Synthesis of Cytotoxic Indenoisoquinoline Topoisomerase I Poisons

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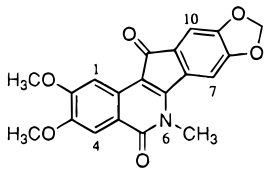
A number of indenoisoquinolines were prepared and evaluated for cytotoxicity in human cancer cell cultures and for activity vs topoisomerase 1 (top1). The two most cytotoxic indenoisoquinolines proved to be *cis*-6-ethyl-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline **(21)** and *cis*-6-allyl-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline **(22)**, both of which displayed submicromolar mean graph midpoints when tested in 55 human cancer cell cultures. Two of the most potent top1 inhibitors were 6-(3-carboxy-1-propyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline **(26)** and 6-ethyl-2,3-dimethoxy-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinolinium chloride **(27)**, both of which also inhibited top2, unwound DNA, and are assumed to be DNA intercalators. However, two additional potent top1 inhibitors, 6-allyl-5,6-dihydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline **(13c)** and 5,6-dihydro-6-(4-hydroxybut-1-yl)-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline **(19a)**, did not unwind DNA and did not affect top2. Some of the DNA cleavage sites detected in the presence of the indenoisoquinolines were different from those seen with the camptothecins. The cleavage sites induced by the indenoisoquinolines were reversed by salt treatment, which is consistent with the reversible trapping of top1 cleavable complexes by the indenoisoquinolines. In general, the potencies of the indenoisoquinolines as top1 inhibitors did not correlate with their potencies as cytotoxic agents, as some of the most cytotoxic agents had little if any effect on top1. On the other hand, the most potent of the indenoisoquinolines vs top1 were not the most cytotoxic. In several cases, moderate activity was observed for both cytotoxicity and activity vs top1.

Some time ago, we reported the synthesis of an indenoisoquinoline **1**.[1] Compound **1** was subsequently found to be cytotoxic in human cancer cell cultures. More recently, a COMPARE analysis[2−4] indicated that the cytotoxicity profile of **1** is similar to that of the topoisomerase 1 (top1) inhibitors camptothecin and saintopin.[5] When tested for activity against top1, compound **1** was in fact found to induce DNA cleavage in



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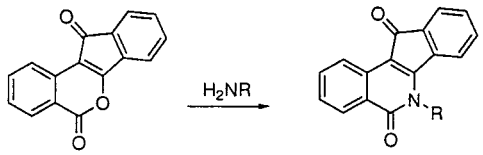
the presence of top1.[5] However, the cleavage site specificity differed from that of camptothecin in that compound **1** did not cleave at all of the sites characteristic of camptothecin, while some DNA cleavage sites were unique to compound **1**. In addition, compound **1** did not produce detectable DNA unwinding, suggesting that in contrast to other noncamptothecin top1 inhibitors, it is not a DNA intercalator. The present study was undertaken in order to investigate the structural parameters associated with the biological activity of **1** and to exploit it as lead compound for the development of new top1 inhibitors and potential anticancer agents.

Scheme 1



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3 R = CH_2CH_3
4 R = $CH_2CH_2CH_3$
5 R = cyclopropyl
6 R = CH_2COOCH_3
7 R = $CH_2CH_2CH_2CH_2OH$
8 R = $CH_2CH_2CH_2CH_2CH_2OH$

Chemistry

A number of indenoisoquinolines **3**−**8** lacking the methylenedioxy and methoxy substituents of **1** were synthesized by reacting commercially available benz-[*d*]indeno[1,2-*b*]pyran-5,11-dione **(2)** with various primary amines (Scheme 1). The reactions were carried out at room temperature in chloroform and the yields were generally high. These compounds were synthesized in order to determine whether simple and readily available

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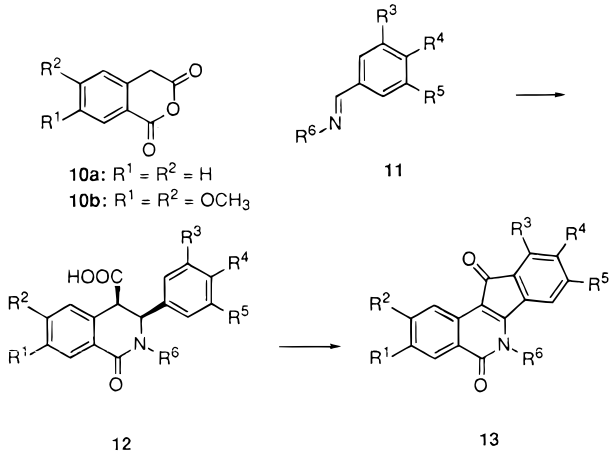
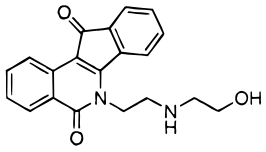
Scheme 2



	R^1	R^2	R^3	R^4	R^5	R^6
a	H	H	H	O-CH_2-O		CH_3
b	H	H	H	O-CH_2-O		$CH_2CH_2CH_2CH_3$
c	OCH_3	OCH_3	H	O-CH_2-O		$CH_2CH{=}CH_2$
d	OCH_3	OCH_3	H	O-CH_2-O		$CH_2CH_2CH_2CH_3$
e	OCH_3	OCH_3	H	O-CH_2-O		CH_2Ph
f	OCH_3	OCH_3	H	O-CH_2-O		C_6H_4-*p*-OCH_3
g	H	H	H	OBn	OBn	CH_3
h	OCH_3	OCH_3	H	OBn	OBn	CH_3
i	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	CH_3
j	H	H	OCH_3	OCH_3	OCH_3	CH_3
k	OCH_3	OCH_3	H	O-CH_2-O		CH_2CH_3

compounds would retain any of the activity of the parent compound **1**. The idea to synthesize these compounds was also given encouragement by the antineoplastic activity recently reported for oracin **(9)**, which has been found to induce G2 cell cycle arrest and apoptosis in Burkitt's lymphoma cells.[6−11]



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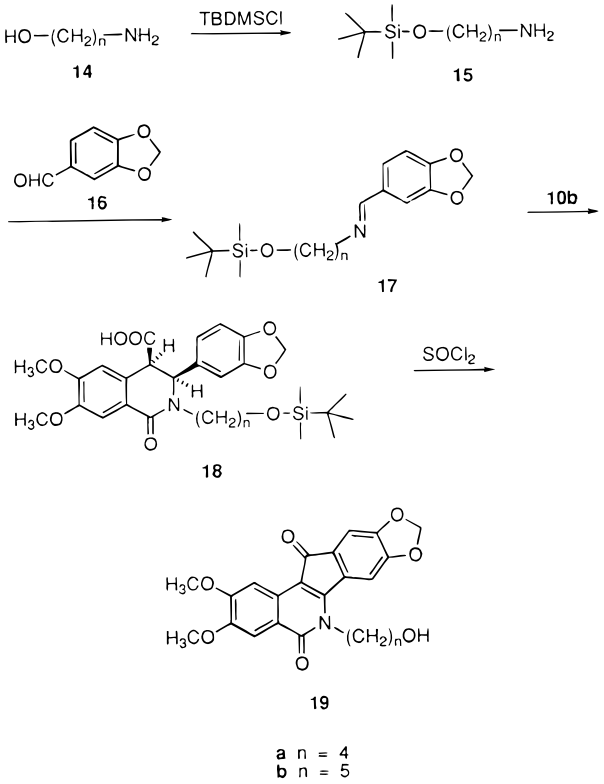
To accommodate additional substituents on the two aromatic rings of the indenoisoquinoline system, an alternative synthesis was executed which was based on the condensation of Schiff bases **11** with homophthalic anhydrides **10** to afford cis substituted isoquinolones **12**, followed by conversion to the desired products **13** in the presence of thionyl chloride (Scheme 2).[1] Using this method, a series of 11 additional indenoisoquinolines **13a**−**k** were synthesized. These compounds incorporate a variety of substituents at C-2, C-3, N-6, C-8, C-9, and C-10 of the ring system.

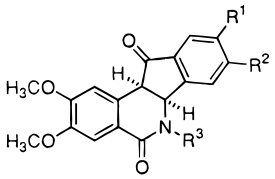
A modification of this route was carried out in order to synthesize compounds containing an alcohol group at the end of an alkyl chain located at N-6 (Scheme 3). Treatment of 4-amino-1-butanol **(14a)** or 5-amino-1-pentanol **(14b)** with *tert*-butyldimethylsilyl chloride according to the procedure of Corey and Venkateswarlu

Scheme 3



afforded the corresponding protected intermediates **15a** and **15b**.[12] The imines **17a** and **17b** were synthesized by treating the *O*-TBDMS protected aminols **15a** and **15b** with piperonal **(16)** in chloroform in the presence of anhydrous magnesium sulfate. Condensation of the Schiff bases **17a** and **17b** with 4,5-dimethoxyhomophthalic anhydride **(10b)** afforded the cis 3,4-disubstituted isoquinolones **18a** and **18b**. The cis stereochemistry of **18a** and **18b** was confirmed by a 6 Hz coupling constant observed for the C-3 and C-4 methine signals.[13] Treatment of **18a** or **18b** with thionyl chloride resulted in deprotection of the terminal alcohol, Friedel−Crafts reaction to form the five-membered ring, and dehydrogenation to afford **19a** and **19b**.

Several dihydro derivatives **20**−**23** were also prepared. The syntheses of **20** and **23** were carried out as described previously.[14,15] Compounds **21** and **22** were prepared by treatment of the acids **12k** and **12c** with Eaton's reagent (10% P_2O_5 in methanesulfonic acid).



20 R^1, R^2 = OCH_2O; R^3 = CH_3
21 R^1, R^2 = OCH_2O; R^3 = CH_2CH_3
22 R^1, R^2 = OCH_2O; R^3 = $CH_2CH{=}CH_2$
23 R^1 = OSO_2CH_3; R^2 = OCH_3; R^3 = CH_3

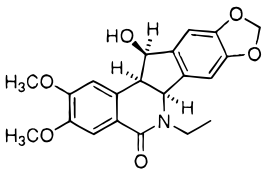
Treatment of **21** with borane-tetrahydrofuran complex in refluxing THF for 1 h resulted in reduction of the ketone to afford **24.** When **21** was treated with the same reagent in refluxing THF for 12 h, reduction of both the

Table 1. Cytotoxicities 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	1.3	35	41	4.2	73	68	37	96	20	++
2	>100	>100	>100	>100	>100	>100	>100	>100	85	0
3										0
4										0
5	61	>100	>100	84	>100	>100	>100	>100	98	0
6			>100	>100	>100	>100	>100	>100	81	0
7	13	3.2	4.6	4.4	74	7.4	>100	>100	16	+
8	4.4	3.9		14.3	93		3.4	41	14	+
13a	22	54		>100	>100	>100	>100		45	+
13b	17	2.9	9.0	8.2	94	37	38	>100	30	+
13c	3.4	2.3	2.2		6.6	2.6	3.2	5.2	4.2	+
13d	16	45		21	78		16	>100	42	+
13e	12	>100		50	>100		4.0	>100	42	±
13f	20	>100	>100	>100	>100	>100	76	>100	70	0
13g	72	>100	>100	93	>100	88	>100	>100	82	0
13h	46	80	39	>100	58	>100	>100	>100	72	0
13i	>100	>100	>100	55	>100	>100	>100	>100	95	0
13j	24		>100		>100	>100	>100		78	0
13k	2.2	2.6	2.0	2.1	3.0	3.6	2.3	2.6	2.4	±
19a	0.70	1.4		0.99	3.7		0.36	10	3.2	+
19b	8.7	>100		>100	>100		5.1	>100	45	+
20	9.4	2.0	3.1	0.42	6.7	2.1	4.1	17	5.0	±
21	0.29	0.29		0.18	0.44		0.42	0.45	0.81	±
22	0.36	0.34		0.38	1.78		0.77	1.4	0.98	0
23										±
24	>100	>100	>100		>100	>100	>100	>100	100	0
25	30	35	50		24	26	41	25	31	0
26	87	40		73	>100	27	48	>100	48	++
27	22	13	56		1.9	2.7	58	14	13	++

[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. The entries with population standard deviations are the results of two determinations, and the ones without population standard deviations are the result of single determinations. [c] ++: greater than 50% of the activity of 1 μM camptothecin; +: between 20 and 50% of the activity of 1 μM camptothecin; ±: less than 20% of the activity of 1 μM camptothecin; 0: inactive. Each drug was tested at concentrations ranging between 0.1 and 300 μM (for example, see Figure 2).

ketone and amide carbonyls occurred to yield **25**. The stereochemistry of the hydroxyl group results from the approach of the reducing reagent to the less sterically hindered, convex surface of the indenoisoquinoline **21**.



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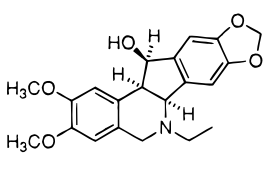

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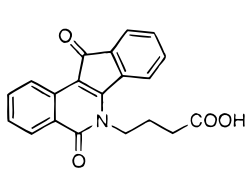
We were interested in obtaining an indenoisoquinoline derivative having an acidic group which might be converted into a more water-soluble salt. The carboxylic acid **26** was obtained by oxidation of indenoisoquinoline **7** with Jones reagent.



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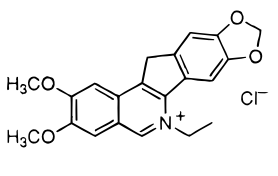


27

Dehydration, as well as dehydrogenation, of the alcohol **25** occurred in the presence of palladium in charcoal in refluxing acetic acid. Treatment of the product with aqueous NaCl provided the indenoisoquinolinium salt **27**.

Biological Results and Discussion

The indenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. The GI_{50} values obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a calculation of the average GI_{50} for all of the cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-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.[4] In addition, the relative activities of the compounds in the top1 cleavage assay are listed in Table 1.

In general, most of the new indenoisoquinolines were even less cytotoxic in human cancer cell cultures than the moderately active (MGM 20 μM) lead compound **1**. However, a few members of the series proved to be more cytotoxic than **1**, including the *N*-allyl analogue **13c** (MGM 4.2 μM), the *N*-ethyl homologue **13k** (MGM 2.4 μM), analogue **19a** (MGM 3.2 μM) having an *N*-(4′-hydroxybutyl) substituent, and the three dihydro derivatives **20** (MGM 5.0 μM), **21** (MGM 0.81 μM), and **22** (MGM 0.98 μM). The *N*-ethylisoquinolinium species **27** (MGM 13 μM) and the relatively simple indenoisoquinolines **7** (MGM 16 μM) and **8** (MGM 14 μM), both lacking substituent on the aromatic rings, were slightly more cytotoxic than **1**. Whereas the isoquinolinium salt **27** was comparable to **1** regarding top1 cleavage activity,

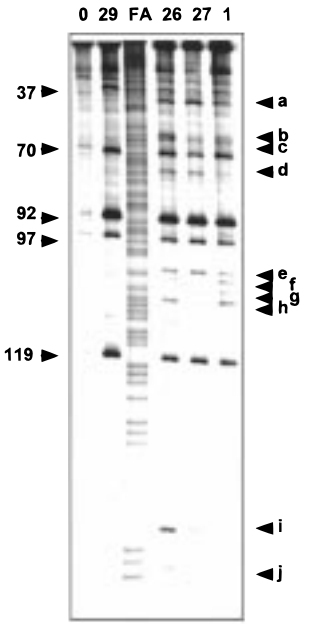
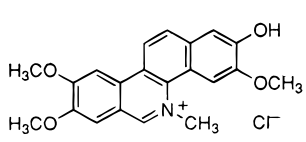


Figure 1. Comparison of top1-mediated DNA cleavage induced by compounds **29**, **26**, **27**, and **1** (NSC 314622).[5] The DNA fragment used corresponds to the 3′-end-labeled PVU II/Hind III fragment of pBluescript SK(−) phagemid DNA. DNA was reacted with top1 in the presence of the indicated drugs (**29**, 1.0 μM; **26** 100.0 μM; **27** and **1**, 10.0 μM). Reactions were at room temperature for 30 min and were stopped by adding 0.5% SDS. DNA fragments were separated on 16% denaturing polyacrylamide gels. FA: purine ladder. Left lane: DNA reacted with top1 in the absence of any drug. The numbers on the left correspond to the camptothecin **(29)** induced cleavage sites, whereas the letters on the right correspond to additional cleavage sites induced by **26**, **27**, and **1**.[5,24]

the other more cytotoxic analogues were significantly less potent than **1** in the top1 cleavage assay. The isoquinolinium salt **27** might be expected to have activity similar to **1** because it is structurally related to the cytotoxic benzophenanthridine alkaloid fagaronine **(28)**, a known inhibitor of both top1 and top2.[16]



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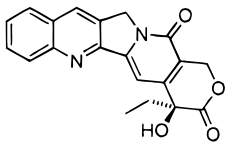

29

The most potent of the new indenoisoquinolines vs top1 proved to be **26** and **27**. Both of these compounds were examined for induction of DNA cleavage in the 3′-end-labeled PvuII/HindIII fragment of pBluescript SK-(−) phagemid DNA in the presence of top1[5] (Figure 1). The results were compared with those for the lead compound **1** and camptothecin **(29)**. Some of the cleavage sites detected in the presence of **26**, **27**, and **1** were different from those induced by camptothecin **(29)**. The indenoisoquinolines **26**, **27**, and **1** induced several top1 cleavage sites that were not observed with camptothecin **(29)**. In Figure 1, the numbers on the left of the gel picture correspond to the camptothecin-induced cleavage sites,[5] while the letters on the right correspond to additional cleavage sites induced by the indenoisoquinolines. The bands corresponding to cleavage at identical sites varied in intensity between the indenoisoquinolines and camptothecin. The DNA cleavage sites induced among the indenoisoquinolines also varied in intensity. For example, the cleavage site labeled "i" was much more intense for **26** than it was for either **27** or **1**.

A wider array of compounds were tested at various concentrations, and an example of the results is displayed in Figure 2. The top1 inhibition data are summarized in Table 1. In general, except for **13k**, which had very weak activity, the indenoisoquinolines induced

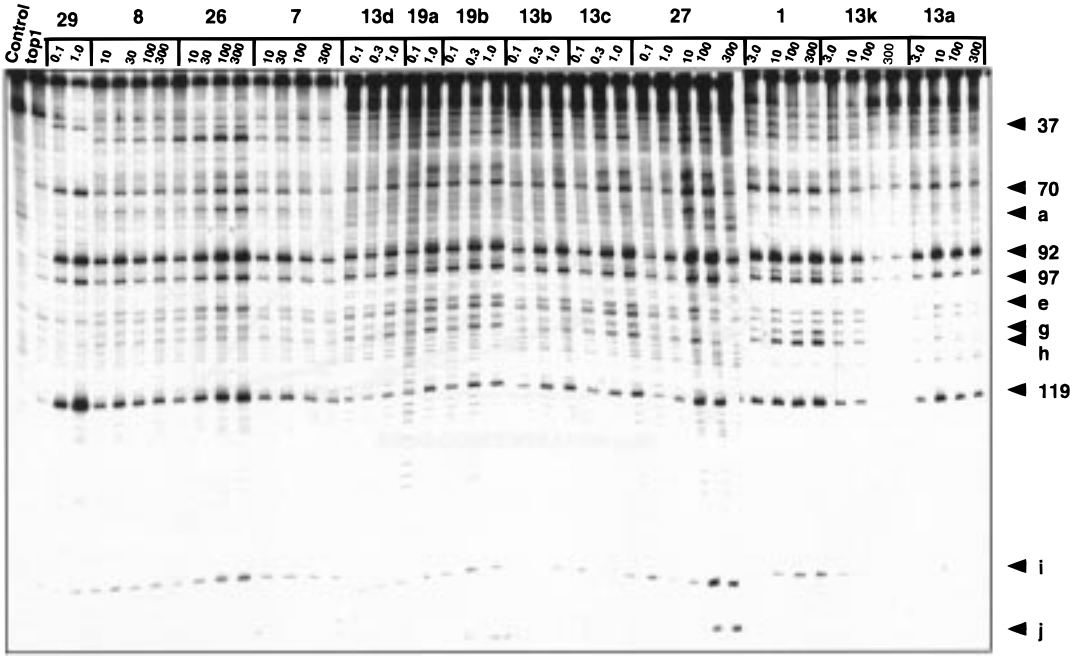

Figure 2. Comparison of top1-mediated DNA cleavages at different drug concentrations induced by the indicated drugs in pBluescript SK(−) phagemid DNA. The indicated drug concentrations are in μM/L. Reactions were at room temperature for 30 min and stopped by adding 0.5% SDS. DNA fragments were separated on 16% denaturing polyacrylamide gels. Top1 was present in all reactions except in the control lane. Control: DNA with neither top1 nor any drug. Numbers and letters to the right are as in Figure 1.

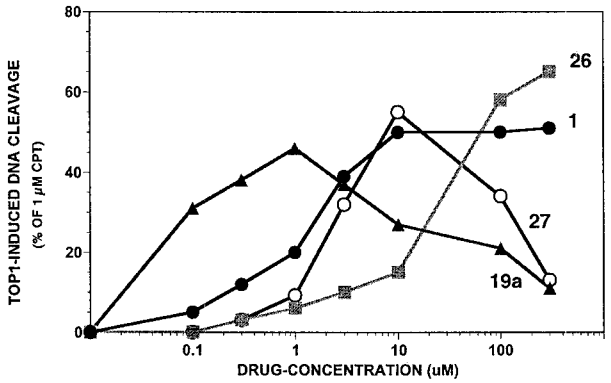

Figure 3. Top1-mediated DNA cleavage at different drug concentrations induced by the drugs indicated on the top on pBluescript SK(−) phagemid DNA. The activity of 1.0 μM camptothecin **(29)** was arbitrarily set to 100% and the activity of the indicated drugs is given in relation to the camptothecin standard. Individual points represent the mean of three to four independent experiments with a 10% variability.

similar cleavage patterns. The indenoisoquinolines were generally less potent than camptothecin. The relative effective concentrations compared with 0.1 μM camptothecin were 10 for **19a**, 50 for **1** and **27**, and 500 for **26**. With some of the compounds (e.g., **27**), the activity seemed to increase initially as the concentration was increased, but then it declined at higher concentrations. This is reflected in Figure 3, which was obtained after a more extensive investigation of the most potent indenoisoquinolines. The increase and then decrease in activity vs concentration indicates that the compound suppresses top1-mediated DNA cleavage at higher drug concentrations, a result which is similar to the bell-shaped curves seen with DNA unwinding or intercalating poisons.[17−19] To investigate the possibility that some of the most potent indenoisoquinolines could be unwinding DNA and thus causing inhibition of top1 activity at higher drug concentrations, they were examined for DNA unwinding activity. The unwinding assay using supercoiled DNA in the presence of top1 is a simple procedure to detect DNA intercalation.[20] The results in Figure 4 show that the indenoisoquinoline **27** in fact does unwind DNA, as does **26** at higher concentrations. On the other hand, the indenoisoquinoline **19a**, like the lead compound **1**, does not appear to unwind DNA. Thus, the reduced efficiency of **19a** for inducing top1-mediated DNA cleavage at high concentration does not appear to be related to DNA unwinding. It is possible that **19a** might interact with DNA in a manner that causes inhibition of top1 activity at higher concentrations but does not induce unwinding. Alternatively, higher concentrations of **19a** may induce a conformational change in the enzyme through a direct interaction. Similar possible effects have been proposed previously to explain the bell-shaped curves produced by saintopin E, which also does not cause DNA unwinding.[18]

Camptothecin **(29)** induces DNA strand breaks by stabilizing the cleavage complexes and inhibiting DNA 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 camptothecin and the indenoisoquinoline derivatives **1**, **13c**, **19a**, **26**, and **27** were reversed by salt treatment (data not shown). This reversibility is consistent with the reversible trapping of top1 cleavage complexes by the indenoisoquinolines.

In general, a planar indenoisoquinoline system appears to be a necessary, although not sufficient, condition for potent activity in the top1 cleavage assay. The nonplanar systems **20**−**25** were all inactive or displayed weak activity vs top1 (Table 1). A direct comparison can be made between the planar indenoisoquinoline **1** and the corresponding nonplanar, cis dihydro compound **20**. Compound **1** displays good activity in the top1 cleavage assay, whereas the activity of **20** is weak. On the other hand, indenoisoquinolines **3**−**6** and **13f**−**j** are all planar ring systems that are inactive as top1 inhibitors.

It is of interest to compare the results obtained with the *N*-(4′-hydroxybutyl) compound **7** with those of the corresponding acid **26** in the top1 cleavage assay. Both of these simple indenoisoquinolines lack substituents in the aromatic rings and differ only in the oxidation state of the terminal carbon of the N-substituent. There is a significant increase in top1 inhibitory activity in going from the alcohol **7** to the corresponding carboxylic acid **26**.

Since a number of top1 poisons also inhibit top2,[22] we tested the induction of top2 cleavage complexes by indenoisoquinolines. Figure 5 shows a representative experiment with the compounds that were the most active against top1. Compound **26** induced top2 cleavage complexes at sites which often did not overlap with the top2 sites induced by VP-16 (etoposide). Compound **27** had only marginal top2 activity at 100 μM, and compounds **13c**, **19a**, and **1** had no effect on top2 cleavage activity (Figure 5). Compounds **7** and **8** also exhibited weak top2 activity, and compounds **13b**, **13k**, **20**, **21**,

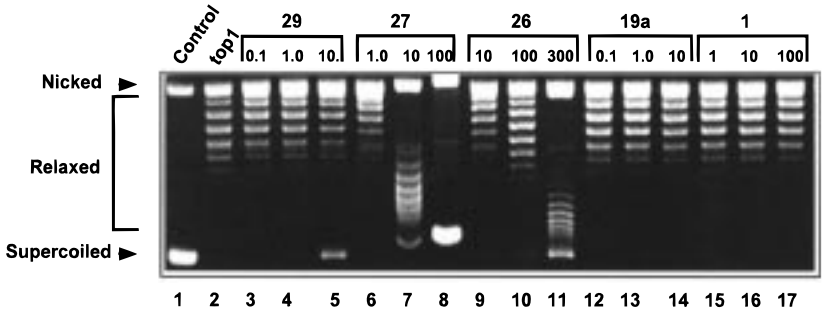

Figure 4. Native supercoiled SV40 DNA (lane 1) was incubated with top1 in the absence of any drug (lane 2) or in the presence of the indicated drugs (concentrations in μM/L) for 30 min at 37 °C. Reactions were stopped with 0.5% SDS (final concentration). The DNA was then separated in a 0.8% agarose gel stained with ethidium bromide.

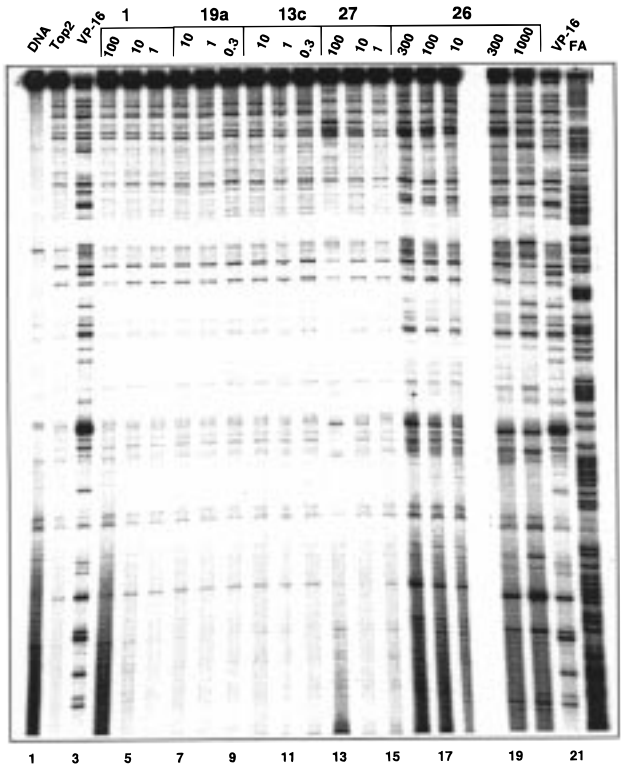

Figure 5. Comparison of top2-mediated DNA cleavages by compounds **1**, **19a**, **13c**, **27**, **26**, and VP-16 (etoposide). The DNA fragment used corresponds to a fragment of the human c-myc proto-oncogene.[23] Reactions were performed at 37 °C for 30 min and stopped by adding SDS (0.5% final concentration). DNA fragments were separated on an 8% denaturing polyacrylamide gel. Top2 was present in all reactions except in lane 1. The indicated drug concentrations above each lane are in μM/L. The concentration of VP-16 was 100 μM. FA indicates the purine ladder.

and **22** had no effect on top2 cleavage (data not shown). These results indicate that the indenoisoquinolines are prominently top1 inhibitors, except for the two derivatives **26** and **27** that are dual top1 and top2 inhibitors and also produce DNA unwinding.

In conclusion, the present study was undertaken in order to maximize the cytotoxicity of the lead compound **1** in human cancer cell cultures as well as its activity as a top1 poison. The first objective was realized in the cytotoxic indenoisoquinolines **7**, **8**, **13c**, **13k**, **19a**, **20**, **21**, **22**, and **27**, all of which displayed a lower MGM than the lead compound **1** (Table 1). With regard to the second objective, several top1 inhibitors were synthesized which rival the topoisomerase activity of **1**, including **13c**, **19a**, **26**, and **27**. One obvious point of further interest is that with the possible exception of **19a** and **13c**, the two activities did not maximize in the same compounds, suggesting that the activity of some of the more cytotoxic compounds may not be due to their activity vs top1. The situation is complicated by such factors as cellular uptake and possible conversion of parent compounds to metabolites which may have increased activity vs top1.

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 micron glass plates. Compounds were visualized with short wavelength UV light or phosphomolybdic acid indicator. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

6-Ethyl-5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline (3). Ethylamine (0.2 mL, 3 mmol) was added to a stirred solution of commercially available (Aldrich) benz[*d*]indeno[1,2-*b*]pyran-5,1l-dione (**2**) (0.49 g, 2 mmol) in $CHCl_3$ (10 mL). The bright orange mixture was stirred overnight. To the reaction mixture $CHCl_3$ (100 mL) was added, and the mixture was washed with H_2O (3 × 25 mL) and brine (1 × 25 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give an orange−red solid (0.43 g, 75%): mp 188−189 °C; IR (thin film) 2986, 1690, 1656, 1611, 1549, 1503, 1430, 1320, 1197, 991 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.66 (d, J = 8.3 Hz, 1 H), 8.32 (d, J = 7.9 Hz, 1 H), 7.69 (dt, J = 8.4 and 1.4 Hz, 1 H), 7.60 (dd, J = 8.0 and 1.4 Hz, 1 H), 7.52 (d, J = 6.9 Hz, 1 H), 7.40 (m, 3 H), 4.56 (q, J = 7.2 Hz, 2 H), 1.53 (t, J = 7.2 Hz, 3 H); CIMS m/z (relative intensity) 276 (MH^+, 100). Anal. Calcd for $C_{18}H_{13}NO_2$: C, H, N.

5,6-Dihydro-5,11-diketo-6-propyl-11*H*-indeno[1,2-*c*]isoquinoline (4). Propylamine (0.3 mL, 3 mmol) was added to a stirred solution of benz[*d*]indeno[1,2-*b*]pyran-5,1l-dione (**2**) (0.49 g, 2 mmol) in $CHCl_3$ (10 mL). The red solution stirred overnight before $CHCl_3$ (75 mL) was added, and the mixture was washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow−orange solid (0.32 g, 55%): mp 166−167 °C; IR (neat) 2967, 1660, 1502, 1427, 1317, 1193, 959 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.69 (d, J = 8.0 Hz, 1 H), 8.33 (d, J = 9.0 Hz, 1 H), 7.70 (dt, J = 9.0 and 3.0 Hz, 1 H), 7.62 (d, J = 6.2 Hz, 1 H), 7.40 (m, 4 H), 4.46 (t, J = 8.0 Hz, 2 H), 1.92 (m, 2 H), 1.12 (t, J = 7.4 Hz, 3 H); CIMS m/z (relative intensity) 290 (MH^+, 100). Anal. Calcd for $C_{19}H_{15}NO_2$: C, H, N.

6-Cyclopropyl-5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline (5). Cyclopropylamine (10 mL) was added to a stirred solution of benz[*d*]indeno[1,2-*b*]pyran-5,1l-dione **(2)** (0.28 g, 1.1 mmol) in $CHCl_3$ (10 mL). The red solution stirred overnight before $CHCl_3$ (50 mL) was added, and the mixture was washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a red solid (0.3 g, 91%): mp 206−208 °C; IR (thin film) 3751, 1665, 1500, 1420, 1311, 1083, 950 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.62 (d, J = 7.7 Hz, 1 H), 8.29 (d, J = 8.4 Hz, 1 H), 7.88 (d, J = 7.0 Hz, 1 H), 7.69 (dt, J = 6.9 and 1.2 Hz, 1 H), 7.59 (dd, J = 6.1 and 1.3 Hz, 1 H), 7.40 (m, 3 H), 3.37 (m, 1 H), 1.45 (q, J = 6.8 Hz, 2 H), 0.99 (m, 2 H); CIMS m/z (relative intensity) 288 (MH^+, 100). Anal. Calcd for $C_{19}H_{13}NO_2$: C, H, N.

5,6-Dihydro-5,11-diketo-6-(methoxycarbonylmethyl)-11*H*-indeno[1,2-*c*]isoquinoline (6). Triethylamine (2.7 mL, 19.4 mmol) was added to a stirred solution of glycine methyl ester hydrochloride (1.57 g, 12.5 mmol) in chloroform (30 mL). After 1 h, benz[*d*]indeno[1,2-*b*]pyran-5, 1l-dione (**2**) (1.24 g, 5.0 mmol) was added to the mixture. The red mixture stirred an additional 4 h before $CHCl_3$ (100 mL) was added, and the mixture was washed with H_2O (3 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give an orange−red solid (1.48 g, 92%): mp 248−251 °C; IR (thin film) 2956, 1735, 1667, 1609, 1502, 1426, 1227, 981 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.68 (d, J = 8.0 Hz, 1 H), 8.32 (d, J = 8.2 Hz, 1 H), 7.73 (dt, J = 7.1 and 1.3 Hz, 1 H), 7.61 (m, 1 H), 7.47 (dt, J = 7.1 and 1.1 Hz, 1 H), 7.37 (m, 2 H), 7.26 (m, 1 H), 5.34 (s, 2 H), 3.79 (s, 3 H); CIMS m/z (relative intensity) 320 (MH^+, 100). Anal. Calcd for $C_{19}H_{13}NO_4$: C, H, N.

5,6-Dihydro-6-(4-hydroxy-1-butyl)-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline (7). 4-Amino-1-butanol (0.891 g, 10 mmol) was added to a chloroform (30 mL) solution of benz[*d*]indeno[1,2-*b*]pyran-5,1l-dione (**2**) (2.48 g, 10 mmol), and the reaction mixture was stirred at room temperature for 2 days.

The reaction mixture turned dark red. The reaction mixture was taken in chloroform (100 mL) washed with water (2 × 50 mL), 0.5 N HCl (50 mL), and brine (100 mL), dried (Na_2SO_4), and concentrated to give the crude product. The product was filtered through a short column of silica gel and the polar fraction concentrated to afford a reddish brown solid which was crystallized from 2-propanol to yield the product (2.56 g, 80%): mp 160−162 °C; IR (KBr) 3300, 1695, 1645, 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.63 (d, J = 8.1 Hz, 1 H), 8.26 (d, J = 8.1 Hz, 1 H), 7.70−7.15 (m, 6 H), 4.51 (t, J = 7.8 Hz, 2 H), 3.77 (t, J = 6.1 Hz, 2 H), 1.99 (p, J = 8.0 and 7.5 Hz, 2 H), 1.83 (s, 1 H, D_2O exchangeable), 1.75 (t, J = 6.6 Hz, 2 H). Anal. Calcd for $C_{20}H_{17}NO_3$: C, H, N.

5,6-Dihydroxy-6-(5-hydroxy-1-pentyl)-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline (8). 5-Amino-1-pentanol (1.03 g, 10 mmol) was added to a chloroform (20 mL) solution of benz-[*d*]indeno[1,2-*b*]pyran-5,1l-dione (**2**) (2.48 g, 10 mmol), and the reaction mixture was stirred at room temperature overnight. The reaction mixture turned dark red. The reaction mixture was taken in chloroform (100 mL), washed with water (2 × 50 mL), 0.5 N HCl (50 mL), and brine (100 mL), dried (Na_2SO_4), and concentrated to give the crude product. The TLC showed traces of starting material. The product was filtered through a short column of silica gel and the polar fraction concentrated to get a reddish-brown solid which was crystallized from 2-propanol to afford the product (2.53 g, 76%): mp 146−148 °C; IR (KBr) 2996, 1698, 1642, 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.63 (d, J = 8.1 Hz, 1 H), 8.27 (d, J = 8.1 Hz, 1 H), 7.67 (d, J = 8.4 Hz, 1 H), 7.56 (d, J = 6.8 Hz, 1 H), 7.45−7.30 (m, 4 H), 4.47 (t, J = 7.9 Hz, 2 H), 3.71 (t, J = 5.9 Hz, 2 H), 1.92 (p, J = 7.9 and 7.4 Hz, 2 H), 1.82 (s, 1 H, D_2O exchangeable), 1.78−1.55 (m, 4 H); CIMS *m/z* (relative intensity) 334 (MH^+, 100). Anal. Calcd for $C_{21}H_{19}NO_3$: C, H, N.

***cis*-4-Carboxy-3,4-dihydro-*N*-methyl-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (12a).** Homophthalic anhydride **(10a)** (0.81 g, 5 mmol) was added to a stirred solution of 3,4-methylenedioxybenzylidenemethylamine **(11a)** (0.82 g, 5 mmol) in chloroform (5 mL). After 30 min, the precipitated product was filtered from the yellow solution and washed with chloroform to give a pale yellow solid (1.2 g, 74%): mp 165−167 °C; ^1H NMR (DMSO-d_6) δ 7.99 (d, J = 7.5 Hz, 1 H), 7.48 (m, 3 H), 6.76 (d, J = 8.0 Hz, 1 H), 6.52 (d, J = 8.0 Hz, 1 H), 6.43 (s, 1 H), 5.93 (s, 2 H), 5.03 (d, J = 6.2 Hz, 1 H), 4.64 (d, J = 6.1 Hz, 1 H), 2.89 (s, 3 H); CIMS *m/z* (relative intensity) 326 (MH^+, 100).

5,6-Dihydro-5,11-diketo-6-methyl-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (13a). Thionyl chloride (8.1 mL) was added with stirring to the cis acid **12a** (0.7 g, 2.1 mmol). The yellowish-brown mixture became orange within 15 min and after 30 min was red. After 4 h, the reaction mixture was diluted with benzene (25 mL) and evaporated to dryness. The brownish-red solid was recrystallized from methanol and passed through a short column (SiO_2) and eluted with chloroform to give a brown solid (0.14 g, 24%): mp 310−312 °C; IR (thin film) 2358, 1652, 1540, 1506, 1292 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.43 (d, J = 8.0 Hz, 1 H), 8.16 (d, J = 8.0 Hz, 1 H), 7.75 (t, J = 7.5 Hz, 1 H), 7.56 (s, 1 H), 7.44 (t, J = 7.6 Hz, 1 H), 7.15 (s, 1 H), 6.19 (s, 2 H), 3.92 (s, 3 H); CIMS *m/z* (relative intensity) 306 (MH^+, 100). Anal. Calcd for $C_{18}H_{11}NO_4$: C, H, N.

3,4-Methylenedioxybezylidenebutylamine (11b). Piperonal (7.5 g, 50 mmol) and *n*-butylamine (6 mL, 75 mmol) were stirred in chloroform (100 mL) in the presence of anhydrous $MgSO_4$ (5 g) at room temperature for 4 h. The mixture was filtered, and the residue was washed with chloroform (20 mL). The combined filtrate was concentrated under reduced pressure to afford a yellow oil (9.8 g, 96%): IR (neat) 1649, 1643, 1604 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.11 (s, 1 H), 7.31 (d, J = 1.2 Hz, 1 H), 7.06 (dd, J = 1.2 and 7.9 Hz, 1 H), 6.79 (d, J = 7.8 Hz, 1 H), 5.95 (s, 2 H), 3.53 (t, J = 6.6 Hz, 2 H), 1.63 (p, J = 7.3 Hz, 2 H), 1.37 (hextet, J = 7.3 Hz, 2 H), 0.91 (t, J = 7.3 Hz, 3 H).

***cis*-*N*-(1-Butyl)-4-carboxy-3,4-dihydro-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (12b).** Homophthalic anhydride **(10a)** (3.24 g, 20 mmol) was added to a chloroform (20 mL) solution of the imine **11b** (4.1 g, 20 mmol), and the mixture was stirred at room temperature for 45 min, after which the TLC showed the complete disappearance of the starting materials. The reaction mixture was concentrated to remove chloroform completely. The residue was dissolved in hot ethyl acetate (100 mL) and left at room temperature for 12 h. The colorless crystals that separated were filtered and dried to give pure **12b** (6.57 g, 89%): mp 178−181 °C; IR (KBr) 1712, 1634, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (dd, J = 1.0 and 7.5 Hz, 1 H), 7.52 (d, J = 7.5 Hz, 1 H), 7.40−7.28 (m, 2 H), 6.51−6.45 (m, 2 H), 6.37 (s, 1 H), 5.75 (dd, J = 1.1 and 6.4 Hz, 2 H), 4.87 (d, J = 6.2 Hz, 1 H), 4.51 (d, J = 6.2 Hz, 1 H), 3.93 (dt, J = 7.2 and 6.6 Hz, 1 H), 2.73 (dt, J = 7.2 and 6.6 Hz, 1 H), 1.52 (p, J = 7.2 Hz, 2 H), 1.26 (hextet, J = 7.3 Hz, 2 H), 0.83 (t, J = 7.2 Hz, 3 H); CIMS *m/z* (relative intensity) 368 (MH^+, 100); EIMS *m/z* (relative intensity) 367 (M^+, 5), 322 (30), 135 (100). Anal. Calcd for $C_{21}H_{21}NO_5$: C, H, N.

6-(1-Butyl)-5,6-dihydro-5,11-diketo-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (13b). Thionyl chloride (30 mL) was added dropwise to the acid **12b** (3.35 g, 0.089 mol) with stirring. The resulting solution was stirred at room temperature for 12 h, after which the solution turned dark pink. Benzene (20 mL) was added to the reaction mixture, and it was concentrated under reduced pressure. The resulting residue was purified by column chromatography (acetone: hexane, 1:4) followed by crystallization (EtOAc/hexane) to obtain pure indenoisoquinoline **13b** (1.37 g, 44%): mp 200−201 °C; IR (KBr) 1691, 1665, 1631 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.50 (d, J = 8.1 Hz, 1 H), 8.21 (d, J = 8 Hz, 1 H), 7.61 (t, J = 8 Hz, 1 H), 7.34 (t, J = 8 Hz, 1 H), 6.98 (s, 1 H), 6.87 (s, 1 H), 6.03 (s, 2 H), 4.34 (t, J = 8 Hz, 2 H), 1.80 (p, J = 8 Hz, 2 H), 1.51 (hextet, J = 8 Hz, 2 H), 1.01 (t, J = 8 Hz, 3 Hz); ^{13}C NMR ($CDCl_3$) δ 189.01, 163.1, 154.85, 151.21, 148.97, 133.58, 132.18, 132.05, 130.50, 128.29, 126.39, 122.82, 122.69, 107.48, 105.12, 104.84, 102.57, 44.13, 31.33, 20.1, 13.73; CIMS *m/z* (relative intensity) 348 (MH^+, 100); EIMS *m/z* (relative intensity) 347 (M^+, 60), 330 (10), 318 (30), 291 (100). Anal. Calcd for $C_{21}H_{17}NO_4$: C, H, N.

3,4-Methylenedioxybenzylideneallylamine (11c). Allylamine (1.71 g, 30 mmol) was added to a solution of piperonal (4.5 g, 30 mmol) in chloroform (30 mL) in the presence of anhydrous magnesium sulfate (3 g), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered, the residue washed with chloroform (10 mL), and the combined filtrate was concentrated under reduced pressure to afford a yellow oil (5.55 g, 98%): IR (neat) 1679, 1639, 1600 and 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05 (s, 1 H), 7.31 (s, 1 H), 7.04 (d, J = 8 Hz, 1 H), 6.10−6.00 (m, 1 H), 5.88 (s, 2 H), 5.04 (m, 2 H), 4.10 (m, 2 H).

***cis*-*N*-Allyl-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (12c).** 4,5-Dimethoxyhomophthalic anhydride **(10b)** (1.11 g, 5 mmol) was added to a chloroform (10 mL) solution of the imine **11c** (0.945 g, 5 mmol) and the mixture was stirred at room temperature for 45 min, after which the TLC showed the complete disappearance of the starting materials and a white precipitate formed in the reaction mixture. The precipitated product was filtered off, washed with chloroform (5 mL), and dried to give pure **12c** (1.43 g, 70%): mp 235−238 °C; IR (KBr) 3000, 1736, 1686, 1615 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.0 (bs, 1 H), 7.52 (s, 1 H), 7.13 (s, 1 H), 6.76 (d, J = 8.5 Hz, 1 H), 6.52 (d, J = 8.5 Hz, 1 H), 6.44 (s, 1 H), 5.94 (s, 2 H), 5.85−5.70 (m, 1 H), 5.16 (dd, J = 3.5 and 17.5 Hz, 2 H), 4.92 (d, J = 6.5 Hz, 1 H), 4.57 (d, J = 6.5 Hz, 1 H), 3.82 (s, 3 H), 3.75 (s, 3 H), 3.20−3.10 (m, 2 H). CIMS *m/z* (relative intensity) 412 (MH^+, 100). Anal. Calcd for $C_{22}H_{21}NO_7$: C, H, N.

6-Allyl-2,3-dimethoxy-5,6-dihydro-5,11-oxo-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinoline (13c). Treatment of **12c** (2.05 g, 5 mmol) with Eaton's reagent (10% P_2O_5 in methanesulfonic acid, 60 mL) at room temperature with stirring in an open flask for 24 h resulted in a mixture of **22** and **13c**. The two products were separated by column chromatography on silica gel (230−400 mesh) using hexane:acetone

(4:1) to afford **22** (842 mg, 43%) and **12c** as a purple solid product (588 mg, 30%) after recrystallization from ethyl acetate−hexane: mp 290−294 °C; IR (KBr) 2370, 1698, 1653, 1551, 1484 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97 (s, 1 H), 7.63 (s, 1 H), 6.99 (s, 1 H), 6.91 (s, 1 H), 6.20−6.05 (m, 1 H), 6.06 (s, 2 H), 5.31 (d, J = 10.5 Hz, 1 H), 5.20−5.00 (m, 3 H), 3.33 (s, 3 H), 3.97 (s, 3 H). Anal. Calcd for $C_{22}H_{17}NO_6$: C, H, N.

***cis*-*N*-(1-Butyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (12d).** 4,5-Dimethoxyhomophthalic anhydride **10b** (2.22 g, 10 mmol) was added to a chloroform (10 mL) solution of the imine **(11b)** (2.1 g, 10 mmol) and the mixture was stirred at room temperature for 45 min, after which the TLC showed the complete disappearance of the starting materials and a white precipitate formed in the reaction mixture. The precipitated product was filtered off, washed with chloroform (5 mL), and dried to give pure **12d** (3.45 g, 81%): mp 242−244 °C; IR (KBr) 1732, 1640, 1610, 1600 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.56 (s, 1 H), 7.08 (s, 1 H), 6.55−6.48 (m, 2 H), 6.40 (s, 1 H), 5.79 (d, J = 2.5 Hz, 2 H), 4.86 (d, J = 6.2 Hz, 1 H), 4.45 (d, J = 6.2 Hz, 1 H), 3.88 (dt, J = 7.4 and 6.1 Hz, 1 H), 3.84 (s, 3H), 3.76 (s, 3 H), 2.71 (dt, J = 7.5 and 6.1 Hz, 1 H), 1.49 (p, J = 7.3 Hz, 2 H), 1.26 (hextet, J = 7.3 Hz, 2 H), 0.83 (t, J = 7.3 Hz, 3 H). Anal. Calcd for $C_{23}H_{25}NO_7$: C, H, N.

6-(1-Butyl)-5,6-dihydro-5,11-diketo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (13d). Thionyl chloride (30 mL) was added dropwise to the acid **12d** (2.135 g, 5 mmol) with stirring. The resulting solution was stirred at room temperature for 12 h, after which the solution turned dark pink. Benzene (20 mL) was added to the reaction mixture and it was concentrated under reduced pressure. Benzene (50 mL) was added to the resulting residue, and the pink solid was filtered off to obtain pure indenoisoquinoline **13d** (1.3 g, 65%): mp 280−284 °C; IR (KBr) 1699, 1653, 1646, 1578 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.99 (s, 1 H), 7.62 (s, 1 H), 7.04 (s, 1 H), 6.92 (s, 1 H), 6.07 (s, 2 H), 4.39 (t, J = 7.6 Hz, 2 H), 4.01 (s, 3 H), 3.96 (s, 3 H), 1.82 (p, J = 7.3 Hz, 2 H), 1.68−1.55 (m, 2 H), 1.02 (t, J = 7.3 Hz, 3 Hz). Anal. Calcd for $C_{23}H_{21}NO_6 \cdot 0.1\ H_2O$: C, H, N.

3,4-Methylenedioxybenzylidenebenzylamine (11e). Piperonal (4.5 g, 30 mmol) and benzylamine (3.21 g, 30 mmol) were stirred in methylene chloride (30 mL) in the presence of anhydrous $MgSO_4$ (5 g) at room temperature for 4 h. The mixture was filtered, the residue was washed with methylene chloride (20 mL), and the combined filtrate was concentrated under reduced pressure to afford a white solid (7.03 g, 98%): mp 69−70 °C; IR (KBr) 1638, 1618, 1602 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (s, 1 H), 7.33 (d, J = 1.3 Hz, 1 H), 7.30−7.10 (m, 5 H), 7.06 (dd, J = 1.3 and 8.0 Hz, 1 H), 6.74 (d, J = 8 Hz, 1 H), 5.90 (s, 2 H), 4.69 (s, 2 H).

***cis*-*N*-Benzyl-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (12e).** 4,5-Dimethoxyhomophthalic anhydride **(10b)** (1.11 g, 5 mmol) was added to a chloroform (10 mL) solution of the imine **11e** (1.19 g, 5 mmol), and the mixture was stirred at room temperature for 2 h, after which the TLC showed the complete disappearance of the starting materials and a white precipitate formed in the reaction mixture. The precipitated product was filtered off, washed with chloroform (5 mL), and dried to give pure **12e** (1.89 g, 82%): mp 262−264 °C; IR (KBr) 1736, 1654, 1647, 1618, 1595, 1575 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.56 (s, 1 H), 7.35−7.20 (m, 5 H), 7.13 (s, 1 H), 6.75 (d, J = 8.3 Hz, 1 H), 6.51 (d, J = 8.1 Hz, 1 H), 6.43 (s, 1 H), 5.93 (s, 2 H), 5.25 (d, J = 15.6 Hz, 1 H), 4.86 (d, J = 5.6 Hz, 1 H), 4.51 (d, J = 5.3 Hz, 1 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.39 (d, J = 15.6 Hz, 1 H).

6-Benzyl-5,6-dihydro-5,11-diketo2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (13e). Thionyl chloride (10 mL) was added dropwise to the acid **12e** (1.15 g, 2.5 mmol) with stirring. The resulting mixture was stirred at room temperature for 5 h, after which the solution turned purple. Benzene (20 mL) was added to the reaction mixture, and it was concentrated under reduced pressure. Carbon tetrachloride was added to the resulting residue, and the undissolved solid was filtered off to obtain pure indenoisoquinoline **13e** (0.716 g, 65%): mp 310−312 °C; IR (KBr) 1695, 1652, 1619, 1578 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (s, 1 H), 7.66 (s, 1 H), 7.4−7.20 (m, 5 H), 7.02 (s, 1 H), 6.74 (s, 1 H), 5.99 (s, 2 H), 5.69 (s, 2 H), 4.04 (s, 3 H), 3.97 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 162.54, 155.03, 148.72, 135.44, 132.52, 130.22, 129.19, 127.67, 125.64, 108.32, 105.24, 103.03, 102.47, 56.31, 47.80, and 56.03. CIMS m/z (relative intensity) 442 (MH^+, 100). Anal. Calcd for $C_{26}H_{19}NO_6 \cdot 0.8H_2O$: C, H, N.

3,4-Methylenedioxybenzylidene-*p*-anisidine (11f). Piperonal (15 g, 0.1 mol) and *p*-anisidine (12.3 g, 0.1 mol) were stirred in methylene chloride (100 mL) in the presence of anhydrous $MgSO_4$ (5 g) at room temperature for 4 h. The mixture was filtered, the residue was washed with methylene chloride (20 mL), and the combined filtrate was concentrated under reduced pressure to afford a yellow solid. The crude product was crystallized in 95% ethanol to give a white crystalline solid (22.38 g, 87%): mp 113−114 °C; IR (KBr) 1636 and 1617 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 7.51 (d, J = 1.2 Hz, 1 H), 7.2−7.10 (m, 3 H), 6.95−6.80 (m, 3 H), 6.01 (s, 2 H), 3.80 (s, 3 H).

***cis*-*N*-(*p*-Anisyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (12f).** 4,5-Dimethoxyhomophthalic anhydride **(10b)** (1.11 g, 5 mmol) was added to a chloroform (10 mL) solution of the imine **11f** (1.275 g, 5 mmol) and the mixture was stirred at room temperature for 12 h, after which the TLC showed the complete disappearance of the starting materials and a white precipitate formed in the reaction mixture. The precipitated product was filtered off, washed with chloroform (5 mL), and dried to afford pure **12f** (1.36 g, 60%): mp >350 °C; IR (KBr) 1644, 1639, 1599 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.60−7.30 (m, 5 H), 7.20−6.80 (m, 4 H), 6.10 (s, 2 H), 5.30 (d, J = 6 Hz, 1 H), 4.77 (d, J = 6 Hz, 1 H), 3.78 (s, 3 H), 3.71 (s, 3 H), 3.01 (s, 3 H).

6-(*p*-Anisyl)-2,3-dimethoxy-5,6-dihydro-5,11-diketo-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (13f). Thionyl chloride (9 mL) was added dropwise to the acid **12f** (0.822 g, 2 mmol) with stirring. The resulting solution was stirred at room temperature for 5 h, after which the solution turned purple. Benzene (20 mL) was added to the reaction mixture, and it was concentrated under reduced pressure. The resulting residue was passed through a short column of silica gel (230−400 mesh), eluting with chloroform. Concentration of the eluent resulted in a pink solid which was crystallized from ethyl acetate to obtain pure indenoisoquinoline **13f** (0.436 g, 53%): mp 360−364 °C; IR (KBr) 1692, 1652, 1625, and 1552 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.94 (s, 1 H), 7.60 (s, 1 H), 7.34 (d, J = 8.1 Hz, 2 H), 7.10 (d, J = 8 Hz, 2 H), 6.88 (s, 1 H), 5.90 (s, 2 H), 5.05 (s, 1 H), 4.02 (s, 3 H), 3.93 (s, 3 H), 3.91 (s, 3 H); CIMS m/z (relative intensity) 458 (MH^+, 100). Anal. Calcd for $C_{26}H_{19}NO_7$: C, H, N.

3,4-Dibenzyloxybenzylidenemethylamine (11g). 3,4-Dibenzyloxybenzaldehyde (7.96 g, 25.0 mmol) was added to a 40% aqueous solution of methylamine (10 mL), and the reaction mixture was stirred at room temperature for 3 h. The mixture was extracted with ether (4 × 75 mL), the ether layers were combined, and the solution washed with saturated aqueous sodium chloride (75 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give an off-white solid (7.7 g, 94%): mp 56−57 °C; IR (KBr) 3031, 2936, 2832, 1648, 1600, 1582, 1509, 1454, 1431, 1267, 1171, 1137, 1017, 735, 696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14 (s, 1 H), 7.35 (m, 11 H), 7.11 (dd, J = 8.1 and 1.0 Hz, 1 H), 6.93 (d, J = 8.1 Hz, 1 H), 5.18 (s, 4 H), 3.46 (s, 3 H); CIMS m/z (relative intensity) 332 (MH^+, 100). Anal. Calcd for $C_{22}H_{21}NO_2$: C, H, N.

***cis*-3-(3′,4′-Dibenzyloxyphenyl)-4-carboxy-3,4-dihydro-*N*-methyl-1-(2*H*)-isoquinolone (12g)**. Homophthalic anhydride **(10a)** (0.81 g, 5 mmol) was added to a stirred solution of 3,4-dibenzyloxybenzylidenemethylamine **(11g)** (1.66 g, 5 mmol) in chloroform (5 mL). After 30 min, ether was added, and the resulting precipitate was filtered and washed with ether to give a pale yellow solid (0.9 g, 36%): mp 170−172 °C; IR (thin film) 3030, 1731, 1625, 1514, 1263, 1137, 1014 cm^{-1}; ^1H NMR

($CDCl_3$) δ 8.19 (dd, J = 6.5 and 1.9 Hz, 1 H), 7.36 (m, 10 H), 7.09 (d, J = 4.9 Hz, 1 H), 6.74 (d, J = 8.9 Hz, 1 H), 6.68 (d, J = 8.9 Hz, 1 H), 6.51 (m, 2 H), 5.03 (d, J = 7.2 Hz, 2 H), 4.92 (d, J = 6.1 Hz, 2 H), 4.8 (d, J = 6.3 Hz, 2 H), 4.5 (d, J = 6.2 Hz, 2 H), 2.98 (s, 3 H); CIMS *m*/*z* (relative intensity) 494 (MH^+, 100). Anal. Calcd for $C_{31}H_{27}NO_5$: C, H, N.

8,9-Dibenzyloxy-5,6-dihydro-5,11-diketo-6-methyl-11*H*-indeno[1,2-*c*]isoquinoline (13g). Thionyl chloride (8.1 mL) was added with stirring to the cis acid **12g** (0.7 g, 2.1 mmol). The result was a yellowish-brown mixture that became orange within 15 min and after 30 min was red. After 4 h, the reaction mixture was diluted with benzene (25 mL) and evaporated to dryness. The brownish-red solid was recrystallized from methanol and passed through a short column (silica gel), eluting with chloroform, to give a brown solid (0.14 g, 24%): mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (d, J = 8.0 Hz, 1 H), 8.16 (d, J = 8.0 Hz, 1 H), 7.75 (t, J = 7.5 Hz, 1 H), 7.39 (m, 13 H), 5.34 (s, 1 H), 5.29 (s, 1 H), 3.93 (s, 1 H); CIMS *m*/*z* (relative intensity) 474 (MH^+, 100). Anal. Calcd for $C_{31}H_{23}NO_4$: C, H, N.

***cis*-3-(3′,4′-Dibenzyloxyphenyl)-4-carboxy-3,4-dihydro-*N*-methyl-6,7-dimethoxy-1-(2*H*)-isoquinolone (12h).** 4,5-Dimethoxyhomophthalic anhydride **(10b)** (0.56 g, 2.5 mmol) was added to a stirred solution of 3,4-dibenzyloxybenzylidenemethylamine **(11g)** (0.83 g, 2.5 mmol) in chloroform (3 mL). After 30 min, the yellow mixture became heterogeneous, and ether was added to further precipitate the product. The light yellow precipitate was collected and washed with chloroform to give a solid (0.59 g, 44%): mp 194−196 °C; ^{1}H NMR ($CDCl_3$) δ 7.49 (s, 1 H), 7.34 (m, 11 H), 7.18 (s, 1 H), 6.91 (d, J = 8.3 Hz, 1 H), 6.79 (s, 1 H), 6.57 (d, J = 8.3 Hz, 1 H), 5.02 (s, 2 H), 4.98 (d, J = 6.1 Hz, 1 H), 4.92 (s, 2 H), 4.50 (d, J = 5.8 Hz, 1 H), 3.78 (s, 3 H), 3.74 (s, 3 H), 2.81 (s, 3 H); FABMS (*m*-NBA) *m*/*z* (relative intensity) 554 (MH^+, 100).

8,9-Dibenzyloxy-5,6-dihydro-5,11-diketo-6-methyl-2,3-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (13h). Thionyl chloride (15 mL) was added with stirring to the cis acid **12h** (1.2 g, 2.2 mmol). The result was an orange mixture that became dark red within 15 min. After 6 h, the reaction mixture was diluted with benzene (25 mL) and evaporated to dryness. Chloroform (7 mL) was added to the purple solid, and the solid was collected and washed with ether to give a light purple solid (0.75 g, 64%): mp 238−240 °C; IR (thin film) 3027, 2963, 1685, 1649, 1493, 1458, 1252, 1203, 1088, 1014 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1 H), 7.62 (s, 1 H), 7.38 (m, 10 H), 7.21 (s, 1 H), 7.11 (s, 1 H), 5.23 (d, J = 5.2 Hz, 4 H), 4.02 (s, 3 H) 3.95 (s, 3 H), 3.81 (s, 3 H); CIMS *m*/*z* (relative intensity) 534 (MH^+, 22). Anal. Calcd for $C_{33}H_{27}NO_6$: C, H, N.

3,4,5-Trimethoxybenzylidenemethylamine (11i). 3,4,5-Trimethoxybenzaldehyde (7.81 g, 40.0 mmol) and a 40% aqueous solution of methylamine (20 mL) were stirred at room temperature for 2.5 h. The mixture was extracted with ether (4 × 75 mL), the ether layers were combined, and the solution was washed with saturated aqueous sodium chloride (75 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a colorless oil (7.94 g, 95%): IR (neat) 2940, 2840, 1646, 1576, 1500, 1453, 1407, 1369, 1323, 1230, 1115, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 1.3 Hz, 1 H), 6.95 (s, 2 H), 3.89 (s, 6 H), 3.87 (s, 3 H), 3.50 (d, J = 1.3 Hz, 3 H); CIMS *m*/*z* (relative intensity) 210 (MH^+, 100). Anal. Calcd for $C_{11}H_{15}NO_3$: C, H, N.

***cis*-4-Carboxy-3,4-dihydro-*N*-methyl-6,7-dimethoxy-3-(3′,4′,5′-trimethoxyphenyl)-1(2*H*)isoquinolone (12i)**. 4,5-Dimethoxyhomophthalic anhydride **(10b)** (0.22 g, 1 mmol) was added to a stirred solution of 3,4,5-trimethoxybenzylidenemethylamine **(11i)** (0.23 g, 1 mmol) in chloroform (5 mL). After 30 min, the bright yellow homogeneous solution was tan, and no solid was observed. Ether was added dropwise and the resulting precipitate was filtered and washed with ether to give a fine white solid (0.1 g, 20%): mp 229−231 °C; IR (neat) 2928, 1743, 1593, 1418, 1329, 1241, 1167, 1119 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 1 H), 7.15 (s, 1 H), 6.38 (s, 2 H), 5.0 (d, J = 5.9 Hz, 1 H), 4.48 (d, J = 5.9 Hz, 1 H), 3.79 (s, 3 H), 3.72 (s, 3 H), 3.59 (s, 9 H); CIMS *m*/*z* (relative intensity) 432 (MH^+, 100). Anal. Calcd for $C_{22}H_{25}NO_8$: C, H, N.

5,6-Dihydro-5,11-diketo-6-methyl-2,3,8,9,10-pentamethoxy-11*H*-indeno[1,2-*c*]isoquinoline (13i). Thionyl chloride (15 mL) was added with stirring to the cis acid **12i** (1.2 g, 2.8 mmol). The result was a yellow mixture that became dark red within 15 min. After 4 h, the reaction mixture was diluted with benzene (25 mL) and evaporated to dryness. The purple solid was dissolved in chloroform, and ether was added to give a precipitate that was collected and washed with ether to give a purple solid (0.75 g, 7.1%): IR (neat) 2944, 1653, 1471, 1255, 1116, 1019 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 7.69 (s, 1 H), 7.02 (s, 1 H), 4.11 (s, 3 H), 4.05 (s, 3 H), 4.02 (s, 3 H), 3.99 (s, 6 H), 3.91 (s, 3 H); CIMS *m*/*z* (relative intensity) 412 (MH^+, 100).

***cis*-4-Carboxy-3,4-dihydro-*N*-methyl-3-(3′,4′,5′-trimethoxyphenyl)-1(2*H*)isoquinolone (12j).** Homophthalic anhydride **(10a)** (0.32 g, 2 mmol) was added to a stirred solution of 3,4,5-trimethoxybenzylidenemethylamine **(11i)** (0.46 g, 2 mmol) in chloroform (5 mL). After 45 min, ether was added dropwise to the homogeneous mixture, and the resulting precipitate was filtered from the yellow solution and washed with ether to give a pale yellow solid (0.43 g, 60%): mp 194−195 °C; IR (neat) 2830, 1620, 1549, 1459, 1185, 1123 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1 H), 7.99 (d, J = 7.2 Hz, 1 H), 7.52 (m, 4 H), 6.32 (s, 2 H), 5.04 (d, J = 5.9 Hz, 1 H), 4.63 (d, J = 6.0 Hz, 1 H), 3.58 (s, 3 H), 3.55 (s, 6 H), 2.94 (s, 3 H); CIMS *m*/*z* (relative intensity) 372 (MH^+, 100). Anal. Calcd for $C_{20}H_{21}NO_6$: C, H, N.

5,6-Dihydro-5,11-diketo-6-methyl-8,9,10-trimethoxy-11*H*-indeno[1,2-*c*]isoquinoline (13j). Thionyl chloride (10 mL) was added with stirring to **12j** (200 mg, 0.5 mmol). After 4 h, the reaction mixture was diluted with benzene (50 mL) and evaporated to dryness. The dark orange solid was dissolved in chloroform, and ether was added to give a dark orange solid (16 mg, 10%): mp 194−195 °C; IR (neat) 2938, 1665, 1463, 1400, 1292, 1125, 1007, 976 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 7.8 Hz, 1 H), 8.32 (d, J = 8.0 Hz, 1 H), 7.68 (t, J = 8.0 Hz, 1 H), 7.45 (t, J = 7.8 Hz, 1 H), 7.04 (s, 1 H), 4.09 (s, 3 H), 4.06 (s, 3 H), 3.97 (s, 3 H), 3.89 (s, 3 H); CIMS *m*/*z* (relative intensity) 352 (MH^+, 100). Anal. Calcd for $C_{20}H_{17}NO_5$: C, H, N.

3,4-Methylenedioxybenzylideneethylamine (11k). Piperonal (20.1 g, 0.14 mol) and a 70% aqueous solution of ethylamine (20 mL) were stirred at room temperature for 3 h. The mixture was extracted with ether (4 × 50 mL). The ether layers were combined, washed with aqueous sodium chloride (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a white crystalline powder (24.56 g, 93%): mp 47−48 °C; IR (KBr) 2963, 2836, 1645, 1603, 1498, 1480, 1441, 1252, 1092, 1031, 959, 926 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 7.32 (d, J = 1.3 Hz, 1 H), 7.09 (dd, J = 1.4 and 6.0 Hz, 1 H), 6.80 (d, J = 8.0 Hz, 1 H), 5.98 (s, 2 H), 3.59 (qd, J = 6.0 and 1.2 Hz, 2 H), 1.26 (t, J = 7.3 Hz, 3 H); CIMS *m*/*z* (relative intensity) 178 (MH^+, 100). Anal. Calcd for $C_{10}H_{11}NO_2$: C, H, N.

***cis*-4-Carboxy-*N*-ethyl-3-(3′,4′-methylenedioxyphenyl)-6,7-dimethoxy-3,4-dihydro-1(2*H*)isoquinolone (12k).** 3,4-Methylenedioxybenzylideneethylamine **(11k)** (0.89 g, 5.0 mmol) was stirred in chloroform (5.0 mL), and 4,5-dimethoxyhomophthalic anhydride **(10b)** (1.11 g, 5.0 mmol) was added. After 30 min, the yellow precipitate was filtered and washed with chloroform to give a pale yellow solid (0.58 g, 29%): mp 231−233 °C (dec); IR (KBr) 2937, 1732, 1615, 1594, 1573, 1254, 1223, 1174, 1089, 1034, 986, 898 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.50 (s, 1 H), 7.15 (s, 1 H), 6.76 (d, J = 7.8 Hz, 1 H), 6.57 (d, J = 8.1 Hz, 1 H), 6.48 (s, 1 H), 5.94 (s, 2 H), 5.03 (d, J = 6.2 Hz, 1 H), 4.51 (d, J = 6.2 Hz, 1 H), 3.79 (dq, J = 6.9 Hz, 1 H), 3.80 (s, 3 H), 3.73 (s, 3 H), 2.96 (dq, J = 6.9 Hz, 1 H), 1.01 (t, J = 6.9 Hz, 3 H); FABMS (*m*-NBA) *m*/*z* (relative intensity) 400 (MH^+, 100).

6-Ethyl-5,6-dihydro-5,11-diketo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indeno[1,2-*c*]isoquinoline (13k). Thionyl chloride (6.0 mL) was added with stirring to the cis acid

12k (0.58 g, 1.5 mmol), and the reaction mixture became dark reddish-purple and heterogeneous. After 4 h, the reaction mixture was diluted with benzene (5 mL) and evaporated to dryness. The brownish-red solid was loaded onto silica gel and passed through a short column of silica gel, eluting with chloroform, to give a brownish-red solid (0.34 g, 60%): mp 291−293 °C; IR 2969, 1694, 1643, 1613, 1555, 1486, 1393, 1308, 1252 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (s, 1 H), 7.65 (s, 1 H), 7.08 (s, 1 H), 7.01 (s, 1 H), 6.08 (s, 2 H), 4.49 (q, J = 7.2 Hz, 2 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 1.50 (t, J = 7.2 Hz, 3 H); CIMS m/z (relative intensity) 366 (MH^+, 4.0). Anal. Calcd for $C_{21}H_{17}NO_6$: C, H, N.

General Procedure for the Synthesis of Imines 17. The *O*-TBDMS protected aminols **15** were synthesized using a reported procedure.[12] The imines **17** were synthesized by treating the *O*-TBDMS protected aminols (9 mmol) with piperonal (9 mmol) in chloroform (20 mL) in the presence of anhydrous magnesium sulfate (2 g) at room temperature for 3 h. The imines were used as such for the next reaction without further purification. The crude yields of the imines **17** were quantitative.

General Procedure for the Synthesis of Isoquinolones 18. 4,5-Dimethoxyhomophthalic anhydride **(10b)** (2.22 g, 10 mmol) was added to a chloroform (20 mL) solution of the imine **17a** or **17b** (10 mmol), and the mixture was stirred at room temperature for 12 h, after which the TLC showed the complete disappearance of the starting materials and a white precipitate formed in the reaction mixture. The precipitated product was filtered off and washed with chloroform (5 mL) and dried to give pure **18a** or **18b**.

***cis*-*N*-(*tert*-Butyldimethylsilyloxybut-1-yl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3′,4′-methylenedioxyphenyl)-1(2*H*)isoquinolone (18a).** The isoquinolone **18a** was isolated in 36% yield: mp 239−240 °C; IR (KBr) 3065, 2944, 1737 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.51 (s, 1 H), 7.11 (s, 1 H), 6.75 (d, J = 8.0 Hz, 1 H), 6.54 (dd, J = 1.3 and 8.1 Hz, 1 H), 6.46 (d, J = 1.2 Hz, 1 H), 5.93 (s, 2 H), 4.98 (d, J = 6.1 Hz, 1 H), 4.55 (d, J = 6.1 Hz, 1 H), 3.81 (s, 3 H), 3.80−8.70 (m, 1 H), 3.74 (s, 3 H), 3.53 (t, J = 5.78 Hz, 2 H), 2.95−2.80 (m, 1 H), 1.60−1.35 (m, 4 H), 0.88 (s, 9 H), −0.98 (s, 6 H); ^{13}C NMR (DMSO-d_6) δ 170.64, 162.47, 151.21, 147.65, 147.00, 146.81, 131.26, 126.84, 121.55, 121.43, 110.85, 109.81, 107.87, 107.71, 101.06, 62.20, 61.01, 55.44, 47.77, 45.25, 29.67, 29.54, 25.81, 24.07, 17.91, −5.37; CIMS m/z (relative intensity) 558 (MH^+, 80). Anal. Calcd for $C_{29}H_{39}NO_8Si$: C, H, N.

***cis*-*N*-(*tert*-Butyldimethylsilyloxypent-1-yl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3′,4′-methylenedioxyphenyl)-1(2H)isoquinolone (18b).** The isoquinolone **18b** was isolated in 57% yield: mp 240−242 °C; IR (KBr) 3054, 2933, 1737 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.90 (bs, 1 H), 7.51 (s, 1 H), 7.09 (s, 1 H), 6.75 (d, J = 8.1 Hz, 1 H), 6.55 (dd, J = 1.6 and 8.1 Hz, 1 H), 6.47 (d, J = 1.3 Hz, 1 H), 5.93 (s, 2 H), 4.97 (d, J = 6.2 Hz, 1 H), 4.53 (d, J = 6.2 Hz, 1 H), 3.81 (s, 3 H), 3.83−8.70 (m, 1 H), 3.74 (s, 3 H), 3.52 (t, J = 6.2 Hz, 2 H), 2.85−2.73 (m, 1 H), 1.60−1.30 (m, 6 H), 0.82 (s, 9 H), − 0.99 (s, 6 H). CIMS m/z (relative intensity) 572 (MH^+, 100). Anal. Calcd for $C_{30}H_{41}NO_8Si$: C, H, N.

General Procedure for the Synthesis of Indenoisoquinolines 19. Thionyl chloride (10 mL) was added dropwise to the acid **18** (2 mmol) with stirring. The resulting solution was stirred at room temperature for 5 h after which the solution turned purple. Benzene (20 mL) was added to the reaction mixture, and it was concentrated under reduced pressure. The resulting residue was passed through a short column of silica gel (230−400 mesh), eluting with chloroform: methanol (95:5). Concentration of the eluent resulted in a pink solid which was crystallized from ethyl acetate to obtain pure indenoisoquinolines **19.** Under the reaction conditions, the deprotection of the *O*-TBDMS group was observed and only the hydroxy compounds were isolated.

5,6-Dihydro-5,11-diketo-6-(4-hydroxybut-1-yl)-2,3-dimethoxy-8,9-methylenedioxy-(11*H*)indeno[1,2-*c*]isoquinoline (19a). The indenoisoquinoline **19a** was isolated in 84% yield: mp 304−308 °C; IR (KBr) 3432, 2929, 1696, 1645, 1610 cm^{-1}; ^1H NMR (DMSO-d_6, 65 °C) δ 7.91 (s, 1 H), 7.53 (s, 1 H), 7.31 (s, 1 H), 7.06 (s, 1 H), 6.17 (s, 1 H), 4.43 (t, J = 7.7 Hz, 2 H), 3.90 (s, 3 H), 3.86 (s, 3 H), 3.45 (t, J = 5.8 Hz, 2 H), 1.88−1.70 (m, 2 H), 1.60−1.50 (m, 2 H); CIMS m/z (relative intensity) 424 (MH^+, 100). Anal. Calcd for $C_{23}H_{21}NO_7 \cdot 0.5H_2O$: C, H, N.

5,6-Dihydro-6-(4-hydroxypent-1-yl)-5,11-diketo-2,3-dimethoxy-8,9-methylenedioxy-11*H*-indenoisoquinoline (19b). The indenoisoquinoline **18b** was isolated in 79% yield: mp 288−290 °C; IR (KBr) 3411, 2929, 1698, 1653, 1582, 1550 cm^{-1}; ^1H NMR (DMSO-d_6, 80 °C) δ 7.91 (s, 1 H), 7.53 (s, 1 H), 7.21 (s, 1 H), 7.07 (s, 1 H), 6.18 (s, 1 H), 4.41 (bs, 2 H), 3.90 (s, 3 H), 3.86 (s, 3 H), 3.60 (bs, 1 H), 3.40 (bs, 2 H), 1.88−1.70 (m, 2 H), 1.60−1.40 (m, 4 H); CIMS m/z (relative intensity) 438 (MH^+, 100). Anal. Calcd for $C_{23}H_{21}NO_7 \cdot 0.3H_2O$: C, H, N.

***cis*-5,6,12,13-Tetrahydro-2,3-dimethoxy-6-methyl-5,11-dioxo-8,9-(methylenedioxy)-(11*H*)indeno[1,2-*c*]isoquinoline (20).** This compound was prepared as described previously.[14]

***cis*-6-Ethyl-5,6,12,13-tetrahydro-2,3-dimethoxy-5,11-dioxo-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinoline (21).** The acid **12k** (3.99 g, 3 mmol) was added slowly under nitrogen to a solution of degassed Eaton's reagent (10% P_2O_5 in methanesulfonic acid, 120 mL) with stirring over a period of 20 min. The reaction mixture was stirred at room temperature for 4 h, after which the mixture was added dropwise to water (600 mL) with stirring. The precipitated white solid was filtered off and dissolved in chloroform (150 mL). The chloroform layer was washed with saturated $NaHCO_3$ solution (2 × 50 mL), water (50 mL) and brine (60 mL) and dried (Na_2SO_4). Concentration of the organic layer gave the crude product, which was purified by column chromatography (4:1, hexane:ethyl acetate) to obtain pure **21** as a white solid (2.39 g, 63%). Neutralization of the bicarbonate layer with concentrated HCl gave the unreacted acid (0.821 g) as a white solid. Thus the yield based on the recovered starting acid is 79.3%. An analytical sample was prepared by recrystallization from EtOAc−hexane (1:1) to yield white prisms: mp 169−170 °C; IR (KBr) 3006, 2994, 1706, 1642, 1601 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.59 (s, 1 H), 7.16 (s, 1 H), 7.06 (s, 1 H), 7.00 (s, 1 H), 6.09 (s, 1 H), 6.04 (s, 1 H), 5.04 (d, J = 6.9 Hz, 1 H), 4.70−4.53 (m, 1 H), 4.21 (d, J = 7.0 Hz, 1 H), 3.94 (s, 3 H), 3.88 (s, 3 H), 3.40−3.26 (m, 1 H), 1.35 (t, J = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 198.8, 162.0, 154.7, 152.0, 150.6, 149.4, 148.5, 128.8, 126.4, 120.3, 110.2, 108.6, 104.2, 102.6, 56.6, 56.0, 55.8, 50.4, 43.3, 13.2. Anal. Calcd for $C_{21}H_{19}NO_6$: C, H, N.

***cis*-6-Allyl-5,6,12,13-tetrahydro-2,3-dimethoxy-5,11-dioxo-8,9-(methylenedioxy)-(11*H*)indeno[1,2-*c*]isoquinoline (22).** Indenoisoquinoline **22** was synthesized in 72% yield from the acid **12c** in a similar procedure for the synthesis of indenoisoquinoline **21**. The treatment of the isoquinolone **12c** (4.11 g, 10 mmol) with Eaton's reagent (120 mL) provided the indenoisoquinoline **22** in 72% (2.83 g) yield: mp 178−180 °C; IR (KBr) 2990, 1708, 1642, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (s, 1 H), 7.17 (s, 1 H), 7.07 (s, 1 H), 7.03 (s, 1 H), 6.09 (s, 1 H), 6.05 (s, 1 H), 6.05−5.90 (m, 1 H), 5.45−5.20 (m, 3 H), 5.16 (d, J = 6.9 Hz, 1 H), 4.19 (d, J = 6.9 Hz, 1 H), 3.94 (s, 3 H), 3.88 (s, 3 H), 3.90−3.80 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 198.8, 162.3, 154.8, 152.3, 150.6, 149.5, 148.6, 132.6, 129.0, 126.6, 120.0, 118.0, 110.4, 108.7, 104.4, 102.7, 102.6, 56.3, 56.1, 55.9, 50.3. Anal. Calcd for $C_{22}H_{19}NO_6$: C, H, N.

5,6-Dihydro-5,11-diketo-2,3,8-trimethoxy-6-methyl-9-[(methylsulfonyl)oxy]-(11*H*)indeno[1,2-*c*]isoquinoline (23). This compound was prepared as described previously.[15]

6-Ethyl-5,6,12α,13α-tetrahydro-11β-hydroxy-2,3-dimethoxy-8,9-(methylenedioxy)-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (24). The indenoisoquinoline **21** (0.381 g, 1 mmol) was heated at reflux with a 1 M solution of borane−tetrahydrofuran complex (4 mL) in dry THF (30 mL) for 1 h. After cooling, the reaction mixture was concentrated, the residue was dissolved in EtOAc (60 mL), and glacial acetic acid was added dropwise until pH 5. The organic layer was washed with saturated sodium bicarbonate (2 × 50 mL) and brine, dried (Na_2SO_4), and concentrated. The residue on chromato-

graphic purification (2% methanol in chloroform as eluent) provided the pure product **24** (0.363 g, 95%). An analytical sample was prepared by recrystallization from EtOAc−hexane (3:1) to yield white prisms: mp 189−192 °C; IR (KBr) 3468, 2919, 1630, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (s, 1 H), 6.95 (s, 1 H), 6.94 (s, 1 H), 6.74 (s, 1 H), 5.99 (s, 1 H), 5.98 (s, 1 H), 4.97 (dd, J = 5.8 and 7.6 Hz, 1 H), 4.89 (d, J = 6.4 Hz, 1 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.90−3.73 (m, 1 H), 3.59 (t, J = 5.8 Hz, 1 H), 3.45−3.30 (m, 1 H), 2.03 (d, J = 7.6 Hz, 1 H, D_2O exchangeable), 1.03 (t, J = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 162.8, 152.1, 148.5, 148.4, 148.3, 138.7, 135.2, 128.2, 122.8, 110.5, 109.2, 106.6, 106.0, 101.5, 77.4, 60.0, 55.9, 55.8, 48.3, 37.9, 12.0. Anal. Calcd for $C_{21}H_{21}NO_6$: C, H, N.

6-Ethyl-5,6,12α,13α-tetrahydro-11β-hydroxy-2,3-dimethoxy-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinoline (25). The indenoisoquinoline **21** (2.391 g, 6.27 mmol) was heated at reflux with a 1 M solution of borane−tetrahydrofuran complex (15 mL) in dry THF (100 mL) for 12 h. After cooling, the reaction mixture was concentrated, the residue was dissolved in EtOAc (100 mL), and glacial acetic acid was added dropwise until pH 5. The organic layer was washed with saturated sodium bicarbonate (2 × 100 mL) and brine, dried (Na_2SO_4), and concentrated. The residue on chromatographic purification (5% ethyl acetate in chloroform as eluent) provided the pure product **25** (2.13 g, 92%). An analytical sample was prepared by recrystallization from 2-propanol to yield white crystals: mp 180−184 °C; IR (KBr) 3479, 2909, 1605, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.76 (s, 1 H), 6.90 (s, 1 H), 6.77 (s, 1 H), 6.66 (s, 1 H), 6.040 (s, 1 H), 6.02 (s, 1 H), 5.34 (dd, J = 3.1 and 6.6 Hz, 1 H), 4.90 (d, J = 8.4 Hz, 1 H), 4.15 (d, J = 16.2 Hz, 1 H), 4.06 (d, J = 16.2 Hz, 1 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.71 (t, J = 7.5 Hz, 1 H), 2.86−2.70 (m, 1 H), 2.20−2.13 (m, 1 H), 2.03 (d, J = 3.1 Hz, 1 H, D_2O exchangeable), 1.02 (t, J = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 149.4, 149.3, 148.4, 137.9, 131.2, 124.3, 123.4, 110.4, 110.1, 109.9, 104.7, 101.6, 74.0, 73.6, 58.0, 56.2, 56.0, 46.8, 45.6, 9.00. Anal. Calcd for $C_{21}H_{19}NO_6 \cdot 1.5\ H_2O$: C, H, N.

6-(3-Carboxy-1-propyl)-5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline (26). The indenoisoquinoline **7** (0.319 g, 1 mmol) was dissolved in acetone (50 mL) and cooled in an ice bath. Jones reagent was added dropwise to the cold solution of the alcohol until the red color of the reagent persisted. The excess Jones reagent was quenched by adding few drops of isopropyl alcohol. 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, 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 in $CHCl_3$ (3 × 50 mL). The combined organic layer was dried (Na_2SO_4) and concentrated to afford the acid as an orange solid. The solid was crystallized from isopropyl alcohol to yield orange crystals (0.320 g, 96%): mp 204−206 °C; IR (KBr) 3000 (b), 1708, 1698, 1654 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 (d, J = 8 Hz, 1 H), 8.30 (d, J = 8 Hz, 1 H), 7.86 (d, J = 7.4 Hz, 1 H), 7.73 (t, J = 8.0 Hz, 1 H), 7.61 (d, J = 7.2 Hz, 1 H), 7.50−7.30 (m, 3 H), 4.60 (t, J = 7.8 Hz, 2 H), 3.71 (s, 1 H), 2.60 (t, J = 7.0 Hz, 2 H), 2.19 (p, J = 7.0 Hz, 2 H). Anal. Calcd for $C_{20}H_{15}NO_4$: C, H, N.

6-Ethyl-2,3-dimethoxy-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinolinium Chloride (27). The amino alcohol **25** (0.738 g, 2 mmol) was heated at reflux with 5% palladium on charcoal (0.265 g) in glacial acetic acid (100 mL) for 20 h. After cooling, the mixture was filtered through a small pad of Celite, and the solvent was evaporated to give a brown residue. The residue was dissolved in water (50 mL) and ethanol (6 mL) to give a light brown solution, to which was added 15% aqueous sodium chloride (10 mL). A yellow product precipitated immediately and was filtered, washed with ice cold water (10 mL), and dried over P_2O_5 under vacuum overnight to yield a yellow powder (0.552 g, 72%). An analytical sample was crystallized from methanol: mp 340−343 °C (dec); IR (KBr) 3382, 1480, 1305 and 1210 cm^{-1}; H NMR (MeOH-d_4) δ 9.27 (s, 1 H), 7.61 (s, 2 H), 7.46 (s, 1 H), 7.30 (s, 1 H), 6.15 (s, 2 H), 5.03 (q, J = 7.2 Hz, 2 H), 4.87 (s, 2 H), 4.15 (s, 3 H), 4.05 (s, 3 H), 1.75 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (MeOH-d_4) δ 189.5, 162.4, 155.7, 155.0, 152.5, 147.3, 133.4, 130.8, 127.9, 123.6, 107.5, 107.3, 106.6, 105.4, 101.5, 57.7, 57.1, 54.8, 15.7. Anal. Calcd for $C_{21}H_{20}NO_4Cl \cdot H_2O$: C, H, N.

Top1-Mediated DNA Cleavage Reactions Using 3′-End-Labeled 161 BP Plasmid DNA. The 161 bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (10 μL reactions) for 1 h at 37 °C and separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161 bp fragment was eluted from the gel slice (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 Hind III 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 units 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 30 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). 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).

Top2-Mediated DNA Cleavage Assays Using 5′-End-Labeled Human C-myc DNA. A 403-base-pair DNA fragment of the human c-myc gene from the junction between the first intron and the first exon was prepared by PCR between positions 2671 and 3073 using the oligonucleotides 5′-TGC-CGCATCCACGAAACTTTGC-3′ as sense primer and 5′-GAACTGTTCAGTGTTTACCCCG-3′ as antisense primer. Single-end labeling of these DNA fragments was obtained by 5′-end labeling of the adequate primer oligonucleotide. Approximately 0.1 μg of the human c-myc DNA that had been restricted by XhoI and XbaI was used as template for PCR.[23] The 5′-end-labeled DNA fragments were equilibrated with or without a drug in 1% dimethyl sulfoxide, 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mM Na_2EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin for 5 min before addition of purified human top2 (40−70 ng) in a 10 μL final reaction volume. The reactions were performed at 37 °C for 30 min and thereafter stopped by adding 1% sodium dodecyl sulfate (SDS) and 0.4 mg/mL proteinase K (final concentrations) followed by an additional incubation at 50 °C for 30 min. Samples were ethanol-precipitated before separation of the top2-cleaved fragments on denaturing polyacrylamide gels. The sequencing gels were made of 7% polyacrylamide in 1X TBE buffer (90 mM Tris borate, 2 mM EDTA, pH 8.3). Electrophoresis was performed at 2500 V (60 W) for 2−5 h. The gels were dried and visualized using a Phosphoimager and ImageQuant software.

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 purified calf thymus top1. Reactions were performed at 37 °C for 30 min and terminated by the addition of SDS (0.5% final concentration), and 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 then 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 of ethidium bromide and visualized by transillumination with UV light (300 nm).

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