Bioorganic & Medicinal Chemistry Letters 19 (2009) 2343–2345

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of boronic acid derivatives of tyropeptin: Proteasome inhibitors

Takumi Watanabe [a,*], Isao Momose [b,*], Masatoshi Abe [a], Hikaru Abe [a], Ryuichi Sawa [a], Yoji Umezawa [a], Daishiro Ikeda [b], Yoshikazu Takahashi [a], Yuzuru Akamatsu [a]

[a] Molecular Structure Research Group, Microbial Chemistry Research Center, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan
[b] Numazu Bio-Medical Research Institute, Microbial Chemistry Research Center, 18-24 Miyamoto, Numazu 410-0301, Japan

ARTICLE INFO

Article history:
Received 19 November 2008
Revised 9 February 2009
Accepted 11 February 2009
Available online 4 March 2009

Keywords:
Proteasome
Inhibitor
Boronic acid
Centrifugal liquid–liquid partition chromatography

ABSTRACT

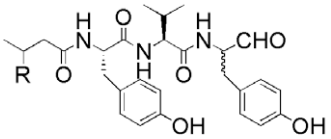
Boronic acid derivatives of tyropeptin were synthesized with TP-110 as the lead compound. Due to the lability of the aminoboronic acid moiety, careful design of the deprotection and coupling sequence was required. Liquid–liquid partition chromatography was found to be a powerful tool for purification of compounds of this class. The obtained derivatives showed potent inhibitory activities against the human 20S proteasome in vitro.

The proteasome, a multicatalytic threonine protease, is responsible for ubiquitin-dependent nonlysosomal proteolysis.[1] This enzyme has three distinct active sites that are individually responsible for the chymotryptic, peptidyl-glutamyl peptide-hydrolyzing (PGPH), and tryptic proteolytic activities.[2] Among these, the chymotrypsin-like activity is of greatest interest, and much research in medicinal chemistry has focused on it.[3,4]

Elevated levels of the proteasome have been implicated in many diseases including cancer. In fact, it has been reported that the anticancer activity of proteasome inhibitors is due to inhibition of the transcriptional factor NF-κB;[5,6] stabilization of p21, p27, and p53;[7,8] and suppression of the unfolded protein response (UPR).[9] Thus, proteasome inhibitors are recognized as promising anticancer agents.[10,11]

Previously, we reported the isolation and structural determination of the novel proteasome inhibitors tyropeptins A and B (**1a** and **1b**) produced by *Kitasatospora* sp. MK993-dF2.[12,13] Tyropeptins have a tripeptide structure with a formyl functionality at the P1 position, which can form a hemiacetal with the nucleophilic hydroxyl group in the catalytic site of the proteasome. An SAR study of tyropeptins was carried out later in which more potent compounds that were selective inhibitors of chymotryptic activity were designed and synthesized.[14] It is noteworthy that TP-110, one of these derivatives, exhibited outstanding growth inhibitory activity against human prostate cancer PC-3 cells. Encouraged by these results, another SAR study was performed in which TP-110 was used as the lead compound (Fig. 1).

In 2003, FDA approved the first clinical use of the proteasome inhibitor bortezomib **4** (also referred to as PS-341, Velcade®) for the treatment of multiple myeloma. The most characteristic structural feature of this compound is the presence of the boronic acid moiety, a functional group found in various bioactive agents.[15,16] A report by Adams et al.[17] showed that in comparison with the antagonistic activity of the aldehyde congener, bortezomib is a >2500-fold more potent antagonist of the chymotrypsin-like activ-



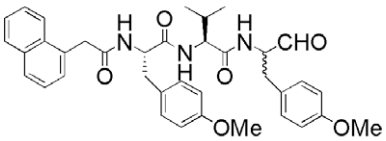
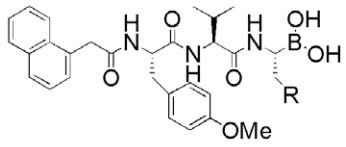


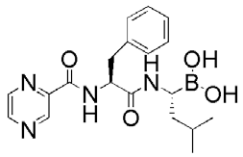
Figure 1. Structure of tyropeptins (**1a**, **1b**) and TP-110 (**2**).

* Corresponding authors. Tel.: +81 3 3441 4173; fax: +81 3 3441 7589 (T.W.), tel.: +81 55 924 0601; fax: +81 55 922 6888 (I.M.).
E-mail addresses: twatanabe@bikaken.or.jp (T. Watanabe), imomose@bikaken.or.jp (I. Momose).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.02.117



3a (R=4-MeO-Ph), **3b** (R=Ph), **3c** (R=*i*-Pr)



Bortezomib (**4**)

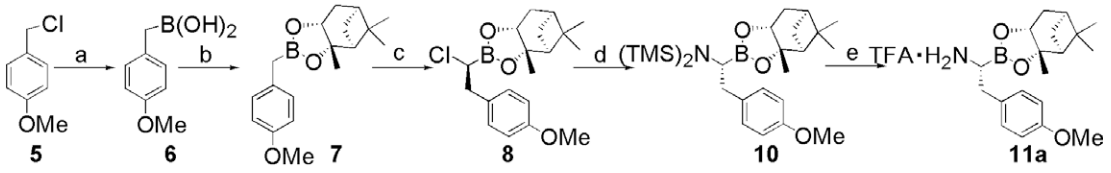
Figure 2. Boronic acid derivatives of tyropeptin (**3a–c**) and bortezomib (**4**).

ity of the proteasome. The boronate moiety can also form covalent bonds with the nucleophilic hydroxyl group in the catalytic site of the proteasome. The remarkable enhancement of inhibition is due to the slow dissociation of oxygen from boron. Inspired by the results of this study, we synthesized tyropeptin boronic acid derivatives, as shown in Figure 2 (**3a–c**). Apart from **3a**, which was designed on the basis of TP-110, other structurally related analogs were prepared that had Leu- or Phe-boronic acid as the P1 residue (**3b** and **3c**, respectively). By comparing the proteasome-inhibitory activities of all these compounds, we expected to clarify the mode of interaction between the S1 cavity of the proteasome and P1 side chains of the inhibitors.

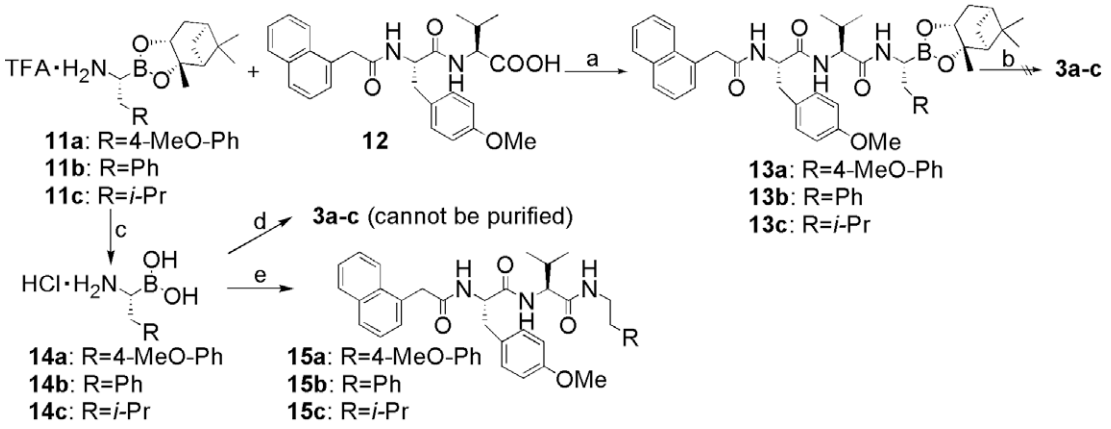
Scheme 1 illustrates the synthesis of Tyr(Me)-boronic acid **11a** from 4-methoxybenzyl chloride **5**.[18,19] The Grignard reagent generated from **5** was treated with triethylborate, and subsequent hydrolysis with H_2SO_4 resulted in 4-methoxybenzylboronic acid **6**. Subsequent esterification with (+)-pinanediol afforded **7**. A stereoselective one-carbon homologation with $LiCHCl_2$ employing the pinane part as the chiral auxiliary resulted in chloride **8** with >98:2 diastereoselectivity. The absolute configuration of the newly introduced stereocenter of the major isomer was determined to be *S* by converting **8** into the reported compound **9** (1-(4-methoxyphenyl)-2-butanol) and comparing their signs of optical rotation.[20,21] The chlorine atom of **8** was then substituted with a nitrogen functionality in an S_N2 manner by treatment with LiHMDS. Acidic desilylation of **10** resulted in the desired Tyr(Me)-boronic acid **11a** in the form of a TFA salt. The Phe- and Leu-boronic acids (**11b** and **11c**, respectively) were prepared according to previously described procedures.[18,19]

The subsequent coupling reaction of **11a–c** and 1-naphtylacetyl-Tyr(Me)-Val-OH **12** proceeded without any difficulty (Scheme 2). The stage was then set for final deprotection of the resultant tripeptides **13a–c**, which led to the target compounds **3a–c**. Previous procedures for the syntheses of peptide boronates generally employed one of two methods: transesterifications with an additive boronic acid such as *i*-BuB$(OH)_2$ (condition A) or hydrolysis of the esters in aqueous media and concomitant oxidative cleavage of the liberated pinanediol to direct equilibrium to the accumulation of free boronic acids (condition B). However, these methods failed to give the final products **3a–c**, presumably because of the limited solubility of the substrates in the media. Modification of the reaction conditions by using various combinations of organic solvents and reagents were also unsuccessful. These discouraging results forced us to examine deprotection at an earlier step. In fact, removal of the pinanediols of **11a–c** under the transesterification conditions resulted in successful synthesis of aminoboronic acid hydrochlorides **14a–c** (Scheme 2). The efficacy of the subsequent peptide bond formation depended on the coupling reagent: the use of WSC·HCl, TBTU, DPPA, or BOP led to boron-proton exchange to give **15a–c**. Only diethyl cyanophosphate resulted in an acceptable amount of the desired product; however, it could not be separated from the byproducts derived from the coupling reagent.

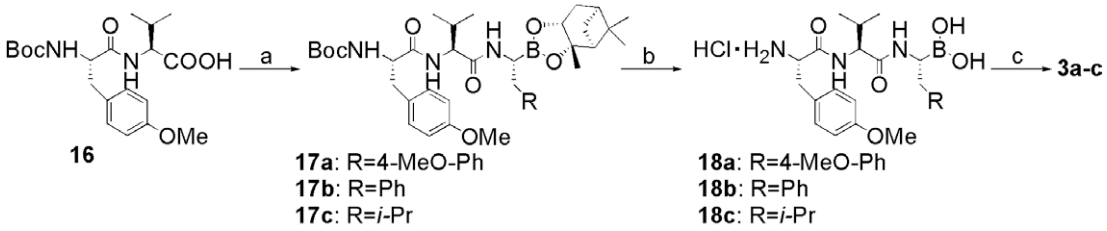
After careful optimization of the reaction sequence and purification method, a synthetic route was finally established; this is



Scheme 1. Reagents and conditions: (a) (i) Mg, THF, then $B(OEt)_3$; (ii) H_2SO_4, H_2O, 41%; (b) (+)-pinanediol, Et_2O, 63%; (c) $LiCHCl_2$, $ZnCl_2$, THF–Et_2O, 98% (>96% de); (d) LiHMDS, THF, 84%; (e) TFA, hexane, 95%.



Scheme 2. Reagents and conditions: (a) WSC·HCl, HOBt, *i*-Pr_2EtN, CH_2Cl_2; (b) condition A: $NaIO_4$, H_2O, organic co-solvent (acetone, THF, or DMF); condition B: *i*-BuB$(OH)_2$, 0.1 M HCl–hexane; (c) *i*-BuB$(OH)_2$, 0.1 M HCl–hexane; (d) **12**, diethyl cyanophosphate, DMF; (e) condition A: **12**, WSC·HCl, HOBt, *i*-Pr_2EtN, CH_2Cl_2; condition B: **12**, TBTU, HOBt, *i*-Pr_2EtN, THF; condition C: **12**, DPPA, TEA, DMF; condition D: **12**, BOP reagent, TEA, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) **11a–c**, WSC·HCl, HOBt, *i*-Pr_2EtN, CH_2Cl_2, 69% for **17a**, 42% for **17b**, 48% for **17c**; (b) (i) TFA, $CHCl_3$; (ii) *i*-$BuB(OH)_2$, 0.1 M HCl–hexane, 88% for **18a**, 62% for **18b**, 80% for **18c**; (c) 1-naphthylacetic anhydride, DMAP, CH_2Cl_2, 72% for **3a**, 72% for **3b**, quant. for **3c**.

Table 1
Proteasome-inhibitory activities[9] of tyropeptin boronic acid derivatives (**3a–c** and **18a–c**), TP-110 (**2**), and bortezomib (**4**)

Compounds	Chymotrypsin-like activity	PGPH activity	Trypsin-like activity
3a	0.0063	>40	5.6
3b	0.0044	7.4	4.6
3c	0.0026	0.94	2.8
18a	0.13	16	3.7
18b	0.10	7.7	4.0
18c	0.021	1.4	1.9
TP-110 (**2**)	0.083	>40	>40
Bortezomib (**4**)	0.024	0.73	>40

The IC_{50} values are in μM.

shown in Scheme 3. Boc-Tyr(Me)-Val-OH (**16**), which was already synthesized in the previous study,[14] was attached to **11a–c** using WSC·HCl to provide the tripeptide intermediates **17a–c**. The Boc group and chiral auxiliary were removed in the usual manner to obtain the tripeptide boronates as HCl salts (**18a–c**). Centrifugal liquid–liquid partition chromatography (CPC) using a solvent system of $CHCl_3$/MeOH/H_2O (5:6:4) in the ascending mode was found to be effective for the purification of the relatively unstable intermediates. Finally, acylation of the amino groups of **18a–c** with 1-naphthylacetyl anhydride provided the desired tyropeptin-boronate analogs **3a–c**, which were also purified by CPC ($CHCl_3$/MeOH/H_2O (5:6:4), in the descending mode).

The synthesized tyropeptin boronic acid derivatives[22] were subjected to in vitro assays for measuring the proteasome-inhibitory activities (Table 1, the human 20S proteasome was purchased from Biomol, Plymouth Meeting, PA).[12] To evaluate the influence of the acyl group at the N-terminus on the inhibitory effect, the tripeptide HCl salts **18a–c** were also tested. Substitution of the formyl group of TP-110 (**2**) with boronic acid (**3a**) resulted in moderate improvement in the inhibition of the chymotryptic proteasome activity. It is noteworthy that compound **3c**, which has an aliphatic side chain at P1, is an approximately ninefold more potent inhibitor of the chymotryptic activity of proteasome than bortezomib. The lower in vitro activity of **3a** and **3b** in comparison to that of **3c** implies that the S1 pocket of proteasome favors aliphatic side chains over benzylic ones. Comparison of the results of **3a** and **3b** suggests that the existence of the methoxy group on the phenyl group in the P1 side chain has only a small impact on the affinity to the catalytic site of the proteasome. Removal of the 1-naphthylacetyl group (**18a–c**) resulted in substantial loss of activity. Moreover, all synthesized tyropeptin derivatives showed selectivity toward the chymotrypsin-like activity.

In summary, boronic acid derivatives of tyropeptin were synthesized in this study, and careful design of the reaction sequence and CPC purification resulted in the desired compounds without loss of the boronate moiety. The tyropeptin derivatives **3a–c** are good potential inhibitors of the chymotryptic activity of proteasome. In particular, the Leu-boronate derivative **3c** displayed an IC_{50} value that was even lower than that of bortezomib. Further investigations on the biological activities of these compounds, including their cytotoxicity and anti-tumor activity, are currently underway and will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.117.

References and notes

1. Ciechanover, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 5944.
2. Orlowski, M.; Cardozo, C.; Michaud, C. *Biochemistry* **1993**, *32*, 1563.
3. Lopes, U. G.; Erhardt, P.; Yao, R.; Cooper, G. M. *J. Biol. Chem.* **1997**, *272*, 12893.
4. An, B.; Goldfarb, R. H.; Siman, R.; Dou, Q. P. *Cell Death Differ.* **1998**, *5*, 1062.
5. Frankel, A.; Man, S.; Elliot, P.; Adams, J.; Kerbel, R. S. *Clin. Cancer Res.* **2000**, *6*, 33719.
6. Sunwoo, J. B.; Chen, Z.; Dong, G.; Yeh, N.; Bancroft, C. C.; Sausville, E.; Adams, J.; Elliot, P.; Van Waes, C. *Clin. Cancer Res.* **2001**, *7*, 1419.
7. Hideshima, T.; Richardson, P.; Chauhau, D.; Palombella, V. J.; Elliot, P. J.; Adams, J.; Anderson, K. C. *Cancer Res.* **2001**, *61*, 3071.
8. Ling, Y.-H.; Liebes, L.; Jiang, J.-D.; Holland, J. F.; Elliot, P. J.; Adams, J.; Muggia, F. M.; Perez-Solar, R. *Clin. Cancer Res.* **2003**, *9*, 1145.
9. Lee, A.-H.; Iwakoshi, N. N.; Anderson, K. C.; Glimcher, L. H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9946.
10. Mitsiades, C. S.; Mitsiades, N.; Hideshima, T.; Richardson, P. G.; Anderson, K. C. *Curr. Drug Targets* **2006**, *7*, 1341.
11. Rajkumar, S. V.; Richardson, P. G.; Hideshima, T.; Anderson, K. C. *J. Clin. Oncol.* **2005**, *23*, 630.
12. Momose, I.; Sekizawa, R.; Hashizume, H.; Kinoshita, N.; Homma, Y.; Hamada, M.; Iinuma, H.; Takeuchi, T. *J. Antibiot.* **2001**, *54*, 997.
13. Momose, I.; Sekizawa, R.; Hirosawa, S.; Ikeda, D.; Naganawa, H.; Iinuma, H.; Takeuchi, T. *J. Antibiot.* **2001**, *54*, 1004.
14. Momose, I.; Umezawa, Y.; Hirosawa, S.; Iinuma, H.; Ikeda, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1867.
15. Yang, W.; Gao, X.; Wang, B. *Med. Res. Rev.* **2003**, *23*, 346.
16. Groziak, M. P. *Am. J. Ther.* **2001**, *8*, 321. See also references cited therein.
17. Adams, J.; Behnke, M.; Chen, S.; Cruickshank, A. A.; Dick, L. R.; Greiner, L.; Klunder, J. M.; Ma, Y.-T.; Plamondon, L.; Stein, R. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333.
18. Matteson, D. S.; Sadhu, K. M.; Lienhard, G. E. *J. Am. Chem. Soc.* **1981**, *103*, 5241.
19. Kettner, C. A.; Shenvi, A. S. *J. Biol. Chem.* **1984**, *259*, 15106.
20. Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2469.
21. Acetti, D.; Brenna, E.; Fuganti, C. *Tetrahedron: Asymmetry* **2007**, *18*, 488.
22. All compounds were characterized by ^1H NMR, ^{13}C NMR, and HRMS. Details of the synthetic procedure and physicochemical data of the unreported compounds are provided as Supplementary data.