Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 989–993

Design, synthesis, and SAR of anthranilamide-based factor Xa inhibitors with improved functional activity

Penglie Zhang,* Liang Bao, Jingmei F. Zuckett, Zhaozhong J. Jia, John Woolfrey, Ann Arfsten, Susan Edwards, Uma Sinha, Athiwat Hutchaleelaha, Joseph L. Lambing, Stanley J. Hollenbach, Robert M. Scarborough and Bing-Yan Zhu*

Millennium Pharmaceuticals, Inc., 256 E. Grand Avenue, South San Francisco, CA 94080, USA

Received 8 July 2003; revised 26 November 2003

Abstract—Compound **2** containing an aminomethylbenzoyl moiety as the S4 binding motif was synthesized in order to modulate hydrophlicity of anthranilamide-based factor Xa inhibitors with substituted biphenyl P4 groups. Structure–activity relationship studies around **2** have led to a series of potent factor Xa inhibitors which are highly active in the human plasma-based thrombin generation assay with 2XTG values less than 1 μM. Compound **55** shows strong antithrombotic activity in our rabbit deep vein thrombosis model, and also exhibits good oral bioavailability and a long half life in rats.

Factor Xa is an attractive target for the development of new orally active anticoagulants to replace warfarin, a vitamin K antagonist. The early factor Xa inhibitors—such as BABCH,[1] DABE,[2] and DX-9065a[3]—were mostly bisamidines. The poor pharmacokinetics, including low oral bioavailability, of these compounds led to the synthesis of a variety of mono-benzamidines as factor Xa inhibitors in which the P1 amidino group is retained because it has been deemed critical for the strong binding of an inhibitor with factor Xa. However, most mono-benzamidines still lack desirable level of oral absorption to be developed as oral anticoagulants.[4]

Anthranilamide-based factor Xa inhibitors have been reported by several groups.[5–7] In the preceding paper, we have described the discovery of compound **1** and its analogues (Fig. 1). These compounds are highly potent in in vitro binding assay (IC_{50} and K_i). However, they display low anticoagulant activity in the human plasma-based thrombin generation assay as demonstrated by their 2XTG values.[8] The low anticoagulant activity is partially attributed to the low hydrophilicity and high plasma protein binding of this type of compound. In an effort to design less lipophilic factor Xa inhibitors, we sought to replace the distal phenyl ring in compound **1** with a *para*-aminomethyl group as in compound **2**. The amino group not only renders compound **2** a decreased cLogD value (2.36), but is also expected to bind to the S4 pocket of factor Xa through a π-cation interaction. We report here the synthesis of compound **2** and its analogues, as well as the structure–activity relationship (SAR) studies leading to factor Xa inhibitors which show improved activity in the thrombin generation assay. Anthranilamide-based factor Xa inhibitors incorporating aminomethyl substituted chlorothiophene moieties as P4 have recently been reported by Berlex scientists.[9]

Compound **2** was synthesized and found to be less potent (IC_{50} = 10 nM, Table 1) than **1** by about one order of magnitude. SAR studies on substitution of the benzylic amino group show that methylation increases potency as demonstrated by compounds **7** and **8**. Bulkier substituents at the amino group reduce in vitro activity as shown by **9** and **10**. Compounds **11**–**16** were prepared to probe additional interactions in the S4

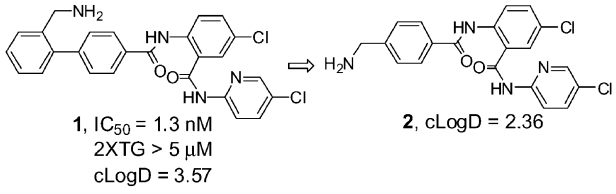
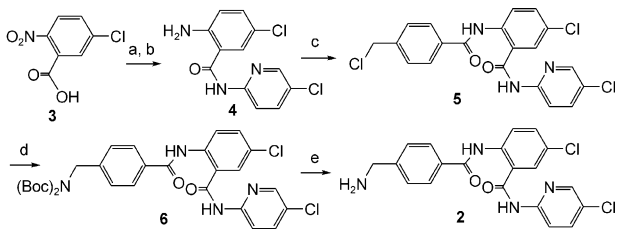


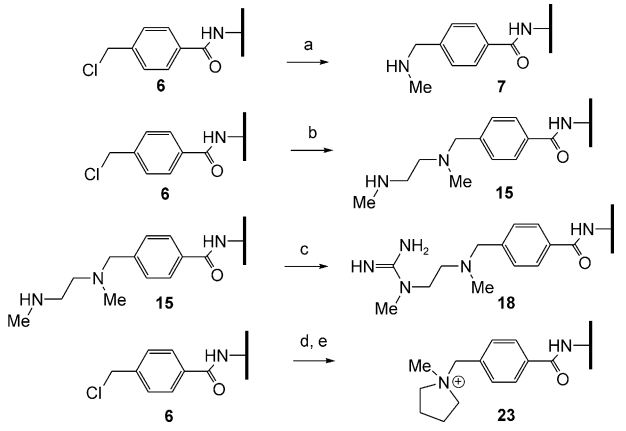
Figure 1.

* Corresponding author. Tel.: +1-650-246-7312 (P.Z.); tel.: +1-650-244-6986 (B.-Y.Z.); e-mail: penglie.zhang@mpi.com; yan.zhu@mpi.com

0960-894X/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2003.11.080



Scheme 1. (a) $POCl_3$ (1.1 equiv), 5-Cl-2-NH_2-pyridine (1 equiv), pyridine, rt, 5 min; (b) $SnCl_2{\cdot}2H_2O$ (4 equiv), EtOAc, reflux, 2 h; (c) 4-chloromethyl-benzoyl chloride (1 equiv), THF, rt, 12 h; (d) $NH(Boc)_2$ (5 equiv), NaH (5 equiv), DMF, rt, 3 h; (e) 4 N HCl in dioxane, rt, 3 h.



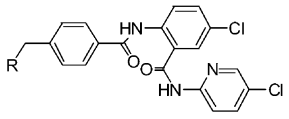
Scheme 2. (a) NH_2Me (5 equiv), DMF, rt, 1 h; (b) $NHMe(CH_2)_2NHMe$ (5 equiv), DMF, rt, 1 h; (c) 1*H*-pyrazole-1-carboxamidine hydrochloride (1 equiv), TEA (5 equiv), EtOH, reflux, 5 h; (d) pyrrolidine (5 equiv), DMF, rt, 1 h; (e) MeI (neat), reflux, 5 h.

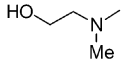
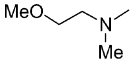
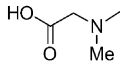
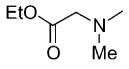
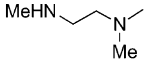
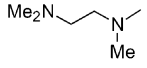
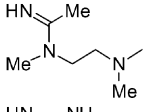
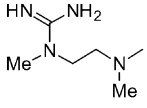
region. The higher potencies of **15** and **16** may result from ionic or hydrogen bonding interactions between the distal amino group and Glu97 at the region extended from factor Xa's S4 pocket. These interactions may also contribute to the enhanced potency of compounds **17** and **18** due to the increased basicity at the P4 terminal. Compound **20** bearing a dimethylhydrazide group is a sub-nanomolar factor Xa inhibitor. The high potency of compounds **22**–**24** may arise from a π-cation interaction between the positive charge and the box-like S4 pocket of Xa formed by Tyr99, Phe174 and Trp215.

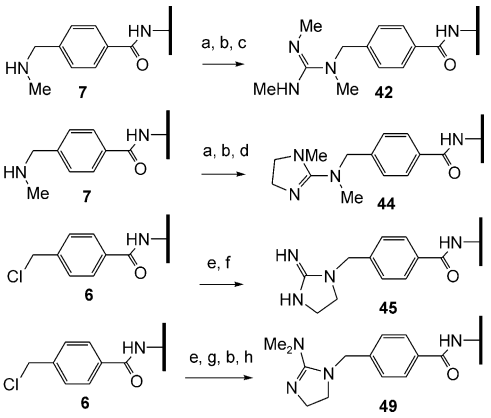
Among the factor Xa Inhibitors containing cyclic amino groups (Table 2), **25** and **26** are equipotent. A 4-amino substituent at the piperidine moiety is tolerated as shown by compound **28**. Compounds **33**–**35** further demonstrate that a basic moiety at the P4 terminus helps retain factor Xa inhibitory activity.

Table 3 summarizes the in vitro binding potency of compounds **36**–**49** which contain an amidino or guanidino group at the P4 position. The potency enhancing effect of N^1-methylation is again observed (**37** and **40** vs **36** and **39**). Compound **38** with a phenyl at the P4 terminal is less potent than the methyl analogue **37**. Stepwise methylation of the guanidine nitrogen atoms retains anti-factor Xa potency as demonstrated by compounds **41** and **42**. Cyclization with an ethylene unit between two guanidine nitrogens results in compounds **43**–**45**. The increase in ring size from five in **45** to six in **46** and seven in **47** leads to slight decreases in potency. Methylation or dimethylation of the exocyclic nitrogen

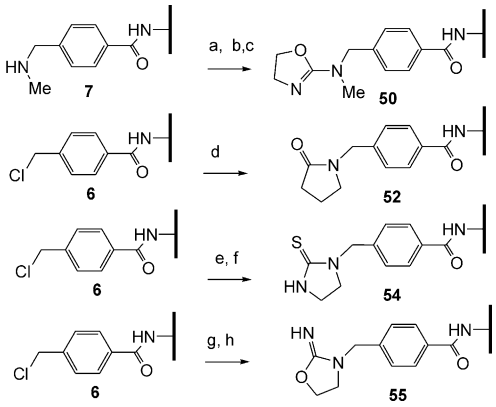
Table 1. Compounds **7**–**24** as factor Xa inhibitors



Compd	R	IC_{50} (nM)
2	NH_2	10
7	NHMe	7.0
8	NMe_2	4.0
9		12
10		35
11		82
12		53
13		156
14		176
15		6.8
16		6.9
17		1.1
18		2.0
19		40
20		0.9
21		40
22		0.8
23		2.3
24		5.8

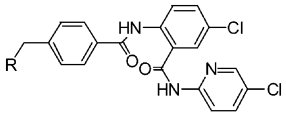


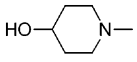
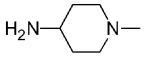
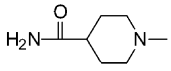
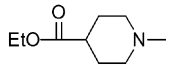
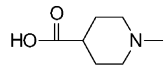
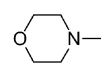
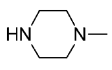
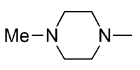
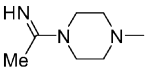
Scheme 3. (a) MeNCS (5 equiv), THF, EtOH, reflux, 12 h; (b) MeI (5 equiv), acetone, reflux, 2 h; (c) NH_2Me (5 equiv), MeOH, reflux, 20 min; (d) $NH_2(CH_2)_2NHMe$ (2 equiv), MeOH, rt, 2 h; (e) $NH_2(CH_2)_2NH_2$ (5 equiv), DMF, rt, 2 h; (f) CNBr (3 equiv), DMF, rt, 12 h; (g) Im_2CS (1 equiv), Et_3N (5 equiv), DMF, 70 °C, 2 h; (h) $NHMe_2$ (5 equiv), MeOH, reflux, 20 min.



Scheme 4. (a) MeNCS (5 equiv), THF, EtOH, reflux, 12 h; (b) MeI (5 equiv), acetone, reflux, 2 h; (c) $NH_2(CH_2)_2OH$ (2 equiv), MeOH, rt, 2 h; (d) 2-pyrrolidinone (2 equiv), K_2CO_3 (5 equiv), DMF, 80 °C; (e) $NH_2(CH_2)_2NH_2$ (5 equiv), DMF, rt, 2 h; (f) Im_2CS (1 equiv), Et_3N (5 equiv), DMF, 70 °C, 2 h; (g) $HO(CH_2)_2NH_2$ (10 equiv), DMF, rt, 2 h; (h) CNBr (3 equiv), DMF, rt, 12 h.

Table 2. Compounds **25**–**35** as factor Xa inhibitors



Compd	R	IC_{50} (nM)
25		16
26		15
27		136
28		15
29		76
30		171
31		230
32		140
33		17
34		15
35		5

of compound **45** leads to compounds **48** and **49** which retain high potency.

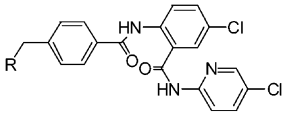
Continued modification of the guanidine moiety was carried out by replacing one or two nitrogens with a carbon, oxygen or sulfur atom to obtain less basic analogues (compounds **50**–**55** in Table 3). The most potent compounds are **50**[10] and **55**. Compound **50** is 7 times more potent than its sulfur analogue, **51**. The least potent compounds in Table 3 are **52**, **53**, and **54**. This observation demonstrates again that more basic P4 motifs give more potent factor Xa inhibitors.

The compounds discussed above show excellent selectivity ($IC_{50} > 11$ μM) against thrombin, trypsin, tissue plasminogen activator (tPA), activated protein C (APC) and plasmin. Lower selectivity against kallikrein is observed as exemplified by some of the most potent compounds (Table 4).

Human plasma-based thrombin generation assay was performed on selected potent compounds. As shown in Table 4, compound **8** doubled the lag time of maximum thrombin generation at a fairly high plasma concentration (2XTG = 3.2 μM) despite its high in vitro binding potency. Compound **20**, which is more potent in vitro and more water soluble than **8**, has a 2XTG = 1.5 μM. Compound **22** bearing a quaternary nitrogen; amidine and guanidine-based compounds **37**, **40**, **41**, **44** and **45**; as well as oxazolidine **55** are even more active in this assay. Their 2XTG values are less than 1 μM.

The pharmacokinetics of compounds in Table 4 was tested in Sprague–Dawley rats. Compound **8** is highly absorbed following oral administration (6 mg/kg). The half life, volume of distribution and clearance of compound **8** are respectively 7.5 h, 32.9 L/kg and 20.6 mL/min/kg (0.4 mg/kg dosed intravenously). The hydrazide **20**; quaternary salt **22**; amidine **37**; guanidines **40**, **41**, **44**, and **45** have poor oral bioavailability. Compound **55** has an F value of 44%. This compound also has a long half life (8.5 h), reasonable volume of distribution (13.6

Table 3. Compounds **36–55** as factor Xa inhibitors



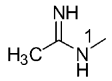
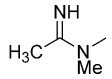
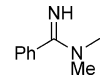
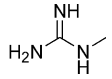
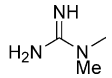
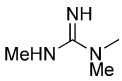
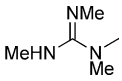
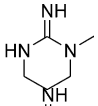
Compd	R	IC_{50} (nM)
36		3.6
37		0.9
38		11
39		1.4
40		1.0
41		0.7
42		0.8
43		2.0
44		0.3
45		1.5
46		3.8
47		6.3
48		3.5
49		1.3
50		1.5
51		10
52		552
53		30

Table 3 (*continued*)

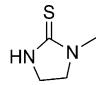
Compd	R	IC_{50} (nM)
54		35
55		0.2

Table 4. Data for selected compounds as factor Xa inhibitors

Compd	Xa K_i (nM)	kallikrein, IC_{50} (μM)	2XTG (μM)	cLogD @pH 7	F (%)
8	1.3	0.50	3.2	3.61	100
20	0.3	0.64	1.5	3.91	4
22	1.0	0.13	0.3	—[a]	<1
37	0.6	0.50	0.5	0.71	<1
40	0.1	0.38	0.38	0.17	<1
41	0.2	0.79	0.79	0.50	—[b]
44	0.3	0.30	0.30	0.04	<1
45	0.5	0.21	0.58	−0.07	<1
55	1.5	0.56	0.56	1.63	44

[a] By definition, a cationic compound does not have a cLogD value.
[b] Not tested.

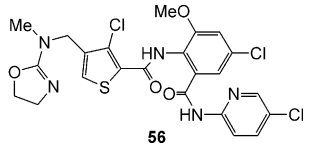
L/kg) and clearance (18.3 mL/min/kg) at an intravenous dose of 0.4 mg/kg. Inhibitor **55** shows 25% and 40% inhibition of thrombus formation at a plasma concentration of 0.25 μM and 0.83 μM, respectively, in our rabbit deep vein thrombosis model.[11] The cLogD of compound **55** (1.63) is in the middle of the range shown in Table 4. The favorable in vivo profile of compound **55** strongly implies that a balance between anticoagulant activity and oral absorption can be achieved by fine tuning the physical chemical properties of the anthranilamide-based factor Xa inhibitors and their in vitro binding potency.

The preparation of compounds in Tables 1 to 3 is exemplified by the synthesis of compounds shown in Schemes 1–4. Other compounds were obtained according to slightly modified procedures.

In summary, in order to modulate the lipophilicity of the previous series of anthranilamide-based factor Xa inhibitors with biphenyl P4 groups, we have designed and synthesized compounds which contain substituted aminomethylbenzoyl moieties as the S4 binding motifs. These compounds are potent against factor Xa in vitro, selective over other serine proteases and more active in the human plasma-based thrombin generation assay. Compound **55** not only shows strong antithrombotic activity in our rabbit deep vein thrombosis model, but also has good oral bioavailability and long half life in rat. The amidine or guanidine containing compounds are poorly absorbed, but exhibit potent activity in the functional thrombin generation assay. Further modifications of this type of compound resulted in P1 neutral, P4 benzamidine-based factor Xa inhibitors which display pharmacokinetic profiles suitable for oral anticoagulants. The details will be reported in due course.

References and notes

1. Sturzebecher, J.; Markwardt, F.; Walsmann, P. *Thromb. Res.* **1976**, *9*, 637.
2. Tidwell, R. R.; Webster, W. P.; Shaver, S. R.; Geratz, J. D. *Thromb. Res.* **1980**, *19*, 339.
3. Nagahara, T.; Yokoyama, Y.; Inamura, K.; Katakura, S.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. *J. Med. Chem.* **1994**, *37*, 1200.
4. (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. *Annu. 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. *Annu. Rep. Med. Chem.* **2000**, *35*, 83.
5. 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.
6. Shrader, W. D.; Young, W. B.; Sprengler, P. A.; Sangalang, J. C.; Elrod, K.; Carr, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1801.
7. (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.
8. Zhang, P.; Bao, L.; Zuckett, J. F.; Goldman, E. A.; Jia, Z. J.; Arfsten, A.; Edwards, S.; Sinha, U.; Hutchaleelaha, A.; Park, G.; Lambing, J. L.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, the preceding paper in this issue. doi: 10.1016/j.bmcl.2003.11.079.
9. (a) For a patent disclosure: Arnaiz, D. O.; Chou, Y.-L.; Karanjawala, R.; Kochanny, M. J.; Lee, W.; Liang, A. M.; Morrissey, M. M.; Phillips, K. L.; Sacchi, K. L.; Sakata, S. T.; Shaw, K. J.; Snider, R. M.; Wu, S. C.; Ye, B.; Zhao, Z.; Griedel, B. D. WO 9932477, 1999. (b) For the crystal structures of two inhibitors in this series bound to factor Xa: Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, G.; Light, D. R.; Biancalana, S.; Whitlow, M. *Biochemistry* **2002**, *41*, 15514.
10. A structurally related compound (**56**) has recently been disclosed by Berlex. See: Ye, B.; Cheeseman, S.; Chou, Y.-L.; Ewing, J.; Fitch, R.; Griedel, B. D.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A.; Morrissey, M. M.; Post, J.; Sacchi, K. L.; Sakata, S.; Shaw, K. J.; Subramannyam, B.; Vergona, R.; Walters, J.; Wang, Y.-X.; White, K. A.; Wu, S. C.; Zhao, Z.; Kochanny, M. J. 221st ACS National Meeting, Anaheim, CA, April 1–5, 2001, MEDI 128. Compound **56** reportedly has a 40% oral bioavailability in dog and shows antithrombotic effect with an $ED_{50} = 0.8$ μM in a rat vena cava stasis model.



56

11. Hollenbach, S.; Sinha, U.; Lin, P. H.; Needham, K.; Frey, L.; Hancock, T. E.; Wong, A.; Wolf, D. L. *Thromb. Haemost.* **1994**, *71*, 357.