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1,2-Disubstituted Indole, Azaindole and Benzimidazole Derivatives Possessing Amine Moiety: A Novel Series of Thrombin Inhibitors

Kumiko Takeuchi,* Jolie A. Bastian, Donetta S. Gifford-Moore, Richard W. Harper, Shawn C. Miller, Jeffrey T. Mullaney, Daniel J. Sall, Gerald F. Smith, Minsheng Zhang† and Matthew J. Fisher

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

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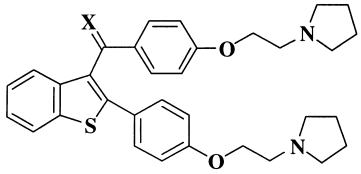
Abstract—A novel series of 1,2-disubstituted indole, azaindole and benzimidazole derivatives possessing an amine moiety was identified as thrombin inhibitors. An indole with basic diamine moieties (**12a**) was the most potent thrombin inhibitor in the series with $K_{ass} = 197 \times 10^6$ L/mol. © 2000 Elsevier Science Ltd. All rights reserved.

Thrombin, a trypsin-like serine protease, catalyzes fibrin formation and activates platelets, thereby playing a pivotal role in the development of thrombotic diseases.[1] Recently we have reported the discovery of specific, active site directed thrombin inhibitors that are based on the 2,3-disubstituted benzo[*b*]thiophenes (e.g., **1a**,**b**).[2–7] Within that effort, we observed by an X-ray analysis of a crystal of the ternary complex between α-thrombin, hirugen, and a compound from this series that the benzo[*b*]thiophene nucleus binds into the hydrophobic S_1 specificity pocket of thrombin.[2,3] Since this binding interaction is thought to be critical for good thrombin inhibitory potency, we elected to examine this relationship in detail. During the initial evaluation of this series, the role of the heterocyclic core was investigated by replacing the benzo[*b*]thiophene nucleus with other heteroaromatic rings such as benzofuran and indole. The initial brief structure–activity relationship (SAR) of the 2,3-disubstituted heteroaromatic compounds seemed to indicate that benzo[*b*]thiophene was the preferred heteroaromatic ring (**1b**–**3**, Table 1).[2] However, we found that the differential positioning of the two side chains profoundly influenced the thrombin inhibitory activity of the nitrogen-containing heteroaromatic compounds. In this report we describe the SAR studies of novel 1,2-disubstituted indole, azaindole and benzimidazole compounds that exhibited unexpected thrombin inhibitory activity.



1a (X = O: K_{ass} = 0.41 x 10^6 L/mol)
b (X = H,H: K_{ass} = 3.43 x 10^6 L/mol)

Chemistry

Synthesis of the compounds **1a**,**b** has previously been reported[2,6] and the compounds **2** and **3** were prepared in a similar manner. In those previous SAR studies for the benzo[*b*]thiophene-derived thrombin inhibitors[2–7] extensive modification of the 2,3-disubstituted heteroaromatic moiety proved to be a labor intensive undertaking due to the two carbon–carbon bond formations. A 1,2-disubstituted indole (or similar nitrogen heterocycles) would allow us to avoid one of the carbon–carbon bond forming reactions for the attachment of the two required side chains. Such substitution pattern would then facilitate rapid SAR development towards the desired thrombin inhibitory potency. Schemes 1–3 depict general syntheses of benzimidazole, azaindole, and indole compounds. Generally, 2-substituted heteroaromatic ring formation, N1-alkylation with 4-carbomethoxybenzyl bromide, followed by modification of the carbomethoxy group to appropriate amine moiety furnished target molecules. In the case of benzimidazoles, N-alkylation preceded the ring formation.

*Corresponding author. Tel.: +1-317-276-6771; fax: +1-317-433-0715; e-mail: ktak@lilly.com
†Current address: Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

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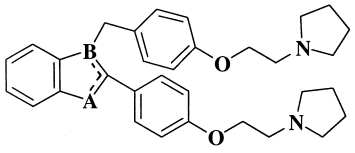
Results and Discussion

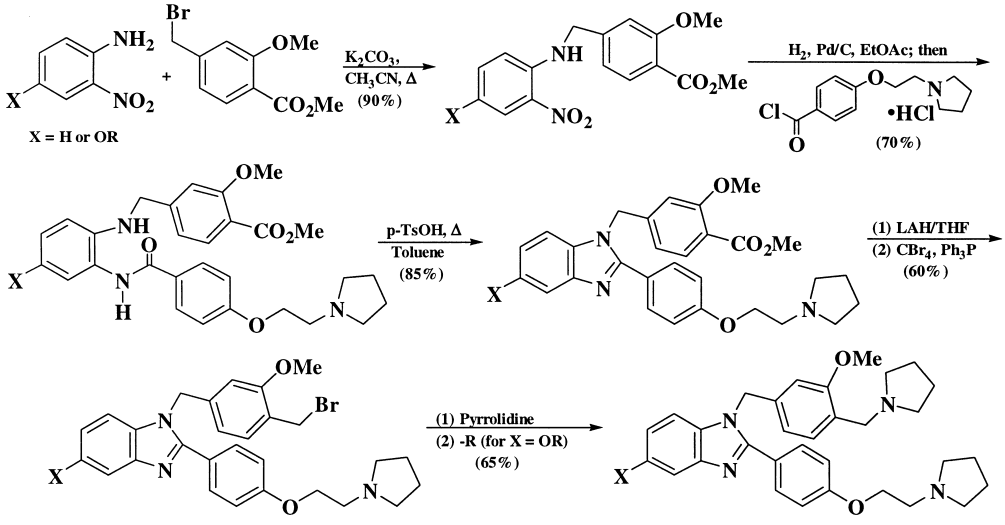
There was a stark contrast in the effects of the disubstitution pattern of the heterocycles on the thrombin inhibitory activity as shown in Table 1. 1,2-Disubstituted indole **4** was 150 times more potent than the 2,3-disubstituted indole **3**. It was also twofold more potent than the 2,3-disubstituted benzo[*b*]thiophene **1b**. 1,2-Disubstituted benzimidazole **5** showed eightfold better thrombin inhibitory activity than the 2,3-disubstituted indole **3**. In contrast, it was 10-fold weaker than the 2,3-disubstituted benzo[*b*]thiophene **1b**. Among the 2,3-disubstituted heterocycles the benzo[*b*]thiophene **1b** was clearly superior to the benzofuran **2** and the indole **3** (**1b** > **2** >> **3**). These results seemed to indicate that the nitrogen-containing heteroaromatic core with the 1,2-disubstition pattern might have a salient feature that would be very different from the 2,3-disubstituted benzo[*b*]thiophene ring. 1,2-Disubstituted indoles and other heteroaromatic compounds could be viable candidates for developing structurally unique, potent thrombin inhibitors. Further SAR studies of 1,2-disubstituted indoles, benzimidazoles, and azaindoles were therefore warranted.

Table 1. SAR on the heteroaromatic core

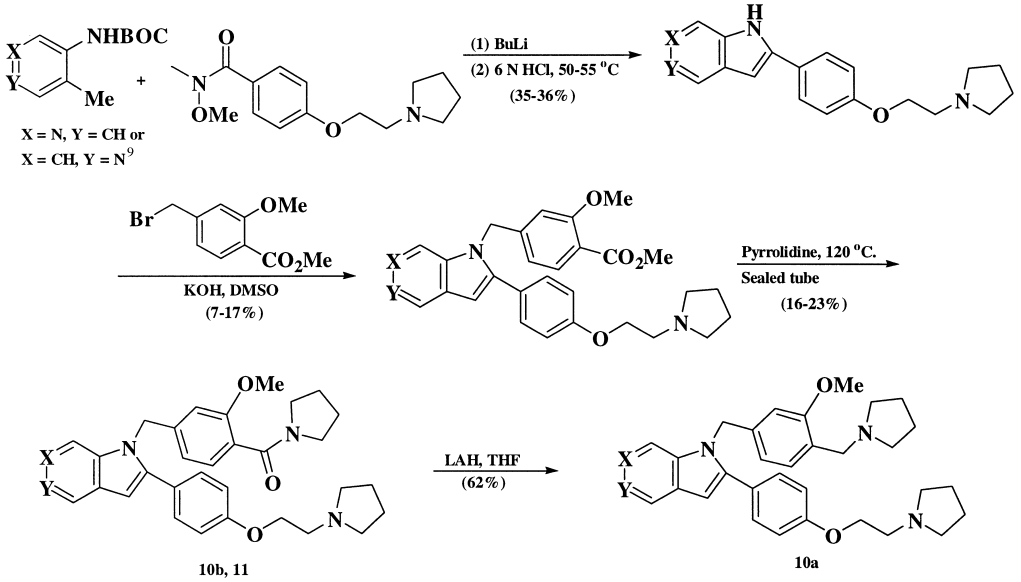


Compound	A	B	K_{ass} ($\times 10^6$ L/mol)[8]
1b	S	C	3.43
2	O	C	0.974
3	NH	C	0.042
4	C	N	6.27
5	N	N	0.332

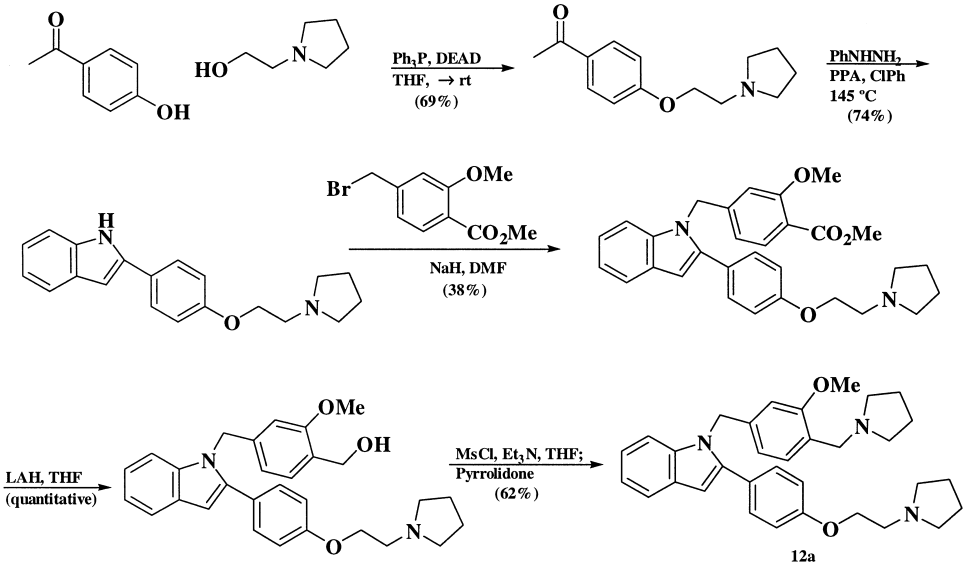
The results of SAR studies on the benzimidazole series are shown in Tables 2–4. Table 2 shows the substituent



Scheme 1. Synthesis of benzimidazole derivatives.



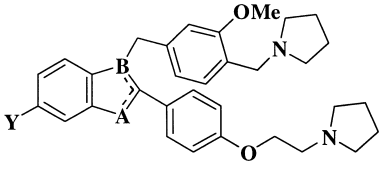
Scheme 2. Synthesis of azaindole derivatives.[9]



Scheme 3. Synthesis of indole derivatives.

effects on the thrombin inhibitory activity of the benzimidazoles. Our extensive SAR studies of benzo[*b*]thiophene derived thrombin inhibitors revealed that the C3′-methoxy substituent[4] on the C3-phenyl ring and the contracted C4′-side chain by a methylene linker[5–7] were extremely beneficial to the thrombin inhibitory activity. In the case of 1,2-disubstituted indole analogues, the N1 side chain could be considered equivalent to the C3-side chain of the benzo[*b*]thiophene in its molecular orientation, and therefore the same substituent pattern at C3′ and C4′ positions of the C3-phenyl ring was applied to the N1 side chain of benzimidazole **5**. This modification proved to be beneficial and improved the thrombin inhibitory activity of benzimidazole 36-fold (from **5** to **6a**). 6-Hydroxy substituent (**7a**) did not have any beneficial effect on the activity in contrast to the benzo[*b*]thiophene series[4] which showed about eightfold increase from K_{ass} = 126 to 815 $\times$ 10^6 L/mol. Longer chain substituents such as methyleneamine (**8**) and methylenehydroxy group (**9**) were detrimental to the inhibitory activity as were seen with benzo[*b*]thiophene series.[2]

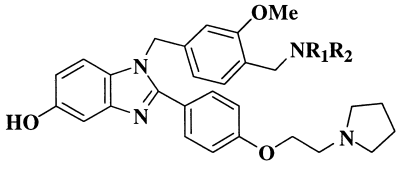
Table 2. Substituent effects on the thrombin inhibitory activity of benzimidazoles versus benzo[*b*]thiophenes



Compound	Y	K_{ass} ($\times 10^6$ L/mol)[8] 1,2-Disubstituted benzimidazole (A, B = N)	K_{ass} ($\times 10^6$ L/mol)[8] 2,3-Disubstituted benzo[*b*]thiophene (A = S; B = C)
6a	H	11.9	126
7a	OH	9.72	815
8	CH_2NH_2	4.49	—
9	CH_2OH	1.93	—

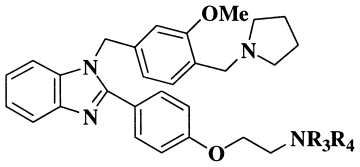
Table 3 shows the effects of the C4′-amine substituents. The basicity of the cyclic amine was not critical to the inhibitory activity (**7a**–**d**) in contrast to the benzo[*b*]thiophene derivatives where a basic amine side chain was required.[2,4] The benzylamine **7e** showed moderate improvement on the activity. These results indicate that benzimidazole compounds behave differently than the benzo[*b*]thiophene series. We also examined the effect of C2-side chain modification (Table 4). As in the case of benzo[*b*]thiophene,[2] the C2-side chain preferred a basic

Table 3. Modification of C4′-amine



Compound	NR_1R_2	K_{ass} ($\times 10^6$ L/mol)[8]
7a	Pyrrolidin-1-yl	9.72
7b	Imidazol-1-yl	9.60
7c	Triazol-1-yl	10.2
7d	Pyrazol-1-yl	12.0
7e	Benzylamino	16.5

Table 4. Modification of C2-side chain

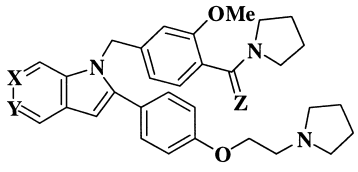


Compound	NR_3R_4	K_{ass} ($\times 10^6$ L/mol)[8]
6a	Pyrrolidin-1-yl	11.9
6b	Pyrrolid-2-on-1-yl	1.50
6c	Pyrazol-1-yl	1.46

amine group for potent thrombin inhibitory activity. Less basic pyrrolidone **6b** and pyrrazole **6c** were eightfold less active than the pyrrolidine **6a**.

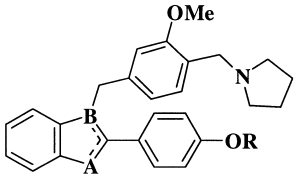
Table 5 shows the thrombin inhibitory activity of 5- or 6-azaindole derivatives. Interestingly, the more rigid and less basic amide moiety of the N1 side chain (**10a**) showed modestly better activity than the methylene amine linkage (**10b**) in the azaindole series. The azaindole **10b** exhibited respectable activity with over sixfold increase from **6a** (benzimidazole), but about twofold less potency than **6a** (benzo[*b*]thiophene) and threefold less than **12a** (indole, Table 6). 5-Azaindole **10a** showed better activity than 6-azaindole **11**. This was possibly due to the better positioning of the ring nitrogen at the 5- rather than the 6-position to interact with the carboxy terminus of Asp189 of thrombin, assuming the orientation of the molecule being the same as that of the 2,3-disubstituted benzo[*b*]thiophene in the thrombin specificity pocket. These results suggest that the positioning of the ring nitrogen influences the thrombin inhibitory activity of disubstituted nitrogen-containing heteroaromatic compounds much more significantly than the basicity of an amine moiety at the C4′-phenyl ring.

Table 5. Azaindole derivatives



Compound	X	Y	Z	K_{ass} ($\times 10^6$ L/mol)[8]
10a	C	N	O	83.7
10b	C	N	H,H	73.9
11	N	C	O	59.7

Table 6. 1,2-Disubstituted indoles versus 2,3-disubstituted benzo[*b*]thiophene



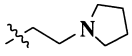
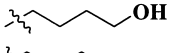
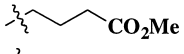
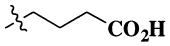
Compd.	X	R	K_{ass} ($\times 10^6$ L/mol)[8] 1,2-Disubstituted Indole (A = C; B = N)	K_{ass} ($\times 10^6$ L/mol)[8] 2,3-Disubstituted Benzo[*b*]thiophene (A = S; B = C)
12a	H		197	126
12b	H		33.7	14.9
12c	H		24.7	7.68
12d	H		6.41	3.91

Table 6 shows the SAR results of indole derivatives. In contrast to the benzimidazole series, 1,2-disubstituted indole **12a** showed potent thrombin inhibitory activity, even better than the benzo[*b*]thiophene series (**6a** benzo[*b*]thiophene). Within the indole series, the compound **12a** containing optimal side chains by the 1,2-substitution pattern was over 30-fold more potent than **4** and almost 4700 times more potent than **3**. As in the case of benzimidazole (Table 4), a basic amine moiety on the C2-side chain was preferred to maintain potent thrombin inhibitory activity for both the indole and benzo[*b*]thiophene series. The inhibitory activity decreased from six- to 30-fold with the indole series and from 15- to 32-fold with the benzo[*b*]thiophene series as the basicity of the C2-side chain was reduced (**12b–d**). Among these compounds, the indole series exhibited better inhibition overall than the benzo[*b*]thiophene series. In contrast, the benzimidazole series lost activity considerably, 10- to 80-fold when compared with benzo[*b*]thiophene (Table 2) and 17-fold when compared with indole (**6a** versus **12a**).

Results in Tables 1–3, 5, and 6 suggest that the nitrogen-containing heteroaromatic derivatives might be interacting with thrombin very differently from the benzo[*b*]thiophene series of thrombin inhibitors. In general, a hydrophobic ring was favored in the narrow hydrophobic cavity of the S_1 specific pocket of thrombin, at the end of which was exposed the carboxy group of Asp189.[10] Among the nitrogen-containing heteroaromatic compounds studied, the most hydrophobic indole ring with one nitrogen was more favored than azaindole or benzimidazole with two nitrogens in the ring in this order. 5-Azaindole was more favored than 6-azaindole possibly due to the better positioning of the ring nitrogen at the 5- rather than the 6-position for its interaction with the Asp189 carboxy terminus. The more hydrophilic benzimidazole ring was less favored possibly due to the electrostatic repulsion by the N3 in the narrow and hydrophobic cavity of the S_1 pocket, which might have prevented the inhibitor from intercalating deep into the cavity. This might explain why the beneficial effect of the hydroxy group observed with the benzo[*b*]thiophene series[11] appeared to be nullified in the case of benzimidazole, where the hydroxy group could not reach deep enough to interact with the Asp189 carboxy terminus of thrombin. The preliminary results of indole and azaindole derivatives presented here suggest that these compounds may provide a novel class of thrombin inhibitors with unique features, different from the benzo[*b*]thiophene series. The indole analogues could offer advantages of synthetic ease and possess desirable thrombin inhibitory potency without a hydroxy substituent, which was required for the benzo[*b*]thiophene series.

In summary, we identified 1,2-disubstituted indole, azaindole and benzimidazole derivatives possessing basic amine moieties as novel series of active site directed thrombin inhibitors with unique features versus the benzo[*b*]thiophene derived thrombin inhibitors. In contrast to the benzo[*b*]thiophenes, the 2,3-disubstituted indoles did not show thrombin inhibitory activity. The preference of 1,2-disubstituted nitrogen-containing heteroaromatic

compounds for thrombin inhibitory activity was in the order: indole (**12a**) > azaindole (**10b**) > benzimidazole (**6a**). These results suggest that positioning of the second nitrogen in the heteroaromatic ring may profoundly influence and change biological activity of these novel thrombin inhibitors. Synthetic ease of these compounds would enable further SAR exploration.

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8. 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} values reported here are preliminary results with the variation in the assay in the range of ±10%.

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10. Specific shape requirements of S_1 pocket for accepting hydrophobic groups based upon X-ray crystallographic studies have been discussed in ref 2.

11. We had examined the substituent effects of the benzo[*b*]thiophene nucleus on the binding interaction and discovered that the 6-hydroxy substituent improved its binding affinity possibly via hydrogen-bond interaction with the carboxy terminus of Asp189 which was exposed at the crevice of the S_1 pocket.[2,4]