Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1023–1028

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Nonbenzamidine Isoxazoline Derivatives as Factor Xa Inhibitors

Mimi L. Quan,* Christopher D. Ellis, Ming Y. He, Ann Y. Liauw, Patrick Y. S. Lam, Karen A. Rossi, Robert M. Knabb, Joseph M. Luettgen, Matthew R. Wright, Pancras C. Wong and Ruth R. Wexler

Bristol-Myers Squibb Co., PO Box 80500, Wilmington, DE 19880-0500, USA

Received 15 October 2002; revised 21 January 2003; accepted 22 January 2003

Abstract—Factor Xa (fXa) is an important serine protease in the blood coagulation cascade. Inhibition of fXa has emerged as an attractive target for potential therapeutic applications in the treatments of both arterial and venous thrombosis. Herein, we describe a series of non-benzamidine isoxazoline derivatives as fXa inhibitors. The chloroaniline group was found to be the most potent benzamidine mimic in this series. Chloroaniline **1** (ST368) has a K_i value of 1.5 nM against fXa and is highly selective for fXa relative to thrombin and trypsin.

Introduction

Thrombotic disorders are a major cause of mortality in Western society. Thrombosis is the result of a blood clot, or a thrombus, in a blood vessel results in occlusion of the blood flow. Thrombin, the final serine protease in the blood coagulation cascade, is responsible for the conversion of fibrinogen to insoluble fibrin eventually leading to blood clotting. Factor Xa (fXa), a trypsin-like serine protease, is a critical enzyme which in combination with fVa and ca^{2+} on a phospholipid surface forms the prothrombinase complex that converts biologically inactive prothrombin to thrombin. The activation of thrombin by fXa is a highly amplified process.[1] As a result, inhibition of fXa may be more effective than direct inhibition of thrombin in regulation of haemostasis. In addition, inhibitors of fXa may have a more favorable efficacy: safety ratio compared to direct inhibitors of thrombin as demonstrated in animal models of thrombosis.[2] Accordingly, fXa has emerged as an important target for the development of new antithrombotic agents.[3]

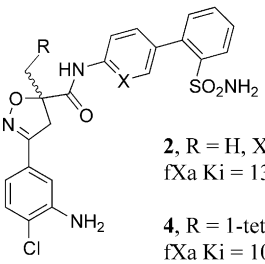
Previous reports from our laboratory have described a series of isoxazoline benzamidine derivatives as fXa inhibitors.[4–7] We have shown that the bisbenzamidine compounds[4] can be elaborated to monobasic compounds[5–7] with improved inhibitory activity, efficacy, and pharmacokinetics. These compounds are potent fXa inhibitors and anticoagulants as shown by SK549 and SM084.[5–7] However, these fXa inhibitors still have a relatively short half-life and low oral bioavailability, in part due to the basicity of the benzamidine group. Therefore, the benzamidine group was replaced with less basic and non-basic benzamidine mimics to try to enhance the pharmacokinetic profile and oral bioavailability of these inhibitors.

Results and Discussion

A variety of benzamidine mimics were employed in an isoxazoline and a pyrazole series, which afforded potent and selective fXa inhibitors.[8–10] Several structure-based P_1 libraries were designed and synthesized. Results from these libraries indicated that chloroaniline was a relatively potent and selective benzamidine mimic, approximately 15–100-fold less potent for fXa than the corresponding benzamidine analogues.[10] As shown in Figure 1, chloroaniline **2** was determined to be approximately 20-fold less potent compared to the benzamidine analogue **3** while chloroaniline **4** was about 60-fold less active than the corresponding benzamidine **5**. These results suggest that the structural activity relationships derived from substitution of the 5-position of the isoxazoline may be different when a chloroaniline group is employed as the P_1 moiety. Therefore a series

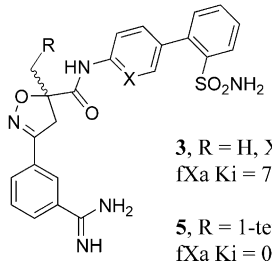
*Corresponding author. Tel.: +1-609-818-5301; fax: +1-609-818-3331; e-mail: mimi.quan@bms.com

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00080-5



2, R = H, X = CH
fXa Ki = 130 nM

4, R = 1-tetrazole, X = N
fXa Ki = 10 nM



3, R = H, X = CH
fXa Ki = 7.2 nM

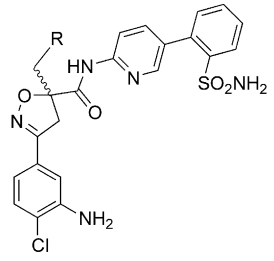
5, R = 1-tetrazole, X = N
fXa Ki = 0.17 nM

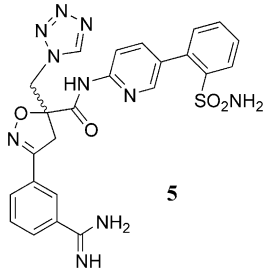
Figure 1. Comparison of benzamidine and chloroaniline in the P_1 position.

of compounds was prepared with chloroaniline in the P_1 position and various substituents at the 5-position of the isoxazoline.

Five-membered ring heterocycles at the 5-position of the isoxazoline were initially evaluated because analogues such as **5** showed good fXa potency in the benzamidine series.[5–6] Tetrazole **4**, pyrazole **6**, and 1,2,4-triazole **7** were found to have similar fXa affinity in the range of 10–35 nM (Table 1). During the synthesis of **4**, a more potent by-product (fXa K_i of 2.5 nM) was isolated and was identified to be urea **8**. To follow up on this lead, the series of compounds shown in Table 1 were prepared.

Table 1. Isoxazoline derivatives with various substitutions at R[5]





5

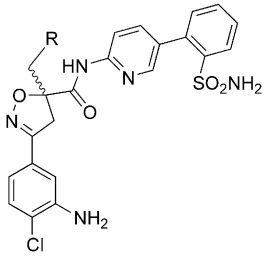
Compd	R	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
5 (±)		0.17	1100	31
4 (±)		10	>21,000	>1600
6 (±)		14	>21,000	>1600
7 (±)		34	>21,000	>4200
8 (±)	**$NHCONH_2$**	**2.5**	**9300**	**>1600**
9 (±)	NHCONHEt	69	>6300	>4200
10 (±)	NHCOMe	11	>21,000	>1600
11 (±)	NHCOH	15	14,000	>8000
12 (±)	$NHSO_2NH_2$	5.5	5900	>8000
13 (±)	**$NHSO_2Me$**	**1.9**	**1300**	**>8000**
14 (±)	$NHSO_2$-nPr	3.7	18,000	>8000
15 (±)	$NHSO_2CH_2CF_3$	3.3	6600	>8000
16 (±)	$NHSO_2Ph$	1.7	>21,000	>8000
17 (±)	$NHSO_2$-thiophen-3-yl	1.2	>21,000	>8000
18 (±)	$NHSO_2$-3-pyridyl	3.0	17,000	>8000
19 (±)	$NHSO_2CH_2Ph$	2.2	6200	>4200

fXa, thrombin, and trypsin K_i values were obtained using human purified enzymes. See ref 11 for assay conditions.

Ethyl substitution at the terminal urea nitrogen decreased fXa affinity significantly (**9**). Replacement of the terminal urea NH_2 group with either a methyl group (**10**) or hydrogen (**11**) gave compounds 4–6 times less active. The sulfamide analogue **12** was 2× less potent. The fXa activity was regained when the terminal NH_2 of **12** was replaced with a methyl moiety (**13**). Other sulfonamides (**14**–**19**) also showed low nanomolar affinity for fXa, although no significant improvement over **13** was observed (Table 1). As previously observed, all of the chloroanilines demonstrated very good selectivity for fXa relative to thrombin and trypsin.[10] This selectivity can be explained by the smaller S_1 pocket in trypsin active site relative to Xa, thereby causing unfavorable interaction by the change from the smaller hydrophobic residue alanine to the somewhat larger and more hydrophilic serine at position 190.

Several sets of enantiomers were obtained by chiral HPLC separation as shown in Table 2. In each case, the negative

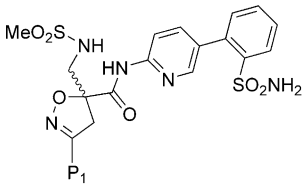
Table 2. Comparison of the enantiomers

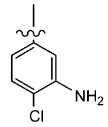
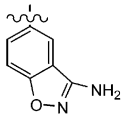
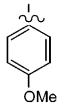


Compd	R	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
20 (−)		7.1	19,000	>1600
21 (+)		320	>21,000	>1600
22 (−)		15	>21,000	>1600
23 (+)		1100	>21,000	>1600
24 (−)	$NHCONH_2$	2.5	800	>1600
25 (+)	$NHCONH_2$	69	>21,000	>6000
1 (−)	$NHSO_2Me$	1.5	800	>8000
26 (+)	$NHSO_2Me$	240	>21,000	>4200

fXa, thrombin, and trypsin K_i values were obtained using human purified enzymes. See ref 11 for assay conditions.

Table 3. Compounds with nonbenzamidine mimics at P_1



Compd	P1	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
13		1.9	1300	>8000
27		4.6	5200	>5200
28		10	>6300	>4200

fXa, thrombin, and trypsin Ki values were obtained using human purified enzymes. See ref 11 for assay conditions.

enantiomer was found to be more potent. This is in agreement with what was observed earlier for the benzamidine series. Methylsulfonamide ST368 (**1**) was the most potent fXa inhibitor prepared in this series (fXa $K_i = 1.5$ nM).

The chloroaniline moiety in **13** was also replaced by other benzamidine mimics[10] as shown Table 3. Aminobenzisoxazole **27** and *p*-methoxyphenyl **28** have fXa K_i values of 4.6 and 10 nM, about two- and five-fold less potent than the corresponding chloroaniline **13**, respectively. These two compounds are also highly selective for fXa related to other serine protease.

Computer modeling studies using Insight II (V.98.0, MSI, San Diego, CA) and the CFF98 Forcefield to compare SM084 and **20** (Fig. 2) have shown that **20** penetrates deeper down into the S1 specificity pocket in order for the aniline group to interact with Asp^{189}. The aniline moiety is capable of interacting with Asp^{189} in a similar way as achieved for SM084; but the NH_2 of **20** is not able to form the additional hydrogen bond with Gly^{218} of the enzyme backbone as observed with SM084. However, the chlorine is capable of forming a lipophilic interaction with Val^{213}, which may have resulted in the enhanced fXa affinity. A nitrogen in the tetrazole group of **20** could potentially form a hydrogen bond with either Arg^{143} or Gln^{192}. Comparing **24** and **1** with **20**, it appears that both the urea CO and the

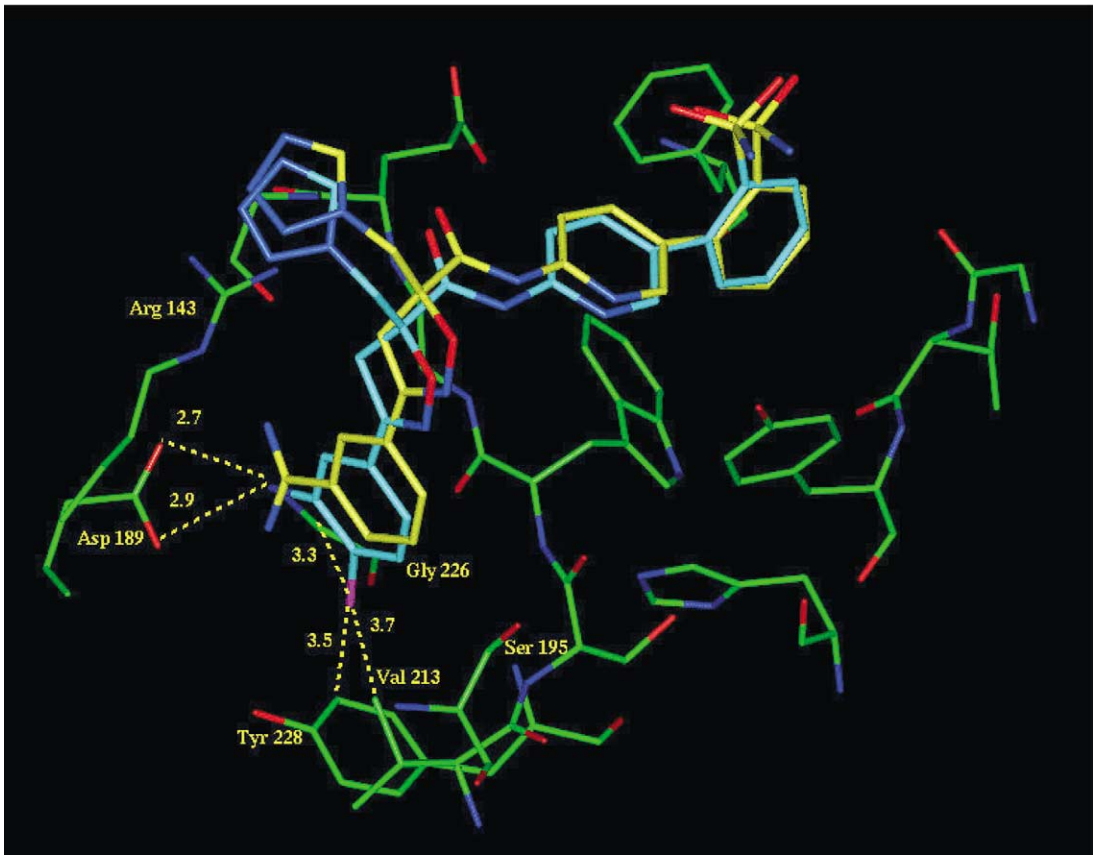


Figure 2. Modeling of SM084 and 20 in the factor Xa active site.

Table 4. PK profiles of selective compounds in dogs

Compd	Dose (mg/kg)	Clearance (L/h/kg)	$t_{1/2}$ (h)
SM084	5.0	1.32	1.1
24	0.5	0.43	2.32
ST368 (**1**)	0.5	0.53	7.25

PK Data for SM084 were obtained by intravenous dosing in a discrete study. Data for other compounds were obtained from intraveneous cassette dosing studies N-in-1 iv studies.

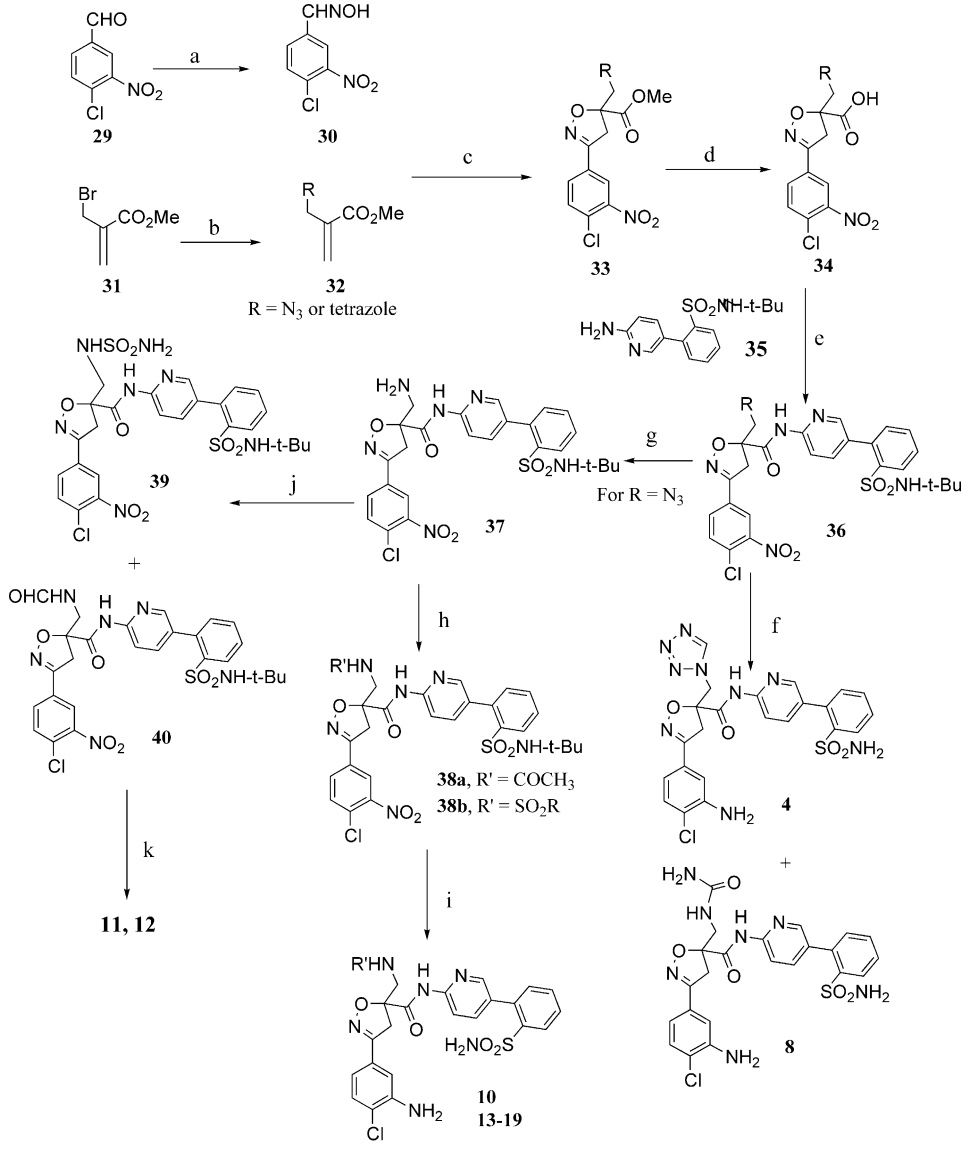
Table 5. Antithrombotic efficacy of selective compounds

Compd	Human fXa K_i (nM)	Rabbit fXa K_i (nM)	ID_{50} (μmol/kg/h)
SM084	0.11	0.11	0.032
24	2.5	4.7	5.5
ST368 (**1**)	1.5	2.3	8.0

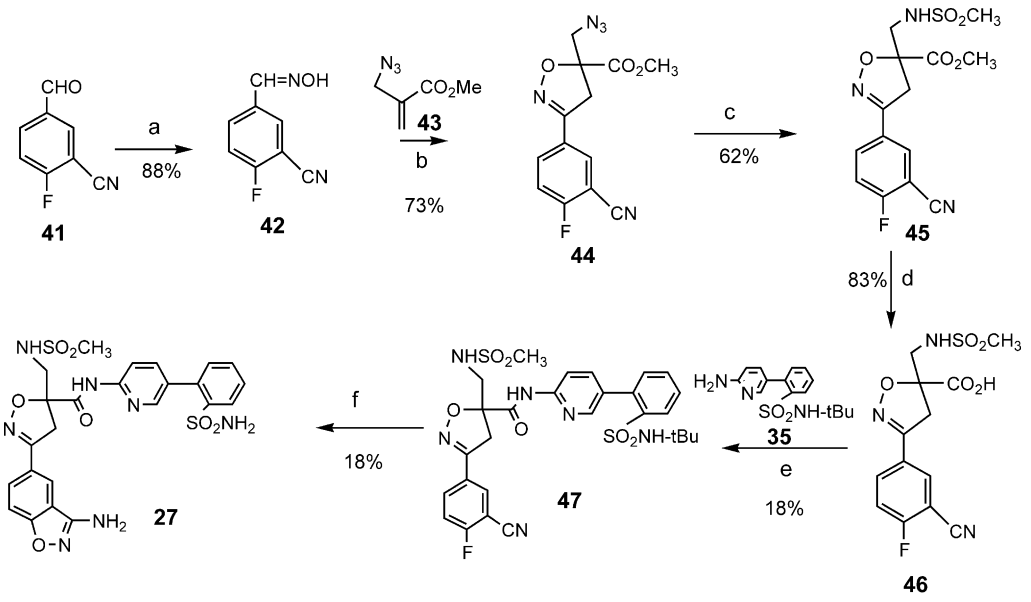
ID_{50} values were determined in the rabbit Arterio-Venous shunt model.[7]

methylsulfonamide SO ought to be capable of forming a hydrogen bond with Arg^{143} or Gln^{192}. Additionally, the NH group is within binding distance of Gly^{218}. The enhanced Xa affinity may be due to the urea and the sulfonamide being better hydrogen bond acceptors than the tetrazole. In addition, the methyl, phenyl, and thiophenyl groups have the potential for a favorable lipophilic interaction with the Cys ^{220}/Cys^{191} disulfide bond.

Computer modeling also shows that the amino group of aminobenzisoxazole can form a hydrogen bond with Gly^{218} and Asp^{189} and the other nitrogen can interact with Asp^{189} through a water molecule. Compared to the chloroaniline, the aminobenzisoxazole group shows similar interactions with the enzyme and therefore is anticipated to have similar affinity. Although the methoxy group of the *p*-methoxyphenyl can interact with the enzyme in a similar way to the chloro group of the chloroaniline, it lacks the potential interaction with



Scheme 1. Synthesis of chloroaniline derivatives. (a) NH_2OH/EtOH/pyridine; (b) NaN_3 or tetrazole/DMSO; (c) bleach/CH_2Cl_2; (d) LiOH/THF; (e) SOCl, **35**/Et_3N/CH_2Cl_2; (f) TFA, $SnCl_2$/EtOAc; (g) $P(OEt)_3$/THF, H^+; (h) RCOCl or RSO_2Cl/Et_3N; (i) TFA, $SnCl_2$/EtOAc; (j) $ClSO_2NCO$/formic acid/Et_3N; (k) TFA, $SnCl_2$/EtOAc.



Scheme 2. Synthesis of aminobenzisoxazole SV27. (a) NH_2OH/EtOH/pyridine; (b) bleach/CH_2Cl_2; (c) $P(OEt)_3$/THF, H^+, CH_3SO_2Cl; (d) LiOH/THF; (e) $(COCl)_2$, **35**/Et_3N/CH_2Cl_2; (f) $(CH_3)_2CNOH$/KO-*t*-Bu, EtOH/H^+, TFA.

Asp[189]. Hence, the 5-fold decrease in fXa affinity is not surprising.

To determine the pharmacokinetic profile of these compounds relative to the corresponding benzamidine, Compounds **24** and ST368 (**1**) were studied in dogs dosed at 0.5 mg/kg intravenously (Table 4). ST368 showed an improved clearance value (0.5 L/kg/h vs 1.32 L/kg/h) and prolonged half-life (7.25 h vs 1.1 h) compared with SM084. These two compounds (**24** and **1**) were evaluated for antithrombotic efficacy in a rabbit arterio-venous shunt thrombosis model[7] and the results relative to SM084 are shown in Table 5. The ID_{50} values were determined to be 5.5 and 8.0 μmol/kg/h, respectively, compared to 0.032 μmol/kg/h for SM084. In the in vitro clotting assay using human plasma, compounds **24** and ST368 double the APTT at 11 and 4.6 μM, respectively.

Although we have improved the pharmacokinetic profile in terms of clearance and half-life with ST368, the A-V shunt and APTT results indicate that ST368 is not potent enough for further preclinical progression. The low efficacy of ST368 may be due to high protein binding. Therefore, compounds discussed in this paper were not evaluated in oral bioavailability studies.

Synthesis

The tetrazole analogues **4** and **20** were prepared utilizing the same methods previously described for the synthesis of SM084,[6] but with the use of 4-chloro-3-nitrobenzaldehyde as the starting material (Scheme 1). The urea **8** was isolated as a by product from the last two steps of the tetrazole formation reactions (TFA, $SnCl_2$). The triazole and pyrazole analogues were prepared in a similar manner as the preparation of the tetrazoles. The other chloroaniline derivatives were synthesized from the azide intermediate **36**. Reduction of the azide group with $P(OEt)_3$ followed by acid hydrolysis afforded amine **37**. Acylation of **37** with acetyl chloride gave **38a**. Reaction with the requisite sulfonyl chloride produced the sulfonamide **38b**. Deprotection of the sulfonamide with TFA followed by reduction provided compounds **10** and **13–19**. Reaction of amine **37** with $ClSO_2NCO$/formic acid/Et_3N afforded **39** and **40**. These two compounds were separated and then carried on the desired targets individually by deprotection followed by reduction of the nitro group with $SnCl_2$.

The synthesis of the aminobenzisoxazole **27** is shown in Scheme 2. 3-Cyano-4-fluorobenzaldehyde was converted to oxime **42** with hydroxylamine. Reaction of **42** with bleach followed by [2 + 3] cycloaddition with **43** gave isoxazoline **44**. Reduction of the azide group with $P(OEt)_3$ followed by sulfonylation with methanesulfonyl chloride produced sulfonamide **45**. The ester of **45** was hydrolyzed to the corresponding acid **46**, which was then coupled with biarylamine **35** to afford amide **47**. Reaction with acetone oxime followed by heating in EtOH/HCl generated the aminobenzisoxazole. Lastly, removal of the *t*-butyl group with TFA gave the desired product **27**. Compound **28** with a methoxyphenyl P_1 was prepared similarly using *p*-methoxybenzaldehyde as the starting material.

Conclusions

We have designed and synthesized a series of isoxazoline compounds employing P_1 benzamidine. The chloroaniline moiety was found to be the best benzamidine replacement in this series. The best compound ST368 (**1**), while a 14-fold less potent than the corresponding benzamidine SM084, was found to have both better selectivity profile for fXa relative to other serine proteases and an improved pharmacokinetic profile in

terms of clearance and half-life. Efforts to further optimize the pharmacokinetic profile of our fXa inhibitors will be reported in due course.

Acknowledgements

We wish to thanks Al Mical and his group for the chiral separations, and Earl Crain and Carol Watson for conducting the rabbit A-V shunt experiments.

References and Notes

1. Elodi, S.; Varadi, K. *Thromb. Res.* **1979**, *15*, 617.
2. Harker, L. A.; Hanson, S. R.; Kelly, A. B. *Thromb. Haemostasis* **1997**, *78*, 736.
3. (a) Betz, A. *Exp. Opin. Ther. Patents* **2001**, *11*, 1007. (b) Rai, R.; Sprengeler, P. A.; Elrod, K. C.; Young, W. B. *Current Medicinal Chemistry* **2001**, *8*, 101.
4. Quan, M. L.; Pruitt, J. R.; Ellis, C. D.; Liauw, A. Y.; Galemmo, R. A., Jr.; Stouten, P. F. W.; Wityak, J.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2813.
5. 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.
6. 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.
7. Wong, P. C.; Quan, M. L.; Crain, E. J.; Watson, C. A.; Wexler, R. R.; Knabb, R. M. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 351.
8. Pinto, D. J.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Wong, P. C.; Knabb, R. M.; Luettgen, J. M.; Aungst, B. J.; Li, L.; Wright, M.; Jona, J. A.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566.
9. Quan, M. L.; Wexler, R. R. *Curr. Top. Med. Chem.* **2001**, *1*, 137.
10. Lam, P. Y. S; Li, R.; Clark, C. G.; Adams, J. J.; Galemmo, R. A.; Fevig, J. M.; Pinto, D. J.; Quan, M. L.; He, M.; Han, Q.; Alexander, R. S.; Rossi, R. A.; Wong, P. C.; Luettgen, J. M.; Aungst, B. J.; Wright, M. R.; Bai, S. A.; Knabb, R. M.; Wexler, R. R. 220th ACS National Meeting, Washington, DC, **2000**, MEDI 329.
11. **Enzyme affinity assays.** FXa, thrombin, and trypsin K_i values were obtained from human purified enzymes. All assays are run in micro-titer plates using a total volume of 250 μL in 0.1 M Sodium Phosphate Buffer containing 0.2 M NaCl and 0.5% polyethylene glycol 6000 at pH 7.0. The compounds are run at 10 μM, 3.16, 1.0, 0.316, 0.1, 0.0316, 0.01, and 0.00316 μM. Plates are read for 30 min at 405 nm. Rates are determined for the controls (no inhibitor) and for the inhibitors. % Enzyme activity is determined from these rates and used in the following formula to determine K_i: $K_i = 1000 \times$ Inhibitor Concentration/((K_m + S)-S×ACT)/(ACT×K_m)-1); where S = Substrate Concentration; ACT = % Enzyme Activity for inhibitor. All compounds were tested in duplicate studies and were compared with the same internal standards. These assays are described in detail in refs 12 and 13.
12. Knabb, R. M.; Kettner, C. A.; Timmermans, P. B. M. W.M.; Reilly, T. M. *Thromb. Haemostasis* **1992**, *67*, 56.
13. Kettner, C. A.; Mersinger, L. J.; Knabb, R. M. *J. Biol. Chem.* **1990**, *265*, 18289.