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Studies of Benzothiophene Template as Potent Factor IXa (FIXa) Inhibitors in Thrombosis†

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FIXa is a serine protease enzyme involved in the intrinsic pathway of the coagulation cascade. The upstream intervention of the coagulation cascade in selectively inhibiting FIXa would leave hemostasis intact via the extrinsic pathway, leading to an optimum combination of efficacy and safety with low incidence of bleeding. We have identified 2-amindinobenzothiophene template as a lead scaffold for FIXa inhibiton based on its homology with urokinase plasminogen activator (uPA). Subsequent SAR work on the template revealed a number of highly potent FIXa inhibitors, though with moderate selectivity against FXa. X-ray study with one of the analogues demonstrated active site binding interaction with the induced opening of the S1 β pocket and a secondary binding at the S2−S4 sites, which is in direct contrast with the previous finding.

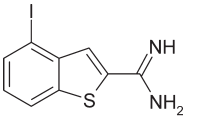
Despite tremendous efforts to prevent and treat thrombotic events, arterial and venous thromboses continue to be the major cause of mortality. Current medical strategy for combating thrombosis involves aspirin as the primary antiplatelet agent for chronic, secondary prevention of arterial thrombosis,[1,2] whereas ticlopidine and clopidgrel add marginal benefits over aspirin.[3,4] Heparin and warfarin are proven anticoagulants for acute and chronic thrombotic disorders, respectively.[5−7] However, these anticoagulants have restricted therapeutic utility due to significant adverse effect, such as bleeding, thrombocytopenia, and need for careful patient monitoring.[7] Therefore, there is a significant medical need for superior antithrombotic agents with a wider therapeutic index. In the past number of years, research efforts have been focused on targeting the specific enzymes of the extrinsic/common pathway in the coagulation cascade, such as thrombin, FXa,[a] and TF/FVIIa.[8] Although many selective and potent thrombin and FXa inhibitors have been advanced to the clinical development, the potential risk of bleeding and a narrow therapeutic window remain for these newer anticoagulants and their safety profiles are yet to be established in clinical trials.[9]

FIXa is a key coagulation factor in the intrinsic pathway of the cascade, essential for amplification of coagulation that results in thrombus formation via FX activation to FXa.[10] Enzymology studies have shown that activation of factor X by IXa/VIIIa is nearly 50 times more efficient than activation by factor VIIa/TF.[11] Furthermore, deficiency in FIXa leads to mild to moderate bleeding tendency in hemophilia B patients.[12] A recent study shows that patients who are heterozygous gene carriers of FIXa have decreased coagulability and are protected from ischemic heart diseases.[13] As FIX is activated both by the stimulation of the intrinsic system and by low level of TF, it is believed that selective inhibition of FIXa would be effective in antithrombosis in low tissue factor sites but not in high tissue factor environments,[14] which will allow for intravascular anticoagulation with maintenance of extravascular hemostasis.[15] Therefore, the upstream inhibition of FIXa at the initiation stage of the cascade would represent an excellent approach for developing selective and safe anticoagulants.

Early approaches to FIXa inhibition have been attempted with the use of antibodies (SB24914),[16] synthetic active site blocked competitive inhibitor,[15] and RNA aptamers.[17] Recently, several groups have reported their small molecule approaches but with limited success, as most of the leads were derived from FXa inhibitor scaffolds.[18] Notably, Transtech has selected a partial FIXa inhibitor, TTP889, for clinical development, although the mechanism of action of this compound has not been published.[19]

We herby report our work in the small molecule approach to the inhibition of FIXa.

As the active sites of FIXa, FXa, and uPA are very similar,[18,20] we evaluated a number of templates reported in literature to seek an initial lead for FIXa inhibitors. After a series of synthetic efforts and molecular modeling studies, we decided to focus on the 2-amidinobenzothiophene template, exemplified by 2-amidino-4-iodobenzothiophene (**1**) as the lead.



(**1**)

Notably, benzothiophene template derived carboxylic acids or their amides have been reported as bronchiodialators.[21]

†PDB code of compound **16** in the FIXa active site is 3LC3.

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[a]Abbreviations: FIXa, factor IXa, a serine protease enzyme within the intrinsic pathway of the blood coagulation cascade; uPA, urokinase plasminogen activator; SAR, structure−activity relationship; FXa, factor Xa, a serine protease enzyme in the blood coagulation cascade; TF/FVIIa, tissue factor and factor VIIa complex.

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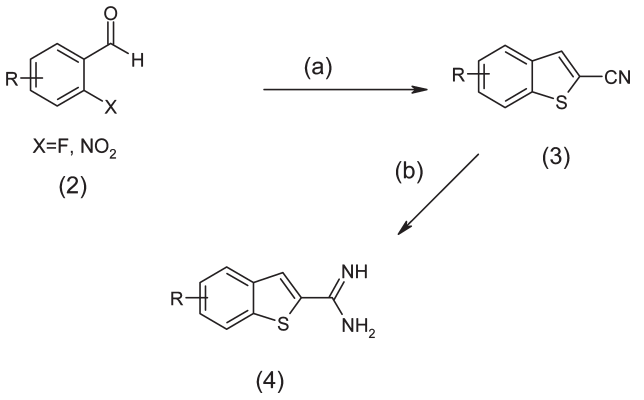
Scheme 1[a]



[a] Reagents: (a) $HSCH_2CN$, DBU, DMF, 60 °C, 71−85%; (b) LiHMDS, THF, 0 °C, then HCl, 99%.

Compound **1** has been reported as a uPA inhibitor with an IC_{50} of 210 nM;[20] it shows a K_i of 0.95 μM against FIXa in our FIXa assay. We envisaged that the FIXa activity and selectivity may be improved through a systematic SAR investigation, as the lead molecule **1** is relatively simple and has a low molecular weight.

The lead optimization strategy for this series was initially aimed at substitutions at the benzo[*b*] ring to establish key functional groups at optimal positions for potency. We decided to leave the selectivity issue to a later stage after gaining further understanding on how the template binds at the FIXa active site. It is known that the S1 sites of uPA and FIXa are structurally very similar to but different from that of FXa, with a serine residue and an alanine residue at position 190, respectively. Our modeling study suggests that substitution at the thiophene ring of the molecule **1** would impede the binding interaction of the amidine group with Asp189 in the smaller S1 pocket.

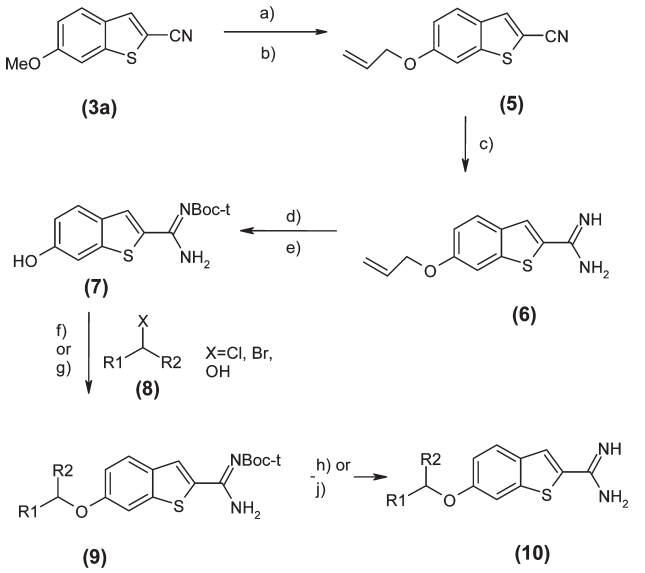
Chemistry

The general synthesis of the substituted benzo[*b*]thiophene-2-carboximidine analogues is shown in Scheme 1. Thus, substituted 2-fluoro-/nitrobenzaldehydes (**2**) were treated with thioacetonitrile (stabilized with amberlyst) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dimethylformamide (DMF) at 60 °C to give the annulated product 2-cyanobenzothiophenes (**3**) in over 70% yield after purification. The conversion of the 2-cyano group to the amidine was readily achieved in high yield by treating compounds **3** with over 1 equiv of lithium hexamethyldisilazide (LiHMDS), followed by the addition of hydrochloric acid (HCl).

To generate a library of O-alkylated analogues efficiently, a more convergent synthetic route with an allyloxy for hydroxy and *t*-BOC for amidine protection strategy was developed. Scheme 2 exemplifies the preparation of 6-alkyoxybenzothiophene analogues from the corresponding 6-methoxybenzothiophene-2-nitrile (**3a**).

Thus, 6-methoxy analogue **3a** was demethylated by boron tribromide (BBr_3) at −78 °C in dichloromethane (DCM), followed by its reprotection by ally bromide in the presence of sodium hydride (NaH) and dimethyl sulfoxide (DMSO) to give the allyloxy intermediate **5**. The 2-amidine compound **6** was furnished with the treatment of **5** by LiHMDS in quantitative yield. The amidine protection with *t*-Boc anhydride and subsequent deprotection of the ally group with phenylsilane

Scheme 2[a]



[a] Reagents: (a) BBr_3, DCM, −78 °C to room temp, 92%; (b) allyl bromide, NaH, DMSO, 0 °C to room temp, 98%; (c) LiHMDS (2 equiv), THF, 0 °C to room temp, then HCl, 100%; (d) *t*-Boc_2O, DIPEA, dioxane−H_2O, 100%; (e) $PhSiH_3$, $Pd(Ph_3P)_4$, DCM, 0 °C, 68%; (f) **8** (when X = Cl, Br), K_2CO_3, DMF, or MeCN; (g) DEAD, Ph_3P, **8** (when X = OH), THF, room temp; (h) TFA, DCM, room temp; (j) $Mg(ClO_4)_2$, MeCN, 80 °C.

and palladium catalyst gave the 6-hydroxy-*t*-Boc amidine intermediate **7**. This key intermediate could be prepared in multigram quantity and coupled with a variety of alkyl halides or alkyl alcohols under Mitsunobu conditions to give the ether linked products **9**. The final removal of the *t*-Boc group was achieved by either trifluoroacetic acid (TFA) or magnesium perchlorate $Mg(ClO_4)_2$.[22]

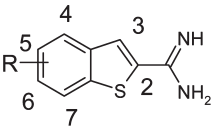
Results and Discussion

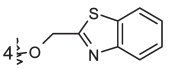
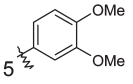
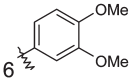
The FIXa inhibitory activities of the analogues prepared according to Schemes 1 and 2 were evaluated in an amidolytic assay using chromogenic substrate S-2366 and human recombinant FIXa (see Experimental Section). FXa assay was carried out as described in the literature.[24] Initial round screening on the analogues substituted with a variety of simple functional groups around the b-ring of the benzothiophene template uncovered a number of active compounds that showed moderate inhibitory activity against FIXa and a limited degree of selectivity against FXa. Some representative examples are illustrated in Table 1.

Although many functional groups were examined, it appeared that only analogues with O- or aryl-linked substitutents showed any degree of activity against FIXa, particularly those substituted at the 4-, 5-, or 6-position. No significant activity was observed for any compounds substituted at the 7-position. This was substantiated by modeling studies that suggest 7-substituted compounds are not well tolerated in the FIXa active site.

At 4-position, the initial SAR indicated a preference for lipophilic groups (compounds **11**−**13**) with gradual enhancement of FIXa activity, though none showed any significant improvement over the lead compound **1**. The phenyl substituted analogue **14** showed the similar potency to compound **12**. The selectivities against FXa were generally poor. At the 5-position, the phenyl substituted analogue **15** was 5-fold less

Table 1. Inhibitory Activities[a] of Substituted Benzothiophene Compounds in FIXa and FXa Assays



Compd	R	Ki(μM) / FIXa[a]	Ki(μM) / FXa[a]
1	4-I	0.95	22.0
11	4-OCH_2COOEt	11.9	ND[b]
12	4-OBn	1.5	6.0
13		0.8	2.6
14	4-Ph	1.6	ND
15	5-Ph	8.0	ND
16		5.9	11.9
17	5-OBn	1.9	3.7
18	6-OBn	3.8	14.8
19	6-OCH_2COOEt	3.8	0.6
20		24.2	ND

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] ND: not determined.

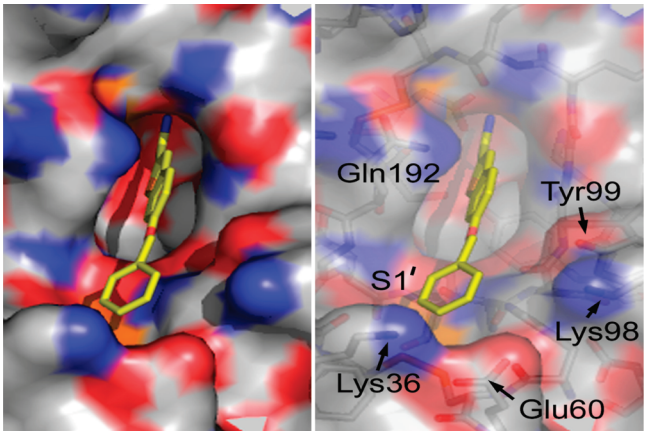
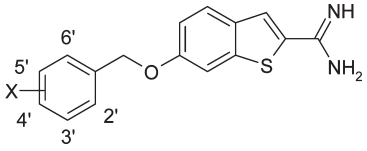


Figure 1. Molecular docking of compound **18** in FIXa active site (1RFN).

Table 2. Inhibitory Activity of Substituted 6-Benzyloxy Compounds in FIXa and FXa Assays



compd	X	K_i (μM), FIXa[a]	K_i (μM), FXa[a]
18	H	3.8	14.8
21	2′-F	23.0	ND[b]
22	3′-F	0.68	6.2
23	4′-F	0.7	7.3
24	2′-Cl	7.2	ND[b]
25	3′-Cl	0.9	4.3
26	4′-Cl	0.45	2.9
27	2′-MeO	26.9	ND[b]
28	3′-MeO	0.45	4.3
29	4′-MeO	0.25	13.1
30	4′-Me	0.9	7.9
31	4′-CO_2Me	11.3	ND[b]
32	3′,4′-F,F	0.45	5.7
33	3′,4′-F,Cl	0.075	0.105
34	3′,4′-Cl,F	0.031	0.055

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] ND: not determined.

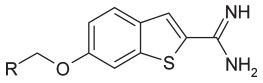
potent than the 4-phenyl analogue **14**. Further exploration on the substitutions of the phenyl ring showed only marginal improvement in FIXa activity, exemplified by 3′,4′-dimethoxyphenyl compound **16**. The 5-benzyloxy analogue **17** showed similar magnitude of activities to the corresponding 4-benzyloxy analogue **12** against both FIXa and FXa. At the 6-position, both O-linked analogues **18** and **19** demonstrated moderate FIXa activities, though with different selectivities against FXa, whereas the 6-phenyl substituted analogue **20** was less potent inhibitor than the 5-phenyl analogue **16**.

A number of 4-, 5-, and 6-substituted analogues were subjected to a series of modeling and docking studies within the FIXa active site, using the published crystal structure of human FIXa.[25] It became apparent that 4- and 5-substituted analogues offered little noticeable interactions except that of the amidine and Asp189. Most of the 4- and 5-substitutents appeared to point toward the solvent front, while the 6-substitutents appeared to point toward the S′ pocket. This is exemplified by our modeling of compound **18** within the FIXa active site, as shown in Figure 1.

Given the potential functional residues, such as Lys98, His57, and Tyr99 within the vicinity of the S′ site available for potential binding interactions, we decided to focus on the SAR work at the 6-position, using analogue **18** as a new lead. Table 2 exemplifies some of the active analogues from our effort in substituting the phenyl ring of compound **18**.

Substitutions at the 2-position of the phenyl ring with halogens (F, Cl) and methoxy groups led to a significant reduction of the FIXa activity (compounds **21**, **24**, **27**), while the same substitutions at either the 3- or 4-position gave more potent FIXa inhibitors (**22**, **23**, **25**, **26**, **28**, **29**) than compound **18**. All these compounds showed a moderate selectivity against FXa, with that of compound **29** reaching greater than 50-fold. Further screening of basic functional groups at the 4-position did not yield many significantly more potent FIXa inhibitors; for example, 4-methyl analogue **32** showed similar

Table 3. Inhibitory Activity for 6-Arylalkyloxy-Substituted Benzothiophene Compounds in FIXa and FXa Assays



compd	R	K_i (μM), FIXa[a]	K_i (μM), FXa[a]
35	$PhCH_2$	21.6	ND[b]
36	3-Py	1.05	3.35
37	2-thiophen	0.35	1.15

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] ND: not determined.

activity to compound **23**, while 4-carboxylate methyl ester **32** showed less activity than the lead molecule **18**. However, analogues with 3,4-disubstitutions appeared to be more potent than the corresponding singly substituted analogues, ranging from moderately improved (compound **32**) to the more dramatically improved (compound **33**, K_i = 75 nM; compound **34**, K_i = 31 nM), though no selectivity improvement was observed against FXa.

Next we also examined the extension of the benzyloxy side chain of compound **18** and the bioisostere replacement of its phenyl ring with various heterocycles. As the majority of those screened did not lead to significant improvement over the lead molecule **18**, Table 3 only shows a few typical examples.

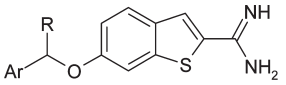
One carbon extension of the benzyloxy side chain on the lead **18** resulted in a compound (**35**) with 6-fold less activity, whereas the 3-pyridyl replacement of the phenyl group gave some improvement of activity (**36**). The more dramatic improvement of activity was observed with the 2-thiophene analogue (**37**), showing over 10-fold greater FIXa activity than the lead molecule (**18**).

As shown in Table 1, compound **19** with an O-linked acetate group showed equipotent FIXa activity to the benzyloxy analogue **18**, and we envisaged that a combination of both groups, preferably with those more optimal aryl groups as shown in Tables 2 and 3, would enhance the activity further. Therefore, we investigated a number of branched analogues at the benzylic position of compound **18**, including those with carboxylate groups. Table 4 shows some selected examples.

Introduction of a methyl or amide group at the benzylic position of compound **18** gave compounds **38** and **39** with much reduced FIXa activity when compared with analogue **23**. However, introduction of methyl carboxylate gave an over 17-fold more potent FIXa inhibitor (**40**) than compound **23**, though it was equipotent against FXa. Its hydrolyzed product acid **41** showed a further 2-fold improvement of FIXa activity over the ester analogue **40** but with 44-fold better selectivity against FXa. Similarly, the corresponding 4-chlorophenyl analogue ester **42** was also 2-fold more potent than its 4-fluoro analogue **40**, but our attempts to assay the corresponding carboxylic acid of this compound failed because of its insolubility. Further SAR studies with the extended esters **43** and **44** based on ester **42** gave a similar magnitude of activities against both FIXa and FXa. Interestingly, introduction of an ethyl carboxylate ester group to the potent 2-thiophene analogue (**37**) gave a very potent FIXa inhibitor (**45**, K_i = 3 nM). Again, no selectivity was observed against FXa. Our attempts to hydrolyze this ester also met with difficulty in solubility, which was difficult to characterize and assay.

In order to understand how this benzothiophene template binds at the FIXa active site, we selected a number of

Table 4. Inhibitory Activity for 6-Substituted Branched Benzothiophene Compounds in FIXa and FXa Assays



Compd.	Ar	R	Ki(μM) FIXa[a]	Ki(μM) FXa[a]
38	4'-F-Ph	Me	11.3	ND[b]
39	4'-F-Ph	$CONH_2$	1.98	45
40	4'-F-Ph	CO_2Me	0.040	0.044
41	4'-F-Ph	CO_2H	0.021	0.91
42	4'-Cl-Ph	CO_2Me	0.020	0.017
43	4'-Cl-Ph	CH_2CO_2Me	0.028	0.033
44	4'-Cl-Ph	$(CH_2)_2CO_2Me$	0.036	0.037
45		CO_2Et	0.003	0.025

[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%. [b] ND: not determined.

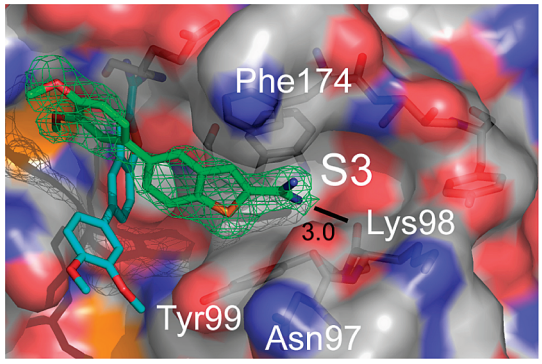


Figure 2. X-ray structure of compound **16** in the FIXa active site (PDB code 3LC3).

analogues substituted at the 4-, 5-, or 6-position for X-ray crystallography studies, using a truncated form of human factor IXa,[26] Compound **16** was successfully cocrystallized with FIXa, yielding a high resolution (1.9 Å) crystal structure (3LC3) as shown in Figure 2.

It appeared that two molecules of compound **16** were present in the crystal structure; one in the primary binding site (S1, S1′), the other at the S3−S4 site. At the S1 site, hydrogen bonds were formed as expected between the amidine moiety and the carboxylate of Asp189 (distance between nitrogen and oxygen, 2.9 and 3.0 Å). The benzothiophene template clearly laid in the hydrophobic S1 pocket. Compound **16** with 5-(3′,4′-dimethoxyphenyl) substitution at the

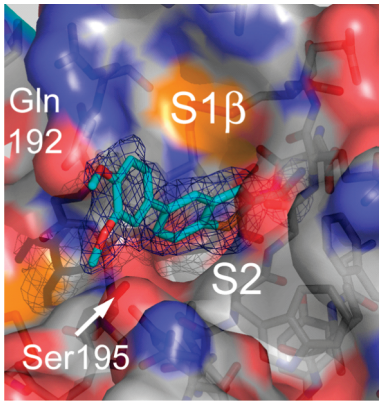


Figure 3. Selected view of compound **16** at primary binding S1 site with an induced opening of S1 β site.

benzothiophene template appeared to have induced the opening of the S1 β pocket as shown more clearly in Figure 3. This is in direct contrast with the previous X-ray crystal structure (1RFN),[25] using *p*-aminobenzamidine as the ligand, where the S1 β site was seen closed.

The secondary binding of compound **16** at S3/S4 site was unexpected, as it was observed previously that S2 or S3/S4 sites are blocked by Tyr99.[25] As shown in Figure 2, the Tyr99 residue appeared to have moved away from its previous reported position because of a dramatic rearrangement of the 99-loop, leaving open the S2−S4 sites for the secondary molecule binding. The amidine group of the second molecule (**16**) formed hydrogen bonding interaction with the carbonyl group of Lys98. The benzothiophene template lay directly within the lipophilic pocket surrounded by Trp215, Phe174, and Tyr99. The shift of Tyr99 residue and secondary binding of molecule **16** at the S3/S4 site is significant, as it has been suggested that blockage of either the S2 or S3/S4 site by Tyr99 may be one of the reasons that no potent low molecular weight active site FIXa inhibitors have been reported so far.[25] Our findings above suggest it is possible to develop potent FIXa inhibitors.

Conclusion

In the present report we have described some synthesis and initial rounds of SAR studies on the benzothiophene template as FIXa inhibitors. Our initial focus on its 6-position led to a number of highly potent FIXa inhibitors, albeit the selectivity against FXa was moderate (up to 50-fold). We have demonstrated that a moderately active compound (**16**) binds at the FIXa active site through X-ray study with an induced S1 β site opening and a secondary binding interaction at the S3/S4 site, due to Tyr99 residue shift and a dramatic 99-loop rearrangement. These findings are in direct contrast with those reported previously in literature,[25] paving the way for our modified optimization strategy in the development of potent and selective FIXa inhibitors, which will be the subject of our next publications.

Experimental Section

Methods and Materials. Reagents, starting materials, and solvents were purchased from common commercial suppliers and used as received or distilled from the appropriate drying agent. Reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen or argon. Precoated aluminum backed silica gel 60 F_{254} plates with a layer thickness of 0.25 mm were used for thin layer chromatography, and the stationary phase for preparative column chromatography using medium pressure was silica gel 60, mesh size 40−60 μm, from E. Merck, Darmstadt, Germany.

NMR spectra were obtained using a Bruker ACF 400 operating at 400 MHz, and the ^{1}H shifts (ppm) were calibrated to that of residual $CHCl_3$ in $CDCl_3$, at 7.26 ppm. Mass spectra were obtained in the indicated mode using a Finnigan SSQ 710L machine. Melting points were determined using an Electrothermal 9100 series apparatus.

The purities of all compounds tested in biological systems were assessed as being >95% using analytical LC−MS, which was performed using a Waters 600E pump, Waters 2767 autosampler, Waters 2487 IEEE UV detector (set at 254 nm), and Waters Micromass ZQ detector (ESI mode). Elution was done with a gradient of 5−95% solvent B in solvent A (solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in acetonitrile) over 11 min through a Gemini 5 μm C18 110A (50 mm × 4.60 mm) column at 1.5 mL min^{-1}. Area % purity was measured at 254 nm. Preparative LC−MS was performed using a Waters 600E pump, Waters 515 makeup pump, Waters 2767 autosampler, Waters 2487 IEEE UV detector (set at 254 nm), and Waters Micromass ZQ detector (ESI mode). Elution was done with a gradient of 5−95% solvent B in solvent A (solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in acetonitrile) over 8 min through a X-Terra Prep MS C_{18} 5 μm (19mm × 50 mm) column at 20 mL min^{-1}. The makeup pump was used with methanol. Isolated fractions yielded pure material as the formate salt after lyophilization.

Microanalyses of representive compounds were performed by MEDAC Ltd. UK. Where analyses are indicated only by the symbols of the elements, results obtained were within 0.4% of the theoretical values. All compounds (**3**−**45**) quoted in this paper have been patented.[23]

General Procedure A for Substituted Benzothiophene-2-carboximindine Analogues (Scheme1). 2-Cyanobenzothiophene (3) Synthesis. To a solution of arylaldehydes (1 equiv) in DMF (3 mL/mmol) and DBU (2 equiv) was added thioacetonitrile (1.2 equiv) dropwise at 0 °C. The solution was stirred at 0 °C for 20 min, then heated to 40 °C for 16−20 h. The reaction mixture was allowed cool to room temperature, the solvent was reduced in vacuo, and the product was purified via silica chromatography (EtOAc/hexane mix), yielding the intermediates **3**.

Amidines (4) Formation. To a solution of nitriles **3** (1 equiv) in THF (3 mL/mmol) at 0 °C under argon was added a solution of lithium hexamethyldisilazide (1 M in THF, 1.2 equiv) dropwise. The solution was stirred at 0 °C for 1.5 h, then allowed to warm room temperature for 45 min.

The reaction was quenched with methanol and the solvent removed. The residue was redissolved in THF (3 mL/mmol). Then HCl (4 M in dioxane, 2−3 equiv) was added slowly. The mixture was stirred at room temperature for 30 min and then filtered and the solid collected to yield desired amidines **4**.

6-Allyloxy-2-cyanobenzothiophene (5). To a solution of 6-methoxy-2-cyano-benzothiophene (**3a**) (17.1 g, 90.5 mmol) in DCM (200 mL) at −78 °C under argon was added BBr_3 (0.1 M, 180.9 mL, 180.9 mmol) dropwise over 1 h. After addition was complete the mixture was stirred for a further 2 h, then allowed to warm to room temperature and stirred overnight. The reaction was quenched with saturated aqueous $NaHCO_3$ (500 mL) and back-extracted with EtOAc. The organic layers were washed with H_2O (2 × 200 mL) and brine (1 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo to afford the intermediate 6-hydroxy-2-cyanobenzothiophene compound as a dark-orange/brown solid, 14.6 g, 92%, which was taken directly to the next step. ^{1}H NMR (MeOH-d_4, 400 MHz): δ 7.93 (s, 1H), 7.74−7.76 (m, 1H), 7.26 (s, 1H), 6.96−7.01 (m, 1H). MS *m*/*z* (ES+) 175.9 $[M + H]^+$.

The above intermediate (14.5 g, 82.8 mmol) was stirred in DMSO (130 mL) at 0 °C under argon, and NaH (60% dispersion, 7.89 mL, 91.1 mmol) was added portionwise. The reaction

mixture was stirred for 15 min before addition of allyl bromide (7.89 mL, 91.1 mmol). The reaction mixture was warmed to room temperature and stirred for 48 h before pouring onto H_2O/ice (600 mL). This was extracted with EtOAc (400 mL), and the organic layer was washed with 1 M aqueous NaOH, H_2O, and brine, dried ($MgSO_4$), and concentrated to give the title compound **5** as a beige solid, 17.6 g, 98%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67−7.72 (m, 2H, ArH), 7.20 (m, 1H, ArH), 7.35 (m, 1H, ArH), 6.01 (m, 1H, CH═), 5.38 (dd, 1H, CH═), 5.27 (dd, 1H, CH═), 4.55 (d, 2H, CH_2O). MS *m*/*z* (ES+) 215.95 $[M + H]^+$.

6-Allyloxybenzothiophene-2-carboximidine (6). To a solution of compound **5** (5.35 g, 25.0 mmol) in THF (70 mL) at −10 °C under argon was added LiHMDS (1.0 M, 27.4 mL, 27.3 mmol) dropwise over 60 min. After the mixture was warmed to room temperature and stirred for 5 h, an additional 1 equiv of LiHMDS was added. HCl in dioxane (4.0 M, 6.84 mL, 27.3 mmol) was added dropwise and the reaction mixture stirred at room temperature overnight. The reaction mixture was concentrated, and addition of ether precipitated the title compound **6** as the HCl salt, 6.68 g, 100%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.77 (s, 1H, ArH), 7.71 (m, 1H, ArH), 7.15 (s, 1H, ArH), 7.01 (m, 1H, ArH), 6.72 (br, 4H, amidine + HCl), 6.08 (m, 1H, CH═), 5.43 (m, 1H, CH═), 5.28 (m, 1H, CH═), 4.64 (s, 2H, CH_2O). MS *m*/*z* (ES+), 233.0 $[M + H]^+$.

6-Hydroxybenzothiophene-2-*t*-Boc-carboximidine (7). To a solution of **6** (7.00 g, 30.1 mmol) in dioxane/H_2O (1:1, 300 mL) at room temperature under argon was added DIPEA (8.50 mL, 60.3 mmol), and the reaction mixture was stirred for 30 min. $(BOC)_2O$ (7.27 g, 33.2 mmol) was added and the mixture stirred for a further 1 h. The reaction mixture was extracted with EtOAc (300 mL), and the organics were washed with H_2O (2 × 200 mL) and saturated aqueous NaCl (2 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo. Trituration with diethyl ether yielded the 6-allyloxybenzothiophene-2-*t*-Boc-carboximidine intermediate (9.77 g, 100%), which was taken directly to the next step. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.08 (s, 2H, NH_2), 8.23 (s, 1H, ArH), 7.79 (m, 1H, ArH), 7.56 (d, 1H, ArH), 7.06 (m, 1H, ArH), 5.45 (m, 1H, CH═), 5.30 (m, 1H, CH═), 5.27 (m, 1H, CH═), 4.67 (s, 2H, CH_2O), 1.46 (s, 9H, *t*-Bu). MS *m*/*z* (ES+) 333 $[M + H]^+$.

To a solution of the above product (9.77 g, 29.4 mmol) in DCM (110 mL) at room temperature under argon was slowly added $Pd(PPh_3)_4$ (847 mg, 0.70 mmol). After being stirred for 10 min, the reaction mixture was cooled to 0 °C and phenylsilane (11.0 mL, 58.9 mmol) was added dropwise. The resulting solution was stirred at room temperature for 30 min. Then the reaction mixture was quenched with H_2O (300 mL) and the aqueous layers were back-extracted with DCM. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by silica chromatography, eluting with a solvent gradient of 15−50% ethyl acetate in hexane, yielded the title compound **7** as a yellow semisolid (6.0 g, 68%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.0 (br, 1H, OH), 9.04 (br, 2H, NH2), 8.18 (s, 1H, ArH), 7.71 (d, 1H, ArH), 7.25 (d, 1H, ArH), 6.91 (m, 1H, ArH), 1.46 (s, 9H, *t*-Bu). MS *m*/*z* (ES+) 293 $[M + H]^+$.

General Procedure B for the Preparation of 6-Alkoxybenzothiophene-2-*t*-Boc-carboximidines (9). To a solution of compound **7** (1.0 equiv, 150 mg, 0.51 mmol) in DMF (5.0 mL per 0.5 mmol of **7**) was added the alkyl bromides **8** (1.1 equiv), followed by K_2CO_3 (1.1 equiv). The reaction mixture was heated under reflux until completion was evident by TLC analysis. The residue was purified by silica chromatography, yielding the title compounds **9**.

General Procedure C for the Preparation of 6-Alkoxybenzothiophene-2-*t*-Boc-carboximidines (9). To a solution of compound **7** (1.0 equiv, 150 mg, 0.51 mmol), the alkyl alcohols (**8**, X = OH) (1.5 equiv), and PPh_3 (1.5 equiv) in THF (2 mL per 0.5 mmol of 7) at room temperature was added a solution of DEAD (1.5 equiv) in THF dropwise. After being stirred at room temperature until completion, the reaction mixture was concentrated in vacuo and purified by silica chromatography, yielding the title compounds **9**.

General Procedure D for the Deprotection of BOC Amidines (9) with TFA Preparation of 6-Alkoxybenzothiophene-2-carboximidines (10). The BOC protected amidines **9** were dissolved in 10−20% TFA/CH_2Cl_2 (10−15 mL/mmol) and stirred at room temperature for 2−6 h. The solvent was removed, the residue triturated with excess diethyl ether and filtered, and the solid collected to give the desired product **10** as the TFA salt. If required, further purification was performed via LC−MS.

General Procedure E for the Deprotection of BOC Amidines (9) with Mg(ClO_4) Preparation of 6-Alkoxybenzothiophene-2-carboximidines (10). To a solution of BOC protected amidine **9** (1 equiv) in acetonitrile (10 mL/mmol) was added $MgClO_4$ (1.5 equiv) under an argon atmosphere, and the mixture was heated to 70 °C for 16−20 h. The mixture was allowed to cool to room temperature and the solvent concentrated in vacuo. The crude residue was purified by silica chromatography (methanol/CH_2Cl_2 mix) to yield the desired products **10**.

General Procedure F for the Hydrolysis of Esters. To a solution of ester **10** (when R1 = CO_2Me) (1 equiv) in THF (10 mL/mmo) was added a solution of aqueous lithium hydroxide (1.0 M, 1 equiv), and the mixture was stirred at room temperature for 3−5 h. The solvent was reduced to low volume. The residue was partitioned between CH_2Cl_2 and H_2O, and the phases were separated. The aqueous layer was washed with CH_2Cl_2, acidified to pH 5 with acetic acid, and then extracted with ethyl acetate (×3). The ethyl acetate extracts were combined, dried over $MgSO_4$, and filtered and the solvent was removed to yield the desired carboxylic acid products (**10**, when R1 = CO_2H).

4-Benzyloxybenzo[*b*]thiophene-2-carboxamidine (12). 12 was synthesized in an analogous fashion to general procedure A to give the title compound. ^{1}H NMR (DMSO-d_6): δ 8.14 (s, 1H, H3) 7.55 (m, 3H, 3ArH), 7.42 (m, 4H, 4ArH), 7.04 (d, 1H, J = 7.70 Hz, Ar-H7), 5.30 (s, 2H, CH_2). MS *m*/*z* (ES+) 283 $[M + H]^+$.

5-(3,4-Dimethoxyphenyl)benzo[*b*]thiophene-2-carboxamidine Hydrochloride (16). 16 was synthesized according to the general procedure A (23% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.59 (s, 2H), 9.22 (s, 2H), 8.41 (s, 1H), 8.33 (s, 1H), 8.25 (d, 1H, J = 8 Hz), 7.92 (dd, 1H, J = 1 Hz and J = 8 Hz), 7.32 (d, 2H, J = 8 Hz), 7.19−7.03 (m, 2H), 3.88 (s, 3H), and 3.82 (s, 3H). MS *m*/*z*: 349.85 $(M + H)^+$.

6-Benzyloxybenzo[*b*]thiophene-2-carboxamidine (18). 18 was synthesized in an analogous fashion to general procedure A to give the title compound. ^{1}H NMR (DMSO-d_6): δ 8.01 (s, 1H, Ar-H3) 7.85 (d, 1H, J = 8.8 Hz, Ar-H5), 7.74 (s, 1H, Ar-H7), 7.49 (d, 1H, J = 7.6 Hz, Ar-H-2′), 7.40 (t, 2H, J = 7.0 Hz, Ar-H3′), 7.34 (d, 1H, J = 6.6 Hz, Ar-H4′), 7.14 (d, 1H, J = 8.8 Hz Ar-H4), 5.20 (s, 2H, CH_2). MS *m*/*z* (ES+) 283 $[M + H]^+$.

6-(3-Chloro-4-fluorobenzyloxy)benzo[*b*]thiophene-2-carboxamidine. Compound with Trifluoroacetic Acid (34). The synthesis was done according to general procedure C followed by general procedure D to yield the title compound as a beige solid, 24% over two steps. ^{1}H NMR (DMSO, 400 MHz): δ 9.5 (bs, 1H, NH), 8.3 (s, 1H, ArH), 8.01 (dd, 1H, ArH), 7.9 (s, 1H, ArH), 7.73 (dd, 1H, ArH), 7.64 (m, 2H, ArH), 7.31 (d, 1H, ArH), and 5.2 (s, 2H, methylene H). LC−MS *m*/*z* (ES^+) 335 $[M + H]^+$.

6-(Thiophen-2-ylmethoxy)benzo[*b*]thiophene-2-carboxamidine (37). 37 was synthesized in an analogous fashion to general procedure A to give the title compound. ^{1}H NMR (DMSO-d_6): δ 8.54 (s, 2H,NH_2), 8.15 (s, 1H, ArH), 7.89 (d, 1H, J = 8.88 Hz, ArH), 7.65 (d, 1H, J = 2.02 Hz, ArH), 7.42 (dd, 1H, J = 1.02 and 5.05 Hz, ArH), 7.22 (m, 2H, 2 ArH), 7.01 (m, 1H, ArH), 5.39 (s, 2H, CH_2). MS *m*/*z* (ES+) 288.9 $[M + H]^+$.

3-(2-Carbamimidoylbenzo[*b*]thiophen-6-yloxy)-3-(4-chlorophenyl)propionic Acid Methyl Ester Trifluoroacetic Acid Salt (43). 43 was synthesized according to general procedures C and D with the preparation of the alcohol as follows. Sodium borohydride was added to a solution of methyl (4-chlorophenyl)-acetate in methanol at room temperature and stirred for 30 min. The mixture was partitioned between ethyl acetate and water,

extracted with ethyl acetate (30 mL × 3), dried over magnesium sulfate, filtered, and solvent was removed. MPLC (4:1 hexane/ethyl acetate) was conducted to yield a colorless oil (489 mg, 2.3 mmol, 48%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.38 (s, 4H), 5.61 (d, 1H, J = 4.9 Hz), 4.97 (m, 1H), 3.58 (s, 3H), 2.64 (m, 2H).

This compound was then used in the general Mitsunobu procedure C to yield a colorless gum (30 mg, 0.062 mmol, 112%). MS m/z: 489.03 $(M + H)^+$. This residue was then BOC deprotected according to the general TFA/DCM procedure D to yield the desired product, a white crystalline solid (1.0 mg, 0.0019 mmol, 3%). ^{1}H NMR (DMSO-d_6 400 MHz): δ 9.14 (br s, 4H), 8.32 (s, 1H), 8.03 (d, 1H, J = 8.8 Hz), 7.84 (s, 1H), 7.68 (d, 2H, J = 8.4 Hz), 7.55 (d, 2H, J = 8.4 Hz), 7.28 (m, 1H), 6.07 (m, 1H), 3.74 (s, 3H), 3.19 (m, 2H). MS m/z: 389.03 $(M + H)^+$.

4-(2-Carbamimidoylbenzo[*b*]thiophen-6-yloxy)-4-(4-chlorophenyl)butyric Acid Methyl Ester Trifluoroacetic Acid Salt (44). Methyl iodide (0.32 mL, 5.2 mmol) was added to a solution of 3-(4-chlorobenzoyl)propionic acid (1.00 g, 4.7 mmol) and potassium carbonate (716 mg, 5.2 mmol) in dry acetonitrile (30 mL) under inert atmosphere and stirred at 85 °C for 16 h. The mixture was partitioned between DCM (10 mL) and NaOH (1.0 mol solution, 10 mL) and extracted with DCM (10 mL × 3). The organics were dried and filtered and solvent was removed to yield a white crystalline solid (429 mg, 1.9 mmol, 40%). This was then dissolved in MeOH (20 mL). $NaBH_4$ (77 mg, 2.1 mmol) was added portionwise, and the mixture was stirred at room temperature for 1.5 h, water (15 mL). DCM (15 mL) was added and the mixture stirred for 30 min. The sample layer was extracted with DCM (10 mL × 3). The organics were dried and filtered, and solvent was removed. MPLC (4:1 hexane/ethyl acetate) was conducted to yield the hydroxyl compound as a colorless oil (259 mg, 1.1 mmol, 60%). MS m/z: 228.92 $(M + H)^+$. This compound was then coupled with **7** via the general Mitsunobu procedure C to yield a yellow oil (43 mg, 0.085 mmol, 33%). ^{1}H NMR (DMSO-d_6 400 MHz): δ 8.88 (br s, 2H), 8.01 (s, 1H), 7.59 (d, 1H), 7.27 (m, 5H), 6.90 (d, 1H), 5.40 (m, 1H), 3.16 (s, 3H), 2.27 (m, 2H), 2.26 (m, 2H).

4-(2-Carbamimidoylbenzo[*b*]thiophen-6-yloxy)-4-(4-chlorophenyl)butyric acid methyl ester trifluoroacetic acid salt (**44**) was synthesized via BOC deprotection of the above oil according to the general TFA/DCM procedure D to yield the desired product, a white crystalline solid (2.6 mg, 0.005 mmol, 6%). ^{1}H NMR (DMSO-d_6 400 MHz): δ 8.99 (br s, 4H), 8.03 (s, 1H), 7.73 (d, 1H), 7.45 (s, 1H), 7.24 (m, 4H), 6.96 (m, 1H), 5.36 (m, 1H), 3.14 (s, 3H), 2.25 (m, 2H), 2.01 (m, 2H). MS m/z: 402.97 $(M + H)^+$.

(2-Carbamimidoylbenzo[*b*]thiophen-6-yloxy)thiophen-2-ylacetic Acid Ethyl Ester (45). 45 was synthesized according to general procedures B and E, yielding the desired product as a colorless oil (9% yield). ^{1}H NMR (CD_3OD): δ 8.07 (s, 1H), 7.85 (d, 1H), 7.5 (d, 1H), 7.4 (dd, 1H), 7.22 (d, 1H), 7.16 (dd, 1H), 6.95 (d, 1H), 6.24 (s, 1H), 4.15 (m, 2H), 1.12 (t, 3H). LC−MS (ES^+) m/z: 361 $[M + H]^+$.

FIXa Assay. Recombinant Factor IX Production. A recombinant form of human factor IX was prepared to overcome potential problems with the use of ethylene glycol in a factor IXa inhibition assay. A DNA fragment encoding the full length mature factor IX protein was amplified from human liver cDNA (Novagen; forward primer 5′-gggaattccatatgtataattcaggtaaattgg-3′ and reverse primer 5′-ggagtcccaagctttcactcgagagtgagctttgttttttccttaatccagttgacataccg-3′) and, after cloning in pUC18 and amplification in JM109 *E.coli*, cloned into pET22b(+) using restriction sites for *Nde*I and *Xho*I. This cloning procedure allowed an N-terminal methionine and C-terminal 6xHis tag to be incorporated to facilitate expression and purification.

The resultant F-FIX protein was expressed in BL21(DE3) *E.coli* with induction by IPTG and purified from inclusion bodies: after sonication of cells, the insoluble fraction was solubilized by addition of 6 M guanidine hydrochloride and F-FIX purified by Ni-NTA affinity chromatography. Protein was refolded over 4 days at 4 °C in a solution containing 500 mM L-arginine and 0.5 mM L-cysteine, then dialyzed into a Tris/NaCl buffer in two overnight steps.

Activation of Purified Factor IX. Recombinant factor IX was activated by overnight incubation at 11 °C with factor XIa (HTI, 0.5% w/v) and then further purified by Ni-NTA affinity chromatography before final dialysis into 20 mM Tris, 50 mM NaCl (pH 8.0).

Assay. The factor IXa amidolytic assay was performed in 50 mM Tris, 100 mM NaCl, 5 mM $CaCl_2$, 0.5% PEG 6000 (pH 7.4) in a VersaMax plate reader. The S-2366 chromagenic substrate (Quadratech) was used at a final concentration of 500 μM, and the change in absorption was recorded at 405 nm using SoftMax software. Test compounds were assayed at least three times at a range of concentrations, and an IC_{50} value was established using Prism software (GraphPad).

Molecular Modeling. Molecular modeling of compounds was performed with Chemical Computing Group's MOE software (Montreal, Canada).

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Supporting Information Available: Experimental and spectroscopic information for additional compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Schror, K. Antiplatelet drugs: a comprehensive review. *Drugs* **1995**, *50*, 7–28.
(2) Wood, A. J. J. Aspirin, heparin and fibrinolytic therapy in suspected acute myocardial infarction. *N. Engl. J. Med.* **1997**, *336*, 847–860.
(3) Feuerstein, G.; Ruffolo, R. R., Jr. A novel anti-platelet drug for prevention of thrombotic disorders. *Expert Opin. Invest. New Drugs* **1994**, *3*, 1163–1170.
(4) Feuerstein, G.; Ruffolo, R. R., Jr. Clopidogrel−pharmacological profile of a novel anti-thrombotic agent. *Expert Opin. Invest. New Drugs* **1995**, *4*, 425–430.
(5) Thomas, D. P. Does low molecular weight heparin cause less bleeding? *Thromb.Haemostasis* **1997**, *78*, 1422–1425.
(6) Hirsch, J.; Sirangusa, S.; Cosmi, B.; Ginsberg, J. S. Low molecular weight heparin (LMWH) in the treatment of patients with acute venous thromboembolism. *Thromb. Haemostasis* **1995**, *74*, 360–363.
(7) Topol, E. J. Toward new frontier in myocardial reperfusion therapy: emerging platelet pre-eminence. *Circulation* **1997**, *97*, 211–218.
(8) (a) Abbenante, G.; Fairlie, D. P. Protease inhibibitors in the clinic. *Med. Chem.* **2005**, *1*, 71–104. (b) Quan, M. L.; Smallheer, J. M. The race to an orally active FXa inhibitor: recent advances. *Curr.Opin. Drug Discovery Dev.* **2004**, *7* (4), 460–469. (c) Frederick, R.; Pochet, L.; Charlier, C.; Masereel, B. Modulators of the coagulation cascade: focus and recent advances in inhibitors of tissue factor, factor VIIa and their complex. *Curr. Med. Chem.* **2005**, *12*, 397–417.
(9) Hirsh, J.; O'Donnell, M.; Weitz, J. I. New anticoagulants. *Blood* **2005**, *105*, 453–463.
(10) Feuerstein, G. Z.; Stern, D. The coagulation factor lottery: Is 9 the winning number? An essay on future oral anticoagulants. *Drug Discovery Today: Ther. Strategies* **2005**, *2* (3), 279–283.
(11) Lawson, J. H.; Mann, K. G. Cooperative activation of human factor IX by the human intrinsic pathway of blood coagulation. *J. Biol. Chem.* **1991**, *266*, 11317–11327.
(12) McGraw, R. A.; Davis, L.; Lundblad, R. L.; Stafford, D. W.; Roberts, H. R. Structure and function of FIX: defects in hemophilia B. *Clin. Hematol.* **1985**, *14*, 359–385.
(13) Sramek, A.; Kriek, M.; Rosendaal, F. R. Decreased mortality of ischaemic heart disease among carriers of haemophilia. *Lancet* **2003**, 351–354.
(14) (a) McKean, M.-L.; Adelman, S. J. Future therapies for the prevention and treatment of venous and arterial thrombosis. *Expert Opin. Invest. Drugs* **1998**, *7*, 687–690. (b) Weitz, J. I.; Bates, S. M. New anticoagulants. *J. Thromb. Haemostasis* **2005**, *3*, 1843–1853.
(15) Spanier, T. B.; Chen, J. M.; Oz, M. C.; Edwards, N. M.; Kisiel, W.; Stern, D. M.; Rose, E. A.; Schmidt, A. M. Selective anticoagulation

with active site-blocked factor IXa suggests separate roles for intrinsic and extrinsic coagulation pathways in cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* **1998**, *116*, 860–869.
(16) Toomey, J. R.; Blackburn, M. N.; Storer, B. L.; Valocik, R. E.; Koster, P. F.; Feuerstein, G. Z. Comparing the antithrombotic efficacy of a humanized anti-factor IX(a) monoclonal antibody-(SB249417) to the low molecular weight heparin enoxaparin in a rat model of arterial thrombosis. *Thromb. Res.* **2000**, *100*, 73–79.
(17) Dyke, C. K.; Steinhubl, S. R.; Kleiman, N. S.; Cannon, R. O.; Aberle, L. G.; Lin, M.; Myles, S. K.; Melloni, C.; Harrington, R. A.; Alexander, J. H.; Becker, R. C.; Rusconi, C. P. First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology. *Circulation* **2006**, *114* (23), 2490–2497.
(18) (a) Batt, D. G.; Qiao, J. X.; Modi, D. P.; Houghton, G. C.; Pierson, D. A.; Rossi, K. A.; Luettgen, J. M.; Knabb, R. M.; Jadhav, P. K.; Wexler, R. R. 5-Amidinoindoles as duel inhibitors of coagulation factors IXa and Xa. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5269–5273. (b) Smallheer, J. M.; Alexander, R. S.; Wang, J.; wang, S.; Nakajima, S.; Rossi, K. A.; Smallwood, A.; Barbera, F.; Burdick, D.; Luettgen, J. M.; Knabb, R. M.; Wexler, R. R.; Jadhav, P. K. SAR and factor IXa crystal structure of a dual inhibitor of factors IXa and Xa. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5263–5267. (c) Qiao, J. X.; Cheng, X.; Modi, D. P.; Rossi, K. A.; Luettgen, J. M.; Knabb, R. M.; Jadhav, P. K.; Wexler, R. R. 5-Amidinobenzo[b]thiophenes as dual inhibitors of factors IXa and Xa. *Bioorg. Med. Chem. Lett.* **2005**, *15* (1), 29–35. (d) Vijaykumar, D.; Sprengeler, P. A.; Shaghafi, M.; Spencer, J. R.; katz, B. A.; Yu, C.; Rai, R.; Young, W. B.; Schultz, B.; Janc, J. Discovery of novel hydroxyl pyrazole based factor IXa inhibitor. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2796–2799.
(19) Rothlein, R.; Shen, J. M.; Naser, N.; Gohimukkula, D. R.; Caligan, T. B.; Andrews, R. C.; Schmidt, A. M.; Rose, E. A.; Mjalli, A. M. M. TTP889, a novel orally active partial inhibitor of FIXa inhibits clotting in two A/V shunt models without prolonging bleeding times. *Blood* (*ASH Annu. Meet. Abstr.*) **2005**, *106*, 1886.
(20) Katz, B. A.; Mackman, R.; Luong, C.; Radika, K.; Martelli, A.; Sprengeler, P. A.; Wang, J.; Chan, H.; Wong, L. Structure basis for selectivity of a small molecule, S1-binding, submicromolar inhibitor of urokinase-type plasminogen activator. *Chem. Biol.* **2000**, *7*, 299–312.
(21) Ho, P. P. K.; Soper, Q. F. Improvements in or Relating to Benzothiophene Derivatives as Antiasthmatic Agents. EP 1984/0139464, A1, **1984**.
(22) Stafford, J. A.; Brakeen, M. F.; Karanewsky, D. S.; Valvano, N. L. A highly selective protocol for the deprotection of BOC-protected amides and carbamates. *Tetrahedron Lett.* **1993**, *34* (49), 7873–7876.
(23) Wang, S.; Blench, T.; Marlin, F.; Beck, R.; Dickinson, R. Heterocyclic Compounds and Their Use in the Treatment of Cardiovascular Diseases. WO 2007/096362 A1, **2007**.
(24) Kettner, C.; Mersinger, L.; Knabb, R. The selective inhibition of thrombin by peptides of boroarginine. *J. Biol. Chem.* **1990**, *265*, 18289–18297.
(25) Hopfner, K.-P.; Lang, A.; Karcher, A.; Sichler, K.; Kopetki, E.; Brandstetter, H.; Huber, R.; Bode, W.; Engh, R. A. Coagulation factor IXa: the relaxed conformation of Tyr99 blocks substrate binding. *Structure* **1999**, *7*, 989–996.
(26) The X-ray crystal structures of the selected compounds in complex with a truncated recombinant form of human factor IXa were elucidated by Proteros Biostructures, GmbH (Martinsried, Germany). Structure coordinates have been deposited with RCSB Protein Data Bank (RCSB ID 057093), PDB code 3LC3.