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Original article

α- and β-hydrazino acid-based pseudopeptides inhibit the chymotrypsin-like activity of the eukaryotic 20S proteasome

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ABSTRACT

We describe the synthesis of a library of new pseudopeptides and their inhibitory activity of the rabbit 20S proteasome chymotrypsin-like (ChT-L) activity. We replaced a natural α-amino acid by an α- or a β-hydrazino acid and obtained inhibitors of proteasome up to a submicromolar range (0.7 μM for molecule **24b**). Structural variations influenced the inhibition of the ChT-L activity. Models of inhibitor/20S proteasome complexes corroborated the inhibition efficacies obtained by kinetic studies.

1. Introduction

The 26S proteasome is a large, multicatalytic threonine protease complex that processively degrades ubiquitinylated proteins to small peptides [1]. The ubiquitin–proteasome pathway plays a central role in the degradation of regulatory proteins that are crucial for many intracellular processes including cell progression, apoptosis and NF-κB activation. The 26S proteasome is composed of a 20S catalytic core particle that is capped at each end by the 19S regulatory complex which is responsible for the recognition, unfolding and translocation of protein substrates into the 20S catalytic core cavity. The eukaryotic 20S proteasome is formed by four stacked rings, and each of the two inner rings is composed of seven different β subunits [2]. Three proteolytic activities are localized in 6 β subunits and are classified as chymotrypsin-like (ChT-L, β5 subunit), trypsin-like (T-L, β2 subunit) and caspase-like or post-acid (PA, β1 subunit) activities since peptide bonds are cleaved on the carboxyl side of hydrophobic, basic and acidic amino acid residues, respectively [3]. The ChT-L activity has been the focus of drug development [4]. Inhibition of ChT-L activity induces cell cycle arrest and selective apoptosis of malignant cells leading to a new category of antineoplastic agents [5]. The dipeptide boronic acid bortezomib (Velcade®) and the epoxyketone carfilzomib (PR-171) [6] have been approved for treating incurable multiple myeloma (both compounds) [7] and mantle lymphoma (bortezomib) [8]. The lactone salinosporamide A (NPI-0052) entered into clinical trials for advanced solid and hematological malignancies [9]. Most natural and synthetic proteasome inhibitors, such as epoxyketones, peptide aldehydes, peptide vinyl sulfones and peptide boronic acids, bear a reactive group that forms a transient or irreversible covalent bond with the catalytic O^{γ} atom of Thr1 of the active sites [10,11]. Although these reactive groups contribute to the inhibitory activity, they can also cause a lack of specificity, excessive reactivity and instability which may increase adverse effects and limit efficacy of proteasome inhibitors *in vivo*. Therefore, non covalent inhibitors

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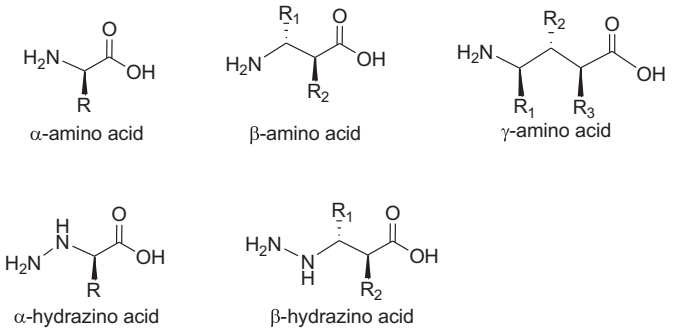

Fig. 1. General structure of amino acids and hydrazino acids. For clarity, each amino acid and hydrazine acid is represented by the residue formula of only one configuration.

that bind reversibly to the active sites may provide an alternative mechanism of inhibition and offer therapeutic advantages. Non covalent inhibitors have been less extensively investigated [12,13]. They include peptidic inhibitors such as ritonavir [14], aminobenzylstatine [15], and 3,4,5-trimethoxy-L-phenylalanine derivatives [16], lipopeptides [17], macrocyclic [18], and linear TMC-95 derivatives [19–22] and sulfonamide compounds [23]. We describe here a new class of non covalent 20S inhibitors based on α- or β-hydrazino acid scaffolds. We postulated that these peptidomimetic elements would mimic the natural α-peptides which are the only elements encountered so far in peptidic proteasome inhibitors. α- and β-hydrazino acid scaffolds are peptidomimetic building blocks that have two nitrogen atoms. They can be considered as analogs of α and β-amino acids, respectively, in which the amine group has been replaced by a hydrazine (Fig. 1). α- and β-hydrazino acid scaffolds can also be considered as analogs of β and γ-amino acids where the C^{β} or C^{γ}-atom is replaced by a nitrogen (Fig. 1) [24]. These structures can mimic the typical secondary structure of native α-peptides, preserving biological activity and enhancing proteolytic stability [25,26] making them useful tools to design new protease inhibitors. Furthermore, the additional nitrogen may allow the formation of additional H-bonds [25,27]. However, to our knowledge, α-hydrazino acid scaffolds have been rarely used in medicinal chemistry and particularly very scarcely introduced within protease inhibitors. α-Hydrazino peptides were reported to inhibit the serine protease leukocyte elastase [25] and retro hydrazino–azapeptoids were recently described as covalent proteasome inhibitors with IC_{50} up to 0.7 μM [28]. Finally, whereas β-amino acids are well documented [29–31] almost nothing is known about the β-hydrazino acid based peptidomimetics.

We previously described preliminary results concerning the first synthesis of CF_3-β-hydrazino acid and the biological evaluation of few compounds based on a central fluorinated β-hydrazino acid scaffold that inhibit the ChT-L activity with IC_{50} values up to 1.6 μM (compounds **1a**, **1b**, **2**, **3a**, **3b**, **4** and **6**, Table 1) [32]. Proteasome inhibition was selective; cytosolic calpain I and lysosomal cathepsin B were not inhibited by compounds **1b**, **3b** and **4** [32]. Using a cell-based chemiluminescent assay, compound **1b** behaved as an inhibitor of the ChT-L activity in human HeLa cells (20% inhibition at 50 μM after 1 h 30 min incubation) [32]. In this present report, we made several changes in the structures of the fluorinated β-hydrazino acid derivatives and we introduced non fluorinated α- and β-hydrazino acid scaffolds. We performed pharmacomodulations around the four moieties of the molecules (Fig. 2) in order to establish structure–activity relationships of this new class of proteasome inhibitors. A library of 40 molecules was designed, synthesized and evaluated on the 20S rabbit proteasome (Fig. 2). At first, we kept in the third moiety the β-hydrazino acid scaffold (C.1. Fig. 2; compounds **5a**, **5b**, **7**, **8**, **9**, **10a**, **10b**, **11a**, **11b**, **12**, **13**, Table 1). We then evaluated the influence of the length of the hydrazino scaffold by replacing the CF_3-β-hydrazino acid scaffold by the

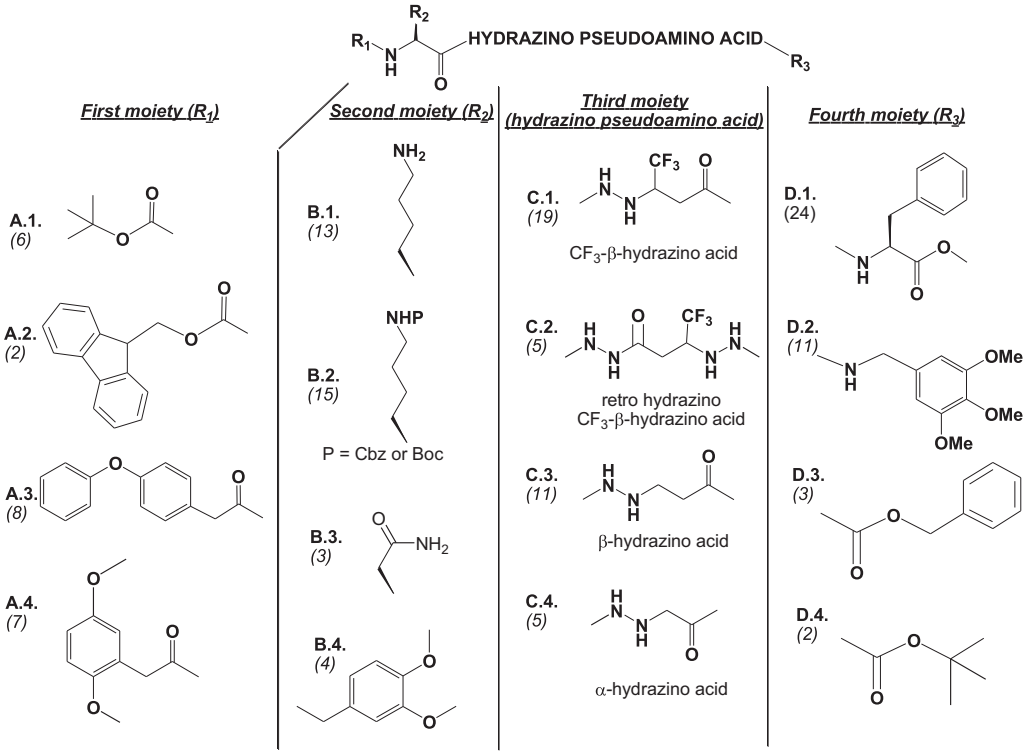

Fig. 2. Schematic representation of hydrazino acid based pseudopeptides. The number of compounds with different A-D groups is indicated in brackets with the numbers of these groups.