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The structure–activity relationships of A-ring-substituted aromathecin topoisomerase I inhibitors strongly support a camptothecin-like binding mode

Maris A. Cinelli [a], Andrew E. Morrell [a], Thomas S. Dexheimer [b], Keli Agama [b], Surbhi Agrawal [b], Yves Pommier [b], Mark Cushman [a,*]

[a] Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, and the Purdue Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA
[b] Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892-4255, USA

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ABSTRACT

Aromathecins are inhibitors of human topoisomerase I (Top1). These compounds are composites of several heteroaromatic systems, namely the camptothecins and indenoisoquinolines, and they possess notable Top1 inhibition and cytotoxicity when substituted at position 14. The SAR of these compounds overlaps with indenoisoquinolines, suggesting that they may intercalate into the Top1-DNA complex similarly. Nonetheless, the proposed binding mode for aromathecins is purely hypothetical, as an X-ray structure is unavailable. In the present communication, we have synthesized eight novel series of A-ring-substituted (positions 1–3) aromathecins, through a simple, modular route, as part of a comprehensive SAR study. Certain groups (such as 2,3-ethylenedioxy) moderately improve Top1 inhibition, and, often, antiproliferative activity, whereas other groups (2,3-dimethoxy and 3-substituents) attenuate bioactivity. Strikingly, these trends are very similar to those previously observed for the A-ring of camptothecins, and this considerable SAR overlap lends further support (in the absence of crystallographic data) to the hypothesis that aromathecins bind in the Top1 cleavage complex as interfacial inhibitors in a 'camptothecin-like' pose.

1. Introduction

Topoisomerase I (Top1) is an enzyme that is critical for efficient DNA replication and cell division. As DNA is highly supercoiled, it must also be relaxed prior to cellular processes such as replication and transcription. The enzyme acts by binding to and nicking double-stranded DNA through the action of a nucleophilic tyrosine residue (Tyr723). Within the covalent Top1-DNA cleavage complex, the scissile DNA strand undergoes 'controlled rotation'[1–3] around the nonscissile strand, relieving the supercoils. The hydroxyl group of the scissile strand's 5′ end then re-ligates the broken strand and the enzyme is released.[1] As it plays a pivotal role in cellular proliferation, Top1 is often overexpressed in human tumors. High levels of this enzyme have been found in lung, colorectal, and ovarian cancers[4–6] and elevated Top1 levels in breast cancers are also associated with poor patient prognosis.[7]

The only FDA-approved Top1 inhibitors are topotecan (**2**) and irinotecan (**3**),[8] drugs based on the pentacyclic antitumor alkaloid camptothecin (**1**), (Fig. 1) which was originally isolated from the Chinese tree *Camptotheca acuminata*.[8,9] Camptothecin binds at the interface of Top1-DNA cleavage complexes in an intercalative mode,[10–12] where it stacks between the base pairs of the Top1-DNA cleavage complex and, stabilized chiefly by *pi–pi* stacking,[13] sterically prevents the re-ligation reaction. It also forms key hydrogen bonds with Top1 amino acid residues.[1,10–12] The resulting covalent Top1-DNA adduct then produces collisions with advancing replication forks and transcription complexes, which triggers irreversible DNA damage and apoptosis.[14,15]

Although the camptothecins are potent and possess high cytotoxicity, they also suffer from well-identified drawbacks, including short duration of action, poor solubility, resistance mutants,[16] and high toxicity.[17,18] Additionally, the E-ring lactone of camptothecin is readily opened to its hydroxycarboxylate form in vivo.[19] This form is less active and binds strongly to human blood proteins.[20]

One promising class of noncamptothecin Top1 poisons is the indenoisoquinolines, such as MJ-III-65 (**4**).[21,12] These compounds possess high anti-Top1 activity, are cytotoxic, and are more stable because they lack the hydroxylactone. Through comprehensive SAR studies,[21–24] two clinical candidates, indotecan (**5**) and indimitecan (**6**) were developed and have begun Phase 1 clinical trials at the National Cancer Institute.[25,26]

We described in two previous communications[27,28] the design, synthesis, and biological evaluation of substituted 12*H*-5,11a-

* Corresponding author. Tel.: +1 765 494 1465; fax: +1 765 494 6790.
E-mail address: cushman@pharmacy.purdue.edu (M. Cushman).

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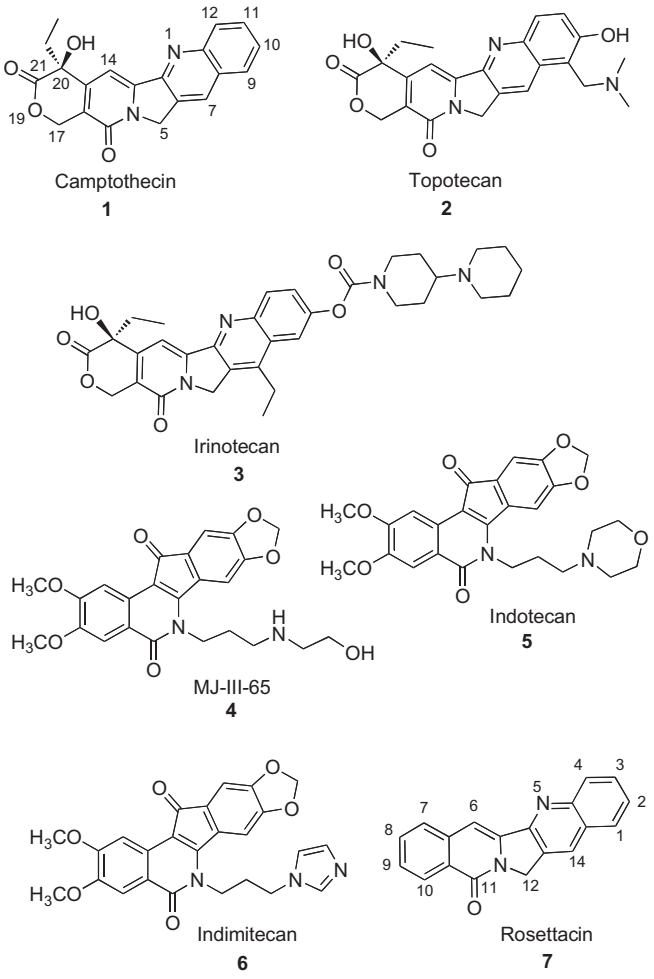


Figure 1. Representative Top1 poisons.

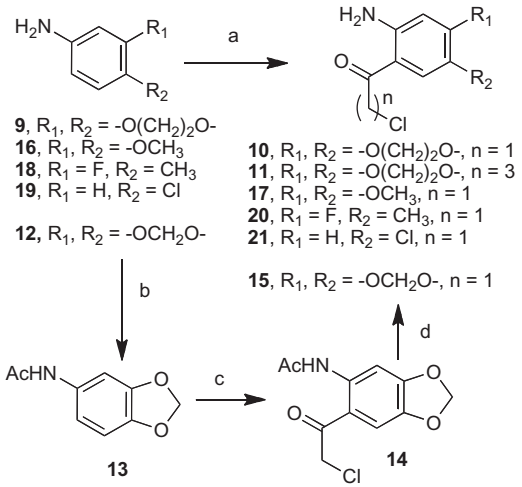
diazadibenzo[*b,h*]fluoren-11-ones, called 'aromathecins'.[27–30] These compounds can be thought of as composites of the camptothecins and indenoisoquinolines, in which the E-ring of the former has been 'aromatized' (replaced by a benzene ring). The majority of these compounds, when substituted at position 14, possess greater Top1 inhibitory and antiproliferative activity than the unsubstituted core compound, rosettacin (**7**).[31] Molecular models indicate that these 14-substituents overlap spatially with the lactam substituents of indenoisoquinolines, which are both proposed to project into the major groove of the DNA-Top1 complex and hydrogen bond to Top1 amino acids and water in the DNA major groove.[27,28] Because of the high degree of SAR overlap at these positions (down to *specific* substituents), it was proposed that 'common' SAR elements are shared between the indenoisoquinolines and aromathecins. The SAR studies also support the hypothesis that aromathecins intercalate in a fashion similar to indenoisoquinolines.[10–12] Due to the overall similarity in shape and structure to camptothecins, it also was suggested that the aromathecins may bind in a distinctively 'camptothecin-like' pose.[13,15] These models are purely hypothetical, however, and because aromathecins are difficult to crystallize with the enzyme, no X-ray structure of any aromathecin in ternary complex with DNA and Top1 is currently available.

The present study was undertaken in order to explore the hypothesis that the biological activity of the aromathecin system could be modulated and eventually improved through substitution at positions other than 14. To this date, no A-ring (positions 1–4)-substituted aromathecins have been evaluated. Using some of the rationale provided by existing camptothecin and indenoisoquinoline SARs and the 'overlapping' SAR hypothesis, we prepared 8 novel series of aromathecins (series **27** through **34**) substituted on both the A-ring and position 14. The A-ring substituents encompass a variety of steric, electronic, and H-bonding properties, allowing for maximum exploration of chemical space and elucidation of those elements required for binding and optimal bioactivity.

2. Chemistry

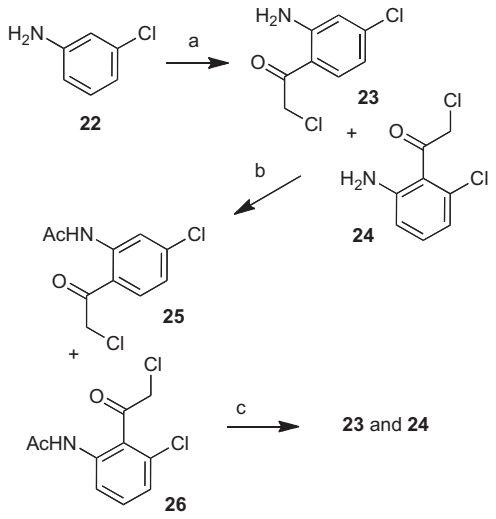
Although there are several routes to rosettacin and aromathecin derivatives,[32,33] our route proceeds through tricyclic synthon **8**.[34] The previously developed route to **8**[28] (see Scheme 3 for structure) proceeds in good yield from commercially available starting materials and can be scaled up readily. This ketone can then be condensed with substituted *o*-aminochloroaceto- or -chlorobutyrophenones to provide versatile aromathecin cores that can be readily functionalized, making the assembly of substituted aromathecins rapid and modular. Following existing camptothecin SARs, a variety of groups (halogens, ethers, the methylenedioxy group, and a methyl group) were chosen for the study. To vary the nature of the A-ring substituent, substituted precursor amino aryl ketones were first prepared.

The preparation of some of these ketone coupling partners is described in Scheme 1. To install the 2,3-ethylenedioxy group, 1,4-benzodiozan-6-amine (**9**) was chloroacetylated[19] using Sugasawa's Friedel–Crafts conditions to yield **10**, and chlorobutyrated likewise to afford **11**.[35–37] As the methylenedioxy group (for 2,3-methylenedioxyaromathecins) is not compatible with the strong Lewis acids utilized in the chloroacetylation, the modified zinc-catalyzed procedure of Luzzio et al.[19] was employed, and beginning with 3,4-methylenedioxyaniline (**12**) the ketone **15** was eventually obtained in low yield but high purity. The chloroacetylation of 4-aminoveratrole (**16**) proceeded in the absence of additional catalyst (due to the electron-donating effects of the methoxy groups) to afford **17**. 3-Fluoro-4-methylaniline (**18**) and 4-chloroaniline (**19**) were also chloroacetylated to afford their respective ketones **20** and **21**, albeit in low yield. The chloroacetylation of 3-chloroaniline (**22**), however, afforded an inseparable mixture of **23** and **24**, which were converted into their respective acetanilides **25** and **26** to aid in purification. These compounds were separated and hydrolyzed to yield **23** and **24**, in a ratio consistent with that re-

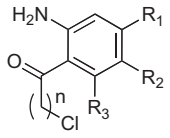


Scheme 1. Reagents and conditions: (a) (i) $BCl_3{\cdot}Me_2S$, 1,2-dichloroethane, 0 °C; (ii) chloroacetonitrile (to afford **10**), 4-chlorobutyronitrile (to afford **11**), $AlCl_3$ (to afford all except **16**), reflux; (iii) 2 M HCl, reflux; (b) AcO_2, Et_3N, H_2O, rt; (c) chloroacetyl chloride, $ZnCl_2$, CH_3NO_2; (d) concd HCl, reflux.

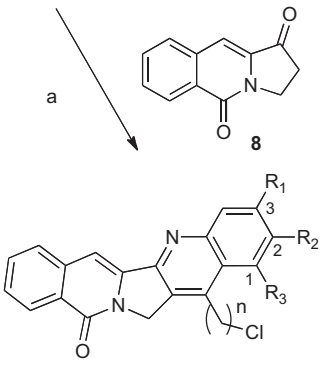
ported by Sugasawa et al.[36] (Scheme 2). Compound **23** was used to prepare the 3-chloroaromathecin series, and **24**, the 1-chloro series.



Scheme 2. Reagents and conditions: (a) (i) $BCl_3{\cdot}Me_2S$, 1,2-dichloroethane, 0 °C; (ii) chloroacetonitrile, $AlCl_3$, reflux; (iii) 2 M HCl, reflux; (b) Ac_2O, 80 °C; (c) concd HCl, EtOH, 0 °C to reflux.



10, R_1, R_2 = -O(CH$_2$)$_2$O-, n = 1
11, R_1, R_2 = -O(CH$_2$)$_2$O-, n = 3
15, R_1, R_2 = -OCH$_2$O-, n = 1
17, R_1, R_2 = -OCH$_3$, n = 1
20, R_1 = F, R_2 = CH$_3$, n = 1
21, R_1 = H, R_2 = Cl, n = 1
23, R_1 = Cl, R_2 = H, n = 1
24, R_1, R_2 = H, R_3 = Cl, n = 1



27a, R_1, R_2 = -O(CH$_2$)$_2$O-, R_3 = H, n = 1
28a, R_1, R_2 = -O(CH$_2$)$_2$O-, R_3 = H, n = 3
29a, R_1, R_2 = -OCH$_2$O-, R_3 = H, n = 1
30a, R_1, R_2 = -OCH$_3$, R_3 = H, n = 1
31a, R_1 = F, R_2 = CH$_3$, R_3 = H, n = 1
32a, R_1, R_3 = H, R_2 = Cl, n = 1
33a, R_1 = Cl, R_2, R_3 = H, n = 1
34a, R_1, R_2 = H, R_3 = Cl, n = 1

Scheme 3. Reagents and conditions: (a) *p*-TsOH, benzene or toluene, AcOH (for **11**), reflux overnight.

The condensation of these ketones with **8** was performed under Friedlander conditions,[27] and the chloroalkylated aromathecin cores **27a**–**34a** were obtained in modest to excellent yield. In the majority of cases, a stoichiometric amount (or less) of *p*-TsOH was sufficient, although several cases did require higher temperatures, longer times, and (in the case of **28a**) catalytic amounts of AcOH (Scheme 3).

Aromathecin analogues **27b**–**i** (2,3-ethylenedioxy), **29b**–**f** (2,3-methylenedioxy), **30b**–**e** (2,3-dimethoxy), **31b**–**g** (2-methyl-3-fluoro), **32b**–**f** (2-chloro) **33b**–**e** (3-chloro) and **34b**–**d** (1-chloro) (Scheme 4) were prepared by simple S_N2 displacement of the unhindered, benzylic chloride by a nucleophile at room or slightly elevated temperature in DMSO or DMF. Many nucleophiles were chosen to explore an assortment of 14-substituents and to search for possible synergistic or antagonistic effects with the A-ring substituents. Nucleophiles available as salts [(*S*)-pyrrolidin-3-ol[38] and *N*,*N*-dimethylamine] were used in the presence of excess triethylamine. This displacement reaction proceeded overnight in most cases to yield the substituted aromathecins in modest to excellent yield, although displacement by imidazole (to prepare **27c**, **30c**, **32f** and **33e**) was performed at a higher temperature. Some ethylenedioxy analogues (**27d** and **27f**) were converted into trifluoroacetate salts to aid in solubility. Likewise, analogues **34b**–**d** were converted to their hydrochloride salts.

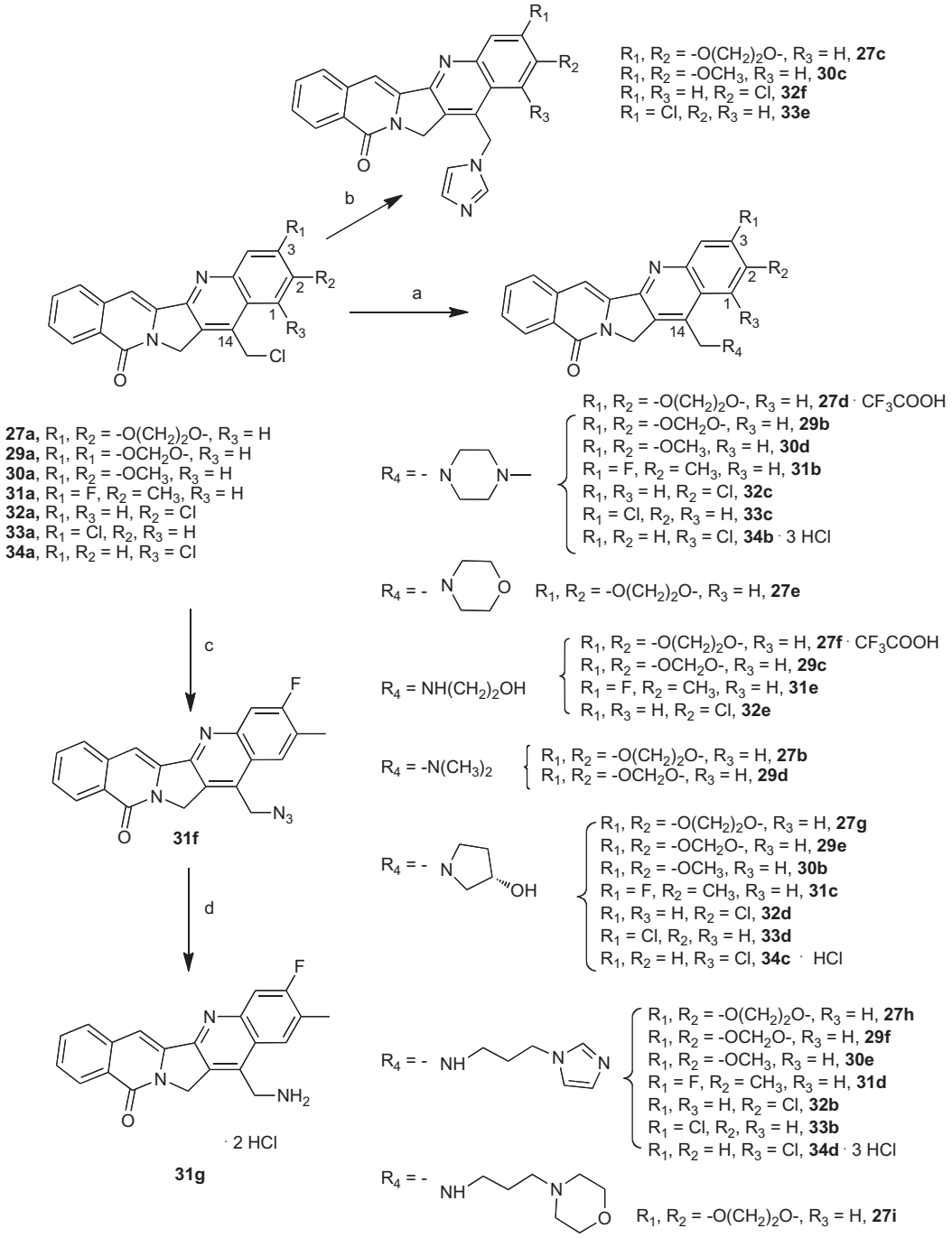
Finally, to synthesize the 'extended' 3′-substituted propyl ethylenedioxy series **28b**–**g** (Scheme 5), an in situ Finkelstein reaction[27] was performed at high temperatures using both an excess of nucleophile and sodium iodide to compensate for the decreased electrophilicity of the terminal alkyl chloride.

3. Results and discussion

Aromathecin analogues (with the exception of chlorinated compounds **29a**, **31a**, and **32**–**34a**, and azides **28f** and **31f**, due to the historically low bioactivities of these compounds) were assayed in the National Cancer Institute's Developmental Therapeutics Assay,[39,40] where they were tested against the eight cancer cell line subpanels described in Table 1 as well as a leukemia panel (approximately 60 cell lines total). After an initial one-dose assay (at 10^{-5} M), selected compounds were tested at five concentrations ranging from 10^{-8} to 10^{-4} M. Cytotoxicity results are reported as GI_{50} values for selected cell lines from each subpanel, and overall antiproliferative potency is quantified as a mean-graph midpoint (MGM) in Table 1. The MGM is a measure of the average GI_{50} against all cell lines tested, where compounds whose GI_{50} values fall outside the concentration range tested (10^{-8} to 10^{-4} M) are assigned GI_{50} values of either 10^{-8} or 10^{-4} M. For comparative purposes, Top1 and antiproliferative activity data for camptothecin (**1**),[17] indenoisoquinoline **4**,[21,41] clinical leads **5** and **6**,[25] and rosettacin (**7**) are included.

Top1 inhibition was measured by a compound's ability to induce enzyme-linked DNA cleavage, and is graded by the following semiquantitative rubric relative to 1 μM camptothecin: 0, no inhibitory activity; +, between 20 and 50% activity; ++, between 50 and 75% activity; +++, between 75% and 95% activity; ++++, equipotent to or more potent. Compounds that are between two scores are delineated with parentheses [i.e., between ++ and +++ is ++(+)].

As can be observed in Figure 2, substitution on the A-ring very easily changes a compound's anti-Top1 activity for a given 14-substituent (here, the aminopropylimidazole side chain), although no 'synergistic' effect was observed with the 14-substituents. Several important SAR trends can be gleaned from these data. The ethylenedioxy group, in general, is well tolerated on the A-ring, and for all except **27g**, anti-Top1 activity is either unchanged or

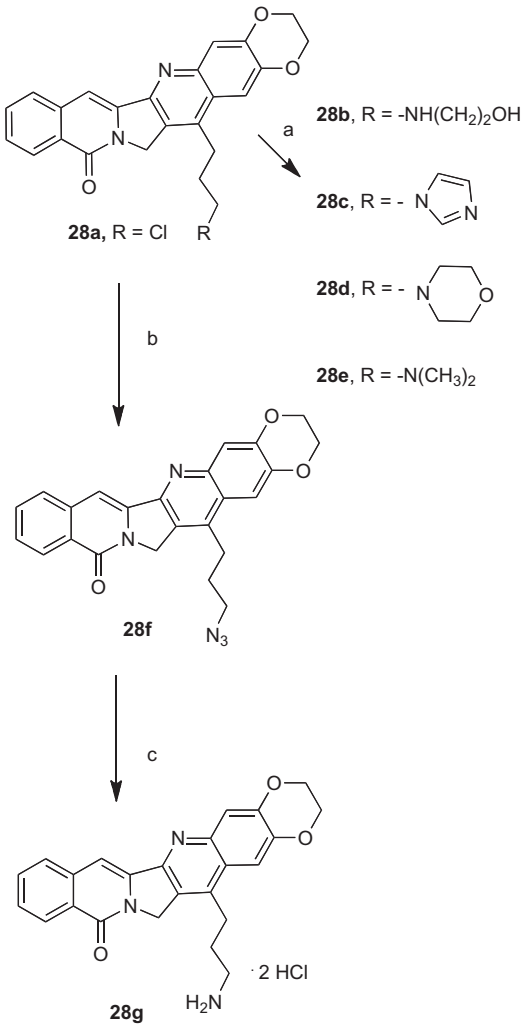


Scheme 4. Reagents and conditions: (a) (i) Amine or amine salt + Et_3N, DMSO or DMF; (ii) HCl/MeOH (series **34**) or CF_3COOH (**27d** and **f**); (b) imidazole, DMSO or DMF, 60–100 °C; (c) for **31a**, NaN_3, DMSO, rt; (d) (i) $(EtO)_3P$, benzene, reflux; (ii) HCl/MeOH, reflux.

improved over the analogous unsubstituted compounds. For the monomethylene compounds **27b**, **27e**, and **27i**, Top1 inhibitory activity is improved, and for **27a**, **27c**, **27d**, **27f** and **27h**, the anti-Top1 activity is unchanged. Although they are potent Top1 poisons, more variability (with the same comparison to the unsubstituted series) is seen in the extended (**28**) series. General variability was also observed for extended unsubstituted compounds alone.[27]

As antiproliferative activity involves factors more complicated than Top1 inhibition alone (as evidenced by low correlation between Top1 inhibition and cytotoxicity[27,28,42]) the GI_{50} values are variable in both the monomethylene and extended series, although improvements in potency on a per-analogue basis are observed in several cases, and many are more potent than clinical candidate **5**. Examining the antiproliferative activity of this entire series of ethylenedioxy compounds (series **27** and **28**), although some were not selected for five-dose testing, all compounds (except for **28a**, which was not tested for cytotoxicity) possessed at least some activity. At a concentration of 10 μM, compound **27a** inhibited cell growth at a mean 10.3%, **27d** at 23%, **27i** at 10.8%, **28e** at 19%, and **27h** at 14.3%.

It is unknown exactly how the ethylenedioxy group may enhance potency, although it is reported that this group improves activity for camptothecin derivatives, such as in the clinical candidate lurtotecan (Fig. 3 and **35**)[1,19,43–45] For camptothecins, placement of ethylenedioxy (or methylenedioxy) groups at the



Scheme 5. Reagents and conditions: (a) Amine, NaI, DMSO or DMF, 100 °C; (b) NaN_3, DMSO, 100 °C (c) (i) $(EtO)_3P$, benzene, reflux; (ii) 3 M HCl, MeOH, reflux.

analogous 10,11 position may improve solubility over the parent compound, although it is clear that improved solubility is not *solely* responsible for improved potency, as racemic 9,10-methylenedioxycamptothecin is only 20% as active as the 10,11-isomer.[45] For hexacyclic camptothecin analogues, hypotheses ranging from 'extended coplanarity'[42,46] to increased positive charge density[47] have been posited. In our earlier proposed binding mode (Fig. 4) the aromatic system (here, of **27h**, which was chosen due to its initial high potency against Top1) intercalates between the base pairs and the A-ring of the aromathecins abuts the nonscissile DNA strand as the ligand in the crystal structures of topotecan and camptothecin does.[27,28,48] The E-ring faces the cleaved strand, and the quinoline nitrogen of the B-ring faces the general direction of Arg364, which it may hydrogen bond to (although it is out of distance in Fig. 4).

Overlaying hypothetical models of aromathecin **27h** with the ligand in a camptothecin crystal structure (Fig. 5) roughly superimposes the A-rings of the two systems. These compounds' ethylenedioxy groups could be positioned similarly and thus exert their effect via similar mechanisms, which could lend support to the proposed binding mode shown in Figure 4.

10,11-Methylenedioxycamptothecins are potent cytotoxic Top1 inhibitors,[45,46,49,50] and are usually more so than the corresponding ethylenedioxycamptothecins, possibly due to the dioxolane ring's ability to better hold coplanarity.[46] Disappointingly, the same effect was not observed for 2,3-methylenedioxyaromathecins. These compounds still retain notable antiproliferative activity (compound **29d** possessed 22.3% inhibition at 10 μM as well) but there is no trend toward improved Top1 inhibition. In our experience, these compounds were very insoluble, and unfavorable physical properties could hinder obtaining significant SAR data.

Other A-ring substituents lend additional support to a proposed camptothecin-like binding mode. In the elegant studies by Monroe Wall and colleagues, it is reported that methoxy substituents, including 10,11-dimethoxy substitution, attenuate bioactivity when placed on the A-ring of camptothecin.[51,52] Remarkably, this trend is also observed for the aromathecins, indicating more SAR overlap. Compounds **30a**–**30e** all possess minimal (all 0 or 0/+) anti-Top1 activity. Aromathecin **30b** has only 14.2% mean growth inhibition at 10 μM, and the remainder of the series was declined for testing by the NCI. The bulkier, free-rotating methoxy groups could simply pose a steric liability toward the nonscissile strand or decrease the number of planar conformations available due to repulsions between the groups. Even before crystallography, it was proposed that the inactivity of dimethoxycamptothecins was caused by a steric 'blocking' effect.[52,53]

The 2-methyl-3-fluoro compounds **31b**–**g** were originally synthesized to mimic fluorinated camptothecins such as exatecan (**36**) and diflomotecan (**37**)[1,17] (Fig. 3). Disappointingly, no increases in Top1 inhibitory activity were seen in this series, and antiproliferative activity generally decreased (neither compound **31c** nor **31e** induced >25% growth inhibition at 10 μM). This is not entirely surprising, as other 10,11-disubstitution patterns are reported to have deactivating effects for camptothecins as well. A comparison between compounds in this series and the clinical candidates in Figure 3 is perhaps unjustified due to the presence of other structural motifs (e.g., chiral amines, homolactones) that aromathecins lack. The increased antitumor activity observed for these fluorinated camptothecins could also be due to their lipophilicity, which increases partitioning to lipids and slows the rate of lactone hydrolysis in vitro and in vivo.[54]

In light of these deactivating disubstitution patterns, it is worth mentioning that substitution at position 11 tends to diminish anti-Top1 activity for camptothecins, regardless of the substituent.[55] This effect is identical for aromathecins, observed in the placement of both a methoxy (series **30**) and fluorine (series **31**) at the analogous 3-position. Placement of chlorine at this position (series **33**) also abolishes most of the Top1 inhibitory activity, indicating the same steric constraints that hinder camptothecins at this position exist for the aromathecin system as well. Modeling compound **33b** (Fig. 6) in ternary complex with Top1 and DNA indicates that when this compound is minimized in our proposed 'camptothecin-like' binding mode (Fig. 4), the bulky, electronegative chlorine could pose steric and electronic liabilities when projected toward the phosphodiester backbone of the nonscissile strand. Upon minimization, the planar aromatic system of this model distorts to possibly avoid these clashes (Fig. 6). Indeed, Staker et al.'s work indicates that this region is also sterically constrained in crystal structures of indenoisoquinolines and indolocarbazoles.[10,11] Additionally, prior to these studies, Wang et al. proposed that modifications at this region of camptothecin (positions 9–11) could greatly affect its biochemical activity due to close proximity to flanking nucleotides.[56] Nonetheless, cyclic groups (e.g., ethylenedioxy) are more restrained and could theoretically be better accommodated, as cyclic groups are also proposed to face the nonscissile strand for indenoisoquinolines.[57] Even if substituted camptothecins bound in an alternative mode to fit these groups, it is likely that aromathecins behave similarly due to the trends in bioactivity. Antiproliferative activity for the 3-chloro series is also variable; (cf. compound **33b** with **33d** and **33e**, the latter two of which did not inhibit cell growth beyond 15% at 10 μM).

Table 1
Antiproliferative potencies and topoisomerase I inhibitory activities of A-ring- and 14-substituted aromathecin analogue series **27**–**32**

Compd	Cytotoxicity[a] (GI_{50} in μM)								MGM[b]	Top1 cleavage[c]
	Lung HOP-62	Colon HCT-116	CNS SF-539	Melanoma UACC-62	Ovarian OVCAR-3	Renal SN12C	Prostate DU-145	Breast MCF-7		
1[17]	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.01	0.0405 ± 0.0187[f]	++++
4[17]	0.02	0.10	0.04	0.03	0.5	<0.01	<0.01	<0.01	0.21 ± 0.19	++++
5[20]	1.78	1.15	0.04	0.03	74.1	0.813	0.155	0.37	4.64 ± 1.25	++++
6[20]	<0.01	<0.01	0.037	<0.01	0.085	<0.01	<0.01	0.01	0.079 ± 0.023	++++
7	>100	57.3	>100	>100	>100	>100	>100	60.3	91.2	++
27a	—	—	—	—	—	—	—	—	—	+
27b	3.31	1.17	2.69	1.66	2.19	2.51	2.19	0.30	2.95	+++
27c	19.5	>100	4.37	21.4	18.6	81.3	>100	0.30	19.05	+++
27d[d]	—	—	—	—	—	—	—	—	—	++(+)
27e	3.80	1.33	0.81	0.99	1.70	2.57	0.93	0.30	1.78 ± 0.165	++(+)
27f	5.69	2.63	4.26	1.16	1.95	5.75	2.04	0.44	2.33 ± 0.24	++(+)
27g	1.01	0.82	0.49	0.46	1.84	0.94	1.02	0.27	1.24 ± 0.21	++
27h[d]	—	—	—	—	—	—	—	—	—	++
27i	—	—	—	—	—	—	—	—	—	++
28a	—	—	—	—	—	—	—	—	—	0
28b	1.01	0.82	0.49	0.46	1.84	0.94	1.02	0.27	2.35 ± 0.215	++
28c	0.57	1.95	1.95	0.93	7.08	12.6	2.88	0.19	3.24	++(+)
28d	0.54	1.42	1.15	1.15	1.84	1.13	0.76	0.06	0.82 ± 0.03	++(+)
28e	—	—	—	—	—	—	—	—	—	+++
28g	2.14	1.95	1.86	1.62	1.95	1.86	1.82	1.05	2.14	+++
29b	17.0	1.45	11.7	1.62	16.2	2.34	51.3	0.38	8.91	++
29c	0.66	0.95	1.35	0.47	1.55	1.91	1.82	0.28	2.00	++(+)
29d	—	—	—	—	—	—	—	—	—	++
29e	0.48	0.85	0.60	0.35	>100	0.79	—	0.07	6.91	++
29f	5.01	1.32	2.14	3.39	4.26	5.89	2.57	0.48	5.50	++
30a	—	—	—	—	—	—	—	—	—	0
30b–**e**[e]	—	—	—	—	—	—	—	—	—	0 or 0/+
31b	20.1	1.80	6.46	16.2	14.6	16.0	2.63	1.80	6.31 ± 0.145	++(+)
31c	—	—	—	—	—	—	—	—	—	++
31d	36.7	3.05	1.10	>100	4.68	1.80	6.24	2.21	11.3 ± 0.95	0
31e	11.2	18.4	9.23	14.2	16.8	20.2	12.2	1.62	14.4 ± 1.81	++(+)
31g	—	—	—	—	—	—	—	—	—	+(+)
32b	—	—	—	—	—	—	—	—	—	++
32c	2.29	0.15	1.58	1.41	1.51	1.78	2.75	3.31	1.85 ± 0.34	+(+)
32d	1.05	1.10	1.26	0.74	1.86	—	1.66	0.37	1.44	+(+)
32e	7.24	2.14	4.36	2.04	6.46	10.2	—	0.23	5.02	++
32f	0.46	0.40	0.39	0.31	0.69	1.54	0.48	0.19	1.02	+
33b	2.00	1.82	1.86	1.91	2.04	2.09	—	1.66	2.51	+
33c	18.1	13.5	15.1	17.0	15.1	19.0	23.4	10.9	14.5	+
33d	—	—	—	—	—	—	—	—	—	+
33e	—	—	—	—	—	—	—	—	—	0
34b	1.86	1.41	1.51	15.5	4.17	13.8	9.77	1.00	4.37	0
34c	1.66	1.55	1.95	3.09	2.63	3.71	3.98	0.37	2.40	+
34d	2.51	1.58	1.90	7.24	4.90	6.03	1.02	0.55	3.09	+++

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition.
[b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested, ranging from 10^{-8} to 10^{-4} molar.
[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% activity; ++++, equipotent to or more potent. Compounds that are ranked between two scores are delineated with parentheses [i.e., between ++ and +++ is ++(+)].
[d] Some compounds such as this were not selected for further testing; refer to text for details.
[e] These compounds were declined for testing by the NCI.
[f] For MGM GI_{50} values in which a standard error appears, the GI_{50} values for individual cell lines are the average of two determinations; values without standard error are from one determination. The values for **1**, **4**, **5**, and **6** are from many determinations.

Halogens improve anti-Top1 potency for camptothecins when placed at positions 9 and 10.[45] The placement of chlorine at position 2 of the aromathecin system is tolerated in some cases but does not confer any advantages. Interestingly, these compounds possess relatively high antiproliferative activity (even **32b**, which was not tested in the 5-dose assay, inhibited cell growth at nearly 30% at 10 μM). Nonetheless, the exact nature of this effect is unknown and several of these compounds may undergo additional testing. Ultimately, action at a second target besides Top1 may be responsible, although preliminary studies with aromathecins indicated that they do not inhibit Top2.[28] Unfortunately, the isomeric 1-chloro series (**34b**–**d**) possessed less anti-Top1 activity in two out of three cases, but molecular models (not shown) did not indicate any obvious steric or electronic encumbrances. Although 9-chlorocamptothecin was among the most potent analogues synthesized in one study,[45] the equivalent 1-chloroaromathecin series cannot be considered an outlier to the overlapping SAR and binding mode hypotheses. Many groups report that, in addition to halogens, a variety of 9-substituents including hydroxyl groups and amines,[45,58] oxyimino moieties,[59] alkyl chains[60] and nitro groups[61] can aid in improving Top1 inhibition, antitumor activity, bioavailability, and pharmacokinetics. Perhaps the effects of the 9-substituent could be more general than previously thought.[59] Due to synthetic difficulties, no additional 1-substituted aromathecins have been prepared, and the full role 1-substitution plays has yet to be determined.

The idea that aromathecins may bind in a manner similar to camptothecins is not entirely novel. The former's 14 substituents, capable of hydrogen bonding, are calculated to project into the major groove of the ternary complex.[27,28] Many 7-substituted

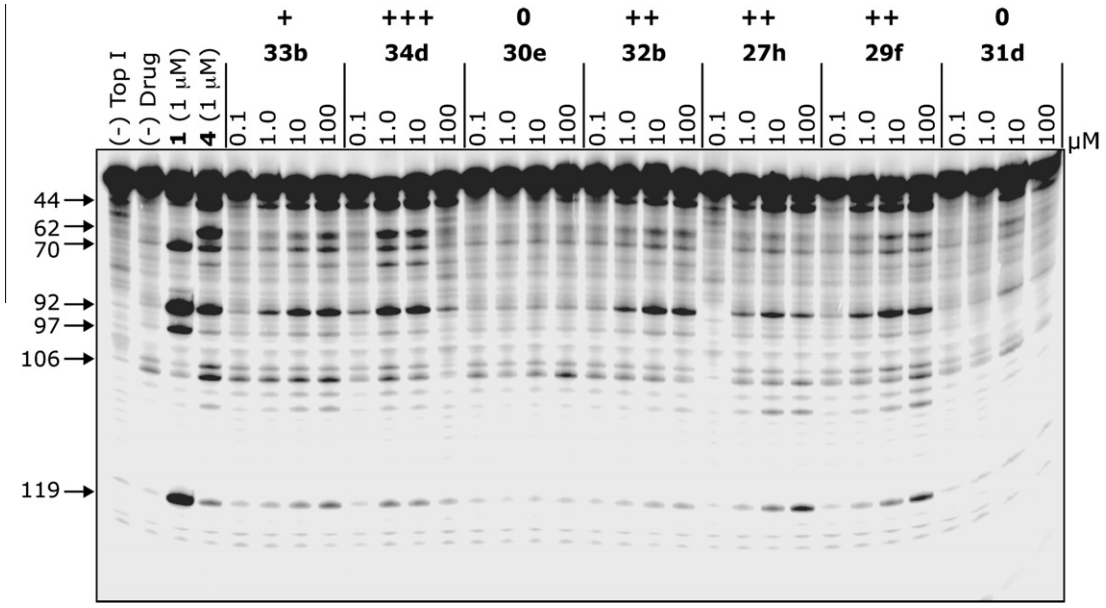


Figure 2. Top1-mediated DNA cleavage induced by aromathecins **33b**, **34d**, **30e**, **32b**, **27h**, **29f**, and **31d**. Lane 1: DNA alone; lane 2: Top1 alone; lane 3: **1**, 1 μM; lane 4: **4**, 1 μM; lanes 5–32: lanes 6–29: **33b**, **34d**, **30e**, **32b**, **27h**, **29f**, and **31d** at 0.1, 1, 10, and 100 μM, respectively, from left to right. Numbers and arrows on left indicate arbitrary cleavage site positions.

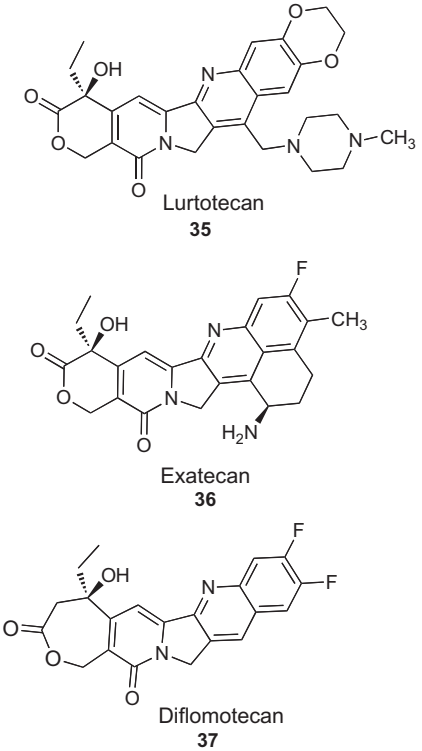


Figure 3. A-ring substituted camptothecins.

camptothecins (Fig. 3) also possess similar substituents that would also sit in the major groove region if they bound in the same pose as camptothecin and topotecan.[10,15]

4. Conclusions

In conclusion, eight novel series of A-ring-substituted aromathecin analogues were prepared by a practical, modular route beginning with commercially available starting materials. These analogues were assayed for Top1 inhibition and antiproliferative activity. Despite the lack of a strong correlation between anti-Top1 activity and cytotoxicity, it has been shown that varying the steric bulk, electronegativity, and hydrogen bonding properties of these A-ring substituents can drastically change the bioactivity of the aromathecin system. The nature of these SAR trends mirror those previously observed for camptothecins (with respect to the ethylenedioxy group, disubstitution, and positions 11 and 3), and these data, coupled with prior knowledge of positions 7 and 14, strongly support the hypothesis that these two systems share many essential SAR elements, which could provide a viable avenue for further optimization of this system. As there is no crystal structure available for aromathecins, these results also provide the only experimental evidence supporting our proposed camptothecin-like binding mode.

5. Experimental

5.1. General procedures

Reagents and solvents were purchased from commercial vendors and were used without further purification. 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 $CHCl_3$ or $CDCl_3$ as the solvent unless 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 or Bruker Avance 500 (QNP probe or TXI 5 mm/BBO 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. APCI-MS was performed using an Agilent 6320 Trap mass spectrometer. Purity of all biologically relevant compounds is $\geq$95% by combustion microanalysis. Combustion microanalyses were performed by Galbraith Laboratories (Knoxville, TN), Midwest Microlab LLC (Indianapolis, IN), or at the Purdue University Microanalysis Laboratory using a Perkin-Elmer Series II CHNS/O model 2400 analyzer. All reported values are within 0.4%

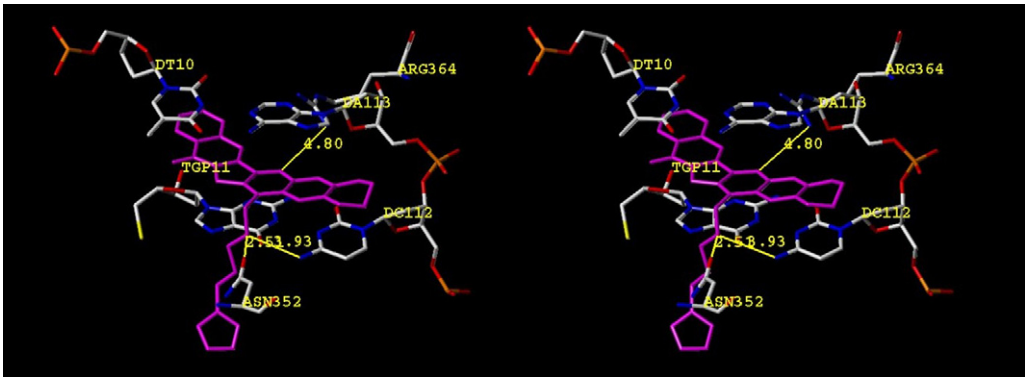


Figure 4. Hypothetical model for binding of 2,3-ethylenedioxyaromathecin **27h** in a Top1-DNA complex. The ligand is colored in magenta and all other relevant structures are labeled and colored by atom type. Distances are from heavy atom to heavy atom; the diagram is programmed for wall-eyed (relaxed) viewing.

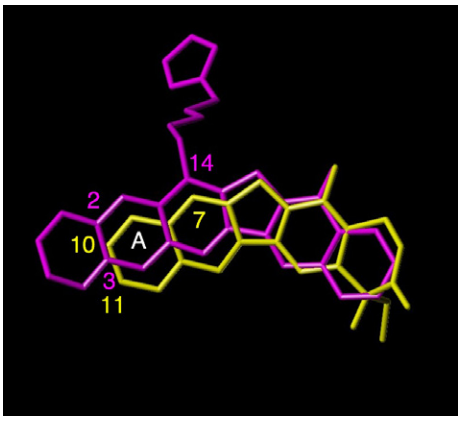


Figure 5. Ligand overlay of aromathecin **27h** (magenta) and camptothecin (**1**, yellow). Relevant positions are indicated in their respective colors and the A-ring is labeled.

of calculated values. Analytical thin-layer chromatography was performed on Baker-flex silica gel IB2-F plastic-backed TLC 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. Precursor compounds **8**[27,28] and **13**, **14**, and **15**[19,62] were prepared according to literature procedures and as depicted in Schemes 1 and 2.

5.2. General procedure for chloroacylation of substituted anilines (10–11, 17, 20–21, and 23–24)[35–37]

Boron trichloride-methyl sulfide complex (approximately 1.80–5.20 g, 10.0–29.0 mmol, or 1.1–1.5 equiv, based on the aniline) was diluted with 1,2-dichloroethane (30–110 mL) and the mixture was cooled to 0 °C under an argon atmosphere. The aniline (1–4 g, ~10–26 mmol) was added dropwise, or, in the case of solid anilines, as a solution in a minimal amount of dichloroethane. The resulting chunky precipitate was stirred for 15 min, and chloroacetonitrile (for compounds **10**, **17**, **20–21**, and **23–24**) or chlorobutyronitrile (for **11**) was added. For all but **17**, aluminum chloride (approximately 1.2 equiv based on the aniline) was then added, and the mixture was warmed to room temperature, stirred for approximately 15 min and heated at reflux for between 1.5–4 h (typical time: 3 h). The mixture was cooled, and 2 M HCl (an amount equivalent to the solvent volume of the reaction) was added. The mixture was heated to reflux for 0.5 h, and then cooled and poured into ice water (>100 mL) and the aqueous and organic layers were separated. The aqueous layer was exhaustively extracted with CH_2Cl_2, and the organic phases were washed with water and satd NaCl and dried over anhydrous sodium sulfate. Concentration afforded crude products that were purified by precipitation, crystallization, or column chromatography.

5.2.1. 2-Amino-α-chloro-4,5-ethylenedioxyacetophenone (10)[19]

Boron trichloride-methyl sulfide complex (1.78 g, 9.92 mmol), compound **9** (1.00 g, 6.61 mmol), chloroacetonitrile (0.624 g, 8.27 mmol) and aluminum chloride (1.32 g, 9.92 mmol), in dichloroethane, provided a yellow solid that was isolated by precipitating with EtOAc–hexanes (0.251 g, 17%): mp 110 °C (lit.[19] mp 130 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (s, 1H), 6.15 (s, 1H), 4.56 (s, 2H), 4.31–4.28 (m, 2H), 4.22–4.19 (m, 2H).

5.2.2. 1-(7-Amino-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-4-chloro-1-butanone (11)

Boron trichloride-methyl sulfide complex (5.23 g, 29.1 mmol), compound **9** (4.00 g, 26.5 mmol), 4-chlorobutyronitrile (3.42 g,

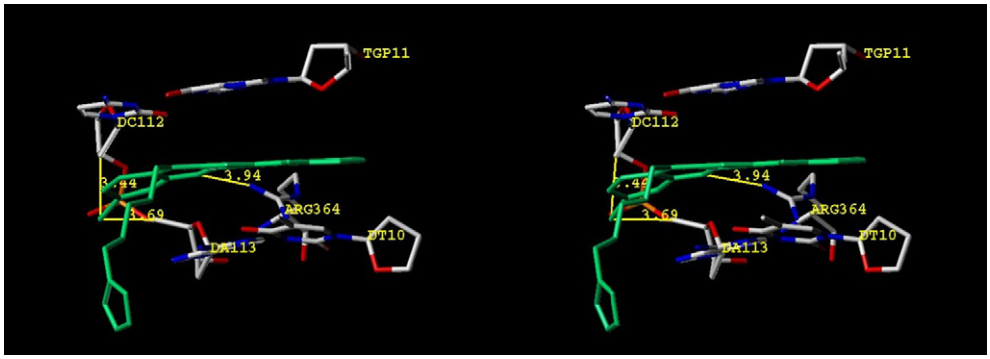


Figure 6. Hypothetical model for binding of 3-chloroaromathecin **33b** in a Top1-DNA complex. The ligand is colored in green and all other relevant structures are labeled and colored by atom type. Distances are from heavy atom to heavy atom. The C, D, and E rings of the aromathecin are coplanar; note the substantial out-of-plane bending to relieve possible steric and/or electronic clashes on the A-ring side. Distances are heavy atom to heavy atom. The diagram is programmed for wall-eyed (relaxed) viewing.

33.1 mmol) and aluminum chloride (3.89 g, 29.1 mmol), in dichloroethane, afforded a residue that was adsorbed onto SiO_2 (7.64 g), and purified by flash column chromatography (SiO_2, 59.6 g), eluting with CH_2Cl_2, to yield a clear-yellow oil. Residual 4-chlorobutyronitrile was removed in vacuo by gentle heating in a water bath (50 °C). The crude product was obtained as a sticky red solid, (2.00 g, 29%) contaminated with a small amount of nitrile. This material was used in the next step without further purification (to prepare **26a**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 1H), 6.49 (s, 1H), 4.31–4.23 (m, 4H), 3.68 (t, J = 6.3 Hz, 2H), 3.06 (t, J = 7.0 Hz, 2H), 2.25–2.10 (m, 2H).

5.2.3. 2-Amino-α-chloro-4,5-(dimethoxy)acetophenone (17)[36]

Boron trichloride-methyl sulfide complex (3.69 g, 20.6 mmol), compound **16** (3.00 g, 19.6 mmol), and chloroacetonitrile (1.78 g, 23.5 mmol), in dichloroethane, afforded a brown semisolid that was dissolved in CH_2Cl_2 and filtered through a pad of SiO_2 to remove black impurities. The pad was rinsed with CH_2Cl_2 (120 mL) until the filtrate ran clear and colorless. The filtrate was concentrated to yield a brown solid (2.33 g, 52%) after washing with hexanes (40 mL): mp 113–115 °C (lit[36] mp 123–124 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (s, 1H), 6.50–6.20 (br s, 2H), 6.12 (s, 1H), 4.58 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H).

5.2.4. 2-Amino-4-fluoro-5-methyl-α-chloroacetophenone (20)

Boron trichloride-methyl sulfide complex (5.16 g, 28.8 mmol), aniline **18** (3.00 g, 23.0 mmol), chloroacetonitrile (2.26 g, 30.0 mmol), and aluminum chloride (4.79 g, 36.0 mmol), in dichloroethane, afforded a residue that was adsorbed onto SiO_2 (8.84 g) and purified by flash column chromatography (SiO_2), eluting with a gradient of 50% hexanes in CH_2Cl_2 to CH_2Cl_2 to yield the title compound. Additional column fractions yielded residue that, after recrystallization from boiling hexanes (100 mL), afforded the product as fine yellow-green needles. A total of 0.781 g (16%) was obtained: mp 111–113 °C. IR (film) 3449, 3341, 2946, 1656, 1639, 1592, 1553, 1229, 1139, 867, 849, 781, 619; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 8.3 Hz, 1H), 6.35 (d, J = 11.5 Hz, 1H), 6.30 (br s, 2H), 4.62 (s, 2H), 2.18 (s, 3H); CIMS m/z (rel intensity) 202 (MH^+, 100).

5.2.5. 5-Chloro-2-amino-α-chloroacetophenone (21)[36]

Boron trichloride-methyl sulfide complex (3.08 g, 17.2 mmol), 4-chloroaniline (**19**, 2.00 g, 15.6 mmol), chloroacetonitrile (1.47 g, 19.5 mmol), and aluminum chloride (2.50 g, 18.7 mmol), in dichloroethane, afforded a black gum. This residue was adsorbed onto SiO_2 (4.17 g) and purified by flash column chromatography (SiO_2, 56.8 g), eluting with 50% hexanes in CH_2Cl_2, to yield an iridescent green solid (0.230, 7.2%): mp 130–133 °C (lit[36] mp 140–141 °C.) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 2.3 Hz, 1H), 7.27–7.23 (m, 1H), 6.67 (d, J = 8.9 Hz, 1H), 6.30 (br s, 1H), 4.64 (s, 2H).

5.2.6. 4-Chloro-2-amino-α-chloroacetophenone (23), 6-chloro-2-amino-α-chloroacetophenone (24), and their respective *N*-acetyl derivates 25 and 26[36]

Boron trichloride-methyl sulfide complex (4.63 g, 25.9 mmol), compound **22** (3.00 g, 23.5 mmol), chloroacetonitrile (2.21 g, 29.5 mmol), and aluminum chloride (3.76 g, 28.2 mmol), in dichloroethane, yielded a green solid. Both **23** and **24** were present by TLC. The residue was adsorbed onto SiO_2 (11.8 g), and was purified by flash column chromatography (SiO_2, 91.4 g), eluting with 25% hexanes in CH_2Cl_2, to remove unwanted by-products.

5.2.6.1. Synthesis and separation of 25 and 26. The obtained residue containing **23** and **24** was diluted with acetic anhydride (10 mL), and the mixture was heated to 80 °C for 30 min, upon which it became a dark red. The mixture was concentrated to remove excess acetic anhydride, and was adsorbed onto SiO_2 (8.52 g). The residue was purified by flash column chromatography (SiO_2, 54.9 g), eluting with a gradient of 10% hexanes in CH_2Cl_2 to 1% MeOH in CH_2Cl_2 to yield, first, **25** (0.780 g, 13%), as white needles after recrystallization from 50% EtOH in CH_2Cl_2 and washing with hexanes: mp 131–132 °C (lit[36] mp 139–140 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.4 (br s, 1H), 8.91 (d, J = 2.0 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.14 (dd, J = 6.9, 1.9 Hz, 1H), 4.73 (s, 2H), 2.26 (s, 3H) and then **26** (0.270 g, 5%), as an orange solid: mp 115–117 °C (lit[36] mp 130–132 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (br s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.44 (t, J = 8.2 Hz, 1H), 7.27 (t, J = 9.5 Hz, 1H), 4.71 (s, 2H), 2.19 (s, 3H).

5.2.6.2. Hydrolysis of the acetanilides: 4-chloro-2-amino-α-chloroacetophenone (23)[36]. Compound **25** (0.780 g, 3.17 mmol) was dissolved in EtOH (40 mL), and concd HCl (5 mL) was added. The mixture was heated to reflux for 1 h, and the resultant dark orange solution was cooled and poured into ice water (150 mL). 2 M NaOH was added until the pH was approximately 9. The mixture was extracted with CH_2Cl_2 (3 × 150 mL), and the organic phase was washed with H_2O (200 mL), dried over anhydrous sodium sulfate, and concentrated to yield **23** as an orange iridescent solid (0.600 g, 93% from **25**, 12% from **22**): mp 126–128 °C (lit[36] mp 134–135 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 8.7 Hz, 1H), 6.71 (d, J = 1.9 Hz, 1H), 6.65 (dd, J = 7.5, 2.0 Hz, 1H), 6.39 (br s, 2H), 4.63 (s, 2H).

5.2.7. 6-Chloro-2-amino-α-chloroacetophenone (24)[36]

Compound **26** (0.270 g, 1.10 mmol) was diluted in EtOH (12 mL), and concd HCl (3 mL) was added. The mixture was heated at reflux for 1 h 15 min, cooled, and poured into ice water (50 mL). 2 M NaOH was added until the pH was approximately 9, and the mixture was extracted with CH_2Cl_2 (2 × 40 mL). The organic layers were washed with H_2O (1 × 100 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was adsorbed onto SiO_2 (5.0 g), and was purified by flash column chromatography (SiO_2, 28.5 g), eluting with CH_2Cl_2 to yield a yellow-green solid (0.158 g, 71% from **26**, 3.3% from **22**): mp 50.5–52 °C (lit[36] mp 60–61 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (t, J = 8.1 Hz, 1H), 6.76 (d, J = 7.9 Hz, 1H), 6.63 (d, J = 8.3 Hz, 1H), 4.97 (br s, 1H), 4.74 (s, 2H).

5.3. Synthesis of aromathecin cores (27a–34a)

5.3.1. 14-Chloromethyl-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (27a)

Compound **8** (0.100 g, 0.502 mmol) and compound **10** (0.114 g, 0.502 mmol) were diluted with benzene (30 mL). *p*-TsOH monohydrate (0.005 g, 0.026 mmol) was added and the solution was heated at reflux for 16 h using a Dean-Stark trap to collect azeotroped water. The solution was concentrated, diluted with $CHCl_3$ (175 mL) and washed with satd $NaHCO_3$ (3 × 50 mL) and satd NaCl (50 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 3% MeOH in $CHCl_3$ to provide a yellow solid (0.107 g, 55%): mp 275 °C (dec). IR (KBr) 1655, 1628, 1605, 1288, 1243, 1226, and 1068 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 8.1 Hz, 1H), 7.79–7.57 (m, 5H), 7.55 (s, 1H), 5.40 (s, 2H), 4.95 (s, 2H), 4.45 (s, 4H); ESIMS m/z (rel intensity) 391/393 (MH^+, 96/33). Anal. Calcd for $C_{22}H_{15}ClN_2O_3 \cdot 0.5H_2O$: C, 66.09; H, 4.03; N, 7.01. Found: C, 66.02; H, 3.97; N, 7.14.

5.3.2. 14-(3′-Chloropropyl)-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (28a)

Compound **11** (0.481 g, 1.88 mmol) was suspended in $CHCl_3$ (5 mL). Compound **8** (0.300 g, 1.50 mmol) was added, followed by *p*-TsOH (0.285 g, 1.50 mmol), glacial acetic acid (2 mL), and

toluene (60 mL). The mixture was heated at reflux for 17.5 h, using a Dean-Stark trap. The mixture was cooled to room temperature, concentrated, and the residue was suspended in a mixture of MeOH (30 mL) and $CHCl_3$ (100 mL). The suspension was washed with satd $NaHCO_3$ (200 mL) and H_2O (200 mL). The aqueous layer was extracted with $CHCl_3$ (50 mL). The organic layers were washed with H_2O (200 mL), satd NaCl (125 mL), dried over anhydrous sodium sulfate, and treated with decolorizing carbon (0.100 g). The mixture was filtered, concentrated, adsorbed onto SiO_2 (3.80 g), and purified by flash column chromatography (SiO_2, 62.8 g), eluting with $CHCl_3$, to yield a yellow amorphous solid (0.275 g, 44%) after washing with MeOH (10 mL): mp 255–258 °C. IR (film) 3401, 2930, 1659, 1627, 1603, 1506, 1440, 1287, 1244, 1068, 915, 870, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 8.2 Hz, 1H), 7.77–7.52 (m, 5H), 7.48 (s, 1H), 5.31 (s, 2H), 4.43 (s, 4H), 3.69 (t, J = 6.0 Hz, 2H), 3.27 (t, J = 7.5 Hz, 2H), 2.27–2.17 (m, 2H). Anal. Calcd for $C_{24}H_{19}ClN_2O_3 \cdot 0.75H_2O$: C, 66.67; H, 4.78; N, 6.48. Found: C, 66.28; H, 4.46; N, 6.40.

5.3.3. 14-Chloromethyl-2,3-methylenedioxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (29a)

Compound **8** (0.200 g, 1.00 mmol) and compound **15** (0.236 g, 1.10 mmol) were diluted with toluene (30 mL) and *p*-TsOH (0.190 g, 1.00 mmol) was added. The mixture was heated at reflux, using a Dean-Stark trap, for 23.5 h. The bright orange suspension was cooled and concentrated, and the residue was suspended in $CHCl_3$ (100 mL) and washed with satd $NaHCO_3$ (100 mL). The aqueous layer was extracted with $CHCl_3$ (5 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated to yield a yellow amorphous solid (0.310 g, 82%) after washing with THF (40 mL) and drying: mp 325–327 °C (dec). IR (film) 2916, 1657, 1619, 1597, 1499, 1462, 1339, 1252, 1034, 866, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.1 Hz, 1H), 7.79–7.70 (m, 2H) 7.60–7.53 (m, 3H), 7.43 (s, 1H), 6.21 (s, 2H), 5.40 (s, 2H), 4.95 (s, 2H); ESIMS *m/z* (rel intensity) 377/379 (MH^+, 100/35).

5.3.4. 14-Chloromethyl-2,3-dimethoxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (30a)

Compound **8** (0.600 g, 3.01 mmol), compound **17** (0.760 g, 3.31 mmol) and *p*-TsOH (0.572 g, 3.01 mmol) were diluted with benzene (80 mL). The mixture was heated at reflux for 17 h using a Dean-Stark trap to collect azeotroped water. The mixture was cooled and the precipitate was filtered. The tan solid collected was washed with MeOH (10 mL), diluted with $CHCl_3$, and the organic phase was washed with satd $NaHCO_3$ (200 mL), H_2O (2 × 200 mL), and dried over anhydrous sodium sulfate. The solution was concentrated and adsorbed onto SiO_2 (7.12 g) and the residue was purified by flash column chromatography (SiO_2), eluting with $CHCl_3$ to 0.5% MeOH in $CHCl_3$, to yield a yellow amorphous solid (0.267 g, 23%) after washing with cold $CHCl_3$ (5 mL), MeOH (10 mL), and ether (20 mL): mp 308–311 °C (dec). IR (KBr) 3427, 1654, 1626, 1606, 1590, 1576, 1507, 1482, 1434, 1358, 1255, 1222, 1012, 844, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 7.9 Hz, 1H), 7.75–7.72 (m, 2H), 7.59–7.54 (m, 3H), 7.32 (s, 1H), 5.42 (s, 2H), 5.01 (s, 2H), 4.10 (s, 6H); ESIMS *m/z* (rel intensity) 393/335 (MH^+, 100/33). Anal. Calcd for $C_{22}H_{17}ClN_2O_3 \cdot 1.5H_2O$: C, 62.94; H, 4.80; N, 6.67. Found: C, 63.04; H, 4.57; N, 6.67.

5.3.5. 14-Chloromethyl-3-fluoro-2-methyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (31a)

Compound **8** (0.400 g, 2.01 mmol), compound **20** (0.506 g, 2.51 mmol) and *p*-TsOH (0.382 g, 2.01 mmol) were diluted with benzene (70 mL) and the mixture was heated at reflux using a Dean-Stark trap. After 16.5 h, another 0.070 g (0.35 mmol) of **20** was added, and reflux was continued for 1 h 20 min. The mixture was cooled, concentrated, and the residue was suspended in $CHCl_3$ (100 mL) and washed with satd $NaHCO_3$ (100 mL). The aqueous layers were extracted with $CHCl_3$ (100 mL). The combined organic layers were washed with H_2O (200 mL), following which the aqueous layer was again extracted with $CHCl_3$ (50 mL). The combined organic layers were washed with satd NaCl (200 mL). The aqueous phase was again extracted with $CHCl_3$ (50 mL). The combined organic layers were dried over anhydrous sodium sulfate, concentrated, and adsorbed onto SiO_2 (8.30 g). Purification by flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 4% MeOH in $CHCl_3$, resulted in precipitation of the compound on the column. Elution was continued with $CHCl_3$. The obtained orange powder was boiled in tetrahydrofuran (80 mL), filtered, and washed with $CHCl_3$ and ether to yield the title compound as a pale orange powder (0.464 g, 63%): mp 300–303 °C (dec). IR (KBr pellet) 3028, 3923, 2363, 2342, 1657, 1631, 1602, 1480, 1435, 1341, 1232, 1222, 1133, 899, 768, 726, 690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.86–7.57 (m, 5H), 5.44 (s, 2H), 5.03 (s, 2H), 2.58 (s, 3H); ESIMS *m/z* (rel intensity) 365 (MH^+, 100).

5.3.6. 2-Chloro-14-chloromethyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (32a)

Compound **8** (0.200 g, 1.00 mmol), compound **21** (0.230 g, 1.12 mmol) and *p*-TsOH (0.190 g, 1.00 mmol) were diluted with toluene (30 mL) and the mixture was heated at reflux for 13.5 h using a Dean-Stark trap. The mixture was cooled, concentrated, and the residue was diluted with $CHCl_3$ (100 mL). The solution was washed with satd $NaHCO_3$ (100 mL). H_2O (100 mL) was added, and the aqueous phase was exhaustively extracted with $CHCl_3$ (1 × 50, 6 × 25 mL). The resultant cloudy suspension was dried over anhydrous sodium sulfate and concentrated to yield a yellow-green solid (0.339 g, 92%) after washing with MeOH (50 mL) and ether (10 mL): mp 276–277 °C (dec). IR (film) 2917, 1665, 1537, 1442, 1343, 1138, 822, 751, 686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 8.0 Hz, 1H), 8.21 (d, J = 9.0 Hz, 1H), 8.15 (d, J = 2.1 Hz, 1H), 7.81–7.59 (m, 5H), 5.46 (s, 2H), 5.01 (s, 2H); ESIMS m/*z* (rel intensity) 367 (MH^+, 100).

5.3.7. 3-Chloro-14-chloromethyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (33a)

Compound **8** (0.200 g, 1.00 mmol) and compound **23** (0.230 g, 1.12 mmol) were diluted with toluene (40 mL) and *p*-TsOH (0.190 g, 1.00 mmol) was added. The mixture was heated at reflux for 17 h using a Dean-Stark trap. The mixture was cooled and concentrated, and the residue was diluted with $CHCl_3$ (100 mL), and the suspension was washed with satd $NaHCO_3$ (100 mL). The aqueous layer was extracted with $CHCl_3$ (4 × 50 mL), and the organic layers were dried over anhydrous sodium sulfate and concentrated to yield a yellow solid (0.314 g, 86%) after washing with MeOH (25 mL): mp 301.5–303 °C (dec). IR (film) 3584, 3369, 2922, 1656, 1622, 1604, 1449, 1342, 1183, 1075, 923, 751, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, J = 7.8 Hz, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.15 (d, J = 9.0 Hz, 1H), 7.80–7.61 (m, 5H), 5.46 (s, 2H), 5.04 (s, 2H); ESIMS *m/z* (rel intensity) 368 (MH^+, 65), 331 (MH–HCl, 100).

5.3.8. 1-Chloro-14-chloromethyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (34a)

Compound **8** (0.175 g, 0.88 mmol) and compound **24** (0.200 g, 0.98 mmol) were diluted with toluene (40 mL) and *p*-TsOH (0.167 g, 0.88 mmol) was added. The mixture was heated to reflux for 4.5 h using a Dean-Stark trap. The mixture was stirred at room temperature for 13 h. The mixture was concentrated, and the residue was diluted with CH_2Cl_2 (150 mL), and the suspension was washed with satd $NaHCO_3$ (150 mL). The aqueous layer was

exhaustively extracted with CH_2Cl_2 (3 × 75, 3 × 50 mL). MeOH was added to the remaining aqueous suspension, which was extracted with CH_2Cl_2 (3 × 50 mL). This suspension was washed with H_2O (2 × 100 mL). The suspension and combined organic layers were dried over anhydrous sodium sulfate and concentrated to yield a yellow amorphous solid (0.305 g, 95%) after washing with MeOH (50 mL) and ether (30 mL): mp 300–302 °C (dec). IR (film) 1657, 1628, 1444, 1380, 1206, 1124, 925, 883, 850, 814, 766, 754, 725, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CF_3COOD$) δ 8.61 (d, J = 8.2 Hz, 1H), 8.47 (dd, J = 6.8, 3.0 Hz, 1H), 8.33 (s, 1H), 8.08–7.90 (m, 5H), 5.78 (s, 2H), 5.57 (s, 2H); APCI-MS m/z (rel intensity) 367 (MH, base-peak).

5.4. General procedure for synthesis of 14-substituted aromathecins (series 27 and 29–34)[27,28]

The chlorinated aromathecin core (between 0.050 and 0.115 g, or 0.140–0.300 mmol) was diluted in DMSO (20–30 mL, for room temperature reactions), or an equivalent amount of dry DMF (for some reactions performed at higher temperature). A nucleophile (between a three- and tenfold excess, typically between 5 and 6 equiv) was then added. In the case of amine salts, approximately 10 equiv of Et_3N were added. The mixture was stirred overnight (between 12 and 24 h). The product was extracted by either diluting the reaction mixture with $CHCl_3$ (between 50 and 100 mL) and washing with water (at least 90 mL) and satd NaCl, or alternatively, by pouring the reaction mixture into H_2O (~100 mL) and extracting with $CHCl_3$ (at least 100 mL). The organic layers were washed with copious water (between 400 and 600 mL) and (when DMF was used), sat. NH_4Cl (~200 mL), and were dried over anhydrous sodium sulfate and concentrated, and the residue was purified by flash column chromatography (loading by either diluting the residue in an appropriate solvent or adsorbing onto between 3 and 5 g of SiO_2) on 20–30 g SiO_2. The obtained solids were then washed with ether or hexane (30–50 mL) and dried. The preparation of salts is described under the subheadings for individual compounds.

5.4.1. 14-*N,N*-Dimethylaminomethyl-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27b)

Compound **27a** (0.090 g, 0.230 mmol), dimethylamine hydrochloride (0.094 g, 1.15 mmol) and Et_3N (0.16 mL), 1.15 mmol), in DMSO yielded the title compound as a brown solid (0.057 mg, 57%) after flash column chromatography (SiO_2, eluting with a gradient of $CHCl_3$ to 4% MeOH in $CHCl_3$) and washing with ether (50 mL): mp 249–251 °C (dec). IR (KBr) 1659, 1632, 1607, 1505, 1287, 1245, 1064 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, J = 7.7 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.81–7.75 (m, 2H), 7.60 (m, 1H), 7.58 (s, 1H), 7.49 (s, 1H), 5.29 (s, 2H), 4.42 (s, 4H), 3.86 (s, 2H), 2.24 (s. 6H); ESIMS m/z (rel intensity) 400 (MH^+, 100). Anal. Calcd for $C_{24}H_{21}N_3O_3$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.08; H, 5.19; N, 10.24.

5.4.2. 2,3-Ethylenedioxy-14-(1′-imidazolylmethyl)-2*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27c)

Compound **27a** (0.116 g, 0.297 mmol) and imidazole (0.060 g, 0.891 mmol) in DMSO at 100 °C for 2 h, yielded the title compound as a yellow solid (0.056 mg, 45%) after flash column chromatography (SiO_2, eluting with a gradient of $CHCl_3$ to 7% MeOH in $CHCl_3$) and washing with diethyl ether (50 mL): mp 270 °C (dec). IR (KBr) 1656, 1627, 1605, 1505, 1291, 1246 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.33 (d, J = 7.7 Hz, 1H), 7.96–7.92 (m, 2H), 7.82–7.76 (m, 1H), 7.65 (s, 1H), 7.61–7.54 (m, 3H), 7.25 (s, 1H), 6.92 (s, 1H), 5.80 (s, 2H), 5.20 (s, 2H), 4.42 (s, 4H); ESIMS m/z (rel intensity) 423 (MH^+, 100). Anal. Calcd for $C_{25}H_{18}N_4O_3 \cdot 0.75H_2O$: C, 68.88; H, 4.51; N, 12.85. Found: C, 69.15; H, 4.30; N, 12.66.

5.4.3. 2,3-Ethylenedioxy-14-(*N*-methylpiperazinylmethyl)-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one trifluoroacetate (27d)

Compound **27a** (0.090 g, 0.230 mmol) and *N*-methylpiperazine (0.069 g, 0.690 mmol) in DMSO yielded the desired compound as a brown solid after flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 7% MeOH in $CHCl_3$. The obtained compound was washed with ether and diluted with $CHCl_3$ (40 mL) and trifluoroacetic acid (2 mL) was added. The reaction mixture was allowed to stir at room temperature for 30 min, concentrated, and the residue was triturated with diethyl ether. The obtained precipitate was filtered and washed with diethyl ether (50 mL) to provide a yellow solid (0.113 g, 86%): mp 238–242 °C. IR (KBr) 3430, 1661, 1505, 1287, 1243, 1181, 1067 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.39 (br s, 1H) 8.36 (d, J = 7.4 Hz, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.82–7.78 (m, 2H), 7.62–7.57 (m, 2H), 7.54 (s, 1H), 5.36 (s, 2H), 4.50–4.00 (br m, 2H), 4.44 (s, 4H), 4.07 (s, 2H), 3.37 (m, 2H), 3.03 (m, 4H), 2.80 (d, J = 4.2 Hz, 3H); ESIMS m/z (rel intensity) 455 (MH^+, 100). Anal. Calcd for $C_{29}H_{27}F_3N_4O_5 \cdot 0.25H_2O$: C, 60.78; H, 4.84; N, 9.78. Found: C, 60.68; H, 4.74; N, 9.56.

5.4.4. 2,3-Ethylenedioxy-14-(1′-morpholinomethyl)-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (27e)

Compound **27a** (0.090 g, 0.230 mmol) and morpholine (0.060 g, 0.690 mmol) in DMSO yielded the desired compound as a brown solid after flash column chromatography (SiO_2), eluting with a gradient of $CHCl_3$ to 2% MeOH in $CHCl_3$. The product was washed with ether (50 mL) and treated with TFA (as described for **27d**), but only the free base was obtained upon drying, as a yellow-brown solid (0.088 g, 86%): mp 150–155 °C (dec). IR (KBr) 1661, 1630, 1507, 1290, 1246, 1200, 1176, 1129, 1068 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 7.9 Hz, 1H), 7.81–7.76 (m, 2H), 7.61–7.56 (m, 2H), 7.50 (s, 1H), 5.34 (s, 2H), 4.43 (s, 4H), 3.96 (s, 2H), 3.56 (br s, 4H), 2.50 (br s, 4H); ESIMS m/z (rel intensity) 442 (MH^+, 100). Anal. Calcd for $C_{26}H_{23}N_2O_4 \cdot 0.5H_2O$: C, 69.32; H, 5.37; N, 9.33. Found: C, 69.46; H, 5.04; N, 9.08.

5.4.5. 14-(*N*-Ethanolaminomethyl)-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one trifluoroacetate (27f)

Compound **27a** (0.090 g, 0.230 mmol) and ethanolamine (0.042 g, 0.691 mmol) in DMSO afforded the desired compound as a dark yellow solid after flash column chromatography (SiO_2, eluting with a gradient of $CHCl_3$ to 7% MeOH in $CHCl_3$) and washing with ether (50 mL). The obtained precipitate was treated with TFA as described for **27d**, and was filtered and washed with diethyl ether (50 mL) to provide a brown-orange solid (0.101 g, 83%): mp 180–183 °C. IR (KBr) 3424, 1658, 1622, 1505, 1290, 1245, 1200 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.92 (br s, 2H), 8.37 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.88 (s, 1H), 7.85–7.79 (m, 1H), 7.64–7.59 (m, 3H), 5.53 (s, 2H), 4.78 (br s, 2H), 4.48 (s, 4H), 3.79 (t, J = 5.51 Hz, 2H), 3.37 (m, 2H); the hydroxyl group is absent due to line-broadening; ESIMS m/z (rel intensity) 416 (MH^+, 100). Anal. Calcd for $C_{26}H_{22}F_3N_3O_6$: C, 58.98; H, 4.19; N, 7.94. Found: C, 59.22; H, 4.21; N, 7.89.

5.4.6. 2,3-Ethylenedioxy-14-[*N*-(*S*)-3′-hydroxypyrrolid inomethyl]-12*H*-5,11a diazadibenzo[*b,h*]fluoren-11-one (27g)

Compound **27a** (0.065 g, 0.166 mmol), (*S*)-pyrrolidin-3-ol hydrogen maleate[38] (0.102 g, 0.499 mmol), and Et_3N (0.168 g, 1.66 mmol) in DMSO afforded the desired product as a tan solid (0.048 g, 64%) after flash column chromatography (SiO_2, eluting with a gradient of $CHCl_3$ to 2% MeOH in $CHCl_3$) and washing with hexanes: mp 235–237 °C (dec). IR (film) 3369, 2930, 1659, 1623, 1603, 1505, 1441, 1287, 1243, 1068, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 7.9 Hz, 1H), 7.77–7.52 (m, 6H), 5.38 (s, 2H), 4.42 (s, 4H), 4.36 (br s, 1H), 4.08 (s, 2H), 2.94–2.90 (m, 1H), 2.70–2.62 (m, 2H), 2.46–2.42 (m, 1H), 2.26–2.20 (m,

1H), 1.95 (bd, J = 7.0 Hz, 1H), 1.79–1.75 (m, 1H); ESIMS m/z (rel intensity) 442 (MH^+, 100). Anal. Calcd for $C_{26}H_{23}N_3O_4{\cdot}H_2O$: C, 67.96; H, 5.48; N, 9.14. Found: C, 68.28; H, 5.22; N, 9.02.

5.4.7. 2,3-Ethylenedioxy-14-[(1′-imidazolyl)propylaminomethyl]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (27h)

Compound **27a** (0.065 g, 0.166 mmol) and 1-(3-aminopropyl)imidazole (0.062 g, 0.499 mmol) in DMSO afforded the desired compound as a bright yellow amorphous solid (0.044 g, 56%) after flash column chromatography (SiO_2, eluting with a gradient of $CHCl_3$ to 3% MeOH, with a few drops of Et_3N) and washing with ether: mp 203–207 °C (dec.) IR (film) 3368, 2929, 1658, 1622, 1602, 1506, 1287, 1245, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 8.0 Hz, 1H), 7.76–7.56 (m, 6H), 7.47 (s, 1H), 7.26 (s, 1H), 6.87 (s, 1H), 5.37 (s, 2H), 4.44 (s, 4H), 4.23 (s, 2H), 4.08 (t, J = 6.9 Hz, 2H), 2.76 (t, J = 6.6 Hz, 2H), 2.01–1.90 (m, 2H); the amine exchanges with residual water; ESIMS m/z (rel intensity) 480 (MH^+, 100). Anal. Calcd for $C_{28}H_{25}N_5O_3{\cdot}1.5H_2O$: C, 66.39; H, 5.57; N, 13.83. Found: C, 66.42; H, 5.57; N, 13.64.

5.4.8. 2,3-Ethylenedioxy-14-(3′-morpholinopropylaminomethyl)-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (27i)

Compound **27a** (0.065 g, 0.166 mmol) and 3-morpholinopropylamine (0.072 g, 0.499 mmol) in DMSO afforded the title compound as a flocculent yellow amorphous solid (0.052 g, 63%) after flash column chromatography (SiO_2, eluting with a gradient of 0.5% Et_3N in $CHCl_3$ to 1.5% MeOH–1% Et_3N in $CHCl_3$) and washing with diethyl ether: mp 202–205 °C. IR (film) 3401, 2921, 1658, 1629, 1603, 1506, 1446, 1289, 1244, 1115, 1068, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 8.0 Hz, 1H), 7.77–7.53 (m, 6H), 5.39 (s, 2H), 4.42 (s, 4H), 4.23 (s, 2H), 3.66 (t, J = 4.5 Hz, 4H), 2.81 (t, J = 6.5 Hz, 2H), 2.42–2.38 (m, 6H), 1.77–1.70 (m, 2H), 1.60–1.50 (br m, obscured by residual water, 1H); ESIMS m/z (rel intensity) 499 (MH^+, 100). Anal. Calcd for $C_{29}H_{30}N_4O_4{\cdot}1.5H_2O$: C, 66.27; H, 6.33; N, 10.66. Found: C, 66.29; H, 6.14; N, 10.63.

5.4.9. 2,3-Methylenedioxy-14-[1′-(*N*-methylpiperazinylmethyl)]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (29b)

Compound **29a** (0.070 g, 0.185 mmol) and *N*-methylpiperazine (0.074 g, 0.743 mmol) in DMSO afforded the title compound as a pale-yellow amorphous solid (0.063 g, 77%) after flash column chromatography (SiO_2, 24.2 g, eluting with 0.5% MeOH in $CHCl_3$ to 5% MeOH in $CHCl_3$) and washing with ether (60 mL): mp 288–291 °C (dec). IR (film) 3369, 2926, 2807, 1662, 1622, 1587, 1501, 1464, 1247, 1034, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 7.9 Hz, 1H), 7.77–7.69 (m, 2H), 7.66 (s, 1H), 7.58–7.54 (m, 2H), 7.48 (s, 1H), 6.18 (s, 2H), 5.39 (s, 2H), 3.93 (s, 2H), 2.70–2.30 (br m, 8H), 2.29 (s, 3H); ESIMS m/z (rel intensity) 441 (MH^+, 100). Anal. Calcd for $C_{26}H_{24}N_4O_3{\cdot}0.25H_2O$: C, 70.18; H, 5.55; N, 12.59. Found: C, 70.07; H, 5.45; N, 12.59.

5.4.10. 14-(*N*-Ethanolaminomethyl)-2,3-methylenedioxy-12*H*-5,11a diazadibenzo[*b*,*h*]fluoren-11-one (29c)

Compound **29a** (0.065 g, 0.172 mmol) and ethanolamine (0.042 g, 0.690 mmol) in DMSO afforded the title compound as a flocculent yellow solid (0.042 g, 61%) after flash column chromatography (SiO_2, 24.8 g, eluting with a gradient of 0.5% MeOH in $CHCl_3$ to 4% MeOH in $CHCl_3$), washing with ether (25 mL) and drying in vacuo: mp 225–228 °C (dec). IR (film) 3401, 2917, 2342, 1655, 1618, 1493, 1462, 1337, 1250, 1038, 847, 757, 687 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.33 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.80 (t, J = 7.0 Hz, 1H), 7.73 (s, 1H), 7.59 (t, J = 7.3 Hz, 1H), 7.52 (s, 1H), 7.45 (s, 1H), 6.26 (s, 2H), 5.37 (s, 2H), 4.55 (t, J = 5.2 Hz, 1H), 4.21 (s, 2H), 3.52 (q, J = 5.4 Hz, 1H), 2.71 (t, J = 5.8 Hz, 2H); the amine proton is not visible due to exchange with residual water; ESIMS m/z (rel intensity) 402 (MH^+, 100). Anal. Calcd for $C_{23}H_{19}N_3O_4{\cdot}1.25H_2O$: C, 65.16; H, 5.11; N, 9.91. Found: C, 65.35; H, 4.78; N, 9.87.

5.4.11. 14-(*N*,*N*-Dimethylaminomethyl)-2,3-methylenedioxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (29d)

Compound **29a** (0.070 g, 0.186 mmol) and *N*,*N*-dimethylamine (0.56 mL, 2 M in THF) in DMSO afforded the title compound as a yellow powder (0.046 g, 64%) after flash column chromatography (SiO_2, 29.4 g, eluting with 0.25% Et_3N in $CHCl_3$) and washing with ether (40 mL): mp 272–277 °C (dec). IR (film) 3585, 2916, 1661, 1623, 1605, 1463, 1337, 1247, 1034 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.13 Hz, 1H), 7.75–7.66 (m, 3H), 7.60–7.50 (m, 2H), 7.48 (s, 1H), 6.16 (s, 2H), 5.38 (s, 2H), 3.84 (s, 2H), 2.33 (s, 6H); ESIMS m/z (rel intensity) 386 (MH^+, 100). Anal. Calcd for $C_{23}H_{19}N_3O_3{\cdot}0.5H_2O$: C, 70.04; H, 5.11; N, 10.65. Found: C, 70.17; H, 5.02; N, 10.27.

5.4.12. 14-[*N*-(*S*)-3′-Hydroxypyrrolidinomethyl]-2,3-methylenedioxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (29e)

Compound **29a** (0.065 g, 0.172 mmol), (*S*)-pyrrolidin-3-ol hydrogen maleate (0.141 g, 0.690 mmol) and Et_3N (0.347 g, 3.44 mmol) in DMSO afforded the title compound as a yellowish-tan powder (0.051 g, 69%) after flash column chromatography (SiO_2, 27.6 g, eluting with a gradient of 0.6% MeOH in $CHCl_3$ to 1.75% MeOH in $CHCl_3$) and washing with ether (25 mL): mp 260–263 °C (dec). IR (film) 3436, 2916, 2343, 1656, 1619, 1601, 1465, 1338, 1248, 1035, 944, 758, 687 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.33 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H). 7.80 (t, J = 7.2 Hz, 1H), 7.76 (s, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.51 (s, 1H), 7.44 (s, 1H), 6.26 (s, 2H), 5.32 (s, 2H), 4.72 (d, J = 4.3 Hz, 1H), 4.20–4.10 (m, 1H) 4.05 (d, J = 2.9 Hz, 2H), 2.80–2.66 (m, 2H), 2.50–2.38 (m, 2H), 2.02–1.96 (m, 1H), 1.60–1.50 (m, 1H); ESIMS m/z (rel intensity) 428 (MH^+, 100). Anal. Calcd for $C_{25}H_{21}N_3O_4{\cdot}0.8H_2O$: C, 67.96; H, 5.16; N, 9.51. Found: C, 67.68; H, 4.78; N, 9.49.

5.4.13. 14-[1′-(Imidazolyl)propylamino]methyl)-2,3-methylenedioxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (29f)

Compound **29a** (0.055 g, 0.146 mmol) and 1-(3-aminopropyl)imidazole (0.091 g, 0.730 mmol) in DMSO afforded the title compound as a yellow amorphous solid (0.052 g, 77%) after flash column chromatography (SiO_2, 28.1 g, eluting with 0.2% MeOH–0.2% Et_3N in $CHCl_3$ to 4% MeOH–0.2% Et_3N in $CHCl_3$) and washing with ether (25 mL): mp 195–199 °C (dec). IR (film) 3436, 2916, 2310 1655, 1622, 1602, 1488, 1465, 1238, 1037, 687 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.80 (t, J = 6.9 Hz, 1H), 7.72 (s, 1H), 7.59–7.53 (m, 3H), 7.46 (s, 1H), 7.13 (s, 1H), 6.86 (s, 1H), 6.27 (s, 2H), 5.40 (s, 2H), 4.21 (s, 2H), 4.04 (t, J = 6.9 Hz, 2H), 2.64 (br m, 2H), 1.92 (m, 2H); the amine proton is not visible due to residual water in the solvent; ESIMS m/z (rel intensity) 466 (MH^+, 100). Anal. Calcd for $C_{27}H_{23}N_5O_3{\cdot}0.75H_2O$: C, 67.60; H, 5.16; N, 14.62. Found: C, 67.90; H, 4.85; N, 14.47.

5.4.14. 14-[*N*-(*S*)-3′-Hydroxypyrrolidinomethyl]-2,3-dimethoxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (30b)

Compound **30a** (0.060 g, 0.153 mmol), (*S*)-pyrrolidin-3-ol hydrogen maleate (0.094 g, 0.458 mmol) and Et_3N (0.154 g, 1.50 mmol) in DMSO afforded the title compound as a yellow amorphous solid (0.051 g, 75%) after flash column chromatography (SiO_2, eluting with 2% MeOH in $CHCl_3$), and washing with ether (20 mL) and hexanes (20 mL): mp 223–226 °C. IR (KBr) 3430, 2933, 2342, 1660, 1627, 1603, 1505, 1478, 1431, 1250, 1167, 846, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 8.1 Hz, 1H), 7.76–7.68 (m, 2H), 7.64 (s, 1H), 7.57–7.52 (m, 3H), 5.39 (s, 2H), 4.40 (br m, 1H), 4.20 (q, J = 13.4 Hz, 2H), 4.09 (s, 3H), 4.05 (s, 3H), 2.94, (q,

J = 5.5 Hz, 1H), 2.80–2.70 (m, 2H), 2.48 (q, *J* = 6.2 Hz, 1H), 2.30–2.20 (m, 1H), 1.90–1.70 (m, 2H); ESIMS *m/z* (rel intensity) 444 (MH^+, 100). Anal. Calcd for $C_{26}H_{25}N_5O_4{\cdot}1H_2O$: C, 67.67; H, 5.90; N, 9.10. Found: C, 67.38; H, 5.76; N, 9.13.

5.4.15. 14-(1′-Imidazolylmethyl)-2,3-dimethoxy-2*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (30c)

Compound **30a** (0.070 g, 0.178 mmol) and imidazole (0.049 g, 0.713 mmol) were diluted with DMSO (25 mL) under an argon atmosphere. The mixture was heated to 60 °C for 4 h. Additional imidazole (0.012 g, 0.178 mmol) was added, and the mixture was stirred at room temperature for 16 h. TLC indicated that the reaction was incomplete, so additional imidazole (0.012 g, 0.178 mmol) was added, and the mixture was heated to 100 °C for 1 h. Extraction (following the general procedure) and flash column chromatography (SiO_2, eluting with a gradient of $CHCl_3$ to 5% MeOH in $CHCl_3$) yielded a yellow amorphous solid (0.056 g, 73%) after washing with ether (20 mL): mp 309–312 °C (dec). IR (KBr) 3468, 3027, 1654, 1627, 1571, 1509, 1482, 1360, 1256, 1170, 1086, 1033, 853, 758, 689 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, *J* = 7.4 Hz, 1H), 7.96 (d, *J* = 6.8 Hz, 1H), 7.94 (s, 1H), 7.82 (t, *J* = 7.9 Hz, 1H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.53 (s, 2H), 7.48 (s, 1H), 7.29 (s, 1H), 6.92 (s, 1H), 5.98 (s, 2H), 5.20 (s, 2H), 3.98 (s, 3H), 3.93 (s, 3H); ESIMS *m/z* (rel intensity) 425 (MH^+, 100). Anal. Calcd for $C_{25}H_{20}N_4O_3{\cdot}1.5H_2O$: C, 66.51; H, 5.13; N, 12.41. Found: C, 66.64; H, 4.89; N, 12.53.

5.4.16. 2,3-Dimethoxy-14-[1′-(*N*-methylpiperazinylmethyl)]-2*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (30d)

Compound **30a** (0.055 g 0.140 mmol), and *N*-methylpiperazine (0.042 g, 0.420 mmol) in DMSO afforded the title compound as a yellow-green amorphous solid (0.046 g, 72%) after flash column chromatography (SiO_2, eluting with a gradient of 1% MeOH in $CHCl_3$ to 5% MeOH in $CHCl_3$), and washing with ether (20 mL): mp 256–259 °C (dec). IR (KBr) 3435, 2926, 2791, 1665, 1636, 1601, 1504, 1480, 1431, 1249, 1167, 842, 816, 686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.0 Hz, 1H), 7.77–7.89 (m, 3H), 7.58–7.56 (m, 2H), 7.53 (s, 1H), 5.40 (s, 2H), 4.09 (s, 3H), 4.07 (s, 3H), 4.01 (s, 2H), 2.70–2.30 (br m, 8H), 2.30 (s, 3H); ESIMS *m/z* (rel intensity) 457 (MH^+, 100). Anal. Calcd for $C_{27}H_{28}N_4O_3{\cdot}0.5H_2O$: C, 69.66; H, 6.28; N, 12.03. Found: C, 69.59; H, 6.21; N, 12.13.

5.4.17. 14-[(1′-Imidazolyl)propylaminomethyl]-2,3-dimethoxy-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (30e)

Compound **30a** (0.050 g, 0.127 mmol) and 1-(3-aminopropyl)imidazole (0.048 g, 0.381 mmol) in DMSO afforded the title compound as a yellow powder (0.040 mg, 66%) after flash column chromatography (SiO_2, eluting with a gradient of 0.5% MeOH–0.1% Et_3N in $CHCl_3$ to 2.5% MeOH–0.2% Et_3N in $CHCl_3$) and washing with ether (20 mL): mp 213–216 °C (dec). IR (film) 3306, 2918, 1657, 1626, 1602, 1507, 1478, 1356, 1253, 1111, 687 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, *J* = 8.0 Hz, 1H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.73 (t, *J* = 7.7 Hz, 1H), 7.57–7.53 (m, 3H), 7.49 (s, 1H), 7.44 (s, 1H), 7.03 (s, 1H), 6.85 (s, 1H), 5.39 (s, 2H), 4.30 (s, 2H), 4.09 (s, 3H), 4.07 (s, 3H), 4.05 (t, *J* = 6.9 Hz, 2H), 2.77 (t, *J* = 6.7 Hz, 2H), 2.01 (pent, *J* = 6.7 Hz, 2H); the amine proton is not visible due to residual water in the solvent; ESIMS *m/z* (rel intensity) 482 (MH^+, 100). Anal. Calcd for $C_{28}H_{27}N_5O_3{\cdot}1H_2O$: C, 67.32; H, 5.85; N, 14.02. Found: C, 66.95; H, 5.73; N, 13.92.

5.4.18. 3-Fluoro-2-methyl-14-[1′-(*N*-methylpiperazinylmethyl)]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (31b)

Compound **31a** (0.070 g, 0.192 mmol) and *N*-methylpiperazine (0.058 g, 0.575 mmol) in DMSO afforded the desired compound as a pale yellow-green amorphous solid (0.053g, 64%) after flash column chromatography (SiO_2, eluting with 5% MeOH in $CHCl_3$) and washing with ether: mp 238–242 °C (dec). IR (KBr pellet) 3435, 2930, 2790, 2764, 2372, 2348, 1670, 1641, 1504, 1441, 1161, 1139, 821, 686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.0 Hz, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 7.80–7.56 (m, 5H), 5.44 (s, 2H), 4.03 (s, 2H), 2.70–2.40 (br m, 8H), 2.54 (s, 3H), 2.30 (s, 3H); ESIMS *m/z* (rel intensity) 429 (MH^+, 100). Anal. Calcd for $C_{26}H_{25}FN_4O{\cdot}0.5H_2O$: C, 71.38; H, 5.99; N, 12.81. Found: C, 71.34; H, 5.67; N, 12.70.

5.4.19. 3-Fluoro-14-[*N*-(*S*)-3′-Hydroxypyrrolidinomethyl]-2-methyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (31c)

Compound **31a** (0.070 g, 0.192 mmol) was diluted with DMSO (25 mL) and (*S*)-pyrrolidin-3-ol hydrogen maleate (0.118 g, 0.576 mmol) was added, followed by Et_3N (0.194 g, 1.92 mmol). The mixture was stirred at room temperature for 16.5 h. TLC indicated incomplete reaction; 0.040 g (0.192 mmol) of additional pyrrolidinol and Et_3N (0.065 g, 0.642 mmol) were added, and the mixture was heated at 50 °C for 45 min. Extraction (by general procedure) and flash column chromatography (SiO_2, eluting with 1% MeOH in $CHCl_3$ to 1.5% MeOH in $CHCl_3$) afforded a cream-colored powder (0.050 g, 62%) after washing with ether: mp 239–242 °C (dec). IR (KBr pellet) 3369, 2903, 2806, 1661, 1614, 1597, 1506, 1482, 1440, 1347, 1229, 1135, 1024, 862, 760, 689 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.37 (dd, *J* = 4.6, 3.9 Hz, 2H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.84–7.78 (m, 2H), 7.63–7.58 (m, 2H), 5.39 (s, 2H), 4.75 (d, *J* = 4.3 Hz, 1H), 4.23–4.18 (m, 3H), 2.85–2.73 (m, 2H), 2.57–2.42 (m, 2H), 2.50 (s, 3H, obscured by solvent peak), 2.02–2.98 (m, 1H), 1.70–1.50 (m, 1H); ESIMS *m/z* (rel intensity) 416 (MH^+, 100). Anal. Calcd for $C_{25}H_{22}FN_3O_2{\cdot}0.25H_2O$: C, 71.50; H, 5.40; N, 10.01. Found: C, 71.15; H, 5.04; N, 9.90.

5.4.20. 3-Fluoro-14-[(1′-imidazolyl)propylaminomethyl]-2-methyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (31d)

Compound **31a** (0.070 g, 0.19 mmol) was diluted with DMSO (25 mL), and 1-(3-aminopropyl)imidazole (0.072 g, 0.08 mmol) was added. The mixture was stirred at room temperature for 16.5 h. TLC indicated the reaction was incomplete, so an additional 0.024 g (0.192 mmol) of 1-(3-aminopropylimidazole) was added, and the mixture was heated at 50 °C for 1 h. Extraction (by general procedure) and flash column chromatography (SiO_2, eluting with a gradient of 1% MeOH in $CHCl_3$ to 6% MeOH–0.2% Et_3N in $CHCl_3$) yielded a bright yellow powder (0.077 g, 89%) after washing with ether: mp 183–187 °C (dec). IR (KBr pellet) 3435, 3248, 3116, 3084, 1947, 2603, 1659, 1633, 1606, 1507, 1439, 1341, 1231, 1137, 912, 862, 759, 689 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 8.37 (d, *J* = 8.0 Hz, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.84–7.77 (m, 2H), 7.63–7.58 (m, 3H), 7.14 (s, 1H), 6.87 (s, 1H), 5.44 (s, 2H), 4.30 (s, 2H), 4.05 (t, *J* = 7.0 Hz, 2H), 2.66 (t, *J* = 6.3 Hz, 2H), 2.50 (s, 3H, obscured by solvent peak), 1.94–1.89 (m, 2H); ESIMS *m/z* (rel intensity) 454 (MH^+, 100). Anal. Calcd for $C_{27}H_{34}FN_5O{\cdot}0.5H_2O$: C, 70.11; H, 5.45; N, 15.14. Found: C, 69.73; H, 5.30; N, 14.97.

5.4.21. 14-(*N*-Ethanolaminomethyl)-3-fluoro-2-methyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (31e)

Compound **31a** (0.070 g, 0.192 mmol) was diluted with DMSO (25 mL) and ethanolamine (0.059 g, 0.960 mmol) was added. The mixture was stirred overnight at room temperature for 17 h. TLC indicated incomplete reaction, so additional ethanolamine (0.029 g 0.480 mmol) was added, and the mixture was heated at 50 °C for 1 h. Extraction (by general procedure) and flash column chromatography (SiO_2, eluting with a gradient of 1% MeOH in $CHCl_3$ to 5% MeOH–0.2% Et_3N in $CHCl_3$) afforded a residue that was recrystallized from boiling $CHCl_3$ (20 mL) to yield a pale yellow amorphous solid (0.036 g, 48%) after washing with ether: mp 212–216 °C (dec) IR (KBr pellet) 3378, 3066, 2925, 1656, 1617, 1602, 1504, 1438, 1348, 1128, 1110, 1056, 878, 689 cm^{-1}; ^1H NMR

(300 MHz, DMSO-d_6) δ 8.35 (t, J = 7.3 Hz, 2H), 7.98 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 10.2 Hz, 2H), 7.62–7.58 (m, 2H), 5.44 (s, 2H), 4.59 (br s, 1H), 4.34 (s, 2H), 3.56 (q, J = 5.4 Hz, 2H), 2.80–2.70 (s, 2H), 2.50 (s, 3H, obscured by solvent peak); ESIMS *m/z* (rel intensity) 390 (MH^+, 100). Anal. Calcd for $C_{23}H_{20}FN_5O_2{\cdot}0.3H_2O$: C, 69.97; H, 5.26; N, 10.64. Found: C, 69.90; H, 5.24; N, 10.57.

5.4.22. 14-Azidomethyl-3-fluoro-2-methyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (31f)

Compound **31a** (0.070 g, 0.19 mmol) and sodium azide (0.037 g, 0.58 mmol) in DMSO afforded the title compound as a pale yellow amorphous solid (0.057 g, 79%) after flash column chromatography (SiO_2, eluting with $CHCl_3$) and washing with ether: mp 225–226 °C (dec). IR (KBr pellet) 3437, 2106, 1661, 1636, 1504, 1430, 1342, 1142, 877, 688 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.35 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.88 (d, J = 10.9 Hz, 1H), 7.83 (t, J = 7.5 Hz, 1H), 5.43 (s, 2H), 4.23 (s, 2H), 2.49 (s, 3H, obscured by solvent peak); ESIMS *m/z* (rel intensity) 372 (MH^+, 100).

5.4.23. 14-Aminomethyl-3-fluoro-2-methyl-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one dihydrochloride (31g)

Compound **31f** (0.047 g, 0.127 mmol) was diluted with benzene (30 mL). Triethyl phosphite (0.063 g, 0.380 mmol) was added, and the mixture was heated at reflux for 20 h. The mixture was cooled, methanolic HCl (3 M, 10 mL) was added, and the mixture was heated at reflux for 3 h. The solution was cooled and concentrated to yield an orange solid (0.053 g, 100%) after washing with ether and drying in vacuo: mp 278–282 °C (dec). IR (KBr pellet) 3428, 2047, 2915, 2866, 2632, 2346, 1902, 1656, 1624, 1603, 1528, 1346, 1254, 1128, 802, 689 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.50–7.40 (m, 2H), 7.36 (d, J = 7.3 Hz, 2H), 7.13 (t, J = 6.6 Hz, 1H), 6.87 (d, J = 10.4 Hz, 1H), 6.59 (s, 1H), 5.00 (s, 2H), 4.47 (s, 2H), 2.19 (s, 3H); the amine is not visible due to exchange with the solvent; ESIMS *m/z* (rel intensity) 346 (MH^+, 100). Anal. Calcd for $C_{21}H_{18}Cl_2FN_3O{\cdot}0.5H_2O$: C, 59.03; H, 4.48; N, 9.83. Found: C, 58.89; H, 4.48; N, 9.63.

5.4.24. 2-Chloro-14-[(1′-imidazolyl)propylaminomethyl]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (32b)

Compound **32a** (0.060 g, 0.163 mmol) and 1-(3-aminopropyl)imidazole (0.102 g, 0.817 mmol) in DMSO afforded the desired compound as a yellow amorphous solid (0.039 g, 53%) after flash column chromatography (SiO_2, 25.8 g, eluting with a gradient of 1% MeOH–0.25% Et_3N in $CHCl_3$ to 2% MeOH–0.25% Et_3N in $CHCl_3$) and washing with ether (100 mL): mp 171–175 °C (dec). IR (film) 3293, 2930, 1659, 1617, 1602, 1497, 1481, 1341, 1089, 825, 752, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 7.8 Hz, 1H), 8.27 (s, 1H), 8.17 (d, J = 9.1 Hz, 1H), 7.79–7.58 (m, 5H), 7.44 (s, 1H), 7.03 (s, 1H), 6.86 (s, 1H), 5.43 (s, 2H), 4.30 (s, 2H), 4.08 (t, J = 6.7 Hz, 2H), 2.78 (t, J = 6.7 Hz, 2H), 2.03–1.98 (m, 2H); the amine proton is not visible due to residual water in the solvent; ESIMS *m/z* (rel intensity) 456 (MH^+, 100). Anal. Calcd for $C_{26}H_{22}ClN_5O{\cdot}2.2H_2O$: C, 63.01; H, 5.37; N, 14.1. Found: C, 63.37; H, 4.99; N, 13.70.

5.4.25. 2-Chloro-14-[1′-(*N*-methylpiperazinylmethyl)]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (32c)

Compound **32a** (0.060 g, 0.163 mmol) and *N*-methylpiperazine (0.066 g, 0.652 mmol) in DMSO afforded the title compound as a pale-yellow amorphous solid (0.055 g, 78%) after flash column chromatography (SiO_2, 22.9 g, eluting with a gradient of 1% MeOH in $CHCl_3$ to 4% MeOH in $CHCl_3$) and washing with ether (30 mL): mp 238–241 °C (dec). IR (film) 2931, 2791, 1662, 1632, 1605, 1496, 1480, 1456, 1338, 1161, 1089, 1011, 826, 731, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, J = 8.0 Hz, 1H), 8.37 (d, J = 2.2 Hz, 1H), 8.16 (d, J = 9.0 Hz, 1H), 7.78–7.59 (m, 5H), 5.46 (s, 2H), 4.02 (s, 2H), 2.70–2.30 (br m, 8H), 2.30 (s, 3H); ESIMS *m/z* (rel intensity) 431 (MH^+, 100). Anal. Calcd for $C_{25}H_{23}ClN_4O{\cdot}0.25H_2O$: C, 68.96; H, 5.44; N, 12.87. Found: C, 68.95; H, 5.66; N, 12.90.

5.4.26. 2-Chloro-14-[*N*-(*S*)-3′-hydroxypyrrolidinomethyl]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (32d)

Compound **32a** (0.060 g, 0.163 mmol), (*S*)-pyrrolidin-3-ol (0.133 g, 0.642 mmol) and Et_3N (0.197 g, 1.96 mmol) in DMSO afforded the title compound as a yellow powder (0.040 g, 59%) after flash column chromatography (SiO_2, 25.6 g, eluting with a gradient of 1% MeOH in $CHCl_3$ to 1.5% MeOH in $CHCl_3$) and washing with ether (30 mL): mp 235–238 °C (dec). IR (film) 3369, 2917, 2797, 1659, 1617, 1602, 1496, 1480, 1340, 1088, 827, 754, 730, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 8.1 Hz, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.15 (d, J = 9.0 Hz, 1H), 7.77–7.58 (m, 5H), 5.45 (d, J = 2.4 Hz, 2H), 4.40 (br s, 1H), 4.15 (s, 2H), 2.95 (q, J = 5.8 Hz, 1H), 2.78–2.72 (m, 2H), 2.53–2.47 (m, 1H), 2.30–2.23 (m, 1H), 1.84–1.62 (m, 2H); ESIMS *m/z* (rel intensity) 418 (MH^+, 100). Anal. Calcd for $C_{24}H_{20}ClN_3O_2{\cdot}0.5H_2O$: C, 67.52; H, 4.96; N, 9.84. Found: C, 67.80; H, 5.03; N, 9.80.

5.4.27. 2-Chloro-14-(*N*-ethanolaminomethyl)-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (32e)

Compound **32a** (0.070 g, 0.191 mmol) and ethanolamine (0.047 g, 0.762 mmol) in DMSO afforded a yellow powder after flash column chromatography (SiO_2, 27.9 g, eluting with a gradient of 1% MeOH–0.2% Et_3N in $CHCl_3$ to 1.5% MeOH–0.2% Et_3N in $CHCl_3$) and washing with ether (100 mL). This solid contained residual $Et_3N{\cdot}HCl$, and was dissolved in $CHCl_3$ (50 mL, with MeOH added for solubility) and washed with dilute NaOH (2 × 25 mL). The aqueous phase was extracted with $CHCl_3$ (5 × 25 mL), and the organic phase was dried over anhydrous sodium sulfate and concentrated to yield a yellow powder (0.027 g, 36%) after washing with ether (30 mL): mp 218–220 °C (dec). IR (film) 3306, 2917, 2833, 1654, 1614, 1494, 1482, 1346, 845, 827, 757, 690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 8.3 Hz, 1H), 8.25 (d, J = 2.2 Hz, 1H), 8.15 (d, J = 9.1 Hz, 1H), 7.76–7.58 (m, 5H), 5.46 (s, 2H), 4.36 (s, 2H), 3.79 (t, J = 4.6 Hz, 2H), 2.96 (t, J = 5.2 Hz, 2H), 2.08 (br s, 1H); the hydroxyl proton is not visible due to residual water in the solvent; ESIMS *m/z* (rel intensity) 392 (MH^+, 100). Anal. Calcd for $C_{22}H_{18}ClN_3O_2{\cdot}1H_2O$: C, 64.47; H, 4.92; N, 10.25. Found: C, 64.35; H, 4.69; N, 9.93.

5.4.28. 2-Chloro-14-(1′-imidazolylmethyl)-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (32f)

Compound **32a** (0.070 g, 0.191 mmol) and imidazole (0.065 g, 0.955 mmol) at 60 °C in DMF afforded the title compound as a yellow-green chalky solid (0.054 g, 70%) after flash column chromatography (SiO_2, 26.7 g, eluting with a gradient of 1.5% MeOH in $CHCl_3$ to 4% MeOH in $CHCl_3$), washing with ether (30 mL) and drying: mp 296–298 °C (dec). IR (film) 2918, 1660, 1632, 1606, 1500, 1341, 1230, 1083, 828, 756, 689 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, J = 2.1 Hz, 1H), 8.31 (d, J = 8.0 Hz, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.98–7.83 (m, 4H), 7.79 (s, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.28 (s, 1H), 6.94 (s, 1H), 5.92 (s, 2H), 5.18 (s, 2H); ESIMS *m/z* (rel intensity) 399 (MH^+, 100). Anal. Calcd for $C_{23}H_{15}ClN_4O{\cdot}0.25H_2O$: C, 68.49; H, 3.87; N, 13.89. Found: C, 68.33; H, 3.90; N, 13.92.

5.4.29. 3-Chloro-14-[(1′-imidazolyl)propylaminomethyl]-12*H*-5,11a-diazadibenzo[*b*,*h*]fluoren-11-one (33b)

Compound **33a** (0.060 g, 0.163 mmol) was diluted with DMSO (20 mL). 1-(3-Aminopropyl)imidazole (0.102 g, 0.817 mmol) was added, and the mixture was stirred at room temperature for 18 h. The reaction was incomplete by TLC, so the mixture was heated to 55 °C for 2 h. The product was extracted (following general procedures) and flash column chromatography (SiO_2, 27.0 g, eluting with

1% MeOH–0.2% Et_3N in $CHCl_3$) yielded a yellow-orange amorphous solid (0.046 g, 61%) after washing with ether (20 mL): mp 163–176 °C (dec). IR (film) 3401, 2922, 1660, 1622, 1604, 1480, 1343, 1230, 1081, 923, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 7.8 Hz, 1H), 8.21 (d, J = 1.5 Hz, 1H), 8.20 (d, J = 8.7 Hz, 1H), 7.78–7.57 (m, 5H), 7.42 (s, 1H), 7.03 (s, 1H), 6.84 (s, 1H), 5.42 (s, 2H), 4.31 (s, 2H), 4.06 (d, J = 6.9 Hz, 2H), 2.76 (t, J = 6.7 Hz, 2H), 2.01–1.97 (m, 2H), 1.60–1.50 (br s, 1H, obscured by solvent peak); ESIMS m/z (rel intensity) 456 (MH^+, 100). Anal. Calcd for $C_{26}H_{22}ClN_5O{\cdot}2.25H_2O$: C, 62.90; H, 5.38; N, 14.11. Found: C, 62.65; H, 4.99; N, 13.94.

5.4.30. 3-Chloro-14-[1′-(*N*-methylpiperazinylmethyl)]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (33c)

Compound **33a** (0.060 g, 0.163 mmol) and *N*-methylpiperazine (0.066 g, 0.652 mmol) in DMSO afforded the title compound as a pale-yellow amorphous solid (0.055 g, 78%) after flash column chromatography (SiO_2, 24.4 g, eluting with a gradient of 1% MeOH in $CHCl_3$ to 5% MeOH in $CHCl_3$) and washing with ether (20 mL): mp 226–228 °C (dec). IR (film) 2931, 2798, 1662, 1631, 1605, 1460, 1341, 1291, 1162, 1077, 1011, 918, 751, 686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.1 Hz, 1H), 8.33 (d, J = 9.1 Hz, 1H), 8.20 (d, J = 2.1 Hz, 1H), 7.78–7.54 (m, 5H), 5.43 (s, 2H), 4.03 (s, 2H), 2.70–2.30 (br m, 8H), 2.30 (s, 3H); ESIMS m/z (rel intensity) 431 (MH^+, 100). Anal. Calcd for $C_{25}H_{23}ClN_4O{\cdot}0.50H_2O$: C, 68.25; H, 5.50; N, 12.73. Found: C, 68.47; H, 5.34; N, 12.71.

5.4.31. 3-Chloro-14-[*N*-(*S*)-3′-hydroxypyrrolidinomethyl]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (33d)

Compound **33a** (0.060 g, 0.163 mmol) and (*S*)-pyrrolidin-3-ol (0.133 g, 0.648 mmol) were diluted with DMSO (25 mL). Et_3N (0.197 g, 1.96 mmol) was added, and the mixture was stirred at room temperature for 17 h. The reaction was incomplete by TLC, so the mixture was heated to 50 °C for 1 h and 30 min. Extraction followed general procedures, and flash column chromatography (SiO_2, 24.9 g, eluting with a gradient of 1% MeOH in $CHCl_3$ to 1.5% MeOH in $CHCl_3$) yielded a pale-yellow amorphous solid (0.046 g, 68%) after washing with ether (30 mL): mp 247–249 °C (dec). IR (film) 3401, 2916, 2799, 1659, 1619, 1603, 1480, 1344, 1190, 922, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 9.1 Hz, 1H), 8.21 (d, J = 2.1 Hz, 1H), 7.79–7.55 (m, 5H), 5.45 (d, J = 1.6 Hz, 2H), 4.39 (br s, 1H), 4.17 (s, 2H), 3.00–2.90 (m, 1H), 2.73 (d, J = 3.7 Hz, 2H), 2.50–2.44 (m, 1H), 2.30–2.20 (m, 1H), 1.84–1.76 (m, 2H); ESIMS m/z (rel intensity) 418 (MH^+, 100). Anal. Calcd for $C_{24}H_{20}ClN_3O_2{\cdot}0.75H_2O$: C, 66.82; H, 5.02; N, 9.74. Found: C, 66.67; H, 4.77; N, 9.50.

5.4.32. 3-Chloro-14-(1′-imidazolylmethyl)-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (33e)

Compound **33a** (0.070 g, 0.191 mmol) and imidazole (0.065 g, 0.955 mmol) in DMF at 80 °C afforded the title compound as a chalky yellow solid (0.054 g, 71%) after flash column chromatography (SiO_2, 27.4 g, eluting with a gradient of 1.5% MeOH in $CHCl_3$ to 3.5% MeOH in $CHCl_3$), and washing with ether (30 mL): mp 279–281 °C (dec). IR (film) 3401, 2917, 1658, 1621, 1599, 1498, 1448, 1228, 1079, 687 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.30–8.29 (m, 2H), 8.18 (s, 1H), 7.97 (d, J = 7.0 Hz, 1H), 7.89 (s, 1H), 7.78–7.64 (m, 4H), 7.24 (s, 1H), 6.89 (s, 1H), 5.90 (s, 2H), 5.17 (s, 2H); ESIMS m/z (rel intensity) 399 (MH^+, 100). Anal. Calcd for $C_{23}H_{15}ClN_4O{\cdot}0.25H_2O$: C, 66.99; H, 4.03; N, 13.59. Found: C, 66.81; H, 3.81; N, 13.39.

5.4.33. 1-Chloro-14-[1′-(*N*-methylpiperazinylmethyl)]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one trihydrochloride (34b)

Compound **34a** (0.070 g, 0.191 mmol) and *N*-methylpiperazine (0.076 g, 0.762 mmol) in DMSO afforded the desired compound as a cream-colored solid after flash column chromatography (SiO_2, 27.6 g, eluting with a gradient of 0.5% MeOH in $CHCl_3$ to 3% MeOH in $CHCl_3$). This solid was washed with ether (25 mL), dissolved in $CHCl_3$ (35 mL) and methanolic HCl (3 M, 5 mL) was added slowly. The mixture was stirred at room temperature for 2 h and concentrated to yield an orange-yellow iridescent solid (0.047 g, 46%) after washing with ether (50 mL) and drying in vacuo: mp 203–206 °C (dec). IR (KBr) 3400, 2599, 2489, 1651, 1618, 1596, 1344, 1127, 821, 759, 692 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.13 (br s, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.18 (dd, J = 7.7, 1.9 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.84–7.77 (m, 3H), 7.70 (s, 1H), 7.65 (t, J = 7.2 Hz, 1H), 5.42 (s, 2H), 4.40 (s, 2H), 3.21–3.28 (m, 2H), 3.00–2.80 (m, 4H), 2.70–2.50 (m, 5H); ESIMS m/z (rel intensity) 431 (MH^+, 100). Anal. Calcd for $C_{25}H_{26}Cl_4N_4O{\cdot}1.3H_2O$: C, 53.26; H, 5.11; N, 9.94. Found: C, 53.63; H, 5.51; N, 9.87.

5.4.34. 1-Chloro-14-[*N*-(*S*)-3′-hydroxypyrrolidinomethyl]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one hydrochloride (34c)

Compound **34a** (0.070 g, 0.191 mmol) and (*S*)-pyrrolidin-3-ol hydrogen maleate (0.157 g, 0.764 mmol) were diluted with DMSO (25 mL) and Et_3N (0.231 g, 0.764 mmol) was added. The mixture was stirred at room temperature for 16 h, and then heated to 55 °C for 1.5 h. Extraction followed general procedures, and flash column chromatography (SiO_2, 25.7 g, eluting with a gradient of 0.25% MeOH in $CHCl_3$ to 1.25% MeOH in $CHCl_3$) afforded a yellow solid. This solid was treated (in $CHCl_3$) with methanolic HCl as described for **34b** to yield a dark-orange iridescent solid (0.031 g, 37%) after washing with ether (50 mL) and drying in vacuo: mp 208–210 °C (dec). IR (KBr) 3400, 2658, 1659, 1627, 1601, 1440, 1377, 1343, 1125, 1102, 926, 815, 758, 688 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.1–10.0 (br m, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.20 (dd, J = 7.8, 3.5 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 7.5 Hz, 1H), 7.86 (td, J = 8.1, 2.7 Hz, 1H), 7.81 (t, J = 7.1 Hz, 1H), 7.70 (s, 1H), 7.62 (t, J = 7.2 Hz, 1H), 5.70–5.30 (m, 4H), 4.10 (br s, 0.5H), 4.52 (br s, 0.5H), 4.39 (br s, 0.5H), 3.68–2.54 (m, 3H), 3.27–3.25 (br m, 0.5H), 2.50–2.30 (br m, 0.5H), 2.10–2.00 (br m, 0.5H), 2.00–1.90 (br m, 0.5H), 1.80–1.70 (br m, 0.5H); ESIMS m/z (rel intensity) 418 (MH^+, 100). Anal. Calcd for $C_{24}H_{21}Cl_2N_3O_2{\cdot}2H_2O$: C, 58.78; H, 5.14; N, 8.57. Found: C, 59.01; H, 4.84; N, 8.26.

5.4.35. 1-Chloro-14-[(1′-imidazolyl)propylaminomethyl]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one trihydrochloride (34d)

Compound **34a** (0.090 g, 0.245 mmol) was diluted with DMSO (25 mL) and 1-(3-aminopropyl)imidazole (0.153 g, 0.1.22 mmol) was added. The mixture was stirred at room temperature for 17 h, and then heated to 50 °C for 2 h. Extraction followed general procedures, and flash column chromatography (SiO_2, 27.0 g, eluting with a gradient of 1% MeOH in $CHCl_3$ to 4% MeOH in $CHCl_3$) afforded a yellow solid. This solid was treated with methanolic HCl as described for **34b** to yield the title compound as a flaky red iridescent solid (0.048 g, 35%) after washing with ether (50 mL) and drying in vacuo: mp 180–185 °C. IR (film) 3400, 2922, 2726, 1658, 1620, 1601, 1463, 1299, 1203, 1103, 818, 758, 683 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.9 (br s, 2H), 9.25 (s, 1H), 8.33 (d, J = 7.9 Hz, 1H), 8.22 (dd, J = 7.8, 1.6 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.89–7.77 (m, 4H), 7.73 (s, 1H), 7.71 (s, 1H), 7.63 (t, J = 7.5 Hz, 1H), 5.80 (s, 2H), 4.99 (s, 2H), 4.41 (t, J = 6.6 Hz, 2H), 3.30–3.20 (br m, 2H), 2.40–2.30 (m, 2H); the protonated secondary amine is not visible due to exchange with residual water in the solvent; ESIMS m/z (rel intensity) 456 (MH^+, 100). Anal. Calcd for $C_{26}H_{25}Cl_4N_5O{\cdot}2.5H_2O$: C, 51.16; H, 4.95; N, 11.47. Found: C, 50.90; H, 5.19; N, 11.11.

5.5. General procedure for synthesis of extended ethylenedioxyaromathecins 28b–g

Compound **28a** (0.180–0.215 mmol) was diluted in DMSO or DMF (25–30 mL), and sodium iodide (6 equiv) and the nucleophile

(between 6 and 12 equiv) were added. The mixture was heated to 100 °C overnight (between 12 and 36 h or until TLC indicated completion of the reaction), and the mixture was poured into H_2O (at least 100 mL) and extracted thoroughly with $CHCl_3$ (at least 150 mL). The organic layers were washed with copious water (between 400 and 600 mL) and (when DMF was used), satd NH_4Cl (~200 mL), and dried over anhydrous sodium sulfate. The solution was concentrated, adsorbed onto SiO_2 (between 3 and 5 g), and purified by flash column chromatography (SiO_2, between 20 and 30 g), to afford the aromathecins as solids after washing with ether or suspending in methanol and precipitating with ether.

5.5.1. 14-[3′-(*N*-Ethanolaminopropyl)]-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (28b)

Compound **28a** (0.090 g, 0.215 mmol), sodium iodide (0.193 g, 1.29 mmol) and ethanolamine (0.079 g, 1.29 mmol) in DMSO afforded the title compound as a yellow solid (0.044 g, 46%) after flash column chromatography (SiO_2, 29.0 g, eluting with a gradient of 1.5% MeOH–0.5% Et_3N in $CHCl_3$ to 6% MeOH–0.5% Et_3N in $CHCl_3$) and precipitation by general procedure: mp 200–205 °C. IR (film) 3584, 2930, 1656, 1621, 1602, 1507, 1441, 1400, 1288, 1246, 1068, 914 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 8.0 Hz, 1H), 7.78–7.51 (m, 6H), 5.31 (s, 2H), 4.42 (s, 4H), 3.74 (t, J = 4.9 Hz, 2H), 3.19 (t, J = 7.5 Hz, 2H), 2.85–2.78 (m, 4H), 2.00–1.95 (m, 2H); the hydroxyl group and amine are not visible due to residual water in the solvent; ESIMS m/z (rel intensity) 444 (MH^+, 100). Anal. Calcd for $C_{26}H_{25}ClN_3O_4 \cdot 1.25H_2O$: C, 67.01; H, 5.95; N, 9.02. Found: C, 66.76; H, 5.67; N, 8.88.

5.5.2. 2,3-Ethylenedioxy-14-[3′-(1′-imidazolylpropyl)]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (28c)

Compound **28a** (0.075 g, 0.179 mmol), sodium iodide (0.160 g, 1.08 mmol), and imidazole (0.073 g, 1.08 mmol) were diluted with anhydrous DMF (30 mL) under an argon atmosphere. The mixture was heated to 100 °C for 21 h. As the reaction was incomplete by TLC, additional imidazole (0.073 g, 1.08 mmol) was added, and the mixture was kept at 100 °C for an additional 3 h, upon which a white precipitate formed (presumably NaCl). The mixture was held at 40 °C for 16.5 h, cooled, and diluted with H_2O (100 mL). The mixture was extracted following general procedures, and the residue was purified by flash column chromatography (SiO_2, 30.0 g), eluting with 1% MeOH–0.5% Et_3N in $CHCl_3$, to yield a yellow amorphous solid (0.040 g, 50%) after washing with ether (25 mL): mp 275–278 °C. IR (film) 3584, 2930, 1657, 1621, 1602, 1506, 1447, 1288, 1246, 1067, 917 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 8.0 Hz, 1H), 7.77–7.53 (m, 6H), 7.20 (s, 1H), 7.17 (s, 1H), 7.01 (s, 1H), 5.23 (s, 2H), 4.42 (s, 4H), 4.17 (t, J = 7.1 Hz, 2H), 3.05 (t, J = 8.0 Hz, 2H), 2.27–2.17 (m, 2H); ESIMS m/z (rel intensity) 451 (MH^+, 100). Anal. Calcd for $C_{27}H_{22}N_4O_3 \cdot 1H_2O$: C, 69.22; H, 5.16; N, 11.96. Found: C, 69.31; H, 5.24; N, 11.77.

5.5.3. 2,3-Ethylenedioxy-14-[3′-(*N*-morpholinopropyl)]-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (28d)

Compound **28a** (0.075 g, 0.179 mmol), sodium iodide (0.160 g, 1.08 mmol) and morpholine (0.188 g, 2.15 mmol) in DMF afforded the title compound as a white amorphous solid (0.060 g, 72%) after flash column chromatography (SiO_2, 30.1 g, eluting with 0.5% MeOH–0.25% Et_3N in $CHCl_3$) and precipitation by the general procedure: mp 259–260 °C. IR (film) 3584, 2929, 2382, 1659, 1630, 1603, 1506, 1448, 1398, 1287, 1244, 1116, 1068, 914 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 7.9 Hz, 1H), 7.78–7.54 (m, 6H), 5.32 (s, 2H), 4.42 (s, 4H), 3.76–3.73 (m, 4H), 3.16 (t, J = 7.3 Hz, 2H), 2.45–2.41 (m, 6H), 1.97–1.93 (m, 2H); ESIMS m/z (rel intensity) 470 (MH^+, 100). Anal. Calcd for $C_{28}H_{27}N_3O_4$: C, 71.62; H, 5.80; N, 8.95. Found: C, 71.35; H, 5.75; N, 8.92.

5.5.4. 14-[3′-(*N,N*-Dimethylaminopropyl)]-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (28e)

Compound **28a** (0.075 g, 0.179 mmol), sodium iodide (0.160 g, 1.08 mmol), and *N,N*-dimethylamine (1.07 mL of a 2 M solution in THF) in DMF afforded the title compound as a yellow powder (0.051 g, 67%) after flash column chromatography (SiO_2, 30.9 g, eluting with a gradient of 0.5% MeOH–0.25% Et_3N in $CHCl_3$ to 1% MeOH–0.5% Et_3N in $CHCl_3$) and precipitation by the general procedure: mp 221–225 °C. IR (film) 3400, 2924, 1658, 1622, 1603, 1507, 1448, 1289, 1247, 1067, 918, 751, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 8.1 Hz, 1H), 7.78–7.51 (m, 6H), 5.30 (s, 2H), 4.43 (s, 4H), 3.13 (t, J = 7.5 Hz, 2H), 2.54 (t, J = 7.1 Hz, 2H), 2.26 (s, 6H), 1.95–1.90 (m, 2H); ESIMS m/z (rel intensity) 428 (MH^+, 100). Anal. Calcd for $C_{26}H_{25}N_3O_3 \cdot 2H_2O$: C, 67.37; H, 6.31; N, 9.07. Found: C, 67.30; H, 6.05; N, 9.00.

5.5.5. 14-(3′-Azidopropyl)-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (28f)

Compound **28a** (0.075 g, 0.179 mmol) and sodium azide (0.058 g, 0.895 mmol) were diluted with anhydrous DMF (25 mL) under an argon atmosphere. The mixture was heated to 100 °C, upon which it became a bright pink solution. After 17 h, the color had discharged to pale orange, and the solution was cooled, sonicated briefly, and poured into H_2O (100 mL). The mixture was extracted with $CHCl_3$ (3 × 50 mL) and the organic layers were washed with H_2O (3 × 200 mL), satd NH_4Cl (200 mL), and dried over anhydrous sodium sulfate. The residue was adsorbed onto SiO_2 (3.07 g) and purified by flash column chromatography (SiO_2, 28.6 g), eluting with $CHCl_3$ to yield a yellow-green microcrystalline solid (0.059 g, 78%) after washing with MeOH (10 mL) and ether (50 mL) and drying in vacuo for 72 h: mp 228–230 °C (dec). IR (film) 3584, 2930, 2097, 1660, 1628, 1604, 1507, 1448, 1289, 1248, 1068, 688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.2 Hz, 1H), 7.78–7.54 (s, 5H), 7.47 (s, 1H), 5.31 (s, 2H), 4.44 (s, 4H), 3.48 (t, J = 6.4 Hz, 2H), 3.19 (t, J = 7.6 Hz, 2H), 2.08 (m, 2H); ESIMS m/z (rel intensity) 426 (MH^+, 100). Anal. Calcd for $C_{24}H_{19}N_5O_3$: C, 67.76; H, 4.50; N, 16.46. Found: C, 67.47; H, 4.47; N, 16.29.

5.5.6. 14-(3′-Aminopropyl)-2,3-ethylenedioxy-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one dihydrochloride (28g)

Compound **28f** (0.050 g, 0.118 mmol) was diluted with benzene (25 mL) and triethyl phosphite (0.059 g, 0.354 mmol) was added. The mixture was heated at reflux for 19 h. TLC indicated complete consumption of the azide and the mixture was cooled to room temperature. Methanolic HCl (10 mL, 5 M) was added, and the yellow-green mixture was heated at reflux for 3 h, cooled, and concentrated to afford a bright yellow amorphous solid (0.055 g, 99%) after washing with ether (30 mL) and drying in vacuo: mp 290–300 °C (dec). IR (KBr) 3445, 3040, 2708, 1665, 1622, 1603, 1501, 1480, 1401, 1299, 1257, 1156, 1062, 920, 761, 686 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 7.20–7.10 (m, 1H), 7.01–6.78 (m, 3H), 6.37 (s, 1H), 6.28 (s, 1H), 6.05 (s, 1H), 4.32 (s, 2H), 4.14–4.10 (m, 4H), 3.10–3.00 (br m, 2H), 2.50–2.40 (br m, 2H), 1.80–1.70 (br m, 2H); the amino group exchanges with the solvent; ESIMS m/z (rel intensity) 400 (MH^+, 100). Anal. Calcd for $C_{24}H_{23}Cl_2N_3O_3 \cdot 0.5H_2O$: C, 59.88; H, 5.03; N, 8.73. Found: C, 60.06; H, 5.29; N, 8.66.

5.6. Topoisomerase I-mediated DNA cleavage reactions

Human recombinant Top1 was purified from Baculovirus as previously described.[63] DNA cleavage reactions were prepared as previously reported[27] (for review see[64]) with the exception of the DNA substrate. Briefly, a 117-bp DNA oligonucleotide (Integrated DNA Technologies) encompassing the previous identified Top1 cleavage sites identified in the 161-bp fragment from pBluescript SK(−) phagemid DNA was employed. This 117-bp oligonucleotide

contains a single 5′-cytosine overhang, which was 3′-end labeled by fill-in reaction with [α-^{32}P]-dGTP in reaction 2 buffer (50 mM Tris–HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 units of DNA polymerase I (Klenow fragment, New England BioLabs). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled DNA substrate was collected. Approximately 2 nM of radiolabeled DNA substrate was incubated with recombinant Top1 in 20 μL of reaction buffer [10 mM Tris–HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 μg/mL BSA] at 25 °C for 20 min in the presence of various concentrations of compounds. The reactions were terminated by adding SDS (0.5% final concentration) followed by the addition of two volumes of loading dye (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). Aliquots of each reaction were subjected to 20% denaturing PAGE. Gels were dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics). For simplicity, cleavage sites were numbered as previously described in the 161-bp fragment.[63]

5.7. Modeling studies

The crystal structure of camptothecin or topotecan in complex with Top1 and a short DNA fragment were downloaded from the Protein Data Bank (PDB codes 1T8I and 1K4T).[10,15] For the topotecan structure, an atom of Hg and one molecule of PEG were deleted. Hydrogens were added and minimized by the Powell method, using the MMFF94s force field and MMFF94 charges, in Sybyl 8.1 (Tripos, Inc). Analogues of aromathecins were constructed, fixed positive charges were assigned to amines using Sybyl atom types, and the structures were energy minimized using a conjugate gradient method, the MMFF94s force field, and MMFF94 charges. The structure of the ligand was aligned onto topotecan or camptothecin using the 'fit atoms' function. The aligned ligand was overlapped in the crystal structure, and the structure of camptothecin or topotecan was deleted. This new ternary complex was re-subjected to energy minimization using a standard Powell method, the MMFF94s force field, and MMFF94 charges, converging to termination at 0.05 kcal/mol Å, with a distance-dependent dielectric function. The structure of the ligand and a sphere with a radius of 5–8 Å were allowed to move during the minimization, and the surrounding structures were frozen in an aggregate. Ligand overlays were performed by aligning the protein backbones in Sybyl.

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