Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors

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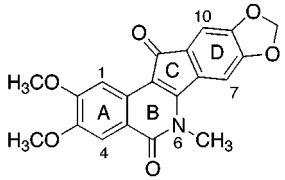
In an attempt to design and synthesize potential anticancer agents acting by inhibition of topoisomerase I (top1), a new series of indenoisoquinolines was prepared and tested for cytotoxicity in human cancer cell cultures and for activity against top1. The synthesis relied on the condensation of substituted Schiff bases with homophthalic anhydrides to produce *cis*-3-aryl-4-carboxyisoquinolones that were cyclized to indenoisoquinolines in the presence of thionyl chloride. Both top1 inhibitory activity and cytotoxicity maximized in a single compound, 6-[3-(2-hydroxyethyl)aminopropyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline hydrochloride (**19a**), which proved to be a very potent top1 inhibitor having a 110 nM mean graph midpoint (MGM) when tested for cytotoxicity in 55 human cancer cell cultures. A number of structurally related indenoisoquinolines were also obtained that had both potent cytotoxicity as well as top1 inhibitory activity. The key feature of the more potent compounds was the presence of an aminoalkyl side chain on the indenoisoquinoline nitrogen atom. The DNA cleavage patterns induced by top1 in the presence of the indenoisoquinolines were different from those seen with camptothecin. Some of the cleavage sites induced by the indenoisoquinolines were different from those seen with camptothecin, and conversely, camptothecin induced unique cleavage sites not apparent with the indenoisoquinolines. However, both camptothecin and the indenoisoquinolines also induced DNA cleavage sites that were the same in both series but varied in intensity. In addition, some of the DNA cleavages seen with the free base of **19a** (compound **18c**) in the presence of top1 were inhibited at higher drug concentrations, suggesting either a direct inhibition of the enzyme or an alternative mechanism involving DNA intercalation. Consistent with intercalation, compound **18c** did unwind DNA.

Introduction

In 1978, we reported the synthesis of the indenoisoquinoline **1** (NSC 314622),[1] which arose from an unexpected transformation during a synthesis of nitidine chloride.[2] Compound **1** was found to have weak antitumor activity and was not investigated further. Twenty years later, during a COMPARE analysis and search for compounds that would likely have a similar mechanism of action as camptothecin (**2**), the indenoisoquinoline **1** resurfaced as a potential topoisomerase I (top1) inhibitor.[3] Subsequent investigations did in fact show that NSC 314622 (**1**) induced DNA cleavage in the presence of top1 and that its cleavage site specificity was not the same as that observed with camptothecin (**2**).[3] Furthermore, the DNA breaks produced in the presence of top1 and NSC 314622 (**1**) were more stable than those produced by camptothecin.[3] Like camptothecin (**2**), NSC 314622 did not unwind DNA, suggesting that it is also not a DNA intercalator.[3]

Although NSC 314622 is not as potent as camptothecin as a top1 inhibitor, it has a number of features that make it attractive as a potential lead compound for development of top1 inhibitors that may overcome some of the limitations of camptothecin, which include instability due to lactone ring opening and rapid reversibility of top1 inhibition upon drug removal. Therefore, a series of indenoisoquinolines were synthesized and evaluated as top1 inhibitors and as cytotoxic agents in human cancer cell cultures.[4] This was met with limited success, as individual compounds were obtained that were more potent than the lead compound **1** as inhibitors of top1 or as cytotoxic agents, but not both, suggesting that the more cytotoxic agents in the series were acting on another, unidentified target.[4] Further effort has therefore been invested in order to synthesize cytotoxic indenoisoquinolines that act by top1 inhibition. As reported herein, this has resulted in very cytotoxic indenoisoquinolines that produce protein-linked DNA breaks in treated cells at nanomolar concentrations.



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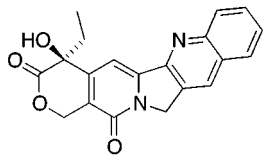

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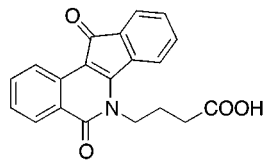
Results and Discussion

Chemistry. One of the most potent top1 inhibitors in the prior series turned out to be the indenoisoquinoline **3** lacking substituents on the two aromatic rings.[4] To determine how changing the substituent on the nitrogen would affect biological activity, a limited number of additional analogues of **3** were prepared with various substituents on the nitrogen atom. The alterations that were examined included shortening the length of the chain by one carbon atom, replacing the carboxylic acid with an alcohol, and replacing the carboxylic acid group with a chloride or bromide that could possibly alkylate the enzyme or the nucleic acid. Indenoisoquinolines **5**−**9** were made by reacting commercially available benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**4**) with various primary amines. The alcohol **7** was converted to the acid **10** by treatment with aqueous chromic acid in acetone. The corresponding sodium salt **11** was prepared from **10** in the presence methanolic sodium hydroxide.



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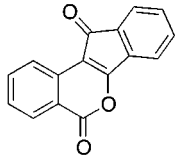

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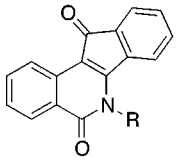
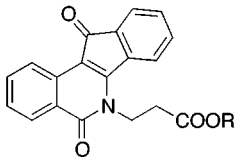


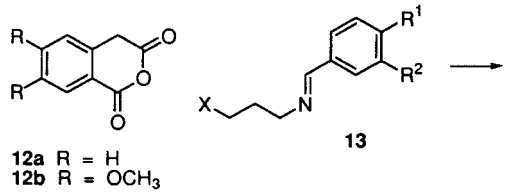
5 R = OH
6 R = CH_2CH_2OH
7 R = $CH_2CH_2CH_2OH$
8 R = CH_2CH_2Cl
9 R = $CH_2CH_2CH_2Br$



10 R = H
11 R = Na

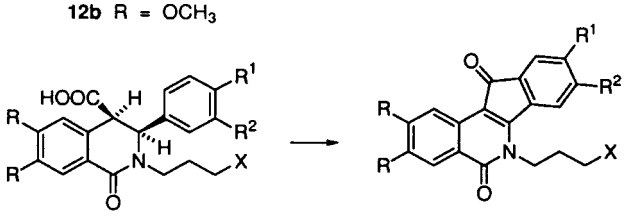
Turning to indenoisoquinolines bearing substituents on the aromatic rings, efforts were also made in this series of compounds to incorporate halogens at the end of N-6 alkyl chains. These compounds could possibly alkylate top1 and, therefore, act as irreversible enzyme inhibitors or as poisons. Since compound **3** was found previously to unwind DNA and therefore possibly act as a DNA intercalator, halogen-containing analogues of **3** might alkylate DNA as well.[4] Covalent bonding of an indenoisoquinoline to the enzyme or to nucleic acid could also facilitate crystallization of a ternary complex for X-ray structure determination.[5,6] In addition, the halides might possibly serve as synthetic intermediates for the incorporation of modified N-6 substituents. As shown in Scheme 1, condensation of the substituted homophthalic anhydrides **12** and imines **13** in chloroform at room temperature resulted in the formation of the cis-substituted isoquinolones **14a**−**e**. The cis relative configurations of the substituents at C-3 and C-4 were determined by the 6-Hz coupling constant observed in the NMR spectrum for the two methine protons at C-3 and C-4. In the corresponding trans diastereomers, both of the substituents at C-3 and C-4 are axial and both diequatorial methine protons appear as singlets ($J = 0$ Hz).[7] Treatment of the isoquinolines **14a**−**e** with thionyl

Scheme 1



12a R = H
12b R = OCH_3

13



14 **15**

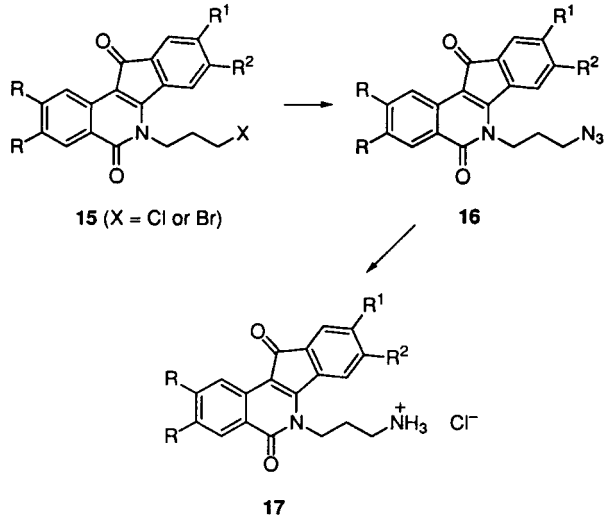
Compd	R	R_1	R_2	X
a	H	O-CH_2-O		Cl
b	H	OCH_3	OCH_3	Cl
c	OCH_3	O-CH_2-O		Cl
d	OCH_3	O-CH_2-O		Br
e	OCH_3	OCH_3	OCH_3	Br

chloride resulted in both intramolecular Friedel−Crafts acylation and dehydrogenation to afford the desired indenoisoquinolines **15a**−**e**.

Two compounds, **17a**,**b**, incorporating an amine in the side chain, were synthesized. The salts were expected to have increased aqueous solubility, thus facilitating formulation. We were also interested in determining the effect of the incorporation of amines on the biological activity, since the related indenoisoquinoline oracin, which also has an amine-containing side chain, has been found to induce G2 cell cycle arrest and apoptosis in Burkitt's lymphoma cells.[8−13] In addition, *N*,*N*-dimethylaminoalkylamino side chains have enhanced the antitumor properties of ellipticines, and this has led to attachment of aminoalkylamino side chains to benzophenanthridines that are structurally related to the present indenoisoquinolines.[14] It is possible that ionic bonding of a protonated amine to the phosphodiester groups of the nucleic acid backbone could position the indenoisoquinoline for intercalation or for bonding within a ternary complex containing the inhibitor, DNA, and protein. As shown in Scheme 2, halide displacement from **15b**, **15d**, or **15e** resulted in the azides **16a**−**c.** The azides **16a**,**b** were reduced to the corresponding amines using the Staudinger reaction, in which the azides were reacted with triethyl phosphite in refluxing benzene, followed by treatment with methanolic hydrochloric acid at reflux, to afford the corresponding amine hydrochlorides **17a**,**b**.[15] Similarly, as shown in Scheme 3, halide displacement from indenoisoquinolines **15** with various amino alcohols resulted in the formation of the products **18a**−**g**, several of which were converted to the corresponding hydrochloride salts **19a**−**d**.

To study the biological effect of adding steric bulk to the D ring, the synthesis displayed in Scheme 4 was carried out. The starting imine **20** was made by treating naphthalene-2-carboxaldehyde with 3-chloropropylamine in the presence of triethylamine. Condensation of the imine **20** with the anhydride **12b** afforded the substituted isoquinolone **21**. As before, the cis relative con-

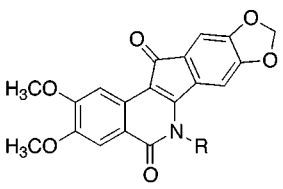
Scheme 2



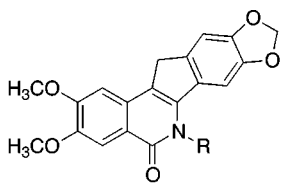
Compd	R	R_1	R_2
a	H	OCH_3	OCH_3
b	OCH_3	O-CH_2-O	
c	OCH_3	OCH_3	OCH_3

figuration of the naphthyl and carboxyl groups were determined by their 6-Hz coupling constant in the ^{1}H NMR spectrum. The isoquinoline **21** was converted exclusively to the cyclized isomer **22** in the presence of thionyl chloride. The regiochemistry of the reaction was apparent based on the two doublets with a 8.5-Hz coupling constant observed in the aromatic region of the ^{1}H NMR spectra. The chloride **22** was displaced by azide to yield **23**, which was reacted with triethyl phosphite in refluxing benzene, followed by treatment with 3 N methanolic hydrochloric acid, to provide the amine hydrochloride **24**.[15] Alternatively, chloride displacement from intermediate **22** with ethanolamine resulted in the formation of the aminol **25**.

To investigate the role of the 11-keto group in the biological activities of the indenoisoquinolines, several analogues lacking this group were synthesized. The desired products **27a**−**c** were obtained in good yields by treating their precursors **1**, and **26a,b** with diborane in refluxing THF.



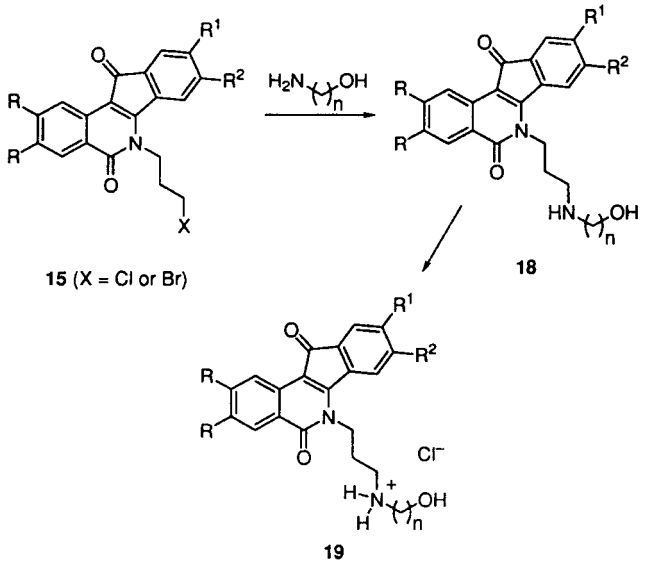
26a R = Et
26b R = *n*-Bu



27a R = Me
27b R = Et
27c R = *n*-Bu

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

Scheme 3

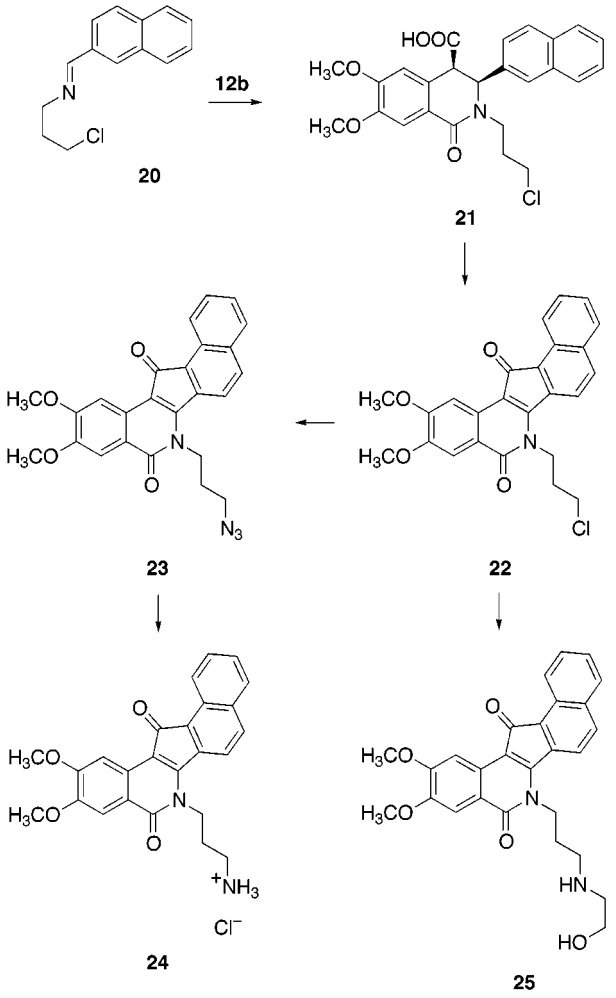


Comp	n	R	R_1	R_2
18a	3	H	O-CH_2-O	
18b	2	H	OCH_3	OCH_3
18c	2	OCH_3	O-CH_2-O	
18d	3	OCH_3	O-CH_2-O	
18e	2	OCH_3	OCH_3	OCH_3
18f	3	OCH_3	OCH_3	OCH_3
18g	4	OCH_3	OCH_3	OCH_3
19a	2	OCH_3	O-CH_2-O	
19b	2	OCH_3	OCH_3	OCH_3
19c	3	OCH_3	OCH_3	OCH_3
19d	4	OCH_3	OCH_3	OCH_3

for all of the cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-4} to 10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.[16] In addition, the relative activities of the compounds in the top1 cleavage assay are listed in Table 1. Compounds **10**, **11**, **16a**,**c**, **22**, and **23** did not have significant growth inhibitory activities in human cancer cell cultures and are therefore not included in the table.

The hydroxylated lactam **5** proved to be slightly more or less active than the lead compound **1**, depending on the cell line tested. Overall, it was slightly less cytotoxic (MGM 29 μM) than the lead compound **1** (MGM 20 μM). However, the indenoisoquinolines **6**−**9** bearing hydroxyalkyl or alkyl halide side chains on the nitrogen were slightly more cytotoxic than the lead compound **1**, with overall potency increasing in the order **6** < **9** < **7** < **8**. The acid **10** and its salt **11** were the least cytotoxic compounds of the group (data not shown). In this series of compounds **5**−**12** lacking substituents on the A and D rings, cytotoxicity did not correlate particularly well with the top1 inhibitory activity in a cell-free system, at least not in any quantitative sense, since the most potent top1 inhibitors were not the most cytotoxic. This is not particularly surprising, since other factors (e.g.

Scheme 4



ability to penetrate the cell membrane and reach the target) also affect cytotoxicity.

Turning to the second group of compounds with methylenedioxy and/or two methoxyl groups on the A and D rings, the alkyl chlorides **15a** (MGM 35 μM), **15b** (MGM 76 μM), and **15c** (MGM 48 μM) were all less potent than the lead compound **1** (MGM 20 μM). A direct comparison of **15c** with **1** allows a determination of the effect of replacing the *N*-methyl group of **1** with a 3′-chloropropyl group, since the two structures are otherwise identical. On the other hand, the two bromides **15d** (MGM 14 μM) and **15e** (MGM 7 μM) were slightly more potent than the lead compound **1** (MGM 20 μM). A comparison of **15c** with **15d** shows that the brominated side chain confers greater activity than the chlorinated side chain, since the two compounds are otherwise identical.

The hydrochlorides of the *N*-(3′-aminopropyl) compounds **17a** (MGM 0.62 μM) and **17b** (MGM 0.16 μM) displayed cytotoxicity GI_{50} values at submicromolar concentrations. Similarly, the amino alcohols **18b**−**g** were highly cytotoxic, each with submicromolar GI_{50}'s. Comparison of the activities of **18a** (MGM 15.5 μM) and **18d** (MGM 0.20 μM) underlines the importance of the two methoxyl groups in the A ring for cytotoxicity, since in other aspects, these two compounds are identical. Comparison of the activity of **18c** (MGM 0.21 μM) with **18e** (MGM 0.51 μM) indicates that the methylenedioxy substituent in the D ring is better than the dimethoxy substitution pattern. This point is also supported by the activities of **18d** (MGM 0.20 μM) and **18f** (MGM 0.55 μM). Weak cytotoxicity was observed for **18a** although it is a strong top1 inhibitor. The hydrochloride salts **19a**−**d** of the amino alcohols were also very cytotoxic. In fact, the most potent compound in the entire series as far as cytotoxicity is concerned was **19a**, which featured an MGM of 0.11 μM. The very high cytotoxicities of these amino alcohols were accompanied by very high activities versus top1, which is consistent with the cytotoxic effects being mediated through top1.

The effect of the length of the linker chain connecting the amine nitrogen to the terminal alcohol can be recognized by comparing the cytotoxicities of **18e** (MGM 0.51 μM), **18f** (MGM 0.55 μM), and **18g** (MGM 0.85 μM). This indicates a slight decrease in biological activity as the length of the linker is increased, which is also apparent in the series consisting of the corresponding hydrochloride salts **19b** (MGM 0.68 μM), **19c** (MGM 1.14 μM), and **19d** (MGM 1.47 μM).

Turning to the naphthalene analogues **22**−**25**, both the chloride **22** and the azide **23** were essentially inactive. In view of the low activity of the *N*-(3′-chloride) **15c** (MGM 48 μM), the 98 μM MGM of the corresponding naphthalene analogue **22** is not surprising. Similarly, activities of the azides **16b** (MGM 12 μM) and **16c** (MGM 56 μM) suggest that the azide-containing naphthalene analogue **23** would not be very potent. In fact, **23** was the least potent compound in the series, displaying an MGM of >100 μM. The naphthalene analogue **24** having a 3′-aminopropyl substituent did have respectable cytotoxicity (MGM 3.7 μM), but it was still much less active than the corresponding analogue **17b** (MGM 0.16 μM) having a methylenedioxybenzene ring. Finally, the naphthalene analogue **25** (MGM 0.19 μM) having an amino alcohol side chain was highly cytotoxic, with activity comparable to that of the corresponding amino alcohols **18c** (MGM 0.21 μM) and **18e** (MGM 0.51 μM) having the same substituent on nitrogen. Overall, the results show that the effect of replacing the methylenedioxybenzene moiety or the dimethoxybenzene ring on the "right-hand side" of the molecule with a naphthalene ring system is variable and depends on the substituent on nitrogen. The cytotoxicity of **25** demonstrates that it is possible to obtain highly active compounds in the naphthalene series. However, it should be noted that compound **25** has only relatively weak activity versus top1 although it is very cytotoxic.

It was anticipated that comparison of the activities of **1** and **26a,b** with the corresponding analogues **27a**−**c**, lacking a ketone carbonyl, would give some insight into the influence of the 11-keto group on biological activity. However, the effect was not consistent, since the activities of the keto compounds **1** (MGM 20 μM)[4] and **26a** (MGM 2.4 μM)[4] were higher than those for **27a** (MGM 44 μM) and **27b** (MGM 85 μM), but the activity of the keto analogue **26b** (MGM 42 μM)[4] was lower than that of the corresponding compound **27c** (MGM 21 μM) lacking a keto group.

In the series of indenoisoquinolines that was published previously, some success was achieved in obtaining compounds that were more cytotoxic than the lead compound **1**, but these more cytotoxic congeners were

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

	cytotoxicity (GI_{50} in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	top1 cleavage[c]
1	1.30	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	++++
Group 1 (unsubstituted on the A and D rings)										
5	56.6	24.3	15.3	21.5	30.2	35.7	40.2	29.1	29.5	+
6	5.62	4.95	12.2	18.2	74.2	16.4	16.4	76.7	14.5 ± 2.48	++
7	4.89	3.64	52.5	16.3	28.8	6.49	9.56	39.7	10.3 ± 2.56	++
8	3.81	3.86	2.48	8.08	5.87	4.39	3.50	3.70	4.37 ± 0.65	±
9	4.59	4.47	4.61	10.1	23.5	7.00	13.0	21.5	13.3 ± 4.80	±
Group 2 (substituted with methylenedioxy and/or two methoxy groups on the A and D rings)										
15a	13.1	74.0	>100	53.8	66.3	>100	12.7	>100	35.1 ± 2.00	−
15b	>100	>100	>100	>100	>100	>100	>100	>100	76.1 ± 13.0	−
15c	40.4	72.6	40.7	68.6	>100	24.0	16.8	80.5	47.9	−
15d	6.22	12.0	4.33	5.02	62.1	5.83	3.20	16.6	13.7 ± 2.50	+
15e	1.87	3.53	2.58	1.91	60.1	3.15	1.92	11.7	6.91 ± 1.00	+
16b	3.58	7.40	3.00	3.13	76.2	4.53	3.19	>100	11.8 ± 0.82	++
17a	0.19	0.35	2.93	1.27	0.85	0.43	0.89	1.05	0.62	++
17b	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21	0.16 ± 0.12	+++
18a	7.17	12.5	16.2	>100	15.0	14.2	16.7	19.9	15.5	++++
18b	0.62	0.34	0.45	0.63	3.64	2.07	0.44	0.97	0.95	++
18c	0.01	0.15	0.09	0.03	1.50	0.01	0.02	0.68	0.21 ± 0.19	++++
18d	0.03	0.04	0.01	0.01	1.19	0.01	0.01	0.47	0.20 ± 0.11	+++
18e	0.05	0.31	0.01	0.38	11.2	0.05	0.02	1.25	0.51 ± 0.09	+++
18f	0.07	0.46	0.08	1.51	1.90	0.15	0.10	1.09	0.55 ± 0.14	+++
18g	0.14	0.71	0.06	1.67	3.52	0.11	0.10	2.36	0.85 ± 0.15	+++
19a	0.02	0.10	0.04	0.03	0.35	<0.01	<0.01	0.79	0.11 ± 0.05	++++
19b	0.15	0.39	0.16	0.73	2.28	0.11	0.07	1.43	0.68 ± 0.23	+++
19c	0.32	0.71	0.42	1.87	3.80	0.23	0.23	2.42	1.14 ± 0.72	++
19d	0.26	0.81	0.59	1.76	5.49	0.28	0.21	2.09	1.47 ± 1.22	+++
Group 3 (having a naphthalene replacement for the D ring)										
24	1.89	1.73	1.53	17.5	3.73	6.54	1.64	2.14	3.72	+
25	0.17	0.18	0.22	0.16	0.27	0.15	0.28	0.42	0.19	+
Group 4 (lacking a C-11 ketone carbonyl)										
27a	11.8	42.9	64.2	50.0	>100	43.6	42.5	>100	43.7	+
27b	>100	>100	>100	>100	>100	>100	>100	>100	85.1	NT[d]
27c	12.0	13.2	3.40	6.51	91.9	21.7	5.37	92.4	21.3 ± 10.3	+

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c] The compounds were tested at concentrations ranging up to 10 μM. The activity of the compounds to produce top1-mediated DNA cleavage was expressed semiquantitatively as follows: +, weak activity; ++, similar activity as the parent compound **1**; +++ and ++++, greater activity than the parent compound **1**; ++++, similar activity as camptothecin (**2**). [d] NT, not tested.

not more potent as top1 inhibitors.[4] Also, certain compounds in the prior series were as potent as **1** as top1 inhibitors, but they were not more cytotoxic than the lead compound **1**. Since the cytotoxicities and top1 inhibitory activities maximized in different compounds, the cytotoxicity did not appear to be due to top1 inhibition. One of the main goals of the present research, therefore, has been to design and synthesize very cytotoxic compounds that were also potent top1 inhibitors. As is evident from the data in Table 1, this goal has now largely been met. In particular, both the amino alcohol **18c** and its hydrochloride salt **19a** were found to be very potent top1 inhibitors, and they were also extremely cytotoxic, with submicromolar GI_{50} values. A number of additional indenoisoquinoline analogues were also obtained that were more potent than the lead compound **1** as top1 inhibitors, including **17b**, **18d**−**g**, and **19b,d**, each of which was significantly more cytotoxic than the lead compound **1**. Some of the other indenoisoquinolines obtained were less potent than the lead compound as top1 inhibitors, including **8**, **9**, **15a**−**e**, **16b**, **25**, and **27c**. Although some of these less potent top1 inhibitors were more potent than the lead compound **1** as cytotoxic agents, none of them except **25** displayed submicromolar GI_{50} values as observed with the more potent top1 inhibitors. The general correlation of top1 inhibitory activity with cytotoxicity observed in the present series argues that the cytotoxicity of these compounds may well be due to their top1 inhibitory activities.

The top1-mediated DNA cleavage patterns in the presence of NSC 314622 (**1**), camptothecin (**2**), **18c**, and **17b** are displayed in Figure 1. As shown in the figure, the indenoisoquinolines **1**, **17b**, and **18c** had similar cleavage patterns. The DNA cleavages produced by the indenoisoquinolines were different from the pattern observed with camptothecin (**2**). The solid (top) wedge to the right of Figure 1 marks a camptothecin cleavage site that is not observed with the indenoisoquinolines. In contrast, the two open wedges show indenoisoquinoline DNA cleavage sites that are not seen with camptothecin. The arrows indicate common cleavage sites that are observed with both camptothecin and the indenoisoquinolines. The bands corresponding to the indenoisoquinoline-stabilized DNA cleavage sites varied in intensity among the compounds. Some of the bands observed with **17b** and to a lesser extent **18c**, but not **1**, were weaker at higher drug concentrations, indicating an increase and then a decrease in DNA cleavage as drug concentration is increased. This indicates that

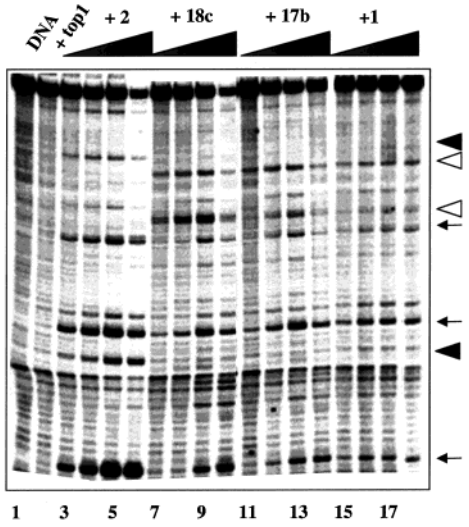


Figure 1. Comparison of the top1-mediated DNA cleavages at different drug concentrations. The DNA used was the pSK-*Hin*dIII PCR fragment, and the four concentrations used for each compound were 0.03, 0.1, 1, and 10 μM. Reactions were at room temperature for 30 min and stopped by adding 0.5% SDS. DNA fragments were separated on 16% polyacrylamide gels. Top1 was present in all reactions except in the control lane. Control: DNA with neither top1 nor any drug.

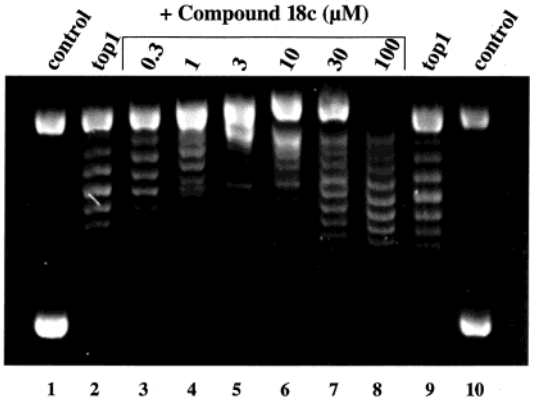


Figure 2. DNA unwinding in the presence of compound **18c**. Native supercoiled SV40 DNA (lanes 1 and 10) was reacted with an excess of top1 in the absence of drug (lanes 2 and 9) or in the presence of the indicated concentrations of **18c** (lanes 3−8) for 30 min at 37 °C. Reactions were stopped with 0.5% SDS followed by proteinase K digestion and run in 1% agarose gel in TBE buffer. DNA was visualized after staining the gel with ethidium bromide.

these compounds suppress top1-mediated DNA cleavage at high concentrations, a result which is similar to the those seen with DNA unwinding or intercalating inhibitors.[17−19] Alternatively, higher concentrations of **17b** or **18c** might suppress top1-mediated DNA cleavage through a direct effect on the enzyme resulting in a conformational change, as has been proposed with saintopin E.[18] To clarify this situation, **18c** was examined in an SV40 DNA unwinding assay in order to determine whether intercalation could be responsible for inhibition of DNA cleavage seen at higher drug concentrations.[20] The results (Figure 2) indicated low-affinity DNA intercalation that could be responsible for suppression of DNA cleavage at higher drug concentration.

Camptothecin (**2**) induces DNA strand breaks by stabilizing the cleavage complexes and inhibiting DNA

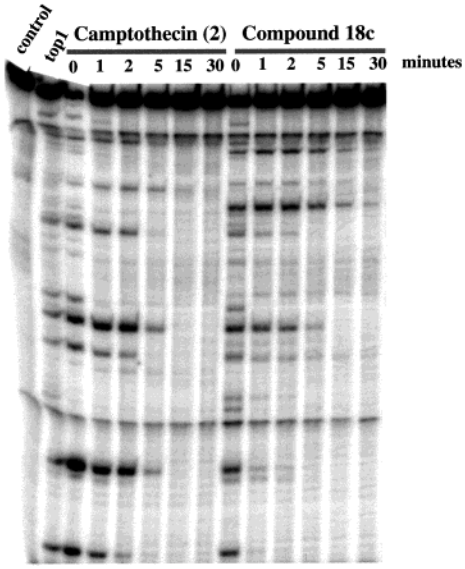


Figure 3. Reversibility of top1 cleavage complexes induced by camptothecin (**2**) and indenoisoquinoline **18c**. Reactions were preformed using the 3′-end-labeled pSK fragment and recombinant top1. Reversal was induced by adding NaCl to a final concentration of 0.35 M. Reversal times are indicated above each lane, and time "0" refers to samples taken immediately before NaCl addition.

religation.[21,22] However, increasing salt concentration can reverse the camptothecin-induced cleavage complexes, and this method has been used to compare the molecular interactions between camptothecin derivatives and top1 cleavage complexes. The cleavage sites induced by both camptothecin and the indenoisoquinoline derivative **18c** were reversed in the presence of increasing salt concentrations (Figure 3), as was the case with a number of other related indenoisoquinolines as reported previously.[4] This is consistent with the reversible trapping of top1 cleavage complexes by the indenoisoquinolines.

In conclusion, the main goal of the present project was to maximize both top1 inhibitory activity and cytotoxicity in the same compound. This strategy would hopefully lead to novel anticancer agents that act through top1 inhibition. As indicated in Table 1, success has been achieved with the free base **18c** and its salt **19a**, which are very potent top1 inhibitors as well as being very cytotoxic in human cancer cell cultures.

Experimental Section

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

5,6-Dihydro-6-hydroxy-5,11-diketo-11*H*-indeno[1,2-*c*]-isoquinoline (5). Hydroxylamine hydrochloride (0.828 g, 12 mmol) was added to chloroform (120 mL), followed by triethylamine (3 mL, 21 mmol), and the solution was stirred for 10 min. The benzopyran **4** (2.48 g, 10 mmol) was added and the reaction mixture was stirred overnight. A reddish brown

precipitate formed in the reaction. The precipitated product was filtered off, washed with chloroform (50 mL), water (60 mL) and dried. The solid thus obtained was heated with 2-propanol (100 mL) and filtered and dried to get a reddish brown solid (2.31 g, 88%): mp 215−220 °C; IR (KBr) 3146, 2451, 1699, 1676, 1636, 1604, 1573 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.37 (d, J = 8.1 Hz, 1 H), 8.10 (d, J = 8.1 Hz, 1 H), 7.91 (d, J = 6.3 Hz, 1 H), 7.68 (t, J = 7.7 Hz, 1 H), 7.44−7.20 (m, 4 H), 5.83 (bs, 1 H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 188.5, 160.7, 153.8, 136.1, 134.8, 133.1, 132.9, 131.2, 131.0, 127.4, 125.9, 124.5, 123.2, 122.4, 122.1, 104.2. Anal. Calcd for $C_{16}H_9NO_3$: C, H, N.

General Procedure for the Synthesis of Indenoisoquinolines 6−9. The amino alcohols or the amino halides were treated with commercially available (Aldrich) benz[*d*]-indeno[1,2-b]pyran-5,11-dione (**4**) in a procedure reported[4] earlier, and the indenoisoquinolines **6**−**9** were isolated as orange solids in 81−98% yield.

5,6-Dihydro-6-(2-hydroxy-1-ethyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (6). The aminol **6** was obtained in 90% yield after crystallization from 2-propanol: mp 200−201 °C; IR (KBr) 3431, 1686, 1663 and 1609 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.59 (d, J = 8.1 Hz, 1 H), 8.26 (d, J = 8.0 Hz, 1 H), 7.70−7.50 (m, 3 H), 7.50−7.35 (m, 3 H), 4.71 (s, 2 H), 4.18 (q, J = 5.5 Hz, 2 H), 2.79 (bs, 1 H, D_2O exchangeable). Anal. Calcd for $C_{18}H_{13}NO_3$: C, H, N.

5,6-Dihydro-6-(3-hydroxy-1-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7). The aminol **7** was obtained in 98% yield after crystallization from 2-propanol: mp 170−171 °C; IR (KBr) 3421, 1686, 1663, 1609 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.70 (d, J = 8.1 Hz, 1 H), 8.33 (d, J = 8.0 Hz, 1 H), 7.78−7.60 (m, 3 H), 7.50−7.35 (m, 3 H), 4.71 (t, J = 6.6 Hz, 2 H), 3.71 (t, J = 5.6 Hz, 2 H), 2.77 (bs, 1 H, D_2O exchangeable), 2.20−2.05 (m, 2 H). Anal. Calcd for $C_{19}H_{15}NO_3$: C, H, N.

6-(2-Chloro-1-ethyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (8). The aminol **8** was isolated in 81% yield as a purple solid: mp 197−199 °C; ^1H NMR ($CDCl_3$) δ 8.72 (d, J = 8.1 Hz, 1 H), 8.33 (d, J = 8.2 Hz, 1 H), 7.85 (d, J = 7.5 Hz, 1 H), 7.72 (dd, J = 7.4 and 7.7 Hz, 1 H), 7.64 (d, J = 7.0 Hz, 1 H), 7.49 (dd, J = 7.3 and 7.6 Hz, 1 H), 7.42 (dd, J = 7.3 and 7.6 Hz, 1 H), 4.82 (dd, J = 7.4 and 7.8 Hz, 2 H), 3.93 (dd, 7.4 and 7.8 Hz, 2 H); LRMS (FAB) m/z 310 (MH^+). Anal. Calcd for $C_{18}H_{12}O_2NCl$: C, H, N.

6-(3-Bromo-1-propyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (9). The bromide **9** was isolated in 60% yield as an orange solid: mp 162−164 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.72 (d, 1 H, J = 8.1 Hz), 8.33 (d, 1 H, J = 8.2 Hz), 7.85 (d, 1 H, J = 7.5 Hz), 7.72 (dd, 1 H, J = 7.4, 7.7 Hz), 7.64 (d, 1 H, J = 7.0 Hz), 7.49 (dd, 2 H, J = 7.3, 7.6 Hz), 7.42 (dd, 1 H, J = 7.3, 7.3 Hz), 4.70 (t, 2 H, J = 7.8 Hz), 3.65 (t, 2 H, J = 6.3 Hz), 2.48 (m, 2 H); LRMS (EI) m/z 368 (M^+), 370. Anal. Calcd for $C_{19}H_{14}O_2NBr$: C, H, N.

6-(2-Carboxy-1-ethyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (10). The indenoisoquinoline **7** (0.305 g, 1 mmol) was dissolved in acetone (50 mL) and cooled in an ice bath. To the cold solution of the alcohol a solution of Jones reagent was added dropwise until the red color of the reagent persisted. The excess Jones reagent was quenched by adding a few drops of 2-propanol. Then the reaction mixture was filtered through a small pad of Celite and the residue was washed with acetone (50 mL). The combined filtrate was concentrated and the residue was dissolved in saturated bicarbonate (100 mL) and the aqueous layer was washed with chloroform (2 × 30 mL). The aqueous layer was neutralized with concentrated HCl and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was dried (Na_2SO_4) and concentrated to afford the acid **10** as an orange solid (0.308 g, 99%): IR (KBr) 3004 (b), 1703, 1690, 1651 and 1576 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.65 (d, J = 8 Hz, 1 H), 8.28 (d, J = 8 Hz, 1 H), 7.80−7.30 (m, 6 H), 4.78 (t, J = 8.2 Hz, 2 H), 2.90 (t, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{13}NO_4 \cdot 0.2H_2O$: C, H, N.

5,6-Dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline-6-(sodium 1-propionate) (11). The acid **10** (0.159, 0.5 mmol) was dissolved in methanol and a methanolic solution of NaOH was added dropwise. The pH of the solution was checked after adding each drop. After the pH was approximately 8, the resulting orange solution was cooled in the freezer for 2 days and the precipitated orange solid was filtered off and dried to afford the product **11** (0.148 g, 96%): mp 323−326 °C; IR (KBr) 3434, 1703, 1657, 1611 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.54 (d, J = 8.1 Hz, 1 H), 8.25−8.15 (m, 2 H), 7.76 (t, J = 7.8 Hz, 1 H), 7.60−7.40 (m, 4 H), 4.61 (t, J = 8.4 Hz, 2 H), 2.52 (t, J = 8.4 Hz, 2 H). Anal. Calcd for $C_{19}H_{12}NO_4Na \cdot 0.5MeOH$: C, H, N.

General Procedure for the Synthesis of Imines 13. The hydrochloride salt of 3-chloropropylamine or 3-bromopropylamine (20 mmol) was stirred with triethylamine (3 mL, 21 mmol) in chloroform (100 mL) at room temperature. After 30 min, the aldehyde (piperonal or 3,4-dimethoxybenzaldehyde; 20 mmol) was added followed by anhydrous magnesium sulfate (5 g) and the reaction mixture was stirred at room temperature for 4−17 h. The reaction mixture was filtered and the residue washed with chloroform (30 mL) and the combined organic layer was washed with water (100 mL), brine (50 mL) and dried (Na_2SO_4). Concentration of the organic layer provided the imines **12** in 85−99% yields.

3,4-Methylenedioxybenzylidene-(3-chloro-1-propylamine) (13a). The imine **13a** was isolated in 89% as a pale yellow oil: IR (neat) 2899, 2843, 1688, 1645, 1606, 1488, 1447, 1390 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.17 (s, 1 H), 7.30 (d, J = 1.1 Hz, 1 H), 7.07 (d, J = 9.1 Hz, 1 H), 6.8 (d, J = 7.9 Hz, 1 H), 5.96 (s, 2 H), 3.70 (t, J = 6.5 Hz, 2 H), 3.59 (t, J = 6.5 Hz, 2 H) and 2.12 (qn, J = 6.5 Hz, 2 H).

3,4-Dimethoxybenzylidene-(3-chloro-1-propylamine) (13b). The imine **13b** was isolated in 99% yield as an oil: IR (neat) 3002, 2958, 2937, 2838, 1644, 1600, 1586, 1513, 1464, 1420 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.20 (s, 1 H), 7.39 (s, 1 H), 7.13 (dd, J = 1.5 and 7.9 Hz, 1 H), 6.86 (d, J = 8.1 Hz, 1 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.71 (t, J = 6.6 Hz, 2 H), 3.60 (t, J = 6.5 Hz, 2 H), 2.14 (qn, J = 6.5 Hz, 2 H).

3,4-Methylenedioxybenzylidene-(3-bromo-1-propylamine) (13d). The imine was isolated in 85% yield as a pale yellow oil: IR (neat) 2898, 2841, 1687, 1643, 1604, 1503 and 1486 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (s, 1 H), 7.31 (d, J = 1.3 Hz, 1 H), 7.08 (dd, J = 1.3 and 7.9 Hz, 1 H), 6.80 (d, J = 7.9 Hz, 1 H), 5.97 (s, 2 H), 3.67 (t, J = 6.5 Hz, 2 H), 3.46 (t, J = 6.5 Hz, 2 H), 2.21 (p, J = 6.5 Hz, 2 H).

3,4-Dimethoxybenzylidene-(3-bromo-1-propylamine) (13e). The imine **13e** was obtained in 98% yield as an oil: IR (neat) 2915, 1732, 1641, 1590, 1538, 1472, 1253, 1082 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.24 (s, 1 H), 7.41 (s, 1 H), 7.16 (dd, J = 1.8 and 8.2 Hz, 1 H), 6.89 (d, J = 8.2 Hz, 1 H), 3.94 (s, 3 H), 3.92 (s, 3 H), 3.70 (t, J = 6.3 Hz, 2 H), 3.48 (t, J = 6.5 Hz, 2 H), 2.26 (m, 2 H); LRPDMS m/z 285 (MH^+), 287 (MH^+); HREIMS calcd for $C_{12}H_{16}NO_2Br$: 285.0364; found: 285.0363.

General Procedure for the Synthesis of Isoquinolones 14. Homophthalic anhydride **(12a)** (1.61 g, 10 mmol) or 4,5-dimethoxyhomophthalic anhydride **(12b)** (2.22 g, 10 mmol) was added to a chloroform (60 mL) solution of the imine **13** (10 mmol) and the mixture was stirred at room temperature. After the complete disappearance of the starting material (TLC), the white precipitate formed in the reaction was filtered off, washed with chloroform (5 mL) and dried to give pure isoquinolones **14** in 43−89% yields.

***cis*-4-Carboxy-*N*-(3-chloropropyl)-3,4-dihydro-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (14a).** The isoquinolone **14a** was isolated in 43% yield: mp 172−173 °C; IR (KBr) 3080, 2970, 1745, 1613, 1595, 1567 and 1485 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.85 (bs, 1 H), 8.18 (d, J = 7.0 Hz, 1 H), 7.53−7.36 (m, 2 H), 6.60−6.47 (m, 2 H), 6.40 (d, J = 1.2 Hz, 1 H), 5.85 (s, 2 H), 4.98 (d, J = 6.2 Hz, 1 H), 4.70 (d, J = 6.3 Hz, 1 H), 4.03−3.93 (m, 1 H), 3.65−3.49 (m, 2 H), 3.22−3.11 (m, 1 H), 2.22−1.98 (m, 2 H). Anal. Calcd for $C_{20}H_{18}NO_5Cl$: C, H, N.

***cis*-4-Carboxy-*N*-(3-chloro-1-propyl)-3,4-dihydro-3-(3,4-dimethoxyphenyl)-1(2*H*)-isoquinolone (14b).** The isoquinolone **14b** was isolated in 65% yield: mp 174−176 °C; IR (KBr) 2996, 1745, 1621, 1598, 1567, 1574, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.0 (bs, 1 H), 8.0 (d, J = 9.0 Hz, 1 H), 7.60−7.47

(m, 2 H), 7.42 (t, J = 9 Hz, 1 H), 6.77 (d, J = 9 Hz, 1 H), 6.56 (d, J = 3 Hz, 1 H), 6.49 (dd, J = 3 and 9 Hz, 1 H), 5.06 (d, J = 6 Hz, 1 H), 4.78 (d, J = 6 Hz, 1 H), 3.90 (qn, J = 6.1 Hz, 1 H), 3.66 (s, 3 H), 3.80−3.55 (m, 2 H), 3.51 (s, 3 H), 3.01 (qn, J = 6 Hz, 1 H), 2.07−1.90 (m, 2 H). Anal. Calcd for $C_{21}H_{22}NO_5Cl\cdot0.3H_2O$: C, H, N.

***cis*-*N*-(3-Chloro-1-propyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (14c).** The isoquinolone **14c** was isolated in 89% yield: mp > 350 °C; IR (KBr) 3091, 2919, 1736, 1622, 1594 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.51 (s, 1 H), 7.12 (s, 1 H), 6.77 (d, J = 7.5 Hz, 1 H), 6.55 (dd, J = 2 and 8.5 Hz, 1 H), 6.45 (d, J = 2 Hz, 1 H), 5.94 (s, 2 H), 5.00 (d, J = 6 Hz, 1 H), 4.66 (d, J = 6 Hz, 1 H), 3.90−3.82 (m, 1 H), 3.81 (s, 3 H), 3.74 (s, 3 H), 3.70−3.58 (m, 2 H), 2.95−2.85 (m, 1 H), 2.05−1.85 (m, 2 H). Anal. Calcd for $C_{22}H_{22}NO_7Cl$: C, H, N.

***cis*-*N*-(3-Bromo-1-propyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (14d).** The isoquinolone **14d** was isolated in 78% yield: mp > 350 °C; IR (KBr) 3093, 2916, 1739, 1622, 1594 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.98 (bs, 1 H), 7.51 (s, 1 H), 7.11 (s, 1 H), 6.78 (d, J = 8.0 Hz, 1 H), 6.55 (d, J = 8.1 Hz, 1 H), 6.45 (s, 1 H), 5.94 (s, 2 H), 5.01 (d, J = 6.3 Hz, 1 H), 4.85−4.75 (m, 1 H), 4.68 (d, J = 6.3 Hz, 1 H), 3.86 (s, 3 H), 3.74 (s, 3 H), 3.60−3.45 (m, 2 H), 2.98−2.88 (m, 1 H), 2.20−1.88 (m, 2 H).

***cis*-*N*-(3-Bromo-1-propyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-dimethoxyphenyl)-1(2*H*)-isoquinolone (14e).** The isoquinolone **14e** was isolated in 51% yield: mp 209−210 °C; ^{1}H NMR (DMSO-d_6) δ 7.25 (s, 1 H), 7.15 (s, 1 H), 6.78 (d, J = 8.4 Hz, 1 H), 6.63 (d, J = 1.9 Hz, 1 H), 6.52 (dd, J = 1.9 and 8.4 Hz, 1 H), 5.03 (d, J = 6.1 Hz, 1 H), 4.66 (d, J = 6.1 Hz, 1 H), 3.89 (m, 1 H), 3.81 (s, 3H), 3.74 (s, 3H), 3.66 (s, 3H), 3.56 (s, 3H), 3.52 (m, 2 H), 2.98 (m, 1 H), 2.11 (m, 1 H), 1.99 (m, 1 H). Anal. Calcd for $C_{23}H_{26}NO_7Br\cdot0.6H_2O$: C, H, N.

General Procedure for the Synthesis of Indenoisoquinolines 15. Thionyl chloride (30 mL) was added to the isoquinolones **14** (2 mmol) and the mixture was stirred at room temperature for 5 h. Benzene was added to the red solution and it was concentrated under reduced pressure. Chloroform was added to the residue and the solution passed through a short column of silica gel. The resulting product was crystallized from chloroform−ethyl acetate to obtain pure indenoisoquinolines **15** in 20−72% yields.

6-(3-Chloro-1-propyl)-5,6-dihydro-5,11-dioxo-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (15a). The indenoisoquinoline **15a** was isolated in 25% yield: mp 222−224 °C; ^{1}H NMR ($CDCl_3$) δ 8.56 (d, J = 8.1 Hz, 1 H), 8.25 (d, J = 8.0 Hz, 1 H), 7.66 (t, J = 7.7 Hz, 1 H), 7.38 (t, J = 7.8 Hz, 1 H), 7.28 (s, 1 H), 7.06 (s, 1 H), 6.07 (s, 2 H), 4.57 (t, J = 7.7 Hz, 2 H), 3.79 (t, J = 6.0 Hz, 2 H), 2.39−2.20 (m, 2 H). Anal. Calcd for $C_{20}H_{14}NO_4Cl$: C, H, N.

6-(3-Chloro-1-propyl)-5,6-dihydro-5,11-dioxo-8,9-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (15b). The indenoisoquinoline **15b** was isolated in 20% yield: IR (KBr) 1693, 1656, 1612, 1575, 1548 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.52 (d, J = 8.1 Hz, 1 H), 8.20 (d, J = 8.0 Hz, 1 H), 7.62 (dd, J = 1.6 and 8.2 Hz, 1 H), 7.35 (dd, J = 1.1 and 7.5 Hz, 1 H), 7.15 (s, 1 H), 7.08 (s, 1 H), 4.56 (t, J = 8.0 Hz, 2 H), 3.97 (s, 3 H), 3.92 (s, 3 H), 3.85 (t, J = 9.4 Hz, 2 H), 2.38−2.29 (m, 2 H). Anal. Calcd for $C_{21}H_{18}NO_4Cl$: C, H, N.

6-(3-Chloro-1-propyl)-5,6-dihydro-5,11-dioxo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (15c). The indenoisoquinoline **15c** was isolated in 72% yield after crystallization in chloroform−ethyl acetate (3:1) as a purple solid: mp 273−276 °C; IR (KBr) 2916, 1694, 1645, 1554, 1486 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (s, 1 H), 7.62 (s, 1 H), 7.26 (s, 1 H), 7.06 (s, 1 H), 6.07 (s, 2 H), 4.60 (t, J = 7.6 Hz, 2 H), 4.03 (s, 3 H), 3.96 (s, 3 H), 3.80 (t, J = 6.2 Hz, 2 H), 2.40−2.30 (m, 2 H). Anal. Calcd for $C_{22}H_{18}NO_6Cl$: C, H, N.

6-(3-Bromo-1-propyl)-5,6-dihydro-5,11-dioxo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (15d). The indenoisoquinoline **15d** was isolated in 57% yield after crystallization from chloroform−ethyl acetate (3:1) as a dark purple solid: mp 273−276 °C; IR (KBr) 2916, 1694, 1645, 1554, 1486 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.19 (s, 1 H), 7.81 (s, 1 H), 7.46 (s, 1 H), 7.44 (s, 1 H), 6.28 (s, 2 H), 4.76 (t, J = 7.6 Hz, 2 H), 4.23 (s, 3 H), 4.17 (s, 3 H), 3.83 (t, J = 6.2 Hz, 2 H), 2.68−2.55 (m, 2 H). Anal. Calcd for $C_{22}H_{18}NO_6Br$: C, H, N.

6-(3-Bromo-1-propyl)-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (15e). The indenoisoquinoline **15e** was isolated in 55% yield as a purple solid: mp 216−217 °C; ^{1}H NMR (DMSO-d_6) δ 7.71 (s, 1 H), 7.36 (s, 1 H), 6.99 (s, 1 H), 6.96 (s, 1 H), 4.45 (m, 1 H), 3.88 (s, 3 H), 3.86 (s, 3 H), 3.82 (s, 6 H), 3.72 (m, 2 H), 2.32 (m, 2 H), 2.03 (m, 1 H). Anal. Calcd for $C_{23}H_{22}NO_6Br\cdot0.3H_2O$: C, H, N.

General Procedure for the Synthesis of Azides 16. The halide **15** (2 mmol) was dissolved in anhydrous DMF (10 mL) and sodium azide (0.153 g, 2.5 mmol) was added. The reaction mixture was heated at reflux for 1−2 h. After the complete disappearance of the starting material (TLC), ice-cold water (100 mL) was added to the reaction mixture and the precipitated solid was filtered off and dried. The product on column purification on silica gel using chloroform as an eluent provided the pure azides **16** in 79−98% yields.

6-(3-Azido-1-propyl)-5,6-dihydro-5,11-dioxo-8,9-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (16a). The chloride **15b** was converted to the azide **16a** in 79% yield: mp 230−232 °C; IR 2943, 2092, 1695, 1648, 1573, 1547, 1492 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.57 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 8.1 Hz, 1 H), 7.66 (t, J = 8.0 Hz, 1 H), 7.38 (t, J = 5.62 Hz, 1 H), 7.25 (d, J = 5.9 Hz, 1 H), 7.16 (s, 1 H), 4.50 (t, J = 8.0 Hz, 2 H), 4.00 (s, 3 H), 3.95 (s, 3 H), 3.68 (t, J = 5.8 Hz, 2 H), 2.20−2.01 (m, 2 H). Anal. Calcd for $C_{21}H_{18}N_4O_4$: C, H, N.

6-(3-Azido-1-propyl)-5,6-dihydro-5,11-diketo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (16b). The azide **16b** was prepared from the bromide **15d** and the product was isolated as dark purple solid in 98% yield after crystallization from ethyl acetate−chloroform: mp 180−184 °C; IR (KBr) 2932, 2912, 2098, 1697, 1646, 1609, 1552 and 1482 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 7.61 (s, 1 H), 7.21 (s, 1 H), 7.04 (s, 1 H), 6.08 (s, 2 H), 4.50 (t, J = 7.6 Hz, 2 H), 4.02 (s, 3 H), 3.96 (s, 3 H), 3.62 (t, J = 6.2 Hz, 2 H), 2.18−2.02 (m, 2 H). Anal. Calcd for $C_{22}H_{18}N_4O_6\cdot0.3H_2O$: C, H, N.

6-(3-Azido-1-propyl)-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (16c). The azide **16c** was synthesized from the bromide **15e** in 88% yield as a purple solid: mp 203−204 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (s, 1 H), 7.59 (s, 1 H), 7.24 (s, 1 H), 7.13 (s, 1 H), 4.51 (t, J = 6.5 Hz, 2 H), 4.06 (s, 3 H), 4.03 (s, 3 H), 3.98 (s, 3 H), 3.96 (s, 3 H), 3.71 (t, J = 6.0 Hz, 2 H), 2.12 (t, J = 6.0 Hz, 2 H); LRCIMS m/z 450 (MH^+). Anal. Calcd for $C_{23}H_{22}N_4O_6$: C, H, N.

General Procedure for the Synthesis of Amine Hydrochlorides 17. The azides **16** (1 mmol) were heated at reflux with triethyl phosphite (0.4 mL) in benzene (60 mL) for 12 h, after which the TLC showed the complete disappearance of the starting azide. The reaction mixture was cooled and methanolic HCl (3 N, 3 mL) was added (an exothermic reaction was observed) and the mixture was heated at reflux for 2 h and cooled. The precipitated solid was filtered and washed with cold methanol (5 mL) and dried under vacuum to afford the hydrochloride salts **17** in 89−99% yields.

6-(3-Amino-1-propyl)-5,6-dihydro-5,11-dioxo-8,9-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (17a). The azide **16a** was converted to the hydrochloride **17a** in 98% yield as a purple solid: mp 284−286 °C; IR 2745, 2596, 2049, 1699, 1648, 1610, 1589, 1574, 1545, 1508, 1492, 1468, 1440, 1429 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.45 (d, J = 8.1 Hz, 1 H), 8.10 (d, J = 8.1 Hz, 1 H), 8.07 (bs, 1 H), 7.74 (t, J = 7.1 Hz, 1 H), 7.41 (t, J = 7.2 Hz, 1 H), 7.15 (s, 1 H), 7.13 (s, 1 H), 4.51 (t, J = 6.9 Hz, 2 H), 4.0 (s, 3 H), 3.85 (s, 3 H), 2.95 (m, 2 H), 2.20−2.05 (m, 2 H). Anal. Calcd for $C_{21}H_{21}N_2O_4Cl$: C, H, N.

6-(3-Amino-1-propyl)-5,6-dihydro-5,11-dioxo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (17b). The azide **16b** was converted to

the hydrochloride **17b** 89% yield as a purple solid: mp 314−316 °C; IR (KBr) 3424, 2913, 1701, 1641, 1580, 1550, 1481 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.00 (bs, 1 H), 7.66 (s, 1 H), 7.34 (s, 1 H), 7.29 (s, 1 H), 6.93 (s, 1 H), 6.17 (s, 2 H), 4.42 (t, J = 8 Hz, 2 H), 3.84 (s, 3 H), 3.81 (s, 3 H), 3.33 (s, 2 H), 2.91 (t, J = 8 Hz, 2 H), 2.10−2.00 (m, 2 H). Anal. Calcd for $C_{22}H_{21}N_2O_6$·Cl·0.5H_2O: C, H, N.

General Procedure for the Synthesis of Aminols 18. A mixture of halide **15** (1 mmol), amino alcohol (3 mmol, ethanolamine or 3-propanolamine or 4-butanolamine) and anhydrous K_2CO_3 (0.31 g) in anhydrous dimethylformamide (15 mL) was heated to 100−140 °C and kept at that temperature for 2−4 h. The hot mixture was filtered and the residue was washed with ethanol (10 mL). The filtrate was cooled in ice and the precipitated product was filtered off and washed with ethanol (10 mL) and dried to provide indenoisoquinolines **18a**−**g** in 36−86% yields.

6-[3-(3-Hydroxypropyl)amino-1-propyl]-5,6-dihydro-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18a). The aminol **18a** was isolated in 81% yield as a dark purple solid by crystallization from ethyl acetate−chloroform (3:1): mp 195−197 °C; IR (KBr) 3457 (bs), 2911, 2825, 1694, 1659, 1607, 1545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.38 (bs, 1 H), 8.09 (d, J = 8.0 Hz, 1 H), 7.70 (s, 1 H), 7.44 (d, J = 7.9 Hz, 1 H), 7.40 (t, J = 7.4 Hz, 1 H), 7.01 (s, 1 H), 6.16 (s, 2 H), 4.38 (bs, 1 H), 3.61 (t, J = 5.4 Hz, 1 H), 3.45 (t, J = 6.4 Hz, 1 H), 2.62 (t, J = 6.0 Hz, 2 H), 2.55 (t, J = 7.0 Hz, 2 H), 1.90−1.70 (m, 2 H), 1.58 (qn, J = 6.6 Hz, 2 H). Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, H, N.

6-[3-(2-Hydroxyethyl)amino-1-propyl]-5,6-dihydro-5,11-dioxo-8,9-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (18b). The aminol **18b** was isolated in 75% yield as a dark purple solid by crystallization in ethyl acetate−chloroform (3:1): mp 179−181 °C; IR 1681,1645, 1549, 1513 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.40 (d, J = 8.1 Hz, 1 H), 8.10 (d, J = 7.9 Hz, 1 H), 7.70 (t, J = 8.3 Hz, 1 H), 7.41 (t, J = 7.9 Hz, 1 H), 7.30 (s, 1 H), 7.11 (s, 1 H), 4.46 (t, J = 6.8 Hz, 2 H), 3.90 (s, 3 H), 3.85 (s, 3 H), 3.41 (t, J = 5.8 Hz, 2 H), 3.41 (d, J = 6.8, 1 H), 2.65 (t, J = 6.3 Hz, 2 H), 2.55 (t, J = 5.8 Hz, 2 H), 1.97−1.80 (m, 2 H). Anal. Calcd for $C_{23}H_{24}N_2O_5$·0.2H_2O: C, H, N.

6-[3-(2-Hydroxyethyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (18c). The aminol **18c** was isolated in 86% yield as a dark purple solid after crystallization in chloroform: mp 231−234 °C; IR (KBr) 3377 (bs), 2926, 1699, 1642, 1580, 1552 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.56 (s, 1 H), 7.40 (s, 1 H), 7.26 (s, 1 H), 6.77 (s, 1 H), 6.11 (s, 2 H), 4.25 (d, J = 8 Hz, 2 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.48 (t, J = 5.9 Hz, 2 H), 2.64 (t, J = 7.3 Hz, 2 H), 2.58 (t, J = 6 Hz, 2 H), 2.19 (s, 1 H), 1.80−1.80 (m, 2 H). Anal. Calcd for $C_{24}H_{24}N_2O_7$·H_2O: C, H, N.

6-[3-(3-Hydroxy-1-propyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (18d). The aminol **18d** was isolated in 76% yield as a dark purple solid after crystallization in chloroform−ethyl acetate: mp 250−252 °C; IR (KBr) 3447, 2926, 1697, 1645, 1551 and 1484 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68 (s, 1 H), 7.49 (s, 1 H), 7.34 (s, 1 H), 6.88 (s, 1 H), 6.13 (s, 2 H), 4.96 (bs, 1 H), 4.30 (m, 2 H), 3.84 (s, 3 H), 3.80 (s, 3 H), 3.60 (bs, 1 H), 3.46 (t, J = 6.3 Hz, 1 H), 2.62 (t, J = 6.2 Hz, 1 H), 2.56 (t, J = 7.0 Hz, 1 H), 1.88−1.70 (m, 4 H), 1.59 (quintet, J = 6.5 Hz, 2 H). Anal. Calcd for $C_{25}H_{26}N_2O_7$: C, H, N.

6-[3-(2-Hydroxyethyl)amino-1-propyl]-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18e). The aminol **18e** was isolated in 36% yield as a dark purple solid after crystallization in chloroform−ethyl acetate: mp 205−206 °C; ^1H NMR ($CDCl_3$) δ 8.05 (s, 1 H), 7.64 (s, 1 H), 7.18 (s, 1 H), 7.16 (s, 1 H), 4.60 (t, J = 7.5 Hz, 1 H), 4.06 (s, 3 H), 3.96 (s, 6 H), 3.93 (s, 3 H), 3.67 (t, J = 5.3 Hz, 2 H), 2.83 (m, 4 H), 2.10 (t, J = 6.8 Hz, 1 H), 1.84 (bs, 2 H). Anal. Calcd for $C_{25}H_{28}N_2O_7$·0.7H_2O: C, H, N.

6-[3-(3-Hydroxypropyl)amino-1-propyl]-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18f). The aminol **18f** was isolated in 75% yield as a dark purple solid after crystallization in chloroform−ethyl acetate: ^1H NMR ($CDCl_3$) δ 7.99 (s, 1 H), 7.59 (s, 1 H), 7.13 (s, 1 H), 7.03 (s, 1 H), 4.33 (t, J = 6.5 Hz, 2 H), 4.03 (s, 3 H), 3.96 (s, 6 H), 3.93 (s, 3 H), 3.80 (t, J = 5.2 Hz, 2 H), 2.88 (t, J = 5.7 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 2.07 (m, 2 H), 1.71 (m, 2 H); LRCIMS m/z 483 (MH^+). Anal. Calcd for $C_{26}H_{30}N_2O_7$·0.8H_2O: C, H, N.

6-[3-(4-Hydroxybutyl)amino-1-propyl]-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18g). The aminol **18g** was isolated in 50% yield as a dark purple solid after crystallization in chloroform−ethyl acetate: ^1H NMR ($CDCl_3$) δ 8.05 (s, 1 H), 7.62 (s, 1 H), 7.14 (s, 1 H), 7.06 (s, 1 H), 4.58 (t, J = 6.6 Hz, 2 H), 4.06 (s, 3 H), 3.99 (s, 3 H), 3.96 (s, 3 H), 3.95 (s, 3 H), 3.60 (t, J = 5.2 Hz, 2 H), 2.79 (t, J = 6.6 Hz, 2 H), 2.72 (m, 2 H), 2.15 (t, J = 6.7 Hz, 2 H), 1.70 (m, 4 H); LREIMS m/z 497 (MH^+). Anal. Calcd for $C_{27}H_{32}N_2O_7$·1.2H_2O: C, H, N.

General Procedure for the Synthesis of Hydrochlorides 19. The aminol **18** (1 mmol) was dissolved in chloroform (100 mL) and an anhydrous solution of HCl in ether (1 M, 30 mL) was added. The mixture was stirred at room temperature for 2 h. The precipitated product was filtered off and washed with methanol (10 mL) and dried over P_2O_5 for 24 h to afford pure hydrochloride salts **19** in 85−99% yields.

6-[3-(2-Hydroxyethyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline Hydrochloride (19a). The aminol **18c** was converted to the hydrochloride **19a** in 89% yield: mp 284−288 °C; ^1H NMR (DMSO-d_6) δ 8.90 (bs, 1 H), 7,73 (s, 1 H), 7.38 (s, 1 H), 7.30 (s, 1 H), 6.99 (s, 1 H), 6.18 (s, 2 H), 5.24 (s, 1 H), 4.42 (m, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H), 3.62 (t, J = 5.2 Hz, 2 H), 3.10 (t, J = 7.3 Hz, 2 H), 2.98 (t, J = 4.4 Hz, 2 H), 2.20−2.18 (m, 2 H). Anal. Calcd for $C_{24}H_{25}N_2O_7Cl$·1.5H_2O: C, H, N.

6-[3-(2-Hydroxyethyl)amino-1-propyl]-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19b). The aminol **18e** was converted to the hydrochloride **19b** in 99% yield: ^1H NMR (DMSO-d_6) δ 7.81 (s, 1 H), 7.42 (s, 1 H), 7.06 (s, 2 H), 4.50 (t, J = 6.4 Hz, 2 H), 3.97 (s, 3 H), 3.89 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.62 (t, J = 5.0 Hz, 2 H), 3.08 (m, 2 H), 2.99 (m, 2 H), 2.21 (m, 2 H); LREIMS m/z 469 (MH^+ − Cl). Anal. Calcd for $C_{25}H_{29}N_2O_7Cl$·0.9H_2O: C, H, N.

6-[3-(3-Hydroxypropyl)amino-1-propyl]-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19c). The aminol **18f** was converted to the hydrochloride **19c** in 99% yield: ^1H NMR (DSMO-d_6) δ 7.80 (s, 1 H), 7.42 (s, 1 H), 7.06 (s, 2 H), 4.51 (t, J = 6.7 Hz, 2 H), 3.97 (s, 3 H), 3.88 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.45 (t, J = 5.9 Hz, 2 H), 3.05 (m, 2 H), 2.95 (t, J = 7.6 Hz, 2 H), 2.19 (m, 2 H), 1.73 (m, 2 H); LREIMS m/z 483 (MH^+ − Cl). Anal. Calcd for $C_{26}H_{31}N_2O_7Cl$·1.8H_2O: C, H, N.

6-[3-(4-Hydroxybutyl)amino-1-propyl]-5,6-dihydro-2,3,8,9-tetramethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19d). The aminol **18g** was converted to the hydrochloride **19d** in 85% yield: ^1H NMR ($CDCl_3$) δ 7.84 (s, 1 H), 7.45 (s, 1 H), 7.09 (s, 2 H), 4.53 (m, 2 H), 3.97 (s, 3 H), 3.90 (s, 3 H), 3.80 (s, 3 H), 3.78 (s, 3 H), 3.38 (t, J = 6.2 Hz, 2 H), 3.04 (m, 2 H), 2.87 (t, J = 7.5 Hz, 2 H), 2.19 (m, 2 H), 1.61 (m, 2 H), 1.43 (m, 2 H); LREIMS m/z 497 (MH^+ − Cl). Anal. Calcd for $C_{27}H_{33}N_2O_7Cl$·H_2O: C, H, N.

2-Naphthylidene-(3-chloro-1-propylamine) (20). The hydrochloride salt of 3-chloro-1-propylamine (2.6 g, 20 mmol) was stirred with triethylamine (3 mL, 21 mmol) in chloroform (100 mL) at room temperature for 30 min. Naphthalene-2-carboxaldehyde (3.13 g, 20 mmol) was added and the reaction mixture was stirred at room temperature for 8 h, after which the reaction mixture was washed with water (100 mL), brine (50 mL) and dried (Na_2SO_4). Concentration of the organic layer provided the product **20**, which on crystallization from hot hexane provided the product (4.26 g, 91%) as a pale white solid: mp 49−50 °C; IR (neat) 2898, 2841, 1687, 1643, 1604, 1503, 1486 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.47 (s, 1 H), 8.04 (s, 1 H), 7.98 (d, J = 8.6 Hz, 1 H), 7.90−7.80 (m, 2 H), 7.53−7.46

(m, 2 H), 3.83 (t, J = 6.6 Hz, 2 H), 3.68 (t, J = 6.4 Hz, 2 H), 2.21 (qn, J = 6.3 Hz, 2 H).

***cis*-*N*-(3-Chloro-1-propyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(2′-naphthyl)-1(2*H*)-isoquinolone (21).** In a similar procedure to that for the synthesis of isoquinolones **14**, the imine **20** (1.16 g, 5 mmol) on treatment with homophthalic anhydride **12b** (1.11 g, 5 mmol) resulted in the isoquinolone **21** (1.27 g, 56%) as a pale yellow solid: mp 214−215 °C; IR (KBr) 3074, 2917, 1733, 1615, 1592 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.90−7.78 (m, 2 H), 7.76 (d, J = 8.6 Hz, 1 H), 7.65 (s, 1 H), 7.58 (s, 1 H), 7.50−7.40 (m, 2 H), 7.10−7.03 (m, 2 H), 5.26 (d, J = 6.42 Hz, 1 H), 4.80 (d, J = 6.3 Hz, 1 H), 3.97−3.88 (m, 1 H), 3.85 (s, 3 H), 3.72 (s, 3 H), 3.70−3.61 (m, 2 H), 3.05−2.96 (m, 1 H), 2.09−1.86 (m, 2 H). Anal. Calcd for $C_{25}H_{24}NO_5Cl$: C, H, N.

12-(3-Chloro-1-propyl)-2,3-dimethoxy-12-hydrobenzo-[1″,2″,5′,6′]benzo[1′,2′,2,1]cyclopenta[3,4-*c*]isoquinoline-5,13-dione (22). In a similar procedure to that for the synthesis of indenoisoquinolines **15**, the isoquinolone **21** (0.91 g, 2 mmol) was converted to the indenoisoquinoline **22** (0.678 g, 78%) by thionyl chloride (50 mL) treatment and crystallization from chloroform−ethyl acetate (3:1): mp 286−288 °C; IR (KBr) 1687, 1652, 1614, 1555 and 1519 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.92 (d, J = 8.5 Hz, 1 H), 8.05 (s, 1 H), 7.87 (d, J = 8.5 Hz, 1 H), 7.79 (d, J = 8.2 Hz, 1 H), 7.69 (d, J = 8.2 Hz, 1 H), 7.61 (s, 1 H), 7.52 (m, 1 H), 7.40 (m, 1 H), 4.70 (t, J = 7.6 Hz, 2 H), 4.06 (s, 3 H), 3.97 (s, 3 H), 3.82 (t, J = 6.0 Hz, 2 H), 2.45−2.38 (m, 2 H). Anal. Calcd for $C_{25}H_{20}NO_4Cl$: C, H, N.

12-(3-Azido-1-propyl)-2,3-dimethoxy-12-hydrobenzo-[1″,2″,5′,6′]benzo[1′,2′,2,1]cyclopenta[3,4-*c*]isoquinoline-5,13-dione (23). The chloride **22** (0.435 g, 1 mmol) was converted to the azide **23** (0.413 g, 93%) in a procedure similar to that for the synthesis of azide **16** and crystallized from chloroform to yield the pure product as a purple solid: mp 179−179 °C; IR (KBr) 2098, 1653, 1553, 1472, 1426 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.90 (d, J = 8.6 Hz, 1 H), 8.01 (s, 1 H), 7.84 (d, J = 8.5 Hz, 1 H), 7.79−7.60 (m, 2 H), 7.58 (s, 1 H), 7.51 (t, J = 6.5 Hz, 1 H), 7.39 (t, J = 6.5 Hz, 1 H), 4.59 (t, J = 7.9 Hz, 2 H), 4.04 (s, 3 H), 3.96 (s, 3 H), 3.63 (t, J = 6.3 Hz, 2 H), 2.21−2.11 (m, 2 H). Anal. Calcd for $C_{25}H_{20}N_4O_4$: C, H, N.

12-(3-Amino-1-propyl)-2,3-dimethoxy-12-hydrobenzo-[1″,2″,5′,6′]benzo[1′,2′,2,1]cyclopenta[3,4-*c*]isoquinoline-5,13-dione Hydrochloride (24). The azide **23** (0.220 g, 0.5 mmol) was converted to the hydrochloride **24** (0.217 g, 96%) in a procedure similar to that for the synthesis of the salts **17** and the hydrochloride salt **24** was isolated as a purple solid: mp 349−351 °C; ^{1}H NMR (DMSO-d_6) δ 8.71 (d, J = 8.6 Hz, 1 H), 8.07 (bs, 1 H), 8.01 (d, J = 7.5 Hz, 1 H), 7.86−7.75 (m, 3 H), 7.53 (t, J = 8.0 Hz, 1 H), 7.42 (t, J = 7.6 Hz, 1 H), 7.39 (s, 1 H), 4.54 (t, J = 6.4 Hz, 2 H), 3.89 (s, 3 H), 3.81 (s, 3 H), 3.33 (s, 2 H), 2.94 (t, J = 8.2 Hz, 2 H), 2.20−2.05 (m, 2 H). Anal. Calcd for $C_{25}H_{23}N_2O_4Cl$: C, H, N.

12-[3-(Hydroxyethyl)amino-1-propyl]-2,3-dimethoxy-12-hydrobenzo[1″,2″,5′,6′]benzo[1′,2′,2,1]cyclopenta[3,4-*c*]isoquinoline-5,13-dione (25). In a similar procedure to that used for the synthesis of **18**, the chloride **22** (0.437 g, 1 mmol) on treatment with ethanolamine provided the aminol **25** (0.367 g, 81%) as a purple solid after crystallization from chloroform−ethyl acetate: mp 195−197 °C; IR (KBr) 3417, 2925, 2825, 1695, 1646, 1584, 1553, 1519, 1474 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.57 (d, J = 8.3 Hz, 1 H), 7.81−7.74 (m, 2 H), 7.68 (t, J = 8.2 Hz, 1 H), 7.60 (d, J = 8.3 Hz, 1 H), 7.46−7.40 (m, 1 H), 7.38−7.32 (m, 1 H), 7.22 (s, 1 H), 4.90 (bs, 1 H), 4.55 (bs, 1 H), 4.36 (t, J = 6.2 Hz, 1 H), 4.30 (t, J = 6.2 Hz, 1 H), 3.79 (s, 3 H), 3.75 (s, 3 H), 3.62 (t, J = 6.3 Hz, 1 H), 3.50 (t, J = 6.2 Hz, 1 H), 2.68 (t, J = 6.2 Hz, 2 H), 2.60 (t, J = 6.3 Hz, 2 H), 1.98−180 (m, 2 H). Anal. Calcd for $C_{27}H_{26}N_2O_5$: C, H, N.

General Procedure for the Synthesis of Indenoisoquinolines 27a−c. The indenoisoquinolines **1**,[1] **26a**,[4] or **26b**[4] (1.5 mmol) were heated at reflux with a 1 M solution of borane−tetrahydrofuran complex (3 mL) in dry THF (30 mL) for 2−12 h. After cooling, the reaction mixture was concentrated and the residue was dissolved in EtOAc (100 mL). Glacial acetic acid was added dropwise until pH 5. The organic layer was washed with saturated sodium bicarbonate (2 × 50 mL), brine, and dried (Na_2SO_4) and concentrated and passed through a short column of silica gel to yield the corresponding reduced products **27a−c**.

5,6-Dihydro-5-keto-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (27a). The isoquinoline **27a** was isolated in 98% yield: mp 288−293 °C; IR (KBr) 3749, 1634, 1555, 1514, 1479 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1 H), 7.37 (s, 1 H), 7.03 (s, 1 H), 6.83 (s, 1 H), 6.03 (s, 2 H), 4.03 (s, 3 H), 4.01 (s, 3 H), 3.99 (s, 3 H), 3.69 (s, 2 H). Anal. Calcd for $C_{20}H_{17}NO_5 \cdot 0.3H_2O$: C, H, N.

6-Ethyl-8,9-methylenedioxy-5,6-dihydro-5-oxo-2,3-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (27b). The isoquinoline **27b** was isolated in 99% yield: mp 280−285 °C; IR (KBr) 1633, 1559, 1514, 1476 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1 H), 7.26 (s, 1 H), 7.05 (s, 1 H), 6.85 (s, 1 H), 6.07 (s, 2 H), 4.60 (q, J = 7.1 Hz, 2 H), 4.04 (s, 3 H), 4.03 (s, 3 H), 3.70 (s, 2 H), 1.53 (t, J = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$) 162.2, 153.6, 148.6, 147.4, 146.2, 139.0, 138.1, 131.0, 130.1, 117.6, 117.5, 108.7, 106.1, 102.2, 102.1, 101.5, 56.1, 56.0, 38.9, 33.3, 14.5. Anal. Calcd for $C_{21}H_{19}NO_5$: C, H, N.

6-(1-Butyl)-8,9-dibenzyloxy-5,6-dihydro-5-oxo-2,3-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (27c). The indenoisoquinoline **27c** was isolated in 97% yield: mp 240−242 °C; IR (KBr) 2960, 1631, 1607, 1580, 1552 and 1479 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1 H), 7.17 (s, 1 H), 7.04 (s, 1 H), 6.86 (s, 1 H), 6.04 (s, 2 H), 4.50 (t, J = 7.7 Hz, 2 H), 4.01 (s, 3 H), 4.00 (s, 3 H), 3.68 (s, 2 H), 1.91−1.81 (m, 2 H), 1.62−1.50 (m, 2 H), 1.02 (t, J = 7.3 Hz, 3 H). Anal. Calcd for $C_{23}H_{23}NO_5$: C, H, N.

Top1-Mediated DNA Cleavage Reactions. Human recombinant top1 was purified from Baculovirus as described previously.[23] The 161 bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease *Pvu*II and *Hin*dIII (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (10 μL reactions) for 1 h at 37 °C, separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161-bp fragment was eluted from the gel slice (centrilutor by Amicon) and concentrated in a Centricon 50 centrifugal concentrator (Amicon, Beverly, MA). Approximately 200 ng of the fragment was 3′-end-labeled at the *Hin*dIII site by fill-in reaction with [α-^{32}P]dCTP and 0.5 mM dATP, dGTP, 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). Labeling reactions were followed by phenol−chloroform extraction and ethanol precipitation. The resulting 161-bp 3′-end-labeled DNA fragment was resuspended in water. Aliquots (approximately 50 000 dpm/reaction) were incubated with top1 at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). Reversibility of cleavage complexes was tested by adding 0.35 M NaCl for in indicated times before terminating the reactions.[3] After ethanol precipitation, the samples were resuspended in loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0), and separated in a denaturing gel (16% polyacrylamide, 7 M urea) run at 51 °C. The gel was dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

SV40 DNA Unwinding Assay. Reaction mixtures (10 μL final volume) contained 0.3 μg of supercoiled SV40 DNA in reaction buffer (10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL bovine serum albumin) and 10 units of human recombinant top1.[20] Reactions were performed at 37 °C for 30 min and terminated by the addition of 0.5% SDS; then 1.1 μL of 10X loading buffer (20% Ficol 400, 0.1 M Na_2EDTA, pH 8, 1.0% SDS, 0.25% bromophenol blue) was added and reaction mixtures were loaded onto a 1% agarose gel made in 1X TBE buffer. After electrophoresis, DNA bands were stained in 10 μg/mL ethidium bromide and visualized by transillumination with UV light (300 nm).[20]

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