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Electronic Supplementary Information：

Two dpa-based Zinc (II) complexes as potential anticancer agents: Nuclease activity, Cytotoxicity and Apoptosis studies

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Table S1 Selected bond lengths (Å) and angles (°) for the complexes **1, 2** and **L**

complex **1**					
Br(2)-Zn(1)	2.4068(15)	Zn(1)-Br(1)	2.4027(15)	Br(4)-Zn(2)	2.4162(14)
Zn(2)-Br(3)	2.4274(15)	Zn(1)-N(1)	2.346(6)	Zn(1)-N(2)	2.121(7)
Zn(1)-N(3)	2.129(6)	Zn(2)-N(4)	2.286(6)	Zn(2)-N(5)	2.163(7)
Zn(2)-N(6)	2.123(6)	Br(1)-Zn(1)-Br(2)	120.16(6)	Br(4)-Zn(2)-Br(3)	112.35(5)
N(2)-Zn(1)-N(1)	76.2(3)	N(3)-Zn(1)-N(1)	73.6(3)	N(2)-Zn(1)-N(3)	148.8(3)
N(2)-Zn(1)-Br(1)	99.4(2)	N(3)-Zn(1)-Br(1)	96.63(19)	N(1)-Zn(1)-Br(1)	135.57(16)
N(2)-Zn(1)-Br(2)	97.01(18)	N(3)-Zn(1)-Br(2)	97.83(19)	N(1)-Zn(1)-Br(2)	104.19(16)
N(6)-Zn(2)-N(4)	75.8(2)	N(6)-Zn(2)-Br(4)	98.17(19)	N(6)-Zn(2)-N(5)	149.2(3)
N(5)-Zn(2)-N(4)	74.4(2)	N(5)-Zn(2)-Br(4)	99.56(18)	N(4)-Zn(2)-Br(4)	139.61(16)
N(6)-Zn(2)-Br(3)	98.90(17)	N(5)-Zn(2)-Br(3)	97.38(16)	N(4)-Zn(2)-Br(3)	108.04(16)
complex **2**					
Zn(1)-N(1)	2.293(6)	Zn(1)-N(2)	2.116(5)	Zn(1)-N(3)	2.139(5)
Zn(1)-Cl(1)	2.240(2)	Zn(1)-Cl(2)	2.228(2)	Zn(2)-N(4)	2.274(5)
Zn(2)-N(5)	2.090(4)	Zn(2)-N(6)	2.121(4)	Zn(2)-Cl(3)	2.2607(19)
Zn(2)-Cl(4)	2.2527(18)	N(2)-Zn(1)-N(1)	75.5(2)	N(3)-Zn(1)-N(1)	73.68(19)
N(2)-Zn(1)-N(3)	147.8(2)	Cl(1)-Zn(1)-N(1)	103.88(15)	Cl(2)-Zn(1)-N(1)	135.30(14)
N(2)-Zn(1)-Cl(1)	98.07(14)	N(2)-Zn(1)-Cl(2)	99.44(18)	N(3)-Zn(1)-Cl(1)	98.03(15)
N(3)-Zn(1)-Cl(2)	95.94(17)	Cl(2)-Zn(1)-Cl(1)	120.72(10)	N(5)-Zn(2)-N(4)	75.64(17)
N(6)-Zn(2)-N(4)	74.27(17)	N(5)-Zn(2)-N(6)	148.8(2)	Cl(3)-Zn(2)-N(4)	106.11(13)

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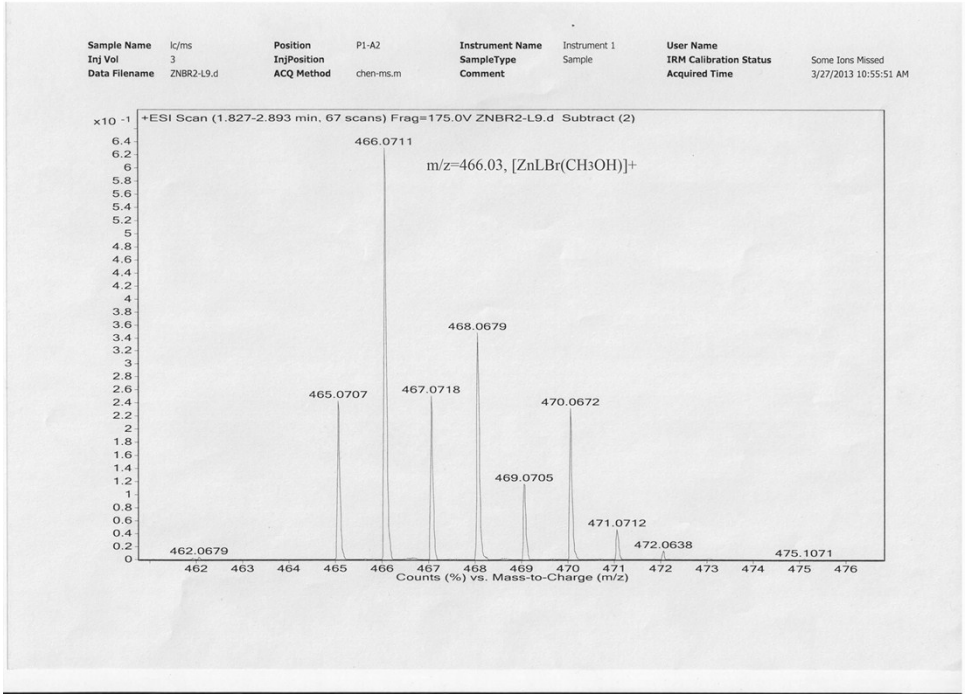
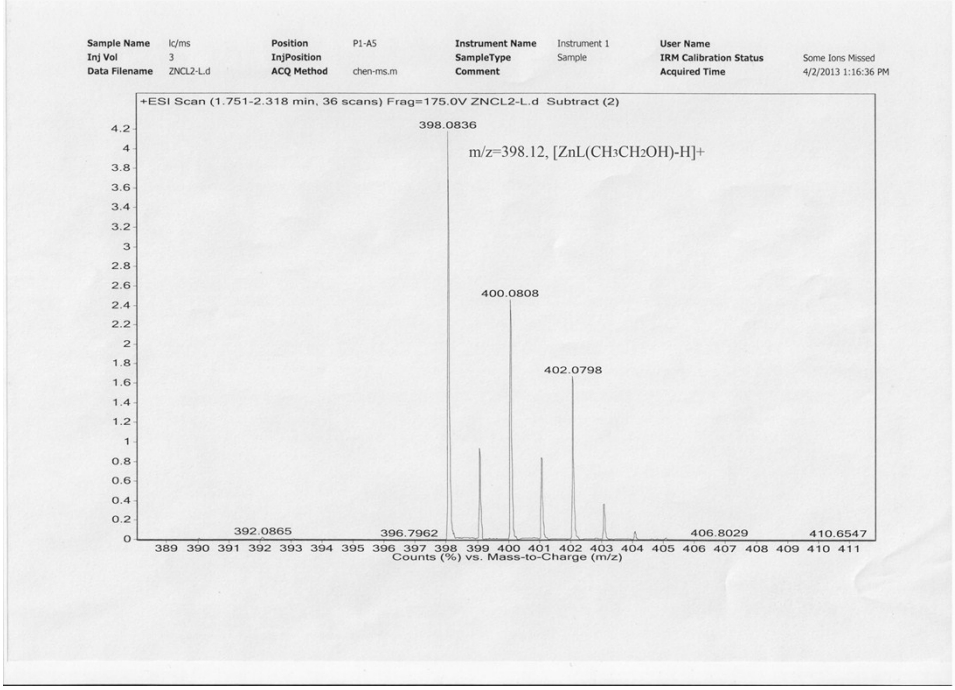
Cl(4)-Zn(2)-N(4)	139.55(13)	N(5)-Zn(2)-Cl(4)	98.60(14)	N(5)-Zn(2)-Cl(3)	98.16(14)
N(6)-Zn(2)-Cl(3)	98.05(14)	N(6)-Zn(2)-Cl(4)	98.76(15)	Cl(4)-Zn(2)-Cl(3)	114.33(7)
Compound **L**					
N(1)-C(5)	1.401(5)	N(1)-C(8)	1.445(5)	N(1)-C(14)	1.438(5)
N(2)-C(9)	1.365(4)	N(2)-C(13)	1.346(5)	N(3)-C(15)	1.340(5)
N(3)-C(19)	1.339(5)	N(4)-C(24)	1.396(4)	N(4)-C(27)	1.446(5)
N(4)-C(33)	1.453(4)	N(5)-C(28)	1.338(4)	N(5)-C(32)	1.338(5)
N(6)-C(34)	1.341(5)	N(6)-C(38)	1.344(5)	C(5)-N(1)-C(8)	119.1(3)
C(5)-N(1)-C(14)	119.9(3)	C(14)-N(1)-C(8)	120.9(3)	C(13)-N(2)-C(9)	120.6(3)
C(19)-N(3)-C(15)	118.1(3)	C(24)-N(4)-C(27)	120.8(3)	C(24)-N(4)-C(33)	119.3(3)
C(27)-N(4)-C(33)	119.2(3)	C(32)-N(5)-C(28)	120.3(3)	C(34)-N(6)-C(38)	119.3(3)



(a)



(b)

Fig. S1(a-b) ESI−MS analysis of complexes **1** and **2**.

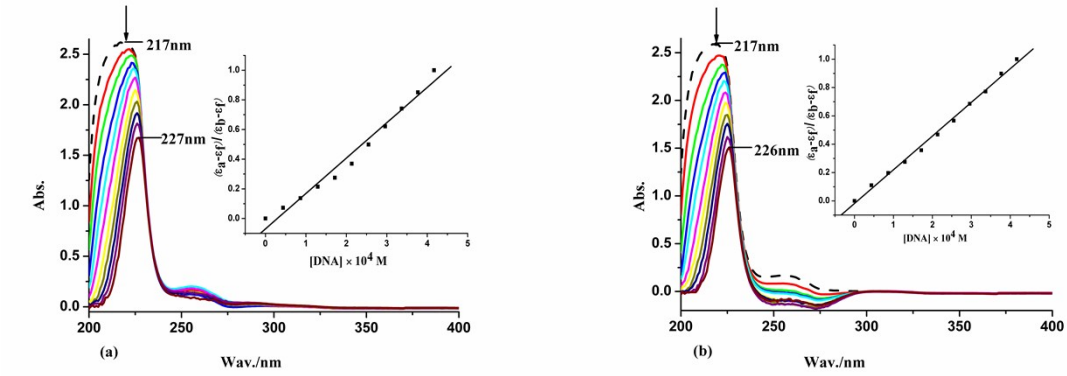


Fig. S2 (a-b) Absorption spectra of complexes **1** and **2** (1.96 μM) in the absence (dashed line) and presence (solid line) of increasing amounts of CT-DNA (44, 87, 129, 172, 214, 255, 296, 337, 377 and 417 μM) in 5 mM Tris-HCl/50 mM NaCl buffer (pH = 7.2). The arrow shows the absorbance changes on increasing DNA concentration. Insert: Plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ versus [DNA] for the titration of DNA to complex.

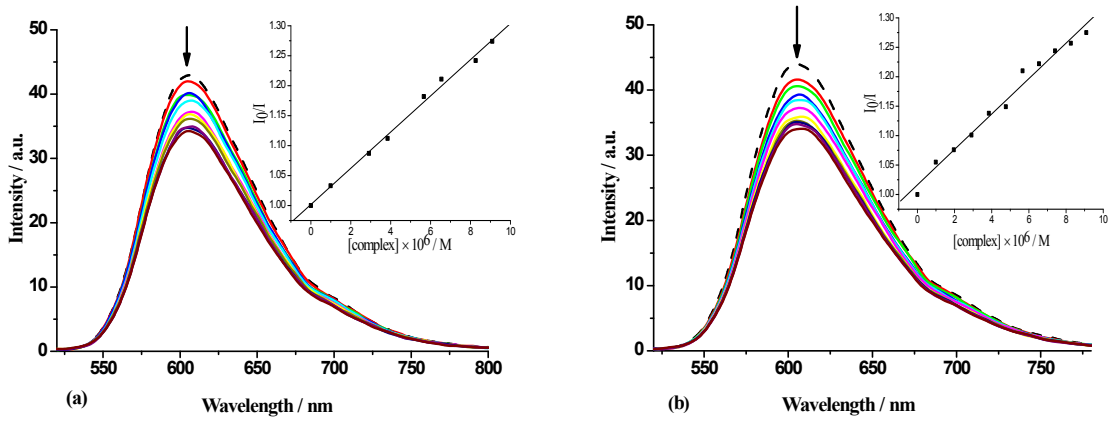


Fig. S3 (a-b) Fluorescence emission spectra of the EB (2.4 μM) bound to CT-DNA (48 μM) system in the absence (dashed line) and presence (solid lines) of complexes **1-2**. Inset: the plot of I_0/I versus the complex concentration.

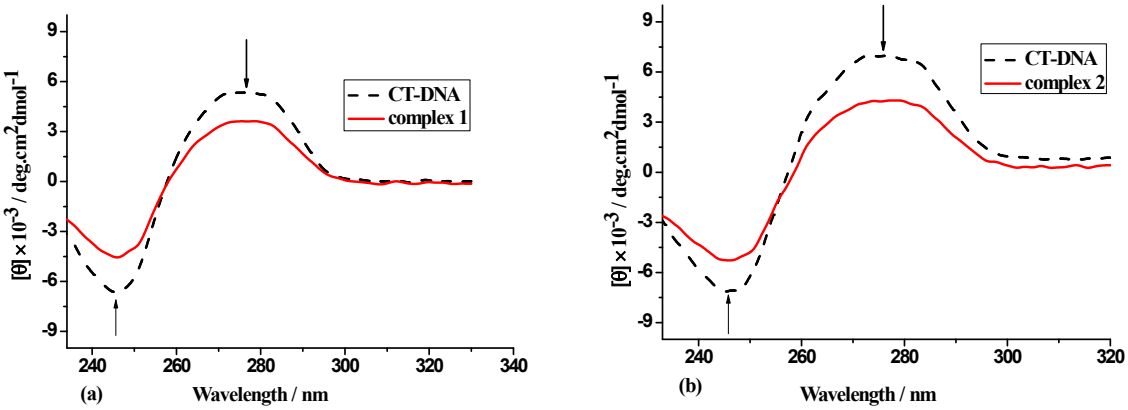


Fig. S4 (a-b) CD spectra of CT-DNA in the buffer solution (Tris-HCl) at 0.66 mM in the absence (dashed line) and presence (solid line)of 0.032 mM complexes **1-2**.

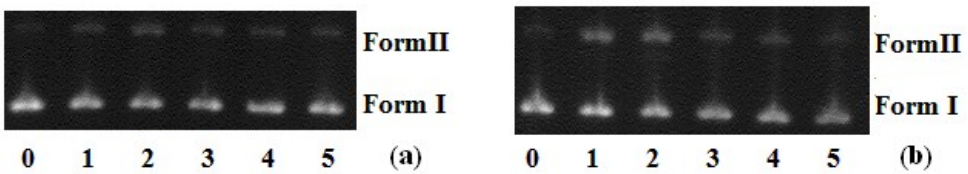


Fig. S5 (a-b) Gel electrophoresis diagram showing the cleavage of pBR322 DNA (0.1 μg/μL) for complexes **1-2** at different concentrations in Tris-HCl/NaCl buffer (pH = 7.2) and 37 ℃.Lane 0: DNA control (4 h); Lane 1-5: DNA + complex (0.005, 0.025, 0.045, 0.065, 0.080 mM), respectively.

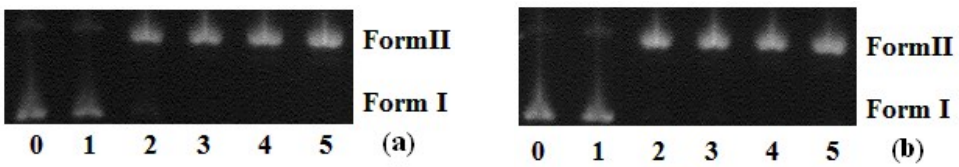


Fig. S6 (a-b) Gel electrophoresis diagrams showing the cleavage of pBR322 DNA (0.1 μg/μL) for complexes **1-2**

at different concentrations in Tris-HCl/NaCl buffer (pH = 7.2) and 37 ℃. Lane 0: DNA control (4 h); Lane 1: DNA + 0.25 mM H_2O_2; Lane 2-5: DNA + H_2O_2 + complex (0.005, 0.025, 0.045, 0.065 mM), respectively.

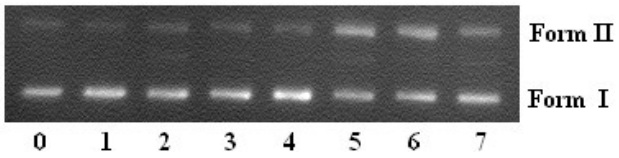


Fig. S7 Gel electrophoresis diagrams showing the cleavage of pBR322 DNA (0.1 μg/μL) for different compounds in Tris-HCl/NaCl buffer (pH = 7.2) and 37 ℃. Lane 0: DNA control (4 h); Lane 1-3: DNA + 0.15 mM **$ZnBr_2$**, **$ZnCl_2$** and **L**. Lane 4: DNA + 0.25 mM H_2O_2; Lane 5-7: DNA + H_2O_2 + 0.15 mM **$ZnBr_2$**, **$ZnCl_2$** and **L**, respectively.

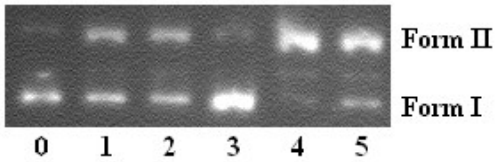


Fig. S8 Cleavage of pBR322 DNA (0.1 μg/μL) by complexes (0.065 mM, 1.3% DMF) incubated for 4 h at pH 7.2 and 33 ℃ in argon atmosphere. Lane 0: DNA control; Lane 1-2: DNA + complexes **1-2**. Lane 3: DNA + 0.25 mM H_2O_2; Lane 4-5: DNA + H_2O_2 + complexes **1- 2**.

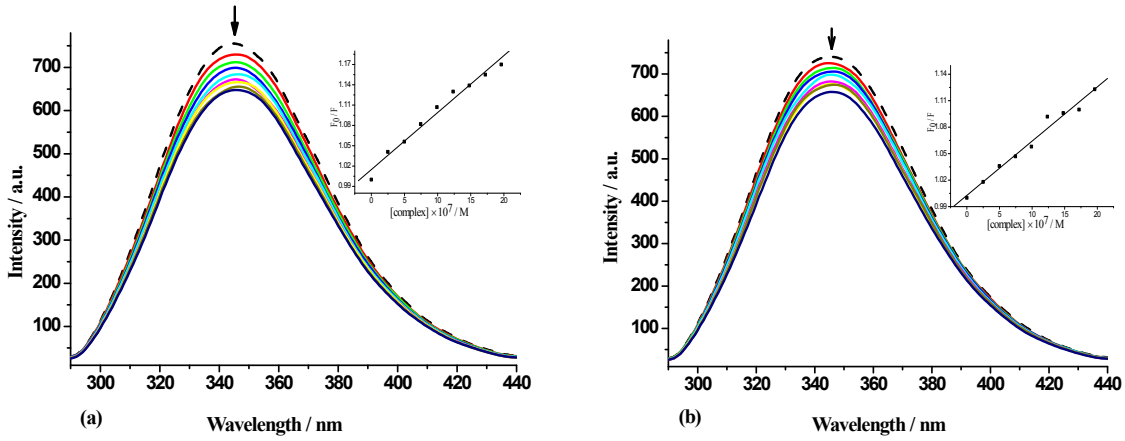


Fig. S9(a-b) Fluorescence emission spectra of the BSA (29.4 μM) system in the absence (dashed line) and presence (solid lines) of complexes **1-2** (0.25, 0.5, 0.74, 0.99, 1.23, 1.48, 1.72, and 1.96 μM, respectively) in phosphate buffer (10 mM, pH = 7.0). Inset: the plot of F_0/F versus the complex concentration.

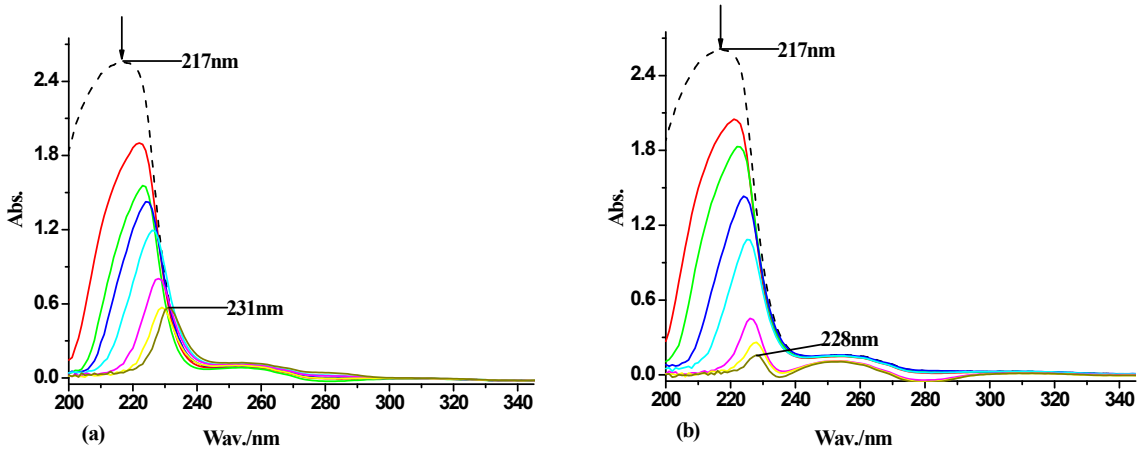


Fig. S10 (a-b) Absorption spectra of complexes **1** and **2** (1.96 μM) in the absence (dashed line) and presence (solid line) of increasing amounts of BSA (0.59, 1.18, 1.76, 2.35, 2.94, 3.53 and 4.12 μM) in phosphate buffer (pH = 7.0).

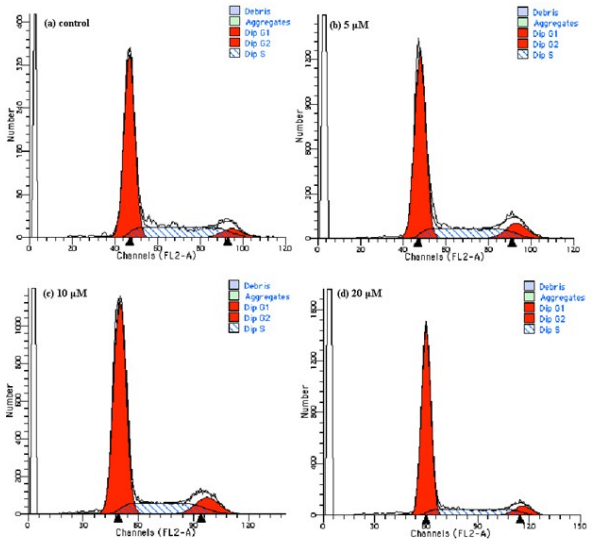


Fig. S11 Effects of complex **1** on the cell cycle progression in MCF-7 cells. The cell cycle distribution was analyzed by ModFit LT software and depicted with the histogram.

Table S2 Cell cycle distribution of cells after treatment for 48 h with various doses of complex **1**

Treatment (μM)	Distribution (% MCF-7cells) for 1		
	G1	S	G2/M
0 (Control)	65.53	28.27	6.2
5	64.58	24.83	10.58
10	68.09	21.15	10.76
20	73.17	19.41	7.42

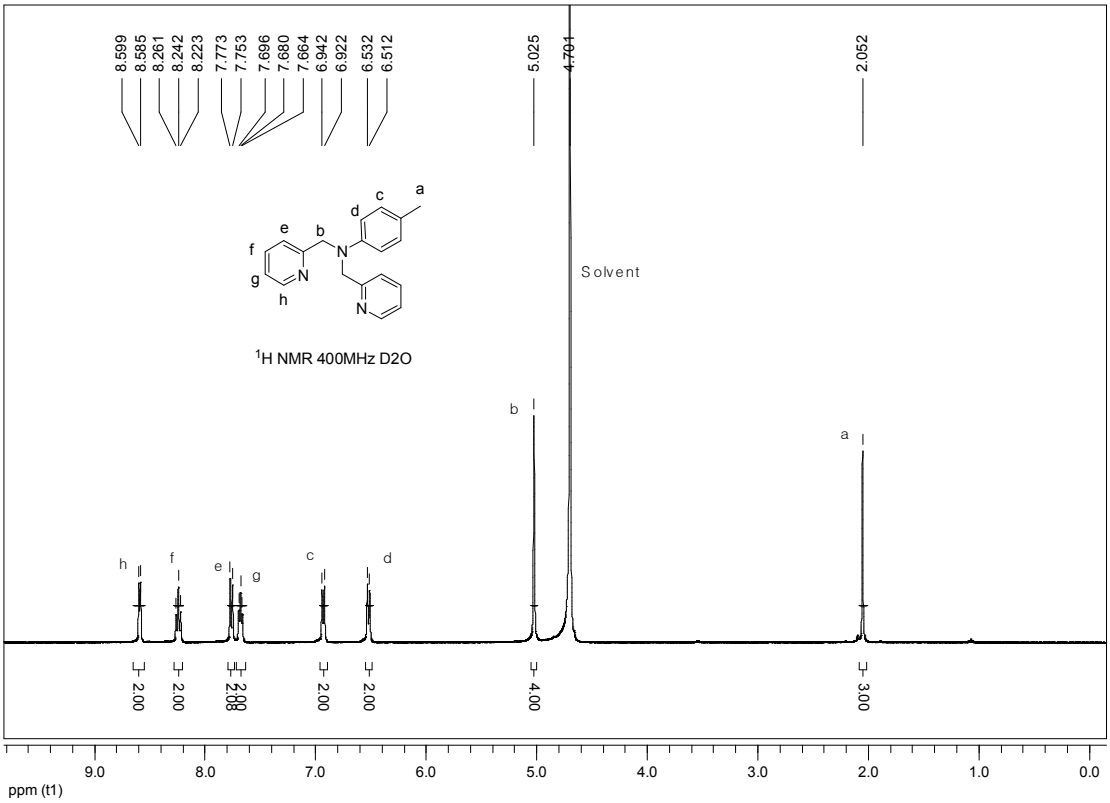


Fig. S12. ^{1}H NMR (400 MHz) spectra of the ligand L in D_2O

Experimental methods

X-ray crystallography

Diffraction data were collected at 293(2) K for the ligand **L** and complexes **1-2** with a Bruker Smart 1000 CCD diffractometer using *Mo-*K_α radiation (λ = 0.71073 Å) with the *ω-2θ* scan technique. The structures were solved by direct methods (SHELXS-97) and refined with full-matrix least-squares technique on F^2 using the SHELXL-97. The hydrogen atoms were added theoretically, and riding on the concerned atoms and refined with fixed thermal factors. The details of crystallographic data and structure refinement parameters are summarized in Table 1, and selected bond angles and distances are listed in Table S1.

DNA-binding and cleavage experiments

The UV absorbance at 260 nm and 280 nm of the CT-DNA solution in 5 mM Tris–HCl/50mM NaCl buffer (pH = 7.2) gives a ratio of 1.8–1.9, indicating that the DNA was sufficiently free of protein.[1] The concentration of CT-DNA was determined from its absorption intensity at 260 nm with a molar extinction coefficient of 6600 M^{-1} cm^{-1}.[2] The absorption spectra of complexes **1** and

2 binding to DNA were performed by increasing amounts of CT-DNA to the complexes in Tris–HCl buffer (pH = 7.2).

The relative bindings of complexes to CT-DNA were studied with an EB-bound CT-DNA solution in 5 mM Tris–HCl/50 mM NaCl buffer (pH = 7.2). The fluorescence spectra were recorded at room temperature with excitation at 510 nm and emission at about 602 nm. The experiments were carried out by titrating complexes into EB–DNA solution containing 2.4×10^{-6} M EB and 4.8×10^{-5} M CT-DNA.

The CD spectra of CT-DNA in the presence or absence of complex were collected in Tris–HCl buffer (pH = 7.2) containing 50 mM NaCl at room temperature. All CD experiments were performed on a JASCO- J715 spectropolarimeter at room temperature from 330 to 230 nm.

The DNA cleavage experiments were done by agarose gel electrophoresis, which was performed by incubation at 37 °C as follows: pBR322 DNA (0.1 μg/μL) in 50 mM Tris–HCl/18 mM NaCl buffer (pH = 7.2) was treated with **1** and **2**. The samples were incubated for 3 or 4 h, and then loading buffer was added. Then the samples were electrophoresed for 2.5 h at 80 V on 0.8 % agarose gel using Tris–boric acid–EDTA buffer. After electrophoresis, bands were visualized by UV light and photographed. The extent of cleavage of the SC DNA was determined by measuring the intensities of the bands using the Gel Documentation System.[3]

Cleavage mechanistic investigation of pBR322 DNA was carried out in the presence of standard radical scavengers and reaction inhibitors. These reactions were carried out by adding standard radical scavengers of DMSO, NaN_3, SOD and EDTA to pBR322 DNA prior to the addition of complex. Cleavage experiment was initiated by the addition of complex and quenched with 2 μL of loading buffer. Further analysis was carried out by the above standard method.

The deoxygenated solutions were stored under an argon atmosphere prior to use. Reaction mixtures were prepared in a glove box by the addition of appropriate volumes of stock solutions to the reaction tubes. The reactions were initiated by quick centrifugation, incubated at room temperature (33 °C) and quenched by the addition to the loading buffer in the glove box. All other conditions were the same as those listed for the aerobic cleavage reactions.

Protein binding studies

The protein binding study was performed by tryptophan fluorescence quenching experiments

using bovine serum albumin stock solution (BSA, 1.5 mM) in 10 mM phosphate buffer (pH = 7.0). A concentrated stock solution of the compounds was prepared as used for the DNA binding experiments, except that the phosphate buffer was used instead of a Tris-HCl buffer for all of the experiments. The fluorescence spectra were recorded at room temperature with excitation wavelength of BSA at 280 nm and the emission at 342 nm by keeping the concentration of BSA constant (29.4 μM) while varying the complex concentration from 0 to 1.96 μM. In addition, Absorption titration experiments were carried out by keeping the concentration of complex constant (1.96 μM) while varying the BSA concentration from 0 to 4.12 μM.

Cytotoxicity assays

Human tumour cell lines HeLa (human cervical cancer cells), MCF-7 (human breast adenocarcinoma cells) and RL952 (endometrial carcinoma cells) were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C in RPMI 1640 medium supplemented with 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 10% fetal bovine serum. The stock solutions used for anticancer experiments were 1 mM complex in phosphate buffer solution containing 10% DMF. During these experiments, DMF was kept < 1% at least in cell culture medium.

MTT assay

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was done to test the ability of complexes **1** and **2** to inhibit cell growth and induce cell death in HeLa, MCF-7 and RL952 cancer cells. Cells were seeded in a 96-well culture plate (1×10^4 cells/100 μL well) for 24 h at 37 °C and 5 % CO_2, and complexes were added to each well in 10 μL of FBS free culture medium and the plates were incubated in a 5 % CO_2 humidified atmosphere for 48 h. Complexes were tested at concentrations ranging between 0 and 80 μM. Then each well was loaded with 0.1 mg of MTT (in 20 μL of PBS pH = 7.4) for 4 h at 37 °C. The formed formazan crystals were then dissolved in 100 μL of DMSO and the absorbance was read at 570 nm using an Enzyme-linked Immunosorbent Assay (ELISA) reader. Experiments were carried out in triplicate, and the percentage of cell viability was calculated according to the following equation:

Cell viability (%) = [A_{570} (sample)/A_{570} (control)] ×100%

Where A_{570}(sample) refers to the reading from the wells treated with zinc complex and A_{570}(control) refers to that from the wells treated with medium containing 10% FBS only.[4]

The IC_{50} value was determined by plotting the percentage viability versus concentration on a logarithmic graph. The average of three duplicate experimental results was taken as the final IC_{50} value.

Clonogenic assay

Clonogenic survival was defined as the ability of the cells to maintain their clonogenic capacity and to form colonies.[5, 6] 300 cells were seeded into 6-well plate to form clones with varying amounts of the complex (0-100 μM) which was added to the plate in the third day, in a humidified atmosphere containing 5% CO_2 at 37 °C. Two weeks later, cells were washed with PBS (pH = 7.4) and fixed with methanol–acetic acid (3:1) solution for 15 min, stained with crystal violet solution (1% crystal violet, 20% ethanol) for 30 min and rinsed with water to remove extra dye, the colonies (containing a minimum of 50 cells) were counted. The number and area of colonies in treated groups were expressed as a percentage of those in control group.

Apoptosis

Hoechst 33342 staining

Apoptosis is the process of programmed cell death, which is important different from necrosis that refers to uncontrolled cell death. The morphology of MCF-7 cell lines after treatment was analyzed with the aid of Hoechst 33342 staining. First, 5×10^4 MCF-7 cell lines were seeded respectively into 24-well plate in 2 mL of medium for 24 h. Then varying amounts of complex **1** were added to the plate, which were incubated for 24 h. After a wash with PBS (Phosphate Buffered Saline), the cells were stained with Hoechst 33342 (1 μg·mL^{-1}) for 15 min at 37 °C. After a final wash in PBS, samples were visualized with the aid of EPI fluorescence microscopy.[7]

Measurement of apoptosis by Annexin V analysis

Cells were seeded in a 6-well culture plate (2×10^5 cells/2 mL well) for 12 h at 37 °C and 5% CO_2, and complex was added to each well respectively at 0, 5, 10 and 20 μM and the plates were incubated in a 5% CO_2 humidified atmosphere for 48 h. Cells were digested in Trypsin, centrifuged, resuspended in 100 μL binding buffer (0.1 M Hepes pH 7.4, 1.4 M NaCl, and 25 mM

$CaCl_2$), and stained with 2.5 μL Annexin V-APC and 2.5 μL 7-AAD (Biolegend) in the dark at room temperature for 15 min. Quantification of apoptosis was measured by a FACStar Plus flow cytometer, and data analysis was carried out with FlowJo software.

Cell cycle analysis

DNA contents and cell cycle analysis were carried out by flow cytometry.[8] Briefly, 5×10^5 MCF-7 cells were incubated with complex **1** respectively at 5, 10 and 20 μM at 37 °C for 48 h. Cells were harvested in cold PBS, centrifuged, resuspended and fixed in 70 % EtOH overnight at 4 °C. After a further washing steps with cold PBS, cells were digested by RNase A (0.25 $mg\cdot mL^{-1}$) at 37 °C for 30 min, and stained with PI (50 $\mu g\cdot mL^{-1}$) in the dark at room temperature for 30 min. The distribution of the cell cycle was measured by a FACStar Plus flow cytometer, and data analysis was carried out with ModFit LT software.

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