Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 1473–1482 **1473**
DOI: 10.1021/jm901476x

Structure Based Drug Design: Development of Potent and Selective Factor IXa (FIXa) Inhibitors†

Shouming Wang,*,‡ Richard Beck,§ Andrew Burd,‡ Toby Blench,‡ Frederic Marlin,‡ Tenagne Ayele,‡ Stuart Buxton,‡ Claudio Dagostin,‡ Maja Malic,‡ Rina Joshi,‡ John Barry,‡ Mohammed Sajad,‡ Chiming Cheung,‡ Shaheda Shaikh,‡ Suresh Chahwala,‡,§ Chaman Chander,§ Christine Baumgartner,∥ Hans-Peter Holthoff,∥ Elizabeth Murray,‡ Michael Blackney,§ and Amanda Giddings§

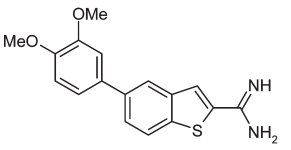
‡*Department of Medicinal Chemistry, and* §*Department of Pharmacology, Trigen Ltd., Emmanuel Kaye Building, 1B Manresa Road, London SW3 6LR, U.K., and* ∥ *Trigen AG, Martinsried, Munich, Germany*

Received October 8, 2009

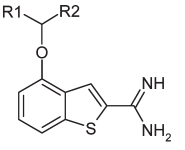
On the basis of our understanding on the binding interactions of the benzothiophene template within the FIXa active site by X-ray crystallography and molecular modeling studies, we developed our SAR strategy by targeting the 4-position of the template to access the S1 β and S2−S4 sites. A number of highly selective and potent factor Xa (FXa) and FIXa inhibitors were identified by simple switch of functional groups with conformational changes toward the S2−S4 sites.

FIXa[a] is a vitamin K-dependent blood coagulation factor that is essential for the amplification or consolidation phase of blood coagulation.[1,2] Its role in maintaining internal hemostasis in the intrinsic pathway of the clotting cascade sets it apart from other serine protease enzymes as an alternative therapeutic target.[3] Development of selective inhibitors of FIXa may provide clinicians safe and effective anticoagulants in attenuating thrombosis, particularly for myocardial infarction, ischemic diseases, and common procedures such as percutaneous coronary intervention, hemodialysis, blood pheresis, cardiac valve replacement, and extracorporeal circulatory support systems that incite coagulation.[4]

Previously, we have reported our early SAR studies of the benzothiophene template, resulting in a number of highly potent FIXa inhibitors, though with moderate selectivities against other serine protease enzymes, such as FXa.[5] X-ray study on one of the analogues, compound **1**, revealed distinctive induced S1 β site opening and a secondary binding of the molecule in the S3−S4 sites which was believed to be closed in previous X-ray studies with different ligands.[6] Close examination of the X-ray structure of the 5-aryl substituted benzothiophene compound **1** revealed the aryl group lies outside of the S1 β pocket; e.g., the pocket is unoccupied even though it has been induced to open by the ligand. After a series of molecular modeling studies on the various substituted analogues derived from benzothiophene template, in combination with the knowledge that the S2−S4 sites may be used for binding interactions, we envisaged that a branched alkyloxy substitution at 4-position on the template, such as compound **2**, may allow the occupation of the S1 β pocket by one of the branched groups (R1), while the other (R2) may point toward the S2−S4 pockets. Notably, simple 4-alkyloxybenzothiophene analogues have been demonstrated to show moderate activity in our early studies. Here, we disclose our identification of highly potent and selective FIXa inhibitors through structure based drug design.



(1)



(2)

Chemistry

The general synthesis of 4-substituted benzo[*b*]thiophene-2-carboximidine analogues is shown in Scheme 1. Thus, 4-hydroxy-2-Boc-amidine intermediate **3**, prepared according to the previous report,[5] was alkylated with methyl α-bromoacetate and K_2CO_3 in DMF to give the branched 4-alkoxy esters **4**, which were either reduced to the corresponding alcohols **5** by $LiAlH_4$ and $NaBH_4$ or converted to the amides **6** with various amines and HATU/HBTU, after ester hydrolysis to the acids. The intermediate alcohols **5** were converted into carbamates **7** via disuccinimidyl carbonate (DSC) coupling with amines, followed by deprotection of the Boc amidines by TFA. They could also be converted to the triflates **8** with trifluoroacetic anhydride and triethylamine, which was displaced with azide, followed by triphenyl phosphine to give the primary amines **9**. Similarly, direct amine displacement of the triflates **8** gave alkylamines **11** after Boc removal by TFA. The primary amines **9** were treated with isocyanates, chlorocarboxamides, or chlorocarboxylates to give ureas or carbamates **10** after deprotection of the *t*-Boc-amidine group by TFA. Scheme 2 describes the synthesis of one example of the amide bioisosteres, oxadiazole **16**. Mandelic acid **12** and hydroxyamidine **13** were heated with

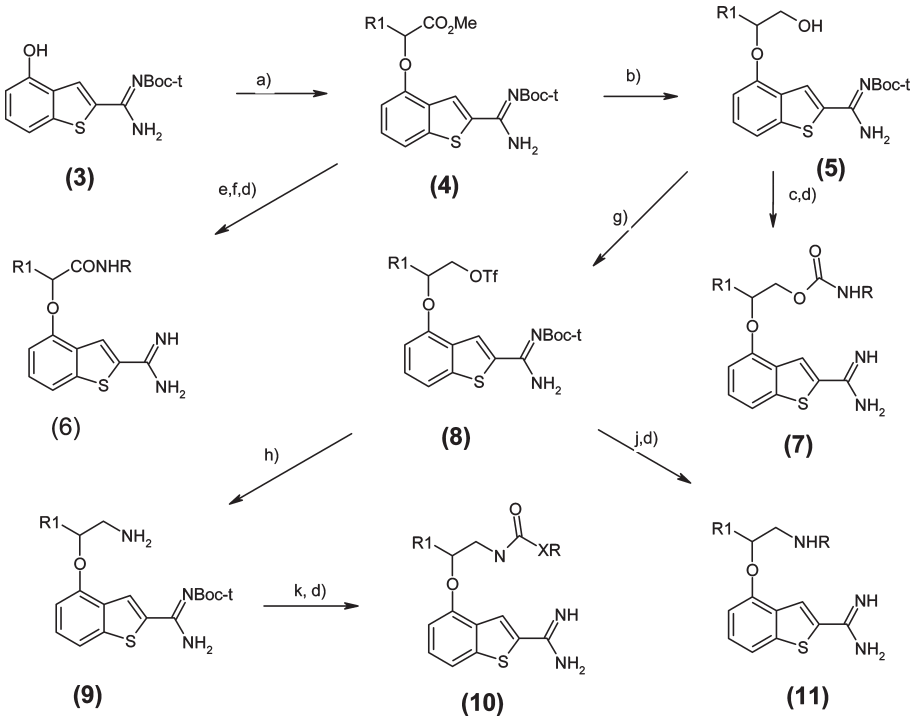
†PDB code for compound **16** cocrystallized with human N-terminally truncated FIXa is 3LC5.

*To whom correspondence should be addressed. Phone/fax: +441628672546. E-mail: shouming_wang@yahoo.co.uk.

[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; aPTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time.

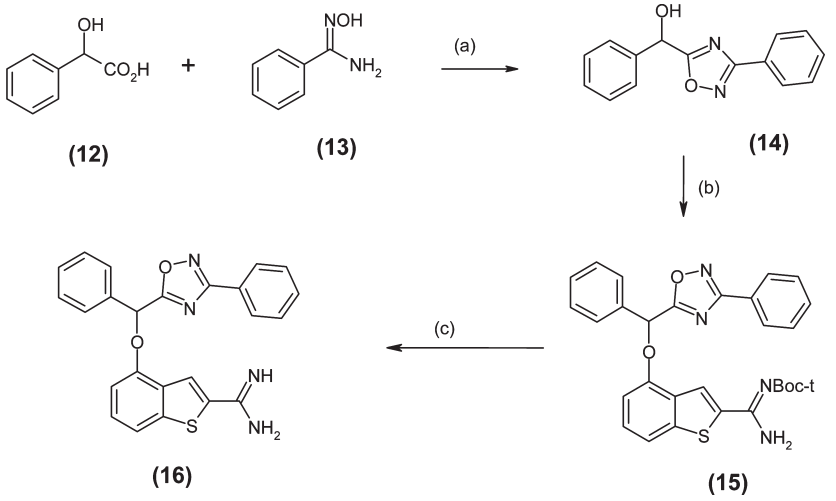
Published on Web 02/02/2010

Scheme 1[a]



[a] Reagents: (a) (R1)CHBrCO$_2$Me, K_2CO_3, DMF, room temp, 86%; (b) (i) $LiAlH_4$, THF, −78 °C; (ii) $NaBH_4$, 97%; (c) RNH_2, DSC, TEA, DCM, 60 °C; (d) TFA, DCM); (e) LiOH, THF; (f) RNH_2, HATU or HBTU, DIPEA, DMF; (g) Tf_2O,TEA, DCM, −78 °C; (h) (i) NaN_3, DMF; (ii) PPh_3, THF, H_2O; (j) RNH_2, THF; (k) RNCO or RXCOCl (X = N, O).

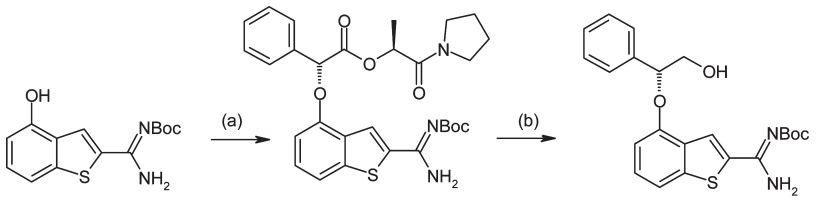
Scheme 2[a]



[a] Reagents: (a) CDI, diglyme, 90 °C; (b) **3**, DEAD, PPh_3, THF, 0 °C to room temp, 15%; (c) TFA, DCM, 68%.

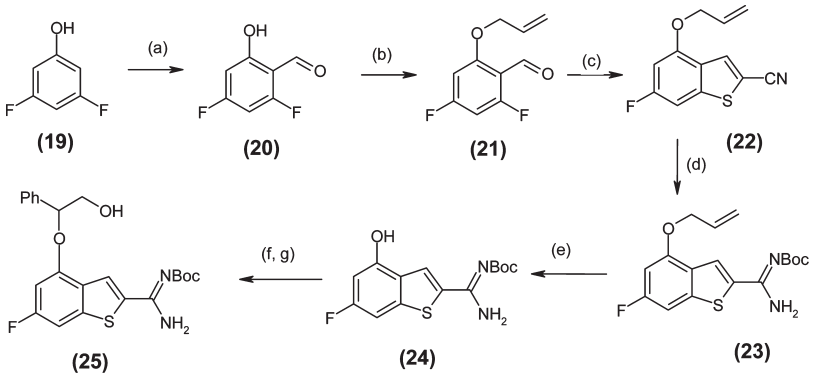
CDI in diglyme to give the hydroxyoxazole intermediate **14**, which was coupled with compound **3** under Mitsunobu condition, yielding compound **15**. Compound **16** was obtained after its treatment with TFA. The corresponding chiral analogues described in Scheme 1 can be accessed from the chiral alcohol **18**, which was prepared according to a chiral auxiliary approach[7] as shown in Scheme 3. Thus, 4-hydroxybenzothiophene intermediate **3** was alkylated with (*S*)-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl 2-bromo-2-phenylacetate **16** and *n*-BuLi to give the chiral ester **17**, which was reduced to the chiral alcohol **18** with $LiAlH_4$ at −78 °C first, followed by $NaBH_4$ in MeOH at 0 °C. Both enantiomers were prepared in high enantiomeric excess using this method.[8] For 4,6-disubstituted analogues, Scheme 4 exemplifies the general approach with the preparation of 4-alkyloxy-6-fluoro analogues via the key intermediate **25**. The formal phenol product **20** from 3,5-difluorophenol **19** was protected with allyl bromide to give compound **21**. Displacement of 3- fluoro group with thioacetonitrile gave the annulated benzothiophene product **22** selectively in situ. Following the established produres[5] of amidination, *t*-Boc protection, and removal of allyl group from compound **23**, 4-hydroxy-6-fluoro intermediate **24** was obtained and reacted with α-bromophenyl acetate. Subsequent reduction of the ester gave the 4,6-disubstituted

Scheme 3[a]



[a] Reagents: (a) (*S*)-1-Oxo-1-(pyrrolidin-1-yl)propan-2-yl 2-bromo-2-phenylacetate (**16**),[7] *n*-BuLi, THF; (b) $LiAlH_4$, −78 °C, THF, $NaBH_4$, 0 °C, MeOH, 78%.

Scheme 4[a]



[a] Reagents: (a) $MgCl_2$, NEt_3, $(CHO)_n$, MeCN; (b) allyl bromide, K_2CO_3, DMF; (c) thioacetonitrile, NaH, THF; (d) (i) LiHMDS, THF, then HCl, dioxane; (ii) Boc_2O, Hunig's base, THF, H_2O; (e) $Pd(PPh_3)_4$, PhSiH, CH_2Cl_2; (f) methyl α-bromophenylacetate, K_2CO_3, DMF; (g) $LiAlH_4$, THF, then MeOH, $NaBH_4$.

alcohol **25**, which can be further manipulated as compound **5** in Scheme 1.

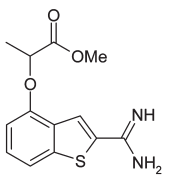
Results and Discussion

The enzymatic assays for FIXa, FXa, and uPA were carried out as described earlier[5] or by a modified procedure.[9] Even though a large number of amides or carboxylates have been prepared from compounds **4**, Table 1 only shows some of the typical examples to highlight some significant SAR. To determine an optimal R1 group for compound **2**, we screened a number of R1 substituted α-halomethyl esters by fixing R2 as the carboxylic ester. Table 1 exemplifies two of the analogues, **26** and **27**. Compound **27** with a phenyl R1 group showed over 10-fold FIXa activity than the methyl analogue **26**. Both compounds showed some degree of selectivity against FXa but not uPA. As the ester group is prone to hydrolysis, we decided to use phenyl group as R1 and screen a library of amides easily derived from the ester **27**. Simple alkylamides with either carboxylic group **28** or amine group **29** showed less FIXa activity than **27**. The anilide analogue **30** was nearly equipotent against all three enzymes FIXa, FXa, and uPA. One carbon-extended benzylamide **31** showed less FIXa activity than **30**. Therefore, we decided to investigate the SAR of substituted anilides. Those with simple functional groups show almost flat SAR for FIXa activity, exemplified by 2′-, 3′-, or 4′-methoxy substituted compounds **32**−**34**, two of which appeared to be moderately selective FXa inhibitors **32**, **34**. Indeed, our further exploration of substituents at the 4′-position led to highly potent and selective FXa inhibitors **35**−**37** against both FIXa and uPA. Notably, the FIXa activity of these compounds remained unimproved over that of compound **30**. Our molecular modeling study of these highly potent and selective FXa inhibitors within the FXa active site did suggest a very good fit of the 4′-substitutents at the S3−S4 sites. Interestingly, as an amide bioisostere, the oxadiazole analogue **16** appeared to show better selectivity (15-fold) against FXa than compound **30**. This compound was then subjected to X-ray crystallography study with human FIXa protein by the same group,[10] which is shown in Figure 1. The cocrystallized structure with 2.62 Å resolution clearly showed the *R*-enantiomer of the racemic compound **16** binding at the active site (PDB code 3LC5). As expected, the amidine group on the benzothiophene template formed the usual S1 interaction with Asp189, the S1 β site was induced open and filled by the phenyl group, and the hydrophobic S3 site surrounded by Phe174, Trp215, and Tyr99 was occupied by the phenyl group on the oxadiazole ring. Moreover, it appeared that one of the oxadiazole N formed an H bond interaction with NαH of Glu218, which was significant because it is a glycine residue at the same position in FXa.

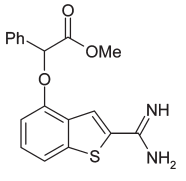
Our molecular modeling study on the corresponding amide compound **30** suggests no binding interaction exists between the amide carbonyl and Glu218 residue. Instead the amide group with a rigid sp^2 conformation appeared to have directed the (substituted) anilide group toward the S3−S4 sites of FXa. In order to pick up the H bond interaction with Glu218 to improve the selectivity, we sought to introduce more flexible sp^3 linked groups in place of the amide functional group. Table 2 shows some of the early examples in this area.

Compound **38** with a primary methylene amine group at the α-position of the benzyloxy substitutent showed a moderate

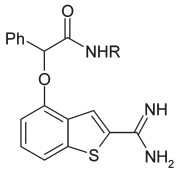
Table 1. Inhibitory Activities[a] of 4-Alkyloxybenzothiophene Compounds in FIXa, FXa, and uPA Assays



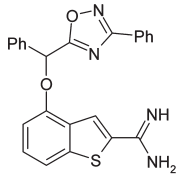
(26)



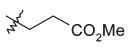
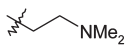
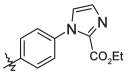
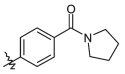
(27)



(28-37)



(16)

Compd	R	Ki(μM) / FIXa[a]	Ki(μM) / FXa[a]	Ki(μM) / uPA[a]
26		5.15	>100	2.27
27		0.5	13.8	0.5
28		8.40	ND[b]	ND[b]
29		1.0	3.40	2.13
30	Ph	0.17	0.15	0.14
31	Bn	0.39	0.95	0.51
32	2'-MeO-Ph	0.14	0.07	0.12
33	3'-MeO-Ph	0.13	0.29	0.17
34	4'-MeO-Ph	0.14	0.052	0.14
35		0.42	0.0026	0.19
36	4'-(Me_2NCO)-Ph	0.37	0.0006	0.16
37		0.29	0.0005	0.13
16		0.27	3.97	0.65

[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.

FIXa activity and selectivity against FXa (28-fold). Capping of the primary amine group by acetyl or benzyl group did not lead to much improved FIXa inhibition or better selectivity (**39**, **40**). However, the carbamate analogue **41** showed a good potency against FIXa (K_i = 50 nM) with 11-fold selectivity against FXa. The corresponding urea analogue **42** showed even better potency against FIXa (K_i = 29 nM) and selectivity against FXa (33-fold). None of the above compounds showed

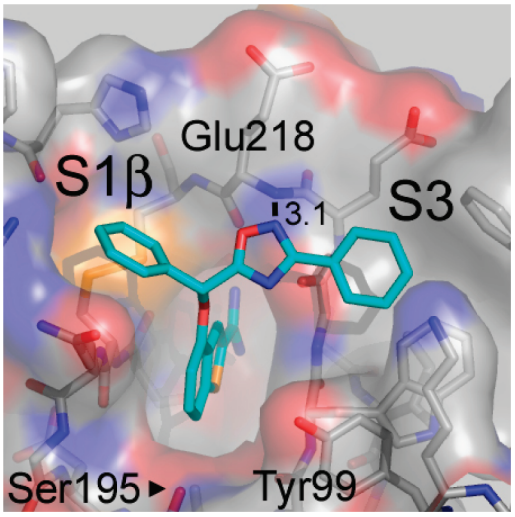
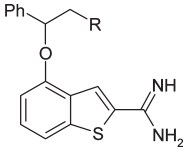


Figure 1. X-ray structure of compound **16** cocrystallized with human N-terminally truncated FIXa (PDB code 3LC5).

Table 2. Inhibitory Activity of α-Branched 4-Benzyloxy Compounds in FIXa, FXa, and uPA Assays



compd	R	K_i (μM), FIXa[a]	K_i (μM), FXa[a]	K_i (μM), uPA[a]
38	NH_2	0.16	4.45	0.27
39	NHCOMe	0.34	1.30	0.22
40	NHBn	0.10	0.25	0.31
41	$NHCO_2Ph$	0.05	0.56	0.085
42	NHCONHPh	0.029	0.96	0.25
43	OH	0.27	1.74	0.14
44	OCONHMe	0.097	0.70	0.21
45	OCONHEt	0.028	0.36	0.15
46	OCONHPh	0.010	1.81	0.44
47	OCONHBn	0.061	0.83	0.51

[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%.

any meaningful selectivity against uPA. The hydroxymethylene analogue **43** also showed a moderate FIXa activity but only 6-fold selectivity against FXa. Its further derivatization to carbamates gave more potent FIXa inhibitors (**44**−**46**), and compound **46** showed over 180-fold selectivity against FXa and 43-fold against uPA. One carbon-extended benzyl carbamate **47** showed less FIXa acivity and selectivity than compound **46**. Molecular modeling on this compound demonstrated possible H bond interaction between the carbamate carbonyl and NαH of Glu218 as shown in Figure 2. Given the superior activity and selectivity of compound **46**, we decided to explore the SAR of the substituted phenyl carbamates, some of which are shown in Table 3.

Substitutions at the phenyl ring were generally well tolerated, leading to a number of highly potent and selective FIXa inhibitors, especially those substituted at 2′- and 4′-positions exemplified by the fluoro substituted analogues **48**−**50**. In a majority of cases, the 3′-substituted analogue tended to give slightly poorer FIXa inhibitor with poor selectivity against

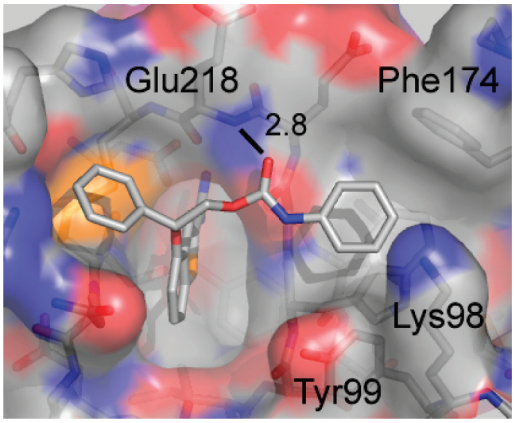
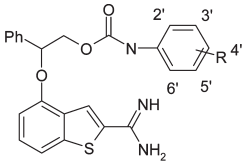
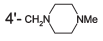
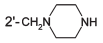


Figure 2. Molecular docking study of compound **46** in human truncated FIXa active site.

Table 3. Inhibitory Activity for 4-Alkyloxy(α-phenyl carbamates)-Substituted Benzothiophene Compounds in FIXa, FXa, and uPA Assays

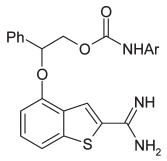


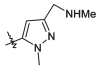
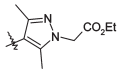
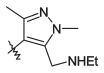
Compd.	R	Ki(μM) FIXa[a]	Ki(μM) FXa[a]	FXa/ FIXa	Ki(μM) uPA[a]	uPA/ FIXa
46	H	0.010	1.81	181	0.44	44
48	2'-F	0.005	0.55	110	0.311	62
49	3'-F	0.025	1.30	52	0.935	37
50	4'-F	0.008	1.70	213	0.78	98
51	2'-Me	0.008	1.184	148	0.308	36
52	4'-MeO	0.005	0.80	160	0.226	45
53	4'-$O(CH_2)_2NMe_2$	0.004	0.872	218	0.201	50
	4'- 					
54		0.010	1.562	156	0.201	20
55	2'-CH_2NH_2	0.004	0.825	206	0.297	74
56	2'-CH_2NHMe	0.003	1.147	383	0.308	103
57	2'-CH_2NHBu-i	0.002	0.583	292	0.733	367
58	2'-CH_2NMe_2	0.008	0.828	104	0.609	76
59	2'- 					
		0.003	0.354	118	0.833	278
60	2',4-F,F	0.007	1.482	212	0.575	82
61	2',6'-F,F	0.007	1.303	186	0.249	34
52-(R)	4'-MeO	0.003	0.60	200	0.089	30
52- (S)	4'-MeO	0.035	3.773	108	1.631	47

[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%.

FXa and uPA **49**. With potent and selective FIXa inhibitors **51** and **52** as leads, further analogues with amino basic groups were explored in an effort to improve solubility and plasma protein binding properties, which led to more potent and

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



Compd	Ar	Ki(μM) FIXa[a]	Ki(μM) FXa[a]	FXa/ FIXa	Ki(μM) uPA[a]	uPA/ FIXa
62		0.012	2.525	210	0.142	12
63		0.010	2.051	205	0.169	17
64		0.009	1.560	173	0.109	12
65		0.005	1.270	254	0.251	50
66		0.008	2.844	360	0.058	7
67		0.011	1.30	118	0.059	5
68		0.005	2.375	475	0.156	31
69		0.003	1.628	542	0.151	50

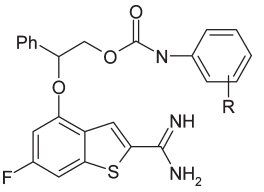
[a] All the IC_{50} values in this text represent the mean of a minimum of three experiments. Variations among the results were less than 10%.

selective FIXa inhibitors either substituted at the 4′-position (**53**, **54**) or 2′-position (**55**−**59**). Compounds **56**, **57**, and **59** demonstrated high selectivity against uPA as well as FXa. Our attempts to look at the additive effect of the disubstituted analogues gave more or less the similar potency and selectivity (**60**, **61**) to those singly substituted (**48**, **50**). However, chiral resolution of the racemic compound **52** demonstrated that the (*R*)-**52** enantiomer was 10 times more potent than the (*S*)-**52** enantiomer, which is consistent with the X-ray structure of compound **16** (Figure 1). As the more potent *R* form was only marginally better than the racemic mixture **52**, we decided to continue our ongoing in vitro analogue studies with the racemic form.

In order to have a wider choice of selection of analogues for our parallel ADME/PK study, we investigated the bioisostere replacement of the anilide moiety attached to the carbamate with large number of heterocyles. Table 4 shows some selected examples.

The dimethyloxazole analogue **62** showed good FIXa activity and was over 200-fold selective against FXa, though the selectivity against uPA was poor. Simple pyrazoles with carbamate at the 4- or 5-position showed similar high FIXa activity and uPA selectivity (**63**, **66**). Substitutions at one of the methyl substitutents of compound **63** with a methoxy or

Table 5. Inhibitory Activity for 4,6-Disubstituted Benzothiophene Carbamate Compounds in FIXa, FXa, and uPA Assays



compd	R	K_i (μM), FIXa[a]	K_i (μM), FXa[a]	K_i(FXa)/ K_i(FIXa)	K_i (μM), uPA[a]	K_i(uPA)/ K_i(FIXa)
70	H	0.0155	1.585	102	2.115	137
71	2′-CH_2NH_2	0.0044	2.115	486	0.828	190
72	2′-CH_2NMe_2	0.0081	1.363	169	0.94	117

[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%.

methylamine group led to slightly improved FIXa inhibitors with selectivity against FXa more or less retained (**64**, **65**). Alkylations at pyrazole N of compound **66** usually gave slight reduction of FIXa activity and selectivity exemplified by compound **67**. However, introduction of alkyamine groups at one of the pyrazole methyl groups gave more potent FIXa inhibitors with over 470-fold selectivity against FXa (**68**, **69**), which was consistent with the SAR in the anilide series shown in Table 3.

As shown by the compounds listed in Tables 2−4, we have largely overcome the selectivity issue against FXa with a number of those showing good selectivity (> 100-fold) against uPA as well. However, a significant number of these compounds are potent dual inhibitors of FIXa and uPA, which could be potentially very useful in treating cardiovascular patients with restenosis, as uPA has been specifically implicated in stimulating neointima formation and inward arterial remodeling.[11]

In our previous communication,[5] we reported SAR studies on the 6-position of the benzothiophene template for S′ site interactions. Given our understanding of the binding interactions within the S1 β and S2−S4 sites with above 4-substituted compounds, we also looked at the potential additive effect of 4,6-disubstituted compounds for FIXa activity and selectivity. Table 5 shows a few preliminary examples with the 6-fluoro substitutent in this aspect.

As shown by compounds **70**−**72**, introduction of an additional fluoro group at the 6-position of the active carbamate compounds led to only marginal reduction of FIXa activity, but significant improvement of uPA selectivity was observed with FXa selectivity more or less retained, compared with the corresponding nonfluorinated analogues **46**, **55**, and **68**. The reason for the improvement of uPA selectivity was unclear, though molecular modeling suggests some slight adjustment of the template vector within the active sites.

In further pharmacodynamic (PD) activity profiling study, a number of selective FIXa inhibitors were selected for activated partial thrombin time (aPTT), prothrombin time (PT), and thrombin time (TT) measurement. It is believed that better efficacy should be expected in a 2× aPTT assay for intrinsic FIXa inhibitors than in 2× PT or 2× TT assays which measure the effect on the extrinsic pathway.[12] Table 6 lists some selected examples.

All compounds shown in Table 6 demonstrated significant pharmacodynamic activity with inhibiting effects on the factors

Table 6. FIXa Compounds in aPTT, PT, and TT Assays

		doubling of PD parameter (μM)			
compd	K_i (nM), FIXa	aPTT	PT	TT	PPB (%)
63	10	12.8	57	60	96
66	8	11.7	63	> 80	95
68	5	7.7	42	56	95
69	3	5.9	29	25	96
59	3	5.0	17	20	95

Table 7. Pharmacokinetic Study by iv Dosing (5 mg/kg) in Anesthetized Rats

compd	C_{max} (μM)	$AUC_{0-\infty}$ (μM·h)	$T_{1/2}$ (h)	CL (mL/min/kg)	HLM (% TO)
50	10.5	5.19	5.5	32.4	11
63	5.19	0.951	2.1	129.3	18

predominantly involved in the intrinsic pathway. This is reflected by nearly 5-fold or more difference of the PD data of aPTT and those of PT and TT for heterocycle based carbamates **63**, **66**, **68**, and **69**. The phenyl carbamate **59** showed more than 3-fold difference in the corresponding measurements. One reason for the significant difference of activities from FIXa enzymatic assays to the micromolar level of pharmacodynamic measurement could be due to the effect of plasma protein binding. As shown in Table 6, all of these five compounds are 95−96% plasma protein bound. The activity data from FIXa assay together with plasma protein binding data correlate very well with the trend of the pharmacodynamic data generated, indicating selective FIXa inhibition. Notably, an early clinical RNA aptamer study by Regado Bioscience showed that 1.1-fold increase in aPTT in response to FIXa inhibition is equivalent to 40% loss of FIX activity while a 1.3-fold increase represents a loss of 80% FIX activity.[13]

As we have obtained potent and selective FIXa inhibitors in both phenyl and heteroaromatic based carbamates as shown in Tables 3 and 4, some preliminary pharmacokinetic (PK) studies were also carried out for both subseries, using anesthetized rats and administering a dose of 5 mg/kg bolus intravenously (iv). The data for two early examples are disclosed in Table 7.

The phenyl carbamate analogue **50** showed a promising profile with more than 10 μM C_{max} and > 5 h half-life, while the pyrazole derived carbamate **63** showed a lower C_{max} and over 2 h half-life. Both compounds showed significant plasma concentrations after several hours. The clearance (CL) for compound **50** is significantly lower than that for compound **63**. Human liver metabolism (HLM) studies for both compounds demonstrated low turnover (TO), though compound **50** appears to be less metabolized than compound **63**, indicating reasonable initial stability for the carbamate functional group present in both molecules. This provided us with a promising platform for our ongoing evaluation and optimization in vivo studies, which will be published in due course.

Conclusion

Through our understanding of the X-ray binding interaction of the benzothiophene analogue **1** within the FIXa active site, we developed a structure based drug design chemistry strategy to explore the binding interactions of S1 β and S2−S4 sites with branched 4-alkyloxy analogues. By modifying the conformation of the linking side chain, we successfully made the switch from potent and selective FXa inhibitors to a series of highly potent and selective FIXa inhibitors, some of which

are also potent and selective dual inhibitors for FIXa and uPA. Further X-ray and molecular modeling studies indicated a new potential H-bond binding interaction of Glu218 residue and carbamate functional group in the highly selective and potent FIXa inhibitors. Our preliminary in vitro PD and PK studies demonstrated promising profiles for these benzothiophene based FIXa inhibitors. Further in vivo studies with more optimized analogues in our ongoing development candidate selection process will be published elsewhere.

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 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 on 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 (19 mm × 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.

General Procedure A for Analogues 4 and 5 (Scheme 1). Methyl 2-(2-(*N*′-(*tert*-Butoxycarbonyl)carbamimidoyl)benzo[*b*]thiophen-4-yloxy)-2-alkylacetates (4) Exemplified by Phenyl Analogue (4a, When R1 = Ph). To a solution of phenol **3** (1.78 g, 6.10 mmol) in DMF (60 mL) under argon was added potassium carbonate (927 mg, 6.71 mmol), followed by methyl α-bromophenylacetate (1.06 mL, 6.71 mmol). The mixture was stirred at room temperature for 4 h before it was quenched by addition of H_2O (50 mL). After extraction with Et_2O (3 × 50 mL), the combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography using 30% EtOAc in hexanes to give the desired product as a white solid (2.27 g, 86% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (s, 1H), 7.62 (m, 1H), 7.47−7.43 (m, 4H), 7.29−7.27 (m, 1H), 6.64 (d, 1H, J = 7.8 Hz), 5.80 (s, 1H), 3.76 (s, 3H), 1.57 (s, 9H). LCMS (ES+, M + H) m/z 441.

***tert*-Butyl Amino(4-(2-hydroxy-1-phenylethoxy)benzo[*b*]thiophen-2-yl)methylenecarbamate (5a).** A solution of ester **4a** (8.56 g, 19.5 mmol) in THF (100 mL) was cooled to −78 °C under argon. Lithium aluminum hydride (2 M in THF, 10.2 mL, 20.4 mmol) was added slowly, and the mixture was stirred at −78 °C for 2 h before the reaction was carefully quenched by addition of MeOH (100 mL). Sodium borohydride (1.47 g, 38.9 mmol) was then added in one portion, and the resulting solution was allowed to warm to room temperature and was stirred at that temperature for 2 h. The reaction mixture was then quenched by addition of aqueous saturated NH_4Cl (50 mL), and the organic solvents were removed under reduced pressure. The resulting aqueous mixture was then diluted with H_2O (100 mL). After extraction with EtOAc (3 × 150 mL), the combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the desired product as a pale-yellow solid, **5a** (7.81 g, 97% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.28 (s, 1H), 7.37−7.27 (m, 6H), 7.16−7.12 (m, 1H), 6.53 (d, 1H, J = 7.8 Hz), 5.43 (dd, 1H, J = 8.1, 3.3 Hz), 4.10 (m, 1H), 3.92 (m, 1H), 2.72 (br s, 1H), 1.57 (s, 9H). LCMS (ES+, M + H) m/z 413.

General Procedure B for Amides 6 Preparation. To a solution of ester **4** (1 equiv) in THF (10 mL/mmol) 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 product acids.

To a solution of the above acids (1 equiv) in DMF (3−5 mL/mmol) were added HATU or HBTU (1.1 equiv) and diisopropylethylamine (1.1 equiv). The solution was stirred for 10−30 min. Then amine (1.1 equiv) was added and the solution stirred at room temperature for 18 h.

The mixture was partitioned between ethyl acetate and water, and then the phases were separated. The organics were washed with brine (×2) and dried ($MgSO_4$), and the solvent was removed. The residue was purified via silica chromatography (EtOAc/hexane or MeOH/CH_2Cl_2 mix), yielding the *t*-Boc-amidine amides, which were deprotected to give the amides **6** by a previously reported procedure.[5]

General Procedure C for Carbamate 7 Formation. To a suspension of **5** (1 equiv) in CH_2Cl_2 (8 mL/mmol) was added triethylamine (2.4 equiv) and disuccinimidyl carbonate (1.2 equiv). The suspension was stirred for 45 min until a yellow solution formed, and then the requisite aniline (2 equiv) was added. The solution was stirred at 60 °C in a sealed tube for 18 h. The solution was then concentrated and the residue purified via silica chromatography (ethyl acetate/hexane mix). The residue was then directly subjected to the general method for deprotection of BOC amidines with TFA.[5]

Preparation of Triflates 8 Exemplified by 8a (When R1 = Ph). To a solution of **5a** (1.0 g, 2.4 mmol) in CH_2Cl_2 (20 mL) at −78 °C under argon was added triethylamine (406 μL, 2.9 mmol), followed by trifluoroactic acid anhydride (407 μL, 2.4 mmol). The solution was stirred at −78 °C for 1 h, then quenched with water, diluted with CH_2Cl_2, and allowed to warm to room temperature. The phases were separated, the organics were dried (Na_2SO_4), and the solvent was removed to yield title compound **8a** (1.4 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.44−7.40 (m, 6H), 7.16−7.14 (m, 1H), 6.49 (d, 1H, J = 8 Hz), 5.69−5.67 (m, 1H), 4.93−4.88 (m, 1H), 4.70−4.67 (m, 1H), and 1.56 (s, 9H).

Preparation of Amines 9 Exemplified by 9a (when R1 = Ph). To a solution of **8a** (1.2 g, 2.2 mmol) in DMF (6 mL) was added sodium azide (184 mg, 2.8 mmol), and the mixture was stirred at room temperature for 18 h. The mixture was partitioned between ethyl acetate and water, and the phases were separated. The organics were washed with water (×1) and brine (×1) and dried ($MgSO_4$), and the solvent was removed. The residue was purified via silica chromatography (20−30% EtOAc/hexane) to

yield the azide intermediate (400 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.41−7.29 (m, 6H), 7.17−7.15 (m, 1H), 6.51 (d, 1H, J = 8 Hz), 6.48−6.46 (m, 1H), 3.84−3.80 (m, 1H), 3.49−3.47 (m, 1H), and 1.56 (s, 9H).

To a solution of the above azide (400 mg, 0.92 mmol) in THF (9 mL) and water (4.5 mL) was added triphenylphosphine (265 mg, 1.01 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was reduced to low volume and the residue partitioned between ethyl acetate and brine. The phases were separated, and the aqueous layer was extracted with ethyl acetate (×2). The combined organics were dried (Na_2SO_4), and the solvent was removed. The residue was triturated with ether and filtered and the solid collected to yield the title compound **9a** (262 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.21 (s, 2H), 8.69 (s, 1H), 7.47−7.44 (m, 3H), 7.39−7.36 (m, 2H), 7.29−7.25 (m, 2H), 6.69 (d, 1H, J = 8 Hz), 6.40 (m, 1H), 3.06−2.94 (m, 2H), and 1.49 (s, 9H).

General Proceduere D for Preparation of 10 by Acylation of Amines 9. To a solution of amines **9** (1 equiv) in CH_2Cl_2 (10−20 mL/mmol) was added diisopropylethylamine (1.1−2.2 equiv) followed by isocyanate or acyl halide (1.1 equiv), and the solution was stirred for 1−18 h. The solvent was removed and the residue purified via silica chromatography (ethyl acetate/hexane mix). Analogues **10** were obtained after TFA treatment of the above *t*-Boc amidines using a previously reported procedure.[5]

Preparation of *N*-Alkylamines 11 Exemplified by 11a (R1 = Ph, R = Bn). To a solution of **8a** (54 mg, 0.1 mmol) in DMF (1 mL) at room temperature was added K_2CO_3 under argon, followed by benzylamine (13 μL, 0.12 mmol) for 18 h . The solvent was removed and the residue triturated with ether and filtered. The solid collected was dissolved in DCM, and TFA was added to deprotect the *t*-Boc amidine[5] to yield the desired product **11a** (22 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.64 (s, 2H, NH + TFA), 9.53 (br s, 2H, NH_2), 9.40 (s, 2H, NH + TFA), 8.72 (s, 1H, ArH), 7.79 (d, 1H, ArH), 7.45−7.62 (m, 11H, ArH), 6.96 (m, 1H, ArH), 6.07 (m, 1H, CHO), 4.44 (t, 2H, CH_2N), 3.48 (m, 2H, CH_2N). MS m/z (ES+) 402 $[M + H]^+$.

Phenyl(3-phenyl[1,2,4]oxadiazol-5-yl)methanol 14. Mandelic acid **12** (0.848 g, 5.58 mmol) and CDI (0.404 g, 5.58 mmol) in dyglime (15 mL) were stirred together for 30 min. *N*-Hydroxybenzenecarboxyimidamide **13** (0.76 g, 5.58 mmol) was added, and the mixture was stirred overnight at room temperature and then heated at 110 °C under reflux for 2 h. The solution was concentrated in vacuo. The residue was applied to a silica chromatography column with EtOAc/hexane (5:1) to give the title compound, 0.150 g, 11% . ^{1}H NMR (DMSO-d_6): δ 8.01 (m, 2H, Ar-H) 7.34 (m, 9H, Ar-H) 6.02 (d, 1H, CH), 3.16 (d, 1H, OH). MS m/z (ES+) 253.0. t_R = 6.57

4-[Phenyl(3-phenyl[1,2,4]oxadiazol-5-yl)methoxy]benzo[*b*]thiophene-2-carboxamidine 16. 16 was synthesized from compound **15** by TFA deprotection of the *t*-Boc group. Compound **15** was prepared from **14** using Mitsunobu condition reported previously.[5] ^{1}H NMR (DMSO-d_6): δ 9.20 (bs, 4H, amidine), 8.47 (s, 1H, ArH), 7.85 (d, 2H, ArH), 7.66 (d, 1H, ArH), 7.65 (d, 2H, ArH), 7.48−7.33 (m, 7H, ArH), 7.32 (s, 1H, CH), 7.06 (d, 1H, Ar). MS m/z (ES+) 427.0 $[M + H]^+$.

(*R*)-(2-*t*-Boc-carbamimidoylbenzo[*b*]thiophen-4-yloxy)phenylacetic Acid (*S*)-1-Methyl-2-oxo-2-pyrrolidin-1-ylethyl Ester 17. A solution of phenol **3** (511 mg, 1.50 mmol) in THF (5 mL) was cooled to 0 °C under argon. *n*-BuLi (1.6 M in heptane, 0.99 mL, 1.58 mmol) was added slowly, and stirring was continued for 20 min at 0 °C. The resulting mixture was added dropwise to a previously prepared cooled (0 °C) solution of bromide **16** (439 mg, 1.50 mmol) in THF (10 mL) under argon. After 4 h at 0 °C, the reaction was quenched by careful addition of aqueous saturated NH_4Cl (10 mL), then H_2O (10 mL). After extraction with EtOAc (3 × 20 mL), the combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography using 50% EtOAc in hexanes to give the desired product as a beige solid (389 mg, 47% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (s, 1H), 7.62 (m, 2H), 7.51 (m, 1H), 7.43 (m, 3H), 7.27 (m, 1H), 6.79 (d, 1H, J = 8.0 Hz), 5.73 (s, 1H), 5.21 (q, 1H, J = 7.0 Hz), 3.63−3.30 (m, 4H), 1.99−1.82 (m, 4H), 1.57 (s, 9H), 1.37 (d, 3H, J = 7.0 Hz). LCMS (ES+, M + H) m/z 552.

(*R*)-*tert*-Butyl Amino(4-(2-hydroxy-1-phenylethoxy)benzo[*b*]thiophen-2-yl)methylenecarbamate 18. To a solution of **17** (47 mg, 0.09 mmol) in THF (2 mL) at −78 °C was added dropwise $LiAlH_4$ (2.0 M solution in THF, 45 μL, 0.089 mmol), and the mixture was stirred at −78 °C for 1 h before quenching the reaction with MeOH (500 μL). More MeOH (250 mL) was added, and the reaction mixture was warmed to 0 °C. $NaBH_4$ (6.5 mg, 0.17 mmol) was added and stirred for 3 h at 0 °C. The mixture was quenched with saturated aqueous NH_4Cl and back-extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Purification by silica chromatography, eluting with 20% EtOAc/hexane, gave the chiral alcohol **18** (20 mg, 57%). ^{1}H NMR and MS results are as for compound **5a.**

2,4-Difluoro-6-hydroxybenzaldehyde 20. To a suspension of 3,5-difluorophenol **19** (3.03 g, 23.3 mmol) in MeCN (100 mL) at room temperature under argon was added magnesium chloride (6.66 g, 69.9 mmol) in one portion, followed by triethylamine (8.12 mL, 58.2 mmol). The mixture was stirred at room temperature for 20 min before paraformaldehyde (3.50 g, 116 mmol) was added in one portion. The mixture was then heated to 80 °C and stirred at that temperature for 3 h, then at room temperature overnight. The mixture was then quenched by pouring onto aqueous HCl (1 N, 80 mL) and extracted with EtOAc (2 × 60 mL). The combined organic extracts were washed with brine (60 mL), dried (Na_2SO_4), filtered, and then concentrated under reduced pressure to give the desired product as a pale-orange oil (3.68 g, 100% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.81 (s, 1H), 10.17 (s, 1H), 6.51−6.39 (m, 2H). LCMS (ES−, M − H) m/z 157.

2-(Allyloxy)-4,6-difluorobenzaldehyde 21. To a solution of **20** (3.68 g, 23.3 mmol) in DMF (50 mL) at room temperature under argon was added K_2CO_3 (3.31 g, 23.9 mmol), followed by allyl bromide (2.07 mL, 23.9 mmol). The mixture was stirred at room temperature for 2 h and 30 min. Then it was quenched by pouring onto aqueous saturated NH_4Cl and extracted with Et_2O (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, and then concentrated under reduced pressure. The residue was purified by flash chromatography using 10% EtOAc in hexanes to give the desired product as a pale-yellow solid (2.12 g, 46% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.39 (s, 1H), 6.52−6.47 (m, 2H), 6.08−6.01 (m, 1H), 5.52−5.47 (m, 1H), 5.41−5.37 (m, 1H), 4.66−4.64 (m, 1H).

4-(Allyloxy)-6-fluorobenzo[*b*]thiophene-2-carbonitrile 22. A solution of thioacetonitrile (778 mg, 10.6 mmol) in THF was cooled to 0 °C under argon before NaH (60% in mineral oil, 426 mg, 10.6 mmol) was added in one portion. The mixture was stirred at 0 °C for 10 min, then at room temperature for 15 min before it was cooled back to 0 °C and **21** was added (2.11 g, 10.6 mmol) in one portion. The mixture was stirred at 0 °C for 30 min, then at room temperature for 30 min before it was cooled back to 0 °C and NaH (60% in mineral oil, 4 × 105 mg, 10.6 mmol) was added portionwise every 10 min. After 1 h at room temperature, the mixture was quenched by addition of aqueous saturated NH_4Cl, and was extracted with with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, and then concentrated under reduced pressure. The residue was purified by flash chromatography using 5% EtOAc in hexanes to give the desired product as a pale-yellow solid (2.08 g, 84% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (d, 1H, J = 0.6 Hz), 7.14−7.11 (ddd, 1H, J = 8.2, 2.0, 0.6 Hz), 6.62−6.59 (dd, 1H, J = 10.9 Hz, 2.0 Hz), 6.13−6.06

(ddt, 1H, J = 18.7, 10.5, 5.3 Hz), 5.51 (ddt, 1H, J = 18.7, 1.3, 1.3 Hz), 5.39 (ddt, 1H, J = 10.5, 1.3, 1.3 Hz), 4.69 (dt, 2H, J = 10.5, 1.3 Hz).

(*E*)-*tert*-Butyl (4-(Allyloxy)-6-fluorobenzo[b]thiophen-2-yl)(amino)-methylenecarbamate 23. 23 was prepared as a beige solid (4.04 g, 100% yield), following the reported procedures.[5] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.98 (s, 1H), 7.11 (dd, 1H, J = 8.4, 1.8 Hz), 6.54 (dd, 1H, J = 11.0, 2.0 Hz), 6.13−6.06 (m, 1H), 5.48 (dd, 1H, J = 17.2, 1.3 Hz), 5.38 (dd, 1H, J = 10.5, 1.3 Hz), 4.66 (dd, 2H, J = 4.0, 1.3 Hz), 1.56 (s, 9H). LCMS (ES+, M + H) m/z 351.

(*E*)-*tert*-Butyl Amino(6-fluoro-4-hydroxybenzo[*b*]thiophen-2-yl)methylenecarbamate 24. 24 was prepared as a brown solid (1.14 g, 61% yield), following the previous procedures.[5] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.13 (s, 1H), 7.02 (m, 1H), 6.45 (m, 1H), 1.56 (s, 9H).

(*E*)-*tert*-Butyl Amino(6-fluoro-4-(2-hydroxy-1-phenylethoxy)-benzo[*b*]thiophen-2-yl)methylenecarbamate 25. 25 was prepared as a white solid (1.1 g, 93% yield), following the same procedure as for compound **5a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.18 (s, 1H), 7.33 (m, 5H), 6.99 (dd, 1H, J = 8.3, 1.8 Hz), 6.30 (dd, 1H, J = 10.7, 1.8 Hz), 5.37 (dd, 1H, J = 8.1, 3.3 Hz), 4.07 (m, 1H), 3.91 (m,1H), 2.80 (br s, 1H), 1.56 (s, 9H).

2-(2-Carbamimidoylbenzo[*b*]thiophen-4-yloxy)propionic Acid Methyl Ester 26 (as TFA Salt). 26 was prepared as a pale-yellow solid (74%) from compound **4b** (when R1 = Me) with TFA, following the previous procedure.[5] ^{1}H NMR (DMSO, 400 MHz): δ 9.21 (s, 4H) 8.44 (s, 1H), 7.74 (d, J = 8 Hz, 1H), 7.52 (d, J = 8 Hz, 1H), 6.89 (d, J = 8 Hz, 1H), 5.27 (q, J = 8 Hz, 1H), 3.71 (s, 3H), 1.64 (d, J = 8 Hz, 3H). MS m/z 279 $[M + H]^+$.

(2-Carbamimidoylbenzo[b]thiophen-4-yloxy)phenylacetic Acid Methyl Ester 27 (as TFA Salt). 27 was synthesized as a beige solid from compound **4a** (when R1 = Ph) with TFA, following the previous procedure.[5] ^{1}H NMR(CD_3OD, 400 MHz): δ 9.52 (s, 2H, NH_2 amidine), 9.08 (s, 2H, NH-TFA amidine), 8.53 (d, 1H, J = 8 Hz, H-Ar), 7.77 (d, 1H, J = 8 Hz, ArH), 7.66−7.56 (m, 2H, ArH), 7.53 (t, 1H, J = 8 Hz, ArH), 7.45−7.41 (m, 3H, ArH), 7.00 (d, 1H, J = 8 Hz, ArH), 6.34 (s, 1H, CH), 3.68 (s, 3H, CH_3). MS m/z (ES^+) 341.00 [M − H]. t_R = 4.93.

PD Measurement. For in vitro coagulation assays, blood was obtained from healthy volunteers by venipuncture and anticoagulated by sodium citrate (9NC Vacutainer, Beckton Dickinson). Plasma was prepared by centrifugation at 2000g for 10 min and kept on ice prior to use. Standard clotting assays were performed in a temperature-controlled automated coagulation analyzer (Sysmex CA-560, Dade Behring, Marburg, Germany) and individual thrombin time (TT), activated partial thromboplastin time (aPTT), and prothrombin time (PT) were determined. For TT, 50 μL of plasma was incubated at 37 °C for 2 min prior to addition of 100 μL of thrombin reagent (Dade Behring OWHM 13), and duration of clot formation was monitored at 660 nm. The aPTT assay was performed by incubating 50 μL of plasma at 37 °C for 1 min before addition of 50 μL of aPTT reagent (FSL, Dade Behring B4219-2) and further incubation at 37 °C for 3 min; 50 μL of 0.025 M $CaCl_2$ (Dade Behring ORHO 37) was added and the clot formation measured at 660 nm. For PT measurement, 50 μL of plasma was incubated for 3 min at 37 °C before addition of 100 μL of PT reagent (Thromborel S, Dade Behring OUHP 29). Clot formation was monitored at 660 nm.

Pharmacokinetic Studies. All animals experiments were performed in accordance with German authorities guidelines. Compounds were administered as bolus intravenous doses (5 mg/kg) over 15 s to anesthetised Sprague−Dawley rats (n = 3 per group, Charles River, Germany). Blood samples (180 μL in sodium citrate, 20 μL) were collected under anesthesia at the following time points: predose, 2, 5, 15, and 30 min and 1, 2, and 3 h. The plasma was prepared from the blood samples by centrifugation (1700g at 20 °C for 5 min) and stored frozen (−80 °C) until required for analysis of drug concentration. Plasma samples were diluted 1.1 with 100% acetonitrile (*Caution*: volatile and precipitate with other substances). After dilution, the sample was centrifuged for 10 min at 13 200 rpm (16100g) (centrifuge 5415D, Eppendorf, Germany) at room temperature. The supernatant was precipitated again with acetonitrile using the same conditions. The second supernatant was utilized for compound concentration measurements. A concentration versus time curve was plotted, and the pharmacokinetic parameters, AUC (area under the curve), and apparent terminal half-life ($t_{1/2}$) were calculated using a noncompartmental analysis.

Acknowledgment. We thank Dr. Roger Dickinson for providing consultancy in this project.

Supporting Information Available: Experimental and spectroscopic information for compounds **28**−**72**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Neuenschwander, P. Coagulation Cascade: Intrinsic Factors. In *Encyclopedia of Respratory Medicine*; Laurant, G., Shapiro, S. Eds.; Elsevier Ltd.: Oxford, U.K., 2006.

(2) Jesty, J.; Nemerson, Y. The Pathways of Blood Coagulation. In *Williams Hematology*, 5th ed.; Beutler, E., Lichman, M. A., Collar, B. S., Kipps, T. J., Eds.; McGraw-Hill, Inc.: New York, 1995.

(3) 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.

(4) Howard, E. L.; Becker, K. C. D.; Kusconi, C. P.; Becker, R. C. Factor IXa inhibitors as novel anticoagulants. *Arterioscler., Thromb., Vasc. Biol.* **2007**, 722–727.

(5) Wang, S.; Blench, T.; Beck, R.; Buxton, S.; Malic, M.; Ayele, T.; Shaikh, S.; Chahwala, S.; Chander, C.; Holland, R.; Merette, S.; Zhao, L.; Blackney, M.; Watts, A. Studies of benzothiophene template as potent factor IXa (FIXa) inhibitors in thrombosis. *J. Med. Chem.* DOI: 10.1021/jm901475e.

(6) (a) Brandstetter, H.; Bauer, M.; Huber, R.; Lollar, P.; Bode, W. X-ray structure of clotting factor IXa: active site and module structure related to Xase activity and hemophilia B. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9796–9800. (b) 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.

(7) (a) Devine, P. N.; Dolling, U.-H.; Heid, R. M., Jr.; Tschaen, D. M. Stereoselective synthesis of 2-aryloxy acids from lactamide derived esters of racemic α-halo carboxylic acids. *Tetrehedron Lett.* **1996**, *37*, 2683–2686. (b) Devine, P. N.; Heid, R. M., Jr.; Tschaen, D. M. Stereoselective synthesis of 2-aryloxy esters: an asymmetric approach to fluoxetine, tomoxetine and nisoxetine. *Tetrahedron* **1997**, *53* (20), 6739–6746.

(8) The enantiomeric excess was determined by Mosher's ester formation of the intermediate alcohol **18**, followed by 400 MHz NMR analysis. No discernible amount of the diastereoisomer was detected in the NMR spectra. Both (*R*)- and (*S*)-**18** were prepared according to ref 7.

(9) uPA assay: Urokinase-type plasminogen activator (uPA) was purchased from Sigma. The amidolytic substrate S-2444 (pyro-Glu-Gly-Arg-pNA) was purchased from Chromogenix. All other chemicals were purchased from Sigma and BDH. Amidolytic activity of uPA (assay concentration of 8.325 ng/mL) was measured with S-2444 (160 μM) in a buffer comprising 100 nM sodium phosphate (pH 7.5), 200 nM NaCl, 0.5% PEG 6000, 0.02% Na azide, and 0.01% BSA. p-NA cleavage was determined at 405 nm at 37 °C in a Versamax plate reader.

(10) 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). The structure has been deposited in RCSB protein Data Bank (RCSB ID 057095, PDB code 3LC5).

(11) (a) Parfyonova, Y.; Plekhanova, O.; Solomatina, M.; Naumov, V.; Bobik, A.; Berk, B.; Tkachuk, V. Contrasting effects of urokinase and tissue type plasminogen activators on neointima formation and vessel remodeling after arterial injury. *J. Vasc. Res.* **2004**, *41*, 268–276. (b) Nicholl, S. M.; Roztocil, E.; Davies, M. G. Urokinase-induced smooth muscle cell responses require distinct signaling

pathways: a role for the epidermal growth factor receptor. *J. Vasc. Res.* **2005**, *41* (4), 672–681.

(12) 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.

(13) 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.