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Design, synthesis, and SAR of anthranilamide-based factor Xa inhibitors incorporating substituted biphenyl P4 motifs

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Abstract—Anthranilamides **4** and **5** were designed and synthesized as selective and orally bioavailable factor Xa inhibitors. Structural modifications aimed at lowering their lipophilicity were performed at the central phenyl ring and at the S4 binding biphenyl region by incorporating water solublizing substituents. The resulting compounds (e.g., **7**, **8**, **14**, **30a**, and **32b**) are highly potent in vitro, and show improved activity in human plasma-based thrombin generation assay.

Thrombotic diseases such as deep vein thrombosis, stroke and pulmonary embolism are a major cause of mortality and morbidity in the developed world. The approaches currently explored to regulate thrombosis include reduction of thrombin generation and inhibition of thrombin activity. Factor Xa is a serine protease that links the extrinsic and intrinsic coagulation pathways of the blood coagulation cascade. It is the enzyme that converts prothrombin to thrombin. The central role of factor Xa in the coagulation cascade has attracted tremendous efforts to develop new anticoagulants as heparin and warfarin replacements.[1]

We have previously reported a series of diaryl ether-based factor Xa inhibitors derived from compound **1** by rigidifying the flexible ethanolamine template (Fig. 1).[2] The diaryl ethers, as represented by compound **2**, are potent against factor Xa in vitro and highly efficacious in our rabbit deep vein thrombosis (DVT) model at submicromolar plasma concentrations. Compound **2**, like many benzamidines, suffers from low oral bioavailability ($F < 5\%$) in rat at a dose of 6 mg/kg. In a related effort to discover factor Xa inhibitors with more favorable oral profiles, we have discovered a series of non-benzamidines as exemplified by compound **3** which contains a 5-bromo-2-aminopyridine P1 and a β-alanine linker.[3] To further explore the structure–activity relationships (SARs) and to optimize in vitro and in vivo properties, structural constraints were introduced to the β-alanine template to give the anthranilamide-based compound **4** as a potential factor Xa inhibitor by following the same underlying design of the diaryl ethers. In this communication, we report the SAR studies around compound **4**, as well as our efforts to modify the physicochemical properties of the resulting compounds in order to improve their activity in antithrombotic functional assay. It should be noted that other anthranilamide-based factor Xa inhibitors have been reported by several groups.[4–6]

As shown in Table 1, compound **4** has an anti factor Xa IC_{50} of 3.4 nM. The conformational restriction in compound **4** likely contributes to its enhanced potency

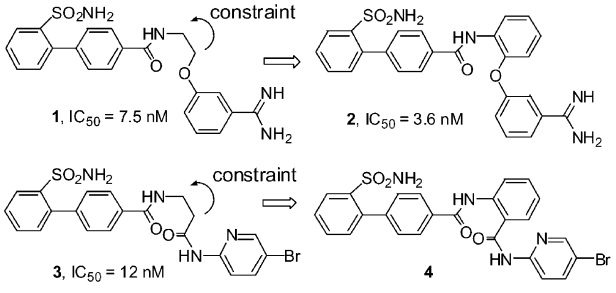

Figure 1. Design of anthranilamide **4** as a factor Xa inhibitor.

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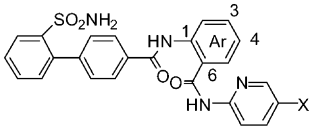
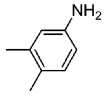
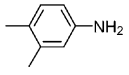
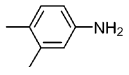
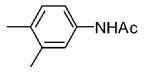
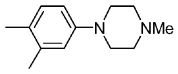
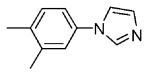
compared to **3**. Compound **4** is also very selective for factor Xa over other serine proteases. The IC_{50} values against thrombin, tissue plasminogen activator (tPA), trypsin, activated protein C (APC) and plasmin are >10 μM, and its anti kallikrein IC_{50} is 2.1 μM. The P1 chloro analogue **5** is slightly less potent than compound **4**. The enzyme selectivity profile of **5** is very similar to that of **4**.

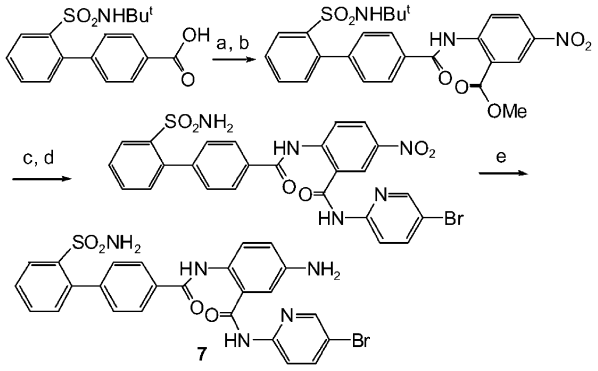
Compounds **4** and **5** exhibit significantly improved pharmacokinetic properties when compared to **2**. For example, **4** and **5** display oral bioavailability of 31% and 35%, respectively, in Sprague–Dawley rats at 6 mg/kg. In the human plasma-based thrombin generation assay,[7] high concentrations of **4** and **5** were required to

Table 1. In vitro data for compounds **4–15** as factor Xa inhibitors

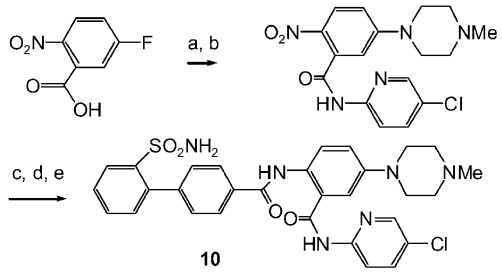


Compd	X		IC_{50} (nM)	2XTG (μM)
4	Br		3.4	>5
5	Cl		6.7	>5
6	Br		6.2	>5
7	Br		4.6	2.8
8	Cl		6.7	3.0
9	Br		370	—[a]
10	Br		1,548	—[a]
11	Br		128	—[a]
12	Cl		48	>5
13	Cl		23	4.9
14	Cl		18	2.2
15	Cl		26	>5

[a] Not tested.



Scheme 1. (a) $SOCl_2$, reflux, 2 h; (b) methyl 2-NH_2-5-NO_2-benzoate (1 equiv), pyridine, CH_2Cl_2, rt, 12 h; (c) 5-Br-2-NH_2-pyridine (4 equiv), $AlMe_3$ (20 equiv), CH_2Cl_2, rt, 12 h; (d) TFA, reflux, 2 h; (e) $SnCl_2{\cdot}2H_2O$ (4 equiv), EtOAc, reflux, 2 h.

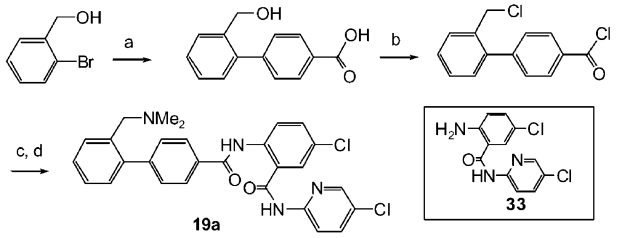


Scheme 2. (a) $POCl_3$ (1 equiv), 5-Cl-2-NH_2-pyridine (1 equiv), pyridine, rt, 5 min; (b) *N*-methylpiperazine (1.5 equiv), Cs_2CO_3 (2 equiv), DMF, 100 °C, 1 h; (c) $SnCl_2{\cdot}2H_2O$ (4 equiv), EtOAc, reflux, 2 h; (d) 4-(2-*tert*-butylaminosulfonyl)phenyl benzoyl chloride (1 equiv), pyridine, CH_2Cl_2, rt, 12 h; (e) TFA, reflux, 2 h.

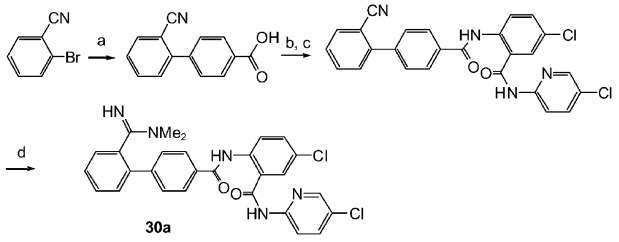
double the lag time of maximum thrombin generation (2XTG > 5 μM). The poor functional activities of **4** and **5** were partially attributed to their high lipophilicity that led to extensive plasma protein binding.[8] The cLogD values of **4** and **5** at pH 7 are 3.97 and 3.78, respectively. Both compounds are 99.8% protein bound in human plasma.[9] The detrimental effect of high plasma protein binding of factor Xa inhibitors on antithrombotic activity is consistent with recent literature disclosures.[4,6c] Similar observations have also been reported in the thrombin inhibitor area.[10,11]

We then focused on chemical modifications of compounds **4** and **5** to enhance their hydrophilicity. The initial attempt involved attaching an amino group to the central phenyl ring as in compounds **6–8**. Table 1 shows these compounds retain the high in vitro binding potency. The 4-amino substituted compounds (**7** and **8**) are markedly more active in the thrombin generation assay (2XTG = 2.8 μM for **7** and 3.0 μM for **8**) than the 3-substituted compound **6** (2XTG > 5 μM). The cLogD value of compound **7** (2.59) is indeed lower than that of compound **6** (3.35). Further modifications of the 4-amino group in **7** led to substantially less potent compounds **9–11**.

In another approach aimed at increasing the water solubility, a nitrogen atom was incorporated into the central phenyl ring. The resulting compounds, **12–15**, are less potent than **5** (Table 1). The cLogD value of



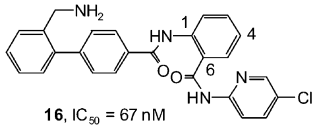
Scheme 3. (a) 4-(CO_2H)-PhB($OH)_2$ (1 equiv), $Pd(PPh_3)_2Cl_2$ (0.05 equiv), 2 M aq K_2CO_3 (5 equiv), dioxane, reflux, 1 h; (b) $SOCl_2$, reflux, 1 h; (c) **33** (1 equiv), THF, rt, 12 h; (d) $NHMe_2$ (10 equiv), DMF, rt, 2 h.



Scheme 4. (a) 4-(CO_2H)-PhB($OH)_2$ (1 equiv), $Pd(PPh_3)_2Cl_2$ (0.05 equiv), 2 M aq K_2CO_3 (5 equiv), dioxane, reflux, 1 h; (b) $SOCl_2$, reflux, 1 h; (c) **33** (1 equiv), THF, rt, 12 h ; (d) $LiNMe_2$ (5 equiv), THF, rt, 10 min.

compound **14** (3.87) is higher than that of compounds **12**, **13** and **15** (3.04, 3.05 and 3.23, respectively). What leads to the higher antithrombotic activity of compound **14** (2XTG = 2.2 μM) remains unclear.

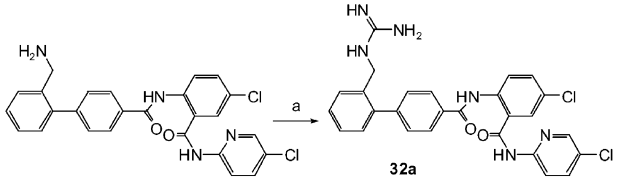
The S4 binding biphenylsulfonamide moiety in compounds **4** and **5** was originally developed by researchers at DuPont. This moiety has been successfully employed in a variety of factor Xa inhibitors.[12] We chose to replace the aminosulfonyl group with an aminomethyl as in compound **16** in order to convert compounds **4** and **5** into more hydrophilic species. As bromo substituted compounds tend to have higher cLogD values than the corresponding chloro analogues, we focused on compounds with a 5-chloro-pyridine as the P1 moiety. Compound **16** is, however, 10 times less potent than **5**.



16, IC_{50} = 67 nM

In a study of factor Xa inhibitors with structures closely related to **16**, a -Cl at the 4-position of the central phenyl ring was shown to significantly improve the anti factor Xa potency.[13] The observation is consistent with recent reports on other anthranilamide-based compounds.[4,5] Incorporation of such -Cl into compound **16** results in **17a**, which regains the lost potency (IC_{50} = 1.3 nM, Table 2) as anticipated.

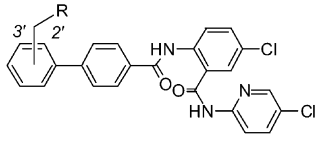
SAR endeavors around **17a** were then carried out. As shown in Table 2, compounds **18a**–**28a** show high anti factor Xa potency with the most potent ones being **19a**, **23a** and **24a**, which bear a dimethylamino, a 4-hydroxypiperidinyl and a 4-carboxypiperidinyl, respectively. Moving the P4 substituents to the 3′-position results in less potent compounds **18b**–**28b**.



Scheme 5. (a) 1*H*-Pyrazole-1-carboxamidine hydrochloride (1 equiv), TEA (5 equiv), EtOH, reflux, 5 h.

Compounds **19a** and **23a** were orally dosed at 6 mg/kg to Sprague–Dawley rats. The oral bioavailability and half life are 100% and 8.3 h for compound **19a**, and 32% and 1.5 h for compound **23a** (Table 4). However, both compounds displayed insignificant functional activity (2xTG values >5 μM). Several more compounds including **17a** and **24a**–**28a** were assayed in the human plasma-based thrombin generation assay. All showed weak anticoagulant activity even though they have K_i values in the range of 12 pM to 2 nM (Table 4).

Table 2. In vitro data for anthranilamides **17**–**28** as factor Xa inhibitors



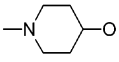
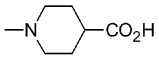
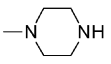
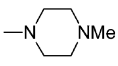
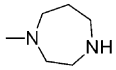
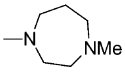
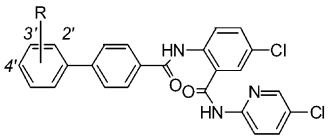
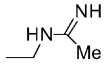
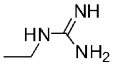
Compd	R	IC_{50} (nM)	
		2′ (**a**)	3′ (**b**)
17	NH_2	1.3	1.9
18	NHMe	4.1	2.3
19	NMe_2	0.9	3.5
20		2.8	5.3
21		2.8	16
22		4.1	74
23		1.0	7.0
24		0.4	1.8
25		4.1	39
26		3.5	23
27		5.6	32
28		3.1	22

Table 3. In vitro data for anthranilamides **29–32** as factor Xa inhibitors



Compd	R	IC_{50} (nM)		
		2′ (**a**)	3′ (**b**)	4′ (**c**)
29		—[a]	1.6	66
30		0.2	2.0	60
31		0.7	4.4	—[a]
32		1.2	9.5	—[a]

[a] Not synthesized.

Table 4. Data for selected anthranilamides as factor Xa inhibitors

Compd	K_i (nM)	2XTG (μM)	cLogD @pH = 7	F
17a	0.2	> 5	3.57	—[a]
19a	0.1	> 5	5.01	100%
23a	0.2	> 5	4.50	32%
24a	0.012	> 5	4.08	—[a]
25a	1.0	> 5	3.77	—[a]
26a	0.9	> 5	4.83	—[a]
27a	1.8	> 5	3.11	—[a]
28a	0.8	> 5	3.80	—[a]
29b	0.7	> 5	2.52	< 1%
30a	0.1	1.4	2.03	—[a]
30b	1.0	5	2.39	—[a]
31a	0.1	> 5	2.40	< 1%
31b	1.8	> 5	2.40	—[a]
32a	0.3	> 5	1.85	< 1%
32b	3.9	2.2	1.85	< 1%

[a] Not tested.

The substituted aminomethyl group was replaced with an amidine or the likes in order to further decrease lipophlicity. Table 3 summarizes the in vitro data for compounds **29–32** with amidino and guanidino substituted P4 biphenyls. The 2′-isomers are again more potent than the 3′-isomers. Further loss of potency is observed with substituents at the 4′-position.

As shown in Table 4, the amidines and guanidines **29–32** have reduced theoretical lipophilicity (cLogD) than the aminomethyl analogues. Certain compounds including **30a** and **32b** showed improved activity in the thrombin generation assay. The remaining ones (e.g., **32a**) do not display expected 2XTG values. The results indicate that the anticoagulant activity of the factor Xa inhibitors discussed above depends not only on their in vitro potency and hydrophilicity but also on other pharmacodynamic parameters including their K_{on} and K_{off} values.

Compounds **29b**, **31a**, **32a**, and **32b** were tested for oral bioavailability in rats at 6 mg/kg. They were all poorly absorbed with F values < 1% (Table 4). This result demonstrates that the improvement in anticoagulant activity would probably be at the expense of oral biovailablity.

Selected compounds described here were synthesized according to Schemes 1 to 5. Other compounds were obtained using slightly modified procedures.

In summary, we have designed and synthesized potent and orally bioavailable anthranilamide-based compounds **4** and **5** as factor Xa inhibitors. Their lack of activity in the human plasma-based thrombin generation assay led to the synthesis of more hydrophilic, and hence less protein bound factor Xa inhibitors. The strategies employed to enhance hydrophilicity included attaching an amino group to the central phenyl ring (**6–11**), substituting the central phenyl ring with a pyridyl (**12–15**), and replacing the sulfonamide moiety in the P4 region with substituted aminomethyl (**18–28**), amidino (**29–30**) or guanidino (**31–32**) groups. These compounds retain in vitro binding potency and some compounds (**7**, **8**, **14**, **30a**, and **32b**) show improved anticoagulant activity with 2XTG values between 1–5 μM. In the accompanying report, we describe our efforts at further reducing the lipophility of this type of compound in order to obtain factor Xa inhibitors which would show antithrombotic activity at therapeutically viable plasma levels.[14]

References and notes

1. (a) Gould, W. R.; Leadley, R. J. *Curr. Pharm. Des.* **2003**, *9*, 2337. (b) Samana, M. *Thromb. Res.* **2002**, *106*, 267. (c) Sanderson, P. E. L. *Ann. Rep. Med. Chem.* **2001**, *36*, 79. (d) Rai, R.; Sprengeler, P. A.; Elrod, K. C.; Young, W. B. *Curr. Med. Chem.* **2001**, *8*, 101. (e) Zhu, B.-Y.; Scarborough, R. M. *Ann. Rep. Med. Chem.* **2000**, *35*, 83.
2. Zhang, P.; Zuckett, J. F.; Woolfrey, J.; Tran, K.; Brian, H.; Wong, P.; Sinha, U.; Park, G.; Reed, A.; Malinowski, J.; Hollenbach, S.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1657.
3. Huang, W.; Wang, L.; Goldman, E.; Zuckett, J. F.; Wu, Y.; Woolfrey, J.; Huang, B.; Wong, P.; Sinha, U., A.; Scarborough, R. M.; Zhu, B.-Y. 223rd ACS National Meeting, Orlando, FL, April 7–11, 2002, MEDI 30.
4. Chou, Y.-L.; Davey, D. D.; Eagen, K. A.; Griedel, B. D.; Karanjawala, R.; Philips, G. B.; Sacchi, K. L.; Shaw, K. J.; Wu, S. C.; Lentz, D.; Liang, A. M.; Trin, L.; Morrissey, M. M.; Kochanny, M. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 507.
5. Shrader, W. D.; Young, W. B.; Sprengler, P. A.; Sangalang, J. C.; Elrod, K.; Carr, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1801.
6. (a) Herron, D. K.; Goodson, T., Jr.; Wiley, M. R.; Weir, L.; Kyle, J. A.; Yee, Y. K.; Tebbe, A. L.; Tinsley, J. M.; Masters, J. J.; Franciskovich, J. B.; Sawyer, J. S.; Beight, D. D.; Ratz, A. M.; Hat, L. A.; Milot, G.; Hall, S. E.; Klimkowski, V. J.; Towner, R. D.; Gifford-Moore, D.; Craft, T. J.; Smith, G. F. *J. Med. Chem.* **2000**, *43*, 859. (b) Yee, Y. K.; Tebbe, A. L.; Linebarger, J. H.; Beight, D. W.; Craft, T. J.; Gifford-Moore, D.; Goodson, T., Jr.; Herron,

D. K.; Klimkowski, V. J.; Kyle, J. A.; Sawyer, J. S.; Smith, G. F.; Tinsley, J. M.; Towner, R. D.; Weir, L.; Wiley, M. R. *J. Med. Chem.* **2000**, *43*, 873. (c) Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Craft, T. J.; Frowlich, L. L.; Gifford-Moore, D.; Hay, L. A.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Metz, J. T.; Ratz, M.; Shuman, R. T.; Smith, G. F.; Smith, T.; Towner, R. D.; Wiley, M. R.; Wilson, A.; Yee, Y. K. *J. Med. Chem.* **2000**, *43*, 2087.

7. Thrombin generation in human plasma assay: Potential factor Xa inhibitors are dissolved in DMSO, and serial dilutions are made in wells of 96 well plates. Reptilase-treated human plasma is added to the inhibitors, followed by substrate (H-β-Ala-Gly-Arg-*p*-nitroanilide) and $CaCl_2$. The thrombin generation reaction is started by addition of 640 pmol/L of recombinant tissue factor. The absorbance is monitored at 405 nm at 37 °C for 22 min. The results are expressed as '2XTG', the concentration of an inhibitor required to double the time of maximum thrombin generation. See also: Hemker, H. C.; Wielders, S.; Kessels, H.; Beguin, S. *Thromb. Haemost.* **1993**, *70*, 617.
8. Sinha, U.; Lin, P. H.; Edwards, S. T.; Wong, P. W.; Zhu, B.-Y.; Scarborough, R. M.; Su, T.; Jia, Z. J.; Song, Y.; Zhang, P.; Clizbe, L. A.; Park, G.; Reed, A.; Hollenbach, S. J.; Malinowski, J.; Arfsten, A. E. *Arterios. Thromb. Vasc. Biol.* **2003**, *23*, 1098.
9. Factor Xa inhibitor is dissolved in DMSO (1 mg/mL) as a stock solution. The stock solution is diluted in 1M HEPES (pH 7.4) to yield a 1 mM working solution. The working solution is added to a human plasma sample (EDTA is used as an anticoagulant) in a ration of 1:100 to yield a final concentration of 10 μM. The mixture is gently mixed and incubated at 37 °C for 30 min. At the conclusion of the incubation three aliquots (450 μL each) are added to a Microcon® YM-10 Centrifugal Filter Device fitted to a 96-well plate. A standard is prepared in protein free human plasma and transferred to the Microcon® YM-10 Centrifugal Filter Device fitted to the same plate. The plate is centrifuged (3690 rpm) for 25 min at 32 °C in a Beckman CS-6R centrifuge. 15 μL each of the filtrate is transferred to a round bottom 96 well plate and 15 μL of ACN including KN1022 (1 μg/mL) as a internal standard is added followed by 60 μL of di-water. The plate is placed on a Multi-Tube Vortexer and vortexed for 30 s. Concentrations in the filtrate are determined by LC/MS/MS and using standard curves prepared in ultra filtered plasma. The plasma protein binding value is expressed as a percentage.
10. Brady, S. F.; Sisko, J. T.; Stauffer, K. J.; Colton, C. D.; Qiu, H.; Lewis, S. D.; Ng, A. S.; Shafer, J. A.; Bogusky, M. J.; Veber, D. F.; Nutt, R. F. *Bioorg. Med. Chem.* **1995**, *3*, 1063.
11. Jones, D. M.; Atrash, B.; Ryder, H.; Teger-Nilsson, A.-C.; Gyzander, E.; Selke, M. *J. Enz. Inhib.* **1995**, *9*, 43.
12. Quan, M. L.; Liauw, A. Y.; Ellis, C. D.; Pruitt, J. R.; Carini, D. J.; Bostrom, L. L.; Huang, P. P.; Harrison, K.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **1999**, *42*, 2752.
13. Zhang, P. et al., unpublished results.
14. Zhang, P.; Bao, L.; Zuckett, J. F.; Jia, Z. J.; Woolfrey, J.; Arfsten, A.; Edwards, S.; Sinha, U.; Hutchaleelaha, A.; Lambing, J. L.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, following paper in this issue. doi: 10.1016/j.bmcl.2003.11.080.