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Synthesis and Biological Activities of Topopyrones

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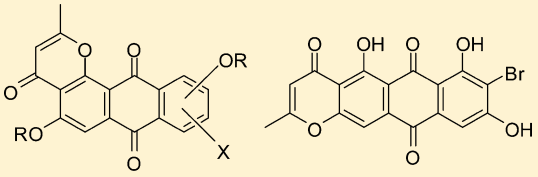
Supporting Information

ABSTRACT: Structure−activity studies were employed to investigate the stabilization of DNA−topoisomerases I and II covalent binary complexes by topopyrone analogues. The synthesis of five new topopyrone derivatives and study of their ability to stabilize DNA−topoisomerase I and DNA−topoisomerase II covalent binary complexes are described. The biochemical assays suggest that the orientation of the fused 1,4-pyrone ring and halogen substituents contribute importantly to the overall potency of the topopyrones as topoisomerase poisons.



The DNA topoisomerases are a family of nuclear enzymes that control the topological state of DNA and are essential to biological processes such as replication, transcription, and chromosome segregation.[1] These enzymes catalyze the relaxation of supercoiled DNA through a breakage−rejoining of the DNA phosphodiester bond.[2,3] DNA relaxation proceeds through the transient formation of a covalent DNA−topoisomerase complex between the active site tyrosine of the enzyme and the DNA phosphodiester bond of the DNA.[4] DNA topoisomerases are classified by the number of polynucleotide strand breaks generated, with type I topoisomerases creating single-strand DNA breaks and type II topoisomerases creating double-strand DNA breaks.[3] The covalent binary complexes formed between DNA and eukaryotic topoisomerases I and II are the targets of a class of antitumor agents referred to as topoisomerase poisons. These compounds stabilize the otherwise transient DNA−topoisomerase covalent binary complex and are believed to produce DNA strand breaks when advancing DNA replication or transcription assemblies collide with the drug-stabilized DNA−topoisomerase covalent binary complex.[5] Camptothecin (CPT) and etoposide are well-characterized topoisomerase I and topoisomerase II poisons, respectively.[6,7]

Other topoisomerase I poisons that have been described include the indenoisoquinolines,[8] calothrixins,[9] and topopyrones.[10−12] The topopyrones are natural products that were first isolated by Kanazawa and co-workers from the culture broths of the fungi *Phoma* sp. BAUA2861 and *Penicillium* sp. BAUA4206.[10] Structurally, the topopyrones are anthraquinone polyphenols that contain a fused 1,4-pyrone ring in an angular or linear orientation and that may also contain a chlorine atom, as in topopyrones A (**1**) and B (**2**).[11] In addition to being topoisomerase I poisons, the topopyrones target and stabilize DNA−topoisomerase II covalent binary complexes.[12] Presently, the preparation of five new topopyrone derivatives (Figure 1) is described, as are initial assay data characterizing the compounds as topoisomerase I and II poisons. Topoisomerase inhibitory activity was found to be dependent on the orientation of the 1,4-pyrone ring moiety as well as the presence and orientation of halogen substituents.

RESULTS AND DISCUSSION

The synthesis of topopyrones A, B, C, and D was reported by Elban *et al.* in 2008 along with the synthesis of two novel topopyrone derivatives (**9** and **10**).[13] In the present study, a similar synthetic approach was adopted to gain access to two new topopyrone C derivatives (**5** and **6**), two new topopyrone A derivatives (**7** and **8**), and one new topopyrone B derivative (**11**). Topopyrones **9** and **10** were prepared in analogy with the procedure reported previously.[13] Highlights of the synthetic route reported here include the Diels−Alder cycloaddition reaction (Scheme 1), Friedel−Crafts acylation reaction at the ortho position of a key phenolic intermediate (Scheme 1), pyrone ring formation using a strong base (Schemes 1 and 2), and regioselective monobromination (Scheme 2).

Diels−Alder addition of the previously reported 1,3-dimethoxy-1-trimethylsiloxy-1,3-butadiene[13,14] and commercially available 2,5-dichloro-1,4-benzoquinone in the presence of pyridine gave quinone **12** (Scheme 1). Protection of the phenol using *p*-toluenesulfonyl chloride (*p*-TsCl) and K_2CO_3 in acetone at reflux afforded tosylate **13** in 90% yield. Pd/C-catalyzed hydrogenation effected reduction of the quinone, which was immediately methylated using dimethyl sulfate and KOH to give **14** in 95% overall yield. Compound **15** was obtained by removal of the tosylate group from **14** (KOH(aq) at reflux). Although this reaction appeared to proceed cleanly, as judged by silica gel TLC analysis, the yield of isolated product was only moderate (56%). An acetyl group was introduced ortho to the free hydroxyl group using $TiCl_4$ and acetyl chloride in 1,2-dichloroethane at reflux, affording

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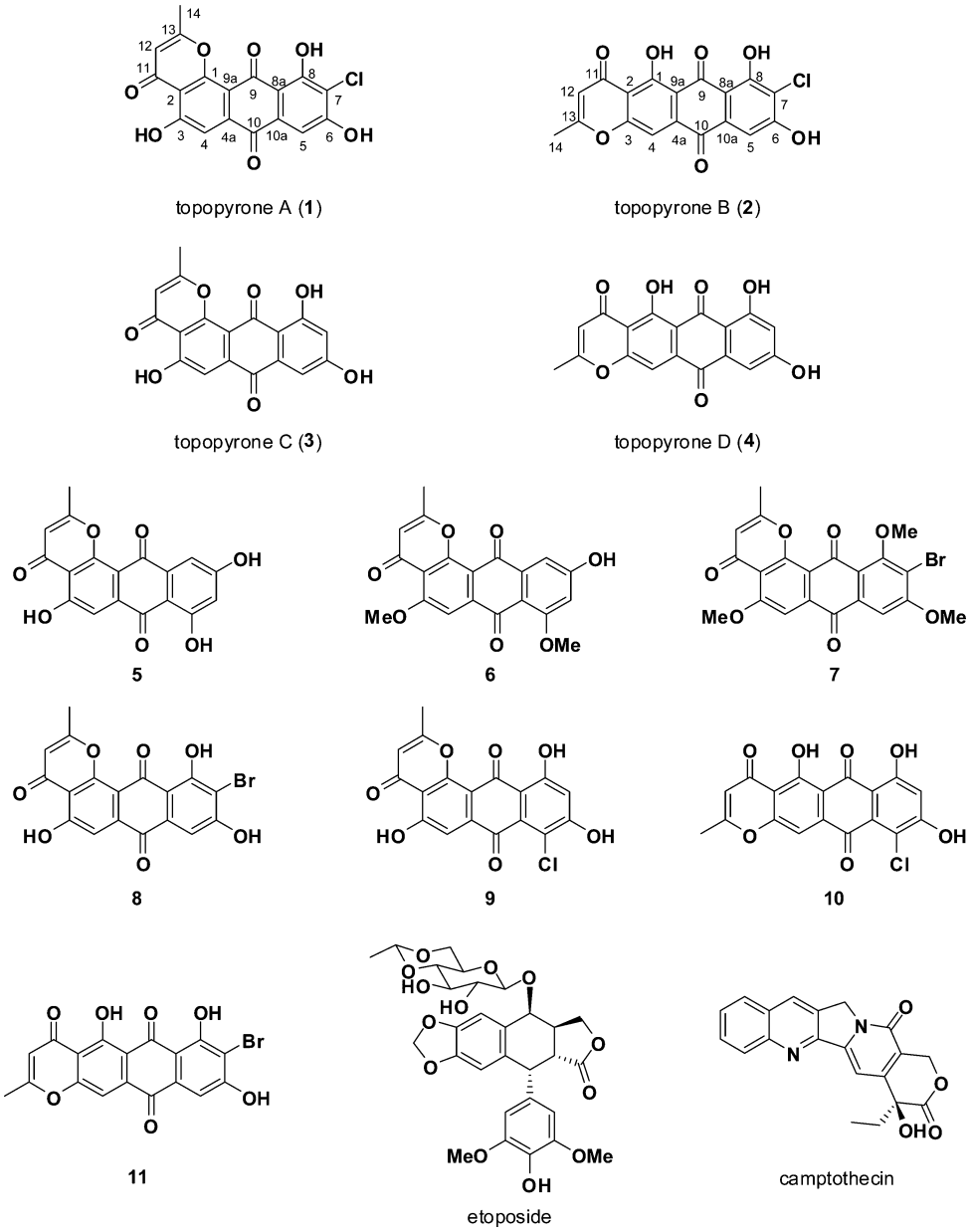

Figure 1. Structures of topopyrones, etoposide, and camptothecin.

compound **16** in 96% yield.[15,16] The pyrone ring was introduced at this stage via base-catalyzed Claisen condensation; compound **16** was treated with NaH (60% dispersion in mineral oil) in freshly distilled ethyl acetate at reflux to afford the β-diketone. This intermediate was subsequently treated with TFA to obtain **17** (56%, two steps). Finally, compound **17** was oxidized to quinone **18** (72% yield) using cerium(IV) ammonium nitrate in CH_3CN. A second Diels–Alder addition reaction was used to obtain the precursors for topopyrones **5** and **6**. Thus quinone **18** was treated with 1-methoxy-1-trimethylsiloxy-3-*tert*-butyldimethylsiloxy-1,3-butadiene (**19**)[13] in the presence of pyridine to facilitate the aromatization of the intermediate Diels–Alder addition product. Compounds **20** and **21** were thereby obtained in 40% and 25% yields, respectively. Finally, compound **20** was treated with tetra-*n*-butylammonium fluoride hydrate (TBAF·H_2O) to remove the tetrabutyldimethylsilyl protecting group, followed by demethylation using 1 M BBr_3 in CH_2Cl_2. This afforded topopyrone C derivative **5** in 23% overall yield. Similarly, topopyrone C derivative **6** was obtained in 50% yield by the treatment of **21** with TBAF·H_2O in THF.

Monobrominated intermediate **23** was obtained in 20% overall yield by treating **22**[13] with *N*-bromosuccinimide (Scheme 2)[17,18] in the presence of $(iPr)_2NH$ at −78 °C in freshly distilled CH_2Cl_2, followed by methylation of the phenolic hydroxyl groups with dimethyl sulfate and K_2CO_3 in acetone. Removal of the tosylate group, followed by *O*-acetylation of the intermediate phenol afforded **24** (52%, two steps). This was achieved using KOH in EtOH–H_2O at reflux followed by treatment with acetic anhydride in the presence of triethylamine and 4-*N*,*N*-dimethylaminopyridine.[13] Treatment of acetylated intermediate **24** with LiH in freshly distilled THF at reflux, followed by treatment with CF_3COOH at 0 °C (Baker–Venkataraman rearrangement)[19,20] led to pyrone ring formation, affording topopyrone **7** in 45% yield for the two steps. Removal of the methyl groups using 1 M $AlCl_3$ in

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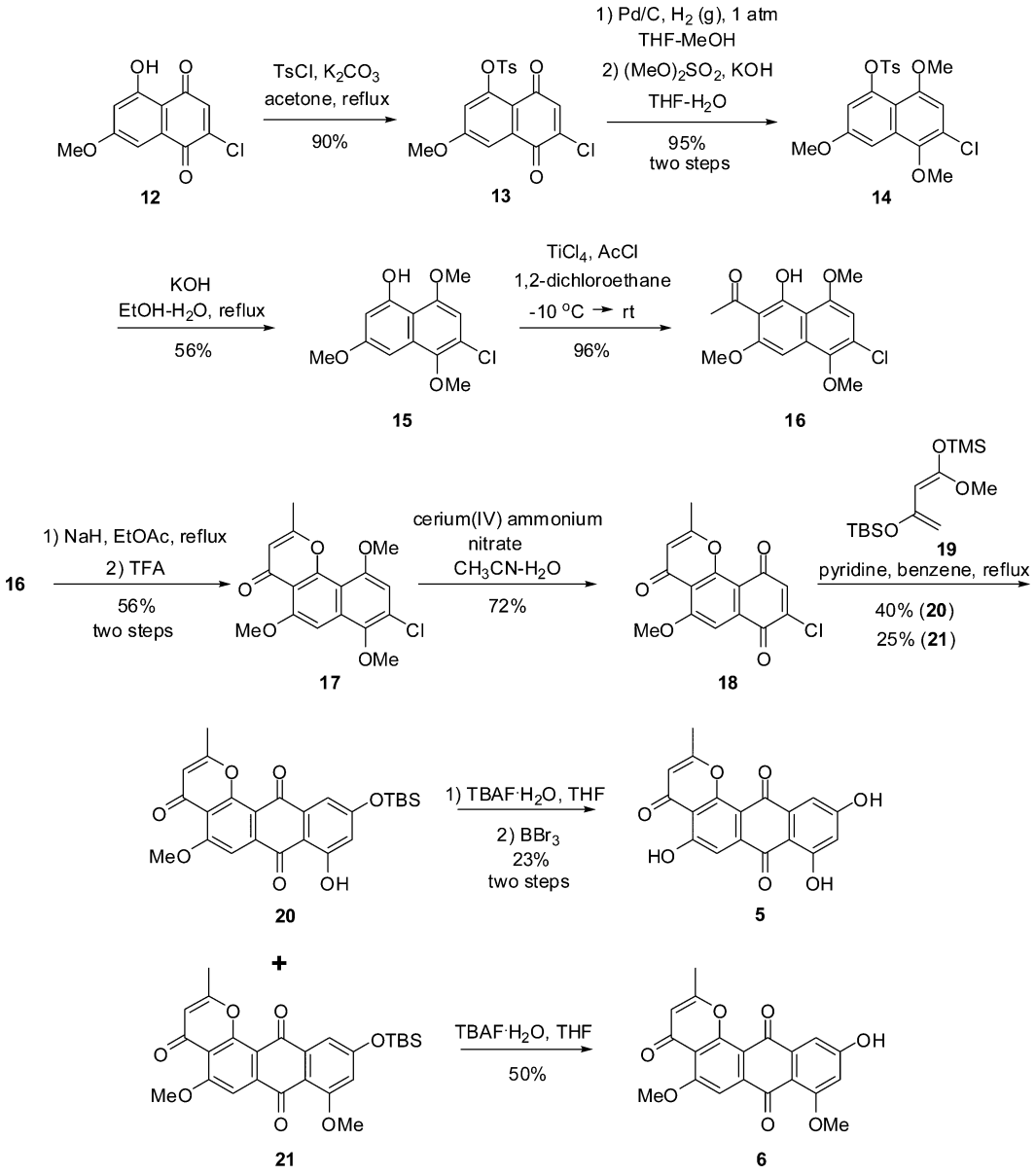
Scheme 1. Synthesis of Topopyrones 5 and 6



nitrobenzene at 90 °C gave brominated topopyrone A derivative **8** in 50% yield.[13] Brominated topopyrone B derivative **11** was obtained by rearrangement of the pyrone ring in **8** using 1% NaOH in MeOH (50% yield).[13]

Biological Evaluation. The ability of novel topopyrones **5** and **6** to stabilize the DNA–topoisomerase I covalent binary complex was investigated using a 23-base pair oligonucleotide substrate that contained a strong topoisomerase I cleavage site having guanosine at the +1 position and thymidine at the −1 position (Figure S1).[21] Polyacrylamide gel electrophoretic analysis indicated that topopyrone C derivatives **5** and **6** both stabilized the DNA–topoisomerase I covalent binary complex with similar efficiencies. Densitometric analysis was used to determine the percentage of DNA cleavage relative to that obtained with 1 μM CPT, which was arbitrarily assigned a value of 100% (Table 1). At 1 and 10 μM concentrations of topopyrone **5**, 6% and 11% DNA cleavage were observed, an amount similar to that obtained with topopyrone **6**. In contrast, brominated topopyrone A derivatives **7** and **8** varied considerably in their efficiencies of stabilization of the DNA–topoisomerase I covalent binary complex (Figure S2 and Table 1). Topopyrone **8** produced 25% DNA cleavage at 10 μM concentration. In sharp contrast, topopyrone **7**, a close structural analogue of **8** containing methylated phenols, failed to stabilize the DNA–topoisomerase I covalent binary complex at 1 or 10 μM concentration. X-ray crystallographic analysis of the binding of other topoisomerase I poisons to the topoisomerase I–DNA binary complex indicates H-bonding between the poisons and the covalent binary complex,[22−24] and it seems likely that H-bonding between one or more phenolic OH groups in **8** contributes to its ability to stabilize the formed ternary complex.

The amounts of the DNA–topoisomerase I covalent binary complexes stabilized by topopyrones **3**, **9**, and **10** were quantified using a nitrocellulose filter binding assay (Table 2).[6] The amounts of the DNA–topoisomerase I covalent binary complexes stabilized in the presence of CPT or the topopyrones were measured by determination of the radio-

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Scheme 2. Synthesis of Topopyrones 7, 8, and 11

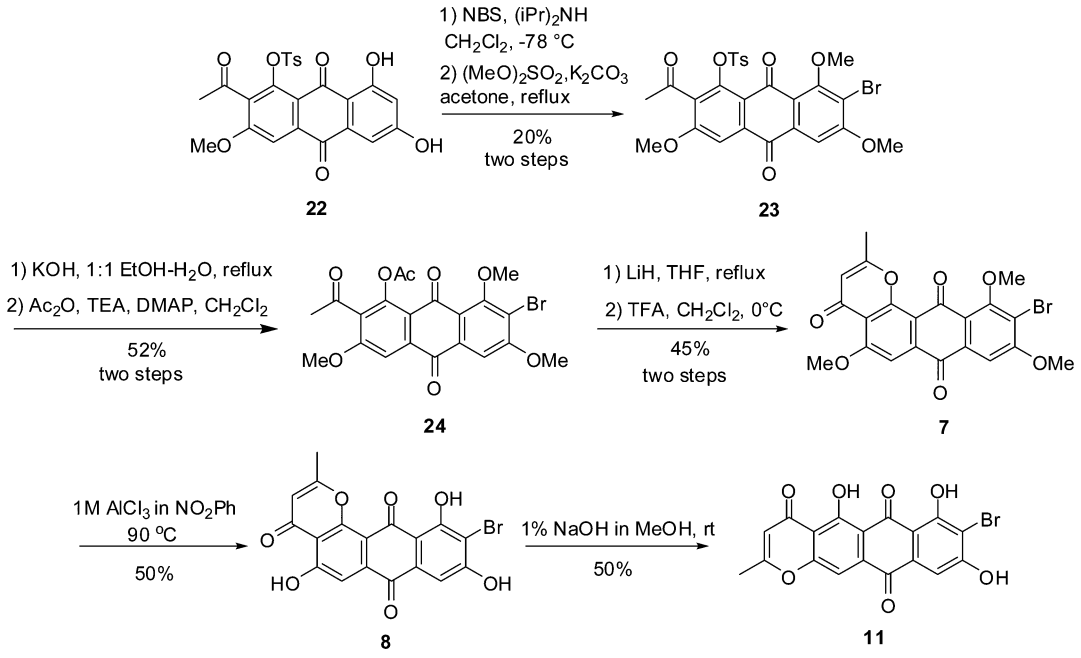

Table 1. Topoisomerase I-Mediated DNA Cleavage by CPT and Topopyrones 5–8 Using a 3′-^{32}P End Labeled 23-Base Pair Oligonucleotide Substrate

topoisomerase I poison	% DNA cleavage[a]
DNA alone	1
topoisomerase I alone	0
1 μM CPT	100
1 μM topopyrone **5**	6
10 μM topopyrone **5**	11
1 μM topopyrone **6**	3
10 μM topopyrone **6**	10
1 μM topopyrone **7**	1
10 μM topopyrone **7**	1
1 μM topopyrone **8**	2
10 μM topopyrone **8**	25

[a]Relative to CPT; determined by densitometric analysis.

Table 2. Nitrocellulose Filter Binding of CPT- or Topopyrone-Stabilized DNA–Topoisomerase Covalent Binary Complexes

topoisomerase poison	DNA covalently bound to topoisomerase (%)[a]
topoisomerase I	
none	1.4 ± 0.5
0.5 μM CPT	39 ± 4.6
10 μM topopyrone C (**3**)	16 ± 4.3
20 μM topopyrone C (**3**)	21 ± 7.8
10 μM topopyrone **9**	6.1 ± 2.4
20 μM topopyrone **9**	5.1 ± 4.4
10 μM topopyrone **10**	11 ± 3.6
20 μM topopyrone **10**	30 ± 5.2
topoisomerase IIα	
none	23 ± 2.7
0.5 μM etoposide	34 ± 4.6
1 μM topopyrone **10**	40 ± 13
5 μM topopyrone **10**	34 ± 2.5

[a]Topoisomerase-mediated cleavage conditions described in the experimental procedures; reaction mixtures contained 6 units of topoisomerase I or 5 units of topoisomerase IIα. Values represent the mean ± SD (n = 3).

activity of the 5′-^{32}P end labeled DNA covalently bound to the enzyme. The topopyrone-stabilized DNA–topoisomerase I covalent binary complexes were trapped by the addition of the protein denaturant sodium dodecyl sulfate (SDS), followed by filtration through a nitrocellulose filter membrane that specifically binds enzyme and not free DNA. Linearized plasmid pBR322 DNA was used as a DNA substrate that contains numerous topoisomerase I binding sites. Topoisomerase I alone covalently bound 1.4 ± 0.5% of the linearized plasmid DNA substrate (Table 2). This increased to 39 ± 4.6% when 0.5 μM CPT was added. Topopyrone C (**3**) was found to stabilize the DNA–topoisomerase I covalent binary complex, possibly in a dose-dependent manner (16% at 10 μM concentration vs 21% at 20 μM concentration), while topopyrone **9** afforded only 5–6% stabilization of the DNA–topoisomerase I covalent binary complex. In contrast, topopyrone **10** afforded stabilization to an extent roughly comparable to that of topopyrone C (**3**) and clearly gave dose-dependent stabilization. Thus, the chlorine substituent at the C5 position of topopyrone **9** is detrimental to stabilization of the DNA–topoisomerase I covalent binary complex (cf. **3** vs **9**), while topopyrone **10**, having the pyrone ring in a linear orientation, restored the activity of the compound as a topoisomerase I poison.

The nitrocellulose filter binding assay was also used to measure stabilization of the DNA–topoisomerase II covalent binary complex by topopyrone **10** (Table 2). Human topoisomerase II exists in two closely related isoforms, topoisomerase IIα and topoisomerase IIβ.[25] Since human topoisomerase IIα is more closely associated with proliferating cells,[25] we used this isoform in our studies. High levels of covalently bound DNA–topoisomerase II complexes were observed even in the absence of any topoisomerase II poison. In the presence of 0.5 μM etoposide, 34 ± 4.6% of the DNA

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was covalently bound to topoisomerase IIα, an amount similar to that achieved with 1 or 5 μM topopyrone **10**.

The cleavage site specificity of the topopyrone-stabilized DNA−topoisomerase I covalent binary complexes was analyzed using a 3′-^{32}P end labeled 158-base pair DNA duplex substrate (Figure 2). The topopyrones were found to stabilize top-

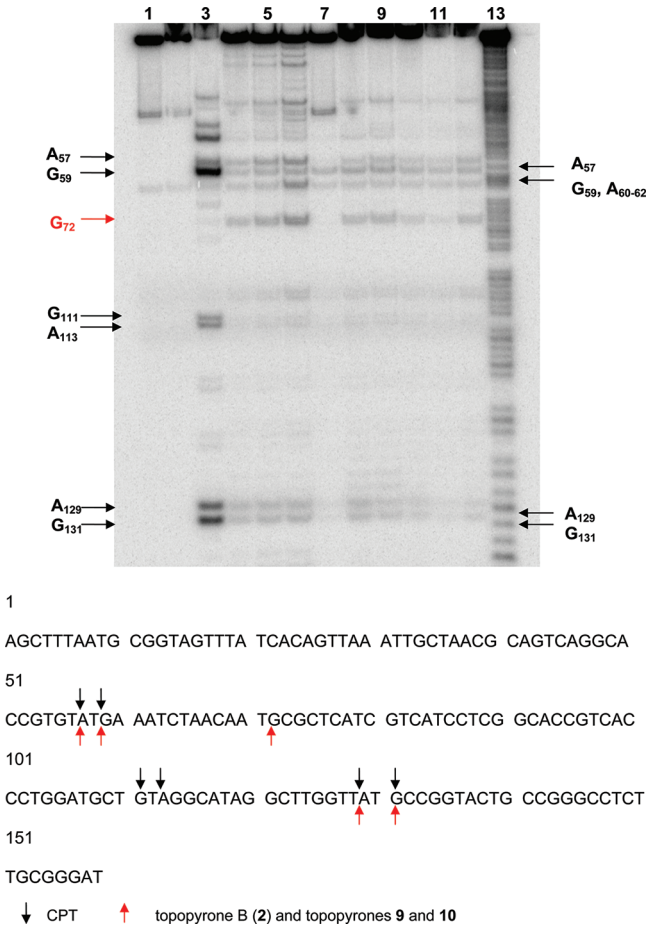

Figure 2. Stabilization of topoisomerase I-mediated DNA cleavage sites on a 3′-^{32}P end labeled 158-base pair DNA duplex substrate by CPT and topopyrones. Upper panel: lane 1, DNA alone; lane 2, DNA + topoisomerase I; lane 3, 1 μM CPT + DNA + topoisomerase I; lane 4, 5 μM topopyrone (**2**) + DNA + topoisomerase I; lane 5, 10 μM topopyrone B (**2**) + DNA + topoisomerase I; lane 6, 20 μM topopyrone B (**2**) + DNA + topoisomerase I; lane 7, 5 μM topopyrone **9** + DNA + topoisomerase I; lane 8, 10 μM topopyrone **9** + DNA + topoisomerase I; lane 9, 20 μM topopyrone **9** + DNA + topoisomerase I; lane 10, 5 μM topopyrone **10** + DNA + topoisomerase I; lane 11, 10 μM topopyrone **10** + DNA + topoisomerase I; lane 12, 20 μM topopyrone **10** + DNA + topoisomerase I; lane 13, G + A lane. Lower panel: DNA cleavage sites stabilized by CPT and topopyrones **2**, **9**, and **10**.

oisomerase I cleavage at sites similar to CPT and with varying efficiencies. Strong stabilization of DNA−topoisomerase I covalent binary complexes by CPT was observed at several 5′-TG-3′ and 5′-TA-3′ sites. Topopyrone B (**2**) displayed dose-dependent stabilization of the DNA−topoisomerase I covalent binary complex (Figure 2, lanes 4−6) and was more potent than topopyrones **9** and **10**. An interesting observation in this experiment was that the three topopyrones, but not CPT, stabilized a topoisomerase I cleavage site at 5′-TG$_{72}$-3′. Additional unique topopyrone-stabilized DNA−topoisomerase I covalent binary complexes were observed at the extreme 5′-end of the DNA duplex substrate, but could not be sequenced.

The distribution of cleavage sites was also examined for topopyrone-stabilized DNA−topoisomerase II covalent binary complexes using a 5′-^{32}P end labeled 158-base pair DNA duplex substrate (Figure 3). Topopyrone B (**2**) and topopyrone **11**

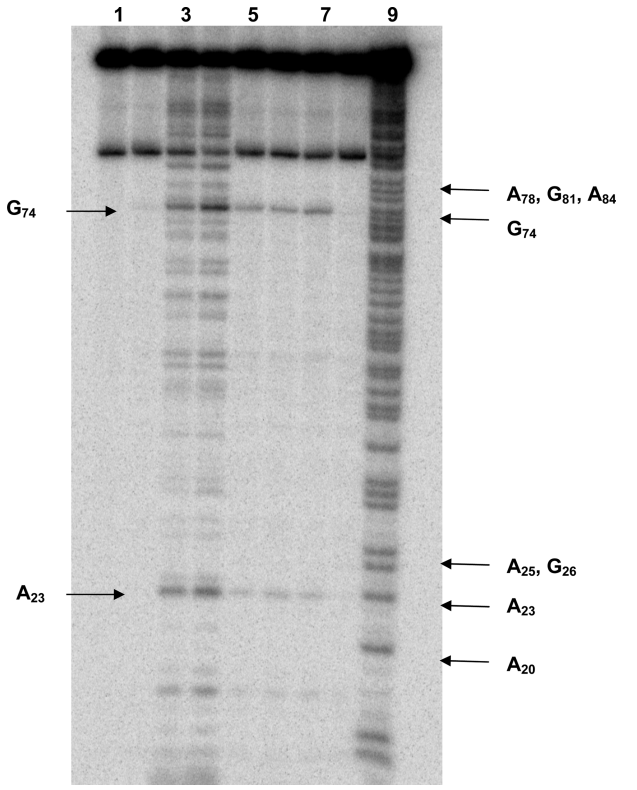

Figure 3. Stabilization of topoisomerase II-mediated DNA cleavage sites on a 5′-^{32}P end labeled 158-base pair DNA duplex substrate by etoposide and topopyrones. Lane 1, DNA alone; lane 2, DNA + topoisomerase IIα; lane 3, 5 μM etoposide + DNA + topoisomerase IIα; lane 4, 20 μM etoposide + DNA + topoisomerase IIα; lane 5, 5 μM topopyrone B (**2**) + DNA + topoisomerase IIα; lane 6, 20 μM topopyrone B (**2**) + DNA + topoisomerase IIα; lane 7, 5 μM topopyrone **11** + DNA + topoisomerase IIα; lane 8, 20 μM topopyrone **11** + DNA + topoisomerase IIα; lane 9, G + A lane.

were found to stabilize the DNA−topoisomerase II covalent binary complex at sites similar to etoposide (5′-CA$_{23}$-3′ and 5′-CG$_{74}$-3′) with varying efficiencies. While topopyrone B (**2**) was able to stabilize the DNA−topoisomerase II covalent binary complex at 5 and 20 μM concentrations, topopyrone **11** was only active at 5 μM concentration (Figure 3, lanes 5−8), possibly due to solubility problems.

EXPERIMENTAL SECTION

General Experimental Procedures. Chemicals and solvents were purchased as reagent grade from Sigma-Aldrich Chemicals and were used without further purification. All the reactions were performed under an argon atmosphere, unless otherwise specified. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F_{254}, 0.25 mm thickness) were used for analytical TLC and were visualized by UV irradiation (254 nm). Flash chromatography was carried out using Silicycle 200−400 mesh silica gel. ^{1}H and ^{13}C NMR spectra were obtained using a Varian 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to residual $CHCl_3$ (δ 7.26 ppm for ^{1}H NMR and δ 77.16

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for ^{13}C NMR) as the internal strandard. Splitting patterns are designated as s, singlet; d, doublet; m, multiplet. High-resolution mass spectra were obtained at the Michigan State Mass Spectrometry Facility or the Arizona State University CLAS High Resolution Mass Spectrometry Facility.

Biochemical Reagents. Terminal deoxynucleotidyl transferase and Sephadex G-25 mini Quick Spin Oligo columns were purchased from Roche Applied Science (Indianapolis, IN, USA). pBR322 plasmid DNA, Klenow fragment (3′→5′ exo$^-$) of DNA polymerase, T4 polynucleotide kinase, and restriction endonucleases *Hin*dIII and *Eco*RV were obtained from New England Biolabs (Ipswich, MA, USA). Thermosensitive alkaline phosphatase and the Gene-Jet PCR purification kit were purchased from Fermentas (Glen Burnie, MD, USA). Human topoisomerase I was purchased from Topogen, Inc. (Access Alley, FL, USA). Human topoisomerase IIα was purchased from USB Products (Santa Clara, CA, USA). Protran B85 nitrocellulose filters were obtained from Whatman (Piscataway, NJ, USA). Liquid scintillation fluid was purchased from RPI Corp (Mount Prospect, IL, USA). All synthetic oligonucleotides, purified by ion exchange, were obtained from Integrated DNA Technologies (Coralville, IA, USA). Radiolabeled nucleotides were purchased from Perkin-Elmer Life Sciences (Waltham, MA, USA). Topopyrone and CPT solutions were freshly prepared in 60% aqueous dimethyl sulfoxide prior to biological evaluation.

Toluene-4-sulfonic Acid 6-Chloro-3-methoxy-5,8-dioxo-5,8-dihydronaphthalen-1-yl Ester (13). To a solution containing 0.41 g (1.7 mmol) of **12**[14] in 100 mL of acetone at room temperature was added 0.65 g (3.4 mmol) of *p*-toluenesulfonyl chloride (*p*-TsCl) and 0.47 g (3.4 mmol) of K_2CO_3. The reaction mixture was heated at reflux for 6 h, and the cooled solution was filtered through a silica gel plug. The silica gel was washed with 100 mL of ethyl acetate, and the combined filtrate was concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (15 × 3 cm). Step gradient elution with 1:1 $CHCl_3$–hexanes → 3:2 $CHCl_3$–hexanes gave **13** as a yellow solid: yield 0.60 g (90%); mp 150–152 °C; silica gel TLC R_f 0.47 (2:1 ethyl acetate–hexanes); mass spectrum (APCI), m/z 393.0199 $(M + H)^+$ ($C_{18}H_{14}ClO_6S$ requires m/z 393.0200).

Toluene-4-sulfonic Acid 6-Chloro-3,5,8-trimethoxynaphthalen-1-yl Ester (14). To a solution containing 1.1 g (2.9 mmol) of **13** in 50 mL of 1:1 MeOH–THF under argon was added ∼70 mg of Pd/C. The reaction mixture was purged with H_2 and stirred under H_2 for 2 h at room temperature, at which time it was filtered through a pad of silica gel and washed with 300 mL of ethyl acetate. The combined filtrate was concentrated under diminished pressure, and the resulting residue was dissolved in 100 mL of 1:1 THF–H_2O. To this solution was added 1.5 g (8.7 mmol) of KOH and 2.6 mL (28.6 mmol) of $(MeO)_2SO_2$. The reaction mixture was stirred for 1 h, then quenched with 200 mL of H_2O, and extracted with three 150 mL portions of ethyl acetate. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (20 × 5 cm). Elution with 1:10 ethyl acetate–hexanes gave **14** as a colorless solid: yield 1.1 g (95%); mp 136–138 °C; silica gel TLC R_f 0.61 (2:1 ethyl acetate–hexanes); mass spectrum (ESI), m/z 423.0676 $(M + H)^+$ ($C_{20}H_{20}ClO_6S$ requires m/z 423.0669).

6-Chloro-1-hydroxy-3,5,8-trimethoxynaphthalene (15). A solution containing 1.4 g (3.3 mmol) of **14** in 100 mL of 1:1 EtOH–H_2O containing 10% (w/v) KOH was heated at reflux for 4 h. EtOH was removed under diminished pressure, and the remaining solution was poured into 6 N HCl (ice cold) and extracted with three 100 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude oil. The residue was purified by flash chromatography on a silica gel column (12 × 3 cm). Elution with 1:7 ethyl acetate–hexanes gave **15** as a colorless solid: yield 0.50 g (56%); mp 115–117 °C; silica gel TLC R_f 0.63 (2:1 ethyl acetate–hexanes); mass spectrum (ESI), m/z 269.0570 $(M + H)^+$ ($C_{13}H_{14}ClO_4$ requires m/z 269.0581).

1-(6-Chloro-1-hydroxy-3,5,8-trimethoxynaphthalen-2-yl)-ethan-2-one (16). To a solution containing 0.50 g (1.9 mmol) of **15** in 20 mL of 1,2-dichloroethane at −10 °C was added 2.1 mL (2.1 mmol) of $TiCl_4$ (1.0 M in CH_2Cl_2). The reaction mixture was stirred for 5 min at −10 °C, and 0.30 mL (3.8 mmol) of acetyl chloride was added. The reaction mixture was heated at reflux for 2 h, then cooled and quenched with 400 mL of 1:1 2 N HCl–saturated aqueous potassium sodium tartrate, filtered through a pad of Celite, and washed with 200 mL of ethyl acetate. The organic phase was separated, and the aqueous phase was extracted with three 300 mL portions of ethyl acetate. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (15 × 3 cm). Elution with 1:10 ethyl acetate–hexanes gave **16** as a yellow solid: yield 0.56 g (96%); mp 128–130 °C; silica gel TLC R_f 0.61 (1:2 ethyl acetate–hexanes); mass spectrum (ESI), m/z 311.0692 $(M + H)^+$ ($C_{15}H_{16}ClO_5$ requires m/z 311.0686).

8-Chloro-2-methyl-5,7,10-trimethoxybenzo[*h*]chromen-4-one (17). To a solution containing 0.40 g (1.29 mmol) of **16** in 20 mL of freshly distilled ethyl acetate was added 0.57 g (14.1 mmol) of NaH (60% dispersion in mineral oil). The reaction mixture was heated at reflux for 1 h, cooled, and quenched with 100 mL of 2 N HCl (ice cold). The solution was extracted with three 200 mL portions of $CHCl_3$, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was dissolved in 15 mL of TFA at 0 °C and stirred for 15 min at 0 °C and then for 30 min at room temperature. The solvent was concentrated under diminished pressure, and the resulting residue was co-evaporated with two 50 mL portions of toluene. The resulting residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Step gradient elution with 1:1 ethyl acetate–hexanes → 20% MeOH in $CHCl_3$ gave **17** as a colorless solid: yield 0.24 g (56%); mp 182–184 °C; silica gel TLC R_f 0.43 (15% MeOH in 1:1 ethyl acetate–hexanes); mass spectrum (ESI), m/z 335.0675 $(M + H)^+$ ($C_{17}H_{16}ClO_5$ requires m/z 335.0686).

8-Chloro-5-methoxy-2-methylbenzo[*h*]chromene-4,7,10-trione (18). To a solution containing 0.24 g (0.71 mmol) of **17** in 50 mL of CH_3CN at 0 °C was added 0.77 g (1.4 mmol) of ceric ammonium nitrate in 8 mL of H_2O. The reaction mixture was warmed to room temperature and stirred for 0.5 h, at which time 150 mL of H_2O was added. The reaction mixture was extracted with four 150 mL portions of $CHCl_3$, and the combined organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with $CHCl_3$ as eluant gave **18** as a yellow solid: yield 0.16 g (72%); mp 257–259 °C; silica gel TLC R_f 0.50 (15% MeOH in 1:1 ethyl acetate–hexanes); mass spectrum (ESI), m/z 305.0213 $(M + H)^+$ ($C_{15}H_{10}O_5Cl$ requires m/z 305.0217).

10-(*tert*-Butyldimethylsilyloxy)-8-hydroxy-5-methoxy-2-methyl-1-oxabenzo[*a*]anthracene-4,7,12-trione (20) and 10-(*tert*-Butyldimethylsilyloxy)-5,8-dimethoxy-2-methyl-1-oxabenzo[*a*]anthracene-4,7,12-trione (21). To a solution containing 0.11 g (0.36 mmol) of **18** in 35 mL of dry benzene was added 0.12 mL (1.5 mmol) of pyridine followed by 1.1 g (3.6 mmol) of freshly prepared **19**.[13] The reaction mixture was heated at reflux for 18 h, after which the cooled reaction mixture was quenched with 350 mL of 2 N HCl (ice cold) and extracted with three 150 mL portions of $CHCl_3$. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude yellow solid. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 2% MeOH in 1:1 ethyl acetate–hexanes gave **20** as an orange solid: yield 67 mg (40%); mp >300 °C dec; silica gel TLC R_f 0.64 (10% MeOH in 1:1 ethyl acetate–hexanes); mass spectrum (ESI), m/z 467.1548 $(M + H)^+$ ($C_{25}H_{27}O_7Si$ requires m/z 467.1526). Further elution with 5% MeOH in 1:1 ethyl acetate–hexanes gave **21** as a yellow solid: yield 42 mg (25%); mp >300 °C dec; silica gel TLC R_f 0.5 (10% MeOH in 1:1 ethyl acetate–hexanes); mass spectrum (ESI), m/z 481.1698 $(M + H)^+$ ($C_{26}H_{29}O_7Si$ requires m/z 481.1683).

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Topopyrone 5. To a solution containing 55 mg (0.12 mmol) of **20** in 10 mL of THF at room temperature was added 34 mg (0.13 mmol) of TBAF·H_2O. The reaction mixture was stirred at room temperature for 0.5 h, quenched with 100 mL of 2 N HCl, and extracted with three 50 mL portions of ethyl acetate and two 25 mL portions of $CHCl_3$. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude orange-yellow solid. The residue was dissolved in 10 mL of CH_2Cl_2 at −78 °C, and 1.18 mL (1.18 mmol) of BBr_3 (1.0 M in CH_2Cl_2) was added. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction was quenched by the addition of 100 mL of 2 N HCl and extracted with three 50 mL portions of ethyl acetate. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude orange-yellow solid. The crude product was purified by flash chromatography on a silica gel column (25 × 2 cm). Elution with 94:5:1 $CHCl_3$−MeOH−AcOH gave **5** as an orange solid: yield 9.0 mg (23%); mp >150 °C dec; silica gel TLC R_f 0.51 (94:5:1 $CHCl_3$−MeOH−AcOH); mass spectrum (APCI), m/z 339.0505 $(M + H)^+$ ($C_{18}H_{11}O_7$ requires m/z 339.0503). All attempts at obtaining NMR spectra of **5** met with failure in a variety of solvent systems due to insufficient solubility. Accordingly, **5** was converted to its 3,5,7-trimethoxy derivative to permit characterization by NMR. The derivative was also characterized by high-resolution mass spectrometry.

To a solution containing 10.0 mg (0.03 mmol) of **5** in 5 mL of acetone was added 0.04 mL (0.30 mmol) of $(MeO)_2SO_2$ and 42 mg (0.30 mmol) of K_2CO_3. The reaction mixture was heated at reflux for 18 h, quenched with 50 mL of 1 N HCl, and extracted with two 20 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, then filtered and concentrated under diminished pressure to afford a crude residue. The crude product was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 2% MeOH in $CHCl_3$ gave the 3,5,7-trimethoxy derivative of topopyrone **5** as an orange solid: yield 5.0 mg (45%); silica gel TLC R_f 0.48 (2% MeOH in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.48 (3H, d, J = 0.4 Hz), 3.98 (3H, s), 4.01 (3H, s), 4.14 (3H, s), 6.20 (1H, d, J = 0.4 Hz), 6.82 (1H, d, J = 2.8 Hz), 7.35 (1H, d, J = 2.4 Hz) and 7.58 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.7, 56.5, 57.2, 57.5, 103.0, 103.5, 106.2, 113.5, 117.6, 118.1, 118.5, 136.3, 137.4, 158.1, 162.4, 163.3, 164.5, 165.9, 177.6, 179.6, and 183.5; mass spectrum (ESI), m/z 381.0983 $(M + H)^+$ ($C_{21}H_{17}O_7$ requires m/z 381.0974).

Topopyrone 6. To a solution containing 52 mg (0.11 mmol) of **21** in 10 mL of THF at room temperature was added 30 mg (0.11 mmol) of TBAF·H_2O. The reaction mixture was stirred for 1 h at room temperature, quenched with 100 mL of 2 N HCl, and extracted with three 50 mL portions of ethyl acetate and two 25 mL portions of $CHCl_3$. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude orange-yellow solid. The crude product was purified by flash chromatography on a silica gel column (20 × 2 cm). Elution with 94:5:1 $CHCl_3$−MeOH−AcOH gave **6** as a yellow solid: yield 20 mg (50%); mp >180 °C dec; silica gel TLC R_f 0.42 (94:5:1 $CHCl_3$−MeOH−AcOH); mass spectrum (ESI), m/z 367.0807 $(M + H)^+$ ($C_{20}H_{15}O_7$ requires m/z 367.0818). All attempts at obtaining NMR spectra of **6** met with failure in a variety of solvent systems due to insufficient solubility. Accordingly, **6** was converted to its 7-acetoxy derivative to permit characterization by NMR. The derivative was also characterized by high-resolution mass spectrometry.

A mixture of 12 mg (33 μmol) of **6**, 3 mL of acetic anhydride, and 1.5 mL of pyridine was stirred at room temperature for 3 h. The reaction mixture was quenched with 50 mL of 2 N HCl and extracted with two 25 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The crude product was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 3% MeOH in $CHCl_3$ gave the 7-acetoxy derivative of topopyrone **6** as a yellow solid: yield 9.0 mg (68%); silica gel TLC R_f 0.50 (2% MeOH in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s), 2.48 (3H, s), 4.04 (3H, s), 4.15 (3H, s), 6.22 (1H, s), 7.07 (1H, d, J = 2.0 Hz), 7.66 (1H, d, J = 2.4 Hz), and 7.69 (1H, s); ^{13}C NMR ($CDCl_3$) δ 19.9, 21.1, 56.8, 57.1, 104.3, 110.4, 113.1, 113.2, 114.4, 116.9, 118.3, 138.4, 139.9, 156.3, 157.7, 161.7, 164.3, 165.1, 168.3, 176.8, 179.0, and 180.2; mass spectrum (ESI), m/z 409.0931 $(M + H)^+$ ($C_{22}H_{17}O_8$ requires m/z 409.0923).

2-Acetyl-7-bromo-3,6,8-trimethoxy-1-tosyloxyanthraquinone (23). To a solution containing 0.05 g (0.10 mmol) of **22**[13] in 10 mL of CH_2Cl_2 at −78 °C was added 0.02 mL (0.15 mmol) of $(iPr)_2NH$ followed by 0.02 g (0.11 mmol) of *N*-bromosuccinimide in 10 mL of CH_2Cl_2. After the additions, the reaction was quenched by the addition of 20 mL of 1:1 H_2O−CH_2Cl_2. The phases were separated, and the aqueous phase was extracted with three 10 mL portions of CH_2Cl_2. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude red solid. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 2% MeOH in 1:1 ethyl acetate−hexanes gave the crude monobrominated product **23** as a yellow solid. The product obtained was dissolved in 4 mL of acetone, and 0.05 g (0.36 mmol) of K_2CO_3 was added, followed by 0.03 mL (0.40 mmol) of $(MeO)_2SO_2$. The reaction mixture was stirred at reflux for 3 h, and the cooled reaction mixture was quenched with 50 mL of 2 N HCl and then extracted with three 50 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude yellow solid. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 1:2 ethyl acetate−hexanes gave **23** as a yellow solid: yield 12 mg (20%); silica gel TLC R_f 0.35 (1:1 ethyl acetate−hexanes); mass spectrum (ESI), m/z 589.0182 $(M + H)^+$ ($C_{26}H_{22}BrO_9S$ requires m/z 589.0168).

1-Acetoxy-2-acetyl-7-bromo-3,6,8-trimethoxyanthraquinone (24). To a solution containing 0.07 g (0.12 mmol) of **23** in 15 mL of 1:1 EtOH−H_2O was added 0.06 g (1.0 mmol) of KOH. The reaction mixture was heated at reflux for 4 h. The cooled solution was poured into 2 N HCl and extracted with three 50 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude yellow solid. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 1:2 ethyl acetate−hexanes afforded the product having a free hydroxy group. This material was dissolved in 5 mL of CH_2Cl_2, and 0.03 mL (0.30 mmol) of acetic anhydride was added followed by 0.04 mL (0.30 mmol) of Et_3N and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine. The reaction mixture was stirred at room temperature for 18 h, quenched with 50 mL of 2 N HCl, and extracted with three 50 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude yellow solid. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 1:2 ethyl acetate−hexanes gave **24** as a yellow solid: yield 30 mg (52%); silica gel TLC R_f 0.29 (1:1 ethyl acetate−hexanes); mass spectrum (ESI), m/z 477.0162 $(M + H)^+$ ($C_{21}H_{18}BrO_8$ requires m/z 477.0185).

Topopyrone 7. To a solution containing 0.04 g (0.08 mmol) of **24** in 10 mL of freshly distilled THF was added 0.02 g (2.5 mmol) of LiH. The reaction mixture was heated at reflux for 18 h, and the cooled reaction mixture was quenched carefully with 50 mL of 2 N HCl. The resulting solution was extracted with three 75 mL portions of ethyl acetate, dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude residue. The residue was dissolved in 5 mL of CF_3COOH. The reaction mixture was stirred at room temperature for 1 h and concentrated under diminished pressure, and the residue so obtained was co-evaporated with two 5 mL portions of toluene. The resulting crude residue was purified by flash chromatography on a silica gel column (7 × 2 cm). Gradient elution with 1:1 ethyl acetate−hexanes → 2% MeOH in 1:1 ethyl acetate−hexanes gave **7** as a yellow solid: yield 17 mg (45%); mp >280 °C dec; silica gel TLC R_f 0.21 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 2.48 (3H, d, J = 0.4 Hz), 4.04 (3H, s), 4.09 (3H, s), 4.16 (3H, s), 6.21 (1H, d, J = 0.8 Hz), 7.55 (1H, s) and 7.59 (1H, s); ^{13}C NMR ($CDCl_3$) δ 19.9, 56.9, 57.0, 61.8, 103.1, 104.9, 112.9, 116.7,

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117.7, 117.9, 122.3, 133.5, 136.8, 157.4, 158.9, 160.3, 163.1, 165.2, 176.8, 178.1, and 182.1; mass spectrum (ESI), m/z 459.0060 $(M + H)^+$ ($C_{21}H_{16}BrO_7$ requires m/z 459.0079).

Topopyrone 8. A solution containing 14 mg (35 μmol) of **7** in 2 mL of 1.0 M $AlCl_3$ in nitrobenzene was heated at 90 °C for 48 h. The reaction was quenched while hot with 20 mL of 6 N HCl and stirred at 90 °C for 1 h. The reaction mixture was extracted with three 50 mL portions of $CHCl_3$, dried over anhydrous $MgSO_4$, and then concentrated under diminished pressure to afford a crude green oil. The crude product was purified by flash chromatography on a silica gel column (15 × 2 cm). Step gradient elution with 4:1 ethyl acetate−hexanes → 95:4:1 $CHCl_3$−MeOH−AcOH gave **8** as a green solid: yield 6.5 mg (50%); mp >300 °C dec; silica gel TLC R_f 0.30 (10% MeOH in $CHCl_3$); mass spectrum (APCI), m/z 416.9618 $(M + H)^+$ ($C_{18}H_{10}O_7Br$ requires m/z 416.9610). All attempts at obtaining NMR spectra of **8** met with failure in a variety of solvent systems due to insufficient solubility.

Topopyrones 9 and 10. These two compounds were synthesized as described previously.[13]

Topopyrone 11. A solution containing 8.0 mg (19 μmol) of **8** in 4 mL of 1% NaOH in MeOH was heated at 60 °C for 48 h. The reaction mixture was neutralized carefully with 1 N HCl and concentrated under diminished pressure. The resulting residue was dissolved in 10 mL of H_2O and extracted successively with three 20 mL portions of $CHCl_3$ and two 10 mL portions of ethyl acetate. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and then concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (10 × 2 cm). Elution with 94:5:1 $CHCl_3$−MeOH−AcOH gave **11** as a green solid: yield 4.0 mg (50%); mp >300 °C dec; silica gel TLC R_f 0.23 (10% MeOH in $CHCl_3$); mass spectrum (ESI), m/z 416.9613 $(M + H)^+$ ($C_{18}H_{10}BrO_7$ requires m/z 416.9610). All attempts at obtaining NMR spectra of **11** met with failure in a variety of solvent systems due to insufficient solubility. Accordingly, **11** was converted to its 1,6,8-trimethoxy derivative to permit characterization by NMR. The derivative was also characterized by high-resolution mass spectrometry.

To a solution containing 6.0 mg (14 μmol) of **11** in 4 mL of acetone at room temperature was added 0.03 mL (0.23 mmol) of $(MeO)_2SO_2$ and 32 mg (0.23 mmol) of K_2CO_3. The reaction mixture was heated at reflux for 18 h, quenched with 50 mL of 1 N HCl, and extracted with two 20 mL portions of ethyl acetate. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The crude product was purified by flash chromatography on a silica gel column (10 × 1 cm). Elution with 2% MeOH in $CHCl_3$ gave the 1,6,8-trimethoxy derivative of topopyrone **11** as an orange solid: yield 4.0 mg (66%); silica gel TLC R_f 0.55 (2% MeOH in $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.49 (3H, s), 4.02 (3H, s), 4.10 (3H, s), 4.17 (3H, s), 6.24 (1H, s), 7.66 (1H, s), and 7.71 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.0, 57.1, 57.3, 61.5, 104.4, 105.9, 113.3, 114.3, 115.9, 119.7, 136.7, 139.6, 157.8, 159.3, 161.7, 164.4, 165.2, 179.19, and 179.21; mass spectrum (ESI), m/z 459.0077 $(M + H)^+$ ($C_{21}H_{16}O_7Br$ requires m/z 459.0079).

3′-^{32}P End Labeling and Purification of Oligonucleotides. 3′-^{32}P end labeling was carried out by adding 10 pmol of template DNA, 0.05 mCi [α-^{32}P] cordycepin 5′-triphosphate (5000 Ci/mmol), and 400 units of terminal deoxynucleotidyl transferase in 40 μL (total volume) of 25 mM Tris-HCl, pH 6.6, containing 200 mM potassium cacodylate, 2.5 mM $CoCl_2$, and 0.25 mg/mL bovine serum albumin (BSA). The reaction mixture was incubated at 37 °C for 1 h. Reactions were terminated by the addition of SDS to a final concentration of 0.5%. The radiolabeled oligonucleotides were purified using Sephadex G-25 Quick Spin Oligo columns.

Topoisomerase I-Mediated DNA Cleavage and Religation of a 23-Base Pair Oligonucleotide Substrate. The 3′-^{32}P end labeled DNA strands were annealed with their complementary unlabeled DNA strands in 10 mM Tris-HCl, pH 7.8, containing 100 mM NaCl and 1 mM EDTA. The annealing process was performed by heating the reaction mixture at 95 °C for 5 min, followed by slow cooling to 25 °C. Duplex DNA substrates (~100 fmol), 6 units of topoisomerase I, and either CPT or a topopyrone at the indicated concentrations were incubated at 25 °C for 30 min. The reaction volume was 10 μL, and the reaction was carried out in 10 mM Tris-HCl, pH 7.9, containing 0.15 M NaCl, 1 mM EDTA, 0.1% BSA, 0.1 mM spermidine, and 5% glycerol. The reactions were stopped by the addition of SDS to a final concentration of 0.5%. A 10 μL amount of denaturing gel loading solution containing 80% (v/v) formamide, 2 mM EDTA, 1% (w/v) bromophenol blue, and 1% (w/v) xylene cyanol was added to the reaction mixture. The resulting solution was heated at 95 °C for 5 min, followed by chilling on ice. A 5 μL portion of each sample was then loaded onto a denaturing (7 M urea) 16% (w/v) polyacrylamide gel and electrophoresed at 50 W for 2 h. Gels were visualized using a phosphorimager (Molecular Dynamics) equipped with ImageQuant version 3.2 software.

3′-^{32}P End Labeling and Purification of a 158-Base Pair DNA Restriction Fragment. Plasmid pBR322 DNA (25 μg) was digested with 50 units of *Hin*dIII in 80 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol (DTT) at 37 °C for 3 h. The linearized DNA was 3′-^{32}P end labeled by incubation with 10 units of the Klenow fragment (3′→5′ exo$^-$) of DNA polymerase in 100 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, and 0.10 mCi [α-^{32}P] dATP (3000 Ci/mmol). The reaction mixture was incubated at 37 °C for 1 h followed by heat inactivation of the enzyme at 75 °C for 20 min. Unincorporated [α-^{32}P] dATP was removed with a GeneJET PCR purification kit. The 3′-^{32}P end labeled DNA was digested at 37 °C for 3 h with 50 units of *Eco*RV in 70 μL (total volume) of 50 mM Tris-HCl, pH 7.9, containing 0.1 M NaCl, 10 mM $MgCl_2$ and 1 mM DTT. This digestion resulted in a 3′-^{32}P end labeled fragment having 158 base pairs. Purification of the restriction fragment was performed essentially as described.[26,27]

Topoisomerase I-Mediated DNA Cleavage of a 158-Base Pair DNA Duplex Substrate. A sample of 3′-^{32}P end labeled 158-base pair DNA (30 000 cpm), 6 units of topoisomerase I, and either CPT or a topopyrone at the indicated concentrations were incubated at 25 °C for 30 min in 10 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 0.15 M NaCl, 1 mM EDTA, 0.1% BSA, 0.1 mM spermidine, and 5% glycerol. The reactions were stopped by addition of SDS to a final concentration of 0.5%. A 10 μL sample of denaturing gel loading solution containing 80% (v/v) formamide, 2 mM EDTA, 1% (w/v) bromophenol blue, and 1% (w/v) xylene cyanol was added to the reaction mixture. The resulting solution was heated at 95 °C for 5 min, followed by chilling on ice. A 5 μL amount of each sample was then loaded onto a denaturing (7 M urea) 16% (w/v) polyacrylamide gel and electrophoresed at 50 W for 2 h. Cleavage sites were confirmed by comparison with the reaction products obtained by the Maxam−Gilbert G + A sequencing protocol.[28] Gels were visualized using a phosphorimager (Molecular Dynamics) equipped with ImageQuant version 3.2 software.

5′-^{32}P End Labeling and Purification of a 158-Base Pair DNA Restriction Fragment. Plasmid pBR322 DNA (25 μg) was digested with 50 units of *Hin*dIII in 80 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol (DTT) at 37 °C for 3 h. The digest was then treated with 25 units of thermosensitive alkaline phosphatase in 120 μL (total volume) of 10 mM Tris-HCl, pH 8.0, containing 5 mM $MgCl_2$, 0.1 M KCl, 0.02% Triton X-100, 1 mM 2-mercaptoethanol, and 0.1 mg/mL BSA. The reaction mixture was incubated at 37 °C for 30 min followed by heat inactivation of the enzyme at 75 °C for 5 min. The reaction mixture was then 5′-^{32}P end labeled using 20 units of T4 polynucleotide kinase in 80 μL (total volume) of 70 mM Tris-HCl, pH 7.6, containing 10 mM $MgCl_2$, 5 mM DTT, and 0.10 mCi [γ-^{32}P] ATP (6000 Ci/mmol). The reaction mixture was incubated at 37 °C for 1 h followed by heat inactivation of the enzyme at 65 °C for 20 min. The radiolabeled DNA restriction fragment was purified on an 8% native polyacrylamide gel, and run at 10 W for 3.5 h. The desired DNA fragment was eluted from the gel slice in 3 M sodium acetate overnight followed by ethanol precipitation of the radiolabeled product.

Topoisomerase II-Mediated DNA Cleavage of a 158-Base Pair DNA Duplex Substrate. A sample of 5′-^{32}P end labeled 158-base pair DNA (30000 cpm), 10 units of DNA topoisomerase IIα, and either etoposide or a topopyrone at the indicated concentrations were incubated at 25 °C for 30 min. The reaction was carried out in 10 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL BSA, and 1 mM ATP. The reactions were stopped by addition of SDS to a final concentration of 0.5%. A 10 μL amount of denaturing gel loading buffer containing 80% (v/v) formamide, 2 mM EDTA, 1% (w/v) bromophenol blue, and 1% (w/v) xylene cyanol was added to the reaction mixture. The resulting solution was heated at 95 °C for 5 min, followed by chilling on ice. A 5 μL portion of each sample was then loaded onto a denaturing (7 M urea) 16% (w/v) polyacrylamide gel and electrophoresed at 50 W for 2 h. Cleavage sites were confirmed by comparison with the reaction products obtained by the Maxam–Gilbert G + A sequencing protocol.[28] Gels were visualized using a phosphorimager (Molecular Dynamics) equipped with ImageQuant version 3.2 software.

Nitrocellulose Filter Binding of DNA–Topoisomerase I Complexes. The nitrocellulose filter binding assay was performed essentially as described.[6] Reaction mixtures contained 5′-^{32}P end labeled linearized pBR322 DNA (8500 cpm) in 20 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 0.15 M NaCl, 1 mM EDTA, 0.1% BSA, 0.1 mM spermidine, 5% glycerol, 6 units of topoisomerase I, and the appropriate concentrations of CPT or topopyrone. The reaction mixtures were maintained at 25 °C for 15 min, followed by the addition of SDS to a final concentration of 0.5%. The reaction mixtures were filtered through a Protran B85 nitrocellulose filter under vacuum in a Whatman Hybri-slot manifold. After filtration, the samples were washed twice with 20 μL of the same buffer noted above. The filters were placed in 10 mL of scintillation fluid, and the radioactivity was quantified using a Beckman Coulter LS6500 scintillation counter. The percentage of DNA covalently bound to topoisomerase I was calculated as follows: (amount of radioactivity bound to filter/amount of radioactivity in reaction mixture) × 100.

Nitrocellulose Filter Binding of DNA–Topoisomerase II Complexes. The same protocol was followed as indicated above. Reaction mixtures contained 5′-^{32}P end labeled linearized pBR322 DNA (8500 cpm) in 20 μL (total volume) of 10 mM Tris-HCl, pH 7.9, containing 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL BSA, 1 mM ATP, 5 units of topoisomerase IIα, and the appropriate concentrations of etoposide or a topopyrone. The reaction mixtures were maintained at 25 °C for 15 min, followed by the addition of SDS to a final concentration of 0.5%. The reaction mixtures were filtered through a Protran B85 nitrocellulose filter under vacuum in a Whatman Hybri-slot manifold. After filtration, the samples were washed twice with 20 μL of the same buffer noted above. The filters were placed in 10 mL of scintillation fluid, and the radioactivity was quantified through a Beckman Coulter LS6500 counter. The percentage of DNA covalently bound to topoisomerase I was calculated as follows: (amount of radioactivity bound to filter/amount of radioactivity in reaction mixture) × 100.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra values for synthetic intermediates, figures illustrating stabilization of topoisomerase I-mediated cleavage of a 23-base pair oligonucleotide substrate by topopyrones, and ^{1}H and ^{13}C NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Wang, J. C. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430–440.
(2) Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665–697.
(3) Champoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369–413.
(4) Tse, Y.-C.; Kirkegaard, K.; Wang, J. C. *J. Biol. Chem.* **1980**, *255*, 5560–5565.
(5) Liu, L. F. *Annu. Rev. Biochem.* **1989**, *58*, 351–375.
(6) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629–4638.
(7) Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560–13566.
(8) Anthony, S.; Jayaraman, M.; Laco, G.; Kohlhagen, G.; Kohn, K. W.; Cushman, M.; Pommier, Y. *Cancer Res.* **2003**, *63*, 7428–7435.
(9) Khan, Q. A.; Lu, J.; Hecht, S. M. *J. Nat. Prod.* **2009**, *72*, 438–442.
(10) Kanai, Y.; Ishiyama, D.; Senda, H.; Iwatani, W.; Takahashi, H.; Konno, H.; Tokumasu, S.; Kanazawa, S. *J. Antibiot.* **2000**, *53*, 863–872.
(11) Ishiyama, D.; Kanai, Y.; Senda, H.; Iwatani, W.; Takahashi, H.; Konno, H.; Kanazawa, S. *J. Antibiot.* **2000**, *53*, 873–878.
(12) Khan, Q. A.; Elban, M. A.; Hecht, S. M. *J. Am. Chem. Soc.* **2008**, *130*, 12888–12889.
(13) Elban, M. A.; Hecht, S. M. *J. Org. Chem.* **2008**, *73*, 785–793.
(14) Savard, J.; Brassard, P. *Tetrahedron* **1984**, *40*, 3455–3464.
(15) Rozenberg, V.; Danilova, T.; Sergeeva, E.; Vorontsov, Z. S.; Lysenko, K.; Belokon, Y. *Eur. J. Org. Chem.* **2000**, *2000*, 3295–3303.
(16) Bensari, A.; Zaveri, N. T. *Synthesis* **2003**, *2*, 267–271.
(17) Krohn, K.; Bernhard, S.; Florke, U.; Hayat, N. *J. Org. Chem.* **2000**, *65*, 3218–3222.
(18) Tietze, L. F.; Gericke, K. M.; Schuberth, I. *Eur. J. Org. Chem.* **2007**, *2007*, 4563–4577.
(19) Baker, W. *J. Chem. Soc.* **1933**, *55*, 1381–1391.
(20) Mahal, H. S.; Venkataraman, K. *J. Chem. Soc.* **1934**, *56*, 1767–1769.
(21) Bonven, B. J.; Gocke, E.; Westergaard, O. *Cell* **1985**, *41*, 541–551.
(22) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B. Jr.; Stewart, L. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 15387–15392.
(23) Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. *J. Med. Chem.* **2005**, *48*, 2336–2345.
(24) Ioanoviciu, A.; Antony, S.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. *J. Med. Chem.* **2005**, *48*, 4803–4814.
(25) Drake, F. H.; Hofmann, G. A.; Bartus, H. F.; Mattern, M. R.; Crooke, S. T.; Mirabelli, C. K. *Biochemistry* **1989**, *28*, 8154–8160.
(26) Leitheiser, C. J.; Smith, K. L.; Rishel, M. J.; Hashimoto, S.; Konishi, K.; Thomas, C. J.; Li, C.; McCormick, M. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 8218–8227.
(27) Katano, K.; An, H.; Aoyagi, Y.; Overhand, M.; Sucheck, S. J.; Stevens, W. C. Jr.; Hess, C. D.; Zhou, X.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 11285–11296.
(28) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499–560.