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POTENT AND SELECTIVE THROMBIN INHIBITORS FEATURING HYDROPHOBIC, BASIC P_3-P_4-AMINOALKYLLACTAM MOIETIES[1]

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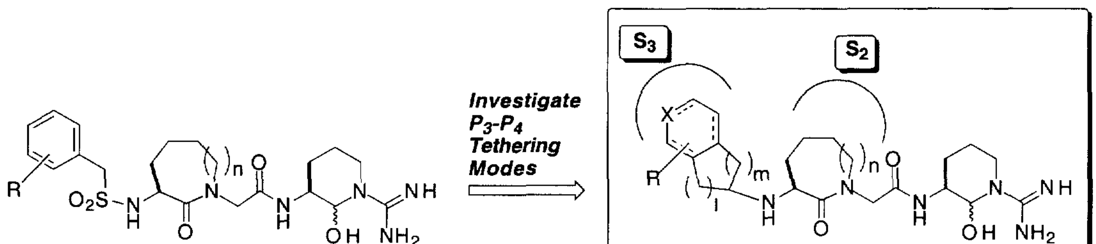
Abstract: Crystal structure and evolving SAR considerations of potent, selective benzylsulfonamide lactam thrombin inhibitors and related serine protease inhibitors have led to the design of novel thrombin inhibitors **1a–g**, featuring hydrophobic, basic, P_4-alkylaminolactam scaffolds that serve as novel types of P_3-P_4 dipeptide mimics. The design, synthesis, and biological activity of these targets is presented.

Thrombin is a multifunctional serine protease with trypsin-like P_1[2] specificity. Amongst a variety of other critical roles it plays in the regulation of hemostasis, it is regarded as the principal mediator of the blood coagulation cascade and is directly responsible for the conversion of fibrinogen into fibrin, a primary component of a blood clot or thrombus. The high incidence of myocardial infarction and cardiovascular disease leading to thrombosis represent leading causes of morbidity and mortality in the industrialized world. Currently, the development of novel, efficacious classes of thrombin inhibitors is a top research objective of several pharmaceutical laboratories.[3,4] Our recently disclosed novel series of peptidomimetic P_3-lactam-P_1-argininals CVS 1578, CVS 1778, and CVS 2097 (Figure 1) are examples of potent, selective, and orally bioavailable transition-state thrombin inhibitors.[5] Further design and SAR studies[6] led us to pursue a series of lactam derivatives **1a–g** which contain basic, hydrophobic P_3-P_4-aminoalkyllactam residues. Such targets were designed to both probe the thrombin S_2, S_3 pockets and to deliver candidates with potentially superior inhibition and selectivity profiles. The design, synthesis, and in vitro biological activity of these targets will be presented in this letter.



CVS 1578 (n = 0, R = H; IC_{50} FIIa 6.2 nM)
CVS 1778 (n = 1, R = H; IC_{50} FIIa 0.71 nM)
CVS 2097 (n = 1, R = 2-CO_2Me; IC_{50} FIIa 0.75 nM)

1a-g, New Targets
X = CH, N; l = no bond,1;
m = 0-3; n = 0, 1

Figure 1: Design of Thrombin Inhibitors Featuring Hydrophobic, Basic P_3-P_4 Peptidomimetic Aminoalkyllactam Moieties.

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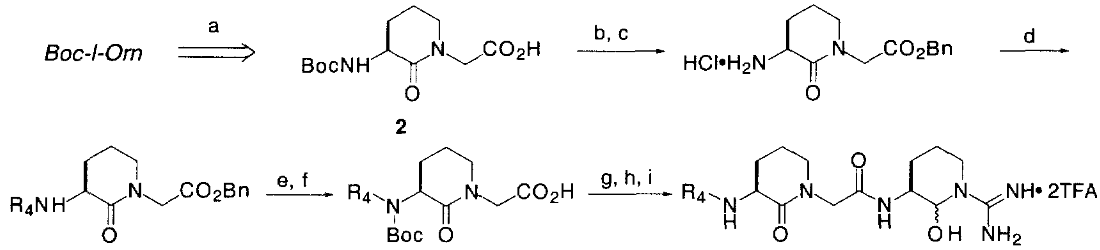
Inhibitor Design Strategy

Our previous SAR studies[5,6] suggested that optimal inhibitor potency and selectivity profiles in the new series **1a–g** may be realized by incorporation of a 2 to 4 atom tether between the P_2-lactam α-center and P_4-aromatic ring. Linkers including the $-CH_2SO_2NH-$ moiety with two or more contiguous tetrahedral centers were preferred and were hypothesized to afford the most potent inhibitors since they allow orientation of the aryl and lactam residues similar to the archetypical d-Phe-Pro template.[6a,c,f] Further X-ray structural information from CVS 1578-thrombin[5a,7] along with structural modeling of the highly potent 7-membered targets CVS 1778 and CVS 2097 led to the design of the new targets **1a–g**, which feature a range of basic, hydrophobic P_3-P_4-aminoalkyllactam residues. These targets were designed to both probe the thrombin S_2 and S_3 pockets and to deliver candidates with potentially superior in vitro profiles. As discussed in our previous communications,[5,6] our P_1-P_4 lactam argininal motifs provide a full complement of important backbone and sidechain interactions at the active site, including antiparallel β-sheet hydrogen bonds to Gly-216, salt bridges, hydrophobic-, edge-to-face and van der Waals interactions. Important interactions commonly found in small molecule thrombin inhibitors are present at the active, S_1, S_2, and S_3 subsites.[8] The synthesis and biological activity of these targets follows.

Synthetic Routes to Lactam Targets

The 6-membered lactam targets **1a–d** were efficiently obtained in ~12 steps from l-α-Boc-ω-Cbz-Orn as outlined in Scheme 1.[9] Intermediate **2** was prepared in >50 gram quantities by modification of Friedinger's method.[10] Reductive amination of **3** with the appropriate aldehyde or ketone proceeded smoothly using $Na(OAc)_3BH$[11] and delivered amines **4a,b,d** in good yield. Elaboration of intermediates, coupling with our recently described P_1-argininal precursor,[12] deprotection, and aminal hydrolysis allowed for efficient construction of targets **1a–d.**



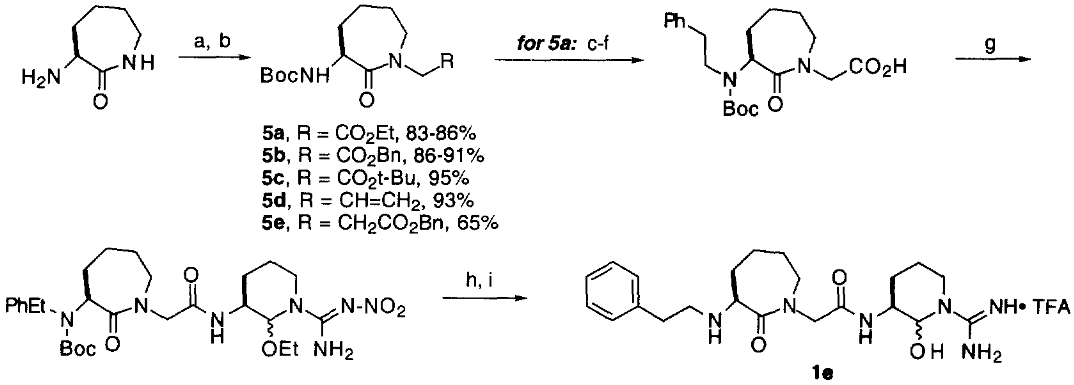
4a, R_4 = PhEt, 66%
4b, R_4 = PhPr, 70%;
4d, R_4 = 2-indanyl, ~quant.

R_4 = H, PhEt, PhPr;
(no Boc-protection necessary for 2-indanyl intermediate)

1a: R_4 = PhPr
1b: R_4 = PhEt
1c: R_4 = H
1d: R_4 = 2-Indanyl

Scheme 1: *Reagents and Conditions:* (a) 4 steps, ref 3 and 8; (b) BnBr, K_2CO_3, DMF, CH_3CN, reflux, 96%; (c) 5 N HCl, EtOAc, rt, 3 h, ~quant.; (d) Aldehyde or ketone deriv., $Na(OAc)_3BH$, Et_3N, DCE, rt, 66%-quant.; (e) Boc_2O, $NaHCO_3$, THF, H_2O, rt,1.5 h, 70-89%; (f) H_2, Pd/C, MeOH, 45 psi, 86%-99%; (g) HCl•Arg(NO_2)-H ethyl aminal, EDC, HOBt, DIPEA, CH_3CN, rt, 74-99%; (h) H_2, Pd/C, EtOH, HOAc, H_2O, 35-45 psi, RP-HPLC, 64-99%; (i) 3 N HCl, rt, ~2-3 h, RP-HPLC, 63-70%.

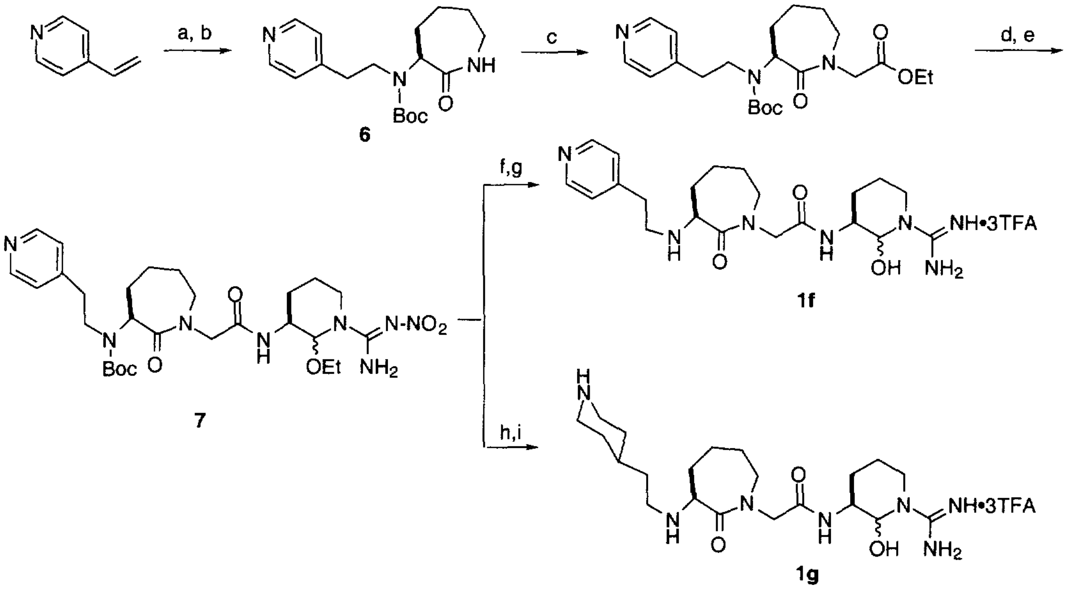
Synthesis of the 7-membered target **1e** proceeded in 9 steps from l-α-amino-ε-caprolactam as outlined in Scheme 2. The versatile intermediates **5a–e** were prepared in multigram quantities by selective alkylation of α-N-Boc-amino-ε-caprolactam. Li $N(TMS)_2$ was the preferred base, and the reactions proceeded very smoothly with



Scheme 2: *Reagents and Conditions:* (a) Boc_2O, $NaHCO_3$, THF, H_2O, 0 °C to rt, 95-99%; (b) $LiN(TMS)_2$,THF, alkylating agent, see text, 0 °C to rt, 83-95%; ***for 5a:*** (c) HCl, EtOH, 0 °C to rt, 96%; (d) $PhCH_2CHO$, $Na(OAc)_3BH$, Et_3N, rt, 69%; (e) Boc_2O, $NaHCO_3$, THF, H_2O, 84%; (f) LiOH, EtOH, H_2O, 0 °C to rt; Dowex H^+, 94%; (g) H-Arg(NO_2)-H ethyl aminal, EDC, HOBt, DIPEA, CH_3CN, rt, 18 h, 71%; (h) H_2, Pd/C, EtOH, HOAc, H_20, 50 psi, ~quant.; (i) 3 N HCl, rt, 3 h, HPLC, 77%.

ethyl-, benzyl-, or *t*-butyl- bromoacetate, or allyl bromide, providing the corresponding products **5a–d** in 83–95% yields. A Michael-type addition with benzyl acrylate afforded the β-N-lactam propionate **5e** in 65% yield. Elaboration of intermediate **5a** via a 7-step process delivered target **1e** in satisfactory overall yield.

Synthesis of the highly polar P_4-heterocyclic 7-membered lactam targets **1f** and **1g** proceeded in 7–8



Scheme 3: *Reagents and Conditions*: (a) l-α-amino-ε-caprolactam, HOAc, rt to reflux; (b) Boc_2O, THF, $NaHCO_3$, rt, 30-40% overall; (c) 1. $LiN(TMS)_2$, THF, rt, 2. $BrCH_2CO_2Et$, -5° to rt, 74-77%; (d) LiOH, EtOH, H_2O, 0° to rt, HCl to pH~6, ~quant.; (e) H-Arg(NO_2)H-OEt aminal, EDC, HOBt, NMM, CH_3CN, rt, 3 d, 50%; (f) H_2, Pd/C, MeOH, 18 psi, 4 days, 93%; (g) 4.5 N HCl, rt, 3 h, RP-HPLC, 24-44%; (h) H_2, Pd/C, EtOH, HOAc, H_2O, 45 psi, 4 d; (i) H_2, PtO_2, 45 psi,1 day, 90% overall.

steps as outlined in Scheme 3. The Michael-type addition of 1-α-amino-ε-caprolactam to 4-vinylpyridine was studied under a variety of conditions. We found this sluggish reaction to proceed best under concentrated conditions of ca. [5 M] in refluxing acetic acid. To expedite purification of the highly polar adduct, the crude mixture was treated directly with Boc_2O and delivered **6** in modest overall yields of 30–40%. Selective lactam N-alkylation and subsequent elaboration led to the advanced intermediate **7**. Hydrogenolysis (Pd/C, HOAc, ~1 atm) and hydrolysis provided P_4-pyridyl lactam **1f**, while a 2-stage hydrogenation protocol (~1 atm, Pd/C; ~3 atm, PtO_2) and hydrolysis furnished the P_4-piperidinyl lactam target **1g**.

Biological Activity

The in vitro biological activity of the targets **1a–g** along with the standards CVS 1578, CVS 1778, and CVS 2097 is shown in Table 1.[13] Most new targets were highly selective against FXa and plasmin. Activity levels on thrombin ranged from excellent to low. The 6-membered derivative **1b** expressed optimal thrombin inhibitory potency, being twice as active as CVS 1578, and showed excellent trypsin selectivity. The seven-membered target **1e** was less active than **1b.** These results contrast the SAR trends found in the P_4-benzylsulfonamide series, where the 7-membered targets CVS 1778 and CVS 2097 expressed highest levels of inhibitory potency.[5] In the six-membered lactam series, activity decreased as a function of the P_4 group in the following order: PhEt > 2-Indanyl > phenylpropyl > H. The P_4-heterocyclic targets **1f** and **1g**, which *a priori* were hypothesized to be interesting FXa inhibitors,[4,8] were relatively poor serine protease inhibitors.

Table 1. In vitro IC_{50} values (nM) of lactam argininals **1a–g** and reference standards against a range of important serine proteases.[a,b]

Compd	MOLNAME	FIIa	FXa	Plasmin	Hu Tryp	FXa/FIIa	Tryp/FIIa
	Reference Compounds:						
CVS 1578	BnSO2-6Lac-G-R-al	6.2	>2500	Inact.	1271	>403	205.0
CVS 1778	BnSO2-7Lac-G-R-al	0.71	22.8	Inact.	152	32.1	214.1
CVS 2097	(2CO2Me)BnSO2-7Lac-G-R-al	0.75	333	>2500	118	445	158
	New Targets:						
1a	PhPr-6Lac-G-R-al	128	Inact	Inact.	1420	-	11.1
1b	PhEt-6Lac-G-R-al	3.09	Inact.	Inact.	1560	very high	504.9
1c	6Lac-G-R-al	1100	Inact.	Inact.	1390	-	1.3
1d	2IndAm-6Lac-G-R-al	113	Inact.	Inact.	294	-	2.6
1e	PhEt-7Lac-G-R-al	53	Inact.	Inact.	821	high	15.5
1f	4PyrEt-7Lac-G-R-al	513	Inact.	>2500	1750	-	3.4
1g	4PprEt-7Lac-G-R-al	>2500	2500	Inact.	2500	-	-

[a]Concentration of **1a–g** and standards necessary to inhibit thrombin (FIIa), FXa, plasmin, and human trypsin cleavage of the chromogenic substrates described in ref 5a by 50%. Reported value for each compound is from a single IC_{50} determination which confirmed initial range values. [b]All target compounds were characterized by ^{1}HNMR, RPHPLC, low/high resolution mass spectroscopy.

The relative potency and selectivity profiles of **1b** may be rationalized from X-ray crystal structure and modeling considerations. The interactions in the S_1, S_2, and S_3 sites appear to be largely conserved between our reference compounds (cf. CVS 1578-thrombin X-ray structure[5a,7]) and models of **1b**. The backbone conformation of **1b** is very similar to CVS 1578 and both slightly differ from the orientation observed with P_2-proline containing inhibitors. Although the phenethyl moiety of **1b** may be more flexible and slightly shorter than the benzylsulfonamide residue of CVS 1578, the tethering of the aromatic ring into S_3 appears more favorable in the former. The phenyl group is efficiently tethered into the hydrophobic S_3 subsite, therefore mimicking the role of the P_3-phenylalanine sidechain in PPACK.[14] The lactam ring and N-methylene groups of **1b** are buried upon binding in the S_2 site by Tyr60A and Trp60D of thrombin's specificity loop, and the argininal sidechain in S_1 participates in a close electrostatic contact with Asp189. Thus, numerous active site interactions, coupled with the tetrahedral nature of the tether, may be of importance for conferring good thrombin inhibitory potency and trypsin selectivity onto this class.

Conclusion

Our rational design considerations, starting with the reference P_3–P_4 lactam sulfonamides and related inhibitors, generated a series of novel P_1-argininals **1a–g** which incorporate peptidomimetic P_3–P_4 hydrophobic, basic lactam moieties as active-site directed transition state analog inhibitors of thrombin. In vitro evaluation against serine proteases involved in the blood coagulation cascade and trypsin revealed inhibitors with variable levels of thrombin (FIIa) inhibitory potency and useful selectivity profiles. The six-membered lactam derivative **1b** was the most interesting candidate prepared. Numerous active site interactions coupled with optimal P_4-tether length and geometry are important for conferring good thrombin inhibitory potency and trypsin selectivity into this class.

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