

SYNTHESIS, SAR AND IN VIVO ACTIVITY OF NOVEL THIENOPYRIDINE SULFONAMIDE PYRROLIDINONES AS FACTOR Xa INHIBITORS

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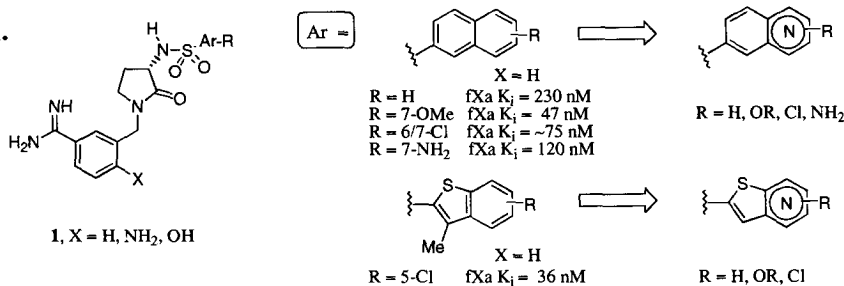
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Abstract: Thienopyridine sulfonamide pyrrolidinones were found to be potent and selective inhibitors of the coagulation cascade enzyme factor Xa. SAR studies led to several compounds that were selected for further in vivo investigation. These novel aryl binding pocket moieties represent a structural modification to a series of fXa inhibitors. Several compounds proved to be efficacious iv antithrombotic agents. © 1999 Elsevier Science Ltd. All rights reserved.

Direct inhibition of the serine protease factor Xa (fXa) offers an attractive point of intervention for the treatment of thrombotic disorders.¹ The activation of fX to fXa lies at the convergence of the intrinsic and extrinsic pathways in the blood coagulation cascade. Factor Xa is the sole enzyme responsible for activation of prothrombin to thrombin which results in fibrin formation, aggregation and clot stabilization, as well as platelet activation.² Inhibition of fXa prevents thrombin production, regardless of the activation pathway. Emerging work in this area has targeted small molecule inhibitors of fXa as potential agents for antithrombotic therapy.¹

Recently, we discovered a series of potent and selective benzamidine inhibitors of fXa derived from sulfonamide pyrrolidinones **1** (Figure 1).^{3,4} Herein, we report findings subsequent to this work that highlight a key modification to the P4 groups. The S4 aryl binding pocket of fXa is unique among serine proteases in that the walls are lined with electron-rich aromatic residues and a Glu97 side chain is located at the terminus; the region is referred to as the cation hole.⁵ Since naphthalenes formed the basis of our initial P4 work, it seemed reasonable to investigate weakly basic azarenes to probe this region of S4. When benzothiophene sulfonamides were also identified as desirable P4 moieties,⁶ the logical extension to thienopyridines was made. Thienopyridines have long been recognized as viable drug motifs, most notably in the areas of antiplatelet therapies,⁷ antibacterials⁸ and as quinoline isosteres.⁹

Figure 1.



In addition to the potential activity enhancements, various substituted quinoline and isoquinoline sulfonamides were explored in an attempt to alter the physicochemical properties of the pyrrolidinone series. While several of the alkoxy- and chloro- derivatives produced submicromolar inhibitors of fXa (Table 1), these compounds resulted in a ≥ 10 -fold decrease in activity when compared to their naphthyl analogs (Fig. 1). One chloroquinoline (**1c**) did retain good in vitro potency towards fXa with only $\sim 1.5X$ loss. As for the weakly basic azarene and aminoazarene sulfonamides, some interesting results were observed. The 2-aminoquinoline **1j** was only 2X less active than its 7-aminonaphthalene analog, whereas the 1-aminoisoquinoline **1f** was slightly better than both ($K_i = 0.09 \mu\text{M}$). Additionally, quinoline **1a** was equipotent to the naphthyl parent ($K_i = 0.23 \mu\text{M}$) and 2-aminoquinoline **1b** was active against fXa. Compounds **1a-f** were better in general than **1g-l**, however, the fXa potency of the 6,6-ring systems was not in a range that warranted further effort. Selectivity over thrombin and trypsin also improved for many azarenes as compared to the naphthalenes.

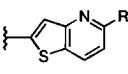
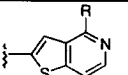
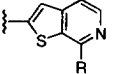
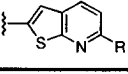
Table 1. Activity¹⁰ of Various Quinoline and Isoquinoline Sulfonamide Pyrrolidinones **1** (X = H).

| Compound | Ar | R | fXa (K_i , μM) | thrombin (K_i , μM) | trypsin (K_i , μM) |
|-----------|----|-----------------|----------------------------------|---------------------------------------|--------------------------------------|
| 1a | | H | 0.20 | >4.0 | >2.9 |
| 1b | | NH ₂ | 0.16 | >4.0 | >2.9 |
| 1c | | Cl | 0.11 | >4.0 | >2.9 |
| 1d | | OEt | >1.2 | >4.0 | >2.9 |
| 1e | | H | 0.38 | >4.0 | >2.9 |
| 1f | | NH ₂ | 0.09 | >4.0 | >2.9 |
| 1g | | H | 0.51 | >4.0 | >2.9 |
| 1h | | NH ₂ | 0.49 | >4.0 | >2.9 |
| 1i | | H | 0.42 | >4.0 | >2.9 |
| 1j | | NH ₂ | 0.24 | >4.0 | >2.9 |
| 1k | | Cl | 0.72 | >4.0 | >2.9 |
| 1l | | OMe | 0.48 | >4.0 | >2.9 |

Applying a similar approach to the related thienopyridine sulfonamides proved to be more fruitful (Table 2). Compounds **1m**, **1o**, **1x**, and **1y** were 4–8X more active against fXa as compared to the quinolinyl analogs (**1i**, **1k**, **1a**, and **1c**). Perhaps a better fit in the S4 pocket is imparted by the 5,6-fused ring systems versus the corresponding 6,6-rings due to a change in the angle of ring fusion. The unsubstituted thienopyridyl sulfonamides (R = H) were generally more active and selective than the respective chloro-substituted analogs (R = Cl). These results may reflect a difference in the binding conformation for these P4 groups since substituents on the thienopyridyl ring could effect the orientation of the groups in S4, i.e., whether the sulfur atom of the thienopyridine ring points up and out to solvent or faces down and in toward the enzyme pocket for these inhibitors.

Previous work had shown that hydroxy or amino substituents *para*- to the amidine moiety enhances in vitro potency;⁴ these groups were incorporated into the thienopyridine series. For the 5-chloro-thieno[3,2-*b*]pyridine sulfonamide, the *para*-amino benzamidine **1p** resulted in a 10X increase and the *para*-hydroxy benzamidine **1q** gave a 20X increase as compared to benzamidine **1o**. Modest increases in both thrombin and trypsin activity is also observed in these derivatives. Interestingly, in the related 5-methoxy thienopyridine analog **1r**, activity towards other serine proteases was enhanced more dramatically. In general, however, the *para*-hydroxy compounds provided many selective ($>10^3$ fold), low nanomolar inhibitors of fXa as illustrated by the in vitro results for **1n**, **1s**, and **1u-w**. The unsubstituted thieno[3,2-*b*]pyridine **1n**, for example, ranks as the most potent and selective for fXa with a 40-fold improvement in anti-fXa activity versus the parent benzamidine **1m**.

Table 2. Activity¹⁰ of Various Thienopyridine Sulfonamide Pyrrolidinones **1** (X = H, NH₂, OH).

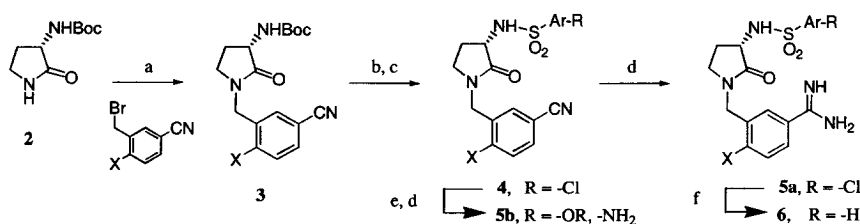
| Compound | Ar | R | X | fXa (K _i , nM) | thrombin (K _i , nM) | trypsin (K _i , nM) | APC (K _i , nM) | plasmin (K _i , nM) | t-Pa (K _i , nM) |
|-----------|---|-----|-----------------|------------------------------|-----------------------------------|----------------------------------|------------------------------|----------------------------------|-------------------------------|
| 1m |  | H | H | 26 | >4,000 | >2,900 | 12,000 | >7,300 | >8,700 |
| 1n | | H | OH | 0.70 | 4,000 | 1,000 | 5,200 | 2,300 | 360 |
| 1o | | Cl | H | 31 | 3,100 | >2,900 | >18,000 | 5,800 | >8,700 |
| 1p | | Cl | NH ₂ | 3.0 | 1,100 | 1,800 | 4,200 | 1,500 | >8,700 |
| 1q | | Cl | OH | 1.5 | 920 | 1,600 | 11,000 | 3,600 | 2,700 |
| 1r | | OMe | NH ₂ | 5.0 | 210 | 860 | 9,500 | >7,300 | >8,700 |
| 1s |  | H | OH | 3.0 | 2,600 | >2,900 | >18,000 | >7,300 | 8,700 |
| 1t | | Cl | H | 39 | >4,000 | >2,900 | --- | --- | --- |
| 1u | | Cl | OH | 2.0 | 4,000 | >2,900 | 8,000 | 4,500 | 8,700 |
| 1v |  | H | OH | 2.0 | 2,000 | >2,900 | 18,000 | 6,200 | >8,700 |
| 1w | | Cl | OH | 5.0 | 2,600 | >2,900 | 5,500 | 7,300 | 7,500 |
| 1x |  | H | H | 88 | >4,000 | 2,900 | --- | --- | --- |
| 1y | | Cl | H | 120 | >4,000 | >2,900 | --- | --- | --- |

As a class, the thienopyridine sulfonamide pyrrolidinones offer a dramatic improvement over the naphthalene series for selectivity across a broad array of serine proteases (Table 2). The unsubstituted 4-, 5- and 6- aza- analogs are also more selective than the chloro- substituted thienopyridines. A comparison of *para*-hydroxy benzamidine analogs **1n/1q** and **1v/1w** illustrates the trend. One of our best *para*-hydroxy benzamidine naphthalene sulfonamides (**1z** where Ar = 7-methoxynaphthalen-2-yl and X = OH)⁴ exhibits a selectivity profile with ratios of K_{i,enz}/K_{i,fXa} in the range of 70-800 (K_{i,fXa} = 3 nM, K_{i,fla} = 206 nM, K_{i,trypsin} = 305 nM, K_{i,APC} = 1970 nM, K_{i,plasmin} = 2350 nM, and K_{i,t-Pa} = 1070 nM). In contrast, the unsubstituted thienopyridine sulfonamides **1n**, **1s**, and **1v** are generally 900-9000 fold selective against these enzymes.

Synthesis of the enantiomerically pure 3-(*S*)-sulfonamide pyrrolidinones proceeded according to the generalized route outlined in Scheme 1.^{3,4} Boc-3-(*S*)-amino-pyrrolidin-2-one **2** is prepared by cyclization (EDC,

HOBt) of N- α -Boc-L-2,4-diaminobutyric acid. Alkylation of the lactam NH with various halomethyl heteroaryl nitriles followed by deprotection and sulfonylation gives the corresponding sulfonamide benzonitriles **4**. For the quinoline and thienopyridine sulfonamides described here, intermediate **4** (where R = Cl) represents a departure point for P4 group manipulation. Halogenated aryl sulfonamide compounds were prepared by Pinner synthesis and ammonolysis on nitrile **4** to give benzamidines **5a**.¹¹ Alternatively, intermediate **4** could be treated with phenol and displaced with an alcohol or ammonia equivalent to yield alkoxy or amino quinolines,¹² which afforded benzamidines **5b** in the usual manner. Furthermore, hydrogenolysis of the halogenated sulfonamide benzamidines **5a** (H₂, Pd-C, MeOH/benzene, KOH, 50 °C)¹³ provided the unsubstituted aryl compounds **6**.

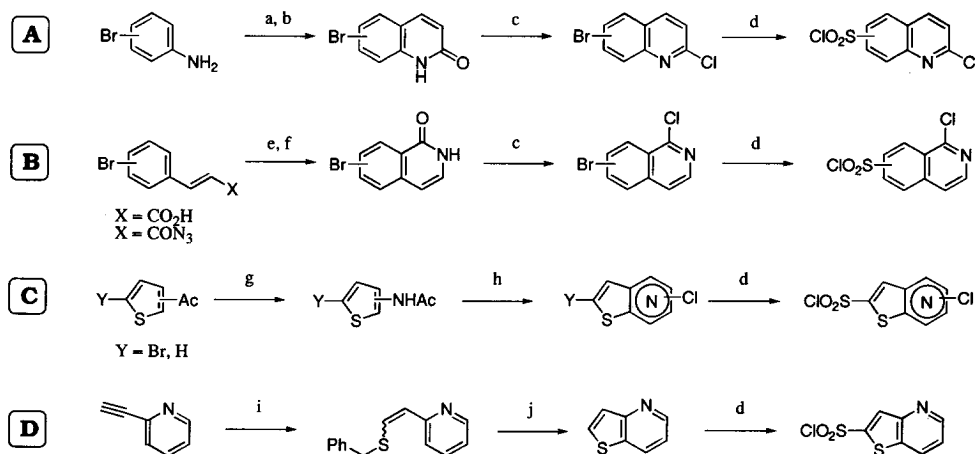
Scheme 1. Synthesis of Quinoline, Isoquinoline and Thienopyridine Sulfonamide Pyrrolidinones.



Reagents: (a) NaH, THF/DMF, 0 °C; (b) HCl/EtOAc; (c) ArSO₂Cl, TEA/DCM; (d) i. HCl/EtOH/DCM; ii. NH₃/MeOH, Δ ; (e) i. PhOH/KOH, 100 °C; ii. NH₄OAc, 80 °C; (f) H₂, Pd-C, MeOH/benzene, KOH, 50 °C.

As for the sulfonyl chlorides used in this work (Scheme 2), synthesis of the azarenes was developed from known procedures for halo- quinoline and isoquinolines¹⁴ (Methods **A** and **B**). A key step employed a one-pot sulfonation¹⁵ of the fused biaryl bromides using sulfur dioxide (gas). Both the 4-aza- and 7-aza-thienopyridines were obtained from literature methods¹⁶ followed by lithiation and sulfonation to provide the chloro- substituted sulfonyl chlorides (Method **C**), whereas the 5-aza- and 6-aza- thienopyridine sulfonyl chlorides were prepared through an adaptation of the isoquinoline synthesis (*cf.* Method **B**; from thiophene aldehydes). When the parent thieno[3,2-*b*]pyridyl sulfonamide displayed superior inhibitory potency for certain analogs, a direct synthesis of the unsubstituted sulfonyl chloride was developed using flash vacuum pyrolysis according to Klemm's method¹⁷ (Method **D**).

Several of our most potent fXa inhibitors from Table 2 were studied in relevant animal models of thrombosis.¹⁸ Of those tested in the rat FeCl₂ arterial thrombosis model,¹⁹ the unsubstituted thienopyridines provided efficacious compounds²⁰ at an iv dose of 300 μ g/kg bolus + 30 μ g/kg/min infusion for 75 min. Compound **1v** (IC₅₀ = 11 nM (rat plasma) and 7 nM (buffer)), for example, reduced thrombus mass by 78% and increased TTO 3.3-fold at this dose. The anti-fXa activity measured *ex vivo* was 95% at 15 min into infusion with a plasma concentration of 2050 nM for **1v**. Furthermore, compound **1v** gave a dose dependent response in

Scheme 2. Synthesis of Quinoline, Isoquinoline and Thienopyridine Sulfonyl Chlorides.

Reagents: (a) $\text{PhCH}=\text{CHCOCl}$, pyr/DCM; (b) AlCl_3 , $\text{C}_6\text{H}_5\text{Cl}$, 100°C ; (c) POCl_3 , 60°C ; (d) i. $n\text{-BuLi}$, THF, -78°C ; ii. $\text{SO}_2(\text{g})$, Et_2O ; iii. SO_2Cl_2 , hexanes; (e) i. EtOCOCl , acetone; ii. NaN_3 , H_2O ; (f) Bu_3N , Ph_2O , Δ ; (g) i. $\text{NH}_2\text{OH}\cdot\text{HCl}$, pyr/MeOH, Δ ; ii. PCl_5 , Et_2O ; (h) POCl_3 , DMF, DCE or TCE; (i) NaOEt , PhCH_2SH , EtOH; (j) FVP 625°C .

the rat model as indicated by the corresponding increases in APTT, PT, anti-fXa and plasma levels. In a canine arterio-venous model of thrombus formation,²¹ the thienopyridine sulfonamide pyrrolidinones tested in vivo demonstrate antithrombotic efficacy²⁰ at an iv dose of $100\text{ }\mu\text{g/kg}$ bolus + $10\text{ }\mu\text{g/kg/min}$ infusion for 240 min. The unsubstituted analog, compound **1v**, reduced thrombus mass by 91–93% and increased TTO 3-fold with anti-fXa activity of 93% and plasma concentration of 736 nM observed after 15 min. It also exhibited a dose dependent response in the dog A/V model.

In summary, we have found that thienopyridine derived pyrrolidinones are potent and selective inhibitors of the coagulation enzyme factor Xa. SAR optimization studies led to many compounds that were selected for further in vivo investigation. These aryl binding pocket moieties represents an extension of a series of fXa inhibitors with a structural modification that provides efficacious intravenous antithrombotic agents. Our work with unique fused biaryl systems continues.

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