Design, Synthesis, and Evolution of a Novel, Selective, and Orally Bioavailable Class of Thrombin Inhibitors: P1-Argininal Derivatives Incorporating P3-P4 Lactam Sulfonamide Moieties[1]

J. Edward Semple,* David C. Rowley, Terence K. Brunck, Theresa Ha-Uong, Nathaniel K. Minami, Timothy D. Owens, Susan Y. Tamura, Erick A. Goldman, Daniel V. Siev, Robert J. Ardecky, Stephen H. Carpenter, Yu Ge, Brigitte M. Richard, Thomas G. Nolan, Kjell Håkanson,† Al Tulinsky,† Ruth F. Nutt, and William C. Ripka

Corvas International, Inc., 3030 Science Park Road, San Diego, California 92121-1102, and Department of Chemistry, Michigan State University, East Lansing, Michigan 48824-1322

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Thrombin is a member of the trypsin class of serine proteases. It is regarded as the key terminal protease of the blood coagulation cascade and regulates normal hemostasis and abnormal intravascular thrombus development. Among its diverse array of biological functions, thrombin stimulates platelet aggregation and catalyzes the conversion of fibrinogen into fibrin; hence, it plays a major role in the development of thrombotic vascular disease.[2] 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 as well as other activities related to the maintenance of vascular function.[3] Thrombotic vascular disease is a major cause of morbidity and mortality in the industrialized world. Established clinical anticoagulant regimens include the administration of coumadin, heparin, or heparin derivatives. The problems associated with these agents are well-recognized.[4] Considerable efforts are being focused on the development of safe new classes of selective inhibitors derived from both natural[5] and synthetic sources,[6,7] which may function by either indirect or direct mechanisms of action.[3b,5a,7] Representative structures thereof are broadly based upon peptide as well as peptidomimetic templates.[8]

Certain peptidyl P1[9]-argininals such as **1**[10] have been found to be highly active as transition-state inhibitors of thrombin and related serine proteases.[11] Our recent work on orally active anticoagulants has led to a novel family of synthetic direct thrombin inhibitors from which the drug candidate PrPent-Asp(OMe)-Pro-Arg-H (**2**, CVS 1123, Figure 1) has emerged.[12] This compound is a potent ($K_i = 1$ nM), selective, and orally bioavailable active site directed inhibitor of thrombin which demonstrated promising pharmacological profiles in a recent phase I clinical trial.[12e]

Although CVS 1123 and other peptidyl argininals[10,11] have shown good thrombin inhibitory activity, oral bioavailability, and selectivity against related serine proteases including plasmin, coagulation factors VIIa (FVIIa) and Xa (FXa), they generally lack selectivity against trypsin. One strategy for achieving enhanced selectivity against trypsin was to focus on improving thrombin binding affinity by increasing conformational rigidity of the peptidic backbone present in inhibitors such as **1** and **2**. We also reasoned that inhibitors which exploit a unique interaction with thrombin's 60 loop might lead to drug candidates with improved selectivity profiles. Since they can mimic partial type II/II′ β-turn conformations,[13e,f] we pursued a series of novel thrombin inhibitors which incorporate lactam moieties as dipeptide surrogates.

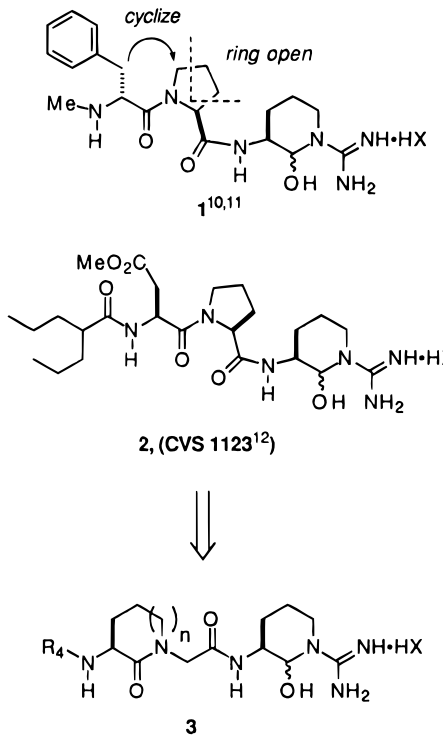
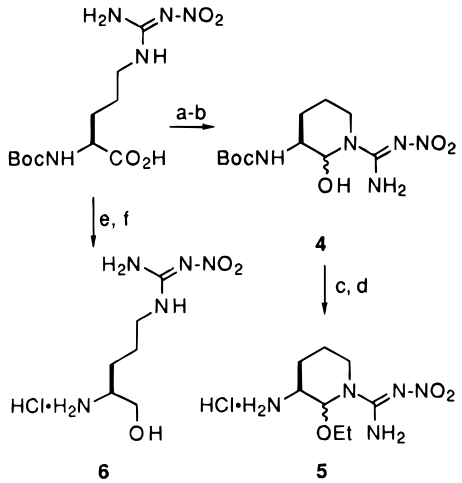


Figure 1. Structures of P1-argininal thrombin inhibitors.

Scheme 1[a]



[a] Key: (a) *i*-BuOCOCl, NMP, THF, −5 °C; Me(OMe)NH, −5 °C to room temperature, 88%; (b) $LiAlH_4$, THF, −78 °C; $KHSO_4$, H_2O, −70 to −30 °C, 74%; (c) EtOH, HCl(cat.) room temperature, 81%; (d) HCl (~12−20 equiv)/EtOH or EtOAc, 0 °C to room temperature, ~quant; (e) BH_3·THF, −78 to −20 °C; MeOH, −78 °C to room temperature, ~quant; (f) HCl, MeOH, 0 °C to room temperature, ~quant.

* Please send all correspondence to: Dr. J. Edward Semple, Corvas International, Inc., 3030 Science Park Rd., San Diego, CA 92121-1102. (619) 455-9800; Fax (619) 455-7895. E-mail address: ed_semple@corvas.com.

† Michigan State University.

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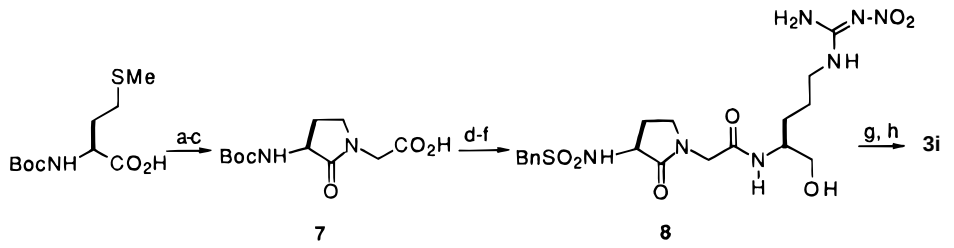
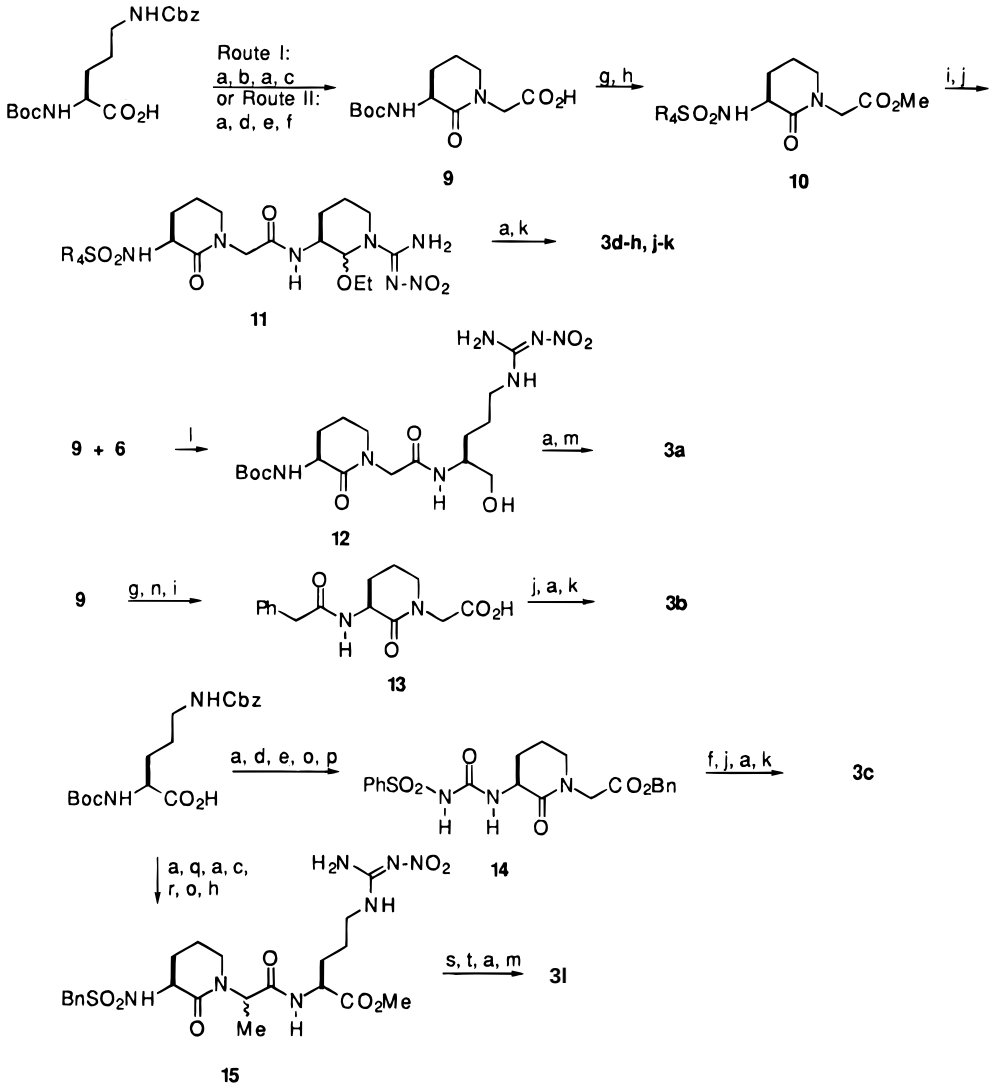
Scheme 2[a]



[a] Key: (a) HCl·Gly-OMe, DCC, HOBt, Et_3N, DMF, room temperature, 90%; (b) MeI, THF, ~quant; (c) NaH, DMF, CH_2Cl_2, 0 °C; H_2O, room temperature, 50%; (d) **6**, HBTU, HOBt, NMM, CH_3CN, room temperature, 70%; (e) HCl, EtOAc, MeOH, 0 °C, 90%; (f) $BnSO_2Cl$, Et_3N, CH_3CN, DMF, 0 °C to room temperature, 85%; (g) H_2, Pd/C, MeOH, HOAc, H_2O, 45 psi, ~quant; (h) EDC, Cl_2CHCO_2H, DMSO, ~5 °C to room temperature, HPLC, 77%.

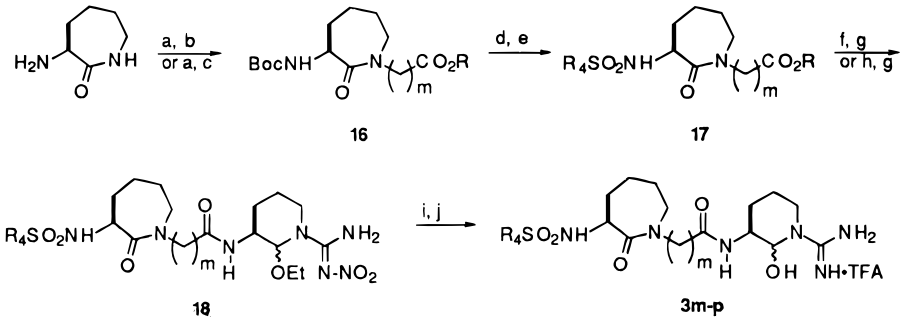
Scheme 3[a]



[a] Key: (a) H_2, Pd/C, MeOH, HOAc, H_2O, 35−50 psi, ~quant; (b) $OHCCO_2H$, room temperature; (c) DMF, 50−80 °C, 52−67% for four steps; (d) EDC, HOBt, NMM, CH_3CN, room temperature, 80%; (e) LiHMDS, THF, $BrCH_2CO_2Bn$, room temperature, 85%; (f) H_2, Pd/C, EtOH, 30 psi, 96−100%; (g) MeOH, HCl, 0 °C to room temperature, ~quant; (h) R_4SO_2Cl, Et_3N, CH_3CN, 0 °C to room temperature, 65−85%; (i) LiOH MeOH, H_2O; Dowex H^+ resin, 93−98%; (j) **5**, EDC, HOBt, DIPEA, CH_3CN, room temperature, 60−80%; (k) 3 N HCl, room temperature, 3 h; HPLC, 50−81%; (l) same as (d), add DMF, 61%; (m) EDC, Cl_2CHCO_2H, DMSO, ~5 °C to room temperature; HPLC, 60%; (n) $C_6H_5CH_2CO_2H$, EDC, HOBt, DIEA, CH_3CN, 75%; (o) HCl, EtOAc, 0 °C to room temperature ~quant; (p) $PhSO_2NCO$, DIEA, CH_3CN, 0 °C to room temperature, 85%; (q) pyruvic acid, room temperature; (r) HCl·Arg(NO_2)OMe, EDC, HOBt, NMM, CH_3CN, room temperature, 69%; (s) $LiBH_4$, THF, EtOH, 0 °C to room temperature, 75%; (t) HPLC separation.

The synthesis of lactam scaffolds as mimetics of peptide bonds and their incorporation into pharmaceutically important target molecules has received increasing attention.[13] Since the pioneering work of Freidinger,[14] α-amino-substituted lactam derivatives have been shown to serve as conformationally restricted dipeptide surrogates. Their potential for improving biological and pharmacokinetic profiles has been exploited in inhibitors of angiotension-converting enzyme,[13a,b] human leukocyte elastase,[13c,d] renin,[15c] and other important enzymes.[13−15] The incorporation of a lactam motif into an active site directed thrombin inhibitor has not been described. In our efforts to prepare P1-argininal thrombin inhibitors which express

Scheme 4[a]



[a] Key: (a) Boc_2O, $NaHCO_3$, THF, 0 °C to room temperature, 95−99%; (b) for $m = 1$: LiHMDS, THF, room temperature; $BrCH_2CO_2R$, 0 °C to room temperature; R = Et, 83−86%; R = Bn, 86−91%; R = t-Bu, 95%; (c) for $m = 2$: LiHMDS, THF, room temperature; $CH_2{=}CHCO_2Bn$, 0 °C to room temperature, 65%; (d) HCl, EtOAc, 0 °C to room temperature, ∼quant; (e) R_4SO_2Cl, Et_3N or NMM, CH_3CN, 0 °C to room temperature; 85−92%; (f) for R = Bn: H_2, Pd/C, EtOH, 15−45 psi; ∼quant; (g) **5** EDC, HOBt, DIPEA, CH_3CN, 54−68%; (h) for R = Et; LiOH, EtOH, H_2O, 0 °C to room temperature; Dowex H^+ resin; 95%; (i) H_2, Pd/C, EtOH, HOAc, H_2O, 45−60 psi, ∼quant; (j) 3 N HCl, room temperature, 3−4 h; HPLC, 74−81%.

selectivity toward related serine proteases encountered in blood coagulation and fibrinolysis, we designed and synthesized a family of dipeptide surrogates incorporating novel P3−P4 lactam sulfonamide moieties.[16] In this communication, we disclose our results on the monocyclic lactams **3a**−**p**. Subsequent reports will describe our efforts on fused bicyclic as well as heterocyclic variants.

Our design considerations for the targets **3** resulted from examination and combination of the salient topographic features[17] of structures **1** and **2**. Curved arrows accompanying structure **1** (Figure 1) are provided to delineate the tethering and/or bridging points from which the new series of lactams **3** were generated. Lactams of ring sizes five to seven afford a range of interesting physical (hydrophobic/size) and geometrical (stereochemistry, conformation, Ψ and Φ dihedral angles[13d,f]) properties. The lactam carbonyl along with the α-amide groups provide the essential hydrogen bond acceptor and donor elements necessary for high affinity antiparallel *β*-hydrogen bonding with the Gly216 residue in the thrombin active site.[17,18] Furthermore, the absence of natural peptide bonds in the P2−P4 region was expected to increase metabolic stability, possibly conveying useful levels of oral bioavailability and duration.[7,13,19]

The P1-argininal precursors **5** and **6** were prepared according to Scheme 1.[20] The 5-, 6-, and 7-membered lactam series were prepared as outlined in Schemes 2, 3, and 4, respectively. Each of the three lactam ring systems was prepared from different precursors which allowed for considerable variety and flexibility during the course of subsequent SAR development.[16] Freidinger's method[14a] was used to produce intermediates **7** and **9** (Schemes 2 and 3, respectively).[21] The 7-membered lactam intermediates **16** were obtained in very good overall yields from commercially available L-(−)-α-amino-ϵ-caprolactam.[22]

A convergent synthetic strategy was adopted for the efficient assembly of all targets. Coupling of an activated P4-acyl, -carbamoyl, -sulfamoyl, or -sulfonyl moiety with α-amino P3-lactam P2-acetic acid ester synthons, followed by P2 deprotection and further coupling with protected P1-argininal precursors led to the penultimate intermediates. Final conversion to the argininal targets **3a**−**p** was accomplished by two independent strategies. The first protocol involved the oxidation of a fully deprotected argininol precursor under mild conditions,[12b,16,20c] while the second and more convenient

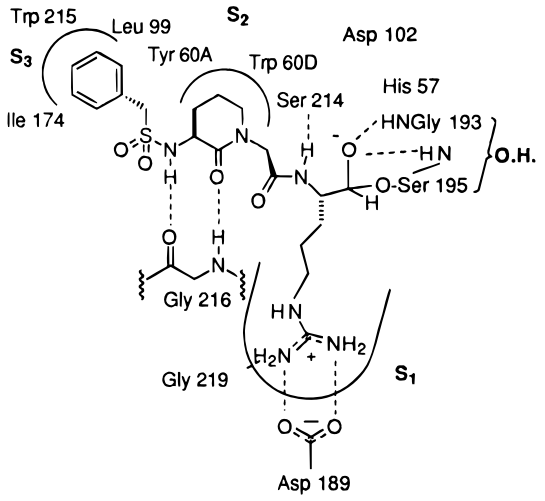


Figure 2. Schematic illustrating the key interactions of **3j** (CVS 1578)−thrombin based on the crystal structure. O.H. denotes oxyanion hole binding site.

method utilized the mild acid hydrolysis of an *O*-ethyl aminal precursor.[12b,20a,c] It is noteworthy that the second method has been successfully employed in our laboratory for the synthesis of up to 250 g quantities of selected argininal-containing serine protease inhibitors.

A preliminary X-ray crystal structure of thrombin-bound inhibitor **3j** was obtained which confirms the originally proposed substrate-like binding mode.[23] Compound **3j** is bound in thrombin's active site, as expected, with the argininal sidechain in S1 making a close electrostatic contact with Asp189. The benzyl group lies in the hydrophobic S3 pocket. The lactam ring and α-methylene occupy S2 and are mostly buried and are slightly shifted relative to other substrate-like inhibitors by Tyr60A and Trp60D of thrombin (Figures 2 and 3). Examining Figure 3, we noted the space available at S2 near thrombin's unique 60 specificity loop.[17b−d] This hydrophobic space suggested to us that substitution on and/or homologation of the P3-lactam ring could be accommodated. Exploration of this strategy led to the more potent 7-membered lactams **3m,n** which also maintained excellent selectivity against trypsin. The importance of geometry and tether length between the P3 and P4 moieties was further investigated by variation of both lactam ring size and P4 moiety. The high selectivity for thrombin may be due to the close interactions with the enzyme S2 and S3 sites as a result of the

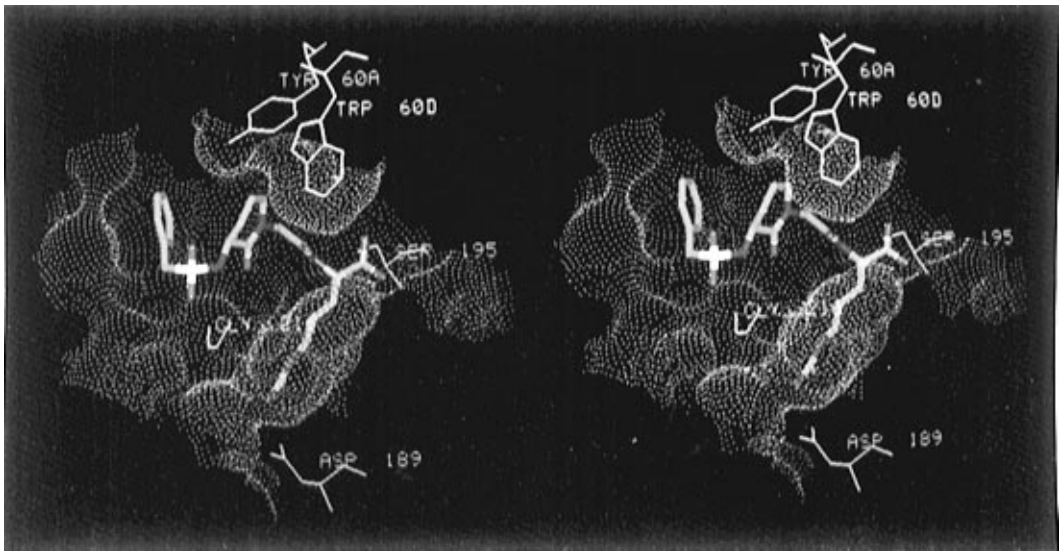
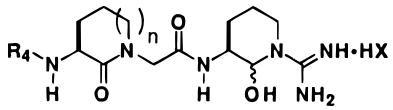


Figure 3. Stereorepresentation of the inhibitor **3j** (CVS 1578) bound in the thrombin active site. The inhibitor backbone is in green. The thrombin active site solvent accessible surfaces and key residues with their labels are in blue.

Table 1. *In Vitro* IC_{50} Values (nM) and Selectivity Ratios of Lactam Sulfonamides **3a**−**p** and Reference Compounds **1** and **2** against Thrombin (FIIa), FXa, and Trypsin[a,b]



compd	P4 substituent	*n*	FIIa[c]	FXa	Trypsin[d]	FXa/FIIa	Try/FIIa
3a	Boc	1	1460	2500	16750	1.7	11.5
3b	$PhCH_2CO$	1	∼2500	inact	>2500(b)		
3c	$PhSO_2NHCO$	1	inact	inact	inact		
3d	$BuSO_2$	1	709	2500	2500(b)	3.5	3.5
3e	1-$NaphSO_2$	1	111	2500	1020(b)	22.5	9.2
3f	$PhSO_2$	1	159	1100	770	6.9	4.8
3g	$PhEtSO_2$ (rac. P3)	1	420	2500	∼2500	∼6	∼6
3h	$ChxNHSO_2$	1	235	1950	147	8.3	0.6
3i	$BnSO_2$	0	125	2500	538(b)	20.0	4.3
3j	$BnSO_2$	1	*6.2 (1.01)*	2500	791	403.2	*127.6*
3k	$BnSO_2$	1(d-)	157	2500	467	15.9	3.0
3l	$BnSO_2$(P2-Ala)	1	286	2500	293(b)	8.7	1.0
3m	$BnSO_2$	2	*0.71 (0.54)*	20.6	74.5	29.0	*104.9*
3n	Bn(2-CO_2Me)SO_2	2	*1.12 (0.85)*	471	137	420	*122*
3o	$BnSO_2$(P2 β-Ala)	2	>2500	inact	>2500		
3p	$PhNHSO_2$	2	14.2	479	16.6	33.7	1.2
1	reference compound	see	*0.7*	>2500	1.52	∼3571	*2.2*
2	reference compound	Figure 1	*1.1*	290	1.36	264	*1.2*

[a] Concentration of **1**, **2**, and **3a**−**p** necessary to inhibit thrombin, FXa, and trypsin cleavage of the chromogenic substrates described in ref 24 by 50%. [b] All new target compounds were characterized by ^{1}H-NMR, RP-HPLC, low and/or high resolution mass spectroscopy. [c] K_i value in parentheses. [d] Human trypsin unless value is followed by (b), signifying bovine trypsin.

rigidity and geometry of the lactam and benzylic sulfonamide moieties.

The key interactions in the **3j** (CVS 1578)-thrombin complex provided by the X-ray crystal structure are shown schematically in Figure 2. As seen in Figures 2 and 3, important interactions commonly found in small molecule thrombin inhibitors are present at the active, S1, S2, and S3 sites.[17b,c] A complete description of the crystallography and comparison to other inhibitors will appear elsewhere.[23]

The *in vitro* IC_{50} data for the lactam sulfonamide inhibitors **3a**−**p** together with the reference compounds **1** and **2** is shown in Table 1.[24] Chromogenic assays were carried out with important serine proteases including thrombin (FIIa), factor Xa (FXa), and trypsin. Selectivity ratios of FXa/FIIa and trypsin/FIIa are tabulated. Six-membered lactams **3a**−**c**, incorporating carbamate, acyl, or sulfonylurea P4-moieties, respectively, were poor inhibitors of all proteases. Entries **3d**−**g** containing P4-tetrahedral sulfonamide functions demonstrated improved levels of activity toward thrombin. Dramatic increases in activity and selectivity, particularly against trypsin, were realized with the benzylsulfonamide inhibitor **3j** (CVS 1578). Replacement of the P4-benzylsulfonamide function with cyclohexyl- or phenylsulfamide moieties led to the less active members **3h** and **3p**, respectively.

We investigated several parameters which influenced the potency and selectivity profiles of this class. Thrombin inhibitory activity as a function of ring size decreases in the order 7 (**3m**) > 6 (**3j**) > 5 (**3i**). In the 6- or 7-membered lactam series, substitution in the ortho and/or meta benzylic P4-positions with small polar or lipophilic groups retains potency and selectivity, cf. **3n**. Lactams **3j,m,n** displayed superior selectivity against trypsin relative to **1** and **2**. This high selectivity may be due to a combination of factors including improved interactions with the thrombin 60 loop[17] and to the

unique interactions mentioned above in the X-ray structure discussion.

The requirement of the α-(*S*)-lactam absolute configuration for maintenance of high activity was confirmed by synthesis of the (*R*)-isomer **3k**.[25] The SAR at P2 was further probed with the preparation of the (*R*)- and (*S*)-alanine-type residues found in target **3l** (data for *S*-isomer shown) as well as the β-alanine unit in **3o**. All targets were significantly less active, suggesting optimal activity for P2-glycine-type residues. This result is consistent with the mode of binding shown by **3j** (Figures 2 and 3). The P2-alanine methyl groups may experience unfavorable steric interactions with His 57 in S2, since the P2-glycine methylene moiety of **5j** is within van der Waals distance of this residue.

The compounds **3j**, **3m**, and **3n** showed absolute oral bioavailability in dogs at levels of 35%, 66%, and 67%, respectively. The high levels of oral activity expressed by the 7-membered lactams is especially noteworthy.[26] Further biological evaluation of these candidates is under active study.

In conclusion, we have designed and evaluated a series of novel peptidomimetic lactam sulfonamide inhibitors. The optimal structures **3j**, **3m**, and **3n** contain 6- and 7-membered lactam sulfonamide P2−P4 moieties onto which a reactive P1-argininal function is strategically appended. These targets express both potent thrombin inhibitory activity and therapeutically interesting selectivity profiles. The lactam sulfonamide motif provides a useful scaffold from which many interesting new systems have emerged. Subsequent communications from our laboratory will describe our results in this area.

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(1) This paper is dedicated to the memory of Geraldine C. Semple.

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(21) In our hands, modification of the reported one-pot, four-step sequence by performing each step *individually* afforded more consistent yields of intermediate **11** (Scheme 3, route I). As outlined in Scheme 3, route II, we found an alternate four-step method to **11** which was operationally superior to that described above.

(22) Available from Sigma Chemical Co.

(23) Håkanson, K.; Tulinsky, A.; Brunck, T. K.; Levy, O. E.; Semple, J. E.; et al. manuscript in preparation.

(24) **Enzyme Assays: IC_{50} Determinations.** Human thrombin was purchased from Enzyme Research Laboratories, Inc. (South Bend, IN); its concentration was predetermined by the supplier from the absorbance at 280 nM and the extinction coefficient. The activity of this material was 3806 NIH units/mg. The potency of inhibitors (IC_{50}) was determined from the inhibition of the enzymatic (amidolytic) activity of thrombin at 22 °C, using the chromogenic substrate Pefachrome tPA (CH3SO2-D-hexahydrotyrosyl-glycyl-arginine-*p*-nitroanilide•HOAc, Pentapharm, Ltd., Basel, Switzerland), obtained from the American distributor Centerchem, Inc. (Tarrytown, NY). The substrate was reconstituted as a 4.0 mM stock with ultrapure water (18 MΩ-cm). The enzymatic reactions were monitored in the wells of a microtitre plate (Dynatech) by measuring the increase in absorbance at 405 nm, using a Thermomax microplate reader; this change in absorbance directly reflected thrombin's cleavage of Pefachrome tPA and the release of pNA. A stock solution of the inhibitor (1 mM) in ultrapure water was diluted to the desired range of 12 concentrations with Hepes-buffered saline containing BSA (HBSA), 10 mM Hepes, 150 mM NaCl, and bovine serum albumin, 0.1%, w/v, pH 7.5. All reactions were performed in triplicate in a final volume of 200 μL of HBSA, containing, at final concentration, 0.5 nM thrombin and 250 μM Pefachrome tPA. To individual wells was added 50 μL of inhibitor, or in the case of the control, 50 μL of HBSA, followed by 50 μL of HBSA and 50 μL of human thrombin. After a 30 min incubation, the reaction was initiated by the addition of 50 μL of Pefachrome tPA. All reactions were under steady-state conditions, where less than 3% of the substrate was consumed. The increasing absorbance was measured at 10 s intervals over 5 min, and the values were stored by a dedicated computer, using Softmax software. From the data, the software allowed for the calculation of the velocity (change in absorbance per min); the averaged velocity for a triplicate sample was plotted against the inhibitor concentration. The data were then fit to a curve described by the four-parameter equation: $Y = (A - D)/(1 + (X/C)^B + D$ where the IC_{50} is represented by term C in the equation. The selectivity of the new inhibitor was also examined against FXa and trypsin. The IC_{50} values were calculated as outlined above for thrombin. Other chromogenic substrates were purchased from Chromogenix. The concentration of enzyme and substrate employed follows: FXa [0.25 nM], S-2765 [250 μM], trypsin [0.5 nM], and S-2222 [250 μM].

(25) Subsequent synthetic investigations and *in vitro* biological evaluation of related P3-lactam derivatives possessing the α-(*R*) absolute stereochemistry corroborated the observation of significantly decreased thrombin inhibitory potency.

(26) The absolute systemic bioavailability (%F) for compounds **3j**, **3m**, and **3n** was determined in fasted, conscious, purpose-bred beagle dogs (two males and two 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 postcolumn 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 %F (AUC-[oral]/AUC[iv] × 100). Further details on the pharmacokinetic and pharmacodynamic profile of these compounds will be published elsewhere.

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