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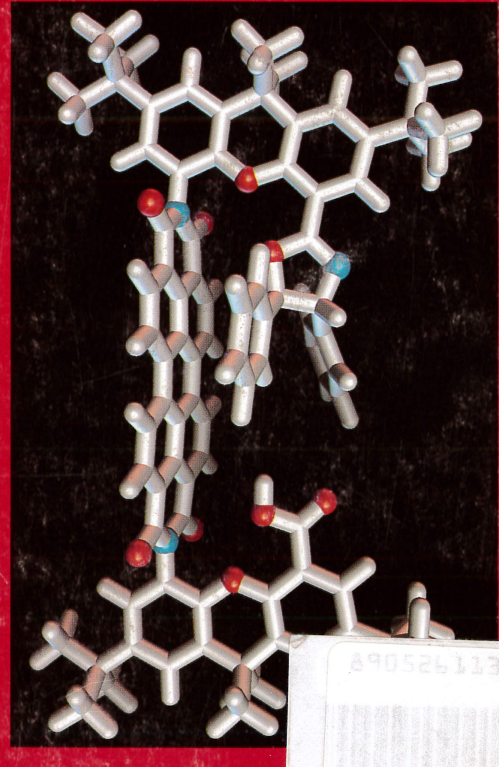

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Contents

[Continued from outside back cover]

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S_1 HETEROCYCLIC THROMBIN INHIBITORS

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R. S. Alexander, C. A. Kettner, and R. R. Wexler.

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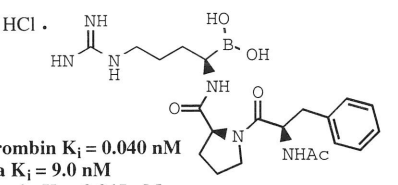
Abstract: A series of boropeptides have previously been described by Kettner et al. to be potent thrombin inhibitors. DuP 714 is a representative of this class of compounds with a K_i = 0.040 nM, but this inhibitor has undesireable side effects. New and selective boronic acid thrombin inhibitors have been developed by replacing the guanidine of the boroarginine side chain with various heterocycles ranging in size and basicity.

Thrombin is involved in the pathogenesis of thrombosis. It functions as the last protease in the blood coagulation cascade hydrolyzing fibrinogen to insoluble fibrin.[2] Boropeptides are highly effective inhibitors of thrombin.[3] DuP 714, Ac-(D)-Phe-Pro-boroArg-OH, is effective in preventing both venous and arterial thrombosis,[2] but it has a low margin of safety. DuP 714 has a K_i of 0.040 nM for thrombin and is two orders of magnitude less reactive with other plasma proteases. However, it readily inhibits trypsin and it is our expectation that compounds with greater selectivity will provide safer inhibitors.

X-ray crystal structures of thrombin and trypsin revealed that the thrombin P_1 pocket is slightly larger than that of trypsin. The Ser^{191} in trypsin narrows the P_1 pocket (Figure 1). We envisioned utilizing the pocket size differences between thrombin and trypsin to obtain selectivity over trypsin and other relevant serine proteases. We also wanted to explore the possibility of decreasing the basicity of the guanidine group of DuP 714 with various heterocycles as the literature suggests that increased toxicity may be attributed to highly basic functionalities.[4] Herein, we report selective 5- and 6-membered heterocycles ranging in size and basicity, and the identification of the butanesulfonamide as a potent replacement of the acetamide group in DuP 714.

DuP 714



Thrombin K_i = 0.040 nM
FXa K_i = 9.0 nM
Trypsin K_i = 0.045 nM

Figure 1. Superimposition of the S_1 Pockets of Thrombin and Trypsin.

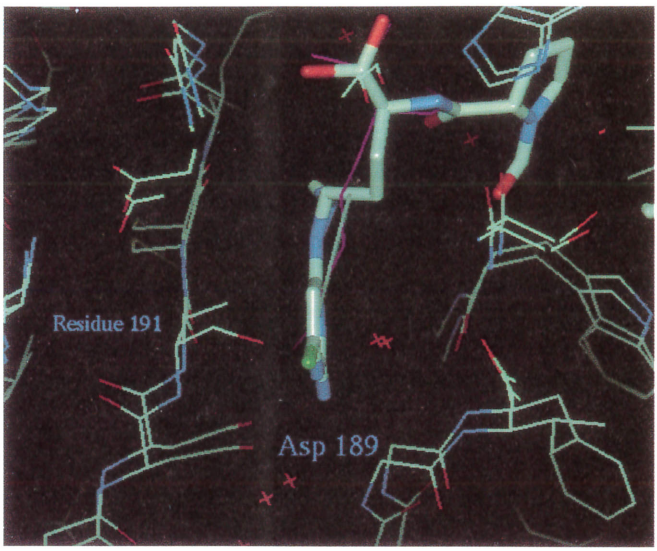
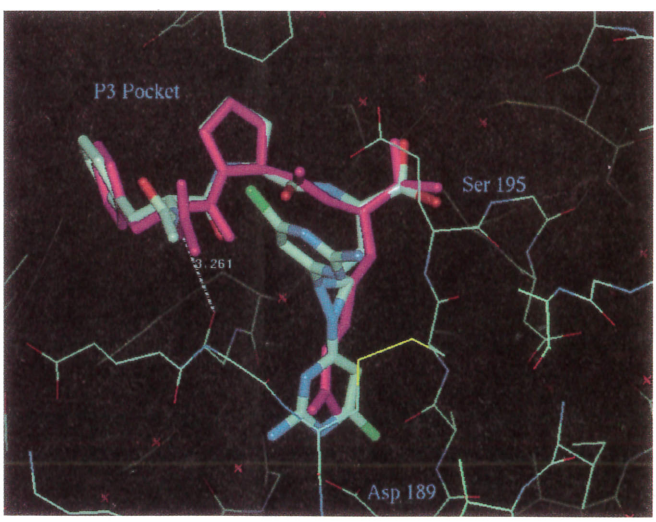


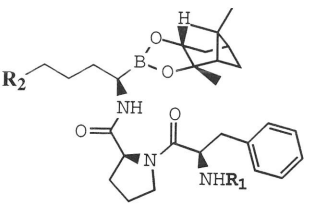
Figure 2. Co-crystal structure of Compound **1** in Thrombin.

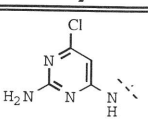
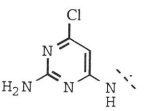
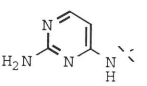
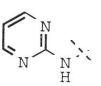


Replacing the guanidine group of DuP 714 with a heterocycle provided selectivity over trypsin and FXa (Table 1, compound **2**). However we lost inhibitory potency against thrombin compared to DuP 714. We also

observed that the butanesulfonamide derivative **2** greatly increased the inhibitory affinity for thrombin compared to the acetyl derivative **1**. Modeling of derivative **2** in the thrombin active site suggests that the increase in affinity is due to the longer bond length of the sulfonamide bond which allows one of the sulfonamide oxygens to form a H-bond with the NH of Gly^{218}.

Table 1. S_1 6-Membered Heterocyclic Thrombin Inhibitors



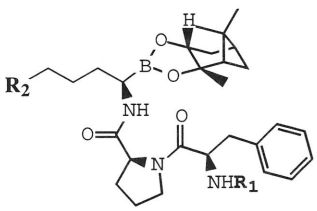
No	R_2	R_1	K_i (nM) Thrombin	Trypsin	FXa*
1		Ac	43	>1200	>6000
2		SO_2Bu	2.1	214	>6000
3		Ac	150	ND	ND
4		Ac	230	>1200	>6000
5		Ac	2000	>1200	ND
6		Ac	600	>1200	>6000
7		SO_2Bu	8.8	ND	ND

ND = Not determined. The K_i's were determined as described by Kettner et al.[3a]
*FXa is another serine protease involved in the coagulation cascade.[3b]

Co-crystallization of compound **1** in thrombin also revealed that the pyrimidine ring was only in the P1 specificity pocket 50% of the time (Figure 2) and yet we obtained nanomolar K_i affinity for thrombin.[5] The X-ray structure of **1** may explain why the thiol analog **5** was not as potent as aminopyrimidine **6**. The loss in potency of compound **5** is probably due to the longer sulfur bond in the thiopyrimidine, which may not be accommodated in the P_1 specificity pocket. Pyrimidine **7** clearly exemplifies how vital the butanesulfonamide

group is to increasing thrombin inhibition compared to acetamide group in compound **5**. Interestingly, 2-aminopyrimidine **3** lost 2.5-fold affinity for thrombin compared to compound **1** and the pyridine derivative **4** also lost affinity for thrombin compared to **3**, suggesting that the chlorine atom of compound **1** and the 2-amino group of compound **1** and **3** must play a role in the binding.

Table 2. S_1 5-Membered Heterocyclic Thrombin Inhibitors



K_i (nM)

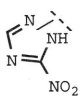
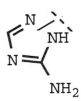
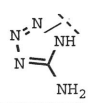
No.	R_2	R_1	Thrombin	Trypsin	FXa
8		Ac	1.7	20	>6000
9		SO_2Bu	3.2	40	970
10		SO_2Bu	15	>1200	6000
11		Ac	650	>1200	>6000
12		Ac	67	27	6000
13		Ac	199	>1200	>6000
14		Ac	507	>1200	>6000
15		Ac	350	>1200	6000

Table 2 illustrates representative examples of the 5-membered S_1 heterocyclic thrombin inhibitors. The 2-aminoimidazole analogs proved to be the most potent of this series even though X-ray results suggest that the amino group does not interact with Asp[189]. These compounds demonstrate that we were indeed able to obtain potent and selective thrombin inhibitors with a less basic P_1 group.

The 2-nitroimidazole analog **10** (Table 2) also exhibited good affinity for thrombin and excellent selectivity over trypsin and FXa. Here again the butanesulfonamide group greatly increased the affinity for thrombin compared to the acetamide group (**11**). The nitro group probably plays a key role in the selectivity over trypsin due the size of the 2-nitroimidazole versus the smaller size of the S_1 pocket of trypsin. This is in contrast to the smaller imidazole compound (**12**), which shows no selectivity over trypsin.

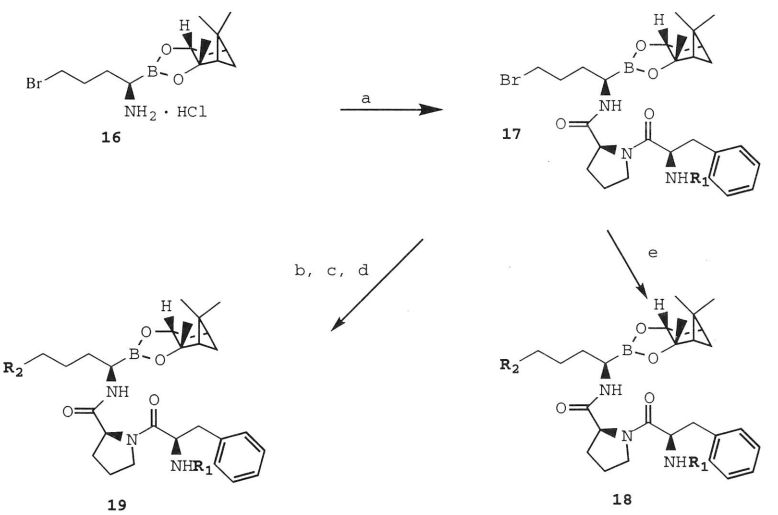
The more acidic heterocycles, such as the triazoles (**13** and **14**), and the tetrazole (**15**) did not exhibit the same affinity for thrombin as the imidazole analogs. Presumably the more basic the P_1 group, the stronger the interaction with Asp^{189} in the specificity pocket of thrombin.

Chemistry

All the guanidine replacements were prepared from the α-aminoboronic acid **16**, which is obtained via Matteson chemistry.[3,6] Peptide coupling with isobutylchloroformate (IBCF), N-methylmorpholine (NMM), and triethylamine in THF at 0 °C gave intermediate **17**. Compounds **5**, **6**, and **8-15** were prepared via alkylation of intermediate **17** with the appropriate heterocycles.[7]

The 2-aminopyrimidin-6-yl compounds were prepared via the ornithine intermediate by azide formation followed by catalytic hydrogenation and chlorine displacement.

Synthesis



Reagents: (a) IBCF, NMM, R_1(D)Phe-Pro-OH, Et_3N, 0 °C, 85%; (b) NaN_3, DMF, 80%; (c) H_2, $Pd(OH)_2$/C, HCl; 90% (d) Et_3N, DMF, R_2-Cl, 60 °C, 50-60%; (e) R_2-H, DMF, K_2CO_3, 60 °C, 60%.

Conclusion

By manipulating the size and basicity of guanidine replacements potent and selective thrombin inhibitors (**2**, **8**, and **9**) have been obtained. Additionally the N-butanesulfonamide group has been identified as a potent replacement for the N-acetyl group of DuP 714. Although the affinity of these compounds is decreased compared to DuP 714, they are more selective for thrombin over other serine proteases.

Acknowledgements

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References and Notes

1. Current address: Schering Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, N.J. 07033.
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3. (a) Kettner, C. A.; Mersinger, L.; Knabb, R. *J. Biol. Chem.* **1990**, *265*, 18289. (b) Radcliffe, R.; Nemerson, Y. In *Methods in Enzymology*; Lorand, L., Ed; Academic: London, 1976, Vol XLV, p 49.
4. (a) Okamoto, S.; Hijikata, A. *Biochim. Biophys. Res. Commun.* **1981**, *101*, 440. (b) Hifijikata-Okunomiya, A.; Okamoto, S. *Thromb. Haemostasis* **1992**, *18*, 135. (c) Angliker, H.; Wilstrom, P.; Shaw, E. *Biochim J.* **1990**, *266*, 829. (d) Elgendy, S.; Deadman, J.; Patel, G.; Green, D.; Chino, N.; Goodwin, C. A.; Scully, M. F.; Kakkar, V. V.; Claeson, G. *Tetrahedron Lett.* **1992**, *33*, 4209. (e) Misra, R. N.; Kelly, Y. K.; Brown, B. R.; Roberts, D. G. G. M.; Chong, S.; Seiler, S. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2165.
5. Data were refined to 2.3 Å resolution and the structure refined to an R_{factor} 0.211.
6. Matteson, D. S.; Jeshi, P. K.; Sadha, K. M. *Organometallics* **1984**, *3*, 1284.
7. Satisfactory spectral data were obtained for all new compounds.

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