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DIBASIC BENZO[*B*]THIOPHENE DERIVATIVES AS A NOVEL CLASS OF ACTIVE SITE DIRECTED THROMBIN INHIBITORS: 4. SAR STUDIES ON THE CONFORMATIONALLY RESTRICTED C3-SIDE CHAIN OF HYDROXYBENZO[*B*]THIOPHENES

Kumiko Takeuchi,* Todd J. Kohn, Daniel J. Sall, Michael L. Denney, Jefferson R. McCowan, Gerry F. Smith, and Donetta S. Gifford-Moore

Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, U.S.A.

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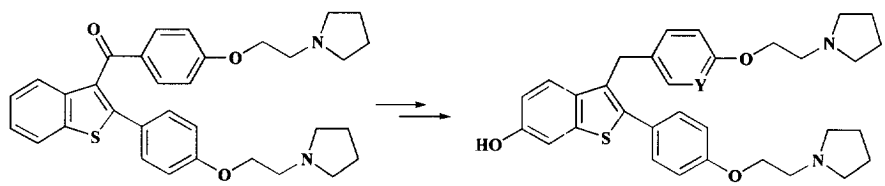
Abstract: A novel series of benzo[*b*]thiophene diamine thrombin inhibitors with a conformationally restricted C3-side chain **3** was investigated. The constrained C3-side chain by a cyclohexyl ring contributed to not only an additive but also a synergistic effect on the thrombin inhibitory activity. The SAR studies resulted in the discovery of a potent thrombin inhibitor **27** that was over 750-fold more potent than the initial lead compound **1**.

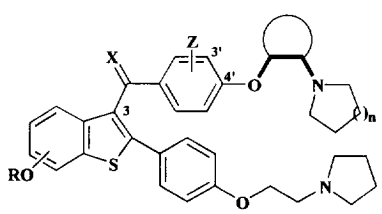
Thrombin, a trypsin-like serine protease, catalyzes fibrin formation and activates platelets, thereby playing a pivotal role in the development of thrombotic diseases.[1,2] Previously we reported a novel class of small organic molecules (e.g., **1**) as active site directed and specific thrombin inhibitors.[3] Our initial structure–activity relationship (SAR) study led to the identification of C3-methylene compounds **2a** and **2b** that were 50-fold more potent than the initial lead compound **1**.



1 (K_{ass} = 0.41 x 10^6 L/mol)

2a (Y = C, K_{ass} = 20.2 x 10^6 L/mol)
b (Y = N, K_{ass} = 24.3 x 10^6 L/mol)

SAR studies of benzo[*b*]thiophene diaminess with conformationally restricted C3-side chain **3** were carried out in this study to improve the thrombin inhibitory activity of this series of compounds. More specifically, the ethylene moiety of the C4'-side chain was constrained as part of a cyclohexyl ring. We also studied the effect and the optimal position of **OR** substituents on the benzo[*b*]thiophene ring and substituent **Z** on the C3-phenyl ring.



3

Compound **4a**, a constrained C3-side chain derivative of **1** by a cyclohexyl ring, showed only a marginal increase in K_{ass}. Ring expansion of the attached pyrrolidine ring to piperidine (**4b**), however, increased the thrombin inhibitory activity threefold. Further ring expansion to the 7-membered ring (**4c**) was not beneficial to the inhibitory activity. The methylene compound **4d** of the piperidine derivative **4b** showed substantial improvement in activity and was equipotent to **2a**, even though **4d** lacked the known beneficial hydroxy

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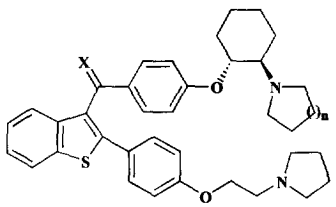
substituent on the benzo[*b*]thiophene ring present in **2a**. We have already observed that SAR modification of various functionalities in the molecule has an additive effect.[3]



4a-d

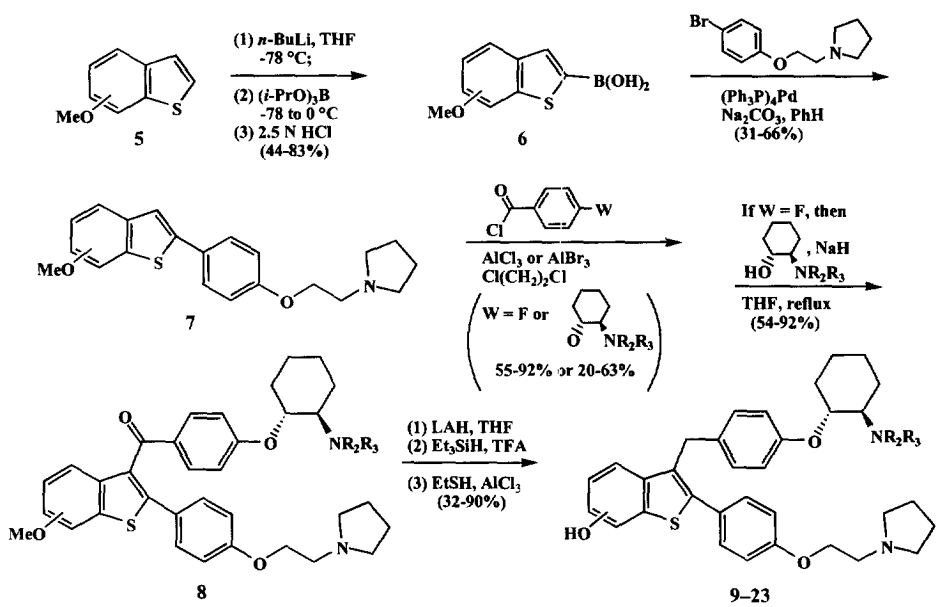
Entry	X	n	K_{ass}[a] (x 10^6 L/mol)
a	O	1	0.51
b	O	2	1.17
c	O	3	0.49
d	H,H	2	20.4

[a]Represents the apparent association constant as measured by the methods of Smith et al.[3] K_{ass} value is the average of two or more determinations, where the variation in the assay is ±10%.

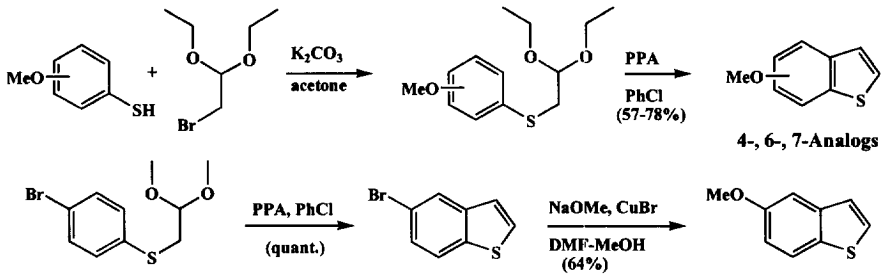
The above results appear to indicate that the conformational changes in the molecule could contribute to not only an additive but potentially a synergistic effect on the thrombin inhibitory activity. We report here the results of SAR studies on the hydroxybenzo[*b*]thiophene compounds with a constrained C3-side chain. The optimal position of the hydroxy group on the benzo[*b*]thiophene ring was also determined in this study (Table 1). We also report the effect of various amino termini on the C3-side chain which was constrained by a cyclohexyl ring (Table 2).

The syntheses of the compounds **9–23** are depicted in Scheme 1. Starting materials, methoxybenzo[*b*]-thiophenes, were prepared as shown in Scheme 2. 4- and 6-Methoxybenzo[*b*]thiophenes were obtained from 3-methoxythiophenol in two steps in a ratio of 1:3.3, which were chromatographically separable with straight hexanes as an eluent. Likewise 7-methoxybenzo[*b*]thiophene was prepared from 2-methoxythiophenol.[4] 5-Methoxybenzo[*b*]thiophene was prepared in two steps: 5-bromobenzo[*b*]thiophene formation by the same route as above, followed by displacement of the bromide with methoxide.[5] The boronic acids **6** were prepared from the methoxybenzo[*b*]thiophenes **5** in three steps by a standard protocol as shown. Suzuki coupling of **6** with 1-

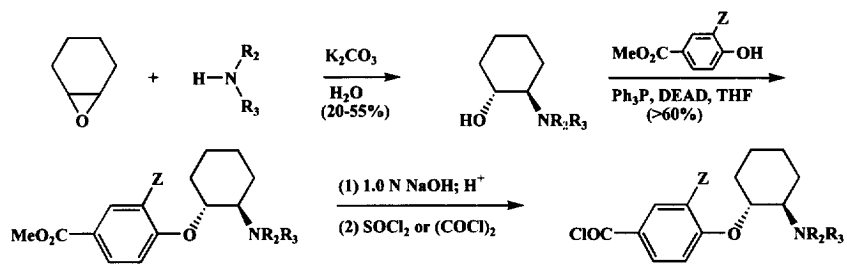
Scheme 1. General Synthetic Route to Analogs 9–23



Scheme 2. Formation of 4-, 5-, 6-, 7-Methoxybenzothiophenes

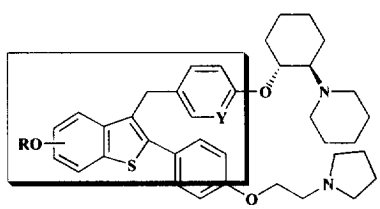


Scheme 3. Synthesis of 4-Alkoxybenzoyl Chlorides



[2-(4-bromophenoxy)ethyl]pyrrolidine afforded **7**. Friedel–Crafts acylation with 4-fluorobenzoyl chloride (followed by alkoxylation) or 4-alkoxybenzoyl chloride (Scheme 3) yielded **8**.[6] Deoxygenation by LAH reduction, followed by treatment of the resultant alcohol with Et_3SiH in TFA, provided compounds **9–11** and demethylation with $AlCl_3$ in EtSH afforded the target molecules **12–23**.

Table 1. Optimization of the Hydroxy Position on the Benzo[*b*]thiophene Ring



Compd	OR	Y	K_{ass} (x 10^6 L/mol)[a]
9	5-OMe	C	36.5
10	6-OMe	C	3.83
11	7-OMe	C	0.118
12	4-OH	C	1.73
13	5-OH	C	13.9
14	6-OH	C	212.4
15	7-OH	C	1.08
16	6-OH	N	96.1

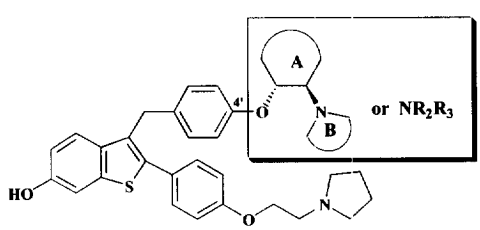
[a]Represents the apparent association constant as measured by the methods of Smith et al.[3] K_{ass} value is the average of two or more determinations, where the variation in the assay is ±10%.

SAR studies of these hydroxybenzo[*b*]thiophene derivatives indicated that 6-hydroxy substitution (entry **14**, Table 1) was optimal and provided potent thrombin inhibition. This could be attributed to the observation that the benzo[*b*]thiophene ring binds in the specificity pocket S_1 of the enzyme according to an X-ray analysis of the co-crystal of thrombin and the compound **2b**,[3] and the hydroxy group at the 6-position could most favorably interact with the carboxyl group of Asp189 via hydrogen bonding. The methoxy compounds also exhibited thrombin inhibition, but were not as potent as the corresponding hydroxybenzo[*b*]thiophenes, since the methoxy group could not provide the favorable hydrogen bonding interaction. The exception of the 5-methoxy derivative (**9** vs **13**) also could be rationalized by the X-ray analysis. The C5-position of the benzo[*b*]thiophene ring is located adjacent to a hydrophobic region of the enzyme and thus the C5-methoxy could take advantage of this proximity. This van der Waal's interaction, however, is not as strong as the 6-OH···Asp189 hydrogen-bond as shown by the inhibitory potency. In contrast to the unconstrained C4'-methylene chain compounds (**2a** vs **2b**), substitution of the C3-phenyl with an electron deficient pyridyl ring was detrimental to the biological activity (**14** vs **16**).

The basicity of the amino termini on the C4'-side chain influenced the thrombin inhibitory activity (Table 2). Less basic morpholine (**22**) and imidazole (**23**) than tertiary amines were detrimental to the thrombin inhibitory activity. Rotationally constrained tertiary amines were more beneficial for the activity than the unconstrained amines (e.g., **17** vs **20**). The sterically most constrained, but not necessarily the bulkiest, pyrrolidine derivative **17** exhibited the most potent activity (vs **18**). C3-Side chain constraint by a smaller cyclopentyl ring was detrimental to the activity (**19** vs **14**). Compound **17** was over 400-fold more potent than **4a** in comparison to 50-fold increase in the thrombin inhibitory activity from **1** to **2a**. Thus, conformational restriction of the C3-side chain appears to contribute to not only an additive but also a synergistic effect on the thrombin inhibitory activity of these benzo[*b*]thiophene derivatives.

X-ray crystallographic studies of **2b**[3] also indicated that the C3-pyridyl ring binds near the opening of the hydrophobic S_2 pocket. Increasing lipophilicity in this region could, therefore, increase interaction at this site and thereby increase the thrombin inhibitory activity. C3-Phenyl substituent effect on the activity of the compound **17**, therefore, was investigated. C3'-Substituted phenyl derivatives **27–35** were prepared as depicted in Scheme 4. 2-Dimethylamino-6-benzyloxybenzo[*b*]thiophene **24** was prepared in two steps:[7] (1) treatment of

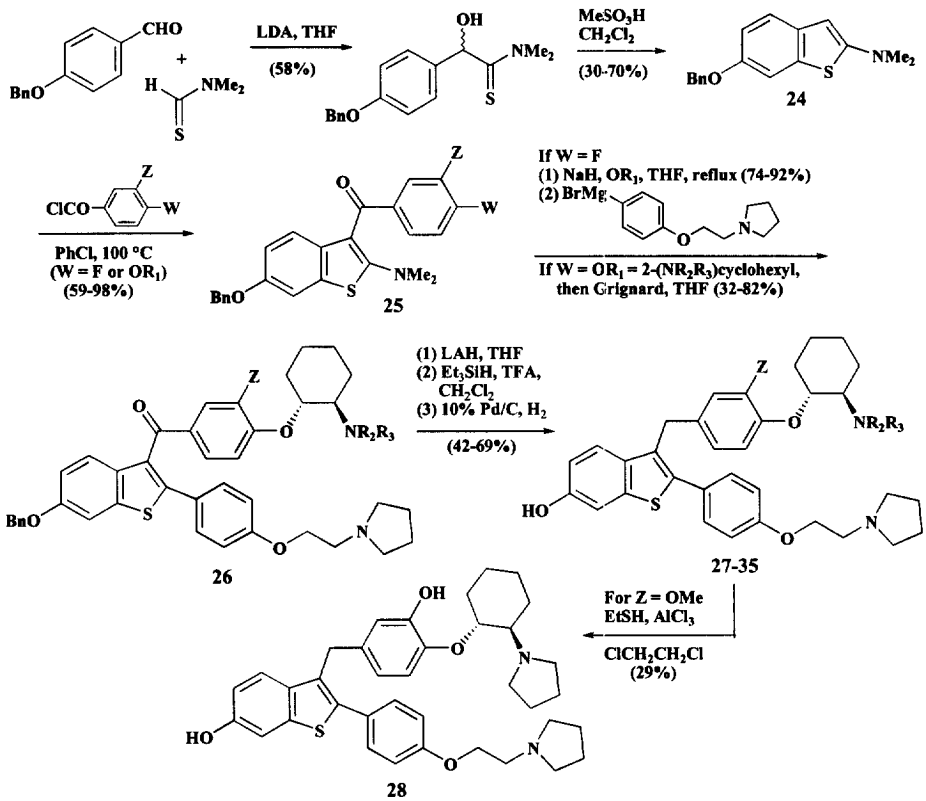
Table 2. Effects of Ring Sizes and Basicity of the C4'-Amino Termini



Compd	A/B	K_{ass} (x 10^6 L/mol)[a]
17	6/5	218.5
14	6/6	212.4
18	6/7	74.8
19	5/6	47.3
20	6/NEt_2	49.8
21	6/NMe_2	51.1
22	6/morpholine	18.1
23	6/imidazole	8.08

[a]Represents the apparent association constant as measured by the methods of Smith et al.[3] K_{ass} value is the average of two or more determinations, where the variation in the assay is ±10%.

Scheme 4. General Synthetic Route to Compounds 27-35



a mixture of 4-benzyloxybenzaldehyde and *N,N*-dimethylthioformamide with LDA in THF at -78 °C and (2) cyclization-aromatization with $MeSO_3H$. Friedel–Crafts acylation of the enaminobenzo[*b*]thiophene **24** with a substituted benzoyl chloride was effected simply by heating the mixture in chlorobenzene without Lewis acid catalysis. Grignard reaction of 3-acyl-2-dimethylaminobenzo[*b*]thiophene **25** proceeded via a Michael addition-elimination sequence.[8] Deoxygenation as above followed by debenzylation of **26** afforded the final compounds **27–35**. Demethylation of the 3-methoxy derivative **27** by a standard method yielded the 3-hydroxy derivative **28**.

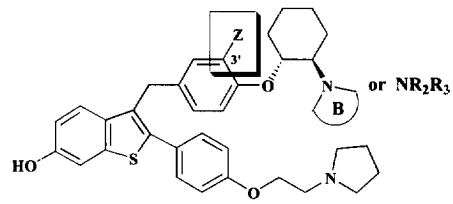
A hydrophobic substituent at the 3'-position of the phenyl ring of the C3-side chain improved the thrombin inhibitory activity possibly due to its favorable interaction with the hydrophobic S_2 subsite of thrombin (Table 3). In particular, the C3'-methoxy derivative **27** was a potent thrombin inhibitor in vitro. This compound also exhibited in vivo clot size reduction in a rat AV shunt model.[9] Like the initial lead **1** and the methylene compounds **2a** and **2b** previously reported,[3] the benzo[*b*]thiophene derivatives with a constrained C3-side chain in the present study also were selective thrombin inhibitors relative to other serine proteases, including trypsin, other coagulation factors such as factor X_a, and the fibrinolytic enzymes. For example, these benzo[*b*]thiophene derivatives were well over three orders of magnitude more selective for thrombin compared to the related serine protease, factor X_a (the compound **27** exhibited K_{ass} for thrombin = 309.3 x 10^6 L/mol in contrast to K_{ass} for X_a= 0.027 x 10^6 L/mol; the ratio of thrombin/X_a= 11,000).

In conclusion, benzo[*b*]thiophene diamine derivatives in which the ethylene chain of the C4'-side chain was constrained by a cyclohexyl ring are a novel class of selective and active site directed thrombin inhibitors. The study of substituent effects on the benzo[*b*]thiophene ring revealed that a hydroxy group at the 6-position

was optimal. This can be attributed to the fact that the benzo[*b*]thiophene ring binds in the specificity pocket S_1 of thrombin, and the hydroxy group favorably interacts with the carboxyl group of Asp189 via hydrogen bonding. We have also found that a hydrophobic substituent at the C3'-position of C3-phenyl ring favorably interacts with the hydrophobic S_2 site of thrombin. Conformational restriction of the C3-side chain uniquely contributes to a synergistic effect on the thrombin inhibitory activity of these benzo[*b*]thiophene diamines. Present SAR studies resulted in the discovery of a potent thrombin inhibitor **27** that was over 750-fold more potent than the initial lead compound **1**.

Acknowledgement: We thank Drs. Ken Kurz and David Snyder for the experiments with a rat AV-shunt model to measure in vivo clot size reduction.

Table 3. C3'-Substituent Effects



Compd	Z	B	K_{ass} (x10^6 L/mol)[a]
17	H	5	218.5
27	OMe	5	309.3
28	OH	5	11.2
29	Me	5	349.0
30	Br	5	279.7
31	F	5	121.0
32	CF_3	5	68.4
14	H	6	212.4
33	OMe	6	116.0
34	F	6	203.0
35	OMe	NMe_2	146.0

[a]Represents the apparent association constant as measured by the methods of Smith et al.[3] K_{ass} value is the average of two or more determinations, where the variation in the assay is ±10%.

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6. Friedel–Crafts acylation of 4- and 7-methoxybenzo[*b*]thiophene derivatives took place at the *para*-position to the methoxy substituent, yielding 7- and 4-acylated products, respectively. Regioselective Friedel–Crafts acylation at the C3 position of the benzo[*b*]thiophenes was effected when the corresponding hydroxy derivatives of **7** were employed. This interesting regioselectivity in the Friedel–Crafts acylation of alkoxybenzo[*b*]thiophene derivatives will be reported elsewhere.
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9. Experiments with the A-V shunt model were performed with slight modifications of the methods of Smith, J. R.; White, A. M. *Br. J. Pharmacol.* **1982**, *77*, 29. Drug or vehicle was infused for 15 min prior to opening the shunt and was continued for an additional 15 min after the shunt was opened. Net clot weights from drug-treated animals were expressed as a percent of the vehicle-treated animals run in the same experiment. Compound **27** significantly reduced the clot size at concentration of 66 μmole/kg/hr iv.