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Original article

# Novel topoisomerase I-targeting antitumor agents synthesized from the *N*,*N*,*N*-trimethylammonium derivative of ARC-111, 5*H*-2,3-dimethoxy-8,9-methylenedioxy-5-[(2-*N*,*N*,*N*-trimethylammonium)ethyl]dibenzo[*c*,*h*][1,6]-naphthyridin-6-one iodide

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ABSTRACT

Several new TOP1-targeting agents were prepared using as an intermediate the *N*,*N*,*N*-trimethyl quaternary ammonium salt **2** of ARC-111. Direct displacement of the quaternary ammonium group with hydroxide, cyclopropylamine, imidazole, 1*H*-1,2,3-triazole, alkylethylenediamines, ethanolamine, and poly-hydroxylated alkylamines provides a convenient means for furthering insight into the structure–activity relationships within this series of non-camptothecin TOP1-targeting agents. The relative TOP1-targeting activities and cytotoxicities were evaluated in RPMI8402 and P388 cells and their camptothecin-resistant variants. Their potential to serve as substrates for the efflux transporters MDR1 and BCRP, which are associated with multidrug resistance, was also assessed.



## 1. Introduction

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 [1–5]. Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proven to be effective in the treatment of cancer. Such agents in effect convert these enzymes into cellular poisons. Camptothecin (CPT) was the first molecule identified as a TOP1-targeting agent. 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 agents have incorporated within their structure the core structure of camptothecin, which includes a δ-lactone. This lactone moiety is susceptible to hydrolysis and its hydrolysis product has 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, the overexpression of MDR1 (P-glycoprotein) and breast cancer resistance protein (BCRP) have been associated with resistance to these camptothecins [9–15]. In view of these observations, several non-camptothecin TOP1-targeting agents have been investigated for their potential to overcome these obstacles which could limit the effective drug concentration within certain tumor types.

Dibenzo[*c*,*h*][1,6]naphthyridinone derivatives have proved to be a particularly promising family of non-camptothecin TOP1-targeting

*Abbreviations:* CPT, camptothecin; BCRP, breast cancer resistance protein; MDR1, P-glycoprotein; TOP1, topoisomerase I; REC, relative effective concentration.

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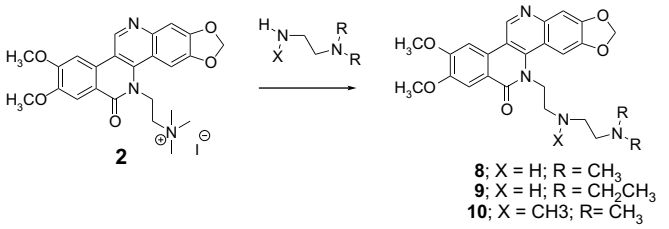
agents [16–18]. 5*H*-2,3-Dimethoxy-8,9-methylenedioxy-5-[2(*N*,*N*-dimethylamino)ethyl]dibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111) represents one of the more extensively investigated members of this group of compounds [19]. Studies have demonstrated that its mechanism of cell-killing is mediated through TOP1. In addition, *in vivo* efficacy studies with tumor-bearing athymic nude mice have demonstrated that it is both potent and efficacious when administered either parenterally or orally. The methodology for the preparation of ARC-111 is not readily amenable to the preparation of certain analogs because of the incompatibility of particular substituents with the reaction conditions that are employed.

Improved pharmacologic properties have been reported for camptothecin derivatives, which have incorporated within their structure polyhydroxylated alkylamino substituents [20]. Of special note was the 7-trihydroxymethylaminomethyl analog of 10,11-methylenecamptothecin. Recent studies on the synthesis and cytotoxicity of polyamine analogs of camptothecin have also demonstrated that these analogs retain TOP1-targeting activity and *in vivo* can inhibit tumor growth [21]. These data prompted our efforts to develop a convenient synthetic approach for preparing derivatives of ARC-111 that would incorporate such functionality. ARC-111 can be readily prepared in overall yields that exceed 58% from 4-hydoxy-6,7-methylenedioxyquinoline. The utility of employing its *N*,*N*,*N*-trimethylammonium derivative as the electrophile was initially reported by our laboratory as a means for the preparation of end-products that would be otherwise problematic [22]. Methods associated with the direct displacement of the quaternary ammonium group with hydroxide, cyclopropylamine, imidazole, 1*H*-1,2,3-triazole, *N*-alkylethylenediamines, ethanolamine, and polyhydroxylated alkylamines are detailed in the present study as a convenient means for furthering insight into the structure–activity relationships within this series of non-camptothecin TOP1-targeting agents.

## 2. Results

### 2.1. Chemistry

The synthesis of the *N*,*N*,*N*-trimethylammonium derivative of **1** (ARC-111) was readily accomplished by addition of methyl iodide to a solution of **1** in 20% methanol in methylene chloride. The trimethylammonium salt **2** was used without further purification. As illustrated in Scheme 1, treatment of this quaternary ammonium salt in anhydrous DMSO with cyclopropylamine, imidazole, or

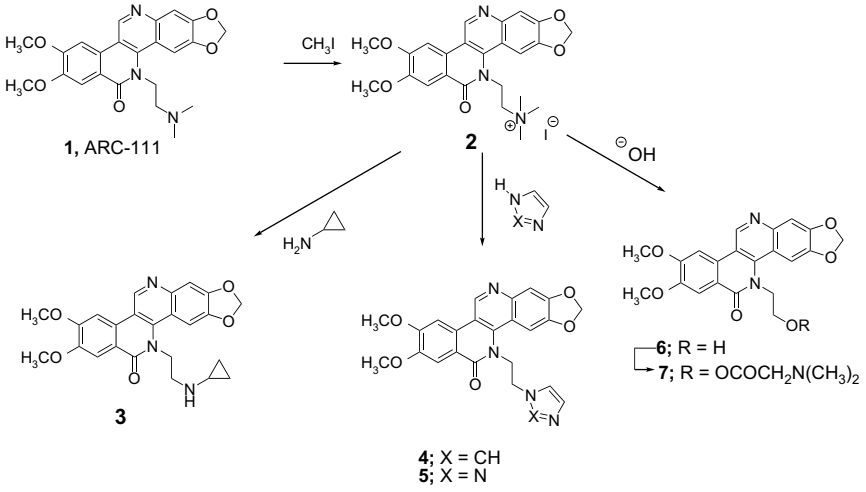



**Scheme 2.** Synthesis of the ethylenediamine derivatives of ARC-111, **8**–**10** from its *N*,*N*,*N*-trimethylammonium iodide derivative, **2**.

1*H*-1,2,3-triazole provided **3**–**5**, respectively in yields that ranged from 19 to 25%. Previous methods for the preparation of the 2-hydroxyethyl derivative, **6**, involved a lengthy consecutive synthetic route and the need for protection and deprotection of the hydroxyl functionality [17]. As a convenient alternative method for generating small quantities of **6**, heating of **2** in DMSO containing approximate 5% water proved to be effective. While **6** had only limited water-solubility, the hydroxyl moiety can serve as a handle for the development of prodrug derivatives with improved solubility. Compound **6** was condensed with *N*,*N*-dimethylglycine to form the more hydrophilic glycinate ester **7** using DCC in the presence of DMAP under similar conditions used for the preparation of 20-glycinate ester of 9-amino-CPT [23].

The preparation of varied 2-[(*N*,*N*-dialkylaminoalkyl)amino] substituents on the ethyl linkage extending from the 5-position of dibenzo[*c*,*h*][1,6]naphthyridin-6-ones is problematic under the current methodology used for the preparation of ARC-111 and related compounds. As illustrated in Scheme 2, treatment of **2** with *N*,*N*-dimethylethylenediamine, *N*,*N*-diethylethylenediamine, and *N*,*N*,*N*′-trimethylethylenediamine did prove to be an effective method for preparing **8**–**10**, respectively. Yields ranged from 25 to 26% using ethylenediamines that retained a primary amine functional group. For the synthesis of **10** *N*,*N*,*N*′-trimethylethylenediamine, a secondary amine, was employed and the yield from this reaction was only 10%. While these yields are not practical for the preparation of large quantities of a select compound, the use of **2** did provide for a convenient method for the preparation and biological assessment of several new analogs related to ARC-111.

We were especially interested in assessing the biological activity of several new analogs of ARC-111 wherein there were hydroxyalkyl groups attached to the amino substituent of a 5-(2-aminoethyl) moiety. Efforts to employ either the tosylate or mesylate of **6** resulted in complex mixtures of products. Conversion of **6** to




**Scheme 1.** Synthesis of compounds **3**–**7** from the *N*,*N*,*N*-trimethylammonium iodide derivative of ARC-111, **2**.

a 2-bromoethyl derivative followed by reaction with the appropriate protected nucleophile also proved problematic. Treatment of **2** with the appropriate ethanolamine derivative as shown in Scheme 3, however, did provide **11**–**14** in modest yield.

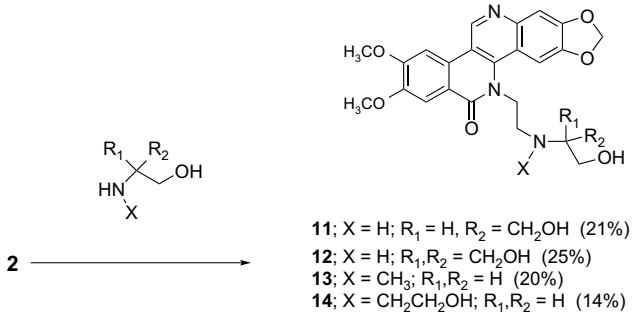
### 2.2. Pharmacology

The TOP1-targeting activities of each of the ARC-111 derivatives that were synthesized are provided in Table 1. The 5-[2-(2-hydroxyethyl)amino]ethyl derivative **13** did exhibit exceptional potency as TOP1-targeting agent with greater intrinsic activity than either CPT or ARC-111. Several compounds, including **3**, **7**, and **11** have similar TOP1-targeting activity to CPT. The relative TOP1-targeting activities of **10** and **14** are somewhat lower than CPT or ARC-111. All of the remaining compounds evaluated are at least an order of magnitude less potent than CPT as TOP1-targeting agents with their order of relative activity being **4**, **12** > **6**, **8**, **9** > **5**.

The cytotoxic activities of **3**–**14** relative to CPT and **1** in RPMI8402 and P388 cell lines are listed in Table 1. Compounds **3** and **13** are among the more cytotoxic analogs, consistent with these analogs being the more potent TOP1-targeting agents. The cytotoxic activity observed for **6**, **7**, **10**, **11**, and **14** ranged from 30 to 54 nM. The one anomaly within this group is the 5-(2-hydroxyethyl) derivative **6**, which despite having relatively poor TOP1-targeting activity, exhibits significant cytotoxicity. Similar results have been observed earlier for this analog [17]. Other compounds with comparatively weak TOP1-targeting activity, such as **4**, **8**, **9**, and **12**, are among the less cytotoxic analogs with $IC_{50}$ values that ranged from 140 to 300 nM. The least active TOP1-targeting agent in this study, the 5-[2-(1*H*-imidazoyl-1-yl)]ethyl derivative **5**, is also the least cytotoxic compound when evaluated in both of these cell lines.

Table 1 also provides cytotoxicity data for CPT-K5 and P388/CPT45 cells, which are the camptothecin-resistant variants of RPMI8402 and P388, respectively. None of the ARC-111 analogs synthesized were as cytotoxic as ARC-111 and CPT in either RPMI8402 or P388 cells. In the CPT-K5 cell line, a mutant form of TOP1 is associated with its resistance to CPT [24]. In the case of P388/CPT45, TOP1 is not expressed [25]. With the exception of **14**, comparative cytotoxicity data between RPMI8402 and CPT-K5 clearly indicate that TOP1-targeting activity is significantly linked to the observed cytotoxic activity of each of the compounds evaluated in this study.

Comparative data between P388 and P388/CPT45 also suggest that for most of the compounds evaluated TOP1-targeting activity is significantly linked to the observed cytotoxic activity. Less than one order of magnitude difference in cytotoxicity activity between this pair of tumor cells was observed for **14**, as well as for **8**–**10**, which possess varied ethylenediamine derivatives attached at the 2-position of the 5-ethyl substituent. These data suggest that mechanisms other than TOP1-targeting activity could contribute to the observed cytotoxic activity of these compounds. Alternatively, it is possible that **8**–**10** may bind to cellular DNA and thereby exert a significant cytostatic effect, which would not be distinguished from cytotoxicity by the MTT assay.




**Scheme 3.** Synthesis of the hydroxyalkylamino derivatives of ARC-111, **11**–**14** from its *N*,*N*,*N*-trimethylammonium iodide derivative, **2**.

**Table 1**
Relative TOP1-targeting activity and cytotoxicity of various 5-substituted derivatives of ARC-111 in human lymphoblastma cells (RPMI8402) and a murine leukemia cell line (P388).

| Compd | TOP1-mediated DNA cleavage[a] | Cytotoxicity $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| | | RPMI8402 | 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 |
| **3** | 0.3 | 0.01 | 0.3 | 0.009 | 0.11 |
| **4** | 2.3 | 0.21 | >10 | 0.20 | >10 |
| **5** | > 10 | 3.5 | >10 | 4.4 | >10 |
| **6**[b] | 4.7 | 0.03 | >10 | 0.03 | 0.9 |
| **7** | 0.3 | 0.03 | 4.6 | 0.027 | 0.39 |
| **8** | 6.4 | 0.14 | 2.6 | 0.075 | 0.34 |
| **9** | 5.2 | 0.15 | 9 | 0.23 | 0.35 |
| **10**[b] | 0.7 | 0.045 | 2.2 | 0.035 | 0.07 |
| **11** | 0.2 | 0.054 | >10 | 0.08 | 2.0 |
| **12**[b] | 2.0 | 0.33 | 7 | 0.33 | 3.5 |
| **13** | 0.04 | 0.015 | 0.58 | 0.012 | 0.33 |
| **14** | 0.48 | 0.04 | 0.32 | 0.03 | 0.25 |

[a] Topoisomerase I cleavage values are reported as REC, Relative Effective Concentration, these are concentrations relative to topotecan, whose value is arbitrarily assumed to be 1, that are able to produce 10% cleavage of the plamid DNA in the presence of human topoisomerase I.
[b] The biological data for these compounds listed in this table have been reported previously [22].

The cytotoxicity data for KB3-1 cells and for the variants KBV-1 and KBH5.0 are listed in Table 2. KBV-1 cells overexpress the efflux transporter MDR1, [26] and KBH5.0 cells overexpress the efflux transporter BCRP [19]. Both irinotecan 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 cells is indicative of substances that are substrates for the efflux transporter MDR1. Similarly, resistance to the cytotoxic effects of a test compound 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. These data suggest that with the exception of **6**, **7**, and **12**, ten of the thirteen new compounds synthesized and evaluated in this study are substrates for MDR1. Fewer compounds, however, were shown to be substrates for BCRP. Comparative data on the cytotoxic activity in KBH5.0 cells relative to KB3-1 cells suggest that **8**, **11** and **14** are substrates for the BCRP efflux transporter.

**Table 2**
Cytotoxic activities of derivatives of ARC-111 in the epidermoid carcinoma cell line KB3-1 and its multidrug-resistant variants, KBV-1 and KBH5.0.

| Compd | Cytotoxicity $IC_{50}$ (μM) | | |
|---|---|---|---|
| | KB3-1 (wt) | KBV-1 ($^+$MDR1) | KBH5.0 ($^+$BCRP) |
| **1** | 0.005 | 0.005 | 0.006 |
| **3** | 0.004 | 0.04 | 0.008 |
| **4** | 0.15 | 1.8 | 0.58 |
| **5** | 0.7 | 10 | 6.0 |
| **6** | 0.027 | 0.04 | 0.04 |
| **7** | 0.032 | 0.035 | 0.05 |
| **8** | 0.06 | 3.4 | 0.75 |
| **9** | 0.04 | 1.2 | 0.3 |
| **10** | 0.05 | 0.6 | 0.21 |
| **11** | 0.026 | 2.0 | 0.75 |
| **12** | 0.25 | 1.8 | 1.8 |
| **13** | 0.006 | 0.06 | 0.05 |
| **14** | 0.023 | 0.35 | 0.28 |

KB3-1 cell line is the parent cell line. KBV-1 is a variant that overexpresses MDR1 (P-glycoprotein) and KBH5.0 is the variant that overexpresses the efflux transporter BCRP.

## 3. Conclusion

The *N*,*N*,*N*-trimethylammonium derivative of ARC-111 **2** proved to be a useful intermediate for the preparation of several structural analogs that are problematic to synthesize using previously developed methodology. A two-carbon linker to an alkylamine moiety had been shown to be associated with potent TOP1-targeting activity and cytotoxicity for analogs of ARC-111. The influence of replacing the dimethylamino substituent on this alkyl side chain with a basic aromatic heterocycle, such as a 1*H*-imidazol-1-yl or 1*H*-1,2,3-triazol-1-yl group, had not been previously assessed. The data obtained for **4** and **5** now indicate these are not favorable structural modifications for enhancement of either TOP1-targeting activity or cytotoxicity. Previous studies have suggested that an *N*-ethylamino or *N*,*N*-diethylamino substituent at the 2-position of the ethyl side chain was associated with significant retention of activity [18]. The results obtained from the cyclopropylamine derivative **3** does confirm these earlier observations that suggested that sterically similar alkylamine derivatives retain potent biological activity. Previous studies revealed that replacement of the dimethylamino group of ARC-111 with a 4-methyl-1-piperazinyl group resulted in a significant loss in activity [17]. By having various ethylenediamines displace the quaternary ammonium group of **2**, a series of compounds were prepared that allowed for a better understanding of how various ethylenediamine derivatives affect biological activity. These ethylenediamine derivatives proved to be more active than the more rigid 4-methylpiperazinyl derivative evaluated previously. In addition, it was discerned that greater activity was observed for the analog **10** wherein both amino substituents were tertiary. As mentioned previously, improved pharmacologic properties have been reported for camptothecin derivatives, which have incorporated within their structure polyhydroxylated alkylamino substituents [20]. In comparing several derivatives prepared by reaction of **2** with various aminoalcohols, only **13** retained potent TOP1-targeting activity and cytotoxicty against the specific cells lines used in this study. It also appears that **13** was less affected by overexpression of MDR1 or BCRP than the other ethanolamine derivatives **11**, **12** or **14**. When the 2-position of the ethyl side chain was substituted as in **6** with only a hydroxyl group or its glycinate ester **7**, overexpression of the efflux transporters did not have an effect on their cytotoxic activity. In summary, compounds **3**, **7** and **13** were identified from these *in vitro* studies as compounds of particular interest. These compounds have solubility properties that allow them to be readily formulated, each exhibits good TOP1-targeting activity and cytotoxicity, and both **3** and **7** are not substrates for the MDR1 and BCRP efflux transporter. Further studies in tumor-bearing mice are needed to assess their *in vivo* efficacy.

The synthetic methodology employed for the preparation of these analogs permits a broad array of varied analogs to be conveniently prepared from a single derivative of ARC-111. Many of these analogs could not be prepared without significant modifications to the synthetic approach typically used in our laboratory for the preparation of 5*H*-2,3-dimethoxy-8,9-methylenedioxy-5-ethyldibenzo[*c*,*h*][1,6]-naphthyridin-6-ones. While this methodology does not represent an optimal synthetic approach to any one of these compounds, it does provide a facile synthetic route that permits one to assess a broad array of analogs and to select those of interest for further evaluation.

## 4. 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. 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 $\delta$ 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 A.C.S. grade or HPLC grade. Methylene chloride was freshly distilled from calcium hydride. All other solvents were used as provided without further purification.

### *4.1. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-(*N,N,N-*trimethylammonium)ethyl]dibenzo[*c,h*][1,6]naphthyridin-6-one iodide (**2**)*

To a solution of **1** (1 mmol) in 100 mL $CH_2Cl_2$–MeOH (4:1) was added methyl iodide (10 mmol) dropwise at room temperature. The resulting suspension was stirred at the same temperature overnight, and then concentrated under reduced pressure. The resulting white powder was pure enough and used in the next step without further purification.

### *4.2. General procedure for the preparation of 2,3-methylenedioxy-8,9-dimethoxy-5-[2-(alkylamino)ethyl]dibenzo[*c,h*][1,6]-naphthyridin-6-one (**3**–**6**, **8**–**14**)*

To a solution of **2** (1.0 mmol equiv) in anhydrous DMSO (8 mL per mmol equiv) was added corresponding nucleophile (10.0 mmol equiv). The reaction mixture was heated to 150 °C in a sealed tube, and then cooled to ambient temperature. The resulting mixture was concentrated by Kugelrohr distillation, dissolved in $CHCl_3$ (20 mL) and then washed with water and brine. The organic layer was dried over $Na_2SO_4$, concentrated and chromtographed in 10:1 $CH_2Cl_2$/MeOH.

#### *4.2.1. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-(*N-*cyclopropylamino)ethyl]dibenzo[*c,h*][1,6]naphthyridin-6-one (**3**)*

Prepared from **2** (100 mg, 0.246 mmol) and cyclopropylamine (140 mg, 2.46 mmol) in 25% yield; reaction time 4 h; mp 237–239 °C; IR (KBr) 1644; $^1H$ NMR ($CDCl_3$) $\delta$ 0.40 (m, 6H), 2.19 (m, 1H), 3.43 (t, 2H, $J = 7.0$), 4.05 (s, 3H), 4.12 (s, 3H), 4.56 (t, 2H, $J = 7.0$), 6.16 (s, 2H), 7.45 (s, 1H), 7.67 (s, 1H), 7.75 (s, 1H), 7.88 (s, 1H), 9.36 (s, 1H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 5.7, 29.4, 47.7, 49.8, 55.4, 55.4, 100.1, 101.0, 101.3, 106.2, 107.8, 110.7, 113.9, 126.7, 140.1, 142.6, 146.4, 146.7, 149.4, 153.3, 163.3; HRMS calcd for $C_{24}H_{23}N_3O_5H$: 434.1716; found 434.1708.

#### *4.2.2. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-(1*H*-imidazo-1-ly)ethyl]dibenzo[*c,h*][1,6]naphthyridin-6-one (**4**)*

Prepared from **2** (100 mg, 0.246 mmol) and imidazole (167 mg, 2.46 mmol) in 23% yield; reaction time 3 h; mp 275–279 °C; IR (KBr) 1647; $^1H$ NMR ($CDCl_3 + CD_3OD$) $\delta$ 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.

#### *4.2.3. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-((1,2,3-triazol-1-yl)amino)ethyl]dibenzo[*c,h*][1,6]naphthyridin-6-one (**5**)*

Prepared from **2** (100 mg, 0.246 mmol) and 1*H*-1, 2, 3-triazole (170 mg, 2.46 mmol) in 19% yield; reaction time 4 h; mp

273–277 °C; IR (KBr) 1651; $^1$H NMR ($CDCl_3 + CD_3OD$) $\delta$ 4.06 (s, 3H), 4.12 (s, 3H), 4.97 (t, 2H, $J = 6.0$), 5.12 (t, 2H, $J = 6.0$), 6.16 (s, 2H), 7.26 (m, 2H), 7.40 (s, 1H), 7.43 (s, 1H), 7.61 (s, 1H), 7.90 (s, 1H), 9.25 (s, 1H); HRMS calcd for $C_{23}H_{19}N_5O_5H$: 446.1464; found 445.1454.

*4.2.4. 2,3-Methylenedioxy-8,9-dimethoxy-5-[(2-hydroxy)ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**6**)*

Prepared from **2** (100 mg, 0.246 mmol) and water (5.0 mL, 278 mmol) in 52% yield; reaction time 3 days. Compound characterization was identical to that reported in the literature [17].

*4.2.5. 2,3-Methylenedioxy-8,9-dimethoxy-5-[[2-[2-(dimethylamino)ethyl]amino]ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**8**)*

Prepared from **2** (100 mg, 0.246 mmol) and *N*,*N*-dimethylethylenediamine (217 mg, 2.46 mmol) in 25% yield; reaction time 4 h; mp 223–226 °C; IR (KBr) 1649; $^1$H NMR ($CDCl_3$) $\delta$ 2.62 (s, 6H), 3.02 (t, 2H, $J = 6.6$), 3.14 (t, 2H, $J = 6.6$), 3.51 (t, 2H, $J = 5.4$), 4.04 (s, 3H), 4.12 (s, 3H), 4.66 (t, 2H, $J = 5.4$), 6.18 (s, 2H), 7.33 (s, 1H), 7.45 (s, 1H), 7.69 (s, 1H), 7.82 (s, 1H), 9.36 (s, 1H); HRMS calcd for $C_{25}H_{28}N_4O_5H$: 465.2138; found 465.2131.

*4.2.6. 2,3-Methylenedioxy-8,9-dimethoxy-5-[[2-[2-(diethylamino)ethyl]amino]ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**9**)*

Prepared from **2** (50 mg, 0.123 mmol) and *N*,*N*-diethylethylenediamine (286 mg, 2.46 mmol) in 26% yield; reaction time 4 h; mp 222–227 °C; IR (KBr) 1655; $^1$H NMR ($CDCl_3$) $\delta$ 1.30 (t, 6H, $J = 7.4$), 3.03 (q, 4H, $J = 7.4$), 3.10 (m, 4H), 3.43 (t, 2H, $J = 5.2$), 4.03 (s, 3H), 4.12 (s, 3H), 4.65 (t, 2H, $J = 5.2$), 6.17 (s, 2H), 7.37 (s, 1H), 7.44 (s, 1H), 7.67 (s, 1H), 7.82 (s, 1H), 9.35 (s, 1H); HRMS calcd for $C_{23}H_{19}N_5O_5H$: 493.2451; found 493.2455.

*4.2.7. 2,3-Methylenedioxy-8,9-dimethoxy-5-[[2-[2-(dimethylamino)ethyl]methylamino]ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**10**)*

Prepared from **2** (50 mg, 0.123 mmol) and *N*,*N*,*N*′-trimethylethylenediamine (251 mg, 2.46 mmol) in 10% yield; reaction time 3 h; mp 217–220 °C; IR (KBr) 1641; $^1$H NMR ($CDCl_3$) $\delta$ 2.31 (s, 3H), 2.79 (s, 6H), 2.86 (t, 2H, $J = 5.8$), 3.07 (t, 2H, $J = 5.8$), 3.12 (t, 2H, $J = 7.2$), 4.02 (s, 3H), 4.11 (s, 3H), 4.62 (t, 2H, $J = 7.2$), 6.17 (s, 2H), 7.42 (s, 1H), 7.60 (s, 1H), 7.67 (s, 1H), 7.79 (s, 1H), 9.33 (s, 1H); HRMS calcd for $C_{26}H_{30}N_4O_5H$: 479.2294; found 479.2285.

*4.2.8. 2,3-Methylenedioxy-8,9-dimethoxy-5-[(2-hydroxy-1-hydroxymethylethyl)aminoethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**11**)*

Prepared from **2** (100 mg, 0.246 mmol) and serinol (224 mg, 2.46 mmol) in 21% yield; reaction time 16 h; mp 237–242 °C; IR (KBr) 1647, 3422; $^1$H NMR ($CDCl_3 + CD_3OD$) $\delta$ 2.64 (m, 1H), 3.29 (t, 2H, $J = 6.6$), 3.49 (m, 4H), 4.04 (s, 3H), 4.12 (s, 3H), 4.47 (t, 2H, $J = 6.6$), 6.06 (s, 2H), 7.30 (s, 1H), 7.48 (s, 1H), 7.61 (s, 1H), 7.71 (s, 1H), 9.24 (s, 1H); HRMS calcd for $C_{23}H_{23}N_3O_6H$: 468.1771; found 468.1761.

*4.2.9. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-[[tris(hydroxymethyl)methyl]amino]ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**12**)*

Prepared from **2** (50 mg, 0.123 mmol) and tris(hydroxymethyl)aminomethane (300 mg, 2.46 mmol) in 25% yield; reaction time 2 h; mp 239–240 °C; IR (KBr) 1637, 3431; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.11 (t, 2H, $J = 5.8$), 3.28 (s, 6H), 3.89 (s, 3H), 4.01 (s, 3H), 4.37 (t, 2H, $J = 5.8$), 6.21 (s, 2H), 7.36 (s, 1H), 7.65 (s, 1H), 7.77 (s, 1H), 7.90 (s, 1H), 9.52 (s, 1H); HRMS calcd for $C_{25}H_{27}N_3O_8H$: 498.1876; found 498.1876.

*4.2.10. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-[N-(2-hydroxyethyl)methylamino]ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**13**)*

Prepared from **2** (100 mg, 0.246 mmol) and 2-methylaminoethanol (185 mg, 2.46 mmol) in 20% yield; reaction time 16 h; mp 263–264 °C; IR (KBr) 1638, 3423; $^1$H NMR ($CDCl_3 + CD_3OD$) $\delta$ 2.24 (s, 3H), 2.56 (t, 2H, $J = 5.4$), 3.11 (t, 2H, $J = 6.6$), 3.54 (t, 2H, $J = 5.4$), 4.05 (s, 3H), 4.12 (s, 3H), 4.65 (t, 2H, $J = 6.6$), 6.17 (s, 2H), 7.47 (s, 1H), 7.68 (s, 1H), 7.85 (s, 1H), 7.88 (s, 1H), 9.38 (s, 1H); HRMS calcd for $C_{25}H_{25}N_3O_6H$: 452.1821; found 452.1819.

*4.2.11. 2,3-Methylenedioxy-8,9-dimethoxy-5-[2-[(bis(2-hydroxyethyl))amino]ethyl]dibenzo [c,h][1,6]naphthyridin-6-one (**14**)*

Prepared from **2** (100 mg, 0.246 mmol) and diethanolamine (224 mg, 2.46 mmol) in 14% yield; reaction time 4 h; mp 216–220 °C; IR (KBr) 1647, 3384; $^1$H NMR ($CDCl_3 + CD_3OD$) $\delta$ 2.62 (t, 4H, $J = 5.2$), 3.01 (t, 2H, $J = 6.2$), 3.46 (t, 4H, $J = 5.2$), 3.98 (s, 3H), 4.07 (s, 3H), 4.58 (t, 2H, $J = 6.2$), 6.12 (s, 2H), 7.36 (s, 1H), 7.63 (s, 1H), 7.65 (s, 1H), 7.77 (s, 1H), 9.27 (s, 1H); HRMS calcd for $C_{25}H_{27}N_3O_7H$: 482.1927; found 482.1916.

*4.3. Glycinate ester of 2,3-methylenedioxy-8,9-dimethoxy-5-[(2-hydroxy)ethyl]dibenzo[c,h][1,6]naphthyridin-6-one (**7**)*

To a solution of **6** (40 mg, 0.10 mmol) in DMF (6 mL) was added DCC (21 mg, 0.10 mmol) and DMAP (5 mg, 0.04 mmol) at room temperature. The resulting mixture was stirred at the same temperature for 30 min and dimethylamino–acetic acid (103 mg, 1 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and diluted with 100 mL dichloromethane, then washed with water and brine. The organic layer was concentrated and chromtographed in 10:1 $CH_2Cl_2$/MeOH. The product was obtained as a white solid in 34% yield: mp 241–245 °C; IR (KBr) 1635, 1751; $^1$H NMR ($CDCl_3 + CD_3OD$) $\delta$ 2.18 (s, 6H), 2.89 (s, 2H), 4.05 (s, 3H), 4.12 (s, 3H), 4.64 (t, 2H, $J = 5.4$), 4.85 (t, 2H, $J = 5.4$), 6.17 (s, 2H), 7.45 (s, 1H), 7.55 (s, 1H), 7.66 (s, 1H), 7.87 (s, 1H), 9.35 (s, 1H); HRMS calcd for $C_{25}H_{25}N_3O_7H$: 480.1771; found 480.1764.

*4.4. Topoisomerase-mediated DNA cleavage assays*

Human topoisomerase I was purified using the baculovirus system as described with slight modification [27]. Human topoisomerase I was expressed in SF9 insect cells and purified from nuclear extracts by hydroxyapatite chromatography as described [28]. Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described [29]. The 3′ endlabeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end filling with Klenow polymerase as previously described [30]. The cleavage assays were performed as previously reported [28,31]. 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 μL 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), which represents 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.

### 4.5. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA). The human lymphoblast RPMI8402 and its camptothecin-resistant variant cell line, CPT-K5 was provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan) [24]. 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, King of Prussia, PA) [25]. The KB3-1 cell line and its multidrug-resistant variant KBV-1 were obtained from K.V. Chin (The Cancer Institute of New Jersey, New Brunswick, NJ) [26]. The KBH5.0 cell line as noted previously 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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