Bioorganic & Medicinal Chemistry 16 (2008) 8598–8606

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

11-Substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine derivatives as novel topoisomerase I-targeting agents

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ARTICLE INFO

Article history:
Received 26 March 2008
Revised 31 July 2008
Accepted 4 August 2008
Available online 7 August 2008

Keywords:
Cytotoxicity
Antitumor agents
Topoisomerase I
Benzo[i]phenanthridines
Multidrug resistance
Photocyclization

ABSTRACT

Several 11-substituted benzo[*i*]phenanthridine derivatives were synthesized, and their TOP1-targeting activity and cytotoxicity were assessed. Comparative data indicate that TOP1-targeting was often the primary molecular target associated with their cytotoxicity. Several 11-aminoalkyl derivatives, 11-aminocarboxy derivatives as well as the 11-[(2-dimethylamino)ethyl]carboxamide of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine were synthesized and did exhibit considerable cytotoxicity with IC_{50} values ranging from 20 to 120 nM in the human lymphoblast tumor cell line RPMI8402.

1. Introduction

Topoisomerases are ubiquitous enzymes that participate in processes such as DNA replication, repair, transcription, and recombination as well as chromosome condensation and segregation.[1,2] Topoisomerase I (TOP1) is the target of several antitumor agents based upon their ability to stabilize the enzyme–DNA cleavage complex, which results in DNA damage and ultimately cell death.[3,4] Camptothecin (CPT) was the first compound identified as a TOP1-targeting agent.[5] Two clinical TOP1-targeting agents, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®), have since been developed. The improved water-solubility of topotecan and irinotecan relative to CPT was critical to their development into the clinic. These agents possess the same ring structure of camptothecin, which includes a δ-lactone. This lactone moiety is susceptible to hydrolysis, and the resulting carboxylic acid has a high affinity for human serum albumin.[6–8] In addition, it is known that both of these clinical agents are susceptible to transporter-mediated cellular efflux, which can limit intracellular accumulation and has been associated with multidrug resistance. Specifically overexpression of MDR1 (P-glycoprotein) and breast cancer resistance protein (BCRP) has been associated with resistance to these camptothecins.[9–15] In view of these observations, non-camptothecin TOP1-targeting agents have been investigated for their potential to overcome these obstacles, which could limit the effective concentration of drug within certain tumor types.

Several 5-(2-aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones have been identified as exceptionally active TOP1-targeting agents with potent antitumor activity.[16,17] One of the more extensively studied of these non-camptothecin TOP1-targeting agents is 5*H*-8,9-dimethoxy-5-(2-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*][1,6]-naphthyridin-6-one (ARC-111), **1** (Fig. 1).[18,19] The 11-aza analog of ARC-111, 11-[2-(dimethylamino)ethyl]-2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquinolin[4,3-c]cinnolin-12-one (ARC-31), **2** (Fig. 1) and related compounds have also been identified as potent TOP1-targeting agents.[16,20] Analogs within both of these series of compounds have proved to be active as antitumor agents in vivo when administered by gavage or parenterally to tumor-bearing mice. The presence of *N*-alkyl substituents within the bay region tends to distort both of these ring systems from planarity. It was considered advantageous for the purpose of comparison to examine whether 11-aminoalkyl substituted benzo[*i*]phenanthridines would possess similar potency as TOP1-targeting agents and comparable cytotoxicity to structurally similar 5-(2-aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones. Specifically targeted for synthesis and biological evaluation were a series of 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]

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doi:10.1016/j.bmc.2008.08.018

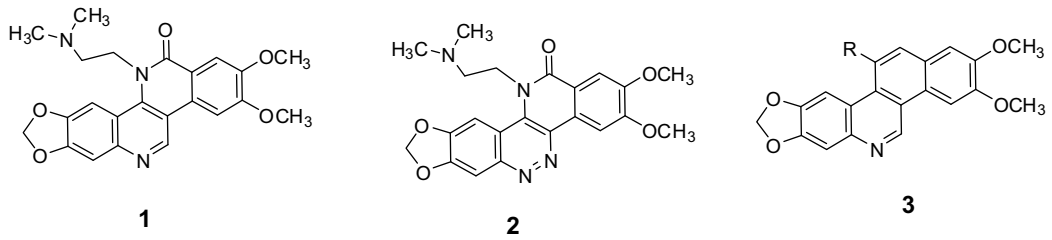


Figure 1. Structure of ARC-111 (**1**), ARC-31 (**2**), and 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridines (**3**).

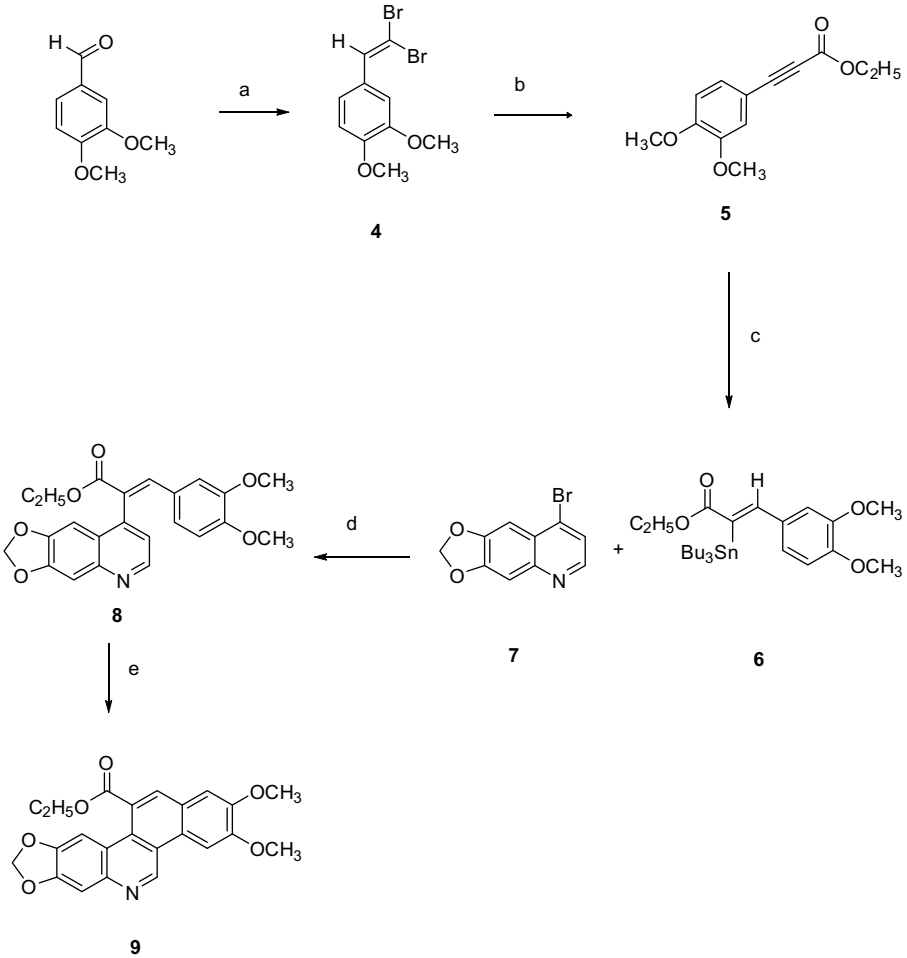
phenanthridines, **3**. In this study, we evaluated several 11-alkylamino and 11-aminocarboxy derivatives together with various 11-substituted benzo[*i*]phenanthridine intermediates that were utilized in their synthesis.

2. Results and discussion

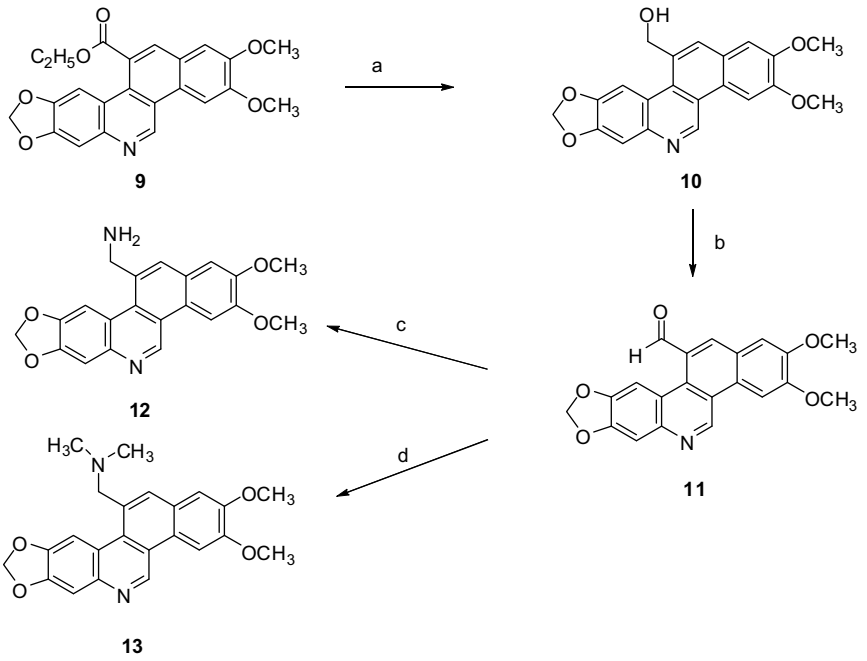
2.1. Chemistry

The key intermediate used for the preparation of 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridines was the 11-ethoxycarbonyl derivative, **9**. The methodology employed for the synthesis of this intermediate is outlined in Scheme 1. Ethyl 3-(3,4-dimethoxyphenyl)propynoate **5** was synthesized from 3,4-dimethoxybenzaldehyde via the dibromo olefin **4** using Corey's procedure.[21,22] Hydrostannation of **5** was accomplished by Bu_3SnH in the presence of AIBN to give (*Z*)-vinylstannane **6** stereoselectively.[22] The Stille cross-coupling reaction of the resulting vinylstannane **6** with bromoquinoline **7**[23] proceeded smoothly to afford compound **8** in good yield.[24] Photocyclization of **8** in the presence of a catalytic amount of iodine resulted in the formation of **9** in 35% yield.[25]

The preparation of 11-aminomethyl- and 11-*N*,*N*-dimethylaminomethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine, **12** and **13** was accomplished as outlined in Scheme 2. Reduction of the ethyl ester **9** to its hydroxymethyl derivative **10** was accomplished using lithium aluminum hydride in THF. Oxidation of the hydroxymethyl group with MnO_2 resulted in the



Scheme 1. Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, 55%; (b) *n*-BuLi, $ClCOOC_2H_5$, THF, 96%; (c) Bu_3SnH, AIBN, benzene, 66%; (d) $PdCl_2(PPh_3)_2$, DMF, 57%; (e) *hv*, I_2 (cat.), benzene, 35%.



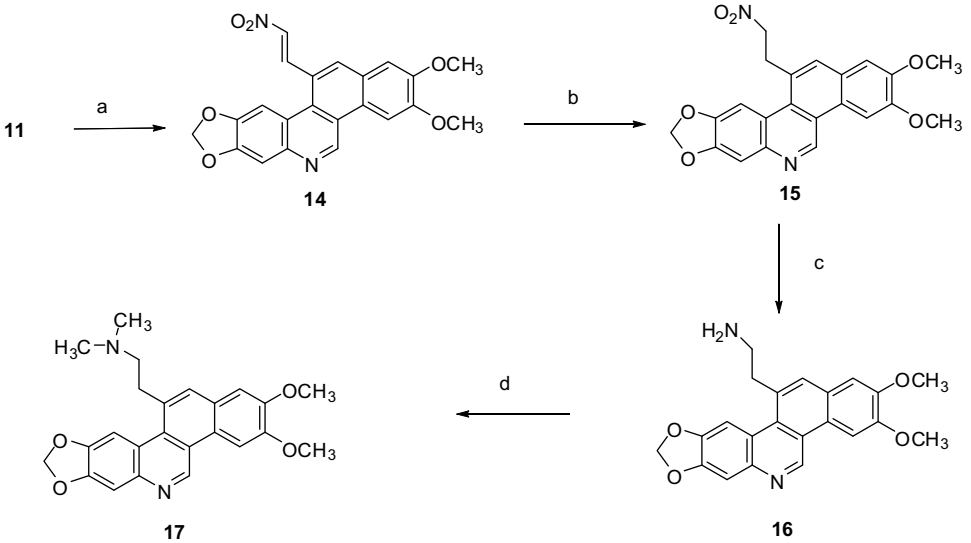
Scheme 2. Reagents and conditions: (a) LAH, THF, 57%; (b) MnO_2, DMF, 62%; (c) ammonium acetate, $NaBH_3CN$, MeOH, 47%; (d) dimethylamine, $NaBH_3CN$, MeOH, 55%.

formation of the 11-formyl derivative **11**. Reductive amination using either ammonium acetate or dimethylamine followed by treatment with sodium triacetoxyborohydride gave **12** and **13**, respectively.

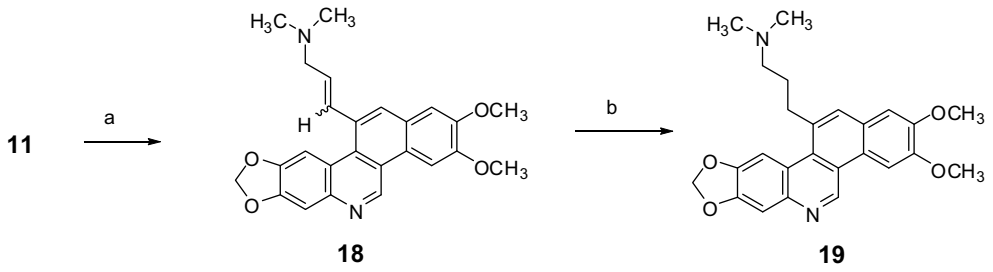
The preparation of the 11-(2-aminoethyl)- and 11-[(2-*N*,*N*-dimethylamino)ethyl]-2,3-dimethoxy-8,9-methylenedioxybenzo-[*i*]phenanthridine **16** and **17** is outlined in Scheme 3. Condensation of the 11-formyl derivative **11** with nitromethane provided the 2-nitroethylene derivative **14**, which could be reduced to the 11-(2-nitroethyl)-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine **15**. In the presence of zinc in acetic acid, the nitro group was reduced to give **16**. Reductive methylation using formalin in the presence of sodium cyanoborohydride provided the *N*,*N*-dimethyl derivative, **17**.

The synthetic approach used in the preparation of 11-[3-(*N*,*N*-dimethylamino)propyl]-2,3-dimethoxy-8,9-methylenedioxybenzo-[*i*]phenanthridine **19** is outlined in Scheme 4. A solution of the formyl intermediate **11** in THF was added to a mixture of 2-(dimethylamino)ethyltriphenylphosphonium bromide and LiHMDS in THF. The resulting product **18** was reduced to the propyl derivative **19** using hydrogen and 10% Pd–C.

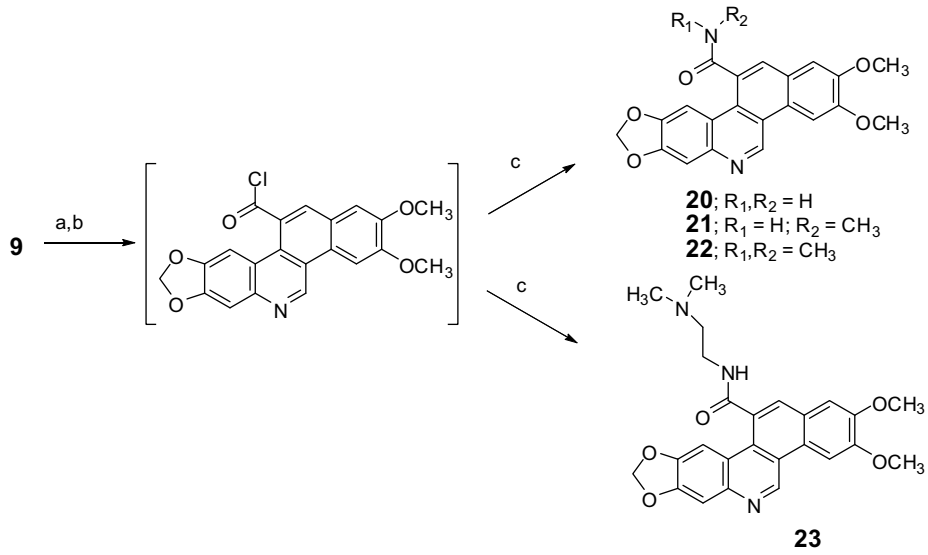
The 11-ethoxycarbonyl derivative **9** was subjected to base hydrolysis in aqueous ethanol, and the carboxylic acid was converted to its acid chloride using thionyl chloride (Scheme 5). This acid chloride was used without further purification for the formation of a series of 11-carboxamides. These included the primary carboxamide, the *N*-methylcarboxamide, and the *N*,*N*-dimethylcarboxamide, **20–22** as illustrated in Scheme 5. Treatment of the



Scheme 3. Reagents and conditions: (a) ammonium acetate, nitromethane, 89%; (b) $NaBH_4$, 1,4-dioxane/EtOH, 69%; (c) Zn, AcOH, 41%; (d) formalin, $NaBH_3CN$, MeOH, 53%.



Scheme 4. Reagents and conditions: (a) 2-(dimethylamino)ethyltriphenylphosphonium bromide, LiHMDS, THF, 47%; (b) Pd/C, H_2, 44%.



Scheme 5. Reagents and conditions: (a) 10% NaOH, EtOH; (b) $SOCl_2$, CH_2Cl_2; (c) RNH_2, Et_3N, CH_2Cl_2, 63–75%.

acid chloride with *N*,*N*-dimethylaminoethylenediamine resulted in the formation of *N*-[(2-*N*,*N*-dimethylamino)ethyl]carboxamide **23**.

2.2. Biological activity

The intrinsic TOP1-targeting activities of the various 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridines that were synthesized are summarized in Table 1. Several compounds had comparable TOP1-targeting activity to CPT or ARC-111. The hydroxymethyl derivative **10** and both the aminomethyl and *N*,*N*-dimethylaminomethyl derivatives **12** and **13** had potent TOP1-targeting activity. While the formyl derivative **11** was less potent, it also had significant TOP1-targeting activity. As had been previously observed with 12-carboxyester derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridines,[26,27] **9** did not exhibit significant TOP1-targeting activity. The nitro precursor compounds **14** and **15** proved to be much less active as TOP1-targeting agents than either the primary or tertiary amino derivatives, **16** and **17**, respectively. The 11-[3-(*N*,*N*-dimethylaminopropyl)] derivative **19** was also much more potent as a TOP1-targeting agent than its 1-propenyl precursor, **18**.

While the primary and secondary carboxamide derivatives **20** and **21** had comparable TOP1-targeting activity, a precipitous drop in activity was observed for the tertiary carboxamide derivative **22**. A significant increase in TOP1-targeting activity was observed among these carboxamides in the case of the 11-[(2-*N*,*N*-dimethylamino)ethylamino]carboxy derivative **23**.

The relative cytotoxic activities of these 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridines in RPMI8402 and P388 cells, as well as their camptothecin-resistant variants, CPT-K5 and P388/CPT-45[28,29] are provided in Table 1.

Table 1
TOP1-targeting activity and cytotoxicity in RPMI8402 and P388 cells and their camptothecin-resistant variants

Compound	TOP1-mediated cleavage[a]	Cytotoxicity IC_{50} (μM)			
		RPMI8402	CPT-K5	P388	P388/CPT45
1	0.3	0.002	0.90	0.001	0.23
9	>10	10	>10	4.0	>10
10	0.2	0.12	>10	0.06	>10
11	0.8	0.1	>10	0.12	10
12	0.2	0.12	2.1	0.026	0.3
13	0.2	0.1	2.7	0.10	2.0
14	>10	0.55	>10	0.18	10
15	>10	0.06	>10	0.07	>10
16	1.0	0.032	0.2	0.014	0.18
17	0.4	0.02	0.33	0.015	0.18
18	>10	0.035	0.35	0.04	0.35
19	0.8	0.06	0.29	0.04	0.2
20	1.1	0.14	>10	0.035	>10
21	0.8	0.28	>10	0.025	>10
22	>10	1.6	>10	0.98	>10
23	0.2	0.035	0.63	0.015	0.26
CPT	0.2	0.004	>10	0.004	>10
Topotecan	1.0	0.21	>10	0.045	>10

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, these are concentrations relative to topotecan, whose value is arbitrarily assumed as 1, that are able to produce 10% cleavage of the plasmid DNA in the presence of human topoisomerase I.[17]

The very weak cytotoxic activity observed with **9** is likely associated with its limited aqueous solubility and its potential for hydrolysis to the carboxylic acid. The cytotoxic activities of **10–13** in RPMI8402 and P388 were lower than anticipated on the basis of their TOP1-targeting activity. The cross-resistance observed in both CPT-K5 and P388/CPT-45 for these compounds clearly indicates that TOP1-targeting is the major mechanism associated with their cytotoxic activity.

The unsaturated nitro derivative **14** was less cytotoxic than the saturated analog **15**. It is possible that the more rigid conformation associated with the *cis*- and *trans*-isomers of **14** may be less favorable. Both **14** and **15** did exhibit significant cross-resistance in the CPT-resistant cell lines despite comparatively weak intrinsic TOP1-targeting activity. The primary and tertiary 11-[2-aminoethyl] derivatives **16** and **17** were among the more cytotoxic 11-substituted benzo[*i*]phenanthridine derivatives that were evaluated. While the 11-[3-dimethylaminoprop-1-enyl] derivative **18** was much less potent than the 11-[3-(dimethylamino)propyl] derivative **19** as a TOP1-targeting agent, these compounds did exhibit similar cytotoxic activity with IC_{50} values ranging from 35 to 60 nM with RPMI8402 and P388 cells. Significant cross-resistance in the CPT-resistant cells CPT-K5 and P388/CPT45 was observed for **16–20**.

The conversion of the primary carboxamide **20** to its secondary carboxamide **21** by the addition of a *N*-methyl substituent did not adversely affect cytotoxic activity. Consistent with its lower TOP1-targeting activity, the tertiary carboxamide derivative **22** was significantly less cytotoxic than either **20** or **21**. A significant increase in activity, however, was observed with the 11-[(2-dimethylamino)ethylamino]carboxy derivative **23**, which is consistent with it being among the more potent TOP1-targeting compounds in this study.

The cytotoxic activities of these 11-substituted benzo[*i*]phenanthridines in KB3-1 cells and its variant cell lines, KBV-1 and KBH5.0, are listed in Table 2. KBV-1 cells overexpress the efflux transporter MDR1,[30] and KBH5.0 cells overexpress the efflux transporter BCRP.[18] CPT-11 and topotecan are substrates for the efflux transporters MDR1 and BCRP. Decreased cytotoxicity against KBV-1 cells relative to the parent cell line KB3-1 is indicative of substances that are substrates for the efflux transporter MDR1. Similarly, resistance to the cytotoxic effects of a substance observed in KBH5.0 cells relative to its parent cell line, KB3-1, is indicative of a compound being a substrate for the BCRP efflux transporter. The data provided in Table 2 clearly indicate that **16**, **18**, and **19** are substrates for the MDR1 efflux transporter with greater than an order of magnitude difference in cytotoxicity observed in KB3-1 cells relative to KBV-1 cells. The primary carboxamide **20** is the only compound that proved to be a very good substrate for the BCRP efflux transporter with almost a 50-fold differential in cytotoxicity observed for KB3-1 and KBH5.0 cells. In addition, this compound also appears to be a weak substrate for the MDR1 transporter. The aminomethyl derivatives **12** and **13** as well as the 11-[(2-dimethylamino)ethylamino]carboxy derivative **23** were weak or marginal substrates for both the MDR1 and BCRP efflux transporters. These data suggest in the case of 11-aminoalkyl benzo[*i*]phenanthridine derivatives that the length of the alkyl linker as well as the degree to which the amino group is substituted can influence their potential to serve as substrates for the MDR1 efflux transporter. In addition, changes associated with carboxamide moiety of 11-aminocarboxy benzo[*i*]phenanthridine derivatives did influence their potential to serve as substrates for the BCRP efflux transporter.

Several of the 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine derivatives evaluated in this study did exhibit potent TOP1-targeting activity, which was comparable to ARC-111 and CPT. These benzo[*i*]phenanthridine derivatives, including those having the more potent TOP1-targeting activity, were generally less cytotoxic than ARC-111, with IC_{50} values being more than one order of magnitude greater. Nonetheless, several of these 11-substituted benzo[*i*]phenanthridines did have cytotoxic activity comparable to topotecan in these various tumor cell lines. The absence of a δ-lactone in their structure and an ability to overcome MDR1- or BCRP-associated multidrug resistance could prove advantageous. Evaluation of their relative efficacy in athymic nude mice with human tumor xenografts will provide for a more complete assessment of their potential clinical utility.

Table 2
Cytotoxicity of 11-substituted 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridines in KB3-1 cells and its variant cell lines, KBV-1 and KBH5.0

Compound	Cytotoxicity IC_{50} (μM)		
	KB3-1	KBV-1	KBH5.0
1	0.005	0.005	0.006
9	>10	>10	>10
10	0.15	0.4	0.4
11	0.13	0.3	0.29
12	0.03	0.16	0.21
13	0.06	0.25	0.29
14	0.18	0.45	0.42
15	0.15	0.36	0.32
16	0.02	0.5	0.11
17	0.021	0.06	0.04
18	0.03	0.33	0.07
19	0.035	0.4	0.13
20	0.055	0.4	2.7
21	0.23	0.42	0.48
22	1.2	4.0	3.6
23	0.027	1.4	0.2
CPT	0.015	0.025	0.026
Topotecan	0.04	0.44	0.44
Irinotecan	0.68	40.0	7.4

3. Experimental

Melting points were determined with a Meltemp capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm, (ICN Biomedicals, Eschwege, Germany) using the solvent systems indicated. Infrared spectral data were obtained using a Thermo-Nicolet Avatar 360 Fourier transform spectrometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in Hertz (Hz). Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO. All starting materials and reagents were purchased from Aldrich. Solvents were purchased from Fisher Scientific, and were ACS grade or HPLC grade. Methylene chloride was freshly distilled from calcium hydride. All other solvents were used as provided without further purification. Compound **7** was prepared as previously described.[23]

3.1. 1,1-Dibromo-2-(3,4-dimethoxyphenyl)ethylene (4)

A mixture of CBr_4 (29.8 g, 0.09 mol) and PPh_3 (47.2 g, 0.18 mol) in dichloromethane (200 mL) was stirred under nitrogen at room temperature for 1 h. To this mixture was added 3,4-dimethoxybenzaldehyde (10.0 g, 0.06 mol) at 0 °C and the mixture was stirred for 16 h at room temperature. The resulting precipitate was filtered

off, and the filtrate was concentrated under reduced pressure. The residue was chromatographed with 10:1 hexanes/EtOAc to provide a colorless oil in 55% yield; IR (KBr) 1600, 2834; ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 3.90 (s, 3H), 6.85 (d, 1H, J = 8.0), 7.10 (dd, 1H, J = 8.0, 2.2), 7.18 (d, 1H, J = 2.2), 7.41 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 55.0, 55.1, 86.5, 110.0, 110.4, 121.1, 127.1, 135.6, 147.8, 148.5.

3.2. Ethyl 3-(3,4-dimethoxyphenyl)propynoate (5)

To a solution of **4** (4.0 g, 12.5 mmol) in dry THF was added BuLi (1.6 M in hexane) (17.2 mL, 27.5 mmol) at −78 °C under nitrogen, and the mixture was warmed up to room temperature with stirring for 1 h. The reaction mixture was then cooled down to −78 °C and ethyl chloroformate (1.62 g, 15.0 mmol) was added. The resulting reaction mixture was stirred at room temperature for 30 min. The reaction was quenched by addition of satd NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed with 20:1 hexanes/EtOAc to provide a colorless oil in 96% yield; IR (KBr) 1704, 2211; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.2), 3.88 (s, 3H), 3.91 (s, 3H), 4.29 (q, 2H, J = 7.2), 6.84 (d, 1H, J = 8.4), 7.06 (d, 1H, J = 2.0), 7.23 (dd, 1H, J = 8.4, 2.0); ^{13}C NMR ($CDCl_3$) δ 13.2, 55.2, 55.1, 61.0, 79.1, 86.1, 110.3, 110.6, 114.5, 126.3, 148.0, 150.7, 153.3.

3.3. Ethyl (*Z*)-3-(3,4-dimethoxyphenyl)-2-(tributylstannyl)propenoate (6)

To a solution of **5** (510 mg, 2.18 mmol) and Bu_3SnH (666 mg, 2.29 mmol) in benzene was added AIBN (9 mg, 0.055 mmol) under nitrogen, and the mixture was stirred at room temperature for 19 h. Solvent was removed and the residue was chromatographed with 5:1 hexanes/EtOAc to provide a colorless oil in 66% yield; ^{1}H NMR ($CDCl_3$) δ 0.70–1.00 (m, 15H), 1.00–1.40 (m, 15H), 3.75 (s, 6H), 4.11 (q, 2H, J = 7.0), 6.69–6.75 (m, 3H), 8.21 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.9, 12.6, 13.4, 26.2, 28.0, 54.9, 54.9, 59.6, 110.0, 110.6, 119.9, 130.7, 136.5, 147.8, 148.8, 152.7, 170.8.

3.4. 2-(6,7-Methylenedioxyquinolin-4-yl)-3-(4,5-dimethoxyphenyl)acrylic acid ethyl ester (8)

A mixture of **6** (500 mg, 0.95 mmol), 4-bromo-6,7-methylenedioxyquinoline, **7** (240 mg, 0.95 mmol), and $PdCl_2(PPh_3)_2$ (33.4 mg, 0.048 mmol) in DMF (15 mL) was stirred under nitrogen at 60 °C for 5 h. A solution of aq KF (276.1 mg, 4.76 mmol) was added, and the reaction mixture was stirred for 1 h. The insoluble material was filtered off and the filtrate was extracted with dichloromethane. The organic layer was washed with brine, dried, and concentrated under reduced pressure. The residue was chromatographed with 1:1 hexanes/EtOAc to provide a light yellow sticky gum in 57% yield; IR (KBr) 1705; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.0), 3.18 (s, 3H), 3.80 (s, 3H), 4.26 (q, 2H, J = 7.0), 6.06 (m, 2H), 6.22 (d, 1H, J = 2.2), 6.68 (d, 1H, J = 8.4), 6.82 (dd, 1H, J = 8.4, 2.2), 7.06 (s, 1H), 7.19 (d, 1H, J = 4.8), 7.44 (s, 1H), 8.05 (s, 1H), 8.76 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 13.3, 54.0, 54.9, 60.4, 99.4, 100.9, 105.3, 109.8, 111.1, 119.7, 123.3, 124.86 125.1, 125.6, 141.2, 141.8, 146.2, 147.3, 147.5, 147.6, 149.7, 150.0, 166.0; HRMS calcd for $C_{23}H_{21}NO_6H$: 408.1447; found: 408.1437.

3.5. 2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-11-carboxylic acid ethyl ester (9)

A solution of **8** (100 mg, 0.245 mmol) and iodine (2 mg) in benzene (250 mL) was irradiated by a Hanovia 450 W medium-pressure lamp through a Pyrex filter for 6 h, with air bubbling through the reaction mixture. The solvent was removed under reduced pressure and the residue was chromatographed with 10:1 $CHCl_3$/MeOH to provide a light yellow powder in 35% yield; mp 240–243 °C; IR (KBr) 1712; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H, J = 7.0), 4.06 (s, 3H), 4.16 (s, 3H), 4.54 (q, 2H, J = 7.0), 6.15 (s, 2H), 7.29 (s, 1H), 7.47 (s, 1H), 7.55 (s, 1H), 8.09 (s, 1H), 8.18 (s, 1H), 9.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.2, 55.2, 55.3, 61.1, 101.0, 101.2, 101.8, 106.3, 107.6, 118.6, 120.6, 124.9, 125.1, 125.5, 127.0, 131.6, 142.7, 144.3, 146.7, 148.3, 149.4, 151.0, 170.0; HRMS calcd for $C_{23}H_{19}NO_6H$: 406.1273; found: 406.1273.

3.6. 11-Hydroxymethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (10)

To a stirred solution of LAH (41.8 mg, 1.1 mmol) in 20 mL THF was added a solution of **9** in THF (223.3 mg, 0.55 mmol) dropwise at 0 °C. The resulting reaction suspension was stirred for 2 h at 0 °C, and then carefully quenched by sequential addition of 0.05 mL water, 0.05 mL of 15% NaOH, and 0.15 mL water. The resulting reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was chromatographed with 10:1 $CHCl_3$/MeOH to provide a yellow powder in 57% yield; mp 270–272 °C; IR (KBr) 3448; ^{1}H NMR (DMSO-d_6) δ 3.95 (s, 3H), 4.07 (s, 3H), 5.08 (s, 2H), 5.88 (s, 1H), 6.25 (s, 2H), 7.54 (s, 1H), 7.56 (s, 1H), 8.19 (s, 1H), 8.37 (s, 1H), 8.57 (s, 1H), 10.13 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 55.5, 55.8, 64.2, 101.7, 102.7, 104.2, 106.3, 107.7, 119.7, 121.5, 124.4, 126.3, 130.3, 132.4, 133.1, 143.1, 146.3, 147.4, 148.1, 149.6, 150.4; HRMS calcd for $C_{21}H_{17}NO_5H$: 364.1185; found: 364.1196.

3.7. 11-Formyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (11)

A solution of **10** (363 mg, 1 mmol), MnO_2 (870 mg, 10 mmol) in 30 mL DMF was stirred for 2 h at room temperature. The resulting reaction mixture was filtered through a Celite bed and the filtrate was concentrated under reduced pressure. The residue was chromatographed with 20:1 CH_2Cl_2/MeOH to provide a brown powder in 62% yield; mp 250–255 °C; IR (KBr) 1685; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 3.99 (s, 3H), 4.11 (s, 3H), 6.11 (s, 1H), 7.36 (s, 1H), 7.39 (s, 1H), 7.49 (s, 1H), 8.05 (s, 1H), 8.43 (s, 1H), 9.79 (s, 1H), 10.51 (s, 1H); HRMS calcd for $C_{21}H_{15}NO_5H$: 362.1028; found: 362.1039.

3.8. 11-Aminomethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (12)

To a solution of **11** (7.2 mg, 0.02 mmol) and ammonium acetate (15.4 mg, 0.2 mmol) in 3 mL MeOH was added a solution of $NaBH_3CN$ (9.4 mg, 0.15 mmol in 0.1 mL MeOH) at 0 °C. The resulting reaction solution was stirred for an additional 30 min at 0 °C, and then two drops of AcOH were added. The reaction mixture was warmed up to 40 °C with stirring for another 2 h. The resulting mixture was quenched by 0.1 mL of 1 N NaOH solution, concentrated, and extracted using $CHCl_3$ (2× 15 mL). The organic layer was concentrated and the residue was chromatographed with 15:1 CH_2Cl_2/MeOH to provide a yellow powder in 47% yield; mp 189–194 °C; ^{1}H NMR ($CDCl_3$) δ 4.07 (s, 3H), 4.15 (s, 3H), 4.61 (s, 2H), 6.17 (s, 2H), 7.26 (s, 1H), 7.60 (s, 1H), 8.05 (s, 1H), 8.12 (s, 1H), 8.56 (s, 1H), 9.94 (s, 1H); HRMS calcd for $C_{21}H_{18}N_2O_4H$: 363.1339; found: 363.1342.

3.9. 11-Dimethylaminomethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (13)

To a solution of **11** (7.2 mg, 0.02 mmol) and dimethylamine (0.2 mmol, 2 M in THF) in 3 mL MeOH was added a solution of

$NaBH_3CN$ (9.4 mg, 0.15 mmol in 0.1 mL MeOH) at 0 °C. The resulting reaction solution was stirred for an additional 30 min at 0 °C, and then two drops of AcOH were added. The reaction mixture was warmed up to 40 °C with stirring for another 2 h. The resulting mixture was quenched by 0.1 mL of 1 N NaOH solution, concentrated, and extracted using $CHCl_3$ (2× 15 mL). The organic layer was concentrated and the residue was chromatographed with 15:1 CH_2Cl_2/MeOH to provide a yellow powder in 55% yield; mp 220–222 °C; ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 6H), 3.93 (s, 2H), 4.08 (s, 3H), 4.15 (s, 3H), 6.16 (s, 2H), 7.28 (s, 1H), 7.58 (s, 1H), 7.92 (s, 1H), 8.14 (s, 1H), 8.93 (s, 1H), 9.93 (s, 1H); HRMS calcd for $C_{23}H_{22}N_2O_4H$: 391.1652; found: 391.1654.

3.10. 11-(2-Nitrovinyl)-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (14)

A suspension of **11** (36 mg, 0.1 mmol), ammonium acetate (38.6 mg, 0.5 mmol) in 2 mL nitromethane was stirred overnight at 80 °C. The resulting reaction mixture was triturated in the presence of a small amount of CH_2Cl_2, and the residue was pure enough and used for the next reaction without further purification; yield: 89%; mp 241–245 °C; IR (KBr) 1509; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 4.08 (s, 3H), 4.18 (s, 3H), 6.19 (s, 2H), 7.33 (s, 1H), 7.63 (s, 1H), 7.67 (s, 1H), 7.76 (d, 1H, J = 13.2), 8.06 (s, 1H), 8.12 (s, 1H), 8.80 (d, 1H, J = 13.2), 9.91 (s, 1H); HRMS calcd for $C_{22}H_{16}N_2O_6H$: 405.1087; found: 405.1089.

3.11. 11-(2-Nitroethyl)-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (15)

To a stirred suspension of $NaBH_4$ (38 mg, 1 mmol) in 10 mL 1,4-dioxane/EtOH (2:1) was added a solution of **14** (80.8 mg, 0.2 mmol) in 1,4-dioxane (5 mL) dropwise at 0 °C. After this addition, the reaction mixture was stirred for an additional 30 min. The resulting reaction mixture was diluted with ice-water and quenched with 50% aqueous AcOH. The resulting suspension was concentrated and then partitioned between sat $NaHCO_3$ and CH_2Cl_2. The organic layer was again concentrated and the residue was chromatographed with 10:1 CH_2Cl_2/MeOH to provide a yellow powder in 69% yield; mp 277–280 °C; IR (KBr) 1554; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 3.99 (s, 3H), 4.08 (s, 3H), 4.16 (t, 2H, J = 7.6), 4.79 (t, 2H, J = 7.6), 6.13 (s, 2H), 7.19 (s, 1H), 7.52 (s, 1H), 7.82 (s, 1H), 7.94 (s, 1H), 8.04 (s, 1H), 9.82 (s, 1H); HRMS calcd for $C_{22}H_{18}N_2O_6H$: 407.1243; found: 407.1231.

3.12. 11-(2-Aminoethyl)-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (16)

To a stirred suspension of **15** (50 mg, 0.12 mmol) in 1.0 mL acetic acid was added Zn power (15.7 mg, 0.24 mmol) portionwise at room temperature. The reaction mixture was stirred for an additional 3 h, diluted with saturated sodium bicarbonate, and then filtered. The filtrate was concentrated, and partitioned between satd $NaHCO_3$ and CH_2Cl_2. The organic layer was again concentrated and the residue was chromatographed with 10:1:0.1 CH_2Cl_2/MeOH/TEA to provide a yellow-green powder in 41% yield; mp 192–198 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 3.27 (t, 2H, J = 6.6), 3.62 (t, 2H, J = 6.6), 4.07 (s, 3H), 4.14 (s, 3H), 6.17 (s, 2H), 7.24 (s, 1H), 7.60 (s, 1H), 7.87 (s, 1H), 8.11 (s, 1H), 8.13 (s, 1H), 9.94 (s, 1H); HRMS calcd for $C_{22}H_{20}N_2O_4H$: 377.1501; found: 377.1497.

3.13. 12-(2-Dimethylamino)ethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (17)

To a solution of **16** (8 mg, 0.02 mmol) and formaldehyde (0.1 mL, 37% solution in H_2O) in 3 mL MeOH was added a solution of $NaBH_3CN$ (9.4 mg, 0.15 mmol in 0.1 mL MeOH) at 0 °C. The resulting reaction solution was stirred for an additional 30 min at 0 °C, and then two drops of AcOH were added. The reaction mixture was warmed up to room temperature with stirring for another 2 h. The resulting mixture was quenched by 0.1 mL 1 N NaOH solution, concentrated, and extracted by $CHCl_3$ (2× 15 mL). The organic layer was concentrated and the residue was chromatographed with 15:1 CH_2Cl_2/MeOH to provide a yellow powder in 53% yield; mp 199–201 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 2.46 (s, 6H), 2.85 (t, 2H, J = 7.8), 3.65 (t, 2H, J = 7.8), 4.07 (s, 3H), 4.14 (s, 3H), 6.17 (s, 2H), 7.28 (s, 1H), 7.60 (s, 1H), 7.89 (s, 1H), 8.10 (s, 1H), 8.18 (s, 1H), 9.94 (s, 1H); HRMS calcd for $C_{24}H_{24}N_2O_4H$: 405.1814; found: 405.1809.

3.14. 11-[(3-Dimethylamino)prop-1-enyl]-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (18)

To a suspension of 2-(dimethylamino)ethyltriphenylphosphonium bromide (40 mg, 0.095 mmol) in 5 mL THF was added LiHMDS (1.0 M in THF, 0.1 mmol) dropwise at room temperature. A solution of **11** (0.08 mmol) in THF was added to the reaction mixture dropwise, and the resulting solution was stirred for 1.5 h, quenched by 0.1 mL water, concentrated, and partitioned into CH_2Cl_2/water. The organic layer was again concentrated under reduced pressure and the residue was chromatographed with 20:1 CH_2Cl_2/MeOH to provide a yellow powder in 47% yield; two inseparable isomers were obtained. The major isomer: mp 220–225 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 2.47 (s, 6H), 3.29 (d, 2H, J = 6.6), 4.08 (s, 3H), 4.16 (s, 3H), 6.18 (s, 2H), 6.35 (dt, 1H, J = 15.8, 6.6), 7.27 (s, 1H), 7.29 (d, 1H, J = 15.8), 7.58 (s, 1H), 7.71 (s, 1H), 8.13 (s, 1H), 8.62 (s, 1H), 9.94 (s, 1H); HRMS calcd for $C_{25}H_{24}N_2O_4H$: 417.1814; found: 407.1801.

3.15. 11-[(3-Dimethylamino)propyl]-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (19)

A suspension of **18** (15 mg, 0.04 mmol) and Pd–C (10 mg) in 10 mL ethanol was shaken under hydrogen (40 psi) for 24 h. The mixture was filtered through Celite and concentrated under reduced pressure. The residue was chromatographed with 20:1 CH_2Cl_2/MeOH to provide a yellow powder in 44% yield; mp 204–209 °C; ^{1}H NMR ($CDCl_3$) δ 2.10 (m, 2H), 2.34 (s, 6H), 2.54 (t, 2H, J = 7.8), 3.49 (t, 2H, J = 7.8), 4.07 (s, 3H), 4.14 (s, 3H), 6.17 (s, 2H), 7.26 (s, 1H), 7.60 (s, 1H), 7.89 (s, 1H), 8.12 (s, 1H), 8.14 (s, 1H), 9.95 (s, 1H); HRMS calcd for $C_{25}H_{26}N_2O_4H$: 419.1971; found: 419.1978.

3.16. 11-Aminocarbonylmethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (20)

A mixture of **9** (41 mg, 0.1 mmol) in 10% NaOH (5 mL) and ethanol (10 mL) was heated to reflux with stirring for 1 h. The reaction mixture was acidified with 2 N HCl to pH 4, and then evaporated to dryness. The residue was suspended in 10 mL dichloromethane and 0.5 mL thionyl chloride was added. The resulting reaction mixture was refluxed for 2 h and then concentrated. The reaction residue was again suspended in dichloromethane and 0.5 mL triethylamine was added. After 15 min, 0.5 mL ammonia solution (2.0 M in THF) was added, and the resulting reaction mixture was refluxed for 1 h. The organic solvent and excess amine were removed under reduced pressure, and the residue was chromatographed with 20:1 CH_2Cl_2/MeOH to provide an off-white powder in 65% yield; mp 265–269 °C; IR (KBr) 1647; ^{1}H NMR (CD_3COOD) δ 3.95 (s, 3H), 4.09 (s, 3H), 6.25 (s, 2H), 7.56 (s, 1H), 7.64 (s, 1H), 7.94 (br, 1H), 8.08 (s, 2H), 8.36 (br, 1H), 8.42 (s, 1H), 10.18 (s, 1H); ^{13}C NMR (CD_3COOD) δ 55.5, 55.9, 101.9, 102.0, 102.7, 106.4,

108.2, 118.8, 120.9, 124.7, 125.6, 130.6, 142.7, 146.1, 147.2, 148.5, 149.8, 150.9, 173.0; HRMS calcd for $C_{21}H_{16}N_2O_5H$: 377.1132; found: 377.1134.

3.17. 11-(*N*-Methylamino)carbonylmethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (21)

A mixture of **9** (41 mg, 0.1 mmol) in 10% NaOH (5 mL) and ethanol (10 mL) was heated to reflux with stirring for 1 h. The reaction mixture was acidified with 2 N HCl to pH 4, and then evaporated to dryness. The residue was suspended in 10 mL dichloromethane and 0.5 mL thionyl chloride was added. The resulting reaction mixture was refluxed for 2 h and then concentrated. The reaction residue was again suspended in dichloromethane and 0.5 mL triethylamine was added. After 15 min, 0.5 mL methylamine (2.0 M in THF) was added and the resulting reaction mixture was refluxed for 1 h. The organic solvent and excess amine were removed under reduced pressure, and the residue was chromatographed with 20:1 CH_2Cl_2/MeOH to provide an off-white powder in 67% yield; mp 271–273 °C; IR (KBr) 1653; ^{1}H NMR (DMSO-d_6) δ 2.91 (d, 3H, J = 4.4), 4.08 (s, 3H), 4.08 (s, 3H), 6.24 (m, 2H), 7.55 (s, 1H), 7.63 (s, 1H), 7.73 (s, 1H), 8.06 (s, 1H), 8.42 (s, 1H), 9.00 (br, 1H), 10.19 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.2, 55.5, 55.9, 101.6, 101.9, 102.7, 106.5, 108.1, 118.7, 120.8, 124.8, 125.6, 130.1, 130.3, 142.8, 146.1, 146.1, 147.2, 148.5, 149.8, 151.0, 171.3; HRMS calcd for $C_{22}H_{18}N_2O_5H$: 391.1288; found: 391.1290.

3.18. 11-(*N*,*N*-Dimethylamino)carbonylmethyl-2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine (22)

A mixture of **9** (41 mg, 0.1 mmol) in 10% NaOH (5 mL) and ethanol (10 mL) was heated to reflux with stirring for 1 h. The reaction mixture was acidified with 2 N HCl to pH 4, and then evaporated to dryness. The residue was suspended in 10 mL dichloromethane and 0.5 mL thionyl chloride was added. The resulting reaction mixture was refluxed for 2 h and then concentrated. The reaction residue was again suspended in dichloromethane and 0.5 mL triethylamine was added. After 15 min, 0.5 mL dimethylamine (2.0 M in tetrahydrofuran) was added, and the resulting reaction mixture was refluxed for 1 h. The organic solvent and the excess amine were removed under reduced pressure, and the residue was chromatographed with 20:1 CH_2Cl_2/MeOH to provide an off-white powder in 75% yield; mp 271–275 °C; IR (KBr) 1621; ^{1}H NMR ($CDCl_3$) δ 2.68 (s, 3H), 3.31 (s, 3H), 4.06 (s, 3H), 4.17 (s, 3H), 6.15 (m, 2H), 7.27 (s, 1H), 7.58 (s, 1H), 7.72 (s, 1H), 7.89 (s, 1H), 8.15 (s, 1H), 9.97 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 34.4, 37.5, 55.2, 55.3, 100.2, 101.0, 101.3, 106.7, 107.1, 118.9, 120.3, 124.7, 125.6, 126.5, 128.5, 128.8, 142.7, 144.8, 147.8, 148.3, 149.5, 150.5, 171.8; HRMS calcd for $C_{23}H_{20}N_2O_5H$: 405.1445; found: 405.1447.

3.19. 2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-11-carboxylic acid 2-(dimethylamino)-ethylamide (23)

A mixture of **9** (41 mg, 0.1 mmol) in 10% NaOH (5 mL) and ethanol (10 mL) was heated to reflux with stirring for 1 h. The reaction mixture was acidified with 2 N HCl to pH 4, and then evaporated to dryness. The residue was suspended in 10 mL dichloromethane and 0.5 mL thionyl chloride was added. The resulting reaction mixture was refluxed for 2 h and then concentrated. The reaction residue was again suspended in dichloromethane and 0.5 mL triethylamine was added. After 15 min, 0.5 mL *N*,*N*-dimethylethylenediamine was added, and the resulting reaction mixture was refluxed for 1 h. The organic solvent and excess amine were removed under reduced pressure, and the residue was chromatographed in 20:1 CH_2Cl_2/MeOH to provide an off-white powder in 63% yield; mp 221–225 °C; IR (KBr) 1635; ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 6H), 2.64 (t, 2H, J = 6.0), 3.71 (m, 2H), 4.03 (s, 3H), 4.11 (s, 3H), 6.09 (s, 2H), 7.15 (br, 1H), 7.18 (s, 1H), 7.38 (s, 1H), 7.66 (s, 1H), 7.85 (s, 1H), 7.94 (s, 1H), 9.61 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 36.9, 44.2, 55.1, 55.2, 56.7, 100.9, 101.1, 101.7, 106.1, 107.1, 118.4, 120.4, 124.8, 125.1, 126.5, 129.3, 129.9, 142.4, 144.3, 146.8, 148.0, 149.3, 150.4, 171.0; HRMS calcd for $C_{25}H_{25}N_3O_5H$: 448.1872; found: 448.1870.

3.20. 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.[31] Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described.[32] 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.[33] The cleavage assays were performed as previously reported.[34,35] The drug and the DNA in the presence of topoisomerase I was incubated for 30 min at room temperature. The reactions were terminated by the addition of 5 mL 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. 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 relative effective concentration (REC), that is, concentrations relative to camptothecin, whose value is arbitrarily assumed as 0.2, that are able to produce the same 10% cleavage on the plasmid DNA in the presence of human topoisomerase I.

3.21. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA). 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).[28] The P388 mouse leukemia cell line and its CPT-resistant TOP1-deficient variant P388/CPT45[29] were obtained from Michael R. Mattern and Randal K. Johnson (GlaxoSmithKline, King of Prussia, PA). The KB3-1 cell line and its multidrug-resistant variant KBV-1[30] were obtained from K.V. Chin (The Cancer Institute of New Jersey, New Brunswick, NJ). The KBH5.0 cell line as noted previously[18] was derived from KB3-1 by stepwise selection against Hoechst 33342. 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 for four 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.

Acknowledgments

This study was supported by the National Cancer Institute, Grants CA098127 (E.J.L.) and CA39662 (L.F.L.), and Genzyme Corporation.

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