Graphical Abstract.

Structure-based design of inhibitors of coagulation factor XIa with novel P1 moieties

Leave this area blank for abstract info.

Donald J. P. Pinto,* Joanne M. Smallheer,* James R. Corte, Erin J. D. Austin, Cailan Wang, Tianan Fang, Leon M. Smith II, Karen A. Rossi, Alan R. Rendina, Jeffrey M. Bozarth, Ge Zhang, Anzhi Wei, Vidhyashankar Ramamurthy, Steven Sheriff, Joseph E. Myers, Jr., Paul E. Morin, Joseph M. Luetzgen, Dietmar A. Seiffert, Mimi L. Quan, and Ruth R. Wexler

FXIa Ki = 6.7 nM

Structure-based design of inhibitors of coagulation factor XIa with novel P1 moieties

Donald J. P. Pinto,* Joanne M. Smallheer,* James R. Corte, Erin J. D. Austin, Cailan Wang, Tianan Fang, Leon M. Smith II, Karen A. Rossi, Alan R. Rendina, Jeffrey M. Bozarth, Ge Zhang, Anzhi Wei, Vidhyashankar Ramamurthy, Steven Sheriff, Joseph E. Myers, Jr., Paul E. Morin, Joseph M. Luetzgen, Dietmar A. Seiffert, Mimi L. Quan, and Ruth R. Wexler

^a Bristol-Myers Squibb Company, Research and Development, 311 Pennington-Rocky Hill Rd. Pennington, NJ 08534

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Factor XIa

anticoagulant

thrombosis

bioavailability

serine protease

ABSTRACT

Compound **2** was previously identified as a potent inhibitor of factor XIa lacking oral bioavailability. A structure-based approach was used to design analogs of **2** with novel P1 moieties with good selectivity profiles and oral bioavailability. Further optimization of the P1 group led to the identification of a 4-chlorophenyltetrazole P1 analog, which when combined with further modifications to the linker and P2' group provided compound **32** with FXIa $K_i = 6.7$ nM and modest oral exposure in dogs.

2009 Elsevier Ltd. All rights reserved.

Arterial thrombosis, leading to myocardial infarction and ischemic stroke, accounts for 25% of all deaths globally¹ and is the leading cause of mortality and morbidity worldwide.² The identification of safe and effective antithrombotic therapies that offer benefits to patients over the standard of care has been a long term goal of cardiovascular drug research.³ The successful development of thrombin⁴ and factor Xa inhibitors⁵ for the prevention of thromboembolic events has provided clinical validation for compounds that target specific serine proteases in the coagulation cascade. Current therapies provide some protection from adverse thrombotic events, but can be limited by increased bleeding risk at optimal therapeutic doses.

Factor XIa (FXIa) is unique among these targets in that it functions to augment the production of factor Xa via the intrinsic pathway, or amplification phase of the coagulation cascade.⁶ Elevated levels of FXI in humans have been identified as a risk factor for thromboembolic disease.⁷ In addition, in both animals⁸ and humans⁹ (Hemophilia C) with genetic deficiencies, a lack of Factor XI does not result in spontaneous bleeding, and FXI deficient mice are protected in multiple thrombosis models.¹⁰ Moreover, selective FXIa inhibitors have been shown to be efficacious in animal models of arterial thrombosis with minimal effects on bleeding time.¹¹ Based on extensive preclinical and recent clinical evidence, targeting the intrinsic pathway of coagulation by inhibition of FXIa has the potential to provide effective antithrombotic agents with reduced potential for bleeding.¹²

We have recently disclosed very potent small molecule inhibitors of FXIa, which include **1**^{11b} and **2**,¹³ that demonstrate efficacy in animal models of arterial thrombosis. A lack of trypsin selectivity, combined with poor oral exposures in animals, precluded the advancement of these molecules as oral agents. Both of these drawbacks can be attributed at least in part to the presence of the highly basic amidine and primary amine P1 groups which play a key role in binding in the S1 region of FXIa through interaction with Asp189. The present work will describe

our efforts to replace the aminomethylcyclohexyl P1 of **2** with less basic and neutral P1 groups with a goal of improving both the overall selectivity profile and oral bioavailability.

The S1 pocket of FXIa, like FXa and thrombin, has an alanine at residue 190 and is slightly larger and more lipophilic than the S1 pocket of trypsin which has a serine in that position.¹⁴ This offers the opportunity to explore larger P1 groups to achieve selectivity over trypsin. This strategy was successfully demonstrated by our labs in the development of orally bioavailable inhibitors of FXa.¹⁵ Thus increasing the size of the P1 group by direct substitution of a 2,2,2-bicyclic system for the cyclohexyl moiety of **2** while maintaining the ionic interaction with Asp189 provided compound **3** (See Table 1), with only a 5-fold decrease in affinity for FXIa and significant improvement in selectivity relative to trypsin (45x). Conversely, benzylamine analogs **4** and **5** retained good affinity for FXIa, but trypsin selectivity was not improved compared to **2**. Molecular modeling suggested that the cyclohexyl moiety positions the basic amine for optimal

Table 1 Bicyclic and Benzylic P1 Replacements

Compd	R	FXIa Ki (nM) ^a	Trypsin Ki (nM) ^a	pKa (calcd)
2		0.31	23	10.2
3		1.49	1040	9.9
4		1.16	59	9.6
5		1.03	58	9.4
6		2.15	1430	7.8
6a		2.6	2800	7.8
6b		2.5	360	7.8
7		19	380	7.8
8		58	1400	3.0
9		125	>5000	0.88

^a Ki values were obtained from purified human enzymes at 25 °C and are averaged from duplicate IC₅₀ determinations.

interaction with Asp189, while allowing the carbonyl to project into the oxyanion hole. With this in mind, 1-amino-5,6,7,8-tetrahydroisoquinoline compound **6** was designed to incorporate a less basic P1 amine (calc. pKa 7.8) to improve permeability while maintaining the orientation at the P1 carbonyl. The increased bulk of this P1 group directly contributes to the much improved selectivity profile over trypsin while maintaining single digit affinity for FXIa (Table 1). The diastereomers of **6** were separated, and although both diastereomers have similar affinity for FXIa (Ki = 2.5 nM), one of the diastereomers (**6a**) showed 7-fold better trypsin selectivity relative to the other diastereomer (**6b**). The importance of the sp³ carbon at the point of attachment to the P1 carboxamide is further illustrated by compounds **7-9** where the tetrahydroisoquinoline P1 is replaced by aminoisoquinoline, aminoindazole and aminobenzisoxazole, respectively.

Neutral P1 groups were successfully exploited in our labs and others in the discovery of thrombin¹⁶ and FXa inhibitors,¹⁷ and we also explored this option in our design of FXIa inhibitors. If appropriate contacts were made in the S1 pocket, it was anticipated that these neutral moieties could prove to be a major step towards achieving our goal of selectivity and bioavailability. A series of neutral P1 analogs of **2** were prepared. Replacement of the basic amine with the corresponding primary amide in compound **10** (Table 2) resulted in a >9,000-fold drop in FXIa affinity. We were therefore pleased to find that simply truncating **2** to the methyl cyclohexyl analog **11** resulted in only a 220-fold

Table 2 Monocyclic P1 Replacements

Compd	P1	FXIa Ki (nM) ^a	Trypsin Ki (nM) ^a
10		2900	>5000
11		68	>6000
12		310	>5000
13		390	>5000
14		200	>5000
15		280	>6000
16		65	>6000
17		530	>5000

^a Ki values were obtained from purified human enzymes at 25 °C and are averaged from duplicate IC₅₀ determinations.

drop in FXIa affinity. Replacement of the basic amine of **4** with a primary amide to provide **12** was less detrimental to FXIa affinity than in the cyclohexyl series, although still significant. In the aromatic series, removal of the amine functionality led to a 1000-fold loss of FXIa affinity in compound **13**, some of which could be recouped by introduction of the *ortho*-fluoro substituent in **14**. Methoxy analog **15** was similar in potency to **14**.

The additive effect of *ortho*-fluoro substitution was evident when comparing the 2-fold difference in FXIa affinity observed for **14** and des-fluoro analog **13** versus the >4-fold difference between **15** and the difluoro analog **16**. This was attributed in part to the fluoro substituent causing the carbonyl of the P1 amide bond to twist slightly out of plane with the aromatic ring such that one F sits in a small lipophilic pocket and thereby allowing a more favorable P1 carbonyl oxygen interaction with the oxyanion hole of FXIa (Figure 1). In the cases of thrombin and FXa, highly potent and selective inhibitors were realized by the adoption of P1 groups to interact with Tyr228.¹⁸ It is interesting to note, however, that a switch from a *p*-OMe to a *m*-Cl group in compound **17**, designed to interact with Tyr228, did not provide the desired boost in binding affinity.

The pharmacologic profiles of a subset of the above compounds were evaluated in dogs using cassette dosing¹⁹ (Table 3). Amino tetrahydroisoquinoline **6a** and aminoisoquinoline **5** had high clearance and poor oral exposure. The carboxamide **12** had moderate clearance, but was also not orally bioavailable. In contrast, the neutral analogs **11**, **14** and **16** showed moderate clearance and volume of distribution and were orally bioavailable in dogs with F% of 26, 30 and 49%, respectively. The potency against FXIa notwithstanding, compounds **11**, **14** and **16** demonstrated that replacing the basic P1 group of **2** with neutral P1 groups provided compounds with significantly reduced affinity for trypsin, greatly improved permeability, and improved the oral bioavailability (25-55%) in dogs. This was further confirmation that an optimal neutral P1 could significantly enable identification of a potent, orally bioavailable FXIa inhibitor.

To address the need for additional binding affinity in the above series, while maintaining neutral P1 moieties, we pursued a strategy of optimizing the interaction with Tyr228 by introducing a suitable P1 group that would extend deeper into the S1 pocket of FXIa. With the aid of molecular modeling and the FXIa bound x-ray structure of **2**, we determined that a chlorophenyl group could occupy the FXIa S1 pocket and interact with the Tyr228 residue. Furthermore, in order to maintain FXIa potency, it was apparent that this substituent would have to be appropriately linked to preserve the P1 amide carbonyl-oxyanion hole interaction. Modeling suggested that this could be achieved by insertion of a 2-atom linker from the *meta*-position of the chlorophenyl group, as exemplified by the urea linked compound **18** (see Table 4) designed to accommodate both prerequisites for binding to the S1 pocket and the oxyanion

Figure 1. Overlay of the S1 pocket of the crystal structure of **2** bound to FXIa with a model of **16**.

hole of the enzyme. Gratifyingly, compound **18** showed modest FXIa affinity (FXIa K_i = 55 nM), improved selectivity against trypsin (K_i > 3000 nM) and provided a 10-fold improvement in XIa affinity over **17**. The addition of the *ortho*-fluoro substituent in **19** and **24** was tolerated, while other larger lipophilic substitutions in this position were less favorable. A 9-fold improvement in FXIa potency was achieved with the polar aminomethyl compound **25** (FXIa K_i = 6 nM), which was also potent in an in vitro clotting assay²⁰ (aPTT_{1.5x} = 3.9 μ M).

Table 4 Urea-Linked P1 replacements

Compd	R ¹	R ²	FXIa K_i (nM) ^a
18	H	H	54
19	F	H	31
20	Me	H	53
21	CF ₃	H	240
22	OMe	H	75
23	H	F	57
24	F	F	23
25	CH ₂ NH ₂	H	5.8

^a K_i values were obtained from purified human enzymes at 25 °C and are averaged from duplicate IC₅₀ determinations.

Table 3 Dog PK Summary^a

Compd	PAMPA pH 7.4 (nm/s)	Cl (mL/min/kg)	Vdss (L/kg)	t _{1/2} (h)	F%
2	0.3	11.2	0.92	1.88	3.6
6a	1.5	34.8	2.8	2.3	<2
7	38	49.5	1.0	0.14	<2
12	4.6	15.4	0.9	1.5	<1
11	—	12.5	1.5	1.4	26
14	547	9.0	1.9	1.8	30
16	234	15.2	1.3	1.2	49

^aCompounds were dosed at 0.5 mg/kg po and 0.4 mg/kg, iv in a cassette format.

^bCompound **2** was dosed discrete at 1.0 mg/kg po and 0.4 mg/kg iv.

A 2.0 Å resolution x-ray crystal structure of **25** bound in the active site of FXIa was obtained (Figure 2).²¹ The binding conformation of the aminoindazole P2' group, the phenylimidazole scaffold and the benzyl P1' substituents were identical to that determined for **2**.¹³ The FXIa bound structure of **25** shows a strong H-bond interaction between the *ortho*-benzylic amine to the backbone carbonyl of Gly216 as well as to the backbone carbonyl of Gly 218, with the methylene able to form a hydrophobic interaction with the disulfide bridge formed by Cys219-Cys191. The chlorine atom sits directly over Tyr228 and forms a strong π -Cl (3.8Å) interaction. The *m*-chlorophenyl P1 moiety is orthogonal to the urea linker, with the urea carbonyl oriented directly into the oxyanion hole and capable of forming strong hydrogen bond interactions with the NH group of Asp194

(3.3 Å), Gly193 (2.9 Å) and Ser195 (3.1 Å). The CH₂NH urea nitrogen protons are within bonding distance (3.3 Å) of the backbone carbonyl of Cys191, and the P1 methylamine interacts with the carbonyl of Gly218. The x-ray structure of **25** also shows an indirect engagement of Gly216 through a network of water molecules. Importantly, the FXIa potency and the x-ray crystal structure of **25** further validates the hypothesis that engaging the P1-S1 Tyr228, an approach that was successfully applied to FXa^{18b, 19, 22} and thrombin¹⁶ inhibitors, is a viable strategy for FXIa inhibitors.

With compound **25** showing good FXIa binding affinity, we sought additional potency enhancements by engaging the structural water molecules in the S1 pocket and the side chain of Lys192, while removing the basic amine to improve the odds for oral bioavailability. With the help of modeling and literature precedent with thrombin inhibitor design,¹⁶ we quickly ascertained that an *N*-linked tetrazole would be an optimal heterocycle to elicit the network water molecule interactions and also Lys192. Thus replacing the aminomethyl group in **25** with a *N*-1-tetrazolyl group led to compound **26** with a FXIa K_i = 1.5 nM, and a 2-fold improvement in the clotting assay (aPTT IC_{1.5x} = 1.8 μM, Table 5) relative to benzylamine **25**. Amongst the *N*-heterocycles evaluated, the *N*-1-tetrazole moiety was by far the most superior in terms of FXIa affinity and potency in the aPTT assay as illustrated by a comparison of tetrazole **26** versus pyrazole **27** and triazole **28**. This was supported by the x-ray structure of **26** bound to FXIa (Figure 3).

Figure 2. 2.0 Å X-ray crystal structure of compound **25** bound in the active site of FXIa.²¹

In general, the inhibitor orientation paralleled that seen for **25**. The tetrazole *N*-2 nitrogen is near the Lys192 backbone NH (3.3 Å) and the disulfide bridge Cys219-Cys192 (3.3-4.0 Å). The acidic tetrazole CH proton appears to be in close proximity to the carbonyl of Gly216 (3.3 Å). The tetrazole *N*-4 nitrogen forms a *H*-bond to a solvent molecule that participates in a *H*-bonding network back to the backbone. It is interesting to note, however,

Table 5. Tetrazole P1 Analogs

Compd	HET	X-Y	Z	R						
					FXIa K _i ^a (nM)	aPTT ²⁰ IC _{1.5x} (μM)	Cl (mL/min/kg)	V _{ss} (L/kg)	T _{1/2} (h)	F%
26	1-tetrazolyl	-CH ₂ NH-	Cl		1.5	1.8	31.2	1.4	2.1	0
27	1-pyrazolyl	-CH ₂ NH-	Cl		450	NT	—	—	—	—
28	1-(1,2,4-triazolyl)	-CH ₂ NH-	Cl		46	34	—	—	—	—
29	1-tetrazolyl	-CH ₂ NH-	Cl		3.4	5.6	34.5	2.8	2.8	—
30	1-tetrazolyl	-CH ₂ CH ₂ -	Cl		1.9	5.2	38.3	4.4	2.6	—
31	1-tetrazolyl	-CH=CH-	Cl		2.7	5.3	9.0	1.6	3.1	~3
32	1-tetrazolyl	-CH=CH-	H		6.7	8.6	20	3.4	2.4	10.4
33	1-tetrazolyl	-CH=CH-	H		5.0 ^b	4.0	—	—	—	—
34	1-tetrazolyl	-CH=CH-	Cl		1.2	3.0	16	0.8	2.2	0

^aK_i values were obtained from purified human enzymes at 25 °C and are averaged from duplicate IC₅₀ determinations.

^bK_i value for compound **33** was measured at 37 °C.

Figure 3. 2.1 Å Resolution X-ray crystal structure of compound **26** bound in the active site of FXIa.²¹

that with such an extensive H-bonding network to the tetrazolyl group, only a 4-fold improvement in binding affinity was seen with compound **26** over compound **25**.

The extensive network of FXIa hydrogen bonding interactions and high polar surface area (PSA) of **26** contributes to its poor oral bioavailability in dogs (Table 5). With the goal of improving the oral bioavailability for these new molecular entities, we therefore focused on lowering the PSA in the next round of analogs. Specifically, we explored replacements for the indazole P2' moiety. The methyl N-phenylcarbamate P2' group was identified as a viable alternative to the 3-aminoindazole moiety.²³ Modeling of this P2' group showed a strong hydrogen bond interaction of the carbamate NH with the carbonyl of His40 and the carbamate carbonyl group interacting with Ile151 through a highly conserved structural water molecule. Compound **29** exhibited good FXIa binding affinity ($K_i = 3.4$ nM) and also demonstrated moderate clotting activity (aPTT $IC_{1.5x} = 5.6$ μ M). A superposition of the FXIa-bound conformation of **29** and the x-ray crystal structure of the corresponding aminoindazole analog **26** bound to FXIa showed a similar binding conformation for these compounds (Figure 4), and confirmed our earlier FXIa model interpretation for compound **29**.

In human liver microsomes, poor metabolic stability was common with urea linked compounds **26** - **29** (HLM $T_{1/2} < 1$ min). To address this issue, the urea was replaced with either a two carbon alkyl or alkenyl amide linker. These linkers were chosen based on modeling allowing for proper fit of the inhibitor in the FXIa active site whereby all key interactions were maintained. Accordingly, compounds **30** and **31** were prepared and demonstrated comparable FXIa affinity to compound **29**, with modest potency in the aPTT assay. An overlay of the x-ray crystal structure of alkenylamide linked analog **33** and urea linked compound **26** bound to FXIa confirmed that these two linkers have comparable conformations and maintain the desired interactions with the enzyme (Figure 5). In dogs, the propionamide compound **30** had a similar PK profile to the urea analogs **26** and **29** (see Table 5). The acrylamide compound **31** showed an improved $T_{1/2}$ of ~ 33 min in HLM assay, and demonstrated low clearance and moderate V_{dss} in a dog PK study, but had low oral bioavailability ($F\% \sim 3\%$). A similar PK profile was observed for the des-chloroimidazole compound **32**, but in this case the oral bioavailability was marginally improved ($F\% = 10\%$).

Synthesis of the aminoindazole compounds described above was carried out as shown in Scheme 1 starting from the previously reported 4-cyano-3-fluorophenylimidazole intermediate **35**.¹³ Heating **35** in the presence of hydrazine hydrate at 160 °C under microwave radiation provided the corresponding aminoindazole. Removal of the Boc protecting group by treatment with TFA, followed by coupling to the appropriate acid using EDC and HOBt or other suitable peptide coupling reagents provided the amide linked compounds in Tables 1-2 and **33-34** in Table 5. Urea linked compounds were similarly prepared from the same amine intermediate by reacting with 4-nitrophenylchloroformate and the requisite benzylamines to give the compounds **18-28**.

The synthesis of the carboxylic acids used to prepare the aminoisoquinolines and tetrahydroisoquinolines in Table 1 is shown in Scheme 2. Commercially available 4-cyano-2-methylbenzonitrile was converted to enamine derivative **36**. Heating this enamine with 2,4-dimethoxybenzylamine in DMPU at 140 °C led to compound **37**, which was hydrolyzed to the corresponding carboxylic acid derivative **38** in good yield. Cleavage of the benzyl protecting group by heating in TFA

Figure 4. Superposition of crystal structure of **26** bound to FXIa (in yellow) and the bound conformation of **29** (in orange).²¹ Crystallographic water molecules shown are conserved in both structures.

Figure 5. Superposition of bound conformation of **26** (in yellow) and the crystal structure of **33** bound to FXIa (in cyan) in the active site of FXIa.²¹

Scheme 1. Reagents and Conditions: a) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, EtOH, 160 °C, microwave; b) TFA, DCM; c) $p\text{-NO}_2\text{PhOCOCl}$, TEA, RNH_2 , 0°C-rt. 24h 5-15% from **35**; d) RCO_2H , EDC, HOBT, NMM, DMF, rt, 24h, >90% from **35**.

Scheme 2. Reagents and Conditions: a) DMF, 75 °C, 95%; b) 2,4-dimethoxybenzylamine, DMPU, 140 °C, 85%; c) 1:1 15% NaOH/MeOH; d) aq HCl, >90%; e) TFA, anisole, 100 °C, 90%; f) H_2 , PtO_2 , TFA, 25% of **40** after prep HPLC.

afforded **39** which was hydrogenated over PtO_2 in TFA²⁴ to provide a mixture of **40** and **41** that was separated by preparative HPLC to furnish **40** in 25% yield.

The tetrazole intermediates required for the synthesis of compounds in Table 5 were prepared as shown in Scheme 3. Intermediates **42a** and **42b** were obtained from commercially available 4-chloro-2-iodoaniline and 2-amino-5-chlorobenzaldehyde using known literature methods.^{18a} The resulting iodo compound **42a** was converted to cinnamate ester **43a** via a Heck cross coupling with ethyl acrylate. Alternately a Horner Emmons Wadsworth condensation of benzaldehyde **42b** and methyl diethyl phosphonoacetate provided the corresponding methyl ester **43b**. Saponification afforded the acrylic acid intermediate **44**. Compound **43b** was alternately hydrogenated

over PtO_2 in ethyl acetate to afford the corresponding propionate, which was subsequently hydrolyzed to the propionic acid derivative **45** in good yield.

Preparation of the key amine intermediate for the synthesis of compounds **29-32** in Table 5 is outlined in Scheme 4 starting from commercially available 4-aminoacetophenone. The amine was converted to the corresponding methylcarbamate by treatment with methylchloroformate, followed by bromination of the methyl ketone to give bromide intermediate **46** in good yield. Condensation of **46** with Boc-L-phenylalanine in the presence of potassium bicarbonate, followed by cyclization with ammonium acetate in xylenes at 160 °C under microwave radiation gave imidazole **47** in moderate yield. Chlorination of the imidazole with NCS in refluxing acetonitrile, followed by removal of the

Scheme 3. Reagents and conditions: a) NaN_3 , $\text{CH}(\text{OMe})_3$, AcOH, rt., 24h 90%; b) ethyl acrylate, TEA, $\text{Pd}(\text{OAc})_2$, CH_3CN 85°C, 24h, 43%; c) NaH, methyl diethylphosphonoacetate, THF, rt., 24h, 57%; d) NaOH, MeOH, water, rt., 94%; e) H_2 (60psi), PtO_2 , EtOAc, 92%

Scheme 4. Reagents and Conditions: a) methylchloroformate, pyridine, DCM, 0 °C to rt, 57%;. b) bromine, CHCl_3 , rt, 77%; c) Boc-Phe, KHCO_3 , DMF 97%; d) ammonium acetate, xylenes, 160 °C, 65%, >95% ee; e) NCS, AcCN, reflux, 91%; f) TFA, DCM, rt, 75%.

Boc group provided amine **48**, which could be substituted for the corresponding aminoindazole analog in Scheme 1 to provide the methyl N-phenylcarbamate analogs in Table 5.

In summary, a structure-based approach to design novel P1 groups in imidazole-based inhibitors of FXIa was successfully explored and resulted in the identification of orally bioavailable analogs of **2** with improved trypsin selectivity. Compound **16**, while lacking sufficient potency, provided 49% oral bioavailability in dogs. The *p*-chlorophenyltetrazole P1 analogs, when appropriately linked either through an extended urea or acrylamide, afforded potent inhibitors of FXIa with good overall selectivity. The aminoindazole P2' of **2** was successfully replaced with a methyl N-phenylcarbamate group in this latter series with retention of potent binding affinity for FXIa. In general the dog pharmacokinetic profiles were moderate for these latter compounds with modest oral exposure observed for the des-chloroimidazole example **32**. A comparison of the selectivity profiles of **2**, **32**, and **34** over a broad range of serine proteases is summarized in Table 6. While we have been quite successful in identifying potent and selective, neutral P1 groups for our phenylimidazole scaffold, further optimization is needed to achieve the ideal balance of potency, selectivity and PK properties for an oral FXIa inhibitor. This paper has primarily focused on modifications to the P1 moiety using the scaffold of potent FXIa inhibitor, **2**. Additional modifications to the P1' and P2' regions of these molecules will be described in due course.

Table 6. Enzyme Selectivity Comparison for **2**, **30** & **34**

Enzyme	Ki (nM)	2	32	34
FXIa		0.3	6.7	1.2
FVIIa		4220	>13000	>13000
FIXa		>34900	>60000	>35000
FXa		6390	1500	>1000
Thrombin		>12610	>15000	>13000
Trypsin		23	>6200	>6200
Plasma Kallikrein		5	240	41
Tissue Kallikrein		650	>30000	1200
Chymotrypsin		>20800	1300	660
Plasmin		8400	>41000	>24000
TPA		>22000	17000	19000

Acknowledgements

The authors would like to thank the BMS Department of Discovery Synthesis and BMS Biocon Research Center for the preparation of synthetic intermediates. Use of the Advanced Photon Source was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357. Use of the IMCA-CAT beamline 17-ID at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Hauptman-Woodward Medical Research Institute.

References and Notes

1. Jackson, S. P. *Nat. Med.* **2011**, *17*, 1423.
2. WHO: Geneva, Switzerland, 2011.
3. a) Fevig, J. M.; Wexler, R. R. In *Ann. Rpts. Med. Chem.*; Doherty, A. M., Ed.; Academic Press: London, 1999; Vol. 34, pp. 81. b) Scarborough, R. M.; Pandey, A.; Zhang, X. In *Ann. Rpts. Med. Chem.*; Doherty, A. M., Ed.; Academic Press: London, 2005; Vol. 40, pp. 85. c) Smallheer, J. M.; Quan, M. L. In *Ann. Rep. Med. Chem.*; Macor, John, E. M., Ed.; Academic Press, 2009; Vol. Volume 44, pp. 189. d) Pinto, D. J.; Smallheer, J. M.; Cheney, D. L.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2010**, *53*, 6243. e) Yeung, J.; Holinstat, M. *J. Blood Med.* **2012**, *3*, 33.

4. Schulman, S.; Kearon, C.; Kakkar, A. K.; Mismetti, P.; Schellong, S.; Eriksson, H.; Baanstra, D.; Schnee, J.; Goldhaber, S. Z. *New Engl. J. Med.* **2009**, *361*, 2342.
5. a) *New Engl. J. Med.* **2010**, *363*, 2499. b) Agnelli, G.; Buller, H. R.; Cohen, A.; Curto, M.; Gallus, A. S.; Johnson, M.; Masiukiewicz, U.; Pak, R.; Thompson, J.; Raskob, G. E.; Weitz, J. I. *New Engl. J. Med.* **2013**, *369*, 799.
6. Gailani, D.; Renne, T. *Arterioscler., Thromb., Vasc. Biol.* **2007**, *27*, 2507.
7. a) Yang, D. T.; Flanders, M. M.; Kim, H.; Rodgers, G. M. *Am. J. Clin. Pathol.* **2006**, *126*, 411. b) Butenas, S.; Undas, A.; Gissel, M. T.; Szuldrzynski, K.; Zmudka, K.; Mann, K. G. *Thromb. Haemost.* **2008**, *99*, 142.
8. a) Gailani, D.; Lasky, N.; Broze, G. *Blood Coag. Fibrinol.* **1997**, *8*, 134. b) Gailani, D.; Renné, T. *J. Thromb. Haemost.* **2007**, *5*, 1106.
9. Franchini, M.; Veneri, D.; Lippi, G. *Hematology* **2006**, *11*, 307.
10. Wang, X.; Cheng, Q.; Xu, L.; Feuerstein, G. Z.; Hsu, M. Y.; Smith, P. L.; Seiffert, D. A.; Schumacher, W. A.; Ogletree, M. L.; Gailani, D. *J. Thromb. Haemost.* **2005**, *3*, 695.
11. a) Wong, P. C.; Crain, E. J.; Watson, C. A.; Schumacher, W. A. *J. Thromb. Thrombolysis* **2011**, *32*, 129. b) Quan, M. L.; Wong, P. C.; Wang, C.; Woerner, F.; Smallheer, J. M.; Barbera, F. A.; Bozarth, J. M.; Brown, R. L.; Harpel, M. R.; Luetzgen, J. M.; Morin, P. E.; Peterson, T.; Ramamurthy, V.; Rendina, A. R.; Rossi, K. A.; Watson, C. A.; Wei, A.; Zhang, G.; Seiffert, D.; Wexler, R. R. *J. Med. Chem.* **2014**, *57*, 955. c) Zhang, H.; Lowenberg, E. C.; Crosby, J. R.; MacLeod, A. R.; Zhao, C.; Gao, D.; Black, C.; Revenko, A. S.; Meijers, J. C.; Stroes, E. S.; Levi, M.; Monia, B. P. *Blood* **2010**, *116*, 4684.
12. a) Schumacher, W. A.; Luetzgen, J. M.; Quan, M. L.; Seiffert, D. A. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 388. b) Isis Pharmaceuticals Reports Positive Phase 2 Data for ISIS-FXI Rx in the Prevention of Venous Thrombosis in Patients Undergoing Total Knee Replacement Surgery. 2014. <http://ir.isispharm.com/phoenix.zhtml?c=222170&p=irol-newsArticle&iid=1933482>.
13. Hangeland, J. *J. Med. Chem.* **2014**, *in press*.
14. Colman, R. W.; Hirsch, J.; Marder, V. J. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*; Philadelphia: Lippincott, 1994.
15. a) 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.; Luetzgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **2005**, *48*, 1729. b) Pinto, D. J. P.; Orwat, M. J.; Koch, S.; Rossi, K. A.; Alexander, R. S.; Smallwood, A.; Wong, P. C.; Rendina, A. R.; Luetzgen, J. M.; Knabb, R. M.; He, K.; et al. *J. Med. Chem.* **2007**, *50*, 5339.
16. Young, M. B.; Barrow, J. C.; Glass, K. L.; Lundell, G. F.; Newton, C. L.; Pellicore, J. M.; Rittle, K. E.; Selnick, H. G.; Stauffer, K. J.; Vacca, J. P.; Williams, P. D.; Bohn, D.; Clayton, F. C.; Cook, J. J.; Krueger, J. A.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; McMasters, D. R.; Miller-Stein, C.; Pietrak, B. L.; Wallace, A. A.; White, R. B.; Wong, B.; Yan, Y.; Nantermet, P. G. *J. Med. Chem.* **2004**, *47*, 2995.
17. a) Lam, P. Y. S.; Clark, C. G.; Li, R.; Pinto, D. J. P.; Orwat, M. J.; Gallemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. S.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luetzgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2003**, *46*, 4405. b) 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 Jr, R.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J. P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 681. c) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernertorfer, J.; Schlemmer, K. H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900.
18. a) Young, M. B.; Barrow, J. C.; Glass, K. L.; Lundell, G. F.; Newton, C. L.; Pellicore, J. M.; Rittle, K. E.; Selnick, H. G.; Stauffer, K. J.; Vacca, J. P.; Williams, P. D.; Bohn, D.; Clayton, F. C.; Cook, J. J.; Krueger, J. A.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; McMasters, D. R.; Miller-Stein, C.; Pietrak, B. L.; Wallace, A. A.; White, R. B.; Wong, B.; Yan, Y.; Nantermet, P. G. *J. Med.*

- Chem.* **2004**, *47*, 2995. b) Perzborn, E.; Roehrig, S.; Straub, A.; Kubitz, D.; Misselwitz, F. *Nat. Rev. Drug Discov.* **2011**, *10*, 61.
19. Pinto, D. J. P.; Orwat, M. J.; Koch, S.; Rossi, K. A.; Alexander, R. S.; Smallwood, A.; Wong, P. C.; Rendina, A. R.; Luetzgen, J. M.; Knabb, R. M.; He, K.; Xin, B.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2007**, *50*, 5339.
20. Activated partial prothrombin time (aPTT) was measured according to the method described in reference 15a. For the aPTT determination with compound 30, Dade Actin FSL was used in place of Sigma Alexin..
21. Crystallographic data for 25, 26 and 30 were collected using laboratory X-ray equipment and processed and refined similar to that described in Quan et al. (see ref. 15a). For 33 data were collected using the Advanced Photon Source beamline 17-ID with a Pilatus 6M detector at 100 K and processed with autoPROC (GlobalPhasing, Ltd.) using XDS (Kabsch, W. *XDS. Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66*, 125.; Kabsch, W. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66*, 133.) with scaling in AIMLESS (Evans, P.R., Murshudov, G.N. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2013**, *69*, 1204-1214.) and the structure was refined using BUSTER/TNT (GlobalPhasing, Ltd.), GRADE (GlobalPhasing, Ltd.) for inhibitor restraint dictionaries, and COOT for modeling as described for re-refinement of structures for Quan et al. (see ref. 15a).

Compound #	25	26	30	33
PDB ID	4X6M	4X6N	4X6O	4X6P
Resolution (Å)	2.40	2.10	2.10	1.93
Final R_{work}	0.191	0.171	0.185	0.164
Final R_{free}	0.230	0.210	0.182	0.197

22. Kohrt, J. T.; Bigge, C. F.; Bryant, J. W.; Casimiro-Garcia, A.; Chi, L.; Cody, W. L.; Dahring, T.; Dudley, D. A.; Filipski, K. J.; Haarer, S.; Heemstra, R.; Janiczek, N.; Narasimhan, L.; McClanahan, T.; Peterson, J. T.; Sahasrabudhe, V.; Schaum, R.; Van Huis, C. A.; Welch, K. M.; Zhang, E.; Leadley, R. J.; Edmunds, J. J. *Chem. Biol. Drug Design* **2007**, *70*, **100**.
23. Details of the work leading to the methyl N-phenylcarbamate P2' will be the subject of a future publication from our labs.
24. Skupinska, K. A.; McEachern, E. J.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2002**, *67*, **7890**.