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N,N-Dialkylated 4-(4-arylsulfonylpiperazine-1-carbonyl)-benzamidines and 4-((4-arylsulfonyl)-2-oxo-piperazin-1-ylmethyl)-benzamidines as potent factor Xa inhibitors

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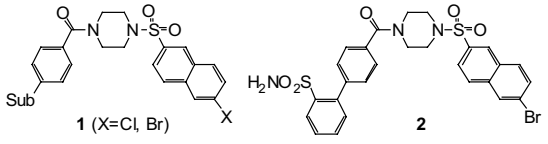
Abstract—A class of N,N-dialkylated 4-(4-arylsulfonylpiperazine-1-carbonyl)-benzamidines and 4-((4-arylsulfonyl)-2-oxo-piperazin-1-ylmethyl)-benzamidines has been discovered as potent factor Xa inhibitors with desirable in vitro and in vivo anticoagulant activity, but with low oral bioavailability. The 5-chloroindole and 6-chlorobenzo[*b*]thiophene groups are optimal as the factor Xa S1 binding elements. The strategy of incorporating a side chain on the piperazine nucleus to enhance binding affinity has been examined.

Factor Xa (fXa) is a pivotal serine protease in the blood coagulation cascade, responsible for the conversion of prothrombin to thrombin. The design and discovery of orally active small molecule human fXa inhibitors as a novel therapy for thromboembolic disorders has been a major focus of the pharmaceutical industry.[1] Since 1998, a variety of substituted (4-(6-halonaphthalene-2-sulfonyl)-piperazine-1-carbonyl)-benzene derivatives (**1**) have been reported in patent publications as fXa inhibitors.[2] Using a biphenylsulfonamide[3] as the S4 binding element, we prepared the 6-bromo-2-naphthyl containing analog **2** based on molecular modeling of this template, which indicated that the 6-bromo-2-naphthyl moiety fits in the S1 pocket of the fXa active site and points into the aromatic ring of Tyr228 at the bottom of the pocket. In vitro, **2** displays a modest fXa IC_{50} of 240 nM with marginal water solubility.



In order to improve the binding affinity and hydrophilicity of our inhibitor, we designed a series of compounds, **3a**–**k**, employing N,N-dialkylated benzamidines which, according to our docking studies, should be tolerated as an S4 binding motif.[4] At physiological pH, these benzamidines would be positively charged and thus would not only increase the inhibitor's hydrophilicity, but could also increase the binding affinity through ionic interactions with the S4 pocket. Unsubstituted benzamidines have long been used in fXa inhibitors as binding elements in both the S1 and S4 pockets. However, most of these benzamidine compounds suffer low oral bioavailability and a short half life. The N,N-dialkylated benzamidines have better oral absorption than the corresponding unsubstituted

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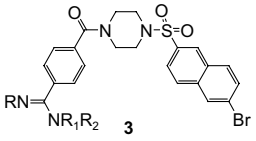
benzamidine, as known in the platelet glycoprotein IIb–IIIa antagonist literature.[5,6] Moreover, researchers at Berlex have reported a compound, ZK-807834,[7] which contains a *N*-methylimidazoline—a cyclized alkyl amidine—group as the S4 binding moiety.

The N,N-dialkylated benzamidine S4 SAR results are given in Table 1. The *N*-methylimidazoline analog **3h** (IC_{50} 31 nM) is the most potent in this group. Any modification from **3h** leads to loss in fXa potency, including de-methylation (**3g**), methyl homologation (**3i**), ring cleavage (**3c**) and ring expansion (**3k**).

To explore the S1 binding region of our inhibitor, we synthesized compounds **4a**–**8** (Table 2). From this series of inhibitors, we found that the 6-chlorobenzo[*b*]thiophene-2-sulfonyl (**4a**: IC_{50} 9 nM) and 5-chloroindole-2-sulfonyl (**7a**: IC_{50} 5 nM) moieties significantly increase fXa activity as the S1 binding ligands. Interestingly, the isomeric 5-chlorobenzo[*b*]thiophene-2-sulfonyl (**5**) and 6-chloroindole-2-sulfonyl (**6**) groups show a significant decrease in potency. It is also clear that the sulfonyl linkage is required as replacement with a carbonyl group (**8**) completely destroys the fXa activity.

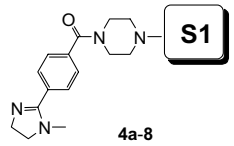
Table 1. S4 SAR of the N,N-dialkylated 4-(4-(6-bromonaphthalene-2-sulfonyl)-piperazine-1-carbonyl)-benzamidines



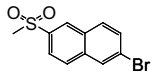
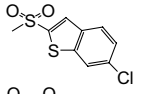
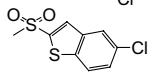
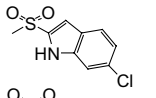
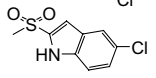
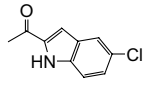
3

Compound		fXa IC_{50} (nM)
3a		64
3b		230
3c		230
3d		390
3e		160
3f		640
3g		530
3h		31
3i		67
3j		1300
3k		430

Table 2. S1 SAR of the 1-(4-arylsulfonylpiperazine-1-carbonyl)-4-(*N*-methylimidazolin-2-yl)-benzenes



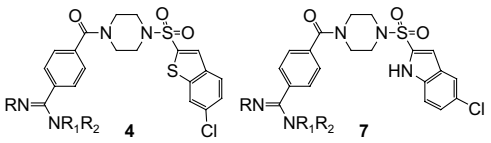
4a-8

Compound	S1	fXa IC_{50} (nM)
3h		31
4a		9
5		270
6		590
7a		5
8		>11,000

After the 6-chlorobenzo[*b*]thiophene-2-sulfonyl and 5-chloroindole-2-sulfonyl were identified as the optimal S1 motifs, we revisited the N,N-dialkylated benzamidine SAR. As shown in Table 3, we discovered the *N*-methylimidazoline (**4a** and **7a**) is still the most potent S4 binding element for this class of inhibitors. We found that less bulky amidines, such as the *N*,*N*-dimethylamidine (**4c**: IC_{50} 27 nM; **7c**: IC_{50} 20 nM) and the azetidine-containing analog (**4e**: IC_{50} 25 nM; **7e**: IC_{50} 24 nM), possess relatively higher fXa activity than larger amidines. Enlargement of the azetidine ring by even one methylene to pyrrolidine resulted in an approximately 3-fold drop in potency (**4f** and **7f**).

The in vitro anticoagulant activity, enzyme selectivity and rat PK profiles of lead inhibitor **7a** are summarized in Table 4. Its fXa K_i is 1.9 nM. In our human plasma based thrombin generation (TG) assay,[8] a concentration of 1.3 μM was needed to double the time of maximum thrombin formation (2×TG). In a rabbit clotting assay, a concentration of 1.6 μM was required to prolong the prothrombin time (PT) by twofold (2×PT). Compound **7a** displays excellent fXa selectivity (>1000-fold by IC_{50}) against thrombin, trypsin, tissue plasminogen activator (t-PA), activated protein C (aPC), plasmin, and kallikrein. To evaluate this fXa inhibitor in vivo, we studied it

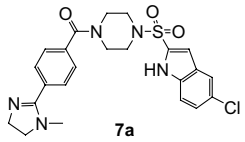
Table 3. In vitro activity of N,N-dialkylated benzamidines



Compound	RN / R_2R_1N	fXa IC_{50} (nM)
4a		9
4b		36
4c		27
4d		90
4e		25
4f		70
4g		71
7a		5
7b		12
7c		20
7d		27
7e		24
7f		84
7g		120

in a rabbit deep vein thrombosis model.[10] Infusion of compound **7a** produced a 37% inhibition of thrombus accretion at a plasma level of 1.1 μM and an ex vivo PT change of 1.26-fold. At a plasma concentration of 2.6 μM, 57% inhibition and 2.09-fold ex vivo PT change were observed. Disappointingly, the oral bioavailability of this fXa inhibitor is only 1.4% in rat.[9] Its half life, volume of distribution and clearance are 7.8 h, 28.9 L/kg, and 42.9 mL/min/kg, respectively.

Table 4. The in vitro anticoagulant activity, enzyme selectivity and PK profiles of fXa inhibitor **7a**



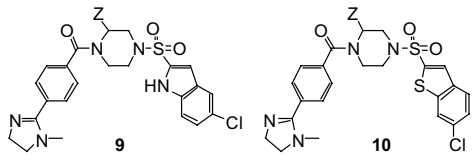
fXa IC_{50} (nM)	5
fXa K_i (nM)	1.9
2×TG (μM)	1.3
2×PT (μM) (rabbit)	1.6
Thrombin IC_{50} (μM)	>10
Trypsin IC_{50} (μM)	>10
t-PA IC_{50} (μM)	>10
aPC IC_{50} (μM)	>10
Plasmin IC_{50} (μM)	>10
Kallikrein IC_{50} (μM)	5.8
F (%)	1.4
$T_{1/2}$ (IV) (h)	7.8
Vd (L/kg)	28.9
CL (mL/min/kg)	42.9

In an attempt to pick up additional binding interactions in the fXa active site, we installed a side chain on the central piperazine nucleus (see Table 5). However, we found that this modification has little effect on fXa activity. Furthermore, we discovered in some cases that this compromises the fXa/thrombin selectivity. For example, the fXa and thrombin IC_{50} values of compound **10h** are 15 nM and 3.2 μM. Its fXa/thrombin selectivity is only about 200-fold, significantly reduced from that of compound **4a** (fXa IC_{50} 9 nM; thrombin $IC_{50} > 10$ μM; >1000-fold selectivity).

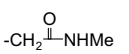
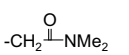
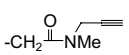
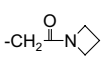
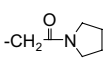
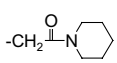
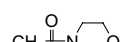
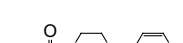
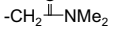
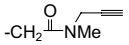
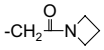
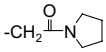
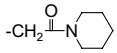
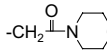
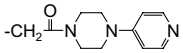
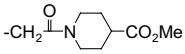
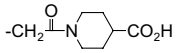
Several series of 4-arylsulfonylpiperazin-2-one-based fXa inhibitors with a variety of S4 motifs have been reported in patent applications[11] and publications.[12] We investigated the possibility of using N,N-dialkylated benzamidines as S4 motifs in the 4-arylsulfonylpiperazin-2-one series by preparing the corresponding analogs **11** and **12**. As shown in Table 6, the resulting amidine series has good potency. Interestingly, *N*-methylimidazoline (**11a** and **12a**) is no longer the optimal S4 motif. The *N*,*N*-dimethylamidine (**11b** and **12b**) and azetidine-containing analog (**11c** and **12c**) show increased potency, with the azetidine substituted amidine being optimum for both the benzothiophene and indole series. Unfortunately, this class of 4-arylsulfonylpiperazin-2-one fXa inhibitors also suffers poor oral bioavailability. For example, the bioavailability for analogs **11b** and **12b** are <1% and 4.1%, respectively.[9]

Scheme 1 details the syntheses of both the 4-(4-arylsulfonylpiperazine-1-carbonyl)-benzamidine and 4-((4-arylsulfonyl)-2-oxo-piperazin-1-ylmethyl)-benzamidine classes of fXa inhibitors. All of the corresponding arylsulfonyl chlorides were synthesized following literature procedures.[11d,e] For preparation of **10h**, racemic

Table 5. Effects of side chains on fXa potency (all compounds racemic)

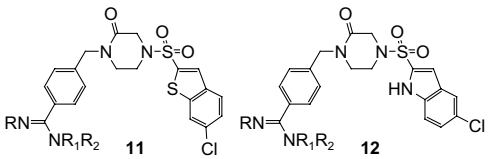


9 **10**

Compounds	Z	fXa IC_{50} (nM)
9a	–CO_2Me	27
9b	–CO_2H	12
9c	–CH_2CO_2Me	3
9d	–CH_2CO_2H	41
9e	–CH_2CONH_2	18
9f		11
9g		10
9h		14
9i		5
9j		7
9k		7
9l		11
9m		8
10a	–CH_2CO_2Me	13
10b	–CH_2CO_2H	29
10c		7
10d		19
10e		10
10f		6
10g		6
10h		15
10i		8
10j		8
10k		8

sulfonylpiperazine **14** was assembled from acylated piperazine **13** and 6-chlorobenzo[*b*]thiophene-2-sulfonyl chloride in pyridine. Treatment of resulting sulfonamide **14** with HCl-saturated methanol produced methyl imidate **15**, which reacted with *N*-methylethylenediamine to furnish the *N*-methylimidazoline functionality. Methyl ester **16** was then converted to amide **10h** by saponification and BOP-assisted coupling with morpholine to furnish the fully elaborated inhibitor.

Table 6. In vitro activity of N,N-dialkylated benzamidines

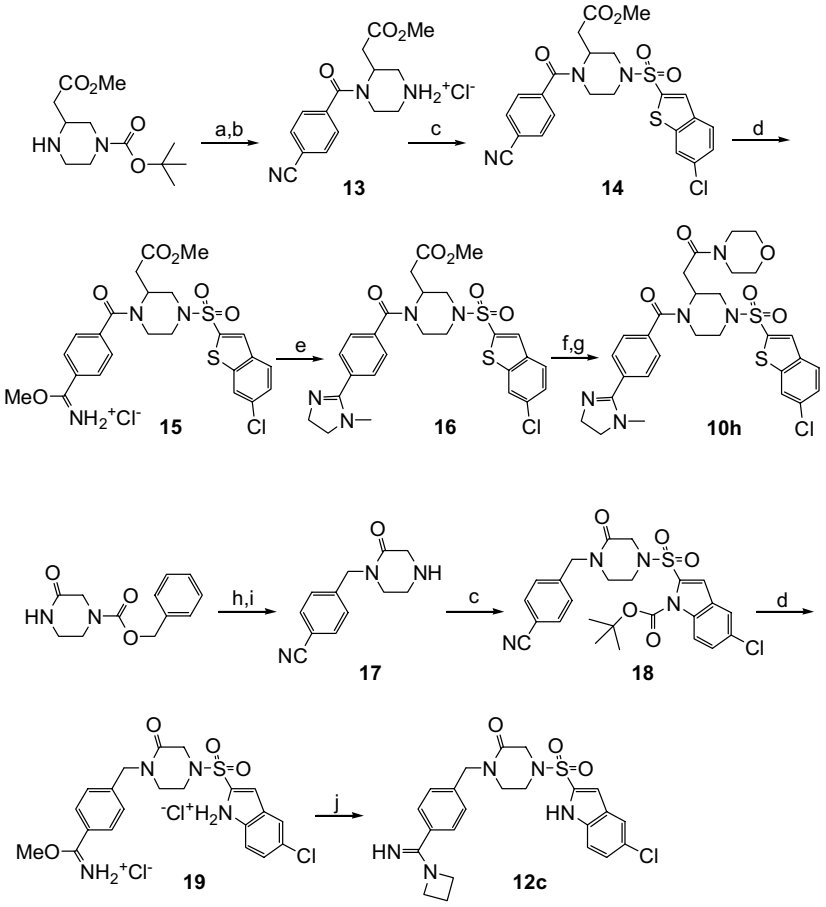


11 **12**

Compounds		fXa IC_{50} (nM)
11a		45
11b		30
11c		11
11d		140
11e		100
12a		57
12b		37
12c		8
12d		240
12e		150

Preparation of inhibitor **12c** started from the nucleophilic displacement of 4-cyanobenzylbromide with Cbz-protected piperazinone in DMF. Deprotection of the benzyl carbamate afforded amine **17**, which was coupled to *N*-Boc-5-chloroindole-2-sulfonyl chloride in pyridine at room temperature. Then the benzonitrile was converted to the benzamidine (**12c**) via methyl imidate **19**.

In conclusion, using N,N-dialkylated benzamidines as S4 motifs and piperazine or piperazinone as the central linker, we have discovered two related series of fXa inhibitors. We found that 6-chlorobenzo[*b*]thiophene and 5-chloroindole groups are the optimal S1 binding elements and that the use of a substituted amidine as the S4 group improved both the potency and physicochemical properties of the inhibitors as compared to the biphenylsulfonamide lead. These compounds have good



Scheme 1. (a) 4-Cyanobenzoyl chloride (1.2 equiv), pyridine, THF, rt; (b) 4 N HCl in dioxane, rt; (c) $ArSO_2Cl$, pyr, rt; (d) MeOH, saturated with HCl (g), 0 °C to rt, overnight; (e) $MeNHCH_2CH_2NH_2$ (3 equiv), MeOH, reflux, 1 h; (f) LiOH, MeOH, water, rt; (g) morpholine (1 equiv), BOP (1 equiv), DIEA (3 equiv), DMF, rt, overnight; (h) 4-cyanobenzyl bromide (1 equiv), Cs_2CO_3 (1 equiv), DMF, rt; (i) H_2 (g), 10% Pd/C, MeOH, rt; (j) azetidine (3 equiv), MeOH, rt.

in vitro and in vivo activity as well as excellent specificity. However, the low oral bioavailability of this class of compounds has made them less attractive for further development.

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References and notes

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