Optimization of the Indenone Ring of Indenoisoquinoline Topoisomerase I Inhibitors

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Two series of indenoisoquinoline topoisomerase I inhibitors have been prepared to investigate optimal substituents on the indenone ring at the 9-position. The more exhaustive series was prepared using a nitrated isoquinoline ring that has been previously demonstrated to enhance biological activity. After preliminary biological evaluation, a more focused series of inhibitors was prepared utilizing a 2,3-dimethoxy-substituted isoquinoline ring. The results of the two series indicate the existence of superior functional groups such as methoxy, fluorine, and cyano for the indenoisoquinoline 9-position. Interestingly, these functional groups coincide with established structure−activity relationships for the 11-position of camptothecin.

Introduction

Camptothecin (**1**, Figure 1) was first discovered in 1966 by Wall and Wani utilizing bioassay-guided fractionation from the tree *Camptotheca acuminata*.[1] Camptothecin was found to be highly cytotoxic toward leukemia cells and a potent tumor inhibitor. Consequently, it served as the structural core for the synthesis of a new class of anticancer agents.[1,2] Research interest in camptothecin and its derivatives increased dramatically after 1985 when it was determined that camptothecin was a selective mammalian topoisomerase I (Top1) inhibitor.[3] Although camptothecin displayed potent biological activity, initial drug development was hindered due to poor aqueous solubility.[4] In an attempt to engineer an improved solubility profile for camptothecin, topotecan and irinotecan were designed.[4−8] Both of these molecules possess basic functionalities that are protonated at physiological pH and serve to enhance the solubility of the camptothecin core structure.[4−8] The engineered solubility profile was a great success for the camptothecins, as irinotecan and topotecan are the only Top1 inhibitors currently approved by the Food and Drug Administration for the treatment of cancer.[4−8]

Unfortunately, camptothecin derivatives are not ideal drug molecules. They are compromised by their inability to completely stabilize the ternary (DNA−enzyme−drug) complex, necessitating long infusion times to achieve maximum activity.[7] Furthermore, the camptothecins are inherently unstable and suffer from lactone ring opening (which is favored at physiological pH) to form a hydroxy-acid that has a high affinity for human serum albumin.[9−12] Additionally, certain cancers have been found to be unresponsive to camptothecin treatment and have either developed Top1 mutations limiting the sensitivity of the enzyme to the drug or have evolved P-glycoprotein drug efflux pumps to remove the drug from the cancer cell.[13,14] As a result of the pharmacokinetic problems with the camptothecins, there is great interest in the development of noncamptothecin Top1 inhibitors as anticancer agents.

In the 1990s, the National Cancer Institute (NCI) developed a COMPARE algorithm to facilitate the prediction of biological targets from data generated by the 60 human cancer cell cytotoxicity screen.[15] Utilizing the COMPARE algorithm, it was predicted that indenoisoquinoline **2** (Figure 1) should be a Top1 inhibitor based upon a comparison of its cytotoxicity profile to that of camptothecin in the NCI cell screen.[16] Subsequent in vitro testing of **2** indicated that it did induce DNA cleavage in the presence of Top1 (albeit at micromolar concentrations), thereby confirming the biological target prediction by the COMPARE analysis.[16] Despite the indenoisoquinoline's relatively modest potency, there were differences between the DNA cleavage products for both the indenoisoquinoline and the camptothecin that warranted the further development of this new class of Top1 inhibitors.[16] Additionally, the Top1 ternary cleavage complex formed in the presence of indenoisoquinoline **2** was more stable than that formed from camptothecin.[16]

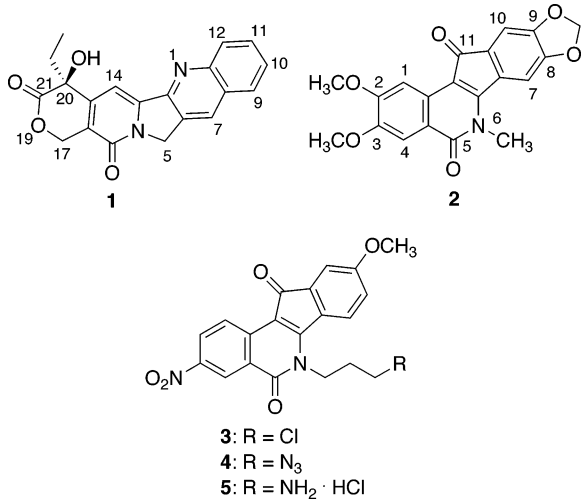

Figure 1. Representative Top1 inhibitors.

Lead optimization of the indenoisoquinolines has provided several compounds with improved cytotoxicity profiles and Top1 inhibitory activities.[17−30] Recently, an investigation was undertaken to explore the improvement in biological activity previously reported for indenoisoquinolines possessing a nitrated isoquinoline ring and a methylenedioxy-substituted indenone ring.[23,30] This investigation led to the identification of a highly potent series of indenoisoquinolines (**3**−**5**) possessing a nitrated isoquinoline ring and a 9-methoxy-substituted indenone ring (Figure 1). Utilizing the nitrated indenoisoquinolines as a

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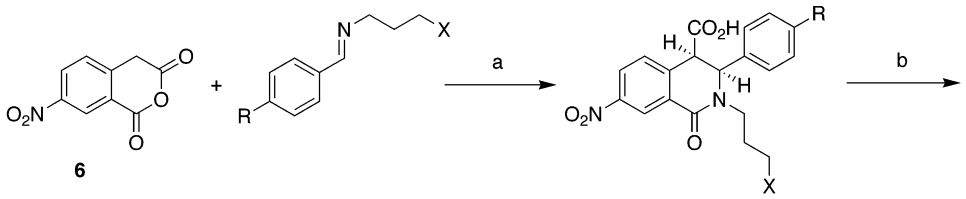
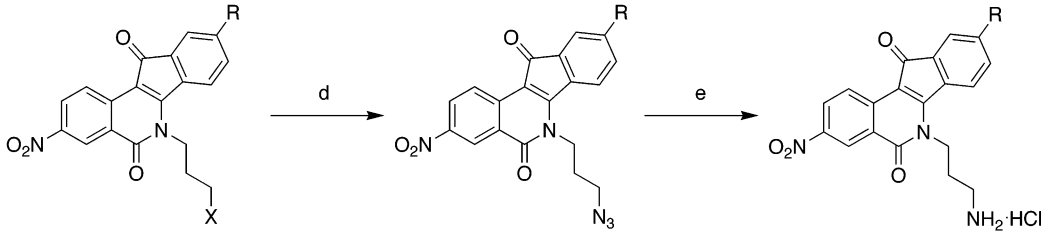
Scheme 1[a]





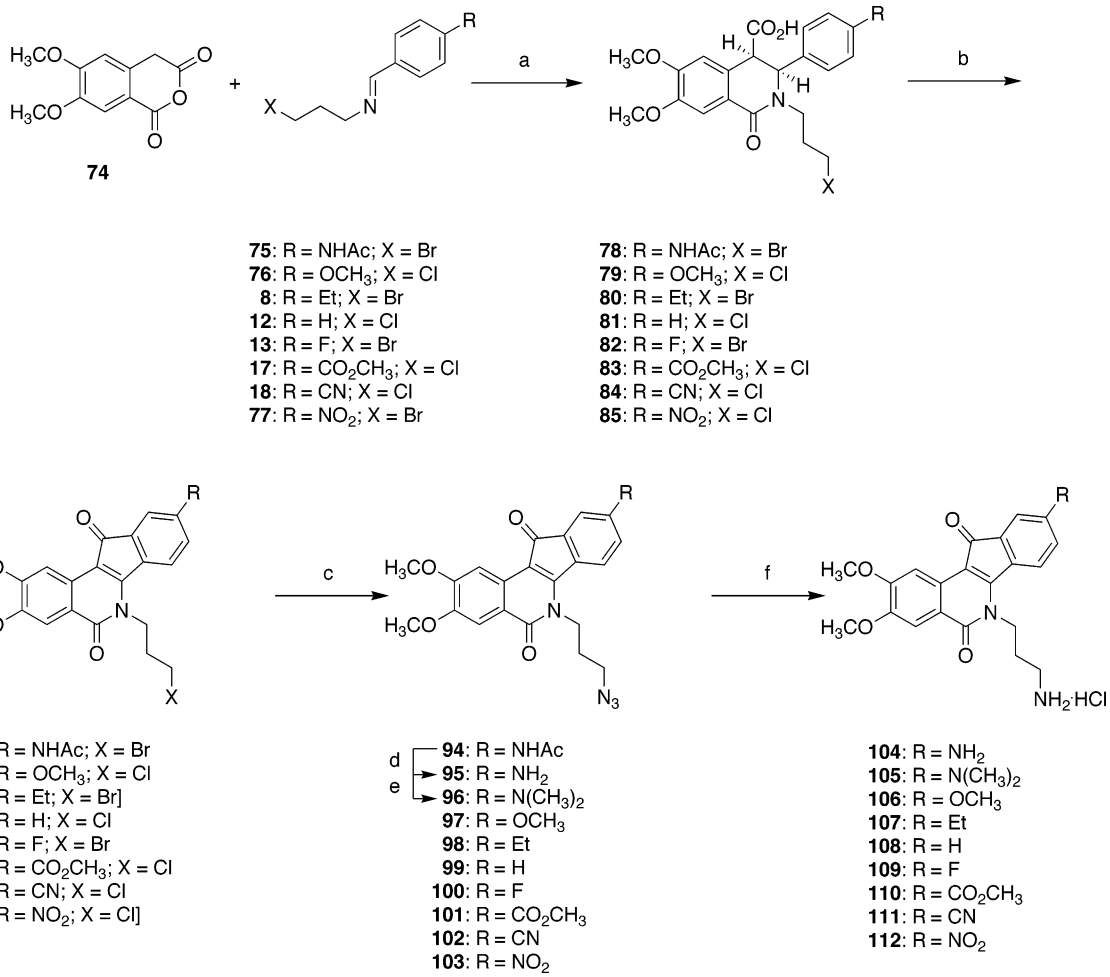
[a] Reagents and conditions**:** (a) $CHCl_3$, rt; (b) (*i*) $SOCl_2$, benzene, reflux; (*ii*) $AlCl_3$, nitrobenzene, 100 °C; (c) MCPBA, $CHCl_3$; (d) NaN_3, DMSO, 100 °C; (e) (*i*) $P(OEt)_3$, benzene, reflux; (*ii*) 3 M HCl in MeOH, benzene, reflux.

platform for lead development, an effort was orchestrated to optimize the 9-position of the indenoisoquinolines in order to discover functionalities that exhibit improved biological activity (both cytotoxicity and Top1 inhibition) similar to that observed for the methoxy substituent at the 9-position. After preliminary biological evaluation and noting the difficulties encountered during the preparation of certain 9-position derivatives (such as strong electron-withdrawing substituents and aniline derivatives), a second series of analogues were prepared utilizing a 2,3-dimethoxy-substituted isoquinoline ring. Collectively, the two series of compounds allow for the identification of new lead substituents at the 9-position of the indenoisoquinolines.

Chemistry

The nitrated indenoisoquinolines utilized in this investigation were prepared according to the conditions outlined in Scheme 1. Treatment of 4-nitrohomophthalic anhydride (**6**)[31] with Schiff bases **7**−**20** provided *cis*-carboxylic acids **21**−**34**. It has previously been demonstrated that the ratio of *cis*- to *trans*-diastereomers afforded by condensations between homophthalic anhydrides and Schiff bases is dictated, in part, by the electronic nature of the Schiff base's substituents.[32] Generally speaking, electron-donating substituents favor the formation of greater amounts of the *cis*-diasteromer, whereas electron-withdrawing substituents increase the formation of the *trans*-product.[32] This was a critical consideration for the successful outcome of analogue syntheses in this study because the oxidative Friedel−Crafts acylation used to convert carboxylic acids **21**−**34** into indenoisoquinolines **35**−**48** only proceeds with the *cis*-diastereomer.[33,34] As a result of diminished formation and difficulty separating the desired *cis*-stereoisomers from their *trans*-diastereomers, *cis*-carboxylic acids **31** and **32** were carried through the subsequent oxidative Friedel−Crafts acylation in crude form. Furthermore, the use of a more electron-deficient substituent (such as a nitro group) on the Schiff base could not

Scheme 2[a]



[a] Reagents and conditions**:** (a) $CHCl_3$, rt; (b) (*i*) $SOCl_2$, benzene, reflux; (*ii*) $AlCl_3$, nitrobenzene, 100 °C; (c) NaN_3, DMSO, 100 °C; (d) 6 M HCl, THF, reflux; (e) H_2CO, $NaCNBH_3$; (f) (*i*) $P(OEt)_3$, benzene, reflux; (*ii*) 3 M HCl in MeOH, benzene, reflux.

be performed at this juncture due to its formation of the *trans*-carboxylic acid and no observed formation of the desired *cis*-stereoisomer.[32]

With an array of *cis*-carboxylic acids prepared, compounds **21**−**34** were subjected to oxidative Friedel−Crafts acylation utilizing thionyl chloride and aluminum chloride to provide indenoisoquinolines **35**−**48**.[23,33,34] Compound **38** was then oxidized with MCPBA to provide analogue **49**, a compound whose synthesis was not envisioned to be amenable to the Schiff base-homophthalic anhydride condensation for the stereoelectronic reasons indicated above. Indenoisoquinolines **35**−**46** were then converted into analogues **50**−**61** by treatment with sodium azide in DMSO and reduced with triethyl phosphite to afford analogues **62**−**73** upon isolation as their respective hydrochloride salts. It is worth noting that compounds **47**−**49** were not elaborated further due to their poor performance in the initial biological assays and undesirable interactions in hypothetical ternary complex models.

The 2,3-dimethoxy-substituted indenoisoquinolines utilized in this study were prepared according to the conditions outlined in Scheme 2. 4,5-Dimethoxyhomophthalic anhydride (**74**)[35] was condensed with Schiff bases **8**, **12**, **13**, **17**, **18**, and **75**−**77** to provide *cis*-carboxylic acids **78**−**85**. Fortunately, *cis*-carboxylic acids with strong electron-withdrawing substituents (including the previously unavailable nitro group) were obtained from the condensation, as the stereoelectronic outcome dictated by the substituents on the Schiff bases was less pronounced than that observed for the reactions involving 4-nitrohomophthalic anhydride.[32] However, there was a definite reduction in the amount of *cis*-carboxylic acid afforded by the condensation of Schiff bases with strong electron-withdrawing substituents.[32] With the desired *cis*-carboxylic acids prepared, compounds **78**−**85** were subjected to oxidative Friedel−Crafts acylation utilizing thionyl chloride and aluminum chloride to provide indenoisoquinolines **86**−**93** (although sufficient microanalyses of compounds **88** and **93** could not be obtained and they were carried through the next transformation in crude form).[23,33,34] Treatment of compounds **86**−**93** with sodium azide in DMSO afforded analogues **94** and **97**−**103**. The aniline amide bond in **94** was then cleaved under acidic conditions to yield the corresponding aniline analogue **95**, which was subjected to reductive amination to provide compound **96**. Reduction of compounds **95**−**103** ultimately afforded analogues **104**−**112** upon isolation as their respective hydrochloride salts.

Biological Results and Discussion

The indenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins.[36,37] 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

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

	cytotoxicity (GI_{50} in μM)[a]									
cmpd	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	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.04	0.0405 ± 0.0187	++++
2	1.3	35	41	4.2	73	68	37	96	20.0	++
3	0.295	0.794	0.027	<0.010	3.39	<0.010	0.036	3.24	0.178 ± 0.012	++++
4	<0.001	<0.001	<0.001		0.155		<0.001	0.251	0.198 ± 0.18	+++
5	<0.010	<0.010	<0.010	<0.010	2.82	<0.010		3.31	0.027 ± 0.008	++++
35	5.75	6.03	5.62	5.75	23.4	11.7	4.27	30.9	15.1	0/+
36	21.4	>50.1	28.2	32.4	>50.1	>50.1	>50.1	>50.1	38.0	0
37	24.5	18.6	>100	26.3	70.8	46.8	50.1	>100	42.0	0
38	1.05	2.24	4.57	3.09	>100	0.891	3.89	30.9	8.91	0
39	18.2	>50.1	16.6	49.0	>50.1	>50.1	>50.1	>50.1	34.5 ± 8.2	0
40	43.6	3.63		20.4	4.90	26.9	40.7	31.6	18.2	++
41	2.29	7.24		2.29	46.8	27.5	3.39	37.2	14.8	++
42	2.45	3.47	3.72	3.47	36.3	2.51	3.98	14.8	7.76	++
43	3.63	3.72	0.661	0.017	0.575	0.019	0.214	0.282	0.241 ± 0.022	++
44										++++
45	43.7	41.7	83.2	27.5	95.5	>100	37.2	>100	66.1	+++
46	2.57	1.82	2.63	1.12	8.71	5.89	2.75	>100	7.08	+++
47	27.5	81.3	56.2	33.9	>100	>100	>100	>100	58.9	0
48	7.59	>100	50.1	23.4	81.3	>100	58.9	>100	42.7	0
49	8.13	7.41	24.0	4.27	28.8	6.92	6.31	83.2	10.2	++
50	3.02	1.82	3.89	2.88	20.0	5.25	3.89	39.8	5.62	0/+
51	>50.1	>50.1	>50.1	>50.1	>50.1	>50.1	>50.1	>50.1	49.0	0
52	72.4	20.4	>100	30.2	>100	58.9	>100	>100	49.0	0
53	0.603	0.776	0.891	0.708	3.89	1.05	1.15	6.03	5.05 ± 2.03	0/+
54	>100	>100	>100	>100	>100	>100	>100	>100	82.3 ± 1.0	0
55	>100	8.13		29.5	NT	72.4	>100	>100	13.5	++
56	0.251	0.309	0.457	0.380	3.47	0.302	0.437	2.88	1.76 ± 0.53	++++
57										0
58	56.2	3.02	>100	>100	>100	>100	14.8	>100	51.3	+++
59	1.82	2.14	2.24	3.31	18.2	3.89	1.78	34.7	6.03	+++
60	25.7	47.9	53.7	37.1	>100	>100	70.8	>100	55.0	0
61	15.1	3.89	>50.1	3.63	>50.1	46.8	7.08	>50.1	18.6	+++
62	0.012	0.174	0.035	0.055	0.457	0.055	0.011	0.200	0.104 ± 0.006	++++
63	1.38	0.575	1.58	1.48	1.58	1.48	0.776	1.55	1.07	+
64	0.191	0.229	0.603		0.324	0.229	0.115	0.316	0.244 ± 0.025	++++
65	<0.010	0.023	<0.010	<0.010	0.170	0.162	<0.010	0.204	0.063 ± 0.010	++++
66	0.186	0.178	1.62	1.51	1.82	0.437	0.200	0.468	0.646 ± 0.096	0
67		<0.005	0.138	0.028	0.083	0.009	0.014	0.123	0.146 ± 0.100	++++
68	<0.010	<0.010	0.028	<0.010	0.052	0.034	<0.010	0.081	0.040 ± 0.004	++++
69	<0.010	<0.010	<0.010	<0.010		<0.010	<0.010		0.021 ± 0.005	+++
70	0.033	0.048	0.011	<0.010	0.295	<0.010	<0.010	0.162	0.030 ± 0.002	++++
71	0.021	<0.010	0.044	<0.010	1.20	<0.010	<0.010	0.224	0.152 ± 0.067	+++
72										++++
73										+++
86	1.29	2.57	3.80	2.09	4.36	13.8	4.68	13.8	6.76	0
87	4.07	8.32	6.76	3.02	70.8	5.50	4.07	37.2	17.2 ± 2.8	0
89	4.47	2.46	14.1	12.3	10.7	>100	>100	>100	23.4	++
90	4.17	5.75	3.55	3.89	24.5	15.5	4.36	43.7	12.3	+
91	>100	>100		>100	>100	>100	>100	>100	81.3	+
92	14.8	15.1	7.24	6.76	12.9	22.9	10.7	>100	11.2	++
94	50.1	50.1	50.1	50.1	50.1	50.1	50.1	50.1	41.7	0
95	0.051	0.047	0.050	0.026	0.214	0.148	0.059	0.214	0.231 ± 0.124	0
96	>100		>100	>100	>100	>100	>100	>100	97.7	++
97	11.5	27.5	56.2	20.9	49.0	64.6	26.3	>100	38.9	0
98	56.2		57.5	91.2	>100	>100	>100	>100	81.3	0
99	3.55	1.82	4.68	2.63	4.57	13.8	28.2	9.77	11.7	++
100	4.17	4.17	3.24	5.25	6.17	8.32	4.17	38.0	12.0	0/+
101	>100	>100	>100	>100	>100	>100	>100	>100	79.4	0
102	14.1	24.0	17.4	12.3	17.0	15.5	33.9	>100	34.7	+
103	89.1	>100	38.9	69.2	>100	>100	34.7	>100	57.5	0/+
104		0.263	0.316	1.38	1.82	2.57	2.40	4.17	2.19	+
105	17.0	3.63	1.90	21.9	17.8	17.0	2.40	17.8	9.77 ± 0	−
106	<0.010	0.054	0.115	0.427	0.389	0.141	<0.010	1.70	0.186 ± 0.042	+++
107	1.82		1.62	1.82	1.51	1.74	1.62	2.00	1.74	0
108	0.151	<0.010	2.82	2.88	0.085	0.794	2.63	0.631	1.35	+++
109	0.603	1.38	1.12	3.39	6.31	4.36	0.575	6.03	2.61 ± 0.21	+++
110	3.72	16.6	15.8	17.4	16.6	15.1	17.8	18.2	13.0 ± 0.45	0/+
111	3.98	15.9		17.0	19.1	16.6	12.3	18.6	10.0	+
112	0.617	1.32	3.72	11.5	16.6	11.7	2.09	15.8	4.40	+

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c] The compounds were tested at concentrations ranging up to 10 μM. The activity of the compounds to produce Top1-mediated DNA cleavage was expressed semiquantitatively as follows: +, weak activity; ++ and +++, modest activity; ++++, similar activity as 1 μM camptothecin.

(approximately 55) in which GI_{50} values below and above the test range (10^{-8} to 10^{-4} molar) are taken as the minimum (10^{-8} molar) and maximum (10^{-4} molar) drug concentrations used in the screening test. For comparison purposes, the activities of the previously reported lead camptothecin (**1**) and compounds **2**−**5** are also included in the table. The relative potencies of the compounds in the production of Top1-mediated DNA cleavage are also listed in the table. These results were expressed semiquantitatively as follows: 0, no detectable activity; +, weak activity; ++, similar activity as compound **2**; +++ and ++++, greater activity than compound **2**; ++++, similar activity as 1 μM camptothecin.

The analogues whose biological data are reported in Table 1 can be readily divided into two categories: nitrated indenoisoquinolines (compounds **3**−**5** and **35**−**73**) and dimethoxy-substituted indenoisoquinolines (compounds **86**−**112**). With such a division, inferences can be made from the data regarding the effects of 9-position substituents on both Top1 inhibition and cytotoxicity for each category of analogues. If one defines the criteria to be considered an "active" compound as an MGM value less than 1 μM or a Top1 inhibitory value of "+++" or greater, then the two categories of analogues can be readily evaluated. In general, the nitrated indenoisoquinolines prepared in this study were usually more cytotoxic and better Top1 inhibitors than the dimethoxy-substituted derivatives. This result was not surprising, however, given the previously observed biological results for nitrated indenoisoquinolines (especially compounds **3**−**5**) and the features imparted by the nitro group believed to be responsible for the enhanced activity (such as improved π-stacking interactions with the DNA base pairs and hydrogen-bonding interactions with Asn722).[23,27,30,38]

Nitroindenoisoquinolines. One of the more surprising results for the nitrated indenoisoquinolines in Table 1 was the determination that the previously identified methoxy substituent is optimally suited for enhancing the biological activity of the indenoisoquinoline 9-position (indenoisoquinolines **3**−**5**). Indeed, the methoxy substituent was the only functional group at the 9-position that consistently produced both cytotoxic and potent Top1 inhibitors regardless of the lactam nitrogen's functionality. Substituents on the lactam nitrogen (such as a propylamino group) have previously been identified to improve the biological activity of the indenoisoquinolines and this improvement is hypothesized to result from more efficient DNA targeting and the formation of hydrogen-bonding interactions within the ternary complex.[24] Thus, the identification of 9-position substituents that can improve the biological activity of the indenoisoquinolines in the absence of enhancement from the lactam nitrogen should in theory be useful for "mixing and matching" with lactam substituents known to confer greater potency than an amino group.[28]

Although the methoxy group was the only substituent identified in this study to enhance both the cytotoxicity and the Top1 inhibition of the analogues, several functional groups were identified that improved Top1 inhibition. A fluorine atom in the 9-position (analogues **41**, **56**, and **68**) provided compounds with potent Top1 inhibition equal in magnitude to camptothecin, regardless of the lactam substituent. Furthermore, when the 9-position fluorine atom was combined with a propylamino-substituted lactam nitrogen, the resulting analogue **68** was equally as cytotoxic as camptothecin. Additional functional groups at the 9-position that resulted in potent Top1 inhibition in the absence of the propylamino lactam substituent's effects included an iodine atom (**44** and **59**), a methyl ester (**45**), a nitrile (**46** and **61**), and a bromine atom (**58**). When the propylamino lactam subsituent's effects are included in the analysis, only compounds **63** and **66**, with their respective 9-position ethyl and phenyl substituents, did not display potent Top1 inhibition.

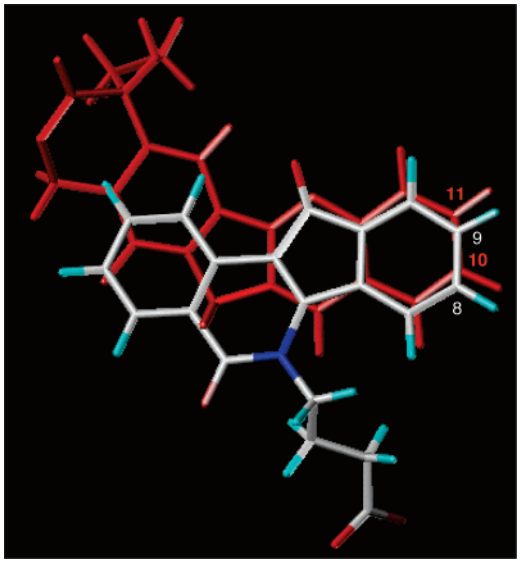


Figure 2. Ligand overlay of the crystal structures of camptothecin (red) and an indenoisoquinoline (colored by atom type). Numbers in red correspond to camptothecin atom numbering and numbers in white correspond to indenoisoquinoline atom numbering.

Even in the absence of increased Top1 inhibition, the analogues in this study indicate important features about the binding pocket in the vicinity of the 9-position of the indenoisoquinolines. A comparison of compounds **3**−**5** (9-position methoxy) and **36**, **51**, and **63** (9-position ethyl) indicate an important feature about the methoxy group. These six compounds differ only by the replacement of an ether oxygen atom with a methylene unit, but this small difference results in a dramatic change in the biological activity of the compounds, with **36**, **51**, and **63** being virtually inactive in the biological assays (relative to **3**−**5**). Therefore, the ether oxygen atom must have an important role in stabilizing the ternary complex. It has previously been postulated that the ether oxygen of the methoxy group contributes to electrostatic charge complementarity with the DNA base pairs in the ternary complex. Additionally, molecular modeling using a hydrated binding pocket has indicated a hypothetical hydrogen bond network between water molecules and the oxygen atom. Given that the analysis of a 9-position ethyl substituent resulted in a complete loss in biological activity, it is evident that the postulated roles of the methoxy group are highly important for stabilizing the ternary complex. Furthermore, analogues possessing 9-position substituents such as methyl and thiomethyl (**37**, **52**, **38**, and **53**) generally displayed poor biological activity until the lactam side chain functionality compensated (compounds **64** and **65**). These results collectively support the role of the 9-position methoxy group regarding charge complementarity and hydrogen bonding. Additionally, electrostatic charge complementarity and the ability to participate in hydrogen-bonding interactions help to rationalize the discovery that fluorine and nitrile substituents at the 9-position tend to confer potent Top1 inhibition.

The inactivity of previously reported 8,9-dimethoxy-substituted indenoisoquinolines was rationalized utilizing a crystal structure overlay of ternary complexes for camptothecin and an indenoisoquinoline and noting the conserved positioning of the aromatic rings of the two ligands (Figure 2).[30] Once again, the use of the crystal structure overlay in Figure 2 illustrates

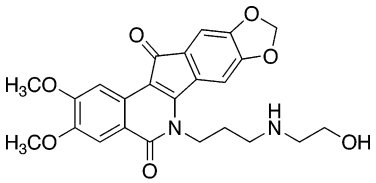
two important points for the camptothecin and indenoisoquinoline classes of Top1 inhibitors. First, the existence of such a conserved overlap for the aromatic rings between the two classes of compounds indicates that the rings are optimally positioned to stabilize the ternary complex and minimize detrimental steric interactions between the ligand and the non-scissile DNA strand. Thus, 9-position substituents that are sterically demanding (such as phenyl, *p*-toluenesulfonyl, and methyl sulfone) would not be expected to demonstrate potent Top1 inhibition. Indeed this is the result as compounds **39**, **54**, **66**, **47**, **48**, and **49** were all rather poor Top1 inhibitors. Furthermore, a close examination of **3** with **35** and **4** with **50** illustrates that even though sterically more demanding substituents have a detrimental effect on biological activity, the negative biological effects can be overcome by incorporation of a more potent functional group on the lactam side chain, illustrated by compound **62**. The second point that Figure 2 indicates is that because the 9-position of the indenoisoquinolines and the 11-position of the camptothecins occupy a similar space in the ternary complex, then substituents at these positions that result in potent Top1 inhibition for one class should demonstrate inhibition in the other class. This is indeed the case given the fact that fluorine (indenoisoquinolines **41**, **56**, and **68**) and nitrile (indenoisoquinolines **46**, **61**, and **73**) substituents at the indenoisoquinoline 9-position or the camptothecin 11-position demonstrated potent Top1 inhibition regardless of the lactam substituent on the indenoisoquinoline.[8,9,39−41] Therefore, in addition to the "steric" and "electrostatic charge complementarity" hypotheses that have been invoked to rationalize the biological results of the indenoisoquinolines, it appears that substituents conferring potent Top1 inhibition (located on the spatially conserved aromatic rings in the ternary complexes for both classes of inhibitors) could theoretically be swapped between classes and still retain Top1 inhibition. Thus, the structure−activity relationships for the conserved rings of both classes should be mutually inclusive. Furthermore, it has been established that indolocarbazoles orient one aromatic ring to occupy the similarly conserved space with the camptothecins and indenoisoquinolines, and the SAR for all three classes of Top1 inhibitors may be applicable to each other and could aid the design of hybrid inhibitors.[8,42]

A comparison of the 9-position substituent's effect on cytotoxicity of the corresponding analogues was equally as intriguing as their effect on Top1 inhibition. In the absence of the propylamino-substituted lactam nitrogen, only a 9-position methoxy group (**3** and **4**) or a bromine atom (**43**) displayed submicromolar MGM values. This result was rather disappointing, especially given that none of the newly examined 9-position substituents that displayed enhanced cytotoxicity also displayed potent Top1 inhibition. This inconsistency has been previously observed and attributed to differential uptake, distribution, and metabolism in a cellular assay.[24] When the effects of the propylamino-substituted lactam nitrogen are considered (analogues **62**−**71**), only compound **63** (with its 9-position ethyl substituent) did not show potent cytotoxicity. Furthermore, several of the compounds (**65** and **68**−**70**) have MGM values comparable to camptothecin. With the exception of compound **66**, all of the analogues displaying potent cytotoxicity were effective Top1 inhibitors and it seems reasonable to attribute their cytotoxicity principally to the inhibition of Top1. Obviously, compound **66** has Top1-independent cytotoxicity, and hypothetical modeling of the compound in ternary complex with DNA and Top1 indicates that there is simply not enough space to accommodate a phenyl ring at the 9-position. Yet again, the results for the propylamino-substituted analogues presumably highlight the importance of effectively targeting DNA in a cellular system and its ability to aid in the stabilization of the ternary complex. Furthermore, it seems reasonable to conclude that the general ability of the propylamino substituent to confer potent Top1 inhibition and cytotoxicity regardless of the 9-position substituent emphasizes that it has a more dominant effect than the 9-position in contributing to the biological activity of the indenoisoquinolines.

Dimethoxyindenoisoquinolines. Due to several problems encountered during the synthesis of nitrated indenoisoquinoline analogues, a second series of compounds was prepared using a 2,3-dimethoxy-substituted isoquinoline ring. Utilizing this platform, analogues **86**−**112** were prepared and included both previously utilized (methoxy, ethyl, hydrogen, fluorine, methyl ester, and nitrile) and novel (acetamide, amino, dimethylamino, and nitro) 9-position substituents. This analogue platform was not envisioned to be as active as the nitrated series of compounds, and the biological results for analogues with novel 9-position substituents were envisioned to guide their incorporation into the nitrated series of analogues. Of the newly examined analogues, only **89**, **99**, **106**, **108**, and **109** demonstrated potent Top1 inhibition. The fact that the 9-position methoxy and fluorine substituents (**106** and **109**) appeared in the analysis of active Top1 inhibitors emphasizes their prominence as superior functional groups important for Top1 inhibition. Demonstrating a consistent theme with the nitrated series of analogues, the substitution of the ether oxygen atom of the methoxy group (compounds **97** and **106**) with a methylene subunit (providing compounds **98** and **107**) had a detrimental effect on Top1 inhibition, and this continues to highlight the importance of electrostatics and hydrogen-bonding interactions in the ternary complex. Reinvestigating the extrapolation of structure−activity relationships between the camptothecin and the indenoisoquinoline classes of Top1 inhibitors, the results for the new 9-position substituents in this current series are not surprising. For camptothecin analogues, 11-position nitro, amino, and dimethylamino substituents are generally not very active.[40] Thus, the absence of Top1 inhibition conferred by these substituents at the indenoisoquinoline 9-position is not surprising and lends support to the hypothesis of a universal nature of SAR for interfacial Top1 inhibitors.

Upon examining the cytotoxicity of the new series of compounds (**86**−**112**), discrepancies between Top1 inhibition and cytotoxicity are evident. Compounds **89**, **99**, **108**, and **109** are poor cytotoxic agents in comparison to their ability to inhibit Top1. Indeed, the only compound to display both potent cytotoxicity and Top1 inhibition in the new series (**86**−**112**) was **106**, with its 9-position methoxy group. Demonstrating a consistent theme with the nitrated series of analogues, the substitution of the ether oxygen atom of the methoxy group (**106**) with a methylene subunit (**107**) resulted in a 10-fold loss in cytotoxicity. Thus, a methoxy group at the 9-position appears to be the most optimal substituent for the 9-position that has thus far been discovered.

The indenoisoquinolines **66** and **95** are inactive as Top1 inhibitors, yet they display cytotoxicities in the submicromolar range. Obviously, their cytotoxicities must be Top1-independent, and this raises the question of whether or not the indenoisoquinolines in the present series that are potent Top1 inhibitors also have additional targets that contribute to their cytotoxicities. This question was previously investigated with the indenoisoquinoline MJ-III-65 **(113**, Figure 3) in the murine cell line P388/CPT45, which does not have any detectable Top1. The results



113

Figure 3. Structure of MJ-III-65.

demonstrated that P388/CPT45 cells showed considerable resistance to compound **113** at low drug concentrations (<1 μM), but not at higher doses.[43] Indenoisoquinoline **113** cytotoxicity is therefore mediated by Top1 inhibition at low drug concentrations, but additional targets are implied at higher concentrations. The potent Top1 inhibitors in the present series are consequently likely to have additional targets that may contribute to their observed cytotoxicities. Compound **113** can intercalate with DNA at high concentrations, and this ability may account for the additional targets that mediate cytotoxicity.[18] Previous studies have shown that some of the indenoisoquinolines can intercalate with DNA in the absence of Top1, while others require the action of Top1 to intercalate.[17]

To compare the 3-nitro and 2,3-dimethoxy series, pairs of compounds that were identical except for 3-nitro versus 2,3-dimethoxy substitution were identified. If one considers the cytotoxicity MGM values of the 10 pairs for which there are complete data (**40**/**89**, **41**/**90**, **46**/**92**, **51**/**98**, **55**/**99**, **56**/**100**, **60**/**101**, **61**/**102**, **63**/**107**, and **68**/**109**), the MGM values are close for nine of them. Only the **68**/**109** pair shows a large difference in cytotoxicity, with the nitro compound **68** being significantly more cytotoxic. This indicates that, in general, changing from the 3-nitro to the 2,3-dimethoxy substitution does not make a large difference in cytotoxicity. On the other hand, the Top1 inhibition data are similar for 8 of the 12 pairs (**40**/**89**, **41**/**90**, **55**/**99**, **46**/**92**, **51**/**98**, **60**/**101**, **63**/**107**, and **68**/**109**), with significant differences evident in the remaining four pairs (**56**/**100**, **61**/**102**, **72**/**110**, and **73**/**111**). Of the pairs that show significant differences in Top1 inhibitory activity, the nitro compounds are more potent in all of the cases. The results are hypothesized to be the consequence of improved charge-complementarity between the nitrated indenoisoquinolines and the DNA base pairs at the site of intercalation and are fully consistent with our models of the ternary complexes formed from the indenoisoquinolines, DNA, and Top1.[30] The lack of a universal, perfect correlation between Top1 inhibition and cellular cytotoxicity is not surprising and is attributed to differential uptake, distribution, and metabolism in a cellular assay, as well as the possibility of additional targets as discussed above.

The DNA cleavage patterns produced by camptothecin **(1**, lane 3 of each gel**)**, the indenoisoquinoline NSC 314622 (**2**), and compounds **5**, **65**, **68**, **69**, **70**, and **106** are displayed in Figure 4. The following points are apparent from inspection of the gels: (1) The potencies of the indenoisoquinolines as Top1 inhibitors are reflected in the intensities of the DNA cleavage bands. The bands produced by compounds **69** (Top1: +++) and **106** (Top1: +++) are slightly weaker in comparison with the other analogues and camptothecin. (2) Top1 inhibitors can be classified as Top1 suppressors, which inhibit DNA cleavage and Top1 poisons, which inhibit the re-ligation reaction after DNA cleavage. Many of the Top1-mediated DNA cleavages are trapped at lower compound concentrations and suppressed at higher concentrations, and therefore, the indenoisoquinolines act as Top1 poisons at lower concentrations and Top1 suppressors at higher concentrations. The suppression could result from binding of the drug to the DNA, rendering it a poorer enzyme substrate at high drug concentration, or from a direct effect on the enzyme to suppress its ability to cleave DNA. Interestingly, all of the analogues presented in Figure 4 demonstrate this effect. (3) There are differences in the cleavage pattern of camptothecin versus the indenoisoquinolines. For example, the cleavage at base pair 44 seen with the indenoisoquinolines is absent with camptothecin. This difference may indicate that the indenoisoquinolines might display antitumor spectra different from camptothecin or its clinically useful derivatives irinotecan and topotecan.

Conclusion

In conclusion, two series of indenoisoquinoline Top1 inhibitors have been prepared to investigate and identify optimal substituents on the indenone ring at the 9-position of the indenoisoquinolines. An extensive series was prepared using a nitrated isoquinoline ring. After preliminary biological evaluation and encountering difficulties preparing 9-position analogues with functional groups such as nitro and aniline derivatives, a more focused series of inhibitors was prepared utilizing a 2,3-dimethoxy-substituted isoquinoline ring. The results of the two series indicate the existence of superior

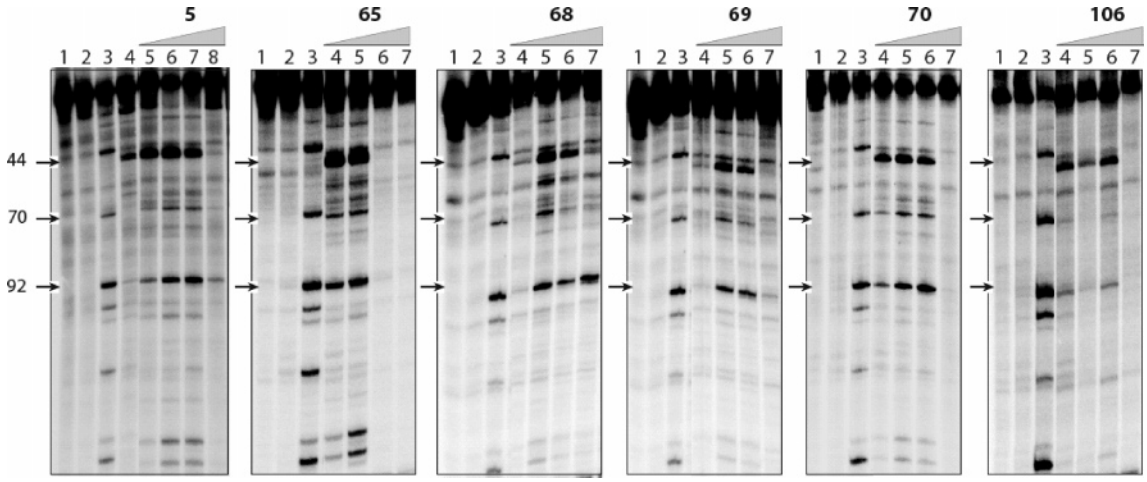


Figure 4. Lane 1, DNA alone; lane 2, Top1 alone; lane 3, +CPT (1 μM); In the first panel, lane 4, Top1 + NSC 314622 (100 μM); lanes 5−8 (for compound **5**) and lanes 4−7 (for compounds **65**, **68**, **69**, **70**, and **106**), Top1 + indicated compound at 0.1, 1, 10, and 100 μM, respectively. Number on left and arrows indicate cleavage site positions.

functional groups such as methoxy, fluoro, and nitrile that have a greater tendency to confer potent Top1 inhibition for the indenoisoquinolines (especially the nitrated series) when substituted at the 9-position. Interestingly, several of the functional groups observed to confer potent Top1 inhibition to the indenoisoquinolines support established structure−activity relationships for the 11-position of camptothecin derivatives. Additionally, indenoisoquinoline 9-position substituents that were relatively inactive (or detrimental to Top1 inhibition) were also observed to be detrimental within the camptothecin class of Top1 inhibitors. The results of the present study with the indenoisoquinolines have been further utilized to help define steric limitations of functional groups protruding toward the nonscissile DNA strand and they also highlight the importance of hydrogen-bonding and substituent electrostatic interactions with the DNA base pairs in the ternary complex. More importantly, the results of this study indicate a universal nature for structure−activity relationships between the camptothecins and the indenoisoquinolines, and this relationship may be useful for the design of hybrid Top1 inhibitors.

Experimental Section

General. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained using $CHCl_3$ as the solvent unless otherwise specified. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using an ARX300 300 MHz Bruker NMR spectrometer. IR spectra were recorded using a Perkin-Elmer 1600 series FTIR spectrometer. Combustion microanalyses were performed at the Purdue University Microanalysis Laboratory and the reported values were within 0.4% of the calculated values. All of the indenoisoquinolines are amorphous solids that in many cases retain water even after prolonged heating in vacuo in a drying pistol. Analytical thin-layer chromatography was carried out on Baker-flex silica gel IB2-F plates and compounds were visualized with short wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

General Procedure for the Preparation of Schiff Bases 7−20 and 75−77. The hydrobromide (or hydrochloride) salt of 3-bromopropylamine (or 3-chloropropylamine; 1.646−9.646 g, 7.520−46.00 mmol, 1.5−2.0 equiv) was treated with triethylamine (1.31−9.85 mL, 9.40−70.7 mmol, 3.0 equiv) in $CHCl_3$ (50−200 mL), and the mixture was allowed to stir at room temperature for 5 min. The appropriate aldehyde (1.454−5.200 g, 6.267−30 mmol, 1 equiv) and magnesium sulfate (4.000−20.00 g) were added, and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was filtered and the filter pad was washed with $CHCl_3$ (50−100 mL). The filtrate was washed with water (3 × 30−100 mL) and satd NaCl (30−100 mL), dried over sodium sulfate, and concentrated to provide the desired compound.

General Procedure for the Preparation of Carboxylic Acids 21−34 and 78−85. 4-Nitrohomophthalic anhydride [(**6**) or 4,5-dimethoxyhomophthalic anhydride (**74**); 1.177−7.300 g, 5.681−33.00 mmol, 1 equiv] was added to a chloroform (50−300 mL) solution of the appropriate Schiff base (2.000−7.200 g, 5.681−33.00 mmol, 1 equiv), and the reaction mixture was allowed to stir at room temperature for 2−72 h. The precipitate was filtered, washed with chloroform (30−100 mL), and dried to provide the desired compound.

General Procedure for the Preparation of Indenoisoquinolines 35−48 and 86−93. Thionyl chloride (1−20 mL) was added to a solution of the appropriate carboxylic acid (0.250−2.000 g, 0.447−4.832 mmol, 1 equiv) in benzene (40 mL). The reaction mixture was heated at reflux for 30 min, allowed to cool to room temperature, and concentrated. The residue was diluted with nitrobenzene (20−100 mL), chilled in an ice bath, and aluminum chloride (0.119−1.288 g, 0.894−9.666 mmol, 2 equiv) was added. The reaction mixture was removed from the bath and the mixture was heated at 100 °C for 1 h. Water (100 mL) was added and the solution was extracted with $CHCl_3$ (3 × 100−200 mL). The combined organic layer was washed with satd $NaHCO_3$ (3 × 50−100 mL) and satd NaCl (50−100 mL) and dried over sodium sulfate. The solution was concentrated, hexanes (800 mL) were added, and the liquid was decanted. The solid was washed with hexanes (100 mL), and the liquid was again decanted. The solid was purified with flash column chromatography (SiO_2), eluting with chloroform, to provide the pure compound.

General Procedure for the Preparation of Indenoisoquinolines 50−61, 94, and 97−103. Sodium azide (0.002−0.242 g, 0.031−3.714 mmol, 1−3 equiv) and the appropriate indenoisoquinoline (0.013−0.529 g, 0.301−1.238 mmol, 1 equiv) were diluted with DMSO (10−100 mL), and the mixture was heated at 100 °C for 1 h. The reaction mixture was diluted with $CHCl_3$ (50−300 mL), washed with water (4 × 50−100 mL) and satd NaCl (50−100 mL), and dried over sodium sulfate. The solution was concentrated to provide a crude solid that was purified by flash column chromatography (SiO_2), eluting with chloroform, to provide the desired compound.

General Procedure for the Preparation of Indenoisoquinolines 62−73 and 104−112. Triethyl phosphite (0.089−0.180 mL, 0.52−1.05 mmol, 2.5 equiv) was added to a solution of the appropriate azido-substituted indenoisoquinoline (0.090−0.167 g, 0.208−0.425 mmol, 1 equiv) in benzene (30−40 mL), and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, 3 M HCl in methanol (10 mL) was added, and the reaction mixture was heated at reflux for 2 h. The reaction mixture was allowed to cool to room temperature and the precipitate was filtered to provide the desired compound.

4-Ethoxybenzylidene-(3-bromo-1-propylamine) (7). The general procedure provided the desired compound as a viscous yellow oil (3.516 g, 99%). IR (film) 1645, 1606, 1511, 1248, 1167, and 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1 H), 7.67 (d, J = 8.6 Hz, 2 H), 6.93 (d, J = 8.6 Hz, 2 H), 4.13 (q, J = 7.0 Hz, 2 H), 3.73 (t, J = 6.1 Hz, 2 H), 3.51 (t, J = 6.4 Hz, 2 H), 2.29 (pent, J = 6.3 Hz, 2 H), 1.48 (t, J = 7.0 Hz, 3 H); ESIMS m/z (rel intensity) 270/272 (MH^+, 100/98). Anal. ($C_{12}H_{16}BrNO$) C, H, N.

4-(Ethyl)benzylidene-(3-bromo-1-propylamine) (8). The general procedure provided the desired compound as a yellow viscous oil (5.655 g, 94%). IR (film) 2965, 2841, 1646, 1609, and 829 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1 H), 7.66 (d, J = 8.1 Hz, 2 H), 7.26 (d, J = 7.9 Hz, 2 H), 3.76 (dt, J = 6.3 and 1.2 Hz, 2 H), 3.51 (t, J = 6.5 Hz, 2 H), 2.72 (q, J = 7.6 Hz, 2 H), 2.30 (pent, J = 6.4 Hz, 2 H), 1.28 (t, J = 7.6 Hz, 3 H); ESIMS m/z (rel intensity) 254/256 (MH^+, 73/73). Anal. ($C_{12}H_{16}BrN$) C, H, N.

4-Methylbenzylidene-(3-bromo-1-propylamine) (9). The general procedure provided the desired compound as a yellow oil (6.320 g, 99%). IR (film) 2840, 1646, 1609, and 813 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1 H), 7.63 (d, J = 8.1 Hz, 2 H), 7.21 (d, J = 7.9 Hz, 2 H), 3.76 (dt, J = 6.3 and 1.2 Hz, 2 H), 3.51 (t, J = 6.5 Hz, 2 H), 2.35 (s, 3 H), 2.21 (pent, J = 6.4 Hz, 2 H); CIMS m/z (rel intensity) 240/242 (MH^+, 43/43), 160 (MH^+ − HBr, 100). Anal. ($C_{11}H_{14}BrN$) C, H, N.

4-(Thiomethyl)benzylidene-(3-bromo-1-propylamine) (10). The general procedure provided the desired compound as a light yellow oil (5.231 g, 98%). IR (film) 1642, 1594, 1091, and 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1 H), 7.65−7.62 (m, 2 H), 7.27−7.24 (m, 2 H), 3.75 (dt, J = 6.3 and 1.2 Hz, 2 H), 3.51 (t, J = 6.5 Hz, 2 H), 2.51 (s, 3 H), 2.21 (pent, J = 6.4 Hz, 2 H); CIMS m/z (rel intensity) 272/274 (MH^+, 70/71), 192 (MH^+ − HBr, 100). Anal. ($C_{11}H_{14}BrNS$) C, H, N.

4-(Phenyl)benzylidene-(3-bromo-1-propylamine) (11). The general procedure provided the desired compound as a yellow viscous oil (4.879 g, 100%). IR (film) 2841, 1644, 1487, 836, 763, and 697 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.38 (s, 1 H), 7.82 (m, 2 H), 7.67 (m, 4 H), 7.49 (m, 2 H), 7.44 (m, 1 H), 3.80 (dt, J = 6.3 and 1.2 Hz, 2 H), 3.54 (t, J = 6.5 Hz, 2 H), 2.33 (pent, J = 6.4 Hz, 2 H); ESIMS m/z (rel intensity) 302/304 (MH^+, 100/94). Anal. ($C_{16}H_{16}BrN$) C, H, N.

Benzylidene-(3-chloro-1-propylamine) (12).[44] The general procedure provided the desired compound as a yellow oil (8.560 g, 100%). ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 7.76 (m, 2 H), 7.46 (m, 3 H), 3.79 (dt, J = 6.3 and 1.2 Hz, 2 H), 3.66 (t, J = 6.4 Hz, 2 H), 2.23 (pent, J = 6.4 Hz, 2 H).

4-Fluorobenzylidene-(3-bromo-1-propylamine) (13). The general procedure provided the desired compound as a yellow oil (5.780 g, 98%). IR (film) 1649, 1602, 1508, 1230, 1152, and 835 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1 H), 7.76 (dd, J = 7.9 and 5.6 Hz, 2 H), 7.14 (m, 2 H), 3.77 (dt, J = 6.3 and 0.9 Hz, 2 H), 3.52 (t, J = 6.4 Hz, 2 H), 2.30 (pent, J = 6.4 Hz, 2 H); CIMS *m*/*z* (rel intensity) 244/246 (MH^+, 38/34), 164 (MH^+ − HBr, 100). Anal. ($C_{10}H_{11}BrFN$) C, H, N.

4-Chlorobenzylidene-(3-bromo-1-propylamine) (14). The general procedure provided the desired compound as a yellow oil (5.375 g, 97%). IR (film) 1646, 1596, 1489, 1088, 1014, and 822 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1 H), 7.69 (dd, J = 8.5 and 1.8 Hz, 2 H), 7.41 (dd, J = 8.5 and 1.8 Hz, 2 H), 3.77 (dt, J = 6.3 and 1.2 Hz, 2 H), 3.51 (t, J = 6.4 Hz, 2 H), 2.30 (pent, J = 6.4 Hz, 2 H); CIMS *m*/*z* (rel intensity) 260/262/264 (MH^+, 71/100/20). Anal. ($C_{10}H_{11}BrClN$) C, H, N.

4-Bromobenzylidene-(3-chloro-1-propylamine) (15). The general procedure provided the desired compound as a yellow oil (5.311 g, 94%). IR (film) 2844, 1646, 1589, 1486, 1295, 1067, 1010, and 818 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1 H), 7.62−7.53 (m, 4 H), 3.77 (dt, J = 6.4 and 1.3 Hz, 2 H), 3.63 (t, J = 6.3 Hz, 2 H), 2.21 (quin, J = 6.4 Hz, 2 H); CIMS *m*/*z* (rel intensity) 260/262/264 (MH^+, 84/100/24). Anal. ($C_{10}H_{11}BrClN$) C, H, N.

4-Iodobenzylidene-(3-bromo-1-propylamine) (16). The general procedure provided the desired compound as a yellow oil (2.182 g, 99%). IR (film) 1645, 1585, 1482, 1006, and 814 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1 H), 7.78−7.74 (m, 2 H), 7.47−7.43 (m, 2 H), 3.76 (dt, J = 6.3 and 1.3 Hz, 2 H), 3.51 (t, J = 6.4 Hz, 2 H), 2.29 (pent, J = 6.4 Hz, 2 H); CIMS *m*/*z* (rel intensity) 352/354 (MH^+, 57/50), 272 (MH^+ − HBr, 100); CIMS *m*/*z* (rel intensity) 352/354 (MH^+, 57/59). Anal. ($C_{10}H_{11}BrIN$) C, H, N.

4-Methoxycarbonylbenzylidene-(3-chloro-1-propylamine) (17). The general procedure provided the desired compound as a yellow oil (7.2 g, 95%). IR (film) 2948, 2601, 2496, 1723, and 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1 H), 8.12−8.07 (m, 2 H), 7.85−7.61 (m, 2 H), 3.93 (s, 3 H), 3.82 (t, J = 6.0 Hz, 2 H), 3.64 (t, J = 6.0 Hz, 2 H), 2.25−2.19 (m, 2 H); ESIMS *m*/*z* 240 (MH^+, 100). Anal. ($C_{12}H_{14}ClNO_2$) C, H, N.

4-Cyanobenzylidene-(3-chloropropylamine) (18). The general procedure provided the desired compound as a yellow oil (6.8 g, 86%). IR (film) 2979, 2601, 2228, 1646, 1480, and 1445 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1 H), 7.84−7.81 (m, 2 H), 7.71−7.68 (m, 2 H), 3.80 (t, J = 6.0 Hz, 2 H), 3.63 (t, J = 6.0 Hz, 2 H), 2.18 (quin, J = 6.0 Hz, 2 H); ESIMS *m*/*z* (rel intensity) 207 (MH^+, 100). Anal. ($C_{11}H_{11}ClN_2$) C, H, N.

4-(Methoxycarbonyloxymethyl)benzaldehyde. Methyl chloroformate (3.363 g, 35.59 mmol) was added to a solution of 4-hydroxymethylbenzaldehyde[6] (3.230 g, 23.72 mmol) in dichloromethane (50 mL) at 0 °C. DMAP (6.522 g, 53.39 mmol) was added, and the solution was allowed to warm to room temperature. After 3 h, the solution was diluted with dichloromethane (150 mL), washed with water (3 × 50 mL) and satd NaCl (50 mL), and dried over sodium sulfate. Concentration provided a crude oil that was purified by flash column chromatography (SiO_2), eluting with a gradient of hexanes to 25% EtOAc in hexanes, to provide a colorless oil (3.057 g, 66%) that solidified upon standing: mp 29−32 °C. IR (film) 1750, 1701, and 1270 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.02 (s, 1 H), 7.91 (dd, J = 6.5 and 1.7 Hz, 2 H), 7.55 (d, J = 8.1 Hz, 2 H), 5.24 (s, 2 H), 3.83 (s, 3 H); CIMS *m*/*z* (rel intensity) 195 (MH^+, 100). Anal. ($C_{10}H_{10}O_4$) C, H.

4-(Methoxycarbonyloxymethyl)benzylidene-(3-bromo-1-propylamine) (19). The general procedure provided the desired compound as a yellow viscous oil (4.375 g, 84%). IR (film) 1749, 1646, 1442, and 1268 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 7.76 (d, J = 7.7 Hz, 2 H), 7.44 (d, J = 8.1 Hz, 2 H), 5.19 (s, 2 H), 3.81 (s, 3 H), 3.77 (t, J = 5.8 Hz, 2 H), 3.51 (t, J = 6.4 Hz, 2 H), 2.29 (pent, J = 6.4 Hz, 2 H); ESIMS *m*/*z* (rel intensity) 314/316 (MH^+, 28/27). Anal. ($C_{13}H_{16}BrNO_3$) C, H, N.

4-(Toluene-4-sulfonyloxy)benzylidene-(3-bromo-1-propylamine) (20). The general procedure provided the desired compound as a yellow viscous oil (4.303 g, 100%). IR (film) 1646, 1598, 1500, 1374, 1198, 1173, 1154, 1093, and 865 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1 H), 7.72 (d, J = 8.4 Hz, 2 H), 7.70 (d, J = 8.6 Hz, 2 H), 7.32 (d, J = 8.4 Hz, 2 H), 7.05 (m, 2 H), 3.76 (dt, J = 6.3 and 1.1 Hz, 2 H), 3.50 (t, J = 6.4 Hz, 2 H), 2.45 (s, 3 H), 2.28 (pent, J = 6.4 Hz, 2 H); ESIMS *m*/*z* (rel intensity) 396/398 (MH^+, 95/100). Anal. ($C_{17}H_{18}BrNO_3S$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3-(4-ethoxyphenyl)-3,4-dihydro-7-nitro-1(2*H*)isoquinolone (21).** The general procedure provided the desired compound as a white solid (4.166 g, 69%): mp 167−171 °C. IR (KBr) 3081, 1741, 1633, 1528, 1348, and 1178 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.92 (d, J = 2.5 Hz, 1 H), 8.41 (dd, J = 8.6 and 2.6 Hz, 1 H), 8.01 (dd, J = 8.6 and 2.9 Hz, 1 H), 6.99 (d, J = 11.7 Hz, 2 H), 6.77 (d, J = 8.7 Hz, 2 H), 5.28 (d, J = 5.9 Hz, 1 H), 4.93 (m, 1 H), 4.04 (m, 1 H), 3.95 (q, J = 7.0 Hz, 2 H), 3.54 (m, 2 H), 3.28 (m, 1 H), 2.30−2.10 (m, 2 H), 1.36 (t, J = 7.0 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 477/479 (MH^+, 24/23). Anal. ($C_{21}H_{21}BrN_2O_6\cdot0.5H_2O$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3-[4-(ethyl)phenyl]-3,4-dihydro-7-nitro-1(2*H*)isoquinolone (22).** The general procedure provided the desired compound as an off-white solid (7.386 g, 79%): mp 157−161 °C. IR (KBr) 3446, 1745, 1636, 1522, 1351, and 1188 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.89 (d, J = 2.5 Hz, 1 H), 8.38 (dd, J = 8.6 and 2.5 Hz, 1 H), 7.98 (dd, J = 8.7 and 1.0 Hz, 1 H), 7.06 (d, J = 8.2 Hz, 2 H), 6.96 (d, J = 8.2 Hz, 2 H), 5.28 (d, J = 6.3 Hz, 1 H), 4.94 (d, J = 6.4 Hz, 1 H), 4.00 (m, 1 H), 3.51 (m, 2 H), 3.26 (m, 1 H), 2.59 (q, J = 7.5 Hz, 2 H), 2.25−2.10 (m, 2 H), 1.16 (t, J = 7.6 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 461/463 (MH^+, 82/81). Anal. ($C_{21}H_{21}BrN_2O_5\cdot0.75H_2O$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-3-(4-methylphenyl)-7-nitro-1(2*H*)isoquinolone (23).** The general procedure provided the desired compound as an off-white solid (6.099 g, 56%): mp 164−168 °C. IR (KBr) 3079, 1740, 1633, 1527, 1347, and 1180 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.89 (d, J = 2.5 Hz, 1 H), 8.38 (dd, J = 8.6 and 2.5 Hz, 1 H), 7.98 (m, 1 H), 7.03 (d, J = 8.0 Hz, 2 H), 6.93 (d, J = 8.3 Hz, 2 H), 5.27 (d, J = 6.2 Hz, 1 H), 4.91 (d, J = 6.2 Hz, 1 H), 4.00 (m, 1 H), 3.51 (m, 2 H), 3.26 (m, 1 H), 2.27−2.07 (m, 2 H), 2.20 (s, 3 H); ESIMS *m*/*z* (rel intensity) 367 (MH^+ − HBr, 100). Anal. ($C_{20}H_{19}BrN_2O_5\cdot0.5H_2O$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-7-nitro-3-[4-(thiomethyl)phenyl]-1(2*H*)isoquinolone (24).** The general procedure provided the desired compound as an off-white solid (6.854 g, 78%): mp 168−170 °C. IR (KBr) 3079, 1737, 1632, 1527, 1492, 1347, and 1176 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.89 (d, J = 2.5 Hz, 1 H), 8.38 (dd, J = 8.7 and 2.56 Hz, 1 H), 7.98 (m, 1 H), 7.10 (d, J = 8.4 Hz, 2 H), 6.98 (d, J = 8.5 Hz, 2 H), 5.28 (d, J = 5.7 Hz, 1 H), 4.87 (m, 1 H), 4.01 (m, 1 H), 3.52 (m, 2 H), 3.29 (m, 1 H), 2.39 (s, 3 H), 2.28−2.10 (m, 2 H); ESIMS *m*/*z* (rel intensity) 355 (MH^+ − HBr − CO_2, 100). Anal. ($C_{20}H_{19}BrN_2O_5S\cdot0.5H_2O$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-3-(4-phenyl)-1(2*H*)isoquinolone (25).** The general procedure provided the desired compound as an off-white solid (5.160 g, 77%): mp 168−171 °C. IR (KBr) 3083, 1740, 1634, 1527, 1487, 1347, and 1175 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.92 (d, J = 2.5 Hz, 1 H), 8.39 (dd, J = 8.6 and 2.6 Hz, 1 H), 8.01 (m, 1 H), 7.54 (m, 4 H), 7.40 (m, 2 H), 7.29 (m, 1 H), 7.14 (d, J = 8.4 Hz, 2 H), 5.37 (d, J = 6.1 Hz, 1 H), 4.99 (d, J = 6.0 Hz, 1 H), 4.06 (m, 1 H), 3.54 (m, 2 H), 3.27 (m, 1 H), 2.29 (m, 1 H), 2.17 (m, 1 H); ESIMS *m*/*z* (rel intensity) 509/511 (MH^+, 100/87). Anal. ($C_{25}H_{21}BrN_2O_5\cdot0.5H_2O$) C, H, N.

***cis*-4-Carboxy-3,4-dihydro-*N*-(3-chloropropyl)-3-phenyl-7-nitro-1(2*H*)isoquinolone (26).** The general procedure provided the desired compound as an off-white solid (5.865 g, 62%): mp 158−159 °C. IR (KBr) 3062, 1745, 1643, 1520, 1485, 1450, 1350, 1191, and 704 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.82 (d, J = 2.5 Hz, 1 H), 8.30 (dd, J = 8.6 and 2.5 Hz, 1 H), 7.89 (dd, J = 8.6 and 1.9 Hz, 1 H), 7.20−7.10 (m, 3 H), 6.98−6.95 (m, 2 H), 5.23 (d, J = 6.0

Hz, 1 H), 4.87 (d, J = 5.8 Hz, 1 H), 3.97−3.88 (m, 1 H), 3.58−3.50 (m, 2 H), 3.21−3.11 (m, 1 H), 2.11−1.90 (m, 2 H).

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-3-(4-fluorophenyl)-7-nitro-1(2*H*)isoquinolone (27).** The general procedure provided the desired compound as an off-white solid (5.687 g, 77%): mp 175−176 °C. IR (KBr) 3060, 1742, 1641, 1520, 1484, 1350, 1236, and 1190 cm^{-1}; ^1H NMR (CD_3OD) δ 8.90 (d, J = 2.5 Hz, 1 H), 8.39 (dd, J = 8.6 and 2.6 Hz, 1 H), 7.97 (dd, J = 8.7 and 1.0 Hz, 1 H), 7.10−7.05 (m, 2 H), 6.98−6.92 (m, 2 H), 5.33 (d, J = 6.4 Hz, 1 H), 4.97 (d, J = 6.4 Hz, 1 H), 4.02 (m, 1 H), 3.53 (m, 2 H), 3.28 (m, 1 H), 2.26 (m, 1 H), 2.10 (m, 1 H); ESIMS *m*/*z* (rel intensity) 406/408 ($M^+ - CO_2$, 34/27), 327 ($M^+ - CO_2 - Br$, 100). Anal. ($C_{19}H_{16}BrFN_2O_5$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3-(4-chlorophenyl)-3,4-dihydro-7-nitro-1(2*H*)isoquinolone (28).** The general procedure provided the desired compound as a yellow solid (7.490 g, 83%): mp 163−165 °C. IR (KBr) 3437, 1744, 1639, 1522, 1354, and 1191 cm^{-1}; ^1H NMR (CD_3OD) δ 8.89 (d, J = 2.4 Hz, 1 H), 8.39 (dd, J = 8.7 and 2.6 Hz, 1 H), 7.96 (m, 1 H), 7.24−7.21 (m, 2 H), 7.05−7.02 (m, 2 H), 5.32 (d, J = 6.4 Hz, 1 H), 4.95 (d, J = 6.8 Hz, 2 H), 4.02 (m, 1 H) 3.53 (m, 2 H), 3.23 (m, 1 H), 2.26−2.11 (m, 2 H); ESIMS *m*/*z* (rel intensity) 343/345 ($MH^+ - HBr - CO_2$, 100/33). Anal. ($C_{19}H_{16}BrClN_2O_5 \cdot 1.0H_2O$) C, H, N.

***cis*-3-(4-Bromophenyl)-*N*-(3-chloropropyl)-4-carboxy-3,4-dihydro-7-nitro-1(2*H*)isoquinolone (29).** The general procedure provided the desired compound as a yellow solid (4.824 g, 85%): mp 169−171 °C. IR (KBr) 3078, 1737, 1638, 1527, 1487, 1407, 1347, 1186, and 742 cm^{-1}; ^1H NMR (CD_3OD) δ 8.89 (d, J = 2.5 Hz, 1 H), 8.37 (dd, J = 8.6 and 2.5 Hz, 1 H), 7.96 (dd, J = 5.6 and 3.1 Hz, 1 H), 7.39 (d, J = 8.5 Hz, 2 H), 6.99 (d, J = 8.5 Hz, 2 H), 5.30 (d, J = 5.6 Hz, 1 H), 4.95 (d, J = 6.1 Hz, 1 H), 4.01 (m, 1 H), 3.65 (m, 2 H), 3.29 (m, 1 H), 2.17 (m, 1 H), 2.05 (m, 1 H); negative ion ESIMS *m*/*z* (rel intensity) 465/467 [$(M - H^+)^-$, 94/100]. Anal. ($C_{19}H_{16}BrClN_2O_5 \cdot 1.0H_2O$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-3-(4-iodophenyl)-7-nitro-1(2*H*)isoquinolone (30).** The general procedure provided the desired compound as a yellow solid (2.390 g, 75%): mp 178−180 °C. IR (KBr) 3082, 1735, 1527, 1484, 1347, and 1186 cm^{-1}; ^1H NMR (CD_3OD) δ 8.88 (d, J = 2.5 Hz, 1 H), 8.38 (dd, J = 8.6 and 2.5 Hz, 1 H), 7.96 (d, J = 8.5 Hz, 1 H), 7.58 (d, J = 8.5 Hz, 2 H), 6.85 (d, J = 8.5 Hz, 2 H), 5.27 (s, 1 H), 4.95 (s, 1 H), 4.02 (m, 1 H), 3.52 (m, 2 H), 3.23 (m, 1 H), 2.26−2.10 (m, 2 H); ESIMS *m*/*z* (rel intensity) 479 ($MH^+ - HBr$, 54), 435 ($MH^+ - HBr - CO_2$, 100). Anal. ($C_{19}H_{16}BrIN_2O_5$) C, H, N.

***cis*-4-Carboxy-*N*-(3-chloropropyl)-3,4-dihydro-3-(4-methoxycarbonylphenyl)-7-nitro-1(2*H*)isoquinolone (31).** The general procedure provided the desired compound as a white solid (5.186 g, 65%). The product was highly insoluble in CD_3OD and required heating to obtain a suitable ^1H NMR sample. The experiment revealed a mixture of *cis*- and *trans*-isomers in a ratio of ∼40:60, respectively. However, it is unclear whether this was the result of sample preparation because the *cis*-isomer readily epimerizes to the *trans*-isomer upon heating. Further complicating matters, the product is readily soluble in DMSO-d_6, but decarboxylates to undermine the stereochemical information at the centers in question. As a result, the material was carried forward in the reaction scheme without characterization.

***cis*-4-Carboxy-*N*-(3-chloropropyl)-3-(4-cyanophenyl)-3,4-dihydro-7-nitro-1(2*H*)isoquinolone (32).** The general procedure provided the desired compound as a white solid (6.083 g, 61%). The product was highly insoluble in CD_3OD and required heating to obtain a suitable ^1H NMR sample. The experiment revealed a mixture of *cis*- and *trans*-isomers, with the major product being the *trans*-isomer. However, it is unclear whether this was the result of sample preparation, because the *cis*-isomer readily epimerizes to the *trans*-isomer upon heating. Further complicating matters, the product is readily soluble in DMSO-d_6 but decarboxylates to undermine the stereochemical information at the centers in question. As a result, the material was carried forward in the reaction scheme without characterization.

***cis*-3-[4-(Methoxycarbonyloxymethylphenyl)]-*N*-(3-bromopropyl)-4-carboxy-3,4-dihydro-1(2*H*)isoquinolone (33).** The general procedure provided the desired compound as an off-white solid (4.059 g, 59%): mp 138−142 °C. IR (KBr) 3438, 1747, 1633, 1522, 1351, and 1276 cm^{-1}; ^1H NMR (CD_3OD) δ 8.90 (d, J = 2.5 Hz, 1 H), 8.39 (dd, J = 8.7 and 2.54 Hz, 1 H), 7.97 (m, 1 H), 7.25 (d, J = 8.3 Hz, 2 H), 7.08 (d, J = 8.3 Hz, 2 H), 5.35 (d, J = 5.8 Hz, 1 H), 5.05 (s, 2 H), 4.96 (d, J = 6.4 Hz, 1 H), 4.02 (m, 1 H), 3.71 (s, 3 H), 3.52 (m, 2 H), 3.26 (m, 1 H), 2.28 (m, 1 H), 2.11 (m, 1 H); ESIMS *m*/*z* (rel intensity) 521/523 (MH^+, 32/31). Anal. ($C_{22}H_{21}BrN_2O_8$) C, H, N.

***cis*-4-Carboxy-*N*-(3-bromopropyl)-3,4-dihydro-3-[4-(toluene-4-sulfonyloxy)phenyl]-1(2*H*)isoquinolone (34).** The general procedure provided the desired compound as an off-white solid (4.678 g, 77%): mp 166−169 °C. The product was insoluble at room temperature in all common NMR solvents, but when heated in deuterated methanol, it epimerized and went into solution. The corresponding NMR spectrum of the *trans*-isomer is reported here: ^1H NMR (CD_3OD) δ 8.86 (d, J = 2.50 Hz, 1 H), 8.39 (dd, J = 8.65 and 2.55 Hz, 1 H), 7.95 (d, J = 8.65 Hz, 1 H), 7.56 (d, J = 8.36 Hz, 2 H), 7.34 (d, J = 8.01 Hz, 2 H), 7.02 (dd, J = 6.71 and 2.01 Hz, 2 H), 6.83 (dd, J = 6.74 and 2.07 Hz, 2 H), 5.29 (s, 1 H), 4.85 (s, 1 H), 3.96 (m, 1 H), 3.49 (m, 2 H), 3.29 (m, 1 H), 2.39 (s, 3 H), 2.24 (m, 1 H), 2.07 (m, 1 H).

6-(3-Bromopropyl)-9-ethoxy-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (35). The general procedure provided the desired compound as a red solid (0.441 g, 46%): mp 230−234 °C. IR (KBr) 1667, 1614, 1507, 1456, 1335, 1337, and 1229 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.16 (d, J = 2.4 Hz, 1 H), 8.80 (d, J = 9.0 Hz, 1 H), 8.48 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.79 (d, J = 8.4 Hz, 1 H), 7.26 (1 H buried under residual solvent), 6.93 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.70 (m, 2 H), 4.19 (q, J = 7.0 Hz, 2 H), 3.69 (t, J = 6.1 Hz, 2 H), 2.51 (m, 2 H), 1.50 (t, J = 7.0 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 457/459 (MH^+, 18/14). Anal. ($C_{21}H_{17}N_2O_5$) C, H, N.

6-(3-Bromopropyl)-9-ethyl-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (36). The general procedure provided the desired compound as an orange solid (0.338 g, 35%): mp 227−231 °C. IR (film) 1671, 1612, 1558, 1501, and 1337 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.18 (d, J = 2.4 Hz, 1 H), 8.86 (d, J = 9.0 Hz, 1 H), 8.50 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.81 (d, J = 7.8 Hz, 1 H), 7.57 (d, J = 1.5 Hz, 1 H), 7.38 (dd, J = 7.8 and 1.7 Hz, 1 H), 4.72 (m, 2 H), 3.69 (t, J = 6.1 Hz, 2 H), 2.78 (q, J = 7.6 Hz, 2 H), 2.52 (m, 2 H), 1.33 (t, J = 7.6 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 441/443 (MH^+, 13/11). Anal. ($C_{21}H_{17}BrN_2O_4 \cdot 0.5H_2O$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-9-methyl-7-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (37). The general procedure provided the desired compound as an orange-red solid (0.479 g, 50%): mp 224−227 °C (dec). IR (film) 1675, 1610, 1556, 1501, and 1331 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.19 (d, J = 2.4 Hz, 1 H), 8.86 (d, J = 9.0 Hz, 1 H), 8.51 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.79 (d, J = 7.8 Hz, 1 H), 7.54 (s, 1 H), 7.36 (d, J = 8.0 Hz, 1 H), 4.73 (m, 2 H), 3.69 (t, J = 6.0 Hz, 2 H), 2.52 (m, 2 H), 2.45 (s, 3 H); CIMS *m*/*z* (rel intensity) 427/429 (MH^+, 96/100). Anal. ($C_{20}H_{15}BrN_2O_4 \cdot 0.5H_2O$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-thiomethyl-11*H*-indeno[1,2-*c*]isoquinoline (38). The general procedure provided the desired compound as a red solid (0.160 g, 33%): mp 242−245 °C. IR (film) 1665, 1610, 1502, 1429, and 1336 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.17 (d, J = 2.4 Hz, 1 H), 8.82 (d, J = 9.0 Hz, 1 H), 8.50 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.80 (d, J = 8.1 Hz, 1 H), 7.54 (d, J = 1.9 Hz, 1 H), 7.31 (dd, J = 8.0 and 2.0 Hz, 1 H), 4.71 (m, 2 H), 3.69 (t, J = 6.1 Hz, 2 H), 2.59 (s, 3 H), 2.49 (m, 2 H); EIMS *m*/*z* (rel intensity) 458/460 (M^+, 35/37), 379 ($M^+ -$ Br, 25). Anal. ($C_{20}H_{15}BrN_2O_4S \cdot 1.0H_2O$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-phenyl-11*H*-indeno[1,2-*c*]isoquinoline (39). The general procedure provided the desired compound as an orange solid that was precipitated cleanly from EtOAc/hexanes (0.168 g, 35%): mp 253−256 °C. IR (film) 1674, 1612, 1498, 1435, and 1338 cm^{-1}; ^1H NMR

($CDCl_3$) δ 9.21 (d, J = 2.4 Hz, 1 H), 8.90 (d, J = 8.9 Hz, 1 H), 8.53 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.99 (d, J = 1.5 Hz, 1 H), 7.98 (d, J = 4.4 Hz, 1 H), 7.80 (dd, J = 8.1 and 1.8 Hz, 1 H), 7.70 (m, 2 H), 7.54 (m, 3 H), 4.78 (m, 2 H), 3.72 (t, J = 6.1 Hz, 2 H), 2.56 (m, 2 H); EIMS *m/z* (rel intensity) 488/500 (M^+, 7/7). Anal. ($C_{25}H_{17}BrN_2O_4 \cdot 0.5H_2O$) C, H, N.

6-(3-Chloropropyl)-5,6-dihydro-5,11-dioxo-3-nitro-11*H*-indeno[1,2-*c*]isoquinoline (40). The general procedure provided the desired compound as an orange solid (1.081 g, 57%): mp 260 °C (dec). IR (film) 1677, 1612, 1505, 1338, and 668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (d, J = 2.4 Hz, 1 H), 8.89 (d, J = 9.0 Hz, 1 H), 8.52 (dd, J = 8.9 and 2.5 Hz, 1 H), 7.90 (d, J = 6.8 Hz, 1 H), 7.75 (dd, J = 6.5 and 1.7 Hz, 1 H), 7.57−7.52 (m, 2 H), 4.77 (m, 2 H), 3.87 (t, J = 6.0 Hz, 2 H), 2.46 (m, 2 H); EIMS *m/z* (rel intensity) 368/370 (M^+, 100/34). Anal. ($C_{19}H_{13}ClN_2O_4 \cdot 0.25H_2O$) C, H, N.

6-(3-Bromopropyl)-9-fluoro-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (41). The general procedure provided the desired compound as a red-orange solid (0.593 g, 41%): mp 265−267 °C (dec). IR (KBr) 1702, 1683, 1611, 1501, 1335, and 1217 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.89 (d, J = 2.4 Hz, 1 H), 8.71 (d, J = 8.9 Hz, 1 H), 8.60 (dd, J = 9.0 and 2.5 Hz, 1 H), 8.00 (dd, J = 8.4 and 4.5 Hz, 1 H), 7.56 (dd, J = 7.0 and 2.5 Hz, 1 H), 7.50 (dt, J = 8.5 and 2.8 Hz, 1 H), 4.65 (t, J = 7.4 Hz, 2 H), 3.79 (t, J = 6.6 Hz, 2 H), 2.39 (t, J = 7.5 Hz, 2 H); ESIMS *m/z* (rel intensity) 431/433 (MH^+, 1.6/1.4). Anal. ($C_{19}H_{12}BrFN_2O_4$) C, H, N.

6-(3-Bromopropyl)-9-chloro-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (42). The general procedure provided the desired compound as a red-orange solid (0.642 g, 45%): mp 273−275 °C. IR (film) 1678, 1614, 1557, 1504, and 1336 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (d, J = 2.4 Hz, 1 H), 8.86 (d, J = 9.0 Hz, 1 H), 8.53 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.89 (d, J = 8.1 Hz, 1 H), 7.69 (d, J = 2.1 Hz, 1 H), 7.53 (dd, J = 8.1 and 2.1 Hz, 1 H), 4.72 (m, 2 H), 3.70 (t, J = 6.0 Hz, 2 H), 2.51 (m, 2 H); EIMS *m/z* (rel intensity) 446/448/450 (M^+, 17/22/6). Anal. ($C_{19}H_{12}BrClN_2O_4$) C, H, N.

9-Bromo-6-(3-chloropropyl)-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (43). The general procedure provided the desired compound as an orange solid (0.367 g, 38%): mp 246−249 °C. IR (film) 1669, 1613, 1500, 1429, and 1339 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.89 (d, J = 2.5 Hz, 1 H), 8.71 (d, J = 9.0 Hz, 1 H), 8.61 (dd, J = 8.9 and 2.5 Hz, 1 H), 7.85 (bs, 2 H), 7.77 (s, 1 H), 4.65 (t, J = 6.3 Hz, 2 H), 3.91 (t, J = 6.5 Hz, 2 H), 2.30 (m, 2 H); MALDIMS *m/z* (rel intensity) 447/449/451 (MH^+, 100/95/45). Anal. ($C_{19}H_{12}BrClN_2O_4$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-9-iodo-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (44). The general procedure provided the desired compound as an orange solid (0.130 g, 54%): mp 252−255 °C. IR (film) 1678, 1613, 1498, 1430, and 1347 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.19 (d, J = 2.4 Hz, 1 H), 8.85 (d, J = 9.0 Hz, 1 H), 8.53 (dd, J = 8.9 and 2.4 Hz, 1 H), 8.01 (d, J = 1.7 Hz, 1 H), 7.96 (dd, J = 8.0 and 1.7 Hz, 1 H), 7.68 (d, J = 8.0 Hz, 1 H), 4.71 (m, 2 H), 3.69 (t, J = 5.9 Hz, 2 H), 2.48 (m, 2 H); EIMS *m/z* (rel intensity) 538/540 (M^+, 47/46), 459 (M^+ − Br, 43). Anal. ($C_{19}H_{12}BrIN_2O_4$) C, H, N.

6-(3-Chloropropyl)-5,6-dihydro-9-methoxycarbonyl-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (45). The general procedure provided the desired compound as a red-orange solid (0.112 g, 12%) upon precipitation with diethyl ether: mp 249−251 °C. IR (KBr) 1722, 1679, 1614, 1504, 1436, and 1342 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.22 (d, J = 2.2 Hz, 1 H), 8.92 (d, J = 8.9 Hz, 1 H), 8.55 (dd, J = 9.0 and 2.4 Hz, 1 H), 8.32 (d, J = 1.4 Hz, 1 H), 8.29 (dd, J = 8.0 and 1.7 Hz, 1 H), 8.02 (d, J = 7.8 Hz, 1 H), 4.79 (m, 2 H), 3.98 (s, 3 H), 3.88 (t, J = 5.8 Hz, 2 H), 2.43 (m, 2 H); ESIMS *m/z* (rel intensity) 427/429 (MH^+, 25/10). Anal. ($C_{21}H_{15}ClN_2O_6 \cdot 1.1H_2O$) C, H, N.

6-(3-Chloropropyl)-9-cyano-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (46). The general procedure provided the desired compound as a yellow-orange solid (0.182 g, 10%) upon precipitation with diethyl ether: mp 272−276 °C. IR (KBr) 1709, 1676, 1613, 1557, 1505, 1339, and 1174 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, J = 2.2 Hz, 1 H), 8.74 (d, J = 8.9 Hz, 1 H), 8.63 (dd, J = 8.9 and 2.5 Hz, 1 H), 8.19 (dd, J = 8.0 and 1.6 Hz, 1 H), 8.10 (d, J = 8.0 Hz, 1 H), 8.07 (d, J = 1.4 Hz, 1 H), 4.68 (m, 2 H), 3.92 (t, J = 6.5 Hz, 2 H), 2.31 (m, 2 H). Anal. ($C_{20}H_{12}ClN_3O_4 \cdot 0.5H_2O$) C, H, N.

9-(Methoxycarbonyloxymethyl)-6-(3-bromopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (47). The general procedure provided the desired compound as an orange solid (0.038 g, 16%) that was precipitated cleanly from EtOAc-hexanes: mp 233−236 °C. IR (film) 1749, 1684, and 1502 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (d, J = 2.3 Hz, 1 H), 8.88 (d, J = 9.0 Hz, 1 H), 8.53 (dd, J = 8.9 and 2.4 Hz, 1 H), 7.94 (d, J = 7.9 Hz, 1 H), 7.72 (s, 1 H), 7.59 (d, J = 7.9 Hz, 1 H), 5.23 (s, 2 H), 4.74 (m, 2 H), 3.84 (s, 3 H), 3.70 (t, J = 6.1 Hz, 2 H), 2.52 (m, 2 H); ESIMS *m/z* (rel intensity) 501/503 (MH^+, 99/100). Anal. ($C_{22}H_{17}BrN_2O_7$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-[4-(toluene-4-sulfonyloxy)phenyl]-11*H*-indeno[1,2-*c*]isoquinoline (48). The general procedure provided the desired compound as an orange solid (0.099 g, 17%) that was precipitated cleanly from EtOAc-hexanes: mp 232−233 °C. IR (film) 1673, 1501, and 1353 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.19 (d, J = 2.4 Hz, 1 H), 8.83 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 8.9 and 2.4 Hz, 1 H), 7.92 (d, J = 8.2 Hz, 1 H), 7.80 (d, J = 8.4 Hz, 2 H), 7.39 (d, J = 8.3 Hz, 2 H), 7.33 (dd, J = 8.2 and 2.3 Hz, 1 H), 7.28 (d, J = 2.4 Hz, 1 H), 4.71 (m, 2 H), 3.69 (t, J = 6.0 Hz, 2 H), 2.48 (s, 3 H), 2.48 (m, 2 H); EIMS *m/z* (rel intensity) 582/584 (M^+, 1/1). Anal. ($C_{26}H_{19}BrN_2O_7S \cdot 0.5H_2O$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-methanesulfonyl-11*H*-indeno[1,2-*c*]isoquinoline (49). 3-Chloroperbenzoic acid (77%; 0.300 g, 1.339 mmol) was added to a solution of 6-(3-bromopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-thiomethyl-11*H*-indeno[1,2-*c*]isoquinoline (**38**; 0.123 g, 0.268 mmol) in $CHCl_3$ (40 mL). After 30 min, the reaction mixture was concentrated, diluted with diethyl ether (50 mL), and filtered. The solid was washed with diethyl ether (100 mL) to provide an orange solid (0.100 g, 76%): mp 240−242 °C (dec). IR (KBr) 1682, 1611, 1504, 1344, 1312, and 1145 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.93 (d, J = 2.5 Hz, 1 H), 8.79 (d, J = 8.9 Hz, 1 H), 8.65 (dd, J = 8.9 and 2.5 Hz, 1 H), 8.18 (s, 2 H), 8.07 (s, 1 H), 4.70 (m, 2 H), 3.80 (t, J = 6.8 Hz, 2 H), 3.38 (s, 3 H), 2.41 (m, 2 H); ESIMS *m/z* (rel intensity) 411 (MH^+ − HBr, 100). Anal. ($C_{20}H_{15}BrN_2O_6S$) C, H, N.

6-(3-Azidopropyl)-9-ethoxy-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (50). The general procedure provided the desired compound as a red solid (0.399 g, 99%): mp 170−173 °C. IR (KBr) 2095, 1666, 1616, 1509, 1337, and 1229 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.17 (d, J = 2.4 Hz, 1 H), 8.81 (d, J = 9.0 Hz, 1 H), 8.48 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.73 (d, J = 8.5 Hz, 1 H), 6.95 (dd, J = 8.3 and 2.4 Hz, 1 H), 4.63 (m, 2 H), 4.19 (q, J = 7.2 Hz, 2 H), 3.69 (t, J = 6.0 Hz, 2 H), 2.17 (m, 2 H), 1.50 (t, J = 7.0 Hz, 3 H); ESIMS *m/z* (rel intensity) 426 [(MLi^+, 13]. Anal. ($C_{21}H_{17}N_5O_5$) C, H, N.

6-(3-Azidopropyl)-9-ethyl-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (51). The general procedure provided the desired compound as a red-orange solid (0.583 g, 90%): mp 190−192 °C. IR (film) 2099, 1670, 1613, 1557, 1502, and 1336 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.19 (d, J = 2.1 Hz, 1 H), 8.86 (d, J = 9.0 Hz, 1 H), 8.50 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.74 (d, J = 8.0 Hz, 1 H), 7.58 (d, J = 1.5 Hz, 1 H), 7.39 (d, J = 7.8 Hz, 1 H), 4.66 (m, 2 H), 3.70 (t, J = 6.0 Hz, 2 H), 2.76 (q, J = 7.6 Hz, 2 H), 2.19 (m, 2 H), 1.33 (t, J = 7.6 Hz, 3 H); EIMS *m/z* (rel intensity) 403 (M^+, 100). Anal. ($C_{21}H_{17}N_5O_4$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-9-methyl-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (52). The general procedure provided the desired compound as a red-orange solid (0.388 g, 80%): mp 225−229 °C. IR (film) 2088, 1671, 1613, 1556, 1503, and 1335 cm^{-1}; ^1H NMR ($CHCl_3$) δ 9.19 (d, J = 2.3 Hz, 1 H), 8.56 (d, J = 8.9 Hz, 1 H), 8.50 (dd, J = 8.9 and 2.4 Hz, 1 H), 7.71 (d, J = 7.8 Hz, 1 H), 7.54 (s, 1 H), 7.37 (d, J = 7.8 Hz, 1 H), 4.66 (t, J = 7.4 Hz, 2 H), 3.69 (t, J = 6.0 Hz, 2 H), 2.45 (s, 3 H), 2.20 (m, 2 H). Anal. ($C_{20}H_{15}N_5O_4$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-thiomethyl-11*H*-indeno[1,2-*c*]isoquinoline (53). The general procedure provided the desired compound as a red solid (0.362 g, 45%): mp 210−213 °C (dec). IR (film) 2093, 1672, 1613, 1504, 1427, and 1336 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.83 (s, 1 H), 8.61 (d, J = 9.1 Hz, 1 H), 8.52 (d, J = 9.2 Hz, 1 H), 7.77 (d, J = 8.6 Hz, 1 H), 7.40−7.38 (m, 2 H), 4.54 (t, J = 6.7 Hz, 2 H), 3.66 (t, J = 6.6 Hz, 2 H), 2.59 (s, 3 H), 2.07 (m, 2 H); ESIMS m/z (rel intensity) 428 (MLi^+, 100). Anal. ($C_{20}H_{15}N_5O_4S\cdot 0.5CH_3OH$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-phenyl-11*H*-indeno[1,2-*c*]isoquinoline (54). Thionyl chloride (10 mL) was added to a solution *cis*-*N*-(3-bromopropyl)-4-carboxy-3,4-dihydro-3-(4-biphenyl)-1(2*H*)isoquinolone (**39**; 1.500 g, 2.945 mmol) in benzene (75 mL). The reaction mixture was heated at reflux for 30 min, allowed to cool to room temperature, and concentrated. The residue was diluted with nitrobenzene (50 mL), chilled in an ice bath, and aluminum chloride (0.785 g, 5.890 mmol) was added. The reaction mixture was removed from the bath and heated at reflux for 1 h. Ice water (100 mL) was added and the solution was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layer was washed with satd $NaHCO_3$ (3 × 100 mL), satd NaCl (100 mL), and dried over sodium sulfate. The solution was concentrated and sodium azide (0.191 g, 2.945 mmol) was added. The reaction mixture was diluted with DMSO (200 mL) and the mixture was heated at 100 °C for 1 h. The reaction mixture was diluted with $CHCl_3$ (500 mL), washed with water (3 × 100 mL) and satd NaCl (100 mL), and dried over sodium sulfate. The solution was concentrated to provide a crude solid that was purified by flash column chromatography (SiO_2), eluting with chloroform, and precipitated from $CHCl_3$−EtOAc-hexanes to afford a red solid (0.641 g, 48%): mp 219−221 °C. IR (film) 2092, 1666, 1612, 1500, 1435, and 1337 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (d, J = 2.4 Hz, 1 H), 8.89 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 8.9 and 2.4 Hz, 1 H), 7.98 (d, J = 1.8 Hz, 1 H), 7.91 (d, J = 8.0 Hz, 1 H), 7.81 (dd, J = 8.0 and 1.8 Hz, 1 H), 7.69 (m, 2 H), 7.54 (m, 3 H), 4.70 (m, 2 H), 3.73 (t, J = 6.0 Hz, 2 H), 2.23 (m, 2 H). Anal. ($C_{25}H_{17}N_5O_4\cdot 0.5H_2O$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (55). The general procedure provided the desired compound as an orange-red solid (0.179 g, 98%): mp 214 °C (dec). IR (film) 2090, 1673, 1614, 1560, 1505, and 1336 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (d, J = 2.4 Hz, 1 H), 8.89 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 9.0 and 2.0 Hz, 1 H), 7.85 (d, J = 7.4 Hz, 1 H), 7.74 (d, J = 6.9 Hz, 1 H), 7.60−7.50 (m, 2 H), 4.69 (t, J = 7.6 Hz, 2 H), 3.71 (t, J = 6.3 Hz, 2 H), 2.20 (m, 2 H); CIMS m/z (rel intensity) 376 (MH^+, 100). Anal. ($C_{19}H_{13}N_5O_4$) C, H, N.

6-(3-Azidopropyl)-9-fluoro-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (56). The general procedure provided the desired compound as an orange-red solid (0.0115 g, 98%): mp 195−200 °C (dec). IR (film) 2101, 1670, 1610, 1503, 1438, and 1338 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.19 (d, J = 2.4 Hz, 1 H), 8.84 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 8.9 and 2.42 Hz, 1 H), 7.88 (dd, J = 9.3 and 4.0 Hz, 1 H), 7.45 (dd, J = 6.7 and 2.4 Hz, 1 H), 7.23 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.65 (m, 2 H), 3.72 (t, J = 5.9 Hz, 2 H), 2.20 (m, 2 H); CIMS m/z (rel intensity) 394 (MH^+, 74). Anal. ($C_{19}H_{12}FN_5O_4\cdot 0.3H_2O$) C, H, N.

6-(3-Azidopropyl)-9-chloro-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (57). The general procedure provided the desired compound as a red-orange solid (0.446 g, 99%): mp 238−240 °C (dec). IR (film) 2091, 1674, 1615, 1556, and 1339 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.20 (d, J = 2.4 Hz, 1 H), 8.86 (d, J = 8.9 Hz, 1 H), 8.53 (dd, J = 8.9 and 2.4 Hz, 1 H), 7.82 (d, J = 8.1 Hz, 1 H), 7.68 (d, J = 2.1 Hz, 1 H), 7.56 (dd, J = 8.1 and 2.1 Hz, 1 H), 4.65 (m, 2 H), 3.72 (t, J = 6.0 Hz, 2 H), 2.17 (m, 2 H). Anal. ($C_{19}H_{12}ClN_5O_4$) C, H, N.

6-(3-Azidopropyl)-9-bromo-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (58). The general procedure provided the desired compound as an orange-red solid (0.243 g, 80%): mp 230 °C (dec). IR (film) 2082, 1671, 1613, 1503, and 1339 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.87 (d, J = 2.4 Hz, 1 H), 8.68 (d, J = 9.0 Hz, 1 H), 8.59 (dd, J = 8.9 and 2.5 Hz, 1 H), 7.85 (m, 2 H), 7.75 (d, J = 1.5 Hz, 1 H), 4.58 (t, J = 6.8 Hz, 2 H), 3.65 (t, J = 6.6 Hz, 2 H), 2.07 (pent, J = 7.2 Hz, 2 H). Anal. ($C_{19}H_{12}BrN_5O_4$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-9-iodo-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (59). The general procedure provided the desired compound as a red solid (0.292 g, 78%): mp 225−227 °C (dec). IR (film) 2099, 1670, 1613, 1503, 1430, and 1338 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.19 (d, J = 2.1 Hz, 1 H), 8.85 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 8.9 and 2.4 Hz, 1 H), 8.01 (d, J = 1.6 Hz, 1 H), 7.97 (dd, J = 8.0 and 1.7 Hz, 1 H), 7.61 (d, J = 8.1 Hz, 1 H), 4.63 (m, 2 H), 3.71 (t, J = 6.0 Hz, 2 H), 2.16 (m, 2 H); ESIMS m/z (rel intensity) 502 (MH^+, 100). Anal. ($C_{19}H_{12}IN_5O_4$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-9-methoxycarbonyl-3-nitro-5,-11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (60). The general procedure provided the desired compound as a red-orange solid (0.083 g, 73%): mp 233−235 °C. IR (KBr) 2092, 1722, 1675, 1615, 1504, and 1343 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.21 (d, J = 2.4 Hz, 1 H), 8.91 (d, J = 8.9 Hz, 1 H), 8.54 (dd, J = 8.9 and 2.4 Hz, 1 H), 8.30 (m, 2 H), 7.97 (d, J = 8.2 Hz, 1 H), 4.69 (m, 2 H), 3.99 (s, 3 H), 3.73 (t, J = 6.0 Hz, 2 H), 2.20 (m, 2 H); ESIMS m/z (rel intensity) 434 (MH^+, 23). Anal. ($C_{21}H_{15}N_5O_6\cdot 0.75H_2O$) C, H, N.

6-(3-Azidopropyl)-9-cyano-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (61). The general procedure provided the desired compound as a red solid (0.155 g, 84%) that was washed with EtOAc and Et_2O: mp 237 °C (dec). IR (KBr) 2088, 1675, 1615, 1504, and 1343 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.83 (s, 1 H), 8.66 (d, J = 8.8 Hz, 1 H), 8.58 (dd, J = 8.7 and 2.1 Hz, 1 H), 8.17 (d, J = 7.9 Hz, 1 H), 8.07 (d, J = 7.9 Hz, 1 H), 8.04 (s, 1 H), 4.59 (t, J = 7.4 Hz, 2 H), 3.66 (t, J = 6.5 Hz, 2 H), 2.07 (pent, J = 6.6 Hz, 2 H). Anal. ($C_{20}H_{12}N_6O_4\cdot 0.5H_2O$) C, H, N.

6-(3-Aminopropyl)-9-ethoxy-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (62). The general procedure provided the desired compound as a red solid (0.126 g, 85%): mp 262−265 °C (dec). IR (KBr) 1672, 1614, 1557, 1505, and 1336 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.85 (d, J = 2.4 Hz, 1 H), 8.65 (d, J = 9.0 Hz, 1 H), 8.55 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.88 (bs, 2 H), 7.81 (d, J = 8.7 Hz, 1 H), 7.15 (d, J = 2.5 Hz, 1 H), 7.06 (dd, J = 8.5 and 2.5 Hz, 1 H), 4.56 (m, 2 H), 4.22 (q, J = 7.0 Hz, 2 H), 3.01 (m, 2 H), 2.15 (m, 2 H), 1.40 (t, J = 6.9 Hz, 3 H); ESIMS m/z (rel intensity) 394 (MH^+, 30), 377 ($MH^+ - NH_3$, 100). Anal. ($C_{21}H_{20}ClN_3O_5\cdot 1.0H_2O$) C, H, N.

6-(3-Aminopropyl)-9-ethyl-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (63). The general procedure provided the desired compound as an orange solid (0.088 g, 85%): mp 268−270 °C (dec). IR (KBr) 3438, 1669, 1614, 1558, 1504, and 1338 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.87 (d, J = 2.4 Hz, 1 H), 8.71 (d, J = 9.0 Hz, 1 H), 8.58 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.93 (bs, 2 H), 7.82 (d, J = 7.9 Hz, 1 H), 7.52 (d, J = 1.5 Hz, 1 H), 7.48 (d, J = 7.8 Hz, 1 H), 4.60 (t, J = 7.0 Hz, 2 H), 3.01 (bs, 2 H), 2.73 (q, J = 7.6 Hz, 2 H), 2.16 (m, 2 H), 1.25 (t, J = 7.6 Hz, 3 H); ESIMS m/z (rel intensity) 378 (MH^+, 100). Anal. ($C_{21}H_{20}ClN_3O_4\cdot 0.75H_2O$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-9-methyl-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (64). The general procedure provided the desired compound as an orange solid (0.078 g, 76%): mp 275 °C (dec). IR (KBr) 3435, 1674, 1615, 1504, and 1338 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, J = 2.4 Hz, 1 H), 8.74 (d, J = 9.0 Hz, 1 H), 8.61 (dd, J = 9.0 and 2.4 Hz, 1 H), 7.80 (d, J = 7.3 Hz, 1 H), 7.44 (bs, 2 H), 7.52 (s, 1 H), 7.47 (d, J = 7.7 Hz, 1 H), 4.58 (m, 2 H), 3.03 (m, 2 H), 2.43 (s, 3 H), 2.11 (m, 2 H); ESIMS m/z (rel intensity) 364 (MH^+, 100). Anal. ($C_{20}H_{18}ClN_3O_4\cdot 0.5H_2O$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-5,11-dioxo-9-thiomethyl-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (65). The general procedure provided the desired compound as a red-brown solid (0.103 g, 93%): mp 267−269 °C (dec). IR (KBr) 3433, 1654, 1613, 1548, 1497, 1431, and 1336 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.88 (d, J = 2.4 Hz, 1 H), 8.70 (d, J = 9.0 Hz, 1 H), 8.59 (dd, J = 9.0 and 2.5 Hz, 1 H), 7.85 (bs, 2 H), 7.81 (d, J = 8.2 Hz, 1 H), 7.49 (d, J =

1.9 Hz, 1 H), 7.43 (dd, $J = 8.2$ and 1.8 Hz, 1 H), 4.59 (t, $J = 7.0$ Hz, 2 H), 3.01 (m, 2 H), 2.62 (s, 3 H), 2.17 (m, 2 H); ESIMS *m/z* (rel intensity) 396 (MH^+, 100). Anal. ($C_{20}H_{18}ClN_3O_4S$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-9-phenyl-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (66). The general procedure provided the desired compound as an orange solid (0.090 g, 88%): mp 260 °C (dec). IR (KBr) 3422, 1668, 1615, 1557, 1501, 1437, and 1336 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.88 (d, $J = 2.4$ Hz, 1 H), 8.75 (d, $J = 9.0$ Hz, 1 H), 8.61 (dd, $J = 9.0$ and 2.5 Hz, 1 H), 8.00 (m, 5 H), 7.82 (m, 2 H), 7.56 (m, 3 H), 4.65 (t, $J = 6.8$ Hz, 2 H), 3.04 (bs, 2 H), 2.20 (m, 2 H); ESIMS *m/z* (rel intensity) 426 (MH^+, 66), 409 ($MH^+ - NH_3$, 100). Anal. ($C_{25}H_{20}ClN_3O_4 \cdot 1.25H_2O$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline Hydrochloride (67). The general procedure provided the desired compound as an orange solid (0.133 g, 86%): mp 268 °C (dec). IR (film) 2843, 1614, 1557, and 1329 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, $J = 2.4$ Hz, 1 H), 8.76 (d, $J = 9.0$ Hz, 1 H), 8.61 (dd, $J = 9.0$ and 2.4 Hz, 1 H), 7.93−7.91 (m, 3 H), 7.70−7.61 (m, 3 H), 4.63 (m, 2 H), 3.05 (m, 2 H), 2.17 (m, 2 H); ESIMS *m/z* (rel intensity) 350 (MH^+, 100). Anal. ($C_{19}H_{16}ClN_3O_4$) C, H, N.

6-(3-Aminopropyl)-9-fluoro-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (68). The general procedure provided the desired compound as a red-orange solid (0.175 g, 100%): mp 248−251 °C (dec). IR (KBr) 2951, 1672, 1614, 1557, 1504, 1440, 1338, and 1219 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, $J = 2.4$ Hz, 1 H), 8.73 (d, $J = 9.0$ Hz, 1 H), 8.63 (dd, $J = 8.9$ and 2.4 Hz, 1 H), 7.99 (dd, $J = 8.6$ and 4.3 Hz, 1 H), 7.87 (bs, 2 H), 7.59 (dd, $J = 7.1$ and 2.6 Hz, 1 H), 7.50 (dt, $J = 8.6$ and 2.7 Hz, 1 H), 4.61 (t, $J = 6.8$ Hz, 2 H), 3.02 (m, 2 H), 2.15 (m, 2 H); negative ion ESIMS *m/z* (rel intensity) 366 [$(M - H^+)^-$, 100]. Anal. ($C_{19}H_{15}ClFN_3O_4 \cdot 1.25H_2O$) C, H, N.

6-(3-Aminopropyl)-9-chloro-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (69). The general procedure provided the desired compound as an orange solid (0.035 g, 34%): mp 265−268 °C (dec). IR (KBr) 3413, 1674, 1615, 1556, 1503, 1432, and 1339 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.89 (d, $J = 2.4$ Hz, 1 H), 8.71 (d, $J = 9.0$ Hz, 1 H), 8.62 (dd, $J = 9.0$ and 2.5 Hz, 1 H), 7.93 (m, 3 H), 7.71−7.69 (m, 2 H), 4.60 (t, $J = 7.1$ Hz, 2 H), 3.02 (m, 2 H), 2.15 (m, 2 H); ESIMS *m/z* (rel intensity) 384/386 (MH^+, 100/33). Anal. ($C_{19}H_{15}Cl_2N_3O_4 \cdot 0.75H_2O$) C, H, N.

6-(3-Aminopropyl)-9-bromo-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (70). The general procedure provided the desired compound as a red-orange solid (0.084 g, 82%): mp 275−277 °C (dec). IR (KBr) 3258, 3071, 2822, 1708, 1662, 1614, 1552, 1499, 1428, 1347, 1200, and 1088 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, $J = 2.4$ Hz, 1 H), 8.73 (d, $J = 8.9$ Hz, 1 H), 8.63 (dd, $J = 8.9$ and 2.4 Hz, 1 H), 7.84−7.80 (m, 5 H), 4.60 (t, $J = 7.3$ Hz, 2 H), 3.02 (m, 2 H), 2.14 (m, 2 H); ESIMS *m/z* (rel intensity) 428/430 (MH^+, 100/96). Anal. ($C_{19}H_{15}BrClN_3O_4$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-9-iodo-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (71). The general procedure provided the desired compound as an orange solid (0.086 g, 80%): mp 270 °C (dec). IR (KBr) 3428, 1661, 1614, 1553, 1499, 1427, and 1347 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, $J = 2.4$ Hz, 1 H), 8.73 (d, $J = 9.0$ Hz, 1 H), 8.63 (dd, $J = 9.0$ and 2.5 Hz, 1 H), 8.04 (d, $J = 8.0$ and 1.6 Hz, 1 H), 7.93 (d, $J = 1.7$ Hz, 1 H), 7.80 (bs, 2 H), 7.69 (d, $J = 8.0$ Hz, 1 H), 4.59 (t, $J = 7.0$ Hz, 2 H), 3.01 (m, 2 H), 2.13 (m, 2 H); negative ion ESIMS *m/z* (rel intensity) 474 [$(M - H^+)^-$, 100]. Anal. ($C_{19}H_{15}ClIN_3O_4$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-9-methoxycarbonyl-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (72). The general procedure provided the desired compound as an orange solid (0.092 g, 100%): mp 257−262 °C (dec). IR (KBr) 3434, 2951, 1726, 1674, 1614, 1504, 1434, and 1342 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.83 (d, $J = 2.1$ Hz, 1 H), 8.67 (d, $J = 9.0$ Hz, 1 H), 8.57 (dd, $J = 8.8$ and 2.2 Hz, 1 H), 8.17 (d, $J = 8.2$ Hz, 1 H), 8.06 (m, 4 H), 7.87 (s, 1 H), 4.59 (m, 2 H), 3.91 (s, 3 H), 3.06 (m, 2 H), 2.15 (m, 2 H); ESIMS *m/z* (rel intensity) 408 (MH^+, 35), 391 ($MH^+ - NH_3$, 100). Anal. ($C_{21}H_{18}N_3O_6 \cdot 1.0H_2O$) C, H, N.

6-(3-Aminopropyl)-9-cyano-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (73). The general procedure provided the desired compound as an orange solid (0.086 g, 93%): mp 275−280 °C (dec). IR (KBr) 1706, 1682, 1615, 1558, 1501, and 1347 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (d, $J = 2.4$ Hz, 1 H), 8.75 (dd, $J = 9.0$ and 3.2 Hz, 1 H), 8.64−8.58 (m, 1 H), 8.17−8.07 (m, 3 H), 8.06−7.95 (m, 3 H), 4.60 (m, 2 H), 3.02 (bs, 2 H), 2.13 (bs, 2 H); negative ion ESIMS *m/z* (rel intensity) 373 [$(M - H^+)^-$, 72]. Anal. ($C_{20}H_{15}ClN_4O_4 \cdot 2.0H_2O$) C, H, N.

4-(Acetamido)benzylidene-(3-bromo-1-propylamine) (75). The general procedure provided the desired compound as an off-white solid (5.149 g, 99%): mp 118−121 °C. IR (film) 3305, 1670, 1643, 1597, 1536, and 1317 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.28 (s, 1 H), 7.71 (d, $J = 8.6$ Hz, 2 H), 7.59 (d, $J = 8.4$ Hz, 2 H), 7.40 (bs, 1 H), 3.76 (dt, $J = 6.3$ and 1.2 Hz, 2 H), 3.51 (t, $J = 6.5$ Hz, 2 H), 2.30 (pent, $J = 6.4$ Hz, 2 H), 2.20 (s, 3 H); ESIMS *m/z* (rel intensity) 283/285 (MH^+, 100/99). Anal. ($C_{12}H_{15}BrN_2O$) C, H, N.

4-Methoxybenzylidene-(3-chloro-1-propylamine) (76).[44] The general procedure provided the desired compound as a yellow viscous oil (4.664 g, 100%). ^1H NMR ($CDCl_3$) δ 8.25 (s, 1 H), 7.68 (dd, $J = 6.0$ and 2.0 Hz, 2 H), 6.94 (dd, $J = 6.8$ and 2.0 Hz, 2 H), 3.84 (s, 3 H), 3.75 (dt, $J = 6.4$ and 1.2 Hz, 2 H), 3.65 (t, $J = 6.4$ Hz, 2 H), 2.20 (pent, $J = 6.38$ Hz, 2 H).

4-(Nitro)benzylidene-(3-bromo-1-propylamine) (77). The general procedure provided the desired compound as a yellow viscous oil (10.700 g, 99%). IR (KBr) 1644, 1602, 1521, and 1346 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.42 (s, 1 H), 8.28−8.25 (m, 2 H), 7.91−7.88 (m, 2 H), 3.84 (dt, $J = 6.3$ and 1.3 Hz, 2 H), 3.53 (t, $J = 6.4$ Hz, 2 H), 2.33 (pent, $J = 6.4$ Hz, 2 H); ESIMS *m/z* (rel intensity) 191 ($MH^+ - HBr$, 100). Anal. ($C_{10}H_{11}BrN_2O_2$) C, H, N.

***cis*-3-(4-Acetamido)-*N*-(3-bromopropyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-1(2*H*)isoquinolone (78).** The general procedure provided the desired compound as an off-white solid (4.548 g, 73%): mp 179 °C. IR (KBr) 3384, 1739, 1690, 1598, 1528, 1285, 1216, and 1183 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.91 (s, 1 H), 7.53 (s, 1 H), 7.40 (d, $J = 8.6$ Hz, 2 H), 7.12 (s, 1 H), 6.95 (d, $J = 8.6$ Hz, 2 H), 5.04 (d, $J = 6.3$ Hz, 1 H), 4.70 (d, $J = 6.1$ Hz, 1 H), 3.89 (m, 2 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.56 (m, 2 H), 2.96 (m, 1 H), 2.13 (m, 1 H), 1.99 (s, 3 H); negative ion ESIMS *m/z* (rel intensity) 503/505 [$(M - H^+)^-$, 44/43]. Anal. ($C_{23}H_{25}BrN_2O_6 \cdot 0.5H_2O$) C, H, N.

***cis*-4-Carboxy-*N*-(3-chloropropyl)-3,4-dihydro-6,7-dimethoxy-3-(4-methoxyphenyl)-1(2*H*)isoquinolone (79).** The general procedure provided the desired compound as an off-white solid (5.659 g, 69%): mp 200−202 °C. IR (KBr) 3073, 1745, 1616, 1595, 1572, 1516, 1486, 1297, 1264, 1189, and 1178 cm^{-1}; ^1H NMR (CD_3OD) δ 7.62 (s, 1 H), 7.23 (s, 1 H), 7.00 (d, $J = 8.8$ Hz, 2 H), 6.76 (d, $J = 8.8$ Hz, 2 H), 5.10 (d, $J = 6.3$ Hz, 1 H), 4.68 (d, $J = 5.8$ Hz, 1 H), 3.97 (m, 1 H), 3.59 (s, 3 H), 3.80 (s, 3 H), 3.71 (s, 3 H), 3.61 (m, 2 H), 3.18 (m, 1 H), 2.12−1.99 (m, 2 H); ESIMS *m/z* (rel intensity) 434/436 (MH^+, 90/28), 398 ($MH^+ - Cl$, 100). Anal. ($C_{22}H_{24}ClNO_6$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3-(4-ethyl)-3,4-dihydro-6,7-dimethoxy-1(2*H*)isoquinolone (80).** The general procedure provided the desired compound as a white solid (2.859 g, 43%): mp 207−211 °C. IR (KBr) 3434, 2967, 1735, 1620, 1595, 1576, 1294, and 1175 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.53 (s, 1 H), 7.14 (s, 1 H), 7.07 (d, $J = 8.1$ Hz, 2 H), 6.95 (d, $J = 8.2$ Hz, 2 H), 5.07 (d, $J = 6.3$ Hz, 1 H), 4.74 (d, $J = 6.2$ Hz, 1 H), 3.91 (m, 1 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.57 (m, 2 H), 2.91 (m, 1 H), 2.55 (q, $J = 7.6$ Hz, 2 H), 2.12−1.99 (m, 2 H), 1.13 (t, $J = 7.6$ Hz, 3 H); ESIMS *m/z* (rel intensity) 396 ($MH^+ - HBr$, 100). Anal. ($C_{23}H_{26}BrNO_5 \cdot 0.2CHCl_3$) C, H, N.

***cis*-4-Carboxy-*N*-(3-chloropropyl)-3,4-dihydro-6,7-dimethoxy-3-phenyl-1(2*H*)isoquinolone (81).** The general procedure provided the desired compound as an off-white solid (2.026 g, 49%). IR (KBr) 2940, 1744, 1615, 1594, 1572, 1488, 1455, 1292, 1186, and 1170 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.54 (s, 1 H), 7.24 (m, 3 H), 7.13 (s, 1 H), 7.05 (m, 2 H), 5.10 (d, $J = 6.3$ Hz, 1 H), 4.74 (d, J

= 6.2 Hz, 1 H), 3.94 (m, 1 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.69 (m, 2 H), 2.98 (m, 1 H), 2.05−1.88 (m, 2 H); CIMS *m*/*z* (rel intensity) 404/406 (MH^+, 38/9), 360/362 ($MH^+ - CO_2$, 100/24). Anal. ($C_{21}H_{22}ClNO_5$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3-(4-fluoro)-3,4-dihydro-6,7-dimethoxy-1(2*H*)isoquinolone (82).** The general procedure provided the desired compound as a white solid (2.420 g, 32%): mp 198−201 °C. IR (KBr) 3455, 2972, 1742, 1593, 1572, 1510, 1296, 1231, 1184, 1170 and 1106 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.53 (s, 1 H), 7.11−7.06 (m, 5 H), 5.13 (d, J = 6.2 Hz, 1 H), 4.75 (d, J = 6.2 Hz, 1 H), 3.90−3.75 (m, 7 H), 3.56−3.50 (m, 2 H), 2.97−2.91 (m, 1 H), 2.14−1.96 (m, 2 H); negative ion ESIMS *m*/*z* (rel intensity) 464/466 [$(M - H^+)^-$, 10/10]. Anal. ($C_{21}H_{21}BrFNO_5$) C, H, N.

***cis*-*N*-(3-Chloropropyl)-4-carboxy-6,7-dimethoxy-3-(4-methoxycarbonylphenyl)-3,4-dihydro-1(2*H*)isoquinolone (83).** The general procedure provided the desired compound as a white solid (7.0 g, 48%): mp 210−211 °C. IR (film) 2932, 1736, 1718, 1622, 1596, and 1574 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.82−7.79 (m, 2 H), 7.53 (s, 1 H), 7.18−7.16 (m, 2 H), 7.07 (s, 1 H). 5.19 (d, J = 6.0 Hz, 1 H), 4.76 (d, J = 6.0, 1 H), 3.91−3.80 (m, 7 H), 3.72 (s, 3 H), 3.66−3.59 (m, 2 H), 2.97−2.91 (m, 1 H), 2.08−1.81 (m, 2 H); ESIMS *m*/*z* 426 (MH^+ − HCl, 100). Anal. ($C_{23}H_{24}ClNO_7$) C, H, N.

***cis*-4-Carboxy-2-(3-chloropropyl)-3-(4-cyanophenyl)-3,4-dihydro-6,7-dimethoxy-1(2*H*)isoquinolone (84).** The general procedure provided the desired compound as a white solid (5.0 g, 35%): mp 220−222 °C. IR (film) 2966, 2227, 1736, 1625, 1597, and 1579 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.74−7.72 (m, 2 H), 7.53 (s, 1 H), 7.22−7.19 (m, 2 H), 7.05 (s, 1 H), 5.22 (d, J = 6.0 Hz, 1 H), 4.80 (d, J = 6.0 Hz, 1 H), 3.91−3.61 (m, 9 H), 2.94−2.89 (m, 1 H), 2.01−1.90 (m, 2 H); ESIMS *m*/*z* 393 (MH^+ − HCl, 100). Anal. ($C_{22}H_{21}ClN_2O_5$) C, H, N.

***cis*-*N*-(3-Bromopropyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-[4-(nitro)phenyl]-1(2*H*)isoquinolone (85).** The general procedure provided the desired compound as an off-white solid (4.438 g, 35%): mp 203−207 °C. IR (KBr) 3449, 2940, 1736, 1623, 1598, 1578, 1520, 1488, 1347, 1293, and 1183 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.14 (d, J = 8.6 Hz, 2 H), 7.55 (s, 1 H), 7.32 (d, J = 8.7 Hz, 2 H), 7.06 (s, 1 H), 5.31 (d, J = 6.3 Hz, 1 H), 4.86 (d, J = 6.1 Hz, 1 H), 3.93 (m, 1 H), 3.88 (s, 3 H), 3.74 (s, 3 H), 3.54 (m, 2 H), 2.96 (m, 1 H), 2.15−1.97 (m, 2 H); ESIMS *m*/*z* (rel intensity) 493/495 (MH^+, 3/3), 413 (MH^+ − HBr, 100). Anal. ($C_{21}H_{21}BrN_2O_7 \cdot 1.0H_2O$) C, H, N.

9-Acetamido-6-(3-bromopropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (86). The general procedure provided the desired compound as crude purple solid that was precipitated cleanly from EtOAc-hexanes (0.070 g, 15%): mp 249−251 °C (dec). IR (film) 3306, 1649, 1548, 1481, 1421, and 1263 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07 (s, 1 H), 7.90 (d, J = 9.5 Hz, 1 H), 7.67 (m, 2 H), 7.47 (d, J = 2.1 Hz, 1 H), 7.33 (s, 1 H), 4.66 (m, 2 H), 4.06 (s, 3 H), 3.99 (s, 3 H), 3.66 (t, J = 6.3 Hz, 2 H), 2.48 (m, 2 H), 2.24 (s, 3 H); ESIMS *m*/*z* (rel intensity) 485/487 (MH^+, 34/45), 405 (MH^+-HBr, 100). Anal. ($C_{23}H_{21}BrN_2O_5 \cdot 0.5H_2O$) C, H, N.

6-(3-Chloropropyl)-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (87). The general procedure provided the desired compound as a purple-red solid that was precipitated from EtOAc-hexanes (0.597 g, 63%): mp 244−245 °C. IR (film) 1650, 1553, 1475, 1424, 1265, and 1023 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H), 7.66 (s, 1 H), 7.63 (dd, J = 7.7 and 2.8 Hz, 1 H), 7.19 (d, J = 2.5 Hz, 1 H), 6.85 (dd, J = 8.3 and 2.7 Hz, 1 H), 4.67 (m, 2 H), 4.06 (s, 3 H), 3.99 (s, 3 H), 3.89 (s, 3 H), 3.83 (t, J = 6.0 Hz, 2 H), 2.41 (m, 2 H); ESIMS *m*/*z* (rel intensity) 414/416 (MH^+, 100/28). Anal. ($C_{22}H_{20}ClNO_5$) C, H, N.

6-(3-Bromopropyl)-9-ethyl-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (88). The general procedure provided the desired compound as a red solid that was precipitated from Et_2O (0.234 g, 49%): mp 219−221 °C. IR (film) 1646, 1481, 1468, and 1266 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.11 (s, 1 H), 7.67 (s, 1 H), 7.61 (m, 1 H), 7.44 (d, J = 1.6 Hz, 1 H), 7.25 (m, 1 H), 4.67 (m, 2 H), 4.07 (s, 3 H), 3.99 (s, 3 H), 3.66 (t, J = 6.3 Hz, 2 H), 2.73 (q, J = 7.6 Hz, 2 H), 2.49 (m, 2 H), 1.30 (t, J = 7.6 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 456/458 (MH^+, 41/38).

6-(3-Chloropropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (89). The general procedure provided the desired compound as a red solid (349 mg, 49%): mp 230−233 °C. IR (film) 1652, 1564, 1500, 1464, 1444, 1253, and 1037 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14 (s, 1 H), 7.74−7.58 (m, 3 H), 7.45−7.35 (m, 2 H), 6.10 (s, 2 H), 4.70 (m, 2 H), 3.83 (t, J = 6.1 Hz, 2 H), 2.42 (m, 2 H); CIMS *m*/*z* (rel intensity) 384/386 (MH^+, 100/30). Anal. ($C_{21}H_{18}ClNO_4$) C, H, N.

6-(3-Bromopropyl)-9-fluoro-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (90). The general procedure provided the desired compound as a red solid that was precipitated from EtOAc-hexanes (0.060 g, 13%): mp 251−253 °C (dec). IR (film) 2937, 1644, 1466, 1432, 1270, 1249, and 1213 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.04 (s, 1 H), 7.71 (dd, J = 4.2 and 8.4 Hz, 1 H), 7.64 (s, 2 H), 7.29 (dd, J = 2.6 and 6.9 Hz, 1 H), 7.26 (s, 1 H), 7.11 (dt J = 2.5 and 8.4, 1 H), 4.64−4.59 (m, 2 H), 4.05 (s, 3 H), 3.99 (s, 3 H), 3.67 (t, J = 6.2 Hz, 2 H), 2.48 (m, 2 H); ESIMS *m*/*z* (rel intensity) 446/448 (MH^+, 100/91). Anal. ($C_{21}H_{17}BrNO_4$) C, H, N.

6-(3-Chloropropyl)-5,6-dihydro-2,3-dimethoxy-9-methoxycarbonyl-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (91). The general procedure provided the desired compound as a purple solid (3.5 g, 52%): mp 253−254 °C. IR (film) 3014, 2950, 1727, 1697, 1648, and 1479 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.16−8.08 (m, 3 H), 7.78 (d, J = 7.7 Hz, 1 H), 7.65 (s, 1 H), 4.70−4.64 (m, 2 H), 4.06 (s, 3 H), 3.99 (s, 3 H), 3.95 (s, 3 H), 3.82 (t, J = 6.0 Hz, 2 H), 2.41−2.35 (m, 2 H); EIMS *m*/*z* (rel intensity) 441 (MH^+, 100). Anal. ($C_{23}H_{20}ClNO_6$) C, H, N.

6-(3-Chloropropyl)-9-cyano-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinolone (92). The general procedure provided the desired compound as a purple solid (0.17 g, 18%): mp 246−249 °C (dec). IR (film) 3585, 3077, 2970, 2229, 1695, 1651, 1609, 1480, 1434, and 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H), 7.88−7.85 (m, 1 H), 7.79−7.76 (m, 2 H), 7.69 (s, 1 H), 4.69−4.64 (m, 2 H), 4.07 (s, 3 H), 4.00 (s, 3 H), 3.84−3.80 (m, 2 H), 2.42−2.33 (m, 2 H); ESIMS *m*/*z* (rel intensity) 380 (MH^+, 100). Anal. ($C_{22}H_{17}ClN_2O_4 \cdot 0.5H_2O$) C, H, N.

6-(3-Bromopropyl)-5,6-dihydro-2,3-dimethoxy-9-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (93). The general procedure provided the desired compound as a purple solid that was precipitated cleanly from Et_2O (0.479 g, 50%): mp 268−272 °C. IR (film) 1655, 1605, 1528, 1508, 1480, 1434, 1338, and 1262 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.39 (dd, J = 8.3 and 2.3 Hz, 1 H), 8.32 (d, J = 2.2 Hz, 1 H), 8.12 (s, 1 H), 7.97 (t, J = 8.0 Hz, 1 H), 7.70 (s, 1 H), 4.73 (m, 2 H), 4.09 (s, 3 H), 4.02 (s, 3 H), 3.86 (t, J = 5.9 Hz, 1 H), 3.70 (t, J = 6.0 Hz, 1 H), 2.50 (m, 2 H); ESIMS *m*/*z* (rel intensity) 473/475 (MH^+, 55/52).

9-Acetamido-6-(3-azidopropyl)-5,6-dihydro-2,3-dimethoxy-5,-11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (94). The general procedure provided the desired compound as a red solid (0.460 g, 57%): mp 178−179 °C (dec). IR (film) 3307, 2097, 1640, 1481, and 1264 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.04 (s, 1 H), 7.87 (d, J = 7.3 Hz, 1 H), 7.64 (s, 1 H), 7.59 (d, J = 8.2 Hz, 1 H), 7.46 (d, J = 2.2 Hz, 1 H), 7.38 (s, 1 H), 4.58 (t, J = 7.3 Hz, 2 H), 4.05 (s, 3 H), 3.99 (s, 3 H), 3.65 (t, J = 6.2 Hz, 2 H), 2.24 (s, 3 H), 2.17 (m, 2 H); negative ion ESIMS *m*/*z* (rel intensity) 446 [$(M - H^+)^-$, 100]. Anal. ($C_{23}H_{21}N_5O_5 \cdot 0.5H_2O$) C, H, N.

9-Amino-6-(3-azidopropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (95). 9-Acetamido-6-(3-azidopropyl)-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**94**; 0.050 g, 0.108 mmol) was diluted with THF (10 mL) and 6 M aq HCl (10 mL). The reaction mixture was heated at reflux for 16 h, allowed to cool to room temperature, and extracted with $CHCl_3$ (3 × 30 mL). The combined organic layer was washed with satd $NaHCO_3$ (3 × 30 mL), dried over sodium sulfate, and concentrated to provide a black solid (0.038 g, 84%): mp 238 °C (dec). IR (film) 3352, 2098, 1644, 1550, 1477, and 1264 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06 (s, 1 H), 7.63 (s, 1 H), 7.39 (d, J = Hz, 1

H), 6.93 (s, 1 H), 6.59 (d, J = Hz, 1 H), 4.56 (m, 2 H), 4.05 (s, 3 H), 3.98 (s, 3 H), 3.60 (m, 2 H), 2.17 (m, 2 H); ESIMS *m*/*z* (rel intensity) 406 (MH^+, 100). Anal. ($C_{21}H_{19}N_5O_4$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-2,3-dimethoxy-9-dimethylamino-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (96). 9-Amino-6-(3-azidopropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (**95**; 0.141 g, 0.336 mmol) was dissolved in HOAc (100 mL). Formaldehyde (15 mL, 37% aq) was added, followed by $NaBH_3CN$ (0.106 g, 1.681 mmol) and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was concentrated, dissolved in $CHCl_3$ (200 mL), washed with satd $NaHCO_3$ (3 × 50 mL) and satd NaCl (50 mL), and dried over sodium sulfate. The solution was concentrated and purified by flash column chromatography (SiO_2), eluting with chloroform, to provide a blue-green solid (0.141 g, 94%): mp 218−220 °C. IR (film) 2097, 1551, 1505, 1394, and 1263 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06 (s, 1 H), 7.62 (s, 1 H), 7.42 (d, J = 8.5 Hz, 1 H), 7.04 (d, J = 2.6 Hz, 1 H), 6.49 (dd, J = 8.3 and 2.6 Hz, 1 H), 4.55 (t, J = 7.1 Hz, 2 H), 4.06 (s, 3 H), 3.98 (s, 3 H), 3.63 (t, J = 6.2 Hz, 2 H), 3.09 (s, 6 H), 2.16 (m, 2 H); ESIMS *m*/*z* (rel intensity) 434 (MH^+, 61). Anal. ($C_{23}H_{23}N_5O_4 \cdot 0.5H_2O$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (97). The general procedure provided the desired compound as a purple-red solid (0.237 g, 93%): mp 222−223 °C. IR (film) 2098, 1649, 1482, 1266, and 1024 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (s, 1 H), 7.65 (s, 1 H), 7.55 (d, J = 8.4 Hz, 1 H), 7.18 (d, J = 2.6 Hz, 1 H), 6.85 (dd, J = 8.4 and 2.6 Hz, 1 H), 4.59 (t, J = 7.7 Hz, 2 H), 4.06 (s, 3 H), 3.99 (s, 3 H), 3.89 (s, 3 H), 3.65 (t, J = 6.2 Hz, 2 H), 2.16 (m, 2 H); ESIMS *m*/*z* (rel intensity) 421 (MH^+, 31). Anal. ($C_{20}H_{20}N_4O_5$) C, H, N.

6-(3-Azidopropyl)-9-ethyl-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (98). The general procedure provided the desired compound as a red solid that was precipitated from Et_2O-hexanes (0.715 g, 80%): mp 170−172 °C (dec). IR (film) 2098, 1650, 1613, 1554, 1481, 1469, and 1266 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 (s, 1 H), 7.68 (s, 1 H), 7.55 (d, J = 7.8 Hz, 1 H), 7.45 (d, J = 1.5 Hz, 1 H), 7.27 (m, 1 H), 4.62 (m, 2 H), 4.07 (s, 3 H), 4.00 (s, 3 H), 3.64 (t, J = 6.2 Hz, 2 H), 2.73 (q, J = 7.5 Hz, 2 H), 2.17 (m, 2 H), 1.30 (t, J = 7.6 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 419 (MH^+, 100). Anal. ($C_{23}H_{22}N_4O_4 \cdot 0.75H_2O$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (99). The general procedure provided the desired compound as a red solid (0.420 g, 98%): mp 200−202 °C (dec). IR (film) 2099, 1652, 1513, 1478, 1429, and 1266 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14 (s, 1 H), 7.69 (s, 1 H), 7.66−7.59 (m, 2 H), 7.46−7.32 (m, 2 H), 4.64 (m, 2 H), 4.06 (s, 3 H), 4.00 (s, 3 H), 3.66 (t, J = 6.2 Hz, 2 H), 2.19 (m, 2 H); ESIMS *m*/*z* (rel intensity) 391 (MH^+, 100). Anal. ($C_{21}H_{18}N_4O_4$) C, H, N.

6-(3-Azidopropyl)-9-fluoro-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (100). The general procedure provided the desired compound as a red solid (0.074 g, 62%) that was precipitated from Et_2O-hexanes: mp 214−218 °C (dec). IR (film) 2097, 1646, 1479, and 1267 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H), 7.68 (s, 1 H), 7.66 (dd, J = 4.2 and 8.5 Hz, 1 H), 7.32 (dd, J = 2.6 and 6.8 Hz, 2 H), 7.14 (dt, 2.7 and 8.4 1H), 4.61−4.56 (m, 2 H), 4.07 (s, 3 H), 4.00 (s, 3 H), 3.67 (t, J = 6.2, 2 H), 2.18−2.11 (m, 2 H); ESIMS *m*/*z* (rel intensity) 409 (MH^+, 100). Anal. ($C_{21}H_{17}FN_4O_4$) C, H, N.

6-(3-Azidopropyl)-5,11-dihydro-2,3-dimethoxy-9-methoxycarbonyl-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (101). The general procedure provided the desired compound as a purple solid (208 mg, 68%): mp 252−254 °C. IR (film) 2950, 2096, 1717, 1650, and 1480 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.16−8.13 (m, 1 H), 8.10 (s, 1 H), 8.07 (s, 1 H), 7.72 (d, J = 7.7 Hz, 1 H), 7.65 (s, 1 H), 4.59 (t, J = 7.7 Hz, 2 H), 4.06 (s, 3 H), 3.99 (s, 3 H), 3.95 (s, 3 H), 3.65 (t, J = 6.0 Hz, 2 H), 2.18−2.11 (m, 2 H); EIMS *m*/*z* (rel intensity) 448 (MH^+, 100). Anal. ($C_{23}H_{20}N_4O_6$) C, H, N.

6-(3-Azidopropyl)-9-cyano-2,3-dimethoxy-5,6-dioxo-5,11-dihydro-11*H*-indeno[1,2-*c*]isoquinoline (102). The general procedure provided the desired compound as a red solid (188 mg, 62%): mp 238−240 °C. IR (film) 2967, 2229, 1695, 1655, 1609 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (s, 1 H), 7.78−7.80 (m, 2 H), 7.76 (s, 1 H), 7.68 (s, 1 H), 4.60−4.55 (m, 2 H), 4.07 (s, 3 H), 4.00 (s, 3 H), 3.69−3.65 (m, 2 H), 2.14−2.09 (m, 2 H); ESIMS *m*/*z* (rel intensity) 422 (MLi^+, 42). Anal. ($C_{22}H_{17}N_5O_4 \cdot 0.25H_2O$) C, H, N.

6-(3-Azidopropyl)-5,6-dihydro-2,3-dimethoxy-9-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (103). The general procedure provided the desired compound as a green solid (0.187 g, 81%) that was precipitated from Et_2O: mp 235−238 °C. IR (film) 2121, 1662, 1605, 1523, 1478, 1432, 1340, and 1276 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.41 (dd, J = 8.3 and 2.3 Hz, 1 H), 8.34 (d, J = 2.2 Hz, 1 H), 8.13 (s, 1 H), 7.91 (d, J = 8.3 Hz, 1 H), 7.71 (s, 1 H), 4.65 (t, J = 8.0 Hz, 2 H), 4.09 (s, 3 H), 4.02 (s, 3 H), 3.71 (t, J = 6.0 Hz, 2 H), 2.17 (m, 2 H); ESIMS *m*/*z* (rel intensity) 436 (MH^+, 100). Anal. ($C_{21}H_{17}N_5O_6 \cdot 0.5H_2O$) C, H, N.

9-Amino-6-(3-aminopropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Dihydrochloride (104). The general procedure provided the desired compound as a black solid (0.100 g, 90%): mp 245−250 °C (dec). IR (KBr) 3432, 2912, 1636, 1552, 1514, 1479, and 1269 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.93 (s, 1 H), 7.90 (bs, 2 H), 7.49 (s, 1 H), 7.40 (d, J = 8.2 Hz, 1 H), 6.87 (d, J = 2.2 Hz, 1 H), 6.58 (dd, J = 8.1 and 2.2 Hz, 1 H), 4.45 (m, 2 H), 3.91 (s, 3 H), 3.85 (s, 3 H), 2.92 (M, 2 H), 2.09 (m, 2 H); ESIMS *m*/*z* (rel intensity) 380 (MH^+, 65). Anal. ($C_{21}H_{23}Cl_2N_3O_4 \cdot 2H_2O$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-2,3-dimethoxy-9-dimethylamino-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Dihydrochloride (105). The general procedure provided the desired compound as a black solid (0.087 g, 88%): mp 200−203 °C (dec). IR (KBr) 3418, 2939, 2627, 1705, 1651, 1552, 1510, 1480, 1433, 1395, and 1267 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99 (bs, 2 H), 7.85 (s, 1 H), 7.45 (d, J = 8.4 Hz, 1 H), 7.43 (s, 1 H), 6.86 (d, J = 2.6 Hz, 1 H), 6.54 (dd, J = 8.5 and 2.5 Hz, 1 H), 4.46 (t, J = 6.4 Hz, 2 H), 3.89 (s, 3 H), 3.83 (s, 3 H), 3.03 (s, 6 H), 2.92 (m, 2 H), 2.11 (m, 2 H); ESIMS *m*/*z* (rel intensity) 408 (MH^+, 100). Anal. ($C_{23}H_{27}Cl_2N_3O_4$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (106). The general procedure provided the desired compound as a light purple solid (0.088 g, 86%): mp 268−270 °C (dec). IR (KBr) 3430, 2942, 1644, 1554, 1482, 1266, 1244, and 1019 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.94 (s, 1 H), 7.82 (bs, 2 H), 7.66 (d, J = 8.4 Hz, 1 H), 7.51 (s, 1 H), 7.07 (d, J = 2.5 Hz, 1 H), 6.99 (dd, J = 8.3 and 2.6 Hz, 1 H), 4.54 (t, J = 6.7 Hz, 2 H), 3.92 (s, 1 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 2.96 (t, J = 7.7 Hz, 2 H), 2.11 (m, 2 H); ESIMS *m*/*z* (rel intensity) 395 (MH^+, 56), 378 ($MH^+ - NH_3$, 100). Anal. ($C_{22}H_{23}ClN_2O_5$) C, H, N.

6-(3-Aminopropyl)-9-ethyl-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (107). The general procedure provided the desired compound as a red solid (0.93 g, 73%): mp 275 °C (dec). IR (KBr) 3438, 1636, 1551, 1482, 1385, and 1269 cm^{-1}; ^1H NMR (DMSO) δ 7.97 (s, 1 H), 7.86 (bs, 2 H), 7.65 (d, J = 7.6 Hz, 1 H), 7.53 (s, 1 H), 7.38−7.35 (m, 2 H), 4.55 (t, J = 6.5 Hz, 2 H), 3.93 (s, 3 H), 3.87 (s, 3 H), 2.96 (m, 2 H), 2.69 (q, J = 7.6 Hz, 2 H), 2.12 (m, 2 H), 1.23 (t, J = 7.6 Hz, 3 H); ESIMS *m*/*z* (rel intensity) 393 (MH^+, 96). Anal. ($C_{23}H_{25}ClN_2O_4$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (108). The general procedure provided the desired compound as an orange solid (0.214 g, 84%): mp 280 °C (dec). IR (KBr) 2974, 1692, 1651, 1556, 1522, 1512, 1478, 1429, 1397, 1265, and 1019 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.02 (s, 1 H), 7.81 (bs, 2 H), 7.77 (d, J = 7.4 Hz, 1 H), 7.57−7.48 (m, 4 H), 4.59 (t, J = 6.1 Hz, 2 H), 3.94 (s, 3 H), 3.89 (s, 3 H), 2.97 (t, J = 6.9 Hz, 2 H), 2.13 (m, 2 H); ESIMS *m*/*z* (rel intensity) 365 (MH^+, 88), 348 ($MH^+ - NH_3$). Anal. ($C_{21}H_{21}ClN_2O_4$) C, H, N.

6-(3-Aminopropyl)-9-fluoro-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (109). The general procedure provided the desired compound as a purple solid (0.090 g, 87%): mp 265−269 °C. IR (KBr) 3438, 1636, 1551, 1482, 1385, and 1269 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.93 (bs, 2

H), 7.87 (s, 1 H), 7.77 (dd, $J = 4.0$ and 13.1 Hz, 1 H), 7.48 (s, 1 H), 7.36−7.29 (m, 2 H), 4.53 (t, $J = 6.9$ Hz, 2 H), 3.91 (s, 3 H), 3.86 (s, 3 H), 2.94 (m, 2 H), 2.12−2.07 (m, 2 H); ESIMS *m*/*z* (rel intensity) 383 (MH^+, 60). Anal. ($C_{21}H_{20}ClFN_2O_4 \cdot 0.75H_2O$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-2,3-dimethoxy-9-methoxycarbonyl-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (110). The general procedure provided the desired compound as a purple solid (0.095 g, 93%): mp 268−270 °C. IR (film) 1719, 1632, 1481, 1269, and 1254 cm^{-1}; ^1H NMR (D_2O) δ 7.54 (d, $J = 6.6$ Hz, 1 H), 7.03 (d, $J = 6.6$ Hz, 1 H), 6.71 (s, 1 H), 6.62 (s, 1 H), 6.34 (s, 1 H), 4.12−4.06 (m, 2 H), 3.77 (s, 3 H), 3.47 (s, 3 H), 3.45 (s, 3 H), 3.11 (t, $J = 7.2$ Hz, 1 H); ESIMS *m*/*z* (rel intensity) 422 (MH^+, 100). Anal. ($C_{23}H_{23}ClN_2O \cdot 1.0H_2O$) C, H, N.

6-(3-Aminopropyl)-9-cyano-2,3-dimethoxy-5,11-dioxo-5,6-dihydro-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (111). The general procedure provided the desired compound as a purple solid (0.066 g, 65%): mp 266−267 °C. IR (film) 1637, 1610, 1511, 1479 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.02−7.79 (m, 6 H), 7.48 (s, 1 H), 4.51−4.49 (m, 2 H), 3.91 (s, 3 H), 3.86 (s, 3 H), 2.96−2.93 (m, 2 H), 2.09−2.08 (m, 2 H); ESIMS *m*/*z* (rel intensity) 391 (MH^+ − HCl, 100). Anal. ($C_{22}H_{20}ClN_3O_4 \cdot 1.25H_2O$) C, H, N.

6-(3-Aminopropyl)-5,6-dihydro-2,3-dimethoxy-9-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (112). The general procedure provided the desired compound as a black solid (0.091 g, 85%): mp 197−200 °C (dec). IR (KBr) 3433, 1643, 1607, 1523, 1478, 1342, and 1273 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.39 (dd, $J = 8.5$ and 2.2 Hz, 1 H), 8.08 (d, $J = 2.3$ Hz, 1 H), 8.01−7.98 (m, 2 H), 7.82 (bs, 2 H), 7.59 (s, 1 H), 4.59 (m, 2 H), 3.96 (s, 3 H), 3.91 (s, 3 H), 2.99 (m, 2 H), 2.10 (m, 2 H); ESIMS *m*/*z* (rel intensity) 410 (MH^+, 100). Anal. ($C_{21}H_{20}ClN_3O_6 \cdot 2.5H_2O$) C, H, N.

Topoisomerase I-Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from Baculovirus as described previously.[45] 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 (50 μL reactions) for 1 h at 37 °C, and separated by electrophoresis in a 1% agarose gel made in 1× TBE buffer. The 161 bp fragment was eluted from the gel slice using the QIAEX II kit (QIAGEN Inc., Valencia, CA). Approximately 200 ng of the fragament was 3′-end-labeled at the Hind III site by fill-in reaction with [alpha-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 unit of DNA polymerase I (Klenow fragment). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled 161 bp fragment was collected. Aliquots (approximately 50 000 dpm/reaction) were incubated with Top1 at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). The samples (10 μL) were mixed with 30 μL of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in denaturing gels (16% polyacrylamine, 7 M urea). Gels were dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

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Supporting Information Available: Elemental analyses for compounds **7**−**11**, **13**−**25**, **27**−**30**, **33**−**73**, **75**, **77**−**87**, **89**−**92**, and **94**−**112**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; A. T. M.; Sim, G. A. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.* **1966**, *88*, 3888−3890.

(2) Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. Plant Antitumor Agents. 18. Synthesis and Biological Activity of Camptothecin Analogues. *J. Med. Chem.* **1980**, *23*, 554−560.

(3) Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L. F. Camptothecin Induces Protein-Linked DNA Breaks via Mammalian DNA Topoisomerase I. *J. Biol. Chem.* **1985**, *260*, 14873−14878.

(4) Kohn, K. W.; Pommier, Y. Molecular and Biological Determinants of the Cytotoxic Actions of Camptothecins. Perspective for the Development of New Topoisomerase I Inhibitors. *Ann. N.Y. Acad. Sci.* **2000**, *922*, 11−26.

(5) Takimoto, C. H.; Wright, J.; Arbuck, S. G. Clinical Applications of the Camptothecins. *Biochim. Biophys. Acta* **1998**, *1400*, 107−119.

(6) Oberlies, N. H.; Kroll, D. J. Camptothecin and Taxol: Historic Achievements in Natural Products Research. *J. Nat. Prod.* **2004**, *67*, 129−135.

(7) Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. Mechanism of Action of Eukaryotic DNA Topoisomerase I and Drugs Targeted to the Enzyme. *Biochim. Biophys. Acta* **1998**, *1400*, 83−106.

(8) Thomas, C. J.; Rahier, N. J.; Hecht, S. M. Camptothecin: Current Perspectives. *Bioorg. Med. Chem.* **2004**, *12*, 1585−1604.

(9) Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. Structure−Activity Study of the Actions of Camptothecin Derivatives on Mammalian Topoisomerase I: Evidence for a Specific Receptor Site and a Relation to Antitumor Activity. *Cancer Res.* **1989**, *49*, 1465−1469.

(10) Minami, H.; Beijnen, J. H.; Verweij, J.; Ratain, M. J. Limited Sampling Model for Area Under the Concentration Time Curve of Total Topotecan. *Clin. Cancer Res.* **1996**, *2* (1), 43−46.

(11) Danks, M. K.; Pawlik, C. A.; Whipple, D. O.; Wolverton, J. S. Intermittent Exposure of Medulloblastoma Cells to Topotecan Produces Growth Inhibition Equivalent to Continuous Exposure. *Curr. Top. Med. Chem.* **1997**, *3* (10), 1731−1738.

(12) Haas, N. B.; LaCreta, F. P.; Walczak, J.; Hudes, G. R.; Brennan, J. M.; Ozols, R. F.; O'Dwyer, P. J. Phase-I Pharmacokinetic Study of Topotecan by 24-Hour Continuous-Infusion Weekly. *Cancer Res.* **1994**, *54* (5), 1220−1226.

(13) Chrencik, J. E.; Staker, B. L.; Burgin, A. B., Jr.; Pourquier, P.; Pommier, Y.; Stewart, L.; Redinbo, M. R. Mechanisms of Camptothecin Resistance by Human Topoisomerase I Mutations. *J. Mol. Biol.* **2004**, *339*, 773−784.

(14) Yoshikawa, M.; Ikegami, Y.; Hayasaka, S.; Ishii, K.; Ito, A.; Sano, K.; Suzuki, T.; Togawa, T.; Yoshida, H.; Soda, H.; Oka, M.; Kohno, S.; Sawada, S.; Ishikawa, T.; Tanabe, S. Novel Camptothecin Analogues that Circumvent ABCG2-Associated Drug Resistance in Human Tumor Cells. *Int. J. Cancer* **2004**, *110*, 921−927.

(15) Paull, K. D.; Hamel, E.; Malspeis, L. Prediction of Biochemical Mechanism of Action from the In Vitro Antitumor Screen of the National Cancer Institute. In *Cancer Chemotherapeutic Agents*; Foye, W. E., Ed.; The American Chemical Society: Washington, DC, 1995; pp 9−45.

(16) Kohlhagen, G.; Paull, K. D.; Cushman, M.; Nagafuji, P.; Pommier, Y. Protein-Linked DNA Strand Breaks Induced by NSC 314622, a Novel Noncamptothecin Topoisomerase I Poison. *Mol. Pharmacol.* **1998**, *54*, 50−58.

(17) Strumberg, D.; Pommier, Y.; Paull, K.; Jayaraman, M.; Nagafuji, P.; Cushman, M. Synthesis of Cytotoxic Indenoisoquinoline Topoisomerase I Poisons. *J. Med. Chem.* **1999**, *42*, 446−457.

(18) Cushman, M.; Jayaraman, M.; Vroman, J. A.; Fukunaga, A. K.; Fox, B. M.; Kohlhagen, G.; Strumberg, D.; Pommier, Y. Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors. *J. Med. Chem.* **2000**, *43*, 3688−3698.

(19) Jayaraman, M.; Fox, B. M.; Hollingshead, M.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of New Dihydroindeno[1,2-*c*]isoquinoline and Indenoisoquinolinium Chloride Topoisomerase I Inhibitors Having High In Vivo Anticancer Acitivity in the Hollow Fiber Animal Model. *J. Med. Chem.* **2002**, *45*, 242−249.

(20) Antony, S.; Jayaraman, M.; Laco, G.; Kohlhagen, G.; Kohn, K. W.; Cushman, M.; Pommier, Y. Differential Induction of Topoisomerase I-DNA Cleavage Complexes by the Indenoisoquinoline MJ-III-65 (NSC 706744) and Camptothecin: Base Sequence Analysis and Activity against Camptothecin-Resistant Topoisomerase I. *Cancer Res.* **2003**, *63*, 7428−7435.

(21) Fox, B. M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline-Camptothecin Hybrids. *J. Med. Chem.* **2003**, *46*, 3275−3282.
(22) Nagarajan, M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Design, Synthesis, and Biological Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Featuring Polyamine Side Chains on the Lactam Nitrogen. *J. Med. Chem.* **2003**, *46*, 5712−5724.
(23) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3659−3663.
(24) Nagarajan, M.; Morrell, A.; Fort, B. C.; Meckley, M. R.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis and Anticancer Activity of Simplified Indenoisoquinoline Topoisomerase I Inhibitors Lacking Substituents on the Aromatic Rings. *J. Med. Chem.* **2004**, *47*, 5651−5661.
(25) Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Aminoalkenylindenoisoquinoline and 11-Diaminoalkenylindenoisoquinoline Topoisomerase I Inhibitors. *Bioorg. Med. Chem.* **2004**, *12*, 5147−5160.
(26) Ioanoviciu, A.; Antony, S.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Synthesis and Mechanism of Action Studies of a Series of Norindenoisoquinoline Topoisomerase I Poisons Reveal an Inhibitor with a Flipped Orientation in the Ternary DNA-Enzyme-Inhibitor Complex As Determined by X-ray Crystallographic Analysis. *J. Med. Chem.* **2005**, *48*, 4803−4814.
(27) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of Benz[*d*]indeno[1,2-*b*]pyran-5,11-diones: Versatile Intermediates for the Design and Synthesis of Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1846−1849.
(28) Nagarajan, M.; Morrell, A.; Ioanoviciu, A.; Antony, S.; Kohlhagen, G.; Hollingshead, M.; Pommier, Y.; Cushman, M. Synthesis and Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Substituted with Nitrogen Heterocycles. *J. Med. Chem.* **2006**, *49*, 6283−6289.
(29) Nagarajan, M.; Morrell, A.; Antony, S.; Kohlhagen, G.; Agama, K.; Hollingshead, M.; Pommier, Y.; Cushman, M. Synthesis and Biological Evaluation of Bisindenoisoquinolines as Topoisomerase I Inhibitors. *J. Med. Chem.* **2006**, *49*, 5129−5140.
(30) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. A Systematic Study of Nitrated Indenoisoquinolines Reveals a Potent Topoisomerase I Inhibitor. *J. Med. Chem.* **2006**, *49*, 7740−7753.
(31) Whitmore, W. F.; Cooney, R. C. The Preparation of Homophthalyl Cyclic Hydrazide and 4-Aminohomophthalyl Cyclic Hydrazide. *J. Am. Chem. Soc.* **1944**, *66*, 1237−1240.
(32) Madaj, E. J.; Cushman, M. A Study and Mechanistic Interpretation of the Electronic and Steric Effects that Determine the Stereochemical Outcome of the Reaction of Schiff Bases with Homophthalic Anhydride and 3- Phenylsuccinic Anhydride. *J. Org. Chem.* **1987**, *52*, 907−915.
(33) Cushman, M.; Cheng, L. Stereoselective Oxidation by Thionyl Chloride Leading to the Indeno[1,2-*c*]isoquinoline System. *J. Org. Chem.* **1978**, *43*, 3781−3783.
(34) Xiao, X.; Morrell, A.; Fanwick, P. E.; Cushman, M. On the Mechanism of Conversion of 4-Carboxy-3,4-dihydro-3-phenyl-1(2*H*)-isoquinolones to Indeno[1,2-*c*]isoquinolines by Thionyl Chloride. *Tetrahedron* **2006**, *62*, 9705−9712.
(35) Potts, K. T.; Robinson, R. Synthetic Experiments Related to the Indole Alkaloids. *J. Chem. Soc.* **1955**, 2675−2686.
(36) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82* (13), 1107−1112.
(37) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(38) Morrell, A.; Jayaraman, M.; Nagarajan, M.; Fox, B. M.; Meckley, M. R.; Ioanoviciu, A.; Pommier, Y.; Antony, S.; Hollingshead, M.; Cushman, M. Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Using a Hollow Fiber Assay. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4395−4399.
(39) Wani, M. C.; Nicholas, A. W.; Wall, M. E. Plant Antitumor Agents. 23. Synthesis and Antileukemic Activity of Camptothecin Analogues. *J. Med. Chem.* **1986**, *29*, 2358−2363.
(40) Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Wall, M. E. Plant Antitumor Agents. 25. Total Synthesis and Antileukemic Activity of Ring A Substituted Camptothecin Analogues. Structure−Activity Correlations. *J. Med. Chem.* **1987**, *30*, 1774−1779.
(41) Fan, Y.; Weinstein, J. N.; Kohn, K. W.; Shi, L. M.; Pommier, Y. Molecular Modeling Studies of the DNA-Topoisomerase I Ternary Cleavable Complex with Camptothecin. *J. Med. Chem.* **1998**, *41*, 2216−2226.
(42) Prudhomme, M. Recent Developments of Rebeccamycin Analogues as Topoisomerase I Inhibitors and Antitumor Agents. *Curr. Med. Chem.* **2000**, *7* (12), 1189−212.
(43) Antony, S.; Kohlhagen, G.; Agama, K.; Jayaraman, M.; Cao, S.; Durrani, F. A.; Rustum, Y. M.; Cushman, M.; Pommier, Y. Cellular Topoisomerase I Inhibition and Antiproliferative Activity by MJ-III-65 (NSC 706744), an Indenoisoquinoline Topoisomerase I Poison. *Mol. Pharmacol.* **2005**, *67*, 523−530.
(44) Yus, M.; Soler, T.; Foubelo, F. A New and Direct Synthesis of 2-Substituted Pyrrolidines. *J. Org. Chem.* **2001**, *66*, 6207−6208.
(45) Pourquier, P.; Ueng, L.-M.; Fertala, J.; Wang, D.; Park, H.-K.; Essigmann, J. M.; Bjornsti, M.-A.; Pommier, Y. Induction of Reversible Complexes Between Eukaryotic DNA Topoisomerase I and DNA-containing Oxidative Base Damages. 7,8-Dihydro-8-Oxoguanine and 5-Hydroxycytosine. *J. Biol. Chem.* **1999**, *274*, 8516−8523.

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