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Substituted Benzo[*i*]phenanthridines as Mammalian Topoisomerase-Targeting Agents

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Abstract—Several benzo[*c*]phenanthridine and protoberberine alkaloids, such as nitidine and berberrubine, are known to induce DNA cleavage in the presence of either topoisomerase I or II. Structure–activity studies performed on various analogues related to benzo[*c*]-phenanthridine and protoberberine alkaloids have provided insights into structural features that influence this topoisomerase-targeting activity. Modifications within the A-ring of benzo[*c*]phenanthridine and protoberberine alkaloids can significantly alter their ability to enhance the cleavable complex formation that occurs between DNA and topoisomerases. Select benzo[*i*]phenanthridines were synthesized as potential bioisosteres of nitidine and its analogues. In the present study, 2,3-methylenedioxy-8,9-dimethoxybenzo[*i*]phenanthridine, 2,3-methylenedioxy-8,9-dimethoxy-5-methylbenzo[*i*]phenanthridine, 2,3,8,9-tetramethoxybenzo[*i*]phenanthridine and 5-methyl-2,3,8,9-tetramethoxybenzo[*i*]phenanthridine were synthesized. These benzo[*i*]phenanthridine derivatives were evaluated for their ability to enhance cleavable complex formation in the presence of topoisomerases and DNA as well as for their cytotoxicity against the human lymphoblastoma cell line, RPMI8402. 2,3-Methylenedioxy-8,9-dimethoxybenzo[*i*]phenanthridine (**4a**) and its 5-methyl derivative (**4b**) are active as topoisomerase I-targeting agents. In contrast to nitidine, the presence of the 5-methyl substituent in the case of **4b** is not associated with enhanced activity. Consistent with previous structure–activity studies on nitidine and protoberberine alkaloids, 2,3,8,9-teramethoxybenzo[*i*]phenanthridine, **5a**, and its 5-methyl derivative, **5b**, are inactive as topoisomerase I-targeting agents. These studies were extended to an evaluation of the relative pharmacological activities of 2,8,9-trimethoxybenzo[*i*]phenanthridine, 3,8,9-trimethoxybenzo[*i*]phenanthridine, and 2,3-methylenedioxy-8,9-methylenedioxybenzo[*i*]phenanthridine.

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-targeting agents 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 agents that target topoisomerase I (TOP1). Recently, bi- and terbenzimidazoles,[7–10] certain benzo[*c*]phenanthridine and protoberberine alkaloids and their synthetic analogues,[11–15] indolocarbazoles,[16] the fungal metabolites, bulgarein[17] and saintopin,[18] as well as derivatives of indenoisoquinoline,[19,20] phenazine[21] and benzophenazine[22] have been identified as TOP1-targeting agents.

Several studies were performed to characterize those structural features of coralyne (**1**), nitidine (**2**), MDD-coralyne (**3**) and related analogues associated with their TOP1-targeting activity.[11,13,23–25] As indicated in Figure 1, a common feature associated with all three of these agents is the presence of a 3-phenylisoquinolinium moi-

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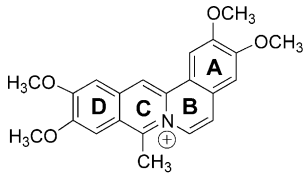
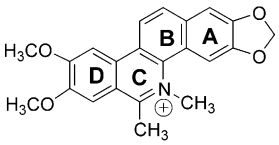
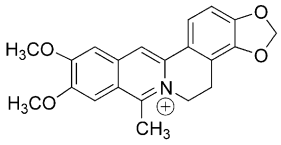


1, Coralyne



2, Nitidine



3, MDD-Coralyne

Figure 1. Structures of coralyne, nitidine and MDD-coralyne.

ety within their structure. It has been speculated in the case of nitidine and related benzo[*c*]phenanthridine alkaloids that the iminium charge is necessary for biological activity.[26–29] Similarly, the charged iminium group is also a common feature of compounds related to the protoberberine alkaloids, such as coralyne, that did exhibit modest antitumor activity.[11,13,21,30,31] Several coralyne analogues with significant TOP1-targeting activity were developed in our laboratory. These analogues also possessed a charged iminium group and proved to be difficult to formulate. Their limited solubility and poor bioavailability hindered efforts to assess their efficacy as antitumor agents in vivo.

Several substituted benz[*a*]acridine and benz[*c*]acridines were synthesized previously in an effort to develop noncharged analogues of coralyne and those protoberberines that are active as topoisomerase-targeting agents.[32] These analogues were prepared in an effort to develop more suitable topoisomerase-targeting agents for evaluation in vivo. While none of the benz[*c*]acridines evaluated in this study was active as either a TOP1- or TOP2-targeting agent, a benz[*a*]acridine derivative did exhibit activity as a TOP1-targeting agent. 5,6-Dihydro-9,10-dimethoxy-3,4-methylenedioxybenz[*a*]-acridine possesses similar TOP1-targeting activity to coralyne. Within this series of compounds, a 3-phenylquinoline moiety replaced the 3-phenylisoquinolinium moiety present in compounds **1**–**3**. These data demonstrated that benz[*a*]acridines could serve as noncharged bioisosteres of protoberberine alkaloids with retention of TOP1-targeting activity.

In the present study, we investigated whether benzo[*i*]-phenanthridines could serve as suitable noncharged bioisosteres for benzo[*c*]phenanthridine alkaloids, such as nitidine. Nitidine is active as a dual toposiomerase-targeting agent, that is, it is capable of stabilizing the cleavable complex that forms between DNA and either TOP1 or TOP2. Benzo[*i*]phenanthridines **4a** and **4b**, which possess a 2,3-methylenedioxy substituent in ring-A and methoxyl groups at positions 8 and 9 in ring-D, were viewed as prime candidates for synthesis and pharmacological evaluation.

Previous structure–activity data associated with several protoberberine alkaloids and related compounds suggest that the replacement of the methylenedioxy moiety within ring-A with 2,3-dimethoxy substituents results in a substantial loss of TOP1-targeting activity.[11,13,23] Similarly, among various benzo[*c*]phenanthridines the presence of 2,3-dimethoxy substituents within ring-A was also associated with a loss of TOP1-targeting activity or cytotoxicity.[25,29–31] We synthesized similarly substituted tetramethoxylated benzo[*i*]phenanthridines, **5a** and **5b,** to probe the extent to which their structure–activity paralleled previous results observed with protoberberine alkaloids and benzo[*c*]phenanthridines. These structure–activity studies were further extended to include the benzo[*i*]phenanthridine derivatives, (**6**–**8**) (Fig. 2).

Chemistry

The method used for the synthesis of the requisite 2-bromo-1-naphthaldhydes is outlined in Scheme 1. These substituted 2-bromo-1-naphthaldehyde and 1-aceto-2-bromonaphthalene derivatives were employed as intermediates for the preparation of variously substituted benzo[*i*]phenanthridines (Schemes 2 and 3).

The synthetic route used for the preparation of compounds **4a** and **5a** is outlined in Scheme 2. The 2-bromo-1-naphthaldehyde intermediates, **13** and **15**, were prepared from appropriately substituted β-tetralones as shown in Scheme 1. Treatment of 6,7-methylenedioxy-β-tetralone (**9**) and 6,7-dimethoxy-β-tetralone (**10**) with dimethylformamide and phosphorus tribromide gave the respective 3,4-dihydro-2-bromonaphthaldehyde derivatives in about 70% yield, which were subsequently oxidized using DDQ in toluene to the respective bromonaphthaldehydes, **13** and **15**. Compounds **13** and **15** could be converted into **14** and **16** respectively by treatment with methylmagnesium bromide followed by PCC oxidation.

Acetals **19** and **20** were obtained in 95% yield by treatment of the respective bromonaphthaldehydes with ethylene glycol in the presence of a catalytic amount of *p*-toluenesulfonic acid as outlined in Scheme 2. A Dean–Stark apparatus was used to remove the water generated during the acetalization reaction. Lithium-halogen exchange was performed by treatment of the acetals **19** and **20** with *n*-butyllithium and then quenched with trimethylborate. Acidic work up of the reaction products resulted in the formation of the boronic acid derivatives, **21** and **22,** respectively. Palladium (0) catalyzed coupling of compounds **21** and **22** with the 2-bromo-4,5-dimethoxynitrobenzene[33] resulted in the formation of the 2-phenylnaphthalene derivatives, **23** and **24,** respectively. Reduction of the nitro groups of **23** and **24** with subsequent intramolecular cyclization resulted in the formation of the desired benzo[*i*]phenanthridines, **4a** and **5a,** respectively, in 54–60% yield.

The 1-aceto-2-bromonaphthalene derivatives, **14** and **16**, were used as intermediates for the preparation of **4b** and **5b**, respectively. The use of *ortho*-nitrophenyl-

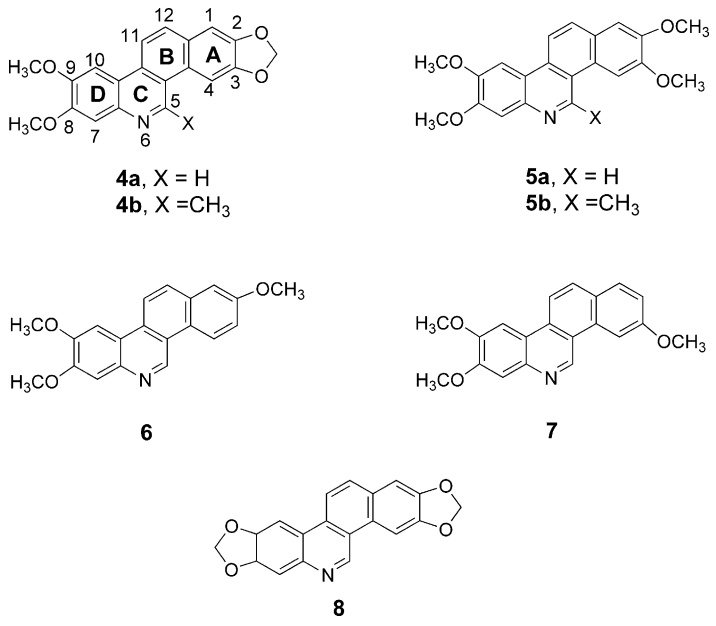
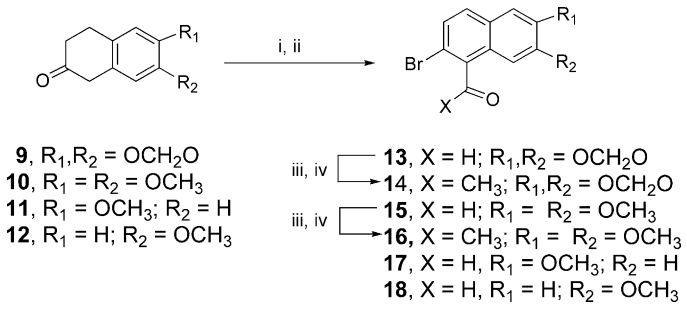
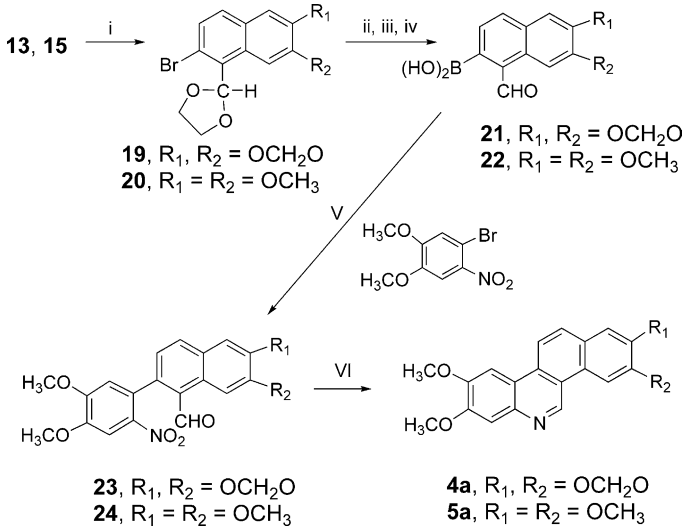


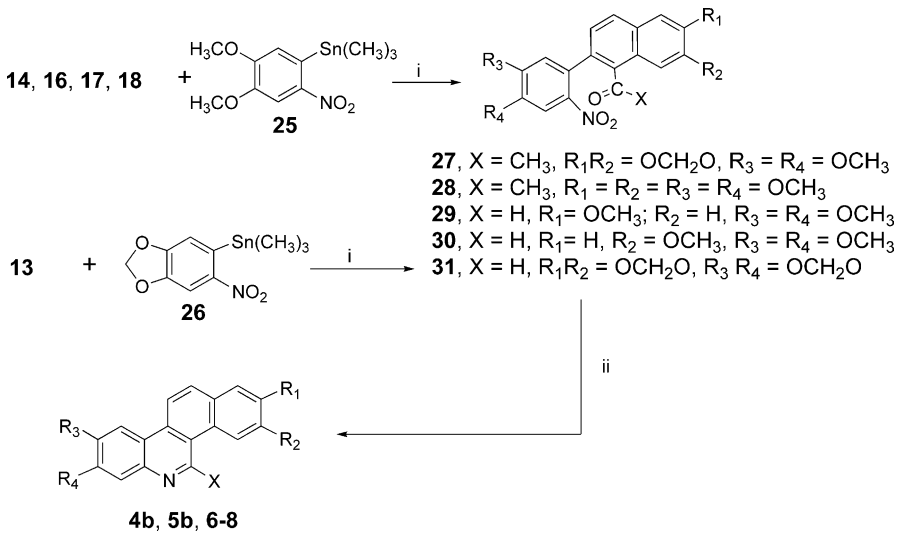
Figure 2. Structures of benzo[*i*]phenanthridine derivatives synthesized and evaluated for pharmacological activity.



Scheme 1. General approach for 2-bromo-1-naphthaldehydes and 2-bromo-1-acetonaphthalene derivatives. (i) DMF, PBr_3, $CHCl_3$; (ii) DDQ, toluene; (iii) CH_3MgBr, THF; (iv) PCC, CH_2Cl_2.



Scheme 2. Synthesis of 8,9-dimethoxy-2,3-methylenedioxy benzo[*i*]phenanthridine, **4a**, and 2,3,8,9-tetramethoxybenzo[*i*]phenanthridine, **5a**. (i) $HOCH_2CH_2OH$; TsOH; (ii) *n*-BuLi; (iii) $B(OCH_3)_3$; (iv) HCl; (v) $Pd(PPh_3)_4$, CuBr, THF; (vi) Zn, HOAc.



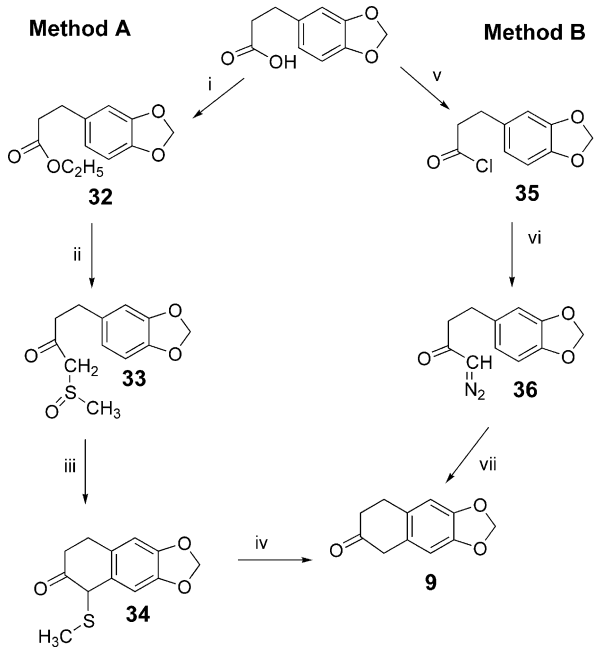
Scheme 3. Preparation of compounds **4b**, **5b**, and **6–8**. (i) $Pd(PPh_3)_4$; CuCN, THF (**27** and **28**) $Pd(PPh_3)_4$; CuBr, THF (**29–31**); (ii) Zn/HOAc.

stannanes has been reported to be a useful method for the preparation of benzo[*i*]phenanthridines.[34] Stille coupling of these ketones with **25**, as outlined in Scheme 3, gave the 1-aceto-2-phenylnaphthalenes **27** and **28,** respectively. Reduction and concomitant cyclization of **27** and **28** occurred on treatment with zinc in acetic acid to provide the desired 5-methyl substituted benzo[*i*]phenanthridines, **4b** and **5b**, respectively.

The synthetic approach outlined in Scheme 3 was also employed for the synthesis of **6–8** based upon the improved overall yields in formation of 2-(2-nitrophenyl)-1-naphthaldehydes observed using the Stille couping reaction. Reaction of trimethyl(2-nitro-4,5-dimethoxyphenyl)stannane, **25**, with **17** and **18** provided the 2-(2-nitrophenyl)-1-naphthaldehydes, **29** and **30**. Trimethyl(2-nitro-4,5-methylenedioxyphenyl)stannane, **26**, was coupled with **13** to form the 2-(2-nitrophenyl)-1-naphthaldehyde, **31**. Treatment of **29–31** with zinc in acetic acid provided the desired benzo[*i*]phenanthridines (**6–8**).

The β-tetralone **9** required for the synthesis of **13** was prepared as illustrated in Scheme 4 using two different routes. In one approach (Method A), 3,4-methylenedioxyphenylpropionic acid was converted to its ethyl ester, **32**. Treatment of **32** with the anion of dimethyl sulfate resulted in the formation of the β-ketosulfoxide, **33**, which cyclized on treatment with trifluoroacetic acid to the 1-methylthio-β-tetralone, **34**.[35] Reductive desulfurization of **34** resulted in the formation of the required 6,7-methylenedioxy-β-tetralone, **9**.

An alternative approach (Method B) for the preparation of **9** involved the formation of the diazoketone, **36**. This method for the preparation of β-tetralones required fewer steps, and resulted in less difficult reaction work-ups and higher overall yield with better reproducibility. As shown in Scheme 4, 3,4-methylenedioxyphenylpropionic acid was treated with thionyl chloride in methylene chloride and a catalytic amount of pyridine was used to form its acid chloride **35**. Treatment of compound **35** with (trimethylsilyl)diazomethane, a commercially available safer alternative to diazomethane,[36] resulted in the formation of diazoketone **36** in good yield. Rhodium(II) acetate catalyzed cyclization led to the formation of 6,7-methlyenedioxy-2-tetralone **9** in about 90% yield (70% after chromatography).[37] It should be noted that the chromatography must be rapid since compound **9** readily oxidizes and substantial loss can occur if this purification step is prolonged.



Scheme 4. Synthetic strategy employed for the preparation of 6,7-methylenedioxy-β-tetralone, **9**. Method A: (i)C_2H_5OH, TMSCl; (ii) $Na^+CH_3SOCH_2$; (iii) CF_3COOH; (iv) H_2, Pd/C. Method B: (v) $SOCl_2$, pyridine, CH_2Cl_2, (vi) $TMSCHN_2$, THF, CH_3CN; (vii) $Rh(OAc)_2CH_2Cl_2$.

Pharmacology

The benzo[*i*]phenanthridine derivatives (**4–8**) synthesized in this study were evaluated for their potential to stabilize the cleavable complex that forms in the presence of either TOP1 or TOP2 and DNA. The results of these assays are summarized in Table 1. The data clearly indicate that appropriately substituted benzo[*i*]phenanthridines can stabilize the cleavable complex formed in the presence of DNA and both TOP1 and TOP2. The more active analogues were those that possessed a methylenedioxy substituent at the 2,3-position. Among the benzo[*i*]phenanthridine evaluated in this study, **4a** and **4b** were the more potent TOP1-targeting derivatives. Both **4a** and **4b** had similar TOP1-targeting activity, exhibiting approximately 10% of the potency of nitidine. There was a notable difference between **4a** and **4b** with regard to their TOP2-targeting activity. While **4a** possesses approximately 10% of the potency of nitidine in stabilizing cleavable complex formed in the presence of DNA and TOP2, **4b** was approximately two orders of magnitude less potent than nitidine. As observed for coralyne derivatives and for various substituted benz[*a*]acridines, the presence of methoxyl substituents at both the 2- and 3-positions of these benzo[*i*]phenanthridines (which are equivalent to the 3 and 4-positions of coralyne and protoberberine alkaloids) was associated with a loss of topoisomerase-targeting activity. Figure 3 illustrates the differences in the potency of **4a** and **4b**, relative to **5a** and **5b** in the TOP1-mediated DNA cleavage assay.

The effect of a single methoxyl group within the A-ring of these benzo[*i*]phenanthridines on activity was also evaluated. In contrast to **5a** and **5b**, the trimethoxylated derivatives, **6** and **7**, did exhibit modest TOP1- and TOP2-targeting activity. While **6** and **7** had similar activities, they were substantially less potent than **4a** or **4b** as TOP1- and TOP2-targeting agents. The presence of both a 2,3- and 8,9-methylenedioxy group on benzo[*i*]phenanthridine, as in **8**, resulted in a derivative that had comparatively poor solubility properties and did not exhibit significant activity as either a TOP1- or TOP2-targeting agent. Figure 4 illustrates the differences in the potency between **6** and **7**, relative to **8** in the TOP1-mediated DNA cleavage assay.

The relative cytotoxic activities of these compounds, determined in the human lymphoblast cell line, RPMI8402, and its camptothecin-resistant variant, CPT-K5, are provided in Table 1. The resistance of CPT-K5 cells to camptothecin is related to a mutant form of TOP1 where aspartate-533 is replaced by glycine.

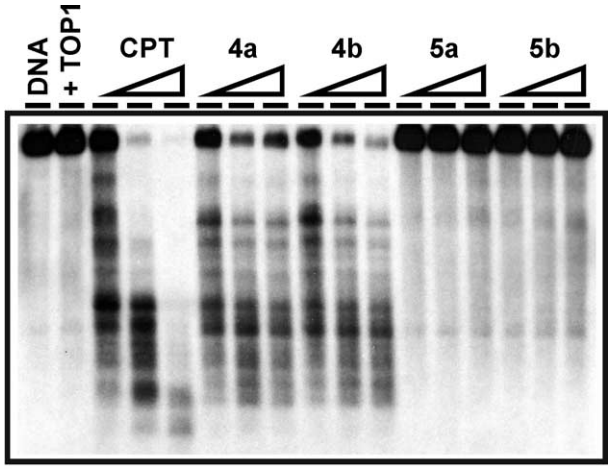


Figure 3. Stimulation of enzyme-mediated DNA cleavage by **4a**, **4b**, **5a**, **5b** and camptothecin (CPT) using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain DNA, human TOP1 and serially (10-fold each) diluted compound from 0.01 to 1.0 μM for CPT and 1.0 to 100 μM for compounds **4a**, **4b**, **5a,** and **5b**. A triangle on top of the lanes is used to indicate the direction of increasing concentration of the compound. The narrow end of the triangle indicates the lowest concentration of the compound.

Table 1. Pharmacological activities of benzo[*i*]phenanthridine derivatives

Compd	TOP1-mediated DNA cleavage[b]	TOP2-mediated DNA cleavage[c]	Cytotoxicity IC_{50} (μM)[a] cell lines	
			RPMI8402	CPT-K5
1	10	(—)[d]	3.9	17
2	1	1	0.4	4.0
3	40	10	5.9	27
4a	10	10	4.5	10.8
4b	10	100	7.6	9.7
5a	(—)[d]	(—)[d]	14	16
5b	(—)[d]	(—)[d]	22	11
6	80	500	5.0	7.5
7	100	500	5.0	8.5
8	(—)[d,e]	(—)[d,e]	>40[e]	>40[e]
CPT	0.1	(—)[d]	0.005	>40
VM-26	(—)[d]	1	0.22	0.28

[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 nitidine, whose value is arbitrarily assumed as 1, that are able to produce approximately 10% cleavage on the plasmid DNA in the presence of human topoisomerase I.
[c]Topoisomerase II cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to VM-26 (teniposide), whose value is arbitrarily assumed as 1, that are able to produce approximately 10% cleavage on the plasmid DNA in the presence of human topoisomerase II.
[d]Compound with a REC value indicating a potency of less than 1% of that of nitidine (**2**) as a TOP1-targeting agent and less than 0.1% of nitidine as a TOP2-targeting agent were designated as not having significant activity, (—).
[e]The extremely poor solubility of this derivative could have limited accurate assessment of its pharmacological activity.

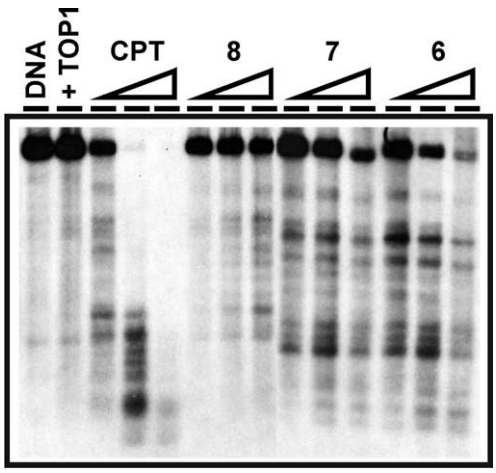


Figure 4. Stimulation of enzyme-mediated DNA cleavage by **6**, **7**, **8** and camptothecin (CPT) using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1, DNA, and serially (10-fold each) diluted compound from 0.01 to 1.0 μM for CPT and 1.0 to 100 μM for compounds **6**, **7**, and **8**. A triangle on top of the lanes is used to indicate the direction of increasing concentration of the compound. The narrow end of the triangle indicates the lowest concentration of the compound.

Camptothecin is not able to stabilize cleavable complex formation between this mutant enzyme and DNA. The least cytotoxic derivatives in RPMI8402 were those that were devoid of any significant topoisomerase-targeting activity. Thus, **5a**, **5b**, and **8** had IC_{50} values $\geq 14\,\mu M$. The differences in topoisomerase-targeting activity among the other benzo[*i*]phenanthridines evaluated, were not reflected in their relative cytotoxicity. As these analogues are able to target both TOP1 and TOP2, major differences in cytotoxicity were not observed for **4a**, **4b**, **6**, and **7** between RPMI8402, and its camptothecin-resistant variant, CPT-K5. The more potent topoisomerase-targeting agents such as nitidine, camptothecin, and VM-26 did exhibit more pronounced cytotoxicity in RPMI8402. In the case of camptothecin was there was a 10,000-fold decrease in its relative cytotoxic potency observed with CPT-K5 relative to RPMI8402 cells. As would be expected in the case of nitidine, the difference in its relative cytotoxicity between these two cell lines is comparatively minor as it can also exert a cytotoxic effect by targeting either TOP1 or TOP2. In the case of VM-26, which acts exclusively as a TOP2-targeting agent, no difference in its relative cytotoxicity was observed between these two cell lines.

Discussion

Protoberberine alkaloids and related compounds can exhibit significant activity as either TOP1- or TOP2-targeting agents. Previous studies have demonstrated that significant specificity for either of these topoisomerases can be achieved by subtle alterations in either substituents or substitution pattern.[11,13,23] Despite the observation that several of these compounds can exhibit similar potency to camptothecin as TOP1-targeting agents, they possess only modest cytotoxic activity.[13,23] In general, there is a poor correlation between cytotoxicity and topoisomerase-targeting activity among these protoberberine derivatives. Several factors could be responsible for this observation. Some coralyne analogues are dual topoisomerase poisons while others are not. In addition, several coralyne analogues possess the iminium moiety. Differences in cellular absorption, therefore, could be a limiting factor associated with the cytotoxicity of various analogues.[38]

The reactivity of the charged iminium moiety toward nucleophilic attack has been proposed as a possible basis for the antileukemic activity of certain benzo[*c*]phenanthridine alkaloids.[26–28,39] It has been successfully demonstrated, however, that stable noncharged analogues of nitidine can be developed that retain activity as topoisomerase poisons and possess potent cytotoxic activity.[19,29] Benz[*a*]acridines structurally-related to protoberberine alkaloids, with the distinct difference of not possessing the cationic iminium group, have been developed that possess topoisomerase I poisoning activity comparable to coralyne.[32] These results prompted our investigation into whether benzo[*i*]phenanthridines could be developed to serve as noncharged analogues of benzo[*c*]phenanthridine alkaloids, such as nitidine, that retain the ability to act as potent dual topoisomerase poisons.

Structure–activity data on coralyne derivatives, protoberberine alkaloids, benzo[*c*]phenanthridines and variously substituted benz[*a*]acridines provided direction in the selection of compounds for evaluation. The 3,4-methylenedioxy substituent on the A-ring of benz[*a*]acridines and protoberberine alkaloids analogues, such as MDD-coralyne, is similarly positioned in regard to molecular topology to a 2,3-methylenedioxy substituent on the A-ring of benzo[*i*]phenanthridines and nitidine. In the case of nitidine and coralyne, the presence of methoxyl groups at the 8,9- and 10,11-positions, respectively, was associated with enhanced TOP1-targeting activity. Based on our studies and previous structure–activity data on benzo[*c*]phenanthridines, it was anticipated that benzo[*i*]phenanthridines with similar substitution and molecular topology would be active as TOP1-targeting agents.

This working hypothesis was validated by the observation that compounds **4a** and **4b**, are able to stabilize the cleavable complex formed between topoisomerase and DNA. As was observed for nitidine, **4a** and **4b** stabilized cleavable complex formation with both TOP1 and TOP2. Previous studies have demonstrated that replacement of the methylenedioxy moiety in similarly substituted protoberberine or benzo[*c*]phenanthridine alkaloids with two methoxyl substituents results in a loss of topoisomerase-targeting activity. The structure–activity relationships observed with these benzo[*i*]phenanthridine derivatives also appears to parallel this phenomenon. Thus, as would be anticipated, evaluation of the 2,3,8,9-tetramethoxy analogues **5a** and **5b** demonstrated that neither of these compounds is active in targeting either TOP1 or TOP2. In contrast to the structure–activity relationships observed with

nitidine analogues, the presence of a 5-methyl substituent in the case of these benzo[*i*]phenanthridine derivatives did not significantly enhance topoisomerase-targeting activity.

It has been suggested that either active transport or hydrolysis of the iminium group of nitidine and coralyne derivatives may be required for cellular absorption.[38,40] In addition, it has been speculated that the cationic charge associated with nitidine and related benzo[*c*]phenanthridine alkaloids is linked to the occurrence of several adverse effects.[40,41] It has also been speculated that the reactivity of the iminium charge on the benzo[*c*]phenantridine ring toward nucleophilic attack may provide an explanation for the weak antitumor activity observed in vivo for various nitidine analogues.[42] As compounds **4a** and **4b** are noncharged, it was hoped that their decreased intrinsic activity as topoisomerase-targeting agents relative to nitidine would be off-set by increased cellular absorption and cytotoxicity. This, however, was not observed in the present studies performed using RPMI8402. Compounds **4a** and **4b** had only one-tenth the relative cytotoxicity of nitidine in this assay. While there were significant differences between the intrinsic topoisomerase-targeting activities of **6** and **7** as compared to **4a,** these derivatives had similar cytotoxicity. These data suggest that in the absence of potent intrinsic topoisomerase-targeting activity other mechanisms may begin to substantially contribute to the observed cytotoxicity. Studies in our laboratory suggest an improved correlation between cytotoxic activity and intrinsic TOP1-targeting activity is generally realized with compounds that (1) possess at least 10% of the intrinsic potency of camptothecin as a TOP1-targeting agent and (2) are selective as TOP1-targeting agents.

Coralyne and nitidine have not proved to be highly effective as antitumor agents in vivo. It is likely that these charged compounds are subject to several dynamics within mammalian systems that limit their absorption and bioavailability. Noncharged compounds, such as substituted benzo[*i*]phenanthridines, may represent an alternative class of TOP1-targeting agents that is free of some of the limitations associated with these positively charged alkaloids. Studies are in progress to develop benzo[*i*]phenanthridines with enhanced intrinsic TOP1-targeting activity and cytotoxicity.

Experimental

Chemistry

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, Eschwege, Ger.) 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 $CDCl_3$ 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. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within ±0.4% of the theoretical value. Compounds **10–12** were purchased from Aldrich Chemical Co. Compounds **25** and **26** were prepared as previously described.[34]

General procedure for the synthesis of benzo[*i*]phenanthridine derivatives (4a, 4b, 5a, 5b, 6, 7, 8). The respective 2-(*ortho*-nitrophenyl)-1-naphthaldehyde or 2-(*ortho*-nitrophenyl)-1-acetonaphthalene (100 mg) was dissolved in glacial acetic acid (8 mL) and heated to reflux with zinc dust (200 mg) for 4 h. Acetic acid was evaporated in vacuo and the resulting residue extracted with chloroform. The chloroform solution was filtered through a Celite bed. The filtrate was washed successively with saturated sodium bicarbonate solution and brine and evaporated to dryness. The residue obtained was chromatographed on 75 g of silica using a 1:1 mixture of hexanes:ethyl acetate as eluent.

2,3-Methylenedioxy-8,9-dimethoxybenzo[*i*]phenanthridine (4a). Prepared from compound **23** in 60% yield; mp >250 °C; IR (Nujol): 1680; UV ($CHCl_3$): 285, 360, 380 nm (log ε = 3.01, 2.21, 2.47); ^1H NMR: δ 4.10 (3H, s), 4.14 (3H, s), 6.15 (2H, s), 7.28 (1H, s), 7.68 (1H, s), 7.83 (1H, s), 7.97 (1H, d, J = 9.2), 8.16 (1H, s), 8.28 (1H, d, J = 9.2), 9.85 (1H, s); ^{13}C NMR: δ 56.7, 100.3, 102.1, 102.2, 106.1, 109.2, 118.4, 127.5, 128.9, 131.2, 131.7, 140.8, 145.6, 145.7, 148.4, 149.9, 150.4, 151.7, 176.1; HRMS m/z calcd for $C_{20}H_{15}NO_4$: 333.1001; found: 333.0999.

8,9-Dimethoxy-5-methyl-2,3-methylenedioxybenzo[*i*]phenanthridine (4b). Prepared from compound **27** in 63% yield; mp >250 °C; IR (Nujol): 1685; UV ($CHCl_3$): 280, 365, 385 nm (log ε = 2.79, 1.90, 2.03); ^1H NMR: δ 3.36 (3H, s), 4.08 (3H, s), 4.13 (3H, s), 6.15 (2H, s), 7.31 (1H, s), 7.51 (1H, s), 7.80 (1H, s), 7.93 (1H, d, J = 9.1 Hz), 8.29–8.35 (2H, m); ^{13}C NMR: δ 31.7, 56.6, 102.1, 105.8, 106.3, 108.8, 118.5, 119.1, 122.9, 127.9, 130.2, 131.3, 132.7, 137.1, 140.3, 147.3, 148.8, 149.7, 151.7, 155.2; HRMS m/z calcd for $C_{21}H_{17}NO_4$: 347.1158; found: 347.1156.

2,3,8,9-Tetramethoxybenzo[*i*]phenanthridine (5a). Prepared from compound **24** in 54% yield; mp 267–268 °C; IR (Nujol): 1621, 1513, 1282; ^1H NMR: δ 4.04 (3H, s), 4.06 (3H, s), 4.10 (3H, s), 4.13 (3H, s), 7.22 (1H, s), 7.55 (1H, s), 7.74 (1H, s), 7.89 (1H, d, J = 8.8 Hz), 8.05 (1H, s), 8.18 (1H, d, J = 8.8 Hz), 9.82 (1H, s); ^{13}C NMR: δ 56.4, 56.6, 102.0, 102.3, 108.5, 109.9, 118.3, 118.4, 120.7, 120.8, 125.7, 127.5, 130.7, 141.5, 145.9, 150.0, 150.1, 150.9, 151.4; HRMS m/z calcd for $C_{21}H_{19}NO_4$: 349.1314; found: 349.1321.

5-Methyl-2,3,8,9-tetramethoxybenzo[*i*]phenanthridine (5b). Prepared from compound **28** in 60% yield; mp 255–257 °C; IR (Nujol): 1620, 1513, 1209; ^{1}H NMR: δ 3.42 (3H, s), 4.08 (6H, s), 4.13 (6H, s), 7.33 (1H, s), 7.52 (1H, s), 7.82 (1H, s), 7.98 (1H, d, J = 8.8 Hz), 8.32 (1H, s), 8.35 (1H, d, J = 8.8 Hz); ^{13}C NMR: δ 31.6, 56.4, 56.5, 56.6, 102.1, 108.7, 108.8, 108.9, 118.6, 119.0, 122.2, 126.5, 129.0, 130.9, 132.6, 140.4, 149.1, 149.6, 149.7, 151.7, 154.9; HRMS m/z calcd for $C_{22}H_{21}NO_4$: 363.1470; found: 363.1471.

2,8,9-Trimethoxybenzo[*i*]phenanthridine (6). Prepared from compound **29** in 50% yield; mp 207–209 °C; IR (KBr): 1620, 1487, 1215; ^{1}H NMR: δ 3.99 (3H, s), 4.10 (3H, s), 4.14 (3H, s), 7.33 (1H, d, J = 2.6 Hz), 7.40 (1H, dd, J = 9.2, 2.6 Hz), 7.62 (1H, s), 7.84 (1H, s), 8.02 (1H, d, J = 9.0 Hz), 8.41 (1H, d, J = 9.0 Hz), 8.77 (1H, d, J = 9.2 Hz), 9.99 (1H, s); ^{13}C NMR: δ 56.0, 56.5, 56.6, 101.9, 108.8, 110.0, 119.5, 120.8, 121.7, 124.0, 125.1, 130.4, 131.3, 133.6, 141.8, 146.0, 151.5, 158.8; HRMS m/z calcd for $C_{20}H_{17}NO_3$ + H: 320.1287; found: 320.1288.

3,8,9-Trimethoxybenzo[*i*]phenanthridine (7). Prepared from compound **30** in 52% yield; mp 224 °C; IR (KBr): 1615, 1502, 1220; ^{1}H NMR: δ 4.07 (3H, s), 4.10 (3H, s), 4.14 (3H, s), 7.30 (1H, dd, J = 8.8, 2.4 Hz), 7.63 (1H, s), 7.86 (1H, s), 7.90 (1H, d, J = 8.8 Hz), 8.03 (1H, d, J = 8.8 Hz), 8.19 (1H, d, J = 2.4 Hz), 8.27 (1H, d, J = 8.8 Hz), 9.97 (1H, s); ^{13}C NMR: δ 56.0, 56.6, 102.2, 103.0, 109.9, 117.6, 117.9, 120.0, 121.5, 127.0, 130.8, 131.6, 132.3, 142.3, 146.1, 150.4, 152.1, 159.9; HRMS m/z calcd for $C_{20}H_{17}NO_3$ + H: 320.1287; found: 320.1293.

2,3 - Methylenedioxy - 8,9 - methylenedioxybenzo[*i*]phenanthridine (8). Prepared from compound **31** in 40% yield; mp > 250 °C; IR (KBr) 1474, 1386, 1279, 1194; ^{1}H NMR: δ 6.17 (2H, s), 6.18 (2H, s), 7.31 (1H, s), 7.58 (1H, s), 7.92 (1H, s), 7.98 (1H, d, J = 9.2 Hz), 8.20 (1H, s), 8.27 (1H, d, J = 9.2 Hz), 9.88 (1H, s); ^{13}C NMR: δ 99.8, 100.4, 102.1, 102.3, 106.2, 107.7, 118.8, 123.9, 127.0, 127.9, 130.9, 131.3, 146.2, 148.3, 149.7, 151.3, 152.0, 152.9, 154.4; HRMS m/z calcd for $C_{19}H_{11}NO_4$: 317.0688: found: 317.0686.

6,7-Methylenedioxy-2-tetralone (9): Method A. A solution of **34** (669 mg, 2.83 mmol) in glacial acetic acid (10 mL) was placed in a hydrogenation flask. To this mixture 460 mg of 10% Pd-C was added and the resulting 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 rotaevaporated to give the crude tetralone, **9**. The crude tetralone was then stirred vigorously with sodium bisulfite (2.0 g in 7.0 mL H_2O and 4.0 mL EtOH) 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 91–92 °C (lit[43] = 88–91 °C).

6,7-Methylenedioxy-2-tetralone (9): Method B. A dichloromethane solution of compound **36** was added dropwise to a solution of rhodium(II) acetate in dichloromethane (15 mL). The resulting mixture was refluxed under nitrogen for 1 h. The reaction mixture was evaporated in vacuo to give **9** as deep yellow crystals in 90% yield, which was typically used without further purification, mp 91–92 °C (lit[43] mp 88–91 °C); IR (Nujol): 1727; ^{1}H NMR: δ 2.52 (2H, t, J = 6.9 Hz), 2.96 (2H, t, J = 6.9 Hz), 3.48 (2H, s), 5.93 (2H, s), 6.60 (1H, s), 6.71 (1H, s); ^{13}C NMR: δ 28.7, 38.7, 45.4, 101.4, 108.7, 118.9, 121.7, 126.6, 130.3, 147.0, 210.9; anal. calcd for $C_{11}H_{10}O_3$: C, 69.46, H, 5.30; found: C, 69.40, H, 5.29.

General procedure for the synthesis of 2-bromo-1-naphthaldehydes (13, 15, 17, 18). Dimethylformamide (3.0 g, 41 mmol) was added dropwise to solution of phosphorus tribromide (3.3 mL, 35 mmol) in dry chloroform (50 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h to give a pale yellow suspension. A solution of the appropriately substituted β-tetralone (2.0 g, 9.7 mmol) in chloroform was added to the yellow suspension and the mixture was heated at reflux for 1 h. The reaction mixture was cooled to 0 °C and saturated aqueous $NaHCO_3$ solution was added dropwise until no effervescence was obtained. The resulting mixture was extracted with dichloromethane, dried (Na_2SO_4) and evaporated to provide the respective 2-bromo-3,4-dihydro-1-naphthaldehyde derivatives as yellow solids. Each of the 2-bromo-3,4-dihydro-1-naphthaldehyde derivatives were chromatographed on silica gel using a 3:1 mixture of hexanes:ethyl acetate as the eluent to give the respective 2-bromo-3,4-dihydro-1-naphthaldehydes as determined by ^{1}H NMR. Without further characterization, these 2-bromo-3,4-dihydro-1-naphthaldehydes (2.4 g, 8.1 mmol) were mixed with DDQ (2.2 g, 97 mmol) in toluene (50 mL) and refluxed for 15 h. After cooling to room temperature, the mixture was subjected to filtration through a Celite bed and the filtrate was evaporated to dryness. The residue obtained was chromatographed on 75 g silica gel using a 3:1 mixture of hexanes–ethyl acetate as eluent to give the respective 2-bromo-1-naphthaldehydes in good yield.

2-Bromo-6,7-methylenedioxy-1-naphthaldehyde (13). Prepared from **9** in 96% yield; mp 165–166 °C; IR (Nujol): 1680; ^{1}H NMR: δ 6.09 (2H, s), 7.05 (1H, s), 7.51 (1H, d, J = 8.7 Hz), 7.63 (1H, d, J = 8.7 Hz), 8.60 (1H, s), 10.68 (1H, s); ^{13}C NMR: δ 102.1, 102.3, 104.6, 127.4, 129.5, 129.6, 130.1, 131.1, 134.6, 148.6, 151.7, 195.5; anal. calcd for $C_{12}H_7O_3Br$: C, 51.60, H, 2.51; found: C, 51.98, H, 2.48.

2-Bromo-6,7-dimethoxy-1-naphthaldehyde (15). Prepared from **10** in 95% yield; mp 141–142 °C; ^{1}H NMR: δ 4.00 (3H, s), 4.05 (3H, s), 7.07 (1H, s), 7.54 (1H, d, J = 8.5 Hz), 7.79 (1H, d, J = 8.5 Hz), 8.71 (1H, s), 10.74 (1H, s); ^{13}C NMR: δ 56.3, 56.6, 104.2, 107.0, 126.5, 128.8, 129.4, 129.8, 129.9, 134.3, 150.4, 153.1, 195.9; HRMS m/z calcd for $C_{13}H_{11}O_3Br$: 293.9891; found: 293.9896.

2-Bromo-6-methoxy-1-naphthaldehyde (17). Prepared from compound **11** in 50% yield; mp 105–107 °C; IR (KBr): 1672; ^{1}H NMR: δ 3.92 (3H, s), 7.09 (1H, d,

$J = 2.6$ Hz), 7.29 (1H, dd, $J = 9.4$, 2.6 Hz), 7.61 (1H, d, $J = 8.8$ Hz), 7.73 (1H, d, $J = 8.8$ Hz), 8.99 (1H, d, $J = 9.4$ Hz), 10.70 (1H, s); ^{13}C NMR: δ 56.8, 106.9, 122.3, 126.8, 127.5, 128.2, 128.7, 131.6, 134.6, 135.1, 158.7, 195.4; HRMS *m/z* calcd for $C_{12}H_9BrO_2$: 263.9786; found: 263.9782.

2-Bromo-7-methoxy-1-naphthaldehyde (18). Prepared from compound **12** in 62% yield; mp 132–134 °C; IR (KBr): 1670; ^{1}H NMR: δ 3.97 (3H, s), 7.21 (1H, dd, $J = 8.9$, 2.6 Hz), 7.54 (1H, d, $J = 8.7$ Hz), 7.71 (1H, d, $J = 8.9$ Hz), 7.77 (1H, d, $J = 8.7$ Hz), 8.69 (1H, d, $J = 2.6$ Hz), 10.77 (1H, s); ^{13}C NMR: δ 56.0, 103.6, 120.5, 126.8, 128.9, 129.1, 130.3, 132.8, 134.2, 135.8, 161.6, 195.7; HRMS *m/z* calcd for $C_{12}H_9BrO_2$: 263.9786; found: 263.9783.

General procedure for the synthesis of 1-aceto-2-bromonaphthalene derivatives (14, 16). A 1.4 M solution of methylmagnesium bromide in tetrahydrofuran (16.8 mL, 16.8 mmol) was added to a solution of the respective naphthaldehydes (**13** and **15**; 715 mg; 2.56 mmol) in dry tetrahydrofuran (20 mL) at 0 °C under nitrogen. The reaction mixture was stirred for 1 h at 0 °C. The reaction was quenched by dropwise addition of water (100 mL) followed by rapid addition of 0.1 N hydrochloric acid (50 mL). The reaction mixture was extracted with ethyl acetate, dried and the solvent was evaporated in vacuo to give an oily residue. The oil was triturated with chloroform to give the respective alcohol derivatives as white needle shaped crystalline solids in 95% and 97% yield respectively. These benzyl alcohols were not characterized, but were treated as formed with pyridinium chlorochromate (857 mg, 3.98 mmol) suspended in dry dichloromethane (10 mL). The respective alcohols (2.4 mmol) were added to this suspension and the resulting mixture was stirred under nitrogen at room temperature for 6 h. Ether (100 mL) was added to the reaction mixture and the suspension obtained was filtered through a Celite bed. The filtrate was evaporated to dryness and the residue obtained was chromatographed on 75 g silica gel using a 1:9 mixture of ethyl acetate–hexanes to give the respective 1-aceto-2-bromonaphthalene derivatives (**14** and **16**) as fluffy bright white needles in 85% and 90% yield, respectively.

1-Aceto-2-bromo-6,7-methylenedioxynaphthalene (14). Prepared from **13** in 90% yield; mp 138 °C; IR (Nujol): 1695; ^{1}H NMR: δ 2.67 (3H, s), 6.06 (2H, s), 6.89 (1H, s), 7.09 (1H, s), 7.41 (1H, d, $J = 8.8$ Hz), 7.56 (1H, d, $J = 8.8$ Hz); ^{13}C NMR: δ 32.4, 101.0, 102.1, 104.9, 113.6, 127.6, 128.4, 129.6, 130.1, 139.8, 148.6, 149.7, 205.2; HRMS *m/z* calcd for $C_{13}H_9O_3Br$: 291.9735; found: 291.9730.

1-Aceto-2-bromo-6,7-dimethoxynaphthalene (16). Prepared from **15** in 85% yield; mp 102–103 °C; IR (Nujol): 1693; ^{1}H NMR: δ 2.70 (3H, s), 3.95 (3H, s), 3.99 (3H, s), 6.84 (1H, s), 7.10 (1H, s), 7.43 (1H, d, $J = 8.4$ Hz), 7.56 (1H, d, $J = 8.4$ Hz); ^{13}C NMR: δ 32.4, 56.4, 103.1, 107.2, 113.3, 126.3, 128.2, 128.7, 129.1, 139.0, 150.4, 151.4, 205.5; HRMS *m/z* calcd for $C_{14}H_{13}O_3Br$: 308.0048; found: 308.0059.

General procedure for the synthesis of 2-bromonaphthaldehyde-1-acetals (19, 20). The respective 2-bromo-1-naphthaldehyde derivatives (1.95 mmol), ethylene glycol (0.7 mL) and *p*-toluenesulfonic acid (10 mg) were dissolved in dry toluene (30 mL). This reaction mixture refluxed under nitrogen in a flask fitted with a Dean-Stark apparatus to remove the water formed during acetal formation. At the end of the reaction (15 h), the solvent from the cooled reaction mixture was evaporated in vacuo and the residue obtained was dissolved in ethyl acetate (50 mL). The ethyl acetate solution was washed with a saturated solution of sodium bicarbonate, dried and the solvent was removed to give the crude acetals. Chromatography on silica gel using a 3:17 mixture of ethyl acetate–hexanes afforded the pure acetals as clear viscous liquids in good yield.

2-Bromo-6,7-methylenedioxynaphthaldehyde-1-ethylacetal (19). Prepared from **13** in 95% yield; IR (Nujol): 1665, 1617; ^{1}H NMR: δ 4.11–4.18 (2H, m), 4.36–4.43 (2H, m), 6.03 (2H, s), 6.56 (1H, s), 7.05 (1H, s), 7.41–7.48 (2H, m), 7.73 (1H, s); ^{13}C NMR: δ 65.6, 101.8, 102.6, 104.8, 106.3, 123.0, 128.0, 129.3, 130.4, 131.1, 131.5, 147.9, 148.6; anal. calcd for $C_{14}H_{11}O_4Br$: C, 52.10, H, 3.41; found: C, 52.52, H, 3.48.

2-Bromo-6,7-dimethoxynaphthaldehyde-1-ethylacetal (20). Prepared from **15** in 95% yield; IR (Nujol): 1660, 1635; ^{1}H NMR: δ 4.02 (3H, s), 4.06 (3H, s), 4.13–4.19 (2H, m), 4.38–4.48 (2H, m), 7.09 (1H, s), 7.54–7.68 (2H, m), 7.72 (1H, s), 7.78 (1H, s); ^{13}C NMR: δ 56.2, 56.6, 65.7, 102.8, 104.6, 106.5, 122.9, 128.4, 129.1, 130.5, 131.2, 131.3, 148.0, 148.5; anal. calcd for $C_{15}H_{15}O_4Br$: C, 53.11, H, 4.48; found: C, 52.93, H, 4.51.

Preparation of 2-(2-nitro-3,4-dimethoxyphenyl)-6,7-methylenedioxy-1-naphthaldehyde, 23, and 2-(2-nitro-3,4-dimethoxyphenyl)-6,7-dimethoxy-1-naphthaldehyde, (24). Acetals (**19** and **20**, 1.4 mmol) were each dissolved in anhydrous tetrahydrofuran (10 mL). This solution was stirred under nitrogen at −78 °C. A hexanes solution of *n*-butyllithium (1.2 mL, 2.8 mmol) was added slowly and the reaction mixture was stirred at −78 °C for 30 min. A pale yellowish brown solution was obtained. To this yellow reaction mixture trimethylborate (0.5 mL, 4.2 mmol) was added and the resulting mixture was stirred at −78 °C for 1 h prior to allowing it to come to room temperature. A 5% solution of hydrochloric acid (20 mL) was added to the reaction mixture and stirred for 30 min at room temperature. The tetrahydrofuran was evaporated in vacuo and the water layer was extracted with dichloromethane. The combined organic layer was washed once with brine, dried and evaporated to give the respective crude boronic acid derivatives (**21** and **22**). In a 3 neck flask, 2-bromo-4,5-dimethoxynitrobenzene (876 mg, 3.34 mmol) was taken with 430 mg of tetrakis(triphenylphosphine)palladium (0) in dimethoxyethane (20 mL) and the resulting solution was stirred at room temperature under nitrogen for 30 min. The solution changed color from brown to yellow. A solution of the respective crude boronates (1.1 mmol) in dimethoxyethane (5 mL) was added to the reaction mixture followed by the addition of 2 M sodium

carbonate (5 mL). The reaction mixture was refluxed for 18 h. The reaction mixture was allowed to come to room temperature and the reaction mixture was filtered through a Celite bed. The filtrate was evaporated to dryness and the residue obtained was column chromatographed on 75 g silica gel using 1:9 mixture of ethyl acetate–hexanes to give the respective 2-phenyl-1-naphthaldehyde derivatives, **23** and **24** in 25 and 22% overall yield respectively, based on the acetals.

2-(3,4-Dimethoxy-6-nitrophenyl)-6,7-methylenedioxy-1-naphthaldehyde (23). Prepared from **19** in 25% yield; mp 225 °C; IR (Nujol): 1670, 1515, 1309; ^{1}H NMR: δ 3.92 (3H, s), 4.04 (3H, s), 6.13 (2H, s), 6.74 (1H, s), 7.13 (1H, d, J = 8.3 Hz), 7.18 (1H, s), 7.77 (1H, s), 7.87 (1H, d, J = 8.3 Hz), 8.76 (1H, s), 10.14 (1H, s); ^{13}C NMR: δ 57.0, 57.1, 102.1, 103.1, 104.7, 108.2, 114.9, 125.7, 127.9, 128.8, 129.5, 131.5, 133.6, 141.2, 144.1, 148.6, 149.2, 151.6, 152.9, 193.6.

2-(3,4-Dimethoxy-6-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (24). Prepared from **20** in 22% yield; mp 228–230 °C; IR (Nujol): 1676, 1513, 1259. ^{1}H NMR: δ 3.93 (3H, s), 4.05 (3H, s), 4.09 (3H, s), 6.76 (1H, s), 7.16 (1H, d, J = 8.1 Hz), 7.19, (1H, s). 7.78 (1H, s), 7.92 (1H, d, J = 8.1 Hz), 8.66 (1H, s), 10.17 (1H, s); ^{13}C NMR: δ 56.3, 56.6, 57.0, 57.1, 105.2, 107.1, 108.2, 115.0, 125.5, 127.0, 127.4, 129.6, 130.3, 133.2, 141.3, 144.3, 149.2, 150.4, 152.9, 153.1, 193.9; HRMS m/z calcd for $C_{21}H_{19}NO_7$-CHO: 368.1134; found: 368.1130.

General procedure for the synthesis of 1-aceto-2-(3,4-dimethoxy-6-nitrophenyl)naphthalene derivatives (27,28), 2-(2-nitro-3,4-dimethoxyphenyl)-1-naphthaldehydes (29–30), and 2-(nitro-3,4-methylenedioxyphenyl)-6,7-methylenedioxy-1-naphthaldehyde (31). A mixture containing either **25** or **26** (0.98 mmol) and the respective 1-aceto-2-bromonaphthalene derivatives (**14** or **16,** 0.89 mmol), $(Ph_3P)_4Pd$ (106 mg) together with copper cyanide (17 mg) or in the case of the 2-bromonaphthaldehdes (**13**, **17** or **18**, 0.89 mmol) with cuprous bromide (10 mg) were refluxed in toluene (20 mL) under nitrogen for 20–24 h. After cooling to room temperature, ethyl acetate (20 mL) was added to the reaction mixture and the organic layer was poured into a separating funnel. The organic layer was washed with distilled water (20 mL). The two phases were allowed to separate as much as possible and the aqueous layer was discarded. The remaining emulsion was passed through a Celite bed. The organic layer was separated from the filtrate and evaporated to dryness. The residue obtained was chromatographed on 75 g silica gel using a 3:2 mixture of hexanes:ethyl acetate.

1-Aceto-2-(3,4-dimethoxy-6-nitrophenyl)-6,7-methylenedioxynaphthalene (27). Prepared from **14** in 20% yield; mp 190–192 °C; IR (Nujol): 1680, 1523, 1300; ^{1}H NMR: δ 2.23 (3H, s), 3.89 (3H, s), 4.01 (3H, s), 6.07 (2H, s), 6.75 (1H, s), 7.06 (1H, d, J = 8.4 Hz), 7.10 (1H, s), 7.16 (1H, s), 7.66 (2H, m); ^{13}C NMR: δ 32.6, 56.9, 57.1, 101.7, 102.6, 105.0, 108.4, 115.6, 125.1, 126.0, 128.7, 129.5, 130.8, 131.5, 133.8, 144.5, 149.6, 150.5, 150.7, 152.8, 207.6.

1-Aceto-2-(3,4-dimethoxy-6-nitrophenyl)-6,7-dimethoxynaphthalene (28). Prepared from **16** in 30% yield; mp 185–187 °C; IR (Nujol): 1689, 1503, 1260; ^{1}H NMR: δ 2.25 (3H, s), 3.90 (3H, s), 3.98 (3H, s), 4.02 (3H, s), 4.03 (3H, s), 6.76 (1H, s), 7.08 (1H, d, J = 8.4 Hz), 7.12 (1H, s), 7.18 (1H, s), 7.70 (1H, s), 7.71 (1H, d, J = 8.4 Hz); ^{13}C NMR: δ 32.7, 56.4, 56.9, 57.0, 57.1, 103.8, 107.2, 108.5, 115.7, 124.9, 125.1, 128.2, 129.5, 130.1, 131.6, 136.9, 141.1, 149.0, 150.5, 151.2, 152.8, 208.0; HRMS m/z calcd for $C_{21}H_{19}NO_7$-C_2H_3O: 368.1134; found: 368.1135.

2-(2-Nitro-3,4-dimethoxyphenyl)-6-methoxy-1-naphthaldehyde (29). Prepared from compound **17** in 70% yield; mp 158–160 °C; IR (KBr): 1677, 1518, 1328; ^{1}H NMR: δ 3.92 (3H, s), 3.97 (3H, s), 4.05 (3H, s), 6.74 (1H, s), 7.20 (1H, d, J = 2.7 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.35 (1H, dd, J = 9.2, 2.7 Hz), 7.77 (1H, s), 7.94 (1H, d, J = 8.4 Hz), 9.15 (1H, d, J = 9.2 Hz), 10.20 (1H, s); ^{13}C NMR: δ 55.8, 57.0, 57.1, 107.0, 108.3, 115.0, 122.3, 126.4, 127.7, 127.9, 128.7, 129.4, 133.5, 135.5, 141.2, 143.0, 149.2, 153.0, 158.7, 193.6; HRMS m/z calcd for $C_{20}H_{17}NO_6$ + H: 368.1134; found: 368.1129.

2-(2-Nitro-3,4-dimethoxyphenyl)-7-methoxy-1-naphthaldehyde (30). Prepared from compound **18** in 70% yield; mp 203–205 °C; IR (KBr): 1677, 1513, 1325; ^{1}H NMR: δ 3.91 (3H, s), 4.00 (3H, s), 4.04 (3H, s), 6.75 (1H, s), 7.15 (1H, d, J = 8.2 Hz), 7.26 (1H, dd, J = 8.9, 2.6 Hz), 7.79 (1H, s), 7.81 (1H, d, J = 8.9 Hz), 8.00 (1H, d, J = 8.2 Hz), 8.81 (1H, d, J = 2.6 Hz), 10.20 (1H, s); ^{13}C NMR: δ 55.5, 56.5, 56.6, 104.0, 107.7, 114.2, 119.9, 124.3, 126.6, 128.9, 129.1, 129.8, 132.3, 134.2, 140.5, 146.3, 148.7, 152.4, 161.0, 193.2; HRMS m/z calcd for $C_{20}H_{17}NO_6$ + H: 368.1134; found: 368.1127.

2-(2-Nitro-3,4-methylenedioxyphenyl)-6,7-methylenedioxy-1-naphthaldehyde (31). Prepared from compound **13** in 36% yield; mp 192–193 °C; ^{1}H NMR: δ 6.12 (2H, s), 6.20 (2H, s), 6.76 (1H, s), 7.11 (1H, d, J = 8.4 Hz), 7.17 (1H, s), 7.66 (1H, s), 7.85 (1H, d, J = 8.4 Hz), 8.73 (1H, s), 10.19 (1H, s); ^{13}C NMR: δ 102.2, 103.1, 103.9, 104.7, 106.0, 112.3, 125.5, 127.9, 128.9, 131.5, 131.6, 133.7, 143.0, 143.6, 148.5, 148.6, 151.6, 151.7, 193.3.

Ethyl 3,4-(methylenedioxy)dihydrocinnamate (32). Trimethylsilyl chloride (2.5 mL, 18.2 mmol) was added to a solution of 3,4-(methylenedioxy)dihydrocinnamic acid (1.5 g, 7.7 mmol) in dry ethanol (70 mL) 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 **32** as a colorless liquid; ^{1}H NMR: δ 1.21 (3H, t), 2.60 (2H, t), 2.87 (2H, t), 4.08 (2H, q, J = 14.1, 7.3 Hz), 5.90 (2H, s), 6.56–6.69 (3H, m); ^{13}C NMR: δ 14.5, 25.1, 34.2, 60.7, 100.8, 106.9, 121.8, 122.2, 122.5, 145.3, 147.1, 173.2; HRMS m/z calcd for $C_{12}H_{14}O_4$: 222.0892; found: 222.0895.

3,4-(Methylenedioxy)phenethylmethylsulfinylmethyl ketone (33). The anion of dimethyl sulfoxide was prepared by adding dry dimethyl sulfoxide (6.0 mL) to

sodium hydride (600 mg) 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. A solution of **32** (1.0 g, 4.54 mmol) in dry dimethyl sulfoxide (6.0 mL) was added dropwise, over a period of 15 min, to the dimethyl sulfoxide anion generated previously. The reaction mixture was slowly allowed to come to room temperature and stirred for 2 h. The reaction mixture was poured into cold water (100 mL) 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 **33** as a low melting buff colored solid. Compound **33** 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 for the synthesis of **34** without further purification; mp 60–62 °C; ^{1}H NMR: δ 2.55 (3H, s), 2.75–2.82 (4H, m), 3.56–3.76 (2H, q, $J_1 = 13.8$, $J_2 = 34.3$ Hz), 5.82 (2H, s), 6.53–6.65 (3H, m); ^{13}C NMR: δ 29.3, 41.4, 47.4, 64.4, 101.3, 108.7, 109.3, 121.6, 134.4, 146.4, 148.1, 202.1.

1,2,3,4-Tetrahydro-1-methylthio-6,7-methylenedioxy-2(1*H*)-napthalenone (34). Trifluoroacetic acid (0.3 mL, 3.7 mmol) was dissolved in benzene (25 mL) and added to the reaction flask containing **34** (470 mg, 1.85 mmol). The reaction mixture was 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 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 **34** in 60% yield; mp 52 °C; ^{1}H NMR: δ 2.05 (3H, s), 2.70–3.10 (4H, m), 4.02 (1H, s), 5.85 (1H, s), 6.65 (1H, d, $J = 8.1$ Hz), 6.72 (1H, d, $J = 8.1$ Hz); ^{13}C NMR: δ 16.3, 21.1, 34.2, 54.1, 101.6, 107.9, 118.5, 123.0, 127.6, 145.1, 147.3, 203.5; HRMS m/z calcd for $C_{12}H_{12}O_3S$: 236.0507; found: 236.0505.

3-(3,4-Methylenedioxyphenyl)propionyl chloride (35). 3-(3,4-Methylenedioxyphenyl)propionic acid (600 mg, 3.0 mmol) was suspended in methylene chloride (10 mL) and a catalytic amount of pyridine (1 drop) was added. Thionyl chloride (0.27 mL, 3.6 mmol) was then added dropwise and the reaction mixture was stirred vigorously under nitrogen at room temperature for 20 h. After the reaction was completed (monitored by TLC), the reaction mixture was evaporated in vacuo to give a quantitative yield of **35** as a yellow oil which was used without further purification. ^{1}H NMR: δ 2.93 (2H, t, $J = 7.3$ Hz), 3.16 (2H, t, $J = 7.3$ Hz), 5.94 (2H, s), 6.63 (1H, d, $J = 7.7$ Hz), 6.68 (1H, s), 6.73 (1H, d, $J = 7.7$ Hz); ^{13}C NMR: δ 31.3, 40.3, 101.5, 108.9, 109.2, 121.8, 132.8, 146.9 148.3 173.5.

1-Diazo-3-(3,4-methylenedioxyphenyl)-2-propanone (36). A solution of compound **35** (600 mg, 3 mmol) in 5 mL THF–acetonitrile (1:1) was added dropwise at 0 °C to a solution of $TMSCHN_2$ (3 mL, 6 mmol, 2M in hexane) in 5 mL THF–acetonitrile (1:1). The resulting mixture was stirred at 0 °C for 4 h. After the reaction was completed, the reaction mixture was evaporated in vacuo to give **36** as a red viscous oil in 95% yield which was used without further purification.

Topoisomerase mediated DNA cleavage assays

Human topoisomerase I was isolated as a recombinant fusion protein using a T7 expression system.[23] DNA topoisomerase II was purified from calf thymus gland as reported previously.[44] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[45] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[46] The cleavage assays were performed as previously reported.[47]

Cytotoxicity assays. The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[48–50] The human lymphoblast RPMI8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan).[51] 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.

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