Synthesis, Cytotoxicity, DNA Interaction, and Topoisomerase II Inhibition Properties of Novel Indeno[2,1-*c*]quinolin-7-one and Indeno[1,2-*c*]isoquinolin-5,11-dione Derivatives

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Indeno[2,1-*c*]quinolin-7-ones and 6*H*-indeno[1,2-*c*]isoquinolin-5,11-diones, bearing two cationic aminoalkyl side chains, were synthesized and evaluated for DNA interaction, topoisomerases inhibition, and cytotoxicity against human cancer cell lines. They displayed strong interaction with DNA and one indeno[1,2-*c*]isoquinolin-5,11-dione bearing side chains at N-6 and C-8 positions (**6a**) was a potent human topoisomerase II inhibitor with high cytotoxicity toward HL60 cells. An increased topoisomerase II inhibition is found with (a) a cationic aminoalkyl side chain at the C-8 rather than at the C-9 position, (b) a dimethylaminoethoxy side chain at the C-8 position introduced on the N-6 monosubstituted derivative, going with suppression of topoisomerase I poisoning, and (c) a dimethylaminoethyl rather than a dimethylaminopropyl side chain at the N-6 position. The cytotoxicity was only partially reduced when using the topoisomerase II-mutated mitoxantrone-resistant HL60/MX2 cell line, suggesting that additional targets are involved in their mechanism of action. These indeno[1,2-*c*]isoquinolin-5,11-dione derivatives represent new DNA−topoisomerase II interfering anticancer molecules.

Introduction

A significant number of DNA-targeted anticancer agents contain a planar intercalating polycycle bearing one aminoalkyl side chain. This is particularly the case for two classes of compounds: (i) indeno[2,1-*c*]quinolin-7-ones represented by **1a** (TAS-103[1]) and (ii) indeno[1,2-*c*]isoquinolin-5,11-diones represented by **2** (MJ-III-65[2]) and **3** (NSC727357[3]) (Chart 1). These compounds display marked cytotoxic properties and, for some of them, potent antitumor activities in xenograft models. Indenoquinolinone **1a** has been advanced to phases I and II clinical trials,[4] and several indeno[1,2-*c*]isoquinolin-5,11-diones are currently in preclinical development.[5] Although the cytotoxic mechanism of these drugs has not been clearly elucidated, they probably act through the inhibition of DNA religation by topoisomerases and are considered as topoisomerase "poisons" with the stabilization of covalent enzyme−DNA complexes.

In the cell, DNA topoisomerases control the topological state of DNA.[6,7] There are two classes of topoisomerase: (i) type I enzymes under- and overwind DNA by generating transient single-stranded breaks in the DNA double helix and (ii) type II enzymes resolve DNA knots and tangles by creating transient double-stranded breaks. Topoisomerase inhibitors designated as "poisons" interact with DNA and/or topoisomerases to form stable ternary complexes, termed "cleavable complexes", causing permanent DNA damage that triggers a series of cellular events finally inducing apoptosis or other types of cell death.[8] DNA topoisomerases are targets for several clinically prescribed anticancer agents: (i) topoisomerase I poisons irinotecan (Campto) and topotecan (Hycamtin) derived from camptothecin[9] and (ii) topoisomerase II poisons including etoposide, doxorubicin, and mitoxantrone. Despite their antitumor potency, the development of resistance mechanisms and the toxicity or reversibility of the covalent complex formation have limited the use of these molecules. Novel molecules are being sought to overcome such limitations and/or expand the anticancer spectrum of the above-cited inhibitors.

Indenoquinolinone **1a** was initially developed as a dual topoisomerase I/topoisomerase II poison,[10] but it was subsequently requalified as a topoisomerase II poison, with a very minor activity against topoisomerase I. Compound **1a** was shown to intercalate into the DNA double helix, and the structural constraints imposed by the intercalative binding block the topoisomerase I-catalyzed DNA relaxation but not the topoisomerase catalytic activity.[11,12] At the cellular level, apoptosis induced by compound **1a** is firmly established. There is a correlation between the G2 cell cycle arrest and changes in mitochondrial membrane potential,[13] hydrogen peroxide formation,[14] and p-300-dependent activation of transcription factor Sp1, enhanced by cellular acidosis.[15]

Indenoisoquinolin-5,11-diones form a class of potent non-camptothecin topoisomerase I poisons endowed with high cytotoxic properties on several tumor cell lines.[16,17] Some compounds have been described as both topoisomerase I and II inhibitors and DNA intercalators: the carboxylic acid derivative **4** (MJ-238,[18,19] Chart 1) and bisindenoisoquinolin-5,11-dione **3**.[3] The representative indenoisoquinolin-5,11-dione **2**[20] was shown to form more stable DNA−topoisomerase I cleavage complexes than camptothecin and maintains a high cytotoxicity on camptothecin-resistant cells, which makes it a good candidate

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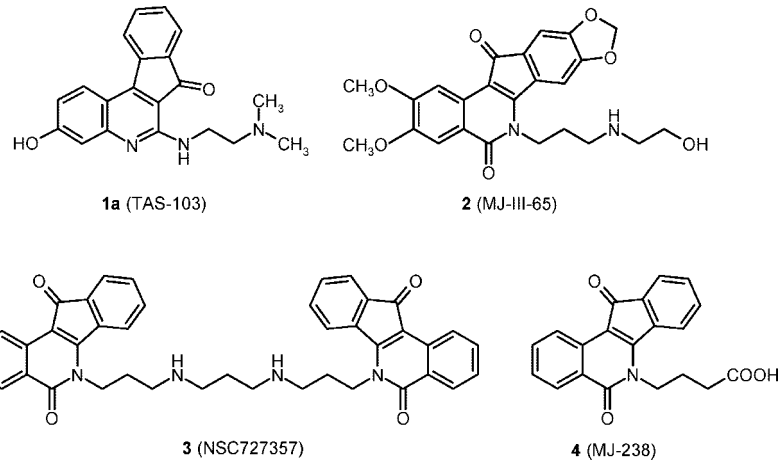
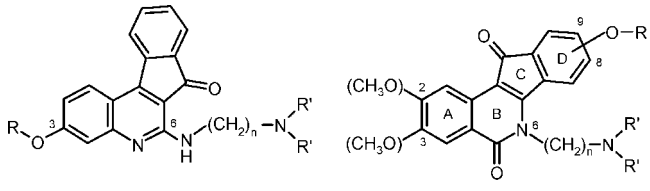
Chart 1. Structures of Indenoquinolinone **1a**, Indenoisoquinolin-5,11-diones **2** and **4**, and Bisindenoisoquinolin-5,11-dione **3**



1a (TAS-103) **2** (MJ-III-65)

3 (NSC727357) **4** (MJ-238)

Chart 2. Structures of Series I and Series II Compounds (Indenoquinolin-7-ones **1a–c** and **5a–c** and Indenoisoquinolin-5,11-diones **6a–g** and **7a**,**b**)



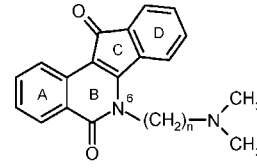
for preclinical development.[2,16] Molecular modeling calculations supported by X-ray diffraction studies performed with the carboxylic acid derivative **4** suggest not only that the indenoisoquinolin-5,11-dione skeleton binds to DNA through an intercalation mode by $\pi-\pi$ stacking with the DNA base pairs flanking the topoisomerase I cleavage site but also that the C-11 ketone is implicated in a network of hydrogen bonds with surrounding amino acid residues, notably Arg364.[19,21–23]

Because DNA affinity appears to be the key determinant involved in the mechanism of indenoquinolin-7-ones and indenoisoquinolin-5,11-diones,[3] we decided to synthesize new derivatives (Chart 2) incorporating a second cationic side chain on the tetracyclic scaffolds.

Not only did we hypothesize that the additional side chain would increase DNA binding through ionic interactions with the negatively charged phosphate backbone, but we also considered that an increased DNA affinity could reduce the dissociation rates of topoisomerase−DNA cleavage complexes. It was also expected that chemical modifications would increase the ionization state of the compounds at physiological pH to enhance their aqueous solubility. We assessed the contribution of these structural modifications to their character as topoisomerase I and II poisons, DNA interaction, and cytotoxicity to be able to elaborate structure–activity relationships in both series of molecules.

In series I, the published biological data[24] on 3-hydroxylated analogues of compound **1a** indicate good cytotoxicity on P388 leukemia cells for derivatives bearing a two-carbon saturated chain, either a dimethylamine or a pyrrolidine at the N-6 position and a hydroxyl at the C-3 position. An amino side chain was introduced at the 3-OH substituent, using the hydroxy intermediates **1a–c** for comparison and indenoquinolinone **1a** as a reference molecule.

Chart 3. Reference Indenoisoquinolin-5,11-diones **8a**,**b**



8a: n = 2
8b: n = 3

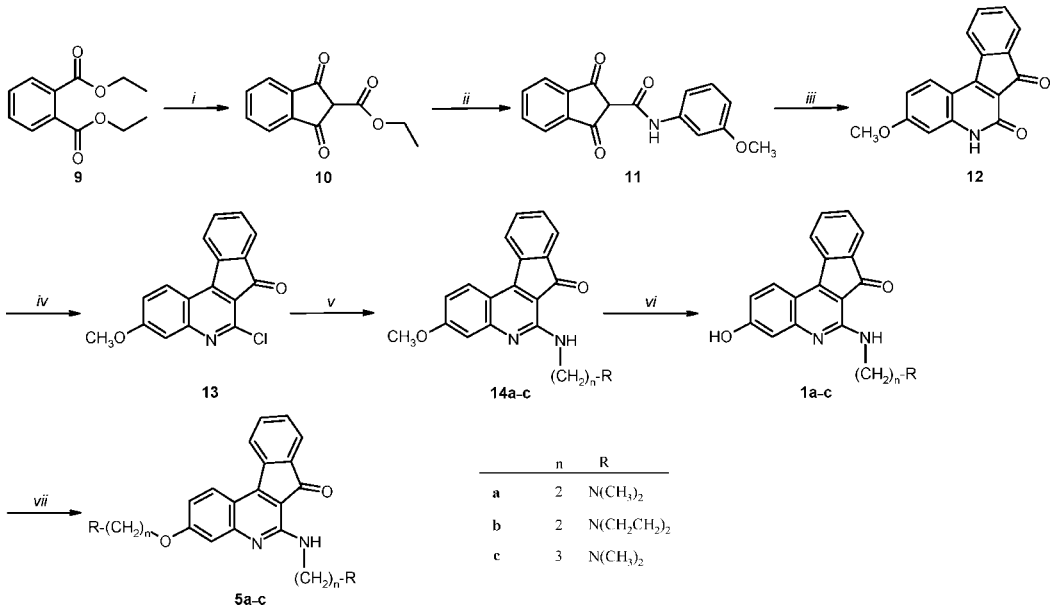
In series II, a cationic side chain was maintained at the N-6 position, as this element has been recognized as important for antiproliferative activity.[18,20] Recently, studies performed with simplified indenoisoquinolin-5,11-diones have shown that linkers with lengths of between two and four atoms are optimal for topoisomerase I inhibition and cytotoxicity,[25] and therefore, analogues bearing an ethyl or a propyl chain on the lactam nitrogen were designed. A 2,3-dimethoxy sequence was introduced on cycle A of some derivatives, as this was reported in previous series[20] to increase the biological activity. However, more recent studies have shown that simplified isoquinolines lacking the 2,3-dimethoxy sequence but bearing an aminoalkyl group on the lactam nitrogen could also be potent cytotoxics.[26] To make a comparison, two simplified indenoisoquinolin-5,11-diones (Chart 3) were used: compound **8b** bearing a dimethylaminopropyl side chain on the lactam nitrogen, previously described as a potent topoisomerase I inhibitor,[26] and its shorter side chain counterpart **8a**.

Chemistry

The synthesis of 3-hydroxyindenoquinolin-7-ones **1a–c** was performed in six steps (Scheme 1) according to a described procedure.[24] The key intermediate **13** was obtained by Claisen−Dieckmann cyclization of diethyl phthalate **9** followed by the reaction of ester **10** with 3-methoxyaniline and cyclization of amide **11** (polyphosphoric acid) into quinolin-6,7-dione **12**. Chlorination of compound **12** ($POCl_3$) yielded 6-chloroquinoline **13**. Compounds **1a–c** were obtained after nucleophilic aromatic substitution of the 6-chloro carbon with appropriate ω-aminoalkylamine (compounds **14a–c)** and O-demethylation (hydrobromic acid). The 3-aminoalkoxy chain was then introduced by nucleophilic substitution with appropriate ω-halogenoalkylamine to give disubstituted quinolines **5a–c**.

Considering indenoisoquinolin-5,11-diones **6a–g** (Scheme 2), the first aminoalkyl side chain was immediately introduced

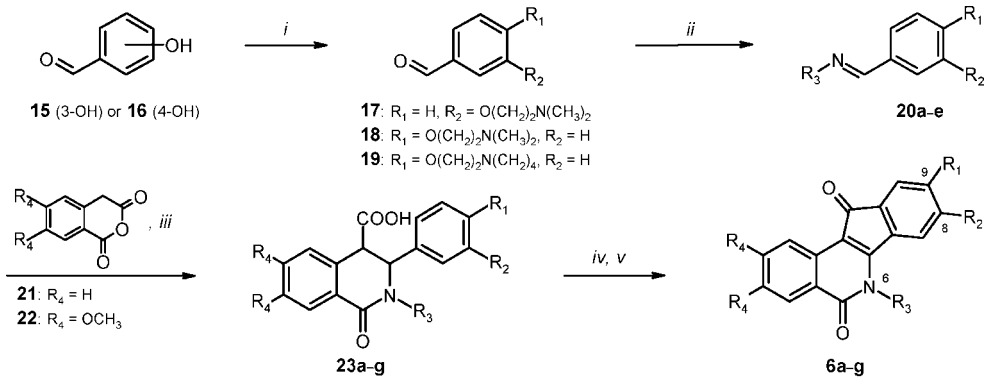
Scheme 1. Synthesis of Indenoquinolin-7-ones **1a–c** and **5a–c** (Series I)[a]



	n	R
a	2	$N(CH_3)_2$
b	2	$N(CH_2CH_2)_2$
c	3	$N(CH_3)_2$

[a] Reagents and conditions: (i) Na, EtOAc, EtOH, 80 °C, 5 h; (ii) *m*-anisidine, toluene, reflux, 30 min; (iii) PPA, 120 °C, 4 h; (iv) $POCl_3$, DMF, reflux, 4 h; (v) appropriate aminoalkylamine, pyridine, 100 °C, 18 h; (vi) 47% HBr, AcOH, 100 °C, 65 h; (vii) appropriate chloroalkylamine, Cs_2CO_3, NaI, DMF, 80 °C, 3 h.

Scheme 2. Synthesis of Indenoisoquinolin-5,11-diones **6a–g** (Series II)[a]

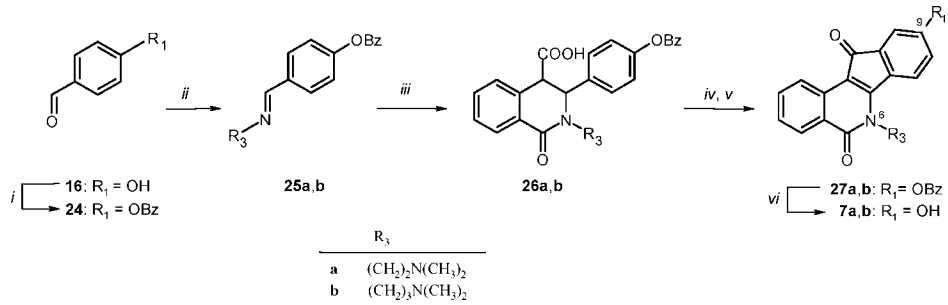


	R_1	R_2	R_3	n	R_4
a	H	$O(CH_2)_2N(CH_3)_2$	$(CH_2)_nN(CH_3)_2$	2	H
b	H	$O(CH_2)_2N(CH_3)_2$	$(CH_2)_nN(CH_3)_2$	3	H
c	$O(CH_2)_2N(CH_3)_2$	H	$(CH_2)_nN(CH_3)_2$	2	H
d	$O(CH_2)_2N(CH_2CH_2)_2$	H	$(CH_2)_nN(CH_2CH_2)_2$	2	H
e	$O(CH_2)_2N(CH_3)_2$	H	$(CH_2)_nN(CH_3)_2$	3	H
f	$O(CH_2)_2N(CH_3)_2$	H	$(CH_2)_nN(CH_3)_2$	2	OCH_3
g	$O(CH_2)_2N(CH_3)_2$	H	$(CH_2)_nN(CH_3)_2$	3	OCH_3

[a] Reagents and conditions: (i) appropriate alkyl chloride, K_2CO_3, DMF, 80 °C, 5 h; (ii) R_3NH_2, $MgSO_4$, $CHCl_3$, room temp, 6 h; (iii) MeOH or THF, room temp, 1 h; (iv) $SOCl_2$, 80 °C, 12 h; (v) $AlCl_3$, dry CH_2Cl_2, 0 °C, 3 h.

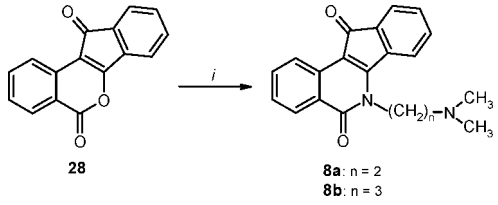
via an etheroxide linkage on 3- or 4-hydroxybenzaldehyde **15** or **16** (compounds **17**–**19**), whereas the second aminoalkyl substituent on the isoquinolone nitrogen resulted from the subsequent formation of imines **20a**–**e** using an appropriate ω-aminoalkylamine R_3NH_2. The sequence of the target compounds **6a**–**g** was pursued using a two-step procedure[18] from appropriate imines **20a**–**e**; condensation with homophthalic anhydride **21** or **22**[27] provided carboxylic acids **23a**–**g** and was followed by a reaction of the cis stereoisomer alone with thionyl chloride to give the final indenoisoquinolin-5,11-diones **6a**–**g**, implying two-electron oxidation followed by an intramolecular Friedel–Crafts cyclization ($AlCl_3$).[28] It was to be noted that isoquinolones **23a**–**g** were obtained as a cis−trans diastereoisomeric mixture that could not be separated by fractionated crystallization or silica gel chromatography. The low yields observed for compounds **6a** and **6b** can be attributable to the formation of their corresponding regioisomers as byproducts (relative to substituents R_1 and R_2) at the intramolecular cyclization step, whereas those observed for dimethoxy derivatives **6f** and **6g** can be explained by their demethylation in the conditions required by the Friedel–Crafts cyclization.

Scheme 3. Synthesis of 9-Hydroxyindenoisoquinolin-5,11-diones **7a**,**b** (Series II)[a]



[a] Reagents and conditions: (i) benzoyl chloride, TEA, dry CH_2Cl_2, 0 °C, 3 h; (ii) R_3NH_2, $MgSO_4$, $CHCl_3$, room temp, 6 h; (iii) THF, room temp, 1 h; (iv) $SOCl_2$, 80 °C, 12 h; (v) $AlCl_3$, CH_2Cl_2, 0 °C, 3 h; (vi) LiOH, CH_2Cl_2/MeOH 1:1, room temp,1 h.

Scheme 4. Synthesis of Indenoisoquinolin-5,11-diones **8a**,**b** (Series II)[a]



[a] Reagents and conditions: (i) $(CH_3)_2N(CH_2)_2NH_2$ or $(CH_3)_2N(CH_2)_3NH_2$, $CHCl_3$, room temp, 18 h.

Synthesis of 9-hydroxyindenoisoquinolin-5,11-diones **7a** and **7b** was performed using a similar strategy (Scheme 3), starting from 4-hydroxybenzaldehyde **16**, whose aromatic hydroxyl was protected as benzoyl ester, resistant to acidic conditions at the cyclization step but removable in alkaline conditions.

Reference compounds **8a**,**b** were synthesized from benz[*d*]indeno[1,2-*b*]pyran-5,11-dione **28**[29] (Scheme 4) according to the procedure previously reported for **8b**.[26]

Results

DNA Interaction. DNA Thermal Denaturation. The ability of the drugs to protect calf thymus DNA (CT[a] DNA, 42% GC bp) against thermal denaturation was used as an indication of their capacity to bind to DNA and to stabilize the DNA double helix. The variations of the T_m values ($\Delta T_m = T_m^{\text{drug–DNA complex}} - T_m^{\text{DNA alone}}$) are presented in Table 1. In series I, ΔT_m values for compounds **5a**–**c** reach 24 °C (drug/DNA ratio = 0.25) whereas ΔT_m values range from 4 to 10 °C for compounds **1a**–**c**. Introduction of a second cationic side chain (compounds **5a**–**c**) was found to strongly stabilize DNA against heat denaturation when compared with their respective hydroxylated single side chain counterparts **1a**–**c**.

A similar behavior was noted in series II where compounds **6c** and **6e**, bearing two cationic side chains, were considerably more efficient at stabilizing the duplex structure of DNA than their 9-hydroxylated counterparts **7a** and **7b**. This could be attributable to additional electrostatic interactions with DNA phosphodiester groups.

Fluorescence Measurements. Binding affinities for compounds in both series were quantified by means of fluorescence using two different methods (Table 1). In series I, the intrinsic fluorescence of the compounds was exploited to determine the apparent binding constant (K_{app}). In series II, since weak fluorescence was observed upon DNA titration, use was made of the conventional fluorescence quenching assay based on DNA competition between the intercalating drug ethidium bromide and the tested molecules.

Table 1. Interaction of Series I and Series II Compounds with DNA

compd	ΔT_m (°C)[a]	K_{app} (10^6 M^{-1})[b]
	Series I, Method 1[c]	
1a	10	1.54 ± 0.12
1b	4	0.51 ± 0.04
1c	7	0.88 ± 0.07
5a	24	1.58 ± 0.14
5b	24	0.46 ± 0.05
5c	27	nd[d]
	Series II, Method 2[e]	
6a	15	1.92 ± 0.21
6b	17	9.61 ± 0.81
6c	17	36.74 ± 2.17
6d	19	24.53 ± 1.14
6e	24	36.11 ± 1.95
6f	19	37.21 ± 2.35
6g	22	46.80 ± 2.86
7a	5	2.57 ± 0.19
7b	8	6.94 ± 0.07
8a	5.9	0.86 ± 0.07
8b	10.8	6.79 ± 0.52

[a] Variation in melting temperature ΔT_m ($T_m^{\text{drug–DNA complex}} - T_m^{\text{DNA alone}}$). Drug/CT DNA ratio = 0.25. [b] Apparent binding constant measured by fluorescence. [c] Intrinsic fluorescence. [Ligand] = 1 μM. [d] nd: not determined. [e] Competition with ethidium bromide (EB). EB/DNA ratio = 1.26.

In series I, the first step to validate the method was to relate the K_{app} value calculated from DNA titration and variation in intrinsic fluorescence measurement for compound **1a** to the previous results.[12] In the second step, K_{app} was calculated for the five derivatives **1a**–**c**, **5a**,**b**, using the same procedure. The two side chain derivatives **5a**,**b** displayed binding constants similar to those of their hydroxyl counterparts **1a**,**b**, whereas they provided better stabilization of the DNA complex against heat denaturation, as mentioned above. These results led to the assumption that despite a comparable affinity for DNA, compounds **1a**,**b** would display a specific mode of binding that would be less efficient at stabilizing the duplex structure of DNA.

In series II, the presence of an additional amino side chain at C-8 (compounds **6a**,**b**) or at C-9 (compounds **6c**,**e**) increased both DNA affinity constant and duplex stabilization when compared to their one side chain counterparts **8a**,**b**. This could be attributable to additional stabilizing interactions, potentially between DNA phosphate backbone and cationic side chains. Moreover, moving the side chain from C-9 (compounds **6c**,**e**) to C-8 (compounds **6a**,**b**) decreased activity for DNA by 18- and 4-fold, respectively. Increasing the length of the N-6 side

[a] Abbreviations: CT, calf thymus; GC, guanine−cytosine; T_m, melting temperature; TkLC, thick layer chromatography.

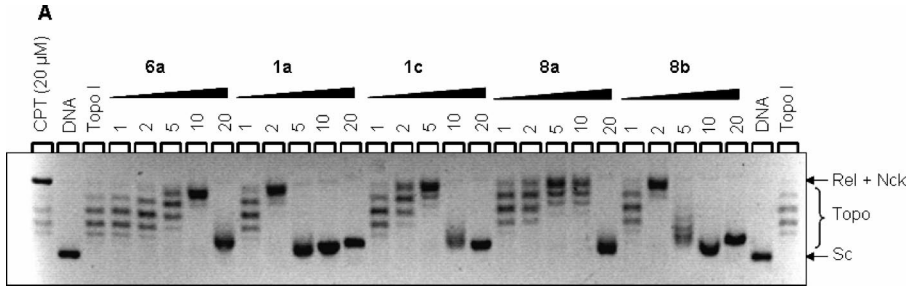


Figure 1. Effect of compounds **1a**,**c**, **6a**, **8a**,**b** on the relaxation of plasmid DNA by human topoisomerase I. Native supercoiled pLAZ (130 ng, lane DNA) was incubated with 4 units of topoisomerase I in the absence (lane Topo I) or presence of tested compound at the indicated concentration (1−20 μM). Camptothecin (CPT) was used at 20 μM. DNA samples were separated by electrophoresis on a 1% agarose gel, which was stained with ethidium bromide after DNA migration. Gels were photographed under UV light: Nck, nicked; Sc, supercoiled; Rel, relaxed; Topo, topoisomer products.

chain (compound **6e** vs **6c**, $n = 3$ vs $n = 2$) or introducing a 2,3-dimethoxy sequence (compound **6f** vs **6c**, $n = 2$) had no effect on DNA affinity. When both modifications were carried out simultaneously by adding a methyl group on the N-6 aminoalkyl side chain and a 2,3-dimethoxy sequence (compound **6g**), a slight increase in DNA affinity was noted compared with its counterparts **6e**,**f**, suggesting that both modifications are required simultaneously to enhance binding to DNA. Finally, replacing the dimethylamino group (**6c**) by a bulkier cyclic amine such as pyrrolidine (**6d**) decreased DNA affinity.

Mode of Binding to DNA. Different binding modes (intercalation and/or groove binding) can account for the melting temperature evaluation. Therefore, to gain an understanding of the drug binding mechanism, a DNA unwinding assay was used (Figure 1) based on the relaxation of supercoiled plasmid DNA in the presence of topoisomerase I for three selected compounds: **1a**, **1c** and **6a**, with compounds **8a**,**b** as references. Supercoiled DNA was treated with topoisomerase I in the presence of increasing concentrations of the tested drug. DNA relaxation products were then identified by agarose gel electrophoresis. As seen in Figure 1, the DNA relaxed by topoisomerase I alone (lane Topo I) generated a family of DNA topoisomers with a slow electrophoretic mobility. The drug was then added while topoisomerase I was maintained in the reaction mixture. When the drug concentration was increased, the closed circular duplex DNA was progressively supercoiled, indicating that the drug intercalates into DNA,[30] the intercalation occurring at micromolar concentrations.

All compounds in series I and II totally inhibit the relaxation of the DNA. In series I, the effects obtained for **1a** were more pronounced than for **1c** (compare the 5 μM lanes) and were consistent with the intercalative mode (unwinding of closed circular duplex DNA). In series II, intercalation is also consistent with previous results obtained from a crystallographic study of the cleavage complex.[22] It is worth mentioning that, as for several intercalating agents, the compounds studied here give no circular dichroism (CD) signals in the drug absorption band in the presence of DNA (data not shown). Therefore, CD could no longer be used to investigate further the mode of binding to DNA.

Sequence Selectivity. The nature of the sequence preferentially recognized by the compounds was determined by the DNase I footprinting methodology, using a radiolabeled 117-bp DNA restriction fragment as a substrate. In all cases, no clear footprints could be detected, whatever the drug concentration tested. Either the affinity of the drugs for DNA was not sufficiently high, or more likely, they formed insufficiently stable drug–DNA complexes to inhibit DNase I cleavage. No preferential binding sequence could be determined with any of the molecules belonging to series I or II.

Topoisomerase Inhibition. A conventional DNA relaxation assay was used to assess the effects of the compounds on the catalytic activity of human topoisomerases I and II. In these experiments (Figure 2), supercoiled plasmid DNA was treated with either topoisomerase I or II in the presence of two concentrations of the test drug (20 and 50 μM) and the DNA relaxation products were then resolved by gel electrophoresis on agarose gel. In either series, no molecule promoted DNA cleavage by topoisomerase I (data not shown). In series I, this observation concords with that previously reported.[11,12]

In contrast, inhibition of topoisomerase II was clearly detected with these compounds. In this case, the reference drug was etoposide, which produced a marked level of DNA double-stranded breaks. Among all the tested molecules (Figure 2A), five compounds were found to stabilize the drug–DNA complex. Their ranking was **6a** > **1c** > **1a** > **1b** > **7a** (data not shown for compounds displaying no detectable DNA cleavage). A net increase in the band corresponding to linear DNA (double-stranded breaks) was noted with **6a**, which appeared to be more potent than compound **1a**. These derivatives clearly promote the cleavage of DNA by topoisomerase II and can be considered as topoisomerase II poisons. The most active topoisomerase II poisons were then compared (Figure 2B) to simplified indenoisoquinolin-5,11-dione reference-compounds **8a**,**b**. The results showed that **8a** is also capable of inducing DNA cleavage but to a lesser extent than its C-8 substituted counterpart **6a**. In series I, none of the compounds bearing two cationic amino side chains generated DNA cleavage in the presence of topoisomerase II, showing that the introduction of an amino side chain into compounds **1a–c** also suppressed the topoisomerase II poison character (data not shown). In series II, results obtained from different experiments indicated that the lactam side chain length is critical for topoisomerase II inhibition. Indeed, aminoethyl derivatives **6a**, **6f**, **7a**, **8a** are more potent than their aminopropyl counterparts **6b**, **6g** (data not shown because no DNA cleavage was detected), **7b**, and **8b**. The position of the second side chain on the tetracycle also seems to be a critical element for topoisomerase II inhibition, as the C-8 substituted derivative **6a** is a potent topoisomerase II poison whereas its C-9 substituted counterpart **6c** displayed no detectable activity. It is interesting to note that indenoisoquinolin-5,11-diones nonsubstituted on cycle D and bearing an aminoalkyl side chain on the lactam nitrogen, such as **8b**, have been described as topoisomerase I poisons.[26] This work reveals that the introduction of a dimethylaminoethoxy side chain at C-8 (compound **6b**) or at C-9 (compound **6e**) suppresses the topoisomerase I

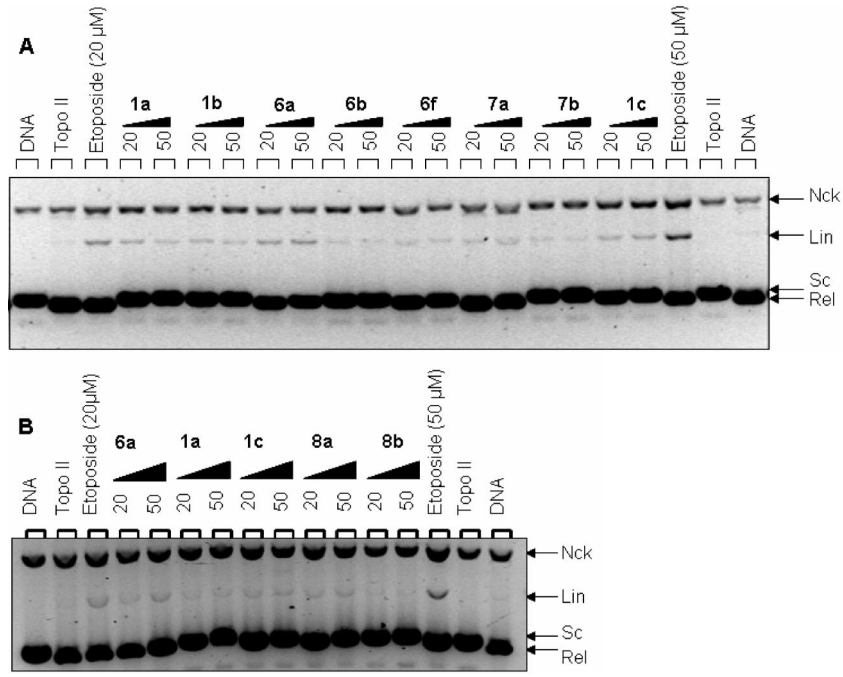


Figure 2. Effect of compounds **1a**−**c**, **6a**,**b**,**f**, **7a**,**b** on the relaxation of plasmid DNA by human topoisomerase II: (A) selected compounds displaying detectable DNA breaks; (B) comparison of most potent compounds with reference compounds **8a**,**b**. Native supercoiled pLAZ (130 ng, lane DNA) was incubated with 4 units of topoisomerase II in the absence (lane Topo II) or presence of tested compound at the indicated concentration (20 and 50 μM). Etoposide was used at 20 (left) and 50 μM (right). DNA samples were separated by electrophoresis on a 1% agarose gel containing 1 μg/mL ethidium bromide. Gels were photographed under UV light: Nck, nicked; Sc, supercoiled; Lin, linear; Rel, relaxed.

inhibitory character. This could probably be the result of unfavorable steric interactions in the cleavage complex with the topoisomerase I pocket.

These results suggest that in series II, topoisomerase I or topoisomerase II preference could be influenced by the nature and position of the cationic side chain. Similar conclusions have been reported for one-side chain-bearing anthraquinone[31] and acridine topoisomerase inhibitors.[32]

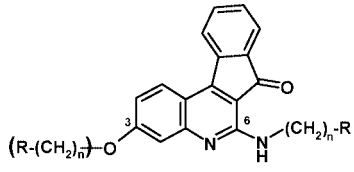
In Vitro Antiproliferative Activity. The antiproliferative activities of the compounds were tested (Table 2) using two human leukemia cell lines, HL60 and HL60/MX2, respectively sensitive and resistant to the antitumor drug mitoxantrone.

To gain insight into the involvement of topoisomerase II inhibition in the cytotoxicity of the molecules, their antiproliferative activity was assessed using the HL60/MX2 cell line resistant to mitoxantrone, which displays altered catalytic activity and reduced levels of topoisomerase II[33] (the values for mitoxantrone and etoposide are given as references). When evaluated on the HL60 cell line, all compounds displayed cytotoxicity in the micromolar and submicromolar ranges. In series I, compounds **1a** and **1b** were highly cytotoxic (IC_{50} = 33 and 88 nM, respectively). Their respective high resistance index (82 and 59) suggests a significant contribution of topoisomerase II inhibition to the cytotoxicity. Their counterparts **5a** and **5b**, bearing two side chains, displayed a significant loss of cytotoxic activity that could be attributable to their lack of topoisomerase II inhibition. In series II, the most cytotoxic compounds were **6a**,**b** and **7a**,**b**, which displayed submicromolar IC_{50}, comparable to that of the compound **8b**. When evaluated on the HL60/MX2 cell line, the strongest topoisomerase II poison **6a** was found to behave as a weaker topoisomerase II poison, such as **6b** and **8b**. The weak resistance index obtained for compound **6a** may be explained by its binding to the enzyme at a site different from that of mitoxantrone or by specific kinetics. No obvious relationship was found between cytotoxicity and topoisomerase II inhibition. This could be the result of several contributions such as differences in cellular uptake between compounds bearing two positive charges compared to their monocationic counterparts and/or involvement of targets and mechanisms different from topoisomerase II-mediated DNA cleavage.

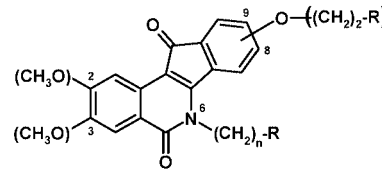
Conclusion

In this study, a second cationic side chain was introduced on known indenoquinolin-7-ones and indenoisoquinolin-5,11-diones. The designed molecules generally show an increased DNA interaction consistent with the formation of additional electrostatic contacts. However, in both series, DNA interaction was not found to correlate either with topoisomerase poisoning or with the antiproliferative activity. In series I, the introduction of a second amino side chain on indenoquinolinone **1a** and on its counterparts (**1b**,**c**) suppressed the topoisomerase II poison character, thereby probably indicating the loss of interaction with the enzyme in the cleavable complex. In series II, the introduction of a second amino side chain on cycle D, as well as a hydroxyl group, suppressed topoisomerase I inhibition compared to the reference compounds. Nevertheless, the introduction of a cationic side chain at C-8 position enhanced topoisomerase II inhibition potency over C-8 nonsubstituted or C-8 hydroxylated derivatives, presumably by promoting favorable interactions within the enzyme active site. The length of the side chain on the lactam nitrogen also influenced topoisomerase inhibition, as an ethyl group provided better inhibition than a propyl group. The best topoisomerase II poison was compound **6a**, displaying better potency than the reference topoisomerase poison **1a**. This compound also displayed a potent cytotoxicity toward HL60 leukemia cells, comparable to that of etoposide. However, the lack of correlation between cytotoxicity and topoisomerase II inhibition for these compounds, together with the low relative resistance index they display

Table 2. Cytotoxicities of Series I and Series II Compounds



Series I



Series II

compd	IC_{50} (μM)[a] HL60	HL60/MX2	RRI[b]
MX[c]	0.063 ± 0.020	1.51 ± 0.08	24
Etop[d]	0.486 ± 0.190	48.5 ± 1.5	100

Series I

compd	C-3	*n*	R	IC_{50} (μM)[a] HL60	HL60/MX2	RRI[b]
1a	OH	2	$N(CH_3)_2$	0.033 ± 0.010	2.70 ± 0.14	82
1b	OH	2	$N(CH_2)_4$	0.088 ± 0.010	5.18 ± 0.11	59
1c	OH	3	$N(CH_3)_2$	2.83 ± 0.22	28.5 ± 1.8	10
5a	$O(CH_2)_nR$	2	$N(CH_3)_2$	4.64 ± 0.47	4.95 ± 0.66	1.1
5b	$O(CH_2)_nR$	2	$N(CH_2)_4$	3.01 ± 1.28	3.81 ± 2.14	1.3
5c	$O(CH_2)_nR$	3	$N(CH_3)_2$	5.44 ± 0.05	9.14 ± 0.58	1.7

Series II

compd	C-8	C-9	*n*	R	C-2,3	IC_{50} (μM)[a] HL60	HL60/MX2	RRI[b]
6a	$O(CH_2)_2R$		2	$N(CH_3)_2$		0.64 ± 0.03	5.26 ± 0.23	8.3
6b	$O(CH_2)_2R$		3	$N(CH_3)_2$		0.87 ± 0.06	6.61 ± 0.68	7.6
6c		$O(CH_2)_2R$	2	$N(CH_3)_2$		1.55 ± 0.19	2.55 ± 0.81	1.7
6d		$O(CH_2)_2R$	2	$N(CH_2)_4$		1.81 ± 0.12	5.25 ± 0.58	2.9
6e		$O(CH_2)_2R$	3	$N(CH_3)_2$		4.62 ± 0.44	4.41 ± 0.61	1.0
6f		$O(CH_2)_2R$	2	$N(CH_3)_2$	OCH_3	4.88 ± 0.21	5.26 ± 0.19	1.1
6g		$O(CH_2)_2R$	3	$N(CH_3)_2$	OCH_3	7.46 ± 1.99	10.80 ± 7.55	1.4
7a		OH	2	$N(CH_3)_2$		0.62 ± 0.14	nd[e]	nd[e]
7b		OH	3	$N(CH_3)_2$		0.52 ± 0.01	2.97 ± 0.27	5.8
8a			2	$N(CH_3)_2$		1.05 ± 0.01	7.35 ± 0.49	7.0
8b			3	$N(CH_3)_2$		0.63 ± 0.07	4.92 ± 0.61	7.8

[a] IC_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Relative resistance index: $IC_{50}^{(MX\text{-}resistant)}/IC_{50}^{(MX\text{-}sensitive)}$. [c] MX: mitoxantrone. [d] Etop: etoposide. [e] nd: not determined.

against mitoxantrone-resistant cells, suggests that additional mechanisms and/or targets could be involved.

Experimental Section

Chemistry. Melting points were determined with a Büchi 535 capillary melting point apparatus and remain uncorrected. Analytical thin-layer chromatography (TLC) was performed on precoated Kieselgel 60F_{254} plates (Merck). The spots were located by UV (254 and 366 nm), and R_f values are given for guidance. Silica gel 60 230–400 mesh purchased from Merck was used for column chromatography. Thick-layer chromatography (TkLC) was performed using silica gel from Merck, from which the compounds were extracted by the solvent system: acetone/aqueous NH_4OH 8:2. The structure of all compounds was supported by IR (FT-Bruker Vector 22 instrument), by ^1H NMR at 300 MHz on a Bruker DRX-300 spectrometer, or by ^{13}C NMR at 75 MHz on a Bruker AC-300 spectrometer. Chemical shifts were reported in ppm using tetramethylsilane as a standard. *J* values are in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; dd, double doublet; td, triple doublet; t, triplet; dt, double triplet; qt, quadruple triplet; q, quadruplet; qt, quintuplet; m, multiplet; b, broad. Analytical HPLC for assessing the purity of final compounds was performed on a Kontron 325 system equipped with a C_{18} reversed-phase column (Kromasil C18) and a UV diode array detector (DAD 440L). Compounds were detected between 200 and 400 nm. Analysis was performed isocratically (eluent H_2O/MeOH/TFA 58:42:0.1). HPLC retention times (t_R) were obtained at a flow rate of 1 mL/min. LC−MS spectra were performed on a Thermo Electron Surveyor MSQ spectrometer. High performance liquid chromatography (ODS column, elution gradient, H_2O/CH_3CN/HCOOH), with a UV detector and with an $APCI^+$ (atmospheric pression chemical ionization) mass detector, was also used to verify the purity of final compounds. Elemental analyses were performed by the "Service Central d'Analyses" at the CNRS, Vernaison (France). Commercially available reagents and solvents were used throughout without further purification. Dichloromethane was dried on calcium chloride.

General Procedure for the Synthesis of Compounds 14a–c. The appropriate aminoalkylamine was added to a suspension of compound **13**[24] (3.0 g, 10.1 mmol, 1 equiv) in pyridine (30 mL), and the mixture was heated while stirring at 100 °C for 18 h. The solvent was removed under reduced pressure, and dichloromethane (50 mL) and water (30 mL) were added to the residue for extraction. The organic layer was washed with 1 N sodium bicarbonate (3 × 20 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 9:1).

3-Methoxy-6-(3-dimethylaminoethylamino)indeno[2,1-*c*]quinolin-7-one (14a). Compound **14a** was obtained as a red oil (1.8 g, 51% yield) from *N*,*N*-dimethylethane-1,2-diamine (5.6 mL, 51 mmol, 5 equiv). R_f = 0.20 (CH_2Cl_2/MeOH 9:1); mp 166–167 °C; IR (neat) 3373, 2930, 2770, 1687, 1618, 1587, 1532, 1460, 1419 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.13 (d, J = 9.3 Hz, 1H, Ar), 7.98 (d, J = 7.6 Hz, 1H, Ar), 7.66 (d, J = 7.1 Hz, 1H, Ar), 7.53 (dd, J = 7.6 Hz, 1H, Ar), 7.42 (dd, J = 7.1 Hz, 1H, Ar), 7.35–7.32 (m, 1H, NH), 7.07 (d, J = 2.7 Hz, 1H, Ar), 6.93 (dd, J = 9.3 Hz, J = 2.7

Hz, 1H, Ar), 3.96 (s, 3H, CH_3), 3.80 (dt, $J = 6.0$ Hz, 2H, CH_2), 2.70 (t, $J = 6.0$ Hz, 2H, CH_2), 2.40 (s, 6H, CH_3).

3-Methoxy-6-(2-pyrrolidin-1-ylethylamino)indeno[2,1-*c*]quinolin-7-one (14b). Compound **14b** was obtained as a red oil (1.7 g, 45% yield) from 2-pyrrolidin-1-ylethylamine (6.5 mL, 51 mmol, 5 equiv). $R_f = 0.25$ (CH_2Cl_2/MeOH 9:1); IR (neat) 3372, 2930, 2778, 1687, 1618, 1588, 1531, 1463, 1419 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.13 (d, $J = 9.2$ Hz, 1H, Ar), 7.98 (d, $J = 7.6$ Hz, 1H, Ar), 7.67 (d, $J = 7.4$ Hz, 1H, Ar), 7.53 (dd, $J = 7.6$ Hz, 1H, Ar), 7.42 (dd, $J = 7.4$ Hz, 1H, Ar), 7.31 (m, 1H, NH), 7.07 (d, $J = 2.1$ Hz, 1H, Ar), 6.94 (dd, $J = 9.2$ Hz, $J = 2.1$ Hz, 1H, Ar), 3.96 (s, 3H), 3.91–3.85 (m, 2H, CH_2), 2.93 (m, 2H, CH_2), 2.76 (m, 4H, CH_2), 1.88 (m, 4H, CH_2).

3-Methoxy-6-(3-dimethylaminopropylamino)indeno[2,1-*c*]quinolin-7-one (14c). Compound **14c** was obtained as a red oil (1.6 g, 43% yield) from *N*,*N*-dimethylpropane-1,3-diamine (6.4 mL, 51 mmol, 5 equiv). $R_f = 0.25$ (CH_2Cl_2/MeOH 9:1); IR (neat) 3360, 2933, 1686, 1619, 1602, 1585, 1536, 1459, 1437, 1427 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (d, $J = 9.4$ Hz, 1H, Ar), 7.95 (d, $J = 7.6$ Hz, 1H, Ar), 7.65 (d, $J = 7.1$ Hz, 1H, Ar), 7.50 (dd, $J = 7.6$ Hz, 1H, Ar), 7.41 (dd, $J = 7.1$ Hz, 1H, Ar), 7.26–7.24 (m, 1H, NH), 7.06 (d, $J = 2.5$ Hz, 1H, Ar), 6.86 (dd, $J = 9.4$ Hz, $J = 2.5$ Hz, 1H, Ar), 3.96 (s, 3H, CH_3), 3.76–3.70 (m, 2H, CH_2), 2.55–2.48 (m, 2H, CH_2), 1.93–1.89 (m, 2H, CH_2).

General Procedure for the Synthesis of Compounds 1a–c. Aqueous hydrobromic acid (47%, 40 mL) was added to a solution of the appropriate compound **14a–c** (8.7 mmol) in acetic acid (40 mL), and the mixture was refluxed for 65 h. The reaction mixture was brought to dryness, water (30 mL) was added to the residue, and the solution was adjusted to pH 8 with aqueous ammonia and subsequently extracted with dichloromethane (6 × 50 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2) and recrystallized from ethanol.

3-Hydroxy-6-(2-dimethylaminoethylamino)indeno[2,1-*c*]quinolin-7-one (1a). Compound **1a** was obtained as a red solid (2.0 g, 69% yield) from compound **14a** (3.0 g, 8.7 mmol). $R_f = 0.35$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 216–217 °C; IR (neat) 3378, 1679, 1613, 1582, 1543, 1458, 1428, 1403 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (d, $J = 9.3$ Hz, 1H, Ar), 7.52–7.49 (m, 1H, NH), 7.45–7.32 (m, 3H, Ar), 7.18 (dd, $J = 7.3$ Hz, 1H, Ar), 7.09 (dd, $J = 7.3$ Hz, 1H, Ar), 6.74–6.71 (m, 1H, Ar), 3.62–3.59 (m, 2H, CH_2), 2.69–2.66 (m, 2H, CH_2), 2.38 (s, 6H, CH_3); HPLC; LC−MS.

3-Hydroxy-6-(2-pyrrolidin-1-ylethylamino)indeno[2,1-*c*]quinolin-7-one (1b). Compound **1b** was obtained as an orange solid (1.9 g, 60% yield) from compound **14b** (3.2 g, 8.7 mmol)). $R_f = 0.35$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 126 °C (dec); IR (neat) 3374, 2822, 1676, 1611, 1587, 1502, 1456, 1414 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (d, $J = 9.5$ Hz, 1H, Ar), 7.50 (d, $J = 6.7$ Hz, 1H, Ar), 7.45 (d, $J = 6.6$ Hz, 1H, Ar), 7.29–7.19 (m, 3H, Ar), 7.05 (bs, 1H, NH), 6.71 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, Ar), 3.73–3.69 (m, 2H, CH_2), 3.02–2.99 (m, 2H, CH_2), 2.91–2.88 (m, 4H, CH_2), 1.96–1.93 (m, 4H, CH_2); HPLC; LC−MS.

3-Hydroxy-6-(3-dimethylaminopropylamino)indeno[2,1-*c*]quinolin-7-one (1c). Compound **1c** was obtained as a red solid (2.0 g, 65% yield) from compound **14c** (3.1 g, 8.7 mmol). $R_f = 0.40$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 110 °C (dec); IR (neat) 3360, 1682, 1615, 1578, 1528, 1457, 1417 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.33–8.25 (m, 2H, Ar), 7.66–7.61 (m, 2H, Ar), 7.54–7.51 (m, 1H, Ar), 7.39–7.36 (m, 1H, NH), 6.93–6.87 (m, 2H, Ar), 3.60 (dt, $J = 6.5$ Hz, 2H, CH_2), 2.42–2.39 (m, 2H, CH_2), 2.25 (s, 6H, CH_3), 1.80–1.77 (m, 2H, CH_2); HPLC; LC−MS.

General Procedure for the Synthesis of Compounds 5a–c. The appropriate chloroalkylamine (2.1 mmol, 1.5 equiv), cesium carbonate (0.46 g, 1.4 mmol, 1 equiv), and sodium iodide (0.31 g, 2.1 mmol, 1.5 equiv) were added to the appropriate compounds **1a–c**, (1.4 mmol, 1 equiv) in DMF (15 mL). The reaction mixture was heated to 80 °C for 3 h. After cooling, the reaction mixture was diluted with dichloromethane (50 mL) and washed with brine (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2).

3-(2-Dimethylaminoethoxy)-6-(2-dimethylaminoethylamino)indeno[2,1-*c*]quinolin-7-one (5a). Compound **5a** was obtained from compound **1a** (0.47 g, 1.4 mmol) and 2-chloroethyldimethylamine hydrochloride (0.30 g, 2.1 mmol). After crystallization from ethyl acetate, compound **5a** was obtained as an orange solid (0.22 g, 38% yield). $R_f = 0.60$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 123–124 °C; IR (neat) 2772, 1692, 1618, 1601, 1585, 1532, 1461, 1423 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.13 (d, $J = 9.1$ Hz, 1H, Ar), 7.99 (d, $J = 7.3$ Hz, 1H, Ar), 7.66 (d, $J = 7.3$ Hz, 1H, Ar), 7.53 (dd, $J = 7.4$ Hz, 1H, Ar), 7.42 (dd, $J = 7.4$ Hz, 1H, Ar), 7.34 (bs, 1H, NH), 7.07 (d, $J = 2.4$ Hz, 1H, Ar), 6.99 (dd, $J = 9.1$ Hz, $J = 2.4$ Hz, 1H, Ar), 4.23 (t, $J = 5.5$ Hz, 2H, CH_2), 3.77 (dt, $J = 6.2$ Hz, 2H, CH_2), 2.82 (dd, $J = 5.5$ Hz, 2H, CH_2), 2.66 (dd, $J = 6.2$ Hz, 2H, CH_2), 2.38 (s, 6H, CH_3), 2.37 (s, 6H, CH_3); HPLC; LC−MS.

3-(2-Pyrrolidin-1-ylethoxy)-6-(2-pyrrolidin-1-ylethylamino)indeno[2,1-*c*]quinolin-7-one (5b). Compound **5b** was obtained from compound **1b** (0.50 g, 1.4 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (0.36 g, 2.1 mmol). After crystallization from ethyl acetate, compound **5b** was obtained as an orange solid (0.21 g, 33% yield). $R_f = 0.60$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 116–117 °C; IR (neat) 2925, 2854, 2763, 1691, 1616, 1599, 1583, 1530, 1458, 1428 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.96 (d, $J = 9.1$ Hz, 1H, Ar), 7.83 (d, $J = 7.4$ Hz, 1H, Ar), 7.56 (d, $J = 7.3$ Hz, 1H, Ar), 7.44–7.39 (m, 1H, Ar), 7.34–7.30 (m, 1H, Ar), 7.23–7.22 (m, 1H, NH), 7.07 (d, $J = 2.3$ Hz, 1H, Ar), 6.98 (dd, $J = 9.0$ Hz, $J = 2.3$ Hz, 1H, Ar), 4.28 (t, $J = 5.8$ Hz, 2H, CH_2), 3.86–3.81 (m, 2H, CH_2), 3.01 (t, $J = 5.6$ Hz, 2H, CH_2), 2.91–2.88 (m, 2H, CH_2), 2.71–2.66 (m, 8H, CH_2), 1.88–1.85 (m, 8H, CH_2); HPLC; LC−MS.

3-(3-Dimethylaminopropoxy)-6-(3-dimethylaminopropylamino)indeno[2,1-*c*]quinolin-7-one (5c). Compound **5c** was obtained from compound **1c** (0.49 g, 1.4 mmol) and 3-chloropropyldimethylamine hydrochloride (0.33 g, 2.1 mmol). After crystallization from ethyl acetate, compound **5c** was obtained as an orange solid (0.21 g, 35% yield). $R_f = 0.65$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 93–94 °C; IR (neat) 2924, 2855, 2764, 1691, 1616, 1599, 1583, 1530, 1458, 1425 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (d, $J = 9.4$ Hz, 1H, Ar), 7.96 (d, $J = 7.3$ Hz, 1H, Ar), 7.65 (d, $J = 6.3$ Hz, 1H, Ar), 7.51 (t, $J = 7.7$ Hz, 1H, Ar), 7.41 (t, $J = 7.1$ Hz, 1H, Ar), 7.26–7.24 (m, 1H, NH), 7.06 (d, $J = 2.4$ Hz, 1H, Ar), 6.91 (dd, $J = 9.1$ Hz, $J = 2.5$ Hz, 1H, Ar), 4.17 (t, $J = 6.3$ Hz, 2H, CH_2), 3.72 (q, $J = 6.6$ Hz, 2H, CH_2), 2.49 (q, $J = 7.6$ Hz, 4H, CH_2), 2.37 (s, 6H, CH_3), 2.36 (s, 6H, CH_3), 2.09–2.06 (m, 2H, CH_2), 1.93–1.90 (m, 2H, CH_2); HPLC; LC−MS.

General Procedure for the Synthesis of Compounds 17–19. A suspension of appropriate hydroxybenzaldehyde **15**, **16** (70 mmol, 2 equiv), and potassium carbonate (14.4 g, 101 mmol, 3 equiv) in DMF (20 mL) was stirred at 80 °C for 30 min. The appropriate alkyl chloride (35 mmol, 1 equiv) was added, and the reaction mixture was heated and maintained at 80 °C for 5 h. After cooling, the reaction mixture was diluted in water (200 mL) and extracted with ethyl acetate (2 × 250 mL). The organic layer was separated, washed with brine (3 × 100 mL), and dried over $MgSO_4$ before the solvent was evaporated to give the desired product.

3-(2-Dimethylaminoethoxy)benzaldehyde (17). Compound **17** was obtained as a brown oil (3.11 g, 46% yield) from aldehyde **15** (8.55 g, 70 mmol) and (2-chloroethyl)dimethylamine (3.76 g, 35 mmol). $R_f = 0.55$ (CH_2Cl_2/MeOH 8:2); IR (neat) 2945, 2822, 2774, 1697, 1596, 1486, 1455; ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, CHO), 7.37–7.33 (m, 3H, Ar), 7.14–7.11 (m, 1H, Ar), 4.04 (t, $J = 5.2$ Hz, 2H, CH_2), 2.65 (t, $J = 5.2$ Hz, 2H, CH_2), 2.27 (s, 6H, CH_3).

4-(2-Dimethylaminoethoxy)benzaldehyde (18). Compound **18** was obtained as a brown oil (2.96 g, 44% yield) from aldehyde **16** (8.55 g, 70 mmol) and (2-chloroethyl)dimethylamine (3.76 g, 35 mmol). $R_f = 0.50$ (CH_2Cl_2/MeOH 8:2); IR (neat) 2900, 2815, 2725, 1677, 1603, 1578, 1509; ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, CHO),

7.83 (d, $J = 8.7$ Hz, 2H, Ar), 7.03 (d, $J = 8.7$ Hz, 2H, Ar), 4.16 (t, $J = 5.7$ Hz, 2H, CH_2), 2.78 (t, $J = 5.7$ Hz, 2H, CH_2), 2.36 (s, 6H, CH_3).

4-(2-Pyrrolidin-1-ylethoxy)benzaldehyde (19). Compound **19** was obtained as a brown oil (5.37 g, 70% yield) from aldehyde **16** (8.55 g, 70 mmol) and 1-(2-chloroethyl)pyrrolidine (4.68 g, 35 mmol). $R_f = 0.50$ (CH_2Cl_2/MeOH 8:2); IR (neat) 2920, 2823, 2750, 1678, 1601, 1578, 1510; ^{1}H NMR ($CDCl_3$) δ 9.84 (s, 1H, CHO), 7.79 (d, $J = 8.7$ Hz, 2H, Ar), 6.99 (d, $J = 8.7$ Hz, 2H, Ar), 4.11 (t, $J = 5.7$ Hz, 2H, CH_2), 2.73 (t, $J = 5.7$ Hz, 2H, CH_2), 2.33–2.29 (m, 8H, CH_2).

4-Formylphenyl Benzoate (24). A solution aldehyde **16** (4.0 g, 32.7 mmol, 1 equiv) and triethylamine (4.6 mL, 32.7 mmol, 1 equiv) in dry CH_2Cl_2 (40 mL) was cooled to 0 °C before benzoyl chloride (4.62 g, 32.7 mmol) was added dropwise, and the reaction mixture was stirred for 3 h at 0 °C. The reaction mixture was diluted with CH_2Cl_2 and washed with 1 M $NaHCO_3$ (2 × 25 mL). Ester **24** was obtained as a white powder after crystallization from ethanol (4.07 g, 55% yield). $R_f = 0.55$ (cyclohexane/AcOEt 8:2); mp 91–92 °C; IR (neat) 1738, 1699, 1597; ^{1}H NMR ($CDCl_3$) δ 10.04 (s, 1H, CHO), 8.22 (d, $J = 7.4$ Hz, 2H, Ar), 8.00 (d, $J = 8.5$ Hz, 2H, Ar), 7.69–7.66 (m, 1H, Ar), 7.57–7.53 (m, 2H, Ar), 7.43 (d, $J = 8.5$ Hz, 2H, Ar).

General Procedure for the Synthesis of Imines 20a–e and 25a,b. The appropriate amine R_3NH_2 (13 mmol, 1 equiv) and $MgSO_4$ (5.0 g) were added to a solution of aldehyde **17**–**19** (13 mmol, 1 equiv) in $CHCl_3$ (25 mL), and the reaction mixture was stirred for 6 h. The reaction mixture was filtered and the filtrate was washed with a solution of 1 M $NaHCO_3$ (2 × 25 mL), dried over $MgSO_4$, and concentrated under reduced pressure to give the desired product.

***N*-[3-(2-Dimethylaminoethoxy)benzylidene]-*N*′,*N*′-dimethylethane-1,2-diamine (20a).** Compound **20a** was obtained as a brown oil (3.35 g, 98% yield) from aldehyde **17** (2.85 g) and *N*,*N*-dimethylethane-1,2-diamine (1.43 mL). IR (neat) 1596, 1266, 1039 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H, CH), 7.31–7.16 (m, 3H, Ar), 6.93 (dd, $J = 6.6$ Hz, $J = 1.3$ Hz, 1H, Ar), 4.05 (t, $J = 7.0$ Hz, 2H, CH_2), 3.87 (t, $J = 5.6$ Hz, 2H, CH_2), 2.68 (t, $J = 5.6$ Hz, 2H, CH_2), 2.59 (t, $J = 7.0$ Hz, 2H, CH_2), 2.28 (s, 6H, CH_3), 2.26 (s, 6H, CH_3).

***N*-[3-(2-Dimethylaminoethoxy)benzylidene]-*N*′,*N*′-dimethylpropane-1,3-diamine (20b).** Compound **20b** was obtained as a brown oil (2.63 g, 73% yield) from aldehyde **17** (2.85 g) and *N*,*N*-dimethylpropane-1,3-diamine (1.64 mL). IR (neat) 1654, 1273, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H, CH), 7.34–7.17 (m, 3H, Ar), 7.00–6.94 (m, 1H, Ar), 4.08 (t, $J = 5.6$ Hz, 2H, CH_2), 3.61 (t, $J = 6.9$ Hz, 2H, CH_2), 2.70 (t, $J = 5.6$ Hz, 2H, CH_2), 2.33–2.29 (m, 8H, CH_3, CH_2), 2.21 (s, 6H, CH_3), 1.84 (qt, $J = 7.2$ Hz, 2H, CH_2).

***N*-[4-(2-Dimethylaminoethoxy)benzylidene]-*N*′,*N*′-dimethylethane-1,2-diamine (20c).** Compound **20c** was obtained as a brown oil (3.01 g, 88% yield) from aldehyde **18** (2.51 g) and *N*,*N*-dimethylethane-1,2-diamine (1.43 mL). IR (neat) 1605, 1246, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H, CH), 7.65 (d, $J = 8.8$ Hz, 2H, Ar), 6.92 (d, $J = 8.8$ Hz, 2H, Ar), 4.08 (t, $J = 5.7$ Hz, 2H, CH_2), 3.69 (t, $J = 6.7$ Hz, 2H, CH_2), 2.72 (t, $J = 5.7$ Hz, 2H, CH_2), 2.61 (t, $J = 6.7$ Hz, 2H, CH_2), 2.32 (s, 6H, CH_3), 2.29 (s, 6H, CH_3).

[4-(2-Pyrrolidin-1-ylethoxy)benzylidene]-(2-pyrrolidin-1-ylethyl)amine (20d). Compound **20d** was obtained as a brown oil (2.51 g, 61% yield) from aldehyde **19** (2.85 g) and 2-pyrrolidin-1-ylethylamine (1.65 mL). IR (neat) 2961, 1785, 1604, 1251, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1H, CH), 7.58 (d, $J = 7.6$ Hz, 2H, Ar), 6.86 (d, $J = 7.6$ Hz, 2H, Ar), 4.50 (t, $J = 6.2$ Hz, 2H, CH_2), 3.68 (t, $J = 7.1$ Hz, 2H, CH_2), 2.85 (t, $J = 6.0$ Hz, 2H, CH_2), 2.80 (t, $J = 7.2$ Hz, 2H, CH_2), 2.54–2.51 (m, 8H, CH_2), 1.75–1.71 (m, 8H, CH_2).

***N*-[4-(2-Dimethylaminoethoxy)benzylidene]-*N*′,*N*′-dimethylpropane-1,3-diamine (20e).** Compound **20e** was obtained as a brown oil (2.45 g, 68% yield) from aldehyde **18** (2.51 g) and *N*,*N*-dimethylpropane-1,3-diamine (1.64 mL). IR (neat) 1604, 1251, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1H, CH), 7.60 (dd, $J = 8.8$ Hz, $J = 2.9$ Hz, 2H, Ar), 6.90 (dd, $J = 8.8$ Hz, $J = 2.9$ Hz, 2H, Ar), 4.07–4.02 (m, 2H, CH_2), 3.58–3.53 (m, 2H, CH_2), 2.71–2.66 (m, 2H, CH_2), 2.29–2.28 (m, 8H, CH_3, CH_2), 2.18 (s, 6H, CH_3).

4-[(2-Dimethylaminoethylimino)methyl]phenyl Benzoate (25a). Compound **25a** was obtained as a light-yellow oil (3.58 g, 93% yield) from aldehyde **24** (2.94 g) and *N*,*N*-dimethylethane-1,2-diamine (1.43 mL). IR (neat) 3341, 1643, 1601, 1580, 1290 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H, CH), 8.22–8.19 (m, 2H, Ar), 7.80 (d, $J = 8.3$ Hz, 2H, Ar), 7.66–7.63 (m, 1H, Ar), 7.54–7.49 (m, 2H, Ar), 7.27 (d, $J = 8.6$ Hz, 2H, Ar), 3.76 (t, $J = 6.8$ Hz, 2H, CH_2), 2.67 (t, $J = 6.8$ Hz, 2H, CH_2), 2.32 (s, 6H, CH_3).

4-[(3-Dimethylaminopropylimino)methyl]phenyl Benzoate (25b). Compound **25b** was obtained as a light-yellow oil (3.83 g, 95% yield) from aldehyde **24** (2.94 g) and *N*,*N*-dimethylpropane-1,3-diamine (1.64 mL). IR (neat) 3340, 1641, 1601, 1578, 1291 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H, CH), 8.23–8.19 (m, 2H, Ar), 7.79 (d, $J = 8.1$ Hz, 2H, Ar), 7.65–7.62 (m, 1H, Ar), 7.53–7.49 (m, 2H, Ar), 7.26 (d, $J = 8.5$ Hz, 2H, Ar), 3.60–3.54 (m, 2H, CH_2), 2.68–2.64 (m, 2H, CH_2), 2.42 (s, 6H, CH_3), 1.90–1.86 (m, 2H, CH_2).

General Procedure for the Synthesis of Carboxylic Acids 23a–g and 26a,b. Appropriate homophtalic anhydride **21** or **22** (11.4 mmol, 1 equiv) was added to a solution of imine **20a**–**e** (11.4 mmol, 1 equiv) in MeOH for compounds **23a**–**e** (60 mL) or in THF for compounds **23f**,**g** and **26a**,**b** (60 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure to give a yellow solid containing a mixture of cis and trans diastereoisomers of acids **23a**–**g** and **26a**,**b**. The cis/trans ratio was calculated by ^{1}H NMR based on the integration of characteristic peaks of each isomer each time it was possible. The crude mixture of diastereoisomers was further reacted without purification.

3-[3-(2-Dimethylaminoethoxy)phenyl]-2-(2-dimethylaminoethyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23a). Carboxylic acid **23a** was obtained from anhydride **21** (1.84 g, 11.4 mmol) and imine **20a** (3.0 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.35 (s, H_{trans}), 5.22 (d, $J = 6.4$ Hz, H_{cis}), 4.63 (d, $J = 6.4$ Hz, H_{cis}), 3.87 (s, H_{trans}), cis/trans ratio = 1:2.

3-[3-(2-Dimethylaminoethoxy)phenyl]-2-(3-dimethylaminopropyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23b). Carboxylic acid **23b** was obtained from carboxylic anhydride **21** (1.84 g, 11.4 mmol) and imine **20b** (3.16 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.43 (s, H_{trans}), 5.29 (d, $J = 6.3$ Hz, H_{cis}), 4.70 (d, $J = 6.3$ Hz, H_{cis}), 3.76 (s, H_{trans}), cis/trans ratio = 1:2.

3-[4-(2-Dimethylaminoethoxy)phenyl]-2-(2-dimethylaminoethyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23c). Carboxylic acid **23c** was obtained from carboxylic anhydride **21** (1.84 g, 11.4 mmol) and imine **20c** (3.0 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.26 (s, H_{trans}), 5.13 (d, $J = 6.0$ Hz, H_{cis}), 4.57 (d, $J = 6.0$ Hz, H_{cis}), 3.83 (s, H_{trans}), cis/trans ratio = 1:2.

1-Oxo-3-[4-(2-pyrrolidin-1-ylethoxy)phenyl]-2-(2-pyrrolidin-1-ylethyl)-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23d). Carboxylic acid **23d** was obtained from carboxylic anhydride **21** (1.84 g, 11.4 mmol) and imine **20d** (3.16 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.20 (s, H_{trans}), 5.15 (d, $J = 5.6$ Hz, H_{cis}), 4.53 (d, $J = 5.6$ Hz, H_{cis}), 3.78 (s, H_{trans}), cis/trans ratio = 1:2.

3-[4-(2-Dimethylaminoethoxy)phenyl]-2-(3-dimethylaminopropyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23e). Carboxylic acid **23e** was obtained from carboxylic anhydride **21** (1.84 g, 11.4 mmol) and imine **20e** (3.16 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.44 (s, H_{trans}), 5.30 (d, $J = 5.7$ Hz, H_{cis}), 4.73 (d, $J = 5.7$ Hz, H_{cis}), 3.82 (s, H_{trans}), cis/trans ratio = 1:3.

6,7-Dimethoxy-3-[4-(2-dimethylaminoethoxy)phenyl]-2-(2-dimethylaminoethyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23f). Carboxylic acid **23f** was obtained from carboxylic anhydride **22**[27] (2.51 g, 11.4 mmol) and imine **20c** (3.0 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.18 (s,

H_{trans}), 5.12 (d, $J = 6.0$ Hz, H_{cis}), 4.52 (d, $J = 6.0$ Hz, H_{cis}), 3.76 (s, H_{trans}), cis/trans ratio = 1:2.

6,7-Dimethoxy-3-[4-(2-dimethylaminoethoxy)phenyl]-2-(3-dimethylaminopropyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (23g). Carboxylic acid **23g** was obtained from carboxylic anhydride **22**[27] (2.51 g, 11.4 mmol) and imine **20e** (3.16 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.34 (s, H_{trans}), 5.25 (d, $J = 6.4$ Hz, H_{cis}), 4.61 (d, $J = 6.4$ Hz, H_{cis}), 3.70 (s, H_{trans}), cis/trans ratio = 2:3.

3-(4-Benzoyloxyphenyl)-2-(2-dimethylaminoethyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (26a). Carboxylic acid **26a** was obtained from carboxylic anhydride **21** (2.51 g, 11.4 mmol) and imine **25a** (3.37 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.41 (s, H_{trans}), 5.35 (bs, H_{cis}), 4.80 (bs, H_{cis}), 3.89 (s, H_{trans}), cis/trans ratio = 1:2.

3-(4-Benzoyloxyphenyl)-2-(3-dimethylaminopropyl)-1-oxo-1,2,3,4-tetrahydroisoquinolin-4-carboxylic Acid (26b). Carboxylic acid **26b** was obtained from carboxylic anhydride **21** (2.51 g, 11.4 mmol) and imine **25b** (3.54 g, 11.4 mmol). Characteristic signals: ^{1}H NMR ($CDCl_3$) δ 5.49 (s, H_{trans}), 4.70 (bs, H_{cis}), 3.88 (s, H_{trans}).

General Procedure for the Synthesis of Indenoisoquinolines 6a–g and 27a,b. Thionyl chloride (10 mL) was added dropwise to the carboxylic acids **23a**–**g** and **26a**,**b** (3 mmol, 1 equiv) while stirring, and the reaction mixture was refluxed at 80 °C for 12 h. After cooling, the reaction mixture was concentrated under reduced pressure, diluted in dry CH_2Cl_2 (20 mL), and cooled to 0 °C. Aluminum chloride was added slowly, and the reaction mixture was stirred for 3 h at 0 °C. A solution of 1 N HCl (200 mL) was then added dropwise, and the aqueous layer was separated and washed with CH_2Cl_2 (3 × 100 mL). The aqueous layer was alkalinized with brine until pH 8 and subsequently extracted with ethyl acetate (3 × 200 mL). The organic layer was washed with brine (3 × 200 mL), dried over $MgSO_4$, and concentrated under reduced pressure to give the crude product, which was purified to obtain indenoisoquinolines **6a**–**g** and **27a**,**b**.

8-(2-Dimethylaminoethoxy)-6-(2-dimethylaminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6a). Compound **6a** was obtained as an orange solid (100 mg, 24% yield from cis isomer) from carboxylic acid **23a** (1.27 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2) followed by crystallization from ethanol. $R_f = 0.80$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 197–198 °C; IR (neat) 1652, 1610, 1273, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.73 (d, $J = 8.0$ Hz, 1H, Ar), 8.35 (d, $J = 8.0$ Hz, 1H, Ar), 7.73 (t, $J = 7.6$ Hz, 1H, Ar), 7.58 (d, $J = 8.0$ Hz, 1H, Ar), 7.48 (t, $J = 7.3$ Hz, 1H, Ar), 7.41 (s, 1H, Ar), 6.81 (dd, $J = 8.0$ Hz, $J = 1.8$ Hz, 1H, Ar), 4.71 (t, $J = 7.5$ Hz, 2H, CH_2), 4.59 (m, 2H, CH_2), 2.92–2.71 (m, 4H, CH_2), 2.42 (m, 12H, CH_3); HPLC; LC−MS.

8-(2-Dimethylaminoethoxy)-6-(3-dimethylaminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6b). Compound **6b** was obtained as an orange solid (84 mg, 20% yield from cis isomer) from carboxylic acid **23b** (1.31 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2) followed by crystallization from ethanol/isopropanol 9:1. $R_f = 0.80$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 160–162 °C; IR (neat) 1654, 1612, 1242, 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.74 (d, $J = 7.8$ Hz, 1H, Ar), 8.34 (d, $J = 8.6$ Hz, 1H, Ar), 7.73 (td, $J = 8.3$ Hz, $J = 1.3$ Hz, 1H, Ar), 7.58 (d, $J = 8.0$ Hz, 1H, Ar), 7.47 (td, $J = 7.2$ Hz, $J = 1.0$ Hz, 1H, Ar), 7.41 (d, $J = 1.8$ Hz, 1H, Ar), 6.77 (dd, $J = 8.0$ Hz, $J = 1.8$ Hz, 1H, Ar), 4.57 (t, 2H, $J = 7.6$ Hz, CH_2), 4.18 (t, $J = 5.5$ Hz, 2H, CH_2), 2.83 (t, $J = 5.5$ Hz, 2H, CH_2), 2.63 (m, 2H, CH_2), 2.41 (s, 6H, CH_3), 2.38 (s, 6H, CH_3), 2.11 (m, 2H, CH_2); HPLC; LC−MS.

9-(2-Dimethylaminoethoxy)-6-(2-dimethylaminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6c). Compound **6c** was obtained as a red solid (185 mg, 45% yield from cis isomer) from carboxylic acid **23c** (1.27 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 7:3:0.2) followed by TkLC (acetone/25% aqueous NH_4OH 95:5). $R_f = 0.85$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 114–115 °C; IR (neat) 1667, 1610, 1225, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.66 (d, $J = 8.1$ Hz, 1H, Ar), 8.31 (d, $J = 8.7$ Hz, 1H, Ar), 7.74–7.65 (m, 2H, Ar), 7.44 (m, 1H, Ar), 7.24 (d, $J = 2.2$ Hz, 1H, Ar), 6.92 (dd, $J = 8.1$ Hz, $J = 2.2$ Hz, 1H, Ar), 4.66 (t, $J = 8.1$ Hz, 2H, CH_2), 4.24 (t, $J = 5.1$ Hz, 2H, CH_2), 2.90 (t, $J = 5.1$ Hz, 2H, CH_2), 2.79 (t, $J = 8.1$ Hz, 2H, CH_2), 2.47 (s, 6H, CH_3), 2.46 (s, 6H, CH_3); HPLC; LC−MS.

9-(2-Pyrrolidin-1-ylethoxy)-6-(2-pyrrolidin-1-ylethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6d). Compound **6d** was obtained as a red solid (263 mg, 57% yield from cis isomer) from carboxylic acid **23d** (1.42 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2). $R_f = 0.80$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 109–110 °C; IR (neat) 1660, 1612, 1250, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.65 (d, $J = 8.1$ Hz, 1H, Ar), 8.31 (d, $J = 7.4$ Hz, 1H, Ar), 7.73–7.63 (m, 2H, Ar), 7.42 (t, $J = 7.4$ Hz, 1H, Ar), 7.22 (d, $J = 3.0$ Hz, 1H, Ar), 6.89 (dd, $J = 8.1$ Hz, $J = 3.0$ Hz, 1H, Ar), 4.66 (t, $J = 8.1$ Hz, 2H, CH_2), 4.22 (t, $J = 7.3$ Hz, 2H, CH_2), 2.96 (m, 4H, CH_2), 2.77 (m, 8H, CH_2), 1.87 (m, 8H, CH_2); HPLC; LC−MS.

9-(2-Dimethylaminoethoxy)-6-(3-dimethylaminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6e). Compound **6e** was obtained as an orange solid (113 mg, 36% yield from cis isomer) from carboxylic acid **23e** (1.32 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 7:3:0.2) followed by TkLC (acetone/25% aqueous NH_4OH 95:5). $R_f = 0.75$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); mp 84–87 °C; IR (neat) 1656, 1608, 1250, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.65 (d, $J = 8.0$ Hz, 1H, Ar), 8.31 (d, $J = 8.3$ Hz, 1H, Ar), 7.73–7.63 (m, 2H, Ar), 7.41 (m, 1H, Ar), 7.23 (d, $J = 2.5$ Hz, 1H, Ar), 6.87 (dd, $J = 8.3$ Hz, $J = 2.5$ Hz, 1H, Ar), 4.56 (t, $J = 7.9$ Hz, 2H, CH_2), 4.17 (t, $J = 5.6$ Hz, 2H, CH_2), 2.79 (t, $J = 5.5$ Hz, 2H, CH_2), 2.53 (t, $J = 6.7$ Hz, 2H, CH_2), 2.38 (s, 6H, CH_3), 2.33 (s, 6H, CH_3), 2.12–1.99 (m, 2H, CH_2); HPLC; LC−MS.

2,3-Dimethoxy-9-(2-dimethylaminoethoxy)-6-(2-dimethylaminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6f). Compound **6f** was obtained as a red oil (91 mg, 16% yield from cis isomer) from carboxylic acid **23f** (1.45 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2). $R_f = 0.70$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH 8:2:0.2); IR (neat) 1649, 1612, 1267, 1023 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1H, Ar), 7.67 (s, 1H, Ar), 7.60 (d, $J = 8.6$ Hz, 1H, Ar), 7.20 (d, $J = 2.6$ Hz, 1H, Ar), 6.88 (dd, $J = 8.4$ Hz, $J = 2.6$ Hz, 1H, Ar), 4.65 (m, 2H, CH_2), 4.21 (t, $J = 5.5$ Hz, 2H, CH_2), 4.07 (s, 3H, CH_3), 4.00 (s, 3H, CH_3), 2.86 (t, $J = 5.5$ Hz, 2H, CH_2), 2.78 (m, 2H, CH_2), 2.46 (s, 6H, CH_3), 2.45 (s, 6H, CH_3); HPLC; LC−MS.

2,3-Dimethoxy-9-(2-dimethylaminoethoxy)-6-(3-dimethylaminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (6g). Compound **6g** was obtained as a red oil (86 mg, 15% yield from cis isomer) from carboxylic acid **23 g** (1.50 g, 3 mmol) and aluminum chloride (2.0 g, 15 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2). $R_f = 0.50$ (CH_2Cl_2/MeOH/25% aqueous NH_4OH); IR (neat) 1648, 1613, 1268, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1H, Ar), 7.67 (s, 1H, Ar), 7.58 (d, $J = 8.6$ Hz, 1H, Ar), 7.20 (d, $J = 2.6$ Hz, 1H, Ar), 6.85 (dd, $J = 8.4$ Hz, $J = 2.6$ Hz, 1H, Ar), 4.58–4.52 (m, 2H, CH_2), 4.17 (t, $J = 5.5$ Hz, 2H, CH_2), 4.07 (s, 3H, CH_3), 4.00 (s, 3H, CH_3), 2.80 (t, $J = 5.5$ Hz, 2H, CH_2), 2.64–2.43 (m, 2H, CH_2), 2.43–2.39 (m, 12H, CH_3), 2.15–2.10 (m, 2H, CH_2); HPLC; LC−MS.

6-(2-Dimethylaminoethyl)-5,11-dioxo-5,11-dihydro-6*H*-indeno[1,2-*c*]isoquinolin-9-yl Benzoate (27a). Compound **27a** was obtained as a red oil (222 mg, 50% from cis isomer) from carboxylic acid **26a** (0.82 g, 3 mmol) and aluminum chloride (3.2 g, 24 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2). $R_f = 0.80$ (CH_2Cl_2/MeOH 9:1); IR (neat) 1737, 1698, 1661, 1610, 1504, 1263, 1207 cm^{-1}; ^{1}H NMR ($CDCl_3$)

δ 8.59 (d, $J = 7.7$ Hz, 1H, Ar), 8.26 (d, $J = 8.2$ Hz, 1H, Ar), 8.18 (d, $J = 7.2$ Hz, 2H, Ar), 7.74–7.64 (m, 3H, Ar), 7.55–7.50 (t, $J = 7.7$ Hz, 2H, Ar), 7.44–7.39 (m, 2H, Ar), 7.28 (dd, $J = 8.2$ Hz, $J = 2.3$ Hz, 1H, Ar), 4.60 (t, $J = 7.9$ Hz, 2H, CH_2), 2.76 (t, $J = 7.9$ Hz, 2H, CH_2), 2.41 (s, 6H, CH_3).

6-(3-Dimethylaminopropyl)-5,11-dioxo-5,11-dihydro-6*H*-indeno[1,2-*c*]isoquinolin-9-yl Benzoate (27b). Compound **27b** was obtained as a red oil (210 mg, 15% yield from cis−trans mixture) from carboxylic acid **26b** (0.85 g, 3 mmol) and aluminum chloride (3.2 g, 24 mmol) after purification by column chromatography (CH_2Cl_2/MeOH/25% aqueous NH_4OH 2:8:0.2). $R_f = 0.80$ (CH_2Cl_2/MeOH 9:1); IR (neat) 1734, 1697, 1661, 1610, 1507, 1261, 1204 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.69 (d, $J = 7.3$ Hz, 1H, Ar), 8.33 (d, $J = 8.1$ Hz, 1H, Ar), 8.22 (d, $J = 7.3$ Hz, 2H, Ar), 7.86 (d, $J = 8.8$ Hz, 1H, Ar), 7.75–7.66 (m, 2H, Ar), 7.57–7.45 (m, 4H, Ar), 7.30 (dd, $J = 8.1$ Hz, $J = 2.5$ Hz, 1H, Ar), 4.59 (t, $J = 7.7$ Hz, 2H, CH_2), 2.56 (t, $J = 6.6$ Hz, 2H, CH_2), 2.34 (s, 6H, CH_3), 2.10–2.02 (m, 2H, CH_2).

General Procedure for Synthesis of Compounds 7a,b. LiOH (33 mg, 1.36 mmol, 2 equiv) was added to a solution of ester **27a**,**b** (0.68 mmol, 1 equiv) in CH_2Cl_2/MeOH 1:1, and the reaction mixture was stirred for 1 h. Then 1 N HCl was added until pH 1 and the aqueous layer was washed with CH_2Cl_2 (3 × 20 mL), alkalinized with NH_4OH until pH 8, and subsequently extracted with ethyl acetate (3 × 40 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The desired products were obtained after crystallization from methanol.

9-Hydroxy-6-(2-dimethylaminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (7a). Compound **7a** was obtained as a red solid (0.14 g, 62% yield) from ester **27a** (0.30 g, 0.68 mmol). $R_f = 0.50$ (CH_2Cl_2/MeOH 9:1); mp 250 °C (dec); IR (neat) 1697, 1660, 1610, 1547, 1505, 1438 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.58 (bs, 1H, OH), 8.59 (d, $J = 8.1$ Hz, 1H, Ar), 8.17 (d, $J = 8.2$ Hz, 1H, Ar), 7.78 (t, $J = 7.1$ Hz, 1H, Ar), 7.56 (d, $J = 8.2$ Hz, 1H, Ar), 7.46 (t, $J = 7.7$ Hz, 1H, Ar), 6.98 (d, $J = 2.7$ Hz, 1H, Ar), 6.86 (dd, $J = 8.2$ Hz, $J = 2.2$ Hz, 1H, Ar), 4.54 (t, $J = 7.1$ Hz, 2H, CH_2), 2.66–2.63 (m, 2H, CH_2), 2.06 (s, 6H, CH_3); HPLC; LC−MS.

9-Hydroxy-6-(3-dimethylaminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (7b). Compound **7b** was obtained as a red solid (0.16 g, 67% yield) from ester **27b** (0.31 g, 0.68 mmol). $R_f = 0.50$ (CH_2Cl_2/MeOH 9:1); mp 243 °C (dec); IR (neat) 1691, 1652, 1613, 1551, 1506, 1475, 1455 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.49 (d, $J = 8.0$ Hz, 1H, Ar), 8.16 (d, $J = 8.0$ Hz, 1H, Ar), 7.76 (t, $J = 7.3$ Hz, 1H, Ar), 7.64 (d, $J = 8.3$ Hz, 1H, Ar), 7.44 (t, $J = 8.0$ Hz, 1H, Ar), 6.96 (d, $J = 2.4$ Hz, 1H, Ar), 6.83 (dd, $J = 8.3$ Hz, $J = 2.4$ Hz, 1H, Ar), 4.43 (t, $J = 7.6$ Hz, 2H, CH_2), 2.41 (t, $J = 6.6$ Hz, 2H, CH_2), 2.16 (s, 6H, CH_3), 1.89–1.84 (m, 2H, CH_2); HPLC; LC−MS.

General Procedure for Synthesis of Compounds 8a,b. Appropriate dimethylaminoalkylamine (1.44 mmol, 1.2 equiv) was added to a stirred solution of indenopyranedione **28**[29] (0.30 g, 1.20 mmol, 1 equiv) in chloroform (40 mL), and the reaction mixture was stirred at room temperature for 18 h.

6-(2-Dimethylaminoethyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (8a). Compound **8a** was obtained as an orange solid (0.15 g, 39% yield) from *N*,*N*-dimethylethane-1,2-diamine (0.13 g, 1.44 mmol) after concentration of the solution (10 mL), filtration, and successive washings of the precipitate with ethyl ether/chloroform 3:1 (20 mL) and ethyl ether (10 mL). $R_f = 0.60$ (CH_2Cl_2/MeOH 8:2); mp 184–187 °C; ^1H NMR ($CDCl_3$) δ 8.71 (d, $J = 7.9$ Hz, 1H, Ar), 8.34 (d, $J = 7.6$ Hz, 1H, Ar), 7.76–7.67 (m, 2H, Ar), 7.64 (dd, $J = 7.0$ Hz, $J = 1.0$ Hz, 1H, Ar), 7.51–7.41 (m, 3H, Ar), 4.66 (t, $J = 8.0$ Hz, 2H, CH_2), 2.76 (t, $J = 8.0$ Hz, 2H, CH_2), 2.41 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 190.48 (C_{quat}), 163.30 (C_{quat}), 155.69 (C_{quat}), 137.05 (C_{quat}), 135.09 (C_{quat}), 133.91 (CH), 133.46 (CH), 132.28 (C_{quat}), 131.03 (CH), 128.43 (CH), 127.22 (CH), 123.52 (CH), 123.37 (C_{quat}), 123.26 (CH), 122.54 (CH), 108.62 (C_{quat}), 57.32 (CH_2), 45.90 (CH_3), 43.23 (CH_2); LC−MS; Anal. ($C_{20}H_{18}N_2O_2$) C, H, N.

6-(3-Dimethylaminopropyl)-6*H*-indeno[1,2-*c*]isoquinolin-5,11-dione (8b). Compound **8b** was obtained as an orange solid (0.24 g, 60% yield) from *N*,*N*-dimethylpropane-1,3-diamine (0.15 g, 1.44 mmol) after concentration of the solution (20 mL), filtration, and successive washings of the precipitate with ethyl ether/chloroform 2:1 (40 mL) and ethyl ether (10 mL). $R_f = 0.45$ (CH_2Cl_2/MeOH 8:2); mp 166–168 °C (lit.[26] 168–171 °C); ^1H NMR ($CDCl_3$) δ 8.71 (d, $J = 8.0$ Hz, 1H, Ar), 8.34 (d, $J = 7.6$ Hz, 1H, Ar), 7.78–7.70 (m, 2H, Ar), 7.64 (dd, $J = 6.9$ Hz, $J = 1.2$ Hz, 1H, Ar), 7.50–7.40 (m, 3H, Ar), 4.60 (t, $J = 8.0$ Hz, 2H, CH_2), 2.53 (t, $J = 6.7$ Hz, 2H, CH_2), 2.31 (s, 6H, CH_3), 2.12–2.00 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 190.60 (C_{quat}), 163.47 (C_{quat}), 155.70 (C_{quat}), 137.15 (C_{quat}), 135.21 (C_{quat}), 133.84 (CH), 133.30 (CH), 132.32 (C_{quat}), 130.96 (CH), 128.38 (CH), 127.12 (CH), 123.50 (CH), 123.43 (C_{quat}), 123.18 (CH), 122.93 (CH), 104.51 (C_{quat}), 56.87 (CH_2), 45.77 (CH_3), 43.57 (CH_2), 27.38 (CH_2); LC−MS.

DNA and Drugs Solutions. Calf thymus DNA (CT DNA, Pharmacia) was deproteinized with sodium dodecyl sulfate (SDS, protein content less than 0.2%) and extensively dialyzed against the required experimental buffer. An extinction coefficient of 6600 M^{-1} cm^{-1} was used to measure the nucleotide concentration of DNA solutions.[34] All synthesized compounds, as well as camptothecin and etoposide (Sigma), were dissolved as 10 mM solutions in DMSO. Further dilutions were made in the appropriate aqueous buffer.

Absorption Spectrometry and Melting Temperature Studies. Absorption spectra and melting curves were obtained using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. Typically, 20 μM of the various drugs were prepared in 1 mL of BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.1) in the presence or absence of 20 μM of CT DNA and transferred into a quartz cuvette of 10 mm path length. The spectra were recorded from 230 to 500 nm and are referenced against a cuvette containing the same DNA concentration in the same buffer. For the absorption titration, CT DNA was added gradually from 1 to 20 μM with a spectrum recorded after each addition. To perform the melting temperature measurement, CT DNA (20 μM) was incubated alone (control T_m) or with increasing concentrations of the tested compound in 1 mL of BPE buffer, thus resulting in a drug/base pair ratio of 0.05, 0.1, 0.25, 0.5. The sample was transferred into a quartz cell, and the absorbance at 260 nm was measured every min over the range 20−100 °C with an increment of 1 °C per min. The T_m values were obtained from first-derived plots.

Fluorescence Measurements. Method 1. The intrinsic fluorescence of analogues of indenoquinolinone **1a** was exploited to determine their apparent binding constants. Fluorescence titration data were recorded at room temperature using a SPEX Fluorolog fluorometer. Excitation was set at 490 nm, and fluorescence emission was monitored over the range 500–700 nm. Samples used for titration experiments were prepared separately at a constant drug concentration of 1 μM and DNA concentration ranging from 0.01 to 10 μM bp. Fluorescence titration data were fitted directly to obtain apparent binding constants using a fitting function incorporated into Prism 3.0 software.

Method 2. Since indeno[1,2-*c*]isoquinolin-5,11-dione derivatives show weak fluorescence variation with DNA titration, the binding studies were carried out through a competitive displacement fluorometry assay using DNA-bound ethidium bromide.[35,36] Excitation was set at 515 nm, and the fluorescence emission was monitored over the range 550–700 nm. Experiments were performed with an ethidium bromide/DNA molar ratio of 12.6:10 and a drug concentration range of 0.01–100 μM in a BPE buffer, pH 7.1. C_{50} values for ethidium bromide displacement were calculated using a fitting function incorporated into Prism 3.0, and the apparent binding constant was calculated as follows: $K_{app} = (1.26/C_{50})K_{ethidium}$, with $K_{ethidium} = 10^7$ M^{-1}.

Topoisomerase Inhibition. The experimental procedure has been previously detailed.[37] Supercoiled pLAZ plasmid DNA (130 ng) was incubated with 4 units of human topoisomerase I or II (TopoGen) at 37 °C for 45 min in 20 μL of relaxation buffer (50

mM tris(hydroxymethyl)aminomethane, pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, and 1 mM ATP) in the presence of graded concentrations (from 1.0 to 50 μM) of the tested compound. Reactions were terminated by adding of SDS to 0.25% and proteinase K to 250 μg/mL and incubating at 50 °C for a further 30 min. An amount of 3 μL of the electrophoresis dye mixture was then added to DNA samples, which were then separated by electrophoresis in a 1% agarose gel containing ethidium bromide (1 μg/mL, topoisomerase DNA cleavage gel) or not (inhibition of the relaxation of DNA) at room temperature for 2 h at 120 V. Gels run without ethidium bromide were then stained using a bath containing ethidium bromide. Both gels were finally washed and photographed under UV light.

Cell Cultures and Antiproliferative Assay. Human HL60 and HL60/MX2 leukemia cells were obtained from the American Tissue Culture Collection. Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 IU/mL), and streptomycin (100 μg/mL). The cytotoxicity of the tested compounds was assessed using a cell proliferation assay developed by Promega (CellTiter 96 AQueous one solution cell proliferation assay). Briefly, 2×10^4 exponentially growing cells were seeded in 96-well microculture plates with various drug concentrations in a volume of 100 μL. After 72 h incubation at 37 °C, 20 μL of the tetrazolium dye was added to each well and the samples were incubated for a further 2 h at 37 °C. Plates were analyzed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

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Supporting Information Available: Analytical data and purity assessment for key compounds; elemental analysis data for compound **8a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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