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Facile formation of hydrophilic derivatives of 5*H*-8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-2,3-methylenedioxydibenzo[*c*,*h*] [1,6]naphthyridin-6-one (ARC-111) and its 12-aza analog via quaternary ammonium intermediates

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ARC-31

ABSTRACT

Several new TOP1-targeting agents were prepared using as intermediates the *N*,*N*,*N*-trimethyl quaternary ammonium salts of either ARC-111 or its 12-aza analog (ARC-31), **3** and **4**, respectively. Direct displacement of the quaternary ammonium group with water, imidazole, alkylethylenediamines, or polyhydroxylated alkylamines provides a convenient means for furthering the structure–activity relationships associated with these non-camptothecin TOP1-targeting agents.

Topoisomerases are enzymes that control the topology of DNA, which is critical for replication and transcription. The two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2) are distinguished based upon differences in their primary sequence and initial mechanisms, wherein either a single- or double-stranded DNA break is involved.[1,2] Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proven to be effective in the treatment of cancer.[3,4] Such agents in effect convert these enzymes into cellular poisons. Camptothecin (CPT) (Fig. 1) was the first molecule identified as a TOP1-targeting agent.[5] Since this discovery, two clinical agents, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®) have been developed. The improved water-solubility of topotecan and irinotecan relative to CPT was critical to their development into the clinic. Both of these compounds incorporate the camptothecin ring system, which has within its structure a δ-lactone. Hydrolysis of this lactone results in an inactive derivative that can possess high affinity for human serum albumin.[6–8] In addition, both topotecan and irinotecan are substrates for efflux transporters associated with multidrug resistance.[9–12] 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 drug concentration as well as the ability to accumulate within certain tumor cells.

Dibenzo[*c*,*h*][1,6]naphthyridin-6-one derivatives have proven to be a particularly promising family of non-camptothecin TOP1-targeting agents.[13–16] 5*H*-2,3-Dimethoxy-8,9-methylenedioxy-5-[(2-dimethylamino)ethyl]dibenzo[*c*,*h*][1,6]naphthyridin-6-one (**1**, ARC-111) represents one of the more extensively investigated

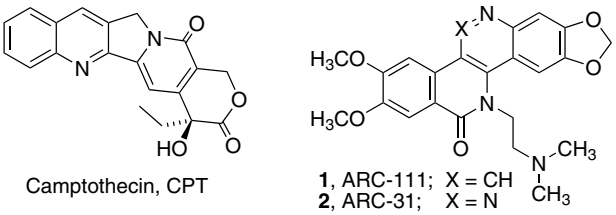

Figure 1. Structure of camptothecin, ARC-111, and ARC-31.

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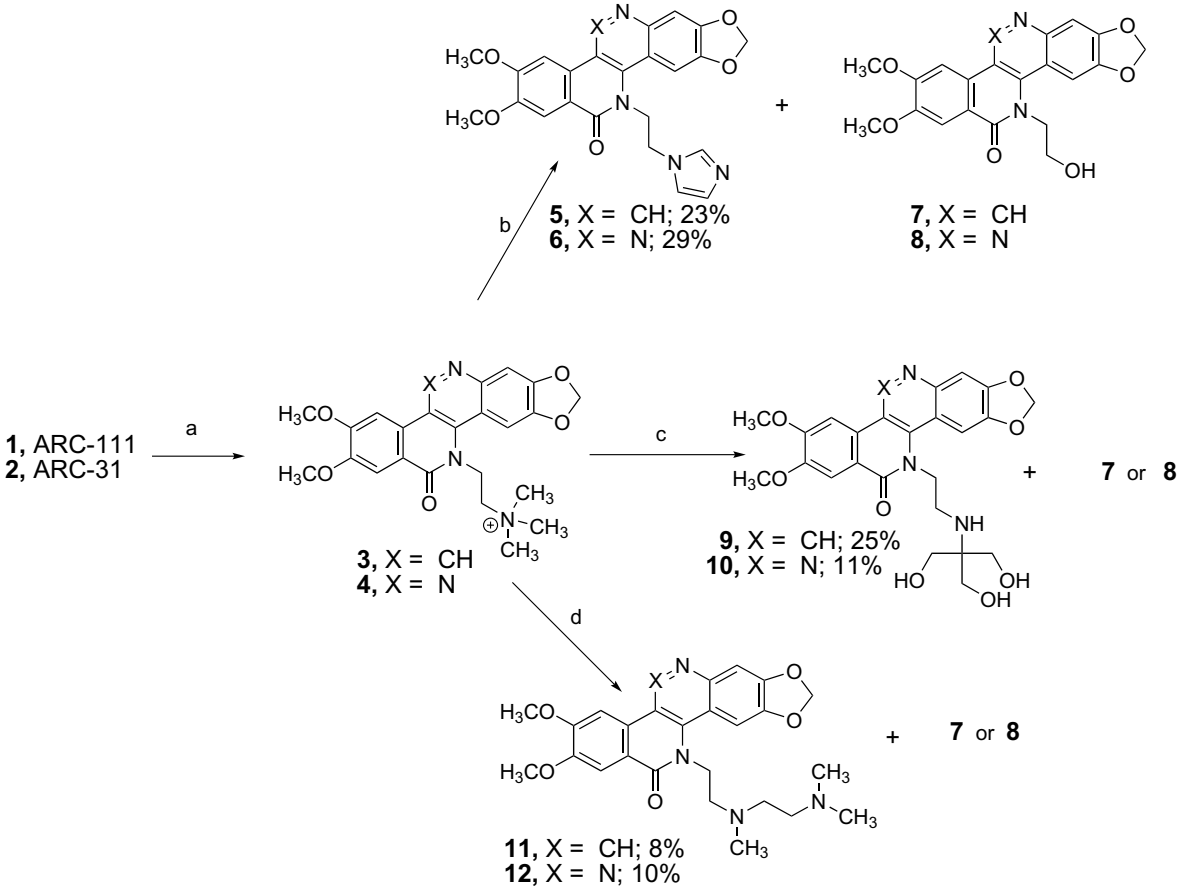
members of this group of compounds.[15] Studies have demonstrated that its mechanism of cell-killing is mediated through TOP1 and in vivo efficacy studies with tumor-bearing athymic nude mice have shown that it is both potent and efficacious when administered either parenterally or orally. Similar results were observed for the 12-aza analogs of ARC-111, 11*H*-Isoquino[4,3-*c*]cinnolin-12-ones.[13,17]

Improved pharmacologic properties have been reported for camptothecin derivatives, which have incorporated within their structure polyhydroxylated alkylamino substituents.[18] Of special note was the favorable biological properties of the 7-trihydroxymethylaminomethyl analog of 10,11-methylenedioxycamptothecin. This analog was associated with remarkable ternary complex stability in the presence of TOP1 and DNA with a half-life an order of magnitude greater than observed previously in studies on various camptothecin analogs.[18] These data prompted our efforts to develop a convenient synthetic approach for preparing derivatives of ARC-111 that would incorporate such functionalities. The synthetic methodology developed for the preparation of **1** or **2** is not readily amenable to the preparation of analogs of ARC-111 with polyhydroxylated alkylamine substituents or aromatic heterocycles at the 2-position of the 5-ethyl substituent. As **1** can be readily prepared in overall yields that exceed 58% from 4-hydroxy-6,7-methylenedioxyquinoline, we investigated the utility of employing the *N,N,N*-trimethylammonium derivatives of **1** and **2** for the preparation of end-products that would be otherwise problematic. Direct displacement of the quaternary ammonium group with water, imidazole, *N,N,N′*-trimethylethylenediamine, or tris(hydroxymethyl)amino-methane was explored as a convenient means for furthering insight into the structure–activity relationships within this series of non-camptothecin TOP1-targeting agents.

The trimethylammonium iodide salt of both ARC-111 (**1**) and ARC-31 (**2**) was readily prepared by addition of methyl iodide to a solution of either **1** or **2** in 20% methanol in methylene chloride (Scheme 1). The excess methyl iodide and solvent were removed under reduced pressure and the resulting trimethylammonium salt, either **3** or **4,** was used without further purification. While not extensively exploited, quaternary ammonium salts have been known to act as leaving groups in substitution reactions.[19] Generally, trimethylammonium salt **3** or **4** was reacted with nucleophile in DMSO to provide the desired product in moderate yield. The reaction was carried out in a sealed tube by heating to 100–150 °C in an oil bath. After allowing the reaction to cool to room temperature, the solvent was removed under reduced pressure and the residue was purified by column chromatography using 1–5% methanol in dichloromethane. In all cases, 2-hydroxyethyl derivative (**7** or **8)** (yields 6–24%) was isolated due to trace amounts of water contained in the commercial reagents. Starting tertiary amine (**1** or **2)** (yields 8–20%) was also obtained as a by-product. The reaction worked smoothly for various nucleophiles, such as imidazole, tris(hydroxymethyl)aminomethane or *N,N,N′*-trimethylethylenediamine. Treatment of the quaternary ammonium salts **3** or **4** with imidazole in anhydrous DMSO provided either **5** or **6**, respectively in yields that ranged from 23% to 29%. Physical and spectral data are provided for these derivatives.[20] In the case of **3**, approximately a 10% yield of the 2-hydroxyethyl derivative **7** was obtained from this reaction. Similar amounts of



Scheme 1. Reagents and conditions: (a) methylene chloride: methanol (4:1), CH_3I, rt, 16 h; (b) imidazole, DMSO, 150 °C, 3 h for **5** and 4 h for **6** ; (c) tris(hydroxymethylaminomethane, DMSO, 150 °C, 2 h for **9** and 4 h for **10**; (d) *N,N,N′*-trimethylethylenediamine, DMSO, 150 °C, 3 h for **11** and 1 h for **12**.

Table 1
TOP1-targeting activity and cytotoxicity of **5**–**12** relative to **1** and **2**

Compound (μM)	TOP1[a]	Cytotoxicity IC_{50}[b]			
		RPMI- 8420	CPT-K5	P388	P388/CPT45
CPT	0.2	0.004	>10	0.004	>10
1	0.3	0.002	0.90	0.001	0.23
2	0.3	0.002	0.74	0.002	0.23
5	2.3	0.21	>10	0.2	>10
6	0.1	0.4	>10	0.19	>10
7	4.7	0.03	>10	0.03	0.9
8	0.3	0.013	1.3	0.012	0.7
9	2.0	0.33	7	0.33	3.5
10	0.2	0.065	5.7	0.04	3.0
11	0.7	0.045	2.2	0.035	0.07
12	0.4	0.033	2.2	0.019	0.3

[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.[14]

[b] The origins of cell lines used in this study and the methods used to assess cytotoxicity have been detailed elsewhere.[15]

8 were obtained when **4** was used as the substrate. Both **3** and **4** were converted to their 2-[tris(hydroxymethyl)methyl-amino]ethyl derivatives **9** and **10** by reacting with tris(hydroxymethyl)aminomethane in yields of 25% and 11%, respectively. Reaction of **3** or **4** with *N,N,N′*-trimethylethylenediamine provided access to the polyamine derivatives **11** and **12** in yields of 8–10%.

The TOP1-targeting activity and cytotoxicity of the hydrophilic derivatives of **1** and **2** synthesized via their quaternary ammonium iodide intermediates are provided in Table 1. While the imidazole derivative **5** was significantly less active as TOP1-targeting agent than **6** and either**1** or **2**, both **5** and **6** were over 100-fold less cytotoxic than **1** and **2**, respectively. The 2-hydroxyethyl derivative **7** was also less active as TOP1-targeting agent than its 12-aza analog **8** based upon the DNA cleavage observed in the presence of the purified enzyme. Both **7** and **8,** however, did have significant cytotoxic activity toward both RPMI8402 and P388 cells with IC_{50} values that ranged from 12 to 30 nM. While the 2-[tris(hydroxymethyl)methylamino]ethyl derivative **9** formed from **3** was much less potent than **1**, its 12-aza derivative **10** did retain comparable TOP1-targeting activity to both camptothecin and ARC-31. Compound **10**, however, was less cytotoxic in RPMI8402 and P388 cells than ARC-31. The polyamine derivatives **11** and **12**, formed from **3** and **4**, respectively, did have comparable TOP1-targeting activity and cytotoxicity to each other. Both of these compounds were less cytotoxic than either **1** or **2** toward RPMI8403 and P388 cells.

CPK-K5 and P388/CPT45 are camptothecin-resistant variant cell lines of RPMI8402 and P388, respectively. The cytotoxicity data observed in these cell lines does suggest that TOP1 is the target associated with the cytotoxicity of compounds evaluated in this study. The relatively weak resistance observed for **11** in P388/CPT45 does suggest that an alternative mechanism may substantially contribute to its cytotoxic activity.

The synthetic methodology used in this study allows for rapid access to several new ARC-111 analogs. Sufficient amounts of these compounds can be prepared to assess their in vivo efficacy in athymic nude mice with human tumor xenografts. Should these data generate further interest in one or more specific compounds, higher yielding methods to specifically synthesize larger quantities of specific agents can be developed in future studies to broaden their pharmacological assessment.

Acknowledgments

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20. *Spectral data are provided for the 2-(1H-imidazol-1-yl)ethyl derivatives* **5** *and* **6***:* 2,3-methylenedioxy-8,9-dimethoxy-5-(*N*-imidazolylaminoethyl)dibenzo-[*c*,*h*]-[1,6]naphthyridin-6-one (**5**), prepared from **3** in 23% yield; mp 275–279 °C(dec); IR (KBr) 1647; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 4.04 (s, 3H), 4.10 (s, 3H), 4.49 (t, 2H, *J* = 6.6), 4.87 (t, 2H, *J* = 6.6), 6.17 (s, 2H), 6.67 (m, 1H), 6.79 (m, 1H), 7.28 (m, 1H), 7.30 (s, 1H), 7.41 (s, 1H), 7.64 (s, 1H), 7.83 (s, 1H), 9.26 (s, 1H); HRMS calcd for $C_{24}H_{20}N_4O_5H$: 445.1512; found 445.1519. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(*1H*-imidazol-1-yl)ethyl]-*11H*-isoquino-[4,3-*c*]cinnolin-12-one (**6**) as prepared from **4** in 29% yield; mp 280–282 °C (dec); IR (neat) 1646; ^{1}H NMR (CD_3COOD): δ 8.69 (s, 1H), 8.20 (s, 1H), 7.72 (s, 1H), 7.60 (s, 1H), 7. 36 (s, 1H), 7.15 (s, 1H), 7.13 (s, 1H), 6.12 (s, 2H), 5.01 (br s, 2H), 4.66 (br s, 2H), 3.89 (s, 3H), 3.81 (s, 3H); HRMS (M^++H) Calcd for $C_{23}H_{19}N_5O_5H$: 446.1464; found: 446.1455.