Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 319–323

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Discovery of a Nonpeptidic Small Molecule Antagonist of the Human Platelet Thrombin Receptor (PAR-1)

Philippe G. Nantermet,[a,*] James C. Barrow,[a] George F. Lundell,[a] Janetta M. Pellicore,[a] Kenneth E. Rittle,[a] MaryBeth Young,[a] Roger M. Freidinger,[a] Thomas M. Connolly,[b] Cindra Condra,[b] Jerzy Karczewski,[b] Rodney A. Bednar,[b] Stanley L. Gaul,[b] Robert J. Gould,[b] Kris Prendergast[c] and Harold G. Selnick[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[b]*Department of Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Molecular Design and Diversity, Merck Research Laboratories, West Point, PA 19486, USA*

Received 17 September 2001; accepted 2 November 2001

Abstract—The synthesis and biological evaluation of a series of nonpeptidic small molecule antagonists of the human platelet thrombin receptor (PAR-1) are described. Optimization of the 5-amino-3-arylisoxazole lead resulted in an approximate 100-fold increase in potency. The most potent of these compounds (**54**) inhibits platelet activation with IC_{50}s of 90 nM against the thrombin receptor agonist peptide (TRAP) and 510 nM against thrombin as the agonist. Further, antagonist **54** fully blocks platelet aggregation stimulated by 1 nM thrombin for 10 min. © 2002 Published by Elsevier Science Ltd.

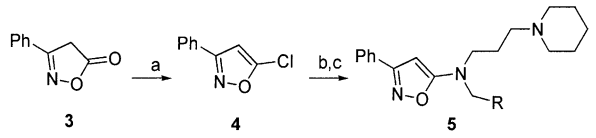
Thrombin plays a key role not only in hemostasis, but also in the activation of a variety of cell types.[1] While the role of this protease in blood coagulation is well defined, the mechanism underlying thrombin's actions upon cells remained unclear until recently. The cloning of a platelet thrombin receptor (PAR-1)[2] has provided some insight into the mechanism of thrombin activation of cells.[3] The thrombin receptor belongs to a family of seven transmembrane domain G-protein-coupled receptors which are activated via a novel mechanism involving proteolytic modification of the extracellular N-terminus. In the case of PAR-1, thrombin cleaves the extracellular domain of the receptor at the Arg41-Ser42 site to expose a new truncated N-terminus bearing the peptide recognition motif SFLLRN, which binds intramolecularly to an undefined extracellular site and acts as a tethered ligand (agonist) for the receptor. Synthetic peptides that mimic this motif, such as the thrombin receptor activating peptide (TRAP, SFLLR-NH_2) have complete PAR-1 agonist properties independent of thrombin activation thus confirming the role of the tethered SFLLRN motif as an activating ligand. Three other receptors that function via a similar mechanism—PAR-2, PAR-3, and PAR-4—have been identified subsequently.[4]

Selective blockade of the intramolecular activation step of the thrombin receptor should result in moderation of platelet activation and aggregation without interfering with the enzymatic activity of thrombin in the coagulation cascade. Therefore, an antagonist of the thrombin receptor may represent a safer antiplatelet agent for the treatment of thrombotic disorders. Thrombin is also known to activate a variety of cell types involved in inflammatory and proliferative disorders presumably via the thrombin receptor; thus suggesting additional potential therapeutic uses[5] of a PAR-1 antagonist.

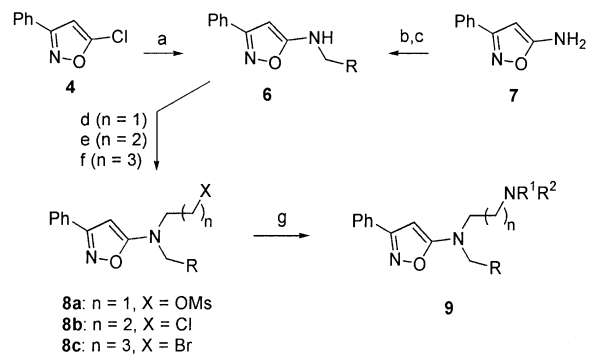
Several reports of PAR-1 antagonists derived from the TRAP peptide[6] as well as nonpeptidic antagonists[7] have been disclosed recently. Earlier reports from these laboratories[8] have described a series of small molecule thrombin receptor antagonists exemplified by **1** (Fig. 1). Directed screening[9] based on **1** resulted in the identification of isoxazole **2**[10] as a structurally novel lead of moderate potency (TRAP $IC_{50} = 9$ μM). Reported herein is a summary of our progress in optimizing **2.**

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

0960-894X/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0960-894X(01)00745-4



Scheme 1. (a) $POCl_3$, Et_3N, 80 °C; (b) *N*-3-aminopropylpiperidine neat, 80 °C; (c) NaH, RCH_2X, DMF, 0 °C to rt.



Scheme 2. (a) $ArCH_2NH_2$; (b) ArCOCl, Et_3N, CH_2Cl_2; (c) $LiAlH_4$, THF; (d) (i) NaH, ethyl bromoacetate, DMF; (ii) $LiAlH_4$, THF; (iii) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (e) NaH, 1-chloro-3-iodopropane, DMF; (f) NaH, 1,4-dibromobutane, DMF; (g) R^1R^2NH.

Initial investigations involved the independent modification of both substituents on the exocyclic amino group. Chlorination of isoxazolone **3** provided **4**, which was reacted with *N*-3-aminopropylpiperidine and further alkylated with alkyl or benzyl bromides to afford derivatives of type **5**, as shown in Scheme 1.

Modification of the piperidine-bearing substituent was accomplished as described in Scheme 2. Intermediates of type **6** could be obtained from chloroisoxazole **4** and benzyl amines or via acylation/reduction of aminoisoxazole **7**. Tethers carrying potential leaving groups were then installed to afford intermediates of type **8**. Nucleophilic displacement with various amines provided derivatives of general structure **9**.

Optimization of the isoxazole aryl substituent was accomplished according to Scheme 3. Acylation of piperonyl amine **10** with acryloyl chloride followed by Michael addition of azepine and reduction lead to the preparation of **12**. Acetophenones and arylbenzoyl chlorides could be converted to β-keto esters of type **13**. Cycloaddition of hydroxylamine and subsequent chlorination afforded chloroisoxazoles **14**. Lithiation of amine **12** in Et_2O at 0 °C followed by addition of **14** provided the target compounds **15**.

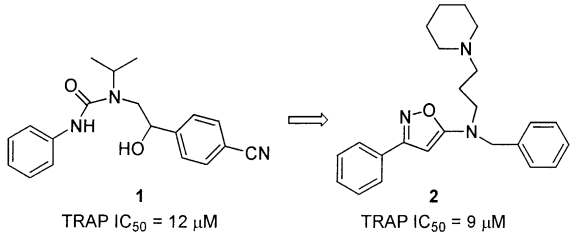
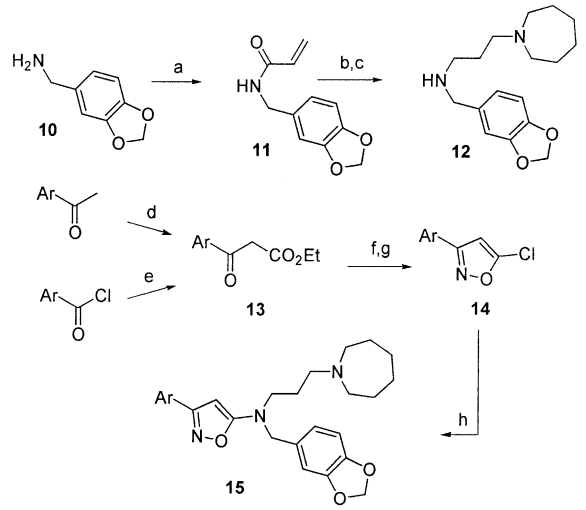


Figure 1. From trisubstituted urea to aminoisoxazole PAR antagonists.



Scheme 3. (a) Acryloyl chloride; (b) azepine; (c) $LiAlH_4$, THF or BH_3–THF, THF; (d) NaH, diethlycarbonate; (e) *n*BuLi, $EtO_2CCH_2CO_2H$; (f) NH_2OH HCl, EtOH; (g) $POCl_3$; (h) **12**, *n*BuLi, Et_2O, 0 °C.

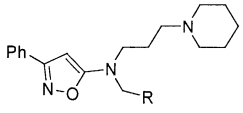
New compounds were tested for their ability to inhibit the activation of platelets by either 3 uM TRAP ($EC_{50}=1$ μM) or 1 nM thrombin ($EC_{50}=0.6$ μM) by measuring the release of ^{3}H-serotonin from activated platelets.[6a] Selected compounds were also examined in a binding assay for their ability to displace a radiolabeled peptide.[11]

The first area of investigation centered around the *N*-benzyl substituent of lead isoxazole **2** (Table 1).

While alkyl replacements are not tolerated (**16** and **17**), up to a 10-fold increase in potency over lead compound **2** can be obtained by selected substitution of the *N*-benzyl substituent. The best groups were 1-naphthyl (**20**) and 3,4-methylenedioxy (**22**) with IC_{50}s of 0.5 and 0.6 μM, respectively, when TRAP was used as the agonist.

Modifications of the tertiary amine side chain were then investigated. Removal of the amine (**23**) resulted in complete loss of activity. Variation of ring size indicated a marked preference for piperidine (**26**) and azepine (**27**) derivatives (Table 2).

Table 1. *N*-Benzyl modifications



R	Compd	IC_{50} (μM)[a]	R	Compd	IC_{50} (μM)[a]
Ph	**2**	9	3-OMe–Ph	**19**	1.6
n-Pr	**16**	>40	1-Naphthyl	**20**	0.5
Cyclohexyl	**17**	>10	2-Naphthyl	**21**	2
3-Cl–Ph	**18**	1	3,4-(OCH_2O)–Ph	**22**	0.6

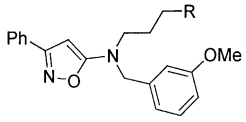
[a]Values represent inhibition of secretion of radiolabeled serotonin from washed human platelets stimulated by 3 μM TRAP peptide ($n>2$, standard error of the mean <10%).

The optimal tether length was demonstrated to be three methylenes (**29**–**31**, Fig. 2).

The final area of investigation centered around the 3-aryl substituent on the isoxazole (Table 3). Substitution at the *ortho* position was detrimental to activity as seen with compounds **32**, **35**, and **38**. *meta*-Substitution had a positive effect on the potency of these isoxazole PAR-1 antagonists. Except for strong electron-withdrawing groups (**44**) and larger groups (**45**), potency could be maintained or slightly improved (**33**, **36**, **39**, **41**, and **43**). *para*-Substitution was beneficial with methyl, methoxy and thiomethyl (**34**, **37**, and **47**). Substitution with chloro or fluoro (**40** and **42**), phenyl (**46**) or polar residues such as methyl sulfoxide (**48**) and sulfone (**49**) were not tolerated. When compared to other 4-halogen derivatives the 4-iodo derivative **50** surprisingly appeared as one of the most active antagonists with an with IC_{50} of 0.09 μM with TRAP used as the agonist.

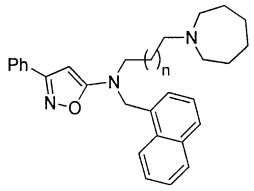
Several compounds demonstrating promising activity when TRAP was used as the agonist were examined in the more physiologically relevant thrombin (1 nM) stimulated secretion assay. Interesting differences appeared in the SAR depending on whether TRAP or thrombin was used as agonist. While *meta*-substitution was generally beneficial to the activity of these antagonists against thrombin as an agonist, *para*-substitution was found to be detrimental to activity with

Table 2. Side-chain modifications



R	Compd	IC_{50} (μM)[a]	R	Compd	IC_{50} (μM)[a]
Cyclohexyl[b]	**23**	>10	*N*-Piperidine	**26**	1.6
N-Azetidine	**24**	>10	*N*-Azepine	**27**	0.5
N-Pyrrolidine	**25**	3.3	*N*-Homoazepine	**28**	>10

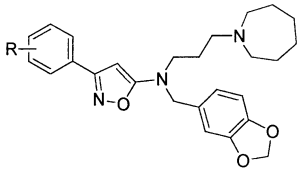
[a]Values represent inhibition of secretion of radiolabeled serotonin from washed human platelets stimulated by 3 μM TRAP peptide ($n>2$, standard error of the mean <10%).
[b]The 3-methoxy phenyl group is replaced by 1-naphthyl in this case.



n = 0: **29** TRAP IC_{50} = 5 μM
n = 1: **30** TRAP IC_{50} = 0.36 μM
n = 2: **31** TRAP IC_{50} = 5 μM

Figure 2. Tether length modifications.

Table 3. 3-Ph modifications



R	Compd	TRAP IC_{50} (μM)[a,c]	Thrombin IC_{50} (μM)[b,c]	Binding IC_{50} (μM)[d]
2-Me	**32**	3.22	nd	nd
3-Me	**33**	0.12	60% @ 4 μM	0.89
4-Me	**34**	0.14	3.2	0.29
2-OMe	**35**	2.22	nd	2.21
3-OMe	**36**	0.13	2.24	0.26
4-OMe	**37**	0.13	60% @ 10 μM	0.25
2-Cl	**38**	2.99	nd	nd
3-Cl	**39**	0.27	0.91	1.16
4-Cl	**40**	1.24	4.25	0.99
3-F	**41**	0.14	1.18	0.77
4-F	**42**	1.31	>10	nd
3-CN	**43**	0.35	1.97	0.6
3-NO_2	**44**	0.83	>10	1.02
3-Ph	**45**	6.42	nd	nd
4-Ph	**46**	4.44	nd	nd
4-SMe	**47**	0.43	>4	nd
4-SOMe	**48**	3.7	nd	nd
4-SO_2Me	**49**	3.2	nd	nd
4-I	**50**	0.09	0.88	0.47
3,4-F_2	**51**	0.5	2.4	2.14
3,4-$(OMe)_2$	**52**	0.4	4.67	1.2
3-F-4-OMe	**53**	0.07	2.14	nd
3,5-F_2	**54**	0.09	0.51	0.15
3,5-$(OMe)_2$	**55**	0.13	1.2	0.52

[a]Values represent inhibition of secretion of radiolabeled serotonin from washed human platelets stimulated by 3 μM TRAP peptide.
[b]Values represent inhibition of secretion of radiolabeled serotonin from washed human platelets stimulated by 1 nM thrombin.
[c]$n>2$, standard error of the mean < 10%.
[d]Values represent displacement of a specific radiolabeled peptide[11] ($n>3$, standard error of the mean < 10%).
nd = not determined.

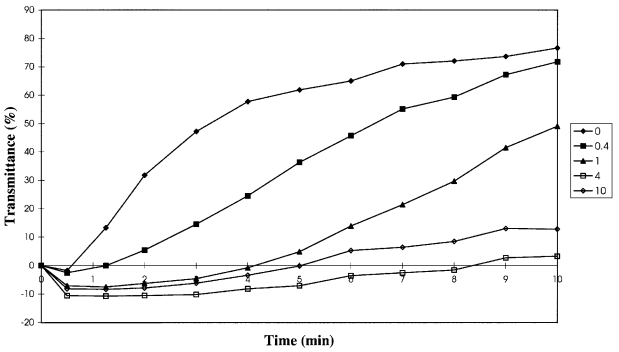


Figure 3. Inhibition of platelet aggregation induced by 1 nM thrombin and measured by change in transmittance, using various concentration of **54** (0, 0.4, 1, 4, 10 μM).

the exception of the 4-iodo derivative **50**. Combination of the previous results lead us to the synthesis of disubstituted antagonists **51**–**55**. Disubstitution at the 3,4-position resulted in compounds with good activity against TRAP but not thrombin-stimulated platelet secretion. The diminished activity of compounds such as **53** against thrombin as the agonist could be due to various factors. The off-rate of these compounds may be too fast, allowing for the tethered ligand to bind and activate platelets. Involvement of PAR-4 on platelets is also a complicating feature although it usually requires a much higher thrombin concentration for activation.[4] Finally, the compounds may exhibit different binding affinities for the cleaved (thrombin activated) and uncleaved (TRAP activated) receptors. Further studies are required to define the importance of these and other potential factors.

In contrast to the previous results, the 3,5-difluoro analogue **54** demonstrates significant potency against both TRAP and 1 nM thrombin stimulated platelet secretion (thrombin $IC_{50} = 0.51$ μM, $n = 10$).

Compound **54** is not an inhibitor of thrombin catalytic activity ($K_i > 10$ μM), nor does it display any inhibition of platelet aggregation induced by ADP or collagen at concentrations up to 20 μM. As observed with most other tested compounds, the binding affinity of **54** for the receptor, as measured by displacement of a specific radiolabeled peptide,[11] is in agreement with the functional data (Table 3). Taken together these results suggest that **54** is acting via selective antagonism of PAR-1 and competing with the intramolecular ligand.

To further evaluate its potential as an antiplatelet agent, **54** was tested for its ability to block human platelet aggregation induced by 1 nM thrombin.[12] As shown in Figure 3, antagonist **54** fully inhibits platelet aggregation stimulated by 1 nM thrombin for the length of the experiment (10 min) at 4 μM, and for 5–6 min at 1 μM, which represents an improvement over our previously reported series.[8]

In conclusion, aminoisoxazole derivatives such as **54** are potent inhibitors of platelet activation. In vitro binding and functional assays with such compounds suggest that they indeed behave as selective antagonists of the thrombin receptor PAR-1, competing with the binding of the tethered ligand to its receptor site rather than interfering with the enzymatic activity of thrombin.

Acknowledgements

The authors thank Mary Becker for help in the preparation of this manuscript and J. Murphy, S. Pitzenberger, S. Varga, A. Coddington, C. Ross, H. Ramjit, K. Anderson, P. Ciecko and M. Zrada for analytical support.

References and Notes

1. Golsack, N. R.; Chambers, R. C.; Dabbagh, K.; Laurent, G. *J. Int. J. Biochem. Cell Biol.* **1998**, *30*, 641.
2. Vu, T. K.; Hung, D. T.; Wheaton, V. I.; Coughlin, S. R. *Cell* **1991**, *64*, 1057.
3. (a) Macfarlane, S. R.; Seatter, M. J.; Kanke, T.; Hunter, G. D.; Plevin, R. *Pharmacol. Rev.* **2001**, *53*, 245. (b) O'Brien, P. J.; Molino, M.; Kahn, M.; Brass, L. F. *Oncogene* **2001**, *20*, 1570. (c) Coughlin, S. R. *Nature* **2000**, *407*, 258. (d) Vergnolle, N. *Aliment. Pharmacol. Ther.* **2000**, *14*, 257. (e) Coughlin, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11023. (f) Dery, O.; Corvera, C. U.; Steinhoff, M.; Bunnett, N. W. *Am. J. Physiol.* **1998**, *274*, C1429. (g) Hou, L.; Howells, G. L.; Kapas, S.; Macey, M. G. *Br. J. Haematol.* **1998**, *101*, 1. (h) Brass, L. F.; Molino, M. *Thromb. Haemostasis* **1997**, *78*, 234. (i) Grand, R. J. A.; Turnell, A. S.; Grabham, P. W. *Biochem. J.* **1996**, *313*, 353. (j) Hollenberg, M. D. *TiPS* **1996**, *17*, 3. (k) Coughlin, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9200.
4. Dery, O.; Bunnett, N. W. *Biochem. Soc. Trans.* **1999**, *27*, 246.
5. (a) Ray, A.; Hedge, L. G.; Gupta, J. B. *Thromb. Res.* **1997**, 87. (b) Ogletree, M. L.; Natarajan, S.; Seiler, S. M. *Perspect. Drug Discov. Des.* **1993**, *1*, 527. (c) Pakala, R.; Liang, C. T.; Benedict, C. R. *Thromb. Res.* **2000**, *100*, 89. (d) Cocks, T. M.; Moffat, J. D. *Trends Pharma. Sci.* **2000**, *21*, 103. (e) Napoli, C.; Cicola, C.; Wallace, J. L.; De Nigris, F.; Standagada, V.; Cliendo, G.; Franconi, F.; Ignarro, L. J.; Cirino, G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3678.
6. (a) Feng, D. M.; Veber, D. F.; Connolly, T. M.; Condra, C.; Tang, M. J.; Nutt, R. F. *J. Med. Chem.* **1995**, *38*, 4125. (b) Andrade-Gordon, P.; Maryanoff, B. E.; Derian, C. K.; Zhang, H. C.; Addo, M. F.; Darrow, A. L.; Eckardt, A. J.; Hoekstra, W. J.; McComsey, D. F.; Oksenberg, D.; Reynolds, E. E.; Santulli, R. J.; Scarborough, R. M.; Smith, C. E.; White, K. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12257. (c) McComsey, D. F.; Hawkins, M. J.; Andrade-Gordon, P.; Addo, M. F.; Oksenberg, D.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1423. (d) Nose, T.; Satoh, Y.; Fijita, T.; Ohno, M.; Nakajima, M.; Inoue, Y.; Ogino, Y.; Costa, T.; Shimohigashi, Y. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1661. (e) Bernatowicz, M. S.; Klimas, C. E.; Hartl, K. S.; Peluso, M.; Allegretto, N. J.; Seiler, S. M. *J. Med. Chem.* **1996**, *39*, 4879. (f) Zhang, H.-C.; Derian, C. K.; Andrade-Gordon, P.; Hoekstra, W. J.; McComsey, D. F.; White, K. B.; Poulter, B. L.; Addo, M. F.; Cheung, W.-M.; Damiano, B. P.; Oksenberg, D.; Reynolds, E. E.; Pandey, A.; Scarborough, R. M.; Maryanoff, B. E. *J. Med. Chem.* **2001**, *44*, 1021.
7. (a) Ahn, H. S. A.; Arik, L.; Boykow, G.; Burnett, D. A.; Caplen, M. A.; Czarniecki, M.; Domalski, M. S.; Foster, C.; Manna, M.; Stamford, A. W.; Wu, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2073. (b) Alexopoulos, K. A.; Matsoukas, J.;

Tselios, T.; Roumelioti, P.; Mavromoustakos, T.; Holada, K. *Amino Acids* **1998**, *15*, 211. (c) Kato, Y.; Kita, Y.; Nishio, M.; Hirasawa, Y.; Ito, K.; Yamanaka, T.; Motoyama, Y.; Seki, H. *Eur. J. Pharmacol.* **1999**, *384*, 197.
8. Barrow, J. C.; Nantermet, P. G.; Glass, K. L.; Ngo, P. L.; Breslin, M. J.; Hutchninson, J. H.; Freidinger, R. M.; Condra, C.; Karczewski, J.; Bednar, R.; Stern, A.; Gould, R.; Connolly, T. M.; Selnick, H. G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2691.
9. Kearsley, S. K.; Sallamack, S.; Fluder, E. M.; Andose, J. D.; Mosley, R. T.; Sheridan, R. P. *J. Chem. Inf. Comp. Sci.* **1996**, *36*, 118.
10. Tatee, T.; Narita, K.; Kurashige, S.; Ito, S.; Miazaki, H.; Yamanaka, H.; Mizukagi, M; Sakamoto, T.; Fukuda, H. *Chem. Pharm. Bull.* **1987**, *35*, 3676.
11. Radiolabeled peptide **1b** within ref 8. Also see ref 6e.
12. Cook, J. J.; Sitko, G. R.; Bednar, B.; Condra, C.; Mellott, M. J.; Feng, D. M.; Nutt, R. F.; Shafer, J. A.; Gould, R. J.; Connolly, T. M. *Circulation* **1995**, *91*, 2961.