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Original article

Identification of a new series of amides as non-covalent proteasome inhibitors

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ABSTRACT

Proteasome inhibition has emerged as an important therapeutic strategy for the treatment of multiple myeloma (MM) and some forms of lymphoma, with potential application in other types of cancers. 20S proteasome consists of three different catalytic activities known as chymotrypsin-like (ChT-L), trypsin-like (T-L), and, post-glutamyl peptide hydrolyzing (PGPH) or caspase-like (C-L), which are located respectively on the β5, β2, and β1 subunits of each heptameric β rings. Currently a wide number of covalent proteasome inhibitors are reported in literature; however, the less widely investigated non-covalent inhibitors might be a promising alternative to employ in therapy, because of the lack of all drawbacks and side-effects related to irreversible inhibition. In the present work we identified a series of amides, two of which (**1b** and **1f**) are good candidates to non-covalent inhibition of the chymotrypsin-like activity of the β5 proteasome subunit. The non-covalent binding mode was corroborated by docking simulations of the most active inhibitors **1b**, **1f** and **2h** into the yeast 20S proteasome crystal structure.

1. Introduction

The eukaryotic 26S proteasome is a large (1.6–2.4 MDa) multi-functional particle, composed of a barrel-shaped 20S catalytic core capped by two 19S regulatory complexes. The 20S proteasome is the proteolytically active key element of the ubiquitin–proteasome system. It is composed by four heptameric rings stacked in a $\alpha_7\beta_7\beta_7\alpha_7$ arrangement and contains three proteolytic subunits, β1, β2 and β5, which have distinct substrate specificities and are responsible, respectively, for the caspase-, trypsin- and chymotrypsin-like activities of proteasome [1]. All the three subunits possess a catalytic site that employs the nucleophilic γ-hydroxyl group of the N-terminal Thr to cleave peptide bonds [2]. The expression of these subunits in normal cells may be replaced by the synthesis of the immunoproteasome catalytic subunits counterparts β1i, β2i and β5i (>50% of identity; also named LMP2, MECL1 and LMP7, respectively) upon exposure to specific stimuli such as the inflammatory cytokines IFN-γ and TNF-α [3].

Proteasome is responsible for the turnover of cellular proteins that regulate cell proliferation and survival pathways, however defects of its proteolytic activity can lead to anarchic cell proliferation and as a consequence to tumor development.

It has been demonstrated that inhibition of the chymotrypsin-like (ChT-L) activity of proteasome represents a valid strategy to induce antineoplastic effects in hematologic tumors [4]. A growing number of studies suggest that proteasome inhibitors may become valuable drugs for the treatment of nontumorous diseases when used in conditions in which the cellular functions are modulated without induction of cell death [5]. Diverse beneficial effects are expected in inflammation [6], neurodegenerative diseases [7], muscular dystrophies [8] and cachexia [9]. Moreover, they have therapeutic potential as antiparasitics in malaria [10] and sleeping syndrome [11] and as antimicrobial agents (tuberculosis) [12].

The majority of 20S proteasome inhibitors, currently reported in literature [13], are peptide-based compounds, endowed with a C-terminal electrophilic warhead that forms covalent adducts with the active site Thr1Oγ (Fig. 1a, for bortezomib). A reversible action

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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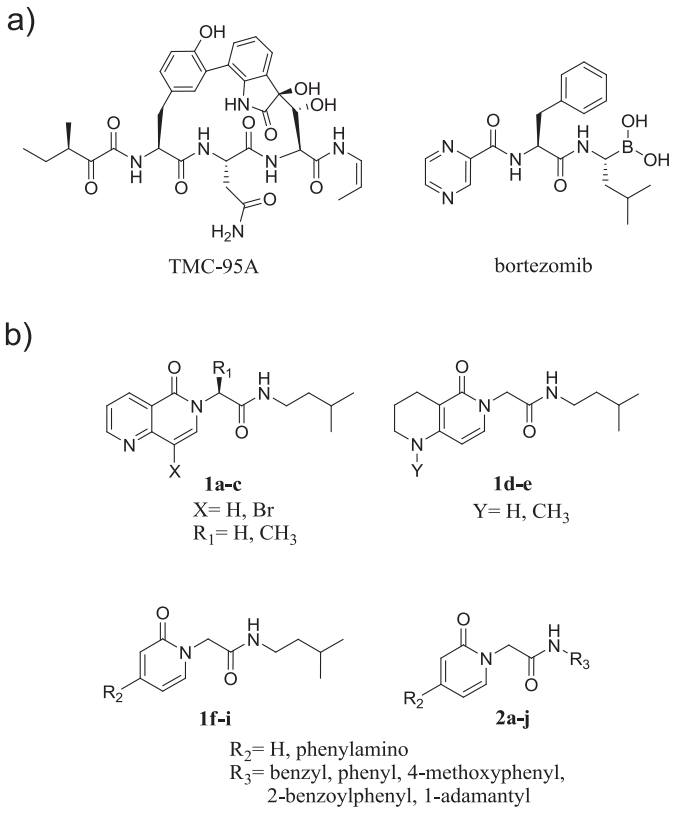
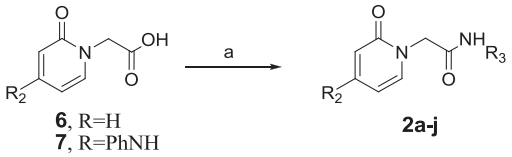


Fig. 1. Structure of representative non-covalent (TMC-95A), covalent (bortezomib) proteasome inhibitors (a) and amides **1**–**2** (b).

characterizes aldehydes, α-keto-aldehydes, α-keto-oxadiazoles, β-lactones, boronates. α′,β′-Epoxyketones are characterized by an irreversible action. Very often this covalent mode of action, together with high reactivity of compounds, may lead to off-target interactions.

In this context, the dipeptide boronate Bortezomib (Velcade®), the first proteasome inhibitor approved by the FDA for treatment of multiple myeloma and mantle cell lymphoma, has been demonstrated to possess low systemic tissue distribution, severe off-target effects due to lack of specificity [14]. Despite the dipeptide boronates reversibly interact with the target enzyme, the formation of a long-lasting inhibitor-proteasome adduct is responsible of its unfavorable pharmacodynamic profile: when it is injected intravenously a big part of administered dose inhibits the chymotrypsin-like activity of 20S proteasome of non-tumor cells, like hematic or liver cells. As a consequence while irreversible blockage of an enzyme should be advantageous for parasitic targets [15], on the contrary in the case of cancer treatment, non-covalent inhibition would be desirable. Thus, there is a recent tendency to identify non-covalent inhibitors, like TMC-95A, (Fig. 1 a) and its linear derivatives [16].



Scheme 2. Reagents and conditions: a) HOBt, EDC·HCl, then suitable amine and DIPEA, CH_2Cl_2, −0 °C → rt, 12 h.

Non-covalent inhibitors, even if less widely investigated, with respect to covalent inhibitors, provide an alternative mechanism for proteasome inhibition and, due to the rapid dissociation from the 20S subunits, may be devoid of all drawbacks related to either irreversible or slowly reversible enzyme inhibition. Their potential advantages are improved selectivity, moderate reactivity and reduced instability, which are often associated with side effects in therapeutics [17]. Taking into account all these reasons non-covalent proteasome inhibitors might be a promising alternative for therapy.

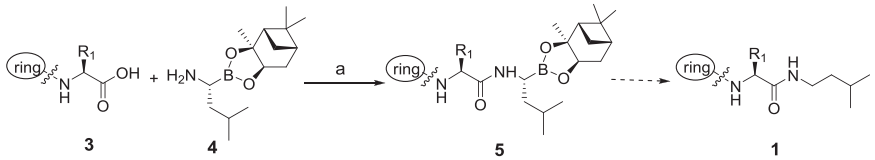
Our research group has been involved in the last years in the development of peptidomimetic vinyl sulfones [18] and boronates [19] as irreversible and pseudo-irreversible 20S proteasome inhibitors, respectively. During the development of peptidomimetic boronates, we identified a number of amides **1** (Fig. 1 b), recovered as by-products of the key step for the introduction of the boronic ester moiety. After a preliminary screening, some of these molecules were supposed to non-covalently inhibit β5 proteasome subunit. Starting from these findings, and on the basis of a docking model, we synthesized and evaluated as potential 20S proteasome inhibitors a second series of amides **2** (Fig. 1 b), in which bulkier substituents directly linked to the amide moiety, in agreement also to literature data [20], have been introduced in order to extend the structure–activity relationship of this class of molecules.

2. Results and discussion

2.1. Chemistry

In our multistep approach for the synthesis of peptidomimetic boronates, carried out with our previously reported procedure [19], we identified compounds **1** as by-products. More in detail the synthetic step leading to the introduction of the pharmacophore portion, realized by coupling the carboxylic acids **3** and pinanediol leucine boronate **4** in the presence of EDC·HCl, HOBt and DIPEA, together with the desired product **5**, led to the formation, as minor reaction product, of amides **1** obtained by spontaneous deboronation of boronic esters **5** (Scheme 1).

The synthesis of amides **2a**–**c**, **2e**–**h** and **2j** (Scheme 2) has been carried out by coupling acid **6** or **7**, synthesized according to a previously reported procedure [19], together with the suitable amines by employing also in this case, HOBt and EDC·HCl as coupling reagents to get the desired amides in high yields. Different conditions were adopted for the synthesis of sterically hindered amides **2d** and **2i**, in this case acid **6** or **7** was first activated to mixed



ring = 1*H*-pyridin-2-one or 1,6-naphthyridin-5(6*H*)-one

Scheme 1. Reagents and conditions: (a) HOBt, CH_2Cl_2, −5 °C, 20 min, then EDC·HCl, DIPEA, **7**, −15 °C → rt, 3 h.