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Discovery of a factor Xa inhibitor (3*R*,4*R*)-1-(2,2-difluoro-ethyl)-pyrrolidine-3,4-dicarboxylic acid 3-[(5-chloro-pyridin-2-yl)-amide] 4-{[2-fluoro-4-(2-oxo-2H-pyridin-1-yl)-phenyl]-amide} as a clinical candidate

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ABSTRACT

A series of (3*R*,4*R*)-pyrrolidine-3,4-dicarboxylic acid amides was investigated with respect to their factor Xa inhibitory activity, selectivity, pharmacokinetic properties, and ex vivo antithrombotic activity. The clinical candidate from this series, R1663, exhibits excellent selectivity against a panel of serine proteases and good pharmacokinetic properties in rats and monkeys. A Phase I clinical study with R1663 has been finalized.

Cardiovascular events caused by thrombosis are still the leading cause of death in developed countries.[1] Anticoagulant therapy has been dominated for decades by parenteral drugs (e.g., low molecular weight heparin) or by vitamin K antagonists such as warfarin. Both therapies have well documented limitations.[2] Extensive efforts have therefore been undertaken in the pharmaceutical industry to discover and develop new antithrombotic agents with less limitations resulting in a better patient compliance.[3]

Over the past decade, factor Xa (fXa) has been the focus of intense research activity.[4] At the convergence of the intrinsic and extrinsic coagulation pathways it plays a pivotal role in the coagulation cascade.[5] The research efforts to identify fXa inhibitors have culminated in the discovery of rivaroxaban (Bayer),[6] which has been approved in the EU for the prevention of venous thromboembolism. In addition, YM150 (Astellas),[7] edoxaban tosilate (Daiichi Sankyo)[8] and apixaban (BMS/Pfizer)[9] are currently undergoing Phase III clinical trials.

In our previous work,[10] we described the identification of 3-aminopyrrolidines as potent and selective factor Xa inhibitors (K_i = 3 nM; 2xPT = 1.5 μM, Fig. 1). However, this series suffered from modest pharmacokinetic properties (e.g., low bioavailability). In search of alternative scaffolds our attention was attracted to the pyrrolidine scaffold **1** which provides a very good vector into the $S_1\beta$-pocket (Fig. 1). This pocket is formed by Arg-143, Gln-192 and the disulfide bridge between Cys-191 and Cys-220 of fXa, and has been shown to provide additional binding affinity if occupied with favorably interacting ligand atoms.[11] Molecular modeling into the binding site of fXa (Fig. 1) of a suitably decorated scaffold **1** revealed favorable interactions of the potential inhibitors with the binding site of fXa and in particular with the $S_1\beta$-pocket. Another advantage of this scaffold is the flexibility of the five-membered ring system which allows a conformational accommodation of the inhibitor to the active site of fXa. A preference for the chlorophenyl moiety instead of the chlorothiophenyl as S_1-pocket motif was suggested. In addition, the chiral synthesis of the orthogonally protected scaffold **1** has been developed at Roche[12] and rapid access to larger quantities of **1** was available. Later, similar series have been described by Qiao et al. (BMS)[13] and Kohrt et al. (Pfizer) resulting in the identification of PD0348292 (Eribaxaban).[14]

Here we describe the synthesis, SAR, pharmacokinetic properties and X-ray structure of potent and selective fXa inhibitors based on the pyrrolidine scaffold **1**.

The first residue which we intended to optimize was the $P_1\beta$-substituent. The synthesis of the compounds described in Table 1 is depicted in Schemes 1 and 2. To synthesize compounds **2**–**23**,

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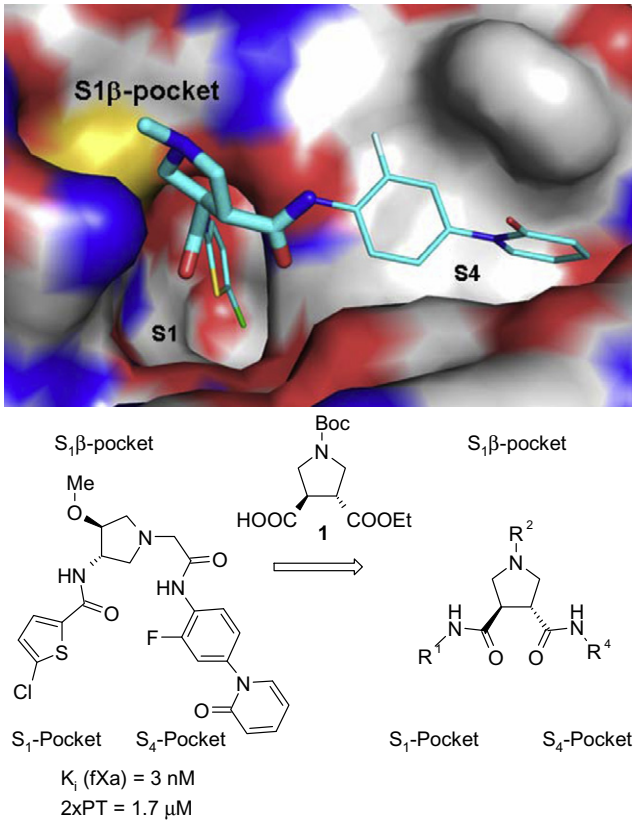


Figure 1. Illustration of design principle of fXa inhibitors using 2vvc.pdb[10] as example.

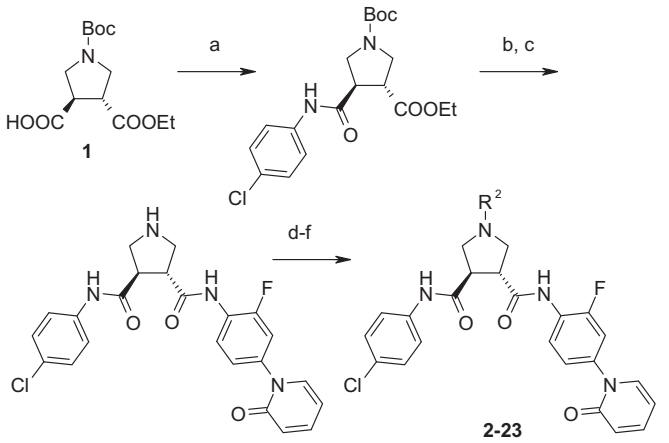
compound **1** was reacted with 4-chloro aniline to afford the corresponding amide (Scheme 1).

Saponification of the ester and treatment of the obtained acid with thionylchloride in presence of the P_4-substituent yielded the Boc-deprotected pyrrolidine bis-amide. After isolation of the free base by extraction, variations on the pyrrolidine nitrogen substituent were achieved by reactions with sulfonyl chlorides, acid chlorides, alkyl bromides, alkyl triflates, and by reductive amination.

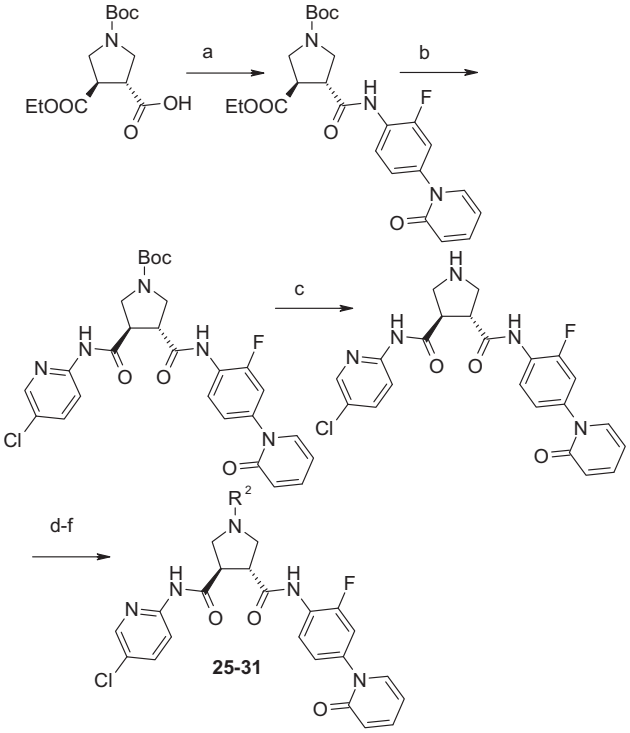
To introduce the corresponding 2-amino-5-chloro pyridine moiety as replacement for the 4-chloro aniline, the reaction sequence started first by amide coupling of the P_4-substituent using BOP-Cl as coupling reagent, followed by Weinreb amide coupling. Boc-cleavage using HCl in dioxane and subsequent variations on the pyrrolidine nitrogen as described in Scheme 1 afforded compounds **25**–**31**.

As listed in Table 1, a large variety of $S_1\beta$-pocket residues are tolerated, leading to low nanomolar binding affinities against fXa. For example, the sulfonamide **2** showed a K_i of 15 nM and a favorable in vitro anticoagulant activity (2xPT = 2.2 μM). In contrast, the unsubstituted pyrrolidine **3** showed only moderate binding (K_i = 120 nM), underlining the importance of an appropriate $P_1\beta$-substituent for good activity. The strongest binding affinity and best anticoagulant activity was demonstrated by the dimethyl sulfamide **13** with K_i = 8 nM and 2xPT = 1.7 μM. In general, compounds with comparable binding affinity showed better anticoagulant activity if their log *D* was lower.

We had concerns about using the 4-chloro aniline moiety as the final P_1-substituent, since severe toxicity is associated with this motif should the amide bond be cleaved in vivo. For this reason we switched to the 2-amino-5-chloro pyridine moiety. Combinations with the most interesting $S_1\beta$-pocket residues (compounds **25**–**31**) revealed that the binding affinity was in general maintained. In addition, due to the increased polarity, the anticoagulant activity was improved (e.g., compound **27** vs **8**).



Scheme 1. Reagents and conditions: (a) MeCN, DIEA, BOP-Cl, 4-chloroaniline, 0 °C, 2 h, 69%; (b) THF/water, LiOH, 25 °C, 18 h, 87%; (c) $SOCl_2$, 1-(4-amino-3-fluorophenyl)-1H-pyridin-2-one, 25 °C, 18 h, 99%; isolation of free base by extraction (compounds **3**). Modification on the pyrrolidine nitrogen with one of the methods d–g: (d) MeCN, sulfonylchlorides or acidchlorides, DIEA, 25 °C, 18 h, 15–45% (compounds **2**, **4**, **5**, **7**, **10**, and **12**–**18**); (e) MeCN, alkylbromides, K_2CO_3, Ag_2O, 80 °C, 18 h, 19–24% (compounds **6**, **8**, **19**, **20**, **22**, and **24**); (f) CH_2Cl_2, trifluoro-methanesulfonic acid 2,2,2-trifluoro-ethyl ester, DIEA, 25 °C, 72 h, 11% (compound **11**); (g) MeOH, $NaCNBH_3$, acetone, acetic acid, 80 °C, 18 h, 5–23% (compounds **9**, **21**, and **23**).



Scheme 2. Reagents and conditions: (a) MeCN, DIEA, BOP-Cl, 1-(4-amino-3-fluorophenyl)-1H-pyridin-2-one, 25 °C, 4 d, 58%; (b) 5-chloro-pyridin-2-ylamine, toluene, $AlMe_3$, 1 h at 25 °C, then reflux for 2 h, 72%; (c) HCl, 1,4-dioxane, 25 °C, 50 min, 60%; isolation of free base by extraction (compound **28**). Modification on the pyrrolidine nitrogen with one of the methods d–f: (d) MeCN, sulfonylchlorides or methylchloroformate DIEA, 25 °C, 18 h, 25–90% (compounds **25**, **26**, and **30**); (e) CH_2Cl_2, trifluoro-methanesulfonic acid 2,2-difluoro ethyl ester or trifluoro-methanesulfonic acid 2,2,2-trifluoro-ethyl ester, DIEA, 25 °C, 72 h, 46–64% (compounds **27** and **31**); (f) MeOH, $NaCNBH_3$, acetone, acetic acid, 80 °C, 18 h, 59% (compound **29**).