Synthesis of New Dihydroindeno[1,2-*c*]isoquinoline and Indenoisoquinolinium Chloride Topoisomerase I Inhibitors Having High in Vivo Anticancer Activity in the Hollow Fiber Animal Model

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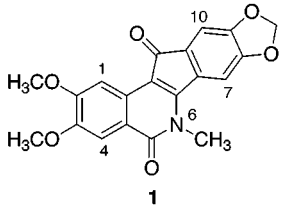
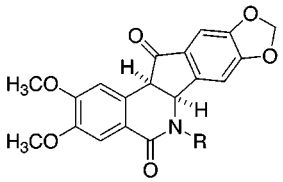
A number of novel dihydroindenoisoquinolines and indenoisoquinolinium salts were synthesized and examined for cytotoxicity in cancer cell cultures and for inhibition of topoisomerase I (top1). The top1-mediated DNA cleavage patterns produced in the presence of several of the new analogues were also investigated, and a few of the more potent compounds were examined for activity in hollow fiber animal models. Very cytotoxic dihydroindenoisoquinoline and isoquinolinium salts were obtained with mean graph midpoints (MGMs) for growth inhibition in the low submicromolar range. Two of the new dihydroindenoisoquinolines were found to be weaker top1 inhibitors than the lead compound **1**, while two of the indenoisoquinolinium salts were more potent. The top1-mediated DNA cleavage patterns of the indenoisoquinolines examined were found to be similar to each other but different from that of camptothecin. Several of the more potent indenoisoquinolines displayed promising anticancer activities in hollow fiber animal models.

Introduction

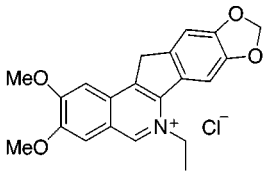
We recently reported the synthesis and biological evaluation of a series of indenoisoquinolines related to the lead compound **1**. Several of these compounds were found to be highly cytotoxic in human cancer cell cultures and to inhibit the enzyme topoisomerase I (top1).[1] The cytotoxicities observed for the dihydro analogues **2a** and **2b**, as well as the isoquinolinium salt **3**, encouraged us to further investigate the structural parameters associated with their biological activities. The goal of the present study was to utilize both **2** and **3** as lead compounds for the design and synthesis of more potent analogues for potential use as anticancer agents. As detailed in the present communication, both dihydroindenoisoquinolines and isoquinolinium salts have now been obtained with mean graph midpoints (MGMs) for growth inhibition in cancer cell cultures in the low submicromolar concentration level. The top1 inhibition and top1-mediated DNA cleavage patterns observed in the presence of a number of the new substances were determined. Several of the compounds have displayed promising anticancer activities in the hollow fiber animal model.



1



2a: R = Et
2b: R = allyl



3

Chemistry

To determine the importance of the central double bond present in the indenoisoquinoline ring system **1** and the effect of enone conjugation on the biological activities of these compounds, we chose to make a series of dihydroindenoisoquinolines. The reduction of the enone double bond in the indenoisoquinolines (e.g., **1**) to afford dihydroindenoisoquinolines (e.g., **2**) would provide insight into the biological effect of converting a planar tetracyclic system to one that is nonplanar. The idea to synthesize these compounds was also encouraged by the antineoplastic activity of the dihydroindenoisoquinolines **2a** and **2b** (Table 1).[1] Accordingly, the substituted isoquinolones **6a**−**i** on treatment with degassed Eaton's reagent[2] (phosphorus pentoxide-methanesulfonic acid, 1:10 by weight) at room temperature under nitrogen atmosphere provided the dihydroindenoisoquinolines **7a**−**i** (Scheme 1) in 18−80% yields. Azide **7j** was synthesized by azide displacement of the bromide in **7i**.

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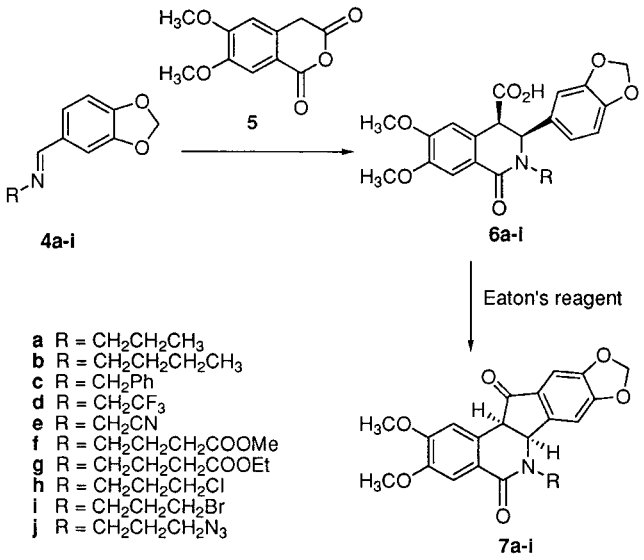
Table 1. Cytotoxicity of Indenoisoquinoline Analogues

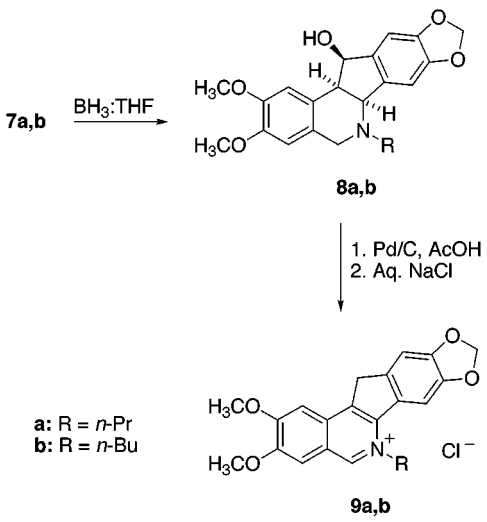
	cytotoxicity[a] (GI_{50} in μM)								
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]
1	1.3	35	41	4.2	73	68	37	96	20
2a	0.29	0.29		0.18	0.44		0.42	0.45	0.81
2b	0.36	0.34		0.38	1.78		0.77	1.4	0.98
3	22	13	56		1.9	2.7	58	14	13
7a	0.59	0.39	0.44	0.26	0.92	0.43	0.31	1.10	0.69 ± 0.29
7b	0.45	0.11	0.32	0.61	1.40			1.60	2.14
7c	2.93	0.54	0.38	1.84	7.17	0.39	0.55		1.18
7d	34.0	14.4	7.63	4.12	7.96	7.87	7.50	7.60	6.60 ± 1.71
7e	5.42	0.60	2.12	1.73	2.02	0.41	0.85	9.04	3.28 ± 0.99
7f	0.82	0.34	0.71	0.53	2.43	0.24	0.63	2.89	1.48 ± 0.19
7g	2.22	1.81		3.04	22.0	2.07	3.07	16.5	7.76
7h	0.08	0.16	0.02	0.02	0.36	0.02	0.03	0.33	0.11 ± 0.01
7i	0.12	0.07	0.04	0.05	0.40	0.03	0.03	0.15	0.12 ± 0.03
7j	0.36	0.40	0.33	0.29	1.27	0.25	0.33	1.27	0.69 ± 0.00
8a	16.0	17.8	21.7	18.7	6.30		33.9	5.80	20.9
9a	3.98	3.55	1.08	2.19	1.00	0.64	10.5	3.37	4.10 ± 1.92
9b	3.06	1.58	2.16	0.51	0.28	0.43	2.69	1.37	1.31 ± 0.24
12a	2.76	6.52	14.7	3.73	1.93	11.6	5.49	2.48	4.22 ± 0.24
12b	1.44	4.02		2.39	1.97	2.35	2.46	2.25	2.95
15	0.30	0.25	0.21	0.15	7.12	0.14	0.22	4.46	0.95 ± 0.17

[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.

Scheme 1



Scheme 2

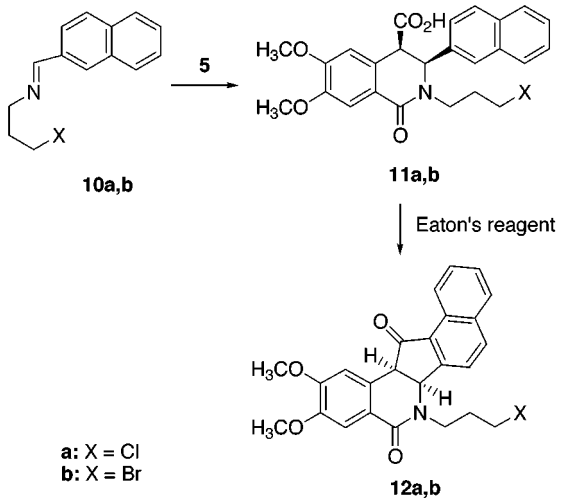


To make ring-contracted analogues of the benzophenanthridine natural products nitidine[3−5] and fagaronine,[6,7] which are both DNA-intercalating antitumor agents having an isoquinolinium substructure, the synthetic route displayed in Scheme 2 was executed. Reduction of the dihydroindenoisoquinolines **7a**,**b** using a borane−tetrahydrofuran complex provided the fully reduced products **8a**,**b** in excellent yields.[8] Dehydration, as well as dehydrogenation, of the alcohols **8a**,**b** occurred in the presence of palladium on charcoal in refluxing acetic acid. Treatment of the product with aqueous sodium chloride provided the indenoisoquinolinium salts **9a** and **9b** in 67% and 72% yields, respectively.

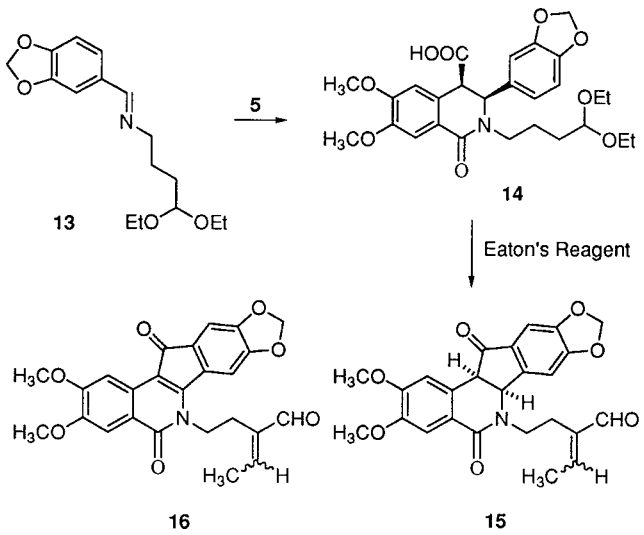
To obtain dihydroindenoisoquinoline analogues with a naphthalene ring in place of the methylenedioxybenzene ring, the route shown in Scheme 3 was executed. The imines **10a** and **10b** were synthesized in 91% and 98% yields, respectively, by treating naphthalene-2-carboxaldehyde with 3-chloropropylamine hydrochloride or 3-bromopropylamine hydrobromide in the presence of triethylamine. Condensation of homophthalic anhydride **5** with the imines **10a** and **10b** in chloroform at room temperature provided the isoquinolones **11a** and **11b** in 56% and 76% yields, respectively. The acids **11a** and **11b** on treatment with Eaton's reagent at room temperature provided the cyclized dihydro products **12a** and **12b** in 60% and 55% yields, respectively, along with traces of the oxidized dehydro products. From the ^{1}H NMR spectrum, it was clear that the products **12a** and **12b** were formed by cyclization exclusively through bond formation to C-1 of the naphthalene ring instead of C-3.

To synthesize an indenoisoquinoline analogue bearing a butyraldehyde side chain on the N-6 nitrogen, the route outlined in Scheme 4 was implemented. The imine **13** was prepared in 99% yield by reacting piperonal with 4-aminobutyraldehyde diethylacetal at room temperature. Condensation of the homophthalic anhydride **5** with the imine **13** in chloroform at room temperature provided the isoquinolone **14** in 62% yield. The acid **14** on treatment with Eaton's reagent at room temperature

Scheme 3



Scheme 4



under nitrogen provided two unexpected products in 25% and 18% yields. The deprotection of the diethylacetal group was clear from the ^{1}H NMR spectra of both products. The ^{1}H NMR spectra of both products showed a quartet at 6.73 and 6.87 ppm, respectively, with a 7.0 Hz coupling constant and integrating for one proton. Both compounds displayed a doublet with a coupling constant of 7.0 Hz at 2.05 and 2.26 ppm, integrating for three protons. The IR spectra of both products showed the carbonyl stretching corresponding to the formyl group at 1677 and 1678 cm^{-1}, respectively, indicating an α,β-unsaturated carbonyl group. On the basis of the spectral data, structures **15** and **16** (Scheme 4) were assigned to the products. The structure of product **15** was further confirmed by elemental analysis and the presence of an appropriate molecular ion peak in the plasma desorption mass spectrum. It is possible that the ethanol liberated from the diethylacetal protecting group undergoes oxidation to provide acetaldehyde, which can then participate in an aldol condensation, followed by dehydration, to give the products.

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}−10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.

Table 2. Topoisomerase I Inhibitory Activities of Indenoisoquinoline Analogues

compd	top1 cleavage[a]	compd	top1 cleavage[a]
1	++	**7i**	++
2a	±	**7j**	+
2b	0	**9a**	+++
3	++	**9b**	+++

[a] Compounds were tested up to a concentration of 100 μM. The activities of the compounds in the production of top1-mediated DNA cleavage is expressed semiquantitatively as follows: +, weak activity; ++, similar activity as the parent compound **1**; +++, greater activity than the parent compound **1**.

As indicated in Table 1, all of the compounds were cytotoxic, with GI_{50} MGM values ranging from 20.9 μM (compound **8a**) to 0.11 μM (compound **7h**). Compound **7a** (MGM 0.69 μM), bearing a propyl group on the N-6 nitrogen, showed a slight increase in the potency relative to the one bearing an ethyl substituent **2a**[1] (MGM 0.81 μM). Both compounds were significantly more cytotoxic than the original lead compound **1**. The butyl homologue **7b** (MGM 2.14 μM) was less potent than **7a** or **2a**, as was the analogue **7c** (MGM 1.18 μM) bearing a benzyl group on the N-6 nitrogen. The trifluoroethyl analogue **7d** (MGM 6.60 μM) and cyanomethyl compound **7e** (MGM 3.28 μM) were slightly less potent than the other new analogues in the series. The methyl ester **7f** (MGM 1.48 μM) was more potent than the corresponding ethyl ester **7g** (MGM 7.76 μM). However, the halides **7h** (MGM 0.11 μM) and **7i** (MGM 0.12 μM), as well as the azide **7j** (MGM 0.69 μM), displayed cytotoxicities at submicromolar concentrations. The halides **7h** and **7i** showed extremely high activity, with some GI_{50} values in the 20−50 nM range, particularly against SF-539 CNS cancer, UACC-62 melanoma, SN-12C renal cancer, and DU-145 prostate cancer. The aminol **8a** (MGM 20.9 μM) had very low activity. The isoquinolinium salts **9a** (MGM 4.1 μM) and **9b** (MGM 1.31 μM) were moderately active. As shown in **12a** (MGM 4.22 μM) and **12b** (MGM 2.95 μM), replacement of the methylenedioxybenzene ring of **7h** and **7i** by a naphthalene ring led to the decrease in the potency. The aldehyde **15** (MGM 0.95 μM) displayed a submicromolar GI_{50} value.

Two representative dihydroindenoisoquinolines **7i** and **7j**, along with two isoquinolinium salts **9a** and **9b**, were examined in topoisomerase I inhibition studies. As shown in Table 2, the dihydroindenoisoquinoline **7i** was as potent as the lead compound **1**, while **7j** was a weaker inhibitor of top1 than the lead compound **1**. In contrast, both of the isoquinolinium compounds **9a** and **9b** were more potent top1 inhibitors than **1**. The weak inhibitory activities of the two dihydro compounds are consistent with observations previously made on other dihydroindenoisoquinolines vs top1.[1] A comparison is shown in

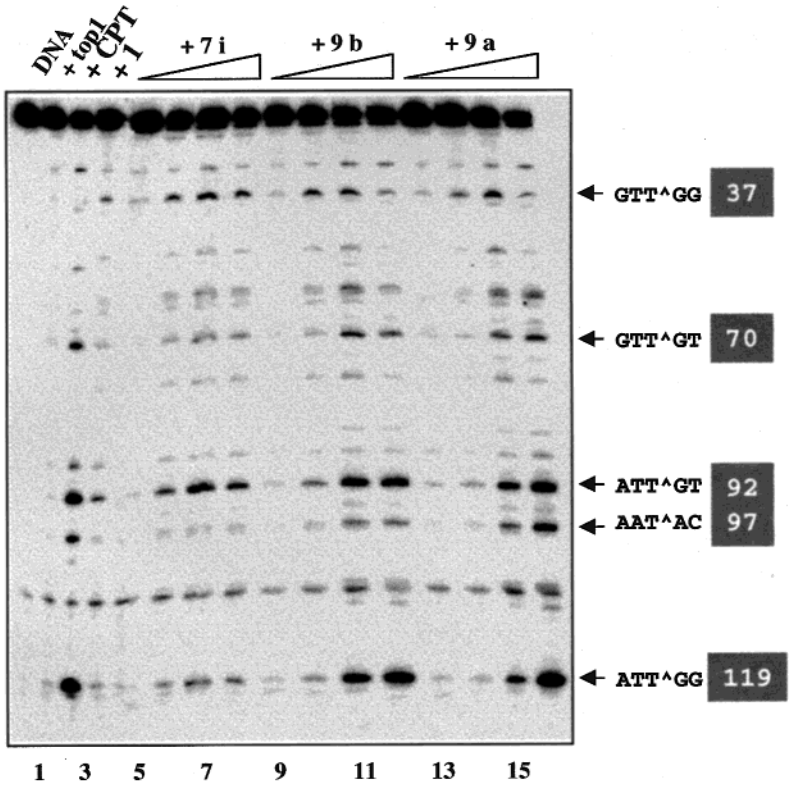


Figure 1. Comparison of the top1-mediated DNA cleavages at different drug concentrations. The DNA used corresponds to the 3′-end-labeled PvuII/HindIII fragment of pBluescript SK(−) phagemid DNA. The four concentrations used for compounds **7i**, **9b**, and **9a** were 0.1, 1.0, 10, and 100 μM. The concentration of compound **1** (lane 4) was 1 μM. Reactions were performed at room temperature for 30 min and stopped by adding 0.5% SDS. DNA fragments were separated on 16% polyacrylamide gels. Top1 was present in all reactions except in the control lane. The control was DNA with neither top1 nor any drug. The sequence of the DNA cleavage sites is indicated to the right (carets correspond to the break sites). Numbers correspond to the positions of the cleavage sites (see ref 1).

Figure 1 of the top1-mediated cleavages produced by the lead compound **1**, the known top1 inhibitor camptothecin, the dihydroindenoisoquinoline **7i**, and the isoquinolinium compounds **9a** and **9b**. The DNA cleavage patterns produced by all of the indenoisoquinolines are similar to each other, but there are differences relative to camptothecin. This is also similar to what was observed previously.[1]

The cleavage pattern of camptothecin shown in Figure 1 is also consistent with previous publications, although there are minor apparent differences due to the migration distances of the DNA fragments in the gel electrophoresis.[1,9,10] The differences in cleavage patterns are related to differences in relative intensities rather than to the presence of drug-specific sites.

Several of the dihydroindenoisoquinoline derivatives and the indenoisoquinolinium chloride **9a** were evaluated as anticancer agents in an in vivo animal model in which polyvinylidene fluoride hollow fibers containing various cancer cell cultures were implanted intraperitoneally (ip) and subcutaneously (sc) into mice and compounds were administered by the ip route.[11] The effects of the compounds on reduction of viable cancer cell mass compared to those of controls were determined. Each compound was tested in the hollow fiber assay against a panel of 12 human tumor cell lines as described previously.[12] The compounds were solubilized in 10% DMSO in saline/Tween-80R and administered intraperitoneally once daily for a total of four doses at each of two dose levels. The day after the last compound dose, the fibers were collected and assessed for viable cell mass. A score of 2 was assigned each time the compound produced a 50% or greater reduction in viable cell mass compared to the vehicle-treated controls. The score for each compound was summed for the intraperitoneal fibers and the subcutaneous fibers. The scores for each of the compounds are given in Table 3. The most active compound, **9a**, reproducibly reduced the viable cell mass of the NCI-H522 lung tumor cells and the OVCAR-3 ovarian tumor cells to less than the original implant concentration (cell kill).

Table 3. Anticancer Activity of Dihydroindenoisoquinoline Derivatives and Indenosoquinoline Hydrochloride **9a** in the Hollow Fiber Assay[a]

compd	ip score	sc score[b]	total score	cell kill[c]
1	2	6	8	Y
2a	2	14	16	N
2b	0	18	18	N
7a	2	4	6	N
7b	2	4	6	N
7c	0	8	8	Y
7h	4	2	6	N
7i	6	2	8	N
7j	4	4	8	N
9a	28	12	40	Y

[a] Polyvinylidene fluoride hollow fibers containing various cancer cell lines were implanted intraperitoneally (ip) or subcutaneously (sc) in mice, and compounds were injected ip using a qd × 4 treatment schedule. A total of 12 ip and 12 sc cell lines were tested in triplicate at two dosage levels, and each cell line with a 50% or greater reduction in viable cell mass was given a score of 2. [b] The ip and sc scores listed are the sums of all of the ip and sc scores for each compound. [c] A net cell kill at one or more of the implant sites is indicated with a Y.

The results listed in Table 3 show that the dihydroindenoisoquinolines **2a**,**b** and **7a**−**c** displayed greater anticancer activity at the sc implant site than at the ip implant site after the compounds were injected ip. Greater activity at the more remote implant site (sc) relative to that at the injection site (ip) is characteristic of an inactive compound that is converted to an active one after administration, which leads us to wonder whether these compounds act as prodrugs that are converted by a two-electron oxidation (dehydrogenation) to the corresponding cytotoxic indenoisoquinolines.

It is noteworthy that although **9a** is the most potent of the compounds tested in the hollow fiber model, its cytotoxicity in cancer cell cultures (MGM 4.10 μM) is relatively unimpressive. This emphasizes the fact that in vitro cytotoxicity studies are not always accurate indicators of how well a compound will perform as an anticancer agent in in vivo efficacy models.

While additional studies are necessary to develop a further understanding of dose, route, and schedule issues, these preliminary results are very encouraging. For the hollow fiber assay, the higher a compound scores the greater the likelihood that it will demonstrate activity in classic subcutaneous xenograft models. The high scores determined for compound **9a** are consistent with those of other compounds with demonstrable xenograft activity, including those currently used in the clinical setting. The relative potencies of topotecan, camptothecin, and SN-38 as topoisomerase I inhibitors have been reported previously, and a comparison of these compounds with selected indenoisoquinolines will be possible once the most active inhibitors have been selected for further development.[13]

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. Infrared spectra were obtained using $CHCl_3$ as the solvent unless otherwise specified. Except where noted, ^{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 obtained 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.

The syntheses and the spectral data for the imines **4a**−**c**, the isoquinolones **6a**−**c**, and the isoquinolines **7a**−**c** were reported earlier.[14]

3,4-Methylenedioxybenzylidene-(2,2,2-trifluoroethylamine) (4d). The hydrochloride salt of 2,2,2-trifluoroethylamine (2.7 g, 20 mmol) was stirred with triethylamine (3 mL, 21 mmol) in chloroform (100 mL) at room temperature for 30 min. Piperonal (3.0 g, 20 mmol) was added, followed by 4 Å molecular sieves (5 g), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered and the residue washed with chloroform (30 mL). The filtrate was washed with water (100 mL) and brine (50 mL) and dried (Na_2SO_4). Concentration of the organic layer provided the product **4d** (4.11 g, 89%) as a white solid: mp 78−79 °C; IR (KBr) 2945, 1643, 1600, 1500, 1443 cm^{-1}; ^{1}H NMR δ 8.35 (s, 1 H), 7.38 (s, 1 H), 7.19 (d, J = 8.0 Hz, 1 H), 6.82 (d, J = 8.0 Hz, 1 H), 6.00 (s, 2 H), 4.05 (q, J = 9.6 Hz, 2 H). Anal. Calcd for $C_{10}H_8NO_2F_3$: C, H, N.

3,4-Methylenedioxybenzylidenecyanomethylamine (4e). The bisulfate salt of aminoacetonitrile (7.7 g, 50 mmol) was stirred with triethylamine (10 mL, 70 mmol) in chloroform (100 mL) at room temperature for 30 min. Piperonal (7.5 g, 50 mmol) was added, followed by 4 Å molecular sieves (5 g), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered, and the residue was washed with chloroform (30 mL) and the combined organic layer washed with water (100 mL) and brine (50 mL) and dried (Na_2SO_4). Concentration of the organic layer provided the product **4e** (9.09 g, 97%) as a pale-yellow solid: mp 132−134 °C; IR (KBr) 2945, 1643, 1600, 1500, 443 cm^{-1}; ^{1}H NMR δ 8.35 (s, 1 H), 7.34 (s, 1 H), 7.16 (d, J = 8.0 Hz, 1 H), 6.85 (d, J = 8.0 Hz, 1 H), 6.02 (s, 2 H), 4.58 (s, 2 H).

General Procedure for the Synthesis of Imines 4f and 4g. The hydrochloride salts of ethyl or methyl 4-aminobutyrate (50 mmol) were stirred with triethylamine (10 mL, 70 mmol) in chloroform (100 mL) at room temperature for 30 min. Piperonal (7.5 g, 50 mmol) was added followed by 4 Å molecular sieves (5 g), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered and the residue washed with chloroform (30 mL). The combined organic layer was washed with water (100 mL) and brine (50 mL) and dried (Na_2SO_4). Concentration of the organic layer provided the products **4f** and **4g** as oils.

3,4-Methylenedioxybenzylidene(3-methoxycarbonyl-1-propylamine) (4f). Yield 89%; IR (neat) 2951, 2901, 2839, 1738, 1688, 1645, 1605, 1485 cm^{-1}; ^{1}H NMR δ 8.15 (s, 1 H), 7.33 (d, J = 1.3 Hz, 1 H), 7.10 (dd, J = 1.3 and 8.0 Hz, 1 H), 6.92 (d, J = 8.0 Hz, 1 H), 5.99 (s, 2 H), 3.66 (s, 3 H), 3.60 (t, J = 7.5 Hz, 2 H), 2.41 (t, J = 7.4 Hz, 2 H), 2.01 (pent, J = 7.1 Hz, 2 H).

3,4-Methylenedioxybenzylidene(3-ethoxycarbonyl-1-propylamine) (4g). Yield 97%; IR (neat) 2979, 2905, 2842, 1731, 1642, 1605, 1485 cm^{-1}; ^{1}H NMR δ 8.15 (s, 1 H), 7.33 (d, J = 1.3 Hz, 1 H), 7.10 (dd, J = 1.3 and 8.0 Hz, 1 H), 6.82 (d, J = 8.0 Hz, 1 H), 5.99 (s, 2 H), 4.12 (q, J = 7.2 Hz, 2 H), 3.60 (t, J = 6.5 Hz, 2 H), 2.39 (t, J = 7.4 Hz, 2 H), 2.01 (pent, J = 7.0 Hz, 2 H), 1.24 (t, J = 7.2 Hz, 3 H).

3,4-Methylenedioxybenzylidene-(3-chloro-1-propylamine) (4h). This compound was prepared as described elsewhere.[10]

3,4-Methylenedioxybenzylidene-(3-bromo-1-propylamine) (4i). This compound was prepared as described elsewhere.[10]

General Procedure for the Synthesis of Isoquinolones 6. 4,5-Dimethoxyhomophthalic anhydride (**5**) (2.22 g, 10 mmol) was added to a chloroform (60 mL) solution of the imine **4** (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 **6** in 57−81% yields.

***cis*-4-Carboxy-*N*-(2,2,2-trifluoroethyl)-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)isoquinolone (6d).** The isoquinolone **6d** was isolated in 73% yield: mp 236−240 °C; IR (KBr) 3093, 2916, 1739, 1622, 1594 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.01 (bs, 1 H), 7.49 (s, 1 H), 7.11 (s, 1 H), 6.74 (d, J = 8.0 Hz, 1 H), 6.52 (dd, J = 1.4 and 8.1 Hz, 1 H), 6.45 (d, J = 1.5 Hz, 1 H), 5.91 (s, 2 H), 5.08 (d, J = 6.0 Hz, 1 H), 4.65 (m, 1 H), 4.57 (d, J = 4.94 Hz, 1 H), 3.79 (s, 3 H), 3.72 (s, 3 H), 3.60 (m, 1 H).

***cis*-4-Carboxy-*N*-(cyanomethyl)-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)isoquinolone (6e).** The isoquinolone **6e** was isolated in 57% yield: mp 210−212 °C; IR (KBr) 3093, 2916, 1739, 1622, 1594 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.88 (bs, 1 H), 7.52 (s, 1 H), 7.12 (s, 1 H), 6.82 (d, J = 8.0 Hz, 1 H), 6.66 (d, J = 8.1 Hz, 1 H), 6.57 (s, 1 H), 5.97 (s, 2 H), 5.21 (d, J = 6.1 Hz, 1 H), 4.58 (d, J = 6.0 Hz, 1 H), 4.45 (d, J = 17.6 Hz, 1 H), 4.39 (d, J = 17.6 Hz, 1 H), 3.84 (s, 3 H), 3.78 (s, 3 H). Anal. Calcd for $C_{21}H_{18}N_2O_7 \cdot 0.1CHCl_3$: C, H, N.

***cis*-4-Carboxy-3,4-dihydro-6,7-dimethoxy-*N*-(3-methoxycarbonyl-1-propyl)-3-(3,4-methylenedioxyphenyl)-1(2*H*)isoquinolone (6f).** The isoquinolone **6f** was isolated in 80% yield: mp 230−234 °C; IR (KBr) 3069, 1731, 1630, 1578 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.0 (bs, 1 H), 7.51 (s, 1 H), 7.11

(s, 1 H), 6.76 (d, J = 8.0 Hz, 1 H), 6.54 (dd, J = 1.4 and 8.0 Hz, 1 H), 6.45 (d, J = 1.3 Hz, 1 H), 5.93 (s, 2 H), 4.98 (d, J = 6.4 Hz, 1 H), 4.62 (d, J = 6.2 Hz, 1 H), 3.81 (s, 3 H), 3.90−3.78 (m, 1 H), 3.74 (s, 3 H), 3.54 (s, 3 H), 2.83−2.70 (m, 1 H), 2.31 (dt, J = 3.0 and 7.2 Hz, 2 H), 1.88−1.63 (m, 2 H). Anal. Calcd for $C_{24}H_{25}NO_9$: C, H, N.

***cis*-4-Carboxy-*N*-(3-ethoxycarbonyl-1-propyl)-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (6g).** The isoquinolone **6g** was isolated in 81% yield: mp 228−232 °C; IR (KBr) 3071, 2974, 1733, 1618, 1593, 1572 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.9 (bs, 1 H), 7.51 (s, 1 H), 7.11 (s, 1 H), 6.76 (d, J = 8.0 Hz, 1 H), 6.54 (dd, J = 1.4 and 8.0 Hz, 1 H), 6.45 (d, J = 1.2 Hz, 1 H), 5.93 (s, 2 H), 4.98 (d, J = 6.3 Hz, 1 H), 4.62 (d, J = 6.2 Hz, 1 H), 4.00 (q, J = 7.2 Hz, 2 H), 3.85 (s, 3 H), 3.90−3.80 (m, 1 H), 3.74 (s, 3 H), 2.79 (dt, J = 6.2 and 7.3 Hz, 1 H), 2.31 (dt, J = 3.0 and 7.4 Hz, 2 H), 1.88−1.62 (m, 2 H), 1.13 (t, J = 7.1 Hz, 3 H). Anal. Calcd for $C_{25}H_{27}NO_9$: C, H, N.

***cis*-4-Carboxy-*N*-(3-chloro-1-propyl)-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)isoquinolone (6h).** This compound was prepared as described elsewhere.[10]

***cis*-*N*-(3-Bromo-1-propyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)isoquinolone (6i).** This compound was prepared as described elsewhere.[10]

General Procedure for the Synthesis of Dihydroindenoisoquinolines 7. The acids **6** (10 mmol) were added slowly under nitrogen to a solution of degassed Eaton's reagent (10% P_2O_5 in methanesulfonic acid, 100 mL) with stirring over a period of 20 min. The reaction mixture was stirred at room temperature for 12 h, after which the mixture was added dropwise to water (600 mL) with stirring. The precipitated white solid was 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 to obtain pure **7**.

***cis*-6-(2,2,2-Trifluoroethyl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7d).** The acid **6d** (1.359 g, 3 mmol) on treatment with degassed Eaton's reagent (100 mL) provided the crude product. Purification by column chromatography (8:2, chloroform/ethyl acetate) and crystallization from ethyl acetate (20 mL) gave pure **7d** (0.822 g, 63%) as a white solid: mp 222−225 °C; IR (KBr) 2920, 1714, 1655, 1596, 1513, 1474 cm^{-1}; ^{1}H NMR δ 7.58 (s, 1 H), 7.14 (s, 1 H), 7.08 (s, 1 H), 6.93 (s, 1 H), 6.10 (s, 1 H), 6.06 (s, 1 H), 5.45−5.30 (m, 2 H), 4.27 (d, J = 6.6 Hz, 1 H), 3.95 (s, 3 H), 3.87 (s, 3 H), 3.85−3.70 (m, 1 H). Anal. Calcd for $C_{21}H_{16}NO_6F_3$: C, H, N.

***cis*-6-(Cyanomethyl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7e).** The acid **6e** (3.01 g, 7.3 mmol) on treatment with degassed Eaton's reagent (100 mL) provided the crude product. Purification by column chromatography (8:2, chloroform/ethyl acetate) and crystallization from ethyl acetate (20 mL) gave pure **7e** as a white solid (0.513 g, 18%): mp 230−234 °C; IR (KBr) 2920, 1714, 1655, 1596, 1513, 1474 cm^{-1}; ^{1}H NMR δ 7.58 (s, 1 H), 7.17 (s, 1 H), 7.15 (s, 1 H), 7.10 (s, 1 H), 6.15 (s, 1 H), 6.12 (s, 1 H), 5.40 (d, J = 6.9 Hz, 1 H), 5.21 (d, J = 17.3 Hz, 1 H), 4.29 (d, J = 17.3 Hz, 1 H), 4.21 (d, J = 6.9 Hz, 1 H), 3.99 (s, 3 H), 3.90 (s, 3 H); ^{13}C NMR δ 197.5, 162.5, 154.8, 153.2, 150.1, 148.9, 147.6, 126.7, 118.2, 115.2, 110.5, 109.8, 105.3, 103.4, 102.9, 57.8, 56.2, 56.0, 50.4, 34.4. Anal. Calcd for $C_{21}H_{16}N_2O_6$: C, H, N.

***cis*-5,6,12,13-Tetrahydro-2,3-dimethoxy-6-(3-methoxycarbonyl-1-propyl)-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7f).** The isoquinolone **7f** was isolated in 53% yield: mp 151−152 °C; IR (KBr) 3000, 1707, 1646, 1601, 1516, 1473 cm^{-1}; ^{1}H NMR δ 7.57 (s, 1 H), 7.16 (s, 1 H), 7.06 (s, 1 H), 7.00 (s, 1 H), 6.08 (s, 1 H), 6.04 (s, 1 H), 5.15 (d, J = 6.9 Hz, 1 H), 4.70−4.55 (m, 1 H), 4.24 (d, J = 6.8 Hz, 1 H), 3.94 (s, 3 H), 3.87 (s, 3 H), 3.61 (s, 3 H), 3.40−3.26 (m, 1 H), 2.58−2.36 (m, 2 H), 2.18−2.00 (m, 2 H). Anal. Calcd for $C_{24}H_{23}NO_8 \cdot H_2O$: C, H, N.

***cis*-6-(3-Ethoxycarbonyl-1-propyl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7g).** The isoquinolone **7g** was isolated in 65% yield as a white solid: mp 170−172 °C; IR (KBr) 2979, 2931, 1724, 1706, 1643, 1601, 1513, 1474 cm^{-1}; ^{1}H NMR δ 7.57 (s, 1 H), 7.16 (s, 1 H), 7.06 (s, 1 H), 7.01 (s, 1 H), 6.08 (s, 1 H), 6.04 (s, 1 H), 5.20 (d, J = 6.9 Hz, 1 H), 4.70−4.56 (m, 1 H), 4.24 (d, J = 6.9 Hz, 1 H), 4.20−4.00 (m, 2 H), 3.94 (s, 3 H), 3.87 (s, 3 H), 3.42−3.30 (m, 1 H), 2.56−2.35 (m, 2 H), 2.20−2.03 (m, 2 H), 1.21 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 198.7, 173.1, 162.5, 154.8, 152.3, 150.5, 149.6, 148.6, 128.9, 126.6, 120.1, 110.3, 108.8, 104.2, 102.7, 102.6, 60.5, 57.4, 56.1, 55.9, 50.4, 47.7, 31.3, 23.2, 14.1. Anal. Calcd for $C_{25}H_{25}NO_8$: C, H, N.

***cis*-6-(3-Chloro-1-propyl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7h).** The isoquinolone **7h** was isolated as white prisms in 55% yield after crystallization from ethyl acetate: mp 180−182 °C; IR (KBr) 2961, 1713, 1650, 1601, 1515, 1475 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54 (s, 1 H), 7.15 (s, 1 H), 7.05 (s, 1 H), 7.00 (s, 1 H), 6.07 (s, 1 H), 6.03 (s, 1 H), 5.22 (d, J = 6 Hz, 1 H), 4.67−4.56 (m, 1 H), 4.22 (d, J = 6 Hz, 1 H), 3.92 (s, 3 H), 3.85 (s, 3 H), 3.70−3.58 (m, 2 H), 3.58−3.45 (m, 1 H), 2.40−2.15 (m, 2 H). Anal. Calcd for $C_{22}H_{20}NO_6Cl$: C, H, N.

***cis*-6-(3-Bromo-1-propyl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7i).** The isoquinolone **7i** was isolated as white prisms after crystallization from ethyl acetate in 47% yield: mp 195−197 °C; IR (KBr) 2966, 1712, 1649, 1601, 1517, 1477 cm^{-1}; ^{1}H NMR δ 7.54 (s, 1 H), 7.15 (s, 1 H), 7.05 (s, 1 H), 7.01 (s, 1 H), 6.07 (s, 1 H), 6.03 (s, 1 H), 5.25 (d, J = 6.9 Hz, 1 H), 4.68−4.53 (m, 1 H), 4.22 (d, J = 6.9 Hz, 1 H), 3.92 (s, 3 H), 3.85 (s, 3 H), 3.55−3.45 (m, 3 H), 2.50−2.15 (m, 2 H); ^{13}C NMR δ 198.5, 162.6, 154.8, 152.4, 150.3, 149.6, 148.7, 128.9, 126.6, 120.0, 110.2, 108.9, 104.2, 102.7, 102.6, 58.4, 56.1, 55.9, 50.4, 47.5, 30.9, 30.8. Anal. Calcd for $C_{22}H_{20}NO_6Br$: C, H, N.

***cis*-6-(3-Azido-1-propyl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (7j).** The bromide **7i** (0.474 g, 1 mmol) was dissolved in chloroform (60 mL), and water (60 mL) was added to the solution, followed by sodium azide (0.65 g, 10 mmol) and tetrabutylammonium bromide (200 mg). The biphasic mixture was stirred at room temperature for 24 h, after which TLC showed the complete disappearance of the starting material. The organic layer was separated, the aqueous layer was washed with chloroform (60 mL), and the combined organic layer was washed with water (100 mL) and brine (60 mL) and dried (Na_2SO_4). The TLC showed two major spots, and the products were separated by flash column chromatography using 5% ethyl acetate in chloroform as the eluent. The desired azide **7j** was isolated as white crystals (0.133 g, 31%) after crystallization from ethyl acetate/chloroform: mp 160−162 °C; IR (KBr) 2931, 2099, 1699, 1647 cm^{-1}; ^{1}H NMR δ 7.54 (s, 1 H), 7.15 (s, 1 H), 7.05 (s, 1 H), 6.98 (s, 1 H), 6.07 (s, 1 H), 6.03 (s, 1 H), 5.16 (d, J = 6.9 Hz, 1 H), 4.68−4.52 (m, 1 H), 4.20 (d, J = 6.9 Hz, 1 H), 3.92 (s, 3 H), 3.85 (s, 3 H), 3.48−3.30 (m, 3 H), 2.13−1.90 (m, 2 H). Anal. Calcd for $C_{22}H_{20}N_4O_6 \cdot 0.2EtOAc$: C, H, N. The major product (0.227 g, 51%) was identified as the dehydro compound from the spectral and analytical data.

5,6,12α,13α-Tetrahydro-11β-hydroxy-2,3-dimethoxy-8,9-(methylenedioxy)-6-(1-propyl)-11*H*-indeno[1,2-*c*]isoquinoline (8a). The indenoisoquinoline **7a** (1.975 g, 5 mmol) was heated at reflux with a 1 M solution of borane−tetrahydrofuran complex (15 mL) in dry THF (60 mL) for 16 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 was attained. 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 **8a** (1.828 g, 96%). An analytical sample was prepared by crystallization from 2-propanol to yield white crystals: mp 180−182 °C; IR (KBr) 3502, 2971, 2320, 2277, 1611, 1521, 1475 cm^{-1}; ^1H NMR δ 7.90 (s, 1 H), 6.91 (s, 1 H), 6.76 (s, 1 H), 6.64 (s, 1 H), 6.03 (s, 1 H), 6.02 (s, 1 H), 5.32 (dd, J = 6.5 and 8.5 Hz, 1 H), 4.85 (d, J = 8.5 Hz, 1 H), 4.15 (d, J = 16.2 Hz, 1 H), 4.02 (d, J = 16.2 Hz, 1 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.69 (t, J = 8.5 Hz, 1 H), 2.75−2.60 (m, 1 H), 1.95 (d, J = 6.5 Hz, 1 H, D_2O exchangeable), 1.94−1.80 (m, 2 H), 1.37−1.20 (m, 1 H), 0.58 (t, J = 7.2 Hz, 3 H); ^{13}C NMR 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; CIMS *m/z* (relative intensity) 383 (MH^+, 100). Anal. Calcd for $C_{22}H_{25}NO_5 \cdot 0.8EtOAc$: C, H, N.

6-(1-Butyl)-5,6,12α,13α-tetrahydro-11β-hydroxy-2,3-dimethoxy-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinoline (8b). In a procedure similar to that used for the synthesis of **8a**, the indenoisoquinoline **7b** (0.818 g, 2 mmol) was converted to the aminol **8b** (0.682 g, 86%): mp 168−170 °C; IR (KBr) 3527, 2957, 2866, 2399, 2335, 2276, 1609, 1518, 1470 cm^{-1}; ^1H NMR δ 7.76 (s, 1 H), 6.90 (s, 1 H), 6.77 (s, 1 H), 6.65 (s, 1 H), 6.03 (s, 2 H), 5.33 (d, J = 6.6 Hz, 1 H), 4.86 (d, J = 8.35 Hz, 1 H), 4.15 (d, J = 15.6 Hz, 1 H), 4.06 (d, J = 16.0 Hz, 1 H), 3.93 (s, 3 H), 3.90 (s, 3 H), 3.70 (t, J = 7.5 Hz, 1 H), 2.78−2.68 (m, 1 H), 2.04 (s, 1 H, D_2O exchangeable), 2.00−1.75 (m, 2 H), 1.30−1.15 (m, 1 H), 1.10−0.80 (m, 2 H), 0.69 (t, J = 7.2 Hz, 3 H); ^{13}C NMR δ 149.4, 149.3, 148.4, 148.3, 137.9, 131.2, 124.3, 123.5, 110.4, 110.1, 109.7, 104.8, 101.7, 74.1, 73.7, 58.8, 56.2, 56.1, 52.1, 45.7, 25.5, 20.4, 13.7. Anal. Calcd for $C_{23}H_{27}NO_5$: C, H, N.

2,3-Dimethoxy-8,9-(methylenedioxy)-6-(1-propyl)-11*H*-indeno[1,2-*c*]isoquinolinium Chloride (9a). The amino alcohol **8a** (0.762 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 (0.566 g, 71%). An analytical sample was crystallized from methanol: mp 218−220 °C (dec); IR (KBr) 2916, 1617, 1497, 1423 cm^{-1}; ^1H NMR (MeOH-d_4) δ 9.28 (s, 1 H), 7.61 (s, 1 H), 7.46 (s, 1 H), 7.43 (s, 1 H), 7.27 (s, 1 H), 6.14 (s, 2 H), 4.94 (t, J = 7.4 Hz, 2 H), 4.21 (s, 2 H), 4.14 (s, 3 H), 4.04 (s, 3 H), 2.20−2.00 (m, 2 H), 1.13 (t, J = 7.4 Hz, 3 H). Anal. Calcd for $C_{22}H_{22}NO_4Cl \cdot 0.4H_2O$: C, H, N.

6-(1-Butyl)-2,3-dimethoxy-8,9-(methylenedioxy)-11*H*-indeno[1,2-*c*]isoquinolinium Chloride (9b). In a procedure similar to that used for the synthesis of **9a**, the aminol **8b** (0.397 g, 1 mmol) was converted to the isoquinolinium chloride **9b** (0.276 g, 67%): mp 186−190 °C; ^1H NMR (MeOH-d_4) δ 9.26 (s, 1 H), 7.71 (s, 1 H), 7.46 (s, 1 H), 7.41 (s, 1 H), 7.26 (s, 1 H), 6.14 (s, 2 H), 4.84 (s, 2 H), 4.13 (s, 3 H), 4.04 (s, 3 H), 3.24−3.00 (m, 1 H), 2.95−2.70 (m, 1 H), 2.10−2.00 (m, 2 H), 1.65−1.50 (m, 2 H), 1.04 (t, J = 7.3 Hz, 3 H). Anal. Calcd for $C_{23}H_{24}NO_4Cl$: C, H, N.

2-Naphthylidene-(3-chloro-1-propylamine) (10a). In a procedure similar to the one used for the synthesis of **4h**, the imine **10a** was isolated as a pale-white solid in 91% yield after crystallization from hot hexane: mp 49−50 °C; IR (KBr) 2898, 2841, 1687, 1643, 1604, 1503, 1486 cm^{-1}; ^1H NMR 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 (quin, J = 6.3 Hz, 2 H).

2-Naphthylidene-(3-bromo-1-propylamine) (10b). In a procedure similar to the one used for the synthesis of **4i**, the imine **10b** was isolated as a white solid in 98% yield after crystallization from hot hexane: mp 48−50 °C; IR (KBr) 2945, 2835, 1634, 1641, 1504, 1448 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.47 (s, 1 H), 8.02 (s, 1 H), 7.98 (d, J = 8.6 Hz, 1 H), 7.90−7.80 (m, 3 H), 7.53−7.46 (m, 2 H), 3.81 (t, J = 6.6 Hz, 2 H), 3.52 (t, J = 6.4 Hz, 2 H), 2.29 (qn, J = 6.4 Hz, 2 H).

***cis*-4-Carboxy-*N*-(3-chloro-1-propyl)-3,4-dihydro-6,7-dimethoxy-3-(2′-naphthyl)-1(2*H*)isoquinolone (11a).** In a procedure similar to that used for the synthesis of isoquinolones **6a**−**i**, the isoquinolone **11a** was isolated in 56% yield: mp 214−215 °C; IR (KBr) 3074, 2917, 1733, 1615, 1592 cm^{-1}; ^1H 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.

***cis*-*N*-(3-Bromo-1-propyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(2′-naphthyl)-1(2*H*)isoquinolone (11b).** In a procedure similar to that used for the synthesis of isoquinolones **6a**−**i**, the isoquinolone **11b** was isolated in 76% yield: mp 234−235 °C; IR (KBr) 3076, 2915, 1723, 1615, 1592 cm^{-1}; ^1H 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), 5.25 (d, J = 6.4 Hz, 1 H), 4.89 (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 $C_{25}H_{24}NO_5Br \cdot H_2O$: C, H, N.

***cis*-6-(3-Chloropropyl)-6,13α,6α-trihydro-2,3-dimethoxy-5,13-dioxobenzo[1″,2″-4′,5′]benzo[1′,2′-2,1]cyclopenta[3,4-*c*]isoquinoline (12a).** In a procedure similar to that used for the synthesis of isoquinolines **7a**−**i**, the indenoisoquinoline **12a** was isolated as white prisms in 60% yield after crystallization from ethyl acetate: mp 156−158 °C; IR (KBr) 2928, 2832, 1710, 1641, 1599, 1516, 1475 cm^{-1}; ^1H NMR δ 9.00 (d, J = 8.3 Hz, 1 H), 8.10 (d, J = 8.4 Hz, 1 H), 7.86 (d, J = 8.1 Hz, 1 H), 7.70−7.64 (m, 2 H), 7.57 (d, J = 8.1 Hz, 1 H), 7.52 (s, 1 H), 7.26 (s, 1 H), 5.43 (d, J = 6.9 Hz, 1 H), 4.76−4.69 (m, 1 H), 4.37 (d, J = 6.9 Hz, 1 H), 3.94 (s, 3 H), 3.81 (s, 3 H), 3.72−3.60 (m, 3 H), 2.48−3.34 (m, 1 H), 2.28−2.12 (m, 1 H). Anal. Calcd for $C_{25}H_{22}NO_4Cl$: C, H, N.

12-(3-Bromo-1-propyl)-12,11α,4α-trihydro-2,3-dimethoxy-5,13-dioxobenzo[1″,2″,-5′,6′]benzo[1′,2′,-2,1]cyclopenta[3,4-*c*]isoquinoline (12b). In a procedure similar to that used for the synthesis of isoquinolines **7a**−**i**, the indenoisoquinoline **12b** was isolated as a pale-yellow solid in 60% yield after crystallization from ethyl acetate: mp 192−194 °C; IR (KBr) 2938, 1710, 1640, 1600, 1516, 1479 cm^{-1}; ^1H NMR δ 9.10 (d, J = 8.3 Hz, 1 H), 8.17 (d, J = 8.4 Hz, 1 H), 7.87 (d, J = 8.1 Hz, 1 H), 7.70−7.64 (m, 2 H), 7.55 (d, J = 8.1 Hz, 1 H), 7.52 (s, 1 H), 7.24 (s, 1 H), 5.44 (d, J = 6.9 Hz, 1 H), 4.76−4.69 (m, 1 H), 4.35 (d, J = 6.9 Hz, 1 H), 3.93 (s, 3 H), 3.81 (s, 3 H), 3.80−3.76 (m, 2 H), 3.72−3.60 (m, 1 H), 2.48−3.34 (m, 1 H), 2.28−2.12 (m, 1 H). Anal. Calcd for $C_{25}H_{22}NO_4Br$: C, H, N.

3,4-Methylenedioxybenzylidene-(4,4-diethoxy-1-butylamine) (13). In a procedure similar to that used for the synthesis of imines **4a**−**i**, the imine **13** was synthesized in 99% yield as a yellow oil: IR (neat) 3385, 3075, 2973, 1691, 1644, 1605, 1505 cm^{-1}; ^1H NMR δ 8.11 (s, 1 H), 7.30 (d, J = 1.2 Hz, 1 H), 7.05 (dd, J = 1.2 and 8.1 Hz, 1 H), 6.78 (d, J = 7.9 Hz, 1 H), 5.96 (s, 2 H), 4.50 (t, J = 5.6 Hz, 1 H), 3.70−3.40 (m, 6 H), 1.80−1.55 (m, 4 H), 1.16 (t, J = 7.2 Hz, 6 H).

***cis*-4-Carboxy-*N*-(4,4-diethoxy-1-butyl)-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)isoquinolone (14).** In a procedure similar to that employed for the synthesis of isoquinolones **6a**−**i**, the isoquinolone **14** was isolated in 62% yield: mp 202−204 °C; IR (KBr) 3074, 2917, 1736, 1617, 1592 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.51 (s, 1 H), 7.12 (s, 1 H), 6.73 (d, J = 8.1 Hz, 1 H), 6.54 (d, J = 1.2 and 8.1 Hz, 1 H), 6.47 (s, 1 H), 5.93 (s, 2 H), 4.99 (d, J = 6.2 Hz, 1 H), 4.56 (d, J = 6.1 Hz, 1 H), 4.39 (t, J = 4.5 Hz, 1 H), 3.81 (s, 3 H), 3.79 (m, 1 H), 3.74 (s, 3 H), 3.55−3.30 (m, 4 H), 2.90−2.80 (m, 1 H), 1.60−1.40 (m, 4 H), 1.06 (t, J = 7.9 Hz, 6 Hz). Anal. Calcd for $C_{27}H_{33}NO_9 \ H_2O$: C, H, N.

***cis*-6-(3-Formylpent-2-ene-5-yl)-5,6,12,13-tetrahydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (15) and 6-(3-Formylpent-2-ene-5-yl)-5,6-dihydro-2,3-dimethoxy-8,9-(methylenedioxy)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (16).** In a pro-

cedure similar to that utilized for the synthesis of isoquinolines **7a**−**i**, the acid **14** (0.515 g, 1 mmol) on treatment with Eaton's reagent (50 mL) yielded the crude product, which was purified by column chromatography (8:2, chloroform/ethyl acetate) to afford the dihydroindenoisoquinoline **15** (0.124 g, 25%) and the oxidized indenoisoquinoline **16** (0.089 g, 18%). Compound **15**: mp 176−178 °C; IR (KBr) 2925, 2831, 2727, 1711, 1677, 1644, 1597, 1466 cm^{-1}; ^1H NMR δ 9.38 (s, 1 H), 7.53 (s, 1 H), 7.12 (s, 1 H), 7.00 (s, 1 H), 6.99 (s, 1 H), 6.73 (q, J = 7.0 Hz, 1 H), 6.04 (s, 1 H), 6.00 (s, 1 H), 5.13 (d, J = 7.0 Hz, 1 H), 4.50−4.41 (m, 1 H), 4.13 (d, J = 7.0 Hz, 1 H), 3.91 (s, 3 H), 3.84 (s, 3 H), 3.33−3.24 (m, 1 H), 2.05 (d, J = 7.0 Hz, 3 H), 2.10−1.90 (m, 2 H); PDMS m/z (relative intensity) 449 (M^+, 100), 366 (60), 340 (78). Anal. Calcd for $C_{25}H_{23}NO_7$: C, H, N. Compound **16**: mp 290−294 °C; IR (KBr) 2915, 2816, 2717, 1697, 1678, 1643, 1613, 1553 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.49 (s, 1 H), 8.03 (s, 1 H), 7.99 (s, 1 H), 7.63 (s, 1 H), 7.05 (s, 1 H), 6.87 (q, J = 7.0 Hz, 1 H), 6.11 (s, 2 H), 4.32 (t, J = 9 Hz, 2 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 2.86 (t, J = 9 Hz, 2 H), 2.25 (d, J = 7.0 Hz, 3 H).

Top1-Mediated DNA Cleavage Reactions. Human recombinant top1 was purified from Baculovirus as described previously.[15] 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 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration).[16] 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).

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