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SOLID-PHASE SYNTHESIS OF N-SUBSTITUTED AMIDINOPHENOXY PYRIDINES AS FACTOR XA INHIBITORS[1]

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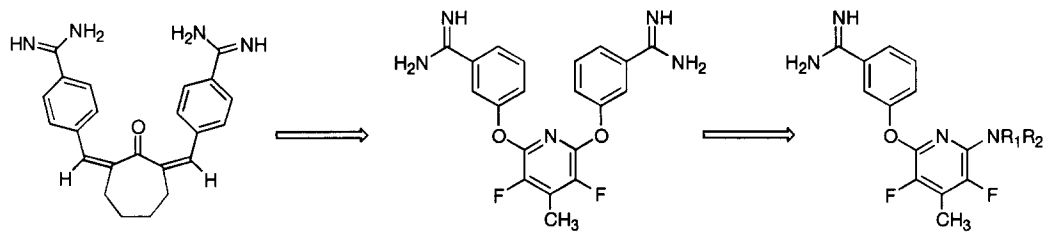
Abstract: An arylamidine linker has been employed for the solid-phase synthesis of N-substituted amidinoaryloxypyridine analogs **2** via nucleophilic substitution on a fluoropyridyl template. Two novel N-substituted amidinoaryloxypyridine derivatives **2a** and **2b** were discovered via this approach.

The prevention of blood coagulation is of primary importance in a number of pathological situations. Factor Xa (FXa) is a coagulant enzyme belonging to the serine protease class. Factor Xa activates prothrombin to generate thrombin, which plays a critical role in thrombosis by not only converting fibrinogen to fibrin for clot formation but also by strongly inducing platelet aggregation.

There are several examples of aryl amidines as potent inhibitors of trypsin-like serine proteases including FXa. These include benzamidine, a competitive inhibitor[2] of FXa (K_i = 410 μM) and 3-amidinobenzyl phenyl ether, a derivative of benzamidine, which was shown to exhibit improved inhibitory potency over FXa (K_i = 6.8 μM).[3] In a series reported by Stürzebecher et al.,[4] a conformationally rigid inhibitor, 2,7-bis-(4-amidinobenzylidene)-cycloheptan-1-one (BABCH), was disclosed that exhibited a K_i value of 13 nM for bovine FXa. The evolution from Z,Z-BABCH (**1a**) to potent and photochemically stable bisamidine compounds that are selective for FXa over other proteases, was achieved by the discovery of ZK-805623 (K_i FXa = 13 nM).[5] Bisamidines, however, possess an inherent liability in terms of oral bioavailability and therefore we embarked upon a program to find a replacement for one of the benzamidine groups; recognizing the fact that benzamidine binds in the S_1 pocket of the enzyme and hence one benzamidine group may be essential to maintain measurable potency.[2]

In order to explore and define a broad scope of functional groups that serve as replacements for the benzamidine moiety, we decided to synthesize a library of monoamidine analogs of ZK-805623 where one benzamidine group was substituted by a series of primary and secondary amines.



Z,Z-BABCH (1a) **ZK-805623** **2**

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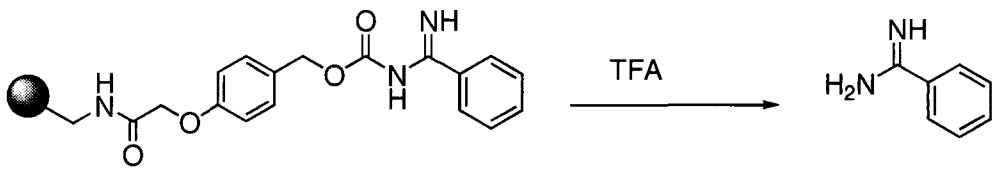
A solid-phase approach to the mono amidine analogs would have to employ a linker that would function as a masked amidine, as the usual chemical steps that are carried out in the Pinner reaction, to effect the transformation of a nitrile to an amidine, (HCl/EtOH, NH_3/EtOH) are not compatible with the solid-phase protocol.

The strategy for attachment of the amidines to the polymer was therefore based on linkers designed for polyamines.[6] We postulated that a 4-alkoxybenzyl carbamate-based linker for the amidine moiety **I** would be susceptible to acidic cleavege with TFA to provide the benzamidine **II**, but stable to the nucleophiles that would be added to the activated pyridine template. Subsequent to our work but prior to this disclosure, a similar linker for amidines was reported in literature.[7]



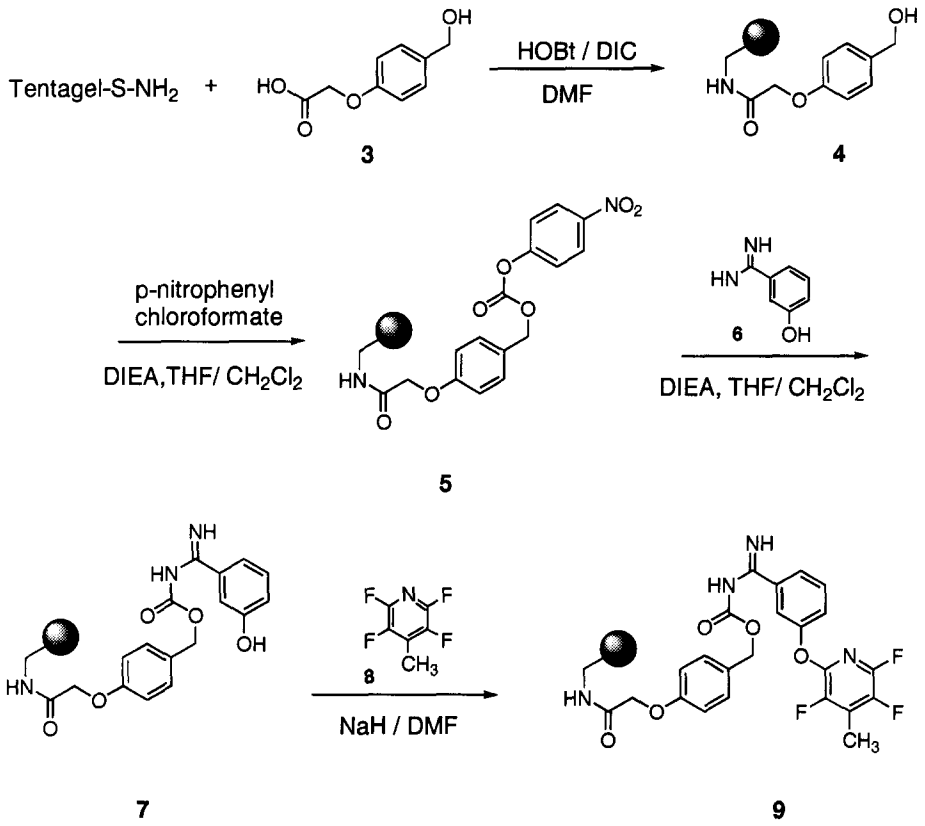
I II

The linker-amidine-bound precursor **9** was assembled as shown in Scheme 1.[8] 4-Hydroxymethylphenoxyacetic acid **3** was attached to the commercially available Tentagel-S-NH_2 resin via a HOBt/DIC mediated coupling using standard solid-phase protocols of washing and double coupling. The polymer bound intermediate **4** was then treated with p-nitrophenyl chloroformate and N,N-diisopropylethylamine (DIEA) in 1:1 CH_2Cl_2/THF (2 cycles) to afford the polymer-bound nitrophenyl carbonate **5**. The carbonate was then treated with 3-hydroxy benzamidine **6** to provide the polymer-bound amidino-phenol intermediate **7**.

Addition of the polymer bound phenol to 4-methyl-tetrafluoropyridine **8** was effected by the generation of the phenoxide on the polymer with sodium hydride in DMF. Although there are few reports of sodium hydride being used in solid-phase organic chemistry, we found that a slurry of sodium hydride in DMF was the most effective method for the generation of the phenoxide anion and subsequent solid-phase nucleophilic aromatic substitution reaction.

To quantitate the loading of the phenol on the polymer, the precursor **7** was treated with TFA and the phenol **6** quantitated via HPLC using an internal standard. It was determined that the template loading on the polymer was 83% relative to the substitution on the starting Tentagel resin. In this manner the polymer bound template **9** was assembled as a precursor to nucleophilic aromatic substitution reactions with amine nucleophiles.

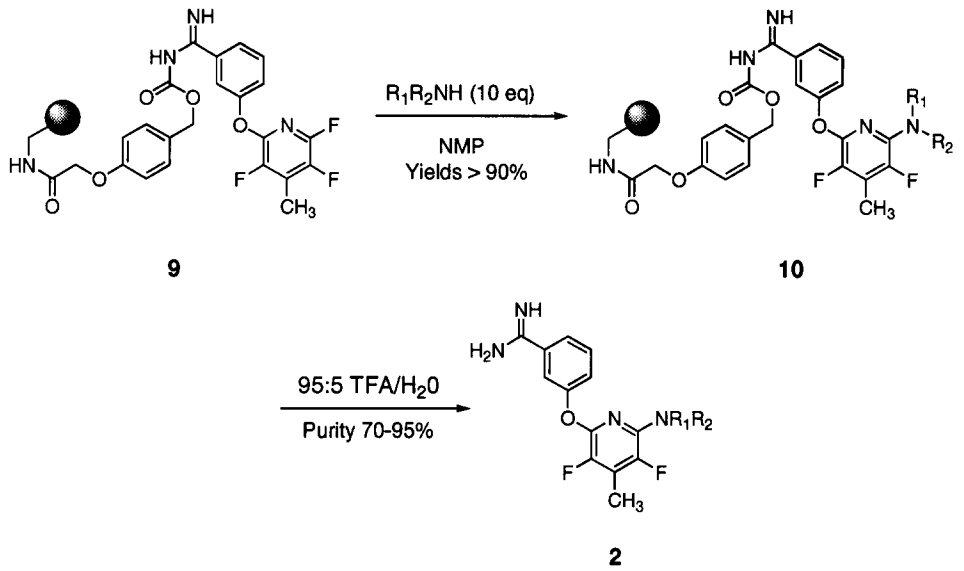
Scheme 1



The nucleophilic aromatic substitution reaction of the template **9** with primary and secondary amines is shown in Scheme 2. The polymer-bound fluoro-pyridine **9** was treated with the amine (10 equiv) in N-methyl pyrrolidine (NMP) as a solvent to afford the polymer-bound product **10**. As expected, acid-mediated cleavage from the polymer afforded the amino-substituted pyridine analogs **2** (Table 1). In this manner, over 400 N-substituted amidinophenoxy pyridine analogs **2** were prepared.[8] All compounds were analyzed for purity by reversed-phase HPLC and quantitated by recovered yield. The recovered yields for the compounds were >90% and purity in the range of 70-95%.

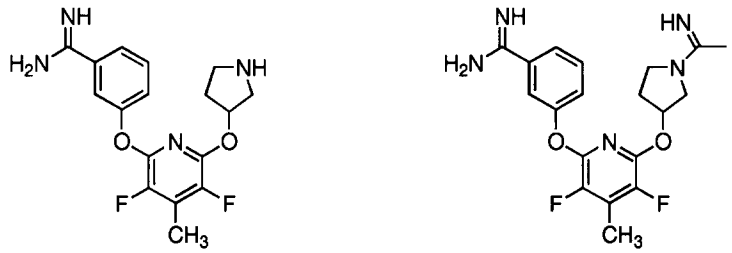
All compounds were assayed for their inhibitory activity against FXa[9] and the results for a representative set of compounds are shown in Table 1. Two compounds, **2a** and **2b**, showed modest activity against FXa with K_i values of 560 and 495 nM, respectively.

Scheme 2



9 10 2

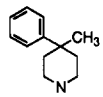
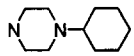
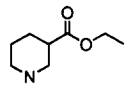
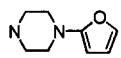
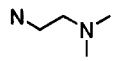
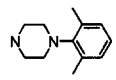
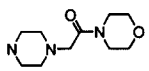
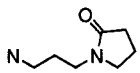
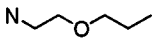
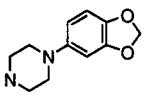
The discovery of piperidine and piperizine motifs as amidine replacements is intriguing based on our knowledge of the general SAR around this template.[5b] In an earlier series of non-benzamidine compounds, analogs **11** and **12** had K_i values >5000 nM against FXa. These compounds contain the pyrrolidinol and the acetimidoyl pyrrolidinol functionalities, that in a different series of FXa inhibitors,[10a] occupy an aryl-binding pocket, defined by Phe174, Tyr99 and Trp215, in the active site of FXa.[10b]



11 12

It is possible that the aryl piperidine and the cyclohexyl piperazine residues provide a better fit in this aryl-binding pocket. However, the activity of the analogs may be limited by the lack of a strongly basic group that fits into the cation hole that exists in the same proximity.[10b] Further efforts to explore this hypothesis are currently underway.

TABLE 1

Compound	NR_1R_2	% Purity[a]	FXa K_i (nM)
2a		90	560
2b		85	495
2c		70	>5000
2d		80	>5000
2e		70	>5000
2f		90	>5000
2g		90	>5000
2h		75	>5000
2i		80	>5000
2j		80	>5000
2k		70	>5000
2l		73	>5000
2m		86	>5000

[a]As determined by reversed-phase HPLC (254 or 220 nM)

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8. **Synthesis of Polymer-Bound Pyridine Precursor.** To 20 g (0.28 meq/g, 5.6 mmol) of Tentagel-S-NH_2 in a 250 mL shaker flask was added 4-hydroxymethylphenoxy acetic acid (3.57 g, 19.6 mmol, 3.5 equiv) and HOBT (3.06 g, 19.6 mmol, 3.5 equiv) in 150 mL DMF. The mixture was shaken for 30 min followed by the addition of N,N-diisopropylcarbodiimide (2.47 g, 3.07 mL, 19.6 mmol, 3.5 equiv). The mixture was stirred for 16 h. The polymer was washed with DMF (3 X 100 mL), isopropanol (3 X 100 mL) and CH_2Cl_2 (3 X 100 mL) to afford the polymer-bound benzyl alcohol **4**. 4-Nitrophenylchloroformate (4.52 g, 22.4 mmol, 4 equiv) in 20 mL of CH_2Cl_2 was then added followed by the addition of DIEA (2.90 g, 3.9 mL, 22.4 mmol, 4 equiv). The mixture was agitated for 2 h and the solvent drained. The process was repeated with the 4-nitrophenylchloroformate and DIEA and agitated for an additional 2 h. The polymer was washed with CH_2Cl_2 (3 X 100 mL), THF (3 X 100 mL), DMF (3 X 100 mL) and finally CH_2Cl_2 (3 X 100 mL). This procedure afforded the nitophenyl carbonate **5**. To the nitrophenylcarbonate-bound polymer **5** (16 g, 4.64 mmol) was added 3-hydroxybenzamidine (3.88 g, 18.56 mmol, 4 equiv) followed by DMF (10 mL) and DIEA (7.20 g, 9.70 mL, 55.6 mmol, 12 equiv). The mixture was agitated for 4 h and washed sequentially with CH_2Cl_2 and DMF to afford the polymer-bound amidine **7**. To the polymer intermediate **7** was added NaH (60% suspension in mineral oil, 452 mg, 11.31 mmol, 3 equiv) suspended in 30 mL DMF followed by 4-methyltetrafluoropyridine (1.6 g, 1.12 mL, 9.8 mmol, 7 equiv) in 30 mL DMF. The reaction was agitated for 2 h then washed sequentially with DMF, isopropanol and methylene chloride to afford the polymer-bound pyridine intermediate **9**. **Synthesis of N-Substituted Pyridines.** The polymer **9** was partitioned into reaction vials, each containing 200 mg of polymer (0.056 mmol) and treated with the primary or secondary amine (5.6 mmol, 10 equiv) in 3 mL N-methylpyrrolidinone (NMP). The reaction mixture was agitated for 24 h and washed sequentially with NMP followed by CH_2Cl_2. The reaction mixture in each vessel was then treated with 1 mL of 95:5 TFA/$H_2$0 solution for 40 min. The cleavage mixture was collected in vials. The resin was washed with an additional 1 mL of the cleavage cocktail and the eluants combined in the respective vials. After transferring 50 μL of each sample into autosampler vials, the solutions were evaporated on a speed vacuum concentrator to afford the target compounds **2**.
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