Electronic Supplementary Information for

Cyclometalated Platinum(II) Complexes as Topoisomerase IIα Poisons

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Experimental Section

Materials. All starting chemicals were purchased from Sigma-Aldrich and used as received unless specified otherwise. Complexes $[(Phpy)Pt^{II}(MeCN)_2](CF_3SO_3)$ (**3**, the precursor for **1a-d**) and $[(Bzqn)Pt^{II}(MeCN)_2](CF_3SO_3)$ (**4**, the precursor for **2a-d**) were synthesized according to reported procedures[1]. Cell lines used in this work include HeLa, NCI-H460, HepG2, CCD-19Lu (American-Type Culture Collection, ATCC) and SUNE1 (generously provided by Prof. S. W. Tsao from Department of Anatomy, The University of Hong Kong, Hong Kong). Other reagents and materials include cell culture medium constituents and phosphate-buffered saline (PBS) (Gibco BRL), cell proliferation kit I (MTT) (Roche), supercoiled DNA and human TopoIIα (TopoGEN), Calf thymus DNA (ctDNA) (purified by phenol/chloroform extractions), anti-P53, anti-Bax, anti-Bcl-2, anti-Bcl-xL, anti-GAPDH, anti-TopIIα antibodies (Santa Cruz Biotechnology), anti-Caspase 3, anti-β-actin, and anti-phospho H_2AX antibodies (Cell Signaling Technology), proteinase inhibitor cocktails (Roche), human TopoIIα siRNA (Santa Cruz Biotechnology), transfection reagent Lipofectamine (Invitrogen) and Opti-MEM medium (Invitrogen).

Instrumentation. ^1H NMR was recorded on a Bruker DPX-300 or 400 FT-NMR spectrometer. UV-vis absorption was recorded on a Perkin-Elmer Lambda 900 UV-visible spectrophotometer. Positive mode mass spectra were recorded on a Finnigan MAT95 mass spectrometer. Elemental analysis was performed by the Institute of Chemistry at the Chinese Academy of Sciences, Beijing. Infrared spectra

were recorded using KBr plates on a Bio-Rad FT-IR spectrometer. Cytotoxicity of Pt(II) complexes and DNA-dependent ATP hydrolysis assay were determined by reading the absorbance at 580 nm and 630 nm respectively on a multiplate reader (Perkin-Elmer FusionTM α-FP).

Emission and Lifetime Measurements.[2] Steady-state emission spectra were recorded on a SPEX 1681 Fluorolog-2 Series F111A1 spectrophotometer. The emission spectra were corrected for monochromator and photomultiplier efficiency and for xenon lamp stability. Samples and standard solutions were degassed for at least three cycles using the freeze-pump-thaw method. With the method provided by Demas and Crosby[2c], the emission quantum yield was determined using $\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$ (where *s* and *r* refer to sample and reference standard solution respectively, *n* is the refractive index of the solvents, *D* is the integrated intensity, and Φ is the luminescence quantum yield), with $[Ru(bpy)_3](PF_6)_2$ (in degassed acetonitrile) as the standard ($\Phi_r = 0.062$). With the values of *A* (absorbance at the excited wavelength) and *L* (optical path length), *B* is calculated by $B = 1 - 10^{-AL}$. Emission lifetime measurements were carried out on a Quanta Ray DCR-3 pulsed Nd:YAG laser system (pulse output 355 nm, 8 ns), with the emission signals detected by a Hamamatsu R928 photomultiplier tube and recorded on a Tektronix model 2430 digital oscilloscope. Errors for λ ($\pm$ 1 nm), τ ($\pm$ 10 %), and Φ ($\pm$ 10 %) were estimated.

Synthesis of Pt(II) Complexes 1–4.

Complex 1a (CF_3SO_3): 2-naphthylisocyanide (1.2 mmol, 185 mg) was added to a stirred suspension of **3** (0.54 mmol, 313 mg) in MeCN (15 mL). After stirring at room temperature for overnight, the mixture was filtered after addition of Et_2O (30 mL) to give pure **1a** as a yellow solid. Yield: 0.29 g, 67%. Anal. Calcd for $C_{34}H_{22}F_3N_3O_3PtS$: C, 50.75; H, 2.76; N, 5.22. Found: C, 50.64; H, 2.85; N, 5.21. FAB-MS (+ve, *m*/*z*): 655 [M^+], 502 [M^+ - (CN-2-Np)], 349 [M^+ - 2(CN-2-Np)]. ^{1}H NMR (300 MHz, CD_3CN): 7.33 (m, 1H), 7.50 (m, 1H), 7.63-7.88 (m, 8H), 7.93-8.15 (m, 9H), 8.45 (s, 1H), 8.53 (s, 1H), 9.05 (s, 1H). IR (KBr): 2189.1 (N≡C), 2212 (N≡C) cm^{-1}.

Complexes 1b–d: The methodology for **1a** was adopted except that 2,6-dimethylphenylisocyanide (**1b**), *n*-butylisocyanide (**1c**) and *tert*-butylisocyanide (**1d**) were used as ligands.

Complex 1b (CF_3SO_3): Yield: 0.28 g, 68%. Anal. Calcd for $C_{30}H_{26}F_3N_3O_3PtS$: C, 47.37; H, 3.45; N, 5.52. Found: C, 47.39; H, 3.60; N, 5.38. FAB-MS (+ve, *m*/*z*): 611 [M+], 480 [M+ - (CN-Xyl)], 349 [M+ - 2(CN-Xyl)]. ^{1}H NMR (300 MHz, CD_3CN): 2.55 (s, 12H, CH_3), 7.25(m, 5H), 7.50(m, 3H), 7.85(m, 2H), 8.05(s, 1H), 8.24(d, 1H, J=7.52), 9.06(s, 1H). IR (KBr): 2178.1 (N≡C), 2208.3 (N≡C) cm^{-1}.

Complex 1c (CF_3SO_3): Yield: 0.25 g, 68%. Anal. Calcd for $C_{22}H_{26}F_3N_3O_3PtS$: C, 39.76; H, 3.94; N, 6.32. Found: C, 39.74; H, 3.94; N, 6.36. FAB-MS (+ve, *m*/*z*): 515

[M^+], 432 [M^+ - (CN-*n*-Bu)], 349 [M^+ - 2(CN-*n*-Bu)]. ^{1}H NMR (300 MHz, CD_3CN): 0.95 (m, 6H, CH_3), 1.54 (m, 4H, CH_2), 1.86 (m, 4H, CH_2), 4.05 (m, 4H, CH_2), 7.38 (m, 2H), 7.45-7.82 (m, 3H), 8.05 (d, 1H, J=7.74), 8.15 (d, 1H, J= 7.47), 8.80 (d, 1H, J= 5.12). IR (KBr): 2221.8 (N≡C), 2245.0 (N≡C) cm^{-1}.

Complex 1d (CF_3SO_3): Yield: 0.28 g, 76%. Anal. Calcd for $C_{22}H_{26}F_3N_3O_3PtS$: C, 39.76; H, 3.94; N, 6.32. Found: C, 39.78; H, 3.92; N, 6.30. FAB-MS (+ve, *m*/*z*): 515 [M^+], 432 [M^+ - (CN-*t*-Bu)], 349 [M^+ - 2(CN-*t*-Bu)]. ^{1}H NMR (300 MHz, CD_3CN): 1.68(m, 18H, CH_3), 7.38 (m, 2H), 7.50 (d, 1H, J= 7.26), 7.70 (d, 1H, J= 5.19), 7.85 (t, 1H, J= 4.39), 8.08 (d, 1H, J= 8.09), 8.25 (d, 1H, J= 7.26), 8.75 (d, 1H, J= 5.81). IR (KBr): 2208.3 (N≡C), 2228.4 (N≡C) cm^{-1}.

Complex 2a (CF_3SO_3): 2-naphthylisocyanide (1.2 mmol, 185 mg) was added to a stirred suspension of **4** (0.54 mmol, 326 mg) in MeCN (15 mL). After stirring at room temperature for overnight, the mixture was filtered after addition of Et_2O (30 mL) to give pure **2a** as a yellow solid. Yield: 0.25 g, 56%. Anal. Calcd for $C_{36}H_{22}F_3N_3O_3PtS$: C, 52.18; H, 2.68; N, 5.07. Found: C, 52.14; H, 2.70; N, 5.08. FAB-MS (+ve, *m*/*z*): 679 [M^+], 526 [M^+ - (CN-2-Np)], 372 [M^+ - 2(CN-2-Np)]. ^{1}H NMR (400 MHz, CD_3CN): 7.19 (s, 1H), 7.29 (d, 1H, J= 8.59), 7.44-7.80 (m, 16H), 7.98 (s, 1H), 8.07 (s, 2H), 8.37(s, 1H). IR (KBr): 2216.1 (N≡C), 2189.1 (N≡C) cm^{-1}.

Complexes 2b-d: The methodology for **2a** was adopted except that

2,6-dimethylphenylisocyanide (**2b**), *n*-butylisocyanide (**2c**) and *tert*-butylisocyanide (**2d**) were used as ligands.

Complex 2b (CF_3SO_3): Yield: 0.20 g, 47%. Anal. Calcd for $C_{32}H_{26}F_3N_3O_3PtS$: C, 48.98; H, 3.34; N, 5.35. Found: C, 49.20; H, 3.48; N, 5.39. FAB-MS (+ve, *m*/*z*): 636 [M^+], 504 [M^+ - (CN-Xyl)], 373 [M^+ - 2(CN-Xyl)]. ^{1}H NMR (400 MHz, CD_3CN): 2.58 (s, 12H, CH_3), 7.32 (t, 4H, J= 5.76), 7.44 (d, 2H, J= 7.73), 7.69 (d, 1H, J= 7.20), 7.78 (t, 1H, J= 5.43), 7.98 (d, 1H, J= 8.76), 8.03 (d, 1H, J= 7.06), 7.83-7.90 (m, 2H), 8.72 (d, 1H, J= 8.07), 9.28 (d, 1H, J= 5.26). IR (KBr): 2206.4 (N≡C), 2187.1 (N≡C) cm^{-1}.

Complex 2c (CF_3SO_3): Yield: 0.30 g, 81%. Anal. Calcd for $C_{24}H_{26}F_3N_3O_3PtS$: C, 41.86; H, 3.81; N, 6.10. Found: C, 41.60; H, 3.68; N, 5.87. FAB-MS (+ve, *m*/*z*): 539 [M^+], 456 [M^+ - (CN-*n*-Bu)], 373 [M^+ - 2(CN-*n*-Bu)]. ^{1}H NMR (400 MHz, CD_3CN): 0.95 (m, 6H, CH_3), 1.56 (m, 4H, CH_2), 1.86 (m, 4H, CH_2), 4.06 (m, 4H, CH_2), 7.64-7.96 (m, 6H), 8.68 (d, 1H, J= 8.06), 9.15 (s, 1H). IR (KBr): 2223.8 (N≡C), 2244 (N≡C) cm^{-1}.

Complex 2d (CF_3SO_3): Yield: 0.27 g, 73%. Anal. Calcd for $C_{24}H_{26}F_3N_3O_3PtS$: C, 41.86; H, 3.81; N, 6.10. Found: C, 41.88; H, 3.84; N, 5.98. FAB-MS (+ve, *m*/*z*): 539 [M^+], 456 [M^+ - (CN-t-Bu)], 373 [M^+ - 2(CN-t-Bu)]. ^{1}H NMR (400 MHz, CD_3CN): 1.94 (m, 18H, CH_3), 7.64-7.96 (m, 6H), 8.65 (d, 1H, J= 8.14), 8.89 (s, 1H). IR (KBr):

2231.5 (N≡C), 2204.5 (N≡C) cm^{-1}.

Absorption Titration and Emission Titration. A TBS/CH_3CN solution (1 mL, 99:1 v/v) containing platinum(II) complex (20 μM) was placed in a thermostatic cuvette and its absorption was recorded each time after a continual addition aliquot of ctDNA stock solution (1 mM) to the sample (1-min equilibration for each aliquot), until the saturation point was reached. Emission spectra (420-800 nm) were recorded the same way as above except that a 3-min equilibration per aliquot was used. DNA binding data from absorption titration and emission titration were evaluated and then fitted with McGhee-Hippel equation.[3]

Viscosity Measurement. Viscosity was measured on a Cannon-Manning Semi-Micro Viscometer immersed in a thermostatically controlled water bath maintained at 25ºC. Titrations were performed by addition of small volume of concentrated stock solutions (4 mM) of **1a** to 1 mM ctDNA in BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.0) in the viscometer. Mixing of the solutions in the viscometer was achieved by bubbling with nitrogen gas.

NMR Titration Experiments on d(CAATCCGGATTG)$_2$. All these experiments were performed with a Bruker DRX500 spectrometer at 298K. Typical acquisition conditions for a ^{1}H NMR spectrum were 458 pulse length, 2.0 s relaxation delay (4 s for determination of formation constants), 16000 data points and 16±32 transients.

The dodecanucleotide d(CAATCCGGATTG)$_2$ was dissolved in phosphate buffer (0.7 mL, 10 mM, pH 7.0) containing NaCl (20 mM) and EDTA (0.1 mM), and a trace of DSS was added as internal reference for reporting chemical shifts. Aliquots of stock solutions of **1a** were titrated directly to the DNA solution in an NMR tube. The spectra were then processed by using an exponential function with a line-broadening coefficient of 0.3 Hz.

Topoisomerase IIα-Mediated DNA Relaxation Assay. Plasmid DNA (0.25 μg/μL) was diluted to the working concentration (12.5 ng/μL) in the reaction buffer (50 mM Tris/HCl pH 7.5, 20 mM KCl, 1 mM EDTA, 0.3 mg/mL BSA, 1 mM dithiothreitol and 2 mM ATP). 4 units of human topoisomerase IIα were used per sample. To a solution containing 250 ng of supercoiled DNA, 2-μL aliquot of Pt(II) complex, Vp-16 or vehicle control was added. This solution was incubated at 37ºC for 30 min and terminated by addition of 2 μL of 10% sodium dodecyl sulfate (SDS) and 2 μL of proteinase K (1 mg/mL). After further incubation at 45ºC for 30 min, the samples were subjected to gel electrophoresis using 1% (w/v) agarose gel. The gel was stained with ethidium bromide after electrophoresis and visualized under UV illumination. Topoisomerase IIα-mediated DNA cleavage was assayed with the same way except that the gel contains 0.5 μg/mL ethidium bromide, which can discriminate the linear and nicked DNA from the relaxed DNA well.

Topoisomerase IIα-mediated kDNA decatenation was assayed in the same way as

that of Topoisomerase IIα-mediated DNA relaxation assay except that kDNA was used instead of plasmid DNA.

Band Depletion Assay for TopoIIα Cleavable Complexes.[4] After treating with Vp-16 or **1a** for 30 min at 37ºC, ~1×10^6 SUNE1 cells were pelleted and lysed with 40 μL alkaline lysis buffer (0.2 M NaOH and 1 mM EDTA). Alkaline lysates were then neutralized with 1/10 volume of 2 M HCl and 1/10 volume of a solution containing 10% NP40, 1 M Tris pH 7.4, 0.1 M $MgCl_2$, 0.1 M $CaCl_2$, 10 mM DTT, 1 mM EGTA and proteinase inhibitor cocktails. After SDS-PAGE of the lysate proteins, immunoblotting was performed using anti-TopoIIα antibody and anti-β-actin antibody.

Alkaline Comet Assay for Cleavable Complexes.[5] **1a**- or Vp-16-treated cells were pelleted and resuspended in 1% low melting agarose. The mixture was loaded onto an agarose pre-coated slide and lysed in a prechilled lysis solution containing 2.5 M NaCl, 100 mM EDTA and 10 mM Tris-base. The slides were immersed in the alkaline buffer (0.2 M NaOH and 1 mM EDTA), subjected to SDS-PAGE and finally stained with ethidium bromide for visualization under a fluorescence microscope.

Topoisomerase IIα-mediated DNA Religation.[6] Reactions occurred in 20 μL of reaction buffer (50 mM Tris/HCl pH 7.5, 20 mM KCl, 1 mM EDTA, 0.3 mg/mL BSA and 1 mM dithiothreitol) containing 4 units of topoisomerase IIα and 250 ng of

negatively supercoiled DNA. Initial DNA cleavage/religation equilibria were established at 37ºC for 6 min. Pre-strand passage cleavage/religation equilibria were established in the absence of nucleotide triphosphate, and post-strand passage equilibria were established in the presence of 4 mM ATP. Topoisomerase IIα-mediated religation of cleaved DNA was induced by cold-induced religation assay, in which samples were rapidly shifted from 37 to 0ºC to initiate the religation and slow down the reaction rate. **1a** or VP-16 was added to the reaction mixtures with different concentrations just before the initiation of DNA religation. Reaction was stopped with the addition of SDS to a final concentration of 1%. After digesting with protease K at 45ºC for 30 min, samples were subjected to 1% (w/v) agarose gel electrophoresis. Religation inhibition was estimated by the loss of linear DNA.

Time-dependence of religation was performed in a total volume of 120 μL of reaction buffer (50 mM Tris/HCl pH 7.5, 20 mM KCl, 1 mM EDTA, 0.3 mg/mL BSA and 1 mM dithiothreitol) containing 1.5 mg of negatively supercoiled DNA and 24 units of purified topoisomerase IIα. After incubation for 6 min at 37ºC, the enzyme-mediated religation was induced by shifting the temperature from 37 to 0ºC. **1a (**4 μM**)** and VP-16 (100 μM) were added to the reaction mixtures just before the initiation of DNA religation. Aliquotes (20 μL) were withdrawn at various time points and the reaction was stopped with the addition of SDS to a final concentration of 1%. The following procedures are same with above.

Electrophoretic-mobility-shift assay (EMSA).[7] TopoIIα-DNA binding was evaluated by an electrophoretic mobility shift assay. Reactions were carried out in 20 μL of reaction buffer (50 mM Tris/HCl pH 7.5, 20 mM KCl, 1 mM EDTA, 0.3 mg/mL BSA and 1 mM dithiothreitol) containing 0.25 μg of supercoiled DNA and 4 unites of human topo IIα in the presence (10 μM) or absence of **1a**. After incubation at 37ºC for 10 min, samples were separated on a 1% agarose gel by electrophoresis. The effect of **1a** on the binding of TopoIIα with DNA was evaluated by comparing the intensity of the shift band, the formation of which is due to the combination of TopoIIα and DNA.

DNA-dependent ATP Hydrolysis Assay.[8] DNA-dependent ATP hydrolysis of human topoisomerase IIα was monitored by measuring the production of inorganic phosphate using acidic molybdate and malachite. Reactions occurred in a buffer (50 mM Tris/HCl pH 7.5, 20 mM KCl, 1 mM EDTA, 0.3 mg/mL BSA and 1 mM dithiothreitol) containing 0.25 μg of supercoiled DNA and 4 units of topoisomerase IIα. **1a,** prepared in DMSO at the indicated concentration, was added serially to the reaction mixture and preincubated for 10 minutes at 37ºC. Reactions were started upon the addition of 400 μM and 2 mM ATP (final concentration) and allowed to proceed for 30 minutes. Reactions were terminated by the addition of 200 μl of malachite green reagent and the production of inorganic phosphate was determined by recording the absorbance at 630 nm immediately on a plate reader (Perkin-Elmer FusionTM α-FP).

Cytotoxicity Test. 1×10^4 cells/well were seeded in 96-well flat bottomed multiwell plate with supplemented culture medium (100 μL/well) followed by incubation with 5% CO_2/95% air at 37ºC for 12 hr. After the addition of the serially diluted solutions of Pt(II) complex, cells were then incubated with 5% CO_2/95% air at 37ºC for another 72 hr. All cytotoxicity tests were run in parallel with a set of negative control, cells in the absence of complex, and a set of positive control using Vp-16 or Cisplatin as cytotoxic agent for comparison. In the end, cytotoxicity was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, in which 10 μL of MTT solution (5 mg/mL in 1 × PBS) was added to each well, followed by a 4-hr incubation. Then 100 μL of solubilization buffer (10% SDS) was added to each well to lyse the cells and solubilize the blue formazan complex formed. The multiwell plate was finally incubated in dark overnight before taking measurement. Formation of formazan was measured by reading the absorbance at 580 nm with a plate reader (Perkin-Elmer FusionTM α-FP). IC_{50} of the complex was determined based on the percentage of cell survived in a dose-dependence manner relative to the negative control.

TopoIIα siRNA Transfection. Transfection was performed by using Lipofectamine transfection reagent (Invitrogen) and following the manufacturer's protocol. Brifely, growth medium was firstly aspirated from cells and replaced by the medium containing no FBS and Penicillin-Streptomycin. Then Lipofectamine/RNA mixture in Opti-MEM medium was added dropwise into the 6-well plates in which cells have

been grown to reach 80% confluency. After gently mixing, cells were incubated in a humidified 37ºC incubator for 6 h. Then medium was aspirated and the cells were incubated in a fresh growth medium for additional 24 h. Finally, 1×10^4 cells/well were seeded in a 96-well flat bottomed multiwell plate with fresh growth medium (100 μL/well). MTT assay was used to evaluate the cytotoxicity of **1a**.

Western Blot Analysis. Total cell lysates were prepared in a RIPA buffer (1% Triton X-100, 10% deoxycholate, 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% SDS, 0.1% PMSF and proteinase inhibitor cocktails). Proteins were fractionated in a 12.5% acrylamide gel, transferred to a PVDF membrane (Amersham Biosciences) and incubated with specific antibodies (anti-p53, anti-caspase 3, anti-Bcl-2, anti-Bcl-xL, anti-Bax, anti-p-H_2AX and anti-β-actin). After treating with the corresponding horseradish peroxidase-conjugated secondary antibodies, proteins of interest were then visualized by chemiluminescent detection.

Flow Cytometric Studies. Flow cytometric analysis was performed with a Coulter EPICS flow cytometer (Coulter, Miami, FL) equipped with 480 long, 525 band and 625 long pass filters. Samples were excited by 15 mW air-cool argon convergent laser at 488 nm. Fluorescence signals were manipulated with Coulter Elite 4.0 software (Coulter) and analyzed by Winlist 1.04 and Modfit 5.11 software (Verity Software House, Topsham, ME).

For cell cycle evaluation, SUNE1 cells were cultured in 6-well plates (5×10^5

cells/well) with 2 mL of fresh growth medium. After incubation for 24 hr, complex **1a** with the concentration equal to its IC_{50} value was added with fresh growth medium and the plates were further incubated for 6, 24, and 48 hr. At the end of each incubation period, cells were trypsinized and washed with PBS. Cells were then fixed with 70% EtOH in 1 × PBS and kept at -20ºC before analysis. Before staining, cells were centrifuged at 3000 rpm for 5 min and the cell pellets were washed with 1× PBS. After being stained with PI, cells were ready for analysis.

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Fig.S1 UV-vis absorption of **1a**, **1b**, **1c**, **1d**, and **3** in CH_3CN at room temperature. (2×10^{-5} M)

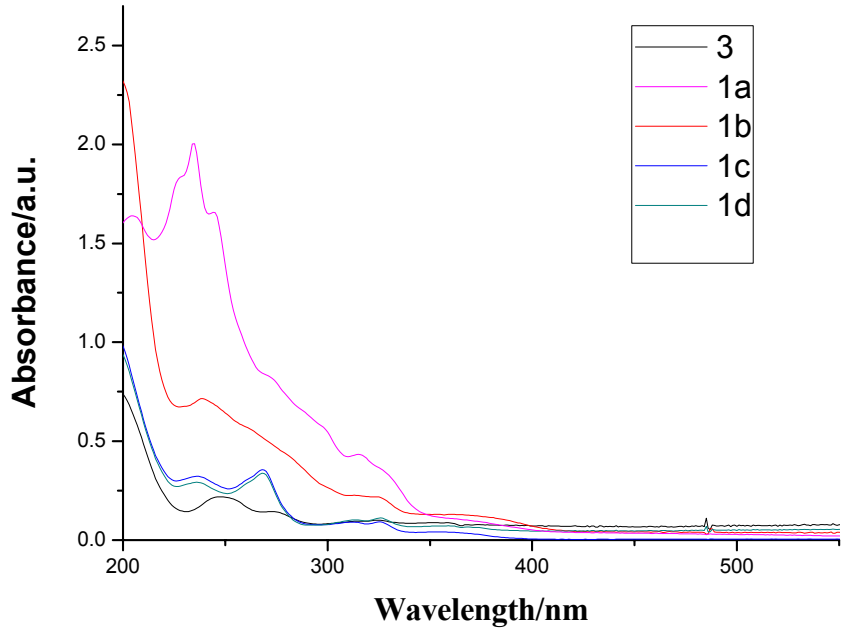


Fig.S2 UV-vis absorption of **2a**, **2b**, **2c**, **2d**, and **4** in CH_3CN at room temperature. (2×10^{-5} M)

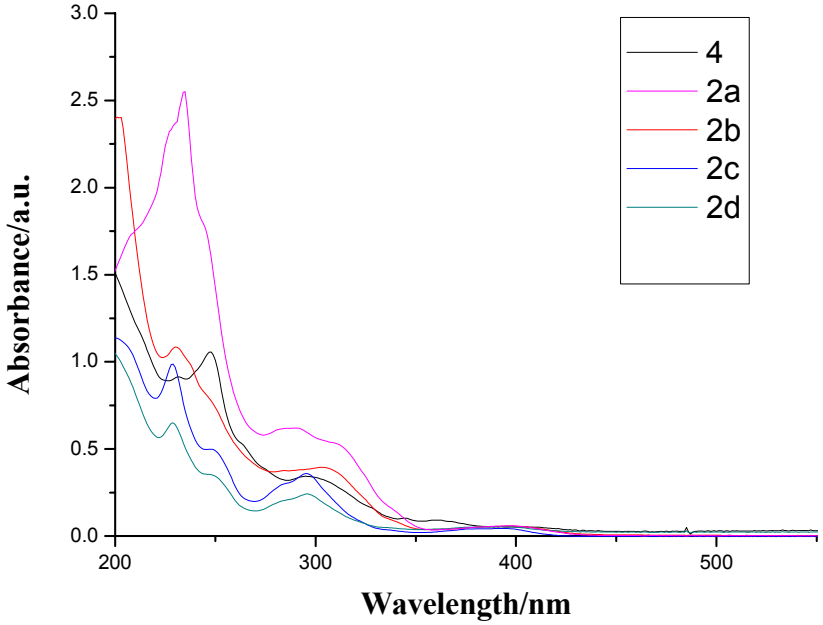


Fig.S3 Emission of **1a**, **1b**, **1c**, and **1d** in CH_3CN at room temperature. (2×10^{-5} M).

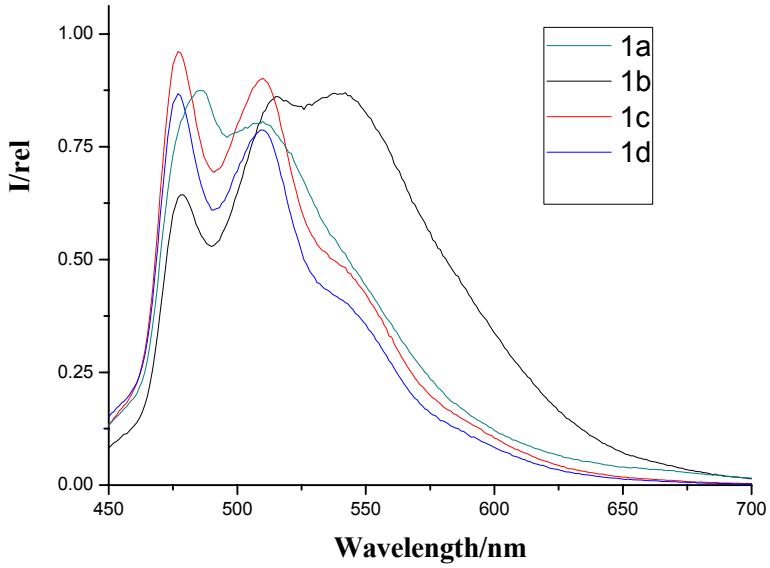


Fig.S4 Emission of **2a**, **2b**, **2c**, and **2d** in CH_3CN at room temperature. (2×10^{-5} M).

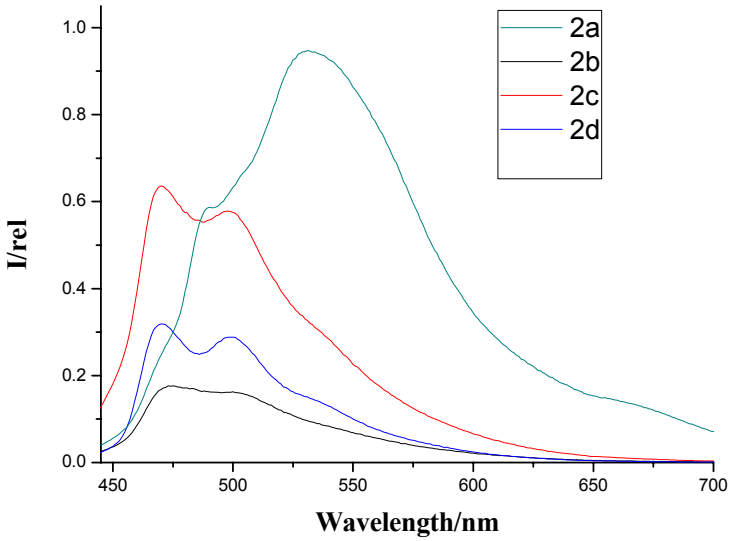
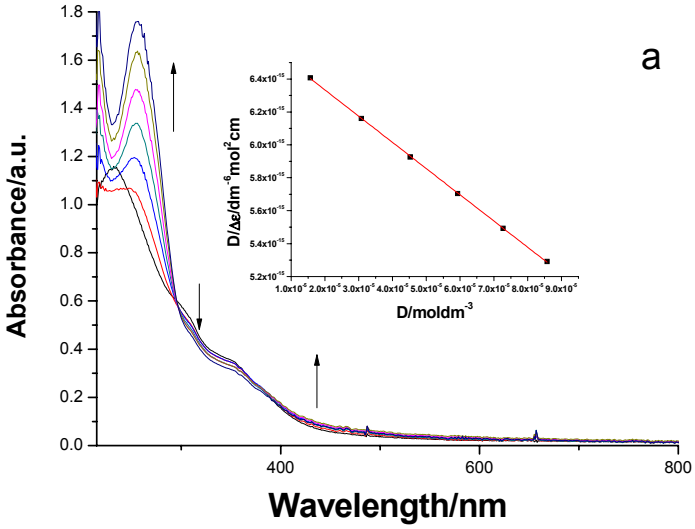


Fig.S5 (a) UV/Vis absorption of **1a** (20 μM) in pH 7.6 Tris-buffered saline with increasing ratio of [DNA]/[**1a**]: 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 at 20˚C. Inset: plot of D/Δε versus D (absorption was monitored at 333 nm); (b) DNA binding decreases the emission of **1a** at 630nm in TBS buffer and increases its emission at 417nm.



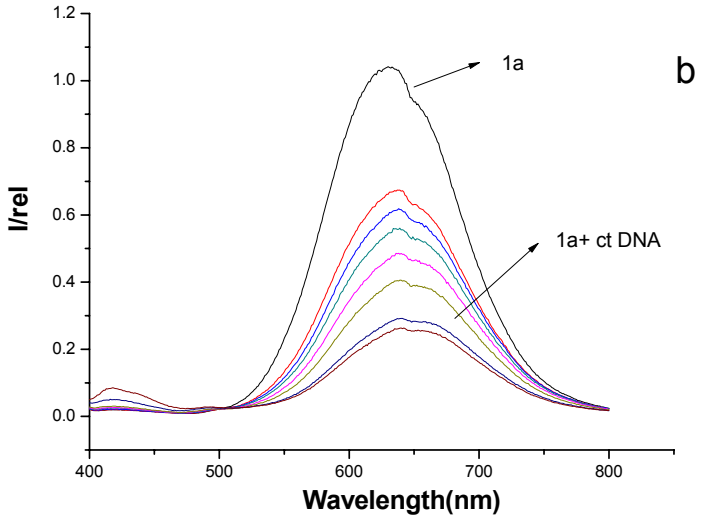


Fig.S6 (a) UV/Vis absorption of **1b** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**1b**]: 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 at 20 ˚C. Inset: plot of D/Δε versus D (absorption monitored at 314 nm); (b) DNA binding increases the emission intensity of **1b** at 490-510nm and decreases its emission at 610nm.

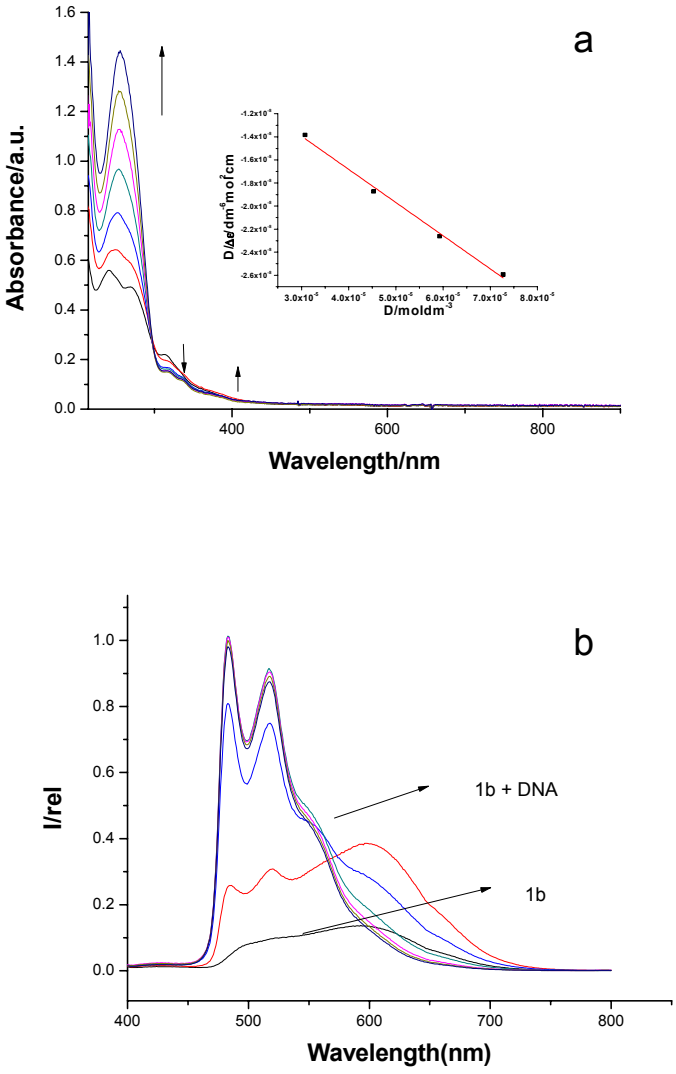


Fig.S7 (a) UV/Vis spectra of **1c** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**1c**]: 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 at 20˚C . Inset: plot of D/Δε versus D (absorption monitored at 352 nm; (b) Emission traces of **1c** (20 μM) in Tris buffer with increasing ratio of [DNA]/[**1c**]: 0.20, 0.39, 0.59, 0.79, 0.98, 1.18, 1.38, 1.57, 1.77, 1.97, 2.16, 2.36, 2.56, 2.75, 2.95, 3.15, 3.34, 3.54, 3.74 at 20 ˚C. Inset: Scatchard plot for the binding of **1c** to ct DNA. The titration data was used to generate the solid line in the figure, which was determined by using McGhee-Hippel Equation[3] and a nonlinear least-squares fitted for the data points.

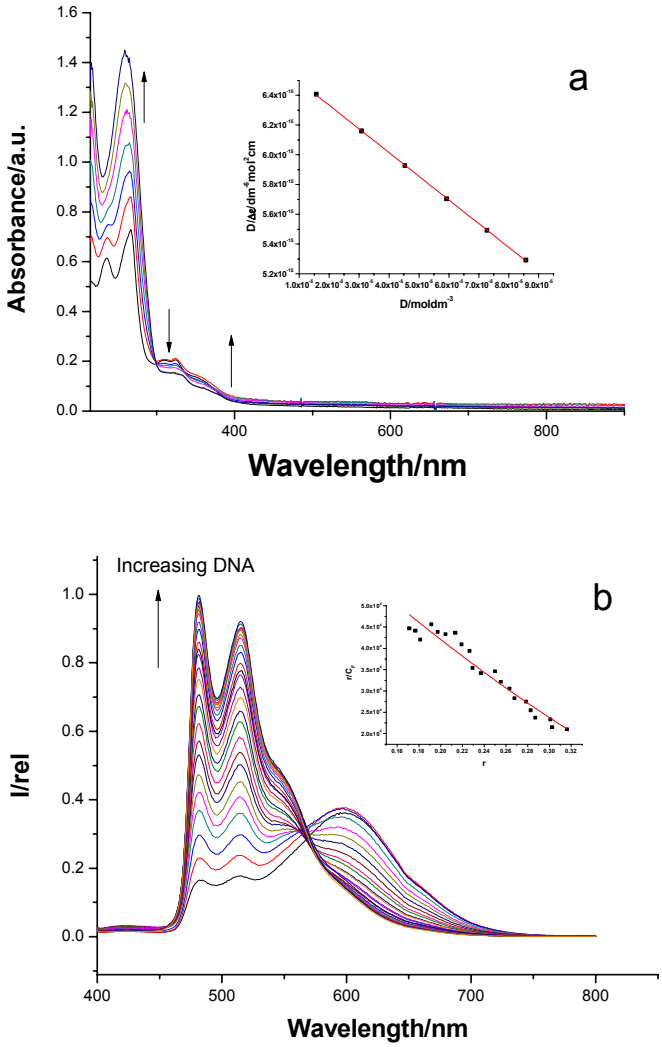
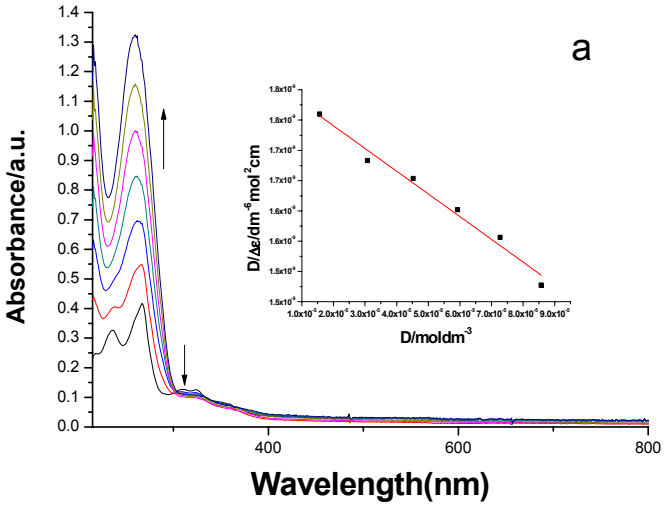


Fig.S8 (a) UV/Vis absorption of **1d** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**1d**]: 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 at 20˚C. Inset: plot of D/Δε versus D (absorption monitored at 317 nm); (b) DNA binding increases the emission of **1d** at 476-510nm.



b)

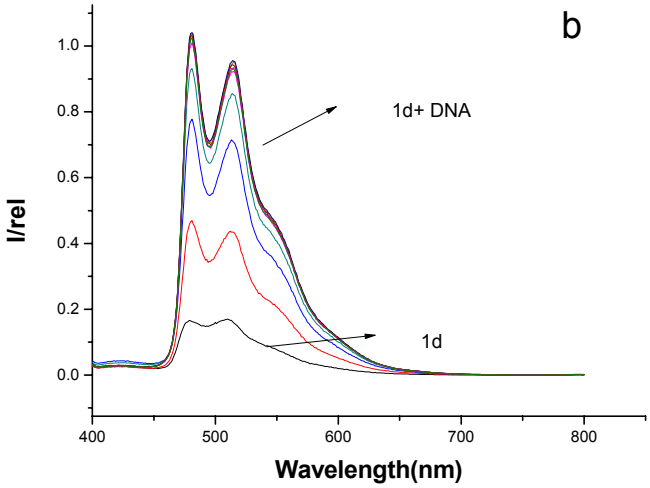
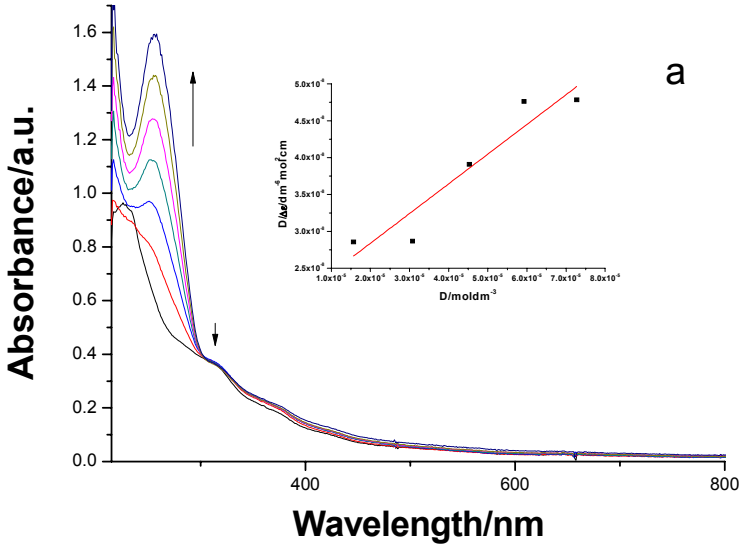


Fig.S9 (a) UV/Vis absorption of **2a** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**2a**]: 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 at 20℃. Inset: plot of D/Δε versus D (absorption monitored at 313 nm); (b) DNA binding decreases the emission intensity of **2a** at 650 nm.



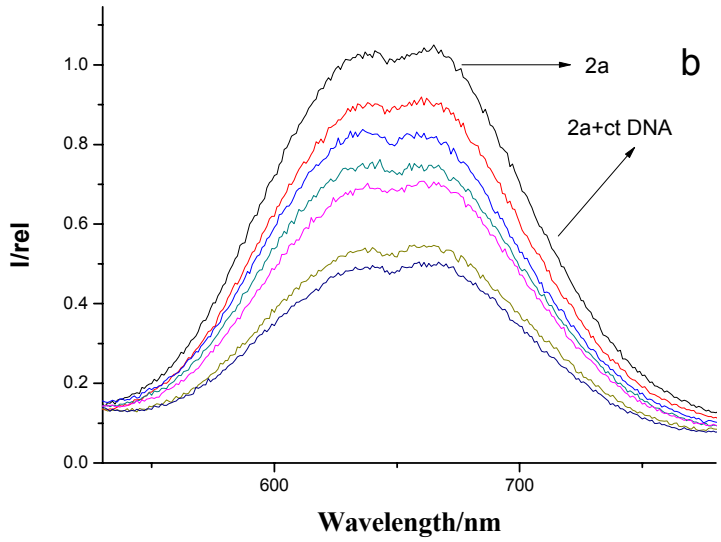
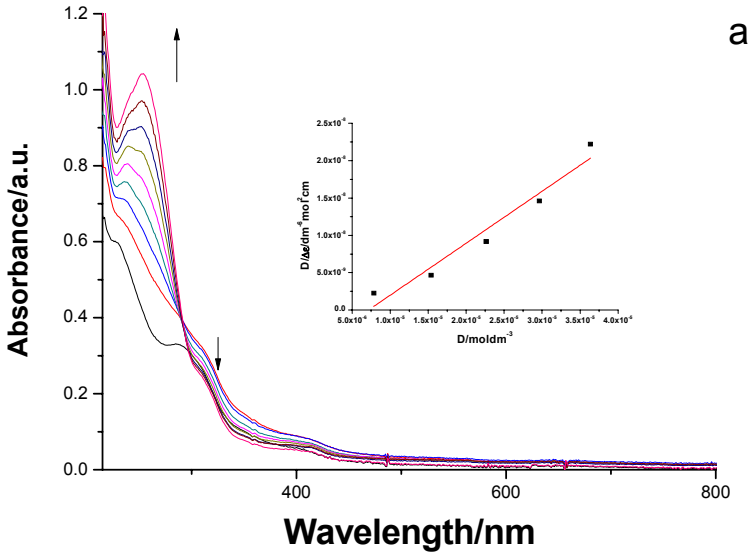


Fig.S10 (a) UV/Vis absorption of **2b** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**2b**]: 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 at 20℃. Inset: plot of D/Δε versus D (absorption monitored at 315 nm; (b) DNA binding decreases the emission intensity of **2b** at 600 nm and increases the emission intensity at 470 nm.



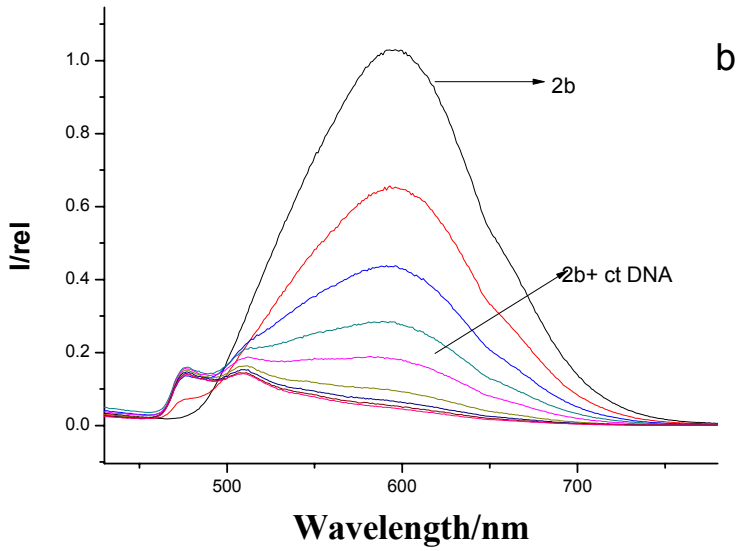


Fig.S11 (a) UV/Vis absorption of **2c** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**2c**]: 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 at 20℃. Inset: plot of D/Δε versus D (absorption monitored at 356 nm; (b) DNA binding decreases the emission intensity of **2c** at 600 nm and increases the emission intensity at 470 nm.

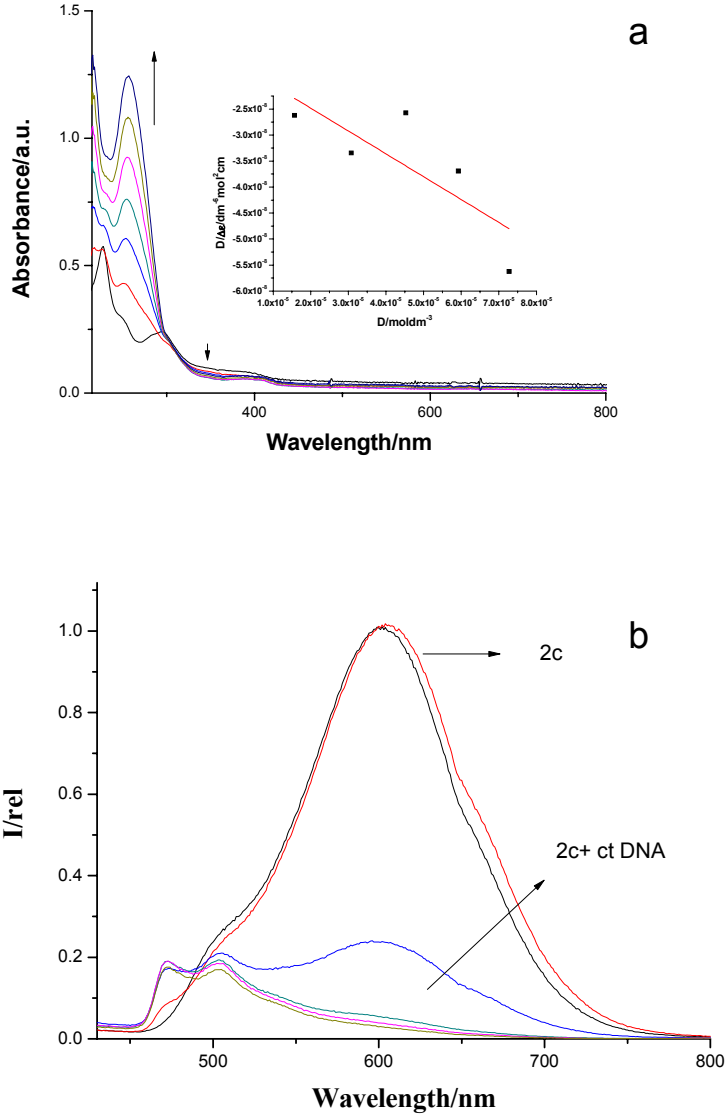


Fig.S12 (a) UV/Vis absorption of **2d** (20 μM) in Tris-buffered saline with increasing ratio of [DNA]/[**2d**]: 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 at 20℃. Inset: plot of D/Δε versus D (absorbance monitored at 298 nm); (b) DNA binding has little impact on the emission of **2d**.

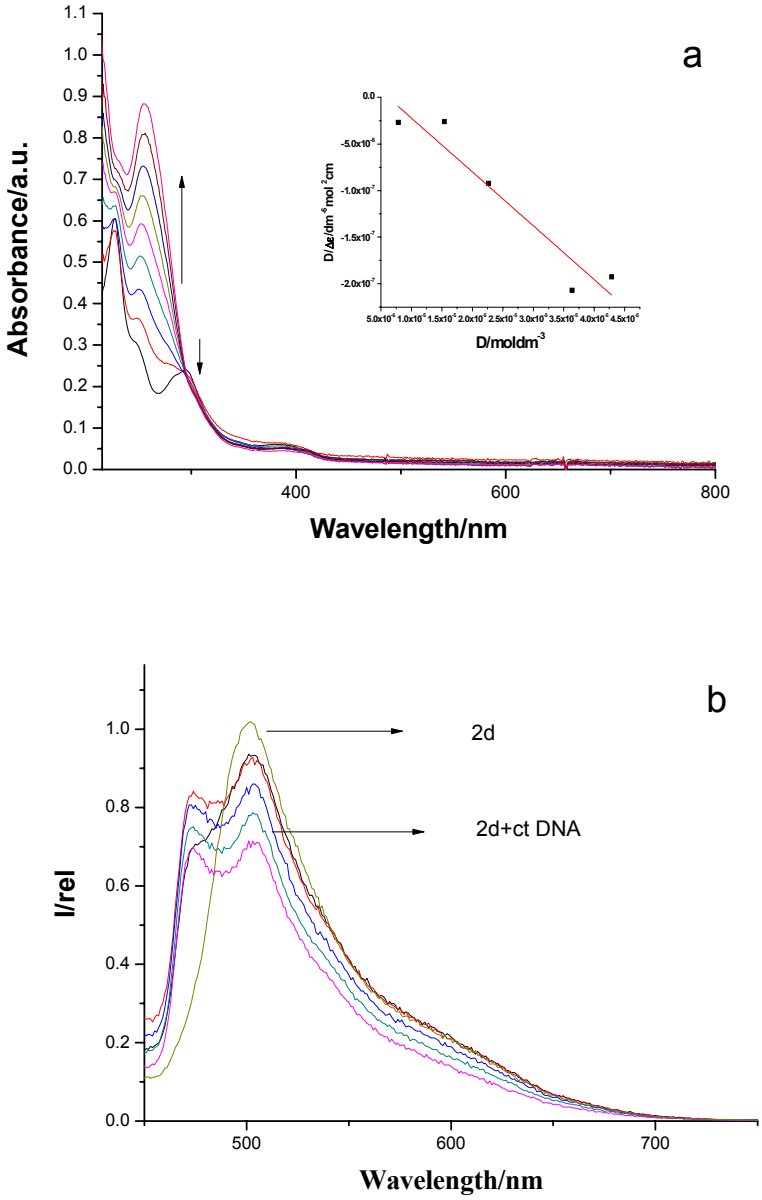


Fig.S13 Relative contour length (L/L_0) of ctDNA in the presence of ethidium bromide, Hoechst 33342, and **1a** as a function of the binding ratio ***r***. "***r***" means the binding ratio of small molecular to duplex DNA; "1+***r***" is the theoretical value of relative contour length (L/L_0) of ctDNA assuming the binding mode of small molecular with DNA is through intercalation; the relationship between the relative solution viscosity (η/η_0) and contour length (L/L_0) is given by $L/L_0=(\eta/\eta_0)^{1/3}$.

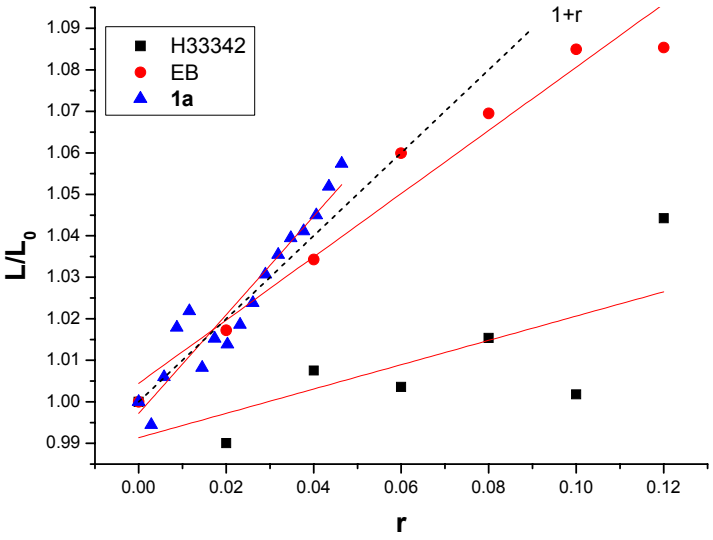


Fig.S14 The effects of Etoposide(VP-16), **1a-d** and **2a-d** on the TopoIIα-mediated supercoiled DNA relaxation assay. Different drug concentrations of **1a-d** and **2a-d** (0.1, 1.0, 10.0 μM; from left to right) and Etoposide (0.1, 1.0, 10.0, 100 μM) were employed. Lane1, supercoiled DNA only; Lane2, supercoiled DNA and TopoIIα. RE, relaxed; NK, nicked; LN, linear; SC, supercoiled; EB, ethidium bromide.

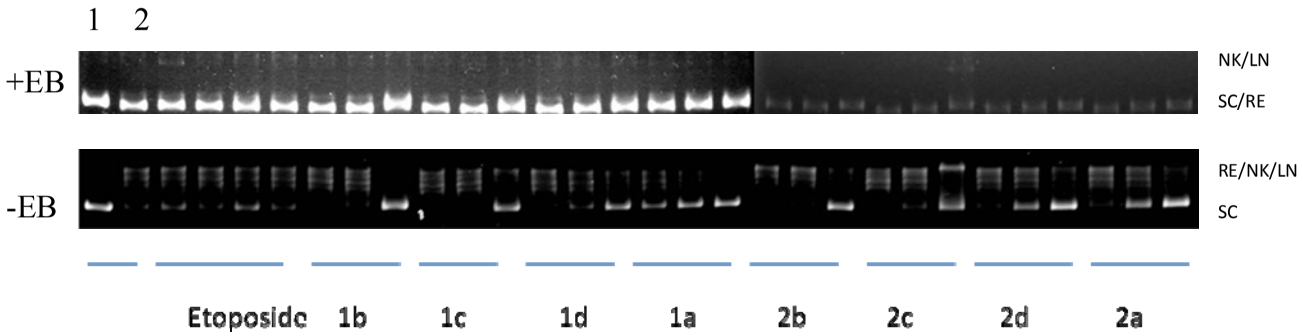


Fig.S15 The effects of Etoposide(VP-16), **1a-d** and **2a-d** on the catalytic activity of human TopoIIα-mediated kDNA decatenation assay. Drug concentration of 0.1, 1.0, 10.0 μM (from left to right) were employed. Lane1, kDNA only; Lane2, kDNA and TopoIIα. **I**, position of kDNA and **II**, position of the decatenated DNA.

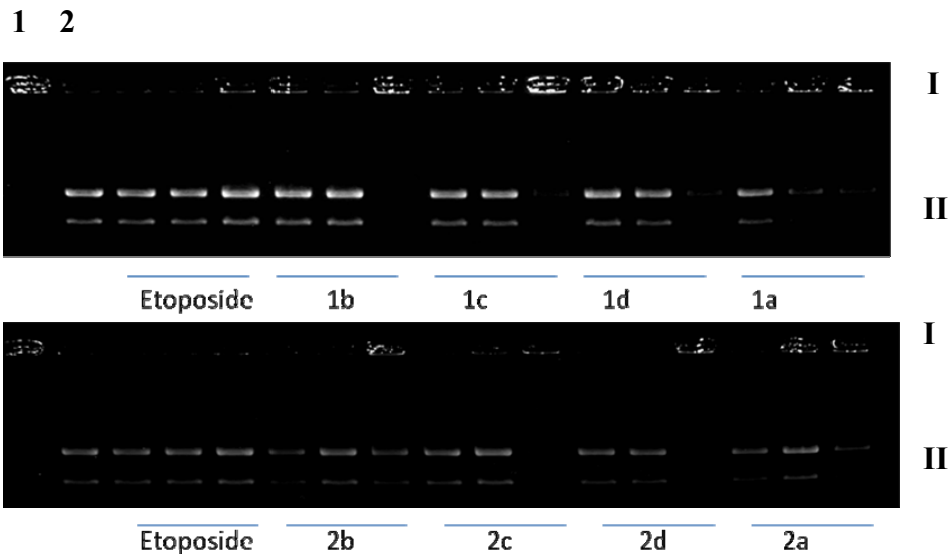
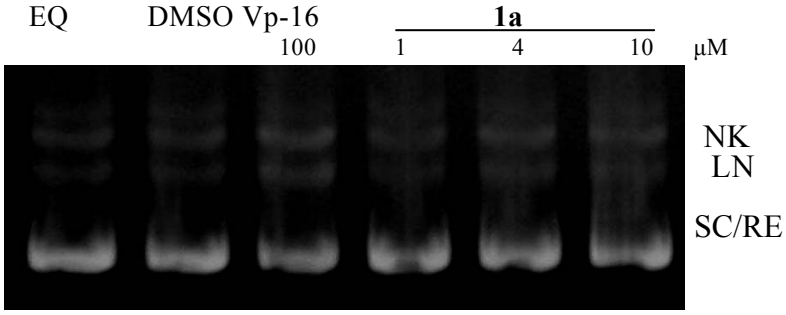


Fig.S16 (a) Dosage dependent inhibition of **1a** on the TopoIIα-mediated pre-strand passage DNA religation. (b) **1a** displays little impact on the TopoIIα-mediated post-strand passage DNA religation. EQ, equilibrium status; RE, relaxed; NK, nicked; LN, linear; SC, supercoiled.

(a)



(b)

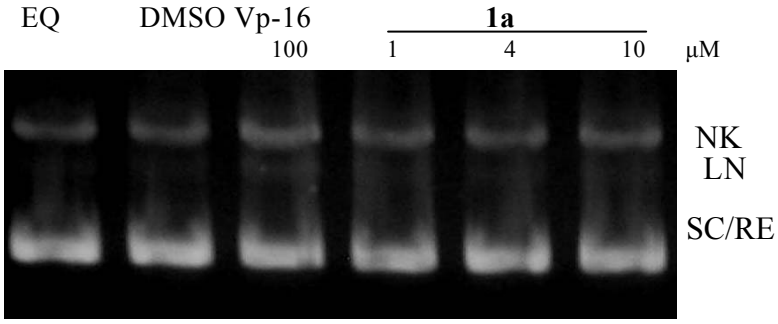


Fig.S17 Quantitation of the comet results. Statistical relationships between groups were determined by Student's t test. *, statistically significant comparisons of drug-treated cells to control ($P < 0.001$); **, statistically significant comparisons of heat reversal-treated cells to nontreated cells ($P < 0.001$).

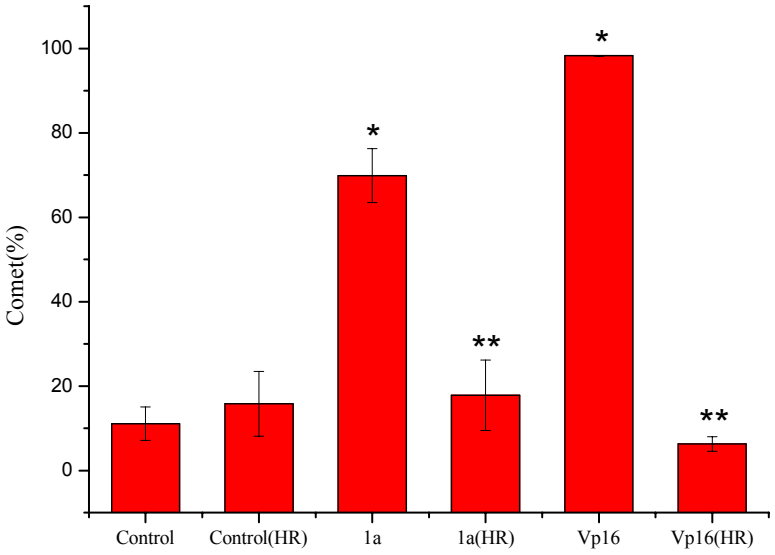


Fig.S18 Increased expression of phosphorylated H_2AX by Vp-16(100 μM) and **1a** (0.4, 1, 4, 10 μM) in SUNE1 cells.

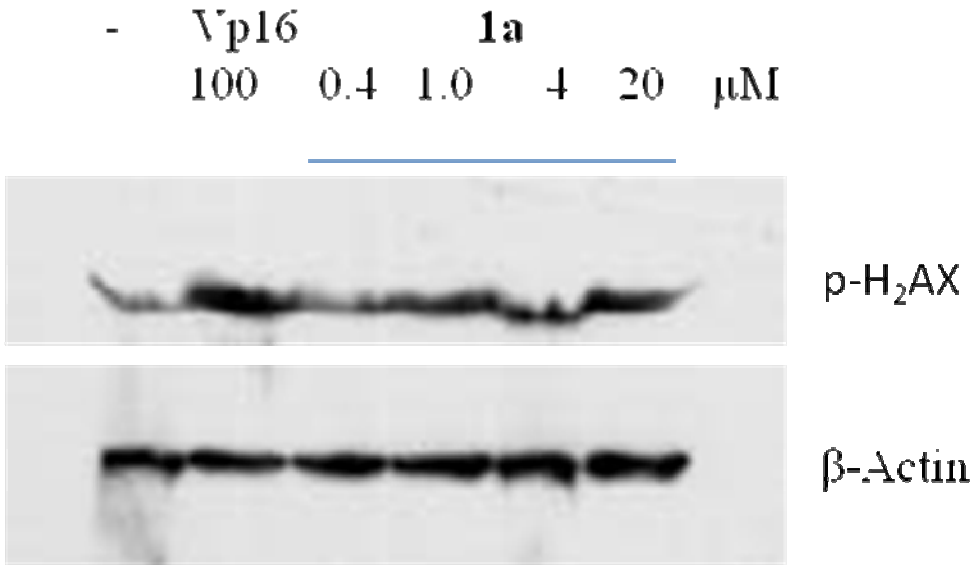


Fig.S19 Complex **1a** induced TopoIIα-cleavable complexes in SUNE1 cells as revealed by a band depletion assay.

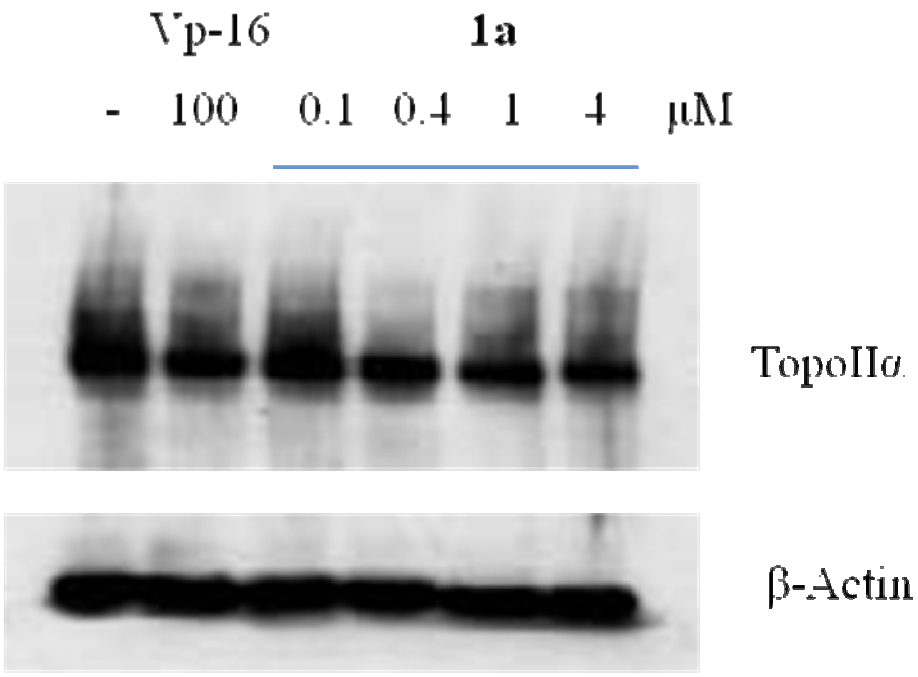


Fig.S20 Electrophoretic mobility shift assay (EMSA) indicates that **1a** (10 μM) has no observed effect on the binding of DNA with TopoIIα. Lane1: DNA only; Lane 2: DNA and 10 μM **1a**; Lane 3: DNA and TopoIIα; and Lane 4: DNA, 10 μM **1a,** and TopoIIα.

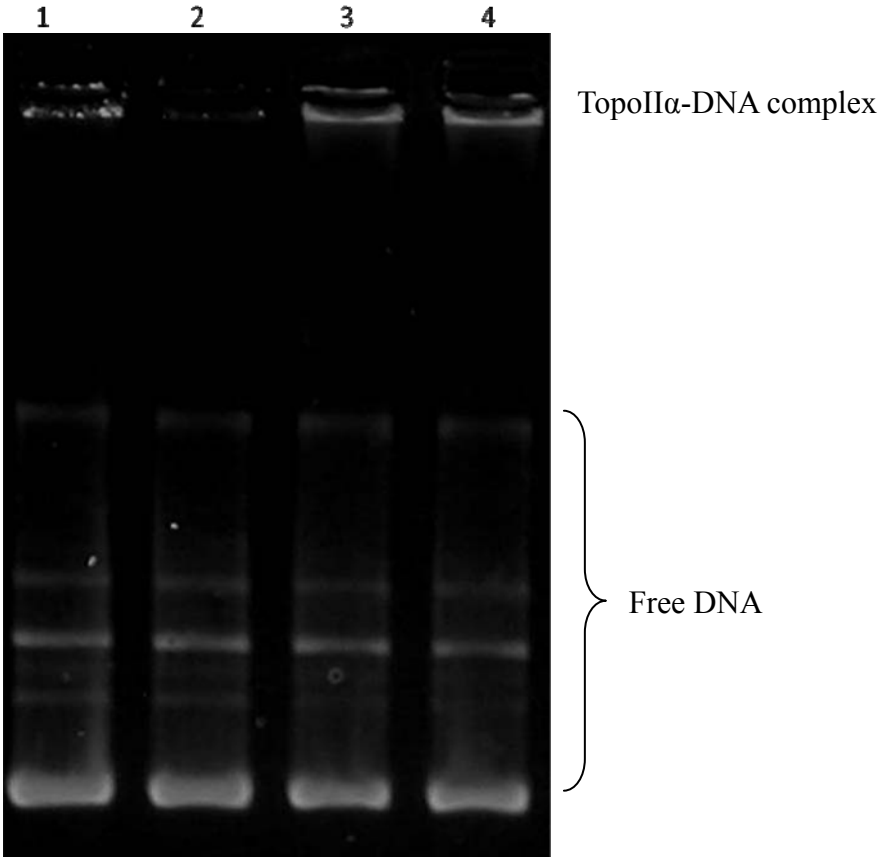


Fig.S21 1a has no remarkable inhibition on the TopoIIα catalysed ATP hydrolysis using different concentrations of ATP in the assay.

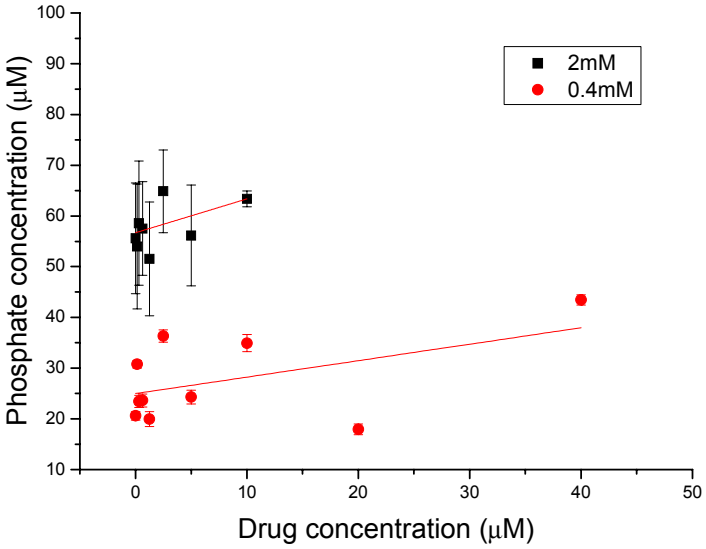


Fig.S22 TopoIIα knockdown had pronounced effects of **1a** (a) and Vp-16 (b) on cell viability. TopoIIα knockdown was realized by transfection of SUNE1 cell with anti-TopoIIα siRNA. After 12 h incubation, MTT assay was utilized to evaluate the cell viability. Western blot was used to confirm the down-expressed TopoIIα after transfection (c).

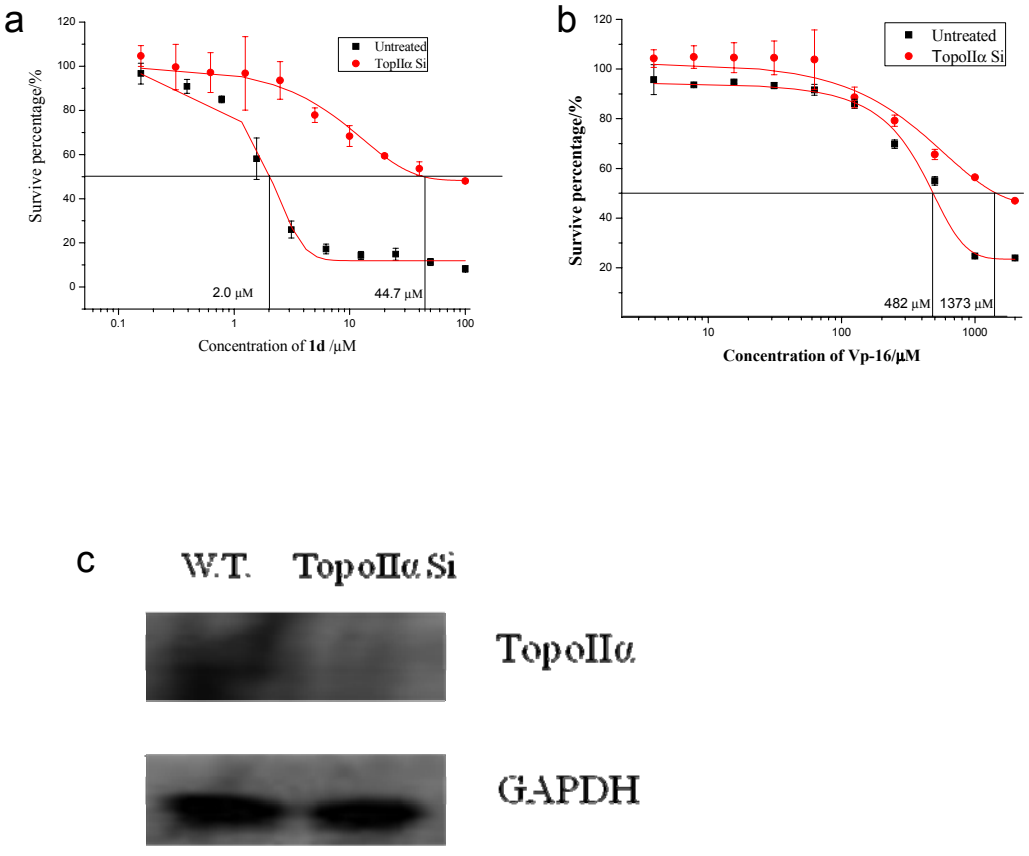


Fig.S23 Western blotting revealed the up-regulation of pro-apoptotic (Bax) protein and the down-regulation of the anti-apoptotic (Bcl-2 and Bcl-xL) protein by **1a** after 48 hr and induction of p53 and activation of a cysteine protease (caspase-3) after 36 hr.

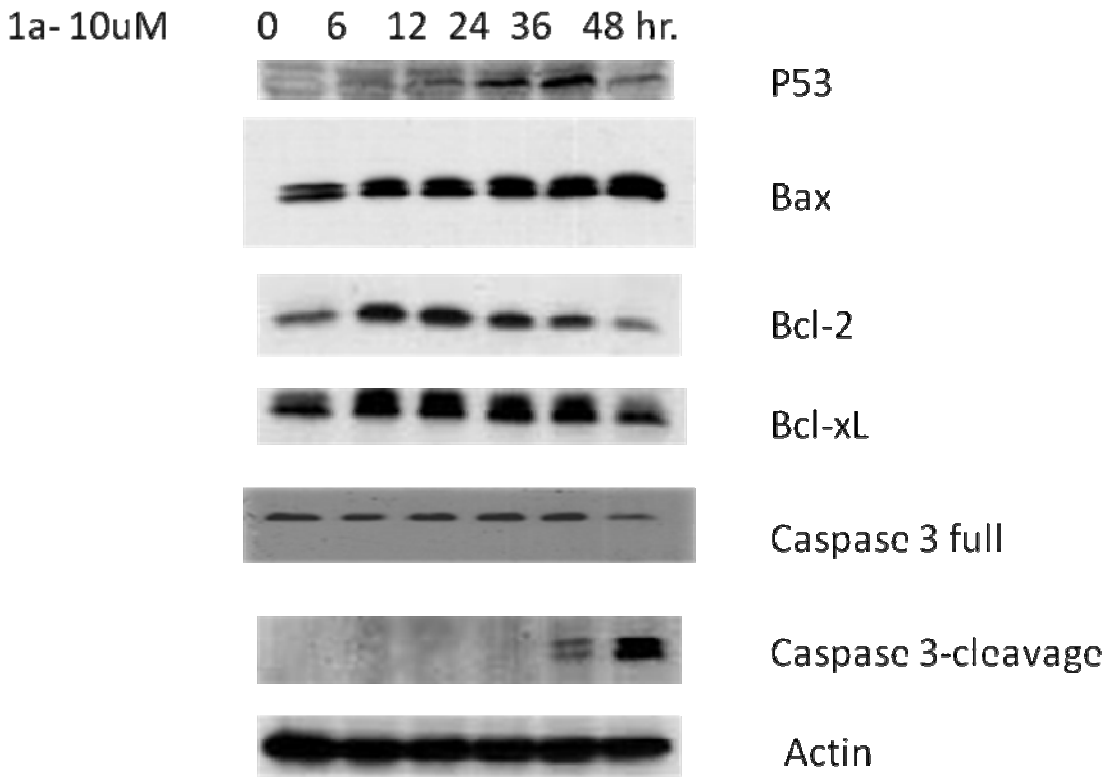


Fig.S24 1a-treated cells tend to arrest at G1-phase after 24 hr treatment as revealed by flow cytometry.

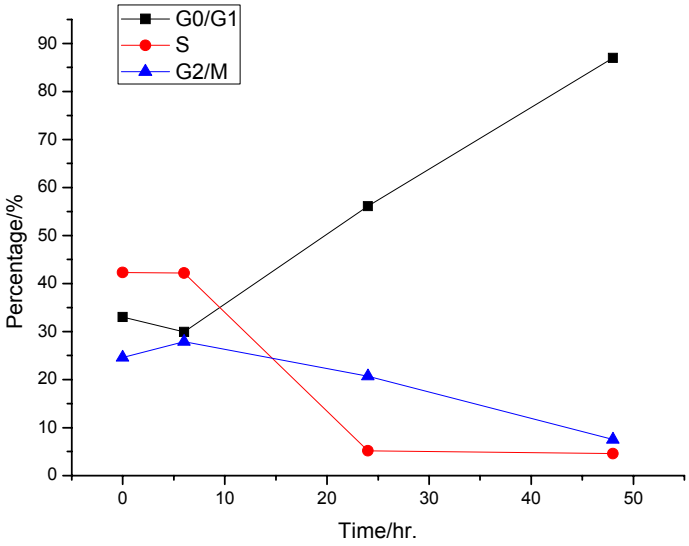


Table S1 Molar absorptivity (λ_{abs}) for compounds **1-4** (2×10^{-5} M) in acetonitrile at room temperature.

Compound	λ_{abs}/nm (εM^{-1} cm^{-1}) (solution ~2×10^{-5} M)
1a	227(sh,91150), 234(100000), 244(sh, 82750), 270(sh, 41600), 315(21500), 369(sh, 4950)
1b	238(35750), 314(10900), 326(sh, 10500), 368(6350)
1c	237(16100), 268(17650), 311(sh, 4475), 324(4580), 359(2050)
1d	236(14600), 268(16800), 313(sh, 5100), 326(5550), 368 (3600)
2a	227(sh, 123500), 234(134500), 245(sh, 88500), 286(31000), 311(sh, 26250), 395(2800)
2b	230(99900), 248(sh, 38550), 300(sh, 19600), 395(3000)
2c	229(49150), 248(sh, 24850), 295(17900), 390(2300)
2d	229(40050), 248(sh, 17650), 283(sh, 10050), 295(12000), 389(2700)
3	245(10900), 273(sh, 7250), 323(sh, 5050), 357 (4400)
4	231(55000), 248(62500), 295(sh, 17000), 345(sh, 5200), 360(4500)

Table S2 Emission of compounds **1-2** (2×10^{-5} M) at room temperature.

Compound	Acetonitrile (298K)	Acetonitrile/TBS buffer(1/99)
	λ_{max}/nm; $\tau_0/\mu s$; Φ_0	λ_{max}/nm; $\tau_0/\mu s$; Φ_0
1a	485(max), 509; 6.69; 0.0025	629(max); 0.18; 0.065
1b	478, 515, 539(max); 0.42; 0.0019	591(max); 0.52; 0.039
1c	476(max), 510, 540(sh); 1.18; 0.011	483, 513, 597(max); 1.55; 0.073
1d	477(max), 509, 542(sh); 0.20; 0.0036	478, 509(max); 0.77; 0.047
2a	488, 530(max), 659(sh); 0.097; 0.011	637, 660(max); 0.24; 0.065
2b	472(max), 499; 0.32; 0.0020	595(max); 1.62; 0.12
2c	470(max), 499; 1.51; 0.0087	603(max); 1.59; 0.16
2d	469(max), 497, 533(sh); 0.23; 0.0035	501(max); 0.89; 0.078

Table S3 DNA Binding constant, hypochromism, and n of complexes **1a-d** and **2a-d** from UV-Vis absorption titration and emission titration assay.

Compound	Absorption Titrition		Emission Titrition	
	K [$mol^{-1}dm^3$] at 20℃	Hypochromicity [%]	K [$mol^{-1}dm^3$] at 20℃	n
1a	1.3×10^6	13%	7.9×10^5	10.5
1b	5.4×10^4	31%	7.6×10^4	1.3
1c	2.4×10^3	25%	8.7×10^4	1.9
1d	1.0×10^4	23%	2.0×10^5	1.7
2a	1.6×10^4	2.7%	1.4×10^5	20
2b	1.4×10^5	23%	3.2×10^5	2.7
2c	4.71×10^5	41%	7.2×10^5	1.0
2d	2.9×10^5	13%	N.A	N.A.

Table S4 ^{1}H NMR chemical shifts [ppm] of d(CAATCCGGATTG)$_2$ and chemical shift differences[a] (in parentheses) induced by addition of **1a** at a [**1a**]/[d(CAATCCGGATTG)$_2$] ratio of 2.

	H8/H6	AH2
C_1	7.524 (**0.017**)	
A_2	8.205 (0.002)	7.252 (-0.003)
A_3	8.080 (-0.001)	7.601 (-0.009)
T_4	6.961 (0.004)	
C_5	7.331 (**0.012)**	
C_6	7.194 (**0.011**)	
G_7	7.659 (0.002)	
G_8	7.601 (-0.009)	
A_9	8.016 (0.001)	7.641 (-0.008)
T_{10}	7.042 (-0.008)	
T_{11}	7.164 (0.000)	
G_{12}	7.791 (**0.029**)	

[a] Significant chemical shift differences (0.01 ppm or more) are shown in bold face and highlight in red color. Positive numbers indicate a downfield shift.

Table S5 Cytotoxicities of **1–4** and Vp-16 in four Human Carcinoma Cell Lines, HeLa, HepG2, SUNE1, NCI-H460, and Normal CCD-19Lu.[a]

	IC_{50}(μM) [72 hr]				
Complex	HeLa	HepG2	SUNE1	NCI-H460	CCD-19Lu
1a	3.9±0.5	17.8±1.5	2.0±0.4/44.7±3.6[b]	4.8±0.4	73.8±4.2
1b	8.1±0.6	14.8±3.0	3.5±0.3	7.9±0.6	3.8±0.2
1c	12.5±2.1	24.4±2.8	7.5±0.9	10.8±1.1	14.8±0.9
1d	4.6±0.5	19.3±1.6	4.8±0.5	7.1±1.4	10.3±0.9
2a	6.3±0.4	52.5±3.8	21.7±1.6	51.6±4.9	78.8±8.1
2b	7.9±1.4	22.1±2.3	5.4±0.9	12.7±1.6	3.5±0.4
2c	18.9±1.6	38.5±4.2	5.4±0.4	14.8±2.5	9.8±1.4
2d	27.4±2.6	21.7±2.3	6.0±0.5	11.3±1.9	7.1±0.9
3	>100	>100	>100	>100	92.8±4.4
4	39.9±3.0	100±5.0	97.1±7.1	>100	49.0±3.6
cisplatin	8.5±1.1	10.5 ± 1.5	15.5± 2.1	3.4± 1.4	166 ± 7.9
Vp-16	77.9±4.8	>100	482±10/1373±57[b]	7.6±0.6	>100

[a] HeLa: human cervical epithelioid carcinoma; SUNE1: human nasopharyngeal carcinoma; HepG2: human hepatocellular carcinoma; NCI-H460: human large-cell lung carcinoma; CCD-19Lu: normal human lung fibroblast cells; values are mean ±SD (μM) from at least three independent experiments; [b] the IC_{50} value of **1a** towards the knockdown SUNE1 cells has been added and highlighted for comparison.