Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1033–1036

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Solid-Phase Parallel Synthesis of Azarene Pyrrolidinones as Factor Xa Inhibitors

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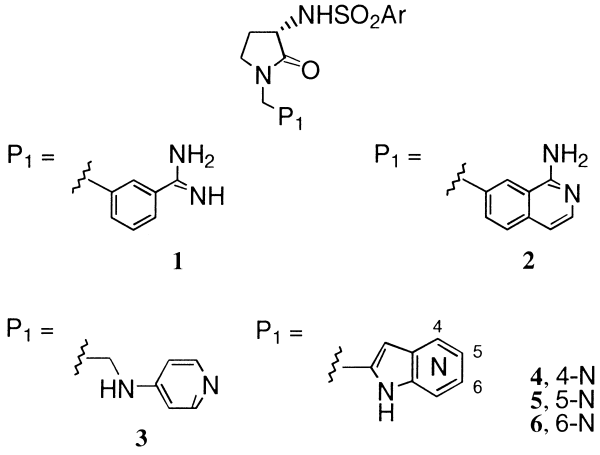
Received 28 January 2000; accepted 1 March 2000

Abstract—A focused library (4×14) prepared from 4-aminopyridine and 4-, 5-, and 6-azoindole templates was synthesized using 14 polymer supported 4-amido-2,3,5,6-tetrafluorophenyl (TFP) sulfonate esters inputs. Several compounds were identified as factor Xa inhibitors ($IC_{50} \leq 0.1$ μM) helping to establish the SAR among these four series of azarene pyrrolidinones.

The serine proteases of the coagulation cascade, namely thrombin (fIIa) and factor Xa (fXa), have been identified as logical targets for the development of antithrombotic agents.[1] The inhibition of fXa, the central enzyme in the cascade, has emerged as a particularly active area of research. Numerous small molecule inhibitors of fXa have been described,[2–4] however, most incorporate highly basic functions which impart poor physicochemical properties for oral administration. Previous results from this laboratory have shown that pyrrolidinone benzamidines **1** are potent inhibitors of fXa.[5–7] The benzamidine group (pK_a 11.6) can be replaced by a less basic aminoisoquinoline **2** (pK_a 7.6) with improved Caco-2 cell permeability and oral bioavailability.[8]

4-Aminopyridine ($pK_a = 9.2$) derivatives have been used to replace benzamidines and guanidines in an effort to improve the oral activity of thrombin inhibitors.[9–11] In the course of our continuing work on novel benzamidine replacements in fXa inhibitors we have prepared 4-aminopyridine pyrrolidinones **3**.[12] As a further extension of this work 4-, 5-, and 6-azaindoles ($pK_a = 6.9$, 8.3, 8.0, respectively) were targeted. These weak bases can be considered as conformationally restricted aminopyridine analogues. Initial results based on this strategy were quite promising. Selected compounds **3a**, **4a**, **5a**, **6a** (Ar = 6-chlorobenzothiophene-2-yl) demonstrated inhibitory activity with anti-fXa K_is of 17, 109, 96, and 43 nM, respectively.[13] This result prompted us to design a focused library using an optimized set of sulfonamide groups identified in the pyrrolidinone benzamidines **1**.



Previous work from our laboratories had demonstrated the ready derivatization of 4-amido-2,3,5,6-tetrafluorophenoxy (TFP) resins (polystyrene/1% divinylbenzene) to form activated sulfonate and carboxylate esters.[14] The resin bound sulfonate esters can be used as electrophilic partners for the formation of sulfonamide linkages (Scheme 2). A variety of amines can serve as nucleophiles and the application of this methodology to the construction of sulfonamido pyrrolidinone libraries is a logical extension of this work. The preparation, analysis and assay of a focused library to quickly expand the SAR of the pyrrolidinone azarenes **3**–**6** is described.

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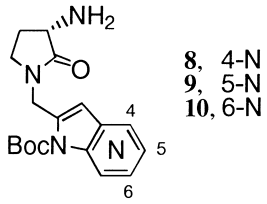
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00151-7

Experimental

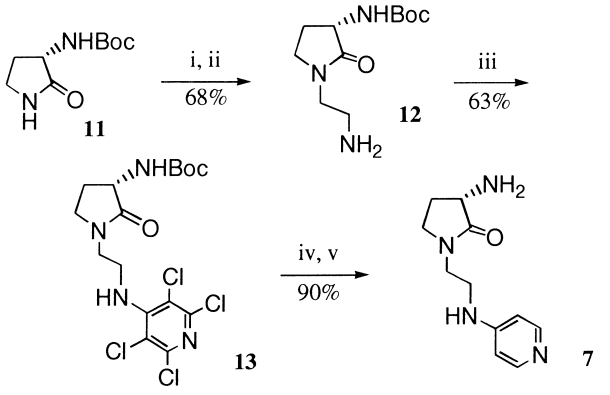
The 4-aminopyridine scaffold **7** was prepared (Scheme 1) by cyanomethylation of pyrrolidinone **11** followed by reduction to amine **12**. Subsequent reaction with 4-nitrotetrachloropyridine yields the 4-substituted regioisomer **13**. Hydrogenolysis followed by deprotection gave scaffold **7**. Scaffolds **8**, **9**, and **10** were prepared as previously described.[13,15]



8, 4-N
9, 5-N
10, 6-N

The aryl sulfonyl chlorides used in this work are commercially available (**14k**–**n**) or have been previously described (**14a**–**i**).[6,7,16] Sulfonyl chloride **14j** was prepared by literature methods.[17] The tetrafluorophenol (TFP) derivatized resin,[18] was loaded with 14 different sulfonyl chlorides **14**. In a typical procedure, TFP resin was agitated with excess **14** and DIEA in CH_2Cl_2 at rt overnight. The loaded resins were washed repeatedly (aq DMF; THF; CH_2Cl_2) and dried in vacuo. Loading was confirmed by ^{19}F NMR on a representative sampling. For example **15c**,**f**,**h** showed: δ −141.7/151.4,−141.8/−151.4 and −142.1/−151.6, respectively, versus −148/−166 for the TFP resin.

Solid-phase sulfonylation of templates was carried out in parallel fashion (Scheme 2). Fifty-six reaction vessels were charged with a combination of four templates (**7**–**10**) and 14 loaded resins **15** (4×14). In a typical reaction, loaded resin and scaffold were agitated in DMF at rt for 3 days. The reaction was monitored for disappearance of starting materials by TLC. Resins were collected by filtration and washed with MeOH. For the reactions with the aminopyridine template **7**, concentration of filtrate gave product **3**. In the case of the azaindole templates **8**–**10**, products **4**–**6** were obtained after removal of the Boc group. Residues were made up to 10 mM DMSO stock solutions, in a microtiter plate and analyzed by LC–MS and ELSD (evaporative light scattering detection).

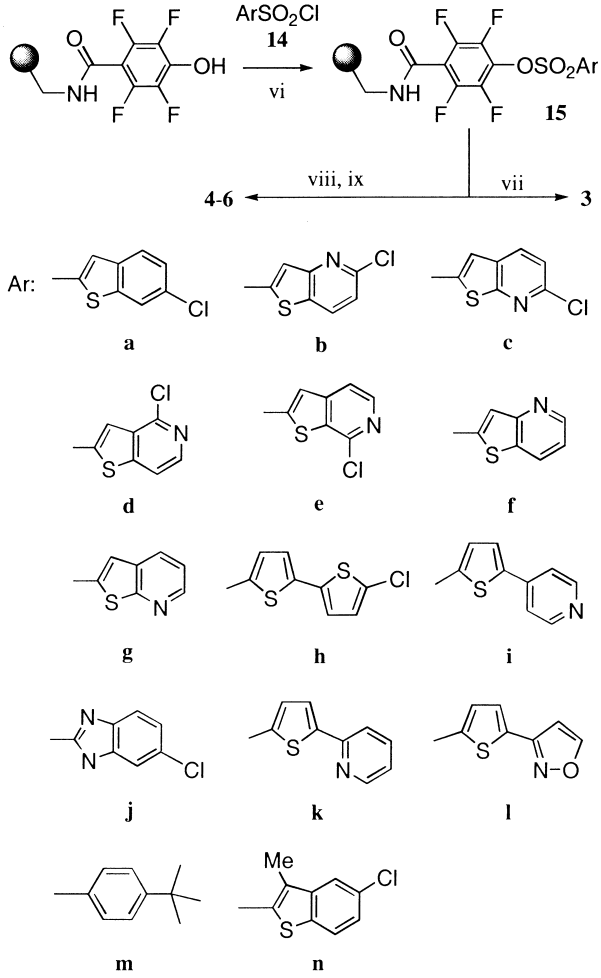


Scheme 1. Preparation of aminopyridine template **7**: (i) NaH, TBAI, $BrCH_2CN$; (ii) PtO_2, H_2; (iii) 4-nitrotetrachloropyridine, NMM; (iv) 10% Pd/C, NaOMe, H_2; (v) TFA/DCM.

Assays were run in triplicate by a modification of the published procedure.[19] Stock solutions were diluted to a final concentration of 1 μM in inhibitor using buffer containing 0.05 M Tris, 0.15 M NaCl and 0.1% of PEG-8000 (pH 7.5). Inhibitor solutions were treated with 1 nM fXa, incubated for 30 min at rt, then assayed with 200 μM of Spectrozyme substrate (American Diagnostica Inc.). The initial rates were read at 405 nm for 5 min with a Molecular Devices microtiterplate reader in kinetic mode. Any compound with ≥65% inhibition at 1 μM in the initial screen was assayed further by serial dilution until ~50% inhibition was observed.

Results and Discussion

The results of the library are given in Table 1; desired product (as determined by MS) was found in every well. The highest purity (as determined by analytical LC using ELSD) was observed for the aminopyridine series **3**; excepting **3i**, the compounds in this series had purities greater than 90% (ELSD). The remaining azaindoles, series **4**–**6**, also had acceptable purity. Overall, better than 70% of the compounds had purities >70%, as determined by ELSD. The average purity of



Scheme 2. Parallel synthesis of azarene sulfonamides **3**, **4**, **5**, and **6**: (vi) DIEA, CH_2Cl_2; (vii) **7**, DMF; (viii) **8**–**10**, DMF; (ix) 20% TFA/CH_2Cl_2.

Table 1. Summary of 56-member library and fXa inhibition assay[19] results

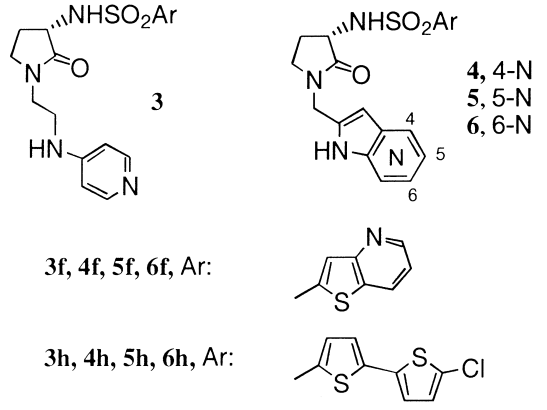
Compd	% Conversion[a]	ELSD[b] purity (%)	LC–MS [M + 1]	Test concn (μM)	Inhibition of fXa (%)
3a	94	99	451	0.1	57
3b	88	99	452	0.4	50
3c	94	99	452	0.1	53
3d	88	99	452	1	39
3e	82	99	452	1	23
3f	99	99	418	0.7	50
3g	99	99	418	1	23
3h	76	95	483	0.1	77
3i	40	50	444	1	41
3j	85	95	435	1	29
3k	99	99	444	1	18
3l	94	99	434	1	22
3m	97	99	417	1	14
3n	96	99	465	0.5	47
4a	81	90	461	0.5	64
4b	90	98	462	0.5	48
4c	87	99	462	1	23
4d	89	99	462	0.5	50
4e	92	99	462	1	45
4f	88	96	428	0.3	58
4g	82	92	428	1	44
4h	36	49	493	1	42
4i	40	47	454	1	35
4j	67	65	444	1	14
4k	38	37	454	1	8
4l	59	73	444	1	31
4m	80	92	427	1	22
4n	65	76	475	1	40
5a	63	77	461	0.5	53
5b	80	92	462	1	58
5c	73	90	462	1	51
5d	83	92	462	1	35
5e	84	95	462	1	25
5f	78	86	428	1	61
5g	67	79	428	1	18
5h	23	28	493	0.1	62
5i	36	27	454	1	21
5j	60	64	444	1	31
5k	26	28	454	1	11
5l	44	54	444	1	17
5m	62	83	427	1	14
5n	49	59	475	1	37
6a	77	86	461	0.1	50
6b	88	97	462	0.3	57
6c	85	96	462	1	50
6d	88	97	462	0.2	52
6e	91	99	462	0.5	48
6f	88	95	428	0.1	52
6g	80	88	428	0.5	43
6h	31	44	493	0.3	53
6i	56	48	454	1	50
6j	69	75	444	1	21
6k	36	37	454	1	17
6l	52	66	444	1	49
6m	76	91	427	1	57
6n	61	76	475	0.5	52

[a]As estimated by analytical LC area% (UV detection).
[b]Determined by analytical LC (evaporative light scattering detection).

this 56-member library is almost 80% with a median of 91%. The major impurity as determined by LC–MS is unconverted starting scaffolds. None of the scaffolds show anti-fXa activity at 1 μM concentration.

Inhibition data show that the four series **3**–**6** were not equally effective fXa inhibitors. Aminopyridines **3** and 6-azaindoles **6** were generally the most effective inhibitors. The 5-azaindole series **5** was the least effective and the 4-azaindole **4** was intermediary in activity. More specifically, chlorobenzothiophene **a** (included as an internal standard) is the only sulfonamide group effective in all four series. The relative potencies for library compounds **3a**, **4a**, **5a**, **6a** tracked that seen for pure inhibitors (vida supra) in that **3a** and **6a** are particularly effective fXa inhibitors.

Closer examination of the table reveals that the structure–activity relationships (SAR) between the series was quite variable. For example entry **h,** (i.e., the chlorobithienyl) ligand is very effective in series **3** and **5**, moderately effective in **6** and weakly active in **4**. In contrast the thienopyridyl **f**, a potent ligand in pyrrolidinone benzamidines **1**, is very effective in series **6**, moderately effective in **3** and **4**, and weakly active in **5**. This suggests that the binding modes for the various scaffold/azarene combinations may differ appreciably.



Compounds **3c**, **f**, **h**, **4f**, **h**, **5f**, **h** and **6f**, **h** were resynthesized; the activities of the pure compounds confirmed the SAR established by the library. For example, in keeping with the library trends, resynthesized samples of **3h**, **6h** and **4h**[20] showed anti-fXa K_is of 15, 60, and 310 nM, respectively.

The details of this work and the implications for inhibitor binding modes in fXa will be published in due course. In summary, a focused 56-member library was prepared using TFP resins. Library purity (70% of wells with greater than 70% by ELSD) was sufficient to establish SAR patterns. New fXa inhibitors (e.g. **3c**, **3h**, **5h**, **6f**) with potencies less than or equal to 0.1 μM were identified.

Acknowledgements

The authors would like to thank Dr. S.-Y. Tang for LC–MS analysis and acknowledge Dr. W. He for the discovery of ligand **j**.

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