Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 28–33
Bioorganic & Medicinal Chemistry Letters

Structure and property based design of factor Xa inhibitors: Biaryl pyrrolidin-2-ones incorporating basic heterocyclic motifs

Robert J. Young,[a,*] Alan D. Borthwick,[a] David Brown,[a] Cynthia L. Burns-Kurtis,[b] Matthew Campbell,[a] Chuen Chan,[a] Marie Charbaut,[a] Máire A. Convery,[a] Hawa Diallo,[a] Eric Hortense,[a,b] Wendy R. Irving,[a] Henry A. Kelly,[a] N. Paul King,[a] Savvas Kleanthous,[a] Andrew M. Mason,[a] Anthony J. Pateman,[a] Angela N. Patikis,[a] Ivan L. Pinto,[a] Derek R. Pollard,[a] Stefan Senger,[a] Gita P. Shah,[a] John R. Toomey,[b] Nigel S. Watson,[a] Helen E. Weston[a] and Ping Zhou[a]

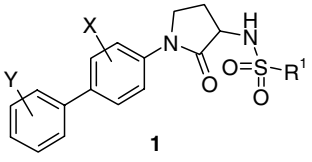
[a]*GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom*
[b]*GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA*

Received 5 September 2007; revised 6 November 2007; accepted 8 November 2007
Available online 13 November 2007

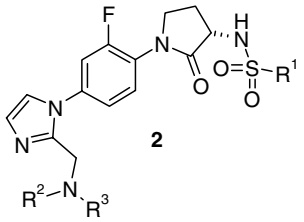
Abstract—Structure and property based drug design was exploited in the synthesis of sulfonamidopyrrolidin-2-one-based factor Xa (fXa) inhibitors, incorporating basic biaryl P4 groups, producing highly potent inhibitors with significant anticoagulant activities and encouraging oral pharmacokinetic profiles.

The quest for safe and efficacious oral anticoagulant therapies has presented numerous challenges to medicinal chemists as the search for potent and selective inhibitors of specific enzymes, such as Factor Xa (fXa), in the blood coagulation cascade has proceeded.[1] In the accompanying paper,[2] we have described the synthesis and evaluation of a series of *N*-biaryl pyrrolidinones **1**, which produced a number of highly potent fXa inhibitors. Whilst this series included molecules with encouraging pharmacokinetic profiles, coagulation was not modulated at therapeutically useful concentrations in spite of the excellent intrinsic potencies secured with many examples.

We have previously reported on the positive impact of reducing hydrophobicity towards securing better translation of intrinsic potency into anticoagulant activity.[3] The effect of a basic substituent, as part of the P4 ligand, was noted as a significant influence on both hydrophobicity and anticoagulant activity in our acyclic alanyl amide series.[4] In this paper, we further develop the theme of a biaryl P4 motif to include the addition of pendant basic functionality,[5] whilst seeking to exploit other key drivers for intrinsic potency and physical property optimisation.



1



2

The good levels of potency achieved with unsubstituted imidazole and pyrazole distal rings, as described in the preceding paper,[2] provided an excellent starting point to make molecules of appropriate topology and reduced hydrophobicity. In particular, a 2-substituted electron deficient imidazole structure offered the opportunity to place the relatively acidic proton on C5 in the correct orientation to better exploit an interaction with the indole fragment of Trp215 established by QSAR analysis. Furthermore, as the presence of a substituent on the opposite 'ortho' position was clearly beneficial for enhancing fXa activity, this was an attractive centre to incorporate a basic substituent. The initial programme of work targeted

Keywords: Factor Xa; Oral inhibitors.
* Corresponding author. Tel.: +44 1438 768372; fax: +44 1438 763620; e-mail: Rob.J.Young@gsk.com

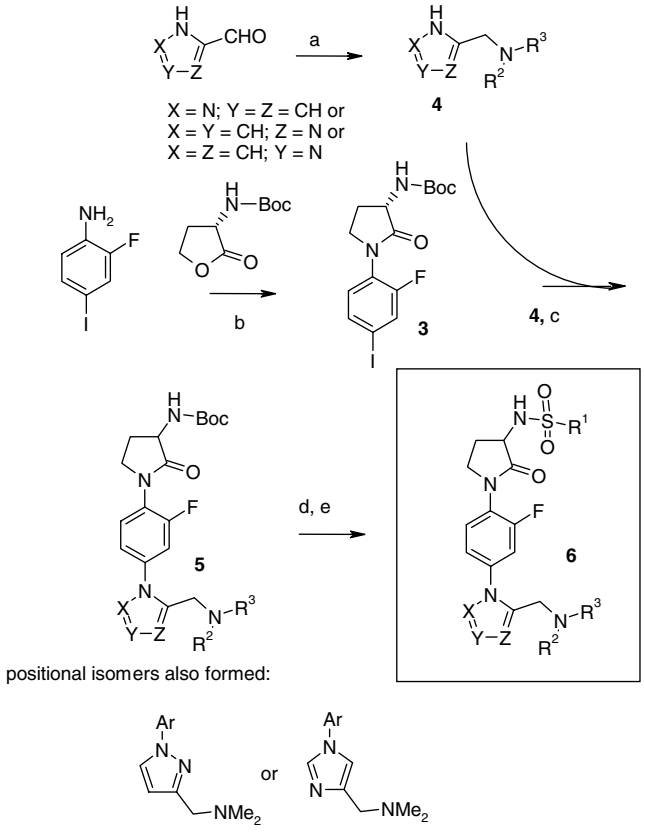
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.019

2-(dimethylamino)methyl-1*H*-imidazol-1-yl analogues **2** and was then expanded to other distal heterocycles to explore structure activity and structure property relationships around this template (Fig. 1).

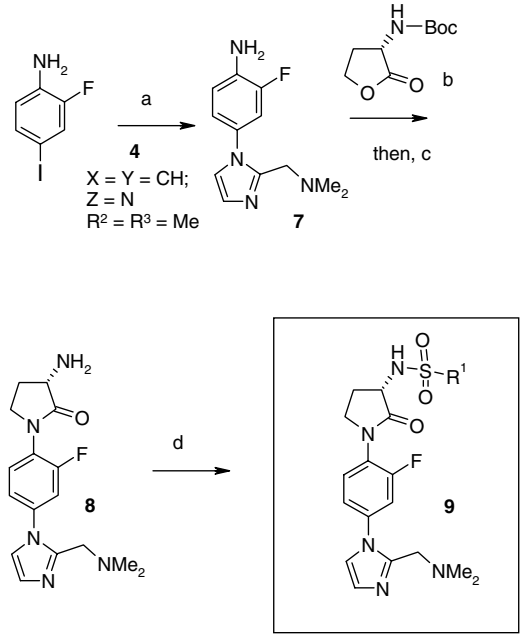
The target molecules were synthesised as outlined in Scheme 1. Thus, 2-fluoro-4-iodo-aniline and *N*-Boc homoserine lactone were converted into the key *N*-aryl pyrrolidinone **3** as described previously, using trimethyl aluminium followed by Mitsunobu methodology.[2] Reductive amination reactions of appropriate amines with commercial heterocyclic aldehydes furnished the *N*-substituted aminomethyl heterocycles **4**, which were cross-coupled to give biaryl compounds **5** (and their positional isomers) under Ullman-type conditions.[6] These intermediates, which had racemised under the forcing conditions, were deprotected by treatment with acid, furnishing amines, which were converted into the requisite sulfonamides **6** using our standard procedure.[2,3]

Alternatively, an asymmetric synthesis was achieved through the reaction of 2-(dimethylamino)methyl-imidazole with 2-fluoro-4-iodo-aniline to form the biaryl aniline **7**, which was carried through the reaction sequence via amine **8** to furnish chiral targets **9** with the preferred (3*S*)-stereochemistry (*vide infra*) (Scheme 2).

Additionally, analogues with related isoxazole carbon-linked motif **10** were synthesised using a key Stille



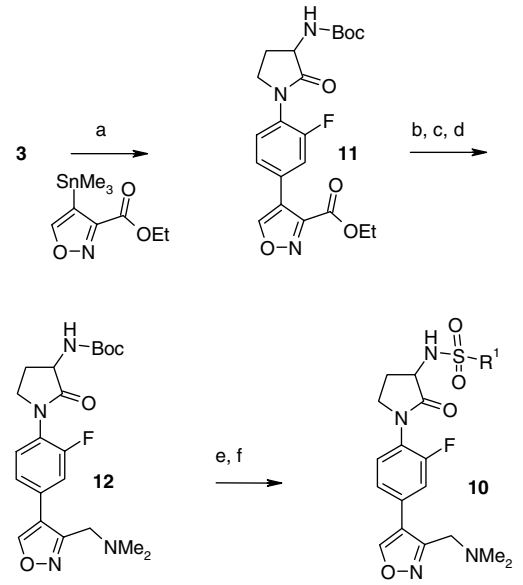
Scheme 1. Reagents and conditions: (a) R^2R^3NH, $NaHB(OAc)_3$, AcOH, THF, rt; (b) Me_3Al, DCM, 0 °C–rt; then DtBAD, Bu_3P, THF, rt; (c) CuI, 8-hydroxyquinoline, K_2CO_3, DMSO, 130 °C; (d) HCl, dioxane, rt; (e) R^1SO_2Cl, pyridine, MeCN, rt.



Scheme 2. Chiral synthesis of target compounds. Reagents and conditions: (a) CuI, 8-hydroxyquinoline, K_2CO_3, DMSO, 130 °C; (b) Me_3Al, DCM, 0 °C–rt; then DtBAD, Bu_3P, THF, rt; (c) HCl, dioxane, rt, (d) R^1SO_2Cl, pyridine, MeCN, rt.

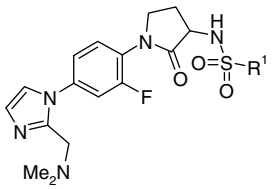
coupling as outlined in Scheme 3. Thus, the iodoaryl intermediate **3** was cross-coupled with ethoxycarbonyl trimethylstannyl isoxazole to give **11**,[7] which was then converted into the requisite amine **12** via hydrolysis, reduction, bromination and displacement. Finally, the racemic target compounds **10** were furnished by acid deprotection and sulfonylation in the usual manner.

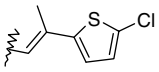
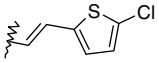
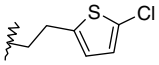
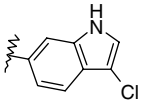
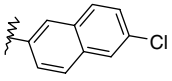
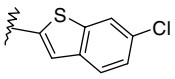
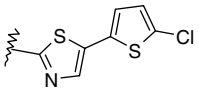
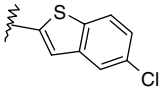
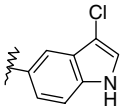
The initial set of 2-(dimethylamino)methyl-1*H*-imidazol-1-yl derivatives, illustrated with fXa[8] and anticoagulant activity (expressed at 1.5× PT)[9] in Table 1, was designed to test



Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, dioxane, Δ; (b) $LiBH_4$, THF, 0 °C; (c) NBS, PPh_3, DCM, rt; (d) Me_2NH, THF, rt; (e) HCl, MeOH, rt; (f) R^1SO_2Cl, pyridine, MeCN, rt.

Table 1. fXa inhibitory activities,[8] anticoagulant potency,[9] measured hydrophobicity[11a] and human serum albumin binding data[11b] for compounds **13** to **21**



Entry	R^1	fXa K_i (nM)	1.5× PT (μM)	CHI $LogD_{7.4}$	%HSA bound
13		0.2	3.3	2.44	nd[a]
14		0.2	4.2	2.54	92.1
15		3.1	1.6	2.32	93.4
16		<1	6.6	1.98	94.6
17		0.6	19.2	2.55	94.7
18		0.8	11.6	2.58	96.8
19		2.3	43.2	2.75	97.9
20		4.5	45.4	2.59	96.3
21		5.9	25.4	1.76	93.2

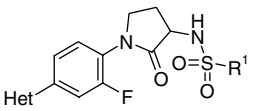
[a] nd, not determined.

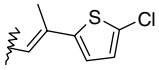
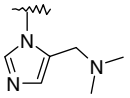
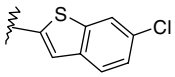
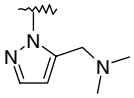
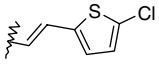
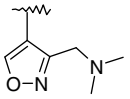
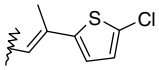
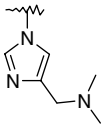
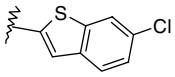
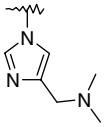
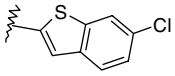
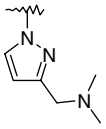
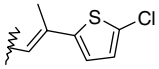
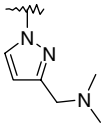
our hypotheses regarding the topology and connectivity of the distal ring, utilising a range of P1 sulfonamides.[3b]

Gratifyingly, sub-nanomolar inhibition of fXa was achieved with many P1 variations, with potencies broadly similar to those achieved with the non-basic biaryl analogues reported in our accompanying paper. However, of greater significance was the translation of this potency into much improved anticoagulant activity. The lower levels of plasma protein binding observed most likely influenced this effect, although there was no simple relationship between the conversion of intrinsic potency into anticoagulant activity and hydrophobicity linked plasma protein binding. It is also worth highlighting that as a result of incorporating the bulky biaryl group, designed to fill the fXa S4 pocket, the 5-chlorothien-2-yl-based analogues **15** and **13**, with ethyl and propenyl linkers, respectively, were potent and selective fXa inhibitors (>1000-fold and >100-fold selective over thrombin, respectively). In contrast, incorporation of these P1 groups in our 1-methyl-2-morpholin-4-yl-2-oxoethyl series resulted in a potent selective inhibitor of thrombin and potent dual inhibitor of thrombin/fXa, respectively.[10]

Encouraged by these promising profiles, further sets of compounds were generated to explore alternative topology and connectivity in the distal rings and the optimal position for the pendant (dimethylamino)methyl substituent (Table 2).

Table 2. fXa inhibitory activities,[8] anticoagulant potency[9] and measured hydrophobicity data[11a] for isomeric diazoles **22** to **28**



Entry	R^1	Distal Het	fXa K_i (nM)	1.5× PT (μM)	CHI $LogD_{7.4}$
22			<0.3	1.6	2.31
23			895	nd[a]	3.35
24			0.5	17.1	3.29
25			29.3	10.1	1.64
26			14.7	37.4	1.82
27			125	>100	2.4
28			14.7	23.5	2.2

[a] nd, not determined.

The SAR generated in Table 2 highlighted that the topology of the ring was very important and supported our hypothesis on the interaction of an acidic proton with Trp215. Most strikingly, including the 2-(dimethylamino)methyl group on the position ortho to the ring junction conferred greater activity where positional isomers were possible. The nature of the opposite 'ortho' position clearly had a bearing on activity in line with QSAR predictions. Thus, the pyrazole **23** presenting a nitrogen at this position was much less active than imidazole **18**, which in turn was possibly eclipsed in activity by the 1*H*-imidazol-5-yl isomer **22**, which would have higher C–H acidity. However, this isomer was not pursued further as it was synthetically much less tractable[12] and carried a greater P450 binding interaction risk as it lacked a 2-substituent on the imidazole ring. The isoxazole analogue **24** (Table 3) was also a potent fXa inhibitor albeit with poorer plasma-based activity.

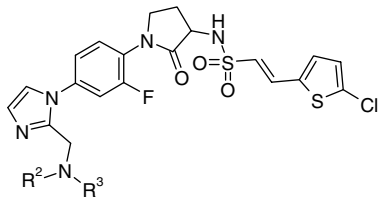
Thus, within the preferred 2-(dimethylamino)methyl-1*H*- imidazol-1-yl series, the 5-chlorothienyl-based analogues **13**, **14** and **15** (Table 1) were identified as potent fXa inhibitors with substantially improved anticoagulant activities compared with compounds described in our accompanying paper.[2] Pharmacokinetic parameters for these analogues were determined in the rat (Table 3)[13] and the profile for the (5-chlorothien-2-yl)ethenyl analogue **14** was particularly attractive as it maintained the highly encouraging profile reported for closely related non-basic analogues,[2] including good oral bioavailability.

Table 3. Pharmacokinetic parameters of compounds **13**, **14** and **15** in male Sprague–Dawley rats following intravenous and oral administration[12]

Compound	$t_{1/2}$[a] (h)	Clp[b] (ml/min/kg)	Vss[c] (L/kg)	F[d] (%)
13	0.8	18	0.7	16
14	1.0	13	0.6	52
15	0.8	39	1.1	10

[a] $t_{1/2}$, half-life of the test compound expressed in hours.
[b] Clp, plasma clearance of the test compound expressed as mL/min/kg.
[c] Vss, steady state volume of distribution of test compound expressed as L/kg.
[d] F, oral bioavailability of test compound expressed as percentage.

Table 4. fXa inhibitory activities,[8] anticoagulant potency[9] and measured hydrophobicity[11a]/human serum albumin binding[11b] and measured/calculated pK_a data[15] for compounds **14** and **29** to **34**



Entry	R^2	R^3	fXa K_i (nM)	1.5× PT (μM)	CHI $LogD_{7.4}$	Meas. pK_a[15]	Calc. pK_a ACD v8.0	%HSA bound
29	Morpholine		1.2	10.9	2.28	5.75	5.8	87.9
30	Pyrrolidine		0.4	2.1	2.21	7.95	8.86	92.0
31	Me	*i*-Pr	0.6	4.4	2.7	—	8.0	95.8
32	Me	Et	0.4	3.4	2.48	—	7.92	93.8
33	3-Fluoro pyrrolidine		16.4	14.5	2.58	—	6.53	94.0
34	Me	H	<1	3.7	1.7	—	8.55	88.6
14	Me	Me	0.2	4.2	2.69	7.26	7.84	92.1

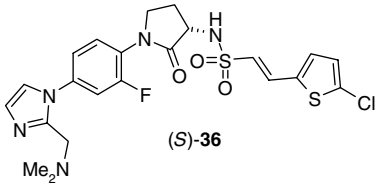
Whist exploring topology in the previous sets, the nature of the distal ring of the P4 ring was clearly having an effect on both the intrinsic hydrophobicity (that of the uncharged species, i.e. logP) and pK_a of the molecules. Thus having now identified 1*H*-imidazol-1-yl as our optimum distal ring we next evaluated a set of amine substituents that, in particular, allowed us to further explore the impact of modulating hydrophobicity through pK_a changes and its impact on structure property relationships (Table 4).[14]

Through these changes a number of compounds were secured that displayed good plasma-based activity. However, the measured hydrophobicity values were in a relatively narrow range and any trends were not clear cut. The overall profiles did not offer any significant advantage over the dimethylamino derivative **14** (Table 4).

The preferred absolute stereochemistry in the promising analogue **14** was established through a combination of approaches. Chiral preparative hplc[16] furnished the individual enantiomers **35** and **36**; both isomers were potent fXa inhibitors with **36** showing significantly greater activity (Table 5). The absolute stereochemistry of **36** was established as the (3*S*)-isomer through asymmetric synthesis (via intermediate **9**, Scheme 1) with further support from an X-ray crystal structure of (3*S*)-**36** bound into factor Xa (Fig. 1).[17] This corroborated the anticipated binding modes, whereby the imidazole 5-proton was located near to the Trp215 residue and the pendant dimethylamino group was located close to the carbonyl oxygen of Glu97, although the electron density was such that this position was not unequivocally defined. This latter observation was perhaps consistent with there being no significant potency increase that may have been attributed to such a hydrogen bonding interaction.

In summary, this work, based on structure and property based design, has led to the identification of a series of aminopyrrolidin-2-one-based fXa inhibitors with biaryl P4 motifs and basic substituents. Through optimising the nature and connectivity of the distal ring and substituent, (3*S*)-**36** in particular was identified as a highly

Table 5. fXa inhibitory activities,[8] for individual enantiomers **35** and **36** of compound **14**



Entry	Stereochemistry	fXa K_i (nM)
14	*RS*	0.2
35	(*R*)-	5
36	(*S*)-	<1

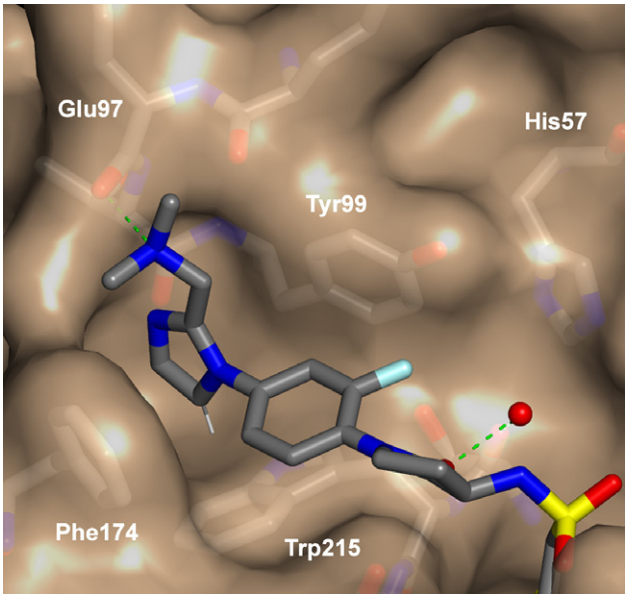


Figure 1. X-ray crystal structure of **36** bound into fXa, showing S4 interactions as described in the text, highlighting the proximity of the ortho hydrogen to an enriched area of electron density on Trp215 and the likely position of the diamino side chain close to the carbonyl of Glu97.

potent and selective[18] fXa inhibitor with significant plasma-based activity and an encouraging oral pharmacokinetic profile.[19] These findings provided the impetus for further studies on the refinement of this template which will be reported in due course.

Acknowledgments

We thank Alan Hill, Pat McDonough, Shenaz Nunhuck and Klára Valkó for constructive discussions and performing the physical measurements reported.

References and notes

1. (a) Quan, M. L.; Smallheer, J. M. *Curr. Opin. Drug Discov. 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; (e) Alexander, J. H.; Singh, K. P. *Am. J. Cardiovasc. Drugs* **2005**, *5*, 279; (f) McBride, B. F. *J. Clin. Pharmacol.* **2005**, *45*, 1004.
2. Young, R. J.; Borthwick, A. D.; Brown, D.; Burns-Kurtis, C. L.; Campbell, M.; Chan, C.; Charbaut, M.; Chung, C.-W.; Convery, M. A.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Pateman, A. J.; Patakis, A. N.; Pinto, I. L.; Pollard, D. R.; Senger, S.; Shah, G. P.; Toomey, J. R.; Watson, N. S.; Weston, H. E. *Bioorg. Med. Chem. Lett.* **2007**, preceding paper.
3. (a) 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; (b) 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.* **2007**, *50*, 1546.
4. Young, R. J.; Campbell, M.; Borthwick, A. D.; Brown, D.; Burns-Kurtis, C. L.; Chan, C.; Convery, M. A.; Crowe, M. C.; Dayal, S.; Diallo, H.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Mordaunt, J. E.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Smith, P. W.; Watson, N. S.; Weston, H. E.; Zhou, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5953.
5. Basic biaryl P4 motifs in other series of fXa inhibitors have been reported, for example (a) Pinto, D. J. P.; Galemmo, R. A.; Quan, M. L.; Orwat, M. J.; Clark, C.; Li, R.; Wells, B.; Woerner, F.; Alexander, R. S.; Rossi, K. A.; Smallwood, A.; Wong, P. C.; Luettgen, J. M.; Rendina, A. R.; Knabb, R. M.; He, K.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5584; (b) Quan, M. L.; Han, Q.; Fevig, J. M.; Lam, P. Y. S.; Bai, S.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1795; (c) Quan, M. L.; Lam, P. Y. S.; Han, Q.; Pinto, D. J. P.; He, M. Y.; Li, R.; Ellis, C. D.; Clark, C. G.; Teleha, C. A.; Sun, J.-H.; Alexander, R. S.; Bai, S.; Luettgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **2005**, *48*, 1729; (d) Jia, Z. J.; Wu, Y.; Huang, W.; Zhang, P.; Clizbe, L. A.; Goldman, E. A.; Sinha, U.; Arfsten, A. E.; Edwards, S. T.; Alphonso, M.; Hutchaleelaha, A.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1221.
6. (a) Oi, R.; Shimakawa, C.; Takenaka, S. *Chem. Lett.* **1988**, *5*, 899; (b) Zhou, J. C; Confalone, P. N.; Li, H.-Y.; Oh, L. M.; Rossano, L. T.; Clark, C. G.; Teleha, C. A. WO 008199 A2, 2002.
7. (a) Sakamoto, T.; Uchiyama, D.; Kondo, Y.; Yamanaka, H. *Chem. Pharm. Bull.* **1993**, *41*, 478; (b) Uchiyama, D.; Yabe, M.; Kameyama, H.; Sakamoto, T.; Kondo, Y.; Yamanaka, H. *Heterocycles* **1996**, *43*, 1301.
8. Factor Xa inhibitory activities were determined using Rhodamine 110, bis-(CBZ-glycylglycyl-L-arginine) amide as fluorogenic substrate; details are described in Ref. 3b.
9. Anticoagulant activities were determined in the prothrombin time (PT) assay; see Ref. 3b.
10. Young, R. J.; Brown, D.; Burns-Kurtis, C. L.; Chan, C.; Convery, M. A.; Hubbard, J. A.; Kelly, H. A.; Pateman, A. J.; Patikis, A.; Senger, S.; Shah, G. P.; Tooomey, J. R.; Watson, N. S.; Zhou, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2927.
11. Hydrophobicity measurements are reported as Chromatographic Hydrophobicity Index (CHI) $\log D_{7.4}$ values, for details see (a) Valkó, K.; Du, C. M.; Bevan, C.; Reynolds, D. P.; Abraham, M. H. *Curr. Med. Chem.* **2001**, *8*, 1137; High throughput human serum albumin binding was measured as described in (b) Valkó, K.; Numhuck, S.; Bevan, C.; Abraham, M. H.; Reynolds, D. P. *J. Pharm. Sci.* **2003**, *92*, 2236.
12. In the Ullmann coupling the ratio of the desired 1*H*-imidazol-5-yl (which led to **22)** to the ultimately less active 1*H*-imidazol-4-yl (e.g., **25**) was ca. 1:3, an outcome compounded by minimal chromatographic separation of the isomeric compounds.
13. The formulation used for both iv and po dosing was a 5:95% (v/v) mixture of DMSO and 50:50 PEG-200:sterile water. Serial blood samples were collected into heparinised containers at various time-points and blood centrifuged to yield plasma. These studies used 2 animals for each (iv/po) leg.
14. The impact of varying analogous substituents on parameters including protein binding and permeation was discussed in Ref. 5a–c, whilst pH-related issues affecting the absorption of Razaxaban were noted in a recent disclosure; Farag Badawy, S. I.; Gray, D. B.; Zhao, F.; Sun, D.; Schuster, A. E.; Hussain, M. A. *Pharm. Res.* **2006**, *23*, 989.
15. Spectroscopic pK_a measurements were performed on a Sirius GLpK_a D-PAS instrument and predicted pK_a values were calculated using Advanced Chemistry Development software v8.0.
16. The enantiomers were separated on a Chiracel AS volume, eluted with 60% ethanol in heptane, with analytical retention times of 4.9 minutes (3*R*)-**35** and 7.1 min (3*S*)-**36**; this was scaled for preparative separations, which furnished individual compounds with >98% ee.
17. The crystal was soaked with the single enantiomer **36**. Electron density for the dimethylaminomethyl portion of the molecules was very poor, the only significant density being at the position where the amino group is modelled. The structure for **36** was refined at 1.7 Å (overall R_{merge} is 0.073) in Refmac5 to a final R_{factor} of 0.189 and R_{free} of 0.215, using procedures described in Ref. 3b. Co-ordinates are deposited in the protein data bank with code 2vh0.
18. Compound **36** was at least >100-fold selective against a panel of trypsin-like serine proteases; indeed such levels of selectivity were observed across this series.
19. The pharmacokinetic profiles of the individual enantiomers **35** and **36** were closely similar to that of the racemate **14**.