Bioorganic & Medicinal Chemistry Letters 19 (2009) 6882–6889

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Aroylguanidine-based factor Xa inhibitors: The discovery of BMS-344577

Yan Shi *, Chi Li, Stephen P. O'Connor, Jing Zhang †, Mengxiao Shi ‡, Sharon N. Bisaha, Ying Wang, Doree Sitkoff, Andrew T. Pudzianowski, Christine Huang, Herbert E. Klei, Kevin Kish, Joseph Yanchunas Jr., Eddie C.-K. Liu, Karen S. Hartl, Steve M. Seiler, Thomas E. Steinbacher, William A. Schumacher, Karnail S. Atwal, Philip D. Stein

Research and Development, Bristol-Myers Squibb Company, PO Box 5400, Princeton, NJ 08543-5400, USA

ARTICLE INFO

Article history:
Received 10 July 2009
Revised 17 October 2009
Accepted 20 October 2009
Available online 23 October 2009

Keywords:
Factor Xa inhibitor
Aroylguanidine
CYP3A4 inhibition
Orally active

ABSTRACT

We report the design and synthesis of a novel class of *N*,*N*′-disubstituted aroylguanidine-based lactam derivatives as potent and orally active FXa inhibitors. The structure–activity relationships (SAR) investigation led to the discovery of the nicotinoyl guanidine **22** as a potent FXa inhibitor (FXa IC_{50} = 4 nM, $EC_{2\times PT}$ = 7 μM). However, the potent CYP3A4 inhibition activity (IC_{50} = 0.3 μM) of **22** precluded its further development. Detailed analysis of the X-ray crystal structure of compound **22** bound to FXa indicated that the substituent at the 6-position of the nicotinoyl group of **22** would be solvent-exposed, suggesting that efforts to attenuate the unwanted CYP activity could focus at this position without affecting FXa potency significantly. Further SAR studies on the 6-substituted nicotinoyl guanidines resulted in the discovery of 6-(dimethylcarbamoyl) nicotinoyl guanidine **36** (BMS-344577, IC_{50} = 9 nM, $EC_{2\times PT}$ = 2.5 μM), which was found to be a selective, orally efficacious FXa inhibitor with an excellent in vitro liability profile, favorable pharmacokinetics and pharmacodynamics in animal models.

Thrombo-embolic disorders are the largest cause of human mortality and morbidity.[1] Current therapeutic drugs such as warfarin and heparin suffer from problems including a narrow therapeutic dose window, slow onset and offset of action, or patient variability and the need for periodic monitoring.[2] Improvement in the treatment and prevention of thrombotic diseases remains an important medical need. Selective inhibitors of the serine proteases in the coagulation cascade have been the targets for anti-thrombotic drug development for some time.[3] Within the coagulation cascade, activated blood coagulation factor X (FXa) is the key physiological activator of prothrombin and is the obligate enzyme in converting prothrombin to thrombin to begin the blood clot formation process. Extensive preclinical and clinical proof-of-principle data show that inhibition of FXa is effective in both venous and arterial thrombosis.[3,4]

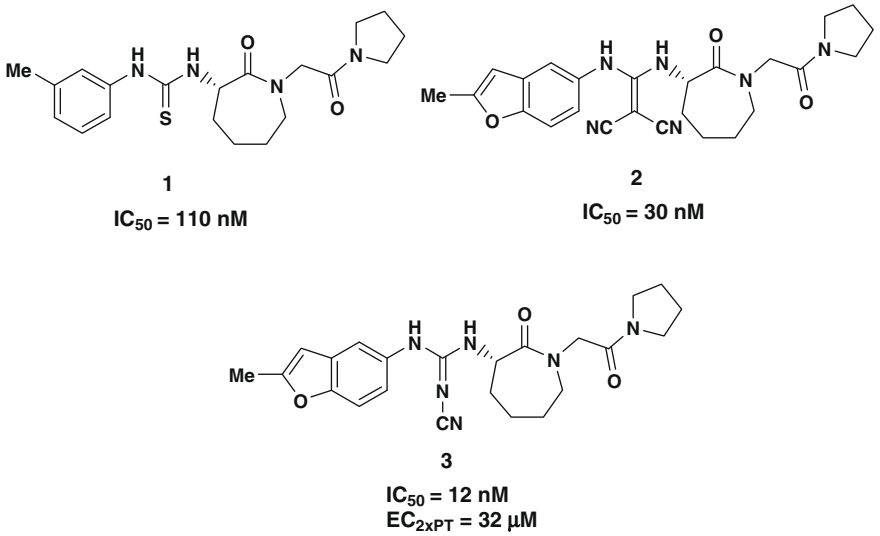
Several selective and orally active FXa inhibitors, such as rivaroxaban,[5] razaxaban and apixaban,[6] LY517717,[7] YM150,[8] DU-176b,[9] have progressed into advanced clinical trials for the prevention and treatment of thromboembolic diseases.

We have previously reported several series of caprolactam based FXa inhibitors containing a thiourea **1**,[10] a ketene aminal **2**,[11] and a cyanoguanidine **3**[12] as linkers of P1 and P4 pharmacophores (Scheme 1). While this group of compounds includes selective and orally active FXa inhibitors, in general their in vivo efficacy has been limited by their moderate in vitro potency and high plasma protein binding.[13] In this Letter, we first describe the new SAR findings for a range of substituents at the central guanidine nitrogen in the *N*,*N*′-disubstituted guanidine series. We then disclose a detailed SAR investigation of aroylguanidines, which led to compound **22** with potent anticoagulation activity (IC_{50} = 4 nM, $EC_{2\times PT}$ = 7 μM).[14] We next present the X-ray structure of **22** bound to FXa and further SAR investigation around this molecule to significantly reduce its CYP3A4 inhibition activity. Finally, we disclose the pharmacokinetic parameters and in vivo characterizations of compound **36** (BMS-344577), an optimized compound in this series which progressed to advanced preclinical development.

The cyanoguanidine compound **3** was chosen as the new starting lead since it has the optimized P1 and P4 pharmacophores attached to the central guanidine-caprolactam core.[12] We first surveyed the SAR of the central guanidine nitrogen substituent and selected analogs are shown in Table 1. In general, the cyano group can be replaced with a variety of electron withdrawing groups (**4**–**12**) without significant loss of inhibitory potency against human FXa (IC_{50}). However, the concentrations to double the prothrombin time ($EC_{2\times PT}$) are on the order of 10^3-fold higher

* Corresponding author. Tel.: +1 609 818 4124; fax: +1 609 818 3450.
E-mail address: yan.shi@bms.com (Y. Shi).
† Present address: Hoffman-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA.
‡ Present address: Wyeth Research, 401N Middletown Road, Pearl River, NY 10965, USA.

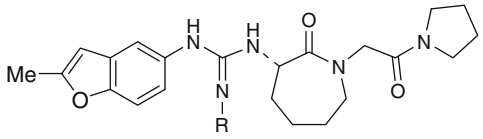
0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.084



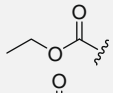
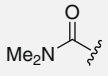
Scheme 1. The thiourea **1**, ketene aminal **2** and cyanoguanidine **3**.

than the corresponding IC_{50}s for these compounds, and all of them are less potent than compound **3** in $EC_{2\times PT}$. For example, although the nitroguanidine **4**, sulfonylguanidines **5** and **7**, and sulfamoylguanidine **6** have similar IC_{50}s against FXa as **3**, their $EC_{2\times PT}$s (all >50 μM) are weaker compared to **3** (32 μM). Compounds with a carbonyl group attached to the central guanidine nitrogen, including acetylguanidine **8**, ethoxylcarbonylguanidine **9**, carbamoylguanidine **10** and **11** are all less potent than **3** both in IC_{50} and $EC_{2\times PT}$; only the benzoylguanidine **12**[15] shows twofold improvement in IC_{50} (6 nM) compared to **3**.

Table 1
General survey of central guanidine nitrogen substituents



3-12

Compd	R	IC_{50}[a] (nM)	$EC_{2\times PT}$[b] (μM)
3	NC–	12	32
4	O_2N–	25	>50
5	MeO_2S–	10	60
6	H_2NO_2S–	12	>50
7	PhO_2S–	24	>50
8		17	70
9		32	34
10		28	60
11		15	55
12		6	48

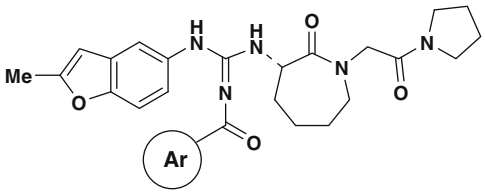
[a] IC_{50} values are measured against Human FXa utilizing the cleavage of a synthetic substrate S-2222.
[b] Concentration of inhibitor required to double the prothrombin based clotting time in human plasma. Data are the average of two independent determinations.

We thus focused on replacing the benzoyl group in **12** with a variety of substituted benzoyl groups and with different heteroaroyls to further improve the anti-FXa activity. Selected analogs are listed in Table 2 to illustrate the SAR. Substituents on the benzoyl group strongly impact the anti-FXa activities both in IC_{50} and $EC_{2\times PT}$. Substituents at the *ortho*-position of the benzoyl group have a detrimental effect and lead to significant losses in anti-FXa activity (data not shown). For *meta*-substituted analogs, we found that while compounds with less polar substituents (**13** and **14**) have similar anti-FXa activities as **12**, the more polar analogs (**15** and **16**) exhibit improved $EC_{2\times PT}$ values but are less potent in IC_{50} compared to **12**. The *para*-substituted benzoylguanidines (compounds **17**–**20**) are the most potent analogs in the substituted benzoylguanidine series with more polar analogs having significantly improved $EC_{2\times PT}$ values compared to **12**. For example, the 4′-methoxycarbonyl benzoylguanidine (**17**, *c*log *P* = 4.6) has an IC_{50} = 6 nM and $EC_{2\times PT}$ = 18 μM; the more polar amide analogs **19** and **20** (*c*log *P* = 3.12 and 3.07) both have IC_{50} = 4 nM and $EC_{2\times PT}$ = 4 and 5 μM, respectively.

Changing the benzoyl group in **12** to a heteroaroyl also resulted in improvement in $EC_{2\times PT}$ (compounds **21**–**27**), although compounds with a nitrogen atom adjacent to the aroyl carbonyl group usually are less potent (compounds **21**, **24** and **28**) in the enzymatic assay (IC_{50}). For example, the picolinoyl guanidine **21** (IC_{50} = 55 nM, $EC_{2\times PT}$ = 16 μM) is about ninefold less potent in IC_{50} than its benzoyl analog **17** (IC_{50} = 6 nM, $EC_{2\times PT}$ = 18 μM), but both exhibit similar $EC_{2\times PT}$ values. The nicotinoyl guanidine **22** (IC_{50} = 4 nM, $EC_{2\times PT}$ = 7 μM) and isonicotinoyl guanidine **23** (IC_{50} = 3 nM, $EC_{2\times PT}$ = 16 μM) are more potent than **12**, with compound **22** having a better $EC_{2\times PT}$. Most of the five-membered heteroaroyl guanidines (**25**, **26** **27**) have similar IC_{50} values as **12**, along with slightly improved $EC_{2\times PT}$ values. The more polar 2-methyl-2*H*-5-tetrazoloyl guanidine **28** is about ninefold less potent in IC_{50} (56 nM) but still has slightly improved $EC_{2\times PT}$ (28 μM) compared to **12**.

To understand the binding mode of aroylguanidines in FXa, an X-ray crystal structure of compound **22** bound to FXa was determined at 1.9 Å resolution (PDB entry 3K9X). The structure is shown in Figure 1.[16] The overall binding motif is similar to that observed for compound **3**.[12] The nicotinoyl guanidine adopts an anti-syn conformation[12] with the nicotinoyl group anti to the 2-methyl

Table 2
SAR of the substituted benzoylguanidines and heteroaroylguanidines



13-27

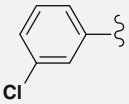
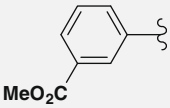
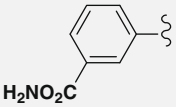
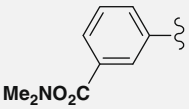
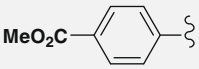
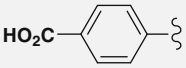
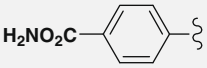
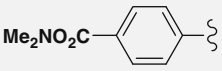
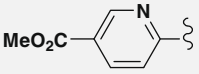
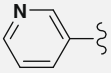
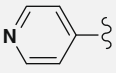
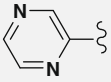
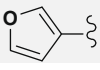
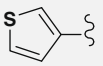
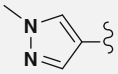
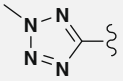
Compd	Ar	IC_{50}[a] (nM)	$EC_{2\times PT}$[b] (μM)
13		7	>50
14		5	41
15		18	7
16		10	8
17		6	18
18		2	5
19		3	5
20		3	4
21		55	16
22		4	7
23		3	16
24		50	18
25		4	33

Table 2 (*continued*)

Compd	Ar	IC_{50}[a] (nM)	$EC_{2\times PT}$[b] (μM)
26		6	19
27		9	11
28		56	28

[a] IC_{50} values are measured against Human FXa utilizing the cleavage of a synthetic substrate S-2222.
[b] Concentration of inhibitor required to double the prothrombin based clotting time in human plasma. Data are the average of two independent determinations.

benzofuran group, and the nicotinoyl group is slightly twisted out of the guanidine plane. Key interactions between **22** and FXa include: (1) the 2-methyl benzofuran group fills the hydrophobic S1 pocket, making contact with Tyr228 at the pocket base (3.4 Å from CH_3 to C–OH); (2) the acylpyrrolidine occupies the hydrophobic S4 pocket bounded by Tyr99, Trp215 and Phe174, with the pyrrolidine ring parallel to the indole of Trp215; (3) the caprolactam carbonyl forms a hydrogen bond with N*H* of Gly216 ($d_{N,O}$ = 3.2 Å); (4) the nicotinoyl carbonyl oxygen contacts the disulfide bond between Cys191 and Cys220, in addition to participating in an intramolecular hydrogen bond with the 2-methyl benzofuran aminal N*H* ($d_{N,O}$ = 2.8 Å) which fixes the anti-syn conformation of the guanidine substituent; (5) the pyridoyl group has an edge to face interaction on one side with the Glu147 side chain; and the opposite side (anti to the carbonyl oxygen) is close to the methylene groups on the caprolactam ring. This is consistent with the observation that compounds with nitrogen at the *ortho*-position (to the carbonyl group) of the aroylguanidine ring (compounds **21**, **24**, **28**) are generally less potent than **12**; there are unfavorable electrostatic interactions between the heteroatom N and the Glu147 side chain if the heteroatom is on the same side

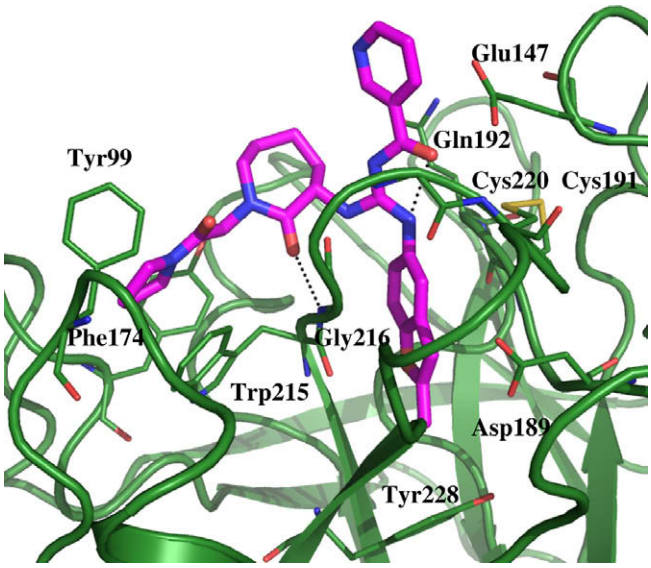


Figure 1. Crystal structure of **22** bound in FXa. The hydrogen bond between the ligand's lactam carbonyl and Gly216 backbone amide N*H* (3.2 Å), and the intramolecular hydrogen bond between the nicotinoyl carbonyl and 2-methyl benzofuran aminal N*H* ($d_{N,O}$ = 2.8 Å) are highlighted. Figure was created using PyMOL (http://www.pymol.org).

as the carbonyl oxygen, and there is an increased desolvation penalty upon binding if the heteroatom is anti to the carbonyl oxygen. Also, the linking amide of the nicotinoyl group has an offset face-to-face interaction with the Gln192 side chain. It appears that substituents at the far end of the aroyl carbonyl group are largely solvent-exposed and variations of these substituents can provide compounds with improved physicochemical properties without strongly impacting their FXa inhibitory activity (IC_{50}) (compounds **17**–**20**).

While compounds **19** and **20** have efficacy similar to **3** in prolonging prothrombin time when administered as an intraduodenal injection in anesthetized rats,[17] compound **22** is much more effective (Fig. 2). A 30 mg/kg intraduodenal injection of **22** increased prothrombin time by a factor of $\geqslant$3.2 for up to 120 min, compared to a factor of $\geqslant$1.8 by compound **3**.[12] However, the improved anticoagulant effect of **22** is accompanied by an unacceptable level of CYP activity (Table 3). Compound **22** is a strong inhibitor of CYP3A4 (IC_{50} = 0.3 μM), along with weaker inhibition activities against CYP2C9 (IC_{50} = 10 μM) and CYP2C19 (IC_{50} = 8 μM). Drugs with CYP inhibition activities may influence the pharmacokinetics of other co-administered drugs and is a major cause for drug–drug interactions. We hypothesized that the CYP inhibition activity of **22** is associated with the pyridine nitrogen-heme interaction, and that substituents adjacent to the pyridine nitrogen may attenuate this interaction either sterically or electronically. Thus, further SAR investigations to eliminate the CYP inhibition activities were concentrated on substituted nicotinoyl groups and results are summarized in Table 3.

As expected from the SAR of substituted benzoylguanidine series and the analyses of the X-ray structure of **22** bound to FXa (vide supra), all the 5- and 6-substituted nicotinoyl guanidines are potent FXa inhibitors (compounds **29**–**36**). The N-oxide **29** has diminished CYP2C9 and CYP2C19 inhibition activity, but remains a potent CYP3A4 inhibitor (IC_{50} = 0.9 μM). The addition of a substituent on the carbon adjacent to the pyridine nitrogen attenuates CYP inhibition activities and the effect appears to be mostly steric. Thus, while the 5-(aminocarbonyl) nicotinoyl guanidine **30** has a similar CYP3A4 inhibition activity (IC_{50} = 0.4 μM) as **22**, the 6-(aminocarbonyl) nicotinoyl guanidine **34** has much weaker CYP3A4 inhibition activity (IC_{50} = 18 μM). In addition, all 6-substituted nicotinoyl guanidines have weaker or diminished CYP3A4 activities. For example, while the less sterically hindered 6-methyl- and 6-chloro-analogs are weaker CYP3A4 inhibitors (IC_{50} = 7 and 8 μM for **31** and **32**, respectively) compared to **22**, the more bulky

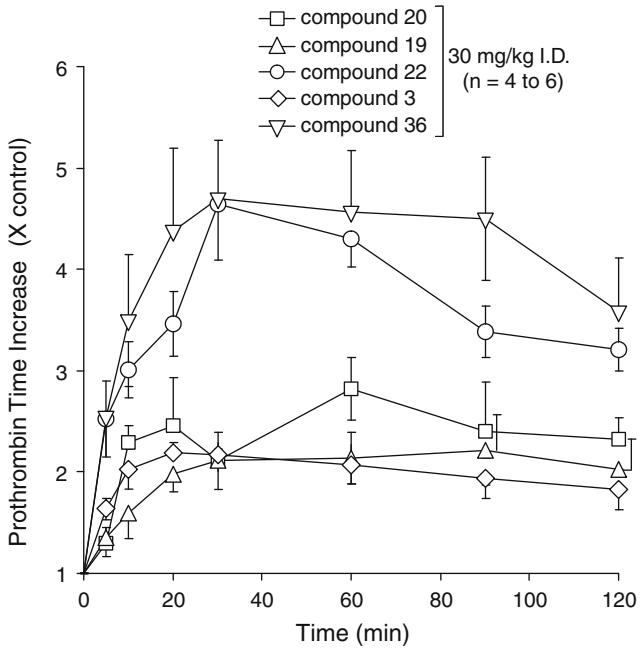
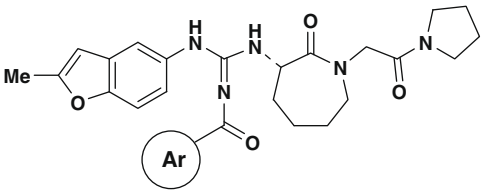
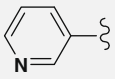
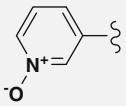
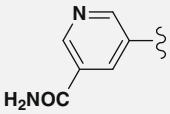
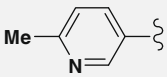
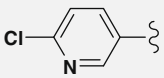
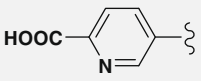
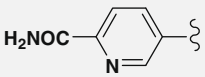
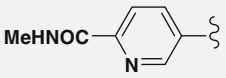
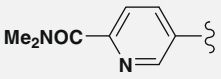


Figure 2. Prolongation of prothrombin time by intraduodenal injection of compounds **3**, **19**, **20**, **22** and **36**.

Table 3
SAR of substituted nicotinoyl guanidines



22, 29-36

Compd	Ar	IC_{50}[a] (nM)	$EC_{2\times PT}$[b] (μM)	IC_{50} for CYP (μM)		
				2C9	2C19	3A4[c] (BFC)
22		4	7	10	8	0.3
29		10	3	122	128	0.9
30		17	7	10	37	0.4
31		4	12	9	27	7
32		5	14	12	22	8
33		3.3	6.8	122	47	87
34		12	5.7	22	13	18
35		6	3	24	12	18
36 (BMS-344577)		9	2.5	43	51	39

[a] IC_{50} values are measured against Human FXa utilizing the cleavage of a synthetic substrate S-2222.
[b] Concentration of inhibitor required to double the prothrombin based clotting time in human plasma. Data are the average of two independent determinations.
[c] 7-Benzyloxy-4-trifluoromethylcoumarin.

Table 4
Pharmacokinetic parameters for **36** (n = 3, fasted)[a].

Parameter (unit)	Rat	Dog	Monkey
iv dose (mg/kg)	10	2	10
CL_{total} (mL/min/kg)	27	1.9	11
V_{dss} (L/kg)	3.3	0.96	2.3
$t_{1/2}$ (h)	1.6	8.6	14
MRT (h)	2	9	4
po dose (mg/kg)	10	2	10
C_{max} (μg/mL)	2.2	2.8	2.1
T_{max} (h)	0.6	1.3	1.8
BA (%)	55	77	28

[a] Dosing vehicle: 10 mg/mL in PEG400/water (1:1, v/v).

Table 5
Selectivity profile of **36**.

Human enzymes	K_i[a] (nM)
Factor Xa	5.2
Trypsin	>10,000
Thrombin	2300
Tryptase	>30,000
Activated protein C	>18,000
Factor XIa	>18,000
Human TF:FVIIa	>15,000
u-PA	>7800
Plasmin	>16,000
t-PA	665

[a] K_i's are calculated from the IC_{50} values assuming competitive inhibition versus the low molecular weight synthetic substrates using the relationship, $K_i = IC_{50}/(1 + [S]/K_m)$, where S is the substrate concentration in the assay and K_m is the Michaelis constant for that substrate. Data are the average of two independent determinations.

6-substituted nicotinoyl guanidines (**33**–**36**) show further attenuated CYP3A4 inhibition activities. The 6-(dimethylcarbamoyl) nicotinoyl guanidine **36** has the best overall anti-FXa activity (IC_{50} = 9 nM, $EC_{2\times PT}$ = 2.5 μM) and CYP activity profiles (IC_{50} for 3A4 (BFC) = 39 μM, IC_{50} >40 μM for 2D6, 2C9, 2C19, 1A2, 3A4 (BzRES)).

In addition to its relatively clean CYP inhibition profile, compound **36** shows no significant activity in hERG, sodium channel, PXR and HHA assays.[18] It is significantly less plasma protein bound (88% in human) compared to compound **3** (98.7%). Furthermore, compound **36** was not mutagenic or clastogenic in an exploratory Ames test,[19] and was inactive in an in vitro assessment of activity against 35 receptors and enzymes at 10 μM (Cerep, Paris, France).[20] It also displays good oral bioavailability and exhibits an excellent pharmacokinetic profile in several animal species. Table 4 shows the pharmacokinetic parameters of **36** in rat, dog and monkey.

Compound **36** is a selective FXa inhibitor relative to related trypsin-like serine proteases as shown in Table 5. It shows excellent selectivity for human FXa: >100-fold versus tissue type plasminogen activator, >440-fold versus thrombin, and >1500-fold relative to other human serine proteases tested. In addition, compound **36** was effective in prolonging prothrombin time in rats ($EC_{2\times PT}$ = 3.6 μM, 72% plasma protein bound in rats).[17] A 30 mg/kg intraduodenal injection of **36** increased prothrombin time by a factor of ⩾3.6 for up to 120 min as shown together with compounds **3**, **19**, **20**, and **22** (Fig. 2). It was efficacious in rat models of venous and arterial thrombosis induced by topical application of $FeCl_2$.[21] In a vena cava thrombosis model, compound **36** inhibited thrombus weight by 90% at an oral dose of 15 mg/kg and had an oral ED_{50} = 2 mg/kg. In a carotid artery thrombosis model, compound **36** was co-administered with aspirin and preserved

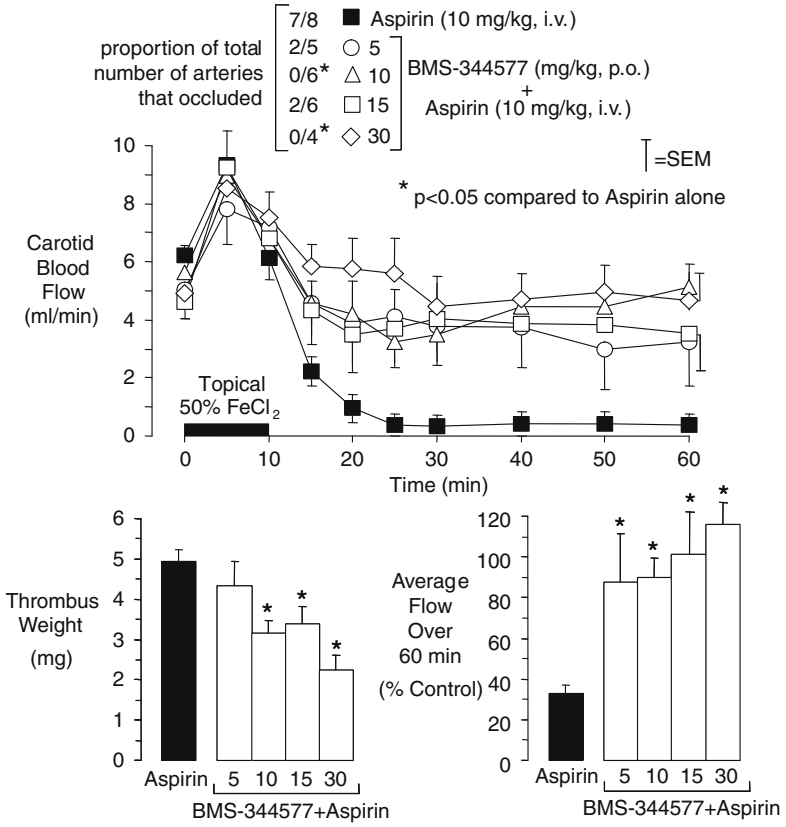
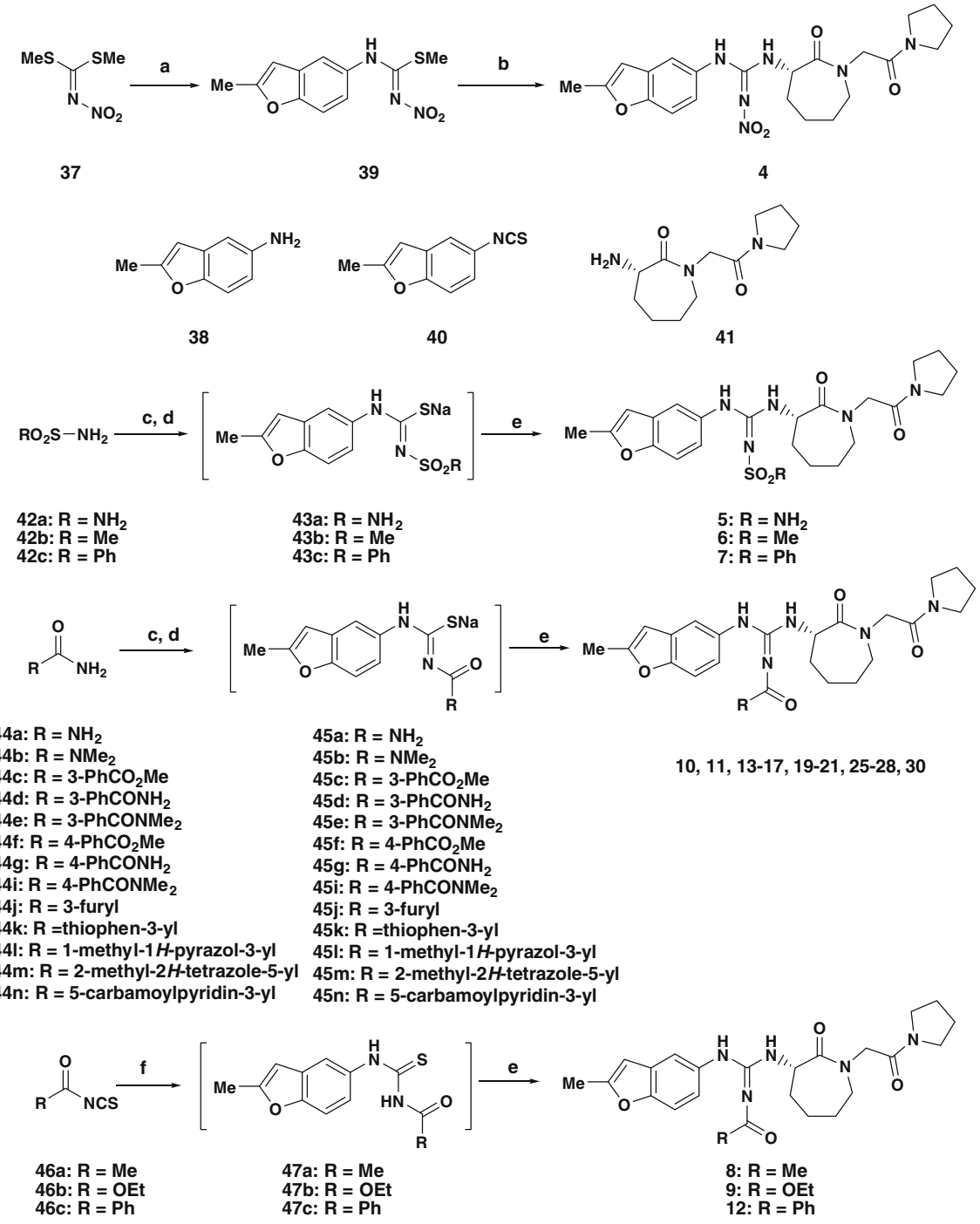


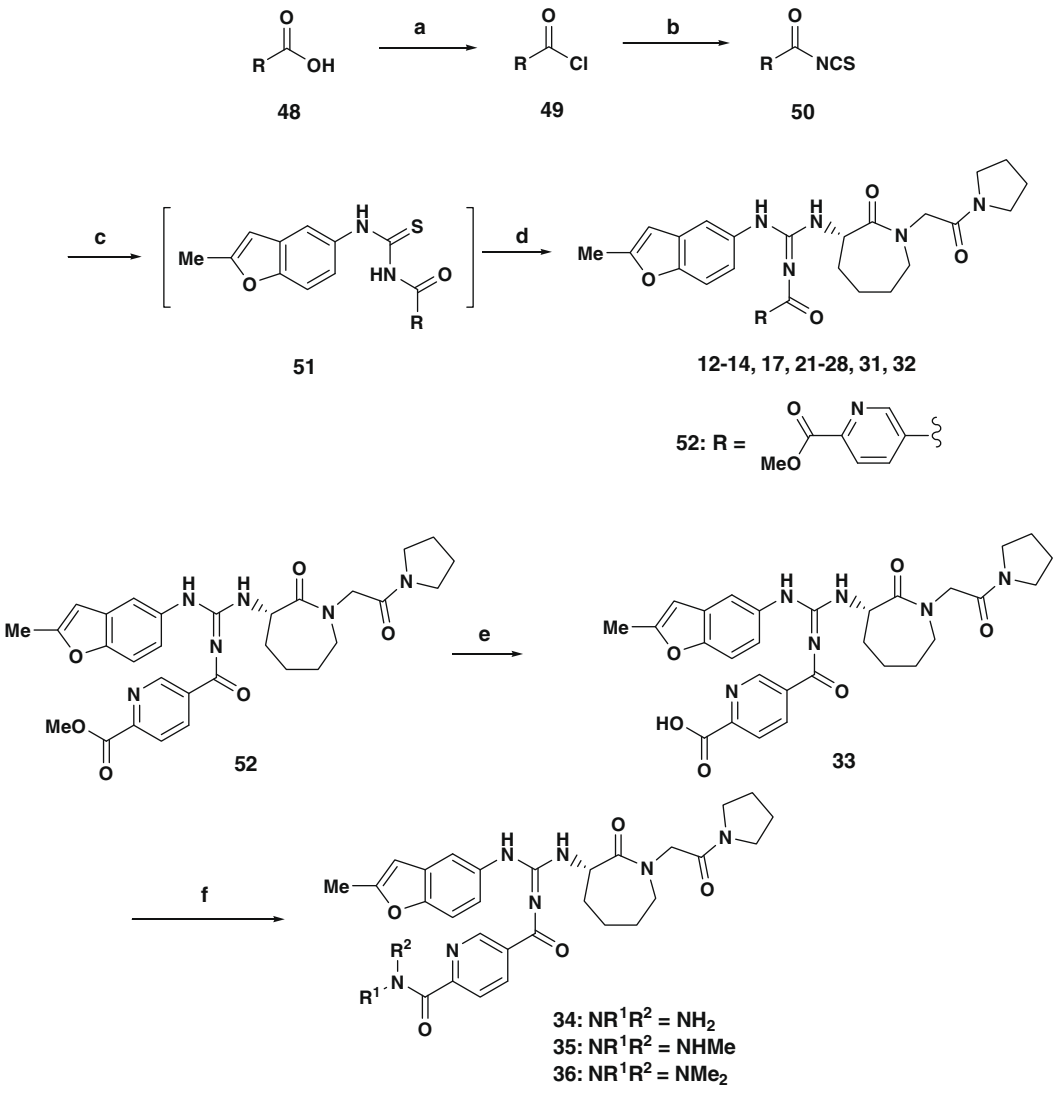
Figure 3. Inhibition of arterial thrombosis in aspirin-treated rats by oral administration of BMS-344577. Vehicle (PEG-400, 1 mL/kg) or BMS-344577 was administered po 1 h before and aspirin was injected iv 10 min before $FeCl_2$ application to the carotid artery. Significant differences relative to vehicle were determined using analysis of variance with Dunnett's test for thrombus weight and average blood flow (area-under-flow curve). The Fisher exact test was used to compare differences in frequency of occlusion.

vessel patency and baseline flow in addition to decreasing thrombus weight (Fig. 3). Oral doses of 10, 15 and 30 mg/kg decreased arterial thrombus weight by 36%, 31% and 54%, respectively. The oral ED_{50} for compound **36** in this artery thrombosis model is 10 mg/kg. On the basis of its potency, its excellent in vitro liability profile, favorable pharmacokinetics and pharmacodynamics in animal models, compound **36** was selected for further evaluation in preclinical animal toxicity studies.

Scheme 2 shows the syntheses of compounds **4**–**17**, **19**–**21**, **25**–**28** and **30**. The nitroguanidine **4** was synthesized by the stepwise displacement of both methylthio groups of dimethyl nitrocarbonimidodithioate **37** in 75% overall yield. Compounds **5**–**7** were synthesized in a one-pot sequence we developed for *N*,*N*′-disubstituted sulfamoylguanidines and sulfonylguanidines.[22] For example, treatment of sulfamide **42a** with sodium hydride and reaction of the resulting anion with 2-methylbenzofuran-7-isothiocyanate **40**[11] afforded an intermediate thioamide anion (**43a**). Subsequent reaction of **43a** with caprolactam amine **41**[10] and EDCI provides **5** (R = NH_2) in 90% yield. Compounds **10** and **11** were obtained through a similar one-pot procedure we developed for *N*,*N*′-disubstituted acylguanidines.[23] Thus the urea **44a** or **44b** was treated with sodium hydride, reacted with 2-methylbenzofuran-7-isothio-



Scheme 2. The synthesis of **4**–**17**, **19**–**21**, **24**–**28**, **30**. Reagents and conditions: (a) **38**, DMF, 30 min; (b) **41**, 60 °C (75% yield over two steps); (c) NaH, DMF; (d) **40**, 60 °C; (e) **41**, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrchloride (EDCI) (10–91% yield over two steps); (f) **38**, 60 °C, DMF.



Scheme 3. The synthesis of aroylguanidines **12**–**14**, **17**, **21**–**28**, **31**–**36**. Reagents and conditions: (a) $(COCl)_2$, DMF (cat), CH_2Cl_2; (b) KSCN, acetone; (c) **38**, 60 °C, DMF; (d) **41**, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrchloride (EDCI) 68% yield over four steps; (e) LiOH, THF, H_2O, 95% yield; (f) NH_4Cl or HNR^1R^2, EDCI (83–95% yield).

cyanate **40** to provide the anion intermediates **45a** or **45b**. Further reaction with caprolactam amine **41** and EDCI provided **10** or **11** in 10% and 55% yield, respectively. Compounds **13**–**17**, **19**–**21**, **25**–**28** and **30** were synthesized through a similar sequence. The compound **18** was obtained by hydrolysis of **17** with LiOH/THF–H_2O in 95% yield. Compounds **8**, **9** and **12** were synthesized from commercial available isothiocyanates **46a–c** through the acylthiourea or aroylthiourea intermediates **47a–c** in 10–91% yields.

Alternatively, aroylguanidines (compounds **12**–**14**, **17**, **21**–**28**, **31** and **32**) can also be prepared from their corresponding acids through aroylthiourea intermediates in a one-pot sequence as illustrated in Scheme 3 for compound **52**. Treatment of the 6-(methoxycarbonyl)nicotinic acid **48** with oxalic chloride generated its acid chloride **49**, which was reacted with potassium isothiocyanate to afford the aroylisothiocyanate **50**. Further reaction of **50** with 2-methylbenzofuran-5-amine **38** provided the aroylthiourea **51**, which was reacted with amine **41** with the assistance of EDCI to furnish the aroylguanidine **52** in 68% yield over four steps. Hydrolysis of **52** afforded the acid **33** in 95% yield, which is then coupled with amines to afford **34**–**36** in 83–95% yield. The N-oxide analog **29** was obtained by mCPBA oxidation of **22** in 65% yield.

In summary, we have discovered a series of aroylguanidine-based lactam derivatives as a novel class of orally bioavailable FXa inhibitors. The SAR investigation led to the discovery of the nicotinoyl guanidine **22** as a potent FXa inhibitor (FXa IC_{50} = 4 nM, $EC_{2\times PT}$ = 7 μM). Further SAR studies to attenuate the unwanted CYP activity of **22** resulted in the discovery of 6-(dimethylcarbamoyl) nicotinoyl guanidine **36** (BMS-344577, IC_{50} = 9 nM, $EC_{2\times PT}$ = 2.5 μM), which was found to be a selective, orally efficacious FXa inhibitor with an excellent in vitro liability profile, favorable pharmacokinetics and pharmacodynamics in animal models.

Acknowledgments

We thank Drs. P. T. Cheng, P. Y. S. Lam, R. R. Wexler for insightful review of this work.

References and notes

1. (a) Murray, C. J. L.; Lopez, A. D. *Lancet* **1997**, *349*, 1269; (b) White, R. H. *Circulation* **2003**, *107*, I4.
2. (a) Hirsh, J. *N. Eng. J. Med.* **1991**, *324*, 1565; (b) van, A. H.; Bode, C.; Darius, H.; Diehm, C.; Encke, A.; Gulba, D. C.; Haas, S.; Hacke, W.; Puhl, W.; Quante, M.; Riess, H.; Scharf, R.; Schellong, S.; Schror, T.; Schulte, K. L.; Tebbe, U. *Clin. Appl. Thromb. Hemost.* **2001**, *7*, 195; (c) Weitz, J. I. *N. Eng. J. Med.* **1997**, *337*, 688.
3. (a) Lassen, M. R.; Laux, V. *Vasc. Health Risk Manag.* **2008**, *4*, 1373; (b) Harenberg, J. *Semin. Thromb. Hemost.* **2008**, *34*, 779; (c) Agnelli, G.; Becattini, C. *Semin. Thromb. Hemost.* **2006**, *32*, 793; (d) Haas, S. *J. Thromb. Thrombolysis*

2008, *25*, 52; (e) Shirk, R. A.; Vlasuk, G. P. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1895.
4. For recent reviews on FXa inhibitors, see: (a) Kunitada, S.; Nagahara, T.; Hara, T. *Handbook Exp. Pharmacol.* **1998**, 397; (b) Vacca, J. P. *Annu. Rep. Med. Chem.* **1998**, *33*, 81; (c) Zhu, B.-Y.; Scarborough, R. M. *Curr. Opin. Cardiov, Pulmon, Ren. Invest. Drugs* **1999**, *1*, 63; (d) Ewing, W. R.; Pauls, H. W.; Spada, A. P. *Drugs Future* **1999**, *24*, 771; (e) Fevig, J. M.; Wexler, R. R. *Annu. Rep. Med. Chem.* **1999**, *34*, 81; (f) Betz, A. *Exp. Opin. Ther. Patents* **2001**, *11*, 1007; (g) Walenga, J. M.; Jeske, W. P.; Hoppensteadt, D.; Fareed, J. *Curr. Opin. Invest. Drugs* **2003**, *4*, 272; (h) Gould, W. R.; Leadley, R. J. *Curr. Pharm. Des.* **2003**, *9*, 2337; (i) Quan, M. L.; Smallheer, J. M. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 460.
5. (a) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900; (b) Eriksson, B. I.; Borris, L.; Dahl, O. E.; Haas, S.; Huisman, M. V.; Kakkar, A. K.; Misselwitz, F.; Kaelebo, P. *J. Thromb. Haemost.* **2006**, *4*, 121; (c) Kubitza, D.; Becka, M.; Wensing, G.; Voith, B.; Zuehlsdorf, M. *Eur. J. Clin. Pharmacol.* **2005**, *61*, 873; (d) Turpie, A. G. G.; Fisher, W. D.; Bauer, K. A.; Kwong, L. M.; Irwin, M. W.; Kalebo, P.; Misselwitz, F.; Gent, M. *J. Thromb. Haemost.* **2005**, *3*, 2479.
6. (a) Pinto, D. J. P.; Orwat, M. J.; Koch, S.; Rossi, K. A.; Alexander, R. S.; Smallwood, A.; Wong, P. C.; Rendina, A. R.; Luettgen, J. M.; Knabb, R. M.; He, K.; Xin, B.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2007**, *50*, 5339; (b) Wong, P. C.; Crain, E. J.; Xin, B.; Wexler, R. R.; Lam, P. Y.; Pinto, D. J.; Luettgen, J. M.; Knabb, R. M. *J. Thromb. Haemost.* **2008**, *6*, 820; (c) Lassen, M. R.; Davidson, B. L.; Gallus, A.; Pineo, G.; Ansell, J.; Deitchman, D. *J. Thromb. Haemost.* **2007**, *5*, 2368; (d) APPRAISE Steering Committee and Investigators *Circulation* **2009**, *119*, 2877; (e) Quan, M. L.; Lam, P. Y. S.; Han, Q.; Pinto, D. J. P.; He, M. Y.; Li, R.; Ellis, C. D.; Clark, C. G.; Teleha, C. A.; Sun, J.-H.; Alexander, R. S.; Bai, S.; Luettgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **2005**, *48*, 1729.
7. (a) Agnelli, G.; Haas, S. K.; Krueger, K. A.; Bedding, A. W.; Brandt, J. T. *ASH Annu. Meeting Abstracts* **2005**, *106*, 278; (b) Hampton, T. *J. Am. Med. Assoc. (JAMA)* **2006**, *295*, 743; (c) Liebeschuetz, J. W.; Jones, S. D.; Wiley, M. E.; Young, S. C. *Struct.-Based Drug Discovery* **2006**, 173.
8. (a) Iwatsuki, Y.; Shigenaga, T.; Moritani, Y.; Suzuki, M.; Ishihara, T.; Hirayama, F.; Kawasaki, T. *Blood* **2006**, *108*, Abst 911; (b) Eriksson, B. I.; Turpie, A. G. G.; Lassen, M. L.; Prins, M. H.; Agnelli, G.; Kalebo, P.; Gaillard, M. L.; Meems, L. *J. Thromb. Haemost.* **2007**, *5*, 1660.
9. (a) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Hirokawa, Y.; Furugori, T.; Nagahara, T. *J. Med. Chem.* **2004**, *47*, 5167; (b) Zafar, M. U.; Gaztanga, J.; Velez, M.; Vorchheimer, D.; Choi, B.; Viles-Gonzalez, J.; Moreno, P.; Fuster, V.; Badimon, J. *J. Am. Coll. Cardiol.* **2006**, *47*, Abst 288A.
10. Bisacchi, G. S.; Stein, P. D.; Gougoutas, J. Z.; Hartl, K. S.; Lawrence, R. M.; Liu, E. C.; Pudzianowski, A. T.; Schumacher, W. A.; Sitkoff, D.; Steinbacher, T. E.; Sutton, J.; Zhang, Z.; Seiler, S. M. *Lett. Drug Des. Discovery* **2005**, *2*, 625.
11. Shi, Y.; Zhang, J.; Stein, P. D.; Shi, M.; O'Connor, S. P.; Bisaha, S. N.; Li, C.; Atwal, K. S.; Bisacchi, G. S.; Sitkoff, D.; Pudzianowski, A. T.; Liu, E. C.; Hartl, K. S.; Seiler, S. M.; Youssef, S.; Steinbacher, T. E.; Schumacher, W. A.; Rendina, A. R.; Bozarth, J. M.; Peterson, T. L.; Zhang, G.; Zahler, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5453.
12. Shi, Y.; Zhang, J.; Shi, M.; O'Connor, S. P.; Bisaha, S. N.; Li, C.; Sitkoff, D.; Pudzianowski, A. T.; Huang, C.; Chong, S.; Klei, H.; Kish, K.; Yanchunas, J.; Liu, E. C.-K.; Hartl, K. S.; Seiler, S. M.; Steinbacher, T. E.; Schumacher, W. A.; Atwal, K. S.; Stein, P. D. *Bioorg. Med. Chem. Lett.* **2009**, doi:10.1016/j.bmcl.2009.06.014.
13. Several literature reports suggest that highly nonpolar coagulation factor inhibitors can have a tendency toward high protein binding, and this tendency could be associated with the relatively high concentrations required to achieve efficacy in plasma-based assays and presumably in vivo. Compound **3** has the concentrations to double the prothrombin time ($EC_{2\times PT}$ = 32 μM) on the order of 10^3 times higher than its corresponding IC_{50} (12 nM), probably due to its high plasma protein bounding value (98.7%) in human plasma. See Ref. 14.
14. For detailed assay methods, see: Shi, Y.; Sitkoff, D.; Zhang, J.; Klei, H. E.; Kish, K.; Liu, E. C.-K.; Hartl, K. S.; Seiler, S. M.; Chang, M.; Huang, C.; Youssef, S.; Steinbacher, T. E.; Schumacher, W. A.; Grazier, N.; Pudzianowski, A.; Apedo, A.; Discenza, L.; Yanchunas, J., Jr.; Stein, P. D.; Atwal, K. S. *J. Med. Chem.* **2008**, *51*, 7541.
15. O'Connor, S. P.; Atwal, K. S.; Li, C.; Liu, E. C.; Seiler, S. M.; Shi, M.; Shi, Y.; Stein, P. D.; Wang, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4696.
16. The X-ray crystal structure coordinates of **22** in human Factor Xa have been deposited in the Protein Data Bank (PDB code 3K9X).
17. Compound testing protocol: male Sprague-Dawley rats (320 to 390 g) were fasted overnight and then anesthetized with sodium pentobarbital (50 mg/kg, ip). The trachea was cannulated with PE-205 tubing to assure airway patency. Catheters (PE-50) were placed in the right carotid artery for blood withdrawal and in the left jugular vein for saline infusion (25 μL/min throughout the experiment) and for iv dosing of test compound. For intestinal delivery (id) of test compound, the small intestine was exposed via a midline laparotomy and a dosing catheter (PE-50) was inserted into the duodenum at the level of the bile duct. Animals received compound by id (30 mg/kg) and iv (10 mg/kg) route in a 1 mg/mL volume of vehicle (10% ethanol/90% PEG300) followed by a 0.3 mL saline flush. Arterial blood samples (0.5 mL) were withdrawn into 3.8% Na-citrate (1/10; v/v) for ex vivo prothrombin time (PT) determination before (0 min control), and at 30, 60, 90 and 120 min after test compound dosing. The PT were was measured using a Amelung KC4A micro coagulation analyzer (Heinrich Amelung GmbH, Lemgo, Germany) and the standard procedure described for Dade Thromboplastin-C reagent (Baxter Healthcare Corp., Miami, FL).
18. Compound **36** has following profile: CYP (inhibition) 3A4-BFC IC_{50} = 39 μM, others (3A4-BZR, 1A2, 2B6, 2C8, 2C9, 2C19, 2D6) all IC_{50}s >40 μM; PXR EC_{50} >25 μM; hERG (flux) IC_{50} >80 μM; HHA (2C19, 2C9, 2D6, 3A4, TC5) all IC_{50} >200 μM.
19. Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, *113*, 173.
20. CEREP in vitro pharmacology screening package including the following assays: A_1 (h), α_1 (non-selective) (r), α_2 (non-selective) (r), β_1 (h), β_2 (h), BZD (central) (r), CCK_A (CCK_1) (h), D_1 (h), D2S (h), AMPA (r), Kainate (r), NMDA (r), H_1 (central) (guinea-pig), H_2 (guinea-pig), MAO-A (r), MAO-B (r), M (non-selective) (r), opiate (non-selective) (r), PCP (r), rolipram (m), 5-HT (non-selective) (r), 5-HT_{1A} (h), 5-HT_{2B} (h), sst (non-selective), GR (h), ERα (h), PR (h), Ca^{2+} channel (L, DHP site) (r), Ca^{2+} channel (L, verapamil site) (r), Na^+ channel (site 2) (r), Cl^- channel (r), NE transporter (h), DA transporter (r), GABA transporter (r), choline transporter (r). For details regarding the specific receptors and experimental conditions see http://www.cerep.fr/Cerep/Users/pages/catalog/binding/catalog.asp.
21. Arterial and venous thrombosis were performed in pentobarbital-anesthetized male Sprague Dawley rats as described in: Schumacher, W. A.; Heran, C. L.; Steinbacher, T. E. *J. Cardiovas. Pharmacol.* **1996**, *28*, 19. Briefly, thrombus weight was determined 1 h after transient topical application of $FeCl_2$ to the vena cava (15% solution for 2 min) or carotid artery (50% solution for 10 min). In both models, Vehicle (PEG-400) or BMS-344577 was administered as a 1-mL/kg gavage at 0 min, rats were anesthetized at 30 min, and $FeCl_2$ was applied at 60 min. In venous thrombosis treatments included Vehicle (n = 5) and BMS-344577 (5, 10, 15, 30 mg/kg; n = 5 per dose level). In arterial thrombosis treatments included Vehicle (n = 8) and BMS-344577 (5, 10, 15, 30 mg/kg; n = 5, 6, 6, 4, respectively) on top of a 10-mg/kg, iv dose of aspirin given 15 min before $FeCl_2$ application. Aspirin was included at its top cyclooxygenase inhibition dose in the rat since it now represents the standard of care for secondary prevention of arterial thrombosis.
22. Zhang, J.; Shi, Y. *Tetrahedron Lett.* **2000**, *41*, 8075.
23. Zhang, J.; Shi, Y.; Stein, P.; Atwal, K.; Li, C. *Tetrahedron Lett.* **2002**, *43*, 57.