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Synthesis and SAR of Benzamidine Factor Xa Inhibitors Containing a Vicinally-Substituted Heterocyclic Core

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Abstract—The selective inhibition of coagulation factor Xa has emerged as an attractive strategy for the discovery of novel antithrombotic agents. Here we describe highly potent benzamidine factor Xa inhibitors based on a vicinally-substituted heterocyclic core.

The serine protease factor Xa (FXa) is central to the regulation of the coagulation cascade because it is situated at the confluence of the intrinsic and extrinsic pathways. FXa combines with factor Va in a Ca^{2+}-dependent manner on phospholipid surfaces to form the prothrombinase complex, which generates thrombin by the limited proteolysis of prothrombin. The inhibition of thrombin generation by selective FXa inhibition is emerging as a promising strategy for the development of effective antithrombotic agents, which may offer advantages relative to existing therapies.[2] Previous reports from our laboratories (Fig. 1) have described the isoxazole benzamidine **1**[3] and the benzamidines **2**–**4** as potent inhibitors of human FXa.[4] These inhibitors differ in the point of attachment of the benzamidine P1 residue to the heterocyclic core, with **1** being '*C*-substituted' on the core and **2**–**4** being '*N*-substituted' on the core. The greater than an order of magnitude range of potency between these inhibitors suggested that the nature of the heterocyclic core is critical to binding potency, and led us to prepare seven additional '*C*-substituted' core analogues **5a**–**g** and five additional '*N*-substituted' core analogues **6j**–**n** (for structures, see Table 1). Although we were primarily interested in preparing five-membered heterocycles, two examples containing a six-membered core, **5h** and **5i**, were prepared for comparative purposes. This manuscript will describe the synthesis and SAR of these analogues in an attempt to further optimize this series of FXa inhibitors.

The general synthesis scheme for the heterocyclic analogues **5a**–**h** and **6j**–**n** is shown in Scheme 1. These analogues were prepared from intermediates **7**, in which the core heterocycle is substituted with an ester or acid functionality, and a nitrile residue serves as the amidine precursor. Direct coupling of the esters **7** (R = Et or Me) with the aluminum reagent[5] derived from 4′-amino-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide[6] produced the

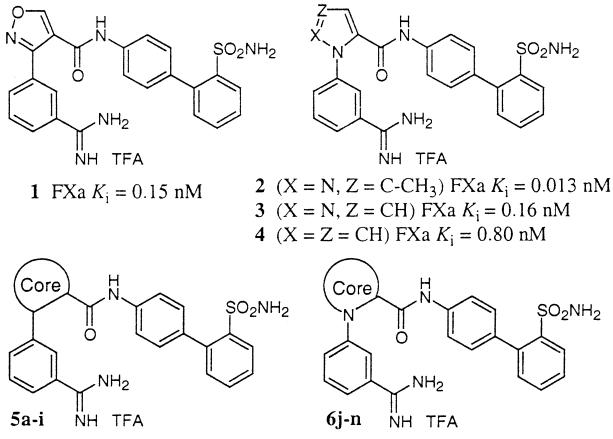

Figure 1.

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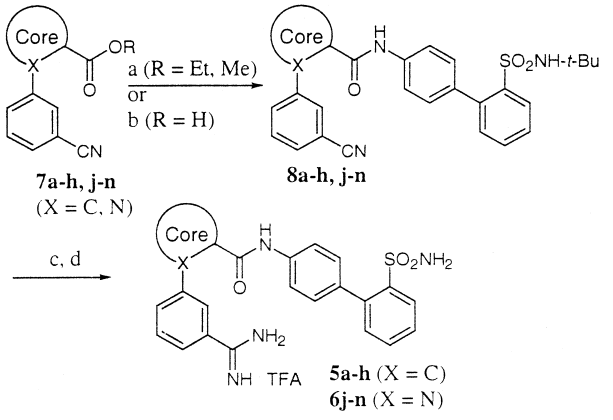
amides **8** in good yields. In the case of the triazole acid **7m**, amide coupling was accomplished via the acid chloride to afford amide **8m**. Standard Pinner reaction on the nitriles **8** gave the corresponding amidines and TFA-catalyzed deprotection afforded the final compounds **5a**–**h** and **6j**–**n**.

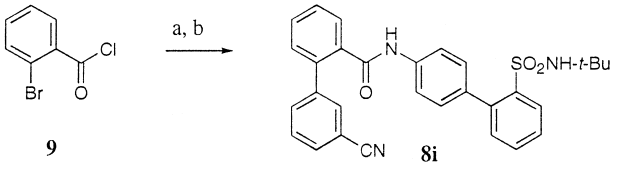
The phenyl core analogue **5i** was prepared as shown in Scheme 2. Amidation of 2-bromobenzoyl chloride **9** with 4′-amino-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide in the presence of DMAP was followed by Suzuki coupling with 3-cyanophenyl boronic acid to afford **8i**. This material was subjected to the Pinner reaction and TFA-catalyzed deprotection as described in Scheme 1 to give the final compound **5i**.

The five-membered heterocyclic *C*-linked compounds **7a**–**g** were prepared as shown in Scheme 3. Treatment of acid chloride **10b** with ethyl isocyanoacetate in the presence of triethylamine afforded the oxazole **7a**. Treatment of methyl ketone **10a** with diethyl carbonate in the presence of sodium hydride gave ketoester **11**. Sequential treatment of **11** with *N,N*-dimethylformamide dimethyl acetal and then with methyl hydrazine led to the *N*-methylpyrazole **7b** as the predominant regioisomer. Bromination of **11** with NBS gave **12**, which was a useful intermediate for preparing several of the required heterocyclic intermediates **7**. Heating **12** with urea in DMF afforded the imidazolone **7c**. Sequential treatment of **12** with potassium acetate in DMF and then with ammonium acetate in refluxing acetic acid gave an approximately 1:3 mixture of imidazole **7d** and oxazole **7e**. Finally, treatment of **12** with thioacetamide in refluxing ethanol afforded the methylthiazole **7f**. The unsubstituted thiazole was prepared by treating **12** with thiourea in refluxing ethanol to furnish the aminothiazole **13**. Treatment of **13** with *tert*-butyl nitrite and copper(II) bromide in refluxing acetonitrile gave a bromothiazole, which was reduced with zinc in refluxing acetic acid to give thiazole **7g**.

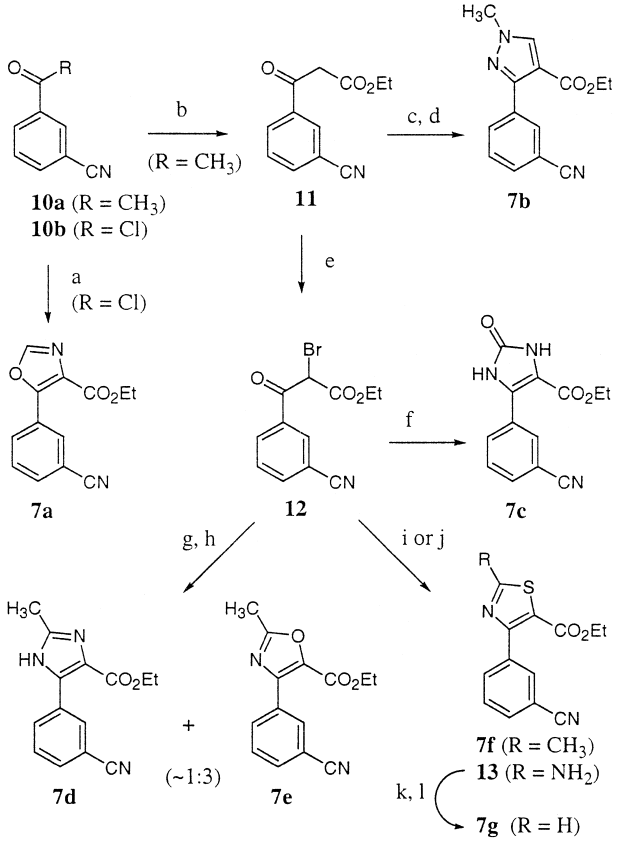
The pyridine core intermediate **7h** was prepared as shown in Scheme 4. Oxidation of 2-bromo-3-methylpyridine **14** with potassium permanganate gave a carboxylic acid, which was esterified with DEAD, triphenylphosphine, and methanol to afford the methyl ester **15**. Suzuki coupling of **15** with 3-cyanophenyl boronic acid furnished the pyridine intermediate **7h**.



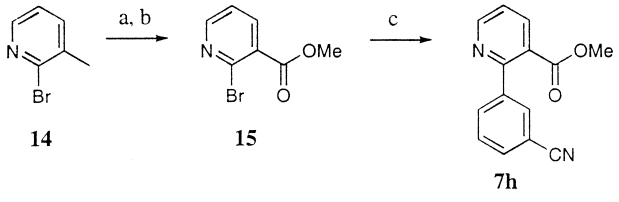
Scheme 1. Reagents: (a) $AlMe_3$, 4′-amino-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide, CH_2Cl_2, 40 °C; (b) (i) $(COCl)_2$, CH_2Cl_2, DMF, (ii) 4′-amino-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide, Et_3N, CH_2Cl_2; (c) (i) HCl (gas), MeOH, 0 °C, (ii) $(NH_4)_2CO_3$, MeOH; (d) TFA, reflux.



Scheme 2. Reagents: (a) 4′-amino-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide, DMAP, $CHCl_3$, 0–25 °C; (b) 3-cyanophenyl boronic acid, $(PPh_3)_4Pd$, TBAB, 2 M Na_2CO_3, toluene, 80 °C.



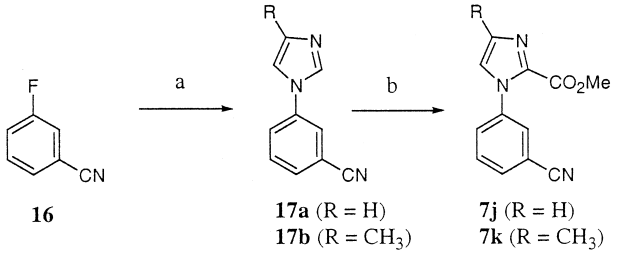
Scheme 3. Reagents: (a) ethyl isocyanoacetate, Et_3N, CH_2Cl_2; (b) NaH, diethyl carbonate, THF, 60 °C; (c) $Me_2NCH(OMe)_2$, 100 °C; (d) methylhydrazine, EtOH, 80 °C; (e) *N*-bromosuccinimide, CCl_4; (f) urea, DMF, 100 °C; (g) KOAc, DMF; (h) NH_4OAc, AcOH, 100 °C; (i) thioacetamide, EtOH, 80 °C; (j) thiourea, EtOH, 80 °C; (k) *tert*-butyl nitrite, $CuBr_2$, CH_3CN, 80 °C; (l) Zn powder, HOAc, 100 °C.



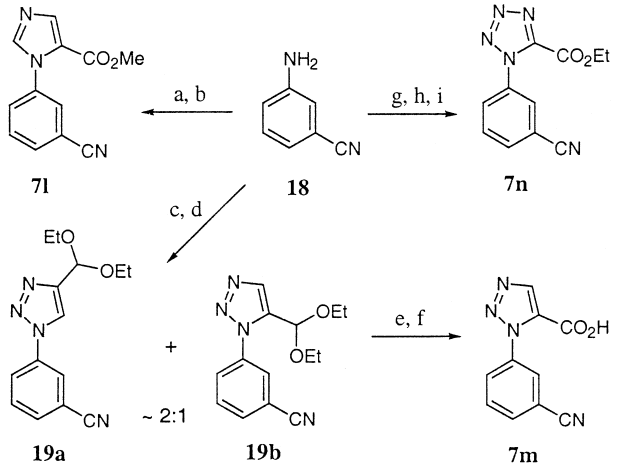
Scheme 4. Reagents: (a) $KMnO_4$, H_2O; (b) DEAD, PPh_3, MeOH; (c) 3-cyanophenyl boronic acid, $Pd(PPh_3)_2Cl_2$, TBAB, 2 M Na_2CO_3, benzene, 80 °C.

The imidazole core intermediates **7j** and **7k** were prepared as shown in Scheme 5. Reaction of 3-fluorobenzonitrile **16** with imidazole or 4-methylimidazole produced **17a** and **17b**, respectively. Regiospecific deprotonation of **17a** and **17b** at C-2 of the imidazole ring was accomplished with *n*-BuLi, and the resulting anions were quenched with methyl chloroformate to afford the required imidazole intermediates **7j** and **7k**, respectively.

The remaining core intermediates **7l–n** were prepared from 3-aminobenzonitrile **18** as shown in Scheme 6. Condensation of **18** with butyl glyoxalate in refluxing ethanol gave an imine which, when treated with tosylmethyl isocyanate (TosMIC) and potassium carbonate in methanol, underwent cyclization and transesterification to give the imidazole methyl ester **7l**. To prepare the triazole **7m**, **18** was diazotized and treated with sodium azide to afford 3-azidobenzonitrile. This azide underwent [3+2] cyclization with 3,3-diethoxy-1-propyne to produce a mixture of the triazoles **19a** and **19b** in an approximately 2:1 ratio. The desired triazole **19b** was hydrolyzed to the aldehyde and subsequently oxidized to the carboxylic acid **7m** with silver nitrate. Finally, the tetrazole **7n** was prepared from **18** by a three step sequence involving initial *N*-acylation with ethyl oxalyl chloride. Treatment of the resulting amide with triphenylphosphine/carbon tetrachloride gave an iminoyl chloride, which upon treatment with sodium azide gave the tetrazole core intermediate **7n**.



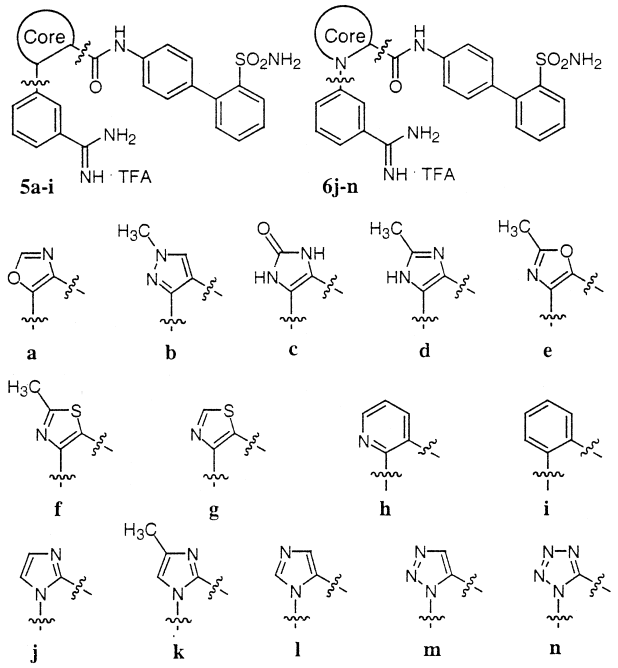
Scheme 5. Reagents: (a) imidazole or 4-methylimidazole, K_2CO_3, DMF, 110 °C; (b) (i) *n*-BuLi, THF, −78 °C, (ii) $ClCO_2Me$, THF, −78 °C.



Scheme 6. Reagents: (a) butyl glyoxalate, ethanol, 80 °C; (b) TosMIC, K_2CO_3, MeOH; (c) $NaNO_2$, TFA; NaN_3, H_2O; (d) 3,3-diethoxy-1-propyne, reflux; (e) 1 N HCl, THF/H_2O; (f) $AgNO_3$, NaOH, H_2O; (g) ethyl oxalyl chloride, Et_3N, CH_2Cl_2; (h) PPh_3, CCl_4, reflux; (i) NaN_3, CH_3CN.

The binding results for the inhibitors **5a–i** and **6j–n** are shown in Table 1, along with data for compounds **1–4**. Several general trends can be observed within this somewhat limited set of 18 compounds. First, the '*N*-substituted' compounds are generally more potent than the '*C*-substituted' compounds. For example, the pyrazole **2** is 4- to 30-fold more potent than the structurally similar '*C*-linked' compounds **5b**, **5e**, and **5f**. Also, the three most potent compounds (**2**, **6m**, and **6n**) are all

Table 1. FXa, thrombin and trypsin inhibition results for **1–4** and core analogues **5a–i** and **6j–n**[a]



Compounds	FXa K_i (nM)[b]	Thrombin K_i (nM)[b]	Trypsin K_i (nM)[b]
1	0.15	2000	21
2	0.013	300	16
3	0.16	900	15
4	0.80	900	56
5a	2.5	3300	261
5b	0.20	2640	220
5c	2.3	6000	390
5d	6.0	>21,000	1100
5e	0.40	3600	190
5f	0.06	1250	60
5g	0.82	>6300	97
5h	1.0	15,400	260
5i	8.2	>6300	780
6j	0.33	800	26
6k	0.30	600	27
6l	1.1	3200	58
6m	0.023	300	3.4
6n	0.037	300	6.4

[a]All compounds were purified by reverse phase HPLC (water/acetonitrile gradient + 0.5% TFA) and isolated as TFA salts following lyophilization. All compounds gave satisfactory spectral and analytical data.

[b]Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were < 30% of the mean. K_i values were measured as described in ref 7.

Table 2. Antithrombotic efficacy of **1**, **2**, **5a**, **5f**, and **6n** in a rabbit arterio-venous (A-V) shunt model[8]

Compound	Human FXa K_i (nM)	Rabbit FXa K_i (nM)	ID_{50} (μmol/kg/h)
1	0.15	0.40	0.31
2	0.013	0.03	0.023
5a	2.5	nd[a]	0.54
5f	0.06	0.08	0.50
6n	0.037	nd[a]	0.13

[a]nd = not determined.

'*N*-linked.' Second, affinity is increased by having a tertiary nitrogen atom at the core position adjacent to the P1 residue. Thus, the pyrazole **3** is 5-fold more potent than the pyrrole **4** and the pyridine **5h** is 8-fold more potent than the phenyl analogue **5i**. Also, this structural feature is shared by the most potent inhibitors. For example, in the '*C*-linked' series **5a**–**g**, only those analogues with this tertiary nitrogen display subnanomolar binding potency. Third, affinity is increased by substitution on the core distal to the P1 and P4 residues, as evidenced by the greater potencies of the 3-methylpyrazole **2** and the 2-methylthiazole **5f** relative to the pyrazole **3** and the thiazole **5g**, respectively. This substitution also results in greater trypsin selectivity, again seen by comparing **2** and **5f** with **3** and **5g**, respectively. Also, a nitrogen atom at this position in the core heterocycle enhances binding affinity. Thus, the triazole **6m** and the tetrazole **6n** are considerably more potent than the pyrazole **3**. However, the nitrogen atom does not lead to greater trypsin selectivity, as the trypsin selectivity of **6m** and **6n** more closely resembles that of pyrazole **3** than that of pyrazole **2**. Finally, five-membered cores are more potent than six-membered cores, as shown by the lower binding affinities of the pyridine **5h** and the phenyl analogue **5i**.

The imidazoles **6j** and **6k** are interesting in that they do not follow the general trends observed for the other compounds. For example, both are quite potent without the tertiary nitrogen adjacent to the P1 residue (compare with **6l**) and the substituted analogue **6k** is equipotent with the unsubstituted analogue **6j**.

The X-ray crystal structure of pyrazole **2** complexed to the structurally similar serine protease trypsin[4] reveals features that might explain some of the FXa binding results of these analogues, although due to space considerations it is not shown here. As expected, the benzamidine is engaged in a bidentate interaction with Asp189 in the S1 specificity pocket and the biphenylsulfonamide residue resides in the S4 aryl-binding pocket. The pyrazole ring is oriented such that the N2 nitrogen interacts with the backbone N-H of Gln192 (3.2 Å) and forms a van der Waals interaction with Cys220 (3.3 Å). Compounds **5c** and **5d**, each of which have a tautomeric form resulting in an N-H in this position, and **5i** and **6l**, which have a C-H projecting toward Gln192 and Cys220, might have lower affinity due to the disruption of these interactions. In the crystal structure, the methyl group of **2** projects toward solvent and appears to have favorable van der Waals interactions with Gly218 and Cys220, which might explain the preference for substitution at this position. The preference for '*N*-linked' over '*C*-linked' heterocycles is unclear without further structural work, but may involve differences in the dihedral angle between the heterocycle and the P1 benzamidine residue, which for **2** is about 70°.

Several of these analogues (**5a**, **5f**, and **6n**) were tested for in vivo antithrombotic efficacy in a rabbit arterio-venous (A-V) shunt model,[8] and the results are shown in Table 2 along with comparative results for **1**[3] and **2**.[4] All of these compounds are potent in this assay, with each having a submicromolar ID_{50}. The most potent compounds are the pyrazole **2** and the tetrazole **6n**.

In conclusion, we have described the SAR of a series of FXa inhibitors in which various heterocyclic core templates are vicinally-substituted with a P1 benzamidine residue and a P4 biphenylsulfonamide residue. This structural motif has resulted in several highly potent FXa inhibitors, especially pyrazole **2**, thiazole **5f**, triazole **6m** and tetrazole **6n**, all of which have a FXa $K_i < 0.1$ nM. These potent templates provide the opportunity to replace the benzamidine with less basic P1 moieties, thereby sacrificing some potency in exchange for improved oral absorption. Efforts along these lines are ongoing and will be reported in due course.[1c,4b,9]

Acknowledgements

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