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Design, Synthesis, and SAR of Monobenzamidines and Aminoisoquinolines as Factor Xa Inhibitors

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Abstract—Monoamidine FXa inhibitors **3** were designed and synthesized. SAR studies and molecular modeling led to the design of conformationally constrained diaryl ethers **4** and **5**, as well as benzopyrrolidinone **7** as potent FXa inhibitors. The monoamidines show high efficacy in a DVT model, but lack desirable oral bioavailability. The benzopyrrolidinone-based aminoisoquinolines **8** do not show significant improvement in oral bioavailability.

Due to the central role of factor Xa (FXa) in both intrinsic and extrinsic pathways in the blood coagulation cascade, the inhibition of FXa is believed to be an effective approach for the treatment of thrombotic disorders.[1,2] Previously disclosed small molecule FXa inhibitors are mostly dibasic.[3] A P1 amidino group in the inhibitor interacts with the Asp189 side chain in the FXa S1 specificity pocket, resulting in a strong ionic interaction and hydrogen bonding. A P4 group, positively charged under physiological conditions, binds to the cation sink in the FXa S4 site. The unfavorable pharmacokinetic properties associated with the strongly basic nature of the amidine moieties have led to the active search for less basic compounds as orally efficacious anticoagulants.[4]

Compound **1** has been reported as a potent FXa inhibitor. The biphenylsulfonamide group interacts with the S4 pocket of FXa as suggested by molecular modeling results.[5] Compound **2**, disclosed by Ajinomoto,[6] has an ethanolamine linker between two benzamidine moieties. Based on the assumption that the *meta*-benzamidine moiety is the S1 binding group, compound **3a** was designed as a FXa inhibitor (Fig. 1). Herein, we report the synthesis and structure–activity relationship (SAR) studies of compound **3**. The initial SAR results and molecular modeling led us to design two types of novel, conformationally constrained FXa inhibitors, as represented by the diaryl ether **4a** and benzopyrrolidinone **7**.

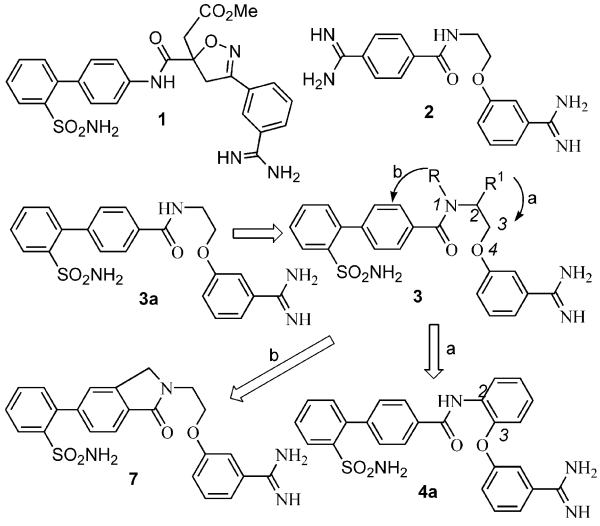

Figure 1. Design of conformationally constrained FXa inhibitors.

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Chemistry

The syntheses of compound **3a** and its analogues are described in Scheme 1. The *N*-Boc protected amino alcohols were reacted with 3-cyanophenol under Mitsunobu conditions.[7] Removal of the Boc protecting group was followed by the coupling with 4-(2-*tert*-butylaminosulfonyl)phenyl benzoic acid. Alkylation of the amide nitrogen was effected with RX (X = OTs, Br, Cl, or I). Pinner conditions[8] followed by ammonolysis gave the desired amidines with the simultaneous removal of the *tert*-butyl group.

The diaryl ethers **4** were synthesized according to Scheme 2. Substituted 1-fluoro-2-nitrobenzenes were reacted with 3-cyanophenol under basic conditions to afford the ether intermediate. The nitro group was reduced with tin(II) chloride hydrate. The coupling of the resulting amines with 4-(2-*tert*-butylaminosulfonyl)-phenylbenzoic acid followed by the amidine formation gave the desired compound **4**.

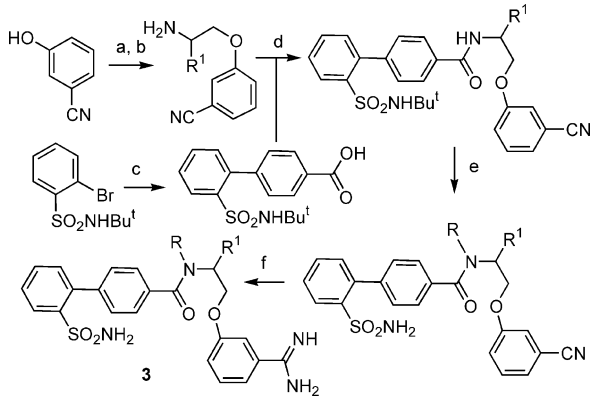
The syntheses of compound **5** is described in Scheme 3. The Weinreb conditions[9] were employed for the amide bond formation. The conversion of the amino group to *N*-acetyl or sulfonamide was performed under standard conditions. The Sandmeyer reaction (^{t}BuONO, CuBr or CuCl, CH_3CN, reflux, 1 h) was employed for the halogenation of the central phenyl ring.[10] The cyano intermediates were then used for the amidine formation. The dimethylamine and methylamines **5d**, **5k** and/or **5l** were found as the major products when **5b** and **5i** were treated with 10% Pd/C under H_2 atmosphere (1 atm) in methanol. Debromination of **5h** was achieved with catalytic hydrogenation to give compound **5a**.

The syntheses of aminoisoquinolines **6** are described in Scheme 4. A unique three step sequence was used for the conversion of isoquinoline to aminoisoquinoline.[11] The *tert*-butyl group was removed with trifluoroacetic acid at reflux.

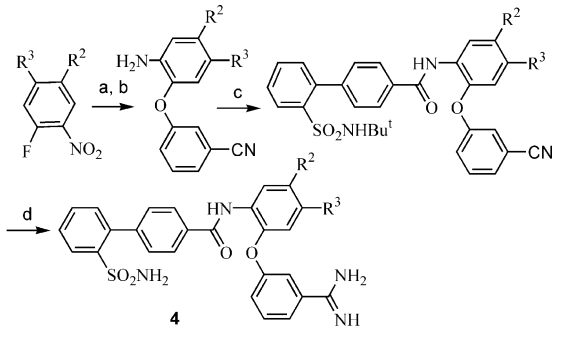
Compound **7** was synthesized according to Scheme 5. The amine intermediate obtained from Scheme 1 (R^1 = H) was reacted with the benzylic bromide to give the lactam in a single step. Scheme 6 describes the syntheses of the aminoisoquinoline compounds **8**.

Results and Discussion

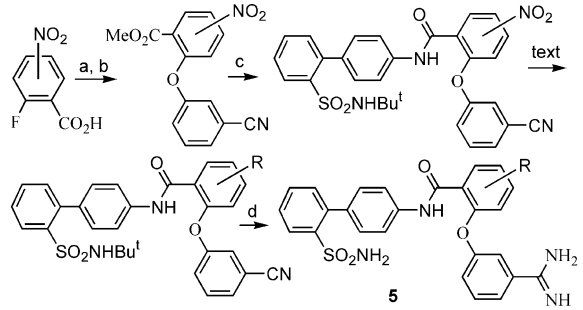
Compound **3a** is a potent FXa inhibitor (IC_{50} = 7.5 nM, Table 1). It is also selective for FXa over other serine proteases. It has IC_{50} values of 321 μM for thrombin, 1.2 μM for trypsin, 109 μM for tissue plasminogen activator (tPA), 3.7 μM for activated protein C (APC), 13 μM for plasmin, and 2.1 μM for kallikrein. Substitution on the ethanolamine template was carried out to



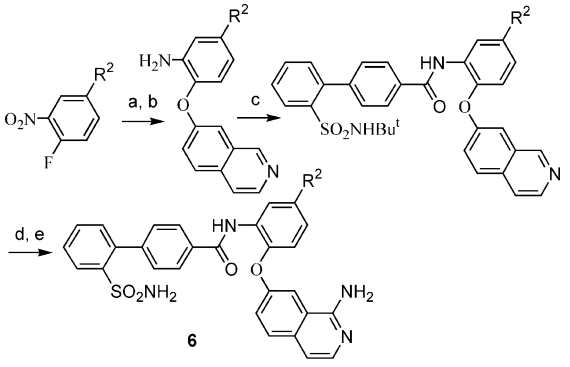
Scheme 1. (a) BocNHCHR^{1}CH$_2$OH, PPh_3, DEAD, THF; (b) TFA, CH_2Cl_2; (c) 4-carboxyphenyl boronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, dioxane, H_2O; (d) BOP reagent, Et_3N, DMF; (e) RX (X = Br, Cl, I, OTs), Cs_2CO_3, DMF; (f) (1) HCl(g), MeOH; (2) NH_4OAc, MeOH.



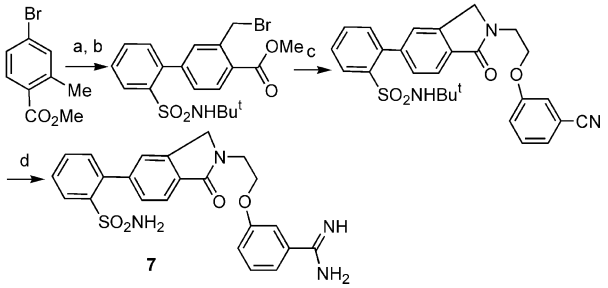
Scheme 2. (a) 3-cyanophenol, K_2CO_3, DMF; (b) $SnCl_2$–$2H_2O$, EtOAc; (c) 4-(2-tert-butylaminosulfonyl)phenylbenzoic acid, BOP reagent, Et_3N, DMF; (d) (1) HCl(g), MeOH; (2) NH_4OAc, MeOH.



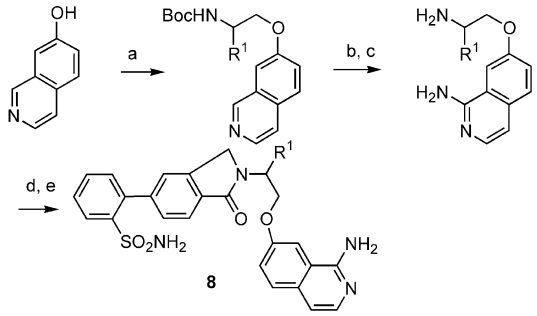
Scheme 3. (a) $TMSCHN_2$, MeOH; (b) 3-cyanophenol, K_2CO_3, DMF; (c) 4-(2-*tert*-butylaminosulfonyl)biphenylamine, $AlMe_3$, CH_2Cl_2; (d) (1) HCl(g), MeOH; (2) NH_4OAc, MeOH.



Scheme 4. (a) 7-hydroxy-isoquinoline, K_2CO_3, DMF; (b) $SnCl_2$–$2H_2O$, EtOAc; (c) 4-(2-tert-butylaminosulfonyl)phenylbenzoic acid, BOP reagent, Et_3N, DMF; (d) (1) MCPBA, acetone; (2) TsCl, –Py; (3) ethanolamine; (e) TFA.

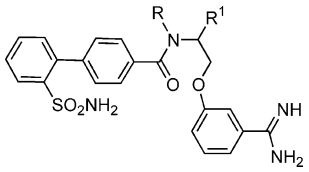


Scheme 5. (a) 2-(*tert*-butylaminosulfonyl)phenyl boronic acid, TEA, $PdCl_2$(dppf), Dioxane; (b) NBS, CCl_4; (c) $NH_2(CH_2)_2O$–(3-CN)Ph, TEA, benzene; (d) (1) HCl(g), MeOH; (2) NH_4OAc, MeOH.



Scheme 6. (a) $BocNHCHR^1CH_2OH$, PPh_3, DEAD, THF; (b) (1) MCPBA, acetone; (2) TsCl, Py; (3) ethanolamine; (c) TFA, CH_2Cl_2; (d) methyl 4-(2-*tert*-butylaminosulfonyl)phenyl-2-bromomethylbenzoate, TEA, benzene; (e) TFA.

Table 1. In vitro potency of ethanolamine-based FXa inhibitors



Compd	R	R^1	IC_{50} (nM)
3a	H	H	7.5
3b	H	Me	16
3c	H	Ph	3.4
3d	H	Bn	5.6
3e	Me	H	24
3f	CH_2Ph	H	14
3g	CH_2-(3-pyridinyl)	H	11
3h	CH_2-(3-furyl)	H	7.8
3i	CH_2CO_2Me	H	13
3j	CH_2CO_2H	H	34

optimize the interaction between the inhibitor and FXa. Compounds **3c** and **3d** containing a bulkier phenyl and benzyl, respectively, are more potent than compound **3b** (racemic) which only bears a methyl group. Compounds **3e–3j** are alkylated at the amide nitrogen and are marginally less potent than compound **3a**. These results clearly show that the alkylation does not jeopardize the anti-FXa activity. As an interesting note, the carboxylic acid compound **3j** is nearly 3 times less potent than the corresponding methyl ester **3i**.

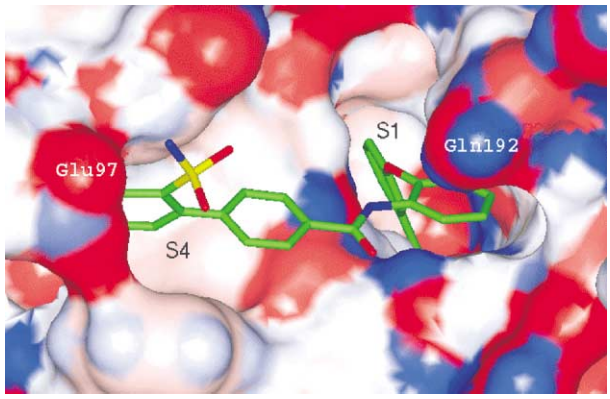
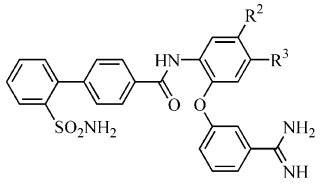


Figure 2. Compound **4a** docked in the FXa active site. The Connolly surface indicates more concentrated red color in areas of increased partial negative charge, and blue color in areas of partial positive charge.

Table 2. In vitro potency of 2-aminophenol-based FXa inhibitors

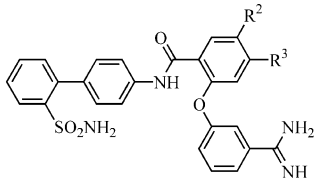


Compd	R^2	R^3	IC_{50} (nM)
4a	H	H	3.6
4b	F	H	4.5
4c	Br	H	1.6
4d	CF_3	H	2.5
4e	SO_2Me	H	1.8
4f	CO_2Me	H	2.5
4g	CO_2H	H	49
4h	H	CH_3	9.9
4i	H	Cl	1.6
4j	H	Br	2.1
4k	H	OCH_3	1.6
4l	H	OH	3.9

Molecular modeling indicates an interaction between the benzamidine moiety of compound **3a** and the carboxylate side chain of Asp189. The biphenyl portion of compound **3a** occupies the FXa S4 pocket. The N^1–C^2 and C^3–O^4 bonds of the central ethanolamine linker are roughly eclipsed in one of the favorable binding conformations. This conformation can be fixed by incorporating the C^2 and C^3 atoms into an aromatic ring (Fig. 1, path a). The diaryl ether compound **4a** with a 2-aminophenol as the central template is projected to fit well in the FXa active site (Fig. 2).

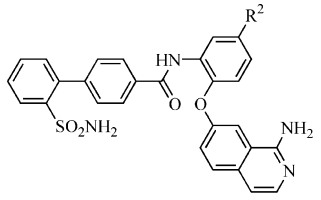
The high potency of compound **4a** ($IC_{50} = 3.6$ nM) against FXa validated our design of diaryl ethers as novel FXa inhibitors (Table 2). Compound **4a** offers diverse opportunities for exploring SARs by substitution in the template region. However, the potencies of its analogues **4b–4f** and **4h–4l** are very close to that of compound **4a**. This indicates that the substitution group at the central ring does not play a critical role in the binding of compounds **4** with FXa. The lone exception is the carboxylic acid compound **4g**, which loses an order of magnitude of potency.

Table 3. In vitro potency of 2-OH-benzoic acid-based FXa inhibitors



Compd	R^2	R^3	IC_{50} (nM)
5a	H	H	7.3
5b	NO_2	H	1.9
5c	NH_2	H	12
5d	$N(CH_3)_2$	H	10
5e	NHAc	H	7.7
5f	$NHSO_2Me$	H	5.4
5g	Cl	H	7.7
5h	Br	H	4.4
5i	H	NO_2	4.8
5j	H	NH_2	19
5k	H	$NHCH_3$	12
5l	H	$N(CH_3)_2$	16
5m	H	NHAc	61
5n	H	$NHSO_2Me$	31
5o	H	Cl	1.5
5p	H	Br	1.2

Table 4. In vitro potency of aminoisoquinolines as FXa inhibitors



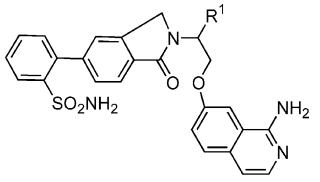
Compd	R^2	IC_{50} (nM)
6a	H	646
6b	F	1320
6c	CF_3	1810
6d	SO_2Me	6060

The reversal of the amide bond in compound **4a** was then investigated to address whether the linkage between the central phenyl ring and P4 moiety is tolerant to changes. To our delight, compound **5a** retains the anti-FXa potency (Table 3). Similar to what was observed in the series of compound **4**, the impact of central ring substitution on potency was negligible, as demonstrated by compound **5b**–**5p**.

The binding conformation of compound **4a** (Fig. 2) indicates that R^2 is mostly solvent exposed, which may explain the minimal effect of R^2 substitution on potency. The R^3 group points to the entrance wall of the FXa S1 pocket. What leads to the flat SARs with R^3 variations is unknown.

The diaryl ethers shown in Tables 2 and 3 have very good enzyme selectivity. For example, compound **4a** has IC_{50} values of $>11\,\mu M$ for thrombin, $0.97\,\mu M$ for trypsin, $18\,\mu M$ for tPA, $>181\,\mu M$ for activated protein C (APC), $43\,\mu M$ for plasmin, and $0.25\,\mu M$ for kallikrein.

Table 5. In vitro potency of aminoisoquinolines as FXa inhibitors



Compd	R^1	IC_{50} (nM)
8a	H	22
8b	Me	25
8c	Ph	623
8d	CH_2Ph	226

The antithrombotic activity of selected compounds in these series was evaluated. Compound **4a** inhibited thrombosis by 41%, which is close to the maximum inhibition (~50%) in our modified rabbit deep vein thrombosis model,[12] at $0.8\,\mu M$ plasma concentration after an intravenous dose of 2 mg/kg. Selected compounds were also evaluated for pharmacokinetic properties in Sprague–Dawley rats. For example, compound **4a** is undetectable after oral administration in rats at a dose of 6 mg/kg in a vehicle of 25% PEG-300. The absolute oral bioavailability of compound **4g** and compound **5i** in rats is only 5.5% and 0.3%, respectively.

The poor pharmacokinetic profile of compound **4a** and its analogues is not unpredicted and is probably associated with the strongly basic amidino group.[3] Less basic derivatives of benzamidines including 2-aminoisoquinolines have been reported as thrombin and FXa inhibitors.[13,14] To explore the effect of an aminoisoquinoline replacement in the diaryl ether series, compounds **6a**–**6d** were synthesized. Unfortunately, they are poor FXa inhibitors (Table 4).

Previously, we noted that the alkylation of the amide nitrogen in compound **3a** gave compounds that retained their potency. This encouraged us to design another type of FXa inhibitor with conformational constraints by tethering the amide nitrogen to the *ortho* position of the proximal phenyl ring of the P4 moiety (Fig. 1, path b). The prototypical benzopyrrolidinone compound **7** is potent against FXa ($IC_{50} = 3.6\,nM$) and it is two times more active than the uncyclized compound **4a**.

The P1 aminoisoquinoline analogues of compound **7** were then pursued to obtain FXa inhibitors with improved oral bioavailability. Compounds **8c** and **8d** with bulkier R^1 groups are >10 times less active than compounds **8a** and **8b** (Table 5). The different potencies of compounds **8a** and **6a** are speculated to arise from their distinctive binding conformations with FXa. Compound **8a** was tested for pharmacokinetic property profiling. At a dose of 6 mg/kg in 25% PEG-300, it has an oral bioavailability of less than 5% in rats.

In summary, monoamidine FXa inhibitors with an ethanolamine template were designed and synthesized. SAR studies and molecular modeling led us to design

two series of novel conformationally constrained FXa inhibitors. The diaryl ethers, as represented by compound **4a**, are very potent and highly efficacious in a rabbit deep vein thrombosis model. The benzopyrrolidinone compound **7** is also highly potent against FXa. The poor pharmacokinetic properties of the benzamidines, especially their low oral bioavailability, led us to synthesize the less basic aminoisoquinolines. Although the diaryl ether-based aminoisoquinolines are not potent FXa inhibitors, the benzopyrrolidinones have decent potency. However, their insufficient oral bioavailability does not warrant further development. We have utilized these SAR results to subsequently design potent, non-benzamidine FXa inhibitors with excellent pharmacokinetic properties. These details will be reported in due course.

References and Notes

1. Davie, E. W.; Fujikawa, K.; Kisiel, W. *Biochemistry* **1991**, *30*, 10363.
2. Vlasuk, G. P.; Ramjit, D.; Fujita, T.; Dunwiddie, C. T.; Nutt, E. M.; Smith, D. E.; Shebuski, R. J. *Thromb. Haemost.* **1991**, *65*, 257.
3. Scarborough, R. M. *J. Enz. Inhib.* **1998**, *14*, 15.
4. (a) Zhu, B.-Y.; Scarborough, R. M. *Curr. Opin. Cardiov. Pulmon. Ren. Investig. Drugs* **1999**, *1*, 63. (b) Zhu, B.-Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83.
5. Quan, M. L.; Ellis, C. D.; Liauw, A. Y.; Alexander, R. S.; Knabb, R. M.; Lam, G.; Wright, M. R.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **1999**, *42*, 2760.
6. Takayanagi, M.; Sagi, K.; Nakagawa, T.; Yamanashi, M.; Karahara, T.; Takehana, S.; Fukuda, Y.; Takahashi, M.; Shoji, M. WO 09831661, 1998.
7. For a review: Hughes, D. L. The Mitsunobu Reaction. *Org. React.* **1992**, *42*, 335. For procedure: Kim, H.-O.; Kahn, M. *Synlett* **1999**, *8*, 1239.
8. Pinner, A. In *Die Imidoaether und ihre Derivate*; Oppenheim: Berlin, 1892; p 1.
9. Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.
10. Boger, D. L.; Kim, S. H.; Miyazaki, S.; Strittmatter, H.; Weng, J.-H.; Mori, Y.; Rogel, O.; Castle, S. L.; McAtee, J. J. *J. Am. Chem. Soc.* **2000**, *122*, 7416.
11. Ueda, I.; Shiokawa, Y.; Manabe, T. U.S. patent 4,673,676, 1987.
12. Hollenbach, S.; Sinha, U.; Lin, P. H.; Needham, K.; Frey, L.; Hancock, T. E.; Wong, A.; Wolf, D. L. *Thromb. Haemost.* **1994**, *71*, 357.
13. (a) Rewinkel, J. B. M.; Lucas, H.; van Galen, P. J. M.; Noach, A. B. J.; van Dinther, T. G.; Rood, A. M. M.; Jenneboer, A. J. S. M.; van Boeckel, C. A. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 685. (b) Rewinkel, J. B. M.; Lucas, H.; Smit, M. J.; Noach, A. B. J.; van Dinther, T. G.; Rood, A. M. M.; Jenneboer, A. J. S. M.; van Boeckel, C. A. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2837.
14. Choi-Sledeski, Y. M.; Becker, M. R.; Green, D. M.; Davis, R.; Ewing, W. R.; Mason, H. J.; Ly, C.; Spada, A.; Liang, G.; Cheney, D.; Barton, J.; Chu, V.; Brown, K.; Colussi, D.; Bentley, R.; Leadley, R. J.; Dunwiddie, C.; Pauls, H. W. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2539.