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Design, Synthesis, and SAR of Substituted Acrylamides as Factor Xa Inhibitors†

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Abstract—Substituted acrylamides were used as templates that bridge P1 and P4 binding elements, resulting in a series of potent (sub-nanomolar) and selective factor Xa inhibitors. In this template, *cis*-geometry of P1 and P4 ligands is highly preferred. SAR on the substituting groups, as well as on modification of P1 and P4 moieties is described. Compounds in this series show good in vivo efficacy in animal models.

Thrombotic diseases are a leading cause of mortality and morbidity.[2] Improvement in the treatment and prevention of thrombotic diseases remains an important medical need. Current therapeutic drugs in the area such as warfarin and heparin suffer from major problems such as patient variability and the need for periodic monitoring, making these drugs undesirable. Factor Xa is a serine protease responsible for conversion of prothrombin to thrombin in the blood coagulation cascade. In recent years factor Xa has emerged as an attractive target for the development of new antithrombotic agents.[3] Our research objective is to develop factor Xa inhibitors for treatment of thrombotic diseases.

The active site of factor Xa, as shown by X-ray structure,[4] contains a deep S1 pocket and a box-like S4 pocket. Going from S1 to S4 pocket requires a 90-degree turn. Most of factor Xa inhibitors in literature contain an S1 binding element (P1) and an S4 ligand (P4), which are connected by a central template (Fig. 1).[5–8] Among these templates, heterocycles such as pyrazole,[5] have been shown to be effective scaffolds that provide Xa inhibitors of high potency as exemplified by DuPont's SN 429 ($K_i = 0.013$ nM). In our search for factor Xa inhibitors, we thought that the heterocycles could be replaced with a substituted double bond. Therefore, we investigated the use of substituted acrylamides as the central template (Fig. 1). As a result, a series of potent and selective factor Xa inhibitors were discovered. In this paper, we will present the structure–activity relationship (SAR) of R^1 and R^2 substitution on the double bond of acrylamide, as well as the selected modifications of P1 and P4 moieties.

We initially synthesized the unsubstituted cinnamamide analogues as pure *Z* and *E* isomers (Fig. 2). The *Z*-isomer **1** showed good Xa activity ($IC_{50} = 17$ nM) in our assays,[9] while the *E*-isomer **2** is 180-fold less active, indicating the importance of *cis*-geometry of P1 and P4 ligands.

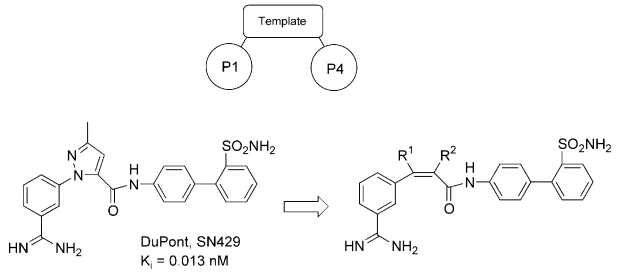

Figure 1. Schematic representation of structure of factor Xa inhibitors.

†For preliminary account of this work, see: ref. 1.

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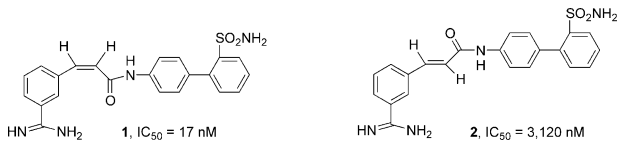


Figure 2. Activity of *E*- and *Z*-isomers.

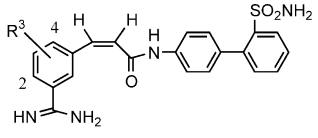
After identification of compound **1**, we studied the effects of substitution on the benzamidine ring. As shown in Table 1, substitution by fluoro and methoxy groups at 4-position (**4** and **6**) was more tolerant than the same substitution at 2-position (**3** and **5**). Actually, 4-fluoro analogue **4** is equally potent as compound **1**, while 4-methoxy analogue **6** is 12-fold less active. Interestingly, 4-methoxyethoxy analogue **7** is 2-fold more potent than compound **1**.

We then set out to study the effects of substituents on the acrylamide double bond. The SAR of R^1 substitution, as shown in Table 2, indicated that alkyl groups at this position generally increase activity (**8**, **9**, and **11**–**13**) except for the isopropyl group (**10**). The methyl substituted analogue **8** gave 4-fold increase of activity over the unsubstituted analogue **1**. Appendages (pyrazolyl and tetrazolyl, **14**–**16**) on the methyl group tended to decrease activity, and 2-furyl analogue **17** gave 11-fold reduction in potency as compared with **1**. The trifluoromethyl compound **12** was 2-fold less active than the methyl analogue **8**.

The SAR of R^2 substitution is shown in Table 3. Generally, substitution at this position with a variety of different groups (**18**–**23**) all resulted in decreased potency. This seemed to suggest that groups larger than hydrogen would decrease activity. This observation led us to try fluorine as R^2, since fluorine is similar to hydrogen in size. The fluoro compound **24** ($IC_{50} = 1$ nM) displays increased potency by 4-fold over **8** (where $R^2 = H$).

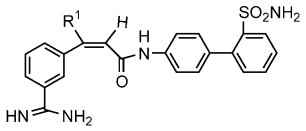
Previous literature has shown that halogen substitution at the biphenyl P4 ligand generally increases anti-Xa activity.[10] A similar trend was observed in our study (**25**–**27**), as shown in Table 4, with fluorine substitution (**25**) giving the best potency ($IC_{50} = 0.2$ nM). These compounds (**24**–**27**) are also selective against other serine proteases such as thrombin (>1000-fold) and trypsin (>360-fold) (Table 4).

Table 1. Substitution on benzamidine ring



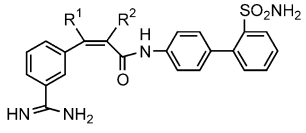
R^3	Compd	FXa (IC_{50}, nM)
H	**1**	17
2-F	**3**	99
4-F	**4**	18
2-CH_3O–	**5**	6470
4-CH_3O–	**6**	215
4-$CH_3OCH_2CH_2O$–	**7**	9

Table 2. SAR of R^1 substitution



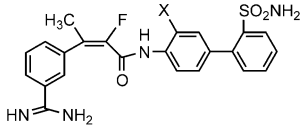
R^1	Compd	FXa (IC_{50}, nM)
H	**1**	17
CH_3	**8**	4
CH_3CH_2–	**9**	7
*i*Pr	**10**	35
*i*Bu	**11**	5
CF_3	**12**	10
CH_3OCH_2–	**13**	7
	**14**	48
	**15**	53
	**16**	245
	**17**	184

Table 3. SAR of R^2 substitution



R^1	R^2	Compd	FXa (IC_{50}, nM)
H	CH_3	**18**	362
H	$-CO_2CH_3$	**19**	759
H		**20**	628
H		**21**	664
CH_3	$-CH_2CONH_2$	**22**	99
CH_3	$-CH_2CO_2H$	**23**	234
CH_3	F	**24**	1

Table 4. Activity and selectivity



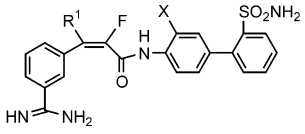
X	Compd	FXa	Ila (IC_{50}, nM)	Trypsin
H	**24**	1	3190	259
F	**25**	0.2	2200	194
Cl	**26**	0.5	650	188
Br	**27**	0.5	503	189

After optimization of R^2 with fluorine, we set out to do further R^1 modification. As shown in Table 5, appending other functional groups (**28**–**31**) to the methyl group at R^1 failed to improve potency. Some polar groups (**28**–**30**) are moderately tolerated at this position, but basic dimethylamino (**31**) is least favored. Again the trifluoromethyl compound **32** is 3-fold less active than the methyl analogue **24**.

With the goal of reducing basicity of benzamidine P1 moiety, electron-withdrawing substituents (nitro, fluoro) were placed on the benzene ring, as shown in Table 6. A nitro group (**33**) at 4-position reduced potency by 75-fold as compared with compound **25**, but reduction of nitro to amine (**34**) regained some activity. However, fluorine at 2-position (**35**–**38**) was well tolerated. Again halogen substitution on the biphenyl increases potency. Compound **37** shows subnanomolar activity with K_i of 0.18 nM.

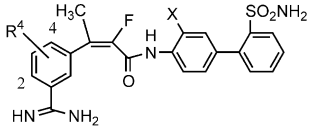
We examined the antithrombotic activity of the compounds in our modified rabbit deep vein thrombosis (DVT) model.[11] For example, compound **37** was found to inhibit thrombus formation by 40%, at an iv bolus dose of 2 mg/kg followed by infusion to maintain an average plasma concentration of 0.66 μM during the 2-h treatment period (giving an average 1.5-fold increase of PT). In comparison, the maximum inhibition caused by low-molecular weight heparin (Enoxaparin) was usually about 45–50% in the model.[11] We also determined oral bioavailability of the compounds in rats. However, they were usually below 3%.

Table 5. Further SAR of R^1 substitution



R^1	X	Compd	FXa (IC_{50}, nM)
CH_3	H	**24**	1
$HOCH_2$–	H	**28**	3.7
$EtSCH_2$–	H	**29**	13
$CH_3SO_2CH_2$–	H	**30**	10
$(CH_3)_2NCH_2$–	F	**31**	191
CF_3	H	**32**	3

Table 6. Modification on benzamidine ring



R^4	X	Compd	FXa (IC_{50}, nM)
4-NO_2	F	**33**	15
4-NH_2	F	**34**	2.6
2-F	H	**35**	3.6
2-F	F	**36**	1.5
2-F	Cl	**37**	0.74 (K_i = 0.18 nM)
2-F	Br	**38**	0.75

Molecular modeling of compound **24** into the active site of Xa crystal structure, as shown in Figure 3, indicated that the amidine group of **24** interacts with the carboxylate of Asp189 in S1 cavity, while the biphenylsulfonamide moiety fits into the box-like S4 pocket defined by Phe174, Tyr99 and Trp215. The vinyl methyl group fits into a pocket defined by the Cys191-Cys220 disulfide bond (yellow surface in Fig. 3). Branching at this carbon results in a reduction of activity, as evidenced by compounds **10** and **11**. The fluorine atom is in close proximity to the Gln192 side chain. It is conceivable that groups larger than fluorine would cause unfavorable interaction with the side chain, resulting in reduced potency, which is consistent with the SAR seen in Table 3.

The acrylamides of this study were generally synthesized according to Scheme 1. An aldehyde or ketone was condensed with an acetate equivalent to give the acrylates, usually as a mixture of *cis* and *trans* isomers. In most cases, the two isomers were separated at this stage. Weinreb amidation of the acrylate with the biphenylamine yielded the acrylamide, which was then converted to the benzamidine product under Pinner conditions.

To synthesize the (*Z*) *cis*-isomers **1** and **3**–**7** in Table 1, the *cis*-cinnamates were prepared, as shown in Scheme 2. Horner–Emmons reaction of the cyanobenzaldehyde with methyl bis(2,2,2-trifluoroethyl)phosphono acetate[12] gave nearly exclusively the *cis*-cinnamate. The (*E*) *trans*-cinnamate leading to compound **2**, was synthesized by

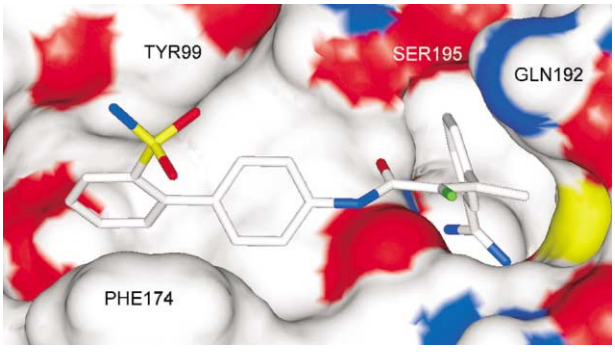
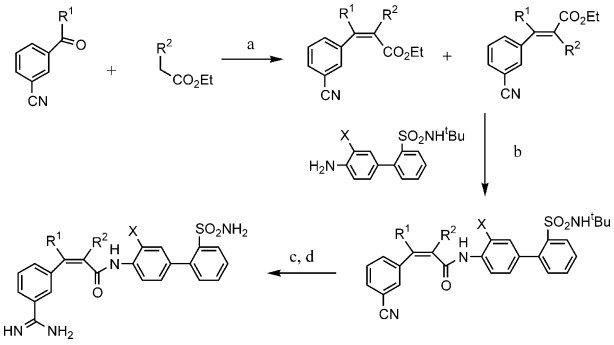
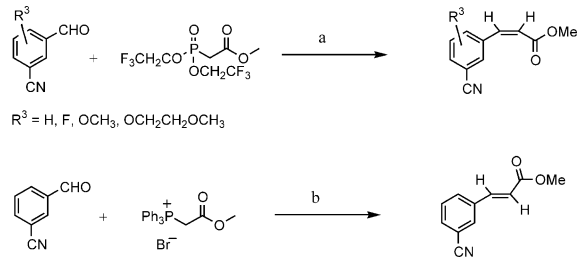


Figure 3. Proposed binding mode of compound **24** with factor Xa. Connolly surface created using InsightII (Accelrys, Inc.). Red, blue, and yellow represent the surface close to oxygen, nitrogen, and sulfur atoms, respectively. The fluorine atom on **24** is green.



Scheme 1. General synthesis. Reagents and conditions: (a) base (e.g., $KN(Me_3Si)_2$, piperidine, KO^tBu); (b) $AlMe_3$, CH_2Cl_2, X = H, F, Cl, Br; (c) MeOH/HCl (g); (d) NH_4OAc.



Scheme 2. Synthesis of compounds **1**–**7**. Reagents and conditions: (a) $KN(Me_3Si)_2$, 18-Crown-6, THF, −78 °C; (b) NaH.

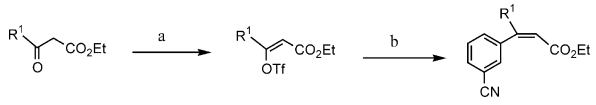
Wittig reaction of 3-cyanobenzaldehyde with (carbomethoxymethyl)triphenyl phosphonium ylide.

Compounds **8**–**14** and **17** in Table 2 were synthesized according to Scheme 3. Ketoacetates were treated with Tf_2O in the presence of TEA to give the enol triflates, which were then coupled with 3-cyanobenzeneboronic acid under Pd(0) catalysis to yield the R^1 substituted cinnamates. The *cis*-geometry of R^1 to the vinyl proton was confirmed by NOE analysis.

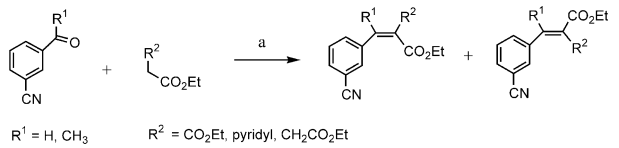
To synthesize compounds **19**–**23** in Table 3, the substituted acrylates were made, as shown in Scheme 4. Knoevenagel reaction of the cyanobenzaldehyde with dimethyl malonate (or pyridylacetate) gave the desired acrylates as a mixture of separable *E*/*Z* isomers. Stubbe condensation of the cyanoacetophenone with dimethyl succinate gave the acrylate as the expected monoester, which gave rise to final products **22** and **23**.

Synthesis of compounds **24**–**27** in Table 4, **33**–**38** in Table 6 began with Horner–Emmons reaction of the cyanoacetophenone **39a** with triethyl 2-fluoro-2-phosphonoacetate, giving the acrylates in favor of the desired isomer (with the cyanophenyl *cis* to the carboxylate) (Scheme 5, Y = H).

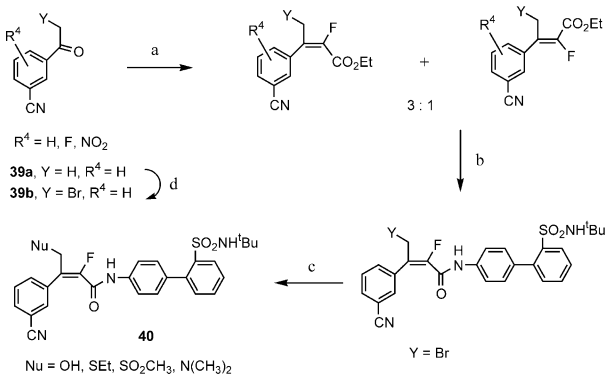
Similarly, Horner–Emmons reaction of the bromoacetophenone **39b**, which was derived from **39a** by bromination with pyridinium tribromide, gave the acrylates as allyl bromides (Scheme 5). Weinreb amidation of these acrylates generated the acrylamides. Substitution of the allyl bromides with nucleophiles (Nu) produced the intermediates **40**, which were then converted to the final



Scheme 3. Synthesis of compounds in Table 2. Reagents and conditions: (a) Tf_2O, Et_3N, CH_2Cl_2, −78 °C; (b) 3-cyanobenzeneboronic acid, $Pd(Ph_3P)_4$, dioxane, K_3PO_4, reflux.



Scheme 4. Synthesis of compounds in Table 3. Reagents and conditions: (a) base (piperidine, or KO^{t}Bu).



Scheme 5. Synthesis of compounds in Tables 4–6. Reagents and conditions: (a) triethyl 2-fluoro-2-phosphonoacetate, $KN(Me_3Si)_2$, −78 °C; (b) biphenylamine, $AlMe_3$; (c) Nu (EtSH for **29**, CH_3SO_2Na for **30**, $(CH_3)_2NH$ for **31**); (d) pyridinium tribromide, HOAc.

products **28**–**31** by Pinner reaction. Compound **28** was obtained as a side product in the preparation of **30**.

In summary, substituted acrylamides were found to be effective templates which gives rise to a series of potent (sub-nanomolar anti-Xa activity) and selective monobenzamidino factor Xa inhibitors. Within this template, *cis*-geometry of P1 and P4 ligands is highly preferred. Compounds in this series show good in vivo efficacy in animal models. However, these compounds suffer from low bioavailability. Replacement of the benzamidine P1 moiety with neutral or less basic groups to achieve good bioavailability will be the subject of additional communications from our laboratories.

References and Notes

1. Song, Y.; Clizbe, L.; Bhakta, C.; Teng, W.; Li, W.; Wu, Y.; Jia, Z. J.; Zhang, P.; Wang, L.; Doughan, B.; Su, T.; Kanter, J.; Woolfrey, J.; Wong, P.; Huang, B.; Tran, K.; Sinha, U.; Park, G.; Reed, A.; Malinowski, J.; Hollenbach, S.; Scarborough, R. M.; Zhu, B-Y. *Books of Abstracts*, *Part 2*, 221th National Meeting of the American Chemical Society, San Diego, CA, April 1–5, 2001; American Chemical Society: Washington, DC, 2001; MEDI 063.
2. (a) Scarborough, R. M. *Annu. Rep. Med. Chem.* **1995**, *30*, 71. (b) Edmunds, J. J.; Rapundalo, S. T. *Ann. Rep. Med. Chem.* **1996**, *31*, 51.
3. (a) Zhu, B.-Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83. (b) Zhu, B.-Y.; Scarborough, R. M. *Curr. Opin. Cardiovasc., Pulm. Renal Invest. Drugs* **1999**, *1*, 63. (c) Sanderson, P. E. *J. Annu. Rep. Med. Chem.* **2001**, *36*, 79.
4. (a) Brandstetter, H.; Kuhne, A.; Bode, W.; Huber, R.; von der Saal, W.; Wirthensohn, K.; Engh, R. A. *J. Biol. Chem.* **1996**, *271*, 29988. (b) Kamata, K.; Kawamoto, H.; Honma, T.; Iwama, T.; Kim, S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6630.
5. Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Luettgen, J. M.; Liang, L.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566.
6. Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Campbell, J. B.; Craft, T. J.; Froelich, L. L.; Gifford-Moore, D. S.; Hay, L. A.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Metz, J. T.; Ratz, A. 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. Phillips, G. B.; Buckman, B. O.; Davey, D. D.; Eagen, K. A.; Guilford, W. J.; Hinchman, J.; Ho, E.; Koovakkat, S.; Liang, A.; Light, D. R.; Mohan, R.; Ng, H. P.; Post, J. M.; Shaw, K. J.; Smith, D.; Subramanyam, B.; Sullivan, M. E.; Trinh, L.; Vergona, R.; Walters, J.; White, K.; Whitlow, M.; Wu, S.; Xu, W.; Morrissey, M. M. *J. Med. Chem.* **1998**, *41*, 3557.
8. Ewing, W. R.; Becker, M. R.; Manetta, V. E.; Davis, R. S.; Pauls, H. W.; Mason, H.; Choi-Sledeski, Y. M.; Green, D.; Cha, D.; Spada, A. P.; Cheney, D. L.; Mason, J. S.; Maignan, S.; Guilloteau, J.-P.; Brown, K.; Colussi, D.; Bently, R.; Bostwick, J.; Kasiewski, C. J.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. *J. Med. Chem.* **1999**, *42*, 3557.
9. The bioassays were performed as described in reference: Sinha, U.; Ku, P.; Malinowski, J.; Zhu, B. Y.; Scarborough, R. M.; Marlowe, C. K.; Wong, P. W.; Lin, P. H.; Hollenbach, S. J. *Eur. J. Pharmacol.* **2000**, *395*, 51 Measurements are average of triplicates with standard deviation of $\pm 20\%$.
10. Han, Q.; Dominguez, C.; Stouten, P. F. W.; Park, J. M.; Duffy, D. E.; Galemmo, R. A. Jr.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Wong, P. C.; Wright, M. M.; Luettgen, J. M.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2000**, *43*, 4398.
11. In our modified model, a cotton thread attached to a copper wire was introduced into the abdominal vena cava via the femoral vein of an anesthetized rabbit. At the same time as the introduction of the cotton thread, compounds were administered intravenously by a bolus dose followed by infusion at a constant rate to maintain the targeted plasma concentration during the 2-h treatment period. Inhibition of thrombus formation was determined by weight of harvested thrombi formed on the cotton thread. In the positive control, Enoxaparin was administered at a dose of 200 U/kg followed by an infusion of 2 U/kg/min, attaining about 45–50% inhibition. For our original model, see: Hollenbach, S.; Sinha, U.; Lin, P. H.; Needham, K.; Frey, L.; Hancock, T. E.; Wong, A.; Wolf, D. L. *Thromb. Haemost.* **1994**, *71*, 357.
12. Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.