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Antibody drug conjugates of cleavable amino-alkyl and aryl maytansinoids

Thomas Nittoli [a,*], Marcus P. Kelly [a], Frank Delfino [a], John Rudge [a], Arthur Kunz [a], Thomas Markotan [b], Jan Spink [b], Zhaoyuan Chen [a], Jing Shan [a], Elizabeth Navarro [a], Michele Tait [a], Kathleen Provoncha [a], Jason Giurleo [a], Feng Zhao [a], Xiaobo Jiang [a], Donna Hylton [a], Sosina Makonnen [a], Carlos Hickey [a], Jessica R. Kirshner [a], Gavin Thurston [a], Nicholas Papadopoulos [a]

[a] Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591, United States
[b] Abzena, 360 George Patterson Blvd, Bristol, PA 19007, United States

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ABSTRACT

Natural products have been used for many medicinal purposes for centuries. Antibody drug conjugates (ADCs) have utilized this rich source of small molecule therapeutics to produce several clinically useful treatments. ADCs based on the natural product maytansine have been successful clinically. The authors further the utility of the anti-cancer natural product maytansine by developing efficacious payloads and linker-payloads for conjugating to antibodies. The success of our approach was realized in the EGFRvIII targeting ADC EGFRvIII-16. The ADC was able to regress tumors in 2 tumor models (U251/EGFRvIII and MMT/EGFRvIII). When compared to a positive control ADC, the efficacy observed was similar or improved while the isotype control ADCs had no effect.

1. Introduction

Maytansine (**1**), an ansa macrolide, was first reported by Kupchan in 1972 and isolated from the African shrub, *Maytenus ovatus* (Fig. 1).[1] Maytansine garnered much interest at the time because of its ability to potently inhibit microtubule growth. The best known anti-cancer microtubule inhibitors at the time, the vinca alkaloids, were 100–1000 times less potent.[2] However, the vinca alkaloids (vincristine, vinblastine, etc.) proved to be more efficacious than maytansine when employed as anti-cancer drugs in the clinic. Maytansine did not progress beyond phase II clinical trials due to dose-limiting gastrointestinal and neurological toxicity, while having low efficacy.[3]

In the early 1990's, in an effort to obviate observed clinical toxicity, maytansine derivatives were employed as payloads for antibody drug conjugates (ADCs).[4] In the ADC format, these potent maytansinoids could be targeted to specific (diseased) cell types, thereby expanding their therapeutic window. Since that time, many other maytansinoids have been developed as antibody payloads.[5–10] Of the 4 FDA approved ADCs (Kadcyla®, Adcetris®, Besponsa®, and Mylotarg®), Kadcyla® employs the maytansinoid developed in the early 1990's for treating HER2+ metastatic breast cancer, the only ADC therapy for a solid tumor target.

In this report, we expand the potential utility of the anti-cancer natural product maytansine. Our goal was to develop maytansinoid payloads, and associated linkers for antibody attachment, that might yield an expanded therapeutic window. We initially focused on two approaches to improve efficacy relative to toxicity: the first by curbing cell penetration through the use of charged and hydrophilic payloads and the second utilizing hydrophobic payloads[11] to increase effects through cell penetration ("bystander effect"[12]). After probing the two different payload classes with the enzymatically cleavable linker assemblies[13,14] deployed in the commercial ADC Adcetris, the more promising of the linker payloads was used in an ADC that demonstrated outstanding efficacy against EGFRvIII expressing tumor xenografts.

2. Results and discussion

2.1. Synthesis of payloads and Linker-Payloads

Keeping the core macrocycle the same, we investigated the bioactivity of substitution at the *N*-methyl alanine nitrogen. To probe the effects of varying the length of the side chain off the macrocycle, as well as differences between linker attachment

* Corresponding author.
E-mail address: thomas.nittoli@regeneron.com (T. Nittoli).

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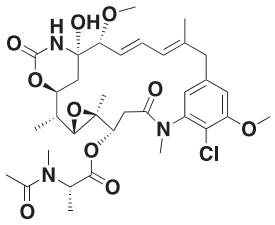

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Fig. 1. Structure of maytansine.

through a primary versus secondary amine, compounds **9**–**12** were synthesized as shown in Scheme 1. These amino-alkyl maytansinoids were synthesized from des-acetyl-maytansine[15] and the corresponding Boc-protected *N*-hydroxysuccinimide activated esters using method A. Compounds **13** and **14** were synthesized to provide more hydrophobic, cell permeable payloads. Maytansinoids **13** and **14** were synthesized from des-acetyl-maytansine (**8**) and the corresponding Boc-protected carboxylic acids using HATU followed by a TFA deprotection (method B).

Of the payloads discussed above, three (**8**, **9** and **14**) were selected, based on activity in *in vitro* cytotoxicity assays (*vide infra*), for attachment to linkers suitable for antibody conjugation. The synthetic routes to these linker-payload combinations are described below.

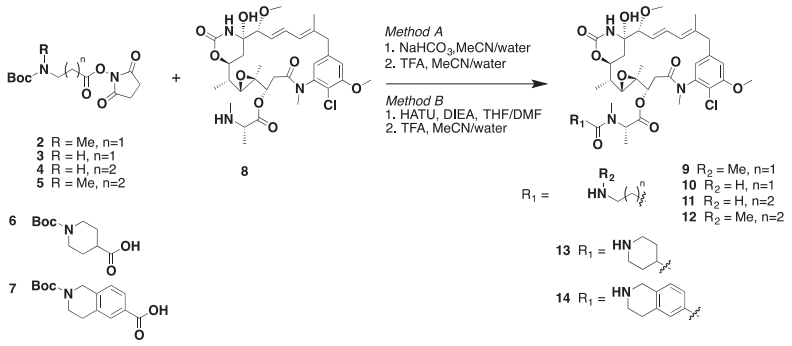
The linker-payloads **15** and **16** were synthesized in one step from des-acetyl-maytansines **8** and **9** using commercially available mc-valine-citrulline-PAB-PNP, basic alumina or $NaHCO_3$ (Scheme 2).

The carbamate **22** was synthesized from the Boc-protected valine-citrulline-PNP-carbonate, **17**, in 4 steps as shown in Scheme 3. Coupling of **17** with carboxylic acid **18**, followed by deprotection and another coupling to maleimide caproic *N*-hydroxysuccinimide active ester **20** furnished **21**. Coupling of the acid **21** with maytansinoid **8** then provided the linker-payload **22**.

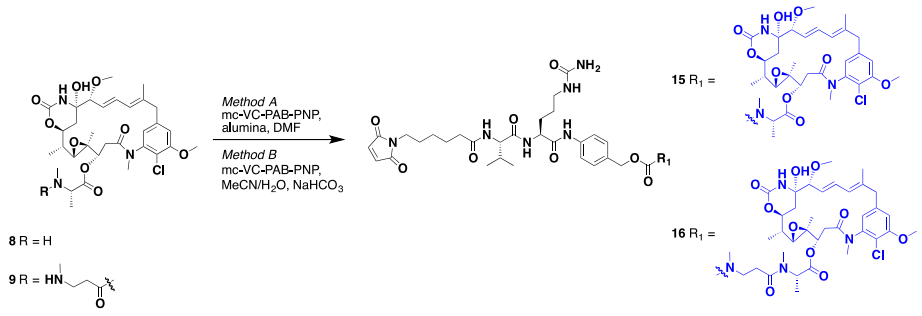
The carbamate **25** was synthesized from valine-citrulline-PABA, **23**, in 5 steps (Scheme 4). A coupling with Boc-protected PEG6 carboxylic acid, PNP-carbonate formation, and another coupling to maytansinoid **14** furnished compound **24**. Deprotection of **24** followed by coupling of the resulting amine with 3-maleimidyl-propionic acid provided the linker-payload **25**.

2.2. In vitro cytotoxicity – payloads

The synthesized payloads, **9**–**14**, were assayed for cytotoxic potency against six different cell lines (Table 1). As expected, the more hydrophilic and charged the maytansinoid payloads (**9**–**12**) were less potent likely due to decreased cell permeability. In support of this conclusion, compound **9** was able to inhibit tubulin formation when assayed in a cell-free system (see Fig. S1 of the supplemental material). This observation has been reported by others for dolastatin derived payloads.[16–18] However, as the hydrophobicity increased (compounds **8**, **13** and **14**), there was a trend towards increasing compound potency (those more likely to possess a "bystander effect"). This trend is evident in the MMT, U251/EGFRvIII, and C4-2 cell lines shown in Table 1 and Fig. 2A–C. These arguments do not take into account the actual binding of each payload to tubulin, which also governs their potency. All of these compounds are assumed to be charged at the physiological pH of the assay (their pKa ~9), and that would further hinder their ability to permeate the cell membrane. Compounds **8** and **14** were similar in potency to maytansine (see Fig. S2 of the supplemental material).



Scheme 1. Synthesis of amino-alkyl maytansinoid payloads **9**–**14**.



Scheme 2. Synthesis of linker-payload **15** and **16**.