Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 6, pp 781–791, 1996

0968-0896/96 $15.00 + 0.00

S0968-0896(96)00054-5

Coralyne and Related Compounds as Mammalian Topoisomerase I and Topoisomerase II Poisons

Darshan Makhey,[a] Barbara Gatto,[b] 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 08855, U.S.A.*

[b]*Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08855, U.S.A.*

Abstract—DNA topoisomerases are nuclear enzymes responsible for modifying the topological state of DNA. The development of agents capable of poisoning topoisomerases has proved to be an attractive approach in the search for novel cancer chemotherapeutics. Coralyne, an antileukemic alkaloid, has appreciable structural similarity to the potent topoisomerase I and II poison, nitidine. Analogues of coralyne were synthesized and evaluated for their activity as topoisomerase I and topoisomerase II poisons. These analogues were also evaluated for cytotoxicity in the human lymphoblast cell line, RPMI 8402, and its camptothecin-resistant variant, CPT-K5. The pharmacological activity of these analogues exhibited a strong dependence on the substitution pattern and the nature of substituents. Several 1-benzylisoquinolines and 3-phenylisoquinolines were also synthesized. These compounds, which incorporate only a portion of the ring structure of coralyne, were evaluated as topoisomerase poisons and for cytotoxicity. These structure–activity studies indicate that the structural rigidity associated with the coralyne ring system may be critical for pharmacological activity. The presence of a 3,4-methylenedioxy substituent on these coralyne analogues was generally associated with enhanced activity as a topoisomerase poison. 5,6-Dihydro-3,4-methylenedioxy-10,11-dimethoxydibenzo[*a*,*g*]quinolizinium chloride was the most potent topoisomerase I poison among the coralyne analogues evaluated, having similar activity to camptothecin. This analogue also possessed exceptional potency as a topoisomerase II poison. Despite the pronounced activity of several of these coralyne derivatives as topoisomerase I poisons, none of these compounds had cytotoxic activity similar to camptothecin. Possible differences in cellular absorption between these coralyne analogs, which possess a quaternary ammonium group, and camptothecin may be responsible for the differences observed in their relative cytotoxicity. Copyright © 1996 Elsevier Science Ltd

Introduction

DNA topoisomerases represent a unique class of nuclear enzymes that alter the topological state of DNA by breaking and rejoining the phosphodiester backbone of DNA.[1–3] Mammalian topoisomerase I is capable of altering the topology of DNA by transiently breaking one DNA strand, while topoisomerase II acts by causing double-strand breaks. Topoisomerase poisoning has recently been recognized as an attractive pharmacological target for the development of novel cancer chemotherapeutic agents.[4–7] Several protoberberine alkaloids and their derivatives have been investigated as potential antitumor agents.[8–11] Berberine (**1**) represents one of the most intensively studied of the naturally-occurring protoberberine alkaloids and is reported to exhibit weak antitumor activity against P388 leukemia in mice.[11] Recent studies have indicated that their ability to act as mammalian topoisomerase I and/or II poisons may be linked to the antitumor activity of certain protoberberine alkaloids.[12] Minor alterations in structure were shown to have a significant effect on the relative activity of these analogues as topoisomerase poisons. Coralyne (**2a**) is a synthetic compound that is structurally similar to the protoberberine alkaloids (Fig. 1). Coralyne has more pronounced antitumor activity relative to berberine, exhibiting significant activity in vivo in mice against L1210 and P388 leukemias.[13,14] While the molecular basis for its antitumor activity has not been identified, it has been speculated that the ability to bind to duplex and triplex DNA may contribute to the observed antileukemic activity.[15,16] The antitumor activity of coralyne, coupled with its relatively low toxicity, prompted studies on the synthesis of a number of derivatives,[13] as well as a practical large-scale synthesis of coralyne itself.[17] Studies performed with coralyne and several of its derivatives suggest that the presence of the methyl substituent at the 8-position and unsaturation at the 5,6-position of coralyne are strongly associated with their antitumor activity against L1210 and P388 leukemias in mice.[13,14,18–21]

Fagaronine (**3**) and nitidine (**4**) represent two of the more potent antitumor agents within the family of benzo[*c*]phenanthridine alkaloids.[18–21] Both of these alkaloids have been shown to be potent inhibitors of mammalian topoisomerases.[21,22] The structural similarities between coralyne and either of these benzo[*c*]phenanthridinium salts, as previously noted by other

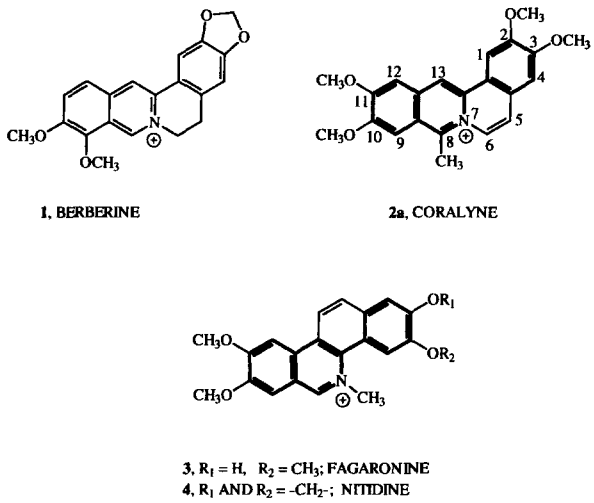
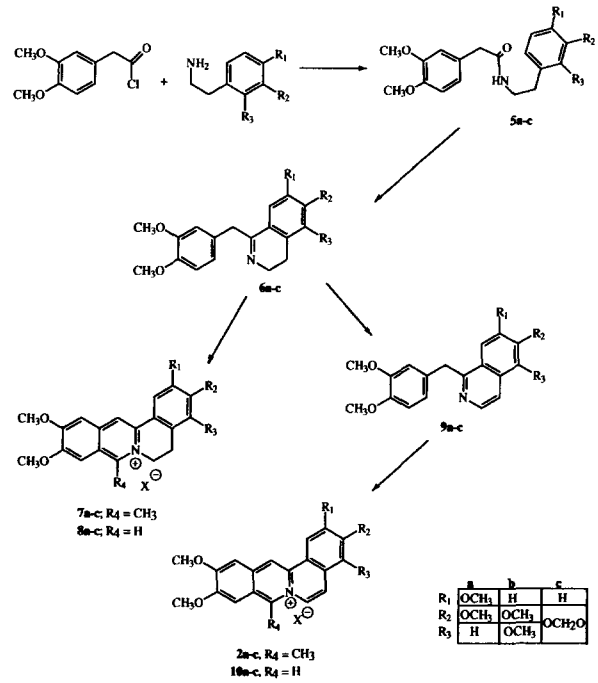


Figure 1. The protoberberine alkaloid, berberine (**1**), the structure and numbering of coralyne (**2a**) and the benzo[*c*]phenanthridine alkaloids, fagaronine (**3**) and nitidine (**4**). The structural features common to coralyne and nitidine are in bold.

investigators,[14] prompted our study into the relative activity of coralyne and select alkoxy-substituted derivatives of coralyne as mammalian topoisomerase poisons (see Fig. 1). The substitution pattern of the alkoxy groups of these coralyne derivatives was modified in the present study to more closely resemble fagaronine or nitidine. Within each series of compounds, we also evaluated the influence of the 8-methyl substituent and unsaturation at the 5,6-position. For all of these coralyne analogues, their relative activity as topoisomerase I and topoisomerase II poisons, as well as cytotoxicity in human lymphoblasts, RPMI 8402 and CPT-K5 cell lines, was determined.

Chemistry

Methods similar to those reported in the literature were used in the preparation of the dibenzo[*a*,*g*]quinolizinium derivatives evaluated in this study.[17] The general synthetic approach employed is outlined in Scheme 1. Reaction of the appropriately substituted dimethoxyphenethylamine with 3,4-dimethoxyphenylacetyl chloride provided the phenylacetamides (**5a–c**), which were cyclized in the presence of phosphorous oxychloride to the 3,4-dihydro-1-benzylisoquinoline intermediates (**6a–c**). These dihydroisoquinoline intermediates could be directly converted to the 5,6-dihydrodibenzo[*a*,*g*]quinolizinium derivatives (**7a–c**) using acetic anhydride in the presence fuming H_2SO_4. Alternatively, **6a–c** could be converted to **8a–c**, under Vilsmeier–Haack conditions.[23] The dihydroisoquinoline intermediates (**6a–c**) could also be oxidized to their 1-benzylisoquinoline derivatives (**9a–c**) using palladium-on-carbon. Treatment of **9a–c** with acetic anhydride in the presence of H_2SO_4 provided the 8-methyldibenzo[*a*,*g*]quinolizinium derivatives (**2a–c**). Under Vilsmeier–Haack conditions, **9a–c** were converted to the dibenzo[*a*,*g*]quinolizinium chloride derivatives (**10a–c**).



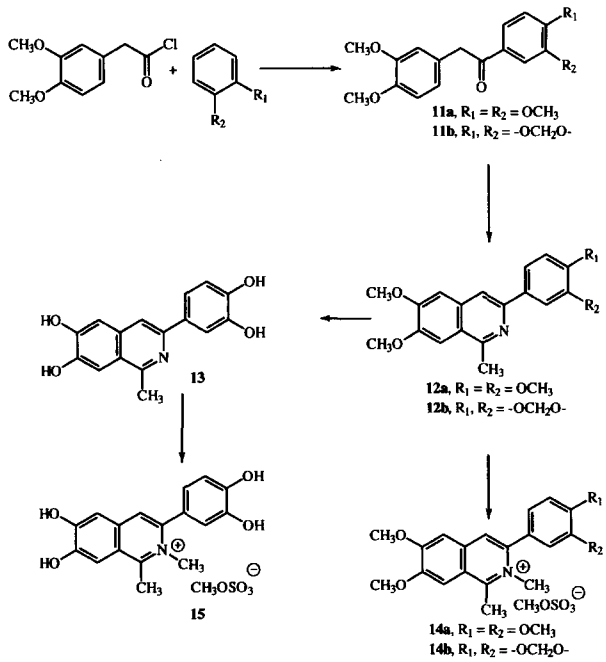
Scheme 1. Synthetic approach for the preparation of the 5,6-dihydrodibenzo[*a*,*g*]quinolizinium derivatives **7a–c** and **8a–c** and the dibenzo[*a*,*g*]quinolizinium derivatives **2a–c** and **10a–c**.

The synthetic methods employed for the preparation of 1-methyl-3-phenylisoquinoline derivatives are outlined in Scheme 2. Friedel–Crafts acylation of 1,2-dimethoxybenzene and 1,2-(methylenedioxy)benzene with 3,4-dimethoxyphenylacetyl chloride using a slight modification of the literature procedure provided the ketone intermediates **11a** and **11b**, respectively.[24] These ketones were converted to 6,7-dimethoxy-1-methyl-3-(3,4-dimethoxyphenyl)isoquinoline (**12a**) and 6,7-methylenedioxy-1-methyl-3-(3,4-dimethoxyphenyl)isoquinoline (**12b**) by reaction with acetonitrile in the presence of P_2O_5.

Cleavage of the methoxy groups of **12a** was accomplished using borontribromide in chloroform to provide the tetrahydroxy derivative (**13**). Compounds **12a**, **12b** and **13** were converted to their 2-methylisoquinolinium derivatives, **14a**, **14b** and **15**, respectively, by treatment with dimethyl sulfate.

Results and Discussion

The relative activity of coralyne and related compounds as inhibitors of mammalian topoisomerase is listed in Table 1. As shown in this table, coralyne and several of its analogues had pronounced activity, in some instances comparable to camptothecin, as mammalian topoisomerase I poisons. Previous studies[13,14] on the influence of structure of a series of coralyne derivatives against L1210 and P388 leukemias in mice revealed that the presence of 8-alkyl substitu-



Scheme 2. Synthetic procedure for the preparation of 1-methyl-3-phenylisoquinolinium derivatives.

tion, planarity and structural rigidity are critical factors for antitumor activity. In this study we evaluated analogues which had methoxy substituents at sites which were equivalent to either the 2,3-positions (**2a**, **7a**, **8a**, **9a** and **10a**) or 3,4 positions (**2b**, **7b**, **8b**, **9b** and **10b**) of coralyne. In addition, 3,4-methylenedioxy-substituted analogues (**2c**, **7c**, **8c**, **9c** and **10c**) were also synthesized and their pharmacological activities evaluated. The methylenedioxy group at the 3,4-position of these coralyne analogues appears to be a common factor associated with their potency as topoisomerase poisons. The methylenedioxy derivatives (**2c**, **7c**, **8c** and **10c**) not only retained topoisomerase I poisoning activity comparable to that of coralyne, but also possessed notable activity as topoisomerase II poisons. As there is no evidence to suggest that the molecular mechanism of topoisomerase I poisoning is related to that responsible for topoisomerase II poisoning, the observation that, for these analogues, enhanced activity is observed for both of these topoisomerases was unanticipated. These results are consistent, however, in view of the fact that this modification in substitution pattern and substituents allows these molecules to more closely resemble the alkoxy substitution pattern on the potent topoisomerase I and II poison, nitidine. In our evaluation of the relative potency of a series of coralyne analogues as topoisomerase poisons, several differences were also noted in those structural requirements which favored activity when compared with the structure–activity relationships reported for the in vivo antitumor activity of varied coralyne derivatives. It is of particular interest that the best topoisomerase I poison in this series of compounds (**8c**) lacks an 8-alkyl substituent, which is not consistent with the structure–activity studies on antitumor activity in mice. The potent activity of **8c** is evident by the total cleavage of DNA observed in the presence of topoisomerase I, which occurred at almost equivalent concentration for **8c** (0.27 μM, 0.1 μg/mL) and camptothecin (0.29 μM, 0.1 μg/mL), see Figures 2 and 3. In addition, **8c** possesses a kink in an otherwise planar molecule because of its 5,6-position being saturated. In previous studies on the antitumor activity of coralyne derivatives in mice, the presence of saturation at this position was associated with diminished activity. All other similarly substituted molecules, **2c**, **7c** and **10c**, which are either planar and/or have an 8-methyl substituent, are 10-fold less active compared with **8c**, as topoisomerase I poisons. Berberine, which is a positional isomer of **8c**, is inactive when assayed under identical conditions as a topoisomerase I poison.[12] Compounds **2b**, **7b**, **8b** and **10b** did not exhibit significant activity as topoisomerase I poisons. Thus, relative to a 3,4-methylenedioxy substituent, dimethoxy substitution at 3,4-position of these coralyne analogues dramatically altered their potency as topoisomerase I poisons. While **8c** did exhibit modest activity as a topoisomerase II poison, several of these analogs, including **2c** and **10c**, did exhibit potent activity as topoisomerase II poisons, see Table 1 and Figure 4.

All of the 2,3-dimethoxy coralyne derivatives, with the exception of **10a**, are selective topoisomerase I poisons with no significant activity as topoisomerase II poisons. In contrast to the results observed with **2a**, **7a** and **8a**, compound **10a** was inactive as a topoisomerase I poison and did exhibit significant activity against topoisomerase II. The basis for this exceptional difference in activity associated with the ability of **10a** as a topoisomerase poison and the other 2,3-dimethoxy coralyne derivatives is unclear at this time.

Structural rigidity may be a critical requirement for retention of activity as topoisomerase poisons within this series of compounds. Compounds **9a**, **9b** and **9c** may be regarded as ring-opened analogues of coralyne which lack the carbon at the 8-position as well as the cationic charge associated with the quaternary ammonium group. All three of these analogues exhibited very weak activity as topoisomerase I or II poisons.

The 1-methyl-3-phenylisoquinoline derivatives **12a** and **12b** and their N-methylated quaternized analogues **14a** and **14b** may be regarded as ring-opened analogues lacking the C_5–C_6 moiety of coralyne did not exhibit significant topoisomerase poisoning activity. Also, compounds **13** and **15** which are polyphenolic analogues of compounds **12a/12b** and **14a/14b**, respectively, were relatively ineffective as topoisomerase I or II poisons.

The cytotoxicity of coralyne and these analogues against the human lymphoblast cell line, RPMI 8402, is summarized in Table 1. No clear correlation was observed between cytotoxicity, as determined using MTA assay, and potency as either a topoisomerase I or II poison. Despite the very pronounced activity of several of these analogues as topoisomerase I poisons,

Table 1. Topoisomerase I and topoisomerase II mediated DNA cleavage of coralyne derivatives and related compounds

Compound	Topo I-mediated DNA cleavage[b]	Topo II-mediated DNA cleavage[c]	Cytotoxicity IC_{50}[a] (μM) cell lines	
			RPMI	CPT-K5
2a	10	>1000	4.9	20
2b	>1000	100	0.4	2.0
2c	10	10	2.0	41
7a	5	>1000	5.9	>20[d]
7b	1000	1000	6.9	14
7c	10	30	0.6	6.1
8a	20	>1000	10	18
8b	>1000	1000	9.0	6.4
8c	1	50	8.1	27
9a	>1000	1000	24	29
9b	>1000	1000	15	15
9c	>1000	>1000	9.3	15
10a	1000	10	13	>32[d]
10b	>1000	100	2.6	5.2
10c	10	2	0.8	6.8
12a	>1000	>1000	13	24
12b	1000	>1000	22	31
13	>1000	>1000	7.1	99
14a	>1000	>1000	43	39
14b	1000	1000	6.7	11
15	1000	>1000	7.3	>122[d]
CPT	1	>1000	0.004	10[d]
VM-26	>1000	1	0.3	0.5

[a]IC_{50} has been calculated after 4 days of continuous drug exposure.
[b]Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to camptothecin (CPT), whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.
[c]Topoisomerase II cleavage values are reported as REC, i.e., concentrations relative to VM-26, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of calf thymus topoisomerase II.
[d]No indication of cytotoxicity were considered indicative of IC_{50} values substantially greater than the highest doses assayed.

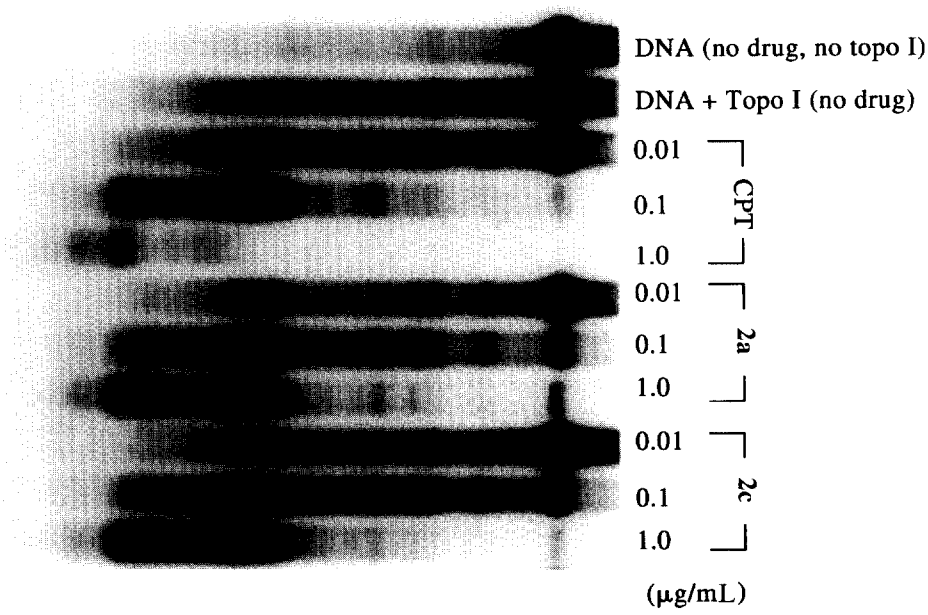


Figure 2. Stimulation of enzyme-mediated DNA cleavage by coralyne analogues using human topoisomerase I. The upper-most lane in each panel is the DNA control without topoisomerase I. The second lane from the top in each panel is the control with topoisomerase I alone. The rest of the lanes are with topoisomerase I and serially (10-fold each) diluted compound from 0.01 to 1.0 μg/mL for compounds **2a** and **2c**. CPT was used as positive control and assayed at concentrations ranging from 0.01 to 1.0 μg/mL.

it is evident that these coralyne analogues are several orders of magnitude less cytotoxic than camptothecin. In contrast to camptothecin, these derivatives exist as cationic quaternary ammonium compounds. On the basis of the intrinsic activity of several of these derivatives to inhibit topoisomerase I, it is possible that the quaternary ammonium functionality associated with the structure of the structurally rigid analogues of coralyne could be a major factor in limiting cellular absorption and attenuating their cytotoxic activity. Fused planar cationic aromatic ring system of coralyne[15,25] and berberine[10,26,27] have been shown to intercalate with DNA. Furthermore, coralyne has been shown to bind to DNA triplexes.[16] The extent to which these processes are related to poisoning of topoisomerases and the role of the cationic moiety in these molecular interactions has not been fully established. Further studies are specifically needed to determine whether the quaternary ammonium group is required for retention of activity as either topoisomerase I or II poisons.

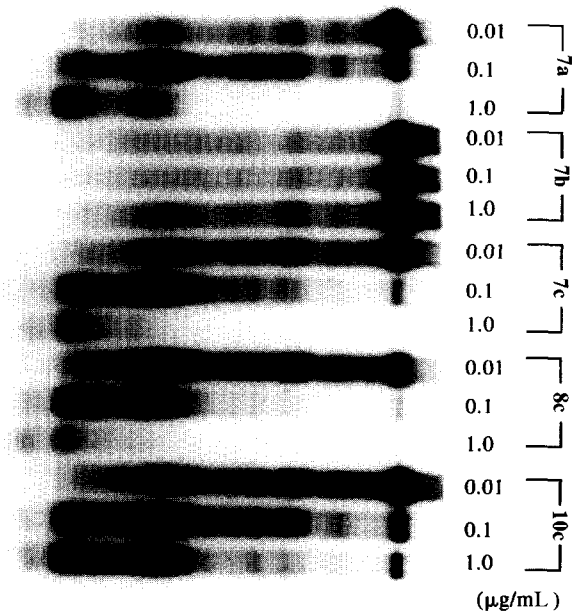


Figure 3. Stimulation of enzyme-mediated DNA cleavage by coralyne analogues using human topoisomerase I. The assays were performed as described in Figure 2 for the comparison of DNA fragmentation patterns. The lanes show topoisomerase I and serially (10-fold each) diluted compound from 0.01 to 1.0 μg/mL for compounds **7a**, **7b**, **7c**, **8c** and **10c**.

Several of the analogues synthesized and evaluated for cytotoxicity in this study which were inactive as topoisomerase poisons did exhibit significant cytotoxicity. Specifically, compounds **9c**, **13**, **14b** and **15** had appreciable cytotoxicity against RPMI 8402 cells. In these instances, the specific pharmacological target associated with their cytotoxicity is unknown.

The cytotoxic activity of these compounds was also evaluated in the camptothecin-resistant cell line, CPT-K5, which contains a mutant form of topoisomerase I. In viewing the cytotoxicity of the more potent topoisomerase I poisons in this study (**2a**, **2c**, **7a**, **7c**, **8a**, **8c** and **10c**), there were differences between the IC_{50} values obtained for CPT-K5 cells as compared with those for RPMI 8402 cells. These data could be viewed as an indication that the pharmacological target associated with the cytotoxicity of these coralyne derivatives is related to their activity as topoisomerase I poisons. In view of similar differences, however, which can be noted for analogues which were inactive as topoisomerase I poison, one cannot ascribe this difference in

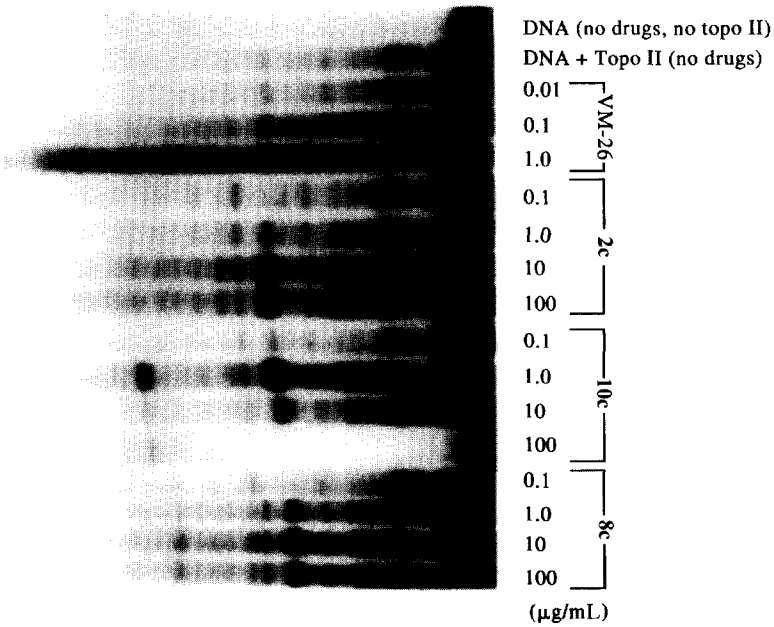


Figure 4. Stimulation of enzyme-mediated DNA cleavage by coralyne analogues using calf thymus topoisomerase II. The upper-most lane in each panel is the DNA control without topoisomerase II. The second lane from the top in each panel is the control with topoisomerase II alone. The rest of the lanes are with topoisomerase II and serially (10-fold each) diluted compound from 0.01 to 1.0 μg/mL for compounds **2c**, **8c** and **10c**. VM-26 was used as positive control and was assayed at concentrations ranging from 0.01 to 1.0 μg/mL.

effect specifically to the mutant form of topoisomerase I in CPT-K5. It also is evident that any difference in cytotoxicity between these cell lines for the compounds evaluated is minor relative to camptothecin.

This study demonstrates that minor structural variation among analogues of coralyne can have a profound impact on their activity as either topoisomerase I or topoisomerase II poisons. Coralyne analogues exhibited enhanced activity as both a topoisomerase I and II poison in those instances where there was a methylenedioxy substituent at the 3,4-position. These results are consistent with the potent activity of nitidine as a topoisomerase II poison and the spatial relationships of nitidine to these coralyne derivatives. In this study the analogues of coralyne which have potent activity as topoisomerase I poisons with little activity as topoisomerase II poisons had methoxy substituents at the 2,3-position. These data suggest that similarly substituted analogues of nitidine (i.e., 1,2-dimethoxy derivatives as opposed to its 2,3-methylenedioxy substituent) could exhibit similar selectivity with regard to its potential to inhibit either mammalian topoisomerase I or II. Studies are in progress to evaluate further those structural features of these alkaloids which influence their potency and selectivity as mammalian topoisomerase poisons.

Experimental

General

Melting points were determined with a Thomas–Hoover Unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin–Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1}. ^1H and ^{13}C NMR were recorded on a Varian Gemini-200 Fourier transform spectrometer at 200 and 50 MHz, respectively. NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in Hz. Mass spectra were obtained from Washington University Resource for Biomedical and Bio-Organic Mass Spectrometry. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm (ICN Biomedicals, Eschwegge, Germany) using the solvent systems indicated. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, Georgia.

General procedure for the synthesis of 8-methyldibenzo-[*a*,*g*]quinolizinium acetosulfates (2a–c)

Fuming (20%) sulfuric acid (1 mL) was added to 4 mL of freshly distilled acetic anhydride, resulting in a vigorous exothermic reaction and the mixture becoming wine-red in color. This mixture was heated at 85–90 °C for 10 min. A solution of 1-benzylisoquinoline (**9a–c**, 2.35 mmol in 1 mL of freshly distilled acetic anhydride) was then added under nitrogen to the wine-red sulfuric acid solution and the resulting mixture was heated at 85–90 °C for 30–60 min. The reaction mixture was then cooled to room temperature and 5 mL methanol was added dropwise and allowed to stir for 30 min. The mixture was then chilled on an ice bath and stirred for an additional 30 min. The solid product obtained was filtered and washed successively, twice with 2 mL distilled water, twice with 2 mL methanol and twice with 10 mL ether. The crude product was then recrystallized from hot methanol to provide 90–95% yield of the desired products as a yellow crystalline material.

8-Methyl-2,3,10,11-tetramethoxydibenzo [*a*,*g*] quinolizinium acetosulfate (2a). Prepared from **9a**; mp 280 °C; IR (Nujol): 1735; ^1H NMR (DMSO-d_6): δ 3.28 (s, 3H), 3.94 (s, 3H), 4.03 (s, 3H), 4.09 (s, 6H), 7.42 (s, 1H), 7.53 (s, 1H), 7.80 (d, 1H, J=8.0), 7.98 (s, 1H), 8.70 (d, 1H, J=8.0), 9.28 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 17.5, 56.2, 56.6, 57.2, 104.1, 105.0, 107.3, 108.1, 116.0, 119.9, 120.8, 121.9, 123.7, 124.7, 133.5, 134.5, 145.4, 151.6, 152.7, 152.9, 156.3; anal. calcd for $C_{24}H_{25}NO_9S$: C, 57.25, H, 5.00, N, 2.78. Found: C, 57.07; H, 5.17; N, 2.72.

8-Methyl-3,4,10,11-tetramethoxydibenzo [*a*,*g*] quinolizinium acetosulfate (2b). Prepared from **9b**; mp 267–269 °C dec (lit.[14] mp 267–269 °C dec); IR (Nujol): 2762, 1702, 1642, 1595, 1546; ^1H NMR (DMSO-d_6): δ 3.40 (s, 3H), 3.43 (s, 3H), 4.00 (s, 3H), 4.09 (s, 3H), 4.13 (s, 6H), 7.77 (s, 1H), 7.85 (d, 2H, J=8.1), 8.01 (d, 1H, J=8.1), 8.75 (d, 1H, J=8.0), 8.88 (d, 1H, J = 8.0), 9.67 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 17.7, 56.8, 56.9, 57.3, 61.6, 104.9, 105.5, 115.1, 116.3, 117.1, 119.5, 121.3, 122.3, 122.9, 126.9, 134.5, 135.5, 147.2, 153.1, 153.7, 156.9; anal. calcd for $C_{24}H_{25}NO_9S$: C, 57.24; H, 5.00; N, 2.78. Found: C, 57.08; H, 5.35; N, 2.75.

8-Methyl-3,4-methylenedioxy-10,11-dimethoxydibenzo [*a*,*g*]quinolizinium acetosulfate (2c). Prepared from **9c**; mp >270 °C dec; IR (Nujol): 3417, 1727, 1648, 1615, 1551; ^1H NMR (DMSO-d_6): 3.41 (s, 3H), 4.13 (s, 3H), 6.46 (s, 2H), 7.70 (d, 1H, J=8.7), 7.76 (s, 1H), 7.81 (d, 1H, J=8.0), 7.89 (s, 1H), 8.58 (d, 1H, J=8.7), 8.84 (d, 1H, J=8.0), 9.64 (s, 1H); anal. calcd for $C_{23}H_{21}NO_9S \cdot 2.25\ H_2O$: C, 52.32; H, 4.44; N, 2.65. Found: C, 52.21; H, 4.24; N, 2.68; HRMS calcd for $C_{21}H_{18}NO_4$: 384.1236; found 348.1237.

General procedure for the synthesis of the phenylacetamides (5a–c)

A solution of 3,4-dimethoxyphenylacetyl chloride (4 mmol) in chloroform (6 mL) was added dropwise under nitrogen to a mixture of the appropriately substituted phenethylamine (4 mmol), chloroform (6 mL) and 2 M sodium carbonate (3 mL) at 0 °C with vigorous stirring. Stirring was continued at 0 °C until the reaction was complete (1–3 h). The reaction mixture was transferred to a separatory funnel using an additional 2 mL chloroform and 2 mL water. The organic phase was separated and the aqueous phase extracted with 5 mL chloroform. The combined chloro-

form extract was washed successively with 5 mL 0.1 N NaOH, 5 mL 0.1 N HCl, and 5 mL saturated NaCl solution. The chloroform extract was dried (Na_2SO_4) and evaporated. The crude product was crystallized from methanol to give 95–100% yields of the pure amide as white needles.

***N*-(3,4-Dimethoxyphenylethyl)-2-(3,4-dimethoxyphenyl) acetamide (5a).** Prepared from 3,4-dimethoxyphenethylamine and 3,4-dimethoxyphenylacetyl chloride; mp 125 °C (lit.[17] 123–125 °C); IR (KBr): 3327, 3064, 3007, 2916, 2840, 1642, 1608, 1591; ^{1}H NMR: δ 2.67 (t, 2H), 3.40–3.47 (m, 4H), 3.83 (s, 6H), 3.86 (s, 3H), 3.88 (s, 3H), 5.30 (br s, 1H), 6.52–6.86 (m, 6H); ^{13}C NMR: δ 35.6, 41.2, 43.5, 56.3, 56.4, 70.5, 111.7, 112.2, 115.8, 121.1, 127.5, 127.9, 128.5, 129.1, 131.1, 131.6, 137.3, 148.1, 149.5, 158.5, 171.8.

***N*-(2,3-Dimethoxyphenylethyl)-2-(3,4-dimethoxyphenyl) acetamide (5b).** Prepared from 2,3-dimethoxyphenethylamine[28] and 3,4-dimethoxyphenylacetyl chloride; mp 80 °C (lit.[29] 79–80 °C); IR: 3315, 2962, 2838, 1636, 1593, 1548; ^{1}H NMR: δ 2.61 (t, 2H), 3.22–3.30 (m, 4H), 3.58 (s, 3H), 3.64 (s, 6H), 3.67 (s, 3H), 6.22 (br s, 1H), 6.44–6.79 (m, 6H); ^{13}C NMR: δ 30.0, 41.0, 43.7, 55.9, 56.2, 56.3, 60.9, 111.2, 111.9, 112.9, 122.0, 122.6, 124.4, 127.9, 133.1, 147.5, 148.5, 149.5, 153.1, 171.9.

***N*-(2,3-Methylenedioxyphenylethyl)-2-(3,4-dimethoxyphenyl)acetamide (5c).** Prepared from 2,3-methylenedioxyphenethylamine[28] and 3,4-dimethoxyphenylacetyl chloride; mp 124 °C; IR (KBr): 3297, 3084, 2935, 1643, 1458; ^{1}H NMR: δ 2.66 (t, 2H), 3.36–3.42 (m, 4H), 3.75 (s, 3H), 3.79 (s, 3H), 5.73 (s, 2H), 5.81 (br s, 1H), 6.61–6.76 (m, 6H); ^{13}C NMR: δ 29.8, 39.7, 43.8, 56.4, 101.0, 107.5, 111.9, 112.9, 120.7, 122.0, 122.1, 123.3, 127.7, 146.0, 147.5, 148.7, 149.6, 171.8; anal. calcd for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.31; H, 6.16; N, 4.07.

General procedure for the synthesis of dihydroisoquinolines (6a–c)

The acetamide (**5a–c**, 2.47 mmol) was refluxed with phosphorus oxychloride (5.69 mmol) and dry toluene (10 mL), under nitrogen, for 20–60 min. The solvent was then carefully evaporated and the residue dissolved in ca. 5 mL methanol. This solution was poured into ca. 10–15 mL of cold water. After washing twice with 10 mL ether, 10 g of ice was added to the aqueous layer. While nitrogen was bubbled through the ice-chilled aqueous layer, the pH was adjusted to pH 10 with concentrated ammonium hydroxide. The aqueous layer was then saturated with sodium chloride and then extracted five times with 20 mL ether. The combined ether extract was dried over anhydrous potassium carbonate, filtered, and evaporated to give the dihydroisoquinolines (**6a–c**). As these compounds readily oxidize, exposure to air was avoided. The dihydroisoquinolines were used without further purification for the preparation of **7a–c**, **8a–c**, and **9a–c**.

General procedure for the synthesis of 5,6-dihydro-8-methyldibenzo[*a*,*g*]quinolizinium acetosulfates (7a–c)

Each of the dihydroisoquinolines (**6a–c**, 2.35 mmol) were dissolved in 1.0 mL of freshly distilled acetic anhydride. A procedure similar to that used for the synthesis of 8-methyldibenzo[a,g]quinolizinium acetosulfates (**2a–c**) was employed. The respective dihydroisoquinoline intermediates were used in place of the corresponding 1-benzylisoquinoline intermediates employed in the previous procedure. The products obtained were crystallized from boiling methanol to yield 85–90% of bright yellow crystalline product.

5,6-Dihydro-8-methyl-2,3,10,11-tetramethoxydibenzo[*a*,*g*]quinolizinium acetosulfate (7a). Prepared from **6a**; mp 278–279 °C (lit.[13] 277–279 °C); IR (KBr): 3450, 2946, 1725, 1611, 1567; ^{1}H NMR (DMSO-d_6): δ 3.18 (t, 2H), 3.23 (s, 3H), 3.40 (s, 3H), 3.89 (s, 3H), 3.94 (s, 3H), 4.08 (s, 6H), 4.75 (t, 2H), 7.13 (s, 1H), 7.63 (s, 2H), 7.80 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 17.8, 26.2, 49.8, 56.1, 56.3, 56.8, 57.3, 106.2, 106.4, 109.1, 110.9, 117.5, 120.0, 122.2, 128.6, 135.6, 139.0, 148.9, 151.6, 152.2, 155.4, 156.9, 167.5.

5,6-Dihydro-8-methyl-3,4,10,11-tetramethoxydibenzo[*a*,*g*]quinolizinium acetosulfate (7b). Prepared from **6b**; mp 255–256 °C; IR (KBr): 3432, 2946, 1723, 1609, 1567, 1502, 1429; ^{1}H NMR (DMSO-d_6): δ 3.20 (t, 2H), 3.22 (s, 3H), 3.82 (s, 3H), 3.94 (s, 3H), 4.07 (s, 6H), 4.74 (t, 2H), 7.26 (d, 1H, J=8.9), 7.67 (s, 1H), 7.78 (s, 1H), 7.87 (d, 1H, J=8.9), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 17.7, 21.0, 49.4, 56.3, 56.8, 57.3, 60.7, 106.3, 112.7, 117.6, 121.3, 122.3, 122.9, 128.9, 135.5, 138.9, 144.7, 152.3, 154.6, 155.3, 156.9, 167.6; anal. calcd for $C_{24}H_{27}NO_9S \cdot 1.25H_2O$: C, 54.59; H, 5.63; N, 2.65. Found: C, 54.59; H, 5.60; N, 2.67; HRMS calcd for $C_{22}H_{24}NO_4$: 366.1705; found: 366.1706.

5,6-Dihydro-8-methyl-3,4-methylenedioxy-10,11-dimethoxydibenzo[*a*,*g*]quinolizinium acetosulfate (7c). Prepared from **6c**; mp >270 °C; IR (KBr): 3434, 2920, 1724, 1617; ^{1}H NMR (DMSO-d_6): δ 3.16 (t, 2H), 3.23 (s, 3H), 3.40 (s, 3H), 4.08 (s, 6H), 4.76 (t, 2H), 6.23 (s, 2H), 7.17 (d, 1H, J=8.0), 7.67 (s, 1H), 7.70 (d, 1H, J=8.0), 7.81 (s, 1H), 8.69 (s, 1H); ^{13}C NMR (DMSO-d_6): 17.9, 20.8, 49.2, 56.8, 57.3, 102.7, 106.3, 106.5, 108.5, 116.2, 118.1, 121.2, 122.4, 122.7, 135.5, 139.0, 144.2, 149.6, 152.4, 155.6, 157.0, 167.6; anal. calcd for $C_{23}H_{23}NO_9S \cdot H_2O$: C, 54.43; H, 4.96; N, 2.76. Found: C, 54.39; H, 4.96; N, 2.74; HRMS calcd for $C_{21}H_{20}NO_4$: 350.1392; found: 350.1384.

General procedure for the synthesis of 5,6-dihydrodibenzo[*a*,*g*]quinolizinium chlorides (8a–c)

Phosphorus oxychloride (7.42 mmol) was added dropwise to chilled (0 °C) dimethylformamide under nitrogen. The mixture was stirred for 15 min at 0 °C. A solution of the respective dihydroisoquinolines (2.7 mmol) in 5.5 mL dimethylformamide was then added and allowed to stir at 0 °C for 1–2 h. The reaction

mixture was then heated at 100 °C for 1–2 h. The reaction mixture was cooled to room temperature and poured into a mixture containing 20 g of ice and 10 mL 6 N HCl. The resulting precipitate was filtered and washed successively twice with 5 mL cold water and twice with 10 mL ether. The final product in each instance was recrystallized from methanol.

5,6-Dihydro-2,3,10,11-tetramethoxydibenzo[*a*,*g*]quinolizinium chloride (8a). Prepared from **6a**; mp 261–262 °C; IR (Nujol): 1664, 1660, 1564; ^{1}H NMR (CD_3OD): δ 3.35 (t, 3H), 3.91 (s, 3H), 4.02 (s, 3H), 4.10 (s, 3H), 4.17 (s, 3H), 5.04 (t, 2H), 7.10 (s, 1H), 7.78 (s, 1H), 8.60 (s, 1H), 9.64 (s, 1H), 10.12 (s, 1H); ^{13}C NMR (CD_3OD): δ 28.1, 56.4, 56.7, 56.9, 57.3, 57.8, 104.4, 106.6, 107.5, 108.1, 108.2, 109.0, 110.2, 112.4, 112.5, 112.8, 115.4, 117.7, 119.6, 130.4, 146.5; anal. calcd for $C_{21}H_{22}NO_4Cl \cdot 0.75H_2O$: C, 62.84; H, 5.90; N, 3.49. Found: C, 62.79; H, 5.84; N, 3.46; HRMS calcd for $C_{21}H_{22}NO_4$: 352.1549; found: 352.1549.

5,6-Dihydro-3,4,10,11-tetramethoxydibenzo[*a*,*g*]quinolizinium chloride (8b). Prepared from **6b**; mp 252 °C; IR (Nujol): 3383, 1632, 1600, 1571; ^{1}H NMR (DMSO-d_6): δ 3.27 (t, 2H), 3.81 (s, 3H), 3.95 (s, 3H), 4.01 (s, 3H), 4.08 (s, 3H), 4.80 (t, 2H), 7.29 (d, 1H, J=8.9), 7.68 (s, 1H), 7.73 (s, 1H), 7.96 (d, 1H, J=8.9), 8.82 (s, 1H), 9.60 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 20.9, 54.4, 56.3, 56.6, 56.9, 60.6, 105.7, 106.7, 112.7, 118.4, 120.4, 122.4, 122.6, 129.1, 136.8, 138.5, 145.2, 145.7, 152.5, 154.9, 157.6; anal. calcd for $C_{21}H_{22}NO_4Cl \cdot 1.5H_2O$: C, 60.79; H, 5.71; N, 3.38. Found: C, 60.79; H, 5.71; N, 3.38; HRMS calcd for $C_{20}H_{18}NO_4$: 336.1236; found: 336.1244.

5,6-Dihydro-3,4-methylenedioxy-10,11-dimethoxydibenzo[*a*,*g*]quinolizinium chloride (8c). Prepared from **6c**; mp >280 °C; IR (Nujol): 2725, 1622, 1574; ^{1}H NMR (CD_3OD): δ 3.27 (t, 2H), 4.07 (s, 3H), 4.13 (s, 3H), 4.62 (t, 2H), 6.16 (s, 2H), 7.03 (d, 1H, J=8.4), 7.60 (s, 1H), 7.63 (s, 1H), 7.75 (d, 1H, J=8.4), 8.60 (s, 1H), 9.34 (s, 1H); ^{13}C NMR (CD_3OD): δ 22.2, 56.0, 57.3, 57.7, 104.3, 106.6, 107.5, 109.6, 117.3, 120.1, 122.3, 122.9, 124.6, 139.2, 146.5, 146.6, 152.0, 155.0, 160.3; anal. calcd for $C_{20}H_{18}NO_4Cl \cdot 2H_2O$: C, 58.89; H, 5.43; N, 3.43. Found: C, 58.74; H, 5.39; N, 3.45; HRMS calcd for $C_{20}H_{18}NO_4$: 336.1236; found: 336.1244.

General procedure for the synthesis of 1-benzyliso quinolines (9a–c)

The appropriate dihydroisoquinoline (3.4 mmol) was refluxed at 220–230 °C with 0.3 g of 10% palladium on carbon in 5 mL tetralin (purged with nitrogen for 20 min prior to use) for 3–4 h. The reaction mixture was then allowed to cool to 180 °C and filtered under nitrogen using a Schlenk-type filtration unit (Aldrich). After cooling to room temperature, the filtrate was chilled to 0 °C and hydrogen chloride (1.0 M in anhydrous ether) was added to adjust the pH of the mixture to pH 1.0. The hydrochloride salt of the 1-benzylisoquinoline precipitated. This precipitate was filtered, washed three times with 10 mL of anhydrous ether, and dried to give the respective 1-benzylisoquinoline hydrochloride salts in 90–100% yields. The dried hydrochloride salts were then dissolved in a minimum quantity of methanol (2–5 mL), to which was added 10 g of ice. Concentrated ammonium hydroxide was added to the ice-cooled aqueous solution to adjust the pH to 10. The aqueous layer was saturated with sodium chloride and then extracted with three 10 mL portions of chloroform. The combined chloroform extract was dried (Na_2SO_4) and evaporated to give **9a–c**.

5,6-Dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline hydrochloride (9b). Prepared from **6b**; mp 206–208 °C (lit.[14] 206–208 °C); IR (Nujol): 2676, 1630, 1625, 1588; ^{1}H NMR (CD_3OD): δ 3.76 (s, 3H), 3.81 (s, 3H), 4.03 (s, 3H), 4.15 (s, 3H), 4.87 (s, 2H), 6.86 (t, 2H), 7.07 (s, 1H), 7.92 (d, 1H, J=1), 8.31 (d, 2H, J=5.7), 8.55 (d, 1H, J=1); ^{13}C NMR (CD_3OD): δ 38.1, 56.9, 57.1, 58.1, 62.5, 113.7, 114.2, 119.2, 120.8, 122.5, 122.8, 127.7, 129.1, 131.2, 135.9, 143.5, 150.5, 151.3, 158.5, 160.2.

5,6-Methylenedioxy-1-(3,4-dimethoxybenzyl)isoquinoline (9c). Prepared from **6c**; mp 111 °C ; IR (KBr): 3429, 3064, 3003, 2930; ^{1}H NMR (CD_3OD): δ 2.93 (s, 3H), 4.03 (s, 3H), 4.04 (s, 3H), 6.01 (s, 2H), 6.91 (d, 1H, J=8.8) 7.07 (s, 1H), 7.25 (s, 1H), 7.60 (m, 2H), 7.68 (s, 1H); ^{13}C NMR (CD_3OD): δ 23.2, 56.5, 76.9, 77.4, 78.2, 101.6, 104.4, 106.0, 107.8, 109.9, 114.1, 121.0, 122.5, 134.0, 135.2, 148.1, 149.2, 150.1, 153.1, 156.2; anal. calcd for $C_{19}H_{17}O_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.85; H, 5.51; N, 4.31.

General procedure for the synthesis of dibenzo[*a*,*g*] quinolizinium chlorides (10a–c)

The procedure employed was similar to that used in the synthesis of 5,6-dihydrodibenzo[a,g]quinolizinium chlorides (**8a–c**). The 1-benzylisoquinoline intermediates were substituted for corresponding dihydroisoquinoline intermediates in the previous procedure. The products obtained were crystallized from a 1:1 mixture of glacial acetic acid and 6 N HCl to give yields ranging from 92 to 98% of **10a–c** as yellow crystalline products.

2,3,10,11-Tetramethoxydibenzo[*a*,*g*]quinolizinium chloride (10a). Prepared from **9a**; mp, IR, ^{1}H NMR and ^{13}C NMR were as previously reported.[12]

3,4,10,11-Tetramethoxydibenzo[*a*,*g*]quinolizinium chloride (10b). Prepared from **9b**; mp 223–225 °C dec; IR (KBr): 3394, 2947, 2843, 1626, 1598, 1561, 1503, 1433; ^{1}H NMR (using a coaxial tube with **9b** dissolved in trifluoroacetic acid in the outer tube and deuterium oxide in the inner tube): δ 4.51 (s, 3H), 4.55 (s, 6H), 4.61 (s, 3H), 7.94 (s, 1H), 7.96 (s, 1H), 8.06 (d, 1H, J=9.2), 8.46 (d, 1H, J=7.3), 8.78 (d, 1H, J=7.3), 9.01 (d, 1H, J=9.2), 9.56 (s, 1H), 9.83 (s, 1H); ^{13}C NMR (using a coaxial tube with the **9b** dissolved in tri-

fluoroacetic acid in the outer tube and deuterium oxide in the inner tube): δ 58.4, 58.8, 59.1, 64.7, 107.0, 107.2, 118.9, 119.2, 119.7, 121.6, 125.0, 126.8, 127.2, 132.0, 139.4, 139.5, 140.0, 145.1, 156.6, 157.9, 161.1; anal. calcd for $C_{21}H_{20}NO_4Cl \cdot 1.25\ H_2O$: C, 61.76; H, 5.55; N, 3.43. Found: C, 60.67; H, 5.37; N, 3.43; HRMS calcd for $C_{21}H_{20}NO_4$: 350.1392; found: 350.1392.

3,4-Methylenedioxy-10,11-dimethoxydibenzo[*a*,*g*]quinolizinium chloride (10c). Prepared from **9c**; mp >270 °C dec; IR (KBr): 3414, 3015, 2928, 1620, 1564, 1488; ^{1}H NMR (using a coaxial tube with the compound dissolved in trifluoroacetic acid in the outer tube and deuterium oxide in the inner tube): δ 4.55 (s, 3H), 4.60 (s, 3H), 6.67 (s, 2H), 7.81 (d, 1H, J=8.5), 7.92 (s, 2H), 8.18 (d, 1H, J=7.7), 8.68 (t, 2H), 9.47 (s, 1H), 9.77 (s, 1H); ^{13}C NMR (using a coaxial tube with the compound dissolved in trifluoroacetic acid in the outer tube and deuterium oxide in the inner tube): δ 58.8, 59.1, 106.7, 107.0, 107.1, 115.2, 115.7, 118.4, 119.8, 121.7, 121.8, 126.6, 131.1, 139.5, 139.9, 140.3, 147.1, 153.6, 156.4, 161.1; anal. calcd for $C_{20}H_{16}NO_4Cl \cdot 1.75H_2O$: C, 59.86; H, 4.90; N, 3.49. Found: C, 59.72; H, 4.93; N, 3.48; HRMS calcd for $C_{20}H_{16}NO_4$: 334.1079; found: 334.1075.

3,4,3′,4′-Tetramethoxydesoxybenzoin (11a). Powdered anhydrous aluminum chloride (0.78 g) was slowly added to a stirred mixture of 1,2-dimethoxybenzene (0.64 g, 4.66 mmol) and 3,4-dimethoxyphenylacetyl chloride (1.0 g, 4.66 mmol) in 10 mL freshly distilled dry dichloromethane. An exothermic reaction occurred. The orange solution became brown as the mixture refluxed. The reaction mixture was heated to reflux for an additional 2 h and then allowed to cool to room temperature. The cooled solution was poured into a mixture containing of 5 g crushed ice and 5.5 mL 7.5 N HCl. The organic phase was separated and the aqueous phase was extracted three times with 10 mL dichloromethane. The combined dichloromethane extract was dried (Na_2SO_4), filtered and evaporated to give a white solid, which was crystallized from ethanol to give pure white needles of **11a** in 98% yield; mp 105–107 °C (lit.[30] mp 104–106 °C); ^{1}H NMR: δ 3.85 (s, 6H), 3.91 (s, 3H), 3.94 (s, 3H), 4.18 (s, 2H), 6.81 (m, 2H), 6.91 (m, 2H), 7.56 (d, 1H, J=2.0), 7.66 (dd, 1H, J = 8.4, 2.0); ^{13}C NMR: δ 45.2, 56.3, 56.4, 56.5, 110.4, 111.1, 111.8, 112.8, 121.3, 121.9, 123.9, 127.9, 130.2, 148.4, 149.5, 153.8, 197.0.

3,4-Dimethoxy-3′,4′-methylenedioxydesoxybenzoin (11b). A solution of 3,4-dimethoxyphenylacetyl chloride (3.25 g, 15 mmol) in 15 mL freshly distilled dry dichloromethane was added dropwise to a stirred mixture of 1,3-benzodioxole (1.83 g, 15 mmol) and tin(IV) chloride (4.6 g, 17.6 mmol) in 15 mL dichloromethane at −10 °C. The mixture was then allowed to rise to room temperature and was stirred for an additional 2 h. The reaction mixture was then poured into 25 mL 6 N HCl and stirred for 16 h. The organic phase was then separated and the aqueous phase was extracted three times with 20 mL aliquots of dichloromethane. The combined dichloromethane extract was washed successively with 20 mL of 0.1 N NaOH and 20 mL distilled water. The dichloromethane extract was then dried (Na_2SO_4), filtered and evaporated to give a cream colored solid. The crude product was crystallized from ethanol to give 3.0 g of white needles of **11b** in 66% yield; mp 110–111 °C; IR (Nujol): 2725, 1675, 1589, 1519; ^{1}H NMR: δ 3.80 (s, 3H), 3.81 (s, 3H), 4.09 (s, 2H), 5.96 (s, 2H), 6.76 (m, 4H), 7.42 (d, 1H, J=1.5), 7.58 (dd, 1H, J=6.4, 1.5); ^{13}C NMR: δ 45.3, 56.3, 102.3, 108.3, 108.7, 111.8, 112.7, 112.9, 121.9, 125.4, 127.7, 131.8, 148.4, 148.6, 149.4, 152.2, 196.4; anal. calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.98; H, 5.32.

General procedure for the synthesis of 1-methyl-3-phenylisoquinolines (12a, 12b)

Anhydrous phosphorus pentoxide (4 mmol) was added in three portions to 10 mL of a solution (100 mM) of the respective desoxybenzoins in acetonitrile. The reaction mixture was stirred under nitrogen for 18–20 h. The reaction mixture was quenched by addition of 10 mL water. The resulting suspension was neutralized with 10% NaOH and extracted with three 10 mL portions of dichloromethane. The combined organic phase was dried (Na_2SO_4), filtered and evaporated to give a cream colored residue. This residue was chromatographed on silica gel using a 95:5 mixture of dichloromethane and ethyl acetate, respectively, as eluent to give the corresponding 1-methyl-3-phenylisoquinolines (**12a** and **12b**) in 75–85% yield.

6,7-Dimethoxy-1-methyl-3-(3,4-dimethoxyphenyl)isoquinoline (12a). Prepared from **11a**; mp 168–169 °C (lit.[31] 168–170 °C); IR (Nujol): 2725, 1621, 1573, 1508; ^{1}H NMR: δ 2.95 (s, 3H), 3.93 (s, 3H), 4.02 (s, 9H), 6.96 (d, 1H, J=8.4), 7.08 (s, 1H), 7.25 (s, 1H), 7.60 (dd, 1H, J=8.4, 2.0), 7.71 (s, 2H); ^{13}C NMR: δ 23.2, 56.4, 104.3, 105.9, 110.4, 111.7, 113.9, 119.5, 122.4, 133.6, 133.9, 149.2, 149.6, 149.7, 150.0, 153.1, 156.2.

6,7-Dimethoxy-1-methyl-3-(3,4-methylenedioxyphenyl)isoquinoline (12b). Prepared from **11b**; mp 174 °C; IR (Nujol): 2727, 1622, 1573, 1504; ^{1}H NMR: δ 2.93 (s, 3H), 4.03 (s, 3H), 4.04 (s, 3H), 6.00 (s, 2H), 6.91 (1H, d, J=8.8), 7.07 (s, 1H), 7.25 (s, 1H), 7.60 (m, 2H), 7.67 (s, 1H); ^{13}C NMR: δ 23.2, 56.5, 101.6, 104.4, 106.0, 107.8, 108.9, 114.1, 121.0, 122.5, 133.9, 135.2, 148.1, 148.6, 149.2, 150.1, 153.1, 156.2; anal. calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.55. H, 5.28, N, 4.30.

6,7-Dihydroxy-1-methyl-3-(3,4-dihydroxyphenyl)isoquinoline (13). Compound **12a** (250 mg, 0.74 mmol) was dissolved in 5 mL dry chloroform and chilled to −50 °C using a mixture of dry ice and acetone. To this mixture was added dropwise 7.4 mL of a 1.0 M solution of boron tribromide in dichloromethane. The reaction mixture was allowed to come to room temperature over a period of 4 h. The precipitate was filtered and washed twice with 2 mL portions of ether. This crude product was recrystallized from ethanol to

give **13** in 98% yield as white needles; mp >280 °C dec; IR (Nujol): 3326, 1602, 1531; ^{1}H NMR (CD_3OD): δ 3.08 (s, 3H), 6.98 (d, 1H, J=8.1), 7.19 (dd, 1H, J=8.1, 1.9), 7.24 (s, 1H), 7.37 (s, 1H), 7.61 (s, 1H), 7.94 (s, 1H); ^{13}C NMR (CD_3OD): δ 17.8, 109.6, 110.6, 116.1, 117.3, 119.6, 121.2, 122.8, 125.6, 138.4, 142.5, 147.7, 149.5, 152.0, 154.3, 158.3; HRMS calcd for $C_{16}H_{13}NO_4$: 283.0844; found: 283.0839.

General procedure for the synthesis of 1,2-dimethyl-3-phenylisoquinolinium derivatives (14a, 14b, and 15)

Each of the respective 1-methyl-3-phenylisoquinoline derivatives (0.67 mmol) was added to 1 mL of dimethyl sulfate. This reaction mixture was then heated at 100 °C for 20–60 min and then allowed to cool to room temperature. Anhydrous ether (8 mL) was added to the cooled reaction mixture and the resulting suspension stirred for 5 min. The precipitate was filtered and recrystallized from methanol to give 95–100% of the corresponding N-methylisoquinolinium salts.

6,7-Dimethoxy-1,2-dimethyl-3-(3,4-dimethoxyphenyl) isoquinolinium methosulfate (14a). Prepared from **12a**; mp 224–226 °C; IR (Nujol): 3508, 1640, 1613, 1548, 1506; ^{1}H NMR (CD_3OD): δ 3.21 (s, 3H), 3.67 (s, 3H), 3.94 (s, 3H), 3.98 (s, 3H), 4.11 (s, 3H), 4.12 (s, 1H), 7.20 (d, 1H, J=8.6), 7.48 (m, 2H), 7.62 (s, 1H), 7.66 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (CD_3OD): δ 18.0, 56.9, 57.1, 57.3, 57.6, 106.0, 107.5, 112.8, 113.5, 120.7, 122.7, 123.2, 126.4, 139.1, 151.5, 170.4, 170.9, 171.3; anal. calcd for $C_{22}H_{27}NO_8S \cdot 1.5H_2O$: C, 53.65; H, 5.83; N, 2.84. Found: C, 53.35; H, 5.52; N, 2.91.

6,7-Dimethoxy-1,2-dimethyl-3-(3,4-methylenedioxyphenyl)isoquinolinium methosulfate (14b). Prepared from **12b**; mp 235–237 °C; IR (Nujol): 3479, 1612, 1568; ^{1}H NMR (DMSO-d_6): δ 3.22 (s, 3H), 3.38 (s, 3H), 4.06 (s, 6H), 6.20 (s, 2H), 7.14 (m, 2H), 7.23 (m, 1H), 7.70 (s, 1H), 7.86 (s, 1H), 8.09 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 18.2, 43.5, 56.8, 56.9, 102.2, 106.2, 106.3, 109.1, 110.0, 122.9, 123.2, 124.1, 127.7, 134.7, 144.9, 147.9, 149.0, 152.5, 156.9, 157.1; anal. calcd for $C_{21}H_{23}NO_8S$: C, 53.11; H, 5.15; N, 3.11. Found: C, 53.03; H, 5.19; N, 2.95.

6,7-Dihydroxy-1,2-dimethyl-3-(3,4-dihydroxyphenyl)isoquinolinium methosulfate (15). Prepared from **13**; mp 118–120 °C; IR (KBr): 3249, 1614, 1528, 1454; ^{1}H NMR (CD_3OD): δ 3.08 (s, 3H), 3.68 (s, 3H), 6.98 (d, 1H, J=8.2), 7.21 (m, 2H), 7.37 (s, 1H), 7.61 (s, 1H), 7.94 (s, 1H); ^{13}C NMR (CD_3CD): δ 17.8, 109.6, 110.6, 116.1, 117.3, 119.6, 121.2, 122.8, 125.6, 138.4, 142.6, 147.7, 149.5, 152.0, 154.3, 158.3; anal. calcd for $C_{18}H_{19}NO_8S$: C, 52.81; H, 4.68; N, 3.42. Found: C, 52.79; H, 4.65; N, 3.40.

Topoisomerase mediated DNA cleavage assays

Human 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.[32] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[33] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[34] The cleavage assays were performed as previously reported.[35]

Cytotoxicity assay

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[36–38] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5, were provided by Dr Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan).[39] 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 and MTT assays were performed at the end of the fourth day.

Acknowledgments

We are grateful to Washington University Resource for Biomedical and Bio-Organic Mass Spectrometry for providing mass spectral data and for the partial support of this facility by the National Institutes of Health (Grant No. P41RR0954). This study was supported by Grant CA 39662 from the National Cancer Institute (L.F.L.) and a fellowship grant from the Johnson & Johnson Discovery Research Fund (E.J.L.).

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(*Received in U.S.A.* 16 *November* 1995; *accepted* 31 *January* 1996)