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NOVEL ACYLGUANIDINE CONTAINING THROMBIN INHIBITORS WITH REDUCED BASICITY AT THE P_1 MOIETY

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Abstract: Replacement of the noragmatine group in thrombin inhibitors with a ß-alanyl-guanidine group resulted in a nearly equipotent and more selective compound **8** despite the fact that the pK_a of this P_1 moiety is five orders of magnitude lower. Further modification resulted in a nonpeptide inhibitor with this ß-alanyl-guanidine group, compound **28**. This is an active and selective thrombin inhibitor and in view of its nonpeptide/low basicity structure selected for further pharmacological studies.

Introduction

In the search for novel antithrombotics thrombin is a major target. The ultimate goal is to find an orally bioavailable compound.[1] The serine protease thrombin expects an arginine as P_1 moiety in its specificity pocket and so most thrombin inhibitors contain a guanidine or amidine moiety.[2] It is generally accepted that these highly basic moieties are also main barriers for oral absorption.[3] A second barrier for oral bioavailability is the peptidic character in the remaining part of the molecule (P_2-P_3 moiety). Our strategy was to find a less basic alternative for this guanidine/amidine moiety and, after that, investigate nonpeptide mimics for the remaining part of the lead molecule. Although several classes of inhibitors exist—peptidomimetics, nonpeptide-TAPAP and NAPAP analogs, and heterocycles—it remains very difficult to replace the basic moiety by a less basic one.[4] In some cases it appears that nonbasic P_1 moieties still result in active thrombin inhibitors but at the expense of in vivo potency. Several reports have now appeared describing this relationship between logP, protein binding, and antithrombotic potency.[5] Our focus has been to generate and evaluate guanidine, benzamidine, and amine isosteres with pK_a's ranging from 6 to 11. In the present study, we used an example of the agmatine-based thrombin inhibitor series, Inogatran[6] (**1**), as a starting point for such P_1 modifications. Novel P_1 substituents that vary in basicity, size and H-bonding capacity have been incorporated in this lead structure.

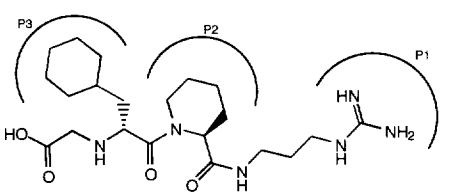

Figure 1. Compound **1** structure and activity.

Measured	Literature[6]
IC_{50} thrombin (IIa) = 1.3 uM	K_i = 15 nM
IC_{50} trypsin = 1.5 uM	K_i = 675 nM

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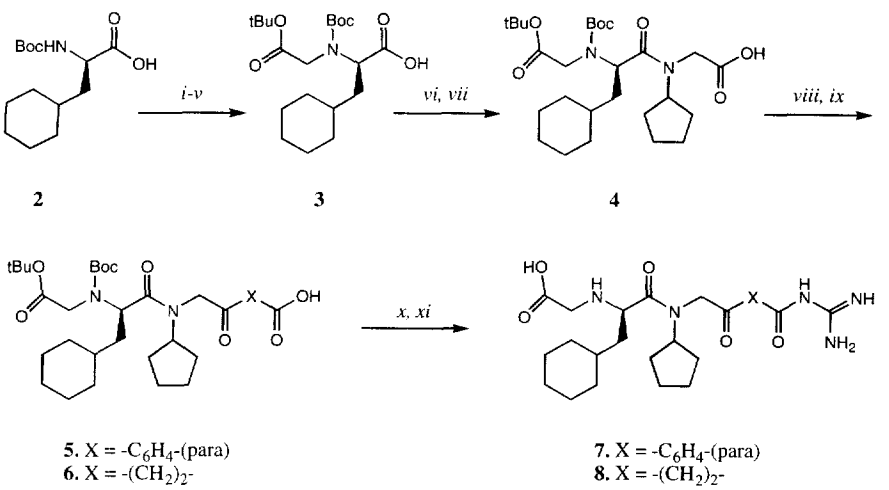


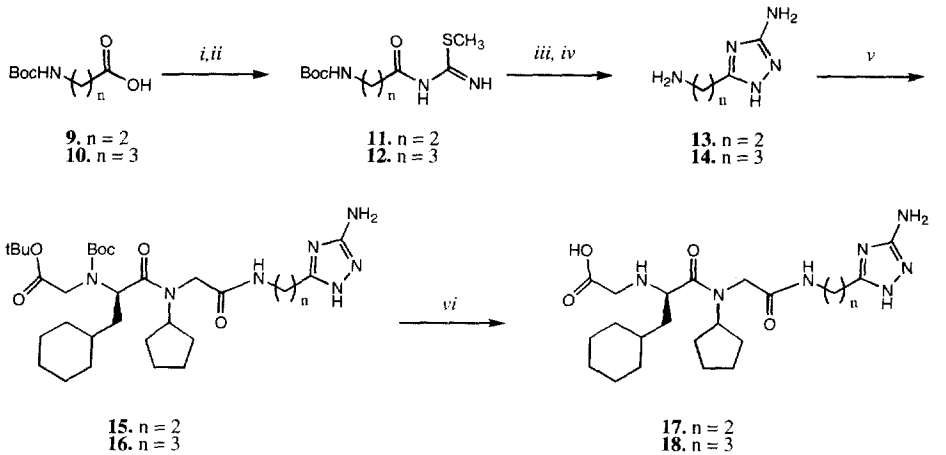
Scheme 1. (i) CH_2Cl_2/MeOH/TBTU/NET_3/rt (95%); (ii) TFA/CH_2Cl_2/rt (quant); (iii) CH_3CN/*t*-Butylbromoacetate/ DIPEA/50 °C (80%); (iv) Boc_2O/NEt_3/MeOH/rt (95%); (v) Dioxane/water/NaOH/rt (quant); (vi) DMF/TBTU/NEt_3/ N-cyclopentyl-Gly-OMe.HCl[7]/rt (61%); (vii) Dioxane/water/NaOH/rt (quant); (viiia) *para*-H_2N-C_6H_4-COOMe/DCC/ Camphorsulfonic acid/CH_2Cl_2/rt (70%); (viiib) H_2N-$(CH_2)_2$-COOMe.HCl/ DMF/NEt_3/TBTU/rt (95%); (ix) Dioxane/ water/NaOH/rt (quant); (x) HCl.H_2N-C(=NH)NHBoc/TBTU/NEt_3/DMF/rt (75%); (xi) TFA/ CH_2Cl_2/rt followed by prep. HPLC-purification (Yield: 30 and 85%, respectively).

Chemistry

The synthesis of the crucial acylguanidine containing inhibitors is outlined in Scheme 1. The alkylated peptide building block **4** can be prepared in seven steps all with high yields. This was followed by either a coupling with *para*-amino benzoic acid methyl ester or the methyl ester of ß-alanine. Subsequently, the crucial conversion to the acylguanidine moieties with mono-boc protected guanidine (*x*) and finally deprotection and purification yielded the end-products **7** and **8**. Scheme 2 outlines the synthetic route towards the aminotriazole compounds **17** and **18.** Here, the crucial step is the S-methyl thiourea derivatisation with the appropriate acids (*ii*). Cyclisation with hydrazine and deprotection yielded intermediates that could be coupled with building block **4** (see Scheme 1). All other compounds were prepared analogous to Scheme 1. The dipeptide mimics described in Table 2 have already been described in the thrombin literature and follow essentially the route of Scheme 1.

Results and Discussion

All compounds were evaluated for their thrombin inhibitory potency and the selectivity was established by measuring the trypsin and factor Xa inhibition.[8a] The results are summarized in Tables 1 and 2. All compounds mentioned in these tables showed IC_{50} values for factor Xa of more than 100 uM and are not reported separately. In view of the reported thrombin inhibitory activity of Inogatran (**1,** K_i = 15 nM, corresponding to an IC_{50} of 1.3 µM in our assay[8b]) this compound underwent additional optimisation. The reason for this was the expectation that less basic P_1 isosteres would result in somewhat less in vitro activity. The result of this drug design cycle was compound **19** (see Table 1) in which the pipecolic acid moiety was replaced by *N*-cyclopentyl-glycine.



Scheme 2. (i) DCC/HO-Suc/CH_2Cl_2/0 °C; (ii) H_2N-C(=NH)SCH_3/CH_2Cl_2/MeOH/rt (78%); (iii) H_2NNH_2.HCl/EtOH/Pyridine/rt (56%); (iv) TFA/CH_2Cl_2/rt (quant); (v) DMF/NEt_3/IBCF/**4**/-15 °C; (vi) TFA/CH_2Cl_2/rt followed by prep. HPLC purification (52%).

The activity was more than tenfold improved compared to the lead compound. Surprisingly, an already optimized compound could still be changed into a more potent one. In the trade-off between intrinsic potency and improved pharmacokinetics we had now obtained a better starting position. Compound **19** was also co-crystallized with thrombin (Figure 2). The crystal symmetry was determined to be P43212 (a = b = 88.4, c = 197.4) and diffracted to 2.7 Å. The arginine analog binding in the S_1 site was first modelled based on the D-Phe-Pro-Arg chloromethylketone inhibited structure of human thrombin; the arginine side chain nearly fitted in the difference density after superimposing the two thrombin structures. However, in later refinement cycles, an altered binding geometry fitted the density better (Figure 2). The two binding geometries are reminiscent of the two (symmetric and asymmetric) binding modes seen in benzamidine based inhibitors.[9] Here, the symmetric binding mode is seen in the D-Phe-Pro-Arg inhibited thrombin, while a different pattern of torsion angles in inhibitor **19** rotates the guanidino group 180 degrees from the symmetric binding mode. This rotation orients the N_ε-H bond away from the 219 CO group and toward the water molecule above the Tyr228 ring.

The peptide amino group linking the P_1 and P_2 equivalent groups forms a hydrogen bond to Ser214CO, while the cyclopentane occupies the S_2 site. Compared to D-Phe-Pro-Arg inhibited thrombin, the Ser214-Trp215 peptide plane is rotated somewhat, and the insertion loop containing Trp60D and Tyr60A is shifted by more than one Å to accommodate the larger residue of **19**. The main chain amino and carbonyl groups of the P_3 equivalent residue, cyclohexylalanine, form the antiparallel hydrogen bonds to Gly216 as is typical for serine proteinase inhibitors, and the cyclohexyl moiety occupies the 'aryl' binding site as well. The acid group at the N-terminus forms a hydrogen bond to the peptide NH of Gly219. Possibly due to repulsion from this acid group, Glu192 of thrombin is found in an extended position, rather than curved to interact with the inhibitor as is sometimes seen otherwise. It is notable that the two extremes of the inhibitor, the guanidinium and acid groups, are each hydrogen bonded to Gly219, enforcing a rather compact shape for the inhibitor that could conceivably be replaced by an alternate and more rigid ring system. This is reinforced by indications that a water molecule may bridge the N-terminal acid, the guanidine, and Gly219CO.

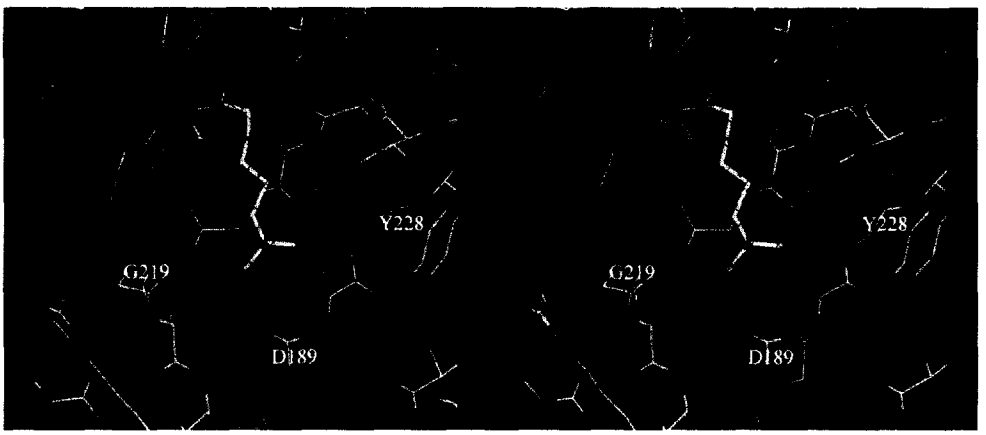


Figure 2. Stereo view of the $2f^o$-f^c electron density map contoured at 1.2 sigma of bovine thrombin in complex with **19**. The yellow and red ball-and-stick plots represent the PPACK-based and altered binding modes discussed in the text, respectively.

These observations prompted the idea of introducing elements in the inhibitor that can engage in additional polar interactions in the S_1 site.

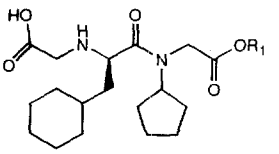
At the same time, we became interested in benzoylguanidines that are used in ion channel mediators in the heart failure field.[10] pK_a's of such moieties were reported in the region of 7–8. A number of differently substituted benzoylguanidines and aliphatic acylguanidines were modelled in the crystal structure of **19.** Despite the apparent fit in the modelling studies only compound **8** proved to be an active and selective thrombin inhibitor. Compound **7** is a representative of the aromatic series and its inactivity may be due to its pK_a of 6.6 or the lack of flexibility in these P_1 moieties.

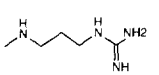
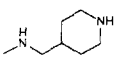
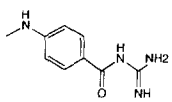
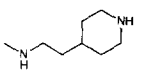
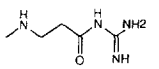
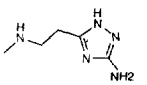
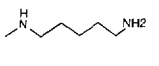
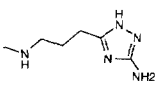
Three amine replacements for the guanidine moiety were synthesized at the same time (**20, 21,** and **22**). It was hoped that these modifications were allowed as other peptidomimetic series had suggested. The results were that most activity was lost in this cluster of analogs (100–1000 times less active than **19**).

We also investigated the effects of making combinations of acylguanidine and benzoylguanidine isosteres. Analog structures like **17** and **18** fullfill these criteria and showed pK_a values between 7 and 8 (see Table 1), which is in a range considered to be compatible with oral absorption. All aminotriazoles and related analogs were inactive, again indicating how demanding this quest is. Although the pK_a values of **17** and **18** are identical with the pK_a of acylguanidine derivative **8** the in vitro activity differs dramatically. Apparently, the orientation or the shape of the aminotriazoles prevents interaction with the Asp 189 in thrombin's active site.

The whole series resulted in one effective isostere for the guanidine moiety: the ß-alanyl-guanidine moiety (**8**). In the thrombin field there only have been few reports on less basic P_1 isosteres. Besides some amines (pK_a> 10.5),[11] mostly aminopyridines (from Boehringer Mannheim,[9] Merck,[12a] Bristol Myers Squibb,[12b] and Eli Lilly[12c]) and imidazoles[13] have been reported. For boronates no basic moieties are required since their effective transition state mimic leads to potent inhibition anyway. Surprisingly, although the pK_a of the acylguanidine is five orders of magnitude less basic, the in vitro activity had decreased only marginally compared to **19**.

Table 1. Optimisation of Inogatran and incorporation of mimics for the basic P_1 guanidine moiety. In vitro activities of thrombin and trypsin.



#	R1 =	IC_{50} IIa uM	IC_{50} Tryp uM	pK_a measured/lit	#	R1 =	IC_{50} IIa uM	IC_{50} Tryp uM	pK_a measured/lit
19		0.11	0.68	12.5 *(lit)*	21		133	215	11.1 *(lit)*
7		100	100	6.6 *(measured)*	22		74	16	11.1 *(lit)*
8		0.24	3.4	7.6 *(measured)*	17		100	100	7.5 *(measured)*
20		13.5	1.2	10.6 *(lit)*	18		100	21	7.5 *(measured)*

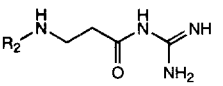
pK_a's at 25 ^{0}C; H_2O, literature.[14]

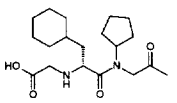
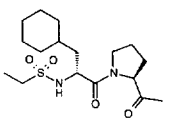
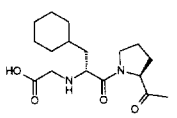
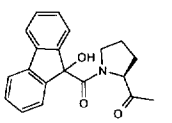
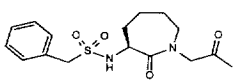
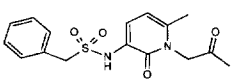
Compound **8** was modelled by modification of the X-ray structure of the thrombin–**19** complex using the Molecular Editor implemented in Quanta/CHARMm 97 (MSI, July 1997, San Diego). Various conformations and orientations of the acylguanidine sidechain were generated by using a simulated annealing approach consisting of a 50 ps constrained molecular dynamics calculation at 500 K. Every 1 ps the structure was energy minimized. No explicit solvation was taken into account. A distant-dependent dielectric $\varepsilon = 4R$ was applied in combination with a 15 Å cut-off. The complex displaying the most favourable interaction energy closely resembles the X-ray structure displayed in Figure 2. The acyl carbonyl is pointing towards the Tyr228 ring. This additional interaction may indeed counteract the reduced basicity of the P_1 functionality.

The selectivity of **8** has substantially improved based on IC_{50} measurements: compared with the starting compound **1,** more than tenfold improvement in the thrombin/trypsin ratio was observed. Both the starting compound as well as **8** showed only very low activity against factor Xa.

In phase three of the design process the other half of compound **8** was modified. The goal was to find a nonpeptide inhibitor while maintaining as much of the intrinsic activity as possible. The results are summarized in Table 2. A diverse set of P_3-P_2 moieties, taken from the thrombin literature, was used to probe this part of the active site. What became clear was that combinations with the acylguanidine moiety resulted in shifts in potency compared to other P_1 groups. Apparently, the two moieties influence each other in their binding modes as can best be described as a lever. The most promising compound is **28,** with the 3-amino-2-pyridinone moiety first used by Zeneca[15] in elastase ligands and later by others in thrombin inhibitors. Compound **28** is still potent enough, although compared to **8** ten times less active, but has lost all peptide character. The intrinsic activity and selectivity are comparable to **1.**

Table 2. Optimisation of the P_3-P_2 moiety in **8**. In vitro activities of thrombin and trypsin.



#	R2 =	IC_{50} IIa uM	IC_{50} Tryp uM	#	R2 =	IC_{50} IIa uM	IC_{50} Tryp uM
23		0.24	3.4	26		10	12
24		1.1	1.2	27		6	14.5
25		73	15	28		1.9	1.9

In conclusion, an interesting new P_1 isostere has been found with a pK_a value in the neutral region. This may contribute significantly towards identifying orally bioavailable antithrombotics. Secondly, in three discrete design cycles an already known thrombin inhibitor (**1**) was transformed from a peptide structure with a highly basic guanidine group to a nonpeptide, achiral, less basic compound with a similar in vitro profile. This end-product requires less expensive building blocks and less synthetic steps which makes it also an interesting compound from a drug development perspective. Further profiling will give answers as to how this compound behaves in animal studies.

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References

1. Lumma, W. C.; Witherop, K. M.; Tucker, T. J.; Brady, S. F.; Sisko, J. T.; Naylor-Olsen, A. M.; Lewis, S. D.; Lucas, B. J.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 1011.
2. Wiley, M. R.; Fisher, M. J. *Exp. Opin. Ther. Patents* **1997**, *7*, 1265.
3. Kimball, S. D. *Blood Coagul. Fibrin.* **1995**, *6*, 511.
4. Ripka, W. C.; Vlasuk, G. P. *Annu. Rep. Med. Chem.* **1997**, *32*, 71.
5. Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R. *J. Med. Chem.* **1997**, *40*,1565.
6. Teger-Nilsson, A.; Bylund, R.; Gustafsson, D.; Gyzander, E.; Eriksson, U. *Thromb. Res.* **1997**, *85*, 133.
7. Stanton, J. L.; Gruenfeld, N.; Babiarz, J. E.; Ackerman, M. H.; Friedman, R. C. *J. Med. Chem.* **1983**, *26*, 1267.
8.(a) Jetten, M.; Peters, C.; Visser, A.; Grootenhuis, P. D. J.; van Nispen, J. W.; Ottenheijm, H. C. J. *Bioorg. Med. Chem.* **1995**, *3*, 1099.(b) IC_{50} values from different labs can not be compared in contrast to K_i's; at Organon an IC_{50} is 50–100 times less than a K_i.
9. Engh, R. A.; Brandstetter, H.; Sucher, G.; Eichinger, A.; Baumann, U.; Bode, W.; Huber, R.; Poll, T.; Rudolph, R.; von der Saal, W. *Structure* **1996**, *4*, 1353.
10. Schmid, A.; Scholz, W.; Lang, H. J.; Popp, R. *Biochem Biophys. Res. Comm.* **1992**, *184*, 112.
11. Lyle, T. A.; Chen, Z.; Appleby, S. D.; Freidinger, R. M.; Gardell, S. J.; Lewis, S. D.; Li, Y.; Lyle, E. A.; Lynch, J. J.; Mulichak, A. M.; Ng, A. S.; Naylor-Olsen, A. M.; Sanders, W. M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 67.
12. (a) Feng, D-M.; Gardell, S. J.; Lewis, S. D.; Bock, M. G.; Chen, Z.; Freidinger, R. M. *J. Med. Chem.* **1997**, *40*, 3726.(b) Misra, R.; Kelly, Y.; Broun, B.; Roberts, D.; Chong, S.; Seiler, S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2165. (c) Eli Lilly, US-5707966, **1998**.
13. Synthelabo, EP-718307, **1996**.
14. Perrin, D. D. In *Dissociation Constants of Organic Bases in Aqueous Solution;* Butterworths: London, 1965; p 473.
15. Andisik, D. W.; Bernstein, P. R.; Bryant, C. B.; Ceccarelli, C.; Damewood, J. R.; Edwards, P. D. *J. Med. Chem.* **1994**, *37*, 1259.