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Optimization of peptidomimetic boronates bearing a P3 bicyclic scaffold as proteasome inhibitors

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ABSTRACT

A new series of pseudopeptide boronate proteasome inhibitors (**2**–**3**) was developed, through optimization of our previously described analogs of bortezomib, bearing a bicyclic 1,6-naphthyridin-5(6*H*)-one scaffold as P3 fragment (**1**). The biological evaluation on human 20S proteasome displayed a promising inhibition profile, especially for compounds bearing a P2 ethylene fragment, which exhibited K_i values in the nanomolar range for the ChT-L activity (e.g. **2a**, K_i = 0.057 μM) and considerable selectivity for proteasome over bovine pancreatic α-chymotrypsin. Docking experiments into the yeast 20S proteasome revealed that the ligands are accommodated predominantly into the ChT-L site and that they covalently bind to the active site threonine residue via boron atom. Within the cellular assays performed against a 60 cancer cell line panel, compounds **3e** and **3f** demonstrated also good antiproliferative activity and compound **3f** emerged as promising lead compound for the development of anticancer agents targeting melanoma and non-small cell lung cancer.

1. Introduction

The 26S proteasome is the most important non-lysosomal, proteolytic complex, structurally composed by a central particle called 20S core, which exerts the catalytic activity, and two regulatory particles named 19S, which are located at both the ends of the catalytic core. The latter is made by four stacked rings, each one composed of seven different subunits. In detail, the two outer rings contain only α subunits and exert structural functions, whereas the two inner rings consist of β subunits and carry out the proteolytic activity [1].

Proteasome presents three different catalytic subunits which are classified on the basis of the amino acid after which they cleave the peptide bond: β1 or post-glutamyl peptidyl hydrolase (PGPH), recently more correctly referred to as caspase-like (C-L) site, which cleaves mainly after acidic amino acids; β2 or trypsin-like (T-L), that cleaves after basic amino acids; β5 or chymotrypsin-like (ChT-L) that cleaves after hydrophobic residues [2]. In all the catalytic subunits, the active site is represented by the N-terminal Thr, whose side chain hydroxyl group performs the nucleophilic attack onto the carbonyl carbon of the peptide bond [3].

Proteasome plays a fundamental role in the protein turnover by degrading misfolded, abnormal or damaged proteins, previously labeled through addition of a polyubiquitin chain. Most of proteasome substrates are involved in cell cycle regulation, angiogenesis and apoptosis, therefore defects of this system can lead to an anarchic cell proliferation [4]. As a consequence, proteasome inhibition has been identified as a promising strategy for anticancer therapy and the great efforts over the past decades led to the introduction in therapy of bortezomib (Velcade®, Fig. 1), the first proteasome inhibitor approved by FDA for the treatment of

Abbreviations: ChT-L, chymotrypsin-like; T-L, trypsin-like; PGPH, post-glutamyl peptide hydrolyzing; C-L, caspase-like; DMSO, dimethyl sulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, *N*-hydroxybenzotriazole; DIPEA, N,N-diisopropylethylamine.

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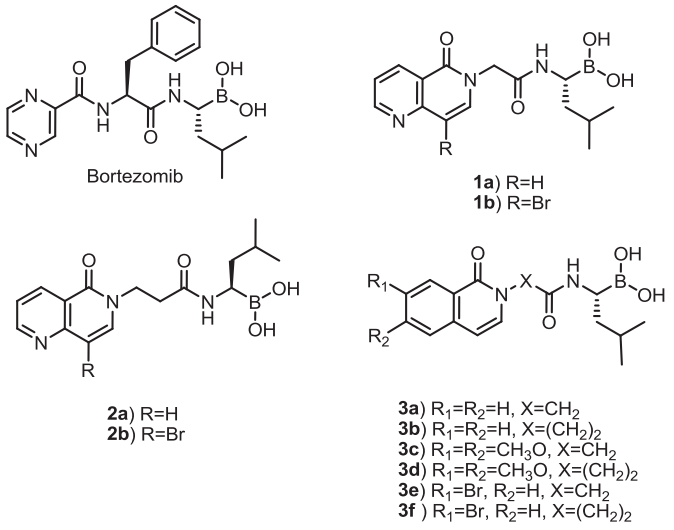

Fig. 1. Structures of bortezomib and compounds **1**–**3**.

relapsed and/or refractory multiple myeloma [5] and mantle cell lymphoma [6].

Although bortezomib has brought many benefits in patients with MM, its clinical use has been limited by its toxic side effects [5], the most serious of which include peripheral neuropathy and hematological toxicity. Considering all these problems, the development of peptidomimetics could lead to many pharmacokinetic and pharmacodynamic advantages. This strategy can be realized, for example, by introducing into a peptide framework unnatural amino acids instead of natural residues, or with the introduction of a non-peptidic scaffold into the peptidic backbone, in order to lock a defined conformation of the peptide [7–9].

Our research group has been involved in the recent past in the development of peptidomimetic 20S proteasome inhibitors [10], in this context, we recently described the synthesis of conformationally constrained pseudopeptide boronates [11], structurally related to bortezomib, bearing a 1,6-naphthyridin-5(6*H*)-one scaffold (e.g. **1a**–**b**, Fig. 1), which exhibited a promising profile of both activity and selectivity.

The N-1 atom of the naphthyridinone scaffold was assumed to reproduce the additional H-bond interaction of N-4 atom of bortezomib pyrazinamide with the side chain hydroxyl group of Asp114, located into the β6 subunit [12]. A docking experiment performed on the most potent compound of this series (**1b**) within the β5 binding site, showed that the inhibitor adopted a folded conformation stabilized by an intramolecular H-bond between the naphthyridinone carbonyl group and one of the hydroxyl moieties of the boronic acid warhead. As a consequence, the P3 naphthyridinone scaffold pointed towards the S2 pocket instead of the originally assumed S3 pocket [11].

On the basis of these findings, we replaced the P2 methylene fragment of the most active compounds (**1a**–**b**) with the ethylene homolog (**2a**–**b**, Fig. 1), assuming that the elongation of the central aliphatic chain should avoid the intramolecular H-bonding, by increasing the distance between the involved groups. This should induce a more similar conformation to that of bortezomib and improve the inhibition efficacy against the ChT-L activity. Our choice of the ethylene chain is justified also by its wide use in the field of peptidomimetics as β-alanine mimetic, since literature data indicate that the replacement of α-amino acids with β-ones allows to an increase the inhibition activity [13]. Furthermore, we developed a panel of inhibitors (**3a-f**) by replacing the naphthyridinone scaffold with the isoquinolin-1(2*H*)-one isostere, that lacks the N-1 atom, with the aim to evaluate its real contribution to the inhibitory activity. The P1 Leu-boronic moiety was not modified since it is essential to reversibly interact with the γ-OH group of N-terminal Thr of 20S proteasome. In this regard, while irreversible blockage of an enzyme is desirable for parasitic targets [14], on the contrary in the case of inhibition of endogenous proteases for cancer treatment, reversible or non-covalent inhibition would be advantageous.

Furthermore, the covalent binding mode of the designed inhibitors was clarified by performing docking analysis using the crystal structure of the yeast 20S proteasome. Moreover, growth inhibitory effects were evaluated at the National Cancer Institute (NCI) against sixty human tumor cell lines.

2. Results and discussion

2.1. Chemistry

Synthesis of the boronic acids **2a**–**b** and **3a**–**f** was performed as outlined in Scheme 1. Ester intermediates **5a**–**h** were synthesized through N-alkylation of the proper bicyclic scaffolds (**4a**–**e**) with ethyl 2-bromoacetate or methyl 3-bromopropionate, in the presence of NaH or KH. The subsequent alkaline hydrolysis gave the corresponding carboxylic acids **6a**–**h** which were coupled to pinanediol leucine boronate [15] **7** in the presence of HOBt, EDCI and DIPEA, to provide the pinanediol esters **8a**–**h**. In this step the corresponding amides **9** were also isolated as by-products. Finally, the pinanediol intermediates underwent a trans-esterification reaction with isobutylboronic acid under acidic conditions, to afford the peptide boronates **2**–**3**.

2.2. Inhibitory effect on the ChT-L, T-L and PGPH activities of human 20S proteasome

The inhibitory profile of peptidomimetic boronates **2**–**3** was evaluated on purified 20S proteasome isolated from human erythrocytes, using the appropriate fluorogenic substrate for each one of the proteolytic activities (i.e. Suc–Leu–Leu–Val–Tyr–AMC for ChT-L; Boc–Leu–Arg–Arg–AMC for T-L; Z-Leu–Leu–Glu–AMC for PGPH) and employing bortezomib as reference compound.

Compounds **2**–**3** underwent a preliminary screening for ChT-L activity at 20 μM, using an equivalent volume of dimethyl sulfoxide (DMSO) as a negative control. Since all compounds inhibited more than 40% of the enzyme activity, continuous assays were then performed at seven different concentrations using the progress curve method to determine the K_i values (Table 1).

Consistently with our initial hypothesis, almost all compounds bearing the ethylene chain as P2 fragment exhibited the highest inhibition potency against the ChT-L activity, with K_i values in the nanomolar range (**2a**, **3b**, **3d, 3f**). On the other hand, the analogs with a methylene P2 fragment demonstrated to be much less active (submicromolar/micromolar K_i values) or completely inactive, with the exception of compound **3e** ($K_i = 0.053$ μM). We may suppose that this result is due to a positive contribution of the bromine atom to enzyme inhibition, whereas, the introduction of the two methoxy groups (i.e. **3c**) resulted to hinder the inhibitory activity. Unexpectedly, no inhibition was recorded for compound **2b**.

All compounds inhibited the PGPH activity in a lesser extent compared to the ChT-L one, whereas no inhibition was shown against T-L activity. This outcome is particularly significant since literature data indicate that the suitable inhibition profile to optimize anticancer efficacy is the co-inhibition of the ChT-L activity together with either the PGPH or T-L ones [16], whereas the inhibition of the sole ChT-L activity produces only moderate effects [17], while the inhibition of all the activities results to be cytotoxic [18].