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Short-step synthesis and structure-activity relationship of cortistatin A analogs

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ABSTRACT

An improved method for synthesizing structurally simplified analogs of cortistatin A (**1**), a novel anti-angiogenic steroidal alkaloid from a marine sponge, was developed. In contrast to previous methods, step- and redox-economical synthesis was achieved using a known α-bromoketone as the starting material. The structure-activity relationship study revealed that the isoquinoline portion was strictly recognized by the target molecule. Surprisingly, the introduction of the acetamide moiety on the A-ring structure dramatically enhanced the selective antiproliferative activity against endothelial cells. This new method can be easily applied to gram-scale synthesis and enabled us to prepare various analogs, which were focused on the participation of the side chain and A-ring structure.

1. Introduction

Marine natural products have garnered considerable attention as a rich and promising source of drug candidates, especially in the field of anticancer drug discovery.[1,2] In most cases, however, the sustainable supply of active compounds has been a challenge for further evaluation and drug development. Generally, only small amounts of bioactive compounds can be isolated from the extracts of marine organisms such as sponges and tunicates. Chemical synthesis of bioactive natural products and their analog compounds can often overcome this drawback. The syntheses of truncated natural products based on structure-activity relationship (SAR) studies would be expected to facilitate the development of more accessible and promising drug leads with optimized activity or chemical stability.[3,4]

In our study of bioactive substances from marine organisms, we have focused on identifying anti-angiogenic substances and isolated cortistatins,[5] a family of novel *abeo*-9(10→19)-androstane-type steroidal alkaloids, from the Indonesian marine sponge *Corticium simplex*. Cortistatin A (**1**, Fig. 1), a major constituent of *C. simplex*, showed a potent and highly selective antiproliferative activity against human umbilical vein endothelial cells (HUVECs). Cortistatin A (**1**) was also revealed to exhibit a potent inhibitory activity against *in vitro* migration and tubular formation of HUVECs induced by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF).[5d] Because of their unique chemical structure and intriguing biological properties, cortistatins are expected to be an emerging novel chemical entity for use as anti-angiogenic drug leads.

However, the extremely scarce supply of natural cortistatins has hampered our validation of the feasibility of their use as potential practical drug leads. There have been a number of reports of the synthesis of cortistatins including six total syntheses [6,7]; however, the yields were low in most cases. Therefore, we engaged in a synthetic study of structurally simplified analog compounds and produced a useful analog, **2** (Fig. 1).[8,9] Analog **2** exhibited a comparable antiproliferative activity to that of cortistatins against HUVECs, good selectivity, and potent *in vivo* antitumor activity following oral administration. To develop a more practical and promising anticancer drug lead based on the core structure of analog **2** as a scaffold, we focused on establishing a more efficient synthetic method than the existing methods. In this report, our second-generation synthetic method for producing cortistatin

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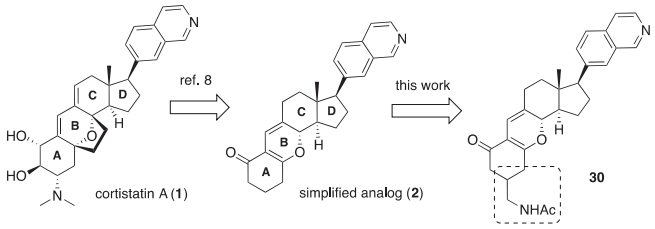


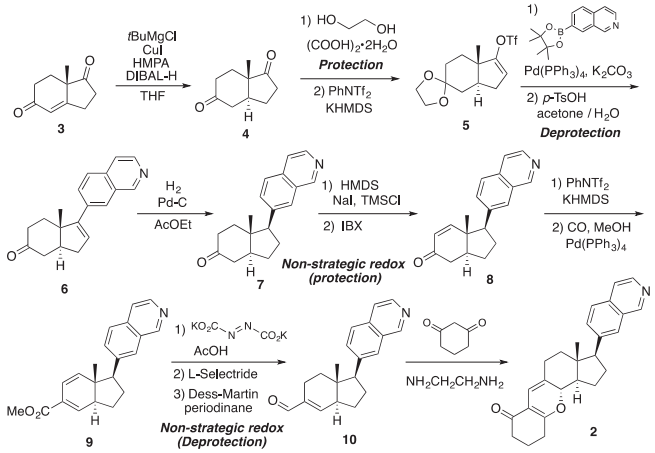
Fig. 1. Chemical structures of cortistatin A (**1**), a simplified analog (**2**), and a potent analog in this work (**30**).

analogs and the generation of a novel and potent analog **30** are presented.

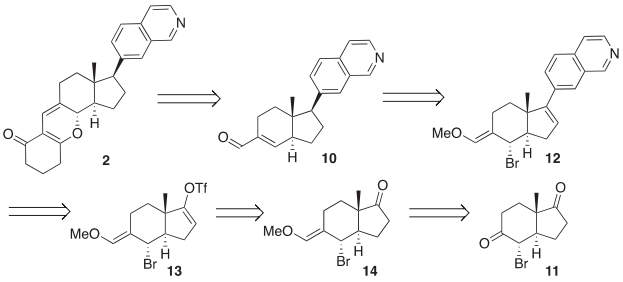
2. Results and discussion

The first-generation synthetic method for producing the cortistatin analog **2** developed by our group is depicted in Scheme 1.[8] We were able to prepare >100 mg of analog **2** using this method without any difficulty to examine its *in vivo* efficacy. However, it was far from being an efficient method[10,11] because it had the following drawbacks: (1) The number of reaction steps and total yield were not satisfactory; in particular, six reaction steps are needed for converting the cyclohexanone **7** to the α,β-unsaturated aldehyde **10** (Scheme 1). (2) The aforementioned problematic steps include iterative redox reactions. (3) The side chain portion of the structure was introduced in an earlier step of the synthesis, and eight reaction steps were needed to prepare each analogous compound for the optimization study of the side chain structure. Therefore, we developed a second-generation synthesis of the cortistatin analog **2** to modify the process for mass production and structural optimization.

The retrosynthetic analysis of the second-generation synthesis is depicted in Scheme 2. The most significant modification was the use of the α-bromoketone **11** as a starting material. Compound **11** can be obtained from Hajos-Parrish ketone (**3**)[12] at a large scale (>100 g) through a *tert*-butyl cuprate (*t*-BuCu)- or SiCu-catalyzed stereoselective conjugate reduction and subsequent bromination using a known method, which was previously used to generate a precursor for the synthesis of vitamin-D_2 and its analogs.[13] The problematic functionalizations encountered in the first-generation synthesis, such as the one-carbon homologation and regioselective introduction of a double bond (from **7** to **10** in Scheme 1), could be resolved through a shortened sequence. Specifically, the one-



Scheme 1. First-generation synthetic method for cortistatin analog (**2**).

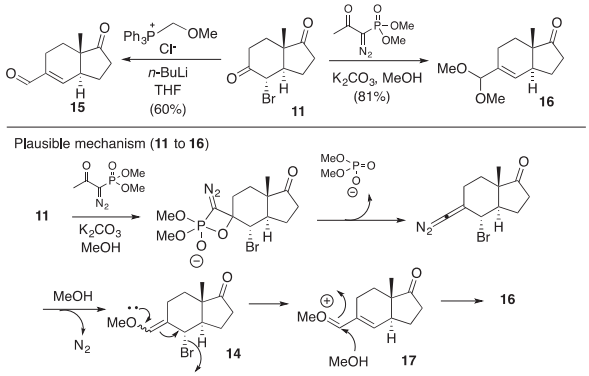


Scheme 2. Retrosynthetic analysis of second-generation synthesis of cortistatin analogs.

carbon homologation through the Wittig reaction giving vinyl ether **14**, and subsequent β-elimination of the bromide (from **12** to **10**) could provide the requisite α,β-unsaturated aldehyde functionality (Scheme 2). The protection/deprotection steps for the carbonyl group were also eliminated, which reduced the overall number of steps.

Initially, a one-carbon homologation reaction of the α-bromoketone **11** was attempted (Scheme 3). A Wittig reaction using (methoxymethyl)triphenylphosphonium chloride provided an α,β-unsaturated aldehyde **15** in place of the desired vinyl ether in moderate yield. In contrast, the use of the Ohira-Bestmann reagent in methanol in the presence of potassium carbonate[14] provided another product in good yield. Spectral analysis revealed that this product was α,β-unsaturated acetal **16**. A plausible reaction mechanism leading to **16** is shown in Scheme 3. The homologation product of the Ohira-Bestmann reaction, methyl vinyl ether **14**, might be formed *in situ*, from which the bromide group would be eliminated as depicted, thereby providing an oxocarbenium ion (**17**). Finally, the nucleophilic addition of the solvent MeOH would yield the α,β-unsaturated acetal **16**. The serendipitous but elegant reaction performed here achieved the requisite transformation around the carbonyl group in a single reaction step.

Next, the introduction of an isoquinoline moiety with the desired stereochemistry was successfully achieved using the Suzuki-Miyaura cross-coupling reaction between isoquinolin-7-yl boronate and the enol triflate **18**, obtained from **16** in good yield, followed by subsequent hydrogenation of the double bond in **19** using the palladium on carbon (Pd-C) catalyst (Scheme 4). To our delight, a complete chemoselectivity between the two double bonds in **19** was fortuitously achieved in this hydrogenation step, providing **20** as the sole product. Following the hydrolysis of the acetal moiety, treatment of the resulting aldehyde **10** with 1,3-cyclohexanedione in the presence of ethylenediamine yielded the desired analog **2**. Thus, the second-generation synthetic method for



Scheme 3. Synthesis of α,β-unsaturated acetal (**16**) and plausible reaction mechanism.