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Solid-phase synthesis of naphthylamidines as factor VIIa/tissue factor inhibitors

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Abstract—Reductive amination followed by acylation of polymer-linked formyl aryl amidines generate combinatorial libraries of aryl amidines **8**–**13**. Potent small molecule naphthylamidine inhibitors **12** (K_i < 100 nM) of FVIIa/TF have been discovered and their activity against other serine proteases in the coagulation cascade is reported.

The prevention of blood coagulation is of primary importance in a number of pathological situations. Factor VIIa (FVIIa) inhibitors selectively target the extrinsic pathway of the coagulation cascade.[1,2] FVIIa binds to its cell membrane-associated cofactor, tissue factor (TF), and activates factor VII, factor IX, and factor X to trigger a protease cascade leading to clot formation. The development of FVIIa/TF inhibitors has emerged as a focus for the treatment and prevention of thrombotic disorders.

A number of naphthylamidines have been reported as FVIIa[2,3] inhibitors. We have described a polymer-based amidine linker that has been utilized to synthesize an aryl amidine library for the discovery of FXa inhibitors.[4] Similar linker strategies have also been employed in the synthesis of FVIIa inhibitors.[3] In this report, we describe the coupling of amidinobenzaldehyde analogs to a polystyrene resin using our linker strategy for amidines. The polymer-bound benzaldehyde derivatives can be reductively alkylated with primary amines to form a diverse library of secondary amines. Acylation of the benzylic secondary amine with acid chlorides or isocyanates provides a library of amides and ureas. Subsequent cleavage from the resin affords the free benzamidine-functionalized library of amides and urea products. In this manner, a library of over 1000 aryl amidines was synthesized from commercially available primary amines, acid chlorides, and isocyanates.

Through this approach, we report the discovery of potent, novel, small molecule naphthylamidine inhibitors (K_i < 100 nM) of FVIIa/TF, and the activity of these compounds against other serine proteases in the coagulation cascade.

As described earlier,[4] Sheppard's linker **1** is coupled to Tentagel using standard DIC coupling procedures to afford derivatized polymer **2** (Scheme 1). Reaction with *p*-nitrophenyl chloroformate provides activated carbonate **3**.

Formyl aryl amidines **4a** and **4b** were synthesized as previously described.[5] Formyl naphthylamidine **4c** was synthesized as in Scheme 2. Debromo-oxidiation of **14**[6] with 2-nitropropane/sodium methoxide gives cyanoaldehyde **15**. Standard Pinner conditions afford formyl naphthylamidine hydrochloride **4c**. Reaction of benzamidines **4** with resin **3** introduces the amidine onto the Sheppard linker to give resin **5**. At this stage, the invariant aryl amidine functionality required in all the products is attached through the acid sensitive Sheppard linker onto the polymer. Resin **5** is now ready for library synthesis.

Reaction of resin-bound aldehyde **5** with a diverse array of commercially available primary amines (20 equiv) followed by $NaB(OAc)_3H$ (10 equiv) in 1% AcOH/MeOH affords polymer bound amine **6**.[7,8] Acylation using a

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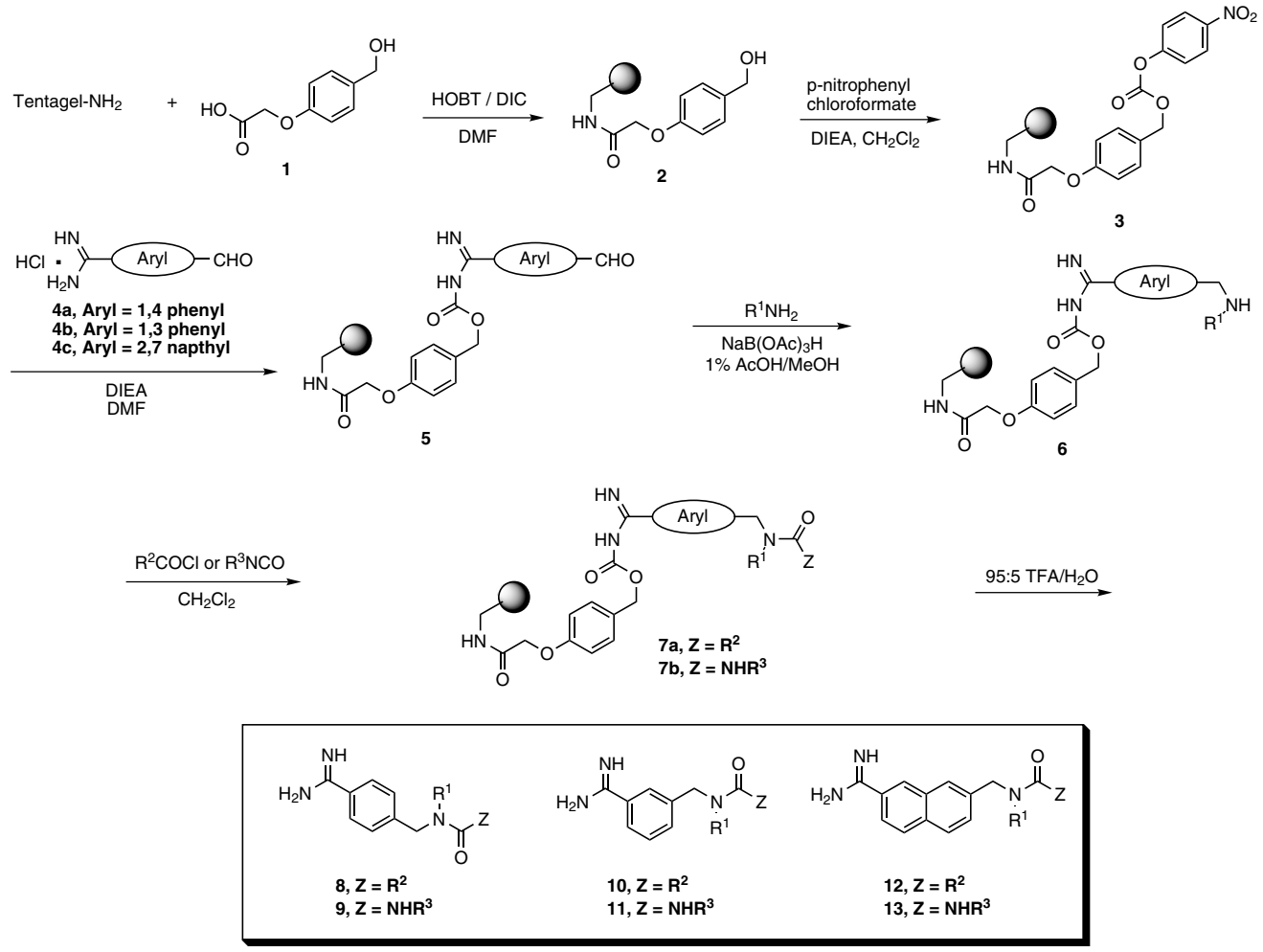
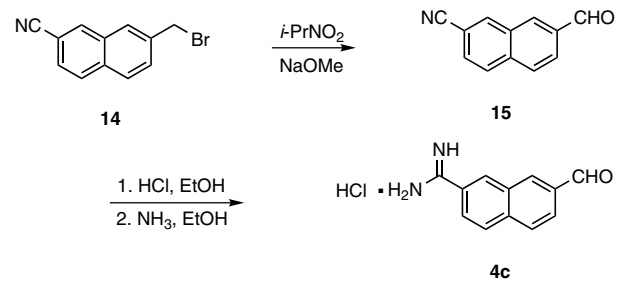


Scheme 1.



Scheme 2.

diverse array of commercially available acid chlorides or isocyanates (5 equiv) affords polymer bound amides **7a** or ureas **7b**, respectively. Cleavage of the amidine from the acid sensitive linker is accomplished with TFA to afford aryl amidines **8**–**13**. In this manner, over 1000 analogs were synthesized. Sampled library members were analyzed by HPLC, NMR, and MS. Generally, >75% of the sampled library members proved to be the correct compound and were found to be 60–95% pure. The isolated yields were generally 60–90% by weight.

Initial screening of the library was performed with the isolated, crude reaction products against human FVIIa/TF at 10 μM (for aryl amidines **8**–**11**) or 1 μM (for naphthylamidines **12** and **13**) concentrations. Neither the benzamidines **8**–**11** nor the naphthylamidine urea compounds **13** showed appreciable activity. Only naphthylamidine compounds **12** were found to inhibit FVIIa/TF >50% at 10 μM. Naphthylamidine compounds **12** were re-synthesized and purified using standard solution-phase techniques from cyanonaphthaldehyde **15**.[9]

The results of the screening of the pure compounds against FVIIa/TF, FXa, thrombin, and trypsin are shown in Table 1.[10] From the pure, active compounds discovered, a clear SAR can be seen. Only naphthylamidines **12** containing 1- or 2-naphthoyl amides, derived from acid chloride diversity element R^2, provided activity <1 μM against FVIIa or FXa. The diversity element derived from the primary amine (R^1) of **12** has an ethyl or propyl spacer followed by a lipophilic (**12a**,**c**,**e**,**g**,**k**) or basic element (**12b**,**d**,**f**,**h**–**j**,**m**).

Several potent inhibitors shown in Table 1 were modeled in the active site of FVIIa/TF to try to understand the SAR for the series.[11] The naphthylamidine moiety was docked into the primary specificity pocket S1, making hydrogen bonds with Asp 189, Ser 190, and Gly 219.

Table 1. Activity of naphthylamidines **12** against human FVIIa/TF, human FXa, human thrombin, and bovine trypsin

Compd	R^1	$R^2 = Z$	FVIIa (K_i, nM)[a]	FXa (K_i, nM)[a]	Thrombin (K_i, nM)[a]	Trypsin (K_i, nM)[a]
12a	$CH_2CH_2CH(CH_3)_2$	1-Naphthyl	93	262	1164	698
12b	$CH_2CH_2CH_2$-1-morpholine	1-Naphthyl	121	442	>5000	378
12c	CH_2CH_2-3,4-dimethoxyphenyl	1-Naphthyl	133	417	2507	105
12d	CH_2CH_2-2-(1-methyl)pyrrolidine	1-Naphthyl	142	111	>5000	433
12e	CH_2CH_2-4-fluorophenyl	1-Naphthyl	194	40	1682	501
12f	CH_2CH_2-2-pyridyl	1-Naphthyl	195	104	4764	299
12g	$CH_2CH_2CH_2$-phenyl	1-Naphthyl	207	632	1196	925
12h	$CH_2CH_2CH_2$-1-imidazole	1-Naphthyl	282	731	>5000	752
12i	CH_2CH_2-4-imidazole	1-Naphthyl	305	264	>5000	378
12j	$CH_2CH_2CH_2$-1-(2-methyl)piperidine	1-Naphthyl	646	137	>5000	367
12k	$CH_2CH_2CH(CH_3)_2$	2-Naphthyl	783	512	486	460
12l	(R)–$CH(COOH)CH_2CH(CH_3)_2$	1-Naphthyl	1011	>5000	>5000	1609
12m	$CH_2CH_2CH_2$-1-(4-methyl)piperazine	1-Naphthyl	1312	136	500	500

[a] K_i values are the mean of multiple determinates ($n > 2$). Standard deviations are <30% of the mean.

The 1-naphthoyl amide group was placed either in the S2 pocket or the S4 pocket, and the R^1 group was placed in S2, S3, or S4. Molecular dynamics calculations[12] revealed a preference for the naphthoyl group at S4, with the R^1 group occupying the S2 site for **12a**. The lowest energy FVIIa-bound conformation of **12a** is shown in Figure 1. The naphthylamidine makes a number of hydrophobic contacts in S1, with Val 213, Ser 214, Trp 215, Cys 191, and Lys 192. It is also in close contact with the active site Ser 195 and His 57. The amidine group forms hydrogen bonds to Asp 189, Ser 190, and Gly 218. In the case of compound **12a**, the 1-naphthoyl group interacts with Trp 215, Thr 99, and Pro 170I, as well as the hydrophobic portion of Gln 217. The isopentyl R^1 group of **12a** interacts with the S2 pocket, making hydrophobic contacts with His 57, Thr 98, Thr 99, and Gly 97 (in yellow). Larger R^1 groups such as the (3,4-dimethoxyphenyl)ethyl group of **12c** can interact with Asp 60, Tyr 94, and Pro 96 of the S2 site.

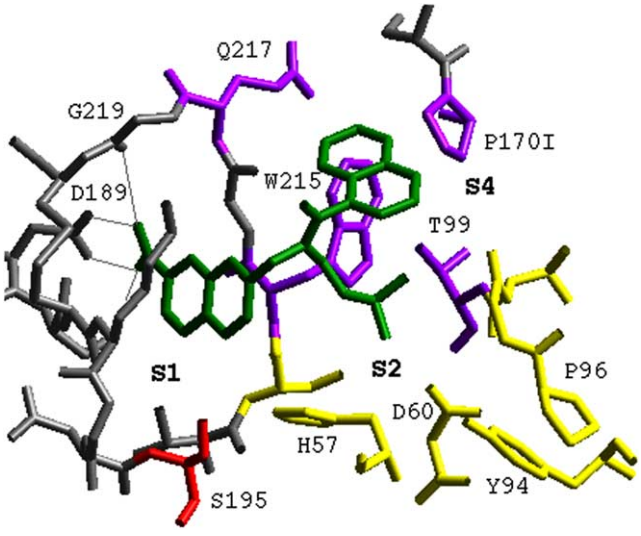


Figure 1. Proposed binding mode of **10a** in FVIIa/TF. The catalytic serine is shown in red, the inhibitor in green, and the S2 and S4 sites are colored yellow and purple, respectively. The Cerius[2] program from MSI was used to create this figure.

In conclusion, a diverse array of aryl amidines has been synthesized via a polymer-bound formyl aryl amidine. From a library of over 1000 compounds, novel, potent naphthylamidine FVIIa and FXa inhibitors have been discovered ($K_i < 100$ nM).

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8. *General experimental procedure*: Resin-bound formyl amidine **5** (100 mg, 0.056 mmol) is slurried in a solution of amine (1.12 mmol, 20 equiv) in 0.5 mL of 1% AcOH in MeOH. The resin is agitated for 20 min and is then drained. A freshly made solution of $NaBH(OAc)_3$ (0.56 mmol, 10 equiv) in 0.5 mL of 1% AcOH in MeOH is added and the resin is agitated for 45 min. The resin is drained, washed with MeOH (3×) and CH_2Cl_2 (3×). CH_2Cl_2 (0.5 mL) is added followed by a solution of the acid chloride or isocyanate (0.28 mmol, 5 equiv). If an acid chloride is added, DIEA (0.28 mmol, 5 equiv) is added prior to the acid chloride solution. After agitation for 45 min, the resin is drained, washed with MeOH (3×), then 1 mL 95:5 TFA/H_2O solution is added. After 5 min, the product is filtered from the resin and evaporated to dryness.
9. All new compounds **12** have MS, ^1H NMR, and elemental analysis consistent with their structure. For **12a** elemental analysis $C_{28}H_{29}N_3O$ ($1.1C_2HF_3O_2 \cdot 0.8H_2O$) found: C 64.63; H 5.40; N 7.39; ^1H NMR (300 MHz, DMSO-*d*6) 0.93 (br s, 6), 1.18 (sept, 1), 1.35 (m, 2), 1.63 (m, 2), 5.07 (br s, 2), 7.41–8.61 (m, 13), 9.19 (m, 2), 9.43 (m, 2).
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