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Solid Phase Chemistry Approach to the SAR Development of a Novel Class of Active Site-Directed Thrombin Inhibitors

Mary George Johnson[1], Duane D. Bronson[1], Jan E. Gillespie[1], Donetta S. Gifford-Moore[2], Kyomi Kalter[1], Michael P. Lynch[1], Jefferson R. McCowan[2], Catherine C. Redick[1], Daniel J. Sall[2], Gerald F. Smith[2], Robert J. Foglesong[1]*

[1]Sphinx Pharmaceuticals, A Division of Eli Lilly and Company, 4615 University Drive, Durham, NC, 27707, and [2]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, 46285

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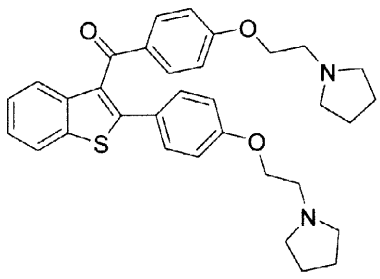
Abstract: A solid phase chemistry approach utilizing Mitsunobu chemistry, amine functionalization, and parallel purification was used to produce a diverse library of benzothiophene analogs. These analogs were used to advance the SAR of this class of molecules and give new directions for future studies.

Introduction

The serine protease thrombin catalyzes several key processes in the coagulation cascade which, when uncontrolled, leads to thrombus formation and life threatening thrombotic diseases[1]. As the last enzyme in the coagulation cascade, thrombin represents an appealing target for therapeutic intervention[2]. Not surprisingly, the design and synthesis of orally active thrombin inhibitors has become a popular theme in the pharmaceutical industry. While a number of very potent and selective inhibitors have been disclosed, few are reported to have the necessary bioavailability to be useful as oral therapies [3]. Recently, we disclosed the identification and characterization of a novel series of active site-directed, orally bioavailable thrombin inhibitors, for example **1**[4] and **2**.[5] Preliminary structure activity relationship studies (SAR) revealed that the basic nitrogens in the C-2 and C-3 side chains were optimal for thrombin inhibitory activity. In order to more rapidly explore the SAR in these two regions, solid phase combinatorial chemistry studies were undertaken with the ultimate goal of improving potency.



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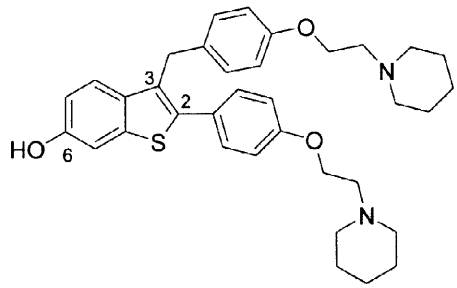

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Our initial plan was to employ suitably protected benzo[*b*]thiophene scaffolds containing the requisite functionality for both attachment to a solid support and for selective introduction of

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structural diversity at either the C-2 or C-3 side chain (**3** and **4**; Figure 1). We chose to investigate single structural modifications in the first phase of the SAR and then incorporate those findings into subsequent rounds of synthesis and screening. In addition, all plate syntheses were performed in a 96-well plate format to complement our biological screening process and in a one compound/well format. Analysis of the lead structure reveals several obvious disconnections for introduction of structural diversity (Figure 1). Since addition of a hydroxyl at the C-6 position imparted up to 20-fold increases in thrombin inhibition in similar analogs[4], it became a convenient handle for resin attachment.

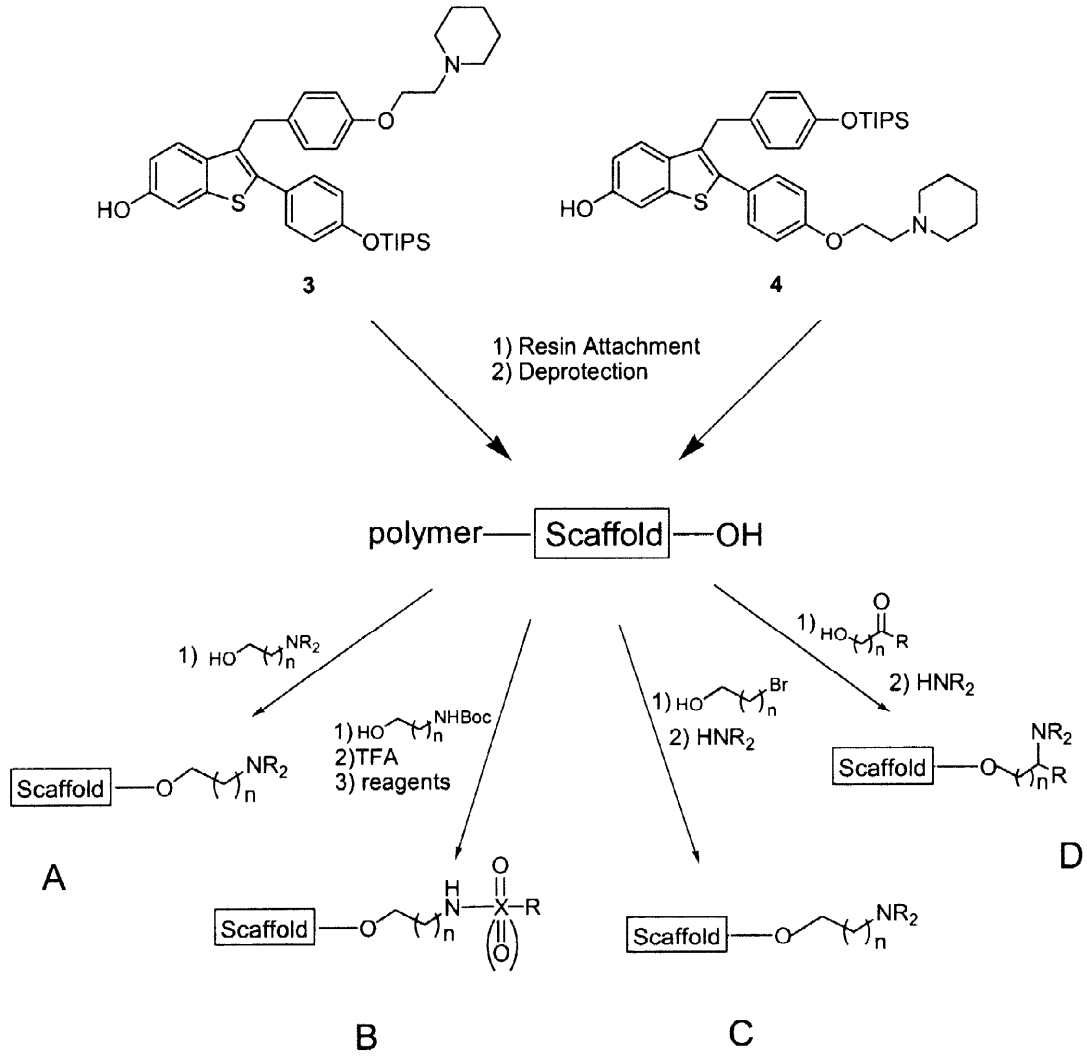


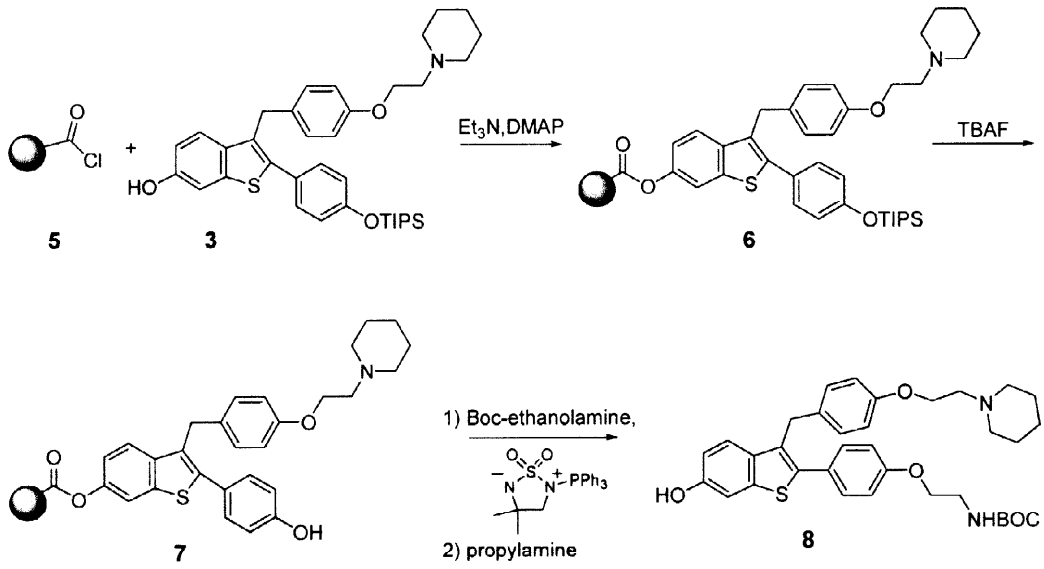
Figure 1. Scaffolds and Potential Routes for Incorporating Structurally Diverse Side Chains at Positions C-2 and C-3.

Each of the routes was considered for the availability of structurally diverse side chains, ease of chemistry and/or purity of products, and time required for development. After some initial experimentation, routes A and B proved to be the most reliable and provided a reasonable sampling of structural diversity in the library. Our selection of potential side chains was influenced by several factors. Guided by the known SAR, we chose diversity elements that would hopefully

improve potency and perhaps bioavailability. We also saw this as an opportunity to find novel structures and new directions for the SAR development of this class of inhibitors.

Synthesis

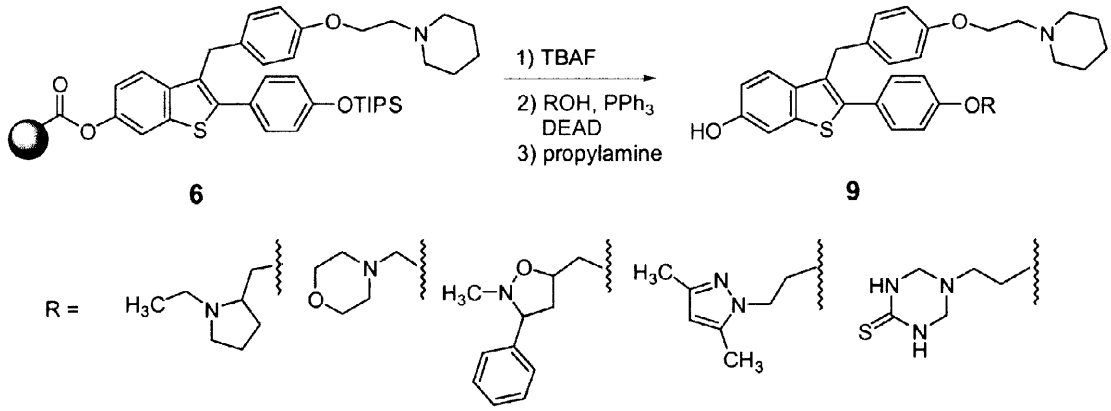
The synthesis began with the TIPS protected benzo[*b*]thiophene, **3**, which was linked to a polystyrene resin by reaction with the styryl acid chloride **5** to afford intermediate **6** (Scheme 1). Deprotection of the TIPS group with buffered tetrabutylammonium fluoride (TBAF) afforded resin bound phenol **7** which was washed with dilute acetic acid to ensure complete removal of the tetrabutylammonium salts. At both steps in the synthesis, the modified benzo[*b*]thiophene scaffolds were cleaved from the resin and analyzed for their percent conversion to product and their purity. Because of the labile nature of phenoxy ester linkages, cleavage of the scaffold from the resin was easily accomplished with excess propylamine which could subsequently be removed either via a stream of nitrogen or under vacuum. A variety of Mitsunobu conditions[6] were studied in an attempt to attach a diverse set of amino alcohols to phenol **7**. Initial experiments using Castro's reagent[7] in the coupling of phenol **7** with *N*-(tert-butoxycarbonyl)ethanolamine proceeded smoothly on a large scale such that cleavage from the resin with propylamine afforded the Boc-aminoethanol derivative, **8**, in quantitative yield with only traces of underivatized starting material.



Scheme 1

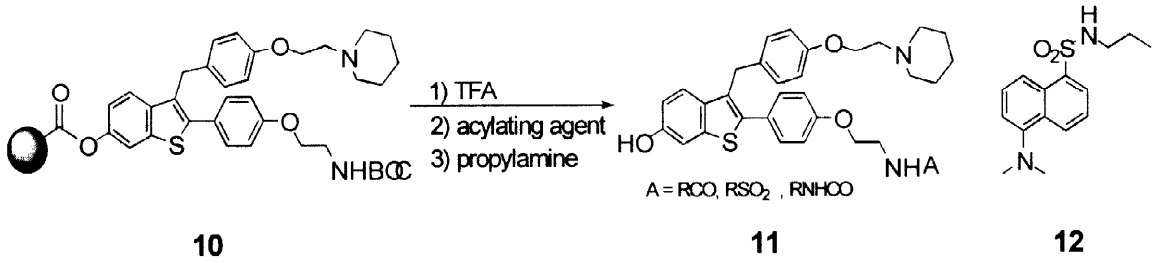
Unfortunately, application of these conditions to a 96-well plate format were less successful. Although small scale trials using several different amino alcohols showed that the method should be amenable to plate preparation, application of the conditions to the entire library gave erratic and unpredictable results. Attempts to determine the source of the inconsistent results were unsuccessful. The more traditional triphenylphosphine and diethylazodicarboxylate (DEAD) reagent system was also tried, but initial results were also inconsistent. Upon further attempts, however, reproducible results were realized using 30 eq. of both the alcohol and

triphenylphosphine, and 22 eq of DEAD. Agitation of the plate during the addition of DEAD also gave more consistent results. Scheme 2 shows the synthetic sequence that was ultimately employed, as well as a few representative examples of the structurally diverse products (**9**) that were generated. In all, a library of over 200 analogs with structurally diverse C-2 side chains was prepared by this route.



Scheme 2

The preparation of additional C-2 modified analogs was accomplished starting from the resin bound, Boc-protected ethanolamine derivative **10** (Scheme 3). Deprotection using trifluoroacetic acid afforded the primary amine which was subsequently treated with an assortment of acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. Cleavage from the resin using propylamine gave products of general structure **11**. In each reaction well no starting material could be detected by TLC analysis. NMR analysis of selected wells, however, showed that each contained a single major by-product whose structures were related to the individual acylating reagents used. One of those side products was isolated and identified as the propyl sulfonamide, **12**. It is possible that these products arise from the addition of propylamine to the mixed anhydrides that are formed between the acylating agents and underivatized carboxylates on the polystyrene resin. Accordingly, it was necessary to employ modified-plate silica gel chromatography to allow parallel purification of this entire library of C-2 analogs.



Scheme 3

The corresponding analogs containing basic amines in the C-3 side chain were prepared in a manner similar to that outlined in Scheme 2 using benzo[*b*]thiophene scaffold **4** and the same set

of amino alcohols. In addition, derivatives possessing *N*-acylated C-3 side chains were synthesized according to the conditions described in Scheme 3. To prevent the formation of by-products of type **12**, the product from coupling the benzo[b]thiophene to the styrene resin was briefly treated with 5% triethylamine in methanol to esterify any unreacted acid chloride residues. The C-3 analogs thus produced were free of any side products and could be directly assayed for biological activity. A total of approximately 140 derivatives with structurally diverse C-3 side chains were prepared by these two routes.

Results and Discussion

The structurally diverse set of 346 analogs that were prepared were evaluated for thrombin inhibitory activity. A multi-tiered testing paradigm was employed which included; 1) rapid screening of all 346 analogs, 2) generation of IC_{50} values on the more structurally novel actives, and 3) resynthesis of actives and determination of association constants (K_{ass}) and *in vitro* anticoagulant activity (thrombin times; TT).

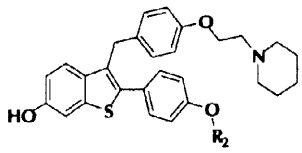
For rapid screening purposes, inhibitory activity was measured using a well characterized thrombin amidase assay.[8] Although a number of reaction wells were not purified and the mass reaction products during plate synthesis not quantified, calibration across plates was achieved by including the synthesis of the known inhibitor **2** at multiple sites in each plate. This provided an indication of the variability that occurred both across and between plates. In order to account for any biological activity that might be attributed to starting materials, designated control wells were loaded with starting materials but left unreacted. From rapid screening, initial results indicated that 70 compounds displayed greater than 50% inhibition at 10 μM. Of those, 15 showed activity comparable to lead compound **2**. Forty-six compounds were selected based on their activity and novelty of structure and their estimated IC_{50} values determined. It was gratifying to note that the estimated IC_{50} for compound **2**, 0.2 μM, was in agreement with the IC_{50} obtained for **2**, 0.13 μM, prepared by traditional synthetic methods[5]. In virtually every case, analogs that displayed similar or better activity to **2** in the rapid screen, displayed equal or better IC_{50} values relative to **2**.

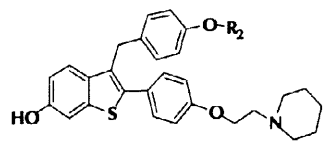
Based on the estimated IC_{50} data, 14 analogs were chosen for resynthesis, purification and characterization, and advanced biological evaluation. Apparent association constants ($K_{ass;}$; Table 1) were obtained for each of these analogs, allowing for their comparison to previously reported benzo[*b*]thiophene-derived thrombin inhibitors.[4] The rank order of data found in the K_{ass} assay mirrored that found in the rapid screen and IC_{50} assays. As would be expected from the earlier assays, the K_{ass} value generated for analog **2** is in agreement with reported values[5], validating the use of the screening data to advance the SAR. Also reported in Table 1 is the selectivity of these agents for thrombin relative to another coagulation factor, Xa. All of the compounds displayed marked selectivity for thrombin.

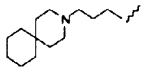
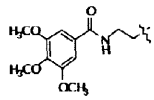
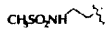
While not all of the data are reported here, some interesting trends did appear from the initial data. The greater number of active analogs in the modified C-2 side chain subset may be indicative of thrombin's greater tolerance for structural diversity in the C-2 side chain relative to C-3. This is consistent with the X-ray crystallographic data which shows that inhibitors of this series bind in a manner in which the C-2 chain branches away from the active site and resides in a solvent-exposed position[4]. In contrast, the C-3 side chain undergoes specific interactions at the S_2 and S_3 binding sites. Therefore, it was surprising that small changes in the C-2 side chain resulted in large differences in activity (**10** vs **11**). Even more interesting was the fact that non-basic side chains resulted in analogs with activity comparable to lead compound **1**, in spite of the preliminary results suggesting the critical nature of the basic amines[3d]. Noteworthy was the activity of the C-3

analog, thiophene sulfonamide, **22**. Although the activity of the non-basic analogs was modest compared to other members of this series, their activity was surprising nonetheless.

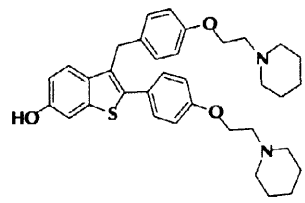
Table 1. Biological Activity of Selected Benzo[b]thiophene Derivatives.





		Protease Inhibition Kass data, L/mole (x10^6)		Coagulation Assays 2x Conc (ng/mL)		dTT/dlipid
		Human Thrombin	Human Xa	TT Citrol	TT Fgn	
10		23	0.33	353	48	0.48
11		11	0.04	748	107	2.15
12		9.8	0.26	1459	159	8.58
13		8.3	0.02	787	144	3.06
14		6.7	0.33	2417	204	6.74
15		5.5	0.28	21161	236	52
16		2.7	0.04	2027	216	5.34
17		2.3	0.005	2846	527	8.3
18		1.5	0.02	27164	1273	6.85
19		1.3	0.03	12516	878	11.78

		Protease Inhibition Kass data, L/mole (x10^6)		Coagulation Assays 2x Conc (ng/mL)		dTT/dlipid
		Human Thrombin	Human Xa	TT Citrol	TT Fgn	
20		19.5	0.01	505	63	2.45
21		13.6	0.01	11367	126	11.21
22		1.02	0.02	>44588	1510	NT



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2, Prepared on resin Protease Inhibition Kass data, L/mole (x10^6)		2, Authentic Sample Protease Inhibition Kass data, L/mole (x10^6)	
Human Thrombin	Human Xa	Human Thrombin	Human Xa
6.2	0.16	8.3	0.16

Each of the compounds in Table 1 was also evaluated in a more physiologically relevant thrombin time (TT) assay, which measures the concentration of the inhibitor necessary to double the time of clot formation in an *in vitro* system. This assay is run in both a buffered system, TT (fibrinogen, Fgn) and in reconstituted plasma, TT (Citrol). In many cases, the rank order of activity from the TT assays parallels the K_{ass} data and for compounds **10** and **20**, it takes relatively low concentrations to double the thrombin time. However, in every case there was a significant loss in activity in going from the buffered Fgn assay to the plasma-derived Citrol assay. Since the Citrol assay contains all the physiological constituents of plasma, it is possible that this discrepancy is due to interaction of the inhibitor with plasma protein and/or lipids, making it less available to inhibit thrombin. To test this theory, each compound was tested in the Fgn (buffered) assay in the presence of increasing concentrations of a representative plasma lipid, phosphatidyl ethanolamine. The last column (dTT/dlipid) in Table 1 shows the slope of the TT versus lipid response curves and is an indication of the sensitivity of the analog to lipid effects. Inspection of column three shows that **10** does indeed show a reduced sensitivity towards lipid effects (dTT/dlipid = 0.48). This is in contrast to **15** and **20** which, although have good K_{ass} values, display more pronounced lipid binding, thereby limiting their potency in the Citrol assay. This phenomenon would also explain why compound **15,** which is more or equipotent than derivative **16** in both the K_{ass} and Fgn (TT) assays, is 10-fold less active than **16** in the Citrol (TT) assay. It is noteworthy, however, that most of these analogs displayed better TT/Fgn values than almost all of the previously reported members of this series and that, for the more active analogs (eg. **10**), the plasma effect was reduced with respect to the lead compounds in the series: only a 7-fold decrease for **10** versus 20 to 50-fold decreases or more for the most active compounds in this class.

Finally, it is interesting to note some of the general SAR features within this set of analogs. The N-ethylpyrrolidines **10** and **13** had better activity (K_{ass}) than the N-methylpyrrolidines **11** and **17** (23 vs. 11 and 8.3 vs. 2.3). This trend was maintained in the lipid sensitivity assays (0.48 vs. 2.15 and 3.06 vs. 8.3). As might be expected, increases in hydrophobicity are paralleled by increases in lipid interference.

Conclusions

Combinatorial chemistry employing the Mitsunobu reaction has been used to prepare directed libraries of derivatives surrounding lead structure **2**. This has resulted in the rapid extension of the SAR of the series and the identification of structurally novel analogs. Analogs were found that were more potent and show the potential for reduced interaction with plasma lipids, thereby enhancing potency in the TT assays. In addition, active analogs containing non-basic side chains were discovered, opening up a potential new direction for SAR studies. Both solution and solid phase combinatorial chemistry have shown great promise as lead generation tools. The studies reported here show that solid phase synthesis used in a high through-put format can also be used to rapidly advance the SAR of a lead compound and reveal new avenues for exploration.

Experimental Section

Preparation of Resin 7:

To a mixture of carboxylated polystyrene[9] (3.3 mmol/g, 10g, 33mmol) in dichloromethane (100ml) containing a small amount of DMF (0.25ml) was added oxalyl chloride (12.6g, 99mmol) dropwise over 5 min. This mixture was allowed to stir for 24 hr at room temperature and under a nitrogen atmosphere. After the appropriate amount of time the solvents were removed under vacuum. Dichloromethane (50ml) was added and then removed under reduced pressure. This step was repeated 3 times. After the final evaporation, the resulting resin, **5**, was stored under high vacuum. Analysis of the resin showed a loading of 3.06 mmol/g, which is essentially quantitative. Polystyrene carbonyl chloride, **5**, (3.06 mmol/g loading, 2.5g, 7.65 mmol) was suspended in dichloromethane (25 ml). The TIPS-protected benzothiophene, **3**, (4.71g, 7.65 mmol) was added in one portion followed by dimethylaminopyridine (100mg) and then triethylamine (1.2g, 1.6ml, 11.5mmol, 1.5 eq.). The mixture was agitated on a rotary shaker for 72 hr. At the appropriate time the mixture was filtered over a sintered glass funnel and washed, in order (with stirring between washes), with: dichloromethane (100ml), methanol (100ml), tetrahydrofuran (100ml), dimethyl formamide (100ml), tetrahydrofuran (100ml), 10% triethylamine/methanol (100ml), methanol (100ml x 2), dichloromethane (100ml x 4). The resulting resin was dried under vacuum until a constant weight was obtained. The total yield was 5.83g (84% of theoretical). Based on the weight gain, the loading was 0.93 mmol/g.

The resin (5.83g, 5.42mmol) obtained above was suspended in tetrahydrofuran (25 ml) and glacial acetic acid (1.63g, 1.6ml, 27.1 mmol, 5eq) added. Tetrabutylammonium fluoride (1M in tetrahydrofuran, 27.1 ml, 27.1 mmol) was then added in one portion and the mixture agitated on a rotary shaker for 48 hr. The mixture was filtered over a sintered glass filter and then washed with 50% acetic acid/water (500ml). The mixture was then washed with tetrahydrofuran (100ml), methanol (100ml), tetrahydrofuran (100ml), methanol (100ml), dichloromethane (100ml x 3). The resin, **7**, was dried under vacuum and used without further manipulation in subsequent chemistry.

*Preparation of Resin bound Boc-amino ethanol benzothiophene, **10**, using Castro's reagent:*

Castro's reagent (4.45g, 10.85mmol) and Boc-aminoethanol (1.75g, 1.68ml, 10.85 mmol) were mixed in tetrahydrofuran (25ml) for 5 min. This mixture was then added to a stirring suspension of resin **7** and the resulting reaction mixture agitated on a rotary shaker for 4 days. A small sample of the resin was cleaved by stirring the resin in 30% propylamine/ tetrahydrofuran for 24 hr. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was analyzed by TLC (9:1 dichloromethane/methanol), NMR and MS. A trace of unreacted starting material was seen on the TLC, but was not detected in the NMR. **8**: 2- (4'-(2-N-tertiarybutoxycarbonylaminoethyloxy)-phenyl)-3-(4'-(2-piperidinoethyloxy)-phenyl)-6-hydroxy benzothiophene: ^{1}H NMR (300 MHz, methanol-d_4) 1.49 (s, 13 H), 1.68 (m, 4 H), 2.61 (m, 4 H), 2.83 (t, J = 6.0 Hz, 2 H), 3.94 (t, J = 6.0 Hz, 2 H), 4.10 (m, 4 H), 5.05 (bt, 1 H), 6.72 (d, J = 6.0 Hz, 4 H), 6.80 (dd, J = 9.0 Hz, 3.2 Hz, 1 H), 7.0 (m, 2 H), 7.11 (m, J = 3.2.0 Hz, 1 H), 7.27 (m, 3 H); mass spectrum *m/z* (PFAB) 602.1 (M^+)

*General Preparation of Analogs **11** (acylation):*

The resin **10** (440mg) was mixed 10% trifluoroacetic acid/dichloromethane and agitated on a rotary shaker for 16 hrs. The reaction mixture was filtered and washed with methanol (10 ml) , dichloromethane (10 ml), tetrahydrofuran (10 ml), 10% triethylamine/ dichloromethane (10 ml), tetrahydrofuran (10 ml), dichloromethane (10 ml x 3). An isopycnic slurry was prepared using 420 mg of the resin and an approximately 2:1 DMF/dichloromethane solution (52.5 ml). To a fritted

reaction plate was added the isopycnic slurry (1 ml). The solvents were allowed to drain and the resin was washed with dichloromethane (1ml x 5). With this method, each well will contain 8 mg of resin (7.44umol/well). The plates were assembled in a plate clamp assembly and a solution of pyridine (5.9mg, 74.4 umol) and dimethylaminopyridine (1mg) in dichloromethane (100 uL) was added to each well. To these wells were then added a solution of the acylating agent in dichloromethane (0.2M, 500uL, 100umol). The wells were capped and agitated for 18hr. After the required time the plates were uncapped, drained and each reaction well was washed with dichloromethane (1ml), methanol (1ml), dimethylformamide (1ml), tetrahydrofuran (1ml), dimethylformamide (1ml), methanol (1ml x 5), and dichloromethane (1ml x 3). The plates were reassembled in the plate clamp and 10% propylamine in 1:1 methanol/tetrahydrofuran (500 uL) was added to each well. The plates were agitated for 18 hr. The filtrates of each reaction well were collected into a final plate, the solvents removed under vacuum, and the residue of each well redissolved. TLC analysis of each well was obtained and the solvents removed. The resulting residue was submitted for biological assays. In the event that purification is required (see text), a 96-well plate was fitted with plugged, 5-inch disposable pipettes and 0.5ml of silica gel was added to each pipette. The reaction mixtures were applied to each column and fractions collected in separate 96 well plates. The pure fractions were combined into one 96-well plate, the solvents removed under vacuum and the resulting material submitted for biological assays.

*General Preparation of Analogs **9** (Mitsunobu):*

The resin, **7**, (10mg, 9.2umol) was placed in a 4ml amber vial and the appropriate aminoalcohol (30 eq., 0.28mmol) was added. To this mixture was added tetrahydrofuran (100uL). If the alcohol has solubility problems, dimethylformamide could be added in conjunction with the tetrahydrofuran. Triphenylphosphine (30eq. 0.28 mol, 68 mg) in tetrahydrofuran (200 uL) was added to each reaction mixture and the vials were agitated on a rotary shaker. After 5 min, a solution of DEAD (22eq. 0.201 mmol, 35mg) in tetrahydrofuran (200uL) was added to each reaction mixture and the mixtures allowed to shake for 40 hr. The suspensions were transferred to a 96-well filter plate and washed with dichloromethane (1ml x 2), methanol (1ml), tetrahydrofuran (1ml), dimethyl-formamide (1ml), tetrahydrofuran (1ml), dimethylformamide (1ml), methanol (1ml x 2), dichloromethane (1ml x 4). The plate containing the resin was then assembled in a plate clamp and each well treated with 10% propylamine in 1:1 methanol/tetrahydrofuran (500 uL) for 18 hr. After the appropriate time, the reaction mixtures were allowed to drain into a collection plate. The solvents were removed, the residues redissolved, and each well analyzed by TLC. The solvents were again removed and the resulting material submitted for biological assays.

*General Preparation of Analogs **9** (resyntheses):*

The resin (100mg, 85umol) and the aminoalcohol, were mixed in tetrahydrofuran (2ml). To this mixture was added triphenylphosphine (668mg, 2.55mmol) in tetrahydrofuran (2ml) and the mixture stirred vigorously. DEAD (325mg, 1.87mmol) in tetrahydrofuran (2ml) was then added dropwise. After stirring for 24 hr., the mixture was filtered and the resin washed with dichloromethane (10ml x 2), methanol (10ml), tetrahydrofuran (10ml), dimethylformamide (10ml), tetrahydrofuran (10ml), dimethyl- formamide (10ml), methanol (10ml), dichloromethane (10ml x 4) and methanol (10ml x 4). The resin was transferred to a roundbottom flask and treated with 10% propylamine in 1:1 methanol/tetrahydrofuran (10ml) for 24 hr. The resin was filtered and washed with 5ml of tetrahydrofuran and 5 ml of methanol. The filtrate was concentrated under vacuum and purified over a reverse phase HPLC column to give the desired compound as its TFA salt.

Representative analytical data for the resynthesized analogs:

3-((4'-[3-(1-(2-pyridylmethyl))piperidine]ethoxy]phenyl)methyl)-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-6-hydroxybenzothiophene, **21**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 1.05-1.5 (m, 10 H), 1.65 (m, 4 H), 1.41 (m, 4 H), 3.30 (m, 6 H), 4.04 (bt, 2 H), 4.11 (s, 2 H), 4.21 (m, 1 H), 4.60 (s, 2 H), 6.62(d, J = 710Hz, 1 H), 6.71 (d, J = 10 Hz, 2 H), 6.84 (d, J = 10 Hz, 2 H), 7.2-7.6 (m, 9 H), 8.75 (bd, 1 H) ; mass spectrum *m/z* (PFAB) 634.0 (M^+)

2-(4-(2-piperidinoethyloxy)phenyl)-3-(4'-(2-N,N-diisopropylaminoethyloxy)phenyl)-6-hydroxy benzothiophene, **20**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 0.85 (d, 12 H), 1.29 (m, 2 H), 1.41 (m, 4 H), 2.33 (m, 4 H), 2.58 (m, 4 H), 2.85 (t, J = 7.5 Hz, 2 H), 3.64 (t, J = 7.5 Hz, 2 H), 3.90 (m, 4 H), 6.55-7.19 (m, 11 H); mass spectrum *m/z* (PFAB) 586.9 (M^+)

2-(4-(2-(methylsulfonylamido)ethyloxy)phenyl)-3-(4'-(2-piperidinoethyloxy)phenyl)-6-hydroxy benzothiophene, **19**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 1.3-1.8 (m, 6 H), 2.78 (s, 3 H), 2.8-2.9 (m, 2 H), 3.25-3.4 (m, 6 H), 3.75-3.91 (m, 4 H), 4.08 (t, J = 6.6 Hz, 2 H), 6.55 (dd, J = 10, 3.6 Hz, 1 H), 6.67 (d, J = 10 Hz, 2 H), 6.77 (d, J = 9 Hz, 2 H), 6.84 (d, J = 9 Hz, 2 H), 6.98 (d, J = 3.6 Hz, 1 H), 7.09 (d, J = 10 Hz, 1 H), 7.18 (d, J = 9 Hz, 2 H); mass spectrum *m/z* (PFAB) 596 (M^+)

2-(4-(2-(3,4,5-trimethoxybenzamido)ethyloxy)phenyl)-3-(4'-(2-piperidinoethyloxy)phenyl)-6-hydroxybenzothiophene, **18**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 1.5-1.8 (m, 6 H), 2.81 (bt, 2 H), 3.3-3.42 (m, 4 H), 3.58 (m, 5 H), 3.65 (s, 6 H), 3.90 (s, 2 H), 4.02 (t, J = 7.2 Hz, 2 H), 4.09 (m, 2 H), 6.55-7.17 (m, 13 H); mass spectrum *m/z* (PFAB) 697.1 (M^++1)

2-(4-(N-methylpiperidino-2-methyloxy)-phenyl)-3-(4'-(2-piperidinoethyloxy)phenyl)-6-hydroxy benzothiophene, **16**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 1.5-1.85 (m, 15 H), 2.7 (s, 3 H) 2.73-2.90 (m, 2 H), 3.28-3.45 (m, 6 H), 3.91 (s, 2 H), 4.08 (t, J = 6.6 Hz, 2 H), 6.56 (dd, J = 10, 3.6 Hz, 1 H), 6.67 (d, J = 10 Hz, 2 H), 6.76 (d, J = 9 Hz, 2 H), 6.84 (d, J = 9 Hz, 2 H), 7.0 (d, J = 3.6 Hz, 1 H), 7.09 (d, J = 10 Hz, 1 H), 7.20 (d, J = 9 Hz, 2 H); mass spectrum *m/z* (PFAB) 587.3 (M^++1)

2-[4-[3-(3-(3-aza-[5,5]-spiroundecane))propyloxy]phenyl]-3-(4'-(2-piperidinoethyloxy)phenyl)-6-hydroxybenzothiophene, **15**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 1.13-2.03 (m, 22 H), 2.78-3.41(m, 12 H) 3.90 (m, 4 H), 4.09 (t, J = 6.6 Hz, 2 H), 6.56 (dd, J = 10, 3.6 Hz, 1 H), 6.69 (d, J = 10 Hz, 2 H), 6.76 (d, J = 9 Hz, 2 H), 6.85 (d, J = 9 Hz, 2 H), 6.99 (d, J = 3.6 Hz, 1 H), 7.10 (d, J = 10 Hz, 1 H), 7.20 (d, J = 9 Hz, 2 H); mass spectrum *m/z* (PFAB) 657.2 (M^++1)

2-(4-(N-methylpiperidino-3-methyloxy)-phenyl)-3-(4'-(2-piperidinoethyloxy)phenyl)-6-hydroxy benzothiophene, **12**: ^{1}H NMR (300 MHz, methanol-d_4) ∂ = 1.2-1.9 (m, 11 H), 2.11 (m, 1 H), 2.7 (s, 3 H) 2.72-2.90 (m, 3 H), 3.30 (t, J = 6.6 Hz, 2 H), 3.35-3.50 (m, 4 H), 3.68-3.88 (m, 2 H) 3.91 (s, 2 H), 4.10 (t, J = 6.6 Hz, 2 H), 6.58 (dd, J = 10, 3.6 Hz, 1 H), 6.69 (d, J = 10 Hz, 2 H), 6.78 (d, J = 9 Hz, 2 H), 6.84 (d, J = 9 Hz, 2 H), 6.98 (d, J = 3.6 Hz, 1 H), 7.09 (d, J = 10 Hz, 1 H), 7.20 (d, J = 9 Hz, 2 H); mass spectrum *m/z* (PFAB) 586.2 (M^+)

General method of determining apparent association constants (K_{ass})
Inhibitor binding affinities for human α-thrombin and other serine proteases were measured as apparent association constants (K_{ass}) which were derived from inhibition kinetics. Briefly, enzyme inhibition kinetics were performed in 96-well polystyrene plates and reaction rates were determined from the rate of hydrolysis of appropriate p-nitroanilide substrates at 405 nm using a Thermomax plate reader from Molecular Devices (San Francisco, CA). The same protocol was followed for all enzymes studied: 50 μl buffer in each well, followed by 25 μl of inhibitor solution and 25 μl enzyme; within two minutes, 150 μl chromogenic substrate was added to start the enzymatic reactions. The rates of benzoyl-Phe-Val-Arg-p-NA hydrolysis reactions provide a linear relationship with human α-thrombin such that free thrombin can be quantitated in reaction mixtures. Data was analyzed directly as rates by the Softmax program to produce [free enzyme] calculations for tight-binding K_{ass} determinations. For apparent K_{ass} determinations, 5.9 nM human thrombin was used to hydrolyze 0.2mM BzPheValArg-pNA; and 1.34 nM human factor Xa with 0.18 mM BzIle-Glu-Gly-Arg-pNA.

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