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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5176–5182
Bioorganic & Medicinal Chemistry Letters

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

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Received 6 June 2006; revised 26 June 2006; accepted 5 July 2006
Available online 25 July 2006

Abstract—Previously, potent factor Xa inhibitors were described based on the 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one bicyclic core and a 4-methoxyphenyl P1 moiety. This manuscript describes 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one and related bicyclic cores with the 3-aminobenzisoxazole P1 moiety. Many of these compounds are potent, selective, and efficacious inhibitors of coagulation factor Xa.
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Thromboembolic diseases remain the leading cause of death and disability in developed countries. This reality, combined with the limitations of current therapies, has led to extensive efforts to develop novel antithrombotic agents.[1] Factor Xa has become a major focus of pharmaceutical intervention in the past decade because of its central role in the blood coagulation cascade.[2] Extensive preclinical and clinical evidence has demonstrated that inhibition of factor Xa is efficacious in both venous and arterial thrombosis.[3,4]

Previously, it was demonstrated that a series of non-benzamidine *N*-arylpyrazole carboxamides, represented by the 3-aminobenzisoxazole P1 analog razaxaban (**1**),[5a] were highly potent, selective, and orally bioavailable small molecule fXa inhibitors (Fig. 1). Razaxaban has been shown to be efficacious in phase II deep vein thrombosis (DVT) clinical trials.[5b] Furthermore, it was

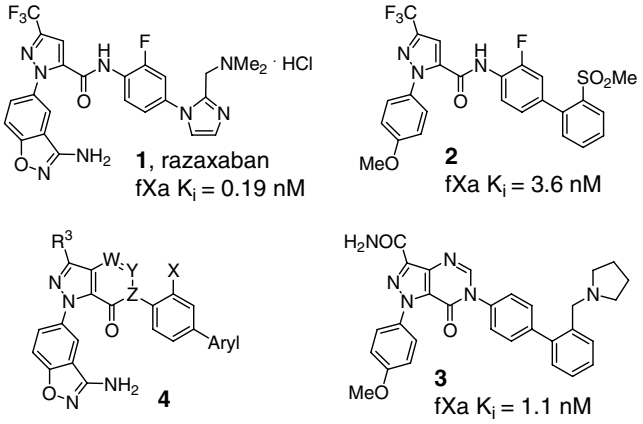

Figure 1.

demonstrated that the 4-methoxyphenyl residue could be an effective P1 group when combined with an optimized pyrazole-P4 subunit, as in compound **2**.[6] Recently, bicyclic core variants of **2**, represented by the 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one **3**,[7] have been shown to retain potent binding affinity for fXa. These bicyclic variants were also expected to be less susceptible to in vivo amide hydrolysis, which in the case of *N*-arylpyrazole carboxamides such as **1** and **2** would liberate a biarylaniline fragment. However, compound **3** was

Keywords: Factor Xa inhibitors; Anticoagulants; Antithrombotic agents; Pyrazole; Bicyclic core; Pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones.
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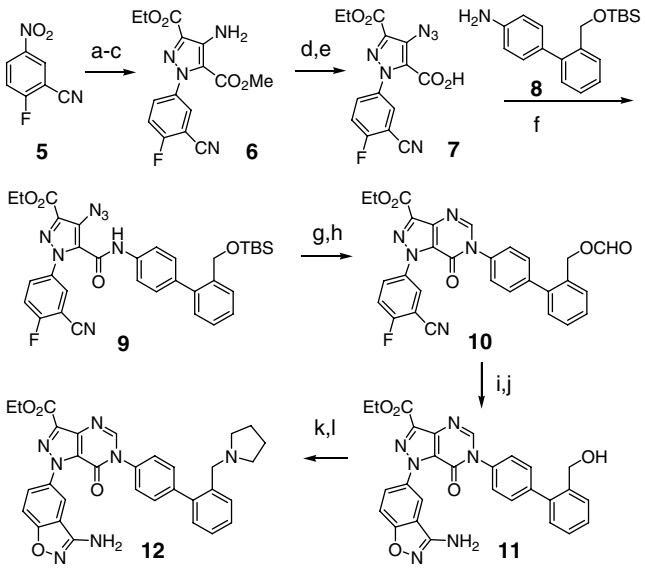
doi:10.1016/j.bmcl.2006.07.002

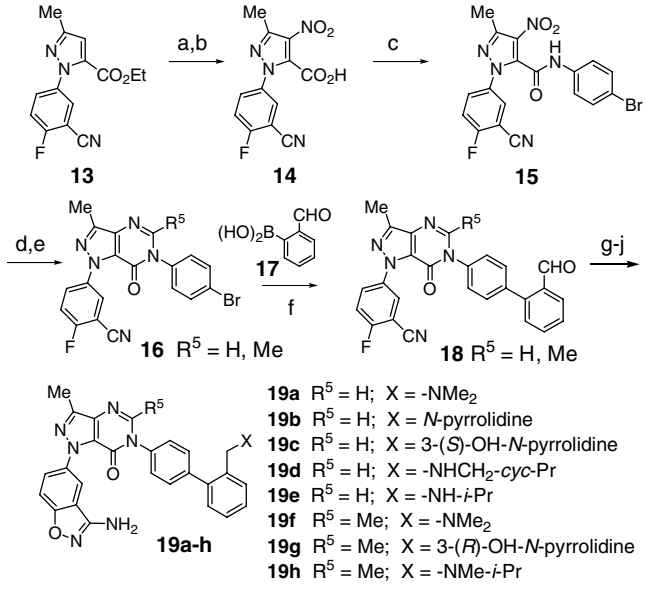
found to have only modest efficacy in a rabbit arteriovenous (A-V) shunt thrombosis model[8] relative to razaxaban. This was rationalized on the basis of **3** being 5-fold less potent than razaxaban.[7] This manuscript describes bicyclic variants in the 3-aminobenzisoxazole P1 series,[9] represented by **4**, which were prepared with the expectation that this P1 series would display greater potency and in vivo efficacy relative to analogs with the 4-methoxyphenyl P1, such as **3**.[10]

The initial bicyclic core example in the 3-aminobenzisoxazole P1 series, the 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one **12**, was prepared as described in Scheme 1, using the synthesis route used for preparing compounds such as **3**.[7] Catalytic hydrogenation of the nitro functionality of **5** afforded an aniline, which upon diazotization and treatment with ethyl cyanoacetate gave the expected hydrazone intermediate. Treatment of this hydrazone with methyl bromoacetate and K_2CO_3 at 90 °C gave the 4-aminopyrazole diester **6** by N-alkylation and in situ ring closure. However, this sequence proceeded in very low and irreproducible yield due to the poor nucleophilicity of the nitrogen in the N-alkylation step. Nevertheless, aminopyrazole **6** was diazotized and treated with sodium azide to form a 4-azidopyrazole. Selective hydrolysis of the methyl ester was accomplished with lithium hydroxide to give **7**. Conversion of **7** to an acid chloride and subsequent treatment with readily available biphenylaniline **8**[11] afforded amide **9**. Reduction of the azide afforded a 4-aminopyrazole-5-carboxamide, which was cleanly cyclized to the 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one bicyclic core by refluxing in 95% formic acid. This treatment also resulted in loss of the TBS group and subsequent conversion of the alcohol to a formate ester, to give **10**. Treatment of **10** with acetohydroxamic acid and K_2CO_3 to form the 3-aminobenzisoxazole P1 moiety[12] was followed by hydrolysis of the formate ester, giving **11**. Bromination of the alcohol and displacement with pyrrolidine afforded the 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one example **12**.

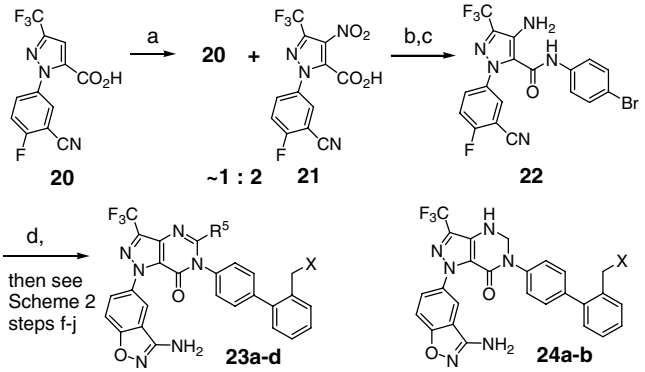
Due to the poor yield of **6** discussed above, additional examples in this bicyclic series were prepared via an alternate route, described in Scheme 2, wherein the pyrazole R^3 substituent was initially a methyl group.[9b] Readily available pyrazole ester **13**[9a] was efficiently and selectively nitrated at the pyrazole 4-position using excess ammonium nitrate and trifluoroacetic anhydride in TFA.[13] Subsequent ester hydrolysis afforded acid **14**, which was coupled with 4-bromoaniline to give **15**. Nitro reduction was unexpectedly difficult, with several reagents ($SnCl_2$, Zn/HCl, and Fe/HCl) resulting in significant decomposition. However, potassium borohydride and CuCl in ethanol afforded in moderate yield the clean 4-aminopyrazole, which was efficiently cyclized to the bicyclic core **16** by refluxing in either *N*,*N*-DMF dimethyl acetal (R^5 = H) or *N*,*N*-dimethylacetamide dimethyl acetal (R^5 = Me). Suzuki coupling with boronic acid **17** readily gave the biphenyl aldehyde **18**. The completion of the synthesis of compounds **19a**–**h** was best accomplished by a four-step sequence. Thus, sodium borohydride reduction of the aldehyde to the alcohol was followed by the introduction of the



Scheme 1. Reagents and conditions: (a) H_2 (1 atm), 10% Pd/C, EtOH (95%); (b) $NaNO_2$, HCl/H_2O, 0 °C; then ethyl cyanoacetate, NaOAc, $MeOH/H_2O$, 0 °C (55%); (c) $BrCH_2CO_2Me$, K_2CO_3, DMF, 90 °C (10–20%); (d) $NaNO_2$, TFA, 0 °C; then NaN_3 (80%); (e) LiOH, THF/H_2O (90%); (f) $(COCl)_2$, CH_2Cl_2; then **8**, DMAP, CH_2Cl_2 (70%); (g) $SnCl_2{\cdot}H_2O$, MeOH, 65 °C; (h) 95% HCO_2H, reflux (70% for two steps); (i) AcNHOH, K_2CO_3, DMF; (j) K_2CO_3, EtOH, 65 °C (45% for two steps); (k) PBr_3, CH_2Cl_2; (l) pyrrolidine, CH_3CN, (50% for two steps).



Scheme 2. Reagents and conditions: (a) NH_4NO_3 (2 equiv), TFAA (7 equiv), TFA (90%); (b) LiOH, $MeOH/H_2O$ (80%); (c) $(COCl)_2$, CH_2Cl_2; then 4-bromoaniline, DMAP, CH_2Cl_2 (80%); (d) KBH_4, CuCl, EtOH, (50%); (e) *N*,*N*-DMF dimethyl acetal, reflux (R = H, 85%), or *N*,*N*-dimethylacetamide dimethyl acetal, reflux (R = Me, 75%); (f) **17**, $Pd(PPh_3)_4$, Na_2CO_3, benzene, H_2O, 80 °C (70–80%); (g) $NaBH_4$, MeOH (50–65%); (h) HO-NHAc, K_2CO_3, DMF (60–70%); (i) MnO_2, CH_2Cl_2 (75-85%); (j) HNRR′, $NaBH(OAc)_3$, HOAc, THF, (50–70%).

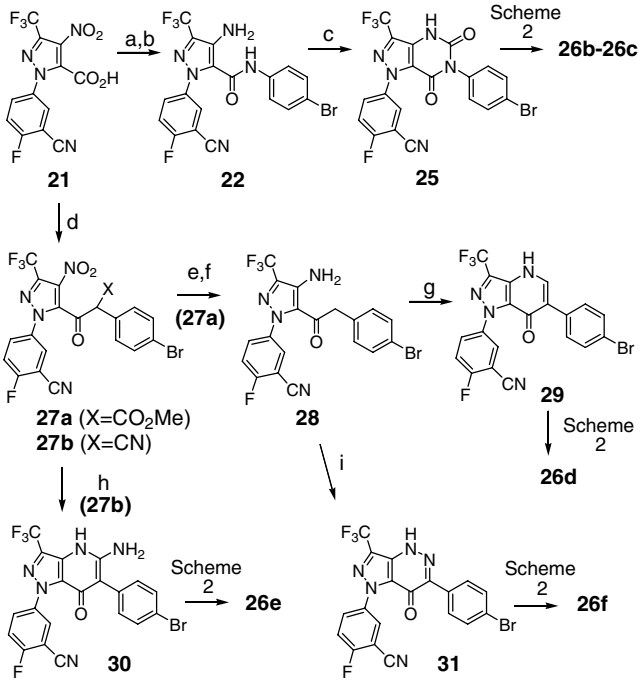


Scheme 3. Reagents and conditions: (a) NH_4NO_3 (2 equiv), TFAA (7 equiv), TFA (60%); (b) $(COCl)_2$, CH_2Cl_2; then 4-bromoaniline, DMAP, CH_2Cl_2 (90%); (c) KBH_4, CuCl, EtOH, (70%); (d) *N*,*N*-DMF dimethyl acetal, (R = H, 90%), or *N*,*N*-dimethylacetamide dimethyl acetal, reflux; then HOAc, reflux (R = Me, 75%).

3-aminobenzisoxazole P1 moiety using acetohydroxamic acid. The alcohol was then re-oxidized to the aldehyde with MnO_2 and treated under standard reductive amination conditions with an appropriate cyclic or acyclic amine, affording compounds **19a**–**h**. Alternatively, the alcohol could be brominated with PBr_3 followed by displacement with the appropriate amine as previously described.

Slight modification of this sequence, as shown in Scheme 3, afforded compounds **23a**–**d**, where the pyrazole R^3 group is trifluoromethyl.[9b] In this case, the pyrazole nitration was best performed on the carboxylic acid **20**.[5a] However, even after using an excess of reagents and prolonged reaction times, it generally afforded only an approximately 2:1 mixture of nitropyrazole **21** along with recovered starting material **20**. These materials could be efficiently separated by prolonged stirring of the solid in water, wherein the desired nitro compound **21** slowly went almost completely into solution. Filtration and extraction afforded clean product in about 60% yield. The starting material **20** could then be recycled. Coupling with 4-bromoaniline and nitro reduction as before gave **22**. Where R^5 = H, formation of the bicyclic core was accomplished as in Scheme 2 by refluxing in *N*,*N*-DMF dimethyl acetal. However, where R^5 = Me, the cyclization was best accomplished by a two-step sequence, wherein **22** was first refluxed in *N*,*N*-dimethylacetamide dimethyl acetal and the resulting acetamidine intermediate was refluxed in glacial acetic acid to effect the final cyclization.[7] Once the CF_3 bicyclic core was established, the synthetic sequence followed that described in Scheme 2, to afford compounds **23a**–**d**. The 5,6-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(4*H*)-one examples **24a**,**b** were prepared from a core reduction side product isolated from the sodium borohydride reduction of the corresponding biphenyl aldehyde, as described in Scheme 2.

Additional bicyclic core analogs were prepared as described in Scheme 4. Aminopyrazole **22** was cyclized by heating with phosgene in a sealed tube to afford the 1*H*-pyrazolo[4,3-*d*]pyrimidin-5,7(4*H*,6*H*)-dione core **25**.

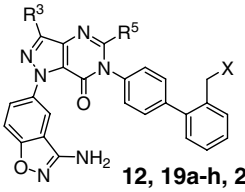


Scheme 4. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2; then 4-bromoaniline, DMAP, CH_2Cl_2 (90%); (b) $SnCl_2$, MeOH, reflux (70%); (c) ClCOCl/PhMe, sealed tube, 110 °C (85%); (d) $(COCl)_2$, DMF, CH_2Cl_2, then methyl *p*-bromophenylacetate, LDA, THF, −78 °C (X = CO_2Me, 60%), or $(COCl)_2$, DMF, CH_2Cl_2, then *p*-bromophenylacetonitrile, LDA, THF, −78 °C (X = CN, 75%); (e) 1.5 N HCl, dioxane, reflux; (f) Fe, 1N HCl, EtOH, reflux (65% for two steps); (g) $HC(OEt)_3$, 120 °C (85%); (h) $SnCl_2$, EtOH, reflux (50%); (i) $NaNO_2$,H_2SO_4, (80%).

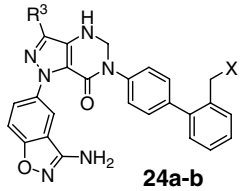
Methods described in Scheme 2 allowed for the preparation of compounds **26b**,**c**. Compound **26a** was prepared in a similar fashion, except that the fully elaborated P4 aniline was used in coupling with the carboxylic acid **21**. For bicyclic cores where the amide nitrogen is replaced by carbon, conversion of **21** to the acid chloride followed by treatment at −78 °C with the lithium anion of methyl *p*-bromophenylacetate and *p*-bromophenylacetonitrile gave good yields of α-ketoester **27a** and α-cyanoketone **27b**, respectively. Acid-catalyzed decarboxylation of **27a** followed by nitro reduction afforded **28**. Heating **28** in neat triethyl orthoformate induced ring closure to the 1*H*-pyrazolo[4,3-*b*]pyridin-7(4*H*)-one core **29**. The α-cyanoketone **27b** was treated with $SnCl_2$ in refluxing ethanol to effect nitro reduction and subsequent ring closure to give the 5-amino substituted 1*H*-pyrazolo[4,3-*b*]pyridin-7(4*H*)-one bicyclic core **30**. Diazotization of **28** was followed by in situ trapping by the adjacent enol to afford the 1*H*-pyrazolo[4,3-*c*]pyridazin-7(4*H*)-one bicyclic core **31**. Compounds **29**–**31** were converted by the methods described previously into **26d**–**f**, respectively.

The SAR for the 1-(3-aminobenzo[*d*]isoxazol-5-yl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one core examples are shown in Table 1. The initial bicyclic example, **12**, was an order of magnitude less potent than razaxaban **1** in the in vitro fXa K_i assay, but was 4-fold more potent and equipotent to **1** in the in vitro aPTT and PT clotting

Table 1. 1*H*-Pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one core



12, 19a-h, 23a-d



24a-b

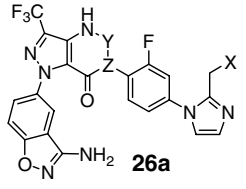
Compound[a]	R^3	R^5	X	fXa K_i[b] (nM)	aPTT IC2x[c] (μM)	PT IC2x[c] (μM)
1	—	—	—	0.19	6.1	2.1
12	CO_2Et	H	*N*-Pyrrolidine	1.6	1.6	2.3
19a	Me	H	NMe_2	0.53	2.1	1.3
19b	Me	H	*N*-Pyrrolidine	1.3	1.6	1.1
19c	Me	H	3-(*S*)-OH-*N*-Pyrrolidine	0.50	4.3	2.1
19d	Me	H	$–NHCH_2$-*c*-Pr	1.7	8.1	3.1
19e	Me	H	–NH-*i*-Pr	1.5	3.0	1.9
19f	Me	Me	NMe_2	0.27	1.7	1.4
19g	Me	Me	3-(*R*)-OH-*N*-Pyrrolidine	0.37	5.8	2.5
19h	Me	Me	–NMe-*i*-Pr	0.35	2.0	4.0
23a	CF_3	H	NMe_2	0.35	3.7	2.6
23b	CF_3	Me	NMe_2	0.17	9.2	4.1
23c	CF_3	Me	$–NHCH_2$-*c*-Pr	0.50	18.6	10.9
23d	CF_3	Me	3-(*R*)-OH-*N*-Pyrrolidine	0.18	28	6.6
24a	CF_3	—	NMe_2	0.25	1.7	1.4
24b	CF_3	—	3-(*R*)-OH-*N*-Pyrrolidine	0.21	2.9	1.6

[a] All final compounds were purified by prep HPLC and gave satisfactory spectral data.
[b] K_i values were obtained from purified human enzyme and were averaged from multiple determinations ($n = 2$), as described in Ref. 5a.
[c] The aPTT (activated partial thromboplastin time) and PT (prothrombin time) in vitro clotting assays were performed in human plasma as described in Ref. 5a.

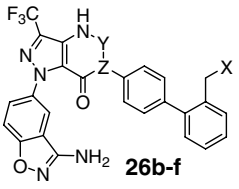
assays, respectively. The 3-methylpyrazole series **19a**–**h** afforded compounds approaching the binding potency of razaxaban, especially when R^5 was also methyl, as in compounds **19f**–**h**. The 3-methyl series also exhibited generally favorable potency in the clotting assays relative to **1**. The 3-trifluoromethylpyrazole series **23a**–**d** provided even more potent compounds, but generally at the expense of lower potency in the in vitro clotting assays, presumably due to the higher protein binding imparted by the CF_3 group. For example, compounds **23b** and **23d** are essentially equipotent with razaxaban in binding potency, but are less potent in the clotting assays. The reduced analogs of this core, the 5,6-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(4*H*)-ones **24a**,**b**, retain excellent potency and now have very favorable clotting assay activity relative to compounds **23a**–**d** and to razaxaban.

The SAR for additional bicyclic core analogs are shown in Table 2. Compounds **26a**–**c** contain the 1*H*-pyrazolo-[4,3-*d*]pyrimidin-5,7(4*H*,6*H*)-dione bicyclic core.[7] These examples are characterized by excellent in vitro binding

Table 2. Examples of other bicyclic cores



26a



26b-f

Compound[a]	Y	Z	X	fXa K_i[b] (nM)	apt IC2x[c] (μM)	PT IC2x[c] (μM)
1	—	—	—	0.19	6.1	2.1
26a	C=O	N	NMe_2	0.35	37.8	14.9
26b	C=O	N	3-(*R*)-OH-*N*-Pyrrolidine	0.11	74.5	24.1
26c	C=O	N	4-OH-*N*-Piperidine	0.22	352	92.6
26d	CH	C	NMe_2	3.2	ND	ND
26e	$C–NH_2$	C	4-OH-*N*-Piperidine	0.77	638	230
26f	N	C	NMe_2	5.5	ND	ND

[a] All final compounds were purified by prep HPLC and gave satisfactory spectral data.
[b] K_i values were obtained from purified human enzyme and were averaged from multiple determinations ($n = 2$), as described in Ref. 5a.
[c] The aPTT (activated partial thromboplastin time) and PT (prothrombin time) in vitro clotting assays were performed in human plasma as described in Ref. 5a.

potency, especially **26b**, but also by uniformly poor activity in the in vitro clotting assays, suggesting that this core contributes to high protein binding. Examples **26d–f** were prepared to determine if it was possible to replace the carboxamide nitrogen with carbon, thus negating the potential for generating an aniline degradant in vivo. Only the 5-amino-1*H*-pyrazolo[4,3-*b*]pyridin-7(4*H*)-one example **26e** retains subnanomolar fXa binding potency. Unfortunately, this compound has poor activity in the clotting assays, again suggesting that it is highly protein bound.

Compounds with the 3-aminobenzisoxazole P1 residue have previously been reported to show high selectivity for fXa versus thrombin, trypsin and related serine proteases.[5a,9,11] Additional selectivity data are shown for selected compounds in Table 3. Compounds **19f**, **19g**, **23b**, and **24b** all showed >1000-fold selectivity relative to trypsin, aPC, factor IXa, factor VIIa, plasmin, tPA, plasma kallikrein, urokinase, and chymotrypsin. They are less selective relative to thrombin, but still show >500-fold selectivity in all cases. The overall selectivity profile for these compounds in most cases is comparable to that of razaxaban **1**.

The pharmacokinetic profiles of several compounds were studied in dogs via a cassette dosing format, with dosing at 0.5 mg/kg intravenously and 0.2 mg/kg orally (Table 4). The 3-methylpyrazole **19a** has high clearance and only 2% bioavailability. Given its good Caco-2 permeability, this low bioavailability is more likely to be related to high first pass metabolism, rather than poor absorption. Addition of the 5-methyl substituent improves the pharmacokinetic profile of this series, with **19f** and **19g** exhibiting lower clearance and higher bioavailability relative to **19a** and a half-life comparable to razaxaban. The PK profile is further improved by the 3-trifluoromethylpyrazole substituent, as shown by **23b** and **23d**. These compounds have lower clearance and longer half-life than razaxaban, together with better bioavailability than the 3-methylpyrazole examples. Compound **24b** has a profile similar to **19f** and **19g**.

Three of these compounds were also studied in the rabbit arterio-venous (A-V) shunt thrombosis model.[8] Upon intravenous dosing, compounds **19f**,**19g**, and **23b** inhibited thrombus formation with an ID_{50} of 2.0, 2.4, and 1.9 μmol/kg/h, respectively (Table 5). Compound **23b** had an IC_{50} in this same assay of 290 nM. This activity compares well to that of razaxaban in this model.

Several examples from the 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one core series were examined for their stability under conditions of varying pH in order to assess their potential for liberation of the P4 aniline via hydrolytic cleavage of the bicyclic core. Data are shown for compounds **19a**, **19f**, and **23a** in Table 6. The results indicate that the pyrimidinone ring of the bicyclic core decomposes at various rates depending on the pH and on the substitution pattern of the bicyclic core. In most cases two major degradants were observed, the identities of which were determined by LC/MS. Compound **II** results from hydrolysis of the C5-N6 bond to afford the *N*-formyl (R^5 = H) or *N*-acetyl (R^5 = Me) derivative. Further hydrolysis affords the free amine **III**. For compound **19a**, **II** was the predominant degradant observed at pH 4.0, while the free amine **III** was the predominant degradant at pH 1.0. Mechanistically, the *N*-formyl **II** was thought to be an intermediate in the formation of **III**. Evidence for this was gathered experimentally by taking one half of the pH 4.0 samples and lowering them to pH 1.0. After one week, the pH 1.0 samples showed

Table 3. Human enzyme selectivity profile

Enzyme K_i (nM)	**19f**	**19g**	**23b**	**24b**	**1**
fXa	0.27	0.37	0.17	0.21	0.19
Thrombin	305	245	130	130	540
Trypsin	>4200	>4200	>4200	>4200	>10,000
aPC	19,000	>76,000	>37,000	>20,000	19,700
fIXa	>41,000	>41,000	37,000	>41,000	9000
Plasmin	>15,000	>15,000	>25,000	>15,000	>15,000
tPA	23,000	>33,000	>40,000	>33,000	>33,000
Urokinase	>13,000	>13,000	>40,000	>13,000	>13,000
Chymotrypsin	960	580	>11,000	3240	8500

All K_i's were obtained from purified human enzymes and are averaged from multiple determinations (n = 2). See Ref. 5a for more details.

Table 4. Dog pharmacokinetic profiles

Compound	Cl (L/h/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	F (%)	Caco-2 ($P_{app} \times 10^{-6}$ cm/s)
19a[a]	3.3	9.5	2.5	2	11
19f[a]	1.7	8.9	4.2	23	6.1
19g[a]	1.2	6.6	3.8	48	4.7
23b[a]	0.7	13	14	63	19
23d[a]	0.6	6.6	8.4	58	ND
24b[a]	0.98	4.2	4.3	27	3.9
Razaxaban[b]	1.1	5.3	3.4	84	5.6

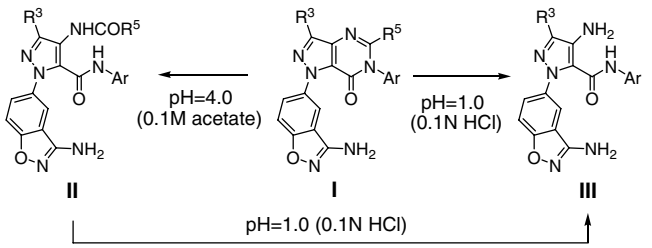
[a] Compounds were dosed as the TFA salts in an N-in-1 format at 0.5 mg/kg iv and 0.2 mg/kg po (n = 2).
[b] Ref. 5a. ND = not determined.

Table 5. Anticoagulant activity in rabbits

Compound	fXa K_i (rabbit) (nM)	PT IC2x (rabbit) (μM)	Rabbit A-V shunt[8] IC_{50} (nM)	Rabbit A-V shunt[8] ID_{50} (μmol/kg/h)	Protein binding (rabbit) (% bound)
19f	0.30	1.8	ND	2.0	ND
19g	0.59	3.4	ND	2.4	91
23b	0.20	2.7	290	1.9	97
Razaxaban[a]	0.19	1.9	340	1.6	93

[a] Ref. 5a. ND = not determined.

Table 6. Stability of 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones



Compound[a]	Conditions[a]	**I** remaining[b] (%)	**II**[b] (%)	**III**[b] (%)
19a	pH 1.0	97.2	0.6	2.4
	pH 4.0	90.1	8.5	0.12
19f	pH 1.0	89.7	5.5	1.4
	pH 4.0	94.7	3.4	—
23a	pH 1.0	83.2	1.3	5.6
	pH 4.0	72.3	23.4	—

[a] pH 1.0 = 0.1 N HCl buffer; pH 4.0 = 0.1 M acetate buffer. Stability studies were conducted at 40 °C with an initial concentration of 5 μg/mL.

[b] Values of % **I**–**III** were determined by HPLC after 2 days. The identities of **II** and **III** were determined by LC/MS.

complete conversion to the amine **III**, while samples remaining at pH 4.0 still showed a preponderance of **II**. Thus, it appears that at both pH 1.0 and 4.0, initial formation of **II** occurs, but only at pH 1.0 is **II** further hydrolyzed appreciably to **III**. The degradation of **19f** is similar to that of **19a**, with **II** (R^5 = Me) being the major component at pH 4.0. At pH 1.0 an appreciable amount of **II** remains, while conversion to **III** is slower. This observation presumably reflects the slower acid hydrolysis of an *N*-acetyl group relative to an *N*-formyl group. The 3-trifluoromethylpyrazole analog **23a** undergoes a more rapid decomposition than the 3-methylpyrazole analogs, with 23% conversion to **II** at pH 4.0 after 2 days. Apparently, the CF_3 group is activating the pyrimidinone ring toward the initial hydrolysis, thereby accelerating the decomposition. The observed formation of **II** and **III** in these studies of **19a**, **19f**, and **23a** leads to the possibility that the free P4 aniline could still be generated in vivo, although this risk is slight, since it would require further hydrolysis of an amide such as **III**, which can be viewed as a vinylogous urea. The free P4 aniline was not observed in these stability studies. Still, advancement of this series would require use of an Ames negative P4 aniline.

In summary, several examples of pyrazole-fused bicyclic core fXa inhibitors in the 3-aminobenzisoxazole P1 series have been described. The 1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one core is especially promising as a means to maintain potent fXa inhibition while also reducing the probability that in vivo amide hydrolysis will liberate a biarylaniline fragment. Within this series, analogs **19f**, **19g**, and **23b** are not only potent fXa inhibitors, but they are also highly selective versus relevant serine proteases. Compound **23b** has a favorable pharmacokinetic profile in dogs, with lower clearance and longer half-life relative to razaxaban. Furthermore, **19f**,**19g**, and **23b** are highly efficacious in the rabbit A-V shunt thrombosis model, with activity comparable to that of the clinical candidate, razaxaban. However, in aqueous solution stability studies, these compounds were found to undergo variable degrees of hydrolytic cleavage of the pyrimidinone ring to generate pyrazole-5-carboxamide degradants. While this core series is still promising, a major emphasis has been geared toward finding bicyclic cores with greater aqueous stability.[9b,14] Further efforts along these lines will be described in due course.

Acknowledgments

The authors thank Bruce Aungst, Frank Barbera, Tracy Bozarth, Earl Crain, Andrew Leamy, Dale McCall, and Carol Watson for technical assistance.

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