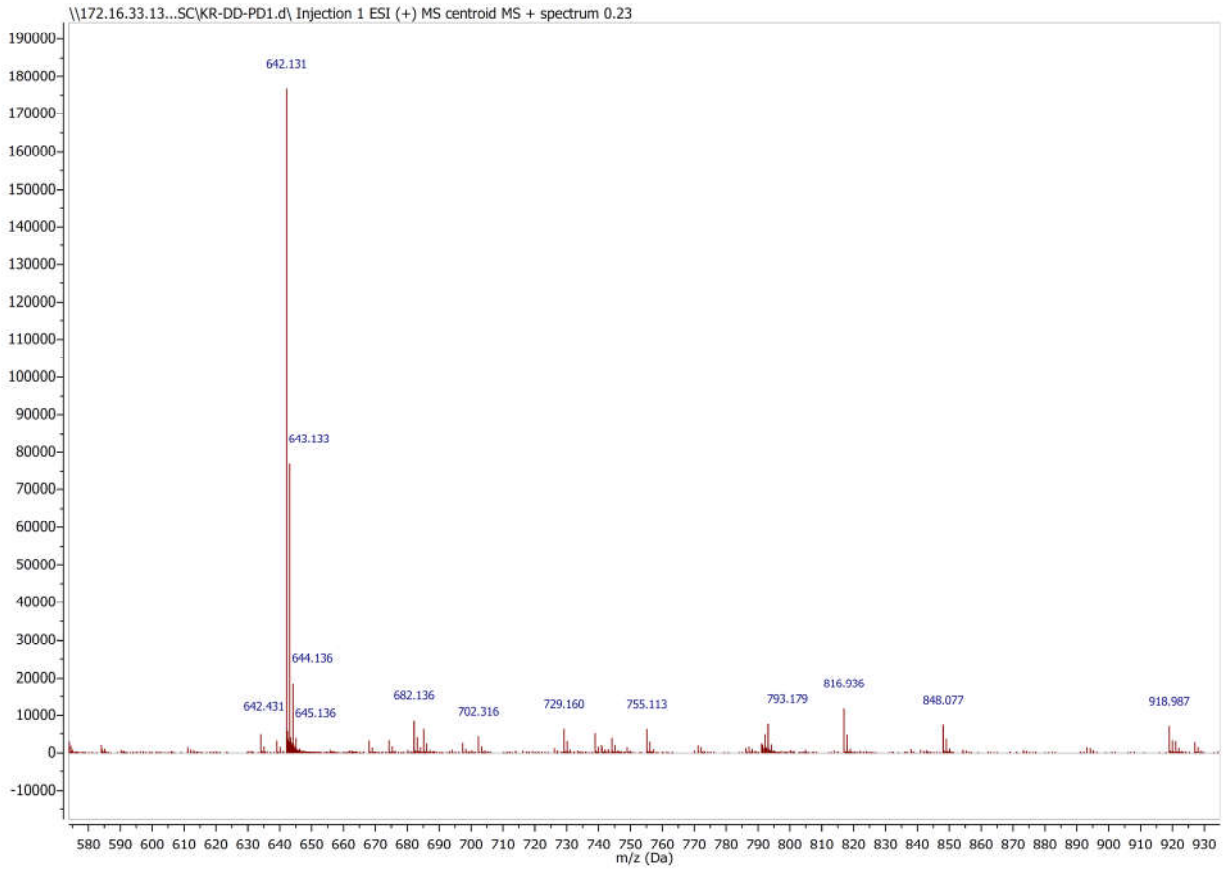


Fig. S4 Mass spectrum of complex **3** in methanol showing the $[M-(OAc)]^+$ peak at m/z = 642.131

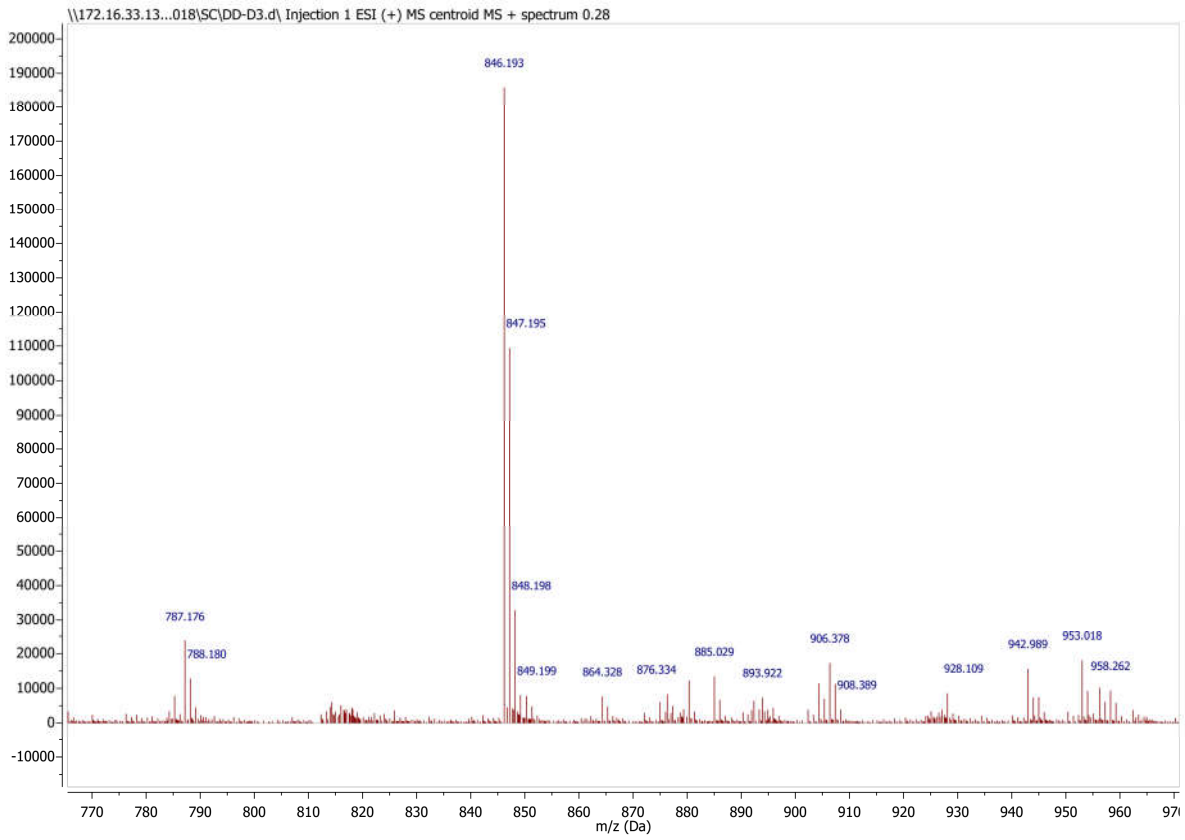


Fig. S5 Mass spectrum of complex **4** in methanol showing the $[M-(OAc)]^+$ peak at m/z = 846.193

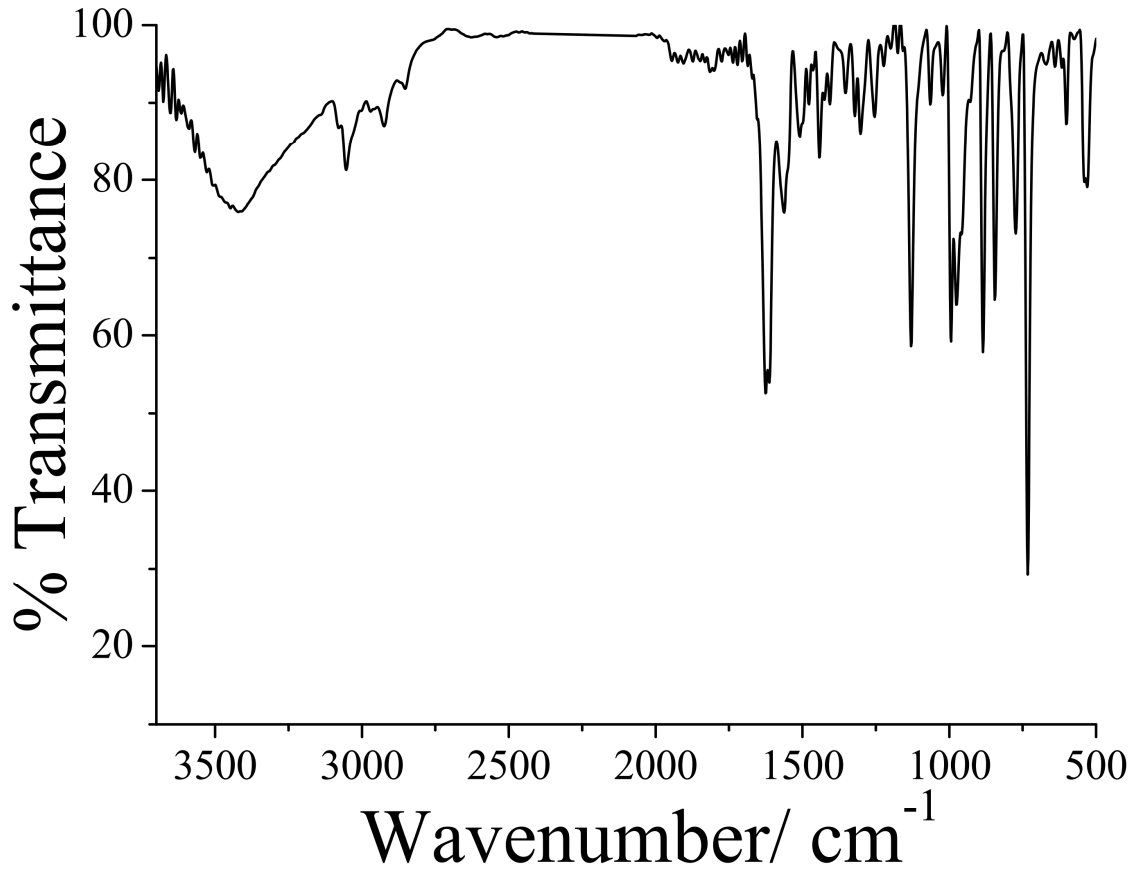


Fig. S6 IR spectrum of complex **1** in solid KBr matrix.

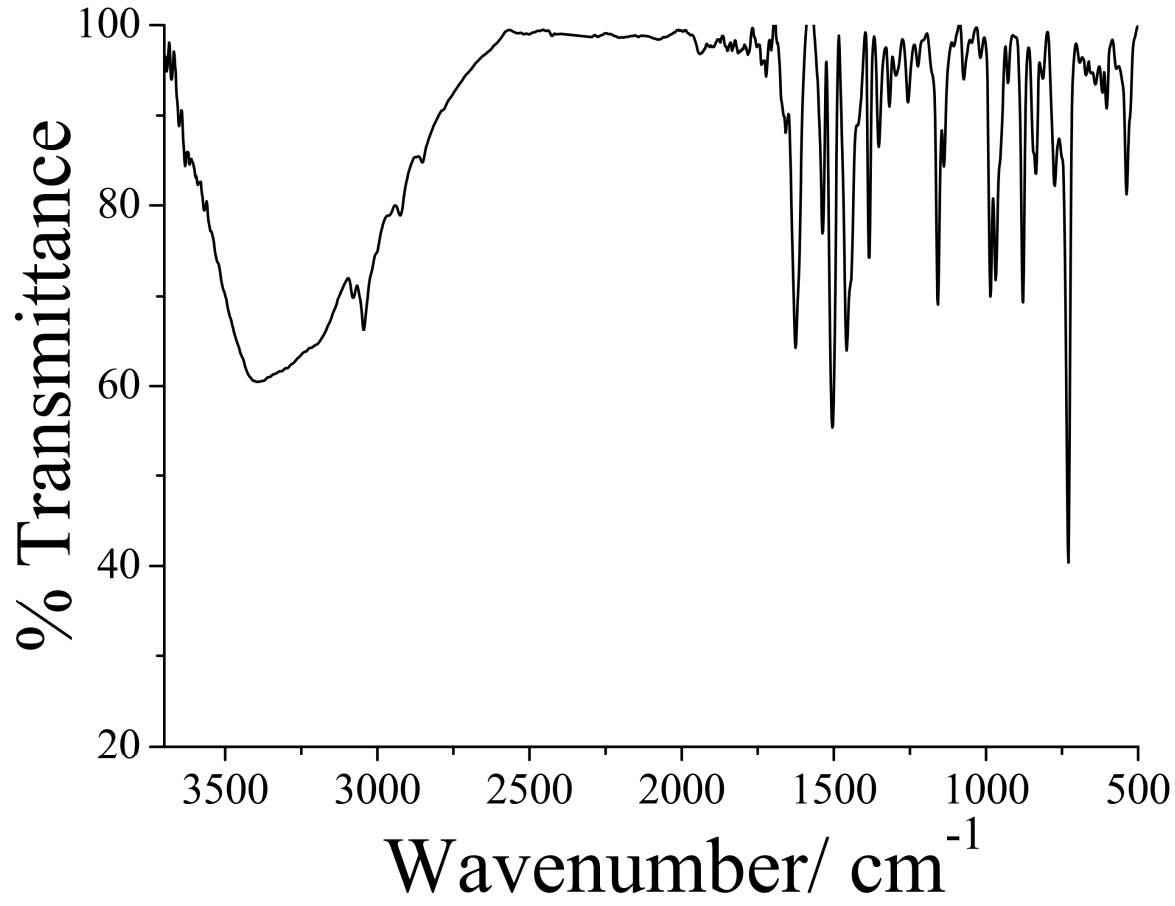


Fig. S7 IR spectrum of complex **2** in solid KBr matrix.

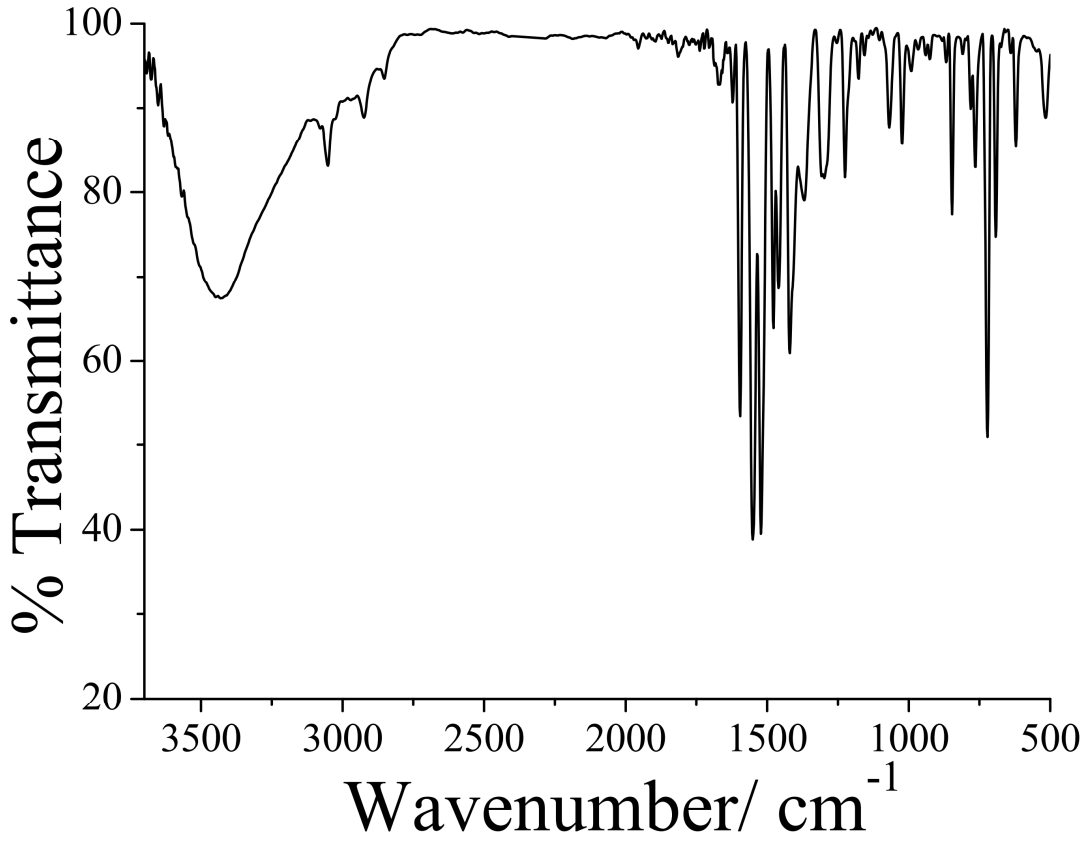


Fig. S8 IR spectrum of complex **3** in solid KBr matrix.

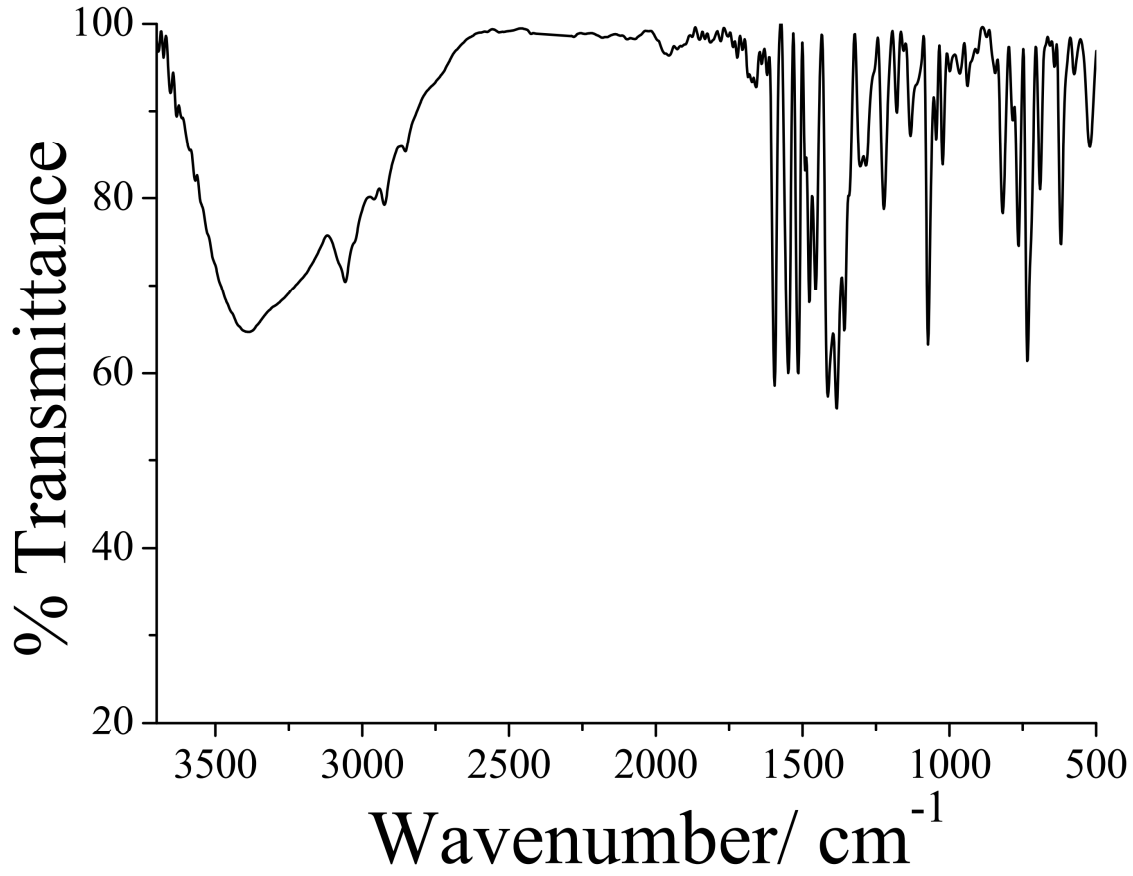


Fig. S9 IR spectrum of complex **4** in solid KBr matrix.

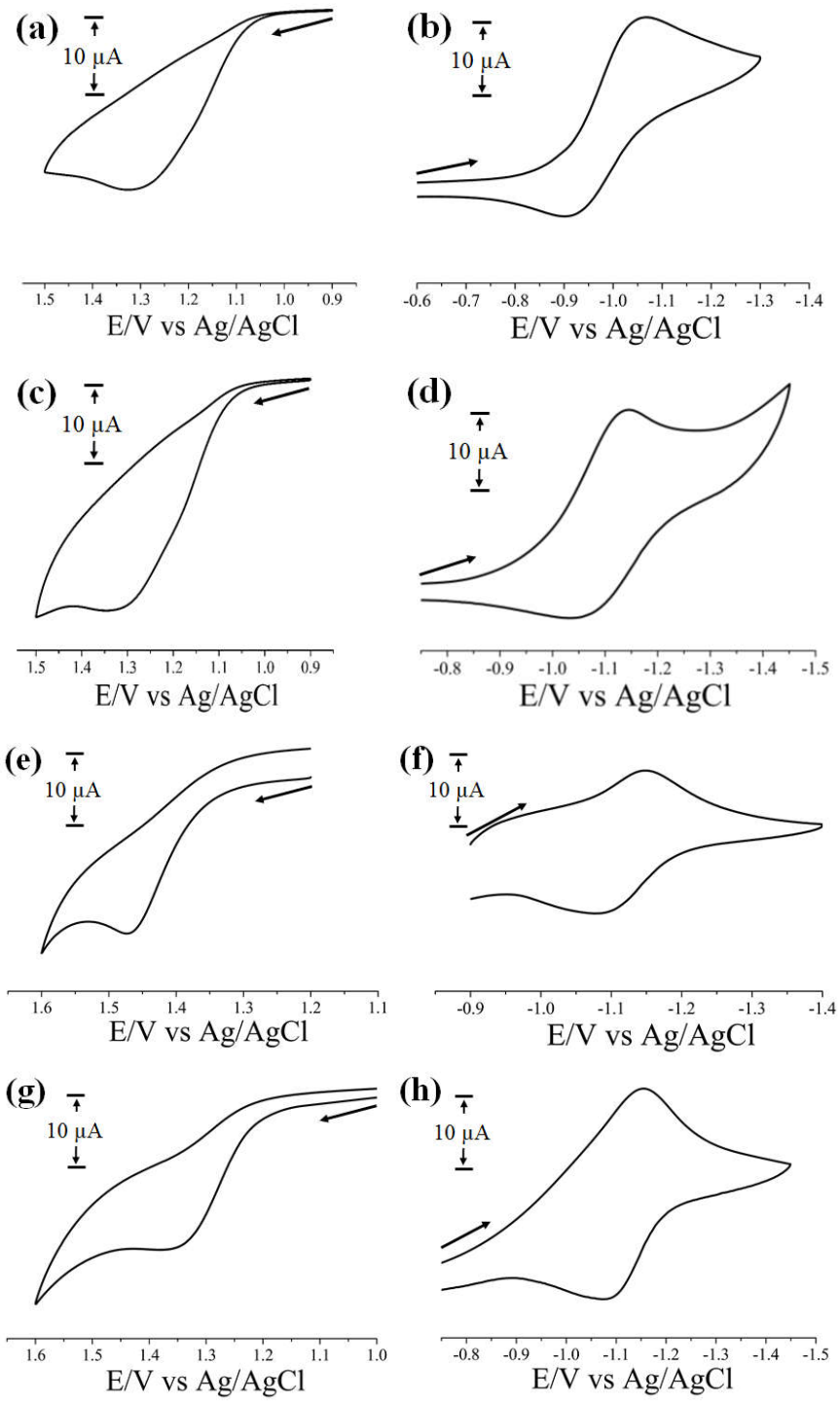


Fig. S10 Cyclic voltammetric responses of complex **1** [(a) and (b)], **2** [(c) and (d)], **3** [(e) and (f)] and **4** [(g) and (h)] in DMF showing cathodic (b, d, f and h) and anodic (a, c, e and g) scans using TBAP (0.1 M) as the supporting electrolyte at a scan rate of 50 mV s^{-1}.

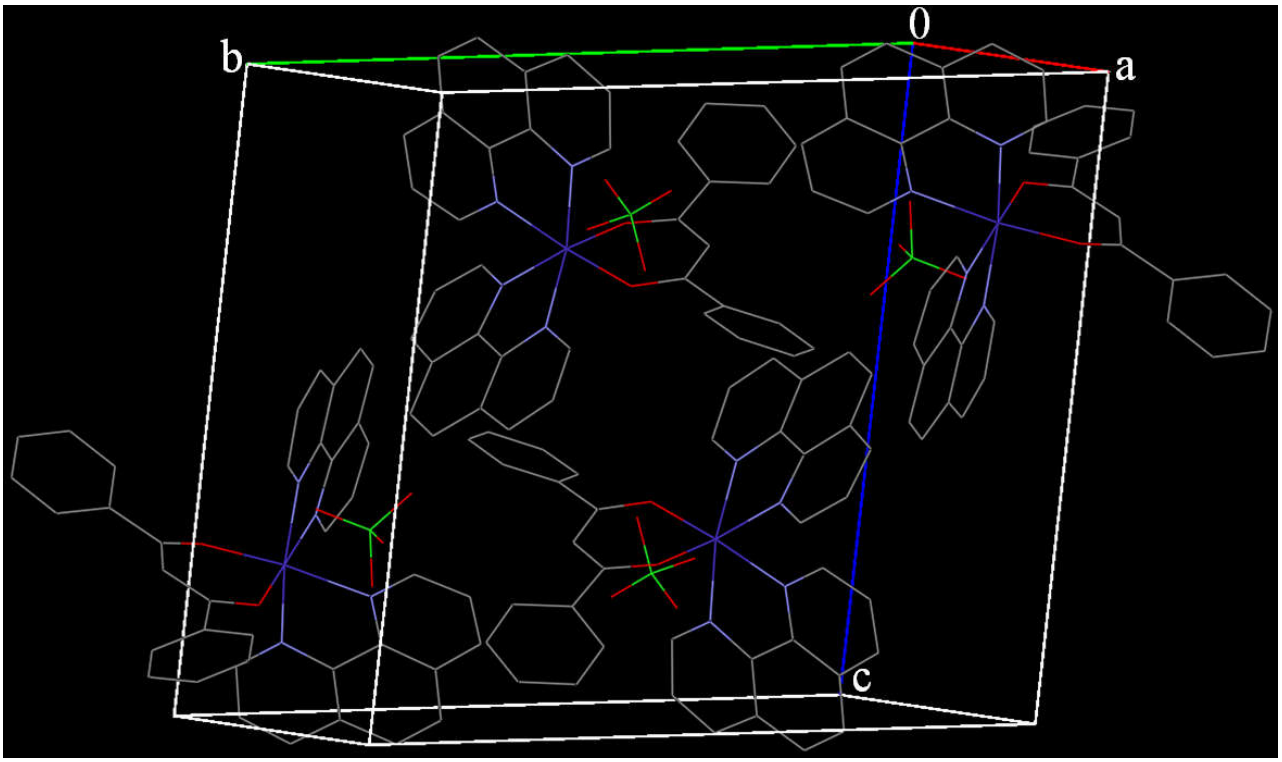


Fig. S11 Unit cell packing diagram for the complex $[Co(dbm)(phen)_2](ClO_4)$ (**3a**). The hydrogen atoms are not shown for clarity. There are four molecules in the unit cell.

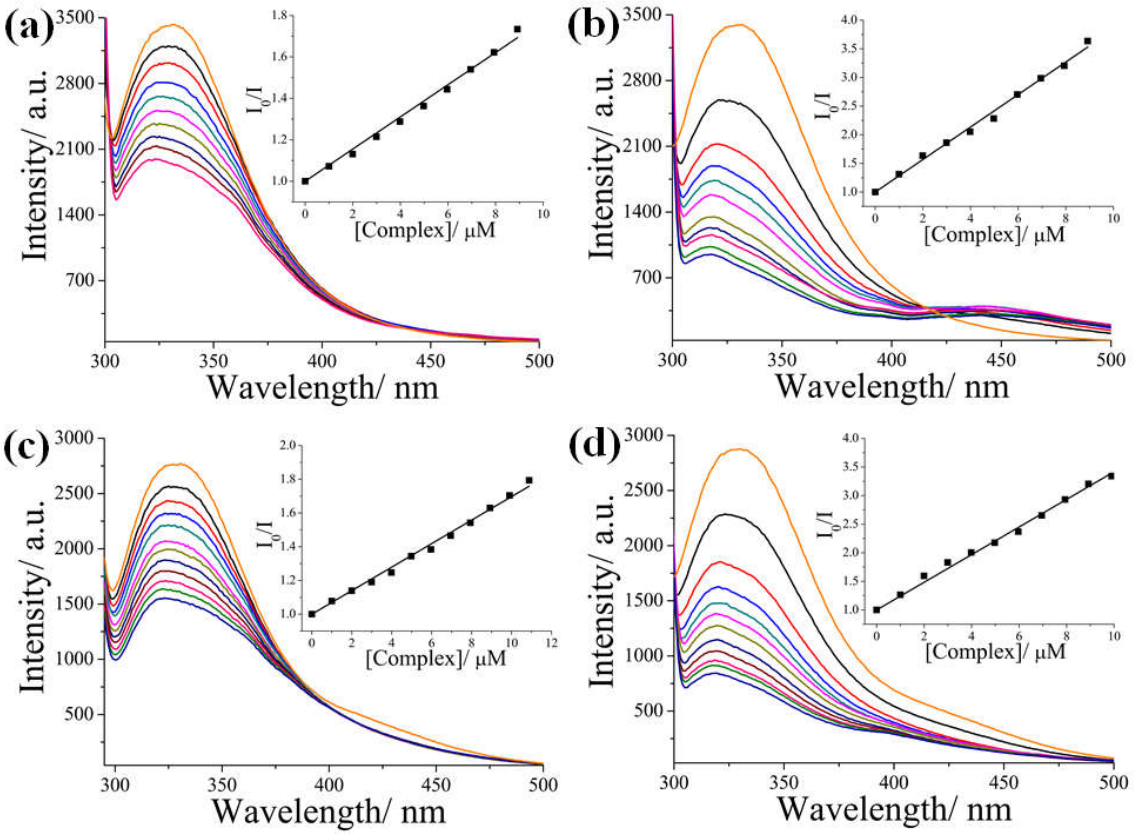


Fig. S12 Emission spectral traces of HSA (2 μM) in the presence of complexes **1** (a), **2** (b), **3** (c) and **4** (d) with the inset showing the plot of (I_0/I) vs. [complex].

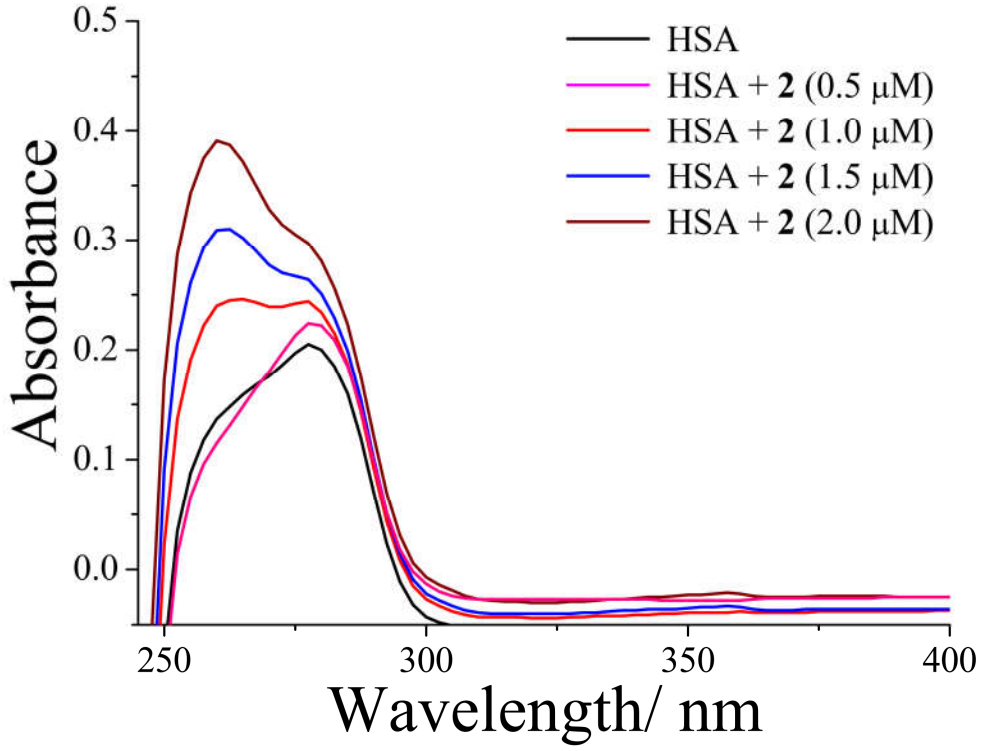
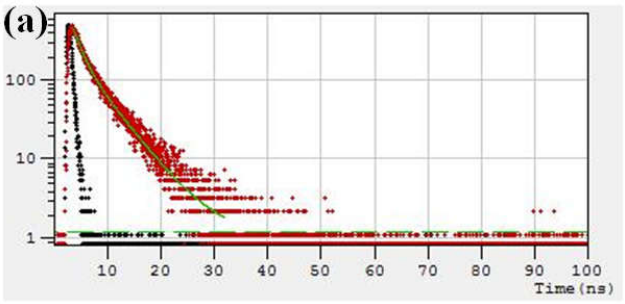
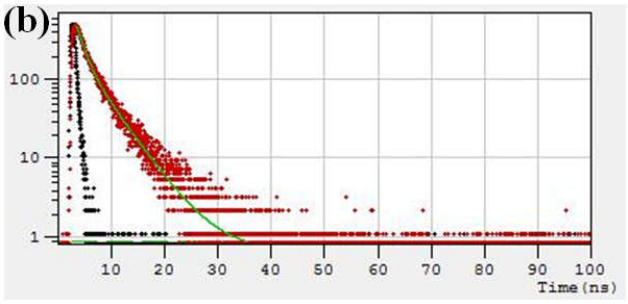
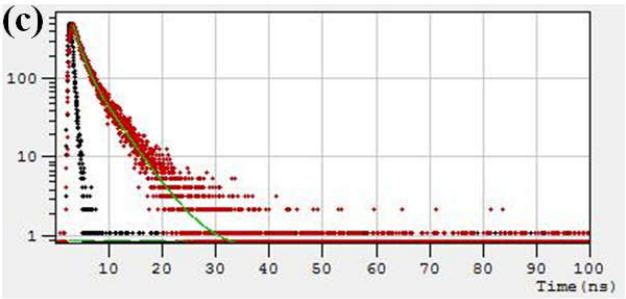


Fig. S13 Absorption spectral traces of HSA alone (5 μM) and in presence of different concentrations of complex **2** (0.5-2.0 μM) in Tris-HCl buffer (pH 7.2).







(d)

Sample	Lifetime (ns)		
	τ_1	τ_2	$<\tau>$
HSA (2 μM)	1.31	4.58	4.07
HSA (2 μM) + **2** (5 μM)	1.29	4.61	4.07
HSA (2 μM) + **2** (10 μM)	1.25	4.38	3.68

Fig. S14 Fluorescence intensity decay profiles of human serum albumin (HSA) in the absence [panel (a)] and presence of 5 and 10 μM of complex **2** [panels (b) and (c) respectively] in Tris-HCl buffer (pH 7.2). The intensity decays were measured at 340 nm (λ_{ex} = 280 nm). Panel (d) shows the life-time of bi-exponential fluorescence decay (τ_1 and τ_2) and average life-time ($<\tau>$).

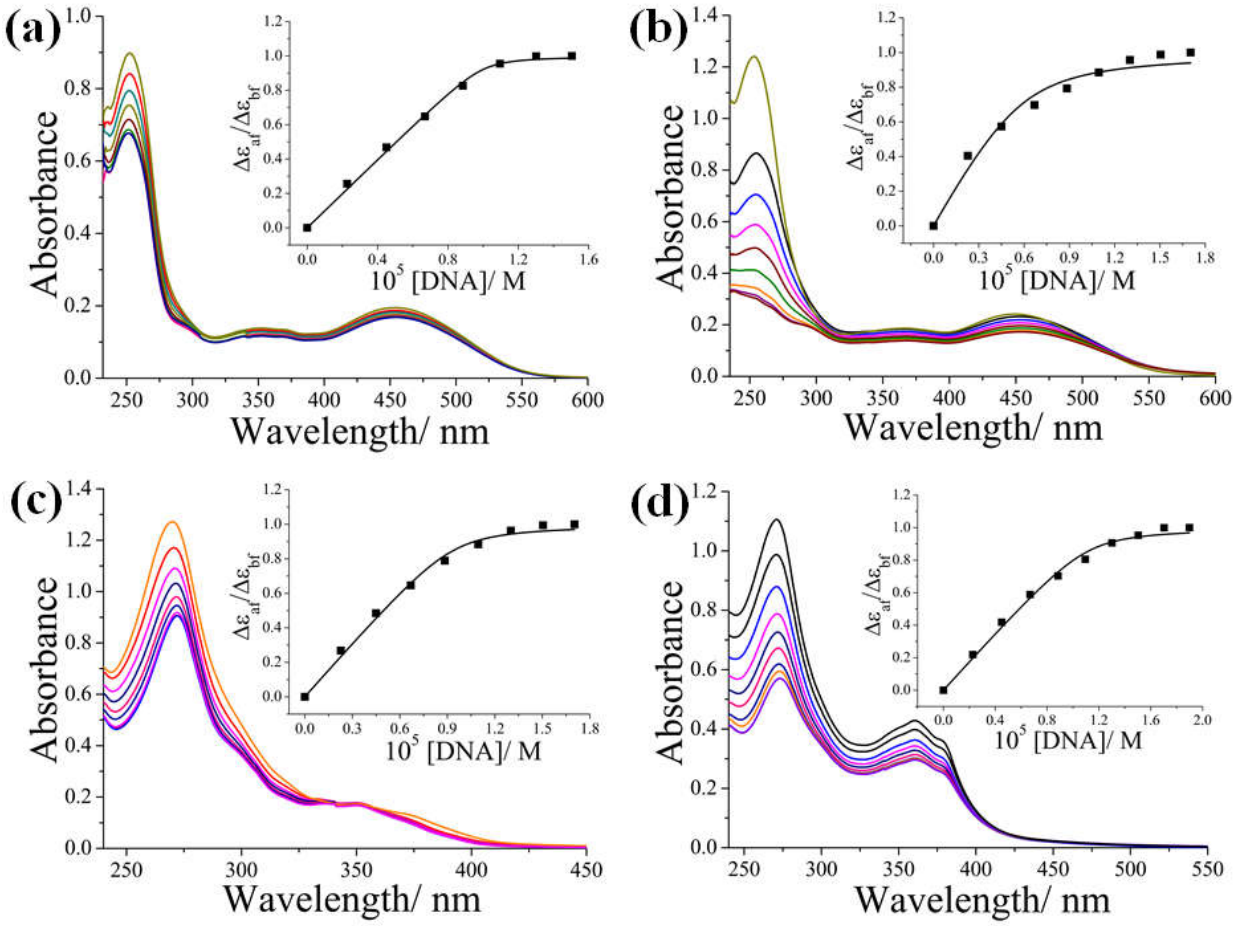


Fig. S15 Absorption spectral traces of the complexes **1-4** [(a)-(d)] in 5 mM Tris-HCl buffer (pH 7.2) on increasing the quantity of calf thymus DNA. The inset shows the least-squares fits of $\Delta\varepsilon_{af}/\Delta\varepsilon_{bf}$ vs. [DNA] for the complexes.

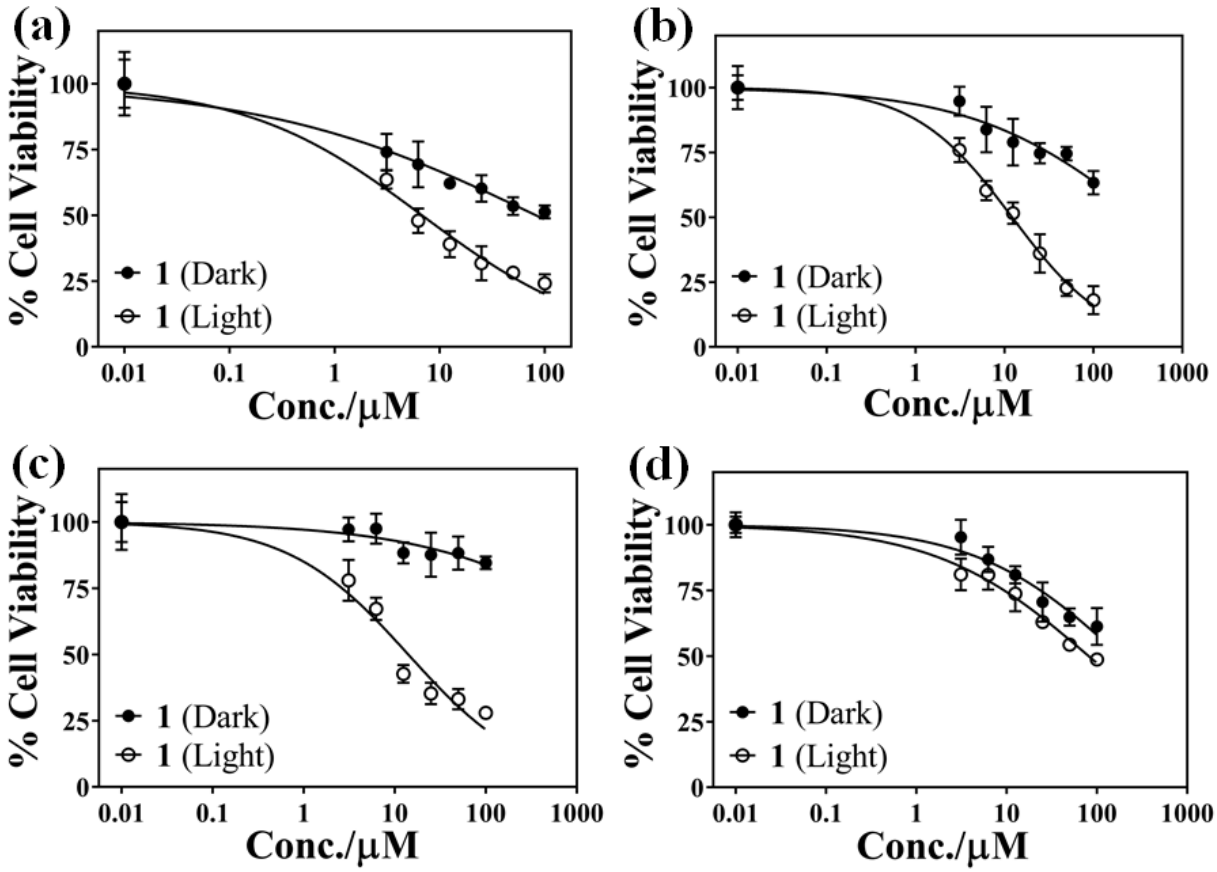


Fig. S16 Cell viability plots showing the cytotoxic effect of complex **1** in (a) HeLa; (b) MCF-7 ; (c) MDA-MB-231 and (d) HPL1D cells in dark (solid symbols) and in the presence of visible light (hollow symbols, 400-700 nm, 10 J cm^{-2}).

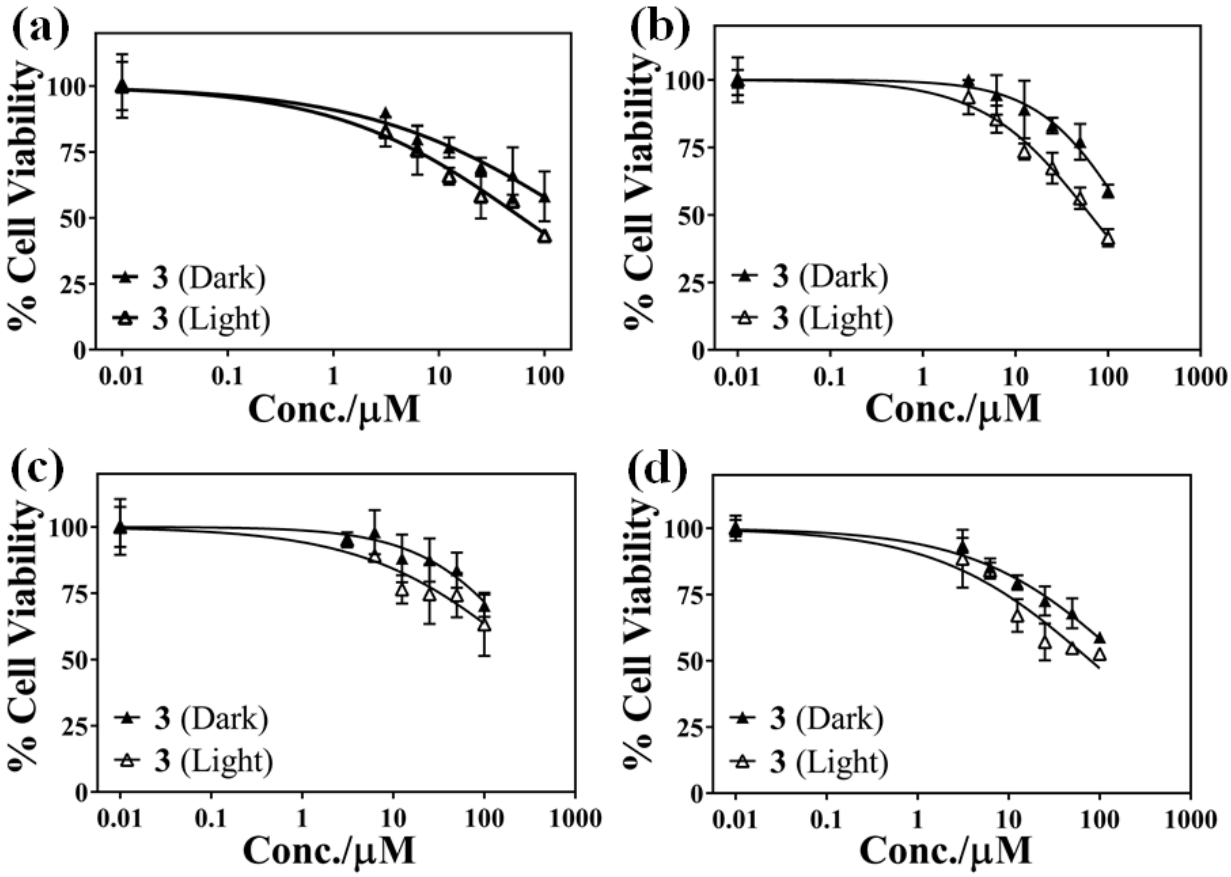


Fig. S17 Cell viability plots showing the cytotoxic effect of complex **3** in (a) HeLa; (b) MCF-7; (c) MDA-MB-231 and (d) HPL1D cells in dark (solid symbols) and in the presence of visible light (hollow symbols, 400-700 nm, 10 J cm^{-2}).

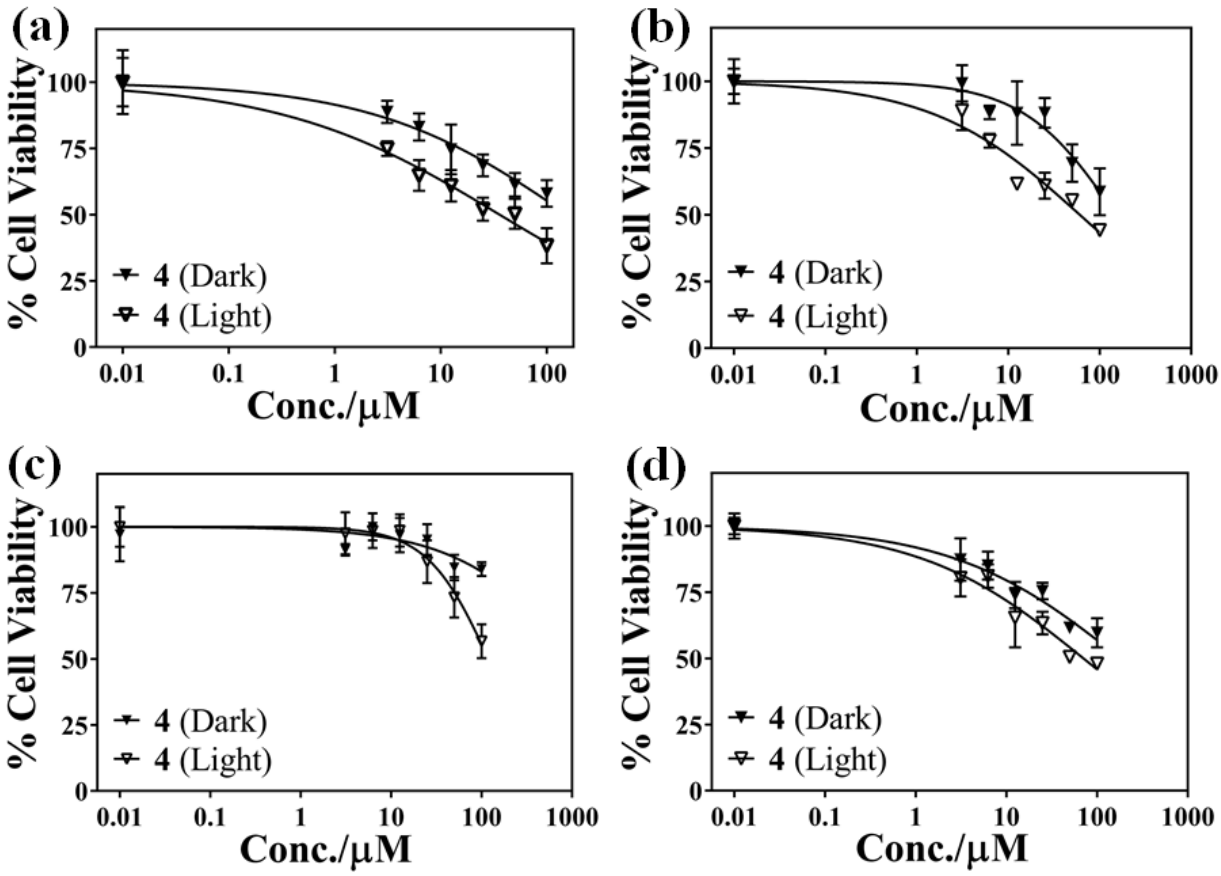


Fig. S18 Cell viability plots showing the cytotoxic effect of complex **4** in (a) HeLa; (b) MCF-7; (c) MDA-MB-231 and (d) HPL1D cells in dark (solid symbols) and in the presence of visible light (hollow symbols, 400-700 nm, 10 J cm^{-2}).

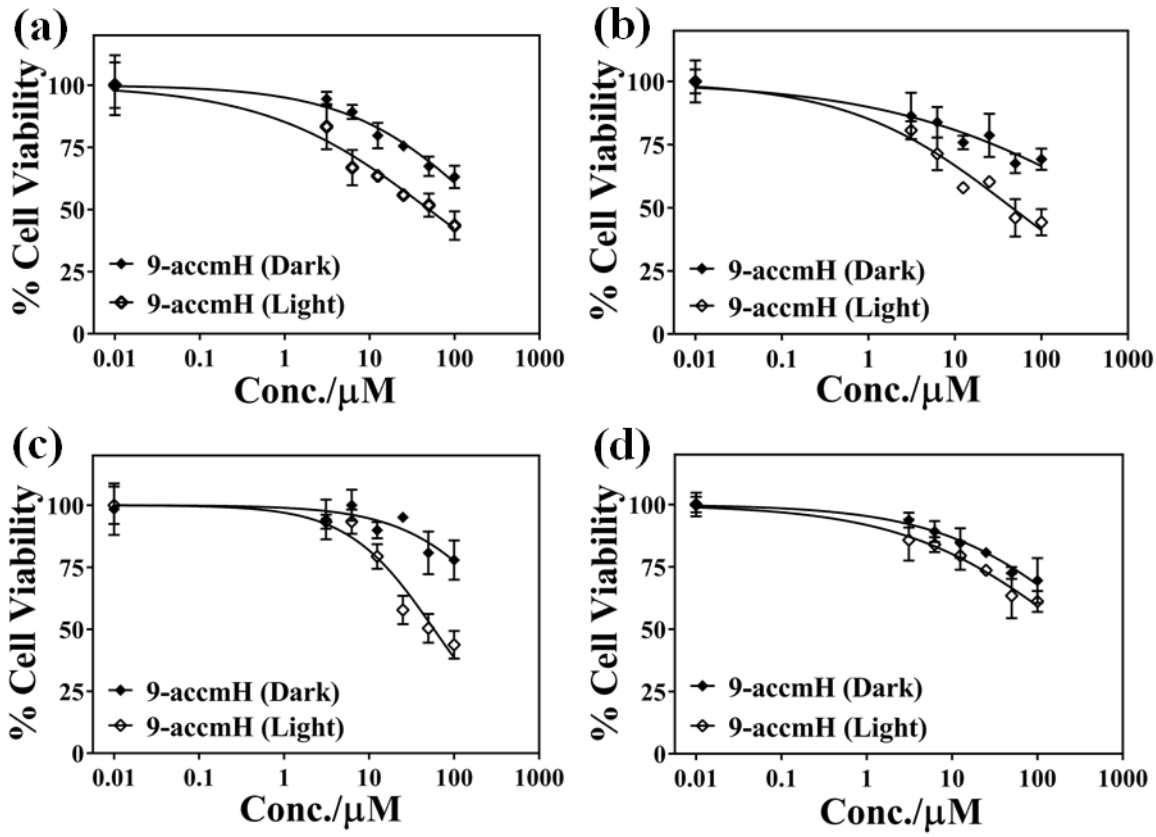


Fig. S19 Cell viability plots showing the cytotoxic effect of 9-accmH ligand in (a) HeLa; (b) MCF-7; (c) MDA-MB-231 and (d) HPL1D cells in dark (solid symbols) and in the presence of visible light (hollow symbols, 400-700 nm, 10 J cm^{-2}).

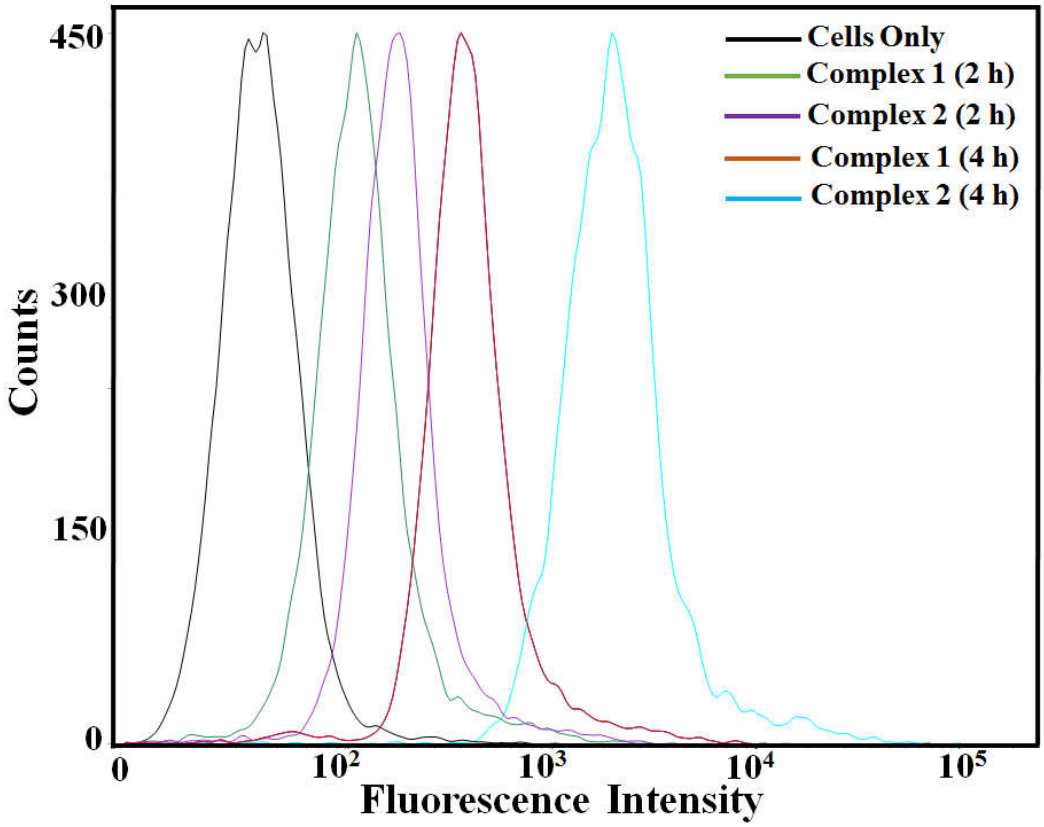


Fig. S20: Uptake of the complexes **1** and **2** (10 μM) at 37 °C for 2 and 4 h incubation time in HeLa cells determined by flow cytometry. The shift in the band position for different incubation time indicates time dependent uptake of the complexes **1** and **2** in HeLa cells.

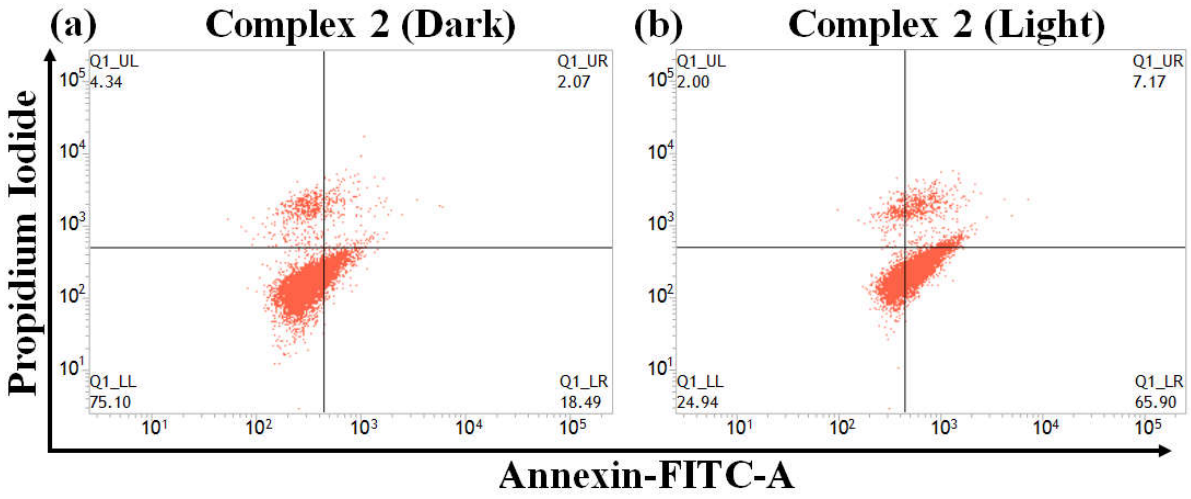


Fig. S21: Flow cytometric profiles of Annexin V-FITC-PI staining of HeLa cells undergoing apoptosis induced by complex **2** (10 μM) in dark and visible light (400-700 nm, 10 J cm^{-2}).

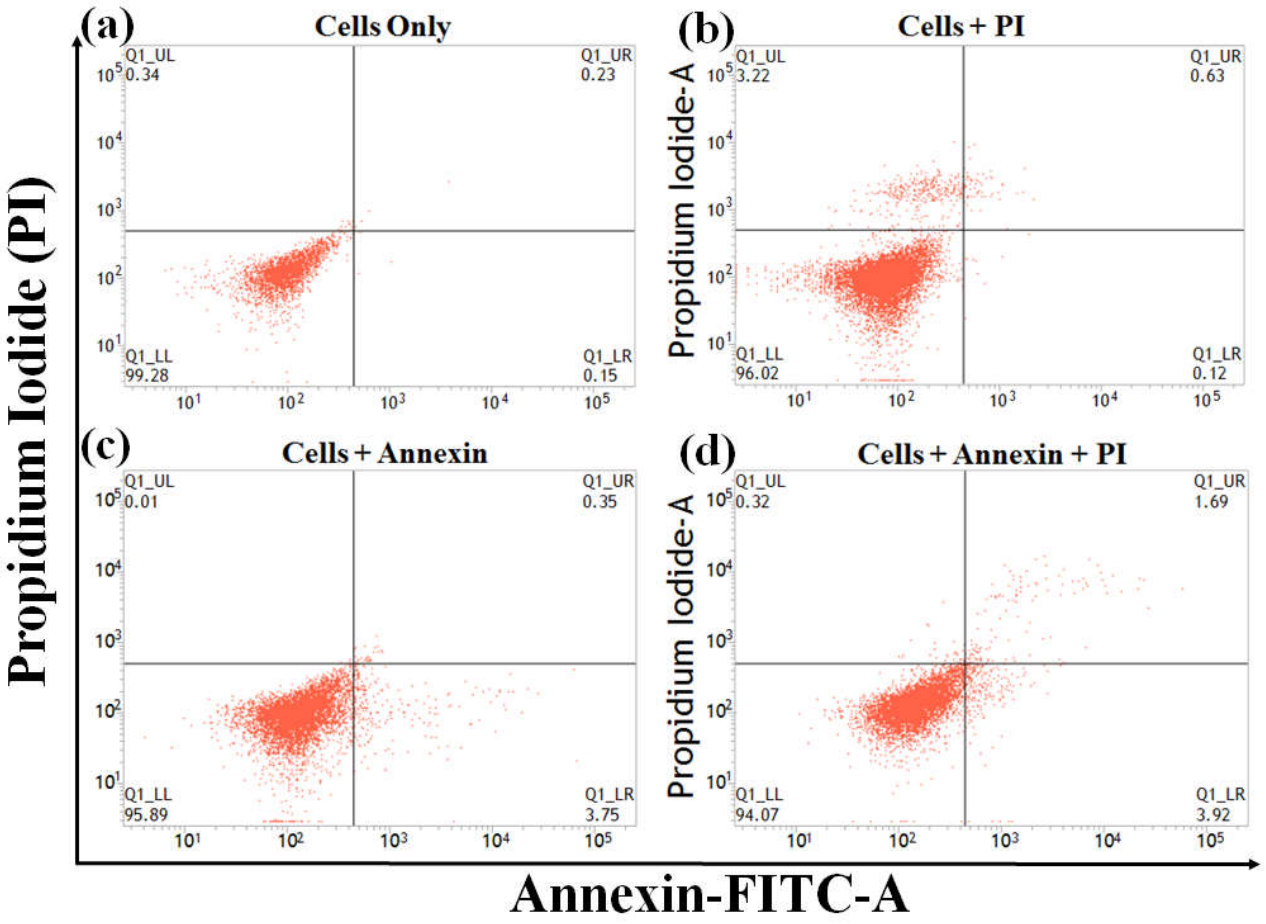


Fig. S22: Control flow cytometric profiles of Annexin V-FITC-PI staining of HeLa cells without addition of any cobalt complex: Panels (a) cells only; (b) cells + PI; (c) cells + Annexin and (d) cells + Annexin + PI.