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DESIGN AND SYNTHESIS OF A NOVEL CLASS OF THROMBIN INHIBITORS INCORPORATING HETEROCYCLIC DIPEPTIDE SURROGATES

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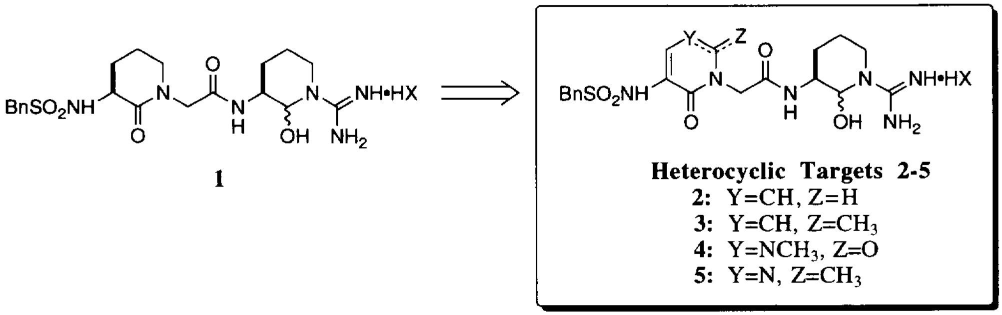
Abstract: Several potent and selective inhibitors of thrombin incorporating novel heterocyclic peptide surrogates in the P_3–P_2 position of peptidyl argininals have been discovered. Illustrated in this article are three classes of heterocycles: pyridones, uracils, and pyrimidinones. The synthesis and biological activities of these unique aromatic heterocyclic derivatives are reported herein. © 1997 Elsevier Science Ltd.

Thrombin, a member of the trypsin class of serine proteases involved in the coagulation cascade, is the primary enzymatic mediator of the coagulation response to vascular injury and regulates normal hemostasis and abnormal 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] Peptidomimetics which inhibit this key enzyme are emerging as potential therapeutic agents for the prevention and treatment of thrombotic vascular disease. Considerable efforts are being focused on the development of selective direct thrombin inhibitors derived from both natural[3] and synthetic sources.[4,5]

Certain peptidyl P_1–argininals have been found to be highly active as transition-state inhibitors of thrombin as well as other serine proteases.[6,7] Previously described peptidyl argininals have shown good thrombin inhibitory activity, although they have generally lacked selectivity against the related serine protease trypsin. Our recent work on orally bioavailable anticoagulants led to a family of lactam-based thrombin inhibitors from which lead candidate **1** emerged.[8] Certain members of these novel constrained P_3–P_2 mimics display high potency as thrombin inhibitors. Based on these considerations, we designed and synthesized a family of heterocyclic replacements of the parent lactam ring of **1** (see Figure 1).



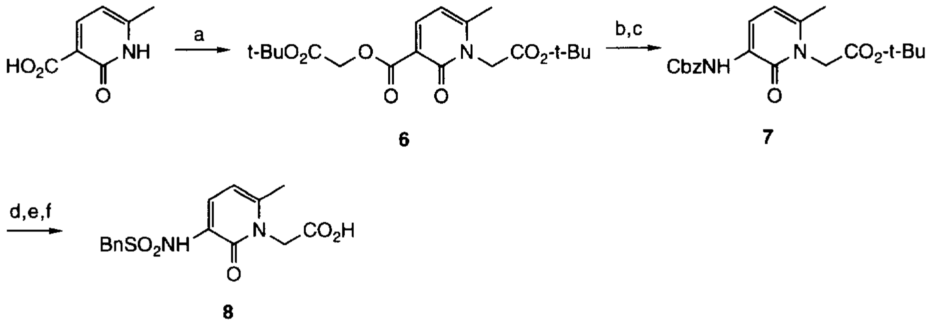
2: Y=CH, Z=H
3: Y=CH, Z=CH_3
4: Y=NCH_3, Z=O
5: Y=N, Z=CH_3

Figure 1. Structures of Prototypical Thrombin Inhibitor and Heterocycles **2–5**.

Aromatic heterocycles serve as useful P_3 scaffolds that, in addition to conferring conformational rigidity, can also possess an interesting range of physical properties including charge density and lipophilicity. Their synthesis and incorporation into pharmaceutically important target molecules have received increasing attention.[9–12] The absence of normal peptide bonds in the P_2–P_4 region was expected to increase metabolic stability and, possibly, oral bioavailability to pharmacologically relevant levels. Herein is described our initial work on peptidomimetic-based thrombin inhibitors containing pyridone-, uracil-, and pyrimidinone-based heterocycles **2–5**, each incorporating key recognition elements of **1** into our nonpeptidic P_1-argininal inhibitors. Notably, **4** is the first example of a uracil derivative to be utilized as a peptide mimic. These peptide surrogates confer upon P_1-argininals significant levels of potency for inhibition of thrombin and selectivity against other serine proteases.

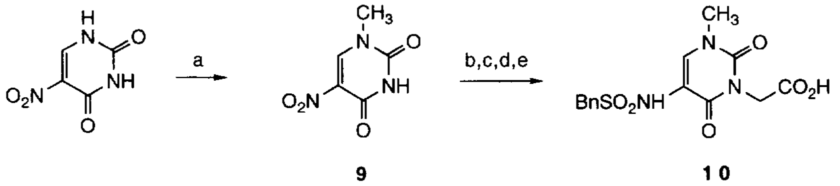
Chemistry[13]

Modeling of pyridone **2** in the active site of thrombin indicated a near optimal fit at the S_2 site. Thus, targets **2–5** were designed with small substituents attached to the heterocyclic nucleus. The pyridone, 6-methylpyridone, uracil, and pyrimidinone synthons **15**, **8**, **10**, and **14**, respectively, were prepared. Synthetic routes to pyridone intermediate **15** and and 6-methylpyridone intermediate **8** paralleled the routes described by Bernstein et al.[9e] with some critical variations for the preparation of **8** (Scheme 1).[14]



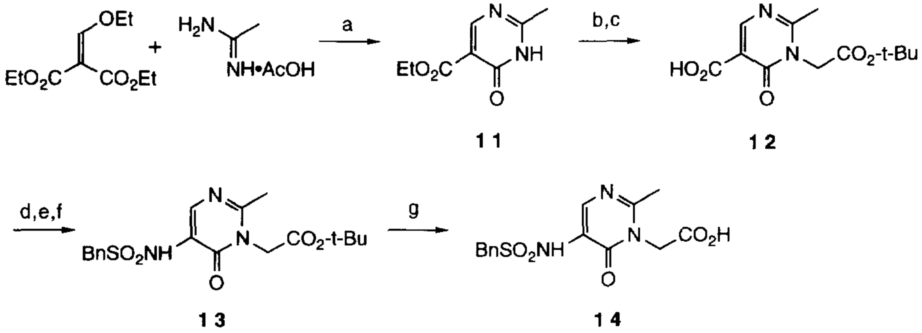
Scheme 1. *Reagents and conditions:* (a) K_2CO_3, $BrCH_2CO_2$-*t*-Bu, DMF, 75%; (b) LiOH, quant. (c) DPPA, Et_3N, dioxane, 110 °C; BnOH, 110 °C, 78%; (d) H_2, Pd/C, EtOH, 82%; (e) $BnSO_2Cl$, collidine, 60%; (f) TFA, CH_2Cl_2, 98%.

The uracil synthon **10** was readily synthesized as the N-methylated analog (Scheme 2). This sequence required the regioselective methylation of 5-nitrouracil at the 1-position, which was accomplished using the modified procedure of Ishikawa et al.[15]



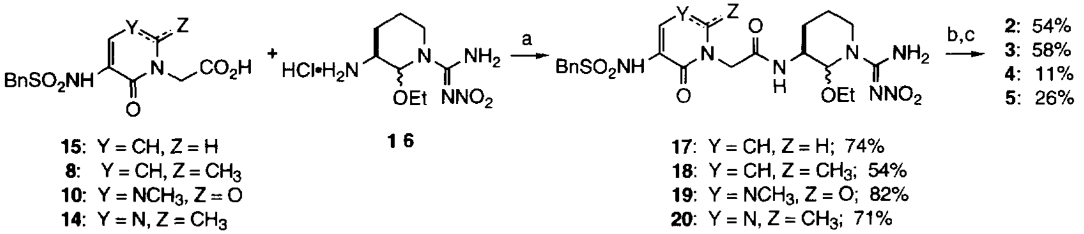
Scheme 2. *Reagents and conditions:* (a) K_2CO_3, CH_3I, DMSO, 77%; (b) NaH, $BrCH_2CO_2$-*t*-Bu, DMF, 59%; (c) H_2, Pd/C, EtOH, 81%; (d) $BnSO_2Cl$, NMM, 94%; (e) TFA, CH_2Cl_2, 88%.

The route to pyrimidinone synthon **14** is depicted in Scheme 3. The alkylation of **11** proved to be problematic: N, C, and O alkylation occurred using a variety of conditions. N-alkylation at the 3-position was enhanced using fluoride ion as base and DME as solvent.[16] The sulfonylation of the 5-aminopyrimidinone intermediate was accompanied by the corresponding bis-sulfonylated byproduct. Treatment of the mixture of mono- and bis-addition products with potassium carbonate in aqueous methanol afforded clean mono-addition product **13** in high yield.



Scheme 3. *Reagents and conditions:* (a) EtOH, reflux, 46%; (b) TBAF, $BrCH_2CO_2$-*t*-Bu, DME, 45%; (c) LiOH, 67%; (d) DPPA, Et_3N, dioxane, 110 °C; BnOH, 110 °C, 53%; (e) H_2, Pd/C, EtOH, quant; (f) $BnSO_2Cl$, NMM; K_2CO_3, aq MeOH, 82%; (g) TFA, CH_2Cl_2, 76%.

A convergent strategy was adopted for the efficient assembly of all targets (Scheme 4). A convenient three step sequence was utilized to prepare targets **2–5**: coupling of P_4-benzylsulfonyl-P_3-heterocycle-P_2-acetic acid synthons **10**, **14**, and **15**, and **8** to the O-ethyl aminal **16**,[17] hydrogenation to remove the nitro protection from the guanidine, and mild acidic hydrolysis to unmask the aldehyde.[18] This method has been successfully employed in our laboratory for the synthesis of multigram quantities of selected argininal-containing serine protease inhibitors.



Scheme 4. *Reagents and conditions:* (a) EDC, HOBt, NMM, CH_3CN, (b) H_2, Pd/C, EtOH, HOAc, H_2O; (c) 3.0 N HCl; HPLC purification.

Results and Discussion

Targets **2–5** were prepared and evaluated for their ability to inhibit various serine proteases (Table 1).[19] They show thrombin inhibitory activities (IC_{50}'s) in the range of 0.5–141 nM and exhibit selectivities against factor Xa and trypsin in the range of 3 to 1000 fold while having minimal effect on thrombolytic enzymes plasmin and tPA. Significantly, pyridones **2** and **3** and pyrimidinone **5** are an order of magnitude more potent than lactam **1**; however, they also exhibit increased potency against trypsin. We have observed that the methyl group ($Z = CH_3$) of compounds **3** and **5** confers selectivity for thrombin versus Factor Xa. Comparatively polar compounds uracil **4** and pyrimidinone **5** exhibit decreased potency against thrombin relative to their pyridone counterparts.

Table 1. In Vitro IC_{50} Values (nM) of Heterocyclic Peptide Mimics Against Thrombin, Factor Xa, Plasmin, Trypsin, and tPA[a]

compound	thrombin	factor Xa	plasmin	trypsin	tPA
1	6.2	2500	>2500	791	>2500
2	0.505	20.9	>2500	26.2	>2500
3	0.467	447	>2500	10.9	>2500
4	141	2500	>2500	396	>2500
5	2.32	905	>2500	160	>2500

[a]Concentration of compounds **1**–**5** necessary to inhibit human enzyme (thrombin, Factor Xa, plasmin, trypsin, and tPA) cleavage of the chromogenic substrates described in ref 16 by 50%.

The relative potencies of heterocycle-containing compounds **2**–**5** towards thrombin may be rationalized by examining the manner in which the constrained P_3/P_2 analogs are postulated to fill the active site of thrombin defined by His57, Tyr60A, Trp60D, and Leu99. Models of the compounds **2**–**5** complexed with thrombin have been constructed from a crystal structure of the parent inhibitor **1** bound to thrombin (Figure 2).[8,20] While the pyridone moiety of **2** is predicted to bind efficiently to the S_2 site, the more lipophilic 6-methylpyridone analog **3** makes an additional key hydrophobic contact. Neither of these compounds introduce polarity into this pocket. According to our model, the second nitrogen atom in the pyrimidinone **5**, directed towards the aromatic face of Tyr60A, has only a slightly deleterious effect. We believe the more polar and bulky uracil **4** lacks potency

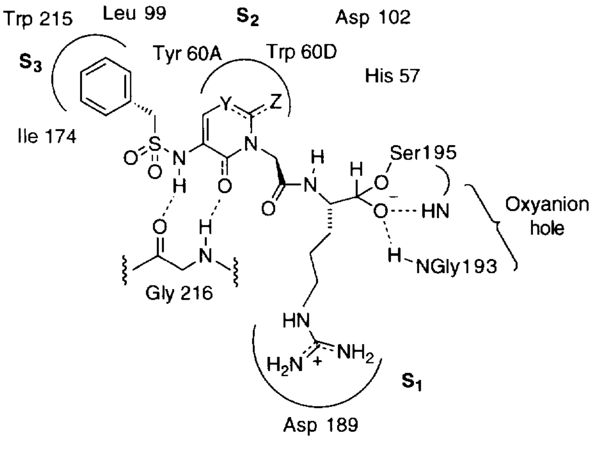


Figure 2. Schematic illustrating the key interactions of **2–5** complexed to thrombin.

potency because the N-methyl group, as well as the probable waters of hydration associated with the adjacent carbonyl, approach too closely to Tyr60A.

Compound **2** demonstrated oral efficacy in a rat arterio-venous shunt model of thrombosis with an ED_{50} of 2.9mg/kg po,[21] and displayed absolute oral bioavailability of 31% in conscious dogs.[22] Further biological evaluation of candidates in this general class, which have exhibited improved oral absorption and duration, is under active study.

Conclusion

A new series of rationally designed P_3-P_2-heterocyclic dipeptide surrogates was linked to a reactive P_1-argininal resulting in the discovery of highly potent and selective thrombin inhibitors. The syntheses of these targets were facilitated by the use of the key intermediate **14**, N^g-nitro-L-argininal ethyl aminal•HCl. Several of these compounds have excellent in vitro potencies against thrombin, with desirable selectivities towards other trypsin-like serine proteases. High oral efficacy and useful levels of oral bioavailability were demonstrated for the pyridone surrogate **2**. The sulfonamide/heterocycle motif provides a useful scaffold from which many interesting new systems have emerged. Further SAR and biological activities of these compounds are currently underway and will be reported in detail elsewhere.

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18. Hydrogenation of the uracil derivative **19** was problematic, resulting in a poorer yield for the final target **4**. The yields shown for compounds **4** and **5** are unoptimized.
19. Enzyme Assays were described in Ref. 8.
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21. The antithrombotic efficacy of compound **2** after oral absorption was evaluated in a rat model of thrombosis. Briefly, the test agent was dissolved in water and administered via gavage needle to unanesthetized rats. After 40 min, the animals were anesthetized and a shunt constructed of PE tubing and containing a silk suture was placed between the carotid artery and the jugular vein. Blood was permitted to flow through the shunt for a period of 15 min, beginning 75 min after oral administration. The ability of the compound to reduce the mass of the resultant thrombus on the suture was assessed over a range of doses. ED_{50} was defined as the dose required to produce a thrombus weight of half of that of control saline treated rats. A minimum of six rats were tested for each dose.
22. The absolute systemic bioavailability (%F) for compound **3** was determined in fasted, conscious, purpose-bred beagle dogs (2 males and 2 females) following separate intravenous (5 mg/kg) and oral (20 mg/kg) administration and collection of plasma samples over a defined time-course covering 6 h. The determination of plasma levels was accomplished using HPLC following post-column fluorogenic derivatization using methodologies that will be published elsewhere. The area under the plasma concentration versus time curves (AUC[0-∞]) for the oral (AUC[oral]) versus the intravenous (AUC[iv]) dosing regimens were calculated by linear trapezoidal estimation using a non-compartmental model, and were used to calculate percent oral bioavailability (%F = [(AUC[oral]/AUC[iv])/(dose iv)/(dose oral)]*100). Further details on the pharmacokinetic, pharmacodynamic and pharmacological profile of this compound will be published elsewhere.

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