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Design, synthesis and biological evaluation of novel tripeptidyl epoxyketone derivatives constructed from β-amino acid as proteasome inhibitors

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ABSTRACT

A series of novel tripeptidyl epoxyketone derivatives constructed from β-amino acid were designed, synthesized and evaluated as proteasome inhibitors. All target compounds were tested for their proteasome inhibitory activities and selected compounds were tested for their anti-proliferation activities against two multiple myeloma (MM) cell lines RPMI 8226 and NCI-H929. Among them, eleven compounds exhibited proteasome inhibitory rates of more than 50% at the concentration of 1 μg/mL and nine compounds showed anti-proliferation activities with IC_{50} values at low micromolar level. Compound **20h** displayed the most potent proteasome inhibitory activities (IC_{50}: 0.11 ± 0.01 μM) and anti-proliferation activities with IC_{50} values at 0.23 ± 0.01 and 0.17 ± 0.02 μM against two tested cell lines. Additionally, the polyubiquitin accumulation in the western blot analysis supported that proteasome inhibition in a cellular system was induced by compound **20h**. All these experimental results confirmed that β-amino acid can be introduced as a building block for the development of proteasome inhibitors.

1. Introduction

The functionally active proteasome is a proteolytic complex that is responsible for digestion of about 80–90% intracellular proteins including misfolded and abnormal proteins as well as various regulatory proteins associated with cell growth, differentiation, and apoptosis into short peptides and amino acids.[1–5] The prominent roles of proteasome in protein degradation has made it a promising anti-cancer drug target.[6–8] To date, various proteasome inhibitors have been identified and developed, and a few of them are extensively evaluated in clinical trials with possibilities for clinical applications.[9]

The approval of proteasome inhibitors bortezomib and carfilzomib (Fig. 1) for the treatment of multiple myeloma (MM) validates the close correlation between this proteolytic particle and tumorigenesis, maintenance and progression.[10,11] Compared to bortezomib, the peptidyl epoxyketone compound carfilzomib causes fewer side effects, especially low rates of peripheral neuropathy.[12,13] In addition, carfilzomib was proved to be able to overcome bortezomib resistance in some cancer models due to its longer and irreversible inhibition of the proteasome.[14] Herein, development of carfilzomib analogue with epoxyketone fragment may lead to proteasome inhibitors with enhanced safety profiles.

To best of our knowledge, most proteasome inhibitors approved or under clinical trials are short peptides sharing a similar α-peptide skeleton. It is reported that adding an extra backbone carbon by introducing a β-amino acid building block could improve the biological activity and enzymatic stability of the compound.[15] Besides, Zhu and colleagues reported some β-amino acid contained dipeptidyl boronic acid compounds with similar proteasome inhibitory activities and even better pharmacokinetic profiles in comparison with bortezomib, which validated the remarkable value of this building block.[16,17] In this manuscript, 33 tripeptidyl epoxyketone proteasome inhibitors (Fig. 2) constructed from β-amino acid (i.e. 3-amino-3-phenylpropionic acid in this paper) were synthesized and evaluated, and structure–activity relationships (SARs) were discussed in detail.

2. Results and discussion

2.1. Chemistry

The epoxyketone fragment **7** was synthesized following the method described in the literature with modifications,[18,19] and

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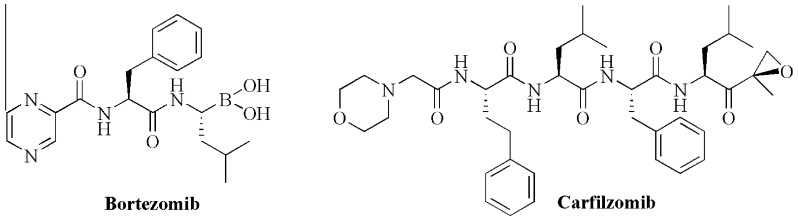


Figure 1. Structures of approved proteasome inhibitors bortezomib and carfilzomib.

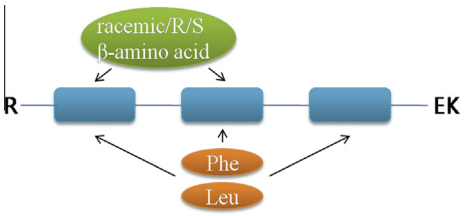


Figure 2. Epoxyketone tripeptidyl compounds constructed from β-amino acid. R: various substitutions at terminal positions of the tripeptides EK: epoxyketone.

the synthetic routes are summarized in Scheme 1. Reaction of *N*-Boc-protected amino acid **1** with *N,O*-dimethylhydroxylamine hydrochloride gave Weinreb amide **2**, which was then treated with isopropenylmagnesium bromide at 0 °C to form the desired α,β-unsaturated ketone **3**.[18] Subsequent reduction of **3** with sodium borohydride and cerium chloride afforded allylic alcohol **4**, which was oxidized into epoxide **5** in the presence of mCPBA.[18,19] The epoxide **5** was not stable enough and was thereby oxidized into epoxyketone **6** directly with Dess–Martin reagent.[18] Afterwards, deprotection of **6** with trifluoroacetic acid resulted in compound **7**.

The synthetic routes for tripeptidyl epoxyketone derivatives are summarized in Schemes 2 and 3. The racemic and enantiomeric *N*-Boc-protected β-amino acids were purchased from commercial sources. Reaction of epoxyketone **7a** or **7b** with corresponding *N*-Boc-protected amino acid furnished dipeptides **8**, **11** or **16**, which were deprotected and treated with various *N*-Boc-protected amino acids again to afford tripeptides **9**, **12**, **14**, **17**, **19** or **21**. Finally, the Boc-protecting group of the tripeptides were removed and the generated products were subjected to reaction with corresponding acid to obtain target compounds **10a–e**, **13a–e**, **15a–e**, **18a–n**, **20f**, **20h**, **22f** and **22h**.

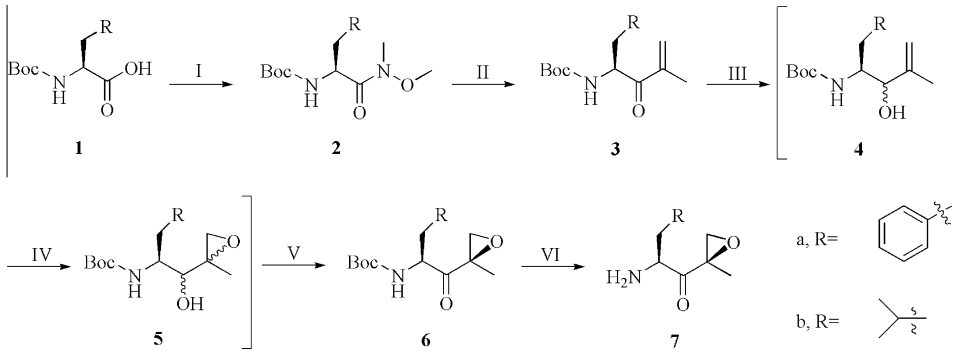
2.2. Proteasome inhibitory activities

The synthesized target compounds were evaluated for their 20S proteasome chymotrypsin-like inhibitory activities in vitro. Bortezomib and carfilzomib were employed as the positive controls. The results are summarized in Tables 1–3.

As shown in Table 1, compounds (**18a–e**) with β-amino acid-Phe-Leu skeleton exhibited the best proteasome inhibitory activities, and four compounds (**18b–e**) showed inhibitory rates of more than 80% at the concentration of 1 μg/mL, with IC_{50} values of 0.34 ± 0.03, 0.44 ± 0.04, 0.38 ± 0.03, and 0.34 ± 0.04 μM, respectively. However, compounds (**10a–e**, **13a–e** and **15a–e**) with β-amino acid in the middle of the tripeptidyl skeleton (Leu-β-amino acid-Phe, Leu-β-amino acid-Leu and Phe-β-amino acid-Leu) displayed weak proteasome inhibitory activities. Therefore, the peptidyl skeleton played important roles in maintaining the activities of this series of compounds.

In order to intensively evaluate the influences of substituents at the end of carboxamide in compounds **18a–e** on proteasome inhibitory activity, compounds **18f–n** with various substituents at the end of carboxamide were synthesized. According to the data of inhibitory activities of compounds **18a–n** (Tables 1 and 2), compounds **18b–h** with phenyl or pyridyl moieties at the end of carboxamide showed more potent activities than that of compounds with five-membered aromatic heterocyclic rings (**18j–k**) and aliphatic heterocyclic rings (**18l–n**) at the end of carboxamide. In addition, various replacements on the phenyl ring (**18c–g**) only have a slight impact on activity.

Further study of the impact of stereo configuration of the β-amino acid on activity was performed with selected two stereo isomers (**20f** vs **22f**; **20h** vs **22h**) of compound **18f** and **18h**. As



Scheme 1. Synthesis of epoxyketone fragments (**7a** and **7b**). Reagents and conditions: (I) HOBt, EDCI, *N,O*-dimethylhydroxylamine hydrochloride, diisopropylethylamine, DCM, 0 °C; (II) isopropenylmagnesium bromide, THF, 0 °C; (III) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, THF, 0 °C–rt; (IV) *m*CPBA, DCM, 0 °C; (V) Dess–Martin periodinane, DCM, 0 °C–rt; (VI) trifluoroacetic acid, DCM, 0 °C–rt.