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Halothiophene benzimidazoles as P1 surrogates of inhibitors of blood coagulation factor Xa

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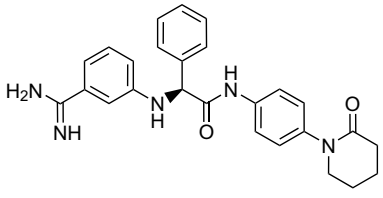
Abstract—Neutral weak halothiophene benzimidazole inhibitors of the serine protease factor Xa were identified via screening of a compound library. The X-ray crystal structure of representative **3a** bound to human fXa confirmed the S1 binding mode. Starting from **3a** a series of halothiophene benzimidazoles was synthesized and investigated for their factor Xa inhibitory activity. This led to potent and selective achiral inhibitors against fXa such as compounds **9k** and **9w**.

1. Introduction

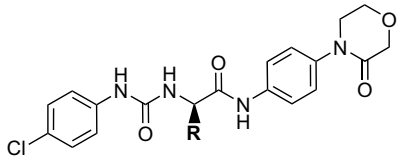
Factor Xa (fXa), a trypsin-like serine protease, is involved in the process of blood coagulation. At the convergence point of the extrinsic and intrinsic coagulation pathways fXa, as a component of the prothrombinase complex, converts prothrombin to thrombin via proteolysis. Due to the specific mechanism within these pathways it is assumed that inhibition of this penultimative enzymatic step will allow the effective control of thrombogenesis with a minimal effect upon bleeding.[1]

As a member of the trypsin-like serine proteases factor Xa has a negatively charged Asp189 at the bottom of the primary specificity (S1) pocket, which is the main anchoring point for positively charged substrates. Most of the known first and second generation factor Xa inhibitors have a benzamidine moiety as a P1 residue. These amidine groups in P1 position engage in a salt bridge with the negatively charged Asp189.[2] At the outset of our research program obtaining active fXa inhibitors, we have synthesized and evaluated L-phenylglycine derivatives such as mono-benzamidine **1** (Fig. 1).[3] An X-ray structure of **1** bound to human fXa is illustrated in Figure 2 and confirmed the depicted binding mode.

Although these benzamidines are potent inhibitors of fXa they contain a polar basic group, which is



1 IC_{50} fXa = 15.0 nM



2a R = Pr IC_{50} fXa = 5.6 nM
2b R = H IC_{50} fXa = 32.0 nM

Figure 1. FXa binding affinities of benzamidine **1**, and chlorophenyl ureas **2a** and **2b**.

Keywords: Anti-coagulants; Factor Xa inhibitors; Halothiophene benzimidazoles; X-ray crystal structures.
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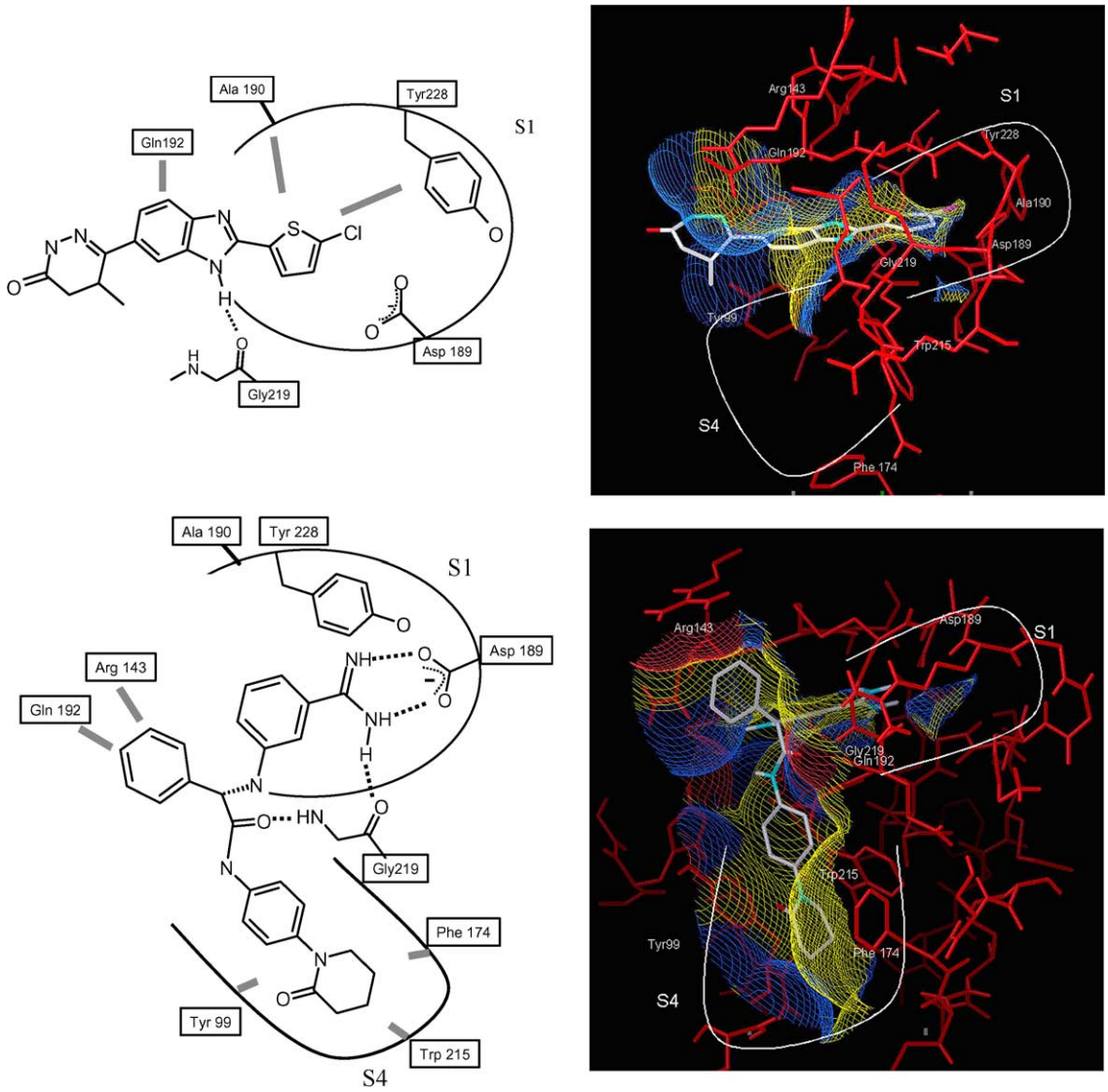


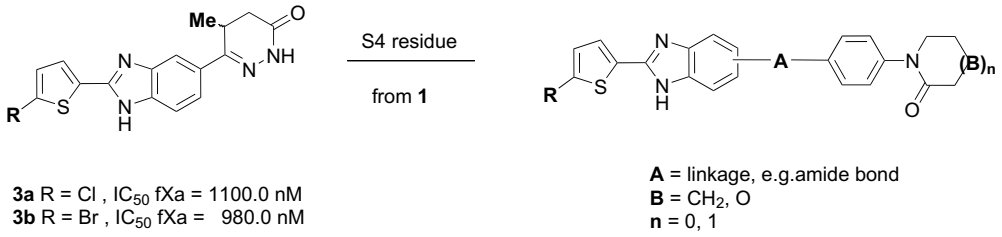
Figure 2. X-ray structures of **3a** and **1** bound to human factor Xa, refined to 2.3 and 2.0 Å, respectively.

associated with poor pharmacokinetic (PK) properties. As part of our research program to obtain inhibitors with optimized PK profiles, the chiral norvaline derivative **2a** was discovered (Fig. 1).[4] A feature of this nonbasic compound is the chlorophenyl as a surrogate for benzamidine in the S1 binding pocket, a D-amino acid as the central scaffold and a phenyl morpholinone as the P4 ligand, respectively. However, the achiral parent analogue **2b** displayed a 6-fold diminished potency in comparison with **2a** indicating that a central branching contributes to the activity significantly.

To search for novel benzamidine mimics a high-throughput screen of our in-house library was undertaken. As a result, neutral compounds **3a** and **3b** as weak inhibitors of factor Xa were identified. These pyridazinones originally turned out to be potent phosphodiesterase-III inhibitors with a positive inotropic action.[5] Both of the compounds are comprised of a 2,3,4,5-tetrahydropyridazin-3-one attached to a halothiophene benzimidazole from which the latter is believed to occupy the S1 pocket.

The current article discusses the design, synthesis, and in vitro structure–activity relationships of potent achiral and nonbasic factor Xa inhibitors containing a halothiophene benzimidazole as P1 residue and tethered cyclic anilino amides as P4 ligands starting from X-ray crystallographic data of compound **3a**.

At the outset of this study an X-ray crystallographic analysis has been performed to confirm our binding hypothesis. Compound **3a** was co-crystallized with human factor Xa (Fig. 2). The chlorothiophene benzimidazole of **3a** replaces the benzamidine group of compound **1** in the S1 pocket. Compound **3a** does not have a charged group that forms a salt bridge with the carboxyl group of Asp189. However, a conserved water molecule within the S1 pocket was displaced by the chlorine and makes a hydrophobic contact with the phenyl ring of Tyr228 (3.7 Å) and the side chain of Ala190 (3.6 Å). Another major interaction is a hydrogen bond between the benzimidazole nitrogen and the carbonyl of Gly219 (3.1 Å). The pyridazinone moiety has no relevant interactions and points out of the binding



3a R = Cl , IC_{50} fXa = 1100.0 nM
3b R = Br , IC_{50} fXa = 980.0 nM

Figure 3. FXa binding affinities of 2,3,4,5-tetrahydro-pyridazin-3-ones **3a**,**b**, and conception of halothiophene benzimidazole analogues.

site. In contrast to benzamidine **1** there are no binding interactions in the S4 pocket. The piperidone part in compound **1** has hydrophobic interactions within this pocket. It is covered between the phenyl rings of Phe174 (4.0 Å), Trp215 (5.0 Å), and Tyr99 (5.0 Å).

It is well precedented that a variety of strategies can be employed to optimize interaction with the S4 pocket. However, many of the S4 residues in our group and in competitor groups as well are substituted anilines. For the design of potent inhibitors based on the chlorothiophene benzimidazole scaffold the L-shaped conformation together with the anilino piperidone as P4 ligand observed in **1** or the aniline morpholinone in **2a** and **2b** has to be build up. For the ease of synthesis a tethered amide linkage between the 5(6)-position of the benzimidazole and the anilino piperidone was envisaged (Fig. 3).

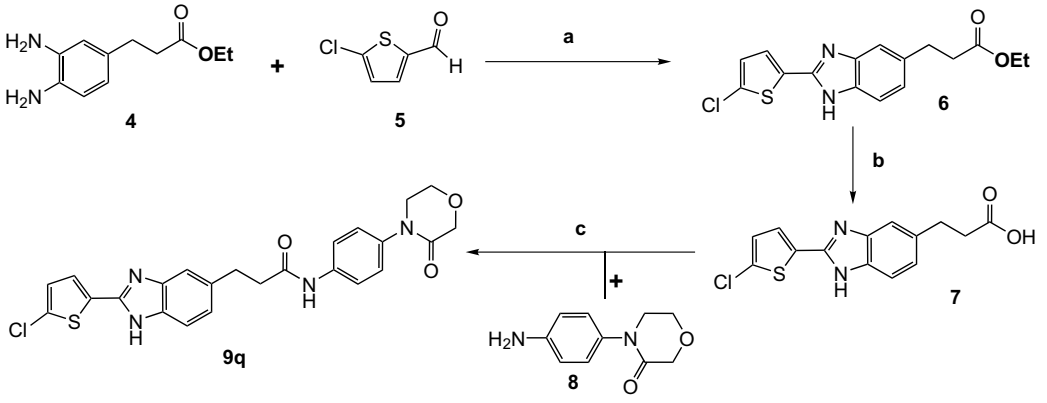
2. Chemistry

The starting diaminoaryl compounds bearing an alkylene carbonyl tether were prepared according to literature procedures or were commercially available. A representative synthesis of the P4 modified inhibitors **9a**–**l** and **9q**–**w** is described in Scheme 1, exemplified by morpholinone **9q**. Condensation of diaminophenyl compound **4**[6] with aldehyde **5** in the presence of pyrosulfite provided benzimidazole ester **6**.[5] Hydrolysis of this ester with sodium hydroxide afforded benzimidazole acid **7**. Coupling of acid **7** and anilino morpholinone **8**[7] with benzotriazole derivative TBTU generated the target amide **9q**.

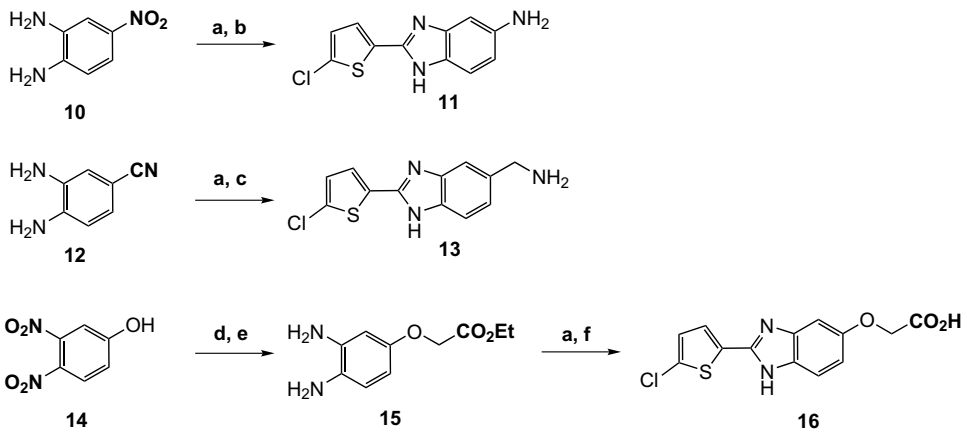
The preparation of key intermediates **11**, **13**, and **16** not described in the literature is outlined in Scheme 2. Benzimidazole **11** was formed in two steps from 4-nitrophenylendiamine **10** and aldehyde **5** with pyrosulfite and subsequent reduction of the nitro group using stannous chloride. Benzimidazole **13** was prepared from 3,4-diamino-benzonitrile **12** and aldehyde **5** again with pyrosulfite whereas the cyano intermediate was reduced to methylamine **13** with lithium aluminum hydride. Starting from 3,4-dinitro-phenol **14** benzimidazole **16** was obtained in four steps. Alkylation of phenol **14** with bromo-acetic acid ethyl ester and hydrogenation of the corresponding nitro groups afforded diamine **15**. Condensation of **15** with aldehyde **5** under pyrosulfite conditions and subsequent saponification of the ethyl ester with sodium hydroxide provided acid **16**. Intermediates **11**, **13**, and **16** were converted with the corresponding acid or amine to the final amides **9m**–**p**, respectively.

3. Results and discussion

The structure–activity relationships surrounding the inhibitors incorporating the different linkages and the modified P4 ligands are outlined in Table 1. Compounds **9a**–**w** were assayed against human fXa, thrombin and trypsin as previously described.[8] Briefly, protease activity was monitored in vitro using protease-specific chromogenic substrates. Anti-protease activity of drugs was calculated from the OD ratio of drug and vehicle

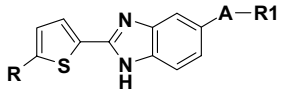


Scheme 1. General synthesis of the target compounds **9a**–**l** and **9q**–**w**, illustrated at **9q**. Reagents and conditions: (a) $NaSO_3H$, NMP, 110 °C, 2 h; (b) NaOH, MeOH, rt, 12 h; (c) TBTU, DMF, rt, 12 h.



Scheme 2. Alternative approaches to some thiophene benzimidazole intermediates. Reagents and conditions: (a) **5**, $NaSO_3H$, NMP, 110 °C, 12 h; (b) $SnCl_2$, EtOH, 90 °C, 24 h; (c) $LiAlH_4$, THF, 0 °C, 2 h; (d) $BrCH_2CO_2Et$, Cs_2CO_3, MeCN, rt, 12 h; (e) Raney-Ni, H_2, THF, rt, 4 h; (f) 1 N NaOH, MeOH, rt, 12 h.

Table 1. Factor Xa binding affinity data of selected 5(6)-substituted (5-halo-thiophen-2-yl)-1*H*-benzimidazoles



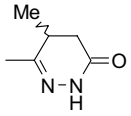
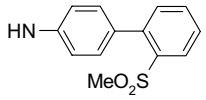
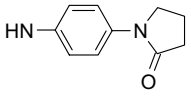
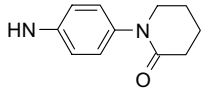
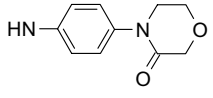
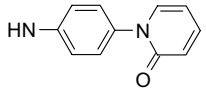
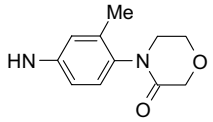
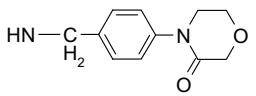
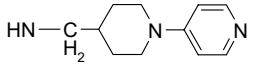
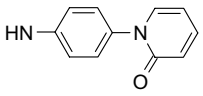
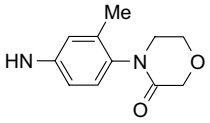
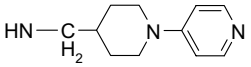
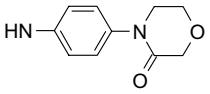
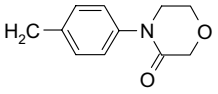
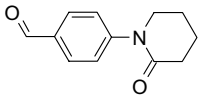
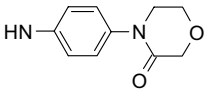
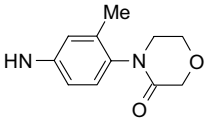
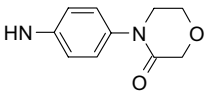
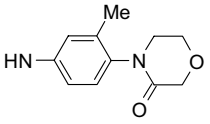
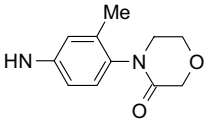
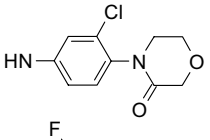
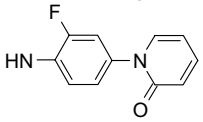
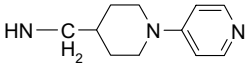
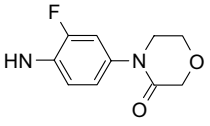
Compound	R	A	R1	IC_{50} fXa (nM)
3a	Cl	—		1100.0
9a	Cl	$CH_2(C{=}O)$		490.0
9b	Cl	$CH_2(C{=}O)$		530.0
9c	Cl	$CH_2(C{=}O)$		260.0
9d	Cl	$CH_2(C{=}O)$		230.0
9e	Cl	$CH_2(C{=}O)$		180.0
9f	Cl	$CH_2(C{=}O)$		120.0
9g	Cl	$CH_2(C{=}O)$		170.0
9h	Cl	$CH_2(C{=}O)$		14.0

Table 1 (*continued*)

Compound	R	A	R1	IC_{50} fXa (nM)
9i	Br	$CH_2(C{=}O)$		63.0
9j	Br	$CH_2(C{=}O)$		41.0
9k	Br	$CH_2(C{=}O)$		5.2
9l	Cl	$C{=}O$		1900.0
9m	Cl	$NH(C{=}O)$		1200.0
9n	Cl	CH_2NH		440.0
9o	Cl	$OCH_2(C{=}O)$		150.0
9p	Cl	$OCH_2(C{=}O)$		20.0
9q	Cl	$CH_2CH_2(C{=}O)$		10.0
9r	Cl	$CH_2CH_2(C{=}O)$		8.2
9s	Br	$CH_2CH_2(C{=}O)$		8.4
9t	Cl	$CH_2CH_2(C{=}O)$		7.0
9u	Cl	$CH_2CH_2(C{=}O)$		7.6
9v	Br	$CH_2CH_2(C{=}O)$		58.0
9w	Cl	$CH_2CH_2(C{=}O)$		5.9

containing assay. For thrombin and trypsin all compounds displayed $IC_{50}>10\,\mu M$.

The initial compound **9a** prepared in this series contained an acetamide in 5(6)-position of the benzimidazole and a methylsulfonyl biphenyl[9] as S4 template, which directly led to a 2-fold increase in potency compared to screening compounds **3a** and **3b**. To explore P4 modification, the methylsulfonyl phenyl was replaced with pyrrolidinone (**9b**), piperidinone (**9c**), morpholinone (**9d**), and pyridinone (**9e**), respectively, where the nitrogen is attached to the benzene ring. As a result, the six-membered rings **9c–e** showed a 2- to 3-fold potency enhancement over biphenylsulfone **9a**, whereas the five-membered **9b** showed no change in potency relative to the corresponding **9a**. Methyl substitution on the phenyl ring increased factor Xa activity further. The methyl substituted morpholinone **9f** displayed a 2-fold increase in potency over the unsubstituted morpholinone **9d**. It is assumed that the methyl group forced the morpholinone ring to twist and adopt a more favorable configuration within the S4 pocket. Homologation of phenyl morpholinone in **9d** generated aminomethyl analogue **9g**, which is marginally more potent. However, replacement of phenyl morpholinone with 1-(4-pyridyl)-piperidinyl[10] resulted in a 16-fold increase in binding affinity (**9h**) indicating that this functionality could reside deeper in the S4 binding site of the enzyme. To investigate the halogen effect observed with the screening compounds **3a** and **3b** chlorine was replaced by bromine in some selected inhibitors. The resulting bromo derivatives **9i**,**j**, and **9k** were 2-fold more active than the corresponding chloro compounds **9e**,**f**, and **9h**, respectively, which led to our first mono-digit nanomolar inhibitor **9k**. It is obvious that the bromo inhibitors gave a better fit in the S4 pocket than did the chlorides.

Further studies on the P4 residue involved the variation on the length of the tether between the S1 and S4 scaffold. If the phenyl morpholinone is directly linked as an amide to the benzimidazole significant decrease of activity was observed. The activity of the amide **9l** is only in the range of the chloro screening compound **3a**. Reversal of the methylene amide bond in **9m** or merely the amide bond in **9n** resulted in decreased fXa inhibitory activity compared to analogue **9d**. Both compounds displayed decreased potency by 5- or 2-fold over **9d**, respectively.

Expansion of the linker by an additional oxygen or methylene group positively affected the potency. The incorporation of an oxygen directly bound to the 5(6)-position of the benzimidazole tended to increase the activity. Although morpholinone ether **9o** had only a minimal effect on potency enhancement over the corresponding **9d** the methyl substituted morpholine ether **9p** increased the potency by 6-fold. The ether linkage in the central region probably to its rotational flexibility may allow a more pronounced interaction of the S4 residue with the lipophilic S4 pocket. However, replacement of the oxygen linker of inhibitor **9o** with a methylene group afforded analogue **9q**, which is more than one order of magnitude potent compared to the parent **9d** indicating that the L-shape conformation and the P4 ligand is nearly optimized. The presence of substituents at the P4 phenyl ring increased the activity further. A methyl group (**9r**) or a chloro substituent (**9t**) afforded low nanomolar inhibitors. In contrast to the acetamide series the substitution of chlorine with a bromo atom at the thienyl ring in morpholinone **9s** did not change the potency relative to the corresponding **9r**. The additional replacement of the phenyl morpholinone with 1-(4-pyridyl)-piperidinyl provided a decrease in potency by one order of magnitude. Bromide **9v** was found to have a 11-fold less affinity than the chloride **9k**. It is assumed that this substitution pattern in combination with the elongated propionamide tether caused unfavorable interactions within the S1 and/or S4 pocket. Interestingly, introduction of a 3-fluoro substituent at the phenyl ring, which increased the activity in other series,[9] showed the same effect in pyridone **9u** and morpholinone **9w**. Fluoride **9w** represented the most potent inhibitor within the propionamide series.

Although this class of compounds displayed good in vitro properties, physicochemical parameters such as solubility ($<10\,\mu g/mL$ in pH 7 phosphate buffer) and oral pharmacokinetic profiles (bioavailability $<20\%$ in rats and dogs) have to be improved.

4. Conclusion

Starting from the X-ray structure of halothiophene benzimidazole **3a** emerging from a screening library, a series of 5(6)-substituted tethered amides was designed resulting in the discovery of neutral and achiral factor Xa inhibitors. Potency depends on the length and nature of the linker between benzimidazole and P4 residue. Modifications of these parameters led to the identification of low nanomolar inhibitors **9k** and **9w**. Physicochemical and pharmacokinetic optimization of these prototype compounds may lead to the discovery of novel anti-coagulation drugs.

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