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Orally active thrombin inhibitors. Part 2: Optimization of the P2-moiety

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Abstract—Synthesis and SAR of orally active thrombin inhibitors of the D-Phe-Pro-Arg type with focus on the P2-moiety are described. The unexpected increase in in vitro potency, oral bioavailability, and in vivo activity of inhibitors with dehydroproline as P2-isostere is discussed. Over a period of 24 h the antithrombin activity of the most active inhibitors with IC_{50}s in the nanomolar range was determined in dogs demonstrating high thrombin inhibitory activity in plasma and an appropriate duration of action after oral administration.
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Thrombin has long been the main focus of thrombosis research. Its role in blood coagulation and its inhibition as a therapeutic opportunity for thromboembolic disorders like deep vein thrombosis (DVT), myocardial infarction, unstable angina, pulmonary embolism, and ischemic stroke have been extensively reviewed.[1]

Although several highly potent and selective thrombin inhibitors have been described, most of them lack suitable oral bioavailability[2] and plasma half-life. The main strategies[1b] pursued to address these problems have been to increase the lipophilicity of the inhibitor and/or to reduce the basicity of the Arg mimicking moiety. Approaches drawing on more lipophilic inhibitors are often hampered by substantial interspecies variations of the oral bioavailability[3] and high plasma protein binding.[4] Attempts to reduce the basicity of the P1-moiety led to prodrug approaches like ximelagatran the double prodrug of the thrombin inhibitor melagatran[5] which have both recently been approved in some European countries for DVT[6] despite some liver related safety concerns.[7]

SAR studies and optimization of our initial leads finally resulted in the development of orally active thrombin inhibitors of the D-Phe-Pro-Arg type with an *N*-(carboxymethyl)-D-cyclohexyl alanine unit as P4–P3-moiety and a heteroarylamidine as P1-moiety.[8] A major part of these studies was concerned with the detailed exploration of the P pocket of thrombin resulting in modifications of the P2-moiety that not only led to inhibitors with improved in vitro potency but also with enhanced oral bioavailability.

The following discussion will be based on structures with the above-mentioned P4–P3-building block, 2-amidino-5-aminomethylpyridine as P1-moiety and variations of the P2-moiety. A detailed discussion of the SAR obtained by the variation of the P4–P3- and P1-moieties has in part been reported earlier[8i] and will be presented separately.[8a,b]

One of the major structural differences between thrombin and the related serine protease trypsin is its Tyr-Pro-Pro-Trp insertion loop at position 60. When the natural substrate fibrinogen is bound, the constrained P pocket is occupied by Val. Bajusz and co-workers[9] introduced Pro as a P2-isostere, a building block that was subsequently used in numerous thrombin inhibitors. Based on these results and our own findings[8] we initiated the following synthesis program directed toward the optimization of the P2-moiety.

The thrombin inhibitors were prepared by a convergent synthesis. A representative example is depicted in

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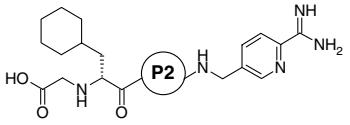
Scheme 1.[10] The dehydroproline synthesis is suitable for scale-up.[8h,11] The P3–P4-building block **4** was easily obtained from Cha-OBn.[8b,h] The two amide bonds of the inhibitor were established with 1-propanephosphonic acid cyclic anhydride (PPA)[12] as coupling reagent and the extremely mild conversion of nitrile **5** to amidine **6** did neither affect the stereocenters nor the double bond of the pyrrolidine ring.[13]

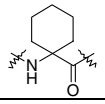
Thrombin inhibitors of the type described above with flexible amino acid building blocks in the P2 position exhibit good to moderate in vitro potency and weak to moderate in vivo activity in rat (Table 1). The alanine derivative **7** had about the same in vitro potency and in vivo activity as melagatran[14] (cf. Table 2).[15,16] The in vitro potency of the epimer **8** was substantially lower. The *N*-methyl alanine derivative **9** was slightly more potent in vitro than **7**. The X-ray structure of **9** in thrombin revealed that the two methyl groups of the P2 unit are engaged in favorable hydrophobic interactions in the P pocket (cf. Fig. 1).[17]

The α-methyl alanine **10** and the inhibitors **11** and **13** containing a 1-amino-1-carboxy-cycloalkyl building block had only low to moderate activities. The disubstituted α-position either leads to an inhibitor conformation unfavorable for binding to the enzyme or the additional α-substituent sterically interferes with the van der Waals surface of the P pocket. The latter is consistent with the low activity observed for D-alanine **8**. An exception is the 1-amino-1-carboxy-cyclopentyl derivative **12** that showed good potency in the chromogenic thrombin assay, although the cyclopentyl ring came in close contact with Cδ-2 of Leu99. Trp60D had to be pushed away by 0.86 Å to accommodate this P2-moiety.[18] The energy required for this rearrangement is somewhat compensated by the large additional hydrophobic contact area between the cyclopentyl ring and the 60 loop of thrombin (cf. Fig. 1).

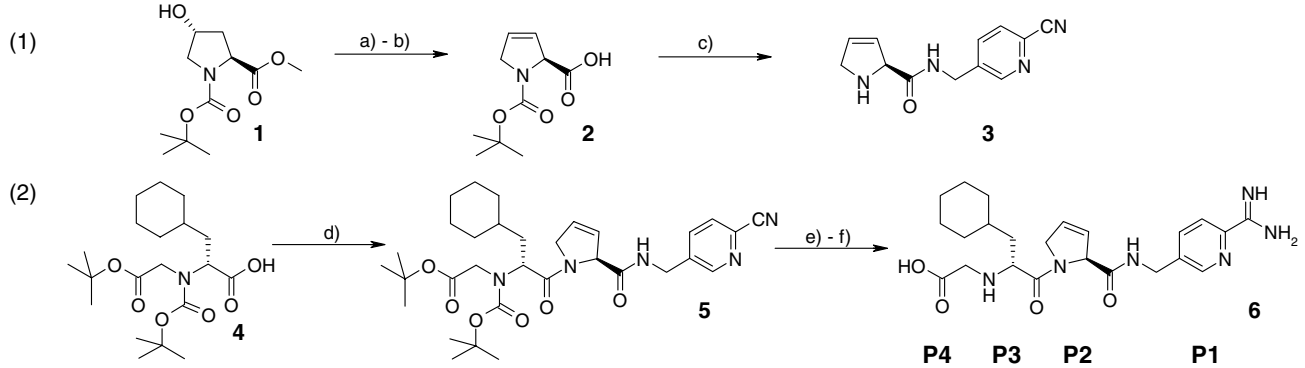
Within this set of inhibitors (Table 1) *N*-methyl alanine **9** displayed the best in vitro potency, but did not exhibit sufficient oral activity. Replacement of this flexible moiety by the more rigid Pro gave the nanomolar thrombin inhibitor **14**,[8d] which also had good oral activity in rat (Table 2). Introduction of dehydroproline (Dhp, compound **6**) in place of Pro slightly increased the in vitro potency and the high in vivo activity was maintained. Although the increase in in vitro potency of the dehydroproline derivative compared to its proline analog is

Table 1. Effect of flexible P2 moieties on the in vitro potency and in vivo activity of the thrombin inhibitors



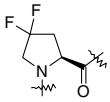
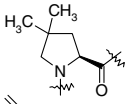
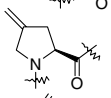
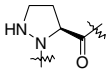
Compound	P2	Chromogenic thrombin assay IC_{50}[a] (nM)	ECT (s), po (rat)[b]	ECT(s), iv (rat)[c]
7		76.8	261	83
8		53600	n.d.	n.d.
9		20.2	153	75
10		581	92	59
11		4090	n.d.	n.d.
12		47.3	80	46
13		103	43	35

n.d., not determined.
[a] See Ref. 15.
[b] Measured 60 min after 21.5 mg × kg^{-1} po, see Ref. 16.
[c] Measured 60 min after 1.0 mg × kg^{-1} iv, see Ref. 16.



Scheme 1. Convergent synthesis of a D-Phe-Pro-Arg type thrombin inhibitor. Reagents and conditions: (a) 1. 1.1 equiv NEt_3, 1.05 equiv MsCl, 1 mol% DMAP, DCM, −10 to 0 °C, 2 h, 98%, 2. 2 equiv aqueous 1 N NaOH, dioxane, 0–5 °C, 2.5 h, 87%; (b) 2.7 equiv $MeOCH_2CH_2OH$, 2.5 equiv NaH, DME, 0 °C to rt, 12 h, 52%; (c) 1. 5-aminomethyl-2-cyanopyridine, 1.3 equiv 50% PPA in EtOAc, 4 equiv DIEA, DCM, 2. HCl, *i*-PrOH, 80% (2 steps); (d) **3**, 1 equiv 50% PPA in EtOAc, 4 equiv DIEA, DCM, 0 °C to rt, 95%; (e) NH_3(g), 1.05 equiv *N*-acetylcysteine, MeOH, Δ; (f) HCl, H_2O, 60 °C, 3 h, 45% (2 steps).

Table 2. Effect of cyclic P2 moieties on the in vitro potency and in vivo activity of the thrombin inhibitors

Compound	P2	Chromogenic thrombin assay IC_{50}[a] (nM)	ECT (s), po (rat)[b]	ECT(s), iv (rat)[c]
14		3.2	800	104
6		1.8	792	138
15		14.8	100	54
16[d]		71.2	100	59
17		290	96	38
18		44.2	55	37
19		31.9	229	59
20[d]		56.3	74	47
21		58.4	516	49
22[d]		16.0	196	77
23		288	141	64
24[d]		1.7	726	201
25[d]		3.4	640	114
Melagatran		69.2	247	98

[a] See Ref. 15.
[b] Measured 60 min after 21.5 mg × kg^{-1} po, see Ref. 16.
[c] Measured 60 min after 1.0 mg × kg^{-1} iv, see Ref. 16.
[d] See Ref. 21.

not significant, it was observed as a general trend in most of the D-Phe-Pro-Arg type thrombin inhibitors studied.[19] The X-ray structure of **6** in complex with human thrombin[20] revealed an optimal fit of the Dhp moiety in the P pocket with distances of 3.92 Å and 3.91 Å from Cγ of Dhp to Cδ-2 of Leu99, and from Cγ of Dhp to Cη-2 of Trp60D, respectively. Ringflip of the pyrrolidine-Cγ in **14** between the two possible ring conformations representing the two local minima of the Pro showed in both cases unfavorable close contacts with either Leu99 or Trp60D when modeled onto the Dhp of **6** leaving

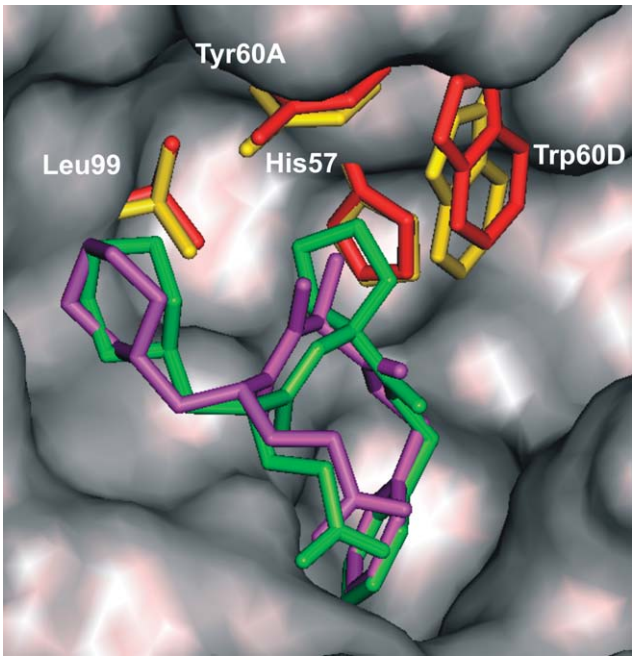


Figure 1. X-ray structure derived structures showing the orientation of the thrombin inhibitors **9** (purple) and **12** (green) in the active site of thrombin. The amino acids Leu99, Tyr60A, Trp60D, and His57 forming the P pocket are highlighted (yellow: complex with **9**; red: complex with **12**).

the coordinates of the backbone Cβ and Cδ unchanged (cf. Fig. 2). The resulting conformational changes in the protein minimize these interactions, but reduce the binding energy of **14** since the hydrophobic contact area of Pro is similar to that of Dhp. This effect is even more pronounced for derivatives **15**[8d] and **16** with the larger pipecolinic and dehydropipecolinic acid moieties.[22] The more flexible piperidine derivative **15** had a 4 times higher in vitro potency than the more rigid dehydropiperidine **16**, which obviously required substantial conformational

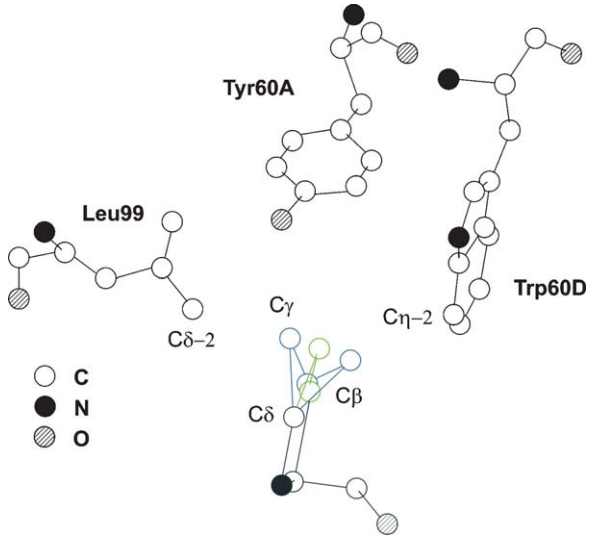


Figure 2. Orientation of the Dhp (Cγ and Cβ in green) and Pro (Cγ and Cβ in blue) moiety of thrombin inhibitors **6** and **14**, respectively, in the P pocket of thrombin based on data from the X-ray structure of the thrombin–inhibitor complex from Dhp derivative **6** and modeling results.

changes in the P pocket to be accommodated. Their in vivo activities, however, were comparable.

Inhibitors with substituents on the proline ring like fluorine (**17**),[23] methyl (**18**)[24] or methylene (**19** and **20**)[25] were less potent than the parent compounds **14** and **6**. The 4-thiaproline **21** exhibited surprisingly high oral activity compared to its moderate potency in the chromogenic thrombin assay. The corresponding 3-thiaproline **22**[26] did not prolong the ecarin clotting time (ECT) to the extent as the better in vitro data would suggest. This could be attributed to its lower metabolic stability. The 4-oxaproline **23**[27] was less potent than its Pro analog **14**. 5-azadehydroproline **24**[28] and 5-azaproline **25**[29] were more potent in vitro. To our surprise they also had a comparatively high oral activity indicating their good metabolic stability. The in vivo activity after oral administration of **24** did, however, not quite match that of dehydroproline **6**.

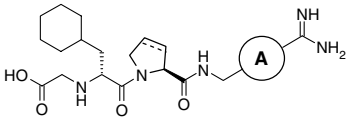
Molecular modeling studies helped to explain the observed in vitro potencies. Cβ of the Pro moiety in **14** forms a van der Waals contact with Cδ-2 of His57 and Cγ of the Pro is located close to the benzene ring of Tyr60A.

Any substituent larger than hydrogen in these positions therefore has a negative effect on the binding energy (cf. **18**–**20**, respectively). Exchange of the γ-methylene group in the Pro moiety of **14** against difluoromethylene, sulfur or oxygen (cf. **17**, **21**, and **23**) increases the electron density in this position introducing an unfavorable interaction with the π-electrons of Tyr60A leading to a loss in potency. In the case of the 4-oxaproline **23**, this effect was especially emphasized since in solution the hydrophilic oxygen can assumed to be hydrated. The bound water molecule has to be removed before the oxazolidine ring can be accommodated in the P pocket. The 3-thiaproline **22** is not involved in such negative interactions. Due to the larger ring size caused by the sulfur atom its in vitro potency, however, is only comparable to that of the slightly less potent pipecolinic acid derivative **15**.

The 5-nitrogen of 5-azadehydroproline **24** and 5-azaproline **25** does neither improve nor hinder the binding to the enzyme[30] because it is exposed to the solvent and not interacting with the protein. The observed in vitro potencies therefore closely resemble those of Dhp derivative **6** and Pro derivative **14**. It is worth noting that the 5-azadehydroproline **24** was slightly more potent in vitro and more active in vivo than the corresponding 5-azaproline **25**.

Pro **14** and Dhp **6**, two of the most potent inhibitors in this series, were chosen for preliminary pharmacodynamic and pharmacokinetic profiling in dog. They displayed an oral bioavailability of 25% and 36%, respectively (Table 3).

Table 3. Effect of Dhp versus Pro in P2 on the in vitro potency, oral bioavailability and in vivo activity of the thrombin inhibitors



Compound	A	P2	Chromogenic thrombin assay IC_{50}[a] (nM)	$AUC_{0-24\,h}$ by $ECT_{mean\pm SD} \times 10^3$ (s)[b] (n = 2–8) (dog)	F_{oral} (%),[c] (dog)
14		Pro	3.2	74.3 ± 3.5	25
6		Dhp	1.8	185.0 ± 68.6*	36
26		Pro	2.7	100.1 ± 26.7	34
27		Dhp	1.7	168.0 ± 38.5*	35
28		Pro	1.0	158.9 ± 70.2	48
29		Dhp	1.0	278.9 ± 47.4*	58
Melagatran			69.2	74.6 ± 27.7	24

[a] See Ref. 15.

[b] After administration of 4.64 mg × kg^{-1}, po. Calculated from data depicted in Figure 3. Asterisk refers to significance ($P < 0.05$, calculated by unpaired t test with Welch's correction) in comparison to the corresponding proline analog.

[c] Calculated from the $AUCs_{0-24\,h}$ after iv and po administration, see Ref. 33.

To study the effect of the Dhp P2-moiety on the in vitro potency, oral bioavailability, and in vivo activity in more detail, a series of thrombin inhibitors as sets of Pro and Dhp pairs with different P1 building blocks was prepared. The benzamidine **26**[31] and thienylamidine **28**[32] have also been described by others. For comparison melagatran was also included in this study. The results of the pharmacodynamic and pharmacokinetic experiments are summarized in Table 3 and Figure 3.

All compounds (**6**, **14**, and **26**–**29**) exhibited higher in vitro potency and in vivo activity higher than those of melagatran. The observed ECTs in particular illustrate the improved oral bioavailability and antithrombin properties of these inhibitors. In all cases, the Dhp derivative matched or surpassed the in vitro potency of the corresponding Pro compound. For the thrombin inhibitors bearing a heterocyclic P1-moiety the Dhp derivatives (**6** and **29**) showed a substantially increased oral bioavailability in dogs compared to the Pro containing compounds (**14** and **28**). In the case of the benzamidine, the measured oral bioavailability was about the same for both derivatives (**26** and **27**).

The activity after oral administration based on the ecarin clotting time of the Dhp derivatives (**6**, **27**, and **29**) was significantly higher than that of the corresponding Pro analogs (**14**, **26**, and **28**; cf. Table 3, Fig. 3). The improvements in ECTs were much higher than the observed differences in in vitro potency would suggest. The cause of this remarkable effect is unknown, but could, e.g., either be due to a higher metabolic stability of the Dhp containing inhibitors and/or could be caused by better active resorption of these analogs possibly favored by the more rigid P2 isostere.

In summary, the synthesis of orally active thrombin inhibitors of the D-Phe-Pro-Arg type with high antithrombin potency has been described. The SAR with respect to the interactions observed for different P2-moieties in the P pocket of thrombin has been discussed. The noteworthy positive effect of the P2-isostere dehydroproline on the in vitro potency and especially the in vivo activity after oral administration has been highlighted.

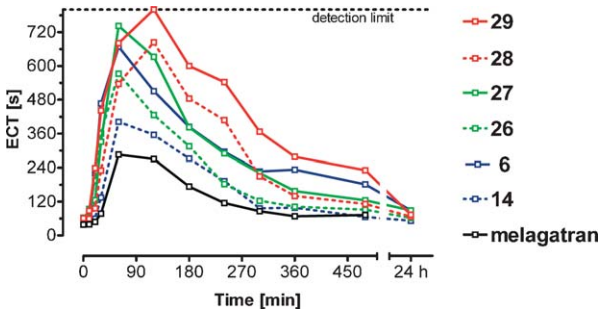


Figure 3. Effect of Dhp versus Pro in P2 on the ecarin clotting time in dogs at a dose of 4.64 mg × kg^{-1}, po.

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References and notes

1. (a) Steinmetzer, T.; Stürzebecher, J. *Curr. Med. Chem.* **2004**, *11*, 2297; (b) Steinmetzer, T.; Hauptmann, J.; Stürzebecher, J. *Exp. Opin. Invest. Drugs* **2001**, *10*, 845; (c) Rewinkel, J. B. M.; Adang, A. E. P. *Curr. Pharm. Des.* **1999**, *5*, 1043; (d) Wiley, M. R.; Fisher, M. J. *Exp. Opin. Ther. Patents* **1997**, *7*, 1265.
2. Menear, K. *Current Med. Chem.* **1998**, *5*, 457.
3. (a) Brady, S. F.; Stauffer, K. J.; Lumma, W. C.; Smith, G. M.; Ramjit, H. G.; Lewis, S. D.; Lucas, B. J.; Gardell, S. J.; Lyle, E. A.; Appleby, S. D.; Cook, J. J.; Holahan, M. A.; Stranieri, M. T.; Lynch, J. J.; Lin, J. H.; Chen, I.-W.; Vastag, K.; Naylor-Olsen, A. M.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 401; (b) Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Sisko, J. T.; Lynch, J. J.; Lyle, E. A.; Baskin, E. P.; Woltmann, R. F.; Appleby, S. D.; Chen, I.-W.; Dancheck, K. B.; Naylor-Olsen, A. M.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 3687.
4. Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I.-W.; Dancheck, K. B.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 1565.
5. Gustafsson, D.; Nyström, J. E.; Carlsson, S.; Bredberg, U.; Eriksson, U.; Gyzander, E.; Elg, M.; Antonsson, T.; Hoffmann, K.; Ungell, A.; Sorensen, H.; Nagard, S.; Abrahamsson, A.; Bylund, R. *Thromb. Res.* **2001**, *101*, 171.
6. Fiessinger, J.-N.; Huisman, M. V.; Davidson, B. L.; Bonameaux, H.; Francis, C. W.; Eriksson, H.; Lundström, T.; Berkowitz, S. D.; Nyström, P.; Thorsén, M.; Ginsberg, J. S. *JAMA* **2005**, *293*, 681.
7. Gurewich, V. *JAMA* **2005**, *293*, 736.
8. (a) Modification of the P1-moiety: Baucke, D.; Hornberger, W.; Lange, U. E. W.; Mack, H.; Seitz W. *Bioorg. Med. Chem. Lett*. preceding manuscript; (b) Modification of the P3-moiety and P4-moiety: Baucke, D.; Hornberger, W.; Lange, U. E. W.; Mack, H.; Seitz W. manuscript in preparation; (c) Böhm, H.-J.; Koser, S.; Mack, H.; Pfeiffer, T.; Seitz, W.; Höffken, H. W.; Hornberger, W. WO9535309, 1995; *Chem. Abstr.* **1995**, *124*, 233168; (d) Böhm, H.-J.; Höffken, H. W.; Hornberger, W.; Koser, S.; Mack, H.; Pfeiffer, T.; Seitz, W.; Zierke, T. WO9625426, 1996; *Chem. Abstr.* **1996**, *125*, 301605; (e) Baucke, D.; Lange, U.; Mack, H.; Seitz, W.; Zierke, T.; Höffken, H.-W.; Hornberger, W. WO9806741, 1998; *Chem. Abstr.* **1998**, *128*, 192940; (f) Baucke, D.; Lange, U.; Mack, H.; Seitz, W.; Höffken, H.-W.; Hornberger, W. WO9937668, 1999; *Chem. Abstr.* **1999**, *131*, 116527; (g) Solid-phase synthesis of thrombin inhibitors: Lange, U. E. W.; Zechel, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1571; (h) Technical scale synthesis of a new and highly potent thrombin inhibitor: Bernard, H.; Bülow, G.; Lange, U. E. W.; Mack, H.; Pfeiffer, T.; Schäfer, B.; Seitz, W.; Zierke, T.

Synthesis **2004**, 2367; (i) Modification of the P1-moiety giving unexpected selectivity: Lange, U. E. W.; Baucke, D.; Hornberger, W.; Mack, H.; Seitz, W.; Höffken, H. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2029.

9. (a) Pozsgay, M.; Szabo, G. C. S.; Bajusz, S.; Simonsson, R. *Eur. J. Biochem.* **1981**, *115*, 491; (b) Bajusz, S.; Barabás, E.; Bragdy, D. In *Proc. 17th Eur. Pept. Symp. 1982*, Blaja, K., Malon, P., Eds.; Walter de Gruyter & Co.: Berlin—New York, 1983, p 643.;
10. All thrombin inhibitors were prepared by this route unless indicated otherwise. Alternatively the linear route described in Ref. 8d,i or the solid-phase synthesis described in Ref. 8g could be used.
11. Mack, H.; Pfeiffer, T.; Seitz, W.; Zierke, T.; Balkenhohl, F.; Lange, U. WO9804523, 1998; *Chem. Abstr.* **1998**, *128*, 154380.;
12. Wissmann, H.; Kleiner, H.-J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 133.
13. (a) Lange, U. E. W.; Schäfer, B.; Baucke, D.; Buschmann, E.; Mack, H. *Tetrahedron Lett.* **1999**, *40*, 7067(b) Less reactive thienyl- and benzonitrile derivatives were converted to the corresponding amidines by the Thio-Pinner reaction described in Ref. 8e.;
14. Gustafsson, D.; Antonsson, T.; Bylund, R. *Thromb. Haemost.* **1998**, *79*, 110.
15. (a) For in vitro assay protocols, see Ref. 8f.; (b) The standard error of the IC_{50} determinations was less than 1% with $r^2 > 0.99$ for the curve fit.
16. (a) Nowak, G. *Pathophysiol. Haemost. Thromb.* **2003**, *33*, 173(b) Measurement of ecarin clotting time (ECT): Venous blood is collected from rats or dogs, respectively, at the desired time after administration of the thrombin inhibitor. One hundred microliters of citrated blood is pipetted into a coagulation cuvette and incubated for 2 min at 37 °C in a coagulometer. After addition of 100 μL of prewarmed (37 °C) ecarin reagent (Pentapharm), the time (ECT) to formation of a fibrin clot is determined.;
17. X-ray structure analysis of thrombin–inhibitor complex of **9**; X-ray crystallographic data have been deposited with the Brookhaven Protein Data Bank. Deposition code 2A2X.
18. X-ray structure analysis of thrombin–inhibitor complex of **12**; X-ray crystallographic data have been deposited with the Brookhaven Protein Data Bank. Deposition code 2ANK.
19. Unpublished results.
20. X-ray structure analysis of thrombin–inhibitor complex of **6**; X-ray crystallographic data have been deposited with the Brookhaven Protein Data Bank. Deposition code 2ANM.
21. The inhibitor was obtained as a mixture of diastereomers due to a racemic P2-building block, the diastereomers were separated by HPLC and tested separately. In Table 2, the biological data of the more active analog are shown. By comparison with very related inhibitors of known absolute configuration we are confident to assign the stereochemistry of the P2-moiety depicted in Table 2.
22. For the synthesis of (*S*)-1,2,3,6-tetrahydropyridine-2-carbonic acid, cf.: Guillena, G.; Najero, C. *J. Org. Chem.* **2000**, *65*, 7310.
23. Compound **17** was prepared by linear synthesis, cf. Ref. 8d examples 32 and 93. For the synthesis of (*S*)-4-difluoroproline building block, cf.: (a) Burger, K.; Rudolph, M.; Fehn, S.; Sewald, N. *J. Fluorine Chem.* **1994**, *66*, 87; (b) O'Neil, I. A.; Thompson, S.; Kalindjian, S. B.; Jenkins, T. C. *Tetrahedron Lett.* **2003**, *44*, 7809.
24. Compound **18** was prepared by linear synthesis, cf. Ref. 8d examples 48 and 93. For the synthesis of (*S*)-4,4-dimethylproline building block, cf.: (a) Hippeli, C.; Reissig, H. U. *Liebigs Ann. Chem.* **1990**, 475; (b) Ezquerra, J.; Pedregal, C.; Rubio, A. *J. Org. Chem.* **1994**, *59*, 4327.
25. For the synthesis of (*S*)-4-methylene- and (*S*)-3-methylene-pyrrolidine-2-carbonic acid building blocks, respectively, cf.: (a) Manfré, F.; Kern, J.-M.; Biellmann, J.-F. *J. Org. Chem.* **1992**, *57*, 2060; (b) Tandon, M.; Wu, M.; Begley, T. P.; Myllyharju, J.; Pirskanen, A.; Kivirikko, K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1139. Compound **19** was prepared by linear synthesis, cf. Ref. 8d examples 32 and 93.
26. For the synthesis of the *rac*-thiazolidine-2-carbonic acid building block, cf.: Johnson, R. L.; Smissman, E. E. *J. Med. Chem.* **1978**, *21*, 165.
27. Compound **23** was prepared by linear synthesis, cf. Ref. 8d examples 32 and 93. Synthesis of the 4-oxaproline building block: Conti, S.; Cossu, S.; Giacomelli, G.; Falorni, M. *Tetrahedron* **1994**, *50*, 13493.
28. Compound **24** was prepared by linear synthesis. Compound **4** was coupled successively with the P2-building block and with 5-aminomethyl-2-cyanopyridine. The standard amidine synthesis and deprotection gave **24**. Synthesis of the 5-azadehydroproline building block: (a) Mish, M. R.; Guerra, F. M.; Carreira, E. M. *J. Am. Chem. Soc.* **1997**, *119*, 8379; (b) Guerra, F. M.; Mish, M. R.; Carreira, E. M. *Org. Lett.* **2000**, *2*, 4265.
29. Compound **25** was prepared by linear synthesis. Compound **4** was coupled successively with the P2-building block and with 5-aminomethyl-2-cyanopyridine. The standard amidine synthesis and deprotection gave **25**. Synthesis of the 5-azaproline building block: Liu, B.; Brandt, J. D.; Moeller, K. D. *Tetrahedron* **2003**, *59*, 8515.
30. The conclusions were drawn by analogy visualizing molecule **6** in the binding pocket of thrombin with QUANTA (Accelry, San Diego, USA 2002).;
31. (a) Antonsson, K. T.; Bylund, R.; Gustafsson, N. D.; Nilsson, N. O. WO9429336, 1994; *Chem. Abstr.* **1995**, *122*, 285553.; (b) Onshima, M.; Iwase, N. Sugiyama, S. EP669317, 1995; *Chem. Abstr.* **1996**, *124*, 56705.;
32. Smith, G. F.; Wiley, M. R.; Schacht, A. L.; Shuman, R. T. WO9523609, 1995; *Chem. Abstr*. **1996**, *124*, 87791.;
33. (a) Plasma levels of thrombin inhibitors were determined in dogs by means of *ex vivo* measurements of ECT in plasma (cf. Ref. 16b). The ECT increases linearly with plasma concentration and therefore provides a suitable method for preliminary pharmacodynamic/pharmacokinetic assessment (cf. Ref. 16a). For the calculation of oral bioavaolability (F) the $AUC_{0-24\,h}$ of the plasma levels were determined after oral administration of $4.64\ mg \times kg^{-1}$ and intravenous administration of $1.0\ mg \times kg^{-1}$, respectively.