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Development of peptidomimetic boronates as proteasome inhibitors

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ABSTRACT

Proteasome inhibition has emerged over the past decade as an effective therapeutic approach for the treatment of hematologic malignancies. It is a multicatalytic complex, whose proteolytic activity relies in three types of subunits: chymotrypsin-like (β5), trypsin-like (β2) and caspase-like (β1). Most important for the development of effective antitumor agents is the inhibition of the β5 subunits. In this context, the dipeptide boronate bortezomib (Velcade®) represents the first proteasome inhibitor approved by the FDA and the lead compound in drug discovery. This paper describes the synthesis and biological evaluation of a series of conformationally constrained pseudopeptide boronates (**1**–**3**) structurally related to bortezomib. The synthesized compounds showed a promising inhibitory profile by blocking primarily the chymotrypsin-like activity of the proteasome with K_i values in submicromolar/micromolar range. These compounds also resulted quite selective since no significant inhibition was recorded in the test against bovine pancreatic α-chymotrypsin. The obtained results were rationalized by means of docking experiments based on a model of the crystal structure of bortezomib bound to the yeast 20S proteasome providing essential insights for further optimization of this class of inhibitors.

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1. Introduction

The 26S proteasome is a multisubunit, multicatalytic threonine protease complex involved in different phases of cellular life-cycle. It is composed of a 20S catalytic core capped by two 19S regulatory complexes. Its proteolytic activity relies in three types of subunit (β1, β2 and β5) positioned into the barrel-like 20S core. These subunits possess chymotrypsin-like (ChT-L) (β5), trypsin-like (T-L) (β2), and post-glutamyl peptide hydrolyzing (PGPH) or caspase-like (β1) activities, so named for the preferred substrate they hydrolyze. Targeting of eukaryotic proteins to this proteolytic system requires their prior marking by polyubiquitin chains. Proteasome is primarily responsible for the turnover of intracellular proteins that regulate cell proliferation and survival pathways. Thus, defects in the proteasome activity can lead to anarchic cell proliferation and tumor development. For these reasons, proteasome inhibition, in particular of the ChT-L activity, has become a significant strategy for drug development in cancer treatment [1–3].

In this context, we designed and synthesized conformationally constrained pseudopeptide boronates (**1**–**3**, Fig. 1) using bortezomib as lead compound, the first proteasome inhibitor approved by FDA in 2003 for the treatment of relapsed and refractory multiple myeloma [4] and mantle cell lymphoma [5]. These constrained surrogates should have in principle several advantages comparing to peptide inhibitors such as stability toward protease degradation, good cell permeability and target-selectivity by stabilizing a biologically active conformation [6]. This strategy has been accomplished by: i) maintaining the Leu-boronic electrophilic warhead at P1 due to its essentiality in binding interaction with the γ-OH group of the N-terminal catalytic Thr; ii) substituting the Phe residue at P2 with less hindered residues such as Gly or Ala due to the fact that there is no much specificity assessed for this site; iii) enclosing the amide moiety at P3 in a 1*H*-pyridin-2-one ring to reduce the peptidic character of the compound as well as to reduce its

Abbreviations: ChT-L, chymotrypsin-like; T-L, trypsin-like; PGPH, post-glutamyl peptide hydrolyzing; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; $Pd_2(dba)_3$, tris(benzylideneacetone)dipalladium; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, *N*-hydroxybenzotriazole; DIPEA, *N*,*N*-diisopropylethylamine.

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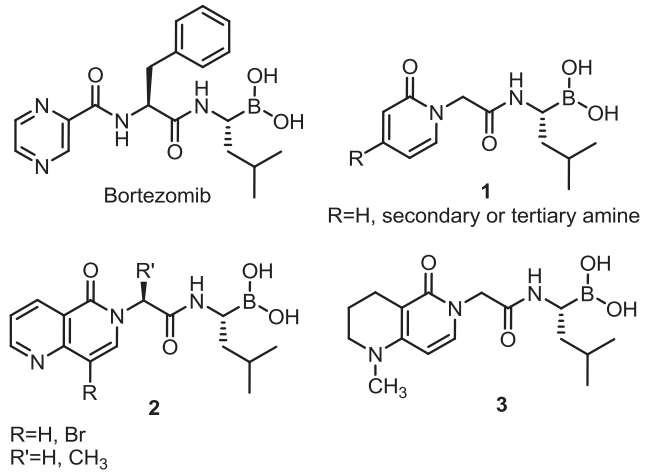


Fig. 1. Structure of bortezomib and compounds **1**–**3**.

conformational freedom; iv) maintaining a basic moiety corresponding to N4 of the pyrazine ring of bortezomib taking into account that the complex bortezomib-β5 subunit is stabilized through an interaction involving this nitrogen and the D114 residue of the adjacent β6 subunit [7].

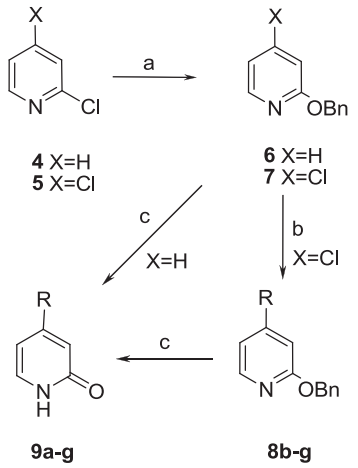
Two series of compounds came out from our design. In the first series (i.e. **1a**–**g**, Scheme 2), the constrained motif is represented by the sole 1*H*-pyridin-2-one scaffold bearing secondary or tertiary amines at C-4 (**1b**–**g**) with the unsubstituted derivative **1a** as reference compound to validate our hypothesis of the interaction with β6 subunit. In the second series [i.e. **2a**–**c** (Scheme 3) and **3** (Scheme 4)], the constrained motif is represented by a bicyclic 1,6-naphthyridin-5(6*H*)-one scaffold.

2. Results and discussion

2.1. Chemistry

The pyridone scaffolds **9a**–**g** (Scheme 1) were synthesized by means of an initial nucleophilic aromatic substitution reaction of 2-chloropyridines (**4**–**5**) with benzyl alcohol in the presence of potassium hydride followed by debenzylation of the resulting *O*-pyridyl benzyl ethers (**6**–**7**) under transfer hydrogenation conditions. The 1*H*-pyridin-2-one **9a** was obtained directly by catalytic debenzylation of 2-benzyloxypyridine **6**, whereas, for compounds **9b**–**g**, the debenzylation reaction was preceded by a microwave-promoted Buchwald–Hartwig amination at position 4 of 4-chloro-2-benzyloxypyridine **7** in presence of $Pd_2(dba)_3$ as a catalyst, the DavePhos ligand and a suitable amine to give intermediates **8b**–**g** (Scheme 1), according to a procedure reported in literature [8]. The obtained 1*H*-pyridin-2-ones **9a**–**g** were *N*-alkylated with ethyl bromoacetate in the presence of NaH to give esters **10a**–**g** (Scheme 2), which were in turn smoothly converted into the corresponding acids **11a**–**g** by alkaline hydrolysis with LiOH. Coupling reactions between acids **11a**–**g** and pinanediol leucine boronate **12** [9] in the presence of EDC·HCl, HOBt and DIPEA gave the pinanediol esters **13a**–**g** (Scheme 2), which were directly used for the successive trans-esterification reaction with isobutylboronic acid under acid conditions to provide the title boronic acids **1a**–**g**.

Synthesis of the boronic acids **2a**–**c** and **3** has been conducted with a similar approach starting from 1,6-naphthyridin-5(6*H*)-ones **14**–**15** and 1-methyl-1,2,3,4-tetrahydro-1,6-naphthyridin-5(6*H*)-one **18** [10], as depicted in Schemes 3 and 4, respectively.



a, R=H
b, R=Et_2N
c, R= morpholin-4-yl
d, R= piperidin-1-yl
e, R= phenylamino
f, R= 4-methylpiperazin-1-yl
g, R= 4-allylpiperazin-1-yl

Scheme 1. Reagents and conditions: (a) BnOH, KH, THF, rt, 18 h, N_2, 87–94%; (b) $Pd_2(dba)_3$, DavePhos, amine, *t*-BuONa, toluene, MW, 120–150 °C, 15 min, N_2, 65–88%; (c) H_2, 10% Pd/C, MeOH/EtOAc (2:1), rt, 2 h, 95–99% (**9b**–**f**) or BBr_3, CH_2Cl_2, −78 °C, 4 h, 99% (**9g**).

2.2. Biological activity

The newly synthesized peptidomimetic boronates **1**–**3** have been tested for their inhibitory properties by using purified 20S proteasome isolated from human erythrocytes and the appropriate fluorogenic substrate for each 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).

First, compounds **1**–**3** underwent a preliminary screening for ChT-L activity at 20 μM using an equivalent volume of dimethyl sulfoxide (DMSO) as a negative control. The screening showed that all compounds, with the exception of **1f**, inhibited more than 40% of the enzyme activity.

Continuous assays were then performed (progress curve method, at seven different concentrations, ranging from those that minimally inhibited to those that fully inhibited the enzyme, Fig. 2) to determine the K_i values (Fig. 3), reported in Table 1.

As can be noticed, all compounds showed promising inhibitory properties toward the ChT-L activity of the proteasome, with K_i values in the submicromolar/micromolar range.

The inhibitory activity toward the other two proteasome activities, as well as the selectivity of these compounds toward the target enzyme, by using bovine pancreatic α-chymotrypsin, was determined with the same method. None of these pseudopeptide boronates exhibited any inhibition in the T-L activity assay at 20 μM. Conversely, all of them but **1g** inhibited the PGPH activity of the proteasome, although to a much lesser extent compared with the ChT-L activity inhibition (generally one or two order of magnitude), with the exception of **1c** which is more potent in PGPH assay (Table 1). This outcome, per se, is extremely important since literature data suggest that the inhibition of only β5 subunits of the proteasome has a moderate effect on inhibition of protein degradation pathways [12], whereas the co-inhibition of β5 subunits with either β1 or β2 subunits exerts the maximal effect that is required to produce an antitumor response [13]. On the other hand, it is also known that the inhibition of all proteasome subunits is

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