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Immunoproteasome inhibition and bioactivity of thiasyrbactins

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ABSTRACT

A family of macrodilactam natural products, the syrbactins, are known proteasome inhibitors. A small group of syrbactin analogs was prepared with a sulfur-for-carbon substitution to enhance synthetic accessibility and facilitate modulation of their solubility. Two of these compounds surprisingly proved to be inhibitors of the trypsin-like catalytic site, including of the immunoproteasome. Their bound and free conformations suggest special properties of the thiasyrbactin ring are responsible for this unusual preference, which may be exploited to develop drug-like immunoproteasome inhibitors. These compounds show greater selectivity than earlier compounds used to infer phenotypes of immunoproteasome inhibition, like ONX-0914.

1. Introduction

The syrbactins are a family of bacterial, macrocyclic, non-ribosomal peptide natural products active against the mammalian proteasome.[1] Their α,β-unsaturated lactam functionality reacts covalently with the catalytic Thr1 residue of proteasome catalytic subunits. Given the clinical success of the proteasome inhibitors bortezomib[2] (BTZ), ixazomib, and carfilzomib[3] against cancers such as multiple myeloma and mantle cell lymphoma,[4] intense investigations into the syrbactins followed their initial discovery.[5,6] Unlike some other proteasome inhibitors, even some used clinically, the syrbactin syringolin A (SylA) is quite selective for proteasome β subunits over other protein targets, as demonstrated by affinity-based protein profiling.[7–9] Several syntheses of the natural syrbactins themselves have been reported, and significant work to prepare analogs has been pursued.[10–13] We recently reported the compound TIR-199 (Chart 1),[14] the first syrbactin to demonstrate activity against tumor cell lines in animal studies. It is most potent against the chymotrypsin-like activity of the β5 constitutive proteasome subunit, which can be ascribed to structural features derived from other syrbactins including the natural product glidobactin A, which was discovered through its intrinsic activity against tumor cell lines.[15] Its key structural features include the macrolactam methyl group and the long unbranched, unfunctionalized side chain. A straight-chain alkyl urea side chain similar to that of glidobactin A was originally applied to syrbactin analogs by Kaiser.[11] Maintaining this feature in the analogs from which TIR-199 emerged enabled SAR for syrbactin macrolactam cores to be readily discerned. Pre-clinical profiling assays of TIR-199 showed it has low aqueous solubility without co-solvents, hampering studies of bioavailability and cellular transport. Approaches to enhance the solubility of syrbactins were therefore of high priority. Both this goal and the desire for synthetic simplification from the 10-step synthesis of TIR-199 drove the study described herein.

The design of the current family of syrbactins referred to informally as NAMs was based on our concise syringolin B analog synthesis[13] that can be applied to any lysine analog. Here it exploits the commercial compound β-aminoethylcysteine, or thialysine. Its sulfur was envisioned to provide a synthetic handle to enable late-stage strategies to enhance solubility. Its TIR-199-based macrodilactam can be accessed synthetically in only a few steps. The specific compounds prepared in this study are shown in Chart 2. Compounds **1** and **2** are inspired by TIR-199, including most of its structure but replacing the isolated alkene. The sulfoxide in **2** is at the same position as the alcohol of glidobactin A and could replicate its stereochemistry. Compounds **3** and **4** are inspired by bortezomib, in that it is a dipeptide with an elec-

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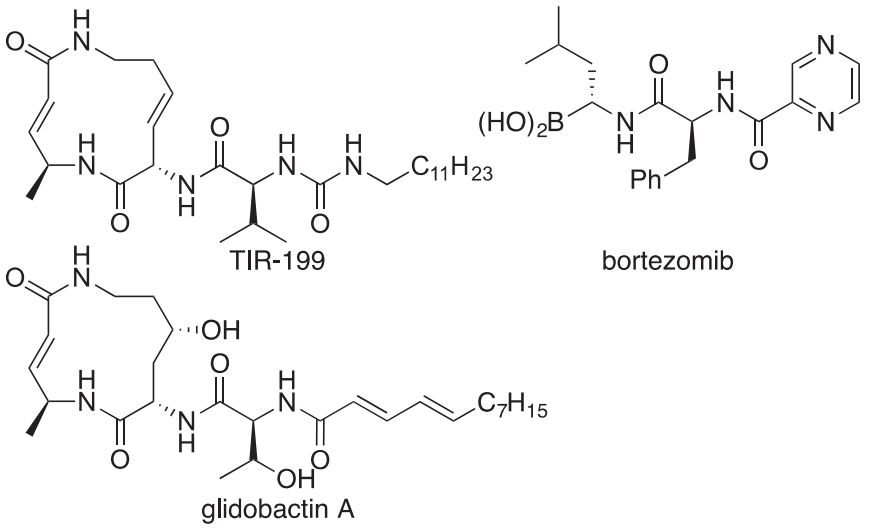

Chart 1. TIR-119, the approved drug bortezomib (Velcade®), and the natural product glidobactin A.

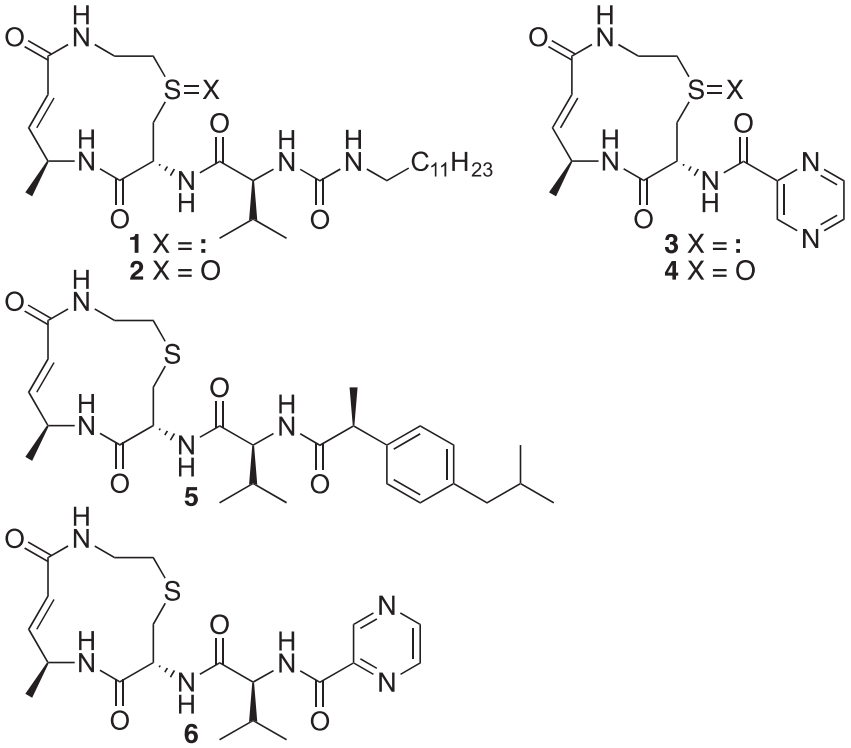

Chart 2. Synthetic syrbactin analogs prepared in this study.

trophilic *C*-terminus and an *N*-terminal pyrazine. The same spatial relationship between the electrophilic carbon and the pyrazine seen in bortezomib is found in the unsaturated amide of **3** and **4**. Compound **5** retains the core of **1** and **2** but adds a branched, saturated, chiral carbon in the side chain, one strategy recommended to enhance solubility.[16–18] Compound **6** likewise retains the macrolactam-valine but terminates it with the more polar pyrazinamide. All were evaluated computationally for their physicochemical properties that affect drug-likeness.[19,20] These results are summarized in Table 1.

2. Results

2.1. Synthesis

The synthesis of the core macrodilactam common to all of the targets prepared here is summarized in Scheme 1. The commercial aminoethylcysteine hydrochloride (**7**, thialysine), was converted in one pot to the ε-phosphonoacetatamide/α-Boc derivative **9** in 83% yield. Small quantities of the doubly Boc-protected and α-phosphonoacetatamide/ ε -Boc amino acids were also formed, and