Factor Xa Inhibitors: S1 Binding Interactions of a Series of *N*-{(*3S*)-1-[(*1S*)-1-Methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides†

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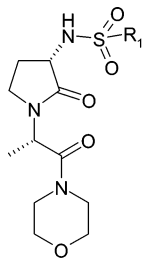
Factor Xa inhibitory activities for a series of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides with different *P*1 groups are described. These data provide insight into binding interactions within the S1 primary specificity pocket; rationales are presented for the derived SAR on the basis of electronic interactions through crystal structures of fXa−ligand complexes and molecular modeling studies. A good correlation between in vitro anticoagulant activities with lipophilicity and the extent of human serum albumin binding is observed within this series of potent fXa inhibitors. Pharmacokinetic profiles in rat and dog, together with selectivity over other trypsin-like serine proteases, identified **1f** as a candidate for further evaluation.

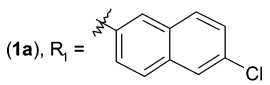
Introduction

Thrombosis is a multifactorial disorder initiated by intravascular blood clotting leading to thrombus formation in an artery or vein. The resultant thrombi can be obstructive or dislodged into smaller blood vessels, depriving vital organs of oxygen (ischemia), which can be catastrophic. Manifestation of these life threatening conditions includes myocardial infarction, pulmonary embolism, and stroke. Drugs that inhibit the blood clotting process can prevent these events, and the development of antithrombotics is a major focus of pharmaceutical research.[1] A number of antithrombotics such as aspirin, heparin, low molecular weight heparins,[2] and warfarin[3] are used clinically, but these drugs suffer from limited efficacy, low oral bioavailability, or a narrow therapeutic window. Indeed, administration of warfarin, a vitamin K epoxide reductase inhibitor, requires individual treatment with continuous dose adjustment and laboratory monitoring because of its interactions with other drugs and dietary vitamin K. Furthermore, warfarin has a slow onset of action.

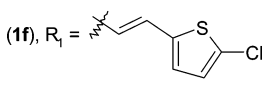
A key driver of thrombus formation is the dysregulation of blood coagulation, which consists of the interconnected intrinsic and extrinsic pathways[4] that converge onto the formation of activated factor X (fXa). When bound to factor Va, calcium, phospholipid, and prothrombin (prothrombinase complex), fXa converts prothrombin into thrombin which in turn proteolytically converts fibrinogen into fibrin. Ultimately, fibrin chains are cross-linked into an insoluble matrix (the blood clot). Thus, thrombin and fXa are attractive targets[5] for pharmacological intervention. Indeed, fondaparinux,[6] a selective indirect fXa inhibitor suitable for subcutaneous administration, has been approved for the prevention of venous thromboembolism in patients undergoing major orthopaedic (hip or knee replacement) surgery. Furthermore, direct thrombin and fXa inhibitors have also shown encouraging results for the prevention of thromboembolic events in clinical studies.[7a−d] Ximelagatran, the first registered, orally active thrombin inhibitor, was approved in Europe for the prevention of thrombosis in patients undergoing hip or knee replacement, but was withdrawn from the market due to concerns over toxicity in the liver.[7e] Currently a number of orally active thrombin [e.g., MCC-977, dabigatran etexilate][8] and fXa [e.g., rivaroxaban, LY-517717, apixaban, and DU-176b][9a−g] inhibitors are being investigated in clinical trials for similar conditions. In addition, DX-9065a is currently in clinical trials for parenteral indications.[9h−i] As part of our program to develop efficacious and orally bioavailable anticoagulants, we have identified highly selective and potent thrombin[10] and fXa inhibitors with good anticoagulant activities and promising profiles for chronic oral administration. Factor Xa inhibitors containing the 3-aminopyrrolidin-2-one scaffold and highly basic benzamidine *P*1 substituents have been disclosed previously.[11a] Our own studies[11b] with this scaffold have led to a nonbasic series of novel and orally active fXa inhibitors incorporating 6-chloro-*N*-[(3*S*)-1-substituted-2-oxo-3-pyrrolidinyl]-2-naphthalenesulfonamides and identified **1a** as our lead compound. The



(**1**)



(**1a**), R_1 =



(**1f**), R_1 =

† PDB ID codes for **1a**, **1b**, **1d**, **1e**, and **1x** complexed with factor Xa are 2cji, 2j95, 2j38, 2j34, and 2j94, respectively.

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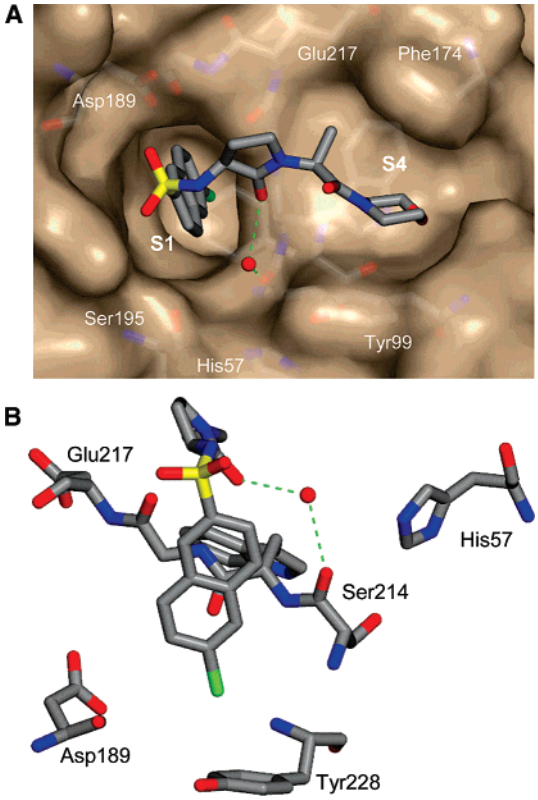


Figure 1. Crystal structure of **1a** bound to fXa showing a water-mediated hydrogen bonding interaction of pyrrolidin-2-one carbonyl to Ser214 carbonyl group. (A) Solvent accessible surface of fXa binding site depicting the 6-chloronaphthyl group binding into the S1 pocket and the alanyl morpholinamide into the S4 pocket while the methyl group binds into a subpocket within S4. (B) Alternative view without the solvent accessible surface showing the bound conformation of 6-chloronaphth-2-ylsulfonamido group in the S1 pocket and the key amino acid residues.

crystal structure of **1a** bound to fXa (Figure 1) clearly showed that the 6-chloronaphth-2-yl group bound in the S1 primary specificity pocket and the morpholino amide occupies the aromatic box (Tyr99, Try215, and Phe174) of the S4 pocket. The pendent methyl group of the alanyl motif binds to a small subpocket formed by Phe174 and the carbon atoms of the side-chain of Glu217. Herein, we extend our study and describe a systematic investigation into the effects of sulfonamido R_1 substituents on fXa inhibitory activities and anticoagulant activities within the *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamide series. Our understanding of the tolerance of sulfonamido groups within the S1 pocket through X-ray crystallography and molecular modeling studies is compared and contrasted with those reported in the literature for alternative series of fXa inhibitors. Correlation of anticoagulant activities with lipophilicity and the extent of human serum albumin (HSA) binding are highlighted together with pharmacokinetic data that led to the identification of **1f** as a candidate for further evaluation.

Chemistry

The key intermediate amine **2** was readily prepared from *N*-CBZ-L-methionine (Scheme 1) by coupling with L-alanine *t*-butyl ester under standard conditions using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxybenzotriazole (HOBt) in the presence of triethylamine to give as an intermediate the protected dipeptide **3**. Activation of the methylthioether moiety of **3** with iodomethane, followed by base-induced cyclization[12] with the hydroxide form of Dowex ion-exchange resin, established the pyrrolidin-2-one system, which was deprotected with TFA in DCM to provide the carboxylic acid **4**. Amide coupling with morpholine using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and *i*-Pr_2NEt, followed by deprotection under palladium-catalyzed hydrogenolysis, thereby yielded the key amine intermediate **2**.

The secondary sulfonamides **1a**−**1h**, **1j**−**1p**, and **1s**−**1y** were prepared by sulfonylation of the (3*S*)-aminopyrrolidin-2-one alanylamide **2** with appropriate sulfonyl chlorides, R_1SO_2Cl, **6**, or suitably protected derivatives thereof under standard conditions (see Method A in General Procedures). R_1s are defined in Table 1. When Boc was used as a protecting group, its removal was effected with TFA in DCM (for **1s** and **1t**). With the exception of **6h**, **6n**, and **6u**−**6y**, all other sulfonyl chlorides were either available commercially (**6m**) or prepared according to literature procedures (**6a**−**6g**,[13,14] **6j**−**6l**,[14,15] **6o**,[16] **6p**,[16] N-Boc of **6s**,[17] and N-Boc of **6t**[17]). Sulfonyl chlorides **6h**, **6n**, and **6u**−**6w** were synthesized from the corresponding chloroheteroaromatics by direct lithiation with *n*-BuLi at −78 °C, followed by sulfinylation with SO_2 and chlorinative oxidation with NCS (Scheme 2). In the case of **6y**, lithiation was carried out by metal−halogen exchange of 5-bromo-2-(5-chloro-2-thienyl)-pyridine[18] with *t*-BuLi, followed by sulfinylation and chlorinative oxidation as above to provide the requisite sulfonyl chloride. The chloro-bis(heteroaryls) were prepared by palladium-catalyzed coupling reactions. Thus, 5-(5-chloro-2-thienyl)-1,3-thiazole, 2-(5-chloro-2-thienyl)-1,3-thiazole, and 5-chloro-2-(2-thienyl)-1,3-thiazole were prepared by Suzuki coupling of either 2- or 5-bromothiazole[19a] with 5-chlorothiophene-2-boronic acid or 2-bromo-5-chlorothiazole[19b] with thiophene-2-boronic acid. 2-(5-Chloro-2-thienyl)furan was synthesized via Stille coupling of the tri-*n*-butyl-2-(furanyl)stannane with 2-bromo-5-chlorothiophene. 3-(5-Chloro-2-thienyl)-1*H*-1,2,4-triazole-5-sulfonyl chloride **6x** was prepared by the reaction of thiourea with 5-chloro-2-thiophenecarbohydrazide[20] at 160 °C in the absence of solvent, followed by careful oxidation of the resultant mercaptotriazolyl derivative with chlorine in AcOH (Scheme 3). The sulfonyl chlorides described above were generally used for sulfonylation with **2** without purification.

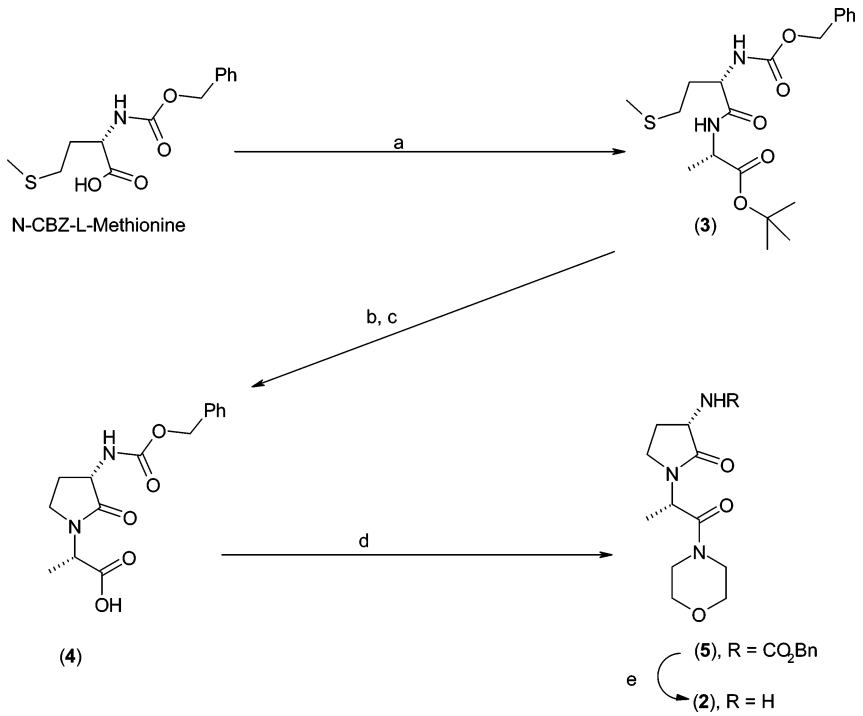
Due to complications in the synthesis of **6q**,[21] an alternative and indirect synthesis for **1q** and **1r** was employed. Thus, *S*-chlorination of the appropriate chloro-1,3-benzothiazole-2(3*H*)-thione[22] using NCS, followed by sulfenylation with **2** and oxidation with $KMnO_4$, provided the 5- and 6-chloro-1,3-benzothiazole-2-sulfonamido derivatives **1q** and **1r** (Scheme 4).

Compound **1i** was synthesized via the sulfonylation of **2** with 4-iodophenylsulfonyl chloride to give **7**. Sonagashira coupling with trimethylsilylacetylene in the presence of Et_3N and catalytic amounts of CuI and $PdCl_2(PPh_3)_2$ in DMF at room temperature, followed by the removal of the trimethylsilyl group with TBAF yielded the acetylenic compound **8**. Hydroborative chlorination[23] with *t*-hexylborane and cupric chloride afforded the required **1i** (Scheme 5).

Results and Discussion

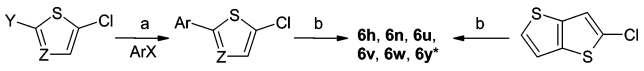
The influence of sulfonamido R_1 substituents on fXa inhibitory activities has been investigated within a series of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}sulfonamides (Table 1). 6-Chloronaphthyl, 5′-chloro-2,2′-bithienyl, 4-chlorostyryl, 5- and 6-chlorobenzothienyl, and 5-chlorothienylethenyl analogues **1a**−**1f** show good to excellent fXa inhibitory activities (K_i = 4−47 nM). Related findings for

Scheme 1[a]



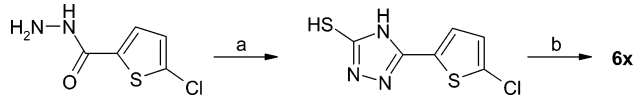
[a] Reagents and conditions: (a) L-alanine *t*-butyl ester, HOBt, Et_3N, EDC; (b) MeI, acetone, then Dowex (HO^- form) ion-exchange resin; (c) TFA, DCM; (d) morpholine, *i*-Pr_2NEt, TBTU; (e) 10% Pd−C, H_2, EtOH.

Scheme 2[a]



[a] Reagents and conditions: (a) for the precursor to **6v**, ArX = 2-bromothiazole, Y = $B(OH)_2$, Z = CH: Pd_2dba_3·$CHCl_3$, PPh_3, Na_2CO_3, H_2O, DME; for the precursor to **6w**, ArX = 5-bromothiazole,[19a] Y = $B(OH)_2$, Z = CH: $PdCl_2dppf$·CH_2Cl_2, K_2CO_3, DME; for the precursor to **6n**, ArX = thiophene-2-boronic acid, Y = Br, Z = N:[19b] $Pd(PPh_3)_4$, Cs_2CO_3, H_2O, DME; for the precursor to **6u**, ArX = tri-*n*-butyl(2-furanyl)stannane, Y = Br, Z = CH: Pd_2dba_3·$CHCl_3$, PPh_3, THF; (b) (i) *n*-BuLi, THF, −78 °C, SO_2; (ii) NCS, DCM; *For **6y**, 5-bromo-2-(5-chloro-2-thienyl)pyridine[18] was used: (i) *t*-BuLi, THF, −78 °C, SO_2; (ii) NCS, DCM.

Scheme 3[a]

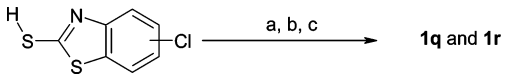


[a] Reagents and conditions: (a) thiourea, 160 °C; (b) Cl_2, AcOH, 10 °C.

these and other chloroheteroaryl *P*1s have been reported[13c,24,25] with other structural classes of fXa inhibitors. Basic *P*1 groups such as 3- and 4-substituted benzamidines in first generation fXa inhibitors[26] have been reported to interact with Asp189 in the S1 pocket. In contrast, **1a**−**1f** are nonbasic and, at the outset of our program, before X-ray crystal structures of our compounds bound to fXa were available, their binding orientation was uncertain. Evidence that chloroaromatic groups could bind in the S1 pocket was obtained by elegant ^{19}F NMR studies of a 6-fluoronaphthyl containing inhibitor bound to fXa with and without Gd-EDTA[a] complex as well as competitive displacement studies with a 6-chloronaphthyl containing fXa inhibitor.[27] Confirmation of a similar binding mode in the present series was established by X-ray crystal structures of these compounds

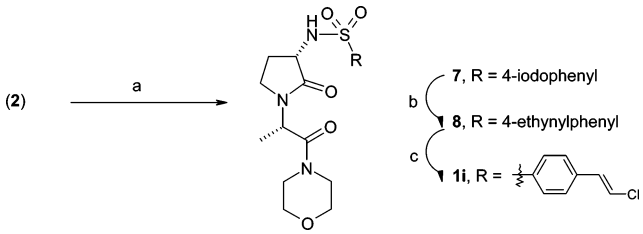
[a] Abbreviations: Gd-EDTA, gadolinium−ethylenediaminetetraacetic acid complexes; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; Mes, 2-morpholinoethanesulfonic acid; PEG, polyethylene glycol; SD, standard deviation.

Scheme 4[a]



[a] Reagents and conditions: (a) NCS, DCM; (b) pyridine, **2**, MeCN; (c) $KMnO_4$.

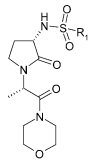
Scheme 5[a]

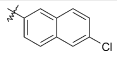
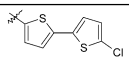
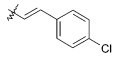
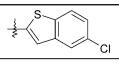
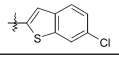
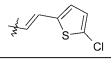
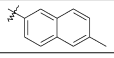
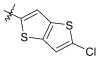
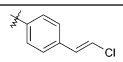
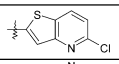
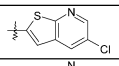
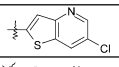
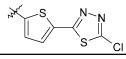
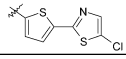
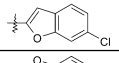
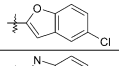
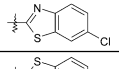
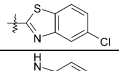
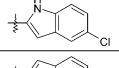
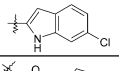
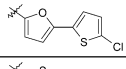
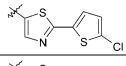
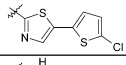
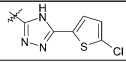
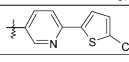


[a] Reagents and conditions: (a) 4-iodophenylsulfonyl chloride, pyridine, MeCN; (b) (i) trimethylsilylacetylene, CuI, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (ii) *n*-Bu_4NF, THF; (c) *t*-hexylborane, $CuCl_2$.[23]

complexed with fXa (Figures 1−3), which showed clearly that the chloroaromatics in **1a**, **1b**, **1d**, and **1e** bound within the S1 pocket. The morpholino group occupied the S4 pocket with the alanyl methyl group bound to a small hydrophobic pocket near to S4. In contrast to a related series[28] in which 3*R* stereochemistry is preferred and the pyrrolidin-2-one C═O is hydrogen-bonded to Gly219,[28] the corresponding carbonyl in our series is indirectly hydrogen-bonded to the carbonyl of Ser214 via a water-mediated interaction. These interactions are similar to that reported for **1a** (Figure 1) in our previous communication.[11b] In contrast to the flat and rigid chlorobenzothienyl and chloronaphthyl *P*1s present in **1d**, **1e**, and **1a**, respectively, the two thienyl rings in **1b** are out of coplanarity (S−C−C−S torsion ca. 159°), which is in good agreement with that reported for the lowest energy conformer of bithiophene.[29] Our crystal structures show the chlorine atom is 3.7−4.2 Å from the centroid

Table 1. Affinities of **1a**−**1y** in the Fluorogenic Factor Xa Assay



Compound	R_1	Ki/nM	SD[a]
1a		6	2
1b		4	2
1c		11	5
1d		47	5
1e		15	3
1f		4	1
1g		109	9
1h		165	35
1i		314	119
1j		163	52
1k		2898	1404
1l		3670	1068
1m		> 15800	-
1n		3225	575
1o		285	14
1p		782	208
1q		4160	1710
1r		112	7
1s		90	20
1t		170	10
1u		1200	80
1v		5	4
1w		2	1
1x		534	80
1y		24	4

[a] SD values are derived from at least n ≥ 2.

of the phenolic ring of Tyr228 in the S1 pocket. This interaction has been reported in the literature as hydrophobic for other series of fXa inhibitors.[24] However, we believe there is an electrostatic component to this interaction based on electrostatic potential maps for these chloroaromatics, which showed a slight positive charge at the center of the Cl atom.[30] Indeed, support for this

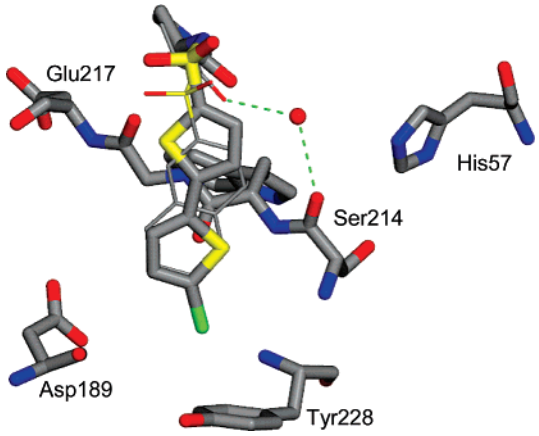


Figure 2. Crystal structure of **1b** bound to fXa showing the conformation of bithienyl *P*1 (thick gray stick) with the 6-chloronaphth-2-yl *P*1 of **1a** (thin gray stick) as comparison.

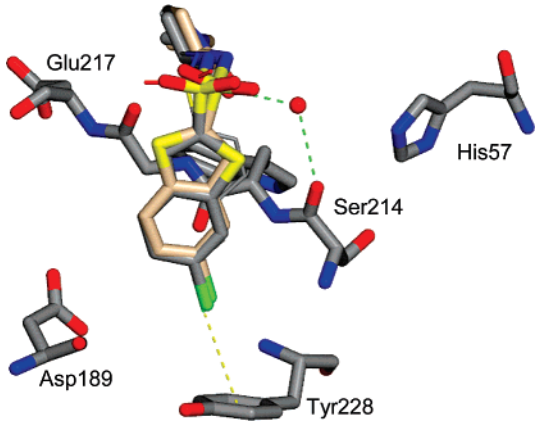


Figure 3. Crystal structures of **1d** (thick wheat stick) and **1e** (thick gray stick) bound to fXa showing the bound conformations of chlorobenzothienyl *P*1s. 6-Chloronaphth-2-yl *P*1 of **1a** is shown (thin gray stick) as reference.

hypothesis arose by replacing the Cl atom with the isosteric methyl group, which resulted in over 10-fold loss in activity [**1g** vs **1a**]. Furthermore, polarization of the aromatic proton facing Asp189 by the adjacent chlorine atom could provide an additional interaction (nonclassical hydrogen bonding),[31] which could augment the binding affinity of chloroaromatics. However, this latter interaction is unlikely to be present in **1h** and **1i**, the thieno[3,2-b]thiophene analogue and the reversed analogue of **1c**, respectively. In the bioactive conformation of **1h**, the sulfur atom in the chlorothieno moiety is expected to occupy the equivalent volume of space that faces Asp189 and, therefore, cannot attain the nonclassical hydrogen bonding interaction mentioned above. When bound to fXa, the proton attached to the same carbon as the Cl atom in the chlorovinyl moiety of **1i** is unlikely to align directly with the carboxylate of Asp189 and is also over 4 Å away to contribute any significant binding affinity. These considerations could provide an explanation for the moderate potency of **1h** and **1i**.

Replacement of the aromatic carbon atom by nitrogen in the chloroaryl ring is generally detrimental to their fXa inhibitory activities (**1j**−**1n**), which may be explained following close analysis of X-ray crystal structures of **1b**, **1d**, and **1e** (Figures 2 and 3) bound to fXa. Analogous to **1a**, the chloroaromatics in **1b**, **1d**, and **1e** are bound to the S1 pocket and display a similar vector for the Cl−Tyr228 interaction. Assuming all chloroheteroaryl analogues maintain this highly favorable Cl−Tyr228 interaction, it is possible to predict the position of the

Table 2. Anticoagulant Activities and Human Serum Albumin Binding of Potent Factor Xa Inhibitors

cmpd	K_i (nM)	1.5XPT (μM)	SD	*n*	logD @ pH 7.4	HSA binding %
1a	6	5.4	0.46	3	1.79	79
1b	4	10.4	0.08	2	2.03	95
1c	11	2.7	0.5	2	1.49	63
1d	47	13.1	0.34	2	2.07	85
1e	15	7.5	0.9	5	1.85	88
1f	4	1.2	0.04	3	1.47	59
1v	5	24.7	1.4	2	2.18	93
1w	2	49.2	4	2	2.1	95
1y	24	27.2	1.3	2	2.24	89

Table 3. Selectivity Profiles of **1a**−**1f** against Other Trypsin-Like Serine Proteases[a]

cmpd	Thr[b]	TF/fVIIa[b]	fXIIa[b]	aPC[b]	Plas[b]	Tryp[b]	Kall[b]
1a	37	>4500	>4500	>1000	>1000	>1000	>300
1b	298	>12 000	>12 000	>25 000	>7000	>2000	>1500
1c	19	>3500	>3500	>1000	>700	>700	>1300
1d	10[c]	>350[c,d]	nd[e]	>350[c,d]	>750[c]	>500[c]	nd[e]
1e	22	nd[e]	nd[e]	>3000[c]	>1500[c]	>1000[c]	nd[e]
1f	90	>30 000	>20 000	>4000	>20 000	>20 000	>1500

[a] Unless otherwise stated, fluorogenic assays were performed on these compounds. [b] Ratio of K_i values (nM) to fXa. TF/fVIIa = tissue factor − factor VIIa; aPC = activated protein C; Plas = plasmin; Tryp = trypsin; and Kall = kallikrein. [c] These ratios were derived from chromogenic assays. [d] These ratios are based on IC_{50} values. [e] nd = not determined.

nitrogen in the chloroheteroaromatic rings of compounds **1j**−**1n**. The lone pair of electrons on the nitrogen atom in the ring of **1k**−**1n** is placed in a highly electron-rich environment formed by the backbone carbonyl of Gly219 and the carboxylate of Asp189. Such a highly unfavorable interaction is reflected in their poor intrinsic activities. In contrast, **1j** is moderately potent (K_i = 163 nM) and is predicted to bind with the nitrogen facing the other side of the S1 pocket, which is likely less electron rich and, therefore, a more favorable environment.

Modifications to the aromatic ring distal to the chlorine atom (i.e., directly attached to SO_2 of the sulfonamido group) are shown in **1o**−**1y**. In the 5,6-fused aromatic systems, **1o**−**1t** are moderate to weakly active. Interestingly, within each isomeric pair (5- and 6-chloro isomers) of benzofurans and benzothiazoles, one member (**1p**, **1q**) is significantly less active than the other (**1o**, **1r**). However, both chloroindolyl analogues (**1s** and **1t**) have similarly moderate activities. Ab initio calculations for the conformational preference of the 5-membered ring heteroaryl-2-sulfonamides, as model systems for **1o**−**1t**, showed[32] that one of the S═O bonds prefers to be coplanar with the C−H, planar N−H, or S in these aromatic systems. When this conformational preference is translated into the 5,6-fused chlorobenzoheteroaryl systems and the important Cl−Tyr228 interaction is maintained, there are significant energy penalties for the bioactive conformations of **1p** and **1q**. Hence, 5-benzofuryl (**1p**) and 6-chlorobenzothiazolyl (**1q**) analogues are less potent than their corresponding isomers **1o** and **1r**. The similar activities of 5- and 6-chloroindolyl isomers (**1s** and **1t**) could also be explained by the little difference in energy between the conformers of the sulfonamido S═O that eclipse the C−H or N−H of the pyrrole ring in 1*H*-pyrrole-2-sulfonamide.

The thienylsulfonamido heteroaromatic ring in the chlorobithienyl *P*1 analogue **1b** is positioned further away from the bottom of the S1 pocket than the ring distal to the chlorine atom in the benzothienyl analogues (vide supra: **1b** in Figure 2 vs **1d** and **1e** in Figure 3). Therefore, this thienyl ring is likely to be further away from potential unfavorable interactions in the lower half of the S1 binding pocket (cf. rationales for **1k**−**1n** above), and replacement of aromatic carbons by nitrogen are expected to be better tolerated. Furthermore, the rotatable bond between the two thienyl rings in **1b** can be twisted to alleviate any possible unfavorable interactions. Indeed both the thiazolyl analogues (**1v** and **1w**) retain potent fXa inhibitory activities (K_i = 5 and 2 nM, respectively) in contrast to the benzothiazolyl analogues (**1q** and **1r**, K_i = 4160 and 112 nM, respectively). Even replacing the thiazolyl ring by a 6-membered pyridyl ring (**1y**) retains good potency (K_i = 24 nM). However, both the furyl (**1u**) and triazolyl (**1x**) analogues are significantly less potent. The weak fXa inhibitory activity of **1u** may be rationalized by the preferred conformation of the *P*1 sulfonamido group in which one of the S═O bonds eclipses the CH of the furan ring (vide supra) while maintaining the highly favorable Cl−Tyr228 interaction.[32] This would position the furyl oxygen atom on the same side as the sulfur atom of the chlorothienyl unit and could change to a less favorable bioactive conformation of **1u** relative to **1b**. A comparison of the crystal structures of

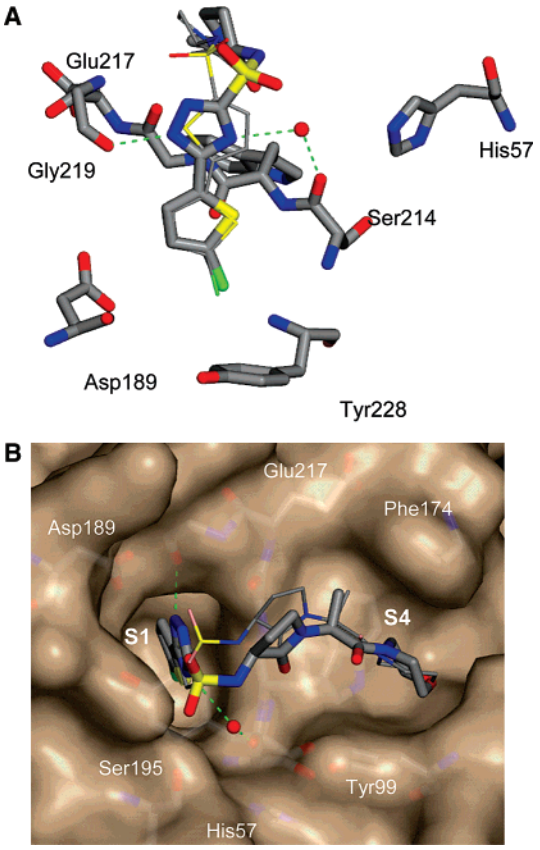


Figure 4. Crystal structure of **1x** (thick stick) in fXa binding site. Compound **1b** (thin stick) is shown for comparison. (A) *P*1 orientation of **1x** showing the perturbed conformation of the triazolyl moiety relative to the bithienyl *P*1 in **1b**. (B) S4 orientation in solvent accessible surface showing major changes in the bound conformation of the 3-sulfonamidopyrrolidin-2-one moiety in **1x** relative to that in **1b**.

Table 4. Pharmacokinetic Parameters of **1f** in Male Rats and Dogs Following Intravenous and Oral Administration at Nominal Doses of 1 mg/kg i.v. and 2.5 mg/kg p.o.

species	$t_{1/2}$[a] (h)	Clp[b] (mL/min/kg)	V_{ss}[c] (L/kg)	F[d] (%)
Sprague Dawley rats	0.7 ± 0.1	8.0 ± 1.7	0.29 ± 0.02	75 ± 23
Beagle dogs	1.2 ± 0.3	4.6 ± 1.7	0.42 ± 0.03	53 ± 9

[a] $t_{1/2}$ = half-life of the test compound expressed in hours. [b] Clp = plasma clearance of the test compound expressed mL/min/kg. [c] V_{ss} = steady-state volume of distribution of test compound expressed as L/kg. [d] F = oral bioavailability of test compound expressed as percentage.

Table 5. Crystallographic and Refinement Data

compound	**1a**[a]	**1b**[a]	**1d**[a]	**1e**[a]	**1x**[b]
RCSB code	2cji	2j95	2j38	2j34	2j94
resolution	2.1	2	2.1	2	2.1
cell dims	56.968	56.914	56.794	56.867	56.739
	72.633	72.731	72.731	73.147	72.563
	79.784	79.901	79.895	80.125	78.924
resolution	2.1	2	2.1	2	2.1
(high shell)	(2.1−2.18)	(2.0−2.07)	(2.1−2.18)	(2.0−2.07)	(2.1-2.21)
Rmerge	0.077	0.050	0.068	0.070	0.109
(high shell)	(0.531)	(0.381)	(0.288)	(0.486)	(0.431)
unique reflection	19 989	22 752	18 209	21 497	18 402
completeness	99	99.5	94.1	94.2	94
multiplicity	4.0	4.0	3.8	3.8	3.8
Rfactor	0.192	0.193	0.211	0.178	0.181
Rfree	0.242	0.242	0.302	0.220	0.234
rms bond	0.016	0.017	0.016	0.017	0.015
rms angle	1.52	1.571	1.697	1.513	1.588
total no. atoms	2397	2443	2485	2462	2441
avg B	31.259	36.95	24.28	24.837	34.27
avg B for catalytic domain	31.128	32.338	24.018	24.57	33.778
avg B for inhibitor	37.337	40.723	31.682	31.588	44.206
avg B for solvent	32.524	36.117	26.585	28.143	39.735

[a] The X-ray intensity data was collected at the Synchrotron Radiation Source (Daresbury, UK) on station 9.6. [b] The X-ray intensity data was collected at the European Synchrtoron Radiation Facility (Grenoble, France) on beamline ID29.

the triazolyl analogue **1x** with **1b** (Figure 4) in fXa revealed such a distortion, which could account for the weaker intrinsic activity of **1x**. While the S atom of the thiophene ring proximal to the sulfonamido group in **1b** is orientated toward Gly219, the N atom at the 1 position of the 1,3,4-triazolyl group in **1x** appears to be indirectly hydrogen-bonded to Ser214 via a water molecule. This distortion is amplified in the binding of the pyrrolidin-2-one and the morpholinoalanylamide in which the previously observed water-mediated interaction of the pyrrolidin-2-one C═O with Ser214 is now absent in **1x**.

The anticoagulant activities of compounds with fXa K_i values ≤47 nM (**1a**−**1f**, **1v**, **1w**, and **1y**) together with their logD values, determined at pH 7.4, and binding to immobilized human serum albumin (HSA) in a high throughput HPLC assay[33] are shown in Table 2. These compounds showed a broad range of anticoagulant activities (1.5xPT 1.2−49.2 μM), which did not correlate with their intrinsic fXa affinities. Indeed the fall off in plasma-based activities relative to their fXa inhibitory activities ranged from 2 to 4 orders of magnitude. The biggest fall off in 1.5xPT values was observed with the bithienyl and related bi(heteroaromatic) *P*1s (**1b**, **1v** and **1w**). In particular, there is over 20 000-fold difference between fXa affinity and anticoagulant activity for **1w**. These more lipophilic analogues (logD 2.03−2.18 at pH 7.4) showed high protein binding (>90% bound to HSA), which could account for their weaker than expected anticoagulant activities (1.5xPT 10.4−49.2 uM). Indeed there is a good correlation of the degree of HSA binding with lipophilicity (logD at pH 7.4) for this series of compounds. Thus, good anticoagulant activities (1.5xPT 1.2−7.5 uM) were observed for compounds (**1a**, **1c**, **1e**, and **1f**) that are more hydrophilic (logD 1.47−1.85 at pH 7.4) and showed <90% HSA binding, while having similar potent fXa inhibitory activities (K_i = 5−16 nM).

Selected compounds (**1a**−**1f**) with good anticoagulant activities were evaluated against a panel of other trypsin-like serine proteases (Table 3). These compounds have excellent selectivity over trypsin, kallikrein, and the fibrinolytic enzyme plasmin. Against other proteases in the coagulation process, thrombin activities were weaker with good selectivities seen for **1a**, **1b**, and **1f**, while no significant activities against activated protein C, TF/factor VIIa, and factor XIIa were detected.

The pharmacokinetic profiles of compounds with good anticoagulant activities were initially ranked using either one to two animals or a cassette dosing regiment with four animals. Compounds **1a**−**1f** showed promising pharmacokinetic properties (plasma clearance 3−18 mL/min/kg, volumes of distribution 0.17−0.6 L/kg, half-lives 0.57−1.5 h) and high oral bioavailabilities (69−100%) in rat. Definitive studies (n = 3) in both rat and dog were then carried out with **1f**, which showed encouraging pharmacokinetic profiles with high oral bioavailabilities (Table 4). Based on a combination of attractive in vitro and pharmacokinetic profiles, **1f** was selected as a candidate for further evaluation. Its antithrombotic profile and further pharmacological evaluation will be reported separately.[34]

Conclusions

Systematic modifications to a series of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}-sulfonamides have resulted in a number of highly potent fXa inhibitors (**1a**−**1f**, **1s**, **1v**, **1w**, and **1y**). X-ray crystallography of selected ligands in fXa and molecular modeling studies has provided rationales for the derived SAR. The importance of both electrostatic and steric interactions of the chlorine atom to the phenolic centroid of Tyr228 has been highlighted. Modifications to the chloroaromatic ring through the replacement of C−H by nitrogen atom are generally detrimental due to the presence of polar groups (e.g., carboxylate of Asp189 and carbonyl backbone of Gly219) which form one side of the S1 pocket. However, there is a greater scope to modify the aromatic ring attached to the sulfonamido group, which is also influenced by the torsional preference of the NSO_2−heteroaryl bond. Relative to their fXa affinities, good anticoagulant activities have been identified for the more hydrophilic analogues that show <90% binding to HSA. Against a panel of other trypsin-like serine proteases, these compounds showed excellent selectivities against trypsin, kallikrein, and the fibrinolytic enzyme, plasmin. With respect to other proteases in the coagulation process, analogues with good selectivities against thrombin and no significant activities against activated protein C, TF/factor VIIa, and factor XIIa have been identified. Together with the good pharmacokinetic profile in rats and dogs, **1f** was selected as a candidate for further evaluation.

Experimental Section

General Procedures. All general purpose solvents used were Fisons analytical reagents. Anhydrous solvents (Sureseal) were purchased from Aldrich and Fluka. All other reagents were usually obtained from Aldrich, Fluka, or Lancaster. All reactions involving organometallic reagents were carried out under nitrogen atmosphere. Reactions were monitored either by LC-MS or TLC using Merck 60 F_{254} silica gel glass backed plates (5 × 10 cm), eluted with suitable solvent mixtures and visualized by UV light, followed by heating with aqueous phosphomolybdic acid. LC-MS were run on a Hewlett-Packard 1050 coupled with a Micromass Platform Series II or Waters ZQ [using electrospray (ES) positive or negative ionization modes] equipped with a Supelcosil ABZ+PLUS column (3 μm, 3.3 cm × 4.6 mm ID). Standard conditions were eluent systems A (H_2O, 0.1% formic acid, 10 mmol ammonium acetate) and B (95% MeCN and 0.05% formic acid in H_2O): gradient 100% A 0.7 min, 100% A−100% B 4.2 min, 100% B 1.1 min, 100−0% B 0.2 min; flow rate = 3 mL/min). Purifications were performed on silica SPE (solid-phase extraction) eluted under suction or using an OPTIX-10 system, Biotage chromatography using prepacked Kieselgel columns, preparative hplc or mass-directed autoprep. SPE refers to the use of cartridges sold by International Sorbent Technology, Ltd. OPTIX-10 refers to the ISCO OPTIX 10 automated chromatography system. This uses pumps to give gradient control of solvents and variable wavelength UV detection and trigger for fraction collection (λ = 254 nm, unless specified). Biotage chromatography refers to purification carried out using equipment sold by Dyax Corporation (either the Flash 40i or Flash 150i) and cartridges prepacked with KPSil. Preparative HPLC refers to methods where the material was purified by high performance liquid chromatography on a Supelcosil ABZ+ PLUS column (5 μm, 10 cm × 21.2 mm i.d.) with 0.1% HCO_2H in water (A) and MeCN (0.5% HCO_2H; B) utilizing the generic gradient elution conditions expressed as "x to y" gradient with a gradient system as follows: 0−1.45 min x %B, 1.45−20 min x to y %B, 20−24 min y to 95% B, 24−30 min 95% B, 32−34 min 95 to x %B at a flow rate of 8 mL/min. The Gilson 233 fraction collector was triggered by UV (254 nm unless specified). Mass directed autoprep refers to methods where the material was purified by high performance liquid chromatography on a HPLCABZ+ 5 μm column (5 cm × 10 mm i.d.) with 0.1% HCO_2H in water and 95% MeCN, 5% water (0.5% HCO_2H) utilizing the following gradient elution conditions: 0−1.0 min 5% B, 1.0−8.0 min 5 to 30% B, 8.0−8.9 min 30% B, 8.9−9.0 min 30−95% B, 9.0−9.9 min 95% B, 9.9−10 min 95−0% B at a flow rate of 8 mL/min (System 2). The Gilson 202-fraction collector was triggered by a Micromass ZMD Mass Spectrometer on detecting the mass of interest. Where appropriate, test compounds were isolated from their aqueous solutions, if necessary, in the presence of 1,4-dioxan, by freeze-drying using Edwards Freeze Dryer Modulyo. GC-MS was performed on a HP5973MSD with CTC Combipal injector, using 1/100 split injection and chemical ionization with ammonia or methane as collision gas, equipped with HP5 5% phenyl methyl siloxane column (30 m × 0.25 mm × 0.25 mm). Standard GC condition was 80−320 °C at 50 °C/min over 10 min using helium as carrier gas at 1.5 mL/min. Mass spectra were run by an electrospray Hewlett-Packard 5989B instrument. All NMR spectra were run on a Bruker DPX 400 MHz instrument in an appropriate deuterated solvent using tetramethylsilane or residual hydrogenated solvent as standard. Elemental microanalyses were determined by Butterworth Laboratories Limited, Teddington, Middlesex, U.K. High-resolution mass spectra were obtained as positive ion accurate mass centroided data using a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 80−1200 Da, with a scan time of 0.95 s, and an interscan delay of 0.07 s, using reserpine as the external mass calibrant ($[M + H]^+$ = 609.2812 Da).

***t*-Butyl *N*-[(Benzyloxy)carbonyl]-L-methionyl-L-alaninate 3.** *N*-(benzyloxycarbonyl)-L-methionine (10 g, 35.3mmol) was dissolved in DMF (200 mL) and EDC (1.2 equiv, 8.13 g, 42.4 mmol) was added followed by HOBt (1.2 equiv, 5.72 g, 42.33 mmol) and triethylamine (4 equiv, 19.7 mL, 141.34 mmol). The mixture was stirred for 1 h, L-alanine *t*-butyl ester (7.7 g, 53 mmol) was then added, and stirring was continued for 18 h. The mixture was concentrated under reduced pressure and partitioned between diethyl ether and water. The separated organic phase was washed with 1 M hydrochloric acid, saturated aqueous sodium bicarbonate solution, and brine, dried ($MgSO_4$), and concentrated under reduced pressure to give **3** (11.9 g, 82%) as an orange oil, which crystallized on standing. ^{1}H NMR ($CDCl_3$): δ 1.37 (d, 3H, J = 7 Hz), 1.46 (s, 9H), 1.92−2.16 (m, 2H), 2.11 (s, 3H), 2.59 (br t, 2H, J = 7 Hz), 4.33−4.48 (m, 2H), 5.11 (s, 2H), 5.55 (br d, 1H, J = 7.5 Hz), 6.62 (br d, 1H, J = 6.5 Hz), 7.28−7.40 (m, 5H).

(2*S*)-2-((3*S*)-3-{[(Benzyloxy)carbonyl]amino}-2-oxopyrrolidin-1-yl) Propanoic Acid 4. A solution of **3** (11.9 g, 29 mmol) in acetone (75 mL) was treated with methyl iodide (10 equiv, 18 mL, 0.29 mol) and stirred at room temperature for 72 h. The reaction mixture was then concentrated under reduced pressure to give an orange solid that was dissolved in MeCN (200 mL). Dowex resin (hydroxide form, 19.42 g) was added, and the mixture stirred for 18 h at room temperature. The mixture was filtered and the resin was washed with EtOAc. The filtrate was concentrated under reduced pressure to afford a yellow oil that was purified by Biotage chromatography (eluting with cyclohexane/EtOAc 3:2) to give the *t*-butyl (2*S*)-2-((3*S*)-3-{[(benzyloxy)carbonyl]amino}-2-oxopyrrolidin-1-yl)propanoate (2.92 g, 28%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.39 (d, 3H, J = 7.5 Hz), 1.45 (s, 9H), 1.86−1.97 (m, 1H), 2.64−2.76 (m, 1H), 3.27−3.36 (m, 1H), 3.48 (t, 1H, J = 9 Hz), 4.25−4.36 (m, 1H), 4.72 (q, H, J = 7 Hz), 5.13 (s, 2H), 5.36 (br s, 1H), 7.28−7.38 (m, 5H).

t-Butyl (2*S*)-2-((3*S*)-3-{[(benzyloxy)carbonyl]amino}-2-oxopyrrolidin-1-yl)propanoate (0.5 g, 1.38 mmol) was dissolved in DCM (7 mL), and TFA (4.7 mL) was added. The mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure to give **4** (0.423 g, 99.9%) as a colorless oil, which after azeotroping with toluene, crystallized to a white solid. ^{1}H NMR (MeOH-d_4): δ 1.43 (d, 3H, J = 7.3 Hz), 1.88−2.02 (m, 1H), 2.37−2.50 (m, 1H), 3.34−3.52 (m, 1H), 4.43 (dd, 1H, J = 10.5 and 9 Hz), 4.67 (q, 1H, J = 7.3 Hz), 5.09 (s, 2H), 7.25−7.50 (m, 5H). LC-MS: t_R 2.43 min; m/z (ES) 307 (MH^+), 305 ($M - H)^-$.

Benzyl (3*S*)-1-[(1*S*)-1-Methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-ylcarbamate 5. Compound **4** (84.5 g, 0.276mol) was dissolved in DMF (2 L) and TBTU (1.8 equiv, 161 g, 0.5 mol) was added, followed by *N*,*N*-diisopropylethylamine (2.4 equiv, 92 mL, 0.66 mol) and morpholine (1.9 equiv, 46 mL, 0.527 mol). The mixture was stirred under nitrogen for 2.5 h, and saturated aqueous ammonium chloride was added. The mixture was stirred for 15 min then partitioned between water and EtOAc. The separated organic phase was washed with aqueous lithium chloride (10% w/v), followed by saturated sodium bicarbonate and brine. The organic layer was dried (over Na_2SO_4) and concentrated under reduced pressure to give **5** (65 g, 63%) as a yellow solid. ^{1}H NMR (MeOH-d_4): δ 1.28 (d, 3H, J = 7.1 Hz), 1.95−2.08 (m, 1H), 2.34−2.44 (m, 1H), 3.31−3.55 (m, 4H), 3.56−3.72 (m, 6H), 4.23 (t, 1H, J = 9.5 Hz), 5.03−5.12 (m, 3H), 5.09 (s, 2H), 7.25−7.39 (m, 5H). LC-MS: t_R 2.39 min; m/z (ES) 376 (MH^+), 374 ($M - H)^-$.

(3*S*)-3-Amino-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-pyrrolidin-2-one 2. A mixture of **5** (20 g, 53.3 mmol), 10% palladium on carbon (10% w/w, 2 g), and ethanol (1.3 L) was stirred under an atmospheric pressure of hydrogen for 16 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to give **2** (12.3 g, 96%) as a pale white oil. ^{1}H NMR ($CDCl_3$): δ 1.31 (d, 3H, J = 7 Hz), 1.64−1.76 (m, 3H), 2.38−2.49 (m, 1H), 3.30 (td, 1H, J = 9.5 and 6.5 Hz), 3.39 (td, 1H, J = 9.5 and 1.7 Hz), 3.47−3.73 (m, 9H), 5.07 (q, 2H, J = 7 Hz).

General Procedures. Method A: Sulfonylation of 2 with Sulfonyl Chlorides 6. To a solution of **2** (typically 50−100 mg) in anhydrous MeCN were added the appropriate sulfonyl chloride **6** (1.05−1.2 equiv) in MeCN or DCM and a mild base (2.2 equiv) such as pyridine or *N*,*N*-di-isopropylethylamine, and the mixture

was stirred at room temperature overnight (typically 18 h). Saturated ammonium chloride solution was added, and the resultant mixture was stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure, and the residue was partitioned between chloroform and a 1:1 mixture of hydrochloric acid (2 M) and water. The organic layer was washed with a 1:1 mixture of saturated aqueous sodium bicarbonate and water and brine. The organic layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure to give the corresponding sulfonamide, which may have required purification by SPE and/or (mass-directed) autoprep before being freeze-dried in an aqueous dioxan solution.

Method B: Preparation of Sulfonyl Chlorides 6 from Parent or Bromo-chloro(hetero)aromatics. *n*-Butyl lithium (1.6 M in hexanes, 1.1 equiv) was added to a cooled (−78 °C) solution of the appropriate chloro(hetero)aromatics (typically 100 mg) in anhydrous THF (typically 5−10 mL) over 15 min. The reaction was stirred for 20 min. Sulfur dioxide gas was then condensed into the reaction for 10 min. The reaction was allowed to warm to room temperature, stirred for 1 h, and then evaporated in vacuo. The resultant residue was stirred at room temperature (typically for 2−18 h) with *N*-chlorosuccinimide (1.23 equiv) in anhydrous DCM (typically 6−10 mL). The solution was filtered, and the filtrate was concentrated under reduced pressure to give the corresponding sulfonyl chloride as a crude product, which was used in Method A without further purification.

(*E*)-2-(5-Chlorothien-2-yl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}ethenesulfonamide 1f. Compound **1f** (19.3 g, 70% yield) as a white solid was synthesized from **2** (14.9 g, 61.8mmol) and (*E*)-2-(5-chloro-2-thienyl)ethenesulfonyl chloride[13c] (16.5 g, 67.9 mmol) using Method A under General Procedures. ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, J = 7 Hz), 1.99−2.12 (m, 1H), 2.59−2.68 (m, 1H), 3.31−3.40 (m, 1H), 3.47−3.74 (m, 9H), 3.96 (ddd, 1H, J = 11, 8 and 4 Hz), 5.02 (br d, 1H, J = 3 Hz), 5.07 (q, 1H, J = 7 Hz), 6.55 (d, 1H, J = 15 Hz), 6.91 (d, 1H, J = 4 Hz), 7.09 (d, 1H, J = 4 Hz), 7.49 (d, 1H, J = 15 Hz). LC-MS: t_R 2.70 min; *m*/*z* (ES) 448/450 MH^+, 446/448 $(M - H)^-$. Anal. ($C_{17}H_{22}ClN_3O_5S_2$): C, H, N.

4-Iodo-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}benzenesulfonamide 7. Compound **7** (3.217 g, 71%) was synthesized from **2** (2.163 g, 8.96 mmol) and 4-iodophenylsulfonyl chloride (1.1 equiv, 2.983 g, 9.86 mmol) using Method A under General Procedures. ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 3H, J = 7 Hz), 1.91−2.05 (m, 1H), 2.45−2.65 (m, 1H), 3.29 (td, 1H, J = 10 and 6 Hz), 3.42−3.74 (m, 9H), 3.82 (dd, 1H, J = 10, and 8 Hz), 5.03 (q, 1H, J = 7 Hz), 6.27 (br s, 1H), 7.64 (d, 2H, J = 8.6 Hz), 7.87 (d, 2H, J = 8.6 Hz). LC-MS: t_R 2.60 min; *m*/*z* (ES) 508 MH^+, 506 $(M - H)^-$.

4-Ethynyl-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}benzenesulfonamide 8. A mixture of **7** (547 mg, 1.08 mmol), CuI (3 mol%, 6.2 mg, 0.032 mmol), dichlorobis(triphenylphosphine)palladium(II) (3 mol%, 22.7 mg, 0.032 mmol), trimethylsilylacetylene (3 equiv, 0.458 mL, 3.24 mmol), and triethylamine (2 mL) in DMF (2 mL) was stirred at room temperature for 22 h. The reaction mixture was evaporated in vacuo to give a dark brown residue that was purified using SPE (20 g SiO_2, 50 to 70% EtOAc-cyclohexane, EtOAc then 5% MeOH−EtOAc) to give *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-4-[(trimethylsilyl)ethynyl]-benzenesulfonamide as a brown foam (514 mg, 99.9%). ^{1}H NMR ($CDCl_3$): δ 0.26 (s, 9H), 1.30 (d, 3H, J = 7 Hz), 1.92−2.05 (m, 1H), 2.49−2.59 (m, 1H), 3.29 (td, 1H, J = 10 and 6 Hz), 3.43−3.77 (m, 10H), 5.01 (q, 1H, J = 7 Hz), 5.56 (bd, 1H, J = 3 Hz), 7.59 (d, 2H, J = 8.6 Hz), 7.84 (d, 2H, J = 8.6 Hz). LC-MS: t_R 3.20 min; *m*/*z* (ES) 478 MH^+, 476 $(M - H)^-$.

To a solution of *N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-4-[(trimethylsilyl)ethynyl]benzenesulfonamide (391 mg, 0.82 mmol) in dry THF (4 mL) at −78 °C, tetra *n*-butylammonium fluoride (1 M in THF, 0.819 mL, 0.82 mmol) was added. The mixture was stirred at −78 °C for 1 h and then at room temperature for another hour and evaporated in vacuo. The resultant residue was dissolved in EtOAc (80 mL) and washed with water (3 × 30 mL), dried ($MgSO_4$), and filtered. Removal of solvent gave a light brown residue that was purified using SPE (10 g SiO_2, EtOAc) to give **8** (276.5 mg, 83%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.30 (d, 3H, J = 7 Hz), 1.95−2.08 (m, 1H), 2.54−2.63 (m, 1H), 3.27 (s, 1H), 3.31 (td, 1H, J = 10 and 6 Hz), 3.43−3.77 (m, 10H), 5.01 (q, 1H, J = 7 Hz), 5.27 (br s, 1H), 7.63 (d, 2H, J = 8.6 Hz), 7.87 (d, 2H, J = 8.6 Hz). LC-MS: t_R 2.46 min; *m*/*z* (ES) 406 MH^+, 404 $(M - H)^-$.

4-[(*E*)-2-Chloroethenyl]-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}benzenesulfonamide 1i. To a slurry of **8** (132.7 mg, 0.327 mmol) in anhydrous THF (4 mL) at 0 °C under nitrogen, *t*-hexylborane (prepared from 0.69 mL of each of THF solution of 1 M borane and 2 M 2-methyl-2-butene according to standard procedure) was added and stirred for 4 h. Copper(II) chloride (2.4 equiv, 105.6 mg, 0.785 mmol) followed by 1,3-dimethyl-2-imidazolidinone (0.5 mL) and water (0.014 mL, 0.785mmol) were added.[23] The mixture was stirred at 0 °C to room temperature for 18 h and quenched with water (5 mL). Extraction with EtOAc (3×) gave a yellow oil that was purified using autopreparative reverse phase HPLC to give **1i** (49.6 mg, 34%) as a creamy white solid. ^{1}H NMR ($CDCl_3$): δ 1.30 (d, 3H, J = 7 Hz), 1.96−2.08 (m, 1H), 2.53−2.64 (m, 1H), 3.26−3.36 (m, 1H), 3.43−3.75 (m, 9H), 3.74 (dd, 1H, J = 10.5 and 8 Hz), 5.01 (q, 1H, J = 7 Hz), 5.15−5.60 (br s, 1H), 6.84 (ABq, 2H, J = 13.6 Hz), 7.44 (d, 1H, J = 8 Hz), 7.86 (d, 1H, J = 8 Hz). LC-MS: t_R 2.70 min, 98% purity; *m*/*z* (ES) 442/444 MH^+, 440/442 $(M - H)^-$. HRMS calcd for $C_{19}H_{23}N_3O_5ClS$ *m*/*z* (MH^+), 442.1203; found, 442.1213.

5-(5-Chloro-1,3-thiazol-2-yl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-2-thiophenesulfonamide 1n. To a mixture of thiophene-2-boronic acid (1.1 equiv, 0.71 g, 5.54 mmol), 2-bromo-5-chlorothiazole[19b] (1 g, 5.04 mmol), and Cs_2CO_3 (2.6 equiv, 4.27 g, 13.1 mmol) under nitrogen, degassed water (12 mL) was added and stirred for 10 min. DME (12 mL) followed by $Pd(PPh_3)_4$ (5 mol%, 291 mg, 0.25 mmol) were then added. The mixture was heated at 70 °C overnight. The crude reaction mixture was extracted with Et_2O (3 × 90 mL), dried, and evaporated to give a dark brown oil (1.152 g). SPE (20 g SiO_2, cyclohexane, 3 to 10% EtOAc−cyclohexane) followed by further purification on OPTIX 10 (2 × 10 g SiO_2, 5% EtOAc−cyclohexane) gave the 5-chloro-2-(2-thienyl)-1,3-thiazole as a yellow solid (527 mg, 51.9%). ^{1}H NMR ($CDCl_3$): δ 7.07 (dd, 1H, J = 5 and 4 Hz), 7.40−7.44 (m, 2H), 7.55 (s, 1H). LC-MS: t_R 3.37 min; *m*/*z* (ES) 202/204 MH^+.

Using Method A under General Procedures, **1n** (3.3 mg) was synthesized from **2** (101.6 mg, 0.42 mmol) and 5-(5-chloro-1,3-thiazol-2-yl)-2-thiophenesulfonyl chloride **6n** [139.5 mg; prepared from 5-chloro-2-(2-thienyl)-1,3-thiazole (104 mg, 0.52 mmol) using Method B under General Procedures and used without further purification]. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, J = 7 Hz), 2.04−2.16 (m, 1H), 2.62−2.73 (m, 1H), 3.32−3.40 (m, 1H), 3.45−3.73 (m, 9H), 3.92 (br t, 1H, J = 9 Hz), 5.03 (q, 1H, J = 7 Hz), 5.48 (br s, 1H), 7.37 (d, 1H, J = 4 Hz), 7.61 (d, 1H, J = 4 Hz), 7.63 (s, 1H). LC-MS: t_R 2.83 min, 95% purity; *m*/*z* (ES) 505/507 MH^+, 503/505 $(M - H)^-$. HRMS calcd for $C_{18}H_{21}ClN_4O_5S_3$ *m*/*z* (MH^+), 505.0441; found, 505.0431.

6-Chloro-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}-1,3-benzothiazole-2-sulfonamide 1q. *N*-Chlorosuccinimide (0.37 g, 2.77 mmol) was added to 6-chloro-1,3-benzothiazole-2(3*H*)-thione[22] (0.5 g, 2.48 mmol) in DCM (15 mL) under nitrogen, and stirred at room temperature for 3 h. A solution of **2** (0.569 g, 2.36 mmol) and triethylamine (1.04 mL, 7.49 mmol) in anhydrous DCM (5 mL) were added, and the resulting mixture was stirred at room temperature under nitrogen for 2 h. The solution was filtered, and the filtrate was diluted with DCM. The organic solution was washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by SPE (silica, eluting with cyclohexane−EtOAc 1:1, increasing polarity to EtOAc−MeOH 19:1) to give (3*S*)-3-{[(6-chloro-1,3-benzothiazol-2-yl)thio]amino}-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]pyrrolidin-2-one (300 mg, 29%) as a white

solid. ^{1}H NMR ($CDCl_3$): δ 1.34 (d, 3H, J = 6.6 Hz), 2.06−2.20 (m, 1H), 2.40−2.51 (m, 1H), 3.30−3.44 (m, 2H), 3.47−3.78 (m, 8H), 4.03 (ddd, 1H, J = 12, 8 and 3.5 Hz), 4.35 (d, 1H, J = 3.5 Hz), 5.10 (q, 1H, J = 6.6 Hz), 7.37 (dd, 1H, J = 9 and 2 Hz), 7.72 (d, 1H, J = 9 Hz), 7.77 (d, 1H, J = 2 Hz). LC-MS: t_R 2.82 min; *m*/*z* (ES) 441/443 MH^+, 439/441 $(M - H)^-$.

(3*S*)-3-{[(6-Chloro-1,3-benzothiazol-2-yl)thio]amino}-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]pyrrolidin-2-one (0.1 g, 0.227 mmol) was stirred at room temperature in anhydrous acetone (3 mL) and 5% aqueous potassium permanganate (1.35 mL) for 3 h, after which additional acetone (3 mL) and 5% aqueous potassium permanganate (1.35 mL) were added. The reaction mixture was stirred for a further 18 h and filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by mass directed preparative HPLC to give **1q** (6.2 mg, 6%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.32 (d, 3H, J = 7 Hz), 2.08−2.21 (m, 1H), 2.70−2.81 (m, 1H), 3.37 (td, 1H, J = 10.5 and 6 Hz), 3.43−3.74 (m, 9H), 4.31 (dd, 1H, J = 10.8 and 8 Hz), 5.01 (q, 1H, J = 7 Hz), 5.86−6.03 (br s, 1H), 7.68 (dd, 1H, J = 8.8 and 2 Hz), 7.97 (d, 1H, J = 2 Hz), 8.10 (d, 1H, J = 8.8 Hz). LC-MS: t_R 2.98 min, 98% purity; *m*/*z* (ES) 473 MH^+. HRMS calcd for $C_{18}H_{21}ClN_4O_5S_2$ *m*/*z* (MH^+), 473.0720; found, 473.0719.

5-Chloro-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}-1*H*-indole-2-sulfonamide 1s. *t*-Butyl 5-chloro-2-[({(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}amino)sulfonyl]-1*H*-indole-1-carboxylate (13 mg, 25%) as a colorless glass was synthesized from **2** (44 mg, 0.18mmol) and 1,1-dimethylethyl 5-chloro-2-(chlorosulfonyl)-1*H*-indole-1-carboxylate[17] (33 mg, 0.094mmol) using Method A under General Procedures. ^{1}H NMR ($CDCl_3$): δ 1.27 (d, 3H, J = 7 Hz), 1.75 (s, 9H), 2.06−2.18 (m, 1H), 2.64−2.73 (m, 1H), 3.32 (td, 1H, J = 10 and 6.5 Hz), 3.41−3.73 (m, 9H), 3.90 (ddd, 1H, J = 11, 8, and 3 Hz), 5.00 (q, 1H, J = 7 Hz), 6.75 (d, 1H, J = 3 Hz), 7.42 (dd, 1H, J = 8 and 2 Hz), 7.42 (s, 1H), 7.62 (d, 1H, J = 2 Hz), 7.95 (d, 1H, J = 8 Hz). LC-MS: t_R 3.41 min; *m*/*z* (ES) 555/557 MH^+, 553/555 $(M - H)^-$.

t-Butyl 5-chloro-2-[({(3*S*)-1-[(1*S*)-1-methyl-2-morpholin-4-yl-2-oxoethyl]-2-oxopyrrolidin-3-yl}amino)sulfonyl]-1*H*-indole-1-carboxylate (11 mg, 0.02 mmol) was dissolved in 1:1 TFA/DCM (0.5 mL) and allowed to stand at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between saturated aqueous sodium bicarbonate and DCM. The separated organic phase was dried ($MgSO_4$) and concentrated under a stream of nitrogen to give **1s** (8.2 mg, 91%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, J = 7 Hz), 2.05−2.17 (m, 1H), 2.58−2.68 (m, 1H), 3.37 (td, 1H, J = 10 and 6.5 Hz), 3.39−3.70 (m, 9H), 4.17−4.28 (m, 1H), 5.03 (q, 1H, J = 7 Hz), 5.69 (d, 1H, J = 6.5 Hz), 7.06 (d, 1H, J = 2 Hz), 7.21−7.30 (m, 2H), 7.63 (d, 1H, J = 1 Hz), 10.9 (s, 1H). LC-MS: t_R 2.95 min, 96% purity; *m*/*z* (ES) 455/457 MH^+, 453/455 $(M - H)^-$. HRMS calcd for $C_{19}H_{23}ClN_4O_5S$ *m*/*z* (MH^+), 455.1156; found, 455.1158.

5-(5-Chloro-2-thienyl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-2-furansulfonamide 1u. A mixture of 2-bromo-5-chlorothiophene (0.307 mL, 2.8 mmol), tri-*n*-butyl(2-furanyl)stannane (0.882 mL, 2.8 mmol), tris(dibenzylideneacetone)dipalladium(0)−chloroform adduct (Pd_2dba_3·$CHCl_3$; 2.5 mol%, 72.5 mg, 0.07 mmol), and PPh_3 (10 mol%, 73.4 mg, 0.28 mmol) in THF (15 mL) was heated under nitrogen at 75 °C for 21.5 h. KF (10 g) and water (5 mL) were added to the cooled reaction mixture and stirred at room temperature for 7 h. Aqueous NaOH (2 M, 5 mL) was then added. The resultant mixture was evaporated in vacuo to remove most THF, diluted with water (20 mL), extracted with ether (3 × 30 mL), and dried ($MgSO_4$). Removal of solvent gave an oily brown solid (1.31 g) that was purified by SPE (20 g SiO_2, cyclohexane) to give 2-(5-chloro-2-thienyl)furan (299 mg, 58%) yellow liquid. ^{1}H NMR ($CDCl_3$): δ 6.43−6.48 (m, 2H), 6.86 (t, 1H, J = 3.5 Hz), 7.01 (t, 1H, J = 3.5 Hz), 7.40 (s, 1H). GC-MS: t_R 7.07 min; *m*/*z* (APCI for C_8H_5ClOS) 184/186 M^+.

Using Method A under General Procedures, **1u** (37 mg, 19% based on **2**) as a light brown foam was synthesized from **2** (97.4 mg, 0.4mmol) and 5-(5-chloro-2-thienyl)-2-furansulfonyl chloride **6u** [226 mg; prepared from 2-(5-chloro-2-thienyl)furan (167.5 mg, 0.91mmol) using Method B under General Procedures and used without further purification]. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, J = 7 Hz), 1.99−2.13 (m, 1H), 2.59−2.68 (m, 1H), 3.35 (td, 1H, J = 10 and 6 Hz), 3.42−3.74 (m, 9H), 3.96 (dd, 1H, J = 10.6 and 8.1 Hz), 5.04 (q, 1H, J = 7 Hz), 5.63 (br s, 1H), 6.50 (d, 1H, J = 3.5 Hz), 6.91 (d, 1H, J = 4 Hz), 7.12 (d, 1H, J = 3.5 Hz), 7.18 (d, 1H, J = 4). LC-MS: t_R 2.93 min; *m*/*z* (ES) 488/490 MH^+, 486/488 $(M - H)^-$. Anal. ($C_{19}H_{22}ClN_3O_6S_2$·$0.55CH_2O_2$): C, H, N.

2-(5-Chloro-2-thienyl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-1,3-thiazole-5-sulfonamide 1v. To a mixture of 2-bromothiazole (0.55 mL, 6.1 mmol) and 5-chlorothiophene-2-boronic acid (991 mg, 6.1 mmol) in DME (10 mL) under nitrogen, a solution of sodium carbonate (1.68 g, 15.86 mmol) in water (10 mL), bubbled with nitrogen for 10 min, followed by Pd_2dba_3·$CHCl_3$ (2.5 mol%, 109 mg, 0.105 mmol) and a solution of PPh_3 (10 mol%, 110.5 mg, 0.421 mmol) in DME (10 mL) were added. The mixture was heated at 80 °C under nitrogen for 16 h and evaporated in vacuo. The resultant aqueous mixture was extracted with DCM (3 × 20 mL), dried ($MgSO_4$), and filtered. Removal of solvent gave a brown oil that was purified by SPE (20 g SiO_2, 3−5% EtOAc-cyclohexane) to give the 2-(5-chloro-2-thienyl)-1,3-thiazole (374 mg, 30%) as a light brown oil. ^{1}H NMR ($CDCl_3$): δ 6.91 (d, 1H, J = 4 Hz), 7.26 (d, 1H, J = 3 Hz), 7.28 (d, 1H, J = 4 Hz), 7.76 (d, 1H, J = 3 Hz). LC-MS: t_R 3.26 min; *m*/*z* (ES) 202/204 MH^+.

Using Method A under General Procedures, **1v** (66 mg, 32%) was synthesized from **2** (98 mg, 0.41 mmol) and 2-(5-chloro-2-thienyl)-1,3-thiazole-5-sulfonyl chloride **6v** [165 mg, prepared from 2-(5-chloro-2-thienyl)-1,3-thiazole (133.2 mg, 0.66 mmol) using Method B under General Procedures and used without further purification]. ^{1}H NMR (MeOH-d_4): δ 1.27 (d, 3H, J = 7 Hz), 1.80−1.93 (m, 1H), 2.39−2.49 (m, 1H), 3.33−3.41 (m, 2H), 3.43−3.70 (m, 8H), 4.26 (dd, 1H, J = 10.4 and 8.6 Hz), 4.99 (q, 1H, J = 7 Hz), 7.08 (d, 1H, J = 4 Hz), 7.66 (d, 1H, J = 4 Hz), 8.16 (s, 1H). LC-MS: t_R 2.96 min; *m*/*z* (ES) 505/507 MH^+, 503/505 $(M - H)^-$. Anal. ($C_{18}H_{21}ClN_4O_5S_3$·$0.6H_2O$): C, H, N.

5-(5-Chloro-2-thienyl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-1,3-thiazole-2-sulfonamide 1w. To a mixture of 5-chlorothiophene-2-boronic acid (1.041 g, 6.41mmol) and 5-bromothiazole[19a] (92% in ether, 1.143 g, 6.41mmol) in DME (30 mL, bubbled with N_2 for 10 min before use), a solution of sodium carbonate (2.6 eq., 1.77 g, 16.67mmol) in water (15 mL, bubbled with N_2 for 20 min before use) followed by tetrakis(triphenylphosphine)palladium(0) (10 mol%, 741 mg, 0.64 mmol) were added. The mixture was heated at 70−80 °C under nitrogen for 5.5 h. The cooled reaction mixture was evaporated in vacuo, extracted with ether (4 × 30 mL), dried ($MgSO_4$), and filtered. Removal of solvent gave a brown oil (1.7 g). Purification on a SPE (20 g SiO_2) and eluted with cyclohexane followed by 5−10% EtOAc−cyclohexane gave the 5-(5-chloro-2-thienyl)-1,3-thiazole as a yellow solid (708 mg, 51%). ^{1}H NMR ($CDCl_3$): δ 6.89 (d, 1H, J = 3.8 Hz), 6.99 (d, 1H, J = 3.8 Hz), 7.90 (s,1H), 8.71 (s, 1H). LC-MS: t_R 3.24 min; *m*/*z* (ES) 202/204 MH^+.

Using Method A under General Procedures, **1w** (140 mg, 66.5%) was synthesized from **2** (100.6 mg, 0.42 mmol) and 5-(5-chloro-2-thienyl)-1,3-thiazole-2-sulfonyl chloride **6w** [175 mg; prepared from 5-(5-chloro-2-thienyl)-1,3-thiazole (103 mg, 0.51 mmol) using Method B under General Procedures and used without further purification]. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, J = 7 Hz), 2.06−2.19 (m, 1H), 2.65−2.74 (m, 1H), 3.37 (td, 1H, J = 10 and 6 Hz), 3.45−3.74 (m, 9H), 4.27 (dd, 1H, J = 10.8 and 8 Hz), 5.04 (q, 1H, J = 7 Hz), 6.20 (br s, 1H), 6.93 (d, 1H, J = 4 Hz), 7.07 (d, 1H, J = 4 Hz), 7.89 (s, 1H). LC-MS: t_R 2.97 min; *m*/*z* (ES) 505/507 MH^+, 503/505 $(M - H)^-$. Anal. ($C_{18}H_{21}ClN_4O_5S_3$·$0.5H_2O$): C, H. For N: calcd, 10.90%; found, 10.34%.

3-(5-Chloro-2-thienyl)-1*H*-1,2,4-triazole-5-sulfonyl Chloride 6x. A mixture of 5-chloro-2-thiophenecarboxylic acid hydrazide

(197 mg, 1.12 mmol) and thiourea (90.3 mg, 1.12 mmol) was heated at 160 °C[20] under nitrogen without solvent for 6.5 h. The cooled mixture was stirred with aqueous NaOH (5%, 20 mL) for 4 h and filtered to remove any insolubles. The basic solution was acidified with acetic acid (1.65 mL) to pH 5. The resultant precipitate was collected and dried in vacuo at 50 °C overnight to give the 5-(5-chloro-2-thienyl)-1,2-dihydro-3*H*-1,2,4-triazole-3-thione as a creamy white solid (198 mg, 82%). ^{1}H NMR (DMSO-d_6): δ 7.69 (d, 1H, J = 3.8 Hz), 7.85 (d, 1H, J = 3.8 Hz). LC-MS: t_R 3.04 min; *m*/*z* (ES) 218/220 MH^+, 216/218 $(M - H)^-$.

To a solution of 5-(5-chloro-2-thienyl)-1,2-dihydro-3*H*-1,2,4-triazole-3-thione (45.5 mg, 0.21 mmol) in acetic acid (2 mL) at about 10 °C (bath temperature), a solution of chlorine in acetic acid (0.11 g/mL, prepared by bubbling chlorine gas into 10 mL of acetic acid in ice−water bath for 15 min, 3.3 equiv, 0.444 mL, 0.63 mmol) was added. The mixture was allowed to warm to room temperature slowly, stirred for 3 h, and evaporated to dryness to afford 3-(5-chloro-2-thienyl)-1*H*-1,2,4-triazole-5-sulfonyl chloride **6x**, which was used without purification. An amount of this crude product was treated with excess methylamine in THF. LC-MS confirmed the formation of the corresponding *N*-methyl sulfonamide [t_R 2.68 min; *m*/*z* (ES) 279/281 MH^+, 277/279 $(M - H)^-$].

3-(5-Chloro-2-thienyl)-*N*-{(3*S*)-1-[(1*S*)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-1*H*-1,2,4-triazole-5-sulfonamide 1x. Compound **1x** (23 mg, 23% based on **2**), as a white solid, was synthesized from **2** (50 mg, 0.21 mmol) and 3-(5-chloro-2-thienyl)-1*H*-1,2,4-triazole-5-sulfonyl chloride **6x** (crude, obtained as above) using Method A under General Procedures. ^{1}H NMR ($CDCl_3$): δ 1.39 (d, 3H, J = 7 Hz), 2.13−2.26 (m, 1H), 2.67−2.76 (m, 1H), 3.39−3.74 (m, 11H), 4.58 (dd, 1H, J = 10.3 and 8.6 Hz), 5.03 (q, 1H, J = 7 Hz), 6.87 (d, 1H, J = 4 Hz), 7.42 (d, 1H, J = 4 Hz). LC-MS: t_R 2.55 min; *m*/*z* (ES) 489/491 MH^+, 487/489 $(M - H)^-$. Anal. ($C_{17}H_{21}ClN_6O_5S_2 \cdot 1.2H_2O$): C, H, N.

FXa Crystal Structures with Bound Ligands. Preparation of the Crystals. Purified des-Gla fXaβ was purchased from Enzyme Research Laboratories (South Bend, IN). The protein was further purified on a weak cation-exchange resin (carboxymethyl (CM) sepharose, Amersham Biosciences, Uppsala, Sweden) equilibrated in a solution containing 25 mM sodium acetate (pH 5.0) from which protein was eluted with a NaCl gradient. Protein was then buffer exchanged into a solution containing 5 mM Mes[a]−NaOH (pH 6.0), 5 mM $CaCl_2$, and up to 1 μM crystallization tool compound RPR128515[35] prior to concentration to approximately 5 mg/mL for crystallization experiments in a Vivaspin ultracentrifugation unit (Vivascience, Hanover, Germany). Crystallizaton was carried out using the hanging drop vapor diffusion method in 2 μl drops containing a 1:1 mixture of protein and well solution. Well solution contained 16−20% PEG[a] 6 K, 50 mM Mes[a]-NaOH (pH 5.7−6.0), 5 mM $CaCl_2$, and 50 mM NaCl. Streak seeding followed by macroseeding, using the derived small crystals, routinely gave needle-shaped crystals (size 200 × 50 × 50 μm) suitable for synchrotron X-ray data collection.

X-ray Data Collection and Processing. Compound was prepared as a 100 mM stock in DMSO for soaking experiments. Crystals were soaked in 20 μl well solution containing 1−5 mM compound and 5−10% DMSO for up to 1 week. Prior to freezing at 100 K, the crystals were briefly transferred to well solution with 20% glycerol included. The X-ray intensity data was collected at either the Synchrotron Radiation Source (Daresbury, UK) on station 9.6 or the European Synchrotron Radiation Facility (Grenoble, France) on beamlines ID29 or ID14 eh4. Data processing and scaling was carried out using DENZO and SCALEPACK,[36] except for **1x**, which was processed using MOSFLM.[37] All crystals have $P2_12_12_1$ symmetry and similar cell dimension, $a \approx 56.8$ Å, $b \approx 72.3$ Å, and $c \approx 79.5$ Å.

Structure Solution and Crystallographic Refinement. The initial structure was solved by molecular replacement using the coordinates of 1ezq,[35] a fXa complex structure from the RCSB Protein Data Bank with ligand removed as the search model. Subsequent structures were solved using difference Fouriers. Model building, including ligand fitting, was carried out in QUANTA2000 (Accelrys). All other data manipulation was carried out using programs from the CCP4 suite,[38] including crystallographic refinement using REFMAC.[39] All nonglycine residues fall within the energetically favorable regions of the Ramachandran plot. All of the atoms of the inhibitors and residues in the vicinity of the active site lie in well-defined electron density. The majority of the rest of the protein is in well-determined electron density, except for the EGF1 domain, which was not visible. The statistics of the crystallographic refinement are shown in Table 5. Atomic coordinates have been deposited with the RCSB Protein Data Bank (2cji for **1a**, 2j95 for **1b**, 2j38 for **1d**, 2j34 for **1e**, and 2j94 for **1x**).

In Vitro Assay for Inhibition of Factor Xa. The ability of test compounds to inhibit human fXa in vitro was determined in a fluorescence assay, using rhodamime 110, bis-(Boc-L-glycylglycyl-L-arginine amide) as the fluorogenic substrate. Test compounds were diluted from a 10 mM DMSO solution to the final assay concentration range of 166 μM to 0.09 nM. Assay was performed at room-temperature using buffer consisting of 50 mM HEPES[a], 150 mM NaCl, and 5 mM $CaCl_2$ at pH 7.4 containing human factor Xa (0.2 nM final concentration). Test compound and enzyme were preincubated for 15 min prior to addition of the substrate (0.01 mM final concentration). Incubation was for 3 h, after which a LJL Analyst (now marketed by Molecular Devices Corporation) was used to monitor the fluorescence at 485/535 nm wavelength. Analysis of the derived data using Activity Base, a curve fitting software from IDBS, gave the pIC_{50} and pK_i values from which K_i values were derived.

Prothrombin Time (PT) Assay.[40] Blood from two female and two male donors, supplied by Biological Specialty Corporation (Colmar, PA), was collected into 3.8% sodium citrate vacutainers and mixed gently. Plasma was generated by centrifugation of the citrated blood samples at 1200 *g* for 20 min at room temperature and stored at −20 °C until use. Prior to assay, plasma from four different human subjects (two male and two female) was thawed and pooled.

PT assays were performed at 37 °C using the Dade Behring BCS Coagulation System (n = 2 trials). An amount equal to 50 μL of plasma containing test compound at concentrations ranging from 0.03 to 100 μM (made from a 100 μM plasma stock containing 1% DMSO and prepared by the coagulation analyzer) was combined with 100 μL of thromboplastin C Plus (Dade Behring, Newark, DE). Absorbance at 405 nm was then monitored and time (sec) to clot formation at each concentration of test compound was determined and compared to time to clot formation of control (human plasma in the absence of test compound) and reported as the fold-increase in PT.

The concentration of test compound, which yield 1.5-fold extension in PT in the human plasma, was estimated using a mathematical model.[41] The extrapolated 1.5× concentration was validated by spiking the plasma with this exact concentration of test compound, and the resulting PT was compared to that of control plasma. The 1.5× concentration was considered accurate if they caused the plasma to clot 1.5-fold slower than control plasma.

Pharmacokinetic Studies. The formulation used for both i.v. and p.o. dosing to rat and dog was a 5:95% (v/v) mixture of DMSO and 50:50 PEG[a]-200/sterile water. For all animal studies, serial blood samples were collected into heparinised containers at various time-points and blood centrifuged to yield plasma.

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Supporting Information Available: Electrostatic potential maps of selected *N*-methyl *P*1 sulfonamide and additional experi-

mental data are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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