Bioorganic & Medicinal Chemistry Letters 27 (2017) 5267–5271

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Macrocyclic pyrrolobenzodiazepine dimers as antibody-drug conjugate payloads

Andrew F. Donnell [a,*], Yong Zhang [a], Erik M. Stang [a], Donna D. Wei [a], Andrew J. Tebben [a], Heidi L. Perez [a], Gretchen M. Schroeder [a], Chin Pan [b], Chetana Rao [b], Robert M. Borzilleri [a], Gregory D. Vite [a], Sanjeev Gangwar [b]

[a] Bristol-Myers Squibb Research & Development, Princeton, NJ 08543, USA
[b] Bristol-Myers Squibb Research & Development, Redwood City, CA 94063, USA

ARTICLE INFO

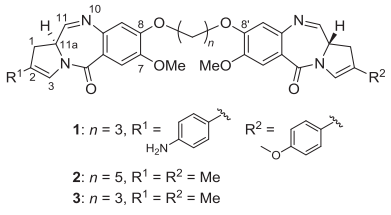
Article history:
Received 28 August 2017
Revised 12 October 2017
Accepted 14 October 2017
Available online 16 October 2017

Keywords:
Antibody-drug conjugates
Pyrrolobenzodiazepines
Macrocycles

ABSTRACT

Macrocyclic pyrrolobenzodiazepine dimers were designed and evaluated for use as antibody-drug conjugate payloads. Initial structure–activity exploration established that macrocyclization could increase the potency of PBD dimers compared with non-macrocyclic analogs. Further optimization overcame activity-limiting solubility issues, leading to compounds with highly potent (picomolar) activity against several cancer cell lines. High levels of in vitro potency and specificity were demonstrated with an anti-mesothelin conjugate.

Cytotoxic drugs have been widely employed in cancer chemotherapy, but, in many cases, their therapeutic index is limited by adverse effects stemming from poor selectivity for cancer cells. Antibody-drug conjugates (ADCs) have the potential to mitigate these side-effects by harnessing the high specificity of antibodies to deliver small-molecule drugs directly to tumor cells.[1,2] Pyrrolobenzodiazepines (PBDs) are a family of sequence-selective DNA minor groove binding natural products whose potent cytotoxic properties make them attractive for use as ADC payloads.[3–5] PBD dimers such as **1** and **2**, formed by joining two PBD monomers via their C8-phenol groups with either a three-carbon or five-carbon spacer, inhibit a variety of cancer cell lines with subnanomolar IC_{50}s. Their high levels of activity have been attributed to an ability to promote intra- and interstrand DNA cross-linking by the formation of covalent aminal linkages between their reactive N10-C11 imine groups and the C2-NH_2 groups of guanine bases.[6] Interest in this class of compounds as ADC payloads is exemplified by vadastuximab talirine, an anti-CD33 conjugate of **1**, which has been advanced into clinic trials by Spirogen and Seattle Genetics.[7,8] Recently, an ADC based on **2** has also been described.[9] Both of these compounds employ a lysosomally-cleavable valine-alanine linker[10,11] for attachment of the payload to the antibody. In the case of **1**, the linker is attached directly to the aniline group. For **2**, the linker is attached to the N10-position of the hemiaminal form of **2** via a self-immolative *p*-aminobenzyl carbamate spacer.



Our interest in these compounds was based on the examination of a model of PBD dimer **3** bound to DNA (Fig. 1). The C11a-stereocenter imparts a conformation that is isohelical with the DNA minor groove, where the compound binds in a mode that enables the imine moieties to covalently bind the NH_2 groups of guanine bases on opposing strands. The C2-methyl groups project further along the groove, which is known to tolerate a variety of substituents, the nature of which can have a dramatic effect on the activity of the compounds.[12] This places the C7/C7′-methoxy groups in an exposed orientation, and we envisioned joining them with a linker to form macrocycles, potentially exploiting conformational restriction to improve potency. Modeling of a macrocyclic analog of **3** with a nine-carbon alkyl chain linking the phenols sug-

* Corresponding author.
E-mail address: andrew.donnell@bms.com (A.F. Donnell).

https://doi.org/10.1016/j.bmcl.2017.10.028
0960-894X/

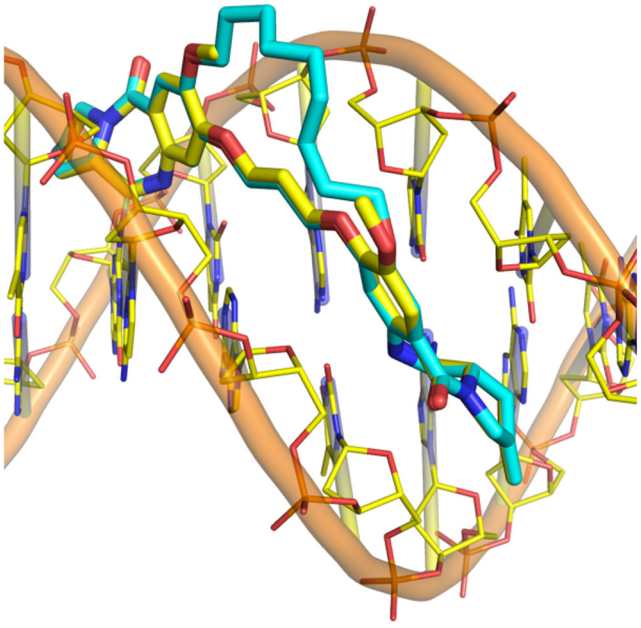


Fig. 1. Model of PBD dimers bound to DNA. PBD dimer **3** is displayed in yellow and a macrocycle formed from **3** using a nine-carbon linker is displayed in cyan. Modeling was carried out using Maestro (Schrödinger, LLC, New York, NY) and the image was generated using PyMOL Molecular Graphics System (Schrödinger, LLC, New York, NY).

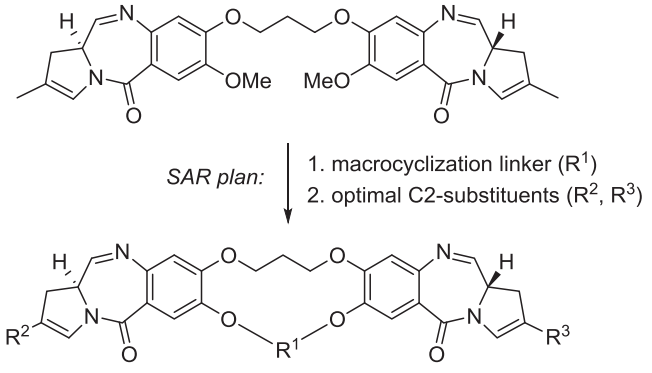


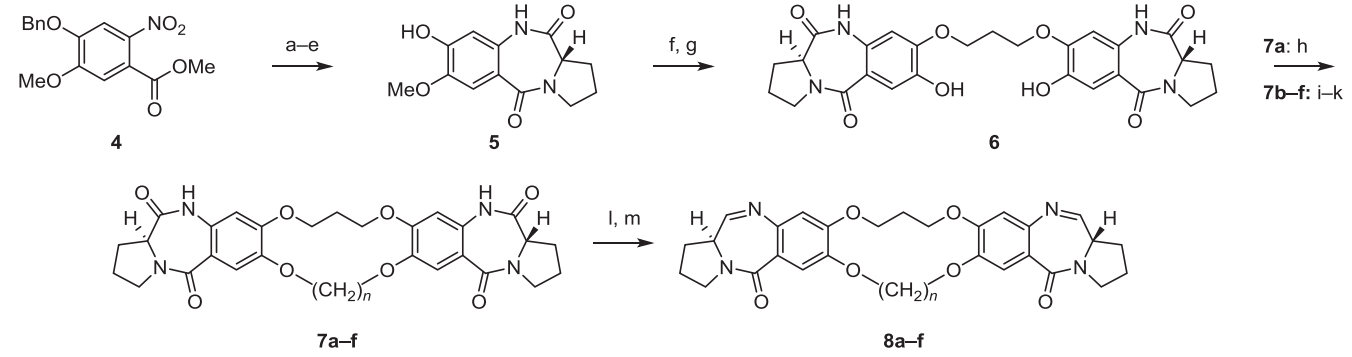
Fig. 2. Areas for SAR exploration.

gested that linkers of this length could be tolerated without distorting the helical binding geometry, although the ideal chain length was not apparent. Thus, as illustrated in Fig. 2, our goals for SAR exploration were, first, to identify an appropriately-sized linker for macrocyclization, and, second, to match the macrocyclic PBD scaffold with suitable C2-substituents.

As an initial test of the tolerability of macrocyclization, we prepared a series of macrocyclic PBD analogs with alkyl linkers ranging in length from 7 to 12 carbons, giving 18- to 23-membered rings. While unsaturation and substitution at the C2-position of the PBD ring system are known to improve activity, we focused our early explorations on the more synthetically-tractable unsubstituted scaffold to establish the feasibility of macrocyclization as well as gain insight into the optimal ring size. We began by constructing the known PBD monomer **5** by adapting a reported five-step sequence starting from methyl 4-(benzyloxy)-5-methoxy-2-nitrobenzoate (**4**) (Scheme 1).[13] Dimerization was then effected by alkylation with 1,3-dibromopropane, and cleavage of the methyl ether groups revealed diphenol **6**.

We explored several routes for constructing macrocycles from **6**. Alkylation of the phenol groups with an appropriately-sized ω-bromo olefin set the stage for ring-closing metathesis, which proceeded in good yield but with by-products arising from olefin isomerization (this is illustrated in Scheme 2 for the compounds derived from 5-bromopent-1-ene). That proved useful at this stage, because we were able to carry the mixtures forward and isolate both the targeted species and its des-methylene congener in sufficient quantities for initial biological evaluation. Compounds **8b–f** were made by this approach (see the Supplementary Data for the full experimental details). However, in many cases, isomer separation was challenging, and we felt that a more selective protocol would be needed to efficiently access larger quantities of material for analog synthesis. Some improvements in the RCM reaction were realized by carrying out the reaction in the presence of reported isomerization suppressors, such as 2,6-dichlorobenzoquinone,[14] although we did not fully optimize this approach because alternate methods for constructing the macrocyclic ring showed promise. For example, ring-closing alkyne metathesis was effective, but synthesis of the requisite internal alkyne precursors added to the overall complexity of the sequence. For many of the analogs, we relied on alkylation of **6** with a dihalo alkane, such as 1,7-dibromoheptane. This method was used to prepare compound **8a** and subsequent analogs. To complete the synthesis, the amide groups were converted to imines by a sequence involving *N*-alkylation with SEM-Cl, hydride reduction, and dehydration of the resultant hemiaminals by stirring with silica gel.[15]

To introduce C2-substitution and unsaturation, we used an alternate synthetic route where we first constructed the macrocyclic skeleton and then assembled the PBD ring system. This chemistry is illustrated in Scheme 3. Methyl 4-hydroxy-3-



Scheme 1. Reagents and conditions: (a) 2.5 M aq. NaOH, THF, 50 °C, quant.; (b) $(COCl)_2$, DMF, THF, rt; (c) L-proline methyl ester hydrochloride, Et_3N, THF, 0 °C to rt, 72% (2 steps); (d) H_2 (50 psi), $Pd(OH)_2$, EtOH, rt; (e) AcOH, MeOH, 80 °C, 71% (2 steps); (f) 1,3-dibromopropane, K_2CO_3, DMSO, rt, 78%; (g) BBr_3, CH_2Cl_2, −78 °C to −5 °C, 33%; (h) 1,7-dibromoheptane, K_2CO_3, DMF, 50 °C; (i) for **7b**: 5-bromopent-1-ene; for **7c** and **7d**: 6-bromohex-1-ene; for **7e** and **7f**: 7-bromohept-1-ene, K_2CO_3, DMF, rt, 33–77%; (j) Grubbs-II, DCE, 75 °C; (k) H_2, 10% Pd/C, MeOH, 64–88% of a mixture of two species (2 steps); (l) NaH, DMF, 0 °C; SEMCl; (m) $LiBH_4$, 1:1 THF–EtOH, 0 °C to rt; silica gel, 1:1 $CHCl_3$–EtOH, 4–37% (2 steps).