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Dimethoxybenzo[*i*]phenanthridine-12-carboxylic acid derivatives and 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones with potent topoisomerase I-targeting activity and cytotoxicity

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Abstract—The exceptional TOP1-targeting activity and antitumor activity of ARC-111, **1**, prompted studies on similarly substituted benzo[*i*]phenanthridine-12-carboxylic ester and amide derivatives. These studies were extended to include 6-substituted 8,9-dimethoxy-2,3-methylenedioxy-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones, which represent reversed lactam analogues of **1**. Several of these analogues retained the potent TOP1-targeting activity and cytotoxicity observed for ARC-111.

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 formed between the enzyme and DNA. This ternary complex effectively converts TOP1 into a cellular poison. Extensive studies on camptothecin and its structurally related analogues have resulted in the development of two clinical TOP1-targeting drugs, topotecan (Hycamptin®) and irinotecan (CPT-11/Camptosar®). These clinical agents have the camptothecin ring system, which includes the presence of a δ-lactone, incorporated within their structures. Hydrolysis of this lactone results in an inactive derivative that possesses high affinity for human serum albumin.[5–7] The 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 further studies on the development of novel TOP1-targeting agents.

Substituted benzo[*i*]phenanthridines and dibenzo[*c*,*h*]-cinnolines can exert TOP1-targeting activity and cytotoxicity against several human tumor cell lines.[12–16] Recent studies in our laboratory have demonstrated that specific 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones possess exceptional TOP1-targeting activity and cytotoxicity.[17–23] The dibenzo[*c*,*h*][1,6]naphthyridin-6-one **1** (ARC-111, topovale) and the isoquino[4,3-*c*]cinnolin-12-one **2** (Fig. 1) were among the analogues that exhibited potent activity. Both compounds **1** and **2** are not substrates for either MDR1 (*p*-glycoprotein) or BCRP, which are efflux

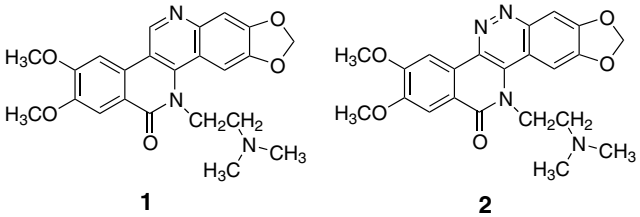

Figure 1. The structures of 8,9-dimethoxy-5-[(2-*N*,*N*-dimethylamino)-ethyl]-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one (**1**) and 8,9-dimethoxy-11-[(2-*N*,*N*-dimethylamino)ethyl]-2,3-methylenedioxyisoquino[4,3-*c*]cinnolin-12-one (**2**).

Keywords: Topoisomerase I; Cytotoxic agents; Anticancer; Benzo[*i*]-phenanthridines; Dibenzo[*c*,*h*]naphthyridines.
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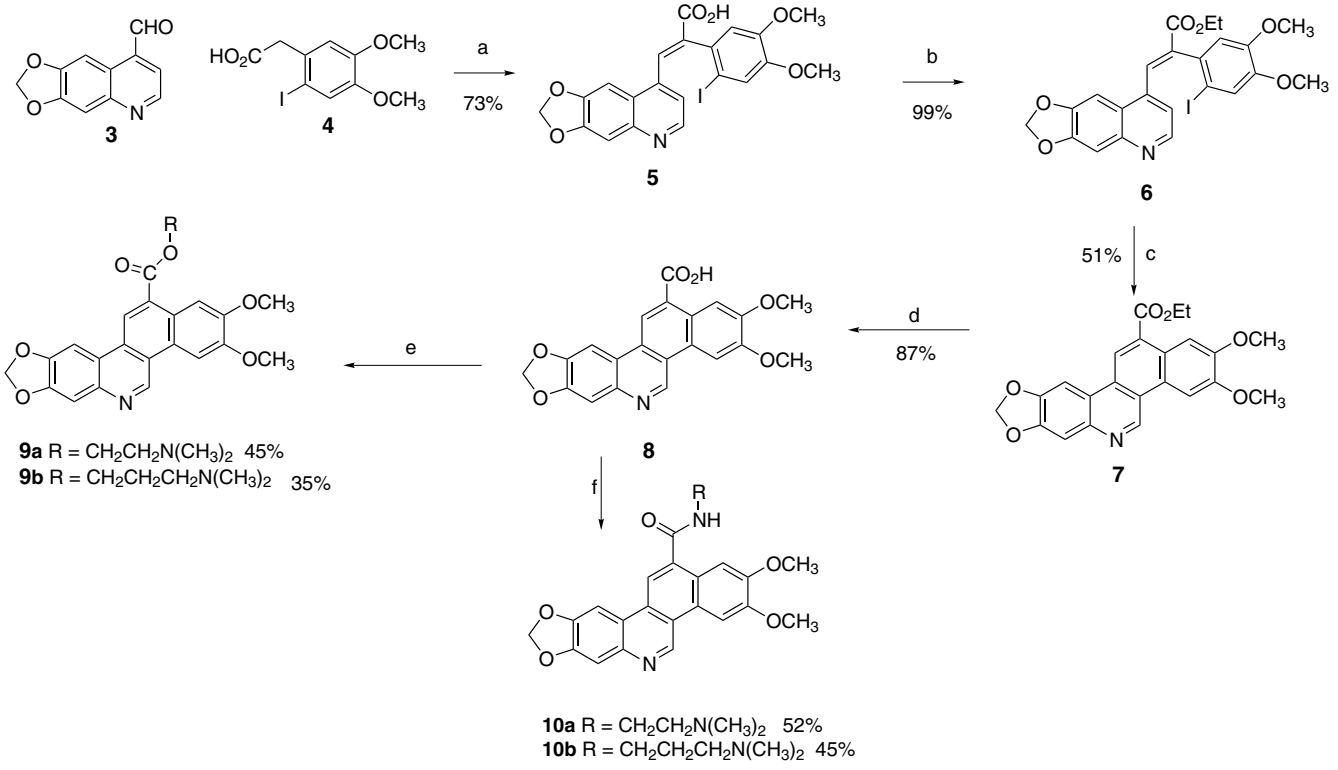
transporters associated with multi-drug resistance.[19,24] Based upon its potent antitumor activity in vivo when administered either orally or parenterally, **1** is viewed as a promising non-camptothecin TOP1-targeting agent.

Further studies were pursued to evaluate compounds with structural similarity to **1**. As the presence of the 2-(*N*,*N*-dimethylamino)ethyl substituent was associated with enhanced cytotoxicity and greater antitumor activity in human tumor xenograft animal models, we initially investigated the TOP1-targeting activity of 2-aminoethyl esters or amides of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid. As an increase in the chain length of the 5-aminoalkyl substituent in the case of **1** reduced its activity, we also synthesized and evaluated the homologous 3-*N*,*N*-dimethylaminopropyl ester and amide derivatives.[17,18] These studies were also extended to the reversed lactam analogues of **1**. Specifically, we synthesized the 6-[(2-*N*,*N*-dimethylamino)ethyl]- and 6-[(3-*N*,*N*-dimethylamino)propyl-8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*][2,6]naphthyridin-6-one derivatives. These structurally related analogues of **1** were assayed for TOP1-targeting activity and cytotoxicity in RPMI8402 and P388 cells, as well as their camptothecin-resistant variants, CPT-K5 and P388/CPT45 cells, respectively.[25,26] In addition, the relative cytotoxic activity of these compounds as compared to the parent cell line, KB3-1 was assessed in KBV-1 cells,[27] which overexpress MDR1 and KBH5.0 cells,[19] which overexpress BCRP.

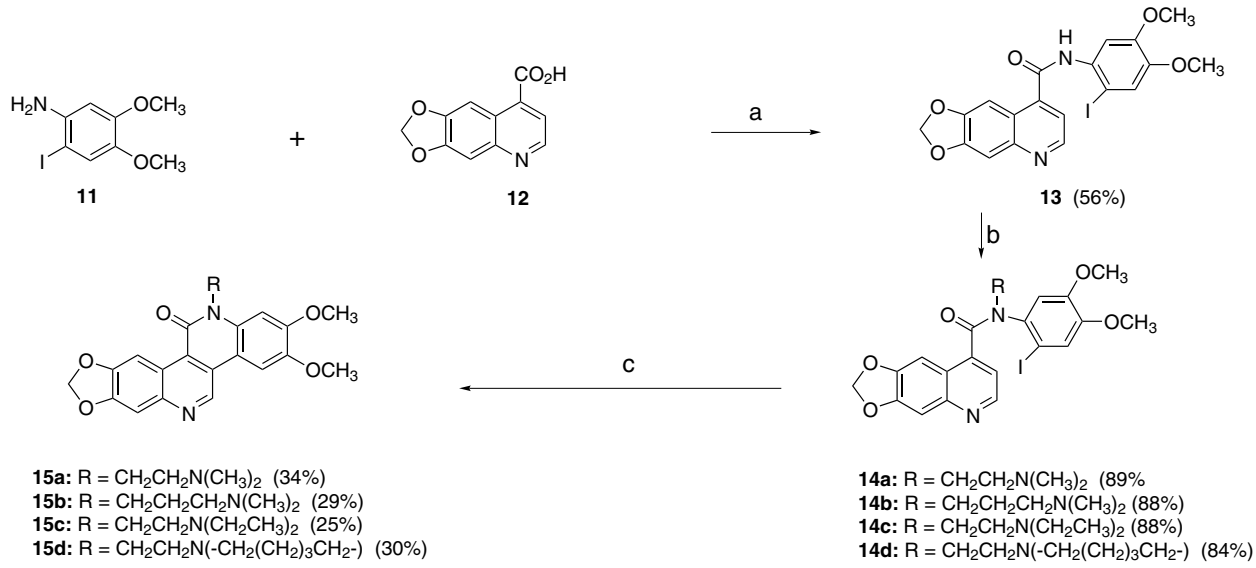
The methods used for the preparation of the ester and amide derivatives of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid are outlined in Scheme 1. Treatment of 3,4-methylenedioxyaniline with methyl vinyl ketone in the presence of $FeCl_3$ in acetic acid provided 4-methyl-6,7-methylenedioxyquinoline.[28]

Compound **3** was prepared in 65% yield by oxidation of this 4-methylquinoline using SeO_2. Refluxing a mixture of **3** and **4**[29] in acetic anhydride and triethylamine provided the stilbene intermediate **5**. The ethyl ester **6** was formed by treatment with thionyl chloride in absolute ethanol. Heck palladium cyclization of either **5** or **6** failed to cleanly provide the desired cyclized products. Photocyclization of **6** in acetonitrile for 30 min did provide the desired cyclized ester **7**. This ester could be readily hydrolyzed with 10% NaOH in ethanol to provide the acid, **8**. Compound **8** was used as a general intermediate for the preparation of both 12-carboxy ester and amide derivatives. Using thionyl chloride, the acid chloride of **8** was prepared. Reaction of this acid chloride with *N*,*N*-dimethylethanolamine and 3-*N*,*N*-dimethylaminopropanol provided the esters **9a** and **9b**, respectively. The amide derivatives **10a** and **10b** were similarly prepared by reaction of the acid chloride of **8** with 2-*N*,*N*-dimethylethylenediamine and 3-*N*,*N*-dimethylaminopropylamine, respectively.

The reversed lactam analogues of **1** were prepared as outlined in Scheme 2. Intermediate **11** was prepared by



Scheme 1. Reagents and conditions: (a) Ac_2O, TEA; (b) $SOCl_2$, EtOH; (c) hυ, MeCN; (d) 10% NaOH in EtOH; (e) $SOCl_2$ then ROH; (f) $SOCl_2$ then RNH_2.



Scheme 2. Reagents and conditions: (a) $SOCl_2$, **12**, then add **11**, CH_2Cl_2, TEA; (b) NaH, DMF, RCl·HCl for **14a**, **14c–d**, and $NaNH_2$, $PhCH_3$, RCl·HCl for **14b**; (c) 2% HCl, hυ.

hydrolysis of its acetamide using aqueous NaOH in ethanol. Intermediate **12** was prepared in 90% yield by treatment of **3** in pyridine with an aqueous solution of $KMnO_4$. Conversion of **12** to the acid chloride was performed using thionyl chloride. Treatment of the acid chloride in CH_2Cl_2 and triethylamine with **11** and allowing this mixture to reflux overnight provided **13** in 56% overall yield. Sodium hydride was added at 0 °C to a solution of **13** and 2-(dimethylamino)ethyl chloride hydrochloride in DMF. The reaction mixture was then allowed to warm to room temperature and stir for 45 min. Compound **14a** was isolated in 89% yield. A similar method was employed for the preparation of **14b–d** in yields of 63%, 88%, and 84%, respectively. An improved yield of **14b** was obtained by treating a solution of **13** and 3-(dimethylamino)propyl chloride hydrochloride with sodium amide in toluene. This reaction mixture was refluxed for 4 h to provide an 88% yield of **14b**. Photolysis of **14a–d** using 2% aqueous HCl through a Vycor filter for 90 min provided yields of the purified cyclized products **15a–d** ranging from 25% to 34%.

The relative TOP1-targeting activity and cytotoxicity of these analogues in varied cell lines are provided in Table 1. The ethyl ester **7** and both alkylamino esters, **9a** and **9b**, were less potent as TOP1-targeting agents and cytotoxic agents. The propyl homologue **9b** was also significantly less cytotoxic than **9a** in RPMI8402, P388, and KB3-1 cells. In light of their diminished cytotoxicity in KBV-1 cells relative to the parent cell line, KB3-1, these data suggest that **7** and **9a** may be marginal substrates for MDR1. In the case of the amide derivative **10a**, comparable TOP1-targeting activity and cytotoxicity relative to **1** was observed in RPMI8402, P388, and KB3-1

Table 1. TOP1-targeting activity and cytotoxicity of 8,9-dimethoxy-2,3-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid derivatives and 6-substituted 8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*][2,6]naphthyridin-5-ones

Compound	TOP1-mediated DNA cleavage[a]	Cytotoxicity IC_{50} values (μM)[b]						
		RPMI8402 wt	CPT-K5 CPT-resist.	P388 wt	P388/CPT45 CPT-resist.	KB3-1 wt	KBV-1 +MDR1	KBH5.0 +BCRP
1	2.5	0.002	0.90	0.001	0.23	0.005	0.005	0.006
7	12	0.085	>10	0.04	3.25	0.041	0.34	0.11
9a	5	0.03	0.73	0.011	0.02	0.034	0.22	0.072
9b	7	0.22	1.5	0.11	0.14	0.50	2.0	0.6
10a	0.5	0.003	1.0	0.002	0.26	0.001	0.10	0.03
10b	30	0.05	2.05	0.03	0.34	0.045	4.5	0.50
15a	3	0.0007	0.21	0.002	0.20	0.002	0.004	0.004
15b	3	0.0005	0.20	0.003	0.020	0.003	0.007	0.004
15c	0.7	0.003	0.90	0.003	0.03	0.003	0.012	0.005
15d	0.1	0.001	1.5	0.003	0.23	0.006	0.006	0.007
CPT	1	0.006	>10	0.014	>10	0.015	0.025	0.026
Topotecan	5	0.021	>10	0.045	>10	0.04	0.44	0.44

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, that is, concentrations relative to camptothecin, 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. Topoisomerase I cleavage assays for determining the REC values were performed as previously described.[18]

[b] The origin of cell lines used in this study and the methods used to assess cytotoxicity have been detailed elsewhere.[19]

cells. The increased cytotoxic activity of **10a** relative to the ester **9a** could also be partly attributed to the anticipated increased stability of **10a** relative to **9a** in cell culture media. The propyl homologue, **10b**, had significantly reduced TOP1-targeting activity and cytotoxicity in these cell lines. It was also evident that both **10a** and **10b**, unlike **1**, were substrates for the efflux transporters MDR1 and BCRP, based upon their significantly reduced toxicity in both KBV-1 and KBH5.0 cells relative to their parent cell line, KB3-1. As anticipated for compounds that principally exert their cytotoxic activity by targeting TOP1, these amide derivatives exhibited decreased cytotoxic activity in CPT-K5 and P388/CPT45 relative to their parent cell lines, RPMI8402 and P388, respectively.

The series of reversed lactams, **15a**–**d**, provided a very different profile of biological activity. Compounds **15a** and **15b** have comparable activity to **1** as TOP1-targeting agents. Compounds **15a** and **15b** were three to four times more cytotoxic in RPMI8402 than **1**, but exhibited similar cytotoxicity in P388 and KB3-1 cells. As was observed with **9a**,**b** and **10a**,**b**, there was no obvious difference in the overall DNA fragmentation pattern obtained in the DNA cleavage assay in the presence of topoisomerase 1 with **15a**–**d** from that observed with camptothecin. In contrast to the ester and amides derivatives, **9a**,**b** and **10a**,**b**, the increase in chain length between **15a** and **15b** did not have an appreciable effect on biological activity. In addition, neither of these reversed lactam analogues of ARC-111 were substrates for MDR1 or BCRP. Compound **15c** is 3 to 4-fold more potent than **1**, **15a**, or **15b** as a TOP1-targeting agent. In terms of intrinsic activity as a TOP1-targeting agent, it is evident that the reversed lactam **15d** is the most potent derivative with 10-fold greater activity than camptothecin. While the difference between the ethyl and propyl homologue was negligible, these data suggest that the presence of the more hydrophobic 2-(*N*,*N*-diethylamino) substituent or 2-(piperidin-1-yl) group in place of the 2-(*N*,*N*-dimethylamino) substituent on the 6-ethyl moiety substantially enhances intrinsic TOP1-targeting activity. Compounds **15c** and **15d** had comparable cytotoxic activity to **1** in RPMI8402, P388, and KB3-1 cells.

These data indicate that esters and amides derived from 8,9-dimethoxy-2,3-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid can possess significant TOP1-targeting activity and cytotoxicity. Unfortunately, all of the 8,9-dimethoxy-2,3-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid derivatives evaluated were substrates for either MDR1 or both MDR1 and BCRP. The reversed lactam analogues of **1** represent a particularly attractive genus of structurally related compounds in that several members within this series of compounds have extraordinary potency as TOP1-targeting agents and possess cytotoxic activity greater than **1** or camptothecin. Several members within this subgroup of non-camptothecin TOP1-targeting agents related to ARC-111 have been shown not to be substrates for efflux transporters associated with multi-drug resistance.

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