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Design, synthesis and biological evaluation of novel non-covalent piperidine-containing peptidyl proteasome inhibitors

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ABSTRACT

A series of novel non-covalent piperidine-containing dipeptidyl derivatives were designed, synthesized and evaluated as proteasome inhibitors. All target compounds were tested for their proteasome chymotrypsin-like inhibitory activities, and selected derivatives were evaluated for the anti-proliferation activities against two multiple myeloma (MM) cell lines RPMI 8226 and MM-1S. Among all of these compounds, eight exhibited significant proteasome inhibitory activities with IC_{50} less than 20 nM, and four are more potent than the positive control Carfilzomib. Compound **28** displayed the most potent proteasome inhibitory activity (IC_{50}: 1.4 ± 0.1 nM) and cytotoxicities with IC_{50} values at 13.9 ± 1.8 nM and 9.5 ± 0.5 nM against RPMI 8226 and MM-1S, respectively. Additionally, the ex vivo blood cell proteasome inhibitory activities of compounds **24** and **27**–**29** demonstrated that the enzymatic metabolism in the whole blood could be well tolerated. All these experiments confirmed that the piperidine-containing non-covalent proteasome inhibitors are potential leads for exploring new anti-cancer drugs.

1. Introduction

The ubiquitin–proteasome pathway (UPP), which regulates various critical mediators of different signaling pathways, is essential for maintaining normal cell function and cellular homeostasis.[1–4] This proteolytic pathway includes several components: ubiquitin, ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), ubiquitin protein ligase (E3), deubiquitinating enzyme (DUB) and the dominant proteasome, most of which have been proved to be potential drug targets.[5–8] The 26S proteasome, a large protein complex with multiple proteolytic activities, is consisted of a 4 stacked ring-formed 20S cylindrical core (α7–β7–β7–α7) and two 19S regulatory particles.[9,10] Three β subunits (β1, β2 and β5) exhibit caspase-like (C-L), trypsin-like (T-L) and chymotrypsin-like (CT-L) activities, respectively.[10] Among all of these potential targets, the validated anti-cancer drug target proteasome has played critical roles in discovery of multiple myeloma (MM) therapy drugs.[11,12] To date, three proteasome inhibitors Bortezomib, Carfilzomib and Ixazomib (Fig. 1) have been approved for the treatment of MM.[13–15] Besides, several other proteasome inhibitors are being extensively evaluated in various clinical trials.[10]

Proteasome inhibitors can be classified into covalent and non-covalent types due to different structure scaffolds and binding modes with proteasome.[16] Most proteasome inhibitors approved or in clinical trials are covalent ones. The firm covalent interactions ensure these small molecular ligands with potent and lasting proteasome inhibitory activities, but may induce severe side effects and limit their tissue distribution for lack of specificity and excessively reactive.[17–19] Non-covalent proteasome inhibitors may offer more therapeutic advantages due to their more widespread tissue distribution compared to the covalent inhibitors.[19,20] Although non-covalent proteasome inhibitors are less well studied, the history of these analogues is as long as that of the covalent inhibitors. CVT-659 (Fig. 2) is the forerunner of this kind of inhibitors with an IC_{50} of 140 nM.[21] Additionally, a trimethoxy-L-phenylalanine-containing dipeptide (**2**, Fig. 2) was reported with potent and selective chymotrypsin-like activity together with moderate cytotoxicity.[22] Besides, Blackburn and colleagues described a series of di- and tripeptides (e.g., **3** and **4**, Fig. 2) with both potent constitutive proteasome and immunoproteasome inhibitory activities.[19]

Since most of the reported non-covalent proteasome inhibitors are short peptides, the enzymatic stability of these compounds

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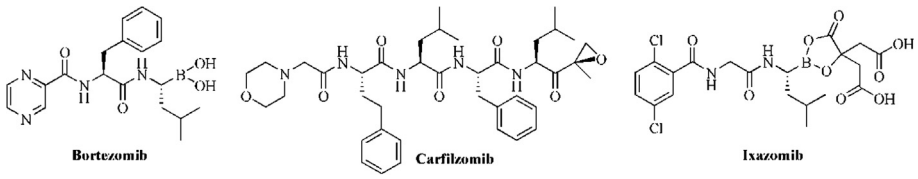

Figure 1. Structures of the three approved proteasome inhibitors.

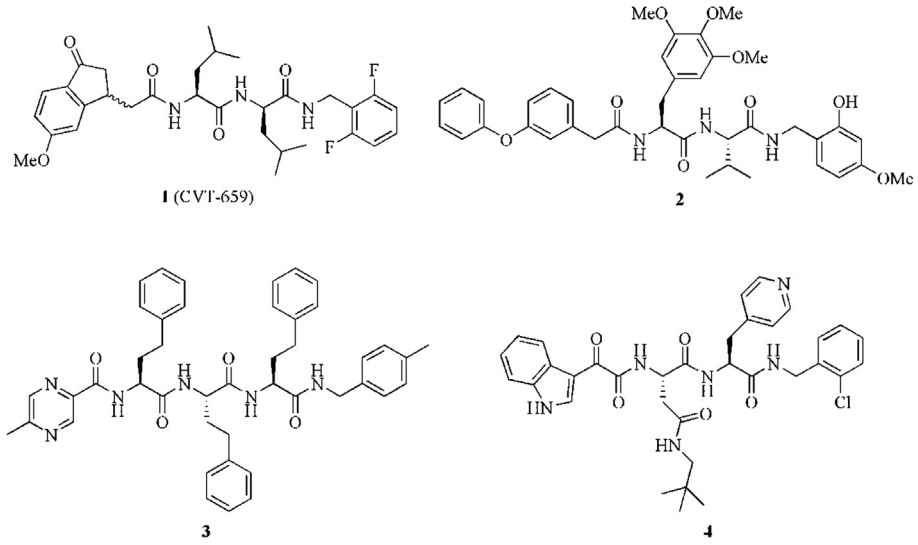

Figure 2. Structures of representative non-covalent proteasome inhibitors.

should be well concerned.[23] Introducing a non-peptide fragment into the peptide skeleton may solve this problem and increase the pharmacokinetic properties of the target compounds. This is mainly owing to the specificity of proteases and peptidases against peptide bond between peptide fragments. In this manuscript, a series of piperidine-containing non-covalent proteasome inhibitors (Fig. 3) were synthesized and evaluated, and structure–activity relationships (SARs) were discussed in detail.

2. Results and discussion

2.1. Chemistry

The synthetic route for piperidine-containing fragments **9** and **10a–g** are summarized in Scheme 1. Fragment **7** can be easily obtained by condensation from pyrazine-2-carboxylic acid with 1-Boc-4-aminopiperidine. However, synthesis of the similar arylcarbamoyl piperidine derivatives **8a–g** were more difficult, in which the *N*-Boc-4-piperidinecarboxylic acid should first be transformed to the acyl chloride at the presence of $SOCl_2$, then reacted with corresponding arylamine **6a–g** to afford the products.[24] Afterwards, deprotection of **7** and **8a–g** with trifluoroacetic acid (TFA) resulted in piperidine TFA salts **9** and **10a–g**.

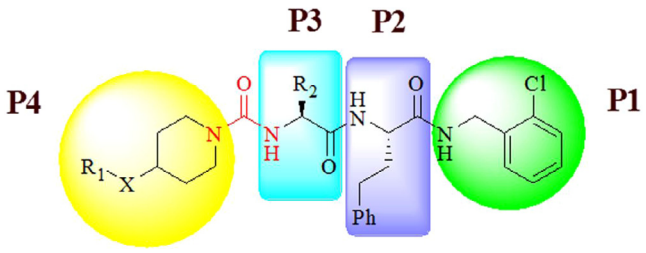

Figure 3. Non-peptide fragment (piperidine) constructed non-covalent proteasome inhibitors.

The target compounds **16–29** were synthesized following the method described in Scheme 2. Reaction of (2-chlorophenyl) methanamine with Boc-L-hPhe furnished compound **12**, which was deprotected, treated with various Boc-protected amino acid and deprotected again to afford dipeptide TFA salts **15a–d**. Subsequently, the primary amine **15a–d** were first transformed to corresponding isocyanate intermediates, which were not stable enough and were thereby reacted with piperidine fragments **9** and **10a–g** to obtain target compounds **16–29**.[25]

2.2. Proteasome inhibitory activities

The synthesized target compounds were evaluated for their 20S proteasome chymotrypsin-like inhibitory activities in vitro. Carfilzomib was employed as the positive control. The results are summarized in Table 1.

As illustrated in Table 1, most target compounds showed potent proteasome inhibitory activities with IC_{50} lower than 100 nM, and 4 compounds were even lower than 10 nM, which indicated that the activities of these compounds were well maintained after introducing the piperidine ring into the peptide skeleton. Different substituents at P2 position (R_2) influenced the activity obviously. Iso-butyl and phenylethyl substituted analogues (**18**, **19**, **22** and **23**) exhibited much more potent activities than methyl and benzyl substituted compounds (**16**, **17**, **20** and **21**), with IC_{50} values of 16.6 ± 1.7 nM, 45.7 ± 1.1 nM, 14.7 ± 2.4 nM and 23.7 ± 3.5 nM, respectively. Phenylethyl showed the superiority and was selected at the P2 position for further optimization. The linker between the