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Proteasome inhibition by new dual warhead containing peptido vinyl sulfonyl fluorides

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ABSTRACT

The success of inhibition of the proteasome by formation of covalent bonds is a major victory over the long held-view that this would lead to binding the wrong targets and undoubtedly lead to toxicity. Great challenges are now found in uncovering ensembles of new moieties capable of forming long lasting ties. We have introduced peptido sulfonyl fluorides for this purpose. Tuning the reactivity of this electrophilic trap may be crucial for modulating the biological action. Here we describe incorporation of a vinyl moiety into a peptido sulfonyl fluoride backbone, which should lead to a combined attack of the proteasome active site threonine on the double bond and the sulfonyl fluoride. Although this led to strong proteasome inhibitors, in vitro studies did not unambiguously demonstrate the formation of the proposed seven-membered ring structure. Possibly, formation of a seven-membered covalent adduct with the proteosomal active site threonine can only be achieved within the context of the enzyme. Nevertheless, this dual warhead concept may provide exclusive possibilities for duration and selectivity of proteasome inhibition.

1. Introduction

The development of proteasome inhibitors has been an outstanding case showing that irreversible inhibitors may provide unique advantages by forming long-lived ties with their target.[1] Depending on the degree of reversibility of this covalent interaction, the putative proteasome inhibitor may therefore display a prolonged interaction and biological action. A prolonged interaction may be beneficial when the undesired proteasome activity is manifest for an extended period.[2,3] Together with covalently reacting kinase inhibitors, which contain Michael acceptor moieties, proteasome inhibitors are part of the important arsenal of presently available crucial anti-cancer drugs. Inhibition of the protein degradation pathway in this manner is currently an effective approach for treatment of blood cancers.[4,5] Increasingly, established proteasome inhibitors are evaluated as anti-inflammatory immunoproteasome inhibitors leading to new therapeutic strategies for treatment of auto-immune diseases such as rheumatoid arthritis and multiple sclerosis[6,7] Recently, in collaboration with Groll et al., we have achieved selective inhibition of the immunoproteasome by crosslinking of the active site effected by a peptido sulfonyl fluoride ligand (PSF).[8]

Most proteasome inhibitors contain a single electrophilic moiety capable of covalently interacting with the threonine active site residue.[9] Especially the vinyl sulfone containing proteasome inhibitors have been subject of many investigations. (Scheme 1).[10] These contain a Michael acceptor as an electrophilic moiety.

However, in contrast to serine proteases in which the attacking nucleophile on the peptide-amide bond is solely the hydroxyl of the serine residue present as part of the catalytic triad, in the proteasome the amino acid involved in scission of the peptide-amide bond is an N-terminal threonine residue containing *two* nucleophiles. As a consequence, very effective and selective inhibition has been achieved by proteasome inhibitors having 'dual' warheads, that is containing two electrophilic sites. This is reflected by the treatment of multiple myeloma in patients with the proteasome inhibitor carfilzomib, containing both an epoxide

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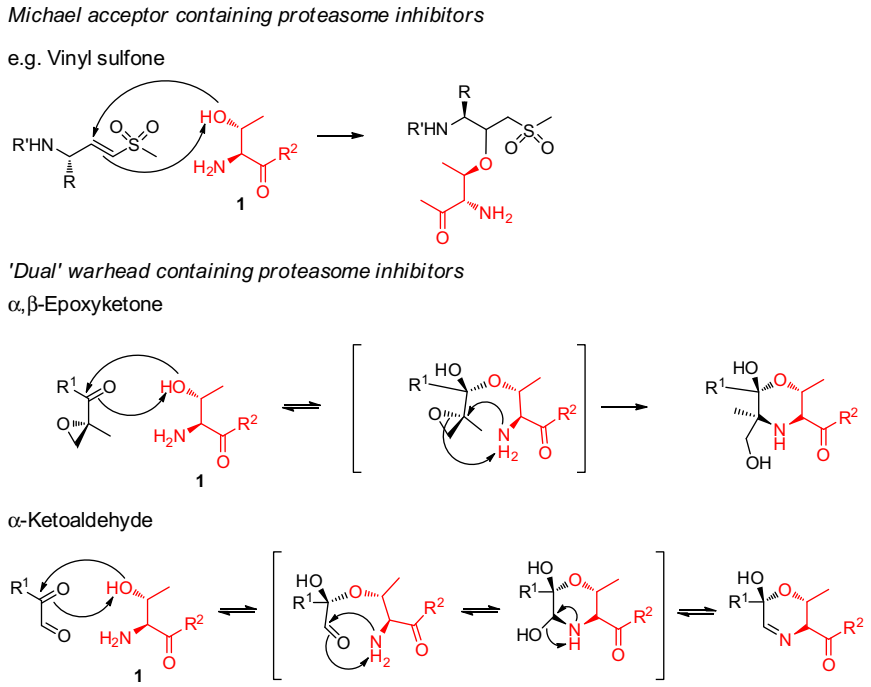

Scheme 1. Mechanisms of covalent inhibition of the proteasome by vinyl sulfones, α-β-epoxyketones and α-ketoaldehydes. The threonine depicted in red represents the N-terminal threonine of the proteasome.

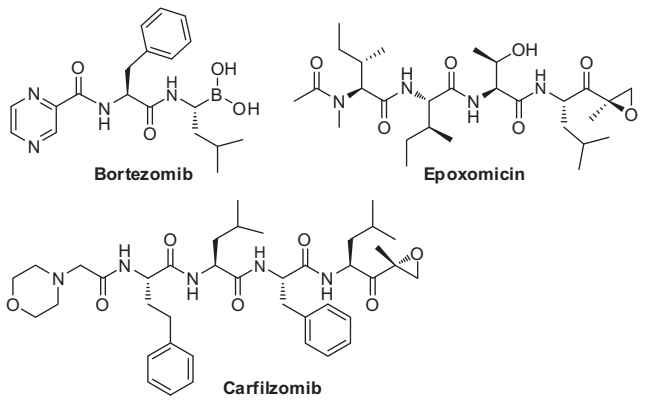

Figure 1. Structures of Bortezomib, Epoxomicin and Carfilzomib.

and carbonyl electrophilic site, after previous treatment with bortezomib, which contains just one electrophilic site (Fig. 1). In our opinion this justifies a quest for dual warhead containing inhibitors such as the one discussed in this research.

Inspired by the dual warhead approach we describe in this paper a new proteasome inhibitor concept in which a Michael electrophilic trap is combined with a sulfonyl fluoride electrophile incorporated into a peptide sequence leading to a peptido vinyl sulfonyl fluoride (PVSF). Both electrophilic traps may then interact with both nucleophilic amino and hydroxyl moieties of the N-terminal threonine residue present in the active site of the proteasome. Other covalently interacting proteasome inhibitors, having two electrophilic sites, including Epoxomicin (Fig. 1) and the alpha keto–aldehyde warhead containing inhibitors, show a similar molecular mechanism of action (Scheme 1).[11,12] However, in the sulfone Michael acceptor containing proteasome inhibitors only the four-position is reacting with the threonine hydroxyl nucleophile (Scheme 1).

2. Results and discussion

2.1. Chemistry

Here we propose the peptido vinyl sulfonyl fluoride (PVSF) as a new and promising *dual* warhead system. It was expected that its molecular structure would allow a Michael reaction leading to a sulfene intermediate followed by an intramolecular reaction of the second nucleophile in the threonine residue leading to a seven-membered ring covalent adduct (Scheme 2).

The synthesis of peptido vinyl sulfonyl fluorides involved employing vinylogous amino sulfonates, which are accessible from amino acid derived aldehydes as was described by Gennari et al. (Scheme 3).[13] Briefly, Cbz-protected leucinol (**2**) was converted into the corresponding amino aldehyde (**3**) by a Swern oxidation. A Wittig–Horner reaction with ethyl diethylphosphoryl methanesulfonate afforded vinyl sulfonate ester **6**, which was cleaved by Bu_4NI. The most efficient conversion of the resulting sulfonate salt (**7**) into the corresponding vinyl sulfonyl fluoride (**8**) was achieved by using XtalFluor-M®[14] in the presence of a catalytic amount of triethylamine trihydrofluoride acting as both a proton and fluoride source.[15] Two PVSF proteasome inhibitors (**10** and **11**, respectively) were obtained after cleavage of the Cbz-group from **8** followed by a coupling reaction with Cbz-Leu_2-OH and Cbz-Leu_3-OH using BOP.

2.2. Biological evaluation

Recently, we described and established the molecular mechanism of action of our peptido sulfonyl fluoride (PSF) proteasome protease inhibitors.[8] It was found that selective inhibition of the immunoproteasome occurred by ligand-induced cross-linking of the active site (Scheme 2). Although PSFs are capable of β5c inhibition, comparison with other warheads highlights the peptido sulfonyl fluoride as a promising motif for β5i targeting. The sequences of inhibitors **10** and **11** were chosen based on earlier results with our most potent PSF proteasome inhibitors **17** and