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# Substituted Benz[*a*]acridines and Benz[*c*]acridines as Mammalian Topoisomerase Poisons

Darshan Makhey,[a] Chiang Yu,[b] Angela Liu,[b] Leroy F. Liu[b] and Edmond J. LaVoie[a,*]

[a]*Department of Pharmaceutical Chemistry, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020, USA*
[b]*Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA*

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**Abstract**—Coralyne and several other synthetic benzo[*a*,*g*]quinolizium derivatives related to protoberberine alkaloids have exhibited activity as topoisomerase poisons. These compounds are characterized by the presence of a positively charged iminium group, which has been postulated to be associated with their pharmacological properties. The objective of the present study was to devise stable noncharged bioisosteres of these compounds. Several similarly substituted benz[*a*]acridine and benz[*c*]acridine derivatives were synthesized and their relative activity as topoisomerase poisons was determined. While the benz[*c*]acridine derivatives evaluated as part of this study were devoid of topoisomerase poisoning activity, several dihydrobenz[*a*]acridines were able to enhance DNA cleavage in the presence of topo I. In contrast to certain protoberberine derivatives that did exhibit activity as topo II poisons, none of the benz[*a*]acridines derivatives enhanced DNA cleavage in the presence of topo II. Among the benz[*a*]acridines studied, 5,6-dihydro-3,4-methylenedioxy-9,10-dimethoxybenz[*a*]acridine, **13e**, was the most potent topo I poison, with comparable potency to coralyne. These data suggest that heterocyclic compounds structurally related to coralyne can exhibit potent topo I posioning activity despite the absence of an iminium cation within their structure. In comparison to coralyne or other protoberberine derivatives, these benz[*a*]acridine derivatives possess distinctly different physicochemical properties and represent a novel series of topo I poisons. 

## Introduction

DNA topoisomerases are nuclear enzymes that catalyze the breaking and rejoining of DNA strands regulating the topological state of DNA.[1–4] Recent studies suggest that topoisomerases are also involved in controlling template supercoiling during RNA transcription.[5,6] The antitumor activity of topoisomerase poisons is associated with their ability to stabilize the enzyme–DNA cleavable complex. This drug-induced stabilization of the enzyme–DNA cleavable complex effectively converts the enzyme into a cellular poison.

Camptothecin and its structurally-related analogues are among the more extensively studied topoisomerase I (topo I) poisons. Recently, bi- and terbenzimidazoles,[7–10] certain benzo[c]phenanthridine and protoberberine alkaloids and their synthetic analogues,[11–13] indolocarbazoles,[14] as well as the fungal metabolites, bulgarein[15] and saintopin,[16] have been identified as topo I poisons.

The exceptional topoisomerase poisoning observed with coralyne (**1**), nitidine (**2**), MDD-coralyne (**3**) and related analogues prompted several studies on those structural features which are associated with their ability to act specifically as either topo I or II poisons.[11,13,17–19] A common feature associated with all three of these agents is the presence of a 3-phenylisoquinolinium moiety within their structure (Chart 1). It has been speculated in the case of nitidine and related benzo[c]phenanthridine alkaloids that the iminium charge is necessary for biological activity.[20–23] Similarly, the charged iminium group is also a common feature of compounds related to the protoberberine alkaloids, such as coralyne, that exhibit antitumor activity.[11,13,17,24,25] It has been reported, however, that 5,6,7,13a-tetrahydrocoralyne, in which the iminium group of 5,6-dihydrocoralyne has been converted to a cyclic tertiary amine, does retain activity as a topo I poison.[18]

*Corresponding author. Tel.: +1-732-445-2674; fax: +1-732-445-6312; e-mail: elavoie@rci.rutgers.edu

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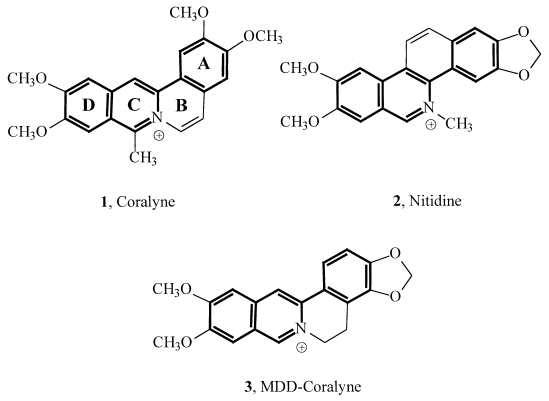



**Chart 1.** Structures of coralyne, nitidine and MDD-coralyne.

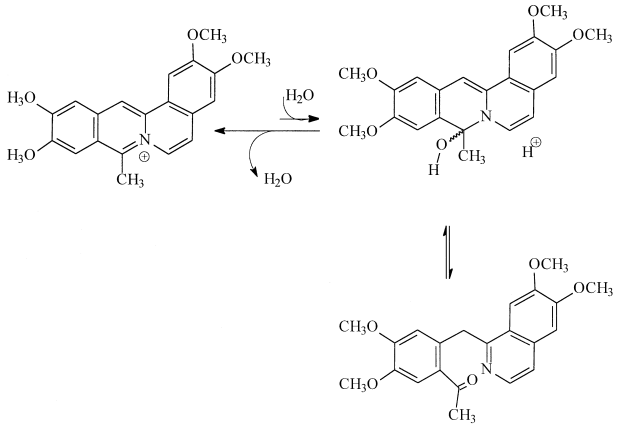


**Chart 2.** Hydration of coralyne.

The reactivity of the iminium moiety toward nucleophilic attack has been proposed as a possible basis for the antileukemic activity of certain benzo[*c*]phenanthridine alkaloids.[26] Janin et al. have successfully demonstrated, however, that noncharged analogues of nitidine can be developed that retain their activity as topoisomerase poisons and possess potent cytotoxic activity.[23]

Protoberberine alkaloids and related compounds can exhibit either topo I or topo II poisoning activity. Previous studies have demonstrated that significant specificity for either of these topoisomerases can be achieved by subtle alterations in either substituents or substitution pattern.[11,13,17] Despite the observation that several of these compounds had similar potency to camptothecin as a topo I poison or similar potency to VM-26 as a topo II poison, they possess only modest cytotoxic activity.[13,17] In general, there has been a poor correlation between cytotoxicity and topoisomerase poisoning activity among these protoberberine derivatives.

The presence of the iminium group could have a substantial influence on cytotoxicity. This charged moiety could impede cellular uptake and, thereby, limit the possibility of observing such a correlation with topoisomerase poisoning activity. In addition, the iminium moiety could be linked to cytotoxic effects unrelated to topoisomerase poisoning. It has been suggested that multiple biochemical effects may contribute to the cytotoxic activity of coralyne.[27,28] In the evaluation of antitumor activity of coralyne and nitidine in laboratory animals, neither compound has exhibited pronounced efficacy. It has been speculated that charged compounds such as coralyne (Chart 2) and nitidine may need to undergo hydrolysis to permit effective transport, with possible intracellular cyclodehydration to reform the tetracyclic structure of parent compound.[29,30]

This possible hindrance to absorption could explain the weak antileukemic activity observed in vivo with these agents. The presence of the iminium moiety within nitidine and coralyne could also be linked to nonspecific toxicity in vitro as well as their relatively weak in vivo antitumor activity.

In the present study a series of substituted benz[*a*]acridines and benz[*c*]acridines (Chart 3) were synthesized as bioisosteres of coralyne and structurally-related protoberberine alkaloids. To evaluate whether a charged iminium moiety was linked to topoisomerase poisoning activity, these benzacridine derivatives, as well as select N-methyl derivatives, were evaluated as topo I and topo II poisons. These compounds were also evaluated for their cytotoxicity against the human lymphoblast cell line, RPMI-8402, and its camptothecin-resistant variant, CPT-K5.

## Chemistry

Scheme 1 outlines the synthetic procedures employed for the preparation of benz[*c*]acridines structurally related to coralyne. Reaction of 2-amino-4,5-dimethoxyacetophenone, **4**, with 6,7-dimethoxy-1-tetralone, **5**, using similar reaction conditions to those previously reported[31] provided 5,6-dihydro-2,3,9,10-tetramethoxybenz[*c*]acridine, **6**, which, when heated as a suspension in decalin at 190 °C in the presence of Pd/C, could be converted to 2,3,9,10-tetramethoxybenz[*c*]acridine, **7**. Both **6** and **7** could be converted to their 12-methyl derivatives by reaction with dimethyl sulfate to form the quaternary ammonium salts, **8** and **9**, respectively.

The preparation of a series of benz[*a*]acridine analogues structurally-related to coralyne was accomplished using the general method outlined in Scheme 2. Knoevenagel condensation of the appropriate *o*-nitrobenzaldehyde with 5,6- or 6,7-disubstituted β-tetralones, provided the 1-(2′-nitrobenzylidene)-2-tetralones, **12a**–**f**. Reduction of **12a**–**f** with zinc in acetic acid gave the desired 5,6-dihydrobenz[*a*]acridine derivatives, **13a**–**f**. Heating **13a**–**d** in decalin at 190 °C in the presence of Pd/C resulted in

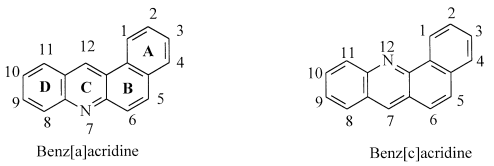
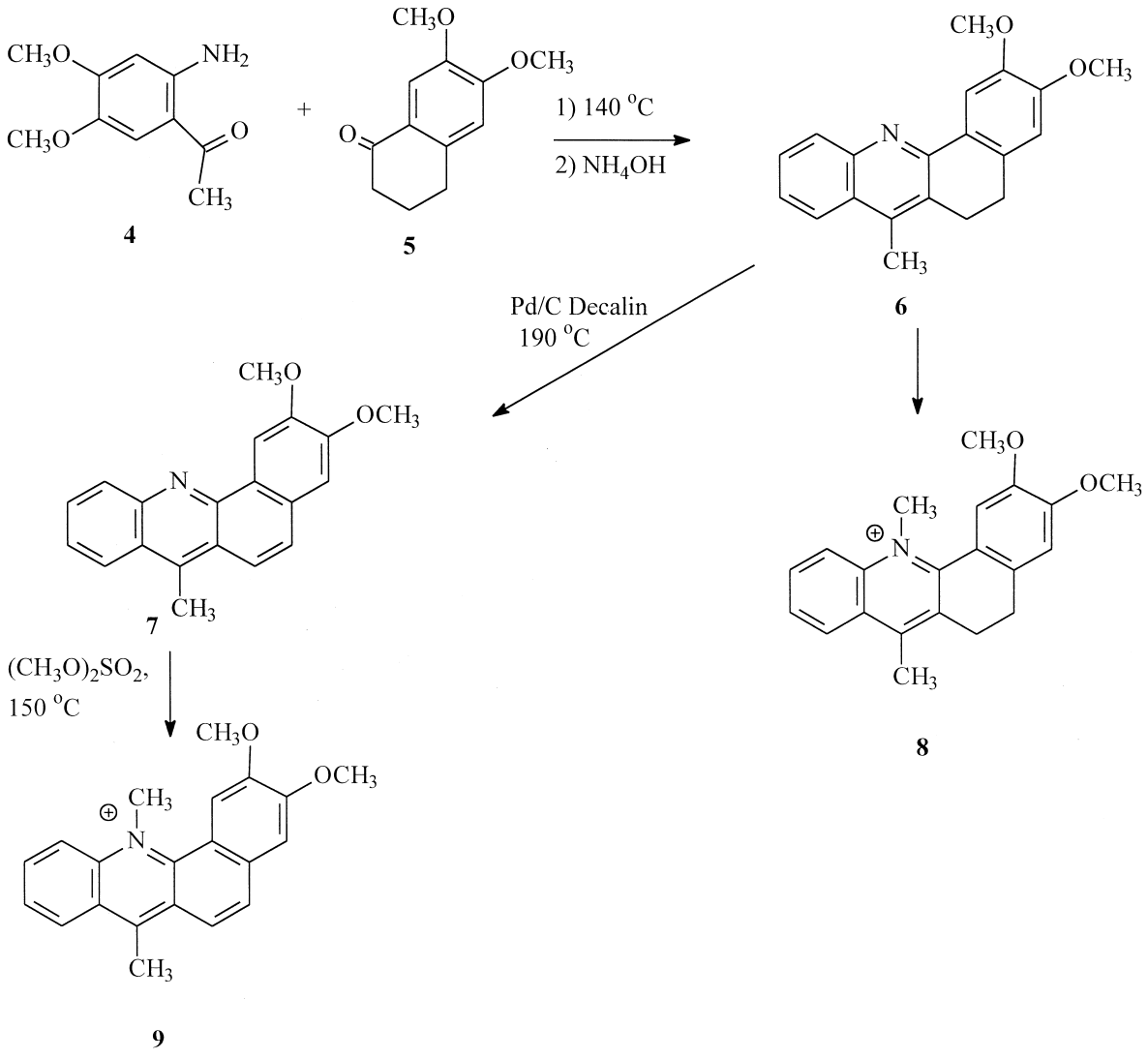



**Chart 3.** Structure, numbering, and ring assignments of benz[*a*]acridine and benz[*c*]acridine.




**Scheme 1.**

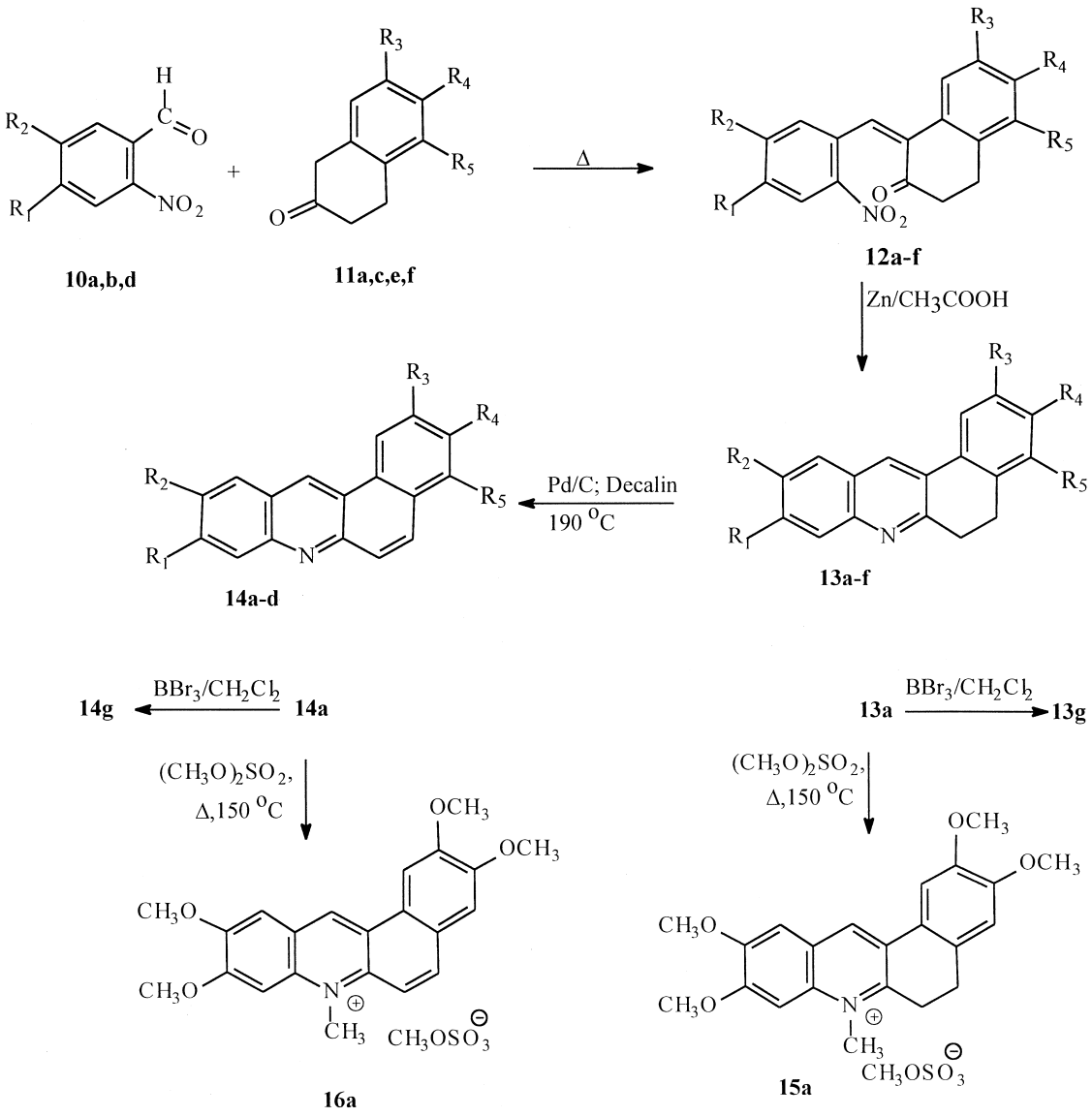
conversion of these dihydro compounds to their benz[*a*]acridine derivatives, **14a–d**. Treatment of either **13a** or **14a** with $BBr_3$ in methylene chloride provided the tetrahydroxy analogues, **13g** and **14g**, respectively. Reaction of **13a** or **14a** with dimethyl sulfate resulted in the formation of their 7-methyl derivatives, **15a** and **16a**.

5,6-Methylenedioxy-2-tetralone (**11e**) was used as the requisite intermediate for the preparation of 5,6-dihydro-9,10-dimethoxy-3,4-methylenedioxybenz[*a*]acridine, **13e**. This tetralone was prepared in six steps as illustrated in Scheme 3. 2,3-Methylenedioxybenzaldehyde was condensed with malonic acid as previously described[32] to give 2,3-methylenedioxycinnamic acid. 2,3-Methylenedioxycinnamic acid was hydrogenated using 10% Pd/C to give the dihydrocinnamic acid derivative, which was then transformed into its ethyl ester, **17**, as previously described.[33] Ethyl-2,3-methylenedioxydihydrocinnamate was then converted to its β-ketosulfoxide, **18**.[34] The β-ketosulfoxide derivative, **18**, when subjected to Pummerer rearrangement by treatment with trifluoroacetic acid, provided 1,2,3,4-tetrahydro-1-methylthio-5,6-methylenedioxy-2(1*H*)-napthalenone, **19**.[35] Hydrogenolysis of **19** using 10% Pd/C in glacial acetic acid gave **11e**.[36]

## Results and Discussion

The relative activity of coralyne (**1**), nitidine (**2**), MDD-coralyne (**3**), the benz[*c*]acridine analogues (**6–9**) and the benz[*a*]acridine derivatives (**13–16**) was determined in a subcellular assay measuring enzyme–DNA cleavable complex formation in the presence of topo I and topo II. In contrast to nitidine and a few select protoberberine derivatives,[13,19] none of the benzacridines synthesized as part of this study was active as a topo II poison.

5,6-Dihydro-2,3,9,10-tetramethoxybenz[*c*]acridine (**6**) and 2,3,9,10-tetramethoxybenz[*c*]acridine (**7**), which have within their A-ring similar methoxyl substitution

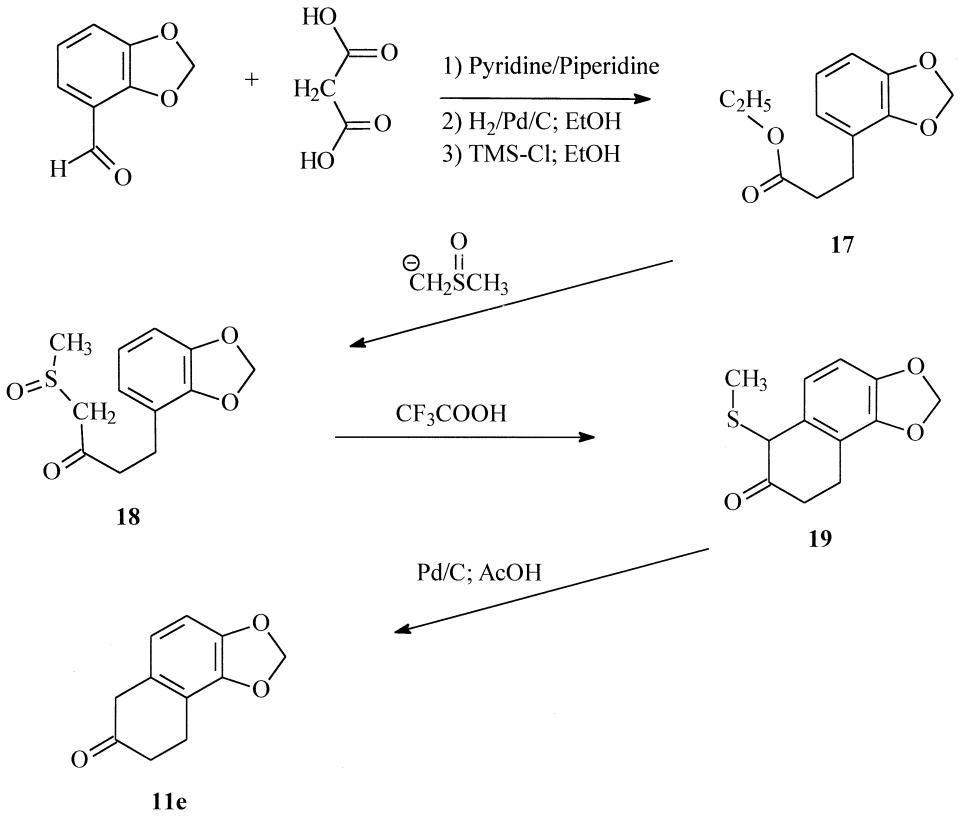



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **a** | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | H |
| **b** | $-OCH_2O-$ | | $OCH_3$ | $OCH_3$ | H |
| **c** | $OCH_3$ | $OCH_3$ | H | H | H |
| **d** | H | H | $OCH_3$ | $OCH_3$ | H |
| **e** | $OCH_3$ | $OCH_3$ | H | $-OCH_2O-$ | |
| **f** | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | $OCH_3$ |
| **g** | OH | OH | OH | OH | H |

**Scheme 2.**

to coralyne, were devoid of any significant activity as topo I poisons. The *N*-methyl quaternary ammonium derivatives of these benz[*c*]acridines, **8** and **9**, were also inactive. These data clearly indicated that relocation of the ring nitrogen of coralyne to its 12 position as in the case of these benz[*c*]acridine derivatives results in a loss of topoisomerase poisoning activity.

Benz[*a*]acridines with similar methoxyl substitution to coralyne within the A-ring such as 5,6-dihydro-2,3,9,10-tetramethoxybenz[*a*]acridine (**13a**) and 2,3,9,10-tetramethoxybenz[*a*]acridine (**14a**) were synthesized and evaluated as topo I poisons (Table 1). While **14a** was inactive as a topo I poison, **13a** did exhibit modest activity. These results parallel the structure–activity relationships observed for 8-desmethyl protoberberine analogues wherein the 5,6-dihydro derivative of an active compound exhibited significantly enhanced potency as topo I poisons. In the case of the benz[*a*]-acridines, saturation at the 5,6-postion was a structural




**Scheme 3.**

**Table 1.** Topo I and topo II mediated DNA cleavage of coralyne derivatives and related compounds

| Compound | Topoisomerase I mediated | Cytotoxicity $IC_{50}$[a] (μM) cell lines | |
|---|---|---|---|
| | DNA cleavage[b] | RPMI | CPT-K5 |
| **1** | 1.0 | 4.9 | 20 |
| **2** | 0.1 | 0.4 | 3.9 |
| **3** | 0.1 | 8.1 | 27 |
| **13a** | 100 | 7.1 | 7.1 |
| **13b** | 100 | 9.0 | 14.9 |
| **13c** | —[c] | 6.9 | 6.9 |
| **13d** | 100 | 1.0 | 8.6 |
| **13e** | 1.0 | 3.0 | 22.4 |
| **13f** | 100 | 14.2 | 14.2 |
| **13g** | 1.0 | 20.3 | 13.6 |
| **15a** | 100 | > 24.2 | > 24.2 |
| **CPT** | 0.1 | 0.004 | > 10 |

[a]$IC_{50}$ has been calculated after 4 days of continuous drug exposure.
[b]Topo I cleavage values are reported as REC (relative effective concentration), i.e. concentrations relative to coralyne, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topo I.
[c]Compound **13c** failed to increase cleavage of the plasmid DNA in the presence of topo I at the concentration range used in this study.

requirement for topo I poisoning activity within this series of compounds. None of the fully unsaturated benz[*a*]acridines evaluated in this study (**14a–d**, **14g**) possessed activity as a topo I poison. In the case of protoberberines derivatives, saturation within the B-ring was associated with an enhancement of topo I poisoning activity.[13,17] While counterintuitive, studies on the DNA binding affinity demonstrated that for 5,6-dihydro protoberberine analogues without a 8-methyl substituents, such as 5,6-dihydro-8-desmethylcoralyne, there was an increase in relative DNA binding affinity relative to the unsaturated analogue.[37] Thus, it is likely that in the case of 5,6-dihydrobenz[*a*]acridines that this increase in DNA binding affinity may be essential for stabilization of the cleaved ternary complex involving DNA, enzyme, and drug. In recent studies we have also shown that substituents on the A-ring of 5,6-saturated protoberberine analogues are engaged in interactions (e.g., hydrogen bonding) with topo I in the ternary drug-DNA–enzyme complex.[37] It is reasonable to suggest that saturation of the 5,6-bond in benz[*a*]acridine may properly position the substituents on the A-ring for these types of drug-enzyme interactions.

Modification of these benz[*a*]acridines by replacing the 9,10-methoxy groups with a 9,10-methylenedioxy substituent provided consistent results where only the 5,6-dihydro derivative, **13b**, was active as a topo I poison. The fully unsaturated analogue, **14b**, was inactive. Replacement of the methoxy groups at positions 2 and 3 of these 5,6-dihydrobenz[*a*]acridines with hydrogen atoms, as in the case of **13c**, resulted in a loss of topo I poisoning activity. The replacement of the methoxy substituents at the 9,10-positions with hydrogen atoms, as in the case of **13d**, did not diminish the activity of this dihydrobenz[*a*]acridine relative to **13a** as a topo I poison.

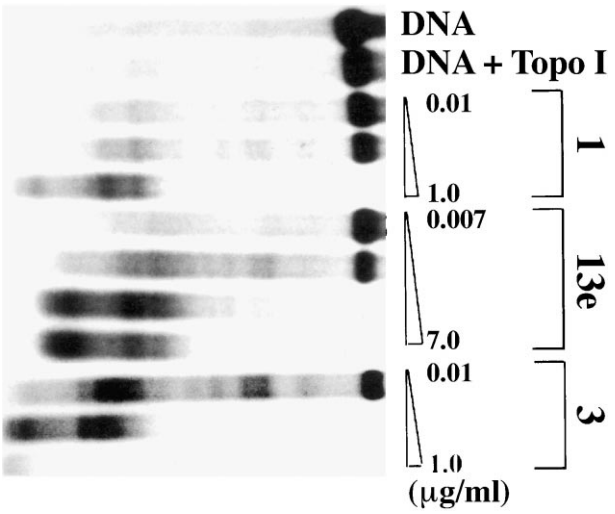



**Figure 1.** Stimulation of enzyme-mediated DNA cleavage by coralyne analogues using human topo I. The top lane is the DNA control without topo I. The second lane from the top is the control with topo I alone. The rest of the lanes are with topo I and serially (10-fold each) diluted coralyne (**1**), MDD-coralyne (**3**), and 5,6-dihydro-3,4-methylenedioxy-9,10-dimethoxybenz[*a*]acridine (**13e**).

The structure–activity relationship observed among the various benz[*a*]acridines did mirror that observed with coralyne and various protoberberine derivatives with regard to their topo I poisoning activity.[11,13] Structure–activity relationships associated with a series of coralyne derivatives, demonstrated that substitution of the A-ring with a 3,4-methylenedioxy substituent was associated with potent topo I poisoning activity.[13] In view of these data, we prepared 5,6-dihydro-3,4-methylenedioxy-9,10-dimethoxybenz[*a*]acridine, **13e**. Consistent with the structure–activity results previously observed with coralyne derivatives, **13e** was the most potent topo I poison within this series of benz[*a*]acridines, exhibiting comparable activity to coralyne (Fig. 1). Previous studies with coralyne derivatives also demonstrated that the presence of 3,4-dimethoxy substituents on the A-ring diminished activity. Consistent with these results, 3,4,9,10-tetramethoxybenz[*a*]acridine, **13f**, had only 1% of the relative potency of **13e** as a topo I poison. Among the protoberberine analogues, it had been observed that demethylation of berberine resulted in an analogue with enhanced activity as a topo I poison.[11] In the case of **13a**, conversion of this analogue to its tetrahydroxy derivative, **13g**, did result in an increase in topo I poisoning activity. Methylation of **13a** to form the *N*-methyl derivative **15a** neither enhanced nor diminished topo I poisoning activity.

A clear correlation between potency as a topo I poison and cytotoxicity among coralyne derivatives was not observed in previous studies. Several factors could be responsible for this observation. Some coralyne analogues are also topo II poisons or dual topoisomerase poisons. In addition, coralyne analogues possess an iminium moiety. Cellular absorption could be viewed as a limiting factor associated with the cytotoxicity of various analogues where only passive diffusion is involved. In certain human tumor cell lines, however, an active transport mechanism for charged compounds related to coralyne appears to be operational.[38]

Several of the benz[*a*]acridine analogues evaluated in this study are noncharged compounds for which enhanced passive diffusion into cells would be expected. In addition, none of these benz[*a*]acridine derivatives are topo II poisons. Nonetheless, the relative cytotoxicity of the various benz[*a*]acridines did not correlate well with their relative potency as topo I poisons. In several instances, such as with **13d** and **13e**, differences in topo I poisoning activity were not reflected in their relative cytotoxic activity. These data suggest that among benz[*a*]acridines there may be factors, in addition to topo I poisoning, that influence cytotoxic activity. In certain instances, differences in physicochemical properties can explain some of the cytotoxicity data. The low cytotoxicity observed for both **13g**, which is a highly polar compound, and **16a**, which contains the iminium moiety, could be associated with their decreased ability to passively diffuse into cells.

In summary, the primary objective of this study was to determine whether the presence of an iminium cation was critical for retaining topoisomerase poisoning activity. In this investigation, two series of potential noncharged bioisosteres structurally related to coralyne and protoberberine alkaloids were investigated. Benz[*a*]acridine and benz[*c*]acridine derivatives were selected for initial evaluation in view of their stability and the absence of any potential for these heterocycles to form an iminium cation by autooxidation. While benz[*c*]acridine derivatives appear unsuitable as bioisosteres, several dihydrobenz[*a*]acridines exhibited activity as topo I poisons. These benz[*a*]acridine derivatives represent a new series of topo I poisons. These data support further research into the development of dihydrobenz[*a*]acridines and related heterocycles, such as benzo[i]phenanthridines, as antitumor agents. Such investigations, using the structure–activity data derived from coralyne and protoberberine derivatives could provide a unique class of topo I poisons that may exhibit a different toxicity profile and an improved potential for efficacy in vivo.

## Experimental

Melting points were determined with a Thomas-Hoover Unimelt capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm (ICN Biomedicals, Eschwegge, Germany), using the solvent systems indicated. Infrared spectral data (IR) were obtained on a Perkin–Elmer 1600 Fourier transform spectrophotometer and are reported in $cm^{-1}$. Proton ($^1H$ NMR) and carbon ($^{13}C$ NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz $^1H$ and 50 MHz $^{13}C$) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are

reported in hertz (Hz). Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO. The purity of all compounds for which HRMS data are provided was determined by analytical reverse-phase HPLC. Compounds were analyzed using both of the following conditions (method A) a Vydac C-18 column (The Separations Group) using methanol:$H_2O$ (80:20) with a flow rate of 1 mL/min; (method B) a Microsorb C-8 column (Rainin Instrument Co., Inc.) using methanol:0.1 M potassium phosphate buffer (pH 7.0) (95:5) with a flow rate of 1 mL/min. HPLC analyses were performed with a Hewlett-Packard 1090 liquid chromatograph equipped with a diode array UV detection monitoring at 254 and 335 nm. The percent purity of these compounds were calculated from the peak area assuming that the extinction coefficient of the compound of interest and the impurity are the same. On the basis of these analyses, all the compounds were found to be 98.0–99% pure in these systems. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within $\pm 0.4\%$ of the theoretical value. Compounds **4** and **5** were purchased from Aldrich Chemical Company (Milwaukee, WI). 5,6-Dimethoxy-2-tetralone, **11f**, was synthesized from 1,6-dibromo-2-hydroxynapthalene.[39]

**5,6-Dihydro-2,3,9,10-tetramethoxy-7-methylbenz[*c*]acridine (6).** 2-Amino-4,5-dimethoxyacetophenone (1.0 g, 5.1 mmol) was dissolved in 10 mL $CH_2Cl_2$ and hydrogen chloride (1.0 M solution, in anhydrous ether) was added with vigorous stirring at room temperature. The hydrochloride salt of the aminoacetophenone precipitated out. The solvent was removed in vacuo and the solid residue obtained dried for 1 h under vacuum. The dry hydrochloride salt was triturated with 6,7-dimethoxy-1-tetralone (1.59 g, 7.68 mmol) and the mixture was transferred into a sealed tube and heated at 140 °C for 1 h. The resulting fused plug was dissolved in boiling methanol (300 mL). This solution was concentrated to 200 mL and left overnight providing needle-shaped crystals. These crystals were filtered and washed with three 5 mL portions of acetone, and dried to give golden yellow needles of the benz[*c*]acridine hydrochloride derivative in 99% yield. The hydrochloride salt was dissolved in 200 mL boiling methanol. After the solution had cooled to room temperature concentrated $NH_4OH$ was added dropwise until pH 10 was obtained. Light yellow crystals began to form. The suspension was then diluted with 200 mL water and extracted thrice with 100 mL portions of $CH_2Cl_2$. The combined extracts were washed once with 50 mL brine, dried using anhydrous $Na_2SO_4$, filtered, and the solvent removed in vacuo to give the free base; mp 240 °C; IR (Nujol): 2922, 1620; $^1$H NMR: δ 2.52 (3H, s), 2.86 (2H s), 3.02 (2H, t), 3.90 (3H, s), 3.98 (3H, s), 4.03 (3H, s), 4.05 (3H, s), 6.70 (1H, s), 7.08 (1H, s), 7.43 (1H, s), 8.04 (1H, s); $^{13}$C NMR: δ 16.5, 25.4, 28.0, 56.6, 56.7, 57.8, 58.3, 102.3, 102.7, 110.9, 111.8, 119.5, 123.2, 127.7, 134.7, 135.6, 146.3, 148.5, 149.7, 151.3, 153.5, 155.2; HRMS calcd for $C_{22}H_{23}NO_4$: 365.1630; found: 365.1628.

**5,6-Methylenedioxy-2-tetralone (11e).** The thioether intermediate, **19**, (0.67 g, 2.83 mM) was taken up in 10 mL glacial acetic acid in a hydrogenation flask. 0.46 g of 10% Pd-C was added and this mixture was shaken in a Parr apparatus at 40 psig of hydrogen for 40 h. The reaction mixture was filtered through a celite bed, which was washed thrice with 5 mL portions of glacial acetic acid. The glacial acetic acid was evaporated in vacuo to give the crude tetralone, **11e**. The crude tetralone was then treated with sodium bisulfite to convert it to the more stable bisulfite adduct. Pure tetralone was generated as required from its bisulfite adduct by treatment with 10% sodium carbonate solution followed by extraction with dichloromethane; mp 90–91 °C (lit.[36] 88–91 °C), IR (Nujol) 1715; $^1$H NMR: δ 2.50 (2H, t), 2.98 (2H, t), 3.50 (2H, s), 5.93 (2H, s), 6.56 (1H, d, $J=6$), 6.65 (1H, d, $J=6$); $^{13}$C NMR: δ 21.6, 37.9, 45.0, 101.5, 107.4, 118.5, 121.1, 127.8, 144.1, 146.2, 211.2. Anal. ($C_{11}H_{10}O_3$) C, H.

### General procedure for the synthesis of 1-(2′-nitrobenzylidene)-2-tetralone derivatives

A glacial acetic acid (10 mL) solution of 2.45 mmol each of the respective 2-tetralone, 2-nitrobenzaldehyde, and sodium acetate was refluxed for 3–8 h under a nitrogen atmosphere. The reaction mixture was then allowed to cool to room temperature. The mixture was carefully loaded on a silica gel (75 g) column and chromatographed using a 1:1 mixture of ethyl ether and hexanes. The yellow colored compound generally eluting fourth from the column was collected to provide the respective tetralone derivatives in 20–25% yield.

**1-(2′-Nitro-4′,5′-dimethoxybenzylidene)-6,7-dimethoxy-2-tetralone (12a).** Prepared from 6,7-dimethoxy-2-tetralone and 6-nitroveratraldehyde; mp 65–66 °C; IR (Nujol): 2855, 1720, 1540; $^1$H NMR: δ 2.71 (2H, t), 2.98 (2H t), 3.35 (3H, s), 3.66 (3H, s), 3.89 (3H, s), 3.97 (3H, s), 6.41 (1H, s), 6.62 (1H, s), 6.73 (1H, s), 7.74 (1H, s), 7.91 (1H, s); $^{13}$C NMR: δ 28.4, 39.7, 56.5, 56.6, 56.8, 56.9, 107.7, 108.6, 111.2, 113.1, 128.1, 129.3, 130.3, 132.2, 133.3, 148.9, 149.0, 150.0, 153.3, 200.7. Anal. ($C_{21}H_{21}NO_7$) C, H, N.

**1-(2′-Nitro-4′,5′-methylenedioxybenzylidene)-6,7-dimethoxy-2-tetralone (12b).** Prepared from 6,7-dimethoxy-2-tetralone and 6-nitropiperonal; mp 76–77 °C; IR (Nujol): 2875, 1723, 1553; $^1$H NMR: δ 2.68 (2H, t), 3.00 (2H, t), 3.41 (3H, s), 3.90 (3H, s), 6.08 (2H, s), 6.37 (1H, s), 6.51 (1H, s), 6.73 (1H, s), 7.68 (1H, s), 7.83 (1H, s); $^{13}$C NMR: δ 28.0, 38.0, 56.1, 56.4, 103.7, 105.9, 110.1, 111.3, 112.6, 124.3, 130.8, 131.1, 132.2, 133.8, 147.6, 148.4, 149.7, 152.5. Anal. ($C_{20}H_{17}NO_7$) C, H, N.

**1-(2′-Nitrobenzylidene)-6,7-dimethoxy-2-tetralone (12c).** Prepared from 6,7-dimethoxy-2-tetralone and 2-nitrobenzaldehyde; mp 63–64 °C; IR (Nujol): 1712, 1540; $^1$H NMR: δ 2.69 (2H, t), 3.01 (2H, t), 3.25 (3H, s), 3.88 (3H, s), 6.26 (1H, s), 6.72 (1H, s), 7.25–7.26 (1H, m), 7.44–7.50 (2H, m), 7.87 (1H, s), 8.12–8.17 (1H, m); $^{13}$C NMR: δ 28.0, 37.9, 55.7, 56.3, 108.6, 111.3, 112.5, 124.2,

124.9, 125.4, 129.3, 130.1, 131.8, 133.5, 133.9, 134.7, 147.5, 149.6, 200.7. Anal. ($C_{19}H_{17}NO_5$) C, H, N.

**1-(2′-Nitro-4′,5′-dimethoxybenzylidene)-2-tetralone (12d).** Prepared from 2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 58–60 °C; IR (Nujol): 1724, 1540; $^1$H NMR: δ 2.71 (2H, t), 3.08 (2H, t), 3.56 (3H, s), 4.02 (3H, s), 6.51 (1H, s), 6.92–6.96 (2H, m), 7.17–7.24 (2H, m), 7.73 (1H, s), 8.00 (1H, s); $^{13}$C NMR: δ 28.3, 27.8, 56.7, 56.9, 108.3, 112.5, 126.7, 127.6, 128.5, 128.8, 129.9, 132.3, 133.4, 134.5, 138.8, 141.6, 149.4, 153.5. Anal. ($C_{19}H_{17}NO_5$) C, H, N.

**1-(2′-Nitro-4′,5′-dimethoxybenzylidene)-5,6-methylenedioxy-2-tetralone (12e).** Prepared from 5,6-methylendioxy-2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 66–68 °C; IR (Nujol): 1710, 1553; $^1$H NMR: δ 2.98 (2H, t), 3.19 (2H, t), 4.02 (3H, s), 4.03 (3H, s), 6.03 (2H, s), 6.82 (1H, d, $J$ = 8.1), 7.07 (1H, s), 7.29 (1H, d, $J$ = 8.1), 7.49 (1H, s), 8.20 (1H, s); $^{13}$C NMR: δ 21.8, 30.8, 56.5, 56.7, 101.8, 105.7, 107.6, 117.9, 119.1, 123.9, 125.9, 126.9, 128.7, 143.4, 145.4, 147.7, 150.1, 152.9, 156.1, 176.4. Anal. ($C_{20}H_{17}NO_7$) C, H, N.

**1-(2′Nitro-4′,5′-dimethoxybenzylidene)-5,6-dimethoxy-2-tetralone (12f).** Prepared from 5,6-dimethoxy-2-tetralone and 2-nitro-4,5-dimethoxybenzaldehyde; mp 70–72 °C; IR (Nujol): 1715, 1550; $^1$H NMR: δ 2.59 (2H, t), 3.06 (2H, t), 3.78 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 3.92 (3H, s), 6.47 (1H, s), 6.77 (1H, d, $J$ = 8.2), 7.41 (1H, d, $J$ = 8.2), 7.67 (1H, s); $^{13}$C NMR: δ 19.7, 32.0, 56.3, 56.7, 56.8, 56.85, 108.1, 121.9, 125.6, 126.1, 128.0, 129.2, 132.1, 132.9, 133.7, 140.3, 141.4, 149.3, 152.9, 153.5, 200.9. Anal. ($C_{21}H_{21}NO_7$) C, H, N.

### General procedure for the synthesis of 5,6-dihydrobenz[*a*]acridine derivatives

The respective 1-(2′-nitrobenzylidene)-2-tetralone derivative (0.3 mmol) was dissolved in 10 mL glacial acetic acid and refluxed with zinc dust (1.64 mmol) under a nitrogen atmosphere for 1–4 h. The reaction mixture was allowed to cool to room temperature and then the entire mixture was loaded carefully on silica gel (100 g) column and chromatographed first with 500 mL of ethyl ether to remove acetic acid followed by elution with a 1:1 mixture of hexanes and ethyl acetate. The polarity of the mobile phase was reduced, if necessary, by mixing suitable proportions of hexanes. The relevant fractions were pooled and concentrated in vacuo to yield 83–95% of corresponding 5,6-dihydrobenz[*a*]acridines.

**5,6-Dihydro-2,3,9,10-tetramethoxybenz[*a*]acridine (13a).** Prepared from 1-(2′-nitro-4′,5′-dimethoxybenzylidene)-6,7-dimethoxy-2-tetralone; mp 182–183 °C; IR (Nujol): 3210, 1615; $^1$H NMR: δ 2.99 (2H, t), 3.23 (2H, t), 3.94 (3H, s), 4.00 (3H, s), 4.02 (3H, s), 4.04 (3H, s), 6.79 (1H, s), 7.10 (1H, s), 7.31 (1H, s), 7.54 (1H, s), 8.17 (1H, s); $^{13}$C NMR: δ 28.8, 32.3, 56.5, 56.7, 105.7, 107.2, 107.5, 111.8, 123.9, 125.8, 127.1, 128.0, 130.5, 143.1, 148.8, 149.6, 152.8, 156.7, 176.7; HRMS calcd for $C_{21}H_{21}NO_4$: 351.1471; found: 351.1475.

**5,6-Dihydro-2,3,-dimethoxy-9,10-methylenedioxybenz-[*a*]acridine (13b).** Prepared from 1-(2′-nitro-4′,5′-methylenedioxybenzylidene)-6,7-dimethoxy-2-tetralone; mp 218–219 °C; IR (Nujol): 2780, 1630; $^1$H NMR: δ 2.97 (2H, t), 3.17 (2H, t), 3.93 (3H, s), 4.00 (3H, s), 6.07 (2H, s), 6.79 (1H, s), 7.07 (1H, s), 7.29 (1H, s), 7.32 (1H, s), 8.07 (1H, s); $^{13}$C NMR: δ 29.0, 33.1, 56.5, 56.7, 102.1, 103.3, 105.7, 107.6, 111.8, 125.2, 125.9, 127.0, 128.0, 130.6, 145.0, 147.9, 148.8, 149.6, 150.8, 156.9; HRMS calcd for $C_{20}H_{17}NO_4$: 335.1158; found: 335.1162.

**5,6-Dihydro-2,3-dimethoxybenz[*a*]acridine (13c).** Prepared from 1-(2′-nitrobenzylidene)-6,7-dimethoxy-2-tetralone; mp 55–56 °C; IR (Nujol): 2815, 1615; $^1$H NMR: δ 2.96 (2H, t), 3.23 (2H, t), 3.90 (3H, s), 3.98 (3H, s), 6.75 (1H, s), 7.30 (1H, s), 7.44 (1H, t), 7.60 (1H, t), 7.80 (1H, d, $J$ = 10.2), 8.0 (1H, d, $J$ = 10.2), 8.19 (1H, s); $^{13}$C NMR: δ 27.3, 28.9, 56.5, 56.6, 106.2, 110.7, 125.2, 125.5, 127.6, 127.8, 131.2, 132.4, 133.7, 133.9, 134.2, 136.3, 151.3, 153.6, 157.8; HRMS calcd for $C_{19}H_{17}NO_2$: 291.1259; found: 291.1246.

**5,6-Dihydro-9,10-dimethoxybenz[*a*]acridine (13d).** Prepared from 1-(2′-nitro-4′,5′-dimethoxybenzylidene)-2-tetralone; mp 95–96 °C; IR (Nujol): 1633, 1516; $^1$H NMR: δ 3.06 (2H, t), 3.24 (2H, t), 4.02 (3H, s), 4.03 (3H, s), 7.10 (1H, s), 7.28–7.37 (3H, m), 7.50 (1H, s), 7.82 (1H, d, $J$ = 7.0), 8.29 (1H, s); $^{13}$C NMR: δ 29.2, 32.3, 56.5, 56.7, 105.9, 107.4, 124.0, 124.3, 127.3, 127.7, 128.5, 128.9, 129.1, 133.8, 137.6, 144.2, 150.5, 153.1; HRMS calcd for $C_{19}H_{17}NO_2$: 291.1259; found: 291.1250.

**5,6-Dihydro-9,10-dimethoxy-3,4-methylenedioxybenz[*a*]-acridine (13e).** Prepared from 1-(2′-nitro-4′,5′-dimethoxybenzylidine)-5,6-methylenedioxy-2-tetralone; mp 220–222 °C; IR (Nujol): 1715, 1532; $^1$H NMR: δ 3.02 (2H, t), 3.19 (2H, t), 4.02 (3H, s), 4.03 (3H, s), 6.03 (2H, s), 6.83 (1H, d, $J$ = 8.1), 7.07 (1H, s), 7.34–7.38 (2H, m), 8.19 (1H, s); $^{13}$C NMR: δ 30.2, 32.3, 56.5, 56.6, 101.8, 105.8, 107.5, 107.8, 117.9, 119.2, 123.8, 126.9, 128.3, 128.7, 143.2, 145.4, 147.6, 149.9, 152.7, 156.3; Anal ($C_{20}H_{17}NO_4$) C, H, N.

**5,6-Dihydro-3,4,9,10-tetramethoxybenz[*a*]acridine (13f).** Prepared from 1-(2′-nitro-4′,5′-dimethoxybenzylidine)-5,6-dimethoxy-2-tetralone; mp 195–196 °C; IR (Nujol): 1730, 1515; $^1$H NMR: δ 2.82 (2H, t), 3.20 (2H, t), 3.86 (3H, s), 3.91 (3H, s), 4.03 (3H, s), 4.04 (3H, s), 6.93 (1H, d, $J$ = 8.6), 7.10 (1H, s), 7.52 (1H, s), 7.56 (1H, d, $J$ = 8.6), 8.27 (1H, s); $^{13}$C NMR: δ 21.8, 31.6, 56.4, 56.6, 56.8, 61.1, 105.8, 106.4, 106.5, 107.6, 111.4, 111.9, 120.4, 124.1, 127.1, 129.3, 131.6, 150.3, 150.4, 153.4, 156.3. Anal. ($C_{21}H_{21}NO_4$) C, H, N.

### General procedure for the synthesis of benz[*a*]acridines and benz[*c*]acridines from their 5,6-dihydro derivatives

The respective 5,6-dihydrobenz[*a*]acridine or 5,6-dihydrobenz[*c*]acridine derivatives (0.22 mmol) were refluxed in 15 mL decalin with 76 mg of 10% palladium on carbon under nitrogen atmosphere for 2–9 h. The reaction mixture was then quickly suction filtered while

hot through a celite bed using a sintered glass funnel. The filter bed was washed thoroughly thrice using 20 mL portions of boiling chloroform followed by two 20 mL portions of boiling ethyl acetate. The combined filtrate was then concentrated in vacuo and dried under vacuum to give the respective benz[*a*]acridine derivatives.

**2,3,9,10-Tetramethoxy-7-methylbenz[*c*]acridine (7).** Prepared from **4**; mp >250 °C; IR (Nujol): 3520, 1633, 1610; $^1$H NMR: δ 2.87 (3H, s), 4.03 (3H, s), 4.04 (3H, s), 4.13 (3H, s), 4.23 (3H, s), 7.17 (1H, s), 7.20 (1H, s), 7.52 (1H, d, $J$=9.2), 7.59 (1H, s), 7.80 (1H, d, $J$=9.2), 8.87 (1H, s); $^{13}$C NMR: δ 14.4, 56.4, 56.7, 101.7, 106.0, 108.2, 108.3, 120.8, 122.1, 122.4, 125.5, 125.8, 126.9, 128.6, 135.7, 139.0, 145.2, 149.8, 150.9, 153.3; HRMS calcd for $C_{22}H_{21}NO_4$: 363.1470; found: 363.1472.

**2,3,9,10-Tetramethoxybenz[*a*]acridine (14a).** Prepared from **13a**; mp 247–249 °C; IR (Nujol): 2810, 1630; $^1$H NMR: δ 3.99 (3H, s), 4.01 (3H, s), 4.03 (3H, s), 4.08 (3H, s), 7.08 (1H, s), 7.12 (1H, s), 7.41 (1H, s), 7.74 (1H, d, $J$=9.3), 7.81 (2H, m), 8.82 (1H, s); $^{13}$C NMR: δ 56.3, 56.4, 56.5, 56.6, 103.9, 104.8, 106.8, 109.2, 122.8, 123.0, 124.5, 126.2, 126.4, 127.7, 130.7, 145.7, 147.2, 149.8, 149.9, 150.3, 153.9; HRMS calcd for $C_{21}H_{19}NO_4$: 349.1314; found: 349.1314.

**2,3-Dimethoxy-9,10-methylenedioxybenz[*a*]acridine (14b).** Prepared from **13b**; mp 245–246 °C; IR (Nujol): 2790, 1630; $^1$H NMR: δ 4.06 (3H, s), 4.16 (3H, s), 6.15 (2H, s), 7.25 (2H, s), 7.48 (1H, s), 7.84 (1H, d, $J$=8.2), 7.99 (1H, s), 8.99 (1H, s); $^{13}$C NMR: δ 56.5, 56.6, 102.3, 102.4, 104.1, 104.9, 109.4, 112.8, 122.9, 124.4, 126.5, 126.7, 128.5, 130.9, 135.7, 147.0, 147.4, 148.4, 150.1, 152.0; HRMS calcd for $C_{20}H_{15}NO_4$ calcd: 333.1002; found: 333.1004.

**2,3-Dimethoxybenz[*a*]acridine (14c).** Prepared from **13c**; mp 190–192 °C; IR (Nujol): 2881, 1632; $^1$H NMR: δ 4.02 (3H, s), 4.13 (3H, s), 7.18 (1H, s), 7.51–7.59 (1H, m), 7.72–7.81 (1H, m), 7.83 (1H, s), 7.89 (1H, s), 7.93–8.22 (2H, m), 8.23 (1H, d, $J$=8.5), 9.13 (1H, s); $^{13}$C NMR: δ 56.5, 56.6, 104.4, 109.5, 124.2, 124.5, 126.3, 126.4, 126.8, 126.9, 128.5, 129.4, 130.0, 130.1, 130.2, 132.3, 148.1, 149.2, 150.1, 150.2; HRMS calcd for $C_{19}H_{17}NO_2$: 289.1104; found: 289.1099.

**9,10-Dimethoxybenz[*a*]acridine (14d).** Prepared from **13d**; mp 181–182 °C; IR (Nujol): 2883, 1621; $^1$H NMR: δ 4.09 (6H, s), 7.26 (1H, s), 7.54 (1H, s), 7.60–7.71 (2H, m), 7.89–7.96 (3H, m), 8.69 (1H, d, $J$=8.1), 9.23 (1H, s); $^{13}$C NMR: δ 56.6, 56.7, 105.1, 107.1, 122.9, 123.3, 123.4, 125.5, 127.6, 128.5, 128.7, 129.3, 130.4, 131.6, 135.7, 146.4, 148.0, 150.6, 154.3; HRMS calcd for $C_{19}H_{17}NO_2$: 289.1100; found: 289.1104.

### General procedure for the synthesis of 5,6-dihydro-2,3,9,10-tetrahydroxybenz[*a*]acridine (13g) and 2,3,9,10-tetrahydroxybenz[*a*]acridine (14g)

The respective benz[*a*]acridine derivatives (0.195 mmol) were dissolved in 2 mL $CH_2Cl_2$ and the solution was chilled to −50 °C using a cooling bath of isopropanol and dry ice. 1.95 mmols of boron tribromide (1.0 M) solution in $CH_2Cl_2$ was added under a nitrogen atmosphere. The reaction mixture was stirred at −50 °C for 1 h and then slowly allowed to come to room temperature over a period of 4 h. The reaction mixture was then cooled to −10 °C and was quenched by addition of 5 mL saturated ammonium chloride solution. The resulting solution was evaporated to dryness and the residue obtained was extracted thrice with 20 mL portions of boiling acetone. The resulting yellow suspensions were filtered each time. The undissolved precipitate was dissolved in 5 mL boiling methanol and set aside overnight. Needle shaped crystals of the respective tetrahydroxybenz[*a*]acridines were formed in 95% yield.

**5,6-Dihydro-2,3,9,10-tetrahydroxybenz[*a*]acridine (13g).** Prepared from **13a**; mp >220 °C; IR (Nujol): 3361, 3164, 2719, 1620; $^1$H NMR ($CD_3OD$): δ 2.97 (2H, t), 3.35 (2H, t), 6.77 (1H, s), 7.45 (3H, m), 8.83 (1H, s); $^{13}$C NMR ($CD_3OD$): δ 27.4, 29.2, 102.9, 110.9, 112.5, 116.6, 122.9, 126.5, 128.5, 129.4, 134.8, 135.6, 146.6, 148.6, 151.1, 152.9, 156.0; HRMS calcd for $C_{17}H_{13}NO_4$: 295.0845; found: 295.0842.

**2,3,9,10-Tetrahydroxybenz[*a*]acridine (14g).** Prepared from **14a**; mp >270 °C; IR (KBr): 3361, 3164, 1620, 1516; $^1$H NMR ($CD_3OD$): δ 7.26 (1H, s), 7.37 (1H, s), 7.56 (1H, s), 7.61 (1H, d, $J$=9.2), 8.05 (2H, m), 9.61 (1H, s); $^{13}$C NMR ($CD_3OD$): δ 101.1, 108.7, 110.3, 114.3, 114.7, 122.9, 124.4, 124.8, 126.7, 137.6, 137.8, 138.3, 139.0, 149.4, 150.7, 150.8, 159.3; HRMS calcd for $C_{17}H_{11}NO_4$: 293.0688; found: 293.0685.

### General procedure for *N*-methylation of benz[*a*]acridines and benz[*c*]acridines

Dimethyl sulfate (4 mL) was added to 0.27 mmol of the respective benz[*a*]acridine or benz[*c*]acridine and the mixture heated under a nitrogen atmosphere in an oil bath at 150 °C for between 20 min and 5 h. Anhydrous ethyl ether (10 mL) was added to the reaction mixture with vigorous stirring after it had cooled to room temperature. The precipitated quaternary salt was suction filtered and washed thrice with 10 mL portions of anhydrous ethyl ether and dried. The quaternary salts were crystallized from boiling methanol in 90% yield.

**5,6-Dihydro-7,12-dimethyl-2,3,9,10-tetramethoxybenz[*c*]-acridinium methosulfate (8).** Prepared from **6**; mp >250 °C; IR (Nujol): 3510, 1645, 1613; $^1$H NMR ($CD_3OD$): δ 2.90 (3H, s), 2.96 (2H, t), 3.08 (2H, t), 3.93 (3H, s), 4.00 (3H, s), 4.10 (3H, s), 4.18 (3H, s), 4.62 (3H, s), 7.17 (1H, s), 7.42 (1H, s), 7.61 (1H, s), 7.63 (1H, s); $^{13}$C NMR ($CD_3OD$): δ 16.6, 27.2, 29.0, 46.2, 57.0, 57.2, 57.3, 57.4, 100.7, 105.5, 112.5, 115.2, 121.2, 125.1, 133.4, 138.5, 139.7, 149.7, 150.5, 152.7, 152.9, 154.8, 157.4; HRMS calcd for $C_{23}H_{26}NO_4+$: 380.1858; found: 380.1856.

**7,12-Dimethyl-2,3,9,10-tetramethoxy-benz[*c*]acridinium methosulfate (9).** Prepared from **7**; mp >240 °C; IR (Nujol): 3495, 1640, 1615; $^1$H NMR (DMSO-$d_6$): δ 2.53

(3H, s), 3.39 (3H, s), 3.97 (3H, s), 4.03 (3H, s), 4.16 (3H, s), 4.17 (3H, s), 7.69 (1H, s), 7.75 (1H, s), 7.91 (1H, s), 7.99 (1H, d, $J=9.5$), 8.24 (1H, d, $J=9.5$); $^{13}$C (DMSO-$d_6$) NMR: δ 15.5, 55.4, 56.4, 56.5, 56.6, 56.7, 102.1, 106.3, 110.3, 111.2, 125.8, 123.2, 122.5, 128.8, 129.0, 130.9, 133.3, 139.0, 141.1, 147.6, 152.1, 153.8, 155.3; HRMS calcd for $C_{23}H_{24}NO_4+$: 378.1698; found: 378.1695.

**5,6-Dihydro-2,3,9,10-tetramethoxy-7-methylbenz[*a*]acridinium methosulfate (15a).** Prepared from **13a**; mp >250 °C; IR (Nujol): 3490, 1620; $^1$H NMR (DMSO-$d_6$): δ 3.06 (2H, t), 3.37 (5H, t), 3.85 (3H, s), 3.93 (3H, s), 4.02 (3H, s), 4.05 (3H, s), 7.04 (1H, s), 7.45 (1H, s), 7.62 (1H, s), 7.63 (1H, s), 9.29 (1H, s); $^{13}$C NMR (DMSO-$d_6$): δ 25.9, 27.8, 53.1, 56.0, 56.1, 56.7, 56.8, 99.6, 106.6, 106.7, 107.9, 112.2, 112.3, 122.0, 124.4, 127.3, 129.1, 148.8, 150.3, 151.1, 153.0, 155.3; HRMS calcd for $C_{22}H_{24}NO_4+$: 366.1706; found: 366.1706.

**7-Methyl-2,3,9,10-tetramethoxybenz[*a*]acridinium methosulfate (16a).** Prepared from **14a**; mp >250 °C; IR (Nujol): 2820, 1620; $^1$H NMR (DMSO-$d_6$): δ 3.39 (3H, s), 3.96 (3H, s), 3.97 (3H, s), 4.03 (3H, s), 4.07 (3H, s), 7.59 (1H, s), 7.67 (1H, s), 7.85 (1H, d, $J=9.2$), 8.25 (1H, s), 8.31 (1H, d, $J=9.2$), 8.75 (1H, s), 10.18 (1H, s); $^{13}$C NMR (DMSO-$d_6$): δ 54.4, 56.8, 56.9, 57.5, 57.9, 105.7, 106.7, 107.9, 110.4, 123.2, 125.3, 125.4, 127.8, 127.9, 128.3, 131.4, 146.8, 149.3, 152.0, 158.5, 159.9; HRMS calcd for $C_{22}H_{22}NO_4+$: 364.1549; found: 364.1542.

**Ethyl-2,3-(methylenedioxy)dihydrocinnamate (17).** This intermediate was prepared in three steps (Scheme 3) from 2,3-(methylenedioxy)benzaldehyde. Malonic 6.93 g (67 mM) acid was placed in a 125 mL 3-neck flask fitted with a reflux condenser, into which a pyridine solution of 5.0 g (67 mM) of 2,3-(methylenedioxy)benzaldehyde was added. The reaction mixture was heated at 80–85 °C for 30 min and then the temperature was raised to 110–115 °C when it began to reflux. The reaction mixture was refluxed for 3 h. After cooling to room temperature the reaction mixture was poured into 150 mL cold water and slowly acidified by dropwise addition of concentrated hydrochloric acid to pH 1. The crystals obtained were collected by filtration under vacuum and washed thrice with 10 mL cold water and air-dried. The crystals were recrystallized from methanol and decolorized using charcoal to give a quantitative yield of the white crystalline cinnamic acid; mp 194–195 °C (lit.[36] 194–195 °C); $^1$H NMR: δ 6.08 (2H, s), 6.66 (1H, d, $J=16.1$), 6.84–6.97 (3H, m), 7.72 (1H, d, $J=16.1$); $^{13}$C NMR: δ 102.0, 110.7, 117.5, 120.2, 122.3, 123.2, 141.9, 147.3, 148.5, 171.9.

2,3-(Methylenedioxy)cinnamic acid 6.0 g (26 mM) was placed in a hydrogenation flask and dissolved in 80 mL absolute ethanol. 0.3 g of 10% Pd-C was added and this mixture was shaken in a Parr apparatus at 60 psig of hydrogen for 5 h. The reaction mixture was filtered through a celite bed to remove the catalyst and the clear filtrate was evaporated in vacuo to give a quantitative yield of the dihydrocinnamic acid. An analytical sample was crystallized from benzene to provide a white crystalline solid; mp 79–80 °C (lit.[36] 79–80 °C); $^1$H NMR: δ 2.69 (2H, t), 2.91 (2H, t,), 5.94 (2H, s), 6.68–6.79 (3H, m), 11.43 (1H, s); $^{13}$C NMR: δ 25.2, 34.2, 101.2, 107.5, 122.0, 122.1, 122.8, 146.0, 147.7, 179.9.

Trimethylsilyl chloride 2.5 mL (18.2 mM) was added to a solution of 1.5 g (7.73 mM) of 2,3-(methylenedioxy)-dihydrocinnamic acid in 70 mL dry ethanol and this mixture was stirred at room temperature under nitrogen for 12 h. The excess ethanol was evaporated in vacuo and the residue obtained was chromatographed on 100 g silica gel using 1:9 mixture respectively of ethyl acetate and hexanes to give a quantitative yield of the ester (**17**) as a colorless liquid; $^1$H NMR: δ 1.23 (3H, t), 2.63 (2H, t), 2.91 (2H, t), 4.14 (2H, q, $J_1=7.1$, $J_2=14.3$), 5.92 (2H, s), 6.64–6.79 (3H, m); $^{13}$C NMR: δ 14.7, 25.5, 34.4, 60.9, 101.1, 107.3, 122.0, 122.4, 122.8, 145.9, 147.6, 173.2.

**2,3-(Methylenedioxy)phenethyl methylsulfinylmethyl ketone (18).** The anion of dimethyl sulfoxide was prepared by adding 6.0 mL dry dimethyl sulfoxide to 0.6 g sodium hydride and heating the mixture at 70–75 °C for 45 min under nitrogen. This reaction mixture was allowed to cool to room temperature and then transferred to a water bath maintained at 5–10 °C. 1.0 g (4.54 mM) of **17** was separately dissolved in 6.0 mL dry dimethyl sulfoxide and this solution was added dropwise, over a period of 15 min, to the dimethyl sulfoxide anion generated previously. The reaction mixture was then slowly allowed to come to room temperature and stirred for 2 h. The reaction mixture was poured into 100 mL cold water and acidified to pH 3–4 using 1.2 N hydrochloric acid and extracted five times with 40 mL portions of chloroform. The combined organic layer was washed twice with 100 mL portions of distilled water, dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to give quantitative yield of **17** as a low melting buff colored solid. Compound **17** was found to be unstable to column chromatography using silica gel, however, $^1$H NMR of the crude compound indicated that it could be used directly in the next step without further purification; mp 62–63 °C; $^1$H NMR: δ 2.60 (3H, s), 2.79–3.01 (4H, m), 3.53–3.82 (2H, q, $J_1=13.8$, $J_2=34.3$), 5.89 (2H, s), 6.59–6.86 (3H, m); $^{13}$C NMR: δ 23.8, 39.4, 45.1, 64.4, 101.1, 107.4, 121.9, 122.0, 123.0, 145.8, 147.6, 201.9.

**1,2,3,4-Tetrahydro-1-methylthio-5,6-methylenedioxy-2(1*H*)-napthalenone (19).** Trifluoroacetic acid (0.3 mL, 3.7 mM) was dissolved in 25 mL benzene and added to the reaction flask containing 0.47 g (1.85 mM) of **18**. The reaction mixture was then heated to reflux for 1.5 h. On cooling to room temperature the reaction mixture was transferred to a separating funnel and washed twice using 10 mL portions of a saturated solution of sodium bicarbonate. The benzene layer was then dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to give a red syrup which was chromatographed over 100 g of silica gel using 1:9 mixture, respectively, of ethyl acetate and hexanes to give **19** in 60% yield; mp 56 °C; $^1$H NMR: δ 2.08 (3H, s), 2.78–3.18 (4H, m), 4.02 (1H, s), 5.89 (1H, s), 6.63 (1H, d, $J=8$), 6.72 (1H, d,

$J=8$); $^{13}C$ NMR: δ 16.5, 21.3, 34.6, 54.3, 101.7, 107.8, 118.8, 122.9, 127.9, 145.2, 147.3, 203.2.

### Topoisomerase mediated DNA cleavage assays

Human topoisomerase was isolated as a recombinant fusion protein using a T7 expression system (unpublished results). DNA topoisomerase I was isolated as a recombinant fusion protein using a T7 expression system (unpublished results). DNA topoisomerase II was purified from calf thymus gland as reported previously.[40] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[41] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[42] The cleavage assays were performed as previously reported.[8] Assessment of relative potency was performed by visual comparison of the extent of cleavage of DNA in the presence of enzyme and varying concentrations of drug. Topo I cleavage values are reported as REC (relative effective concentrations), i.e., concentrations relative to coralyne, whose value is arbitrarily assumed as 1. Potencies were determined at doses that were required to cause conversion of approximately 10% of the DNA substrate into the cleavage products.

### Cytotoxicity assay

The cytotoxicity was determined using the as MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[43–45] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Insitute, Nagoya, Japan).[46] The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% $CO_2$ and maintained by regular passage in RPMI medium supplemented with 10% heat inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of $IC_{50}$, cells were exposed continuously with varying concentrations of drug concentrations and MTT assays were performed at the end of the fourth day.

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