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Aminobenzisoxazoles with biaryl P4 moieties as potent, selective, and orally bioavailable factor Xa inhibitors

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Abstract—We have previously reported on a series of aminobenzisoxazoles as potent, selective, and orally bioavailable factor Xa inhibitors, which culminated in the discovery of razaxaban. Herein, we describe another approach to improve factor Xa inhibitory potency and pharmacokinetic profile by incorporating basic and water soluble functionalities on the terminal ring of the P4 biaryl group found in our earlier Xa inhibitors. This approach resulted in a series of potent, selective, and orally bioavailable factor Xa inhibitors.

Thrombosis remains the major cause of cardiovascular disorders in the Western countries. The continued impact of thrombotic diseases on morbidity and mortality has led to extensive efforts to discover and develop new antithrombotic agents.[1] Factor Xa has become a major focus of pharmaceutical intervention in the past decade because of its central and unique position in the coagulation cascade.[2] Preclinical and clinical studies have shown that inhibitors of factor Xa are effective in both venous and arterial thrombosis.[3,4]

Our efforts to discover and develop orally active inhibitors of factor Xa led to our first clinical candidate DPC423.[5] In an effort to improve trypsin selectivity, the *m*-benzylamine P1 moiety in DPC423 was replaced with 3-aminobenzisoxazole which resulted in compound **1** with >4000-fold selectivity. However, compound **1** demonstrated poor solubility and permeability which resulted in very low oral bioavailability in dogs. Re-optimization of the P4 moiety by replacing the terminal ring with solubilizing and less lipophilic heterocycles culminated in the discovery of clinical candidate razaxaban.[6a] Razaxaban was studied in phase II clinical trials and was shown to be efficacious in the treatment of deep vein thrombosis.[6b] Herein, we describe another approach to improve the factor Xa potency and pharmacokinetic profile of **1** by incorporating basic and water soluble functionalities on the terminal ring. This approach resulted in a series of potent, selective, and orally bioavailable factor Xa inhibitors (**2**) as shown in Figure 1.

The general synthesis for compounds with structure **2** is described in Scheme 1. Pyrazole-4-carboxylic acid **3**[5] was converted to the acyl chloride with thionyl chloride

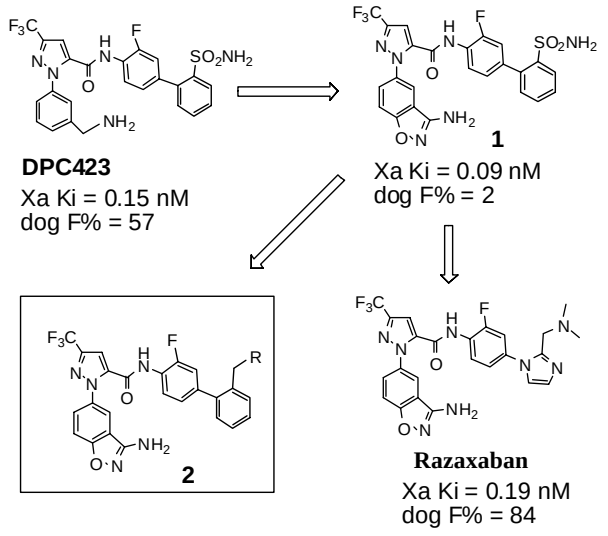

Figure 1.

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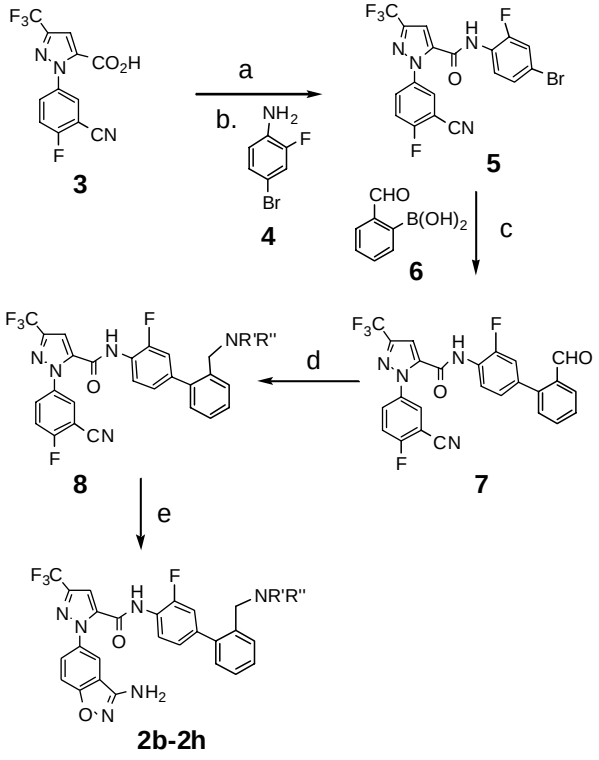
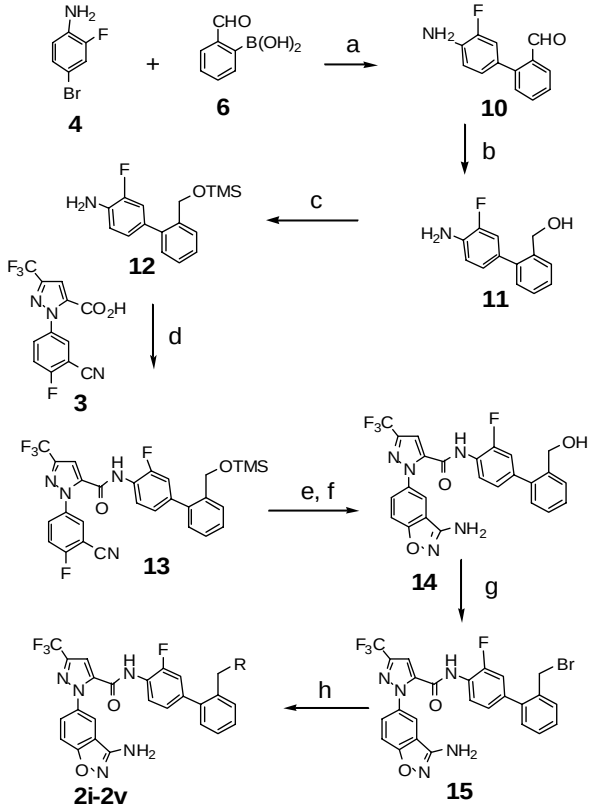


Scheme 1. Reagents and conditions: (a) $SOCl_2$/rt; (b) **4**, Et_3N/CH_2Cl_2, two steps: 80–90%; (c) $Pd(PPh_3)_4$, 2 M Na_2CO_3, H_2O–THF, reflux, 4 h, 80–90%; (d) NHR′R″, $NaBH(OAc)_3$, THF, 70–80%; (e) $CH_3CONHOH$, K_2CO_3, DMF/H_2O, 80–90%.



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, 2 M Na_2CO_3, H_2O–THF, reflux, 4 h, 80–90%; (b) $NaBH_4$, 95%; (c) TMSCl, imidazole, 90%; (d) 1—$SOCl_2$; 2—Et_3N/CH_2Cl_2, 85%; (e) $CH_3CONHOH$, K_2CO_3, DMF/H_2O, 80–90%; (f) HCl/EtOH, reflux 3 h, 80%; (g) PPh_3, CBr_4, 0 °C to rt, 85%; (h) excess R_1R_2NH, 40–50%.

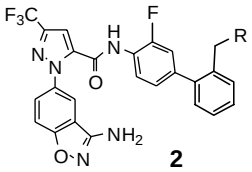
and then coupled with 4-bromo-2-fluoroaniline (**4**) to give amide **5**. Suzuki–Miyaura coupling of **5** with boronic acid **6** using $Pd(PPh_3)_4$ as the catalyst produced biaryl compound **7**. Reductive amination with various amines generated compound **8**. Aminobenzisoxazole formation[6a] with acetohydroxamic acid and potassium carbonate afforded the final product **2b**–**2h**.

Compounds **2a**, **2i**–**2v** were prepared by the sequence shown in Scheme 2. 4-Bromo-2-fluoroaniline **4** was coupled with boronic acid **6** under Suzuki–Miyaura conditions to yield biaryl aldehyde **10**. Aldehyde **10** was reduced with sodium borohydride to give alcohol **11**, which was then protected with TMSCl to give compound **12**. Compound **12** was coupled with acid **3** using the same conditions described previously to give amide **13**. Aminobenzisoxazole formation, followed by de-protection of the TMS group, produced alcohol **14**. Compound **14** was converted to the corresponding bromide **15**, which was reacted with pyrrolidine, 2(*S*)-CH_2OH-pyrrolidine, 3(*R*)-OH-pyrrolidine, 3(*S*)-OH-pyrrolidine, piperidine, morpholine, 4-methylpiperazine, imidazole, pyridine, *N*-methylmorpholine, and triethylamine to yield the final compounds **2i**–**2l**, **2o**–**2q**, and **2s**–**2v**, respectively, or reacted with 3(*R*)-Boc-aminopyrrolidine, 3(*S*)-Boc-aminopyrrolidine, and Boc-protected piperazine, followed by de-protection of the Boc group with TFA, to give compounds **2m**, **2n**, and **2r**, respectively. Compound **2a** was prepared from reacting bromide **15** with sodium azide, followed by Staudinger reduction.[7]

The SAR for this series is shown in Table 1. Replacing the aminosulfonamide moiety of **1** with an aminomethyl group resulted in compound **2a** with a Xa K_i of 2 nM, which represents a 20-fold loss in Xa affinity. However, alkylation of the amino group in **2a** regained Xa potency to subnanomolar affinity, except for **2r**. Compared with **2a**, mono- and di-alkylation of the amino group increased Xa affinity by 5- to 20-fold. Cyclic amines also retained Xa potency. The most potent compounds pyrrolidine **2i** and piperidine **2o** showed a 15- to 20-fold enhancement in Xa potency. Hydroxymethyl, hydroxyl, and amino-substitutions on the pyrrolidine ring were all well tolerated (**2j**–**n**). Piperidine is more potent than morpholine and piperazine. Methylation of the piperazine (**2q**) did not result in improved Xa potency. An aromatic heterocycle such as imidazole (**2s**) was found to be threefold less potent compared with alkylamines.

Interestingly, quaternary amines (**2t**–**2v**) were the most potent Xa inhibitors in this series with double digit picomolar affinity as demonstrated by their K_i values. They also exhibited the most potent in vitro anticoagulant activity with an $EC_{2\times}$ of 300 nM in the aPTT assay. However, the quaternary amines are less permeable than other compounds in this series as exemplified by compounds **2t** and **2v** with permeability of 0.35×10^{-6} and 0.25×10^{-6} cm/s in the Caco-2 assay, respectively.

Table 1. In vitro activity



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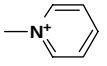
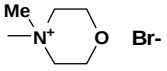
Compound	R	Xa K_i (nM)	Caco-2 $\times 10^{-6}$ (cm/s)	aPTT $EC_{2\times}$ (μM)
2a	$-NH_2$	2.0	nd	6.0
2b	–NHMe	0.27	3.48	4.5
2c	$-NHCH_2$-*c*-Pr	0.25	nd	19.1
2d	–NH-*i*-Pr	0.34	nd	5.5
2e	–NH-*c*-Bu	0.20	0.83	10.9
2f	–NH-*c*-Pen	0.30	nd	12.6
2g	$-NMe_2$	0.21	4.20	2.8
2h	Aziridinyl	0.40	nd	10.6
2i	Pyrrolidinyl	0.10	3.84	15
2j	2(*S*)-CH_2OH-Pyrrolidinyl	0.20	nd	3.9
2k	3(*R*)-OH-Pyrrolidinyl	0.16	3.7	7.3
2l	3(*S*)-OH-Pyrrolidinyl	0.53	nd	16
2m	3(*R*)-NH_2-Pyrrolidinyl	0.52	4.86	12.0
2n	3(*S*)-NH_2-Pyrrolidinyl	0.54	4.64	7.2
2o	Piperidinyl	0.15	8.89	8.7
2p	Morpholinyl	0.55	nd	60.8
2q	4-Me-Piperazinyl	0.86	2.32	6.4
2r	Piperazinyl	1.14	nd	10.0
2s	1*H*-Imidazolyl	0.66	nd	19.4
2t		0.08	0.35	0.29
2u		0.02	nd	0.35
2v	$-N^+(Me)_3Br^-$	0.05	0.25	0.29
Razaxaban		0.19	5.56	6.1

Xa K_is obtained from purified human enzymes and are averaged from multiple determinations ($n = 2$). See Ref. 6a for details on the Xa, Caco-2, and aPTT assays.
nd, no data.

The permeability and in vitro anticoagulant activity of these compounds varied depending on the substituents. For compounds with similar K_i values, the anticoagulant activity difference in the aPTT assay is probably due to the difference in protein binding of the compounds.

We earlier reported that compounds with the aminobenzisoxazole P1 moiety exhibit excellent selectivity relative to many key serine proteases.[6a] As anticipated, compounds in the current series also were very selective. As exemplified in Table 2, compounds **2g**, **2i**, and **2k** showed >10,000-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 >1000-fold selectivity. The overall selectivity profile for these compounds is very similar to razaxaban.

The pharmacokinetic profile of selected potent compounds with good permeability in the Caco-2 assay was studied in dogs via a cassette dosing format (Table 3). Compounds **2g**, **2i**, and **2k** were dosed at 0.4 mg/kg intravenously and 0.2 mg/kg orally. Compared with razaxaban, these compounds have a slightly lower permeability in the Caco-2 assay and therefore it is not surprising that they showed lower bioavailability. The clearance for these compounds is similar to razaxaban, but these compounds do show a higher volume of distribution, which resulted in a longer halflife. Overall these compounds are very much comparable to razaxaban in terms of the pharmacokinetic profile in that they have halflife in the range of 5–10 h and show good oral bioavailability.

These three compounds were also studied in the rabbit arterio-venous (A-V) shunt thrombosis model.[5b] Upon intravenous dosing, compounds **2g**, **2i**, and **2k** inhibited thrombus formation in a dose-dependent manner with an IC_{50} of 920, 180, and 50 nM, respectively (Table 4). The correlation between in vitro anticoagulant activity and in vivo antithrombotic activity is not very good. However, the in vivo antithrombotic activity observed

Table 2. Human enzyme selectivity profile

Enzyme K_i (nM)	**2g**	**2i**	**2k**	Razaxaban
Xa	0.21	0.10	0.16	0.19
Thrombin	300	250	400	540
Trypsin	>1600	>2500	>2500	>10,000
aPC	15,000	13,000	5700	19,700
IXa	>12,000	>12,000	>12,000	9000
VIIa	>15,000	>15,000	>15,000	>15,000
Plasmin	>15,000	>15,000	>27,000	>15,000
tPA	>33,000	>33,000	>35,000	>33,000
Plasma Kallikrein	>2300	>2300	nd	>2300
Urokinase	>13,000	>13,000	nd	>13,000
Chymotrypsin	1500	1700	nd	8500

All K_is obtained from purified human enzymes and are averaged from multiple determinations ($n = 2$). See Ref. 6a for more details.
nd, no data.

Table 3. Dog pharmacokinetic profiles

Compound	Cl (L/kg/h)	V_{dss} (L/kg)	$t_{1/2}$ (h)	F (%)
2g	1.1	9.0	6.6	38
2i	1.7	23	10	64
2k	0.76	5.8	6.5	43
Razaxaban	1.1	5.3	3.4	84

Compounds were dosed as the TFA salts in an N-in-1 format at 0.4 mg/kg iv and 0.2 mg/kg po ($n = 2$).

Table 4. Anticoagulant activity in rabbits

Compound	Xa K_i (rabbit) nM	aPTT/PT (rabbit) $EC_{2\times}$, μM	Rabbit A-V shunt[5b] IC_{50} (nM)	Protein binding[8] (rabbit)
2g	0.22	5.3/1.5	920	94.4
2i	0.46	7.5/0.8	180	82.0
2k	0.17	7.1/1.4	50	68.0
Razaxaban	0.19	3.5/1.9	340	93.4

in the rabbit A-V shunt model correlates with the rabbit protein binding shown in Table 4. With similar Xa affinity, compound **2g** has higher protein binding and is less potent in the rabbit A-V shunt thrombosis model. Compound **2k** has 32% free fraction and, therefore, exhibits the most potent anticoagulant activity in the A-V shunt model (IC_{50} of 50 nM). Overall, compound **2k** is six times more potent than razaxaban in the rabbit A-V shunt thrombosis model.

In summary, optimization of the P4 moiety by incorporating basic and water solubilizing groups on the terminal ring of the biphenyl group has led to a series of potent, selective, and orally bioavailable factor Xa inhibitors. Compounds **2g**, **2i**, and **2k** show comparable potency, selectivity, and pharmacokinetic profile to razaxaban. Compound **2k** showed a sixfold enhancement in potency in the rabbit A-V shunt thrombosis model compared to razaxaban.

Acknowledgments

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