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Bicyclic Pyridones as Potent, Efficacious and Orally Bioavailable Thrombin Inhibitors

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Abstract—A new class of conformationally constrained thrombin inhibitors is described. These compounds contain a unique bicyclic pyridone scaffold which serves as a P3P2 dipeptide surrogate. The synthesis and antithrombotic activity of these inhibitors is reported.

Thrombus formation is the culmination of a series of enzymatic reactions in which the serine protease thrombin plays an essential and multifaceted role. For example, thrombin acts directly to promote clot formation by catalyzing the hydrolysis of the soluble plasma protein fibrinogen to insoluble fibrin. Thrombin is also a potent stimulator of platelet aggregation and is required for the biosynthesis of other coagulation factors. Defects in the balance between these coagulation factors and their endogenous inhibitors can have serious consequences, such as the formation of thrombi in blood vessels.[1] These thromboembolic events can contribute to a number of health conditions such as angina, ischemia, myocardial infarction, and stroke. For these reasons, inhibition of thrombin has been viewed as an attractive mechanism for the treatment and prevention of both venous and arterial thrombosis. Over the last 10 years there has been considerable progress in the area of thrombin inhibitor research.[2] Initial ventures focused on the synthesis of peptide inhibitors based on a Phe-Pro-Arg template. Recent work has focused on nonpeptide inhibitors as a strategy to improve pharmacological and pharmacokinetic properties. In this context, several groups have reported inhibitors which incorporate a pyridone or pyrazinone as a P2 amino acid surrogate within the tripeptide scaffold.[3–6] This work has led to the emergence of potent, efficacious and bioavailable thrombin inhibitors such as **1**[5] and has spawned interest in more novel structures. As part of our program aimed at developing structurally diverse thrombin inhibitors, we have investigated a number of compounds which contain a novel bicyclic pyridone P3P2 skeleton. This communication describes the design, synthesis, and selected biological results of these unique dipeptide mimics.

In the context of a thrombin inhibitor containing a bicyclic pyridone relating to inhibitor **1**, we envisioned the constraint of the P3 benzyl sulfonamide side chain by cyclization to C-4 of the pyridone ring. Topographic considerations within the thrombin active site required an *R* configuration for the newly formed chiral center adjacent to the lactam carbonyl group in order to access to the distal hydrophobic S3 binding pocket occupied by the phenyl ring in structure **1**. Therefore, template **2** became an attractive target for synthesis since its rigid structure would preorganize the inhibitor for optimal interaction between its lipophilic P3 ligand and the enzyme while maintaining the essential hydrogen bonding elements required for interaction with the Gly 216 residue present in the thrombin backbone (Fig. 1).

The chemistry employed for the synthesis of this new class of compounds is illustrated in Scheme 1. The success of the reaction scheme hinged on our ability to

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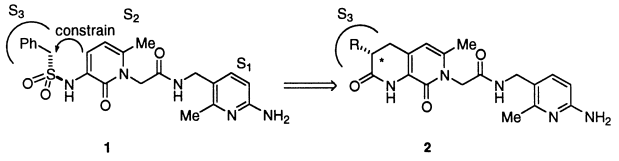
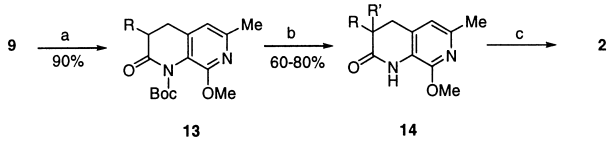


Figure 1. Conformational restriction of pyridone **1** to generate bicyclic template **2**.

prepare the versatile nitroaldehyde **7**, in order to maximize analogue production. The desired aldehyde was synthesized in five steps from commercially available ethyl nitroacetate (**3**). Reaction of ester **3** with ethanolic ammonia afforded nitroacetamide **4**. Construction of the pyridone nucleus was accomplished by heating an aqueous solution of acetamide **4** with commercially available ethyl 2,4-dioxovalerate in the presence of piperdinium acetate. This procedure reproducibly afforded nitro ester **5** in 28% yield and was routinely run in 100 g batches. Protection of the pyridone nucleus was necessary as the highly electron deficient pyridone was susceptible to electron transfer reactions when exposed to a number of organometallic reagents. Therefore, pyridone **5** was masked as its methoxypyridine analogue by reaction with Meerwein's salt in warm dichloromethane.[7] The oxidation state of the C-4 carbonyl group was adjusted via a standard two-step reduction/oxidation protocol to afford the pivotal aldehyde **7** in 20% overall yield from ester **3**. Horner–Emmons olefination of aldehyde **7** with an appropriately substituted phosphonoacetate derivative provided mixtures of *cis* and *trans* trisubstituted enoates **8**, which bore the latent P3 substituent.

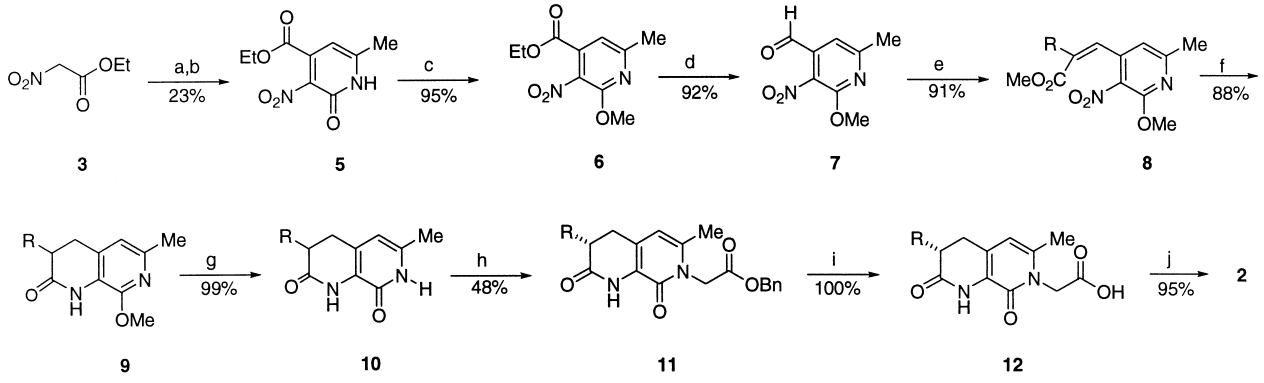
The key step in the reaction (Scheme 1) was a one-pot, double reductive lactamization of nitroenoates **8**, which provided access to the bicyclic lactam core structures. The pyridone rings were then regenerated upon brief exposure of methoxypyridines **9** to a melt of pyridinium hydrochloride.[8] Alkylation of the newly formed bicyclic pyridones **10** with one equivalent of benzyl bromoacetate afforded equal mixtures of *N*- and *O*-alkylated products which were easily separated by column chromatography. Attempts to bias product distribution in favor of *N*-alkylation using standard strategies were unsuccessful. However, we were able to perform iterative recyclizations of the unwanted glycolate byproducts to the back to pyridones **10** using the dealkylation procedure described above. Chiral preparative HPLC proved to be a successful method for separating the enantiomers of lactams **11**.[9] Hydrogenolysis of benzyl esters **11** afforded carboxylic acids **12** in quantitative yield. The resulting acids were coupled to 2-amino-5-aminomethyl-6-methylpyridine[6] to provide target compounds **2**.

For the synthesis of α,α-dialkylated lactam derivatives, the lactam NH of intermediates **9** was protected using Boc anhydride and catalytic DMAP to afford **13** (Scheme 2). Initial attempts to directly alkylate **9** with various bases resulted in low yields of lactams **14**. Moreover, treatment of protected lactams **13** with a number of different bases resulted in products which arose from deprotonation of the pyridine methyl group. This problem was circumvented by using potassium *tert*-butoxide as the base in the presence of equimolar sodium hydride. Employment of these reaction conditions afforded, after work up, good yields of the deprotected, dialkylated lactam **14**. Presumably sodium hydride acts to irreversibly trap the ^{t}BuOH which is formed in the reaction. Exclusion of NaH in favor of excess KO^{t}Bu or NaO^{t}Bu resulted in no reaction and complete recovery of **13**.



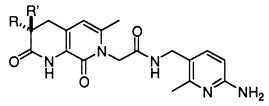
Scheme 2. (a) Boc_2O, DMAP, MeCN; (b) NaH, ^{t}BuOK, TBAI, R′X, THF, 50 °C; (c) Scheme 1, steps (g)–(j).

The inhibition constants (K_i values) found for bicyclic pyridones **2** versus thrombin and the closely related protease, trypsin, are given in Table 1. The compounds listed in this table are potent inhibitors of thrombin and are selective over trypsin. These compounds do not inhibit other serine proteases such as factor Xa, plasmin and tissue plasminogen activator (K_i >80 μM). Nanomolar binding affinities are easily obtained by incorporating small alkyl groups at P3 as demonstrated by



Scheme 1. (a) NH_3, EtOH, 80 °C, 3 h; (b) ethyl 2,4-dioxovalerate, piperdinium acetate, H_2O; (c) $Me_3O^+BF_4^-$, DCM; (d) (i) Dibal-H, DCM; (ii) $(ClCO)_2$, DMSO, Et_3N; (e) $(MeO)_2P(O)CHRCO_2Me$, NaH, THF; (f) H_2, Pd(C), MeOH, 55 °C; (g) pyridinium HCl, 155 °C, 5 min; (h) (i) Cs_2CO_3, $BrCH_2CO_2Bn$, DMF, ii. Chiralcel-OD column; (i) H_2, Pd(C), MeOH; (j) EDC, HOBT, NMM, 2-amino-5-aminomethyl-6-methylpyridine.

Table 1. Inhibition constants (K_i) versus thrombin and trypsin, in vitro anticoagulant potency (2× aptt) and in vivo antithrombotic efficacy.



Compd	R	R′	K_i (nM)		2×APTT (nM)[a]	rat $FeCl_3$[b]	Final plasma conc (nM)
			Thrombin	Trypsin			
2a	H	H	350	101,000	nd[c]		
2b	Et	H	1.6	4200	310		
2c	*n*-Pr	H	0.33	510	180		
2d	H	*n*-Pr	36	22,000	nd		
2e	*c*-$PrCH_2^-$	H	0.21	770	150	(1/6)	185
2f	*c*-$BuCH_2^-$	H	0.084	420	190	0/6	596
2g	$PhCH_2^-$	H	0.36	12,700	200		
2h	*i*-Bu	H	0.40	580	220	0/6	255
2i	*i*-Bu	Me	0.24	860	160		
2j	*i*-Bu	*i*-Bu	0.27	1500	390		

[a]Human plasma.
[b]Occlusions after iv infusion at 10 μg/kg/min. Parenthesis infusion at 3 μk/kg/min.
[c]nd = not determined.

the ethyl substituted lactam **2b** ($K_i = 1.6$ nM). Further SAR about the P3 substituent reveals that binding affinity is enhanced by the incorporation of small cycloalkyl ligands (e.g., **2e**,**f**). Molecular modeling of the *n*-propyl substituted lactam **2c** in the enzyme active site shows a preference for a single enantiomer to achieve optimal binding. This bias is supported experimentally when comparing the K_i value of lactam **2c** ($K_i = 0.33$ nM) to the K_i value of its antipode **2d** ($K_i = 36$ nM). It is interesting to note that alkylated lactams bearing an *S*-configuration are still 10-fold more potent than the unsubstituted parent lactam **2a**. This result suggests that additional binding energy is gained by substituting at the β face of these inhibitors. Indeed, the addition of either a methyl (**2i**) or isobutyl (**2j**) group α-to the lactam carbonyl in **2h** results in an increase potency. Furthermore, these dialkylated lactams display better trypsin selectivity than the corresponding monoalkylated analogues.

Table 1 illustrates the remarkably low 2× APTT values inherent to these bicyclic inhibitors. This in vitro assay measures the concentration of inhibitor that is required to double clotting times and is dependent upon both the potency and protein binding of the inhibitor. The protein binding of **2e** and **2f** in human plasma is 59 and 81%, respectively. Since cyclopropyl analogue **2e** has a higher free fraction, it is understandable that it has a lower 2× APTT value despite being 2.5-fold less potent than its homologue **2f**. Table 1 also lists the results from the rat ferric chloride model of arterial thrombosis.[10] As suggested by the intrinsic potencies of these bicyclic inhibitors, rats dosed with 10 μg/kg/min iv of **2f** or **2h** experienced no arterial clotting. In fact, when **2e** was challenged at a 3 μg/kg/min iv dose, good efficacy was maintained and the final plasma concentrations remained above the 2× APTT concentration.

Pharmacokinetic parameters for compounds **1**, **2h** and **2i** after oral administration in dogs are shown in Table 2. A brief comparison is given to illustrate that subtle changes at P3 have a pronounced effect on the overall absorption profile of a compound in this series without greatly affecting the potency or efficacy. For example, lactam **2h** displays inferior absorption properties relative to **1**, but maintains a comparable half-life. On the other hand, the α-methyl homologue, **2i**, shows similar plasma levels as **1** but a shorter terminal half-life.

Table 2. Oral absorption kinetics for compounds **1**, **2h** and **2i** in dogs[a]

Compd	C_{max} (μM)	t_{max} (min)	$t_{1/2}$	AUC (μM h)
1	0.99	75	154	4.42
2h	0.18	70	141	0.75
2i	1.37	30	90	2.97

[a]Dose of 1 mg/kg; $n = 2$.

In summary, we have rationally designed a new series of thrombin inhibitors based on the 3-amino-2-pyridone acetamide template **1**. These compounds include a unique conformationally constrained peptidomimetic scaffold. Since the synthesis described is versatile, it is amenable to SAR work at both the P3 and P1 regions of the molecule. Second and third generation non-lactam containing bicyclic inhibitors which show improved pharmacokinetics have been synthesized and the results of the studies will be disclosed in subsequent publications.

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