Investigation of the Lactam Side Chain Length Necessary for Optimal Indenoisoquinoline Topoisomerase I Inhibition and Cytotoxicity in Human Cancer Cell Cultures

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Indenoisoquinolines with lactam substituents such as ethylamino, propylamino, and butylamino have previously demonstrated potent biological activity, but an optimal length has never been established. In the present study, a series of simplified indenoisoquinoline analogues possessing a linker spacing of 0−12 carbon atoms between the lactam nitrogen and the terminal amino group have been prepared, determining that 2−4-atom lengths are optimal for topoisomerase I inhibition and cytotoxicity. Using these lengths, analogues were prepared with the amino group and portions of the linker replaced by a pyridine ring. A three-carbon spacer within the pyridine series still demonstrated potent topoisomerase I inhibition.

Introduction

Topoisomerases are nature's solution to the topological problems associated with manipulating double-stranded, helical DNA during essential cellular processes.[1−4] The main classification of topoisomerases is dependent upon the enzyme's ability to create a single-stranded (type I) or double-stranded (type II) nick in DNA to alleviate torsional strain.[1−4] Interest in topoisomerase I (top1) as a potential therapeutic target originated from the characterization of camptothecin (**1**; Figure 1) as a selective mammalian topoisomerase I inhibitor.[5−7] Validation of drug development for this target occurred with the approval of two camptothecin derivatives, topotecan and irinotecan, for the treatment of various forms of cancer.[7,8] However, these drugs are less than optimal, each possessing a hydrolytically unstable lactone ring that when hydrolyzed affords a product with high affinity for serum albumin.[9−12] Therefore, novel, hydrolytically stable top1 inhibitors would represent an improvement in top1-targeted anticancer chemotherapy.[8]

The top1 inhibitory activity of indenoisoquinoline **2** (Figure 1) was determined after a COMPARE analysis of its cytotoxicity profile in the National Cancer Institute's (NCI's) 60-cell screen indicated a high degree of correlation with camptothecin.[13] Subsequent experimentation confirmed the activity of **2** as a top1 inhibitor with modest potency. The mechanism of action of the indenoisoquinolines involves the stabilization of the ternary cleavage complex by intercalation between the DNA base pairs upon single-strand cleavage by top1 and formation of a network of hydrogen bonds with top1, thus preventing religation of the broken phosphodiester backbone, a mechanism identical to that of the camptothecins. Recent crystallographic analyses elegantly demonstrate the inhibition of top1 by these compounds and illustrate the general features of interfacial top1 inhibition by intercalation and specific contacts with top1 amino acid residues.[14−17] In addition, ab initio calculations have underscored the primary importance of π-stacking interactions in the stabilization of the ternary complex.[18]

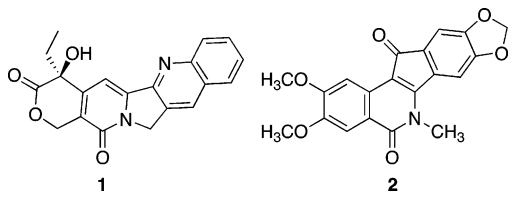

Figure 1. Representative topoisomerase I inhibitors.

Previously, it was demonstrated that ethylamino-, propylamino-, and butylamino-substituted lactam side chains conferred potent biological activity for unsubstituted indenoisoquinolines.[19] Indeed, the influence of these three substituents on biological activity has made them a standard functionality for incorporation into variously substituted indenoisoquinoline analogues. Furthermore, the 2−4-carbon spacing has been, and is currently, utilized for the development of highly potent indenoisoquinolines with various functional groups at the end of this linker.[19−30] However, the determination of optimal spacing between the terminal amino substituent and the lactam nitrogen has not yet been confirmed experimentally, and its establishment could lead to the improvement of indenoisoquinoline top1 inhibitors. Therefore, a complete series of simplified indenoisoquinoline homologues possessing a linker spacing of 0−12 carbon atoms between the lactam nitrogen and the terminal amino group have been prepared and evaluated for both cytotoxicity and top1 inhibition in an effort to determine the optimal chain length for biological activity.

Chemistry

Preparation of analogues for this study began with the monoprotection of diaminoalkanes **3**−**11** with Boc_2O according to standard methods (Scheme 1). With compounds **12**−**20** suitably protected, analogues **25**−**36** were prepared by the treatment of **12**−**24** with benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**21**)[29] (Scheme 2). Through experimentation, it was determined that the monoprotection of diaminoalkanes with lengths of less than seven carbons was not entirely necessary. Employment of excess diaminoalkanes provided a suitable method to prevent large quantities of dimerized product from forming, and the diaminoalkanes were sufficiently water soluble to allow their

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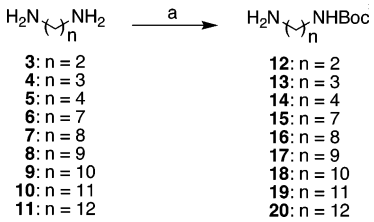
Scheme 1. Preparation of Mono-Boc-Protected Diaminoalkanes[a]



[a] Reagents and conditions: (a) Boc_2O (1 equiv), $CHCl_3$, room temperature.

removal during aqueous workup. Hence, compounds **25**, **29**, and **30** were prepared without utilization of mono-Boc-protected diaminoalkanes. Beyond a six-carbon length, however, aqueous solubility of the unprotected diaminoalkanes was negligible, and purification of **31**−**36** was greatly facilitated by the use of a *tert*-butyl carbamate to protect the terminal amine on the lactam side chain. Deprotection of carbamates **26**−**28** and **31**−**36** readily occurred upon exposure to hydrochloric acid, providing the remainder of the analogues (**37**−**47**) for this study.

Upon evaluation of the analogues prepared in Scheme 2, the optimal chain length for lactam substituents was determined and utilized in the preparation of pyridine-substituted indenoisoquinolines **48**−**51** (Scheme 3). Unlike analogues **37**−**47**, compounds **48**−**51** should not be protonated at physiological pH, and their biological activities, when compared to those of analogues **37**−**39**, should be different due to the different roles in hydrogen bonding (donor versus acceptor) of the analogue side chains. Treatment of **21**[29] with the appropriate aminoalkylpyridine derivatives readily provided compounds **48**−**51**, with the isolation of **49** and **50** greatly facilitated by the formation of their corresponding hydrochloride salts.

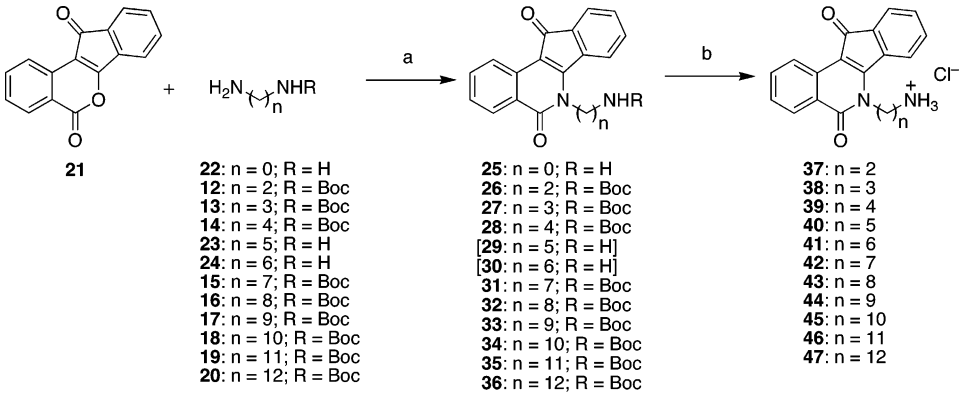
Biological Results and Discussion

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.[31,32] 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 taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. For comparison purposes, the activities of camptothecin (**1**), parent indenoisoquinoline **2**,[13] and previously reported compounds **37**−**39**[19] are included in the table. The relative potencies of the compounds in the production of top1-mediated DNA cleavage are listed in the table and summarized as follows: +, weak activity; ++, activity similar to that of the parent compound **2**; +++ and ++++, activity greater than that of the parent compound **2**; ++++, activity similar to that of 1 μM **1**.

From the biological data presented in Table 1, several general features are evident from the investigation of optimal lactam chain length. First, carbamate-protected indenoisoquinolines such as compounds **31**−**36** demonstrated poor cytotoxicity and top1 inhibition relative to those of **1** and poor top1 inhibition relative to that of the parent indenoisoquinoline **2**. This structure−activity relationship has been previously identified with carbamate-protected bisindenoisoquinolines[33] and is believed to arise from inefficient targeting of DNA (relative to that of the deprotected, positively charged derivatives), detrimental steric interactions in the ternary complex, and general hydrophobicity of the molecules relative to that of the deprotected derivatives.

In general, potent biological activity was observed for the deprotected, (alkylamino)indenoisoquinolines **37**−**47** relative to their carbamate-protected precursors (Table 1). From the data reported in Table 1, it is clear that biological activity is greatly improved, for both cytotoxicity and top1 inhibition, with the utilization of shorter lactam side chains. Indeed, maximum biological activity was observed for the previously reported[19] compounds **37**−**39**, with 2−4-carbon chain lengths. Thus, the historical use of two- and three-carbon lactam substituent chain lengths has been validated and experimentally confirmed to be optimal for developing potent indenoisoquinoline top1 inhibitors. Interestingly, there are two anomalies in the generalization that "a shorter chain length is better" for biological activity. First, compound **25**, with a hydrazine-derived lactam side chain was poorly cytotoxic and an inactive top1 inhibitor (MGM = 39.8 μM; top1 inhibition 0). This result is believed to be due to inefficient targeting of DNA (since the side chain of **25** should not have a fixed positive charge at physiological pH). Furthermore, hypothetical binding models of **25** in a ternary complex with DNA and top1 did not show any stabilizing hydrogen-bonding interaction between the amino group and either the DNA base pairs at the site of intercalation or top1. This finding was in stark contrast to that of the terminal amines of compounds **37**−**39**, which displayed stabilizing hydrogen-bonding interactions in hypothetical models.[19] The second anomaly worth mentioning is the discrepancy between the cytotoxicity of **40** and its top1 inhibition (MGM = 0.471 μM; top1 inhibition 0).

Scheme 2. Preparation of Indenoisoquinoline Analogues[a]



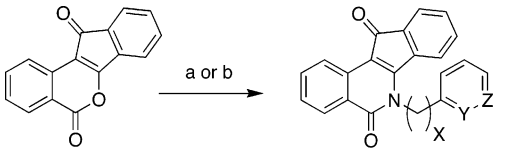
[a] Reagents and conditions: (a) $CHCl_3$, reflux; (b) 3 M HCl in MeOH, room temperature.

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

	cytotoxicity (GI_{50}, μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-268	melanoma UACC-62	ovarian OVCAR -3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	top1 cleavage[c]
1	0.010	0.030	0.010	0.010	0.220	0.020	0.010	0.040	0.0405 ± 0.0187	++++
2	1.30	35.0	NT	4.20	73.0	68.0	37.0	96.0	20.0	++
25	24.5	>50.1	28.2	>50.1	>50.1	>50.1	>50.1	>50.1	39.8	0
31	NT	17.4	20.9	20.0	33.9	74.1	31.6	81.3	30.9	0
32	10.7	25.7	8.71	15.5		>50.1	>50.1	>50.1	19.9	0
33	24.5	NT	17.8	17.4	28.8	77.6	>100	>100	50.1	
34	28.8	60.3	42.7	43.6	>100	>100	97.7	>100	52.5	0
35	20.9	35.5	29.5	24.0	70.8	>100	91.2	>100	42.6 ± 5.35	
36	32.4	>100	20.4	27.5	>100	>100	91.2	>100	51.3	
37	0.620	0.270	0.210	0.920	0.710	0.490	0.760	0.920	0.530 ± 0.320	+++
38	0.200	0.180	0.25	0.26	1.38	0.160	0.22	0.78	0.32 ± 0.23	+++
39	0.08	0.10	0.10	0.05	0.52	0.04	0.01	0.84	0.16 ± 0.01	+++
40	0.288	0.200	0.871	1.35	0.708	0.398	0.347	1.35	0.471 ± 0.054	0
41	1.29	0.912	1.23	1.62	2.00	1.32	0.603	2.04	1.32	++
42	1.20	1.26	1.78	2.00	1.70	1.66	0.832	2.24	1.66 ± 0.155	0
43	2.14	1.66	2.82	3.80	3.47	3.47	3.39	5.62	3.71	+
44	6.76	6.46	9.77	9.33	9.55	8.51	6.03	9.55	8.13	0
45	4.79	2.75	1.82	13.8	10.7	3.31	3.47	11.7	5.50	0
46	2.19	1.91	2.04	1.70	2.09	2.00	4.57	11.0	5.13	+
47	17.0	NT	18.2	14.8	17.8	13.5	19.1	19.5	18.2	0/+
48	11.2	NT	12.3	10.7	13.8	28.2	13.5	3.09	15.1	0
49	7.59	NT	6.76	8.71	13.8	13.8	13.8	42.7	11.5	+++
50	21.4	NT	17.4	27.5	>100	77.6	74.1	5.13	53.7	0
51	28.8	NT	27.5	89.1	>100	>100	81.3	4.57	44.7	0
52	2.8	0.9		2.6	1.7	2.5	1.2	2.7	2.4	++++
53	2.9	1.3		1.9	8.1	1.7	4.6	3.4	3.0	++
54		23		37	20	20	52	38	41	++++
55		43		44	0.88	33	>100	68	59	++

[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 100 μM. The activity of the compounds to produce top1-mediated DNA cleavage was expressed semiquantitatively as follows: +, weak activity; ++, activity similar to that of the parent compound **2**; +++ and ++++, activity greater than that of the parent compound **2**; ++++, activity similar to that of 1 μM **1**.

Scheme 3. Pyridine-Substituted Indenoisoquinolines[a]



[a] Reagents and conditions: (a) aminoalkylpyridine, Et_3N, $CHCl_3$, reflux; (b) (i) aminoalkylpyridine, Et_3N, $CHCl_3$; (ii) 3 M HCl in MeOH.

This result is not entirely without precedence, and it is obvious that the cytotoxicity of this molecule is not related to its ability to inhibit top1, but must be dependent upon another, unknown biological target.[19,33]

The DNA cleavage patterns produced by **1** (lane 3 of each gel**)**, the indenoisoquinoline NSC 314622, and compounds **39**, **41**, **43**, and **45** are displayed in Figure 2. The following points are apparent from inspection of the gels: (1) The potencies of the indenoisoquinolines as top1 inhibitors are reflected in the intensities of the DNA cleavage bands. The bands produced by compound **39** (top1 inhibition +++) are slightly weaker in comparison with those produced by camptothecin, but stronger in intensity than the bands produced by **41** (top1 inhibition ++), **43** (top1 inhibition +), and **45** (top1 inhibition 0). Thus, a comparison of the gels in Figure 2 indicates that as the lactam side chains of the indenoisoquinolines increase, a corresponding decrease in the ability to inhibit top1 is observed. (2) Top1 inhibitors can be classified as top1 suppressors, which inhibit DNA cleavage, and top1 poisons, which inhibit the religation reaction after DNA cleavage. As compund **39** illustrates (lanes 7 and 8), the predominant top1 cleavage site (marker 44) is trapped at lower compound concentrations and suppressed at higher concentrations, and therefore, indenoisoquinoline **39** acts as a top1 poison at lower concentrations and top1 suppressor at higher concentrations. The suppression could result from binding of the drug to the DNA, rendering it a poorer enzyme substrate at high drug concentration, or from a direct effect on the enzyme to suppress its ability to cleave DNA. (3) There are differences in the cleavage pattern of camptothecin vs the indenoisoquinolines. For example, the cleavage at base pair 44 seen with the indenoisoquinolines is absent with camptothecin. This difference may indicate that the indenoisoquinolines might display antitumor spectra different from those of camptothecin or its clinically useful derivatives irinotecan and topotecan.

Although the knowledge that a shorter alkyl side chain is important for furthering drug development efforts for indenoisoquinolines, the cause of this result is equally important. Molecular modeling of compounds **37**−**47** in a ternary complex with DNA and top1 (PDB code 1SC7)[15] indicated that the majority of these molecules should exert similar top1 inhibitory activities, since the side chains project out into the major groove and can be readily accommodated sterically.[15] However, the results from Table 1 show that, as the length of the lactam side chain increases, there is a general decrease in top1 inhibition after a four-carbon atom length. Rationalization of the biological data was therefore initially sought without the use of molecular models of the ternary complexes. Upon further inspection of Table 1 regarding only the compounds assumed to be protonated at physiological pH (**37**−**47)**, it was realized that, as the lactam chain length increased, there was an increase in the MGM values of the compounds (as well as the previously observed decrease in top1 inhibition). Since these compounds differed only by the

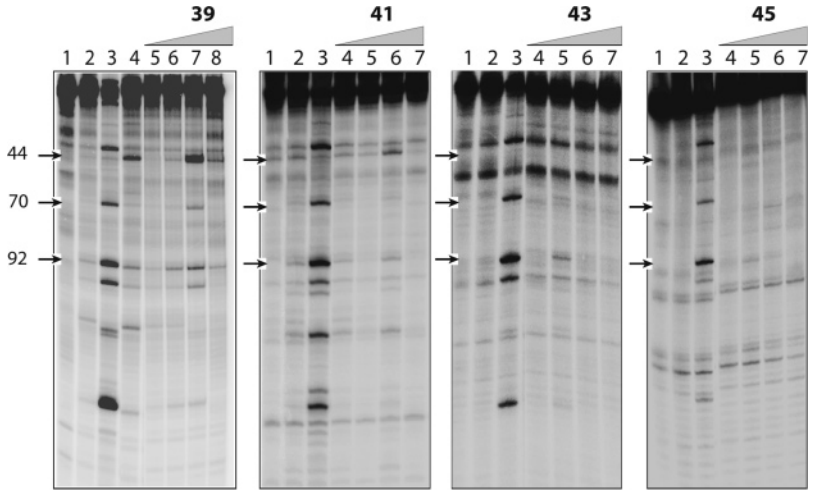


Figure 2. Key: lane 1, DNA alone; lane 2, top1 alone; lane 3, + CPT (1 μM); first panel, lane 4, top1 + NSC 314622 (100 μM); lanes 5−8 (for compound **39**) and lanes 4−7 (for compounds **41**, **43**, and **45**), top1 + indicated compound at 0.1, 1, 10, and 100 μM, respectively. Numbers on the left and arrows indicate cleavage site positions.

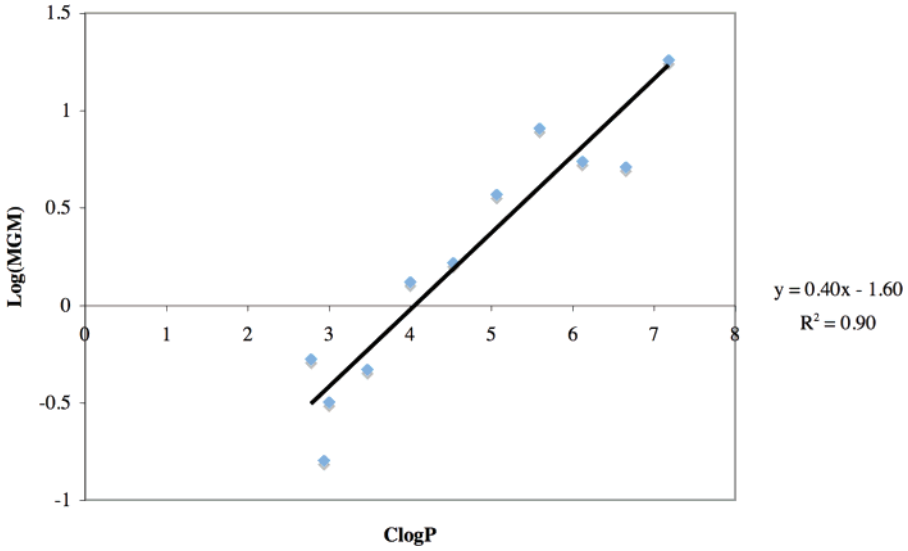


Figure 3. *C* log *P* vs log(MGM).

incremental increase of methylene units, the effect could possibly be hydrophobic in nature. Indeed, calculation of the *C* log *P* values of compounds **37**−**47** (performed in ChemDraw Ultra, version 9.0.1) and plotting against log(MGM) resulted in a linear dependence with an r^2 value of 0.90, indicating a high degree of correlation (Figure 3). Thus, for this series of compounds general hydrophobicity, as measured by *C* log *P* values, appears to be predictive of cytotoxicity in the NCI's 60-cell screen. Inspection of the ternary complex of topotecan bound to DNA and top1 (PDB code 1K4T)[14] reveals the presence of a hydrated binding pocket and appears to provide an essential clue supporting both the data in Table 1 and the relationship between *C* log *P* and cytotoxicity (Figure 3). A hypothetical model was subsequently developed utilizing the topotecan crystal structure and the experimentally determined structural overlay between topotecan and the indenoisoquinolines (Figure 4).[14,15,17] Overlaying the hypothetical models for compounds **37**, **39**, **41**, **43**, **45**, and **47** (colored by atom type) in a ternary complex with DNA (red) and top1 (blue) allows the visualization of the extensive hydrophilic binding pocket (water molecules are indicated in green) for the indenoisoquinoline side chain. Increasing the number of methylene units beyond four in the lactam side chain of the indenoisoquinolines results in an increasingly hydrophobic linker with increasingly negative interactions with the hydrated binding pocket. In agreement with this hypothesis, polyamine-substituted indenoisoquinolines **52**−**55** retain significant top1 inhibitory activity in the ++ to ++++ range.[24]

Having experimentally confirmed that the optimal lactam chain length for cytotoxicity and top1 inhibition for the indenoisoquinolines was 2−4 carbon atoms between the lactam nitrogen and the terminal amino group, this relationship was further scrutinized by preparing analogues **48**−**51** that utilize a pyridine ring in place of a portion of the alkyl linker and the terminal amino group. However, the newly designed analogues still maintained a 2−4-carbon atom linker between the lactam nitrogen and the nitrogen atom in the pyridine ring. Although all of the analogues were less cytotoxic than their corresponding alkyl chain analogues, compound **49** displayed potent top1 inhibition equal in magnitude to that of compounds **37**−**39**. With the discrepancy between cytotoxicity and top1 inhibition, it seems reasonable to infer that protonation of the terminal amino group of unsubstituted indenoisoquinolines is important for cellular cytotoxicity (perhaps by improved DNA targeting) but is not necessarily a requirement for potent top1 inhibition. Indeed, this result has been observed in another indenoisoquinoline study involving heterocycles appended to the lactam side chain, and the present study of pyridine heterocycles

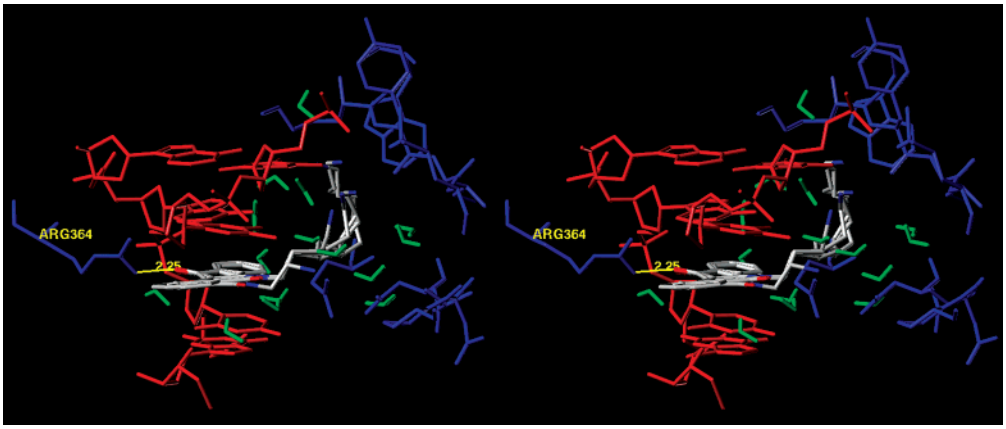
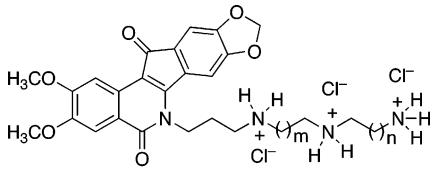


Figure 4. Overlay of the hypothetical models derived from PDB code 1K4T for compounds **37**, **39**, **41**, **43**, **45**, and **47** (colored by atom type) in a ternary complex with DNA (red) and top1 (blue). Water molecules are indicated in green, and the hydrogen bond between the analogues and Arg364 is shown in yellow. The figure is programmed for wall-eyed (relaxed) viewing.

reaffirms previously derived structure−activity relationships.[34] However, the absence of protonation of the pyridine nitrogen at physiological pH should not be construed as the only reason for the discrepancies in biological activity between compounds **37**−**39** and **48**−**51**. Furthermore, the pyridine-substituted analogues do not provide strong support of the currently established optimal length of the linker chain. This could be a result of several factors including, but not limited to, reduced flexibility of the side chain, increased steric bulk, disrupted hydrogen-bonding networks with the aqueous environment, and the aforementioned protonation state of the pyridine nitrogen at physiological pH and DNA targeting.

Compounds **37**−**39** are more cytotoxic than compounds **52**−**55** and **48**−**51**. Thus, incorporating polar motifs (such as polyamino groups) on the lactam side chain of the indenoisoquinolines does not necessarily translate into increased cytotoxicity but does improve top1 inhibition. Additionally, structural changes to the lactam side chain that reduce its flexibility, such as the present case for the pyridine-containing analogues, result in discrepancies between enzyme inhibition and cytotoxicity. The presently observed relationship between *C* log *P* and cytotoxicity indicates that drug design and molecular modeling of the indenoisoquinolines should consider the environment of the lactam side chain in the ternary complex. Multiple variables in addition to lipophilicity are expected to be involved in cellular uptake, distribution, and ultimately cytotoxicity of the indenoisoquinolines (as well as other DNA-targeting drug molecules). However, lipophilicity has previously been demonstrated to affect the nonspecific binding of anthracycline antitumor drugs to serum albumin, and it seems reasonable to assume that nonspecific binding (or unintended localization) could occur intracellularly with the indenoisoquinolines and result in discrepant biological activities.[35,36] Differences in structure, such as the replacement of the indenoisoquinoline side chain with a pyridine ring or polyamino groups, are also expected to result in different rates of uptake, distribution, metabolism, and excretion within the cancer cell. Hence, discrepancies will ultimately arise between enzyme-based assays and the NCI 60-cell-line screen for cytotoxicity.



52: m = 1, n = 1
53: m = 1, n = 2
54: m = 2, n = 1
55: m = 2, n = 2

In conclusion, a series of indenoisoquinolines have been prepared to determine the optimal carbon chain length between the lactam nitrogen and the terminal amine of the side chain. The results of this study confirmed that the optimal carbon linker for biological activity (both top1 inhibition and cytotoxicity) is 2−4 atoms, thereby validating the use of this length in past and future efforts to develop indenoisoquinolines as top1 inhibitors. Interestingly, the study indicated a strong correlation between *C* log *P* values and MGM values for indenoisoquinolines with protonated terminal amines on the lactam side chain. Furthermore, the substitution of a pyridine ring in indenoisoquinoline **49** in place of a portion of the linker and the terminal amino group resulted in a discrepancy between top1 inhibition and cytotoxicity, indicating that a protonated terminal amino group on the indenoisoquinolines is more important for maintaining cytotoxicity than top1 inhibition.

Experimental Section

General Procedures. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained using $CHCl_3$ as the solvent unless otherwise specified. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using an ARX300 300 MHz Bruker NMR spectrometer. IR spectra were recorded using a Perkin-Elmer 1600 series FTIR spectrometer. Combustion microanalyses were performed at the Purdue University Microanalysis Laboratory, and the reported values were within 0.4% of the calculated values. Analytical thin-layer chromatography was carried out on Baker-flex silica gel IB2-F plates, and the compounds were visualized with short-wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel. Compounds **12**−**14**, **26**−**28**, and **37**−**39** were prepared as previously described.[19]

General Procedure for the Preparation of Mono-Boc-Protected Diamines 12−**20.** Boc_2O (0.500 g, 2.291 mmol) was dissolved in $CHCl_3$ (10 mL), and the solution was added dropwise to a solution of diamine (11.45 mmol) in $CHCl_3$ (50 mL). The reaction mixture was allowed to stir at room temperature for 24 h, concentrated, and purified by flash column chromatography (SiO_2), eluting with a solution of 1% Et_3N/10% MeOH in $CHCl_3$, to provide the mono-Boc-protected diamine.

Mono-Boc-1,7-diaminoheptane (15).[37] The general procedure provided the desired compound as a colorless semisolid (0.473 g, 90%): ^{1}H NMR ($CDCl_3$) δ 4.52 (br s, 1 H), 3.12 (q, J = 6.2 Hz, 2 H), 2.70 (t, J = 6.8 Hz, 2 H), 1.43−1.23 (m, 19 H).

Mono-Boc-1,8-diaminooctane (16).[38] The general procedure provided the desired compound as a colorless semisolid (0.492 g,

88%): ^{1}H NMR ($CDCl_3$) δ 4.51 (br s, 1 H), 3.12 (q, J = 6.5 Hz, 2 H), 2.69 (t, J = 6.8 Hz, 2 H), 1.43−1.23 (m, 21 H).

Mono-Boc-1,9-diaminononane (17).[39] The general procedure provided the desired compound as a colorless semisolid (0.125 g, 21%): ^{1}H NMR ($CDCl_3$) δ 4.50 (br s, 1 H), 3.12 (q, J = 6.5 Hz, 2 H), 2.70 (t, J = 6.8 Hz, 2 H), 1.44−1.22 (m, 23 H).

Mono-Boc-1,10-diaminodecane (18).[39] The general procedure provided the desired compound as a colorless semisolid (0.192 g, 31%): ^{1}H NMR ($CDCl_3$) δ 4.50 (br s, 1 H), 3.13 (q, J = 6.3 Hz, 2 H), 2.71 (t, J = 6.9 Hz, 2 H), 1.44−1.18 (m, 27 H).

Mono-Boc-1,11-diaminoundecane (19). The general procedure provided the desired compound as a colorless solid (0.555 g, 85%): mp 30−34 °C; IR (film) 3370, 2919, 2851, 1687, 1522 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.49 (br s, 1 H), 3.11 (q, J = 6.5 Hz, 2 H), 2.71 (t, J = 6.8 Hz, 2 H), 1.44−1.27 (m, 29 H); ESIMS m/z (rel intens) 287 (MH^+, 100). Anal. ($C_{16}H_{34}N_2O_2$) C, H, N.

Mono-Boc-1,12-diaminododecane (20).[39,40] The general procedure provided the desired compound as a colorless semisolid (0.191 g, 28%): ^{1}H NMR ($CDCl_3$) δ 4.48 (br s, 1 H), 3.11 (q, J = 6.2 Hz, 2 H), 2.76 (t, J = 6.9 Hz, 2 H), 1.44−1.26 (m, 31 H).

6-Amino-5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline (25). Benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**21**)[29] (0.150 g, 0.604 mmol) was treated with hydrazine (**22**) (0.255 g, 7.964 mmol) in $CHCl_3$ (50 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, diluted with $CHCl_3$ (150 mL), and washed with saturated $NaHCO_3$ (2 × 50 mL). The solution was dried over sodium sulfate and concentrated to provide a red-orange solid (0.120 g, 76%): mp 272−274 °C; IR (film) 3448, 3305, 1686, 1663, 1610, 1507, 1312, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.54 (d, J = 7.8 Hz, 1 H), 8.51 (d, J = 7.2 Hz, 1 H), 8.24 (d, J = 7.4 Hz, 1 H), 7.85 (m, 1 H), 7.60−7.45 (m, 4 H), 6.19 (s, 2 H); EIMS m/z (rel intens) 262 (M^+, 100). Anal. ($C_{16}H_{10}N_2O_2 \cdot 0.25H_2O$) C, H, N.

General Procedure for the Preparation of Mono-Boc-Protected Indenoisoquinolines 31−36. Mono-Boc-protected diamine (2.054 mmol) was added to a solution of **21**[29] (0.255 g, 1.027 mmol) in $CHCl_3$ (100 mL). The reaction mixture was heated at reflux for 24 h, concentrated, and purified by flash column chromatography (SiO_2), eluting with $CHCl_3$, to provide the mono-Boc-protected indenoisoquinoline.

6-(7′-*tert*-Boc-aminoheptyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (31). The general procedure provided the desired compound as a yellow-orange solid (0.451 g, 95%): mp 112−116 °C; IR (film) 3369, 1697, 1664, 1503, 1172 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 8.2 Hz, 1 H), 7.84−7.79 (m, 1 H), 7.71 (d, J = 7.7 Hz, 1 H), 7.63−7.50 (m, 4 H), 6.76 (m, 1 H), 4.50 (t, J = 7.4 Hz, 2 H), 2.90 (q, J = 6.2 Hz, 2 H), 1.77 (m, 2 H), 1.46−1.28 (m, 17 H); ESIMS m/z (rel intens) 483 (MNa^+, 100). Anal. ($C_{28}H_{32}N_2O_4$) C, H, N.

6-(8′-*tert*-Boc-aminooctyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (32). The general procedure provided the desired compound as a yellow-orange solid (0.466 g, 97%): mp 140−143 °C; IR (film) 3368, 2929, 1698, 1665, 1504, and 1172 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 7.9 Hz, 1 H), 8.23 (d, J = 8.1 Hz, 1 H), 7.84−7.78 (m, 1 H), 7.71 (d, J = 7.5 Hz, 1 H), 7.63−7.47 (m, 4 H), 6.75 (m, 1 H), 4.50 (t, J = 7.4 Hz, 2 H), 2.91 (q, J = 6.6 Hz, 2 H), 1.78 (m, 2 H), 1.46−1.26 (m, 19 H); ESIMS m/z (rel intens) 497 (MNa^+, 100). Anal. ($C_{29}H_{34}N_2O_4$) C, H, N.

6-(9′-*tert*-Boc-aminononyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (33). The general procedure provided the desired compound as an orange solid (0.145 g, 77%): mp 91−95 °C; IR (film) 3371, 2928, 1698, 1666, 1504, 1172 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 8.0 Hz, 1 H), 8.23 (d, J = 7.4 Hz, 1 H), 7.84−7.79 (m, 1 H), 7.71 (d, J = 7.4 Hz, 1 H), 7.63−7.50 (m, 4 H), 6.74 (m, 1 H), 4.50 (t, J = 7.3 Hz, 2 H), 2.91 (q, J = 6.6 Hz, 2 H), 1.78 (m, 2 H), 1.47−1.24 (m, 21 H); ESIMS m/z (rel intens) 511 (MNa^+, 100). Anal. ($C_{30}H_{36}N_2O_4$) C, H, N.

6-(10′-*tert*-Boc-aminodecyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (34). The general procedure provided the desired compound as a yellow-orange solid (0.220 g, 78%): mp 135−137 °C; IR (film) 3368, 2927, 1698, 1666, 1504, 1172 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 7.9 Hz, 1 H), 8.23 (dd, J = 8.1 and 0.7 Hz, 1 H), 7.84 (dt, J = 7.2 and 1.4 Hz, 1 H), 7.71 (d, J = 7.5 Hz, 1 H), 7.63−7.47 (m, 4 H), 6.76 (m, 1 H), 4.50 (t, J = 7.4 Hz, 2 H), 2.90 (q, J = 6.5 Hz, 2 H), 1.77 (m, 2 H), 1.46−1.23 (m, 23 H); ESIMS m/z (rel intens) 525 (MNa^+, 100). Anal. ($C_{31}H_{38}N_2O_4$) C, H, N.

6-(11′-*tert*-Boc-aminoundecyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (35). The general procedure provided the desired compound as a yellow-orange solid (0.445 g, 86%): mp 111−114 °C; IR (KBr) 3364, 2918, 2850, 1678, 1660, 1534, 1505, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 7.4 Hz, 1 H), 7.84−7.79 (m, 1 H), 7.71 (d, J = 7.5 Hz, 1 H), 7.62−7.50 (m, 4 H), 6.74 (m, 1 H), 4.50 (t, J = 7.4 Hz, 2 H), 2.90 (q, J = 6.5 Hz, 2 H), 1.78 (m, 2 H), 1.46−1.22 (m, 25 H); ESIMS m/z (rel intens) 539 (MNa^+, 100). Anal. ($C_{32}H_{40}N_2O_4$) C, H, N.

6-(12′-*tert*-Boc-aminododecyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (36). The general procedure provided the desired compound as a yellow-orange solid (0.177 g, 66%): mp 129−134 °C; IR (film) 3369, 2926, 1698, 1666, 1504, 1172 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 8.0 Hz, 1 H), 8.22 (d, J = 7.4 Hz, 1 H), 7.84−7.78 (m, 1 H), 7.71 (d, J = 7.5 Hz, 1 H), 7.62−7.50 (m, 4 H), 6.74 (m, 1 H), 4.50 (t, J = 7.3 Hz, 2 H), 2.90 (q, J = 6.6 Hz, 2 H), 1.77 (m, 2 H), 1.46−1.22 (m, 27 H); ESIMS m/z (rel intens) 553 (MNa^+, 100). Anal. ($C_{33}H_{42}N_2O_4$) C, H, N.

6-(5-Aminopentyl)-5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (40). Lactone **21**[29] (0.100 g, 0.403 mmol) was treated with diamine **23** (0.206 g, 2.014 mmol) in $CHCl_3$ (40 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature and washed with water (3 × 15 mL). The solution was dried over sodium sulfate, filtered, and treated with 2 M HCl in Et_2O (5 mL). After 30 min, the reaction mixture was filtered, and the filter pad was washed with $CHCl_3$ (50 mL) and hexanes (50 mL) to provide an orange solid (0.122 g, 82%): mp 265−268 °C; IR (film) 3432, 3077, 2856, 1707, 1635, 1611, 1549, 1504 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.59 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 8.1 Hz, 1 H), 7.85−7.80 (m, 3 H), 7.74 (d, J = 7.4 Hz, 1 H), 7.63−7.51 (m, 4 H), 4.52 (t, J = 7.3 Hz, 2 H), 2.81 (m, 2 H), 1.83 (m, 2 H), 1.65−1.52 (m, 4 H); ESIMS m/z (rel intens) 333 (MH^+, 100). Anal. ($C_{21}H_{21}ClN_2O_2 \cdot 0.75H_2O$) C, H, N.

6-(6-Aminohexyl)-5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]-isoquinoline Hydrochloride (41). Lactone **21**[29] (0.100 g, 0.403 mmol) was treated with diamine **24** (0.234 g, 2.014 mmol) in $CHCl_3$ (40 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature and washed with water (3 × 25 mL). The solution was dried over sodium sulfate, filtered, and treated with 2 M HCl in Et_2O (5 mL). After 30 min, the reaction mixture was filtered, and the filter pad was washed with $CHCl_3$ (50 mL) and hexanes (50 mL) to provide an orange solid (0.125 g, 81%): mp 195 °C dec; IR (film) 3435, 1660, 1630, 1610, 1504 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.59 (d, J = 7.8 Hz, 1 H), 8.23 (d, J = 8.1 Hz, 1 H), 7.85−7.71 (m, 4 H), 7.61−7.51 (m, 4 H), 4.52 (t, J = 7.3 Hz, 2 H), 2.78 (m, 2 H), 1.79 (m, 2 H), 1.59−1.39 (m, 6 H); ESIMS m/z (rel intens) 347 (MH^+, 100). Anal. ($C_{22}H_{23}ClN_2O_2 \cdot 0.5H_2O$) C, H, N.

General Procedure for the Preparation of Indenoisoquinoline Hydrochloride Salts 42−47. HCl (3 M) in MeOH (10 mL) was slowly added to a solution of mono-Boc-protected indenoisoquinoline (0.100 g, 0.188−0.217 mmol) in $CHCl_3$ (50 mL) at room temperature. After 2 h, the reaction mixture was concentrated, and the residue was triturated with Et_2O. Filtration of the obtained solid provided the indenoisoquinoline as a hydrochloride salt.

6-(7-Aminoheptyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline Hydrochloride (42). The general procedure provided the desired compound as a yellow-orange solid (0.085 g, 99%): mp 228−231 °C; IR (KBr) 3436, 2931, 1702, 1650, 1611, 1549, 1504, 759 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.60 (d, J = 8.1 Hz, 1 H), 8.24 (d, J = 8.2 Hz, 1 H), 7.85−7.80 (m, 1 H), 7.73 (d, J = 7.4 Hz, 1 H), 7.63−7.49 (m, 6 H), 4.53 (t, J = 7.0 Hz, 2 H), 2.78

(t, $J = 7.1$ Hz, 2 H), 1.80 (m, 2 H), 1.55−1.35 (m, 8 H); ESIMS *m*/*z* (rel intens) 361 (MH^+, 100). Anal. ($C_{23}H_{25}ClN_2O_2 \cdot 0.5H_2O$) C, H, N.

6-(8-Aminooctyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (43). The general procedure provided the desired compound as an orange solid (0.083 g, 95%): mp 182−185 °C; IR (KBr) 3436, 2930, 1661, 1505, 761 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60 (d, $J = 8.5$ Hz, 1 H), 8.24 (d, $J = 7.0$ Hz, 1 H), 7.86−7.81 (m, 1 H), 7.73 (d, $J = 7.6$ Hz, 1 H), 7.63−7.52 (m, 6 H), 4.52 (t, $J = 7.9$ Hz, 2 H), 2.78 (t, $J = 7.3$ Hz, 2 H), 1.79 (m, 2 H), 1.50 (m, 4 H), 1.31 (m, 6 H); ESIMS *m*/*z* (rel intens) 375 (MH^+, 100). Anal. ($C_{24}H_{27}ClN_2O_2 \cdot 0.75H_2O$) C, H, N.

6-(9-Aminononyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (44). The general procedure provided the desired compound as an orange solid (0.082 g, 94%): mp 204−207 °C; IR (KBr) 3435, 2927, 1702, 1662, 1610, 1549, 1504, 1427, 759 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60 (d, $J = 8.3$ Hz, 1 H), 8.24 (d, $J = 9.3$ Hz, 1 H), 7.83−7.81 (m, 1 H), 7.73 (d, $J = 7.8$ Hz, 1 H), 7.63−7.51 (m, 6 H), 4.52 (t, $J = 8.3$ Hz, 2 H), 2.78 (t, $J = 7.3$ Hz, 2 H), 1.79 (m, 2 H), 1.51 (m, 4 H), 1.28 (m, 8 H); ESIMS *m*/*z* (rel intens) 389 (MH^+, 100). Anal. ($C_{25}H_{29}ClN_2O_2 \cdot 0.75H_2O$) C, H, N.

6-(10-Aminodecyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (45). The general procedure provided the desired compound as an orange solid (0.087 g, 91%): mp 189−192 °C; IR (KBr) 3443, 2925, 2851, 1705, 1646, 1611, 1550, 1504, 1467, 759 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60 (d, $J = 7.9$ Hz, 1 H), 8.23 (d, $J = 7.5$ Hz, 1 H), 7.83 (m, 1 H), 7.73 (d, $J = 8.0$ Hz, 1 H), 7.63−7.51 (m, 6 H), 4.52 (t, $J = 7.4$ Hz, 2 H), 2.76 (m, 2 H), 1.79 (m, 2 H), 1.49 (m, 4 H), 1.27 (m, 10 H); ESIMS *m*/*z* (rel intens) 403 (MH^+, 100). Anal. ($C_{26}H_{31}ClN_2O_2 \cdot 0.5H_2O$) C, H, N.

6-(11-Aminoundecyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (46). The general procedure provided the desired compound as an orange solid (0.085 g, 88%): mp 125−129 °C; IR (KBr) 3436, 2922, 2851, 1662, 1610, 1549, 1504, 1426, 758 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60 (d, $J = 8.0$ Hz, 1 H), 8.23 (d, $J = 7.5$ Hz, 1 H), 7.85 (m, 1 H), 7.72 (d, $J = 7.5$ Hz, 1 H), 7.63−7.51 (m, 6 H), 4.51 (t, $J = 8.0$ Hz, 2 H), 2.77 (t, $J = 7.6$ Hz, 2 H), 1.78 (m, 2 H), 1.48 (m, 4 H), 1.25 (m, 12 H); ESIMS *m*/*z* (rel intens) 417 (MH^+, 100). Anal. ($C_{26}H_{31}ClN_2O_2 \cdot H_2O$) C, H, N.

6-(12-Aminododecyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (47). The general procedure provided the desired compound as a yellow solid (0.087 g, 91%): mp 175−178 °C; IR (KBr) 3435, 2927, 2850, 1704, 1644, 1506, 1466, 762 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.59 (d, $J = 7.8$ Hz, 1 H), 8.23 (d, $J = 7.5$ Hz, 1 H), 7.83 (m, 1 H), 7.72 (d, $J = 7.8$ Hz, 1 H), 7.63−7.51 (m, 6 H), 4.51 (t, $J = 7.5$ Hz, 2 H), 2.76 (m, 2 H), 1.78 (m, 2 H), 1.51 (m, 4 H), 1.24 (m, 14 H); ESIMS *m*/*z* (rel intens) 431 (MH^+, 100). Anal. ($C_{28}H_{35}ClN_2O_2 \cdot 1.25H_2O$) C, H, N.

5,6-Dihydro-5,11-dioxo-6-(2-pyridylmethyl)-11*H*-indeno[1,2-*c*]isoquinoline (48). 2-(Aminomethyl)pyridine (0.054 g, 0.504 mmol) was added to a solution of **21**[29] (0.100 g, 0.403 mmol) in $CHCl_3$ (50 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, washed with H_2O (3 × 25 mL) and saturated NaCl (25 mL), dried over sodium sulfate, and concentrated. The residue was washed with EtOAc and hexanes, and dried to provide a yellow solid (0.110 g, 81%): mp 240−242 °C; IR (KBr) 1698, 1655, 1618, 1501, 1427, 755 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.62 (d, $J = 8.0$ Hz, 1 H), 8.56 (d, $J = 4.9$ Hz, 1 H), 8.22 (d, $J = 8.1$ Hz, 1 H), 7.97 (dt, $J = 7.8$ and 1.7 Hz, 1 H), 7.89 (m, 1 H), 7.61−7.37 (m, 7 H), 5.91 (s, 2 H); ESIMS *m*/*z* (rel intens) 339 (MH^+, 100). Anal. ($C_{22}H_{14}N_2O_2$) C, H, N.

5,6-Dihydro-5,11-dioxo-6-(3-pyridylmethyl)-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (49). 3-(Aminomethyl)pyridine (0.054 g, 0.504 mmol) was added to a solution of **21**[29] (0.100 g, 0.403 mmol) in $CHCl_3$ (50 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, washed with H_2O (3 × 25 mL) and saturated NaCl (25 mL), dried over sodium sulfate, and concentrated. The residue was diluted with $CHCl_3$ (40 mL), 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was concentrated, and the residue was washed with $CHCl_3$ to provide a pink solid (0.146 g, 97%): mp 274 °C dec; IR (KBr) 2343, 2106, 1695, 1655, 1610, 1551, 1501, 754 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.98 (s, 1 H), 8.78 (d, $J = 5.2$ Hz, 1 H), 8.64 (d, $J = 8.1$ Hz, 1 H), 8.36 (d, $J = 9.1$ Hz, 1 H), 8.23 (d, $J = 7.5$ Hz, 1 H), 7.90 (m, 2 H), 7.59−7.39 (m, 5 H), 5.89 (s, 2 H); ESIMS *m*/*z* (rel intens) 339 (MH^+, 100). Anal. ($C_{22}H_{15}ClN_2O_2$) C, H, N.

5,6-Dihydro-5,11-dioxo-6-(2-pyridylethyl)-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (50). 2-(2-Aminoethyl)pyridine (0.098 g, 0.806 mmol) was added to a solution of **21**[29] (0.100 g, 0.403 mmol) in $CHCl_3$ (50 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, washed with H_2O (3 × 25 mL) and saturated NaCl (25 mL), dried over sodium sulfate, and concentrated. The residue was diluted with $CHCl_3$ (40 mL), 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was concentrated, and the residue was washed with $CHCl_3$ to provide a yellow solid (0.146 g, 93%): mp 240 °C dec; IR (KBr) 2307, 1698, 1659, 1610, 1548, 1504, 1429, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.78 (d, $J = 5.5$ Hz, 1 H), 8.57 (d, $J = 8.0$ Hz, 1 H), 8.33 (m, 1 H), 8.02 (d, $J = 8.0$ Hz, 1 H), 7.97 (d, $J = 8.0$ Hz, 1 H), 7.90 (d, $J = 8.0$ Hz, 1 H), 7.80 (m, 2 H), 7.61−7.44 (m, 4 H), 4.92 (t, $J = 6.5$ Hz, 2 H), 3.59 (t, $J = 6.3$ Hz, 2 H); ESIMS *m*/*z* (rel intens) 353 (MH^+, 100). Anal. ($C_{23}H_{17}ClN_2O_2$) C, H, N.

5,6-Dihydro-5,11-dioxo-6-(3-pyridylethyl)-11*H*-indeno[1,2-*c*]isoquinoline (51). 3-(2-Aminoethyl)pyridine (0.172 g, 0.604 mmol) was added to a solution of **21**[29] (0.100 g, 0.403 mmol) in $CHCl_3$ (50 mL). Triethylamine (0.224 mL, 1.612 mmol) was added, and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, washed with H_2O (3 × 25 mL) and saturated NaCl (25 mL), dried over sodium sulfate, and concentrated. The obtained precipitate was washed with EtOAc and hexanes and dried to provide an orange solid (0.140 g, 99%): mp 220 °C dec; IR (KBr) 1691, 1660, 1609, 1549, 1504, 1424, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (s, 1 H), 8.73 (d, $J = 5.6$ Hz, 1 H), 8.59 (d, $J = 8.1$ Hz, 1 H), 8.44 (d, $J = 7.9$ Hz, 1 H), 8.10 (d, $J = 8.1$ Hz, 1 H), 7.88 (m, 3 H), 7.61−7.48 (m, 4 H), 4.87 (t, $J = 6.7$ Hz, 2 H), 3.37 (t, $J = 6.3$ Hz, 2 H); ESIMS *m*/*z* (rel intens) 353 (MH^+, 100). Anal. ($C_{23}H_{16}N_2O_2 \cdot 0.55H_2O$) C, H, N.

Top1-Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from baculovirus as described previously.[41] The 161 bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonucleases *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 1× 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 [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 unit of DNA polymerase I (Klenow fragment). Unincorporated [^{32}P]dGTP was removed using miniature Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled 161 bp fragment was collected. Aliquots (approximately 50000 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). 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). The 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).[14] One molecule of PEG and the topotecan carboxylate form were deleted. Several 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 indenoisoquinolines **37**−**47**, constructed in Sybyl and energy minimized with the MMFF94s force field and MMFF94 charges, was overlapped with the structure of topotecan according to the proposed structural similarity[16,17] 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 for compounds **19**, **25**, **31**−**36**, and **40**−**51**. This material is available free of charge via the Internet at http://pubs.acs.org.

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