Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1199–1202

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Diamino Benzo[*b*]thiophene Derivatives as a Novel Class of Active Site Directed Thrombin Inhibitors. Part 6: Further Focus on the Contracted C4′-Side Chain Analogues

Kumiko Takeuchi,* Todd J. Kohn, Richard W. Harper, Ho-Shen Lin, Donetta S. Gifford-Moore, Michael E. Richett, Daniel J. Sall, Gerald F. Smith and Minsheng Zhang†

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 13 January 2000; accepted 23 March 2000

Abstract—Novel benzo[*b*]thiophene diamine thrombin inhibitors were investigated, focusing on a contracted C4′-side chain series. SAR studies identified compounds with either a pyrrolidino or morpholino group as potent, active site directed thrombin inhibitors when the amino group was connected to the C3-phenyl ring with a methylene linker at the C4′ position of the phenyl ring.

Thrombin, a trypsin-like serine protease, catalyzes fibrin formation and activates platelets, thereby playing a pivotal role in the development of thrombotic diseases. Less than ideal antithrombotic therapeutics such as coumadin and heparins have triggered an intense search for more efficacious and less liable anticoagulants. We have identified through our broad screening efforts a novel small organic molecule, 2,3-disubstituted benzo[*b*]thiophene **1** (eq (1)), as an active site directed thrombin inhibitor.[1]

Extensive structure–activity relationship (SAR) studies based upon the initial lead compound **1** have been undertaken, highlighting the importance of new synthetic methodology to enable continued SAR exploration for identification of a suitable clinical candidate (eq (2)).[2]

The SAR studies on the C3-side chain led us to discover a novel class of potent, active site directed thrombin inhibitors **2** (where X = OMe or Br) which possessed a contracted C4′-side chain.[3,4] We report here our exploration of further SAR studies focused on the contracted C4′ side chain.

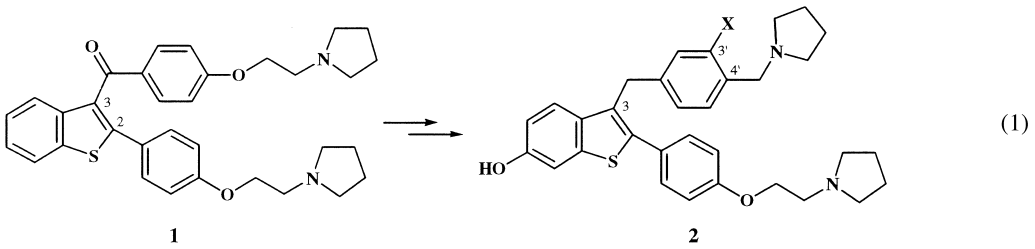
We have reported the effect of C3-side chain length on the thrombin inhibitory activity of the 2,3-disubstituted benzo[*b*]thiophene diamine derivatives.[3] A correlation of the thrombin inhibitory activity in K_{ass} with the C4′-side chain length is depicted in Figure 1, which shows a clear advantage of one methylene linker (L = CH_2) over a shorter or a longer chain between the C3-phenyl and pyrrolidine ring. Fixing the C4′-side chain length between the phenyl and the amino group with a methylene linker, we report here three aspects of substituent effects on the various parts of the molecule.

First of all, the C3′ substituent effect on the thrombin inhibitory activities is shown in Table 1. Methoxy and bromo compounds **2b** and **2j** have been synthesized by the methods previously reported.[4] All the compounds reported in the present study were synthesized similarly with minor modifications when needed. Compounds **2d**, **2g** and **2i** were prepared from commercially available 3-amino-, 3-nitro, and 3-chloro-4-methyl benzoic acid, respectively. Displacement reactions of the bromide **2j** with an appropriate substituent afforded compounds **2a**, **2c** and **2h**. Compound **2f** was derived from reduction of **2h**. Compound **2e** was obtained from demethylation of **2b**. Table 1 shows that hydrophobic substituents with disregard to their electron donating (OMe, Me) or withdrawing (Br) nature were beneficial to the thrombin inhibitory activity, possibly due to their favorable interaction with the hydrophobic S_2 subsite of thrombin, as previously reported,[1,2] whereas the hydrophilic substituents (OH, CH_2NH_2, NMe_2) were detrimental to the inhibitory activity. This contrast is clearly seen

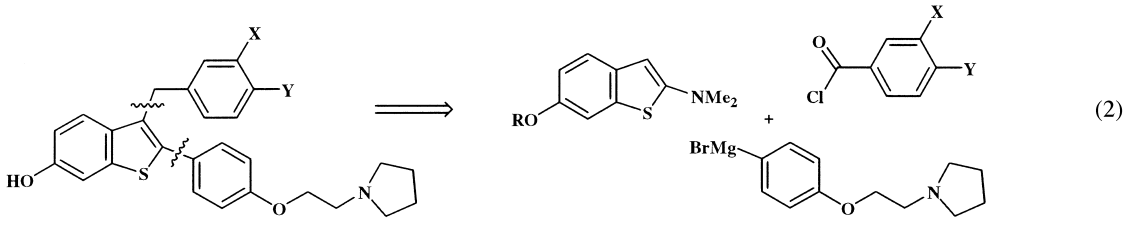
*Corresponding author. Tel.: +1-317-276-6771; fax: +1-317-433-0715; e-mail: ktak@lilly.com

†Current address: Bristol-Meyers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-5400, USA.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00211-0



(1)



(2)

between compounds **2b** (OMe) and **2e** (OH) or between **2b** (OMe) and **2d** (NHMe). The low inhibitory activity of compound **2g** (NMe_2) could be also due to its steric bulkiness.

Second, the effects of the substituent **Y** on the benzo[*b*]thiophene ring is shown in Table 2. X-ray crystallographic analysis of the disubstituted benzo[*b*]thiophene thrombin inhibitors disclosed that this portion of the molecule was inserted into the S_1 specificity pocket of thrombin.[1] A substituent which facilitates interaction with Asp189 at the S_1 site via a hydrogen bond was considered to be beneficial for the inhibitory activity, and 6-hydroxybenzo[*b*]thiophene was identified as a favorable moiety.[2] Here we explored the effects of amino and fluoro substituents to probe further the hydrogen bond interaction[6] between the inhibitor and the thrombin. A 6-fluoro substituent was detrimental to the thrombin inhibitory activity possibly due to its more lipophilic nature than hydroxy or even hydrogen and also because it is a hydrogen acceptor rather than a donor, indicating no favorable interaction with hydrophilic carboxy terminus of Asp189. A considerable 6-fold decrease in the thrombin inhibitory activity of the 6-amino derivative **7a**,[7] compared to **2b** with 6-hydroxy substituent, was somewhat surprising since one could expect a good hydrogen bond interaction between the amino group and the carboxylate of Asp189. This decrease in the inhibitory activity could be attributed to the less acidic anilinic proton of **7a** than the phenolic proton of **2b**. It could also be due to the interaction between the narrow cavity of the S_1 specificity pocket and the tri-substituted benzo[*b*]thiophene ring, which might not sterically accommodate the two amino hydrogens well, in contrast to one hydrogen of the hydroxy group with more flexibility. The decreased inhibitory activity of the

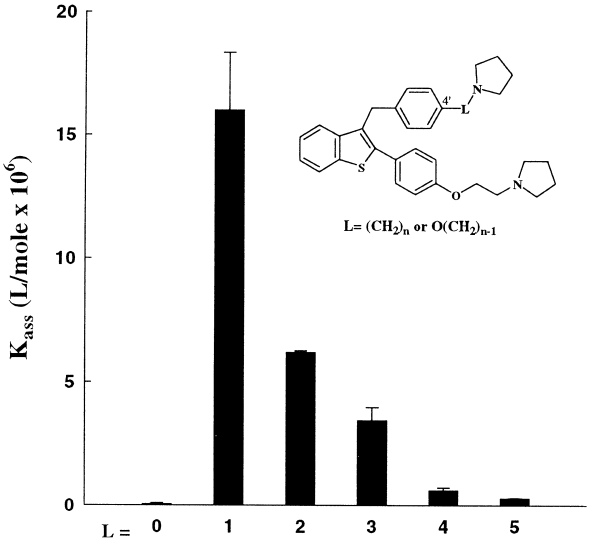
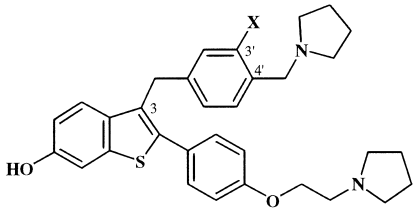


Figure 1. Effects of the C4′-side chain length on the thrombin inhibitory activity.

Table 1. Thrombin inhibitory activity of the compounds



2

Compounds	X	K_{ass} (L/mol×10^6)[5] thrombin
2a	H	81.2
2b	OMe	815
2c	Me	679
2d	NHMe	126
2e	OH	39.1
2f	CH_2NH_2	30.0
2g	NMe_2	2.31
2h	CN	25.8
2i	Cl	234
2j	Br	1240

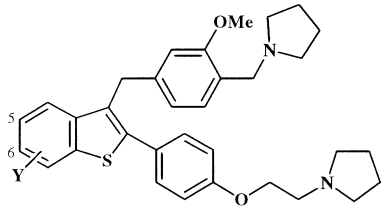
methylsulfonamide derivative **8** could also be explained by its bulky size in the specific pocket of the enzyme. The 5-methoxy derivative **5** showed respectable activity, attributable to its favorable interaction with the hydrophobic region of the enzyme,[2] in contrast to 6-methoxy derivative **6** that was disfavored both sterically and without a hydrogen at the S_1 site.

The third modification of the molecule was focused on the substituent effects on the C4′-pyrrolidine ring (Table 3). (*S*)-Proline derivatives **10a**, **10b** and **10c** were prepared from (*S*)-proline *t*-butyl ester. As Table 3 indicates, a substituent on the pyrrolidine ring was detrimental. Earlier we reported[1] that a basic amine functionality was needed for the thrombin inhibitory activity. We observed here that the inhibitory activity decreased as the basicity of the compounds was reduced. The 2-pyrrolidone derivative **9** lost thrombin inhibitory activity ca. 30%, compared to **2c**. The (*S*)-proline derived compound **10c** which could neutralize the amine basicity lost its activity almost 4-fold.

Further modification of the pyrrolidine ring was to replace it with other cyclic or acyclic amines (Table 4). Morpholine replacement **11b** retained respectable thrombin inhibitory activity with modest loss, compared to **2b**. Acyclic analogue **12** lost its activity about 2-fold, though still maintaining potent inhibitory activity. The smaller acyclic dimethyl compound **13**, however, lost its activity considerably. This indicates the conformational rigidity and steric bulk in this region of the molecule is required for maintaining good inhibitory activity. These results are interesting in comparison with our earlier work,[2] in that the same substituents had very different effects on the thrombin inhibitory activity when the C4′-side chain was conformationally restricted. Particularly notable is the example of the morpholine compounds. The inhibitory activity was well maintained in the current analogue **11b** (with decrease in $K_{ass} = 24\%$ from **2b**) in contrast to an over 10-fold loss of activity from the pyrrolidino to the morpholino group when the C4′-side chain was constrained by a cyclohexyl ring.[2] The C3-side chain as a whole resides in between the S_2 and S_3 hydrophobic regions of thrombin,[8] almost sandwiched, but the reason for this difference in the thrombin inhibitory activity between these two hydrophobic C4′ groups is not clear.

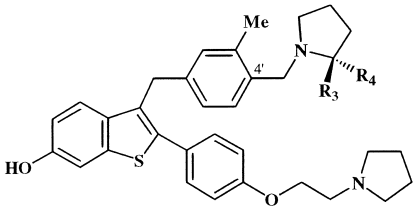
The thrombin inhibitory activity of various morpholine derivatives (Table 5) may be compared with the pyrrolidines' activity in Table 1. The results in Tables 1 and 5 show that compounds from the two series with the same substituent patterns (**2b**, **2c** and **2j** versus **11a**, **11b** and

Table 2. Effects of substituent Y



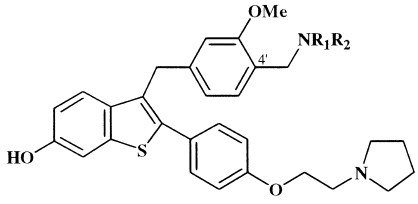
Compounds	Y	K_{ass} (L/mol×10⁶)[5] thrombin
2b	6-OH	815
3a	H	126
4	6-F	23.1
5	5-OMe	221
6	6-OMe	6.04
7a	6-NH_2	139
8	6-$NHSO_2Me$	2.94

Table 3. Substituent effects on the C4′-pyrrolidine



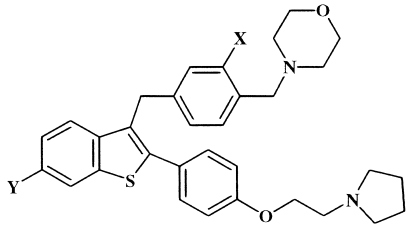
Compounds	R_3,R_4	K_{ass} (L/mol×10⁶)[5] thrombin
2c	H,H	679
9	O	525
10a	(*S*)-CH_2OH	395
10b	(*S*)-CH_2OCH_3	353
10c	(*S*)-CO_2H	192

Table 4. Modification of C4′-dialkylamino group



Compounds	NR_1R_2	K_{ass} (L/mol×10⁶)[5] thrombin
2b	Pyrrolidino	815
11b	Morpholino	617
12	NEt_2	415
13	NMe_2	50

Table 5. Thrombin inhibitory activity of morpholine derivatives



Compounds	X	Y	K_{ass} (L/mol×10⁶)[5] thrombin
3b	OMe	H	124
7b	OMe	NH_2	152
11a	OMe	OH	617
11b	Me	OH	564
11c	Br	OH	1160

11c; **3a** versus **3b**; **7a** versus **7b**) correspond well in their thrombin inhibitory activities, of which the pyrrolidine derivatives exhibited moderately better activity than the morpholine series, except for the 6-amino derivatives. Thus, we have identified both the pyrrolidine and morpholine series as potent, active site directed thrombin inhibitors.

In conclusion, novel benzo[*b*]thiophene diamine thrombin inhibitors were investigated, focusing on a contracted C4′-side chain series. The SAR studies identified compounds with either a pyrrolidino or morpholino group as potent, active site directed thrombin inhibitors when the amino group was connected to the C3-phenyl ring via a methylene linker at the C4′ position of the phenyl ring.

References and Notes

1. Sall, D. J.; Bastian, J. A.; Briggs, S. L.; Buben, J. A.; Chirgadze, N. Y.; Clawson, D. K.; Denney, M. L.; Gierra, D. D.; Gifford-Moore, D. S.; Harper, R. W.; Hauser, K. L.; Klimkowski, V. J.; Kohn, T. J.; Lin, H.-S.; McCowan, J. R.; Palkowitz, A. D.; Smith, G. F.; Takeuchi, K.; Thrasher, K. J.; Tinsley, J. M.; Utterback, B. G.; Yan, S.-C. B.; Zhang, M. *J. Med. Chem.* **1997**, *40*, 3489.

2. Takeuchi, K.; Kohn, T. J.; Sall, D. J.; Denney, M. L.; McCowan, J. R.; Smith, G. F.; Gifford-Moore, D. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 759.

3. Zhang, M.; Bailey, D. L.; Bastian, J. A.; Briggs, S. L.; Chirgadze, N. Y.; Clawson, D. K.; Denney, M. L.; Gifford-Moore, D. S.; Harper, R.; Johnson, L. M.; Klimkowski, V. J.; Kohn, T. J.; Lin, H.-S.; McCowan, J. R.; Richett, M. E.; Sall, D. J.; Smith, A. J.; Smith, G. F.; Snyder, D. W.; Takeuchi, K.; Utterback, B. G.; Yan, S.-C. B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 775.

4. Sall, D. J.; Bailey, D. L.; Bastian, J. A.; Buben, J. A.; Chirgadze, N. Y.; Clemens-Smith, A. J.; Denney, M. L.; Fisher, M. J.; Giera, D. D.; Gifford-Moore, D. S.; Harper, R.; Johnson, L. M.; Klimkowski, V. J.; Kohn, T. J.; Lin, H.-S.; McCowan, J. R.; Richett, M. E.; Smith, G. F.; Snyder, D. W.; Takeuchi, K.; Toth, J. E.; Zhang, M. *J. Med. Chem.* **2000**, *43*, 649.

5. K_{ass} represents the apparent association constant as measured by the methods of Smith, G. F.; Gifford-Moore, D.; Craft, T. J.; Chirgadze, N.; Ruterbories, K. J.; Lindstrom, T. D.; Satterwhite, J. H. In *New Anticoagulants for the Cardiovascular Patient*; Pifarre, R., Ed.; Hanley & Belfus: Philadelphia, 1997; pp 265–300. K_{ass} value is the average of two or more determinations, where the variation in the assay is in the range of $\pm 10\%$.

6. For general discussion on the hydrogen bonding, see March, J. *Advanced Organic Chemistry*, 4th ed.; John Wiley & Sons: New York, 1992; Chapter 6, pp 75–79 and references therein.

7. Compound **7a** was prepared from **2b** via Smiles' rearrangement reported by Bayles, R.; Johnson, M. C.; Maisey, R. F.; Turner, R. W. *Synthesis* **1977**, 33.

8. Bastian, J. A.; Chirgadze, N. Y.; Denney, M. L.; Gifford-Moore, D. S.; Sall, D. J.; Smith, G. F.; Wikel, J. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 363.