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DIAMINO BENZO[*B*]THIOPHENE DERIVATIVES AS A NOVEL CLASS OF ACTIVE SITE DIRECTED THROMBIN INHIBITORS: 3. ENHANCING ACTIVITY BY IMPOSING CONFORMATIONAL RESTRICTION IN THE C-4" SIDE CHAIN[1]

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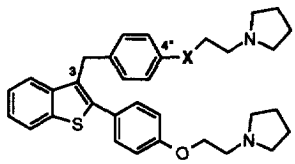
Abstract: The preparation and biological evaluation of a series of benzo[*b*]thiophene diamine thrombin inhibitors possessing conformationally restricted C-4" linkers are reported. Compared to the parent compounds **1a/b**, the unsaturated derivatives **3a/b** exhibited a modest twofold increase in thrombin inhibitory activity, while the more lipophilic carbocyclic ring containing analogs **4a/b** affected an eightfold enhancement in potency.

The serine protease thrombin plays an integral role in the blood coagulation cascade by catalyzing the cleavage of the plasma protein fibrinogen to insoluble fibrin monomers and promoting platelet activation.[2] Under normal physiological conditions these two processes lead to controlled thrombus formation, but aberrant coagulation (thrombosis) can ultimately lead to blood vessel occlusion. Since heart disease resulting from thrombosis is the leading cause of morbidity and mortality in humans, the search for selective, direct acting, oral thrombin inhibitors has become intensely competitive.[3] Previously, we reported structurally unique nonpeptidal oral thrombin inhibitors (e.g., **1a/b**) lacking a typical active site directing amidino or guanidino functionality.[4] X-ray crystallographic studies have shown that the benzo[*b*]thiophene nucleus of **1a** occupies the thrombin specificity pocket (S_1) while the C-3 side chain spans the hydrophobic proximal (S_2) and distal (S_3) pockets (Figure 1).[5a] Specifically, the C-3 phenyl ring is situated at the opening of the S_2 binding site while the pyrrolidine ring binds in the S_3 pocket. It has been reported that the



1a: X = O
1b: X = CH_2

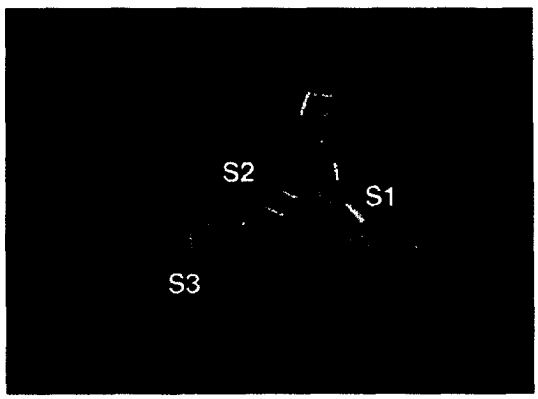


Figure 1. The X-ray crystal structure of inhibitor **1a** bound in the active site of human α-thrombin.[5a] The C-3 side chain of **1a** extends along the S_2 pocket, which consists of the thrombin residues Trp215, Leu99, His57, Tyr60A and Trp60D, and the S_3 binding site, which consists of residues Trp215, Ile174 and Glu97A-Leu99.[5b]

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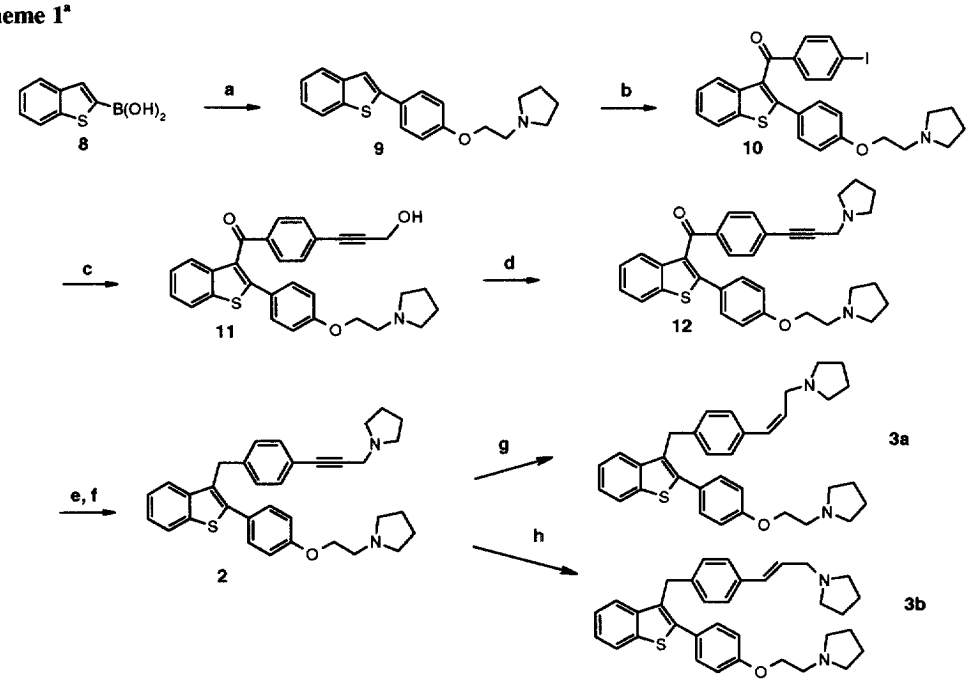
thrombin inhibitory activity of this class of molecules can be increased by incorporating C-3" substituents, which are presumed to interact at the S_2 binding site.[6] The current studies are directed at increasing the interaction of the C-3 pyrrolidine ring with the S_3 pocket by imposing conformational restraint in the otherwise freely flexible C-4" linker of the C-3 side chain as an alternative means to enhance thrombin inhibition. Two ways to conformationally restrict the C-4" side chain are to introduce unsaturation or carbocyclic rings. Preparation and evaluation of the acetylenic and olefinic derivatives (**2** and **3a/b**, respectively; Table 1) and the 5- and 6-membered ring analogs (**4a/b**; Table 1), as well as studies focused on the hydrophobicity of the side chain were completed. This paper will summarize enhancement of the thrombin inhibitory activity of **1a/b** through these modifications to the C-4" side chain.

Chemistry

The benzo[*b*]thiophene analogs tested in this study were prepared as outlined in Schemes 1 and 2.[7] We employed a strategy in which the acetylene **2** and olefins **3a/b** were derived from a common synthetic route (Scheme 1). Preparation of the acetylenic deriviative **2** began with Suzuki coupling[8] of benzothiophene-2-

Scheme 1[a]

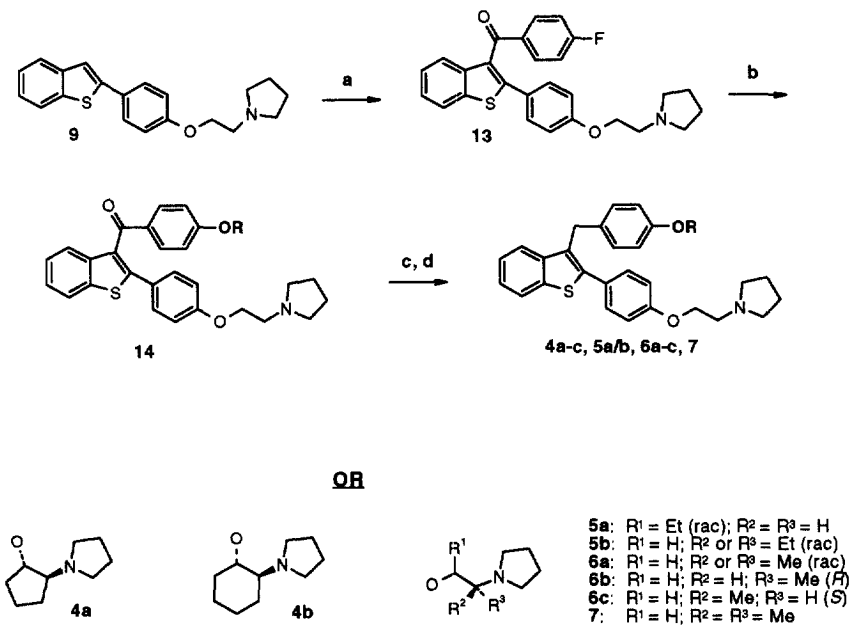


[a]*Reagents*: (a) 1-[2-(4-bromophenoxy)ethyl]pyrrolidine, $Pd(PPh_3)_4$, 2 N aq Na_2CO_3 (38%); (b) 4-iodobenzoic acid, $SOCl_2$, then **9** and $TiCl_4$ (79%); (c) propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, TEA (quantitative); (d) MsCl, K_2CO_3, TEA (cat), then pyrrolidine (51%); (e) DIBAL-H; (f) Et_3SiH, TFA (2 steps: 76%); (g) H_2, Lindlar catalyst (44%); (h) DIBAL-H (61%).

boronic acid (**8**) and 1-[2-(4-bromophenoxy)ethyl]pyrrolidine to afford the 2-arylbenzothiophene **9**. Friedel–Crafts acylation with 4-iodobenzoyl chloride followed by Pd-mediated coupling to propargyl alcohol by the method of Sonogashira[9] gave the 2,3-disubstituted intermediate **11**. Mesylation of alcohol **11** followed directly by treatment with pyrrolidine yielded diamine **12**. Selective reduction of the ketone, in the presence of the acetylene, with DIBAL-H at 0 °C, and deoxygenation with TFA/Et_3SiH gave the methylene derivative **2**. The acetylene functionality of **2** was selectively reduced with H_2/Lindlar's catalyst or DIBAL-H/40 °C to afford the *cis* and *trans* olefins, **3a** and **3b**, respectively.[10]

The C-4" ether linked derivatives (**4–7**) were prepared according to Scheme 2. Acylation of 2-arylbenzothiophene **9** with 4-fluorobenzoyl chloride gave the common 2,3-disubstituted intermediate **13**. Installation of the C-4" side chains (-OR) was accomplished by displacement of the fluoride ion by the appropriate sodium alkoxide[11] resulting in intermediates **14**. Reductive-deoxygenation of the C-3 ketones as described previously afforded the ethers **4–7**.

Scheme 2[a]

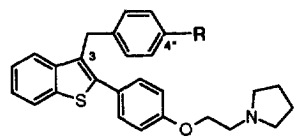


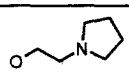
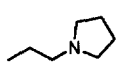
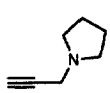
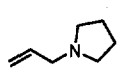
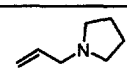
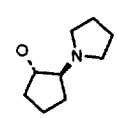
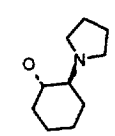
[a]*Reagents*: (a) 4-fluorobenzoyl chloride, $TiCl_4$ (65%); (b) NaH, ROH (30-96%); (c) DIBAL-H; (d) TFA, Et_3SiH (2 steps: 37-94%).

Results and Discussion

It has been reported that substitution of the ether oxygen at C-4" by a carbon atom is well tolerated by the enzyme (**1a** *vs* **1b**).[4] Replacement of the freely flexible C-4" saturated tether of **1b** by an acetylenic linker (**2**) results in comparable activity. Reduction of the acetylene leads to the more potent olefinic derivatives **3a/b**. In fact, the *trans* isomer **3b** exhibits twofold more inhibitory activity than the saturated parent. While the acetylenic and olefinic linkers of analogs **2**, **3a/b** reduce the conformational mobility of the C-4" side chain, molecular modeling studies indicate that they can still occupy a number of low energy conformations. However, carbocyclic ring systems are more conformationally rigid, and incorporation of the ethylene linker of **1a** into the racemic cyclopentyl and cyclohexyl ring containing analogs (**4a/b**; Table 1) affords an eightfold increase in thrombin inhibition. Accompanied by the data for analogs **2** and **3a/b**, it appears that enhanced thrombin inhibition can be achieved through conformational restriction in the C-4" side chain.

Table 1. The Effects of Conformational Restriction in the C-4" Side Chain on Biological Activity.



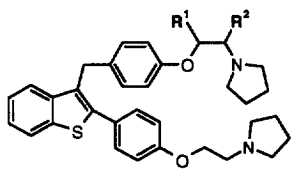
Compd	R	K_{ass}[a] (x 10^6 L/mol)
1a		3.43 ± 0.55
1b		6.70 ± 0.76
2		4.84 ± 0.13
3a (*cis*)		9.80 ± 1.06
3b (*trans*)		13.23 ± 0.41
4a		27.40 ± 2.45
4b		23.97 ± 1.37

[a]Represents the apparent association constant as measured by the methods of Smith et al.[12] K_{ass} values are the mean of n = 3, showing the standard deviation.

In addition to conformational restriction, the 5- and 6-membered rings in **4a/b** also impart increased lipophilicity to the inhibitor, and the S_2 and S_3 binding sites of thrombin are known to be hydrophobic in nature (see Figure 1). The racemic, branched acyclic analogs **5a/b**, which mimic the lipophilicity of analogs **4a/b** by

incorporating ethyl groups in the same proximity as the carbocyclic rings but retain conformational mobility, were also prepared and evaluated (Table 2). Indeed, ethyl substitution *alpha* to the ether (**5a**) or amine (**5b**) functionality affects a four- or eightfold increase in potency, respectively. Examination of the X-ray crystal structure of **1a** with thrombin (Figure 1) suggests that this boost in activity may derive from new hydrophobic interactions between the ethyl substituents and thrombin residues Leu99 (for **5a**) and Trp215 (for **5b**). The more active analog **5b** is equipotent to the cycloalkyl derivatives **4a/b** suggesting that increased lipophilicity in this region of the inhibitor may have a greater impact on binding than conformational restraint. Relevant to this finding, racemate **6a** with the smaller methyl group partially maintains the hydrophobic interactions of the ethyl substituents. While potency is lower in relation to **5**, the methyl substituted analog is still twofold more active than the unsubstituted **1a**. Additionally, a stereochemical effect is seen with the (*S*)-enantiomer (**6c**) being twice as potent as the (*R*)-enantiomer (**6b**). The gem dimethyl derivative **7** removes the stereocenter resulting from substitution on the C-4" ethoxy linker; however, its activity is only comparable to that of **6b**, the (*R*)-methyl compound.

Table 2. Effects of Lipophilic Branching on the C-4" Ethoxy Linker on Biological Activity.



Compd	R^1	R^2	K_{ass}[a] (x 10^6 L/mol)
1a	H	H	3.43 ± 0.55
5a	Et	H	12.3 ± 2.0
5b	H	Et	28.1 ± 5.7
6a	H	Me	7.34 ± 2.13
6b	H	Me (*R*)	4.17 ± 0.32
6c	H	Me (*S*)	9.07 ± 1.70
7	H	gem diMe	4.69 ± 0.36

[a]Represents the apparent association constant as measured by the methods of Smith et al.[12] K_{ass} values are the mean of n = 3, showing the standard deviation.

This study shows that greater thrombin inhibition can be attained by conformationally restricting the freely flexible C-4" side chains of parent benzo[*b*]thiophenes **1a/b**. Moreover, it appears that lipophilic interactions in this region of the inhibitor may play an important role in increasing the thrombin inhibitory activity as well. Future communications will describe the utility of these results in designing more potent and efficacious thrombin inhibitors within this series of diamino benzo[*b*]thiophene derivatives.

References and Notes

1. Preliminary accounts of this work have been presented: Sall, D. J.; Bastian, J. A.; Denney, M. L.; Gifford-Moore, D. S.; Harper, R. W.; Kohn, T. J.; Lin, H.-S.; Rampersaud, A. A.; Smith, G. F.; Takeuchi, K.; Zhang, M. *Abstracts of Papers*, 214th National American Chemical Society Meeting: Las Vegas, NV, Sept. 8-11th, 1997; MEDI-058.
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5. (a) A detailed account of the X-ray crystallography studies performed on this series of thrombin inhibitors will be provided in a future publication. (b) The numbering scheme for the thrombin residues is based on topological equivalence to chymotrypsin.
6. Sall, D. J.; Briggs, S. L.; Chirgadze, N. Y.; Clawson, D. K.; Gifford-Moore, D. S.; Klimkowski, V. J.; McCowan, J. R..; Smith, G. F.; Wikel, J. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2527.
7. The structure of all intermediates and final products were verified by ^{1}H NMR, MS, and IR. The experimentally determined elemental percentages of C, H, and N were within 0.4% of theoretical values. Yields were not optimized.
8. For a review of Suzuki coupling reactions: Suzuki, A. *Pure Appl. Chem.* **1985**, *57*, 1749.
9. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.
10. By ^{1}H NMR integration of olefinic protons, both *cis* (J = 11.8 Hz) and *trans* (J = 15.9 Hz) **3** were greater than 90% isomerically pure.
11. The pyrrolidinyl alcohols used in the fluoride displacement reactions were prepared according to the following general methods: (a) (±)-*trans*-(1-Pyrrolidinyl)cyclopentan-2-ol (for **4a**) and (±)-*trans*-(1-pyrrolidinyl)-cyclohexan-2-ol (for **4b**) were prepared by treating the appropriate cycloalkene oxide with pyrrolidine in K_2CO_3/H_2O. (b) (±)-1-(1-Pyrrolidinyl)butan-2-ol (for **5a**) was prepared by treating 1-bromo-2-butanone with pyrrolidine in K_2CO_3/DMF, followed by reduction to the alcohol with $LiAlH_4$. (±)-2-(1-Pyrrolidinyl) butanol (for **5a**) was prepared similarly from ethyl 2-bromobutyrate. (c) The methyl substituted pyrrolidinyl alcohols (for **6a–c**, **7**) were prepared by treatment of the appropriate methyl substituted 1° amino alcohol ((±)-2-amino-1-propanol for **6a**; (*R*)-(+)-2-amino-1-propanol for **6b**; (*S*)-(-)-2-amino-1-propanol for **6c**; and 2-amino-2-methyl-1-propanol for **7**) with 1,4-dibromobutane in K_2CO_3/THF.
12. Inhibitor binding affinities for human α-thrombin were measured as apparent association constants (K_{ass}) which were derived from inhibition kinetics as previously described: Smith, G. F.; Gifford-Moore, D. S.; Craft, T. J.; Chirgadze, N. Y.; 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.