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Abstract—In an effort to identify a potential back-up to apixaban (Eliquis®), we explored a series of diversified P4 moieties. Several analogs with substituted gem-dimethyl moieties replacing the terminal lactam of apixaban were identified which demonstrated potent FXa binding affinity (FXa K_i), good human plasma anticoagulant activity (PT EC_{2x}), cell permeability, and oral bioavailability.

Thromboembolic diseases remain the leading cause of death and disability in developed countries. Anticoagulants are key agents for the prophylaxis and treatment of thromboembolic disorders. For many years, the standard of care has been limited to warfarin (Coumadin®)[1] which has the limitations of a narrow therapeutic index, dietary restrictions, and the need for regular monitoring.[2] For decades a replacement for warfarin had been sought. The most prominent of these approaches included targeting key enzymes of the coagulation cascade, namely, factor Xa (FXa) and thrombin. Recent regulatory approvals in order of approval include thrombin inhibitor, dabigatran (Pradaxa®),[3] and FXa inhibitors rivaroxaban (Xarelto®),[4] apixaban (Eliquis®),[5] and edoxaban (Lixiana®)[6] (Figure 1), for venous thromboembolism (VTE) prevention and stroke prevention in atrial fibrillation have validated both mechanisms as novel replacements for warfarin. Efficacy and bleeding results in preclinical and clinical studies have suggested that FXa inhibition may have a wider therapeutic index than thrombin inhibition. The newer agents have the potential to address several warfarin limitations, which include eliminating the need for dietary restrictions, regular monitoring, eliminating the concern around multiple drug-drug interactions (DDIs) and long half-life.

A deep back-up strategy was adopted early on at Bristol-Myers Squibb to identify FXa inhibitors with superior profiles. These efforts resulted in the identification of apixaban (Eliquis®) which has proven to be safe and effective in patients with nonvalvular atrial fibrillation (AF) and venous thromboembolism (VTE).[7] Bristol-Myers Squibb's strategy for small-molecule FXa inhibitors spanned over a decade and led to multiple clinical candidates. Early clinical candidates included DPC423[8] and razaxaban,[9] which, provided clinical proof of concept for the FXa inhibitor class and also helped with clinical dose selection for apixaban.

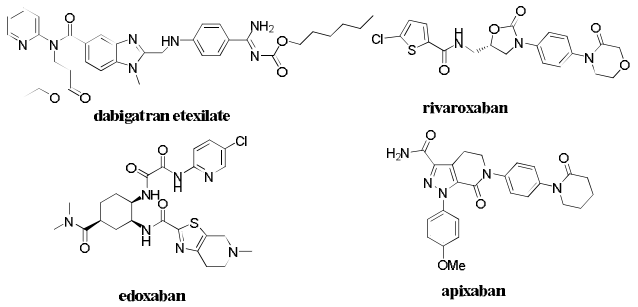
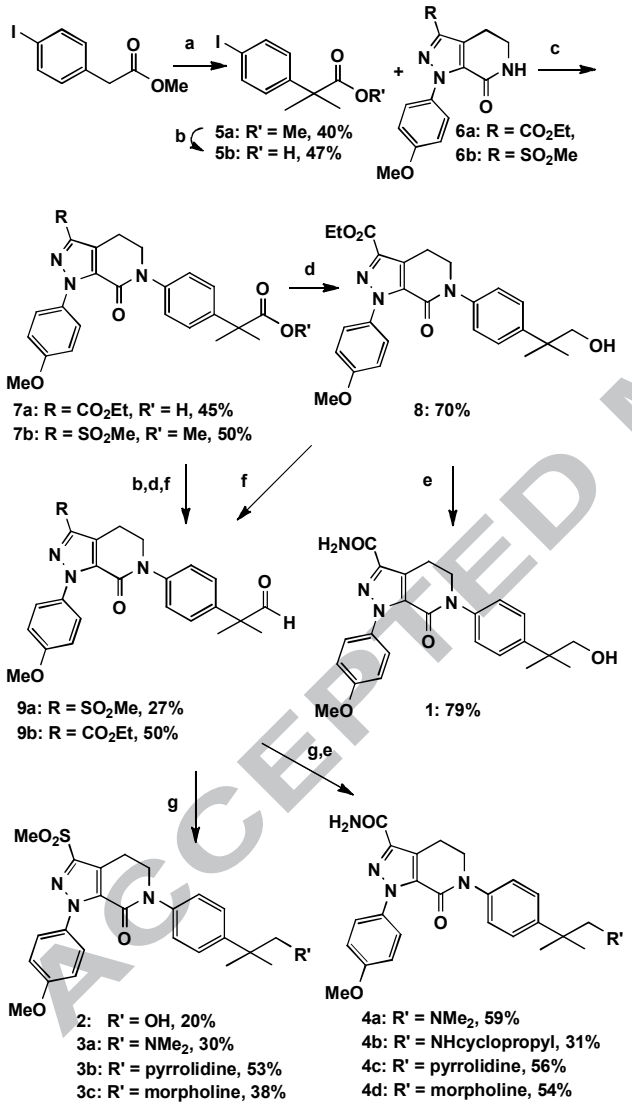


Figure 1: Recently approved oral anticoagulants

Given the promise that the mechanism held for delivering superior medicines, our back-up strategy focused on structural diversity with the goal of a

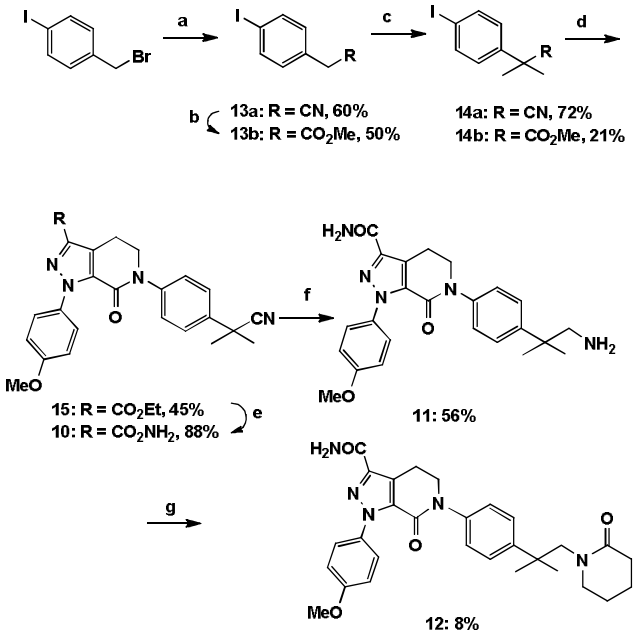
differentiated profile from previous candidates. Considerable effort was directed at finding alternative diverse P4 groups. This paper aims to highlight such an approach which culminated in the discovery of geminal methyl P4 groups which retained potent inhibition of FXa, good overall protease selectivity, and oral bioavailability. Recently, we described novel cyclopropyl P4 entities that served as an alternative group to the lactam P4 moiety of apixaban.[10] We also reasoned that while the cyclopropyl group incorporated a certain degree of rigidity in the S4 region, there was sufficient capacity to also include P4 groups with a varied degree of conformational freedom. The focus of this paper is to highlight additional strategies that would mimic the cyclopropyl P4 group.



Scheme 1. Synthesis of Compounds **1**, **2**, **3a-c**, **4a-d**. Reagents and Conditions: a) MeI, NaH, DMF; b) LiOH, water, THF; c) K_2CO_3, CuI, 1,10-phenanthroline, DMSO, 130 °C; d) BH_3, THF; e) NH_3, ethylene glycol, seal tube, 80 °C; f) PCC, NaOAc; g) R'= amine, $ZnCl_2$, $NaBH_3CN$.

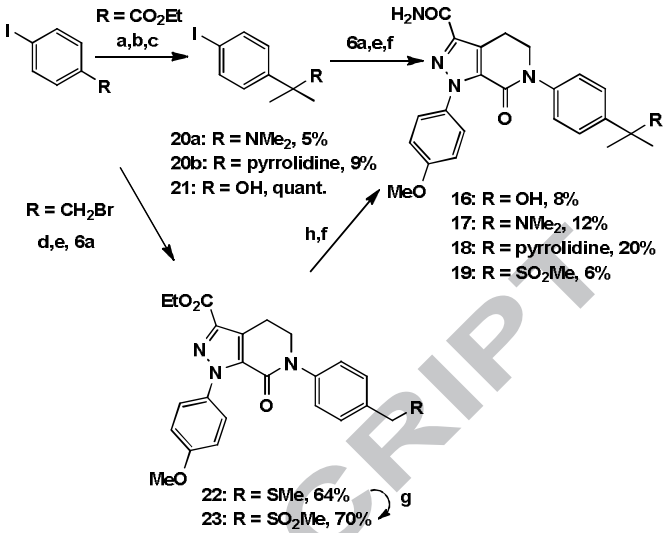
The syntheses of compounds listed in Table 1-2 are shown in Schemes 1-3. In Scheme 1, compound **5a** was prepared in 40% yield through bis-alkylation of methyl 2-(4-iodophenyl) acetate with methyl iodide using sodium hydride as the base. Ester hydrolysis (lithium hydroxide) of **5a** afforded carboxylic acid **5b** in 47% yield. Ullman coupling of **5b** with ester **6a**[10] afforded **7a**, which was reduced to alcohol **8** in a 70% yield. Amidation of **8** (ammonia saturated ethylene glycol in a sealed tube at 80 °C) provided **1** in 79% yield. Ullman coupling of compound **5a** with sulfone **6b**[10] afforded ester **7b** in 50% yield. Ester hydrolysis (lithium hydroxide) followed by reduction and subsequent oxidation with pyridinium chlorochromate and sodium acetate afforded aldehyde **9a** in 27% overall yield. Reductive amination of **9a** with sodium cyanoborohydride in the presence of zinc chloride and the appropriate amine provided compounds **3a-c** in 30-53% yields. Compound **2** was obtained as a by-product of the reductive amination of **9a** in 20% yield. Alternatively, aldehyde **9b** was obtained by the oxidation of **8** and converted to amines via reductive amination, followed by amidation (as described previously), to afford **4a-d** in good yields.

In a two- and three-step sequence starting from 1-(bromomethyl)-4-iodobenzene, intermediates **14a** and **14b** were prepared, respectively (Scheme 2). Ullman coupling of compound **14a** with pyrazole precursor **6a**[10] provided key pyrazole compound **15** in 45% yield. Amidation of **15** provided carboxamide analog **10** in 88% yield. Hydrogenation of **10** provided compound **11** in 56% yield. Further lactamization via acylation with 4-bromovaleryl chloride and cyclization with potassium *t*-butoxide afforded lactam analog **12**.



Scheme 2. Synthesis of Compounds **11** and **12**.
Reagents and Conditions: a) KCN, MeOH, H_2O b) TMSCl, MeOH;[11] c) NaH, MeI, THF; d) **6a**, K_2CO_3, CuI, 1,10-phenanthroline, DMSO, 130 °C; e) NH_3, ethylene glycol, 80 °C; f) H_2, 10% Pd/C, HCl, EtOH; g) 4-bromovaleryl chloride, TEA, THF, then KO*t*Bu, 0 °C to rt.

In Scheme 3, compounds **20a** and **20b** were readily obtained, although in low yields, by treatment of ethyl 4-iodobenzoate with dimethylamine or pyrrolidine with trimethylaluminum, followed by the addition of titanium tetrachloride and methylmagnesium bromide (2.5 eq.).[12] The utilization of excess methylmagnesium bromide (2.5 eq.) on ethyl 4-iodobenzoate in THF afforded the tertiary alcohol **21** in quantitative yield. Compound **22** was obtained by the treatment of 4-iodobenzyl bromide with sodium thiomethoxide followed by Ullman coupling with **6a**[10] in 64% yield. Oxidation of **22** with *m*CPBA afforded **23** in 70% yield. Compounds **16-19** were then arrived at by the procedures outlined in Scheme 3.



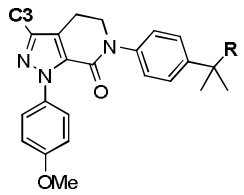
Scheme 3. Synthesis of Compounds **16**-**19**.
Reagents and Conditions: a) Me_3Al, Me_2N or pyrrolidine, THF; b) $TiCl_4$, THF; c) 2.5 eq. MeMgBr, THF;[12] d) NaSMe, DMF, quant.; e) K_2CO_3, CuI, 1,10-phenanthroline, DMSO, 130 °C; f) NH_3, ethylene glycol, 80 °C; g) *m*CPBA, DCM; h) MeI, NaH, DMF.

The strategy at BMS for follow-on compounds was specifically designed for incorporation of structural diversity with the goal of achieving significant differentiation from previous clinical candidates such as apixaban. Towards this end a concerted effort was undertaken to look for diversity at the P4 position using the general apixaban template. Table 1 lists examples of several geminally substituted methyl P4 groups. Compounds that contained a basic P4 group, such as compounds **3a-c**, **4a-d**, **11**, **17** and **18**, were significantly more potent (FXa K_i < 0.1 nM) and had good *in vitro* clotting activity (PT EC_{2x} < 4 μM). Neutral compounds, such as compounds **1**, **2**, **10**, **12**, **16**, and **19** were less active. Impressively, several analogs, **3c**, **4d**, and **10**, showed good permeability in the Caco-2 assay (>10 x 10^{-6}nm/s). Pyrazolo C3 carboxamide and methanesulfonyl groups were evaluated. Consistent with the SAR in discovery of apixaban, pyrazolo C3 carboxamide was the preferred group. It is interesting to note that tertiary amino groups, such as **17** and **18** were more potent (FXa K_i <0.2 nM) than sulfone analog **19** (FXa K_i = 0.48 nM).

While intriguing, it is not surprising to see basic P4 moieties having potent FXa binding affinity and good clotting activity, an observation seen with earlier clinical candidates, such as razaxaban.[9] Polar neutral P4 groups are also tolerated, as illustrated by compound **1**, **10** and **19**. These groups interact with the highly charged residues in the S4 pocket, in a similar manner, as razaxaban.[9]

The excellent FXa inhibition and cell permeability demonstrated by these analogs allowed us to further evaluate several of these analogs in dogs via cassette PK studies (Table 2).[8,9] In general, compounds bearing basic P4 moieties demonstrated moderate clearance (Cl > 1 L/Kg/h), large volume of distribution (V_{dss} > 5 L/Kg), short to moderate half-life (< 5 h), and with modest oral bioavailability (< 50%). Compound **3c** had good permeability (Caco-2 P_{app} 17 $x10^{-6}$ nm/sec), but low oral bioavailability in dogs (F% = 13) due to its poor intrinsic PK profile (moderate Cl = 1.28 L/Kg/h and high V_{dss} = 10.1 L/Kg). However, neutral P4 analogs such as compounds **1** and **19**, showed improved pharmacokinetics in dogs such as moderate to low Cl (0.98 and 0.32 L/Kg/h), low V_{dss} (3.02 and 0.51 L/Kg) and moderate $t_{1/2}$ (4.95 and 1.70 h), and good oral bioavailability (50 and 79%). Additionally, these compounds have excellent selectivity profiles as shown for compound **1** (Table 3).

Table 1: *In Vitro* Profiles



Compound	C3	R	FXa K_i[a] (nM)	PT[b] EC_{2x} (μM)	Caco-2 P_{app}[c] (10^{-6}nm/s)
apixaban	-	-	0.08	3.8	9
1	$CONH_2$	CH_2OH	0.66	3.8	5.8
2	SO_2Me	CH_2OH	1.01	3.9	3.6
3a	SO_2Me	CH_2NMe_2	0.11	0.67	4.1
3b	SO_2Me	CH_2-*N*-pyrrolidinyl	0.24	0.92	4.3
3c	SO_2Me	CH_2-*N*-morpholinyl	0.32	3.7	17
4a	$CONH_2$	CH_2NMe_2	0.07	0.45	5.9
4b	$CONH_2$	CH_2-*N*-cyclopropyl	0.05	0.68	2.8
4c	$CONH_2$	CH_2-*N*-pyrrolidinyl	0.05	0.73	5.6
4d	$CONH_2$	CH_2-*N*-morpholinyl	0.06	3.8	17
10	$CONH_2$	CN	1.22	3.4	18
11	$CONH_2$	CH_2NH_2	0.15	0.69	0.7
12	$CONH_2$	CH_2-*N*-piperidine-2-one	1.5	7.4	8.3
16	$CONH_2$	OH	9.27	na[d]	na[d]
17	$CONH_2$	NMe_2	0.20	0.96	1.1
18	$CONH_2$	*N*-pyrrolidinyl	0.07	0.81	1.7
19	$CONH_2$	SO_2Me	0.48	3.90	1.0

[a]K_i values are obtained from purified human FXa enzyme and are averaged from two experiments (n=2). [b]PT values are measured according to Refs 8 and 9. [c]Caco-2 permeability (A-to-B). [d] na = not available.

Table 2: *In Vivo* Dog Pharmacokinetic Profiles[a]

Compound	Cl (L/Kg/h)	V_{dss} (L/Kg)	$t_{1/2}$ (PO)(h)	F%
apixaban	0.02	0.20	5.80	58
1	0.98	3.02	4.95	50
3a	3.86	7.3	1.86	34
3b	2.20	5.50	1.40	10
3c	1.28	10.1	4.18	13
4a	5.04	6.57	1.04	41
4d	1.76	5.01	2.80	10
11	3.36	11.8	3.33	44
18	7.60	12.0	na[b]	na[b]
19	0.32	0.51	1.70	79

[a]Dog phamacokinetics: compounds were dosed (iv/PO) as TFA salts in different experiments in a cassette dosing N-in-one format at 0.5 mg/kg iv and 0.2 mg/kg PO (n = 2) according to refs 8 and 9. [b]na = not available.

Table 3: Enzyme selectivity data for Compound **1**

Human enzyme	K_i (nM)
FXa	0.66
Trypsin	>15,000
Thrombin	2,578
Plasma Kallikrein	>11,000
Chymotrypsin	>40,000
Activated Protein C	>37,600
Factor IXa	>15,000
Factor VIIa	>15,000
Factor XIa	>15,000
Urokinase	>13,000
tPA	>43,000

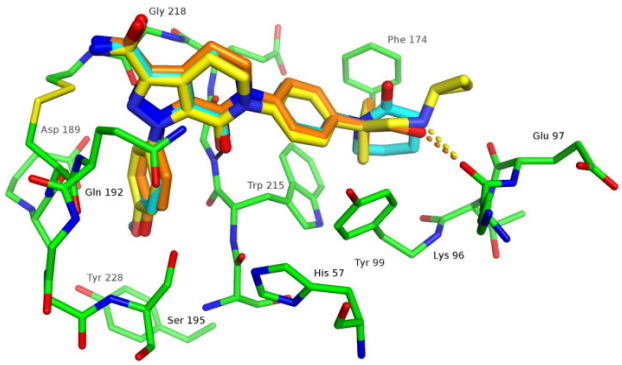


Figure 2: Overlay models of hydroxymethylene **1** (orange) cyclopropylamino **4b** (yellow), and apixaban (blue).

Figure 2 shows a CADD overlay of compound **1** (FXa K_i = 0.66 nM), **4b** (FXa K_i = 0.05 nM), and apixaban (FXa K_i = 0.08 nM) in human FXa. In general, the bound orientation for these analogs is similar to that seen for apixaban. In the P4 region the hydroxymethylene moiety of **1** adopts a perpendicular configuration in the S4 pocket and does not have the same stacking arrangement that the phenyl lactam P4 group of apixaban has with the S4 residues: Phe174, Tyr99, and Trp215. This configuration, in part, may explain the five-fold decrease in binding affinity. The basic P4 substituents with ring appendages such as the cyclopropyl group in **4b** appear to elicit lipophilic interactions with the backbone Glu97 residue. The combination of a charge interaction coupled with the lipophilic interactions of the substituents afford a greater degree of FXa binding affinity. The hydroxyl group of analog **1** interacts weakly with the carbonyl of Lys96 and is not as strong as that observed with basic P4 groups. In part, this could also explain some loss in binding affinity of the less charged P4 group relative to the charged basic P4 groups.

In summary, structural diversity at the P4 position was successfully incorporated using the pyrazole scaffold of apixaban. Novel tertiary substituted P4 moieties were identified which demonstrated potent inhibition of FXa and with good translation into the clotting activity. In general, basic P4 moieties provided enhanced FXa binding affinities compared to neutral P4 analogs. Good oral bioavailability was observed for several of these analogs, however, their overall pharmacokinetic properties were moderate to poor (moderate Cl > 1 L/Kg/h and high V_{dss} > 5 L/Kg). The neutral hydroxy methyl analog **1** and methylsulfone analog **19** provided the best balance of FXa affinity, clotting activity, and good dog pharmacokinetics in terms of low clearance, low volume of distribution, moderate half life and oral bioavailability. However, these analogs did not positively differentiate from apixaban and were not considered for further evaluation.

Acknowledgements

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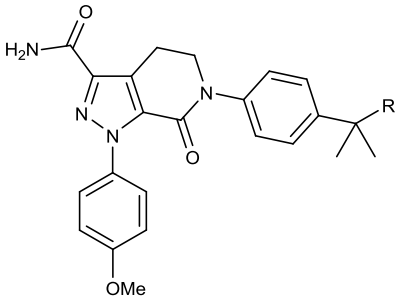

1, R = CH_2OH, fXa = 0.66 nM
19, R = SO_2Me, fXa = 0.48 nM