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Novel Bicyclic Lactam Inhibitors of Thrombin: Highly Potent and Selective Inhibitors

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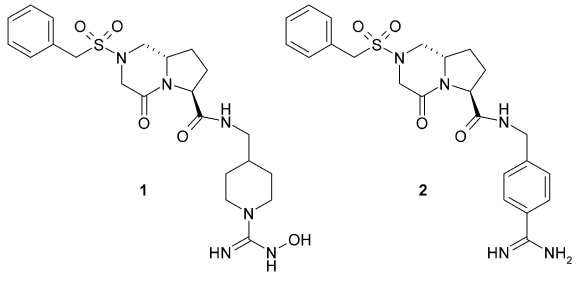
Abstract—The potency and selectivity of a previous series of low molecular weight thrombin inhibitors were improved through modifications of the P1 and P3 residues. Introduction of diphenyl substituted sulfonamides in the P3 moiety led to highly efficacious compounds. By correctly selecting the combination of P1 and P3 residues, high levels of potency, selectivity and in vivo efficacy were obtained.

The development of low molecular weight thrombin inhibitors has been an active area of research due to the many limitations of anticoagulants that are approved for the treatment of thrombosis and related diseases. An ideal inhibitor should be potent, highly selective and bioavailable. It should also involve minimum levels of bleeding and provide predictable levels of coagulation, which would translate into a reduced need to monitor patients.

We have recently reported a series of bicyclic lactam based low molecular weight thrombin inhibitors displaying low nanomolar potency, high selectivity and in vivo efficacy (**1** and **2**).[1] Despite the attractive biological profile of these inhibitors, they did not display high levels of oral absorption (F ~0.5%)[2] which was a key requirement of the program. In this paper, we report further optimization of inhibitors using novel P3 and P1 residues (Table 1), in an attempt to optimize for improved bioavailability.



The syntheses of the bicyclic template **6**[3] and of P1 moieties **P**, **Q**, **S**, **V**, **W** and **Z**,[1] as well as **X** and **Y**,[4] (Table 1) have been published elsewhere. P3 moieties **A** and **B** are commercially available as acids. The coupling methods of the P3 and P1 moieties to **6** have already been reported,[1,3] except for those described below.

The diphenylethylsulfonamide **C**, diphenylmethyl-sulfonamide **D**, and phenylethylsulfonamide **E** were prepared according to Scheme 1, eqs 1, 2, and 3, respectively. Thus, 2,2-diphenylethanol **3** was first converted to thioacetate **4** by a Mitsunobu reaction. The thioacetate was then hydrolyzed and oxidized in one pot using 30% H_2O_2 in acetic acid. The sulfonic acid thus obtained was converted to the sulfonyl chloride **5** using thionyl chloride in DMF (Scheme 1, eq 1). A similar approach was attempted to prepare the diphenylmethylsulfonyl chloride, but failed to yield the desired product. An alternative, shorter sequence was used, based on the method of Kloosterziel et al.[5] Diphenyldiazomethane[6] was mixed with bicyclic amine **6** in ether, and SO_2 was bubbled through the solution to give sulfonamide **7** in good yield (Scheme 1, eq 2). The purity of the

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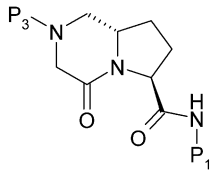
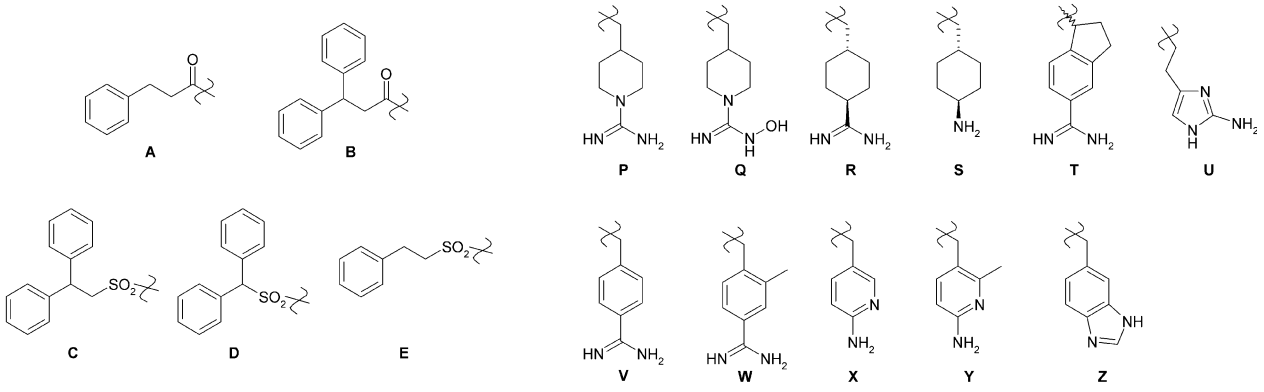
Table 1. In vitro and in vivo data



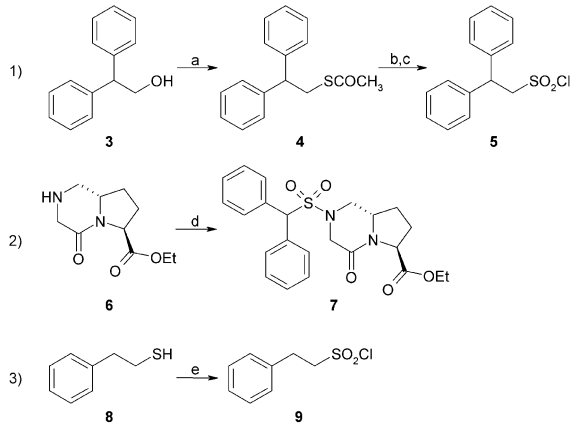
Compd[a]	P_3	P_1	K_{ithr} (nM)	Sel[b]	Rat arterial thrombosis model[c]			% Bio
					MOT[d]	aPTT[e]	TT[f]	
21	**A**	**P**	180	nd[g]	nd	nd	nd	
22a	**B**	**P**	80	125	21±1	29±2	85±10	
22b	**B**	**Q**	25	306	35±16	23±2	63±8	
23a	**C**	**P**	8	471	36±10	35±2	214±25	2.2
23b	**C**	**Q**	2	921	45±14	26±3	148±14	0.7
23c	**C**	**S**	5	>2174	37±14	35±4	150±19	1.5
23d	**C**	**V**	1	24	>60	74±14	636±100	
23e	**C**	**X**	35	137	30±18	19±1	51±1	
23f	**C**	**Y**	48	354	nd	nd	nd	
24a	**D**	**P**	9	38	52±9	28±1	154±5	
24b	**D**	**Q**	2	469	46±17	28±7	230±45	1.9
24c	**D**	**S**	5	2000	>60	44±2	216±13	
24d	**D**	**V**	1	65	51±11	54±7	631±123	
24e	**D**	**W**	2	38	>60	42±4	354±73	
24f	**D**	**Y**	10	1214	>60	27±2	102±7	
24g	**D**	**Z**	75	11	nd	nd	nd	
24h	**D**	**U**	135	9	nd	nd	nd	
24i	**D**	**R**	2	189	44±10	54±8	724±94	0.1
24j	**D**	**T**	75	37	nd	nd	nd	
25	**E**	**Q**	14	200	49±13	25±1	146±36	2.7

[a]All new target compounds were characterized by ^{1}H NMR, reverse-phase HPLC and mass spectroscopy.
[b]Selectivity: $K_{i\ trypsin}/K_{i\ thrombin}$.
[c]Dose: intravenous bolus dose (0.75 mg/kg) followed by an infusion (50 μg/kg/min).
[d]Mean occlusion time in min (control: 17 to 19 min).
[e]Activated partial thromboplastin time in seconds (control: 20 to 22 s).
[f]Thrombin time in seconds (control: 40 to 45 s).
[g]nd, Not determined.

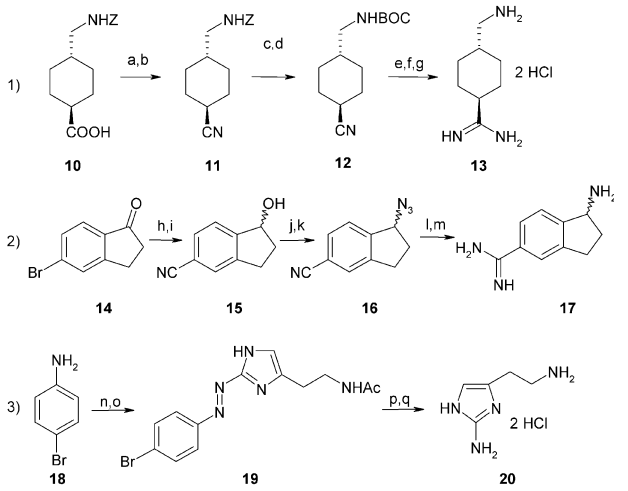


diphenyldiazomethane was very important for the reaction to proceed in high yield.[7] Finally, phenylethyl mercaptan **8** was converted to phenylethylsulfonyl chloride **9** by oxidation with SO_2Cl_2 and KNO_3 (Scheme 1, eq 3).[8]

We describe in Scheme 2 the preparation of P1 moieties **R**, **T**, and **U** (eqs 1, 2 and 3, respectively). Protected methylaminocyclohexyl-carboxylic acid **10** was first converted into an amide, which was then dehydrated to nitrile **11**. The CBZ protecting group was then exchanged for a BOC group, and nitrile **12** was converted into amidine **13** through a three-step sequence involving the formation of the amidoxime, the hydrogenolysis of its *O*-acetate [9] and the removal of the BOC group (Scheme 2, eq 1). In eq 2, bromo-indanone **14** was converted to cyanoalcohol **15** by treatment with CuCN, and subsequent reduction of the resulting ketone to the alcohol with $NaBH_4$. Alcohol **15** was mesylated followed by displacement with NaN_3 to form azide **16**. The nitrile group was further transformed into the amidine,[10] and the azide was reduced to amine **17** through hydrogenolysis. Aminohistamine **20** was prepared by the diazotization of histamine acetate.[11] Azo compound **19**



Scheme 1. (a) Ph_3P, DIAD, CH_3COSH, THF, 82%; (b) H_2O_2, CH_3CO_2H, 70 °C, 100% (**CAUTION**: a shield must be used at all times during the reaction and evaporative workup); (c) $SOCl_2$, DMF, 50%; (d) diphenyldiazomethane, SO_2, Et_2O, 85%; (e) SO_2Cl_2, KNO_3, MeCN, quant.



Scheme 2. (a) isopropyl chloroformate, NMM, THF, −20 °C, 1 h then NH_3 45 min, 100%; (b) $SOCl_2$, NMM, DMF, 0 °C, 90%; (c) H_2, Pd/C 10%, MeOH; (d) $(BOC)_2O$, CH_2Cl_2, 88% (two steps); (e) $NH_2OH{\cdot}HCl$, Na_2CO_3, EtOH, Δ, 13%; (f) Ac_2O, H_2, Pd/C 10%, AcOH, 97%; (g) 4 N HCl in dioxane, 100%; (h) CuCN, *N*-methylpyrrolidone, 150 °C, 18 h, 59%; (i) $NaBH_4$, MeOH, THF, −78 °C (1 h), then rt (1 h), 84%; (j) $SOCl_2$, CH_2Cl_2, 0 °C, 50%; (k) NaN_3, DMF, 60 °C, 71%; (l) HCl gaz, EtOH, 0 °C, 48 h, then NH_3 gaz, EtOH, 18 h; (m) H_2, Pd/C 10%, MeOH, 100% (two steps); (n) $NaNO_2$, 2.3 N HCl, 0 °C; (o) *N*-Ac-histamine, Na_2CO_3, then 1 N NaOH, 30%; (p) H_2, PtO_2, EtOH, 81%; (q) 6 N HCl, Δ, quant.

was then hydrogenolyzed, and the acetate group was hydrolyzed with 6 N HCl (Scheme 2, eq 3). The two amidino-amines **13** and **17**, as well as aminoimidazole-amine **20**, were coupled using the previously reported procedure[1,3] to the diphenyl-sulfonyl bicyclic template without any protection of their basic functional groups.

Inhibition of the amidolytic activity of thrombin (K_i) and in vivo coagulation parameters in the rat arterial thrombosis model, such as the mean occlusion time (MOT), the activated partial thromboplastin time (aPTT) and the thrombin time (TT), were measured according to an already published procedure.[12] Oral bioavailability (%F) was determined in conscious rats by comparing the areas under the curves for TT values versus time following administration of compounds by oral (30 mg/kg) and iv bolus (0.15 mg/kg) routes.

We have previously demonstrated that the replacement of the P3 acetamides with the sulfonamides yielded generally a 2- to 3-fold increase in potency.[3] The addition of a second phenyl ring in P3 also improved the potency by 2-fold (cf., **21** with **22a** in Table 1). It is interesting to note that the S3 subsite of thrombin is capable of accommodating large hydrophobic groups.

The above results prompted us to explore the possibility of preparing diphenyl substituted sulfonamides. Inhibitors **23a** and **24a** were prepared and found to be considerably potent. These two compounds not only exhibited good binding to thrombin but also displayed good efficacy in vivo, with a significant extension of the mean occlusion times.

We then decided to turn our attention to the P1 moiety, keeping both sulfonamides **C** and **D** constant. The goal was to reduce the basicity of the guanidine of P1 moiety **P**, while retaining its ability to form H-bonds with Asp189 at the bottom of the S1 subsite of thrombin. Substituents **Q**, **S**, **V**, **W**, and **Z** had already been used in our initial exploration.[1] As expected, substituent **Q** improved the binding of inhibitors **22b**, **23b**, and **24b** by a factor of four, and also demonstrated a good efficacy in vivo. Residue **W** was prepared in order to regain the selectivity lost with **V**. By adding a more hydrophobic substituent to the phenyl ring, it was hoped that favorable interactions would be gained in the S1 subsite of thrombin. However, even with the more bulky moiety **T** (**24j**), the selectivity over trypsin was not comparable to the compounds with the cyclohexyl or piperidine residues. Other less basic aromatic substituents, such as **U** and **Z** (**24g** and **24h**, respectively), were not potent enough and did not exhibit a significant increase in selectivity to be considered for in vivo testing. However, compounds **23e**, **23f**, and **24f** showed a high level of selectivity. Indeed, the size of these two residues should be comparable (if not smaller) to the phenylamidines.

Selected potent compounds were tested for oral bioavailability. Unfortunately, only modest levels of absorption were observed.

We have demonstrated that the S3 subsite of thrombin can accommodate large substituents such as the diphenyl carboxamides and sulfonamides in this series of inhibitors. By carefully choosing the combination of P1 and P3 residues attached to the central bicyclic template, high levels of potency, selectivity and in vivo efficacy could be obtained. Attempts to reduce the overall polarity of the molecules by increasing the hydrophobicity of the P3 moiety and reducing the basicity of the P1 moiety failed to render the inhibitors orally bioavailable.

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