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Structure–activity relationship and pharmacokinetic profile of 5-ketopyrazole factor Xa inhibitors

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Abstract—Efforts to further optimize the clinical candidate razaxaban have led to a new series of pyrazole-based factor Xa (fXa) inhibitors. Designed to prevent the potential formation of primary aniline metabolites in vivo, the nitrogen of the carboxamido linker between the pyrazole and proximal phenyl moiety of the razaxaban scaffold was replaced with a methylene group. The resulting ketones demonstrated excellent potency and selectivity for fXa but initially had poor oral bioavailability. Optimization by conversion from a P1 aminobenzisoxazole to a P1 *p*-methoxyphenyl residue, replacing the 3-trifluoromethylpyrazole with a 3-amidopyrazole, and employing a pyridone P4 group provided a fXa inhibitor with a potency and pharmacokinetic profile equivalent to that of razaxaban and improved selectivity over thrombin.

A direct inhibitor of fXa is an attractive therapeutic target for prevention and treatment of thrombotic diseases.[1] Since small quantities of fXa can lead to the production of large quantities of thrombin, the agent responsible for catalyzing the conversion of fibrinogen to fibrin and activating platelets during clot formation, inhibition of fXa is highly efficient.[2] Inhibitors of fXa are also expected to have a wider therapeutic window compared to thrombin inhibitors in a clinical setting.[3]

Recently we described two new series of pyrazole-based fXa inhibitors as part of our efforts to further optimize our clinical candidate razaxaban.[4] While the biaryl-aniline P4 moiety of razaxaban tested negative in the Ames assay and was not observed in vivo, both series were designed to eliminate possible formation of a potentially mutagenic primary biarylaniline.[5] The tetrahydropyrazolo-pyridinone series culminated in BMS-740808, which demonstrated high affinity for fXa and favorable in vivo pharmacokinetic and antithrombotic profiles.[6] The indoline series produced potent compounds that had good clotting activity in vitro but poor bioavailability.[7] Herein we describe an alternative series that replaces the carboxamido linker between the pyrazole and proximal phenyl group of the razaxaban scaffold with a ketone moiety, thus obviating potential formation of aniline metabolites altogether.

Initial leads for the ketone series are presented in Table 1. The effects of both methylene substitution (R_1) and fluorine incorporation at the 2′-position of the proximal phenyl ring (R_2) were explored. Sulfone **1** and sulfonamide **2** were 2- to 3-fold less active than razaxaban in terms of fXa binding affinity but demonstrated better selectivity over trypsin. Anticoagulation activity in both prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays was low and permeability was negligible in the Caco-2 assay for **2**. Fluorine incorporation was attempted to improve fXa affinity, in vitro anticoagulation activity, and permeability for these compounds.[8a] Compared to their non-fluorinated analogs, binding affinity for fXa was increased by 10-fold for 2′-fluoro sulfone **3** and 3-fold for 2′-fluoro sulfonamide **4**. The sulfonamide (**4**) was also more active in the anticoagulation assays and exhibited greater permeability than its desfluoro analog **2**. To determine the effect of substitution alpha to the ketone, we methylated several compounds. This methylation alpha to the carbonyl, as exemplified by comparing **8** with **4**, was found

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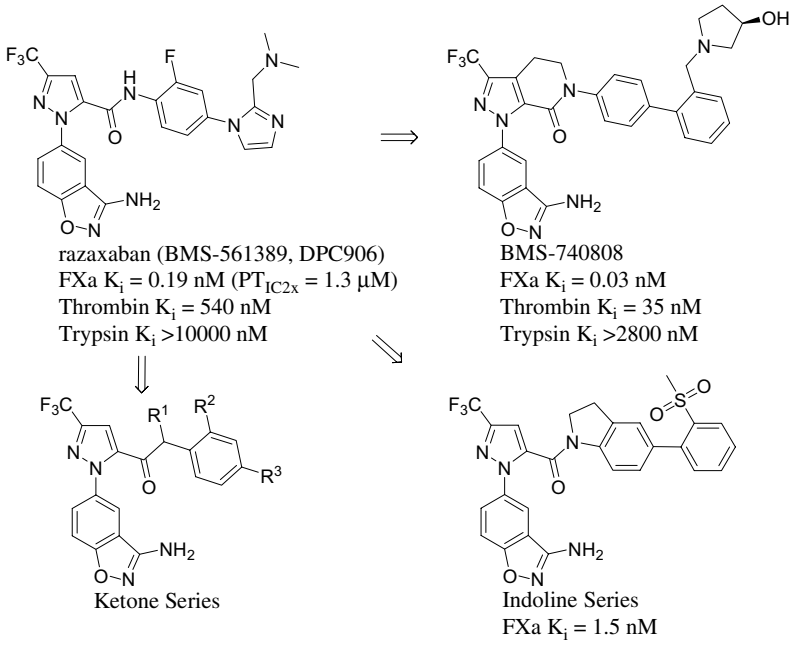
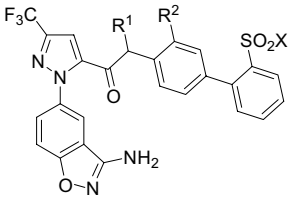


Table 1. Factor Xa and trypsin binding affinities, anticoagulation activity, and in vitro permeability for initial leads[a]



Compound	R^1	R^2	X	fXa K_i (nM)	Trypsin K_i (nM)	PT IC_{2x} (μM)	aPTT IC_{2x} (μM)	Caco-2 Papp ($\times 10^{-6}$ cm/s)
1	H	H	CH_3	0.68	>20,000	—	—	—
2	H	H	NH_2	0.53	>20,000	18	33	bql
3	H	F	CH_3	0.07	>20,000	9.0	9.3	bql
4	H	F	NH_2	0.16	>20,000	12	18	31
5[b]	CH_3	H	CH_3	4.7	>20,000	—	—	—
6[b]	CH_3	H	NH_2	22	>20,000	—	—	—
7[b]	CH_3	F	CH_3	2.8	>20,000	—	—	—
8[b]	CH_3	F	NH_2	2.3	>20,000	112	30	bql

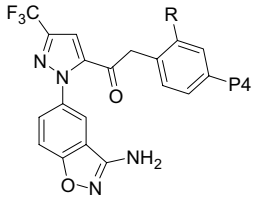
[a] K_is were obtained from purified human enzymes. K_is, PT, aPTT, and Caco-2 values were measured according to Ref. 4,8.
[b] Compounds are racemic.

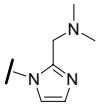
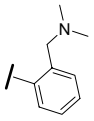
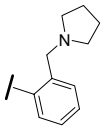
to decrease binding affinity, in vitro clotting activity, and surprisingly cellular permeability.

Conversion of the carboxamido linker to a ketone in the razaxaban scaffold initially afforded inhibitors with subnanomolar binding affinity for fXa but low in vitro anticoagulation activity and poor in vitro permeability. To address these issues, P4 moieties that historically have given good in vitro activity, attenuated protein binding, and favorable pharmacokinetic profiles were evaluated.[4,8–10] All compounds of Table 2 had subnano-molar binding affinities for fXa and high selectivity over trypsin. Imidazole **9** was 5-fold less active toward fXa than razaxaban with 8-fold lower selectivity over thrombin. However, **9** had significant activity in the PT assay and comparatively high in vitro permeability (razaxaban Caco-2 Papp = 5.6×10^{-6} cm/s). Benzylamines **10** and **11** were 2- and 3-fold more potent, respectively, than imidazole **9** and also had good activity in the PT assay. The low Caco-2 data for pyrrolidine **11** are consistent with previous observations for this P4 group.[4,9] Pyridone analogs **12** and **13** had very potent fXa binding affinity, with pyridone **13** being significantly more potent than its saturated counterpart. The relative lack of activity in the PT assay for **12** and **13** compared to **9**–**11**, given their good binding affinity for fXa, suggests that the pyridone analogs have greater protein binding than their amine-containing P4 counterparts, thus decreasing their antithrombotic efficacy in vitro.

Compounds **9**–**11** met our in vitro criteria for fXa binding affinity and antithrombotic efficacy. As a result, these P4 amines were tested in a dog pharmacokinetic model and were found to have high clearance rates, short half-lives, and poor bioavailability (Table 3). To address these problems we revisited studies by our group

Table 2. Factor Xa, thrombin, and trypsin binding affinities, clotting activity, and in vitro permeability for P4 modifications[a]



Compound	R	P4	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)	PT IC_{2x} (μM)	Caco-2 Papp ($\times 10^{-6}$ cm/s)
9	H		0.97	350	>4200	2.0	24 ± 4
10	F		0.57	—	>4200	5.8	—
11	H		0.32	310	>4200	3.2	0.9 ± 0.1
12	H		0.34	—	>20,000	8.1	23
13	F		0.033	349	>15,000	11.6	7.6

[a] K_is were obtained from purified human enzymes. K_is, PT, aPTT, and Caco-2 values were measured according to Ref. 4,8.

Table 3. Ketone pharmacokinetic profiles[a]

Compound	Caco-2 Papp ($\times 10^{-6}$ cm/s)	Cl (L/h/kg)	Vdss (L/Kg)	$T_{1/2}$ (po)	F% (po)
Razaxaban	5.56	1.1	3.4	5.3	84
9	24 ± 4	2.5	4.5	1.6	16
10	—	2.0	11	—	bql
11	0.85 ± 0.07	2.3	12	3.6	4.9

[a] Compounds were dosed in an *N*-in-1 format at 0.5 mg/kg iv and 0.2 mg/kg po ($n = 1$).[4,8]

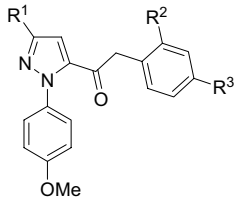
indicating that oral absorption, in addition to clearance rates and volumes of distribution, could be improved, albeit at the cost of fXa binding affinity, by incorporating neutral P1 substituents.[11] We employed this strategy by replacing the P1-aminobenzisoxazole of the ketone series with a neutral *p*-methoxyphenyl group.

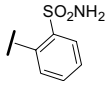
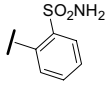
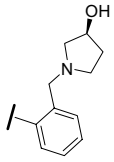
The *p*-methoxyphenyl analogs are presented in Table 4. As seen previously for this P1 substituent, these compounds exhibited good selectivity over thrombin and trypsin.[10,11] The P4 *ortho*-phenyl sulfonamides **14** and **15**were less active than their P1 aminobenzisoxazole analogs (**2** and **4**) by 23- and 43-fold, respectively. This is also consistent with data obtained by our group indicating that the P1 *p*-methoxyphenyl functionality decreases fXa binding affinity relative to the P1 aminobenzisoxazole by $\geqslant$7-fold.[9] In vitro anticoagulation activity was also decreased for both PT and aPTT assays in comparing **14**–**2**. Fluorine incorporation imparted a modest 2-fold increase in binding for sulfonamide **15**. Both hydroxypyrrolidine **16** and tetrahydropyridone **17** were more active than **14** in both binding (4-fold) and clotting assays (2-fold). As seen previously with aminobenz-isoxazoles **12** and **13**, pyridone **18** was 10-fold more active than **17**. However, no impact on PT efficacy was observed. To attenuate the lipophilicity of what was perceived to be a highly protein-bound molecule, the 3-trifluoromethyl functionality of the pyrazole of **18** was replaced with a C-linked primary amide.[10] It was thought that this modification would provide an increase in clotting activity due to reduced protein binding. Gratifyingly, the resulting 3-amidopyrazole (**19**) improved potency in the PT and aPTT assays by 3- and 6-fold, respectively.

Table 5 illustrates the high selectivity of pyridone **19** for factor Xa over other human enzymes. Compared to razaxaban, **19** is much more selective with respect to thrombin and less selective over chymotrypsin.

Pyridone **19** met our in vitro criteria for advancement and was evaluated in a dog pharmacokinetic model (Table 6). Bioavailability was high, and, despite a low volume of distribution, the half-life of compound **19** was equivalent to that of razaxaban because of a very low rate of clearance (0.09 L/h/Kg).

Table 4. Binding affinities and in vitro activity for *p*-methoxyphenyl P1 analogs[a]



Compound	R^1	R^2	R^3	fXa K_i (nM)	Thrombin, K_i (nM)	Trypsin K_i (nM)	PT IC_{2x} (μM)	aPTT IC_{2x}(μM)
14	CF_3	H		12	>21,000	>2500	40	65
15	CF_3	F		6.9	>21,000	>6000	—	—
16	CF_3	H		3.3	>6300	>4200	16	44
17	CF_3	H		1.0	>15,000	>15000	17	34
18	CF_3	F		0.09	>15,000	>15000	22	30
19	$CONH_2$	F		0.11	>15,000	>15000	5.2	5.1

[a] K_is were obtained from purified human enzymes. K_is, PT, aPTT, and Caco-2 values were measured according to Ref. 4,8.

Table 5. Enzyme selectivity profile of pyridone **19**

Human enzyme (K_i)	Razaxaban (μM)	Pyridone **19** (μM)
Factor Xa	0.00019	0.00011
Trypsin	>10	>15
Thrombin	0.540	>15
Plasma Kallikrein	>2.3	8.9
APC	>20	>38
Factor IXa	>9	>15
Factor XIa	>12	>15
Chymotrypsin	>8.5	1.7
Plasmin	>15	>28
tPa	>33	>43

All K_i values were obtained according to Ref. 4.

Table 6. Pharmacokinetic profile of *p*-methoxyphenyl P1 analogs[a]

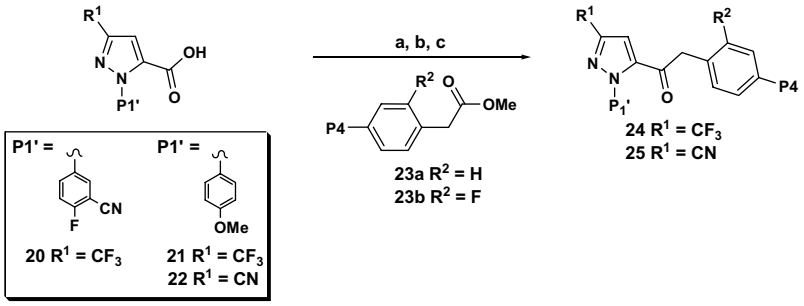
Compound	Caco-2 Papp ($\times 10^{-6}$cm/s)	Cl (L/h/kg)	Vdss (L/Kg)	$T_{1/2}$ (po)	F% (po)
Razaxaban	5.56	1.1	3.4	5.3	84
19	2.2	0.09	0.65	5.4	117

[a] Compounds were dosed in an *N*-in-1 format at 0.5 mg/kg iv and 0.2 mg/kg po ($n = 1$).[4,8]

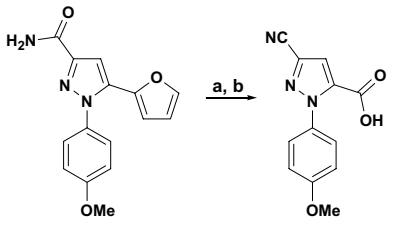
The general strategy for preparing compounds from the ketone series is shown in Scheme 1. Carboxylic acids **20**–**22** were first converted to either the corresponding acid chloride or pivaloyl mixed anhydride. Treatment with the enolates of esters **23a**,**b**, the preparation of which is shown in Scheme 3, afforded the corresponding beta-keto esters. Hydrolysis and decarboxylation under acidic conditions generated ketones **24**–**25.**

The chemistry used to prepare acids **20**and **21** has previously been described.[4,11] Acid **22** was prepared from amide **26**, which was generated from commercially available 1-(4-methoxyphenyl)hydrazine hydrochloride according to the procedures of Ref. 7. Dehydration upon treatment with trifluoroacetic anhydride and pyridine was followed by oxidation to the acid to give **22** (Scheme 2).

Esters used in the generation of the ketone scaffolds in Scheme 1 were prepared according to Scheme 3. Commercially available toluene **27** was brominated under radical conditions using 2,2′-azobis(2-methylpropionitrile) (AIBN) and *N*-bromosuccinimide. Displacement of the bromide with sodium cyanide, hydrolysis under basic conditions, and methylation with trimethylsilyldiazomethane afforded ester **29a**. Ester **29b**was similarly prepared by methylating commercially available acetic acid **28**. Pyridone **30** and imidazole **31**[4] were installed using Ullmann conditions in average yield to give esters **33** and **34**, respectively. Coupling of **29a**,**b** with boronic acid **32a**– **c**[4] under Suzuki conditions afforded the corre-



Scheme 1. Reagents: (a) $(COCl)_2$, DMF, CH_2Cl_2 or PivCl, Et_3N, CH_2Cl_2; (b) ester **23a** or **23b**, *n*BuLi, DIA, THF, 65–88% (two steps); (c) 4 M H_2SO_4/MeOH, 40–88%.

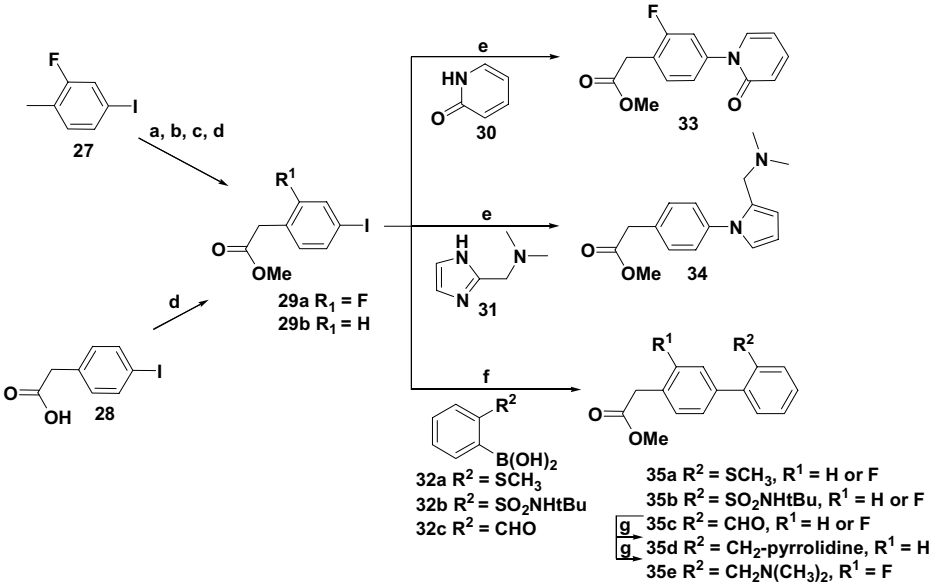


Scheme 2. Reagents and condition: (a) TFAA, py, dioxane, 73%; (b) $KMnO_4$, *t*BuOH, 5% NaH_2PO_4, H_2O, 60 °C, 71%.

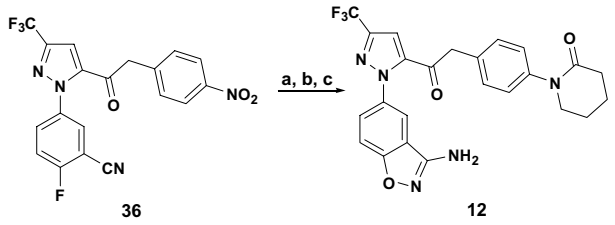
sponding biphenyl analogs **35a**–**c**. Reductive amination of benzaldehyde **35c** with the appropriate amine generated the desired P4 benzyl amines.

Compounds containing tetrahydropyridone P4 groups were prepared according to the procedure of Scheme 4. Ketone **36** was synthesized by coupling commercially available ethyl 2-(4-nitrophenyl)acetate with acid **20** using the chemistry shown in Scheme 1. Reduction of the nitro group with stannous chloride dihydrate was followed by synthesis of the lactam employing 5-bromopentanoyl chloride and two equivalents of potassium *tert*-butoxide. Conversion to the aminobenz-isoxazole was carried out using acetohydroxamic acid and potassium carbonate in low yield. The same chemistry was employed when the P1 substituent was a *p*-methoxyphenyl group.

Compounds containing methyl substituents alpha to the ketone carbonyl (**5**–**8**) were prepared by converting acids **27** and **28** to the corresponding *tert*-butyl-dimethylsilyl esters (TBSCl, Im, DMF, 55–80%). Coupling with acid **20** according to Scheme 1 was followed by methylation with lithium hexamethyl-disilazide and excess iodomethane. The biphenyl P4 group was then prepared via Suzuki couplings with boronic acids **32a**,**b** according to Scheme 3 and conversion to the aminobenz-isoxazole according to Scheme 4, albeit in greater yield (33–80%). Sulfone and sulfonamide P4 moieties were generated from their corresponding thioanisole and *tert*-butyl sulfonamide analogs upon treatement with *meta*-chloroperoxy-benzoic acid (CH_2Cl_2, 30–80%) and trifluoroacetic acid (6–90%), respectively.



Scheme 3. Reagents and conditions: (a) NBS, AIBN, acetone, reflux, 73% (b) NaCN, TBAB, tol/H_2O, reflux, 54%; (c) NaOH, 2:1 EtOH/H_2O, reflux, 96%; (d) $TMSCH_2N_2$, C_6H_6, MeOH, 66%; (e) **30** or **31**, CuI, K_2CO_3, DMSO, 30–40%; (f) $Pd_2(dba)_3$, PPh_3, Na_2CO_3, tol/H_2O/EtOH, 33–90%; (g) pyrrolidine or dimethylamine, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 50–72%.



Scheme 4. Reagents: (a) $SnCl_2{\cdot}2H_2O$, EtOAc/EtOH, 86%; (b) $ClC(O)(CH_2)_4Br$, KOtBu (2.0 equiv), THF, 45%; (c) $CH_3CONHOH$, K_2CO_3, DMF/H_2O, 2%.

Pyridone **19** was prepared from the product of the coupling of acid **22** and ester **33**. Subsequent hydration (H_2O_2, K_2CO_3, DMSO, 13%) of the nitrile substituent of the pyrazole afforded the corresponding 3-amidopyrazole **19** in low yield.

In conclusion, modification of the razaxaban pyrazole-based scaffold by converting the carboxamido linker of the pyrazole and proximal phenyl moieties to a ketone has resulted in a new series of fXa inhibitors. Compounds of this series consistently demonstrated high binding affinity for factor Xa and good selectivity over thrombin and trypsin. Good activity was obtained in clotting assays with compounds containing protonatable nitrogens in the P4 group, however, oral bioavailability in in vivo studies remained low. Replacement of the P1-aminobenzisoxazole with a *p*-methoxyphenyl residue in order to improve pharmacokinetic properties afforded compounds with good fXa binding affinity but low activity in clotting assays. Subsequent conversion from a 3-trifluoromethyl to a 3-amidopyrazole provided pyridone **19**, a fXa inhibitor with a fXa K_i of 0.11 nM and activity in PT and aPTT assays of approximately 5 μM. Pyridone **19** had a low rate of clearance, high oral bioavailability, a half-life equivalent to that of razaxaban, was highly selective over a number of human enzymes, and represents a successful optimization of the 5-ketopyrazole scaffold.

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