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DESIGN AND CONSTRUCTION OF NOVEL THROMBIN INHIBITORS FEATURING P_3-P_4 QUATERNARY LACTAM DIPEPTIDE SURROGATES

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Abstract: Potent serine protease inhibitor **1a** featuring a hybrid P_3-P_4 quaternary lactam dipeptide surrogate was prepared based upon SAR and molecular modeling investigations and in order to further probe the S_2/S_3 thrombin and FXa subsites. An efficient and concise synthetic route to the key aminolactam intermediate **4** was developed. The design, synthesis, and biological activity of this target and its P_3-P_4 diastereomer **1b** is presented.

The high incidence of myocardial infarction and cardiovascular disease caused by thrombosis represents a leading cause of morbidity and mortality in the industrialized world. Accordingly, the development of safe, selective thrombin (FIIa) and FXa inhibitors as potential antithrombotic drugs is an area of intense current interest in the pharmaceutical industry.[1-3] Thrombin (FIIa) and Factor Xa (FXa) are trypsin-like serine proteases involved in the initiation and propagation of the coagulation response to vascular injury. They play key roles in the regulation of normal hemostasis and abnormal intravascular thrombus development (thrombosis). Recently in our laboratories, the novel peptidomimetic P_3-P_4 lactam sulfonamide derivative **CVS 1578**[4] and the quaternary amino variant **CVS 1897**[5] were identified as potent transition-state thrombin inhibitors, which demonstrated good oral bioavailability and selectivity profiles. Improved thrombin binding affinity relative to previous peptidic inhibitors led to enhanced selectivity against trypsin and was achieved in part by exploiting a unique lactam-S_2 interaction with thrombin's 60 insertion loop. Employing X-ray structural information from the lead candidate **CVS 1578** complexed with thrombin, along with topological considerations of the quaternary lactam **CVS 1897**, a pair of novel P_1-argininal targets **1a,b** were designed which feature a P_3-P_4 quaternary lactam moiety[6] containing an ester residue (Figure 1). Such scaffolds may be considered as novel hybrid types of Phe/Asp(or nor-Asp)-Pro dipeptide mimics. The design, synthesis, and biological activity of these targets will be presented in this letter.

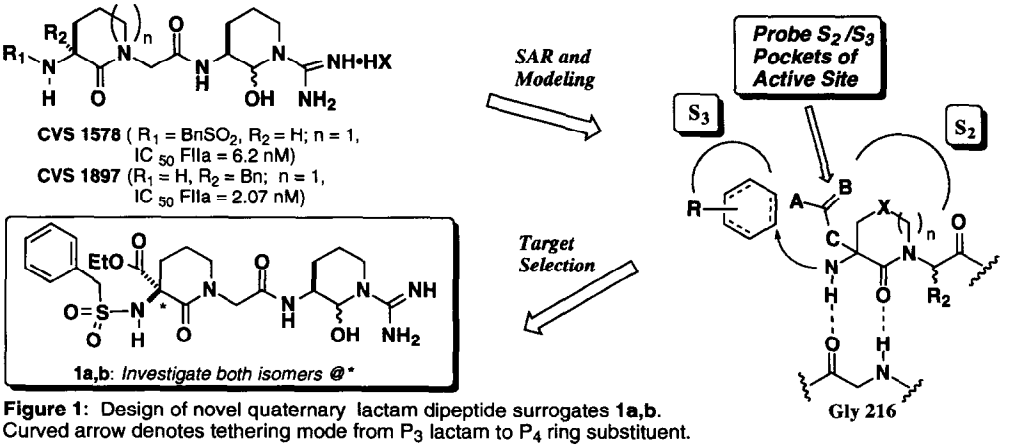

Figure 1: Design of novel quaternary lactam dipeptide surrogates **1a,b**. Curved arrow denotes tethering mode from P_3 lactam to P_4 ring substituent.

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Inhibitor Design Strategy

Perusal of crystal structures[4,7] and topographic examination of **CVS 1578**, (n = 1, FIIa IC_{50} = 6.2 nM) and the quaternary lactam system **CVS 1897** (n = 1, FIIa IC_{50} = 2.07 nM) suggested an attractive series of second generation targets featuring the generic A = B - C residue which provides a functional handle for probing either the S_2 and/or S_3 specificity pockets of thrombin or FXa (Figure 1). In principle, the A = B - C functionality could present tethered cationic, anionic, polar, or hydrophobic groups or combinations thereof at the active site to enhance inhibitor binding affinity. In turn, this could result in inhibitors with increased potency and selectivity profiles. Modeling of the lactam targets **1a,b** indicated that the quaternary ethyl ester residue may provide both a hydrogen bond to Tyr-60A and a hydrophobic interaction with Leu-99. *A priori*, the α–(*R*)-lactam **1a** would be the biologically preferred candidate.

A schematic illustrating the modeled **1a**-thrombin complex based upon the **CVS 1578**-thrombin crystal structure[4,7] is shown in Figure 2. Important interactions commonly found in small molecule thrombin inhibitors are present at the active, S_1, S_2, and S_3 subsites. As discussed in our previous communications,[1d,e,4,5] our P_1-P_4 lactam argininal motifs provide a full complement of important backbone and side-chain 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. Tethering the aromatic ring to the S_3 site is accomplished employing a variety of linkers, preferably of a tetrahedral nature.[4] Thus, important interactions commonly found in small molecule serine protease inhibitors are present at the active, S_1, S_2, and S_3 subsites. Herein, we describe a synthetic approach to the quaternary targets **1a,b** featuring an α-benzylsulfonamido-α-ester residue.

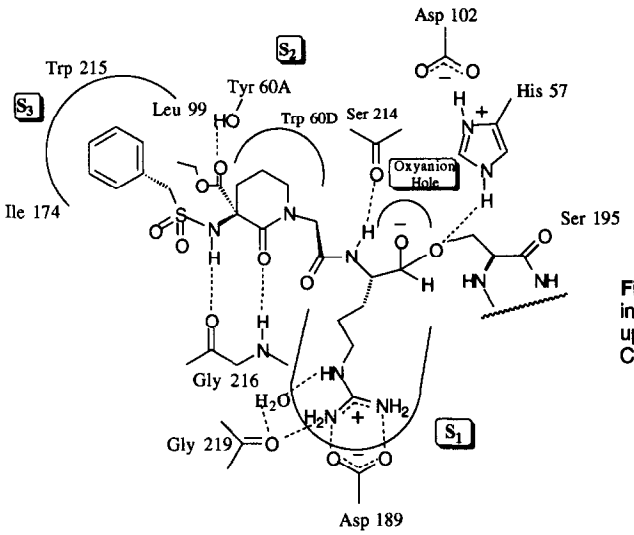
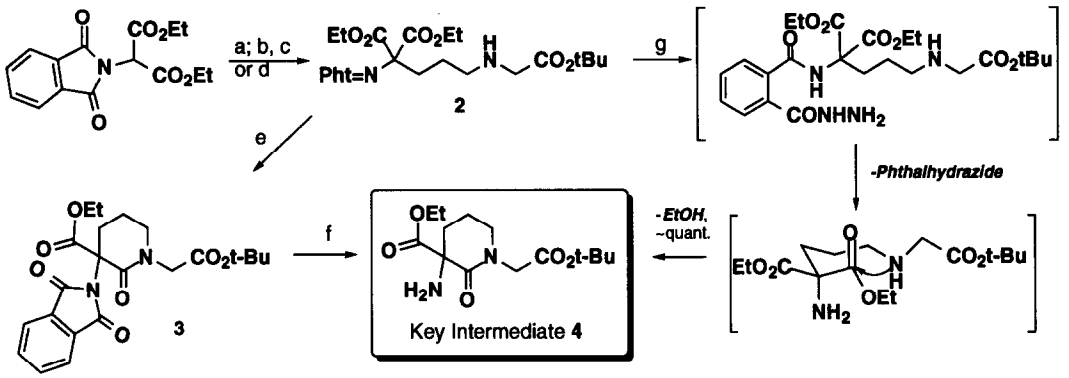


Figure 2: Schematic illustrating the key interactions of modeled **1a**-thrombin based upon the crystal structure of the reference CVS 1578-thrombin complex.

Synthetic Chemistry

Our synthetic foray to the key quaternary α-amino lactam intermediate **4** is outlined in Scheme 1.[8] A convenient Michael addition of diethyl phthalimidomalonate with acrolein led to multigram quantities of the corresponding 3-substituted propionaldehyde derivative. Three reductive amination procedures were next examined employing the preceding aldehyde and glycine *tert*-butyl ester, and we determined that the $Na(OAc)_3BH$

protocol[9a] was the safest and most convenient route to amine **2**. Thermal cyclization of sterically encumbered intermediate **2** to the desired quaternary lactam intermediate **3** was investigated under a wide variety of conditions. This proved to be a difficult, slow reaction and of the 20 variations investigated, we found 2-propanol or *tert*-butanol solvents in the presence of 10 equivalents of anhydrous sodium acetate,[6d] optionally in the presence of catalytic acetic acid in a sealed tube at 120–140 °C, to be optimal conditions for providing modest (40–46%) yields

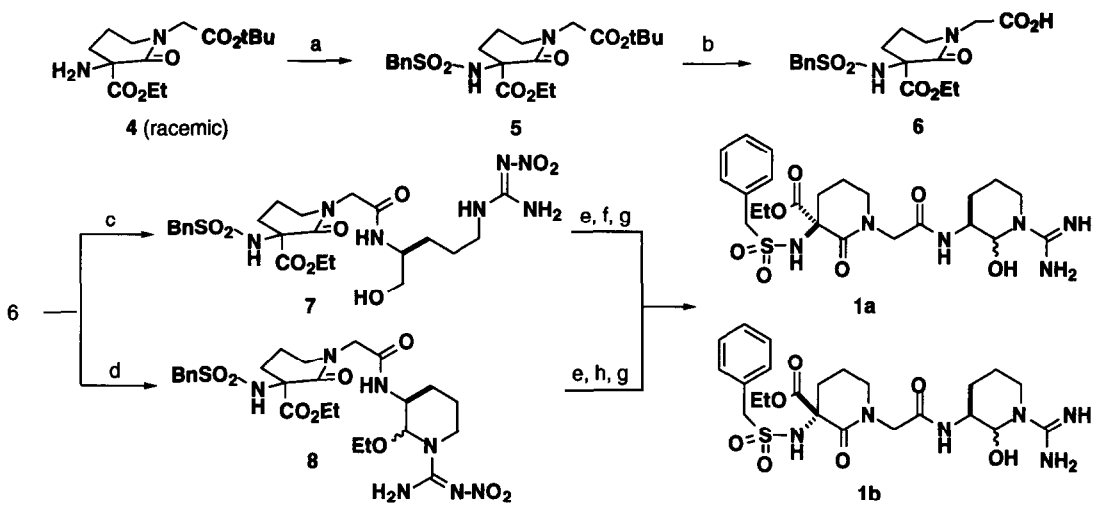


Scheme 1: *Reagents and Conditions:* (a) 1. Acrolein, DIPEA, EtOH, 0 °C to rt, 3 days; 2. HOAc, 72 %, 20-25 g scale *or* 1. Acrolein, NaOEt (cat.), EtOH, 0 °C to rt, 1 day; 2. HOAc, 38%, 5-10 g scale; (b)1. HCl• GlyO-t-Bu, Et_3N, EtOH, rt; 2. H_2, Pd/C, 1 atm, ~27-40%; (c) HCl•GlyO-t-Bu, $NaCNBH_3$, EtOH, rt, 79 %; (d) HCl•GlyO-t-Bu, $Na(OAc)_3BH$, DCE, Et_3N, rt, 65-68%, 9-15 g scale; (e) 2-PrOH, NaOAc (10 equiv), HOAc (cat.),120 ° C, 63 h, 140 °C, 14 h, sealed tube, 46%; (f) $NH_2NH_2•H_2O$ (1.1 equiv), EtOH, rt to reflux, ~90-95%; (g) $NH_2NH_2•H_2O$ (1.1 equiv.), EtOH, rt to reflux, ~quant

of product **3**. The corresponding phthalimido ester ring-opened byproducts were identified as the major byproducts in all cases employing alcoholic solvents. Hydrazinolysis of **3** under standard conditions delivered the desired key intermediate **4** in high yield.

An alternate approach to the quaternary α-amino lactam intermediate **4** is also outlined in Scheme 1. Cognizant that the difficulties of the thermal cyclization process to afford **3** were due to the sterically hindered nature of precursor **2**, we were intrigued by the possibility of removing the bulky phthalimido moiety first and determining the fate of the resulting amino intermediate. We were delighted to find that hydrazinolysis of **2** led via the intermediates depicted in Scheme 1 directly to the key lactam **4** in essentially quantitative yield. The reaction proceeds under mild conditions and gives >95% isolated yields of product on multigram scales.

Synthesis of the targets **1a,b** proceeded in 6 additional steps from the key intermediate α-aminolactam **4** as outlined in Scheme 2. The sterically encumbered environment of intermediate **4** caused its reaction with benzylsulfonyl chloride to be rather slow and capricious, providing **5** in yields ranging from 45–62%. The conditions summarized below in the scheme were optimal for the formation of product **5**. Treatment of **5** with TFA under standard conditions delivered the carboxylic acid derivative **6** in good yield. In order to prepare potentially resolvable late-stage diastereomers, amide bond couplings of **6** with both side-chain protected P_1-argininol[4] and P_1-argininal acetal[4,10] fragments were investigated and afforded the corresponding advanced intermediates **7** and **8**, respectively. Although formation of the diastereomeric pairs **7** and **8** proceeded smoothly and in good yield, our efforts at isomer separation via either tlc or HPLC protocols were unrewarding.



Scheme 2: *Reagents and Conditions*: (a) $BnSO_2Cl$, Et_3N, DMAP, CH_3CN, 0 °C to rt, 62%; (b) TFA, CH_2Cl_2, 0 °C to rt, 82-85%; (c) HCl•Arg(NO_2)-ol, EDC, HOBt, DIPEA, CH_3CN, rt, 54%; (d) HCl•H-Arg(NO_2)-H (OEt cyclol), EDC, HOBt, DIPEA, CH_3CN, rt, 63%; (e) H_2, Pd/C, 50 psi, EtOH, HOAc, H_2O, ~quant.; (f) EDC, DCAA, DMSO, toluene, 5 °C to rt; (g) RP-HPLC isomer separation, 63% from **7**, 73% from **8**; (h) 3 N HCl, rt, 2-3 h.

Fortunately, we successfully resolved the targets at the last stage during the HPLC purification step. Thus, hydrogenolysis of advanced intermediate **7**, oxidation, and RP-HPLC purification delivered the isomerically puretargets **1a,b** in good overall yield. Alternately, hydrogenolysis of intermediate **8**, mild acidic hydrolysis of the ethyl aminal moiety, and RP-HPLC purification delivered the targets **1a,b** in 73% combined yield. The final separation of the diastereomeric lactam target **1a** from **1b** by preparative RP-HPLC, although quite tedious due to the existence of three distinct interconverting P_1-argininal forms[4,5,10] for each diastereomer, provided good yields of pure samples of the biologically active α-(*R*)-lactam **1a** as well as the less interesting α-(*S*)-lactam **1b**. The lactam chirality assignments are tentative and were assigned by modeling considerations of the steric requirements of inhibitor binding into the S_3-thrombin subsite and by the relative biological activity profiles.[4,5] In turn, the observed activity in the new quaternary lactam series correlated well with results obtained in our previous studies where both (*R*)- and (*S*)-P_3-P_4 lactam sulfonamide (**CVS 1578** series) as well as the α-benzyl quaternary lactam (**CVS 1897** series) thrombin inhibitors were compared by in vitro evaluation.

Biological Activity

The in vitro biological activity of the targets **1a,b** along with three standards **CVS 1578**, **α-(*R*)-CVS-1897** and **α-(*S*)-CVS 1897** is shown in Table 1. The assays were carried out using a range of important human serine protease enzymes including trypsin, the procoagulants thrombin (FIIa) and factor Xa (FXa), as well as the thrombolytic enzyme plasmin. The new target **1a** (α-(*R*)-lactam) was selective against plasmin while expressing broad spectrum activity on thrombin, FXa and trypsin. It was more potent, but less selective than the standards. Although isomer **1b** (α-(*S*)-lactam) showed improved selectivity towards trypsin and plasmin, it was an order of magnitude less active against FIIa and FXa. In accord with our previous SAR studies, quaternary lactams of the (*R*)-absolute configuration are biologically preferred.[5] These results suggest the quaternary P_3-P_4 lactam scaffold binds in the thrombin active S_2 and S_3 subsites in a normal substrate-like mode.[1d,e;4,5] Based on

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

Cmpd	MOLNAME	FIIa	FXa	Plasmin	Hu Trypn	FXa/FIIa	Tryp/FIIa
	Reference Compounds:						
CVS 1578	$BnSO_2$-6Lac-G-R-al	6.2	2500	>2500	791	403.2	127.6
CVS 1897 α-(*R*)	6Lac(alphaBn)-G-R-al; α-(*R*)-isomer	2.07	Inact.	Inact.	139	very high	67.1
CVS 1897 α-(*S*)	6Lac(alphaBn)-G-R-al; α-(*S*)-isomer	137.5	Inact.	Inact.	2500	-	18.2
	New Quat. Lactam Targets:						
1a α-(*R*)	$BnSO_2$-6Lac(alphaCO_2Et)-G-R-al; α-(*R*)-isomer	1.1	5.78	271	2	5.3	1.8
1b α-(*S*)	$BnSO_2$-6Lac(alphaCO_2Et)-G-R-al; α-(*S*)-isomer	64.4	43.2	>2500	202	0.7	3.1

[a]Concentration of **1a,b** and reference standards necessary to inhibit thrombin (FIIa), FXa, plasmin, and human trypsin cleavage of the chromogenic substrates described in ref 4a by 50%. Reported value for each compound is the average from two IC_{50} determinations which confirmed initial range values. [b]All target compounds were characterized by ^{1}HNMR, R-P HPLC, low/high resolution mass spectroscopy.

modeling, we surmise that the P_3-P_4 quaternary ethyl ester residue provides both a hydrogen bond to Tyr-60A and a hydrophobic interaction with Leu-99 at the S_2 and S_3 subsites. These additional active site interactions possibly contributed to increased inhibitor activity profiles.[4,7] Other important backbone and sidechain inhibitor-active site interactions were conserved. Although the quaternary ester residue afforded a more potent serine protease inhibitor, it also resulted in a less selective inhibitor class.

Conclusion

Consideration of the P_3–P_4 lactam sulfonamide **CVS 1578** and quaternary lactam inhibitor **CVS 1897** reference scaffolds led to the rational design and synthesis of the novel P_1-argininals **1a,b.** These targets incorporate peptidomimetic P_3–P_4 quaternary lactam moieties featuring α-benzylsulfonamido-α-ester probes as active-site directed transition state analog inhibitors of important human serine proteases. Structurally, such a residue may be regarded as a hybrid Phe/Asp(or nor-Asp)-Pro dipeptide surrogate. In vitro evaluation against serine proteases involved in the blood coagulation cascade and trypsin revealed that **1a** expressed high thrombin (FIIa), FXa and trypsin inhibitory potency but lacked useful selectivity profiles. Quaternary P_3-lactams tentatively possessing the α-(*R*)-configuration showed the greatest biological activity. Numerous active site interactions coupled with the rigidity and geometry of the quaternary lactam center are important for conferring good inhibitory potency onto this class. However, subtle variations at the P_3-P_4 moieties may affect the selectivity profiles of such systems.

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