Bioorganic & Medicinal Chemistry 22 (2014) 3091–3095

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Development of a new class of proteasome inhibitors with an epoxyketone warhead: Rational hybridization of non-peptidic belactosin derivatives and peptide epoxyketones

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ARTICLE INFO

Article history:
Received 27 March 2014
Revised 15 April 2014
Accepted 15 April 2014
Available online 24 April 2014

Keywords:
Proteasome inhibitor
Epoxyketone
Belactosin A

ABSTRACT

Proteasome inhibitors are currently a focus of increased attention as anticancer drug candidates. We recently performed systematic structure–activity relationship studies of the peptidic natural product belactosin A and identified non-peptidic derivative **2** as a highly potent proteasome inhibitor. However, the cell growth inhibitory effect of **2** is only moderate, probably due to the biologically unstable β-lactone warhead. Peptide epoxyketones are an important class of proteasome inhibitors exhibit high potency in cellular systems based on the efficient α,β-epoxyketone warhead. Importantly, belactosin derivatives bind primarily to the primed binding site, while peptide epoxyketones bind only to the non-primed binding site of proteasome, suggesting that hybridization of them might lead to the development of a new class of proteasome inhibitors. Thus, we successfully identified a novel chemotype of proteasome inhibitors **3** and **4** by rational structure-based design, which are expected to bind to both the primed and non-primed binding sites of proteasome.

1. Introduction

The major pathway for systematic degradation of intracellular proteins is the ubiquitin–proteasome system,[1] which is involved in many physiologically important cellular processes, such as signal transduction,[2] immune responses,[3] the unfolded protein response (UPR),[4] and cell cycle progression.[5] Proteasome inhibition causes cell cycle arrest and induces apoptosis, making proteasomes attractive target molecules for drugs to fight cancer and autoimmune disorders.[6] In fact, the US Food and Drug Administration recently approved the proteasome inhibitors bortezomib and carfilzomib (Fig. 1) for the treatment of multiple myeloma,[7] and several other proteasome inhibitors are currently in clinical trials.[8]

Belactosin A, comprising L-alanine, 3-(*trans*-2-aminocyclopropyl)-L-alanine (*trans*-3,4-methano-L-ornithine), and a chiral carboxy-β-lactone moiety, is a naturally occurring tripeptide metabolite produced by *Streptomyces* sp. (Fig. 1),[9] It inhibits proteasome chymotrypsin-like (ChT-L) activity[10] by acylating the active site Thr residue via its strained β-lactone-opening, as confirmed by X-ray crystallographic analysis of belactosin derivatives in complex with proteasome (Fig. 2a).[11] Importantly, belactosin A and its derivatives are the only known proteasome inhibitors that bind to both the primed and non-primed substrate binding sites of proteasome[11] and they are thus attractive lead compounds for the development of unique proteasome inhibitors.

As shown in Figure 3, we performed systematic structure–activity relationship studies of belactosin A and developed the highly potent derivative **1**.[11a] Furthermore, by the topology-based scaffold hopping of **1** based on our structure–activity relationship (SAR) and binding-mode analyses results,[12] we identified significantly simplified non-peptide inhibitor **2**.[13] Despite its significant proteasome inhibitory activity, however, the cell growth inhibitory effects were only moderate. We hypothesized that these contradictory results were due to its unstable β-lactone warhead under biological

Abbreviations: AMC, aminomethylcoumarin; Boc, *t*-butoxycarbonyl; ChT-L, chymotrypsin-like; DIEA, *N*,*N*-diisopropylethylamine; HBTU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; Piv, pivaloyl; Suc, succinyl; TFA, trifluoroacetic acid; UPR, unfolded protein response.

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http://dx.doi.org/10.1016/j.bmc.2014.04.032
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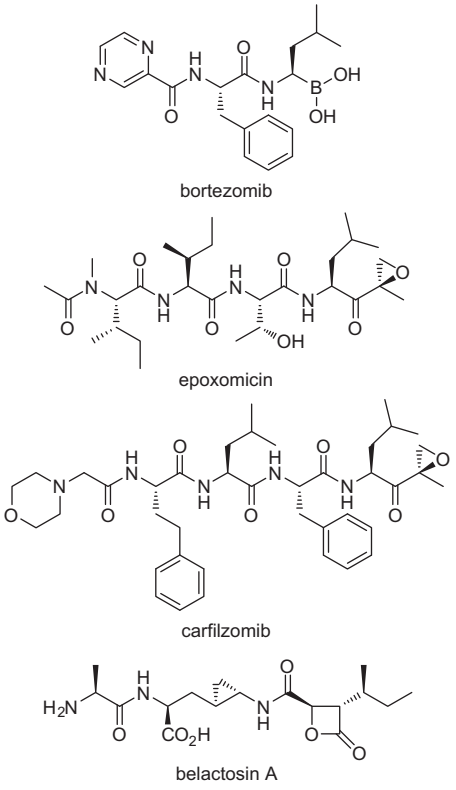

Figure 1. Known proteasome inhibitors.

conditions,[14] and therefore, planned to replace it with a more stable and irreversible warhead to develop potent cell growth inhibitors.

Peptide proteasome inhibitors with an α,β-epoxyketone as the reactive warhead comprise one of the most extensively studied classes of proteasome inhibitors, which inhibit proteasome covalently by forming a morpholino adduct (Fig. 2b).[6a,15] This class of proteasome inhibitors binds only to the non-primed binding site of the proteasome and exhibits remarkable cell growth inhibitory effects, as represented by the clinical drug carfilzomib.[16,14] We planned to produce a new class of non peptidic proteasome inhibitors by replacing the β-lactone moiety of the non-peptidic belactosin A derivatives with an α,β-epoxyketone residue. These hybrid compounds were expected to bind to both the primed and non-primed binding sites of proteasomes like belactosin A and to exhibit potent cell growth inhibitory effects like carfilzomib due to the α,β-epoxyketone warhead. Here, we describe the design, synthesis, and biological activity of this new class of proteasome inhibitors.

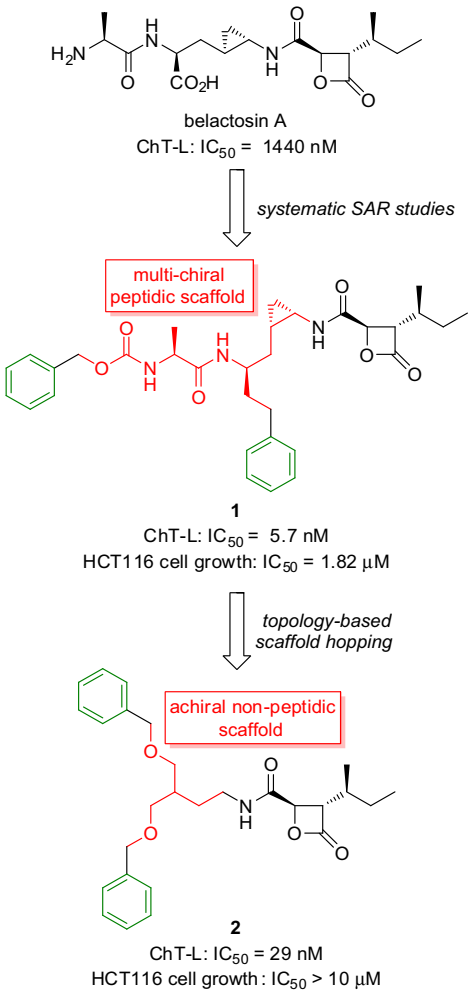

Figure 3. Belactosin A derivatives previously developed by us.

2. Results and discussion

2.1. Design of compounds

Figure 4 is a superposition of the two X-ray crystal structures of proteasome in complex with epoxomicin,[17] a peptidic proteasome inhibitor with an α,β-epoxyketone, and belactosin A derivative **1**,[11a] which clearly shows that epoxomicin binds to the non-primed binding site and its P2 side-chain is directed to the vacant primed binding site of proteasome. Therefore, we assumed that elongating

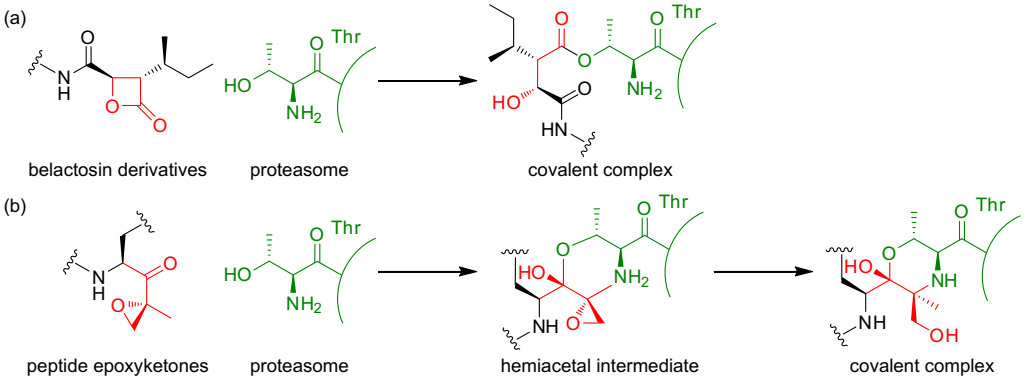

Figure 2. Inhibitory mechanism of covalent proteasome inhibitors: (a) belactosin derivatives (b) peptide epoxyketones.