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DIARYLSULFONAMIDES AS SELECTIVE, NON-PEPTIDIC THROMBIN INHIBITORS[1]

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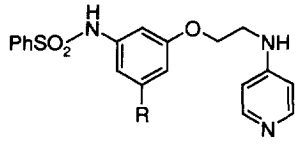
Abstract: Based on the structures of aminopyridine thrombin inhibitors (**1**), a series of aminoalkyl- and guanidinoalkyl-substituted diarylsulfonamides were prepared. The most potent derivative, *N*-[3-(4-guanidinobutoxy)-5-methyl-phenyl]-benzenesulfonamide (**6c**) had Ki = 0.18 μM for thrombin and did not inhibit trypsin, plasmin, or factor Xa. Comparison of the X-ray structures of the thrombin / **1b** and the thrombin / **6c** complexes revealed important aspects which govern the binding of such diarylsulfonamides to thrombin.

Introduction

Thrombin occupies a central position in the blood coagulation cascades[2] and has therefore become the primary target for the development of inhibitors as novel anticoagulants.[3,4] The best studied inhibitors are derived from the peptide sequence D-Phe-Pro-Arg.[5] Recently, we discovered a structurally unrelated class of inhibitors, i.e. the diarylsulfonamides **1**.[6]



	R
1a	H
1b	Me

The X-ray structure of the thrombin / **1b** complex revealed that the pyridine residue resides in the S1 pocket of thrombin.[7] It was thought to be responsible for the selectivity of these inhibitors (Table 1) but seemed to

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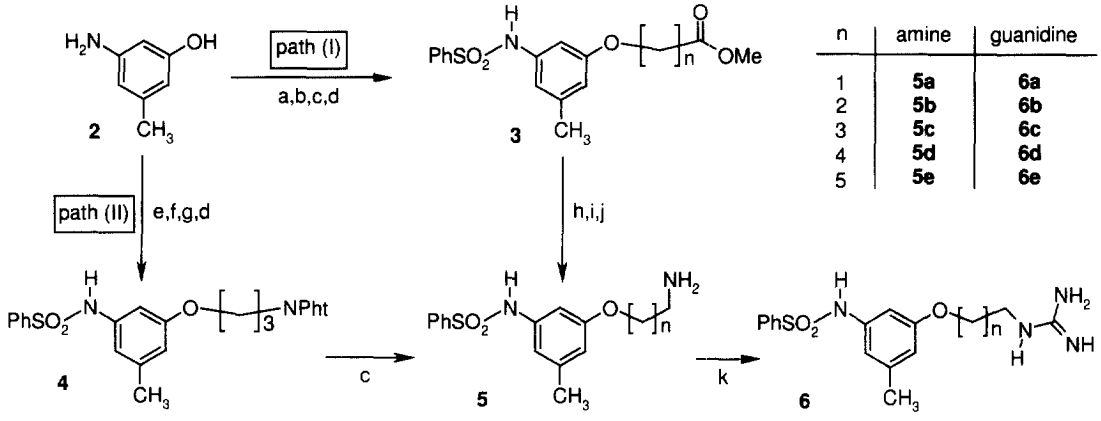
contribute little to the total binding energy.[6] In order to test whether the potency of **1b** can be improved further, we replaced its alkylaminopyridine moiety by the P1 residue of the conventional D-Phe-Pro-Arg derived inhibitors, i.e. by guanidinoalkyl residues.

Results and Discussion

Several aminoalkyl (**5a-e**) and guanidinoalkyl (**6a-e**) based analogues of **1b** were synthesized in order to determine the ideal spacer length between the basic moiety (P1 residue) and the central aryl fragment (P2 residue). The synthetic routes leading to **5a-e** and **6a-e** are depicted in Scheme 1. Starting with 3-amino-5-methyl-phenol (**2**), compounds **5a** and **5c-e** were prepared in 7 steps following path (I). This method failed for compound **5b**, since the reaction of the phthalimido-protected **2** with bromopropionate (step b) led to tarry material only, most probably due to elimination of HBr followed by polymerization of the resulting acrylic ester. Hence, compound **5b** was synthesized in 5 steps according to path (II). The guanidinoalkyl derivatives (**6a-e**) were obtained from their amine precursors (**5a-e**) upon guanylation using S-methylisothiouronium sulfate (overall yield 7-45%).

Scheme 1: Synthesis of the aminoalkyl (**5a-e**) and guanidinoalkyl (**6a-e**) analogues of **1b**



(a) phthalic anhydride, CH_3COOH, reflux, 4 h, 75%; (b) $Br(CH_2)_nCO_2Et$ (n = 1, 3, 4, 5), K_2CO_3, DMF, 130°C, 3 h, 63-93%; (c) N_2H_4-H_2O, EtOH, r.t., 3 h, 81-99%; (d) $PhSO_2Cl$, NEt_3, CH_2Cl_2, 0°C, 1 h, 85-98%; (e) $(Boc)_2O$, CH_2Cl_2, NEt_3, 5°C, 1h, 95%; (f) NaH, phthalimide-N-$(CH_2)_3$-O-Tos, THF, 50 °C, 16 h, 41%; (g) CF_3COOH, r.t., 1 h, 89%; (h) KOH, EtOH, 90°C, 2 h, 66-96%; (i) $ClCO_2CH_2CHMe_2$, N-methyl morpholine, CH_2Cl_2, NH_3/MeOH, -40°C, 0.25 h, 64-84%; (j) $LiAlH_4$, THF, 60°C, 5 h, 30-84%; (k) S-methylisothiouronium sulfate, EtOH abs., reflux, 16 h, 63-93%.

Table 1 compares the results of the amines **5**, the guanidines **6**, and the 4-aminopyridines **1** in the inhibition assays. The amines **5** proved to be less potent when their inhibition constants, Ki, are compared to those of the analogous guanidines **6**. This reflects the preference of thrombin for arginine over lysine in substrates, and has been noted in D-Phe-Pro-Arg derived inhibitors as well.[8] However, this trend becomes less pronounced with increased chain lengths, and the compounds with the longest chains (hexyl; **5e**, **6e**) are almost equipotent. The most potent compounds in each series are those with an intermediate chain length (butyl; **5c**, **6c**), which presumably allows optimized binding both in the S1 and S2 pocket of thrombin. The results of the thrombin-induced plasma coagulation assay confirm the inhibition constants. However, the differences in potencies (expressed as ratios of the concentrations needed to double the thrombin time, IC_{200}) tend to be smaller. Whereas the inhibition constants suggest that the guanidinobutyl derivative **6c** is 3.5 times more potent than the guanidinopropyl derivative **6b**, the compounds are equipotent in the thrombin time assay. This slight discrepancy between the inhibition constants and the results of the coagulation assay may be attributed to nonspecific plasma protein binding which in turn reduces effective inhibitor concentrations. A similar trend was observed previously in the 4-aminopyridine derived thrombin inhibitors.[9] This interpretation fails, however, when Ki and IC_{200} values of compounds from different series are compared. For instance, the guanidinoalkyl derived inhibitor **6c** is twice as potent as the aminopyridine based compound **1a** (in terms of Ki) but it shows only $1/5^{th}$ of the activity in the coagulation assay, while the lipophilicity of **1a** is higher by one order of magnitude. This comparison indicates that effects other than lipophilicity may also play a role.

The selectivity profile of **6c** is similar to that of the parent aminopyridine **1b** (no inhibition of trypsin, plasmin and factor Xa: Ki > 500 μM each, see Table 1). The anti-thrombin activity, however, is one order of magnitude lower. The reason for this is illuminated by comparison of the X-ray structures of **1b**[7] and **6c** (resolution: 2.8 Å; R-value: 26.2%, space group: $P4_12_12$) in thrombin. Figure 1 shows a stereo view of the thrombin / **1b** complex[7] superimposed with **6c** from the thrombin / **6c** structure. The thrombin conformation in the active site is almost identical in both structures. As expected the inhibitors bind to thrombin in a similar manner. The basic moieties (**1b**: aminopyridine; **6c**: alkylguanidine) interact with the enzyme's recognition site (S1), each forming two approximately equidistant salt bridges to Asp189 (**1b**: 3.6 Å, 3.8 Å; **6c**: 3.0 Å, 3.3 Å) at the bottom of the specificity pocket. The central tolyl fragments occupy the hydrophobic S2 site and the aryl-sulfonyl moieties extend into the aryl binding site (S4), in which the phenyl group arranges in a perpendicular orientation with respect to Trp215. The tolyl fragment of **1b** binds perfectly in the hydrophobic pocket. The importance of this interaction becomes apparent when the inhibition constant, Ki, of **1b** is compared to that of **1a**, which lacks the methyl group. The P2/S2 interaction of **6c** is much less ideal. Here, the central phenyl group seems to be pushed downward by about 1 Å into the direction of the Trp215-Gly216-Ser217 sequence which forms the bottom of the S2 site. This leaves some unfilled space in the S2 site between enzyme and inhibitor.

Table 1: Pharmacological and analytical data of the aminoalkyl (**5a-e**) and guanidinoalkyl derivatives (**6a-e**)

	inhibition constants: Ki [μM][a]		*thrombin time:*	*lipophilicity*	*^{13}C-NMR (dmso-d_6):*	*EI-MS [m/z]:*
	thrombin	*trypsin/plasmin/factor Xa*	*IC_{200} [μM]*[b]	*log$P_{calc.}$*[c]	*δ [ppm]*	(M^+)
1a	0.30	> 500	2.3	3.47	-	-
1b	0.023	> 500	0.29	3.97	-	-
5a	240	> 500	>250	2.31	21.3; 48.5; 69.0; 103.4; 106.9; 113.5; 126.3; 128.3; 130.7; 137.9; 138.1; 139.6; 158.7	306
5b	170	> 500	>250	2.28	21.2; 38.3; 44.5; 65.7; 104.2; 110.1; 113.3; 126.6; 128.9; 132.1; 138.2; 139.1; 139.7; 159.2	320
5c	6.5	> 500	179	2.81	21.2; 25.9; 27.2; 59.6; 66.8; 103.3; 108.8; 112.8; 126.4; 128.8; 131.8; 138.8; 139.0; 141.4; 158.8	334
5d	9.9	> 500	>250	3.34	21.3; 22.5; 22.6; 26.7; 38.1; 67.0; 103.4; 110.5; 112.6; 126.7; 129.3; 132.9; 138.7; 139.6; 139.7; 159.0	348
5e	9.0	> 500	>250	3.87	20.2; 21.3; 25.0; 25.6; 26.9; 38.7; 67.1; 103.4; 110.5; 112.5; 126.7; 129.3; 132.9; 138.7; 139.6; 139.7; 159.0	362
6a	2.0	> 500	38	1.69	21.5; 38.0; 65.3; 103.7; 104.3; 114.9; 126.2; 129.3; 129.8; 137.4; 137.9; 139.6; 157.1; 158.3	348
6b	0.63	> 500	10	1.44	21.4; 28.4; 37.9; 63.8; 103.7; 104.3; 114.4; 126.0; 127.9; 129.3; 137.3; 138.1; 140.0; 156.8; 158.5	362
6c	0.18	> 500	10	1.97	21.3; 25 8; 26.3; 40.5; 66.9; 103.4; 110.4; 112.6; 126.7; 129.3; 132.9; 138.7; 139.6; 139.7; 157.0; 158.9	376
6d	6.4	> 500	188	2.50	21.2; 22.6; 25.2; 28.1; 40.0; 67.1; 103.3; 110.2; 112.2; 126.6; 129.0; 132.5; 138.8; 139.2; 139.7; 156.9; 158.9	390
6e	7.0	> 500	237	3.03	21.3; 25.1; 25.8; 28.4; 28.5; 40.7; 67.2; 103.4; 110.5; 112.5; 126.7; 129.3; 132.9; 138.7; 139.6; 139.7; 157.0; 159.0	404

[a] Inhibition constants were determined in phosphate buffer, pH 7.5, 25°C, as described in: von der Saal, W.; Engh, R. A.; Eichinger, A.; Gabriel, B.; Kucznierz, R.; Sauer, J. *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 73.

[b] IC_{200} is the concentration which causes a doubling of the thrombin clotting time of human plasma. IC_{200} values were determined using the equation $y = a + b \times x^c$ where y is the experimental coagulation time [sec], x is the concentration of the inhibitor [μM], a is the coagulation time in the absence of the inhibitor, b and c are parameters which determine the shape and the location on the x-axis of the coagulation time / concentration curve. These parameters were determined from the experimental coagulation times using a nonlinear regression with the starting values b = 10 and c = 0.65.

[c] LogP values were calculated using the CLOGP program.

Simultaneously, the sulfonamide group has moved about 2.5 Å towards Gly216, and its amino group is now within hydrogen bonding distance of the carbonyl group of Gly216. This type of interaction is common to most thrombin inhibitors of the D-Phe-Pro-Arg type,[10] but is generally more tight (2.8 Å) than in the present case (3.6 Å). Thus, it cannot compensate for the lost hydrophic interaction in the S2 pocket, which is most probably the cause of the reduced inhibitory potency of **6c** compared to **1b**. Another unfavourable aspect of the novel thrombin inhibitors is the flexibility of the alkyl chains of **5** and **6** compared to their more rigid counterpart in **1**, since becoming fixed in the thrombin inhibitor complex is entropically unfavourable. In the **6c** / thrombin complex the C4 chain is disordered, i.e. the flexibility is not completely lost. However, a molecular modeling study showed that it is impossible to fix the guanidino group and the central tolyl group in their respectively optimized positions[11] and simultaneously connect them via an alkyl chain which has all carbon atoms in staggered conformations.

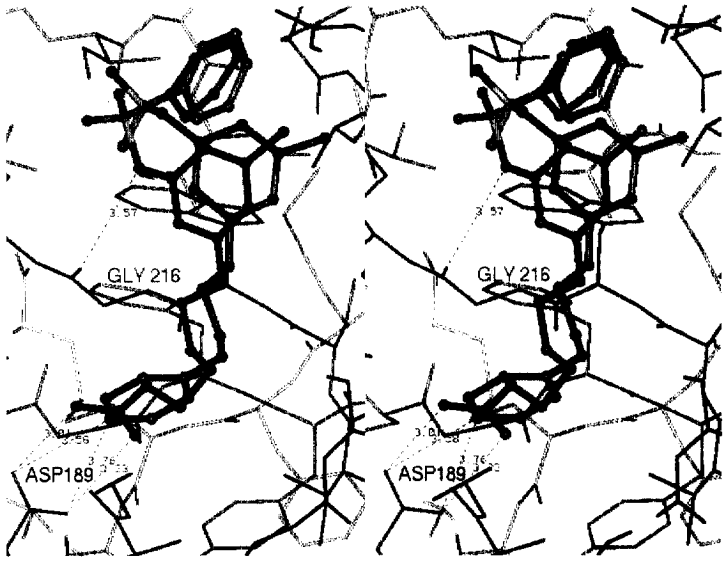


Figure 1: Superposition of the X-ray structures of **1b** (brown) and **6c** (green) in thrombin

Conclusion

The series of aminoalkyl (**5**) and guanidinoalkyl (**6**) derivatives of the diarylsulfonamide thrombin inhibitors **1** highlight several important features which govern the potencies of this recently discovered novel class of anticoagulants. (i) The chain lengths of the amino- and guanidinoalkyl groups play a crucial role, with

an optimum at C4 (**5c**, **6c**). (ii) Concomitant with the substrate preference of arginine over lysine, the guanidines (**6**) are more potent than the amines (**5**). (iii) Even the most potent compound of the series presented here, i.e. **6c**, is an order of magnitude less potent than the aminopyridine analogue **1b**. The X-ray structures of the thrombin / **1b** and thrombin / **6c** complexes revealed that the central moiety of **6c** binds less favourably than that of **1b**, most probably because optimal binding of both the guanidino group at the bottom of the S1 pocket and the tolyl group in the S2 pocket cannot be realized with alkyl chains connecting these two crucial residues.

References and Notes

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11. For the modelling study, the guanidino group was fixed in the position it occupies in the thrombin / **6c** complex, and the central tolyl group was fixed in its position of the thrombin / **1b** complex. Both residues were connected via a butyl chain.