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SOLUTION-PHASE AND SOLID-PHASE SYNTHESIS OF NOVEL TRANSITION STATE INHIBITORS OF COAGULATION ENZYMES INCORPORATING A PIPERIDINYL MOIETY

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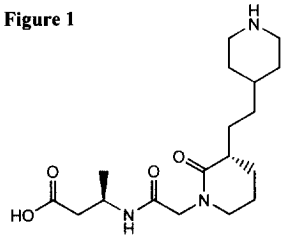
Abstract: 2-Amino-3-piperidin-4-yl-propionic acid containing peptidomimetics are potent protease inhibitors when combined with an appropriate keto-thiazole or keto-carboxylic acid moiety. A novel P_1 residue in factor Xa and thrombin inhibitors has been found resulting in IC_{50} values as low as 0.048 μM, a factor of ten more potent than Argatroban. Starting with non-chiral synthetic routes, a new stereospecific route was developed as well as a new solid-phase method. © 1999 Elsevier Science Ltd. All rights reserved.

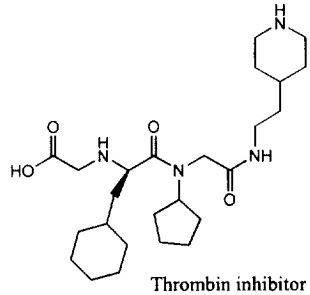
Introduction

The majority of inhibitor structures for serine proteases involved in blood coagulation—like thrombin, factor Xa, and factor VIIa—show a guanidine or a (benz)amidine moiety.[1] This can be explained by the fact that most investigators have used an arginine or *p*-amidino-phenylalanine as starting points for drug discovery. In order to obtain orally active drug candidates that can be used in thrombotic diseases, it is felt that the basicity (pK_a>12.5) or the polarity of these inhibitor structures should be lowered.[2] Less basic isosteres of this so-called P_1 residue have been reported: primary amines (pK_a 10.6),[3] 4-aminopyridines (pK_a 9.2),[4] imidazoles pK_a 6.9),[5] and acylguanidines (pK_a 6.6-7.6).[6] Although in some publications efficient in vitro inhibition was reported only limited information exists on their in vivo behaviour. In their quest for orally active inhibitors of thrombin some groups found compounds that were absorbed orally but did not show substantial antithrombotic potency. This was thought to be caused by high first-pass elimination or plasma protein binding.[7] Finding the right balance evidently requires a more diverse set of less basic P_1 isosteres. In one of our approaches in the thrombin/factor Xa area, comparisons could be made with other drug discovery projects like the GPIIb/IIIa antagonists.

Figure 1



GPIIb/IIIa antagonist
L-734,217 **1**



Thrombin inhibitor
2

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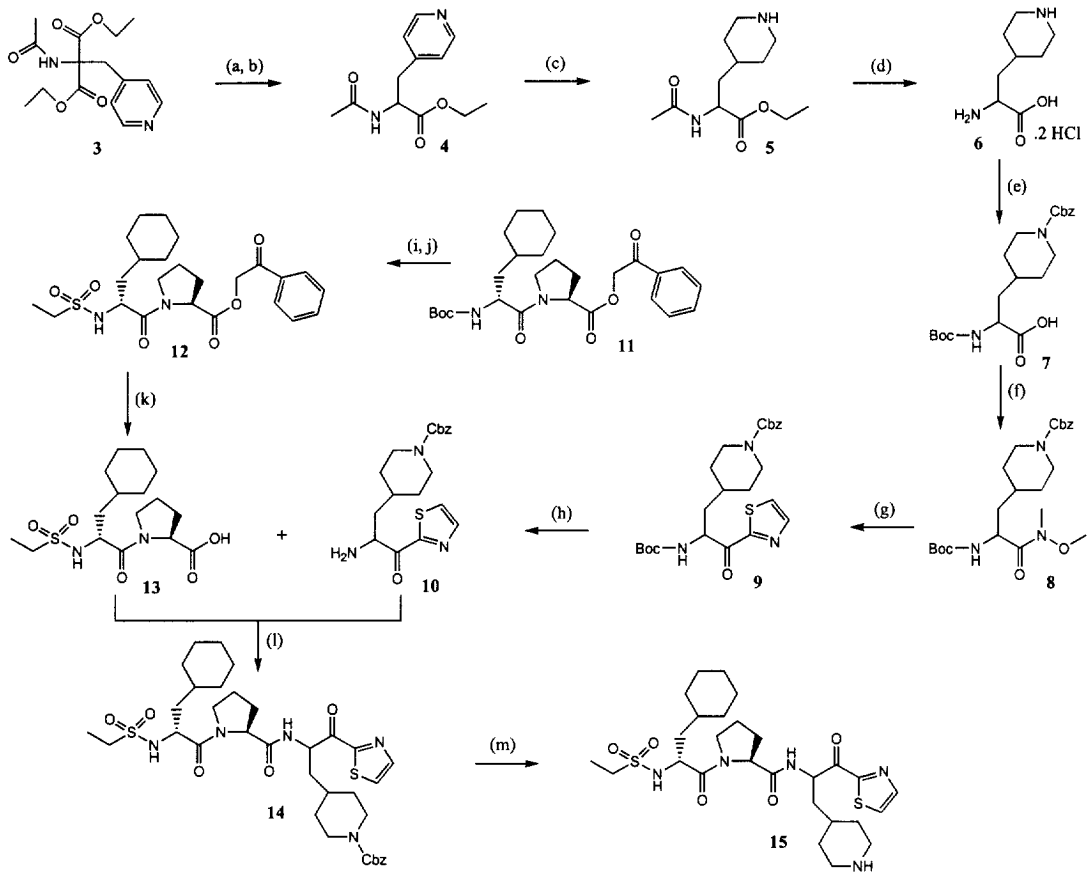
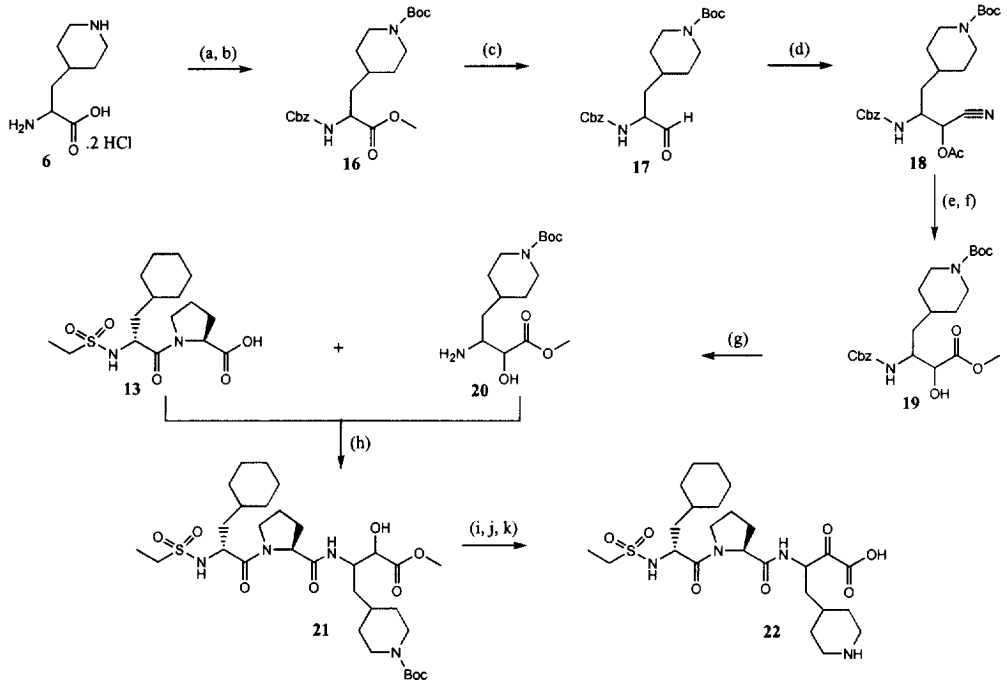
The similarities with inhibitors of thrombin, factor Xa, etc. are obvious: peptidomimetics, an arginine in the lead structure and tripeptide size of the ligand.[8] By replacing this arginine residue with a piperidinyl moiety, orally active compounds could be identified in the GPIIb/IIIa field (e.g., **1**).[9] We previously showed that introduction of a piperidinyl moiety in non-transition state thrombin inhibitors, like **2**, did not result in inhibitory activity (see Fig. 1).[6] Apparently, it is not a straightforward switch but requires different lead structures with different orientations in thrombin. After examining all thrombin and factor Xa lead series once more and combining this with our expertise in transition state analogue inhibitors, several series were designed based on 3-(4-piperidinyl)-alanine derived ketones. A number of synthetic routes were developed that yielded diastereomers and later on optically pure compounds. In addition, a solid-phase method has been developed which facilitates the synthesis of larger series.

Chemistry



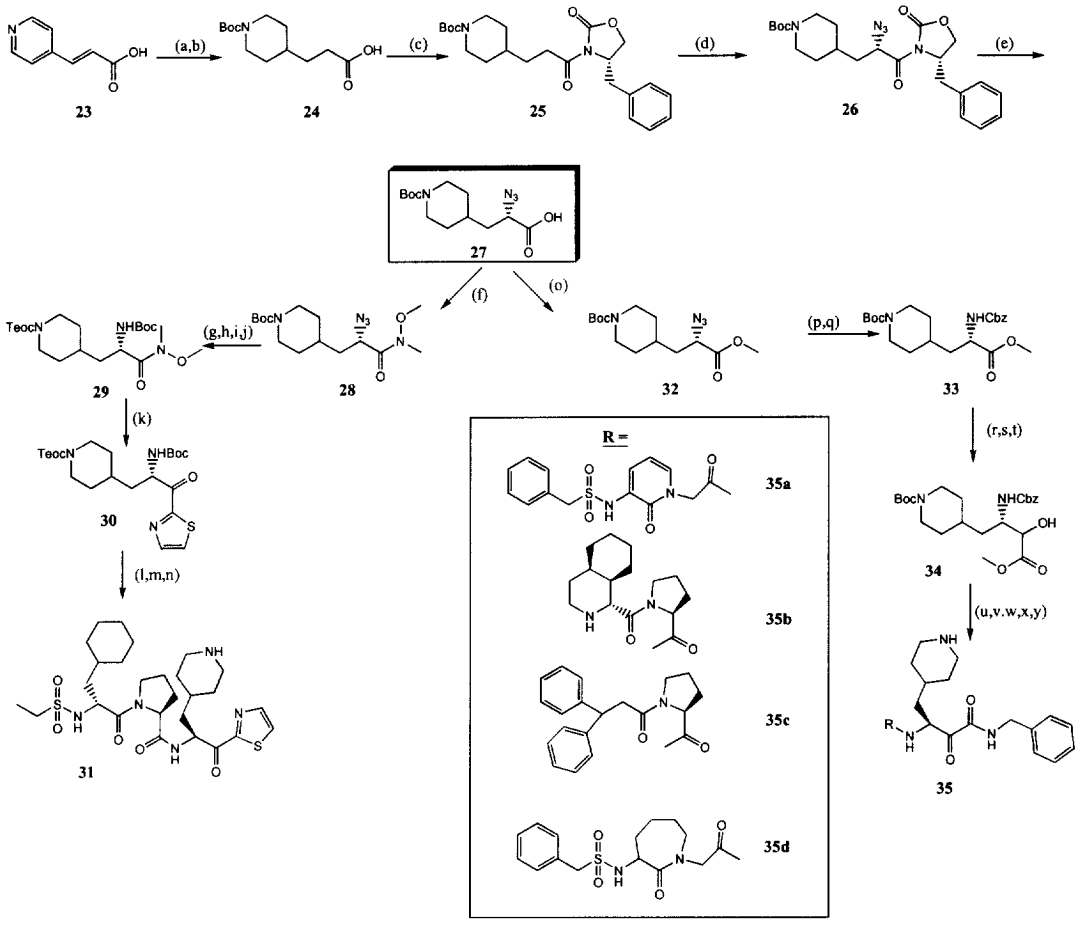
Scheme 1 Synthesis of a diastereomeric 3-(4-piperidinyl)-alanine-(2-thiazolyl) containing thrombin inhibitor. (a) EtOH/H_2O (7:3)/KOH/1 h/rt (quant); (b) DMF/reflux/1.5 h (67%); (c) EtOH/Pd(C) 10%/1 N HCl/H_2O (quant); (d) 6 N HCl/reflux/4 h (quant); (e) 1. dioxane/H_2O (2:3)/$CuSO_4$/pH 9/ZONSu/16 h/rt; 2. Boc_2O/pH 12.5/20 h/rt (89%); (f) CH_2Cl_2/N,O-dimethylhydroxylamine/TBTU/Et_3N (pH 8.5)/2h/rt (62%); (g) -78 °C/1 M BuLi in ether/dry/2-bromo thiazole/THF (20%); (h) TFA/thioanisole (10:1)/4h/rt (quant); (i) CH_2Cl_2/TFA (1:1)/0.5 h/rt (quant); (j) CH_2Cl_2/-78°C/$EtSO_2Cl$/Et_3N (pH 8)/3h/0 °C (80%); (k) THF/dry/1 M TBAF/40 min/rt (90%); (l) DMF/HOBT/DCCI/0 °C/0.5 h/16 h/rt (95%); (m) TFA/thioanisole (10:1)/4h/rt/purified by prep. HPLC (80%).



Scheme 2 Synthesis of a diastereomeric 3-(4-piperidinyl)-alanine-(2-carboxyl) containing thrombin inhibitor. (a) 1. dioxane/H_2O (3:2)/$CuSO_4$/pH 9; 2. Boc_2O/16 h/rt/crystals dissolved in dioxane/pH 12.5/ZONSu/16 h/rt (quant); (b) CH_2Cl_2/MeOH (9:1)/TBTU/pH 8.5/1.5 h/rt (80%); (c) CH_2Cl_2/dry/-78 °C/DIBAL-H/1 h/-78 °C (quant); (d) CH_2Cl_2/H_2O/NaCN/Ac_2O/TEBAC/0.5 h/0 °C (quant); (e) Ether/MeOH (9:1)/-20 °C/HCl g/16 h/0-5 °C (89%); (f) DMF/Boc_2O/Et_3N (pH 8.5)/1h/rt (45%) (g) DMF/Pd(C) 10%/H_2/rt (quant); (h) DMF/0 °C/HOBT/DCCI/0.5 h/0 °C/16 h/rt (88%); (i) CH_2Cl_2/1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one/0.5 h/rt (quant); (j) dioxane/H_2O (9:1)/pH 11.5/rt (quant); (k) TFA/CH_2Cl_2 (1:1)/1h/rt/purified by prep. HPLC (36%).

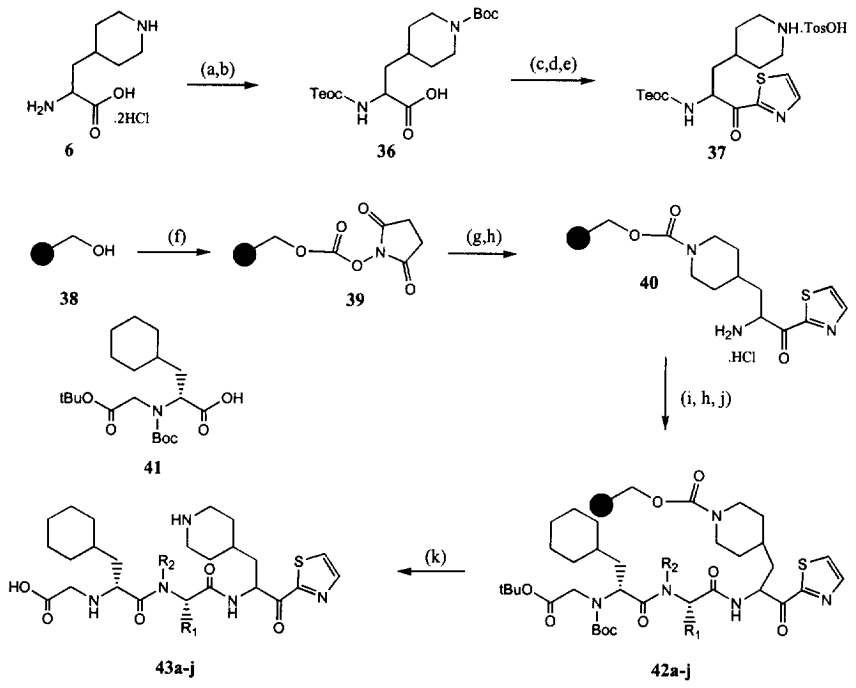
Compound **3** was prepared from acetamidomalonate and 4-picolylchloride in NaOEt. Via saponification of one ethylester, a decarboxylation (**4**), a hydrogenation (**5**) and a deprotection step the 4-piperidine-amino acid **6** was obtained (prepared before by two other groups).[10] **7** was prepared in one pot via pH-controlled Cu-complex formation. Hereafter, the methoxymethylamide (**8**) was synthesized and transformed into the thiazolidine **9**. After removal of the Boc-group the 4-piperidineketothiazole **10** was coupled with the dipeptide **13** to give tripeptide **14**. Dipeptide **13** was prepared by the amide coupling of Boc-Pro-OH with H-Pro-OPac (**11**), followed by Boc-deprotection, ethylsulphonylation and the removal of the Opac-group by TBAF. Finally the tripeptide **14** was deprotected and purified by HPLC to provide ethylsulphonyl-D-Cha-Pro-D/L-4-Pip-KTZ (**15**).

Compound **16** was prepared in a similar manner as compound **7**. The cyanoacetate (**18**) was prepared from the crude aldehyde **17**, esterified to the methylester **19** and deprotected to yield the 4-piperidine building block **20** which was coupled to the ethylsulphonyldipeptide **13**. The tripeptide **21** was oxidized to the ketocarboxylic acid by a Dess-Martin oxidation and saponificated and deprotected to give ethylsulphonyl-D-Cha-Pro-D/L-4-Pip-(CO)-OH (**22**) which was purified by HPLC.



Scheme 3 Stereoselective synthesis route towards ketothiazole and ketocarboxylate containing thrombin inhibitors. (a) H_2/Pd(C) 10%/EtOH (97%); (b) Boc_2O/Et_3N/DMF (90%); (c) pivaloyl chloride at -70 °C then S-4-benzyl-2-oxazolidinone/Buli/THF (70%); (d) KHMDS/Trisylazide/THF (69%); (e) $LiOH/H_2O_2$/THF/H_2O (2:1) (96%); (f) N,O-dimethylhydroxylamine/TBTU/Et_3N/CH_2Cl_2 (100%); (g) HCl g in dioxane (99%); (h) TeocONSu/DMF/Et_3N (99%); (i) H_2/Pd(C) 10% (99%); (j) Boc_2O/Et_3N/DMF (94%); (k) 2-bromothiazole/Buli/ether (55%); (l) *p*-Toluenesulfonic acid hydrate/ether/EtOH (85%); (m) Isobutyl chloroformate/**13**/DMF/Et_3N (65%); (n) HCl g in dioxane, purified by prep. HPLC (31%); (o) CH_2Cl_2/MeOH/TBTU/Et_3N (100%); (p) H_2/Pd(C) 10%/DMF (89%); (q) ZONSu/DMF/Et_3N (90%); (r) DIBAL-H/CH_2Cl_2; (s) NaCN/Ac_2O/TEBAC/CH_2Cl_2/H_2O (64%); (t) HCl g in MeOH/Boc_2O (26%); (u) NaOH/dioxane:H_2O (7:3) (100%); (v) Benzylamine/EDCI/HOBT/NMM/DMF (91%); (w) H_2/Pd(C) 10% followed by DCCI/HOBT/NMM/DMF/acid R-OH with R see **35a-d** (98%); (x) 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one/CH_2Cl_2 (93%); (y) HCl g in dioxane, purified by prep. HPLC (52%).

The synthetic routes leading to chirally pure 3-(4-piperidinyl)-alanine containing thrombin inhibitors **31** and **35a–d** are depicted in Scheme 3. The synthesis of S-2-azido-3-(N-Boc-piperidyl)-propionic acid (**27**) was prepared in 5 steps from 3-(4-piperidyl)-acrylic acid (**23**) using Evans chiral auxiliary. Using compound **27** as a starting material, **30** was prepared in six steps. Chiral HPLC with a Chiralpak AD column showed one enantiomer ($[\alpha]_D$ +31.9^0 , c = 0.45 in chloroform; racemate prepared separately showed two peaks). Selective deprotection, mixed anhydride coupling and preparative HPLC gave compound **31**. Preparation of **34** from S-2-azido-3-(N-Boc-piperidyl)-propionic acid (**27**) was accomplished in six steps. Saponification of the methylester, amidation with benzylamine and hydrogenolysis gave the free amine. Finally, amine **34** was coupled with four different acids, resulting, after oxidation and deprotection, in four end-products **35a–d.**



Scheme 4 Solid phase synthesis of a series of 3-(4-piperidinyl)-alanine containing protease inhibitors. (a) dioxane/H_2O (3:2)/$CuSO_4$/pH 9/Boc_2O/16 h/rt; (b) dioxane/H_2O (3:2)/pH 12.5/Teoc-Cl/16 h/rt (81%); (c) CH_2Cl_2/N,O-dimethylhydroxylamine/TBTU/DIPEA/1 h/rt (85%); (d) THF/BuLi/2-bromothiazole/1 h/-78 °C (85%); (e) Ether/1 eq. TosOH/ether/2 h/35 °C (quant); (f) CH_3CN/CH_2Cl_2 (1:1)/Hydroxymethyl polystyrene/$CO(OSu)_2/Et_3N$/2 h/rt (quant); (g) CH_3CN/CH_2Cl_2 (1:1)/**37**/Et_3N/16 h (90%); (h) 1. TFA/CH_2Cl_2 (1:1)/30 min/rt; 2. 1 M HCl/dioxane/1 min; (i) NMP/**a-j**/HOBt/DIPC/2 h; (j) 1. TFA/CH_2Cl_2 (1:1)/30 min/rt; 2. NMP/**41**/HOBt/DIPC/2 h; (k) TFA/thioanisole (9:1)/4 h/rt.

a-j	R_1	R_2
Boc-L-Phe	Bn	H
Boc-D-Phe	Bn	H
Boc-Gly	H	H
Boc-Sar	H	Me
Boc-Thr	$CH_2CHOHCH_3$	H
Boc-Gln	$CH_2CH_2CONH_2$	H
Boc-Glu(OtBu)	CH_2COOH	H
Boc-Ile	$CH_2CH(CH_3)_2$	H
Boc-Pro	$(CH_2)_3$	
Boc-Cha	$CH_2C_6H_{12}$	H

Next, the solid-phase synthesis of 3-(4-piperidine)-alaninyl ketothiazole containing derivatives **43a–j** was investigated. In view of the structural features of the target structures we considered attachment of the piperidyl moiety to the resin as most appropriate. Starting from **6** we first prepared compound **36** in a similar fashion as described for **9** but using Teoc-Cl instead of CBz-Cl. The Boc protective group could be removed selectively by treatment with 1 equiv TosOH to give **37**. Hydroxymethyl polystyrene was functionalized with di-succinimidyl carbonate to give **39**. Condensation of **37** with **39** was readily achieved in the presence of triethylamine to give immobilized **40** in almost quantitative yield.

Removal of the Boc protective group by standard conditions, exchange of trifluoracetate by chloride, followed by DIPC-HOBt mediated reaction with an appropriate Boc-protected amino acid gave the corresponding immobilized dipeptide. Removal of the Boc protective group and coupling with **41** gave **42a–j**. Finally, cleavage of the tripeptides from the resin was achieved by treatment with TFA-thioanisole. Purification by Sephadex chromatography afforded pure **43a–j** in an overall yield of 40–60%. Using this solid phase method in total approximately 150 compounds could be obtained in a short period of time.

Biology **Table 1.**

Compound	IC_{50} Thrombin [μM]	IC_{50} factor Xa [μM]
15	**0.16**	**4.1**
22	**0.22**	**16**
31	**0.12**	**2.5**
35a	**0.59**	**3.6**
35b	**0.048**	**2.9**
35c	**0.26**	**54**
35d	**1.4**	**5.2**
43a	**3.1**	**7.8**
43c	**19.4**	**1.1**
43d	**4.8**	**0.43**
Reference compound:		
Argatroban	**0.55**	**134**

The biological assays to determine the IC_{50} values have been described and validated elsewhere in detail.[11] Argatroban was used as a reference compound by us and other investigators. Within the series depicted in Table 1 activities may be compared. It is clear that a whole spectrum of potent thrombin inhibitors came out of the synthetic work. **15** and **22** were first generation compounds that were prepared racemically. Both compounds were active thrombin inhibitors but the diastereomers could not be separated. These results prompted work on a stereoselective route that yielded **31** and **35a–d**. Interestingly, the P_3-P_2 moieties that were used by others in the field, in our hands resulted in a range of activities, indicating that the piperidinyl moiety binds differently from straight chain amines and guanidines (X ray data confirming this will be reported separately). A third approach was to find more selective factor Xa compounds and a new solid phase method was developed for that purpose. Compounds **43c** and **43d** were the first examples with a preference for factor Xa and formed the basis for a library of these piperidinyl containing protease inhibitors.

In summary, a new and efficient P_1 isostere in the serine protease area was introduced. It only exerts its function if proper moieties are placed next to it. Apparently, the conformational restriction in the P_1 group limits the degree of freedom in the other active site pockets including the TSA part. Further pharmacological studies with these compounds are in progress and will be reported in future.

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11. (a) Jetten, M.; Peters, C. A. M.; Visser, A.; Grootenhuis, P. D. J.; van Nispen, J. W.; Ottenheijm, H. C. J. *Bioorg Med Chem.* **1995**, *3*, 1099. (b) Using a large data set, an accurate correlation between IC_{50} and K_i could be established, confirming the reported K_i for Argatroban in our assay.