Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2527–2532

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# DIBASIC BENZO[*b*]THIOPHENE DERIVATIVES AS A NOVEL CLASS OF ACTIVE SITE DIRECTED THROMBIN INHIBITORS. 2. EXPLORING INTERACTIONS AT THE PROXIMAL ($S_2$) BINDING SITE.[1]

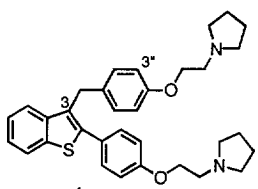
Daniel J. Sall, Stephen L. Briggs, Nickolay Y. Chirgadze, David K. Clawson, Donetta S. Gifford-Moore, Valentine J. Klimkowski, Jefferson R. McCowan, Gerald F. Smith, and James H. Wikel.

*Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*

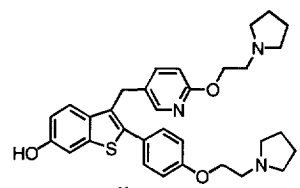
Received 19 June 1998; accepted 29 July 1998

**Abstract**: In an effort to increase the thrombin inhibitory activity of a novel series of inhibitors (i.e., **1a**), substituents were incorporated at the C-3" position of the C-3 aryl ring (**2**). Consistent with the X-ray crystallography studies, small hydrophobic groups at the C-3" site (Br and Me) enhanced thrombin inhibitory activity by 8-fold. However, a few more hydrophilic substituents ($NO_2$ and OMe) also enhanced the potency of the series. The biological results are discussed in terms of molecular modeling studies. 

The trypsin-like, serine protease thrombin catalyzes fibrin formation and activates platelets, thereby playing a pivotal role in the development of thrombotic complications.[2] Accordingly, the search for orally active thrombin inhibitors, for use in the treatment of chronic thrombotic disorders, has intensified.[3] Recently, we described a series of 2,3-disubstituted benzo[*b*]thiophene derivatives (i.e., **1a**) as a structurally novel class of active-site directed thrombin inhibitor.[4] Subsequent structure activity relationship (SAR) studies led to the identification of analog **1b,** which was 50-fold more potent than the parent (**1a**). X-ray crystallographic studies revealed that the hydrophobic benzo[*b*]thiophene nucleus binds in the specificity pocket ($S_1$) of human α-thrombin, while the C-3 side chain spans the proximal ($S_2$) and distal binding sites.[5]




**1a**




**1b**

In an attempt to enhance activity of the series, we sought to optimize the interaction of the C-3 side chain with the proximal and distal binding sites. X-ray crystallography studies indicated that the C-3 aryl ring binds near the opening of the hydrophobic $S_2$ pocket, which is defined by thrombin residues Trp86, Leu132, His79, and Tyr83. Accordingly, we incorporated substituents onto the C-3 aryl ring in order to probe enzyme/inhibitor interactions in this region of the active site. The C-3" position was selected as the site of substitution based on the disposition of the C-3 aryl ring relative to the $S_2$ binding site. Although the $S_2$ pocket is known to be hydrophobic in nature, we wanted to study a variety of substituents to determine if subtle, hydrophilic binding interactions were possible, in addition to the expected lipophilic interactions. In order to explore broad structural space with a minimal number of substituents, a molecular diversity analysis was conducted using three general QSAR parameters: calculated molecular volume (cMR), sigma-meta ($\sigma_m$), and pi ($\pi$).[6,7] Fifteen substituents spanning a range of these parameters (cMR, 0.92 to 37.9; $\sigma_m$, -0.24 to 0.43; and $\pi$, -1.23 to 2.13) were chosen. This paper will describe the synthesis and thrombin inhibitory activity of analogs **2a-o** (Table 1), and provide an explanation of the biological data using molecular modeling.

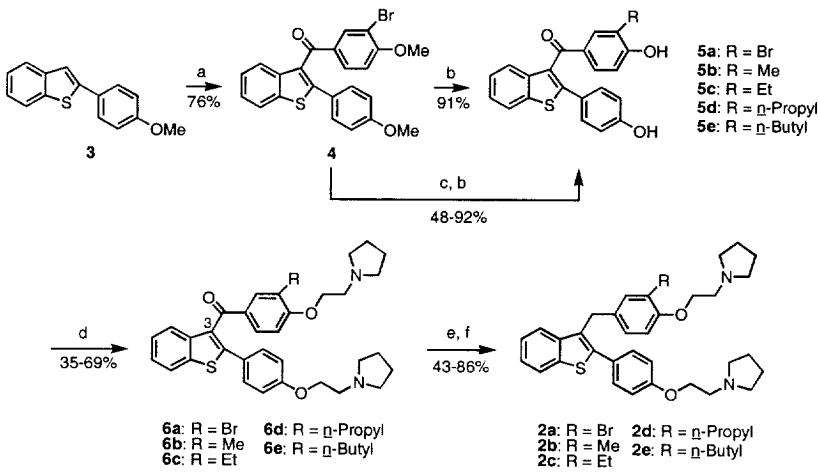
## Chemistry

The syntheses of the benzo[*b*]thiophene derivatives used in this study (**2a-o**; Table 1) were accomplished according to Schemes 1-4.[8] The bromo and alkyl substituted analogs (**2a-e**; Scheme 1) were derived from the

0960-894X/98/$ - see front matter 
*PII:* S0960-894X(98)00447-8

common intermediate **4**, which was prepared by acylating 2-(4-methoxyphenyl)benzo[*b*]thiophene (**3**)[9] with 3-bromo-4-methoxybenzoyl chloride. Bis demethylation using EtSH/$AlCl_3$ afforded the bromo diphenol **5a**. For the alkyl derivatives, bromide **4** could be subjected to Stille conditions (step c)[10] followed by deprotection to give the C-3" alkylated diphenols **5b-e**. Dialkylation of **5a-e** with 1-(2-chloroethyl)pyrrolidine gave derivatives **6a-e**. Deoxygenation of the C-3 ketone was accomplished using a two step procedure involving initial reduction to the secondary alcohol (step e; $LiAlH_4$) and subsequent deoxygenation (step f; $Et_3SiH$/TFA) to the desired methylene products **2a-e**.

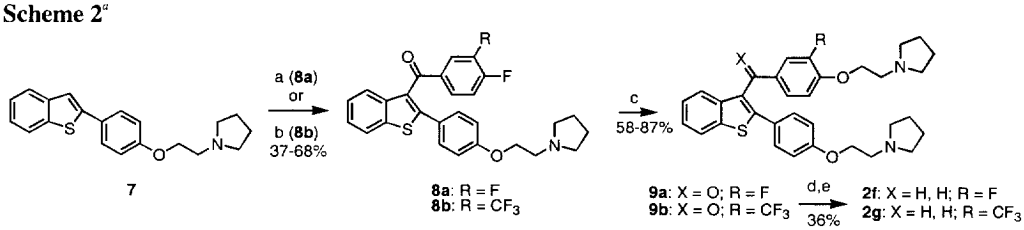
**Scheme 1**[a]




[a]Reagent: (a) 3-bromo-4-methoxybenzoyl chloride (1 eq), $TiCl_4$ (4 eq), 3 h, $ClCH_2CH_2Cl$; (b) EtSH (12 eq), $AlCl_3$ (10 eq), 16 h, $ClCH_2CH_2Cl$; (c) $R_4Sn$ (2.5 eq), $Pd(Ph_3P)_4$ (0.5 mole %), 130 °C, 16 h, toluene; (d) 1-(2-chloroethyl)pyrrolidine hydrochloride (4 eq), $Cs_2CO_3$ (6 eq), 80 °C, DMF; (e) $LiAlH_4$ (6 eq), 16h, THF; (f) $NaBH_4$ (2.2 eq), 3 h, TFA.

The preparation of the fluoro and trifluoromethyl substituted analogs **2f** and **2g** began with 2-[4-(2-(1-pyrrolidinyl)ethoxy)phenyl]benzo[*b*]thiophene (**7**; Scheme 2).[9] Acylation with the appropriate *m*-fluoro- or *m*-trifluoromethyl substituted *p*-fluorobenzoyl chloride afforded the 2,3-disubstituted benzo[*b*]thiophene products **8a** and **8b**. Installation of the basic C-3 side chain was accomplished by fluoride displacement with the sodium

**Scheme 2**[a]

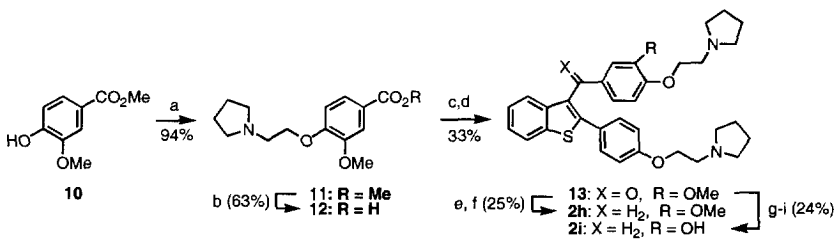



[a]Reagents: (a) 3,4-difluorobenzoyl chloride (1 eq), $TiCl_4$ (4 eq), 5 hr, 0°, $ClCH_2CH_2Cl$; (b) 3-trifluoromethyl-4-benzoyl chloride (4 eq), $TiCl_4$ (4 eq), 4 hr, $ClCH_2CH_2Cl$; (c) NaH (2eq), 1-(2-hydroxyethyl)pyrrolidine (2eq), 3 hr, THF; (d) DIBAL-H, (5eq), 0 °C, 1h, THF; (e) $NaBH_4$ (2.5 eq), 2 hr, TFA.

alkoxide of 1-(2-hydroxyethyl)pyrrolidine to afford analogs **9a** and **9b**. Deoxygenation of the C-3 ketone afforded the desired **2f** and **2g**.

The oxygen substituted derivatives **2h** and **2i** were prepared according to Scheme 3. Alkylation of commercially available methyl vanillate (**10**) gave the 2-(1-pyrrolidinyl)ethoxy substituted product **11**, which could be hydrolyzed to provide the necessary C-3 side chain (**12**). Conversion of the acid to the corresponding acid chloride, and its subsequent use in the acylation of intermediate **7** (Scheme 2), afforded the 2,3-disubstituted benzo[*b*]thiophene product **13**. Direct deoxygenation afforded the methoxy substituted product **2h**. Alternatively, demethylation ($EtSH/AlCl_3$) followed by deoxygenation gave the phenolic product **2i**.

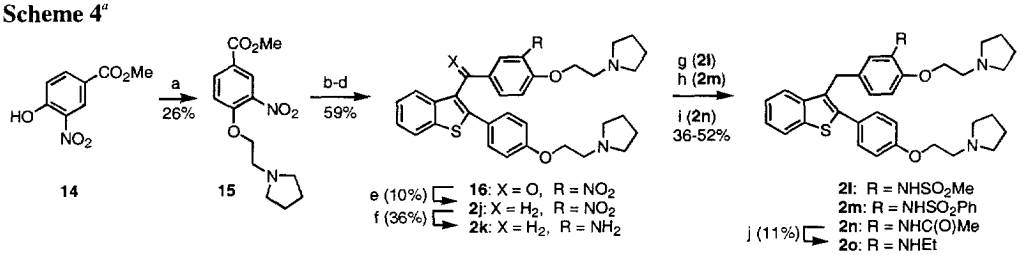
**Scheme 3**[a]




[a]Reagents: (a) 1-(2-chloroethyl)pyrrolidine hydrochloride (2eq), $K_2CO_3$ (4eq), 85 °C, DMF; (b) 5 N aq HCl, Δ, 16h; (c) $SOCl_2$ (18 eq), DMF (cat), Δ, 16h, $ClCH_2CH_2Cl$; (d) $AlCl_3$ (4 eq), **7** (1 eq), 0 °C, 5h, $ClCH_2CH_2Cl$; (e) $LiAlH_4$ (1.1 eq), 3 h, THF; (f) $Et_3SiH$ (10 eq), TFA (7 eq), 0 °C, 6 h; (g) EtSH (8 eq), $AlCl_3$ (4 eq), 0 °C, 1h, $CH_2Cl_2$; (h) DIBAL-H (5 eq), 0 °C, 1h, $CH_2Cl_2$; (i) $NaBH_4$ (2 eq), 1 h, TFA.

The nitrogen containing analogs (**2j-o**) were generated from methyl 4-hydroxy-3-nitrobenzoate (**14**; Scheme 4) following chemistry similar to that outlined in Scheme 3. Mitsunobu alkylation afforded product **15** which was converted to the acid chloride and used in the acylation of intermediate **7** (step d) to afford the 2,3-disubstituted benzo[*b*]thiophene **16**. Deoxygenation afforded the nitro product **2j** which could be reduced to the aniline **2k**. Derivatization of aniline **2k** gave the sulfonamide and acetamide analogs (**2l-n**). Subsequent reduction of acetamide **2n** afforded the secondary amine **2o**.

**Scheme 4**[a]

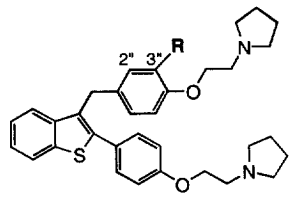



[a]Reagents: (a) DEAD (1.2 eq), $Ph_3P$ (1.2 eq), 1-(2-hydroxyethyl)pyrrolidine (1.2 eq), 60 h, THF; (b) 5 N aq HCl, Δ, 16h; (c) $SOCl_2$ (40 eq), DMF (cat), Δ, 2h, $ClCH_2CH_2Cl$; (d) $TiCl_4$ (8 eq), **7** (1 eq), 0 °C, 8h, $ClCH_2CH_2Cl$; (e) $NaBH_4$ (4 eq), 16h, TFA; (f) $H_2$ (1 atm), 10% Pd/C, 16 h, EtOH, AcOH; (g) $MeSO_2Cl$ (1 eq), 0 °C, 8h, $CH_2C1_2$; (h) $PhSO_2Cl$ (1 eq), 0 °C, 8h, $CH_2Cl_2$; (i) $Ac_2O$ (30 eq), 16h, pyridine; (j) $LiAlH_4$ (2 eq), Δ, 3 h, THF.

**Results and Discussion**

The thrombin inhibitory activity for compounds **1a** and **2a-o**,[11] as well as the substituent values of cMR, $\sigma_m$, and $\pi$ are shown in Table 1.[7] Several trends are evident from the biological activity. As predicted by X-ray crystallographic studies,[5] the incorporation of lipophilic substituents at C-3" enhances the thrombin inhibitory activity of the series, presumably through increased interactions at the hydrophobic $S_2$ binding site. Small groups such as Br and Me (**2a** and **2b**, respectively) increase potency by up to 8-fold. However, this activity enhancement erodes as the size of the hydrophobic substituent increases (**2c-e**).

**Table 1.** QSAR Parameters and Thrombin Inhibitory Activity of Analogs **1a** and **2a-p.**




1a, 2a-o

| Compound | R | cMR[a] | $\sigma_\mu$[a] | $\pi$[a] | $K_{ass}$[c] (L/mol x$10^6$) |
|---|---|---|---|---|---|
| 1a | H | 1.03 | 0.00 | 0.00 | 3.43 ± 0.55 |
| 2a | Br | 8.88 | 0.39 | 0.86 | 27.50 ± 1.84 |
| 2b | Me | 5.65 | -0.07 | 0.56 | 24.2 ± 3.21 |
| 2c | Et | 10.3 | -007 | 1.02 | 7.27 ± 0.69 |
| 2d | n-Propyl | 15.0 | -0.06 | 1.55 | 1.02 ± 0.05 |
| 2e | n-Butyl | 19.6 | -0.08 | 2.13 | 0.42 ± 0.09 |
| 2f | F | 0.92 | 0.34 | 0.14 | 4.87 ± 0.40 |
| 2g | $CF_3$ | 5.02 | 0.43 | 0.88 | 2.40 ± 0.30 |
| 2h | OMe | 7.87 | 0.12 | 0.02 | 12.23 ± 0.90 |
| 2i | OH | 2.85 | 0.12 | 0.07 | 1.10 ± 0.01 |
| 2j | $NO_2$ | 7.36 | 0.71 | -0.28 | 10.20 ± 1.49 |
| 2k | $NH_2$ | 5.42 | -0.16 | -1.23 | 1.10 ± 0.17 |
| 2l | $NHSO_2Me$ | 18.2 | 0.20 | -1.18 | 0.41 ± 0.06 |
| 2m | $NHSO_2Ph$ | 37.9 | 0.16 | 0.45 | 0.22 ± 0.06 |
| 2n | NHAc | 14.9 | 0.12 | -0.22 | 0.31 ± 0.04 |
| 2o | NHEt | 15.0 | -0.24 | 0.08 | 0.29 ± 0.04 |
| 2p | 2"-Br[d] | | | | 0.27 ± 0.01 |

[a]see refs 7 and 8. [b]see ref 12. [c]see ref 11. [d]see ref 15.

Figure 1 illustrates how the Br substituent (**2a**) may project into and fill the spherical $S_2$ pocket, providing a stable fit.[12] As might be predicted, fluorine, which is smaller than a hydrogen but is more hydrophobic, results in comparable activity relative to the unsubstituted parent (**2f** versus **1a**). The decrease in activity for the larger substituents (**2c-e**) is likely due to unfavorable steric interactions in the $S_2$. An additional explanation for the

decreased activity of analogs **2c-e** is the less than optimal positional/conformational changes imparted on the basic C-4" side chain by the additional steric bulk. Alternatively, the C-3 phenyl ring may assume a conformation that orients the C-3" substituents in the opposite direction, into the solvent, leaving the $S_2$ site unoccupied.

**Figure 1.** Molecular modeling representation of the interaction of derivative **2a** in the active site of human α-thrombin (aliphatic hydrogens and the water molecules are not displayed). The C-3 side chain of the inhibitor spans the proximal ($S_2$) and distal binding sites.[12] The bromo substituent at C-3" (purple) binds in the hydrophobic S2 pocket defined by residues Trp86, Leu132, His79, and Tyr83.

Structurally, the $CF_3$ substituent is generally considered to be very similar to a $CH_3$ group. However incorporation of a $CF_3$ in **2g** decreases the activity 10-fold compared to the $CH_3$ (**2b**) analog. To better understand this result, the geometry and electronic nature of the $CH_3$ and $CF_3$ groups were investigated in more detail using the model system *p*-methoxy toluene, substituted at the *meta* position by $CF_3$ and $CH_3$ respectively.[13] It was found that the center of the $CF_3$ group is slightly more extended (1.95 Å) from the phenyl carbon than the corresponding center of the $CH_3$ group (1.77 Å). This extension would normally be thought to enhance activity (as in the **2a** case when R is Br (1.87 Å), however for **2g** the more electronegative fluorine atoms (-0.34) are pushed deeper into the face of the hydrophobic aromatic residues that make up the $S_2$ pocket. The corresponding hydrogens of the Me group of **2b** carry only a small positive charge (0.09).

Even though the more hydrophilic substituents $NO_2$ and OMe might not be predicted to enhance activity through interaction at the lipophilic $S_2$ binding site, they were selected based on molecular diversity analysis. Both the $NO_2$ and OMe analogs (**2j** and **2h**, respectively) were more active than the unsubstituted parent (**1a**). In the case of the derivative **2j**, molecular modeling suggests that one of the oxygens of the nitro substituent lies within hydrogen bonding distance of the phenolic hydrogen of TYR 83, thereby increasing the interaction at $S_2$. The OMe analog (**2h**) is also more potent than the Et derivative (**2c**). In this case however, modeling studies do not suggest any apparent enhanced interactions. It is possible that a combination of subtle geometrical and electronic factors, resulting from replacement of the methylene with the ether oxygen, come into play. Although ill-defined at this point, the poor activity of analogs **2i** and **2k-o** relative to the parent **1a**, could be due to unfavorable electronic and/or steric interactions.

Based on X-ray crystallography studies, the C-3" position was initially chosen as the site for incorporation of substituents based on its position relative to the opening of the $S_2$ pocket. To prove that this initial prediction was valid, the C-2" bromo derivative **2p** was prepared and evaluated.[14] Its diminished activity relative to analog **2a** supports our prediction that the C-3" site allows better substituent access to the hydrophobic $S_2$ pocket.

The purpose of this work was to study the potential interaction of C-3" substituents with the proximal $S_2$ binding region within the active site of thrombin, in the hopes of increasing the intrinsic thrombin inhibitory activity of this novel class of inhibitors. In fact, incorporation of small hydrophobic substituents (ie. Br and Me)

increase the potency of the parent compound by 8-fold. This is consistent with the hydrophobic nature of the $S_2$ binding site. However, more hydrophilic substituents such as $NO_2$ and OMe also enhance inhibitory activity, suggesting that one may be able to take advantage of more subtle interactions within this region as well. Utilization of the results of this study in the design of thrombin inhibitors that are efficacious in animal models of thrombosis will be the topic of future communications.

**References:**


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. *Abstract of Papers*, 214th National Meeting of the American Chemical Society, Las Vegas, NV; American Chemical Society: Washington, DC, 1997.
2. (a) Machovich, R. In *The Thrombin*; Machovich, R., Ed.; CRC Press, Inc: Boca Rotan, FL, 1984; Vol. 1, pp 1-22. (b) Hirsh, J.; Salzman, E. W.; Marder, V. J.; Colman, R. W. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 2nd ed.; Colman, R.W.; Hirsh, J.; Marder, V. J.; Salzman, E. W., Eds.; J. B. Lippincott Co.: Philadelphia, 1987; pp 1063-1072. (c) Hirsh, J.; Salzman, E. *Ibid.* pp 1199-1207. d) Barnett, H. J. M. *Ibid.* pp 1301-1315.
3. For recent reviews on approaches to the design of active-site directed thrombin inhibitors see (a) Edmunds, J. J.; Rapundalo, S.T.; *Ann. Rep. Med. Chem.* **1996**, *31*, 51-60. (b) Scarborough, R. M. *Ann. Rep. Med. Chem.* **1995**, *30*, 71-80. (c) Wiley, M. R.; Fisher, M. J. *Exp. Opin. Ther. Patents* **1997**, *7*, 1265-1282.
4. Sall, D. J.; Bastian, J. A.; Briggs, S. L.; Buben, J. A.; Chirgadze, N. Y.; Clawson, D. K.; Denney, M. L.; Giera, 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-CB.; Zhang, M. *J. Med. Chem.*, 1997, 40, 3489-3493.
5. A manuscript describing the X-ray crystallographic studies conducted with this series of thrombin inhibitors is in preparation.
6. A discussion of the QSAR parameters cMR, $\sigma_m$, and $\pi$, is contained in Martin, YC. Quantitative Drug Design: A Critical Introduction. New York: Marcel Dekker, Inc., 1978: 11-31.
7. Numerical values for the QSAR parameters cMR, $\sigma_m$ and $\pi$ were taken from Hansch, C.; Leo, A. Substituent Constants for Correlation Analysis in Chemistry and Biology. New York: Wiley, 1979.
8. 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.
9. Bastian, J. A.; Chirgadze, N. Y.; Denney, M. L.; Foglesong, R. J.; Harper, R. W.; Johnson, M. G.; Klimkowski, V. J.; Kohn, T. J.; Lin, H-S.; Lynch, M. P.; McCowan, J. R.; Palkowitz, A. D.; Richett, M. E.; Sall, D. J.; Smith, G. F.; Takeuchi, K.; Tinsley, J. M.; Zhang, M. WO 97/25033, 1997.
10. Stille, J. K. *Angew. Chem. Int. Ed. Engl.*, **1986**, *25*, 508-524.
11. 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.; Craft, T. J.; Chirgadze, N.; Ruterbories, K. J.; Lindstrom, T. D.; Satterwhite, J. H. Efegatran: A New Cardiovascular Anticoagulant. In: Pifarre, R, editor. New Anticoagulants for the Cardiovascular Patient. Philadelphia: Hanley & Belfus, Inc., 1997:265-300.
12. The X-ray crystal structure of α-thrombin complexed with **1b**[4,5] was used as the starting point for all molecular modeling, which was performed using QUANTA/CHARMm (versions 96 and 23.2 respectively, Molecular Simulations Inc., San Diego, CA 92121). Each analog was built from the X-ray structure of **1b**, as extracted from the experimental complex. Both amine nitrogens were protonated, and the initially assigned charges were smoothed over all carbon and hydrogen atoms for a net +2 charge. All hydrogens were added and refined for the protein and crystallographic waters by the HBUILD procedure. The ligand binding region was solvated with a 20 Å sphere of TIPS3P water, centered at a point central to the active site. Each analog was processed by reinserting into the active site, deleting all waters having a oxygen within 2.0 Å of a ligand atom, and the complex energy was minimized (ABNR) to convergence (gradient tolerance 0.01 kcal/mole•Å). With respect to the active site center, the region minimized included all protein residues within 14 Å, all waters within 20 Å, and the considered analog. The nonbonded interactions were treated using a 13 Å cutoff and smoothed to 0.0 by a force switch function for electrostatics (applied between 8 Å to 12 Å), and a shift function for the van der Waals.
13. For the model system para-methoxy toluene, respectively the meta-methyl and the meta-trifluromethyl analogs were built and processed with SPARTAN 5.0.1 (Wavefunction Inc., Irvine, CA 92612). For each, the geometry was optimized using the AM1 Hamiltonian. Then for each, electrostatic potential fit atomic charges were calculated with the 3-21G(*) basis set.
14. The C-2" bromo derivative **2p** was prepared by essentially the same route as the C-3" bromide **2a** (Scheme 1) except that 2-bromo-4-methoxybenzoyl chloride was employed as the acylating reagent in the first step.