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RATIONAL DESIGN, SYNTHESIS, AND SERINE PROTEASE INHIBITORY ACTIVITY OF NOVEL P_1-ARGININOYL HETEROCYCLES

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Abstract: Peptidomimetic derivatives featuring a P_1-argininoyl heterocycle were designed. The preparation of two key building blocks containing benzoxazole or benzimidazole rings and their incorporation into thrombin and factor Xa specific sequences is described. The serine protease inhibitory activity of these targets was evaluated. Molecular modeling of two representative structures is presented. © 1997 Elsevier Science Ltd.

Thrombin, Factor VIIa, and Factor Xa are key members of the trypsin class of serine protease enzymes involved in the blood coagulation cascade. They play a vital role in the regulation of normal hemostasis and abnormal intravascular thrombus development.[1] Recent advances in the elucidation of the structure and function of human thrombin have led to an increased understanding of the pivotal role played by this multifunctional enzyme in the regulation of hemostatic processes and maintenance of vascular function.[2] Peptidomimetic inhibitors of this key enzyme are emerging as potential therapeutic agents for the prevention and treatment of thrombotic vascular disease.[3]

Peptidoyl heterocycles that inhibit various serine proteases such as human neutrophil elastase, prolyl endopeptidase, and thrombin are of current interest as therapeutic targets for the treatment of emphysema,[4,5] amnesia,[6] and thrombotic vascular disease,[7] respectively. Prompted by the recent report of Maryanoff et al.[7] on peptidoyl heterocycle containing thrombin inhibitors, we report herein our work in the area of antithrombotic drugs, which has led to the design of related argininoyl heterocycles.

We investigated the argininoyl heterocycles **3a–c** as a novel class of direct serine protease inhibitors (Figure 1). The new targets incorporate an arginine-based α-ketoheterocycle as the electrophilic transition-

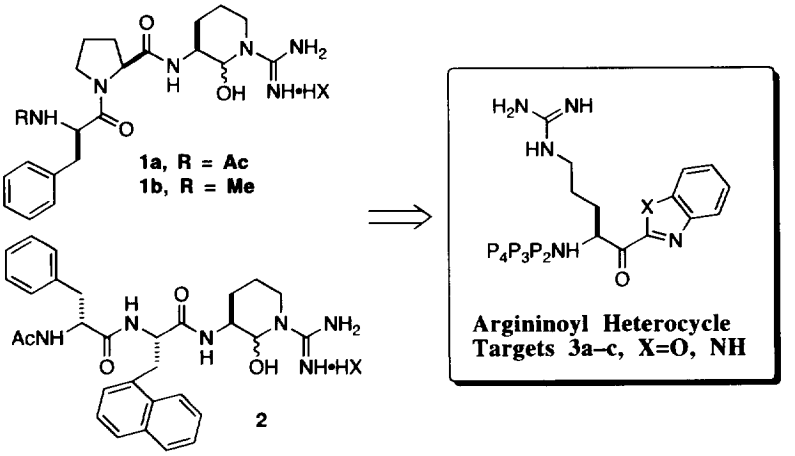


Figure 1. Structures of Prototypical Thrombin and Factor Xa Inhibitors and Argininoyl Heterocycles **3a–c**.

state analog functionality at P_1 into known thrombin[3] and Factor Xa[8] specific sequences. Examination of the S_1' site of trypsin-like serine proteases suggested that the heterocycles of interest should contain at least one basic nitrogen atom to hydrogen-bond with the His 57 in the tetrahedral enzyme-inhibitor complex. A second hydrogen-bond acceptor in the heterocycle might also have favorable interactions with the Gly 193 NH. Such stabilizing interactions are very important in diverse classes of serine protease inhibitors.[3e,4–6] The absence of a carboxy terminus could impart increased levels of metabolic stability, which in turn may afford drug candidates with useful pharmacological profiles.[9]

Chemistry[10]

Methods for the synthesis of valinoyl or prolinoyl benzoxazoles[4–6] were not applicable to the preparation of argininoyl benzoxazoles or benzimidazoles. In order to study these potentially interesting inhibitors, we have developed novel, efficient, and general synthetic routes to these peptidyl argininoyl heterocycles. A convergent strategy was envisioned, which involved the preparation of argininoyl heterocycle precursors **4a** and **4b**, followed by segment coupling to a dipeptide. The coupled intermediates were deprotected with HF and oxidized by using modified Moffatt conditions to furnish the desired targets **3a–c**.

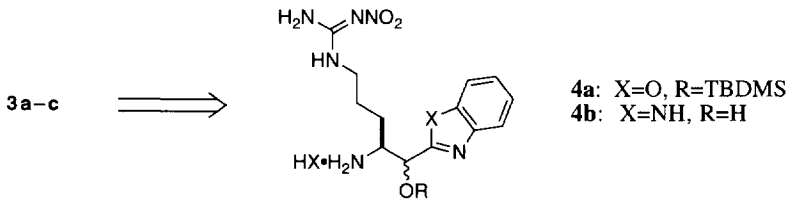
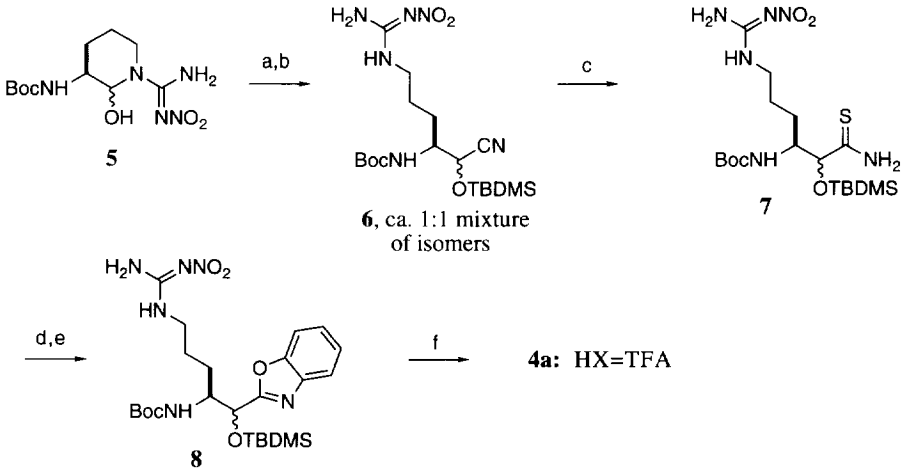


Figure 2. Synthetic Approach to Argininoyl Heterocycles **3a–c**.

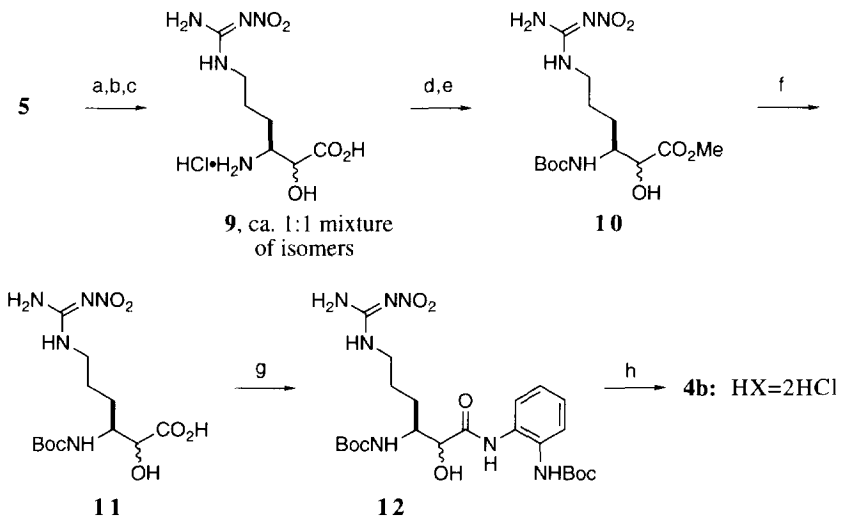
The preparation of the intermediate **4a** proved to be challenging. The literature preparation of peptidyl benzoxazoles which relied on the condensation of *o*-aminophenol with a peptidyl imidate was not applicable to our targets containing the nitroarginine side chain.[11] A new methodology, which is compatible with acid



Scheme 1. *Reagents and conditions:* (a) KCN, $KHCO_3$, THF, H_2O; (b) TBDMSCl, imidazole, 67%; (c) H_2S, pyridine, Et_3N, 100%; (d) MeI; (e) *o*-aminophenol, EtOH, AcOH, reflux, 63% for 2 steps; (f) 50% TFA/CH_2Cl_2, 90%.

sensitive functionalities, was developed (Scheme 1). Boc nitroarginal **5**[12,13] was converted to the *t*-butyldimethylsilyl cyanohydrin **6**, which was isolated a 1:1 mixture of stereoisomers,[14] in two steps by treatment with potassium cyanide followed by TBDMS chloride (67% overall yield). Using a classical procedure,[15a] thioamide **7** was produced in quantitative yield. Formation of the thioimidate, followed by condensation with *o*-aminophenol, produced the desired heterocycle **8** in 63% overall yield.[15b,c] Deprotection with TFA proceeded smoothly to give intermediate **4a** in 90% yield.

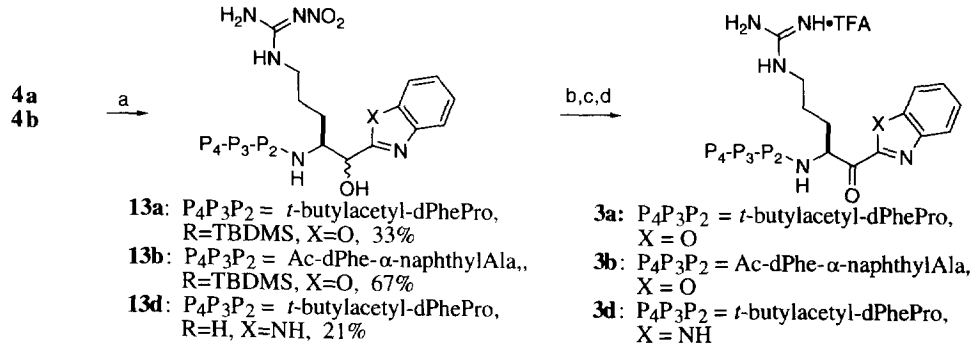
The thioimidate methodology developed successfully to prepare **4a** was applied to the preparation of benzimidazole **4b**. Interestingly, the thioimidate failed to react with 1,2-phenylenediamine under similar conditions. Alternatively (Scheme 2), α-hydroxy-β-amino acid **9**, which was isolated as a 1:1 mixture of stereoisomers,[14,16] was prepared from Boc nitroarginal **5** in three steps: formation of the cyanohydrin, conversion to the imidate, and acidic hydrolysis of the imidate with concomitant removal of the Boc protection. Compound **10** was converted to the Boc hydroxy acid **11** by Fischer esterification, followed by reprotection of the β-amino group with Boc anhydride. Overall yield for the five step protocol was 55%. Hydrolysis of the ester efficiently afforded the Boc hydroxy acid in 95% yield. Coupling of the hydroxy acid **11** to the monoprotected 1,2-phenylenediamine[17] proceeded in fair yield (41%, unoptimized) using HBTU/HOBt to give hydroxy amide **12**. Under the acidic conditions both Boc protecting groups were removed and cyclization occurred to produce the benzimidazole **4b** in 86% crude yield.



Scheme 2. *Reagents and conditions:* (a) KCN, $KHCO_3$, THF, H_2O; (b) MeOH, HCl, 0°C-rt; (c) 6 N HCl, reflux; (d) MeOH, HCl, reflux; (e) Boc_2O, $NaHCO_3$, THF, H_2O, 55% from **2**; (f) LiOH, aqueous MeOH; Dowex cation exchange resin, 95%; (g) mono-Boc-1,2-phenylenediamine, HBTU, HOBt, 4-methylmorpholine, 41%; (h) 4 N HCl, reflux, 86%.

The intermediates **4a** and **4b** were used in the syntheses of peptidoyl heterocycles **3a–c** (Scheme 3). Elaboration with t-butylacetyl-d-PhePro-OH[17] or Ac-dPhe-α-naphthylAla-OH[18] was carried out using HBTU/HOBt/NMM. Yields for the coupling were 33% (**13a**), 67% (**13b**), and 21% (**13c**). HF cleavage of intermediates **13a–c** went smoothly to give the hydroxyheterocycles in 50–70% yield. Oxidation of the hydroxybenzoxazole using a modified Moffatt protocol,[4] followed by HPLC purification, gave the desired

argininoyl benzoxazoles **3a** and **3b** and argininoyl benzimidazole **3c**.



Scheme 3. *Reagents and conditions:* (a) *t*-BuAc-dPhePro-OH, or Ac-dPhe-α-naphthylAla-OH, HBTU, HOBt, 4-methylmorpholine; (b) HF, anisole; (c) DMSO, EDC, dichloroacetic acid; (d) reverse-phase HPLC purification.

Biological Activity

The target argininoyl heterocycles were evaluated for their ability to inhibit a range of important serine proteases, including thrombin (FIIa), factor Xa (FXa), trypsin and plasmin (Table 1).[19] In general, the SAR of the benzoxazoles parallels that of the corresponding aldehydes.

Table 1. In vitro IC_{50} values (μM) of argininoyl heterocycles **3a–3c** and reference standards **1a** and **2** against a range of important serine proteases

cmpd	$P_4P_3P_2$	P_1' moiety	FIIa	FXa	trypsin	plasmin
3a	*t*-BuAc-dPhePro	benzoxazole	0.068	0.11	0.0023	1.32
3b	Ac-dPhe-α-naphthylAla	benzoxazole	>2.5	0.029	0.0064	0.0142
3c	*t*-BuAc-dPhePro	benzimidazole	0.0921	>2.5	1.01	>2.5
reference standards:						
1a	Ac-dPhePro	H	0.284	>2.5	0.156	>2.5
2	Ac-dPhe-α-naphthylAla	H	>2.5	0.025	0.025	<0.025

The most intriguing comparison in the in vitro assays is that of the benzoxazole **3a** to the benzimidazole **3c**. Although both possess the same $P_4P_3P_2$ segment, *t*-butylacetyl-dPhePro, the enzyme selectivities of these two inhibitors are dramatically different. Relative to **3a**, **3c** appears to have similar potency against thrombin, but lost most of its potency against both Factor Xa and trypsin, trends that parallel those of related argininoyl heterocycles.[7]

Models of the compounds **3a** and **3c** complexed with thrombin (Figures 2–4) have been constructed from a crystal structure of thrombin.[20] Placement of **3a** in the active site of thrombin in a fashion analogous to the peptidyl α-ketobenzoxazoles in elastase[4] produced favorable thrombin interactions of the benzoxazole NH and O with His 57 and Gly 193, respectively, which would also be possible in trypsin and factor Xa. Placement of **3c** in the active site of thrombin in the same fashion presents the favorable His 57 interaction, but the NH of the benzimidazole and its sphere of hydration has two unfavorable steric interactions between the conserved Gly 193 and the conserved Hα of residue 192 of the trypsin class of serine proteases. The benzimidazole is

postulated to rotate to fit into the enzyme, similar to the rotation of the heterocycle in the benzothiazole:thrombin X-ray structure reported by Costanzo et al.[7] According to our model, this twist may result in a favorable interactions of the 60 insertion loop (Trp 60d, Lys 60f) unique to thrombin. Thus, **3c** retains the overall binding energy in the S_1' site of thrombin, but not in other trypsin-like serine proteases.

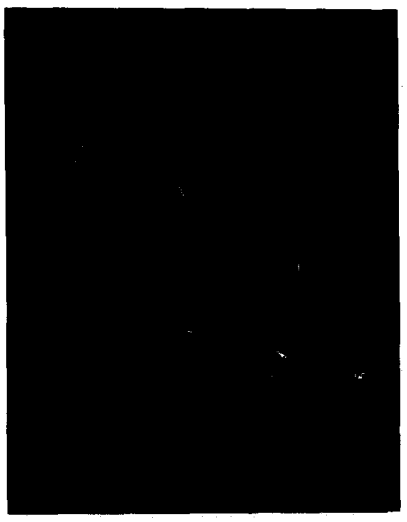


Figure 2. Inhibitor **3a** docked into the active site of thrombin.

Figure 3. Inhibitor **3c** docked into the active site of thrombin.

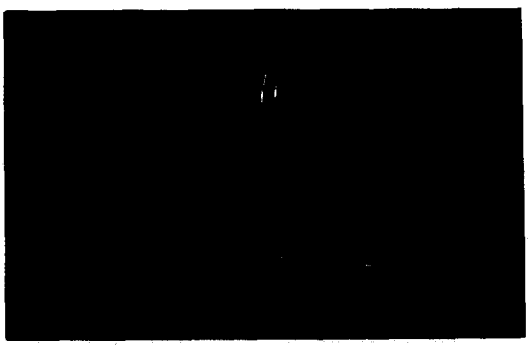


Figure 4. Superposition of inhibitors **3a** and **3c** in the active site of thrombin.

Conclusion

A convenient synthesis of new argininoyl benzoxazoles and argininoyl benzimidazoles was developed and utilized for the preparation of a series of related argininoyl heterocycle targets as potential inhibitors of thrombin and factor Xa. These compounds expressed interesting levels of biological activity with varying selectivities towards related serine proteases. Further investigations of related argininoyl heterocycles will be reported at a later date.

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11. Attempts to convert *t*-butylacetyl-dPhePro-nitroargininal cyanohydrin to the corresponding peptidoyl benzoxazole via the imidate employing the standard protocol[4] were unsuccessful in our laboratory.
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18. *t*-Butylacetyl-dPheProOH and Ac-dPheProOH, prepared using standard solution phase peptide coupling techniques, were spectroscopically and chromatographically homogeneous (≥95% by ^{1}H NMR, and TLC).
19. In vitro enzyme assays were performed according to the protocols described in the following article: Semple, J. E.; Rowley, D. C.; Brunck, T. K.; Ha-Uong, T.; Minami, N. K.; Owens, T. D.; Tamura, S. Y.; Goldman, E. A.; Siev, D. V.; Ardecky, R. A.; Carpenter, S. H.; Ge, Y.; Richard, B. M.; Nolan, T. G.; Håkanson, K.; Tulinsky, A.; Nutt, R. F.; Ripka, W. C. *J. Med. Chem.* **1996**, *39*, 4531.
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