Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2771–2775
Bioorganic & Medicinal Chemistry Letters

P_2 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]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[b]*Department of Biological Chemistry, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[c]*Department of Drug Metabolism, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[d]*Department of Molecular Systems, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[e]*Department of Pharmacology, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*
[f]*Department of Structural Biology, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*

Received 6 January 2005; revised 28 March 2005; accepted 29 March 2005
Available online 29 April 2005

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 P_1 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 μM, $t_{1/2}$ = 4.5 h).

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 therapies[2] 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 bioavailable 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

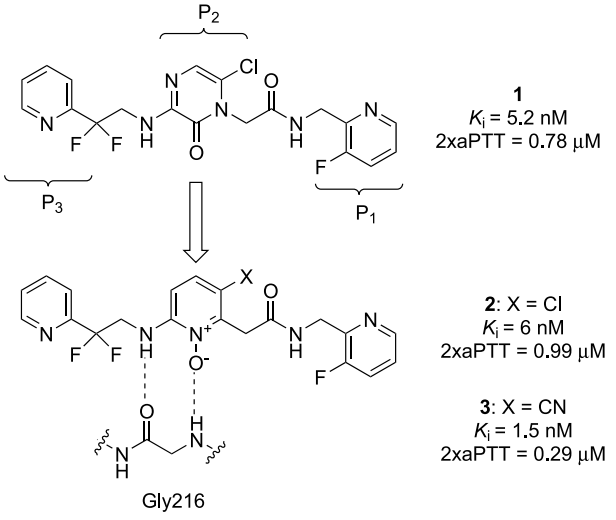


Figure 1. From pyrazinone to pyridine N-oxide.

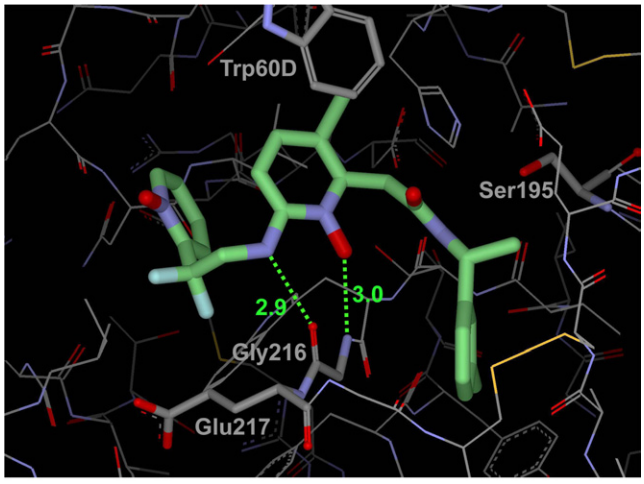
Keywords: Thrombin; Peptidomimetic.
* Corresponding authors. Tel.: +1 215 652 0945; fax: +1 215 652 3971; e-mail addresses: philippe_nantermet@merck.com; christopher_burgey@merck.com

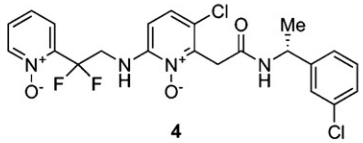
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.110

identify alternative P_2 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 P_1 groups recently discovered in our laboratories into the P_2 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 P_1 benzyl amide **5** (K_i = 150 nM) functions as the benchmark compound. Installation of the N-linked triazole at the *ortho*-position of the P_1 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

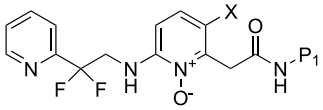


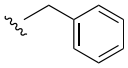
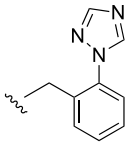
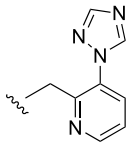
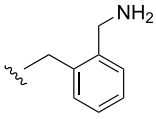
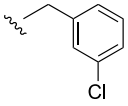
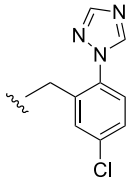
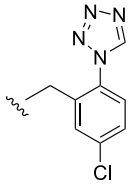
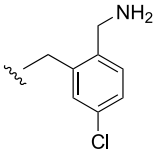


4
K_i = 0.66 nM
2xaPTT = 0.43 μM

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

Table 1. P_1 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.

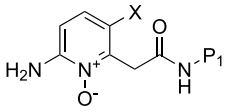
Having completed an investigation of the P_1 SAR, we turned our attention to the evaluation of the pharmacokinetic profile of members of this series (Table 2). Upon oral dosing of aminomethyl derivative **9** (0.65 mpk) to dogs, a 0.82 μM maximum plasma concentration (C_{max}) and a 1.5 h plasma half-life ($t_{1/2}$) were achieved. The *meta*-chloro analog **17** similarly displayed a promising pharmacokinetic profile (C_{max} = 0.70 μM, $t_{1/2,}$ = 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.

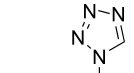
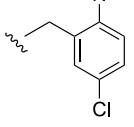
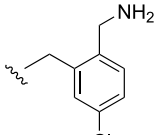
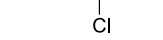
Metabolism studies involving human and dog microsomal incubations revealed that the desired objective of minimizing the extent of P_2 metabolism had been achieved; however, P_1 benzylic site oxidation and subsequent N-dealkylation now arose as the primary metabolic pathway. In an attempt to attenuate 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 μM, $t_{1/2}$ = 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-

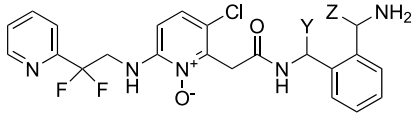
Table 3. Des-P_3 analogs



Compd	X	P_1	K_i (nM)[a]	2xaPTT (μM)[b]
25	Cl		140	—
26	CN		18	1.6
27	Cl		26	—
28	CN		10	0.56

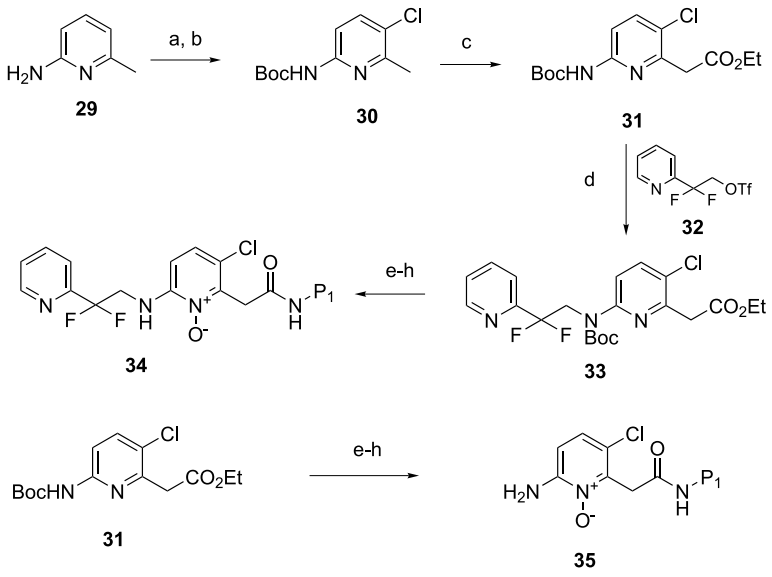
[a] K_i values are the average of at least two determinations, standard error of the mean <10%.

Table 2. P_1 benzylic substitution



Compd	Y	Z	K_i (nM)[a]	2xaPTT (μM)	C_{max} (μM)	$t_{1/2}$ (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_2OH[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) Boc_2O; (b) NCS, DCE; (c) LDA, diethyl carbonate, THF; (d) NaH, DMF, **32**;[5] (e) 1 N LiOH, THF; (f) P_1-NH_2, 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 P_3 2,2-difluoro-2-(2-pyridyl)ethyl-trifluoromethanesulfonate **32**[5] completes the assembly of the key P_3–P_2 subunit **33**. Ester hydrolysis and P_1 amide coupling are followed by deprotection and then pyridine oxidation[12] 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-P_3 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-P_3 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 P_1 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

We thank the analytical chemistry, mass spectroscopy, and NMR analysis groups for their assistance.

References and notes

1. (a) Hendriks, D.; Scharpe, S.; van Sande, M.; Lommaert, M. P. *J. Clin. Chem. Clin Biochem.* **1989**, *27*, 277–285; (b) Campbell, W.; Okada, H. *Biochem. Biophys. Res. Commun.* **1989**, *162*, 933–939; (c) Eaton, D. L.; Malloy, B. E.; Tsai, S. P.; Henzel, W.; Drayna, D. *J. Biol. Chem.* **1991**, *266*, 21833–21838; (d) Bajzar, L.; Manuel, R.; Nesheim, M. E. *J. Biol. Chem.* **1995**, *270*, 14477–14484.
2. Parenteral administration of low molecular weight heparin and intense patient monitoring required with warfarin limit their chronic utility.
3. Adang, A. E. P.; Rewinkel, J. B. M. *Drugs Future* **2000**, *25*, 369.
4. Coburn, C. A. *Exp. Opin. Ther. Patents* **2001**, *11*, 1; Sanderson, P. E. J. *Ann. Reports Med. Chem.* **2001**, *36*, 79; Vacca, J. P. *Ann. Reports Med. Chem.* **1998**, *33*, 81.
5. Burgey, C. S.; Robinson, K. A.; Lyle, T. A.; Sanderson, P. E. J.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Lyle, E. A.; Williams, P. D.; Coburn, C. A.; Dorsey, B. D.; Barrow, J. C.; Stranieri, M. T.; Holahan, M. A.; Sitko, G. R.; Cook, J. J.; McMasters, D. R.; McDonough, C. M.; Sanders, W. M.; Wallace, A. A.; Clayton, F. C.; Bohn, D.; Leonard, Y. M., Jr.; Detwiler, T. J., Jr.; Lynch, J. J., Jr.; Yan, Y.; Chen, Z.; Kuo, L.; Gardell, S. J.; Shafer, J. A.; Vacca, J. P. *J. Med. Chem.* **2003**, *46*, 461–473.
6. Singh, R.; Silva Elipe, M. V.; Pearson, P. G.; Arison, B. H.; Wong, B. K.; White, R.; Yu, X.; Burgey, C. S.; Lin, J. H.; Baillie, T. A. *Chem. Res. Toxicol.* **2003**, *16*, 198–207; Subramanian, R.; Lin, C. C.; Ho, J. Z.; Pitzenberger, S. M.; Silva-Elipe, M. V.; Gibson, C. R.; Braun, M. P.; Yu, X.; Yergey, J. L.; Singh, R. *Drug Metab. Disposition* **2003**, *31*, 1437–14347.
7. After completion of this work a patent publication appeared disclosing a similar concept: South, M. S.; Case, B.; Garland, D. J.; Hayes, M. J.; Huang, H-C.; Huang, W.; Jones, D. E.; Neumann, W. L.; Parlow, J. J.; Reitz, D. B.; Rueppel, M. L.; Webber, R. K. WO 02/42272 A2.
8. (a) Young, M. B.; Barrow, J. C.; Glass, K. L.; Lundell, G. F.; Newton, C. L.; Pellicore, J. M.; Rittle, K. E.; Selnick,

H. G.; Stauffer, K. J.; Vacca, J. P.; Williams, P. D.; Bohn, D.; Clayton, F. C.; Cook, J. J.; Krueger, J. A.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; McMasters, D. R.; Miller-Stein, C.; Pietrak, B. L.; Wallace, A. A.; White, R. B.; Wong, B.; Yan, Y.; Nantermet, P. G. *J. Med. Chem.* **2004**, *47*, 2995–3008; (b) Rittle, K. E.; Barrow, J. C.; Cutrona, K. J.; Glass, K. L.; Krueger, J. A.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; McMasters, D. R.; Morissette, M. M.; Nantermet, P. G.; Newton, C. L.; Sanders, W. M.; Yan, Y.; Vacca, J. P.; Selnick, H. G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3477–3482.

9. Lewis, S. D.; Ng, A. S.; Lyle, E. A.; Mellott, M. J.; Appelby, S. D.; Brady, S. F.; Stauffer, K. S.; Sisko, J. T.; Mao, S.-S.; Veber, D. F.; Nutt, R. F.; Lynch, J. J.; Cook, J. J.; Gardell, S. J.; Shafer, J. A. *Thromb. Haemost.* **1995**, *74*, 1107.
10. (a) Lumma, W. C.; Witherup, K. M.; Tucker, T. J.; Brady, S. F.; Sisko, J. T.; Naylor-Olsen, A. M.; Lewis, S. D.; Lucas, B. J.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 1011–1013; (b) Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I.-W.; Dancheck, K. B.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 1565–1569.
11. Nantermet, P. G.; Selnick, H. G.; Barrow, J. C.; Coburn, C.; Burgey, C. S.; Robinson, K. A.; Lyle, T. A. US 2003/0158218 A1.
12. Oxidation was P_2 regiospecific in the case of the chloro derivatives but in the case of the cyano derivatives careful monitoring of oxidant stoichiometry and reaction time was necessary to avoid over-N-oxidation at the P_3 pyridyl.