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# Design and Synthesis of Glycolic and Mandelic Acid Derivatives as Factor Xa Inhibitors†

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**Abstract**—A series of glycolic and mandelic acid derivatives was synthesized and investigated for their factor Xa inhibitory activity. These analogues are highly potent and selective inhibitors against fXa. In a rabbit deep vein thrombosis model, compound **26** showed significant antithrombotic effects (81% inhibition of thrombus formation) at 1.1 μM plasma concentration following intravenous administration. © 2001 Published by Elsevier Science Ltd.

Factor Xa, a trypsin-like serine protease, is located at the convergence point of the intrinsic and extrinsic pathways in the coagulation cascade. Once assembled in the prothrombinase complex with factor Va and calcium ions on a phospholipid surface, factor Xa converts prothrombin to thrombin. Thrombin, in turn, converts fibrinogen into fibrin, the insoluble matrix of blood clots. Factor Xa's central position in coagulation cascade and the fact that thrombin has other important functions in cellular and neurological processes[2] have made factor Xa an alternative (to thrombin) target for drug discovery for thromboembolic diseases.[3]

Considerable efforts are being focused on synthetic, low-molecular-weight dibasic fXa inhibitors.[3] We previously reported the design and synthesis of various bis-benzamidine derivatives using hydroxymandelate as a template (Fig. 1).[4] To discover novel inhibitors with improved bioavailability and duration of action, we focused our synthetic efforts on reducing the overall basicity and enhancing potency of this template. During the course of this investigation, several laboratories published potent monobenzamidine fXa inhibitors. Most of these inhibitors consist of one benzamidine moiety and one neutral group linked by spacers of appropriate length and orientation.[5–8] In this report, the design, synthesis and in vitro structure–activity relationships of glycolic and mandelic acid-based monobasic noncovalent factor Xa inhibitors will be discussed.

The synthesis of compounds **6**–**14** is exemplified in Scheme 1. Treatment of 3-cyanophenol with *tert*-butyl bromoacetate and potassium carbonate, followed by removal of the Boc-protecting group with TFA, BOP coupling of resulting 2-(3-cyanophenoxy) acetic acid with methyl 4-aminobenzoate, and hydrolysis of the methyl ester afforded key intermediate **2**. Nitrile **2** was converted to benzamidine **3** using the hydroxyl amine

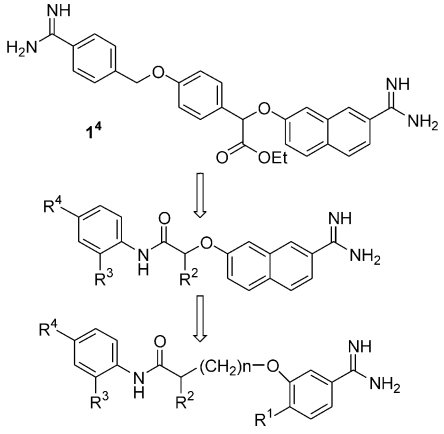


**Figure 1.** From dibenzamidine **1** to monobasic factor Xa inhibitors using mandelic acid as a template.

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

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addition route. PyBOP coupling of various amines with carboxylic acid **3** afforded inhibitors **6**–**14**. Compound **17** was synthesized employing similar procedures using 3-hydroxy-4-(phenylmethoxy)benzenecarbonitrile.

The synthesis of compounds **23**–**25** is exemplified in Scheme 2. Methyl α-bromophenylacetate was treated with 3-cyanophenol and potassium carbonate to yield **4**. Weinreb[9] amidation of ester **4** and substituted or non-substituted 3,4′-amino[1,1′-biphenyl]-2-*tert*-butylsulfonamide,[7] followed by amidine formation[10] and final removal of *t*-butyl group afforded free sulfonamides **23**–**25**. Compounds **19**–**22** were synthesized employing similar procedures using commercially available biaryl-aniline. Compound **15** was synthesized using *tert*-butyl-bromoacetate. Compound **16** was synthesized using 3-hydroxy-4-(phenylmethoxy) benzenecarbonitrile and *tert*-butylbromoacetate.

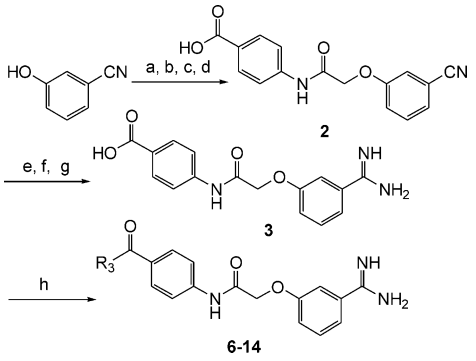
The synthesis of pyrrolamide **27** is shown in Scheme 3. The key intermediate **5** was prepared by treating pyrrolidine with 4-nitrobenzoyl chloride followed by reduction of nitro group and bromination of the phenyl ring. Weinreb amidation, followed by standard Pinner[10] conditions gave **27**. All mandelic acids derivatives described above were prepared as racemates.

When preparing **18**, several attempts failed when treating 3-cyanophenol with 3-bromopropionic acid. The alternative procedure shown in Scheme 4 afforded the desired product.

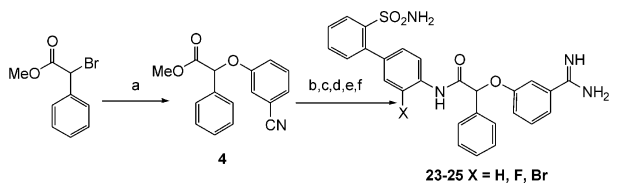
The enzyme inhibition constants of compounds **6**–**27** toward fXa and the structurally related serine proteases thrombin and trypsin are summarized in Tables 1–2.[11,12]

In the first series, a variety of amides of the 2-(3-cyanophenoxy) acetic acid were prepared to explore the P4 binding element. Pyrrolidine-containing analogue such as **7** appeared to be optimal since contracting the ring (**6**) decreased fXa inhibitory activity 3-fold, and enlargement of the ring (**8**–**11**) decreased fXa inhibitory activity 17- to 20-fold. The bulky rings apparently cannot enter the S4 binding site. The placement of a hydrogen acceptor group O– in the ring (**10**) does not influence the affinity. Placement of a tertiary nitrogen group within the ring (**11**) is also unfavorable for fXa inhibition. Introduction of a double bond in pyrrolidine ring (**12**) resulted in a 10-fold increase in potency toward fXa. Addition of a *t*-butyl ester (**14**) to the pyrrolidine ring reduced potency toward fXa by 44-fold. More significantly, substitution of pyrrolidine ring with a carboxylic acid group (**13**) reduced potency toward fXa by >166-fold. This and the observed ring size effect demonstrate the strict steric requirements in the P4 element in this series. In a second series, compounds containing biaryl or heteroaryl amides were prepared based on reports by Quan et al.,[7] in which biphenylsulfonamide moiety of their potent fXa inhibitors were designed to interact with the S4 aryl-binding domain of the fXa active site. Incorporation of biphenylsulfonamide in our series resulted in **15** with an $IC_{50}$ of 0.022 μM. Analogues **7** and **15** were chosen for further optimization due to their good potency and ease of synthesis.

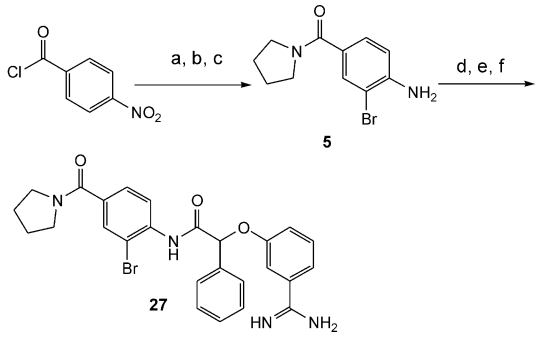
Addressing the P1 element, several groups have described the potency enhancing effects of incorporating a hydroxyl group *para* to the amidino group on the phenyl ring.[6] Introduction of a hydroxyl group in our two most potent compounds **7** and **15** increased potency by 10-fold in **16** and 94-fold in **17**. Homologation of **15**



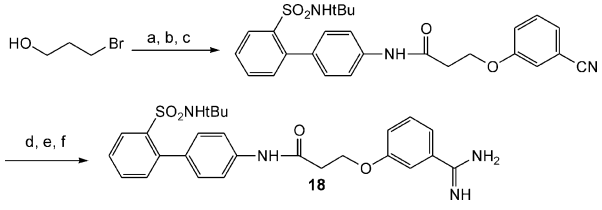

**Scheme 1.** Synthesis of compounds **6**–**14**. (a) $BrCH_2CO_2tBu$, $K_2CO_3$; (b) TFA; (c) methyl 4-aminobenzoate, BOP, DIEA, DMF, rt.; (d) aq LiOH; (e) $NH_2OH{\cdot}HCl$, TEA; (f) AcOH, $Ac_2O$; (g) 10% Pd/C, $H_2$; (h) amine, PyBOP, DIEA, DMF.




**Scheme 2.** Synthesis of compound **23**: (a) 3-cyanophenol, $K_2CO_3$, $CH_3CN$, reflux; (b) 3,4′-amino[1,1′-biphenyl]-2-*tert*-butylsulfonamide, $AlMe_3$, DCM; (c) $H_2S$, TEA/Pry; (d), MeI, acetone, reflux; (e) $NH_4OAc$, MeOH, reflux; (f) neat TFA, rt.




**Scheme 3.** Synthesis of **27**: (a) pyrrolidine, $Et_3N$, DCM; (b) $H_2$, 10%Pd/C; (c) pyridinium tribromide, AcOH; (d) **4**, $AlMe_3$, DCM, (e) HCl, MeOH; (f) $NH_4OAc$, MeOH.

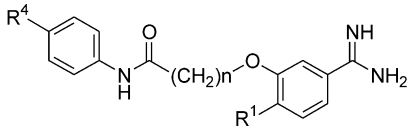



**Scheme 4.** Synthesis of **18**: (a) 3-cyanophenol, $Cs_2CO_3$, DMF; (b) PDC, DMF; (c) 3,4′-amino[1,1′-biphenyl]-2-*tert*-butylsulfonamide, $AlMe_3$, DCM; $AlMe_3$, DCM, (d) HCl, MeOH; (e) $NH_4OAc$, MeOH; (f) TFA.

at the central unit also afforded a 3-fold more potent analogue **18**. SAR studies with various side-chain substitution groups in our amino acid-based monobenzamidine fXa inhibitors[13] demonstrated that a phenyl group is optimal for the inhibitory activity. A 36-fold increase in potency occurred when the glycolic acid central unit of **15** was replaced with mandelic acid unit in **23**. More significantly, 366-fold increase in potency was achieved in **26** when a phenyl group was incorporated into **7**. Further optimization at P4 did not improve the activity in analogues **19**–**22**. Interestingly, halogen substitution at the $R^3$ position of the proximal phenyl ring (see Fig. 1), which increased the activity in other series, reduced potency in our series (analogues **24**, **25**, and **27**).

As shown in Tables 1 and 2, all compounds displayed good selectivity for fXa over thrombin and trypsin. High selectivity against a panel of serine proteases including tissue plasminogen activator (tPA), activated protein C (APC) and plasmin, was also observed for these compounds (data not shown). For example, compound **23** has $IC_{50}$ values of 0.55 nM for Xa, 21.2 μM for tPA, 181 μM for APC and 83.4 μM for plasmin. **26** has $IC_{50}$ values of 0.18 nM for Xa, >11 μM for tPA, >11 μM for APC and >11 μM for plasmin. In a rabbit deep vein thrombosis model,[11] compound **23** showed significant antithrombotic effects (56% inhibition of thrombus formation) at 0.132 μM plasma concentration following intravenous administration. Compound **26** also showed significant antithrombotic effects (81% inhibition of thrombosis formation) at 1.1 μM plasma

**Table 1.** In vitro activity for compounds **6**–**18**



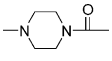
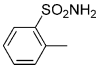
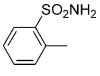
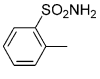
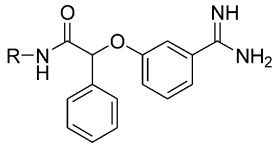
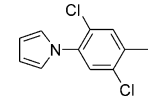
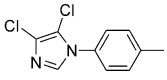
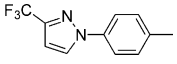
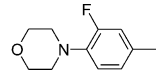
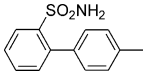
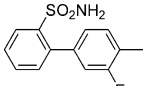
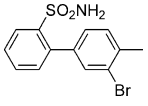
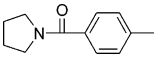
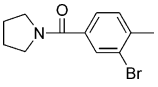
| Compd | $R^4$ | *n* | $R^1$ | $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|
| | | | | fXa | Thromb | Tryp |
| **6** |  | 1 | H | 0.227 | >10 | 2.05 |
| **7** |  | 1 | H | 0.066 | >10 | 2.7 |
| **8** |  | 1 | H | 1.37 | >10 | >10 |
| **9** |  | 1 | H | 1.19 | >10 | >10 |
| **10** |  | 1 | H | 1.12 | >10 | >10 |
| **11** |  | 1 | H | >10 | >10 | >10 |
| **12** |  | 1 | H | 0.0063 | >10 | 0.875 |
| **13** |  | 1 | H | >10 | >10 | >10 |
| **14** |  | 1 | H | 2.93 | >10 | 6.64 |
| **15** |  | 1 | H | 0.021 | >10 | 3.37 |
| **16** |  | 1 | OH | 0.0022 | >10 | 2.29 |
| **17** |  | 1 | OH | 0.0007 | >10 | 0.283 |
| **18** |  | 2 | H | 0.0064 | >10 | 2.57 |

**Table 2.** In vitro activity for compounds **19**–**27**



| Compd | R | $IC_{50}$ (μM) | | |
|---|---|---|---|---|
| | | fXa | Thromb | Tryp |
| **19** |  | 0.093 | 4.09 | 8.78 |
| **20** |  | 0.014 | >10 | 5.33 |
| **21** |  | 0.17 | >10 | >10 |
| **22** |  | 0.0044 | >10 | >10 |
| **23** |  | 0.00055 | >10 | 1.25 |
| **24** |  | 0.013 | >10 | 9.82 |
| **25** |  | 0.0032 | 8.64 | 4 |
| **26** |  | 0.00018 | >10 | 0.174 |
| **27** |  | 0.00099 | >10 | 0.521 |

concentration following intravenous administration. The anticoagulant activity of compounds **23** and **26** is also demonstrated by the results of the plasma coagulation assays since they doubled the activated partial thromboplastin time (aPTT) at 1.03 and 1.6 μM, respectively.

The pharmacokinetic properties of analogues **23** and **26** were evaluated in Sprague–Dawley rats. The oral bioavailability of **23** and **26** were found to be less than 5%. Although we were not successful in identifying oral fXa inhibitors with high bioavailability within this series of compounds, the glycolic and especially the mandelic acid templates have afforded potent and selective fXa inhibitors. Further SAR studies on improving pharmacokinetic properties of these inhibitors will be the subject of additional communications from our laboratories.

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