Design, Synthesis, and Biological Evaluation of 14-Substituted Aromathecins as Topoisomerase I Inhibitors

Maris A. Cinelli,[†] Andrew Morrell,[†] Thomas S. Dexheimer,[‡] Evan S. Scher,[‡] Yves Pommier,[‡] and Mark Cushman*[,†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, and the Purdue Cancer Center, Purdue University, West Lafayette, Indiana 47907, and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892-4255

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The aromathecin or “rosettacin” class of topoisomerase I (top1) inhibitors is effectively a “composite” of the natural products camptothecin and luotonin A and the synthetic indenoisoquinolines. The aromathecins have aroused considerable interest following the isolation and total synthesis of 22-hydroxyacuminatine, a rare cytotoxic natural product containing the 12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one system. We have developed two novel syntheses of this system and prepared a series of 14-substituted aromathecins as novel antiproliferative topoisomerase I poisons. These inhibitors are proposed to act via an intercalation and “poisoning” mechanism identical to camptothecin and the indenoisoquinolines. Many of these compounds possess greater antiproliferative activity and anti-top1 activity than the parent unsubstituted compound (rosettacin) and previously synthesized aromathecins, as well as greater top1 inhibitory activity than 22-hydroxyacuminatine. In addition to potentially aiding solubility and localization to the DNA−enzyme complex, nitrogenous substituents located at the 14-position of the aromathecin system have been proposed to project into the major groove of the top1−DNA complex and hydrogen-bond to major-groove amino acids, thereby stabilizing the ternary complex.

Introduction

Topoisomerase I (top1) is an enzyme that is crucial for DNA replication and transcription. Through these normal cellular processes, duplex DNA acquires a considerable degree of both positive and negative supercoiling. Top1 solves the topological problems supercoiling causes to allow efficient replication and transcription. Mechanistically, top1 acts through a nucleophilic tyrosine residue that nicks a single strand of the phosphodiester backbone of DNA and allows a “controlled rotation” of the DNA about the nonscissile strand, thus relaxing the double helix.[1,2]

Because top1 is preferentially expressed in the S-phase of the cell cycle and has been found in high levels in several solid human tumors,[3,4] it has long been considered an attractive target for the design of cancer chemotherapeutics.[1,5–7] In 1966, Wall and Wani isolated the cytotoxic alkaloid camptothecin (**1**) from the Chinese tree *Camptotheca acuminata*.[8] Camptothecin and its semisynthetic analogues such as topotecan (**2**) and irinotecan (**3**) inhibit top1 by intercalating into the DNA−enzyme complex. The steric bulk of the inhibitors prevents top1’s religation of the nicked DNA, thus “poisoning” the cleavage complex and triggering apoptosis.[1,9,10]

Efforts to improve the solubility and potency of camptothecin[5,11–18] have provided **2** and **3**,[1,5,11,12] the only FDA-approved top1 inhibitors for the treatment of cancer. Despite the clinical success of these compounds, camptothecin derivatives still suffer from poor solubility, reversibility of cleavage−complex formation, and dose-limiting toxicity.[1,3,12,19] Another flaw of the camptothecins is in the E-ring lactone, which exists in equilibrium with its ring-open, hydroxycarboxylate form in vivo.[1,3] While the hydroxyacid form retains some of its potency, it possesses a high affinity for human serum albumin.[20,21]

* To whom correspondence should be addressed. Phone: 765-494-1465. Fax: 765-494-6790. E-mail: cushman@pharmacy.purdue.edu.

[†] Purdue University.

[‡] National Cancer Institute, NIH.

The indenoisoquinolines (including **4** and **5**), a class of noncamptothecin top1 poisons based on the lead compound **6** (NSC 314622),[22] were developed as an alternative to the camptothecins.[22–25] Preclinical development of several indenoisoquinolines has recently begun.[26] The success of the camptothecins and the indenoisoquinolines has led to consideration of other heterocyclic systems that might combine the best features of both series.

The aromathecin[27–30] class of top1 poisons, previously described as stable hybrids of indenoisoquinolines and camptothecins,[27] also bear similarity to the natural product luotonin A (**7**), a weaker top1 poison isolated from *Peganum nigellastrum*.[31] Several series of modified and substituted luotonins have been published, and some analogues have greater antiproliferative activity than the parent compound.[32–34] 22-Hydroxyacuminatine (**8**), a rare natural product isolated from *Camptotheca acuminata*,[28,35] contains the 12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one system, of which the unsubstituted core has been named “rosettacin”[30] (**9**), with substituted compounds being named “aromathecins.”[28,29] Because of the similarity in structure and proposed mechanism of action of aromathecins to camptothecins, luotonins, indenoisoquinolines, and 22-hydroxyacuminatine, the aromathecin system is more accurately described as a composite of many of these heteroaromatic structures.[29] Representative top1 inhibitors, including the relevant indenoisoquinolines **4**−**6**, are shown in Figure 1, while the clinically useful camptothecin derivatives irinotecan and topotecan are shown in Figure 2.

Initial efforts to develop aromathecins focused on the synthesis of **9** and **10**.[27] Unfortunately, these compounds were weak top1 poisons and displayed poor growth inhibition.[27] We have discovered in the present study that substitution of the 14-position of aromathecins with amines, amino alcohols, and nitrogenous heterocycles confers both improved antiproliferative potency and top1 inhibition over both **9** and aromathecin **10**

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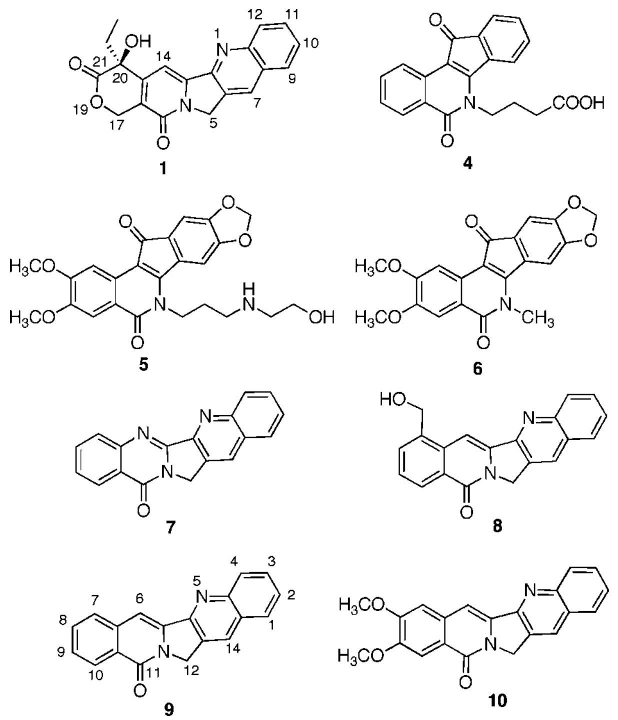


Figure 1. Representative top1 inhibitors.

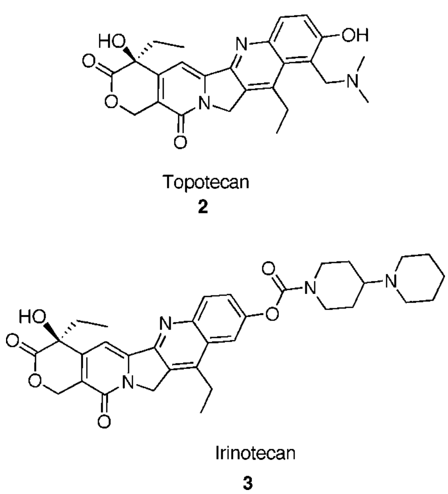


Figure 2. Structures of irinotecan and topotecan.

and confers improved top1 inhibitory activity over 22-hydroxyacuminatine (**8**).

As reported in the present communication, a new series of 14-substituted aromathecins have been synthesized via two novel routes that proceed through the known tricyclic ketone **23**,[36] building on structure–activity relationships established for both camptothecins and indenoisoquinolines. Substitution of the analogous 7-position of camptothecin with hydrogen bond donor–acceptor groups capable of increasing solubility improves biological activity over the parent compound.[3,14,16,17] For indenoisoquinolines, groups such as amino, imidazole, morpholine, and *N,N*-dimethylamine, located on the lactam nitrogen at a distance of 2–3 methylene units from the aromatic core, confer superior top1 inhibition and antiproliferative activity.[23,37] In addition to likely solubilizing the aromatic core, molecular modeling studies indicate that substituents at the 14-position of the aromathecin core and camptothecin's 7-position occupy the same region in space as the lactam substituents of indenoisoquinolines, projecting out into the major groove of the

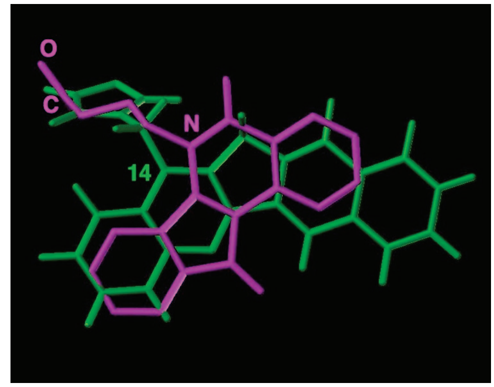


Figure 3. Ligand overlay of aromathecin **27d** (green) and indenoisoquinoline **4** (magenta). The positions of the lactam nitrogen and 14-position are indicated in their respective colors. The carboxylate group of **4** is perpendicular to the plane of the ring system.

DNA–top1 complex. These studies are consistent with the solved crystal structures of indenoisoquinolines in ternary complex with DNA and top1.[10] Hypothetically, these substituents hydrogen-bond with water and major-groove amino acids and increase the stability of the ternary complex. A ligand overlay of the crystal structure of the indenoisoquinoline (**4**)–top1–DNA ternary complex[10] and a hypothetical model of the aromathecin **27d**–top1–DNA ternary complex, showing this substituent overlap, is displayed in Figure 3. On the basis of this hypothesis, mono- and trimethylene analogues **27a–k** and **28a–g** were designed and synthesized.

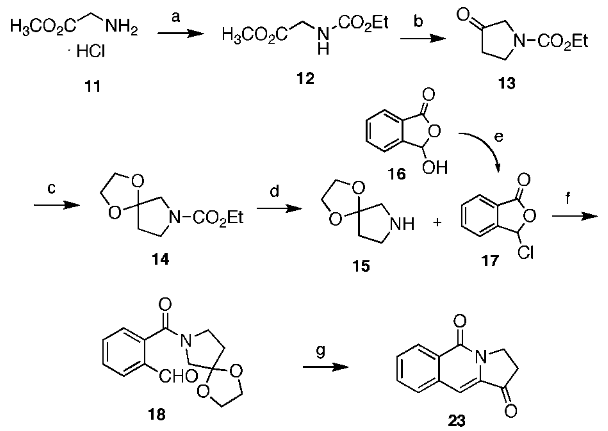
Chemistry

Several routes to aromathecin derivatives have been developed. 22-Hydroxyacuminatine (**8**), rosettacin (**9**), and compound **10** were previously synthesized in our laboratory via the condensation of a pyrroloquinoline with appropriately substituted phthalide derivatives.[27,28] Notable routes to 22-hydroxyacuminatine and the 12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one system include pyridone benzannulation and Heck coupling[38] and aza-Diels–Alder reactions.[39] Recently, Pin et al. have published a novel route to rosettacin and 14-methyl- and 14-phenylaromathecin, employing an N-amidoacylation/aldol condensation with a benzotriazole ester to form the key intermediate.[29]

14-Substituted aromathecins **27a–k** and **28a–g** were prepared from oxatricyclic ketone **23** (Scheme 1). Ketone **23** was previously prepared in Shamma and Novak's attempted synthesis of camptothecin, which was also the first reported synthesis of rosettacin.[36] This compound was prepared by two new routes, both beginning with commercially available amino acids. The first route is outlined in Scheme 1. Beginning with the ethyl carbamate **12** of methyl glycinate **(11)**, 3-pyrrolidinone ethylene ketal (**15**) was prepared via a one-pot Michael addition–Dieckmann condensation and decarboxylation, followed by ketalization of carbamate **13** to yield **14**.[40–42] Removal of the carbamate functionality and reaction with chlorophthalide **17**, readily prepared from 2-carboxybenzaldehyde (**16**),[43] yielded ketal **18**. Ketal **18** was cyclized directly to **23** using a combination of polyphosphoric acid and 85% phosphoric acid, following the final step of Shamma and Novak's work.

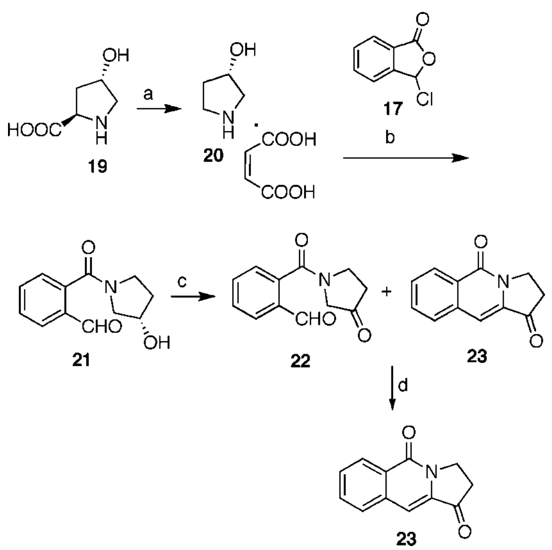
Because of variable yields and loss of material associated with protection–deprotection steps, the more elegant route depicted in Scheme 2 was developed as an alternative, protecting group-free pathway to the key intermediate. Preparation of **23** began with catalytic decarboxylation of commercially available *trans*-4-hydroxy-L-proline (**19**) to yield the amino alcohol **20**

Scheme 1[a]



[a] Reagents and conditions: (a) EtOCOCl, $CHCl_3$, reflux; (b) (i) NaH, benzene, reflux, (ii) methyl acrylate, reflux, (iii) 0 6 M HCl, reflux; (c) ethylene glycol, cat. *p*-TsOH, benzene, reflux; (d) KOH, H_2O, reflux; (e) cat. $FeCl_3$, $SOCl_2$, reflux; (f) THF, Et_3N, room temp; (g) polyphosphoric acid, 85% phosphoric acid, CH_2Cl_2, 100 °C.

Scheme 2[a]



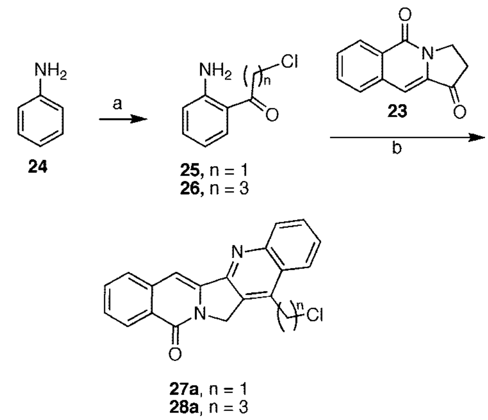
[a] Reagents and conditions: (a) (i) cat. 2-cyclohexen-1-one, cyclohexanol, reflux, (ii) maleic acid, EtOAc, room temp; (b) MeOH, Et_3N, room temp; (c) PDC, CH_2Cl_2, reflux; (d) polyphosphoric acid, $CHCl_3$, reflux.

as its hydrogen maleate salt.[44] Condensation of **20** with **17** provided hydroxyamide **21**, which gave a mixture of the oxidation products **22** and **23** upon reaction with pyridinium dichromate. This mixture was completely cyclized to **23** by treatment with polyphosphoric acid. Although this step is modest-yielding (30−45%), it provided **23** in high purity.

14-Chloromethylaromathecin **27a** and 14-chloropropylaromathecin **28a** were prepared by Friedlander condensation of **23** with aminoacetophenone **25** and aminobutyrophenone **26**, respectively (Scheme 3). These ketones were prepared from aniline (**24**) and chloroacetonitrile or 4-chlorobutyronitrile via the aminohaloborane modification of the Friedel−Crafts acylation, as reported by Sugasawa et al.[45,46] It is anticipated that the synthesis of future aromathecin derivatives can become, in essence, modular, enabling access to numerous substituted aromathecins through **23** and various substituted acetophenones.

The benzylic chloride of intermediate **27a** is easily substituted by a variety of nucleophiles in DMSO. Displacement of the chloride by sodium azide yielded **27b**, which was readily converted to amine **27c** by Staudinger reduction. Although

Scheme 3[a]



[a] Reagents and conditions: (a) (i) $BCl_3 \cdot Me_2S$, 1,2,-dichloroethane, 0 °C, (ii) chloroacetonitrile (for synthesis of **25**), 4-chlorobutyronitrile (for synthesis of **26**), $AlCl_3$, reflux; (iii) 2 M HCl, reflux; (b) *p*-TsOH, benzene, reflux.

substitution of **27a** with imidazole to provide **27d** required higher temperatures, displacement by the remaining amines at room temperature readily afforded analogues **27e**−**k** (Scheme 4).

Amine **28c** was prepared from azide **28b** using the Staudinger methodology as described above (Scheme 5). Because of the decreased electrophilicity of the terminal chloride of **28a**, increased reaction temperatures were required for substitution. Additionally, substitution with the desired amines required adding sodium iodide along with excess amine. The in situ Finkelstein reaction, followed by displacement of the resulting iodides with the required amines, yielded analogues **28d**−**g**, which were isolated as their trifluoroacetate salts.

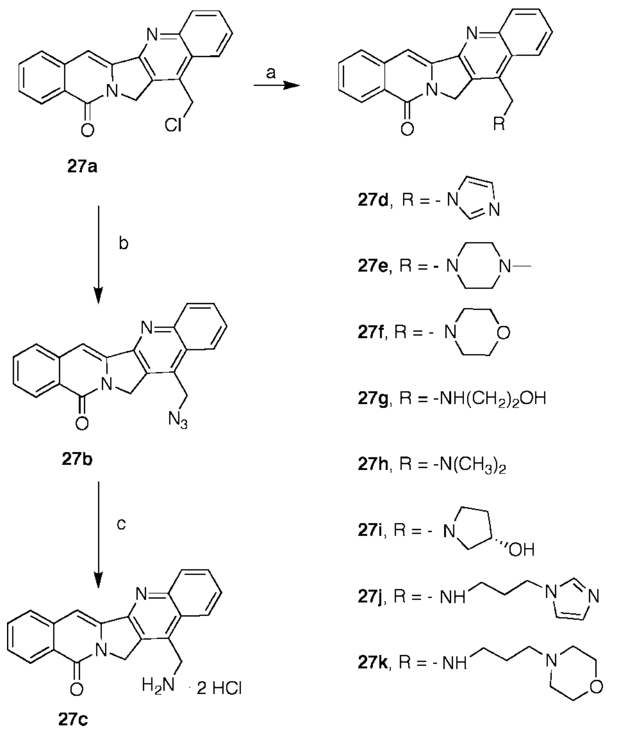
Biological Results and Discussion

All aromathecin analogues were assayed for antiproliferative activity in the National Cancer Institute's Developmental Therapeutics screen. Each compound was evaluated against approximately 60 cell lines originating from various human tumors.[47,48] After an initial one-dose assay, selected compounds were tested at five concentrations encompassing the range from 10^{-8} to 10^{-4} M. Results are reported as GI_{50} values for selected cell lines from each subpanel, and overall antiproliferative effects are quantified as a mid-graph midpoint (MGM) in Table 1. The MGM is a measure of the average GI_{50} against all cell lines tested. For completeness and comparison, the activities of camptothecin (**1**), indenoisoquinolines **5**[1,23,49] and **6**,[22] rosettacin (**9**), and dimethoxyaromathecin (**10**),[27] in addition to the top1 inhibitory activity of 22-hydroxyacuminatine (**8**), are reported.

Top1 inhibition was assayed by measurement of top1-mediated DNA cleavage, and inhibition data are expressed semiquantitatively as follows: 0, no inhibitory activity; +, between 20% and 50% the activity of 1 μM camptothecin (**1**); ++, between 50% and 75% the activity of 1 μM camptothecin; +++, between 75−100% the activity of 1 μM camptothecin; and ++++, equipotent to or more potent than 1 μM camptothecin. Top1 inhibitory data for aromathecins and comparative compounds are also included in Table 1.

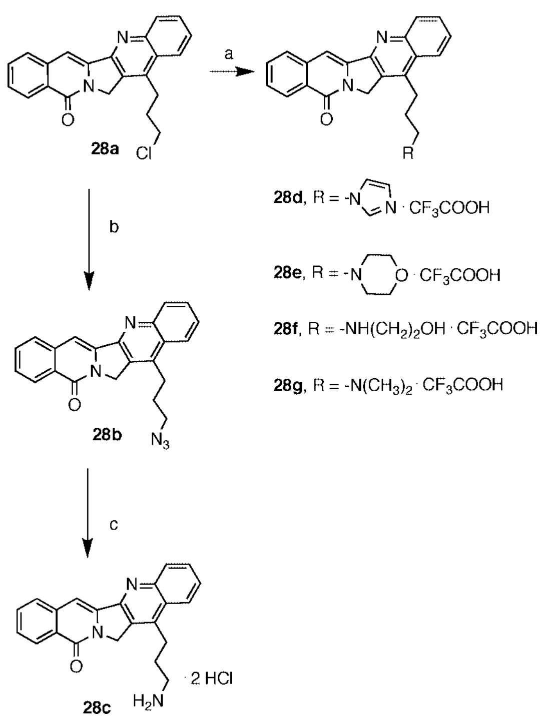
Compounds **27b**, **27k**, and **28a**,**b** are inactive against top1. Nonetheless, compounds **27b**, **27k**, and **28a** were tested in the National Cancer Institute's 60-cell line screen at an initial high dose (10 μM) and were not selected for further testing because of their low activities. Compounds **27f**, **27k**, and **28g**, although not selected for five-dose testing, induced 14%, 25.5%, and

Scheme 4[a]



[a] Reagents and conditions: (a) amine or amine salt + Et_3N, DMSO, room temp, 62−100 °C (**27d**); (b) NaN_3, DMSO; (c) (i) $(EtO_3)P$, benzene, reflux, (ii) 3 M HCl, MeOH, reflux.

Scheme 5[a]



[a] Reagents and conditions: (a) amine, NaI, DMSO, 100 °C; (b) NaN_3, DMSO, 100 °C; (c) (i) $(EtO_3)P$, benzene, reflux, (ii) 3 M HCl, MeOH, reflux.

21.4% average growth inhibition, respectively, in the presence of the inhibitor at a concentration of 10 μM across all cell lines tested.

In general, among the monomethylated aromathecin series **27a**−**j**, substitution at the 14-position of the aromathecin "core" with solubilizing groups capable of forming hydrogen bond donor–acceptor interactions improves top1 inhibitory activity relative to **9** and the 14-unsubstituted dimethoxyaromathecin **10**. In addition, most aromathecins are better top1 inhibitors than **8** (a one "+" inhibitor). Figure 4 indicates the presence of top1-mediated DNA breaks induced by aromathecins **27a**,**d**,**g**,**i** and **28d**,**f**. Interestingly, the cleavage patterns resemble both those induced by camptothecins and by indenoisoquinolines. The most active aromathecin (**28f**) displays a predominant top1 cleavage site at position 62 (Figure 4, lanes 27−30), also observed for the identically substituted indenoisoquinoline **5**.[23,49]

MGM values were improved over **9** and **10** for the majority of 14-substituents tested. These groups vary considerably in size and conformational flexibility, indicating a moderate tolerance by the ternary complex at this position. Especially effective at improving anti-top1 activity in the aminomethylene series is the imidazolyl moiety of **27d**. The chiral hydroxypyrrolidinyl group of **27i**, a group not previously investigated in the development of camptothecins or indenoisoquinolines, also conferred increased anti-top1 activity relative to rosettacin.

It is difficult to compare activity of aromathecins to compounds other than **9** and **10**, however. Luotonin A (**7**) was not tested in the National Cancer Institute assay but has displayed top1 inhibitory activity[31,32,34] and antiproliferative activity against the human lung carcinoma line H460.[31] 22-Hydroxyacuminatine (**8**) was also not tested in the National Cancer Institute assay, although previous studies report activity against murine leukemia KB and P388 cell lines.[35,39] However, it was determined in 2006 that 22-hydroxyacuminatine's cytotoxicity did not appear to be top1 dependent.[28]

The improved top1 inhibitory activity and antiproliferative potency of 14-substituted aromathecins over the parent compound may be due in part to improved solubility as the substituents at the 7-position of camptothecin and the substituents of irinotecan and topotecan greatly enhance activity through solubilizing the aromatic core.[1,3,14,16,17] For the aromathecins, this hypothesis may be corroborated in part by the inactivity of compounds **27b** and **28a**,**b**. No definite correlation between growth inhibition, top1 inhibition, or cLogP has been observed for the aromathecin class, although it has been observed for certain indenoisoquinolines.[37] Compound **27e**, which has a higher cLogP (3.67) than **9** (3.37), inhibits cell growth nearly 10 times as well and is also a more potent top1 inhibitor. Conversely, compound **27k** has a lower cLogP (3.32) but is inactive against top1 compared to rosettacin **9**.

It has also been hypothesized[1,5,10,23] that side chains of top1 inhibitors that project toward the major groove of the DNA−enzyme ternary complex (as seen in Figure 3) may aid in stabilization through hydrogen bonding interactions with water or top1 amino acids found in the major groove. We have previously published a hypothetical model of **9** merged into a DNA−camptothecin crystal structure, hypothesizing that **9** intercalated in a manner similar to camptothecin.[27] Figure 5 shows a hypothetical model of **27d** merged into the DNA−top1 crystal structure. The construction of this model was aided by the availability of the crystal structures of camptothecin (**1**) and indenoisoquinolines in ternary complex with top1 and DNA[10] (see Experimental Section for modeling details). The aromatic core of compound **27d** is calculated to intercalate between the base pairs without obvious steric hindrance and is likely stabilized by π-stacking interactions. The imidazolyl group projects on the outer range of H bonding distance with Asn352 (heavy-atom distance of 3.77 Å). It appears the imidazolyl group may be able to rotate somewhat to make this contact. Other models indicate the monomethylene analogues make hydrogen

Table 1. Antiproliferative and Topoisomerase I Inhibitory Activities of 14-Substituted Aromathecin Analogues

	cytotoxicity (GI_{50} in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	top 1 cleavage[c]
1	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.04	0.0405	++++
5	0.02	0.10	0.04	0.03	0.5	>0.01	>0.01	0.79	0.11	++++
6	1.3	35	41	4.2	73	68	37	96	20.0	++
8[d]										+
9	68.2	32.7	66.7	97.2	39.8	>100	>100	41.8	58.9	++
10	>100	57.3	>100	>100	>100	>100	>100	>100	91.2	+
27a	26.5	43.4	7.4	>100	>100	>100	>100	>100	40.7	+
27c	9.8	1.5	4.9	11.7	2.9	2.9	2.8	9.4	3.9	++
27d	10.0	13.2	4.7	3.4	17.0	21.5	11.5	>100	12.6	+++
27e	>100	1.9	1.8	>100		>100	4.9	2.1	6.2	++(+)
27f[e]										++
27g	3.5	3.6	3.9	2.6	6.3	2.7	2.8	>100	5.2	++(+)
27h	>100		>100	>100	>100	>100	>100	>100	46.8	++(+)
27i	28.6	9.0	5.4	2.7	5.5	16.9	7.0	10.3	7.6	++(+)
27j	4.7	3.2	3.8	6.4	4.4	3.5	3.2	7.0	3.5	++
28c[f]										+
28d	2.0	1.6	0.74	1.5	5.0	4.5	3.3	4.2	1.6	+++
28e	5.0	3.9	3.5	1.4	10.6	5.9	20.3	>100	5.9	++
28f	1.2	1.6	12.9	1.8	6.3	4.1	13.2	1.7	2.8	++++
28g[e]										+++

[a] 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, ranging from 10^{-8} to 10^{-4} M. [c] Compound-induced DNA cleavage due to top1 inhibition is graded by the following rubric relative to 1 μM camptothecin: 0, no inhibitory activity; +, between 20% and 50% activity; ++, between 50% and 75% activity; +++, between 75% and 95% of activity; ++++, equipotent. [d] 22-Hydroxyacuminatine was not tested in the National Cancer Institute assay. [e] These compounds were not selected for further testing; refer to text for details. [f] Currently undergoing five-dose testing.

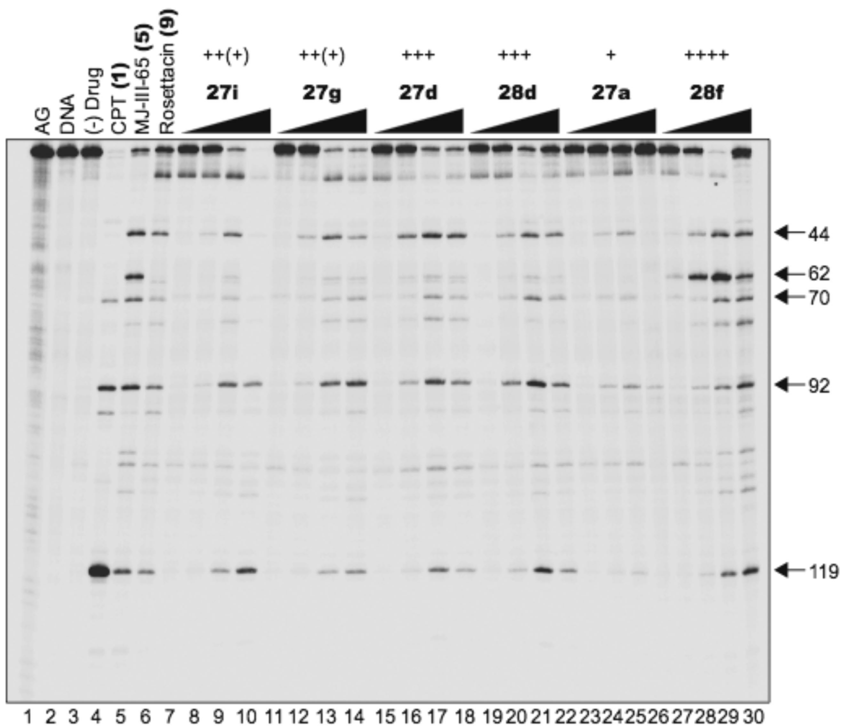


Figure 4. Top1-mediated DNA cleavage induced by aromathecins **27a**,**d**,**g**,**i**, **28d**, and **28f**: (lane 1) AG; (lane 2) DNA alone; (lane 3) top1 alone; (lane 4) camptothecin (**1**), 1 μM; (lane 5) **5**, 1 μM; (lane 6) rosettacin (**9**), 100 μM; (lanes 7−30) (for compounds **27a**,**d**,**g**,**i**, **28d**, and **28f**) top1 + indicated compound at 0.1, 1, 10, and 100 μM, respectively.

bonding contacts with Asn352, Thr747, and the carbonyl of Ile427 (structures not shown). The pyridine nitrogen of the aromathecin core faces the minor groove, where it appears to interact with Arg364, identical to the camptothecin class of top1 inhibitors. The presence of a lone pair of electrons at this position has also proven to be critical for many classes of top1 inhibitors, such as indenoisoquinolines and indolocarbazoles.[10]

To further probe the characteristics of the 14-position and investigate interactions deeper within the major groove, analogues **28a**−**g** were prepared. It was demonstrated in several studies of indenoisoquinolines that approximately three methylene units between the aromatic core and a "distal" functionality were ideal for biological activity.[23,37] Unfortunately, the results of this modification proved rather variable for the aromathecins with regard to top1 inhibition and antiproliferative activity. A notable exception is compound **28f**, which is the most potent aromathecin top1 inhibitor synthesized to this date. It is not known why this substituent confers such potent activity, although certain amino alcohol substitutions conferred similar activity upon indenoisoquinolines.[50] It is also possible that **27f**

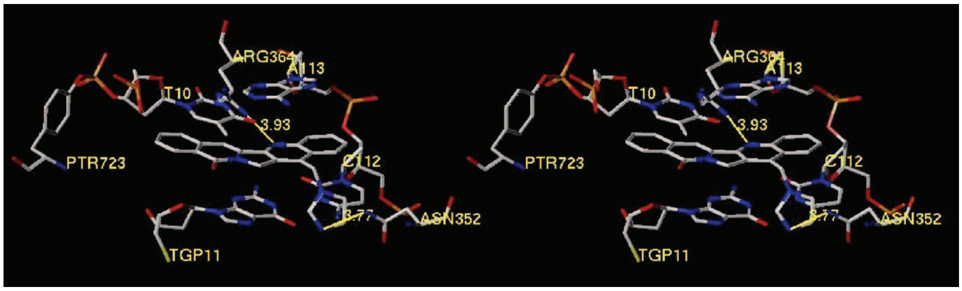


Figure 5. Hypothetical model for the binding of aromathecin **27d** in the ternary complex of DNA, top1, and the inhibitor. The diagram is programmed for wall-eyed (relaxed) viewing.

acts in a manner similar to indenoisoquinoline **5**, which bears an identical side chain.[23,49]

It is worth noting the inconsistencies observed between antiproliferative activity and top1 inhibition with certain aromathecin analogues. Compound **27a**, despite its poor anti-top1 activity, has greater antiproliferative potency than rosettacin (**9**). In addition, preliminary assays indicate intense cytotoxicity for compound **28c** (−47.5% cell growth in the presence of 10 μM inhibitor, indicating a net cell kill). Clearly, the antiproliferative activity of these two compounds is not due to inhibition of top1. It is unknown how these compounds exert their cytotoxic effect. Perhaps the mechanism is similar to that originally observed with **8**. Also, differences in compound metabolism and uptake by cells have previously been proposed for certain indenoisoquinolines showing similar disparities.[23] A formal COMPARE[48,51,52] analysis, as was performed for **6**,[22] will be performed on these "targetless" aromathecins pending further data.

For many classes of DNA-binding drugs, the addition of amine substituents, especially polyamine substituents, facilitates localization to DNA in addition to aiding solubility. These nitrogenous substituents are protonated at physiological pH and increase the drugs' Coulombic attraction to the negatively charged DNA.[50,53,54]

Conclusion

In conclusion, two novel synthetic routes to aromathecin analogues have been developed from inexpensive, commercially available precursors. Two series of 14-substituted aromathecins have been prepared via these routes, bearing substituents separated from the aromatic core by short "linker" regions. These novel "composite" structures of camptothecin, luotonins, and indenoisoquinolines have been evaluated against human top1 and numerous human tumor cell lines with promising results. These results establish that 14-substitution, on a whole, serves to improve top1 inhibitory and antiproliferative potency. This effect is likely a combination of increased solubility (as seen with 7-substituted camptothecins), charge complementarity with DNA, and hydrogen bonding (as proposed for indenoisoquinolines). Although increasing side chain length to that optimal for indenoisoquinolines did result in the discovery of a top1 inhibitor equipotent to camptothecin, the results of side chain elongation were largely variable with respect to both top1 inhibition and antiproliferative activity. Nonetheless, these data have served to reinvigorate interest in a class of compounds previously thought to be inactive and have since become the impetus for developing further favorable 14-substitutions.

Experimental Section

General Procedures. Melting points were determined in capillary tubes using a Mel-Temp apparatus and are not corrected. Infrared spectra were obtained as films on salt plates using $CHCl_3$ as the solvent except where otherwise specified, using a Perkin-Elmer Spectrum One FT-IR spectrometer, and are baseline-corrected. ^1H NMR spectra were obtained at 300 or 500 MHz, using a Bruker ARX300 and Bruker Avance 500 (TXI 5 mm probe), respectively. Mass spectral analyses were performed at the Purdue University Campus-Wide Mass Spectrometry Center. ESIMS was performed using a FinniganMAT LCQ Classic mass spectrometer system. EI/CIMS was performed using a Hewlett-Packard Engine or GCQ FinniganMAT mass spectrometer system. Combustion microanalyses were performed at the Purdue University Microanalysis Laboratory using a Perkin-Elmer Series II CHNS/O model 2400 analyzer; reported values are within 0.4% of calculated values. Analytical thin-layer chromatography was performed on Baker-flex silica gel IB2-F plastic-backed TLC plates. Preparative thin-layer chromatography was performed on Analtech silica gel 1500 μm glass plates. Compounds were visualized with both short- and long-wavelength UV light. Silica gel flash chromatography was performed using 40−63 μm flash silica gel.

Methyl *N*-Ethoxycarbonylglycinate (12).[40] The hydrochloride salt of methyl glycinate (**11**) (12.6 g, 0.1 mol) was diluted with $CHCl_3$ (200 mL). Triethylamine (25.26 g, 0.250 mol) was added, and the reaction mixture was cooled to 0 °C. Ethyl chloroformate (22.21 g, 0.205 mol) was then added, and the mixture was heated at reflux for 24 h. The solution was washed sequentially with H_2O (2 × 150 mL), 10% aqueous HCl (100 mL), and saturated NaCl (100 mL), dried over anhydrous sodium sulfate, and concentrated to provide an orange oil (14.8 g, 92%). ^1H NMR (300 MHz, $CDCl_3$) δ 5.15 (bs, 1 H), 4.17 (q, J = 7.1 Hz, 2 H), 3.98 (d, J = 5.6 Hz, 2 H), 3.76 (s, 3 H), 1.30 (t, J = 7.1 Hz, 3 H).

***N*-Ethoxycarbonyl-3-pyrrolidinone (13).**[41,42] Compound **12** (8.350 g, 29.59 mmol) was diluted with benzene (50 mL). Sodium hydride (1.420 g, 59.18 mmol) was added, and the mixture was heated at reflux for 30 min. Methyl acrylate (3.057 g, 35.51 mmol) was added, and the reaction mixture was heated at reflux for 8 h and then allowed to stir at room temperature for 16 h. Then 6 M HCl (30 mL) was added, and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, and the aqueous and organic phases were separated. The aqueous phase was extracted with EtOAc (3 × 30 mL), and the combined organic layer was washed with saturated $NaHCO_3$ (3 × 30 mL) and saturated NaCl (30 mL). The organic layer was dried over sodium sulfate and concentrated to provide a yellow oil (3.089 g, 73%). ^1H NMR (300 MHz, $CDCl_3$) δ 4.39−4.01 (m, 2 H), 3.82−3.78 (m, 4 H), 2.60 (t, J = 7.8 Hz, 2 H), 1.27 (q, J = 7.2 Hz, 3 H); CIMS m/z (rel intensity) 158 (MH^+, 100).

***N*-Ethoxycarbonyl-3-pyrrolidinone Ethylene Ketal (14).**[41] Compound **13** (2.858 g, 18.18 mmol) was diluted with benzene (50 mL). Ethylene glycol (2.257 g, 36.37 mmol) was added, followed by *p*-TsOH (0.346 g, 1.82 mmol). A Dean−Stark trap was affixed, and the reaction mixture was heated at reflux for 20 h, allowed to cool to room temperature, and washed with water (3 × 25 mL) and saturated NaCl (25 mL). The organic layer was dried over sodium sulfate and concentrated to provide a yellow oil (3.108 g, 81%). ^1H NMR ($CDCl_3$) δ 4.14 (q, J = 7.1 Hz, 2 H), 3.99−3.94

(m, 4 H), 3.56 (q, $J = 7.5$ Hz, 2 H), 3.43 (d, $J = 7.6$ Hz, 2 H), 2.05−2.00 (m, 2 H), 1.28 (t, $J = 7.1$ Hz, 3 H).

3-Pyrrolidinone Ethylene Ketal (15).[36] Compound **14** (3.108 g, 14.71 mmol) was diluted with water (25 mL). Potassium hydroxide (2.800 g, 49.91 mmol) was added, and the solution was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature and extracted with CH_2Cl_2 (4 × 30 mL), and the combined organic layer was washed with saturated NaCl (30 mL). The organic layer was dried over sodium sulfate and concentrated to provide a light-yellow oil (0.887 g, 44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97−3.86 (m, 4 H), 3.07 (t, $J = 7.2$ Hz, 2 H), 2.89 (s, 2 H), 2.15 (s, 1 H), 1.98 (t, $J = 7.4$ Hz, 2 H).

3-Chloro-1(3*H*)-isobenzofuranone (17).[43] 2-Carboxybenzaldehyde (**16**, 10.00 g, 66.61 mmol) and ferric chloride (0.030 g, 0.185 mmol) were diluted with thionyl chloride (25 mL) and the mixture was heated at reflux for 1 h. The reaction mixture was allowed to cool to room temperature and concentrated to provide a brown oil. The oil was diluted with hexanes (20 mL) and concentrated to provide a brown solid. The solid was extracted with boiling hexanes (5 × 50 mL), and concentration of the extract provided a white solid (10.96 g, 98%): mp 52−56 °C (lit.[43] mp 57−59 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95−7.93 (m, 1 H), 7.82 (t, $J = 7.4$ Hz, 1 H), 7.68−7.60 (m, 2 H), 7.10 (s, 1 H).

2,3-Dihydropyrrolo[1,2-*b*]isoquinoline-1,5-dione (23, Method 1).[36] Compound **15** (2.167 g, 15.69 mmol) was diluted with THF (30 mL) and triethylamine (10 mL). Compound **17** was then added, and the solution was allowed to stir at room temperature for 30 min. The reaction mixture was concentrated, diluted with water (50 mL), and extracted with $CHCl_3$ (4 × 40 mL). The combined organic layers were washed with saturated NaCl (40 mL) and dried over sodium sulfate. The solution was filtered and concentrated to provide compound **18**, which was used without further purification in the next step. Compound **18** was diluted with polyphosphoric acid (10.00 g), dichloromethane (5 mL), and phosphoric acid (85%, 5 mL). The reaction mixture was heated at 100 °C for 3 h and then allowed to cool to room temperature. The reaction mixture was diluted with ice−water (100 mL) and extracted with $CHCl_3$ (7 × 100 mL). The combined organic layer was washed with saturated NaCl (100 mL), dried over sodium sulfate, and concentrated to provide the product **23** as an orange-brown solid (2.117 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, $J = 7.9$ Hz, 1 H), 7.79−7.64 (m, 3 H), 7.27 (s, 1 H), 4.42 (t, $J = 6.9$ Hz, 2 H), 7.98 (t, $J = 7.3$ Hz, 2 H).

(*S*)-Pyrrolidin-3-ol Hydrogen Maleate (20).[44] *trans*-4-Hydroxy-L-proline (**19**) (10.00 g, 76.26 mmol) was diluted with cyclohexanol (60 mL). 2-Cyclohexen-1-one (1.0 mL) was added, and the mixture was heated at reflux for 3 h. After the dark red solution was cooled to room temperature, maleic acid (8.950 g, 77.09 mmol) was added portionwise over a 30 min period, keeping the internal temperature below 35 °C. Ethyl acetate (140 mL) was then added dropwise over 1 h to precipitate a pale-orange amorphous solid (12.46 g, 80%) after filtration: mp 83−86 °C (lit.[44] mp 90−91 °C). ^{1}H NMR (300 MHz, CD_3OD) δ 6.25 (s, 2 H), 4.60−4.50 (m, 1 H), 3.30−3.29 (bm, 2 H), 3.22−3.10 (bm, 2 H), 2.10−2.00 (m, 2H).

***N*-(*o*-Formylbenzoyl)-(*S*)-pyrrolidin-3-ol (21).** Compound **20** (3.000 g, 14.62 mmol) was diluted with MeOH (30 mL) and Et_3N (13 mL). Compound **17** (2.096 g, 12.43 mmol) was added, and the mixture was stirred overnight at room temperature for 22 h. The solution was concentrated, and the brown residue dissolved in H_2O (20 mL) and extracted with $CHCl_3$ (4 × 50 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated, and the resulting brown oil was flash chromatographed (SiO_2), eluting with 20:1 CH_2Cl_2−MeOH, to afford an orange-yellow amorphous solid (2.160 g, 79%): mp 71−73 °C. IR (film) 3379, 2947, 2886, 1698, 1613, 1597, 1439 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 10.0 (s, 1 H), 8.01 (d, $J = 7.7$ Hz, 1 H), 7.75 (dt, $J = 12.0$ and 6.7 Hz, 2 H), 7.49 (dt, $J = 6.4$ and 1.3 Hz), 4.50 (m, 0.5 H), 4.35 (m, 0.5 H), 3.79−3.65 (m, 2 H), 3.31−3.00 (m, 2 H), 2.20−1.80 (m, 2 H); CIMS *m/z* (rel intensity) 220 (MH^+, 100).

2,3-Dihydropyrrolo[1,2-*b*]isoquinoline-1,5-dione (23, Method 2).[36] Pyridinium dichromate (5.147 g, 13.68 mmol) was diluted with anhydrous CH_2Cl_2 (30 mL) under an argon atmosphere. A solution of **21** (2.00 g, 9.12 mmol) in anhydrous CH_2Cl_2 (15 mL) was added, and the mixture was heated at reflux for 19.5 h. The mixture was cooled and filtered, and the dark-brown filter cake was washed with $CHCl_3$ (4 × 30 mL). The filtrate was filtered through a pad of Celite, and the pad was washed with $CHCl_3$ (3 × 30 mL). The filtrate was concentrated to yield a dark-brown oil that was then diluted with $CHCl_3$ (40 mL). Polyphosphoric acid (6.15 g) was added, and the mixture was heated at reflux for 2 h and 10 min. The mixture was cooled and poured into ice−water (100 mL). The residue in the flask was stirred with ice−water (3 × 40 mL). The aqueous mixture was extracted with $CHCl_3$ (5 × 100, 1 × 50 mL). The organic layers were washed with saturated NaCl (250 mL), dried over anhydrous sodium sulfate, adsorbed onto SiO_2 (7.273 g), and purified by flash column chromatography (SiO_2), eluting with 1% MeOH in $CHCl_3$. The solvent was evaporated and the resulting solid was recrystallized from boiling EtOH (20 mL) to yield an iridescent orange solid (0.590 g, 33%): mp 180−184 °C (lit.[36] mp 191−192 °C). The ^{1}H NMR spectrum was identical to compound **23** prepared by method 1 above.

2-Amino-α-chloroacetophenone (25).[45] Boron trichloride−methyl sulfide complex (1.059 g, 5.906 mmol) was diluted with dichloroethane (15 mL) and cooled to 0 °C. Aniline (**24**, 0.500 g, 5.369 mmol) was added dropwise, and the solution was allowed to stir at 0 °C for 10 min. Chloroacetonitrile (0.507 g, 6.711 mmol) was added, followed by aluminum chloride (0.787 g, 5.906 mmol), and the solution was allowed to gradually warm to room temperature. After 10 min, the reaction mixture was heated at reflux for 3 h. The solution was allowed to cool to room temperature, 2 M HCl (15 mL) was added, and the reaction mixture was heated at reflux for 30 min. The reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with saturated NaCl (25 mL) and dried over sodium sulfate. Concentration provided a yellow solid (0.194 g, 21%) that was isolated by washing with hexanes: mp 106−109 °C (lit.[45] mp 112−113 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (dd, $J = 8.2$ and 1.4 Hz, 1 H), 7.34−7.28 (m, 1 H), 6.72−6.64 (m, 2 H), 4.70 (s, 2 H).

1-(*o*-Aminophenyl)-4-chloro-1-butanone (26).[46] Boron trichloride−methyl sulfide complex (6.353 g, 35.43 mmol) was diluted with dichloroethane (70 mL) and cooled to 0 °C. Aniline (**24**, 3.000 g, 32.21 mmol) was added dropwise, and the solution was allowed to stir at 0 °C for 10 min. 4-Chlorobutyronitrile (4.170 g, 40.27 mmol) was added, followed by aluminum chloride (4.724 g, 35.43 mmol), and the solution was allowed to gradually warm to room temperature. After 10 min, the reaction mixture was heated at reflux for 2.5 h. The solution was allowed to cool to room temperature, 10% aqueous HCl (70 mL) was added, and the reaction mixture was heated at reflux for 30 min. The reaction mixture was allowed to stir at room temperature for 24 h, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with saturated NaCl (50 mL) and dried over sodium sulfate. Concentration provided a crude yellow-brown oil that was purified by flash column chromatography (SiO_2), eluting with a gradient of hexanes to 50% EtOAc−hexanes. The solvent was evaporated, and the resulting product was diluted with Et_2O (50 mL) and treated with 3 M HCl in MeOH (10 mL) and allowed to stir at room temperature for 20 min. The salt was filtered and washed with Et_2O (50 mL) to provide a white solid. The solid was dissolved in saturated $NaHCO_3$ (150 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with saturated NaCl (50 mL), dried over sodium sulfate, filtered, and concentrated to provide a yellow oil (2.022 g, 32%) that solidified upon standing: mp 51−55 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (dd, $J = 8.5$ and 1.6 Hz, 1 H), 7.31−7.25 (m, 1 H), 6.70−6.65 (m, 2 H), 3.70 (t, $J = 6.3$ Hz, 2 H), 3.18 (t, $J = 7.1$ Hz, 2 H), 2.25 (pent, $J = 6.9$ Hz, 2 H).

14-Chloromethyl-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27a). Compound **23**(0.176 g, 0.884 mmol) and compound **25** (0.150 g, 0.884 mmol) were diluted with benzene (100 mL). *p*-Toluenesulfonic acid monohydrate (0.168 g, 0.884 mmol) was added, and the solution was heated at reflux for 24 h using a Dean−Stark trap to collect the azeotroped water. The solution was concentrated, diluted with $NaHCO_3$ (150 mL), and extracted with $CHCl_3$ (6 × 100 mL) and saturated NaCl (100 mL). The organic layer was dried over sodium sulfate, concentrated, and purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 5% MeOH in $CHCl_3$, to provide a yellow solid (0.174 g, 59%) after washing with MeOH: mp 270 °C (dec). IR (KBr) 1661, 1638, 761, 754, and 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.1 Hz, 1 H), 8.27 (d, J = 7.6 Hz, 1 H), 8.19 (d, J = 8.4 Hz, 1 H), 7.86−7.69 (m, 4 H), 7.65 (s, 1 H), 7.62−7.56 (m, 1 H), 5.44 (s, 2 H), 5.05 (s, 2 H); ESIMS *m/z* (rel intensity) 333/335 (MH^+, 100/35). Anal. ($C_{20}H_{13}ClN_2O$) C, H, N.

14-Azidomethyl-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27b). Compound **27a** (0.070 g, 0.210 mmol) and sodium azide (0.022 g, 0.346 mmol) were diluted with DMSO (20 mL), and the mixture was stirred at room temperature for 19 h. The solution was diluted with $CHCl_3$ (30 mL), and more $CHCl_3$ was then added until the organic phase was clear. The organic layer was washed with H_2O (4 × 25 mL) and saturated NaCl (25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was washed and filtered with MeOH, redissolved in $CHCl_3$, and purified by flash column chromatography (SiO_2), eluting with $CHCl_3$ to provide a pale-yellow amorphous solid (0.051 g, 71%) after washing with MeOH: mp 210−212 °C (dec). IR (film) 2918, 2102, 1665, 1639, 1605, 745, 685 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, J = 7.6 Hz, 1 H), 8.28 (d, J = 8.2 Hz, 1 H), 8.11 (d, J = 8.3 Hz, 1 H), 7.84−7.60 (m, 5 H), 7.67 (s, 1 H), 5.50 (s, 2 H), 5.01 (s, 2 H); ESIMS *m/z* (rel intensity) 340 (MH^+, 100). Anal. ($C_{20}H_{13}N_5O \cdot 0.6H_2O$) C, H, N.

14-Aminomethyl-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one Dihydrochloride (27c). Compound **27b** (0.056 g, 0.165 mmol) was diluted with benzene (20 mL), triethyl phosphite (0.069 g, 0.413 mmol) was added, and the solution was heated at reflux for 19 h. The solution was cooled to room temperature, 3 M methanolic HCl (10 mL) was added, and the dark-orange solution was heated at reflux for 3 h. The precipitate was vacuum filtered to yield a bright red flaky solid (0.046 g, 73%) after washing with MeOH: mp 278−284 °C (dec, salt), 265−269 °C (dec, free base). IR (film) 2920, 1656, 1633, 1597, 751, 690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, CD_3OD, Et_3N) δ 8.43 (d, J = 7.4 Hz, 1 H), 8.17 (t, J = 8.0 Hz, 2 H), 7.76−7.54 (m, 5 H), 7.70 (s, 1 H), 5.42 (s, 2 H), 4.37 (2 H); ESIMS *m/z* (rel intensity) 314 (MH^+, 100). Anal. ($C_{20}H_{17}Cl_2N_3O$), C, H, N.

14-(1-Imidazolylmethyl)-2*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27d). Compound **27a** (0.085 g, 0.225 mmol) and imidazole (0.052 g, 0.766 mmol) were diluted with DMSO (25 mL) and heated to 62 °C for 3 h, and the resultant clear pale-orange solution was subsequently stirred at room temperature for 15 h. TLC showed incomplete reaction, so additional imidazole (0.255 mmol) was added, and the solution was heated at 100 °C for 1 h. The solution was diluted with $CHCl_3$ (50 mL) and washed with H_2O (4 × 40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to $CHCl_3$−4% MeOH, to yield a yellow amorphous solid (0.041 g, 44%) after washing with diethyl ether: mp 294−297 °C (dec). IR (film) 1660, 1631, 1600, 762, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 8.0 Hz, 1 H), 8.28 (d, J = 8.4 Hz, 1 H), 8.02 (d, J = 8.5 Hz, 1 H), 7.82−7.57 (m, 5 H), 7.65 (s, 1 H), 7.60−7.47 (m, 1 H), 7.09 (bs, 1 H), 6.90 (bs, 1 H), 5.67 (s, 2 H), 5.26 (s, 2 H); ESIMS *m/z* (rel intensity) 365 (MH^+, 100). Anal. ($C_{23}H_{16}N_4O \cdot 0.75H_2O$) C, H, N.

14-[1-(*N*-Methylpiperazinylmethyl)]-2*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27e). Compound **27a** (0.075 g 0.225 mmol) was diluted with DMSO (25 mL), and *N*-methylpiperazine (0.068 g, 0.675 mmol) was added. The solution was stirred at room temperature for 19 h. The solution was diluted with $CHCl_3$ (50 mL) and washed with H_2O (4 × 40 mL) and saturated NaCl (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (SiO_2), eluting with 7% MeOH in $CHCl_3$ to yield a pale-yellow amorphous solid (0.058 g, 65%) after washing with hexanes: mp 204−207 °C. IR (film) 2932, 2789, 1661, 1633, 1604, 753, 687 cm-[1]; ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 8.4 Hz, 1 H), 8.36 (d, J = 8.6 Hz, 1 H), 8.23 (d, J = 7.9 Hz, 1 H), 7.78−7.57 (m, 5 H), 7.62 (s, 1 H), 5.47 (s, 2 H), 4.06 (s, 2 H), 2.61 (bs, 4 H), 2.47 (bs, 4 H), 2.29 (s, 3 H); ESIMS *m/z* (rel intensity) 397 (MH^+, 80), 297 ($MH^+ - C_5H_{11}N_2$, 100). Anal. ($C_{25}H_{24}N_4O \cdot 0.6H_2O$) C, H, N.

14-(1-Morpholinomethyl)-2*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27f). Compound **27a** (0.064 g, 0.192 mmol) was diluted with DMSO (25 mL), and morpholine (0.050 g, 0.576 mmol) was added. The solution was stirred at room temperature for 20 h. The solution was diluted with $CHCl_3$ (40 mL) and then washed with H_2O (4 × 40 mL) and saturated NaCl (40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the resulting residue was purified by flash column chromatography (SiO_2), eluting with EtOAc, which afforded a pale-yellow amorphous solid (0.056 g, 77%) after washing with hexanes: mp 242−244 °C (dec). IR (film) 2929, 1661, 1602, 756, 689 cm^{-1}; ^1H NMR (300 MHZ, $CDCl_3$) δ 8.58 (d, J = 8.0 Hz, 1 H), 8.39 (d, J = 8.3 Hz, 1 H), 8.24 (d, J = 8.5 Hz, 1 H), 7.82−7.58 (m, 5 H), 7.66 (s, 1 H), 5.27 (s, 2 H), 4.06 (s, 2 H), 3.73 (t, J = 4.2 Hz, 4 H), 2.59 (t, J = 4.3 Hz, 4 H); ESIMS *m/z* (rel intensity) 384 (MH^+, 100). Anal. ($C_{24}H_{21}N_3O_2$) C, H, N.

14-(*N*-Ethanolaminomethyl)-2*H*-5,11a-diazadibenzo[*b,h*]fluoren-11one (27g). Compound **27a** (0.065 g, 0.195 mmol) was diluted with DMSO (25 mL), and ethanolamine (0.048 g, 0.781 mmol) was added. The mixture was stirred at room temperature for 20 h, poured into $CHCl_3$ (40 mL), and washed with H_2O (4 × 40 mL). A small amount of methanol was added to aid solubility. The organic layers were dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (SiO_2), eluting with a gradient of 6% MeOH in $CHCl_3$ to 9% MeOH in $CHCl_3$, to provide a fine yellow powder (0.029 g, 42%) after washing with hexanes: mp 198.5−204 °C (dec). IR (film) 2925, 1656, 1618, 1599, 753, 689 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.38−8.31 (m, 2 H), 8.14 (d, J = 8.1 Hz, 1 H), 7.97 (d, J = 7.9 Hz, 1 H), 7.81 (q, J = 7.2 Hz, 2 H), 7.69−7.56 (m, 3 H), 5.44 (s, 2 H), 4.57 (t, J = 5.3 Hz, 1 H), 4.32 (s, 2 H), 3.54 (q, J = 5.6 Hz, 2 H), 2.73 (t, J = 5.7 Hz, 2 H); ESIMS *m/z* (rel intensity) 358 (MH^+, 100). Anal. ($C_{22}H_{19}N_3O_2 \cdot 1.25H_2O$) C, H, N.

14-(*N,N*-Dimethylaminomethyl)-2*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27h). Compound **27a** (0.050 g, 0.150 mmol) was diluted with DMSO (25 mL), and *N,N*-diethylamine hydrochloride (0.035 g, 0.429 mmol) and Et_3N (0.045 g, 0.445 mmol) were added. The solution was stirred at room temperature for 20 h and then diluted with $CHCl_3$ (40 mL) and then washed with H_2O (4 × 40 mL) and saturated NaCl (40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. Flash column chromatography of the residue (SiO_2), eluting with EtOAc, yielded a very pale yellow amorphous solid (0.033 g, 64%) after washing with hexanes: mp 201−202.5 °C. IR (film) 2942, 2819, 2768, 1661, 1634, 1604, 753, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$,) δ 8.58 (d, J = 8.1, 1 H), 8.37 (d, J = 8.3 Hz, 1 H), 8.23 (d, J = 8.9 Hz, 1 H), 7.80−7.58 (m, 5 H), 7.63 (s, 1 H), 5.45 (s, 2 H), 3.97 (s, 2 H), 2.35 (s, 6 H); ESIMS *m/z* (rel intensity) 345 (MH^+, 100). Anal. ($C_{22}H_{19}N_3O \cdot 0.3H_2O$) C, H, N.

14-[*N*-(*S*)-3-Hydroxypyrrolidinomethyl]-12*H*-5,11a diazadibenzo[*b,h*]fluoren-11-one (27i). Compound **27a** (0.060 g, 0.180 mmol) and compound **20** (0.111 g, 0.541 mmol) were diluted with DMSO (25 mL), and Et_3N (0.182 g, 1.80 mmol) was added. The solution was stirred at room temperature for 19 h, diluted with $CHCl_3$ (40 mL), and washed with H_2O (4 × 30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 4% MeOH in $CHCl_3$, to yield a flocculent yellow solid (0.048 g, 69%) after washing with hexanes: mp 220 °C (dec). IR (film) 2918, 2849, 1658, 1601, 1619, 1479, 1347, 1125, 755, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, $J = 8.0$ Hz, 1 H), 8.36 (d, $J = 8.5$ Hz, 1 H), 8.20 (d, $J = 8.0$ Hz, 1 H), 7.78−7.51 (m, 5 H), 7.63 (s, 1 H), 5.43 (s, 2 H), 4.35 (bs, 1 H), 4.18 (s, 2 H), 2.92−2.90 (m, 1 H), 2.73−2.71 (m, 2 H), 2.50−2.48 (m, 2 H), 2.23−2.13 (m, 1 H), 1.78−1.74 (m, 1 H); ESIMS *m/z* (rel intensity) 384 (MH^+, 100). Anal. ($C_{24}H_{21}N_3O_2 \cdot 0.25H_2O$) C, H, N.

14-[(1-Imidazolyl)propylaminomethyl]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (27j). Compound **27a** (0.060 g, 0.180 mmol) was diluted with DMSO (25 mL), and 1-(3-aminopropyl)imidazole (0.068 g, 0.505 mmol) was added. The solution was stirred at room temperature for 17 h, diluted with $CHCl_3$ (40 mL), and washed with H_2O (4 × 30 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated, and the resultant dark yellow solid was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 6% MeOH−1% Et_3N in $CHCl_3$, to yield a pale-yellow solid (0.045 g, 60%) after washing with hexanes: mp 138−140 °C (dec). IR (film) 3413, 3292, 1656, 1620, 1600, 1507, 1451, 1343, 755, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, $J = 8.0$ Hz, 1 H), 8.26 (d, $J = 8.7$ Hz, 2 H), 7.83−7.56 (m, 5 H), 7.67 (s, 1 H), 7.43 (s, 1 H), 7.03 (s, 1 H), 6.84 (s, 1 H), 5.45 (s, 2 H), 4.36 (s, 2 H), 4.07 (t, $J = 6.9$ Hz, 2 H), 2.78 (t, $J = 6.6$ Hz, 2 H), 2.04−1.95 (m, 2 H); ESIMS *m/z* (rel intensity) 422 (MH^+, 100). Anal. ($C_{26}H_{23}N_5O \cdot 0.75H_2O$) C, H, N.

14-(3-Morpholinopropylaminomethyl)-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (27k). Compound **27a** (0.055 g, 0.165 mmol) was diluted with DMSO (25 mL), and 3-morpholinopropylamine (0.119 g, 0.826 mmol) was added. The solution was stirred at room temperature for 19 h, diluted with $CHCl_3$ (40 mL), and washed with H_2O (4 × 30 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated, and the resultant dark yellow solid was purified by flash column chromatography (SiO_2), eluting with a gradient of 1% Et_3N in $CHCl_3$ to 1% MeOH−1% Et_3N in $CHCl_3$ to yield a flaky yellow solid (0.040 g, 55%) after washing with diethyl ether: mp 172−175 °C. IR (film) 3445, 3302, 2929, 1656, 1619, 1600, 1451, 1344, 1117, 753, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, $J = 8.3$ Hz, 1 H), 8.28 (t, $J = 8.5$ Hz, 2 H), 7.82−7.58 (m, 5 H), 7.67 (s, 1 H), 5.48 (s, 2 H), 4.37 (s, 2 H), 3.65 (t, $J = 4.4$ Hz, 4 H), 2.81 (bm, 2 H), 2.42−2.37 (m, 6 H), 1.78−1.68 (m, 2 H); ESIMS *m/z* (rel intensity) 441 (MH^+, 100). Anal. ($C_{27}H_{28}N_4O_2 \cdot 0.5H_2O$) C, H, N.

14-(3-Chloropropyl)-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (28a). Compound **23** (1.000 g, 5.020 mmol) and compound **26** (1.091 g, 5.522 mmol) were diluted with benzene (125 mL). *p*-Toluenesulfonic acid monohydrate (0.955 g, 5.020 mmol) was added, and the solution was heated at reflux for 5 h using a Dean−Stark trap to collect the azeotroped water. The solution was concentrated, and the precipitate was washed with Et_2O (50 mL). The precipitate was dissolved in $CHCl_3$ (500 mL) and washed with saturated $NaHCO_3$ (3 × 200 mL). The combined aqueous layer was then extracted with $CHCl_3$ (3 × 200 mL). The organic layers were combined, dried over sodium sulfate, concentrated, and purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 4% MeOH in $CHCl_3$, to provide an off-white solid (1.642 g, 91%) after washing with Et_2O (50 mL): mp 235−237 °C. IR (KBr) 3465, 1654, 1619, 1601, 756, and 689 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.37 (d, $J = 8.0$ Hz, 1 H), 8.29 (d, $J = 7.6$ Hz, 1 H), 8.18 (dd, $J = 8.5$ and 1.0 Hz, 1 H), 8.00 (d, $J = 7.8$ Hz, 1 H), 7.88−7.67 (m, 3 H), 7.64 (s, 1 H), 7.63−7.58 (m, 1 H), 5.40 (s, 2 H), 3.88 (t, $J = 6.3$ Hz, 2 H), 3.37−3.31 (m, 2 H), 2.19−2.14 (m, 2 H); ESIMS *m/z* (rel intensity) 361/363 (MH^+, 100/33). Anal. ($C_{22}H_{17}ClN_2O \cdot 0.5H_2O$) C, H, N.

14-(3-Azidopropyl)-2*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (28b). Compound **28a** (0.150 g, 0.416 mmol) and sodium azide (0.081 g, 1.25 mmol) were diluted with DMSO (35 mL), and the mixture was heated at 100 °C for 16 h. The mixture was diluted into H_2O (100 mL) and extracted with $CHCl_3$ (1 × 100 mL, 1 × 80 mL, 1 × 50 mL). The combined organic layers were washed with H_2O (3 × 200 mL), dried over anhydrous sodium sulfate, and concentrated to afford an off-white amorphous solid, which was isolated by washing with ether and MeOH and purified by preparative TLC (SiO_2, $CHCl_3$) to yield a pale-yellow amorphous solid (0.062 g, 40%) after washing with ether: mp 185−188 °C (dec). IR (film) 2918, 2108, 1659, 1626, 1604, 1506 1441, 1341, 1287, 1244, 1067, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, $J = 7.9$ Hz, 1 H), 8.24 (d, $J = 8.2$ Hz, 1 H), 8.11 (d, $J = 8.3$ Hz, 1 H), 7.79−7.58 (m, 6 H), 5.35 (s, 2 H), 3.50 (t, $J = 6.3$ Hz, 2 H), 3.29 (t, $J = 7.7$ Hz, 2 H), 2.20−2.00 (m, 2 H); ESIMS *m/z* (rel intensity), 368, (MH^+, 100). Anal. ($C_{22}H_{17}N_5O \cdot 0.5H_2O$) C, H, N.

14-(3-Aminopropyl)-2*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one Dihydrochloride (28c). Compound **28b** (0.040 g, 0.109 mmol) was diluted with benzene (25 mL), and triethyl phosphite (0.045 g, 0.272 mmol) was added. The solution was heated at reflux for 19 h and cooled, and methanolic HCl (3 M, 15 mL) was added, followed by heating at reflux for another 3 h. The mixture was cooled and concentrated to afford a bright-yellow amorphous solid (0.040 g, 88%) after washing with $CHCl_3$ and ether and drying in vacuo: mp 290−295 °C (dec). IR (KBr) 3434, 2923, 2874, 1658, 1620, 1601, 1478, 1343, 755, 689 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.14−7.11 (m, 3 H), 7.00−6.80 (m, 4 H), 6.60−6.40 (m, 1 H), 6.25 (s, 1 H), 4.26 (s, 2 H), 3.10 (t, $J = 7.3$ Hz, 2 H), 2.60−2.40 (m, 2 H), 1.80−1.60 (bm, 2 H); ESIMS *m/z* (rel intensity), 342 (MH^+, 100). Anal. ($C_{22}H_{21}Cl_2N_3O \cdot H_2O$) C, H, N.

14-[3-(1-Imidazolylpropyl)]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one Trifluoroacetate (28d). Compound **28a** (0.100 g, 0.277 mmol), sodium iodide (0.249 g, 1.662 mmol), and imidazole (0.113 g, 1.66 mmol) were diluted with DMSO (30 mL), and the reaction mixture was heated at 100 °C for 24 h and then allowed to cool to room temperature. The reaction mixture was diluted with $CHCl_3$ (150 mL) and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$−1% Et_3N to 3% MeOH in $CHCl_3$−1% Et_3N, to provide a yellow solid. The solid was diluted with $CHCl_3$ (2 mL), and trifluoroacetic acid (5 mL) was added. The reaction mixture was allowed to stir at room temperature for 2 h and concentrated, and the residue was triturated with diethyl ether. The precipitate was filtered and washed with diethyl ether (50 mL) to provide a yellow solid (0.081 g, 58%): mp 215−220 °C. IR (KBr) 1661, 1631, 1201, and 1128 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.10 (s, 1 H), 8.38 (d, $J = 8.1$ Hz, 1 H), 8.27 (d, $J = 8.4$ Hz, 1 H), 8.18 (d, $J = 7.6$ Hz, 1 H), 8.01 (d, $J = 7.9$ Hz, 1 H), 7.86−7.79 (m, 3 H), 7.75−7.62 (m, 4 H), 5.40 (s, 2 H), 4.45 (t, $J = 7.3$ Hz, 2 H), 3.27 (t, $J = 7.4$ Hz, 2 H), 2.28 (bs, 2 H); ESIMS *m/z* (rel intensity) 393 (MH^+, 100). Anal. ($C_{27}H_{21}F_3N_4O_3 \cdot 0.25H_2O$) C, H, N.

14-[3(1-Morpholinopropyl)]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one Trifluoroacetate (28e). Compound **28a** (0.100 g, 0.277 mmol), sodium iodide (0.250 g, 1.66 mmol), and morpholine (0.144 g, 1.66 mmol) were diluted with DMSO (30 mL), and the reaction mixture was heated at 100 °C for 48 h and then allowed to stir at room temperature for 16 h. The reaction mixture was diluted with $CHCl_3$ (150 mL) and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$−1% Et_3N to 5% MeOH in $CHCl_3$−1% Et_3N, to provide a yellow solid. The solid was diluted with $CHCl_3$ (2 mL), and trifluoroacetic acid (5 mL) was added. The reaction mixture was allowed to stir at room temperature for 2 h and concentrated, and the residue was triturated with diethyl ether. The precipitate was filtered and washed with diethyl ether (50 mL) to provide a yellow solid (0.140 g, 79%): mp 190−195 °C. IR (KBr) 1662, 1632, 1197, 1134 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, $J = 8.1$ Hz, 1 H), 8.33 (d, $J = 8.6$ Hz, 1 H), 8.20 (d, $J = 7.5$ Hz, 1 H), 8.02 (d, $J = 8.0$ Hz, 1 H), 7.90−7.72 (m, 3 H), 7.71 (s, 1 H), 7.65−7.60 (m, 1 H), 5.43 (s, 2 H), 3.98 (d, $J = 12.5$ Hz, 2 H), 3.63 (t, $J = 11.9$ Hz, 2 H), 3.44−3.25 (m, 6 H), 3.16−3.03 (m, 2

H), 2.11 (m, 2 H); ESIMS *m/z* (rel intensity) 412 (MH^+, 100). Anal. ($C_{28}H_{26}F_3N_3O_4$) C, H, N.

14-(3-*N*-Ethanolaminopropyl)-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one Trifluoroacetate (28f). Compound **28a**(0.100 g, 0.277 mmol), sodium iodide (0.249 g, 1.66 mmol), and ethanolamine (0.100 mL, 1.66 mmol) were diluted with DMSO (30 mL), and the reaction mixture was heated at 100 °C for 16 h and then allowed to cool to room temperature. The reaction mixture was diluted with $CHCl_3$ (150 mL) and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$−1% Et_3N to 9% MeOH in $CHCl_3$−1% Et_3N, to provide a white solid. The solid was diluted with $CHCl_3$ (3 mL), and trifluoroacetic acid (5 mL) was added. The reaction mixture was allowed to stir at room temperature for 2 h and concentrated, and the residue was triturated with diethyl ether. The precipitate was filtered and washed with diethyl ether (50 mL) to provide a yellow solid (0.101 g, 73%): mp 225−227 °C (dec). IR (KBr) 3383, 1661, 1621, 1602, 1201, 1132 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.48 (bs, 2 H), 8.39 (d, J = 8.1 Hz, 1 H), 8.34 (d, J = 8.1 Hz, 1 H), 8.19 (d, J = 8.4 Hz, 1 H), 8.02 (d, J = 8.0 Hz, 1 H), 7.89−7.80 (m, 2 H), 7.76−7.71 (m, 1 H), 7.70 (s, 1 H), 7.65−7.60 (m, 1 H), 5.40 (s, 2 H), 3.66 (t, J = 5.0 Hz, 2 H), 3.32 (t, J = 7.1 Hz, 2 H), 3.16 (bs, 2 H), 3.02 (bs, 2 H), 2.06 (bs, 2 H); ESIMS *m/z* (rel intensity) 386 (MH^+, 100). Anal. ($C_{26}H_{24}F_3N_3O_4 \cdot 0.5H_2O$) C, H, N.

14-[3-(*N,N*-Dimethylaminopropyl)]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one Trifluoroacetate (28g). Compound **28a** (0.100 g, 0.277 mmol), sodium iodide (0.249 g, 1.66 mmol), and dimethylamine (2 M in THF, 1.67 mL, 3.32 mmol) were diluted with DMSO (30 mL), and the reaction mixture was heated at 100 °C for 16 h and then allowed to cool to room temperature. The reaction mixture was diluted with $CHCl_3$ (150 mL) and washed with water (3 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$−1% Et_3N to 4% MeOH in $CHCl_3$−1% Et_3N, to provide a yellow oil. The oil was diluted with $CHCl_3$ (3 mL), and trifluoroacetic acid (5 mL) was added. The reaction mixture was allowed to stir at room temperature for 2 h and concentrated, and the residue was triturated with diethyl ether. The precipitate was filtered and washed with diethyl ether (50 mL) to provide a light-yellow solid (0.095 g, 71%): mp 210 °C (dec). IR (KBr) 3434, 1666, 1633, 1199, 1128 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.38 (d, J = 7.9 Hz, 1 H), 8.33 (d, J = 8.2 Hz, 1 H), 8.20 (d, J = 7.6 Hz, 1 H), 8.02 (d, J = 8.0 Hz, 1 H), 7.90−7.80 (m, 2 H), 7.76−7.71 (m, 1 H), 7.70 (s, 1 H), 7.65−7.60 (m, 1 H), 5.43 (s, 2 H), 3.28−3.23 (m, 4 H), 2.78 (s, 6 H), 2.09−2.07 (m, 3 H); ESIMS *m/z* (rel intensity) 370 (MH^+, 100). Anal. ($C_{26}H_{24}F_3N_3O_3 \cdot H_2O$) C, H, N.

Topoisomerase I-Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from Baculovirus as described previously.[55] 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 mixtures) 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 fragment was 3′-end labeled at the Hind III site by fill-in reaction with [α-^{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 topoisomerase I 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% polyacrylamide, 7 M urea). Gels were dried and visualized by using a phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Modeling Studies. The structure of the ternary complex, containing topoisomerase I, DNA, and camptothecin, was downloaded from the Protein Data Bank (PDB code 1T8I).[10,27] Several of the atoms were then fixed according to the Sybyl atom types. Hydrogens were added and minimized using the MMFF94s force field and MMFF94 charges. Modeled analogues were constructed in Sybyl 7.3, energy minimized with the MMFF94s force field and MMFF94 charges, and overlapped with the crystal structure ligand in the ternary complex, and the crystal structure ligand was then deleted. The new complex was subsequently subjected to energy minimization using MMFF94s force field with MMFF94 charges. During the energy minimization, the structure of the aromathecin and a surrounding 5 Å sphere were allowed to move, while the structures of the remaining protein and nucleic acids were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/(mol Å) energy gradient convergence criterion and a distance-dependent dielectric function. Ligand overlays were constructed using the indenoisoquinoline crystal structure 1SC7.[10]

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Supporting Information Available: Elemental analysis results for compounds **27a−k** and **28a−g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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