Supporting Information

For

Cyclometalated Gold(III) Complexes with *N*-Heterocyclic Carbene Ligands as Topoisomerase I Poisons

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Part A: Experimental Section.

Materials. All chemicals, unless otherwise noted, were purchased from Sigma-Aldrich. All solvents were purified according to conventional methods. Calf-thymus DNA (ctDNA) was purified by phenol/chloroform extractions. Cell Proliferation Kit I (MTT) was purchased from Roche.

Instrumentation. ^{1}H NMR spectra were recorded on a DPX-300 Bruker FT-NMR spectrometer with chemical shift (in ppm) relative to tetramethylsilane. Mass spectra (Fast Atom Bombardment, FAB) of **1**, **2** and **4** were recorded on a Finnigan MAT95 mass spectrometer using 3-nitrobenzyl alcohol (NBA) as matrix. Mass spectrum of **3** was recorded on an Electrospray Ionisation-Time-of-Flight Mass Spectrometer (ESI-qTOP-MS, Q-Tof Premier, Waters). Gold analysis was undertaken using an Agilent 7500 Inductively coupled plasma mass spectrometer. All absorption spectra were recorded on a Perkin-Elmer Lambda 900 UV-visible spectrophotometer. Elemental analyses were performed by the Institute of Chemistry at Chinese Academy of Sciences, Beijing.

Synthesis. The synthesis and characterization of **1** have been reported previously (V. K.-M. Au, K. M.-C. Wong, N. Zhu and V. W.-W. Yam, *J. Am. Chem. Soc.*, 2009, **131**, 9076).

Complex **1**: Au(C^N^C)Cl (Fig. S1, 40.00 mg, 0.087 mmol), *N,N'*-dimethylimidazolium iodide (20.61 mg, 0.092 mmol) and KO^{t}Bu (11.20 mg, 0.100 mmol) were refluxed in 25 mL CH_3CN overnight under an inert atmosphere. After 24 hours, a saturated $LiOSO_2CF_3$ solution in CH_3CN was added and the mixture was stirred at room temperature for another 30 minutes. The mixture was gravity filtered and the filtrate was collected. The filtrate was concentrated to about 5 mL,

excess Et_2O was added and the mixture was kept <10 ℃ for 1 day. Pale yellow solid was formed. Yield: 49.32 mg, 82.6%. Anal. Calcd for $C_{23}H_{19}N_3O_3F_3SAu$: C, 41.13; H, 2.83; N, 6.26. Found: C, 41.06; H, 3.01; N, 6.56. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 3.84 (s, 6H, -CH_3), 6.94 (d, 2H, *J* = 7.09 Hz), 7.31 (t, 2H, *J* = 6.70), 7.37 (t, 2H, *J* = 7.18), 7.83 (s, 2H), 7.99 (d, 2H, *J* = 7.51 Hz), 8.06 (d, 2H, *J* = 8.03), 8.25 (t, 1H, *J* = 8.01). ^{19}F NMR (400 MHz, $(CD_3)_2SO$): δ -79.32. FAB-MS (+ve, *m/z*): 522 [M^+].

Complex **2:** Au(C^N^C)Cl (100 mg, 0.217 mmol), 1,1′-methylene bis(3-*n*-butylimidazolium) diiodide (56.20 mg, 0.109 mmol) and KO^{t}Bu (25.80 mg, 0.230 mmol) were refluxed in 20 mL CH_3CN under an inert atmosphere overnight. A yellow colored solution was formed. The solution was treated in a similar manner to that of complex **1,** yellow precipitates were crystallized out. Yield: 0.1081 g, 72.5 %. Anal. Calcd for $C_{51}H_{46}N_6O_6F_6S_2Au_2$: C, 43.41; H, 3.29; N, 5.96. Found: C, 43.62; H, 3.33; N, 6.08. ^{1}H NMR (400 MHz, CD_3CN): δ 0.62 (t, 6H, *J* = 7.36, -^{n}Bu), 1.03 (q, 4H, *J* = 7.53 ,-^{n}Bu), 1.62-1.66 (m, 4H, -^{n}Bu), 4.03 (t, 4H, *J* = 7.11, -^{n}Bu), 6.73 (d, 4H, *J* = 7.34), 6.83 (s, 2H), 6.91 (t, 4H, *J* = 7.39), 7.14 (t, 4H, *J* = 7.60), 7.41 (d, 4H, *J* = 7.73), 7.47 (d, 4H, *J* = 8.04), 7.69 (s, 2H), 8.02 (t, 2H, *J* = 8.02 Hz), 8.09 (s, 2H). ^{19}F NMR (400 MHz, (CD_3CN): δ -79.33. FAB-MS (+ve, *m/z*): 1261 $[M + OSO_2CF_3]^+$.

Complex **3:** Au(C^N^C)Cl (100 mg, 0.217 mmol), 1,2-propylene bis (3-*n*-butylimidazolium) diiodide (57.73 mg, 0.109 mmol) and KO^{t}Bu (25.80 mg, 0.230 mmol) were refluxed in 20 mL CH_3CN under an inert atmosphere overnight. A yellow colored solution was formed. The solution was treated in a similar manner to that of complex **1,** yellow precipitates were crystallized out. Yield: 0.1163 g, 78 %. Anal. Calcd for $C_{53}H_{50}N_6O_6F_6S_2Au_2$: C, 44.23; H, 3.50; N, 5.84. Found: C, 43.96; H, 3.49; N, 5.90. ^{1}H NMR (400 MHz, CD_3CN): δ 0.69 (t, 6H, *J* = 5.64, -^{n}Bu), 1.13 (q, 4H, -^{n}Bu), 1.66-1.67 (m, 4H, -^{n}Bu), 2.43 (t, 2H, *J* = 6.28), 4.04 (t, 4H, *J*=5.00), 4.19 (s, 4H), 6.83 (s, 4H), 7.14 (s, 4H), 7.23 (d, 4H, *J* = 5.90), 7.47(s, 4H),7.69 (s, 4H), 7.74 (s, 4H), 8.14-8.15 (m, 2H). ^{19}F NMR (400 MHz, (CD_3CN): δ -79.311. ESI-Q-TOF-MS (+ve, *m/z*): 570 $[M]^{2+}$.

Complex **4:** The preparation of complex **4** was similar to that of **1**, using Au(S^N^C)Cl (Fig. S1, 96.6 mg, 0.1777 mmol), *N,N*′-dimethylimidazolium iodide (41.8 mg, 0.1866 mmol) and KO^{t}Bu (21.5 mg, 0.1900 mmol). A pure orange-brown solid was formed. Yield: 93.1 mg, 69.4 %. Anal. Calcd for $C_{27}H_{21}N_3O_3F_3S_2Au\cdot(0.5H_2O)$: C, 42.53; H, 2.90; N, 5.51. Found: C, 42.18; H, 2.81; N,

5.52. ^{1}H NMR (400 MHz, CD_3CN): δ 3.85 (s, 6H, -CH_3), 7.02-7.07 (m, 2H), 7.11-7.23 (m, 7H), 7.31 (d, 4H, J = 6.17), 8.37 (s, 2H). ^{19}F NMR (400 MHz, ($CDCl_3$): δ -79.33. ESI-Q-TOF-MS (+ve, *m/z*): 604 $[M]^+$.

Absorption-Titration Experiments. A solution of gold(III) complex in TBS/DMSO (9:1) solution (1 mL) was placed in a thermostatic cuvette and its absorption spectrum was recorded. Aliquots of a millimolar stock solution of ctDNA were added to the sample solution. The absorption spectra were recorded after equilibration for 1 min per aliquot until saturation point was reached. The binding constant was determined by applying the Scatchard equation: $[DNA]/\Delta\varepsilon_{ap} = [DNA]/\Delta\varepsilon + 1\ [1/(\Delta\varepsilon \times K_b)]$, in which $\Delta\varepsilon_{ap} = | \varepsilon_A - \varepsilon_B |$ where $\varepsilon_A = A_{obs}/[complex]$, and $\Delta\varepsilon = | \varepsilon_B - \varepsilon_F |$ where ε_B and ε_F correspond to the extinction coefficients of the DNA-bound and -unbound complex, respectively.

Emission-Titration Experiments. A solution of gold(III) complex in TBS/DMSO (9:1) solution (1 mL) was placed in a thermostatic cuvette and its absorption spectrum was recorded. Aliquots of a millimolar stock solution of ctDNA were added to the sample solution. The emission spectra were recorded in the 300-750 nm range after equilibration for 5−10 minutes per aliquot until saturation point had been reached.

Gel-Mobility-Shift Assay. 100-bp DNA ladder (corresponding to 15 μM in base pairs) was incubated with ethidium bromide or **1** in a 1:1 ratio of DNA base pair to the complex for 30 min. The mixtures were analyzed by gel electrophoresis using a 2% (w/v) agarose gel and tris-acetate-EDTA (TAE) buffer. The gel was immersed ethidium bromide solution after electrophoresis, and visualized using UV transillumination.

Viscosity Measurement. The method used by Suh and Chaires was employed in this study [D. Suh, J. B. Chaires, *Bioorg. Med. Chem.*, 1995, **3**, 723]. Viscosity experiments were performed using a Cannon-Manning Semi-Micro Viscometer, immersed in a thermostated water bath maintained at 27^{o}C. Titrations of **1**, ethidium bromide (EB) and Hoechst 33342 (H33342) were carried out by the addition of small volumes of concentrated stock solutions to the DNA sample in BPE buffer (Na_2HPO_4, 6 mM; NaH_2PO_4, 2 mM; Na_2EDTA, 1 mM) at pH 7.0 in the viscometer. Solutions in the viscometer were mixed by bubbling nitrogen through the solution. DNA concentrations of approximately 1 mM (in base pairs) were used.

Topoisomerase I-Mediated DNA Relaxation Assay. Plasmid DNA (2.9 kb) was purchased from Promega (Madison, WI, USA) at a concentration of 1 μg/mL. It was diluted to the working concentration in reaction buffer (50 mM Tris/HCl (pH 7.5), 20 mM KCl, 1 mM EDTA, 0.3 mg/mL BSA and 1 mM dithiothreitol). Recombinant human topoisomerase I (TopoI) was kindly provided by Prof. Y. C. Cheng (Pharmacology, Yale University). Supercoiled (SC) plasmid DNA was diluted by using reaction buffer to a concentration of 25 ng/μL. To a 75 ng of SC DNA dilution, aliquot of **1** (1 μL) dissolved in PBS-EtOH (19:1, v/v), CPT or vehicle control was added. The mixtures were incubated for 20 min at room temperature and Topo I solution (2 μL) was added. This solution was incubated at 37ºC for 2 h. The reaction was terminated by addition of a 20% sodium dodecyl sulfate (SDS, 1.25 μL) solution and proteinase K (2.5 μL, 1 mg/mL). After incubation at 45ºC for 1 h, the samples were analyzed by gel electrophoresis using 1% (w/v) agarose gels containing 0.1% (w/v) SDS. The gel was stained with ethidium bromide after electrophoresis and was visualized under UV illumination.

TopoI-Mediated DNA Cleavage. pBR322 DNA was cut with *Hind*III restriction endonuclease and then 3′-end-labeled with [α-32P]dATP using the large fragment of DNA polymerase I and unlabeled dCTP/dGTP/dTTP. The reaction mixture containing TopoI, ^{32}P-end-labeled pBR322 DNA, and **1** was incubated at 37^{o}C for 20 min. The reactions were terminated by the addition of SDS and proteinase K. DNA samples were analyzed by electorphoresis in 6% polyacrylamide gel. Gels were autoradiographed after electrophoresis.

Alkaline Comet Assay. Drug-treated KB cells (~5×10^{5}) were pelleted and re-suspended in 1 mL of ice-cold PBS buffer. 50 μl of re-suspended cells were mixed with 0.5 mL of pre-warmed 0.7% low melting point agarose. This mixture (0.1 mL) was loaded onto a fully frosted slide that had been pre-coated with 0.7% agarose. The slides were kept for 10 min at 4^{o}C, and then submerged in pre-chilled lysis solution [1% N-lauryl sarcosine, 1% Triton X-100, 2.5 M NaCl, and 10 mM EDTA (pH 10.5)] for 1h at 4^{o}C. After soaking with electrophoresis buffer (0.3 N NaOH and 1 mM EDTA), the slides were subjected to electrophoresis for 10 min at 14 Volts in cold room. After electrophoresis, slides were stained with SYBR green stain and nuclei were visualized under a fluorescence microscope.

Band Depletion Assay. KB cells (10^{6}/sample) were treated with drug for 15min at 37^{o}C. Cells were pelleted and lysed immediate (without washing) using the alkaline lysis procedure. Briefly, cell pellets were lysed with 0.2 N NaOH containing 1mM

EDTA. Alkaline lysates were then neutralized with one-tenth volume of 2 N HCl and one-tenth 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, and 1 mM EGTA , and 100 μg/ml each of leupeptin, pepstatin, and aprotinin. To the neutralized lysates, half volume of 3× SDS-PAGE sample buffer was added. SDS lysates were analyzed by SDS-PAGE. Immunoblotting analysis of cell lysates was carried out using anti-TopoI and anti-β-actin antibody (Santa Cruz).

Cell Lines and Cell Culture. Human normal lung fibroblast (CCD-19Lu) and MS1 (CRL-2279) cell lines were commercially obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Human nasopharyngeal carcinoma cells (HONE1, SUNE1 and its cisplatin-resistant variant, CNE1) were generously provided by Prof. S. W. Tsao (Department of Anatomy, The University of Hong Kong). KB and KB-CPT-100 were kindly provided by Prof. Y. C. Cheng (Department of Pharmacology, Yale University). Cell-culture flasks and 96-well microtitre plates were purchased from Nalge Nunc. Culture medium, other medium constituents, and phosphate-buffered saline (PBS) were purchased from Gibco BRL.

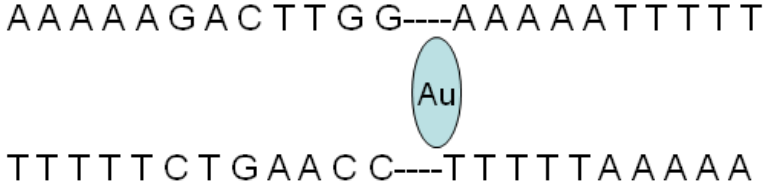
The CCD-19Lu cells were maintained in a minimum essential medium (MEM) with Earle's balanced salts. The HepG2 and MS1 cells were maintained in a minimum essential medium (DMEM) with *D*-glucose content of 4,500 mg/L. The SUNE1, CNE1 and HONE1 cells were maintained in RPMI 1640 medium. All the medium were supplemented with L-glutamine (2 mM) and fetal bovine serum (10%). Penicillin (100 UmL^{-1}) and streptomycin (100 μgmL^{-1}) were added to all media. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air, and were sub-cultured trice weekly.

Cytotoxicity Evaluation. Assays of cytotoxicity were conducted in 96-well, flat-bottomed microtitre plates. The supplemented culture medium (90 μL) with cells (1×10^5 cells per mL) was added to the wells. Gold(III) complexes were dissolved in the culture medium with 1% DMSO to concentrations of 0.5 − 1 μM, and aliquots of the solutions were subsequently added to a set of wells. Cells for control experiments were treated with supplemented media with 1% DMSO (100 μL). The microtitre plates were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air for a further 3 days. All the assays were run in parallel with a negative control (i.e., vehicle control) and a positive control, in which cisplatin was used as a cytotoxic agent.

Assessment of cytotoxicity was carried out by using a modified method of the Mosmann-based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At the end of each incubation period, MTT solution (10 μL, Cell Proliferation Kit I, Roche) was added into each well and the cultures were incubated further for 4 h at 37°C in a humidified atmosphere of 5% CO_2/95% air. A solubilizing solution (100 μL) was added into wells to lyse the cells and to solublize the formazan complex formed. The microtitre plates were maintained in a dark, humidified chamber overnight. The formation of formazan was measured by using a microtitre plate reader at 550 nm and the percentages of cell survival were determined. The cytotoxicity was evaluated based on the percentage cell survival in a dose-dependent manner relative to the negative control.

Cellular-Uptake Experiments. Cellular-uptake experiments were conducted according to the literature method. In general, SUNE1 cells (5×10^4 cells) were seeded in 60-mm tissue-culture dishes with culture medium (2 mL/ well) and incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air for 24 h. The culture medium was removed and replaced with medium containing gold(III) complexes. After exposure to the gold(III) complexes for 2 h, the medium was removed and the cell monolayer was washed four times with ice-cold PBS. Milli-Q water (500 μL) was added and the cell monolayer was scraped off from the culture dish. Samples (300 μL) were digested in 70% HNO_3 (500 μL) at 70°C for 2 h then diluted 1:100 in water for inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Molecular Docking. A molecular model study on the interaction between the gold complex and TopoI-DNA was performed. This was done using Gaussian 03. The gold complex was optimized using DFT with a LanL2MB basis set [(a) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* 1985, **82**, 270; (b) Wadt, W. R.; Hay, P. J. *J. Chem. Phys.* 1985, **82**, 284; (c) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* 1985, **82**, 299]. The DNA sequence used is shown as follows:



The optimized structure of the gold complex was used to do the docking. Molecular docking was performed using ICM-Pro 3.6-1d program (Molsoft, Totrov, M.; Abagyan, R. *Proteins, Suppl.* 1997, **1**, 215). According to ICM method, the molecular system was described using internal coordinates as variables. Energy calculations were based on the ECEPP/3 force field with a distance-dependent dielectric constant. The biased probability Monte Carlo (BPMC) minimization procedure was used for global energy optimization. The BPMC global energy optimization method consists of the following steps: (1) a random conformation change of the free variables according to a predefined continuous probability distribution; (2) local energy minimization of analytical differentiable terms; (3) calculation of the complete energy including non-differentiable terms such as entropy and solvation energy; (4) acceptance or rejection of the total energy based on the Metropolis criterion and return to step 1. A series of five grid potential representations of the receptor were automatically generated and superimposed that accounted for the hydrophobicity, carbon-based and hydrogen-based van der Waals boundaries, hydrogen-bonding profile, and electrostatic potential. The binding between the gold complex and TopoI-DNA was evaluated by binding energy, including grid energy, continuum electrostatic, and entropy terms. The crystal structure for TopoI (PDB code: 1K4T, Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin Jr., A. B.; Stewart, L. J. *Proc. Natl. Acad. Sci. U.S.A.* 2002, **99**, 15387) was downloaded from protein data bank. Hydrogen and missing heavy atoms were added to the receptor structure followed by local minimization using the conjugate gradient algorithm and analytical derivatives in the internal coordinates. The ICM docking was performed to find the most favorable orientation. The resulting gold complex-TopoI-DNA trajectories were energy minimized, and the interaction energies were computed. Each gold complex was docked three times and a minimum of the three interaction energies was used. As a reference, molecular docking of well-known TopoI inhibitor – topotecan showed a binding energy of –11.64 kcal/mol.

The *in vivo* experiment was performed with approval from the Committee on the Use of Live Animals for Teaching and Research, The University of Hong Kong. SPF grade four-week-old female BALB/c AnN-nu mice (nude mice, 16 – 18 g) were purchased. PLC tumor cells (5×10^6) resuspended in RPMI were implanted by subcutaneous injection on the right flank of the mice. When tumors were approximately 50 mm^3 in size, animals were randomly separated into 3 groups to receive treatment of twice-a-week intraperitoneal injection of 10% PET vehicle control (where 10% PET = 6% polyethylene glycol 400, 3% ethanol, 1% Tween 80 and 90% PBS), complex **1**

(100 mg/kg) or cyclophosphamide (30 mg/kg) for 8 times. After 30 days, the mice were sacrificed and the tumors were isolated and weighted.

Part B: Figures and Table.

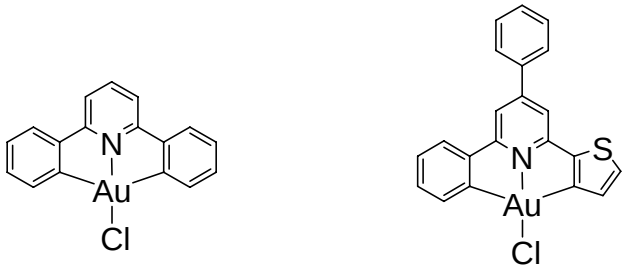


Figure S1. Au(C^N^C)Cl and Au(S^N^C)Cl complexes

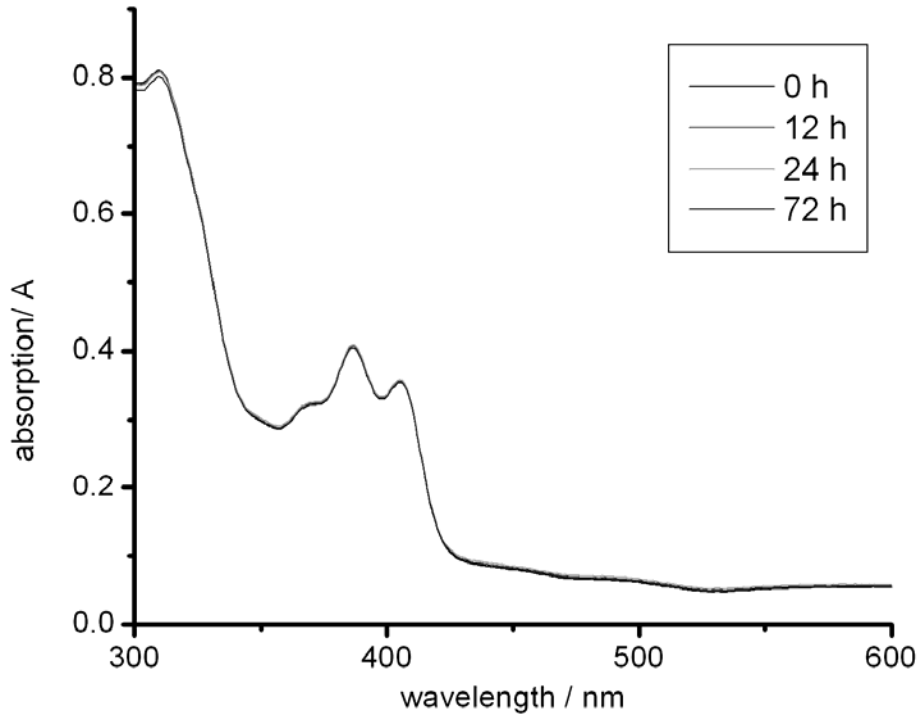


Figure S2. Stability evaluation of **1** in DMSO/PBS (1:19) containing 2 mM GSH.

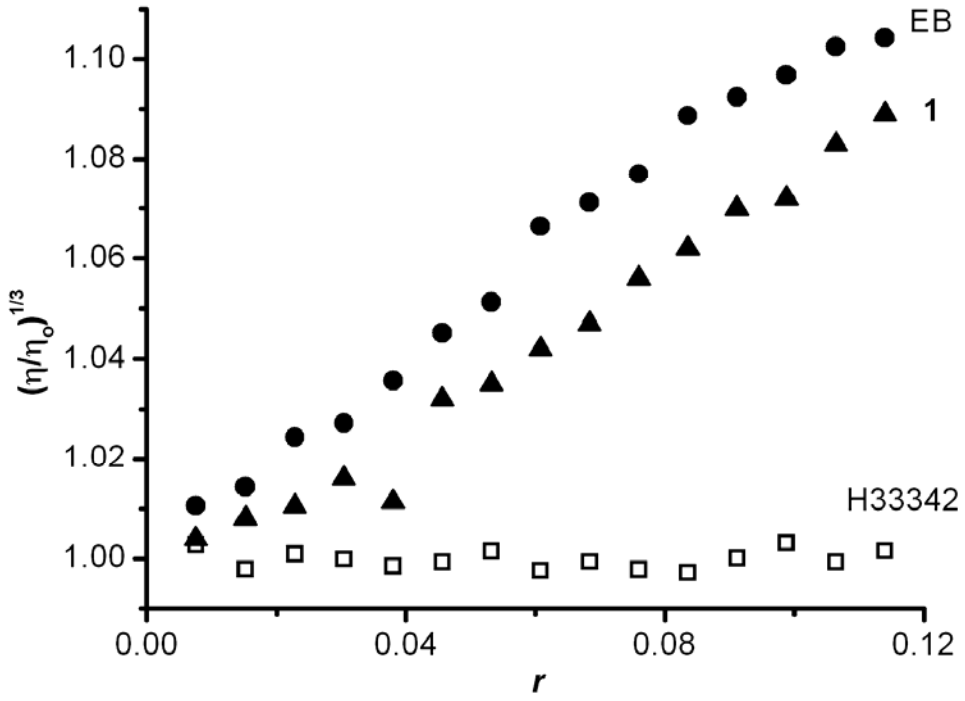


Figure S3. Relative viscosity of ctDNA in the presence of ethidium bromide (EB), Hoechst33342 (H33342), or **1**, shown as a function of the binding ratio (*r*).

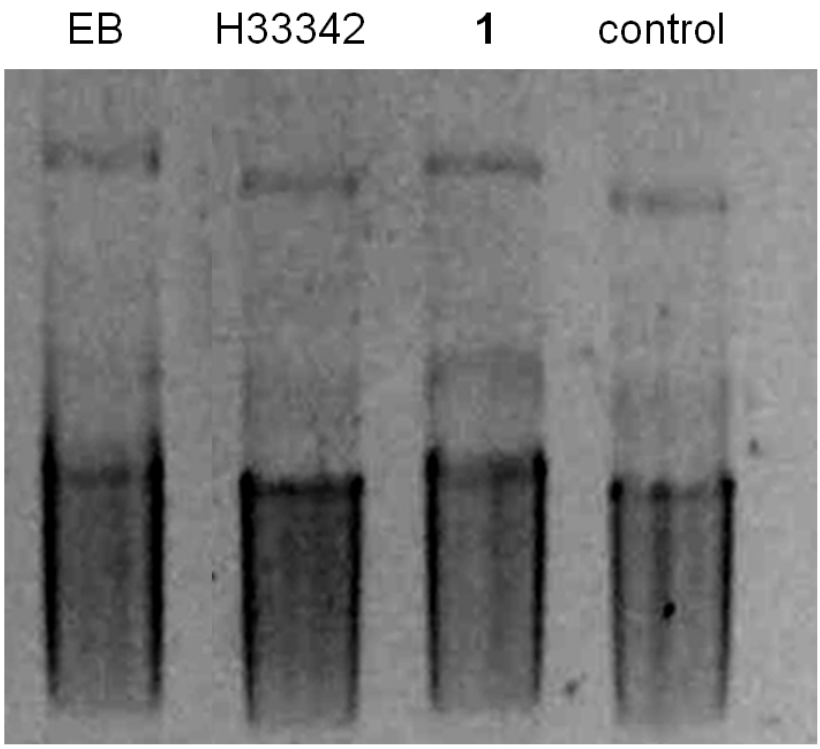


Figure S4. Gel electrophoresis of 100-bp DNA ladder (15.2 μM in base pairs) in 2% (w/v) agarose gel showing the mobility of the DNA in the absence (control) or presence of ethidium bromide (EB), Hoechst33342 (H33342), **1** in 1:1 ratio of DNA base pairs to the complex.

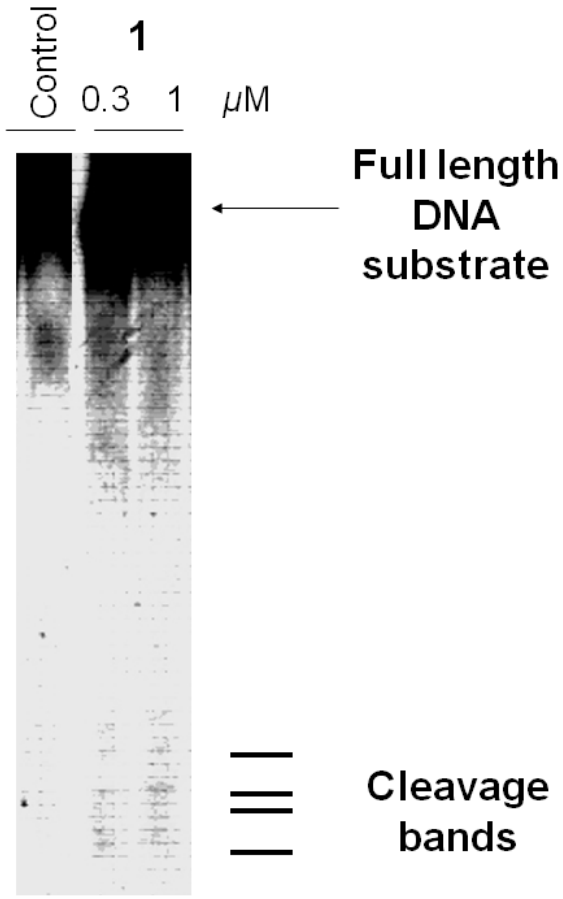


Figure S5. Gel electrophoresis of ^{32}P-end-labeled pBR322 DNA shows that **1** at 0.3 and 1 μM induce DNA cleavage in 6% denaturing PAGE gel.

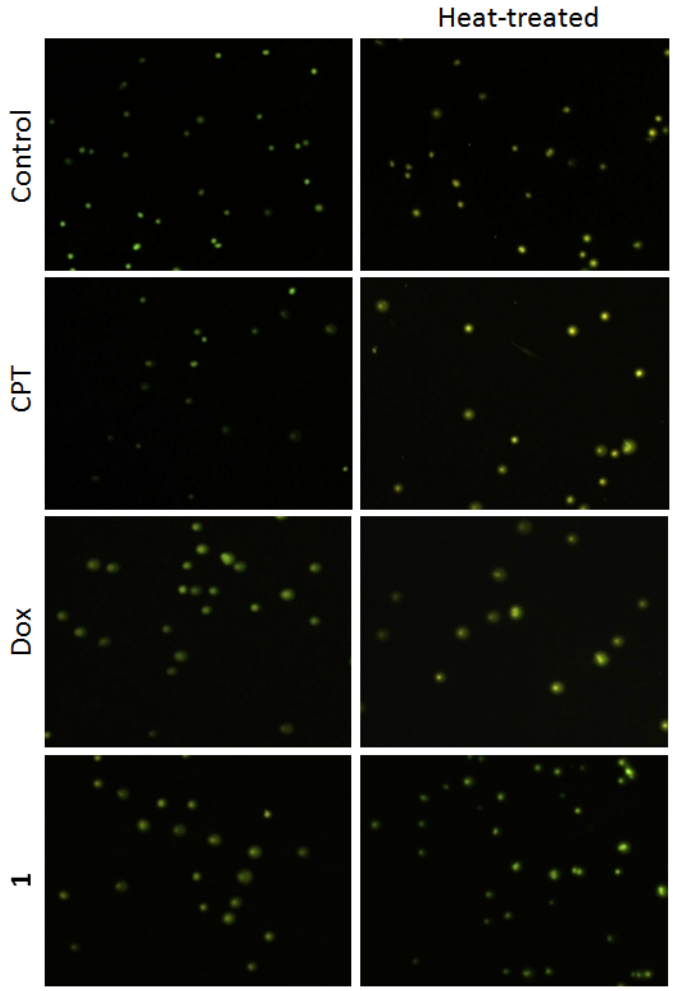


Figure S6. Complex **1** and CPT, but not doxorubicin, induced reversible chromosomal DNA strand breaks in KB cells as revealed by alkaline comet assay. Tails of damaged DNA were observed after treatment with **1**, CPT and doxorubicin. Reversal of strand breaks was observed in cells treated with **1** and CPT.

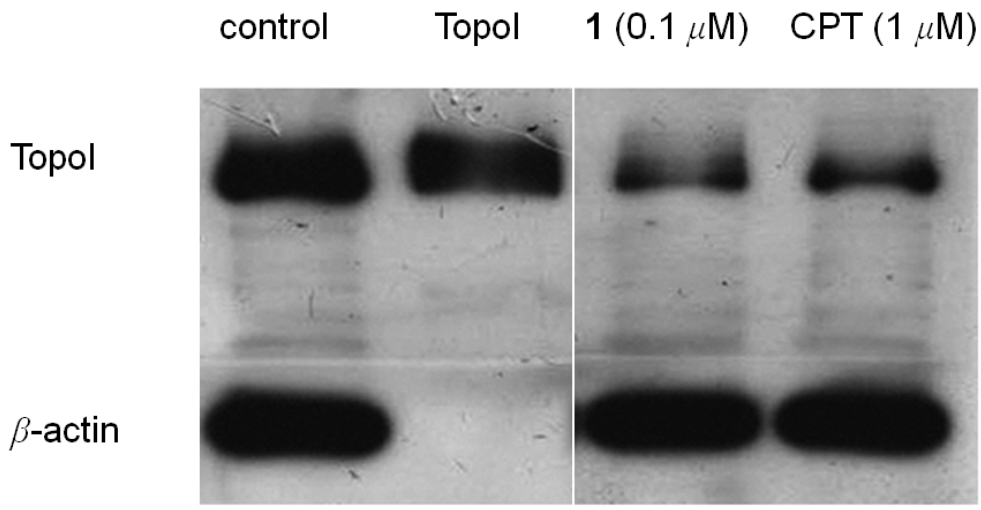


Figure S7. Complex **1** and CPT induced TopoI-cleavable complexes in KB cells as revealed by a band depletion assay.

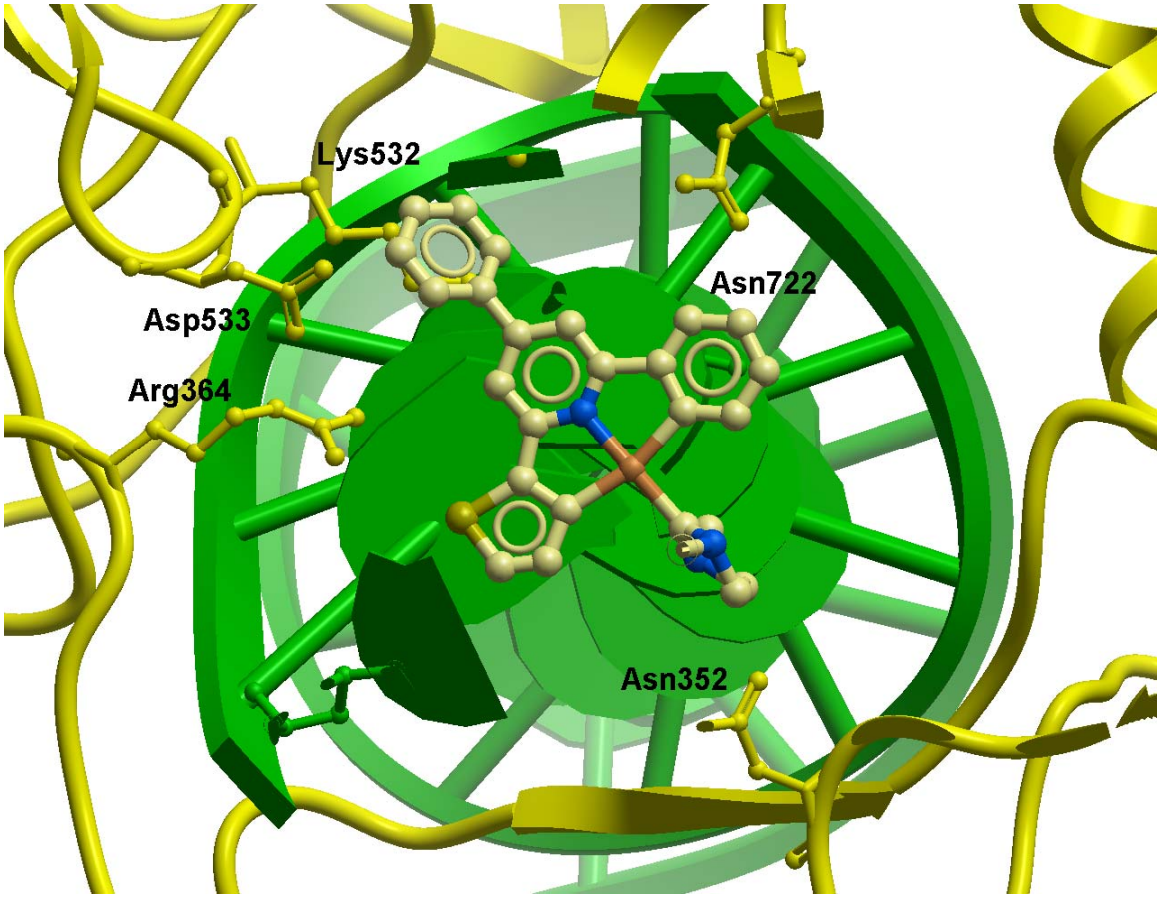


Figure S8. Molecular modeling of **4** interacting with TopoI-DNA, **4** binds to TopoI-linked DNA with carbene side chain pointing into the major groove (binding energy = –5.84 kcal/mol). The TopoI protein is in the ribbon representation and colored in yellow, while DNA is colored in green and **4** is in a ball and stick model.

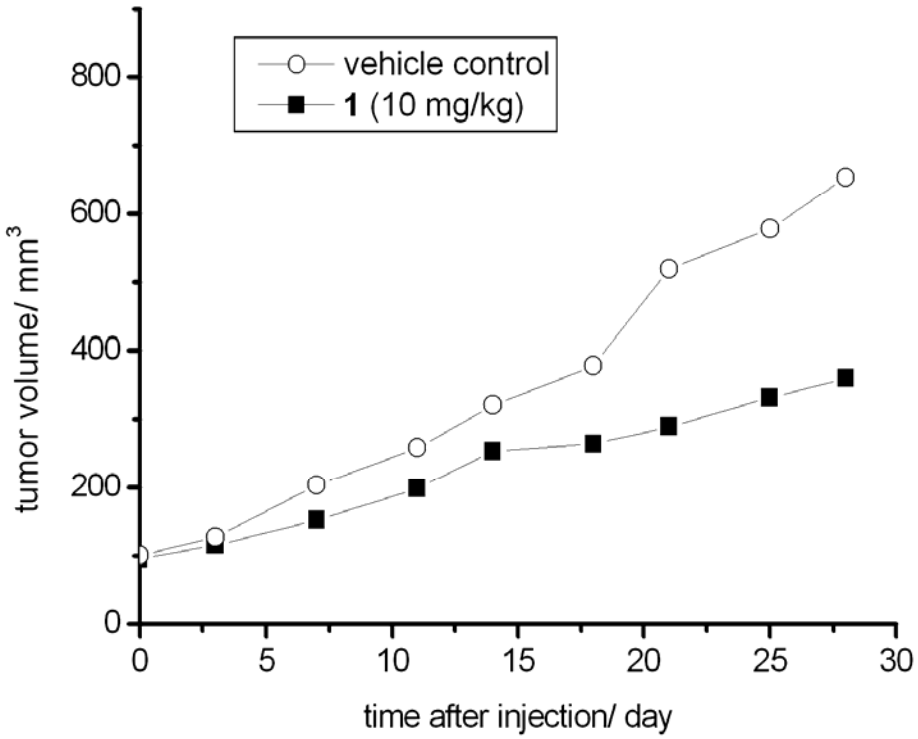


Figure S9. *In vivo* anti-tumor activity of **1** against PLC tumor.

Table. S1. Emission data ($\lambda_{ex} \geq 360$ nm in dichloromethane) for **1**–**4**.

complex	λ_{max}/nm (τ_0/μs)	Φ_{em}
1	481 (0.62), 513, 562	6.1×10^{-3}
2	490 (0.82), 528, 572	2.5×10^{-3}
3	487 (0.68), 521, 536	2.7×10^{-3}
4	475 (0.52)	1.8×10^{-2}