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P1 Phenethyl Peptide Boronic Acid Inhibitors of HCV NS3 Protease

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Abstract—A series of peptide boronic acids containing extended, hydrophobic P1 residues was prepared to probe the shallow, hydrophobic S1 region of HCV NS3 protease. The *p*-trifluoromethylphenethyl P1 substituent was identified as optimal with respect to inhibitor potency for NS3 and selectivity against elastase and chymotrypsin.

Hepatitis C virus (HCV) chronically infects approximately 170 million persons worldwide and causes serious progressive liver disease. In a substantial fraction of cases, HCV infection leads to cirrhosis and hepatocellular carcinoma.[1] The best treatment for HCV infection is a combination of pegylated interferon and ribavirin, which produces sustained virological response in only 54% of patients and causes significant side effects, such as anemia, neutropenia, and depression.[2] The unsatisfactory efficacy and safety profile of current therapies, together with the highly variable course of disease progression, makes clinical treatment of HCV infection difficult.[1] There is a clear need for substantially improved antiviral therapies for HCV.

Although HCV replication is poorly characterized due to an inability to grow the virus in cell culture, the steps involved in processing the viral polyprotein are understood in reasonable detail.[3] HCV has a 9.5 kb RNA genome that encodes a polyprotein of 3010 to 3040 aa. Processing of the polyprotein by cellular and viral proteases generates at least ten mature viral structural and nonstructural (NS) proteins. One of these, NS3, is a serine protease that is responsible for cleaving the four carboxy terminal cleavage sites in the polyprotein. NS3 is well characterized biochemically,[4] and has been crystallized alone,[5] in complex with its cofactor peptide NS4A,[6,7] and with peptide inhibitors.[8] Experiments in the chimpanzee disease model[9] have demonstrated that functional NS3 is required for HCV infectivity,[10] validating the enzyme as a target for drug discovery.

Early in the evolution of our HCV NS3 protease medicinal chemistry program, we focused on optimizing the P1 residue[11] in peptide boronic acid inhibitors.[12] Literature reports indicated that the protease prefers small, hydrophobic P1 side chains (ethyl, allyl, CH_2SH) in peptide substrates[13,14] and inhibitors.[15–17] Inspection of the three-dimensional structure of NS3 protease shows that the S1 site is very hydrophobic, as it is encompassed by the side chains of Phe 154, Ala 157, Lys 136, Val 132, and Leu 135. Moreover, S1 is not well defined, but rather exists as a broad surface extending to S3. In order to further probe structure/activity relationships in this region of the protease active site, we have prepared peptide boronic acids with extended, hydrophobic P1 substituents. Initially, a series of linear alkyl, terminally branched alkyl, and aralkyl P1 residues was investigated. Subsequently, an extensive series of substituted phenethyl substituents were examined. We report that a *p*-trifluoromethylphenethyl P1 residue affords optimal inhibitor potency for NS3 and specificity with respect to other serine proteases.

Peptide boronic acid inhibitors were synthesized using the methodology for asymmetric homologation of boronic acid pinanediol esters developed by Matteson and co-workers.[18,19] As shown in Scheme 1, reaction of a Grignard reagent with triisopropyl borate, followed by esterification with (+)-pinanediol affords a boronic

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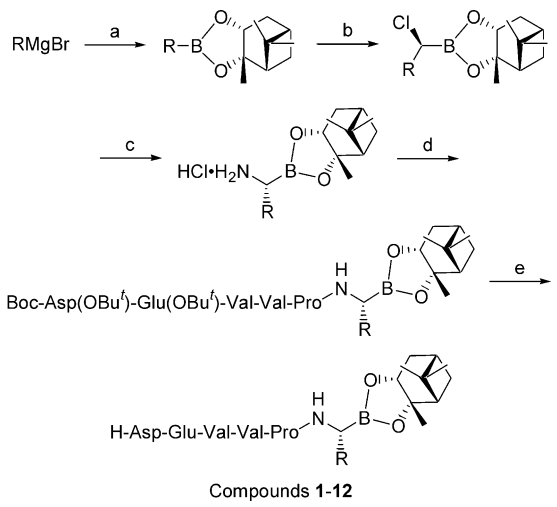
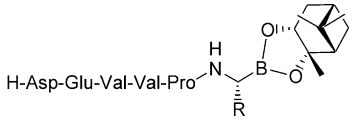
ester. Homologation with dichloromethyllithium[20] diastereoselectively provides an (*S*)-α-chloroboronic ester. Displacement of chloride by lithium bis(trimethylsilyl)amide,[21] followed by acidolysis gives the (*R*)-α-aminoboronic ester as a stable hydrochloride salt. Coupling to the protected pentapeptide Boc-Asp(OBu^t)-Glu(OBu^t)-Val-Val-Pro-OH and deprotection with trifluoroacetic acid afforded the desired peptide boronic acids **1**–**12** (See Table 1 for definition of the R groups).[22]

A series of substituted phenethyl containing peptides was prepared as shown in Scheme 2. In this case, the



Scheme 1. Synthesis of **1**–**12**. Reagents and conditions: (a) triisopropyl borate, Et_2O, −78 to 25 °C; H_2SO_4, 0 °C; (+)-pinanediol; (21–76%); (b) Cl_2CHLi, THF, −100 to 25 °C; (63–83%) (c) LHMDS, THF, −78 to 25 °C; 4 N HCl/dioxane, −78 °C; (22–90%); (d) Boc-Asp(OBu^t)-Glu(OBu^t)-Val-Val-Pro-OH, PyAOP, DIEA, DMF; (23–62%); (e) 90% TFA, 5% triisopropylsilane, 5% CH_2Cl_2; (87–95%). Compounds **2** and **9** were prepared as pinacol esters, and thus are a 1:1 mixture of diastereomers at P1. The synthesis of **8** began by esterification of commercially available phenylboronic acid with (+)-pinanediol.

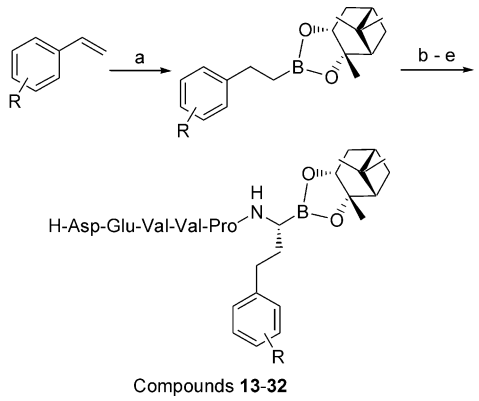
Table 1. Inhibition of NS3 protease, human leukocyte elastase, and human pancreatic chymotrypsin by peptide boronic acids[a]



Compd	R	NS3 K_i (μM)	Elastase IC_{50} (μM)	Chymotrypsin IC_{50} (μM)
1	Ethyl	0.008	0.020	>60
2[b]	*n*-Butyl	0.011	NT	2.1
3	*n*-Pentyl	0.012	NT	0.38
4	*n*-Hexyl	0.013	NT	0.42
5	*i*-Butyl	0.008	0.060	NT
6	*i*-Amyl	0.039	NT	0.30
7	4-Methylpentyl	0.007	7.3	0.28
8	Phenyl	0.9	NT	NT
9[b]	Benzyl	0.5	NT	0.070
10	Phenethyl	0.008	3.5	0.075
11	Phenpropyl	0.20	NT	NT
12	Phenbutyl	0.010	0.4	1.9

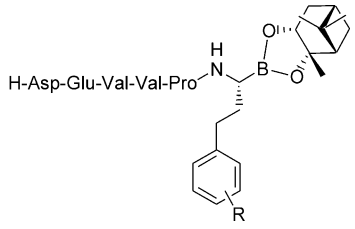
[a]Each K_i is the average of at least three independent determinations, while each IC_{50} is the average of two determinations.
[b]Compound is a 1:1 mixture of diastereomers at P1.

required boronates were prepared by hydroboration of a substituted styrene with catecholborane, followed by transesterification with (+)-pinanediol.[23] Subsequent homologation, nitrogen substitution, and peptide coupling afforded hexapeptides **13**–**32** (See Table 2 for definition of the R groups).



Scheme 2. Synthesis of **13**–**32**. Reagents and conditions: (a) catecholborane, 70 °C; (+)-pinanediol, THF; (32–82%); (b) Cl_2CHLi, THF, −100 °C; $ZnCl_2$, −100 to 25 °C; (14–92%); (c) LHMDS, THF, −78 to 25 °C; 4 N HCl/dioxane, −78 °C; (10–95%); (d) Boc–Asp(OBu^t)-Glu(OBu^t)-Val-Val-Pro-OH, PyAOP, DIEA, DMF, or EDC, HOAt, $NaHCO_3$, CH_2Cl_2/DMF (5:1), 0 °C; (8–56%); (e) 90% TFA, 5% triisopropylsilane, 5% CH_2Cl_2; (87–95%). Compound **30** was prepared from 4-*tert*-butoxystyrene.

Table 2. Inhibition of NS3 protease, human leukocyte elastase, and human pancreatic chymotrypsin by P1 phenethyl peptide boronic acids[a]



Compd	R	NS3 K_i (μM)	Elastase IC_{50} (μM)	Chymotrypsin IC_{50} (μM)
10	H	0.008	3.5	0.075
13	2-Methyl	0.82[b]	NT	NT
14	3-Methyl	0.034	5.7	NT
15	4-Methyl	0.017	5.0	3.7
16	2,4-Dimethyl	0.53[b]	NT	NT
17	2,5-Dimethyl	1.0[b]	NT	NT
18	2-Fluoro	0.018	NT	NT
19	3-Fluoro	0.009	NT	NT
20	4-Fluoro	0.006	0.8	0.050
21	2,6-difluoro	0.93[b]	NT	NT
22	3-Trifluoromethyl	0.025	NT	NT
23	4-Trifluoromethyl	**0.002**	**1.8**	**16**
24	4-Chloro	0.002	1.4	0.065
25	4-Bromo	0.004	1.6	NT
26	4-Phenyl	0.007	0.9	48
27	4-Isopropyl	0.005	0.45	>60
28	4-Cyclohexyl	0.003	0.40	>60
29	4-*tert*-Butyl	0.003	0.34	>60
30	4-Hydroxy	0.008	0.9	NT
31	4-Methoxy	0.003	0.56	20
32	4-Phenoxy	0.003	0.22	>60

[a]Each K_i is the average of at least three independent determinations, while each IC_{50} is the average of two determinations.
[b]Indicates inhibition data is from an IC_{50} measurement, not K_i.

Our initial experiments focused on peptides containing linear alkyl (**1**–**4**), terminally branched alkyl (**5**–**7**), and aralkyl (**8**–**12**) P1 residues. Table 1 shows enzyme inhibition data for these compounds against the catalytic domain of NS3 protease and two other serine proteases, human leukocyte elastase and human pancreatic chymotrypsin.[24–27]

The extended linear alkyl residues (**2**–**4**) each give comparable potency to ethyl (**1**) for NS3 protease, and have only modest selectivity over chymotrypsin (30- to 190-fold). The branched alkyls isobutyl (**5**) and 4-methylpentyl (**7**) also afford inhibitors with potency comparable to **1**, while isoamyl (**6**) induces a 5-fold loss in potency. In addition, **7** has 1000-fold selectivity over elastase and 40-fold selectivity over chymotrypsin. The aralkyls display a more defined structure–activity relationship than either the linear or branched alkyls. Peptides containing P1 phenyl (**8**), benzyl (**9**), and phenpropyl (**11**) are 25- to 100-fold less potent than (**1**), while the phenethyl (**10**) and phenbutyl (**12**) peptides maintain low nanomolar potency. Compound **10** has more than 400-fold selectivity over elastase, but only 9-fold selectivity over chymotrypsin. Inhibition of other serine proteases has been previously observed by peptide boronic acids with P1 phenethyl residues.[28,29]

We subsequently investigated the properties of a series of peptides with substituted phenethyl residues at P1 in order to improve the potency and selectivity of the lead structure **10** (Table 2). Substitution of the phenethyl group at the *para* position is clearly preferred over *ortho* substitution and equivalent or superior to *meta* substitution (compare **13**, **14**, and **15**; **18**, **19**, and **20**; **22** and **23**). Disubstitution is unacceptable, even with a *para* substituent (**16**, **17**, and **21**). At the *para* position, all substituents tested afforded potent inhibitors, including alkyl (**15**, **27**, **28**, and **29**), aryl (**26**), electron-withdrawing (**20**, **23**, **24**, and **25**), and electron-donating (**30**, **31**, and **32**). Compounds with para substituted P1 residues show modest potency for elastase ($IC_{50} = 0.2$–5 μM) and highly variable potency for chymotrypsin ($IC_{50} = 0.050$ to > 60 μM). Two conclusions regarding chymotrypsin inhibition may be drawn: A *para* halogen substituent improves chymotrypsin affinity (**20** and **24**) and a sterically bulky *para* substituent abrogates chymotrypsin binding (**26**, **27**, **28**, **29**, and **32**). Finally, the *p*-trifluoromethylphenethyl residue (**23**) appears optimal in this series in terms of NS3 potency (4-fold more potent than **1**) and selectivity (900-fold versus elastase and 8000-fold vs chymotrypsin).

In summary, we have investigated a series of peptide boronic acids containing extended P1 residues as inhibitors of HCV NS3 protease. The 4-trifluoromethylphenethyl residue was identified as optimal with respect to inhibitor potency and selectivity. Within the P1 phenethyl series, substantial effects on inhibitor potency and selectivity were observed with changes in the position and identity of the aromatic ring substituents. A clear understanding of the observed structure–activity relationships awaits a crystal structure of one of these peptides bound to NS3 protease. Nonetheless, our results will facilitate the design of next-generation HCV protease inhibitors based on both cyclic peptide and peptidomimetic structures.

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References and Notes

1. Lauer, G. M.; Walker, B. D. *N. Engl. J. Med.* **2001**, *345*, 41.
2. Manns, M. P.; McHutchison, J. G.; Gordon, S. C.; Rustgi, V. K.; Shiffman, M.; Reindollar, R.; Goodman, Z. D.; Koury, K.; Ling, M.-H.; Albrecht, J. K. and the International Hepatitus Interventional Therapy Group *Lancet* **2001**, *358*, 958.
3. Bartenschlager, R.; Lohmann, V. *J. Gen. Virol.* **2000**, *81*, 1631.
4. Bartenschlager, R. *J. Viral Hepatitis* **1999**, *6*, 165.
5. Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z. *Cell* **1996**, *87*, 331.
6. Kim, J. L.; Morgenstern, K. A.; Lin, C.; Fox, T.; Dwyer, M. D.; Landro, J. A.; Chambers, S. P.; Markland, W.; Lepre, C. A.; O'Malley, E. T.; Harbeson, S. L.; Rice, C. M.; Murko, M. A.; Caron, P. R.; Thomson, J. A. *Cell* **1996**, *87*, 343.
7. Yao, N.; Reichert, P.; Taremi, S. S.; Prosise, W. W.; Weber, P. C. *Structure* **1999**, *7*, 1353.
8. Di Marco, S.; Rizzi, M.; Volpari, C.; Walsh, M. A.; Narjes, F.; Colarusso, S.; De Francesco, R.; Matassa, V. G.; Sollazzo, M. *J. Biol. Chem.* **2000**, *275*, 7152.
9. Kolykhalov, A. A.; Agapov, E. V.; Blight, K. J.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *Science* **1997**, *277*, 570.
10. Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *J. Virol.* **2000**, *74*, 2046.
11. For a description of the protease nomenclature, see:- Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
12. Kettner, C. A.; Shenvi, A. B. *J. Biol. Chem.* **1984**, *259*, 15106.
13. Urbani, A.; Bianchi, E.; Narjes, F.; Tramontano, A.; De Francesco, R.; Steinkühler, C.; Pessi, A. *J. Biol. Chem.* **1997**, *272*, 9204.
14. Zhang, R.; Durkin, J.; Windsor, W. T.; McNemar, C.; Ramanathan, L.; Le, H. V. *J. Virol.* **1997**, *71*, 6208.
15. Llinàs-Brunet, M.; Bailey, M.; Fazal, G.; Goulet, S.; Halmos, T.; Laplante, S.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1713.
16. Llinàs-Brunet, M.; Bailey, M.; Déziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2719.
17. Ingallinella, P.; Altamura, S.; Bianchi, E.; Taliani, M.; Ingenito, R.; Cortese, R.; De Francesco, R.; Steinkühler, C.; Pessi, A. *Biochemistry* **1998**, *37*, 8906.
18. Matteson, D. S.; Majumdar, D. *J. Am. Chem. Soc.* **1980**, *102*, 7588.
19. Matteson, D. S.; Ray, R. *J. Am. Chem. Soc.* **1980**, *102*, 7590.

20. Matteson, D. S.; Majumdar, D. *Organometallics* **1983**, *2*, 1529.
21. Matteson, D. S.; Sadhu, K. M. *J. Am. Chem. Soc.* **1981**, *103*, 5241.
22. All intermediates gave satisfactory NMR and mass spectral data. Peptide boronic acids **1**–**32** were characterized by high resolution mass spectrometry and analytical HPLC.
23. Brown, H. C.; Gupta, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 5249.
24. Compounds were tested for enzyme inhibition as their pinanediol esters, as it has been demonstrated that boronic acid pinanediol esters are rapidly hydrolyzed to the free boronic acid in dilute aqueous solution (see ref 12).
25. Inhibition of NS3 protease activity was determined using a modification of the reported method (Taliani, M. et al. *Anal. Biochem.* **1996**, *240*, 60). The fluorescence-based continuous assay contained 50 mM Tris pH 7.0, 5 mM DTT, 50% glycerol, 2% CHAPS, 10 μM NS4A cofactor peptide and 4 nM NS3 protease. Inhibitors were serially diluted into the reaction mixture followed by a 15 min preincubation with the enzyme. Catalysis was initiated by the addition of the fluorogenic ester substrate Ac-DED(EDANS)EEAbuΨ[COO]ASK(DABCYL)-NH2 (final concentration = 5 μM). Assays were conducted at ambient temperature. Enzymatic activity was monitored with a Perkin Elmer LS 50B luminescence spectrometer (excitation = 360 nm, emission = 530 nm, both slits were set to 10 nm). Inhibition constants were determined from a nonlinear least squares fit of the data to the equation $V[I]/Vo = 1/(1 + [I]/K_{i,app})$. The thermodynamic K_i was determined from $K_{i,app}$ via the relationship for competative kinetics: $K_i = K_{i,app}/(1 + [S]/K_m)$ where $[S] = 5$ μM and $K_m = 20$ μM.
26. Human neutrophil elastase was obtained from ART Biochemicals, Athens, Georgia. Stock solutions of lyophilized enzyme (1 mg/mL) were prepared in PBS buffer containing 10% glycerol and stored at −20 °C. Human neutrophil elastase was assayed with the Meo-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (Sigma) as a substrate (ref 12). The hydrolysis of substrate was monitored at 405 nm on a Hewlett-Packard spectrophotometer. Kinetic parameters were determined in PBS buffer at room temperature with concentration of DMSO did not exceed 2%.
27. Human pancreatic chymotrypsin was obtained from Calbiochem, San Diego, CA. Stock solutions of lyophilized enzyme (20 μM) were prepared in 1 mM hydrochloric acid and stored at −20 °C. Human pancreatic chymotrypsin was assayed with the Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (Calbiochem) as a substrate. The hydrolysis of substrate was monitored at 405 nm on a Titertek Multiscan MCC/340 plate reader. Kinetic parameters were determined in 0.1 M Tris, pH 7.8, 10 mM $CaCl_2$ buffer at room temperature with a concentration of DMSO that did not exceed 2%.
28. Wienand, A.; Ehrhardt, C.; Metternich, R.; Tapparelli, C. *Bioorg. Med. Chem.* **1999**, *7*, 1295.
29. Deadman, J. J.; Elgendy, S.; Goodwin, C. A.; Green, D.; Baban, J. A.; Patel, G.; Skordalakes, E.; Chino, N.; Claeson, G.; Kakkar, V. V.; Scully, M. *F. J. Med. Chem.* **1995**, *38*, 1511.