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Parallel synthesis and structure–activity relationships of a series of highly potent, selective, and neutral factor Xa inhibitors

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Abstract—Parallel synthesis and iterative optimization led to the discovery of a series of potent and specific factor Xa inhibitors demonstrating excellent in vitro activity with promising pharmacokinetics.

1. Introduction

Thrombotic diseases are the leading cause of mortality and morbidity in North America. Warfarin, the only oral anticoagulant currently prescribed, requires careful and costly monitoring due to its narrow therapeutic index, which is exacerbated by patient variability and drug–drug or drug–food interactions. Factor Xa (fXa) is a trypsin-like serine protease that converts prothrombin to active thrombin in the blood coagulation cascade. In the past few years, inhibition of fXa has emerged as an attractive target in the development of orally active antithrombotic agents to replace warfarin.[1] The recent clinical trials of an indirect fXa inhibitor, fondaparinux sodium, for the prevention of venous thromboembolism is direct evidence that specific fXa inhibitors are more beneficial than nonspecific fXa inhibitors, such as low molecular weight heparins.[2]

Design strategy: Molecular modeling studies of mono-benzamidine FXa inhibitor SN-429[3] based on the crystal structures of two amidine inhibitors complexed with fXa[4] showed that the molecule adopts an L-shape conformation at the active site of fXa, with the biphenyl group binding to the S4 hydrophobic site and the benzamidine fitting in the S1 pocket forming a twin–twin ionic interaction with Asp189 side chain. In addition to Asp189, the bottom of the S1 binding pocket is also comprised of the hydrophobic side chain of residue Tyr228. Thrombin, the final serine protease in the blood coagulation cascade, also shows a high degree of sequence homology with fXa in the S1 region. It has been demonstrated in the thrombin inhibitor area that a neutral moiety can form hydrophobic interactions with Tyr228 to compensate for the lack of ionic interaction with Asp189.[5] Therefore, we hypothesized that an aromatic nonamidine motif might fit in the S1 pocket by hydrophobic interactions. Based on this hypothesis, our design strategy was to retain the biphenylsulfonamide as a S4 binding moiety, utilize a number of flexible linkers in the center of the molecule, and explore various non-benzamidine moieties as S1 binding motifs.

Keywords: Factor Xa; SAR; Amino acid; Aspartic acid.
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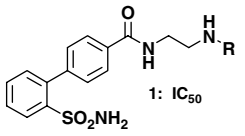
Table 1. SAR of ethylenediamine based compounds



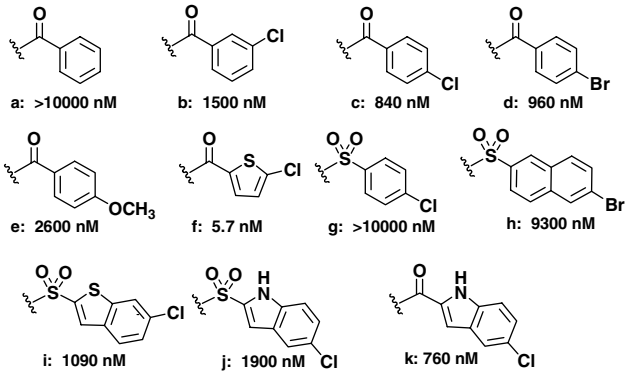
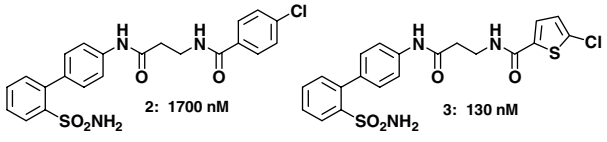


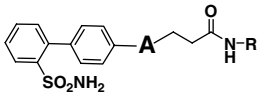
Table 1 shows the in vitro fXa binding affinity of a series of inhibitors containing an ethylenediamine linker. The unsubstituted phenyl S1 binding group (**1a**) did not show appreciable binding activity. Installing a chloro (**1b** and **1c**) or bromo (**1d**) substituent on the phenyl group improved fXa binding affinity to around 1 μM, with a slight preference for the *para*-geometry. The 4-methoxy substitution (**1e**), however, showed less binding affinity than the halogens. When the chlorophenyl ring of **1b** was replaced with a similarly substituted chlorothiophene ring (**1f**) a nearly 150-fold improvement was realized. The 5-chlorothiophene fragment has also been utilized by the Bayer scientists as a S1 moiety in their fXa inhibitors.[6]

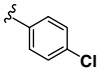
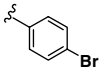
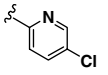
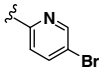
Changing the amide connecting the S1 chlorophenyl moiety with a sulfonamide (**1g**) resulted in a significant loss in potency. However, compounds **1h**–**j** with fused-bicyclic sulfonamides, such as the bromonaphthalene, chloroindole,[7] and chlorobenzothiophene,[8] displayed measurable binding affinity. Interestingly, when the sulfonamide linkage of chloroindole **1j** was replaced with an amide (**1k**) the potency improved 2.5-fold.



We next reversed the biphenyl amide linkage of the two most potent analogs from our initial series: chlorophenyl and chlorothiopheneyl analogs **1c** and **1f**. As shown above, chlorophenyl compound **2** lost 2-fold activity and chlorothiopheneyl compound **3** was substantially less active than in the previous case. Modeling studies indicated that in order for the chlorine atom to interact optimally with Tyr228, compound **3** has to adopt a conformation with increased torsional strain at its linker region (data not included).

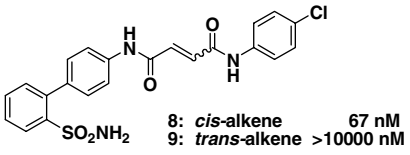
Table 2. SAR of reversed S1 linkage and biphenyl amide linkages



Compd	R =	IC_{50} (nM)	
		A = –CONH– (a)	–NHCO– (b)
4a/b		17	12
5a/b		29	65
6a/b		19	37
7a/b		12	20

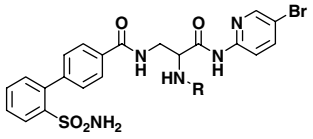
Data for the final series of ethylene linked inhibitors is given in Table 2, which shows the effect of reversing the S1 amide for both biphenyl amide linkages. Addressing the S1 haloarene amide first, we found that changing the orientation of the amide bond results in a 50-fold and a 33-fold improvement for the chloro (**4a**) and bromo (**5a**) cases, respectively. This improvement is retained in changing the S1 phenyl ring to a similarly substituted pyridyl ring (**6a** and **7a**). The pyridyl substitution offers a modest increase in hydrophilicity. Modification of the S4 biphenyl amide bond's orientation (**4b**–**7b**) has little effect on potency with the corresponding values being within experimental error. 4-Halo-anilide or 5-halo-2-aminopyridyl are also utilized as P1 motifs in several other series of factor Xa inhibitors.[9]

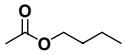
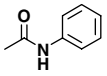
Our final examination of unsubstituted linkers consisted of the synthesis and evaluation of structurally constrained analogs **8** and **9**. Here, we found that locking the template with the two groups appended in a *cis*-orientation (**8**) resulted in a 5-fold loss of potency as compared with the saturated system **4b**. *trans*-Isomer **9** did not show measurable activity within the limits of our assay, which is not surprising considering that it would be unable to form the 90° turn at the binding site of fXa, and is consistent with results observed for the mono-benzamidine inhibitors.[10]



With the linker region of the molecule investigated we decided to further optimize our template by adding

Table 3. SAR of side chain adjacent to the S1 amide



Compd	R	IC_{50} (nM)
10a	–H	1650
10b	$–SO_2CH_3$	1250
10c	$–COCH_3$	805
10d		1246
10e		8500

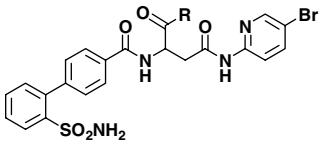
functionality to the linker region in an attempt to pick up additional interactions with the fXa active site. As the bromopyridyl S1 motif was the most active we decided to use it for subsequent investigations.

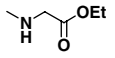
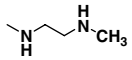
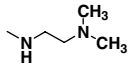
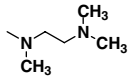
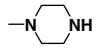
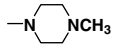
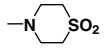
Elaboration of the template adjacent to the S1 aminopyridinyl amide resulted in a significant loss of potency (Table 3). Modification of the basic amino group (**10a**) to a methanesulfonamide (**10b**), acetamide (**10c**), carbamate (**10d**), or urea (**10e**) all resulted in a loss of potency as compared to unsubstituted analog **7a**. Based on our modeling studies of the linear template we reasoned that this loss in potency was likely due to an unfavorable steric interaction with the side chain of the inhibitor with the protein as the aminopyridinyl amide binds in the S1 pocket.

We next modified the linker adjacent to the biphenyl amide. Here, we discovered analogs equipotent or better than **7a** and which tolerated diverse functionality (Table 4). The carboxylic acid **11a** showed a nearly 3-fold increase in potency as compared to unsubstituted inhibitor **7a**. This gain was lost, however, when the carboxylic acid was converted to either methyl ester **11b** or methyl amide **11c**. We found that the potency was retained when ethyl glycinate was attached (**11d**) to the carboxylic acid. Attaching a basic amino side chain to carboxamide **11c** reduced activity by about 3-fold, as seen in compounds **11e**–**g**. Turning our attention to cyclic functional groups we synthesized a variety of cyclic and exocyclic linked amides (**11h**–**p**) of which the six-membered piperidinyl ring (**11k**) and seven-membered hexamethyleneiminyl (**11l**) were the most active. Further examination of **11a** and **11k** revealed that, while the analogs are quite similar with respect to their IC_{50} values, their in vitro activity differs more than 10-fold with 2XPT values of 74 and 7 μM, respectively. Further investigation revealed a difference in the plasma protein binding of these two inhibitors: 98.8% for **11a** and 96.1% for **11k**, which likely contributes to the difference in activity. It should be noted, however, that this modification offers a considerable improvement over lead inhibitor **7a**, which has a 2XPT of 100 μM.

We next turned our attention to reversing the amide linker used to attach the side chain to the linear template

Table 4. SAR of side chain modification adjacent to the S4 amide

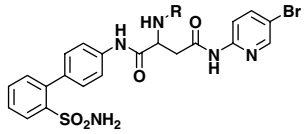


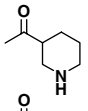
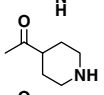
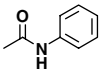
Compd	R	IC_{50} (nM)
11a	–OH	4.37
11b	–OMe	24.7
11c	$–NHCH_3$	17.4
11d		3.3
11e		43.4
11f		49.7
11g		45.5
11h		15.9
11i		26.5
11j		14.5
11k		7.6
11l		4.98
11m		90.9
11n		26.5
11o		22.9
11p		40.1

(Table 5). While this necessitated reversing the S4 amide bond our previous experience suggested that this would have a negligible, if not beneficial effect (vide supra). Unfortunately, all members of this series showed significantly lower potencies than in the previous case. Most potent were ethyl carbamate **12b** and phenyl urea **12l**, which were 36.5 and 37 nM, respectively. Weakly basic groups were found to be better than strongly basic moieties as demonstrated by pyridines **12i** and **12j** as compared to nipecotic and isonipecotic amides **12g**–**h** and compounds **12a**, **12e**, and **12f**.

At this point, we decided to investigate the effect of the chiral center on activity, as well as the addition of more polar S4 moieties in an attempt to increase the hydrophilicity of our inhibitors. From the aforementioned studies we concluded that the aspartic acid linker oriented as described in Table 4 would be the best choice for further studies, with the piperidinyl amide used as

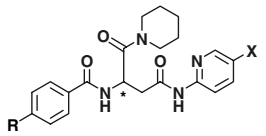
Table 5. SAR of side chain modification adjacent to the S4 amide, continued

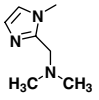
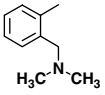
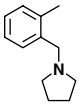
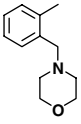
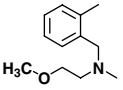


Compd	R	IC_{50} (nM)
12a	–H	145
12b	$–CO_2CH_2CH_3$	36.5
12c	$–COCH_3$	182
12d	$–COCF_3$	200
12e	$–COCH_2NH_2$	150
12f	$–COCH_2N(CH_3)_2$	215
12g		442
12h		194
12i		47.2
12j		43.8
12k		149
12l		37.0

the side chain functional group. To this end, the 2-(aminosulfonyl)phenyl group was replaced with either a 2-dialkylaminomethyl aryl group[11] or a 1-methyl-2-imadazolinyl group.[12] These analogs also have the advantage of potentially improving the binding affinity due to the electrostatic interactions with protein backbone carbonyl oxygens in the S4 pocket. At the same time, the effect of the S1 halogen (chloro vs bromo) would also be investigated.

Table 6. SAR of S1 halogen and S4 biaryl modifications and effect of chirality on aspartic acid central template



Compd	R	X = Cl, IC_{50} (nM)		X = Br, IC_{50} (nM)	
		*=(*S*) [a]	*=(*R*) [b]	*=(*S*) [c]	*=(*R*) [d]
13		24	12	—	4.6
14		28	26	—	8.2
15		9.2	7.6	330	3.1
16		2.9	1.7	360	0.65
17		2.7	2.0	230	1.0
18		3.6	1.8	290	1.1
19		9.3	4.8	940	1.3
20		—	—	6000	—

As shown in Table 6, the stereochemistry of the attached side chain does have an effect on potency. This is most dramatic in the case of analogs with a bromopyridinyl S1 where those containing an (*S*)-stereocenter (**15c**–**19c**) are 30–1000-fold less potent than their (*R*)-counterparts (**15d**–**19d**). Interestingly, this effect is considerably less dramatic for analogs containing a chloropyridyl S1 group where there is only a 2-fold preference for the (*R*)-isomer. We have tried to rationalize these data through docking experiments and have had no success in developing an explanation for the increased sensitivity to chirality demonstrated by the bromopyridinyl containing analogs. Because of the striking effect the S1 halogen has regarding the effect of the chiral center a direct comparison to illustrate the effect of the larger bromine as compared to the chlorine is not possible. In looking at the more potent (*R*)-isomers there does seem to be a roughly 2-fold increase in potency in going from chloro to bromo—a result similar to what was observed for the unsubstituted linker (**7b**).

Turning our attention to the S4 moieties; there appears to be no drop in potency in going from neutral 2-(aminosulfonyl)phenyl **11k** to a dimethylamidinyl group (**13d**) or a 1-methyl-2-imidazolinyl group (**14d**). A 2–3-fold gain in potency was observed, however, in changing the S4 group to the 2-dimethylaminomethylimidazolyl group (**15a**, **15b**, and **15d**) and the potency is improved again in going to the 2-(dialkyl aminomethyl)phenyl S4. The charged amino group appears necessary for potency, however, as a 20-fold loss in potency was observed in going from dimethylamino **16c** to hydroxyl **20c**. An array of amines was examined in the biphenyl series, which demonstrated a tolerance for polar and nonpolar functionality as well as cyclic and acyclic moieties. The most potent substituent in the biphenyl series was the dimethylaminomethyl group for analogs containing either a chloropyridyl or bromopyridinyl S1 with IC_{50} values of 1.7 and 0.65 nM, respectively. These analogs also show significant improvement over uncharged analogs in the functional assays (Table 7).

Table 7. Preliminary in vivo and in vitro data for selected inhibitors

Compd	7a	11a	11k	16a	16b	16d
Factor Xa IC_{50} (nM)	12	4.37	7.6	2.9	1.7	0.65
Factor Xa K_i (nM)	7.55	1.72	1.36	1.0	0.40	0.14
2X PT(μM)	ND	74	7	3.6	1.74	1.98
TG 2X lag (μM)	14	ND	4.6	0.96	1.0	0.42
F%	2.67	4.0	2.7	7.9	25.9	ND
$t_{1/2}$ (h)	1.77	1.0	1.35	6.1	1.18	ND
Cl (mL/min/kg)	35	6.7	50.6	124	178	ND
Vz (L/kg)	5.4	0.6	5.9	59.2	14	ND

ND: Not determined.

We screened this class of fXa inhibitors (**13**–**19**) against a panel of related trypsin-like serine proteases, and found that they retained the excellent enzyme selectivity of their parent compounds. None of them inhibits thrombin, trypsin, tPA, activated protein C, plasmin, and plasma kallikrein at IC_{50} values below 10 μM.

The in vitro anticoagulant activity of selected potent fXa inhibitors were measured in clotting assay and thrombin generation inhibition assays (Table 7). With slightly higher fXa binding affinity and lower plasma protein binding, compound **11k** displayed much improved in vitro anticoagulant potency compared to compound **7a**. The binding kinetics of **11k** were investigated and we found that it does not behave as a slow binding inhibitor[13] ($K_{on} = 27\,\mu M^{-1}\,S^{-1}$, $K_{off} = 0.024\,S^{-1}$). The lack of desired in vitro efficacy by compound **11k**, which has a K_i value of 1 nM and hPPB of 96%, illustrates the significant challenges of oral fXa inhibitor program.

Our move to inhibitors containing a charged S4 group demonstrates the effect that hydrophilicity can have on efficacy. Comparing the ratio of TG to K_i, we observed a more than 4-fold improvement in going from **7a** to the more hydrophilic **11k**; analogs with nearly identical K_i values.

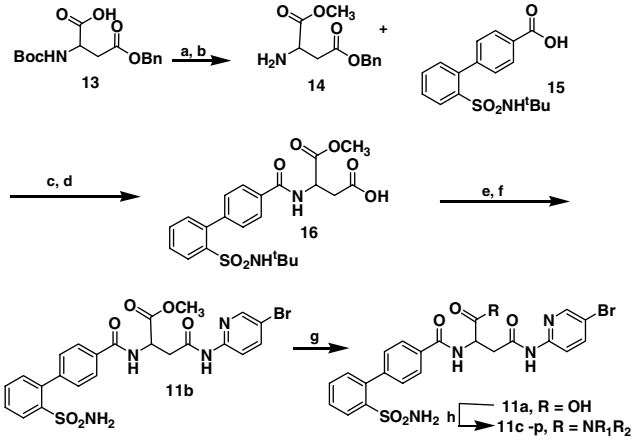
Additional in vivo characterization of these compounds included IV and oral pharmacokinetic profiles in Sprague–Dawley rats. Analogs containing a neutral S4 group showed low absorption in the rat with the best inhibitor **3a** (containing an acidic side chain) showing $F = 4\%$. Turning our attention to inhibitors containing a basic S4 group we found that analogs **16a** and **16b** both offered improved absorption with the fraction absorbed being 7.9% and 25.9%, respectively, a >3-fold difference for these two enantiomers. Still more surprising is the difference in half-life for these two chiral inhibitors (Table 7).

2. Chemistry

While a number of different syntheses are required for the research described above, the following synthetic protocol details many of the transformations, which were key to this work. First, the free carboxylic acid of deprotected aspartic acid **13** was first converted to the methyl ester using methylchloroformate and DMAP followed by deprotection of the *t*-butyl carbamate using HCl in dioxane affording amine **14**. This was then coupled to *t*-butylsulfonamide containing carboxylic acid **15** using BOP in DMF followed by hydrogenolysis of the benzyl protected ester using Pd/C to give carboxylic acid **16**. The carboxylic acid was then converted to the acid chloride using oxalyl chloride with catalytic DMF, then coupled to 2-amino-5-bromopyridine using pyridine as a solvent. The *t*-butyl sulfonamide was then deprotected via treatment with TFA to give unsubstituted sulfonamide **11b**. Further elaboration of the side chain was accomplished by saponifying the methyl ester using LiOH followed by coupling to a suitable amine using BOP in DMF to afford the final compound after reverse phase HPLC purification.

3. Conclusions

In summary, we have designed and synthesized a series of novel neutral S1 factor Xa inhibitors by executing parallel synthesis and systematic SAR. Neutral inhibitors **11a** and **11k** with an aspartic acid central template exhibited improved binding affinity over unsubstituted ethylene linked compound **7a** while retaining excellent enzyme selectivity. From these results we then focused on analogs containing a charged S4 moiety in an attempt to further increase hydrophilicity. This led to the



Scheme 1. (a) MeOCOCl, DMAP, CH_2Cl_2; (b) 4 N HCl in dioxane; (c) BOP, Et_3N, DMF; (d) H_2 (1 atm), 10% Pd/C, MeOH; (e) (1) $(COCl)_2$, DMF (cat.), CH_2Cl_2; (2) 2-amino-5-bromopyridine, pyridine; (f) TFA; (g) LiOH, H_2O, MeOH; (h) HNR_1R_2, BOP, Et_3N, DMF.

series of analogs shown in Table 6 the best of which—inhibitors **16a**, **16b**, and **16d**—show better potency and more importantly, better activity in our functional assay. From this series we also determined the best stereochemical geometry of the side chain and discovered an interesting effect regarding the S1 halogen and its effect toward sensitivity to the chiral center (vide supra). It is clear that at the given K_i values, increasing hydrophilicity significantly enhances the anticoagulant potency of fXa inhibitors. The SAR obtained here helped us design other series of compounds with improved anticoagulant and pharmacokinetic properties, which eventually led to the discovery of an oral factor Xa inhibitor development candidate. Those results will be reported in due course (Scheme 1).

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