Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors: A Systematic Study and Optimization

Andrew Morrell,[†] Michael Placzek,[†] Seth Parmley,[†] Smitha Antony,[‡] Thomas S. Dexheimer,[‡] Yves Pommier,[‡] and Mark Cushman*,[†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, and the Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907, and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892-4255

Received March 27, 2007

The biological activity of indenoisoquinoline topoisomerase I (Top1) inhibitors can be greatly enhanced depending on the choice of substituents on the aromatic rings and lactam side chain. Previously, it was discovered that a 3-nitro group and a 9-methoxy group afforded enhanced biological activity. In the present investigation, indenoisoquinoline analogues were systematically prepared using combinations of nitro groups, methoxy groups, and hydrogen atoms in an effort to understand the contribution of each group toward cytotoxicity and Top1 inhibition. Analysis of the biological results suggests that the nitro group is important for Top1 inhibition and the methoxy group improves cytotoxicity. In addition, previously identified structure−activity relationships were utilized to select favorable lactam side chain functionalities for incorporation on the aromatic skeleton of analogues in this study. As a result, this investigation has provided optimal Top1 inhibitors equipotent to camptothecin that demonstrate low nanomolar cytotoxicities toward cancer cells.

Introduction

DNA topoisomerases are important for the successful replication, transcription, and recombination of DNA, as well as chromatin remodeling.[1−5] Thus, the proliferation and survival of eukaryotic cells are dependent on topoisomerases, which are therefore potential therapeutic targets for anticancer therapy. The cellular role of topoisomerase I is to relieve torsional strain in DNA by creating a single strand nick in the phosphodiester backbone, a process that renders the enzyme covalently ligated to the DNA until strain is relieved.[1] Thus, a potential therapeutic agent could inhibit topoisomerase I (Top1) by two distinct pathways: (1) "suppressing" its ability to nick the DNA strand and (2) "poisoning" the enzyme by trapping it as a covalent complex with DNA.

The identification of indenoisoquinoline **1** as a novel lead molecule for the inhibition of Top1 resulted from a COMPARE analysis of its cytotoxicity profile demonstrating a similarity to that of the known Top1 inhibitor, camptothecin (**2**).[6] These molecules intercalate between the DNA base pairs at the site of single strand cleavage and effectively stabilize a ternary complex consisting of the drug molecule, DNA, and Top1. This mechanism of action classifies these inhibitors as Top1 poisons. Recently published X-ray crystal structures for camptothecins,[7,8] indenoisoquinolines,[8,9] and an indolocarbazole[8] elegantly demonstrate this mechanism of action and illustrate the general features for poisoning Top1 by intercalation at the cleavage site.[10]

Clinical validation of Top1 as a drug target has occurred, with the camptothecin derivatives topotecan and irinotecan representing the only Top1 inhibitors currently approved by the U.S. Food and Drug Administration for anticancer therapy.[11] However, the camptothecins suffer from pharmacokinetic problems, most notably the hydrolysis of their lactone ring, which results in a hydroxy acid product that has high affinity for human serum albumin.[12−15] Indenoisoquinoline **1**, unlike the camptothecins, is hydrolytically stable but alternatively suffers from intrinsically low biological activity.[6] As a result, considerable research has been expended to improve the biological activity of the indenoisoquinolines.[9,16−26] Novel insights have been gleaned regarding the contributions of the indenone ring, isoquinoline ring, and lactam side chain toward biological activity.[20,22,27−30] Recent efforts have indicated that compounds **3**−**5** (Figure 1) represent novel lead compounds with potencies comparable to the potencies of camptothecins and with cytotoxicities approximately 1000 times more potent than that of the original lead molecule **1**.[30]

In the present study, second-generation analogues were systematically prepared to examine the contributions of the nitro group and methoxy group toward Top1 inhibition and cytotoxicity. Previously observed structure−activity relationships have indicated that lactam substituents such as amino, morpholino, dimethylamino, ethanolamino, and imidazolyl afford excellent biological activity for unsubstituted, dimethoxy-substituted, and methylenedioxy-substituted indenoisoquinolines.[17,20,22,24,26,29,31] Thus, it seemed prudent to select these functionalities for incorporation in the present study along with analogues possessing nitrated and methoxy-substituted aromatic rings. Figure 2 indicates previously synthesized analogues of interest to the present study.[17,22,26] Previously, it has been demonstrated that indenoisoquinolines possessing a 3-nitro group display enhanced biological activity, especially when combined with a 9-methoxy group.[21,30,32] This enhancement of biological activity has been hypothesized to result from favorable hydrogen-bonding interactions and electrostatic charge complementarity between Top1, the indenoisoquinolines, and the DNA base pairs in the cleavage complex.[21,30] However, a systematic examination of the independent effects that these substituents may exert on the biological activity of the indenoisoquinolines has not been conducted. As a result, the current study has been designed in such a way as to improve the current level of understanding regarding the effects of the nitro and methoxy groups on the

* To whom correspondence should be addressed. Phone: 765-494-1465. Fax: 765-494-6790. E-mail: cushman@pharmacy.purdue.edu.
[†] Purdue University
[‡] National Cancer Institute, NIH.

10.1021/jm070361q CCC: $37.00
Published on Web 08/15/2007

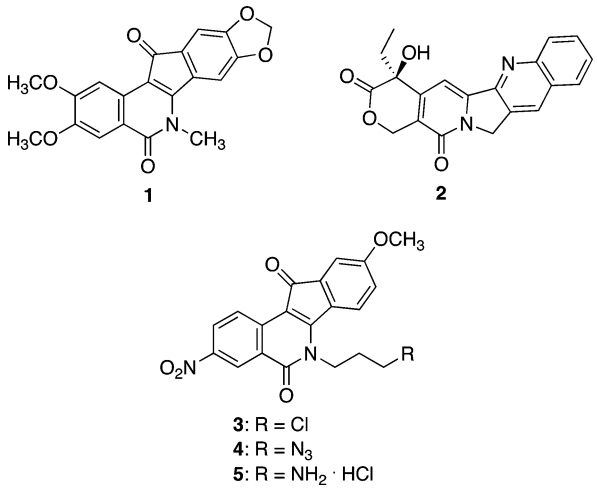


Figure 1. Representative topoisomerase I inhibitors.

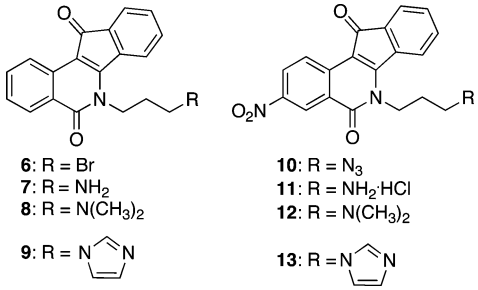


Figure 2. Indenoisoquinolines included in the current study.

biological activity of the indenoisoquinolines. Furthermore, the study has been conducted utilizing lactam side chains expected to confer enhanced biological activity to the analogues in an effort to provide new lead molecules for anticancer therapy.

Chemistry

The syntheses of advanced indenoisoquinoline analogues **20** and **22**−**25** are described in Scheme 1. Condensation of homophthalic anhydrides **14**[33] and **15** with Schiff bases **16**[34] and **17** provided carboxylic acids **18** and **19**. The cis stereochemical relationship was established by the observed coupling constant of ∼6 Hz for the two methine protons.[35] These carboxylic acids were subjected to oxidative Friedel−Crafts ring closure with thionyl chloride[36] and aluminum chloride[21,30] to provide indenoisoquinolines **20** and **21**. Indenoisoquinoline **3** was readily available from a previous synthetic endeavor.[30]

It was quickly recognized that the choice of halide leaving group on the lactam side chain was critical for successful S_N2 displacement with organic amines such as dimethylamine, ethanolamine, morpholine, and imidazole. Azide, however, was a suitably active nucleophile for displacement of the alkylchloride present in **21** to provide analogue **22**, which was subsequently reduced to provide compound **23** upon isolation as the hydrochloride salt. Unsuccessful efforts to displace the alkyl chloride motifs present in compounds **3** and **21** using organic amines as nucleophiles prompted their conversion to the corresponding alkyl iodides **24** and **25**, thereby providing advanced intermediates amenable to the preparation of the desired analogues. Subsequent experiments determined that lactam-substituted alkyl bromides, such as **20**, were suitably functionalized for nucleophilic displacement to provide the desired analogues.

Benz[*d*]indeno[1,2-*b*]pyran-5,11-dione **26**[26] was treated with the corresponding primary amine to provide the desired target compound (**27**, Scheme 2). This procedure was also utilized, as previously described, for the preparation of compounds **6**−**13**.[17,22,26]

With compounds **20**, **24**, **25**, and **6** appropriately functionalized for analogue synthesis, target compounds were then prepared according to Scheme 3. The alkyl halide side chain present in compound **6** was initially converted into the corresponding alkylazide **28** by treatment with sodium azide in dimethylsulfoxide. Compound **28** was not subjected to reduction (analogous to **22**) because the corresponding derivative **7** was previously prepared by an alternative method.[22] Furthermore, the corresponding azido- and amino-substituted analogues derived from **20** and **26** have been previously prepared.[21,30]

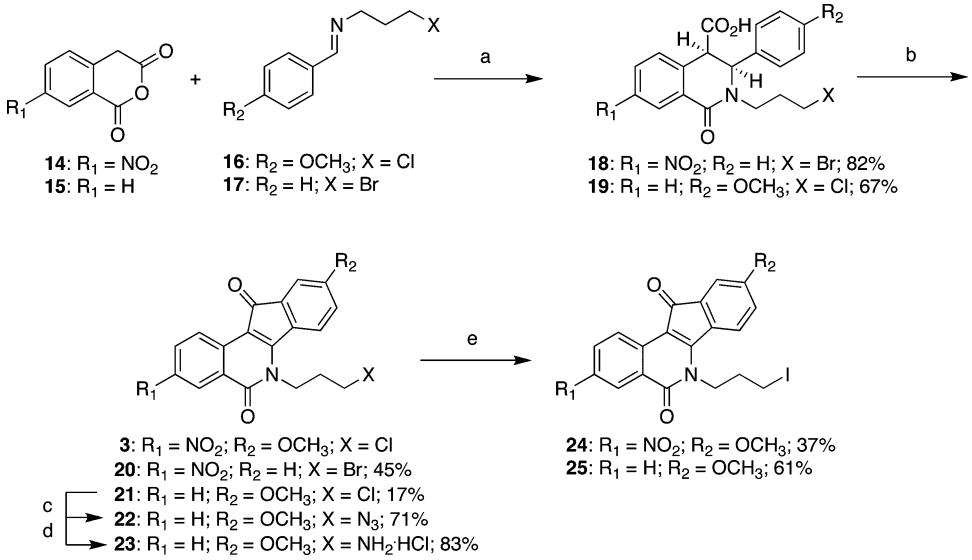
Compounds **20**, **24**, and **25** were treated with morpholine in refluxing 1,4-dioxane to provide target compounds **29**−**31**. Compounds **32**−**35** were prepared by treatment of **20**, **24**, **25**, and **6** with ethanolamine in DMSO and isolated as their respective hydrochloride salts. Compounds **24** and **25** were treated with dimethylamine in refluxing 1,4-dioxane to afford target compounds **36** and **37**, which were isolated as their respective hydrochloride salts. Finally, target compounds **38** and **39** were prepared by treatment of **24** and **25** with imidazole in refluxing 1,4-dioxane.

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.[37,38] 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}−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 the previously reported lead compound **1**, camptothecin (**2**), and compounds **3**−**5** are also included in the table.[6,30] The relative potencies of the compounds in the production of topoisomerase I mediated DNA cleavage are also listed in the table. These results were expressed semiquantitatively as follows: 0, no detectable activity; +, weak activity; ++, similar activity as compound **1**; +++ and ++++, greater activity than compound **1**; ++++, similar activity as 1 μM camptothecin.

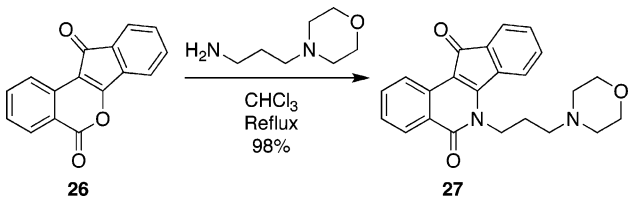
Examination of the results in Table 1 indicates that the substitution pattern at the indenoisoquinoline 3-position, 9-position, and the lactam side chain has a pronounced effect on the biological activities of the molecules. If an "active" compound is defined as one that displays an MGM value of less than 1 μM or Top1 inhibitory activity of +++ or greater, then the trends in substitution pattern can be readily evaluated. Four groups of compounds can be categorized by evaluating the 3- and 9-positions: group 1, hydrogen-substituted (**6**−**9**, **27**, **28**, and **35**); group 2, 3-position hydrogen and 9-methoxy (**21**−**23**, **31**, **34**, **37**, and **39**); group 3, 3-nitro and 9-position hydrogen (**10**−**13**, **20**, **29**, and **32**); group 4, 3-nitro and 9-methoxy (**3**−**5**, **24**, **30**, **33**, **36**, and **38**). These four groups all possess optimized lactam side chains and differ only in the presence or absence of a nitro or methoxy substituent at the 3- or 9-positions. The enhancing effect of a 3-nitro group and a 9-methoxy group on biological activity has previously been identified, and an electrostatic charge complementarity hypothesis has been offered to help rationalize the effects.[30] However, a detailed analysis

Scheme 1[a]



[a] Reagents and conditions: (a) $CHCl_3$, room temp; (b) (i) $SOCl_2$, benzene, reflux; (ii) $AlCl_3$, nitrobenzene, 100 °C; (c) NaN_3, DMSO, 100 °C; (d) (i) $P(OEt)_3$, benzene, reflux; (ii) 3 M HCl in MeOH, reflux; (e) NaI, acetone, reflux.

Scheme 2



of the extent to which each substituent modulates the biological activity of the indenoisoquinolines has not been performed. Thus, examination of these groups in the context of this study should provide insight into the importance of each substituent regarding cytotoxicity and Top1 inhibition.

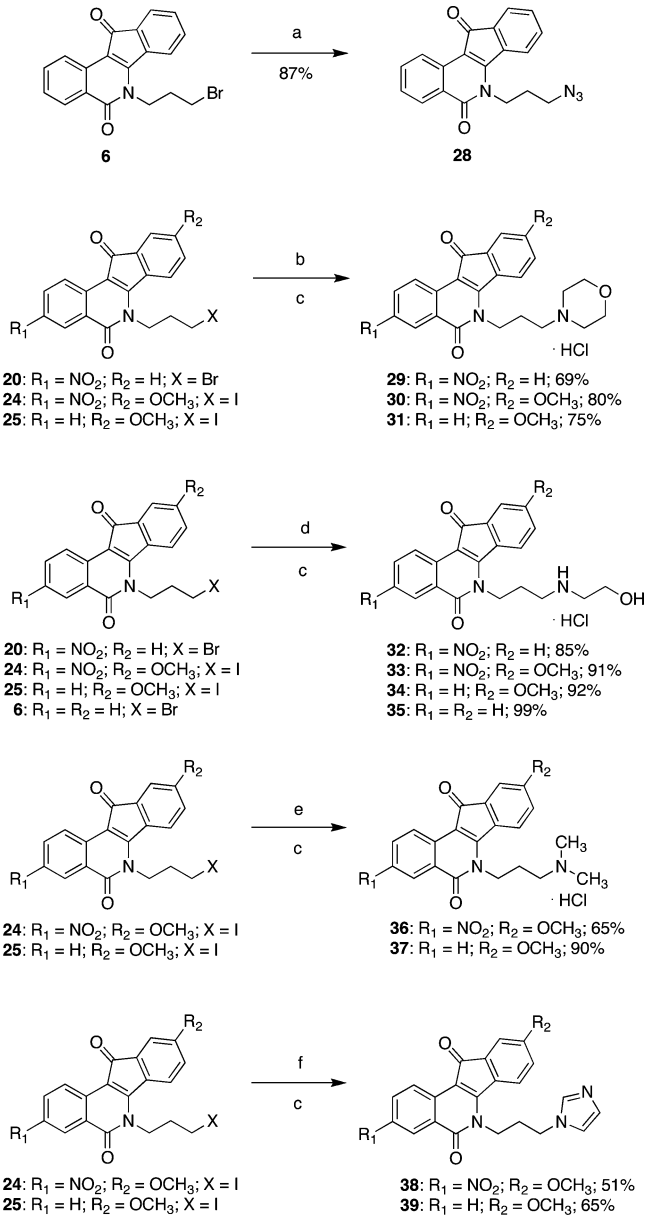
Investigation of the results for group 1 (3- and 9-positions substituted with hydrogen) reveals that only two compounds (**7** and **35**) satisfy the criterion for active compounds as defined by having an MGM value of less than 1 μM. Furthermore, four compounds (**7**, **8**, **9**, and **35**) possessed Top1 inhibitory activities of +++ or greater. However, one can readily conclude from Table 1 that substituting the 3- or 9-position with hydrogen atoms is less optimal (relative to the other compounds in Table 1) for biological activity. It is noted that the compounds designed and evaluated in group 1 were not intended to be the most active compounds because this distinction was intended for group 4 on the basis of previously gleaned structure−activity relationships.[21,30] However, the biological results for the compounds in group 1 do serve to provide a benchmark for evaluating the efficacy of the nitro and methoxy substituents at the 3- and 9-positions in the other groups.

In contrast to group 1, the analogues classified according to group 2 (3-position hydrogen atom and 9-methoxy group) displayed improved cytotoxicity profiles. Four compounds (**23**, **34**, **37**, and **39**) possessed submicromolar MGM values (a 2-fold improvement versus group 1) and four compounds (**21**, **22**, **31**, and **39**) demonstrated potent Top1 inhibition (+++). Therefore, it can be concluded that the 9-methoxy substituent contributes greatly to the improvement in cellular cytotoxicity relative to the hydrogen-substituted analogues in group 1. Interestingly, only analogue **39**, with its imidazolyl-substituted lactam side chain, fits the criterion established for an "active" compound in both assays. This implies that Top1 inhibition and cytotoxicity in the NCI 55 cell screen are not always strongly correlated within the indenoisoquinoline class of Top1 inhibitors and several of these molecules may be interacting with other cellular targets leading to the observed cytotoxicity.

Previously derived structure−activity relationships predicted that the group 3 analogues, with their 3-nitro substituent, would greatly outperform the group 2 analogues in the biological assays.[21,30] Interestingly, the magnitude of this trend was less than predicted. In group 3, four compounds (**11**, **13**, **29**, and **32**) displayed submicromolar MGM values. This was the same number of compounds as that observed for group 2, thereby indicating that the presence of the 3-nitro group did not improve the relative cytotoxic nature of the analogues to a greater extent than the 9-methoxy group. Five compounds (**11**, **12**, **13**, **29**, and **32**) displayed Top1 inhibition of +++ or greater. This was a slight improvement over the groups 1 and 2 analogues and suggests a role for the 3-nitro group in improving Top1 inhibition. Furthermore, group 3 demonstrated an improvement for both cytotoxicity and Top1 inhibition relative to group 1. Thus, it appears that the 3-postion nitro group and the 9-methoxy group are both independently capable of affording potent biological activity. If this speculation is correct, then preparing analogues with the 3- and 9-position both substituted with a nitro and methoxy group, respectively, should provide more potent analogues than in situations in which only one of the two substituents is present.[30]

Analysis of the analogues in group 4 appears to confirm this hypothesis. In group 4 all eight analogues (**3**, **4**, **5**, **24**, **30**, **33**, **36**, and **38**) possessed submicromolar MGM values. Additionally, all of the compounds in group 4 displayed potent Top1 inhibition greater than or equal to +++. Furthermore, compounds **5**, **30**, **33**, **36**, and **38** were all 2−3 times more cytotoxic than camptothecin (**2**). Except for compound **4**, all of the group 4 analogues were at least equipotent to camptothecin in their abilities to inhibit the enzyme. A comparison of the analogues in group 4 with those in group 1 provides an even more striking example of the substituents' ability to modulate biological activity. The possession of both the nitro and methoxy substituents provided 4 times as many "active" analogues based on their MGM values. Additionally, those molecules were 20−1000 times more cytotoxic. Thus, when it comes to the

Scheme 3[a]



[a] Reagents and conditions: (a) NaN_3, DMSO, 100 °C; (b) morpholine, K_2CO_3, 1,4-dioxane, reflux; (c) 3 M HCl in MeOH; (d) ethanolamine, DMSO, room temp; (e) dimethylamine, K_2CO_3, 1,4-dioxane, reflux; (f) imidazole, K_2CO_3, 1,4-dioxane, reflux.

biological activity of these indenoisoquinoline analogues, a definite synergistic benefit results from possessing both a 3-nitro group and a 9-methoxy group.

The results in Table 1 also provide for an alternative method of analysis by evaluating analogues with the same lactam side chain but different 3- and 9-position substituents. From this method, it follows that there are five groups represented in Table 1 that differ by their respective lactam side chains (each of which has been proven to improve the biological activity of the indenoisoquinolines)[17,20,22,24,26,29,31] and four members in each group. Compounds **5** (MGM, 0.027 μM; Top1, ++++), **7** (MGM, 0.320 μM; Top1, +++), **11** (MGM, 0.143 μM; Top1, ++++), and **23** (MGM, 0.048 μM; Top1, +) all possess propylamino-substituted lactam nitrogens but have different aromatic ring substituents. Thus, evaluating the differences in the biological activities between these molecules should allow conclusions to be drawn regarding specific contributions to the biological activity by the 3- and 9-position substituents. Using compound **7** as the benchmark (since it possesses hydrogen atoms substituted at the 3- and 9- positions), one can clearly see that substituting a nitro group at the 3-position (analogue **11**) resulted in a 2-fold increase in cytotoxicity with a concomitant 20% increase in Top1 inhibition. Substituting a methoxy group at the 9-position (analogue **23**) resulted in a 7-fold increase in cytotoxicity but at the cost of Top1 inhibition. Aromatic rings with both substituents (analogue **5**) resulted in a 12-fold increase in cytotoxicity and a 60% increase in Top1 inhibition. Therefore, it is possible to make the generalization that the 3-nitro group tends to improve Top1 inhibition to a greater extent than cytotoxicity and the 9-methoxy group improves cytotoxicity more than Top1 inhibition.

Compounds **8** (MGM, 1.86 μM; Top1, +++), **12** (MGM, 9.77 μM; Top1, +++), **36** (MGM, 0.020 μM; Top1, ++++), and **37** (MGM, 0.300 μM; Top1, 0) all possess dimethylaminopropyl-substituted lactam nitrogens and displayed biological activities similar to the previous group. Surprisingly, the trend observed for the previous group with aminopropyl-substituted lactam nitrogens was not exactly similar to the current group, especially given the similarity of the lactam side chains. In the current group, substituting the 3-position with a nitro group was not as beneficial to the cytotoxicity (analogue **12**) because it was 5 times less active than the benchmark compound **8**. However, it did retain Top1 inhibition. Substituting the 9-position with a methoxy group (analogue **37**) resulted in a compound that was 6 times more cytotoxic but provided a poor Top1 inhibitor. This was similar to the previously identified trend for the 9-methoxy group. Accordingly, when the 3- and 9-positions were substituted with the nitro and methoxy groups, respectively, analogue **36** demonstrated a 93-fold improvement in cytotoxicity and provided a potent Top1 inhibitor.

From continuation of the analysis, compounds **27** (MGM, 15.1 μM; Top1, ++), **29** (MGM, 0.632 μM; Top1, +++), **30** (MGM, 0.014 μM; Top1, ++++), and **31** (MGM, 2.70 μM; Top1, +++) all possess morpholine-substituted lactam nitrogens. By return to a previously identified trend, substituting a nitro group at the 3-position (analogue **29**) resulted in a 24-fold increase in cytotoxicity relative to the benchmark compound **27**. Interestingly, the 9-methoxy-substituted analogue **31** did not display the typical large increase in cytotoxicity as previously observed, although a small increase in activity did occur and good Top1 inhibition was maintained. Exactly as before, substituting both the 3- and 9-positions resulted in an exceedingly potent compound **30** that was over 1000 times more cytotoxic than **27** and was a potent Top1 inhibitor.

The DNA cleavage patterns produced by camptothecin (**2**, lane 3), the indenoisoquinoline NSC 314622 (lane 4), and compounds **27** and **29**−**31** are displayed in Figure 3. 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 compounds **27** (Top1, ++), **29** (Top1, +++), and **31** (Top1, +++) are slightly weaker in comparison with **30** (Top1, ++++) and camptothecin (Top1, ++++). (2) Top1 inhibitors can be classified as Top1 suppressors, which inhibit DNA cleavage, and as Top1 poisons, which inhibit the religation reaction after DNA cleavage. Many of the Top1-mediated DNA cleavages are trapped at lower compound concentrations and suppressed at higher concentrations, and therefore, the indenoisoquinolines act as Top1 poisons at lower concentrations and as Top1 suppressors at higher concentrations. The suppression could result from binding of the drug to the DNA, rendering it a poorer enzyme substrate at high drug

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

	cytotoxicity (GI_{50} in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b] (μM)	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	++++
3	0.295	0.794	0.027	<0.010	3.39	<0.010	0.036	3.24	0.178 ± 0.012	++++
4	<0.001	<0.001	<0.001		0.155		<0.001	0.251	0.198 ± 0.18	+++
5	<0.010	<0.010	<0.010	<0.010	2.82	<0.010		3.31	0.027 ± 0.008	++++
6	4.59	4.47	4.61	10.1	23.5	7	13	21.5	13.3 ± 4.80	+
7	0.20	0.18	0.25	0.26	1.38	0.16	0.22	0.78	0.32 ± 0.23	+++
8	1.74	0.58	1.86	0.51	1.7	0.91	1.32	2.82	1.86	+++
9	2.69	1.41	2.34	0.79	1.66	1.66	1.41	2.75	1.86	++++
10	>100	8.13	79.4	44.7		72.4	>100	>100	13.5	++
11	0.275	0.085	0.302	0.372	0.112	0.102	0.148	0.832	0.143 ± 0.097	++++
12	5.62	6.46		7.08	25.7	4.17	5.62	>100	9.77	+++
13	0.19	0.274	0.016	0.012	0.864	0.015	0.017	2.17	0.370 ± 0.28	++++
20		3.47	>100	>100	>100	>100	>100	>100	40.0	0
21										+++
22	33.9	26.9	44.7	75.9	52.5	>100	61.7	64.6	38.9	+++
23	<0.010	<0.010	0.038		0.028	<0.010	0.014	0.059	0.048 ± 0.024	+
24		0.046	0.058	0.148	3.02	0.309	0.034	1.48	0.328 ± 0.046	++++
27	3.72		3.47	5.37	16.6	21.4	7.24	>100	15.1	++
28	7.59	4.90		19.5	7.94	25.1	29.5	7.76	12.3	0
29	0.021	0.038	0.095	0.380		0.309	0.085	1.23	0.632 ± 0.029	+++
30	<0.010	<0.010		<0.010	<0.010	<0.010		<0.010	0.014 ± 0.001	++++
31	1.41	1.26	1.95	1.58	2.69	4.07	2.29	4.68	2.70 ± 0.125	+++
32	0.031	0.027	>100	0.200	1.35	0.229	>100	1.07	0.296 ± 0.067	++++
33	<0.010		<0.010	<0.010	<0.010	0.012	<0.010	<0.010	0.016	++++
34	0.026	0.044	0.112	0.550	0.417	0.158	0.055	0.389	0.124 ± 0.014	0
35	0.195		0.550	0.178	0.550	0.269	0.174	0.490	0.339	++++
36	<0.010	<0.010	<0.010	<0.010	0.028	<0.010	<0.010	<0.010	0.020 ± 0.001	++++
37	0.078	0.102	0.240	1.00	0.427	0.245	0.257	0.617	0.300 ± 0.072	0
38	<0.010	<0.010	<0.010	<0.010	0.020	<0.010	<0.010	<0.010	0.019 ± 0.004	++++
39	0.056	0.110	0.178	0.071	1.66	0.676	0.204	0.646	0.416 ± 0.134	+++

[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; ++ and +++, modest activity; ++++, similar activity as 1 μM camptothecin.

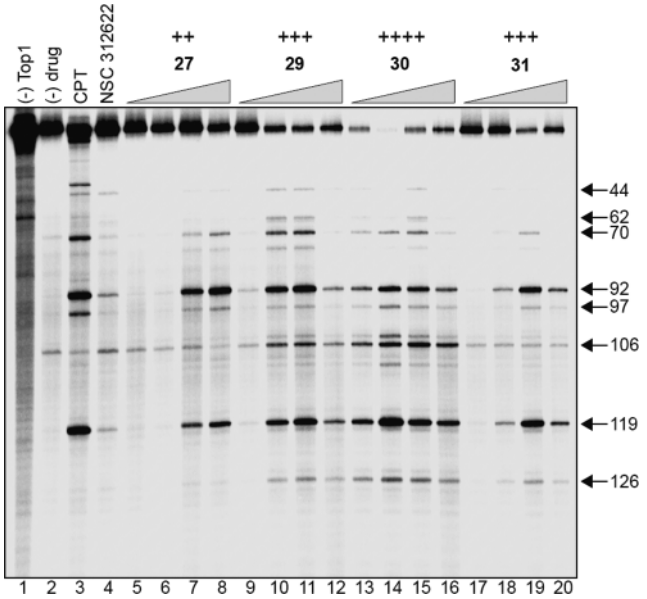


Figure 3. Lane 1: DNA alone. Lane 2: Top1 alone. Lane 3: +CPT (1 μM). Lane 4: Top1 + NSC 314622 (100 μM). Lanes 5−20 (for compounds **27**, **29**, **30**, and **31**): Top1 + indicated compound at 0.1, 1, 10, and 100 μM, respectively. Number on right and arrows indicate cleavage site positions.

concentration, or from a direct effect on the enzyme to suppress its ability to cleave DNA. Interestingly, compound **27** (the least potent analogue of the series) was the only analogue presented in Figure 3 that did not demonstrate this effect. (3) There are differences in the cleavage pattern of camptothecin vs the indenoisoquinolines. For example, the cleavages at base pairs 44 and 126 seen with the indenoisoquinolines are absent with camptothecin. Alternatively, the cleavage at base pair 97 is much more intense for camptothecin than for any of the indenoisoquinoline analogues. These differences may indicate that the indenoisoquinolines might display antitumor spectra different from camptothecin or its clinically useful derivatives irinotecan and topotecan.

For compounds **32** (MGM, 0.296 μM; Top1, ++++), **33** (MGM, 0.016 μM; Top1, ++++), **34** (MGM, 0.124 μM; Top1, 0), and **35** (MGM, 0.339 μM; Top1, ++++), all possessing an ethanolamino-substituted lactam nitrogen, the trends in biological activity were less pronounced than the previous groups because of the increased potency of the benchmark compound **35**. Substituting the 3-position with a nitro group (analogue **32**) had very little effect on the cytotoxicity, whereas substituting the 9-position with a methoxy group resulted in the typical increase in cytotoxic activity (although diminished relative to the previously examined groups). Yet again, substituting both the 3- and 9-position with the nitro and methoxy groups yielded an exceedingly potent compound **33** that is 21-fold more cytotoxic than the benchmark compound **35**. This compound is even more interesting because of the ethanolamine-containing side chain, which is envisioned to be a viable handle for formulating the indenoisoquinoline via pegylation to improve its solubility profile for advanced biological testing.

The final group of compounds to examine are those that possess imidazolyl-substituted lactam nitrogens. Analogues **9** (MGM, 1.86 μM; Top1, ++++), **13** (MGM, 0.370 μM; Top1, ++++), **38** (MGM, 0.019 μM; Top1, ++++), and **39** (MGM,

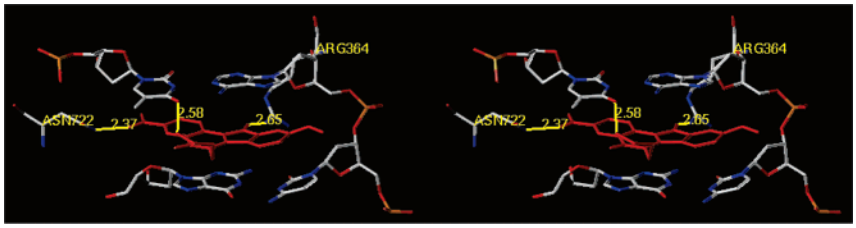


Figure 4. Hypothetical model of compound **5** (red) in ternary complex with DNA and Top1. Top1 amino acid residues not involved in bonding interactions have been removed to improve clarity. The stereoview is programmed for wall-eyed (relaxed) viewing.

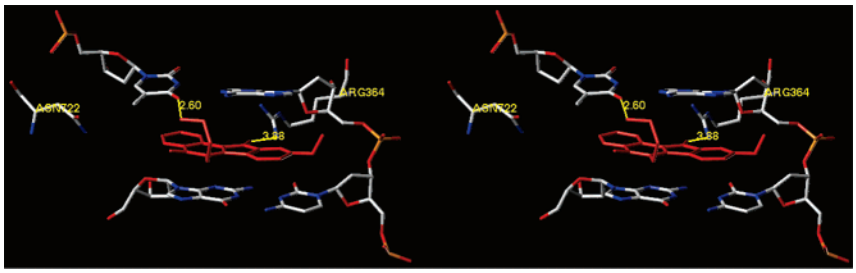


Figure 5. Hypothetical model of compound **23** (red) in ternary complex with DNA and Top1. Top1 amino acid residues not involved in bonding interactions have been removed to improve clarity. The stereoview is programmed for wall-eyed (relaxed) viewing.

0.416 μM; Top1, +++) were in general very potent compounds, performing well in both the cytotoxicity and Top1 inhibition assays. This tendency has been previously observed and was a major determinant for the inclusion of the imidazole group in the present study.[20,26,29] Unlike the previously analyzed group of compounds (those possessing an ethanolamino-substituted lactam nitrogen), introduction of a nitro group at the 3-position had an improving effect on cytotoxicity, providing a compound that was 5 times more cytotoxic than the benchmark compound **9**. However, the Top1 inhibition remained unchanged (yet equal in magnitude to camptothecin). In the present case, introducing a methoxy group at the 9-position (analogue **39**) resulted in a less pronounced effect on cytotoxicity, providing a compound only 4-fold more active than the benchmark analogue. Interestingly, Top1 inhibition remained potent for **39** (Top1, +++), which is in contrast to the other analogues with a similar aromatic ring substitution investigated in this study. Thus, the result highlights the ability of the imidazole group on the lactam side chain to confer potent Top1 inhibition in the absence of aromatic ring substituents that tend to promote such activity. Last, substituting both the 3- and 9-positions with nitro and methoxy groups, respectively, afforded an exceedingly potent compound **38** similar to those in the previously observed groups.

In an attempt to further understand the contribution toward Top1 inhibition by the 3-nitro group, hypothetical binding models were developed using compounds **5** and **23** and a previously reported crystal structure[8] of an indenoisoquinoline in ternary complex with DNA and Top1 (Figures 4 and 5). Compound **5** is capable of making three hydrogen-bonding contacts to enhance the stabilization of the ternary complex, illustrated as yellow lines in Figure 4. One oxygen atom of the nitro group is shown to participate in hydrogen bonding with the amide protons of Asn722 (heavy atom distance of 2.37 Å), thereby providing one potential explanation for the increased Top1 inhibition of indenoisoquinoline **5** relative to compound **23**. The amino group on the lactam side chain of **5** is suitably positioned in the hypothetical model for hydrogen bonding with an oxygen atom of thymine (heavy atom distance of 2.58 Å). However, compound **23** is capable of interacting in a similar manner (heavy atom distance 2.60 Å, Figure 5). This implies that the lactam side chain interactions are not responsible for the difference in biological activities between **5** and **23** and that the effect results solely from the presence or absence of the nitro group. Last, a hydrogen bond is proposed to exist between the 11-position carbonyl oxygen of **5** and the guanidine protons of Arg364 (heavy atom distance of 2.65 Å). Interestingly, compound **23** displays a similar hydrogen-bonding interaction with Arg364, but the interaction is envisioned to be weaker (relative to **5**) because of the increased heavy atom distance (3.88 Å). This increase, along with the absence of a hydrogen-bonding interaction with Asn722, allows compound **23** to orient in such a way as to allow the 9-methoxy group to achieve a nonplanar orientation extending into the nonscissile DNA strand. Prior observations with camptothecins and indenoisoquinolines have confirmed the detrimental effects on Top1 inhibition for analogues possessing nonplanar substituents protruding into the nonscissile area of the cleavage complex.[12,30,39] Thus, the present observations are significant because they rationalize the data in Table 1 where Top1 inhibition is typically decreased for analogues possessing a 3-position hydrogen atom and a 9-methoxy group. Alternatively, the observations help to explain why Top1 inhibition is significantly enhanced when a nitro group replaces the 3-position hydrogen atom. From examination of the data for compound **7** in Table 1, it appears to fit these hypotheses because its cytotoxicity was less potent than **5** and **23** (predicted because it lacks the 9-methoxy group) and it was a less potent topoisomerase I inhibitor than **5** (predicted because **7** lacks the 3-nitro group) but a more potent inhibitor than **23** (a less predictable result but still in line with the observation that the 9-methoxy group is not the sole determining factor for improving, or limiting, enzyme inhibition).

Previous molecular modeling studies have indicated that π-stacking plays a definitive role in the binding of indenoisoquinolines in the ternary complex.[27] Utilizing charge complementarity to gain insight into the π-stacking interactions, a hypothetical binding model has been developed that emphasizes another role for the 3-nitro group (Figures 6 and 7). The models illustrated in Figures 6 and 7 were constructed from the energy-minimized complexes of **5** and **23** in ternary complex with DNA and Top1. Replacement of the aminopropyl lactam side chains present in compounds **5** and **23** with a methyl group was performed in order to improve the overall contrast between areas of net negative and positive charge in the model. This simplification seemed justified because the present emphasis is

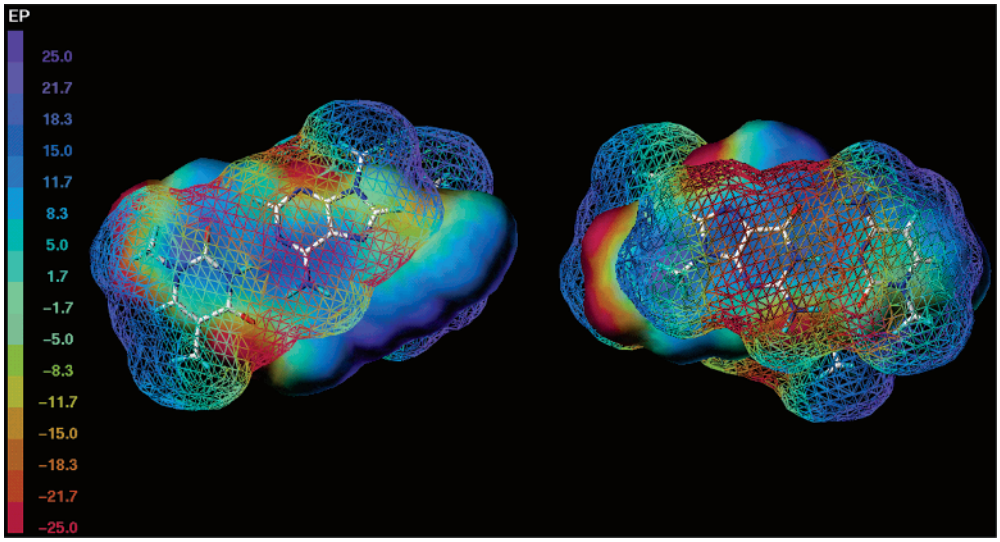


Figure 6. Electrostatic potential surfaces for truncated analogue **5** (solid surface) intercalated between DNA base pairs (line surface). The left model is a view of the −1 base pair, and the right model is a view of the +1 base pair. Red indicates regions of negative charge, and blue indicates regions of positive charge. All generated surfaces were globally scaled to a range of +25 to −25 kcal/mol and used MMFF94 charges.

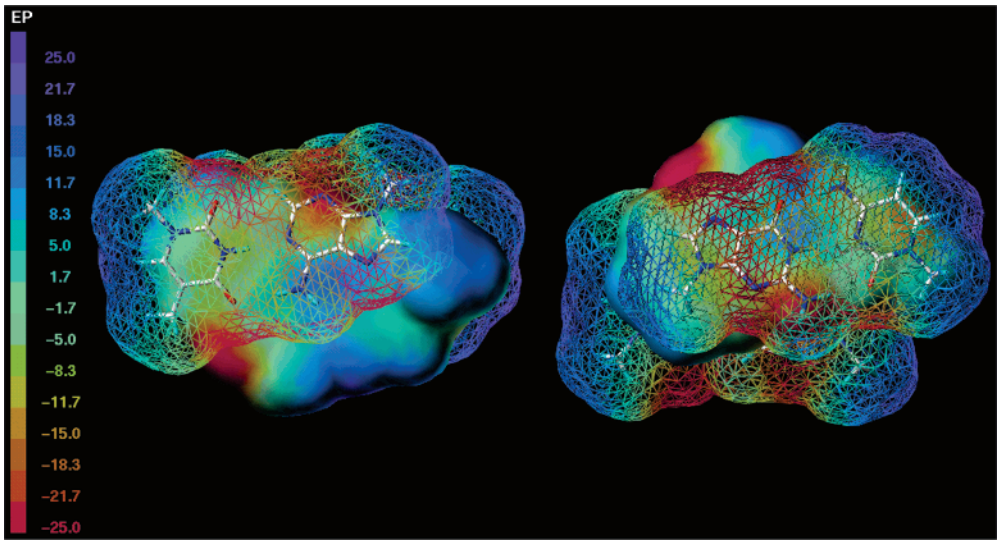


Figure 7. Electrostatic potential surfaces for truncated analogue **23** (solid surface) intercalated between DNA base pairs (line surface). The left model is a view of the −1 base pair, and the right model is a view of the +1 base pair. Red indicates regions of negative charge, and blue indicates regions of positive charge. All generated surfaces were globally scaled to a range of +25 to −25 kcal/mol and used MMFF94 charges.

on the aromatic ring substituents and not the lactam side chains, which would be expected to have similar interactions for both compounds in the ternary complex. Last, the deoxyribose sugars of the DNA base pairs at the site of intercalation were replaced with methyl groups, a modification previously demonstrated to still allow accurate calculations predicting the binding of an indenoisoquinoline.[27] Electrostatic potential surfaces [globally scaled to a range of −25 kcal/mol (red) to +25 kcal/mol (blue)] were then generated in Sybyl 6.9 using MMFF94 charges for the DNA base pairs (illustrated as lines) and **5** and **23** (both shown as opaque). Each figure depicts orientations that allow viewing of charge complementarity between the indenoisoquinoline and the −1 base pair (left) and +1 base pair (right).

In Figure 6, the nitro group illustrates two beneficial effects toward Top1 inhibition. First, the electron-rich oxygen atoms of the nitro groups present in **5** complements the electron-deficient outer edges of the scissile strand DNA base pair (left edge of both orientations). Second, the electron-withdrawing nature of the nitro group results in a relatively electron-deficient indenoisoquinoline aromatic ring system (compared to the DNA base pairs). This deficiency results in good electrostatic complementarity of **5** with the DNA base pairs and contributes to the stabilization of the complex from π-stacking interactions. This rationale helps to explain the substantial loss in Top1 inhibition reported in Table 1 from the replacement of the 3-nitro group with a hydrogen atom (compound **23**, Figure 7). Examination of Figure 7 indicates that there is a substantial decrease in beneficial electrostatic complementarity between the isoquinoline ring (left edge of both orientations) and the DNA base pairs in the cleavage complex. Thus, electrostatic charge complementarity, in conjunction with hydrogen-bonding to Asn722, appears to rationalize the enhanced biological activity of Top1 inhibitors that possess a nitro substituent at the 3-position.

In conclusion, the present study provides a detailed analysis of the effects of the 3-nitro group and the 9-methoxy group. Trends observed from the biological activities reported in Table 1 indicate that the 9-methoxy group typically improves the cytotoxicity of the indenoisoquinoline analogues at the expense of Top1 inhibition. Alternatively, the 3-nitro group was found to improve Top1 inhibition to a greater extent than cytotoxicity

(although many compounds exhibited significant increases in both assays). Furthermore, a comparison of analogues **5** and **23**, utilizing hypothetical models, indicates the importance of the 3-nitro group in both hydrogen-bonding interactions with Top1 and π-stacking interactions with the DNA base pairs in the cleavage complex. Thus, by systematic examination of the nitro and methoxy groups and by observation of differences in the hypothetical models of the selected compounds, the enhanced biological activity previously observed for nitrated indenoisoquinolines has been rationalized.[21,26,28,30] The resulting models will be useful for the future design of effective topoisomerase I inhibitors. Additionally, the use of optimal lactam side chains that tend to confer enhanced biological activity to the indenoisoquinolines has succeeded in providing new lead compounds with exceptionally potent biological activity.

Experimental Section

General. 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 compounds were visualized with short-wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

Benzylidene-(3-bromo-1-propylamine) (17). The hydrobromide salt of 3-bromopropylamine (5.364 g, 24.50 mmol) was treated with triethylamine (4 mL) in $CHCl_3$ (100 mL), and the reaction mixture was allowed to stir at room temperature for 5 min. Benzaldehyde (2.000 g, 18.85 mmol) and magnesium sulfate (6.000 g) were added, and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was filtered, and the filter pad was washed with $CHCl_3$ (50 mL). The filtrate was washed with water (3 × 50 mL) and saturated NaCl (50 mL), dried over sodium sulfate, and concentrated to provide a yellow oil (4.262 g, 100%). IR (film) 1645, 754, and 693 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1 H), 7.76−7.71 (m, 2 H), 7.44−7.40 (m, 3 H), 3.78 (dt, J = 6.3 and 1.3 Hz, 2 H), 3.52 (t, J = 6.5 Hz, 2 H), 2.31 (pent, J = 6.4 Hz, 2 H); ESIMS m/z (rel intensity) 226/228 (MH^+, 100/91). Anal. ($C_{10}H_{12}BrN$) C, H, N.

***cis*-4-Carboxy-3,4-dihydro-*N*-(3-bromopropyl)-3-phenyl-7-nitro-1(2*H*)-isoquinolone (18).** 4-Nitrohomophthalic anhydride (**14**) (3.664 g, 17.69 mmol) was added to a chloroform (125 mL) solution of benzylidene-(3-bromo-1-propylamine) (**17**) (4.000 g, 17.69 mmol), and the reaction mixture was allowed to stir at room temperature for 1.25 h. The precipitate was filtered, washed with chloroform (150 mL), and dried to provide a yellow solid (6.278 g, 82%): mp 158−160 °C. IR (KBr) 3435, 3061, 1743, 1638, 1520, 1349, and 1191 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.90 (d, J = 2.5 Hz, 1 H), 8.38 (dd, J = 8.7 and 2.6 Hz, 1 H), 7.98−7.95 (m, 1 H), 7.25−7.19 (m, 3 H), 7.07−7.03 (m, 2 H), 5.32 (d, J = 6.2 Hz, 1 H), 4.96 (d, J = 6.2 Hz, 1 H), 3.99−3.96 (m, 1 H), 3.52−3.46 (m, 2 H), 3.31−3.22 (m, 1 H), 2.26−2.12 (m, 2 H); negative ion ESIMS m/z (rel intensity) 431/433 [$(M - H^+)^-$, 12/9]. Anal. ($C_{19}H_{17}BrN_2O_5 \cdot 1.0H_2O$) C, H, N.

***cis*-4-Carboxy-*N*-(3-chloropropyl)-3,4-dihydro-3-(4-methoxyphenyl)-1(2*H*)-isoquinolone (19).** Homophthalic anhydride (**15**) (3.065 g, 18.90 mmol) was added to a chloroform (125 mL) solution of 4-methoxybenzylidene-(3-chloro-1-propylamine) (**16**)[34] (4.000 g, 18.90 mmol), and the reaction mixture was allowed to stir at room temperature for 3 h. The precipitate was filtered, washed with chloroform (100 mL), and dried to provide an off-white solid (4.723 g, 67%): mp 180−181 °C. IR (KBr) 3437, 2957, 1740, 1622, 1598, 1573, 1514, 1479, 1258, and 1173 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.10 (dd, J = 7.6 and 1.4 Hz, 1 H), 7.63 (d, J = 7.6 Hz, 1 H), 7.55 (dt, J = 7.4 and 1.5 Hz, 1 H), 7.50−7.43 (m, 1 H), 6.97−6.95 (m, 2 H), 6.75−6.71 (m, 2 H), 5.13 (d, J = 6.3 Hz, 1 H), 4.76 (d, J = 6.2 Hz, 1 H), 3.98−3.92 (m, 1 H), 3.70 (s, 3 H), 3.61−3.57 (m, 2 H), 3.22−3.17 (m, 1 H), 2.13−2.01 (m, 2 H); ESIMS m/z (rel intensity) 374/376 (MH^+, 100/33). Anal. ($C_{20}H_{20}ClNO_4$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-5,11-dioxo-3-nitro-11*H*-indeno[1,2-*c*]isoquinoline (20). Thionyl chloride (5 mL) was added to a solution of *cis*-4-carboxy-3,4-dihydro-*N*-(3-bromopropyl)-3-phenyl-7-nitro-1(2*H*)isoquinolone (**18**) (1.000 g, 2.308 mmol) in benzene (50 mL). The reaction mixture was heated at reflux for 30 min, allowed to cool to room temperature, and concentrated. The residue was diluted with nitrobenzene (30 mL) and chilled in an ice bath, and aluminum chloride (0.616 g, 4.616 mmol) was added. The reaction mixture was removed from the bath and heated at 100 °C for 1 h. Ice−water (100 mL) was added, and the solution was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layer was washed with saturated $NaHCO_3$ (3 × 50 mL) and saturated NaCl (50 mL) and dried over sodium sulfate. The solution was concentrated, hexanes (900 mL) were added, and liquid was decanted. The obtained solid was washed with hexanes (100 mL), and the liquid was again decanted. The crude solid was purified by flash column chromatography (SiO_2), eluting with chloroform, to provide an orange solid (0.432 g, 45%): mp 258−260 °C (dec). IR (film) 1672, 1612, 1560, 1503, 1428, and 1337 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.20 (d, J = 2.4 Hz, 1 H), 8.89 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.92−7.90 (m, 1 H), 7.75−7.72 (m, 1 H), 7.57−7.52 (m, 2 H), 4.76−4.70 (m, 2 H), 3.70 (t, J = 6.2 Hz, 2 H), 2.54−2.48 (m, 2 H); CIMS m/z (rel intensity) 413/415 (MH^+, 100/82). Anal. ($C_{19}H_{13}BrN_2O_4$) C, H, N.

6-(3-Chloropropyl)-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (21). Thionyl chloride (2 mL) was added to a solution of *cis*-4-carboxy-*N*-(3-chloropropyl)-3,4-dihydro-3-(4-methoxyphenyl)-1(2*H*)isoquinolone (**19**) (0.510 g, 1.364 mmol) in benzene (40 mL). The reaction mixture was heated at reflux for 30 min, allowed to cool to room temperature, and concentrated. The residue was diluted with nitrobenzene (20 mL) and chilled in an ice bath, and aluminum chloride (0.364 g, 2.728 mmol) was added. The reaction mixture was removed from the bath and heated at 100 °C for 1.5 h. Ice−water (100 mL) was added, and the solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (3 × 50 mL) and saturated NaCl (50 mL) and dried over sodium sulfate. The solution was concentrated, hexanes (250 mL) were added, and liquid was decanted. The obtained solid was washed with hexanes (100 mL), and the liquid was again decanted. The solid was purified by flash column chromatography (SiO_2), eluting with chloroform, to provide a purple-red solid (0.082 g, 17%) that was precipitated from EtOAc/hexanes: mp 195−198 °C. IR (KBr) 1662, 1611, 1505, 1481, 1432, and 1299 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 8.1 Hz, 1 H), 8.31 (dd, J = 8.2 and 0.7 Hz, 1 H), 7.73−7.71 (m, 1 H), 7.66 (d, J = 8.4 Hz, 1 H), 7.45−7.40 (m, 1 H), 7.22 (d, J = 2.6 Hz, 1 H), 6.86 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.67−4.62 (m, 2 H), 3.89 (s, 3 H), 3.83−3.79 (m, 2 H), 2.43−2.36 (m, 2 H); CIMS m/z (rel intensity) 354/356 (MH^+, 100/30). Anal. ($C_{20}H_{16}ClNO_3$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (22). Thionyl chloride (2 mL) was added to a solution of *cis*-4-carboxy-*N*-(3-chloropropyl)-3,4-dihydro-3-(4-methoxyphenyl)-1(2*H*)isoquinolone (**21**) (0.500 g, 1.337 mmol) in benzene (40 mL). The reaction mixture was heated at reflux for 30 min, allowed to cool to room temperature, and concentrated. The residue was diluted with CH_2Cl_2 (30 mL) and chilled in an ice bath, and aluminum chloride (0.357 g, 2.675 mmol) was added. The reaction mixture was removed from the bath and heated at reflux for 1.5 h. Ice−water (100 mL) was added, and the solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (3 × 50 mL) saturated NaCl (50 mL) and dried over sodium sulfate. The solution was concentrated, and sodium azide (0.087 g, 1.337 mmol) was added. The reaction mixture was diluted with DMSO (50 mL) and heated

at 100 °C for 2 h. The reaction mixture was diluted with $CHCl_3$ (175 mL), washed with water (4 × 30 mL) and saturated NaCl (30 mL), and dried over sodium sulfate. The solution was concentrated to provide a crude solid that was purified by precipitation from EtOAc/hexanes to afford a red solid (0.340 g, 71%): mp 172−173 °C. IR (KBr) 2099, 1661, 1611, 1505, 1481, 1432, and 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 (d, J = 8.1 Hz, 1 H), 8.32 (d, J = 7.3 Hz, 1 H), 7.74−7.68 (m, 1 H), 7.61 (d, J = 8.4 Hz, 1 H), 7.46−7.40 (m, 1 H), 7.24 (d, J = 2.6 Hz, 1 H), 6.89 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.61 (t, J = 7.5 Hz, 2 H), 3.90 (s, 3 H), 3.66 (t, J = 6.1 Hz, 2 H), 2.19−2.10 (m, 2 H); EIMS *m*/*z* (rel intensity) 360 (M^+, 4). Anal. ($C_{20}H_{16}N_4O_3$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (23). Triethyl phosphite (0.12 mL) was added to a solution of 6-(3-azidopropyl)-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**22**) (0.100 g, 0.277 mmol) in benzene (50 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, 3 M HCl in methanol (10 mL) was added, and the reaction mixture was heated at reflux for 3 h. The reaction mixture was allowed to cool to room temperature, and the obtained precipitate was filtered to provide an orange solid (0.085 g, 83%): mp 272−275 °C (dec). IR (KBr) 3430, 1705, 1639, 1611, 1480, 1435, 1305, and 1229 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.54 (d, J = 8.1 Hz, 1 H), 8.20 (d, J = 8.1 Hz, 1 H), 7.88 (bs, 2 H), 7.83 (dt, J = 6.9 and 1.3 Hz, 1 H), 7.52 (t, J = 8.0 Hz, 1 H), 7.15 (d, J = 2.6 Hz, 1 H), 7.02 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.56 (t, J = 6.8 Hz, 2 H), 3.89 (s, 3 H), 2.99−2.94 (m, 2 H), 2.13−2.08 (m, 2 H); ESIMS *m*/*z* (rel intensity) 335 (MH^+, 100). Anal. ($C_{20}H_{19}ClN_2O_3$) C, H, N.

5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (24). Sodium iodide (1.300 g, 8.673 mmol) and 6-(3-chloropropyl)-5,6-dihydro-9-methoxy-3-nitro-5,-11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**3**) (0.200 g, 0.502 mmol) were diluted with acetone (125 mL), and the mixture was heated at reflux for 48 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (300 mL), and filtered. The filtrate was concentrated and purified by flash column chromatography (SiO_2), eluting with chloroform, to provide a red solid (0.090 g, 37%): mp 243−246 °C. IR (film) 1678, 1609, 1554, 1479, 1432, 1336, and 1232 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.81 (d, J = 2.2 Hz, 1 H), 8.60 (d, J = 8.9 Hz, 1 H), 8.51 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.83 (d, J = 8.5 Hz, 1 H), 7.14 (d, J = 2.6 Hz, 1 H), 7.07 (dd, J = 8.5 and 2.6 Hz, 1 H), 4.52 (t, J = 7.6 Hz, 2 H), 3.90 (s, 3 H), 3.50 (t, J = 7.1 Hz, 2 H), 2.35−2.30 (m, 2 H); ESIMS *m*/*z* (rel intensity) 363 (MH^+ − HI, 100). Anal. ($C_{20}H_{15}IN_2O_5$) C, H, N.

5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (25). Sodium iodide (2.338 g, 15.60 mmol) and 6-(3-chloropropyl)-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**21**) (0.311 g, 0.879 mmol) were diluted with acetone (500 mL), and the mixture was heated at reflux for 48 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (300 mL), and filtered. The filtrate was concentrated and purified by flash column chromatography (SiO_2), eluting with chloroform, to provide a red solid (0.240 g, 61%): mp 206−209 °C. IR (KBr) 1651, 1610, 1478, 1433, 1300, and 1228 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.67 (d, J = 7.8 Hz, 1 H), 8.31 (dd, J = 8.2 and 0.7 Hz, 1 H), 7.73 (dt, J = 7.2 and 1.4 Hz, 1 H), 7.64 (d, J = 8.4 Hz, 1 H), 7.45−7.43 (m, 1 H), 7.22 (d, J = 2.6 Hz, 1 H), 6.87 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.59−4.54 (m, 2 H), 3.90 (s, 3 H), 3.39 (t, J = 6.7 Hz, 2 H), 2.45−2.40 (m, 2 H); ESIMS *m*/*z* (rel intensity) 446 (MH^+, 45). Anal. ($C_{20}H_{16}INO_3$) C, H, N.

5,6-Dihydro-6-(3-morpholinyl-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (27). 4-(3-Aminopropyl)-morpholine (0.35 mL, 2.417 mmol) was added to a solution of benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**26**) (0.200 g, 0.806 mmol) in $CHCl_3$ (50 mL). The reaction mixture was allowed to stir at room temperature for 48 h. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with H_2O (3 × 25 mL) and saturated aqueous NaCl (25 mL), dried over Na_2SO_4, and concentrated. The obtained residue was diluted with $CHCl_3$ (50 mL), and 3M HCl in MeOH (10 mL) was added dropwise. The reaction mixture was allowed to stir at room temperature for 24 h, and the precipitate was filtered to provide an orange solid (0.338 g, 98%): mp 310−315 °C. IR (film) 3434, 2929, 2559, 2462, 1695, 1663, 1609, 1549, 1504, 758 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.80 (s, 1 H), 8.60 (d, J = 8.0 Hz, 1 H), 8.24 (d, J = 7.0 Hz, 1 H), 7.87−7.79 (m, 2 H), 7.60−7.49 (m, 4 H), 4.58−4.54 (m, 2 H), 3.96 (d, J = 11.0 Hz, 2 H), 3.77 (t, J = 11.9 Hz, 2 H), 3.36−3.30 (m, 2 H), 3.11−3.06 (m, 2 H), 2.32−2.28 (m, 2 H); ESIMS *m*/*z* (rel intensity) 375 (MH^+, 100). Anal. ($C_{23}H_{23}ClN_2O_3 \cdot 1.0H_2O$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (28). A solution of 6-(3-bromopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**6**) (0.256 g, 0.695 mmol) and sodium azide (0.090 g, 1.390 mmol) in DMSO (20 mL) was allowed to stir at room temperature for 20 h. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with water (3 × 50 mL) and saturated NaCl (50 mL), and dried over sodium sulfate. The solution was concentrated and the crude solid was purified by flash column chromatography (SiO_2), eluting with chloroform, to provide an orange solid (0.199 g, 87%): mp 144−145 °C. IR (film) 2099, 1698, 1662, 1610, 1503, and 1318 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.58 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 8.2 Hz, 1 H), 7.84−7.80 (m, 2 H), 7.61−7.50 (m, 4 H), 4.58 (t, J = 7.6 Hz, 2 H), 3.63 (t, J = 6.5 Hz, 2 H), 2.07−2.02 (m, 2 H); CIMS *m*/*z* (rel intensity) 331 (MH^+, 100). Anal. ($C_{19}H_{14}N_4O_2$) C, H, N.

5,6-Dihydro-6-(3-morpholinyl-1-propyl)-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (29). 6-(3-Bromopropyl)-5,6-dihydro-5,11-dioxo-3-nitro-11*H*-indeno[1,2-*c*]isoquinoline (**20**) (0.200 g, 0.484 mmol) and K_2CO_3 (0.201 g, 1.452 mmol) were diluted with 1,4-dioxane (50 mL). Morpholine (0.21 mL, 2.42 mmol) was added, and the reaction mixture was heated at reflux for 16 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (150 mL), and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The obtained solid was dissolved in $CHCl_3$ (50 mL), 3 M HCl in MeOH was added, and the reaction mixture was allowed to stir at room temperature for 1 h. The precipitate was filtered and washed with $CHCl_3$ and hexanes to provide an orange solid (0.152 g, 69%): mp 280−285 °C (dec). IR (KBr) 3428, 2346, 1697, 1679, 1616, 1560, 1506, 1431, and 1339 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.85 (d, J = 2.4 Hz, 1 H), 8.70 (d, J = 9.0 Hz, 1 H), 8.58 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.91 (d, J = 7.5 Hz, 1 H), 7.68−7.56 (m, 3 H), 4.60 (t, J = 6.8 Hz, 2 H), 3.96 (d, J = 12.0 Hz, 2 H), 3.80 (t, J = 12.0 Hz, 2 H), 3.41−3.34 (m, 4 H), 3.08−3.04 (m, 2 H), 2.32−2.30 (m, 2 H); ESIMS *m*/*z* (rel intensity) 420 (MH^+, 100). Anal. ($C_{23}H_{22}ClN_3O_5 \cdot 0.75H_2O$) C, H, N.

5,6-Dihydro-9-methoxy-6-(3-morpholinyl-1-propyl)-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (30). 5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**24**) (0.200 g, 0.408 mmol) and K_2CO_3 (0.169 g, 1.224 mmol) were diluted with 1,4-dioxane (50 mL). Morpholine (0.18 mL, 2.04 mmol) was added, and the reaction mixture was heated at reflux for 16 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (200 mL), and washed with water (4 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The obtained solid was dissolved in $CHCl_3$ (100 mL), 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. The precipitate was filtered and washed with $CHCl_3$ and hexanes to provide a red solid (0.159 g, 80%): mp 213−215 °C (dec). IR (KBr) 3422, 1682, 1611, 1554, 1504, 1480, 1435, and 1335 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.52 (bs, 1 H), 8.85 (d, J = 2.4 Hz, 1 H), 8.66 (d, J = 9.0 Hz, 1 H), 8.56 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.82 (d, J = 8.6 Hz, 1 H), 7.20 (d, J = 2.5 Hz, 1 H), 7.08 (dd, J = 8.5 and 2.6 Hz, 1 H), 4.56−4.54 (m, 2 H), 3.97−3.91 (m, 5 H), 3.75 (t, J = 11.6 Hz, 2 H), 3.40−3.26 (m, 4 H), 3.08−3.06 (m, 2 H), 2.29−2.26 (m, 2 H); ESIMS *m*/*z* (rel intensity) 450 (MH^+, 100). Anal. ($C_{24}H_{24}ClN_3O_6 \cdot 1.0H_2O$) C, H, N.

5,6-Dihydro-9-methoxy-6-(3-morpholinyl-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (31). 5,6-Di-

hydro-6-(3-iodopropyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (**25**) (0.200 g, 0.449 mmol) and K_2CO_3 (0.620 g, 4.492 mmol) were diluted with 1,4-dioxane (50 mL). Morpholine (0.39 mL, 4.492 mmol) was added, and the reaction mixture was heated at reflux for 2 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (150 mL), and washed with water (4 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The obtained solid was dissolved in $CHCl_3$ (100 mL), 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. The obtained precipitate was filtered and washed with $CHCl_3$ and hexanes to provide a red solid (0.149 g, 75%): mp 265 °C (dec). IR (KBr) 3428, 1659, 1506, 1479, 1458, 1430, 1301, 1227, and 1126 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.65 (bs, 1 H), 8.54 (d, J = 7.8 Hz, 1 H), 8.20 (d, J = 8.1 Hz, 1 H), 7.83−7.78 (m, 1 H), 7.71 (d, J = 8.4 Hz, 1 H), 7.52−7.47 (m, 1 H), 7.15 (d, J = 2.6 Hz, 1 H), 7.01 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.53−4.51 (m, 1 H), 3.95−3.89 (m, 5 H), 3.76−3.68 (m, 2 H), 3.42−3.35 (m, 4 H), 3.09−3.06 (m, 2 H), 2.28−2.25 (m, 2 H); ESIMS *m*/*z* (rel intensity) 405 (MH^+, 100). Anal. ($C_{24}H_{25}ClN_2O_4 \cdot 0.75H_2O$) C, H, N.

5,6-Dihydro-6-[3-(2-hydroxyethyl)amino-1-propyl]-3-nitro-5,-11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (32). Ethanolamine (0.222 g, 3.630 mmol) was added to a solution of 6-(3-bromopropyl)-5,6-dihydro-5,11-dioxo-3-nitro-11*H*-indeno[1,2-*c*]isoquinoline (**20**) (0.100 g, 0.242 mmol) in DMSO (100 mL), and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with $CHCl_3$ (200 mL), washed with water (4 × 100 mL) and saturated NaCl (100 mL), and dried over sodium sulfate. The solution was concentrated and diluted with $CHCl_3$ (100 mL). Then 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated and the solid residue was washed with $CHCl_3$ and filtered to provide an orange solid (0.088 g, 85%): mp 265 °C (dec). IR (KBr) 3414, 1698, 1677, 1615, 1559, 1504, 1428, and 1339 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.92 (d, J = 2.4 Hz, 1 H), 8.77 (d, J = 8.9 Hz, 1 H), 8.63 (bs, 1 H), 8.60 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.94 (d, J = 7.0 Hz, 1 H), 7.71−7.62 (m, 3 H), 5.27 (t, J = 4.8 Hz, 1 H), 4.63 (t, J = 7.2 Hz, 2 H), 3.65 (q, J = 5.2 Hz, 2 H), 3.15 (bs, 2 H), 3.00 (bs, 2 H), 2.26−2.22 (m, 2 H); ESIMS *m*/*z* (rel intensity) 394 (MH^+, 100). Anal. ($C_{21}H_{20}ClN_3O_5 \cdot 0.75H_2O$) C, H, N.

5,6-Dihydro-6-[3-(2-hydroxyethyl)amino-1-propyl]-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (33). Ethanolamine (0.187 g, 3.060 mmol) was added to a solution of 5,6-dihydro-6-(3-iodopropyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**24**) (0.100 g, 0.204 mmol) in DMSO (100 mL), and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with $CHCl_3$ (250 mL), washed with water (3 × 100 mL) and saturated NaCl (50 mL), and dried over sodium sulfate. The solution was concentrated and diluted with $CHCl_3$ (100 mL). Then 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 solid residue was washed with $CHCl_3$ and filtered to provide a red solid (0.086 g, 91%): mp 255−258 °C (dec). IR (KBr) 3347, 2941, 2786, 1679, 1611, 1556, 1504, 1480, 1433, 1335, and 1229 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.81 (d, J = 2.4 Hz, 1 H), 8.76 (bs, 1 H), 8.61 (d, J = 9.0 Hz, 1 H), 8.52 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.80 (d, J = 8.5 Hz, 1 H), 7.14 (d, J = 2.5 Hz, 1 H), 7.06 (dd, J = 8.4 and 2.5 Hz, 1 H), 5.26 (bs, 1 H), 4.55 (t, J = 6.4 Hz, 2 H), 3.90 (s, 3 H), 3.65 (bs, 2 H), 3.15 (bs, 2 H), 2.99 (bs, 2 H), 2.23−2.21 (m, 2 H); ESIMS *m*/*z* (rel intensity) 424 (MH^+, 100). Anal. ($C_{22}H_{22}ClN_3O_6 \cdot 1.0H_2O$) C, H, N.

5,6-Dihydro-6-[3-(2-hydroxyethyl)amino-1-propyl]-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (34). Ethanolamine (0.137 g, 2.250 mmol) was added to a solution of 5,6-dihydro-6-(3-iodopropyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**25**) (0.100 g, 0.225 mmol) in DMSO (50 mL), and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with $CHCl_3$ (250 mL), washed with water (3 × 50 mL) and saturated NaCl (50 mL), and dried over sodium sulfate. The solution was concentrated and diluted with $CHCl_3$ (100 mL). Then 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated and the solid residue was washed with $CHCl_3$ and filtered to provide an orange solid (0.086 g, 92%): mp 275 °C. IR (KBr) 3426, 1704, 1637, 1610, 1477, 1437, 1308, and 1232 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.60 (bs, 2 H), 8.57 (d, J = 8.3 Hz, 1 H), 8.21 (d, J = 8.1 Hz, 1 H), 7.84−7.79 (m, 1 H), 7.73 (d, J = 8.4 Hz, 1 H), 7.53−7.48 (m, 1 H), 7.17 (d, J = 2.6 Hz, 1 H), 7.03 (dd, J = 8.4 and 2.5 Hz, 1 H), 5.26 (t, J = 4.8 Hz, 1 H), 4.56 (t, J = 6.4 Hz, 2 H), 3.89 (s, 3 H), 3.64−3.60 (m, 2 H), 3.12−3.10 (m, 2 H), 3.01−2.98 (m, 2 H), 2.22−2.19 (m, 2 H); ESIMS *m*/*z* (rel intensity) 379 (MH^+, 100). Anal. ($C_{22}H_{23}ClN_2O_4 \cdot 1.25H_2O$) C, H, N.

5,6-Dihydro-6-[3-(2-hydroxyethyl)amino-1-propyl]-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (35). Ethanolamine (0.333 g, 5.445 mmol) was added to a solution of 6-(3-bromopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**6**) (0.150 g, 0.363 mmol) in DMSO (100 mL), and the reaction mixture was allowed to stir at room temperature for 35 h. The reaction mixture was diluted with $CHCl_3$ (200 mL), washed with water (4 × 100 mL) and saturated NaCl (100 mL), and dried over sodium sulfate. The solution was concentrated and diluted with $CHCl_3$ (100 mL). Then 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated, and the solid residue was washed with $CHCl_3$ and filtered to provide an orange solid (0.140 g, 99%): mp 280−283 °C (dec). IR (KBr) 3439, 2944, 2798, 1712, 1638, 1610, 1550, and 1503 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.61 (d, J = 8.0 Hz, 1 H), 8.25 (d, J = 8.1 Hz, 1 H), 7.88−7.81 (m, 2 H), 7.63−7.53 (m, 4 H), 5.22−5.19 (m, 1 H), 4.60 (t, J = 6.7 Hz, 2 H), 3.64−3.61 (m, 2 H), 3.12−3.09 (m, 2 H), 3.00−2.98 (m, 2 H), 2.22−2.18 (m, 2 H); ESIMS *m*/*z* (rel intensity) 349 (MH^+, 100). Anal. ($C_{21}H_{21}ClN_2O_3 \cdot 0.25H_2O$) C, H, N.

5,6-Dihydro-9-methoxy-6-(3-dimethylamino-1-propyl)-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (36). 5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**24**) (0.121 g, 0.246 mmol) and K_2CO_3 (0.155 g, 1.12 mmol) were diluted with 1,4-dioxane (50 mL). Dimethylamine (2 M solution in THF) (1.12 mL, 2.24 mmol) was added, and the reaction mixture was heated at reflux. After 2 h, dimethylamine (2 M solution in THF) (1.12 mL, 2.24 mmol) was added and the reaction mixture was heated at reflux for 16 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (200 mL), and washed with water (4 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$/1% Et_3N to 6% MeOH/$CHCl_3$/1% Et_3N. The obtained solid was dissolved in $CHCl_3$ (50 mL), 3 M HCl in MeOH (10 mL) was added, and the reaction mixture was allowed to stir at room temperature for 3 h. The precipitate was filtered and washed with $CHCl_3$ and hexanes to provide an orange solid (0.072 g, 65%): mp 280−285 °C (dec). IR (KBr) 1681, 1609, 1554, 1478, and 1333 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.99 (bs, 1 H), 8.85 (d, J = 2.4 Hz, 1 H), 8.67 (d, J = 9.0 Hz, 1 H), 8.57 (dd, J = 8.9 and 2.4 Hz, 1 H), 7.82 (d, J = 8.6 Hz, 1 H), 7.21 (d, J = 2.6 Hz, 1 H), 7.08 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.57 (t, J = 6.6 Hz, 2 H), 3.92 (s, 3 H), 3.28−3.16 (m, 2 H), 2.77 (s, 3 H), 2.75 (s, 3 H), 2.27−2.22 (m, 2 H); ESIMS *m*/*z* (rel intensity) 408 (MH^+, 100). Anal. ($C_{22}H_{22}ClN_3O_5 \cdot 1.0H_2O$) C, H, N.

5,6-Dihydro-9-methoxy-6-(3-dimethylamino-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (37). 5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**25**) (0.200 g, 0.449 mmol) and K_2CO_3 (0.620 g, 4.492 mmol) were diluted with 1,4-dioxane (50 mL). Dimethylamine (2 M solution in THF) (2.25 mL, 4.492 mmol) was added, and the reaction mixture was heated at reflux for 4 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (200 mL), and washed with water (4 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The

obtained solid was dissolved in $CHCl_3$ (50 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 precipitate was filtered and washed with $CHCl_3$ to provide a purple solid (0.155 g, 90%): mp 275 °C (dec). IR (KBr) 3427, 1670, 1611, 1504, 1481, 1456, 1294, and 1243 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.35 (bs, 1 H), 8.53 (d, J = 7.8 Hz, 1 H), 8.20 (d, J = 8.0 Hz, 1 H), 7.83 (t, J = 7.3 Hz, 1 H), 7.72 (d, J = 8.4 Hz, 1 H), 7.51 (t, J = 7.4 Hz, 1 H), 7.14 (d, J = 2.4 Hz, 1 H), 7.01 (dd, J = 8.2 and 2.4 Hz, 1 H), 4.53−4.50 (m, 2 H), 3.89 (s, 3 H), 3.36−3.27 (m, 2 H), 2.75−2.74 (m, 6 H), 2.22−2.18 (m, 2 H); ESIMS *m*/*z* (rel intensity) 363 (MH^+, 100). Anal. ($C_{22}H_{23}ClN_2O_3 \cdot 1.75H_2O$) C, H, N.

5,6-Dihydro-6-(3-imidazolyl-1-propyl)-9-methoxy-3-nitro-5,-11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (38). 5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**24**) (0.135 g, 0.275 mmol) and K_2CO_3 (0.190 g, 1.377 mmol) were diluted with 1,4-dioxane (50 mL). Imidazole (0.094 g, 1.377 mmol) was added, and the reaction mixture was heated at reflux for 16 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (200 mL), and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$/1% Et_3N to 4% MeOH/$CHCl_3$/1% Et_3N, to provide a red solid (0.061 g, 51%): mp 215 °C (dec). IR (KBr) 1678, 1610, 1554, 1504, 1478, 1432, and 1335 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.85 (s, 1 H), 8.62−8.61 (m, 1 H), 8.54 (d, J = 9.2 Hz, 1 H), 7.74 (s, 1 H), 7.29 (s, 1 H), 7.25−7.23 (m, 1 H), 7.18 (s, 1 H), 6.97 (s, 1 H), 6.94 (d, J = 7.2 Hz, 1 H), 4.46−4.44 (m, 2 H), 4.25 (t, J = 6.8 Hz, 2 H), 3.90 (s, 3 H), 2.25−2.22 (m, 2 H); ESIMS *m*/*z* (rel intensity) 431 (MH^+, 100). Anal. ($C_{23}H_{18}N_4O_5 \cdot 1.25H_2O$) C, H, N.

5,6-Dihydro-6-(3-imidazolyl-1-propyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (39). 5,6-Dihydro-6-(3-iodopropyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**25**) (0.200 g, 0.449 mmol) and K_2CO_3 (0.620 g, 4.492 mmol) were diluted with 1,4-dioxane (50 mL). Imidazole (0.306 g, 4.492 mmol) was added, and the reaction mixture was heated at reflux for 4 h. The reaction mixture was concentrated, diluted with $CHCl_3$ (150 mL), and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$/1% Et_3N to 6% MeOH/$CHCl_3$/1% Et_3N, to provide a red solid (0.112 g, 65%): mp 145−150 °C. IR (KBr) 1650, 1610, 1505, 1479, 1459, 1431, 1299, 1226, and 788 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.63 (d, J = 8.1 Hz, 1 H), 8.29 (d, J = 8.2 Hz, 1 H), 7.72−7.66 (m, 2 H), 7.44 (t, J = 7.1 Hz, 1 H), 7.19 (s, 1 H), 7.15 (d, J = 2.5 Hz, 1 H), 7.07 (s, 1 H), 6.66 (dd, J = 8.4 and 2.5 Hz, 1 H), 6.59 (d, J = 8.3 Hz, 1 H), 4.50 (t, J = 7.3 Hz, 2 H), 4.25 (t, J = 6.4 Hz, 2 H), 3.85 (s, 3 H), 2.39−2.32 (m, 2 H); ESIMS *m*/*z* (rel intensity) 386 (MH^+, 63). Anal. ($C_{23}H_{19}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Topoisomerase I Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from baculovirus as described previously.[40] The 161 base pair (bp) fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (50 μL mixtures) 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 fragament was 3′-end-labeled at the Hind III site by fill-in reaction with [α-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 unit 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 topoisomerase I 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% polyacrylamine, 7 M urea). Gels were dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The results of the assay were expressed semiquantitatively as follows: 0, no detectable activity; +, weak activity; ++, similar activity as compound **1**; +++ and ++++, greater activity than compound **1**; ++++, similar activity as 1 μM camptothecin.

Molecular Modeling. The structure of the ternary complex, containing topoisomerase I, DNA, and an indenoisoquinoline, was downloaded from the Protein Data Bank (PDB code 1SC7).[8] Several of the atoms were then fixed according to the Sybyl atom types. Hydrogens were added and minimized using the MMFF94s force field and MMFF94 charges. Modeled analogues were constructed in Sybyl 7.1, energy-minimized with the MMFF94s force field and MMFF94 charges, and overlapped with the crystal structure ligand in the ternary complex, and the crystal structure ligand was then deleted. The new complex was subsequently subjected to energy minimization using MMFF94s force field with MMFF94 charges. During the energy minimization, the structure of the indenoisoquinoline was allowed to move while the structures of the protein and nucleic acids were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal mol^{-1} $Å^{-1}$ energy gradient convergence criterion and a distance-dependent dielectric function. Molecular surfaces were created using the MOLCAD module implemented in Sybyl 6.9 with MMFF94 charges.

Acknowledgment. This work was made possible by the National Institutes of Health (NIH) through support of this work with Research Grant UO1 CA89566 and Training Grant ST32 CA09634-12 and by an ACS Medicinal Chemistry Predoctoral Fellowship sponsored by Pfizer Global Research and Development (A.M.). The in vitro testing was conducted through the Developmental Therapeutics Program, DCTD, NCI, under Contract NO1-CO-56000. This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. This research was conducted in a facility constructed with support from Research Facilities Improvement Program Grant C06-14499 from the National Center for Research Resources of the National Institutes of Health.

Supporting Information Available: Elemental analysis results for compounds **17**−**23** and **25**−**39**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wang, J. C. DNA Topoisomerases. *Annu. Rev. Biochem.* **1996**, *65*, 635−692.
(2) Nitiss, J. L. Investigating the Biological Functions of DNA Topoisomerases in Eukaryotic Cells. *Biochim. Biophys. Acta* **1998**, *1400*, 63−81.
(3) Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. Mechanism of Action of Eukaryotic DNA Topoisomerase I and Drugs Targeted to the Enzyme. *Biochim. Biophys. Acta* **1998**, *1400*, 83−106.
(4) Champoux, J. J. DNA Topoisomerases: Structure, Function, and Mechanism. *Annu. Rev. Biochem.* **2001**, *70*, 369−413.
(5) Wang, J. C. Cellular Roles of DNA Topoisomerases: A Molecular Perspective. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430−440.
(6) Kohlhagen, G.; Paull, K. D.; Cushman, M.; Nagafuji, P.; Pommier, Y. Protein-Linked DNA Strand Breaks Induced by NSC 314622, a Novel Noncamptothecin Topoisomerase I Poison. *Mol. Pharmacol.* **1998**, *54*, 50−58.
(7) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analog. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387−15392.
(8) Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. Structures of Three Classes of Anticancer Agents Bound to the Human Topoisomerase I−DNA Covalent Complex. *J. Med. Chem.* **2005**, *48*, 2336−2345.

(9) Ioanoviciu, A.; Antony, S.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Synthesis and Mechanism of Action Studies of a Series of Norindenoisoquinoline Topoisomerase I Poisons Reveal an Inhibitor with a Flipped Orientation in the Ternary DNA−Enzyme−Inhibitor Complex as Determined by X-ray Crystallographic Analysis. *J. Med. Chem.* **2005**, *48*, 4803−4814.
(10) Marchand, C.; Antony, S.; Kohn, K. W.; Cushman, M.; Ioanoviciu, A.; Staker, B. L.; Burgin, A. B.; Stewart, L.; Pommier, Y. A Novel Norindenoisoquinoline Structure Reveals a Common Interfacial Inhibitor Paradigm for Ternary Trapping of the Topoisomerase I−DNA Covalent Complex. *Mol. Cancer Ther.* **2006**, *5*, 287−295.
(11) Takimoto, C. H.; Wright, J.; Arbuck, S. G. Clinical Applications of the Camptothecins. *Biochim. Biophys. Acta* **1998**, *1400*, 107−119.
(12) Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. Structure−Activity Study of the Actions of Camptothecin Derivatives on Mammalian Topoisomerase I: Evidence for a Specific Receptor Site and a Relation to Antitumor Activity. *Cancer Res.* **1989**, *49*, 1465−1469.
(13) Minami, H.; Beijnen, J. H.; Verweij, J.; Ratain, M. J. Limited Sampling Model for Area under the Concentration Time Curve of Total Topotecan. *Clin. Cancer Res.* **1996**, *2* (1), 43−46.
(14) Danks, M. K.; Pawlik, C. A.; Whipple, D. O.; Wolverton, J. S. Intermittent Exposure of Medulloblastoma Cells to Topotecan Produces Growth Inhibition Equivalent to Continuous Exposure. *Curr. Top. Med. Chem.* **1997**, *3* (10), 1731−1738.
(15) Haas, N. B.; LaCreta, F. P.; Walczak, J.; Hudes, G. R.; Brennan, J. M.; Ozols, R. F.; O'Dwyer, P. J. Phase-I Pharmacokinetic Study of Topotecan by 24-Hour Continuous-Infusion Weekly. *Cancer Res.* **1994**, *54* (5), 1220−1226.
(16) Strumberg, D.; Pommier, Y.; Paull, K.; Jayaraman, M.; Nagafuji, P.; Cushman, M. Synthesis of Cytotoxic Indenoisoquinoline Topoisomerase I Poisons. *J. Med. Chem.* **1999**, *42*, 446−457.
(17) Cushman, M.; Jayaraman, M.; Vroman, J. A.; Fukunaga, A. K.; Fox, B. M.; Kohlhagen, G.; Strumberg, D.; Pommier, Y. Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors. *J. Med. Chem.* **2000**, *43*, 3688−3698.
(18) Jayaraman, M.; Fox, B. M.; Hollingshead, M.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of New Dihydroindeno[1,2-*c*]isoquinoline and Indenoisoquinolinium Chloride Topoisomerase I Inhibitors Having High in Vivo Anticancer Activity in the Hollow Fiber Animal Model. *J. Med. Chem.* **2002**, *45*, 242−249.
(19) Fox, B. M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline−Camptothecin Hybrids. *J. Med. Chem.* **2003**, *46*, 3275−3282.
(20) Nagarajan, M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Design, Synthesis, and Biological Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Featuring Polyamine Side Chains on the Lactam Nitrogen. *J. Med. Chem.* **2003**, *46*, 5712−5724.
(21) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3659−3663.
(22) Nagarajan, M.; Morrell, A.; Fort, B. C.; Meckley, M. R.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis and Anticancer Activity of Simplified Indenoisoquinoline Topoisomerase I Inhibitors Lacking Substituents on the Aromatic Rings. *J. Med. Chem.* **2004**, *47*, 5651−5661.
(23) Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Aminoalkenylindenoisoquinoline and 11-Diaminoalkenylindenoisoquinoline Topoisomerase I Inhibitors. *Bioorg. Med. Chem.* **2004**, *12*, 5147−5160.
(24) Antony, S.; Kohlhagen, G.; Agama, K.; Jayaraman, M.; Cao, S.; Durrani, F. A.; Rustum, Y. M.; Cushman, M.; Pommier, Y. Cellular Topoisomerase I Inhibition and Antiproliferative Activity by MJ-III-65 (NSC 706744), an Indenoisoquinoline Topoisomerase I Poison. *Mol. Pharmacol.* **2005**, *67*, 523−530.
(25) Xiao, X.; Miao, Z.-H.; Antony, S.; Pommier, Y.; Cushman, M. Dihydroindenoisoquinolines Function as Prodrugs of Indenoisoquinolines. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2795−2798.
(26) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of Benz[*d*]indeno[1,2-*b*]pyran-5,11-diones: Versatile Intermediates for the Design and Synthesis of Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1846−1849.
(27) Xiao, X.; Antony, S.; Pommier, Y.; Cushman, M. On the Binding of Indeno[1,2-*c*]isoquinolines in the DNA-Topoisomerase I Cleavage Complex. *J. Med. Chem.* **2005**, *48*, 3231−3238.
(28) Morrell, A.; Jayaraman, M.; Nagarajan, M.; Fox, B. M.; Meckley, M. R.; Ioanoviciu, A.; Pommier, Y.; Antony, S.; Hollingshead, M.; Cushman, M. Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Using a Hollow Fiber Assay. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4395−4399.
(29) Nagarajan, M.; Morrell, A.; Ioanoviciu, A.; Antony, S.; Kohlhagen, G.; Hollingshead, M.; Pommier, Y.; Cushman, M. Synthesis and Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Substituted with Nitrogen Heterocycles. *J. Med. Chem.* **2006**, *49*, 6283−6289.
(30) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. A Systematic Study of Nitrated Indenoisoquinolines Reveals a Potent Topoisomerase I Inhibitor. *J. Med. Chem.* **2006**, *49*, 7740−7753.
(31) Antony, S.; Jayaraman, M.; Laco, G.; Kohlhagen, G.; Kohn, K. W.; Cushman, M.; Pommier, Y. Differential Induction of Topoisomerase I−DNA Cleavage Complexes by the Indenoisoquinoline MJ-III-65 (NSC 706744) and Camptothecin: Base Sequence Analysis and Activity against Camptothecin-Resistant Topoisomerase I. *Cancer Res.* **2003**, *63*, 7428−7435.
(32) Ruchelman, A. L.; Singh, S. K.; Liu, A.; Zhou, N.; Liu, L. F.; LaVoie, E. J. Cytotoxicity of 5*H*-Dibenzo[*c*,*h*][1,6]naphthyridin-6-ones and 6*H*-Indeno[1,2-*c*]isoquinoline-5,11-diones in Tumor Cells Sensitive and Resistant to Camptothecin Analogs. *Lett. Drug Des. Discovery* **2004**, *1*, 198−202.
(33) Whitmore, W. F.; Cooney, R. C. The Preparation of Homophthalyl Cyclic Hydrazide and 4-Aminohomophthalyl Cyclic Hydrazide. *J. Am. Chem. Soc.* **1944**, *66*, 1237−1240.
(34) Yus, M.; Soler, T.; Foubelo, F. A New and Direct Synthesis of 2-Substituted Pyrrolidines. *J. Org. Chem.* **2001**, *66*, 6207−6208.
(35) Cushman, M.; Cheng, L. Total Synthesis of Nitidine Chloride. *J. Org. Chem.* **1978**, *43* (2), 286−288.
(36) Cushman, M.; Cheng, L. Stereoselective Oxidation by Thionyl Chloride Leading to the Indeno[1,2-*c*]isoquinoline System. *J. Org. Chem.* **1978**, *43*, 3781−3783.
(37) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82* (13), 1107−1112.
(38) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In-Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(39) Fan, Y.; Weinstein, J. N.; Kohn, K. W.; Shi, L. M.; Pommier, Y. Molecular Modeling Studies of the DNA−Topoisomerase I Ternary Cleavable Complex with Camptothecin. *J. Med. Chem.* **1998**, *41*, 2216−2226.
(40) Pourquier, P.; Ueng, L.-M.; Fertala, J.; Wang, D.; Park, H.-K.; Essigmann, J. M.; Bjornsti, M.-A.; Pommier, Y. Induction of Reversible Complexes between Eukaryotic DNA Topoisomerase I and DNA-Containing Oxidative Base Damages. 7,8-Dihydro-8-oxoguanine and 5-Hydroxycytosine. *J. Biol. Chem.* **1999**, *274*, 8516−8523.

JM070361Q