Enhanced photodynamic effect of cobalt(III) dipyridophenazine complex on thyrotropin receptor expressing HEK293 cells

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Electronic Supporting Information

Experimental Section.

DNA binding experiments. The CT DNA concentration was measured from its absorption intensity at 260 nm using molar absorption coefficient (ε_{260}) value of 6600 M^{-1} cm^{-1}.[1] Absorption titration experiments were performed using different concentrations of CT DNA in 50 mM Tris-HCl buffer keeping the complex (dissolved in DMF) concentration as constant. Due correction was made for the absorbance of the CT DNA itself. The samples were equilibrated before recording each spectrum. The equilibrium binding constant (K_b) values of the complexes were determined from a non-linear fit of the plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ *vs.* [DNA] applying McGhee-von Hippel (MvH) method and using the expression of Bard and coworkers:

$(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2K_b^2 C_t[\mathrm{DNA}]_t/s)^{1/2})/2K_bC_t$ … (1)

where $b = 1 + K_bC_t + K_b[\mathrm{DNA}]/2s$, K_b is the binding constant, C_t is the total concentration of the metal complex, and s is the fitting constant giving an estimate of the binding site size (in base pairs) of the metal complex interacting with the DNA, ε_f, ε_a and ε_b are, respectively, the molar extinction coefficients of the free complex in solution, complex bound to DNA at a definite concentration and the complex in completely bound form with CT DNA.[2] The non-linear least-squares analysis was done using Origin Lab., version 6.1.

The apparent binding constant (K_{app}) values of the complexes **1-3** were obtained by fluorescence spectral technique using ethidium bromide (EB) bound CT DNA solution in Tris-HCl/NaCl buffer (pH, 7.2). The fluorescence intensities of EB at 600 nm (546 nm excitation) were monitored with an increasing amount of the ternary complex concentration. EB was non-emissive in Tris-buffer medium due to fluorescence quenching of the free EB by the solvent molecules.[3,4] In presence of CT DNA, EB showed enhanced emission intensity due to its binding to DNA. A competitive binding of the diamagnetic cobalt(III) complexes to CT DNA could result displacement of the bound EB to the solution thus decreasing its emission intensity. The K_{app} values were obtained from the equation: K_{app} x $[\mathrm{complex}]_{50} = K_{EB}$ x [EB], where K_{app} is the apparent binding constant of the complex studied, $[\mathrm{complex}]_{50}$ is the concentration of the complex at 50% quenching of DNA bound EB emission

intensity, K_{EB} is the binding constant of EB (K_{EB} = 1.0 x 10^7 M^{-1}) and [EB] is the concentration of EB (1.3 μM).[5] DNA melting experiments were carried out by monitoring the absorbance of CT DNA (260 nm) at various temperatures in the absence and presence of the complexes in a 15:1 molar ratio of the CT DNA and the complex with a ramp rate of 0.5 °C min^{-1} in 5 mM phosphate buffer medium (pH 6.85) using Cary 300 bio UV-visible spectrometer with Cary temperature controller.

DNA photocleavage experiments. The photo-induced cleavage of supercoiled (SC) pUC19 DNA by the cobalt(III) complexes **1-3** was investigated by agarose gel electrophoresis. The reactions were carried out using a UV-A lamp of 365 nm (6 W, sample area of illumination: 45 mm^2) and in red light of 676 nm using a continuous-wave (CW) Argon-Krypton laser of 50 mW laser power, laser beam diameter of 1.8 mm with a beam divergence of 0.70 mrad of the Spectra Physics Water-Cooled Mixed-Gas Ion Laser Stabilite® 2018-RM. The power of the laser beam at the sample position was measured using Spectra Physics CW Laser Power Meter (Model 407A). Eppendorf and glass vials were used for respective UV-A and visible light experiments in a dark room at 25 ºC using SC DNA (1 μL, 30 μM) in 50 mM Tris-HCl buffer (pH 7.2) containing 50 mM NaCl and the complex (2 μL) with varied concentrations. The concentration of the complexes in DMF or the additives in buffer corresponded to the quantity in 2 μL stock solution after dilution to the 20 μL final volume using Tris-HCl buffer. The solution path length in the sample vial was ~5 mm. After the photoexposure, the samples were incubated for 1 h at 37 ºC, followed by its addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (3 μL). The solutions were finally loaded on 1% agarose gel containing 1.0 μg mL^{-1} ethidium bromide. Electrophoresis was carried out in a dark room for 2 h at 45 V in Tris-acetate-EDTA (TAE) buffer. The bands were visualized by UV light and photographed. The extent of DNA cleavage was determined by measuring the intensities of the bands using a UVITECH Gel Documentation System. Due corrections were made for the presence of minor quantity of nicked circular (NC) DNA in the original SC DNA sample and for the low affinity of ethidium bromide binding to SC compared to NC and linear forms of DNA.[6] The extent of experimental error in measuring the SC and NC forms of DNA from the gel diagrams varied from

3-5%. The inhibition reactions for the photo-induced DNA cleavage studies were carried out using different reagents (NaN_3, 0.5 mM; DMSO, 4 μL; KI, 0.5 mM; TEMP, 0.5 mM; DABCO, 0.5 mM; catalase, 4 units; superoxide dismutase, 4 units; distamycin-A, 50 μM) prior to the addition of the complexes. For the D_2O experiment, this solvent was used for dilution of the stock solution to 20 μL.

References.

(1) M. E. Reichman, S. A. Rice, C. A. Thomas and P. Doty, *J. Am. Chem. Soc.,* 1954, **76**, 3047-3053.

(2) (*a*) J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.,* 1974, **86**, 469-489. (*b*) M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc.,* 1989, **111**, 8901-8911.

(3) M. Waring, *J. Mol. Biol.,* 1965, **13**, 269-282.

(4) J.-B. LePecq and C. Paoletti, *J. Mol. Biol*., 1967, **27**, 87-106.

(5) M. Lee, A. L. Rhodes, M. D. Wyatt, S. Forrow and J. A. Hartley, *Biochemistry,* 1993, **32**, 4237-4245.

(6) J. Bernadou, G. Pratviel, F. Bennis, M. Girardet and B. Meunier, *Biochemistry,* 1989, **28**, 7268-7275.

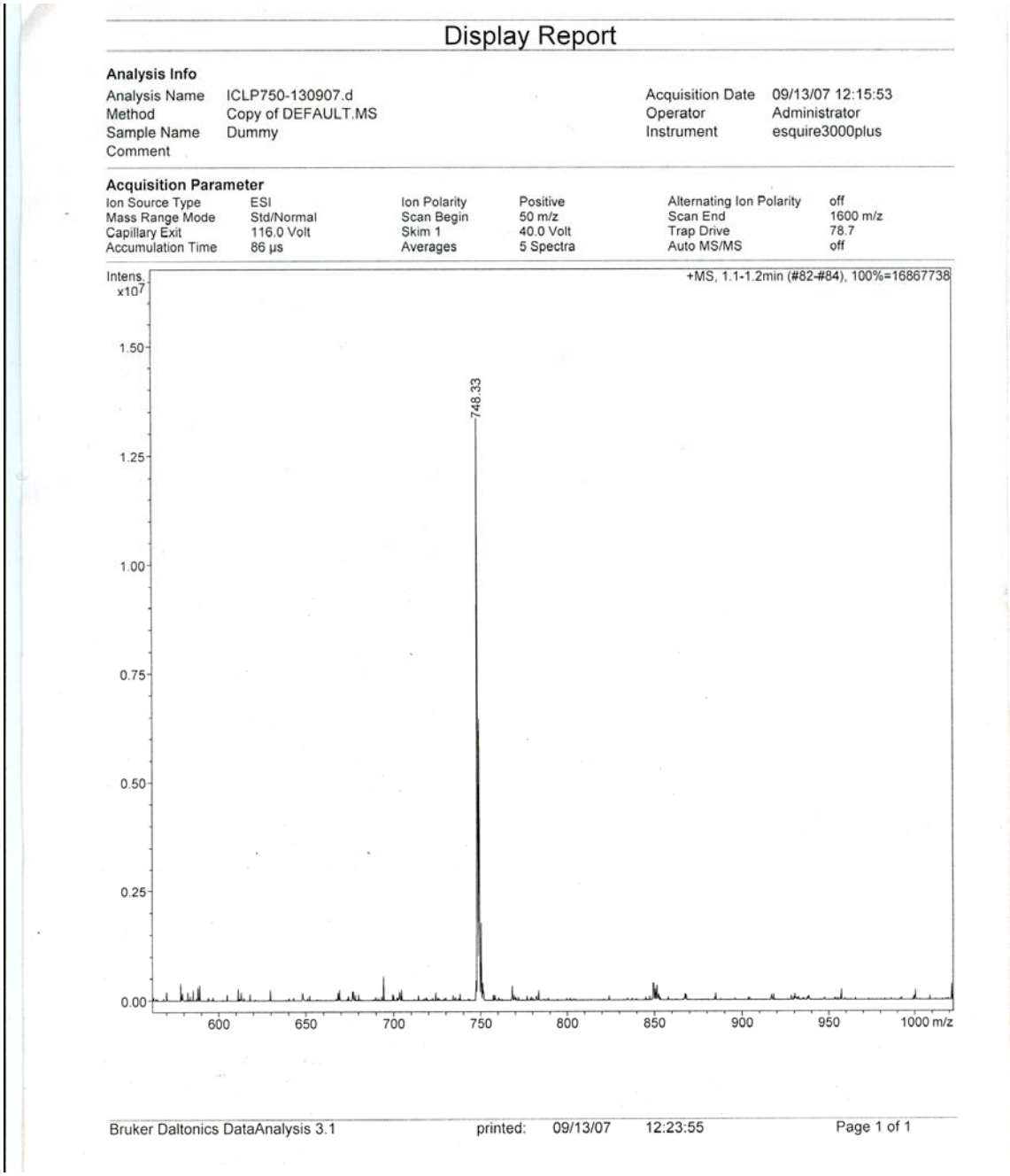

Fig. S1ESI (+ve) mass spectrum of [CoL(phen)] (**1**) in MeOH.

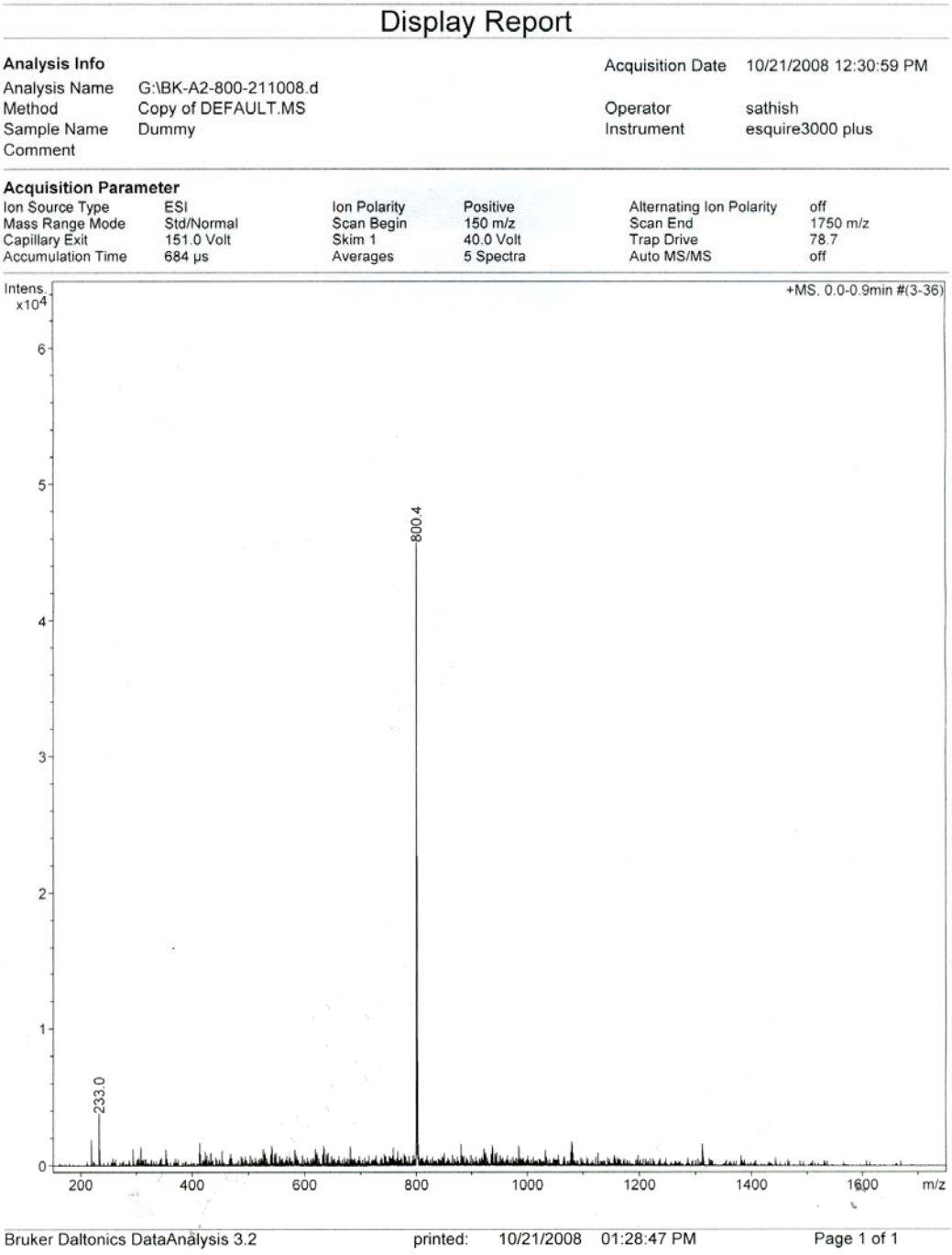

Fig. S2 ESI (+ve) mass spectrum of [CoL(dpq)] (**2**) in MeOH.

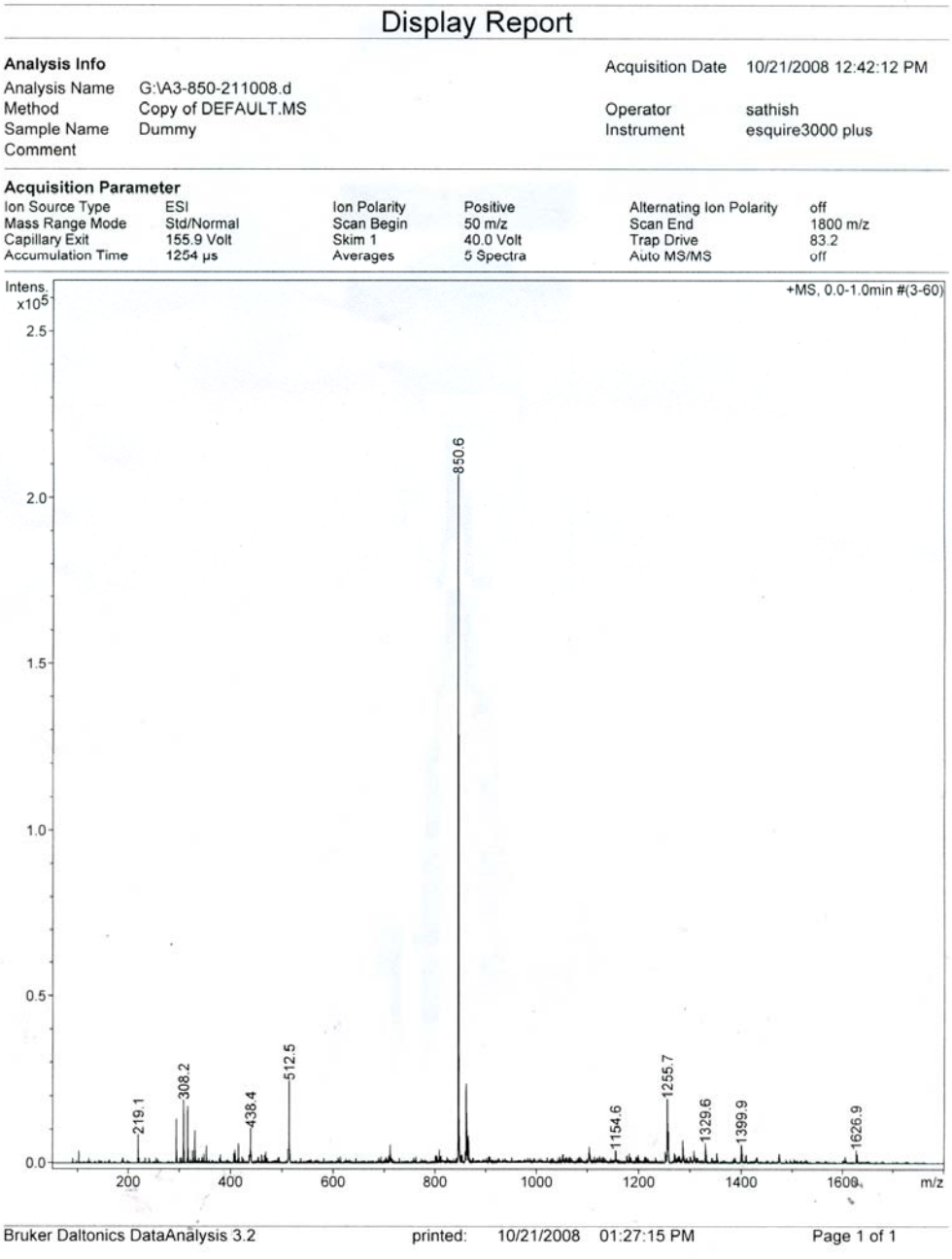

Fig. S3 ESI (+ve) mass spectrum of [CoL(dppz)] (**3**) in MeOH.

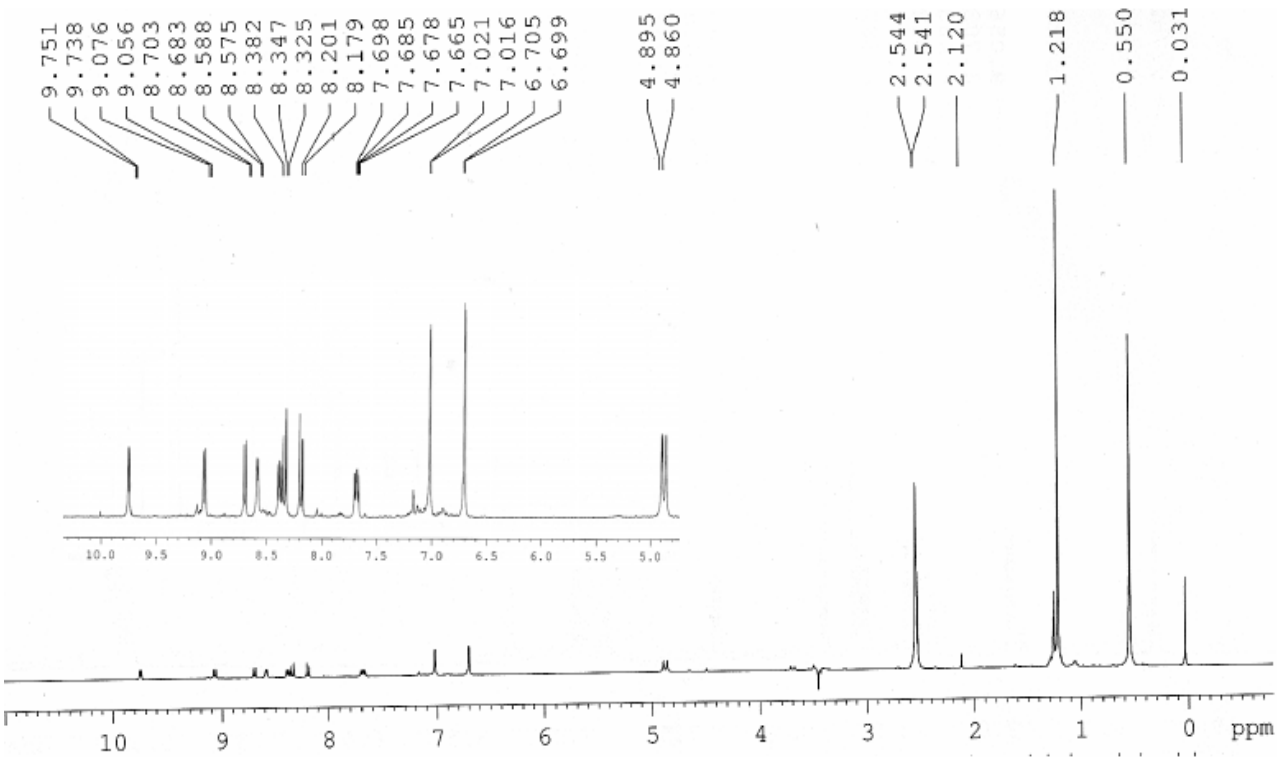

Fig. S4 ^{1}H NMR spectrum (400 MHz) of [CoL(phen)] (**1**) in DMSO-d_6. Four CH_2 hydrogen atoms are buried in the suppressed H_2O in DMSO peak (3.0-4.0 ppm).

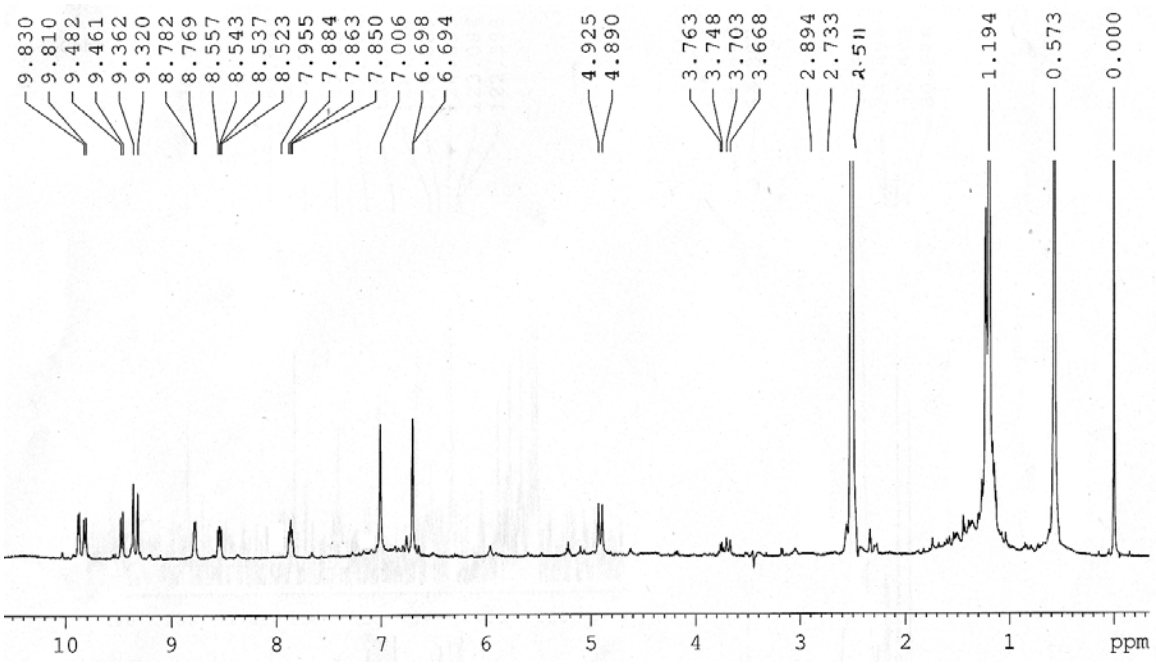

Fig. S5 ^{1}H NMR spectrum (400 MHz) of [CoL(dpq)] (**2**) in DMSO-d_6. Four CH_2 hydrogen atoms are buried in the suppressed H_2O in DMSO peak (3.0-4.0 ppm).

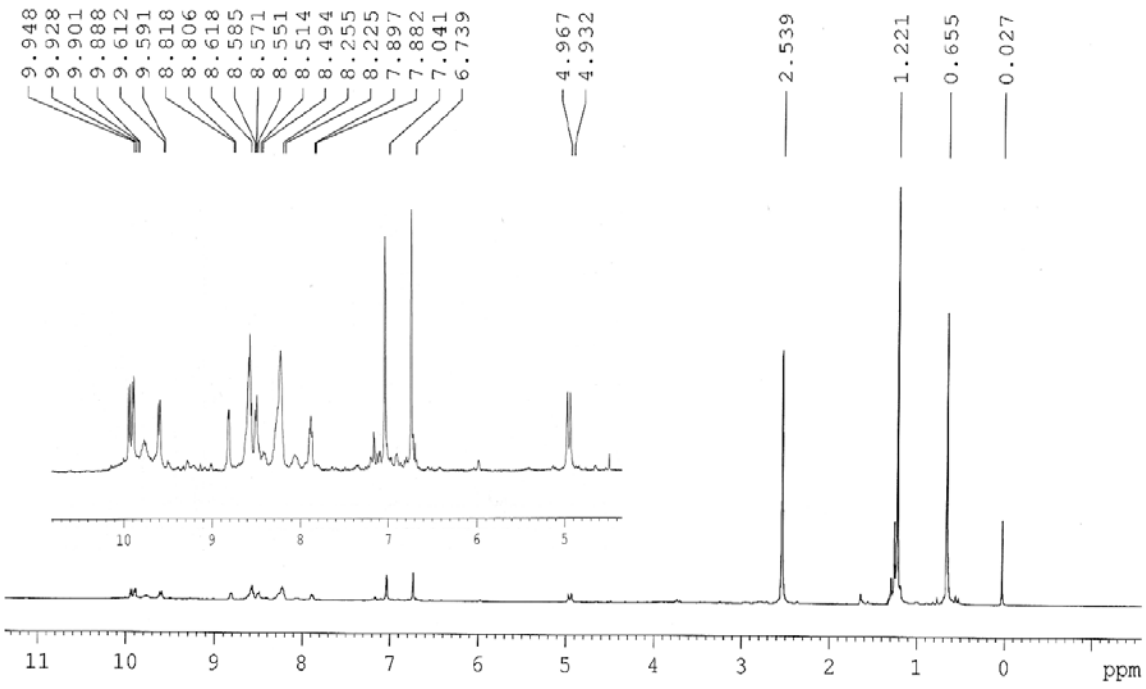

Fig. S6 ^{1}H NMR spectrum (400 MHz) of [CoL(dppz)] (**3**) in DMSO-d_6. Four CH_2 hydrogen atoms are buried in the suppressed H_2O in DMSO peak (3.0-4.0 ppm).

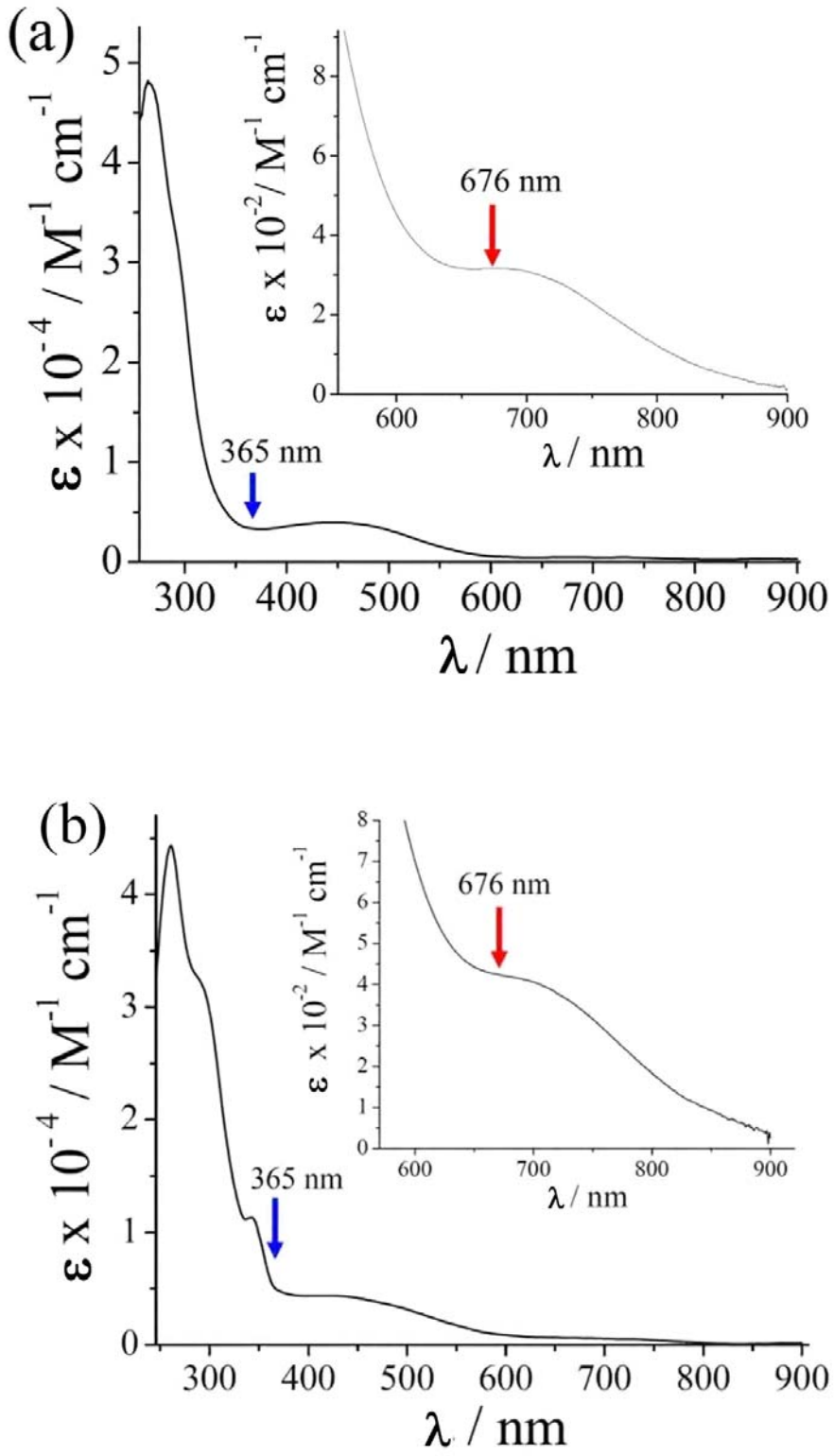

Fig. S7 UV-visible spectra of (a) [CoL(phen)] (**1**) and (b) [CoL(dpq)] (**2**) in 6% DMF/ 5 mM Tris-HCl buffer (pH 7.2). The DNA photocleavage wavelengths are shown by arrow.

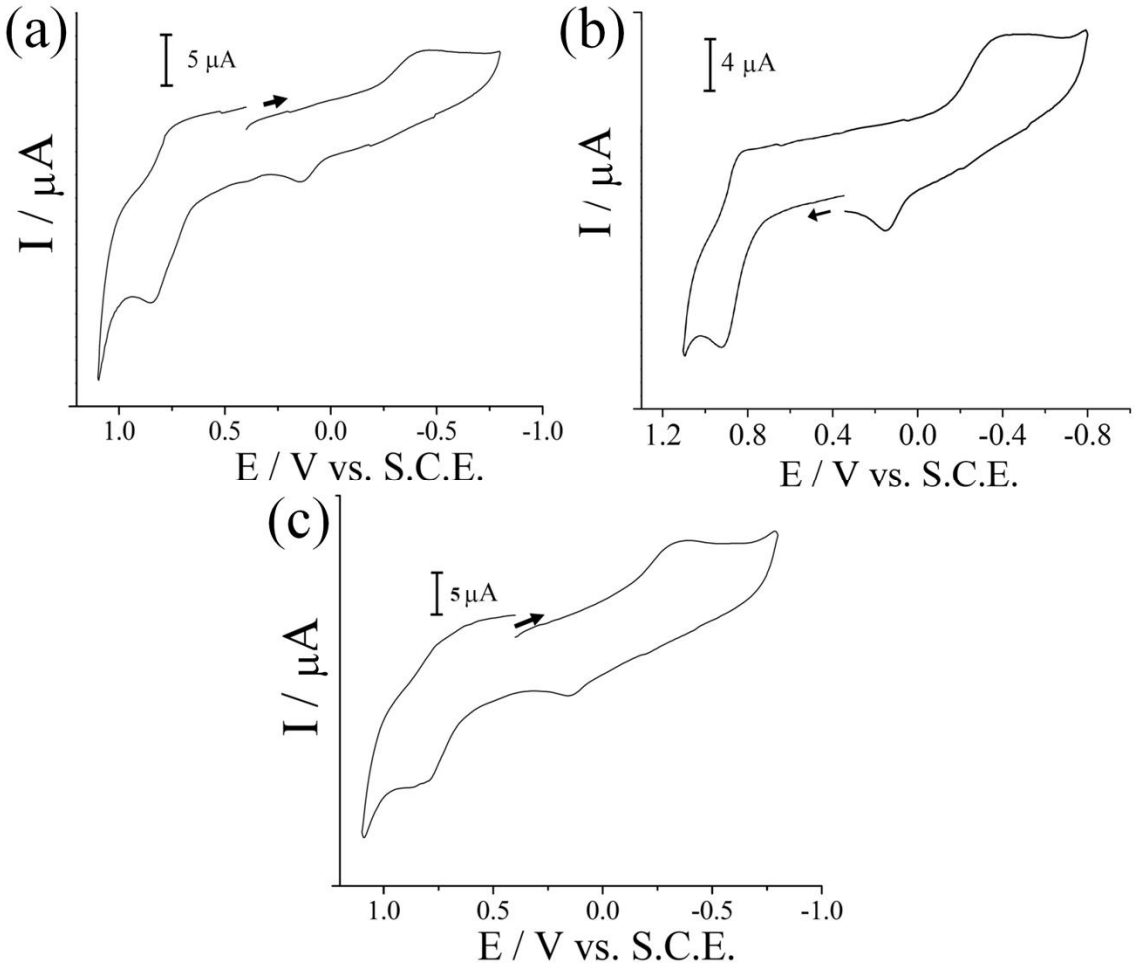

Fig. S8 Cyclic voltammograms for the complexes **1-3** in DMF with 0.1 M TBAP as supporting electrolyte at a scan rate of 50 mV/s: (a) [CoL(phen)], (b) [CoL(dpq)] and (c) [CoL(dppz)].

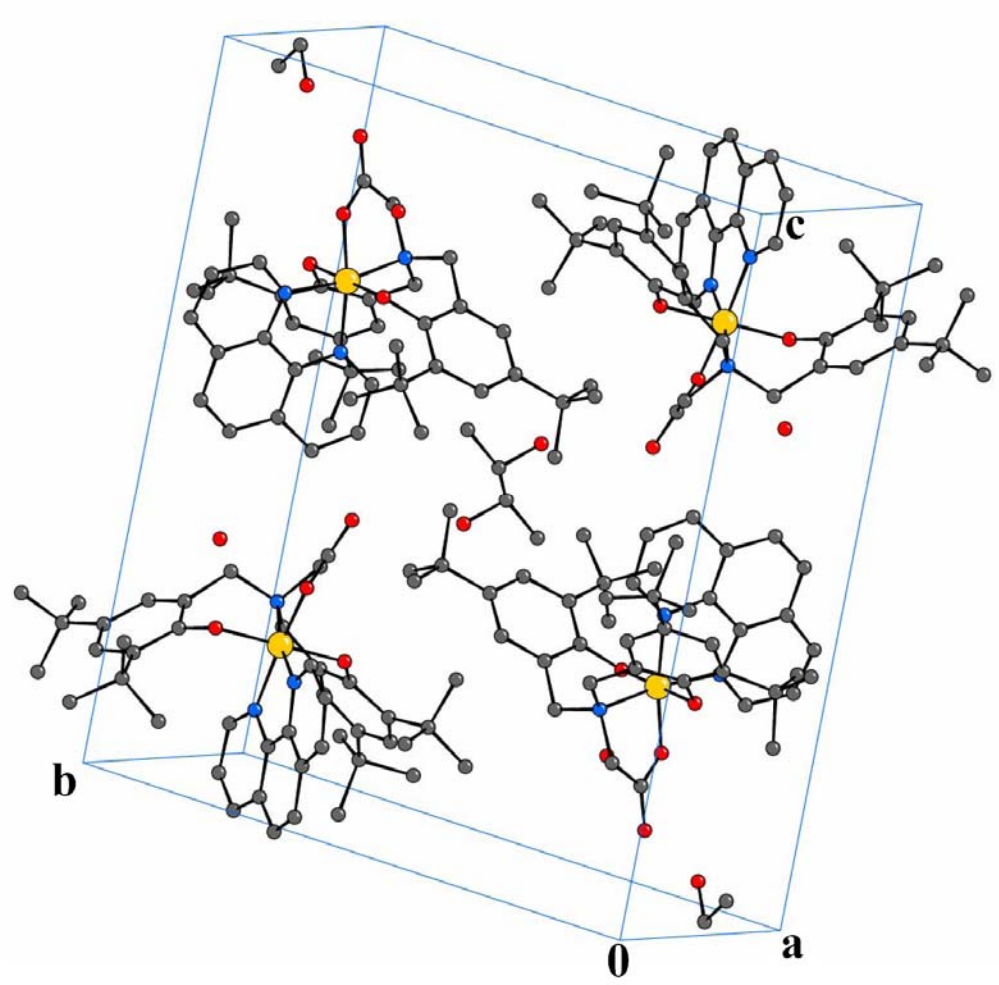

Fig. S9 Unit cell packing diagram for **1**·0.5EtOH·H_2O in $P2_1/c$ space group with four molecules in the unit cell. The hydrogen atoms are omitted for clarity.

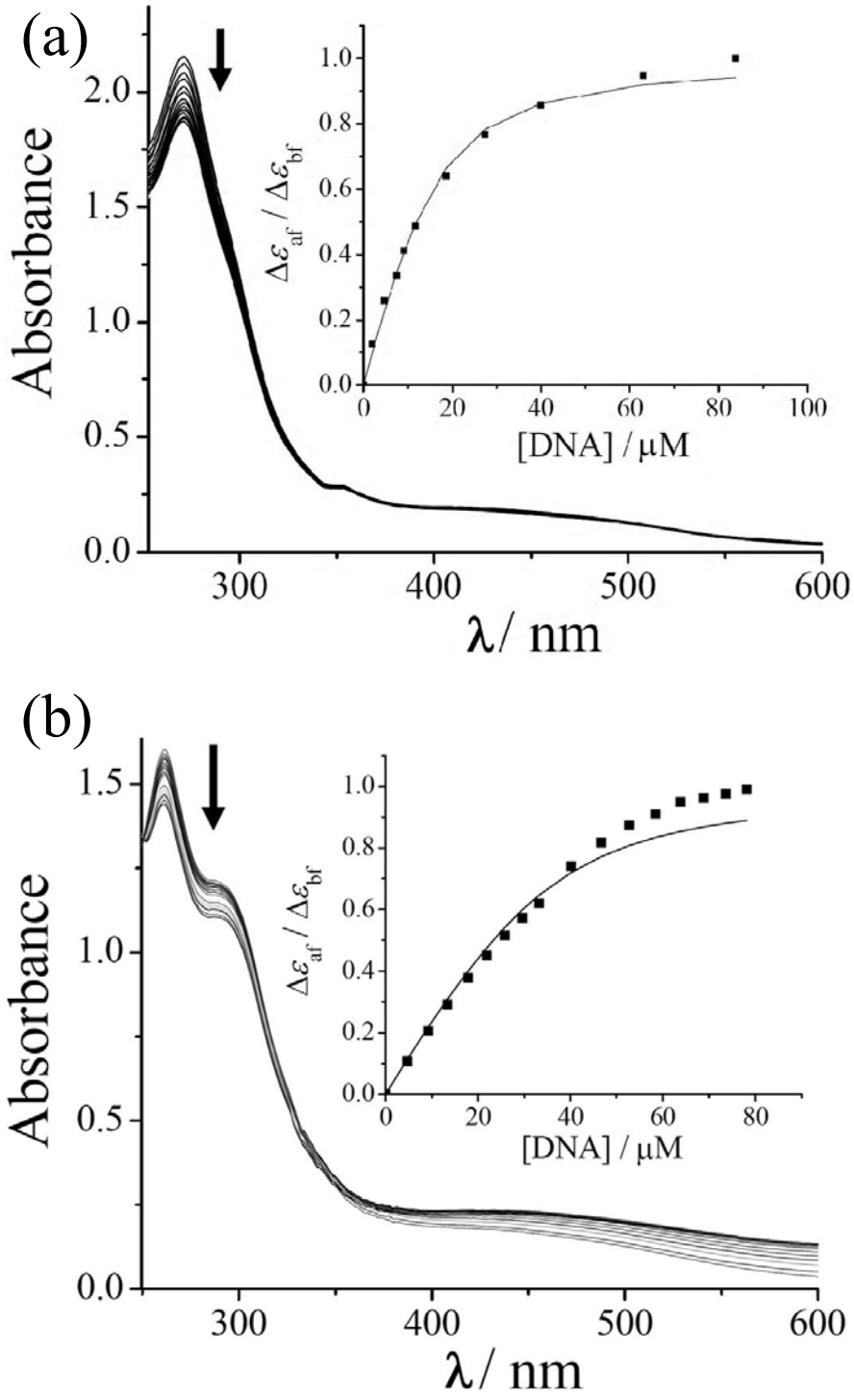

Fig. S10 Absorption spectral traces of (a) complex **1** (40 μM) and (b) complex **2** (40 μM) in 5 mM Tris-HCl buffer (pH 7.2) containing 6% DMF on increasing the quantity of CT DNA (241 μM NP). The insets show the least-squares fit of $\Delta\varepsilon_{af}/\Delta\varepsilon_{bf}$ vs. [DNA] using MvH equation (vide text).

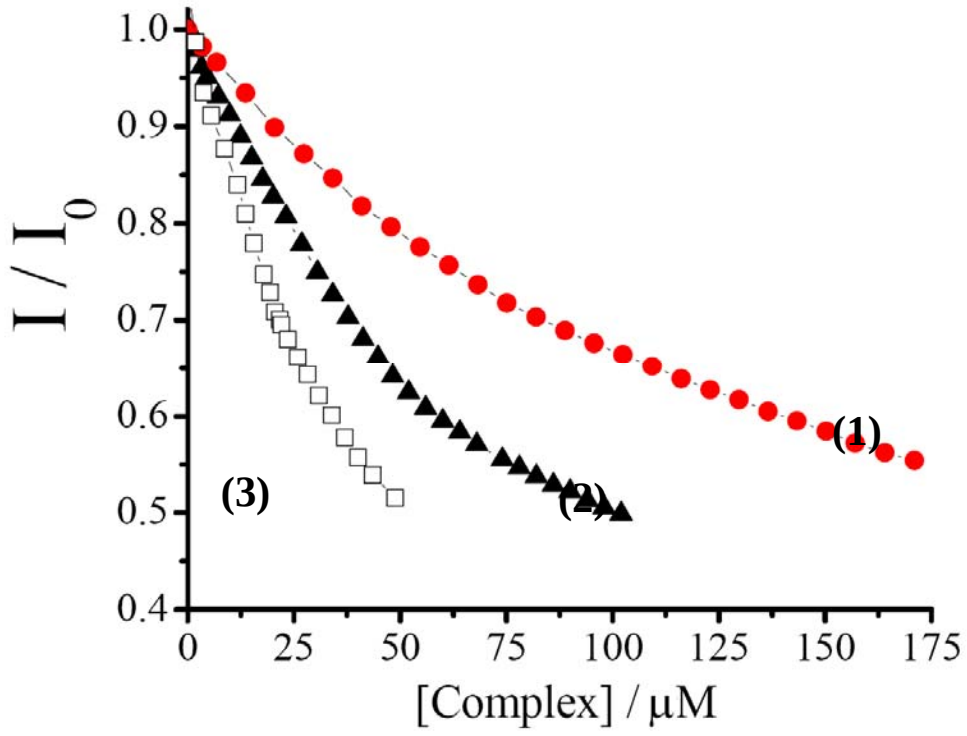

Fig. S11 Ethidium bromide displacement assay for determining the apparent DNA binding constant (K_{app}) for the complexes **1-3** in 5 mM Tris HCl buffer (pH 7.2). The plot shows the decrease in I/I_0 with increasing complex concentrations, where I_0 is the fluorescence intensity of ethidium bromide (saturated with CT DNA) and I is the fluorescence intensity of ethidium bromide (saturated with CT-DNA) after addition of the complexes.

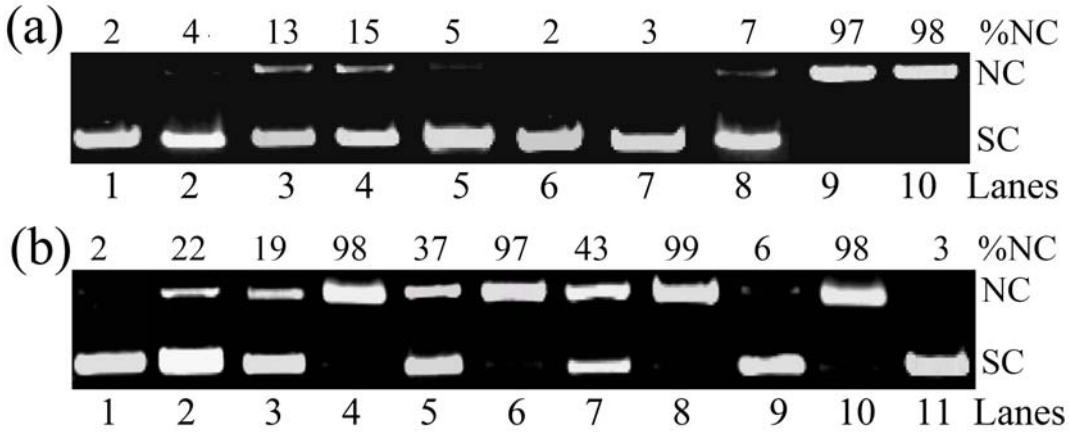

Fig. S12 Photocleavage of SC pUC19 DNA (0.2 μg, 30 μM) by [CoL(B)] (5 μM; B = phen, **1**; dpq, **2**; dppz, **3**) in Tris-HCl/NaCl buffer (pH 7.2) containing 10% DMF on photo-exposure to UV-A light of 365 nm (6 W) for 2 h: (a) lane 1, DNA control; lane 2, DNA + $Co(NO_3)_2.6H_2O$ (30 μM); lane 3, DNA + dpq (30 μM); lane 4, DNA + dppz (30 μM); lane 5, DNA + H_3L (30 μM); lane 6, DNA + **2** (5 μM) (in dark); lane 7, DNA + **3** (5 μM) (in dark); lane 8, DNA + **1** (5 μM); lane 9, DNA + **2** (5 μM); lane 10, DNA + **3** (5 μM) and (b) lane 1, DNA control; lane 2, DNA + distamycin-A (50 μM); lane 3, DNA + distamycin-A (50 μM) + **2** (5 μM); lane 4, DNA + distamycin-A (50 μM) + **3** (5 μM); lane 5, DNA + methyl green (50 μM); lanes 6, DNA + methyl green (50 μM) + **2** (5 μM); lane 7, DNA + methyl green (50 μM) + **3** (5 μM); lane 8, DNA + **2** (5 μM); lane 9, DNA + **2** (5 μM) (argon atmosphere); lane 10, DNA + **3** (5 μM); lane 11, DNA + **3** (5 μM) (argon atmosphere).

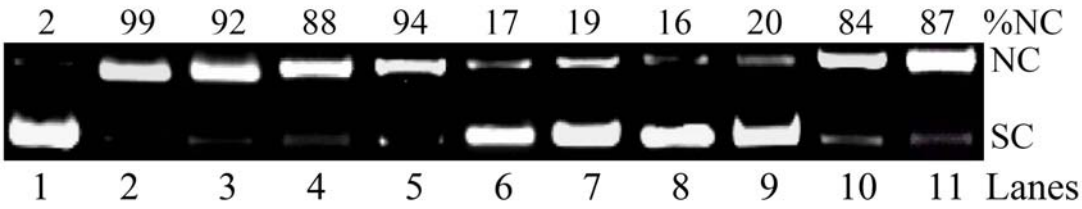

Fig. S13 The gel electrophoresis diagram showing the mechanistic aspect of photocleavage of SC pUC19 DNA (0.2 μg, 30 μM bp) by **3** (5 μM) in 50 mM Tris-HCl / NaCl buffer medium (pH 7.2) containing 6% DMF on irradiation with UV light of 365 nm (6 W) having the sample at a distance of 5 cm from the source. The reaction conditions and the cleavage data are given below in a tabular form.

Lane No.	Reaction conditions	λ / nm (t / h)	%NC form
1	DNA control	365 (2)	2
2	DNA + **3** (5 μM)	365 (2)	99
3	DNA + **3** (5 μM) + TEMP (500 μM)	365 (2)	92
4	DNA + **3** (5 μM) + NaN_3 (500 μM)	365 (2)	88
5	DNA + **3** (5 μM) + DABCO (500 μM)	365 (2)	94
6	DNA + **3** (5 μM) + SOD (4 units)	365 (2)	17
7	DNA + **3** (5 μM) + DMSO (4 μL)	365 (2)	19
8	DNA + **3** (5 μM) + KI (500 μM)	365 (2)	16
9	DNA + **3** (5 μM) + Catalase (4 units)	365 (2)	20
10	DNA + **3** (5 μM)	365 (1.5)	84
11	DNA + **3** (5 μM) + D_2O (16 μL)	365 (1.5)	87

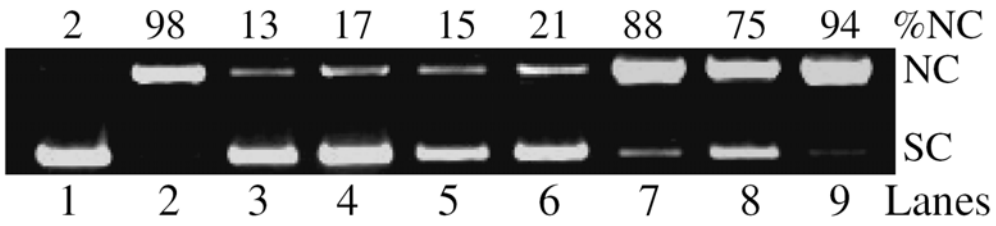

Fig. **S14** The gel electrophoresis diagram showing mechanistic aspects of photocleavage of SC pUC19 DNA (0.2 μg, 30 μM bp) by **3** (30 μM) in Tris-HCl/NaCl buffer medium (pH 7.2) containing 6% DMF on irradiation at 676 nm (50 mW) wavelength using a CW Ar-Kr laser source. The reaction conditions and the cleavage data are given below in a tabular form. **DNA photocleavage experiments:** The visible light source used was from a tunable wavelength Spectra Physics Water-Cooled Mixed-Gas Ion Laser StabiliteÒ 2018-RM with Laser Power Meter Model 407A (50 mW, CW beam diameter at $1/e^2$ 1.8 mm ± 10%, beam divergence 0.7 mrad ± 10%). The sample was irradiated at a distance of 10 cm from the aperture of the instrument. The power of the emerging laser beam was directly measured at a distance of 10 cm from the aperture with a thermo-couple based Laser Power Meter (model 407A).

Lane No.	Reaction conditions	λ / nm (t / h)	%NC form
1	DNA control	676 (2)	2
2	DNA + **3** (30 μM)	676 (2)	98
3	DNA + **3** (30 μM) + KI (500 μM)	676 (2)	13
4	DNA + **3** (30 μM) + DMSO (4 μL)	676 (2)	17
5	DNA + **3** (30 μM) + Catalase (4 units)	676 (2)	15
6	DNA + **3** (30 μM) + SOD (4 units)	676 (2)	21
7	DNA + **3** (30 μM) + TEMP (500 μM)	676 (2)	88
8	DNA + **3** (30 μM) + NaN_3 (500 μM)	676 (2)	75
9	DNA + **3** (30 μM) + DABCO (500 μM)	676 (2)	94

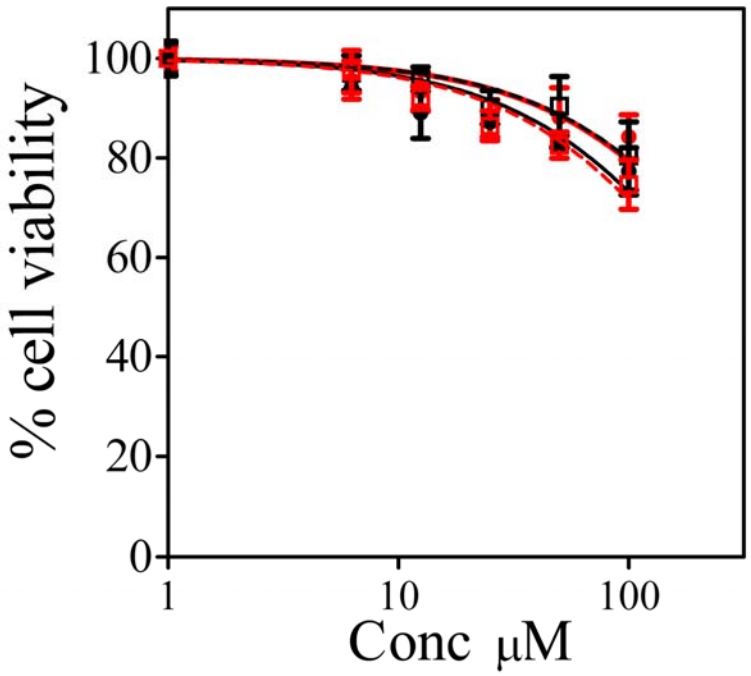

Fig. S15 Plot of % cell viability vs. concentration of [CoL(dpq)] (**2**) showing no significant reduction in cell viability upon treatment of HEK293 cells (■) and HEK293-hTSHR cells (□) with complex **2** in dark, after exposure to visible light (10 *J* cm^{-2}) as obtained by MTT assay. The black line represents non-linear fitting of the data for light unexposed cells, the red line represents fitting of the data for irradiation the red line represents the fitting for the visible light treated cells. The error bars correspond to mean±s.d. (n=3).

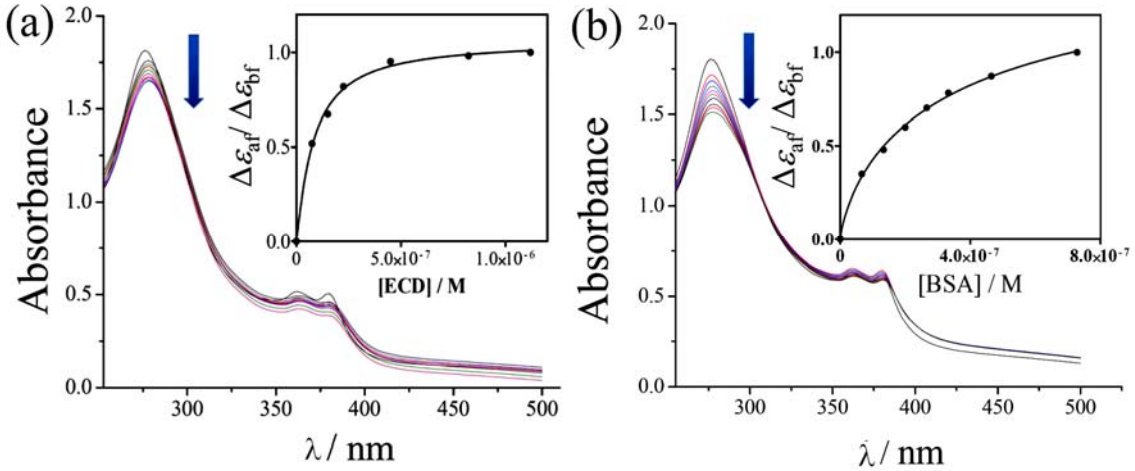

Fig. S16 UV-visible titration of complex **3** with (a) hTSHR-ECD and (b) BSA in 6% DMF/5 mM phosphate buffer pH 7.4. The arrows show the decrease in absorbance. The inset shows $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ vs. [protein] and the fitting of the data by non-linear regression analysis hyperbolic saturation binding for determination of dissociation constant (K_d).

Table S1: Selected bond distances(Å) and bond angles (°) data for the complex **1**·0.5EtOH·H_2O

Co(1)-O(1)	1.898(5)	O(2)-Co(1)-O(3)	89.7(2)
Co(1)-O(2)	1.930(5)	O(2)-Co(1)-N(1)	90.3(3)
Co(1)-O(3)	1.896(6)	O(2)-Co(1)-N(2)	90.4(3)
Co(1)-N(1)	1.984(7)	O(2)-Co(1)-N(3)	96.0(2)
Co(1)-N(2)	1.944(7)	O(3)-Co(1)-N(1)	170.7(3)
Co(1)-N(3)	1.980(7)	O(3)-Co(1)-N(2)	88.3(3)
O(1)-Co(1)-O(2)	178.5(2)	O(3)-Co(1)-N(3)	84.3(3)
O(1)-Co(1)-O(3)	89.5(2)	N(1)-Co(1)-N(2)	82.4(3)
O(1)-Co(1)-N(1)	90.3(3)	N(1)-Co(1)-N(3)	104.9(3)
O(1)-Co(1)-N(2)	88.3(3)	N(2)-Co(1)-N(3)	170.2(3)
O(1)-Co(1)-N(3)	85.2(2)		

Table S2. Selected Crystallographic Data for the Complex **1**·0.5EtOH·H_2O

Complex	**1**·0.5EtOH·H_2O
Empirical formula	$C_{45}H_{59}N_3O_{5.5}Co$
Fw	788.88
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions:	
a, Å	13.205(4)
b, Å	16.068(4)
c, Å	21.477(6)
α, deg	90.0
U, Å^3	4391(2)
Z	4
T, K	293(2)
Dc, Mg m^{-3}	1.193
λ, Å (Mo-K_α)	0.71073
μ, mm^{-1}	0.437
Data / restraints / parameters	7922 / 2 / 460
F(000)	1684
Goodness-of-fit	1.03
R (F_o)[a], I>2σ(I) / *wR* (F_o)[b]	0.1025 / 0.2441
R (all data)/*wR* (all data)	0.2535 / 0.3310
Largest diff. peak and hole (e Å^{-3})	1.038, -0.668

[a] $R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, [b] $wR = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o)^2]\}^{1/2}$; $w = [\sigma^2(F_o)^2 + (AP)^2 + BP]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$, A = 0.1599, B = 0.0000