

P₂ pyridine N-oxide thrombin inhibitors: a novel peptidomimetic scaffold

Philippe G. Nantermet,^{a,*} Christopher S. Burgey,^{a,*} Kyle A. Robinson,^a Janetta M. Pellicore,^a Christina L. Newton,^a James Z. Deng,^a Harold G. Selnick,^a S. Dale Lewis,^b Bobby J. Lucas,^b Julie A. Krueger,^b Cynthia Miller-Stein,^c Rebecca B. White,^c Bradley Wong,^c Daniel R. McMasters,^d Audrey A. Wallace,^e Joseph J. Lynch, Jr.,^e Youwei Yan,^f Zhongguo Chen,^f Lawrence Kuo,^f Stephen J. Gardell,^b Jules A. Shafer,^b Joseph P. Vacca^a and Terry A. Lyle^a

^aDepartment of Medicinal Chemistry, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

^bDepartment of Biological Chemistry, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

^cDepartment of Drug Metabolism, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

^dDepartment of Molecular Systems, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

^eDepartment of Pharmacology, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

^fDepartment of Structural Biology, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA

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Abstract—In this study, we have demonstrated that the critical hydrogen bonding motif of the established 3-aminopyrazinone thrombin inhibitors can be effectively mimicked by a 2-aminopyridine N-oxide. As this peptidomimetic core is more resistant toward oxidative metabolism, it also overcomes the metabolic liability associated with the pyrazinones. An optimization study of the P₁ benzylamide delivered the potent thrombin inhibitor **21** ($K_i = 3.2$ nM, 2xaPTT = 360 nM), which exhibited good plasma levels and half-life after oral dosing in the dog ($C_{max} = 2.6$ μ M, $t_{1/2} = 4.5$ h).

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Thrombosis-related disorders such as deep vein thrombosis, pulmonary embolism, and thromboembolic stroke remain a major cause of morbidity worldwide.¹ The limitations associated with current therapies² have driven the search for small-molecule direct inhibitors of specific enzymes involved in the coagulation cascade.³ In this regard, inhibitors of both thrombin and factor Xa have attracted considerable recent attention.⁴ In our laboratories, the search for potent and orally bio-available direct thrombin inhibitors has led to the evaluation of pyrazinone based small molecule peptidomimetics (**1**, Fig. 1).⁵ Detailed metabolic studies indicated a significant degree of oxidative metabolism around the pyrazinone core.⁶ As a result, we sought to

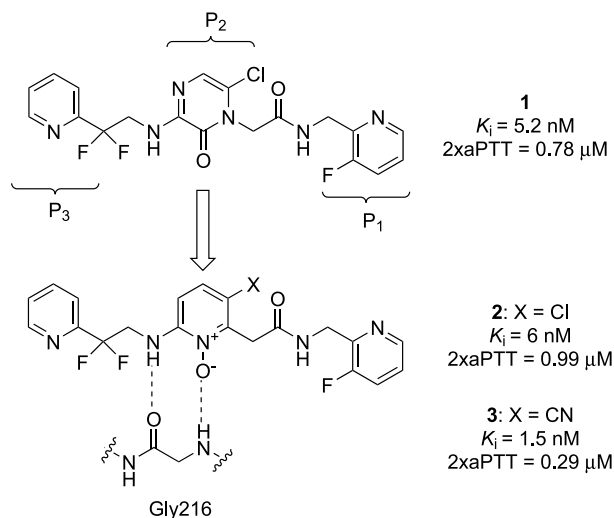


Figure 1. From pyrazinone to pyridine N-oxide.

Keywords: Thrombin; Peptidomimetic.

* Corresponding authors. Tel.: +1 215 652 0945; fax: +1 215 652 3971; e-mail addresses: philippe_nantermet@merck.com; christopher_burgey@merck.com

identify alternative P₂ peptidomimetic cores that would retain the classical H-bond network with glycine-216 of the enzyme (Fig. 1); one such alternative envisioned was a pyridine N-oxide scaffold.⁷

Preparation of pyridine N-oxide derivative **2**, the direct analog of pyrazinone **1**, afforded a 6 nM thrombin inhibitor and provided support for this strategy. A cyano group at the 5-position provided a further potency enhancement (Fig. 1, **3**). As anticipated, the corresponding neutral pyridine derivatives are significantly less potent against thrombin than their parent compounds (pyridine of **2**: K_i = 620 nM; pyridine of **3**: K_i = 103 nM; structures not shown), consistent with the N-oxide oxygen serving as an H-bond acceptor. Additionally, X-ray crystallographic analysis of a related inhibitor **4** bound in the active site of thrombin (Fig. 2, PDB Code: 1Z71) clearly demonstrates the H-bond network with glycine-216.

An initial study focused on incorporating the optimized P₁ groups recently discovered in our laboratories into the P₂ pyridine N-oxide template (Table 1).⁸ Compounds were evaluated for their thrombin inhibitory potency and their functional ability to double the activated partial thromboplastin time (2xaPTT) in human plasma.⁹ The unsubstituted P₁ benzyl amide **5** (K_i = 150 nM) functions as the benchmark compound. Installation of the N-linked triazole at the *ortho*-position of the P₁ benzyl or pyridyl group^{8a} yields inhibitors (**6–8**) with both good intrinsic and functional potency. Introduction of a 2-aminomethyl substituent^{8b} also results in a significant potency improvement, as illustrated by compounds **9** and **10**. Incorporation of a meta-chloro substituent affords a significant improvement in intrinsic potency

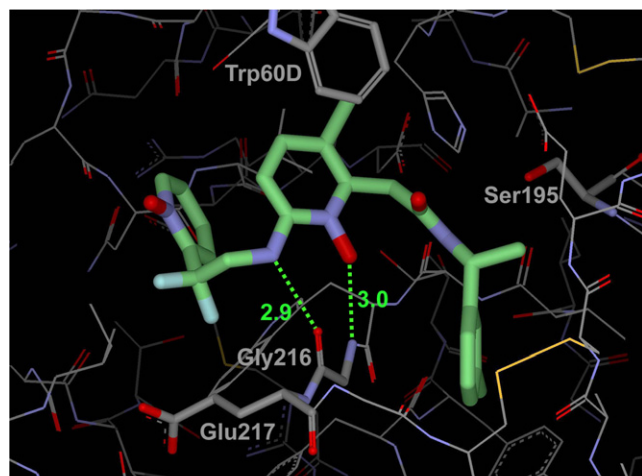


Figure 2. X-ray crystal structure of **4** bound in the thrombin active site.

Table 1. P₁ SAR

Compd	X	P ₁	K_i (nM) ^a	2xaPTT (μM)
5	CN		150	—
6	Cl		2.60	0.46
7	Cl		2.50	0.40
8	CN		0.23	0.15
9	Cl		0.40	0.13
10	CN		<0.01	0.09
11	Cl		0.42	0.73
12	CN		0.08	0.20
13	Cl		0.05	0.23
14	H		1.50	0.43
15	H		0.14	0.20
16	CN		0.002	0.10
17	Cl		<0.01	0.15
18	CN		0.001	0.07
19	H		0.04	0.10

^a K_i values are the average of at least two determinations, standard error of the mean <10%.

(e.g., **12** vs **5**), but, as noted in earlier studies, the concomitant increase in lipophilicity is detrimental to functional activity.¹⁰ The combination of the *ortho*-azole and the *meta*-chloro substituents affords exquisitely potent thrombin inhibitors (**13–16**); for example, compound **16** displays a 2 pM K_i against thrombin, with a 2xaPTT of 100 nM. Similarly, the potency-enhancing aminomethyl and chlorine substituents can be merged

in an additive fashion to yield extremely potent thrombin inhibitors (**17–19**). The most potent compound produced from this study is **18** ($K_i = 1$ pM), displaying a 2xaPTT of 70 nM.

Having completed an investigation of the P_1 SAR, we turned our attention to the evaluation of the pharmacokinetic profile of members of this series (Table 2). Upon oral dosing of aminomethyl derivative **9** (0.65 mpk) to dogs, a 0.82 μ M maximum plasma concentration (C_{max}) and a 1.5 h plasma half-life ($t_{1/2}$) were achieved. The *meta*-chloro analog **17** similarly displayed a promising pharmacokinetic profile ($C_{max} = 0.70$ μ M, $t_{1/2} = 1.3$ h). As the tri- and tetrazole analogs (**13–16**) had inferior pharmacokinetic profiles, our efforts focused on the optimization of the P_1 aminomethyl series.

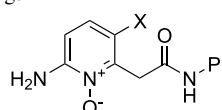
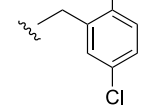
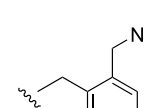
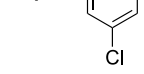
Metabolism studies involving human and dog microsomal incubations revealed that the desired objective of minimizing the extent of P_2 metabolism had been achieved; however, P_1 benzylic site oxidation and subsequent N-dealkylation now arose as the primary metabolic pathway. In an attempt to attenuate this metabolism, a study to substitute the benzylic sites was initiated (Table 2). Methylation of the benzylic position alpha to the P_1 amide group (Table 2, Y = Me) and resolution gave access to inhibitors **20** and **21**. The more active enantiomer **21** displays an 8-fold loss in binding potency (vs **9**) yet maintains good anticoagulant activity ($K_i = 3.2$ nM, 2xaPTT = 360 nM); notably, this modification resulted in a significant improvement in the pharmacokinetic profile (**21**, $C_{max} = 2.6$ μ M, $t_{1/2} = 4.5$ h). Methylation at the other benzylic site was also tolerated, but did not lead to improved dog pharmacokinetics (**22–23**). Inspection of the X-ray crystal structure of the related inhibitor **4** bound in the active site of thrombin (Fig. 2) suggested that strategic placement of a hydrogen bonding group at the P_1 benzylic position could potentially gain access to an additional interaction with residues contained within the active site (e.g., Ser-195). This analysis is supported by the 5-fold

potency increase (vs **21**) attained with the hydroxymethyl analog **24**.

The high levels of potency demonstrated by the *ortho*-aminomethyl and tetrazole analogs (Table 1, **15–19**) offered the possibility that significant truncation of these molecules could still deliver potent thrombin inhibitors. Accordingly, complete excision of the P_3 binding element afforded inhibitors in the low to mid nanomolar range (Table 3, **25–28**); most notable is compound **28** with a $K_i = 10$ nM and a 2xaPTT = 560 nM (MW = 346). Unfortunately, removal of the P_3 group afforded no significant advantage regarding pharmacokinetic profile.

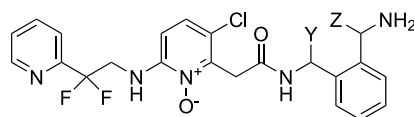
The synthesis of these P_2 pyridine N-oxide inhibitors is outlined in Scheme 1.¹¹ Protection of 2-amino-6-methyl-

Table 3. Des- P_3 analogs

				
Compd	X	P_1	K_i (nM) ^a	2xaPTT (μ M) ^b
25	Cl		140	—
26	CN		18	1.6
27	Cl		26	—
28	CN		10	0.56

^a K_i values are the average of at least two determinations, standard error of the mean <10%.

Table 2. P_1 benzylic substitution

						
Compd	Y	Z	K_i (nM) ^a	2xaPTT (μ M)	C_{max} (μ M)	$t_{1/2}$ (h)
9	H	H	0.40	0.13	0.82 ^d	1.5
20	Me ^b	H	260	—	—	—
21	Me ^b	H	3.2	0.36	2.6 ^e	4.5
22	H	Me ^b	20	5.9	—	—
23	H	Me ^b	0.45	0.27	0.1 ^f	2.0
24	CH ₂ OH ^c	H	0.48	0.23	0.34 ^g	1.5

^a K_i values are the average of at least two determinations, standard error of the mean <10%.

^b **20/21** and **22/23** are enantiomeric pairs.

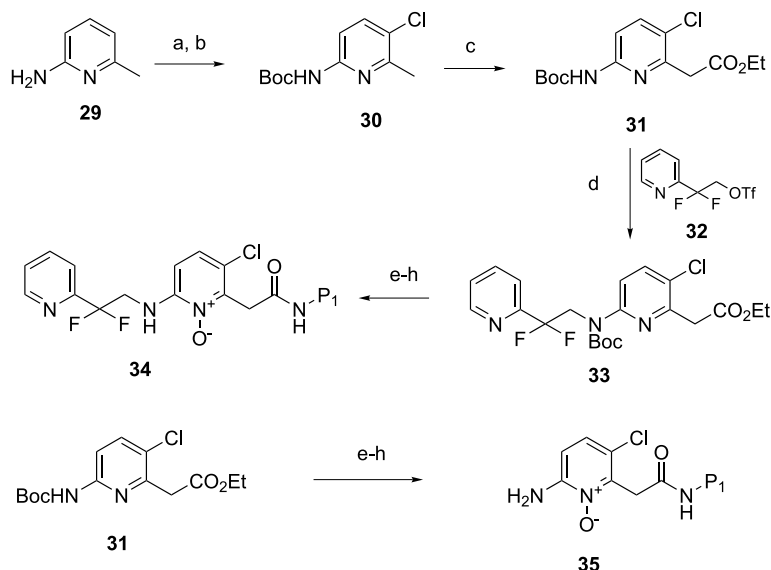
^c Single enantiomer, absolute configuration not established.

^d po dose = 0.65 mpk.

^e po dose = 0.95 mpk.

^f po dose = 0.7 mpk.

^g po dose = 0.85 mpk.



Scheme 1. Reagents and conditions: (a) Boc₂O; (b) NCS, DCE; (c) LDA, diethyl carbonate, THF; (d) NaH, DMF, 32;⁵ (e) 1 N LiOH, THF; (f) P₁-NH₂, EDC, HOAt, DMF; (g) TFA, DCM; (h) mCPBA, DCE.

pyridine with di-tert-butyl dicarbonate, followed by regioselective chlorination affords **30**. The pyridylacetate **31** is produced via benzylic metalation of **30** with LDA and subsequent quenching with diethyl carbonate. Alkylation of **31** with the P₃ 2,2-difluoro-2-(2-pyridyl)ethyl-trifluoromethanesulfonate **32**⁵ completes the assembly of the key P₃-P₂ subunit **33**. Ester hydrolysis and P₁ amide coupling are followed by deprotection and then pyridine oxidation¹² with mCPBA to afford the final products **34**. The cyano pyridines are prepared via an analogous route starting from 2-amino-5-cyano-6-methylpyridine.

The des-P₃ analogs can be assembled in a similar manner from key intermediate **31**. Base hydrolysis is followed by EDC mediated amide formation. Amine deprotection and final pyridine N-oxidation deliver the des-P₃ thrombin inhibitors.

In conclusion, we have demonstrated that the critical hydrogen bonding motif of the established 3-aminopyrazinone thrombin inhibitors can be effectively mimicked by a 2-aminopyridine N-oxide. As this peptidomimetic core is more resistant toward oxidative metabolism, it also overcomes the metabolic liability associated with the pyrazinones. An optimization study of the P₁ benzylamide delivered the potent thrombin inhibitor, **21** which exhibited good plasma levels and half-life after oral dosing in the dog. Studies to explore the generality of 2-aminopyridine N-oxides as peptidomimetics are underway.

Acknowledgments

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