Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1060–1064
Bioorganic & Medicinal Chemistry Letters

The discovery of fluoropyridine-based inhibitors of the factor VIIa/TF complex—Part 2

Jeffrey T. Kohrt,* Kevin J. Filipski, Wayne L. Cody, Cuiman Cai, Danette A. Dudley, Chad A. Van Huis, J. Adam Willardsen, Lakshmi S. Narasimhan, Erli Zhang, Stephen T. Rapundalo, Kamlai Saiya-Cork, Robert J. Leadley and Jeremy J. Edmunds

Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48105, USA

Received 6 September 2005; revised 21 October 2005; accepted 21 October 2005
Available online 11 November 2005

Abstract—The activated factor VII/tissue factor complex (FVIIa/TF) is known to play a key role in the formation of blood clots. Inhibition of this complex may lead to new antithrombotic drugs. A fluoropyridine-based series of FVIIa/TF inhibitors was discovered which utilized a diisopropylamino group for binding in the S2 and S3 binding pockets of the active site of the enzyme complex. In this series, an enhancement in binding affinity was observed by substitution at the 5-position of the hydroxybenzoic acid sidechain. An X-ray crystal structure indicates that amides at this position may increase inhibitor binding affinity through interactions with the S1′/S2′ pocket.

The activated factor VII/tissue factor complex (FVIIa/TF) has been shown to play a key role in the formation of blood clots.[1] Theoretically, inhibition of the FVIIa/TF complex formation or inhibition of its active site which is responsible for cleavage of factor X (FX) to activated factor X (FXa) will disable the key extrinsic coagulation pathway, but keep intact the intrinsic pathway for the maintenance of normal hemostasis. This may lead to enhanced antithrombotic drugs with decreased bleeding side effects.[2] Several strategies exist for disruption of the FVIIa/TF complex formation and function,[3] but work still remains incomplete for the development of small molecule, FVIIa/TF active-site inhibitors as anticoagulant therapies.[4]

In a previous publication, we reported on the discovery of a fluoropyridine-based series of FVIIa/TF inhibitors containing 5-substituted hydroxybenzoic acid sidechains.[5] Molecular modeling suggested that a substituent at the 5-position of this sidechain increased inhibitor binding affinity by occupying the S1′/S2′ pocket of the enzyme complex (Fig. 1).[5] This series of inhibitors also utilized a racemic 1-methyl-3-phenyl-propylamino pharmacophore for binding in the S2 and S3 binding pockets, respectively, in the active site of the enzyme complex.

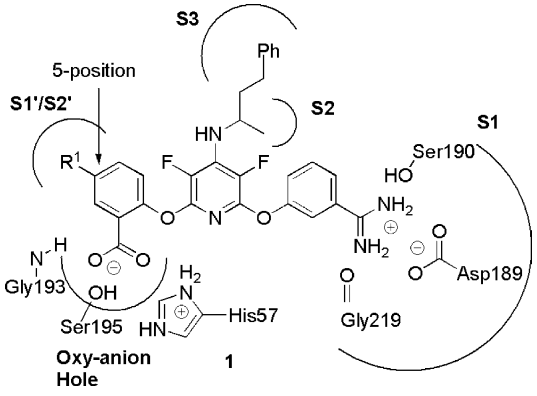


Figure 1. Proposed binding mode for the fluoropyridine-based inhibitors in the active site of the FVIIa/TF complex.

The presence of this racemic amine complicated the isolation of analogs that also contained chiral 5-amidohydroxybenzoic acid sidechains due to the formation of a mixture of diastereomeric final products that could not be easily separated. Also, synthetic complications

Keywords: Factor VIIa inhibitor; Tissue factor; Serine protease inhibitor.
* Corresponding author. Fax: +1 734 622 3909; e-mail: jeffrey.kohrt@pfizer.com

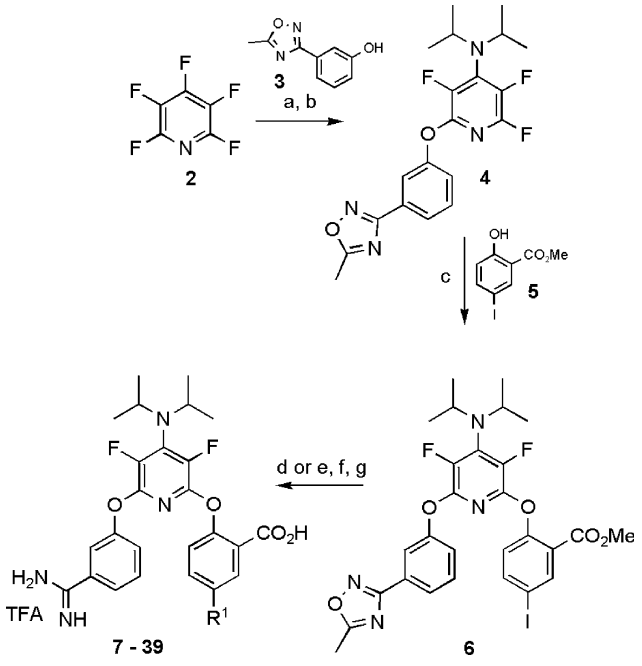
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.076

resulted from the need to protect this primary amine with a *para*-methoxybenzyl-protecting group. This protecting group was necessary so that a more facile route to new TF/VIIa inhibitors with elaborated hydroxybenzoic acid sidechains could be investigated via palladium-catalyzed cross coupling reactions.[5]

Herein, we report the discovery of a series of FVIIa/TF inhibitors based on a fluoropyridine template that utilizes diisopropylamine as a pharmacophore capable of binding in both the S2 and S3 pockets of the FVIIa/TF enzyme complex. This modification extended our ability to identify potent FVIIa/TF inhibitors via the introduction of a wider range of 5-substituted hydroxbenzoic acid sidechains that may bind in the S1′/S2′ pocket of the active site of the FVIIa/TF complex.

The formation of the aryl ether intermediate **4** utilized a two-step procedure where diisopropylamine was first added to pentafluoropyridine **2** in the presence of triethylamine followed in a separate step by the addition of 3-(5-methyl-[1,2,4]oxadiazol-3-yl)-phenol **3**[6] with potassium carbonate (Scheme 1). Subsequent addition of 5-iodo-methyl salicylate **5**[7] and cesium carbonate produced the penta-substituted pyridine **6**. This was the key substrate for the introduction of further functionality onto the 5-position of the hydroxybenzoic acid sidechain.

The formation of the 5-aryl or heteroaryl analogs relied upon a Suzuki coupling of **6** and aryl or heteroarylboronic acids.[8] Alternatively, when the desired boronic acid was not available, Stille coupling conditions using aryl or heteroarylstannanes were utilized.[9] The benzyl sidechain was formed via a Negishi coupling procedure employing benzylzinc chloride.[10] Finally, benzamide formation relied upon the palladium-catalyzed carboxylation of **6** in the presence of carbon dioxide[11] to form a 5-carboxylic acid intermediate, which was subjected to a HATU-mediated[6] coupling reaction with primary amines. Hydrogenation of the oxadiazole intermediate over Raney nickel under acidic conditions revealed the amidine.[6] Saponification of the methyl ester with lithium hydroxide provided analogs **7**–**39**. All inhibitors were isolated by reverse-phase preparative HPLC as their trifluoroacetate salts.



Scheme 1. Reagents and conditions: (a) diisopropylamine, Et_3N, CH_2Cl_2, rt; (b) **3**, K_2CO_3, DMSO, 70 °C; (c) **5**, Cs_2CO_3, DMSO, 80 °C; (d) R^1 = amide; i—CO_2, $Pd(OAc)_2$, K_2CO_3, DMF/H_2O (4:1), 110 °C; ii—amine, HATU[6], Et_3N, DMF; (e) R^1 = aryl: $ArB(OH)_2$, K_3PO_4, $Pd(PPh_3)_4$, DMF, 110 °C or $ArSn(Bu)_3$, $PdCl_2(PPh_3)_4$, CuI, THF, 70 °C or R^1 = benzyl: $PhCH_2ZnCl$, $Pd(PPh_3)_4$, THF, 60 °C; (f) H_2, Raney nickel, MeOH, acetic acid, water; (g) LiOH, MeCN, H_2O, rt.

In a search for new fluropyridine-based FVIIa/TF inhibitors containing non-chiral amines as a P2/P3 pharmacophore, we identified **7** (IC_{50} = 240 nM) as a potential lead (Table 1). This new inhibitor contained diisopropylamine as an alternative for the 1-methyl-3-phenyl-propylamino group that was shown in a previous series to fill the S2 and S3 pockets of the enzyme active site.[5] Although this initial lead was a weak FVIIa/TF inhibitor, it was anticipated that the binding affinity of this series could be enhanced by incorporation of a substituent at the 5-position of the hydroxybenozic acid sidechain. From previous experience, this type of modification led to better binding of the benzoic acid sidechain in the S1′/S2′ pocket of the enzyme (Fig. 1) and provided inhibitors with enhanced inhibition of the FVIIa/TF complex.[5]

Replacing the 5-methoxy substituent of **7** with dimethylamino **8** (IC_{50} = 130 nM) resulted in an increase in binding affinity. When a methylene group was used as a linker to extend the dimethylamino residue deeper into the S1′/S2′ pocket, a large loss in potency was observed for **9**. A much more potent inhibitor was realized by replacing the methyldimethylamino group of **9** with the benzyl group of **10** (73 nM). Further increases in binding affinity were observed with phenyl **11** (36 nM). It was observed that binding affinity was reduced from phenyl **11** when the distal phenyl substituent was replaced with other heteroaryl rings as exemplified in **12**–**15**.

From molecular modeling, it was predicted that the addition of substituents at the 3-position of the distal phenyl ring of **11** would increase binding affinity for this series of inhibitors by extending residues deeper into the S1′/S2′ pocket. Contrary to our model, the 3-substituted analogs **16**–**18** displayed less potency with respect to **11**. Binding affinity was further decreased with analogs such as 2-toluyl **19** and 2-naphthyl **20**.

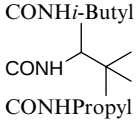
In order to enhance the binding affinity of this series of inhibitors, analogs with amide substitution at the 5-position of the hydroxybenzoic acid sidechain were investigated (Table 2).[5,6] The benzyl-amide **21** (IC_{50} = 47 nM) showed a slight decrease in binding affinity in comparison to the bi-phenyl **11**. Substituted benzyl-amides such as 3-methoxybenzyl-amide **24** (17 nM) and 4-chloroben-

Table 1. FVIIa/TF inhibitors with 5-substituted benzoic acid sidechains

FVIIa/TF inhibitors	R^1	FVIIa/TF IC_{50}[12] (μM)	FIIa IC_{50}[13] (μM)	FXa IC_{50}[14] (μM)[a]
7	OMe	0.24	0.33	62%
8	NMe_2	0.13	0.34	54%
9	CH_2NMe_2	2.4	ND	ND
10	CH_2Ph	0.073	3.1	33%
11	Ph	0.036	0.50	78%
12	3-Thienyl	0.067	0.17	47%
13	2-Pyridyl	0.097	0.81	83%
14	4-Pyridyl	0.19	ND	ND
15	2-Pyrimidyl	0.29	ND	ND
16	Ph (3-CH_3)	0.048	3.1	38%
17	Ph (3-CH_2OH)	0.12	1.1	44%
18	Ph (3-*i*Pr)	0.13	18	39%[b]
19	Ph (2-CH_3)	0.21	1.4	47%
20	2-Naphthyl	0.57	74%[a]	54%

ND, not determined.
[a] All percent inhibitions are at 66 μM inhibitor concentration unless otherwise indicated.
[b] Percent inhibition at 200 μM inhibitor concentration.

Table 2. FVIIa/TF inhibitors with 5-amido-benzoic acid sidechains

FVIIa/TF inhibitors	R^1	FVIIa/TF IC_{50} (μM)	FIIa IC_{50} (μM)	FXa IC_{50} (μM)[a]
21	$CONHCH_2Ph$	0.047	0.67	62%
22	$CONHCH_2$ (2-thienyl)	0.15	ND	ND
23	$CONHCH_2$ (2-furyl)	0.18	ND	ND
24	$CONHCH_2Ph$ (3-OMe)	0.017	69	62%
25	$CONHCH_2Ph$ (2,4-diMe)	0.027	2.5	66%
26	$CONHCH_2Ph$ (2,6-diMe)	0.031	1.8	49%
27	$CONHCH_2Ph$ (2-Cl)	0.18	ND	ND
28	$CONHCH_2Ph$ (2-OMe)	0.59	ND	ND
29	$CONHCH_2CH_2Ph$ (3-OMe)	0.38	ND	ND
30	$CONHCH_2Ph$ (4-Cl)	0.016	0.97	52%
31	CONH*i*-Butyl	0.020	1.2	71%
32		0.016	2.1	46%[c]
33	CONHPropyl	0.092	1.3	31%[b]
34	CONHPentyl	0.11	1.6	58%
35	$CONHCH_2$Cyclohexyl	0.60	ND	ND
36	$CONHCH_2$ (2-THP)	1.6	3.7	ND
37	CONHCyclopentyl	0.043	2.7	97%
38	CONHCyclohexyl	0.063	4.1	56%
39	CONHCyclopropyl	0.17	1.5	2.6

ND, not determined. THP, tetrahydropyran.
[a] All percent inhibitions are at 200 μM inhibitor concentration unless indicated.
[b] Percent inhibition at 66 μM inhibitor concentration.
[c] Racemic compound.

zyl-amide **30** (16 nM) showed a 2.5-fold increase in potency from **21**. Disubstituted benzyl-amides like 2,4-dimethylbenzyl-amide **25** and 2,6-dimethylbenzyl-amide **26** showed smaller increases in potency from **21**. Replacing the benzyl group with either a methylene-2-thienyl **22** or methylene-2-furyl **23** showed significant losses in binding potency, while extending the alkyl chain to the phenethyl-amide **29** also decreased potency significantly.

On examination of the SAR of inhibitors containing 5-alkyl-amide sidechains, it was observed that replacing the benzyl group of **21** with cycloalkylmethyl groups, such as cyclohexylmethyl-amide **35** and tetrahydropyranylmethyl-amide **36**, resulted in a large loss of binding affinity. An increase in potency was recovered with the deletion of the methylene linker as exemplified by cyclo-alkyl-amides **37–39**. The most potent inhibitors in this non-aromatic series of amides were identified as branched alkyl-amides. For example, both isobutyl-amide **31** (20 nM) and 1,2,2-trimethyl-propyl-amide **32** (16 nM) were more potent than the straight-chained alkyl-amides **33** (92 nM) and **34** (110 nM).

In order to determine whether the 5-amido group of our series was actually occupying the proposed S1′/S2′ binding pocket, an X-ray crystal structure of **31** in the active site of the enzyme complex was obtained (Fig. 2).[15] Analysis of these data showed that the phenylamidine was hydrogen bonding to Asp189 and Gly219 in the S1 pocket of the FVIIa/TF active site. The benzoic acid was interacting through hydrogen bonding with Gly193 and His57 in the oxy-anion hole.[16] The diisopropylami-

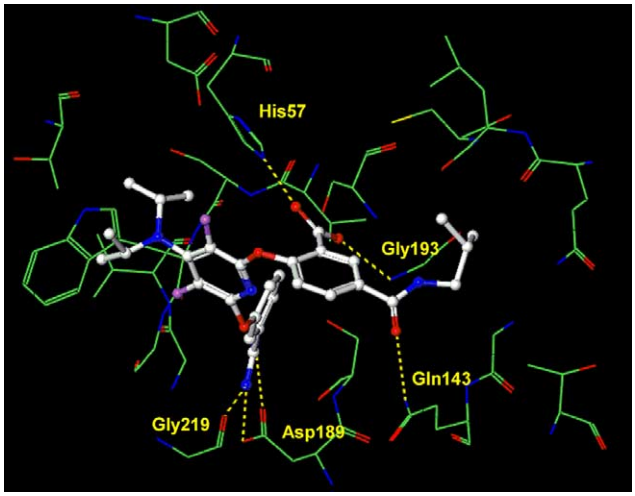


Figure 2. Binding interactions of **31** in the FVIIa/TF active site.

no sidechain was shown to be filling the S2 and S3 binding pockets through hydrophobic interactions. Finally, the isobutyl-amide sidechain filled the S1′/S2′ pocket. A hydrogen bond between the amide carbonyl and the nitrogen atom of the Gln143 allowed the alkyl group to adopt a confirmation that allowed for maximum van der Waals interactions with this solvent-exposed, shallow pocket (Fig. 3). Others have reported inhibitors with similar alkyl-amide sidechains which may also bind in this S1′/S2′ pocket.[4b,5,6]

Screening these TF/FVIIa inhibitors for selectivity against FXa and thrombin (FIIa) showed that in all cases, the selectively for TF/FVIIa was maintained over FXa. Most analogs were also selective for TF/FVIIa over thrombin with **7**, **8**, and **12** being notable exceptions. Molecular modeling experiments with **12** in the active site of thrombin indicated that the moderate binding activity could potentially be due to an alternate binding mode from that of TF/FVIIa. In the thrombin active site, the phenylamidine of **12** continued to bind

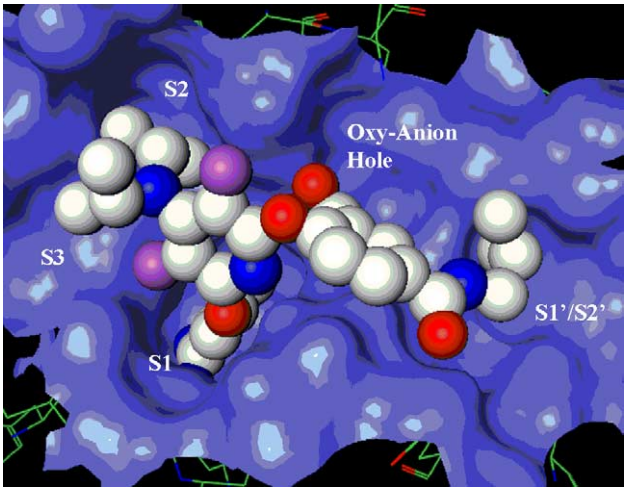


Figure 3. Alignment of **31** in the binding pockets of the FVIIa/TF active site.

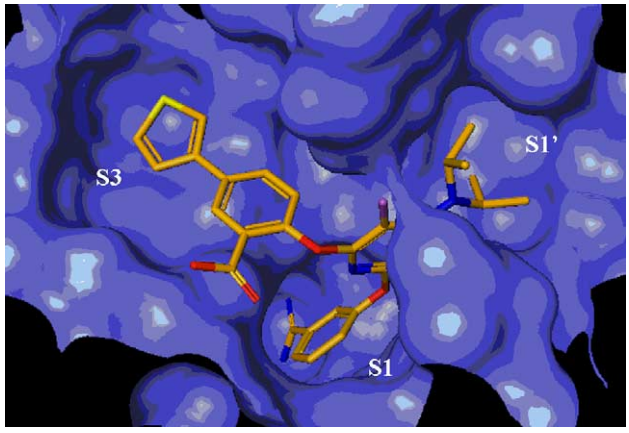


Figure 4. Potential binding mode of **12** in the active site of thrombin.

in the S1 pocket forming hydrogen bonds with Asp189, while the 5-substituent of the hydroxybenzoic acid sidechain now filled the distal S3 hydrophobic pocket and the diisopropylamine occupied the S1′ pocket (Fig. 4).[17]

A fluoropyridine series of FVIIa/TF inhibitors has been discovered which utilized a diisopropylamino sidechain for binding in the S2 and S3 binding pockets of the active site of the enzyme complex. An increase in binding affinity was obtained by the incorporation of substitutions at the 5-position of the hydroxybenzoic acid sidechain. An X-ray crystal structure indicated that a 5-amido substituted analog increased binding affinity through hydrophobic interactions with the S1′/S2′ pocket of the enzyme complex. Work is ongoing to enhance the binding and pharmacological activity of this series and will be reported in due course.

References and notes

1. (a) Petersen, L. C.; Valentin, S.; Hedner, U. *Thromb. Res.* **1995**, *79*, 1; (b) Kirchhofer, D.; Nemerson, Y. *Curr. Opin. Biotechnol.* **1996**, *7*, 386; (c) Bajaj, S. P.; Joist, J. H. *Semin. Thromb. Haemostasis* **1999**, *25*, 407.
2. (a) Rapaport, S. I.; Rao, V. M. *Thromb. Haemost.* **1995**, *74*, 7; (b) Rao, L. V. M.; Exban, M. *Blood Coag. Fibrinolysis* **2000**, *11*, 135; (c) Morrissey, J. H. *Haemostasis* **1996**, *26*, 66; (d) Himber, J.; Refina, C. J.; Burcklen, L.; Roux, S.; Kirchhofer, D. *Thromb. Haemost.* **2001**, *85*, 475; (e) Suleymanov, O. D.; Szalony, J. A.; Salyers, A. K.; Lachance, R. M.; Parlow, J. J.; South, M. S.; Wood, R. S.; Nicholson, N. S. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 1115; (f) Szalony, J. A.; Suleymanov, O. D.; Salyers, A. K.; Panzer-Knodle, S. G.; Blom, J. D.; LaChance, R. M.; Case, B. L.; Parlow, J. J.; South, M. S.; Wood, R. S.; Nicholson, N. S. *Thromb. Res.* **2004**, *112*, 167.
3. (a) Girard, T. J.; Nicholson, N. S. *Curr. Opin. Pharmacol.* **2001**, *1*, 159; (b) Gallagher, K. P.; Mertz, T. E.; Chi, L.; Rubin, J. R.; Uprichard, A. C. G. *Handbook Exp. Pharmacol.* **1999**, *132*, 421; (c) Key, N. S.; Bach, R. R. *Thromb. Hemostasis* **2001**, *85*, 375.
4. For a general review see: (a) Lazarus, R. A.; Olivero, A. G.; Eigenbrot, C.; Kirchhofer, D. *Curr. Med. Chem.* **2004**, *11*, 2275; (b) Schweitzer, B. A.; Neumann, W. L.; Rahman, H. K.; Kusturin, C. L.; Sample, K. R.; Poda,

G. E.; Kurumbail, R. G.; Stevens, A. M.; Stegeman, R. A.; Stallings, W. C.; South, M. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3006; (c) Buckman, B. O.; Chou, Y.-L.; McCarrick, M.; Liang, A.; Lentz, D.; Mohan, R.; Morrissey, M. M.; Shaw, K. J.; Trinh, L.; Light, D. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2249; (d) Sagi, K.; Fujita, K.; Sugiki, M.; Takahashi, M.; Takehana, S.; Tashiro, K.; Kayahara, T.; Yamanashi, M.; Fukuda, Y.; Oono, S.; Okajima, A.; Iwata, S.; Shoji, M.; Sakurai, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1487; (e) Groebke Zbinden, K.; Banner, D. W.; Ackermann, J.; D'Arcy, A.; Kirchhofer, D.; Ji, T.-H.; Tschopp, T. B.; Wallbaum, S.; Wever, L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 817; (f) Klingler, O.; Matter, H.; Schudok, M.; Donghi, M.; Czech, J.; Lorenz, M.; Nestler, H. P.; Szillat, H.; Schreuder, H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3715; (g) Parlow, J. J.; Kurumbail, R. G.; Stegeman, R. A.; Stevens, A. M.; Stallings, W. C.; South, M. S. *J. Med. Chem.* **2003**, *46*, 4696; (h) Olivero, A. G.; Eigenbrot, C.; Goldsmith, R.; Robarge, K.; Artis, D. R.; Flygare, J.; Rawson, T.; Sutherlin, D. P.; Kadkhodayan, S.; Beresini, M.; Elliott, L. O.; DeGuzman, G. G.; Banner, D. W.; Ultsch, M.; Marzec, U.; Hanson, S. R.; Refino, C.; Bunting, S.; Kirchhofer, D. *J. Biol. Chem.* **2005**, *280*, 9160.

5. Kohrt, J. T.; Filipski, K. J.; Cody, W. L.; Cai, C.; Dudley, D. A.; Van Huis, C. A.; Willardsen, J. A.; Rapundalo, S. T.; Saiya-Cork, K.; Leadley, R. J.; Narasimhan, L. S.; Zhang, E.; Whitlow, M.; Adler, M.; McLean, K.; Chou, Y.-L.; McKnight, C.; Arnaiz, D. O.; Shaw, K. J.; Light, D. R.; Edmunds, J. J. *Biorg. Med. Chem. Lett.* **2005**, *15*, 4752.
6. (a) Kohrt, J. T.; Filipski, K. J.; Rapundalo, S. T.; Cody, W. L.; Edmunds, J. J. *Tetrahedron Lett.* **2000**, *41*, 6041; (b) Menzler, S.; Bikker, J. A.; Horwell, D. C. *Tetrahedron Lett.* **1998**, *39*, 7619.
7. Kometani, T.; Watt, D. S.; Ji, T.; Fitz, T. *J. Org. Chem.* **1985**, *50*, 5384.
8. Watanabe, T.; Miyaura, N.; Suzuki, A. *Synlett* **1992**, 208.
9. Li, J.-J.; Yue, W. S. *Tetrahedron Lett.* **1999**, *40*, 4507.
10. Zhu, L.; Wehmeyer, R. M.; Rieke, R. D. *J. Org. Chem.* **1991**, *56*, 1445.
11. Bumagin, N. A.; Nikitin, K. V.; Beletskaya, I. P. *J. Organomet. Chem.* **1988**, *358*, 563.
12. The ability of compounds to act as inhibitors of the catalytic activity of the human FVIIa/TF complex was assessed by determining the concentration of a test substance that inhibited by 50% (IC_{50}) the ability of a complex of human recombinant FVIIa/TF to cleave the fluorogenic substrate SN-17c. SN-17c (California Peptide) is a short peptide (13 mer) linked to an ANS (6-amino-1-naphthalene-(*n*-butyl) sulfonamide) fluorophore. Typically, the reaction consisted of a mixture of 5 nM FVIIa (NovoNordisk), 25 nM human recombinant TF (Berlex Biosciences), and 200 μM SN-17c in a buffer of 20 mM Hepes, 20 mM $CaCl_2$ and 0.1% BSA in a total assay volume of 45 μl at pH 7.5. The rate of change in relative fluorescent units (RFUs) was determined at 37 °C by measuring the fluorescence change over 15 min. using a SpectraMax Gemini spectrophotometer at an excitation wavelength of 455 nm and read at 450 nm.
13. Chi, L.; Peng, Y.-W.; Gibson, G.; Hicks, G.; Mertz, T. E.; Rapundalo, S.; Janiczek, N.; Edmunds, J. J.; Leadley, R. *J. Cardiovasc. Pharmacol.* **2004**, *44*, 493.
14. Chi, L.; Gibson, G.; Peng, Y.-W.; Potoczak, R.; Bousley, R.; Brammer, D.; Riley, J.; Kreuser, S.; Rekhter, M.; Chen, J.; Leadley, R. *J. Thromb. Haemost.* **2004**, *2*, 85.
15. X-ray crystallographic data was deposited in the RCSB database (www.rcsb.org) as PDB ID code 2c4f.
16. Eigenbrot, C. *Curr. Protein Peptide Sci.* **2002**, 287.
17. (a) Srivastava, S.; Goswami, L. N.; Dikshit, D. K. *Med. Res. Rev.* **2005**, *25*, 66; (b) Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426; (c) Matthews, J. H.; Krishnan, R.; Costanzo, M. J.; Maryanoff, B. E.; Tulinsky, A. *Biophys. J.* **1996**, *71*, 2830.