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Design, synthesis and docking studies of novel dipeptidyl boronic acid proteasome inhibitors constructed from αα- and αβ-amino acids

Jingmiao Shi [a,b,†], Meng Lei [c,†], Wenkui Wu [d], Huayun Feng [c], Jia Wang [e], Shanshan Chen [e], Yongqiang Zhu [d,*], Shihe Hu [e], Zhaogang Liu [e], Cheng Jiang [a,b,*]

[a] Jiang Su Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 210009, China
[b] Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China
[c] College of Science, Nanjing Forestry University, Nanjing 210037, China
[d] College of Life Science, Nanjing Normal University, Nanjing 210023, China
[e] Jiangsu Chia Tai Fenghai Pharmaceutical Co. Ltd, Nanjing 210037, China

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ABSTRACT

A series of novel dipeptidyl boronic acid proteasome inhibitors constructed from αα- and αβ-amino acids were designed and synthesized. Their structures were elucidated by ^{1}H NMR, ^{13}C NMR, LC–MS and HRMS. These compounds were evaluated for their β5 subunit inhibitory activities of human proteasome. The results showed that dipeptidyl boronic acid inhibitors composed of αα-amino acids were as active as bortezomib. Interestingly, the activities of those derived from αβ-amino acids lost completely. Of all the inhibitors, compound **22** (IC_{50} = 4.82 nM) was the most potent for the inhibition of proteasome activity. Compound **22** was also the most active against three MM cell lines with IC_{50} values less than 5 nM in inhibiting cell growth assays. Molecular docking studies displayed that **22** fitted very well in the β5 subunit active pocket of proteasome.

The ubiquitin-proteasome pathway (UPP) plays a critical role in recognizing and degrading abnormal and misfolded proteins.[1,2] In this pathway, the 26S proteasome is the main proteolytic component, which contains two ATP-dependent 19S regulatory particles (RPs) and one 20S core particle (CP). The catalytic 20S CP consists of 28 protein subunits which arrange in $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$ four stacked rings.[3,4] In eukaryotic proteasomes, three potent proteolytic activities are harbored in β-subunits and are classified as caspase-like (PGPH, β1 subunit), trypsin-like (T-L, β2 subunit), and chymotrypsin-like (CT-L, β5 subunit), respectively. All these active centers are related to N-terminal threonine residue (O^{γ}-Thr1), which acts as a nucleophile in peptide bond hydrolysis.[5]

Bortezomib (Fig. 1), a dipeptidyl boronic acid proteasome inhibitor, covalently interact with the nucleophilic oxygen lone pair of the residue O^{γ}-Thr1 of 20S proteasome. It is noteworthy to point out that bortezomib prefers to target β5 active site rather than β2 and β1 active sites (β5 > β2>>β1),[6] showing reversible inhibition of CT-L activity. Although bortezomib is now used in clinics for the treatment of relapsed and refractory multiple myeloma (MM) patients, it can cause some severe side effects in peripheral nerve, cardiovascular system and gastrointestinal tract.[7–10] Therefore, our effort has been devoted to design and synthesis of a series of dipeptidyl boronic acid proteasome inhibitors containing αα- and βα-amino acid building blocks in the past few years, hoping to overcome the reported side effects. Three potent drug-like candidates **A**, **B** and **C** were screened (Fig. 1).

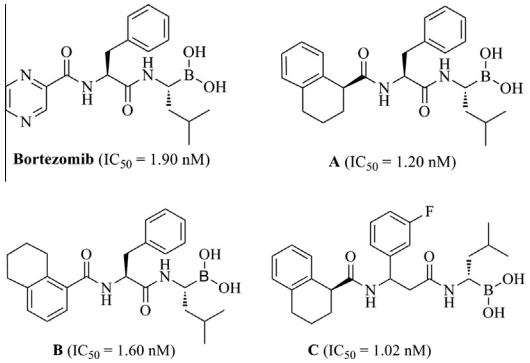

Figure 1. Structures of bortezomib and drug-like candidates **A**, **B**, and **C**.

In our previous study, compounds constructed from βα-peptides (such as candidate **C**) showed longer half-life and less toxicity than

* Corresponding authors. Tel.: +86 25 85891591.
E-mail addresses: zhyqscu@hotmail.com (Y. Zhu), jc@cpu.edu.cn (C. Jiang).
† These two authors contributed equally to this work.

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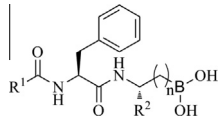


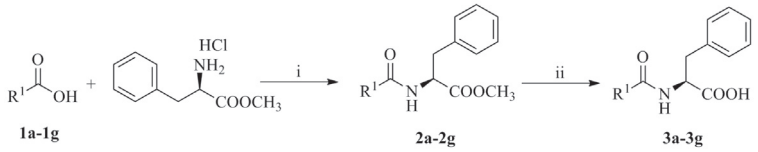
Figure 2. General structure of final compounds (n = 1 or 0).

bortezomib because of the employment of β-unnatural amino acid in the backbone.[11,12] To further explore the structure–activity relationship (SAR) of such kind of compounds, we designed a series of dipeptidyl boronic acid proteasome inhibitors containing αβ-amino acids (Fig. 2, n = 1). Most of our previous work focused on the aromatic-substituted analogues for R^1 position (Fig. 2); but in one case, the substitution of pyrazinyl group on bortezomib by a methyl group maintained inhibitory activity.[13] Encouraged by this, we designed a series of dipeptidyl boronic acid proteasome inhibitors constructed from αα-amino acids with aliphatic substitutions at R^1 position (Fig. 2, n = 0). Herein we report the synthesis and in vitro inhibitory activity of these new compounds. In order to understand ligand–protein interaction mode, molecular docking study was carried out.

As shown in Scheme 1, compounds with aromatic substitutions at R^1 position (**2a**–**2b**) were readily synthesized by common peptide synthesis methods using 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDCI) as a coupling agent and *N*,*N*-diisopropylethylamine (DIPEA) as a base. Compounds substituted with aliphatic groups at R^1 position (**2c**–**2g**) were prepared from corresponding acyl chloride with triethylamine as a base. After saponification and acidification of methyl esters **2a**–**2g**, acids **3a**–**3g** were obtained in good to excellent yields.

The synthesis of β-amino boronates **10a**–**10d** hydrochlorides was critical steps in the preparation of target compounds (Scheme 2). Corresponding boronic acids reacted with (+)-pinanediol to form the corresponding borate ester **5a**–**5c** in 92–98% yields. Then **5a**–**5c** underwent a Matteson homologation–alkylation reaction[14,15] to give the desired intermediates **6a**–**6c** in excellent yields. Sodium azide was used to build α-azide containing borate esters **7a** and **7b** in 92% and 91% yields, respectively. With **7a** and **7b** in hand, β-azide containing borate esters **8a** and **8b** were obtained through a second Matteson homologation–alkylation reaction in 81% and 70%, respectively, which were then reduced to **9a** and **9b** in high yields. After reduction of azide, β-amino boronates **10a** and **10b** were obtained in low to moderate yields. However, for phenyl substituted intermediate **10c**, an attempt to prepare it according to the same method as **10a** and **10b** was not successful. So we employed another synthetic route (Scheme 2). Treatment of monochlorosubstituted boronate **6c** with lithium bis(trimethylsilyl) amide gave protected amine **7c**, which was directly used for the next step without further purification. Insertion of a methylene in compound **7c** was performed at −78 °C with chloroiodomethane to produce **8c** according to a reported method.[16] Deprotection of the trimethylsilyl group in **8c** afforded amino boronate **10c**. And α-amino boronate **10d** was obtained from commercial source.

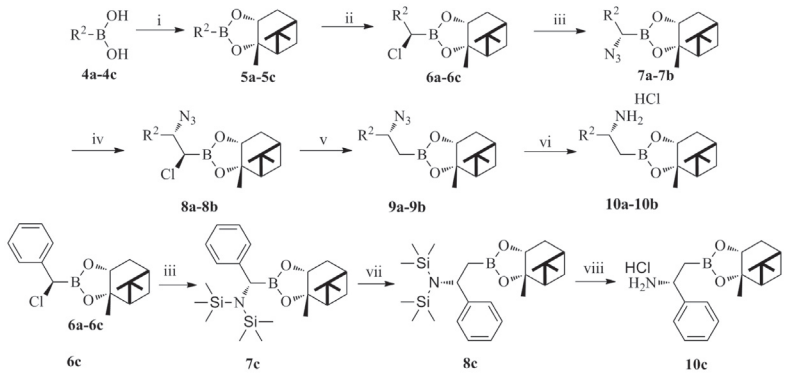
Coupling of amino boronates **10a**–**10d** with various acids **3a**–**3g** in the presence of EDCI, HOBt and DIPEA gave dipeptidyl boronates



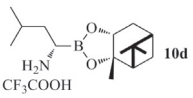
1a-1g **2a-2g** **3a-3g**

1a-3a: R^1=Phenyl; **1b-3b**: R^1=Pyrazin-2-yl; **1c-3c**: R^1=Methoxy methyl; **1d-3d**: R^1=Methoxy ethyl; **1e-3e**: R^1=n-Propyl; **1f-3f**: R^1=Neopentyl; **1g-3g**: R^1=Cyclopentyl.

Scheme 1. Synthesis of N-terminal-protected α-amino acids **3a**–**3g**. Reagents and conditions: (i) **2a**–**2b**: EDCI, HOBt, DIPEA, DCM, −15 °C to rt, 10 h; **2c**–**2g**: $SOCl_2$, Et_3N, DCM, −15 °C to rt, 4 h; (ii) (1) $LiOH{\cdot}H_2O$, $MeOH/H_2O$, rt, 14 h; (2) 1 M HCl, rt.



4a-10a: R^2 = Isobutyl; **4b-10b:** R^2 = Phenethyl; **4c-6c**: R^2= Phenyl.



Scheme 2. Synthesis of β-amino boronates **10a**–**10c** hydrochlorides. Reagents and conditions: (i) (+)-pinanediol, EA, rt, 7 h; (ii) *n*-BuLi, anhydrous DCM, anhydrous $ZnCl_2$, THF, −78 °C to rt, 2 h; (iii) **7a**–**7b**: NaN_3, $(Bu)_4N^+Br^-$, DCM/H_2O, rt, 10 h; **7c**: $LiN(SiMe_3)_2$, THF, −78 °C to rt, 20 h; (iv) *n*-BuLi, anhydrous DCM, anhydrous $ZnCl_2$, THF, −78 °C to rt, 2 h; (v) $LiBH(C_2H_5)_3$, THF, 0 °C to rt, 7 h; (vi) (1) $LiAlH_4$, THF, −78 °C to rt, 20 h. (2) 4.5 M HCl in Et_2O, −20 °C to rt, 3 h; (vii) *n*-BuLi, ICH_2Cl, THF, −78 °C to rt, 5 h; (viii) 4.5 M HCl in Et_2O, −78 °C to rt, 5 h.

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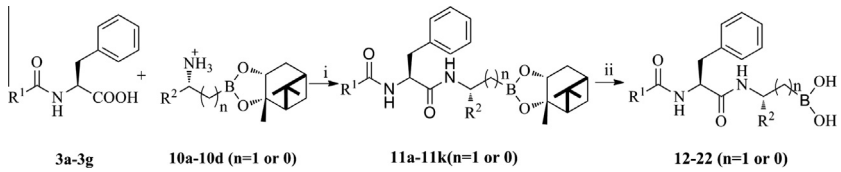
11a–**11k**, which were used directly for acid-catalyzed transesterification with isobutyl boronic acid to provide target compounds **12**–**22** in good yields (Scheme 3).

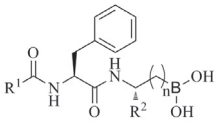
The CT-L inhibitory activities of 20S human proteasome of target compounds were evaluated and bortezomib was used as a control (results shown in Table 1). To our great surprise, dipeptides constructed from αβ-amino acids ($n = 1$) exhibited no inhibitory activities whether substituents at R^2 position with aliphatic groups such as isopropyl (**12**, **13**), phenylethyl (**14**, **15**) or aromatic phenyl groups (**16**, **17**). So an α-amino acid substituent (generally leucine) at R^2 position was critical for the activities, which was consistent with previous report.[13] So we next investigated the activities of the aliphatic substituents at R^1 position with isopropyl group at R^2 position. Some groups, such as alkoxys (methoxymethyl in compound **18** and methoxyethyl in compound **19**), linear alkyl (*n*-propyl in compound **20**), branched alkyl (neopentyl in compound **21**), naphthenic hydrocarbon (cyclopentyl in compound **22**) were selected to investigate SAR. The data in Table 1 revealed that compounds with alkyl groups at R^1 position (**18**–**22**) were as active as bortezomib (all IC_{50} values less than 10 nM). These results demonstrated that aliphatic substituents were also active building blocks for dipeptidyl boronic acid proteasome inhibitors.

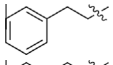
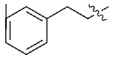
Next the effects of compounds **18**–**22** in inhibiting cells growth were tested in three multiple myeloma cell lines U266, RPMI8226 and ARH77 (Table 2). The result showed that the compounds exhibited effective cytotoxicities with IC_{50} less than 10 nM and



Scheme 3. Synthesis of dipeptidyl boronic acids **12**–**22**. Reagents and conditions: (i) EDCI, HOBt, DIPEA, DCM, −15 °C to rt, 10 h; (ii) isobutylboronic acid, 1 M HCl, MeOH/hexane, rt, 6 h.

Table 1
CT-L inhibitory activities of compounds **12**–**22** and cytotoxicities of compounds **18**–**22**



Compds	*n*	R^1	R^2	Enzymatic assays	Cellular assays[b] (IC_{50}, nM)		
				IC_{50} (nM)[a]	U266	RPMI8226	ARH77
12	1			NA[c]			
13	1			NA			
14	1			NA			
15	1			NA			
16	1			NA			
17	1			NA			
18	0			6.31	6.04	4.56	5.72
19	0			8.47	11.5	7.32	9.03
20	0			6.94	8.51	5.61	7.87
21	0			4.69	8.01	4.54	8.31
22	0			4.82	2.48	2.29	3.87
Bortezomib[d]	0			7.09	5.73	3.88	6.07

[a] Each enzymatic IC_{50} determination was performed with eight concentrations, and each assay point was determined in duplicate.
[b] Each cellular IC_{50} determination was performed with ten concentrations, and each assay point was determined in triplicate.
[c] NA, not active.
[d] IC_{50} value obtained for bortezomib under our experimental conditions.

Table 2
Results of covalent docking between proteasome and bortezomib, compounds **13** and **22**

Compds	Glide Gscore (absolute value)	Cdock affinity (absolute value)	B–O$^{\gamma}$ bond distance (Å)
13	4.78	5.54	3.39
22	8.03	6.62	2.26
Bortezomib	8.20	7.07	2.17

correlated well with enzymatic results, suggesting that these were probably not off-target effects. Due to its excellent enzymatic and cellular activities, compound **22** was selected for further devaluation for its in vivo efficacy in animal models.

In order to better understand the binding mode of these two types of compounds with proteasome, molecular docking was performed for compounds **13** and **22** using Covalent Dock module in Schrodinger.[17] The structure of proteasome was downloaded from the PDB (code: 2F16). In covalent docking calculation, the reaction residue and reaction type were set as 'Thr1' and 'boronic acid addition reaction', respectively. The output parameters were shown in Table 2.

Compared with bortezomib and compound **22**, inhibitor **13** had the lowest Cdock affinity (5.54) and longest B–O$^{\gamma}$ bond distance (3.39 Å), which was not a covalent bond. Furthermore, compound **13** had an additional methylene group between boronic acid pharmacophore and R^2 substituent, which made the peptide backbone much longer and changed the interaction mode of phenyl and pyrazine-2-carboxyl moieties with the residues in the β5 subunit (Fig. 3A), while the two moieties of compound **22** almost took the same interaction mode as bortezomib (Fig. 3B). Moreover, as shown in Figure 3C, five hydrogen bonds were formed between proteasome and compound **13**, four of which were formed between pharmacophore boronic acid and residues (Thr1, Thr168 and Lys33) and only one hydrogen bond was bridged between peptide backbone moiety and residue Thr21O, which could not fix the backbone in an effective interaction mode.

For compound **22**, the boron atom covalently interacted with the nucleophilic oxygen lone pair of the residue O$^{\gamma}$-Thr1 to form a tetrahedral adduct (B–O$^{\gamma}$ 2.26 Å). And the two hydroxyl groups of boronic acid formed hydrogen bonds with residues Arg19, Thr1 and Lys33 to strengthen the tetrahedral adduct. Figure 3D showed that the backbone of compound **22** was also stabilized by other four hydrogen bonds with the conserved residues

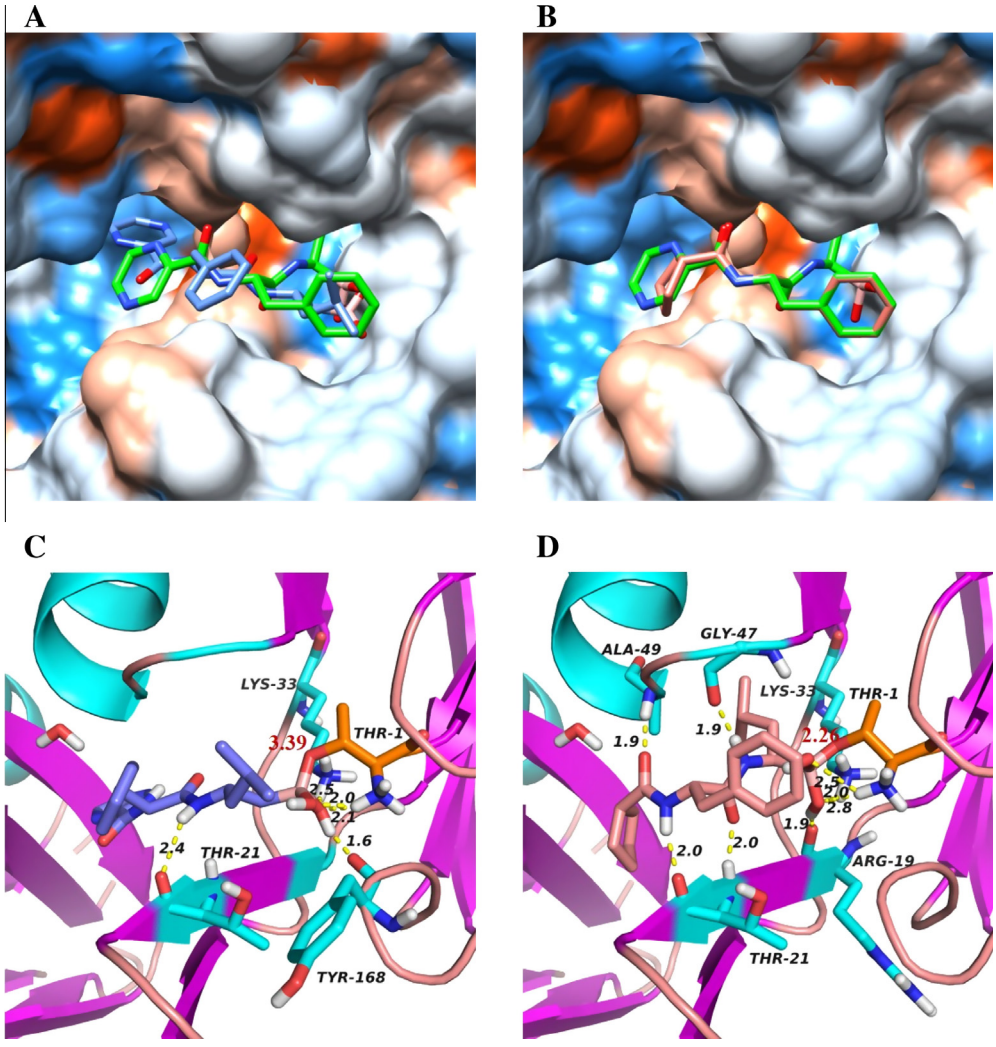


Figure 3. Binging models of bortezomib (green), compound **13** (slate) and compound **22** (pink) in the active site of β5 subunit of proteasome. For A and B: Hydrophobic surface was shown in pink, hydrophilic surface in blue. For C and D: Thr1 was shown in orange, and other key residues were shown in cyan, hydrogen bonds in yellow; compounds and key residues were shown in stick, protein in cartoon. (A) binding of bortezomib and compound **13**; (B) binding of bortezomib and compound **22**; (C) interactions between compound **13** and residues; (D) interactions between compound **22** and residues.

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(Gly47, Thr21N, Thr21O and Ala49). Compared with bortezomib, compound **22** adopted cyclopentyl instead of the pyrazine-2-carboxyl at R^1 position, and this variation did maintain the hydrophobicity, which made compound **22** form strong hydrophobic interactions with the residues Ala20, Ala22, Val26, and Ala27 of the deep hydrophobic pocket. All these interactions made compound **22** fit very well in the active pocket, which was consistent with the results of inhibitory activity. All the theoretical results displayed that the interactions between proteasome and compound **13** were much weaker than **22**, explaining the fact that compound **13** was less active than bortezomib and compound **22**.

In conclusion, a series of dipeptidyl boronic acid derivatives constructed from αα- and αβ-amino acids were synthesized and evaluated for their proteasome inhibition. The inhibitors containing αβ-amino acids were inactive, while those with αα-ones were as potent as bortezomib. Molecular docking studies demonstrated that pharmacophore boronic acid of proteasome inhibitors constructed from αβ-amino acids could not form covalent bond with O^{γ}-Thr1 and the backbone of αβ-dipeptidyl boronic acid inhibitors was prolonged and could not form more effective hydrogen bonds than those derived from αα-amino acids. Among all of the new compounds, inhibitor **22** with aliphatic substituent at R^1 group showed excellent activities in both proteasome inhibition and cytotoxicities against three MM tumor cell lines, deserving further evaluation for its in vivo efficacy in animal models.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.007.

References and notes

1. Groll, M.; Huber, R. *Methods Enzymol.* **2005**, *398*, 329.
2. Hershko, A.; Ciechanover, A. *Annu. Rev. Biochem.* **1998**, *67*, 425.
3. Groll, M.; Ditzel, L.; Lowe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463.
4. Lowe, J.; Stock, D.; Jap, B.; Zwickl, P.; Baumeister, W.; Huber, R. *Science* **1995**, *268*, 533.
5. Brannigan, J. A.; Dodson, G.; Duggleby, H. J.; Moody, P. C.; Smith, J. L.; Tomchick, D. R.; Murzin, A. G. *Nature* **1995**, *378*, 416.
6. Berkers, C. R.; Verdoes, M.; Lichtman, E.; Fiebiger, E.; Kessler, B. M.; Anderson, K. C.; Ploegh, H. L.; Ovaa, H.; Galardy, P. J. *Nat. Methods* **2005**, *2*, 357.
7. Orlowski, R. Z.; Zeger, E. L. *Expert Opin. Invest. Drugs.* **2006**, *15*, 117.
8. Dorsey, B. D.; Iqbal, M.; Chatterjee, S.; Menta, E.; Bernardini, R.; Bernareggi, A.; Cassara, P. G.; D'Arasmo, G.; Ferretti, E.; De Munari, S.; Oliva, A.; Pezzoni, G.; Allievi, C.; Strepponi, I.; Ruggeri, B.; Ator, M. A.; Williams, M.; Mallamo, J. P. *J. Med. Chem.* **2008**, *51*, 1068.
9. Richardson, P. G.; Barlogie, B.; Berenson, J.; Singhal, S.; Jagannath, S.; Irwin, D.; Rajkumar, S. V.; Srkalovic, G.; Alsina, M.; Alexanian, R.; Siegel, D.; Orlowski, R. Z.; Kuter, D.; Limentani, S. A.; Lee, S.; Hideshima, T.; Esseltine, D. L.; Kauffman, M.; Adams, J.; Schenkein, D. P.; Anderson, K. C. *N. Engl. J. Med.* **2003**, *348*, 2609.
10. Ludwig, H.; Khayat, D.; Giaccone, G.; Facon, T. *Cancer* **2005**, *104*, 1794.
11. Zhu, Y.; Zhu, X.; Wu, G.; Ma, Y.; Li, Y.; Zhao, X.; Yuan, Y.; Yang, J.; Yu, S.; Shao, F.; Li, R.; Ke, Y.; Lu, A.; Liu, Z.; Zhang, L. *J. Med. Chem.* **2010**, *53*, 1990.
12. Zhu, Y.; Wu, G.; Zhu, X.; Ma, Y.; Zhao, X.; Li, Y.; Yuan, Y.; Yang, J.; Yu, S.; Shao, F.; Lei, M. *J. Med. Chem.* **2010**, *53*, 8619.
13. Zhu, Y.; Zhao, X.; Zhu, X.; Wu, G.; Li, Y.; Ma, Y.; Yuan, Y.; Yang, J.; Hu, Y.; Ai, L.; Gao, Q. *J. Med. Chem.* **2009**, *52*, 4192.
14. Matteson, D. S.; Ray, R. *J. Am. Chem. Soc.* **1980**, *102*, 7590.
15. Matteson, D. S.; Sadhu, K. M. *J. Am. Chem. Soc.* **1983**, *105*, 2077.
16. Gorovoy, A. S.; Gozhina, O.; Svendsen, J. S. *J. Pept. Sci.* **2013**, *19*, 613.
17. Zhu, K.; Borrelli, K. W.; Greenwood, J. R. *J. Chem. Inf. Model.* **2014**, *54*, 1932.