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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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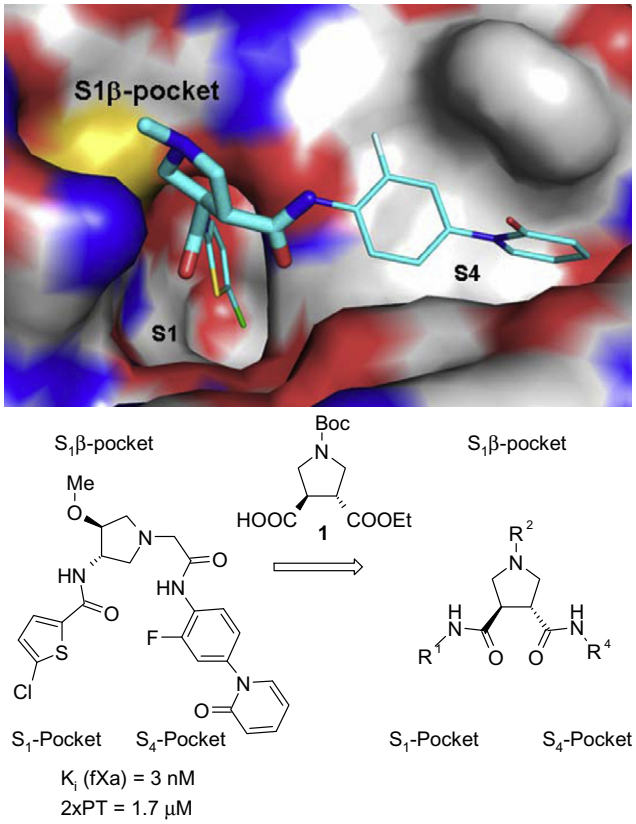


Figure 1. Illustration of design principle of fXa inhibitors using 2vvc.pdb[10] as example.

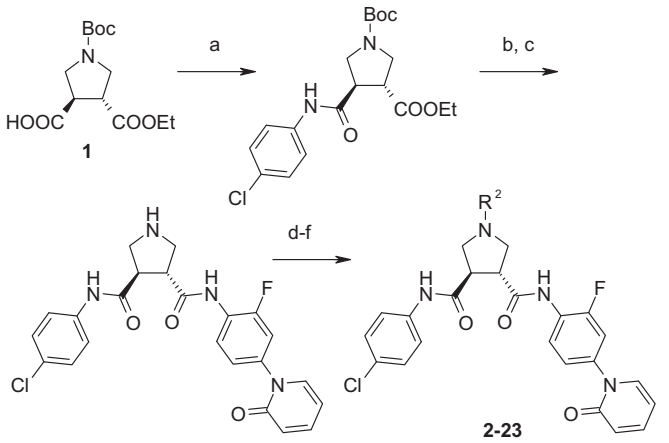
compound **1** was reacted with 4-chloro aniline to afford the corresponding amide (Scheme 1).

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.

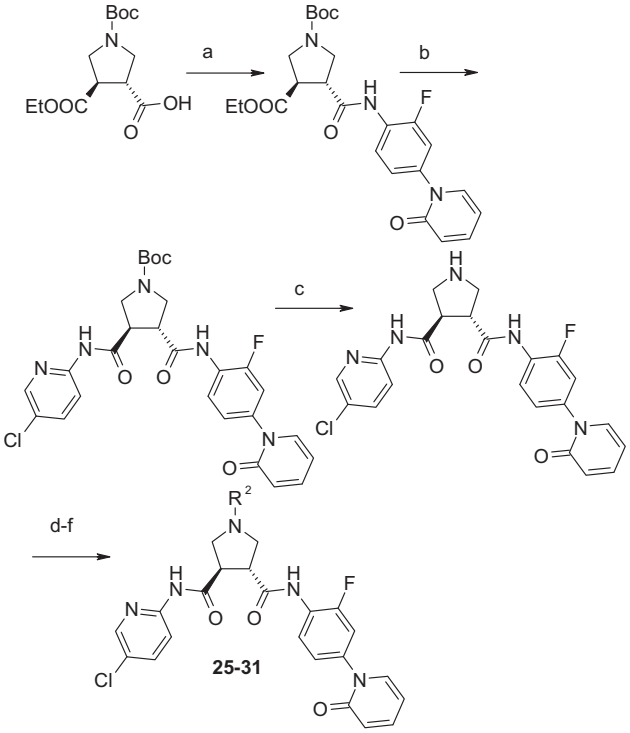
To introduce the corresponding 2-amino-5-chloro pyridine moiety as replacement for the 4-chloro aniline, the reaction sequence started first by amide coupling of the P_4-substituent using BOP-Cl as coupling reagent, followed by Weinreb amide coupling. Boc-cleavage using HCl in dioxane and subsequent 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**).

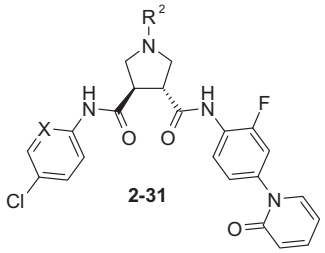


Scheme 1. Reagents and conditions: (a) MeCN, DIEA, BOP-Cl, 4-chloroaniline, 0 °C, 2 h, 69%; (b) THF/water, LiOH, 25 °C, 18 h, 87%; (c) $SOCl_2$, 1-(4-amino-3-fluorophenyl)-1H-pyridin-2-one, 25 °C, 18 h, 99%; isolation of free base by extraction (compounds **3**). Modification on the pyrrolidine nitrogen 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**).

Table 1
$P_1\beta$-substituent SAR



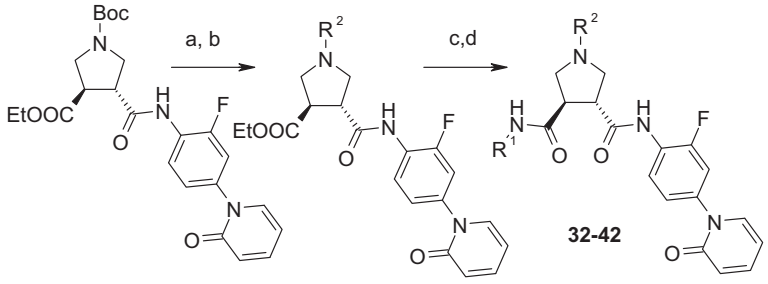
Compound	R^2	X	fXa K_i [a,b] (μM)	2xPT (μM)	log *D* (pH 7.4)
2	SO_2Me	CH	0.015	2.2	2.17
3	H	CH	0.120	5.3	n.d.[c]
4	Ac	CH	0.028	1.8	1.80
5	SO_2Et	CH	0.011	2.0	2.44
6	CH_2–CH_2–OH	CH	0.024	2.7	1.80
7	C(O)OMe	CH	0.022	2.6	2.43
8	CH_2–CHF_2	CH	0.010	2.9	2.69
9	*i*Propyl	CH	0.017	2.0	n.d.[c]
10	SO_2NH_2	CH	0.010	1.8	1.83
11	CH_2–CF_3	CH	0.009	2.9	3.20
12	$SO_2CH_2CF_3$	CH	0.017	3.2	2.68
13	SO_2NMe_2	CH	0.008	1.7	2.62
14	SO_2*i*Propyl	CH	0.010	2.0	2.81
15	C(O)OEt	CH	0.054	5.2	2.57
16	C(O)Pyrrolidine	CH	0.071	5.2	2.78
17	C(O)O(*n*)-Propyl	CH	0.047	6.3	3.06
18	SO_2*n*-Propyl	CH	0.018	2.2	2.94
19	CH_2-Cyclopropyl	CH	0.046	8.1	n.d.[c]
20	CH_2–CH_2F	CH	0.021	5.4	n.d.[c]
21	CH_2-4-Pyridyl	CH	0.034	5.9	n.d.[c]
22	CH_2–CH_2–OMe	CH	0.044	4.7	n.d.[c]
23	CH_2-2-Thiophenyl	CH	0.011	2.1	n.d.[c]
24	CH_2–CN	CH	0.042	7.6	n.d.[c]
25	SO_2Me	N	0.020	1.7	1.46
26	SO_2Et	N	0.024	3.8	1.77
27	**CH_2–CHF_2**	**N**	**0.007**	**1.5**	**2.20**
28	H	N	0.055	6.9	0.63
29	*i*Propyl	N	0.015	1.7	1.86
30	C(O)OMe	N	0.027	1.6	2.02
31	CH_2–CF_3	N	0.008	1.4	2.62

[a] Values are means of three experiments.
[b] All compounds exhibit high selectivity versus thrombin (K_i > 25 μM).
[c] n.d., not determined.

We note that all compounds listed in Table 1 demonstrated excellent selectivity versus thrombin, with K_i values greater than 25 μM.

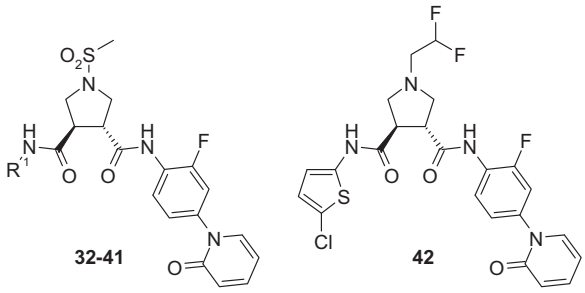
In search of alternative P_1-motifs, we elaborated a further alternative synthetic route (Scheme 3). In analogy to Scheme 2, the reaction sequence started with the introduction of the P_4-substituent, followed by Boc-cleavage, reaction with methanesulfonyl chloride or alternatively with 2,2-difluoroethyl triflate, saponification of the ester to the corresponding acid, and finally coupling of various aniline derivatives using BOP-Cl as coupling reagent. The aniline derivatives which were introduced by this method are listed in Table 2 in comparison to the 4-chlorophenyl (compound **2**) and 5-chloropyridin-2-yl (compound **25**) analogs.

With the exception of the 4-chloro-3-fluorophenyl (compound **39**; K_i = 62 nM) and the 5-chlorothiophenyl moieties (compound **42**; K_i = 36 nM), significantly weaker binding affinity towards fXa



Scheme 3. Reagents and conditions: (a) 6 N HCl in isopropanol, 25 °C, 2 h, 100%; (b) MeCN, methanesulfonyl chloride, DIEA, 25 °C, 18 h, 99%; or CH_2Cl_2, trifluoromethanesulfonic acid 2,2-difluoro ethyl ester, DIEA, 25 °C, 72 h, 46%; (c) 1,4-dioxane/water, LiOH, 25 °C, 24 h, 45%; (d) MeCN, BOP-Cl, DIEA, anilines, 25 °C, 24 h, 3–78%.

Table 2
P_1-substituent SAR



Compound	R^1	fXa K_i[a] (μM)	2xPT (μM)	log *D* (pH 7.4)
2	4-Chlorophenyl	0.015	2.2	2.17
25	5-Chloropyridin-2-yl	0.020	1.7	1.46
32	5-Indolyl	0.345	22.4	1.07
33	4-Methoxyphenyl	0.463	12.5	1.19
34	3-Chloro-4-methoxypheny	0.810	35.7	1.90
35	3-Fluoro-4-methoxyphenyl	0.192	5.9	0.56
36	4-Trifluormethyloxyphenyl	17.30	n.d.[b]	2.36
37	4-Chloro-2-fluorophenyl	0.130	4.7	n.d.[b]
38	4-Chlorophenylmethyl	16.59	n.d.[b]	n.d.[b]
39	4-Chloro-3-fluorophenyl	0.062	7.9	2.37
40	5-Indazolyl	1.247	n.d.[b]	0.50
41	2-Amino-4-chlorophenyl	0.421	12.3	n.d.[b]
42	5-Chlorothiophenyl	0.036	3.6	n.d.[b]

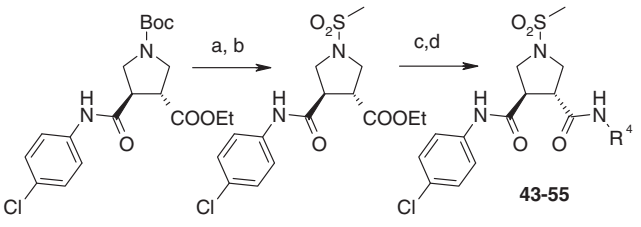
[a] Values are means of three experiments.
[b] n.d., not determined.

was observed. This is also reflected by a reduction of the anticoagulant activity.

A direct comparison of the 2,2-difluoroethyl derivatives **8**, **27**, and **42** demonstrated that the binding affinities and anticoagulant activities are clearly in favor of the six-membered (hetero)aromatic moieties of compounds **8** and in particular **27** as suggested by our initial modeling studies. Furthermore, the amide coupling of the 2-amino-5-chlorothiophene residue turned out to be problematic, as a rapid decomposition of the thiophene derivative was observed.

Finally, optimization of the P_4-substituent was investigated. For this purpose the sequence outlined in Scheme 1 was adapted slightly: the $P_1\beta$-motif was introduced first and the P_4-substituent was attached by BOP-Cl mediated amide coupling at the last reaction step (Scheme 4).

Table 3 summarizes all evaluated S_4-pocket residues with their corresponding activities. When compared to compound **2** no significant improvement in terms of binding affinity was achieved. Compounds **47** and **55** showed a better anticoagulant activity than compound **2** with 2xPT = 1.8 μM and 2xPT = 1.4 μM, respectively. Both compounds, however, where not profiled further. Compound **47** showed a twofold higher clearance in rat microsomes (data not shown) than compound **2**. Compound **55** was characterized by a high polar surface area of 106 Å^2 and an associated low passive permeation through artificial membranes with a permeation coefficient of $P_{eff} = 0.46 \times 10^{-6}$ cm s^{-1}, which was threefold lower than for compound **2** with a PSA of 96 Å^2. Therefore, oral bioavailability of compound **55** was expected to be lower than for compound **26**, which is analogous to compound **2** (see Table 4).

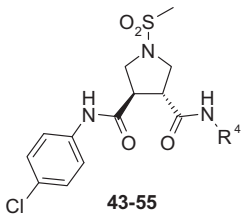


Scheme 4. Reagents and conditions: (a) 6 N HCl in isopropanol, 25 °C, 2 h, 100%; (b) MeCN, methanesulfonyl chloride, DIEA, 25 °C, 18 h, 55%; (c) 1,4-dioxane/water, LiOH, 25 °C, 24 h, 84%; (d) MeCN, BOP-Cl, DIEA, anilines, 25 °C, 24 h, 14–61%.

In support of the modeling and chemistry efforts, X-ray co-crystal structures of fXa with compounds **2**, **11**, **12**, and **27** were determined. The novel pyrrolidine scaffold proved to be an excellent framework to direct substituents into the S_1, $S_1\beta$, and S_4 pockets of factor Xa. While the S_1 pocket is deeply buried, the S_4 and $S_1\beta$ sites are more solvent-exposed, explaining the much steeper SAR for P_1-motifs. The chlorine atom of the P_1 pyridine substituent sits on top of the aromatic ring of Tyr-228 and the P_4 phenylpyridone group is favorably aligned with the aromatic residues in the S_4 pocket as observed by others (e.g., PDB code 2p93).[13] The $P_1\beta$-substituent clearly offered better opportunities to optimize molecular properties without sacrificing binding affinity. The crystal structure (Fig. 2) shows that the polar atoms of **27** interact through several direct or water-mediated hydrogen bonds with fXa binding site residues. The difluoroethyl moiety attached to the pyrrolidine nitrogen nicely fits into the $S_1\beta$-pocket and the terminal fluorine atoms are engaged in both hydrophobic (sulfur of Cys-191-220 bridge, Cβ of Gln-192) and cation-dipole (guanidine of Arg-143) interactions. The $S_1\beta$-pocket in factor Xa with its electropositive character appears to be a fluorophilic binding region as also seen, for example, in neprilysin.[15]

Finally, the 2-amino-5-chloro pyridine derivatives **26**, **27**, **29**, and **30** were chosen for further in-depth profiling (Table 4). All compounds showed good binding affinities and anticoagulant activities as well as excellent selectivity versus a panel of serine proteases. The clearance after incubation in human microsomes or human hepatocytes was low for all compounds and no issues were detected in the Ames, MNT and phototoxicity assays. Large differences were observed for the protein binding determined in human plasma. The basic compound **29** with the isopropyl $P_1\beta$-motif showed a high protein binding with less than 1% free fraction and also exhibited hERG inhibition with IC_{50} of 12 μM and further caused marginal phospholipidosis in the in vitro assay. In contrast,

Table 3
P_4-substituent SAR



43-55

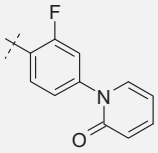
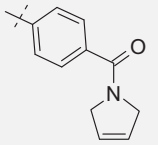
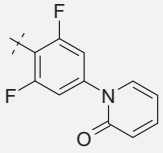
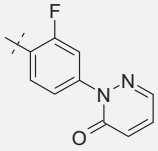
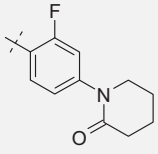
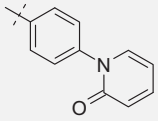
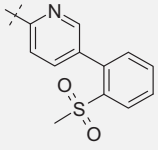
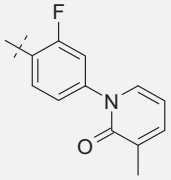
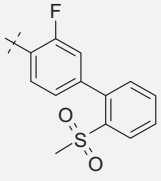
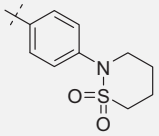
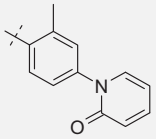
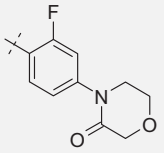
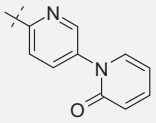
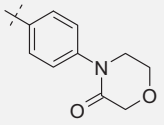
Compound	R^4	fXa K_i (μM)	2xPT (μM)
2		0.015	2.2
43		0.092	8.5
44		0.031	2.2
45		1.779	n.d.
46		0.124	4.5
47		0.014	1.8
48		0.042	4.4
49		0.015	3.5
50		0.040	8.1

Table 3 (*continued*)

Compound	R^4	fXa K_i (μM)	2xPT (μM)
51		0.201	12.0
52		0.022	2.1
53		0.046	3.9
54		0.040	4.5
55		0.024	1.4

the moderately basic difluoroethyl derivative **27** showed only modest protein binding with a free fraction of 23%, no significant hERG inhibition and no phospholipidosis in vitro. When tested in rat PK studies, compound **26** showed only low oral bioavailability and a relatively short half-life. This trend was even more pronounced when tested in a cynomolgus monkey PK study. Bioavailability was decreased down to 9%, disqualifying the compound from further development. In contrast to compound **26**, all other derivatives showed good oral bioavailability in cynomolgus monkeys and half-lives in a favorable range. In addition, all compounds exhibited a relatively low volume of distribution.

Compounds **27** and **30** were profiled in more detail. fXa and fVa form on charged phospholipid surfaces the prothrombinase complex, which is responsible for the rapid thrombin generation necessary for clot formation. Compound **27** inhibited peptidase activity of the prothrombinase complex[16] with K_i = 1.3 nM, and compound **30** with K_i = 6.6 nM. Interestingly, compound **27** showed one order of magnitude faster on- and off-rates when compared to compound **30**, resulting in similar K_D values of 3.7 or 4.3 nM, respectively. The faster binding kinetics of **27** may be associated with the more flexible difluoroethyl $P_1\beta$-motif when compared with the more rigid methylcarbamate $P_1\beta$-motif of **30**. Both molecules showed full reversibility of binding. When tested in a human whole blood flow system for ex vivo anticoagulant activity[17] compound **27** demonstrated inhibition of fibrin deposition in a dose-dependent manner with IC_{50} values in the range of 310 nM when coagulation was triggered by collagen or 840 nM when coagulation was triggered by J82 cells expressing tissue factor. Compound **30** turned out to be slightly weaker with an IC_{50} value of 1260 nM in the latter assay.

Both compounds showed comparable fXa activity for the human and rabbit enzymes. Compounds **27** and **30** were therefore profiled in rabbit in vivo models of anticoagulation. The results will be reported elsewhere.

Table 4
Pharmacological, safety, and pharmacokinetic data

Compound	**26**	**27** R1663	**29**	**30**
fXa K_i (nM)	24	7	15	27
2xPT (μM)	3.8	1.5	1.7	1.6
Prothrombinase K_i (nM)	n.d.[b]	1.3	1.8	6.6
fXa K_i (nM) rabbit	n.d.[b]	3.3	n.d.[b]	17
fXa K_i (nM) rat	n.d.[b]	100	n.d.[b]	n.d.[b]
fXa K_D (nM) human	n.d.[b]	3.7	n.d.[b]	4.3
k_{on} (M^{-1} s^{-1})	n.d.[b]	3.6×10^7	n.d.[b]	9.8×10^5
k_{off} (s^{-1})	n.d.[b]	1.3×10^{-1}	n.d.[b]	3.9×10^{-2}
Human whole blood ex vivo anticoagulation				
IC_{50} (nM) (collagen)	n.d.[b]	310	n.d.[b]	n.d.[b]
IC_{50} (nM) (J82)	n.d.[b]	840	n.d.[b]	1260
Selectivity[a] (μM)	>33	>33	>33	>33
f_u human (%)	14	23	<0.6	6.8
CL human microsomes (ml/min/kg)	5.3	4.7	10	0.01
CL human hepatocytes (ml/min/kg)	0.09	1.1	n.d.[b]	0.2
hERG IC_{50} (μM)	>10	>10	12	>10
Ames/MNT/Phototox.	Negative	Negative	Negative	Negative
pK_a/phospholipidosis	2.99/negative	4.79/negative	7.55/marginal	2.75/negative
Rat PK				
F (%)	24	100	39	100
$t_{1/2}$ (h) po	1.1	1.3	2.9	1.5
CL (ml/min/kg)	1.3	2.1	4.2	7.3
V_{ss} (L/kg)	0.040	0.18	0.47	0.42
AUCpo/D (ng h/ml)	3300	10,000	900	2300
Cyno. monkey PK				
F (%)	9	65	80	64
$t_{1/2}$ (h)	1.9	6.3	4.7	5.0
CL (ml/min/kg)	5.5	6.4	17	1.8
V_{ss} (L/kg)	0.46	0.54	1.6	0.44
AUCpo/D (ng h/ml)	230	1700	820	6700

[a] Selectivity was determined versus a panel of serine proteases consisting of TF/fVIIa, thrombin, fIXa, trypsin, fXIa, aPC, plasmin, kallikrein.
[b] n.d., not determined.

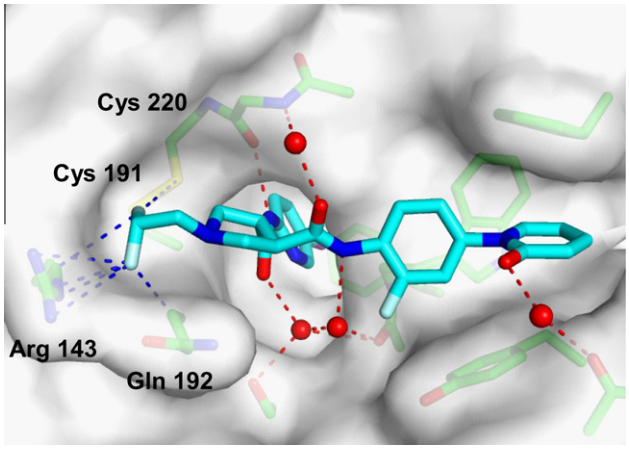


Figure 2. X-ray co-crystal structure of compound **27** bound to the active site of fXa (resolution 1.66 Å, PDB code 2xbv).[18] Hydrogen bonds of the ligand are shown in red and protein-ligand interactions in the S_1β-pocket in blue dashed lines.

After carefully reviewing all data, compound **27** (R1663) was selected for further clinical development.

In summary, we have discovered potent, selective and orally bioavailable fXa inhibitors based on the pyrrolidine scaffold **1**. By modulating in particular the S_1β-pocket residue, the pharmacokinetic properties of the previously published series could be significantly improved. Within this series compound **27** (R1663) was selected for further clinical development and a Phase I trial has been finalized.

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Coordinates have been deposited at the Protein Data Bank with the PDB codes: 2xbv (**27**), 2xbw (**10**), 2xbx (**2**), 2xc0 (**35**), 2xc4 (**11**), 2xby, and 2xc5 (other compounds).
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