Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5022–5027

Synthesis and in vitro biological evaluation of aryl boronic acids as potential inhibitors of factor XIa

Tsvetelina I. Lazarova,[a,*] Lei Jin,[b] Michael Rynkiewicz,[b] Joan C. Gorga,[c] Frank Bibbins,[c] Harold V. Meyers,[a] Robert Babine[b] and James Strickler[c]

[a]*Medicinal Chemistry, Daiichi Asubio Medical Research Laboratories, LLC, One Kendall Square, Building 700, Cambridge, MA 02139, USA*
[b]*Structural Chemistry, Daiichi Asubio Medical Research Laboratories, LLC, One Kendall Square, Building 700, Cambridge, MA 02139, USA*
[c]*Biochemistry, Daiichi Asubio Medical Research Laboratories, LLC, One Kendall Square, Building 700, Cambridge, MA 02139, USA*

Received 6 June 2006; accepted 14 July 2006
Available online 28 July 2006

Abstract—A series of functionalized aryl boronic acids were synthesized and evaluated as potential inhibitors of factor XIa. Crystal structures of the protein–inhibitor complexes led to the design and synthesis of second generation compounds showing single digit micromolar inhibition against FXIa and selectivity against thrombin, trypsin, and FXa.

Thromboembolitic diseases are a major cause of death and disability in the western world.[1] Current antithrombotic therapies rely on the use of anticoagulant agents, such as heparin, low molecular weight heparins (LMWH), and warfarin, as well as antiplatelet agents such as aspirin. Some of the limitations of these therapies are a lack of oral bioavailability (heparin, LMWH) and the need to constantly monitor blood parameters (warfarin).[2] Most of the current research efforts have focused on the development of inhibitors of thrombin and factor Xa (FXa), and much progress has been reported in these research areas, including compounds in advanced clinical development.[3–5]

In our laboratories, we have focused on a relatively unexplored target in the blood coagulation cascade, namely factor XIa (FXIa).[6] Factor XI is a serine protease expressed as a zymogen that is converted to its active form, FXIa, by factor XIIa and by thrombin. Upon activation, FXIa promotes coagulation by activating factor IX. There is evidence that a high level of FXIa increases the risk for venous thrombosis,[7] although currently there are few reports concerning FXIa as a potential target for the development of small molecule antithrombotic drugs.[8] Since FXIa plays a role in the amplification pathway in coagulation and not in initiation of clotting, an inhibitor specific for FXIa rather than for the other coagulation pathway proteases, such as FXa and thrombin, may be effective at reducing the risk of thrombosis without a significant risk of bleeding. Indeed, in a recent publication, FXIa null mice (fXI−/−) were found to be resistant to clot induction by $FeCl_3$ in an arterial thrombosis model, but had normal bleeding times.[9] In this letter, we report the synthesis and in vitro biological activity of aryl boronic acid derivatives as potential inhibitors of FXIa.

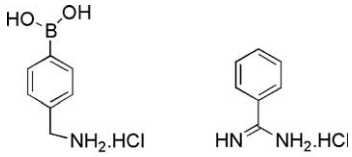
Aryl boronic acids have found applications in diverse chemical areas, such as organometallic chemistry, organic synthesis, host–guest chemistry, and medicinal chemistry. Simple phenyl boronic acids,[10] as well as some functionalized arylboronic acids[11] have been shown to have weak serine protease inhibitory activity. More potent protease inhibition has been observed for boronic acids incorporated within a peptidomimetic framework.[12] These boronic acid peptidomimetics can function as covalent reversible or irreversible inhibitors of the protease by forming a tetrahedral boronate ester with the serine hydroxyl of the catalytic triad. A number of peptidyl boronate inhibitors have been reported,[13] the

Keywords: Aryl boronic acids; Factor XIa; Factor Xa; Thrombin; Trypsin.
* Corresponding author. Tel.: +1 617 470 6276; fax: +1 781 435 1112; e-mail: tlazarova@medchempartners.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.043

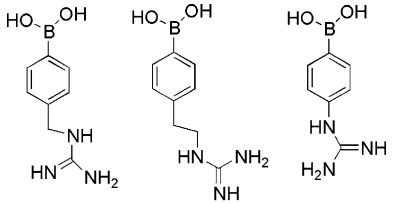
most promising of which is Velcade®, a selective boronate proteosome inhibitor approved as an antineoplastic agent.[14]

In our laboratory, we began the investigation of aryl boronic acids as potential inhibitors of FXIa with the screening of commercially available substituted phenyl boronic acids. We selected compounds with hydrogen bond donor substituents on the phenyl ring that could make an electrostatic interaction with aspartate 189 of the S1 specificity pocket. All compounds were tested in an in vitro enzyme inhibition assay[15] against FXIa, and boronic acid **1** emerged as the only compound with an IC_{50} value in the range of that of the control compound benzamidine **2** (IC_{50} **1** = 77.3 μM, IC_{50} **2** = 120 μM, respectively).



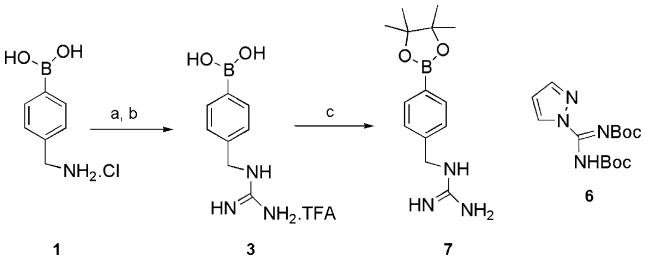
1 2

We selected compound **1** for further studies. In order to maximize the interaction of **1** with aspartate 189, the methylene amine group was converted to a guanidinium group. At the same time, the length of the linker between the guanidinium group and the phenyl ring was varied in order to find the optimum spatial arrangement for interaction with both serine 195 and aspartate 189. For that purpose, we prepared boronic acids **3**, **4**, and **5**. For ease of manipulation, we chose to isolate the compounds as boron pinacol esters. This functional group modification was not expected to interfere with the ability of the boron to form the covalent complex with the active site serine 195, since X-ray crystallographic studies had previously shown that the ester moiety is hydrolyzed prior to binding (due to the rapid equilibration between the boronic ester and the free boronic acid in solution).[16]



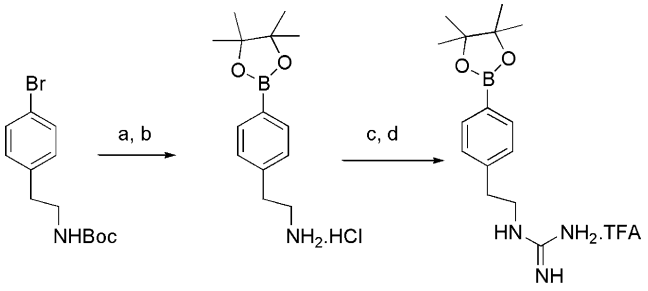
3 4 5

The syntheses of the pinacol esters of acids **3**, **4**, and **5** are shown in Schemes 1–3, respectively. In Scheme 1, boronic acid **1** was treated with 1 equiv of the bis BOC-protected guanidinylation reagent **6** and 1 equiv of diisopropylethyl amine in dimethylformamide (DMF) for 18 h at ambient temperature, followed by deprotection with trifluoroacetic acid (TFA) for 5 h at ambient temperature to give the desired compound **3** as a TFA salt in 78% overall yield. Treatment of **3** with 1 equiv of pinacol and 1 equiv of diisopropylethyl amine in dioxane for 18 h at 60 °C afforded the pinacol ester **7** in 91% yield.



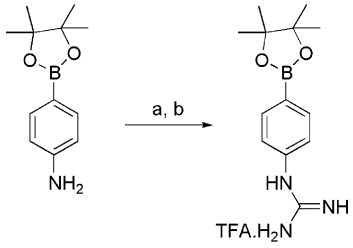
1 3 7 6

Scheme 1. Reagents and conditions: (a) 1 equiv **6**, 1 equiv *i*-Pr_2EtN, DMF, rt, 18 h, 90%; (b) TFA, rt, 5 h, 87%; (c) 1 equiv pinacol, 1 equiv *i*-Pr_2EtN, dioxane, 60 °C, 18 h, 91%.



8 9 10

Scheme 2. Reagents and conditions: (a) 1 equiv dipinacolato diboron, 3 equiv KOAc, 0.1 equiv Pd(dppf)Cl_2, DMSO, 80 °C, 18 h, 67%; (b) 4 N HCl, rt, 30 min, 96%; (c) 1 equiv **6**, 1 equiv *i*-Pr_2EtN, DMF, rt, 18 h, 88%; (d) TFA, rt, 5 h, 94%.



11 12

Scheme 3. Reagents and conditions: (a) 1 equiv **6**, 1 equiv *i*-Pr_2EtN, DMF, rt, 18 h, 86%; (b) TFA, rt, 5 h, 90%.

The preparation of the pinacol ester of boronic acid **4** is outlined in Scheme 2. Miyaura boronylation[17] of the commercially available phenyl bromide **8** with 1 equiv of dipinacolato diboron, 3 equiv of potassium acetate, and 10 mol% palladium diphenylphosphoferrocene dichloride (Pd(dppf)Cl_2) in dimethylsulfoxide (DMSO) for 18 h at 80 °C afforded the boron pinacol ester in 67% yield after a flash chromatography purification. A subsequent treatment of the pinacol ester with 4 N hydrochloric (HCl) acid for 30 min at ambient temperature gave the amine salt **9** in 96% yield. The introduction and deprotection of the guanidinium group was accomplished in a two-step procedure, similar to that for com-

pound **3** (Scheme 1) and the pinacol ester **10** was isolated as the TFA salt in 83% overall yield for the last two-steps.

Scheme 3 shows the preparation of the pinacol ester of boronic acid **5**. Commercially available 4-amino phenyl boronic pinacol ester **11** was converted to the target pinacol ester **12** in 77% overall yield by the same two-step procedure used in the preparation of compound **3** (Scheme 1).

Pinacol esters **7**, **10**, and **12**, as well as boronic acid **3** were tested in in vitro enzyme inhibition assays against FXIa, FXa, thrombin, and trypsin.[15] The results are shown in Table 1. Compounds **10** and **12** showed the greatest potency against FXIa with IC_{50} values of 7.3 and 5.9 μM, respectively, while compounds **3** and **7** were less potent (IC_{50} **3** = 22 μM and IC_{50} **7** = 24.7 μM), but showed a promising selectivity trend, especially against FXa and trypsin (approximately 10-fold). Both compounds **10** and **12** had good selectivity against FXa, but no selectivity against thrombin and trypsin. As expected, there was no difference in the IC_{50} values for boronic acid **3** and its pinacol ester **7**, since the pinacol moiety was hydrolyzed prior to binding.[18] We decided to obtain the crystal structures of compounds **3**, **10**, and **12** with the FXIa catalytic domain rhaFXI$_{370–607}$-S434A, T475A, C482S, K437A (FXIac)[19] in order to rationalize the observed selectivity and look for opportunities to introduce additional points of contact with the enzyme.

In the crystal structure[20] (Fig. 1A–C), all three compounds showed a glycerol boronate ester, instead of the original pinacol ester for compounds **10** and **12** and the free boronic acid for compound **3**. Since glycerol was only present in the cryo-protecting solutions, the transesterification reaction could have occurred when the crystal was prepared for X-ray analysis. The formation of the glycerol boronate ester had no effect on the biological properties of compounds **3**, **10**, and **12**, since there was no change in their IC_{50} values in the presence of glycerol. All three compounds bound in the active site with the guanidine group interacting with aspartate 189 in the S1 pocket of the FXIac. The boron atom was tetracoordinate and covalently attached to serine 195. There were structural differences between the ligands in order to accommodate their different lengths. The two ends (guanidine and glycerol boronate) of compounds **3** and **10** were superimposed on each other. To accommodate the extra methylene group in compound **10**, the phenyl ring tilted away from the position it occupied in compound **3**. The guanidine group in compound **12** was in a different position than the guanidine groups in compounds **3** and **10** and formed only one hydrogen bond with aspartate 189 instead of two, yet the location of the nitrogen involved in the hydrogen bonding was very close to the location of the nitrogen atoms in compounds **3** and **10**. The phenyl ring of compound **12** adopted a third orientation in the S1 pocket. This structural information suggested that the meta position of the phenyl ring (relative to the boronic ester) or the benzylic carbon in compounds **3** and **10** provided good opportunities to access a small pocket (located just above the disulfide bond between cysteine 191 and cysteine 219) near the S1 subsite that is specific for FXIa.

Table 1. In vitro biological data

Compound	IC_{50} (μM) FXIa	IC_{50} (μM) FXa	IC_{50} (μM) Thrombin	IC_{50} (μM) Trypsin
3	22	>200	146	>200
7	24.7	>200	129	>200
10	7.3	175.4	30.8	20.3
12	5.9	129	5.5	2.3

We chose to modify the benzylic position in compound **10**. Since such a modification would create a chiral center, we decided to prepare the racemic compound and let the biologically active enantiomer co-crystallize selectively with the protein.

The synthesis of boronic ester **13**, a representative member of the new series of compounds, is shown in Scheme 4. Commercially available amine **14** was treated with 1 equiv of *tert*-butylcarbonyl (Boc) anhydride, 3 equiv of triethyl amine in dichloromethane (DCM) for 18 h at ambient temperature to provide the protected Boc derivative in 96% yield. The ketone was then reduced to the alcohol with excess sodium borohydride in methanol for one hour at ambient temperature in 83% yield. Reaction of the alcohol intermediate with 1 equiv of nicotinoyl chloride and 3 equiv of diisopropylethyl amine in DCM for 18 h at ambient temperature gave the nicotinoyl ester **15** in 76% yield. The boronic pinacol ester was introduced by treating **15** with 1 equiv of dipinacolato diboron, 3 equiv of potassium acetate, and 0.1 equiv of Pd(dppf)Cl_2 in DMSO at 80 °C for 18 h and isolated in 73% yield after a flash chromatography purification. The Boc group was removed by treatment with 4 N HCl for 30 min (97% yield). The resulting free amine was converted to a guanidinium group by reaction with 1 equiv of pyrazole reagent **6** and 1 equiv of diisopropylethyl amine in DMF for 18 h at ambient temperature. Final deprotection with excess TFA for 5 h at ambient temperature gave the target compound **13** as a racemate in 78% overall yield for the last two-steps.

The in vitro biological data for compound **13** and its precursor **10** are shown in Table 2. The IC_{50} value for the racemic **13** against FXIa was 1.4 μM. Compound **13** also showed an improved selectivity against trypsin (approximately 200-fold) as compared to the precursor compound **10** (3-fold). There was no significant change in the selectivity against FXa (approximately 30-fold for compound **13** vs 25-fold for compound **10**). There was also a modest improvement in the selectivity against thrombin (8-fold for compound **13** as compared to 4-fold for compound **10**). In order to find a possible explanation for the observed results, as well as find out, which of the two enantiomers is responsible for the biological activity, we solved the crystal structure of compound **13** with FXIac.

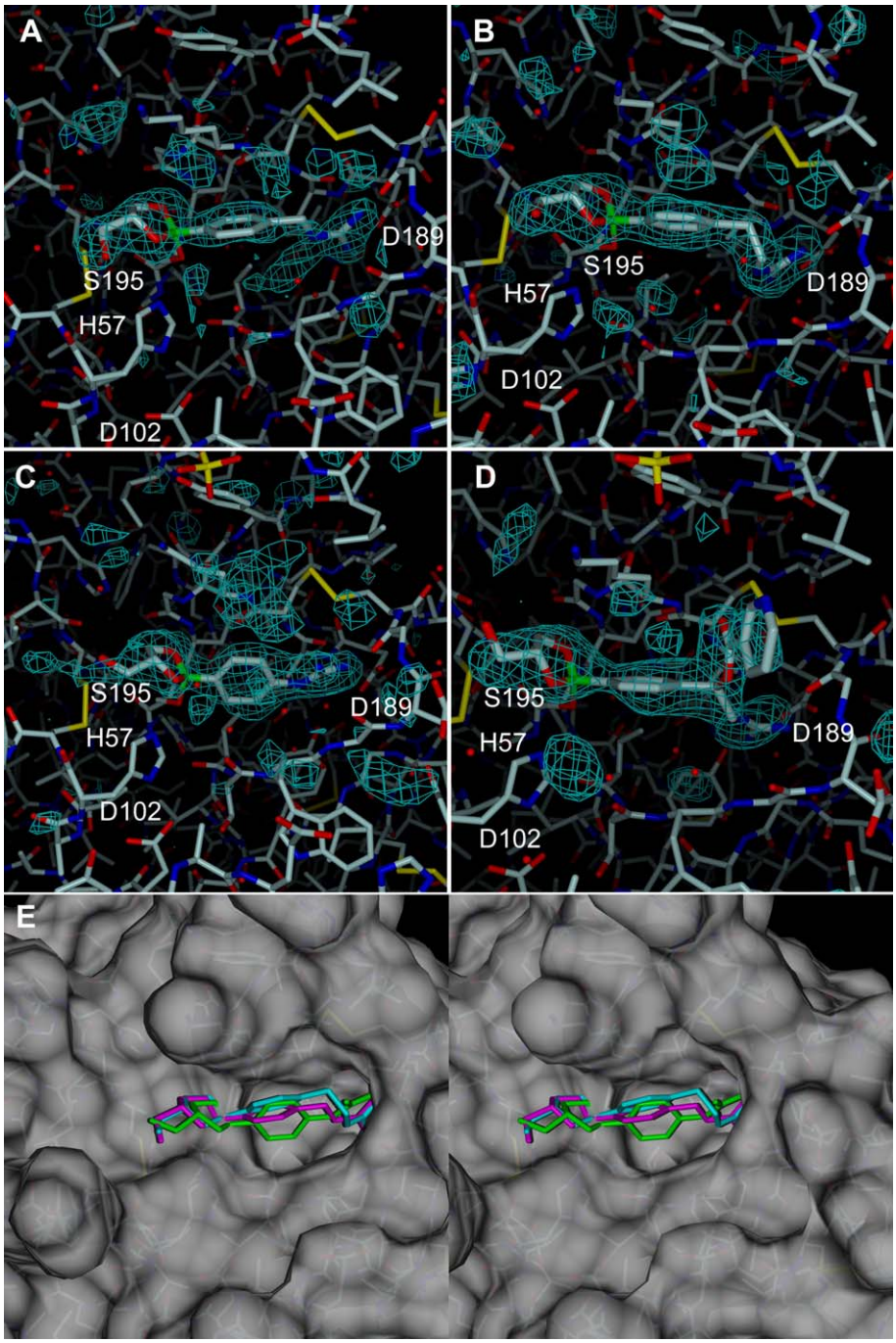
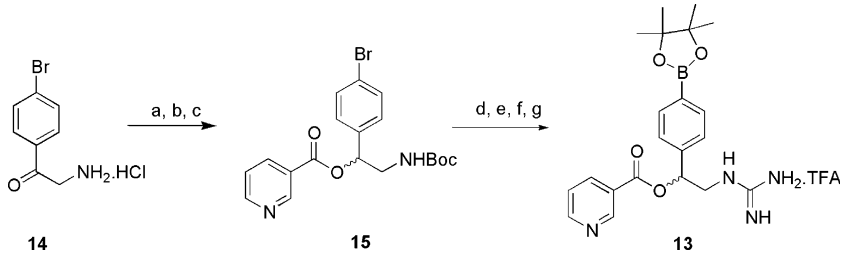


Figure 1. Panels A–D are electron density maps calculated using Fourier coefficients F_{obs}–F_{calc} using phases from the final refined models omitting the atoms for the ligand and contoured at 2.5 sigma for FXIa complexes with compounds **3**, **10**, **12**, and **13**, respectively. Panel E is a wall-eyed stereodiagram of the superposition of the bound conformations of compounds **3** (magenta), **10** (cyan), and **12** (green) in the active site of FXIa. The solvent accessible protein surface is from the FXIa-compound **3** complex.

The crystal structure (Fig. 1D) showed that the S enantiomer of compound **13** was bound in the active site. Assuming only this enantiomer is active against FXIa, this effectively results in a submicromolar IC_{50} for compound **13**. As previously seen, the compound crystallized as the glycerol boronate ester and formed a covalent bond between its boron atom and the oxygen of serine 195, as well as a salt bridge between its guanidine group and the side-chain carboxylate of aspartate 189. There are, however, differences between the FXIa-bound structures of compound **13** and its precursor, compound **10** (Fig. 1B). The ester carbonyl in compound **13** is within hydrogen bonding distance (3.0 Å) of and oriented toward the backbone nitrogen of lysine 192. The pyridyl group makes van der Waals contacts with the side-chain of leucine 146, and this binding mode results in a shift of the position of residues 143–147 of up to 1.7 Å in the FXIa-compound **13** structure compared to the FXIa-compound **10** structure (Fig. 1B). While the hydrogen bond made by the ester



Scheme 4. Reagents and conditions: (a) 1.0 equiv $(Boc)_2O$, 2.5 equiv Et_3N, DCM, rt, 18 h, 96%; (b) 5 equiv $NaBH_4$, methanol, rt, 1 h, 83%; (c) 1.0 equiv nicotinoyl chloride, 3 equiv *i*-Pr_2EtN, DCM, rt, 18 h, 76%; (d) 1 equiv dipinacolato diboron, 3 equiv KOAc, 0.1 equiv Pd(dppf)Cl_2, DMSO, 80 °C, 18 h, 73%; (e) 4 N HCl, rt, 30 min, 97%; (f) 1 equiv **6**, 1 equiv *i*-Pr_2EtN, DMF, rt, 18 h, 85%; (g) TFA, rt, 5 h, 92%.

Table 2. In vitro biological data

Compound	IC_{50} (μM) FXIa	IC_{50} (μM) FXa	IC_{50} (μM) Thrombin	IC_{50} (μM) Trypsin
10	7.3	175.4	30.8	20.3
13	1.4	43.6	12.3	>200

carbonyl is probably not contributing much to specificity since the other proteases tested can make this interaction as well, the steric clash between leucine 146 and the pyridyl group of **13** is not likely to be accommodated well by all serine proteases. This observation can explain the gain in selectivity observed with compound **13** compared to compound **10**. The pyridyl ring is also positioned so that additional substituents on the *meta*- or *ortho*-positions (relative to the boronic acid) could make additional interactions in both the S2 subsite and a flexible pocket near the mouth of the S1 pocket that is formed by lysine 192, cysteine 191, cysteine 219, and residues 143–148. Compounds that optimize interactions in these subsites, in addition to the covalent interaction with ser195 and the electrostatic interaction with asp 189, may very well advance both the potency and selectivity of this compound series.

In conclusion, we have synthesized single digit micromolar aryl boronic acid inhibitors of FXIa with selectivity against trypsin, thrombin, and FXa. X-ray studies of the inhibitors with the catalytic domain of FXIa identified several subsites where additional interactions with the enzyme may lead to the design of more potent and selective compounds. Synthesis and biological evaluation of second generation aryl boronic acid inhibitors has been initiated.

References and notes

1. Ruef, J.; Katus, H. A. *Expert Opin. Ther. Patents* **2003**, *12*, 781.
2. Hirsh, J.; Fuster, V. *Circulation* **1994**, *89*, 1449.
3. (a) Coburn, C. A. *Expert Opin. Ther. Patents* **2001**, *11*, 1; (b) Sanderson, P. E. J. *Annu. Rep. Med. Chem.* **2001**, *36*, 79; (c) Vacca, J. P. *Annu. Rep. Med. Chem.* **1998**, *33*, 81; (d) Ilies, M. A.; Scozzafava, A.; Supuran, C. T. *Expert Opin. Ther. Patents* **2002**, *12*, 1181.
4. (a) Rewinkel, J. B. M.; Adang, A. E. P. *Curr. Pharm. Des.* **1999**, *5*, 1043; (b) Steinmetzer, T.; Hauptmann, J.; Sturzebecher, J. *Expert Opin. Ther. Patents* **2001**, *11*, 845.
5. (a) Gould, W. R.; Leadley, R. J. *Curr. Phar. Des.* **2003**, *9*, 2337; (b) Samana, M. *Throm. Res.* **2002**, *106*, 267; (c) Rai, R.; Sprengeler, P. A.; Elrod, K. C.; Young, W. B. *Curr. Med. Chem.* **2001**, *8*, 101.
6. Jin, L.; Pandey, P.; Babine, R. E.; Gorga, J. C.; Seidl, K. J.; Gelfand, E.; Weaver, D. T.; Abdel-Meguid, S. S.; Strickler, J. E. *J. Biol. Chem.* **2005**, *6*, 4704.
7. Salomon, O.; Zivelin, A.; Livnat, T.; Dardik, R.; Loewenthal, R.; Avishal, O.; Steinberg, D. M.; Rosove, M. H.; O'Connell, N.; Lee, C. A.; Seligsohn, U. *Blood* **2003**, *101*, 4783.
8. Ries, U. J.; Wienen, W. *Drugs Future* **2003**, *28*, 355.
9. Wang, X.; Cheng, Q.; Xu, L.; Feuerstein, G. Z.; Hsu, M.-Y.; Smith, P. L.; Seiffert, D. A.; Schumacher, W. A.; Ogletree, M. L. *J. Thrombosis Haemostasis* **2005**, *3*, 695.
10. (a) Weston, G. S.; Blazquez, J.; Baquero, F.; Shoichet, B. K. *J. Med. Chem.* **1998**, *41*, 4577; (b) Wasserthal, P. S.; Martichonok, V.; Keller, T. H.; Chin, B.; Martin, R.; Jones, J. B. *Bioorg. Med. Chem. Lett.* **1994**, *2*, 35; (c) Patent WO 92/19707 1992 to Procter and Gamble Company, US.
11. (a) Deadman, J. J.; Spencer, J.; Greenidge, P. A.; Goodwin, C. A.; Kakkar, V. V.; Scully, M. F. WO 2002057273 A1; (b) Spencer, J.; Burd, A. P.; Goodwin, C. A.; Merette, S. A. M.; Scully, M. F.; Adatia, T.; Deadman, J. J. *Tetrahedron* **2002**, *58*, 1551; (c) De Nanteuil, G.; Gloanec, P.; Lila, C.; Portevin, B.; Verbeuren, T.; Rupin, A.; Simonet, S. EP 792883 A1.
12. (a) Bukhtiyarova, M.; Rizzo, C. J.; Kettner, C. A.; Korant, B. D.; Scarnati, H. T.; King, R. W. *Antiviral Chem. Chemother.* **2002**, *12*, 367; (b) Jagannathan, S.; Forsyth, T. P.; Kettner, C. A. *J. Org. Chem.* **2001**, *66*, 6375; (c) Stoll, V. S.; Eger, B. T.; Hynes, R. C.; Martichonok, V.; Jones, J. B.; Pai, E. F. *Biochemistry* **1998**, *37*, 451; (d) Davis, J. H.; Agard, D. A.; Handel, T. M.; Basus, V. J. *J. Biomol. NMR* **1997**, *10*, 21; (e) Amparo, E. C.; Miller, W. H.; Pacofsky, G. J.; Wityak, J.; Weber, P. C.; Duncia, J. J. V.; Santella, J. B., III. WO 9620689 A2; (f) Pacofsky, G. J.; Pruitt, J. R.; Weber, P. C. WO 9509859 A1; (g) Tian, Z.-Q.; Brown, B. B.; Mack, D. P.; Hutton, C. A.; Bartlett, P. A. *J. Org. Chem.* **1997**, *62*, 514; (h) Coutts, S. J.; Kelly, T. A.; Snow, R. J.; Kennedy, C. A.; Barton, R. W.; Adams, J.; Krolikowski, D. A.; Freeman, D. M.; Cambell, S. J. *J. Med. Chem.* **1996**, *39*, 2087.
13. (a) Coburn, C. A. *Expert Opin. Ther. Patents* **2001**, *11*, 721; (b) Vacca, J. P. *Curr. Opin. Chem. Biol.* **2000**, *9*, 355; (c) Kettner, C.; Mersinger, I.; Knobb, R. *J. Biol. Chem.* **1990**, *265*, 18289; (d) Tarrapelli, C.; Metternich, R.; Ehrhardt, C. *J. Biol. Chem.* **1993**, *268*, 4734; (e) Weber, P. C.; Lee, S. I.; Levandowski, F. A.; Schadt, M. C.; Chang, C. W.; Kettner, C. A. *Biochemistry* **1995**, *34*, 3750.
14. Velcade is a trademark of Millenium Pharmaceuticals, Inc. (a) Adams, J.; Kauffman, M. *Cancer Invest.* **2004**,

22, 304; (b) Adams, J. *Expert Opin. Ther. Patents* **2003**, *13*, 45.
15. Peptidolytic activities for FXIa, FXa, thrombin, and trypsin were measured using a fluorogenic reporter group, 7-methyl-4-aminocoumarin (AMC), attached via an amide linkage to the tripeptide pyroglutamyl-prolyl-arginyl (Glp-Pro-Arg). The assay was carried out in 20 mM Tris–HCl, 150 mM NaCl, and 0.02% Tween 20 at pH 7.4. Enzyme and substrate concentrations were 0.25 nM and 50 μM, respectively, for factor XIa. The assay was incubated at 30 °C for 15–30 min and then released AMC measured (emission 460 nm, excitation 355 nm) on a Wallac 1420 Multilabel Counter. The data were fit to a one-site dose response using non-linear least squares to determine the IC_{50} value.
16. Von Matt, A.; Ehrhardt, C.; Burkhard, P.; Metternich, R.; Walkinshaw, M.; Tarrapelli, C. *Bioorg. Med. Chem.* **2000**, *8*, 2291.
17. Ishiyama, T.; Miyaura, N. *Chem. Rec.* **2004**, *3*, 271.
18. Pinacol ester **10** was completely converted to the corresponding boronic acid **4** after 30 min incubation in the assay buffer, as shown by LS/MS.
19. Jin, L.; Pandey, P.; Babine, R. E.; Gorga, J. C.; Seidl, K. J.; Gelfand, E.; Weaver, D. T.; Abdel-Meguid, S. S.; Strickler, J. E. *J. Biol. Chem.* **2005**, *6*, 4704.
20. Crystallographic data for structures are on file with CCDC. Deposition numbers: compound **3** 1ZMJ, compound **10** 1ZML, compound **12** 1ZMN, compound **13** 1ZMJ.