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NEW THROMBIN INHIBITORS BASED ON D-CHA-PRO-DERIVATIVES

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A series of new analogs with modifications in the C-terminal residue were prepared based on the known thrombin inhibitor D-Phe-Pro-agmatine. These include several compounds alkylated at the N^{δ}-, N^{ω}- and $N^{\omega'}$-atoms of the guanidino group and a number of inhibitors derived from commercially available diamines. All analogs with alkylation of the guanidino group showed very poor activity. In contrast, the most potent and selective inhibitor with a cyclic and basic residue in the P1-position was found to be Ph–CH_2–SO_2–D-Cha-Pro–4-(amidomethyl) amidinopiperidine **11** with a K_i of 0.27 nM. In addition, a number of compounds were synthesized, in which the basic amidino group of the P1-residue was replaced by a hydroxyl group. Although the inhibition constants of these phenol derivatives showed still remarkable potency (**16**, $K_i = 130$ nM), their activity in clotting assays was strongly reduced.

Keywords: Thrombin; Enzyme inhibitors; Anticoagulants; Tripeptide; Synthesis

INTRODUCTION

Thrombin is the terminal protease in both the extrinsic and intrinsic way of the coagulation cascade. It reacts with several substrates, e.g. fibrinogen, factors V, VIII, XIII, protein C and the thrombin receptor. It is well

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accepted that the design of small, potent and selective thrombin inhibitors is a promising way for the development of antithrombotic agents.[1,2]

A simple classification of the known active site thrombin inhibitors is the division into covalent and non-covalent inhibitors. To the first group belong irreversible inhibitors, like PPACK,[3] acylating agents,[4] mechanism-based inhibitors[4] and the transition state analogs.[5–7] Unfortunately, very often they lack selectivity, contain reactive groups in the molecule, are prone to racemization of the P1-residue, show an unwanted slow binding behaviour[8] and sometimes they are difficult to synthesize. Therefore, non-covalent thrombin inhibitors are probably more useful for drug development and at present there exist several lead structures in this group, e.g. MD-805,[9] NAPAP[10] or Napsagatran.[11]

A different basic structure is the D-Phe-Pro-agmatine[12] (Figure 1), which was first described by Bajusz in 1982. However, recently new analogs have been developed from this agmatine derivative, with remarkable activity, like Inogatran,[13] D-Phe-Pro–p-amidinobenzylamine[14] (Figure 1) and several inhibitors from Merck.[15–17]

One approach to improve the affinity was achieved by incorporation of more hydrophobic P3- and P4-residues, e.g. Ph–CH_2–SO_2–D-3,3-diphenylalanine.[15] A second important strategy was the substitution of the flexible P1-agmatine by more rigid and more hydrophobic basic residues such as amidinobenzylamine,[14] trans-4-(aminomethyl)cyclohexylamine[15,16] or 3-(aminomethyl)-6-aminopyridine.[17]

Within this group, we wanted to investigate the influence of modifications on the C-terminal agmatine residue. The first series includes alkylations of the N^{δ}-, N^{ω}- and $N^{\omega'}$-atoms of the guanidino group. A similar approach has been described recently in a series of thrombin inhibitors containing 4-amidinophenylalanine where the affinity could be slightly enhanced by substitution of the amidino moiety by a methyl-amidino group.[18] An additional

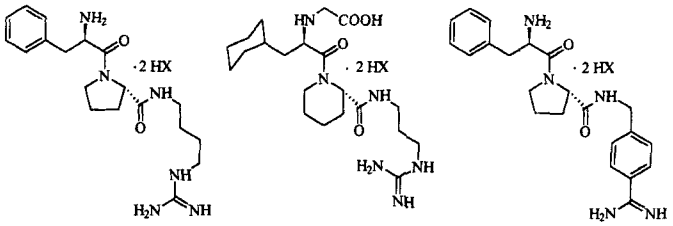

FIGURE 1 Structure of D-Phe-Pro-agmatine, Inogatran and D-Phe-Pro–p-amidinobenzylamine.

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advantage of this thrombin inhibitor was the *ca.* 100-fold higher selectivity versus trypsin. We assumed that these substitutions should have a negative influence on the hydrogen bonding network of the P1-residue; however alkylation maintains the basic character of the guanidino group and should allow the formation of additional hydrophobic interactions between thrombin and the inhibitor, which may compensate for fewer hydrogen bonds.

In a second series cyclic diamines more rigid than the skeleton of agmatine were used as starting compounds for the synthesis of new inhibitor structures with increased hydrophobicity in the P1-residue.

There exist only a few examples of potent thrombin inhibitors without a basic P1-residue, like some peptidyl boronates[19] or the recently described benzo[*b*]thiophenes.[20] Among this series, the most potent inhibitor contains a hydroxyl substituent at the C-6 position of the A-ring of the benzo[*b*]thiophene. The X-ray structure of the enzyme–inhibitor complex shows a distance of 2.7 Å between the C-6 hydroxyl and the Asp189 of thrombin, suggesting the possible formation of a hydrogen bond. Therefore, some analogs derived from D-Phe-Pro-agmatine containing 4-(aminoalkyl)phenol derivatives in P1-position were synthesized. The synthesis and kinetic analysis of these analogs is described in this paper.

MATERIALS AND METHODS

Assays

The kinetic and clotting assays were performed as described previously.[21,22]

Chemistry

All of the inhibitors synthesized are shown in Table I together with the results of their analysis by Maldi mass spectrometry and their retention times found by analytical reversed-phase HPLC. Inhibitors **1–6** were synthesized by the route depicted in Scheme 1. Boc–D-Cha-Pro–OH was preactivated with PyBop/DIEA and reacted with a 15-fold excess of diamino butane in a similar manner to that described for the synthesis of Boc–NH–$(CH_2)_4$–NH_2.[23] The resulting intermediate was guanylated by pyrazol-1-carboxamidin[24] (for the synthesis of **1**) or by the appropriate *S*-methylisothiouronium iodide salts (for **2–5**), which were prepared as guanylation reagents from the N,N′-alkylated thioureas by reaction with methyl iodide.[25] The reactions with the *N*-monomethyl- and monoethyl-*S*-methylisothiouronium iodide salts were nearly finished after two days, in

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TABLE I Structures and analytical data of the synthesised thrombin inhibitors* of the general formula R_1-Pro-R_2

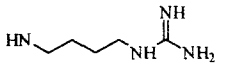
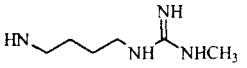
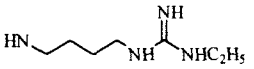
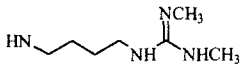
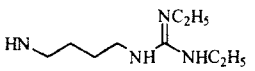
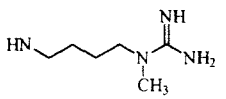
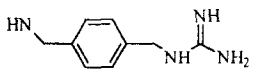
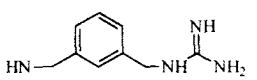
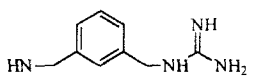
No.	R_1	R_2	*HPLC* t_R (min)	*Mol. Wt. Calc./found* MH^+
1	D-Cha		15.5	380.5/381.0
2	D-Cha		16.4	394.6/395.2
3	D-Cha		16.8	408.6/409.3
4	D-Cha		17.6	408.6/409.6
5	D-Cha		19.4	436.6/437.3
6	D-Cha		16.5	394.6/394.7
7	D-Phe		13.2	422.5/423.4
8	D-Phe		13.1	422.5/423.3
9	D-Cha		18.39	428.6/429.1

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TABLE I (*Continued*)

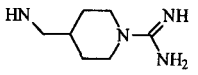
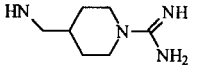
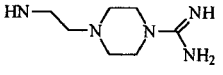
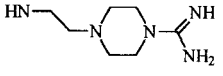
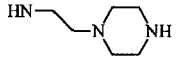
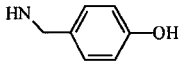
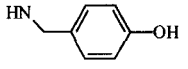
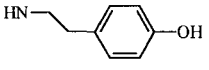
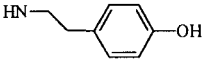
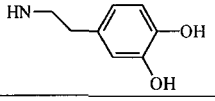
No.	R_1	R_2	*HPLC* t_R (min)	*Mol. Wt. Calc./found* MH^+
10	D-Cha		17.1	406.6/407.9
11	Ph–CH_2–SO_2–D-Cha		35.6	560.8/561.3
12	D-Cha		15.2	421.6/423.0
13	Ph–CH_2–SO_2–D-Cha		31.3	575.8/576.4
14	Ph–CH_2–SO_2–D-Cha		31.1	532.7/533.7
15	D-Cha		22.1	373.5/373.7
16	Ph–CH_2–SO_2–D-Cha		40.9	527.7/550.1†
17	D-Cha		23.5	387.5/388.8
18	Ph–CH_2–SO_2–D-Cha		42.1	541.7/564.7†
19	D-Cha		20.8	403.5/403.5

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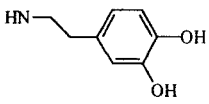
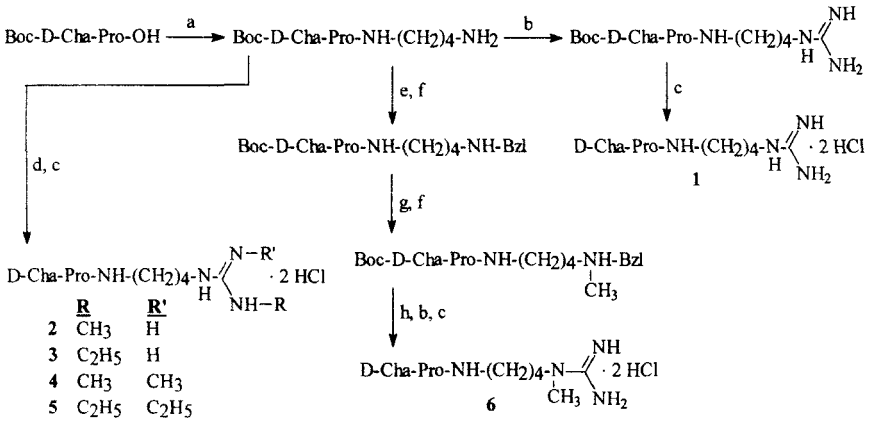
TABLE I (*Continued*)

No.	R_1	R_2	*HPLC* t_R (min)	*Mol. Wt. Calc./found* MH^+
20	Ph–CH_2–SO_2–D-Cha		39.3	557.7/581.7[†]

[*] The final inhibitors were purified to more than 97% by semipreparative HPLC: Shimadzu LC-8A, Vydac C_{18}, 5 µM reversed phase column (250 × 25 mm). Solvents: A, 0.1% aqueous TFA; B, 0.1% TFA in acetonitrile, gradient 10% B to 55% B in 120 min, flow rate 10 ml/min, monitored at 220 nm. Analytical HPLC: Shimadzu LC-10A, Vydac C_{18}, 5 µM reversed phase column (250 × 4 mm). Solvents: A, 0.1% aqueous TFA; B, 0.1% TFA in acetonitrile, gradient 10% B to 60% B in 50 min, flow rate 1 ml/min, monitored at 220 nm. MS: Perseptive Biosystems Maldi-Tof Laser Tec Research mass spectrometer, [†] highest peak $[M\text{-}Na]^+$.



SCHEME 1 Synthesis of alkylated agmatine derivatives **1–6**. (a) 1 eq. PyBop, 2 eq. DIEA in DCM, 0°C, 10 min, 15 eq. 1,4-diaminobutane, 60 min; (b) 1.5 eq. 1H-pyrazole-1-carboxamidine · HCl, 3 eq. DIEA in DMF, 12 h; (c) 1 M HCl in acetic acid, 30 min; (d) 2 eq. TEA, 1.5 eq. CH_3–S–C(=NR′)NHR · HI in DMF, 6 d, 20°C; (e) 2 eq. benzaldehyde in methanol, 45 min, 20°C; (f) $NaBH_4$, 2 h; (g) 1.2 eq. formaldehyde in methanol, 45 min; (h) saturated ammonium formate in methanol/H_2O 9 : 1, Pd(II)-acetate, 24 h.

contrast to reactions with the sterically more hindered analogs which needed a much longer time of up to one week. The synthesis of the N^{δ}-alkylated inhibitor **6** required a different procedure. Direct methylation attempts of the N^{δ}-atom of Boc–D-Cha-Pro–NH–$(CH_2)_4$–NH_2 by methyl iodide or formaldehyde followed by reduction with $NaBH_4$ resulted only in an undefined mixture of non-, mono- and di-methylated products. Therefore, a procedure described by Quitt[26] was used for selective mono-methylation. The first step was reaction of the amine with benzaldehyde and reduction of the azomethine by $NaBH_4$. In the second step, the resulting benzylamine was

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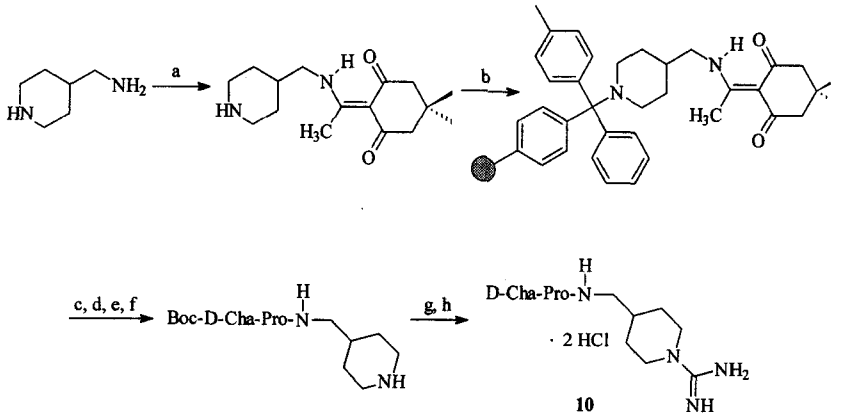
treated first with formaldehyde, followed by reduction with $NaBH_4$ to give the *N*-methyl-*N*-benzylamine. The *N*-debenzylation was performed by transfer hydrogenolysis[27] using palladium acetate in a saturated solution of ammonium formate in a methanol/water 9 : 1 mixture. The intermediate was guanylated by pyrazol-1-carboxamidin as described above. Finally, the Boc-groups were removed by 1M HCl in acetic acid to give **1–6**. In analogy to the synthesis of **1** we used also *p*- and *m*-diaminoxylene as starting materials instead of diamino butane to obtain *p*- and *m*-(guanidinomethyl)-benzylamide derivatives **7–9** (Table I).

A combination of solution and solid phase peptide synthesis was utilized to synthesize **10** starting from 4-(aminomethyl)piperidine (Scheme 2). The first step was the selective reaction of 2-acetyl-dimedone with the primary amino group of 4-(aminomethyl)piperidine, which leads to 4-(Dde-amino-methyl)piperidine.[28] The remaining steps were performed by solid phase synthesis. Compared to solution synthesis this method allowed a more ready separation of the 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazole cleavage side product after Dde removal by 2% hydrazine in DMF. Therefore, 4-(Dde-aminomethyl)piperidine was attached to 4-methyltrityl chloride resin, after removing the Dde group, Fmoc–Pro–OH and Boc–D-Cha–OH were coupled with HBTU/DIEA using a standard protocol. The peptide was cleaved from the resin by treatment with 30% hexafluoroisopropanol in



SCHEME 2 Combined solution–solid phase synthesis of **10**. (a) 1 eq. 2-acetyldimedone, 2 h reflux in ethanol; (b) 0.33 eq. 4-methyltrityl chloride resin, 2 eq. DIEA, 4 h, DCM; (c) 3 × 2 min 2% hydrazine in DMF; (d) 4 eq. Fmoc–Pro–OH/HBTU/DIEA; (e) 4 eq. Boc–D-Cha–OH/HBTU/DIEA; (f) 30% hexafluoroisopropanol in DCM, 45 min; (g) 1.5 eq. 1H-pyrazole-1-carboxamidine · HCl, 3 eq. DIEA in DMF, 12 h; (h) 1 M HCl in acetic acid, 30 min.

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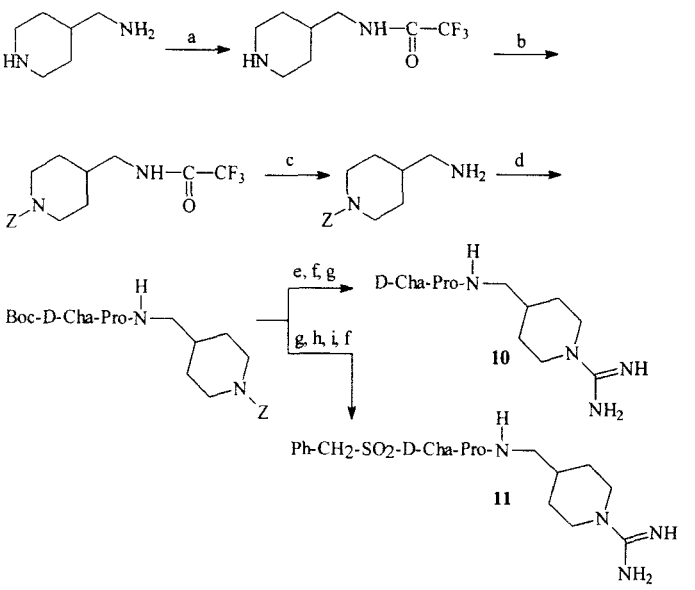
DCM and after guanylation and cleavage of the Boc-group, **10** was obtained. Because of its high activity, inhibitor **10** was resynthesized by a pure solution synthetic method as described in Scheme 3. Initially, 4-(aminomethyl)piperidine was treated with ethyl trifluoroacetate which reacts selectively with the primary amino group.[29] In a second step, the piperidine moiety was protected by reaction with Z-OSu. The trifluoroacetyl group was removed by treatment with 2 M NaOH in dioxane/water. The synthesis of inhibitors **10** and **11** was completed by standard procedures as depicted in Scheme 3. Compounds **12–14** were prepared in the same way using *N*-(aminoethyl)piperazine as starting material.

Inhibitors **15**, **17** and **19** were prepared by DCC/HOBt mediated coupling of Boc–D-Cha-Pro–OH with the appropriate 4-(aminoalkyl)phenoles, followed by deprotection with 1 M HCl/HAc. Dopamine and tyramine were commercially available, *p*-hydroxybenzylamine was prepared by reduction of *p*-cyanophenol with $LiAlH_4$.[30] Reaction with Ph–CH_2–SO_2Cl resulted in **16**, **18** and **20**.



SCHEME 3 Solution synthesis of **10** and **11**. (a) 1.1 eq. ethyl trifluoroacetate in DCM, 3 h at RT; (b) 1.05 eq. Z-OSu in dioxane·H_2O, DIEA pH 9; (c) 1 M NaOH/dioxane 1 : 1, 3 h, 40°C; (d) Boc–D-Cha-Pro–OH, mixed anhydride procedure in THF; (e) H_2 and Pd/C; (f) 1.5 eq. 1H-pyrazole-1-carboxamidine·HCl, 3 eq. DIEA in DMF, 12 h; (g) 1 M HCl in acetic acid; (h) 1.1 eq. Ph-CH_2–SO_2Cl in DMF, 2.2 eq. DIEA; (i) 33% HBr in acetic acid, 1.5 h at RT.

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RESULTS AND DISCUSSION

Alkylation of the Agmatine Residue

The inhibition constants for the first series of compounds are summarized in Table II. All compounds described in this paper showed competitive inhibition kinetics. Inhibitor **1** was synthesized as a reference molecule since it is known that only the substitution of D-Phe by D-Cha in the P3-position should result in a 10–20-fold improvement in binding affinity.[13,31] As expected, the K_i for **1** was improved ~18 times compared with D-Phe-Pro-agmatine.[12,32] In contrast, all attempts to increase the affinity through additional hydrophobic interactions based on alkylation of the guanidino group failed. The single alkylation on the N^{ω}-atom of the agmatine residue in **2** and **3** resulted in a reduction in affinity by nearly three orders of magnitude; the N^{ω}- and $N^{\omega'}$-di alkylated compounds **4** and **5** were totally inactive. Because the basic properties of the guanidino group of these compounds still remained, the reason for the lower affinity seems to be due to steric hindrance by the alkyl substitutions, which probably leads to an elimination of the salt bridge to Asp189 in the S1-pocket of thrombin. A less pronounced effect was found after methylation of the N^{δ} of the agmatine, however, also in this case a ~20-fold reduction in the K_i-value of **6** compared with **1** was obtained, probably due to an elimination of the hydrogen bond between the carbonyl oxygen of Gly219 and the N^{δ} of the guanidino group, which exists in the D-Phe-Pro-Arg based thrombin-inhibitor complexes.[33]

TABLE II Inhibition of thrombin*, trypsin, factor X_a and plasmin by agmatine analogs, K_i values in μM

No.	*Thrombin*	*Trypsin*	*FX_a*	*Plasmin*
1	0.01	0.70	30	>1000
2	5.1	>1000	>1000	>1000
3	10.0	>1000	>1000	>1000
4	>1000	>1000	>1000	>1000
5	>1000	>1000	>1000	>1000
6	0.18	20	>1000	>1000

* The following enzymes and substrates (supplied by Pentapharm Ltd, Basel, Switzerland) were used at the final concentrations indicated: bovine thrombin (2262 U/mg, final concentration 0.45 U/ml), substrate $MeSO_2$-D-hexahydrotyrosyl-Gly-Arg-pNA (final concentrations 0.18 and 0.09 mM); bovine pancreatic trypsin (42 U/mg, 0.0038 U/ml, Serva, Heidelberg, Germany), substrate $MeSO_2$-D-hexahydrotyrosyl-Gly-Arg-pNA (final concentrations 0.18 and 0.06 mM); bovine FX_a (5 U/vial, 0.11 U/ml, Diagnostic Reagents Ltd., Thame, UK), substrate $MeSO_2$-D-Nle-Gly-Arg-pNA (final concentrtions 0.36 and 0.18 mM); human plasmin (0.67 CTA-U/mg, 0.06 CTA-U/ml, Behringwerke AG, Marburg, Germany), substrate Tos-Gly-Pro-Lys-pNA (final concentrations 0.18 and 0.09 mM).

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Inhibitors with a Cyclic and Basic P1-Residue

Table III summarizes the inhibition constants of all inhibitors with a cylic and basic P1-residue. The *p*- and *m*-(guanidinomethyl)benzylamide derivatives **7** and **8** contain a D-Phe in the P3-position and therefore they are generally less active than compounds with D-Cha at the N-terminus. The meta-analog **8** is slightly more potent than **7**, however, compared with D-Phe-Pro-agmatine ($K_i = 0.18\,\mu M$[32]) the affinity is decreased by one order of magnitude. Unexpected was the only small improvement in affinity by incorporation of D-Cha in compound **9** compared with **8**. Therefore, we assume that this C-terminal *m*-(guanidinomethyl)benzylamide probably induces a different binding mode for the P3 amino acid than in the classical D-Phe-Pro-Arg peptides.

Previously developed thrombin inhibitors based on (*S*)-3-(aminomethyl) amidinopiperidine incorporated at the C-terminus of β-naphthylsulfonyl-aspartyl analogs[11] or compounds derived from *N*-(amidino)nipecotic acid, like [*S*-(*R**,*R**)]-1-aminoiminomethyl)-*N*-[[1-[*N*-[(2-naphthalenylsulfonyl)-L-seryl]-2-pyrrolidinyl]-methyl]]-3-piperidinecarboxamide (BMS-189090, $K_i = 3.64\,nM$)[34] demonstrated, that the S1-site of thrombin is well suited to accommodate a *N*-(amidino)piperidine group. Therefore, we incorporated 4-(aminomethyl)amidinopiperidine in the D-Cha-Pro-Arg structure. In contrast to the extensive synthesis of the (*S*)-3-(aminomethyl)piperidine intermediate,[11] the 4-(aminomethyl)piperidine is a commercially available starting material without a chiral centre. The resulting inhibitors **10** and **11** ($K_i = 2.8$ and $0.27\,nM$, respectively) were very potent and showed a much higher selectivity versus trypsin than our reference inhibitor **1**. During this work the incorporation of 4-(aminomethyl) amidinopiperidine in a series of new pyridinone based thrombin inhibitors was reported, the most potent compound having a K_i of $0.5\,nM$ and showing also a very high selectivity versus trypsin and other related serine proteases.[35]

TABLE III Inhibition by inhibitors with a cyclic and basic P1 residue, K_i values in μM

No.	*Thrombin*	*Trypsin*	*FX_a*	*Plasmin*
7	7.05	>1000	>1000	>1000
8	2.1	>1000	>1000	>1000
9	1.45	>1000	>1000	>1000
10	0.0028	0.81	>1000	83
11	0.00027	0.053	1.4	0.62
12	0.17	19	>1000	>1000
13	0.011	1.9	33	3.9
14	0.46	5.7	>1000	46

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A generally lower affinity was observed with compounds **12** and **13**, containing a *N*-(aminoethyl)N′-amidinopiperazine as P1-residue. Compared with the 4-(aminomethyl)*N*-amidinopiperidine this residue is more flexible because of the insertion of one methylene group; in addition, the hydrophilic piperazine moiety probably leads to reduced hydrophobic interactions in the S1-site of thrombin. A further ~40-fold reduction in activity was observed with the shorter piperazine **14**, indicating the importance of the strong basic guanidino groups for potency.

Inhibitors with C-terminal 4-(aminoalkyl)phenoles

The third series includes compounds with 4-(aminoalkyl)phenol derivatives in the P1-position. The K_i-values of inhibitors **15** and **16** are ~500 times higher compared with the amidinopiperidine analogs, however this affinity was achieved without any basic P1-residue being present (Table IV). Because the 4-(aminomethyl)phenol is slightly shorter than the appropriate amidinopiperidine we tried to extend the length by introducing an additional methylene group in compounds **17–20**. As seen in Table IV, this modification had nearly no influence on the K_i values for thrombin. At this point it is not possible to determine, if the phenolic hydroxyl group is able to make a hydrogen bond to Asp189 as suggested for the benzo[*b*]thiophene derivatives.[20] Although these phenol derivatives have only a moderate potency as seen from their K_i-values, they could be an interesting starting point for further development, because it was shown that more lipophilic thrombin inhibitors show better oral bioavailability.[16,17] On the other hand it was described also that very lipophilic compounds perform relatively poor as anticoagulants during *in vivo* studies compared with more polar and less protein-bound inhibitors.[16] Therefore, we performed different clotting assays with these analogs and compared the data with selected inhibitors from the series with basic P1 residues. As seen in Table V, no differences in the clotting assays could be found between the most potent inhibitors

TABLE IV Inhibition by 4-(aminoalkyl)phenol derivatives, K_i values in μM

No.	*Thrombin*	*Trypsin*	*FX_a*	*Plasmin*
15	1.02	>1000	>1000	>1000
16	0.13	78	23	>1000
17	1.5	>1000	>1000	>1000
18	0.23	>1000	>1000	>1000
19	3.8	>1000	>1000	>1000
20	0.43	>1000	100	>1000

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TABLE V Comparison of K_i and IC_{200}* values for selected inhibitors, all data in μM

No.	*K_i thrombin*	*TT*	*aPTT*	*PT*
10	0.0028	0.038	0.33	0.75
11	0.00027	0.037	0.44	0.65
12	0.17	0.32	3.1	7.1
13	0.011	0.065	0.9	1.5
15	1.02	3.4	27	36
16	0.13	3.3	13	20
17	1.5	6.0	55	90
18	0.23	6.0	26	39
19	3.8	15	84	200
20	0.43	6.5	24	48

*The IC_{200} values are the concentration of the inhibitor in human plasma, which causes a doubling of clotting times (thrombin time = TT; activated partial thromboplastin time = aPTT and prothrombin time = PT).

10 and **11**. Because of the relatively high thrombin concentrations present in these clotting systems, it is not possible to detect significant differences among inhibitors with K_i values below 10 nM.[8] However, a reasonable correlation was found between inhibitors **12** and **13**, the lower K_i value of **13** based on the N-terminal benzylsulfonyl group resulting also in a significant improvement in clotting tests. In contrast, nearly zero or only minor effects were found on comparing the appropriate inhibitor pairs **15** and **16**, **17** and **18** or **19** and **20**. Despite the ~10-fold improved K_i values of **16**, **18** and **20**, they showed no improved activity in clotting assays, probably due to unspecific plasma protein binding, which could lead to a reduced free inhibitor concentration in the test system. This negative effect also becomes evident, if these analogs were compared with **12**, which has a similar K_i, but much better IC_{200} value.

CONCLUDING REMARKS

Several series of thrombin inhibitors, derived from the D-Phe-Pro-agmatine structure, were synthesized. The results of the inhibition assays showed that substitution of the nitrogen atoms of the agmatine guanidino group by alkyl residues resulted in compounds with poor activity compared to the reference inhibitor **1**. Among a second series with cyclic and basic P1 residues, potent thrombin inhibitors were obtained by the substitution of the agmatine for a 4-(aminomethyl)amidinopiperidine group. The resulting compounds **10** and **11** were also very selective towards thrombin compared to trypsin and related serine proteases. In addition, they are easy to synthesize from commercially available starting materials. All of the prepared analogs are fast

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binding inhibitors and have no reactive group in the molecule, which could lead to unwanted side reactions. The third series with C-terminal 4-(aminoalkyl)phenoles showed moderate activity in their inhibition constants, but they were poor anticoagulants in clotting assays. These relatively hydrophobic inhibitors have probably a strong tendency for unspecific protein binding. This demonstrates the importance of retaining some polar groups in the molecule and to find the perfect balance between affinity, selectivity and physical properties for the design of useful thrombin inhibitors.[16]

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