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Discovery of novel 20S proteasome inhibitors by rational topology-based scaffold hopping of bortezomib

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ABSTRACT

A series of structurally novel proteasome inhibitors **1–12** have been developed based rational topology-based scaffold hopping of bortezomib. Among these novel proteasome inhibitors, compound **10** represents an important advance due to the comparable proteasome-inhibitory activity (IC_{50} = 9.7 nM) to bortezomib (IC_{50} = 8.3 nM), the remarkably higher BEI and SEI values and the effectiveness in metabolic stability. Therefore, compound **10** provides an excellent lead suitable for further optimization.

In eukaryotic cells, there are two different pathways for protein degradation: the lysosomal pathway and the ubiquitin-proteasome pathway (UPP). The lysosomal pathway can provide amino acids as building materials for protein synthesis by breaking down endogenous and exogenous proteins in a relatively nonspecific manner. In contrast, the UPP is the major pathway of endogenous proteins degradation. The UPP, which plays a critical role in recognizing and degrading abnormal and misfolded proteins, is responsible for maintenance of an intracellular protein expression.[1,2] In this pathway, the 26S proteasome is the main proteolytic component that consists of two subcomplexes with different functions: the 19S regulatory particles (RPs) and the 20S core particle (CP). The 19S RPs consists of multiple subunits and caps the 20S CP at one or both ends. The barrel-shaped catalytic 20S CP is composed of 28 protein subunits in $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$ arrangement (Fig. 1A).[3,4] In 20S proteasome, three different catalytic subunits are harbored in β-subunits and are classified on the basis of the amino acid after which they cleave the peptide bond: caspase-like (C-L, β1 subunit), which cleaves mainly after acidic amino acids; trypsin-like (*T*-L, β2 subunit), which cleaves after basic amino acids; chymotrypsin-like (CT-L, β5 subunit), which cleaves after hydrophobic residues.[5] In vertebrate cells, there exist three different types of proteasome subtype: constitutive proteasome, immunoproteasome, and thymoproteasome.[6] These three proteasome subtypes differ by the incorporation of three distinct sets of catalytic subunits: the constitutive proteasome incorporates subunits β1, β2, and β5, the immunoproteasome incorporates subunits β1i, β2i, and β5i, and the thymoproteasome incorporates subunits β1i, β2i, and β5t (Fig. 1B).[7] All of these hydrolytic activities are manifested through a threonine residue (O^{γ}-Thr1), which acts as a nucleophile in peptide bond hydrolysis.[8] Protein degradation by the UPP is initiated by the labeling of targeted proteins with polyubiquitin chains. Then, polyubiquitin-labeled proteins are taken up to a hole inside the 20S proteasome and were hydrolyzed by proteasomal catalytic active sites.[9]

The 20S proteasome is validated as a valuable anticancer target because proteasome inhibition causes an accumulation of unnecessary proteins and induces apoptosis.[10] Proteasome inhibitor bortezomib (Velcade), a dipeptide boronic acid, is a frontline drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of relapsed multiple myeloma (MM).[11] The FDA approval of tetrapeptidyl epoxyketone carfilzomib (Kyprolis) for the treatment of relapsed MM patients who have received at least two prior therapies further confirmed that targeting the UPP is a viable route for the treatment of human cancers.[12] Besides the bortezomib and carfilzomib, several structurally diverse proteasome inhibitors have been discovered and developed by screening chemical libraries containing synthetic and natural small molecules and by chemical modifications of lead compounds (Fig. 2).[13] However, despite initial clinical successes of bortezomib and carfilzomib, these two drugs have several shortcomings such as side effects, drug resistance and unsatisfactory pharmacokinetic profiles.[14] Therefore, there is an urgent need for novel proteasome inhibitors as potential chemotherapeutic agents.

As we all know, scaffold hopping has been identified as a promising strategy for discovering structurally novel compounds.[15] Recently, Sun and co-workers classified scaffold hopping approaches into four major

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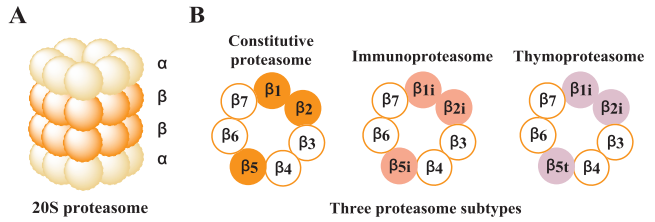

Fig. 1. (A) The 20S proteasome. (B) Schematic representation of subunit composition of three proteasome subtypes.

categories by focusing on the degree of change associated with the original parent molecule.[16] However, there are very few success examples by using topology-based scaffold hopping approach, which is the highest hop level of scaffold hopping approaches. Therefore, our effort has been devoted to design and synthesis of a series of structurally novel highly potent 20S proteasome inhibitors based topology-based scaffold hopping of bortezomib in the past few years, hoping to overcome the reported shortcomings.

The X-ray crystal structure of 20S proteasome in complex with bortezomib is depicted in Fig. 3 (PDB ID: 2F16), which clearly shows that bortezomib only binds to the nonprimed binding site of proteasome.[17] Moreover, boronic acid warhead of bortezomib covalently binds to Thr hydroxyl group of β5 subunit, which is the most important binding site in its interaction to keep the biological activities. The *P*1-leucine side chain is found to occupy the S1 pocket deeply and fully. The phenylalanine moiety in *P*2 position points into the vacant primed binding site. The pyrazine-2-carboxyl group in *P*3 position makes bortezomib form strong hydrophobic interactions with the S3 deep hydrophobic pocket. This structural information suggests that the antiparallel β sheet conformation and the boronic acid warhead of bortezomib are essential for the strong binding to the β5 subunit of 20S proteasome. Furthermore, according to the literature, the boronic acid warhead is very important to reversibly interact with the hydroxyl group of *N*-terminal Thr in β5 subunit. On this point, reversible inhibition of endogenous proteases is advantageous for cancer treatment, while irreversible blockage of parasitic proteases is desirable.[18]

Based on the structural information and previous studies, we designed and synthesized the structurally novel proteasome inhibitors **1–12**, in which the pyrazine-2-carboxyl group in *P*3 position of bortezomib was replaced with aryl ether/amine group (short-linker scaffold, **1–6**) and aryl methyl ether/amine group (long-linker scaffold, **7–12**) (Fig. 4A). The results of alignment were depicted in Fig. 4B and C, these compounds can be superimposed onto the binding conformation of bortezomib and almost took the same interaction mode as bortezomib. Furthermore, the boronic acid warhead, the *P*1-leucine side chain and the *P*2-phenylalanine moiety of the newly designed compounds were well superimposed on each other and had no steric repulsion with the β5 subunit of 20S proteasome. Compared with bortezomib, these compounds adopted aryl ether/amine group and aryl methyl ether/amine group instead of the pyrazine-2-carboxyl group at *P*3 position, and these variations did maintain the strong hydrophobic interactions of *P*3 moiety with the S3 deep hydrophobic pocket. All the theoretical results displayed that these newly designed compounds can retain the strong inhibitory activity of bortezomib.

The designed compounds **1–12** were synthesized following the method described in the literature with modifications,[19–24] and the synthetic routes are summarized in Schemes 1–5. The amino boronate hydrochloride **19** was the key intermediate in the total synthetic route (Scheme 1). The highly optical (+)-pinanediol **13** was synthesized from

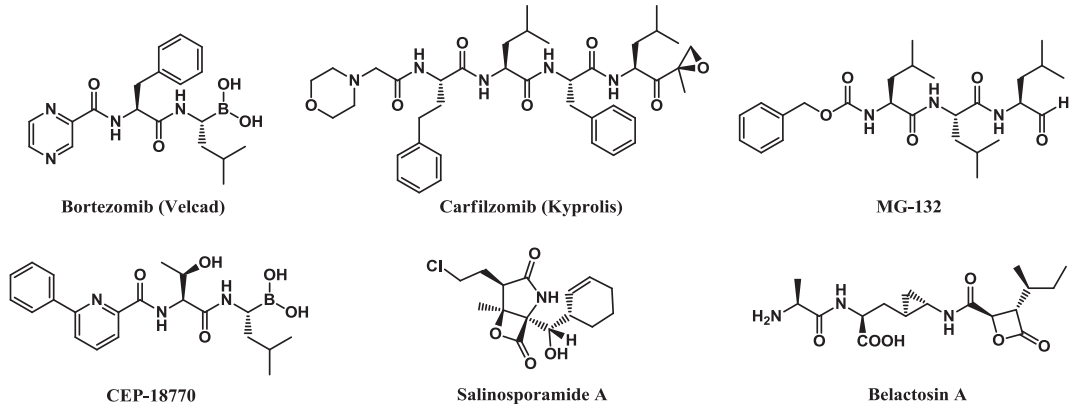

Fig. 2. Structures of approved and currently developed proteasome inhibitors.

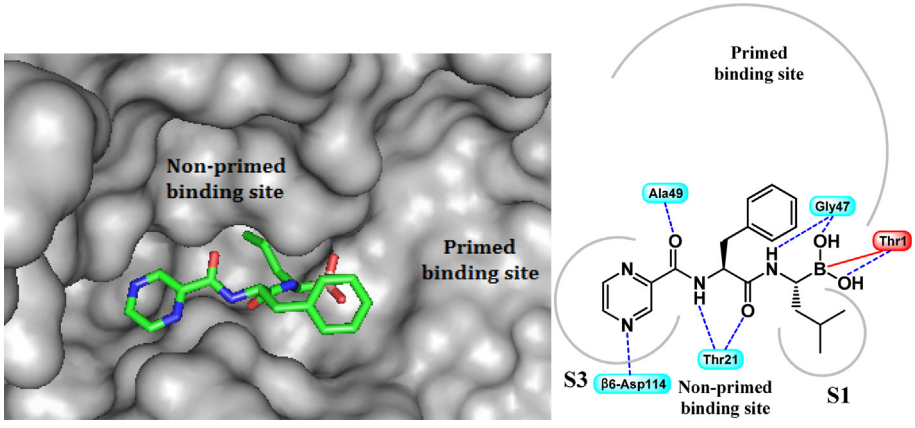

Fig. 3. X-ray crystal structures of 20S proteasome in complex with bortezomib (green tube, PDB ID: 2F16).