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Structure- and property-based design of factor Xa inhibitors: Pyrrolidin-2-ones with acyclic alanyl amides as P4 motifs

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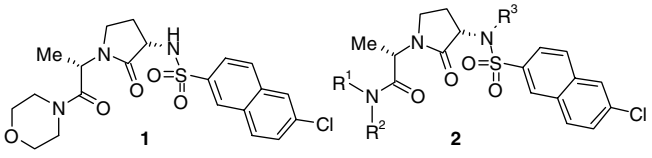
Abstract—Structure-based drug design was exploited in the synthesis of 3-(6-chloronaphth-2-ylsulfonyl)aminopyrrolidin-2-one-based factor Xa (fXa) inhibitors, incorporating an alanylamide P4 group with acyclic tertiary amide termini. Optimized hydrophobic contacts of one amide substituent in P4 were complemented by hydrophobicity-modulating features in the second, producing potent fXa inhibitors including examples with excellent anticoagulant properties.

In the search for anticoagulant therapies with improved efficacy/safety profiles, coagulation Factor Xa (fXa) has provided a major focus.[1] The pivotal role of fXa in the blood coagulation cascade makes it an attractive therapeutic target and emerging data support this hypothesis.[2] The primary specificity (S1) pocket of fXa recognizes a basic residue, in common with other such trypsin-like serine proteases. Consequently, many fXa inhibitors include a basic amine or amidine P1 motif to facilitate binding to Asp189 at the base of this pocket; such charged features limit permeation and generally engender poor oral pharmacokinetics.

We recently described a series of non-basic compounds exploiting an alternative interaction in S1, specifically that between an aryl chloride and Tyr228.[3] Optimisation of this aminopyrrolidin-2-one-based series led to molecules, for example, **1**, which displayed good anticoagulant potency and attractive oral pharmacokinetics.[4] Other structural classes of neutral or weakly basic fXa inhibitors, which utilize similar interactions in the S1 pocket, have been reported.[5]

The application of structure- and property-based design is described here in the evaluation of a series of acyclic analogues **2** exploiting the alanyl amide P4 ligand.



Impetus for this work arose from inspection of the crystal structure of **1** bound into fXa;[3] one edge of the morpholine ring made hydrophobic contacts with aromatic residues forming S4, but without filling this space. Initial molecular modelling supported the hypothesis that a small aliphatic R^1 group, as one substituent on a tertiary acyclic amide, could better occupy this space (Fig. 1). It was envisaged that the second amide substituent might be used to introduce hydrophobicity-modulating features to enhance pharmacokinetic and anticoagulant profiles as discussed in our earlier publications.[3,4]

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Furthermore, distance calculations supported the idea of R^2 substituents forming additional H-bonds with backbone residues.

Available structural information indicated a clear preference for molecules with the S,S stereochemistry, so synthesis of the orthogonally protected intermediate **3** (Scheme 1) exploited the established route from CBZ-Met-OH and H-Ala-OtBu.[3] Hydrogenolysis and sulfonylation afforded ester **4**, acid deprotection of which gave chiral acid **5**. Alkylation of **4** with 2-bromoacetamide and deprotection of the *tert*-butyl ester provided the tertiary sulfonamide intermediate **6**, introducing a particular modification that significantly enhanced activity in the cyclic series.[3]

The targeted acyclic compounds **2** were produced by amide coupling of primary or secondary amine substrates[6] with **5** or **6** (Scheme 2). Terminal amines **8** and their homologues were produced via *N*-Boc-protected intermediates **7**, followed by acid deprotection. Sulfonamides **9** and ureas **10** were readily derived from these amines.

A first matrix of compounds investigated the SAR of lower alkyl combinations and showed a clear preference for tertiary amides with enhanced activity when one substituent was isopropyl (Table 1). This was ascribed to good hydrophobic contacts of the isopropyl group with the aromatic S4 residues, consistent with modelling predictions.

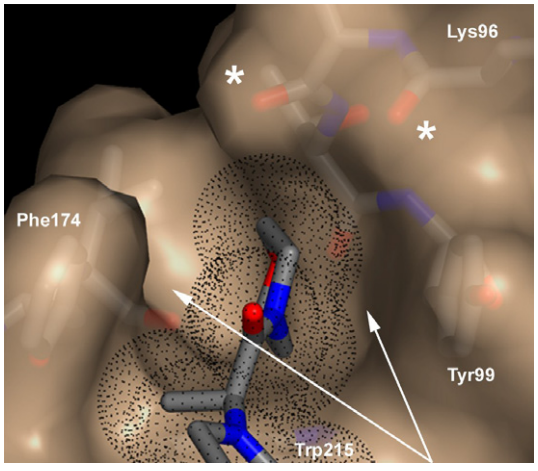
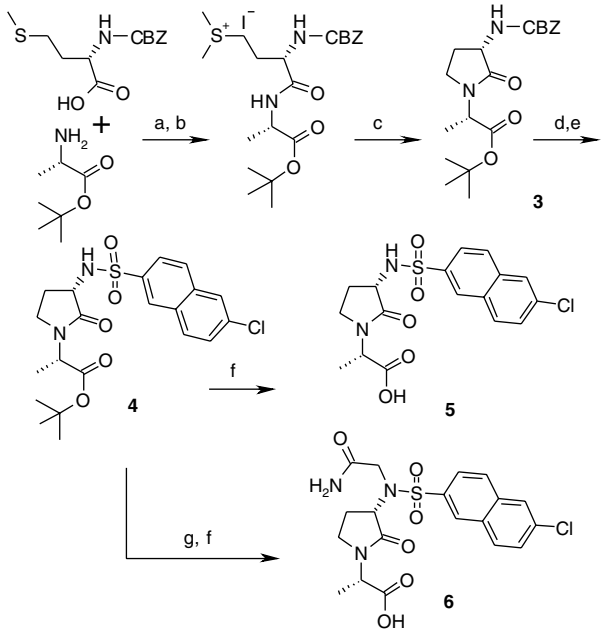
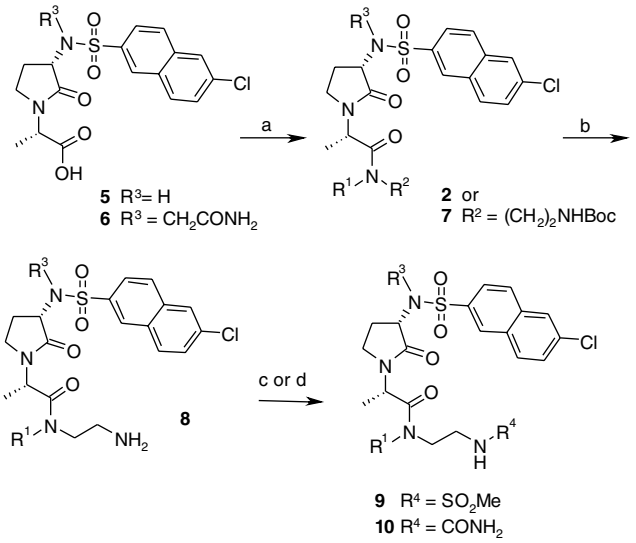


Figure 1. X-ray crystal structure of **1** bound into fXa,[3] highlighting potential S4 interactions for an acyclic structure: (i) better filling of the S4 'aromatic box' (arrowed) and (ii) accessible backbone residues around Lys96 (as starred∗).

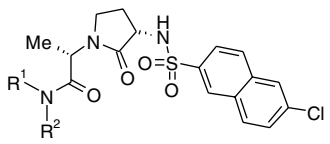


Scheme 1. Reagents and conditions: (a) EDC, 1-hydroxybenzotriazole (HOBT), Et_3N, DMF, rt; (b) MeI, acetone, rt; (c) DOWEX $[OH]^-$, MeCN; (d) H_2, Pd–C, EtOH, rt; (e) 6-chloronaphth-2-ylSO_2Cl, pyridine, DCM, rt; (f) TFA, DCM; (g) $BrCH_2CONH_2$, K_2CO_3, DMF, rt.



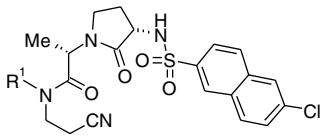
Scheme 2. Reagents and conditions: (a) R^1R^2NH, EDC, HOBT, Et_3N, DMF, rt; (b) TFA, DCM; (c)$MeSO_2Cl$, pyridine DCM, rt; (d) H_2NCO_2Ph, NMM, THF, Δ.

Table 1. fXa inhibitory activities[7a] for compounds **11**–**16**



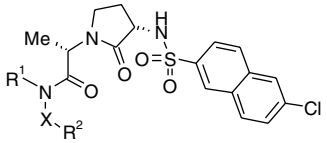
Compound	R^1	R^2	fXa K_i/nM
11	Me	Me	346
12	Et	Me	56
13	Et	Et	24
14	*i*-Pr	H	2720
15	*i*-Pr	Me	22
16	*i*-Pr	Et	11

Table 2. fXa inhibitory activities[7a] for compounds **17–23**



Compound	R^1	fXa K_i/nM
17	*i*-Pr	5
18	*i*-Bu	136
19	*c*-Pr	57
20	CH_2*c*-Pr	3
21	*c*-Bu	15
22	*c*-Pentyl	11
23	*c*-Hexyl	73

Table 3. fXa inhibitory activities[7a] for compounds **24–29**



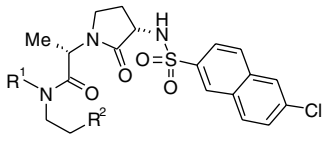
Compound	R^1	X	R^2	fXa K_i/nM
24	Et	CH_2	4-Pyridinyl	27
25	*i*-Pr	CH_2	Ph	24
26	*i*-Pr	CH_2	2-Pyridinyl	11
27	*i*-Pr	CH_2	3-Pyridinyl	9
28	*i*-Pr	CH_2	4-Pyridinyl	6
29	*i*-Pr	CH_2CH_2	2-Pyridinyl	13

A subsequent subset of compounds, derived from available monomers incorporating a common cyanoethyl group, provided further empirical evidence to support the hypothesis concerning the required hydrophobic contacts (Table 2). The high potency of isopropyl and cyclopropylmethyl analogues corroborated the likely optimum size and fit of the R^1 substituent.

The finding that good intrinsic activity could be maintained in analogues containing a hydrogen bond accepting nitrogen was also demonstrated in a series of alkyl(pyridinylalkyl)amines (Table 3). As expected, this gave compounds with enhanced potency with the isopropyl R^1 group; no clear preference for the pyridine orientation was apparent. Members of these series showed only moderate anticoagulant activities, as assessed in the prothrombin time (PT) assay;[8] for example, **17** K_i = 5 nM, 1.5 × PT 9.4 μM, *c*log$D_{7.4}$[9] 2.3; **27** K_i = 9 nM, 1.5 × PT 18.7 μM, *c*log$D_{7.4}$ 3.0; **28**, K_i = 6 nM, 1.5 × PT 8.8 μM, *c*log$D_{7.4}$ 3.0.

At this stage, the incorporation of more hydrophilic R^2 substituents was investigated, to capitalise on previous findings that reducing hydrophobicity[9] promoted a better translation of intrinsic potency into plasma-based activity.[3,4] Gratifyingly, both intrinsic potency and anticoagulant activity were enhanced (Table 4), with amino substituted analogues having particularly attractive profiles (e.g., **33**, K_i = 4 nM, 1.5 × PT 1.3 μM, *c*log$D_{7.4}$ 0.6; **42** K_i = 2 nM, 1.5 × PT 1.6 μM, *c*log$D_{7.4}$ 1.3). Results in this matrix also supported isopropyl as the R^1 group of choice; related cyclopropylmethyl derivatives gave broadly similar fXa activities that did not translate as

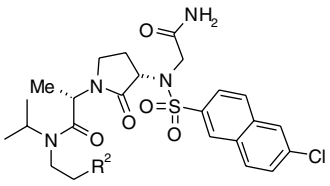
Table 4. fXa inhibitory activities,[7a,b] anticoagulant potency[8] and hydrophobicity calculations[9] for compounds **30–48**



Compound	R^1	R^2	*c*log$D_{7.4}$[9]	fXa K_i/nM	1.5 × PT/μM
30	*i*-Pr	OH	2.3	6	9.7
31	CH_2*c*-Pr	OH	2.3	7	16.7
32	H	NH_2	—	4100[a]	—
33	*i*-Pr	NH_2	0.6	4	1.3
34	CH_2*c*-Pr	NH_2	0.6	15	2.8
35	*i*-Pr	$CONH_2$	1.5	11	8.0
36	*i*-Pr	$NHCONH_2$	1.5	2	8.1
37	CH_2*c*-Pr	$NHCONH_2$	1.5	7	16
38	H	$NHSO_2Me$	—	39,000[a]	—
39	*i*-Pr	$NHSO_2Me$	1.8	1	2.5
40	CH_2*c*-Pr	$NHSO_2Me$	1.8	2	10.5
41	*i*-Pr	SO_2NH_2	1.4	3	4.8
42	*i*-Pr	NMe_2	1.3	2	1.6
43	*i*-Pr	Piperidine	2.4	2	1.7
44	*i*-Pr	Morpholine	2.2	4	5.8
45	*i*-Pr	Azepine	3	2	1.6
46	*i*-Pr	CH_2NH_2	0	2	2.2
47	*i*-Pr	$CH_2NHCONH_2$	1.7	5	6.7
48	*i*-Pr	CH_2NHSO_2Me	2	3	9.5
1	Morpholine		2.2	6	5.0

[a] Data secured using a fluorogenic assay.[7b]

Table 5. fXa inhibitory activities[7a,7b] and anticoagulant potency[8] for tertiary sulfonamides **49**–**55**



Compound	R^2	fXa K_i/nM	1.5 × PT/μM
49	OH	1[a]	1.9
50	NH_2	<1	0.8
51	$NHCONH_2$	1	1.5
53	$NHSO_2Me$	<1	1.9
53	Piperidine	1	0.6
54	Morpholine	1	1.7
55	Azepine	<1	0.4

[a] Data secured using a fluorogenic assay.[7b]

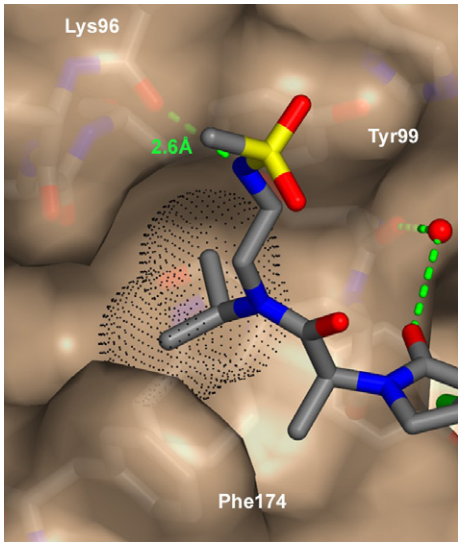


Figure 2. X-ray crystal structure of **39** bound into fXa, showing S4 interactions as described in the text.

well into plasma-based potency. Furthermore the preference for tertiary amides was supported by the poor activity seen with **32** and **38**.

Mirroring a trend seen in the cyclic alanyl amide series for tertiary sulfonamides,[3] a series of tertiary glycinamides showed enhanced affinity with good levels of anticoagulant activity (Table 5) when compared with their secondary analogues (Table 4).

Pharmacokinetic studies in the rat with selected examples in this series generally revealed poor profiles characterised by increased plasma clearance and poorer oral exposure compared with related cyclic morpholine-based analogues.[10] Increased flexibility and high polar surface areas have both been implicated as factors that impact negatively on oral bioavailability[11] and both parameters may have contributed to the lower oral exposure of the more hydrophilic acyclic alanyl amides compared with that seen with morpholine-based analogues.[12]

An X-ray structure of **39** bound into fXa confirmed binding in the manner predicted, with the molecule unambiguously fitted to the Fo–Fc electron density map.[13] In S4, the isopropyl group clearly fitted well into the aromatic box formed by the residues Tyr99, Phe174 and Trp215 and, gratifyingly, the sulfonamide NH makes a hydrogen bond with the carbonyl of Lys96 (Fig. 2).[14]

In summary, the synthesis, activities and structure–property relationships have been explored within a rationally designed series of 3-(6-chloronaphth-2-ylsulfonyl)aminopyrrolidin-2-one-based fXa inhibitors incorporating an acyclic alanyl amide P4 motif. Using structure- and property-based approaches has provided a novel series with excellent intrinsic and anticoagulant activities. These studies gave clear insight into optimal features for incorporation into further series of fXa inhibitors. Findings from these studies will be reported in separate publications.[15]

References and notes

1. (a) Quan, M. L.; Smallheer, J. M. *Curr. Opin. Drug Disc. Dev.* **2004**, *7*, 460; (b) Gould, W. R.; Leadley, R. *J. Curr. Pharm. Des.* **2003**, *9*, 2337; (c) Hirsh, J.; O'Donell, M.; Weitz, J. I. *Blood* **2005**, *105*, 453; (d) Golino, P.; Loffredo, F.; Riegler, L.; Renzullo, E.; Cocchia, R. *Curr. Opin. Invest. Drugs* **2005**, *6*, 298.
2. (a) Rajagopal, V.; Bhatt, D. L. *J. Thromb. Haem.* **2005**, *3*, 436; (b) Viles-Gonzalez, J. F.; Gaztanaga, J.; Zafar, U. M.; Fuster, V.; Badimon, J. J. *Am. J. Cardiovasc. Drugs* **2004**, *4*, 379.
3. Watson, N. S.; Brown, D.; Campbell, M.; Chan, C.; Chaudry, L.; Convery, M. A.; Fenwick, R.; Hamblin, J. N.; Haslam, C.; Kelly, H. A.; King, N. P.; Kurtis, C. L.; Leach, A. R.; Manchee, G. R.; Mason, A. M.; Mitchell, C.; Patel, C.; Patel, V. K.; Senger, S.; Shah, G. P.; Weston, H. E.; Whitworth, C.; Young, R. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3784.
4. Chan, C.; Borthwick, A. D.; Brown, D.; Campbell, M.; Chaudry, L.; Chung, C.-W.; Convery, M. A.; Hamblin, J. N.; Johnstone, L.; Kelly, H. A.; Kleanthous, S.; Kurtis, C. L.; Patikis, A.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Toomey, J. R.; Watson, N. S.; Weston, H. E.; Whitworth C.; Young, R. J.; Zhou, P.; *J. Med. Chem.*, submitted for publication.
5. 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.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J-P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 681, and selected citations in Ref 1a.
6. The majority of amine substrates employed were available from the GSK monomer collection; or prepared as described in Borthwick, A. D.; Campbell, M,; Chan, C.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Senger, S.; Smith, P. W.; Watson, N. S.; Young, R. J. WO 03043981 A1, 2003.
7. (a) FXa inhibitory activities were determined using *N*-α-Z-D-Arg-Gly-Arg-*p*-nitroanilide as chromogenic substrate; (b) Rhodamine 110, bis-CBZ-glycylglycyl-L-arginine amide as fluorogenic substrate; details are described in Ref. 4. For compounds tested in both assays, comparable levels of activity were observed.
8. Anticoagulant activities were determined in the prothrombin time (PT) assay; see Ref. 4.

9. Hydrophobicity predictions, expressed as $c\log D_{7.4}$, were calculated using Advanced Chemistry Development software v8.0. These correlated well with measured Chromatographic Hydrophobicity Index (CHI) $\log D_{7.4}$ vales; see Valko, K.; Du, C. M.; Bevan, C.; Reynolds, D. P.; Abraham, M. H. *Curr. Med. Chem.* **2001**, *8*, 1137.
10. 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. For example, compound **1** has Clp of 13 mL/min/kg, compared with values of 27.5, 42.6 and 46.3 for compounds **30**, **36** and **45**, respectively.
11. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
12. Representative examples: compound **1** rotatable bonds 3 and PSA 96 Å^2, $F = 91\%$; **33**, RB 6, PSA 113 Å^2, $F = 2\%$; **39**, RB 7, PSA 133 Å^2, $F = 3\%$.
13. The structure for **39** was refined at 1.8 Å (overall Rmerge is 0.055) in Refmac5 to a final Rfactor of 0.191 and Rfree of 0.233, using procedures described in Ref. 4. Co-ordinates are deposited in the protein data bank with code 2j4i.
14. Preliminary data for the complex structure with **45** revealed that the protonated amine was also likely to be in position to make an H-bond interaction with the backbone carbonyl of Lys96.
15. Watson, N. S. et al, manuscripts in preparation.