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RATIONAL DESIGN, SYNTHESIS, AND SERINE PROTEASE INHIBITORY ACTIVITY OF A NOVEL P_1-ARGININAL DERIVATIVE FEATURING A CONFORMATIONALLY CONSTRAINED P_2-P_3 BICYCLIC LACTAM MOIETY[1]

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Abstract: Based on molecular modeling and judicious combination of the salient topographic features of the recently discovered P_3-lactam derivative **1** with the P_2-prolyl derivatives **2a,b**, the novel thrombin inhibitor **3a** was designed. Inhibitor **3a** incorporates a fused bicyclic lactam as a novel type of P_2-P_3 dipeptide surrogate. The synthesis and biological activity of this potent serine protease inhibitor is presented.

The coagulation proteases thrombin and factor Xa are members of the trypsin class of serine protease enzymes. They are critically involved with the initiation and propagation of the coagulation response to vascular injury and thus play a vital role in the regulation of normal hemostasis and abnormal intravascular thrombus development.[2] Thrombotic vascular disease is a major cause of morbidity and mortality in the industrialized world. Accordingly, inhibition of thrombosis has become a major focus of drug discovery over recent years.[3] Considerable advances have been made in the design and synthesis of selective inhibitors of thrombin (FIIa) as well as related key serine proteases.[4] Such inhibitors may function by either indirect or direct mechanisms of action,[5] and representative examples can incorporate novel peptidomimetic[6] as well as more traditional peptide[7] motifs.

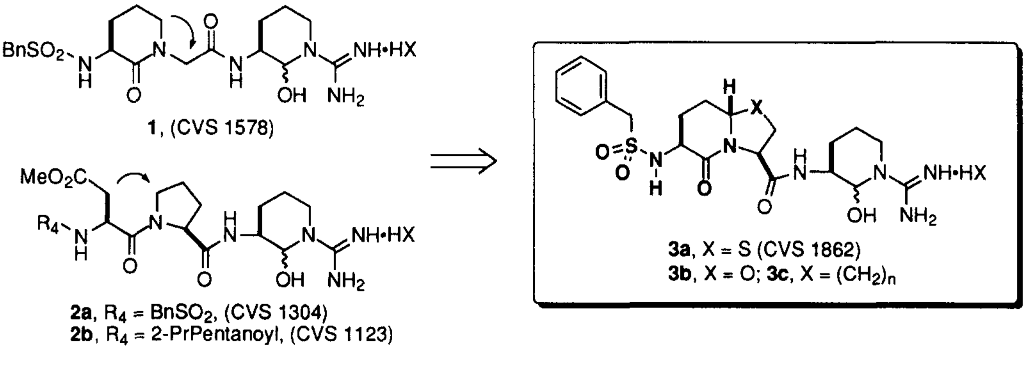


Figure 1. Conformational Restriction of Reference Compounds **1** and **2a,b** to Generate Novel Targets **3a-c**.

The application of mono- and bicyclic lactam scaffolds as peptide surrogates and their incorporation into pharmaceutically interesting target molecules is currently an area of active investigation.[8] In this context, lactams are being employed for the preparation of peptidomimetics of the i + 1 and i + 2 residues of type II' β-turn conformations.[9] They are considered as useful templates since their backbone structures usually maintain or restrict biologically relevant dihedral angle, conformational, and stereochemical information derived from the parent peptide array. They can incorporate critical hydrogen bond donor and acceptor elements such as amide NH and carbonyl groups. Scaffolds such as these can therefore display many useful structural features which will help stabilize a potential inhibitor in the active site of a biologically important enzyme target.

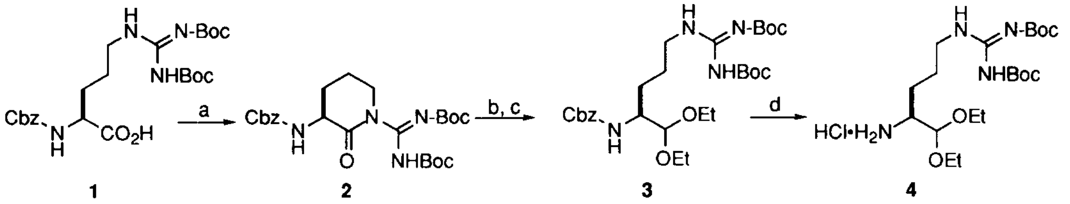
We recently described the design, synthesis, and evolution of a new class of potent and selective thrombin inhibitors which incorporated P_3-P_4 lactam sulfonamides as a novel type of dipeptide surrogate.[10] Subsequent optimization in this series was facilitated by the availability of an x-ray structure of the lead candidate **1** (CVS 1578, Figure 1) crystallized into the thrombin active site. Using crystal structure information from both this family and the P_2-proline argininal inhibitors **2a** (CVS 1304[11]) and **2b** (CVS 1123[11,12]), we designed the new series of potential inhibitors of formula **3a-c**. The curved arrows accompanying structures **1** and **2a,b** delineate the bridging or tie-back points from which the targets **3a-c** were generated. Structures **3a-c** represent a topographically novel type of P_2-P_4 dipeptide surrogate that can be considered as a hybrid resulting from the fusion of the P_2-proline residues with the P_3-lactam moieties. Examination and docking of models revealed that such fused bicyclic lactam systems could be easily accommodated in the thrombin active site. The spatial features of such an assembly provide inhibitors which are able to effectively undergo all the traditional energetically favorable interactions and also exploit the unique interaction at the 60 loop in the thrombin active site.[13,14] These new targets retain the argininal function as the electrophilic transition-state analog functionality at P_1, an important feature that often imparts high levels of oral bioavailability to thrombin inhibitors.[10,15]

Although we were intrigued by all three structures (**3a-c**), our initial studies focused on the novel fused thiazolidine lactam target **3a**, which was accessible via a stereocontrolled synthetic approach. The new motif nicely simulated the backbone structures found in inhibitors **1** and **2a,b**. The appended carbonyl and amide N-H functions, thought to provide essential hydrogen bond acceptor and donor elements necessary for high affinity antiparallel β-hydrogen bonding with the Gly 216 residue in the thrombin active site,[10,14] are appropriately positioned. We and others have found such stabilizing interactions to be very important in various classes of thrombin inhibitors.[11,13,15] The absence of normal peptide bonds in this molecule could impart increased levels of metabolic stability, which in turn may afford drug candidates with relevant pharmacological profiles.[16]

Chemistry[17]

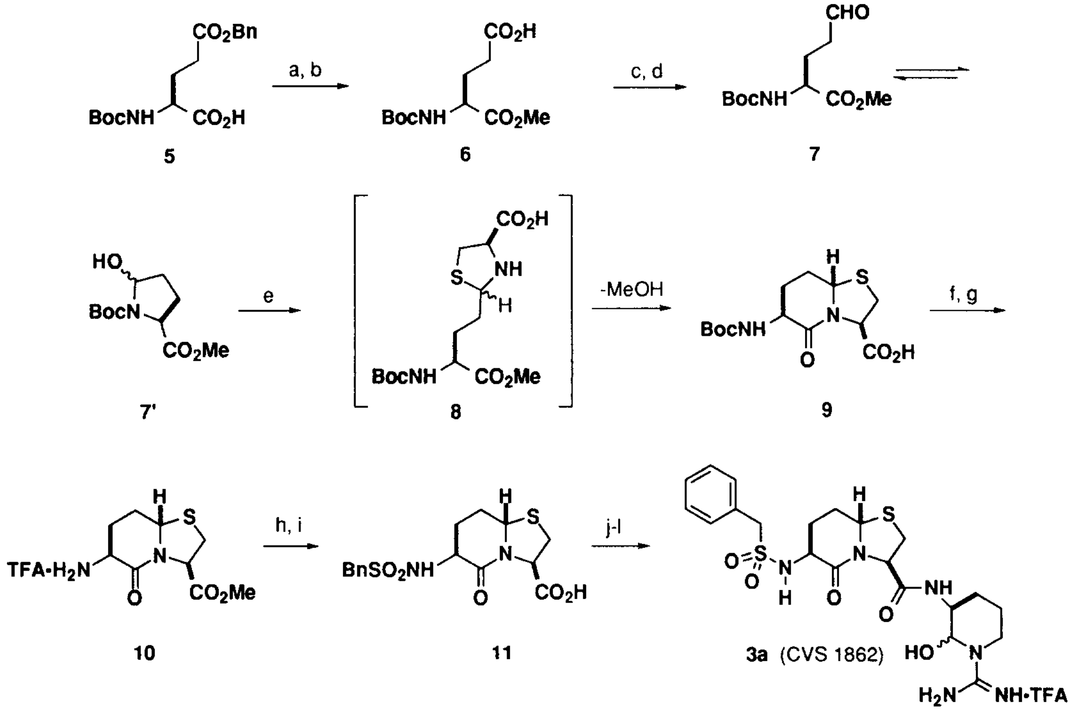
The synthesis of the P_1-argininal precursor is outlined in Scheme 1. Due to the presence of the sulfur atom in our final target, we needed to avoid any late-stage hydrogenolytic deprotection protocols. This consideration precluded the use of our recently established methodology, which proceeds via a penultimate hydrogenolysis of a ω-nitroarginine aminal derivative.[18] Commercially available Cbz-Arg(Boc_2)-OH **1** was intramolecularly dehydrated by treatment with EDC and HOBt under standard conditions and generated the lactam **2** in excellent yield. Controlled low temperature $LiAlH_4$ reduction and very careful acidic quenching afforded a labile aldehyde intermediate in high yield that was immediately treated with anhydrous ethanol and hydrogen chloride to provide the diethyl acetal **3**. Hydrogenation of **3** in the presence of one equivalent of HCl afforded the aminoacetal salt **4** in 74% overall yield.

To address our concerns regarding racemization of the sensitive P_1-methine center during both the acetalization and final hydrolytic steps, we developed a rapid and convenient analytical HPLC method that examined the formation and separation of diastereomeric pairs. Coupling of **4** with the chiral P_2-P_4 synthon PrPent-Asp(OMe)-Pro-OH and elaboration afforded the inhibitor **2b** and its corresponding P_1-isomer. Routine reversed-phase HPLC analysis clearly showed the desired P_1-α-(S)-argininal form along with the corresponding α-(R)-diastereomer. By application of this protocol, we typically obtained reference compound **2b** in ≥90% d.e., implying that both the crucial acetalization and final hydrolytic steps proceeded with satisfactory retention of optical integrity.



Scheme 1. *Reagents and conditions:* (a) EDC, HOBt, CH$_3$CN, 0° C to rt, 94%; (b) LiAlH$_4$, THF, -60° C; KHSO$_4$, H$_2$O, -70° C to -30° C, 95%; (c) EtOH, HCl, -20° C to rt; (d) H$_2$, Pd/C, 1 equiv. HCl, EtOH, 74% for two steps.

Construction of the bicyclic lactam intermediates and subsequent assembly of the target **3a** is outlined in Scheme 2. By an economical two-step protocol, Boc-Glu(OBn)-OH **5** was elaborated into glutamic acid derivative **6**.[19] Formation of the corresponding thioester followed by ionic hydrogenation[20] delivered the



Scheme 2. *Reagents and conditions:* (a) CDI,THF, 0° C to rt; MeOH, 0° C to rt, 93%; (b) H$_2$, Pd/C, THF, quantitative; (c) EtSH, EDC, DMAP, CH$_3$CN, rt, 78%; (d) Et$_3$SiH, Pd/C, acetone, rt, 2 h, 75%; (e) Cysteine, pyr, 4Å molecular sieves, rt, 4 h, reflux, 18 h, 78%; (f) repeat step a, 75%; (g) TFA, CH$_2$Cl$_2$, 0° C to rt, 81%; (h) BnSO$_2$Cl, collidine, 0° C to rt, 50%; (i) NaOH, MeOH, H$_2$O, 0° C, H$^+$, 0° C, 89%; (j) **4**, EDC, HOBt, NMM, CH$_3$CN, 81%; (k) 3 N HCl, CH$_3$CN, rt; (l) HPLC, 32%.

protected glutamic semialdehyde **7**, which, by NMR analysis and chemical reactivity profiles, exists predominately in the hemiaminal form **7'**. Following a modification of Baldwin's protocol,[21] condensation of **7** with cysteine led, via intermediate **8**, to the protected bicyclic lactam derivative **9** in a gratifying overall yield of 78%. The thiazolidine intermediate **8** is formed as a mixture of diastereomers at the heterocyclic function, but under the reaction conditions, equilibration to the more thermodynamically stable isomer occurs, and generates the desired stereochemistry depicted in product **9**.[21a,b] Formation of the methyl ester via the acyl imidazole intermediate followed by acid-catalyzed Boc-deprotection afforded the amine salt **10** in good overall yield. Coupling with benzylsulfonyl chloride in the presence of collidine and subsequent ester hydrolysis afforded the advanced intermediate carboxylic acid **11** in satisfactory overall yield. Amidation of **11** with amino acetal **4** under standard EDC, HOBt conditions delivered the penultimate intermediate in good yield. Hydrolysis with dilute hydrochloric acid in acetonitrile simultaneously deblocked both guanidino-Boc groups and hydrolyzed the ethyl aminal moiety. A final reverse phase HPLC purification delivered the target **3a** (CVS 1862) in an unoptimized yield of 32%.

Biological Activity

The biological activity of the target **3a** along with the standards **1** and **2a,b** is shown in Table 1. The in vitro 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 enzymes plasmin and tissue plasminogen activator (tPA).[22] The new target **3a** was selective against FXa, plasmin, and tPA while expressing potent activity on thrombin. It was slightly less active than the standards and the observed activity/selectivity profile is more reminiscent of the P_2-proline systems **2a,b** than the monocyclic lactam **1**. This result suggests that the P_2-P_3 bicyclic lactam scaffold binds in the thrombin active S_2 subsite in a normal substrate-like mode.[13]

Table 1. In vitro IC_{50} values (nM) of bicyclic lactam argininal **3a** and reference standards **1** and **2a,b** against a range of important serine proteases.[a,b]

Cmpd	FIIa	FXa	Plasmin	Trypsin	tPA
1	6.2	>2500	Inact.	791	Inact.
2a	5.01	29.7	14.8	2.58	N.D.
2b	1.1	290	315	1.36	Inact.
3a	16.4	>2500	923	11.6	Inact.

[a] Concentration of inhibitors **1**, **2a,b**, and **3a** necessary to inhibit thrombin (FIIa), FXa, plasmin, human trypsin, and tPA cleavage of the chromogenic substrates described in ref. 10a by 50%. [b] All target compounds were characterized by ^{1}HNMR, RPHPLC, low/high resolution mass spectroscopy.

Information gleaned from the X-ray crystal structure of thrombin-bound inhibitor **1** also confirmed a nearly substrate-like binding mode. As opposed to inhibitor **3a**, however, the monocyclic lactam ring and adjacent α-methylene occupy S_2 with subtle conformational differences. It is mostly buried and slightly shifted, relative to other substrate-like inhibitors, by Tyr60A and Trp60D of thrombin's unique 60 specificity loop.[10a,13b,d] This unique binding mode is one of the important contributing factors leading to the observed trypsin selectivity, and apparently is not available in target **3a**. The P_2-thiaproline portion of the fused bicyclic system may also experience unfavorable steric interactions with His 57 in S_2, since the P_2-glycine methylene moiety of **1** is within van der Waals distance of this residue.[10a]

Based upon the X-ray structural data for compound **1** and the biological results, in Figure 2 we depict a model representative of **3a** in the thrombin active site. The key binding interactions at S_1, S_2, and S_3 are also shown. The tetrahedral P_4-benzylsulfonamido moiety, an important residue discovered from our studies on the monocyclic lactam family, is retained in this new bicyclic series. Tethered off the lactam amino group, it mimics a *d*-phenylalanine residue and strategically positions the phenyl ring into the S_3 specificity pocket of thrombin.[10a,13b]

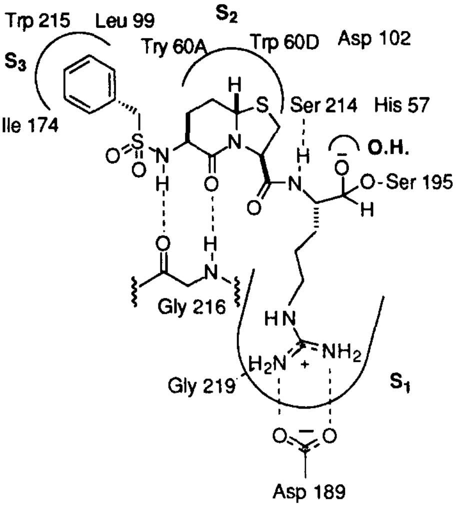


Figure 2. Schematic illustrating the key interactions of **3a** (CVS 1862)-thrombin based on the crystal structure of reference inhibitor **1**. O.H. denotes oxyanion hole binding site.

Conclusion

A convenient protocol for the synthesis of an acid-labile P_1-argininal synthon has been developed. New synthetic methodology is described for the construction of a novel benzylsulfonamide-containing fused bicyclic thiazolidine lactam scaffold which efficiently serves as P_2-P_4 dipeptide surrogate. Incorporation of a P_1-argininal into this manifold generates a novel class of rationally designed and biologically active serine protease inhibitors exemplified by **3** which expresses useful selectivity profiles. The incorporation of this new type of P_2-P_4 dipeptide surrogate into other classes of pharmaceutically important target molecules may be of interest.

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22. The in vitro enzyme assays were performed according to the protocols described in reference 10a.

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