Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Luminescent europium(III)-platinum(II) heterometallic complex as theranostic agent: a proof-of-concept study

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1. Experimental

1.1. Materials

All the reagents and chemicals ($Eu(NO_3)_3 \cdot 6H_2O$, 3-Aminoquinoline, diethylene triaminepentaacetic acid (DTPA) and K_2PtCl_4 were purchased from commercial sources (Alfa Aesar, India; Sigma-Aldrich, USA) and used as such without further purifications. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), trypsin–EDTA, Dulbecco's modified eagle's medium (DMEM, Gibco® Life Technologies, Bengaluru, India), penicillin–streptomycin antibiotic, bisBenzimide H33258 and gelatin (from cold water fish skin) were purchased from Sigma-Aldrich (Bengaluru, India) and used as received. Solvents used were either HPLC grade or were purified by standard procedures.[S1] *cis*-$[PtCl_2(DMSO)_2]$ was prepared according to literature procedure.[S2] Diethylenetriamine-*N*,*N*′,*N*″-triacetic-*N*,*N*″-dianhydride (DTPA-bis(anhydride)) was synthesized using previously reported procedure.[S3] Supercoiled (SC) pUC19 DNA (cesium chloride purified) was obtained from Merck Millipore (India). Calf thymus DNA (CT-DNA), bovine serum albumin (BSA), ethidium bromide (EB), agarose (molecular biology grade), gel loading solution containing 0.25% bromophenol blue, 0.25% xylene cyanol FF and 40% sucrose in water were purchased from Sigma (USA). Tris(hydroxymethyl)aminomethane−HCl (Tris-HCl) buffer solution (pH 7.2) was prepared from Milli-Q water of resistivity of 18.2 MΩ.cm.

1.2 Physical Methods.

The elemental microanalyses for C, H and N were performed using a Perkin-Elmer 2400 Series II elemental analyzer. FT-IR spectra were recorded with a Perkin-Elmer Model 1320 FT-IR spectrometer (KBr disk, 4000-400 cm^{-1}). Electronic spectra were measured at 298 K using a Perkin-Elmer Lambda 25 UV-vis spectrophotometer. ^{1}H-NMR spectra were recorded at 298 K on a JEOL-ECX 500 FT (500 MHz) instrument with chemical shift referenced to tetramethylsilane (TMS = Me_4Si) as the internal standard. Electrospray ionization mass spectral (ESI-MS) measurements were carried out using a WATERS Q-TOF Premier mass spectrometer. The fluorescence and time-delayed luminescence spectral data were recorded using Agilent Cary eclipse fluorescence spectrophotometer at 298 K. The circular dichroism (CD) spectroscopy of the complexes with CT-DNA were done by using JASCO J-815 spectropolarimeter equipped with a peltier temperature control device under continuous flow of nitrogen purging. Isothermal titration

calorimetry (ITC) studies were performed using MicroCal iTC200 system from GE Healthcare Life Sciences. Lifetime measurements for Eu-Pt_2 complex in absence and presence of CT-DNA were done in 5 mM Tris-HCl buffer (pH 7.2) at 298 K using a pulsed xenon lamp at λ_{ex} =330 nm and λ_{em} =616 nm with a delay time and gate time of 0.1 ms. Decay curves were fitted by non-linear least square method and values obtained were within standard deviation of ± 10%. The overall luminescence quantum yields ($\emptyset_{overall}$) were measured in Tris-HCl buffer (pH=7.2) at 298 K at room temperature according to a reported procedure using quinine sulfate as reference using following equation:[S4]

$$\emptyset_{overall}=\emptyset_{ref}A_{ref}In^2/AI_{ref}n^2_{ref}$$

where A, I and n represent the respective absorbance at the excitation wavelength, area under the emission spectral curve and refractive index of the solvent respectively. The ϕ_{ref} represents the quantum yield of the standard quinine sulfate solution. Fluorescence microscopic investigations were carried out using model LSM780NLO (Carl Zeiss GmbH) confocal laser scanning fluorescence microscope.

1.3. Synthesis of Compounds.

1.3.1. Synthesis of *N,N''*-Bis(3-amidoquinolyl)diethylenetriamine-*N,N',N''*-triacetic acid (H_3L).

3-aminoquinone (0.323 g, 2.2 mmol) was added in portion to the solution of DTPA-bis(anhydride) (0.400 g, 1.1 mmol) in dry DMF (20 mL). The reaction mixture was stirred for 5 h at 70 ^{0}C. The solvent was removed in *vacuo* to obtain a bright yellow colored solid product. The compound was washed with acetonitrile (3x20 mL) and diethyl ether (3x20 mL) three times and was dissolved in 5 mL of water and was adjusted to pH 6 by dropwise addition of NaOH. The aqueous solution was washed with (3x20 mL) dichloromethane to get rid of organic impurities. Water was then evaporated completely and dried in vauum overnight to gain a yellow powder as desired product (yield: 0.650 g, 90%). ESI-MS in CH_3OH (*m/z*): $[M+H]^+$ calcd. for $C_{32}H_{35}N_7O_8$: 646.26 (100%). Found: 646.25 (100%). ^{1}H-NMR (500 MHz, Methanol-d_3) δ ppm 2.86 (4H, s), 2.99 (4H, s), 3.66 (6H, s), 3.74 (4H, s), 7.39 - 7.46 (3H, m), 7.46 - 7.56 (3H, m), 7.60 (4H, d), 7.71 (3H, d), 8.53 (2H, d), 8.86 (2H, d); FT-IR (KBr pellet, ν_{max}, cm^{-1}): 3260 (w), 1667 (s, $\nu_{C=O}$ of COOH), 1608 (s, $\nu_{C=O}$ of CONH), 1558 (m), 1491 (w), 1468 (s), 1368 (m), 1286 (m, ν_{C-O} of COOH), 1220 (w), 1093 (m),

991 (m), 902 (s), 783 (m) (vs, very strong; s, strong; m, medium; w, weak; br, broad). UV-vis (DMF, 298 K), λ_{max} in nm (ε in M^{-1} cm^{-1}): 265 (15,500), 318 (7000) and 331 (7000).

1.3.2. Synthesis of [Eu(L)(H_2O)].

To a 10 mL aqueous solution of $Eu(NO_3)_3 \cdot 6H_2O$ (0.300 g, 0.672 mmol) was added dropwise an aqueous solution (15 mL) of H_3L (0.434 g, 0.672 mmol) pretreated with NaOH (0.081 g, 2.016 mmol) for 15 min. The reaction mixture was refluxed for 3 h with stirring to form a light yellow precipitate. The precipitate was filtered and washed successively with cold methanol (3 x 10 mL) and diethyl ether (3 x 10 mL) and finally dried in vacuum over P_4O_{10} to obtain desired final product (yield: 0.437 g, ~80%). Anal. Calc. for $C_{32}H_{34}N_7O_9Eu$: C, 47.30; H, 4.22; N, 12.07. Found: C, 47.12; H, 4.13; N, 11.96. ESI-MS in DMF: *m/z* $[M\text{-}H_2O+H]^+$ calcd. for $C_{32}H_{33}N_7O_8Eu$: 796.16; Found: 796.15. FT-IR (KBr pellet, ν_{max}, cm^{-1}): 3431 (w), 1607 (s, ν_{asym} CO_2^-), 1589 (s, $\nu_{C=O}$ of CONH), 1496 (m), 1468 (m), 1388 (s, ν_{sym} CO_2^-), 1350 (m), 1294 (m), 1091 (m), 914(m), 870 (m), 784 (s). UV-visible (DMF, 298 K): λ_{max}, nm (ε, M^{-1} cm^{-1}): 263 (17,330), 318 (5,040) and 331 (5,020). Molar conductivity in 10% aqueous DMF at 298 K [Λ_M/S cm^2 mol^{-1}]: 12.1.

1.3.3. Synthesis of [{*cis*-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)] (Eu-Pt_2 (1)).

To a DMF solution (5 mL) of [Eu(L)(H_2O)] (0.250 g, 0.308 mmol), was added *cis*-[$Pt(DMSO)_2Cl_2$] (0.260 g, 0.616 mmol). The reaction mixture was stirred at 50 ^{0}C in the dark for 48 h. The pale yellowish color clear solution was then filtered. Addition of excess acetone (120 mL) to the filtrate gave a pale yellow precipitate. The resulting precipitate was filtered off, and washed with cold water and diethyl ether, then dried in vacuum over P_4O_{10} to obtain the desired final product, Eu-Pt_2 (**1**). Yield: (0.368 g, 80%). Anal. Calc. for $C_{36}H_{46}Cl_4EuN_7O_{11}Pt_2S_2$ is C, 28.81; H, 3.09; N, 6.53; Found: C, 29.01; H, 2.98; N, 6.48; FT-IR (KBr pellet, ν_{max}, cm^{-1}) 3426 (br, -OH), 3244 (br), 3016 (m), 1602 (vs, ν_{asym} CO_2^-), 1578 (s, $\nu_{C=O}$ of CONH), 1500 (m), 1387 (s, ν_{sym}, CO_2^-), 1325 (m), 1152 (m), 1134 (m, $\nu_{S=O}$), 1093 (m), 1025 (m), 966 (w), 926 (m), 863 (m), 778 (m), 753 (m), 591 (w), 526 (w), 440 (m, $\nu_{Pt\text{-}S}$). UV-visible (DMF, 298 K): λ_{max}, nm (ε, M^{-1} cm^{-1}): 273 (23,450), 318 (13,980), 332 (14,930) and 348 (11,250). ESI-MS in DMF-H_2O (1:1): *m/z* 550.55-554.55 [**1**-Pt(DMSO)Cl_2-H_2O-Cl+H]$^{2+}$; 712.52-718.53 [**1** - 2Cl]$^{2+}$; 1103.09 [**1**-Pt(DMSO)Cl_2-H_2O-Cl]$^+$ (See Fig. S1 for detailed mass spectral analysis).

2. DNA Binding Methods.

Binding experiments of the $EuPt_2$ complex **1** with CT-DNA were studied by UV-visible absorption titration, ethidium bromide displacement assay, circular dichroism (CD) and isothermal titration calorimetry (ITC).

2.1. Absorption Spectral Studies.

DNA binding experiments were carried out in Tris-HCl/NaCl buffer (5 mM Tris-HCl, 5 mM NaCl in water, pH 7.2) using a DMF solution of the complex. The ratio of UV absorbance at 260 nm and 280 nm was approximately 1.9:1, suggesting that DNA was sufficiently free from protein.[S5] The concentration CT-DNA was determined from its absorbance intensity at 260 nm and its known molar absorption coefficient value 6600 M^{-1} cm^{-1}.[S6] Absorption spectral titration experiments were performed by varying the concentration of CT-DNA while keeping the complex concentration constant. Due correction was made for the absorbance of DNA itself. Before each measurement sample were equilibrated with CT-DNA for 5 min. The absorbance of the complex were measured after each successive addition of CT-DNA. The intrinsic DNA binding constant (K_b) was obtained using the equation:

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/ K_b(\varepsilon_a-\varepsilon_f)$$

where [DNA] is the concentration of DNA in the base pairs, ε_a is the apparent extinction coefficient observed for the complex, ε_f corresponds to the extinction coefficient of the complex in its free form, and ε_b refers to the extinction coefficient of the complex when fully bound to DNA.[S7] Data were plotted using Origin Lab, version 8.0 to obtain the $[DNA]/(\varepsilon_a-\varepsilon_f)$ vs. [DNA] linear plots. The ratio of the slope to intercept from the linear fit gives the value of the intrinsic binding constant (K_b).

2.2. Ethidium bromide Displacement Assay.

The apparent binding constant (K_{app}) of the complex to CT DNA was determined from the emission spectral measurements using ethidium bromide (EthB) as a spectral probe in 5 mM Tris-HCl/NaCl buffer (pH 7.2) at 298 K. EthB showed no apparent emission in Tris-buffer medium because of fluorescence quenching of free EthB by solvent molecules. The emission intensity gets significantly enhanced due to its intercalative binding to duplex DNA. A competitive binding of the complex to DNA is found to reduce the EthB emission intensity. The relative binding

propensity of the complex to DNA was estimated from the reduction of the emission intensity. The values of the apparent binding constant (K_{app}) were obtained by using the following equation:

$$K_{app} \times [\text{Complex}]_{50} = K_{EthB} \times [\text{EthB}]$$

where K_{app} is the apparent binding constant of the complex studied, $[\text{Complex}]_{50}$ is the concentration of the complex at 50% quenching of DNA-bound ethidium bromide emission intensity, K_{EthB} is the binding constant of the EthB ($K_{EthB} = 1.0 \times 10^7\ M^{-1}$), and [EthB] is the concentration of ethidium bromide (4.0 μM).[S8]

2.3. Circular Dichroism Studies.

The circular dichroism (CD) spectroscopy of CT-DNA in presence of the complexes were done by using JASCO J-815 spectropolarimeter equipped with a Peltier temperature control device at 298 K under continuous flow of nitrogen purging. All the experiments were done in a 1-mm path length quartz cell. CD spectra of CT-DNA in absence and presence of the complexes at various molar ratio of [complex]/[CT-DNA] were recorded after incubation with CT-DNA at 37 ^{0}C. Each spectra was collected in 5 mM Tris-HCl/NaCl buffer (pH 7.2) by the accumulations of three average spectra and subtracting the data of the buffer with a scan speed 100 nm min^{-1}.

2.4. Isothermal Titration Calorimetry (ITC) Study.

ITC experiment was done to study the interaction of the $EuPt_2$ complex with CT- DNA in Tris-HCl/NaCl buffer (5mM Tris-HCl, 5mM NaCl, pH 8.5).The experiment was performed at 30 ^{0}C using MicroCal iTC200 system. All solutions were thoroughly degassed before being used. The instrument consist of a reference cell that has heat capacity like the sample cell solution. The sample cell before being used for experiment was thoroughly washed with Tris-HCl/NaCl buffer. The sample cell was loaded with 0.01 mM CT-DNA. The heat released by dilution of CT- DNA in the cell is negligible. $EuPt_2$ complex was dissolved in Tris-HCl/NaCl buffer (5 mM Tris-HCl, 5 mM NaCl, pH 8.5) was injected with a concentration 0.1 mM in the sample cell. Titration was performed by using a 40 μL syringe filled with the complex solution, with stirring at 1000 rpm. Injections were initiated after baseline stability was achieved. A titration experiment consisted of 20 consecutive injections of 2 μL volume and 4 sec duration each, with a filter period of 5 sec. The reference power was set at 5 μcal/sec with an initial delay of 60 sec. The resulting data were

fitted by sequential binding site model (number of sites =2) using MicroCal® ORIGIN software supplied with the instrument.

2.5. Gel electrophoretic mobility shift assay.

A gel electrophoretic mobility shift assay was used to determine the unwinding of closed circular, supercoiled pUC19 plasmid DNA induced by the complexes that differ in their coordination mode with the DNA double helix. Supercoiled pUC19 DNA (0.2 μg) was treated with varying concentration of the respective complexes in 50 mM Tris-HCl/NaCl buffer (pH 7.2) to a dilution to 20 μL final volume using Tris-HCl buffer. The mixtures were incubated in dark at 37 ^{0}C for 16 h, and quenched by adding 2 μL of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol FF and 40% sucrose. The solutions were finally loaded on 1% agarose gel containing 1.0 μg/mL ethidium bromide an electrophoresed in a dark room for 2.0 h at 60 V in TAE gel running buffer. After electrophoresis, the bands were visualized by UV-A light and photographed using UVITEC FireReader V4 gel documentation system.

3. BSA binding Studies.

Serum albumin proteins constitutes a major component in blood plasma proteins and plays important roles in drug transport and metabolism. The interaction of the $EuPt_2$ complex with bovine serum albumin (BSA), a structural homolog with human serum albumin (HSA) has been studied from tryptophan emission quenching experiment. Emission intensity of BSA at λ = 340 nm decreases gradually with increasing the complex concentration, which confirms that the interaction between the complex and BSA have occurred (Fig. S9). The complex solutions were gradually added to the solution of BSA (2 μM) in 5 mM Tris-HCl/NaCl buffer (pH 7.2) and the quenching of the emission signals at 340 nm (λ_{ex} = 295 nm) were recorded. The quenching constant (K_{BSA}) has been determined quantitatively by using Stern-Volmer equation. Stern-Volmer plots of I_0/I vs. [complex] were made using the corrected fluorescence data taking into account the effect of dilution (Fig. S9a). Linear fit of the data using the equation:

$$I_0/I = 1 + K_{BSA}[Q] = 1 + k_q \tau_0[Q]$$

where I_0 and I are the emission intensities of BSA in the absence and in the presence of quencher of concentration [Q], gave the quenching constant (K_{BSA}) using Origin Pro 8.0 software.[S9] k_q is the

quenching rate constant, τ_0 is the average lifetime of the tryptophan in BSA without quencher reported as 1 x 10^{-8} s.[S10] For such static quenching interaction, the binding constant (K) and the number of binding sites (n) can be determined according to the Scatchard equation:[S11]

$\log (I_0 - I)/ I = \log K + n \log[Q]$

The n and K can be calculated by the slope and the intercept of the double logarithm regression curve of $\log(I_0 - I)/ I$ *versus* $\log[Q]$ (Fig. S9b). The various binding parameters for interaction of $EuPt_2$ complex with BSA are listed in following table below:

Complex	K_{BSA} **(M^{-1})**	k_q **($M^{-1} s^{-1}$)**	K **(M^{-1})**	n
$EuPt_2$ (1)	1.5×10^5	1.5×10^{13}	10.0×10^6	1.39

4. Cell Proliferation Assay

The cytotoxicicty or cell viability of the $EuPt_2$ complex **1** was studied using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) metabolic assay. The MTT assay is based on the ability of mitochondrial dehydrogenases in the viable cells to break the tetrazolium rings of MTT forming dark blue membrane impermeable crystals of formazan, which upon solubilization can be estimated spectrophotometrically.[S12]. The level of the formazan formed gave a measure of the number of viable cells. In vitro studies of $EuPt_2$ complex **1** was carried out with HeLa (human cervical carcinoma) and H460 (human lung carcinoma) cells. Cells were maintained with Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator (37 °C and 5% CO_2). Cells (10^4 cells/well) were plated onto multiple glass bottom 96-well tissue culture plates at an initial confluence of ~70%. The cells were then treated with $EuPt_2$ complex dissolved in DMEM containing 1% DMSO at various concentrations for overnight (16 h) at 37 °C in a 5% CO_2 humidified incubator. An amount of 0.5 mg/mL of MTT in Basal DMEM was prepared in a dark environment. After discarding the old media, 200 μL of the freshly prepared MTT solution was added to each of the cell containing wells, followed by incubation for 4-5 h in dark at 37 °C, in a 5% CO_2 humidified incubator. After incubation, culture medium was discarded and 200 μL of DMSO was added to solubilize formazan crystals by slowly shaking the plates for 30 min. The cell proliferation was determined by measuring the absorbance at 570 nm in microtiter plate reader (Multiskan spectrum, Thermo scientific). All the in vitro cytotoxicity experiments were performed in triplicate. All the values for

percentage cell proliferation were normalized relative to untreated cells and a graph was drawn between percantage cell proliferation and concentration of the complex to determine IC_{50} values.

5. Cellular uptake studies

$EuPt_2$ complex (25 μM) was added to the respective cell culture media, DMEM and sterilized by filtration with 0.22 micron filter. Sterilized glass coverslips containing 24 well plate (13 mm, 0.2% gelatin coated) was used to seed the HeLa cells (1 x 10^4 cells/well) till the confluency reached to ~ 70%. The cells were incubated with $EuPt_2$ complex for 4 h at 37 °C in a 5% CO_2-humidified incubator. Then, the treated cells were washed with 1X PBS buffer three times in an interval of 5 min to remove the debris, followed by fixing the cells with 4% formaldehyde solution for 20 min at room temperature. Subsequently, fixed slides were washed with 1X PBS buffer three times to remove the excess formaldehyde. After this treatment, Hoechst 33258 was used to stain the nuclei of the cells for 15 min at room temperature and then washed with 1X PBS buffer in similar manner to remove the excess stain. Slides were coated with buffered mounting medium to prevent fading and drying followed by mounted with the cover slips. The slides was observed and images were acquired using a Carl Zeiss LSM780NLO confocal laser scanning microscope (CLSM) at 40X magnification using appropriate filters for blue and red emission from Hoechst 33258 and Eu-Pt_2 complex using λ_{ex} = 405 nm.

References:

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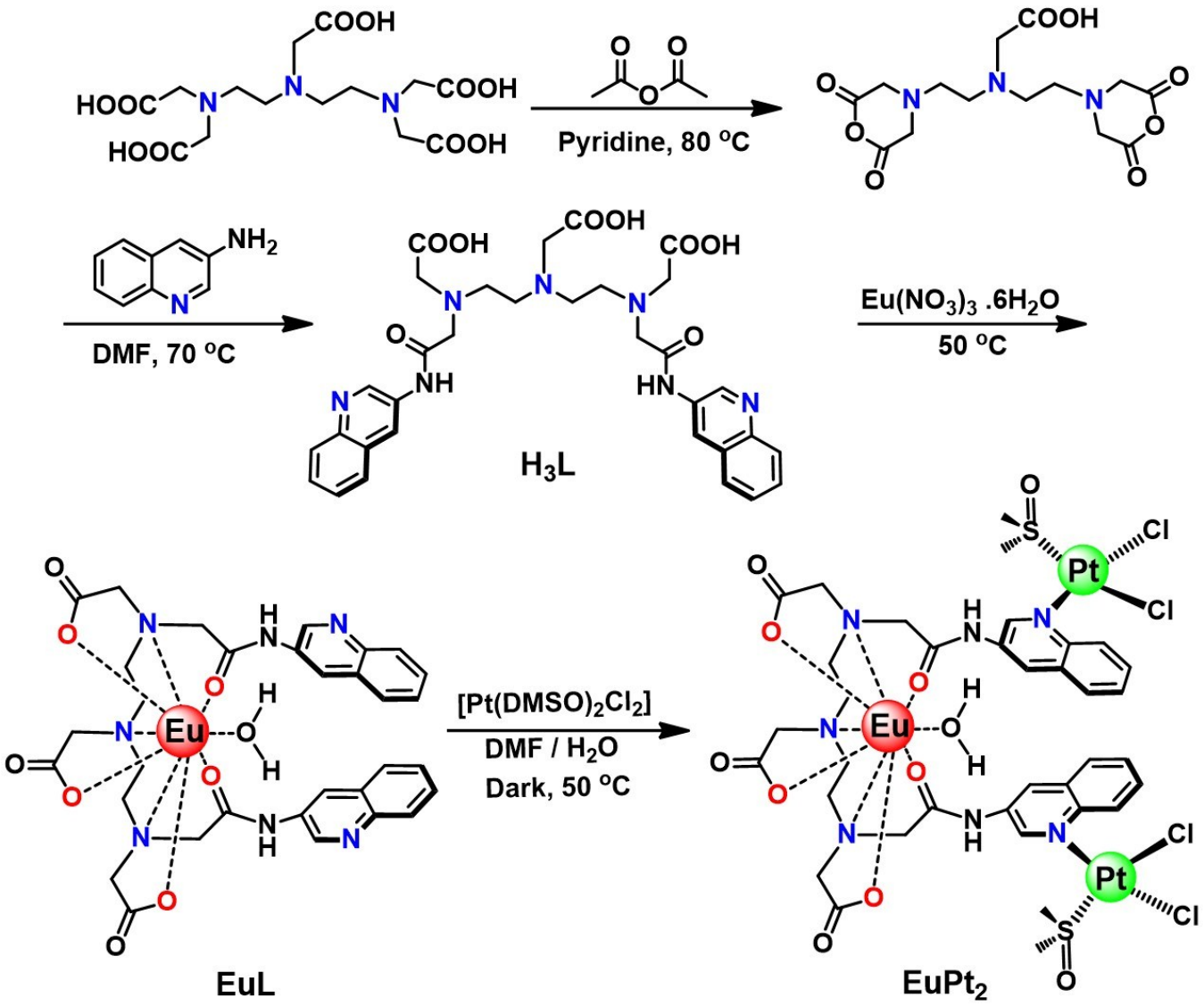
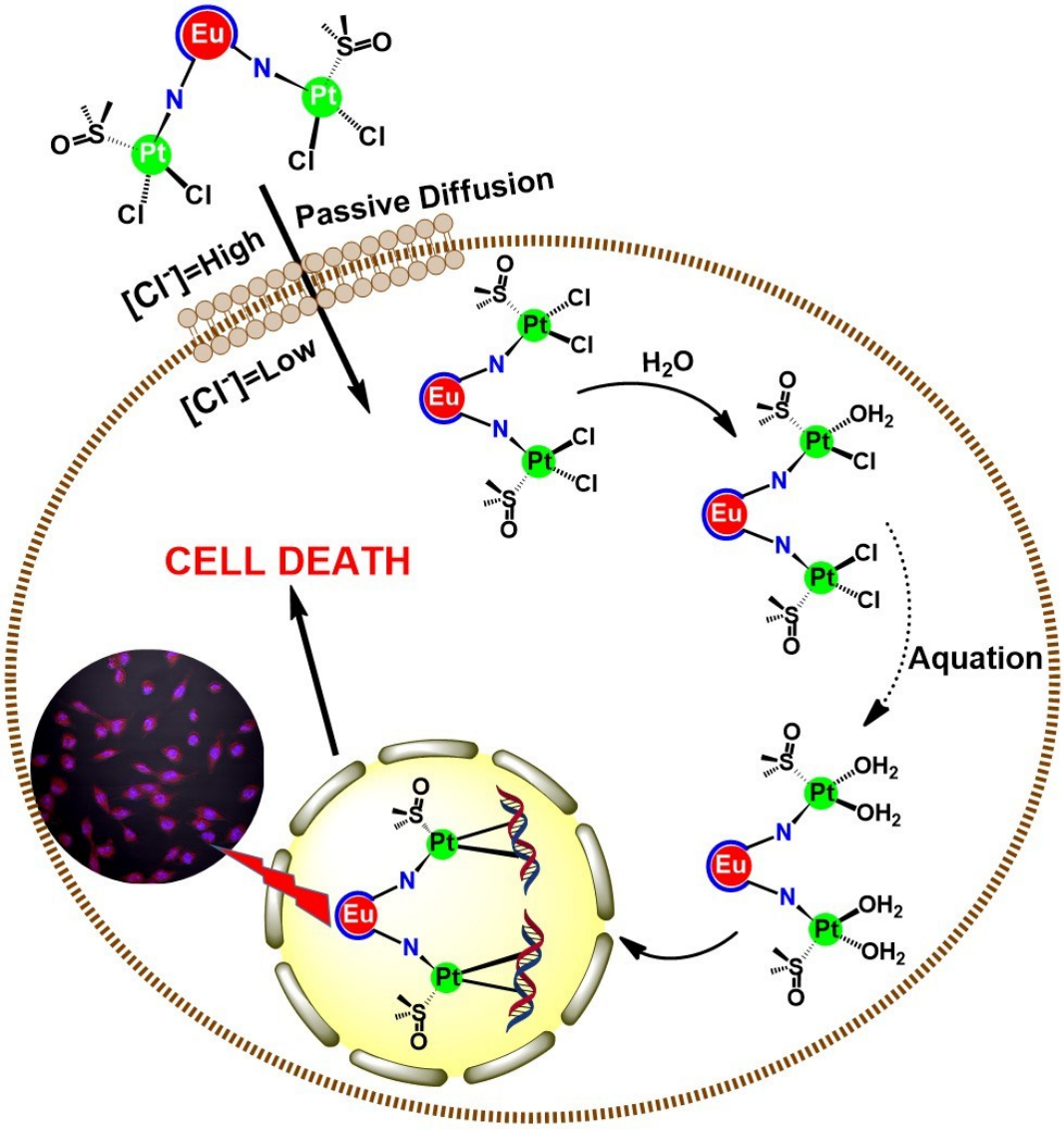


Scheme S1. Synthetic scheme for the preparation of ligand H_3L, EuL and Eu-Pt_2 complex **1**.



Scheme S2. Possible mechanism of cellular internalization and activation of Eu-Pt_2 complex **1** through activation by aquation, binding with DNA as evidenced from confocal fluorescence microscopy and cell death.

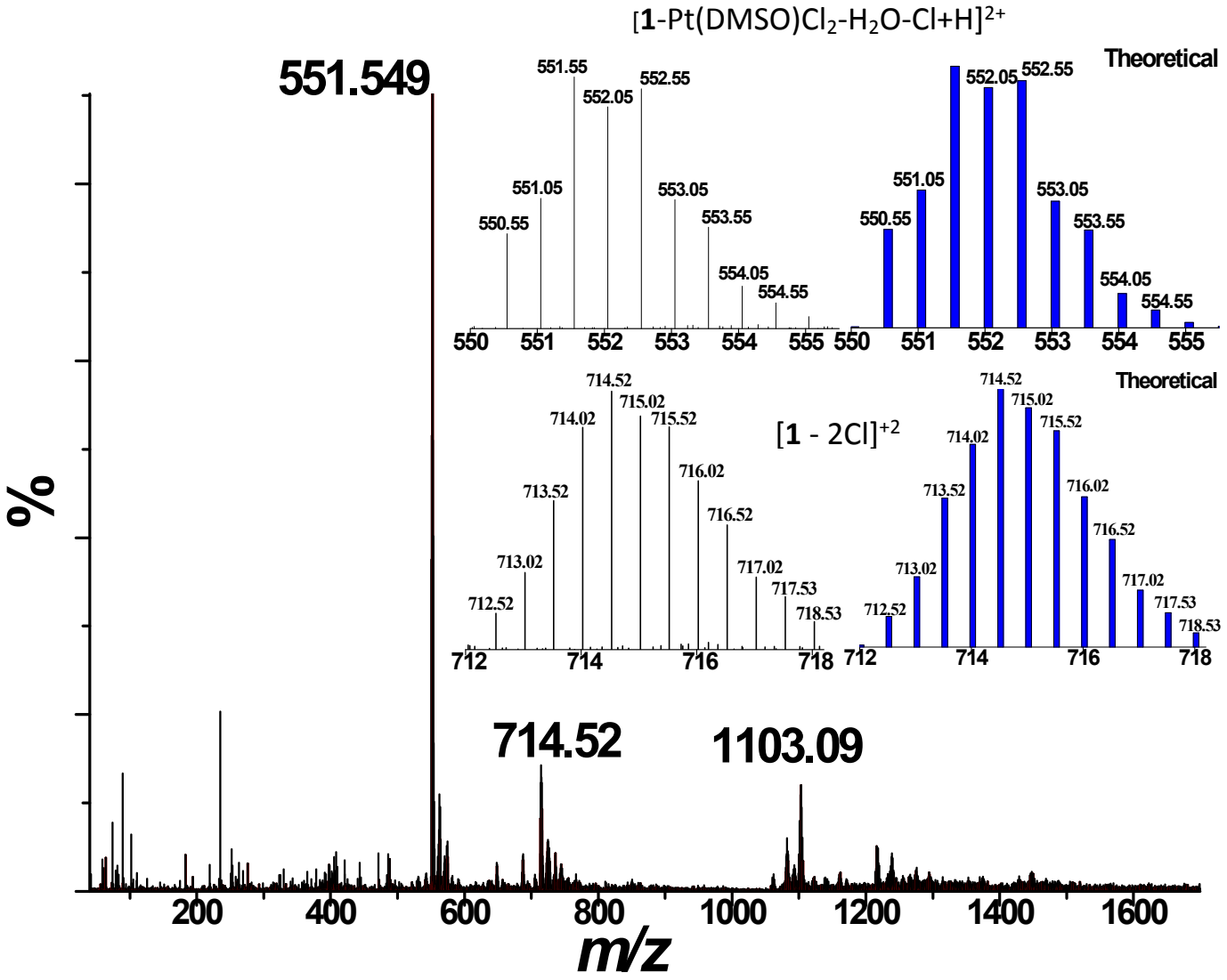


Figure S1. ESI-MS spectra of Eu-Pt_2 complex **1** in DMF-H_2O (1:1). Inset shows the experimental and theoretically simulated isotopic distribution patterns of the observed peaks.

Table S1. Assignments of the major peaks observed in the ESI-MS spectra of Eu-Pt_2 complex.

Complex	Observed *m/z*	Attribution	Formula	Calculated *m/z*
Eu-Pt_2 (1)	550.55-554.55	[**1**-Pt(DMSO)Cl_2-H_2O-Cl+H]$^{2+}$	$C_{34}H_{39}ClEuN_7O_9PtS$	551.55
	712.52-718.53	[**1** - 2Cl]$^{2+}$	$C_{36}H_{46}Cl_2EuN_7O_{11}Pt_2S_2$	714.52
	1103.09	[**1**-Pt(DMSO)Cl_2-H_2O-Cl]$^{+}$	$C_{34}H_{38}ClEuN_7O_9PtS$	1103.10

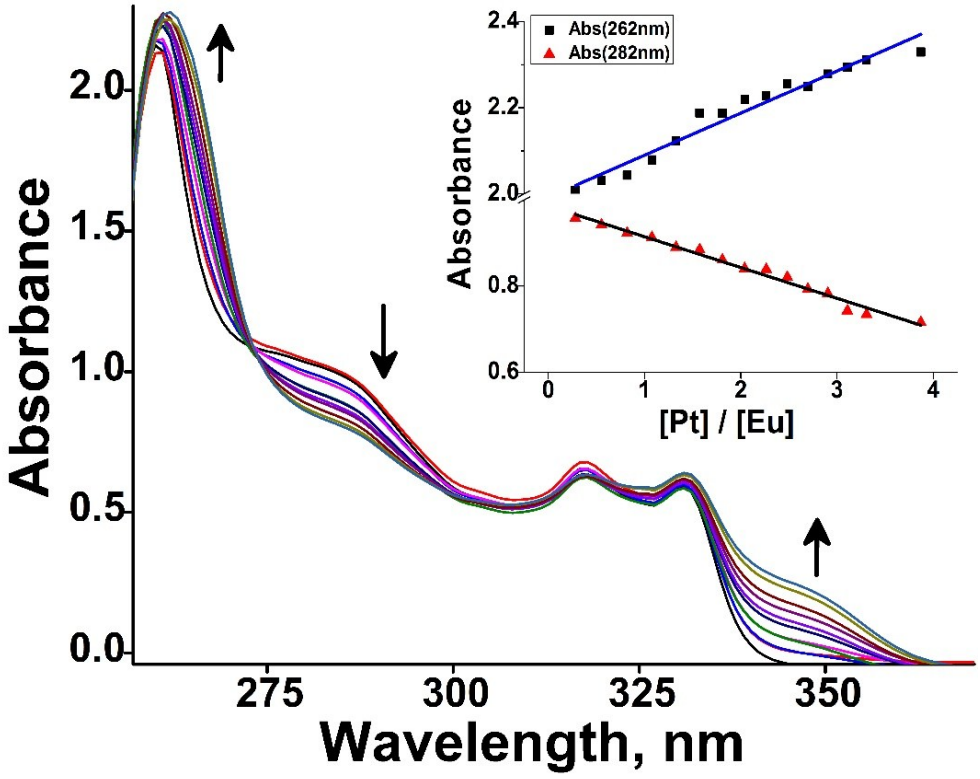


Figure S2. Evolution of the UV-visible absorption spectra of a 0.1 mM solution of [Eu(L)(H_2O)] upon addition of [*cis*-$PtCl_2(DMSO)_2$] in DMF at 298 K. Inset: Evolution of the absorbance's at 262 nm and 282 nm during the titration.

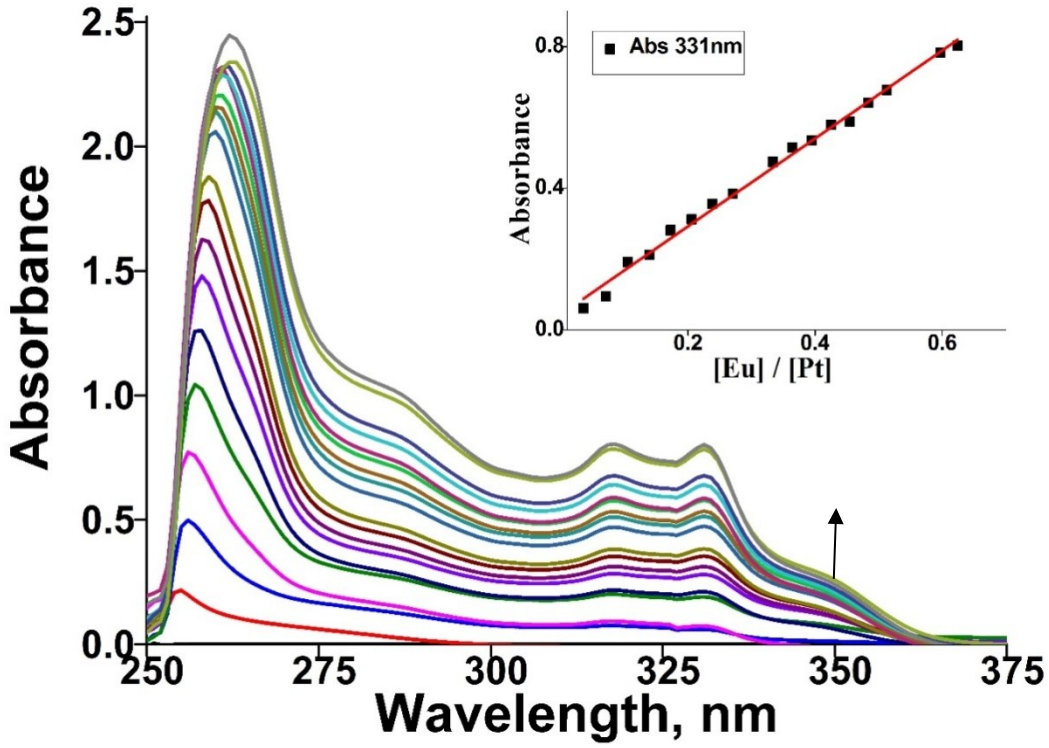


Figure S3. UV-Vis spectrophotometric titration of 0.1 mM solution *cis*-$[PtCl_2(DMSO)_2]$ with increasing concentration of EuL in DMF at 298 K. Inset: Evolution of the absorbances at 331 nm during the titration.

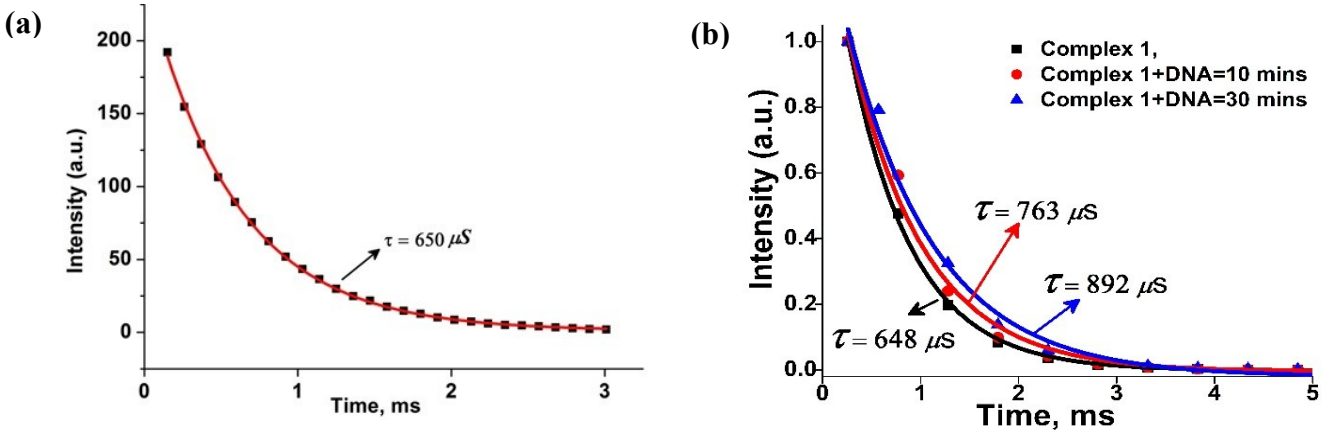


Figure S4. (a) Luminescence decay data showing the single-exponential character of the decays of Eu-Pt_2 complex in DMF-H_2O (1:1) and **(b)** Luminescence decay profile of Eu-Pt_2 complex (50 μM) in 5 mM Tris-HCl buffer (pH=7.2) in presence of CT-DNA (450 μM) at 298 K with different incubation times with CT-DNA. Delay time = 0.1 ms, gate time = 0.1 ms. All lifetime listed are within standard deviation of ± 10%.

Figure S5. Agarose gel containing CT-DNA bound Eu-Pt_2 complex showing red emission originated from f-f transition from Eu(III) in [{cis-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)] (300 μM) bound to CT-DNA (60μM) when excited at UV-A light of 365 nm.

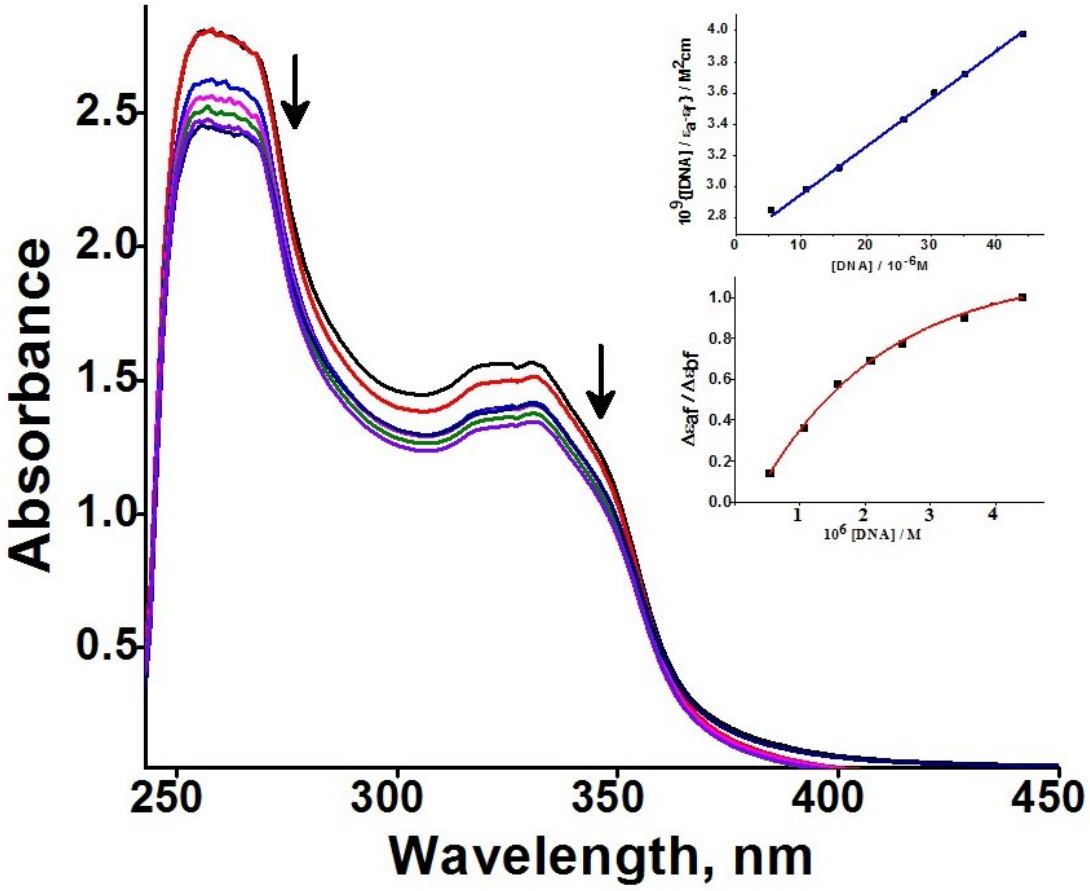


Figure S6. Absorption spectral traces of Eu-Pt_2 complex in 5 mM Tris-HCl buffer (pH 7.2) with increasing concentration of CT-DNA (280 μM) at room temperature. Inset: Figure shows plot of [DNA]/ ($\varepsilon_a - \varepsilon_f$) versus [DNA] and $\{(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)\}$ versus [DNA] of Eu-Pt_2 complex.

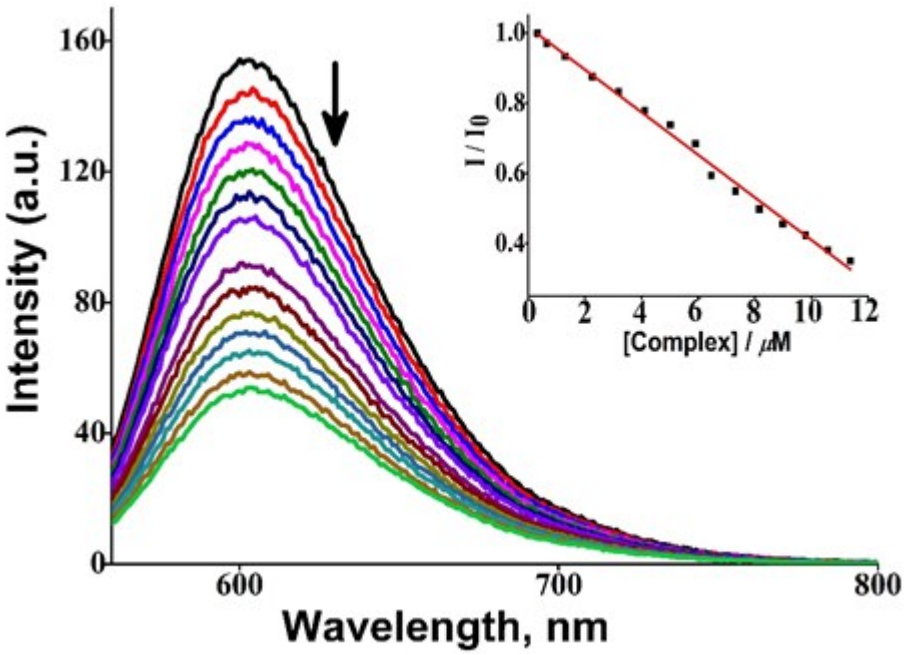


Figure S7. Fluorescence quenching curves of ethidium bromide (EthB) bound to CT-DNA with increasing concentration of Eu-Pt_2 complex in 5 mM Tris-HCl/NaCl buffer (pH 7.2). The arrow shows the intensity decreases on increasing the complex concentration. λ_{ex}= 546 nm, λ_{em} = 603 nm, [DNA] = 280 μM, [EthB] = 4 μM. Inset: The inset shows the plot of I/I_0 *vs.* [Eu-Pt_2].

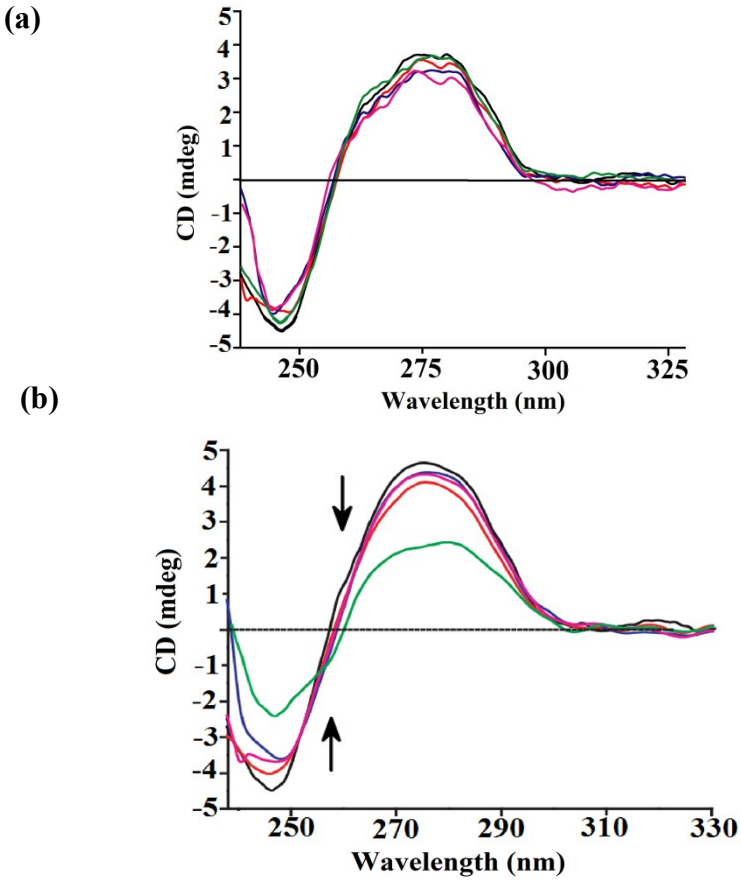


Figure S8. Evolution of circular dichroism (CD) spectra of CT-DNA (280 μM) in the presence of (a) EuL and (b) Eu-Pt_2 complexes in 5 mM Tris-HCl/NaCl buffer (pH 7.2) having [complex]/[CT-DNA] in different mole ratio ([complex]/[DNA] = 0.0, 0.1, 0.3, 0.5 and 0.8

equiv.) at 298 K. Arrow shows direction of change in CD signal with increasing complex concentration.

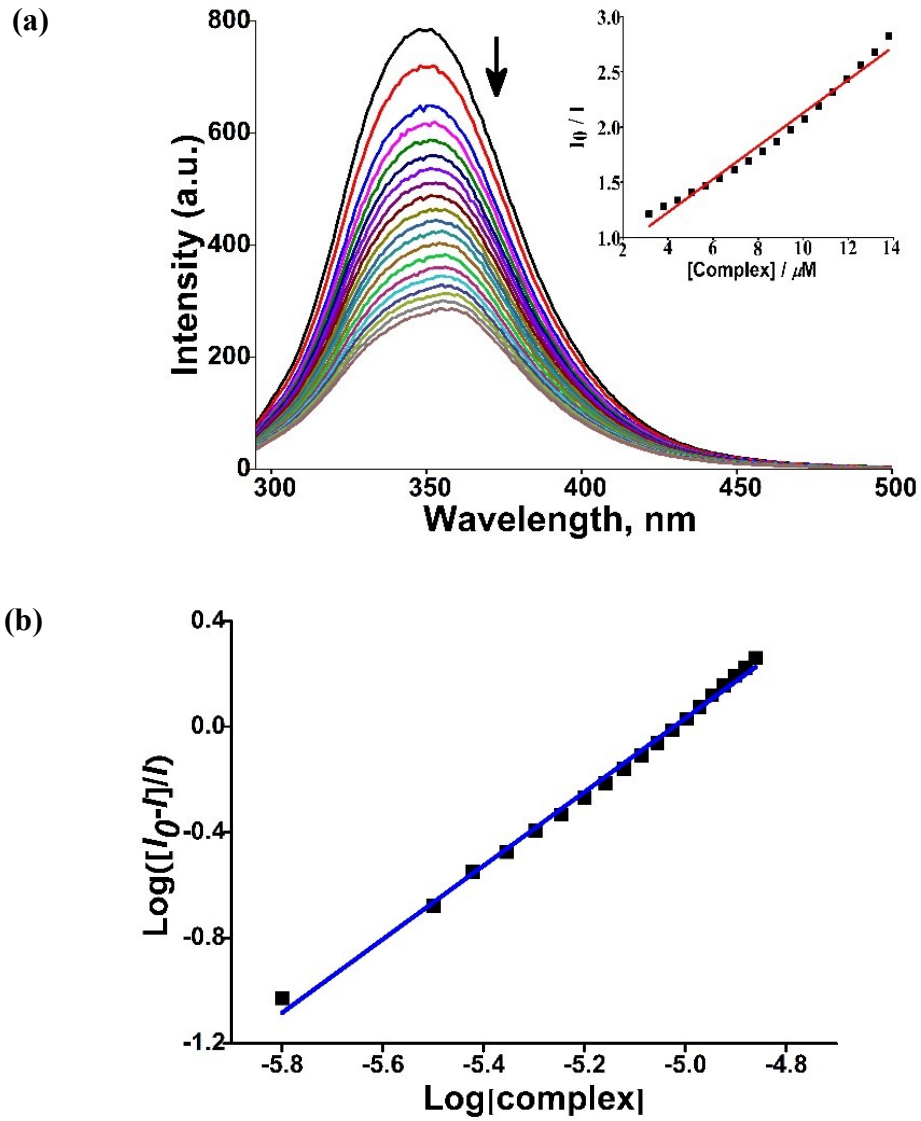


Figure S9. (a) Emission spectral traces of bovine serum albumin (BSA) protein (2 μM) with increasing concentration of $EuPt_2$ complex (**1**) (shown by arrow) in 5 mM Tris-HCl/NaCl buffer medium (pH 7.2). λ_{ex} = 295 nm, λ_{em} = 340 nm. The inset shows the plot of (I_0 /I) vs. [$EuPt_2$]. (b) Scatchard plot of log(I_0 −I)/I vs. log[$EuPt_2$] for BSA in the presence of Eu-Pt_2 complex **1**.

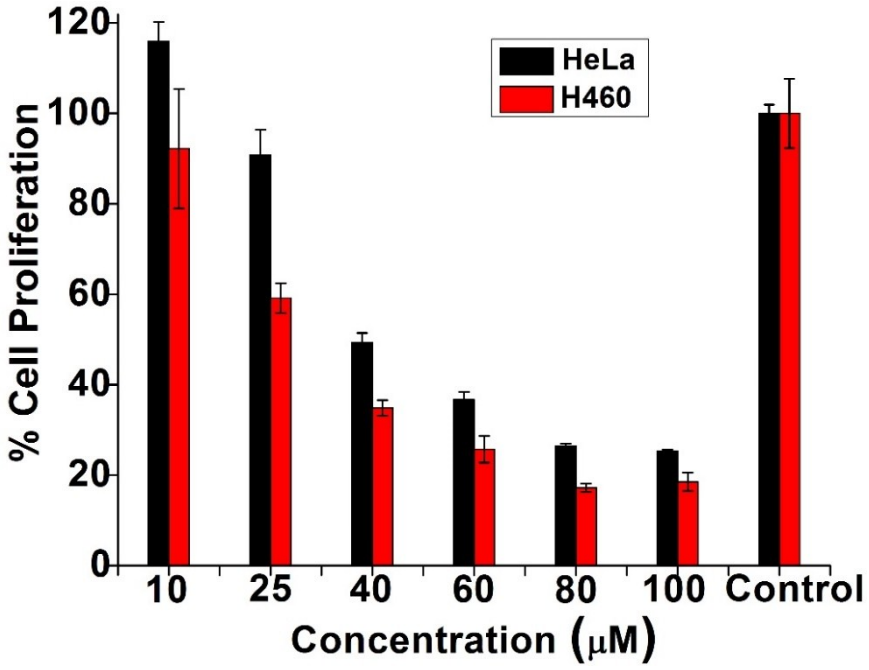


Figure S10. MTT cytotoxicity assay of Eu-Pt_2 complex **1** at various concentration with HeLa and H460 cells upon incubation for 16 h.

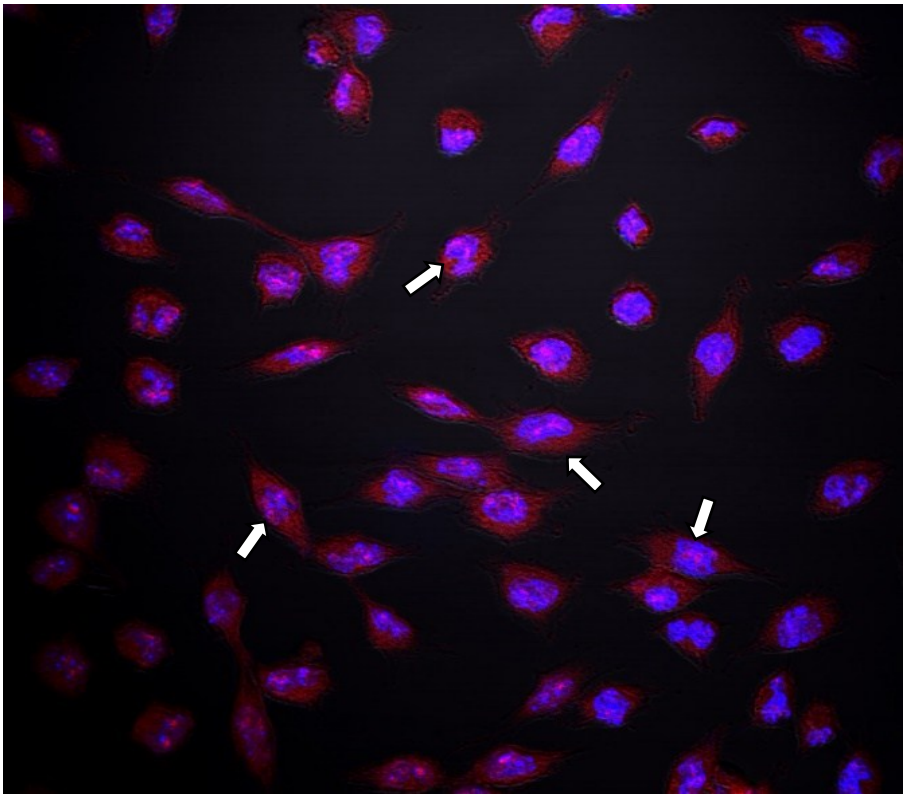


Figure S11. Confocal laser scanning microscopic image of the HeLa cancer cells treated with Eu-Pt_2 complex (25 μM) and Hoechst 33258 dye (5 μg mL^{-1}) on 4 h incubation, showing cellular internalization of Eu-Pt_2 complex (red emission). Arrow head indicating the red spots in nuclei originated from Eu^{3+}-based luminescence from Eu-Pt_2 complex indicating its nuclear localization.