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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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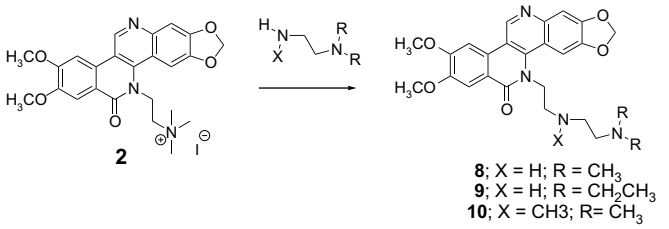
agents [16–18]. 5*H*-2,3-Dimethoxy-8,9-methylenedioxy-5-[2(*N*,*N*-dimethylamino)ethyl]dibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111) represents one of the more extensively investigated members of this group of compounds [19]. Studies have demonstrated that its mechanism of cell-killing is mediated 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

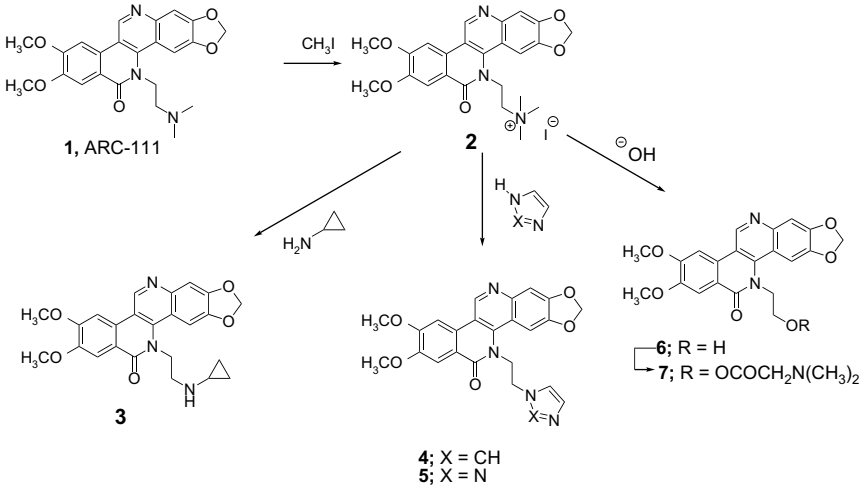


Scheme 2. Synthesis of the ethylenediamine derivatives of ARC-111, **8**–**10** from its *N*,*N*,*N*-trimethylammonium iodide derivative, **2**.

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**.