Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3721–3725

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and X-ray Crystal Structures of Substituted Fluorobenzene and Benzoquinone Inhibitors of the Tissue Factor VIIa Complex

John J. Parlow,[a,*] Ravi G. Kurumbail,[b] Roderick A. Stegeman,[b] Anna M. Stevens,[b] William C. Stallings[b] and Michael S. South[a]

[a]*Department of Medicinal and Combinatorial Chemistry, Pharmacia Corporation, 800 North Lindbergh Boulevard, St. Louis, MO 63167, USA*

[b]*Structure and Computational Chemistry, Pharmacia Corporation, 700 Chesterfield Village Parkway, St. Louis, MO 63198, USA*

Received 11 April 2003; revised 4 August 2003; accepted 5 August 2003

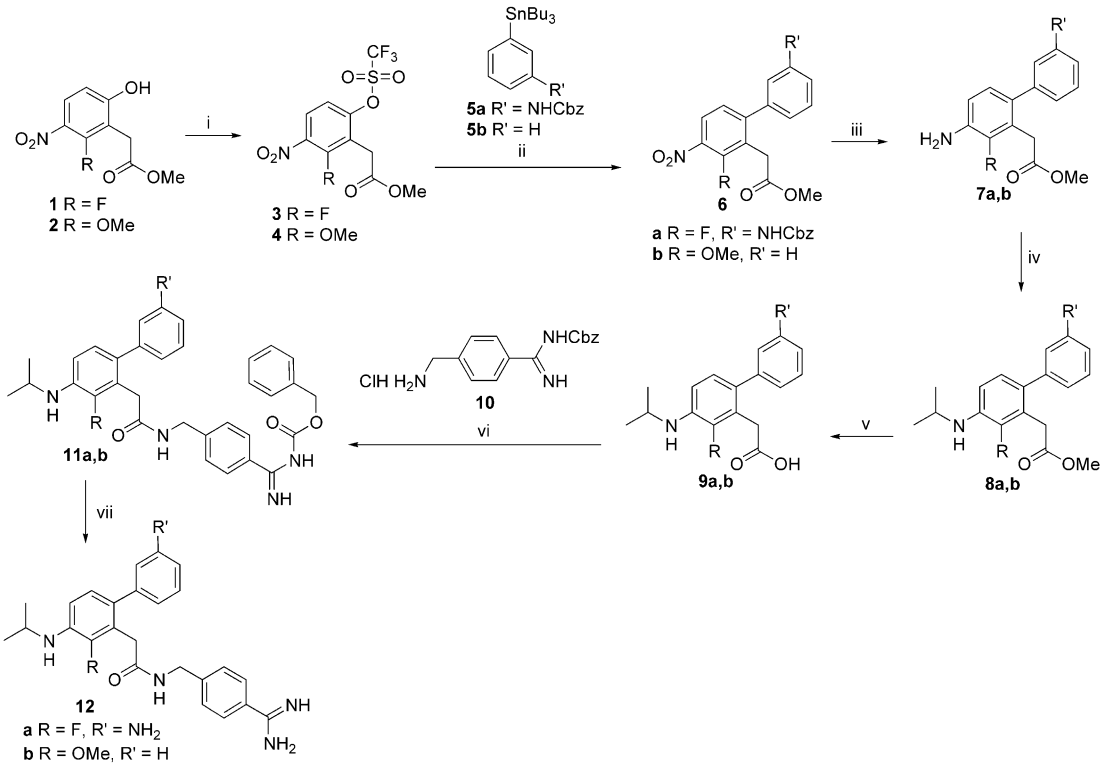
Abstract—Multistep syntheses of substituted benzenes and benzoquinone inhibitors of tissue Factor VIIa are reported. The benzene analogues were designed such that their substitution pattern would occupy and interact with the S_1, S_2, and S_3 pockets of the tissue Factor VIIa (TF/VIIa) enzyme. The compounds exhibited modest potency on TF/VIIa with selectivity over Factor Xa and thrombin. The X-ray crystal structures of the targeted fluorobenzene **12a** and benzoquinone **14** inhibitors bound to TF/VIIa were obtained and will be described.

Cardiovascular disease is the most common cause of mortality in the Western world.[1] The disease is characterized by the acute coronary syndromes (ACS) unstable angina and myocardial infarction, which often lead to sudden death. Often, the root cause for ACS is due to deposition of a thrombus in coronary arteries. This inappropriate thrombus formation is initiated via the extrinsic coagulation cascade caused by a plaque rupture, which exposes cell surface tissue factor (TF) to the serine protease VIIa in circulating blood forming the TF/VIIa complex. This cascade is critical in normal hemostasis, but is also involved in the pathogenesis of various thrombotic diseases. Under normal conditions, TF expressed in the sub-endothelium of healthy blood vessels is not exposed to blood. However, in a disease state or during injury, TF comes in contact with Factor VIIa and the ensuing TF/VIIa complex activates Factors X and IX to Xa and IXa, respectively. The complex of Factor Xa and Factor Va on a membrane surface converts prothrombin to thrombin, leading to fibrin formation, deposition and subsequent thrombus formation.[2] Effective and safe antithrombotics are needed to combat cardiovascular diseases. Most research has focused on thrombin and Factor Xa inhibitors as potentially valuable therapeutic agents for these diseases.[3] More recently, small molecule inhibitors of tissue Factor VIIa have been the point of much research effort because of their potential to inhibit the coagulation cascade while minimizing the risk of bleeding side effects.[4]

We previously reported the preparation of pyrazinone analogues as tissue Factor VIIa inhibitors.[5] These pyrazinone compounds are active-site inhibitors for TF/VIIa exhibiting potency at the nanomolar level with excellent selectivity over thrombin (IIa) and Factor Xa. In an effort to increase the potency and influence the pharmacokinetic properties, other core ring systems were evaluated. Depicted in Figure 1 is the general pyrazinone structure **I**. Focusing on the central ring, one of the exercises was to replace the nitrogens with carbons on the pyrazinone ring, resulting in benzene **II** and benzoquinone as central ring systems.[6,7] Based on the X-ray crystal structures of TF/VIIa,[5,8] several key interactions were crucial for both potency for TF/VIIa and selectivity over thrombin and Factor Xa. It was

*Corresponding author. Tel.: +1-314-274-3494; fax: +1-314-274-1621; e-mail: john.j.parlow@pfizer.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.002



Scheme 1. Synthesis of substituted benzene TF/VIIa inhibitors. Reagent and conditions: (i) triflic anhydride, Et_3N, DCM, −10 °C–rt; (ii) $Pd(PPh_3)_4$, LiCl, dioxane, 85 °C; (iii) Fe, $MeCO_2H$, 80 °C; (iv) Me_2CO, $NaHB(OAc)_3$, DCM/THF; (v) NaOH, MeOH, 65 °C; (vi) HOBt, NMM, PS-CD, DCM/DMF, then PS-aldehyde and PS-polyamine resins; (vii) H_2, Pd/C, MeOH.

found in the pyrazinone series that the benzamidine binds, as expected, in the S_1 pocket mediated by an ion-pair between the basic amidine moiety and the carboxylate of Asp 189. The substituted *meta*-aminophenyl ring occupies the S_2 pocket and the amino group at the 3-position of the pyrazinone occupies and interacts with the S_3 pocket. In addition, the ketone at the 2-position on the pyrazinone ring forms a hydrogen bond with the peptide backbone of Gly 216. We explored the possibility of replacing the pyrazinone core with a simple benzene ring system that could orient the various substituents in the correct spatial arrangement to probe the S_1, S_2, and S_3 pockets. In addition, the benzene ring could be substituted at the 2-position with various hydrogen bond accepting groups to engage Gly 216. Reported herein is the discovery, synthesis, biological activity, and X-ray structures of novel substituted benzene and benzoquinone analogues as tissue Factor VIIa inhibitors.

A key feature required in replacing the pyrazinone ring with a benzene ring system was to have a hydrogen bond accepting group at the 2-position. Thus, a substituted benzene ring with a fluorine in the 2-position was designed with the fluorine acting as the hydrogen bond acceptor to interact with Gly 216. The fluorobenzene target **12a** was prepared as shown in Scheme 1. Compound **1** was used as the starting material[9] allowing for substitution at the 6-position while maintaining the fluorine at the 2-position. The fluoro compound **1** was reacted with triflic anhydride to afford the triflate **3**. The triflate **3** underwent a Stille coupling with benzyl 3-(tributylstannyl)phenylcarbamate **5a** using *tetrakis* (triphenylphosphine)palladium(0) and lithium chloride in dioxane at 85 °C to afford the 6-phenyl derivative **6a**.[10] Iron reduction of the nitro group afforded the desired amine **7a**. Reductive amination of the amino compound **7a** with acetone using sodium triacetoxyborohydride afforded the isopropylamine **8a**. Hydrolysis of the methyl ester using sodium hydroxide afforded the carboxylic acid **9a**. The amide coupling was accomplished by treating the acid **9a** with polymer-bound carbodiimide, hydroxybenzotriazole, and *N*-methylmorpholine as base followed by addition of the amine **10**, benzyl amino[4-(aminomethyl)phenyl]-methylcarbamate, to yield the Cbz protected product **11a**. Hydrogenation deprotection of the Cbz groups was used to afford the desired 2-fluorobenzene product **12a**.

A benzoquinone core maintains the ketone in the same position as the pyrazinone **I** for hydrogen bonding with Gly 216. The precursor to prepare a benzoquinone ring system was synthesized as described above and shown in Scheme 1. 2-Methoxybenzene **2** was used as the starting

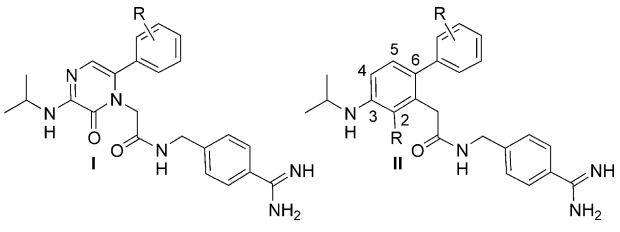
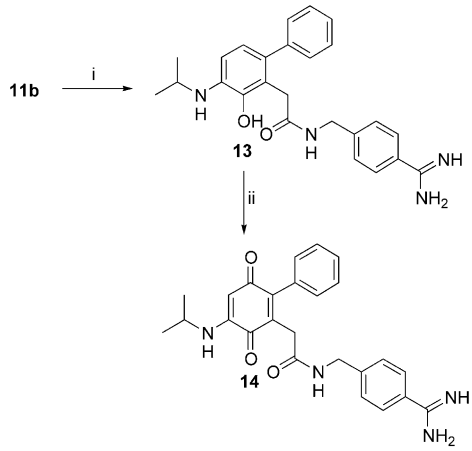


Figure 1. Pyrazinone and benzene core structures.



Scheme 2. Benzoquinone synthesis. Reagent and conditions: (i) BBr_3, DCM, −10 °C; (ii) $(KSO_3)_2NO$, H_2O/THF.

material to afford the benzoquinone precursor **11b**. Compound **11b** was designed with the isopropylamine at the 3-position and the unsubstituted phenyl ring at the 6-position. The less potent unsubstituted phenyl ring was chosen over the aminophenyl ring at the 6-position to avoid quinone formation of that phenyl ring in the final step of the synthesis. The synthesis was carried out as shown in Scheme 2. The methoxybenzene **11b** was treated with boron tribromide to afford the phenol **13**. Boron tribromide was used as the reagent to provide concomitant deprotection of the methyl ether and Cbz group affording the desired phenol **13** with the 2-hydroxy serving as the hydrogen bond accepting group to interact with Gly 216. Reacting the phenol **13** with Fremy's salt[11] afforded the targeted benzoquinone **14**.

The target compounds were screened for potency on TF/VIIa and for other enzymes affecting coagulation to determine specificity (Table 1). Each enzyme assay consists of the specific enzyme and chromogenic substrate for that enzyme. Enzyme activity was determined by monitoring the increase in absorbance at 405 nm caused by the release of *p*-nitroaniline when the substrate is hydrolyzed. Inhibition of the enzyme reduces the change in absorbance with the data reported as IC_{50} values. Compound **12a** was the most potent compound ($IC_{50} = 340$ nM) on TF/VIIa, having the amino substitution on the P_2 phenyl ring. The benzoquinone **14**, which maintains the ketone at the 2-position from the pyrazinone core, also exhibited activity on TF/VIIa with excellent selectivity over both Factor Xa and thrombin. The 2-hydroxybenzene **13** and 2-methoxybenzene **12b** analogues were both active against TF/VIIa with the 2-hydroxy compound having potency comparable with the benzoquinone **14**. However, these analogues are less potent against TF/VIIa than their corresponding pyrazinone analogues.[5]

Table 1. IC_{50} values

Compd	IC_{50} (uM)		
	VIIa	Xa	Thrombin
12a	0.34	> 30	0.95
12b	14.7	> 30	7.9
13	2.5	> 30	> 30
14	2.8	> 30	> 30

The crystal structure of compound **12a** bound to TF/VIIa is shown in Figure 2. The bound conformation of fluorobenzene inhibitor **12a** in the active site of VIIa resembles that of pyrazinone inhibitors that have been reported previously.[5] The benzamidine moiety engages the carboxylate of Asp 189 in the S_1 site, similar to the interactions observed in the crystal structures of other serine proteases. The peptide nitrogen of the acetate linker forms a hydrogen bond (3.2 Å) with the carbonyl oxygen of Ser 214. The anilino nitrogen attached to the central fluorobenzene core donates a hydrogen bond (3.4 Å) to the main chain oxygen of Gly 216. As anticipated, the fluorine atom in the central ring accepts a hydrogen bond (3.4 Å) from the amide nitrogen of Gly 216. Some of these hydrogen bonds are longer and perhaps indicate non-optimized interactions of the inhibitor in the active site. This might explain the relatively weaker binding affinity of this class of inhibitors compared to that of pyrazinone inhibitors.

The amino group attached to the P_2 phenyl ring forms tight interactions with Asp 60, Tyr 94, and the carbonyl oxygen of Thr 98. Potential collision of the phenyl group of the inhibitor at P_2 with the side chain of Tyr 99 in Factor Xa is probably why it does not inhibit Factor Xa.

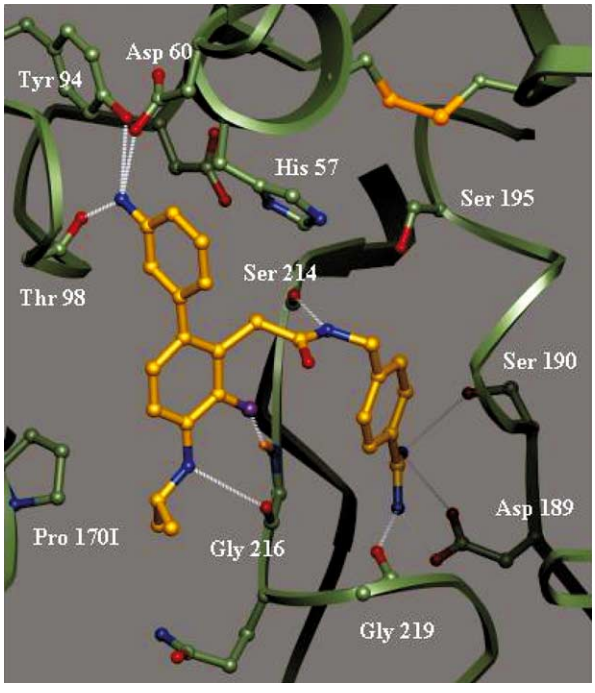


Figure 2. Crystal structure of **12a** (fluorobenzene) bound in the active site of TF/VIIa complex. Crystals of TF/VIIa complex were obtained by slight modification of the procedure described by Banner et al.[8a] The structure has been refined to an R_{free} of 29.1% at 2.4 Å resolution ($R_{crystal}$: 23.7%). Some of the key side chains of Factor VIIa are displayed (C: green, N: dark blue, O: red, S: yellow and H: orange). The inhibitor is represented with carbon, nitrogen, oxygen, and fluorine atoms displayed in gold, blue, red, and purple, respectively. The hydrogen bonds formed by the inhibitor are shown in dotted white lines.

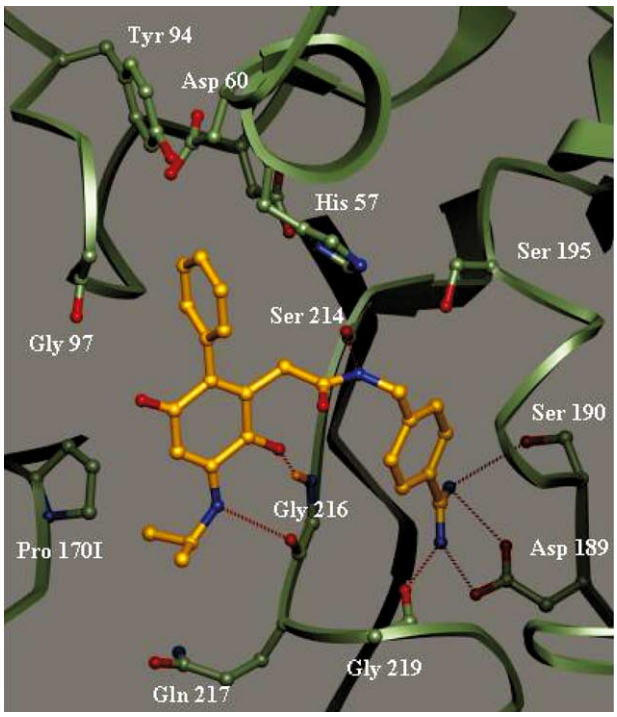


Figure 3. Crystal structure of **14** (benzoquinone) bound in the active site of TF/VIIa. The structure was refined to an R_{free} of 28.0% at 2.2 Å resolution ($R_{crystal}$: 22.5%). The atoms are colored as in Figure 2. Some of the key side chains of Factor VIIa are displayed. The hydrogen bonds are shown as dotted lines (magenta). One of the quinone oxygen atoms accepts a hydrogen bond from the amide nitrogen of Gly 216 as observed in the structures of pyrazinone inhibitors.

The crystal structure of compound **14** bound to TF/VIIa is shown in Figure 3. The binding orientation of the benzoquinone inhibitor **14** is similar to that of the fluorobenzene **12a**. The ion-pair that is formed by the amidine moiety of the inhibitor with the carboxylate of Asp 189 functions as the main anchor for the inhibitor in the enzyme active site. In addition to this, the amidine group also forms hydrogen bonds to the main chain carbonyl of Gly 219 and the hydroxyl group of the side chain of Ser 190. Three other hydrogen bonds are formed by the inhibitor with the peptide backbone of residues Ser 214-Gly 216 of VIIa. The amide nitrogen of the acetate linker interacts with the main chain oxygen of Ser 214 (3.2 Å) while the secondary nitrogen attached to the quinone scaffold donates a hydrogen bond (3.0 Å) to the carbonyl oxygen of Gly 216. One of the quinone oxygen atoms accepts a hydrogen bond (3.3 Å) from the peptide nitrogen of Gly 216 as anticipated in our design. The other quinone oxygen forms van der Waals interactions (3.5 Å) with the carbonyl oxygen of Gly 97.

In summary, we have prepared novel benzene analogues and a benzoquinone designed to occupy and interact with the S_1, S_2, and S_3 pockets of the TF/VIIa enzyme. The substituted benzene analogues were successfully prepared via a multistep synthesis and further derivatized to prepare a benzoquinone **14**. These compounds exhibit modest potency for TF/VIIa and some of them display excellent selectivity over Factor Xa and thrombin. The X-ray crystal structures of the targeted fluorobenzene **12a** and benzoquinone **14** inhibitors bound to TF/VIIa were obtained. The crystal structure of the fluorobenzene **12a** analogue clearly shows that the fluorine acts as the hydrogen bond acceptor and engages with Gly 216. Similarly, one of the ketones of the benzoquinone **14** registers with the peptide backbone of Gly 216 via a hydrogen bond. The progression of the synthesis of substituted benzene analogues from the discovery of the lead compound to the development of potent analogues will be the topic of a future publication.

Acknowledgements

The authors thank Rhonda M. Lachance for the biological evaluation and Dr. Huey Shieh for some of the early crystallographic refinements of the TF-VIIa structure. Diffraction data for the TF-VIIa complex with the inhibitors were collected at beamline 17-ID in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. IMCA-CAT facilities are supported by the corporate members of the IMCA and through a contract with Illinois Institute of Technology (IIT), executed through the IIT's Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

References and Notes

1. Braunwald, E.; Califf, R. M.; Cannon, C. P.; Fox, K. A.; Fuster, V.; Gibler, W. B.; Harrington, R. A.; King, S. B.; Kleiman, N. S.; Theroux, P.; Topol, E. J.; Van de Werf, F.; White, H. D.; Willerson, J. T. *Am. J. Med.* **2000**, *108*, 41.
2. (a) Houston, D. S. *Expert Opin. Ther. Targets* **2002**, *6*, 159. (b) Golino, P. *Thromb. Res.* **2002**, *106*, V257. (c) Bajaj, M. S.; Birktoft, J. J.; Steer, S. A.; Bajaj, S. P. *Thromb. Haemost.* **2001**, *86*, 959. (d) Kaiser, B.; Hoppensteadt, D. A.; Fareed, J. *Expert Opin. Investig. Drugs* **2001**, *10*, 1925. (e) Kirchhofer, D.; Nemerson, Y. *Curr. Opin. Chem. Biotech.* **1996**, *7*, 386. (f) Girard, T. J. Tissue Factor Pathway Inhibitor' In *New Therapeutic Agents in Thrombosis and Thrombolysis* Eds.; Sasahara A.A., and Loscalzo J., Atherosclerosis Shannon, Irel. 1998; Vol 139, p 199.
3. For reviews see: (a) Sanderson, P. E. J. *Annu. Rep. Med. Chem.* **2001**, *36*, 79. (b) Betz, A. *Expert Opin. Thera. Pat.* **2001**, *11*, 1007. (c) Zhu, B. Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83. (d) Vacca, J. P. *Curr. Opin. Chem. Biol.* **2000**, *4*, 394. (e) Sanderson, P. E. J. *Med. Res. Rev.* **1999**, *19*, 179. (f) Fevig, J. M.; Wexler, R. R. *Annu. Rep. Med. Chem.* **1999**, *34*, 81. (g) Ewing, W. R.; Pauls, H. W.; Spada, A. P. *Drugs. Future* **1999**, *24*, 771.
4. For reviews see: (a) Robinson, L. A.; Saiah, E. M. K. *Annu. Rep. Med. Chem.* **2002**, *37*, 85. (b) Girard, T. J.; Nicholson, N. S. *Curr. Opin. Pharmacol.* **2001**, *1*, 159. (c) Carroll, A. R.; Pierens, G. K.; Fechner, G.; de Almeida Leone, P.; Ngo, A.; Simpson, M.; Hyde, E.; Hooper, J. N. A.; Boström, S.-L.; Musil, D.; Quinn, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 13340. (d) Hanessian, S.; Margarita, R.; Hall, A.; Johnstone, S.; Tremblay, M.; Parlanti, L. *J. Am. Chem. Soc.* **2002**, *124*, 13342. (e) Hanessian, S.; Therrien, E.; Granberg, K.; Nilsson, I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2907. (f) Young, W. B.;

Kolesnikov, A.; Rai, R.; Sprengeler, P. A.; Leahy, E. M.; Shrader, W. D.; Sangalang, J.; Burgess-Henry, J.; Spencer, J.; Elrod, K.; Cregar, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2253. (g) Kohrt, J. T.; Filipski, K. J.; Rapundalo, S. T.; Cody, W. L.; Edmunds, J. J. *Tetrahedron Lett.* **2000**, *41*, 6041. (h) Jakobsen, P.; Horneman, A. M.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2803. (i) Jakobsen, P.; Ritsmar Pedersen, B.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2095. (j) Roussel, P.; Bradley, M.; Kane, P.; Bailey, C.; Arnold, R.; Cross, A. *Tetrahedron* **1999**, *55*, 6219. (k) Wang, D.; Girard, T. J.; Kasten, T. P.; LaChance, R. M.; Miller-Wideman, M. A.; Durley, R. C. *J. Nat. Prod.* **1998**, *61*, 1352. (l) Semple, J. E.; Rowley, D. C.; Brunck, T. K.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 315. (m) Uchiba, M.; Okajima, K.; Abe, H.; Okabe, H.; Takatsuki, K. *Thromb. Res.* **1994**, *74*, 155. (n) Butenas, S.; Ribarik, N.; Mann, K. G. *Biochemistry* **1993**, *32*, 6531. (o) Van der Woerd-De Lange, J. A.; Van Dam-Mieras, M. C. E.; Hemker, H. C. *Haemostasis* **1981**, *10*, 315.

5. (a) Parlow, J. J.; Case, B. L.; Dice, T. A.; Fenton, R. L.; Hayes, M. J.; Jones, D. E.; Neumann, W. L.; Wood, R. S.; Lachance, R. M.; Girard, T. J.; Nicholson, N. S.; Clare, M.; Stegeman, R. A.; Stevens, A. M.; Stallings, W. C.; Kurumbail, R. G.; South M. S. *J. Med. Chem.* **2003**, In press. (b) South, M. S.; Case, B. L.; Wood, R. S.; Jones, D. E.; Hayes, M. J.; Girard, T. J.; Lachance, R. M.; Nicholson, N. S.; Clare, M.; Stevens, A. M.; Stegeman, R. A.; Stallings, W. C.; Kurumbail, R. G.; Parlow, J. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2319.

6. (a) South, M. S.; Parlow, J. J. WO 0168605, **2001**. (b) South, M. S.; Parlow, J. J. WO 0179155, 2001.

7. Others have used a similar approach for thrombin inhibitors, Pan, W.; Lu, T.; Markotan, T. P.; Tomczuk, B. E. WO 0228825, 2002.

8. (a) Banner, D. W.; D'Arcy, A.; Chene, C.; Winkler, F. K.; Guha, A.; Konigsberg, W. H.; Nemerson, Y.; Kirchhofer, D. *Nature* **1996**, *380*, 41. (b) Pike, A. C.; Brzozowski, A. M.; Roberts, S. M.; Olsen, O. H.; Persson, E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8925. (c) Kemball-Cook, G.; Johnson, D. J.; Tuddenham, E. G.; Harlos, K. *J. Struct. Biol.* **1999**, *127*, 213.

9. Synthesis of compounds **1** and **2** will be described in a future publication.

10. Echavarren, A. M.; Stille, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 5478.

11. Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.