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Discovery of 3-Amino-4-Chlorophenyl P1 as a Novel and Potent Benzamidine Mimic Via Solid-Phase Synthesis of an Isoxazoline Library†

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Abstract—In an effort to identify orally bioavailable factor Xa inhibitors, two isoxazolines libraries were prepared to scan for novel P1 ligands. From this work, 4-chloro-3-aniline was identified as a novel and potent benzamidine mimic.

Factor Xa (FXa) is the crucial enzyme at the convergent point of the intrinsic and extrinsic coagulation pathways.[1–3] Together with FVa, calcium and phospholipids, a prothrombinase complex is formed to convert prothrombin to thrombin. Thrombin, in turn, converts fibrinogen to fibrin and activates platelets, eventually leading to the formation of thrombus or blood clots. Inhibition of FXa has been demonstrated to result in antithrombosis in both animal models[4] and clinical settings.[5] Thus FXa has emerged as a promising target for the discovery of oral anticoagulants. While several extremely potent FXa inhibitors containing benzamidine as the P1 residue have been identified, poor oral absorption and short duration of action have precluded the development of these compounds as oral agents. The poor pharmacokinetics and low permeability are the result of the high basicity and insufficient lipophilicity of the benzamidine (p*K*a 11.6). In order to develop orally-bioavailable FXa inhibitors with improved pharmacokinetics, many research groups have focused on less basic, or neutral replacements for the benzamidine moiety.[6–8] We have embarked on a comprehensive and rational design of benzamidine mimics.[9] To accompany the rational approach, we have also undertaken a random library approach to search for novel benzamidine mimics. Herein, we would like to report on the discovery of 3-amino-4-chlorophenyl P1 as a novel and potent benz-

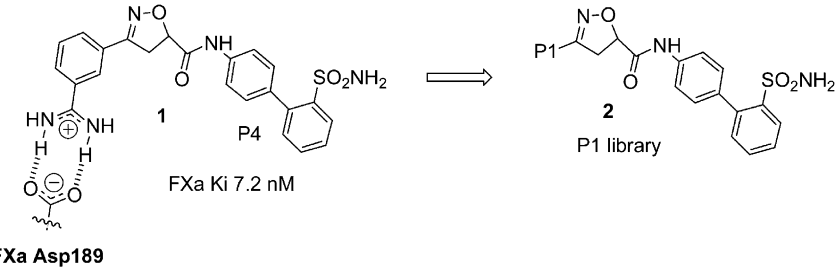

Figure 1. Nonbenzamidine P1 library of isoxazoline FXa inhibitors.

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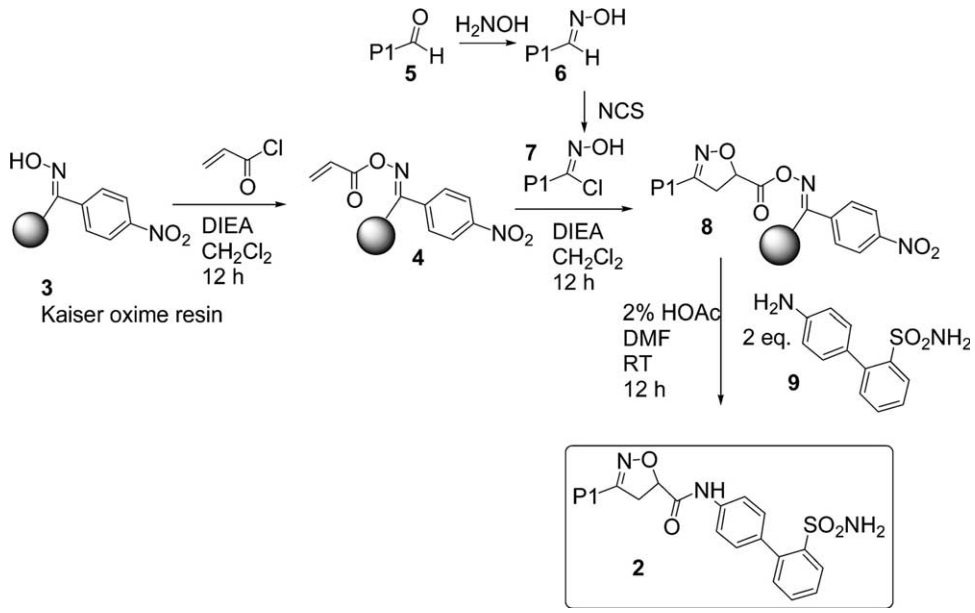
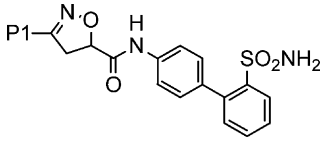


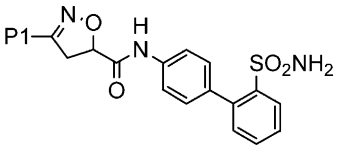
Figure 2. Solid phase synthesis (SPS) of nonbenzamidine library.

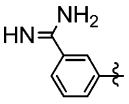
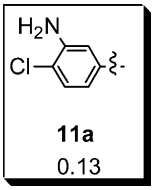
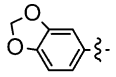
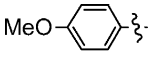
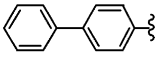
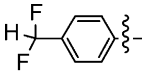
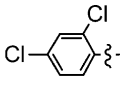
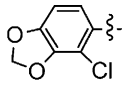
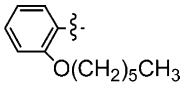
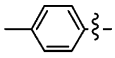
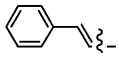
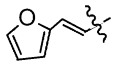
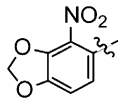
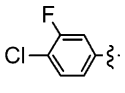
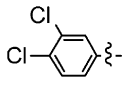
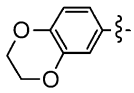
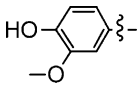
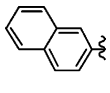
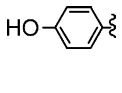
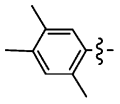
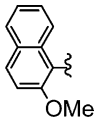
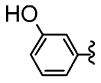
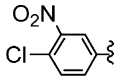
Table 1. First nonbenzamidine P1 library



P1 FXa K_i, μM	**2a** 1.0	**2b** 1.9	**2c** 4.1	**2d** 4.5	**2e** 4.6	**2f** 5.9
P1 FXa K_i, μM	**2g** 7.5	**2h** 7.8	**2i** 10	**2j** 11	**2k** 15	
P1 FXa K_i, μM	**2l** 17	**2m** 17	**2n** 24	**2o** >30	**2p** >30	**2q** >30
P1 FXa K_i, μM	**2r** >30	**2s** >30	**2t** >30	**2u** >30	**2v** >30	

Table 2. Second nonbenzamidine P1 library



P1 FXa K_i, μM							
						
1 0.0072	**11a** 0.13	**11b** 0.49	**11c** 0.7	**11d** 1.1	**11e** 1.3	**11f** 1.6
P1 FXa K_i, μM							
						
11g 1.7	**11h** 1.7	**11i** 1.7	**11j** 1.8	**11k** 2.1	**11l** 2.3	**11m** 2.3
P1 FXa K_i, μM							
						
11n 2.4	**11o** 2.5	**11p** 2.7	**11q** 13.2	**11r** 20	**11s** 20	**11t** 22
P1 FXa K_i, μM							
						
11u 26	**11v** >3	**11w** >3	**11x** >30			

amidine mimic that resulted from our solid-phase library synthesis approach.

We have recently reported the discovery of a potent series of monobasic isoxazoline FXa inhibitors which incorporate a benzamidine ligand in the P1 position[10] as represented by **1** (Fig. 1). The benzamidine ligand of **1** interacts via bidentate hydrogen bonding with Asp189 in the S1 pocket of FXa. To facilitate the search for novel, less basic P1 ligands, a series of benzamidine mimics was explored in an isoxazoline library, which was synthesized using solid-phase synthesis (SPS).[11]

The synthesis[12] of the nonbenzamidine library (Fig. 2) was initiated by condensing acrolyl chloride with Kaiser oxime resin **3**[13] to provide acryloyl oxime **4**.[14] A diverse set of aldehydes **5** was condensed with hydroxylamine followed by chlorination with *N*-chlorosuccinimide to give hydroximoyl chloride **7**. 1,3-Dipolar cycloaddition of the nitrile oxide dipole, generated from **7** by the action of base, and acryloyl dipolarophile **4** provided isoxazoline **8** regiospecifically.[15] Cleavage of the isoxazoline from the resin with concomitant amidation with biarylaniline **9**[16] was accomplished in 2% acetic acid in dimethylformamide at room temperature to yield isoxazoline **2**.

From the first diverse nonbenzamidine P1 library (22 members, Table 1), we found that a relatively short alkyl chain (**2d**) has better FXa affinity than a long alkyl chain (**2m**). 3-Carboxyphenyl **2s** is inactive, probably due to the electrostatic repulsion with Asp189 of FXa. The 4-halophenylisoxazolines appear to be more active than 3-halophenylisoxazolines (**2a**–**c** vs **2f**, **h**, **I**, **o**) and 4-chlorophenyl **2a** is the most potent compound from the first library. This result directed us to bias our second library towards 4-substituted analogues.

Methylenedioxyphenyl **11b** was found to have better FXa affinity than the corresponding ethylenedioxyphenyl derivative **11q** (Table 2). Thiophene **11e** is found to be more potent than phenyl compound **2j** (Table 1). Styryl **11l** is equivalent to the corresponding furan analogue **11m**. The flexible phenethyl **2q** exhibits poor binding affinity compared with the more conformationally rigid styryl **11l**. It was interesting to note that the large 4-biphenyl **11d** can be accommodated as

the P1 moiety. In terms of *para* substituents, *p*-methoxy **11c** has the best FXa K_i (97× weaker than benzamidine **1**). The *p*-methoxy derivative **11c** is more potent than difluoromethyl **11f**, methyl **11k** and hydroxy **11t**. From the library the most potent P1 ligand was found to be 3-amino-4-chlorophenyl **11a**.[17] Remarkably, compared with benzamidine **1**, it is only 18-fold weaker in FXa K_i. This places 3-amino-4-chlorophenyl P1 as one of the best benzamidine mimics reported.[6,7,9]

In summary, we have designed a versatile SPS route to make libraries based on diverse nonbenzamidine P1 substituents and discovered a novel 3-amino-4-chlorophenyl P1 as one of the most potent benzamidine mimics reported (loss of only 18× in FXa K_i).[8] The optimization of this novel benzamidine mimic will be described elsewhere.[18,19]

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11. **Representative procedure:** 4-Chloro-3-nitrobenzaldehyde (279 mg, 1.5 mmol) was placed in a 4 mL plastic vial with an internal filter and a drain plug. Ethanol (2.5 mL) was added followed by the addition of an aqueous hydroxylamine hydrochloride solution (0.50 mL, 1.8 mmol, 3.74 M) and an aqueous sodium acetate solution (0.79 mL, 1.2 mmol, 1.52 M). The vial was rotated for 12 h on a Barnstead/Thermolyne Labquake Rotator. The ethanol and water are removed under a stream of nitrogen leaving a solid that was washed once with water and dried under dynamic vacuum to give 4-chloro-3-nitro-benzaldehyde oxime.

4-Chloro-3-nitro-benzaldehyde oxime was placed in a 4 mL plastic vial with an internal filter and anhydrous chloroform (2 mL) and THF (1 mL) are added. *N*-chlorosuccinimide (200 mg, 1.5 mmol) was added followed by pyridine (15 μL, 0.1% mol) and the solution was rotated for two h to give 4-chloro-3-nitro-*N*-hydroxy-benzimoyl chloride.

The acryloyl oxime resin[14] **4** (200 mg, 0.15 mmol) was added to the 4 mL plastic vial containing 4-chloro-3-nitro-*N*-hydroxy-benzimoyl chloride followed by dropwise addition of *N,N*-diisopropylethylamine (0.26 mL, 1.50 mmol) and the vial was rotated for 2 h. The solution darkened in color over this time. The vial was placed on a Baker-21 extraction system SP24. Utilizing the drain plug, the resin was washed with DMF (3×), methanol (4×), ethyl acetate (4×), and lastly with dichloromethane (4×), and dried under a stream of air. IR: ν 1772 cm^{-1} (C=O), no 1400 cm^{-1} peak was detected.

4′-Amino-biphenyl-2-sulfonic acid amide[17] **9** (74 mg, 0.30 mmol) and *N,N*-dimethylformamide (2 mL) were added to the vial. The vial was capped, agitated for 60 s, and acetic acid (40 μL) was added and the solution was rotated for 14 h at room temperature. The vial was placed onto the Baker-21 extraction system, the solvent was collected into a test tube and the resin was washed with DMF (2×7 mL) and collected into the same test tube. The collected solvent was placed in a separatory funnel and partitioned with ethyl acetate and a 5% HCl solution. The organic phase was washed with 5% HCl solution (5×), saturated sodium bicarbonate solution (1×), brine (1×), dried over sodium sulfate and the solvent was evaporated under reduced pressure. The residue was gravity filtered through a silica filled tube (Supelco Supelclean™ LC-Si SPE tube) using an ethyl acetate/hexane gradient to give 46 mg of 3-(4-chloro-3-nitro-phenyl)-4,5-dihydro-isoxazole-5-(2′-sulfamoyl-biphenyl-4-yl)-carboxamide **11x** (61% yield). The product is judged to be >95% pure by NMR. ^1H NMR ($CDCl_3$) δ 8.71 (s, 1H), 8.18 (d, 1H, $J=1.8$ Hz), 8.09 (d, 1H, $J=8.1$ Hz), 7.85–7.28 (m, 9H), 5.38 (dd, 1H, $J=11.1, 4.8$ Hz), 4.58 (bs, 2H), 3.87–3.78 (m, 2H). MS (CI)$(M+NH_4)^+$ 518/520 Cl pattern (20%). The recovered resin showed no C=O at 1772 cm^{-1} (IR).
12. Preparation of acryloyl oxime resin **4**: To a 3-necked 2 L round bottomed flask equipped with a mechanical stirrer, septum capped inlet and a nitrogen bubbler was added *p*-nitrobenzophenone oxime polystyrene (Kaiser) resin **3** (40 g, 0.76 mmol/g, 30.4 mmol). Dry methylene chloride (800 mL) and *N,N*-diisopropylethylamine (20.9 mL, 120 mmol) were added and the flask cooled in an ice bath to 0 °C. To the stirred suspension of the resin was added acryloyl chloride (7.4 mL, 90 mmol) dropwise via a syringe. The ice bath was removed and the stirring continued for 12 h. The resin was isolated by filtration and washed in the filter funnel successively with methylene chloride (2×), methanol (2×), ether (1×), and methylene chloride (2×) and then dried under dynamic vacuum for 14 h. IR: ν 1756 (C=O), 1632 (C=C) cm^{-1}.
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14. IR: ν 1772 (C=O) cm^{-1}.
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16. Preparation of 4′-amino-biphenyl-2-sulfonic acid amide **9**: 4′-Amino-biphenyl-2-sulfonic acid *tert*-butylamide[18] (5.00 g, 16.4 mmol) was heated to reflux in trifluoroacetic acid (80 mL) for 7 h. The solvent was evaporated and the solid judged to be of sufficient purity for further use.
17. **11a** was obtained from **11x** via the use of $SnCl_2 \cdot 2H_2O$ in refluxing ethyl acetate.
18. (a) Quan, M. L.; Ellis, C. D.; He, M. Y.; Liauw, A. Y.; Woerner, F. J.; Alexander, R. S.; Knabb, R. M.; Lam, P. Y. S.; Luettgen, J. M.; Wong, P. C.; Wright, M. R.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 369. (b) Quan, M. L.; Ellis, C. D.; He, M. Y.; Liauw, A. Y.; Lam, P. Y. S.; Rossi, K. A.; Knabb, R. M.; Luettgen, J. M.; Wright, M. R.; Wong, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett* **2003**, *13*, 1023.
19. The 3-amino-4-chlorophenyl P1 moiety was incorporated into an analogous series and the pharmacokinetic profile examined in dog. This compound was found to be highly bioavailable (71%).