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 **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]

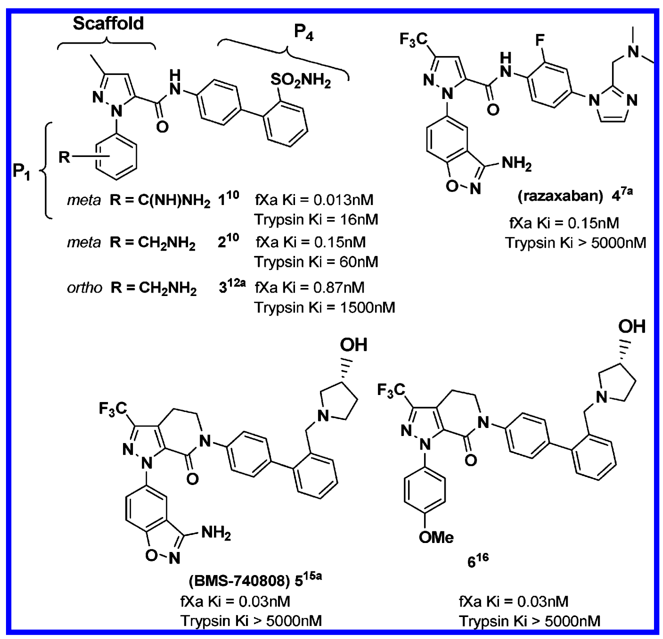

Figure 1. Schematic of important pyrazole fXa compounds.

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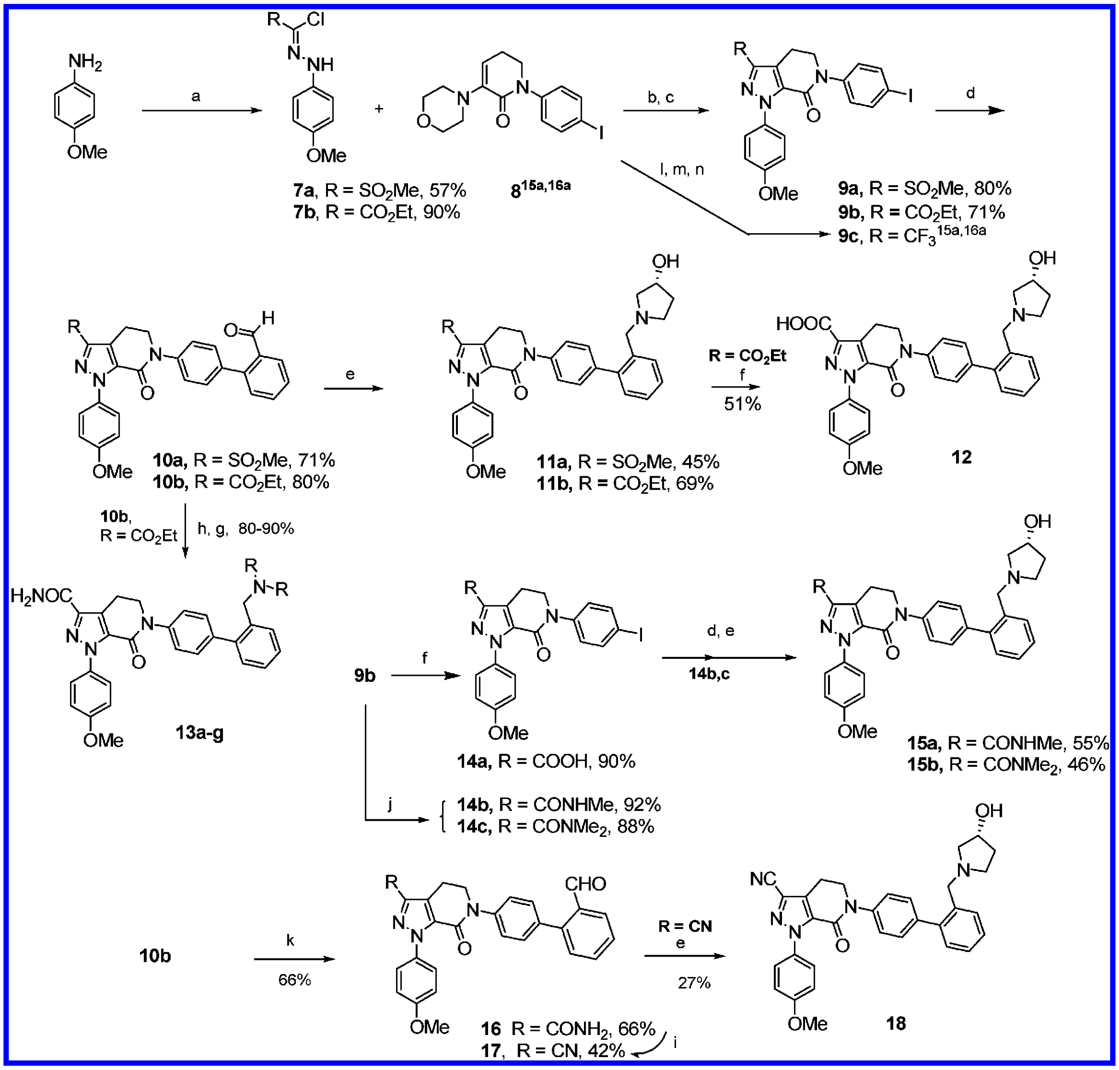
Scheme 1. Syntheses of C-3-carboxypyrazolo-pyridinone Analogues[a]



[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 group at a higher pH. In the bacterial reverse mutation (AMES)[13] assay, the aniline moiety of **3** tested positive, which was further 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 optimization strategy that resulted in the identification of compound **40**, a structurally novel and neutral bicyclic pyrazole fXa inhibitor (currently in phase III trials) with a superior pharmacokinetic profile (low clearance and volume of distribution) compared to compound **4**.

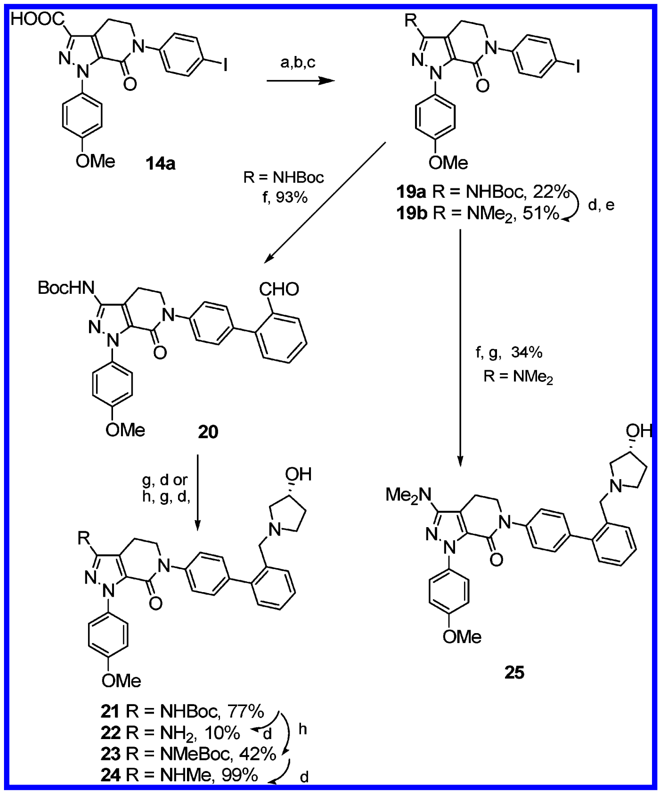
Chemistry

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.

Tetrazolyl compounds **27** and **28** were prepared according to Scheme 3. Treatment of **14b** with lutidine and triflic 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.

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

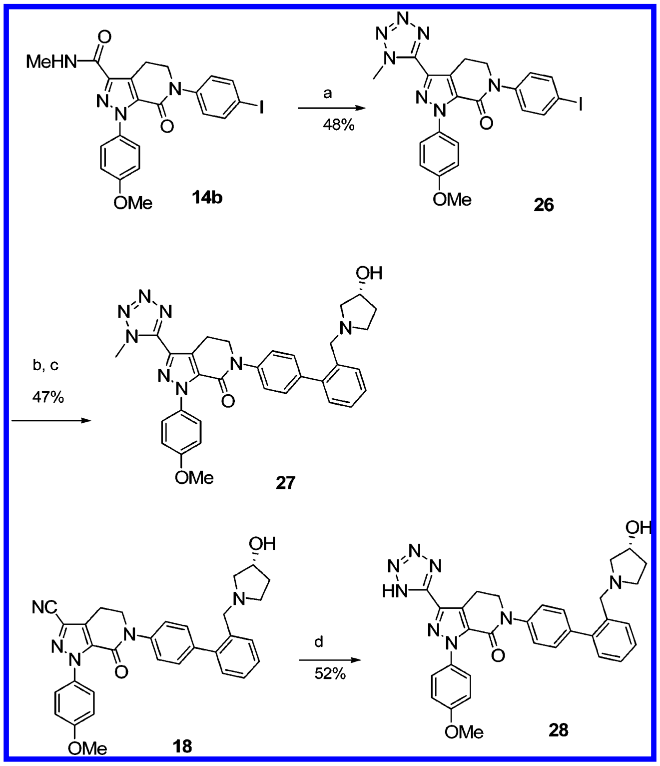


[a] (a) Oxalylchloride, DCM, catalyst DMF; (b) NaN_3, water, acetone 0 °C; (c) toluene, 80 °C, ^{t}BuOH; (d) TFA, DCM; (e) formaldehyde (37%, excess), $ZnCl_2$ (0.5 M/THF), $NaBH_3CN$, MeOH; (f) 2-formylphenylboronic acid, $(Ph_3P)_4Pd$, Na_2CO_3 (2 N), 4:1 toluene/EtOH, reflux; (g) 3-(*R*)-OH-pyrrolidine,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

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 idodomethane and potassium carbonate provided compound **36e** in 47% yield.

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.

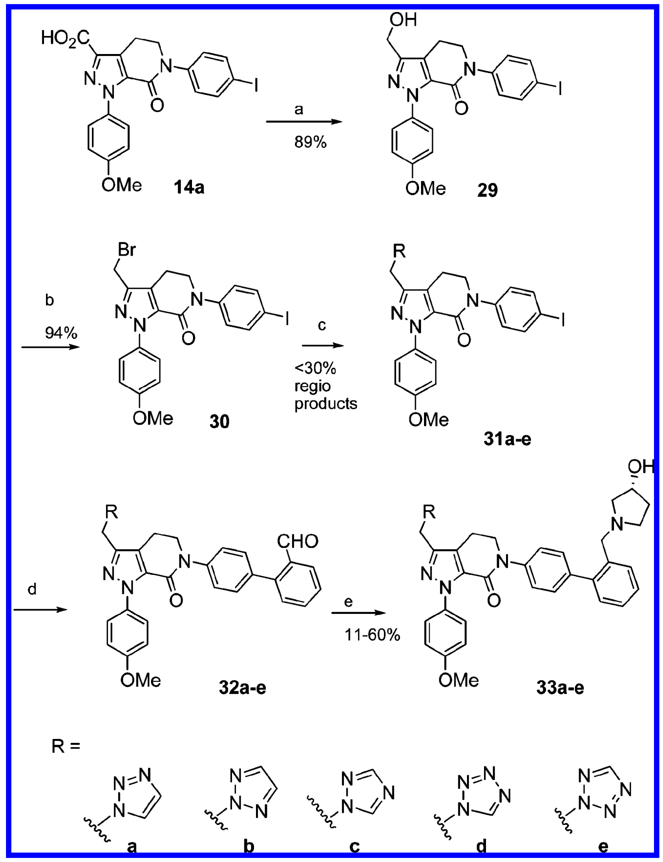
Analogues in which the P_4 moiety is either the phenylpiperidinyl or the corresponding phenyllactam groups were accessed according to the methods outlined in Scheme 6. Ullmann coupling[22] (K_2CO_3, CuI, 1,10-phenanthroline in DMSO, 130 °C) of compound **9c** with excess piperidine in a sealed tube provided 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

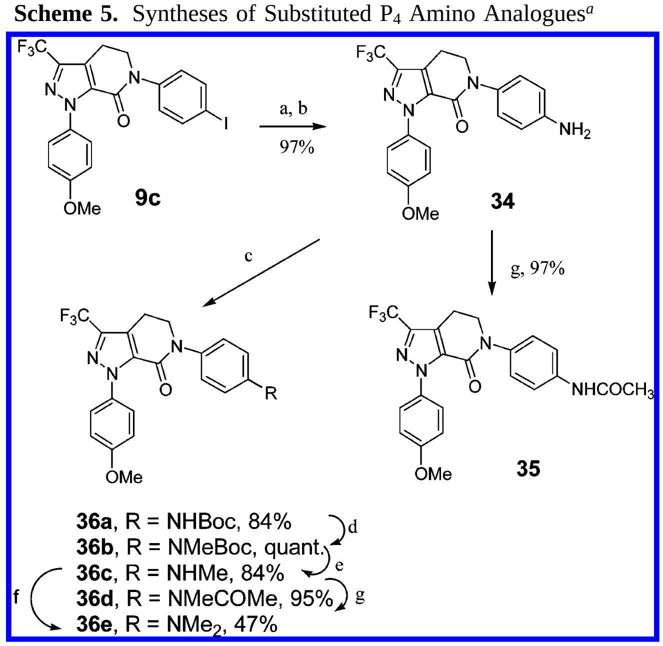
Because of the enhancement in potency seen with the tetrahydropyrazolopyridone scaffold, efforts to extend the SAR to include neutral P_1 groups such as the *p*-methoxyphenyl that previously showed reduced fXa binding in the monocyclic pyrazole series proved to be successful.[16a−b] Although the compounds with this P_1 group showed potent fXa inhibition in

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.

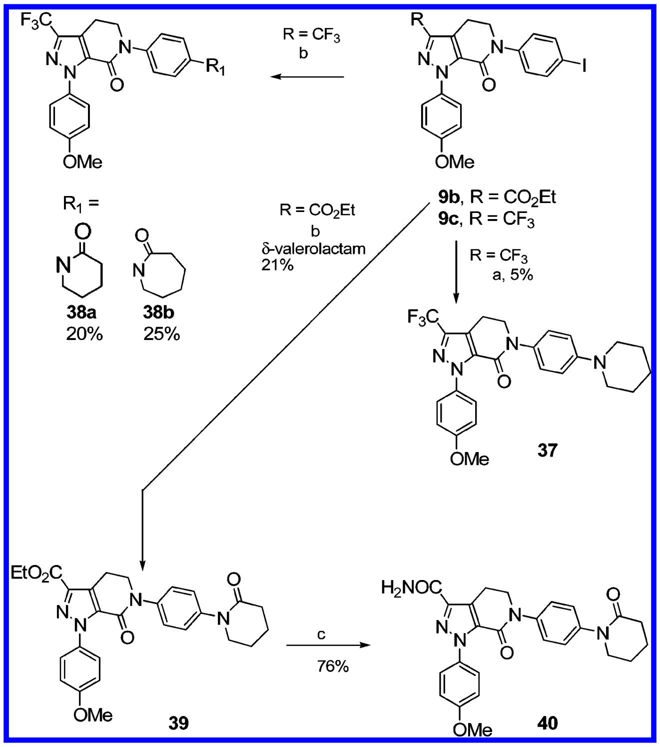
Scheme 5. Syntheses of Substituted P_4 Amino Analogues[a]



[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.

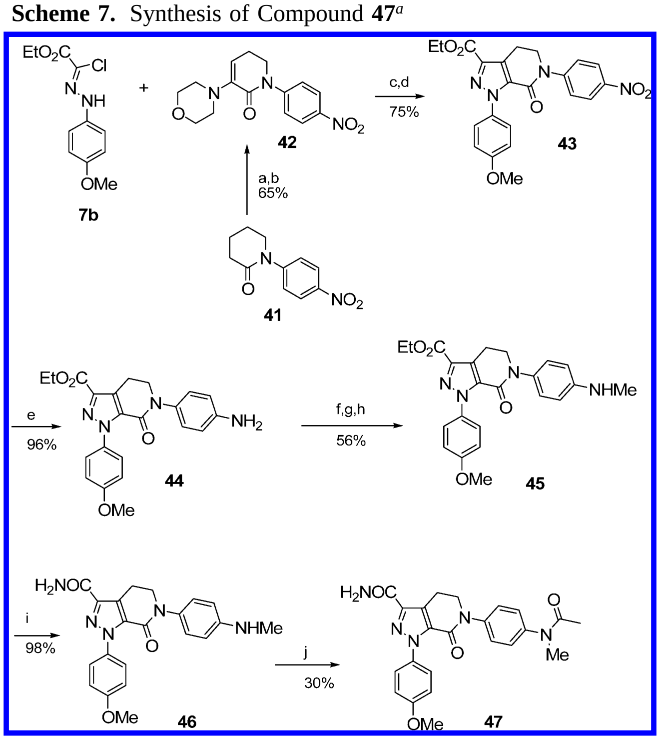
the binding assay, the in vitro clotting activity as measured by the prothrombin time (PT) assay of these compounds was moderate to high. Further optimization of the *p*-methoxyphenyl

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.

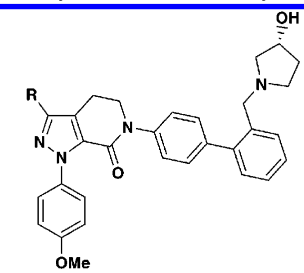
Scheme 7. Synthesis of Compound **47**[a]

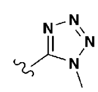
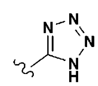
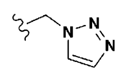
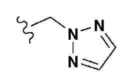
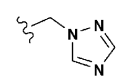
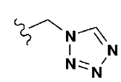
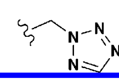


[a] (a) 3 equiv of PCl_5, $CHCl_3$, reflux; (b) morpholine reflux; (c) TEA, toluene, reflux; (d) TFA, DCM; (e) H_2, Pd/C (10%), MeOH; (f) Boc_2O, NaH, THF; (g) NaH, THF, MeI; (h) TFA, DCM; (i) ammonia, MeOH/ethylene glycol, 120 °C, 4 h; (j) acetyl chloride, NaOH (1 N), DMC.

bicyclic pyrazole series required careful adjustment for potency and polarity at the C-3 pyrazole position for possible alternatives to the lipophilic trifluoromethyl substituent (Table 1). In the course of our investigation of the C-3 pyrazole position, we

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 inhibitory activity was seen for the methylsulfonyl compound **11a** (fXa K_i = 0.25 nM), the carboxamido compound **13f**

Table 2. Comparative Permeability and Dog Pharmacokinetic Parameters[a]

Compd.	Cl L/Kg/h	Vdss L/Kg	$T_{1/2}$ (po) h	F% (po)	Caco-2 P_{app} X 10^{-6}cm
13f	0.32	1.6	5.6	100	2.3
33a	2.47	4.9	2.8	24	1.6
33d	2.58	3.7	1.8	7	0.9

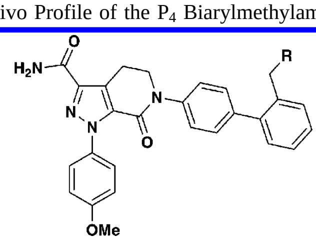
[a] Compounds were dosed (po/iv) as TFA salts in a cassette dosing N-in-one format[7a,34a−c] at 0.4 mg/kg iv and at 0.2 mg/kg po (n = 2).[10] Caco-2 and dog PK parameters were measured according to refs 7a and 11.

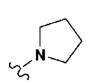
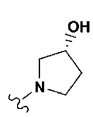
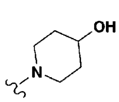
(fXa K_i = 0.07 nM), the nitrile compound **18** (fXa K_i = 0.33 nM), and the dimethylamino compound **25** (fXa K_i = 0.31 nM). The binding affinity and clotting activity of the carboxamide analog **13f** showed significant improvement when compared to the corresponding trifluoro-methyl compound **6**. Compared to the parent carboxamide **13f**, the substituted carboxamides **15a** and **15b**, though significantly less potent in the binding assay, were only about 2- to 3-fold less potent in the clotting (PT) assay. The ester analogue **11b** and its corresponding carboxylic acid **12** were less potent in both assays. Among the C-3 amino analogues investigated, the order of fXa potency was NMe_2 > NHMe ≥ N(Me)Boc ≥ NH_2, NHBoc. The unsubstituted amino analogue **22** and the *N*-methylamino compound **24** demonstrated acceptable activity in the clotting assay. Amongst the tetrazole analogs, the tetrazole compound **27** showed moderate clotting activity, though less potent in the binding assay, and the reverse was true for tetrazole **28**. Triazolylmethyl and tetrazolylmethyl analogues **33a−d** exhibited good potencies in both assays. Overall, the compounds shown in Table 1 were highly selective (>1000-fold) for fXa relative to other serine proteases such as thrombin and trypsin.

Table 2 lists the pharmacokinetic profile in dogs of a representative set of the most optimized C-3 substituted compounds. The carboxamide **13f** demonstrated an excellent pharmacokinetic profile, with low clearance (Cl = 0.32 L kg^{-1} h^{-1}), moderate volume of distribution (V_{dss} = 1.6 L kg^{-1}), and a half-life ($T_{1/2}$) of 5.6 h. The high oral bioavailability (F = 100%) exhibited by **13f** was consistent with the high apparent permeability (P_{app} = 2.3 × 10^{-6} cm s^{-1}) of this compound in the Caco-2 assay.[23] In contrast, the pharmacokinetic profiles of the triazole analogue **33a** and the 1,2,3,4-tetrazole analogue **33d** were poor with high clearance, moderate volume of distribution, and poor oral bioavailability.

Given the excellent fXa activity exhibited by compound **13f** and its high oral bioavailability, we shifted the focus on further P_4 optimization (Table 3). In general, the compounds retained subnanomolar fXa binding affinity and potent clotting activity, good selectivity (trypsin/thrombin, >100-fold), and showed good permeability (P_{app}) in the Caco-2 assay. The unsubstituted amino compound **13a** (fXa K_i = 0.97 nM) was the least potent, whereas the substituted amino compounds **13b−d** exhibited

Table 3. C-3 Carboxamido Pyrazoles: In Vitro and in Vivo Profile of the P_4 Biarylmethylamino Moieties[a]



Compd.	R	[h]fXa K_i nM	[h]PT EC_{2X} μM	Rabbit AVShunt IC_{50} nM	Caco-2[a] P_{app} X 10^{-6} cm/sec	Cl[b] l/Kg/h (dogs)	Vdss[b] L/Kg (dogs)	$T_{1/2}$[b] (po) h (dogs)	F%[b] (po) (dogs)
13a	NH_2	0.97	2.0	NT	1.2	NT	NT	NT	NT
13b	NHMe	0.14	1.2	445	1.7	1.3	7.4	7.3	56
13c	NMe_2	0.24	0.9	175	2.3	2.0	6.1	3.6	56
13d	NEt_2	0.08	1.4	NT	2.3	2.4	6.5	3.7	20
13e		0.30	1.1	NT	3.5	1.5	5.6	3.5	53
13f		0.07	1.2	120	2.3	0.3	1.6	5.6	100
13g		0.29	1.8	180	4.9	0.1	0.99	4.3	55
13h	$N[(CH_2)_2OH]_2$	0.36	2.3	NT	0.9	0.5	1.09	1.8	15

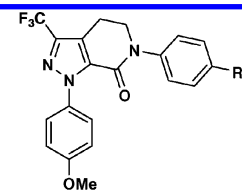
[a] K_i values were obtained from purified human enzymes and are averaged from two experiments (n = 2).[28,29] Prothrombin time (PT) values are measured according to refs 7a and 11. Human trypsin K_i values for all compounds listed in Table 2 are >3000 nM. Caco-2 and dog PK parameters were measured according to refs 7a and 11. [b] Compounds were dosed (po/iv) as TFA salts in a cassette dosing N-in-one format at 0.4 mg/kg iv and 0.2 mg/kg po (n = 2).[34a−c] NT indicates "not tested."

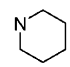
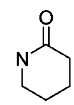
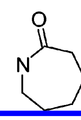
potent fXa inhibitory activity (fXa K_i < 0.3 nM) and good clotting activity (PT $EC_{2\times}$ < 1.5 μM). As was observed with the 3-(*R*)-hydroxypyrrolidine compound **13f**, the 4-hydroxypiperidinyl analogue **13g** also demonstrated low clearance, moderate volume of distribution, and half-life in the same range as observed with **13f**. Again, the high dog oral bioavailability seen for **13g** (F = 55%) correlated well with the observed Caco-2 (P_{app}) permeability value. In the rabbit arteriovenous shunt (AVShunt) thrombosis model,[6g] compounds **13b**, **13c**, **13f**, and **13g** inhibited thrombus formation in a dose-dependent manner with IC_{50} values of 445, 175, 120, and 180 nM, respectively, and with the exception of **13b** were slightly more potent than compound **4** (AVShunt IC_{50} = 340 nM)[7a] and were in the same range when compared to compound **5** (AVShunt IC_{50} = 140 nM).[15a] Of the compounds in this series, **13b** had the longest half-life in dogs, albeit with relatively high clearance and high volume of distribution. Interestingly, these data did not correlate well with the observed **13b** half-life in the human liver microsome (HLM) assay[24] ($T_{1/2}$ > 100 min). In the same assay, the HLM half-life for compounds **4** and **5** was 38 and 42 min, respectively. Taken together therefore, the carboxamide pyrazole analogue **13f** emerged as a potent alternative to compound **4**, with excellent potency both in vitro and in vivo and a good pharmacokinetic profile in dogs.

In a parallel effort, the compounds containing P_4 nitrogen atom (as the point of attachment) were also explored (Table 4).[12c] This strategy proved to be highly successful in that a potent compound **36d** bearing a *N*-methylacetyl group was quickly identified. This discovery was significant in that it differed in structure from all our previous pendent P_4 groups we had explored. The compound, though more potent than the aniline derivatives **34**, **36c**, and **36e**, was weak in the clotting assay, suggesting high protein binding. The high level of potency exhibited by compound **36d** (fXa K_i = 0.50 nM) suggested that the orientation of the P_4 *N*-methylacetyl substituent in the S_4 region of the fXa active site is very important. This type of observation was unique in the fXa literature at the time it was discovered. To explain this finding, a closer look at the model of **36d** in the active site of fXa clearly showed the *N*-methyl P_4 group forming a lipophilic π interaction with the bottom S_4 Trp215 residue,[12d] and thus positioning the acetyl carbonyl functionality perpendicular to the inner P_4 phenyl ring, thereby forming a hydrophobic interaction with the other residues in this region. The importance of the orientation of the *N*-methyl group was confirmed by the loss in fXa affinity with the acetamide analogue **35** (fXa K_i = 180 nM) where the planarity of this group positioned it in an unfavorable orientiation in the S_4 region of the enzyme. The dimethylamino analogue **36e** (fXa K_i = 6.0 nM) and piperidinyl **37** (fXa K_i = 2.1 nM) were also less potent, suggesting a planar orientation with these moieties in the S_4 region as well. Cyclization of the P_4 *N*-methyl acetyl group in **36d** to form lactam analogues **38a** and **38b** retains the subnanomolar fXa binding affinity. Unfortunately, lactam analogues **38a** (PT = 23 μM) and **38b** (PT = 26 μM) exhibited poor anticoagulant activity. This could be explained by the high lipophilicity (cLogP > 7) and high human serum protein binding (>99%)[25] exhibited by these compounds.

In order to modulate the lipophilicity of **36d** and **38a**, we reintroduced the polar C-3 carboxamido moiety (Table 5) that was shown to be important in compounds **13a**−**h**. The carboxamidopyrazole analogue **40** (fXa K_i = 0.08 nM, PT = 3.8 μM) and **47** (fXa K_i = 0.61 nM, PT = 3.1 μM) not only maintained subnanomolar fXa binding affinity but also demonstrated much improved potency in the clotting (PT) assay,

Table 4. In Vitro Profile of P_4 Substituents with Imbedded Nitrogen[a]



Compd.	R	[h]fXa K_i nM	[h]Thrombin K_i nM	[h]PT [a]EC_{2X} μM
34	NH_2	1600	> 6300	NT
35	NHCOMe	180	> 6300	NT
36c	NHMe	610	> 6300	NT
36d	**N(Me)COMe**	**0.5**	**> 6300**	**8.2**
36e	NMe_2	6	> 13400	40.6
37		2.1	3900	3.7
38a		**0.23**	**4400**	**36**
38b		0.47	3300	26

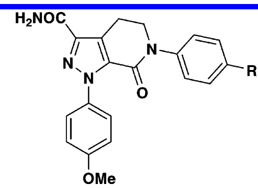
[a] K_i values were obtained from purified human enzymes and are averaged from two experiments (n = 2).[28,29] Prothrombin time (PT) values are measured according to refs 7a and 11. Human trypsin K_i values for all compounds listed in Table 2 are >3000 nM. NT indicates "not tested".

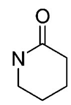
especially when compared to the related trifluoromethylpyrazole analogues **38a** and **36d.**

Selectivity and Liability Profiling. Compound **40** shows a high degree of selectivity versus other proteases (see supplemental section), even compared to compounds **4**[7a] and **5**.[15a] Additionally, the compound shows weak activity against various P_{450} isozymes (IC_{50} > 25μM) and weak activity against the hERG potassium channel (IC_{50} > 25 μM, patch clamp assay).[26a−e] The solubility of compound **40** was shown to be approximately 40−50 μg/mL.[27] In the human liver microsome assay, compound **40** was very stable with a $T_{1/2}$ of >100 min (the HLM $T_{1/2}$ of **47** was not measured). The Caco-2 permeability values for compounds **40** (P_{app} = 0.9 × 10^{-6} cm s^{-1}) and **47** (P_{app} = 2.5 × 10^{-6} cm s^{-1}) were moderate to high.

Dog Pharmacokinetics and Rabbit Antithrombotic Efficacy. As a result of the excellent in vitro potency and selectivity of compounds **40** and **47**, the pharmacokinetic profiles of both compounds were studied in dogs using a cassette dosing paradigm ("N-in-one" study, Table 6).[7a,34a,b] The acetylated *N*-methyl analogue **47** was orally bioavailable; but showed high clearance (Cl = 2.8 L kg^{-1} h^{-1}), moderate volume of distribution (V_{dss} = 1.7 L kg^{-1}), and unacceptable half life. The dog pharmacokinetics for compound **40** was outstanding with very low clearance (Cl = 0.02 L kg^{-1} h^{-1}), and low volume of distribution (V_{dss} = 0.2 L kg^{-1}). These values were significantly

Table 5. Optimization of the Amido and Lactam P_4 Moieties[a]



Compd.	R	[a]fXa K_i nM	[a]Thrombin K_i nM	PT[b] EC_{2X} μM	APTT[b] EC_{2X} μM	Caco-2[b] P_{app} X10^{-6} cm/sec	Solubility[b] μg/ml
40		0.08	3100	3.8	5.1	0.9	50
47	N(Me)COMe	0.61	2520	3.12	5.6	2.5	NT

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

Table 6. Comparative in Vitro and in Vivo Profiles of Compounds **40**, **47**, **4**, and **5**[a]

Compd.	[h]fXa K_i nM	[r]fXa K_i nM	[h]Serum P.B. %	Rabbit AVShunt IC_{50} nM	Cl[a] L/Kg/h	Vdss[a] L/Kg	$T_{1/2}$[a] (po) h	F%[a] (po)
47	0.54	2.6	NT	NT	2.8	1.7	0.7	56
Apixaban (40)	**0.08**	**0.17**	**87**	**329**	**0.02**	**0.2**	**5.8**	**58**
Razaxaban (4) [7a]	0.19	0.19	91	340	1.1	3.4	5.3	84
5[15a]	0.03	0.06	97	140	0.35	1.6	5.1	82

[a] "*h*" and "*r*" refer to human and rabbit species, respectively. K_i values were obtained from purified human enzymes and are averaged from two experiments ($n = 2$). Compounds were dosed in a cassette dosing (po/iv) N-in-one format[7a,34a−c] *a* refers to the cassette dosing (po/iv) dog pharmacokinetic parameters. P.B. refers to serum protein binding. NT indicates "not tested".

lower than those observed with compounds **4** and **5**. FXa being a vascular target, the pharmacokinetic profile for compound **40** was viewed as highly desirable and less likely to have nontarget-related adverse effects. Importantly, compound **40** had a moderate half-life ($T_{1/2} = 5.8$ h) and good oral bioavailability ($F = 58\%$). The human serum protein binding as measured by equilibrium dialysis for **40** was 87%.[25] In the rabbit AVShunt thrombosis model (Figure 4), compound **40** inhibited thrombus formation in a dose-dependent manner with an IC_{50} value of 329 nM.[15d,e] This is comparable to the antithrombotic potency obtained for compound **4** ($IC_{50} = 340$ nM) in the same model.

X-ray Crystallography of Compound 40. The X-ray structure for compound **40** bound to fXa (Figure 2, 2.3 Å resolution with an R value of 0.229 and an R_{free} of 0.277) shows a tight inhibitor−enzyme complex[30−33] and shows a similar binding mode compared to compound **2**[11] and **4**.[7a] The *p*-methoxy group in the S_1 specificity pocket does not appear to interact with any specific residue in this region of the enzyme, and is oriented in a planar manner relative to the phenyl P_1 moiety. Other interactions that were found to be similar to those observed for compounds **4**[7a] and **5**[15a] included the pyrazole N-2 nitrogen atom interaction with the backbone of Gln192 (3.2 Å) and the carbonyl oxygen (scaffold carboxamide) interaction with the NH of Gly216 (2.9 Å). The pyrazole C-3 carboxamido moiety shows the NH within bonding distance to the Glu146 carbonyl oxygen (3.1 Å) with the carbonyl oxygen solvent exposed. The orientation of the pendant P_4 phenyllactam of **40** in the S_4 region shows an edge to face interaction with Trp215 and is appropriately positioned between the Tyr99 and Phe174

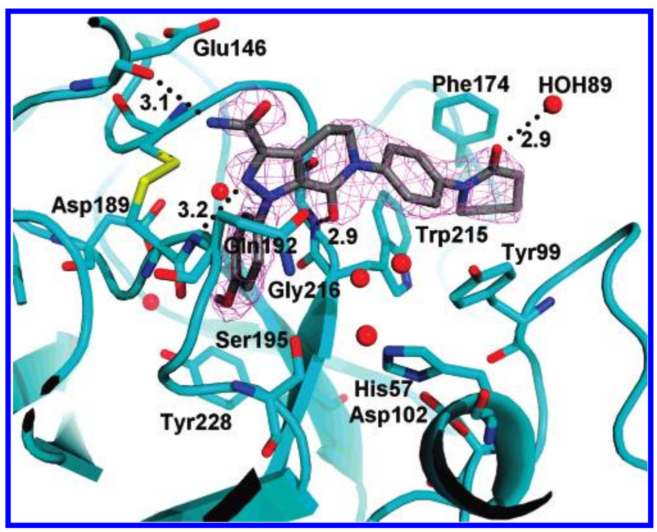


Figure 2. X-ray structure of fXa bound to compound **40**. Atomic coloring is used with cyan for fXa cartoon and carbon atoms and gray for compound **40** carbon atoms. Water molecules are shown as red spheres. Initial electron density ($2F_o - F_c$ contoured at 1σ) is shown in magenta. Hydrogen bonds between protein and ligand are shown as dashed black lines. The figure was created using PyMol.[35]

residues. The X-ray structure does not appear to show the carbonyl oxygen group of the pendent lactam moiety directly interacting with any residues in the S_4 pocket but appears to show it interacting with a water molecule. Importantly, the P_4 lactam carbonyl brings about a conformational bias toward

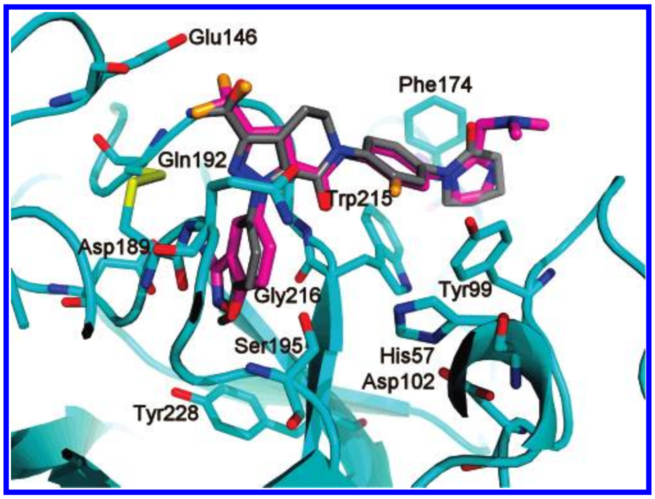


Figure 3. Superposition X-ray structures of fXa bound to compounds **40** and **4**. Atomic coloring is used with cyan for fXa cartoon and carbon atoms, gray for compound **40** carbon atoms, magenta for compound **4** carbon atoms, and orange for compound **4** fluorine atoms. The orientation is the same as in Figure 2. The figure was created using PyMol.[35]

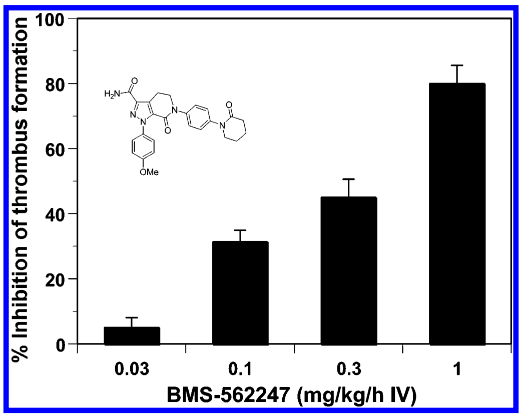


Figure 4. Rabbit arteriovenous shunt (AVShunt) profile of compound **40** (BMS-562247).

orthogonality to optimally position itself between the S_4 enzyme residues Trp215, Tyr99, and Phe174. Overall, compound **40** fits into the fXa enzyme active site in a highly complementary manner. Figure 3 shows an overlay of the X-ray structures of the fXa-bound compounds **40** and **4**.

Conclusions

Several bicyclic pyrazolopyridinone analogues bearing the *p*-methoxyphenyl P_1 moiety have been identified with potent inhibitory activity against coagulation fXa. A diverse set of C-3 substituents were identified that retained potent fXa activity and possessed good clotting (PT) activity. Most optimal in terms of in vitro potency and potency in the clotting assay is the C-3 carboxamido pyrazole moiety. Combination of this moiety with the relatively polar and neutral lactam P_4 moiety resulted in compound **40**, with high metabolic stability, ultralow clearance, and low volume of distribution. Compound **40** is a potent, selective, and orally bioavailable fXa inhibitor that demonstrates antithrombotic efficacy similar to compound **4** in the rabbit arteriovenous shunt thrombosis model (Figure 4). Importantly, compound **40** (apixaban, BMS-562247) is a significant improvement when compared to compound **4** based on its excellent pharmacokinetic profile and has been advanced to human clinical trials for the prevention and treatment of venous and arterial thrombosis.

Table 7. Diffraction Data for Compound **40** (BMS-562247)

(A) Data Collection Statistics		
space group	$P2_12_12_1$	
unit cell parameters		
a, Å	56.4	
b, Å	72.8	
c, Å	79.4	
	overall	highest shell
diffraction limits, Å	19.4−2.3	2.4−2.3
no. of unique reflections	14544	1645
data completeness	96.8	94.9
(B) Refinement Statistics		
no. of non-H atoms in protein		2238
no. of non-H atoms in inhibitor		34
no. of refined water molecules		122
rmsd bond distances from ideal, Å		0.008
rmsd angles from ideal, deg		1.5
crystallographic residual *R* value		0.229
crystallographic residual R_{free}		0.277
test set, % of all reflections		5.9

Experimental Section

All reactions were run under an atmosphere of dry nitrogen unless otherwise noted. Solvents and reagents were obtained from commercial vendors in the appropriate grade and used without further purification unless otherwise indicated. NMR spectra (^{1}H, ^{13}C, ^{19}F) were obtained on VXR or Unity 300 MHz instruments (Varian Instruments, Palo Alto, CA) with chemical shift in ppm downfield from TMS as an internal reference standard. ^{1}H assignment abbreviations are as follows: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), broad singlet (bs), doublet of doublets (dd), doublet of triplets (dt), and multiplet (m). Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ, and were within 0.4% of the theoretical values. Mass spectra were measured with a HP 5988A mass spectrometer with a particle beam interface using NH_3 for chemical ionization or a Finnigan MAT 8230 mass spectrometer with NH_3-DCI or VG TRIO 2000 for ESI. High-resolution mass spectra were measured on a VG 70-VSE instrument with NH_3 ionization. Flash chromatography was carried out using EM Science silica gel 60 (230−400 mesh). Preparative thin layer chromatography was done on EM Science 60 plates F_{254} (2 mm, 20 cm × 20 cm). HPLC purification was performed on a Jasco 900 series instrument or a Rainin Dynamax SD200 using a C18 reverse-phase column with acetonitrile/H_2O (containing 0.05% TFA) as a mobile phase. All compounds were found to be >95% pure by HPLC analysis unless otherwise noted.

X-ray Crystallographic Studies. The factor Xa/compound **40** crystals were obtained from GLA-Domainless *β*-Factor Xa (Haematologic Technologies) that had been fractionated on a Amersham mono-Q 10/10 column equilibrated with 50 mM Tris, pH 8.0, 100 mM NaCl, and 1 mM $CaCl_2$ and eluted with a 20-bed volume gradient from 0 to 500 mM NaCl. Inhibitor was added to the fractions as they were collected. Protein of interest eluted at about 100 mM NaCl. This solution was incubated for 12 h and concentrated to 6 mg/mL using a Vivaspin 6 mL concentrator with a 5000 MWCO membrane. The crystals were grown at 4 °C using hanging drop vapor diffusion, with 18% PEG 6000 and 200 mM NaOAc (pH 5.5) in the reservoir. Hanging drops contained 4 *μ*L of protein solution and 4 *μ*L of reservoir solution. The drops were microseeded with a crushed crystal from a previous crystal growth. Cryoprotectant was introduced to the factor Xa crystals by first transferring the crystals to a 2 *μ*L drop of the soaking solution and then bridging the small drop into a 20 *μ*L drop of solution containing 22% PEG 200, buffered with 200 mM Na acetate (pH 5.5) and 20% ethylene glycol. After about a minute in this solution, the crystals were frozen. Data for the fXa/**40** complex were collected at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) located at Sector 5 of the Advanced Photon Source (APS). DND-CAT is supported by E.I. DuPont de Nemours & Co., The Dow Chemical Company and the State of Illinois. Use of the APS was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-

AC02-06CH11357. Data were collected at a wavelength of 1.0 Å at 100 K using an Oxford cryosystems cooling device. Data frames of 1° rotation were collected. Data were 99% complete. Raw data were processed with the program HKL.[30] The program EPMR[31] was used to determine the initial model for refinement using the PDB coordinates 1FJS (minus the inhibitor and solvent molecules) as the search model. The CNX (Accelrys) program was used for crystallographic refinement. Simulated annealing (at a maximum temperature of 3000 K) was followed by *B*-factor refinement. The inhibitor was built with the program QUANTA (Accelrys). Peaks in the difference electron density map that were greater than 3σ and that were less than 4 Å away from the protein were built in as solvent molecules. No major adjustments to the protein model were needed during the course of the refinements. Final *R* values as well as other relevant data collection statistics are found in Supporting Information. Coordinates for the enzyme inhibitor structure have been deposited with the Protein Data Bank with the code 2P16.

Enzyme Affinity Assays. All enzyme K_i values were obtained from purified human enzymes. All fXa assays were run in microtiter plates using a total volume of 250 μL in 0.1 M sodium phosphate buffer containing 0.2 M NaCl and 0.5% polyethylene glycol 6000 at pH 7.0. The compounds were run at 10, 3.16, 1.0, 0.316, 0.1, 0.0316, 0.01, and 0.003 16 μM. Plates were read for 30 min at 405 nm. Rates were determined in the presence of the controls (no inhibitor) and for the inhibitors. Percent enzyme activity was determined from these rates and used in the following formula to determine K_i:

$$K_i = \frac{\text{InhibitorConcentration}}{\dfrac{K_m + S - S^*\ \text{ACT}}{\text{ACT}^* K_m} - 1}$$

where *S* is the substrate concentration and ACT is the fraction of percent enzyme activity for inhibitor rates. All compounds were tested in duplicate studies and were compared with the same internal standards. The intraassay and interassay variabilities are 5% and 20%, respectively. These assays are described in detail in refs 28 and 29. All of the enzyme assays were conducted in pH 7.4 buffer at room temperature. All enzymes were purified from human tissues and were obtained from commercially available sources. Individual enzyme and substrate K_m were determined in separate experiments and were close to values established in the literature. Steady-state inhibition of enzyme activity was determined by incubating a range of inhibitor concentrations (1 nM to 50 μM, in duplicate) with fixed enzyme (0.1−100 nM) and peptide substrate (200−1000 μM) concentration for up to 30 min. The K_i was calculated, assuming competitive inhibition and one-site binding, either from the IC_{50} or from the extent of inhibition at each inhibitor concentration.

In Vitro Coagulation Assays (PT/APTT). Standard clotting assays were performed in a temperature-controlled automated coagulation device (Sysmex 6000, Dade-Behring). Blood was obtained from healthy volunteers by venipuncture and anticoagulated with $^1/_{10}$ volume of 0.11 M buffered sodium citrate (Vacutainer, Becton Dickinson). Plasma was obtained after centrifugation at 2000*g* for 10 min and kept on ice prior to use. An initial stock solution of the inhibitor at 10 mM was prepared in DMSO. Subsequent dilutions were done in plasma. Plasma solutions containing inhibitor were kept on ice prior to assay. Clotting time was determined on control plasma and plasma containing five to seven different concentrations of inhibitor. Determinations at each plasma concentration were done in duplicate. The clotting time at each concentration was compared with the control clotting time for each pooled plasma. The prothrombin time test was performed using Dade Thromboplastin C Plus according to the reagent instructions. Plasma (50 μL) was warmed to 37 °C for 3 min before adding Dade Thromboplastin C Plus (100 μL). The activated partial thromboplastin time (aPTT) was performed using AlexinTM (Sigma Diagnostics) according to the reagent instructions. Plasma (50 μL) was warmed to 37 °C for 1 min before adding aPTT reagent (50 μL). Three minutes later calcium chloride (50 μL) was added.

Dog "N-in-One" Pharmacokinetic Study. Pharmacokinetic study protocols were approved by the site Animal Care and Use Committee. Compounds were dissolved in *N*,*N*-dimethylacetamide (DMAC) to a concentration of 20 mg/mL. Compounds were combined in a final dosing solution containing 0.2 mg/mL of each compound in 10:10:10:70 % v/v DMAC/ethanol/propylene glycol/water. Beagle dogs were administered 2.5 mL kg^{-1} h^{-1} for 1 h by intravenous infusion or 1 mL kg^{-1} by oral gavage. At timed intervals, blood samples were drawn into $^1/_{10}$ volume of 3.2% sodium citrate and placed on ice. Plasma was obtained after centrifuging blood at 2000*g* for 10 min at 4 C. Urine was collected up to 24 h after dosing. Plasma and urine were frozen on dry ice and stored at −70 °C for later analysis. Samples from the pharmacokinetic studies were analyzed with LC−MS/MS methods. High-throughput technologies such as the turbulent-flow column-switching technique and direct plasma sample injection were applied to some studies. In general, the analytical methods were specific and sensitive with a quantification level of 1 nM. The intraday variability was less than 30%. Average run time was about 6 min for each sample.[34a−c]

Syntheses. Preparation of (*Z*)-*N'*-(4-Methoxyphenyl)-1-(methylsulfonyl)methanehydrazonoyl Chloride (7a). To *p*-anisidine **7** (4.39 g, 3.6 mmol) in concentrated HCl (9.2 mL) and water (20 mL) at 0 °C was slowly added sodium nitrite (2.58 g, 3.7 mmol) in water (20 mL). The mixture was stirred cold (−5 °C) for 0.5 h. The above mixture was poured into a mixture of commercially available 3-chloromethanesulphonylacetone (6.1 g, 3.5 mmol), acetone (50 mL), sodium acetate (6.7 g, 8.2 mmol), and water (100 mL). The mixture was stirred 4 h at room temperature. The precipitate was filtered off and dried to afford the desired product **7a** as a red solid (5.28 g, 57%). ^{1}H NMR ($CDCl_3$) δ: 8.05 (s, 1H), 7.12 (d, *J* = 9.2 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 3.80 (s, 3H), 3.23 (s, 3H) ppm. The product obtained was used as is in the [3 + 2] cycloaddition step.

Preparation of (*Z*)-Ethyl 2-Chloro-2-(2-(4-methoxyphenyl)-hydrazono)acetate (7b). *p*-Anisidine (16 g, 0.129 mol) was dissolved in a solution of concentrated HCl (40 mL) and water (100 mL) and cooled to −5 °C. To this solution was added dropwise an aqueous solution (H_2O, 60 mL) of sodium nitrite (9.4 g, 0.136 mol) so as to maintain the temperature below −5 °C. Once the addition was complete, the diazotized product was stirred for 20 min at 0 °C followed by the sequential addition of ethyl chloroacetoacetate (22 g, 0.133 mol), ethanol (100 mL), sodium acetate (32 g, 0.389 mol), and water (400 mL). The reaction mixture was gradually warmed to room temperature and stirred for an additional 2 h. At this point the product that precipitated as a black solid was filtered, washed with excess water, and dried in vacuo to afford the desired product (30 g, 90%). ^{1}H NMR ($CDCl_3$) δ: 8.28 (s, 1H), 7.18 (d, *J* = 9.1 Hz, 2H), 6.90 (d, *J* = 9.2 Hz, 2H), 4.41(q, *J* = 7.0 Hz, 2H), 3.80 (s, 3H), 1.42 (t, *J* = 7.3 Hz, 3H) ppm. The crude product obtained by this methodology was used as is in the [3 + 2] cycloaddition step.

Preparation of 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydro-7*H*-pyrazolo[3,4-*c*]pyridin-7-one (9a). Compound **7a** (2.60 g, 10 mol) was stirred with morpholine compound **8** (3.80 g, 10 mol), triethylamine (2.76 mL, 20 mol), and toluene (30 mL). The reaction mixture was heated at 70 °C under N_2 for 12 h and cooled to 5 °C, and HCl (4 N, 12.4 mL) was added dropwise. The cooling bath was removed, and the mixture was stirred at room temperature for 4 h. Hexane (5 mL) and water (10 mL) were added. The precipitate formed was filtered, washed with water and hexane, and dried to afford 4.15 g (80%) of the desired product **9a**. ^{1}H NMR ($CDCl_3$) δ: 7.71 (d, *J* = 8.8 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 9.1 Hz, 2H), 4.13 (t, *J* = 7.0 Hz, 2H), 3.82 (s, 3H), 3.36 (t, *J* = 6.6 Hz, 2H), 3.31 (s, 3H) ppm. LRMS *m*/*z* 523.9 $(M + H)^+$.

Preparation of Ethyl 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxylate (9b). To toluene (400 mL) was added compound **7b** (30 g, 0.117 mol), compound **8** (29.9 g, 0.078 mol), and triethylamine

(74 mL, 0.53 mol), and the mixture was gently heated at the reflux temperature for 24 h. The reaction mixture was cooled, quenched with water (200 mL), extracted with ethyl acetate (2 × 200 mL), washed with brine (100 mL), and dried (Na_2SO_4). Evaporation of the solvent afforded a black oil, which was directly purified by silica gel column chromatography (1:1 hexane/ethyl acetate). The cycloadduct morpholine intermediate obtained was directly treated with trifluoroacetic acid (50 mL) in CH_2Cl_2 (500 mL) for 24 h. The reaction mixture was concentrated and quenched with water (100 mL), and the organics were extracted with ethyl acetate (2 × 100 mL), washed with brine (100 mL), dried (Na_2SO_4), and concentrated to the desired compound, which was pure enough for the next step (28.8 g, 71%). ^{1}H NMR ($CDCl_3$) δ: 7.70 (d, J = 8.80 Hz, 2H), 7.46 (d, J = 8.80 Hz, 2H), 7.08 (d, J = 8.80 Hz, 2 H), 6.92 (d, J = 9.20 Hz, 2H), 4.49 (q, J = 6.90 Hz, 2H), 4.12 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.35 (t, J = 6.60 Hz, 2H) ppm. LRMS *m*/*z* 517.9 (M + H)$^+$.

Preparation of 4′-[3-Methanesulfonyl-1-(4-methoxyphenyl)-7-oxo-1,4,5,7-tetrahydropyrazolo[3,4-*c*]pyridin-6-yl]biphenyl-2-carbaldehyde (10a). To the methylsulfone pyrazole **9a** (0.41 g, 0.78 mmol), 2-formylbenzeneboronic acid (0.18 g, 1.10 mmol), and 2 M Na_2CO_3 (0.8 mL 1.4 mmol) were added toluene (30 mL) and EtOH (20 mL). After the mixture was degassed with N_2, tetrakistriphenylphosphinepalladium(0) (0.05 g, 0.004 mmol) was added and the mixture was heated at the reflux temperature for 24 h. The mixture was cooled and concentrated. The residue was dissolved in EtOAc (50 mL), washed with water (25 mL) and brine (25 mL), and dried ($MgSO_4$). Purification by chromatography on silica gel with 1:1 hexanes/EtOAc afforded 0.28 g (80%) of aldehyde **10a** as a gray solid. ^{1}H NMR ($CDCl_3$) δ: 9.99 (s, 1H), 8.04 (m, 1H), 7.67 (dt, J = 1.40, 7.30 Hz, 1H), 7.53 (m, 1H), 7.50 (d, J = 9.20 Hz, 2H), 7.44 (m, 5H), 6.96 (d, J = 9.20 Hz, 2H), 4.24 (t, J = 6.60 Hz, 2H), 3.83 (s, 3H), 3.41 (t, J = 6.60 Hz, 2H), 3.33 (s, 3H) ppm. ESIMS *m*/*z* 524.0 (M + Na)$^+$.

Preparation of Ethyl 6-(2′-Formylbiphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxylate (10b). To the pyrazole ester **9b** (2.1 g, 4.0 mmol), was added 2-formylbenzeneboronic acid (0.91 g, 6.1 mmol), 2 M Na_2CO_3 (4 mL, 8.0 mmol), toluene (50 mL), and EtOH (10 mL). The mixture was degassed with nitrogen for 0.25 h followed by the addition of tetrakis(triphenylphosphine)palladium(0) (0.1 g, 0.09 mmol), and the reaction mixture was heated at the reflux temperature for 24 h. The mixture was cooled and concentrated in vacuo. The residue was dissolved in EtOAc (50 mL) and washed with water and brine (50 mL) and dried ($MgSO_4$). Purification by silica gel chromatography (1:1 hexanes/EtOAc) afforded the title biarylaldehyde compound **10b** (1.6 g, 80%) as an orange solid. ^{1}H NMR ($CDCl_3$) δ: 9.97 (s, 1H), 8.03 (dd, J = 1.10,7.70 Hz, 1H), 7.66 (dt, J = 1.10, 7.30 Hz, 1H), 7.52 (m, 2H), 7.50 (d, J = 8.80 Hz, 2H), 7.43 (d, J = 9.80 Hz, 2H), 7.42 (m, 2H), 6.94 (d, J = 8.80 Hz, 2H), 4.51 (q, J = 7.0 Hz, 2H), 4.43 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.39 (t, J = 6.60 Hz, 2H), 1.46 (t, J = 7.30 Hz, 3H) ppm. ESIMS *m*/*z* 496.18 (M + H)$^+$.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)-biphenyl-4-yl)-1-(4-methoxyphenyl)-3-(methylsulfonyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4H)-one (11a). To the biarylcarboxaldehyde intermediate **10a** (96 mg,0.19 mmol) in MeOH (4 mL) was added 3-(*R*)-pyrrolidinol (50 mg, 0.57 mmol), and the mixture was stirred for 15 min. A solution of 0.5 M $ZnCl_2$ in THF (0.19 mL) and sodium cyanoborohydride (12 mg, 0.19 mmol) were added sequentially, and the reaction mixture was stirred at room temperature for 24 h. The mixture was concentrated, and the residue was quenched with water (25 mL). The aqueous layer was extracted with EtOAc (2 × 25 mL), washed with water (25 mL) and brine (25 mL), and dried ($MgSO_4$). Filtration, concentration, and purification by reverse-phase HPLC (acetonitrile/water/0.05% TFA) followed by lyophilization of the pure fraction afforded the title compound **11a** as a colorless solid (61 mg, 46.9%). HPLC purity, >95%. ^{1}H NMR (MeOD) δ: 7.57 (bs, 1H), 7.45−7.41 (m, 6 H), 7.29 (m, 3 H), 6.93 (d, J = 9.09 Hz, 2 H), 4.47 (m, 1 H), 4.34 (m, 2 H), 4.13 (t, J = 6.60 Hz, 2H), 3.75 (s, 3H), 3.49 (m 2H), 3.26 (t, J = 6.60 Hz, 2H), 3.22 (s, 3H), 3.11−2.74 (m, 2H), 2.07−1.73 (m, 2H) ppm. HRMS calculated for $C_{31}H_{33}N_4O_5S$ (M + H)$^+$ 573.2172; found 573.2180. Anal. Calcd for $C_{31}H_{32}N_4O_5S$•1.5TFA: C, 54.91, H, 4.54, N, 7.53. Found: C, 54.84, H, 4.78, N, 7.63.

Preparation of (*R*)-Ethyl 6-(2′-((3-Hydroxypyrrolidin-1-yl)-methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxylate (11b). To an EtOH (1 mL) solution of compound **10b** (62 mg, 0.13 mmol) was added 3-(*R*)-pyrrolidinol (10 mg, 0.13 mmol), and the reaction mixture was stirred for 0.25 h. To this mixture was added $ZnCl_2$ (0.5 M inTHF, 0.1 mL) followed by sodium cyanoborohydride (5 mg, 0.07 mmol), and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was quenched with water (25 mL), and the organics were extracted with EtOAc (2 × 25 mL), washed with water (25 mL) and brine (25 mL), and dried ($MgSO_4$). Purification by reverse-phase HPLC (acetonitrile/water/0.05% TFA) and lyophilization of the pure fraction afforded the desired compound **11b** as a colorless solid (51 mg, 69%). HPLC purity, >95%. ^{1}H NMR (MeOD) δ: 7.57 (s, 1H), 7.45−7.39 (m, 6H), 7.31 (m, 3H), 6.92 (d, J = 9.1 Hz, 2 H), 4.46−4.38 (m, 2H), 4.36 (q, J = 7.0 Hz, 2H), 4.12 (t, J = 6.6 Hz, 2H), 3.74 (s, 3H), 3.48−3.28 (m, 3H), 3.27 (t, J = 6.6 Hz, 2H), 3.16−2.75 (m, 2H), 2.06−1.75 (m, 2H), 1.34 (t, J = 7.0 Hz, 3H) ppm. HRMS calculated for $C_{33}H_{35}N_4O_5$ (M + H)$^+$ 567.2607; found 567.2612. Anal. Calcd for $C_{33}H_{34}N_4O_5$•1.0TFA•1.0H_2O: C, 60.17, H, 5.34, N, 8.02. Found: C, 59.99, H, 5.47, N, 8.21.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)-biphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxylic Acid (12). Compound **11b** (0.12 g, 0.2 mmol) was dissolved in THF (6 mL). To this solution was added MeOH (2 mL) and water (0.5 mL) followed by LiOH (20 mg, 0.43 mmol), and the reaction mixture was stirred at room temperature for 24 h. The mixture was concentrated and purified by reverse-phase HPLC (acetonitrile/water/0.05% TFA), and lyophilization of the pure fraction afforded the title compound **12** as a colorless solid (70 mg, 50.7%). HPLC purity, >95%. ^{1}H NMR (MeOD) δ: 7.57 (bs, 1H), 7.45 (t, J = 3.5 Hz, 2H), 7.41 (m, 4H), 7.31 (m, 3H), 6.92 (d, J = 9.1 Hz, 2 H), 4.43−4.24 (m, 2H), 4.12 (t, J = 6.6 Hz, 2H), 3.74 (s, 3H), 3.28 (t, J = 6.6 Hz, 2H), 3.11−2.77 (m, 2H), 2.07−1.75 (m, 2H) ppm. HRMS calculated for $C_{31}H_{31}N_4O_5$ (M + H)$^+$ 539.2294; found 539.2282. Anal. Calcd for $C_{31}H_{30}N_4O_5$•1.4TFA•1.1H_2O: C, 56.40, H, 4.73, N, 7.78. Found: C, 56.60, H, 4.23, N, 7.73.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)-biphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (13f). To the biarylaldehyde intermediate **10b** (0.266 g, 0.53 mmol) were added EtOH (3 mL) and 3-(*R*)-pyrrolidinol (0.132 g, 1.0 mmol), and the mixture was stirred at room temperature for 0.5 h. Sodium cyanoborohydride (34 mg, 0.53 mmol) and THF (5 mL) were added, and the mixture was stirred at room temperature for 24 h. The mixture was concentrated in vacuo to afford the crude intermediate **11b**. ESIMS *m*/*z* 567 (M + H)$^+$. The crude **11b** was transferred to a sealed tube, and concentrated NH_4OH (5 mL) and ethanol (5 mL) were added. The reaction mixture was sealed and stirred at room temperature for 4 days. Evaporation of the solvents in vacuo afforded an oil, which was dissolved in EtOAc (50 mL), washed with water (25 mL) and brine (25 mL), and dried (Na_2SO_4). Purification by reverse-phase HPLC (acetonitrile/water with 0.05% TFA) and lyophilization afforded the desired compound **13f** as a colorless solid (0.126 g, 45%). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 7.78 (s, 2H), 7.53 (m, 6H), 7.39 (d, J = 8.40 Hz, 2H), 7.34 (m, 1H), 7.02 (d, J = 7.0 Hz, 2H), 4.49 (d, J = 8.40 Hz, 2H), 4.38 (m, 1H), 4.30 (m, 1H), 4.14 (t, J = 6.30 Hz, 2H), 3.81 (s, 3H), 3.50 (m, 2H), 3.26 (t, J = 6.20 Hz, 2H), 3.20−2.85 (m, 3H), 2.10−1.85 (m, 3H) ppm. ESIMS *m*/*z* 538.12 (M + H)$^+$. HRMS calculated for $C_{31}H_{32}N_5O_4$ (M + H)$^+$ 538.2454; found 538.2456.

Preparation of 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxylic Acid (14a). To a methanol (15 mL) and THF (20 mL) solution of the pyrazole ester compound **9b** (12 g, 23.0 mmol) was added 1 N NaOH (25 mL), and the mixture was stirred for 24 h. The organic solvents were stripped, and the aqueous solution was extracted with Et_2O (2 × 100 mL). The aqueous layer was acidified with concentrated HCl and extracted with EtOAc (2 × 100 mL). The EtOAc layer was dried with (Na_2SO_4), filtered, and concentrated to a brown solid (10.2 g, 90%). ^1H NMR ($CDCl_3$) δ: 7.68 (d, J = 8.40 Hz, 2H), 7.49 (d, J = 8.80 Hz, 2H), 7.10 (d, J = 8.80 Hz, 2H), 6.91 (d, J = 8.70 Hz, 2H), 4.12 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.34 (t, J = 6.60 Hz, 2H) ppm. ESIMS m/z 490.26 $(M + H)^+$.

Preparation of 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-*N*-methyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (14b). To a cold (0 °C) solution of methylamine (2 M in THF, 0.64 mL, 1.29 mmol) in CH_2Cl_2 (10 mL) was added trimethylaluminum (2 M in toluene, 0.64 mL, 1.29 mmol), and the reaction mixture was stirred cold for 0.25 h. To this solution was added compound **9b** (0.16 g, 0.32 mmol), and the reaction mixture was gradually allowed to warm to room temperature and stirred at this temperature for 72 h. The reaction was quenched with HCl (1 N, 50 mL), and the mixture was extracted with CH_2Cl_2 (2 × 50 mL), washed with brine (50 mL), and dried (Na_2SO_4). Filtration followed by purification via silica gel column chromatography (EtOAc/hexanes, 1:4) afforded compound **14b** (53 mg, 92%). ^1H NMR ($CDCl_3$) δ: 7.66 (d, J = 8.59 Hz, 2H), 7.45 (d, J = 8.84 Hz, 2H), 7.08 (d, J = 8.59 Hz, 2H), 6.95 (d, J = 8.84 Hz, 2H), 4.09 (t, J = 6.60 Hz, 2H), 3.82 (s, 3H), 3.40 (t, J = 6.60 Hz, 2H) ppm. ESIMS m/z 518.0 $(M + H)^+$.

Preparation of 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-*N*,*N*-dimethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (14c). To a cold (0 °C) dimethylamine (2 M in THF, 0.68 mL, 1.3 mmol) solution were added CH_2Cl_2 (10 mL) and trimethylaluminum (2 M in toluene, 0.68 mL, 1.3 mmol), and the mixture was stirred cold for 0.25 h. To this cold solution was added compound **9b** (0.17 g, 0.34 mmol), and the mixture was gradually allowed to warm to room temperature and stirred at this temperature for 72 h. The reaction mixture was quenched with HCl (1 N), and the organics were extracted with CH_2Cl_2 (2 × 50 mL), washed with brine (50 mL), and dried (Na_2SO_4). Filtration, concentration, and purification via silica gel chromatography using (EtOAc/hexanes, 9:1, as eluent) afforded the desired compound **14c** (0.154 g, 88%). ^1H NMR ($CDCl_3$) δ: 7.68 (d, J = 8.58 Hz, 2H), 7.46 (d, J = 8.84 Hz, 2H), 7.09 (d, J = 8.55 Hz, 2H), 6.93 (d, J = 8.84 Hz, 2H), 4.09 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.44 (s, 3H), 3.30 (t, J = 6.60 Hz, 2H), 3.13 (s, 3H) ppm. ESIMS m/z 517.1 $(M + H)^+$.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)-biphenyl-4-yl)-1-(4-methoxyphenyl)-*N*-methyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (15a). To a DME/water solution (4:1, 2 mL) were added compound **14b** (45 mg, 0.089 mmol), 2-formylphenylboronic acid (27 mg, 0.18 mmol), and potassium carbonate (49 mg, 0.35mmol). The mixture was degassed with nitrogen for 0.25 h followed by the addition of tetrakistriphenylphosphinepalladium (5 mg), and the reaction mixture was heated at 65 °C for 24 h. The reaction mixture was quenched with water (25 mL), and the aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with water (25 mL) and brine (25 mL) and dried ($MgSO_4$). The crude product was purified via silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:9, as eluent) to afford the requisite biarylcarboxaldehyde intermediate (34 mg, 79%). ^1H NMR ($CDCl_3$) δ: 9.99 (s, 1H), 8.02 (d, J = 7.84 Hz, 2H), 7.64 (t, J = 7.58 Hz, 1H), 7.51−7.37 (m,7H), 6.97 (m, 1H), 6.95 (d, J = 8.84 Hz, 2H), 4.20 (t, J = 6.60 Hz, 2H), 3.82 (s, 3H), 3.46 (t, J = 6.60 Hz, 2H), 3.01 (d, J = 5.05 Hz, 3H) ppm. ESIMS m/z 481.16 $(M + H)^+$. To 6-(2′-formylbiphenyl-4-yl)-1-(4-methoxyphenyl)-*N*-methyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide obtained as described above (34 mg, 0.07 mmol) in MeOH (4 mL) was added 3-(*R*)-pyrrolidinol (12 mg, 0.13 mmol), and the reaction mixture was stirred for 0.25 h. To this solution was added sodium cyanoborohydride (5 mg, 0.079 mmol) and stirring was continued for an additional 24 h. The mixture was concentrated and quenched with water (25 mL), and the organics were extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with water (25 mL) and brine (25 mL) and dried ($MgSO_4$). Filtration and concentration afforded an oil, which was purified via reverse-phase HPLC (acetonitrile/water/0.05% TFA). Lyophilization of the pure fraction afforded the desired product **15a** as a colorless solid (25 mg, 55%). HPLC purity, >95%. ^1H NMR (MeOD) δ: 7.69 (m, 1H), 7.58−7.48 (m, 6 H), 7.41−7.39 (m, 3H), 7.00 (d, J = 8.84 Hz, 2 H), 4.39 (m, 2 H), 4.19 (t, J = 6.60 Hz, 2H), 3.86 (s, 3H), 3.50 (m 2H), 3.40 (t, J = 6.60 Hz, 2H), 3.27 (m, 3H), 3.00 (m, 3H), 2.25−1.80 (m, 3H) ppm. HRMS calculated for $C_{32}H_{34}N_4O_4$ 552.2611 $(M + H)^+$; found 552.2599.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)-biphenyl-4-yl)-1-(4-methoxyphenyl)-*N*,*N*-dimethyl-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (15b). To a DME/water solution (4:1, 2 mL) were added compound **14c** (76 mg, 0.147 mmol), 2-formylphenylboronic acid (44 mg, 0.29 mmol), and potassium carbonate (81 mg, 0.59 mmol). The reaction mixture was degassed under nitrogen for 0.25 h followed by the addition of tetrakistriphenylphosphinepalladium (5 mg). The combined solution was heated (65 °C) for 24 h. The reaction was quenched with water (50 mL), and the organics were extracted with EtOAc (2 × 50 mL), washed with brine (50 mL), and dried ($MgSO_4$). The crude product was purified via silica gel column chromatography (EtOAc/hexanes, 1:9, as eluent) to afford the corresponding carboxaldehyde intermediate (61 mg, 84.7%). ^1H NMR ($CDCl_3$) δ: 9.99 (s, 1H), 8.02 (d, J = 8.84 Hz, 1H), 7.65 (t, J = 8.80 Hz, 1H), 7.51−7.37 (m, 8H), 6.95 (d, J = 8.84 Hz, 2H), 4.20 (t, J = 6.60 Hz, 2H), 3.82 (s, 3H), 3.46 (s, 3H), 3.36 (t, J = 6.60 Hz, 2H), 3.15 (s, 3H) ppm. ESIMS m/z 495.18 $(M + H)^+$. To the carboxaldehyde product (61 mg, 0.12 mmol) in MeOH (4 mL) was added 3-(*R*)-pyrrolidinol (22 mg, 0.25 mmol), and the mixture was stirred for 0.25 h. To this solution was added sodium cyanoborohydride (8 mg, 0.127 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water (25 mL), and the organics were extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (25 mL) and dried ($MgSO_4$). Filtration and concentration afforded an oil, which was purified via reverse-phase HPLC (acetonitrile/water/0.05% TFA) followed by lyophilization of the pure fraction to afford the desired product **15b** as a colorless solid (38.2 mg, 45.7%). HPLC purity, >95%. ^1H NMR (MeOD) δ: 7.69 (m, 1H), 7.58−7.48 (m, 6 H), 7.41−7.39 (m, 3H), 7.00 (d, J = 8.84 Hz, 2 H), 4.39 (m, 2 H), 4.19 (t, J = 6.60 Hz, 2H), 3.86 (s, 3H), 3.50 (m 2H), 3.40 (s, 3H), 3.25 (t, J = 6.60 Hz, 2H), 3.15 (s, 3H), 3.00 (m, 3H), 2.20−1.80 (m, 3H) ppm. HRMS calculated for $C_{33}H_{36}N_5O_4$ 556.2767 $(M + H)^+$; found 556.2773. Anal. Calcd for $C_{33}H_{36}N_6O_4$•1.2TFA•1.0H_2O: C, 59.01, H, 5.34, N, 9.72. Found: C, 59.05, H, 4.98, N, 9.65.

Preparation of 6-(2′-Formylbiphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (16). Compound **10b** (200 mg, 0.40 mmol) was dissolved in methanol (50 mL). This solution was saturated with ammonia, and the reaction mixture was sealed tight. The reaction mixture was stirred at 80 °C overnight and quenched with water (75 mL). The product precipitated out and was filtered and washed with excess water and dried under vacuum to afford the desired product (120 mg, 66%). ^1H NMR ($CDCl_3$) δ: 9.97 (s, 1H), 8.03 (dd, J = 1.10, 7.70 Hz, 1H), 7.66 (dt, J = 1.10, 7.30 Hz, 1H), 7.52 (m, 2H), 7.50 (d, J = 8.80 Hz, 2H), 7.43 (d, J = 9.80 Hz, 2H), 7.42 (m, 2H), 6.94 (d, J = 8.80 Hz, 2H), 6.50 (bs, 1H), 5.85 (bs, 1H), 4.43 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.39 (t, J = 6.60 Hz, 2H) ppm. ESIMS m/z 467 $(M + H)^+$.

Preparation of 6-(2′-Formylbiphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carbonitrile (17). To an acetonitrile (5 mL) solution was added oxalyl chloride (0.070 mL, 0.8 mmol), and the mixture was cooled in an ice bath. DMF (70 mg, 0.96 mmol) was added, and the mixture was stirred until gas evolution ceased. To this mixture were added compound **16** (0.15 g, 0.33 mmol) and pyridine (0.127 g, 1.6mmol), and the mixture turned deep-red and was stirred at room temperature for 2 h. The solvents were removed in vacuo, and the residual oil was directly purified via silica gel column chromatography (MeOH/CH_2Cl_2, 90.5:9.5, as eluent) to afford the desired product **17** as a tan solid (64 mg, 42%). ^{1}H NMR ($CDCl_3$) δ: 9.92 (s, 1H), 7.95 (d, J = 6.57 Hz, 1H), 7.43 (t, J = 7.58 Hz, 1H), 7.42 (m, 4H), 7.40 (m, 5H), 6.89 (d, J = 8.80 Hz, 2H), 4.17 (t, J = 6.60 Hz, 2H), 3.75 (s, 3H), 3.16 (t, J = 6.60 Hz, 2H) ppm. ESIMS *m/z* 449.04 $(M + H)^+$.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)-biphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carbonitrile (18). To a MeOH (6 mL) and THF (4 mL) solution were added compound **17** (60 mg, 0.12 mmol) and 3-(*R*)-pyrrolidinol (22 mg, 0.25 mmol), and the mixture was stirred for 1 h. To this solution was added sodium cyanoborohydride (11 mg, 0.16 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water (25 mL), and the organics were extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with water (25 mL) and brine (25 mL) and dried ($MgSO_4$). Filtration followed by concentration afforded an oily residue, which was purified via reverse-phase HPLC (acetonitrile/water/0.05%TFA) followed by lyophilization of the pure fraction to afford the desired product **18** as a colorless solid (22 mg, 26.8%). HPLC purity, >95%. ^{1}H NMR (MeOD) δ: 7.68 (m, 1H), 7.57−7.48 (m, 6 H), 7.41 (m, 3H), 7.00 (d, J = 8.80 Hz, 2 H), 4.40 (m, 2 H), 4.23 (t, J = 6.60 Hz, 2H), 3.85 (s, 3H), 3.50 (m 2H), 3.22 (t, J = 6.60 Hz, 2H), 3.00 (m, 3H), 2.20−1.80 (m, 3H) ppm. HRMS calculated for $C_{31}H_{30}N_5O_3$ 520.2349 $(M + H)^+$; found 520.2350.

Preparation of *tert*-Butyl 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridin-3-ylcarbamate (19a). To a CH_2Cl_2 (25 mL) solution of the pyrazole acid **14b** (1.19 g, 2.4 mmol) were added oxalyl chloride (0.3 mL, 3.4 mmol) and 2 drops of DMF, and the reaction mixture was stirred at room temperature for 24 h. The mixture was concentrated and then dissolved in acetone (20 mL) and added to a cold (0 °C) solution of NaN_3 (0.47 g, 7.3 mmol) in water (20 mL). After 20 min the acylazide was extracted with EtOAc (2 × 25 mL), washed with brine (25 mL), and dried (Na_2SO_4). The acylazide intermediate was dissolved in toluene (20 mL) and heated gently at 80 °C for 1 h. To this hot solution was then syringed in *tert*-butyl alcohol (25 mL), and the reaction mixture was heated at 80 °C for an additional 24 h. The reaction mixture was cooled, concentrated, and partitioned between EtOAc (25 mL) and water (25 mL). The layers were separated, and the aqueous layer was further extracted with EtOAc (25 mL) and dried (Na_2SO_4). Purification via silica gel column chromatography (2:1 hexanes/EtOAc as eluent) afforded the title compound **19a** as a tan foam (0.3 g, 22%). ^{1}H NMR ($CDCl_3$) δ: 7.68 (d, J = 8.40 Hz, 2H), 7.41 (d, J = 9.20 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 9.20 Hz, 2H), 6.75 (s, 1H), 4.07 (t, J = 6.60 Hz, 2H), 3.80 (s, 3H), 3.15 (t, J = 6.60 Hz, 2H), 1.52 (s, 9H) ppm. ESIMS *m/z* 561.2 $(M + H)^+$.

Preparation of 3-(Dimethylamino)-6-(4-iodophenyl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (19b). To a CH_2Cl_2 (20 mL) solution of **19a** (0.45 g, 0.80 mmol) was added TFA (4 mL), and the reaction mixture was stirred for 24 h at room temperature. The solvents were evaporated under high vacuum to dryness. The crude deprotected pyrazole amine was dissolved in MeOH/THF (1:1, 6 mL). To this solution was added formaldehyde (37% in water, 0.24 mL, 2.40 mmol), and the mixture was stirred for 15 min. Then zinc chloride in THF (0.5 M, 0.8 mL, 0.4 mmol) was added followed by sodium cyanoborohydride (50 mg, 0.80 mmol), and the reaction mixture was stirred for 24 h. The solvent was evaporated, and the residue was dissolved in EtOAc (100 mL), washed with water (50 mL) and brine (50 mL), and dried ($MgSO_4$). Purification by silica gel column chromatography (1:1 hexanes/ethyl acetate as eluent) afforded the desired product **19b** as a yellow foam (0.20 g, 51%). ^{1}H NMR ($CDCl_3$) δ: 7.66 (d, J=8.80 Hz, 2H), 7.42 (d, J=4.04 Hz, 2H), 7.08 (d, J= 8.80 Hz, 2H), 6.89 (d, J=9.10 Hz, 2H), 4.05 (t, J= 6.60 Hz, 2H), 3.78 (s, 3H), 3.06 (t, J= 6.60 Hz, 2H), 2.91 (s, 6H) ppm. ESIMS *m/z* 489.2 $(M + H)^+$.

Preparation of *tert*-Butyl 6-(2′-Formylbiphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]-pyridin-3-ylcarbamate (20). To a toluene (20 mL) and EtOH (10 mL) solution were added compound **19a** (0.30 g, 0.53 mmol), 2-formylbenzeneboronic acid (0.12 g, 8.0 mmol), and 2 M Na_2CO_3 (1.1 mL, 2.0 mmol). The reaction mixture was degassed with nitrogen for 0.25 h, followed by the addition of tetrakistriphenylphosphinepalladium (30 mg, 0.03 mmol). The reaction mixture was heated at the reflux for 24 h, cooled, and concentrated in vacuo. The resulting residue was dissolved in EtOAc (50 mL), washed with water (25 mL) and brine (25 mL), and dried ($MgSO_4$). Purification by silica gel chromatography (1:1 hexanes/ EtOAc) afforded the title compound **20** as a yellow solid (27 g, 93%). HPLC purity, >95%. ^{1}H NMR ($CDCl_3$) δ: 10.11 (s, 1H), 8.03 (dd, J = 1.1, 7.7 Hz, 1H), 7.64 (dt, J = 1.4, 7.3 Hz, 1H), 7.49 (t, J = 7 Hz, 1H), 7.42 (m, 7H), 6.92 (d, J = 9.2 Hz, 2H), 6.79 (s, 1H), 4.17 (t, J = 6.6 Hz, 2H), 3.81 (s, 3H), 3.17 (t, J = 6.6 Hz, 2H), 1.53 (s, 9H) ppm. ESIMS *m/z* 539.29 $(M + H)^+$.

Preparation of (*R*)-3-Amino-6-(2′-((3-hydroxypyrrolidin-1-yl)-methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (22). To the pyrazole intermediate **20** (58 mg, 0.10 mmol) were added MeOH (3 mL) and 3-(*R*)-pyrrolidinol (0.132 g, 0.10 mmol), and the mixture was stirred for 15 min. Zinc chloride (0.5M in THF, 0.1 mL, 0.05 mmol) was added followed by sodium cyanoborohydride (7 mg, 0.10 mmol), and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated in vacuo, and the residue was dissolved in EtOAc (25 mL), washed with water (25 mL) and brine (25 mL), and dried (Na_2SO_4). Purification by reverse-phase HPLC (acetonitrile/water/0.05%TFA gradient) followed by freeze-drying afforded compound **21** as a colorless solid (60 mg, 77%). HPLC purity, >95%. ^{1}H NMR ($CDCl_3$)δ: 7.90 (m, 1H), 7.50−7.42 (m, 7H), 7.27 (m, 1H), 7.24 (d, J = 8.10 Hz, 2H), 6.93 (d, J = 8.80 Hz, 2H), 6.90 (s, 1H), 6.80 (s, 1H), 4.45 (m, 3H), 4.14 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.75 (m, 1H), 3.45 (m, 1H), 3.20 (t, J = 6.60 Hz, 2H), 2.6−2.10 (m, 3H), 1.53 (s, 9H) ppm. ESIMS *m/z* 610.12 $(M + H)^+$. HRMS calculated for $C_{35}H_{40}N_5O_5$ $(M + H)^+$ 610.3025; found 610.3025. Compound **21** (20 mg, 0.03 mmol) was dissolved in dichloromethane (20 mL), and to this solution was added TFA (0.5 mL). The reaction mixture was stirred at room temperature for 1 h. The solution was concentrated in vacuo, and the residue was directly purified directly via reverse-phase HPLC (acetonitrile/water/0.05%TFA). Lyopilization of the pure fraction afforded the desired product **22** as a colorless solid (1.5 mg, 10%). HPLC purity, >95%. ^{1}H NMR (MeOD) δ: 7.45 (m, 1H), 7.44−7.27 (m, 9H), 6.85 (d, J = 9.10 Hz, 2 H), 4.35−4.29 (m, 2H), 4.07 (t, J = 6.60 Hz, 2H), 3.71 (s, 3H), 3.39−3.29 (m, 3H), 3.08 (m,-1H), 2.87(t, J = 6.60 Hz, 2H), 2.06−1.78 (m, 2H) ppm. HRMS calculated for $C_{30}H_{32}N_5O_3$ 510.2505 $(M + H)^+$; found 510.2507.

Preparation of (*R*)-*tert*-Butyl 6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl(methyl)carbamate (23). To a DMF (4 mL) solution stirred at 0 °C was added the pyrazole intermediate **20** (0.2 g, 0.37 mmol), followed by NaH (60%, 22 mg, 0.55 mmol). After the mixture was stirred for 0.5 h, iodomethane (0.051 mL, 0.82 mmol) was added. The reaction mixture was stirred at room temperature for 24 h, then quenched with water (50 mL), extracted with EtOAc (2 × 25 mL), washed with water (25 mL) and brine (25 mL), and dried ($MgSO_4$). The crude methylated product was obtained as a colorless oil. MS *m/z* 575.3 $(M + H)^+$. The aldehyde was subjected to reductive amination with 3-(*R*)-pyrrolidinol as previously described with sodium cyanoborohydride, and the product obtained was purified directly via reverse-phase HPLC (acetonitrile/water/0.05%TFA). Lyophilization of the product afforded the title compound **23** as a colorless solid (115 mg, 42%). HPLC purity, >95%. ^{1}H NMR ($CDCl_3$) δ: 7.90 (m, 1H), 7.50−7.42 (m, 7H), 7.27 (m, 1H), 7.24 (d, J = 8.10 Hz, 2H), 6.93 (d, J = 8.80 Hz, 2H), 4.45 (m, 3H), 4.10 (m, 2H), 3.81 (s, 3H), 3.75 (m, 1H), 3.45 (m, 1H), 3.37 (s, 3H), 3.05 (t, J = 6.60 Hz, 2H), 2.6−2.10 (m, 3H), 1.53 (s, 9H) ppm. ESIMS *m/z* 624.12 $(M + H)^+$. HRMS calculated for $C_{36}H_{42}N_5O_5$ $(M + H)^+$ 624.3186;

found 624.3175. Anal. Calcd for $C_{36}H_{41}N_5O_5$·1.2TFA: C, 60.42, H, 5.59, N, 9.21. Found: C, 60.82, H, 5.31, N, 8.99.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-3-(methylamino)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (24). Compound **23** was stirred in dichloromethane (5 mL) with TFA (0.5 mL) until the deprotection was complete. The mixture was concentrated in vacuo and directly purified via reverse-phase HPLC (acetonitrile/water/0.05% TFA) and lyophilized to the title compound **24** as a colorless solid (86 mg, 99%). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 7.74 (m, 1H), 7.53 (m, 2H), 7.48 (d, J = 8.40 Hz, 2H), 7.38 (m, 6H), 6.93 (d, J = 8.80 Hz, 2H), 4.49 (d, J = 5.50 Hz, 1H), 4.35 (m, 1H), 4.30 (m, 1H), 4.10 (t, J = 6.60 Hz, 2H), 3.77 (s, 3H), 3.60−2.95 (m, 4H), 2.10−1.75 (m, 3H) ppm. ESIMS m/z 524.16 (M + H)$^+$. HRMS calculated for $C_{31}H_{34}N_5O_3$ (M + H)$^+$ 524.2662; found 524.2656. Anal. Calcd for $C_{31}H_{33}N_5O_3$·1.0TFA: C, 63.86, H, 5.38, N, 11.22. Found: C, 63.59, H, 5.39, N, 11.11.

Preparation of (*R*)-3-(Dimethylamino)-6-(2′-((3-hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (25). The title compound was prepared in 34% yield from compound **19b** following the procedure employed for compound **21**. HPLC purity, >95%. ^{1}H NMR (CDCl$_3$) δ: 7.80 (m, 1H), 7.48 (m, 7H), 7.28 (m, 2H), 6.93 (dd, J = 7.0, 8.80 Hz, 2H), 4.48−4.21 (m, 3H), 4.18 (q, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.44 (m, 1H), 3.39 (m, 1H), 3.18 (t, J = 6.60 Hz, 2H), 3.08 (s, 6H), 2.80 (m, 1H), 2.70 (m, 1H), 2.20 (m, 1H), 2.05 (m, 1H), 1.85 (m, 1H) ppm. ESIMS m/z 538.19 (M + H)$^+$. HRMS calculated for $C_{32}H_{36}N_5O_3$ (M + H)$^+$ 538.2818; found 538.2813. Anal. Calcd for $C_{32}H_{35}N_5O_3$·2.1TFA: C, 55.95, H, 4.81, N, 9.01. Found: C, 55.89, H, 4.83, N, 9.18.

Preparation of 6-(4-Iodophenyl)-1-(4-methoxyphenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (26). To a dichloromethane (20 mL) solution of **14b** (0.5 g, 0.99 mmol) stirred at 0 °C was added lutidine (0.23 mL, 1.98 mmol), followed by the addition of triflic anhydride (0.33 mL, 1.98 mmol). The reaction mixture was stirred at this temperature for 1 h followed by gradual warming at room temperature for 4 h, at which point the reaction was judged to be complete by TLC. Quenching with cold water (50 mL), followed by extraction of the organics with dichloromethane (2 × 25 mL), drying with MgSO$_4$, and evaporation afforded the crude iminotriflate, which was redissolved in DMF (10 mL). To this solution was then added excess sodium azide (2 g, 30.7 mmol), and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with cold (0 °C) water (100 mL), and the organics were extracted with EtOAc (2 × 50 mL), washed with brine (50 mL), and dried (MgSO$_4$). Evaporation of the solvents gave a crude product, which was directly purified via silica gel column chromatography using hexane/ethyl acetate as eluent (1:1). Compound **26** was obtained as a brown solid (0.25 g, 48%). ^{1}H NMR (400 MHz, MeOD) δ: 7.62 (d, J = 8.3 Hz, 2H), 7.52 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.34 Hz, 3 H), 7.05 (d, J = 9.09 Hz, 2 H), 4.47 (s, 3 H), 4.29 (t, J = 6.82 Hz, 2 H), 3.88 (s, 3 H), 3.50 (t, J = 6.60 Hz, 2 H). ESIMS m/z 528.2 (M + H)$^+$.

Preparation of 6-(2′-(((*R*)-3-Hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (27). The title compound was prepared in 47% yield from compound **26** following the procedure employed for compound **21**. HPLC purity, >95%. ^{1}H NMR (400 MHz, MeOD) δ: 7.69 (bs, 1H), 7.52−7.62 (m, 6 H), 7.44 (d, J = 8.34 Hz, 3 H), 7.05 (d, J = 9.09 Hz, 2 H), 4.60 (bs, 1 H), 4.47 (s, 3 H), 4.40−4.46 (m, 2 H), 4.29 (t, J = 6.82 Hz, 2 H), 3.88 (s, 3 H), 3.50 (t, J = 6.60 Hz, 2 H), 3.38−3.61 (m, 2 H), 2.86−3.28 (m, 2 H), 1.84 2.24 (m, 2 H). ESIMS m/z 577.2 (M + H)$^+$. HRMS calculated for $C_{32}H_{33}N_8O_3$ (M + H)$^+$ 577.2601; found 577.2633.

Preparation of (*R*)-6-(2′-((3-Hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-3-(1*H*-tetrazol-5-yl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (28). To compound **18** (18 mg, 0.03 mmol) in DMF (1 mL) was added sodium azide (5 mg, mmol) and ammonium chloride (5 mg, mmol), and the mixture was heated to 80 °C for 72 h. The mixture was concentrated and directly purified by reverse-phase HPLC (acetonitrile/water/0.05% TFA). The pure fraction was lyophilized to afford the desired product **28** as a white solid (10 mg, 52%). HPLC purity, >95%. ^{1}H NMR (MeOD) δ: 7.59 (m, 1H), 7.48−7.41 (m, 5 H), 7.83−7.81 (m, 3H), 6.93 (d, J = 8.84 Hz, 2H), 4.40 (m, 2H), 4.30 (m, 1H), 4.80 (t, J = 6.60 Hz, 2H), 3.75 (s, 3H), 3.38 (t, J = 6.60 Hz, 2H), 3.10 (m, 2H), 2.01−1.76 (m, 2H) ppm. HRMS calculated for $C_{31}H_{31}N_8O_3$ (M + H)$^+$ 563.2519; found 563.2529.

Preparation of 3-(Hydroxymethyl)-6-(4-iodophenyl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (29). To a cold (0 °C) THF (30 mL) solution was added pyrazole 3-carboxylic acid derivative **14a** (4.66 g, 9.50 mmol) followed by the addition of borane tetrahydrofuran solution (1 M, 14.0 mL, 14.0 mmol). The mixture was allowed to stir at room temperature for 18 h and quenched with AcOH (0.5 mL) and water (20 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 75 mL). The combined organic layers were washed with brine (50 mL) and dried (Na$_2$SO$_4$). Filtration and concentration afforded compound **29** (4 g, 89%). ^{1}H NMR (CDCl$_3$) δ: 7.68 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 9.2 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 4.79 (s, 2H), 4.10 (t, J = 6.6 Hz, 2H), 3.80 (s, 3H), 3.09 (t, J = 6.6 Hz, 2H) ppm. ESIMS m/z 476.16(M + H)$^+$.

Preparation of 3-(Bromomethyl)-6-(4-iodophenyl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (30). To a suspension of alcohol **29** (3 g, 6.30 mmol) in CH$_2$Cl$_2$ (50 mL), was added PBr$_3$ (0.89 mL, 8.8 mmol) dropwise. The mixture was stirred at room temperature for 1 h, quenched with cold (0 °C) water (100 mL), extracted with CHCl$_3$ (2 × 50 mL), washed with brine (50 mL), dried (Na$_2$SO$_4$), and concentrated in vacuo to afford the desired product **30** (3.2 g, 94%). ^{1}H NMR (CDCl$_3$) δ: 7.69 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 4.58 (s, 2H), 4.13 (t, J = 6.6 Hz, 2H), 3.82 (s, 3H), 3.08 (t, J = 6.6 Hz, 2H) ppm. The crude material obtained was used directly in the next reaction.

Preparation of 3-((1*H*-1,2,3-Triazol-1-yl)methyl)-6-(4-iodophenyl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (31a) and 3-((2*H*-1,2,3-Triazol-2-yl)methyl)-6-(4-iodophenyl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (31b). To DMF (5 mL) was added 1*H*-1,2,3-triazole (0.1 mL, 2.0 mmol) and NaH (0.1 g, 2.56 mmol) followed by the pyrazole bromide **30** (0.92 g, 1.7 mmol). The mixture was gently heated to 50 °C for 24 h, cooled, quenched with water (25 mL), and extracted with ethyl acetate (2 × 25 mL). The organic layer was dried (MgSO$_4$), filtered, and concentrated to the desired product as a mixture of isomers **31a** and **31b** (89 mg). ESIMS m/z 527.1 (M + H)$^+$. The crude product containing the isomeric triazoles were carried to the next step without purification.

Preparation of (*R*)-3-((1*H*-1,2,3-Triazol-1-yl)methyl)-6-(2′-((3-hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (33a) and (*R*)-3-((2*H*-1,2,3-Triazol-2-yl)methyl)-6-(2′-((3-hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (33b). To the crude mixture of isomeric triazoles **31a** and **31b** (0.89 g, 1.70 mmol) were added 2-formylbenzeneboronic acid (0.38 g, 2.6 mmol), 2 M Na$_2$CO$_3$ (1.7 mL, 3.4 mmol), toluene (30 mL), and EtOH (10 mL). The reaction mixture was vigorously stirred and degassed with nitrogen for 0.25 h, followed by the addition of tetrakis-triphenylphosphinepalladium catalyst (58 mg, 0.03 mmol). The reaction mixture was heated at reflux for 24 h, cooled, and concentrated in vacuo. The residue was dissolved in EtOAc (50 mL), washed with water (25 mL) and brine (25 mL), and dried (MgSO$_4$). Purification by silica gel column chromatography (1:3 hexanes/EtOAc as eluent) afforded the isomeric triazole biarylcarboxaldehyde intermediates **32a**,**b**. The triazole biarylcarboxaldehyde derivatives were subjected to reductive amination with 3-(*R*)-pyrrolidinol, zinc chloride, and sodium cyanoborohydride in anhydrous THF (10 mL) as previously described, to obtain after reverse-phase HPLC purification (acetonitrile/water/0.05% TFA) and lyophilization the desired product **33a**

as a colorless solid (20 mg, 28%). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 8.24 (d, J = 1.0 Hz, 1H), 7.78 (d, J = 1.10 Hz, 1H), 7.76 (m, 1H), 7.53 (m, 6H), 7.38 (d, J = 8.50 Hz, 2H), 7.33 (m, 1H), 6.99 (d,J = 8.70 Hz, 2H), 5.77 (s, 2H), 4.49 (d, J = 5.90 Hz, 1H), 4.38 (m, 1H), 4.28 (m, 1H), 4.10 (t, J = 6.60 Hz, 2H), 3.79 (s, 3H), 3.60−2.90 (m, 3H), 2.91 (t, J = 6.60 Hz, 2H), 2.10−1.75 (m, 3H) ppm. ESIMS m/z 576.19 (M + H)$^+$. HRMS calculated for $C_{33}H_{34}N_7O_3$ (M + H)$^+$ 576.2536; found 576.2726. Anal. Calcd for $C_{33}H_{33}N_7O_3$•1.0TFA•1.0H_2O: C, 59.4, H, 5.13, N, 13.85. Found: C, 59.67, H, 4.87, N, 13.83. Similarly, purification by reverse-phase HPLC (acetonitrile/water/0.05% TFA) and lyophilization afforded the desired product **33b** as a colorless solid (160 mg, 55%). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 7.85 (s, 1H), 7.53 (m, 1H), 7.52−7.38 (m, 5H), 7.37 (d, J = 8.1 Hz, 2H), 7.32 (m, 1H), 6.99 (d, J = 8.8 Hz, 2H), 5.78 (s, 2H), 4.48 (d, J = 5.5 Hz, 1H), 4.38 (m, 1H), 4.28 (m, 1H), 4.09 (t, J = 6.6 Hz, 2H), 3.79 (s, 3H), 3.50 (m, 2H), 3.10−2.80 (m, 3H), 2.87 (t, J = 6.6 Hz, 2H), 2.15−1.75 (m, 3H) ppm. ESIMS m/z 576.19 (M + H). HRMS calculated for $C_{33}H_{34}N_7O_3$ (M + H)$^+$ 576.2723; found 576.2532. Anal. Calcd for $C_{33}H_{33}N_7O_3$•1.0TFA•1.0H_2O: C, 59.40, H, 5.13, N, 13.85. Found: C, 59.87, H, 5.00, N, 13.76.

Preparation of (*R*)-3-((1*H*-1,2,4-Triazol-1-yl)methyl)-6-(2′-((3-hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (33c). The title compound **33c** was also prepared according to the procedure outlined for compounds **33a** and **33b**. Displacement of the pyrazole bromide **30** (0.48 g, 0.89 mmol) in DMF (5 mL) with 1,2,4-triazole (74 mg, 1.0 mmol) and NaH (54 mg, 1.3 mmol) in DMF afforded after purification via silica gel column chromatography compound **31c** (0.42 g crude). ESIMS m/z 527.1 (M + H)$^+$. Treatment of triazole **31c** (0.42 g of crude) with 2-formylphenylboronic acid under Suzuki conditions described previously afforded the requisite biarylcarboxaldehyde intermediate **32c**, which was readily converted to compound **33c** via the reductive amination procedure employed for **33a**,**b**. Purification by reverse-phase HPLC (acetonitrile/water/0.05% TFA) and lyophilization afforded the desired product **33c** as a colorless solid (70 mg, 11% over two steps). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 8.70 (s, 1H), 8,02 (s, 1H), 7.76 (m, 1H), 7.53 (m, 6H), 7.37 (d, J = 8.1 Hz, 2H), 7.33 (m, 1H), 6.99 (d, J = 9.1 Hz, 2H), 5.56 (s, 2H), 4.49 (d, J = 5.5 Hz, 1H), 4.38 (m, 1H), 4.29 (m, 1H), 4.10 (t, J = 6.6 Hz, 2H), 3.79 (s, 3H), 3.55 (m, 2H), 3.10 (m, 1H), 2.91 (d, J = 6.6 Hz, 2H), 2.85 (m, 1H), 2.10−1.80 (m, 3H) ppm. ESIMS m/z 576.19 (M + H)$^+$. HRMS calculated for $C_{33}H_{34}N_7O_3$ (M + H)$^+$ 576.2723; found 576.2714.

Preparation of (*R*)-3-((1*H*-Tetrazol-1-yl)methyl)-6-(2′-((3-hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (33d) and (*R*)-3-((2*H*-Tetrazol-2-yl)methyl)-6-(2′-((3-hydroxypyrrolidin-1-yl)methyl)biphenyl-4-yl)-1-(4-methoxyphenyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (33e). The title compounds **33d and 33e** were also prepared according to the procedure outlined for compounds **33a** and **33b**, via the displacement of the bromide **30** (1.0 g, 1.8 mmol) with 1*H*-tetrazole (0.16 g, 4.4 mmol) and NaH (60%, 0.176 g, 4.30 mmol) in DMF (15 mL). Purification by silica gel column chromatography (1:3 hexanes/EtOAc as eluent) afforded the 1-tetrazole intermediate **31d** (44 mg) and the 2-tetrazole intermediate **31e** (25 mg) for a combined yield of 70%. The tetrazole derivatives **31d**,**e** were converted to the desired products **33d** and **33e** according to the procedures previously described for compounds **33a**,**b**. Purification by reverse-phase HPLC (acetonitrile/water/0.05% TFA) and lyophilization gave the desired product **33d** as a colorless solid (85 mg, 18% over two steps). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 9.58 (s, 1H), 7.76 (m, 1H), 7.53 (m, 4H), 7.45 (d, J = 8.40 Hz, 2H), 7.38 (d, J = 8.50 Hz, 2H), 7.33 (m, 1H), 6.99 (d, J = 8.70 Hz, 2H), 5.88 (s, 2H), 4.49 (d, J = 5.50 Hz, 1H), 4.38 (m, 1H), 4.30 (m, 1H), 4.12 (t, J = 6.60 Hz, 2H), 3.79 (s, 3H), 3.55 (m, 2H), 3.10−2.85 (m, 2H), 2.96 (t, J = 6.60 Hz, 2H), 2.10−1.74 (m, 3H) ppm. ESIMS m/z 577.12 (M + H)$^+$. HRMS calculated $C_{32}H_{33}N_8O_3$ (M + H)$^+$ 577.2588; found 577.2698. Compound **33e** was similarly obtained as a colorless solid after lyophilization (145 mg, 63% over two steps). HPLC purity, >95%. ^{1}H NMR (DMSO-d_6) δ: 9.04 (s, 1H), 7.75 (m, 1H), 7.53 (m, 6H), 7.38 (d, J = 8.00 Hz, 2H), 7.33 (m, 1H), 6.99 (d, J = 9.20 Hz, 2H), 6.12 (s, 2H), 4.49 (d, J = 5.50 Hz, 1H), 4.38 (m, 1H), 4.30 (m, 1H), 4.12 (t, J = 6.60 Hz, 2H), 3.79 (s, 3H), 3.55 (m, 2H), 3.10−2.85 (m, 2H), 2.96 (t, J = 6.60 Hz, 2H), 2.10−1.74 (m, 3H) ppm. ESIMS m/z 577.12 (M + H)$^+$. HRMS calculated for $C_{32}H_{33}N_8O_3$ (M + H)$^+$ 577.2676; found 577.2686. Anal. Calcd for $C_{32}H_{32}N_8O_3$•1.0TFA•1.1H_2O: C, 57.04, H, 4.91, N, 15.56. Found: C, 57.00, H, 4.82, N, 15.58.

Preparation of 6-(4-Aminophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (34). To a slurry consisting of compound **9c** (3.52 g, 6.86 mmol) was added diphenylmethanimine (1.49 g, 8.22 mmol), BINAP catalyst (0.341 g, 5.14 mmol), and sodium *tert*-butoxide (1.99 g, 20.7 mmol). The combined slurry was degassed over nitrogen for 0.5 h followed by the addition of the $Pd_2(dba)_3$ catalyst (0.314 g, 0.343 mmol). The mixture was heated at reflux for 4 h, cooled, and quenched with water (100 mL). The organics were extracted with ethyl acetate (100 mL), dried ($MgSO_4$), and concentrated to an oil. The oil was dissolved in methanol (400 mL), and to this solution was added solid hydroxylamine hydrochloride (1.47 g, 21.15 mmol) and sodium acetate (3.47 g, 42.3 mmol). The reaction mixture was stirred at room temperature for 3 h. Concentration of the reaction in vacuo afforded a thick oil, which was quenched in water (100 mL). The organics were extracted with dichloromethane (2 × 100 mL), dried ($MgSO_4$), filtered, concentrated, and purified directly via silica gel column chromatography (dichloromethane/methanol (9.5:0.5) as eluent) to afford the desired product **34** as a tan solid (2.67 g, 97%). HPLC purity, >95%. ^{1}H NMR ($CDCl_3$) δ: 7.48 (d, J = 8.80 Hz, 2H), 7.07 (d, J = 8.40 Hz, 2H), 6.94 (d, J = 9.20 Hz, 2H), 6.67 (d, J = 8.40 Hz, 2H), 4.07 (t, 2H), 3.82 (s, 2H), 3.68 (bs, 2H), 3.15 (t, 2H) ppm. ^{19}F NMR($CDCl_3$) δ: −61.66 (CF_3). ESIMS m/z 403 (M + H)$^+$.

Preparation of *N*-(4-(1-(4-Methoxyphenyl)-7-oxo-3-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-6(7*H*)-yl)phenyl)-acetamide (35). Compound **34** hydrochloride (0.25 g, 0.56 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0 °C. To this solution was added acetic anhydride (0.063 g, 0.60 mmol) followed by triethylamine (1 mL). The reaction mixture was stirred at room temperature overnight and quenched with HCl (1 N, 25 mL). The organics were extracted with EtOAc (2 × 50 mL), dried ($MgSO_4$), filtered, and concentrated to an oil. Purification via silica gel column chromatography (dichloromethane/methanol (9.5:0.5) as eluent) afforded compound **35** as a brown solid (0.236 g, 97%). HPLC purity, >95%. ^{1}H NMR ($CDCl_3$) δ: 7.66 (bs, 1H), 7.49 (m, 4H), 7.20 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 4.15 (t, 2H), 3.81 (s, 3H), 3.81 (s, 3H), 3.17 (t, 2H), 2.05 (s, 3H) ppm. ESIMS m/z 445.2 (M + H)$^+$.

Preparation of *tert*-Butyl 4-(1-(4-Methoxyphenyl)-7-oxo-3-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-6(7*H*)-yl)phenylcarbamate (36a) and *tert*-Butyl 4-(1-(4-Methoxyphenyl)-7-oxo-3-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-6(7*H*)-yl)phenyl(methyl)carbamate (36b). To dichloromethane (1 mL) was added compound **34** (0.85 g, 2.13mmol) followed by di-*tert*-butyl dicarbonate (0.67 g, 3.19 mmol). The reaction mixture was heated gently to 80 °C overnight, cooled, redissolved in dichloromethane (2 mL), and purified via silica gel column chromatography (dichloromethane/methanol (9.5:0.5) as eluent) to afford compound **36a** as a tan solid (0.89 g, 84%). ^{1}H NMR ($CDCl_3$) δ: 7.48 (d, J = 8.80 Hz, 2H), 7.38 (d, J = 9.00 Hz, 2H), 7.26 (d, J = 9.20 Hz, 2H), 6.94 (d, J = 8.80 Hz, 2H), 4.10 (t, 2H), 3.81 (s, 3H), 3.16 (t, 2H), 1.56 (s, 9H) ppm. ESIMS m/z 503 (M + H)$^+$. HRMS calculated for $C_{25}H_{26}F_3N_4O_4$ 503.1868 (M + H)$^+$; found 526.1873. Compound **36a** (0.89 g, 1.78 mmol) was dissolved in anhydrous THF (20 mL) and cooled to 0 °C. To this cold solution was added NaH (60%, 0.13 g), and the reaction mixture was stirred for 0.5 h followed by the addition of iodomethane (0.37 g, 2.67mmol). The mixture was stirred at 0 °C for 2.5 h and quenched with cold water (100 mL). The organics were extracted with ethyl acetate (2 × 50 mL), dried ($MgSO_4$), filtered, and concentrated to a tan foam. The crude was purified via silica gel column chromatography (DCM/methanol, 9.5:0.5, as eluent) to afford compound **36b** as a tan solid (0.92 g, 100%). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.48 (d, J = 8.80 Hz, 2H), 7.27 (m, 4H), 6.94 (d, J = 8.80 Hz, 2H), 4.15 (t, 2H), 3.81 (s, 3H), 3.81 (s, 3H), 3.23 (s, 3H), 3.18 (t, 2H), 1.45 (s, 9H) ppm. ESIMS m/z 539 (M + Na)$^+$ and 517 (M + H)$^+$. HRMS calculated for $C_{26}H_{28}F_3N_4O_4$ (M + H)$^+$ 517.2132; found 517.2157.

Preparation of 1-(4-Methoxyphenyl)-6-(4-(methylamino)-phenyl)-3-(trifluoromethyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (36c) and *N*-(4-(1-(4-Methoxyphenyl)-7-oxo-3-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-6(7*H*)-yl)phenyl)-*N*-methylacetamide (36d). TFA (1 mL) was added to a dichloromethane (10 mL) solution of compound **36b** (0.80 g, 1.56 mmol). The reaction mixture was stirred at room temperature for 1 h, concentrated, and quenched with a saturated sodium bicarbonate solution (50 mL). The organics were extracted with EtOAc (2 × 50 mL), dried ($MgSO_4$), filtered, and concentrated to a brown oil. The crude oil was purified via silica gel column chromatography (dichloromethane/methanol (9.5:0.5) as eluent) to afford compound **36c** as a brown solid (0.52 g, 84%). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.48 (d, J = 8.80 Hz, 2H), 7.27 (m, 4H), 6.94 (d, J = 8.80 Hz, 2H), 4.15 (t, 2H), 3.81 (s, 3H), 3.81 (s, 3H), 3.23 (s, 3H), 3.18 (t, 2H) ppm. ESIMS m/z 417 (M + H)$^+$. HRMS calculated for $C_{21}H_{20}F_3N_4O_2$ 417.1682 (M + H)$^+$; found 417.1700. A portion of compound **36c** (0.38 g, 0.91mmol) was dissolved in dichloromethane (10 mL), and to this solution was added acetic anhydride (0.5 mL) followed by triethylamine (1 mL). The reaction mixture was stirred at room temperature overnight and quenched with HCl (1 N, 25 mL). The organic material was extracted with EtOAc (2 × 50 mL), dried ($MgSO_4$), filtered, concentrated, and purified via silica gel column chromatography (dichloromethane/methanol (9.5:0.5) as eluent) to afford compound **36d** (0.31 g, 95%) as a brown solid. HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.66 (bs, 1H), 7.49 (m, 4H), 7.20 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 4.15 (t, 2H), 3.81 (s, 3H), 3.18 (s, 3H), 3.17 (t, 2H), 2.05 (s, 3H) ppm. ESIMS m/z 459 (M + H)$^+$. HRMS calculated for $C_{23}H_{22}F_3N_4O_2$ (M + H)$^+$ 459.1714; found 459.1728.

Preparation of 6-(4-(Dimethylamino)phenyl)-1-(4-methoxy-phenyl)-3-(trifluoromethyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (36e). Compound **36c** (0.25 g, 0.62 mmol) was dissolved in DMF (2 mL), followed by the addition of potassium carbonate (1 g, 7.24 mmol) and iodomethane (1 mL). The reaction mixture was capped and stirred at room temperature for 24 h, then quenched with water (25 mL). The organic material was extracted with EtOAc (2 × 25 mL), dried ($MgSO_4$), filtered, and concentrated to an oil. The compound was purified via reverse-phase HPLC (acetonitrile/water/0.05% TFA), and lyophilization of the desired fraction afforded **36e** as a colorless solid (0.125 g, 47%). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.66 (bs, 1H), 7.49 (m, 4H), 7.20 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 4.15 (t, 2H), 3.81 (s, 3H), 3.18 (s, 6H), 3.17 (t, 2H) ppm. ESIMS m/z 431 (M + H)$^+$. HRMS calculated for $C_{25}H_{22}F_3N_4O_2$ (M + H)$^+$ 431.4289; found 431.4280.

Preparation of 1-(4-Methoxyphenyl)-6-(4-(piperidin-1-yl)-phenyl)-3-(trifluoromethyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (37). In a sealed tube was added DMSO (1 mL), piperidine (0.5 mL), compound **9c** (0.2 g, 0.38 mmol), potassium carbonate (0.16 g, 1.16mmol), 1,10-phenanthroline (0.001 g), and copper iodide (0.005 g). The mixture was sealed and heated at 130 °C for 24 h, cooled, and quenched with water (50 mL). The organics were extracted with ethyl acetate (2 × 50 mL), dried ($MgSO_4$), and evaporated to a brown oil. Purification via reverse-phase HPLC followed by lyophilization of the pure fraction afforded the desired product **37** as a colorless solid (9 mg, 5%). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.63 (d, J = 9.0 Hz, 2H), 7.49 (m, 4H), 6.96 (d, J = 9.5 Hz, 2H), 4.19 (t, J = 6.6 Hz, 2H), 3.84 (s, 3H), 3.50 (m, 4H), 3.23 (t, J = 6.6 Hz, 2H), 2.15 (m, 4H), 1.77 (m, 2H) ppm. ESIMS m/z 471.27 (M + H)$^+$. HRMS calculated for $C_{25}H_{26}F_3N_4O_2$ (M + H)$^+$ 471.2008; found 471.1980.

Preparation of 1-(4-Methoxyphenyl)-6-(4-(2-oxopiperidin-1-yl)phenyl)-3-(trifluoromethyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]-pyridin-7(4*H*)-one (38a). The pyrazole derivative **9c** (0.08 g, 0.16 mmol) was dissolved in DMSO (1 mL), and to this solution was added δ-valerolactam (0.05 g, 0.47 mmol), 1,10-phenanthroline (1 mg), and K_2CO_3 (0.07 g, 0.47 mmol). The reaction mixture was degassed for 0.5 h followed by the addition of CuI (1 mg) and heated to 130 °C for 24 h. The reaction mixture was quenched with water (50 mL), and the organics were extracted with ethyl acetate (2 × 50 mL), washed with water (50 mL), and dried ($MgSO_4$). The solvent was concentrated to a tan solid, which was dissolved in methanol and purified directly via reverse-phase HPLC (acetonitrile/water/0.05% TFA gradient), and lyophilization afforded the desired product **38a** as a colorless solid (15 mg, 20%). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.50 (d, J = 10.2 Hz, 2H), 7.37 (d, J = 11.5 Hz, 2H), 7.28 (d, J = 9.1 Hz, 2H), 6.97 (d, J = 9.2 Hz, 2H), 4.18 (t, J = 6.5 Hz, 2H), 3.85 (s, 3H), 3.02 (t, J = 7.6 Hz, 2H), 3.20 (t, J = 3.3 Hz, 2H), 2.65 (t, J = 6.1 Hz, 2H), 2.01 (m, 4H) ppm. ESIMS m/z 508.2 (M + Na)$^+$. HRMS calculated for $C_{25}H_{24}F_3N_4O_3$ (M + H)$^+$ 485.1801; found 485.1810. Anal. Calcd for $C_{25}H_{23}F_3N_5O_3$: C, 61.98, H, 4.79, N, 11.56. Found: C, 61.88, H, 4.88, N, 11.67.

Preparation of 1-(4-Methoxyphenyl)-6-(4-(2-oxoazepan-1-yl)-phenyl)-3-(trifluoromethyl)-5,6-dihydro-1*H*-pyrazolo[3,4-*c*]pyridin-7(4*H*)-one (38b). The title compound **38b** was prepared following the Ullmann procedure employed for compound **38a**, by reacting compound **9c** (0.08 g, 0.16 mmol) with azepan-2-one (0.05 g, 0.47 mmol) and potassium carbonate (0.07 g, 0.47 mmol) in the presence of catalytic CuI (1 mg) in DMSO (1 mL). A colorless solid was obtained after purification via reverse-phase HPLC (22 mg, 25% yield). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.45 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.8 Hz, 2H), 7.24 (d, J = 6.8 Hz, 2H), 6.91 (d, J = 6.8 Hz, 2H), 4.11 (t, J = 6.6 Hz, 2H), 3.79 (s, 3H), 3.72 (m, 2H), 3.14 (t, J = 7.5 Hz, 2H), 2.71 (m, 2H), 1.81 (m, 6H) ppm. ESIMS m/z 499.16 (M + H)$^+$. HRMS calculated for $C_{26}H_{26}F_3N_4O_3$ (M + H)$^+$ 499.1875; found 499.1866. Anal. Calcd for $C_{26}H_{25}F_3N_5O_3$: C, 62.64, H, 5.05, N, 11.24. Found: C, 62.50, H, 5.11, N, 11.39.

Preparation of 1-(4-Methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidinyl)phenyl-4,5,6,7-tetrahydro-1*H*-pyrazole-[3,4-*c*]pyridine-3-carboxamide (40). To the pyrazole compound **9b** (25 g, 0.048 mol) was added δ-valerolactam (6.7 g, 0.067 mol), K_2CO_3 (8 g, 58.0 mmol), and DMSO (100 mL). The reaction mixture was degassed with nitrogen for 0.5 h followed by the addition of CuI (1.84 g, 9.0 mmol). The mixture was heated to 130 °C for 24 h, cooled, and quenched with water (50 mL). The organics were extracted with EtOAc (2 × 100 mL) and dried ($MgSO_4$). Purification by silica gel column chromatography (MeOH/DCM, 1:9, as eluent) afforded the intermediate **39** as a tan foam (5 g, 21%). ^1H NMR ($CDCl_3$) δ: 7.49 (d, J = 9.20 Hz, 2H), 7.35 (d, J = 8.80 Hz, 2H), 7.26 (d, J = 8.10 Hz, 2H), 6.92 (d, J = 8.80 Hz, 2H), 4.49 (q, J = 7.30 Hz, 2H), 4.13 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.59 (m, 2H), 3.39 (t, J = 6.60 Hz, 2H), 2.55 (m, 2H), 1.91 (m, 4H), 1.45 (t, J = 7.30 Hz, 3H) ppm. ESIMS m/z 477 (M + H)$^+$. To the ester intermediate **39** (4.8 g, 0.009 mol) was added 5% NH_3 in ethylene glycol (40 mL), and the mixture was heated to 120 °C for 4 h in a sealed vessel. The mixture was cooled, and the reaction was quenched with water (50 mL). The solid was precipitated, filtered, and dried under vacuum. Purification by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:9, as eluent) afforded the desired product **40** as a colorless solid (3.5 g, 76%). HPLC purity, >95%. ^1H NMR ($CDCl_3$) δ: 7.49 (d, J = 8.80 Hz, 2H), 7.37 (d, J = 9.10 Hz, 2H), 7.26 (d, J = 8.80 Hz, 2H), 6.98 (s, 1H), 6.95 (d, J = 9.20 Hz, 2H), 6.28 (s, 1H), 4.14 (t, J = 6.60 Hz, 2H), 3.81 (s, 3H), 3.61 (m, 2H), 3.39 (t, J = 6.60 Hz, 2H), 2.63 (t, J = 6.20 Hz, 2H), 1.96 (m, 4H) ppm. HRMS calculated for $C_{25}H_{26}N_5O_4$ (M + H)$^+$ 460.1985; found 460.1984. Anal. Calcd for $C_{25}H_{25}N_5O_4 \cdot 1.5H_2O$: C, 61.72, H, 5.80, N, 14.34. Found: C, 61.62, H, 5.74, N, 14.30.

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Supporting Information Available: Complete experimental procedures for compounds **6**, **41**−**47**, and **13a**−**e**,**g**−**h** and Table 8 detailing the enzyme selectivity profile of compound **40** compared to its predecessor compounds **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Hyers, T. M. Management of venous thromboembolism. Past present and future. *Arch. Intern. Med*. **2003**, *163*, 759−768. (b) Stein, P. D.; Grandison, D.; Hua, T. A. Therapeutic level of anticoagulation with warfarin in patients with mechanical prosthetic heart valves; review of literature and recommendations based on internal normalized ratio. *Postgrad. Med. J.* **1994**, *70* (Suppl. 1), S72−S83. (c) Hirsh, J.; Poller, L. The international normalized ratio. A guide to understanding and correcting its problems. *Arch. Intern. Med.* **1994**, *154*, 282−288.

(2) Weitz, J. I. Low molecular weight heparins. *New Engl. J. Med*. **1997**, *337*, 688−689.

(3) Turpie, A. G. G.; Antman, E. M. Low-molecular weight heparins in the treatment of acute coronary syndromes. *Arch. Intern. Med.* **2001**, *161*, 1484−1490.

(4) (a) Adang, A. E. P; Rewinkel, J. B. M. A new generation of orally active antithrombotics: comparing strategies in the GPIIb/IIIa, thrombin and factor Xa areas. *Drugs Future* **2000**, *25*, 369−383. (b) Rewinkel, J. B. M.; Adang, A. E. P. Strategies and progress towards the ideal orally active thrombin inhibitor. *Curr. Pharm. Des.* **1999**, *5*, 1043−1075.

(5) Samama, M. M.; Gerotziafas, G. T. Evaluation of the pharmacological properties and clinical results of the synthetic pentasaccharide (fondaparinux). *Thromb. Res*. **2003**, *109*, 1−11.

(6) (a) Walenga, J. M.; Jeske, W. P.; Hoppensteadt, D.; Fareed, J. Factor Xa inhibitors: today and beyond. *Curr. Opin. Invest. Drugs* **2003**, *4* (3), 272−281. (b) Samama, M. M. Synthetic direct and indirect factor Xa inhibitors. *Thromb. Res.* **2002**, *106*, V267−V273. (c) Kaiser, B. Visions & reflections. Factor Xa, a promising target for drug development. *Cell. Mol. Life Sci.* **2002**, *59*, 189−192. (d) Leadley, R. J., Jr. Coagulation factor Xa inhibition: biological background and rationale. *Curr. Top. Med. Chem*. **2001**, *1*, 151−159. (e) Hauptmann, J.; Stürzebecher, J. Synthetic inhibitors of thrombin and factor Xa: from bench to bedside. *Thromb. Res.* **1999**, *93*, 203−241. (f) Wong, P. C.; Crain, E. J.; Watson, C. A.; Zaspel, A. M.; Wright, M. R.; Lam, P. Y. S.; Pinto, D. J.; Wexler, R. R.; Knabb, R. M. Nonpeptide factor Xa inhibitors III: effects of DPC423, an orally-active pyrazole antithrombotic agent on arterial thrombosis in rabbits. *J. Pharmacol. Exp. Ther*. **2002**, *303*, 993−1000. (g) Wong, P. C.; Pinto, D. J.; Knabb, R. M. Nonpeptide factor Xa inhibitors: DPC423, a highly potent and orally bioavailable pyrazole antithrombotic agent. *Cardiovasc. Drug Rev.* **2002**, *20* (2), 137−152. (h) Wong, P. C.; Quan, M. L.; Crain, E. J.; Watson, C. A.; Wexler, R. R.; Knabb, R. M. Nonpeptide factor Xa inhibitors: I. studies with SF303 and SK549, a new class of potent antithrombotics. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 351−357.

(7) (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.; Luettgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. Discovery of 1-(3′-aminobenzisoxazol-5′-yl)-3-trifluoromethyl-*N*-[2-fluoro-4-[(2′-dimethylamino-methyl)imidazol-1-yl]phenyl]-1*H*-pyrazole-5-carboxyamide hydrochloride (razaxaban), a highly potent and selective, orally bioavailable factor Xa inhibitor. *J. Med. Chem.* **2005**, *48*, 1729−1744. (b) Wong, P. C.; Crain, E. J.; Watson, C. A.; Wexler, R. R.; Lam, P. Y. S.; Quan, M. L.; Knabb, R. M. Razaxaban, a direct factor Xa inhibitor, in combination with aspirin and/or clopidogrel improves low-dose antithrombotic activity without enhancing bleeding liability in rabbits. *J. Thromb. Thrombolysis* **2007**, *24* (1), 43−51. (c) Lassen, M. R.; Davidson, B. L.; Gallus, A.; Pineo, G; Ansell, J.; Deitchman D. A phase II randomized, double-blind, five-arm, parallel-group, dose-response study of a new oral directly-acting factor Xa inhibitor, razaxaban, for the prevention of deep vein thrombosis in knee replacement surgery on behalf of the razaxaban investigators. *Blood* **2003**, *102* (11), Abstract 41.

(8) (a) Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E.; Roehrig, S. Discovery of the novel antithrombotic agent 5-chloro-*N*-({(5)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1-3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide (Bay-59-7939): an oral, direct factor Xa inhibitor. *J. Med. Chem.* **2005**, *48*, 5900−5908. (b) Eriksson, B. L.; Borris, L.; Dahl, O. E.; Haas, S.; Huisman, M. V.; Kakkar, A. K. ODIXa-HIP study investigators. Oral, direct factor Xa inhibition with Bay-59-7939 for the prevention of venous thromboembolism after total hip replacement. *J. Thromb. Haemostasis* **2006**, *4* (1), 121−128.

(9) (a) Liebeschuetz, J. W.; Jones, S. D.; Wiley, M. E.; Young, S. C. Iterative structure-based screening of virtual chemical libraries and factor Xa: finding the orally available antithrombotic candidate LY517717. *Structure-Based Drug Discovery* **2006**, 173−192. (b) Hampton T. New oral anticoagulants show promise. *JAMA, J. Am. Med. Assoc.* **2006**, *295* (7), 743−744.

(10) (a) McBride, B. F. A preliminary assessment of the critical differences between novel oral anticoagulants currently in development. *J. of Clin. Pharm*. **2005**, *45* (9), 1004−1017. (b) Saiah, E.; Soares, C. S. Small molecule coagulation cascade inhibitors in the clinic. *Curr. Top. Med. Chem*. **2005**, *5* (16), 1677−1695.

(11) 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.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam P. Y. S. Discovery of 1-[3-aminomethyl)phenyl]-*N*-[3-fluoro-2′-(methylsulfonyl)-[1,1′-biphenyl]-4-yl]-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamide (DPC423), a highly potent, selective, and orally bioavailable inhibitor of coagulation factor Xa. *J. Med. Chem*. **2001**, *44*, 566−578.

(12) (a) Pruitt, J. R.; Pinto, D. J. P; Galemmo, R, A.; Alexander, R. S.; Rossi, K. A.; Wells, B. L.; Drummond, S.; Bostrom, L. L.; Burdick, D.; Bruckner, R.; Chen, H.; Smallwood, A.; Wong, P. C.; Wright, M. R.; Bai, S.; Luettgen, J. M.; Knabb, R. M.; Lam, P. Y. S. Wexler, R. R. Discovery of 1-(2-aminomethylphenyl)-3-trifluoromethyl-*N*-[3-fluoro-2′-(aminosulfonyl)[1,1′-biphenyl)]-4-yl]-1*H*-pyrazole-5-carboxyamide (DPC602), a potent, selective, and orally bioavailable factor Xa inhibitor. *J. Med. Chem.* **2003**, *46*, 5298−5315. (b) Lam, P. Y. S.; Clark, C. G.; Li, R.; Pinto, D. J.; Orwat, M. J.; Galemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. A.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luettgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. Structure-based design of novel guanidine/benzamidine mimics: potent and orally bioavailable factor Xa inhibitors as novel anticoagulants. *J. Med. Chem*. **2003**, *46*, 4405−4418. (c) A similar approach was used in the following: Jia, Z. J.; Wu, Y.; Huang, W.; Zhang, P.; Clizbe, L. A.; Scarborough, R. M.; Zhu, B.-Y. 1-(2-Naphthyl)-1*H*-pyrazole-5-carboxamides as potent factor Xa, inhibitors. Part 2: a survey of P4 motifs. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1221−1227. (d) A similar interaction was seen by the Berlex group: Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, H.; Light, D. R.; Biancalana, S.; Whitlow, M.; Crystal structures of two potent nonamidine inhibitors bound to factor Xa. *Biochemistry* **2002**, *41*, 15514−15523.

(13) (a) Ames, B. N.; McCann, J.; Yamasaki, E. Methods for detecting carcinogen and mutagenesis with the salmonella/mammalian-microsome mutagenicity test. *Mutat. Res*. **1975**, *31*, 347−364. (b) For a review, see the following: Quillardet, P.; Hofnung, M. The SOS chromotest: a review. *Mutat. Res*. **1993**, *297*, 235−279.

(14) (a) Obe, G.; Pfeiffer, P.; Savage, J. R. K.; Johannes, C.; Goedecke, W.; Jeppesen, P.; Natarajan, A. T.; Martinez-Lopez, W.; Olle, G. A.; Drets, M. E. Chromosomal aberrations: formation, identification and distribution. *Mutat. Res*. **2002**, *504*, 17−36. (b) Krishna, G.; Hayashi, M. In vivo rodent micronucleus assay: protocol, conduct and data interpretation. *Mutat. Res*. **2000**, *455*, 155−166.

(15) (a) Pinto, D. J. P.; Orwat, M.; Quan, M. L.; Galemmo, R. A., Jr.; Amparo, E.; Wells, B.; Ellis, C.; He, M. Y.; Alexander, R. S.; Rossi, K. A.; Smallwood, A.; Wong, P. C.; Luettgen, J. M.; Rendina, A. R.; Knabb, R. M.; Mersinger, L.; Kettner, C.; Bai, S.; He, K.; Wexler, R. R.; Lam, P. Y. S. 1-[3-Aminobenz-isoxazol-5′-yl]-3-trifluoromethyl-6-[2′-(3-(*R*)-hydroxy-*N*-pyrrolidinyl)methyl-[1,1′]-biphen-4-yl]-1,4,5,6-tetrahydropyrazolo-[3,4-*c*]-pyridin-7-one (BMS-740808) a highly potent, selective, efficacious and orally bioavailable inhibitor of blood coagulation factor Xa. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4141−4147. (b) Li, Y.-L.; Fevig, J. M.; Cacciola, J.; Buriak, J.; Rossi, K. A.; Jona, J.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Bai, S. A.; Wexler, R. R.; Lam, P. Y. S. Preparation of 1-(3-aminobenzo-[d]isoxazol-5-yl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones as potent, selective, and efficacious inhibitors of coagulation factor Xa. *Bioorg. Med. Chem. Lett.* **2006**, *16* (19), 5176−5182. (c) Fevig, J. M.;

Cacciola, J.; Buriak, J.; Rossi, K. A.; Knabb, R. M.; Luettgen, J. M.; Wong, P. C.; Bai, S. A.; Wexler, R. R.; Lam, P. Y. S. Preparation of 1-(4-methoxyphenyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones as potent, selective and bioavailable inhibitors of coagulation factor Xa. *Bioorg. Med. Chem. Lett.* **2006**, *16* (14), 3755−3760. (d) Wong, P. C.; Watson, C. A.; Crain, E. J. C.; Luettgen, J. M.; Ogletree, M. L.; Wexler, R. R.; Lam, P. Y. S.; Pinto, D. J.; Knabb, R. M. Effects of the factor Xa inhibitor apixaban on venous thrombosis and hemostasis in rabbits. *Blood* **2006**, *108*, 275a (Abstract 917). (e) Wong, P. C.; Watson, C. A.; Crain, E. J. C.; Ogletree, M. L.; Wexler, R. R.; Lam, P. Y. S.; Pinto, D. J.; Knabb, R. M. Potent antithrombotic activity of apixaban, a direct factor Xa inhibitor, with minimum bleeding time effect in rabbit models of arterial thrombosis and hemostasis. *J. Thromb. Haemostasis* **2007**, *5* (Suppl. 1), Abstract 1315.

(16) (a) Pinto, D. J. P.; Galemmo, R. A., Jr.; Quan, M. L.; Orwat, M. J.; Clark, C.; Li, R.; Wells, B.; Woerner, F.; Alexander, R. A.; 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. Discovery of potent, efficacious and orally bioavailable inhibitors of blood coagulation factor Xa with neutral P_1 moieties. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4141−4147. (b) Lam, P. Y. S.; Clark, C. G.; Li, R.; Pinto, D. J.; Orwat, M. J.; Galemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. A.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luettgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. Structure-based design of novel guanidine/benzamidine mimics: potent and orally bioavailable factor Xa inhibitors as novel anticoagulants. *J. Med. Chem*. **2003**, *46*, 4405−4418.

(17) Phillips, R. R. The Japp−Klingmann reaction, a review. *Org. React.* **1959**, *10*, 143−178.

(18) Basha, A.; Lipton, M.; Weinreb, S. M. A mild, general method for the conversion of esters to amides. *Tetrahedron Lett.* **1977**, *18* (48), 4171−4174.

(19) Capson, T. L.; Poulter, D. C. A facile synthesis of primary amines from carboxylic acids by the Curtius rearrangement. *Tetrahedron Lett.* **1984**, *25* (33), 3515−3518.

(20) Brown, H. C.; Heim, P.; Yoon, N. M. Selective reductions. XV. Reaction of diborane in tetrahydrofuran with selected organic compounds containing representative functional groups. *J. Am. Chem. Soc.* **1970**, *92* (6), 1637−1646.

(21) Wolfe, J. P.; Ahman, J.; Sadighi, J. P.; Singer, R. A.; Buchwald, S. L. An ammonia equivalent for the palladium-catalyzed amination of aryl halides and triflates. *Tetrahedron Lett*. **1997**, *38* (36), 6367−6370.

(22) (a) For a review on Cu-catalyzed cross-coupling reactions, see the following: Lindley, J. *Tetrahedron* **1984**, *40*, 1435−1456. (b) Kametani, T.; Ohsawa, T.; Ihara, M. Examples of CuI-mediated intramolecular amidations under relatively mild conditions. *Heterocycles* **1980**, *14*, 277−280.

(23) Hilgers, A. R.; Conradi, R. A.; Burton, S. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm. Res.* **1990**, *7*, 902−910.

(24) Obach, R. S.; Baxter, J. G.; Liston, T. E.; Silber, B. M.; Jones, B. C. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab. Dispos.* **1999**, *27* (11),1350−1359.

(25) Pacific, G. M.; Viani, A. Methods of determining plasma and tissue binding of drugs. *Clin. Pharmacokinet.* **1992**, *23*, 449−468.

(26) (a) Andrews, B.; Chen, S.; Zhu, M.; Moulin, F.; Flint, O. Evaluation of cytochrome P450-mediated hepatotoxicity using a high throughput robotic system, *Drug Metab. Rev.* **2003**, *35* (Suppl. 2), 207. (b) Crespi. C. L.; Vaughn, P. M.; Penman, B. W. Microtiter plate assay for inhibition of human, drug-metabolizing cytochromes P450. *Anal. Biochem.* **1997**, *248*, 188−190. (c) Rampe, D.; Roy, M. L.; Dennis, A.; Brown, A. M. A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett.* **1997**, *417* (1), 28−32. (d) Bianchi, L.; Shen, Z.; Dennis, A. T.; Priori, S. G.; Napolitano, C.; Ronchetti, E.; Bryskin, R.; Schwartz, P. J.; Brown, A. M. Cellular dysfunction of LQT5-minK mutants: abnormalities of IKs, IKr and trafficking in long QT syndrome. *Hum. Mol. Genet.* **1999**, *8* (8), 1499−507. (e) Bertilsson, G.; Heidrich, J.; Svensson, K.; Svensson, K.; Åsman, M.; Jendeberg, L.; Sydow-Bäckman, M.; Ohlsson, R.; Postlind, H.; Blomquist, P.; Berkenstam, A. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95* (21), 12208−12213.

(27) Thermodynamic equilibrium aqueous solubility was measured at ambient temperature in 0.9% saline solution.

(28) Knabb, R. M.; Kettner, C. A.; Timmermans, P. B. M. W. M.; Reilly, T. M. In vivo characterization of a new synthetic thrombin inhibitor. *Thromb. Haemostasis* **1992**, *67*, 56−59.

(29) Kettner, C. A.; Mersinger, L. J.; Knabb, R. M. The selective inhibition of thrombin by peptides of boroarginine. *J. Biol. Chem.* **1990**, *265*, 18289−18297.

(30) Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. In *Macromolecular Crystallography. Part A*; Carter, C. W., Jr., Sweet, R. M., Eds.; Methods in Enzymology, Vol. 276; Academic Press: New York, 1997; pp 307−326.

(31) Kissinger, C. R.; Gehlhaar, D. K.; Fogel, D. B. Rapid automated molecular replacement by evolutionary search. *Acta Crystallogr.* **1999**, *D55*, 484−491.

(32) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.

(33) Diffraction data for factor Xa inhibitor compound **40** are shown in Table 7.

(34) (a) Compounds were studied in the N-in-one dog PK experiments in which cassettes of 10 compounds were dosed together to each dog ($n = 2$). (b) Wu, J. T.; Zeng, H.; Qian, M.; Brogdon, B. L.; Unger, S. E. Direct plasma sample injection in multiple-component LC−MS−MS assays for high-throughput pharmacokinetic screening. *Anal. Chem*. **2000**, *72*, 61−67. (c) Zeng, H.; Wu, J. T.; Unger, S. E. The investigation and the use of high flow column-switching LC/MS/MS as a high-throughput approach for direct plasma sample analysis of single and multiple components in pharmacokinetic studies. *J. Pharm. Biomed. Anal*. **2002**, *27*, 967−982.

(35) DeLano, W. L. *The PyMol Molecular Graphics System*; DeLano Scientific: San Carlos, CA, 2002; http://www.pymol.org.

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