Thiophene-Anthranilamides as Highly Potent and Orally Available Factor Xa Inhibitors[1]

Bin Ye,* Damian O. Arnaiz, Yuo-Ling Chou, Brian D. Griedel, Rushad Karanjawala, Wheeseong Lee, Michael M. Morrissey, Karna L. Sacchi, Steven T. Sakata, Kenneth J. Shaw, Shung C. Wu, Zuchun Zhao, Marc Adler, Sarah Cheeseman, William P. Dole, Janice Ewing, Richard Fitch, Dao Lentz, Amy Liang, David Light, John Morser, Joseph Post, Galina Rumennik, Babu Subramanyam, Mark E. Sullivan, Ron Vergona, Janette Walters, Yi-Xin Wang, Kathy A. White, Marc Whitlow, and Monica J. Kochanny*

Berlex Biosciences, Post Office Box 4099, Richmond, California 94804-0099

Received January 31, 2007

There remains a high unmet medical need for a safe oral therapy for thrombotic disorders. The serine protease factor Xa (fXa), with its central role in the coagulation cascade, is among the more promising targets for anticoagulant therapy and has been the subject of intensive drug discovery efforts. Investigation of a hit from high-throughput screening identified a series of thiophene-substituted anthranilamides as potent nonamidine fXa inhibitors. Lead optimization by incorporation of hydrophilic groups led to the discovery of compounds with picomolar inhibitory potency and micromolar in vitro anticoagulant activity. Based on their high potency, selectivity, oral pharmacokinetics, and efficacy in a rat venous stasis model of thrombosis, compounds ZK 814048 (**10b**), ZK 810388 (**13a**), and ZK 813039 (**17m**) were advanced into development.

Introduction

Thrombotic disorders remain the leading cause of mortality and morbidity in Western society.[2] Anticoagulants are the primary therapy for the treatment and prevention of thrombotic disease, but have properties that limit their use. Heparin and low molecular weight heparin must be dosed parenterally, and heparin is associated with increased bleeding risk.[3] Coumadin (warfarin), although orally available, has a slow onset of action and a narrow therapeutic window, resulting in the need for careful monitoring.[4] There remains an unmet clinical need for orally active, safe, and efficacious anticoagulants for long-term antithrombotic therapy.

One major area of focus in the effort to develop an oral anticoagulant has been the development of inhibitors of factor Xa (fXa[a]).[5] fXa, a trypsin-like serine protease located at the convergent point of the intrinsic and extrinsic pathways, plays a pivotal role in the blood coagulation cascade. Together with factor Va and calcium, fXa forms the prothrombinase complex, which catalyzes the formation of thrombin via proteolysis of prothrombin. Thrombin, the terminal enzyme in the cascade, has several procoagulant functions including activation of platelets, regulation of factors in the cascade, and the conversion of fibrinogen to fibrin, which polymerizes to form the insoluble matrix of a blood clot or thrombus. Because inhibition of fXa prevents thrombin formation but does not affect pre-existing thrombin, fXa inhibitors are predicted to cause less impairment of hemostasis than direct thrombin inhibitors, leading to a wider therapeutic window.[6] Both proteinaceous and small molecule inhibitors of fXa have been shown to be effective and safe in preclinical animal thrombosis models.[7] Data from animal models has demonstrated a higher therapeutic ratio (antithrombotic efficacy versus bleeding) for fXa inhibitors than for direct thrombin inhibitors, leading to the expectation of a superior clinical profile.[8]

A wide variety of novel, potent, and selective small molecule fXa inhibitors have been reported.[9] Early small molecule fXa inhibitors invariably contained a benzamidine or naphthylamidine group, initially thought to be necessary for binding in the S1 pocket. The suboptimal oral absorption and/or pharmacokinetic properties often associated with amidines directed efforts to remove this functionality. Numerous reports of the identification of potent, nonamidine, small molecule fXa inhibitors have appeared in recent literature.[10]

High-throughput screening of our compound library identified the anthranilamide compound **1** (Chart 1) as a novel, potent ($K_{i,app}$ = 10 nM), nonamidine fXa inhibitor.[11] Initial optimization of this template resulted in the discovery of compound **2**,[12] with improved fXa potency ($K_{i,app}$ = 1.0 nM) and micromolar anticoagulant activity, as measured by the concentration of compound required to cause a doubling of the prothrombin time (PT) in vitro in human plasma (2×PT = 12 μM). In this paper, we present further optimization of template **2**, leading to the discovery of orally available fXa inhibitors with picomolar potency.[13]

Chemistry. The synthesis of compounds **3a**−**c** was similar to that reported previously for compound **2**.[12] The synthesis of **3b** and **3c** is outlined in Scheme 1. For compound **3c**, commercially available 3-methoxy-2-nitrobenzoic acid **4** was converted to the acid chloride and reacted with 5-chloro-2-aminopyridine **5b** to afford amide **6b** in 97% yield. Following reduction of the nitro group with sodium hydrosulfite, chlorination with *N*-chlorosuccinimide (NCS) occurred exclusively at the 5-position of the anthranilamide ring as a result of the directing effect of the aniline. The substitution pattern was confirmed by ^{1}H NMR. The thiophene-acid chloride **8**[12] was coupled with aniline **7b** to afford **9b** in 85% yield. Addition of exactly one equivalent of base was critical in this coupling step to avoid displacement of the halide. Reaction of **9b** with excess *N*-methylpiperazine afforded the desired product **3c**. Similarly, displacement of the chlorine with other amines or nitrogen nucleophiles afforded compounds **10a**−**p**, **17a**−**g**, and **17j**−**p**.

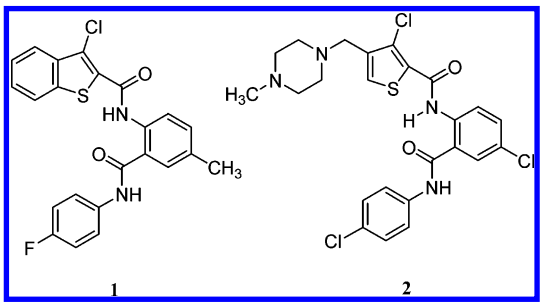
Methylamino derivative **10b** was further reacted to form sulfonamide and urea analogs (Scheme 2). Treatment with the

* To whom correspondence should be addressed. E-mail: rickbinye@yahoo.com (B.Y.); mkochanny@sbcglobal.net (M.J.K.).

[a] Abbreviations: fXa, factor Xa; fIIa, factor IIa (thrombin); PT, prothrombin time; 2×PT, concentration required to give a doubling of the prothrombin time; tPA, tissue plasminogen activator; aPC, activated protein C; EGF, epidermal growth factor; des-GLA-EGF1-factor Xaβ, factor Xa construct with the EGF 2 domain and the serine protease domain.

10.1021/jm070125f CCC: $37.00
Published on Web 05/31/2007

Chart 1



appropriate sulfonyl chloride or isocyanate afforded compounds **12a**−**c** directly, and **12f** was prepared by hydrolysis of an intermediate ester. Hydroxyethyl analog **12d** was prepared by the formation of an intermediate trichloromethyl carbamate and displacement with 2-aminoethanol. Treatment of **10b** with 2-bromoethyl isocyanate in THF at 0 °C afforded urea **11** in quantitative yield. Displacement of the bromide using excess pyrrolidine gave the substituted urea **12e** in low yield. The major product arose from intramolecular cyclization to afford oxazoline **13a**. Modification of the reaction conditions failed to improve the yield of urea **12e**. When a tertiary amine was used instead of a secondary amine, **13a** was obtained as the sole product. Other oxazoline (**13b**−**e**), oxazine (**13h**), keto-oxazoline (**13i**), and thiazoline (**13j**) analogs were prepared from the corresponding amines and isocyanates or thioisocyanates.

Structure−**Activity Results and Pharmacological Activity.** Despite nanomolar fXa binding affinity, compound **2** is a weak anticoagulant (2×PT = 12 μM). Anticoagulant activity of fXa inhibitors as well as thrombin inhibitors has been observed to be a function not only of potency, but also of lipophilicity and protein binding.[14] Early optimization studies on this series showed that the 4-chloroaniline could be replaced with 2-amino-5-chloropyridine, and a polar substituent such as methoxy could be added at C-3 of the central anthranilamide ring.[11a] Incorporation of these changes into inhibitor **2** afforded compounds **3a** and **3b**, with similar or slightly improved fXa inhibitory and anticoagulant activities compared to compound **2** (Table 1). When both changes were made simultaneously, the effects were additive, affording compound **3c** with a further improvement in fXa potency and low micromolar anticoagulant activity. All compounds were selective for fXa versus thrombin and trypsin. Experimental logD values did not differ substantially between compounds **2**, **3a**, and **3c**, suggesting that the improved PT potency for **3c** is primarily a function of increased potency against fXa.

Given the improved activity of **3c**, we returned to the optimization of the thiophene ring C-4 substituent. Our previous work indicated that this portion of the template binds in the S4 pocket of fXa and that amine substituents were preferred for fXa potency.[12] Initially, we investigated simple aliphatic and alicyclic amines (Table 2). For aliphatic monoamines, adding a small alkyl substituent increased fXa potency by up to 5-fold compared to that of the unsubstituted compound (**10b**, **10c**, vs **10a**), while a branched alkyl substituent (**10d**) decreased potency relative to the unbranched amines. Lower dialkylamine substituents had similar fXa potency to the corresponding monoalkylamine compounds (**10e**, **10f**). Alicyclic monoamines gave similar (**10h**) or slightly reduced fXa potency compared to piperazine and aliphatic amines (**10g**, **10i**). These results are consistent with previous observations for analogs of compound **2**.[12]

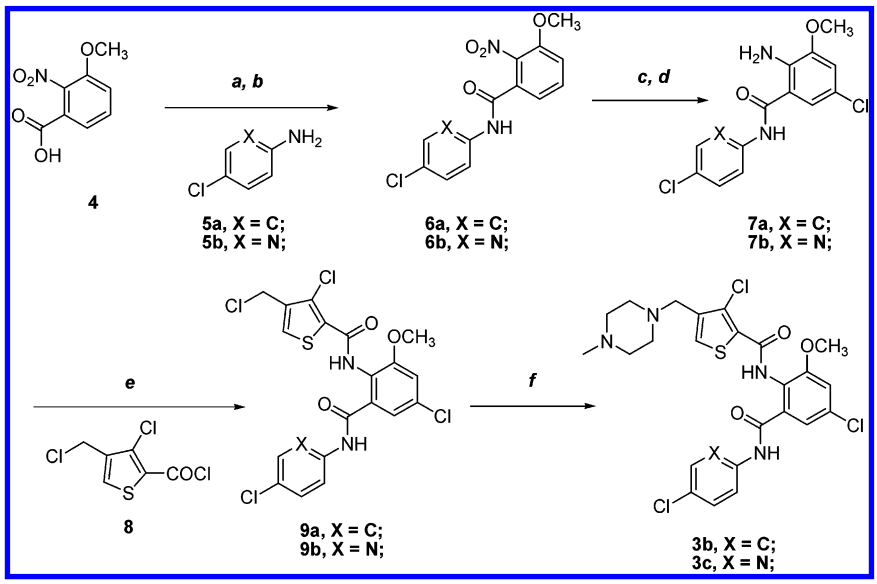
We then examined the effect of aliphatic amine substituents bearing an additional hydrophilic or basic group. Straight or branched chain hydroxy-substituted amines were investigated (**10j**−**10n**), but none gave a significant change in fXa activity compared to the aliphatic amines **10e** and **10f**. Aliphatic diamines, however, gave an unexpected result. Compared to the corresponding hydroxy-substituted amines **10k** and **10j**, diamine compounds **10o** and **10p** showed a 10-fold improvement in fXa potency, affording low picomolar fXa inhibitors. A corresponding 6- to 7-fold improvement in PT potency was also observed, giving the first submicromolar anticoagulants in this series. This result is in contrast to the phenyl series, where substitution of an amine for a hydroxy caused only about a 2-fold increase in fXa potency.[12] The reason for this difference between the two series is unclear. Compounds **10o** and **10p** were 5- to 10-fold more potent against thrombin relative to the monoamines ($K_{i,app}$ = 200−300 nM), but retained at least 10 000-fold selectivity for fXa.

Simple sulfonamide (**12a**) and urea (**12b**, **12c**) substituents gave a 3- to 10-fold increase in fXa potency relative to the closest amine analogs (**10b**, **10e**), also affording picomolar inhibitors (Table 3). However, in spite of their fXa potency, these compounds were an order of magnitude less potent than the diamino compounds **10o** and **10p** in the PT assay. A similar disconnect between fXa potency and anticoagulant activity was seen for our early neutral inhibitors in this series and was attributed to poor solubility and high nonspecific protein binding.[11] To improve PT potency, we tried adding basic and hydrophilic substituents to the urea (**12d**−**f**). These changes were largely unsuccessful, affording only minor improvements in PT potency in the best case. The pyrrolidinoethyl urea **12e** had 5-fold better PT potency than the corresponding ethyl urea **12c**, consistent with the predicted lower lipophilicity of **12e**. However, hydroxy (**12d**) or acid (**12f**) substitution had no effect on PT.

As outlined in Scheme 2, the major product of the reaction to form the substituted urea **12e** was the cyclized oxazoline, **13a**. Compound **13a** was found to be surprisingly potent, having single digit picomolar fXa activity and submicromolar anticoagulant activity (Table 4). The potency of **13a** led us to explore modifications of the oxazoline substituent. Extension of the *N*-methyl group to *N*-ethyl had little effect (**13b**), however, replacement with trifluoroethyl caused a large drop in potency (**13c**). Larger groups like *t*-butyl or 2-methoxyethyl also significantly decreased potency (**13d**, **13e**). Substitution on the oxazoline ring decreased fXa and PT potency, although the effect was smaller at C-4 than at C-5 (**13f**, **13g**). Replacement of the oxazoline with a number of closely related ring systems also afforded potent fXa inhibitors (**13h**−**l**). However, only the six-membered-ring analog **13h** and the highly basic pyrroline **13l** retained submicromolar PT potency. Compounds **13i** and **13k** are uncharged at neutral pH, and their PT potency is comparable to that of the neutral sulfonamide and urea analogs (**12a**, **12b**). The reason for the reduced anticoagulant potency of the thiazoline analog **13j**, however, is unclear. Compounds **13a** and **13i**−**k** were more potent inhibitors of thrombin ($K_{i,app}$ = 50−100 nM) than other compounds from this template, but remained highly selective for fXa.

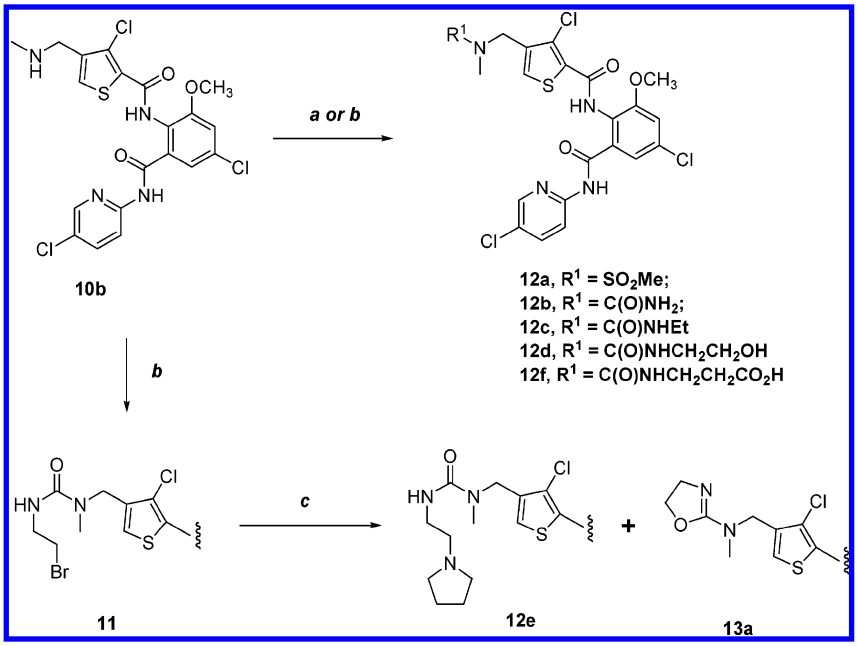
The results for the urea and oxazoline analogs (Tables 3 and 4) suggest a preference for planar groups off the methylthiophene. We explored this further by preparing a variety of compounds in which the nitrogen substituent is sp^2 hybridized (Table 5). Consistent with our hypothesis, all compounds were highly potent inhibitors of fXa, many with single digit picomolar $K_{i,app}$ values. In general, compounds in which the linking nitrogen was part of a ring had better selectivity for fXa versus

Scheme 1[a]



[a] Reagents and conditions: (a) $(COCl)_2$, DMF (cat.), CH_2Cl_2, 0 °C to rt; (b) pyridine, DMAP (cat), 0 °C to rt; (c) $Na_2S_2O_4$, THF/dioxane/H_2O, rt; (d) NCS, benzene, 50 °C; (e) pyridine, CH_2Cl_2, 0 °C to rt; (f) *N*-methylpiperazine, DMF, rt.

Scheme 2[a]

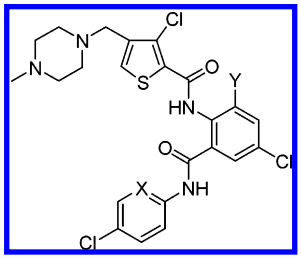


[a] Reagents and conditions: (a) $MeSO_2Cl$, pyridine, 0 °C to rt (for **12a**); (b) isocyanate, dioxane or MeOH, rt (for **11**, **12b**,**c**,**e**); $(Cl_3CO)_2C(O)$, CH_2Cl_2, then 2-aminoethanol, rt (for **12d**); O═C═$NCH_2CH_2CO_2Et$, dioxane, rt; then LiOH.H_20, water (for **12f**); (c) pyrrolidine, DMF, rt or NEt_3, THF, 0 °C to rt (to give **13a** exclusively).

thrombin than compounds with an exocyclic linking nitrogen (e.g., **17a** vs **13a**, **17b** vs **13j**, and **17g** vs **17h**). While the compounds containing nonaromatic amine substituents were highly potent fXa inhibitors and anticoagulants, most of the substituents are highly basic (cpKa 10−14). This was expected to lead to poor pharmacokinetic properties, given the experience with amidine and guanidine containing fXa inhibitors. In the search for substituents with moderate basicity, we prepared imidazole derivatives **17j** and **17k**. These compounds were also found to be highly potent fXa inhibitors, but were weaker anticoagulants ($2\times PT = 1.4$ and 2.0 μM) than the corresponding imidazolines **17f** and **17g**. Adding an amino group at C-2 to increase hydrophilicity maintained fXa activity and improved anticoagulant activity (**17l**, $2\times PT = 0.77$ μM). Alkylation of the exocyclic amine (**17m**−**o**) also afforded highly potent fXa inhibitors. The anticoagulant potency dropped off slightly as substituent size increased. The substituted imidazoles were among the most potent fXa inhibitors and anticoagulants we identified. Furthermore, selectivity against thrombin was significantly improved for these compounds relative to the oxazoline series.

Pharmacokinetic profiles were determined for selected inhibitors (Table 6). Compounds were dosed in conscious beagle dogs intravenously at 1 mg/kg and by oral gavage at 10 mg/kg, and plasma samples were taken at intervals after dosing. Inhibitor concentrations were determined ex vivo using an established chromogenic assay measuring fXa inhibition[15,16] or by HPLC, with peak area measured by UV absorption and compared to a standard solution of known inhibitor concentration. The simple methylamino compound **10b** was among the best compounds

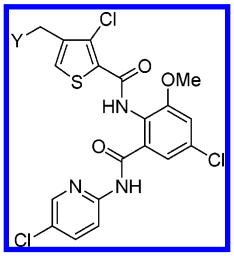
Table 1. Addition of Hydrophilic Substituents



			$K_{i,app}$[a,b] (nM)			
cmpd	X	Y	fXa	fIIa	2×PT[c] (μM)	logD (7.4)
2	C	H	0.76	840	12	2.96
3a	N	H	0.36	680	6.9	3.11
3b	C	OMe	0.43	1300	4.2	ND[d]
3c	N	OMe	0.16	1500	1.6	2.75

[a] $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m. $K_{i,app}$ values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean. [b] All compounds had $K_{i,app}$ values for bovine trypsin of >5000 nM. [c] Concentration that gives a 2-fold extension of human prothrombin time in vitro. [d] ND = not determined.

Table 2. Effect of Amine-Substituted Thiophenes on Enzyme Inhibition and Anticoagulant Activity

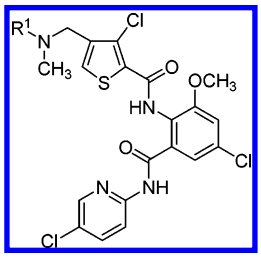


		$K_{i,app}$[a,b] (nM)		
cmpd	Y	fXa	fIIa	2×PT[c] (μM)
3c	4-methyl-1-piperazine	0.16	1500	1.6
10a	$-NH_2$	1.0	>5000	4.2
10b	$-NHCH_3$	0.21	3700	1.6
10c	$-NHCH_2CH_3$	0.40	2600	2.2
10d	$-NHCH(CH_3)_2$	1.4	5400	ND[d]
10e	$-N(CH_3)CH_2CH_3$	0.22	1700	1.4
10f	$-N(CH_2CH_3)_2$	0.32	1500	2.4
10g	1-morpholine	1.4	1400	4.9
10h	1-pyrrolidine	0.52	1400	1.6
10i	1-(4-hydroxy)piperidine	2.2	770	3.2
10j	$-N(CH_3)CH_2CH_2OH$	0.24	1400	1.6
10k	$-N(CH_3)-(CH_2)_3-OH$	0.18	1100	1.5
10l	$-N(CH_3)CH_2CH(CH_3)OH$	0.40	2100	1.5
10m	$-N(CH_3)CH_2C(CH_3)_2OH$	0.71	1100	5.3
10n	$-N(CH_3)CH_2CH(OH)CH_2OH$	0.26	1200	3.2
10o	$-N(CH_3)-(CH_2)_3-N(CH_3)_2$	0.024	300	0.24
10p	$-N(CH_3)CH_2CH_2$-(1-pyrrolidine)	0.019	190	0.24

[a] $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m. $K_{i,app}$ values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean. [b] All compounds had $K_{i,app}$ values for bovine trypsin of >5000 nM. [c] Concentration that gives a 2-fold extension of human prothrombin time in vitro. [d] ND = not determined.

in this series, affording high plasma levels and maintaining levels close to peak out to at least 12 h. Adding a hydroxy substituent (**10j**) led to slightly lower oral exposure, while the potent diamino compound **10o** gave no measurable plasma levels after oral dosing. The highly potent oxazoline compound **13a** and imidazole **17m** also gave quite promising results, with plasma concentration profiles similar to the aliphatic monoamines (**10b**, **10j**). Anticoagulant activity was determined ex vivo by PT

Table 3. Effect of Sulfonamide and Urea Substitution on Enzyme Inhibition and Anticoagulant Activity



		$K_{i,app}$[a,b] (nM)		
cmpd	R^1	fXa	fIIa	2×PT[c] (μM)
10b	-H	0.21	3700	1.6
10e	$-CH_2CH_3$	0.22	1700	1.4
12a	$-SO_2CH_3$	0.045	340	2.2
12b	$-C(O)NH_2$	0.026	340	2.6
12c	$-C(O)NHCH_2CH_3$	0.020	450	5.8
12d	$-C(O)NHCH_2CH_2OH$	0.050	360	4.1
12e	$-C(O)NHCH_2CH_2$-(1-pyrrolidine)	0.036	140	1.1
12f	$-C(O)NHCH_2CH_2CO_2H$	0.022	630	7.0

[a] $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m. $K_{i,app}$ values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean. [b] All compounds had $K_{i,app}$ values for bovine trypsin of >5000 nM. [c] Concentration that gives a 2-fold extension of human prothrombin time in vitro.

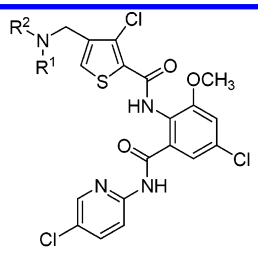
measurement. After a 10 mg/kg oral dose, compound **13a** prolonged PT 3.2-, 5.0-, and 4.0-fold at 1, 2, and 6 h. Compounds **10b** and **17m** prolonged PT to a lesser extent following oral dosing (data not shown), but were also weaker anticoagulants in vitro in dog plasma (Table 7).

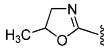
Selectivity data against a panel of human proteases are shown in Table 8. Compounds **10b**, **13a**, and **17m** all had >20 000-fold selectivity relative to trypsin, tPA, factor XIa, urokinase, aPC, plasmin, chymotrypsin, cathepsin G, and neutrophil elastase. Compound **17m** was also highly selective against thrombin, while thrombin selectivity for **10b** and **13a** was somewhat lower (>17 000- and >12 000-fold). The lowest selectivity observed was against plasma kallikrein, however, all three compounds retained >4000-fold selectivity versus this enzyme.

Compounds **10b**, **13a**, and **17m** were tested in the rat vena cava stasis model of thrombosis. The results are shown in Figure 1. All three compounds exhibited a dose-dependent inhibition of experimental vena cava thrombosis in anesthetized rats. For compounds **13a** and **17m**, inhibition was statistically significant at 1 mg/kg, and no clot was detected in any of the animals at 3 mg/kg. For compound **10b**, inhibition was statistically significant only at the highest dose of 10 mg/kg. Estimated ED_{50} values in this model were approximately 4.4 mg/kg, i.v., for **10b**, 0.4 mg/kg, i.v., for **13a**, and 0.36 mg/kg, i.v., for **17m**. In general, these data are consistent with in vitro data for these compounds, which show that compound **10b** is a substantially weaker inhibitor of rat fXa than **13a** and **17m** and also requires higher concentrations to prolong PT in rat plasma (Table 7). Based on their potency, selectivity, efficacy, and pharmacokinetic profiles, compounds **10b** (ZK 814048), **13a** (ZK 810388), and **17m** (ZK 813039) were all identified as candidates meeting the selection criteria for development.[17]

Crystallography. The crystal structure of compound **17m** bound to fXa is shown in Figure 2A (pdb code 2P3T). The inhibitor binds in an L-shaped conformation with the chloropyridine buried in the S1 pocket and the imidazole substituent

Table 4. SAR of Oxazoline Analogs



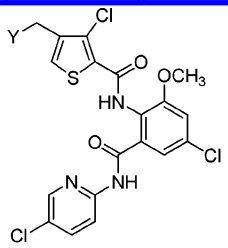
Cpd	R^1	R^2	$K_{i,app}$ (nM)[a,b] fXa	fIIa	2xPT (μM)[c]
13a	$-CH_3$		0.007	90	0.36
13b	$-CH_2CH_3$		0.012	470	0.57
13c	$-CH_2CF_3$		2.2	>5000	28
13d	t-Bu		0.12	1700	ND[d]
13e	$-CH_2CH_2OMe$		0.26	2000	1.1
13f	$-CH_3$		0.12	410	1.2
13g	$-CH_2CH_3$		0.031	580	3.8
13h	$-CH_3$		0.012	160	0.22
13i	$-CH_3$		0.026	90	2.2
13j	$-CH_3$		0.024	100	1.8
13k	$-CH_3$		0.059	60	4.2
13l	$-CH_3$		0.004	970	0.40

[a] $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m. $K_{i,app}$ values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean. [b] All compounds had $K_{i,app}$ values for bovine trypsin of >5000 nM. [c] Concentration that gives a 2-fold extension of human prothrombin time in vitro. [d] ND = not determined.

extending into the S4 binding site. The chlorine atom makes contact with Tyr228 in the S1 pocket. This chloro binding mode has been reported for other fXa inhibitors,[18] as well as for inhibitors of thrombin,[14b] trypsin,[19] and urokinase.[20] Compound **17m** forms only one well-defined hydrogen bond to fXa between the amide NH attached to the chloropyridine ring and the carbonyl of Glycine 218. There are no direct charge−charge interactions.

The crystal structures of compounds in this series provide insight into the observed SAR (pdb entries 1MQ5,[21] 1MQ6,[21] and 2P3T). Figure 2B shows the methyl group of **13a** (1MQ6) is buried deep in the S4 pocket, where it is in close contact with the indole ring of Trp215 (3.5 Å). The methyl group also makes contact with a water molecule (HOH722 in 1MQ6, 3.4 Å). This water donates two hydrogen bonds to the backbone carbonyls of Thr98 (2.9 Å) and Ile175 (2.7 Å). Similar waters have been seen in other fXa structures.[21,22] Replacing methyl with ethyl (**13b**) may force this water to shift away from the S4 pocket, but it still should maintain the hydrogen bonds to Thr98 and Ile175 (see HOH16 in 1EZQ[22a]), consistent with the similar potency of **13a** and **13b**. More bulky groups such as *t*-butyl (**13d**) or $CH_2CH_2OCH_3$ (**13e**) would entirely displace this water molecule. For these compounds, however, the

Table 5. SAR of sp^2 Hybridized Nitrogen Substituents



Cpd	Y	$K_{i,app}$ (nM)[a,b] fXa	fIIa	2xPT (μM)[c]
17a		≤0.010	1100	0.26
17b		≤0.010	2600	1.4
17c		≤0.010	1500	0.27
17d		0.034	340	3.3
17e		0.004	2200	0.53
17f		≤0.010	3300	0.32
17g		0.004	3300	0.26
17h		≤0.010	920	0.25
17i		≤0.010	630	0.40
17j	1-imidazole	0.022	510	2.0
17k	2-methyl-1-imidazole	≤0.010	440	1.4
17l	2-amino-1-imidazole	0.006	3500	0.77
17m	2-(methylamino)-1-imidazole	0.005	990	1.2
17n	2-(ethylamino)-1-imidazole	0.005	1100	1.8
17o	2-(isopropylamino)-1-imidazole	0.008	1200	2.3
17p		0.020	860	1.8

[a] $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m. $K_{i,app}$ values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean. [b] All compounds had $K_{i,app}$ values for bovine trypsin of >5000 nM. [c] Concentration that gives a 2-fold extension of human prothrombin time in vitro.

space between Phe174 and Trp215 may have insufficient volume to accommodate the larger residues, resulting in the observed 10-fold loss of potency. Electronegative groups such CH_2CF_3 (**13c**) would have unfavorable interactions with the carbonyls of Thr98 and Ile175. In **17m**, part of the imidazole ring occupies the same space as the methyl group of **13a**. Most of the compounds shown in Table 5 could maintain the same contacts with Trp215 and HOH722. However, the oxygen of **17d** would have unfavorable contacts with HOH722, accounting for the 6-fold loss in potency for this inhibitor. Planar substituents likely have a better fit in the space between Tyr99 and Phe174, accounting for the high potency of the compounds in Table 5.

It has been hypothesized that basic substituents in the S4 pocket interact with electronegative groups of residues 96−98

Table 6. Pharmacokinetic Profile in Dogs

cmpd	Cl[a] (mL/min/kg)	V_{ss}[a] (L/kg)	$t_{1/2}$[a] (h)	C_{max}[b] (μg/mL)	%F[c]
10b[d]	1.6	1.4	10.5	5.1	75
10j[d]	27	4.4	2.1	1.2	>100
10o[d]	ND[f]	ND	ND	BLQ[g]	ND
13a[d]	5.8	1.6	3.2	1.9	56
17m[e]	4.4	1.0	3.4	3.7	98

[a] Cl, V_{ss}, and $t_{1/2}$ values were determined based on a 1 mg/kg iv dose (10% DMSO/saline, dosing volume 1 mL/kg). [b] C_{max} was based on a 10 mg/kg po dose (40% w/w hydroxypropyl-β-cyclodextrin/water, dosing volume 1 mL/kg). [c] %F was calculated by normalizing the AUC after 10 mg/kg po and dividing by the AUC after 1 mg/kg iv. AUC values were calculated based on extrapolation to infinity. [d] Inhibitor concentrations were determined by chromogenic assay. [e] Inhibitor concentrations were determined by HPLC-UV. [f] ND = not determined. [g] BLQ indicates that levels were below the limit of quantitation for the method employed.

(Figure 2B). The positive charge on the imidazole ring of **17m** interacts with the backbone carbonyls of residues 96−98. For compounds **10o** and **10p**, the tertiary amine at the end of the chain may directly interact with the carboxylic acid of Glu97. It is harder to explain the relative potency of other modifications in the S4 binding region (e.g., **12a**−**f** and **13f**−**l**, Tables 3 and 4). Potent inhibitors with neutral amide groups at this position such as **12b** and **12c** may form hydrogen bonds to the backbone carbonyls that line the S4 pocket. However, there is no easy explanation for the 45 pM potency of compound **12a**. This inhibitor may adopt a unique conformation that places the sulfonamide group outside of the S4 pocket. The X-ray structures of fXa complexes provide important insight into the activity of this thiophene template. However, the electrostatic interactions in the S4 pocket remain difficult to predict.

Conclusions

Replacement of the chlorobenzene ring of **2** with chloropyridine and addition of a methoxy substituent to the anthranilamide ring afforded compound **3c** with improved potency and anticoagulant activity. Modification of the thiophene C-4 substituent led to the discovery of a series of highly potent and orally available fXa inhibitors. Pharmacokinetic studies in dogs identified the picomolar inhibitors **13a** and **17m** as having low clearance and moderate to high oral bioavailability. Compound **10b**, while a weaker inhibitor, gave high plasma levels after oral dosing and had a prolonged half-life. All three compounds had good selectivity against a panel of human serine proteases and were efficacious in a venous thrombosis model in rats. Compound **13a** was initially selected for development, but was terminated for reasons that included insufficient chemical stability of the oxazoline moiety. Following the termination of **13a**, compounds **10b** and **17m** were advanced into development.

Experimental Section

All reactions were run under an atmosphere of dry nitrogen. All starting materials not described below were purchased from commercial sources or synthesized following the noted literature procedure. All reagents and solvents were used as received from commercial sources without additional purification. Elemental analysis and logD determinations were performed by Robertson Microlit Laboratories; Madison, NJ, and elemental analysis results were within ±0.4% of the calculated values. NMR spectra were obtained with a Varian XL-300 spectrometer and were consistent with the assigned structures. HPLC was performed with a Rainin SD-1 Dynamax system and a C-18 reverse phase Dynamax 60 A column using a gradient of acetonitrile (0.1% TFA) in water (0.1% TFA).

Human fXa and human fIIa were from Enzyme Research Lab., South Bend, IN, and bovine trypsin was from Boehringer Mannheim Corp., Indianapolis, IN. All peptide-*p*-nitroanilide substrates were purchased from Kabi Pharmacia Hepar, Inc., Franklin, OH. TrisHCl, NaCl, and $CaCl_2$ were from J. T. Baker Inc., Jackson, TN, and polyethylene glycol 6000 was from BDH Laboratory Supplies, Poole, England.

***N*-(5-Chloro-2-pyridinyl)-3-methoxy-2-nitrobenzamide (6b).** To a suspension of 3-methoxy-2-nitrobenzoic acid **4** (197 g, 1.0 mol) in CH_2Cl_2 (1 L) at 0 °C were added several drops of DMF, followed by oxalyl chloride (114 mL, 1.3 mol). The cooling bath was removed, and the reaction was stirred at room temperature. After 16 h, the mixture was concentrated to give the crude acid chloride. The solid was pulverized and dried under vacuum. 3-Methoxy-2-nitrobenzoyl chloride (216 g, quant.) was obtained as a light yellow solid, mp 83−85 °C: ^{1}H NMR ($CDCl_3$) δ 7.8 (d, 1H), 7.6 (t, 1H), 7.4 (d, 1H), 3.9 (s, 3H).

To a stirred solution of 2-amino-5-chloropyridine, **5b** (129 g, 1.0 mol), in dry pyridine (1 L) at 0 °C was added a small amount of 4-dimethylaminopyridine, followed by 3-methoxy-2-nitrobenzoyl chloride (215 g, 1.0 mol). The reaction was allowed to warm to room temperature and stirred overnight. The mixture was poured into water (6 L) and filtered. The filter cake was collected, slurried in water (3 L), and filtered. The solid was dried to give **6b** (301 g, 97%) as an off-white powder, mp 215−217 °C: ^{1}H NMR ($CDCl_3$) δ 8.8 (br s, 1H), 8.3 (d, 1H), 8.1 (s, 1H), 7.7 (d, 1H), 7.5 (t, 1H), 7.3 (m, 2H), 3.9 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.4, 151.4, 151.0, 147.2, 138.8, 132.7, 130.4, 126.9, 121.2, 117.3, 116.3, 57.7. Anal. ($C_{13}H_{10}ClN_3O_4$) C, H, N.

***N*-(4-Chlorophenyl)-3-methoxy-2-nitrobenzamide (6a).** Compound **6a** was prepared from 4-chloroaniline and **4** by a similar procedure as for compound **6b** (4.4 g, 48%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.8 (s, 1H), 7.7 (m, 3H), 7.5 (d, 1H), 7.4 (t, 2H), 3.9 (s, 3H), 3.4 (br s, 1H).

2-Amino-5-chloro-*N*-(5-chloro-2-pyridinyl)-3-methoxybenzamide (7b). To a stirred solution of sodium hydrosulfite (300 g, 1.7 mol) in water (4 L) was added **6b** (140 g, 0.45 mol). THF (2 L) and 1,4-dioxane (2 L) were added, and the reaction became homogeneous. The reaction was stirred overnight, then potassium carbonate was added until basic, at which point the organic and aqueous phases separated. The organic phase was concentrated to give an off-white solid. The solid was slurried in water, filtered, and dried under vacuum at 40 °C to give the aniline, 2-amino-*N*-(5-chloro-2-pyridinyl)-3-methoxybenzamide (111 g, 88%): ^{1}H NMR ($CDCl_3$) δ 8.8 (br s, 1H), 8.3 (d, 1H), 8.1 (s, 1H), 7.7 (dd, 1H), 7.1 (dd, 1H), 6.8 (d, 1H), 6.6 (t, 1H), 5.9 (br s, 1H), 3.9 (s, 3H).

To a stirred solution of the aniline (39.1 g, 140 mmol) in benzene (2 L) was added NCS (20 g, 148 mmol). The reaction was heated at 50−55 °C for 24 h, then cooled to room temperature and concentrated. The crude solid was dissolved in EtOAc (1 L), washed with water (3 × 100 mL), dried over Na_2SO_4, and concentrated. The crude product was recrystallized from benzene to afford **7b** (40 g, 90%) as off-white needles, mp 146−148 °C: ^{1}H NMR ($CDCl_3$) δ 8.6 (br s, 1H), 8.3 (m, 2H), 7.7 (dd, 1H), 7.1 (d, 1H), 6.8 (d, 1H), 5.9 (br s, 1H), 3.9 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 149.8, 148.3, 146.6, 139.3, 137.9, 126.7, 120.1, 118.0, 114.7, 113.9, 112.9, 56.0. Anal. ($C_{13}H_{11}Cl_2N_3O_2$) C, H, N.

2-Amino-5-chloro-*N*-(4-chlorophenyl)-3-methoxybenzamide (7a). Compound **7a** was prepared from **6a** by a similar procedure as for compound **7b** (1.4 g, 61%): ^{1}H NMR ($CDCl_3$) δ 7.7 (br s, 1H), 7.4 (dd, 4H), 7.1 (d, 1H), 6.8 (d, 1H), 5.8 (br s, 2H), 3.9 (s, 3H).

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-chloromethyl-2-thiophenecarboxamide (9b). To a stirred solution of 3-chloro-4-chloromethyl-2-thiophenecarbonyl chloride (**8**;[12] 60 g, 260 mmol) in dry CH_2Cl_2 (450 mL) at 0 °C was added **7b** (63 g, 200 mmol) in portions, followed after 5 min by pyridine (33 mL, 330 mmol). The reaction changed from a bright yellow suspension to a homogeneous brown solution within a few minutes after the pyridine addition, and a precipitate began to form within 1 h. After 17 h at room temperature, the reaction was concentrated and dried under vacuum. The crude

Table 7. Species Specificity of fXa Inhibition and Anticoagulant Activity

	fXa $K_{i,app}$[a] (nM)			2×PT[b] (μM)		
cmpd	human	dog	rat	human	dog	rat
10b	0.21 ± 0.07	1.7 ± 0.0	2.5 ± 0.2	1.64 ± 0.11	4.35 ± 0.05	7.3 ± 0.28
13a	0.007 ± 0.001	0.15 ± 0.05	0.29 ± 0.17	0.36 ± 0.02	0.35[c]	0.93[c]
17m	0.005 ± 0.001	0.31 ± 0.02	0.06 ± 0.01	1.25 ± 0.07	4.2[c]	2.56[c]

[a] $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m. $K_{i,app}$ values are averaged from multiple determinations ($n \geq 2$). [b] Concentration that gives a 2-fold extension of the PT in vitro. Values are averaged from multiple determinations unless otherwise indicated. [c] $n = 1$.

Table 8. Human Protease Selectivity Profiles

enzyme K_i[a] (nM)	**10b**	**13a**	**17m**
fXa	0.21[b]	0.007[b]	0.005[b]
thrombin	3700	90	990
trypsin	>10 000	>10 000	>5000
tPA	>5000	>5000	>5000
kallikrein	1000	37	45
factor XIa	>10 000	>10 000	>5000
urokinase	>5000	>5000	>5000
aPC	>10 000	>10 000	>5000
plasmin	>10 000	>10 000	>5000
chymotrypsin	>10 000	>10 000	>5000
cathepsin G	>10 000	>10 000	>5000
neutrophil elastase	>10 000	>10 000	>5000

[a] K_i values are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean. [b] $K_{i,app}$ value. $K_{i,app}$ is the apparent K_i value, defined as $IC_{50}/2$ when the IC_{50} value is determined at a substrate concentration equal to the K_m.

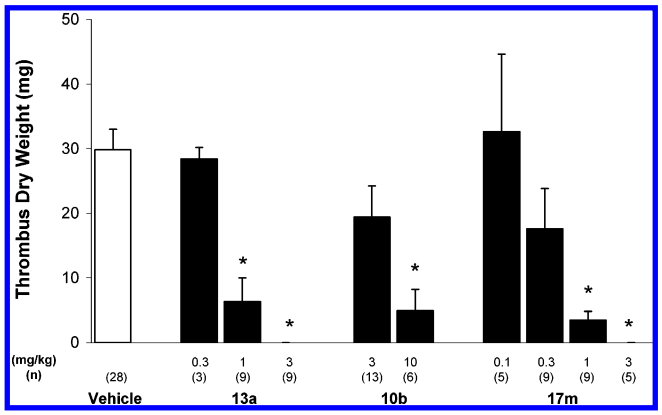


Figure 1. The effects of compounds on vena cava thrombosis after i.v. dosing in anesthetized rats. *$p < 0.05$ compared to the vehicle control group.

solid was ground, triturated with water (4 × 100 mL) and dried under vacuum to give **9b** (113 g, 85%) as a light brown powder, mp 196−198 °C: ^{1}H NMR ($CDCl_3$) δ 9.3 (br s, 1H), 9.1 (br s, 1H), 8.3 (d, 1H), 8.0 (d, 1H), 7.7 (d, 1H), 7.6 (s, 1H), 7.2 (d, 1H), 7.0 (d, 1H), 4.6 (s, 2H), 3.9 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.9, 158.5, 153.4, 149.9, 146.3, 137.9, 136.8, 132.9, 132.5, 129.7, 126.8, 123.9, 121.7, 119.8, 115.1, 113.9, 56.6, 38.6. Anal. ($C_{19}H_{13}Cl_4N_3O_3S$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(4-chlorophenyl)amino]carbonyl]-6-methoxyphenyl]-4-chloromethyl-2-thiophenecarboxamide (9a). Compound **9a** was prepared from **7a** by a similar procedure as for compound **9b** (0.27 g, 67%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.4 (s, 1H), 9.5 (s, 1H), 8.1 (s, 1H), 7.7 (d, 2H), 7.3−7.4 (m, 4H), 4.8 (s, 2H), 3.9 (s, 3H).

General Method for Preparation of Amine-Substituted Compounds from Chloromethylthiophenes. To a 0.1 M solution of a chloromethylthiophene intermediate (e.g., **9a** or **9b**) in dry DMF at 0 °C was added an amine (5 equiv), the mixture was stirred for 0.5 h at 0 °C, and then the mixture was warmed to room temperature. After 4−18 h, the reaction mixture was poured into water, the resulting solid was collected by filtration or extracted into a solvent such as EtOAc, and the organic solution was dried over Na_2SO_4 and concentrated. The crude product was purified by

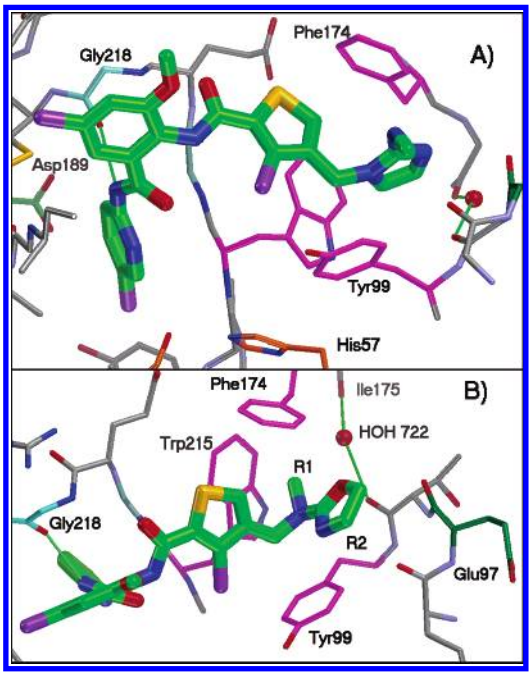


Figure 2. (A) The conformation of **17m** bound to fXa (PDB entry 2P3T). Hydrogen bonds are shown as green lines. The carbon atoms of **17m** are colored green and the chlorines are displayed in purple. Asp189 at the base of the S1 pocket is dark green and Gly218 is light blue. The hydrophobic residues that line the S4 pocket, Tyr99, Phe174, and Trp215, are shown with magenta carbons. Two residues from the active site triad, His57 and Ser195, are gold. (B) A detailed view of the S4 pocket from the fXa/**13a** complex (PDB entry 1MQ6). Glu97 is shown in dark green. R_1 and R_2 refer to the substitution scheme from Table 4.

flash chromatography. Alternatively, the product was purified by HPLC using a Dynamax column and a gradient of CH_3CN in H_2O with 0.1% TFA. The resulting fractions were combined, and the solvent was removed by lyophilization to afford the product. In the case of HPLC purification, the product was obtained as the trifluoroacetic acid salt.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]phenyl]-4-[(4-methyl-1-piperazinyl)methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (3a). The aniline precursor, 2-amino-5-chloro-*N*-(5-chloro-2-pyridinyl)benzamide, was prepared from 5-chloro-2-nitrobenzoic acid and 2-amino-5-chloropyridine following the coupling and reduction procedures reported above for compounds **6b** and **7b**. Reaction with **8** according to the procedure for compound **9b** afforded the chloromethylthiophene intermediate, 3-chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]-carbonyl]phenyl]-4-chloromethyl-2-thiophenecarboxamide, (2.9 g, 90%): ^{1}H NMR (DMSO-d_6/TFA) δ 11.4 (s, 1H), 11.0 (s, 1H), 7.6−8.4 (m, 7H), 4.8 (s, 2H).

This chloromethylthiophene intermediate was reacted with 1-methylpiperazine according to the general method to afford compound **3a**, purified by HPLC, giving a white solid (0.38 g, 64%): ^{1}H NMR (DMSO-d_6/TFA) δ 11.4 (br s, 1H), 11 (s, 1H), 8.4 (d, 1H), 8.3 (s, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 8.0 (m, 2H), 7.8 (dd, 1H), 4.4 (s, 2H), 3.1−3.8 (m, 8H), 2.9 (s, 3H). Anal. ($C_{23}H_{22}Cl_3N_5O_2S\cdot3.0C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(4-chlorophenyl)amino]carbonyl]-6-methoxyphenyl]-4-[(4-methyl-1-piperazinyl)methyl]-2-thiophenecarboxamide (3b). Compound **3b** was prepared from **9a** and 1-methylpiperazine and purified by flash chromatography, giving a white solid (0.77 g, 64%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.4 (s, 1H), 9.5 (s, 1H), 8.1 (s, 1H), 7.6 (d, 2H), 7.3 (m, 4H), 4.3 (s, 2H), 3.8 (s, 3H), 3.1−3.8 (m, 8H), 2.9 (s, 3H). Anal. ($C_{24}H_{23}Cl_3N_4O_3S\cdot0.4CH_2Cl_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(4-methyl-1-piperazinyl)methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (3c). Compound **3c** was prepared from **9b** and 1-methylpiperazine and purified by HPLC, giving a white solid (0.1 g, 35%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (d, 1H), 8.2 (s, 1H), 8.1 (d, 1H), 7.8 (dd, 1H), 7.3 (d, 2H), 4.4 (s, 2H), 3.8 (s, 3H), 3.1−3.8 (m, 8H), 2.9 (s, 3H). Anal. ($C_{24}H_{24}Cl_3N_5O_3S\cdot3.4C_2HF_3O_2\cdot0.5\ H_2O$) C, H, N.

4-Aminomethyl-3-chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-2-thiophenecarboxamide (10a). Compound **10a** was prepared from **9b** and ammonia and purified by flash chromatography, giving a white solid (0.51 g, 53%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.4 (s, 1H), 8.1 (d, 1H), 7.9 (s, 1H), 7.8 (dd, 1H), 7.3 (m, 2H), 4.0 (m, 2H), 3.8 (s, 3H). Anal. ($C_{19}H_{15}Cl_3N_4O_3S$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(methylamino)methyl]-2-thiophenecarboxamide (10b). Compound **10b** was prepared from **9b** and methylamine and purified by flash chromatography, giving a white solid (22 g, 74%), mp 166−168 °C: ^{1}H NMR (DMSO-d_6/TFA) δ 11.0 (s, 1H, C(O)N*H*), 9.33 (s, 1H, C(O)N*H*), 9.1 (br s, 1H, N*H*), 8.23 (d, 1H, Ar-*H*), 8.03 (d, 1H, Ar-*H*), 8.01 (s, 1H, Ar-*H*), 7.79 (dd, 1H, Ar-*H*), 7.24 (d, 1H, Ar-*H*), 7.22 (d, 1H, Ar-*H*), 4.06 (s, 2H, CH_2-thiophene), 3.81 (s, 3H, OCH_3), 2.55 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6/TFA) δ 166.4, 159.6, 155.8, 151.2, 146.2, 140.1, 135.2, 133.6, 133.1, 132.9, 132.5, 127.2, 125.9, 123.3, 121.4, 116.8, 115.4, 57.6, 45.6, 33.3. Anal. ($C_{20}H_{17}Cl_3N_4O_3S$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(ethylamino)methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (10c). Compound **10c** was prepared from **9b** and ethylamine, giving a yellow solid (22 g, 57%). A sample was further purified by HPLC, giving a white solid: ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H), 9.40 (s, 1H), 8.80 (br s, 2H), 8.75 (d, 1H), 8.20 (d, 1H), 8.10 (s, 1H), 7.80 (dd, 1H), 7.48 (d, 1H), 7.60 (d, 1H), 4.15 (s, 2H), 3.85 (s, 3H), 3.05 (br s, 2H), 1.20 (t, 3H). Anal. ($C_{21}H_{19}Cl_3N_4O_3S\cdot1.25C_2HF_3O_2\cdot0.5H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(1-methylethyl)amino]methyl]-2-thiophenecarboxamide (10d). Compound **10d** was prepared from **9b** and isopropylamine and purified by flash chromatography, giving a white foam (0.56 g, 53%): ^{1}H NMR ($CDCl_3$) δ 9.1 (s, 1H), 8.9 (s, 1H), 8.3 (d, 1H), 8.1 (d, 1H), 7.6 (dd, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 7.05 (s, 1H), 3.9 (s, 3H), 3.8 (s, 2H), 2.9 (m, 1H), 1.0 (d, 6H). Anal. ($C_{22}H_{21}Cl_3N_4O_3S$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(ethylmethylamino)methyl]-2-thiophenecarboxamide (10e). Compound **10e** was prepared from **9b** and *N*-ethylmethylamine and purified by flash chromatography, giving a white solid (0.24 g, 47%): ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H), 9.38 (s, 1H), 8.30 (d, 1H), 8.20 (d, 1H), 7.90 (dd, 1H), 7.70 (s, 1H), 7.40 (d, 1H), 7.25 (d, 1H), 3.90 (s, 3H), 3.30 (s, 2H), 2.40 (q, 2H), 2.10 (s, 3H), 1.00 (t, 3H). Anal. ($C_{22}H_{21}Cl_3N_4O_3S\cdot0.05CH_2Cl_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(diethylamino)methyl]-2-thiophenecarboxamide (10f). Compound **10f** was prepared from **9b** and diethylamine and purified by flash chromatography, giving a white solid (0.42 g, 54%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.5 (s, 1H), 9.4 (s, 1H), 8.4 (s, 1H), 8.2 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.3 (s, 1H), 7.2 (s, 1H), 4.2 (s, 2H), 3.8 (s, 3H), 3.1 (br s, 4H), 1.2 (m, 6H). Anal. ($C_{24}H_{23}Cl_3N_4O_3S\cdot1.2H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-(4-morpholinylmethyl)-2-thiophenecarboxamide (10g). Compound **10g** was prepared from **9b** and morpholine and purified by flash chromatography, giving a white solid (0.68 g, 62%): ^{1}H NMR ($CDCl_3$) δ 9.1 (s, 1H), 8.7 (s, 1H), 8.2 (d, 1H), 8.1 (s, 1H), 7.6 (dd, 1H), 7.4 (s, 1H), 7.2 (d, 1H), 7.0 (s, 1H), 3.9 (s, 3H), 3.7 (br s, 4H), 3.5 (s, 2H), 2.5 (br s, 4H). Anal. ($C_{23}H_{21}Cl_3N_4O_3S\cdot0.4C_4H_8O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-(1-pyrrolidinylmethyl)-2-thiophenecarboxamide (10h). Compound **10h** was prepared from **9b** and pyrrolidine and purified by flash chromatography, giving a white solid (0.90 g, 84%): ^{1}H NMR ($CDCl_3$) δ 9.1 (s, 1H), 8.7 (s, 1H), 8.3 (d, 1H), 8.1 (s, 1H), 7.6 (d, 1H), 7.5 (s, 1H), 7.2 (s, 1H), 7.0 (s, 1H), 3.9 (s, 3H), 3.8 (s, 2H), 2.6 (m, 4H), 1.8 (m, 4H). Anal. ($C_{23}H_{21}Cl_3N_4O_3S\cdot0.1CH_2Cl_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(4-hydroxy-1-piperidinyl)methyl]-2-thiophenecarboxamide (10i). Compound **10i** was prepared from **9b** and 4-hydroxypiperidine and purified by flash chromatography, giving a white solid (1.0 g, 59%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.5 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.2 (d, 1H), 8.1 (s, 1H), 7.8 (d, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.3 (m, 2H), 3.9 (s, 3H), 3.8 (br s, 1H), 3.4 (m, 1H), 3.2 (m, 2H), 3.0 (m, 1H), 1.8 (m, 4H). Anal. ($C_{24}H_{23}Cl_3N_4O_4S\cdot0.22\ CH_2Cl_2\cdot0.5H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(2-hydroxyethyl)methylamino]methyl]-2-thiophenecarboxamide (10j). Compound **10j** was prepared from **9b** and 2-(methylamino)ethanol and purified by flash chromatography, giving a white solid (0.48 g, 57%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.9 (s, 1H), 9.4 (s, 1H), 8.4 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.8 (s, 1H), 7.4 (s, 1H), 7.2 (s, 1H), 4.4 (t, 1H), 3.9 (s, 3H), 3.5 (m, 4H), 3.3 (d, 2H), 2.4 (m, 3H). Anal. ($C_{22}H_{21}Cl_3N_4O_3S\cdot0.3C_4H_8O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(3-hydroxypropyl)methylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (10k). Compound **10k** was prepared from **9b** and 3-methylamino-1-propanol and purified by HPLC, giving a white solid (0.96 g, 29%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (br s, 1H), 9.6 (br s, 1H), 7.2−8.5 (m, 6H), 4.2−4.5 (m, 4H), 3.9 (s, 3H), 3.0−3.4 (m, 2H), 2.7 (s, 3H), 2.2 (m, 2H). Anal. ($C_{23}H_{23}Cl_3N_4O_4S\cdot2.45C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(2-hydroxypropyl)methylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (10l). Compound **10l** was prepared from **9b** and 1-methylamino-2-propanol and purified by HPLC, giving a white solid (2.2 g, 68%): ^{1}H NMR (DMSO-d_6/TFA) δ 11.1 (s, 1H), 9.6 (br s, 1H), 9.5 (s, 1H), 8.3 (s, 1H), 8.2 (m, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.4 (d, 1H), 4.1−4.4 (m, 2H), 3.8 (s, 3H), 3.14 (m, 1H), 2.74 (m, 2H), 2.4 (s, 3H), 1.0 (d, 3H). Anal. ($C_{23}H_{23}Cl_3N_4O_4S\cdot2.1C_2HF_3O_2\cdot1.0H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(2-hydroxy-2-methylpropyl)methylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (10m). Compound **10m** was prepared from **9b** and 2-methyl-1-(methylamino)-2-propanol and purified by HPLC, giving a white solid (2.3 g, 62%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.6 (s, 1H), 7.2−8.5 (m, 6H), 4.2−4.6 (m, 2H), 3.9 (s, 3H), 3.0−3.3 (m, 2H), 2.9 (s, 3H), 1.3 (s, 3H), 1.2 (s, 3H). Anal. ($C_{24}H_{25}Cl_3N_4O_4S\cdot2.7C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(2,3-dihydroxypropyl)methylamino]methyl]-2-thiophenecarboxamide (10n). Compound **10n** was prepared from **9b** and 3-(methylamino)-1,2-propanediol and purified by flash chromatography, giving a white solid (0.60 g, 35%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.5 (s, 1H), 8.4 (s, 1H), 8.2 (m, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.3 (s, 1H), 7.2 (s, 1H), 4.0−4.4 (m, 3H), 3.9 (s, 3H), 3.0−3.5 (m, 3H), 2.5 (s, 3H). Anal. ($C_{23}H_{23}Cl_3N_4O_5S\cdot0.5H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[[3-(dimethylamino)propyl]methylamino]methyl]-2-thiophenecarboxamide (10o). Compound **10o** was prepared from **9b** and *N*,*N*,*N*′-trimethyl-1,3-propanediamine and purified by flash chromatography, giving a white solid (0.76 g, 33%): ^{1}H NMR (DMSO-d_6) δ 8.3 (d, 1H), 8.1 (d, 1H), 7.9 (dd, 1H), 7.7 (s, 1H), 7.4 (d, 1H), 7.2 (d, 1H), 3.9 (s, 3H), 3.4 (s, 2H), 2.4 (t, 2H), 2.2 (t, 2H), 2.1 (s, 3H), 2.0 (s, 6H), 1.5 (m, 2H). Anal. ($C_{25}H_{28}Cl_3N_5O_3S$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[methyl[2-(1-pyrrolidinyl)ethyl]amino]methyl]-2-thiophenecarboxamide (10p). Compound **10p** was prepared from **9b** and 1-(2-methylaminoethyl)pyrrolidine (prepared by reductive amination of *N*-(2-aminoethyl)pyrrolidine with 37% formaldehyde using $NaBH_3CN$) and purified by flash chromatography, giving a white solid (0.48 g, 37%): ^{1}H NMR (DMSO-d_6/TFA) δ 11.2 (s, 1H), 10.8 (s, 1H), 10.1 (br s, 1H), 8.4 (d, 1H), 8.3 (s, 1H), 7.9 (s, 1H), 7.7 (d, 2H), 7.6 (d, 1H), 7.4 (d, 2H), 4.4 (s, 2H), 3.6 (m, 2H), 3.4 (br s, 6H), 2.8 (s, 3H), 2.0 (br s, 4H). Anal. ($C_{26}H_{28}Cl_3N_5O_3S\cdot 1.0H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-iminotetrahydrooxazol-3-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17a).** Compound **17a** was prepared from **9b** and 2-aminooxazoline and purified by HPLC, giving a white solid (0.80 g, 30%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.6 (br s, 1H), 9.4 (s, 1H), 9.2 (br s, 1H), 7.2−8.3 (m, 6H), 4.7 (t, 2H), 4.6 (s, 2H), 3.8 (s, 3H), 3.7 (t, 2H). Anal. ($C_{22}H_{18}Cl_3N_5O_4S\cdot 1.6C_2HF_3O_2\cdot 0.5H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-iminotetrahydrothiazol-3-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17b).** Compound **17b** was prepared from **9b** and 2-aminothiooxazoline and purified by HPLC, giving a white solid (0.16 g, 4%): ^{1}H NMR (DMSO-d_6/TFA) δ 8.3 (s, 1H), 8.1 (d, 1H), 7.8 (s, 1H), 7.8 (dd, 1H), 7.3 (dd, 2H), 4.7 (s, 2H) 3.9 (t, 2H), 3.8 (s, 3H) 3.5 (t, 2H). Anal. ($C_{22}H_{18}Cl_3N_5O_3S\cdot 2.3C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-(methylthio)imidazolin-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17e).** Compound **17e** was prepared from **9b** and 2-thiomethyl-4,5-dihydroimidazole and purified by HPLC, giving a white solid (0.15 g, 37%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 10.1 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (d, 1H), 7.9 (s, 1H), 7.8 (d, 1H), 7.3 (s, 1H), 7.2 (s, 1H), 4.6 (s, 2H), 3.8 (s, 3H), 3.8 (s, 4H), 2.6 (s, 3H). Anal. ($C_{23}H_{20}Cl_3N_5O_3S_2\cdot 3.5C_2HF_3O_2\cdot 2.0H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-methylimidazolin-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17f).** Compound **17f** was prepared from **9b** (2.00 g, 4 mmol), 2-methyl-4,5-dihydroimidazole (1.50 g, 17.8 mmol), and K_2CO_3 (2.50 g, 18.1 mmol) and purified by HPLC, giving a white solid (1.0 g, 33%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 10.2 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (d, 1H), 8.0 (s, 1H), 7.8 (d, 1H), 7.3 (s, 1H), 7.2 (s, 1H), 4.6 (s, 2H), 3.8 (s, 3H), 3.7 (s, 4H), 2.3 (s, 3H). Anal. ($C_{23}H_{20}Cl_3N_5O_3S\cdot 1.7C_2HF_3O_2\cdot 0.5\ H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-aminoimidazol-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide (17l).** Compound **17l** was prepared from **9b** and 2-aminoimidazole and purified by flash chromatography, giving a white solid (6.1 g, 87%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 10.4 (s, 1H), 8.3 (d, 1H), 8.1 (d, 1H), 7.9 (dd, 1H), 7.8 (br s, 1H), 7.6 (s, 1H), 4 (d, 1H), 7.3 (d, 1H), 6.9 (dt, 1H), 5.0 (s, 2H), 4.8 (s, 3H). Anal. ($C_{22}H_{17}Cl_3N_6O_3S\cdot 0.4CH_2Cl_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-(ethylamino)imidazol-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17n).** Compound **17n** was prepared from **9b** and 2-(ethylamino)imidazole and purified by HPLC, giving a white solid (0.31 g, 21%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (d, 1H), 8.3 (d, 1H), 8.1 (d, 2H), 7.8 (d, 1H), 7.6 (s, 1H), 7.35 (s, 1H), 7.3 (s, 1H), 7.0 (s, 1H), 6.9 (s, 1H), 5.1 (s, 2H), 3.9 (s, 3H), 3.2 (m, 2H), 1.2 (t, 3H). Anal. ($C_{24}H_{21}Cl_3N_6O_3S\cdot 1.6C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-(isopropylamino)imidazol-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide (17o).** Compound **17o** was prepared from **9b** and 2-(isopropylamino)imidazole and purified by flash chromatography, giving a white solid (1.16 g, 49%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (br d, 1H), 8.38 (s, 1H), 8.1 (d, 2H), 7.8 (d, 1H), 7.42 (s, 1H), 7.35 (s, 2H), 7.0 (s, 1H), 6.5 (s, 1H), 6.45 (s, 1H), 5.43 (d, 1H), 4.94 (s, 2H), 3.9 (s, 3H), 3.2 (m, 2H), 1.2 (d, 6H). Anal. ($C_{25}H_{23}Cl_3N_6O_3S\cdot 0.1CH_2Cl_2\cdot 0.5H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-{[(5-chloro-2-pyridinyl)amino]carbonyl}-6-methoxyphenyl]-4-{2-(methylamino)-1*H*-imidazol-1-yl]-methyl}-2-thiophenecarboxamide (17m) and 3-Chloro-*N*-[4-chloro-2-{[(5-chloro-2-pyridinyl)amino]carbonyl}-6-methoxyphenyl]-4-[(1*H*-imidazol-2-ylmethylamino)methyl]-2-thiophenecarboxamide (17p). To a suspension of **9b** (14.7 g, 29 mmol, 1 equiv) in DMSO (50 mL) was added a solution of 2-(methylamino)imidazole hydrochloride (17.5 g, 131 mmol, 4.5 equiv) in DMSO (30 mL), followed by K_2CO_3 (26.1 g, 190 mmol, 6.5 equiv, powdered form, −325 mesh). The reaction mixture was stirred at room temperature for 16 h and then poured into water (500 mL). The pink solid was collected by filtration and washed with water (2 L). The solid was dissolved in CH_2Cl_2 (500 mL), containing a small amount of MeOH, and washed with water. The aqueous layer was extracted with CH_2Cl_2 (2 × 500 mL), and the extracts were combined with the original organic layer. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was dissolved in 20:1 (v/v) EtOAc−MeOH and purified by flash chromatography, eluting with 20:1 EtOAc−MeOH, then 9:1 EtOAc−MeOH, to afford 13 g of a light red oil. The oil was dissolved in CH_2Cl_2 and added dropwise into stirred hexane. Filtration and drying afforded **17m** as a white solid (9 g, 55%): ^{1}H NMR (DMSO-d_6/TFA) δ 12.46 (br s, 1H, N*H*), 10.85 (s, 1H, C(O)N*H*), 9.36 (s, 1H, C(O)N*H*), 8.28 (d, 1H, Ar-*H*), 8.08 (d, 1H, Ar-*H*), 7.83 (dd, 1H, Ar-*H*), 7.57 (s, 1H, Ar-*H*), 7.31 (d, 1H, Ar-*H*), 7.24 (d, 1H, Ar-*H*), 7.04 (d, 1H, Ar-*H*), 6.94 (d, 1H, Ar-*H*), 4.99 (s, 2H, -C*H*$_2$-thiophene), 3.83 (s, 3H, OC*H*$_3$), 2.85 (s, 3H, NC*H*$_3$); ^{13}C NMR (DMSO-d_6/TFA) δ 165.2, 158.6, 155.2, 150.8, 147.3, 146.6, 138.3, 134.9, 134.4, 133.0, 132.1, 128.4, 126.0, 123.7, 122.5, 120.5, 117.4, 115.7, 114.7, 113.3, 57.0, 43.3, 29.6. Anal. ($C_{23}H_{19}Cl_3N_6O_3S\cdot 0.1C_6H_{14}\cdot 1.2H_2O$) C, H, N.

Also isolated by chromatography was **17p** (white solid, 0.20 g, 1%): ^{1}H NMR (DMSO-d_6) δ 10.9 (br s, 2H), 9.3 (br s, 1H), 8.4 (d, 1H), 8.1 (d, 1H), 7.9 (dd, 1H), 7.6 (s, 1H), 7.4 (s, 1H), 7.2 (s, 1H), 6.6 (s, 2H), 4.4 (s, 2H), 3.9 (s, 3H), 2.9 (s,3H). Anal. ($C_{23}H_{19}Cl_3N_6O_3S\cdot 0.05C_6H_{14}\cdot 0.8H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-imino-tetrahydroimidazol-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17c).** Reaction of **9b** with 1,2-diaminoethane afforded *N*-(5-chloropyridin-2-yl)-2-[((4-((*N*′-(2-aminoethyl)amino)methyl)-3-chlorothiophen-2-yl)carbonyl)-amino]-3-methoxy-5-chlorobenzamide (0.97 g, 93%), which was used without purification: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (d, 1H), 9.2 (br s, 1H), 8.4 (d, 1H), 8.2 (d, 1H), 8.1 (s, 1H), 7.8−8.0 (m, 2H), 7.4 (d, 1H), 7.3 (d, 1H), 4.3 (s, 2H), 3.9 (s, 3H), 3.2−3.4 (m, 4H).

To a solution of this amine (0.57 g, 1.1 mmol) in MeOH (20 mL) were added NaOAc (0.18 g, 2.2 mmol) and cyanogen bromide (0.26 mL, 5 M solution in CH_3CN, 1.3 mmol). After stirring for 3 h at room temperature, the reaction mixture was concentrated and saturated $NaHCO_3$ (aq) was added. The reaction mixture was extracted with CH_2Cl_2, and the combined extracts were dried over Na_2SO_4. Purification by HPLC afforded **17c** (0.37 g, 24%) as a white solid: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (d, 2H), 8.0 (br s, 1H), 7.8 (dd, 1H), 7.75 (s, 1H), 7.3 (d, 2H), 4.5 (s, 2H), 3.8 (s, 3H), 3.5 (s, 4H). Anal. ($C_{22}H_{18}Cl_3N_6O_3S\cdot 1.8C_2HF_3O_2\cdot 1.7H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-imino-4-oxoimidazolin-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide (17d).** Prepared by the reaction of **9b** with

2-aminoacetoamide, followed by cyanogen bromide, in a similar manner as for **17c**, and purified by HPLC, giving a white solid (0.37 g, 15%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.3 (s, 1H), 8.3 (d, 1H), 8.1 (m, 2H), 7.7 (d, 1H), 7.6 (d, 1H), 7.5 (s, 1H), 7.3 (d, 1H), 7.2 (d, 1H), 4.4 (s, 2H), 4.3 (s, 2H), 3.8 (s, 3H). Anal. ($C_{22}H_{17}Cl_3N_6O_4S\cdot0.25C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((imidazolin-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17g).** To a stirred solution of **9b** (1.5 g, 3.0 mmol) in DMF (15 mL) was added 1,2-diaminoethane (0.9 g, 15 mmol) at room temperature. After 2 h, the reaction was poured into water and extracted with EtOAc, dried (Na_2SO_4) and concentrated to afford the crude amine intermediate. To the amine was added triethyl orthoformate (1.33 g, 9 mmol) in acetic acid (20 mL). After stirring at room temperature for 1 h, the reaction was poured into water and extracted with EtOAc. The organic layer was dried (Na_2SO_4), concentrated, and purified by HPLC to afford **17g** as a white solid (0.87 g, 39%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 10.3 (s, 1H), 9.4 (s, 1H), 8.6 (d, 2H), 8.3 (d, 1H), 8.1 (d, 1H), 7.8 (dd, 1H), 7.3 (d, 1H), 7.2 (d, 1H), 4.6 (s, 2H), 3.7−3.9 (m, 7H). Anal. ($C_{22}H_{18}Cl_3N_5O_3S\cdot1.6C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((imidazol-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17j).** A solution of **9b** (0.87 g, 1.73 mmol) and imidazole (0.35 g, 5.18 mmol) in DMF (6 mL) was heated at 45 °C for 15 h. Additional imidazole (0.25 g, 3.67 mmol) was added, and heating continued for 5 days. Acidification with trifluoroacetic acid (0.5 mL) and purification by HPLC afforded **17j** as a white solid (0.77 g, 50%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.85 (s, 1H), 9.40 (s, 1H), 9.20 (s, 1H), 8.25 (d, 1H), 8.05 (d, 1H), 7.95 (s, 1H), 7.80 (dd, 1H), 7.70 (s, 1H), 7.60 (s, 1H), 7.30 (d, 1H), 7.20 (d, 1H), 5.40 (s, 2H), 3.80 (s, 3H). Anal. ($C_{22}H_{16}Cl_3N_5O_3S\cdot2.0C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((2-methylimidazol-1-yl)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (17k).** A mixture of **9b** (0.8 g, 1.6 mmol) and 2-methylimidazole (0.39 g, 5.4 mmol) in DMF (7 mL) was heated at 45 °C for 2 days. Acidification with trifluoroacetic acid (0.5 mL) and purification by HPLC afforded **17k** as a white solid (0.70 g, 59%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.85 (s, 1H), 9.40 (s, 1H), 8.25 (d, 1H), 8.05 (d, 1H), 7.80 (m, 2H), 7.50 (s, 2H), 7.30 (d, 1H), 7.20 (d, 1H), 5.30 (s, 2H), 3.80 (s, 3H), 2.60 (s, 3H). Anal. ($C_{23}H_{18}Cl_3N_5O_3S\cdot1.7C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[methyl(methylsulfonyl)amino]methyl]-2-thiophenecarboxamide (12a). To a solution of **10b** (1.3 g, 2.5 mmol) in pyridine (20 mL) at 0 °C was added methanesulfonyl chloride (0.20 mL, 2.8 mmol). The solution was allowed to warm to room temperature with stirring. After 16 h, the mixture was concentrated, and the resulting oil was purified by flash chromatography to afford **12a** (1.1 g, 75%) as a white solid: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.8 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.2 (s, 2H), 3.9 (s, 3H), 3.0 (s, 3H), 2.7 (s, 3H). Anal. ($C_{21}H_{19}Cl_3N_4O_5S_2\cdot0.42C_4H_8O_2$) C, H, N.

4-[[(Aminocarbonyl)methylamino]methyl]-3-chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (12b). A solution of potassium cyanate (0.70 g, 8.6 mmol) in MeOH (4 mL) was added dropwise to a solution of **10b** (0.10 g, 0.20 mmol) in acetic acid (1.5 mL), and the mixture was stirred at room temperature for 20 h. Concentration and purification by HPLC afforded **12b** as a white solid (0.090 g, 58%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (br s, 1H), 9.3 (s, 1H), 8.2 (s, 1H), 8.1 (s, 1H), 7.8 (d, 1H), 7.5 (s, 1H), 7.2 (s, 2H), 4.4 (s, 2H), 3.8 (s, 3H), 2.9 (s, 3H). Anal. ($C_{21}H_{18}Cl_3N_4O_4S\cdot2.0C_2HF_3O_2\cdot1.0H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[[(ethylamino)carbonyl]methylamino]-methyl]-2-thiophenecarboxamide (12c). To a solution of **10b** (1.0 g, 2.0 mmol) in 1,4-dioxane (20 mL) was added ethyl isocyanate (0.18 mL, 2.2 mmol), and the reaction was stirred at room temperature. After 16 h, the mixture was concentrated. The residual solid was purified by flash chromatography to afford **12c** (0.85 g, 74%) as a white solid: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.4 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.5 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.4 (s, 2H), 3.9 (s, 3H), 3.1 (q, 2H), 2.8 (s, 3H), 1.0 (t, 3H). Anal. ($C_{23}H_{22}Cl_3N_5O_4S\cdot0.5H_2O\cdot0.19CH_2Cl_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[[[(2-hydroxyethyl)amino]carbonyl]-methylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (12d). To a solution of bis(trichloromethyl) carbonate (0.15 g, 0.51 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added **10b** (0.10 g, 0.20 mmol), and the mixture was stirred for 0.5 h. Ethanolamine (0.40 mL, 6.6 mmol) was then added, and the mixture was stirred at room temperature for 4 h. Concentration and purification by HPLC afforded **12d** as a white solid (0.080 g, 22%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (br s, 1H), 9.3 (s, 1H), 8.3 (d, 1H), 8.1 (d, 1H), 7.8 (dd, 1H), 7.7 (br s, 1H), 7.5 (s, 1H), 7.3 (s, 1H), 7.2 (s, 1H), 4.4 (s, 2H), 3.8 (s, 3H), 3.4 (t, 2H), 3.1 (t, 2H), 2.8 (s, 3H). Anal. ($C_{23}H_{22}Cl_3N_5O_5S\cdot1.2C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((*N*′-methyl-*N*″-(2-carboxyethyl)ureido)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (12f).** To a solution of *N*-(5-chloropyridin-2-yl)-2-[((4-((methylamino)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide **10b** (0.42 g, 0.87 mmol) in 1,4-dioxane (5 mL) was added ethyl 3-isocyanatopropionate (0.15 mL, 1.0 mmol), and the mixture was stirred at room temperature. After 0.5 h, water (2 mL) was added. Excess LiOH·H_2O was added, and the mixture was stirred for 2 h. Concentration and purification by HPLC afforded **12f** as a white solid (0.39 g, 62%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.5 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.3 (s, 2H), 3.9 (s, 3H), 3.3 (t, 2H), 2.8 (s, 3H), 2.4 (t, 2H). Anal. ($C_{24}H_{22}Cl_3N_5O_6S\cdot0.9C_2HF_3O_2\cdot0.5H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[methyl[[[2-(1-pyrrolidinyl)ethyl]amino]carbonyl]amino]methyl]-2-thiophenecarboxamide (12e). To a stirred solution of **10b** (0.7 g, 1.4 mmol) in THF (10 mL) at 0 °C was added 2-bromoethylisocyanate (0.23 g, 1.54 mmol), and the mixture was stirred for 0.5 h. Concentration afforded bromoethylurea **11** (0.91 g, quant.), which was used without purification.

To a stirred solution of **11** (0.91 g, 1.4 mmol) in DMF (4 mL) at room temperature was added pyrrolidine (0.50 g, 7 mmol). After 1 h, the reaction was diluted with EtOAc (60 mL) and washed with brine (2 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated. Purification by flash chromatography afforded **13a** as a white solid (0.48 g, 60%) and impure **12e**. Compound **12e** was further purified by HPLC to afford a white solid (0.20 g, 22%).

12e: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.5 (br s, 1H), 9.4 (s, 1H), 8.4 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.5 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 6.8 (br s, 1H), 4.4 (s, 2H), 3.9 (s, 3H), 3.6 (m, 2H), 3.4 (m, 2H), 3.2 (m, 2H), 3.0 (m, 2H), 2.8 (s, 3H), 2.0 (m, 2H), 1.8 (m, 2H). Anal. ($C_{27}H_{29}Cl_3N_6O_4S\cdot3.3C_2HF_3O_2$) C, H, N.

Optimized Conditions for the Synthesis of 3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-2-oxazolyl)methylamino]methyl]-2-thiophenecarboxamide (13a). To a stirred solution of **10b** (0.40 g, 0.8 mmol) in THF (5 mL) was added 2-bromoethylisocyanate (0.144 g, 0.96 mmol) at 0 °C. After 2 h, Et_3N (0.16 g, 1.6 mmol) was added. The reaction was allowed to warm to room temperature, stirred overnight, and concentrated. Purification by flash chromatography afforded **13a** as a white solid (0.40 g, 89%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H, C(O)N*H*), 10.3 (br s, 1H, N*H*), 9.4 (s, 1H, C(O)N*H*), 8.3 (d, 1H, Ar-*H*), 8.0 (dd, 2H, Ar-*H*), 7.9 (dd, 1H, Ar-*H*), 7.4 (d, 1H, Ar-*H*), 7.3 (s, 1H, Ar-*H*), 4.8 (t, 2H, oxazoline CH_2O), 4.5 (s, 2H, CH_2-thiophene), 3.9 (t, 2H, oxazoline CH_2N), 3.8 (s, 3H, OCH_3), 3.0 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6/TFA) δ 164.8, 161.1, 158.4, 154.7, 150.5, 146.3, 137.8, 136.9, 134.4, 131.9, 131.4, 127.34, 125.5, 123.6, 122.3, 120.0, 115.3, 114.3, 68.4, 56.8, 52.4, 47.6, 35.4. Anal. ($C_{23}H_{20}Cl_3N_5O_4S\cdot0.2CH_2Cl_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-2-oxazolyl)ethylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (13b). Compound **13b** was prepared from **10c** (1.53 g, 2.97 mmol) in a similar manner as for **13a** and purified by HPLC, giving a white solid (0.56 g, 23%): ^{1}H NMR ($CDCl_3$) δ 9.0 (s, 1H), 8.9 (s, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.6 (dd, 1H), 7.4 (s, 1H), 7.3 (d, 1H), 7.0 (d, 1H), 4.4 (s, 2H), 4.3 (t, 2H), 3.9 (s, 3H), 3.8 (t, 2H), 3.3 (q, 2H), 1.1 (t, 3H). Anal. ($C_{24}H_{22}Cl_3N_5O_4S\cdot 2.15C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-2-oxazolyl)(2,2,2-trifluoroethyl)amino]methyl]-2-thiophenecarboxamide (13c). To a stirred solution of **9b** (2.1 g, 4.1 mmol) in DMF (10 mL) was added 2,2,2-trifluoroethanamine (2.0 g, 20 mmol) at room temperature. The reaction was heated at 75 °C overnight, then cooled to room temperature, and poured into ice water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 40 mL), and the organic phase was dried over Na_2SO_4. Concentration and purification by flash chromatography afforded the trifluoroethylamino intermediate (2.1 g, 90%). ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (d, 1H), 8.1 (d, 2H), 7.8 (dd, 1H), 7.2 (d, 2H), 4.3 (s, 2H), 4.1 (m, 2H), 3.9 (m, 1H), 3.8 (s, 3H).

The intermediate (2.1 g, 3.68 mmol) was reacted in a similar manner as for **13a**. Purification by flash chromatography afforded **13c** (1.1 g, 43%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (d, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.75 (d, 1H), 7.2 (d, 2H), 4.9 (t, 2H), 4.7 (s, 2H), 4.4 (br s, 2H), 3.9 (t, 2H), 3.8 (s, 3H). Anal. ($C_{24}H_{19}Cl_3F_3N_5O_4S\cdot 0.1C_4H_8O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-2-oxazolyl)(1,1-dimethylethyl)amino]methyl]-2-thiophenecarboxamide Trifluoromethyl Acetic Acid Salt (13d). To a stirred solution of **9b** (2.83 g, 5.5 mmol) in DMF (10 mL) was added *tert*-butylamine (2.0 g, 28 mmol) at room temperature. After 12 h, the reaction mixture was poured into ice water (70 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL), and the organic phase was dried over Na_2SO_4. Concentration and purification by flash chromatography afforded the *tert*-butylamino intermediate (2.5 g, 83%).

The intermediate (2.43 g, 4.5 mmol) was reacted in a similar manner as for **13a**. Purification by HPLC afforded **13d** as a white solid (1.49 g, 39%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.9 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.7 (s, 1H), 7.4 (d, 2H), 7.3 (s, 1H), 4.9 (t, 2H), 4.6 (s, 2H), 3.9 (s, 3H), 3.8 (t, 2H), 1.4 (s, 9H). Anal. ($C_{26}H_{26}Cl_3N_5O_4S\cdot 2.0C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-2-oxazolyl)(2-methoxyethyl)amino]methyl]-2-thiophenecarboxamide Trifluoromethyl Acetic Acid Salt (13e). To a stirred solution of **9b** (2.0 g, 4.0 mmol) in DMF (10 mL) was added 2-methoxyethanamine (1.46 g, 20 mmol) at 0 °C. The ice bath was removed, and the reaction was stirred at room temperature overnight. The solvent was removed, and the resulting residue was purified by flash chromatography to afford the methoxyethylamino intermediate (1.2 g, 55%).

The intermediate (1.2 g, 2.2 mmol) was reacted in a similar manner as for **13a**. Purification by HPLC afforded **13e** (0.58 g, 28%): ^{1}H NMR (DMSO-d_6/TFA) δ 11.0 (s, 1H), 10.3 (d, 1H), 9.4 (s, 1H), 7.2−8.4 (m, 6H), 4.8 (m, 2H), 4.6 (s, 2H), 3.9 (s, 6H), 3.5 (s, 2H), 3.4 (s, 2H), 3.2 (d, 2H). Anal. ($C_{25}H_{24}Cl_3N_5O_5S\cdot 2.9C_2HF_3O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(5,6-dihydro-4*H*-1,3-oxazin-2-yl)methylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (13h). Compound **13h** was prepared from **10b** (1.5 g, 3.0 mmol) and 3-bromopropyl isocyanate (0.59 g, 3.6 mmol) in a similar manner as for **13a** and purified by HPLC, giving a white solid (0.56 g, 40%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 9.3 (br s, 1H), 8.3 (s, 1H), 8.1 (d, 1H), 7.9 (dd, 1H), 7.4 (s, 1H), 7.2 (s, 1H), 4.5 (m, 2H), 4.4 (s, 2H), 3.8 (s, 3H), 3.3 (m, 2H), 3.0 (s, 3H), 2.1 (m, 2H), 1.3 (m, 2H). Anal. ($C_{24}H_{22}Cl_3N_5O_4S\cdot 1.8C_2HF_3O_2\cdot 0.5H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-4-oxo-2-oxazolyl)methylamino]methyl]-2-thiophenecarboxamide (13i). Compound **13i** was prepared from **10b** (0.5 g, 1.0 mmol) and bromoacetylisocyanate (0.59 g, 3.6 mmol; prepared by reaction of 2-bromoacetamide with oxalyl chloride) in a similar manner as for **13a** and purified by flash chromatography, giving a white solid (0.12 g, 21%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (s, 1H), 8.1 (dd, 1H), 7.9 (dd, 1H), 7.7 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.8 (m, 2H), 4.6 (m, 2H), 3.8 (s, 3H), 3.0 (s, 3H). Anal. ($C_{23}H_{18}Cl_3N_5O_5S\cdot 0.4CH_2Cl_2\cdot 0.3C_4H_8O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-2-thiazolyl)methylamino]methyl]-2-thiophenecarboxamide (13j). Compound **13j** was prepared from **10b** (1 g, 2 mmol) and 2-bromoethylisothiocyanate (1 g, 6 mmol) in a similar manner as for **13a** and purified by flash chromatography, giving a white solid (0.97 g, 87%): ^{1}H NMR (DMSO-d_6/TFA) δ 11.0 (s, 1H), 10.1 (br d, 1H), 9.5 (s, 1H), 7.3−8.5 (m, 6H), 4.7 (m, 2H), 4 (m, 2H), 3.9 (s, 3H), 3.6 (m, 2H), 3.2 (s, 3H). Anal. ($C_{23}H_{20}Cl_3N_5O_3S_2\cdot 0.05C_4H_8O_2$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-5-methyl-2-oxazolyl)methylamino]methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (13f). To a stirred solution of 1-amino-2-propanol (2.0 g, 27 mmol) in THF (20 mL) was added thiocarbonyldiimidazole (5.3 g, 27 mmol) at room temperature. After 3 h, the reaction mixture was concentrated and purified by flash chromatography to afford 5-methyl-2-oxazolidinethione (2.9 g, 93%).

The 5-methyl-2-oxazolidinethione (2.7 g, 23 mmol) was dissolved in $POCl_3$ (40 mL), and PCl_5 (4.8 g, 23 mmol) was added at room temperature. The reaction was heated at 100 °C for 3 h, then cooled to room temperature, concentrated, and dried under vacuum. The resulting product, 2-chloro-4,5-dihydro-5-methyloxazole (2.7 g, 100%), was used without purification.

To a stirred solution of 2-chloro-4,5-dihydro-5-methyloxazole (0.36 g, 3 mmol) in DMF (5 mL) was added **10b** (1.5 g, 3 mmol), followed by Et_3N (0.57 g, 5.6 mmol) at room temperature. After 16 h, the reaction mixture was concentrated and purified by HPLC to afford **13f** (0.66 g, 31%) as a white solid: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.4 (d, 1H), 8.1 (d, 1H), 7.9 (dd, 1H), 7.5 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.4 (s, 2H), 4.2 (m, 1H), 3.8 (s, 3H), 3.2 (m, 2H), 2.8 (s, 3H), 1.4 (d, 3H). Anal. ($C_{24}H_{22}Cl_3N_5O_4S\cdot 0.9C_2HF_3O_2\cdot 0.8H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[[(4,5-dihydro-4-methyl-2-oxazolyl)-ethylamino]methyl]-2-thiophenecarboxamide (13g). Reaction of **10c** with 2-chloro-4,5-dihydro-4-methyloxazole (prepared by a similar method as for **13f**) afforded **13g**, which was purified by flash chromatography, giving a white solid (0.12 g, 47%): ^{1}H NMR ($CDCl_3$) δ 9.1 (s, 1H), 8.7 (s, 1H), 8.3 (d, 1H), 8.2 (s, 1H), 7.7 (d, 1H), 7.5 (s, 1H), 7.3 (s, 1H), 4.4 (s, 2H), 4.3 (m, 1H), 4.1 (m, 1H), 3.9 (s, 3H), 3.8 (t, 1H), 3.3 (m, 2H), 1.3 (d, 3H), 1.2 (t, 3H). Anal. ($C_{25}H_{24}Cl_3N_5O_4S\cdot 0.3H_2O$) C, H, N.

3-Chloro-*N*-[4-chloro-2-[[(5-chloro-2-pyridinyl)amino]carbonyl]-6-methoxyphenyl]-4-[(methyl-2-oxazolylamino)methyl]-2-thiophenecarboxamide Trifluoroacetic Acid Salt (13k). To a suspension of **12d** (0.10 g, 0.18 mmol) in EtOH (10 mL) was added chloroacetaldehyde diethylacetal (0.28 g, 1.84 mmol). The reaction mixture was refluxed for 4 days. After cooling to room temperature, water and saturated $NaHCO_3$ (aq.) were added. The solid was collected by filtration and purified by HPLC to afford **13k** (0.03 g, 24%) as a white solid: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.4 (s, 1H), 8.1 (dd, 1H), 7.9 (dd, 1H), 7.8 (s, 1H), 7.6 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 7.0 (s, 1H), 4.4 (s, 2H), 3.9 (s, 3H), 3.6 (m, 2H), 3.0 (s, 3H). Anal. ($C_{23}H_{18}Cl_3N_5O_4S\cdot 1.0C_2HF_3O_2\cdot 1.0H_2O$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[[(4-((*N*′-methyl-*N*′-(3,4-dihydro-2*H*-pyrrol-5-yl)amino)methyl)-3-chlorothiophen-2-yl)carbonyl)-amino]-3-methoxy-5-chlorobenzamide Trifluoroacetic Acid Salt (13l).** To a solution of **10b** (3.0 g, 6.0 mmol) in DMF (30 mL) were added *N*,*N*-diisopropylethylamine (1.94 g, 15 mmol) and

2-methylthioimidazoline hydroiodide (1.9 g, 7.8 mmol). The mixture was heated at 90 °C for 20 h. The cooled mixture was poured into water, extracted with EtOAc, dried ($MgSO_4$) and concentrated. Purification by HPLC afforded **13l** (1.3 g, 27%) as a white solid: ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.7 (d, 1H), 9.4 (d, 1H), 8.3 (m, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.7 (d, 1H), 7.3 (d, 1H), 7.2 (d, 1H), 4.6 (d, 2H), 3.9 (s, 3H), 3.6 (m, 2H), 2.9−3.2 (m, 5H), 2.2 (m, 2H). Anal. ($C_{24}H_{22}Cl_3N_5O_3S\cdot1.65C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-((*N*′-(1-iminoethyl)-*N*′-methylamino)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide (17h).** To a stirred solution of **10b** (0.70 g, 1.4 mmol) in MeOH (30 mL) was added NEt_3 (3 mL, 22 mmol) and ethyl acetimidate hydrochloride (large excess). The reaction was stirred at room temperature for 16 h and then concentrated. Purification of the residual oil by HPLC afforded **17h** as a white solid (0.80 g, 75%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 9.3 (br s, 1H), 8.6 (br s, 1H), 8.4 (s, 1H), 8.1 (d, 1H), 7.9 (d, 1H), 7.8 (s, 1H), 7.4 (s, 1H), 7.3 (s, 1H), 4.6 (s, 2H), 3.9 (s, 3H), 3.1 (s, 3H), 2.3 (s, 3H). Anal. ($C_{22}H_{20}Cl_3N_5O_3S\cdot0.5H_2O\cdot1.9C_2HF_3O_2$) C, H, N.

***N*-(5-Chloropyridin-2-yl)-2-[((4-(((amidino)(methyl)amino)methyl)-3-chlorothiophen-2-yl)carbonyl)amino]-3-methoxy-5-chlorobenzamide (17i).** To a stirred solution of **10b** (0.10 g, 0.20 mmol) in DMF (3 mL) were added NEt_3 (0.28 mL, 2.0 mmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (0.30 g, 2.0 mmol). The mixture was stirred at room temperature for 15 h and then heated at 45 °C for 3 h. The cooled mixture was acidified with trifluoroacetic acid and purified by HPLC to afford **17i** as a white solid (0.080 g, 56%): ^{1}H NMR (DMSO-d_6/TFA) δ 10.9 (s, 1H), 9.4 (s, 1H), 8.3 (d, 1H), 8.1 (d, 1H), 7.8 (dd, 1H), 7.6 (s, 1H), 7.4 (br s, 4H), 7.3 (s, 1H), 7.2 (s, 1H), 4.5 (s, 2H), 3.8 (s, 3H), 2.9 (s, 3H). Anal. ($C_{21}H_{19}Cl_3N_6O_3S\cdot1.5C_2HF_3O_2$) C, H, N.

Enzyme Assay Procedures.[23] The activities of human fXa, human thrombin and bovine trypsin were determined kinetically as the initial rate of cleavage of a peptide p-nitroanilide by the enzyme. The assay was performed at room temperature in flat-bottom microtiter plates in a final volume of 200 μL. The reaction mixture consisted of 50 mM Tris-HCl, 150 mM NaCl, 2.5 mM $CaCl_2$, and 0.1% polyethylene glycol 6000, pH 7.5, with enzyme and substrate at the following concentrations: (1) fXa assay, 0.04−1 nM fXa and 164 μM S-2222; (2) thrombin assay, 16 nM thrombin and 300 μM S-2302; and (3) trypsin assay, 16 nM bovine trypsin and 127 μM S-2266. Standard techniques with at least four substrate dilutions were used to determine the K_m for a given enzyme and substrate. The substrate concentration listed is equal to the K_m. Controls without the test inhibitors or with a reference compound were also run in each assay plate. Enzyme was incubated with test compounds for 10 min; the reaction was then started by the addition of the substrate. Reaction rates were determined by measuring the rate of the absorbance change at 405 nm in a ThermoMax microplate reader (Molecular Devices Corp., Sunnyvale, CA). Assay conditions used to determine the selectivity for the remaining proteases shown in Table 8 have been previously published.[11b]

Data Analysis Methods. IC_{50} values for inhibitors were determined from the dose response curve by fitting the data to the Hill equation with an automated analysis method using a computer spreadsheet. $K_{i,app}$ values were calculated as the $IC_{50}/2$, with the IC_{50} value determined at a substrate concentration equal to K_m ($K_{i,app} = IC_{50}/(1 + [S]/K_m) = IC_{50}/2$). For inhibitors with $K_{i,app}$ values less than 3 nM, IC_{50} values were determined by fitting data to a modification of the Morrison equation to correct for the proportion of inhibitor bound to the enzyme relative to the free inhibitor.[24] $K_{i,app}$ values are the mean of multiple determinations ($n \geq 2$). Standard deviations are <30% of the mean.

Prothrombin Time (PT) Assay. For in vitro coagulation studies, pooled, lyophilized human plasma (SARP, Helena Laboratories, Beaumont, TX) was used following reconstitution. Thromboplastin HS (Sigma, St. Louis, MO) was used for PT assays. For construction of concentration−response curves, plasma was serially diluted at half-log increments ranging from 0.01 to 100 μg/mL. Clot time for all coagulation assays was measured by taking the average of two measurements using an Electra 1400C automated coagulometer (Medical Laboratory Automation, Inc., Pleasantville, NY).

Pharmacokinetic Studies in Dogs. Fasted (18 h) male beagle dogs were instrumented with indwelling jugular catheters on the day of the experiment for the purpose of withdrawing blood samples. Dogs were allowed water ad lib during the experiment. Drugs were administered orally by gavage via a feeding tube temporarily inserted into the stomach or intravenously by a bolus (ca. 30 s) injection via the cephalic vein. For oral dosing, the compound was dissolved in a 40% w/w solution of hydroxypropyl-β-cyclodextrin in water acidified to pH 5 with 1 N HCl. Final compound concentration was 10 mg/mL, and dosing volume was 1 mL/kg. For intravenous dosing, the compound was dissolved in a 10% v/v solution of DMSO in unbuffered saline, acidified to pH 5 with 0.1 N HCl. Final compound concentration was 1 mg/mL, and the dosing volume was 1 mL/kg.

Blood samples were drawn at predetermined time intervals out to 12 h. For each time point, 2.7 mL of blood was collected from the jugular vein catheter and placed in a test tube containing 0.3 mL of a 5% sodium citrate solution in phosphate buffered saline (without calcium or magnesium). The blood samples were kept on ice until centrifuged ($1000 \times g$ for 10 min at 4 °C) to obtain plasma. The plasma samples were kept frozen (−20 °C) until assayed for determination of drug levels.

Anti-fXa activity of the test compound in plasma was determined ex vivo using a chromogenic assay.[15] The assay was performed at room temperature in flat-bottom microtiter plates in a final volume of 200 μL. The assay buffer consisted of 50 mM Tris-HCl, 150 mM NaCl, and 0.1% polyethylene glycol 6000, pH 7.5. Solutions of human fXa (6 nM in assay buffer) and substrate S-2222 (948 μM in assay buffer) were prepared. A standard curve was prepared with five dilutions of test compound in pooled dog plasma. Plasma samples were thawed and diluted 1:100 with assay buffer. To each well of a 96-well microtiter plate were added 20 μL of diluted plasma sample, 30 μL of buffer, and 50 μL of substrate solution. Two wells of each plate received pooled dog plasma instead of diluted plasma sample (negative control), and four wells received a diluted solution of a standard fXa inhibitor of known activity (positive control). The plate was shaken for 30 s, then 100 μL of fXa solution added. Reaction rates were determined by measuring the rate of the absorbance change at 405 nm at 10 s intervals for 2 min. Plasma samples and standards were tested in duplicate. The rate of reaction in mOD/min was calculated based on the kinetic readings. The rate was converted to compound concentration (μM) based on the standard curve using log−logit curve fit analysis.

Thrombosis in Rats: Vena Cava Stasis Model. Male Wistar rats were anesthetized with 90 mg/kg, i.p., Nembutal. Five minutes prior to the ligature of the vena cava, the test compound was administered by intravenous bolus injection. Three minutes prior to application of the ligature, thromboplastin (Thromborel, Behring Werke, Marburg, Germany) was infused over 5 min at a dose of 100 μL/kg/min. The vena cava was ligated immediately below the bifurcation of the left vena renalis. The thrombi were excised 60 min following ligation, and thrombus wet weight was determined. Venous blood samples for the determination of coagulation parameters were taken immediately before and five and 60 min following drug administration. Statistical analysis was performed by a one-way analysis of variance and subsequent comparison of the treatment groups with controls employing Dunn's method, as the data are not normally distributed. $P < 0.05$ was taken as a significant difference.

X-ray Crystallography. Crystals of des-GLA-EGF1-factor Xaβ were grown using protocols described previously.[21] This reference describes a proprietary inhibitor used to grow the initial crystals. This proprietary inhibitor is compound **17m**.

Supporting Information Available: Combustion analysis data for newly synthesized target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Some of this data has been previously reported: (a) Ye, B.; Cheeseman, S.; Chou, Y.-L.; Ewing, J.; Fitch, R.; Griedel, B. D.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A.; Morrissey, M. M.; Post, J.; Sacchi, K. L.; Sakata, S. T.; Shaw, K. J.; Subramanyam, B.; Vergona, R.; Walters, J.; Wang, Y.-X.; White, K. A.; Wu, S. C.; Zhao, Z.; Kochanny, M. J. Design, synthesis and biological activity of novel non-amidine factor Xa inhibitors. 4. Structure−activity relationship of substituted benzithiophene-anthranilamides. *Abstracts of Papers*, 221st American Chemical Society National Meeting, April 1−5, 2001, San Diego, CA; American Chemical Society: Washington, DC, 2001; Abstract MEDI -128. (b) Kochanny, M. J.; Adler, M.; Cheeseman, S.; Chou, Y. L.; Davey, D. D.; Eagen, K. A.; Ewing, J.; Fitch, R.; Griedel, B. D.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A.; Morrissey, M. M.; Phillips, G. B.; Post, J.; Sacchi, K. L.; Sakata, S. T.; Shaw, K. J.; Snider, R. M.; Subramanyam, B.; Trinh, L.; Vergona, R.; Walters, J.; Wang, Y. X.; White, K. A.; Whitlow, M.; Wu, S. C.; Ye, B.; Zhao, Z. Development of highly potent, selective and orally available non-amidine factor Xa inhibitors. *Abstracts of Papers*, 221st American Chemical Society National Meeting, April 1−5, 2001, San Diego, CA; American Chemical Society: Washington, DC 2001; Abstract MEDI-16.

(2) Vlasuk, G. P. Direct factor Xa inhibitors. In *New Therapeutic Agents in Thrombosis and Thrombolysis*, 1st ed; Sasahara, A. A., Loscalzo, J., Eds.; Marcel Dekker: New York, 1997; pp 261−283.

(3) (a) Lefkovits, J.; Topol, E. Direct thrombin inhibitors in cardiovascular medicine. *Circulation* **1994**, *90*, 1522−1568. (b) Berry, C. N.; Girardot, C.; Lecoffre, C.; Lunven, C. Effects of synthetic thrombin inhibitor argatroban on fibrin or clot-incorporated thrombin: comparison with heparin and recombinant hirudin. *Thromb. Haemost.* **1994**, *72*, 381−386. (c) Wittig, J. I.; Bourdon, P.; Brezniak, D. V.; Maraganore, J. M.; Fenton, J. W., II. Thrombin-specific inhibition by and slow cleavage of hirulog-1. *Biochem. J.* **1992**, *283*, 737−43. (d) Kelly, A. G.; Marzee, W. M.; Krupski, W.; Bass, A.; Cadroy, Y.; Hanson, S. R.; Harker, L. A. Hirudin interruption of heparin-resistant arterial thrombus formation in baboons. *Blood* **1991**, *77*, 1006−1012.

(4) (a) Stein, P. D.; Grandison, D.; Hua, T. A. Therapeutic level of anticoagulation with Warfarin in patients with mechanical prosthetic heart valves; review of literature and recommendations based on International Normalized Ratio. *Postgrad. Med.* **1994**, *70* (Suppl. 1), S72−S83. (b) Hirsh, J.; Poller, L. The International Normalized Ratio. A guide to understanding and correcting its problems. *Arch. Int. Med.* **1994**, *154*, 282−288.

(5) (a) Walenga, J. M.; Jeske, W. P.; Hoppensteadt, D.; Fareed, J. Factor Xa inhibitors: Today and beyond. *Curr. Opin. Invest. Drugs* **2003**, *4*, 272−281. (b) Kaiser, B. Factor Xa−A promising target for drug development. *Cell Mol. Life Sci.* **2002**, *59*, 189−192.

(6) (a) Mann, K. G.; Butenas, S.; Brummel, K. The dynamics of thrombin formation. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 17−25. (b) Samama, M. M.; Gerotziafas, G. T. Evaluation of the pharmacological properties and clinical results of the synthetic pentasaccharide (fondaparinux). *Thromb. Res.* **2003**, *109*, 1−11.

(7) (a) Wong, P. C.; Crain, E. J.; Watson, C. A.; Zaspel, A. M.; Wright, M. R.; Lam, P. Y. S.; Pinto, D. J.; Wexler, R. R.; Knabb, R. M. Nonpeptide factor Xa inhibitors III: Effects of DPC423, an orally-active pyrazole antithrombotic agent, on arterial thrombosis in rabbits. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 993−1000. (b) McClanahan, T. B.; Hicks, G. W.; Morrison, A. L.; Peng, Y. W.; Janiczek-Dolphin, N.; Mertz, T. E.; Sullivan, M. E.; Morser, J.; Juneau, P. L.; Leadley, R. The antithrombotic effects of CI-1031 (ZK-807834) and enoxaparin in a canine electrolytic injury model of arterial and venous thrombosis. *Eur. J. Pharmacol.* **2001**, *432*, 187−194. (c) Rebello, S. S.; Kasiewski, C. J.; Wang, W.; Bentley, R. G.; Morgan, S. R.; Chu, V.; Colussi, D.; Brown, K.; Perrone, M. H.; Leadley, R. J. Role of short-term inhibition of factor Xa by FXV673 in arterial passivation: A study in a chronic model of thrombosis in conscious dogs. *J. Cardiovasc. Pharmacol.* **2001**, *38*, 288−297. (d) Sinha, U.; Ku, P.; Malinowski, J.; Zhu, B. Y.; Scarborough, R. M.; Marlowe, C. K.; Wong, P. W.; Lin, P. H.; Hollenbach, S. J. Antithrombotic and hemostatic capacity of factor Xa versus thrombin inhibitors in models of venous and arteriovenous thrombosis. *Eur. J. Pharmacol.* **2000**, *395*, 51−59. (e) Leadley, R. J., Jr.; Morgan, S. R.; Bentley, R.; Bostwick, J. S.; Kasiewski, C. J.; Heran, C.; Chu, V.; Brown, K.; Moxey, P.; Ewing, W. R.; Pauls, H.; Spada, A. P.; Perrone, M. H.; Dunwiddie, C. T. Pharmacodynamic activity and antithrombotic efficacy of RPR120844, a novel inhibitor of coagulation factor Xa. *J. Cardiovasc. Pharmacol.* **1999**, *34*, 791−799. (f) Sato, K.; Kawasaki, T.; Hisamichi, N.; Taniuchi, Y.; Hirayama, F.; Koshio, H.; Matsumoto, Y. Antithrombotic effects of YM-60828, a newly synthesized factor Xa inhibitor, in rat thrombosis models and its effects on bleeding time. *Br. J. Pharmacol.* **1998**, *123*, 92−96.

(8) (a) Leadley, R. J., Jr. Coagulation factor Xa inhibition: Biological background and rationale. *Curr. Top. Med. Chem.* **2001**, *1*, 151−159. (b) Hauptmann, J.; Stuerzebecher, J. Synthetic inhibitors of thrombin and factor Xa: From bench to bedside. *Thromb. Res.* **1999**, *93*, 203−241.

(9) Reviews: (a) Quan, M. L.; Smallheer, J. M. The race to an orally active factor Xa inhibitor: Recent advances. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 460−469. (b) Gould, W. R.; Leadley, R. J. Recent advances in the discovery and development of direct coagulation factor Xa inhibitors. *Curr. Pharm. Des.* **2003**, *9*, 2337−2347. (c) Rai, R.; Sprengler, P. A.; Elrod, K. C.; Young, W. B. Perspectives on factor Xa inhibition. *Curr. Med. Chem.* **2001**, *8*, 101−109. (d) Sanderson, P. E. J. Anticoagulants: Inhibitors of thrombin and factor Xa. *Ann. Rep. Med. Chem.* **2001**, *36*, 79−88. (e) Zhu, B. Y.; Scarborough, R. M. Factor Xa inhibitors: Recent advances in anticoagulant agents. *Ann. Rep. Med. Chem*., **2000**, *35*, 83−102. (f) Vacca, J. P. New advances in the discovery of thrombin and factor Xa inhibitors. *Curr. Opin. Chem. Biol.* **2000**, *4*, 394−400. (g) Ewing, W. R.; Pauls, H. W.; Spada, A. P. Progress in the design of inhibitors of coagulation factor Xa. *Drugs Future* **1999**, *24*, 771−787.

(10) (a) Young, R. J.; Campbell, M.; Borthwick, A. D.; Brown, D.; Burns-Kurtis, C. L.; Chan, C.; Convery, M. A.; Crowe, M. C.; Dayal, S.; Diallo, H.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Mordaunt, J. E.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Smith, P. W.; Watson, N. S.; Weston, H. E.; Zhou, P. Structure- and property-based design of factor Xa inhibitors: Pyrrolidin-2-ones with acyclic alanyl amides as P4 motifs. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5953−5957. (b) Pinto, D. J. P.; Orwat, M. J.; Quan, M. L.; Han, Q.; Galemmo, R. A., Jr.; Amparo, E.; Wells, B.; Ellis, C.; He, M. Y.; Alexander, R. S.; Rossi, K. A.; Smallwood, A.; Wong, P. C.; Luettgen, J.M.; Rendina, A. R.; Knabb, R. M.; Mersinger, L.; Kettner, C.; Bai, S.; He, K.; Wexler, R. R.; Lam, P. Y. S. 1-[3-Aminobenzisoxazol-5′-yl]-3-trifluoromethyl-6-[2′-(3-(*R*)-hydroxy-*N*-pyrrolidinyl)methyl-[1,1′]-biphen-4-yl]-1,4,5,6-tetrahydropyrazolo-[3,4-*c*]-pyridin-7-one (BMS-740808), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4141−4147. (c) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K. H.; Reinemer, P.; Perzborn, E. Discovery of the novel antithrombotic agent 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide (BAY 59−7939): An oral, direct factor Xa inhibitor. *J. Med. Chem.* **2005**, *48*, 5900−5908. (d) Quan, M. L.; Lam, P. Y. S.; Han, Q.; Pinto, D. J. P.; He, M. Y.; Li, R.; Ellis, C. D.; Clark, C. G.; Teleha, C. A.; Sun, J. H.; Alexander, R. S.; Bai, S.; Luettgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. Discovery of 1-(3′-Aminobenzisoxazol-5′-yl)-3-trifluoromethyl-*N*-[2-fluoro-4-[(2′-dimethylaminomethyl)imidazol-1-yl]phenyl-1*H*-pyrazole-5-carboxyamide hydrochloride (Razaxaban), a highly potent, selective, and orally bioavailable factor Xa inhibitor. *J. Med. Chem.* **2005**, *48*, 1729−1744. (e) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Hirokawa, Y.; Furugori, T.; Nagahara, T. Synthesis and conformational analysis of a non-amidine factor Xa inhibitor that incorporates 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine as S4 binding element. *J. Med. Chem.* **2004**, *47*, 5167−5182. (f) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Nagata, T.; Hirokawa, Y.; Nagahara, T. Design, synthesis and biological activity of non-amidine factor Xa inhibitors containing pyridine N-oxide and 2-carbamoylthiazole units. *Bioorg. Med. Chem.* **2004**, *12*, 5579−5586. (g) Nazare, M.; Essrich, M.; Will, D. W.; Matter, H.; Ritter, K.; Urmann, M.; Bauer, A.; Schreuder, H.; Dudda, A.; Czech, J.; Lorenz, M.; Laux, V.; Wehner, V. Factor Xa inhibitors based on a 2-carboxyindole scaffold: SAR of neutral P1 substituents. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4191−4195. (h) Nazare, M.; Essrich, M.; Will, D. W.; Matter, H.; Ritter, K.; Urmann, M.; Bauer, A.; Schreuder, H.; Czech, J.; Lorenz, M.; Laux, V.; Wehner, V. Novel factor Xa inhibitors based on a 2-carboxyindole scaffold: SAR of P4 substituents in combination with a neutral P1 ligand. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4197−4201. (i) Zhang, P.; Bao, L.; Zuckett, J. F.; Goldman, E. A.; Jia, Z. J.; Arfsten, A.; Edwards, S.; Sinha, U.; Hutchaleelaha, A.; Park, G.; Lambing, J. L.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B. Y. Design, synthesis and SAR of anthranilamide-based factor Xa inhibitors incorporating substituted biphenyl P4 motifs. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 983−987. (j) Choi-Sledeski, Y. M.; Kearney, R.; Poli, G.; Pauls, H.; Gardner, C.; Gong, Y.; Becker, M.; Davis, R.; Spada, A.; Liang, G.; Chu, V.; Brown, K.; Collussi, D.; Leadley, R., Jr.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J.-P.; Mikol, V. Discovery of an orally efficacious inhibitor of coagulation factor Xa which incorporates a neutral P1 ligand. *J. Med. Chem.* **2003**, *46*, 681−684. (k) Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Craft, T. J.; Froelich, L. L.; Gifford-Moore, D. S.; Hay, L. A.; Herron, D. K.; Klimkowski, V. J.; Kurz,

K. D.; Metz, J. T.; Ratz, A. M.; Shuman, R. T.; Smith, G. F.; Smith, T.; Towner, R. D.; Wiley, M. R.; Wilson, A.; Yee, Y. K. Non-amidine-containing 1,2-dibenzamidobenzene inhibitors of human factor Xa with potent anticoagulant and antithrombotic activity. *J. Med. Chem.* **2000**, *43*, 2087−2092. (l) Herron, D. K.; Goodson, T., Jr.; Wiley, M. R.; Weir, L. C.; Kyle, J. A.; Yee, Y. K.; Tebbe, A. L.; Tinsley, J. M.; Mendel, D.; Masters, J. J.; Franciskovich, J. B.; Sawyer, J. S.; Beight, D. W.; Ratz, A. M.; Milot, G.; Hall, S. E.; Klimkowski, V. J.; Wikel, J. H.; Eastwood, B. J.; Towner, R. D.; Gifford-Moore, D. S.; Craft, T. J.; Smith, G. F. 1,2-Dibenzamidobenzene inhibitors of human factor Xa. *J. Med. Chem.* **2000**, *43*, 859−872.

(11) (a) Chou, Y. L.; Davey, D. D.; Eagen, K. A.; Griedel, B. D.; Karanjawala, R.; Phillips, G. B.; Sacchi, K. L.; Shaw, K. J.; Wu, S. C.; Lentz, D.; Liang, A.; Trinh, L.; Morrissey, M. M.; Kochanny, M. J. Structure−activity relationships of substituted benzothiophene-anthranilamide factor Xa inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 507−511. (b) Liang, A. M.; Light, D. R.; Kochanny, M.; Trinh, L.; Rumennik, G.; Lentz, D.; Post, J.; Morser, J.; Snider, M. Discovery and characterization of a potent and selective non-amidine inhibitor of human factor Xa. *Biochem. Pharmacol*. **2003**, *65*, 1407−1418.

(12) Kochanny, M. J.; Adler, M.; Ewing, J.; Griedel, B. D.; Ho, E.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A. M.; Morrissey, M. M.; Phillips, G. B.; Post, J.; Sacchi, K. L.; Sakata, S. T.; Subramanyam, B.; Vergona, R.; Walters, J.; White, K. A.; Whitlow, M.; Ye, B.; Zhao, Z.; Shaw, K. J. Substituted thiophene-anthranilamides as potent inhibitors of human factor Xa. *Bioorg. Med. Chem*. **2007**, *15*, 2127−2146.

(13) Several other laboratories have also investigated anthranilamide-derived inhibitors of fXa. See reference 10k and the following. (a) Yee, Y. K.; Tebbe, A. L.; Linebarger, J. H.; Beight, D. W.; Craft, T. J.; Gifford-Moore, D.; Goodson, T., Jr.; Herron, D. K.; Klimkowski, V. J.; Kyle, J. A.; Sawyer, J. S.; Smith, G. F.; Tinsley, J. M.; Towner, R. D.; Weir, L.; Wiley, M. R. N^2-Aroylanthranilamide inhibitors of human factor Xa. *J. Med. Chem.* **2000**, *43*, 873−882. (b) Shrader, W. D.; Young, W. B.; Sprengeler, P. A.; Sangalang, J. C.; Elrod, K.; Carr, G. Neutral inhibitors of the serine protease factor Xa. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1801−1804. (c) Zhang, P.; Bao, L.; Zuckett, J. F.; Jia, Z. J.; Woolfrey, J.; Arfsten, A.; Edwards, S.; Sinha, U.; Hutchaleelaha, A.; Lambing, J. L.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B. Y. Design, synthesis and SAR of anthranilamide-based factor Xa inhibitors with improved functional activity. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 989−993.

(14) See references 10i, 10k, 11a, 12, 13c, and the following: (a) Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I.-W.; Dancheck, K. B.; Vacca, J. P. Potent noncovalent thrombin inhibitors that utilize the unique amino acid D-dicyclohexylalanine in the P3 position. Implications on oral bioavailability and antithrombotic efficacy. *J. Med. Chem.* **1997**, *40*, 1565−1569. (b) Tucker, T. J.; Brady, S. F.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Naylor-Olsen, A. M.; Yan, Y.; Sisko, J. T.; Stauffer, K. J.; Lucas, B. J.; Lynch, J. J.; Cook, J. J.; Stranieri, M. T.; Holahan, M. A.; Lyle, E. A.; Baskin, E. P.; Chen, I. W.; Dancheck, K. B.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. Design and synthesis of a series of potent and orally bioavailable noncovalent thrombin inhibitors that utilize nonbasic groups in the P1 position. *J. Med. Chem.* **1998**, *41*, 3210−3219.

(15) Aurell, L.; Friberger, P.; Karlsson, G.; Claeson, G. A new sensitive and highly specific chromogenic peptide substrate for factor Xa. *Thromb. Res.* **1977**, *11*, 595−609.

(16) Although this method has a shortcoming in that active metabolites could alter results, the speed at which we were able to generate data to influence the design process outweighed this shortcoming.

(17) Arnaiz, D. O.; Chou, Y. L.; Griedel, B. D.; Karanjawala, R. E.; Kochanny, M. J.; Lee, W.; Liang, A. M.; Morrissey, M. M.; Phillips, G. B.; Sacchi, K. L.; Sakata, S. T.; Shaw, K. J.; Snider, R. M.; Wu, S. C.; Ye, B.; Zhao, Z. *ortho*-Anthranilamide derivatives as anticoagulants. U.S. Patent 6,140,351, October 31, 2000.

(18) See reference 10c and the following: (a) Maignan, S.; Guilloteau, J. P.; Choi-Sledeski, Y. M.; Becker, M. R.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. Molecular structures of human factor Xa complexed with ketopiperazine inhibitors: Preference for a neutral group in the S1 pocket. *J. Med. Chem.* **2003**, *46*, 685−690. (b) Nazaré, M.; Will, D. W.; Matter, H.; Schreuder, H.; Ritter, K.; Urmann, M.; Essrich, M.; Bauer, A.; Wagner, M.; Czech, J.; Lorenz, M.; Laux, V.; Wehner, V. Probing the subpockets of factor Xa reveals two binding modes for inhibitors based on a 2-carboxyindole scaffold: A study combining structure−activity relationship and X-ray crystallography. *J. Med. Chem.* **2005**, *48*, 4511−4525. (c) Kohrt, J. T.; Filipiski, K. J.; Cody, W. L.; Bigge, C. F.; La, F.; Welch, K.; Dahring, T.; Bryant, J. W.; Leonard, D.; Bolton, G.; Narasimhan, L.; Zhang, E.; Peterson, J. T.; Haarer, S.; Sahasrabudhe, V.; Janiczek, N.; Desiraju, S.; Hena, M.; Fiakpui, C.; Saraswat, N.; Sharma, R.; Sun, S.; Maiti, S. N.; Leadley, R.; Edmunds, J. J. The discovery of glycine and related amino acid-based factor Xa inhibitors. *Bioorg. Med. Chem.* **2006**, *14*, 4379−4392.

(19) Stubbs, M. T.; Reyda, S.; Dullweber, F.; Moller, M.; Klebe, G.; Dorsch, D.; Mederski, W. W.; Wurziger, H. pH-Dependent binding modes observed in trypsin crystals: Lessons for structure-based drug design. *ChemBioChem* **2002**, *3*, 246−249.

(20) (a) Katz, B. A.; Sprengeler, P. A.; Luong, C.; Verner, E.; Elrod, K.; Kirtley, M.; Janc, J.; Spencer, J. R.; Breitenbucher, J. G.; Hui, H.; McGee, D.; Allen, D.; Martelli, A.; Mackman, R. L. Engineering inhibitors highly selective for the S1 sites of Ser190 trypsin-like serine protease drug targets. *Chem. Biol.* **2001**, *8*, 1107−1121. (b) Mackman, R. L.; Katz, B. A.; Breitenbucher, J. G.; Hui, H. C.; Verner, E.; Luong, C.; Liu, L.; Sprengeler, P. A. Exploiting subsite S1 of trypsin-like serine proteases for selectivity: Potent and selective inhibitors of urokinase-type plasminogen activator. *J. Med. Chem.* **2001**, *44*, 3856−3871.

(21) A detailed discussion of the crystal structure of compound **13a** bound to fXa is described elsewhere: Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, G.; Light, D. R.; Biancalana, S.; Whitlow, M. Crystal structures of two potent nonamidine inhibitors bound to factor Xa. *Biochemistry* **2002**, *41*, 15514−15523.

(22) (a) Maignan, S.; Mikol, V. The use of 3D structure data in the design of specific factor Xa inhibitors. *Curr. Top. Med. Chem.* **2001**, *1*, 161−174. (b) Adler, M.; Davey, D. D.; Phillips, G. B.; Kim, S.-H.; Jancarik, J.; Rumennik, G.; Light, D. R.; Whitlow, M. Preparation, characterization, and the crystal structure of the inhibitor ZK-807834 (CI-1031) complexed with factor Xa. *Biochemistry* **2000**, *39*, 12534−12542.

(23) Lottenberg, R.; Christensen, U.; Jackson, C. M.; Coleman, P. L. Assay of coagulation proteases using peptide chromogenic and fluorogenic substrates. *Methods Enzymol*. **1981**, *80*, 341−361.

(24) (a) Jordan, S. P.; Waxman, L.; Smith, D. E.; Vlasuk, G. P. Tick anticoagulant peptide: kinetic analysis of the recombinant inhibitor with blood coagulation factor Xa. *Biochem.* **1990**, *29*, 11095−11100. (b) Morrison, J. F. Kinetics of the reversible inhibition of enzyme-catalysed reactions by tight-binding inhibitors. *Biochim. Biophys. Acta* **1969**, *185*, 269−286.

JM070125F