Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline−Camptothecin Hybrids

Brian M. Fox,[†] Xiangshu Xiao,[†] Smitha Antony,[‡] Glenda Kohlhagen,[‡] Yves Pommier,[‡] Bart L. Staker,[§] Lance Stewart,[§] and Mark Cushman*,[†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892-4255, and BioStructures Group, deCODE genetics, Inc., 7869 N.E. Day Road, Bainbridge Island, Washington 98110

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The indenoisoquinolines are a novel class of topoisomerase I (top1) inhibitors that are cytotoxic in cancer cell cultures and are therefore under development as potential anticancer agents. As inhibitors of the DNA religation reaction occurring after DNA cleavage by the enzyme, they are classified as top1 poisons, similar to the camptothecins. Two strategies were employed in order to further develop the structure−activity relationships of the indenoisoquinolines and enhance their therapeutic potential. The first strategy involved the synthesis of indenoisoquinoline−camptothecin hybrid molecules to take advantage of a proposed structural analogy between the indenoisoquinolines and camptothecin. The desired hybrids were synthesized by reaction of halogenated phthalides with a dihydropyrroloquinoline. The second strategy involved the attachment of various alkenyl substituents to the C-11 position of the indenoisoquinolines, which were assumed to project into the DNA minor groove. The required C-11-substituted indenoisoquinolines were synthesized by McMurry reactions of 11-ketoindenoisoquinolines with aldehydes, and the geometries of the resulting alkenes were established by nuclear Overhauser effect difference NMR spectroscopy. All of the new indenoisoquinolines were examined for cytotoxicity in human cancer cell cultures as well as for activity vs top1. Although the indenoisoquinoline−camptothecin hybrid molecules proved to be less cytotoxic and displayed less activity against top1, an analogue incorporating a 3′-aminoalkenyl substituent at the C-11 position of the indenoisoquinoline system was significantly more potent than the prototype indenoisoquinoline in both assays. These results indicate that C-11 aminoalkyl substituents that are assumed to project into the minor groove enhance the cytotoxicity and top1 inhibitory activity of the parent indenoisoquinoline system.

Introduction

As previously described in 1978, treatment of the cis-substituted isoquinolone **1** with thionyl chloride unexpectedly afforded the indenoisoquinoline **2** instead of the acid chloride of **1**.[1] The product **2** was found to be moderately active as an anticancer agent, but its limited potency did not warrant further investigations. A later COMPARE analysis of the cytotoxicity profile of the topoisomerase I (top1) inhibitor camptothecin (**3**) revealed that it was similar to the cytotoxicity profile produced by the indenoisoquinoline **2** (referred to as NCS314622), suggesting that compound **2** might also be a top1 inhibitor.[2−4] Subsequently, the indenoisoquinoline **2** was indeed found to inhibit top1, and its ability to stabilize the "cleavage complexes" by inhibition of the DNA religation reactions after top1-catalyzed single-strand breakage classified it as a top1 "poison" as opposed to a top1 "suppressor".[2,3] In this respect, the indenoisoquinoline **2** was found to resemble camptothecin (**3**). However, the DNA single-strand breaks induced by the indenoisoquinoline **2** were more stable than those induced by camptothecin (**3**), and the cleavage site specificity of **2** was different from that of camptothecin (**3**).[2]

Although several camptothecin (**3**) derivatives such as irinotecan and topotecan are clinically useful anticancer agents, they suffer from several limitations resulting from instability due to lactone ring opening and rapid reversibility of the cleavage complexes after drug removal. Consequently, there is a present need for additional therapeutic agents that inhibit top1, like the camptothecins, but induce novel DNA cleavage patterns, have modified toxicity profiles and extended durations of action, and display different antitumor spectra relative to the camptothecins themselves. Therefore, a number of analogues of the indenoisoquinoline **2** have been synthesized with the aim of improving their anticancer and enzyme inhibitory potencies.[5−7] Among the modifications that were tried in order to achieve these goals, the replacement of the *N*-methyl group with 3-bromopropyl and 3-aminopropyl substituents produced the desired increase in potency. For example, the 3-bromopropyl analogue **4**[6] was moderately more potent than the parent compound **2**,[5] and the 3-bromopropyl

* To whom correspondence should be addressed. Phone: 765-494-1465. Fax: 765-494-1414. E-mail: cushman@pharmacy.purdue.edu.
[†] Purdue University
[‡] National Cancer Institute, NIH.
[§] deCODE Genetics, Inc.

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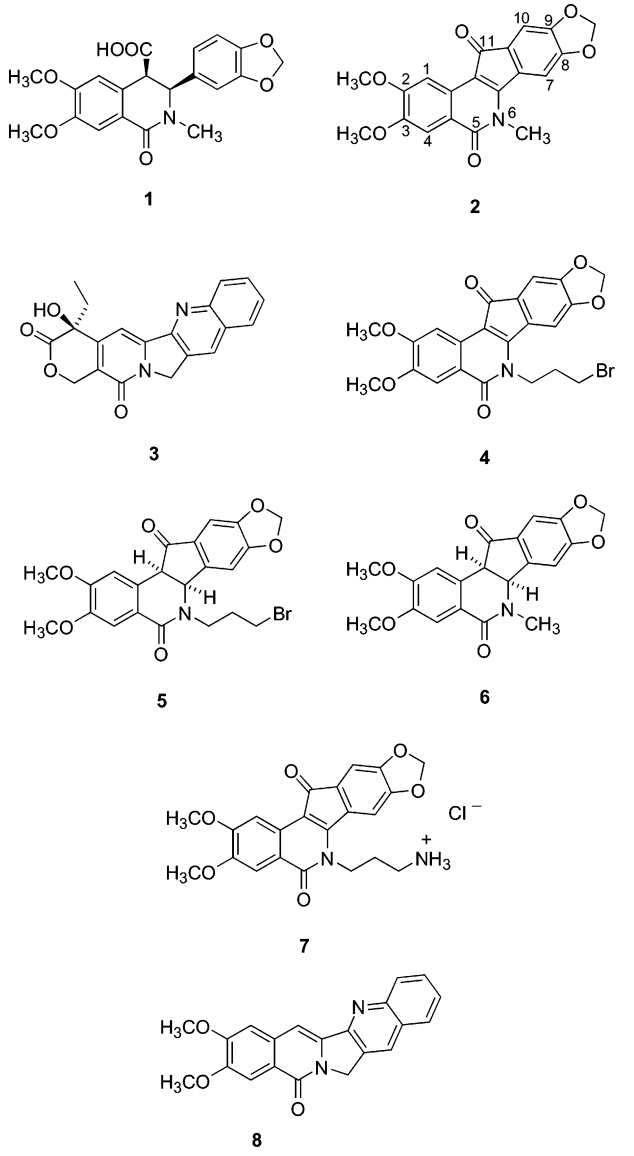


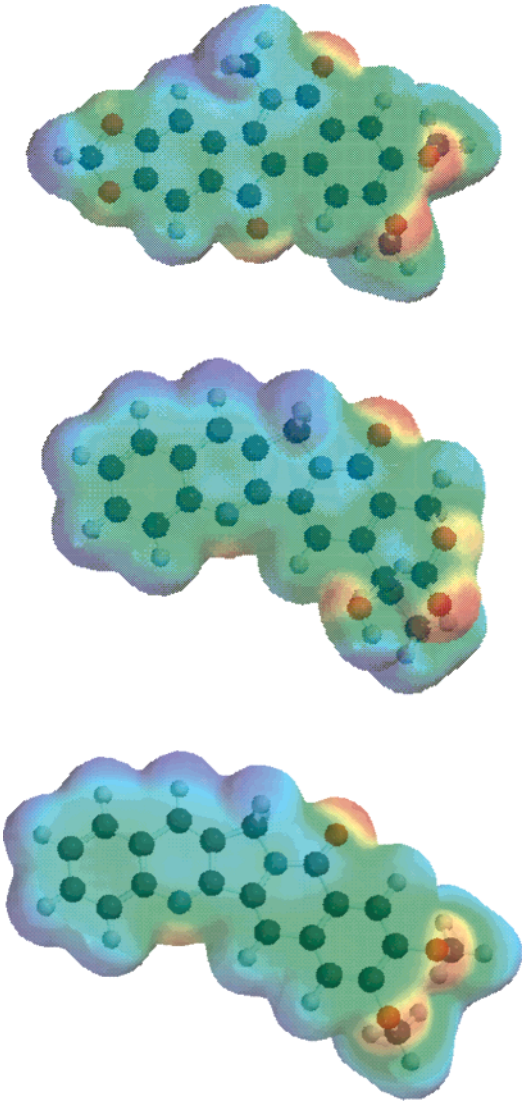


Figure 1. Electrostatic potential surfaces for indenoisoquinoline **2** (top), camptothecin **3** (middle), and the hybrid structure **8** (bottom). The values are color-coded onto the total electron density surface, with colors toward red indicating electron-rich regions of the molecule.

derivative **5**[7] of the cis-fused analogue **6**[5] was significantly more potent than **6** as a cytotoxic agent and as a top1 inhibitor. Likewise, the hydrochloride **7**[6] of the *N*-3-aminopropyl derivative was significantly more potent as an enzyme inhibitor and as a cytotoxic agent in cancer cell cultures than the parent compound **2**.

In the present investigation, an attempt was made to take advantage of a possible structural relationship between the lead compound **2** and camptothecin (**3**), which is drawn here in an orientation that emphasizes its resemblance to **2**. In this view, the lactam of the lead indenoisoquinoline **2** would correspond to the lactam of camptothecin (**3**), and the two methoxyl oxygens of **2** would correspond to the two lactone oxygens of **3**. We reasoned that if the structural correspondence were indeed responsible for the activity of **2**, then a more potent analogue of **2** might be synthesized by making a compound having a hybrid structure that would more closely resemble camptothecin (**3**). Compound **8** was therefore proposed as a logical hybrid structure based on the calculated electrostatic potential surfaces of **2**, **3**, and **8**, which are displayed in Figure 1.

A second strategy that was pursued involved the attachment of haloalkenyl and aminoalkenyl side chains to C-11, since, as stated above, similar substituents had already proven to be effective in enhancing the anticancer activity and top1 inhibitory activity when attached to the lactam nitrogen of the indenoisoquinolines. Prior DNA unwinding studies had indicated that *N*-3-aminoalkyl derivatives of the indenoisoquinoline ring system can intercalate.[6] If one assumes a similar orientation of the indenoisoquinoline **7** relative to DNA as was observed in the recently published X-ray structure of the topotecan ternary complex,[8] taking into consideration the structural analogy between the two ligands discussed above, the hypothetical model shown in Figure 2 can be constructed. In this proposal, the cationic side chain on the indenoisoquinoline nitrogen would project into the major groove toward the Asn352 residue of the protein. According to this model, the attachment of alkenyl side chains using the C-11 ketone as a reactive functional group would afford indenoisoquinolines having side chains that would project into the minor groove, toward the Arg364 and Asp533 residues of the enzyme. The present study was therefore undertaken in order to determine the biological effects

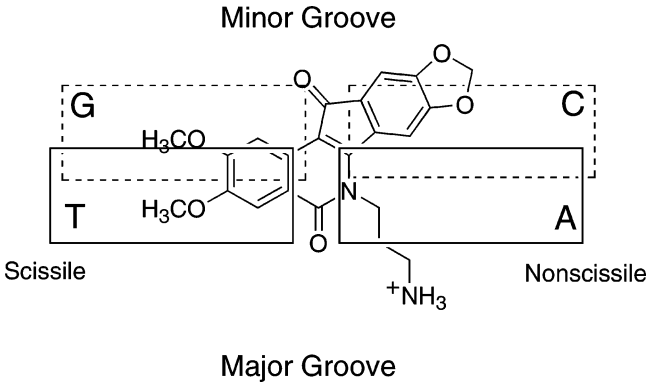
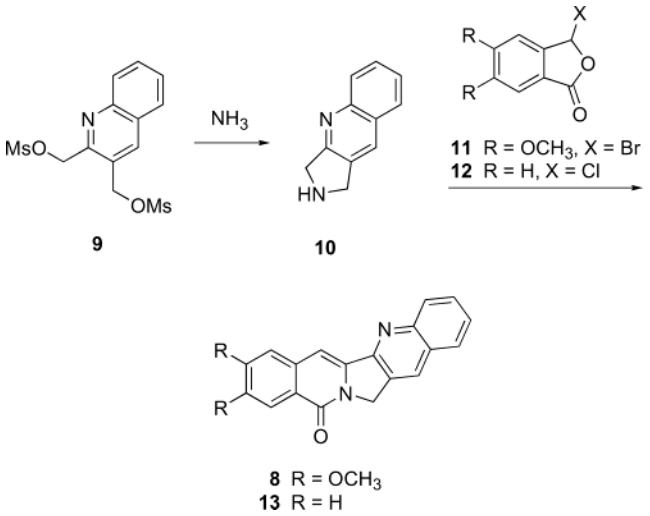


Figure 2. Hypothetical model of the orientation of indenoisoquinoline **7** relative to DNA in the ternary complex containing top1, DNA, and the inhibitor **7**.

Scheme 1



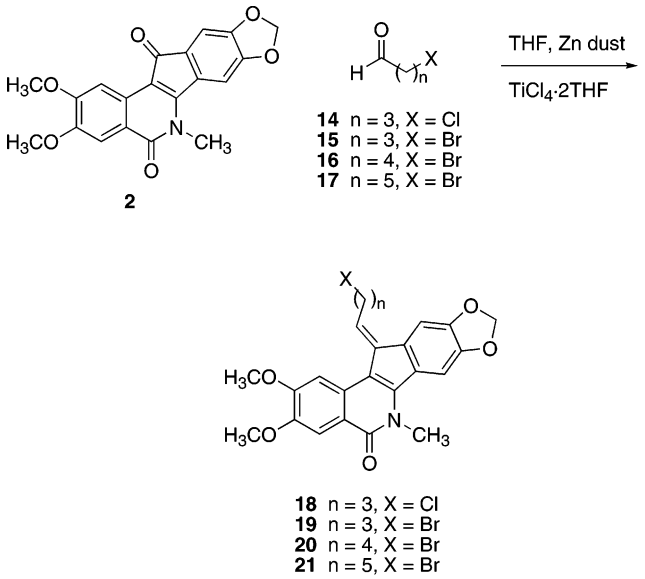
resulting from the introduction of a variety of substituents at C-11 that would presumably project into the minor groove of DNA.

Chemistry

Syntheses of the indenoisoquinoline−camptothecin analogue **8** and the corresponding compound **13**,[9] which lacks the two methoxyl groups of **8**, are outlined in Scheme 1. Treatment of a THF solution of the dimesylate **9**[10] with liquid ammonia afforded the 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline (**10**),[11] which was reacted in situ with bromide **11**[12] to afford the desired compound **8**. Similarly, reaction of **10** with the chloride **12**[13] yielded the corresponding unsubstituted derivative **13**.[9]

As portrayed in Scheme 2, the 11-indenoisoquinolines **18**−**21** were prepared using a McMurry reaction of the ketone **2**[1] with the haloaldehydes **14**−**17**. In each case, the production of a single double-bond isomer was observed. The stereochemistry of the double bond was determined by obtaining nuclear Overhauser effect (NOE) difference spectra of compound **19**. To determine the stereochemistry of the double bond of indenoisoquinoline **19** by NOE difference spectra, unambiguous assignments of the signals in the ^{1}H NMR spectrum of **19** (Figure 3) were essential. We were confident in assigning the H_B, H_E, L, F, G, and H protons to the resonances at 7.89, 6.86, 6.08, 3.00, 2.28, and 3.61 ppm, respectively (see Figure 3). The resonance at 7.89 ppm

Scheme 2



was assigned to H_B because this proton is adjacent to the amide carbonyl, which would deshield H_B and cause it to shift farthest downfield with respect to the other aromatic protons. The resonance at 6.68 ppm was assigned to the vinylic proton H_E because this is the only triplet that should integrate for one proton. Furthermore, the resonance at 6.08 ppm was assigned to the L protons because they are the only protons that should appear as a two-proton singlet. The assignment of protons at F, G, and H was accomplished by evaluating their chemical shifts. The protons at H are adjacent to a bromide and are farthest downfield at 3.60 ppm. The allylic protons at F are further upfield at 3.00 ppm, and finally the protons at G are farthest upfield at 2.28 ppm.

The remaining aromatic resonances were assigned using NOE difference spectrometry. Irradiation of the L protons (6.08 ppm) resulted in enhancement of the resonances at 7.39 and 7.36 ppm, corresponding to H_D and H_C. Because we know that the resonance at 7.89 ppm is for H_B, we can assign the resonance at 7.44 ppm to H_A by the process of elimination. Irradiation of H_E (6.86 ppm) resulted in a strong enhancement of the resonance at 7.44 ppm corresponding to H_A instead of H_D. Therefore, the double bond at C-11 of indenoisoquinoline **4** can be unambiguously assigned the *E* stereochemistry.

The assignments of the remainder of the resonances in the NMR spectrum of **19** (Figure 3) were accomplished in the same manner and supported the assignments made above. Specifically, irradiation of the F protons (3.00 ppm) caused an enhancement of the resonance at 7.36 ppm. Therefore, the resonance at 7.36 ppm corresponds to H_D. Through the process of elimination, the resonance at 7.39 ppm must belong to H_C. Irradiation of H_C (7.39 ppm) resulted in enhancement of the resonance at 4.040 ppm, resulting in assignment of this resonance to the protons of the methylamine K. Finally, irradiation of H_B (7.89 ppm) resulted in enhancement of the resonance at 4.023 ppm. Therefore, the resonance at 4.023 ppm corresponds to the protons at J, and by process of elimination, the resonance at 4.044 belongs to the protons at I.

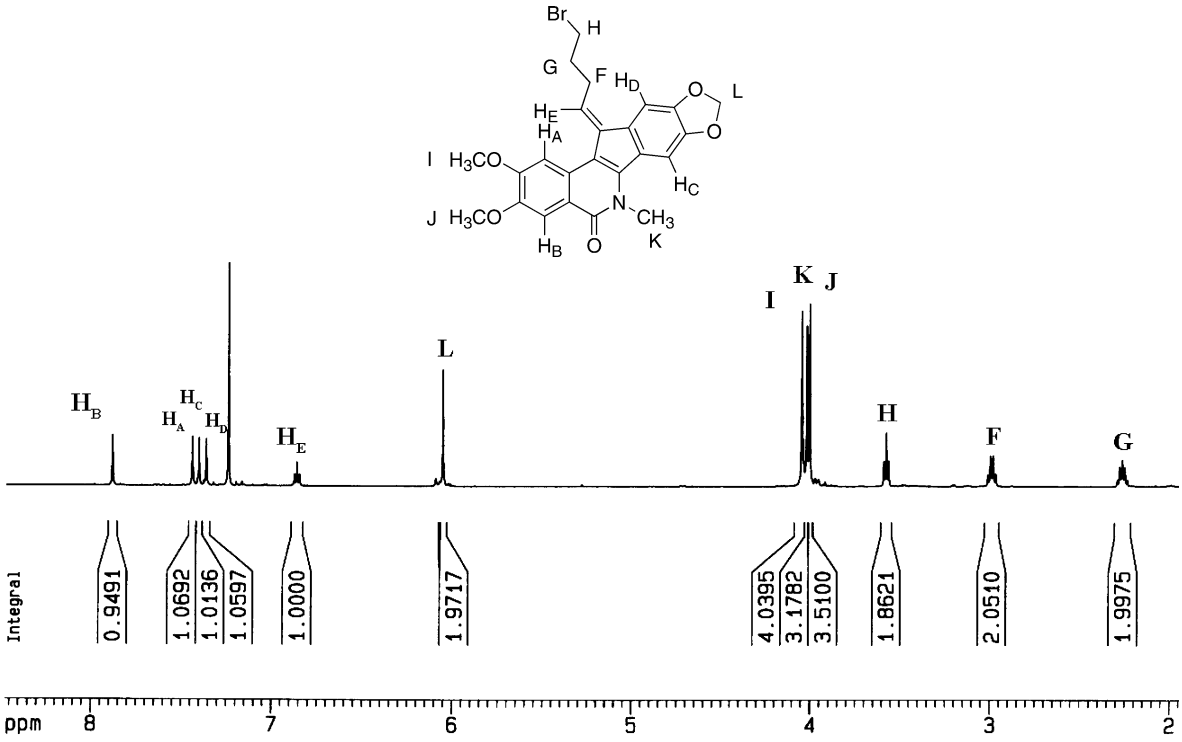
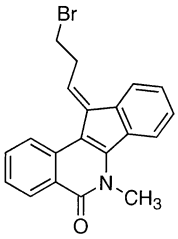


Figure 3. ^{1}H NMR spectrum of indenoisoquinoline **19**.

The fact that only one double-bond isomer having the *E* geometry is produced in each case seems reasonable because inspection of molecular models suggests that the allylic carbon is closer to C-1 in the *Z* isomer than it is to C-10 in the *E*-isomer (see structure **23** for numbering scheme). The steric clash between the hydrogens attached to the allylic carbon of the *Z* isomers with the hydrogen attached to the C-1 carbon atom should therefore raise its energy relative to the *E* isomers and make the *Z* isomers less accessible during the McMurry coupling reaction. To investigate this point further, the global energy minima of the *E* and *Z* isomers **22** and **23** were calculated with the Sybyl 6.8 program using the Tripos force field and Gasteiger–Hückel charges. The results obtained using the grid



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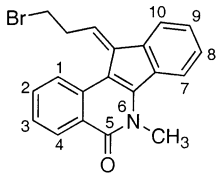

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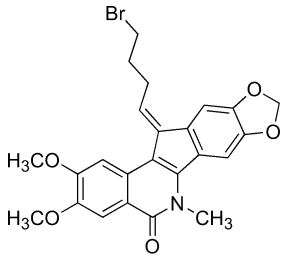
search routine indicated a global minimum energy of 32.50 kcal/mol for the *E* isomer **22** and 72.48 kcal/mol for the *Z* isomer **23**. The calculated energy differences are therefore significant and could explain why none of the *Z* isomer was detected after the McMurry reaction.

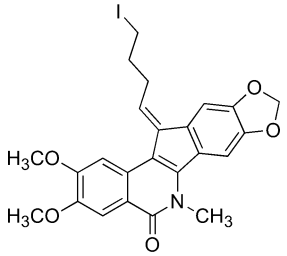
The 4-iodobutenyl compound **24** was derived from the bromide **19** using the Finkelstein reaction (Scheme 3). As with the haloalkene derivatives, the McMurry reaction of the lead compound **2** with Boc-protected β-alaninal (**25**)[14] afforded the desired 3-aminopropenyl compound **26** after acidic workup (Scheme 4).

Scheme 3



19

NaI, Me_2CO



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Biological Results and Discussion

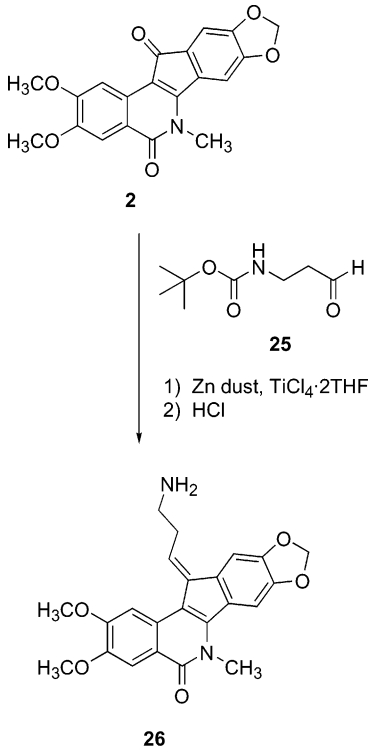
The indenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. The GI_{50} values obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a

Table 1. Cytotoxicities and Topoisomerase I Inhibitory Activities of Indenoisoquinoline Analogues

	cytotoxicity (GI_{50} in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	top1 cleavage[c]
8	>100	57.3	>100	>100	>100	>100	>100	>100	91.2	+
13	68.2	32.7	66.7	97.2	39.8	>100	>100	41.8	58.9	++
18	17.5	40.4	NT[d]	33.3	42.3	>100	35.9	>100	33.1	+
19	19.4	37.8	30.0	11.4	58.1	>100	67.8	>100	27.8	0
20	26.6	9.5	7.1	>100	>100	>100	4.5	>100	33.1	±
21	3.1	6.1	5.7	3.9	23.6	5.7	5.5	19.2	7.8	+
24	27.6	0.56	4.3	3.5	22.8	8.8	28.7	5.2	16.8	0
26	0.071	0.028	0.42	0.20	0.56	0.58	0.37	1.8	0.34	+++
2	1.3	35	41	4.2	73	68	37	96	20	++
7	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21	0.16	+++

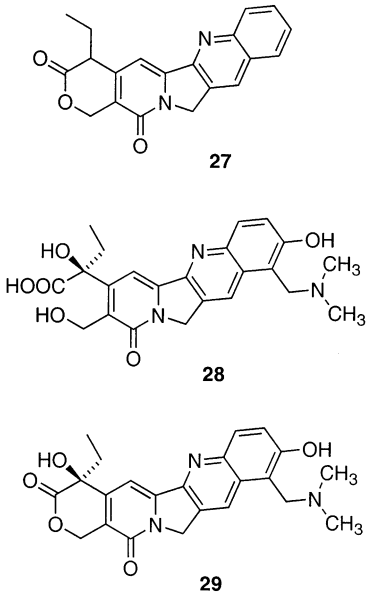
[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c] The compounds were tested at concentrations up to 10 μM. The activity of the compounds to produce top1-mediated DNA cleavage was expressed semiquantitatively as follows: +, weak activity; ++, similar activity as the parent compound **2**; +++ and ++++, greater activity than the parent compound **2**; ++++, similar activity as camptothecin **(3)**. [d] NT = not tested.

Scheme 4



calculation of the average GI_{50} for all of the cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-8}–10^{-4} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. In addition, the relative activities of the compounds in the top1-mediated DNA cleavage assay are listed in Table 1. For comparison purposes, the activities of the previously reported lead compound **2**[2] and its more potent *N*-3′-aminoalkyl derivative **7**[6] are also included in the table.

Judging from the mean graph midpoints of the indenoisoquinoline–camptothecin hybrid molecules **8** (MGM = 91.2 μM) and **13** (MGM = 58.9 μM) relative to the lead compound **2** (MGM = 20.0 μM), making the indenoisoquinolines more "camptothecin-like" was not a particularly good strategy for increasing their cytotoxicity in cancer cells, at least as evidenced by these two examples. Both compounds were generally less cytotoxic than the lead compound **2**. The hybrid molecule **13** and the lead compound **2** displayed similar potencies as top1 inhibitors. Interestingly, the pattern of top1-mediated DNA cleavage sites induced by **13** resembles more that of the lead compound **2** than that of camptothecin (data not shown). The only difference between camptothecin (**3**) and analogues **8** and **13** is the replacement of the lactone ring of camptothecin (**3**) by either a dimethoxybenzene ring in **8** or a benzene ring in **13**. A recently documented hydrogen-bonding interaction between the hydroxyl group of the camptothecin analogue topotecan and the carboxyl group of Asp533 of the enzyme contributes to the binding of the camptothecin ring system, and an analogous interaction is not possible with **8** and **13**, which may help to explain why these analogues are not as potent as camptothecin.[8] The importance of the hydroxyl group for camptothecin activity is also emphasized by the lack of activity displayed by 20-deoxycamptothecin (**27**) in a variety of biological systems.[15–18] In addition, the recently published X-ray crystallography studies show that the carboxylate and hydroxyl groups of the ring-opened hydroxy acid form of the lactone **28** of topotecan (**29**) also contribute to its binding in the ternary complex,[8] and these interactions would obviously not be possible with **8** and **13**.



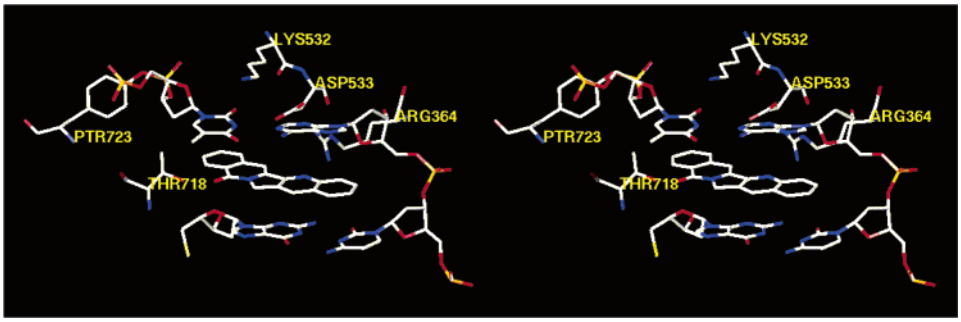


Figure 4. Model of the binding of the camptothecin−indenoisoquinoline hybrid **13** in the ternary complex consisting of DNA, top1, and the inhibitor. The diagram is programmed for wall-eyed viewing.

To investigate whether steric factors might play a role in the lower potency of **13**, a model was constructed by overlapping the structure of the hybrid **13** with the structure of topotecan in the published ternary complex and then deleting the camptothecin structure (Figure 4).[8] The synthetic double-stranded DNA in the crystalline ternary complex contained a phosphorothiolate at the cleavage site.[8] As shown in Figure 4, the lower activity of **13**, in comparison to camptothecin, as a top1 inhibitor does not appear to be due to steric factors, since it can be modeled into the camptothecin binding site in the ternary complex without any obvious steric constraints. This result re-emphasizes the importance of the hydroxylated lactone moiety in camptothecin (**3**), since the only difference in the structure of the hybrid **13** relative to **3** is the replacement of the substituted lactone ring of **3** with a benzene ring in **13**.

Turning to the halogenated 11-alkenyl side chain derivatives **18**−**21** and **24**, they were all approximately in the same range of cytotoxicity as the lead compound **2**, although the analogues **18**−**20** were slightly less cytotoxic, while compounds **21** and **24** were slightly more cytotoxic than **2**. The four-carbon alkenyl halides **19** and **24** were inactive as top1 inhibitors, while the five-carbon bromide **20** and the six-carbon bromide **21** showed increasing activity against top1. However, both **20** and **21** were less potent as top1 inhibitors than the lead compound **2**.

The most potent compound in the present series of new indenoisoquinolines, both in terms of cytotoxicity and top1 inhibitory activity, was the 11-(3′-aminopropenyl) derivative **26**. The introduction of the 11-(3′-aminopropenyl) substituent into the lead compound **2** resulted in a decrease in the cytotoxicity MGM from 20 to 0.34 μM, indicating a large increase in overall cytotoxicity in cancer cell cultures. In addition, the resulting compound **26** was more potent as a top1 poison than **2**. These effects on activity are very close to what was previously observed upon replacement of the *N*-methyl group of the lead compound **2** with a 3′-aminopropyl substituent, resulting in compound **7**.[6] In the present case, the 3′-aminopropenyl side chain is assumed to project into the minor groove, where it could interact with Asp533 or Arg364 or with a stacked base residue of the DNA.[8] The enhanced cytotoxicities of the amino compounds **26** and **7** may also be due to their altered solubility properties, facilitated cellular uptake, and/or the electrostatic attraction of the positively charged ammonium cation to the negatively charged DNA phosphodiester backbone prior to intercalation into the cleavage complex.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. Infrared spectra were obtained using $CHCl_3$ as the solvent unless otherwise specified. Except where noted, ^{1}H NMR spectra were obtained using $CDCl_3$ as solvent and TMS as internal standard. ^{1}H NMR spectra were determined at 300 MHz. Microanalyses were performed at the Purdue University Microanalysis Laboratory. Analytical thin-layer chromatography was carried out on Analtech silica gel GF 1000 μm glass plates. Compounds were visualized with short-wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

8,9-Dimethoxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (8). A solution of **9**[10] (200 mg, 0.58 mmol) in anhydrous THF (20 mL) was degassed by bubbling argon through the solution for 30 min. Liquid NH_3 was added via coldfinger for 5 min at approximately 1 drop per 5 s. The coldfinger was removed, and the reaction mixture was allowed to warm to room temperature under argon. The reaction mixture was stirred at room temperature for 12 h, at which point argon was bubbled through the solution for 1.5 h to remove excess NH_3. Anhydrous THF (10 mL) and NEt_3 (3 mL) were added, and the reaction mixture was stirred at room temperature for 30 min. Bromide **11**[12] was added, and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo and replaced with 10% NaOAc/AcOH (30 mL). The reaction mixture was stirred at room temperature for 24 h, at which point the solvent was removed in vacuo. The resulting solid was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were pooled, washed with saturated aqueous $NaHCO_3$ (1 × 100 mL) and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a brown solid. Purification (silica gel, $CHCl_3$) provided **8** (106 mg, 53%) as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (s, 1 H), 8.12 (m, 2 H), 7.84 (m, 1 H), 7.71 (s, 1 H), 7.82 (m, 1 H), 7.61 (s, 1 H), 7.50 (s, 1 H), 5.32 (s, 2 H), 3.94 (s, 3 H), 3.91 (s, 3 H); ESIMS *m/z* (rel intensity) 345.2 (100, MH^+). Anal. ($C_{21}H_{16}N_2O_3 \cdot 0.5H_2O$) C, H, N.

12*H*-5,11a-Diazadibenzo[*b,h*]fluoren-11-one (13). A solution of **9**[10] (200 mg, 0.58 mmol) in anhydrous THF (20 mL) was degassed by bubbling argon through the solution for 30 min. Liquid NH_3 was added via coldfinger for 5 min at approximately 1 drop per 5 s. The coldfinger was removed, and the reaction mixture was allowed to warm to room temperature under argon. The reaction mixture was stirred at room temperature for 12 h, at which point argon was bubbled through the solution for 1.5 h to remove excess NH_3, affording a solution of intermediate **10**. Anhydrous THF (10 mL) and NEt_3 (3 mL) were added, and the reaction mixture was stirred at room temperature for 30 min. Chloride **12**[13] (195 mg, 1.16 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo and replaced with 10% NaOAc/AcOH (30 mL). The reaction mixture was stirred at room temperature for 24 h, at which point the solvent was removed in vacuo. The resulting solid was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were pooled, washed with saturated aqueous $NaHCO_3$ (1 × 100 mL) and brine, dried

($MgSO_4$), filtered, and concentrated in vacuo to provide a brown solid. Purification (silica gel, $CHCl_3$) provided **13** (90 mg, 55%) as a yellow solid: IR (film) 3062, 2952, 2839, 1714, 1619, 1566, 1456, 1438, 1256, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 7.56 Hz, 1 H), 8.34 (s, 1 H), 8.23 (d, J = 8.64 Hz, 1 H), 7.91 (d, J = 8.19 Hz, 1 H), 7.90−7.71 (m, 3 H), 7.68 (s, 1 H), 7.66−7.56 (m, 2 H), 5.38 (d, J = 1.07 Hz, 2 H); ESIMS m/z (rel intensity) 285.2 (100, MH^+). Anal. ($C_{19}H_{12}N_2O\cdot 0.25H_2O$) C, H, N.

11-(4′-Chlorobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (18). $TiCl_4$·2THF (508 mg, 1.52 mmol), Zn dust (199 mg, 3.04 mmol), and dry THF (15 mL) were added to a flame-dried two-necked flask equipped with a magnetic stir bar and reflux condenser. The suspension was heated at reflux under argon for 3 h, after which a solution of 4-chlorobutanal (**14**)[19] (108.1 mg, 1.01 mmol) and indenoisoquinoline **2**[1] (185 mg, 0.51 mmol) in dry THF (15 mL) was introduced by syringe. The reaction mixture was heated at reflux for 2.5 h, after which 4 N HCl (20 mL) was added. The solution was stirred at room temperature for 1 h and then allowed to stand for 3 h. The resulting orange precipitate was collected by vacuum filtration. The solid was purified by flash chromatography (silica gel, 5:1 $CHCl_3$/hexanes) to provide **18** (96.1 mg, 43%) as an orange solid: mp 196−201 °C; IR (film) 2926, 1636, 1610, 1517, 1483, 1255, 1034 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1 H), 7.78 (s, 1 H), 7.40 (s, 1 H), 7.37 (s, 1 H), 6.88 (t, J = 7.19 Hz, 1 H), 6.04 (s, 2 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.02 (s, 3 H), 3.74 (t, J = 6.32 Hz, 2 H), 3.00 (dt, J = 7.30 and 7.45 Hz, 2 H), 2.20 (qn, J = 6.90 Hz, 2 H); ESIMS m/z (rel intensity) 440.7 (100, MH^+), 442.6 (43, MH^+). Anal. ($C_{24}H_{22}ClNO_5$) C, H, N.

11-(4′-Bromobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (19). A 100 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, septa, and argon line was charged with zinc dust (537 mg, 8.21 mmol) and was flame-dried. THF (30 mL) and a 1 M solution of $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added. The suspension was heated at reflux for 5 h, at which point a suspension of **2**[1] (500 mg, 1.37 mmol) and 4-bromobutanal (**15**)[20,21] (413 mg, 2.74 mmol) in THF (30 mL) was added by pipet. The reaction mixture was heated at reflux for 1 h, and then the reaction was quenched with 4 N HCl (40 mL). The solution was stirred for 1 h and then cooled at 0 °C for 2 h. The orange precipitate was collected by vacuum filtration to provide an orange solid. This was purified by flash chromatography (silica gel, $CHCl_3$) to provide **19** (161.6 mg, 30%) as an orange solid: mp 197−199 °C; IR (film) 2921, 1610, 1517, 1483, 1381, 1296, 1253, 1207, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.44 (s, 1 H), 7.40 (s, 1 H), 7.36 (s, 1 H), 6.83 (t, J = 7.11 Hz, 1 H), 6.05 (s, 2 H), 4.00 (s, 6 H), 3.97 (s, 3 H), 3.57 (t, J = 6.33 Hz, 2 H), 2.97 (q, J = 7.23 Hz, 2 H), 2.25 (qn, J = 6.80 Hz, 2 H); ESIMS m/z (rel intensity) 486.2 (97, MH^+), 484.2 (100, MH^+). Anal. ($C_{24}H_{22}BrNO_5$) C, H, N.

11-(5′-Bromopentylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (20). A 100 mL two-necked round-bottomed flask equipped with a reflux condenser and magnetic stir bar was charged with zinc dust (537 mg, 8.21 mmol) and flame-dried. THF (30 mL) and 1 M $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added to the round-bottomed flask, and the mixture was heated at reflux for 6 h. THF (30 mL), 5-bromopentanal (**16**)[21] (452 mg, 2.74 mmol), and **2**[1] (500 mg 1.37 mmol) were added to the reaction mixture, which was heated at reflux for 2 h. The reaction mixture was cooled to room temperature, 4 N HCl (40 mL) was added, and this mixture was stirred for 30 min and cooled in a −20 °C freezer overnight. The resulting yellow precipitate was collected by vacuum filtration and purified by flash chromatography to provide **20** (133.7 mg, 33%) as an orange solid: mp 196−197 °C; IR (film) 2932, 1632, 1612, 1517, 1483, 1254, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.89 (s, 1 H), 7.46 (s, 1 H), 7.41 (s, 1 H), 7.32 (s, 1 H), 6.86 (t, J = 6.83 Hz, 1 H), 6.05 (s, 2 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.01 (s, 3 H), 3.49 (t, J = 6.47 Hz, 2 H), 2.86 (q, J = 7.15 Hz, 2 H), 2.06 (m, 2 H), 1.88 (m, 2 H); ESIMS m/z (rel intensity) 500.2 (95, MH^+), 498.2 (100, MH^+). Anal. ($C_{25}H_{24}BrNO_5$) C, H, N.

11-(6′-Bromohexylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (21). A 100 mL two-necked round-bottomed flask equipped with a reflux condenser and magnetic stir bar was charged with zinc dust (537 mg, 8.21 mmol) and flame-dried. THF (30 mL) and 1 M $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added to the round-bottomed flask, and the mixture was heated at reflux for 4 h. Anhydrous THF (30 mL), 6-bromohexanal (**17**)[21] (491 mg, 2.74 mmol), and **2**[1] (500 mg 1.37 mmol) were added to the reaction mixture, which was heated at reflux for 2 h. The reaction mixture was cooled to room temperature, 4 N HCl (40 mL) was added, and this mixture was stirred for 30 min and then cooled in a −20 °C freezer overnight. The resulting yellow precipitate was collected by vacuum filtration and purified by flash chromatography to provide **21** (118.0 mg, 17%) as an orange solid: mp 182−185 °C; IR (film) 2929, 1636, 1610, 1516, 1482, 1296, 1255, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.46 (s, 1 H), 7.40 (s, 1 H), 7.32 (s, 1 H), 6.88 (t, J = 6.96 Hz, 1 H), 6.05 (s, 2 H), 4.06 (s, 3 H), 4.02 (s, 3 H), 4.00 (s, 3 H), 3.44 (t, J = 6.62 Hz, 2 H), 2.84 (q, J = 7.13 Hz, 2 H), 1.95 (qn, J = 7.04 Hz, 2 H), 1.74 (m, 2 H), 1.66 (m, 2 H); ESIMS m/z (rel intensity) 514.2 (100, MH^+), 512.2 (91, MH^+). Anal. ($C_{26}H_{26}BrNO_5$) C, H, N.

11-(4′-Iodobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (24). NaI (217 mg, 1.45 mmol) was added to a suspension of bromide **19** (70 mg, 0.15 mmol) in acetone (15 mL). The reaction mixture was heated at reflux for 12 h, after which the resulting orange precipitate was collected by vacuum filtration and purified by flash chromatography (silica gel, $CHCl_3$) to provide **24** (73.0 mg, 95%) as an orange solid: mp 173−174.5 °C; IR (KBr) 2944, 1612, 1522, 1481, 1254, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.43 (s, 1 H), 7.40 (s, 1 H), 7.36 (s, 1 H), 6.84 (t, J = 7.22 Hz, 1 H), 6.05 (s, 2 H), 4.08 (s, 3 H), 4.04 (s, 3 H), 4.00 (s, 3 H), 3.35 (t, J = 6.69 Hz, 2 H), 2.95 (q, J = 7.32 Hz, 2 H), 2.19 (m, 2 H); ESIMS m/z (rel intensity) 532.1 (100, MH^+). Anal. ($C_{24}H_{22}INO_5$) C, H, N.

11-(3′-Aminopropylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (26). $TiCl_4$−THF (1:2) complex (730 mg, 2.19 mmol) and zinc dust (284 mg, 4.37 mmol) were put in a three-necked round-bottomed flask. THF (30 mL) was added. The resulting suspension was heated under reflux for 4 h. At this point, a mixture of aldehyde **25**[14] (189 mg, 1.09 mmol) and indenoisoquinoline **2** (266 mg, 0.73 mmol) in THF (30 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 4 h. Then 3 N HCl (10 mL) was added after the mixture was cooled to room temperature. The mixture was stirred at room temperature for 1 h, followed by 0 °C for 2 h, and finally at room temperature overnight. The mixture was cooled to 0 °C, and solid $NaHCO_3$ was added to neutralize HCl. The solvents were evaporated, and the residue was subjected to flash chromatography, eluting with $CHCl_3$−MeOH (4:1) to provide **26** as a yellow powder (121 mg, 41%): mp >180 °C (dec); ^1H NMR (300 MHz, DMSO-d_6) 7.68 (s, 1 H), 7.62 (s, 1 H), 7.51 (s, 1 H), 7.48 (s, 1 H), 6.94 (t, J = 6.0 Hz, 1 H), 6.15 (s, 2 H), 4.01 (s, 3 H), 3.95 (s, 3 H), 3.87 (s, 3 H), 3.12−3.18 (m, 4 H). ESIMS m/z (rel intensity) 407.0 (100, MH^+). Anal. ($C_{23}H_{22}N_2O_5\cdot 0.9CHCl_3$) C, H, N.

Top1-Mediated DNA Cleavage Reactions. Human recombinant top1 was purified from Baculovirus as described previously.[22] The 161 bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (50 μL reaction mixtures) for 1 h at 37 °C and separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161 bp fragment was eluted from the gel slice using the QIAEX II kit (QIAGEN Inc., Valencia, CA). Approximately 200 ng of the fragment was

3′-end-labeled at the Hind III site by fill-in reaction with [α-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 units of DNA polymerase I (Klenow fragment). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled 161 bp fragment was collected. Aliquots (approximately 50 000 dpm/reaction) were incubated with top1 at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration).[2] The samples (10 μL) were mixed with 30 μL of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in denaturing gels (16% polyacrylamide, 7 M urea). Gels were dried and visualized by using a phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

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