Cyclopent[*a*]anthraquinones as DNA-Intercalating Agents with Covalent Bond Formation Potential: Synthesis and Biological Activity

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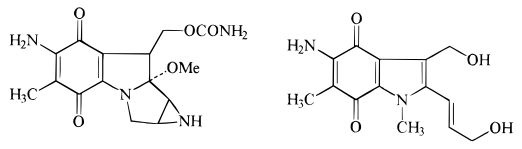
A series of mitomycin C (MMC) analogues, namely cyclopentanthraquinone derivatives, were synthesized *via* Diels−Alder cyclization of naphthoquinone with 1-vinylcyclopent-1-enes. These new compounds are planar structures, like MMC, and bear an aziridine ring and a methyl carbamate side chain. After bioreduction, they are anticipated to be capable of intercalating into double-stranded DNA and bind covalently. Structure−activity relationships were studied. Of these compounds, 2,3-aziridino-4-[[(methylamino)carbonyl]methyl]cyclopent[*a*]anthracene-6,11-dione (**4**) was shown to have inhibitory activity against several leukemic and solid tumor cell lines. Mice (BDF_1) bearing Lewis lung adenocarcinoma were treated with **4** and MMC (ip, QD × 5). At a dose of 30.0 mg/kg, compound **4** was as effective as MMC (0.8 mg/kg). Compound **4** appears to be less toxic than MMC. DNA unwinding assay indicated that **4** is able to intercalate into DNA double strands and is also a topoisomerase II inhibitor.

Introduction

When searching for new drug molecules with improved selectivity toward major solid tumors, identifying the biochemical and functional differences between normal and neoplastic cells facilitates rationale drug design. Hypoxia, for example, has been recently recognized as a potential tool for the development of a more selective form of anticancer therapy. Bioreductive alkylating agents activated to more cytotoxic metabolites under hypoxic conditions may provide a physiological means of preferential cell kill in poorly vascularized solid tumors. The bioreductive mitomycin C (MMC, **1**, Figure 1)[1] and its analogue, indoloquinone E09[2] (**2**, Figure 1), are the prototype of compounds exploiting this approach. It is known that MMC can serve as a bifunctional alkylating agent that cross-links to DNA.[3] After bioreduction, MMC readily forms intermediate mitosene through elimination of methanol. The opening of the aziridine ring and elimination of the carbamate moiety of MMC generates the two reactive sites at C-1 and C-10. This enables MMC to bind covalently to the minor groove of double-stranded DNA. It has been reported by Tomasz et al.[4] that MMC forms interstrand cross-links with two diagonally opposed dG residues in a CpG sequence.

Indoloquinone E09, a fully synthetic bioreductive DNA cross-linking agent, was found to be at least 10 times more cytotoxic against tumor cell lines *in vitro* under hypoxic conditions. *In vivo*, E09 was inactive against P388 murine leukemia, but it did exert significant antiproliferative effects against several other murine and human solid tumors, including the generally resistant MAC mouse colon tumors and gastric, ovarian, and breast xemografts.[5] E09 is bioactivated by the enzyme DT-diaphorase and may exhibit particularly good activity against tumors rich in this enzyme.[2,6]



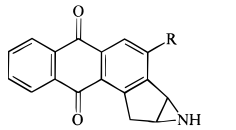


Figure 1.

As described above, both MMC and E09 are DNA cross-linking agents, but are not able to intercalate into DNA double strands. DNA-alkylating antitumor agents, in general, do not selectively inhibit cancerous cell growths. On the other hand, the DNA-intercalating agents cannot bind to DNA persistently. DNA-intercalating agents with DNA cross-linking potential are, therefore, expected to be ideal anticancer drugs and should have improved therapeutic selectivity. Several anticancer agents, linking DNA-intercalating ligands (such as chrysophanol, emodin, or acridine derivatives) with alkylating mustard, have been designed and synthesized with this concept.[7−9]

One of our ongoing research projects is to exploit new DNA-cross-linking agents with intercalating potential as potent antitumor agents. Previously, we reported the synthesis of 2,3-dihydro-1*H*-cyclopent[*a*]anthracene-6,-11-dione (cyclopentanthraquinone), **3**, bearing a mustard side chain at C-4 and an aziridine ring at the cyclopentene moiety.[9] Compound **3** can be considered as a MMC analogue. The planar anthraquinone molecule of **3** is expected to intercalate into DNA double strands and, like MMC or E09, covalently cross-link to the macromolecule after bioreductive activation of the aziridine function under hypoxic conditions.

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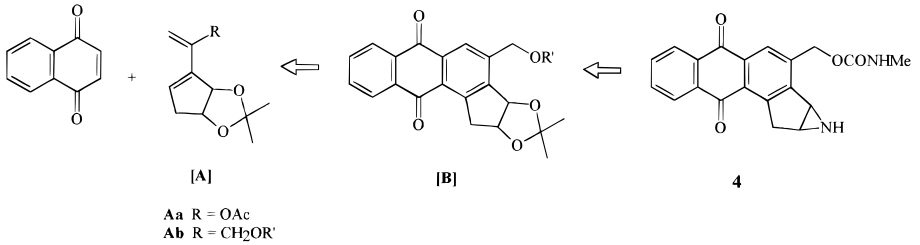
Scheme 1



The preliminary *in vitro* studies have showed that compound **3** is a less potent inhibitor against both leukemic L1210 and HL-60 cell growth than MMC. We also found that **3** is a more potent inhibitor of topoisomerase II (Topo II) k-DNA decatination than MMC.[9] The previous structure−activity relationship studies of these derivatives demonstrated that both mustard side chain and aziridine moieties play a significant role in the drug−DNA interaction. However, while further evaluating the antitumor activity of compound **3**, we found that after 3 months of refrigeration this agent decomposed, probably due to the instability of the mustard side chain. Our newly synthesized cyclopentaanthraquinone derivatives bearing an aziridine ring and a carbamate side chain (e.g. compound **4**) are structurally even more similar to MMC, but more stable than their precursors (e.g. compound **3**). Compound **4** is, therefore, expected to be more effective against solid tumors under hypoxic conditions.

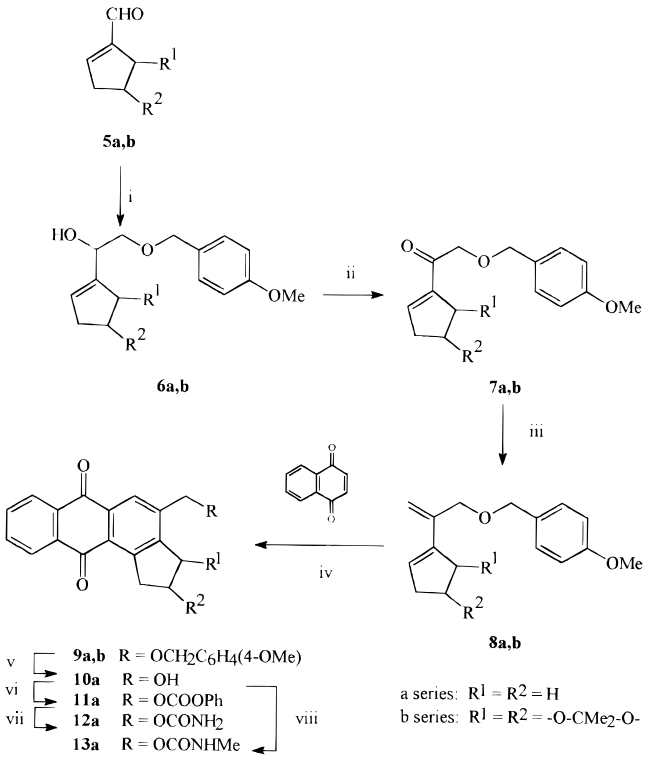
We here describe the synthesis and the structure−activity relationships of the new cyclopentanthraquinone derivatives.

Chemistry

Previously, we have synthesized 4-hydroxy-substituted cyclopentanthraquinones by a Diels−Alder reaction of 1,4-naphthoquinone with a diene **Aa**.[9] To apply this reaction to the synthesis of the targeted compound **4**, we needed to prepare a diene with a protected hydroxymethyl group (e.g. [**Ab**], R = $CH_2OCH_2C_6H_4$(4-OMe)). Diels−Alder cycloaddition of the diene [**Ab**] with naphthoquinone should give 4-O-protected hydroxymethyl cyclopentanthraquinone derivative [**B**] as shown in Scheme 1. Before a carbamate function to 4-CH_2OH is introduced, suitable protection of the hydroxy function is necessary, i.e. different conditions should be used to remove the 4-CH_2OH protecting group and the di-*O*-isopropylidene group at C2,3. We chose a 4-methoxybenzyl function to protect the 4-CH_2OH group because it can be selectively removed by treatment with dichlorodicyanoquinone (DDQ).[10] Conversion of the 4-CH_2OH into its carbamate derivative following the construction of an aziridine ring a C2,3 should afford the target compound [**C**].

As shown in the Scheme 2, the initial step was to synthesize dienes **8a** from the known aldehyde **5a**.[11] Treatment of **5a** with [[(4-methoxybenzyl)oxy]methyl]-lithium (readily available by transmetalation of *n*-$Bu_3SnCH_2OCH_2Ph$ with *n*-BuLi)[12,13] by following previously described procedures[14,15] afforded **6a**. Attempts to oxidize **6a** to the corresponding ketone **7a** resulted in a poor yield. Among the oxidizing agents tried, we found that the best yield of the ketone **7a** (78%) was obtained when using Swern reagent [$(CF_3CO)]_2O$/DMSO] at −50 °C. Wittig reaction of ketone **7a** with

Scheme 2[a]



[a] (i) $Bu_3SnCH_2OCH_2C_6H_4$(4-OMe)/*n*-BuLi/THF, −78 °C, 1 h (yield 40%[a], 54%[b]); (ii) Swern oxidation, −50 °C, 1 h (78%[a], 98%[b]); (iii) Wittig reaction (67%[a], 68%[b]); (iv) (1) anthraquinone/toluene, reflux 20 h, (2) 10% Pd/C, 1-octene/toluene, reflux, 48 h (52%[a], 33%[b]); (v) DDQ/CH_2Cl_2, rt, 4 h (68%[a]); (vi) PhOCOCl/pyridine, rt, 5 h (85%[a]); (vii) liquid NH_3, −78 °C, 4 h (83%[a]); (viii) MeNCO/pyridine, 24 h (29%[a]) (where superscripts a and b refer to the a and b series, respectively).

methyltriphenylphosphonium iodide and *s*-BuLi in dry THF at −78 °C afforded a diene **8a** in a 67% yield. Cycloaddition of diene **8a** and naphthoquinone followed by treatment with 10% Pd/C in refluxing 1-octene formed cyclopentanthraquinone **9a** in 51% yield. The 4-methoxybenzyl group was removed by treatment with DDQ, and the product **10a** was then directly converted into its *N*-methylcarbamate **13a** by treatment with methyl isocyanate. On the other hand, treatment of **10a** with phenyl chloroformate in pyridine yielded an ester derivative **11a**. By following a procedure developed by Islam et al.,[16] compound **11a** was converted into its carabmate **12a** by treatment with an excess of liquid ammonia.

After successfully synthesizing the model compounds (**13a** and **12a**), we applied the same strategy to the preparation of cyclopentanthraquinone **4**. The known aldehyde **5b**[9] was converted into a mixture of diastereomers **6b** in a ratio of 2:3 by following the same procedure as described above as shown in Scheme 2. Diene **8b** was than smoothly synthesized from **6b**. Diel−Alders

Table 1. Inhibition of Cell Growth by Cyclopentanthraquinone Derivatives and MMC

tumor cell lines	inhibitory concentration (IC_{50})			
	MMC	**4**	**12a**	**13a**
human promyelocytic leukemia (HL-60)	0.050	6.03	123.4	273.7
human tetratocarcinoma (833K)	0.328	23.9	ND[a]	ND
human mammary adenocarcinoma (SKBr-3)	0.993	19.9	ND	ND
Chinese hamster lung cells (DC-3F)	0.082	10.4	ND	ND
Chinese hamster lung cells resistant to actinomycin D (DC-3F/AD-II)	0.110	3.6	ND	ND

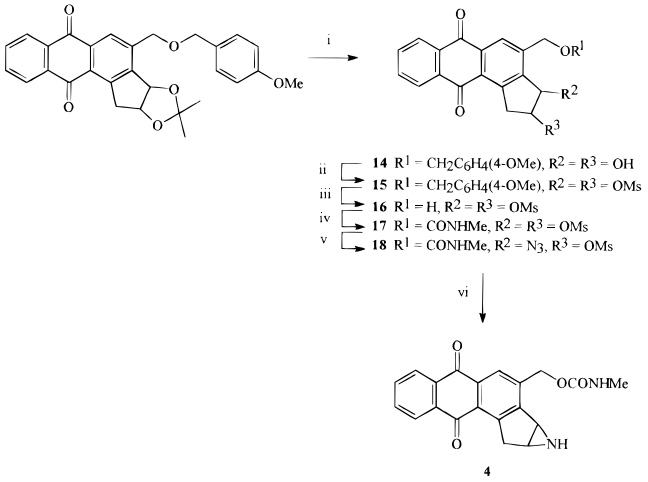
[a] ND: not determined.

Table 2. Anticancer Activity of Compound **4** in Mice Bearing Lewis Lung Carcinoma[a]

compound	dose (mg/kg)	average weight change (mg/kg)			average tumor volume (T/C)		
		day 7	day 10	day 14	day 7	day 10	day 14
control	0	−1	−0.1	+0.5	1	1	1
mitomycin C	0.4	−1.8	−1.2	+0.6	0.49	0.411	0.515
	0.8	−2.2	−1.8	+0.2	0.356	0.363	0.437
	2.0[b]	−2.7			0.169		
4	10	−1.5	−0.2	+2.0	1.011	0.303	0.529
	30	−2.3	−0.4	+0.5	0.529	0.205	0.496

[a] Tumor (4×10^6 cells) was inoculated subcutaneously (sc). Treatment started on day 3, ip, QD × 5. Control had four mice and each dose of drugs had three mice. Tumor sizes were evaluate on days 7, 10, and 14 after the beginning of treatment. [b] All three mice died on day 8 or 9.

Scheme 3[a]



[a] (i) Pyridinium toluenesulfonate/MeOH, reflux, 4 h (44%); (ii) $MeSO_2)_2O$/pyridine (91%); (iii) DDQ/CH_2Cl_2, rt, 4 h (56%); (iv) MeNCO/CH_2Cl_2, rt, 24 h (32%); (v) NaN_3/DMF, 0 °C, 2 h (79%); (vi) Et_3N/THF/H_2O, Ph_3P, rt, 1.5 h (49%).

reaction of diene **8b** with 1,4-naphthoquinone followed by treatment with 10% Pd/C in refluxing 1-octene afforded cyclopentanthraquinone **9b** in good yield. Usually, removal of the isopropylidene protecting group can be easily achieved by treatment with trifluoroacetic acid at room temperature. However, we found that this reagent cleaved both *O*-(4-methoxybenzyl) and 2,3-isopropylidenedioxy groups of **9b** simultaneously under various conditions. After several trials, we found that by using pyridinium *p*-toluenesulfonate (PPPT),[17] the 2,3-di-*O*-isopropylidene group of **9b** could be selectively removed to give **14** (Scheme 3). Compound **14** was then allowed to react with methanesulfonic anhydride in pyridine, and the product, 2,3-*cis*-bis(mesyloxy) derivative **15**, was obtained in almost quantitative yield. Compound **15** was treated with DDQ in a mixture of CH_2Cl_2/H_2O at room temperature afforded 4-hydroxymethyl derivative **16**, which was then further converted into *N*-methylcarbamate **17** in rather low yield (32%) by treatment with an excess of methyl isocyanate.

Treatment of **17** with NaN_3 in DMF at 0 °C selectively displaced the benzylic 3-mesyloxy function giving an approximate 80% yield of the 3-azido-substituted product **18**. Upon treatment of **18** with triethylamine and triphenylphosphine in a mixture of THF and water, the target compound aziridine derivative **4** was prepared in moderate yield.

Biological Activity

The preliminary *in vitro* cytotoxicity evaluation of compounds **4**, **12a**, and **13a** against various cancer cells, such as human promyelocytic leukemic (HL-60), human teratocarcinoma (833K), human mammary adenocarcinoma (SKBr-3), Chinese hamster lung cells (DC-3F), and Chinese hamster lung cells resistant to actinomycin D (DC-3F/AD-II), was compared with MMC. It was shown that the cyclopentanthraquinone derivatives were less potent than MMC (Table 1). Among cyclopentanthraquinones, compounds **12a** and **13a** without the aziridine ring were less cytotoxic than compound **4** with this functionality (Table 1). The same observation has been noted previously.[9] It is worthwhile to note that compound **4** was more potent to Chinese hamster lung cells resistant to actinomycin D (DC-3F/AD-II) (drug concentration required to reduce the cell density to 50% of control value, $IC_{50} = 3.6\ \mu M$) than that of the sensitive cell line (DC-3F) ($IC_{50} = 10.4\ \mu M$). On the contrary, MMC appeared to be less cytotoxic to the resistant cell line than to the sensitive one.

Mice (BDF_1) bearing Lewis lung adenocarcinoma were treated with compounds **4** and MMC by intraperitoneal injection, single dose for 5 days (ip, QD × 5). At a dose of 30 mg/kg compound **4** was as effective as MMC (0.8 mg/kg) (Table 2). All three mice died on day 8 or day 9 after treatment with MMC at dose of 2.0 mg/kg. On the basis of these this preliminary study, compound **4** appears to be less toxic than MMC.

As described above, the cyclopentanthraquinone derivative **4** was designed as a DNA-intercalating agent with covalent bond formation potential. We have proved that **4** exhibits DNA-intercalating property as shown by the DNA unwinding assay.[18] The ability of compound **4** to form covalent bonds with macromolecu-

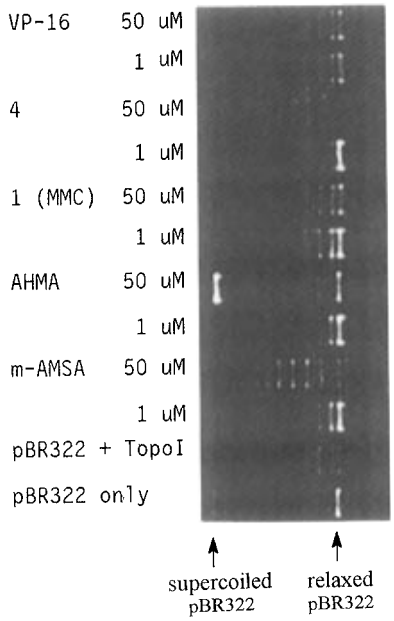


Figure 2. DNA unwinding assay; compound **4** was compared with known DNA-intercalating agents (m-AMSA and AHMA) and with DNA-nonintercalating agents (VP-16 and MMC).

lar DNA will be examined later. Compound **4** was compared with known DNA-intercalating agents [amsacrin (*m*-AMSA)[19] and 3-(acridin-9-ylamino)-5-(hydroxymethyl)aniline (AHMA, a potential antitumor DNA intercalator)[20]] and with nonintercalating DNA-reactive agents (VP-16 and MMC) as shown in Figure 2. Gel electrophoresis showed that the band pattern of compound **4** was quite similar to that of *m*-AMSA and AHMA and was different from the patterns of VP-16 and MMC. Thus, the DNA unwinding assay indicated that compound **4**, like *m*-AMSA and AHMA, was able to intercalate into DNA double strands.

Topo II-mediated cleavage of double-stranded DNA is a common mechanism of DNA-intercalating agents. When comparing compound **4** with *m*-AMSA and MMC, we studied the inhibitory effect of these compounds on Topo II as detected by an assay based on decatenation of k-DNA by Topo II.[21] Figure 3 shows that compound **4** and *m*-AMSA are potent inhibitors of Topo II, while MMC is a weaker inhibitor.

Experimental Section

Melting points are uncorrected and were determined on a Thomas-Hoover capillary apparatus. Column chromatography was performed on silica gel G60 (70-230 mesh, ASTM, Merck). Thin-layer chromatography was performed on Analtech Uniplates and visualized with short-wavelength UV light. Elementary analyses were carried out by MHW Laboratories, Phoenix, AZ. ^{1}H-NMR spectra were recorded on Bruker ACF-250 or Bruker AMX-400 spectrometer with Me_4Si as the internal standard. Values reported for coupling constants are first order. Mass spectra were recorded on Delsi Nermag Instruments GC/MS spectrometer with ammonia as the carrier.

1-(1-Cyclopenten-1-yl)-2-[(4-methoxybenzyl)oxy]ethanol (6a). To a solution of tributyl[(4-methoxybenzyl)oxy]-methyltin[11] (16.5 g, 37.5 mmol) in dry THF (250 mL) at −78 °C was added dropwise 15.5 mL of *n*-BuLi (2.5 M in hexane, 37.5 mmol). The resulting yellow solution was stirred at −78 °C for 10 min, followed by addition of 1-cyclopentenecarbaldehyde (**5a**)[12] (3 g, 31.3 mmol) in 20 mL of dry THF. After 1 h of stirring, the mixture was quenched with saturated NH_4Cl solution, extracted with ether (100 mL × 3), dried over $MgSO_4$, and evaporated in vacuo to dryness. The residue was chromatographed on a silica gel column (3 × 30 cm) using toluene/EtOAc (10:1 v/v) as the eluent. The main fraction was collected and evaporated in vacuo to dryness to give 3.1 g (40%) of **6a** as syrup: ^{1}H-NMR ($CDCl_3$) δ 1.83−1.91 (2H, m, CH_2), 2.23−2.35 (4H, m, CH_2), 2.56 (1H, br, exchangeable, OH), 3.35−3.62 (2H, m, 2-CH_2), 3.80 (3H, s, OMe), 4.44 (1H, d, J = 7.73 Hz, CH), 4.50 (2H, s, $PhCH_2$), 5.67 (1H, s, CH=), 6.88 and 7.26 (each 2H, d, J = 8.48 Hz, ArH); MS *m/e* 266 (MNH_4^+), 246 (M − 2). Anal. ($C_{21}H_{20}O_3$) C, H.

1-[4,5-(Isopropylidenedioxy)-1-cyclopenten-1-yl]-2-[(4-methoxybenzyl)oxy]ethanol (6b). By following the same procedure as described above, **6b** (7.48 g, 54%) was prepared from 4,5-(isopropylidenedioxy)-1-cyclopentenecarbaldehyde (**5b**, 7.58 g, 43.2 mmol)[5] as a syrup (a pair of diastereomers were observed in a ratio of 3:2 from its ^{1}H NMR): ^{1}H-NMR ($CDCl_3$) δ 1.33 and 1.39 (each 3H, s, 2 × Me), 2.49−2.55 (2H, m, CH_2), 2.93 ($^1/_3$H, brs, exchangeable, OH), 3.02 ($^2/_3$H, brs, exchangeable, OH), 3.48 ($^2/_3$H, dd, J = 7.51, J = 9.68 Hz, 2-CH_2), 3.59 ($^4/_3$H, d, J = 5.42 Hz, 2-CH_2), 3.72 (1H, dd, J = 1.92, J = 3.72 Hz, 1-CH), 3.79 (3H, s, OMe), 4.50 ($^2/_3$H, s, $PhCH_2$), 4.51 ($^4/_3$H, s, $PhCH_2$), 4.43−4.76 (1H, m, CH), 5.03 ($^1/_3$H, d, J = 5.93 Hz, CH), 5.08 ($^2/_3$H, d, J = 5.91 Hz, 5′-CH), 5.71 (1H, d, J = 0.56 Hz, CH=), 6.86 and 7.25 (each 2H, d, J = 8.49 Hz, ArH); MS *m/e* 338 (MNH_4^+), 319 (M − 1). Anal. ($C_{18}H_{24}O_5$) C, H.

1-Cyclopenten-1-yl [(4-Methoxybenzyl)oxy]methyl Ketone (7a). To a cooled (ca. −55 °C) solution of trifluoroacetic anhydride (2.7 mL, 14.8 mmol) in dry CH_2Cl_2 (100 mL) was added slowly 2.1 mL (30 mmol) of DMSO. After being stirred for 5 min, **6a** (2.82 g, 11.4 mmol) in CH_2Cl_2 (20 mL) was added dropwise into the mixture. The reaction mixture was stirred at the same temperature for additional 30 min and then quenched with triethylamine (8 mL). The mixture was warmed to room temperature, washed with ice−water (10 mL × 3), dried ($MgSO_4$), and evaporated in vacuo to dryness. The residue was chromatographed on silica gel (3 × 25 cm) using toluene/EtOAc (9:1 v/v) as the eluent to give 2.17 g (78%) of **7a** as a pale yellow syrup: ^{1}H NMR ($CDCl_3$) δ 1.86−1.94 (2H, m, CH_2), 2.52−2.60 (4H, m, CH_2), 3.80 (3H, s, OMe), 4.39 (2H, s, $COCH_2$), 4.55 (2H, s, $PhCH_2$), 6.78 (1H, dd, J = 1.72, J = 1.93 Hz, CH=), 6.88 and 7.30 (each 2H, d, J = 8.70 Hz, ArH); MS *m/e* 264 (MNH_4^+), 245 (M − 1). Anal. ($C_{15}H_{18}O_3$) C, H.

4,5-(Isopropylidenedioxy)-1-cyclopenten-1-yl [(4-Methoxybenyzyl)oxy]methyl Ketone (7b). Compound **7b**, 7.38 g (99%), was prepared from **6b** (7.48 g, 23.4 mmol) in a similar manner: ^{1}H NMR ($CDCl_3$) δ 1.36 and 1.38 (each 3H, s, Me), 2.70−2.74 (2H, m, CH_2), 3.81 (3H, s, OMe), 4.39 (2H, dd, J = 6.54 Hz, $COCH_2$), 4.56 (2H, s, $PhCH_2$), 4.79−4.80 (1H, m, CH), 5.33−5.34 (1H, m, 5′-CH), 6.85 (1H, m, CH=), 6.88 and 7.29 (each, 2H, J = 8.66 Hz, ArH); MS *m/e* 336 (MNH_4^+), 317 (M − 1). Anal. ($C_{18}H_{22}O_5$) C, H.

2-(1-Cyclopenten-1-yl)-3-[(4-methoxybenzyl)oxy]-1-propene (8a). To a mixture of methyltriphenylphosphonium iodide (6.79 g, 16.8 mmol) in dry THF (100 mL) was added slowly 12.3 mL (16.8 mmol) of *s*-BuLi (1.3 M in hexane) at −78 °C. After the solution was stirred for 30 min, a solution of **7a** (1.97 g, 8 mmol) in dry THF (10 mL) was added to the mixture. The mixture was stirred at 0 °C for 1 h and then was stirred at room temperature for an additional 3 h. The mixture was treated with saturated aqueous NH_4Cl solution (20 mL) and extracted with Et_2O (100 mL × 4). The combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated in vacuo to dryness. The product **8a** (1.32 g, 67%), as syrup, was purified by column chromatography (silica gel, toluene/EtOAc, 10:1 v/v): ^{1}H NMR ($CDCl_3$) δ 1.84−1.92 (2H, m, CH_2), 2.42−2.51 (4H, m, CH_2), 3.77 (3H, s, OMe), 4.17 (2H, d, J = 0.40 Hz, 1-CH_2), 4.46 (2H, s, $PhCH_2$), 5.07 and 5.20 (each 1H, s, CH_2=), 5.82−5.84 (1H, m, CH=), 6.87 and 7.26 (each 2H, d, J = 8.67 Hz, ArH); MS *m/e* 262 (MNH_4^+), 245 (M + 1). Anal. ($C_{16}H_{20}O_2$) C, H.

2-[4,5-(Isopropylidenedioxy)-1-cyclopenten-1-yl]-3-[(4-methoxybenzyl)oxy]-1-propene (8b). By following the same procedure, **8b** (5.02 g, 68%) was prepared from **7b** (7.38 g, 23.1 mmol) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.38 and 1.43 (each 3H, s, Me), 2.60−2.66 (2H, m, CH_2), 3.80 (3H, s, OMe), 4.18 (2H, dd, J = 4.84 Hz, 1-CH_2), 4.15 (2H, s, $PhCH_2$),

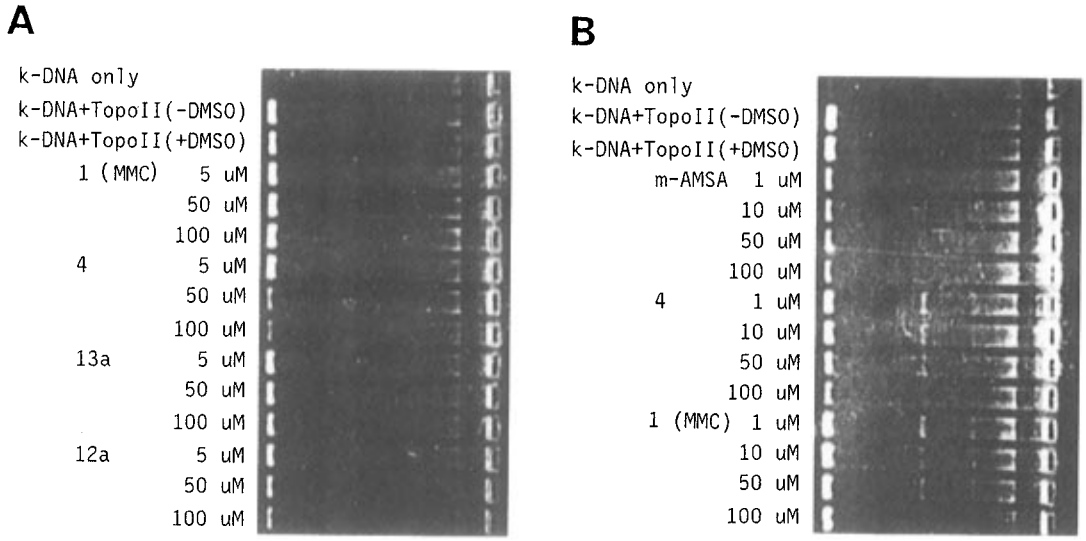


Figure 3. Inhibition of Topo II-mediated k-DNA decatenation by (a) MMC, compounds **4**, **13a**, and **12a**; (b) m-AMSA, **4**, and MMC, using purified Topo II from calf thymus.

4.78 (1H, t, J = 5.60 Hz, CH), 5.25 (1H, d, J = 5.60 Hz, CH), 5.33 and 5.51 (each 1H, s, CH_2=), 5.84 (1H, t, J = 0.50 Hz, CH=), 6.88 and 7.26 (each 2H, d, J = 8.70 Hz, ArH); MS *m/e* 334 (MNH_4^+), 316 (M). Anal. ($C_{19}H_{24}O_4$) C, H.

2,3-Dihydro-4-[[(4-methoxybenzyl)oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (9a). A mixture of **8a** (1.25 g, 5 mmol) and 1,4-naphthoquinone (0.87 g, 5.5 mmol) in toluene (30 mL) was heated under reflux. The reaction was monitored by TLC (silica gel, Et_2O/hexane, 1:5 v/v), which indicated that the starting materials were consumed and one major product was formed after refluxing for 20 h. To the mixture was then added 10% Pd/C (400 mg, 100 mg per 12 h) and 1-octene (10 mL), and refluxing continued for an additional 48 h. The mixture was allowed to cool to room temperature and filtered through a bed of Celite, and the filter cake was washed with ether. The combined filtrate and washings were evaporated in vacuo to dryness. The residue was crystallized from ethanol to give **9a** (945 mg, 51%) as yellow crystals: mp 166−167 °C; ^{1}H NMR ($CDCl_3$) δ 2.17−2.24 (2H, m, 2-H), 2.94 (2H, t, J = 7.70 Hz, 3-H), 3.51 (2H, t, J = 7.70 Hz, 1-H), 4.82 (3H, s, OMe), 4.56 and 4.59 (each 2H, s, CH_2), 6.91 and 7.32 (each 2H, d, J = 8.55 Hz, ArH), 7.74−7.85 (2H, m, ArH), 8.25 (1H, s, 5-H), 8.26−8.30 (2H, m, ArH). Anal. ($C_{26}H_{22}O_4$) C, H.

2,3-Dihydro-2,3-(isopropylidenedioxy)-4-[[(4-methoxybenzyl)oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (9b). In a similar manner, **9b** was prepared from **8b** (5.0 g, 15.8 mmol): yield 3.1 g (33%) as yellow needles; mp 119−120 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.13 and 1.36 (each 3H, s, Me), 3.50 (1H, dd, $J_{1a,2}$ = 5.63, $J_{1a,1b}$ = 19.4 Hz, 1-Ha), 3.79 (3H, s, OMe), 3.79 (1H, d, $J_{1a,1b}$ = 19.4 Hz, 1-Hb), 4.55 (2H, s, $PhCH_2$), 4.78 (2H, dd, J = 13.4 Hz, CH_2), 5.01 (1H, t, J = 5.60 Hz, 2-H), 5.57 (1H, d, J = 5.60 Hz, 3-H), 6.84 and 7.28 (each 2H, d, J = 8.60 Hz, ArH), 7.70−7.73 (2H, m, ArH), 8.18−8.24 (2H, m, ArH), 8.33 (1H, s, 5-H). Anal. ($C_{29}H_{26}O_6$) C, H.

2,3-Dihydro-2,3-dihydroxy-4-[[(4-methoxybenzyl)oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (14). A mixture of **9b** (2.48 g, 6.0 mmol) and pyridinium *p*-toluenesulfonate (1.52 g, 6.0 mmol) in dry MeOH (500 mL) was heated at reflux for 4 h. The solvent was removed by vacuum distillation, and the residue was chromatographed on a silica gel column (3 × 25 cm) using CH_2Cl_2/MeOH (200:1 v/v) as the eluent to give 1.15 g (44%) of **14**: mp 165−166 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.56−3.66 (2H, m, 1-H), 3.81 (3H, s, OMe), 4.59 (2H, s, $PhCH_2$), 4.57−4.61 (1H, m, 2-H), 4.80 (2H, dd, J = 13.5 Hz, CH_2), 5.15 (2H, d, J = 5.36 Hz, 3-H), 6.90 and 7.29 (each 2H, d, J = 8.60 Hz, ArH), 7.75−7.79 (2H, m, ArH), 8.13 (1H, s, 5-H), 8.19−8.25 (2H, m, ArH). Anal. ($C_{26}H_{22}O_6$) C, H.

2,3-Dihydro-2,3-*cis*-bis(mesyloxy)-4-[[(4-methoxybenzyl)oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (15). A mixture of **14** (1.15 g, 2.66 mmol) and methanesulfonic anhydride (6.96 g, 40.0 mmol) in pyridine (70 mL) was stirred at room temperature for 30 min. The mixture was concentrated in vacuo, and the residue was coevaporated several times with toluene to remove all traces of pyridine. The residue was chromatographed on a silica gel column (3 × 20 cm) using CH_2Cl_2/MeOH (100:1 v/v) as the eluent to give 1.43 g (91%) of **15** as yellow crystals: mp 73−74 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.15 and 3.20 (each 3H, s, OMs), 3.76 (1H, dd, $J_{1a,2}$ = 7.15, $J_{1a,1b}$ = 18.0 Hz, 1-Ha), 3.81 (3H, s, OMe), 4.11 (1H, dd, $J_{1b,2}$ = 7.01, $J_{1a,1b}$ = 18.0 Hz, 1-Hb), 4.62 (2H, dd, J = 11.3 Hz, $PhCH_2$), 4.79 (2H, dd, J = 12.8 Hz, CH_2), 5.29−5.37 (1H, m, 2-H), 6.32 (1H, d, J = 5.17 Hz, 3-H), 6.90 and 7.33 (each 2H, d, J = 8.46 Hz, ArH), 7.80−7.82 (2H, m, ArH), 8.23−8.30 (2H, m, ArH), 8.33 (1H, s, 5-H). Anal. ($C_{28}H_{26}O_{10}S_2$) C, H, S.

2,3-Dihydro-4-(hydroxymethyl)-1*H*-cyclopent[*a*]anthracene-6,11-dione (10). To a vigorously stirred mixture of **9a** (800 mg, 2.0 mmol), CH_2Cl_2 (60 mL), and H_2O (3 mL) was added DDQ (676 mg, 3.0 mmol) at room temperature. After being stirred for 4 h, the mixture was washed with saturated aqueous solution of $NaHCO_3$, followed with water, dried ($MgSO_4$), and evaporated in vacuo to dryness. The residue was chromatographed on a silica gel column (3 × 25 cm) using CH_2Cl_2/MeOH (200:1 v/v) as the eluent to give 406 mg (75%) of **10**: mp 191−192 °C (hexane/MeOH); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.17−2.25 (2H, m, 2-H), 2.92 (2H, t, J = 7.66 Hz, 3-H), 3.47 (2H, t, 1-H), 4.72 (2H, d, J = 2.18 Hz, CH_2OH), 5.07 (1H, brs, exchangeable, OH), 7.77−7.80 (2H, m, ArH), 8.22−8.31 (2H, m, ArH), 8.31 (1H, s, 5-H). Anal. ($C_{18}H_{14}O_3$) C, H.

2,3-Dihydro-2,3-*cis*-bis(mesyloxy)-4-(hydroxymethyl)-1*H*-cyclopent[*a*]anthracene-6,11-dione (16). By following the same procedure for the synthesis of **10**, compound **16** was prepared from **15** (1.40 g, 2.38 mmol): yield 0.63 g (56%); mp 137−138 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.15 and 3.18 (3H, s, OMs), 3.67 (1H, dd, $J_{1a,2}$ = 6.76, $J_{1a,1b}$ = 18.0 Hz, 1-Ha), 3.99 (1H, dd, $J_{1b,2}$ = 6.99, $J_{1a,1b}$ = 18.0 Hz, 1-Hb), 4.56 (1H, brs, exchangeable, OH), 4.90 (2H, dd, J = 4.25 Hz, CH_2OH), 5.35 (1H, dt, $J_{1a,2}$ = 6.76, $J_{1b,2}$ = 6.99, $J_{2,3}$ = 5.66 Hz, 2-H), 6.35 (1H, d, $J_{2,3}$ = 5.22 Hz, 3-H), 7.73−7.76 (2H, m, ArH), 8.13−8.21 (2H, m, ArH), 8.22 (1H, s, 5-H). Anal. ($C_{20}H_{18}O_9S_2$) C, H, S.

4-[[(Phenoxycarbonyl)oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (11). A mixture of **10** (200 mg, 0.72 mmol) and phenyl chloroformate (0.8 mL) in dry pyridine (10 mL) was stirred in an ice bath for 30 min and then at room temperature for 2 h and then evaporated in vacuo to dryness. The residue was dissolved in $CHCl_3$ (50 mL), washed with water (20 mL × 2), dried ($MgSO_4$), and evaporated in vacuo to dryness. The residue was crystallized from hexane/$CHCl_3$/MeOH to give **11**: 152 mg (53%); mp 155−156 °C; ^{1}H NMR ($CDCl_3$) δ 2.23−2.31 (2H, m, 2-H), 3.06 and 3.57 (each 2H, t,

J = 7.70 Hz, 3-H and 1-H), 5.38 (2H, s, CH_2), 7.20−7.38 (3H, m, ArH), 7.38−7.42 (2H, m, ArH), 7.80−7.82 (2H, m, ArH), 8.31 (1H, s, 5-H), 8.27−8.32 (2H, m, ArH). Anal. ($C_{25}H_{18}O_5$) C, H.

4-[(Carbamoyloxy)methyl]-2,3-dihydro-1*H*-cyclopent-[*a*]anthracene-6,11-dione (12). To a solution of **11** (100 mg, 0.25 mmol) in a mixture of CH_2Cl_2/MeOH (9:1 v/v) was added liquid NH_3 (10 mL) in a dry ice/acetone bath. After being stirred for 30 min, the temperature was allowed to warm to room temperature over a period of 3 h. The solvent was evaporated and the solid residue crystallized from a mixture of hexane/CH_2Cl_2 to give **13**: 66 mg (83%); mp 229−230 °C; ^{1}H NMR (DMSO-d_6) δ 2.19−2.27 (2H, m, 2-H), 3.02 (2H, t, J = 7.70 Hz, 3-H), 3.47 (2H, t, J = 7.70 Hz, 1-H), 5.19 (2H, s, CH_2), 7.96−8.01 (2H, m, ArH), 8.10 (1H, s, 5-H), 8.22−8.26 (2H, m, ArH). Anal. ($C_{19}H_{15}NO_4$) C, H, N.

2,3-Dihydro-4-[[(methylcarbamoyl)oxy]methyl-1*H*-cyclopent[*a*]anthracene-6,11-dione (13). A mixture of **10** (100 mg, 0.36 mmol) and excess of MeNCO (2 mL) in acetone (20 mL) was stirred at room temperature for 1 day. The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column (2 × 25 cm) using CH_2Cl_2/MeOH (200:1 v/v) as the eluent to give **13**: 32 mg (27%); mp 232−233 °C (CH_2Cl_2/EtOH): ^{1}H NMR ($CDCl_3$) δ 2.20−2.27 (2H, m, 2-H), 2.86 (3H, d, J = 4.85 Hz, NH*Me*), 2.98 (2H, t, J = 7.60 Hz, 3-H), 3.53 (2H, t, J = 7.50 Hz, 1-H), 4.85 (1H, brs, exchangeable, NH), 5.20 (2H, s, CH_2), 7.75−7.80 (2H, m, ArH), 8.19 (1H, s, 5-H), 8.25−8.57 (2H, m, ArH). Anal. ($C_{20}H_{17}NO_4$) C, H, N.

In a similar manner, compound **17** was synthesized.

2,3-*cis*-Bis(mesyloxy)-2,3-dihydro-4-[[(methylcarbamoyl)oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (17) was prepared from **16** (400 mg, 0.85 mmol): yield 142 mg (32%); mp 161−162 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.80 (3H, d, J = 4.80 Hz, NH*Me*), 3.26 and 3.32 (each 3H, s, OMs), 3.84 (1H, dd, $J_{1a,2}$ = 6.71, $J_{1a,1b}$ = 18.5 Hz, 1-Ha), 4.13 (1H, dd, $J_{1b,2}$ = 7.70 Hz, $J_{1a,1b}$ = 18.5 Hz, 1-Hb), 5.14 (1H, brs, exchangeable, NH), 5.37 (2H, dd, J = 14.5 Hz, CH_2), 5.40−5.45 (1H, m, 2-H), 6.38 (1H, d, $J_{2,3}$ = 5.15 Hz, 3-H), 7.82−7.86 (2H, m, ArH), 8.26−8.32 (2H, m, ArH), 8.34 (1H, s, 5-H). Anal. ($C_{22}H_{21}NO_{10}S_2$) C, H, N, S.

3-Azido-2,3-*trans*-dihydro-2-(mesyloxy)-4-[[(methylcarbamoyl]oxy]methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (18). A mixture of **17** (190 mg 0.36 mmol) and NaN_3 (24 mg, 0.36 mmol) in DMF (15 mL) was stirred at 0 °C for 2 h. The mixture was evaporated in vacuo to dryness, and the residue was chromatographed on a silica gel column (2 × 25 cm) using CH_2Cl_2/MeOH (200:1 v/v) as the eluent to give 134 mg (70%) of **18** as syrup: ^{1}H NMR ($CDCl_3$) δ 2.81 (3H, d, J = 4.83 Hz, NH*Me*), 3.84 (1H, d, $J_{1a,1b}$ = 16.0 Hz, 1-Ha), 4.01 (1H, dd, $J_{1b,2}$ = 6.10, $J_{1a,1b}$ = 16.0 Hz, 1-Hb), 5.31 (1H, s, 3-H), 5.33 (2H, d, J = 13.8 Hz, CH_2), 5.45 (1H, d, J = 6.10 Hz, 2-H), 7.78−7.81 (2H, m, ArH), 8.23−8.29 (2H, m, ArH), 8.32 (1H, s, 5-H); MS *m*/*e* 488 (MNH_4^+). Anal. ($C_{21}H_{18}N_4O_7S$) C, H, N, S.

2,3-Imino-2,3-dihydro-4-[[(methylcarbamoyl)oxy]-methyl]-1*H*-cyclopent[*a*]anthracene-6,11-dione (4). To a mixture of **18** (134 mg, 0.284 mmol) and Et_3N (0.5 mL) in THF (10 mL) containing H_2O (0.5 mL) was added triphenylphosphine (118 mg, 0.446 mmol). The mixture was stirred at room temperature for 1.5 h and then diluted with EtOAc (30 mL). The solution was washed with H_2O (15 mL × 3), dried ($MgSO_4$), and evaporated in vacuo to dryness. The residue was chromatographed on a silica gel column (2 × 25 cm) using CH_2Cl_2/MeOH (200:1 v/v) as the eluent to give **4**: 51 mg (49%); mp 198−199 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.85 (3H, d, J = 4.96 Hz, NH*Me*), 3.32 (1H, brs, 2-H), 3.55 (1H, brs, 3-H), 3.62 (1H, brd, 1-Ha), 3.82 (1H, d, $J_{1a,1b}$ = 19.7 Hz, 1-Hb), 4.84 (1H, brs, NH), 5.40 (2H, dd, J = 12.90 Hz, CH_2), 7.70−7.80 (2H, m, ArH), 8.23 (1H, s, 5-H), 8.24−8.29 (2H, s, ArH). Anal. ($C_{20}H_{16}N_2O_4$) C, H, N.

Biological Assay: Evaluation of Antitumor Activity in Cultured Cells. The effects of the compounds on cell growth were determined in HL-60 (human promyelocytic leukemia) cells and for compound **4** also in 833K (human teratocarcinoma) and SKBr-3 (human mammary adenocarcinoma) cells or Chinese hamster lung cells sensitive (DC-3F) or resistant to actinomycin D (DC-3F/AD-II). For HL-60 cells, assays were carried out by XTT−tetrazolium microculture assay after 72 h of incubation as described by Scudiero *et al.*[22] After incubation with phenazine methosulfate−XTT solution at 37 °C for 6 h, absorbance at 450 and 630 nm was detected with microplate reader (EL 340; Bio-Tek Instruments Inc., Winooski, VT). Other solid tumor cells in monolayers were assayed using SRB protein-staining methods as described by Skehan *et al.*[23] Six to seven concentrations of each compound were used. The IC_{50} and dose−effect relationships of compounds for antitumor activity were calculated by a median-effect plot.[24,25]

Inhibition of k-DNA Decatenation. k-DNA was isolated from *C. fasciculate* according to previously published methods.[21] The standard reaction mixture for k-DNA decatenation assay contained 50 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$, 50 mM KCl, 0.5 mM DTT, 30 μg/mL BSA, 5 mM ATP, 1.5 μg of k-DNA, and 5 μg of protein of nuclear extracts in 50 μL of 10% SDS and 1 mg/mL proteinase K, and the mixture was further incubated at 37 °C for 30 min.[25] Reaction was terminated by the addition of 5 μL of 10% SDS and 1 mg/mL proteinase K, and the mixture was further incubated at 37 °C for 30 min, followed by electrophoresis on 1% agarose with TBE buffer. After ethidium bromide staining (5 μg/mL), gels were photographed under UV illumination, using Polaroid type 55 film.

DNA Unwinding Assay. To measure the DNA intercalating activity, DNA unwinding assay was carried out using the method described by Fisher et al.[18] Supercoiled pBR322 (Boehringer Mannheim) (0.5 μg) was relaxed with 2.2 units of calf thymus DNA Topo I (Bethesda Research Labs) in 100 μL of relaxation buffer (10 mM Tris, 50 mM KCl, 100 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA, and 30 μg/mL bovine serum albumin, pH 7.9) for 1.5 h at 37 °C. Various concentrations of drugs in DMSO were added, and the solution was mixed and incubated at 37 °C for 30 min. Rections were stopped by adding 5 μL of 20% SDS. Samples were extracted twice with equal volumes of equilibrated phenol and once with chloroform. Five microliters of gel-loading buffer (0.5% bromophenol blue, 0.25% xylene cyanol, 1.5% ficol type 400) was added to 20 μL of the sample. Electrophoresis was carried out through 1% agarose in 40 mM Tris base and 1 mM EDTA and titrated to pH 7.2 with acetic acid at 2 V/cm.

Antitumor Activity in Tumor-Bearing Mice. BDF_1 mice bearing E0771 mammary adenocarcinoma or Lewis lung carcinoma were injected (ip) daily for 5 days beginning day 3 after tumor inoculation (subcutaneous implant with 0.2 mL brei). Body weight changes, mortality, and average tumor volume were recorded on days 7, 10, and 14. Day 14 results are reported. Drugs were dissolved in dimethyl sulfoxide (DMSO). Untreated controls were injected with DMSO alone. Two Topo II inhibitors, etoposide (VP-16) and *m*-AMSA, were also dissolved in DMSO and served as treated controls for comparing the therapeutic efficacy.

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