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3-Amino-4-sulfonylpyridinone Acetamide and Related Pyridothiadiazine Thrombin Inhibitors

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Abstract—We describe a series of highly potent and efficacious thrombin inhibitors based on a 3-amino-4-sulfonylpyridinone acetamide template. The functionally dense sulfonyl group stabilizes the aminopyridinone, conformationally constrains the 4-substituent, and forms a hydrogen bond to the insertion loop tyrosine OH. We also describe a related series of fused bicyclic dihydrothiadiazinedioxide derivatives, of which one had improved pharmacokinetics in dogs after oral dosing.

The number of patents and papers describing the use of the 3-aminopyridinone acetamide template for the synthesis of thrombin inhibitors is growing rapidly.[1] We have noted that simple 3-aminopyridinones are unstable[2] and have described three methods for their stabilization. The first is simply to derivatize the 3-amino group as a sulfonamide or amide.[2] The second method is to incorporate a nitrogen in the ring at the 4-position to give a pyrazinone.[2] A third is to substitute an electron withdrawing group such as trifluoromethyl at the 4-position (structure **1**).[3] This last method gave compounds with low nanomolar potency against thrombin despite the lack of a group to fill the 'distal binding pocket'. However, these 4-trifluoromethylpyridinones were not sufficiently efficacious to warrant further development. To improve upon them, while using the same stabilization method, we reasoned that it should be possible to stabilize the pyridinone and fill the 'distal binding pocket' using a 4-sulfonyl substituent. Modelling of a 3-amino-4-sulfonylpyridinone template **2** in the active site of thrombin showed that one of the sulfonyl oxygens could form a hydrogen bond to either the tryptophan indole NH or the tyrosine phenol of thrombin's insertion loop. Simultaneously, the other oxygen would form an intramolecular hydrogen bond to the 3-amino group, locking the sulfone in a limited number of conformations, one of which points the sulfone substituent directly into the 'distal binding pocket'.

In this communication we describe the synthesis and properties of a variety of 3-amino-4-sulfonylpyridinones **2** in which the 4-sulfonyl group serves as a conformationally constraining electron withdrawing linker.[4,5] We also describe a related series of 4-aminosulfonylpyridinones **3** and their bicyclic thiadiazine derivatives **4**.[6] The latter offer an achiral alternative to the chiral bicyclic lactam template **5** reported previously.[7,8]

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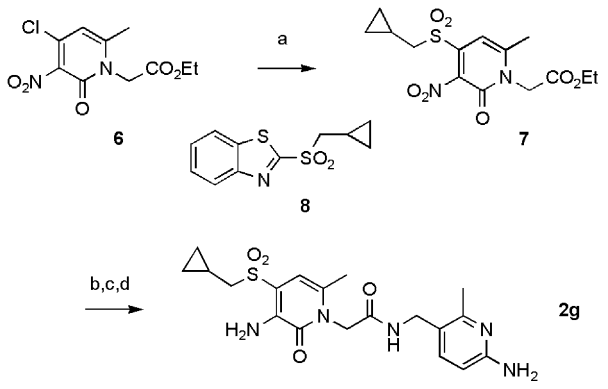
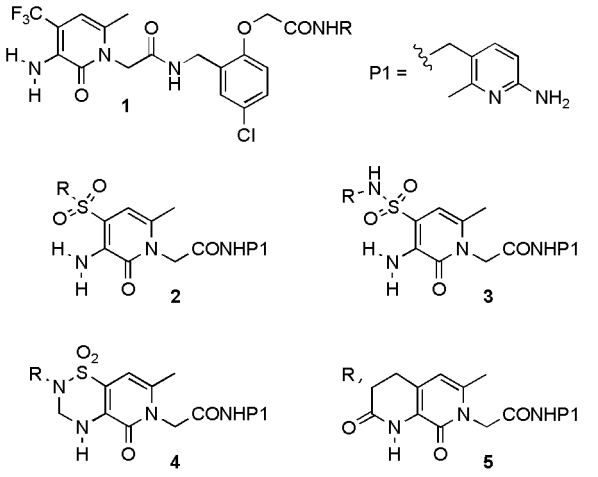


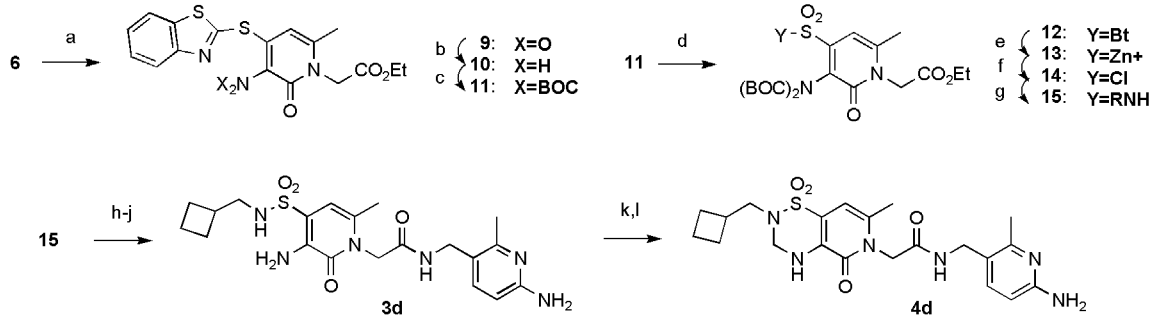
Scheme 1. Synthesis of compound **2g**: (a) **8**, $NaBH_4$, EtOH, 2 h, then add acetic acid to pH 4–5 and **6**, 2 h; (b) H_2, 10% Pd/C, EtOH, 3 h; (c) LiOH, THF, MeOH, H_2O, 2 h, 73% over 3 steps; (d) P1.2HCl,[2] EDC, HOBT, NMM, DMF, 16 h, 65%.



The synthetic route to the 4-sulfones starts from 4-chloropyridinone **6**[9,10] and is exemplified by the synthesis of compound **2g** (Scheme 1).[4] This direct route involves nucleophilic addition of the preformed side chain as its sulfinate salt to give the 3-nitro-4-sulfonylpyridinone **7**. In general, the sulfinate salts were prepared either by reaction of the corresponding organolithium with sulfur dioxide, by reduction of the sulfonyl chloride with sodium sulfite, or as illustrated in Scheme 1, by sodium borohydride reduction of the benzothiazole (Bt) derivative (**8**).[11] This last method is convenient because the 2-sulfonylbenzothiazole is prepared under mild conditions and the reduction and subsequent nucleophilic addition is done in one pot. Reduction of the nitro group followed by ester hydrolysis and coupling to 2-amino-5-aminomethyl-6-methylpyridine[2] gave thrombin inhibitor **2g**.

The route to the 4-aminosulfonylpyridinones and derived pyridothiadiazine dioxides is exemplified by the synthesis of compounds **3d** and **4d** (Scheme 2).[6] Addition of 2-mercaptobenzothiazole to 4-chloropyridinone **6** gave thioether **9**. The highly deactivated sulfur in **9** was inert to oxidation under standard conditions. On the other hand, oxidation of the amine **10** was uncontrollable, so protection as its bis-BOC derivative **11** was necessary. Oxidation was then accomplished using potassium permanganate to give sulfone **12**.[11] Zinc in ethanolic acetic acid was used to reduce the 2-sulfonylbenzothiazole to the sulfinate.[12] Reaction of zinc sulfinate **13** with NCS then gave sulfonyl chloride **14**.[13] Coupling of **14** with cyclobutylmethylamine gave sulfonamide **15** which was deprotected, hydrolyzed and coupled to the P1 group to give thrombin inhibitor **3d**. Cyclization to the bicyclic thiadiazine dioxide **4d** was then accomplished with aqueous ethanolic formaldehyde with ammonia,[14] followed by treatment with warm dilute hydrochloric acid to break down the dimer which formed under the cyclization conditions.

The biological results for the sulfones **2a–i** are given in Table 1. They are significantly more potent than the 4-trifluoromethylpyridinones **1**.[3] However, they are slightly less potent than the fused bicyclic lactams **5**, although this is offset by lower protein binding (e.g., sulfone **2h** is 45% free in human plasma whereas the corresponding lactam **5** with the same cyclobutylmethyl side chain is 19% free).[7] Compound **2h** is clearly the most potent sulfone derivative ($K_i = 0.2$ nM, $2\times$APTT $= 0.23$ μM) and it has good selectivity against trypsin ($K_i = 0.55$ μM) and activated protein C, t-PA, plasma kallikrein and plasmin ($K_i > 100$ μM). Cyclopropylmethylsulfone **2g** is 9-fold less potent than **2h** in the enzyme assay but only 2-fold in the APTT assay, presumably a result of its lower protein binding. Both **2g** and **2h** are fully efficacious in the rat ferric chloride arterial thrombosis model.[16]



Scheme 2. Synthesis of **3d** and **4d**: (a) BtSH, Et_3N, EtOH, reflux, 15 min, 91%; (b) H_2, 10% Pd/C, EtOAc, 16 h, 86%; (c) $(BOC)_2O$, DMAP, CH_2Cl_2, 16 h, 73%; (d) $KMnO_4$, AcOH, H_2O, 16 h, 56%; (e) Zn, AcOH, EtOH, 16 h; (f) NCS, CH_2Cl_2, −5 °C- RT, 45 min; (g) (c-C_4H_7)CH_2NH_2, CH_2Cl_2, NMM, 16 h; (h) HCl, EtOAc, 0 °C–rt, 3 h, 44% over 4 steps; (i) LiOH, THF, MeOH, H_2O, 1 h, 78%; (j) P1.2HCl,[2] EDC, HOAT, NMM, DMF, 16 h, 40%; (k) formaldehyde, H_2O, EtOH, NH_4OH, reflux, 6 h; (l) warm 1 M HCl, 40% over 2 steps.

Table 1. Inhibition constants,[a] in vitro anticoagulant potency (2×APTT),[b] efficacy in the rat ferric chloride thrombosis model[c] and pharmacokinetic parameters in dogs after oral administration at 1 mg/kg

Compd	R	Thrombin inhibition K_i, nM	Trypsin inhibition K_i, nM	2×APTT μM	Protein binding % free[d]	Rat $FeCl_3$[e]	Final plasma concn (μM)[f]	Dog C_{max} (μM)	$t_{1/2}$ (min)	AUC (μM h)
2a	$PhCH_2$	1.6	1600	0.47	22	1/6	0.25	0.45	63	0.62
2b	Ph	3.8	500	0.66	19	4/6	0.57	—	—	—
2c	4-MePh	94	800	—	—	—	—	—	—	—
2d	2-Thienyl	1.7	340	0.39	40	3/6	0.43	—	—	—
2e	c-C_6H_{11}	3	260	0.75	38	1/6	0.65	—	—	—
2f	c-C_3H_5	17	2800	1.64	—	—	—	—	—	—
2g	(c-C_3H_5)CH_2	1.8	1900	0.4	70	0/5	0.55	0.37	66	0.8
2h	(c-C_4H_7)CH_2	0.2	550	0.23	45	0/6	0.68	0.57	47	2.58
2i	(c-C_5H_9)CH_2	1.2	630	0.4	29	2/6	0.53	—	—	—
3a	c-C_3H_5	7.4	2900	0.85	—	—	—	—	—	—
3b	(c-C_3H_5)CH_2	4.5	1000	0.75	53	2/6	0.54	—	—	—
3c	c-C_4H_7	1.7	1100	0.35	45	1/6[g]	0.86	—	—	—
3d	(c-C_4H_7)CH_2	0.75	740	0.47	23	0/6	0.64	—	—	—
4a	c-C_3H_5	19	10000	0.75	—	5/6	—	—	—	—
4b	(c-C_3H_5)CH_2	2.1	6100	0.79	25	1/6[g]	0.65	0.59	136	2.14
4c	c-C_4H_7	10	6300	1.11	—	—	—	—	—	—
4d	(c-C_4H_7)CH_2	3.9	1600	0.92	—	—	—	—	—	—

[a]See ref 15.
[b]Concentration of compound required to double the human activated partial thromboplastin time.
[c]See ref 16.
[d]Human plasma.
[e]Occlusions at 10 μg/kg/min iv infusion.
[f]Arithmetic average at the end of the infusion.
[g]One reflow.

The X-ray crystal structure **2g** bound in thrombin's active site is shown in Figure 1 and it confirmed the essential features of the modelling.[17] The aminopyridine and the pyridinone bind in the expected manner.[2] There is a long hydrogen bond (3.5 Å) between one of the sulfone oxygens and the OH of the insertion loop tyrosine, a hydrogen bond between the other sulfone oxygen and the 3-amino group, and the cyclopropyl group occupies the 'distal binding pocket'. The energetic contribution of the sulfone hydrogen bonds is impossible to determine independent of the other factors which affect binding. However, it is interesting to note that there is a large difference in potency between benzylsulfone **2a** and its benzylthioether analogue (1.6 vs 79 nM, respectively).[4]

The 4-aminosulfonylpyridinones **3a** and **3c** are less potent in the enzyme assay than their alkylsulfone counterparts **2g** and **2h**. However **3c** (2×APTT = 0.35 μM) is efficacious in the rat, as is its homologue **3d**. The constraint imposed on the side chain of derived thiadiazines **4a** and **4c** significantly affects their potency. Insertion of a methylene to compensate for this constraint (**4b** and **4d**) improves the activity against the enzyme in each case. Compound **4b** was studied in further depth and it retains the selectivity of

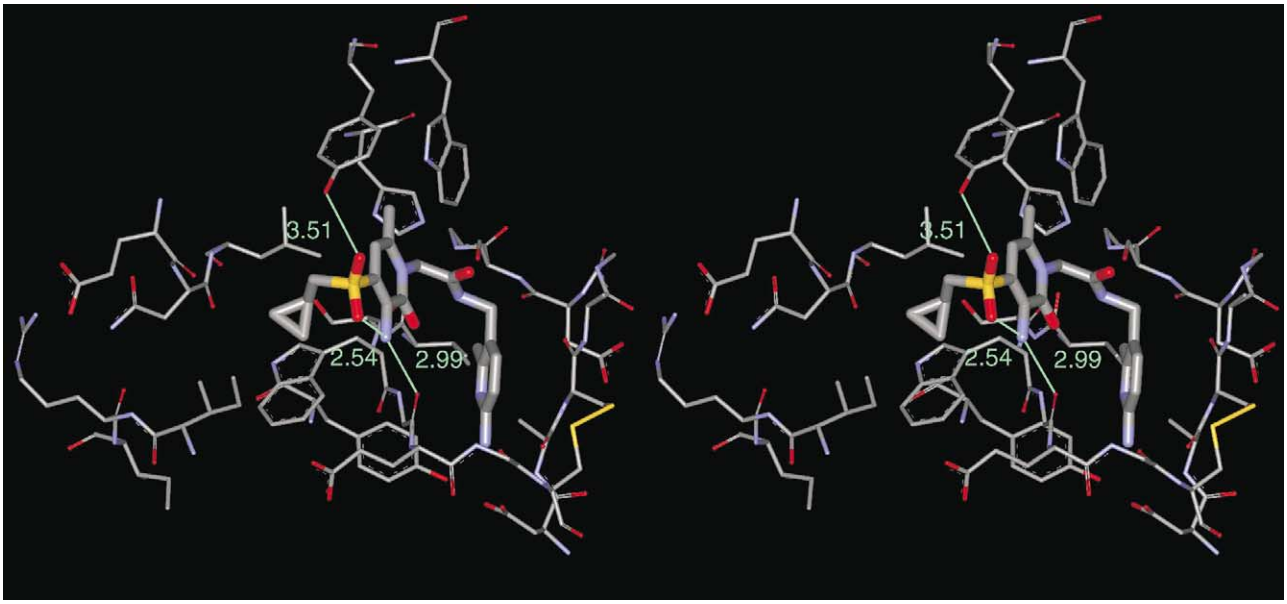


Figure 1. The crystal structure of **2g** in the thrombin active site.

2h and is efficacious in the rat. Furthermore, while the half-lives of sulfones **2a**, **2g** and **2h** were between 47 and 66 min after oral administration to dogs, **4b** had a longer half-life (136 min) with an AUC of 2.14 μM h.

In summary, we designed and synthesized a series of highly potent and efficacious 3-amino-4-sulfonylpyridinone acetamide thrombin inhibitors. The functionally dense sulfone stabilizes the aminopyridinone, conformationally constrains the 4-substituent via a hydrogen bond to the adjacent amino group, and hydrogen bonds to the insertion loop tyrosine. We prepared a related series of bicyclic dihydrothiadiazinedioxides from the corresponding 4-aminosulfonylpyridinones. The cyclopropylmethyl derivative **4b** is efficacious in rats and has improved pharmacokinetics in dogs after oral dosing compared to the 4-sulfonylpyridinones **2** and bicyclic lactams **5**, making the pyridothiadiazine template an attractive, achiral alternative to the bicyclic lactam template.

Acknowledgements

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