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2,3-Dimethoxybenzo[*i*]phenanthridines: Topoisomerase I-Targeting Anticancer Agents

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Abstract—Appropriately substituted benzo[*i*]phenanthridines structurally related to nitidine, a benzo[*c*]phenanthridine alkaloid with antitumor activity, are active as topoisomerase I-targeting agents. Studies on benzo[*i*]phenanthridines have indicated analogues that possess a 2,3-methylenedioxy moiety and at least one and preferably two methoxyl groups at the 8- and 9-positions, such as 8,9-dimethoxy-2,3-methylenedioxybenzo[*i*]phenanthridine, **2**, are active as topoisomerase I-targeting agents. Tetramethoxylated benzo[*i*]phenanthridines, wherein the 2,3-methylenedioxy moiety is replaced with methoxyl groups at the 2- and 3-position, are inactive as a topoisomerase I-targeting agent. These results initially suggested that the 2,3-methylenedioxy moiety was critical to the retention of potent activity. Further studies revealed that 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine, **7a**, is more potent than **2** as a topoisomerase I-targeting agent. The observation that 2,3-dimethoxylated benzo[*i*]phenanthridines can actually exhibit enhanced activity prompted the present study in which several 8-substituted 2,3-dimethoxybenzo[*i*]phenanthridines were prepared and their pharmacological activities evaluated. The influence of NH_2, CN, CH_2OH, OBn, OCH_3, OH, and $NHCOCH_3$ substituents at the 8-position on the relative activity of these 2,3-dimethoxybenzo[*i*]phenanthridines was examined. Relative to these derivatives, **7a** was the most potent topoisomerase I-targeting agent, possessing similar cytotoxicity to that of nitidine in the human lymphoblast tumor cell line, RPMI8402.

Introduction

The topological state of DNA is regulated by DNA topoisomerases that catalyze the breaking and rejoining of DNA strands.[1–4] These nuclear enzymes are also involved in controlling template supercoiling during RNA transcription.[5,6] There are two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2) based upon differences in their initial mechanisms wherein a single or double-stranded DNA break is implicated. 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 changes these essential components of the replication machinery of the cell into a cellular poison.

Camptothecin and its structurally-related analogues that target TOP1 have been extensively studied. 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] and indenoisoquinolines,[19,20] phenazines[21] and benzophenazines[22] have been identified as TOP1-targeting agents. Recently, benz[*a*]acridine and benzo[*i*]phenanthridine derivatives have been identified as TOP1-targeting agents.[23,24]

Initial studies with a select series of benzo[*i*]phenanthridines indicate that 2,3-methylenedioxy-8,9-dimethoxybenzo[*i*]phenanthridine **2** (Fig. 1), was more active than 5,6-dihydro-9,10-dimethoxy-3,4-methylenedioxybenz[*a*]-anthracene **1** as a TOP1-targeting agent. While **3** and **4** (Fig. 1) target TOP1, these derivatives are less active than **2**. Compound **6**, which possesses both a 2,3- and an 8,9-methylenedioxy group does not exhibit significant

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TOP1-targeting activity. The presence of methoxyl groups at both the 2- and 3-position of benzo[*i*]phenanthridine, such as in the case of **5** is associated with a loss of topoisomerase-targeting activity. The observation, therefore, that **7a** has greater TOP1-targeting activity than **2** was initially unexpected and prompted the synthesis and evaluation of other 2,3-dimethoxybenzo[*i*]phenanthridine derivatives.

Results

Chemistry

The synthetic approach employed for the preparation of **7a** and the 8-substituted 2,3-dimethoxybenzo[*i*]phenanthridines **7b–7h** is outlined in Scheme 1. Treatment of 6,7-dimethoxy-β-tetralone under bromo-Vilsmeier conditions provided **8** in 80% yield.[25] Treatment of **8** with

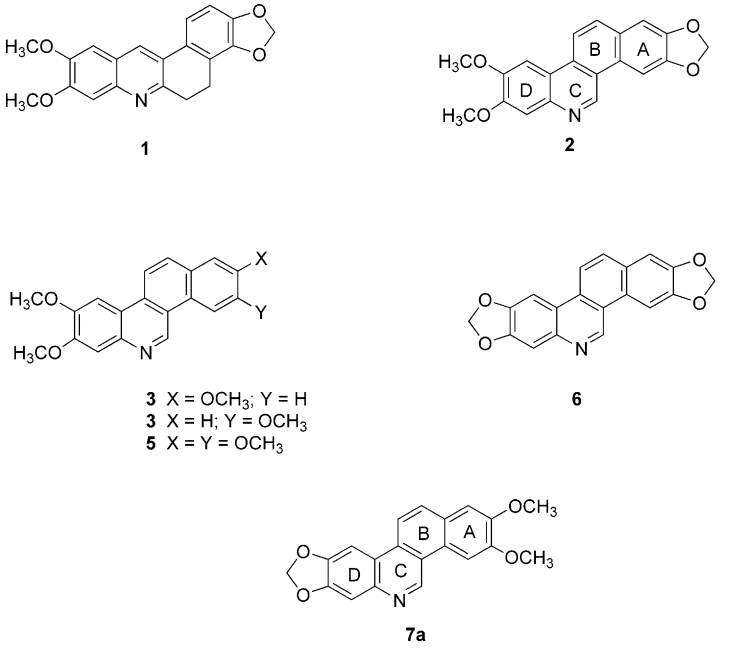
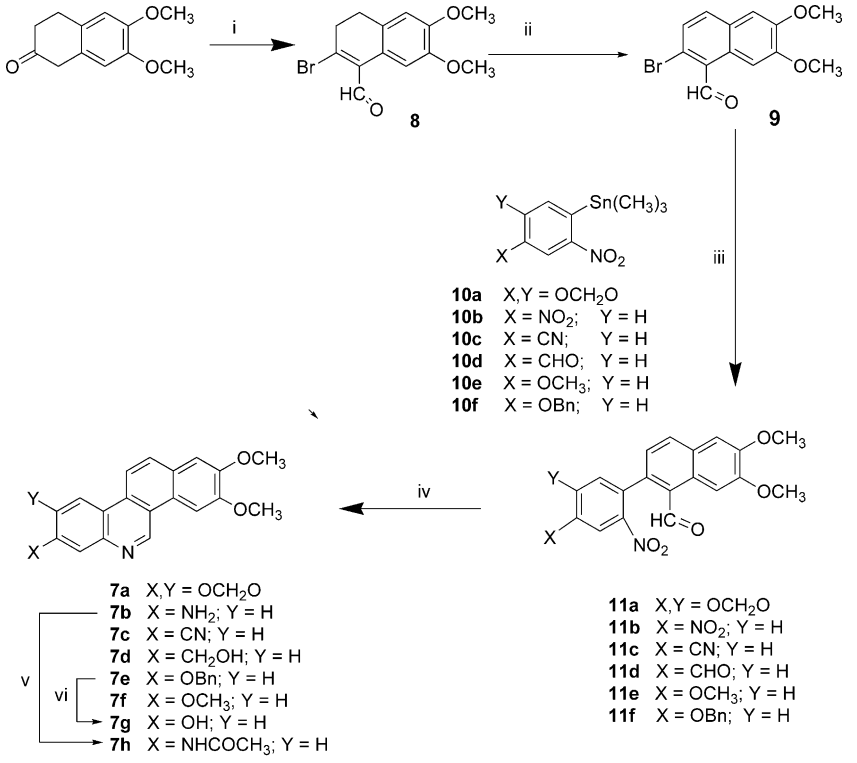


Figure 1. Benz[*a*]acridine and benzo[*i*]phenanthridine derivatives evaluated as TOP1-targeting agents.



Scheme 1. (i) DMF, PBr_3,$CHCl_3$, reflux; (ii) DDQ, toluene, reflux; (iii) $(CH_3)_3SnSn(CH_3)_3$, $Pd(PPh_3)_4$, THF, reflux; (iv) Zn, AcOH, reflux; (v) Ac_2O, pyridine; (vi) H_2, 45 psi, Ra–Ni, MeOH; EtOAc/THF.

DDQ in toluene provided 3-bromo-6,7-dimethoxy-1-naphthldehyde **9** in 95% yield. Trimethyl(*ortho*-nitrophenyl)stannanes have proved useful intermediates for the preparation of phenanthridines and benzo[*i*]phenanthridines by reaction with the appropriate benzaldehyde or naphthaldehyde.[26] Stille coupling of **9** with various trimethyl(*othro*-nitrophenyl)stannanes **10a–f** gave the **11a–f** in yields ranging from 70 to 95%. Treatment of **11a–f** with zinc dust in acetic acid provided the benzo[*i*]phenanthridines **7a–f**. Hydrogenolysis of **7e** using H_2 and Raney–Ni gave **7g** in good yield. Reaction of **7b** with acetic anhydride in pyridine provided the acetamide derivative **7h**.

Pharmacology

The relative TOP1-targeting activities of the various 2,3-dimethoxybenzo[*i*]phenanthridines **7a–h** are provided in Table 1. Of the benzo[*i*]phenanthridines evaluated in this study, **7a** was the most potent TOP1-targeting agent. This result was unanticipated in view of previous studies with benzo[*i*]phenanthridine derivatives, such as **5**, wherein the presence of methoxyl groups at the 2- and 3-positions was associated with a loss of topoisomerase-targeting activity. Of the 8-substituted 2,3-dimethoxybenzo[*i*]phenanthridines evaluated, only **7c**, **7f** and **7g** were active in stabilizing the cleavable complex formed between TOP1 and DNA. Steric factors associated with substituents at the 8-position appear to influence TOP1-targeting activity as the 8-hydroxy derivative **7g** was more active than the methoxy derivative **7f** which in turn was more active than the benzyloxy derivative **7e**. A representative gel map of the TOP1-mediated DNA cleavage assay observed with compounds **4**, **7a**, **7d** and **7g** is illustrated in Figure 2. Each of the compounds was also evaluated for TOP2-targeting activity. Nitidine and VM-26, which have comparable potency as TOP2-targeting agents, were used as positive controls in these assays. Compound **2** did exhibit activity in stabilizing the cleaved complex formed between TOP2 and DNA.

Table 1. TOP1-targeting activity and cytotoxicity of **1**, **2**, and the 2,3-dimethoxybenzo[*i*]phenanthridines **7a–h**

Compound	TOP1-mediated DNA cleavage[a]	Cytotoxicity IC_{50} (μ M)[b] cell lines	
		RPMI8402	CPT-K5
1	5	6.8	22
2	10	4.5	11
7a	1	0.4	0.4
7b	(–)	2.3	5.6
7c	80	8.0	>40
7d	(–)	13	40
7e	(–)	8.5	23
7f	10	3.9	25
7g	2	1.6	14
7h	(–)	4.0	30
Nitidine	1	0.4	4.0
CPT	0.1	0.005	>40
VM-26	(–)	0.2	0.3

[a]IC_{50} was calculated after 4 days of continuous drug exposure.
[b]TOP1 cleavage values are reported as REC, relative effective concentration, that is, 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 TOP1.

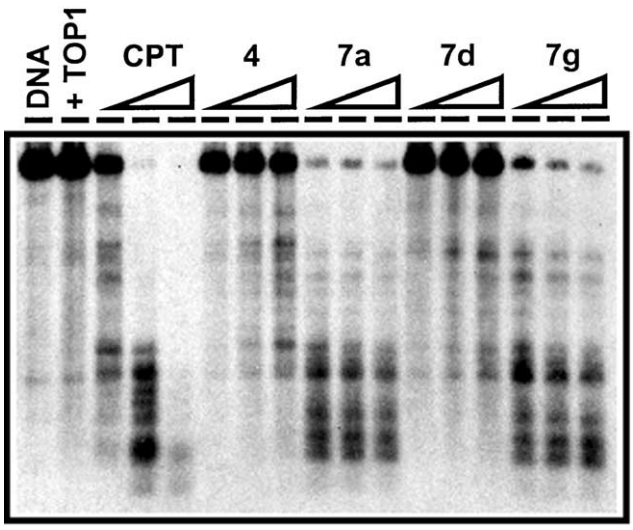


Figure 2. Stimulation of enzyme-mediated DNA cleavage by **4**, **7a**, **7d**, **7g** and camptothecin (CPT) using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.01 to 1.0 μM for CPT and 1.0 to 100 μM for compounds **4**, **7a**, **7d** and **7g**.

It had approximately 10% of the potency of nitidine as a TOP2-targeting agent (data not shown). None of the other benzo[*i*]phenanthridine derivatives was active as TOP2-targeting agents.

The cytotoxic activities of **7a–h** are provided in Table 1. Compound **7a** had similar cytotoxicity to nitidine in the human lymphoblastoma cell line RPMI8402. CPT-K5 is the camptothecin-resistant variant of RPMI8402. The basis for this resistance is a mutant but functional TOP1, which does not form a stabilized cleaved complex in the presence of camptothecin.[27] While nitidine exhibited marginal cross-resistance, **7a** did not exhibit cross-resistance in CPT-K5.

Discussion

Previous studies indicate that the presence of methoxyl substituents at the 2- and 3-positions of benzo[*i*]phenanthridines was associated with a loss of TOP1-targeting activity. The fact that **7a** is not only active as a TOP1-targeting agent, but also an order of magnitude more potent than **2** was, therefore, unexpected. This result prompted the study of a series of 2,3-dimethoxy-8-substituted benzo[*i*]phenanthridines. Comparative studies on the relative potency of the 2,3-dimethoxybenzo[*i*]phenanthridines synthesized and evaluated as TOP1-targeting agents showed the relative potencies to be **7a** >>**7g** >**7f** >>**7c** >>**7b**,**7d**,**7e**,**7h**.

Consistent with its greater potency as a TOP1-targeting agent, **7a** was also an order of magnitude more cytotoxic than **2**. Compound **7a** had an IC_{50} of 400 nM in the human lymphoblastoma cell line RPMI8402, while compound **2** had an IC_{50} of 4.5 μM. In addition to **7a**, only **7f** and **7g** had greater or comparable TOP1-targeting

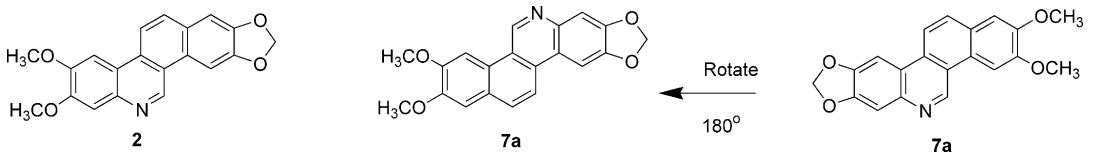


Figure 3. Comparison of the two-dimensional structures of **2** and **7a** after alignment of similar functionality and molecular topology.

activity to **2**. While **7g** had 5-fold greater intrinsic potency as a TOP1-targeting agent than **7f**, it had only slightly above a 2-fold greater cytotoxic activity in the human lymphoblastoma cell line RPMI402, with an IC_{50} of 1.6 μM. The IC_{50} of **7f** in RPMI8402 cells was 3.9 μM.

Structure–activity data had initially suggested that **2** was among the more potent benzo[*i*]phenanthridine derivatives as TOP1-targeting agents. In addition, replacement of the methylenedioxy moiety of **2** with two methoxy groups results in a complete loss of activity. These data raised the speculation that 2,3-dimethoxylated benzo[*i*]phenanthridines would not possess potent TOP1-targeting activity. The exceptional TOP1-targeting activity and cytotoxicity of **7a** proved this is not the case. The data in this study do suggest that the methylenedioxy moiety in the case of both **2** and **7a** is a significant structural component for maintaining potent TOP1-targeting activity. In light of these results, the optimal molecular topology for effective TOP1-targeting activity among these benzo[*i*]phenanthridines may consist of a methylendioxy moiety at one end of the molecule with dimethoxy groups on the benzo-ring distal to this substituent. Arranging both **2** and **7a** such that methylenedioxy moieties are on the same side for each molecule (which requires rotating **7a** clockwise by 180° as illustrated in Fig. 3), so that each adopts a similar two-dimensional molecular topology. In comparing these two molecules, as illustrated in Figure 3, the only obvious difference between **2** and **7a** is the location of the nitrogen heteroatom. In the case of **7a**, it appears that having the nitrogen heteroatom adjacent to the benzo-ring containing the methylenedioxy substituent results in an enhancement of the TOP1-targeting activity of these benzo[*i*]phenanthridines.

It is our intention to use **7a** as a molecular template for further studies to gain additional insight into those structural features that are associated with enhancement of the TOP1-targeting activity of substituted benzo[*i*]-phenanthridines and related compounds.

Experimental

General

Melting points were determined with a Thomas-Hoover Unimelt capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm, (ICN Biomedicals, Eschwegge, Germany) using the solvent systems indicated. Infrared spectral data (IR) were obtained on a Perkin-Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1}. Proton (^1H NMR, 200 MHz) and carbon (^{13}C NMR, 50 MHz) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer on compounds dissolved in deuterated chloroform, unless otherwise indicated, with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). Mass spectra were obtained by the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO, USA. Tetrahydrofuran was freshly distilled from sodium and benzophenone prior to use. Tetrakis(triphenylphosphine)palladium(0) was purchased as bright yellow powder or crystal from Aldrich Chemical Company (Milwaukee, WI, USA) or Acros Organics (Fisher Scientific, Pittsburgh, PA, USA). Compound **10a** was synthesized as previously described.[26]

General procedure for the preparation of benzo[*i*]phenanthridines (7a–f)

The appropriate 2-(*o*-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (100 mg, 0.26 mmol) was dissolved in glacial acetic acid (15 mL) and heated to reflux with zinc dust (160 mg, 2.5 mmol) for 2-3.5 h. Acetic acid was evaporated in vacuo and the residue was dissolved in chloroform. The solution was filtered through a Celite 545 bed and the filtrate washed successively with saturated aqueous sodium bicarbonate and brine. The organic layer was dried (anhyd. Na_2SO_4) and evaporated in vacuo. The residue was chromatographed using 50 g of silica gel and 1:1 ethyl acetate/hexanes to give as follows.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (7a). This was prepared from **11a** (100 mg, 0.26 mmol) and isolated as a beige solid after chromatography using 1:1 ethyl acetate/hexanes in 80% yield (40 mg), mp 171–173 °C; IR (KBr) 2226, 1610, 1461, 1277; ^1H NMR δ 4.06 (3H, s), 4.15 (3H, s), 6.15 (2H, s), 7.27 (1H, s), 7.54 (1H, s), 7.85 (1H, s), 7.94 (1H, d, $J=6.7$) 8.09 (1H,s), 8.15 (1H, d, $J=8.7$), 9.88 (1H, s); ^{13}C NMR δ 56.4, 56.6, 99.7, 102.3, 102.4, 107.7, 108.5, 118.5, 121.0, 121.2, 125.6, 127.7, 130.8, 131.2, 142.8, 145.9, 148.8, 149.7, 150.1, 151.0; HR-MS calcd for $C_{20}H_{15}NO_4+H$: 334.1079; found: 334.1079.

8-Amino-2,3-dimethoxybenzo[*i*]phenanthridine (7b). This was prepared from **11b** (100 mg, 0.26 mmol) and isolated as an orange–yellow solid after chromatography using a 1:8 mixture of methanol/ethyl acetate in 50%

yield (40 mg); mp 224–225 °C; ^{1}H NMR (CD_3OD) δ 3.95 (3H, s), 4.04 (3H, s), 7.15 (1H, dd, J = 8.8, 2.3), 7.23 (1H, d, J = 2.3), 7.26 (1H, s), 7.88 (1H, d, J = 9.0), 7.98 (1H, s), 8.20 (1H, d, J = 9.0), 8.32 (1H, d, J = 8.8), 9.68 (1H, s); ^{13}C NMR (CD_3OD) δ 59.3, 59.5, 105.8, 112.4, 112.9, 120.9, 121.4, 122.6, 123.2, 127.7, 129.7, 131.2, 135.7, 136.2, 149.5, 151.3, 153.5, 153.7, 155.1; HR-MS calcd for $C_{19}H_{16}N_2O_2$: 304.1212; found: 304.1206.

8-Cyano-2,3-dimethoxybenzo[*i*]phenanthridine (7c). This was prepared from **11c** (80 mg, 0.22 mmol) and isolated as a light yellow solid as after chromatography using 2:1 mixture of ethyl acetate/hexanes in 40% yield (28 mg); mp >250 °C; IR (KBr) 2226, 1610, 1516; ^{1}H NMR (DMSO-d_6) δ 4.00 (3H, s), 4.12 (3H, s), 7.67 (1H, s), 8.06 (1H, d, J = 8.0), 8.31 (1H, d, J = 8.9), 8.51 (1H, s), 8.67 (1H, s), 8.73 (1H, d, J = 8.0), 9.01 (1H, d, J = 8.9), 10.46 (1H, s); ^{13}C NMR (DMSO-d_6) δ 56.0, 56.4, 103.4, 108.6, 110.9, 118.5, 119.0, 122.3, 124.9, 125.3, 127.5, 128.6, 128.9, 129.4, 132.2, 134.9, 143.5, 150.6, 151.1, 156.2; HR-MS calcd for $C_{20}H_{14}N_2O_2$: 314.1055; found: 314.1067.

2,3-Dimethoxy-8-hydroxymethylbenzo[*i*]phenanthridine (7d). This was prepared from **11d** (80 mg, 0.22 mmol) and isolated as a yellow solid after chromatography using ethyl acetate in 50% yield (35 mg); mp >250 °C IR (KBr) 3429, 2366, 1732; ^{1}H NMR (DMSO-d_6) δ 3.98 (3H, s), 4.11 (3H, s), 7.64 (1H, s), 7.73 (1H, d, J = 8.4), 8.16 (1H, s), 8.24 (1H, d, J = 8.8), 8.49 (1H, s), 8.66 (1H, d, J = 9.0), 8.83 (1H, d, J = 8.8), 10.36 (1H, s); ^{13}C NMR (DMSO-d_6) δ 55.9, 56.3, 65.4, 103.1, 108.6, 118.3, 121.2, 123.7, 123.8, 125.0, 127.0, 128.0, 128.9, 130.1, 131.7, 136.8, 144.4, 149.4, 150.1; HR-MS calcd for $C_{20}H_{17}NO_3$: 319.1208; found: 319.1196.

8-Benzoxyl-2,3-dimethoxybenzo[*i*]phenanthridine (7e). This was prepared from **11e** (100 mg, 0.23 mmol) and isolated as a white solid after chromatography using a 2:1 mixture of ethyl acetate/hexanes in 56% yield (51 mg); mp 227–228 °C ^{1}H NMR δ 4.08 (3H, s), 4.18 (3H, s), 5.29 (2H, s), 7.33 (1H, s), 7.36–7.48 (5H, m), 7.55 (1H, dd, J = 7.9, 1.7), 7.73 (1H, d, J = 1.7), 8.04 (1H, d, J = 8.9), 8.17 (1H, s), 8.41 (1H, d, J = 8.8), 8.54 (1H, d, J = 8.9), 10.04 (1H, s); ^{13}C NMR δ 56.5, 56.6, 70.8, 102.3, 108.6, 110.9, 118.4, 119.5, 119.6, 120.7, 124.2, 125.8, 127.7, 128.2, 128.6, 129.2, 131.4, 131.7, 137.1, 146.8, 148.6, 150.1, 151.2, 159.5; HR-MS calcd for $C_{26}H_{21}NO_3$: 395.1521; found: 395.1522.

2,3,8-Trimethoxybenzo[*i*]phenanthridine (7f). This was prepared from **11f** (70 mg, 0.19 mmol) as a light yellow solid after chromatography using ethyl acetate as eluting solvent in 70% yield (42 mg); mp 200–201 °C; ^{1}H NMR δ 4.02 (3H, s), 4.08 (3H, s), 4.18 (3H, s), 7.32 (1H, s), 7.35 (1H, dd, J = 9.2, 2.7), 7.64 (1H, d, J = 2.7), 8.03 (1H, d, J = 8.8), 8.16 (1H, s), 8.40 (1H, d, J = 8.8), 8.52 (1H, d, J = 9.2), 10.04 (1H, s); ^{13}C NMR δ 56.1, 56.5, 56.6, 102.3, 108.6, 109.7, 118.4, 119.2, 119.3, 120.7, 124.2, 125.8, 127.7, 131.4, 131.7, 146.9, 148.6, 150.1, 151.1, 160.4; HR-MS calcd for $C_{20}H_{17}NO_3$: 319.1208; found: 319.1204.

2,3-Dimethoxy-8-hydroxybenzo[*i*]phenanthridine (7g). Compound **7e** (15 mg, 0.038 mmol) was dissolved 40 mL of 3:3:2 methanol/ethyl acetate/THF. Raney–Ni (40 mg) was added to this solution. The mixture was shaken in a Parr® apparatus for 40 h at 45 psi of hydrogen. The reaction mixture was then concentrated in vacuo and the residue was chromatographed using 50 g of silica gel and a 1:10 methanol/ethyl acetate as a light-yellow powder in 86% yield (10 mg); mp 238–240 °C ^{1}H NMR (CD_3OD) δ 4.03 (3H, s), 4.12 (3H, s), 7.30 (1H, dd, J = 9.0, 2.4), 7.44 (1H, s), 7.47 (1H, d, J = 2.4), 8.09 (1H, d, J = 9.0), 8.22 (1H, s), 8.43 (1H, d, J = 9.0), 8.58 (1H, d, J = 9.0), 9.95 (1H, s); HR-MS calcd for $C_{19}H_{15}NO_3$: 305.1052; found: 305.1060.

8-Acetamino-2,3-dimethoxybenzo[*i*]phenanthridine (7h). A solution of **7b** (15 mg, 0.049 mmol) in pyridine (6 mL) was treated dropwise with acetic anhydride (1.5 mL) at 0 °C. The mixture was gradually warmed up to room temperature and stirred for 2 h. The solvent was evaporated and the residue was taken up in methanol (15 mL). Evaporation of the solvent afford **7h** (15 mg) as a light yellow solid in mp >250 °C 90% yield. ^{1}H NMR (CD_3OD) δ 2.24 (3H, s), 4.03 (3H, s), 4.13 (3H, s), 7.47 (1H, s), 7.86 (1H, dd, J = 8.8, 1.9), 8.12 (1H, d, J = 9.2), 8.24 (1H, s), 8.47 (1H, d, J = 8.8), 8.48 (1H, d, J = 1.9), 8.63 (1H, d, J = 9.2), 10.00 (1H, s); ^{13}C NMR (DMSO-d_6) δ 24.5, 55.8, 56.3, 103.0, 108.7, 117.4, 118.1, 119.9, 120.1, 120.4, 123.8, 125.1, 127.5, 130.3, 131.4, 139.6, 145.3, 149.4, 149.8, 150.9, 169.0; HR-MS calcd for $C_{21}H_{18}N_2O_3$: 346.1317; found: 346.1319.

2-Bromo-3,4-dihydro-6,7-dimethoxy-1-naphthaldehyde (8). Dimethylformamide (1.6 mL, 21.4 mmol) was added dropwise to a solution of phosphorus tribromide (1.6 mL, 16.9 mmol) in dry chloroform (20 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h to give a pale yellow suspension. A solution of 6,7-dimethoxy-2-tetralone (1.0 g, 4.85 mmol) in dry chloroform (20 mL) 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 made basic with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with dichloromethane, dried (anhyd Na_2SO_4), and evaporated in vacuo. The residue was chromatographed over 100 g of silica gel using 1:3 ethyl acetate/hexanes to give **8** (1.15 g) as a yellow crystalline solid in 80% yield; mp 82–83 °C ^{1}H NMR δ 2.83 (2H, t, J = 7.6), 3.01 (2H, t, J = 7.6), 3.88 (3H, s), 6.66 (1H, s), 7.71 (1H, s), 10.31 (1H, s); ^{13}C NMR δ 28.9, 38.5, 56.4, 56.5, 110.0, 111.1, 123.1, 128.0, 132.7, 144.2, 147.7, 149.1, 193.6; HR-MS calcd for $C_{13}H_{13}BrO_3$: 296.0048; found: 296.0042.

2-Bromo-6,7-dimethoxy-1-naphthaldehyde (9). 2-Bromo-3,4-dihydro-6,7-dimethoxy-1-naphthaldehyde (**8**) (960 mg, 3.23 mmol) and DDQ (880 mg, 3.88 mmol) was refluxed in toluene (45 mL) for 12 h. After cooling to room temperature, the mixture was filtered through a Celite 545 bed and the filtrate was evaporated to dryness. The residue obtained was chromatographed on 75 g of silica gel using 1:3 ethyl acetate/hexanes to give **9** (902 mg) as a light yellow solid in 95% yield; mp 141–142 °C IR (KBr) 1671; ^{1}H NMR δ 4.01 (3H, s), 4.06 (3H, s), 7.09

(1H, s), 7.56 (1H, d, $J=8.6$), 7.72 (1H, d, $J=8.6$), 8.74 (1H, s), 10.76 (1H, s); ^{13}C NMR δ 56.3, 56.6, 104.2, 107.0, 126.5, 128.9, 129.5, 129.8, 129.9, 134.4, 150.4, 153.1, 196.0; HR-MS calcd for $C_{13}H_{11}BrO_3$: 293.9892; found: 293.9896.

Trimethyl(3,4-methylenedioxy-6-nitro)stannane (10a). This was prepared from 1-bromo-3,4-methylenedioxy-6-nitrobenzene[28] as previously detailed.[26]

General procedure for the preparation of trimethylnitroarylstannanes (10b–10e)

Trimethyl(2,4-dinitrophenyl)stannane (10b). A mixture of hexamethylditin (1.0 g, 3.1 mmol), 1-iodo-2,4-dinitrobenzene (588 mg, 2.0 mmol) and $Pd(PPh_3)_4$ (95 mg) in anhydrous THF (25 mL) was heated to reflux for 10 h. After cooling to room temperature, THF was evaporated and methylene chloride (30 mL) was added to the residue. Aqueous potassium fluoride (7.0 M, 2 mL) was added dropwise to this mixture with vigorous stirring. The mixture was passed through a Celite 545 bed and the filtrate was washed with brine. The methylene chloride layer was dried (anhyd. Na_2SO_4), filtered and evaporated in vacuo. The residue was chromatographed over 100 g of silica gel using 1:6 ethyl acetate/hexanes to give **10b** (350 mg) in 53% yield; ^{1}H NMR δ 0.43 (9H, s), 7.95 (1H, d, $J=8.0$), 8.44 (1H, dd, $J=8.0$, 2.0), 9.11 (1H, d, $J=2.0$); ^{13}C NMR δ −6.9, 119.2, 127.2, 139.0, 149.4, 150.0, 154.1; HR-MS calcd for $C_9H_{12}N_2O_4Sn$–CH_3: 316.9584; found: 316.9591.

Trimethyl(4-cyano-2-nitrophenyl)stannane (10c). This was prepared from 4-chloro-3-nitrobenzonitrile (460 mg, 2.5 mmol) and hexamethylditin (1.0 g, 3.1 mmol) as a yellow solid in 40% yield; ^{1}H NMR δ 0.40 (9H, s), 7.87 (2H, s), 8.57 (1H, s); ^{13}C NMR δ −6.9, 114.2, 117.5, 127.5, 135.9, 138.9, 147.9; HR-MS calcd for $C_{10}H_{12}N_2O_2Sn$–CH_3: 296.9686; found: 296.9693.

Trimethyl(4-formyl-2-nitrophenyl)stannane (10d). This was prepared from 4-chloro-3-nitrobenzaldehyde (490 mg, 2.5 mmol) and hexamethylditin (1.0 g, 3.1 mmol) as a light yellow solid in 70% yield; mp 97–98 °C; ^{1}H NMR δ 0.30 (9H, s), 7.90 (1H, d, $J=7.4$), 8.09 (1H, d, $J=7.4$), 8.76 (1H, s), 10.11 (1H, s); ^{13}C NMR δ −7.0, 125.2, 133.1, 138.1, 138.8, 149.1, 154.2, 190.7; HR-MS calcd for $C_{10}H_{13}NO_3Sn$–CH_3: 299.9683; found: 299.9684.

Trimethyl(4-benzyloxy-2-nitrophenyl)stannane (10e). This was prepared from 4-benzyloxy-1-bromo-2-nitrobenzene (462 mg, 1.5 mmol) and hexamethylditin (1.0 g, 3.1 mmol) as a light yellow oil in 70% yield; ^{1}H NMR δ 0.32 (9H, s), 5.16 (2H, s), 7.27 (1H, dd, $J=8.1$, 2.5), 7.36–7.65 (5H, m), 7.58 (1H, d, $J=8.1$), 7.95 (1H, d, $J=2.5$); ^{13}C NMR δ −7.3, 70.9, 110.4, 121.8, 128.0, 128.8, 129.2, 130.7, 136.4, 138.2, 154.6, 160.3.

Trimethyl(4-methoxy-2-nitrophenyl)stannane (10f). This was prepared from 4-bromo-3-nitroanisole (1.6 g, 6.9 mmol) in 55% yield; mp 93–95 °C; ^{1}H NMR δ 0.32 (9H, s, [^{119}Sn-H, d, $^2J_{Sn\text{-}H}=54$]), 3.89 (3H, s), 7.21 (1H, dd, $J=8.0$, 2.6), 7.57 (1H, d, $J=8.0$), 7.86 (1H, d, $J=2.6$); ^{13}C NMR δ −7.1, 56.7, 107.7, 117.3, 133.9, 146.8, 149.6, 154.1; HR-MS calcd for $C_{10}H_{15}NO_3Sn$–CH_3: 301.9839; found: 301.9832.

4-Benzyloxy-1-bromo-2-nitrobenzene. A solution of 4-bromo-3-nitrophenol (1.0 g, 4.6 mmol) and benzyl bromide (0.82 mL, 7.0 mmol) in 40 mL of 2:1 acetonitrile/acetone was treated with potassium carbonate (970 mg, 7.0 mmol). The resulted mixture was heated under reflux for 17 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was dissolved in ethyl acetate and the solution was washed successively with H_2O (30 mL), 1 M HCl (30 mL × 3) and brine. The organic layer was dried (anhyd Na_2SO_4) and evaporated in vacuo. The residue was chromatographed using 75 g of silica gel and 1:5 ethyl acetate/hexanes to give 4-benzyloxy-1-bromo-2-nitrobenzene (1.34 g) as a yellow crystalline solid in 95% yield; mp 46–47 °C; ^{1}H NMR δ 5.11 (2H, s), 7.05 (1H, dd, $J=9.0$, 3.0), 7.36–7.45 (5H, m), 7.47 (1H, d, $J=3.0$), 7.60 (1H, d, $J=9.0$); ^{13}C NMR δ 71.4, 105.4, 112.4, 121.1, 128.1, 128.9, 129.1, 129.3, 129.5, 135.8, 136.0, 138.2, 158.7; HR-MS calcd for $C_{13}H_{10}BrNO_3$: 306.9844; found: 306.9846.

4-Bromo-3-nitrophenol. A solution of 4-bromo-3-nitroanisole (2.32 g, 10 mmol) in dichloromethane (25 mL) was added dropwise to a solution of boron tribromide (BBr_3, 1.0 M in dichloromethane, 25 mL) at −78 °C. The solution was gradually brought to room temperature and stirred for 30 h. H_2O (30 mL) was added dropwise and resulting precipate was taken up in ethyl acetate (40 mL). The aqueous layer was extracted twice with ethyl acetate (25 mL × 2). The combined organic layer was washed successively with saturated aqueous sodium bicarbonate and brine. The organic layer was dried (anhyd Na_2SO_4) and evaporated in vacuo. The residue was chromatographed using 75 g of silica gel and a 1:3 mixture of ethyl acetate/hexanes to give 4-bromo-3-nitrophenol (1.52 g) as a yellow solid in 70% yield; mp 144–145 °C; ^{1}H NMR δ 6.95 (1H, dd, $J=8.8$, 2.9), 7.36 (1H, d, $J=2.9$), 7.58 (1H, d, $J=8.8$); ^{13}C NMR δ 105.2, 113.4, 116.4, 121.4, 136.3, 155.9; HR-MS calcd for $C_6H_4BrNO_3$: 216.9375; found: 216.9380.

General procedure for the synthesis of 11a–11f

2-(2,4-Dinitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (11b). $Pd(Ph_3P)_4$ (60 mg, 0.05 mmol) and cuprous bromide (10 mg, 0.07 mmol) were added to a solution of **10b** (345 mg, 1.0 mmol) and **9** (186 mg, 0.63 mmol) in THF (20 mL) at room temperature. The mixture was heated to reflux under N_2 for 15 h. After cooling, THF was evaporated and ethyl acetate (30 mL) was added to the residue. The solution was washed with water (20 mL). The organic layer was separated and passed through a Celite 545 bed to remove suspended particles. The organic layer was washed with brine, dried (anhyd Na_2SO_4) and evaporated in vacuo. The residue was chromatographed using 75 g of silica gel and 1:3 ethyl acetate/hexanes to give **11b** (229 mg) in 95% yield; mp 185–186 °C; ^{1}H NMR δ 4.06 (3H, s), 4.10 (3H, s), 7.12 (1H, d, $J=8.3$), 7.24 (1H, d, $J=9.5$), 7.66 (1H, d,

$J=8.3$), 7.98 (1H, d, $J=8.3$), 8.53 (1H, dd, $J=8.3$, 2.2), 8.63 (1H, s), 8.97 (1H, d, $J=2.2$), 10.30 (1H, s); ^{13}C NMR δ 56.4, 56.7, 104.3, 107.1, 120.5, 124.5, 126.9, 128.0, 131.0, 133.4, 134.8, 139.3, 142.2, 148.0, 149.3, 151.0, 153.4, 191.9; HR-MS calcd for $C_{19}H_{14}N_2O_7$: 382.0801; found: 382.0787.

2-(3,4-Methylenedioxy-6-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (11a). This was prepared from **10a** (122 mg, 0.37 mmol) and **9** (100 mg, 0.34 mmol in 83% yield). Mp 223–225 °C; IR (KBr) 1677, 1502, 1256; ^{1}H NMR δ 4.04 (3H, s), 4.09 (3H, s), 6.21 (2H, s), 6.78 (1H, s), 7.13 (1H, d), $J=8.4$), 7.18 (1H, s), 7.66 (1H, s), 7.91 (1H, d, $J=8.4$) 8.84 (1H, s), 10.22 (1H, s); ^{13}C NMR δ 56.3, 56.6, 103.9, 105.2, 105.9, 107.1, 112.3, 125.4, 127.0, 127.5, 130.4, 131.6, 133.3, 143.8, 148.5, 150.1, 151.7, 153.1, 193.6; HR-MS calcd for $C_{20}H_{15}NO_7+$ H: 382.0927; found: 382.0927.

2-(4-Cyano-2-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (11c). This was prepared from **10c** (150 mg, 0.51 mmol) and **9** (147 mg, 0.50 mmol) in 95% yield; mp 186–187 °C; ^{1}H NMR (DMSO-d_6) δ 3.99 (6H, s), 7.27 (1H, d, $J=8.3$), 7.56 (1H, s), 7.78 (1H, d, $J=8.1$), 8.14 (1H, d, $J=8.3$), 8.27 (1H, d, $J=8.1$), 8.54 (1H, s), 8.78 (1H, s), 10.31 (1H, s); ^{13}C NMR (DMSO-d_6) δ 55.8, 55.9, 103.5, 107.6, 112.5, 117.2, 124.9, 126.2, 127.0, 128.6, 130.2, 133.1, 134.1, 136.5, 139.0, 139.6, 149.0, 150.2, 152.5, 193.1; HR-MS calcd for $C_{20}H_{14}N_2O_5$: 362.0903; found: 362.0918.

2-(4-Formyl-2-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (11d). This was prepared from **10d** (157 mg, 0.50 mmol) and **9** (147 mg, 0.50 mmol) as a bright yellow solid in 80% yield; mp 175–176 °C; ^{1}H NMR (DMSO-d_6) δ 3.88 (6H, s), 7.29 (1H, d, $J=8.2$), 7.56 (1H, s), 7.79 (1H, d, $J=7.9$), 8.15 (1H, d, $J=8.2$), 8.28 (1H, d, $J=7.9$), 8.56 (1H, s), 8.69 (1H, s), 10.22 (1H, s), 10.30 (1H, s); ^{13}C NMR (DMSO-d_6) δ 55.9, 103.6, 107.6, 124.9, 125.4, 126.2, 126.9, 130.1, 132.9, 133.1, 134.1, 136.8, 139.9, 140.1, 149.2, 150.1, 152.5, 191.8, 193.0; HR-MS calcd for $C_{20}H_{15}NO_6$: 365.0899; found: 365.0909.

2-(4-Benzyloxy-2-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (11e). This was prepared from **10e** (320 mg, 0.82 mmol) and **9** (204 mg, 0.69 mmol) as a light yellow solid in 92% yield; mp 143–144 °C; ^{1}H NMR δ 4.05 (3H, s), 4.09 (3H, s), 5.21 (2H, s), 7.14 (1H, d, $J=8.3$), 7.18 (1H, s), 7.29 (1H, d, $J=2.3$), 7.30 (1H, s), 7.42–7.49 (5H, m), 7.72 (1H, d, $J=2.3$), 7.90 (1H, d, $J=8.3$), 8.86 (1H, s), 10.18 (1H, s); ^{13}C NMR δ 56.3, 56.6, 71.4, 105.2, 107.1, 110.9, 114.1, 120.0, 123.9, 125.8, 126.4, 127.1, 127.4, 128.1, 129.1, 129.4, 130.3, 133.2, 134.7, 135.9, 150.3, 150.9, 168.8, 194.0; HR-MS calcd for $C_{26}H_{21}NO_6$: 443.1369; found: 443.1370.

2-(4-Methoxy-2-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (11f). This was prepared from **10f** (348 mg, 1.1 mmol) and **9** (295 mg, 1.0 mmol) as a orange solid in 70% yield; mp 200–202 °C; IR (KBr) 1672, 1523, 1256; ^{1}H NMR δ 3.95 (3H, s), 4.04 (3H, s), 4.09 (3H, s), 7.14 (1H, d, $J=8.3$), 7.17 (1H, s), 7.21 (1H, dd, $J=8.6$, 2.6), 7.31 (1H, d, $J=8.6$), 7.62 (1H, d, $J=2.6$), 7.89 (1H, d, $J=8.3$), 8.85 (1H, s), 10.17 (1H, s); ^{13}C NMR δ 56.3, 56.4, 56.6, 105.2, 107.1, 109.8, 119.4, 125.9, 126.8, 127.5, 130.3, 133.2, 134.7, 143.2, 149.8, 150.4, 153.1, 160.4, 194.0; HR-MS calcd for $C_{20}H_{17}NO_6$: 367.1056; found: 367.1050.

Topoisomerase mediated DNA cleavage assays

Human topoisomerase I was isolated as a recombinant fusion protein using a T7 expression system.[29] DNA topoisomerase II was isolated and purified as previously described.[30] Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described.[31] The 3′ end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[32] The cleavage assays were performed as previously reported.[33,34] The reactions were terminated by the addition of 5 μL of 5% SDS and 1 mg/mL protein kinase K with an additional 1 h of incubation at 37 °C. Samples were then alkali denatured by the addition of NaOH, EDTA, sucrose, and bromophenol blue to final concentrations of 75 mM, 2.5%, and 0.05 mg/mL, respectively, prior to loading onto a neutral agarose gel.

Cytotoxicity assay

Cytotoxicity was determined using the MTT-microtiter plate tetrazolinium assay (MTA).[35–37] 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).[27] 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). Each well was plated with either 2000 RPMI8402 cells or 4000 CPT-K5 cells. 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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