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Synthesis and evaluation of novel dolastatin 10 derivatives for versatile conjugations

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ABSTRACT

Dolastatin 10 (**1**) is a highly potent cytotoxic microtubule inhibitor (cytotoxicity IC_{50} < 5.0 nM) and several of its analogs have recently been used as payloads in antibody drug conjugates. Herein, we describe the design and synthesis of a series of novel dolastatin 10 analogs useful as payloads for conjugated drugs. We explored analogs containing functional groups at the thiazole moiety at the *C*-terminal of dolastatin 10. The functional groups included amines, alcohols, and thiols, which are representative structures used in known conjugated drugs. These novel analogs showed excellent potency in a tumor cell proliferation assay, and thus this series of dolastatin 10 analogs is suitable as versatile payloads in conjugated drugs. Insights into the structure–activity relationships of the analogs are also discussed.

1. Introduction

Dolastatin 10 (**1**) is an anti-microtubule agent that was isolated from the sea hare *Dolabella auricularia* in the Indian Ocean in 1987 (Fig. 1).[1] Dolastatin 10 (**1**) was reported to exhibit exceptionally potent anti-proliferative activity against various cancer cell lines and is considered to be a promising anti-cancer drug.[2] However, neither dolastatin 10 (**1**) nor any of its analogs have been approved as anti-cancer agents to date due to their strong side-effects.[3–6] Recently, however, these potent cytotoxic compounds have attracted attention as payloads for conjugated drugs.[7] Monomethyl auristatin E (MMAE) (**2**) is a representative example of a payload and is used in an approved antibody drug conjugate (ADC), brentuximab vedotin.[8] In this ADC, MMAE (**2**) is attached to a monoclonal antibody which delivers the cytotoxic agent to cancer cells expressing a specific antigen, thereby reducing the side-effects of the cytotoxic agent. The success of ADC suggests that conjugating dolastatin 10 analogs to targeting units is a promising strategy for utilizing their potential.

Conjugated drugs usually consist of a payload, a linker, and a targeting unit. The combinations of these components are critically important for the efficacy of the resulting conjugated drug.[7,9] Among the components, the linker unit must be optimized to meet several requirements, such as stability in plasma, the ability to release the payload at the target site, and appropriate physicochemical properties. Various linker technologies have been developed and the structure of the linker is critically influenced by the functional group(s) in the payload. Several dolastatin 10 derivatives have been designed for conjugated drugs, including MMAE (**2**), monomethyl auristatin F (MMAF) (**3**),[10] and PF-0638101 (**4**).[11] Typically, the primary or secondary aliphatic amine on the *N*-terminal is used for conjugation with a linker, and the hydroxyl group of MMAE (**2**) has also been used for linkage.[12] Aliphatic amines and alcohols are representative functional groups used for generating conjugated drugs, and other functional groups such as thiols, anilines, and phenols can be also used to link payloads to other components.[13–17] However, there are no reports investigating the installation of these groups on the dolastatin 10 structure, hampering the utility of dolastatin 10 analogs in conjugated drugs. To expand the scope of the conjugation chemistry of dolastatin 10 derivatives, we explored new analogs with various functional groups (thiols, aromatic amines, and phenols) in addition to reported aliphatic amines and alcohols. Herein, we report novel derivatives of dolastatin 10 that can be used for versatile conjugations.

2. Compound design

Dolastatin 10 (**1**) is composed of the five amino acids dolavaline (Dov), valine (Val), dolaisoleuine (Dil), dolaproine (Dap), and dolaphenine (Doe). Previous structure-activity relationship (SAR) studies suggested that the *N*-terminal Dov moiety and *C*-terminal Doe moiety are relatively tolerant toward chemical modifications.[2] Maderna et al. reported the co-crystal structure of the PF-06380101 (**4**) and (Tc) 2R tubulin constructs.[11] According to the

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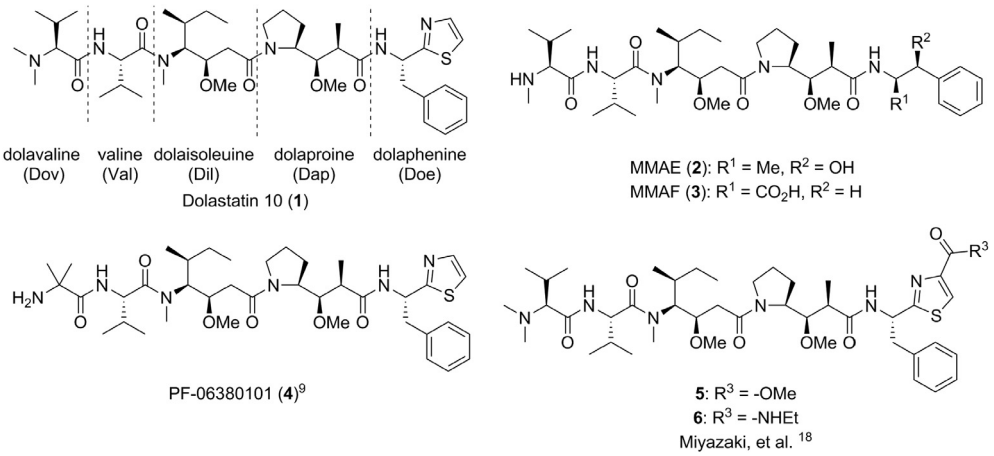

Fig. 1. Dolastatin 10 (**1**) and selected derivatives.

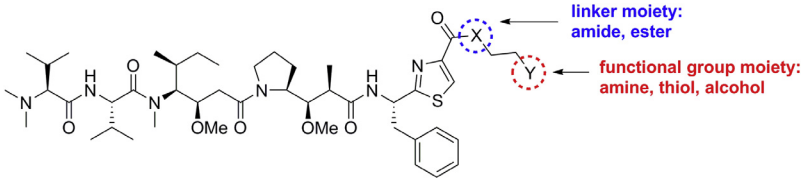

Fig. 2. Plan for the exploration of novel dolastatin 10 derivatives.

reported structure, an amino group at the *N*-terminal forms hydrogen bond networks with two amino acid residues of tubulin. However, there appears to be space for the installation of an additional functional group at the *C*-terminal and thus we considered modification of the *C*-terminal Doe moiety. We examined earlier reports of *C*-terminal modifications, in particular the methyl ester derivative **5** and the ethyl amide derivative **6** reported by Miyazaki et al. in 1993 in which a carbonyl functionality was installed at the 2-position of the thiazole of Doe.[18] Both derivatives **5** and **6** were reported to possess activity equipotent to that of dolastatin 10 (**1**), although no specific description of their cytotoxicity value (IC_{50}) was provided. Given the tolerance of the Doe moiety against chemical modification, we hypothesized that the installation of functional groups at the termini of these functionalities would have little impact on the cytotoxicity of dolastatin 10 (**1**). Thus, we planned to synthesize and evaluate the derivatives shown in Fig. 2. This plan is advantageous from the synthetic point of view in that peptide synthesis is usually performed in the *C*-terminal to *N*-terminal direction. Typically, derivatization of the *C*-terminal residue is less straightforward than that of the *N*-terminal residue, but in the case of the planned derivatives shown in Fig. 2, functional groups at the *C*-terminal can be introduced more easily by preparing the precursor with a carboxylic acid at the Doe moiety, as they are selected in the final step of the synthetic route.

3. Results and discussion

3.1. Synthesis

The synthesis of the new derivatives commenced by preparing carboxylic acid precursor **13** in a convergent manner (Scheme 1). Dov,[19] Dil,[20] and Dap[21] units were synthesized as previously reported. A Doe unit with an ethoxycarbonyl group at the 2-position of the thiazole was prepared using a modification of Hantzsch thiazole synthesis.[22] The left-hand Dov-Val-Dil unit **9** was synthesized by two cycles of Cbz deprotection and condensation of the corresponding amino acid units, followed by deprotection of the *t*-Bu ester. The right-hand Dap-Doe-CO_2Et unit **11** was prepared by coupling of the Dap and Doe units and Boc-deprotection. Next, these two units were coupled using HATU with diisopropyl ethyl amine to provide the ethyl ester derivative **12**, yielding the equivalent of the methyl ester derivative **5**. Then, the ethyl ester of Doe was saponified to give the carboxylic acid precursor **13**.

New derivatives with various functional groups were synthesized from precursor **13** by condensation of the corresponding amines and alcohols, followed by deprotection of the protecting groups to provide functional groups at the terminal when needed. For amide formation, HATU with diisopropyl ethyl amine was used for the condensation of amines and **13**. For ester formation, the procedure reported by Siina et al.[23] gave the desired esters in good yields. Boc, THP, and Trt protecting groups were used for terminal amines, alcohols, and thiols, respectively. These protecting groups were removed by HCl, TsOH, or TFA/Et_4SiH at the final step, followed by typical procedures. Thirteen novel derivatives (**14a**–**g**, **15a**–**d**, and **16a**–**c**) with terminal functional groups were synthesized, and *N*-ethyl amide derivative **6** was also prepared for comparison with the other amide derivatives.

3.2. Biological evaluation

The cytotoxicities of the compounds were evaluated against SKOV-3 (human ovarian cancer), A549 (human adeno carcinoma derived from lung cancer), and L1210 (mouse lymphocytic leukemia) cell lines (Table 1). MMAE (**2**) and the ethyl amide derivative **6** showed excellent potency for each cell line. The ethyl ester derivative **12** which is comparable to the methyl ester derivative **5** had stronger cytotoxicity against SKOV-3 than those of the two compounds. However, the amine derivatives with a primary amide linker (**14a**, **14b**) showed drastically decreased potency against all three cell lines. The alcohol derivative **14c** exhibited one order of magnitude higher IC_{50} values compared to those of the ethyl amide derivative **6**. The thiol derivative **14d** showed equivalent or higher