Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 14 (2006) 4379–4392
Bioorganic & Medicinal Chemistry

The discovery of glycine and related amino acid-based factor Xa inhibitors

Jeffrey T. Kohrt,[a,*] Kevin J. Filipski,[a] Wayne L. Cody,[a] Christopher F. Bigge,[a] Frances La,[a] Kathleen Welch,[a] Tawny Dahring,[a] John W. Bryant,[a] Daniele Leonard,[a] Gary Bolton,[a] Lakshmi Narasimhan,[a] Erli Zhang,[a] J. Thomas Peterson,[a] Staci Haarer,[a] Vaishali Sahasrabudhe,[a] Nancy Janiczek,[a] Shrilakshmi Desiraju,[b] Mostofa Hena,[b] Charles Fiakpui,[b] Neerja Saraswat,[b] Raman Sharma,[b] Shaoyi Sun,[b] Samarendra N. Maiti,[b] Robert Leadley[a] and Jeremy J. Edmunds[a]

[a]*Pfizer Global Research and Development, Michigan Labs, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*
[b]*Naeja Pharmaceutical Inc., #2 Taiho Alberta Center, 4290-91A Street, Edmonton, Alberta, Canada T6E 5V2*

Received 21 January 2006; revised 17 February 2006; accepted 21 February 2006
Available online 10 March 2006

Abstract—Herein, we report on the identification of three potent glycine and related amino acid-based series of FXa inhibitors containing a neutral P1 chlorophenyl pharmacophore. A X-ray crystal structure has shown that constrained glycine derivatives with optimized N-substitution can greatly increase hydrophobic interactions in the FXa active site. Also, the substitution of a pyridone ring for a phenylsulfone ring in the P4 sidechain resulted in an inhibitor with enhanced oral bioavailability.

1. Introduction

It has been well established that there is a need for the replacement of the long-standing antithrombotic warfarin for the treatment and prophylaxis of primary and secondary venous and arterial thromboembolisms.[1] While serving as an effective therapy for the prevention of blood clot formation, warfarin's nonspecific activity, delay in time to reach efficacy, narrow therapeutic window, and the necessity for constant monitoring leave considerable room for the discovery of new oral antithrombotics.[1]

Orally bioavailable factor Xa (FXa) inhibitors are currently being evaluated preclinically and clinically because they have the potential to address all the limitations noted above. This key serine protease is at the central junction of both the intrinsic and extrinsic coagulation pathways involved in the formation of blood clots, and the inhibition of FXa has been clinically demonstrated to be a therapeutically safe and effective target for the reduction of the formation of blood clots.[1,2]

The inhibition of FXa by small molecules has been aggressively pursued by the pharmaceutical industry.[1a,3] Initial successes have led to the clinical development of low molecular weight heparins[1b] and fondaparinux[4a,b] both of which are indirect inhibitors of FXa. In addition, a direct FXa inhibitor, DX-9065a, has also been advanced to clinical trials.[4c] While these drugs demonstrated an improvement in antithrombotic development, their subcutaneous route of administration may limit their use in chronic thrombosis disease management.[4b] Thus, a continued search for new orally active FXa inhibitors persists.

Early efforts to develop oral FXa inhibitors were hindered by the need to synthesize molecules that contained highly charged, strongly basic pharmacophores like phenylguanidine and phenylamidine moieties.[5] These basic pharmacophores anchored the inhibitor in the key S1 subsite of FXa through hydrogen bonding with the carboxylic acid of Asp189.[1a,6] While these highly basic compounds were often very potent inhibitors of FXa,

Keywords: Factor Xa inhibitor; Antithrombotics.
* Corresponding author. Tel.: +1 734 622 1371; fax: +1 734 622 3909; e-mail: jeffrey.kohrt@pfizer.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.02.040

they usually possessed undesirable pharmacokinetic properties such as poor oral absorption and short duration of action.[7]

In order to improve the pharmacokinetic properties of these highly basic FXa inhibitors, efforts have focused on the discovery of compounds with less-basic P1 phenylamidine mimetics such as an aminobenzisoxazole exemplified in DuPont's Razaxaban[8f] or an indole used in Lilly's LY-517717.[8g] The other alternative to improve pharmacological properties is the use of neutral P1 sidechains.[8–11]

A key step in the development of neutral P1 groups was a patent in 1996 by Zeneca disclosing piperazine naphthylsulfonamide compounds containing chloronaphthalene and piperidinylpyridine sidechains.[8a] While not specifically stated in the patent, the chloronaphthene sidechain was assumed in reviews to be binding in the S4 subsite.[6b,8b] Subsequently it was reported that molecular modeling studies on this and other related FXa inhibitors suggested a preferred 'reversed' binding mode in which the chloronaphthylene and other chloroheterocylic sidechains were occupying the S1 pocket with the basic sidechains binding in the S4 pocket.[8c] Both DuPont and Lilly added to this body of knowledge with disclosures of the replacement of a P1 3-amidinophenyl group with 4-methoxyphenyl and other neutral P1 groups in FXa inhibitors.[8d,e] Crystal structures of these inhibitors were not published at this time confirming this stated binding mode with the enzyme.

Recently, a crystal structure from the Aventis group showed conclusively that a phenylamidine FXa inhibitor did not necessarily need this basic group for binding in the S1 pocket of the enzyme.[9] In a series of piperazinone-based inhibitors, the phenylamidine portion of the molecules was shown to bind in the S4 pocket with a neutral chlorobenzothiophene group occupying the S1 pocket.[9a] A subsequent report disclosed an example of a crystal structure of FXa complexed with a piperazinone inhibitor containing a neutral chlorothiophene group bound in the S1 pocket and a weakly basic azaindole group filling the S4 pocket.[9b] These results opened the door to the identification and design of new templates utilizing neutral P1 sidechains as phenylamidine replacements in the discovery of FXa inhibitors. The results of these efforts have been highlighted by recent reports of the use of the orally active FXa inhibitor Bay-59-7939 with a chlorothiophene S1 sidechain in clinical trials.[10]

As part of a structure-based drug design program to discover orally active FXa inhibitors, we investigated a proline-based series of inhibitors that contained multiple chiral stereocenters.[11d] Syntheses of these chiral amino acid templates limited rapid analog development and resulted in inhibitors of high molecular weight. These issues led us to investigate lower molecular weight non-chiral amino acids as the core for new inhibitors (Fig. 1). Herein, we report on the discovery of three series of glycine-based FXa inhibitors, in which we have incorporated a neutral chlorophenyl P1 sidechain.

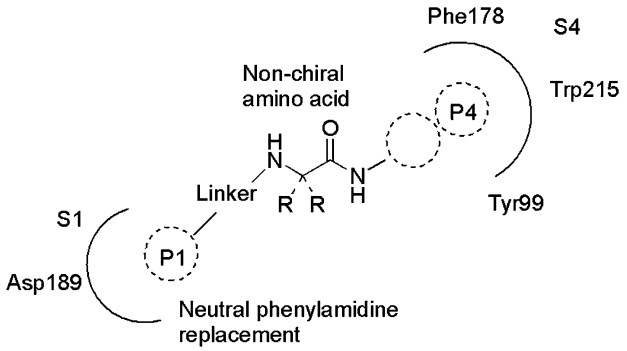


Figure 1. Inhibitor rational for the discovery of non-chiral FXa inhibitors.

2. Chemistry

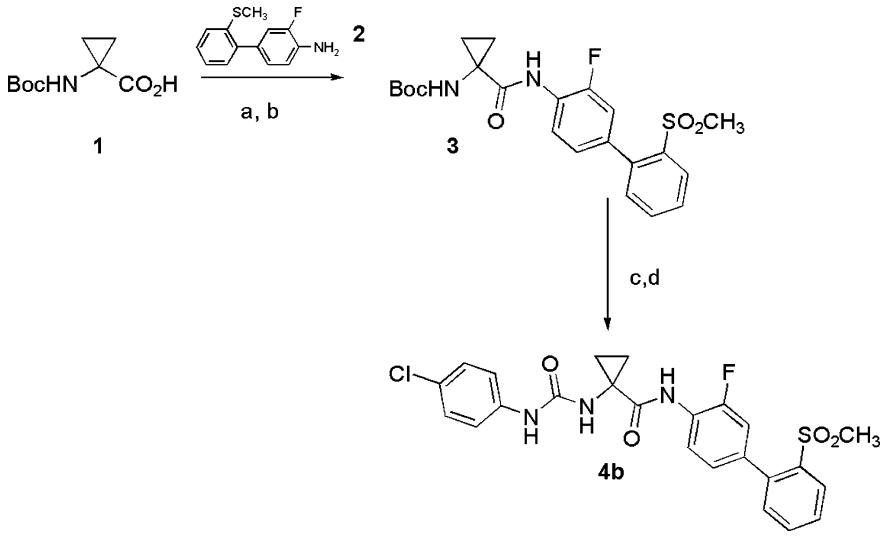
The synthesis of glycine-based inhibitors related to *N*-alkylglycine, cycloalkylglycine, and *N*-alkylcycloalkylglycine is exemplified by the three examples below (Schemes 1–3). The synthesis of the cyclopropylglycine analog **4b** was initiated from *N*-Boc-cyclopropylglycine carboxylic acid **1**, 3-fluoro-2′-methylsulfanyl-biphenyl-4-ylamine **2**, and 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ)[12] to prepare an amide intermediate. The biphenylmethylsulfide group was then oxidized with *meta*-chloroperbenzoic acid (*m*-CPBA) to provide the methylsulfone **3**. Deprotection of the Boc-group with trifluoroacetic acid in dichloromethane and then the addition of 4-chlorophenylisocyanate in the presence of triethylamine afforded final compound **4b**.

Synthesis of the *N*-(methoxyethyl)glycine analog **8i** proceeded from bromoacetyl bromide that was allowed to react with aniline **5** to form amide **6**. The bromo group was then displaced by methoxyethylamine to form **7**. The urea derivative was made via the addition of 4-chlorophenylisocyanate as before to produce the derivative **8i**.

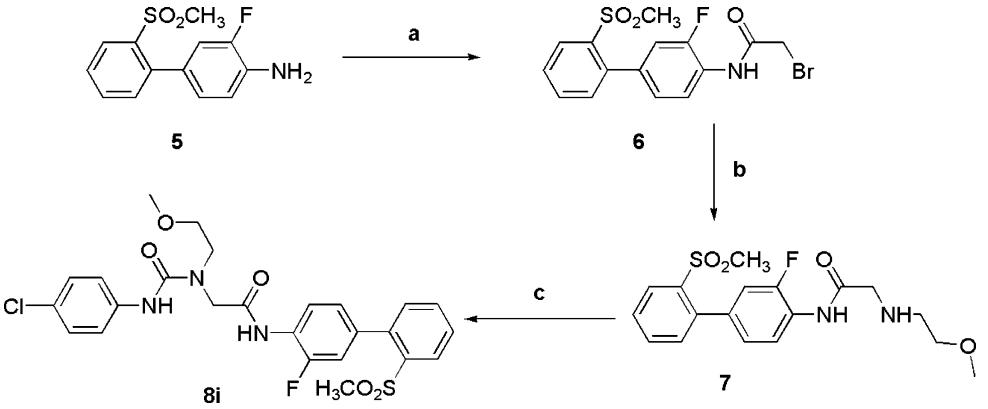
Finally, the synthesis of *N*-(cyclopropylmethyl)cyclopropylglycine analog **11d** started from the aminocyclopropylglycine amide **9**. Reductive amination utilizing cyclopropylaldehyde, titanium isopropoxide, and sodium cyanoborohydride provided the substituted amine **10**.[13] Addition of the 4-chlorophenylisocyanate as before produced the urea derivative **11d**.

3. Results and discussion

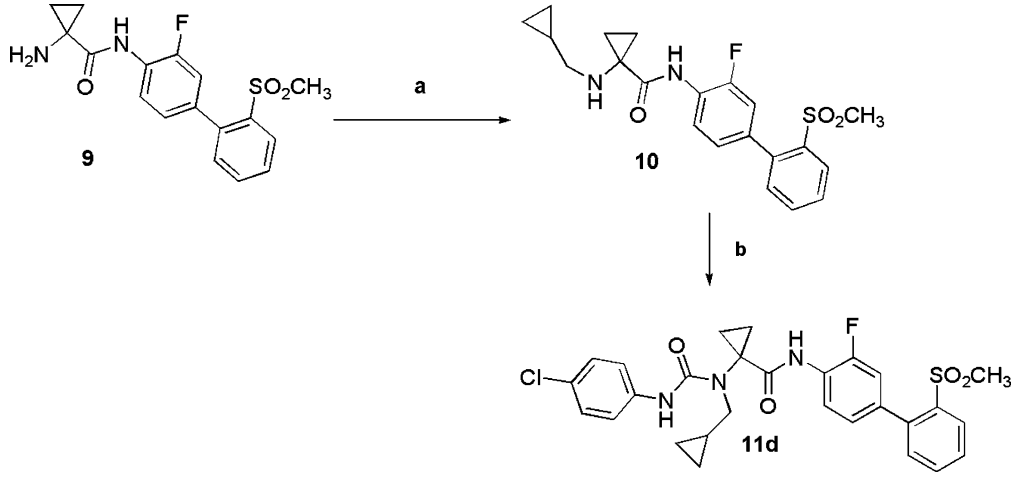
The design hypothesis for our initial series of FXa inhibitors focused on the use of non-chiral, glycine-based templates such as the α-disubstituted 1-amino-1-cyclopropanecarboxylic acid in combination with neutral P1 and P4 pharmacophores. The S4 binding pocket of FXa contains three aromatic amino acid sidechains (Phe178, Trp215, and Tyr99) that have been shown to form favorable interactions with either neutral or positively charged aromatic groups.[6,9] An amino-biphenylsulfonamide was chosen as the initial P4 sidechain due to reports of its use in other FXa inhibitors.[6]



Scheme 1. Synthesis of cycloalkylglycine analog **4b**. Reagents and conditions: (a) **2**, EEDQ, triethylamine, $CHCl_3$, reflux, 20 h; (b) *m*-CPBA, ethyl acetate, rt, 2.5 h; (c) trifluoroacetic acid, CH_2Cl_2, rt, 1.5 h; (d) 4-chlorophenylisocyanate, Et_3N, CH_2Cl_2, rt, 2 h.



Scheme 2. Synthesis of *N*-alkylglycine analog **8i**. Reagents and conditions: (a) bromoacetylbromide, CH_2Cl_2, rt, 15 h; (b) methoxyethylamine, $(i\text{-Pr})_2EtNH$, CH_2Cl_2, rt, 2 h; (c) 4-chloro-phenylisocyanate, Et_3N, CH_2Cl_2, rt, 2 h.



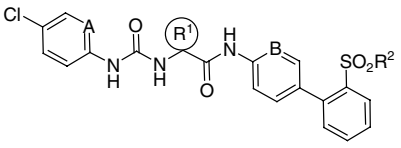
Scheme 3. Synthesis of *N*-alkylcycloalkylglycine analog **11d**. Reagents and conditions: (a) cyclopropanecarbaldehyde, $Ti(Oi\text{-Pr})_4$, CH_2Cl_2, $NaBH_3CN$, methanol, rt, 20 h; (b) 4-chlorophenylisocyanate, Et_3N, CH_2Cl_2, rt, 2 h.

The 4-chloroaniline group was incorporated as a neutral P1 sidechain. From molecular modeling studies on a related series of FXa inhibitors, the 4-chloroaniline substituent tethered to the amino acid core via a urea linkage was suggested as an optimal P1 binding element for the active site of FXa.[11d]

Gratifyingly, from the combination of the above pharmacophores, the first cyclopropylglycine inhibitor **4a** (IC_{50} = 0.033 μM) provided a good lead to investigate structure–activity relationship studies for this series of inhibitors (Table 1). In an examination of the P4 sidechain, addition of a fluoro group to the aniline ring of **4a** provided the more potent inhibitor **4b** (IC_{50} = 0.022 μM). Replacement of the phenylmethylsulfone of **4b** with a phenylsulfonamide resulted in the preparation of **4c** (IC_{50} = 0.036 μM) displaying potency similar to that of the initial lead **4a**. As the chlorophenyl sidechain was considered an optimal for this template, only its replacement with 4-chloroaminopyridine[14] was examined. This weakly basic sidechain could potentially alter the physicochemical and pharmacological properties of this series. Unfortunately, this pyridine replacement led to inhibitor **4d** (IC_{50} = 0.255 μM) with greatly diminished potency from **4c**.

The replacement of the cyclopropyl ring with larger rings into the glycine core led to changes in enzyme inhibition (Table 1). For example, the cyclohexyl analog **4e** (IC_{50} = 0.016 μM) was the most potent inhibitor in this series. Incorporation of a sulfur or oxygen into the cyclohexyl ring for analogs **4f** and **4g**, respectively, led to 4- and 15-fold decreases in potency with respect to analog **4e**. Replacing the cyclohexyl ring for either the cyclopentyl or cyclopentenyl rings in derivatives **4h** or **4i**, respectively, also led to inhibitors with decreased potency.

Table 1. Cycloalkylglycine and dialkylglycine binding data

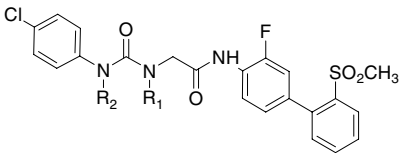


Compound	R^1	R^2	A	B	IC_{50} (μM)	±SEM
4a		CH_3	CH	CH	0.033	0.007
4b		CH_3	CH	CF	0.022	0.001
4c		NH_2	CH	CF	0.036	0.002
4d		CH_3	N	CF	0.26	0.021
4e		CH_3	CH	CH	0.016	0.001
4f		NH_2	CH	CF	0.065	0.004
4g		NH_2	CH	CF	0.25	0.022
4h		CH_3	CH	CH	0.038	0.005
4i		NH_2	CH	CF	0.066	0.004
4j	$(CH_3)_2$	CH_3	CH	CF	0.02	0.003
4k	$(CH_3)_2$	NH_2	CH	CF	0.044	0.001
4l	$(CH_2OH)_2$	NH_2	CH	CH	0.029	0.002

Replacement of the cyclic glycine core with a dialkyl amino acid such as 2-amino-2-methyl-propionic acid provided inhibitors **4j** and **4k** with activities similar to those of the corresponding cyclic compounds **4b** and **4c**, respectively (Table 1). In addition, 2-amino-3-hydroxy-2-hydroxymethyl-propionic acid was used to synthesize inhibitor **4l** that was shown to possess less than a 2-fold change in potency (IC_{50} = 0.029 μM) from compound **4j**.

Interestingly, incorporating glycine as the core amino acid resulted in analog **8a** (IC_{50} = 0.053 μM) with only a 2-fold decrease in potency with respect to the related cyclopropyl analog **4c** (Table 2). This small loss of activity encouraged us to investigate whether glycine N-substitution could be used to increase the potency of this glycine series. Molecular modeling suggested that N-substitution could provide inhibitors with increased binding by optimizing interactions in a disulfide containing, hydrophobic pocket in the FXa active site (Fig. 2). Previous reports have shown that chiral amino acids such as phenylglycine can access this binding pocket.[15] Following this idea, a more potent analog was realized with respect to **8a** with the incorporation of sarcosine

Table 2. *N*-Alkylglycine inhibitor binding data



Compound	R^1	R^2	IC_{50} (μM)	±SEM
8a	H	H	0.053	0.0030
8b	CH_3	H	0.020	0.0020
8c	H	CH_3	>1.0	
8d	Et	H	0.021	0.0020
8e	Pr	H	0.010	0.002[a]
8f	Bu	H	0.005	0.0001
8g	*i*-Bu	H	0.110	0.0050
8h	CH_2-*t*-Bu	H	0.380	0.0350
8i	$CH_2CH_2OCH_3$	H	0.027	0.0200
8j	$CH_2CH_2N(CH_3)_2$	H	>1.0	
8k	CH_2–*c*-Pr	H	0.004	0.00001
8l	*c*-Pr	H	0.490	0.0370
8m	CH_2–*c*-Bu	H	0.013	0.0010
8n	CH_2–*c*-Pent	H	0.017	0.0005
8o	CH_2–*c*-Hx	H	0.120	0.0050
8p	CH_2-3-THP	H	0.140	0.0280
8q	CH_2-2-furyl	H	0.003	0.0005
8r	CH_2-2-thienyl	H	0.008	0.0004
8s	CH_2-2-oxazolyl	H	0.014	0.0010
8t	CH_2-3-thienyl	H	0.027	0.0030
8u	CH_2Ph	H	0.030	0.0010
8v	3-CH_3O–$PhCH_2$	H	0.021	0.0010
8w	3-F–$PhCH_2$	H	0.023	0.0010
8x	3-CH_3PhCH_2	H	0.026	0.0030
8y	2-CH_3O–$PhCH_2$	H	0.040	0.003[a]
8z	2-Cl–$PhCH_2$	H	0.064	0.0030
8aa	4-CH_3O–$PhCH_2$	H	0.080	0.0070
8bb	4-Cl–$PhCH_2$	H	0.120	0.0040

c-Pr, cyclopropyl; *c*-Bu, cyclobutyl; *c*-Pent, cyclopentyl; *c*-Hx, cyclohexyl; THP, tetrahydropyranyl.

[a] Standard deviation for $n = 2$.

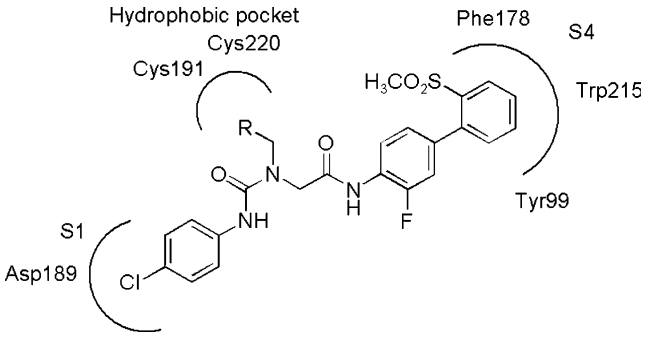


Figure 2. Proposed *N*-alkyl interactions in the hydrophobic pocket.

in analog **8b** (IC_{50} = 0.020 μM). Not unexpectedly, substitution of a *N*-methyl on the aniline portion of the urea of inhibitor **8c** (IC_{50} > 1 μM) greatly diminished potency as a result of the loss of a critical hydrogen bond interaction with the protein. Based on these results, further studies were undertaken to increase the potency of the N-substituted glycine derivatives.

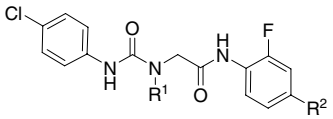
Thus, the *N*-methyl was replaced by larger alkyl groups in the inhibitors **8d**–**8f**. It was shown that the *N*-butyl analog **8f** (IC_{50} = 0.005 μM) was the most potent *N*-alkyl inhibitor. Increasing the alkyl branching or incorporation of a heteroatom into the sidechain resulted in analogs **8g**–**8j** with significantly reduced potency. Incorporation of *N*-(cycloalkyl)methyl sidechains led to the preparation of analog **8k** (IC_{50} = 0.004 μM) as the most potent cycloalkyl derivative. Inhibitors with larger cycloalkyl rings such as cyclobutyl, cyclopentyl, and cyclohexyl analogs **8m**–**8o** showed a decrease in potency from compound **8k**.

A series of *N*-benzylic and *N*-heteroaromatic methylglycine inhibitors were then prepared as replacements for the cyclopropylmethyl group of analog **8k**. The most potent heterocyclic derivative was the 2-furyl analog **8q** (IC_{50} = 0.003 μM). The 2-thienyl analog **8r** and 2-oxazolyl analog **8s** were less potent than **8q** by approximately 3- and 5-fold, respectively. In addition, the 3-thienyl analog **8t** was the least potent heteroaromatic derivative.

In general, the *N*-heteroaromatic methyl analogs were more potent than the *N*-benzylic analogs. The parent *N*-benzyl **8u** (IC_{50} = 0.030 μM) was as potent as the least potent heteroaromatic derivative **8t**. No significant increase in potency was observed with the preparation of the 3-substituted phenyl analogs **8v**–**8x**. Substitution at the 2-position of the phenyl ring of analogs **8y** and **8z** reduced potency with respect to **8u**, while substitution at the 4-position of the phenyl ring led to the weakest binding benzylic analogs **8aa** and **8bb**.

In order to reduce the overall molecular weight of this series and potentially increase their absorption and oral bioavailability,[16] various heterocycles and amides[17] were used as replacements for the P4 phenylsulfone sidechain. From molecular modeling studies, it was hypothesized that *N*-aryl-substituted piperidones and pyridones[18] would make good phenylsulfone replacements (Table 3). The piperidinone analog **12** (IC_{50} = 0.225 μM) reduced potency from its parent **4i** by nearly 10-fold, but interestingly the pyridone analog **13** (IC_{50} = 0.005 μM) was essentially equipotent to the P4 phenylsulfone **8k**. It is known that sidechains that are better able to π-π stack with the aromatic amino acids in the S4 pocket of the enzyme can lead to more potent inhibitors.[6,9] Due to these interactions, the large increase in potency of the pyridone ring is speculated to be due to the increased aromatic nature of this sidechain over that of the non-aromatic piperidone ring.

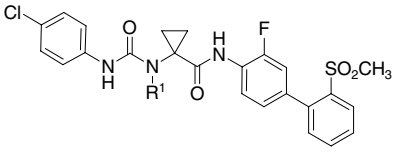
Table 3. *N*-Alkylglycine binding data



Compound	R^1	R^2	FXa IC_{50} (μM)	SEM
12	$CH_2CH_2OCH_3$		0.225	0.01
13	CH_2–*c*-Pr		0.005	0.001

While modest increases in potency were obtained by N-substitution of the glycine core, it was realized that many of the inhibitors while possessing different N-substitutions had very similar potencies. It was hypothesized that interactions in the disulfide containing hydrophobic pocket may not be responsible for all of the observed changes in potency. Modeling suggested that rotation of the inhibitor's glycine core could move the *N*-substituent out of the hydrophobic pocket and into the solvent, thus providing for only a small range of activities for differently N-substituted analogs. This may help explain why *N*-benzylic derivatives such as **8v** can have the same potency as *N*-methyl analog **8b**. In order to reduce the potential for rotation and possibly orient the N-substituent into the disulfide pocket, a new series of inhibitors with a constrained core based on cyclopropylglycine[19] was prepared (Table 4).

Table 4. *N*-Alkylcyclopropylglycine binding data



Compound	R^1	FXa IC_{50} (μM)	SEM
11a	Bu	0.0008	0.0001
11b	CH_3	0.017	0.001
11c	Pr	0.039	0.005
11d	CH_2–*c*-Pr	0.068	0.002
11e	CH_2–*c*-Hex	>1	
11f	CH_2–*c*-Pent	>1	

In most cases, the use of this constrained amino acid resulted in inhibitors **11b**–**11f** that were equal to or less potent than the corresponding glycine analogs from Table 2. This proposed constraint of rotation[19] appeared to lead to N-substituted inhibitors with reduced or different interactions within the disulfide hydrophobic pocket and/or increased interactions with solvent. One exception to this observation was the almost 7-fold increase in potency observed for analog **11a** (IC_{50} = 0.8 nM) as compared to its glycine homolog **8f**. This was the first glycine-based inhibitor that displayed subnanomolar potency.

Since analog **11a** was our most potent inhibitor, an X-ray crystal structure was obtained of it bound in the FXa active site (Fig. 3). As expected the 4-chlorophenyl sidechain was positioned in the S1 pocket of the enzyme, while the phenylsulfone sidechain was bound into the aromatic S4 pocket. Strong hydrogen bonds were observed between both the carbonyl of Gly219 and the 4-chlorophenylurea NH (3.3 Å) and between the carbonyl of Gly216 and the cyclopropylglycine amide NH (3.2 Å). Also, the carbonyl of the cyclopropylglycine formed a hydrogen bond with a water molecule (2.8 Å) that interacts with the sidechains of Ser195 (3.2 Å), His57 (3.1 Å) and the carbonyl of Ser214 (2.7 Å). The constrained amino acid core did help to position the *N*-butyl sidechain in the hydrophobic pocket and not allow rotation into solvent. These non-bonding interactions in the hydrophobic disulfide pocket and a reduction in entropy due to a rigidified template may account for the increased potency of compound **11a** over the *N*-butylglycine analog **8f**.

3.1. Anticoagulant activity, selectivity, and pharmacokinetics

Twelve compounds from the three series of inhibitors were further evaluated for selectivity against serine proteases related to FXa. All compounds tested were highly selective for FXa. In studies utilizing human trypsin, none of the compounds had affinity less than 10 μM. While, in a human thrombin (FIIa) assay, only two compounds **4l** (IC_{50} = 5.7 μM) and **13** (IC_{50} = 7.7 μM) had potency better than 10 μM (Table 5).

Next, the ex vivo anticoagulant activity of key compounds was evaluated via prolongation of fibrin clot formation in human plasma as measured by the doubling of prothrombin time (2× PT). Evaluation of the inhibitors in the 2× PT assay was disappointing. In most cases, the concentrations necessary to achieve the doubling time for fibrin formation (Table 5) were greater than 2 μM, including compound **11a**, the most potent in vitro

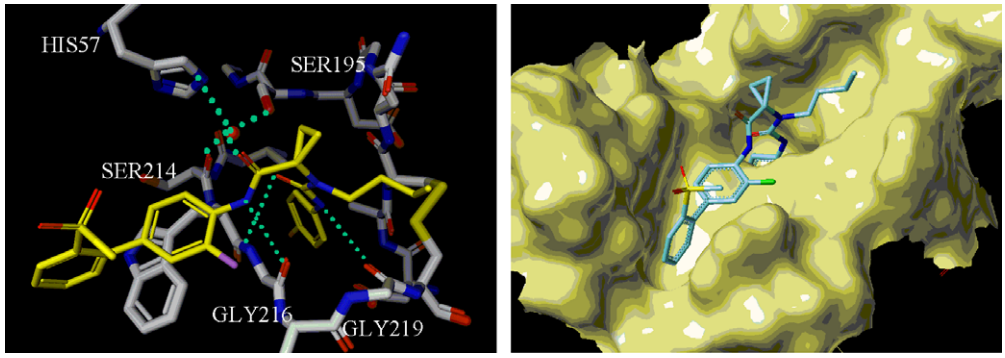


Figure 3. X-ray crystal structure of **11a** in the active site of FXa.

Table 5. Selectivity, activity, and pharmacokinetic data

Compound	IC_{50} (μM)		2× PT (μM)	FXa % inhibition[a]	C_{max}[b] (nM)	T_{max}[c] (h)	Auc[d]	App $T_{1/2}$[e] (h)
	Trypsin	FIIa IC_{50} (μM)						
4b	>10	>10	14.0	1.0	43.0	1	—	—
4e	>10	>10	ND	0.0	0.0	—	—	—
4j	>10	>10	13.0	0.0	130.0	4	—	—
4l	>10	5.7	4.5	2.0	—	—	—	—
8f	>10	>10	7.0	10.0	—	—	—	—
8k	>10	>10	7.6	20.0	250.0	0.5	590	1.5
8q	>10	>10	9.1	30.0	230.0	0.5	670	1.6
11a	>10	>10	28.0	0.0	13.0	4	—	—
13	**>10**	**7.7**	**1.7**	**90.0**	**440.0**	**0.5**	**13,000**	**1.6**

[a] FXa % inhibition, maximum percent inhibition of FXa over 4 or 6 h study.
[b] C_{max}, maximum plasma concentration of inhibitor.
[c] T_{max}, time measured for maximum concentration of inhibitor in plasma.
[d] Auc, area under the curve—plasma exposure of inhibitor nM/h.
[e] App $T_{1/2}$: apparent half-life; ND, not determined.

binding analog. The only compound that demonstrated a lower 2× PT concentration was the pyridone inhibitor **13**.

Select FXa inhibitors were tested in a conscious rat pharmacokinetic-pharmacodynamic (PK–PD) model for an initial evaluation of anti-FXa activity, plasma exposure, and apparent half-life. Test compounds were administered intragastrically by oral gavage at a dose of 5 mg/kg. Plasma samples were taken at set intervals up to 4 or 6 h post-dose. FXa inhibition and plasma concentrations were determined by using an ex vivo FXa chromogenic assay.[20] Pharmacokinetic parameters of plasma exposure and apparent half-life were determined from a plot of plasma concentrations versus time. Only one class of compounds, the N-substituted glycine inhibitors, displayed anti-FXa activity in this assay. Compounds **8k** and **8q** were the first compounds to display modest anti-FXa potency, albeit with low plasma exposures (Table 5). It was not until the phenylsulfone group of **8f** was replaced with the pyridone group of **13** that an inhibitor displayed good anti-FXa activity and high plasma exposure indicating increase in absorption and oral bioavailability. In all cases, the absorbed analogs displayed a short apparent half-life.

Differences in physicochemical properties may play a role in the substantially increased absorption of compound **13** over the related analog **8f**. In direct comparison to phenylsulfone analog **8f**, the pyridone compound **13** showed decreases in log *P* (3.8 and 2.8, respectively), polar surface area (91 and 78, respectively), number of rotatable bonds (11 and 10, respectively), and molecular weight (530 and 469 g/mol, respectively). Each of these properties has been shown to be key factors that influence absorption and oral bioavailability.[16,21]

4. Conclusions

In conclusion, we have identified three potent glycine-based series of FXa inhibitors containing a neutral P1 sidechain. An X-ray crystal structure has shown that rigid glycine derivatives with optimized N-substitution can greatly increase inhibitor potency. Also, the substitution of a P4 pyridone ring for a phenylsulfone has led to an inhibitor with enhanced oral bioavailability. Continued optimization of the physicochemical and pharmacological properties of these inhibitors may provide improved oral FXa anticoagulants.

5. Experimental

5.1. General

All chemicals, reagents, and solvents were purchased from commercial sources (e.g., Aldrich Chemical Co., Inc., Milwaukee, WI; Mallinckrodt Baker, Inc., Paris, KY, etc.) where available and used without further purification. Compound **4l** was prepared as outlined in Ref. 22. All intermediates were characterized by proton nuclear magnetic spectroscopy (^{1}H NMR) and mass spectrometry (MS) using atmospheric pressure chemical ionization (APCI) or electron scatter (ES) ionization sources. All final compounds were determined to be consistent with the proposed structure by ^{1}H NMR, MS. All final compounds also were greater than 95% pure as determined by analytical RP-HPLC on a Vydac218TP54 C18 column utilizing a gradient of 0.1% trifluoroacetic acid (TFA) in acetonitrile to 0.1% TFA in water (22–80% over 22 min—method A) or by a Altech Altima C18 column replacing TFA with 0.1% formic acid in both water and acetonitrile (22–80% over 10 min—method B) and were within theoretical limits for elemental analyses (C, H, N) or high resolution mass spectrometry (HRMS).

5.1.1. 1-[3-(4-Chloro-phenyl)-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (4b). *Step 1.* 1-*tert*-Butoxycarbonylamino-cyclopropanecarboxylic acid **1** (1.00 g, 4.97 mmol), 3-fluoro-2′-methylsulfanyl-biphenyl-4-ylamine **2** (1.15 g, 4.97 mmol), and 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) (1.47 g, 5.96 mmol) were dissolved in dry chloroform (20 mL). Triethylamine (1.04 mL, 7.45 mmol) was added and the solution was heated at reflux for 20 h. The reaction was allowed to cool, and an excess of ethyl acetate was added. The solution was washed sequentially with 10% aqueous citric acid, 1 N NaOH, water, and then brine before drying the solution over $MgSO_4$. Concentration of the solution under reduced pressure and purification of the crude product by flash chromatography revealed amide as a white solid (2.10 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.00 (m, 1H), 7.91 (s, 1H), 7.74 (m, 1H), 7.38–7.12 (m, 5H), 2.35 (s, 3H), 1.39 (s, 9H), 1.34 (m, 2H), 1.00 (m, 2H); MS APCI (AP+): 417.1 $(M+1)^+$.

Step 2. To a mixture of the amide (1.80 g, 4.32 mmol) in ethyl acetate (43 mL) was added *m*-CPBA (70%, 4.26 g, 17.3 mmol). The solution was stirred for 2.5 h at room temperature before diluting with excess ethyl acetate. The solution was washed sequentially with saturated aqueous $Na_2S_2O_3$, two portions of saturated aqueous $NaHCO_3$, water and then brine before drying over $MgSO_4$. Filtration followed by concentration of the solution under reduced pressure and purification of the crude product by flash chromatography revealed **3** as a white solid (1.72 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38, 9.10 (2s, 1H), 8.56 (s, 1H), 8.06 (m, 1H), 7.77–7.64 (m, 2H), 7.43 (m, 1), 7.38 (m, 1H), 7.22 (ddd, 1H, J = 2, 9, 9 Hz) 2.89 (s, 3H), 1.67 (m, 2H), 1.50 (s, 9H), 1.39 (m, 2H); MS APCI (AP+): 449.1 $(M+1)^+$.

Step 3. Into a solution of **3** (0.230 g, 0.515 mmol) in dry dichloromethane (5 mL) was added trifluoroacetic acid (TFA) (3 mL), and the solution was stirred at room temperature for 1.5 h. The solution was then concentrated under reduced pressure and dried under vacuum. The crude product was dissolved in dry THF (10 mL) and cooled to 0 °C in an ice bath. Triethylamine (0.359 mL, 2.58 mmol) was then added followed by 4-chlorophenyl-isocyanate (0.079 g, 0.515 mmol). The reaction mixture was stirred at room temperature for 2 h before concentrating under reduced pressure. The

resulting crude product was purified by flash chromatography to reveal **4b** as a white solid (0.162 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.48 (s, 1H), 8.78 (s, 1H), 8.05 (dd, 1H, J = 1, 8 Hz), 7.83 (t, 1H, J = 8 Hz), 7.74 (dt, 1H, J = 1, 8 Hz), 7.65 (dt, 1H, J = 1, 8 Hz), 7.45–7.38 (m, 3H), 7.31–7.24 (m, 3H), 7.17 (dd, 1H, J = 1, 8 Hz), 7.07 (s, 1H), 2.84 (s, 3H), 1.41 (m, 2H), 1.04 (m, 2H); MS APCI (AP+) 502.1 $(M+1)^+$; CHN calcd for $C_{24}H_{21}ClFN_3O_4S$: C, 57.36; H, 4.22; N, 8.36. Found: C, 56.97; H, 4.07; N, 8.05.

5.1.2. 2-[3-(4-Chloro-phenyl)-1-(2-methoxy-ethyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8i). *Step 1.* To a solution of **5** (5.0 g, 18.9 mmol) in dichloromethane (200 mL) at room temperature was added bromoacetyl bromide (4.20 g, 20.8 mmol) dropwise, and the mixture was stirred overnight. It was then diluted with ethyl acetate (200 mL), washed with 5% aqueous HCl (30 mL) and then brine. The organics were concentrated to obtain **6** (7.1 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.30 (br s, 1H), 8.10 (dd, 1H, J = 1, 8 Hz), 8.03 (t, 1H, J = 8 Hz), 7.80–7.65 (m, 2H), 7.44 (dd, 1H, J = 1, 8 Hz), 7.37 (dd, 1H, J = 2, 13 Hz), 7.25 (dd, 1H, J = 2, 13 Hz), 4.20 (s, 2H), 2.95 (s, 3H).

Step 2. To a mixture of **6** (1.0 g, 2.6 mmol) and diisopropylethylamine (0.50 g, 3.9 mmol) in dichloromethane (20 mL) was added 2-methoxy-ethylamine (0.39 g, 5.2 mmol) at room temperature. The mixture was stirred for 2 h, concentrated under reduced pressure, and purified by flash chromatography to obtain **7** (0.70 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.85 (br s, 1H), 8.55 (t, 1H, J = 8 Hz), 8.20 (dd, 1H, J = 1, 8 Hz), 7.70–7.50 (m, 2H), 7.40–7.30 (m, 2H), 7.20 (d, 1H, J = 8 Hz), 3.55 (t, 2H, J = 5 Hz), 3.45 (s, 2H), 3.40 (s, 3H), 2.90 (t, 2H, J = 5 Hz), 2.70 (s, 3H), 1.60 (br s, 1H).

Step 3. To a solution of **7** (0.60 g, 1.57 mmol) and triethylamine (0.31 g, 3.14 mmol) in dichloromethane (10 mL) was added 4-chlorophenylisocyanate (0.31 g, 1.88 mmol) at room temperature. The mixture was stirred for 2 h, quenched with an excess of water, and extracted with ethyl acetate (3× 20 mL). The combined organic extracts were washed with 5% aqueous HCl (4 mL), brine, dried over $MgSO_4$, evaporated, and purified by column chromatography to obtain **8i** as a white solid (0.47 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 8.60 (s, 1H), 8.40 (t, 1H, J = 8 Hz), 8.20 (dd, 1H, J = 1, 8 Hz), 7.70–7.65 (m, 1H), 7.60–7.50 (m, 1H), 7.35–7.28 (m, 6H), 7.20 (d, 1H, J = 8 Hz), 4.15 (s, 2H), 3.75–3.60 (m, 4H), 3.55 (s, 3H), 2.65 (s, 3H); MS (ES): 535.41 $(M+1)^+$; CHN calcd for $C_{25}H_{25}ClFN_3O_5S$: C, 56.23; H, 4.72; N, 7.87. Found: C, 56.33; H, 4.75; N, 7.70.

5.1.3. 1-[3-(4-Chloro-phenyl)-1-cyclopropylmethyl-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11d). *Step 1.* To a solution of **9** (0.40 g, 1.15 mmol) in dichloromethane were added cyclopropanecarbaldehyde (0.08 g, 1.15 mmol) and titanium isopropoxide (0.41 g, 1.44 mmol) successively at room temperature. The mixture was stirred for 1 h, diluted with methanol (2 mL), and treated with sodium cyanoborohydride (0.050 g, 0.81 mmol). The resulting mixture was stirred for 20 h, quenched with water (2 mL), and ethyl acetate (100 mL) was added. The precipitated solid was filtered through Celite, the filtrate was dried over $MgSO_4$, evaporated, and purified by flash chromatography (ethyl acetate/hexane, 15:85) to obtain **10** as a white solid (0.21 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.40 (br s, 1H), 8.55 (t, 1H, J = 8 Hz), 8.20 (t, 1H, J = 8 Hz), 7.70–7.55 (m, 2H), 7.40–7.35 (m, 2H), 7.15 (d, 1H), 2.70 (s, 3H), 2.55–2.45 (m, 2H), 1.50–1.40 (m, 2H), 1.35 (br s, 1H), 1.30–1.20 (m, 1H), 1.00–0.95 (m, 2H), 0.55–0.45 (m, 2H), 0.20–0.10 (m, 2H).

Step 2. To a solution of **10** (0.40 g, 1.00 mmol) and triethylamine (0.33 g, 3.20 mmol) in dichloromethane (10 mL) was added 4-chlorophenylisocyanate (0.14 g, 0.90 mmol) at room temperature. The mixture was stirred for 2 h, quenched with water, and extracted with ethyl acetate (3× 20 mL). The combined extracts were washed with 5% aqueous HCl (4 mL), brine, dried over $MgSO_4$, concentrated under vacuum, and purified by column chromatography to obtain **11d** as a white solid (0.30 g, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (br s, 1H), 8.40 (t, 1H, J = 8 Hz), 8.20 (dd, 1H, J = 1, 8 Hz), 7.70–7.55 (m, 2H), 7.40–7.20 (m, 7H), 7.00 (s, 1H), 3.90 (dd, 1H, J = 5, 8 Hz), 2.90 (dd, 1H, J = 5, 8 Hz), 2.70 (s, 3H), 2.15–2.05 (m, 1H), 1.90–1.80 (m, 1H), 1.55–1.30 (m, 2H), 1.30–1.20 (m, 1H), 0.70–0.55 (m, 2H), 0.45–0.35 (m, 2H); MS (ES) 557.24 $(M+1)^+$; CHN calcd for $C_{28}H_{27}ClFN_3O_4S$: C, 60.48; H, 4.89; N, 7.56. Found: C, 60.67; H, 5.10; N, 7.38.

The following compounds were prepared using the general procedure for **4b**.

5.1.4. 1-[3-(4-Chloro-phenyl)-ureido]-cyclopropanecarboxylic acid (2′-methanesulfonyl-biphenyl-4-yl)-amide (4a). White solid 0.27 g, 80%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.700 (s, 1H), 8.7 (s, 1H), 8.06 (dd, 1H, J = 1, 8 Hz), 7.72–7.63 (m, 2H), 7.67 (d, 2H, J = 9 Hz), 7.45 (d, 2H, J = 9 Hz), 7.38 (1H, m), 7.32 (d, 1H, J = 9 Hz), 7.26 (d, 1H, J = 9 Hz), 6.86 (s, 1H), 2.76 (s, 3H), 1.44–1.43 (m, 1H), 1.02–1.01 (m, 1H); MS APCI (AP+) 484 $(M+1)^+$; CHN calcd for $C_{24}H_{22}ClN_3O_4S+0.42H_2O$: C, 58.64; H, 4.68; N, 8.55. Found: C, 58.25; H, 4.71; N, 8.34.

5.1.5. 1-[3-(4-Chloro-phenyl)-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-sulfamoyl-biphenyl-4-yl)-amide (4c). White solid 0.15 g, 18%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.41 (s, 1H), 8.78 (s, 1H), 7.99 (dd, 1H, J = 1, 8 Hz), 7.80 (t, 1H, J = 8 Hz), 7.57 (m, 2H), 7.43 (m, 2H), 7.30 (dd, 1H, J = 2, 8 Hz), 7.24 (m, 5H), 7.13 (dd, 1H, J = 2, 8 Hz), 7.08 (s, 1H), 1.41 (dd, 2H, J = 5, 8), 1.03 (dd, 2H, J = 4, 8); MS APCI (AP+) 503 $(M+1)^+$; CHN calcd for $C_{23}H_{20}ClFN_4O_4S+0.40H_2O+0.45TFA$: C, 51.13; H, 3.81; N, 9.98. Found: C, 50.75; H, 3.58; N, 9.76.

5.1.6. 1-[3-(5-Chloro-pyridin-2-yl)-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (4d). White solid 0.16 g, 8%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (s, 1H), 9.32 (s, 1H), 8.23 (dd, 1H, J = 0.5, 3 Hz), 8.13 (s, 1H), 8.05 (dd,

1H, J = 1, 8 Hz), 7.80–7.62 (m, 5H), 7.38 (dd, 1H, J = 1, 8 Hz), 7.28 (dd, 1H, J = 2, 11 Hz), 7.16 (dd, 1H, J = 2, 8 Hz), 2.84 (s, 3H), 1.43 (dd, 2H, J = 5, 8 Hz), 1.06 (dd, 2H, J = 4, 8 Hz); MS APCI (AP+) 503 $(M+1)^+$; CHN calcd for $C_{23}H_{20}ClFN_4O_4S$: C, 54.93; H, 4.01; N, 11.14. Found: C, 54.68; H, 3.80; N, 10.98.

5.1.7. 1-[3-(4-Chloro-phenyl)-ureido]-cyclohexanecarboxylic acid (2′-methanesulfonyl-biphenyl-4-yl)-amide (4e). White solid 0.14 g, 56%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (s, 1H), 8.88 (s, 1H), 8.03 (dd, 1H, J = 1, 8 Hz), 7.72–7.58 (m, 2H), 7.64 (d, 2H, J = 9 Hz), 7.37 (d, 2H, J = 9 Hz), 7.38 (1H, m), 7.27 (d, 1H, J = 9 Hz), 7.22 (d, 1H, J = 9 Hz), 6.29 (s, 1H), 2.76 (s, 3H), 2.04–2.01 (m, 2H), 1.75–1.69 (m, 2H), 1.59–1.57 (m, 2H), 1.46–1.43 (m, 2H), 1.23–1.20 (m, 1H); MS APCI (AP+) 526 $(M+1)^+$; CHN calcd for $C_{27}H_{28}ClN_3O_4S$+0.42H_2O: C, 61.65; H, 5.37; N, 7.99. Found: C, 61.53; H, 5.30; N, 7.90.

5.1.8. 4-[3-(4-Chloro-phenyl)-ureido]-tetrahydro-thiopyran-4-carboxylic acid (3-fluoro-2′-sulfamoyl-biphenyl-4-yl)-amide (4f). White solid 0.030 g, 61%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.47 (s, 1H), 8.97 (s, 1H), 8.08 (d, 1H, J = 7 Hz), 7.86 (t, 1H, J = 8 Hz), 7.70–7.62 (m, 2H), 7.48 (d, 1H, J = 9 Hz), 7.40–7.27 (m, 6H), 7.22 (d, 1H, J = 8 Hz), 6.68 (s, 1H), 2.98–2.84 (m, 2H), 2.65–2.39 (m, 4H), 2.17–2.11 (m, 2H); MS APCI (AP+) 563 $(M+1)^+$; HRMS calcd for $C_{25}H_{24}ClFN_4O_4S_2$: 563.0990. Found: 563.0994; HPLC (method A) t_R = 16.9 min (95.4%).

5.1.9. 4-[3-(4-Chloro-phenyl)-ureido]-tetrahydro-pyran-4-carboxylic acid (3-fluoro-2′-sulfamoyl-biphenyl-4-yl)-amide (4g). White solid 0.20 g, 69%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.45 (s, 1H), 8.90 (s, 1H), 8.00 (dd, 1H, J = 1, 8 Hz), 7.81 (t, 1H, J = 8 Hz), 7.62–7.54 (m, 2H), 7.40 (m, 2H), 7.32–7.22 (m, 6H), 7.14 (dd, 1H, J = 2, 8 Hz), 6.75 (s, 1H), 3.76 (m, 2H), 3.58 (t, 2H, J = 11 Hz), 2.05 (m, 2H), 1.97 (m, 2H); MS APCI (AP+) 547 $(M+1)^+$; CHN calcd for $C_{25}H_{24}ClFN_4O_5S$+0.15H_2O+0.10TFA: C, 53.94; H, 4.38; N, 9.99. Found: C, 54.01; H, 4.41; N, 9.78.

5.1.10. 1-[3-(4-Chloro-phenyl)-ureido]-cyclopentanecarboxylic acid (2′-methanesulfonyl-biphenyl-4-yl)-amide (4h). White solid 0.12 g, 80%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.64 (s, 1H), 8.70 (s, 1H), 8.04 (dd, 1H, J = 1, 8 Hz), 7.72–7.59 (m, 2H), 7.65 (d, 2H, J = 9 Hz), 7.38 (m, 1H), 7.37 (d, 2H, J = 9 Hz), 7.29 (d, 1H, J = 9 Hz), 7.22 (d, 1H, J = 9 Hz), 6.50 (s, 1H), 2.76 (s, 3H), 2.21–2.14 (m, 2H), 1.87–1.82 (m, 2H), 1.74–1.67 (m, 4H); MS APCI (AP+) 512 $(M+1)^+$; CHN calcd for $C_{26}H_{26}ClN_3O_4S$: C, 60.99; H, 5.12; N, 8.21. Found: C, 60.94; H, 5.22; N, 7.89.

5.1.11. 1-[3-(4-Chloro-phenyl)-ureido]-cyclopent-3-enecarboxylic acid (3-fluoro-2′-sulfamoyl-biphenyl-4-yl)-amide (4i). White solid 0.11 g, 43%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, 1H), 8.77 (s, 1H), 7.99 (dd, 1H, J = 1, 8 Hz), 7.72–7.59 (app t, 1H, J = 8 Hz), 7.61–7.52 (m, 2H), 7.40–7.36 (m, 2H), 7.30–7.21 (m, 4H), 7.14 (dd, 1H, J = 2, 9 Hz), 6.85 (s, 1H), 5.67 (s, 2H), 3.07 (app d, 2H), 2.57 (app d, 2H); MS APCI (AP+) 529 $(M+1)^+$; CHN calcd for $C_{25}H_{22}ClN_4O_4S$: C, 56.76; H, 4.19; N, 10.59. Found: C, 56.71; H, 4.13; N, 10.21.

5.1.12. 2-[3-(4-Chloro-phenyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-2-methyl-propionamide (4j). White solid 0.29 g, 81%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.39 (s, 1H), 8.77 (s, 1H), 8.03 (dd, 1H, J = 1, 8 Hz), 7.77 (app t, 1H, J = 8 Hz), 7.71 (ddd 1H, J = 1, 8, 8 Hz), 7.63 (ddd, 1H, J = 1, 8, 8 Hz), 7.38–7.34 (m, 3H), 7.28–7.20 (m, 3H), 7.60 (s, 1H), 7.15 (dd, 1H, J = 2, 8 Hz), 2.84 (s, 3H), 1.46 (s, 6H); MS APCI (AP+) 504.1 $(M+1)^+$; CHN calcd for $C_{24}H_{23}ClFN_3O_4S$: C, 57.20; H, 4.60; N, 8.34. Found: C, 57.05; H, 4.35; N, 8.17.

5.1.13. 2-[3-(4-Chloro-phenyl)-ureido]-*N*-(3-fluoro-2′-sulfamoyl-biphenyl-4-yl)-2-methyl-propionamide (4k). White solid 0.17 g, 24%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.34 (s, 1H), 8.79 (s, 1H), 7.99 (m, 1H), 7.77 (t, 1H, J = 8 Hz), 7.61–7.53 (m, 2H), 7.38 (m, 2H), 7.31–7.21 (m, 6H), 7.13 (dd, 1H, J = 2, 8 Hz), 6.63 (s, 1H), 1.49 (s, 6H); MS APCI (AP+) 505 $(M+1)^+$; CHN calcd for $C_{23}H_{22}ClFN_4O_4S$+0.15H_2O+0.30TFA: C, 52.31; H, 4.20; N, 10.34. Found: C, 52.10; H, 4.09; N, 10.25.

5.1.14. 2-[3-(4-Chloro-phenyl)-ureido]-3-hydroxy-2-hydroxymethyl-*N*-(2′-sulfamoyl-biphenyl-4-yl)-propionamide (4l). See Refs. 11e and 22. White solid 0.14 g, 56%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.60 (s, 1H), 9.17 (s, 1H), 7.98 (dd, 1H, J = 1, 8 Hz), 7.62 (d, 2H, J = 8 Hz), 7.59–7.49 (m, 2H), 7.39 (d, 2H, J = 8 Hz), 7.29–7.20 (m, 3H), 7.16 (s, 2H), 6.40 (s, 1H), 5.13 (t, 2H, J = 6 Hz) 4.02–3.60 (m, 4H); MS APCI (AP+) 519.5 $(M+1)^+$; HRMS calcd for $C_{23}H_{23}ClN_4O_6SNa$ adduct: 541.0925. Found: 541.0925. HPLC (method A) t_R = 12.7 min (97.0%). The following compounds were prepared using the general procedure for **8i**.

5.1.15. 2-[3-(4-Chloro-phenyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8a). White solid 0.38 g, 55%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.95 (s, 1H), 8.98 (s, 1H), 8.05 (dd, 1H, J = 1, 8 Hz), 7.98 (app t, 1H, J = 13 Hz), 7.73 (ddd, 1H, J = 1, 8, 8 Hz), 7.65 (ddd, 1H, J = 1, 8, 8 Hz), 7.42–7.39 (m, 3H), 7.31 (dd, 1H, J = 2, 11 Hz), 7.24 (d, 2H, J = 13 Hz), 7.17 (dd, 1H, J = 2, 8 Hz), 6.48 (t, 1H, J = 5 Hz), 4.01 (d, 1H, J = 5 Hz), 2.84 (s, 3H); MS APCI (AP+) 476.1 $(M+1)^+$; CHN calcd for $C_{22}H_{19}ClFN_3O_4S$: C, 55.52; H, 4.02; N, 8.83. Found: C, 55.44; H, 3.89; N, 8.63.

5.1.16. 2-[3-(4-Chloro-phenyl)-1-methyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8b). White solid 0.26 g, 77%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 (s, 1H), 8.39 (app t, 1H, J = 8 Hz), 8.21 (dd, 1H, J = 1, 8 Hz), 7.73 (ddd, 1H, J = 1, 8, 8 Hz), 7.56 (ddd, 1H, J = 1, 8, 8 Hz), 7.38–7.25 (m, 6H), 7.18 (br d, 1H, J = 8 Hz), 6.59 (s, 1H), 4.17 (s, 2H), 3.21 (s, 3H), 2.69 (s, 3H); MS APCI (AP-) 488.1 $(M-1)^+$; CHN calcd for $C_{23}H_{21}ClFN_3O_4S$+0.03H_2O: C, 56.32; H, 4.33; N, 8.57; H_2O, 0.11. Found: C, 56.11; H, 4.27; N, 8.30; H_2O, 0.47.

5.1.17. 2-[3-(4-Chloro-phenyl)-3-methyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8c). The compound **8c** was formed via the addition to a dichloromethane (10 mL) solution of amine (0.20 g, 0.406 mmol), (4-chloro-phenyl)-methyl-carbamoyl chloride[23] (0.083 g, 0.406 mmol), triethylamine (0.226 mL, 3.14 mmol), and DMAP (10 mg). The mixture was stirred for 2 h at room temperature, quenched with an excess of water, and extracted with ethyl acetate. The organic extract was washed with 5% aqueous HCl (4 mL), brine, dried over $MgSO_4$, evaporated, and purified by column chromatography to obtain **8c** as a white solid, which was recrystallized from ethyl acetate/hexanes to get white crystals, 0.160 g, 70%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1H), 8.36 (app t, 1H, J = 8 Hz), 8.21 (dd, 1H, J = 1, 8 Hz), 7.64 (ddd, 1H, J = 1, 8, 8 Hz), 7.56 (ddd, 1H, J = 1, 8, 8 Hz), 7.43 (m, 2H), 7.35–7.25 (m, 5H), 7.15 (br d, 1H, J = 8 Hz), 4.96 (t, 1H, J = 5 Hz), 4.01 (d, 2H, J = 4H), 3.30 (s, 3H), 2.69 (s, 3H); MS APCI (AP-): 488.1 $(M-1)^+$; CHN calcd for $C_{23}H_{21}ClFN_3O_4S$: C, 56.28; H, 4.33; N, 8.56. Found: C, 55.99; H, 4.14; N, 8.31.

5.1.18. 2-[3-(4-Chloro-phenyl)-1-ethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8d). White solid, 0.210 g, 44%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.94 (s, 1H), 8.54 (s, 1H), 8.09 (dd, 1H, J = 1, 8 Hz), 8.06 (t, 1H, J = 8 Hz), 7.79–7.67 (m, 2H), 7.53 (d, 2H, J = 9 Hz), 7.45–7.33 (m, 2H), 7.28 (d, 2H, J = 9 Hz), 7.20 (dd, 1H, J = 1, 8 Hz), 4.24 (s, 2H), 3.46 (q, 2H, J = 7 Hz), 2.89 (s, 3H), 1.14 (t, 3H, J = 7 Hz); MS (ES) 501.70 $(M-H)^+$; CHN calcd for $C_{24}H_{23}ClFN_3O_4S$: C, 57.20; H, 4.60; N, 8.34. Found: C, 57.53; H, 4.54; N, 8.14.

5.1.19. 2-[3-(4-Chloro-phenyl)-1-propyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8e). White solid 0.130 g, 51%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (s, 1H), 8.38 (t, 1H, J = 8 Hz), 8.21 (dd, 1H, J = 1, 8 Hz), 7.67–7.55 (m, 2H), 7.37–7.27 (m, 2H), 7.36 (d, 2H, J = 9 Hz), 7.28 (d, 2H, J = 9 Hz), 7.20 (br d, 1H, J = 8 Hz), 6.68 (s, 1H), 4.16 (s, 2H), 3.41 (t, 2H, J = 8 Hz), 2.71 (s, 3H), 1.76 (m, 2H), 1.02 (t, 3H, J = 7 Hz); MS (ES) 517.75 $(M+H)^+$; CHN calcd for $C_{25}H_{25}ClFN_3O_4S$: C, 56.02; H, 5.08; N, 7.84. Found: C, 56.57; H, 4.55; N, 7.75.

5.1.20. 2-[1-Butyl-3-(4-chloro-phenyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8f). White solid 0.040 g, 55%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (br s, 1H), 8.40 (t, 1H, J = 8 Hz), 8.20 (dd, 2H, J = 1, 8 Hz), 7.68–7.56 (m, 1H), 7.60 (t, 1H), 7.38–7.24 (m, 4H), 7.20 (d, 1H, J = 8 Hz), 6.60 (s, 3H), 4.75 (s, 2H), 4.10 (s, 2H), 3.40 (t, 2H, J = 5 Hz), 2.62 (s, 3H), 1.75–1.40 (m, 2H), 1.42–1.38 (m, 2H), 1.05–1.00 (m, 3H); MS (ES) *m/e* 531.0 $(M+1)^+$; CHN calcd for $C_{29}H_{24}ClF_2\ N_3O_4S$: C, 58.68; H, 5.12; N, 7.90. Found: C, 58.33; H, 4.87; N, 7.66.

5.1.21. 2-[3-(4-Chloro-phenyl)-1-(2,2-dimethyl-propyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8h). White solid 0.074 g; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.97 (s, 1H), 8.40 (t, 1H, J = 8 Hz), 8.22 (d, 1H, J = 8 Hz), 7.75–7.50 (m, 2H), 7.40–7.24 (m, 6H), 7.19 (d, 1H, J = 8 Hz), 6.91 (s, 1H), 4.20 (s, 2H), 3.35 (s, 2H), 2.73 (s, 3H), 1.10 (s, 9H); MS (ES) 545.77 $(M+H)^+$; CHN calcd for $C_{27}H_{29}ClFN_3O_4S$: C, 59.40; H, 5.32; N, 7.70. Found: C, 59.12; H, 5.30; N, 7.61.

5.1.22. 2-[3-(4-Chloro-phenyl)-1-cyclopropylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8k). White solid, 0.040 g, 55%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.69 (s, 1H), 8.33 (s, 1H), 7.86–7.81 (m, 2H), 7.54–7.42 (m, 2H), 7.30–7.27 (m, 2H), 7.19–6.94 (m, 6H), 4.11 (s, 2H), 2.64 (s, 3H), 2.26–2.25 (m, 2H), 0.81–0.71 (m, 1H), 0.27–0.22 (m. 2H), 0.04–0.01 (m, 2H); MS APCI (AP+) 530.0 $(M+1)^+$; HRMS: calcd for $C_{26}H_{25}ClFN_3O_4S$: 530.1272. Found: 530.1310.

5.1.23. 2-[3-(4-Chloro-phenyl)-1-cyclopropyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8l). White powder 0.180 g, 75%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H), 8.53 (s, 1H), 8.09 (dd, 1H, J = 1, 8 Hz), 8.03 (t, 1H, J = 8 Hz), 7.79–7.67 (m, 2H), 7.60 (d, 2H, J = 9 Hz), 7.44–7.31 (m, 2H), 7.30 (d, 2H, J = 9 Hz), 7.19 (dd, 1H, J = 1, 8 Hz), 4.21 (s, 2H), 2.89 (s, 3H), 2.92–2.84 (m, 1H), 0.97–0.90 (m, 2H), 0.81–0.74 (m, 2H); MS (ES) 515.78 $(M+H)^+$; CHN calcd for $C_{25}H_{23}ClFN_3O_4S$: C, 58.19; H, 4.49; N, 8.14. Found: C, 58.07; H, 4.22; N, 7.99.

5.1.24. 2-[3-(4-Chloro-phenyl)-1-cyclobutylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8m). White solid 0.160 g, 59%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.12 (s, 1H), 8.39 (t, 1H, J = 8 Hz), 8.22 (dd, 1H, J = 1, 8 Hz), 7.68–7.54 (m, 2H), 7.38–7.27 (m, 2H), 7.36 (d, 2H, J = 9 Hz), 7.28 (d, 2H, J = 9 Hz), 7.19 (br d, 1H, J = 8 Hz), 6.65 (s, 1H), 4.15 (s, 2H), 3.47 (d, 2H, J = 7 Hz), 2.78–2.64 (m, 1H), 2.71 (s, 3H), 2.20–2.08 (m, 2H), 2.06–1.76 (m, 4H); MS (ES) 543.8 $(M+H)^+$; CHN calcd for $C_{27}H_{27}ClFN_3O_4S$: C, 59.61; H, 5.00; N, 7.72. Found: C, 59.60; H 5.03; N, 7.62.

5.1.25. 2-[3-(4-Chloro-phenyl)-1-cyclopentylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8n). White solid 0.060 g, 44%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (br s, 1H), 8.40 (t, 1H, J = 8 Hz), 8.20 (dd, 1 H, J = 1, 8 Hz), 7.68–7.60 (m, 1H), 7.60–7.52 (m, 1H), 7.38–7.20 (m, 6H), 7.19 (br d, 1H, J = 9 Hz), 6.60 (s, 1H), 4.20 (s, 2H), 3.41 (d, 2H, J = 5 Hz), 2.75 (s, 3H), 2.32–2.20 (m, 1H), 1.90–1.80 (m, 2H), 1.76–1.52 (m, 4H), 1.35–1.20 (m, 2H); MS (ES) 557.0 $(M)^+$; CHN calcd for $C_{28}H_{29}ClFN_3O_4S$: C, 60.26; H, 5.24; N, 7.53. Found: C, 60.21; H, 5.45; N, 7.08.

5.1.26. 2-[3-(4-Chloro-phenyl)-1-cyclohexylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8o). Pale yellow solid 0.350 g, 57%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.94 (s, 1H), 8.50 (s, 1H), 8.09 (dd, 1H, J = 1, 8 Hz), 8.06 (t, 1H, J = 8 Hz), 7.79–7.67 (m, 2H), 7.50 (d, 2H, J = 9 Hz), 7.45–7.32 (m, 2H), 7.28 (d, 2H, J = 9 Hz), 7.20 (dd, 1H, J = 1, 8 Hz), 4.26 (s, 2H), 3.27 (d, 2H, J = 7 Hz), 2.88 (s,

3H), 1.75–1.55 (m, 6H), 1.25–1.08 (m, 3H), 1.03–0.88 (m, 2H); MS (ES) 571.8 $(M+H)^+$; CHN calcd for $C_{29}H_{31}ClFN_3O_4S$: C, 60.88; H, 5.46; N, 7.34. Found: C, 60.72; H, 5.44; N, 7.23.

5.1.27. 2-[3-(4-Chloro-phenyl)-1-(tetrahydro-furan-3-ylmethyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8p). White solid 0.110 g, 50%; ^1H NMR (400 MHz, $CDCl_3$): δ 9.40 (br s, 1H), 8.38 (t, 1H, $J = 8$ Hz), 8.22 (d, 1H, $J = 8$ Hz), 7.80 (s, 1H), 7.70–7.56 (m, 2H), 7.40–7.16 (m, 2H), 7.36 (d, 2H, $J = 9$ Hz), 7.24 (d, 2H, $J = 9$ Hz) 4.40 (d, 1H, $J = 8$ Hz), 4.15–4.05 (m, 1H), 3.95–3.85 (m, 2H), 3.64–3.55 (m, 1H), 3.38–3.30 (m, 2H), 2.95 (s, 1H), 2.90 (s, 1H), 2.78 (s, 3H), 2.18–2.08 (m, 1H), 1.70–1.60 (m, 1H), 1.25 (t, 1H, $J = 8$ Hz); MS (ES) 559.78 $(M+1)^+$; CHN calcd for $C_{27}H_{27}ClFN_3O_5S$: C, 57.91; H, 4.86; N, 7.50. Found: C, 58.14; H, 4.63; N, 7.78.

5.1.28. 2-[3-(4-Chloro-phenyl)-1-furan-2-ylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8q). White solid 0.50 g, 55%; ^1H NMR (400 MHz, $CDCl_3$): δ 9.08 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 8$ Hz), 7.68–7.58 (m, 2H), 7.60–7.52 (m, 1H), 7.46 (d, 1H, $J = 1$, 8 Hz), 7.40–7.26 (m, 7H), 7.20 (dd, 1H, $J = 1$, 8 Hz), 6.40 (dd, 1H, $J = 1$, 8 Hz), 4.60 (s, 2H), 4.21 (s, 2H), 2.68 (s, 3H); MS (ES) 555.0 $(M)^+$; CHN calcd for $C_{27}H_{23}ClFN_3O_5S$: C, 58.33; H, 4.17; N, 7.56. Found: C, 58.21; H, 4.30; N, 7.07.

5.1.29. 2-[3-(4-Chloro-phenyl)-1-thiophen-2-ylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8r). White solid 0.58 g, 61%; ^1H NMR (400 MHz, $CDCl_3$): δ 8.70 (br s, 1H), 8.40 (t, 1H, $J = 9$ Hz), 8.21 (dd, 1H, $J = 1$, 8 Hz), 7.70–7.53 (m, 2H), 7.58 (t, 1H), 7.40–7.00 (m, 10H), 4.82 (s, 2H), 4.23 (s, 2H), 2.71 (s, 3H); MS (ES) 572.27 $(M+1)^+$; CHN calcd for $C_{27}H_{23}ClFN_3O_4S_2$: C, 56.69; H, 4.05; N, 7.35. Found: C, 56.84; H, 3.93; N, 7.35.

5.1.30. 2-[3-(4-Chloro-phenyl)-1-oxazol-2-ylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8s). White solid 0.060 g, 29%; ^1H NMR (400 MHz $CDCl_3$): δ 10.60 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 8.26 (br s, 1H), 8.22 (d, 1H, $J = 8$ Hz), 7.75 (s, 1H) 7.68–7.56 (m, 2H), 7.45–7.18 (m, 3H), 7.42 (d, 2H, $J = 9$ Hz), 7.28 (d, 2H, $J = 9$ Hz), 4.82 (s, 2H), 4.30 (s, 2H), 2.75 (s, 3H); MS (ES) 556.69 $(M+1)^+$; CHN calcd for $C_{26}H_{22}ClFN_4O_5S$: C, 56.07; H, 3.98; N, 10.06. Found: C, 56.09; H 4.18; N, 8.83.

5.1.31. 2-[3-(4-Chloro-phenyl)-1-thiophen-3-ylmethyl-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8t). White solid 0.110 g, 58%; ^1H NMR (400 MHz $CDCl_3$): δ 8.78 (br s, 1H), 8.38 (t, 1H, $J = 8$ Hz), 8.22 (dd, 1H, $J = 1$, 8 Hz), 7.70–7.56 (m, 2H) 7.46–7.42 (m, 1H), 7.38–7.20 (m, 8H), 7.10 (dd, 1H, $J = 1$, 8 Hz), 6.92 (br s, 1H) 4.70 (s, 2H), 4.25 (s, 2H), 2.72 (s, 3H); MS (ES) 571.67 $(M+1)^+$; CHN calcd for $C_{27}H_{22}ClFN_3O_4S_2$: C, 56.69; H, 4.05; N, 7.35. Found: C, 56.35; H, 3.82; N, 6.89.

5.1.32. 2-[3-(4-Chloro-phenyl)-1-(3-methoxy-benzyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8v). White solid 0.18 g, 32%; ^1H NMR (400 MHz, $CDCl_3$): δ 8.80 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.65–7.60 (m, 1H), 7.60–7.52 (m, 1H), 7.40–7.30 (m, 2H), 7.23–7.17 (m, 7H), 6.98–6.80 (m, 3H), 4.62 (s, 2H), 4.20 (s, 2H), 3.80 (s, 3H), 2.75 (s, 3H); MS (ES) 596 $(M+1)^+$; HRMS calcd for $C_{30}H_{28}ClFN_3O_5S$: 596.1422. Found: 596.1435. HPLC (method B) $t_R = 6.8$ min (97.8%).

5.1.33. 2-[3-(4-Chloro-phenyl)-1-(3-fluoro-benzyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8w). White solid 0.20 g, 33%; ^1H NMR (400 MHz, $CDCl_3$): δ 8.68 (br s, 1H), 8.36 (t, 1H, $J = 8$ Hz), 8.20 (dd, 2H, $J = 1$, 8 Hz), 7.68–7.56 (m, 2H), 7.44–7.04 (m, 10H), 6.90 (br s, 1H), 4.75 (s, 2H), 4.24 (s, 2H), 2.70 (s, 3H); MS (ES) 584.12 $(M+1)^+$; HRMS calcd for $C_{29}H_{25}ClF_2N_3O_4S$: 584.1222 Found: 584.1248; HPLC (method B) $t_R = 7.0$ min (98.7%).

5.1.34. 2-[3-(4-Chloro-phenyl)-1-(3-methyl-benzyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8x). White solid 0.18 g, 41%; ^1H NMR (400 MHz, $CDCl_3$): δ 8.84 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 8.20 (dd, 2H, $J = 1$, 8 Hz), 7.68–7.54 (m, 2H), 7.38–7.15 (m, 11H), 6.82 (br s, 1H), 4.70 (s, 2H), 4.30 (s, 2H), 2.70 (s, 3H), 2.40 (s, 3H); MS (ES) 579.64 $(M+1)^+$; HRMS calcd for $C_{30}H_{28}ClFN_3O_4S$: 580.1473. Found: 580.1461; HPLC (method B) $t_R = 7.5$ min (98.3%).

5.1.35. 2-[3-(4-Chloro-phenyl)-1-(2-methoxy-benzyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8y). White solid 0.23 g, 42%; ^1H NMR (400 MHz, $CDCl_3$); δ 9.40 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.94 (br s, 1H), 7.68–7.52 (m, 1H), 7.60–7.52 (m, 1H), 7.40–7.23 (m, 8H), 7.20 (br d, 1H, $J = 8$ Hz), 7.10–6.90 (m, 2H), 4.60 (s, 2H), 4.15 (s, 2H), 4.01 (s, 3H), 2.75 (s, 3H); MS (ES) 596 $(M+1)^+$; CHN calcd for $C_{30}H_{27}ClFN_3O_5S$: C, 60.45; H, 4.57; N, 7.05. Found: C, 60.20; H, 4.40; N, 6.94.

5.1.36. 2-[1-(4-Chloro-benzyl)-3-(4-chloro-phenyl)-ureido]-*N*-(3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-acetamide (8bb). White solid 0.22 g, 24%; ^1H NMR (400 MHz, DMSO-d_6): δ 9.80 (br s, 1H), 8.70 (s, 1H), 8.12–8.04 (m, 1H), 7.80–7.66 (m, 1H), 7.56–7.28 (m, 4H), 7.54 (d, 2H, $J = 8$ Hz), 7.42 (d, 2H, $J = 8$ Hz), 7.36 (d, 2H, $J = 8$ Hz), 7.30 (d, 2H, $J = 8$ Hz), 7.20 (dd, 1H, $J = 9$ Hz), 4.60 (s, 2H), 4.20 (s, 2H), 2.80 (s, 3H); MS (ES) 599.58 $(M+1)^+$; CHN calcd for $C_{29}H_{24}ClFN_3O_4S+0.5\ H_2O$: C, 57.16; H, 3.97; N, 6.89. Found: C, 57.12; H, 3.89; % N 6.79.

The following compounds were prepared using the general procedure for **11d**.

5.1.37. 1-[1-Butyl-3-(4-chloro-phenyl)-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11a). White solid 0.20 g, 60%; ^1H NMR (400 MHz, $CDCl_3$): δ 8.55 (br s, 1H), 8.35 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.70–7.55 (m, 2H), 7.40–7.20 (m, 7H), 6.95 (s, 1H), 3.70–3.55 (m,

1H), 3.40–3.30 (m, 1H), 2.75 (s, 3H), 2.05–1.65 (m, 4H), 1.55–1.20 (m, 4H), 1.00 (t, 3H, $J = 8$ Hz); MS (ES) 557.24 $(M+1)^+$; CHN calcd for $C_{28}H_{29}ClFN_3O_4S$: C, 60.26; H, 5.24; N, 7.53. Found: C, 60.98; H 5.15; N, 7.14.

5.1.38. 1-[3-(4-Chloro-phenyl)-1-methyl-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11b). White solid 0.060 g, 32%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (br s, 1H), 8.30 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.70–7.58 (m, 2H), 7.40–7.18 (m, 6H), 6.90 (br d, 1H, $J = 8$ Hz), 3.20 (s, 3H), 2.68 (s, 3H), 1.82 (br s, 1H), 1.60–1.54 (m, 2H), 1.24–1.22 (m, 2H); MS (ES) 516 $(M+1)^+$; CHN calcd for $C_{25}H_{23}ClFN_3O_4S$: C, 58.19; H, 4.49; N, 8.17. Found: C, 57.83; H, 4.59; N, 7.39.

5.1.39. 1-[3-(4-Chloro-phenyl)-1-propyl-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11c). White solid 0.19 g, 59%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (br s, 1H), 8.35 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.65–7.55 (m, 2H), 7.40–7.20 (m, 7H), 6.95 (s, 1H), 3.65–3.55 (m, 1H), 3.35–3.25 (m, 1H), 2.70 (s, 3H), 2.10–2.00 (m, 1H), 1.85–1.80 (m, 2H), 1.80–1.65 (m, 1H), 1.55–1.35 (m, 2H), 1.00 (t, 3H, $J = 8$ Hz). MS (ES) 543.33 $(M+1)^+$; CHN calcd for $C_{27}H_{27}ClFN_3O_4S$: C, 59.61; H, 5.00; N, 7.72. Found: C, 59.71; H, 4.92; N, 7.64.

5.1.40. 1-[3-(4-Chloro-phenyl)-1-cyclopropylmethyl-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11d). White solid 0.30 g, 54%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.70–7.55 (m, 2H), 7.40–7.20 (m, 7H), 7.00 (s, 1H), 3.90 (dd, 1H, $J = 5$, 8 Hz), 2.90 (dd, 1H, $J = 5$, 8 Hz), 2.70 (s, 3H), 2.15–2.05 (m, 1H), 1.90–1.80 (m, 1H), 1.55–1.30 (m, 2H), 1.30–1.20 (m, 1H), 0.70–0.55 (m, 2H), 0.45–0.35 (m, 2H). MS (ES) 557.24 $(M+1)^+$; CHN calcd for $C_{28}H_{27}ClFN_3O_4S$: C, 60.48; H, 4.89; N, 7.56. Found: C, 60.67; H 5.10; N, 7.38.

5.1.41. 1-[3-(4-Chloro-phenyl)-1-cyclohexylmethyl-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11e). White solid 0.21 g, 40%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (br s, 1H), 8.30 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.65–7.55 (m, 2H), 7.45–7.15 (m, 7H), 7.05 (s, 1H), 3.45–3.15 (m, 2H), 2.70 (s, 3H), 2.05–1.35 (m, 11H), 1.35–0.85 (m, 4H); MS (ES) 598.29 $(M+1)^+$; HRMS calcd for $C_{31}H_{33}ClFN_3O_4S$: 598.1942; Found: 598.1954. HPLC (method B) $t_R = 6.2$ min (99.2%).

5.1.42. 1-[3-(4-Chloro-phenyl)-1-cyclopentylmethyl-ureido]-cyclopropanecarboxylic acid (3-fluoro-2′-methanesulfonyl-biphenyl-4-yl)-amide (11f). White solid 0.27 g, 45%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (br s, 1H), 8.35 (t, 1H, $J = 8$ Hz), 8.20 (dd, 1H, $J = 1$, 8 Hz), 7.70–7.50 (m, 2H), 7.40–7.20 (m, 7H), 7.00 (s, 1H), 3.75–3.65 (m, 1H), 3.20–3.15 (m, 1H), 2.70 (s, 3H), 2.35–2.25 (m, 1H), 2.00–1.40 (m, 8H), 1.35–1.20 (m, 2H), 0.90–0.80 (m, 2H); MS (ES) 584.31 $(M+1)^+$; CHN calcd for $C_{30}H_{31}ClFN_3O_4S$: C, 61.69; H, 5.35; N, 7.19. Found: C, 62.20; H, 5.51; N, 6.97.

5.1.43. 2-[3-(4-Chloro-phenyl)-1-(2-methoxy-ethyl)-ureido]-*N*-[2-fluoro-4-(2-oxo-piperidin-1-yl)-phenyl]-acetamide (12). White solid 0.030 g, 70%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.73 (s, 1H), 8.53 (s, 1H), 7.81 (dd, 1H, $J = 8$, 8 Hz), 7.43–7.30 (m, 2H), 7.25–7.18 (m, 3H), 7.03 (dd, 1H, $J = 7$, 8 Hz), 4.19 (s, 2H), 3.55–3.44 (m, 6H), 3.20 (s, 3H), 2.32 (t, 2H, $J = 6$ Hz), 1.82–1.73 (m, 4H); MS (AP+) 477.1 $(M+1)^+$; HRMS calcd for $C_{23}H_{26}ClFN_4O_4$: 477.1660. Found: 477.1709.

5.1.44. 2-[3-(4-Chloro-phenyl)-1-cyclopropylmethyl-ureido]-*N*-[2-fluoro-4-(2-oxo-2*H*-pyridin-1-yl)-phenyl]-acetamide (13). White solid 0.47 g, 98%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (br s, 1H), 8.40 (t, 1H, $J = 8$ Hz), 7.50–7.40 (m, 1H), 7.40–7.15 (m, 7H), 7.15 (d, 1H, $J = 8$ Hz), 6.65 (d, 1H, $J = 8$ Hz), 6.25 (t, 1H, $J = 8$ Hz), 4.20 (s, 2H), 3.35 (d, 2H, $J = 8$ Hz), 1.50 (quin, 1H, $J = 8$ Hz), 0.65 (dt, 2H, $J = 6$, 8 Hz), 0.35 (dt, 2H, $J = 6$, 8 Hz). MS (ES) 470.36 $(M+1)^+$; CHN calcd for $C_{24}H_{22}ClFN_4O_3$: C, 61.47; H, 4.73; N, 11.95. Found: C, 61.30; H, 4.81; N, 11.93.

5.2. Crystallization, data, and structural refinement

Binary complex crystals of Human des Gla factor Xaβ protein with **11a** were produced by hanging drop vapor diffusion using a reservoir solution composed of 25% PEG 600 (Fluka), 0.3 M NaCl (Sigma), and 0.1 M MES (2-morpholinoethanesulfonic acid, Sigma) buffer (pH 5.9). Des Gla factor Xaβ protein (8 mg/mL) complexed with 0.1 mM **11a** was mixed with an equivalent volume of reservoir solution and equilibrated over 250 μL of reservoir solution. Rod-shaped, single crystals ($60 \times 60 \times 240$ μm) were obtained in six weeks as a result of microseeding for two days. Crystals were quickly immersed in oil (70% Paratone-N oil and 30% light, white mineral oil, Hampton Research) and flash-cooled in liquid nitrogen before data collection.

X-ray diffraction intensity data were obtained under cryogenic conditions at the IMCA-CAT 17-ID beamline at the Advanced Photon Source (Argonne National Laboratories, Argonne, IL). The crystals diffracted to a resolution of 1.9 Å and are a member of the space group $P2_12_12_1$ with one molecule per asymmetric unit. The structure of the FXa–**11a** binary complex was refined starting with previously refined FXa model structures, without their inhibitor ligand, with program package CNX.[24] A rigid-body rotation–translation refinement was initially performed to align the modeled structure accurately in the FXa–**11a** unit cell. Further crystallographic refinement was performed by conjugated-gradient minimization and individual B factor refinement with CNX, and model/ligand building was performed with the program 'QUANTA.' There is no electron density observed for the first EGF domain, thus models for the EGF1 domain were not included in subsequent refinement steps. The final model included 279 residues, 99 water molecules, 2 Ca^{2+} ions, and **11a**.

5.3. Enzyme inhibition assays

Compounds were tested for the inhibition of thrombin and trypsin according to a modified protocol of Chu et al.[27a] All enzymes were purchased from Enzyme Research Laboratories (South Bend, Indiana) including purified human FXa, thrombin, and trypsin. Compounds were tested for the inhibition of FXa via a fluorogenic assay for which the fluorogenic substrate for FXa, FS-2765, was purchased from California Peptide (Napa, California).[27b] The chromogenic substrates for thrombin and trypsin were purchased from Roche Molecular Biochemicals (Mannheim, Germany) and Chromogenix (Molndal, Sweden), respectively. Buffer A containing 10.0 mM HEPES, 150 mM sodium chloride, and 0.1% (bovine serum albumin) BSA at pH 7.4 was prepared for use in the FXa and trypsin assays. Thrombin was assayed in Buffer B containing 10.0 mM HEPES, 100 mM NaCl, 0.1% PEG 8000, and 0.05% BSA at pH 7.4. Factor Xa was tested at a final concentration of 30 pM. Thrombin and trypsin were both tested at 500 pM. Compounds were serially diluted to final concentrations of 1.0 μM–900 fM for the FXa inhibition assay and 200 μM–3.4 nM in the selectivity assays. The reactions were initiated by the addition of prewarmed substrate and immediately placed in the microplate reader (SpectraMAX Gemini XS fluorometric plate reader and SpectraMAX 190 chromogenic plate reader) prewarmed to 37 °C. Concentration curves were run in triplicate and IC_{50}s were calculated by following the 4-parameter logistic curve fitting equation. IC_{50}s are reported as means ± SEM (standard error of the mean).

5.3.1. Prothrombin time (PT) assay. Citrated, pooled normal human plasma was purchased from George King Bio-Medical Inc. Inhibitors were serially diluted in DMSO and spiked into the pooled human plasma at final assay concentrations of 13.2–0.26 μM. Samples were run in duplicate using the recombinant thromboplastin reagent Innovin (Dade Behring Inc, Deerfield, Illinois), and PT was measured on the ACL™ 9000 System (Beckman Coulter Inc, Fullerton, California) according to the manufacturers' protocol.[25] The results were reported as the concentration of inhibitor that doubled the time (2× PT) to initial fibrin strand formation.

5.3.2. In vivo assay—rat PK–PD. Rats were housed in an AALAC accredited facility in climate-controlled rooms on a fixed light cycle and had access to normal rat chow and water for one to seven days before the study. This study protocol was approved by the Pfizer Ann Arbor Laboratories Animal Care and Use Committee, and conforms to the guide for the Care and Use of Laboratory Animals[26] published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Surgically prepared conscious rats were dosed intragastrically with 5 mg/kg of test compound ($n = 2$–4). Drug vehicle consisted of a 2% Tween 80 solution mixed 50–50 with a 1% methylcellulose solution (Sigma) with a 5 mL/kg dosing volume. Blood samples were drawn from a carotid artery catheter for the determinations of plasma concentration at 0, 0.5, 1, 2, and 4 or 0, 0.5, 1, 4, and 6 h post-dose. 0.9 mL of blood was drawn into a syringe containing 0.1 mL of 3.8% sodium citrate. Samples were centrifuged twice and the final plasma was stored at −80 °C until analyzed. At the conclusion of the study rats were euthanized with an overdose of Beuthanasia D (Schering Plough, Kennilworth, NJ).

Ex vivo anti-FXa activity of test compounds was determined by a chromogenic assay previously described.[20] In brief, antithrombin III buffer (0.05 M Tris–HCl, 0.175 M sodium chloride, and 7.5 mM EDTA, pH 8.4), bovine FXa (20 nKat),[28] and SPECTROZYME® FXa (methoxycarbonyl-D-cyclohexyglycyl-L-glycyl-D-arginine *p*-nitroanilide acetate, 4 μM) were prepared according to the manufacturer's instructions (American Diagnostica Inc.). A standard curve was prepared with fixed concentrations of test compounds in rat plasma with sodium citrate. Plasma samples were thawed and kept at 4 °C. Plasma samples and standards (20 μL of each) were added to each well of a 96-well microplate. Antithrombin III buffer (60 μL), bovine FXa (60 μL), and SPECTROZYME® FXa (60 μL) were added via pipette into the wells of the plate with 60 s intervals of mixing at room temperature between additions of each reagent. Immediately following the addition of the substrate, the plate was read kinetically for 5 min at 405 nm on a SPECTRAmax Plus[384] spectrophotometer (Molecular Devices Corporation) set at 37 °C. SOFTmax® Pro software (Molecular Devices Corporation) was used to record and analyze the maximal velocity of the reaction for each well. Factor Xa inhibition was calculated for each plasma sample or standard using the following equation: $(1 - V_{max}^{sample}/V_{max}^{control}) \times 100$. The control value ($V_{max}^{control}$) was the predose sample for an individual animal or the no drug standard for the standard curve. SOFTmax® Pro interpolated the final assay concentration for each sample from the standard curve. Values were reported as percent inhibition and plasma concentration (nM). All plasma samples and standards were run in duplicate.

Acknowledgments

The authors thank Mehran Jalaie for help in preparing the X-ray crystallographic binding diagrams used in the manuscript, Michael Stier for his help in compiling experimental data and procedures, Wayne Cody for his help in the editing of the manuscript, and Dana DeJohn for the HRMS determinations.

References and notes

1. (a) Leadley, R. J.; Gould, W. R. *Curr. Pharm. Des.* **2003**, *9*, 2337; (b) Ruef, J.; Katus, H. A. *Expert Opin. Investig. Drugs* **2003**, *12*, 781.
2. (a) Leadley, R. J. *Curr. Top. Med. Chem.* **2001**, *1*, 151; (b) Tan, K. T.; Maken, A.; Lip, G. YH. *Expert Opin. Investig. Drugs* **2003**, *12*, 799; (c) Wieland, H. A.; Laux, V.; Kozian, D.; Lorenz, M. *Curr. Opin. Investig. Drugs* **2003**, *4*, 264.
3. Walenga, J. M.; Jeske, W. P.; Hoppensteadt, D.; Fareed, J. *Curr. Opin. Investig. Drugs* **2003**, *4*, 272.

4. (a) Davidson, B. L. *J. Thromb. Thrombolysis* **2003**, *16*, 49; (b) Porcari, A. R.; Chi, L.; Leadley, R. J. *Expert Opin. Investig. Drugs* **2000**, *9*, 1596; (c) Kaiser, B. *Cardiovasc. Drug Rev.* **2003**, *21*, 91.
5. Al-Obeidi, F.; Ostrem, J. A. *Expert Opin. Ther. Patent* **1999**, *9*, 931.
6. (a) Rewinkel, J. B.; Adang, A. E. *Curr. Pharm. Des.* **1999**, *5*, 1043; (b) Ewing, W. R.; Pauls, H. W.; Spda, A. P. *Drugs Future* **1999**, *24*, 771; (c) Quan, M. L.; Liauw, A. Y.; Ellis, C. D.; Pruitt, J. R.; Carini, D. J.; Bostrom, L. L.; Huang, P. P.; Harrison, K.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **1999**, *42*, 2752; (d) Brandstetter, H.; Kuhne, A.; Bode, W.; Hubert, R.; von der Saal, W.; Wirthensohn, K.; Engh, R. A. *J. Biol. Chem.* **1986**, *271*, 29988.
7. Maignan, S.; Guilloteau, J.-P.; Pouzieux, S.; Choi-Sledeski, Y. M.; Becker, M. R.; Klein, S. I.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. *J. Med. Chem.* **2000**, *43*, 3226.
8. (a) Faull, A. W.; Mayo, C. M.; Preston, J.; Stocker, A. WO09610022, 1996(b) Zhu, B.-Y.; Scarborough, R. M. *Curr. Opin. Cardiovasc. Pulm. Renal Invest. Drugs* **1999**, *1*, 63; (c) Zhu, B.-Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83; (d) Galemmo, R. A.; Dominguez, C.; Fevig, J. M.; Han, Q.; Lam, P. Y. S.; Pinto, D. J. P.; Pruitt, J. R.; Quan, M. L. WO09857937, 1998.; (e) Beight, D. W.; Craft, T. J.; Franciskovich, J. B.; Goodson, T. J.; Hall, S. E.; Herron, D. K.; Klimkowski, V. J.; Kyle, J. A.; Master, J. J.; Mendel, D.; Milot, G.; Sawyer, J. S.; Shuman, R. T.; Smith, G. F.; Tebbe, A. L.; Tinsley, J. M.; Weir, L. C.; Wikel, J. H.; Wiley, M. R.; Yee, Y. K. WO09900121, 1999; (f) 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. *J. Med. Chem.* **2005**, *48*, 1729; (g) Kuo, F.; Clodfelter, D. K.; Priest, T. R.; Kau, D. L. K. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 599.
9. (a) 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. *J. Med. Chem.* **2003**, *46*, 681; (b) Maignan, S.; Guilloteau, J.-P.; Choi-Sledeski, Y. M.; Becker, M. R.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 685; (c) Choi-Sledeski, Y. M.; McGarry, D. G.; Green, D. M.; Mason, H.; Becker, M. R.; Davis, R. S.; Ewing, W. R.; Dankulich, W. P.; Manetta, V. E.; Morris, R. L.; Spada, A. P.; Cheney, D. L.; Brown, K. D.; Colussi, D. J.; Chu, V.; Heran, C. L.; Morgan, S. R.; Bentley, R. G.; Leadley, RJ.; Maignan, S.; Guilloteau, J.-P.; Dunwiddie, C. T.; Pauls, H. W. *J. Med. Chem.* **1999**, *42*, 3572.
10. Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900.
11. (a) Pruitt, J. R.; Pinto, D. J. P.; Galemmo, R. A., Jr.; Alexander, R. S.; Rossi, K. A.; Wells, B. L.; Drummond, S.; Bostrom, L. L.; Burdick, D.; Bruckner, R.; Chen, H.; Smallwood, A.; Wong, P. C.; Wright, M. R.; Bai, S.; Luettgen, J. M.; Knabb, R. M.; Lam, P. T. S.; Wexler, R. R. *J. Med. Chem.* **2003**, *46*, 5298; (b) Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Campbell, J. B.; 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. *J. Med. Chem.* **2000**, *43*, 2087; (c) Lam, P. S.; Clark, C. G.; Li, R.; Pinto, D. J. P.; Orwat, M. J.; Galemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. S.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luettgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2003**, *46*, 4405(d) Bigge, C. F.; Dudley, D. A.; Edmunds, J. J.; Van Huis, C. A.; Casimiro-Garcia, A.; Filipski, K. J.; Kohrt, J. T. WO03045912, 2003.; (e) Bolton, G. L.; Filipski, K. J.; Kohrt, J. T.; La, F. T.; Leonard, D. M. US20040167131, 2004.
12. Hyun, H. M.; Kang, M. H.; Han, S. C. *Tetrahedron Lett.* **1999**, *40*, 3435.
13. Bhattacharyva, S.; Chatterjee, A.; Williamson, J. S. *Synlett* **1995**, 1079.
14. Chou, Y.-L.; Davey, D. D.; Eagen, K. A.; Griedel, B. D.; Daranjawala, R.; Phillips, G. B.; Sacchi, K. L.; Shaw, K. J.; Wu, S. C.; Lentz, D.; Liang, A. M.; Trinh, L.; Morrissey, M. M.; Kochanny, M. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 507.
15. Su, T.; Wu, Y.; Doughan, B.; Jia, Z. J.; Woolfrey, J.; Huang, B.; Wong, P.; Sinha, U.; Scarborough, R. M.; Zhu, B-Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2947.
16. Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235.
17. Su, T.; Wu, Y.; Doughan, B.; Kane-Maguire, K.; Marlowe, C. K.; Kanter, J. P.; Woolfrey, J.; Huang, B.; Wong, P.; Sinha, U.; Park, G.; Malinowski, J.; Hollenbach, S.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2002**, *11*, 2279.
18. During the preparation of this manuscript similar compounds have been disclosed: Mederski, W. W. K. R.; Dorsch, D.; Anzali, S.; Gleitz, J.; Cezanne, B.; Tsaklakidis, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3763.
19. (a) Valle, G.; Crisma, M.; Toniolo, C.; Holt, E. M.; Tanura, M.; Bland, J.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1989**, *34*, 56; (b) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004.
20. (a) Teien, A. N.; Lie, M.; Abildgaard, U. *Thromb. Res.* **1976**, *8*, 413; (b) Teien, A. N.; Lie, M. *Thromb. Res.* **1977**, *10*, 399; (c) Heran, C.; Morgan, S.; Kasiewski, C.; Bostwick, J.; Bentley, R.; Klein, S.; Chu, V.; Brown, K.; Colussi, D.; Czekaj, M.; Perrone, M.; Leadley, R., Jr. *Eur. J. Pharmacol.* **2000**, *389*, 201.
21. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
22. (a) Schmidt, U.; Riedl, B. *Synthesis* **1993**, 815; (b) Scattergood, A.; McLearn, A. L. *J. Am. Chem. Soc.* **1949**, 4153.
23. Hansen, J. B.; Fink-Jensen, A.; Christensen, B. V.; Gronvald, F. C.; Jeppesen, L.; Mogensen, J. P.; Nielsen, E. B.; Scheideler, M. A.; White, F. J.; Zhang, X.-F. *Eur. J. Med. Chem.* **1998**, *33*, 839.
24. Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuzewski, J.; Nilges, N.; Pannu, N. S.; Read, J. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr.* **1998**, *D54*, 905.
25. Beckman Coulter Inc. Instrumentation Laboratory ACL™ 9000 System Training Manual; 1st ed.; Miami Lakes, Florida: Beckman Coulter; 2000.
26. US National Institutes of Health, NIH Publication No. 85-23, revised 1996.
27. (a) Chu, V.; Brown, K.; Colussi, D.; Jingbo, G.; Bostwick, J.; Kasiewski, C.; Ross, V.; Morgan, S.; Guertin, K.; Pauls, H.; Gong, Y.; Zulli, A.; Perrone, M.; Dunwiddie, C.; Leadley, R. J. *Thromb. Res.* **2001**, *103*, 309; (b) Gould, W. R.; Cladera, E.; Harris, M. S.; Zhang, E.; Narasimhan, L.; Thorn, J. M.; Leadley, R. J. *Biochemistry* **2005**, *44*, 9280.
28. Dybkaer, R. *Pure Appl. Chem.* **2001**, *73*, 927.