Discovery of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (Apixaban, BMS-562247), a Highly Potent, Selective, Efficacious, and Orally Bioavailable Inhibitor of Blood Coagulation Factor Xa

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Efforts to identify a suitable follow-on compound to razaxaban (compound **4**) focused on modification of the carboxamido linker to eliminate potential in vivo hydrolysis to a primary aniline. Cyclization of the carboxamido linker to the novel bicyclic tetrahydropyrazolopyridinone scaffold retained the potent fXa binding activity. Exceptional potency of the series prompted an investigation of the neutral P_1 moieties that resulted in the identification of the *p*-methoxyphenyl P_1, which retained factor Xa binding affinity and good oral bioavailability. Further optimization of the C-3 pyrazole position and replacement of the terminal P_4 ring with a neutral heterocycle culminated in the discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (apixaban, compound **40**). Compound **40** exhibits a high degree of fXa potency, selectivity, and efficacy and has an improved pharmacokinetic profile relative to **4**.

Introduction

Thrombotic diseases remain the leading cause of death in developed countries despite the availability of anticoagulants such as warfarin,[1a−c] heparin and low molecular weight heparins,[2,3] and antiplatelet agents such as aspirin and clopidogrel. The oral anticoagulant warfarin inhibits the post-translational maturation of coagulation factors VII, IX, and X and prothrombin and has proven effective in both venous and arterial thrombosis. However, warfarin's usage is limited because of its narrow therapeutic index, slow onset of therapeutic effect, numerous dietary and drug interactions, and a need for monitoring and dose adjustment.[4a,b] This not withstanding, warfarin remains the standard orally administered anticoagulant available in the United States. Patients on warfarin therapy require regular monitoring in part because of its narrow therapeutic index and interactions with food and other drugs. Injectable agents that are also widely used include low molecular weight heparins and the synthetic pentasaccharide fondaparinux.[5] Thus, discovering and developing safe and efficacious oral anticoagulants for the prevention and treatment of a wider range of thrombotic diseases has become increasingly important.

A key strategy for the discovery and development of new anticoagulants has been the targeting of specific enzymes within the blood coagulation cascade. One approach is to inhibit thrombin generation by targeting the inhibition of coagulation factor Xa (fXa).[5,6a−h] Factor Xa, a trypsin-like serine protease, is crucial to the conversion of prothrombin to thrombin, the final enzyme in the coagulation cascade that is responsible for fibrin clot formation. Preclinical animal models have suggested that inhibiting fXa has the potential for providing excellent antithrombotic efficacy with minimal bleeding risk when compared to direct thrombin inhibitors.[6a−g] Recent disclosures from clinical studies with direct fXa inhibitors such as compound

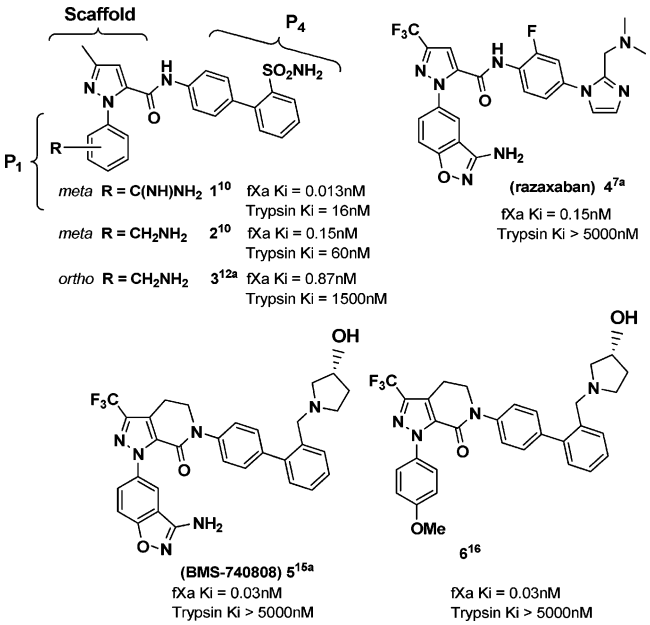

Figure 1. Schematic of important pyrazole fXa compounds.

4,[7a−c] rivaroxaban (BAY 59-7939),[8a,b] 1*H*-indole-5-carboxylic acid {(*R*)-2-[4-(4-methylpiperazin-1-yl)-piperidin-1-yl]-2-oxo-1-phenylethyl}amide (LY-517717)[9] and the indirect parenteral fXa inhibitor fondaparinux[5] have confirmed the preclinical findings.[10]

The discovery of the pyrazole scaffold, illustrated by SN429 (compound **1**, Figure 1, fXa K_i = 13 pM, trypsin K_i = 16 nM),[11] was a significant milestone in our search for molecules targeting coagulation fXa and proved to be crucial in the evolution of orally bioavailable fXa inhibitors such as DPC423 (compound **2**, fXa K_i = 0.15 nM, trypsin K_i = 60 nM),[11] DPC602 (compound **3**, fXa K_i = 0.87 nM, trypsin K_i = 1500 nM),[12a] and razaxaban (compound **4**, fXa K_i = 0.15 nM, trypsin K_i >

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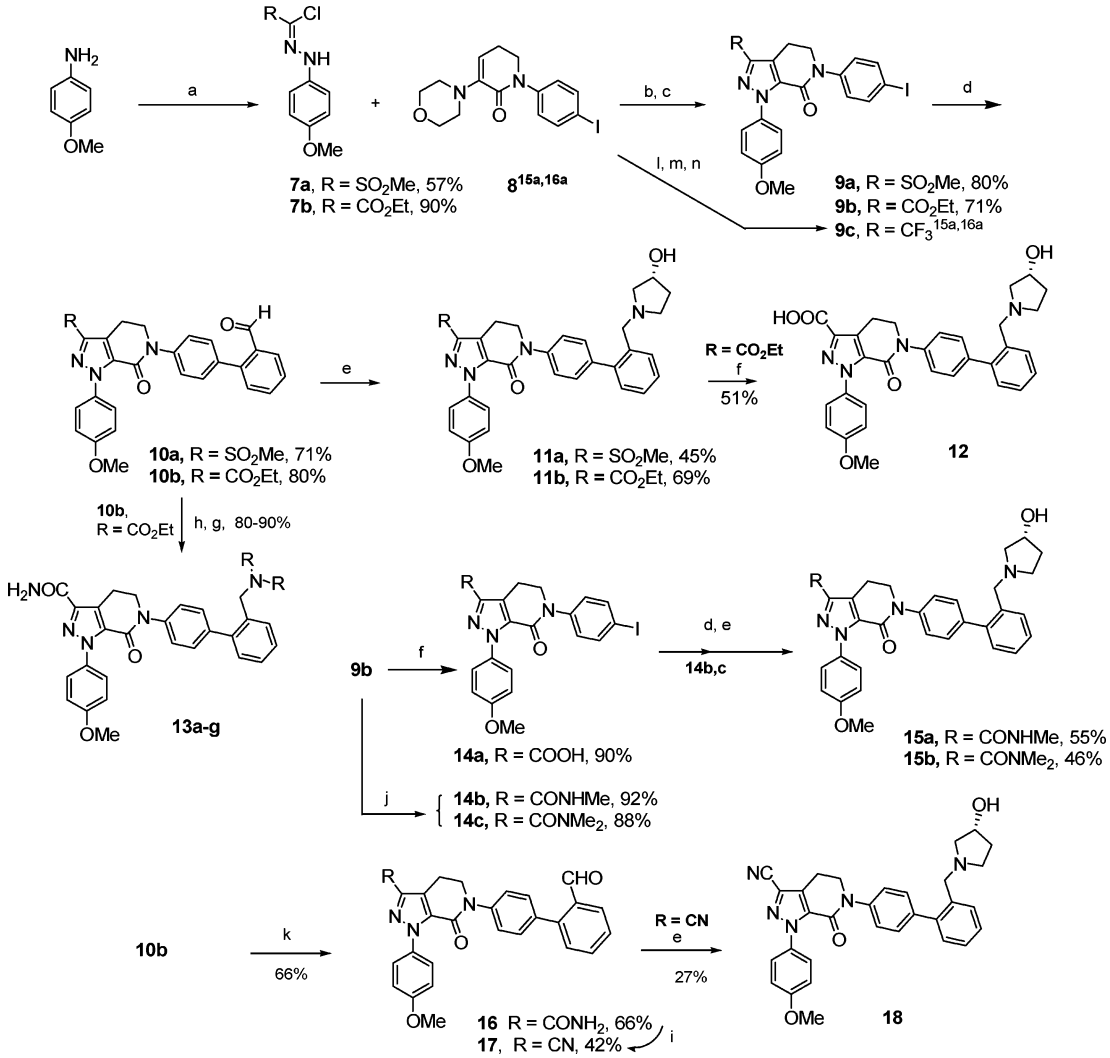
Scheme 1. Syntheses of C-3-carboxypyrazolo-pyridinone Analogues[a]



[a] (a) $NaNO_2$, HCl, 0 °C, NaOAc, EtOH, ethyl 2-chloroacetoacetate; (b) Et_3N/toluene, reflux; (c) 3 N HCl or TFA, DCM; (d) 2-formylphenylboronic acid, $(Ph_3P)_4Pd$, toluene/EtOH or DME/water (4:1), Na_2CO_3 (2 N), reflux; (e) 3-(*R*)-OH-pyrrolidine (2 equiv), $NaCNBH_3$, $ZnCl_2$ (0.5 N, in THF), MeOH; (f) LiOH or NaOH (1 N), MeOH/water; (g) NH_4OH, EtOH, 80 °C; (h) amine, $NaCNBH_3$, $ZnCl_2$ (0.5 N, in THF), MeOH; (i) oxalyl chloride, DMF; (j) $MeNH_2$ or $NHMe_2$, trimethylaluminum (1 N), DCM, 0 °C to room temp; (k) ammonia/MeOH, 50 °C; (l) DMAP, TFAA; (m) ether, 20% aq. HCl; (n) p-methoxyphenylhydrazine, MeOH reflux.

5000 nM).[7a] Compounds **2** and **4** were advanced to clinical trials. Subsequently, compound **4** was further advanced to a phase II trial for the prevention of venous thromboembolism (VTE) after knee replacement surgery and was shown to be highly efficacious when compared to enoxaparin.[7c]

Consistent with our strategy of developing and advancing key follow-on candidates, our focus was directed toward the identification of novel entities that would be significantly differentiated from previous candidates in terms of improving on potential liabilities of earlier compounds. A common structural feature that is present with compound **4** and its predecessor candidates was the presence of the 5-carboxamido linker that connects the pyrazole scaffold to the P_4 moiety. In the advancement of potential candidates for preclinical evaluations, it was necessary to determine the susceptibility of the amide linker to metabolic cleavage because this could potentially liberate a aniline fragment. Fortunately, for compound **4** and its predecessor clinical compound **2** the amide linker was stable to metabolic hydrolysis; however, this was not the case with our preclinical compound **3**, which liberated the biarylamino confirmed in follow-up assays for mutagenicity.[14] Therefore, as part of our optimization strategy, we sought to modify the carboxamido portion of the molecule to obviate the need for mutagenicity studies on potential aniline degradants. Toward this end, we recently disclosed several series of bicyclic pyrazole scaffolds[15a−c,16a] in which the carboxamido linker was cyclized into the pyrazole ring, some of which showed similar or better fXa potency compared with the previously disclosed monocyclic pyrazole analogues.[7a,b,11] The optimization strategy with the bicyclic pyrazole scaffold led to the identification of BMS-740808 (compound **5**, fXa K_i = 0.03 nM, trypsin K_i > 5000 nM, Figure 1),[15a] which was advanced to preclinical safety evaluation. Importantly, the discovery of the potent bicyclic scaffold set the stage for exploratory work employing additional P_1 moieties,[7c,16] many of which demonstrated subnanomolar fXa binding affinities and moderate to high clearance (Cl) and volume of distribution (V_{dss}) in dogs. However, the lack of adequate differentiation from compound **4** in terms of improvement in the overall pharmacokinetic profile made them less attractive for further development. In this paper, we report an

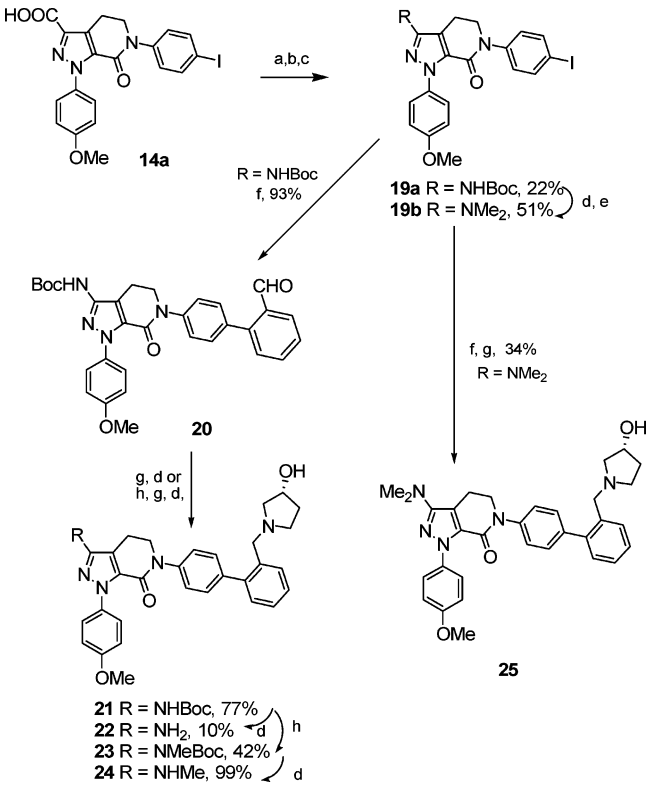
fXa inhibitor (currently in phase III trials) with a superior pharmacokinetic profile (low clearance and volume of distribution) compared to compound **4**.

Chemistry

The synthesis of the C-3 trifluoromethylpyrazole analogue **6** was accomplished via the cyclization methodology previously described.[15,16a] Scheme 1 illustrates the general synthetic methodology utilized to prepare diversified pyrazole C-3 analogues. Commercially available 4-methoxyaniline was diazotized ($NaNO_2$, concentrated HCl, 0 °C) and condensed in situ with either 1-chloro-1-(methylsulfonyl)propan-2-one or ethyl 2-chloroacetoacetate in the presence of sodium acetate[17] to provide the requisite *p*-methoxyphenylchlorohydrazones **7a** in 57% yield and **7b** in 90% yield. Treatment of the chlorohydrazones **7a** and **7b** with compound **8**[15] using excess triethylamine afforded the requisite [3 + 2] cycloadducts which, when treated with TFA in dichloromethane, led to compounds **9a** (80% yield) and **9b** (71% yield) respectively. Suzuki coupling of **9a**,**b** with 2-formylbenzeneboronic acid as illustrated for compound **5**[15] afforded the biaryl *o*-carboxaldehyde intermediates **10a** in 71% yield and **10b** in 80% yield, respectively. Subsequent reductive amination with 3-(*R*)-hydroxypyrrolidine[15,16a] provided the bicyclic pyrazole compounds **11a** (45% yield) and **11b** (69% yield). Hydrolysis (LiOH in THF and water) of the ester group in **11b** gave the desired C-3 carboxylic acid compound **12** in 51% yield. Compounds **13a**−**h** were prepared in a two-step sequence by the reductive amination of **10b** followed by carboxamide formation as described above in yields that ranged between 80% and 90%. Alternatively, treatment of compound **11b** with ammonium hydroxide in ethanol at 80 °C for 4 days provided the carboxamidopyrazole analogue **13f** in 45% yield. Hydrolysis (NaOH (1 N) in THF/water) of **9b** gave carboxylic acid intermediate **14a** (90% yield). Treatment of the pyrazole ester **9b** under the Weinreb amide conditions (methylamine or dimethylamine, trimethylaluminum (1 N) in DCM at 0 °C to room temperature)[18] provided **14b** (92% yield) and **14c** (88% yield). The compounds were subsequently converted to **15a**,**b** in 55% and 46% yield, respectively, following the Suzuki and reductive amination procedures. To prepare the cyanopyrazole compound **18**, compound **10b** was first converted to the carboxamidobiarylcarboxaldehyde **16** in 66% yield by treatment with ammonia in methanol at 80 °C. Dehydration (oxalyl chloride in DMF) to **17** (42% yield) followed by reductive amination gave the desired cyano compound **18** (27% yield).

The aminopyrazole compounds **20**−**25** were accessed according to the methodologies outlined in Scheme 2. Curtius rearrangement[19] of the pyrazolecarboxylic intermediate **14a** provided the Boc protected aminopyrazole intermediate **19a** in 22% yield. Biarylcarboxaldehyde formation (**20**, 93% yield) followed by reductive amination with 3-(*R*)-hydroxypyrrolidine afforded compound **21** in 77% yield. Treatment of compound **21** with TFA provided compound **22** in 10% yield. Alternatively, compound **21** was alkylated with sodium hydride and iodomethane in anhydrous DMF to afford **23** in 42% yield. Treatment of **23** with TFA in dichloromethane afforded compound **24** in 99% yield. To prepare compound **25**, pyrazole derivative **19a** was deprotected with TFA and reductively aminated with formaldehyde (37%) and sodium cyanoborohydride in the presence of zinc chloride (0.5 M in THF) to afford the dimethylaminopyrazole compound **19b** in 51% yield. Biarylcarboxaldehyde formation followed by reductive amination with 3-(*R*)-hydroxypyrrolidine afforded compound **25** in 34% yield.

Scheme 2. Syntheses of 3-Aminopyrazole Analogues[a]



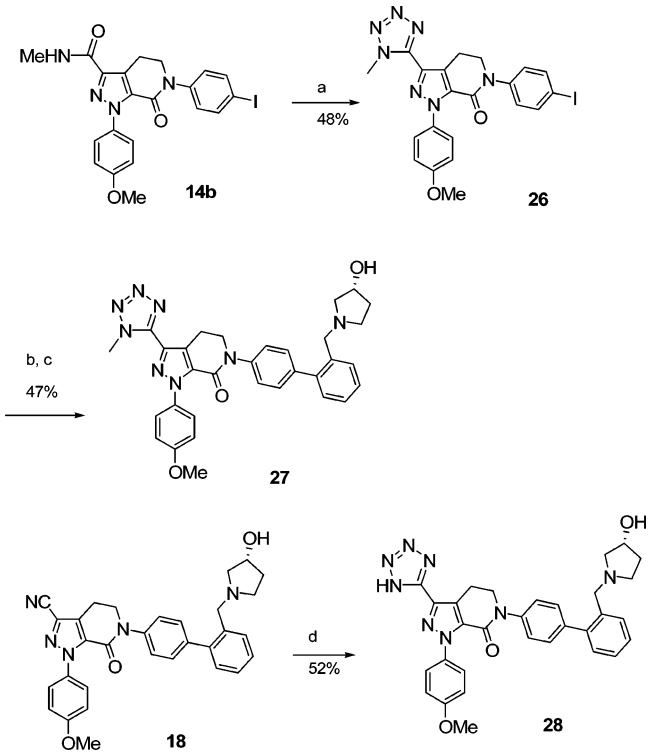
[a] (a) Oxalylchloride, DCM, catalyst DMF; (b) NaN_3, water, acetone 0 °C; (c) toluene, 80 °C, ^{t}BuOH; (d) TFA, DCM; (e) formaldehyde (37%, excess), $ZnCl_2$ (0.5 M/THF), $NaBH_3CN$, MeOH; (f) 2-formylphenylboronic acid, $(Ph_3P)_4Pd$, Na_2CO_3 (2 N), 4:1 toluene/EtOH, reflux; (g) 3-(*R*)-OH-pyrrolidine, $NaCNBH_3$, $ZnCl_2$ (0.5 N, in THF), MeOH; (h) NaH, DMF, MeI, room temp.

anhydride generated the iminotriflate, which was directly treated with excess sodium azide to give the tetrazole derivative **26** in 48% yield. Suzuki coupling with 2-formylboronic acid and reductive amination with 3-(*R*)-hydroxypyrrolidine led to **27** in 47% yield. The tetrazole compound **28** was prepared in 52% yield by heating compound **18** with sodium azide in DMF.

Heteroarylalkyl compounds **33a**−**e** were synthesized according to procedures outlined in Scheme 4. Borane reduction of carboxylic acid[20] intermediate **14a** afforded the alcohol intermediate **29** in 89% yield, which was subsequently converted to the bromomethyl intermediate **30** by treatment with phosphorus tribromide (PBr_3) in dichloromethane in 94% yield. Displacement of the crude bromide **30** with 1,2,3-triazole, 1,2,4-triazole, or 1*H*-tetrazole afforded mixtures of regioisomeric triazolemethyl or tetrazolylmethyl compounds **31a**−**e**, which were subsequently converted to biarylcarboxaldehyde compounds **32a**−**e** and later to the desired compounds **33a**−**e**.

Variably substituted P_4 anilino compounds **34**, **35**, and **36a**−**e** were prepared according to the methods outlined in Scheme 5. Aryl amination of compound **9c** according to the Buchwald amination methodology[21] afforded compound **34** in 97% yield. Acetylation of **34** with acetic anhydride and triethylamine gave the acetyl derivative **35** in 97% yield. Alternatively, aniline **34** was converted to the Boc protected derivative **36a** by treatment with Boc anhydride (neat) at 80 °C in 84% yield. Alkylation with iodomethane provided **36b** in quantitative yield (100%). Removal of the Boc protecting group afforded **36c** was acetylated to afford compound **36d**. Alkylation of **36c** with

Scheme 3. Syntheses of C-3-Cyano, 3-Tetrazole Analogues[a]



[a] (a) Triflic anhydride, lutidine, NaN_3, DMF; (b) 2-formylphenylboronic acid, $(Ph_3P)_4Pd$, Na_2CO_3 (2 N), 4:1 toluene/EtOH, Na_2CO_3 (2 N), reflux; (c) 3-(*R*)-OH-pyrrolidine (2 equiv), $NaCNBH_3$, $ZnCl_2$ (0.5 N, in THF), MeOH; (d) NaN_3, DMF, heat.

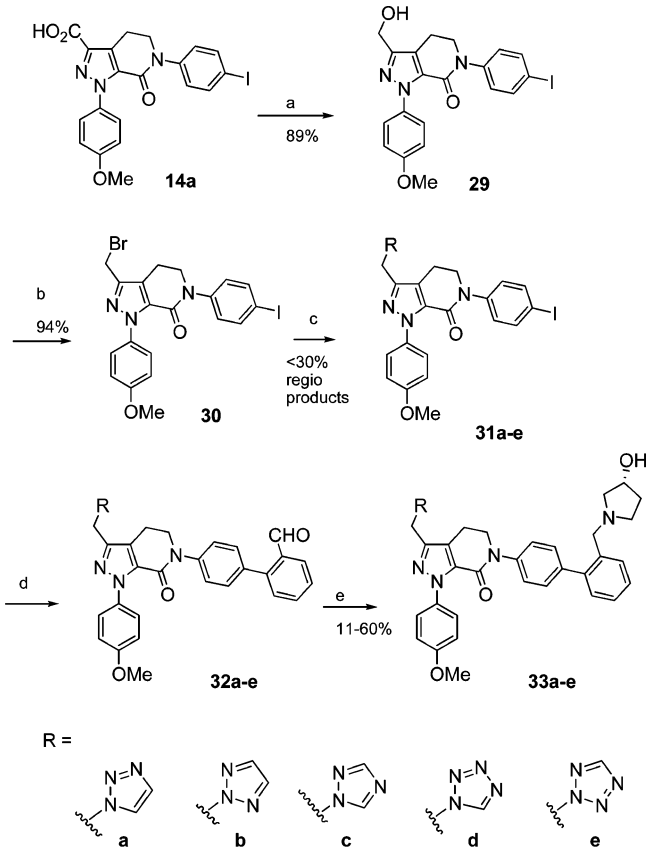
Analogues in which the P_4 moiety is either the phenylpiperidinyl or the corresponding phenyllactam groups were accessed according to the methods outlined in Scheme 6. Ullmann coupling[22] (K_2CO_3, CuI, 1,10-phenanthroline in DMSO, 130 °C) of compound **9c** with excess piperidine in a sealed tube provided compound **37** in 5% yield. In a similar manner, the Ullmann coupling of **9c** with δ-valerolactam or caprolactam led to the P_4 phenyllactam analogues **38a**,**b** in 20−25% yield. Likewise, treatment of pyrazole **9b** with δ-valerolactam under similar Ullmann conditions provided compound **39** in 21% yield, which on aminolysis with ammonia in ethylene glycol at 120 °C provided compound **40** in 76% yield.

The preparation of compound **47** is outlined in Scheme 7. Cycloaddition of chlorohydrazone compound **7b** and morpholine derivative **42** (prepared in 65% yield from lactam **41**) with triethylamine in toluene under reflux conditions followed by treatment with TFA afforded the bicyclic pyrazole **43** in 75% yield. Hydrogenation (palladium on carbon in methanol) provided aniline **44** in 96% yield. Boc protection of **44** (Boc_2O, NaH in THF) followed by alkylation (NaH and iodomethane) and removal of the Boc group with TFA provided the *N*-methylaniline derivative **45** in 56% yield. Aminolysis of **45** with ammonia in ethylene glycol at 120 °C led to compound **46**, which was acetylated (acetyl chloride in the presence of sodium hydroxide (1 N) in DCM) to compound **47** in 30% yield.

Results and Discussion

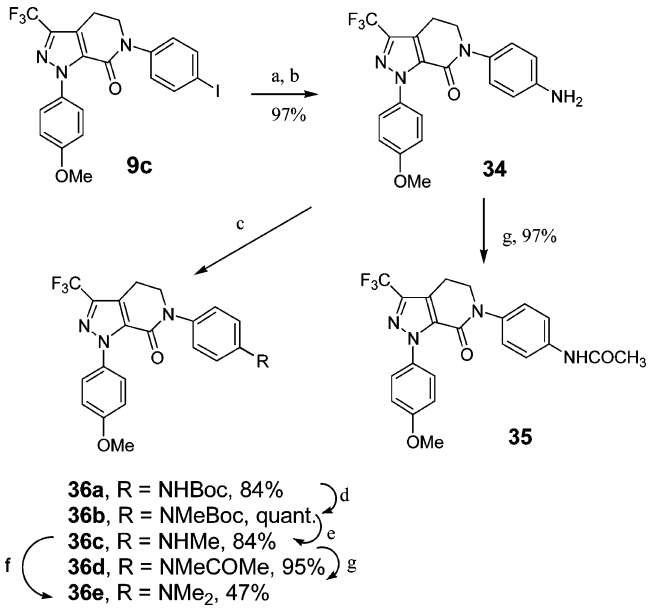
Because of the enhancement in potency seen with the tetrahydropyrazolopyridone scaffold, efforts to extend the SAR to include neutral P_1 groups such as the *p*-methoxyphenyl that previously showed reduced fXa binding in the monocyclic

Scheme 4. Syntheses of Substituted C-3 Heteroalkyl Analogues[a]



[a] (a) BH_3. THF, room temp; (b) PBr_3, DCM, room temp; (c) NaH, 1,2,3-triazole or 1,2,4-triazole or 1*H*-tetrazole, DMF; (d) 2-formylphenylboronic acid, $(Ph_3P)_4Pd$, Na_2CO_3 (2 N), 4:1 toluene/EtOH, reflux; (e) 3-(*R*)-hydroxypyrrolidine, $NaCNBH_3$, $ZnCl_2$ (0.5 N in THF), MeOH.

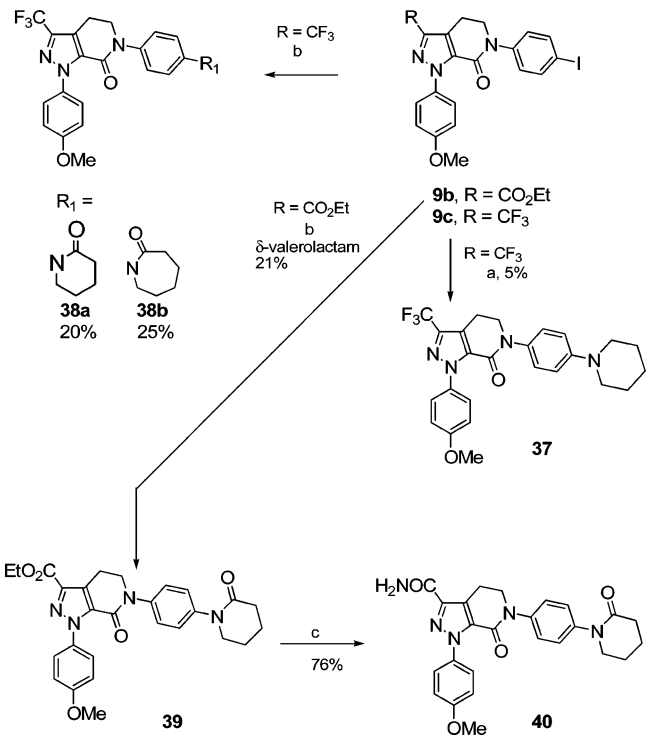
Scheme 5. Syntheses of Substituted P_4 Amino Analogues[a]



[a] (a) Diphenylmethanimine, BINAP, NaO^{t}Bu, $Pd_2(dba)_3$, toluene, reflux; (b) hydroxylamine hydrochloride, NaOAc, MeOH; (c) Boc_2O, neat, 80 °C; (d) NaH, MeI, DMF; (e) TFA, DCM; (f) MeI, DMF, K_2CO_3, room temp; (g) Ac_2O, TEA, DCM, room temp.

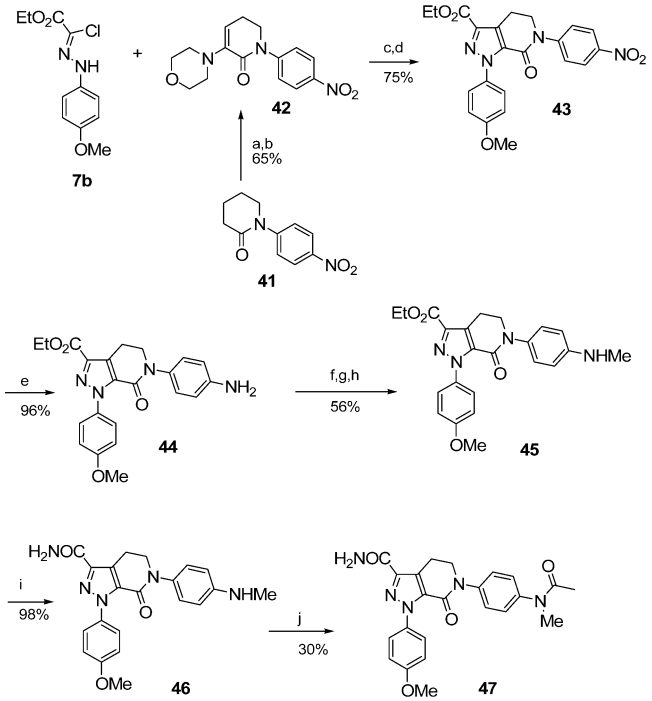
the binding assay, the in vitro clotting activity as measured by

Scheme 6. Syntheses of P_4 Lactam Analogues and Compound **37**[a]



[a] (a) 1.5 equiv of piperidine, K_2CO_3, catalyst CuI, DMSO, sealed tube, 130 °C, 24 h; (b) δ-valerolactam or azepan-2-one, K_2CO_3, catalyst CuI, catalyst 1,10-phenanthroline, DMSO, 130 °C 24 h; (c) ammonia in ethylene glycol, 120 °C, 4 h.

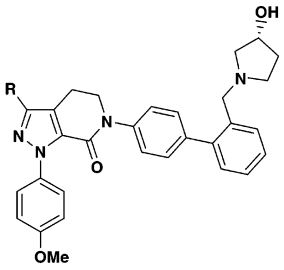
Scheme 7. Synthesis of Compound **47**[a]

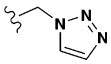
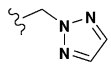
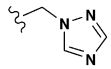
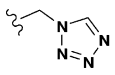
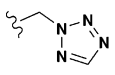


[a] (a) 3 equiv of PCl_5, $CHCl_3$, reflux; (b) morpholine reflux; (c) TEA, toluene, reflux; (d) TFA, DCM; (e) H_2, Pd/C (10%), MeOH; (f) Boc_2O, NaH, THF; (g) NaH, THF, MeI; (h) TFA, DCM; (i) ammonia, MeOH/ethylene glycol, 120 °C, 4 h; (j) acetyl chloride, NaOH (1 N), DMC.

bicyclic pyrazole series required careful adjustment for potency and polarity at the C-3 pyrazole position for possible alternatives

Table 1. In Vitro Activity for Substituted C-3 Pyrazolopyridinones[a]



Compd.	R	[h]fXa K_i nM	[h]Thrombin K_i nM	[h]PT[a] EC_{2X} μM
6[16]	CF_3	0.18	330	33.1
11a	SO_2Me	0.25	180	1.5
11b	CO_2Et	3.9	980	6.1
12	COOH	7.6	>20000	25
13f	$CONH_2$	0.07	140	1.3
15a	CONHMe	4.8	7000	3.5
15b	$CONMe_2$	1.7	11000	2.7
18	CN	0.33	100	2.8
21	NHBoc	9.6	4600	NT
22	NH_2	6.7	9400	4.7
23	N(Me)Boc	2.0	950	10.6
24	NHMe	1.7	4800	3.1
25	NMe_2	0.31	1800	NT
27		2.3	>2500	4.6
28		0.63	12000	12.4
33a		0.67	12000	2.0
33b		0.48	980	5.4
33c		0.25	1900	2.1
33d		0.85	970	1.9
33e		1.10	1000	8.6

[a] K_i values were obtained from purified human enzymes and are averaged from two experiments ($n = 2$).[28,29] PT values are measured according to refs 7a and 11. Human trypsin K_i values for all compounds above are >3000 nM. NT indicates "not tested".

were gratified to see the breadth of substitutions that were readily accommodated in this region of the fXa active site. For example, in addition to the trifluoromethyl analogue **6**,[16a] subnanomolar

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