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Design and synthesis of orally active pyrrolidin-2-one-based factor Xa inhibitors

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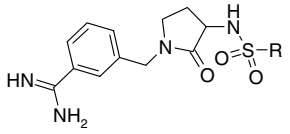
Abstract—A series of novel, non-basic 3-(6-chloronaphth-2-ylsulfonyl)aminopyrrolidin-2-one-based factor Xa (fXa) inhibitors, incorporating an alanylamide P4 group, was designed and synthesised. Within this series, the *N*-2-(morpholin-4-yl)-2-oxoethyl derivative **24** was shown to be a potent, selective fXa inhibitor with good anticoagulant activity. Moreover, **24** possessed highly encouraging rat and dog pharmacokinetic profiles with excellent oral bioavailabilities in both species.

Factor Xa (fXa) plays a central role in the coagulation cascade where it combines with factor Va and calcium ions on membrane surfaces to form the prothrombinase complex that activates prothrombin to thrombin. A key action of thrombin is to convert fibrinogen into fibrin leading to clot formation.[1] In the search for orally active anticoagulants with improved efficacy/safety profiles, direct thrombin inhibitors have been investigated by ourselves[2] and others.[3] More recently, fXa has been recognised as an alternative attractive target for development of new antithrombotic agents.[4]

fXa is a trypsin-like serine protease and thus exhibits a preference for basic groups to bind in its primary (S1) specificity pocket. First-generation fXa inhibitors incorporated highly basic P1 moieties (e.g., benzamidine) and whilst such compounds provide high affinity, they generally suffer from poor pharmacokinetic (PK) profiles and low oral bioavailabilities. Our strategy has centred on eliminating this developability risk by identifying novel series of non-amidine-based inhibitors with profiles suitable for chronic oral therapy.

fXa inhibitors incorporating a 3-amino-2-pyrrolidinone scaffold and bearing benzamidine-based P1 motifs **1** have been disclosed[5] and our in-house programme centred on identifying alternative N-substituted pyrrolidin-2-one motifs that retained attractive in vitro potency and promoted good in vivo profiles.



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We report here the identification of a non-basic series of amides (Table 1) and studies to optimise their profiles. Scheme 1 illustrates the generalised synthetic approach. The pyrrolidinone **8** was prepared from CBZ-Met-OH and the *tert*-butyl ester of the appropriate amino acid adapting a previously reported approach from our laboratories.[7] Removal of the CBZ group followed by sulfonylation of the resultant amine provided a late stage

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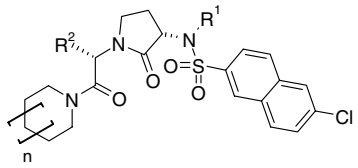
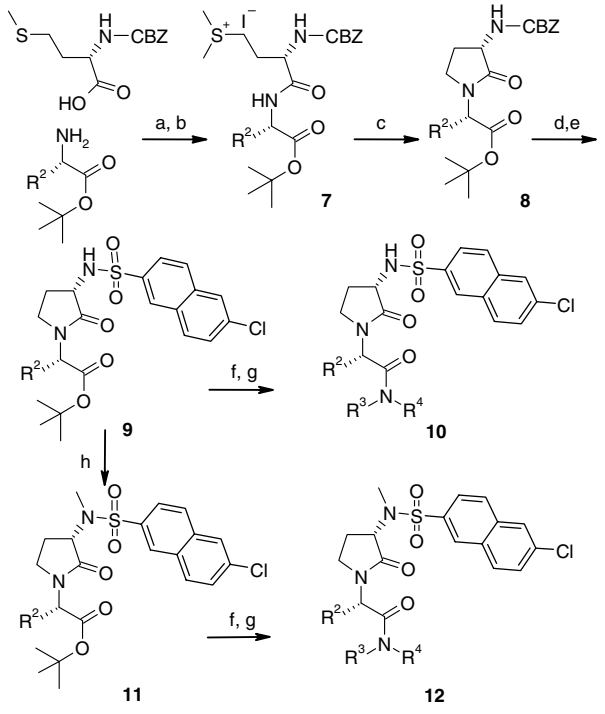
Table 1. fXa inhibitory activities[6a] for compounds **2**–**6**



Compound	R^1	R^2	*n*	fXa K_i (nM)
2	Me	H	2	60
3	Me	H	1	72
4	H	H	1	63
5	H	Me	1	1
6	Me	Me	1	2



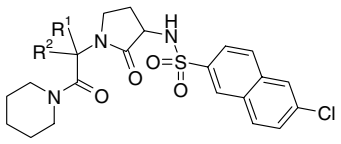
Scheme 1. Reagents and conditions: (a) EDC, 1-hydroxybenzotriazole (HOBT), Et_3N, DMF, rt; (b) MeI, acetone, rt; (c) DOWEX $[OH]^-$, MeCN; (d) H_2, Pd–C, EtOH, rt; (e) 6-chloronaphth-2-yl-SO_2Cl (RSO_2Cl), pyridine, MeCN, rt; (f) TFA, DCM; (g) R^3R^4NH, EDC, HOBT, Et_3N, DCM, rt; (h) MeOTs, $[(Me)_3Si]_2NLi$, THF, −78 °C to rt.

intermediate **9**; deprotection of the *tert*-butyl ester followed by coupling with a range of amines provided the final products **10**. Alternatively, N-alkylation of intermediate **9** to provide **11** followed by deprotection and amide coupling as described above provided a series of related tertiary sulfonamides **12**.

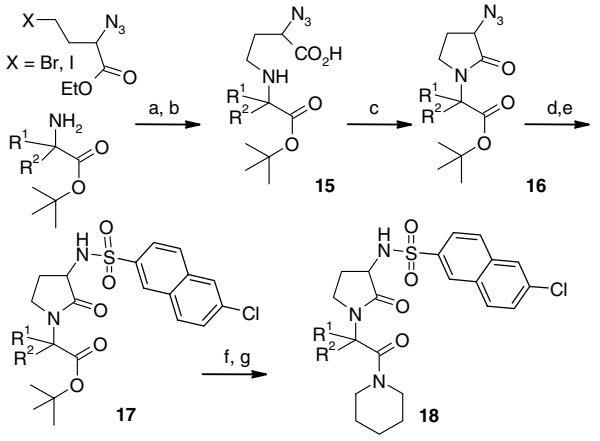
Analogues **13**[8] and **14** (Table 2) were prepared as racemates by an alternative route (Scheme 2).

Alkylation of the appropriate amino *tert*-butyl ester with ethyl azido-4-halobutanoate[9] followed by selective saponification of the ethyl ester gave the acid **15** which was cyclised in the presence of diphenylphosphoryl azide

Table 2. fXa inhibitory activities[6a] for compounds **13** and **14**



Compound	R^1	R^2	fXa K_i (nM)
13a	Et	H	320
13b	Et	H	1020
14	Me	Me	10,600



Scheme 2. Reagents and conditions: (a) Et_3N, MeCN, 50 °C; (b) LiOH, THF–H_2O, rt; (c) $(PhO)_2P(O)N_3$, Et_3N, DMF, rt; (d) H_2, Pd–C, EtOH, rt; (e) RSO_2Cl, $^iPr_2NEt$, DCM, rt; (f) TFA, DCM, rt; (g) piperidine, EDC, HOBT, Et_3N, DCM, rt.

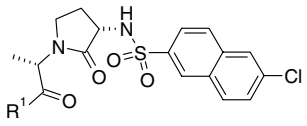
to give the racemic pyrrolidinone **16**. Catalytic hydrogenation of the azido group followed by sulfonylation of the derived amine gave intermediates **17** which were converted into the desired products **18** using procedures analogous to those in Scheme 1.

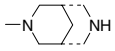
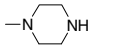
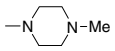
Focusing on the glycyl linker in **4**, a series of branched-chain analogues (Tables 1 and 2) showed that the α-methyl-(alanyl) group conferred potent fXa inhibitory activity in both the secondary and tertiary sulfonamide series (**5** and **6**, respectively); related α-ethyl- and α,α-di-methyl-analogues (**13** and **14**, respectively) showed significantly reduced potency.

Retaining the alanyl linker, a wide range of terminal amides was assessed and a representative subset is shown in Table 3. The piperidine amide **5** was more potent than the related pyrrolidine analogue **19** as were the bridged analogues, **20** and **21**.[10] In contrast, the piperazine **22** and *N*-methylpiperazine **23** derivatives[10] showed significantly reduced activities. A range of substituents at C2 and C3 on the piperidine ring was also well tolerated.[11a] All analogues tested from these series possessed poor rat PK profiles characterised by moderate to high plasma clearance and short half-lives.

A key finding from these studies was that reducing hydrophobicity resulted in a better correlation between

Table 3. fXa inhibitory activities[6a] for compounds **5** and **19–23**

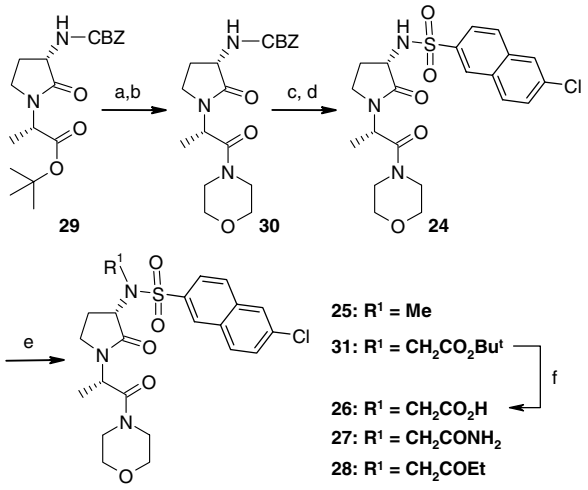


Compound	R^1	fXa K_i (nM)
5		1
19		50
20		3
21		13
22		320
23		2060

intrinsic and anticoagulant potency as illustrated by the in vitro profiles for the lipophilic bridged analogue **20** [CHI log*D* (pH 7.4) 2.94, 1.5× PT 21.7 μM] and the more hydrophilic bispidine derivative **21** (CHI log*D* (pH 7.4) 1.47, 1.5× PT 2.5 μM).[6b,11–13] A difference in the plasma protein binding of hydrophobic and more hydrophilic analogues is believed to be a contributory factor to this observation.[14a]

Building upon the benefits of reducing log*D*, a major breakthrough was achieved by replacing the piperidine with a morpholine ring **24** [CHI log*D* (pH 7.4) 2.2]. Not only did **24** retain good fXa inhibitory activity (Table 4), it also showed promising anticoagulant activity and a rat PK profile characterised by low plasma clearance and good oral bioavailability (85%). A range of tertiary sulfonamides was assessed[11b] and a subset **25–28** is shown in Table 4. Whilst these retained good in vitro profiles, these analogues generally possessed poorer rat PK profiles (Table 4).

Reverse exploitation of the orthogonally protected intermediate **29** provided **24** with high (>98%) diastereomeric purity and provided a preferred route to this analogue (Scheme 3); tertiary sulfonamides **25–28** were derived using standard procedures.



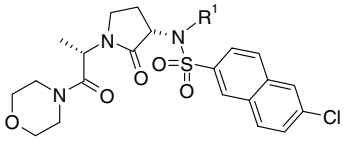
Scheme 3. Reagents and conditions: (a) TFA, DCM, rt; (b) morpholine, EDC, HOBT, Et_3N, DCM, rt; (c) H_2, Pd–C, EtOH, rt; (d) RSO_2Cl, pyridine, MeCN, rt; (e) MeOTs or R^1-Br, $[(Me)_3Si]_2NLi$, THF, −78 °C to rt; (f) TFA, DCM, rt.

The preferred absolute stereochemistry in the highly promising secondary sulfonamide **24** was unambiguously established utilising the *tert*-butyl esters of D- or L-alanine and D- or L-CBZ-Met-OH in the route shown in Scheme 1. Evaluation of the four derived stereoisomers **24** and **32–34** demonstrated that the 1*S*,3*S* isomer **24** conferred the greatest potency (Table 5); similar findings were established for the related piperidine analogues (compare **5** with **35–37**, Table 5).

Without a basic P1 motif, a key question was the binding mode of this series. A combination of approaches[14a] shed light on this issue culminating in a high resolution X-ray crystal structure of **24** bound in the active site of fXa. The molecule could be unambiguously fitted to the $F_o - F_c$ electron density map establishing the binding mode shown in Figure 1.[15]

Thus, the chloronaphthyl group binds in S1 where the chlorine atom makes a key interaction with Tyr228.[14a,16] The carbonyl group in the pyrrolidinone template makes a water-mediated interaction with Ser214. Additional affinity is achieved with the (*S*)-methyl group that binds

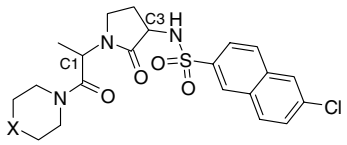
Table 4. fXa inhibitory activities,[6a] anticoagulant potency[6b] and rat PK profiles[a] for **24** and a series of tertiary sulfonamides **25–28**



Compound	R^1	fXa K_i (nM)	1.5× PT (μM)	$t_{1/2}$ (h)	Cl_p (mL/min/kg)	V_{ss} (L/kg)
24	H	6	5	0.9	13	0.6
25	Me	10	—	0.5	15	0.4
26	CH_2CO_2H	2	7	0.2	37	0.3
27	CH_2CONH_2	3	3	0.3	24	0.5
28	CH_2COEt	1	4	0.5	37	1.1

[a] Compounds were administered either iv as a bolus or po via gavage to male Han Wistar rats at nominal doses of 1 mg/kg iv and 2.5 mg/kg po.

Table 5. Influence of stereochemistry on fXa activities[6a] in morpholine- and piperidine-alanylamide series



Compound	X		fXa K_i (nM)
24	O	1*S*,3*S*	6
32	O	1*R*,3*R*	82
33	O	1*S*,3*R*	53
34	O	1*R*,3*S*	160
5	CH_2	1*S*,3*S*	1
35	CH_2	1*R*,3*R*	40
36	CH_2	1*S*,3*R*	16
37	CH_2	1*R*,3*S*	82

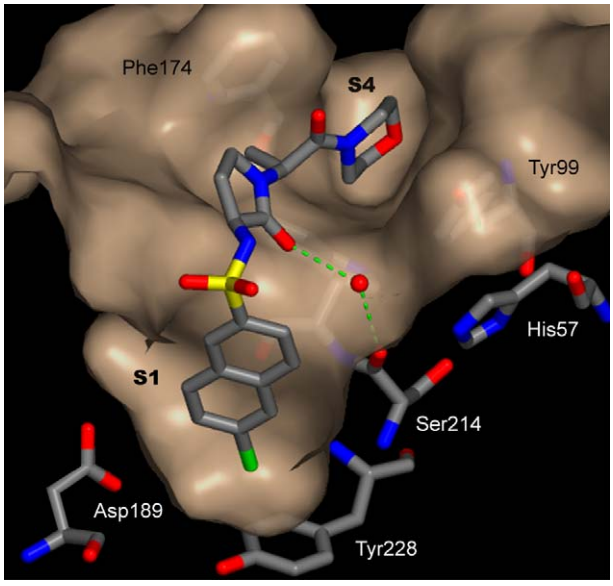


Figure 1. X-ray crystal structure of **24** complexed with fXa.

in a small pocket so providing a rationale for the increased potency seen with alanyl- compared with glycyl-based analogues (see Table 1). The morpholine-alanylamide group fits into the S4 pocket formed by Phe174, Tyr99 and Trp215 but makes no specific H-bonding interactions with the protein. An alternative view of this X-ray crystal complex (Fig. 2) illustrates that the sulfonamide group provides the appropriate geometry for both the chloronaphthyl group to access S1 and the rest of the inhibitor to be directed into S4.

While some residual activity against thrombin (37-fold selectivity) was apparent, morpholinamide **24** showed good selectivity (>100-fold) over all other trypsin-like serine proteases tested (fVIIa, fIXa, fXIIa, aPC, plasmin, kallikrein, trypsin and elastase). Furthermore, **24** possessed an encouraging PK profile in the dog ($t_{1/2}$, 2.1 h; Cl_p, 2.7 ml/min/kg; V_{ss}, 0.44 L/kg) with excellent oral bioavailability (100%).[17] On the basis of this promising profile, the 2-(morpholin-4-yl)-2-oxoethyl series

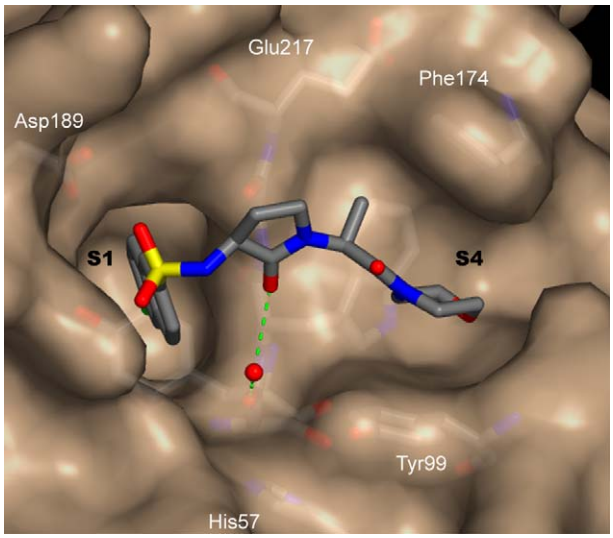


Figure 2. X-ray crystal structure of **24** complexed with fXa (alternative view).

was selected for further evaluation and findings from these studies will be reported in separate publications.[14]

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6. (a) fXa inhibitory activities were determined using *N*-α-Z-D-Arg-Gly-Arg-*p*-nitroanilide as chromogenic substrate; details are described in Ref. 11; (b) Anticoagulant activities were determined in the prothrombin time (PT) assay; details (test 2) are described in Ref. 11.
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8. Compounds **13a** and **13b** were prepared as racemates from the corresponding diastereomeric intermediates **16** which

were separated by chromatography; the relative stereochemistry of the diastereomers **13a** and **13b** have not been determined.

9. (a)2-Azido-4-iodobutanoate was derived from the 4-bromo derivative by reaction with sodium iodide in acetone; 2-azido-4-bromobutanoate was prepared from the 2,4-dibromo derivative (Ref. 9b) by the method reported for the corresponding methyl ester. *J. Org. Chem*. **1982**, *47*, 1319; (b) Wasserman, H. H.; Lipshutz, B. H.; Tremper, A. W.; Wu, J. S. *J. Org. Chem.* **1981**, *46*, 2991.
10. Compounds **21**–**22** were derived by deprotection of the corresponding Boc-protected derivatives by treatment with TFA in DCM. Compound **23** was prepared as a 1:1 mixture of two diastereomers by the route outlined in Scheme 2 utilising the *tert*-butyl ester of L-alanine.
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13. Compound **21** shows good selectivity over other trypsin-like serine proteases tested, including thrombin.
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15. The fXa complex crystal structure with **24** was obtained using an adaptation of the method outlined in Maignan, S.; Guilloteau, J.-P.; Pouzieux, S.; Choi-Sledeski, S.; Becker, M. R.; Klein, S. I.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. *J. Med. Chem.* **2000**, *43*, 3226, Further details are described in Ref. 14a. The structure was refined at 2.1 Å (overall R_{merge} is 0.077) in Refmac5 to a final R_{factor} of 0.192 and R_{free} of 0.242. Coordinates are deposited in the protein data bank with code 2cji.
16. Similar findings have been reported recently for other series of fXa inhibitors (a) Choi-Sledeski, Y. M.; Kearney, R.; Poli, G.; Pauls, H.; Gardner, C.; Gong, Y.; Becker, M.; Davis, R.; Spada, A.; Liang, G.; Chu, V.; Brown, K.; Collussi, D.; Leadley, R., Jr.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J.-P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 681; (b) Jia, Z. J.; Su, T.; Zuckett, J. F.; Wu, Y.; Goldman, E. A.; Li, W.; Zhang, P.; Clizbe, L. A.; Song, Y.; Bauer, S. M.; Huang, W.; Woolfrey, J.; Sinha, U.; Arfsten, A. E.; Hutchaleelaha, A.; Hollenbach, S. J.; Lambing, J. L.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2073; (c) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900.
17. Compound **24** was administered iv (1 mg/kg) as a slow bolus over 5 min and po (1 mg/kg) via gavage to female Beagle dogs. The dosing vehicle was a 5:95% (v/v) mixture of DMSO and 50:50 PEG-200:sterile water.