Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2851–2853

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Potent, Low Molecular Weight Thrombin Receptor Antagonists

Samuel Chackalamannil,* Darío Doller, Keith Eagen, Michael Czarniecki, Ho-Sam Ahn, Carolyn J. Foster and George Boykow

Schering-Plough Research Institute, 2015 Galloping Hill Rd., Kenilworth, NJ 07033, USA

Received 20 March 2001; accepted 6 August 2001

Abstract—Several benzimidazole derivatives have been identified as potent thrombin receptor (PAR-1) antagonists as represented by compound **1h**, which showed an IC_{50} of 33 nM.

Thrombin is a serine proteinase that plays a key role in hemostasis and wound healing.[1] In addition to its pivotal role in hemostasis, thrombin also stimulates potent proliferative and inflammatory processes in a variety of cell types by direct cellular activation.[2] Cellular actions of thrombin are mediated by the activation of specific cell surface receptors known as protease activated receptors (PAR) that comprise a small family of G-protein coupled receptors.[3] It has been postulated that thrombin binds to the cellular receptor through its anion binding exocyte and subsequently cleaves the peptide at Arg_{41}-Ser_{42}. The newly unmasked serine amino terminus acts as a 'tethered ligand' binding intramolecularly to the proximal receptor which elicits *trans*-membrane signaling. The prototype of this new class of receptors, known as protease activated receptor-1 (PAR-1) or the thrombin receptor, is present in a variety of human cell types including platelets, fibroblasts, endothelial cells, and cardiac myocytes.[4]

The potential pathophysiological role of the thrombin receptor in thrombosis, atherosclerosis, and restenosis has been well recognized.[4] As such, a thrombin receptor antagonist may have considerable therapeutic utility in the treatment of these disorders, especially restenosis for which no effective treatment is currently available. Since a thrombin receptor antagonist is specific for the cellular actions of thrombin and does not interfere with the coagulation cascade, such agents are likely to confer added safety margin with regard to hemorrhagic side effects which is a complicating factor in the currently available antithrombotic treatment.[5] Several peptide agonists and antagonists of PAR-1 based on the amino acid sequence of the 'tethered ligand' have been reported.[6] Recently, there have been reports of nonpeptide PAR-1 antagonists.[7] However, these compounds, in general, have only modest affinity for the thrombin receptor. Herein we wish to report the identification and structure activity relationship studies of 2-aminobenzimidazole derivatives that are potent thrombin receptor antagonists. Benzimidazole derivative **1h**, with an IC_{50} value of 33 nM, is the most potent nonpeptide thrombin receptor antagonist reported todate.

The benzimidazole derivative **1b** (Table 1) was identified as a thrombin receptor antagonist lead through high throughput screening. This sparked our interest in the SAR exploration of this class of compounds. The required 1,3-disubstituted benzimidazole derivatives represented by structure **1** were readily prepared according to the literature procedure[8] by sequential *N*-1 and *N*-3 alkylation of 2-aminobenzimdazole (**2**) in methanol or acetone using commercially available phenacyl bromides and alkyl halides (Scheme 1). In the first step, use of the alkylating agent as the limiting reagent minimized dialkylation. The second alkylation often required refluxing conditions and the product could be easily isolated by crystallization from the reaction mixture. IC_{50} determinations were carried out on PAR-1 receptors on human platelets according to the published procedure.[9] The structure–activity relationship data are presented in Table 1.

The monosubstituted benzimidazole derivative **1a** showed an IC_{50} of 1500 nM. Systematic variation of the *N*-3 substitution pattern revealed that a lower alkyl or

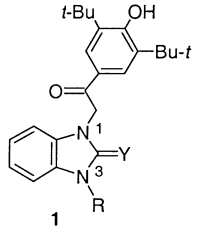
*Corresponding author. Tel.: +1-908-740-3474; fax: +1-908-740-7152; e-mail: samuel.chackalamannil@spcorp.com

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00555-8

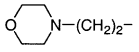
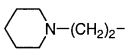
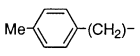
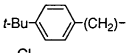
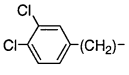
benzyl group yielded optimum activity. For example, the *N*-3-butyl derivative **1i** gave an IC_{50} value of 359 nM whereas the *N*-3 methyl derivative (**1b**) and *N*-3 heptyl derivative (**1j**) were less active. Compound **1c** bearing 2-(1-piperidinyl)ethyl group was somewhat less potent ($IC_{50}=2866$ nM) whereas the corresponding morpholine derivative **1f** was quite potent ($IC_{50}=98$ nM). Compound **1g** bearing *N*-3 benzyl substituent gave an IC_{50} of 65 nM. Phenyl substituent effect on the *N*-3 benzyl group was also studied (**1d**–**1e** and **1h**). Among the various *N*-3 benzyl derivatives examined, compound **1h**, bearing a *p*-tolyl group, showed the best activity ($IC_{50}=33$ nM).

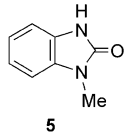
The effect of substituent at *N*-1 was also examined. A representative example of the variations employed is presented in Table 2. Presence of the 3,5-bis-*tert*-butyl-4-hydroxy phenacylmethyl substitution at the *N*-1 ring nitrogen was found to be essential for thrombin receptor antagonist property. All the compounds represented by the structure **8**, devoid of this motif, were inactive. The 1,3-bis-*tert*-butyl phenol derivative **7** per se did not show any inherent thrombin receptor inhibition.

Table 1. Structure–activity relationship of substituted benzimidazoles (**1**)

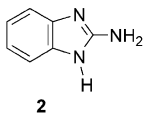


1

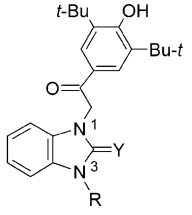
Entry	R	PAR-1 IC_{50} (nM)	Entry	R	PAR-1 IC_{50} (nM)
1a	H	1500	**1f**		98
1b	Me	900	**1g**	Benzyl	65
1c		2866	**1h**		33
1d		734	**1i**	*n*-C_4H_9	359
1e		1324	**1j**	*n*-C_7H_{15}	964

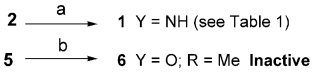


5



2





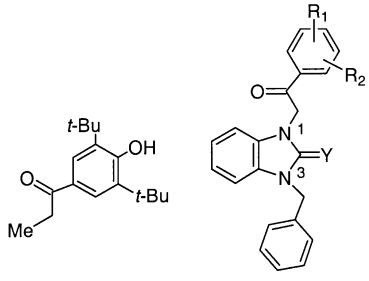
Scheme 1. Reagents and conditions: (a) (i) $BrCH_2(CO)Ar$ (**3**) (1 equiv), acetone, 18 h; (ii) R-X, EtOH, reflux; (b) NaH, DMF, **3**.

Finally, the requirement of the 2-imino group was established by examining a series of compounds represented by the structure **6** (Scheme 1). Compound **6** as well as a number of its close analogues that we examined (not shown) were uniformly inactive.

Several compounds in the benzimidazole series were also tested in functional platelet aggregation assay employing either the PAR-1 selective agonist, high affinity thrombin receptor agonist peptide (ha-TRAP), or thrombin to induce aggregation.[10] The results for compounds **1f**–**1i** are shown in Table 3. These compounds were effective inhibitors of ha-TRAP-induced platelet aggregation, although the IC_{50} values for blocking the aggregation were consistently higher than those observed in the binding assay.[11] The compounds also inhibited thrombin-induced platelet aggregation. The rank order of potency paralleled that for ha-TRAP inhibition, but higher concentrations of the compounds were needed to produce inhibition.

In conclusion, we have identified a new series of benzimidazole derivatives as potent thrombin receptor antagonists. Optimal activity was seen when the two ring nitrogen atoms of benzimidazole were substituted, respectively, by a 3,5-bis-*tert*-butyl-4-hydroxy phenacylmethyl group and a lower alkyl or benzyl group.

Table 2.



7 Inactive

8 Inactive

R_1	R_2
3-OH	4-OH
2-OMe	5-OMe
4-Me	H
2-Me	4-Me
4-CN	H
4-Br	H
2-Cl	4-Cl
3-Me	4-Cl
4-F	H

Table 3. Inhibition of human platelet aggregation

Entry	PAR-1 IC_{50} (nM)	Platelet aggregation IC_{50} (nM)[10]	
		ha-TRAP	Thrombin
1f	98	2000	>10,000
1g	65	265	600
1h	33	575	1500
1i	359	825	10,000

Acknowledgements

The authors would like to thank Drs. Catherine Strader, William J. Greenlee, Ashit Ganguly, and Michael Graziano for helpful discussions.

References and Notes

1. (a) Coleman, R. W.; Marder, V. J.; Salzman, E. W.; Hirsch, J. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 3rd ed.; Coleman, R. W., Hirsch, J., Marder, V. J., Salzman, E. W., Eds.; J. B. Lippincott: Philadelphia, 1994 (b) Stubbs, M. T.; Bode, W. *Curr. Opin. Struct. Biol.* **1994**, *4*, 823. (c) Bazan, J. F. *Nature* **1996**, *380*, 21. (d) Tapparelli, C.; Metternich, R.; Ehrhardt, C.; Cook, N. S. *Trends Pharm. Sci.* **1993**, *14*, 366.
2. DeCaterina, R.; Sicari, R. *Pharmacol. Res.* **1993**, *27*, 1.
3. (a) Coughlin, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11023. (b) Vu, T.-K. H.; Hung, D. T.; Wheaton, V. I.; Coughlin, S. R. *Cell* **1991**, *64*, 1057. (c) Coughlin, S. R.; Vu, T.-K. H.; Hung, D. T.; Wheaton, V. I. *J. Clin. Invest.* **1992**, *89*, 351. (d) Coughlin, S. R. *Trends Cardiovasc. Med.* **1994**, *4*, 77. (e) Hollenberg, M. D. *Trends Pharm. Sci.* **1996**, *17*, 3.
4. (a) Nanevicz, T.; Ishii, M.; Wang, L.; Chen, M.; Chen, J.; Turck, C. W.; Cohen, F. E.; Coughlin, S. R. *J. Biol. Chem.* **1995**, *270*, 21619. (b) Tapparelli, C.; Metternich, R.; Cook, N. S. *Trends Pharm. Sci.* **1993**, *14*, 426.
5. (a) Baykal, D.; Schmedtje, J. F.; Runge, M. S. *Am. J. Cardiol.* **1995**, *75*, 82B. (b) Ogletree, M. L.; Natarajan, S.; Seiler, S. M. *Perspect. Drug Disc. Design* **1994**, *1*, 527. (c) Brass, L. F. *Thromb. Hemost* **1995**, *74*, 499. (d) Brass, L. F. *Coron. Art. Dis.* **1997**, *8*, 49.
6. (a) Bernatowicz, M. S.; Klimas, C. E.; Hartl, K. S.; Peluso, M.; Allegretto, N. J.; Seiler, S. M. *J. Med. Chem.* **1996**, *39*, 4879. (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) Elliot, J. T.; Hoekstra, W. J.; Maryanoff, B. E.; Prestwich, G. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 279. (d) For peptide agonists, see: Gerszten, R. E.; Chen, J.; Ishii, M.; Ishii, K.; Wang, L.; Nanevicz, T.; Turck, C. W.; Vu, T.-K. H.; Coughlin, S. R. *Nature* **1994**, *368*, 648. (e) McComsey, D. F.; Hecker, L. R.; Andrade-Gordon, P.; Addo, M. F.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 255.
7. (a) Ahn, H.-S.; 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) Kato, Y.; Kita, Y.; Nishio, M.; Hirasawa, Y.; Kiyotaka, I.; Yamanaka, T.; Motoyama, Y.; Seki, J. *Eu. J. Pharmacol.* **1999**, *384*, 197.
8. (a) Ogura, H.; Takayanagi, H.; Yamazaki, Y.; Yonezawa, S. *J. Med. Chem.* **1972**, *15*, 923. (b) Simonov, A. M.; Anisimova, V. A. *Chem. Het. Comp. (Engl. Transl.)* **1968**, *4*, 801.
9. (a) Ahn, H.-S.; Foster, C.; Boykow, G.; Arik, L.; Smith-Torhan, A.; Hesk, D.; Chatterjee, M. *Mol. Pharmacol.* **1997**, *51*, 350 Binding assays were performed using human thrombin receptor activating peptide (ha-TRAP) at a concentration of 10 nM.
10. Bednar, B.; Condra, C.; Gould, R. J.; Connolly, T. M. *Thromb. Res.* **1995**, *77*, 453.
11. The concentration of ha-TRAP was 300 nM in the platelet aggregation inhibition assay. The use of a 30-fold higher concentration of the ligand in the platelet aggregation inhibition assay, compared to the binding assay, may explain the higher IC_{50} values. Thrombin-induced aggregation was conducted at the thrombin concentration of 0.1 U/mL. The IC_{50} values were determined at 2 min, and in all cases, aggregation proceeded to completion by 6 min.