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

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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 12H-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 nssisside 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 camptothecin5,11–18 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

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 aromathecins27–30 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 12H-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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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, 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 the stability of the ternary complex. A ligand overlay of the crystal structure of the indenoisoquinoline (4)−top1−DNA ternary complex 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), rossettacin (9), and compound 10 were previously synthesized in our laboratory via the condensation of a pyrroloquinoline with appropriately substituted phthalide derivatives. Notable routes to 22-hydroxyacuminatine and the 12H-5,11a-diazadibenzo[b,h]fluoren-11-one system include pyridone benzannulation and Heck coupling and aza-Diels−Alder reactions. Recently, Pin et al. have published a novel route to rossettacin and 14-methyl- and 14-phenylaromathecin, employing an N-amidoacylation/aldol condensation with a benzotriazole ester to form the key intermediate.

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 rossettacin. 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. Removal of the carbamate functionality and reaction with chlorophthalide 17, readily prepared from 2-carboxybenzaldehyde (16), 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.
The aminohaloborane modification of the Friedel-Crafts alkylation, as reported by Sugasawa et al. 45,46 It is anticipated that aniline, respectively (Scheme 3). These ketones were prepared from oxidation products and various substituted acetophenones.

Reagents and conditions: (a) EtOCOCl, CHCl₃, reflux; (b) (i) NaH, benzene, reflux; (ii) maleic acid, EtOAc, room temp; (b) MeOH, Et₃N, room temp; (c) PDC, CH₂Cl₂, reflux; (d) polyphosphoric acid, CHCl₃, reflux. 23

as its hydrogen maleate salt. 44 Condensation of provided hydroxyamide, which gave a mixture of the oxidation products and 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 aminobutyrophene 26, respectively (Scheme 3). These ketones were prepared from aniline (24) and chloroacetanilide or 4-chlorobutynitrile via the aminohaloboran modification of the Friedel–Crafts acylation, as reported by Sugawara et al. 35,46 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 benzoyl 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.

Reagents and conditions: (a) i) cat. 2-cyclohexen-1-one, cyclohexanol, reflux, (ii) maleic acid, EtOAc, room temp; (b) MeOH, Et₃N, room temp; (c) PDC, CH₂Cl₂, reflux; (d) polyphosphoric acid, CHCl₃, reflux.

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The benzoyl 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 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 GI₅₀ 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 GI₅₀ against all cell lines tested.

For completeness and comparison, the activities of camptothecin (1), indenoisoquinolines 5,6,23,49 and 6,72 rossetatin (9), and dimethoxyaromathecin (10), 72 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
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. 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 hydroxyoxypyrrolidinyl 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 activity31,32,34 and antiproliferative activity against the human lung carcinoma line H460. The 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. 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 hypothesized1,5,10,23 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 (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

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**Scheme 4**

![Scheme 4](image)

Reagents and conditions: (a) amine, NaI, DMSO, room temp, 62–100 °C (27d); (b) NaN₃, DMSO; (c) (i) (EtO₃)P, benzene, reflux, (ii) 3 M HCl, MeOH, reflux.

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**Scheme 5**

![Scheme 5](image)

Reagents and conditions: (a) amine, NaI, DMSO, 100 °C; (b) NaN₃, DMSO, 100 °C; (c) (i) (EtO₃)P, benzene, reflux, (ii) 3 M HCl, MeOH, reflux.
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...
acts in a manner similar to indenoisoquinoline 5, which bears an identical side chain.\textsuperscript{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.\textsuperscript{21} A formal \textsc{Compare}\textsuperscript{51,52} analysis, as was performed for 6,\textsuperscript{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.\textsuperscript{40,53,54}

**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 antiproliferative potency 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.\textsuperscript{21} A formal \textsc{Compare}\textsuperscript{51,52} analysis, as was performed for 6,\textsuperscript{22} will be performed on these “targetless” aromathecins pending further data.

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**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\textsubscript{3} as the solvent except where otherwise specified, using a Perkin-Elmer Spectrum One FT-IR spectrometer, and are baseline-corrected. \textsuperscript{1}H 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-Ethoxy carbonylglycinate (12).** The hydrochloride salt of methyl glycinate (11) (12.6 g, 0.1 mol) was diluted with CHCl\textsubscript{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\textsubscript{2}O (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 (11.4 g, 92%).\textsuperscript{27a} \textsuperscript{1}H NMR (CDCl\textsubscript{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 (s, J = 7.1 Hz, 3 H).

**N-Ethoxy carbonyl-3-pyrrolidinone (13).**\textsuperscript{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 acetate (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\textsubscript{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%).\textsuperscript{27c} \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{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\textsuperscript{+}, 100).

**N-Ethoxy carbonyl-3-pyrrolidinone Ethylene Ketol (14).**\textsuperscript{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%). \textsuperscript{1}H NMR (CDCl\textsubscript{3}) δ 4.14 (q, J = 7.1 Hz, 2 H), 3.99–3.94
2.3-Dihydropyrrolo[1,2-b]isoquinoline-1,5-dione (23, Method 2). Pyridinium dichromate (5.147 g, 13.68 mmol) was diluted with anhydrous CH₂Cl₂ (30 mL) under an argon atmosphere. A solution of 21 (2.00 g, 9.12 mmol) in anhydrous CH₂Cl₂ (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 CHCl₃ (4 × 30 mL). The filtrate was filtered through a pad of Celite, and the pad was washed with CHCl₃ (3 × 30 mL). The filtrate was concentrated to yield a dark-brown oil that was then diluted with CHCl₃ (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 CHCl₃ (5 × 100, 1 × 50 mL). The organic layers were washed with saturated NaCl (250 mL), dried over anhydrous sodium sulfate, and concentrated. The resulting brown oil was flash chromatographed (SiO₂), eluting with a gradient of hexanes to 50% MeOH, to afford an orange-yellow amorphous solid (0.590 g, 33%): mp 180–184 °C (lit.36 mp 191–192 °C). The ¹H NMR spectrum was identical to compound 23 prepared by method 1 above.

2-Amino-a-chloroacetophenone (25). 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. Chloroacetanilide (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 CHCl₃ (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). ¹H NMR (300 MHz, CDCl₃) δ 7.56 (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). 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-Chlorobutynitrile (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 CH₂Cl₂ (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 (SiO₂), eluting with a gradient of hexanes to 50% EtOAc–hexanes. The solvent was evaporated, and the resulting product was diluted with Et₂O (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 Et₂O (50 mL) to provide a white solid. The solid was dissolved in saturated NaHCO₃ (150 mL) and extracted with CHCl₃ (3 × 50 mL). The combined organic layers were washed with saturated NaCl (50 mL) and dried over sodium sulfate, filtered, and concentrated to provide a yellow oil (2.022 g, 32%) that solidified upon standing: mp 51–55 °C. ¹H NMR (300 MHz, CDCl₃) 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-12H-5,11a-diazadibenzo[b,h]fluoren-11-one (27a). Compound 23b (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 2 h using a Dean–Stark trap to collect the azeotropic water. The solution was concentrated, diluted with NaHCO₃ (150 mL), and extracted with CHCl₃ (6 × 100 mL) and saturated NaCl (100 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (SiO₂), eluting with a gradient of CHCl₃ to 5% MeOH in CHCl₃, 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, CDCl₃) δ 8.56 (d, J = 8.1 Hz, 1H), 8.27 (d, J = 7.6 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.86–7.69 (m, 4H), 7.65 (s, 1H), 7.62–7.56 (m, 1H), 5.44 (s, 2H), 5.05 (s, 2H); ESIMS m/z (rel intensity) 333/335 (MH⁺, 100%).

14-Azidomethyl-12H-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 CHCl₃ (30 mL), and more CHCl₃ was then added until the organic phase was clear. The organic layer was washed with H₂O (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 CHCl₃, and purified by flash column chromatography (SiO₂), eluting with CHCl₃ to provide a pale-yellow amorphous solid (0.051 g, 71%) after washing with MeOH: mp 212–212 °C (dec). IR (film) 2918, 2102, 1665, 1639, 1605, 745, 685 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ 8.58 (d, J = 7.6 Hz, 1H), 8.28 (d, J = 8.2 Hz, 1H), 8.11 (d, J = 8.3 Hz, 1H), 7.84–7.60 (m, 5H), 7.67 (s, 1H), 5.50 (s, 2H), 5.01 (s, 2H); ESIMS m/z (rel intensity) 340 (MH⁺, 100%).

14-(N-Ethanolamino)methyl-12H-5,11a-diazadibenzo[b,h]fluoren-11-one (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 CHCl₃ (40 mL), and washed with H₂O (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 (SiO₂), eluting with a gradient of 6% MeOH in CHCl₃ to 9% MeOH in CHCl₃, 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-d₆) δ 8.38–8.31 (m, 2H), 8.14 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.81 (q, J = 7.2 Hz, 2H), 7.69–7.56 (m, 3H), 5.44 (s, 2H), 4.57 (t, J = 5.3 Hz, 1H), 4.32 (s, 2H), 3.54 (q, J = 5.6 Hz, 2H), 2.73 (t, J = 5.7 Hz, 2H); ESIMS m/z (rel intensity) 358 (MH⁺, 100%).

14-(N,N-Dimethylamino)methyl-12H-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-dimethylamine hydrochloride (0.035 g, 0.429 mmol) and Et₂N (0.045 g, 0.445 mmol) were added. The solution was stirred at room temperature for 20 h and then diluted with CHCl₃ (40 mL) and then washed with H₂O (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 (SiO₂), 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, CDCl₃) δ 8.58 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 8.3 Hz, 1H), 8.23 (d, J = 8.9 Hz, 1H), 7.80–7.58 (m, 5H), 7.63 (s, 1H), 5.45 (s, 2H), 3.97 (s, 2H), 2.35 (s, 6H); ESIMS m/z (rel intensity) 345 (MH⁺, 100%).

14-(N-S)-3-Hydroxypropyrolidonin-12H-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 Et₂N (0.182 g, 1.80 mmol) was added. The solution was stirred at room temperature for 19 h, diluted with CHCl₃ (40 mL), and washed with H₂O (4 × 30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (SiO₂), eluting...
with a gradient of CHCl₃ to 4% MeOH in CHCl₃, 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, CDCl₃) 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) 364 (MH⁺, 100). Anal. (C₁₁H₁₀N₂O₂) 0.25H₂O C, H, N.

14-[(1-Morpholinyl)methyl]-12F-5,11a-diazadibenzo[b,h]fluorone-11-one (27a). Compound 27a (0.060 g, 0.180 mmol) was diluted with DMSO (25 mL), and 1-(3-aminopropyl)morpholine (0.068 g, 0.505 mmol) was added. The solution was stirred at room temperature for 17 h, diluted with CHCl₃ (40 mL), and washed with H₂O (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 (SiO₂), eluting with a gradient of CHCl₃ to 6% MeOH in CHCl₃, 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, 1375, 755, 688 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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. (C₁₁H₁₀N₂O₂+0.75H₂O) C, H, N.

14-[(3-Morpholinyl)methyl]-12F-5,11a-diazadibenzo[b,h]fluorone-11-one (27b). Compound 27b (0.055 g, 0.166 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 CHCl₃ (40 mL), and washed with H₂O (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 (SiO₂), eluting with a gradient of 1% Et₂N in CHCl₃ to 1% MeOH–1% Et₂N in CHCl₃, 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, 1541, 1344, 1117, 753, 687 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.85 (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. (C₁₆H₁₄N₄O₂+0.5H₂O) C, H, N.

14-(3-Chloropropyl)-12F-5,11a-diazadibenzo[b,h]fluorone-11-one (28a). Compound 23 (1.000 g, 5.020 mmol) and compound 26 (1.091 g, 5.522 mmol) were dissolved with benzene (125 mL), t-BuOCl (0.3855 g, 5.020 mmol) was added, and the solution was heated at reflux for 5 h using a Dean–Stark trap to collect the azeotropic water. The solution was concentrated, and the precipitate was washed with Et₂O (50 mL). The precipitate was dissolved in CHCl₃ (500 mL) and washed with saturated NaHCO₃ (3 × 200 mL). The combined aqueous layer was then extracted with CHCl₃ (3 × 200 mL). The organic layers were combined, dried over sodium sulfate, concentrated, and purified by flash column chromatography (SiO₂), eluting with a gradient of CHCl₃ to 4% MeOH in CHCl₃, to provide an off-white solid (1.642 g, 91%) after washing with Et₂O (50 mL): mp 235–237 °C. IR (KBr) 3465, 1654, 1619, 1601, 756, and 689 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) 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. (C₁₄H₁₂ClN₂O₂+0.5H₂O) C, H, N.

14-(3-Azidopropyl)-12F-5,11a-diazadibenzo[b,h]fluorone-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 H₂O (100 mL) and extracted with CHCl₃ (1 × 100 mL, 1 × 80 mL, 1 × 50 mL). The combined organic layers were washed with H₂O (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 (SiO₂, CHCl₃) 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, 1504, 1441, 1341, 1287, 1244, 1067, 687 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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), 6.35 (s, 2 H), 3.50 (J, 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. (C₁₄H₁₂N₄O₂+0.5H₂O) C, H, N.
14-(3-N,N-Dimethylaminopropyl)-12H-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 CHCl3 (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 (SiO2, eluting with a gradient of CHCl3–1% Et3N to 9% MeOH in CHCl3–1% Et3N, to provide a white solid. The solid was diluted with CHCl3 (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. The residue was triturated with diethyl ether (50 mL) 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 (0.095 g, 71%): mp 210 °C (dec).

H NMR (300 MHz, DMSO-d6) 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. (C28H26F3N3O4)C, H, N.

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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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