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Structure and property based design of factor Xa inhibitors: Pyrrolidin-2-ones with biaryl P4 motifs

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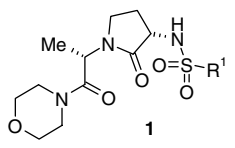
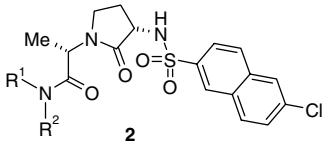
Abstract—Structure and property based drug design was exploited in the synthesis of sulfonamidopyrrolidin-2-one-based factor Xa (fXa) inhibitors, incorporating biaryl P4 groups, producing highly potent inhibitors with encouraging oral pharmacokinetic profiles and significant but sub-optimal anticoagulant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

The quest for anticoagulant therapies with improved efficacy/safety profiles has included considerable efforts to discover small molecule inhibitors of factor Xa (fXa),[1] which plays a pivotal role in the coagulation cascade. Clinical data are emerging to support this approach,[2] although the pursuit of molecules suitable for chronic oral administration has presented significant medicinal chemistry challenges, including identifying compounds with both acceptable pharmacokinetic profiles and good anticoagulant activities.

Classes of molecules that meet these challenges are beginning to emerge, including neutral or weakly basic molecules as represented by our sulfonamidopyrrolidin-2-one-based inhibitors **1**,[3] which include examples displaying attractive anticoagulant properties as well as good oral exposure. Effective utilisation of X-ray structural information led to a series of highly potent acyclic analogues **2**,[4] and as part of a programme of work to build on these latter findings, we sought to exploit structural information to increase intrinsic fXa affinity, whilst expanding our understanding of structure property relationships to promote good pharmacokinetic and anticoagulant profiles. In this communication, the design, synthesis and evaluation of a series of sulfonamidopyrrolidin-2-one-based inhibitors **3** featuring substituted biaryl P4 ligands is described.



1



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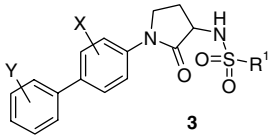

3

A FLO docking study[5] using our available structural information provided a strong impetus to expand our synthetic programme to include biaryl motifs as P4 ligands.[6] This identified the opportunity to secure good hydrophobic contacts between the distal ring and the aromatic residues in the S4 pocket.

Keywords: Factor Xa; Oral inhibitors; QSAR analysis.
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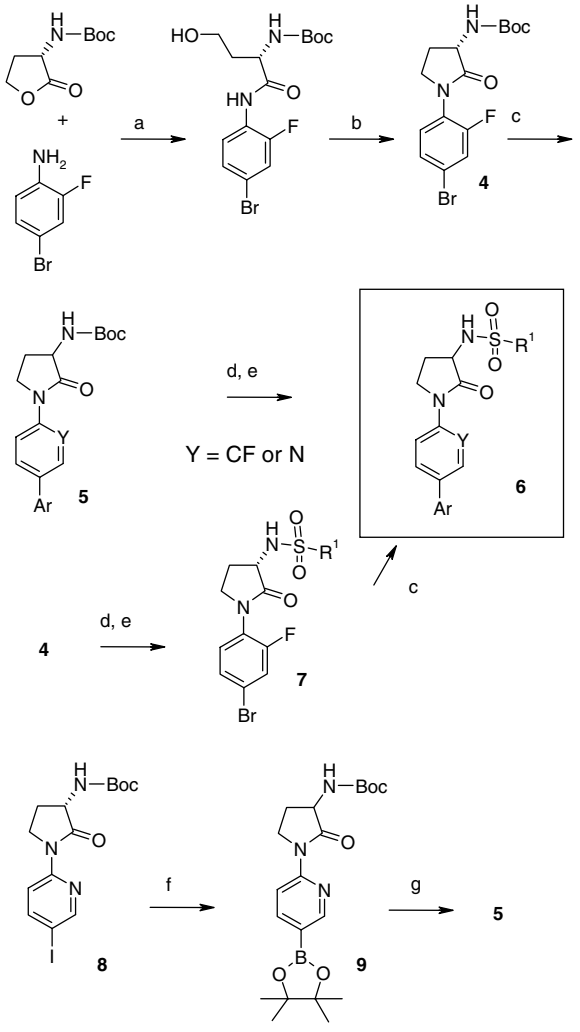
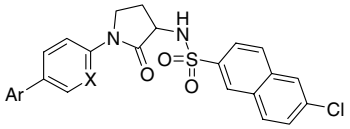
The target molecules were accessed as outlined in Scheme 1, utilising the reaction of the appropriate anilines, activated as trimethylaluminium complexes, with *N*-Boc homoserine lactone as the key step.[7] The resulting homoserine amide was converted into the required *N*-Boc aminopyrrolidin-2-one **4** using Mitsunobu conditions.[8] In this manner, the monoaryl bromide intermediate **4** was accessed from 4-bromo-2-fluoroaniline. Subsequent cross couplings of **4** with aryl boronic acids[9] or heterocycles gave the requisite biaryl intermediates **5** (Y = CF); however the C-3 stereocentre was racemised under these conditions.[10] The final compounds **6** were accessed by removal of the Boc protection, followed by sulfonylation under standard conditions. Alternatively, the target compounds **6** were generated as racemates by conversion of **4** into the corresponding sulfonamides 7 followed by cross-coupling with aryl boronic acids.[10] The pyridines **6** (Y = N) were accessed using a variation on this route starting from 2-amino-5-iodopyridine via the aryl iodide **8**, which was converted into the pinacol boronate ester **9**. This was cross coupled with the requisite aryl bromides,[9] to yield the biaryl intermediates **5** (Y = N) en route to the target compounds **6** (Y = N).

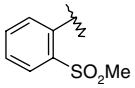
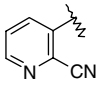
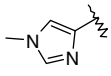
An initial set of compounds explored a variety of distal aromatic rings attached to the 4-position of a 2-fluoro aniline.[11] Gratifyingly, potent inhibition of fXa[12] was achieved with a number of these biaryl motifs, which gave insight into the optimal requirements for activity, with subtle changes producing significant shifts in activity (Table 1). Incorporation of an ortho substituent on the distal ring was generally associated with increased potency, with the 2-methylsulfonyl analogue **10** being particularly noteworthy. In addition, various heterocyclic distal rings were tolerated. Interestingly, retaining a terminal phenyl ring and incorporating a proximal 2-pyridyl ring was well tolerated (cf. **19** and **20**). However,



Scheme 1. Reagents and conditions: (a) Me_3Al, DCM, 0 °C to rt; (b) DtBAD, Bu_3P, THF, rt; (c) $ArB(OR)_2$,$Pd[P(Ph)_3]_4$, DME, aq Na_2SO_4, 80 °C or CuI, trans-1,2-diaminocyclohexane, K_3PO_4, DMSO, 110 °C; (d) HCl, dioxane, rt; (e) R^1SO_2Cl, pyridine, MeCN, rt; (f) bis(pinacolato)diboron, $PdCl_2$dppa, KOAc, 80 °C; (g) ArBr, $Pd[P(Ph)_3]_4$, DME, aq K_2CO_3, 80 °C.

Table 1. fXa inhibitory activities[12] anticoagulant potency[13] for compounds **10**–**20**



Entry	X	Ar	fXa K_i (nM)	1.5× PT (μM)
10	C–F		<0.1	51.7
11	C–F		1.1	54.5
12	C–F		0.7	64.3
13	C–F		8.9	92.9
14	C–F		2.7	>100
15	C–F		4.4	>100
16	C–F		6.3	>100
17	C–F		15.5	>100
18	C–F		74.6	>100
19	C–F		215	>100
20	N		80	>100

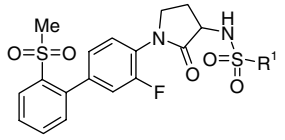
in spite of the very high fXa potency of some of these compounds, very poor anticoagulant activity was achieved, as assessed in the PT assay.[13]

To try to address this very poor conversion of excellent potency into plasma-based activity, the effect of modulating the overall hydrophobicity of the molecule through changes to the P1 ligand was investigated—an approach used with success in our previously reported 1-methyl-2-morpholin-4-yl-2-oxoethyl series **1**.[3b]

Thus, retaining the highly potent 2′-methylsulfonyl biaryl P4 group, a series of P1 sulfonamides was generated (Table 2). Mirroring SAR established for the 1-methyl-2-morpholin-4-yl-2-oxoethyl series **1**, molecules with reduced hydrophobicity were identified (e.g. **21**) that showed better anticoagulant activity, even though they had marginally lesser intrinsic potency.

The activity of **20** (Table 1), the 2-pyridyl analogue, offered a further opportunity to investigate the potential benefits of reducing hydrophobicity, based on *c*log *D* values.[14a] Thus, SAR were investigated in compounds incorporating this 2-pyridyl proximal ring, whilst retaining the preferred (5-chlorothien-2-yl)ethenyl P1 group (Table 2) and making systematic changes to the ortho substituent on the distal phenyl ring in the P4 group (Table 3). A wide range of intrinsic activities was observed. However, the expected benefit of the reduced hydrophobicity was not apparent in the measured values (CHI log *D* values[14b] for **21** and **29** were 3.07 and 3.04, respectively) contributing to the poorer translation of high intrinsic potency into plasma-based activity. This is evident in other fXa series incorporating biaryl S4 ligands and has been attributed to protein binding[3b,6b] and/or substrate-dependent inhibition mechanisms.[6b,15]

Table 2. fXa inhibitory activities[12] anticoagulant potency[13] and hydrophobicity calculations[14a] for compounds **10**, **21**–**26** and human serum albumin binding data[14c] for compounds **10**, **21**, **25**



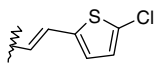
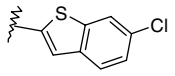
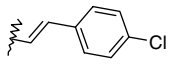
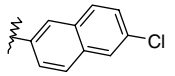
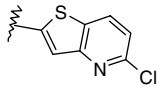
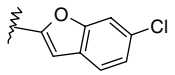
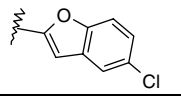
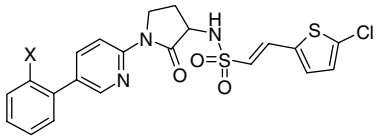
Entry	R^1	fXa K_i (nM)	1.5× PT (μM)	*c*log $D_{7.4}$	% HSA bound
21		0.2	21.3	3	97.6
22		0.1	28.7	4.5	
23		<0.4	30	3.1	
10		<0.1	51.7	3.7	98.8
24		2	99.5	3.1	
25		1.1	>100	2.5	99.6
26		6.2	>100	2.5	

Table 3. fXa inhibitory activities,[12] anticoagulant potency,[13] hydrophobicity calculations[14a] and plasma protein binding figures[14c] for compounds **27** to **37**



Entry	X	fXa K_i (nM)	1.5× PT (μM)	*c*log $D_{7.4}$	% HSA bound
27	SO_2NH_2	<0.4	17	1.1	98.0
28	SO_2NMe	<0.3	25	1.7	
29	SO_2Me	<0.3	38.9	1.3	97.6
30	NSO_2Me	(14)[a]	58.4	1.7	98.2
31	SO_2NMe_2	0.4	65.1	2.1	
32	CN	1.8	73.7	2.2	
33	$CONMe_2$	11.8	78.6	0.9	
34	CF_3	6	>100	1.8	
35	$NMeSO_2Me$	9.1	>100	3.5	
36	OiPr	180	>100	4.7	
37	tBu	(2334)[a]	—	3.7	

[a] fXa chromogenic assay data.[4]

As part of the concerted process of using both property and structure based design, a crystal structure of **21** bound into fXa (Fig. 1) revealed very clear density for the compound in initial difference electron density maps.[16] This structure confirmed the binding mode predicted by modelling studies, including the anticipated preference for the 3-*S* stereochemistry of the lactam ring substituent. The (5-chlorothien-2-yl)ethenyl group sits in the S1 pocket, the central pyrrolidinone makes a water mediated interaction with the main chain carbonyl of Ser214 and the biaryl group sits with the rings orthogonal to each other in the P4 pocket. The methylsulfonyl group sits above the S4 pocket and does not make any direct interactions with the protein. This P4 group is significantly longer than those for which structures have previously been determined[3,4] and this additional length is accommodated by rearrangement of the water structure bridging Tyr99 and Phe174.

This X-ray structure substantiated the modelled binding mode and supported the idea that locking the two rings into a preferred orthogonal conformation would confer good levels of activity. However, this could not explain the range of potencies observed with various ortho substituents of comparable bulk on the distal ring (Table 3). The proximity of the opposite ortho hydrogen of the distal ring to the indole fragment of Trp215 suggested that the partial charge on this hydrogen may be significant. An electrostatic potential map of the indole ring indicated a significantly enriched area of electron density

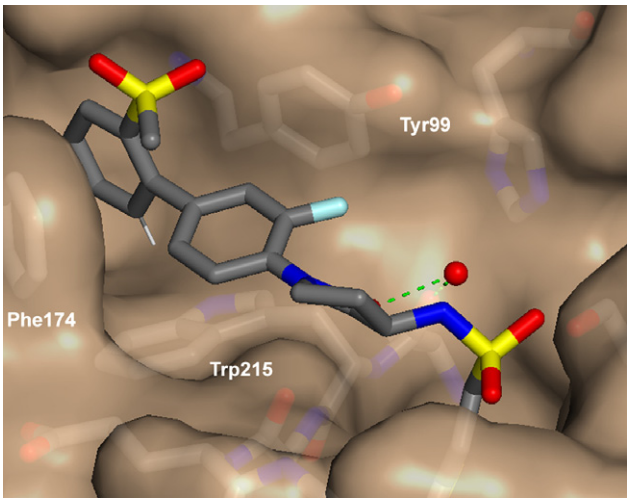


Figure 1. X-ray crystal structure of the *S*-enantiomer of **21** bound into fXa, showing S4 interactions as described in the text, highlighting the proximity of the ortho hydrogen to an enriched area of electron density on Trp215.

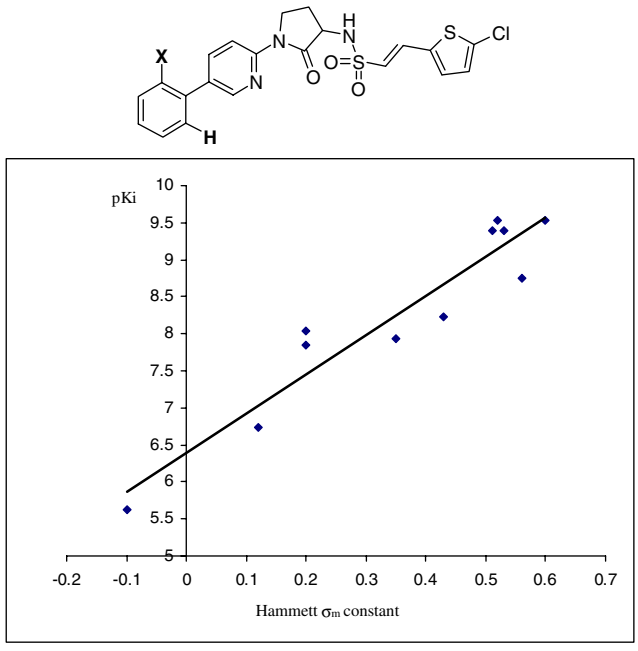


Figure 2. QSAR analysis of measured pK_i values for all 11 compounds in Table 3 versus the Hammett σ_m constant for the ortho substituents **X**

in close proximity to the hydrogen, the charge on which would be influenced by the σ-electron withdrawing effect of the substituent on the opposite ortho position. This hypothesis was substantiated by a plot of the Hammett σ_m constant[17] versus fXa pK_i, in which a clear trend was observed towards increased potency with increased electron withdrawing power (Fig. 2). The trend line for this analysis showed for this series that $pK_i = 5.28 \times \sigma_m + 6.39$, with an R^2 value of 0.897.

Pharmacokinetic studies with selected compounds were carried out in rats[18] (Table 4) and demonstrated that encouraging profiles with significant oral exposure were achievable in this series.

Table 4. Pharmacokinetic parameters of compounds **10**, **23** and **29** in male Sprague–Dawley rats following intravenous and oral administration[17]

Compound	$t_{1/2}$[a] (h)	Clp[b] (mL/min/kg)	Vss[c] (L/kg)	F[d] (%)
23	0.8	9.2	0.5	41
29	0.6	20	0.7	29
10	0.9	20	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.

In summary, the synthesis, activities and structure property relationships have been explored within a rationally designed series of sulfonamidopyrrolidin-2-one-based fXa inhibitors incorporating non-basic biaryl P4 motifs. This work has identified several highly potent examples with encouraging oral pharmacokinetic profiles and significant but sub-optimal anticoagulant activities. X-ray structural data confirmed the binding mode anticipated from modelling studies and quantitative analysis suggested a plausible explanation of the nature of the distal ring interaction. These findings, taken together with our increased in-house knowledge, gave clear indications for our ongoing programme of work; this is developed in a second generation series, described in the accompanying paper.

References and notes

1. (a) Quan, M. L.; Smallheer, J. M. *Curr. Opin. Drug Discovery Devel.* **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.
2. (a) Saiah, E.; Soares, C. *Curr. Top. Med. Chem.* **2005**, *5*, 1677; (b) Weitz, J. I.; Linkins, L.-A. *Exp. Opin. Invest. Drugs* **2007**, *16*, 271; (c) Spyropoulos, A. C. *Exp. Opin. Invest. Drugs* **2007**, *16*, 431.
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. McMartin, C. FLO/QXP Molecular Modeling software. Thistlesoft, Colebrook, CT, USA.
6. Other groups have reported independently on series of fXa inhibitors incorporating biaryl P4 ligands, for example (a) Kohrt, J. T.; Filipski, K. J.; Cody, W. L.; Bigge, C. F.; La, F.; Welch, K.; Dahring, T.; Bryant, J. W.; Leonard, D.; Bolton, G.; Narasimhan, L.; Zhang, E.; Peterson, J. T.; Haarer, S.; Sahasrabudhe, V.; Janiczek, N.; Desiraju, S.; Hena, M.; Fiakpui, C.; Saraswat, N.; Sharma, R.; Sun, S.; Maiti, S. N.; Leadley, R.; Edmunds, J. J. *Bioorg. Med. Chem.* **2006**, *14*, 4379; (b) 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; (c) Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Luettgen, J. M.; Liang, L.; Aunggst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566; (d) for further examples see Ref. 1 and cited references.
7. Bell, I. M.; Beshore, D. C.; Gallicchio, S. N.; Williams, T. M. *Tetrahedron Lett.* **2000**, *41*, 1141.
8. Typically this reaction, in our hands, furnished products in other series with ee's comparable to those reported in Ref. 7 as determined by ^{19}F NMR of Mosher amide derivatives of particular deblocked amines analogous to **7** or chiral hplc analysis of specific final compounds.
9. The majority of aryl bromides and boronic acids combinations employed were available from the GSK monomer collection; or prepared as described in Borthwick, A. D.; Chan, C.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Pinto, I. L.; Pollard, D. R.; Senger, S.; Shah, G. P.; Watson, N. S.; Young, R. J. WO 03053925 A1, 2003.
10. The stereochemistry of the 3-amino linkage we believe was most probably racemised under the strongly basic cross-coupling conditions. However, the preferred (*3S*)-enantiomer, anticipated from docking studies, was clearly evident in the structures bound within fXa when co-crystallised from the racemates. A fuller exploration of the preferred stereochemistry of similar biaryl molecules is discussed in more detail in the accompanying paper.
11. The fluorine substituent was chosen to promote non-planarity of the proximal aryl ring and pyrrolidin-2-one ring. An intermolecular C—F $\cdots$ C=O interaction has been suggested when fluorine atoms are located within the range of 3–4 Å; Olsen, J. A.; Banner, D. W.; Seiler, P.; Obst Sander, U.; D'Arcy, A.; Stihle, M.; Müller, K.; Diederich, *Angew. Chem. Int. Ed.* **2003**, *42*, 2507. The intramolecular C—F $\cdots$ C=O distances observed in the X-ray structures determined with various fXa bound inhibitors of this type in our work often falls within this distance range and could be consistent with such a fluorophilic interaction, although the occupancy of this particular ring orientation appeared to be not quite complete.[16].
12. Factor Xa inhibitory activities were largely determined using Rhodamine 110, bis-(CBZ-glycylglycyl)-L-arginine amide as fluorogenic substrate, with the others using *N*-α-*Z*-D-Arg-Gly-Arg-*p*-nitroanilide as chromogenic substrate; details are described in Ref. 3b. For compounds tested in both assays, comparable levels of activity were observed.
13. Anticoagulant activities were determined in the prothrombin time (PT) assay; see Ref. 3b.
14. (a) Hydrophobicity predictions, expressed as *c*log $D_{7.4}$, were calculated using Advanced Chemistry Development software v8.0; Hydrophobicity measurements are reported as Chromatographic Hydrophobicity Index (CHI) log $D_{7.4}$ values, for details see (b) 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 (c) Valkó, K.; Numhuck, S.; Bevan, C.; Abraham, M. H.; Reynolds, D. P. *J. Pharm. Sci.* **2003**, *92*, 2236.
15. As part of a broader investigation, a number of examples from this and other related fXa series were subjected to an in depth analysis of their protein binding and fXa kinetics, using the Biacore® surface plasmon resonance technique. These experiments indicated that biaryl-based molecules, in comparison to other structural classes we have investigated,[3,4] were generally more tightly bound to specific human serum albumin sites and had reduced fXa on-rates, consistent with their relatively poorer translation of intrinsic potency into plasma-based activity. Chung, C.W. et al. *manuscript in preparation*.
16. The only ambiguity in the electron density was regarding the orientation of the proximal ring. A small percentage of molecules in the crystal contain the ring rotated through 180°. The structure for **21** was refined at 1.95 Å (overall R_{merge} is 0.049) in Refmac5 to a final R_{factor} of 0.196 and R_{free} of 0.241, using procedures described in Ref. 3b. Co-ordinates are deposited in the protein data bank with code 2vh6.
17. Values for σ_m were used from Hansch, C.; Leo, A.; Taft, W. *Chem. Rev.* **1991**, *91*, 165.
18. 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 two animals for each (iv/po) leg.