Synthesis and Anticancer Activity of Simplified Indenoisoquinoline Topoisomerase I Inhibitors Lacking Substituents on the Aromatic Rings

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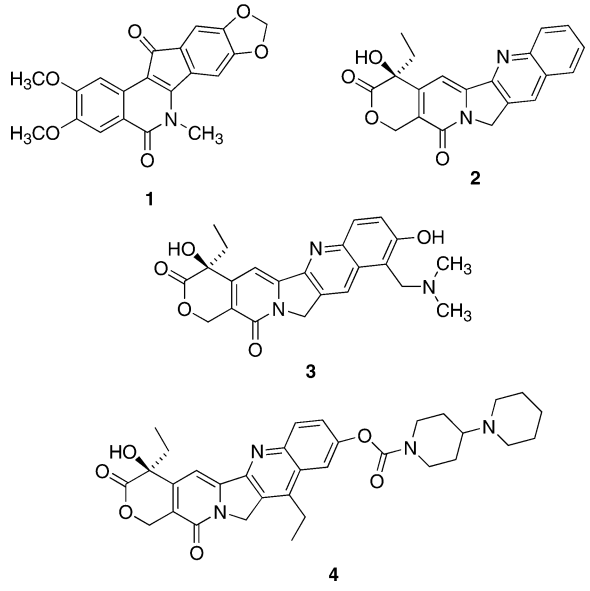
The indenoisoquinolines are a class of cytotoxic topoisomerase I inhibitors that offer certain advantages over the camptothecins, including the greater stabilities of the compounds themselves, as well as the greater stabilities of their drug−enzyme−DNA cleavage complexes. To investigate the possible biological roles of the di(methoxy) and methylenedioxy substituents present on the aromatic rings of the previously synthesized indenoisoquinoline topoisomerase I inhibitors, a series of compounds lacking these substituents was synthesized and tested for both cytotoxicity in cancer cell cultures and for enzyme inhibitory activity. The results indicate that the aromatic substituents make a small, but consistently observable contribution to the biological activity. Molecular models derived for the binding of the unsubstituted indenoisoquinolines in ternary complex with DNA and topoisomerase I indicate that the substituents on the lactam nitrogen project out of the major groove, and the carbonyl group is directed out of the minor groove, where it is involved in a hydrogen bonding interaction with the side chain guanidine group of Arg364. The DNA cleavage patterns observed in the presence of topoisomerase I and various indenoisoquinolines were similar, although significant differences were detected. There were also variations in the DNA cleavage pattern seen with camptothecin vs the indenoisoquinolines, which indicates that these two classes of topoisomerase I inhibitors are likely to target the cancer cell genome differently, resulting in different spectra of anticancer activity. The most cytotoxic of the presently synthesized indenoisoquinolines has a 4-amino-*n*-butyl group on the lactam nitrogen.

Introduction

The topoisomerase I (top1) inhibitory activity of NSC 314622 (**1**)[1] was discovered after a COMPARE analysis of its cytotoxicity profile revealed a strong resemblance to that of other known top1 inhibitors, including camptothecin (**2**) and the clinically useful anticancer drug topotecan (**3**).[2] The indenoisoquinoline **1**, like **2** and **3**, stabilizes DNA-top1 cleavage complexes by intercalating at the DNA cleavage site, resulting in inhibition of the religation reaction.[2−4] These inhibitors are therefore classified as top1 "poisons" as opposed to top1 "suppressors", which inhibit the DNA cleavage reaction. However, there are differences in the biological activities of NSC 314622 (**1**) in comparison with camptothecin (**2**) and the clinically useful anticancer drugs topotecan (**3**) and irinotecan (**4**) that warrant further development of the indenoisoquinolines. First, the DNA cleavage site specificity of **1** is different from that of camptothecin (**2**), so different genes could be targeted and provide a different antitumor spectrum.[2] The cytotoxic activity of topoisomerase inhibitors is indeed dependent on the production of DNA breaks, which inhibit (damage) certain regions of the genome in a sequence-specific or sequence-dependent manner, and which activate death



1 **2** **3** **4**

response pathways by forming irreparable DNA lesions and/or inducing apoptosis. It is known that the clinically useful topoisomerase II (top2) inhibitors have preferential activity for different cancers, and it can be expected that different top1 inhibitors will display different spectra of anticancer activity as well.[5] Second, the cleavage complexes induced by the indenoisoquinoline **1** are more stable than those formed in the presence

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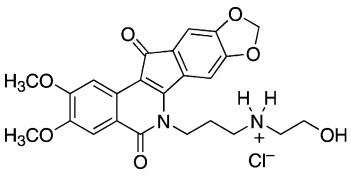
of camptothecin (**2**).[2] The reversibility of camptothecin-induced cleavage complexes imposes long infusion times for maximum activity, so noncamptothecin top1 inhibitors with enhanced cleavage complex stabilities may offer the advantage of shorter administration times.[6] For the camptothecins, SN-38 produces more stable cleavage complexes and is also more active as an antitumor agent in tumor models. Third, the camptothecins, in contrast to the indenoisoquinolines, are chemically unstable due to lactone ring opening to form an inactive hydroxy acid.[7] The lactone is in equilibrium with the hydroxy acid, but the equilibrium is shifted toward the inactive hydroxy acid as the carboxylate of the ring-open form binds tightly to serum albumin. Although these reasons recommend the further development of the top1 inhibitor NSC 314622 (**1**) as an anticancer agent, the usefulness of compound **1** itself is limited by its moderate potency, both as a cytotoxic agent in cancer cells and as a top1 inhibitor.[8] Therefore, a number of additional indenoisoquinolines related to **1** have been synthesized and evaluated, with most of the more active compounds having di(methoxy) or methylenedioxy substituents on the two aromatic rings.[8−11]

The present investigation was undertaken in order to determine whether a simplified system lacking the usual di(methoxy) and methylenedioxy substituents on the two aromatic rings of the indenoisoquinoline system could retain significant cytotoxicity and top1 inhibitory activity. A limited number of compounds fitting this description were reported early on, and they were found to be generally less active than the parent compound **1**, or to have comparably moderate activity.[8,12] In the meantime, several developments have occurred that have led to a reinvestigation of the series of compounds lacking aromatic substituents. A crystal structure of an intercalation complex containing topotecan (**3**), human top1, and duplex DNA has been reported.[4] Models of various indenoisoquinolines in ternary complex with top1 and cleaved duplex DNA have been generated using the reported coordinates of the topotecan complex, assuming that the lactam rings of both systems are oriented similarly in their cleavage complexes.[11] These models do not suggest any critical role of the substituents on the two aromatic rings of the indenoisoquinolines that would preclude binding of their unsubstituted analogues. An additional development is that a number of indenoisoquinolines have been synthesized that contain aminoalkyl substituents on the lactam nitrogen, and some of these compounds have displayed top1 inhibitory activity commensurate with that of camptothecin. In addition, they are very cytotoxic in cancer cells. For example, the indenoisoquinoline **5**, also known as MJ-III-65 or NSC 706744, has a mean-graph midpoint in the NCI cytotoxicity screen of 0.11 μM.[9] Furthermore, the rate constant for cleavage complex reversal with indenoisoquinoline **5** was recently determined to be about one-fourth that of camptothecin at 25 °C, while the rate constant for cleavage complex formation with **5** was approximately twice that of camptothecin.[13] Compound **5** also displayed activity vs camptothecin-resistant topoisomerases I.[13] The results suggest that the simplified indenoisoquinolines lacking aromatic substituents could hypothetically be potent top1 inhibitors and cytotoxic agents if the right substitutions were made on the lactam nitrogen. This hypothesis is also supported by the antineoplastic activity reported for oracin (**6**), which has been reported to induce G2 cell cycle arrest and apoptosis in Burkitt's lymphoma cells.[14−19]



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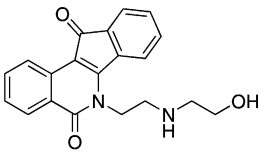

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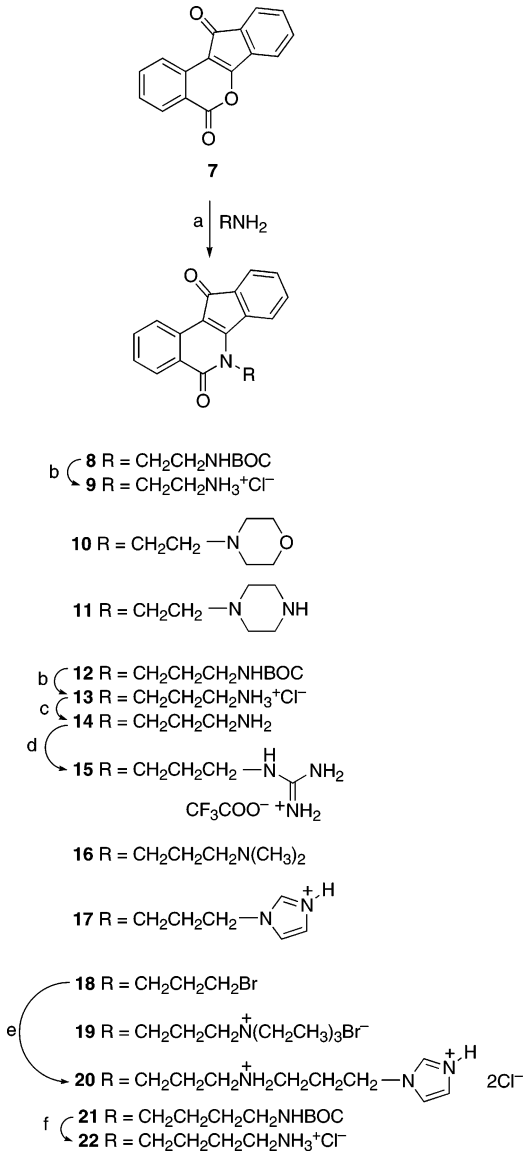
Chemistry

The indenoisoquinolines **8**−**22** were synthesized directly or indirectly by reacting commercially available benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**7**) with various primary amines as outlined in Scheme 1. Treatment of chloroform solutions of the BOC-protected amines **8**, **12**, and **21** with hydrochloric acid in ether resulted in their conversions to the deprotected amine hydrochlorides **9**, **13**, and **22**, respectively. The guanidine trifluoroacetate derivative **15** resulted from treatment of the free base **14** with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-pseudourea and mercuric chloride in aqueous THF, followed by trifluoroacetic acid. The bromide **18**[9] was obtained along with the triethylammonium salt **19** after reaction of **7** with bromopropylamine hydrobromide and triethylamine in chloroform. A nucleophilic displacement reaction between the bromide **18**[9] and 1-(3-aminopropyl)-imidazole yielded the expected product, which was isolated as the di(hydrochloride) salt **20**. The remaining products displayed in Scheme 1 were obtained directly through treatment of lactone **7** with the required primary amines.

Three indenoisoquinolines **31**, **32**, and **33**, which are unsubstituted on the isoquinolone part of the indenoisoquinoline ring system, but substituted with methylenedioxy on the indenone moiety, were obtained as outlined in Scheme 2. This synthesis relies on the condensation of Schiff bases with homophthalic anhydrides as the key step to afford substituted isoquinolines.[20] Treatment of piperonal (**23**) with ethylamine, propylamine, or 3-bromopropylamine afforded the corresponding Schiff bases **24**, **25**, and **26**, which were reacted with homophthalic anhydride (**27**) to afford the substituted isoquinolones **28**, **29**, and **30**. The 6−7 Hz coupling constant observed for the two methine protons in the ^{1}H NMR spectra of the products **28**−**30** is consistent with the assigned cis stereochemistry.[21] In contrast, these two protons usually appear as broad singlets in the ^{1}H NMR spectra of the trans diastereomers in closely related systems.[21] The (methylenedioxy)phenyl ring and the carboxyl group are both pseudoaxial due to A$^{(1,2)}$ strain in the trans diastereomers, involving a nonbonded interaction between the *N*-alkyl and the adjacent phenyl substituents, which results in both methine protons being predominantly pseudoequatorial.[22,23] In the cis diastereomers, one pseudoaxial proton (most likely at C-4) and one pseudoequatorial proton (most likely at C-3) are present. The desired indenoisoquinolines **31**, **32**, and **33** were obtained after treatment of **28**, **29**, and **30** with thionyl

Scheme 1



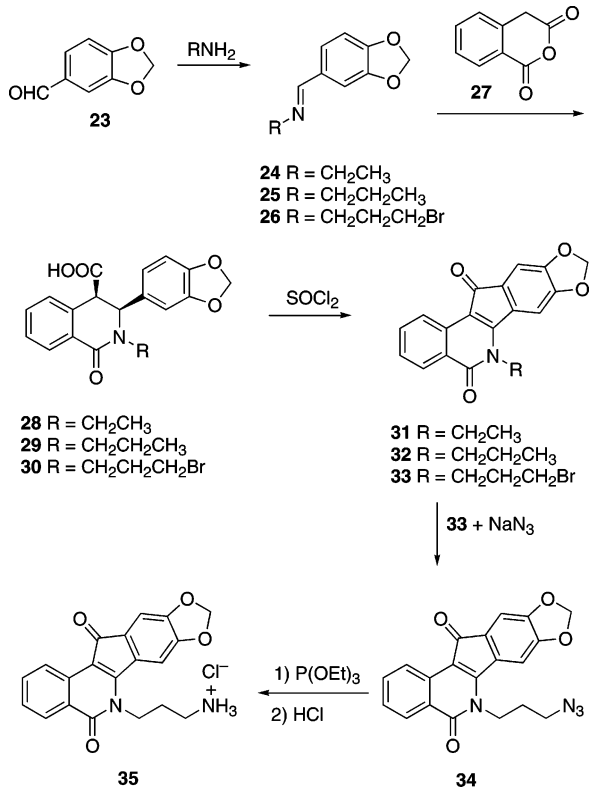
[a] Reagents and conditions: (a) $CHCl_3$, 23 °C; (b) HCl, $CHCl_3$, Et_2O, 23 °C (72 h); (c) NaOH, Et_2O; (d) 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-pseudourea, $HgCl_2$, THF, 50 °C (2 h), 23 °C (5 h); (e) 1-(3-aminopropyl)imidazole, K_2CO_3, 1,4-dioxane, 100 °C (4 h); (f) HCl, $CHCl_3$, Et_2O, 23 °C (5 h).

chloride.[1] Nucleophilic displacement of the bromide from **33** with azide afforded intermediate **34**, which was converted to the amine using the Staudinger reduction.[24] The amine was isolated as its hydrochloride salt **35**.

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 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} to 10^{-4} M) are

Scheme 2



taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. Therefore, the MGM value represents an overall assessment of toxicity of the compound across numerous cell lines. For comparison purposes, the activities of the previously reported lead compound **1**[8] and its more potent derivative **5**[9] are also included in the table, along with the cytotoxicity data for camptothecin (**2**). The relative potencies of the compounds in the production of topoisomerase I-mediated DNA cleavage are also listed. The activity of the compounds to produce top1-mediated DNA cleavage was expressed semiquantitatively as follows: +: weak activity; ++: similar activity as the parent compound **1**; +++ and ++++: greater activity than the parent compound **1**; ++++: similar activity as 1 μM camptothecin.

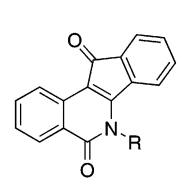
It is important to note that as an approximate average cytotoxicity, the MGM value can be misleading, because it fails to indicate the degree of selective cytotoxicity in particular cell lines. For example, it is possible that a particular drug scaffold may be highly effective for many cell lines and yet have a high MGM due to low cytotoxicity in a relatively small number of cell lines. Conversely, a particular compound may be of interest because it is highly cytotoxic in one cell line or a small number of cell lines, and still have a high MGM. Therefore, it is important to look at the individual cytotoxicity measurements for each cell line with each compound. For example, compound **15** is significantly more cytotoxic than **11** in the SN12C renal cancer cell line, yet **15** has a higher MGM value than **11** (Table 1).

Several of our previously synthesized compounds are included in Table 1 for the sake of comparison with the presently reported top1 inhibitors. These include the indenoisoquinolines **36**,[9] **37**,[9] **38**,[9] **39**,[11] **40**,[9] **41**,[9] **42**,[11] and **43**.[8] The most cytotoxic of the previously reported

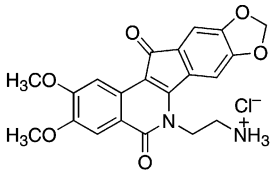
Table 1. Cytotoxicities and Topoisomerase I Inhibitory Activities of Indenoisoquinoline Analogues

	cytotoxicity (GI_{50} in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNSS F-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	top1 cleavage[c]
1	1.3	35	41	4.2	73	68	37	96	20.0	++
2	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.04	0.0405 ± 0.0187	++++
5	0.02	0.10	0.04	0.03	0.35	>0.01	>0.01	0.79	0.11 ± 0.05	++++
6	1.62	1.12	1.65	1.42	3.85	0.95	1.28	2.56	1.90 ± 0.80	+
9	0.62	0.27	0.21	0.92	0.71	0.49	0.76	0.92	0.53 ± 0.32	+++
10	>100	36.3	85.1	29.5	81.3	93.3	>100	>100	67.6	++++
11	1.91	0.58	1.99	1.38	1.12	2.19	3.09	1.95	1.86	+
13	0.20	0.18	0.25	0.26	1.38	0.16	0.22	0.78	0.32 ± 0.23	+++
15	0.69	22.4	0.37	11.0	18.2	0.23	7.59	25.7	9.77	++++
16	1.74	0.58	1.86	0.51	1.7	0.91	1.32	2.82	1.86	+++
17	2.69	1.41	2.34	0.79	1.66	1.66	1.41	2.75	1.86	+++++
20	0.41	1.58	1.07	6.92	1.78	2.00	0.41	1.45	1.00 ± 0.31	++
22	0.08	0.10	0.10	0.05	0.52	0.04	0.01	0.84	0.16 ± 0.01	+++
31	36.3	95.5	40.7	20.0	89.1	>100	>100	>100	51.3	+++
32	>100	30.2	>100	>100	20.9	>100	>100	>100	66.1	++
33	0.69	22.4	0.37	11.0	18.2	0.23	7.59	25.7	9.77	+
35	0.28	0.68	0.43	0.18	1.45	0.19	0.06	1.82	0.25 ± 0.05	+
36	3.81	3.86	2.24	8.08	5.87	4.39	3.50	3.70	4.37 ± 0.65	±
37	5.62	4.95	12.2	18.2	74.2	16.4	16.4	76.7	14.5 ± 2.48	++
38	4.89	3.64	52.5	16.3	28.8	6.49	9.56	39.7	10.3 ± 2.56	++
39	0.58	0.068	1.3	0.14	0.86	0.36	0.40	1.04	0.34 ± 0.11	++++
40	0.19	0.35	2.93	1.27	0.85	0.43	0.89	1.05	0.62	++
41	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21	0.16 ± 0.12	+++
42	0.018	0.12	0.19	0.54	1.4	0.9	0.14	0.65	0.35	++++
43	2.2	2.6	2.0	2.1	3.0	3.6	2.3	2.6	2.4	±

[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 ranging 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 **1**; +++ and ++++: greater activity than the parent compound **1**; ++++: similar activity as 1 μM camptothecin.



36 R = CH_2CH_2Cl
37 R = CH_2CH_2OH
38 R = $CH_2CH_2CH_2OH$



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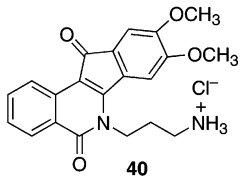

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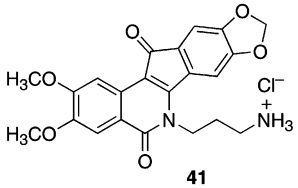

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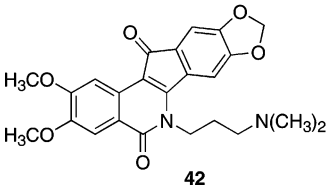

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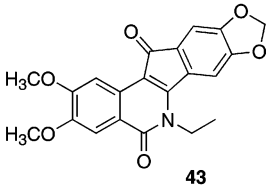

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indenoisoquinolines lacking substituents on the two aromatic rings was compound **36**, which displayed an MGM value of 4.4 μM and a relative top1 inhibitory potency of (±) (Table 1). On the other hand, the two most potent top1 inhibitors of the previously reported indenoisoquinolines lacking substituents on the two aromatic rings were **37**, which displayed a relative potency of (++), and its higher homologue **38**, having a 3-hydroxypropyl substituent on the nitrogen, which also showed top1 inhibitory potency of (++).[12] Compounds **37** and **38** had MGMs of 14.5 μM and 10.3 μM, respectively. The two most cytotoxic of the presently synthesized indenoisoquinolines lacking aromatic substituents are **22** (MGM 0.16 μM), having a 4-amino-*n*-butyl substituent on the lactam nitrogen, and its lower homologue **13** (MGM 0.32 μM). The most potent top1 inhibitor of the new indenoisoquinolines was the 3-imidazolyl-1-propyl compound **17**, having a relative top1 inhibitory potency of (+++++). It can therefore be concluded that there is nothing inherent in the indenoisoquinolines lacking aromatic substituents that would preclude potent cytotoxicity and top1 inhibitory activity. Very cytotoxic agents and potent top1 inhibitors can be obtained provided the right substituents are present on the lactam nitrogen. However, obviously, in this particular set of indenoisoquinolines, cytotoxicity and top1 inhibitory activity are not maximized in the same compound.

The reasons for the enhanced cytotoxicities of indenoisoquinolines bearing aminoalkyl substituents are not clear. It is possible that the positively charged ammonium cations of these indenoisoquinolines increase affinity for DNA by electrostatic attraction to the negatively charged phosphodiester linkages of DNA prior to intercalation as has been established with polyamine derivatives related to spermine and spermidine.[25−28] Another possibility is that the aminoalkyl derivatives of the indenoisoquinolines could be actively transported into cells similarly to the polyamines.[25−31]

Several cases allow the comparison of substituted and unsubstituted indenoisoquinolines having the same substituents on the lactam nitrogen. For example, comparison of the unsubstituted 2-aminoethyl compound **9** (MGM 0.53, top 1 +++) with the substituted

compound **39** (MGM 0.34 μM, top1 ++++)[11] documents a relatively small contribution of the methylenedioxy and di(methoxy) substituents to the biological activity in this case. A similar trend can be observed in the comparison of the unsubstituted compound **13** (MGM 0.32 μM, top1 +++) with the substituted compound **41** (MGM 0.16 μM, top1 +++).[9] Likewise, the unsubstituted indenoisoquinoline **16** (MGM 1.86 μM, top1 +++), having a 3-dimethylaminopropyl substituent on the lactam nitrogen, is less active than the corresponding substituted compound **42** (MGM 0.35 μM, top1 ++++).[11] In all of these cases, a similar trend is observed indicating a small, but discernible and consistent contribution of the two methoxy and methylenedioxy groups to the cytotoxicity.

Given the trend toward small increases in cytotoxicity with greater substitution on the two aromatic rings, it was of interest to see how compound **35**, having an intermediate level of substitution, would fare in the cytotoxicity assay. Interestingly, the MGM of **35** (0.25 μM) is intermediate between the unsubstituted compound **13** (MGM 0.35 μM) and the fully substituted compound **41** (MGM 0.16 μM).[9] Although the differences are very small, the same general trend is observed in this case as well. It is also interesting that the *N*-ethyl compound **31** (MGM 51.3 μM), having an intermediate level of substitution, is less active than the fully substituted *N*-ethyl compound **43** (MGM 2.4 μM).[8]

The availability of the 2-aminoethyl-substituted unsubstituted indenoisoquinoline **9** (MGM 0.53 μM, top1 +++) and its higher homologues **13** (MGM 0.32 μM, top1 +++) and **22** (MGM 0.16 μM, top1 +++) make an examination of the effect of lengthening the aminoalkyl side chain straightforward. The trend toward a slight increase in cytotoxicity as the length of the aminoalkyl side chain is lengthened is apparent.

The primary amino group at the end of the aminoalkyl chain attached to the lactam nitrogen seems to confer the highest cytotoxic activity. Conversion of the primary amino group of **13** (MGM 0.32 μM, top1 +++) to the dimethylamino group present in **16** (MGM 1.86 μM, top1 +++) resulted in a decrease in cytotoxicity that is in the same direction as that observed in going from the 3-aminopropyl compound **41**[9] (MGM 0.16 μM) to the corresponding dimethylamino analogue **42** (MGM 0.35 μM). Likewise, the incorporation of the amino group of **13** into an imidazole system (**17**, MGM 1.86 μM, top1 +++++) or a guanidine system (**15**, MGM 9.77 μM, top1 ++++) resulted in a decrease in cytotoxicity, even though both of those modifications increased the top1 inhibitory potency. The lack of correlation between the cytotoxicity and top1 inhibitory activity of the derivatives tested (Table 1) suggests a different mechanism of action than top1 inhibition in some of the cases. A similar finding was reported previously with anthracene and acridine polyamine conjugates and topoisomerase II inhibition.[32] Overall, the results demonstrate that there is not an exact correlation between cytotoxicity in cancer cell cultures and top1 inhibitory activity. Differences in cellular uptake, distribution within the cell, and additional targets within the cell may all play a role, thus making the correlation of cytotoxicity with top1 inhibitory activity less than perfect. To investigate the possibility of additional biological targets in more detail, the cytotoxicities of several indenoisoquinolines were investigated in top1-deficient P388/CPT45 cells.[33] The four compounds tested (**11**, **13**, **35**, and **42**) did show antiproliferative activity in the top1-deficient P388/CPT45 cells, implicating the existence of additional mechanisms for cell growth inhibition besides top1 targeting. However, the cells were partially resistant to compounds **11** and **13**, indicating that top1 contributes at least in part to the antiproliferative activity, while compound **42** was associated with considerable resistance, indicating that top1 is likely to be the main target. The top1-deficient cells were not resistant to indenoisoquinoline **35**, which is consistent with the potent cytotoxicity observed for this compound in the presence of relatively weak top1-inhibitory activity (Table 1).

All of the compounds were examined for induction of DNA cleavage in the 3′-end-labeled *Pvu*II/*Hind*III fragment of pBluescript SK(−) phagemid DNA in the presence of top1.[2] The resulting cleavage patterns of some of the most potent indenoisoquinolines are displayed in Figure 1. The results were compared with camptothecin (**2**) and the lead compound **1** (NSC 314622). Some, but not all, of the DNA cleavage sites observed with the indenoisoquinolines were different from each other, and there were also some differences relative to camptothecin. For example, the camptothecin band at site 37 was not seen with the indenoisoquinolines in Figure 1, and the band at site 44 was observed with the indenoisoquinolines but not with camptothecin. Also, the bands observed for cleavage at identical sites varied in intensity among the indenoisoquinolines, as well as in comparison with camptothecin. These differences are important because they indicate that different cancer cell genes could be targeted more selectively with one indenoisoquinoline vs another indenoisoquinoline. Also, similar to the situation with other anticancer drugs that share a single target (e.g. top2 and tubulin inhibitors), it can be expected that different top1 inhibitors will have different spectra of antitumor activity.[34] Similar conclusions have been reached in prior top1-DNA cleavage studies involving other indenoisoquinolines.[8,9,35,36] Finally, some of the cleavage bands were more intense at intermediate drug concentrations, but became more faint at higher concentrations. For example, very little cleavage was seen at the highest concentrations of the indenoisoquinoline **35**, even though the bands at lower concentrations were relatively intense. This could be due to inhibition of the cleavage activity of the enzyme at higher drug concentrations, or alternatively, to intercalation of the drug into DNA at higher concentrations, unwinding the DNA and thereby making it a poorer substrate for the DNA-cleaving activity of the enzyme.

As mentioned above, prior molecular modeling of the ternary complexes formed between various di(methoxy)- and methylenedioxy-substituted indenoisoquinolines, DNA, and top1 has not revealed any critical role of these indenoisoquinoline substituents that would necessarily detract from the binding of the presently reported indenoisoquinolines lacking these substituents.[10,11] To investigate this question in more detail, a molecular model has been constructed of the binding of the most potent of the presently reported unsubstituted indeno-

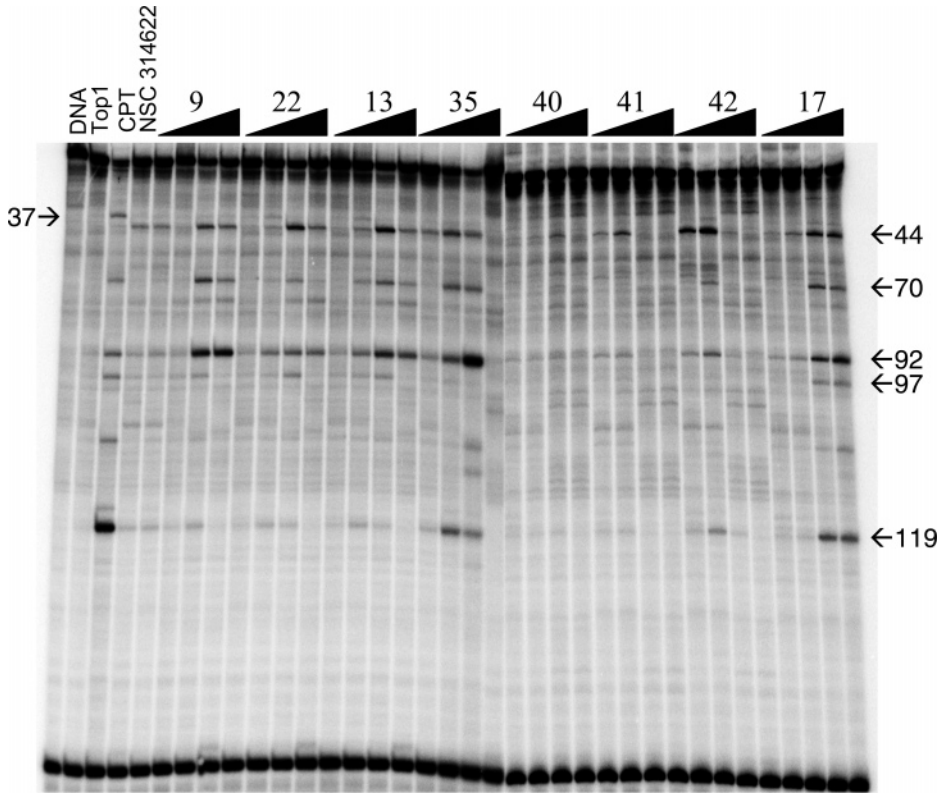

Figure 1. Comparison of the top1-mediated DNA cleavages at different drug concentrations. The DNA used corresponds to the 3′-end-labeled PvuII/HindIII fragment of pBluescript SK (−) phagemid DNA. The four concentrations of the inhibitors used were 0.1, 1.0, 10, and 100 μM. Reactions were performed at room temperature for 30 min and stopped by adding 0.5% SDS. DNA fragments were separated on 16% polyacrylamide gels. Top1 was present in all reaction mixtures except in the control lane. Control: DNA with neither top1 nor any drug. The figure is comprised of two gels, one for compounds **9**, **22**, **13**, and **35**, and another one for **40**, **41**, **42**, and **17**, that are placed side by side to facilitate comparison.

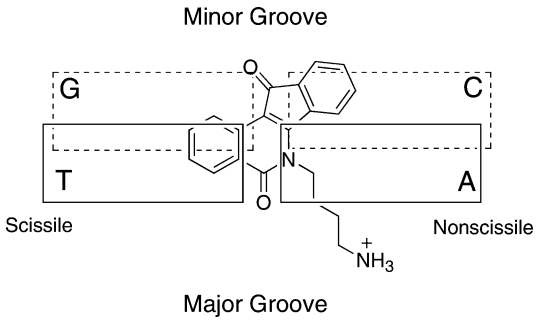

Figure 2. Hypothetical model of the orientation of indenoisoquinoline **22** relative to DNA in the ternary complex containing top1, DNA, and the inhibitor **22**.

isoquinolines, the primary amine **22**, in ternary complex with DNA and top1. To create the model, the first important decision to be made is how to orient the indenoisoquinoline with respect to the DNA. If one makes the assumption that the lactam moiety of the indenoisoquinoline **22** is oriented in the ternary complex similarly to the lactam of the clinically useful camptothecin derivative topotecan (**3**),[4] then the orientation of **22** with respect to DNA would project the aminobutyl substituent out of the duplex toward the major groove, and the ketone could point toward the minor groove (Figure 2). Using this orientation, the hypothetical model shown in Figure 3 can be fashioned starting from the published coordinates of the topotecan ternary complex.[4] To build the model, the structure of **22** was first overlapped with that of topotecan (**2**) with the lactam rings superimposed. The structure of topotecan (**2**) was then deleted, and the energy of the new indenoisoquinoline ternary complex was minimized with Sybyl, employing the MMFF94s force field and MMFF94 charges. During energy minimization, the structures of DNA, the protein, and the surrounding water molecules were frozen, and the indenoisoquinoline **22** was allowed to move. As shown in Figure 3, the indenoisoquinoline ketone moiety of the energy-minimized structure is within hydrogen bonding distance (2.2 Å) to the closest nitrogen of the guanidine group of Arg364 in the minor groove. No other important hydrogen bonding contacts of the indenoisoquinoline with the surrounding nucleic acid or protein structure are evident. As with topotecan (**2**), it is assumed that a major stabilizing force of the inhibitor on the ternary complex is its stacking interactions with the neighboring DNA bases.[4] The aminobutyl substituent is hydrogen bonded to several of the surrounding water molecules, but does not bind to the nucleic acid or protein structure. It is clear from the model that the indenoisoquinoline is capable of replacing a base pair in the DNA duplex at the cleavage site, thereby lengthening the distance between the guanine and the phosphotyrosylthymine residues and thus inhibiting the religation reaction. This would be very similar to the mechanism established for topotecan.[4]

In conclusion, a new series of indenoisoquinolines lacking aromatic substituents has been synthesized in order to probe the importance of the di(methoxy) and methylenedioxy substituents in the prior series of compounds. The results indicate that these substituents are not absolutely necessary for potent cancer cell

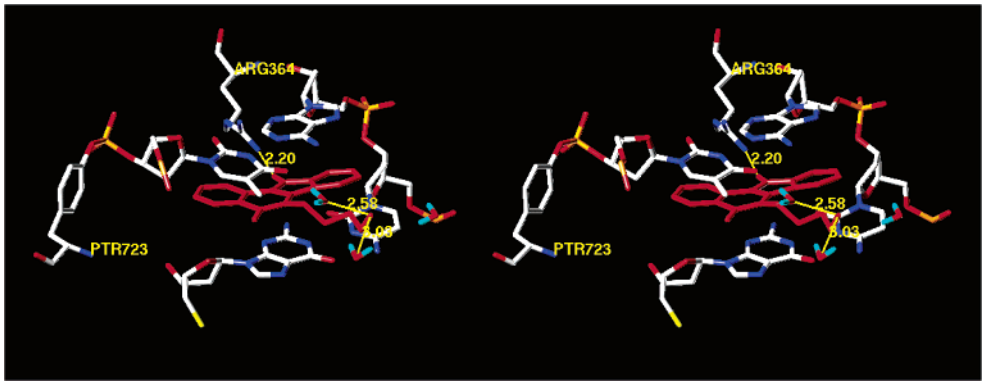

Figure 3. Hypothetical model of the binding of the indenoisoquinoline **22** in the ternary complex consisting of DNA, top1, and the inhibitor. The diagram is programmed for wall-eyed viewing.

cytotoxicity and top1 inhibitory activity, but they do consistently make a small contribution to the overall potency. Molecular modeling indicates that the unsubstituted indenoisoquinolines readily fit into ternary complexes consisting of the inhibitor, DNA, and top1, with the aminoalkyl group pointing out of the duplex into the major groove and the ketone hydrogen bonded with Arg364 in the minor groove. The unsubstituted indenoisoquinolines could offer the potential advantage of greater metabolic stability relative to their substituted counterparts, since they obviously would not be substrates for oxidative O-demethylation. Ongoing preclinical studies of the indenoisoquinolines are attempting to identify the best candidate for further development in the treatment of cancer in humans.

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, ^1H NMR spectra were obtained using $CDCl_3$ as solvent and TMS as internal standard. ^1H NMR spectra were determined at 300 or 500 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. Benz[*d*]-indeno-[1,2-*b*]pyran-5,11-dione **7** was obtained commercially from Aldrich.

6-(2-Aminoethyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline Hydrochloride (9). 2-(*tert*-BOC-amino)-ethylamine (0.366 g, 2.22 mmol) was added to a stirred solution of compound **7** (0.501 g, 2.02 mmol) in $CHCl_3$ (10 mL). The reaction mixture was stirred for 72 h. Chloroform (100 mL) was added to the reaction mixture, and the mixture was washed with distilled H_2O (3 × 25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange-red solid. Purification via flash chromatography (silica gel, chloroform and 5% MeOH/$CHCl_3$) yielded crude 5,6-dihydro-5,11-dioxo-5-(ethyl-3′-*tert*-butoxycarbamoyl)-11*H*-indeno[1,2-*c*]isoquinoline (**8**), which was used in the next step. Crude intermediate **8** (82 mg, 0.21 mmol) was dissolved in chloroform (20 mL), an anhydrous solution of HCl in ether (1.5 mL, 3 mmol) was added, and the solution was stirred for 72 h. The precipitated product was filtered, washed with chloroform, and dried over P_2O_5 to yield the desired product **9** (45 mg, 65%): mp 274−278 °C. IR (KBr pellet) 3444, 2973, 2900, 1708, 1667, 1576, 1551, 1504 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 8.59 (d, J = 8.0 Hz, 1 H), 8.24 (d, J = 8.1 Hz, 1 H), 8.06 (s, 3 H), 7.87 (m, 2 H), 7.55 (m, 4 H), 4.76 (t, J = 6.2 Hz, 2 H), 3.24 (m, 2 H); CIMS *m/z* (rel intensity) 291 (MH^+, 77), 274 ($MH^+ - NH_3^+$, 100). Anal. ($C_{18}H_{15}ClN_2O_2 \cdot 0.44H_2O$) C, H, N, Cl.

5,6-Dihydro-6-(2-*N*-morpholinyl-1-ethyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (10). 4-(2-Aminoethyl)morpholine (0.3 mL, 2.2 mmol) was added to a stirred solution of compound **7** (0.502 g, 2.01 mmol) in $CHCl_3$ (10 mL). The reaction mixture was stirred for 72 h. Chloroform (100 mL) was added, and the mixture was washed with distilled H_2O (3 × 25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange-red solid. Purification via precipitation from chloroform yielded the desired compound **10** (0.360 g, 49%): mp 193−196 °C. IR (film) 3407, 1689, 1660, 1577, 1550, 1504 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.71 (d, J = 7.78 Hz, 1 H), 8.33 (d, J = 8.1 Hz, 1 H), 7.75 (m, 3 H), 7.49 (m, 3 H), 4.70 (t, J = 7.6 Hz, 2 H), 3.72 (s, 4 H), 2.80 (s, 2 H), 2.60 (s, 4 H); CIMS *m/z* (rel intensity) 361 (MH^+, 100), 274 ($MH^+ - C_4H_8NO$, 57). Anal. Calcd. for ($C_{22}H_{20}N_2O_3 \cdot 0.125CHCl_3$) C, H, N, Cl.

5,6-Dihydro-5,11-dioxo-6-(2-*N*-piperazine-1-ethyl)-11*H*-indeno[1,2-*c*]isoquinoline (11). 1-(2-Aminoethyl)piperazine (0.3 mL, 2.2 mmol) was added to a stirred solution of compound **7** (0.495 g, 2.01 mmol) in $CHCl_3$ (10 mL). The reaction mixture stirred for 120 h. Chloroform (100 mL) was added, and the mixture was washed with distilled H_2O (3 × 25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange-red solid. Purification via flash chromatography (silica gel, 6% MeOH/$CHCl_3$) yielded the desired compound **11** (0.084 g, 12%): mp 179−185 °C. IR (film) 3399, 1694, 1663, 1575, 1550, 1504 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, J = 7.8 Hz, 1 H), 8.33 (d, J = 8.2 Hz, 1 H), 7.74 (m, 2 H), 7.63 (m, 1 H), 7.48 (m, 3 H), 4.69 (t, J = 7.6 Hz, 2 H), 2.94 (t, J = 4.7 Hz, 4 H), 2.81 (t, J = 7.9 Hz, 2 H), 2.61 (d, J = 4.3 Hz, 4 H), 1.79 (s, 1 H); CIMS *m/z* (rel intensity) 360 (MH^+, 100), 274 ($MH^+ - C_4H_9N_2$, 58). Anal. ($C_{22}H_{21}N_3O_2 \cdot 0.1CHCl_3$) C, H, N, Cl.

6-(3′-*tert*-BOC-aminopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (12). 3-(*tert*-BOC-amino)-propylamine (0.383 g, 2.22 mmol) was added to a stirred solution of compound **7** (0.500 g, 2.01 mmol) in $CHCl_3$ (10 mL). The reaction mixture stirred for 48 h. Chloroform (100 mL) was added, and the mixture was washed with distilled H_2O (3 × 25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a crude orange-red solid. Purification via flash chromatography (silica gel, chloroform) yielded the desired compound **12** (0.665 g, 81%): mp 189−190 °C. IR (film) 3362, 3065, 2977, 1697, 1576, 1550, 1504 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.72 (d, J = 8.3 Hz, 1 H), 8.34 (d, J = 8.1 Hz, 1 H), 7.74 (t, J = 8.2 Hz, 1 H), 7.63 (d, J = 6.6 Hz, 1 H), 7.54 (d, J = 7.3 Hz, 1 H), 7.46 (m, 3 H), 5.35 (s, 1 H), 4.63 (t, J = 6.6 Hz, 2 H), 3.27 (m, 2 H), 2.07 (m, 2 H), 1.46 (s, 9 H); CIMS *m/z* (rel intensity) 405 (MH^+, 17), 305 ($MH^+ - CO_2C(CH_3)_3$, 100). Anal. ($C_{24}H_{24}N_2O_4 \cdot 0.7H_2O$) C, H, N

6-(3-Aminopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline Hydrochloride (13). 6-(3′-*tert*-BOC-aminopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**12**) (0.077 g, 0.19 mmol) was dissolved in chloroform (20 mL), an anhydrous solution of 2.0 M HCl in diethyl ether

(1.5 mL, 3 mmol) was added, and the mixture was stirred for 72 h. The precipitated product was filtered, washed with chloroform, and dried over P_2O_5 for 72 h to yield the desired compound (0.065 g, 65%): mp 281−283 °C. IR (KBr pellet) 3444, 3136, 2969, 1711, 1634, 1591, 1570, 1548, 1504 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 8.59 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 8.2 Hz, 1 H), 7.93 (s, 3 H), 7.86 (t, J = 8.4 Hz, 2 H), 7.62 (m, 4 H), 4.59 (t, J = 8.7 Hz, 2 H), 3.00 (t, J = 7.2 Hz, 2 H), 2.15 (m, 2 H); CIMS *m/z* (rel intensity) 305 ($MH^+ - Cl^-$, 100), 288 ($MH^+ - NH_3{}^+Cl^-$, 52). Anal. ($C_{19}H_{17}ClN_2O_2 \cdot 0.3H_2O$) C, H, N

6-(3-Guanidinyl-1-propyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (15). A solution of 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-pseudourea (0.23 g, 0.79 mmol) in dry THF (60 mL) was added dropwise to a stirred solution of amine **14** (0.12 g, 0.40 mmol) and $HgCl_2$ (10 mg) in THF−H_2O (40 mL, 20:1, v/v) at 50 °C, and the mixture was stirred for 2 h at that temperature and further stirred at room temperature for 5 h. The reaction mixture was concentrated and diluted with $CHCl_3$ (50 mL). The organic portion was washed with 10% aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The organic residue was loaded on a silica gel column (60 g, 60−200 mesh) and eluted with a 19:1 gradient of $CHCl_3$−Et_3N mixture to give the 3-guanidinyl compound (0.14 g, 0.26 mmol, 65%) as an oil, which was further treated with neat CF_3COOH (20 mL) at room temperature for 2 h. The reaction mixture was concentrated and diluted with chloroform (50 mL), and the resulting solid was filtered through a sintered glass funnel, washed with chloroform (20 mL) and dichloromethane (20 mL), and dried to provide indenoisoquinoline **15** (110 mg, 62%) as a pale red solid: mp 232−234 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.58 (d, J = 8.3 Hz, 1 H), 8.22 (d, J = 7.8 Hz, 1 H), 7.83 (t, J = 7.0 Hz, 1 H), 7.74 (d, J = 7.5 Hz, 1 H), 7.61−7.49 (m, 4 H), 7.16 (bs, 3 H, 3 × −NH-), 4.53 (bs, 2 H), 3.36 (bs, 2 H), 2.00 (m, 2 H); ESIMS *m/z* (rel intensity) 348 (22), 347 ($MH^+ - 3 \times CF_3COOH$, 100), 288 (21). Anal. ($C_{22}H_{19}F_3N_4O_4 \cdot 1.2H_2O$) C, H, N.

5,6-Dihydro-6-(3-(dimethylamino)-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (16). 3-(Dimethylamino)-propylamine (0.25 g, 2.4 mmol) was added to a stirred solution of compound **7** (0.5 g, 2.0 mmol) in chloroform (50 mL), and the reaction mixture was stirred at room temperature for 12 h. The precipitated product was filtered off, washed with ether−chloroform (50 mL, 2:1) and dried to obtain pure indenoisoquinoline **16** (0.47 g, 70%) as an orange solid: mp 168−171 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 8.1 Hz, 1 H), 8.32−8.29 (dt, J = 0.7 and 8.1 Hz, 1 H), 7.75−7.67 (m, 2 H), 7.60 (dt, J = 1.0 and 6.6 Hz, 1 H), 7.46−7.33 (m, 4 H), 4.56 (t, J = 7.9 Hz, 2 H), 2.50 (t, J = 6.6 Hz, 2 H), 2.29 (s, 6 H), 2.07−1.98 (m, 2 H); ESIMS *m/z* (rel intensity) 334 (23), 333 (MH^+, 100), 289 (10), 288 (48). Anal. ($C_{21}H_{20}N_2O_2 \cdot 0.4CH_2Cl_2$) C, H, N.

5,6-Dihydro-6-(3-imidazolyl-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (17). 1-(3-Aminopropyl)imidazole (0.23 g, 1.8 mmol) was added to a stirred solution benz[*d*]-indeno[1,2-*b*]pyran-5,11-dione **7** (0.5 g, 2.0 mmol) in chloroform (50 mL), and the reaction mixture was stirred at room temperature for 12 h. The reddish-brown precipitate was dissolved in chloroform (150 mL), and 2 M HCl in ether (10.0 mL, 20.0 mmol) was added slowly to the reaction mixture, which was stirred at room temperature for 5 h. The product was filtered off through a sintered glass funnel, washed with chloroform (50 mL), and dried to obtain pure indenoisoquinoline hydrochloride **17** (0.65 g, 82%) as a pale red solid: mp 266−268 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 9.18 (s, 1 H), 8.57 (d, J = 8.1 Hz, 1 H), 8.20 (d, J = 8.1 Hz, 1 H), 7.85−7.80 (m, 2 H), 7.70−7.66 (m, 2 H), 7.60−7.48 (m, 4 H), 4.55 (t, J = 6.6 Hz, 2 H), 4.40 (t, J = 7.2 Hz, 2 H), 2.39 (m, 2 H); ESIMS *m/z* (rel intensity) 357 (23), 356 ($MH^+ - HCl$, 100), 289 (9), 288 (45). Anal. ($C_{22}H_{18}ClN_3O_2 \cdot 0.4H_2O$) C, H, N.

6-(3-Bromo-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18) and 5,6-Dihydro-6-[(3-triethylammonium)-1-propyl]-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Bromide (19). Triethylamine (0.85 mL, 6.1 mmol) was added to a stirred solution of 3-bromopropylamine hydrobromide (0.53 g, 2.4 mmol) in chloroform (100 mL), and the reaction mixture was stirred at room temperature for 30 min. Then compound **7** (0.5 g, 2.0 mmol) was added to the reaction mixture at room temperature, and the reaction mixture was stirred at the same temperature for 60 h. The crude reaction product was directly loaded on a silica gel column (60−210 mesh, 70 g) and eluted with a 0−1% gradient of methanol in chloroform to afford indenoisoquinoline bromide **18**[9] (0.32 g, 43%) and indenoisoquinoline ammonium bromide **19** (0.46 g, 49%) as orange solids. Compound **19**: mp 142−144 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, J = 8.3 Hz, 1 H), 8.33 (d, J = 7.5 Hz, 1 H), 7.75−7.66 (m, 2 H), 7.63−7.60 (dd, J = 1.0 and 6.7 Hz, 1 H), 7.49−7.35 (m, 3 H), 4.70 (t, J = 6.6 Hz, 2 H), 3.70 (q, J = 5.9 Hz, 2 H), 3.16−3.07 (m, 6 H), 2.16−2.08 (m, 2 H), 1.42 (t, J = 7.3 Hz, 9 H); ESIMS *m/z* (rel intensity) 389 ($MH^+ - Br$, 19), 364 (15), 363 (MH^+,-CH_2CH_3, 100), 347 (31). Anal. ($C_{25}H_{29}N_2O_2Br \cdot 0.2CHCl_3$) C, H, N.

5,6-Dihydro-6-[3-(3-imidazolylpropyl)amino-1-propyl]-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Di(hydrochloride) (20). A mixture of indenoisoquinoline bromide **18** (0.25 g, 0.68 mmol), 1-(3-aminopropyl)imidazole (0.43 g, 3.4 mmol), and anhydrous K_2CO_3 (0.28 g, 2.0 mmol) in anhydrous 1,4-dioxane (20 mL) was heated to 100 °C and kept at that temperature for 4 h. The reaction mixture was cooled and concentrated on a rotary evaporator. The reaction mixture was diluted with chloroform (100 mL), washed with 1% HCl solution (50 mL), water, and brine, and dried (Na_2SO_4). The reaction mixture was concentrated, loaded on a silica gel column, and eluted with a 0−5% gradient of methanol in chloroform to provide indenoisoquinoline (180 mg, 0.44 mmol, 64%), which was further treated with 2 M HCl in ether (4.4 mL, 8.80 mmol) in chloroform (30 mL) at room temperature for 4 h to afford indenoisoquinoline hydrochloride **20** (170 mg, 80%) as a pale orange solid: mp 264−266 °C (dec). ^1H NMR (300 MHz, DMSO-d_6) δ 9.09 (bs, 2 H, −NH- and imidazole H), 8.59 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 9.0 Hz, 1 H), 7.83 (d, J = 7.0 Hz, 1 H), 7.76 (bs, 1 H), 7.61−7.52 (m, 5 H), 4.58 (bs, 2 H), 4.32 (bs, 2 H), 3.08 (bs, 2 H), 2.87 (bs, 2 H), 2.19 (bs, 4 H); ESIMS *m/z* (rel intensity) 414 (27), 413 ($MH^+ - 2 \times HCl$, 100), 346 (16), 345 (63), 289 (12), 288 (58). Anal. ($C_{25}H_{26}Cl_2N_4O_2 \cdot 0.5CHCl_3$) C, H, N.

6-[(4-*tert*-BOC-amino)-5,6-dihydro-1-butyl]-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (21). 4-(*tert*-BOC-amino)-butylamine (0.5 g, 2.7 mmol) was added to a stirred solution of compound **7** (0.6 g, 2.4 mmol) in chloroform (50 mL), and the reaction mixture was stirred at room temperature for 12 h. The reaction product was directly loaded on a silica gel column (60−210 mesh, 70 g) and eluted with a 0−1% gradient of methanol in chloroform to afford indenoisoquinoline **21** (0.91 g, 78%) as a orange solid: mp 158−160 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 8.1 Hz, 1 H), 8.30 (d, J = 8.0 Hz, 1 H), 7.70 (t, J = 7.3 Hz, 1 H), 7.60 (d, J = 6.2 Hz, 1 H), 7.44−7.34 (m, 4 H), 4.66 (bs, 1 H, −NH), 4.51 (t, J = 7.3 Hz, 2 H), 3.21 (m, 2 H), 1.92−1.87 (m, 2 H), 1.73−1.68 (m, 2 H), 1.41 (s, 9 H); ESIMS *m/z* (rel intensity) 442 (23), 441 (MNa^+, 100), 419 (MH^+, 24), 320 (11), 319 ($MH^+ - Boc$, 56), 311 (48), 302 (28). Anal. ($C_{25}H_{26}N_2O_4$) C, H, N.

6-(4-Amino-1-butyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline Hydrochloride (22). Boc-protected indenoisoquinoline **21** (0.5 g, 1.2 mmol) was dissolved in chloroform (50 mL), and an anhydrous solution of 2.0 M HCl in ether (2 M, 11.5 mL, 23.0 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. The precipitated product was filtered off, washed with chloroform (50 mL), and dried over P_2O_5 for 24 h to afford pure indenoisoquinoline hydrochloride **22** (0.38 g, 88%) as a red solid: mp 268−270 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.55 (d, J = 8.0 Hz, 1 H), 8.20 (d, J = 8.3 Hz, 1 H), 7.93 (bs, 2 H, $-NH_2$), 7.81 (t, J = 7.6 Hz, 1 H), 7.72 (d, J = 7.3 Hz, 1 H), 7.61−7.47 (m, 4 H), 4.51 (bs, 2 H), 2.82 (bs, 2 H), 1.85 (bs, 2 H), 1.72 (m, 2 H); ESIMS *m/z* (rel intensity) 320 (22), 319 ($MH^+ - HCl$, 100). Anal. ($C_{20}H_{19}N_2O_2Cl \cdot 0.5H_2O$) C, H, N.

***cis*-4-Carboxy-*N*-ethyl-3,4-dihydro-3-(3,4-methylenedioxyphenyl)-1-(2,*H*)-isoquinolone (28).** Imine **24**[8] (2.73 g, 15.4 mmol) was placed in a 100 mL round-bottomed flask and dissolved in $CHCl_3$ (25 mL). The solution was cooled in an ice bath to 0 °C. Homophthalic anhydride **(27)** (2.50 g, 15.4 mmol) was added to the mixture over 1 h in portions every 20 min. The mixture was then allowed to return to room temperature while being stirred for 12 h. The solid was filtered off and purified by precipitation from $CHCl_3$, producing **28** as a white solid (0.273 g, 5%): mp 178−180 °C. IR (film) 2979, 1735, 1627, 1490, 1253, 1039 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.99 (d, J = 6.6 Hz, 1 H), 7.55 (d, J = 7.5 Hz, 1 H), 7.50 (dt, J = 1.5 and 7.5 Hz, 1 H), 7.42 (t, J = 6.9 Hz, 1 H), 6.74 (d, J = 8.1 Hz, 1 H), 6.53 (dd, J = 1.5 and 7.8 Hz, 1 H), 6.43 (d, J = 1.5 Hz, 1 H), 5.92 (s, 2 H), 5.06 (d, J = 6.3 Hz, 1 H), 4.63 (d, J = 6.3 Hz, 1 H), 3.76 (m, 1 H), 3.00 (m, 1 H), 1.03 (t, J = 7.0 Hz, 3 H); ESIMS *m/z* (rel intensity) 340 (MH^+, 100). Anal. ($C_{19}H_{17}NO_5 \cdot 0.13CHCl_3$) C, H, N.

***cis*-4-Carboxy-*N*-propyl-3,4-dihydro-3-(3,4-methylenedioxyphenyl)-1-(2,*H*)-isoquinolone (29).** Imine **25**[37] (2.88 g, 15.1 mmol) was placed in a 250 mL round-bottomed flask and dissolved in $CHCl_3$ (75 mL). Homophthalic anhydride **(27)** (2.44 g, 15.1 mmol) was added to the mixture in one portion. The mixture was then stirred at room temperature for 16 h and concentrated to ~35 mL and the product precipitated upon addition of hexanes (20 mL). The solid was filtered off and washed with hexanes (50 mL) and diethyl ether (50 mL), producing **29** as an off-white solid (2.65 g, 50%): mp 175−177 °C. IR (film) 2966, 1743, 1621, 1598, 1572, 1505, 1489, 1445, 1252, 1039, and 749 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.25 (dd, J = 6.6 and 1.7 Hz, 1 H), 7.53−1.45 (m, 3 H), 6.63 (d, J = 8.0 Hz, 1 H), 6.56 (dd, J = 8.0 and 1.83 Hz, 1 H), 6.44 (d, J = 1.7 Hz, 1 H), 5.89 (dd, J = 5.1 and 1.4 Hz, 2 H), 4.96 (d, J = 6.3 Hz, 1 H), 4.73 (d, J = 6.3 Hz, 1 H), 4.01−3.93 (m, 1 H), 2.90−2.82 (m, 1 H), 1.71−1.61 (m, 2 H), 0.97 (t, J = 7.3 Hz, 3 H); CIMS *m/z* (rel intensity) 354 (MH^+, 100). Anal. ($C_{20}H_{19}NO_5 \cdot 0.11CHCl_3$) C, H, N

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (30).** Imine **26**[38] (2.50 g, 9.26 mmol) was added to a 100 mL round-bottomed flask along with $CHCl_3$ (75 mL). The solution was cooled in an ice bath to 0 °C. Homophthalic anhydride (**27**) (2.00 g, 9.26 mmol) was added to the mixture over 1 h in portions every 20 min. The mixture was then allowed to return to room temperature while being stirred for 12 h. The precipitate was collected by vacuum filtration before washing the solid four times with $CHCl_3$, leaving a white solid (0.653 g, 16%): mp 177−180 °C. IR (neat) 2939, 1746, 1615, 1597, 1571, 1489, 1503, 1446, 1258, and 1040 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.98 (bs, 1 H), 7.95 (d, J = 7.5 Hz, 1 H), 7.48 (m, 2 H), 7.38 (t, J = 17.0 Hz, 1 H), 6.71 (d, J = 8.1 Hz, 1 H), 6.46 (dd, J = 1.4 and 8.03 Hz, 1 H), 6.35 (d, J = 1.4 Hz, 1 H), 5.88 (s, 2 H), 5.00 (d, J = 6.2 Hz, 1 H), 4.75 (d, J = 5.9 Hz, 1 H), 3.82 (m, 1 H), 3.48 (m, 1 H), 2.90 (m, 1 H), 2.08 (m, 1 H), 1.96 (m, 1 H); ESIMS *m/z* (rel intensity) 352 (100 MH^+ − HBr). Anal. ($C_{20}H_{18}BrNO_5 \cdot 0.8H_2O$) C, H, N.

6-(3-Ethyl)-5,6-dihydro-8,9-methylenedioxy-5,11-dioxo-11*H*−indeno[1,2-*c*]isoquinoline (31). Imine **24**[8] (2.73 g, 0.015 mol) was placed in a 250 mL round-bottomed flask before dissolving it in $CHCl_3$ (100 mL). The solution was cooled in an ice bath to 0 °C. Next, homophthalic anhydride (**27**) (2.50 g, 0.0154 mol) was added to the mixture over 1 h in portions every 20 min. The mixture was then allowed to return to room temperature while being stirred for 12 h. The mixture was concentrated in vacuo to produce a viscous yellow oil, suggesting a mixture of cis and trans acids. The cis acid was unable to be separated, and therefore the crude yellow oil was placed into the next reaction. Subsequently, the oil was dissolved in $SOCl_2$ (100 mL) before stirring for 4 h. Next, the solution was concentrated in vacuo before redissolving in toluene three times (3 × 100 mL). After each dissolution, the solution was concentrated in vacuo to form a solid. The liquid was purified by flash chromatography (silica gel, $CHCl_3$) to provide a solid. This solid was further purified by precipitation from $CHCl_3$. The mixture was cooled for 12 h before filtering the solid (0.325 g, 7.0%) out of the solvent: mp 263−265 °C. IR (film) 1660, 1504, 1429, 1309, 1270, and 788 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.60 (d, J = 8.1 Hz, 1 H), 8.30 (d, J = 7.8 Hz, 1 H), 7.69−7,63 (m, 1 H), 7.41−7.37 (m, 1 H), 7.11 (s, 1 H), 7.04 (s, 1 H), 6.09 (s, 2 H), 4.54 (q, J = 7.2 Hz, 2 H), 1.51 (t, J = 7.2 Hz, 3 H); CIMS *m/z* (rel intensity) 414 (100, MH^+). Anal. ($C_{19}H_{13}NO_4 \cdot 0.3H_2O$) C, H, N.

5,6-Dihydro-8,9-methylenedioxy-5,11-dioxo-6-propyl-11*H*-indeno[1,2-*c*]isoquinoline (32). Imine **25**[35] (3.32 g, 17.4 mmol) was placed in a 250 mL round-bottomed flask and dissolved in $CHCl_3$ (125 mL). The solution was cooled in an ice bath to 0 °C. Homophthalic anhydride **(27)** (2.815 g, 17.4 mmol) was added to the mixture over 1 h in portions every 20 min. The mixture was then allowed to return to room temperature while being stirred for 12 h. The mixture was concentrated in vacuo to produce a viscous yellow oil, and NMR analysis suggested a mixture of cis acid **29** and the corresponding trans acid. The oil was dissolved in $SOCl_2$ (100 mL) and the mixture stirred for 4 h. The solution was concentrated in vacuo before dissolving it in toluene (3 × 100 mL). The mixture was concentrated, dissolved in toluene (100 mL), and concentrated to yield a solid that was removed by filtration. This procedure was repeated twice, resulting in removal of additional solid. The remaining liquid was purified by flash chromatography (silica gel, $CHCl_3$) to provide a solid, which was precipitated from $CHCl_3$ to provide the product as a solid (0.234 g, 4%): mp 229−230 °C. IR (film) 1662, 1500, 1427, 1310, and 751 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, J = 8.1 Hz, 1 H), 8.28 (d, J = 7.5 Hz, 1 H), 7.66 (dt, J = 1.5 and 7.8 Hz, 1 H), 7.39 (dt, J = 1.2 and 7.5 Hz, 1 H), 7.11 (s, 1 H), 6.94 (s, 1 H), 6.09 (s, 2 H), 4.38 (t, J = 8.1 Hz, 2 H), 1.90 (m, 2 H), 1.11 (t, J = 7.5 Hz, 3 H); EIMS *m/z* (rel intensity) 333 (M^+, 57), 291 ($M^+ - C_3H_6$, 100). Anal.($C_{20}H_{15}NO_4 \cdot 0.4H_2O$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (33). Cis acid **30** (2.890 g, 6.690 mmol) was added to a 250 mL round-bottomed flask along with $SOCl_2$ (160 mL). The solution was capped with a glass stopper and stirred for 4 h. The solution was concentrated in vacuo to form a solid. The compound was purified by flash chromatography (silica gel, $CHCl_3$) to provide a purple solid. This product was further purified by precipitation from $CHCl_3$, yielding a solid (0.288 g, 10%): mp 214−216 °C. IR (neat) 1657, 1610, 1502, 1483, 1426, 1376 1297, 1035, and 786 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, J = 8.1 Hz, 1 H), 8.27 (d, J = 7.2 Hz, 1 H), 7.68 (dt, J = 1.5 and 8.4 Hz, 1 H), 7.40 (dt, J = 1.2 and 8.1 Hz, 1 H), 7.34 (s, 1 H), 7.12 (s, 1 H), 6.09 (s, 2 H), 4.59 (t, J = 7.8 Hz, 2 H), 3.63 (t, J = 6.3 Hz, 2 H), 2.44 (m, 2 H); CIMS *m/z* (rel intensity) 414 (100, MH^+). Anal. ($C_{20}H_{14}BrNO_4 \cdot 0.2H_2O$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (34). A solution of **33** (0.090 g, 0.218 mmol) and NaN_3 (0.043 g, 0.66 mmol) in DMSO (10 mL) was stirred at room temperature for 26 h. The solution was diluted with $CHCl_3$ (100 mL) and washed with H_2O (3 × 25 mL) and sat. NaCl (25 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to provide a red-brown solid (0.079 g, 96%): mp 197 °C (dec). IR (film) 2092, 1655, 1546, 1508, 1478, 1425, 1296, and 1037 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, J = 8.0 Hz, 1 H), 8.19 (d, J = 8.0 Hz, 1 H), 7.80 (t, J = 7.6 Hz, 1 H), 7.49 (m, 2 H), 7.17 (s, 1 H), 6.22 (s, 2 H), 4.52 (t, J = 7.7 Hz, 2 H), 3.63 (t, J = 6.5 Hz, 2 H), 2.03 (m, 2 H); CIMS *m/z* (rel intensity) 375 (100, MH^+). Anal. Calcd for ($C_{20}H_{14}N_4O_4 \cdot 0.05CHCl_3$) C, H, N

6-(3-Aminopropyl)-5,6-dihydro-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride Salt (35). A 50 mL flame-dried flask was charged with **34** (0.100 g, 0.268 mmol), anhydrous benzene (25 mL), and triethyl phosphite (0.114 mL, 0.665 mmol), and the solution was heated at reflux for 16 h. The solution was cooled to room temperature, and 3 N HCl in methanol (10 mL) was added. The solution was heated at reflux for 2 h. The solution was cooled to room temperature, followed by additional chilling at

0 °C for 1 h. The precipitate was filtered and washed with cold methanol (2 mL) to provide a red solid (0.087 g, 84%): mp 277 °C (dec); IR (film) 2910, 1549, 1483, 1428, 1378, 1302, 1034, and 785 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.47 (d, J = 8.0 Hz, 1 H), 8.16 (d, J = 7.8 Hz, 1 H), 7.86 (bs, 2 H), 7.78 (t, J = 7.3 Hz, 1 H), 7.47 (m, 1 H), 6.22 (s, 2 H), 4.51 (t, J = 6.7 Hz, 2 H), 2.94 (bs, 2 H), 2.09 (t, J = 7.4 Hz, 2 H); ESIMS m/z (rel intensity) 349 ($MH^+ - Cl$, 100). Anal. ($C_{20}H_{17}ClN_2O_4 \cdot 1.0H_2O$) C, H, N.

Top1-Mediated DNA Cleavage Reactions. Human recombinant top1 was purified from Baculovirus as described previously.[12] The 161 bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease *Pvu*II and *Hin*dIII (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (50 μL reactions) 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 *Hin*dIII site by fill-in reaction with [alpha-^{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).

Molecular Modeling. The structure of the ternary complex, containing topoisomerase I, DNA, and topotecan, was downloaded from the Protein Data Bank (PDB code 1K4T).[4] One molecule of PEG and the topotecan carboxylate form were deleted. All of the atoms were then fixed according to Sybyl atom types. Hydrogens were added and minimized using the MMFF94s force field and MMFF94 charges. The structure of the indenoisoquinoline **2**, constructed in Sybyl and energy minimized with the Tripos force field and Gasteiger-Hückel charges, was overlapped with the structure of topotecan according to the proposed structural similarity[10] in the ternary complex, and the structure of topotecan was then deleted. The new whole complex was subsequently subjected to energy minimization using the MMFF94s force field with MMFF94 charges. During energy minimization, the structure of the indenoisoquinoline was allowed to move while the structures of the protein, nucleic acid, and water molecules were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/mol·Å energy gradient convergence criterion and a distance-dependent dielectric constant.

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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