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Esters and amides of 2,3-dimethoxy-8,9-methylenedioxy-benzo[*i*]phenanthridine-12-carboxylic acid: Potent cytotoxic and topoisomerase I-targeting agents

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Abstract—The exceptional topoisomerase I-targeting activity and antitumor activity of 5-(2-*N*,*N*-dimethylamino)ethyl-8,9-dimethoxy-2,3-methylenedioxy-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111, topovale) prompted studies on similarly substituted benzo[*i*]phenanthridine-12-carboxylic ester and amide derivatives. Among the benzo[*i*]phenanthridine-12-carboxylic esters evaluated, the 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-dimethylamino)-1-methylethyl, and 2-(*N*,*N*-dimethylamino)-1,1-dimethylethyl esters possessed similar cytotoxicity, ranging from 30 to 55 nM in RPMI8402 and KB3-1 cells. Several of the carboxamide derivatives possess potent topoisomerase I-targeting activity and cytotoxicity. The 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-diethylamino)ethyl, and 2-(pyrrolidin-1-yl)ethyl amides were among the more cytotoxic benzo[*i*]phenanthridine-12-carboxylic derivatives, with IC_{50} values ranging from 0.4 to 5.0 nM in RPMI8402 and KB3-1 cells.

1. Introduction

Topoisomerase I (TOP1) is an effective molecular target for the development of clinically useful anticancer agents.[1–4] TOP1-targeting agents, such as camptothecin, stabilize the cleaved complex, which forms between the enzyme and DNA. Stabilization of this ternary complex effectively converts TOP1 into a cellular poison. Extensive studies on camptothecin and its structurally related analogues have resulted in two clinical TOP1-targeting drugs, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®), Chart 1. These clinical agents have the camptothecin ring system that has incorporated within its structure a δ-lactone. Hydrolysis of this lactone results in an inactive derivative that possesses high affinity for human serum albumin.[5–7] Metabolic instability of this lactone and the observation that both topotecan and irinotecan are substrates for efflux transporters associated with multidrug resistance[8–11] have prompted studies on the development of novel TOP1-targeting agents.

Substituted benzo[*i*]phenanthridines and dibenzo[*c*,*h*]cinnolines have been identified as TOP1-targeting agents with cytotoxic activity to several human tumor cell lines.[12–16] Studies in our laboratory have demonstrated that dibenzo[*c*,*h*][1,6]naphthyridin-6-one **1** (ARC-111, topovale) and the isoquino[4,3-*c*]cinnolin-12-one **2** (Fig. 1) possess exceptional TOP1-targeting activity and cytotoxicity.[17–25] Our laboratory has recently reported on the TOP1-targeting activities and cytotoxicities of both the 2-(*N*,*N*-dimethylamino)ethyl ester **7b** and amide **9a** of 2,3-dimethoxy-8,9-methylenedioxy-benzo[*i*]phenanthridine-12-carboxylic acid, as well as on the biological activity of their propyl homologues.[26] These derivatives bear significant structural similarity to **1**, Figure 1. We provide, herein, insight into the structure–activity relationships observed for these benzo[*i*]phenanthridine-12-carboxylic acid derivatives and detail the synthetic methods used for their preparation.

Keywords: Topoisomerase I; Cytotoxic; Antitumor; Benzo[*i*]-phenanthridine.
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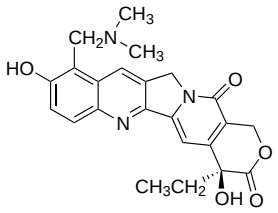
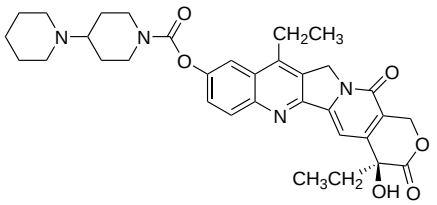
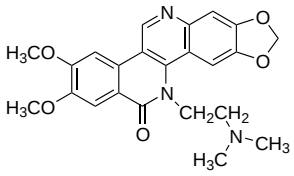


Topotecan (Hycamtin®)

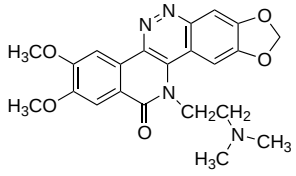


Irinotecan (Camptosar®)

Chart 1. Structures of topotecan and irinotecan.



1



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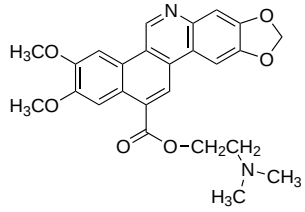
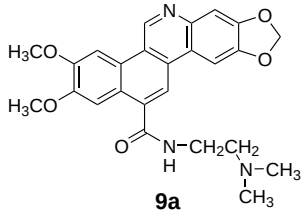


7b



9a

Figure 1. The structures of 8,9-dimethoxy-5-[(2-*N*,*N*-dimethylamino)ethyl]-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one, ARC-111 (topovale), and 2,3-dimethoxy-11-[(2-*N*,*N*-dimethylamino)ethyl]-8,9-methylenedioxyisoquino[4,3-*c*]cinnolin-12-one, **2** and the 2-(*N*,*N*-dimethylamino)ethyl ester **7b** and amide **9a** of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid.

2. Chemistry

Several derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid were synthesized using 3-(6,7-methoxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid, **5**, as the universal intermediate. Compound **5** was prepared, as illustrated in Scheme 1. 6,7-Methylenedioxy-4-methylquinoline **3** was prepared from commercially available 3,4-methylenedioxyaniline. Oxidation of **3** with SeO_2 provided 4-formyl-6,7-dimethoxyquinoline, **4**. Condensation of **4** with 2-iodo-4,5-dimethoxyphenylacetic acid in acetic anhydride in the presence of triethylamine[26] provided **5** in good yield.

Two methods were utilized for the synthesis of targeted ester derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid, which are shown in Scheme 2. Method A involved the formation of the acid chloride of **5** using thionyl chloride and having this intermediate react with the requisite alcohol to form **6a–d**. The resulting ester was then photocyclized in acetonitrile to provide benzo[*i*]phenanthridines **7a–d**. Alternatively, one could use **7a** as a common intermediate (Method B) and by transesterification prepare various ester derivatives. Acceptable yields were obtained using Method B when the transesterification was performed using a primary alcohol.

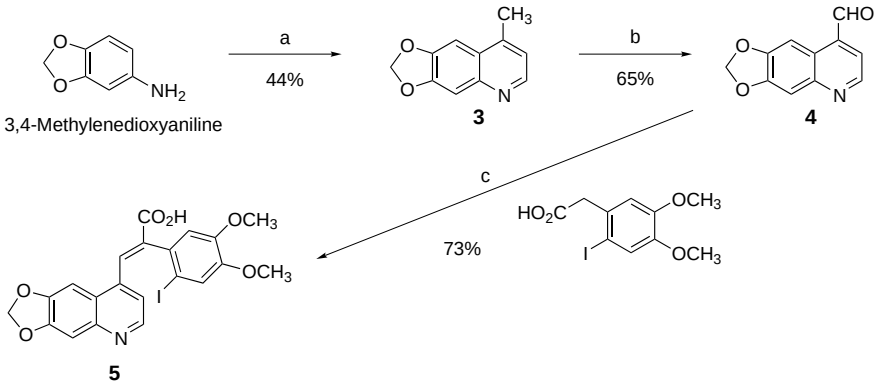
Initial studies using the Heck reaction for cyclizing **6a** and **6b** resulted in the formation of several products. In view of the difficulties encountered in separating the desired benzo[*i*]phenanthridine derivative from these undesired by-products, photocyclization was primarily employed in the preparation of these benzo[*i*]phenanthridine derivatives. In contrast to intermediates **6a–d** as well as **7a**, the cyclized esters **7b–e** were notably more labile and significant losses were observed during column chromatographic purification.

Three methods, as outlined in Scheme 3, were examined for the preparation of select amide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid. Method C involved the reaction of the acid chloride of **5** with the appropriate amine to form the acrylamides **8a–d**, which were then photocyclized to provide the benzo[*i*]phenanthridine derivatives **9a–d**. Alternatively, the 12-carboxyethyl ester benzo[*i*]phenanthridine derivative **7a** was heated in the presence of various ethylamines (Method D) to form the benzo[*i*]-phenanthridine 12-carboxamides **9a**, **9e–j**. The ethyl ester **7a** could be readily converted to the carboxylic acid **10** in almost quantitative yields. While the poor solubility of this acid limited its versatility as an intermediate in these syntheses, it could be converted in neat $SOCl_2$ to form the acid chloride. As outlined for Method E, this acid chloride was allowed to react with *N*,*N*-dimethylethylenediamine or *N*,*N*-dimethyl-1,3-propanediamine to form **9a** and **9k**, respectively.

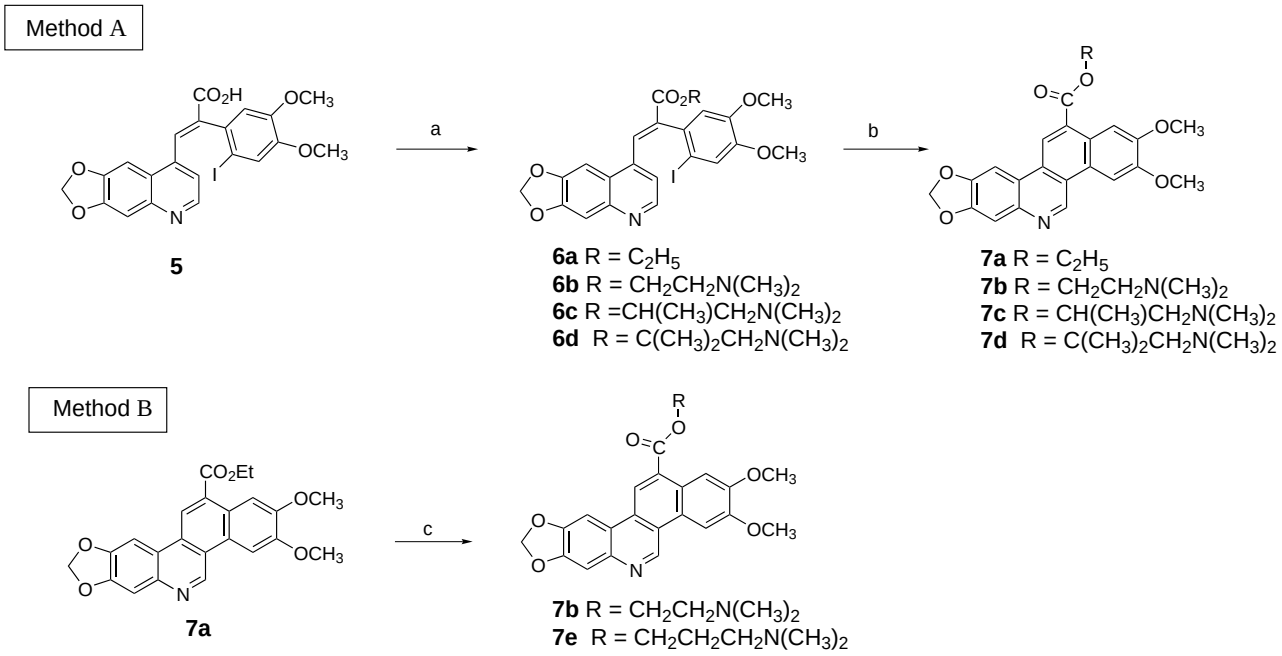
The preparation of select tertiary amides was carried out using either *N*,*N*,*N*′-trimethylethylenediamine, as illustrated in Method C for the preparation of **9d** or by alkylation of the benzo[*i*]phenanthridine carboxamide **9a**, as illustrated in Scheme 4 to form the bis-2-(*N*,*N*-dimethylamino)ethyl derivative **9l**.

3. Pharmacology

The TOP1-targeting activity and cytotoxicity of these varied ester and amide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid are given in Table 1. In general, the ester derivatives were significantly less potent than topotecan as TOP1-targeting agents. Of the five esters that were synthesized, only **7c** had comparable activity to topotecan as a TOP1-targeting agent. The other ester derivatives



Scheme 1. Reagents: (a) Methyl vinyl ketone, $FeCl_3$, AcOH (b) SeO_2, dioxane, H_2O (c) Ac_2O, TEA.

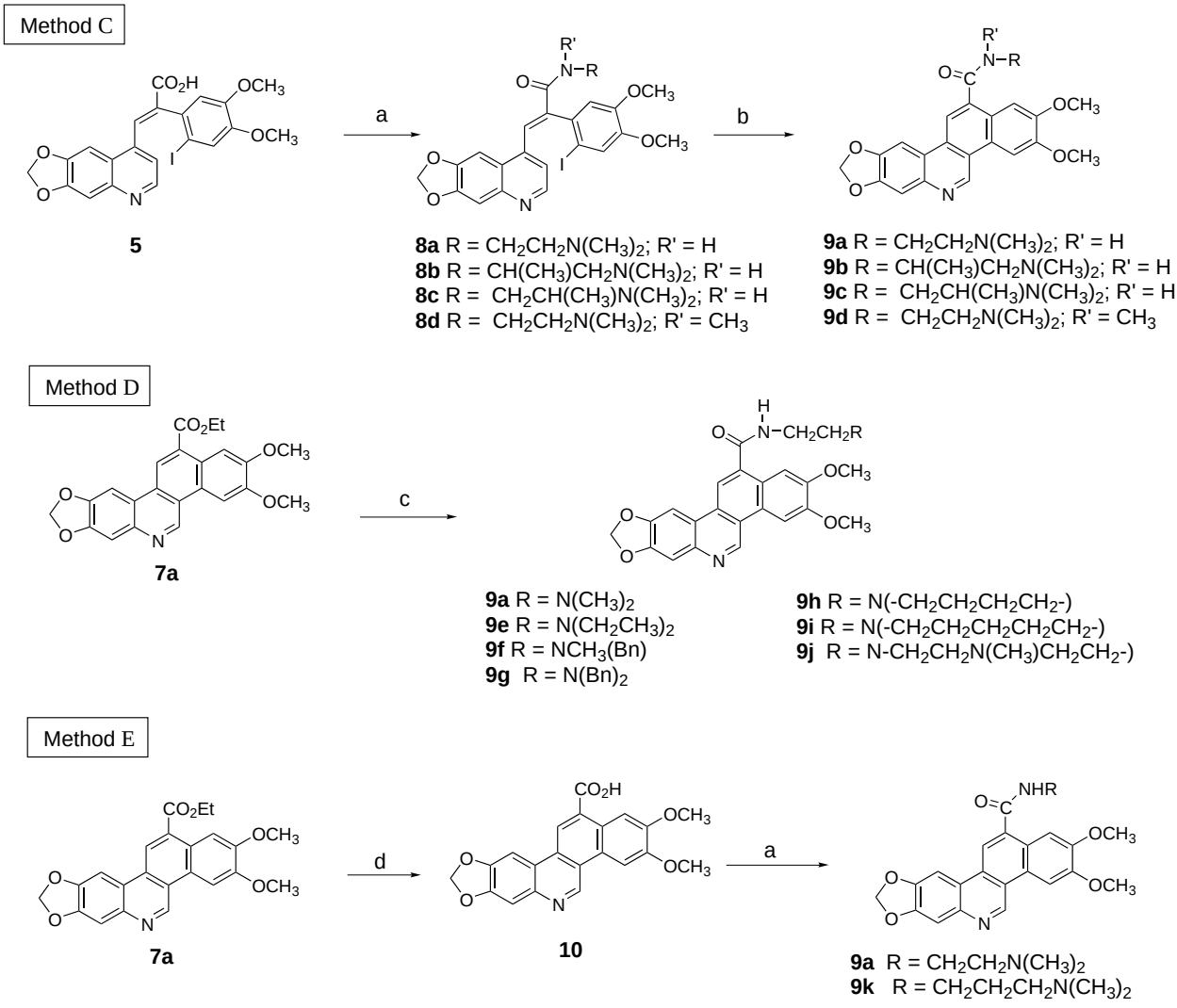


Scheme 2. Reagents: (a) $SOCl_2$, then ROH (b) *hv*, MeCN (c) ROH neat.

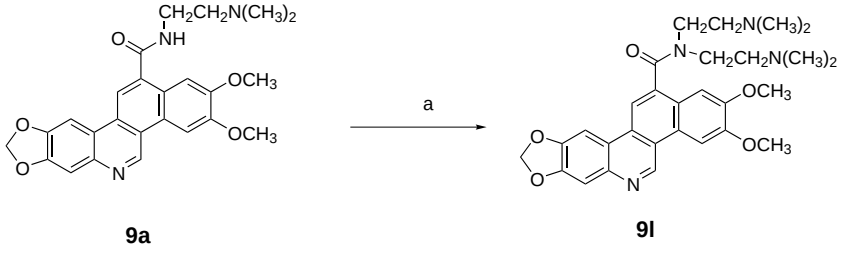
7a, **7b**, **7d**, and **7e** were 5- to 10-fold less potent in their ability to stabilize the cleavable complex formed between TOP1 and DNA. A representative gel of the stimulation of enzyme-mediated DNA cleavage observed with **7b** and **7c**, relative to topotecan, is provided in Figure 2. Among the 12-carboxamides evaluated, **9c** was the most potent TOP1-targeting agent with potency greater than that of topotecan. As in the case of **7c**, this amide derivative was substituted with a 2-(*N*,*N*-dimethylamino)-1-methylethyl substituent. Several other secondary alkyl amide derivatives exhibited TOP1-targeting activity equal to or greater than that of topotecan, including **9a**, **9b**, and **9e**. Tertiary amide derivatives of **9a**, where there is either the addition of a *N*-methyl substituent **9d** or a second *N*-(2-*N*,*N*-dimethylamino)ethyl substituent **9l** on the amide nitrogen atom, had significantly diminished activity as TOP1-targeting agents. Figure 2 shows the enzyme-mediated DNA cleavage associated with the presence of **9a**, **9d**, and topotecan.

Previous studies performed with various *N*-alkyl 5-(2-aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones structurally related to ARC-111 demonstrated that the presence of one or more *N*-benzyl substituents on the 2-(amino)ethyl moiety significantly reduces TOP1-targeting activity.[25] A similar loss in activity was observed for the 2-(*N*-benzylamino)ethyl and 2-(*N*,*N*-dibenzylamino)ethyl amides **9f** and **9g**, respectively. It is of interest to note that extension of the alkyl side chain from 2-carbons to 3-carbons in the case of ARC-111 resulted in a significant loss of biological activity. In the case of amide derivatives, a similar loss of activity was observed when comparing the relative potency of **9a** to that of the 3-(*N*,*N*-dimethylamino)propyl amide **9k**. These data suggest that the relationship between the distance between the amide nitrogen and the tertiary alkylamino substituent may be more critical than the absolute spatial distance of these substituents, relative to the backbone of their polycyclic structures.

The TOP1-targeting activities of both 2-(pyrrolidin-1-yl)ethyl and 2-(4-methylpiperazin-1-yl)ethyl substituted 12-carboxamides, **9h** and **9j**, of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine were greater than



Scheme 3. Reagents: (a) $SOCl_2$, then RNH_2 (b) *hv*, MeCN (c) RNH_2, (d) NaOH, H_2O, EtOH.

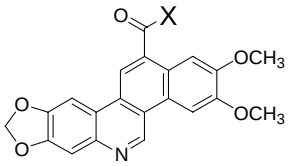


Scheme 4. Reagents: (a) NaH, NaI, DMF, $ClCH_2CH_2N(CH_3)_2 \cdot HCl$.

that observed for topotecan. The 2-(piperdin-1-yl)ethyl **9i** was somewhat less potent as a TOP1-targeting agent than topotecan. These data suggest that the presence of a piperazinyl substituent does not appear to negatively impact TOP1-targeting activity to the extent observed previously in a comparison of various heterocycles attached to the 2-position of the ethyl substituent of 5-ethyldibenzo[*c*,*h*][1,6]naphthyridin-6-ones.[25]

The more cytotoxic of the 12-carboxy esters of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine evaluated were **7c** and **7d**. While these derivatives had cytotoxicity comparable to that of topotecan in RPMI8402 and P388 cells, their relative cytotoxicity in these cell lines was significantly less than that observed for ARC-111. The cytotoxic activities of these varied 12-carboxy esters in RPMI8402, U937, and P388 cells were compared to those observed with their camptothecin-resistant variants. In the case of RPMI8402 and U937, a mutant form of TOP1 has been attributed to camptothecin resistance in their variant cell lines, CPT-K5 and U937/CR, respectively.[27,28] The lack of expression of topoisomerase in P388/CPT45 has been associated with its resistance to camptothecin, relative to its parent cell line P388.[29] Cross-resistance to these cell lines by a cytotoxic agent is indicative of TOP1 as a principal target asso-

Table 1. TOP1-targeting activity and cytotoxicity of ester and amide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid



Compound	X	TOP1	Cytotoxicity IC_{50} (μM)								
			RPMI8402 wt	CPT-K5 CPT-resist.	P388	P388/CPT45 CPT-resist.	U937	U937/CR CPT-resist.	KB3-1 wt	KBV-1 + MDR1	KBH5.0 + BCRP
1		**0.3**	0.002	0.90	0.001	0.23	0.004	0.15	0.005	0.005	0.006
7a	OCH_2CH_3	**10**	0.085	>10	0.04	3.25	0.085	>10	0.06	0.31	0.12
7b	$OCH_2CH_2N(CH_3)_2$	**5.0**	0.03	0.73	0.014	0.033	0.035	0.14	0.034	0.22	0.072
7c	$OCH(CH_3)CH_2N(CH_3)_2$	**1.0**	0.03	1.0	0.02	0.02	0.007	0.03	0.055	0.10	0.065
7d	$OC(CH_3)_2CH_2N(CH_3)_2$	**8**	0.04	2.25	0.04	0.13	0.038	0.40	0.045	0.23	0.11
7e	$OCH_2CH_2CH_2N(CH_3)_2$	**6.5**	0.22	1.5	0.27	0.25	0.6	1.35	0.47	2.3	0.52
9a	$NHCH_2CH_2N(CH_3)_2$	**0.5**	0.003	1.0	0.003	0.32	0.006	2.15	0.005	0.22	0.06
9b	$NHCH(CH_3)CH_2N(CH_3)_2$	**0.6**	0.0004	3.0	0.0007	0.77	0.002	0.35	0.003	0.32	0.02
9c	$NHCH_2CH(CH_3)N(CH_3)_2$	**0.4**	0.002	0.75	0.002	0.54	0.003	0.66	0.006	0.11	0.05
9d	$N(CH_3)CH_2CH_2N(CH_3)_2$	**10**	0.5	5.0	0.15	0.3	0.22	0.85	0.45	1.8	0.9
9e	$NHCH_2CH_2N(CH_2CH_3)_2$	**0.7**	0.006	1.75	0.004	0.37	0.033	1.3	0.03	0.47	0.07
9f	$NHCH_2CH_2NCH_3(Bn)$	**12**	0.034	10	0.035	2.75	0.045	3.6	0.06	0.68	0.35
9g	$NHCH_2CH_2N(Bn)_2$	**60**	0.45	>10	0.35	6.5	0.5	>10	0.5	8.0	6.5
9h	$N(–CH_2CH_2CH_2CH_2–)$	**0.6**	0.003	2.2	0.003	0.33	0.004	>1.0	0.003	0.15	0.05
9i	$N(–CH_2CH_2CH_2CH_2CH_2–)$	**1.5**	0.055	6.5	0.01	1.0	0.011	1.8	0.018	0.18	0.04
9j	$N(–CH_2CH_2N(CH_3)CH_2CH_2–)$	**0.3**	0.065	5.0	0.045	1.35	0.075	2.25	0.1	7.5	1.5
9k	$NHCH_2CH_2CH_2N(CH_3)_2$	**5**	0.05	2.1	0.03	0.34	0.16	2.78	0.045	4.5	0.5
9l	$N[CH_2CH_2N(CH_3)_2]_2$	**3**	0.17	2.0	0.13	2.6	0.13	2.8	0.25	2.0	0.65
TPT		**1**	0.021	>10	0.045	>10	0.024	0.21	0.038	0.47	0.55

Topoisomerase I cleavage values are reported as REC, relative effective concentration, i.e., 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.

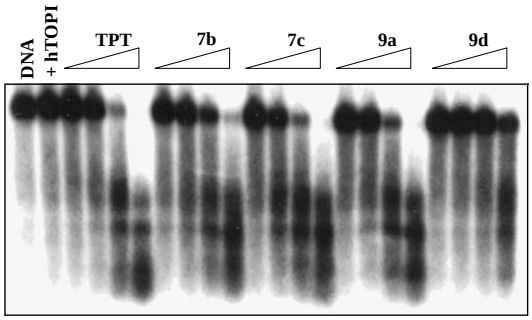


Figure 2. Stimulation of enzyme-mediated DNA cleavage by topotecan (TPT), **7b**, **7c**, **9a**, and **9d** using human TOP1. The first lane is the DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

ciated with its cytotoxic activity. The ethyl ester **7a** exhibited the greatest cross-resistance in all three of these pairs of cell lines. While **7a** has the lowest intrinsic TOP1-targeting activity of these ester derivatives, the fact that it is over 80-fold more cytotoxic in P388 than in P388/CR cells indicates that its cytotoxicity is primarily associated with its TOP1-targeting activity. The much lower relative resistance observed for **7b**, **7c**, **7d**, and **7e** suggests that mechanisms not associated with TOP1 may contribute significantly to their cytotoxic activities. The cytotoxic activities of the 12-carboxy esters of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine were also assessed in KB3-1, and its two variants KBV-1 and KBH5.0. The overexpression of the efflux transporters MDR1 (p-glycoprotein) and BCRP has been observed in KBV-1 and KBH5.0, respectively.[19,30] The overexpression of these efflux transporters has been associated with multidrug resistance. While none of these 12-carboxy esters of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine appeared to be an exceptionally good substrate for either of these efflux transporters, the cytotoxic activities of these analogues in KBV-1 cells were lower. The IC_{50} values obtained in this variant cell line that overexpresses MDR1, with the exception of **7c**, were five to seven times higher than in the parent cell line, KB3-1.

Several of the 12-carboxamide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine had greater intrinsic TOP1-targeting activity and cytotoxicity than either the 12-carboxyester derivatives or topotecan. In RPMI8402, U937, P388, and KB3-1 cell lines, **9a–c** and **9h** were consistently among the more cytotoxic analogues, with IC_{50} values ranging from 1 to 6 nM. While the 2-(piperidin-yl)ethyl amide **9i** and the 2-(4-methylpiperazin-yl)ethyl amide **9j** did exhibit good TOP1-targeting activity, they were not as cytotoxic as the 2-(pyrrolidin-1-yl)ethyl amide **9h**. In comparing the 2-(*N*,*N*-dimethylamino)ethyl ester **7b** with the 2-(*N*,*N*-dimethylamino)ethyl amide **9a**, it is evident that the amide derivative is significantly more cytotoxic in each of these parent cell lines. Particularly noteworthy is the fact that several of the secondary amides clearly mediate their cytotoxic activity primarily through interaction with TOP1, as indicated in differential cytotoxicity observed in P388, relative to P388/CPT45. In the case of the tertiary amides **9d** and **9l** and the *N*-benzyl derivatives **9f** and **9g**, these analogues were significantly less cytotoxic, which correlates with their decreased activity as TOP1-targeting agents. In the case of **9d**, it is also of significance that this *N*-methyl tertiary amide exhibited the least differential cytotoxicity between P388 and its P388/CPT45 variant. As observed with the 12-carboxy ester **7e**, elongation of the alkyl chain from ethyl to propyl, as in **9k**, with these varied aminoalkyl amides of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine was associated with diminished cytotoxicity. While the 2-(piperidin-1-yl)ethyl amide **9i** and the 2-(4-methylpiperazin-1-yl)ethyl amide **9j** did exhibit a good TOP1-targeting activity, they were not as cytotoxic as the 2-(pyrrolidin-1-yl)ethyl amide **9h**.

The cytotoxicity of these amides in KB3-1 and its variants KBV-1 and KBH5.0 clearly demonstrates that, unlike ARC-111, these 12-carboxamide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine are, in general, good substrates for MDR1. Among the more cytotoxic derivatives, the relative difference in cytotoxicity ranged from one to two orders of magnitude. Among the notable exceptions were the tertiary amide derivatives **9d** and **9l**. Several of the carboxamide derivatives were also substrates for the efflux transporter BCRP. The diminished toxicity of **9a**, **9g**, **9h**, **9i**, **9j**, and **9k** in KBH5.0 as compared to KB3-1 suggests that these derivatives are substrates for this efflux transporter.

4. Discussion

The ester derivatives 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid were, in general, isolated in lower yield, exhibited greater chemical instability, were less active as TOP1-targeting agents, and were less cytotoxic than several of the amide derivatives that were evaluated. The comparative cytotoxicity observed in P388 and P388/CPT45 for these esters also suggests that mechanisms other than TOP1-targeting activity contribute to their cytotoxic activity. These factors, in addition to the likelihood that these esters could be extensively hydrolyzed in vivo, diminished interest in extending the structure–activity studies of this series of compounds further. In contrast, several of the amide derivatives did exhibit potent TOP1-targeting activity and cytotoxicity. The comparative cytotoxic activities observed in P388 and P388/CPT45 did reveal for several of the more cytotoxic derivatives (with IC_{50} values ranging from 1 to 30 nM) that TOP1-targeting activity was clearly associated with their cytotoxic activity. These amides also exhibited good chemical stability. These more cytotoxic amides, in contrast to ARC-111, were substrates for MDR1 and, with the exception of **9e**, were substrates for the efflux transporter BCRP.

Thus, these carboxamides, as is the case for topotecan and irinotecan, are substrates for efflux transporters that have been associated with multidrug resistance. Studies will be performed to assess the relative efficacy of these carboxamides in vivo as novel non-camptothecin TOP1-targeting antitumor agents. The absence of a labile

δ-lactone, as in the case of topotecan and irinotecan, that can limit the amount of active drug and limit distribution by virtue of the protein binding of their hydrolysis product to serum albumin provides a basis for examining their potential therapeutic advantages. Thus, amide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine represent a series of compounds that have the potential for further development as novel and clinically useful TOP1-targeting agents.

5. 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, Ger.) using the solvent systems indicated. The photolysis apparatus employed consisted of a 1000 ml reactor with a 450 W mercury-vapor lamp with a 131.5 mm arc length as purchased from Ace Glass, Inc. 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 of ACS grade or HPLC grade. Methylene chloride was freshly distilled from calcium hydride. All other solvents were used as provided without further purification. The various substituted amino alcohols and diamines employed, with the exception of *N*,*N*-dimethyl-1,1-dimethylethanolamine,[31] *N*-methyl-*N*-benzylethylenediamine,[25] and 2-(*N*,*N*-dimethylamino)propylamine, were commercially available. Lithium aluminum hydride reduction of 2-(dimethylamino)propionitrile[32] provided 2-(*N*,*N*-dimethylamino)propylamine.

4-Methyl-6,7-methylenedioxyquinoline (3). Iron (III) chloride (54.2 g, 0.2 mol) was dissolved in glacial acetic acid (600 ml) with warming to 60 °C. 3,4-Methylenedioxyaniline (27.4 g, 0.2 mol) was added and the mixture was stirred for 5 min. Methyl vinyl ketone (17.4 ml, 0.21 mol) was added dropwise over 5 min. Following the completion of addition, the mixture was heated to reflux with stirring for 1.5 h. The mixture was cooled and the precipitate was filtered and washed with additional acetic acid. This material was then basified by addition to cold 30% NaOH, and the resulting mixture was filtered and air-dried. The crude material was then extracted with chloroform (7 × 200 ml), and the combined extracts were washed with 10% K_2CO_3 (3 × 300 ml), dried ($MgSO_4$), and concentrated under vacuum. The resulting material was recrystallized from ethyl ether, yielding 16.6 g as a fluffy light beige solid, in 44% yield; mp: 100.5–101.5 °C; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H), 6.04 (s, 2H), 7.02 (d, 1H, $J = 4.4$), 7.13 (s, 1H), 7.32 (s, 1H), 8.52 (d, 1H, $J = 4.4$); ^{13}C NMR ($CDCl_3$) δ 19.1, 99.3, 101.7, 106.3, 120.6, 125.0, 142.9, 146.3, 147.8, 147.9, 150.2; HRMS calcd for $C_{11}H_9O_2N$, 187.0633; found: 187.0627.

4-Formyl-6,7-methylenedioxyquinoline (4). A mixture of **3** (5.01 g, 27 mmol) in 30 ml dioxane was heated to 75 °C and then a solution of SeO_2 in 5:1 dioxane–H_2O (36 ml) was added dropwise. The mixture was heated to reflux with stirring for 4.5 h, filtered, and the filtrate was evaporated. The residue was dissolved in chloroform (50 ml), washed with water (3 × 50 ml), dried ($MgSO_4$), and evaporated. The residue was chromatographed, eluted with $CHCl_3$, yielding 3.48 g as a beige solid in 65% yield; mp: 146.0–147.5 °C; IR ($CHCl_3$) 1702; ^1H NMR ($CDCl_3$) δ 6.18 (s, 2H), 7.45 (s, 1H), 7.63 (d, 1H, $J = 4.4$), 8.41 (s, 1H), 8.96 (d, 1H, $J = 4.4$), 10.35 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 100.4, 102.3, 106.3, 121.4, 124.7, 135.7, 148.0, 148.3, 150.8, 151.0, 193.4; HRMS calcd for $C_{11}H_7O_3N$, 201.0426; found: 201.0437.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid (5). A mixture of **4** (400 mg, 2.0 mmol) and 2-iodo-4,5-dimethoxyphenylacetic acid[33] (966 mg, 3.0 mmol) in acetic acid (3.5 ml) and TEA (0.31 ml) was heated to reflux with stirring for 90 min. The mixture was cooled to about 70 °C, poured into water, and the resulting mixture was stirred for 30 min with no additional heating. The entire mixture was then evaporated under vacuum and the residue was chromatographed eluting with 97:3 chloroform–methanol, to provide 725 mg as a yellow solid, in 73% yield; mp: 270.5–271.5 °C; IR (KBr) 1704; ^1H NMR (DMSO-d_6) δ 3.47 (s, 3H), 3.72 (s, 3H), 6.24 (s, 2H), 6.67 (s, 1H), 6.83 (d, 1H, $J = 4.7$), 7.23 (s, 1H), 7.33 (s, 1H), 7.41 (s, 1H), 8.15 (s, 1H), 8.44 (d, 1H, $J = 4.7$); HRMS calcd for $C_{21}H_{16}INO_6H$, 506.0101; found: 506.0110.

5.1. General method for the preparation of esters of 3-(6,7-methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid ethyl ester (6a). Thionyl chloride (5 ml) was added dropwise to a mixture of **5** (1.51 g, 3.0 mmol) in absolute ethanol (125 ml), and the mixture was refluxed for 5 h with stirring. The mixture was cooled and evaporated under vacuum. The residue was dissolved in chloroform (250 ml) and washed with satd $NaHCO_3$ (3 × 250 ml), dried ($MgSO_4$), evaporated, and chromatographed eluting with chloroform, to provide 1.59 g in 99% yield, as an orange oil; IR ($CHCl_3$) 1713; ^1H NMR ($CDCl_3$) δ 1.31 (t, 3H, $J = 7.0$), 3.47 (s, 3H), 3.80 (s, 3H), 4.31 (q, 2H, $J = 7.0$), 6.07 (d, 2H), 6.39 (s, 1H), 6.71 (d, 1H, $J = 4.6$), 7.18 (s, 1H), 7.29 (s, 2H), 8.20 (s, 1H), 8.38 (d, 1H, $J = 4.6$); ^{13}C NMR ($CDCl_3$) δ 14.3, 55.9, 56.1, 61.8, 88.2, 99.6, 102.0, 106.4, 113.5, 119.1, 121.4, 123.6, 132.4, 136.4, 139.2, 140.2, 146.8, 147.5, 148.5, 149.3, 150.6, 166.0; HRMS calcd for $C_{23}H_{20}INO_6H$, 534.0419; found: 534.0412.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid 2-(*N*,*N*-dimethylamino)ethyl ester (6b). Prepared from the crude acid chloride of **5** (250 mg, 0.50 mmol) and *N*,*N*-dimethylethanolamine (443 mg, 4.98 mmol) in 98% yield (280 mg) as an oil; IR (CH_2Cl_2) 1714; ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 6H), 2.68 (t, 2H, J = 5.8), 3.55 (s, 3H), 3.85 (s, 3H), 4.41 (t, 2H, J = 5.8), 6.14 (s, 2H), 6.45 (s, 1H), 6.77 (d, 1H, J = 4.8), 7.22 (s, 1H), 7.35 (s, 1H), 7.36 (s, 1H), 8.28 (s, 1H), 8.44 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 45.9, 56.0, 56.2, 57.6, 64.1, 88.3, 99.6, 102.0, 106.5, 113.5, 119.1, 121.4, 123.7, 132.4, 136.7, 139.0, 139.9, 146.9, 147.6, 148.5, 149.3, 150.7, 166.0; HRMS calcd for $C_{25}H_{25}IN_2O_6H$, 577.0836; found: 577.0836.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid 2-(*N*,*N*-dimethylamino)-1-methylethyl ester (6c). Prepared from the crude acid chloride of **5** (150 mg, 0.30 mmol) and 1-(*N*,*N*-dimethylamino)-2-propanol (0.3 ml) in 80% yield (140 mg) as an oil; IR (CH_2Cl_2) 1709; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 3H, J = 6.2), 2.31 (s, 6H), 2.55 (m, 2H), 3.57 (s, 3H), 3.87 (s, 3H), 5.28 (m, 1H), 6.16 (s, 2H), 6.49 (s, 1H), 6.79 (d, 1H, J = 4.8), 7.23 (s, 1H), 7.38 (s, 1H), 7.40 (s, 1H), 8.29 (s, 1H), 8.46 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 18.5, 46.1, 56.0, 56.2, 63.8, 70.4, 88.2, 99.7, 102.0, 106.5, 113.5, 119.1, 121.3, 123.7, 132.6, 136.5, 139.1, 140.2, 146.9, 147.6, 148.5, 149.3, 150.7, 165.5; HRMS calcd for $C_{26}H_{27}IN_2O_6H$, 591.0992; found: 591.1007.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid 2-(*N*,*N*-dimethylamino)-1,1-dimethylethyl ester (6d). Prepared from the crude acid chloride of **5** (505 mg, 1.0 mmol) and 1-(*N*,*N*-dimethylamino)-2-methyl-2-propanol (0.7 ml) in 83% yield (500 mg) as an oil; IR (CH_2Cl_2) 1709; ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 6H), 2.15 (s, 6H), 2.48 (s, 2H), 3.54 (s, 3H), 3.85 (s, 3H), 6.14 (s, 2H), 6.42 (s, 1H), 6.77 (d, 1H, J = 4.4), 7.22 (s, 1H), 7.36 (s, 2H), 8.19 (s, 1H), 8.43 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 24.1, 47.7, 56.0, 56.2, 68.7, 85.0, 88.3, 99.6, 102.0, 106.5, 113.5, 119.1, 121.3, 123.8, 133.0, 135.6, 139.3, 141.5, 146.8, 147.6, 148.4, 149.2, 149.3, 150.6, 165.0; HRMS calcd for $C_{27}H_{29}IN_2O_6H$, 605.1149; found: 605.1156.

5.2. General procedures for preparation of alkylamino ester derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid: (Method A and Method B)

5.2.1. Method A. Cyclization of 3-(6,7-methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic esters.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid ethyl ester (7a). A solution of **6a** (450 mg, 0.85 mmol) in acetonitrile (800 ml) was transferred to the photoreactor apparatus and was degassed by nitrogen purge for 30 min. The solution was then irradiated through a Vycor filter for 45 min. The mixture was removed from the photoreactor, and an equal portion of **6a** (450 mg, 0.85 mmol) in acetonitrile (800 ml) was reacted according to the same procedure. The cyclized product, which had precipitated out during the course of the reaction, was isolated by filtration and washed with additional acetonitrile. Thorough drying provided **7a** in 51% yield (348 mg) as a yellow solid; mp: 250–251 °C (dec.); IR (KBr) 1716; ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 1.48 (t, 3H, J = 7.1), 3.98 (s, 3H), 4.13 (s, 3H), 4.53 (q, 2H, J = 7.1), 6.26 (s, 2H), 7.73 (s, 1H), 7.93 (s, 1H), 8.11 (s, 1H), 8.18 (s, 1H), 8.70 (s, 1H), 10.24 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 14.2, 55.9, 56.6, 62.5, 99.5, 100.0, 102.9, 103.8, 106.6, 120.4, 121.2, 123.2, 124.7, 126.8, 131.8, 132.7, 135.0, 139.8, 151.4, 151.5, 152.3, 152.4, 166.7; HRMS calcd for $C_{23}H_{19}NO_6H$, 406.1290; found: 406.1270.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)ethyl ester (7b). Prepared from **6b** (220 mg, 0.38 mmol) in acetonitrile (900 ml), reaction time (35 min), in 46% yield (79 mg) as a yellow solid; mp: 216.5–217.5 °C; IR (KBr) 1715; ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 6H), 3.00 (t, 2H, J = 5.7), 4.12 (s, 3H), 4.18 (s, 3H), 4.70 (t, 2H, J = 5.7), 6.21 (s, 2H), 7.56 (s, 1H), 7.92 (s, 1H), 8.14 (s, 1H), 8.50 (s, 1H), 8.98 (s, 1H), 9.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.8, 45.6, 56.0, 57.8, 62.8, 97.3, 99.3, 102.1, 106.7, 107.4, 120.8, 122.3, 123.2, 124.9, 126.2, 128.5, 128.9, 142.6, 145.2, 148.9, 149.6, 150.3, 150.5, 167.5; HRMS calcd for $C_{25}H_{24}N_2O_6H$, 449.1703; found: 449.1692.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)-1-methylethyl ester (7c). Prepared from **6c** (220 mg, 0.37 mmol) in acetonitrile (900 ml), reaction time (35 min), in 24% yield (41 mg) as a light yellow solid; mp: 211.0–211.5 °C; IR (KBr) 1704; ^{1}H NMR ($CDCl_3$) δ 1.54 (d, 3H, J = 6.2), 2.41 (s, 6H), 2.55 (m, 1H), 2.85 (m, 1H), 4.11 (s, 3H), 4.19 (s, 3H), 5.54 (m, 1H), 6.21 (s, 2H), 7.57 (s, 1H), 7.92 (s, 1H), 8.17 (s, 1H), 8.46 (s, 1H), 8.94 (s, 1H), 9.92 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.8, 46.3, 56.1, 56.3, 64.6, 70.0, 99.3, 102.1, 106.8, 107.5, 120.9, 122.2, 122.7, 124.9, 126.2, 129.1, 129.7, 142.6, 145.3, 148.8, 149.6, 150.2, 150.5, 167.4; HRMS calcd for $C_{26}H_{26}N_2O_6H$, 463.1869; found 463.1868.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)-1,1-dimethylethyl ester (7d). Prepared from **6d** (220 mg, 0.36 mmol) in acetonitrile (900 ml), reaction time (30 min), in 25% yield (43 mg) as a yellow solid; mp: 211–212 °C (dec.); IR (KBr) 1716; ^{1}H NMR δ 1.79 (s, 6H), 2.47 (s, 6H), 2.89 (s, 2H), 4.12 (s, 3H), 4.18 (s, 3H), 6.19 (s, 2H), 7.55 (s, 1H), 7.90 (s, 1H), 8.15 (s, 1H), 8.41 (s, 1H), 8.84 (s, 1H), 9.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.9, 45.9, 48.2, 56.0, 56.1, 85.1, 99.3, 102.1, 106.8, 107.4, 120.9, 122.0, 122.2, 124.8, 126.2, 129.2, 131.2, 142.5, 145.3, 148.8, 149.6, 150.1, 150.4, 167.4; HRMS calcd for $C_{27}H_{28}N_2O_6Li$, 483.2107; found: 483.2126.

5.2.2. Method B. Transesterification of 5a to various ester derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)ethyl ester (7b). A mixture of **7a** (30 mg, 0.074 mmol) and *N*,*N*-dimethylethanolamine (10 ml); reaction temperature 134 °C; reaction time 12 h; 45% yield (15 mg) as a yellow solid; mp: 216.5–

217.5 °C (dec.); IR (KBr) 1715; ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 6H), 3.00 (t, 2H, J = 5.7), 4.12 (s, 3H), 4.18 (s, 3H), 4.70 (t, 2H, J = 5.7), 6.21 (s, 2H), 7.56 (s, 1H), 7.92 (s, 1H), 8.14 (s, 1H), 8.50 (s, 1H), 8.98 (s, 1H), 9.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.8, 45.6, 56.0, 57.8, 62.8, 97.3, 99.3, 102.1, 106.7, 107.4, 120.8, 122.3, 123.2, 124.9, 126.2, 128.5, 128.9, 142.6, 145.2, 148.9, 149.6, 150.3, 150.5, 167.5; HRMS calcd for $C_{25}H_{24}N_2O_6H$, 449.1703; found: 449.1692.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 3-(*N*,*N*-dimethylamino)propyl ester (7e). Prepared from **7a** (30 mg, 0.074 mmol) and 3-*N*,*N*-dimethylaminopropanol (15 ml); reaction temperature 164 °C; reaction time 16 h; 35% yield (12 mg) as a yellow solid; mp: 226.5–227.5 °C (dec.); IR (KBr) 1709; ^{1}H NMR ($CDCl_3$) δ 2.15–2.23 (m, 2H), 2.42 (s, 6H), 2.67 (t, 2H, J = 7.6), 4.12 (s, 3H), 4.19 (s, 3H), 4.60 (t, 2H, J = 6.2), 6.21 (s, 2H), 7.59 (s, 1H), 7.94 (s, 1H), 8.19 (s, 1H), 8.52 (s, 1H), 8.98 (s, 1H), 9.94 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.2, 29.8, 45.6, 56.1, 56.1, 56.5, 64.1, 99.3, 102.1, 106.7, 107.6, 120.9, 122.3, 123.0, 125.0, 126.3, 128.9, 129.0, 142.6, 145.4, 149.0, 149.7, 150.3, 150.6, 167.6; HRMS calcd for $C_{26}H_{26}N_2O_6H$, 463.1870; found: 463.1857.

5.3. General procedure for the preparation of 3-(6,7-methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic amides

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid 2-(*N*,*N*-dimethylamino)ethyl amide (8a). The crude acid chloride of **5** (250 mg, 0.5 mmol) was formed from thionyl chloride (4.0 ml) by heating to reflux for 3 h. Excess thionyl chloride was removed under vacuum. The residue was dissolved in CH_2Cl_2 (5.0 ml) and TEA (2.0 ml), and then was treated with *N*,*N*-dimethylethylenediamine (402 mg, 4.55 mmol). The suspension was heated to reflux overnight and then cooled down to room temperature. More CH_2Cl_2 was added, and the mixture was washed by satd $NaHCO_3$, water, and brine. The organic phases were dried over sodium sulfate and evaporated, and the residue was purified by chromatography to provide 280 mg of **8a** as a sticky glue in 97% yield; IR (CH_2Cl_2) 1664; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 6H), 2.43 (t, 2H, J = 6.2), 3.42 (t, 2H, J = 6.2), 3.59 (s, 3H), 3.87 (s, 3H), 6.12 (s, 2H), 6.53 (s, 1H), 6.71 (d, 1H, J = 4.4), 7.25 (s, 1H), 7.34 (s, 1H), 7.41 (s, 1H), 8.28 (s, 1H), 8.41 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 37.7, 45.2, 56.0, 56.3, 57.6, 88.6, 99.9, 102.0, 106.3, 113.5, 118.8, 121.8, 124.0, 132.0, 133.4, 139.9, 142.0, 146.8, 147.5, 148.4, 149.7, 149.8, 150.6, 165.0; HRMS calcd for $C_{25}H_{26}IN_3O_5Li$, 582.1087; found: 582.1102.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid 2-(*N*,*N*-dimethylamino)-1-methylethyl amide (8b). Prepared from the crude acid chloride of **5** (250 mg, 0.5 mmol) and 1-*N*,*N*-dimethylamino-2-propylamine (396 mg, 3.88 mmol) to provide 280 mg **8b** as a sticky glue in 98% yield; IR (CH_2Cl_2) 1665; ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3H, J = 5.0), 2.17 (s, 6H), 2.30 (m, 2H), 3.60 (s, 3H), 3.86 (s, 3H), 4.15 (m, 1H), 6.11 (s, 2H), 6.56 (s, 1H), 6.71 (d, 1H, J = 4.6), 7.24 (s, 1H), 7.32 (s, 1H), 7.43 (s, 1H), 8.26 (s, 1H), 8.40 (d, 1H, J = 4.6); ^{13}C NMR ($CDCl_3$) δ 19.0, 44.3, 45.7, 56.0, 56.2, 64.2, 88.6, 100.0, 102.0, 106.3, 113.6, 118.8, 121.8, 124.0, 132.1, 133.2, 139.9, 142.2, 146.8, 147.5, 148.3, 149.6, 149.7, 150.6, 164.8; HRMS calcd for $C_{26}H_{28}IN_3O_5Li$, 596.1234; found: 596.1264.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid 2-(*N*,*N*-dimethylamino)propyl amide (8c). Prepared from the crude acid chloride of **5** (250 mg, 0.5 mmol) and 2-(*N*,*N*-dimethylamino)propylamine (250 mg, 2.44 mmol) to provide 280 mg of **8c** as a sticky glue in 92% yield; IR (CH_2Cl_2) 1665; ^{1}H NMR ($CDCl_3$) δ 0.95 (d, 3H, J = 6.6), 2.12 (s, 6H), 2.73 (m, 1H), 3.04 (m, 1H), 3.50 (m, 1H), 3.62 (s, 3H), 3.89 (s, 3H), 6.14 (s, 2H), 6.41 (s, 1H), 6.56 (s, 1H), 6.77 (d, 1H, J = 4.5), 7.27 (s, 1H), 7.36 (s, 1H), 7.45 (s, 1H), 8.29 (s, 1H), 8.44 (d, 1H, J = 4.5); ^{13}C NMR ($CDCl_3$) δ 10.3, 40.0, 42.7, 56.1, 56.3, 57.7, 88.6, 97.2, 100.0, 102.0, 106.4, 113.5, 118.9, 121.7, 124.0, 133.3, 133.1, 140.0, 142.1, 146.8, 147.5, 148.4, 149.6, 149.8, 150.6, 164.9; HRMS calcd for $C_{26}H_{28}N_3O_5IH$, 590.1152; found: 590.1139.

3-(6,7-Methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic acid *N*-methyl-*N*-[2-(dimethylamino)ethyl] amide (8d). Prepared from the crude acid chloride of **5** (250 mg, 0.5 mmol) and *N*-methyl-*N*-[2-(dimethylamino)ethylamine (408 mg, 4.00 mmol) to provide 280 mg **8d** as a sticky glue in 96% yield; IR (CH_2Cl_2) 1627; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H), 2.46 (s, 3H), 2.65 (br, 2H), 3.54 (s, 3H), 3.66 (t, 2H, J = 6.6), 3.82 (s, 3H), 6.11 (s, 2H), 6.71 (s, 1H), 6.73 (br, 1H), 7.14 (s, 1H), 7.31 (br, 2H), 7.35 (s, 1H), 8.41 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 38.7, 45.8, 46.5, 49.9, 55.9, 56.2, 86.3, 99.6, 102.0, 106.5, 114.7, 119.5, 121.9, 123.7, 130.5, 132.9, 140.2, 142.6, 147.0, 147.5, 148.4, 149.4, 149.4, 150.6, 169.6; HRMS calcd for $C_{26}H_{28}IN_3O_5Li$: 596.1234; found: 596.1249.

5.4. General procedures for the preparation of alkylamino amides of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid: Method C, Method D, and Method E

5.4.1. Method C. Cyclization of 3-(6,7-methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic amides. A solution of 3-(6,7-methylenedioxyquinolin-4-yl)-2-(2-iodo-4,5-dimethoxyphenyl)acrylic amides in acetonitrile was transferred to the photoreactor apparatus and was degassed by nitrogen purge for 30 min. The solution was then irradiated through a Vycor filter. The solvent was removed under vacuum, and the residue was purified by chromatography to provide the cyclized amides in 58–70% yield.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)ethyl amide (9a). A solution of **8a** (220 mg, 0.38 mmol) in acetonitrile (900 ml) was irradiated through a Vycor filter for 30 min to give 100 mg of **9a** as a yellow solid in 59% yield; mp: 226.4–227.1 °C; IR (KBr) 1654; ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 2.34 (s, 6H), 2.69 (t, 2H, J = 6.2), 3.70 (t,

2H, $J = 6.2$), 4.01 (s, 3H), 4.10 (s, 3H), 6.11 (s, 2H), 7.35 (s, 1H), 7.75 (s, 1H), 7.79 (s, 1H), 7.93 (s, 1H), 8.29 (s, 1H), 9.59 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 37.5, 45.2, 55.9, 56.0, 58.3, 99.3, 102.0, 102.1, 106.2, 106.3, 117.7, 120.8, 120.9, 123.9, 125.7, 129.6, 136.1, 141.6, 144.7, 148.7, 149.6, 149.9, 150.6, 170.0; HRMS calcd for $C_{25}H_{25}N_3O_5H$, 448.1872; found: 448.1865.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)-1-methylethyl amide (9b). A solution of **8b** (220 mg, 0.37 mmol) in acetonitrile (900 ml) was irradiated for 30 min to give 120 mg of **9b** in 70% yield as a yellow solid; mp: 220.6–221.2 °C (dec.); IR (KBr) 1644; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 1.30 (d, 3H, $J = 6.6$), 2.79 (s, 6H), 2.99 (m, 1H), 3.43 (m, 1H), 3.75 (m, 1H), 3.82 (s, 3H), 3.95 (s, 3H), 5.95 (s, 2H), 7.10 (s, 1H), 7.53 (s, 1H), 7.88 (s, 1H), 7.94 (s, 1H), 8.37 (s, 1H), 9.55 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 19.6, 42.0, 44.0, 56.1, 56.9, 61.9, 100.5, 103.0, 103.9, 107.1, 107.4, 119.0, 121.0, 121.4, 124.3, 126.1, 129.6, 136.5, 142.9, 146.8, 149.1, 150.1, 150.2, 151.3, 169.3; HRMS calcd for $C_{26}H_{27}N_3O_5Li$, 468.2111; found: 468.2095.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)propyl amide (9c). A solution of **8c** (220 mg, 0.37 mmol) in acetonitrile (900 ml) was irradiated for 30 min to give 110 mg of **9c** in 60% yield as a yellow solid; mp: 220.3–220.7 °C (dec.); IR (KBr) 1652; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 1.19 (d, 3H, $J = 6.6$), 2.43 (s, 6H), 3.07 (m, 1H), 3.51 (m, 1H), 3.77 (m, 1H), 4.04 (s, 3H), 4.14 (s, 3H), 6.12 (s, 2H), 7.36 (s, 1H), 7.74 (s, 1H), 7.76 (s, 1H), 7.96 (s, 1H), 8.24 (s, 1H), 9.64 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 11.1, 40.4, 42.5, 55.7, 55.9, 58.6, 99.1, 102.0, 102.1, 106.0, 117.4, 120.7, 120.7, 123.7, 125.6, 129.4, 136.2, 141.3, 144.5, 148.7, 149.6, 149.8, 150.5, 170.2; HRMS calcd for $C_{26}H_{27}N_3O_5H$, 462.2029; found: 462.2034.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid *N*-methyl-*N*-[2-(*N*,*N*-dimethylamino)ethyl]amide (9d). A solution of **8d** (220 mg, 0.37 mmol) in acetonitrile (900 ml) was irradiated for 45 min to give 115 mg of **9d** in 67% yield as a beige solid; mp: 224.5 °C (dec.); IR (KBr) 1619; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 2.77 (s, 6H), 2.85 (s, 3H), 3.27 (br, 2H), 3.72 (br, 2H), 3.87 (s, 3H), 4.02 (s, 3H), 6.02 (s, 2H), 6.99 (s, 1H), 7.31 (s, 1H), 7.93 (s, 1H), 8.03 (s, 1H), 8.32 (s, 1H), 9.67 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 32.9, 37.6, 44.2, 55.2, 55.8, 56.0, 99.7, 102.1, 102.6, 104.8, 105.8, 116.9, 120.6, 121.0, 122.9, 125.8, 130.3, 141.6, 144.6, 149.0, 150., 150.4, 151.1, 171.2; HRMS calcd for $C_{26}H_{27}N_3O_5Li$, 468.2111; found: 468.2097.

5.4.2. Method D. Amide formation from 2,3-dimethoxy-8,9-methylenedioxy-benzo[*i*]phenanthridine-12-carboxylic acid ethyl ester (7a).

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dimethylamino)ethyl amide (9a). A solution of **7a** (100 mg, 0.25mmol) in *N*,*N*-dimethylethylenediamine (30 ml) was heated to 110 °C for 5 days. TLC was used to monitor the reaction. After the reaction was completed, the residual amine was removed under vacuum. The residue was dissolved in CH_2Cl_2 (30 ml) and washed with water (3 × 10 ml) and brine (10 ml). The organic layers were dried over sodium sulfate, evaporated, and the residue was chromatographed eluting with 3–4.5% methanol–dichloromethane to provide 60 mg of **9a** in 60% yield as a yellow solid; mp: 226.4–227.1 °C; IR (KBr) 1654; ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 2.34 (s, 6H), 2.69 (t, 2H, $J = 6.2$), 3.70 (t, 2H, $J = 6.2$), 4.01 (s, 3H), 4.10 (s, 3H), 6.11 (s, 2H), 7.35 (s, 1H), 7.75 (s, 1H), 7.79 (s, 1H), 7.93 (s, 1H), 8.29 (s, 1H), 9.59 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 37.5, 45.2, 55.9, 56.0, 58.3, 99.3, 102.0, 102.1, 106.2, 106.3, 117.7, 120.8, 120.9, 123.9, 125.7, 129.6, 136.1, 141.6, 144.7, 148.7, 149.6, 149.9, 150.6, 170.0; HRMS calcd for $C_{25}H_{25}N_3O_5H$, 448.1872; found: 448.1865.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-diethylamino)ethyl amide (9e). A solution of **7a** (100 mg, 0.25 mmol) in *N*,*N*-diethylethylenediamine (6.0 ml) was heated to 115 °C for 2 days to provide 43 mg of **9e** in 49% yield as a yellow solid; mp: 200.3–200.7 °C (dec.); IR (KBr) 1633; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 6H, $J = 7.4$), 2.84 (q, 4H, $J = 7.4$), 3.00 (t, 2H, $J = 6.2$), 3.82 (t, 2H, $J = 6.2$), 4.09 (s, 3H), 4.18 (s, 3H), 6.14 (s, 2H), 7.46 (s, 1H), 7.76 (s, 1H), 7.85 (s, 1H), 8.02 (s, 1H), 8.26 (s, 1H), 9.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.3, 37.4, 47.4, 53.5, 56.0, 56.1, 99.3, 102.0, 102.2, 106.4, 107.1, 117.8, 120.6, 121.1, 124.0, 125.9, 129.3, 142.2, 145.1, 148.6, 149.4, 149.9, 150.6, 169.8; HRMS calcd for $C_{27}H_{29}N_3O_5H$, 476.2186; found: 476.2169.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*-benzyl-*N*-methylamino)ethyl amide (9f). A solution of **7a** (30 mg, 0.074 mmol) in *N*-benzyl-*N*-methylethylenediamine (3.0 ml) was heated to 140 °C for 4 days to provide 21 mg of **9f** in 54% yield as a yellow solid; mp: 233.4–233.8 °C (dec.); IR (KBr) 1635; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 2.37 (s, 3H), 2.76 (t, 2H, $J = 6.2$), 3.61 (s, 2H), 3.73 (t, 2H, $J = 6.2$), 3.99 (s, 3H), 4.15 (s, 3H), 6.17 (s, 2H), 7.20 (m, 5H), 7.47 (s, 1H), 7.74 (s, 1H), 7.75 (s, 1H), 8.02 (s, 1H), 8.20 (s, 1H), 9.73 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 37.5, 42.3, 55.4, 56.0, 56.1, 62.5, 99.2, 102.1, 106.3, 106.8, 117.3, 120.7, 121.1, 124.0, 125.9, 127.5, 128.5, 129.1, 129.5, 136.5, 138.3, 141.8, 145.0, 148.7, 149.6, 150.0, 150.8, 169.7; HRMS calcd for $C_{31}H_{29}N_3O_5Li$, 523.2267; found: 523.2241.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(*N*,*N*-dibenzyl)ethyl amide (9g). A solution of **7a** (70 mg, 0.17 mmol) in *N*,*N*-dibenzylethylenediamine (5.0 ml) was heated to 145 °C for 4 days to provide 35 mg of **9g** in 34% yield as a yellow solid; mp: 254.3–254.8 °C (dec.); IR (KBr) 1635; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 2.78 (t, 2H, $J = 6.2$), 3.65 (br, 6H), 3.93 (s, 3H), 4.17 (s, 3H), 6.21 (s, 2H), 7.11 (m, 5H), 7.24 (m, 5H), 7.53 (s, 1H), 7.64 (s, 1H), 7.78 (s, 1H), 8.14 (s, 2H), 9.84 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 37.6, 51.8, 56.0, 56.2, 58.7, 99.3, 102.1, 106.3, 106.8, 117.1, 120.8, 121.2, 124.0, 126.0, 127.4, 128.5, 128.9, 129.7, 136.7, 139.0,

142.0, 145.1, 148.9, 149.8, 150.1, 151.0, 169.6; HRMS calcd for $C_{37}H_{33}N_3O_5Li$, 606.2580; found: 606.2567.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(pyrrolidin-1-yl)ethyl amide (9h). A solution of **7a** (40 mg, 0.10 mmol) in 2-(pyrrolidin-1-yl)ethylamine (3 ml) was heated to 130 °C for 2 days to provide 31 mg of **9h** in 47% yield as a yellow solid; mp: 250.3–250.6 °C (dec.); IR (KBr) 1636; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 1.86 (m, 4H), 2.73 (m, 4H), 2.93 (t, 2H, J = 6.2), 3.75 (t, 2H, J = 6.2), 4.03 (s, 3H), 4.13 (s, 3H), 6.14 (s, 2H), 7.44 (s, 1H), 7.82 (s, 1H), 7.86 (s, 1H), 8.03 (s, 1H), 8.37 (s, 1H), 9.71 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 23.4, 38.6, 54.2, 55.2, 56.0, 56.1, 99.3, 102.1, 106.3, 106.5, 117.8, 120.9, 121.1, 124.1, 125.9, 129.7, 136.1, 141.8, 144.9, 148.8, 149.7, 150.0, 150.7, 170.0; HRMS calcd for $C_{27}H_{27}N_3O_5Li$, 480.2111; found: 480.2114.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(piperidin-1-yl)ethyl amide (9i). A solution of **7a** (40 mg, 0.10 mmol) in 2-(piperazin-1-yl)ethylamine (3 ml) was heated to 140 °C for 2 days to provide 35 mg of **9i** in 73% yield as a light beige solid; mp: 267.3–267.7 °C (dec.); IR (KBr) 1658; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 1.53 (m, 6H), 2.60 (m, 6H), 3.70 (br, 2H), 4.00 (s, 3H), 4.10 (s, 3H), 6.11 (s, 2H), 7.38 (s, 1H), 7.76 (s, 1H), 7.79 (s, 1H), 8.00 (s, 1H), 8.29 (s, 1H), 9.66 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 24.0, 25.5, 36.7, 54.5, 55.9, 56.0, 57.8, 99.2, 102.2, 106.3, 117.6, 120.9, 121.1, 124.0, 125.8, 129.6, 136.2, 141.6, 144.8, 148.9, 149.7, 149.9, 150.7, 170.1; HRMS calcd for $C_{28}H_{29}N_3O_5H$, 488.2185; found: 488.2189.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(4-methylpiperazin-1-yl)ethyl amide (9j). A solution of **7a** (40 mg, 0.10 mmol) in 2-(4-methylpiperidin-1-yl)ethylamine (3 ml) was heated to 135 °C for 2 days to provide 35 mg of **9j** in 71% yield as a light pink solid; mp: 269.3–269.8 °C (dec.); IR (KBr) 1663; ^{1}H NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 2.30 (s, 3H), 2.76 (m, 10H), 3.76 (t, 2H, J = 6.2), 4.04 (s, 3H), 4.12 (s, 3H), 6.11 (s, 2H), 7.39 (s, 1H), 7.62 (s, 1H), 7.71 (s, 1H), 7.90 (s, 1H), 8.11 (s, 1H), 9.57 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD) δ 36.8, 45.7, 52.7, 54.9, 56.0, 57.0, 98.9, 102.0, 102.2, 106.2, 106.6, 117.3, 120.6, 120.8, 123.9, 125.6, 129.3, 136.2, 141.5, 144.7, 148.7, 149.5, 149.9, 150.6, 170.0; HRMS calcd for $C_{28}H_{30}N_4O_5Li$, 509.2376; found: 509.2378.

5.4.3. Method E. Formation of amides from the acid chloride of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid (10).

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 2-(dimethylamino)ethyl amide (9a). The acid **8** (30 mg, 0.08 mmol) was added without further purification to thionyl chloride (10 ml) and the mixture was heated to reflux for 4 h. The excess thionyl chloride was removed under vacuum and *N*,*N*-dimethylethylenediamine (5 ml) was added. The mixture was stirred at ambient temperature for 30 min. The *N*,*N*-dimethylethylenediamine was evaporated and the residue was dissolved in chloroform (25 ml), washed with satd $NaHCO_3$ (3 × 25 ml), and extracted with dilute aq HCl (3 × 25 ml). The combined aqueous extracts were washed with chloroform (2 × 25 ml), were basified (30% NaOH), and back-extracted into ethyl acetate (3 × 30 ml). The organic phases were dried ($MgSO_4$) and evaporated, and the residue was chromatographed eluting with 96:4 chloroform–methanol, providing 18 mg as a yellow solid, in 52% yield; mp: 226.4–227.1 °C; IR (KBr) 1654; ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 2.34 (s, 6H), 2.69 (t, 2H, J = 6.2), 3.70 (t, 2H, J = 6.2), 4.01 (s, 3H), 4.10 (s, 3H), 6.11 (s, 2H), 7.35 (s, 1H), 7.75 (s, 1H), 7.79 (s, 1H), 7.93 (s, 1H), 8.29 (s, 1H), 9.59 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 37.5, 45.2, 55.9, 56.0, 58.3, 99.3, 102.0, 102.1, 106.2, 106.3, 117.7, 120.8, 120.9, 123.9, 125.7, 129.6, 136.1, 141.6, 144.7, 148.7, 149.6, 149.9, 150.6, 170.0; HRMS calcd for $C_{25}H_{25}N_3O_5H$, 448.1872; found: 448.1865.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid 3-(dimethylamino)propyl amide (9k). Prepared from the crude acid chloride of **10** (60 mg, 0.159 mmol) and *N*,*N*-dimethyl-1,3-propylanediamine (11 ml) in 45% yield as a yellow solid; mp: 220.6–221.3 °C (dec.); IR (KBr) 1635; ^{1}H NMR ($CDCl_3$ + 6 drop CD_3OD) δ 2.03 (m, 2H), 2.49 (s, 6H), 2.77 (t, 2H, J = 7.4), 3.63 (t, 2H, J = 6.6), 4.02 (s, 3H), 4.13 (s, 3H), 6.14 (s, 2H), 7.42 (s, 1H), 7.81 (s, 1H), 7.91 (s, 1H), 8.06 (s, 1H), 8.34 (s, 1H), 9.75 (s, 1H); ^{13}C NMR ($CDCl_3$ + 6 drop CD_3OD) δ 26.3, 38.4, 44.6, 44.8, 55.9, 56.1, 57.3, 99.2, 102.1, 106.3, 106.4, 117.4, 120.8, 121.0, 124.0, 125.8, 129.6, 136.3, 141.7, 144.8, 148.8, 149.7, 150.0, 150.7, 170.1; HRMS calcd for $C_{26}H_{27}N_3O_5H$, 462.2030; found: 462.2006.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid *N*,*N*-bis[2-(*N*,*N*-dimethylamino)ethyl] amide (9l). A mixture of **9a** (20 mg, 0.045 mmol), 2-(*N*,*N*-dimethylamino)ethyl chloride hydrochloride (8 mg, 0.055 mmol), and NaI (10 mg, 0.067 mmol) in DMF (2 ml) was cooled to 0 °C, and then NaH 60% in mineral oil (6 mg, 0.15 mmol) was added in small portions. The mixture was allowed to warm to room temperature, stirred for 45 min, and then transferred to an oil bath, which was preheated to 60 °C. The reaction mixture was then heated for 3 h, allowed to cool to room temperature, and quenched by water (10 ml). Solvent was removed under vacuum. The residue was diluted by CH_2Cl_2 (20 ml) and washed with water (10 ml × 3) and brine (10 ml). Organic layers were dried over $MgSO_4$ and evaporated, and the residue was chromatographed eluting with 96:4 to 94:6 chloroform–methanol, providing 18 mg as a light yellow solid, in 78% yield; mp: 208.7–209.2 °C (dec.); IR (KBr) 1624; ^{1}H NMR ($CDCl_3$ + 12 drops of CD_3OD) δ 1.59 (s, 6H), 2.16 (m, 2H), 2.24 (s, 6H), 2.64 (m, 2H), 2.99 (m, 2H), 3.57 (m, 2H), 3.78 (s, 3H), 3.93 (s, 3H), 5.95 (s, 2H), 6.99 (s, 1H), 7.21 (s, 1H), 7.68 (s, 1H), 7.91 (s, 1H), 8.07 (s, 1H), 9.55 (s, 1H); ^{13}C NMR ($CDCl_3$ + 12 drops of CD_3OD) δ 42.5, 44.7, 45.0, 46.6, 55.6, 55.8, 56.2, 57.3, 99.1, 102.1, 102.4, 105.1, 105.7, 116.1, 120.3, 120.7, 123.1, 125.6, 129.9, 136.4, 141.5, 144.6, 148.9, 149.9, 150.1, 151.0, 171.3;

HRMS calcd for $C_{29}H_{34}N_4O_5H$, 519.2607; found: 519.2597.

2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid (10). A mixture of **7a** (40 mg, 0.1 mmol) in 10% NaOH (45 ml) and ethanol (10 ml) was heated to reflux with stirring overnight. The mixture was concentrated to dryness and water (30 ml) was added. The resulting mixture was acidified by the addition of acetic acid and the free acid was then isolated by filtration. After complete drying, 32 mg (87%) was obtained as a yellow solid; mp: 277.7–278.6 °C (dec.); IR (KBr) 1706; HRMS calcd for $C_{21}H_{15}NO_6Li$, 384.1059; found: 384.1061.

5.5. 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.[34] Plasmid YepG was purified by the alkali lysis method, followed by phenol deproteination and CsCl/ethidium bromide isopycnic centrifugation method as described.[35] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme, followed by end-filling with Klenow polymerase as previously described.[36] The cleavage assays were performed, as previously reported.[34,37] The drug and the DNA in the presence of topoisomerase I were 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, i.e., concentrations relative to topotecan, whose value is arbitrarily assumed as 1.0 that produces the same cleavage on the plasmid DNA in the presence of human topoisomerase I.

5.6. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[38–40] 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 P388 mouse leukemia cell line and its CPT-resistant TOP1-deficient variant P388/CPT45 were obtained from Michael R. Mattern and Randal K. Johnson (GlaxoSmithKline, PA).[29] The U937 cell line and its CPT-resistant variant U937/CR were obtained from Dr. Eric H. Rubin (The Cancer Institute of New Jersey, NJ).[28] The KB3-1 cell line and its multidrug-resistant variant KBV-1 were obtained from K. V. Chin (The Cancer Institute of New Jersey, NJ).[30] The KBH5.0 cell line was derived from KB3-1 by stepwise selection against Hoechst 33342.[19] 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.

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References and notes

1. Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665.
2. Liu, L. *Annu. Rev. Biochem.* **1989**, *58*, 351.
3. Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191.
4. Haiang, Y.; Lihou, M.; Liu, L. *Cancer Res.* **1989**, *49*, 5077–5082.
5. Wall, J. G.; Burris, H. A.; Von-Hoff, D. D.; Rodriquez, G.; Kneuper-Hall, R.; Shaffer, D.; O'Rourke, T.; Brown, T.; Weiss, G.; Clark, G., et al. *Anticancer Drugs* **1992**, *3*, 337.
6. Burke, T. G.; Mi, Z. *J. Med. Chem.* **1993**, *36*, 2580.
7. Burke, T. G.; Mi, Z. *Anal. Biochem.* **1993**, *212*, 285.
8. Chen, A. Y.; Yu, C.; Potmesil, M.; Wall, M. E.; Wani, M. C.; Liu, L. F. *Cancer Res.* **1991**, *51*, 6039.
9. Chu, X.; Kato, Y.; Sugiyama, Y. *Cancer Res.* **1997**, *57*, 1934.
10. Kawabata, S.; Oka, M.; Shiozawa, K.; Tsukamoto, K.; Nakatomi, K.; Soda, H.; Fududa, M.; Ikegami, Y.; Sugahara, K.; Yamada, Y.; Kamihira, S.; Doyle, L. A.; Ross, D. D.; Kohno, S. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1216.
11. Yang, C. H.; Schneider, E.; Kuo, M. L.; Volk, E. L.; Roccchi, E.; Chen, Y. C. *Biochem. Pharmacol.* **2000**, *60*, 831.
12. Makhey, D.; Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 1809.
13. Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 521.
14. Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 3795.
15. Yu, Y.; Singh, S. K.; Liu, A.; Li, T.-K.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 1474.
16. Singh, S. K.; Ruchelman, A. L.; Zhou, N.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Med. Chem. Res.* **2003**, *12*, 1.
17. Ruchelman, A. L.; Singh, S. K.; Wu, X.; Ray, A.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3333.
18. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2061.
19. Li, T.-K.; Houghton, P. J.; Desai, S. D.; Daroui, P.; Liu, A. A.; Hars, E. S.; Ruchelman, A. L.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **2003**, *63*, 8400.
20. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2004**, *12*, 795.
21. Singh, S. K.; Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2003**, *46*, 2254.
22. Kerrigan, J. E.; Pilch, D. S.; Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3395.
23. Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2004**, *2*, 3731.

24. Ruchelman, A. L.; Liu, A.; Zhou, N.; Liu, L. F.; LaVoie, E. J. *Lett. Drug Des. Discovery* **2004**, *1*, 198.
25. Ruchelman, A. L.; Liu, A.; Zhou, N.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2005**, *48*, 792.
26. Ruchelman, A. L.; Zhu, S.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5585.
27. Andoh, T.; Ishii, K.; Suzuki, Y.; Ikegami, Y.; Kusunoki, Y.; Takemoto, Y.; Okada, K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5565.
28. Rubin, E.; Pantazis, P.; Bharti, A.; Toppmeyer, D.; Giovanella, B.; Kufe, D. *J. Biol. Chem.* **1994**, *269*, 2433.
29. Woessner, R. D.; Eng, W. K.; Hofmann, G. A.; Rieman, D. J.; McCabe, F. L.; Hertzberg, R. P.; Mattern, M. R.; Tan, K. B.; Johnson, R. K. *Oncol. Res.* **1992**, *4*, 481.
30. Gervasoni, J. E., Jr.; Fields, S. Z.; Krishna, S.; Baker, M. A.; Rosado, M.; Thuraisamy, K.; Hindenburg, A. A.; Taub, R. N. *Cancer Res.* **1991**, *51*, 4955.
31. Anwander, R.; Munck, F. C.; Priermeier, T.; Scherer, W.; Runte, P.; Herrann, W. A. *Inorg. Chem.* **1997**, *36*, 3545.
32. Luten, D. B. *J. Org. Chem.* **1938**, *3*, 588.
33. Olivera, R.; SanMartin, R.; Churruca, F.; Domínguez, E. *J. Org. Chem.* **2002**, *67*, 7215.
34. Gatto, B.; Sanders, M. M.; Yu, C.; Wu, H.-Y.; Makhey, D.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **1996**, *56*, 2795–2800.
35. Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982, pp 149–185.
36. Tewey, K. M.; Rowe, T. C.; Yang, L.; Hallogan, B. C.; Liu, L. F. *Science* **1984**, *226*, 466.
37. Wang, H.; Mao, Y.; Chen, A.; Zhou, N.; LaVoie, E. J.; Liu, L. F. *Biochemistry* **2001**, *40*, 3316.
38. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
39. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
40. Denizot, F.; Lang, R. J. *J. Immunol. Methods* **1986**, *89*, 271.