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8,9-Methylenedioxybenzo[*i*]phenanthridines: Topoisomerase I-Targeting Activity and Cytotoxicity

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Abstract—Substituted benzo[*i*]phenanthridines that have incorporated within their structure an 8,9-methylenedioxy group can exhibit topoisomerase I-targeting activity. Structure–activity studies were performed to examine the influence of saturation at the 11,12-positions of several substituted 8,9-methylenedioxybenzo[*i*]phenanthridines. The activities of these dihydro analogues were compared to those of their unsaturated analogues. In addition, the influence of varying substituents at the 2- and 3-positions within the A-ring of these 8,9-methylenedioxybenzo[*i*]phenanthridines on their relative potency as topoisomerase I-targeting agents and cell proliferation as determined using the MTT assay was investigated. 2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine and its 11,12-dihydro derivative were among the more potent analogues evaluated with regard to topoisomerase I-targeting activity and cytotoxicity.

Introduction

DNA topoisomerases are nuclear enzymes that catalyze the breaking and rejoining of DNA strands regulating the topological state of DNA.[1–6] Studies suggest that topoisomerases are also involved in controlling template supercoiling during RNA transcription.[7,8] The antitumor activity of topoisomerase-targeting agents is associated with their ability to stabilize the enzyme–DNA cleavable complex. Stabilization of the enzyme–DNA cleavable complex by such agents effectively converts the enzyme into a cellular poison.

The two camptothecin-based topoisomerase I-targeting agents in clinical use are topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®). These drugs have incorporated within their structure the camptothecin-ring system, which includes the presence of a δ-lactone. Hydrolysis of this lactone moiety results in an inactive derivative that possesses high affinity for human serum albumin.[9–11] The metabolic instability of this lactone and the observation that both topotecan and irinotecan are substrates for efflux transporters associated with resistance have prompted further studies on the development of novel TOP1-targeting agents.[8–16]

Benzo[*i*]phenanthridine derivatives that possess significant topoisomerase I (TOP1)-targeting activity have been identified.[17,18] These compounds were developed on the basis of the structure–activity relationships observed for analogues of coralyne[12,19] and benz[*a*]acridine.[20] These initial studies lead to the discovery of TOP1-targeting agents, such as MDD-coralyne, **1**, and 3,4-methylenedioxy-9,10-dimethoxy-5,6-dihydroacridine, **2** (Fig. 1).

While **2** represents a noncharged analogue that is structurally-related to coralyne and protoberberine alkaloids and retains significant TOP1-directed activity, its relative cytotoxic potency against the human lymphoblast tumor cell line, RPMI8402 was disappointing. Benzo[*i*]phenanthridine derivatives with a 2,3-methylenedioxy substituent within the A-ring and methoxyl

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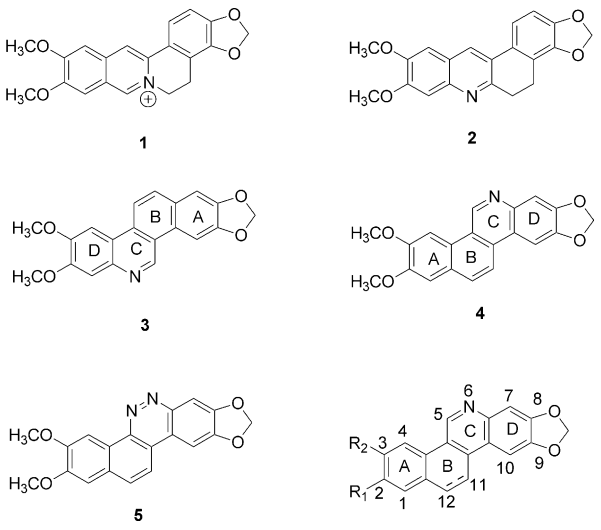


Figure 1. Structures of MDD-coralyne: **1**, 2,3-methylenedioxy-9,10-dimethoxy-5,6-dihydrobenz[*a*]acridine; **2**, 2,3-dimethoxy-9,10-dimethoxybenzo[*i*]phenanthridine; **3**, 2,3-dimethoxy-8.9-methylenedioxybenzo[*i*]phenanthridine; **4**, 2,3-dimethoxy-8.9-methylenedioxydibenzo[*c*,*h*]cinnoline; **5**, and the structure and numbering of 8.9-methylenedioxybenzo[*i*]phenanthridine derivatives.

groups at the 8- and 9-positions, such as **3**, were identified as a second generation of noncharged TOP1-targeting agents related to both protoberberine and benzo[*c*]phenanthridine alkaloids.[12,19,21–25] The initial structure–activity data associated with the TOP1-targeting activity of benzo[*i*]phenanthridines suggested that the presence of 2,3-dimethoxyl substituents within the A-ring results in a dramatic loss in potency. Based upon these initial findings, it was surprising that 2,3-dimethoxy - 8,9 - methylenedioxybenzo[*i*]phenanthridine, **4**, exhibited exceptional TOP1-targeting activity and significantly greater cytotoxicity than **3**. The likely explanation for this apparent contradiction is that **4** adopts a similar molecular topology to **3**, as illustrated in Figure 1. In adopting this orientation, the principle difference in the molecular arrangement of these two molecules is in the relative location of the nitrogen heteroatom. In the case of **4**, the nitrogen heteroatom is immediately adjacent to the ring that contains the methylenedioxy moiety. This orientation of the ring heteroatom appears to be linked to increased TOP1-targeted activity and greater cytotoxicity.

These structure–activity studies were recently extended to a series of dibenzo[*c*,*h*]cinnolines.[26] Among the 8,9-methylenedioxydibenzo[*c*,*h*]cinnolines evaluated in this study, **5** exhibited greater TOP1-targeting activity and cytotoxicity in both RPMI8402 and U937 cell lines than **4**. While **5** exhibits cross-resistance to the camptothecin-resistant variants, CPT-K5 and U937/CR,[26] no cross-resistance is observed with **4**. The ability of benzo[*i*]-phenanthridines to overcome camptothecin-resistance in these cell lines suggests that either they exert their cytotoxic activity by forming a ternary complex in a manner that differs from camptothecin or that this series of TOP1-targeting agents have, in addition, the potential to affect cell proliferation by a mechanism that does not involve TOP1.

These results prompted further study of benzo[*i*]phenanthridines specifically focusing on those derivatives that possess a 8,9-methylenedioxy moiety. Earlier studies with both protoberberine analogues and benz[*a*]acridines suggest that saturation within their ring systems, with a consequential loss of planarity, was associated with increased TOP1-targeting activity and cytotoxicity. Based upon these observations, several 8,9-methylenedioxybenzo[*i*]phenanthridines with varied substituents in the A-ring as well as their 11,12-dihydro derivatives were selected for synthesis and pharmacological evaluation.

The relative importance of each of the methoxy groups of **4** and their 11,12-dihydroderivatives was specifically investigated. Phenolic derivatives such as **10e** and **10f** have the potential to be modified into prodrugs that could significantly enhance the water solubility of these benzo[*i*]phenanthridine derivatives. Therefore, the potential of these phenolic derivatives to retain TOP1-targeting activity was also examined.

Chemistry

Scheme 1 outlines the general approach that was employed for the preparation of these 8,9-methylenedioxybenzo[*i*]phenanthridines. Appropriately substituted β-tetralones were converted to their requisite 2-bromo-3,4-dihydronaphthaldehydes, **6a–e**, as described.[27]

Compounds **6a–c** were prepared from commercially available 6-methoxy-β-tetralone, 7-methoxy-β-tetralone, and 6,7-dimethoxy-β-tetralone, respectively. The benzyloxy β-tetralone intermediates for the preparation of **6d** and **6e** were prepared as outlined in Scheme 2.

Treatment of **6b–d** with DDQ in toluene provided **7b–d** in good yield. Stille coupling of **6a–e** and **7b–d** with trimethyl-(2-nitro-4,5-methylenedioxy)stannane,[28] **8**, provided **9a–e** and **11b–d**, respectively. Treatment of **9a–e** or **11b–d** with zinc dust in acetic acid resulted in reduction of their nitro substituents and subsequent cyclization to the desired benzo[*i*]phenanthridines, **10a–e** and **12b–d**. The benzyloxy derivative **10d** proved to be resistant to hydrogenolysis using palladium-on-carbon. It could be converted to **10f** using Raney nickel and hydrogen at 40–45 psi.

The benzyloxy β-tetralone intermediates **21** and **22** required for the preparation of **6d** and **6e** were prepared as outlined in Scheme 2. The requisite cinnamic acid was reduced by catalytic hydrogenation to form compounds **13** and **14**. The free phenolic group of **13** and **14** was protected by benzyl bromide in refluxing acetone with potassium carbonate (K_2CO_3) as a base. Both the phenolic group and the carboxylic group were benzylated under these conditions. The benzyl ester formed in this step was then subsequently hydrolyzed using KOH in methanol and water. Acidic work up of the reaction resulted in the formation of the benzyloxy substituted phenylpropanoic acid, either **15** or **16** in good yield. Treatment of **15** and **16** with oxalyl chloride provided

the requisite acid chlorides, **17** and **18**. Reaction of these acid chlorides with (trimethylsilyl)diazomethane resulted in the formation of diazoketones **19** and **20**.[29] Rhodium(II) acetate catalyzed cyclization using similar conditions to those previously reported provided the benzyloxy substituted 2-tetralones **21** and **22** in 50–55% yield.[30]

Pharmacology

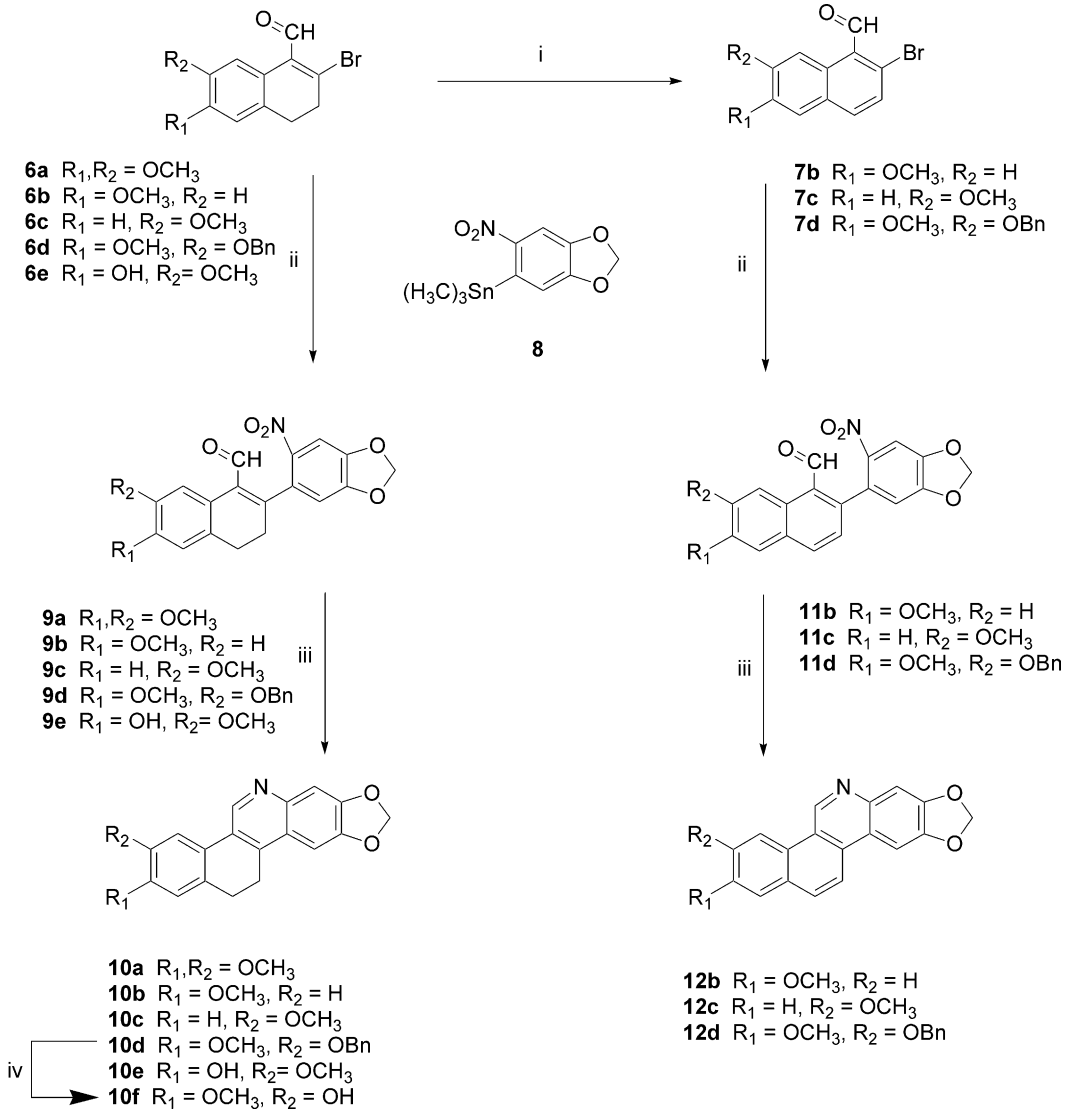
The relative topoisomerase-targeting activities (both TOP1 and TOP2) of 11,12-dihydrobenzo[*i*]phenanthridines, **10a–f** and the benzo[*i*]phenanthridines, **4** and **12b–d**, are listed in Table 1.

Compounds were evaluated for cytotoxicity toward the human lymphoblastoma tumor cell line, RPMI8402 and its camptothecin-resistant variant, CPT-K5.[31] These data are provided in Table 1. In addition, these compounds were evaluated for their effects on cell proliferation in U937 and its camptothecin-resistant variant U937/U937CR,[32] P388 and its camptothecin-resistant variant P388/P388CPT45,[33] and KB3-1 and its variant, KBV-1,[34] which overexpresses *p*-glycoprotein (MDR1). These data are provided in Table 2.

A representative gel associated with the assays performed to determine TOP1-targeting activity, which provides an index of the relative potency of individual compounds to enhance cleavable complex formation between the enzyme and DNA, is illustrated in Figure 2.

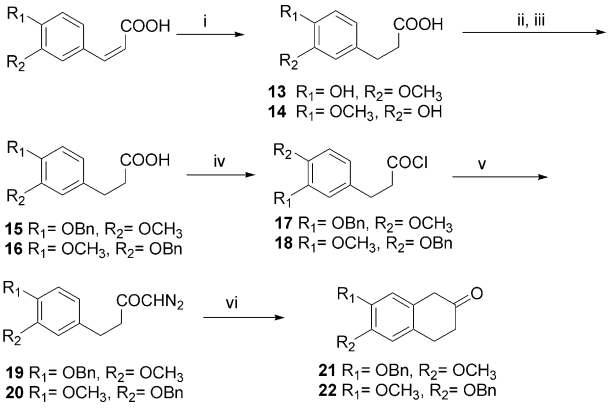
Results and Discussion

The relative TOP1-targeting activity of the various 8,9-methylenedioxybenzo[*i*]phenanthridines evaluated in this study indicates that the presence of methoxyl substituents at both the 2- and 3-positions is associated with increased potency relative to those analogues that possess a single methoxyl group at either of these positions. In the case of 8,9-methylenedioxybenzo[*i*]phenanthridines, **4** clearly exhibits enhanced TOP1-targeting



Scheme 1. Synthetic approach to the preparation of 8,9-methylenedioxydi-benzo[*i*]phenanthridine derivatives, **10a–10f** and **12b–12d**: (i) DDQ, toluene; (ii) $Pd(PPh_3)_4$, CuBr, THF, reflux; (iii) Zn dust, AcOH, reflux; (iv) Ra–Ni, H_2 at 44 psi.

activity relative to either **12b** or **12c**. Among the 11,12-dihydrobenzo[*i*]phenanthridines, **10a** exhibits significantly greater TOP1-targeting activity than either **10b** or **10c**. There was a slight increase in TOP1-targeting activity observed with 1**0a** relative to its unsaturated derivative, **4**. These data parallel the results obtained for coralyne and its dihydro derivative, MDD-coralyne, in that the dihydro derivative exhibited enhanced TOP1-targeting activity and diminished potency as a TOP2-targeting agent. This trend was not clearly reflected in the studies performed with the mono-methoxylated derivatives, **10b**, **10c**, **12b**, and **12c**. While **10b** was more active as a TOP1-targeting agent than **12b**, it was also more potent as a TOP2-targeting agent. In the case of **10c**, it was actually slightly less active than its unsaturated analogue, **12c**, as a TOP1-targeting agent and had similar TOP2-targeting activity.



Scheme 2. Preparation of the β-tetralone intermediates used in the synthesis of **6d** and **6e**: (i) 10% Pd/C; H_2, 40 psi; (ii) BnBr, K_2CO_3; (iii) KOH, H_2O, CH_3OH; (iv) $(COCl)_2$; (v) $TMSCHN_2$; (vi) $Rh(OAc)_2$.

The replacement of the 3-methoxy group of either **10a** or **4** with a benzyloxy group, as in the case of **10d** or **12d**, results in a loss of both TOP1- and TOP2-targeting activities. While replacement of the 2-methoxyl group of **10a** with a hydroxyl group, as in **10e**, results in a major loss of TOP1-targeting activity, substitution of the 3-methoxyl group of **10a** with a hydroxyl group, as in **10f**, did not have a significant impact on TOP1-targeting activity. Thus, **10f** could be used to develop a prodrug that would allow modification of its physico-chemical properties.

Table 2. Pharmacological activity of 8,9-methylenedioxybenzo[*i*]phenanthridine derivatives

Compd	Cytotoxicity IC_{50} (μM)[a]					
	U937	U937/CR	P388	P388/CPT45	KB3-1	KBV-1
1	8	40	1.5	9.0	1.3	>10
2	—[b]	—[b]	0.7	2.2	1.0	1.0
3	2.9	5.0	0.45	0.46	5.5	1.8
4	0.1	0.1	0.23	0.26	0.3	0.2
5	0.06	7.5	0.33	>10	0.05	0.12
10a	0.04	0.05	0.19	0.18	0.16	0.24
10b	0.2	0.5	0.16	0.25	0.3	0.4
10c	34	>100	4.5	22	7.0	2.2
10d	0.09	0.15	—	—	0.18	0.24
10e	10	40	2.4	19.0	3.0	0.3
10f	1.0	0.8	0.36	0.90	0.75	0.36
12b	0.04	0.06	0.29	0.52	0.23	0.27
12c	8.0	60	2.65	2.65	13	2.3
12d	0.29	0.17	0.1	0.18	0.19	0.25
Nitidine	0.8	3.5	0.006	0.63	0.06	2.5
CPT	0.006	0.65	0.13	>10	0.01	0.03
VM-26	0.03	0.01	0.006	0.001	0.02	2.25

[a]IC_{50} has been calculated after 4 days of continuous drug exposure.
[b]Not determined.

Table 1. Pharmacological activity of 8,9-methylenedioxybenzo[*i*]phenanthridine derivatives

Compd	Topo I-mediated DNA cleavage[b]	Topo II-mediated DNA cleavage[c]	Cytotoxicity IC_{50} (μM)[a] cell lines	
			RPMI8402	CPT-K5
1	400	10	5.9	27
2	50	>1000	6.8	22
3	100	10	4.5	11
4	10	>1000	0.4	0.4
5	8	500	0.07	5.5
10a	8	>1000	0.4	0.2
10b	200	500	0.6	0.8
10c	200	500	33	70
10d	>1000	>1000	0.16	0.14
10e	500	500	7.6	25
10f	10	>1000	1.4	1.1
12b	>1000	>1000	0.2	0.2
12c	100	500	18	>100[d]
12d	>1000	>1000	0.3	0.3
Nitidine	10	1	0.4	4.0
Topotecan	1.0	>1000	0.012	>10
Camptothecin	0.5	>1000	0.005	>60
VM-26	>1000	1	0.2	0.3

[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, that is concentrations relative to topotecan, 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, relative effective concentration, that is 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 human topoisomerase II.
[d]IC_{50} values were substantially greater than the highest doses assayed.

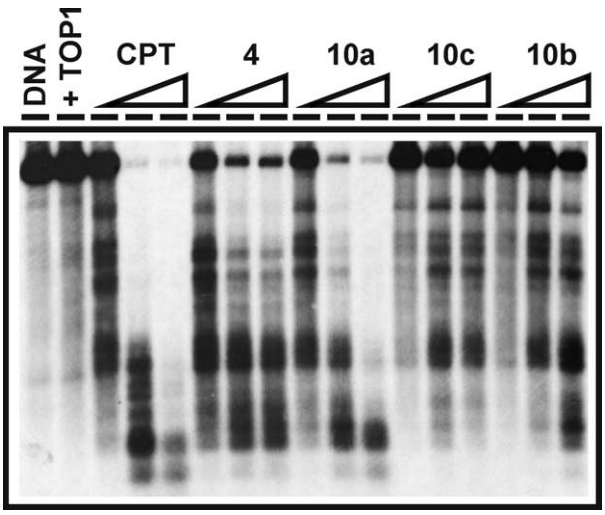


Figure 2. Stimulation of enzyme-mediated DNA cleavage by camptothecin (CPT), **4**, **10a**, **10c**, and **10b** using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted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

The influence of structural modification on the cytotoxicity of these 8,9-methylenedioxybenzo[*i*]phenanthridines as determined using the MTT assay was also assessed. Previous studies in our laboratory have noted that cytotoxic activity and TOP1-targeting activity do not correlate well unless the compound is exclusively a TOP1-targeting agent (i.e., does not exhibit significant TOP2-targeting activity), its potency as a TOP1-targeting agent is at least 10% of that of camptothecin, and there are no significant differences in their potential for cellular absorption. The three more potent TOP1-targeting benzo[*i*]phenanthridines were **4**, **10a** and **10f**. The IC_{50} values obtained for **4** and **10a** ranged from 40 to 500 nM and for **10f** from 360 to 1400 nM. As previously observed for **4**, there was no significant cross-resistance ($\geq$10-fold difference) observed between either RPMI8402 and its camptothecin-resistant variant CPT-K5 or the leukemia cell line, U937 and its camptothecin-resistant variant U937/CR with any of the benzo[*i*]phenanthridines evaluated. Both of these camptothecin-resistant cell lines have a functional, but mutant form of TOP1 that forms a cleavable complex with DNA that is not stabilized by the presence of camptothecin.[31,32] Therefore, the potential role of TOP1-targeting being associated with the effects observed with these benzo[*i*]-phenanthridines in these camptothecin-resistant cell lines cannot be ruled out. These data are, however, in marked contrast to the results obtained with similarly substituted benzo[*c*,*h*]cinnolines, which are 5-aza analogues of benzo[*i*]phenanthridine. In this series of TOP1-targeting agents, there is clear evidence of cross-resistance observed in both CPT-K5 cells and in U937/CR cells.[26] In light of these differences benzo[*i*]phenanthridines were also evaluated for their effect on P388 murine leukemia cells and its camptothecin-resistant variant, P388/CPT45. As the P388/CPT45 cell line does not functionally express topoisomerase-I, these cells provide a good indication as to whether or not TOP1-targeting activity is likely to play a dominant role on cell proliferation as determined using the MTT assay. These data as outlined in Table 2 indicate that mechanisms other than TOP1-targeting activity are associated with the effects that several benzo[*i*]phenanthridines have on cell proliferation (some of these derivatives have modest TOP2-targeting activity). Three of the benzo[*i*]phenanthridines evaluated in this study, **10d**, **12b**, and **12d**, did not exhibit significant TOP1- or TOP2-targeting activity. Nonetheless, these derivatives had significant cytotoxicity (IC_{50} values ranging from 40 nmol to 300 nM). These data again suggest that mechanisms other than stabilization of TOP1-cleavable complex formation do contribute to the cytotoxicity observed with substituted benzo[*i*]phenanthridines.

Several cytotoxic agents are resistant to cells that overexpress the efflux transporter *p*-glycoprotein or MDR1. In cells that overexpress *p*-glycoprotein, much higher concentrations of the drug are required to achieve lethality if it is a substrate for this efflux transported. The absence of a significant difference between KB3-1 cells and its variant, KBV-1, which is known to overexpress *p*-glycoprotein, indicates that these benzo[*i*]phenanthridines are not substrates for this efflux transporter and are capable of overcoming this form of multidrug resistance.

Conclusions

Benzo[*i*]phenanthridines represent a series of novel topoisomerase I-targeting agents. Unlike the camptothecin-based TOP1-targeting agents available in the clinic, these agents do not have an unstable lactone moiety incorporated within their structures. Improved potency has been observed for certain 11,12-dihydrobenzo[*i*]phenanthridines as TOP1-targeting agents. Benzo[*i*]phenanthridines with a 8,9-methylenedioxy moiety in the D-ring together with a 2-methoxyl group and either a hydroxyl or a second methoxyl group at the 3-position of the A-ring are among the more potent analogues. The data also suggest that mechanisms other than TOP-1 targeting contribute significantly to the effects observed by several benzo[*i*]phenanthridines on cell proliferation as obtained using the MTT assay. In contrast to results obtained with 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline, **5**, several benzo[*i*]phenanthridines did not exhibit significant differences with respect between their effect on parent and variants cell lines, which have either mutant topoisomerase I or do not express topoisomerase I. Studies are in progress to develop derivatives with increased potency as TOP1-targeting agents that specifically exert their cytotoxic effect through stabilization of the TOP1 cleavable complex.

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) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in the deuterated 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 from 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). It is very sensitive to air-oxidation and the resulting Pd(II) with brown color loses catalytic activity. 6-Methoxy-, 7-methoxy-, and 6,7-dimethoxy-β-tetralone were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Compounds **4** and **8** were prepared as previously described.[24]

General procedure for the preparation of 2-bromo-3,4-dihydro-1-naphthaldehydes (6a–e)

2-Bromo-3,4-dihydro-6,7-dimethoxy-1-naphthaldehyde (6a). 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 $NaHCO_3$ solution. 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 a 1:3 mixture of ethyl acetate/hexanes to give **72** (1.15 g) as yellow crystalline solid in 80% yield; mp 82–83 °C; ^1H 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; HRMS calcd for $C_{13}H_{13}BrO_3$: 296.0048; found: 296.0042.

2-Bromo-3,4-dihydro-6-methoxy-1-naphthaldehyde (6b). Prepared from 6-methoxy-2-tetralone (950 mg, 5.4 mmol) in 50% yield (718 mg); ^1H NMR δ 2.88 (2H, t, $J=6.5$), 3.01 (2H, t, $J=6.5$), 3.81 (3H, s), 6.70 (1H, d, $J=2.7$), 6.78 (1H, dd, $J=8.5$, 2.7), 7.95 (1H, d, $J=8.5$), 10.30 (1H, s); ^{13}C NMR δ 29.2, 37.8, 55.3, 111.3, 113.8, 123.0, 127.3, 132.5, 136.8, 142.5, 159.5, 193.0; HRMS calcd for $C_{12}H_{11}BrO_2$: 265.9942; found: 265.9924.

2-Bromo-3,4-dihydro-7-methoxy-1-naphthaldehyde (6c). Prepared from 7-methoxy-2-tetralone (1.0 g, 5.7 mmol) in 73% yield (1.11 g); mp 69–70 °C; ^1H NMR δ 2.83 (2H, t, $J=7.3$), 3.02 (2H, t, $J=7.3$), 3.81 (3H, s), 6.79 (1H, dd, $J=8.3$, 2.7), 7.06 (1H, d, $J=8.3$), 7.64 (1H, d, $J=2.7$), 10.32 (1H, s); ^{13}C NMR δ 28.0, 38.4, 55.5, 111.5, 114.1, 126.7, 128.2, 130.8, 132.6, 146.2, 158.4, 192.8; HRMS calcd for $C_{12}H_{11}BrO_2$: 265.9942; found: 265.9946.

7-Benzyloxy-2-bromo-3,4-dihydro-6-methoxy-1-naphthaldehyde (6d). Prepared from 6-methoxy-7-benzyloxy-2-tetralone, **21** (950 mg, 3.4 mmol) in 20% yield; (254 mg); mp 88–89 °C; ^1H NMR δ 2.84 (2H, t, $J=7.6$), 2.98 (2H, t, $J=7.6$), 3.90 (3H, s), 5.18 (2H, s), 6.69 (1H, s), 7.34–7.53 (5H, m), 7.80 (1H, s), 10.29 (1H, s); ^{13}C NMR δ 28.5, 38.1, 56.1, 71.2, 111.3, 112.2, 122.6, 127.8, 128.0, 128.2, 128.7, 132.2, 137.2, 143.7, 146.4, 149.4, 193.1; HRMS calcd for $C_{19}H_{17}BrO_3$: 372.0361; found: 372.0380.

2-Bromo-3,4-dihydro-6-hydroxy-7-methoxy-1-naphthaldehyde (6e). Prepared from 6-methoxy-7-benzyloxy-2-tetralone **22** (950 mg, 3.4 mmol in 50% yield (481 mg); mp >250 °C ^1H NMR δ 2.82 (2H, t, $J=7.2$), 3.00 (2H, t, $J=7.2$), 3.93 (3H, s), 5.71 (1H, s), 6.74 (1H, s), 7.72 (1H, s), 10.33 (1H, s); ^{13}C NMR δ 28.2, 38.1, 56.3, 109.1, 113.8, 128.5, 130.5, 132.3, 143.9, 144.9, 145.5, 193.3; HRMS calcd for $C_{12}H_{11}BrO_3$: 281.9892; found: 281.9887.

General procedure for the synthesis of 2-bromo-1-naphthaldehydes (7b, 7c, 7d)

The appropriate 2-bromo-3,4-dihydro-1-naphthaldehyde (1.0 mmol) and DDQ (272 mg, 1.2 mmol) was refluxed in toluene (40 mL) for 15 h. After cooling to room temperature, the mixture was filtered through a Celite bed and the filtrate was evaporated to dryness. The residue obtained was chromatographed on 75 g silica gel using a 1:5 mixture of ethyl acetate/hexanes.

2-Bromo-6-methoxy-1-naphthaldehyde (7b). Prepared from **6b** (370 mg, 1.39 mmol) in 80% yield (294 mg); mp 105–107 °C; IR (KBr) 1672; ^1H NMR δ 3.92 (3H, s), 7.09 (1H, d, $J=2.6$), 7.29 (1H, dd, $J=9.4$, 2.6), 7.61 (1H, d, $J=8.8$), 7.73 (1H, d, $J=8.8$), 8.99 (1H, d, $J=9.4$), 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 calcd for $C_{12}H_9BrO_2$: 263.9786; found: 263.9782.

2-Bromo-7-methoxy-1-naphthaldehyde (7c). Prepared from (**6c**) (850 mg, 3.18 mmol) in 85% yield as yellow solid (714 mg); mp 132–134 °C; IR(KBr) 1670; ^1H NMR δ 3.97 (3H, s), 7.21 (1H, dd, $J=8.9$, 2.6), 7.54 (1H, d, $J=8.7$), 7.71 (1H, d, $J=8.9$), 7.77 (1H, d, $J=8.7$), 8.69 (1H, d, $J=2.6$), 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 calcd for $C_{12}H_9BrO_2$: 263.9786; found: 263.9783.

7-Benzyloxy-2-bromo-6-methoxy-1-naphthaldehyde (7d). Prepared from **6d** (190 mg, 0.51 mmol) in 84% yield as light yellow powder (160 mg); mp 147–148 °C; ^1H NMR

δ 4.02 (3H, s), 5.36 (2H, s), 7.12 (1H, s), 7.34–7.42 (4H, m), 7.57 (1H, d, $J=8.6$), 7.60 (1H, d, $J=5.2$), 7.73 (1H, d, $J=8.6$), 8.86 (1H, s), 10.75 (1H, s); ^{13}C NMR δ 55.9, 70.7, 105.4, 106.8, 126.1, 127.9, 128.0, 128.2, 128.7, 129.1, 129.4, 129.6, 134.0, 136.2, 150.3, 151.7, 195.5; HRMS calcd for $C_{19}H_{15}BrO_3$: 370.0205; found: 370.0187.

General procedure for the synthesis of 9a–9e and 11b–11d

Tetrakis(triphenylphosphine)palladium (0) (60 mg, 0.05 mmol) and cuprous bromide (10 mg, 0.07 mmol) was added to a solution of **8** (400 mg, 1.2 mmol) and the appropriate 2-bromo-3,4-dihydronaphthalene (1.0 mmol) in THF (25 mL) at room temperature. The mixture was refluxed for 48 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 bed to remove suspended particles. The organic layer was then washed with brine, dried (anhyd Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using 75 g of silica gel and a 1:4 mixture of ethyl acetate/hexanes.

3,4-Dihydro-2-(3,4-methylenedioxy-6-nitrophenyl)-6,7-dimethoxy-1-naphthaldehyde (9a). Prepared from **6a** in 90% yield as a bright yellow solid (234 mg); mp 188–189 °C ^{1}H NMR δ 2.69 (2H, t, $J=5.9$), 2.82 (2H, t, $J=5.9$), 3.92 (3H, s), 3.93 (3H, s), 6.20 (2H, s), 6.71 (1H, s), 6.74 (1H, s), 7.76 (1H, s), 7.90 (1H, s), 9.70 (1H, s); ^{13}C NMR δ 28.2, 33.5, 56.4, 56.5, 104.0, 106.6, 110.6, 111.0, 111.2, 122.9, 129.2, 132.1, 132.5, 141.8, 147.6, 148.9, 149.1, 152.7, 157.9, 191.2; HRMS calcd for $C_{20}H_{17}NO_7$: 383.1005; found: 383.1014.

3,4-Dihydro-2-(4,5-methylenedioxy-2-nitrophenyl)-6-methoxy-1-naphthaldehyde (9b). Prepared from **6b** (267 mg, 1.0 mmol) in 84% yield as golden solid (297 mg); mp 156–157 °C; ^{1}H NMR δ 2.62–3.25 (4H, m), 3.86 (3H, s), 6.21 (2H, s), 6.72 (1H, s), 6.79 (1H, s), 6.82 (1H, dd, $J=8.6$, 3.0), 7.77 (1H, s), 8.14 (1H, d, $J=8.6$), 9.72 (1H, s); ^{13}C NMR δ 28.5, 32.8, 55.4, 103.7, 106.2, 110.2, 111.3, 113.7, 122.8, 128.2, 131.9, 132.2, 138.0, 148.4, 152.3, 156.7, 159.5, 190.7; HRMS calcd for $C_{19}H_{15}NO_6$: 353.0899; found: 353.0916.

3,4-Dihydro-2-(4,5-methylenedioxy-2-nitrophenyl)-7-methoxy-1-naphthaldehyde (9c). Prepared from **6c** (270 mg, 1.0 mmol) in 45% yield as yellow solid; mp 133–134 °C; ^{1}H NMR δ 2.62–3.22 (4H, m), 3.86 (3H, s), 6.22 (2H, s), 6.71 (1H, s), 6.84 (1H, dd, $J=8.2$, 2.6), 7.14 (1H, d, $J=8.2$), 7.78 (1H, s), 7.84 (1H, d, $J=2.6$), 9.74 (1H, s); ^{13}C NMR δ 27.3, 33.4, 55.5, 103.7, 106.2, 109.9, 112.3, 114.2, 127.9, 128.2, 130.7, 131.9, 132.1, 141.3, 148.5, 152.4, 158.3, 159.8, 190.5; HRMS calcd for $C_{19}H_{15}NO_6$: 353.0899; found: 353.0893.

7-Benzyloxy-3,4-Dihydro-6-methoxy-2-(4,5-methylenedioxy-2-nitrophenyl)-1-naphthaldehyde (9d). Prepared from **6d** (100 mg, 0.27 mmol) in 73% yield as yellow solid (90 mg); mp 151–152 °C ^{1}H NMR δ 2.65–3.30 (4H, m), 3.93 (3H, s), 5.20 (2H, s), 6.21 (2H, s), 6.70 (1H, s), 6.77 (1H, s), 7.31–7.43 (5H, m), 7.76 (1H, s), 7.98 (1H, s), 9.68 (1H, s); ^{13}C NMR δ 27.8, 33.1, 56.1, 71.3, 103.7, 106.2, 110.2, 111.4, 113.1, 122.5, 127.8, 128.2, 128.6, 129.4, 131.6, 132.1, 137.4, 148.5, 149.4, 152.3, 157.3, 157.4, 190.8; HRMS calcd for $C_{26}H_{21}NO_7$: 459.1318; found: 459.1304.

3,4-Dihydro-6-hydroxy-7-methoxy-2-(4,5-methylenedioxy-2-nitrophenyl)-1-naphthaldehyde (9e). Prepared from **6e** (50 mg, 0.18 mmol) in 38% yield as orange solid (25 mg); mp >250 °C ^{1}H NMR δ 2.60–2.88 (4H, m), 3.95 (3H, s), 5.73 (1H, s), 6.22 (2H, s), 6.72 (1H, s), 6.80 (1H, s), 7.77 (1H, s), 7.90 (1H, s), 9.70 (1H, s); ^{13}C NMR δ 27.5, 33.0, 56.3, 103.6, 106.2, 110.2, 113.8, 129.7, 131.8, 132.1, 138.1, 144.9, 145.6, 148.4, 148.6, 152.3, 157.5, 190.9; HRMS calcd for $C_{19}H_{15}NO_7$: 369.0849; found: 369.0856.

2-(4,5-Methylenedioxy-2-nitrophenyl)-6-methoxy-1-naphthaldehyde (11b). Prepared from **8** (350 mg, 1.1 mmol) and **7b** (260 mg, 1.0 mmol) as yellow solid in 84% yield; mp 153–154 °C ^{1}H NMR δ 3.98 (3H, s), 6.23 (2H, s), 6.78 (1H, s), 7.22 (1H, s), 7.25 (1H, d, $J=8.8$), 7.38 (1H, d, $J=9.6$), 7.70 (1H, s), 7.97 (1H, d, $J=9.6$), 9.15 (1H, d, $J=8.8$), 10.28 (1H, s); ^{13}C NMR δ 55.5, 103.5, 105.6, 106.6, 111.9, 121.9, 126.1, 127.3, 128.3, 131.1, 133.2, 135.2, 142.0, 142.7, 148.2, 151.4, 158.4, 192.9; HRMS calcd for $C_{19}H_{13}NO_6$: 351.0743; found: 351.0753.

2-(4,5-Methylenedioxy-2-nitrophenyl)-7-methoxy-1-naphthaldehyde (11c). Prepared from **8** (350 mg, 1.1 mmol) and **7c** (300 mg, 1.1 mmol) as light yellow solid in 40% yield; mp 142–143 °C ^{1}H NMR δ 4.02 (3H, s), 6.23 (2H, s), 6.78 (1H, s), 7.14 (1H, d, $J=8.0$), 7.28 (1H, dd, $J=9.2$, 2.6), 7.70 (1H, s), 7.82 (1H, d, $J=9.2$), 8.00 (1H, d, $J=8.0$), 8.81 (1H, d, $J=2.6$), 10.26 (1H, s); ^{13}C NMR δ 55.6, 103.5, 104.2, 105.6, 111.7, 120.1, 124.3, 126.8, 129.1, 130.0, 131.2, 132.5, 134.4, 142.5, 145.9, 148.2, 151.4, 161.2, 193.0; HRMS calcd for $C_{19}H_{13}NO_6$: 351.0743; found: 351.0752.

7-Benzyloxy-6-methoxy-2-(4,5-methylenedioxy-2-nitrophenyl)-1-naphthaldehyde (11d). Prepared from **8** (126 mg, 0.38 mmol) and **7d** (130 mg, 0.35 mmol) as yellow-green solid in 90% yield; mp 192–193 °C ^{1}H NMR δ 4.05 (3H, s), 5.37 (2H, s), 6.22 (2H, s), 6.78 (1H, s), 7.13 (1H, d, $J=8.0$), 7.20 (1H, s), 7.30–7.63 (5H, m), 7.68 (1H, s), 7.91 (1H, d, $J=8.0$), 8.94 (1H, s), 10.20 (1H, s); ^{13}C NMR δ 56.0, 70.8, 103.5, 105.6, 106.3, 106.9, 112.0, 124.9, 126.6, 126.9, 128.0, 128.2, 128.7, 130.0, 131.2, 132.9, 136.4, 142.7, 143.3, 148.1, 150.4, 151.3, 151.8, 193.2; HRMS (FAB) calcd for $C_{26}H_{19}NO_7$+ Li: 464.1322; found: 464.1315.

General procedure for the preparation of 11,12-dihydro-2,3-dimethoxy-8,9methylenedioxybenzo[*i*]phenanthridines (10a, 10b, 10c, 10d) and 2,3-dimethoxy-8,9methylenedioxybenzo[*i*]phenanthridines (12b, 12c, 12d)

The appropriate 3,4-dihydro-2-(6-nitrophenyl)-1-naphthaldehyde (0.26 mmol) was dissolved in glacial acetic acid (15 mL) and heated under reflux with zinc dust

(180 mg, 2.8 mmol) for 2.5 h. Acetic acid was evaporated in vacuo and the residue was dissolved in chloroform. The solution was filtered through a Celite bed and the filtrate washed successively with saturated sodium bicarbonate solution 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 a 1:1 mixture of ethyl acetate/hexanes.

11,12-Dihydro-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]-phenanthridine (10a). Prepared from **9a** in 60% yield as pale yellow solid (52 mg); mp 210–211 °C ^{1}H NMR δ 2.91 (2H, t, J = 7.8), 3.13 (2H, t, J = 7.8), 3.94 (3H, s), 3.99 (3H, s), 6.10 (2H, s), 6.81 (1H, s), 7.30 (1H, s), 7.37 (2H, s), 9.03 (1H, s); ^{13}C NMR 24.4, 28.0, 56.5, 56.7, 99.5, 102.2, 106.9, 107.5, 111.9, 123.8, 125.3, 126.1, 129.6, 140.1, 144.5, 145.8, 148.8, 149.4, 150.3; HRMS calcd for $C_{20}H_{17}NO_4$: 335.1158; found: 335.1145.

11,12-Dihydro-2-methoxy-8,9-methylenedioxybenzo[*i*]-phenanthridine (10b). Prepared from **9b** (100 mg, 0.28 mmol) in 35% yield as pale yellow solid (30 mg); mp 187–188 °C ^{1}H NMR δ 2.95 (2H, t, J = 6.6), 3.13 (2H, t, J = 6.6), 3.87 (3H, s), 6.10 (2H, s), 6.84 (1H, d, J = 2.6), 6.85 (1H, s), 6.90 (1H, dd, J = 8.4, 2.6), 7.37 (1H, s), 7.79 (1H, d, J = 8.4), 9.06 (1H, s); ^{13}C NMR δ 23.8, 28.4, 55.5, 99.2, 101.8, 106.5, 112.6, 113.9, 123.4, 124.8, 125.3, 125.7, 138.1, 139.5, 144.3, 145.3, 148.3, 149.8, 159.5; HRMS (FAB) calcd for $C_{19}H_{15}NO_3$ + H: 306.1130; found: 306.1129.

11,12-Dihydro-3-methoxy-8,9-methylenedioxybenzo[*i*]-phenanthridine (10c). Prepared from **9c** (70 mg, 0.20 mmol) in 50% yield as pale yellow solid (30 mg); mp 190–191 °C ^{1}H NMR δ 2.94 (2H, t, J = 7.8), 3.17 (2H, t, J = 7.8), 3.91 (3H, s), 6.14 (2H, s), 6.86 (1H, dd, J = 8.4, 2.6), 7.24 (1H, d, J = 8.4), 7.36 (1H, s), 7.42 (1H, s), 7.44 (1H, s), 9.11 (1H, s); ^{13}C NMR δ 24.2, 27.1, 55.6, 99.3, 101.9, 106.5, 109.6, 113.0, 125.7, 128.6, 129.0, 133.5, 141.2, 144.6, 145.9, 148.4, 150.3, 151.5, 159.1; HRMS (FAB) calcd for $C_{19}H_{15}NO_3$ + H: 306.1130; found: 306.1143.

3-Benzyloxy-11,12-dihydro-2-methoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (10d). Prepared from **9d** (80 mg, 0.17 mmol in 20% yield as pale yellow solid (15 mg); ^{1}H NMR δ 2.90 (2H, t, J = 8.0), 3.12 (2H, t, J = 8.0), 3.96 (3H, s), 5.27 (2H, s), 6.10 (2H, s), 6.84 (1H, s), 7.35–7.56 (8H, m), 8.86 (1H, s); ^{13}C NMR δ 24.0, 27.7, 56.2, 71.7, 99.1, 101.8, 106.5, 110.4, 111.9, 123.4, 124.8, 125.7, 127.5, 128.1, 128.8, 129.8, 137.3, 139.7, 144.1, 145.4, 147.4, 148.4, 149.7, 149.9; HRMS calcd for $C_{26}H_{21}NO_4$: 411.1471; found: 411.1471.

11,12-Dihydro-2-hydroxy-3-methoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (10e). Prepared from **9e** (15 mg, 0.04 mmol) was dissolved in glacial acetic acid (10 mL) and heated under reflux with zinc dust (25 mg, 0.38 mmol) for 2 h. Trifluoroacetic acid (5 mL) was added until the suspension turned clear. The solution was filtered through a Celite bed and the filtrate was evaporated in vacuo. The residue was recrystallized using ethanol/hexanes to give **10e** (9 mg) as yellow solid in 70% yield; mp >250 °C ^{1}H NMR (DMSO-d_6) δ 2.80 (2H, t, J = 7.0), 3.20 (2H, t, J = 7.0), 3.89 (3H, s), 6.28 (1H, s), 6.78 (1H, s), 7.43 (1H, s), 7.56 (1H, s), 7.70 (1H, s), 9.24 (1H, s); ^{13}C NMR (DMSO-d_6) δ 24.2, 26.9, 56.7, 100.3, 103.0, 104.0, 109.0, 109.2, 115.9, 122.6, 124.0, 126.6, 129.9, 144.5, 145.0, 145.4, 147.7, 149.3, 151.0; HRMS calcd for $C_{19}H_{15}NO_4$: 321.1001; found: 321.0992.

2-Methoxy - 8,9 - methylenedioxybenzo[*i*]phenanthridine (12b). Prepared from **11b** in 50% yield as pale yellow solid (29 mg); mp 232–233 °C; ^{1}H NMR (DMSO-d_6) δ 3.95 (3H, s), 6.27 (2H, s), 7.42 (1H, dd, J = 9.2, 2.6), 7.54 (1H, s), 7.60 (1H, d, J = 2.6), 8.20 (1H, d, J = 9.2), 8.31 (1H, s), 8.68 (1H, d, J = 9.2), 9.00 (1H, d, J = 9.2), 10.08 (1H, s); ^{13}C NMR (DMSO-d_6) δ 55.9, 100.4, 102.6, 107.0, 109.1, 119.5, 120.8, 121.4, 121.7, 124.4, 124.7, 130.4, 131.6, 133.6, 142.7, 145.0, 146.1, 148.8, 149.7; HRMS calcd for $C_{19}H_{13}NO_3$: 303.0895; found: 303.0899.

3-Methoxy - 8,9 - methylenedioxybenzo[*i*]phenanthridine (12c). Prepared from **11c** (50 mg, 0.14 mmol) in 56% yield as pale yellow solid (24 mg); mp 239–240 °C ^{1}H NMR (DMSO-d_6) δ 4.05 (3H, s), 6.28 (2H, s), 7.36 (1H, dd, J = 8.8, 1.8), 7.56 (1H, s), 8.05 (1H, d, J = 8.8), 8.19 (1H, d, J = 9.2), 8.32 (1H, s), 8.46 (1H, d, J = 1.8), 8.54 (1H, d, J = 9.2), 10.18 (1H, s); ^{13}C NMR (DMSO-d_6) δ 56.3, 100.7, 102.7, 103.6, 107.0, 118.4, 118.5, 120.6, 126.8, 130.9, 131.4, 131.7, 131.8, 132.4, 143.3, 146.7, 148.8, 150.0, 159.8; HRMS calcd for $C_{19}H_{13}NO_3$: 303.0895; found: 303.0892.

3-Benzyloxy-2-methoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (12d). Prepared from **11d** (100 mg, 0.22 mmol) as white solid in 50% yield (45 mg); ^{1}H NMR δ 4.10 (3H, s), 5.47 (2H, s), 6.18 (2H, s), 7.34 (1H, s), 7.38–7.48 (3H, m), 7.57 (1H, s), 7.58–7.62 (2H, m), 7.91 (1H, s), 7.99 (1H, d, J = 9.2), 8.19 (1H, s), 8.25 (1H, d, J = 9.2), 9.76 (1H, s); ^{13}C NMR δ 56.1, 71.3, 99.4, 101.9, 104.7, 107.2, 108.4, 118.3, 120.8, 124.8, 125.0, 127.4, 128.3, 129.0, 130.5, 130.8, 131.1, 136.7, 142.3, 145.5, 148.4, 149.4, 149.6, 150.2; HRMS calcd for $C_{26}H_{19}NO_4$: 409.1314; found: 409.1300.

11,12-Dihydro-3-hydroxy-2-methoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (10f). To a solution of **10d** (70 mg, 0.17 mmol) in a mixture of methanol (20 mL) and ethyl acetate (15 mL) was added Raney–Nickel (70 mg) and the resulted solution was shaken in a Parr® apparatus at 44 psi of hydrogen for 18 h. A magnetic rod was used to get rid of most of the nickel. Trifluoroacetic acid (5 mL) was added dropwise to the reaction mixture until the suspension turned to clear yellow solution. The solution was filtered through a Celite bed and the filtrate was evaporated in vacuo to give **10f** (54 mg) as yellow solid in quantitative yield; mp >250 °C; ^{1}H NMR (CD_3OD) δ 2.99 (2H, t, J = 7.0), 3.40 (2H, t, J = 7.0), 3.93 (3H, s), 6.34 (2H, s), 6.96 (1H, s), 7.36 (1H, s), 7.41 (1H, s), 7.77 (1H, s), 9.07 (1H, s); ^{13}C NMR (DMSO-d_6) δ 24.2, 26.9, 56.7, 100.3, 103.0, 104.0, 109.0, 116.0, 122.6, 124.0, 126.6, 129.9, 147.6, 147.8, 149.2, 149.5, 151.0; HRMS calcd for $C_{19}H_{15}NO_4$: 321.1001; found: 321.0987.

3-(4-Hydroxy-3-methoxyphenyl)propionic acid (13). To a solution of *trans*-4-hydroxy-3-methoxy cinnamic acid (5.0 g, 25.7 mmol) in ethyl acetate (20 mL) was carefully added palladium on carbon (10%, 500 mg). Methanol (20 mL) was then added. The reaction mixture was shaken in a Parr® apparatus for 5 h at 40 psi of hydrogen. The solution was passed through a Celite bed and the catalyst was washed carefully with methanol. Concentration in vacuo gave pure **13** (5.0 g) as white solid in quantitative yield; ^{1}H NMR (CD_3OD) δ 2.56 (2H, t, J=7.3), 2.83 (2H, t, J=7.3), 3.83 (3H, s), 6.62–6.81 (3H, m); ^{13}C NMR (CD_3OD) δ 32.0, 37.5, 56.6, 113.3, 116.4, 121.9, 134.0, 146.1, 149.2, 177.3.

3-(3-Hydroxy-4-methoxyphenyl)propionic acid (14). To a solution of 3-hydroxy-4-methoxy cinnamic acid (1.0 g, 5.1 mmol) in ethyl acetate (20 mL) was carefully added palladium on carbon (10%, 500 mg). Methanol (30 mL) was then added. The reaction mixture was shaken in a Parr® apparatus for 5 h at 42 psi of hydrogen. The solution was passed through a Celite bed and the catalyst was washed carefully with methanol. Concentration in vacuo gave pure **14** (1.0 g) as white solid in quantitative yield; mp 143–144 °C; ^{1}H NMR (CD_3OD) δ 2.55 (2H, t, J=7.4), 2.83 (2H, t, J=7.4), 3.82 (3H, s), 6.64-6.71 (2H, m), 6.83 (1H, d, J=8.0); ^{13}C NMR (CD_3OD) δ 30.1, 35.7, 55.1, 111.6, 115.1, 119.1, 133.8, 146.2, 175.7; HRMS calcd for $C_{10}H_{12}O_4$: 196.0736; found: 196.0734.

3-(4-Benzyloxy-3-methoxyphenyl)propionic acid (15). A solution of **13** (1.0 g, 5.1 mmol), benzyl bromide (3.0 mL, 25.2 mmol), and potassium carbonate (2.0g, 14.5 mmol) in acetone (15 mL) and acetonitrile (15 mL) was refluxed for 15 h. The reaction mixture was concentrated in vacuo. To the residue was added methanol (3 mL), water (30 mL) and potassium hydroxide (4.0 g, 71 mmol). The mixture was heated at 70 °C for 10 h. After cooling to room temperature, the reaction mixture was washed with chloroform (30 mL×3) and concentrated hydrochloric acid (HCl) was added dropwise until the pH of the solution was 2.0. The mixture was extracted with chloroform (40 mL×3) and the organic layer was washed with water (20 mL×2) and brine (30 mL), dried (anhyd Na_2SO_4), and evaporated in vacuo. The residue was triturated with hexanes several times and **15** (1.26 g) was obtained in 86% yield as a beige solid; mp 94–95 °C ^{1}H NMR (CD_3OD) δ 2.57 (2H, t, J=7.0), 2.86 (2H, t, J=7.0), 3.84 (3H, s), 5.06 (2H, s), 6.72 (1H, dd, J=8.1, 1.8), 6.87 (1H, s), 6.89 (1H, d, J=8.1), 7.29–7.46 (5H, m); ^{13}C NMR (CD_3OD) δ 31.9, 37.2, 56.8, 72.8, 114.2, 116.4, 121.8, 129.0, 129.1, 129.7, 136.2, 139.2, 148.2, 151.5, 177.1; HRMS calcd for $C_{17}H_{18}O_4$: 286.1205; found: 286.1218.

3-(3-Benzyloxy-4-methoxyphenyl)propionic acid (16). A solution of **14** (1.0 g, 5.1 mmol), benzyl bromide (3.0 mL, 25.2 mmol), and potassium carbonate (2.0 g, 14.5 mmol) in acetone (15 mL) and acetonitrile (15 mL) was refluxed for 25 h. The reaction mixture was concentrated in vacuo. To the residue was added methanol (3 mL), water (30 mL) and potassium hydroxide (4.0 g, 71 mmol). The mixture was heated at 70 °C for 10 h. After cooling to room temperature, the reaction mixture was washed with chloroform (30 mL×3) and concentrated hydrochloric acid (HCl) was added dropwise until the pH of the solution was 2.0. The mixture was extracted with chloroform (40 mL×3) and the organic layer was washed with water (20 mL×2) and brine (30 mL), dried (anhyd Na_2SO_4), and evaporated in vacuo. The residue was triturated with hexanes several times and 16 (1.02 g) was obtained in 70% yield as a beige solid; mp 115–116 °C; ^{1}H NMR δ 2.62 (2H, t, J=7.0), 2.88 (2H, t, J=7.0), 3.88 (3H, s), 5.15 (2H, s), 6.76–6.87 (3H, m), 7.31–7.49 (5H, m); ^{13}C NMR δ 30.2, 35.8, 56.2, 71.2, 112.1, 114.7, 120.9, 127.5, 127.9, 128.6, 132.8, 137.3, 148.2, 148.4, 178.8; HRMS calcd for $C_{17}H_{18}O_4$: 286.1205; found: 286.1194.

7-Benzyloxy-6-methoxy-2-tetralone (21). To a refluxing solution of rhodium (II) acetate dimer (6 mg) in dichloromethane (15 mL) was added dropwise a solution of diazoketone (**19**) (400 mg, 1.3 mmol) in dichloromethane (10 mL). The reaction mixture was refluxed for 1 h and was then concentrated in vacuo. The residue obtained was chromatographed over 75 g of silica gel and a 1:3 mixture of ethyl acetate/hexanes to give **21** (183 mg) in 50% yield; ^{1}H NMR δ 2.56 (2H, t, J=7.0), 3.01 (2H, t, J=7.0), 3.47 (2H, s), 3.91 (3H, s), 5.14 (2H, s), 6.67 (1H, s), 6.79 (1H, s), 7.35–7.44 (5H, m); ^{13}C NMR δ 28.3, 38.6, 44.3, 56.3, 71.4, 111.8, 114.3, 125.2, 127.4, 128.0, 128.7, 129.3, 137.2, 147.2, 148.6, 210.9.

3-(4-Benzyloxy-3-methoxyphenyl)-1-diazo-butan-2-one (19). To a solution of trimethylsilyldiazomethane (2.0 M in hexanes, 1.5 mL), in THF and acetonitrile (1:1, 10 mL) was added dropwise a solution of **17** (400 mg, 1.3 mmol) in THF and acetonitrile (1:1, 10 mL) at 0 °C. The reaction mixture was stirred for 4 h and evaporated in vacuo. The crude diazoketone was used for the next step without further purification and identification.

3-(4-Benzyloxy-3-methoxyphenyl)propionic acid chloride (17). A solution of **15** (500 mg, 1.75 mmol) in dichloromethane (30 mL) was treated with oxalyl chloride (2.5 mL of 2.0 M solution in dichloromethane, 5.0 mmol) and the resulted mixture was stirred vigorously at room temperature for 20 h. The solution turned to dark yellow-green. The reaction mixture was then concentrated in vacuo and the crude acid chloride was used for the next step without further purification; ^{1}H NMR δ 2.97 (2H, t, J=7.9), 3.20 (2H, t, J=7.9), 3.91 (3H, s), 5.15 (2H, s), 6.65–6.86 (5H, m), 7.35–7.49 (3H, m).

6-Benzyloxy-7-methoxy-2-tetralone (22). To a refluxing solution of rhodium (II) acetate dimer (5 mg) in dichloromethane (15 mL) was added dropwise to a solution of diazoketone (**20**) (400 mg, 1.3 mmol) in dichloromethane (10 mL). The reaction mixture was refluxed for 1 h and was then concentrated in vacuo. The residue obtained was chromatographed over 75 g of silica gel and a 1:3 mixture of ethyl acetate/hexanes to give **21** (202 mg) in 55% yield; ^{1}H NMR δ 2.54 (2H, t, J=7.0), 2.96 (2H, t, J=7.0), 3.53 (2H, s), 3.89 (3H, s), 5.17 (2H, s), 6.67 (1H, s), 6.79 (1H, s), 7.36–7.51 (5H, m); ^{13}C NMR δ 28.1, 38.7, 44.4, 56.3, 71.4, 112.1, 114.1,

125.9, 127.4, 128.0, 128.6, 128.7, 137.2, 147.0, 148.8, 210.9; HRMS calcd for $C_{18}H_{18}O_3$: 282.1256; found: 282.1263.

3-(3-Benzyloxy-4-methoxyphenyl)-1-diazo-butan-2-one (20). To a solution of trimethylsilyldiazomethane (2.0 M in hexanes, 1.5 mL), in THF and acetonitrile (1:1, 10 mL) was added dropwise a solution of **18** (400 mg, 1.3 mmol) in THF and acetonitrile (1:1, 10 mL) at 0 °C. The reaction mixture was stirred for 4 h and evaporated in vacuo. The crude diazoketone was used for the next step without further purification and characterization.

3-(4-Benzyloxy-3-methoxyphenyl)propionic acid chloride (18). A solution of **16** (400 mg, 1.40 mmol) in dichloromethane (30 mL) was treated with oxalyl chloride (1.4 mL of 2.0 M solution in dichloromethane, 2.8 mmol) and the resulting mixture was stirred vigorously at room temperature for 20 h. The reaction mixture was then concentrated in vacuo and the crude acid chloride was used for the next step without further purification; ^{1}H NMR δ 2.92 (2H, t, $J = 7.0$), 3.14 (2H, t, $J = 7.0$), 3.89 (3H, s), 5.16 (2H, s), 6.74–6.88 (3H, m), 7.32-7.49 (5H, m).

Topoisomerase-mediated DNA cleavage assays

Human topoisomerase I was expressed in *Escherichia coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously.[35] DNA topoisomerase II was purified as reported previously.[36] Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described.[37] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[38] The cleavage assays were performed as previously reported.[39] The drug and the DNA in presence of topoisomerase I was incubated for 30 min at 37 °C. After development of the gels, typically 24-h exposure was used to obtain autoradiograms outlining the extent of DNA fragmentation. Topoisomerase I-mediated DNA cleavage values are reported as REC, Relative Effective Concentration, that is, concentrations relative to topotecan for TOP1 and VM-26 for TOP2, whose value is arbitrarily assumed as 1.0, that are able to produce the same cleavage on the plasmid DNA in the presence of either TOP1 or TOP2. Relative potency was based upon the amount of drug needed to induce approximately 10% DNA fragmentation.

Inhibition of cell growth: MTT-microtiter plate tetrazolinium cytotoxicity assay (RPMI8402, CPT-K5, U937, U/CR Cells)

The cytotoxicity was determined by using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[40–42] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Anchi Cancer Research Institute, Nagoya, Japan). cytotoxicity assays were performed using 96-well microtiter plates and 2000 cells/well, in 200 μL of growth medium. 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 for 3–4 days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in six replicate wells.

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