Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 10, pp. 1283-1288, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00210-2

DERIVATIVES OF 4-AMINO-PYRIDINE AS SELECTIVE THROMBIN INHIBITORS

Wolfgang von der Saal,* Ralf Kucznierz, Herbert Leinert, and Richard A. Engh#

Department of Chemistry, Boehringer Mannheim GmbH, Sandhofer Str. 116, D-68305 Mannheim, Germany
 # Max-Planck-Institut für Biochemie, D-82152 Martinsried, Germany

Abstract: During screening for novel thrombin inhibitors it was discovered that the 4-aminopyridine derivative **1** inhibits human α-thrombin competitively and selectively. The 4-aminopyridine moiety itself is most likely the major determinant of the selectivity due to the increased hydrophobicity of the S1 pocket in thrombin compared to trypsin and plasmin. Optimization led to the selective thrombin inhibitor **14** with an inhibition constant, Ki of 70 nM. © 1997 Elsevier Science Ltd.

Introduction:

The serine protease thrombin occupies a central position in the blood coagulation cascade. The inhibition of thrombin catalyzed activation of fibrinogen has therefore become a primary target for the development of novel anticoagulants.[1] A therapeutically useful protease inhibitor should be potent, selective, and orally bioavailable. The potency of thrombin inhibitors has been the focus of much attention because this property can be most directly optimized based on the crystal structure of thrombin.[2] Optimization of selectivity is also possible but requires comparison of a larger number of crystal structures; thrombin has several unique features at the binding site which distinguish it from related enzymes. The best studied inhibitors are mostly derived from the peptide sequence D-Phe-Pro-Arg[3] or benzamidine analogs thereof.[4] The strong bases which comprise the P1 residues of these inhibitors remain completely protonated under all physiological conditions. This property hampers the passive diffusion across the intestinal barriers, which is the main route of absorption for most drugs.[5] The replacement of the strongly basic groups in potent thrombin inhibitors by less basic amines is usually accompanied by a decreased potency,[6] reflecting the substrate preference of thrombin for arginine over lysine.

Given this limited success with early thrombin inhibitors, we pursued an inverse approach. We screened our in-house library of compounds for possible lead structures bearing moieties with acidity constants $pKa < 10$. Such compounds can cross the intestinal barriers more easily than their more basic analogs. We reasoned that they could be converted to potent and selective thrombin inhibitors by exploiting the potential of structure based drug design.[7]

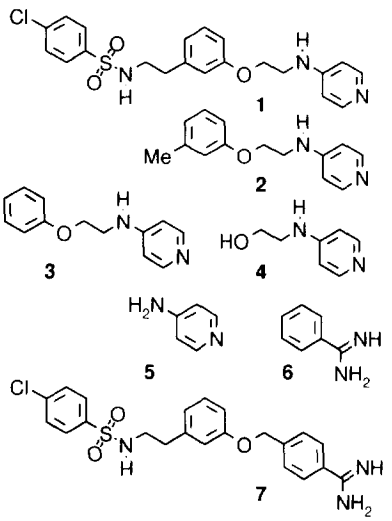
E-mail: wolfgang_von-der-saal@bmg.boehringer-mannheim.com
Fax: +49-621-759 4161

Results and Discussion:

During screening, we discovered that the 4-aminopyridine derivative **1** is a competitive thrombin inhibitor with the critical p*K*a of 9.2. Furthermore, **1** is surprisingly selective: it neither inhibits trypsin nor the fibrinolytic enzyme plasmin (Table 1). We modeled the binding of **1** to the active site region of thrombin using the crystalstructure of the NAPAP / thrombin complex:[8] 4-Aminopyridine is protonated at the aromatic nitrogen atom,[9] which makes it a suitable partner for a salt bridge to Arg189 in the selectivity pocket.[10] In the model, the central and the terminal phenyl groups of **1** occupy the proximal and distal pockets, and the sulfonamide nitrogen atom is hydrogen bonded to the carbonyl oxygen atom of Gly216.

In order to improve the potency of **1** without impairing the selectivity we investigated the contributions of the different parts of the lead compound **1** to the binding energy. The inhibition constant increases considerably when the arylsulfonamide moiety is removed (**2**, **3**), while compounds which lack the central phenyl group (**4**, **5**) do not inhibit thrombin at all. This is in stark contrast to benzamidine (**6**), which derives its binding energy solely from the occupation of the selectivity pocket. Therefore, we assumed that the potency towards thrombin inhibition would increase when the 4-aminopyridine moiety of the lead compound was replaced by benzamidine. However, the resulting compound **7** was only slightly more potent then **1**. The selectivity profile of **7** was inferior to that of **1**, which reflects the preference of benzamidine for trypsin, and underscores the significance of 4-aminopyridine for the selectivity of the lead compound **1**.

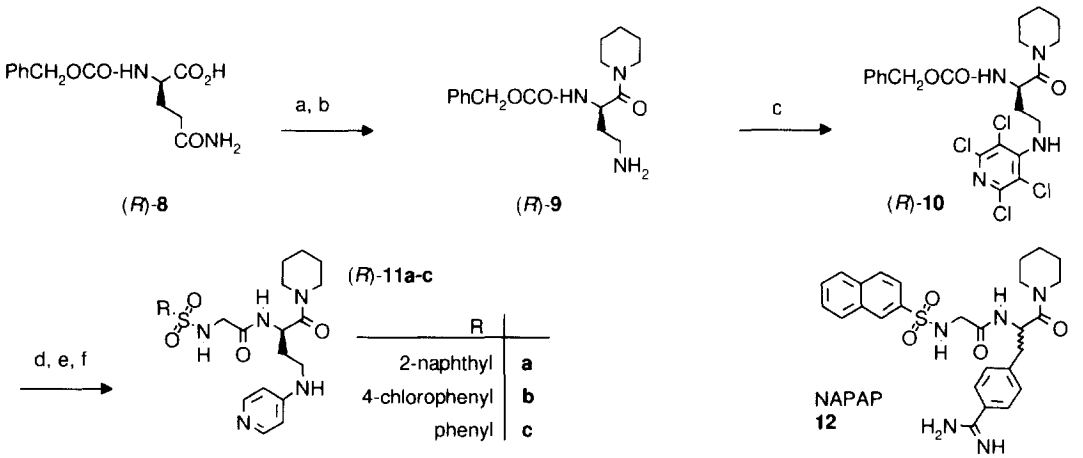
Table 1. Inhibition of the catalytic activity of human α-thrombin, bovine trypsin, and human plasmin at 25 °C and pH = 7.5



	Inhibition constants Ki [μM][11]		
	thrombin	trypsin	plasmin
1	2.8	>500	>500
2	30	>500	>500
3	120	>500	>500
4	>500	>500	>500
5	>2000	>2000	>2000
6[12]	220	35	350
7	1.5	1.7	34

In the inverse experiment, we replaced the benzamidine moiety of the potent thrombin inhibitor NAPAP (**12**)[13] by 4-aminopyridine to give **11a** (Scheme 1). Both enantiomers of this compound were prepared starting from a suitably protected glutamine derivative, e.g. (*R*)-**8**. Hofmann degradation using a hypervalent iodine reagent converted the amide to the amino group (**9**). Initial attempts to introduce the pyridine moiety by reacting **9** with pentachloropyridine lead to mixtures of the 2- and 4-aminopyridine derivatives. Also, 4-chloropyridine reacted only at elevated temperatures with concomitant racemization and production of tarry material. Finally, 4-nitro-tetrachloro-pyridine[14] proved to be the reagent of choice. It reacted smoothly at low temperatures and gave the tetrachloro derivative (*R*)-**10** in good yield. The chlorine atoms were removed by hydrogenolysis in the final step.

Scheme 1. Synthesis of the 4-aminopyridine congeners (*R*)-**11a-c** of NAPAP (**12**)



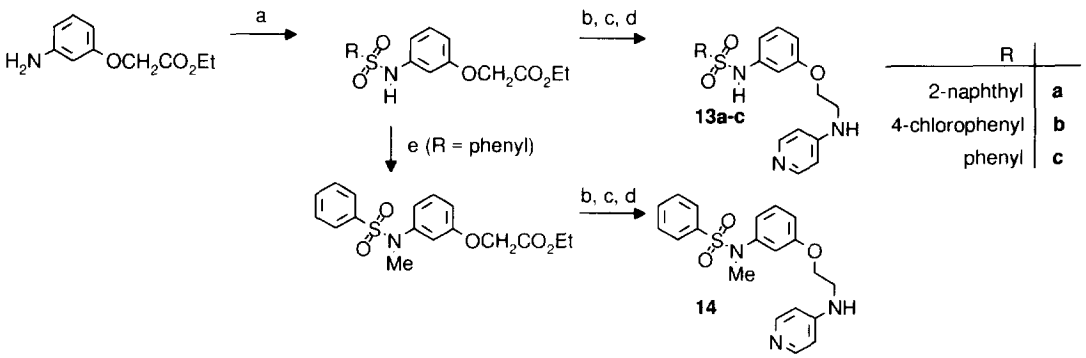
(a) (i) N-hydroxy succinimide, DCC, r.t., 20h, (ii) piperidine, r.t., 20 h, (50%); (b) $PhI(OCOCF_3)_2$, r.t., 16h (67%); (c) 4-nitro-tetrachloropyridine, NMM, r.t., 3h (60%); (d) HBr, HOAc, r.t., 16 h (74%); (e) R-sulfonyl-glycyl chloride, NMM, r.t., 2h (50%); (f) H_2, Pd/C, NaOMe in MeOH (45%).

Again, the 4-aminopyridine derivative (*R*)-**11a** was less active, although more selective, than NAPAP (**12**) (Table 2). (*S*)-**11a** was also prepared and tested, and was found to be devoid of anti-thrombin activity. In NAPAP, too, the (*R*)-enantiomer is one thousand times more active.[10] Two more congeners ((*R*)-**11b**, **c**) were prepared along the same route and tested to investigate whether or not the 4-aminopyridine series would behave differently from the parent NAPAP series. Both series behaved similarly, i.e. the potency declined with the size of the sulfonyl substituent.[13] The X-ray analysis of the (*R*)-**11** / thrombin complex revealed that the 4-aminopyridine did indeed reside in the selectivity pocket of thrombin with the aromatic nitrogen atom in hydrogen-bonding distance to Asp189.[15]

These initial experiments suggested that 4-aminopyridine derivatives would provide only mediocre thrombin inhibitors, because 4-aminopyridine seems to lack the potent interactions of benzamidine in the

selectivity pocket. On the other hand, tosyl-agmatine (3-(4-methylphenyl-sulfonylamino)-propylguanidine) is two orders of magnitude less active than benzamidine,[16] but agmatine derivatives include potent thrombin inhibitors.[17] Hence, we tried to improve the lead compound **1** by modifying its binding to the lipophilic pockets. We realized that it is possible to maintain the two phenyl rings in the proximal and distal pocket of thrombin even when the tether between them was shortened. Consequently, we prepared the diaryl sulfonamides **13a-c** (Scheme 2) which have the advantage of binding to thrombin without the compromise of the ethylene bridge to be immobilized.[7]

Scheme 2. Synthesis of the diaryl sulfonamide thrombin inhibitors



(a) RSO_2Cl, Et_3N, DMF; (b) KOH, MeOH; (c) (i) $ClCO_2CH_2CHMe_2$, Et_3N, (ii), 4-aminopyridine, Et_3N; (d) $LiBH_4$, TMSCl; (e) MeI, K_2CO_3, DMF.

Table 2. Inhibition of the catalytic activity of human α-thrombin, bovine trypsin, and human plasmin at 25 °C and pH = 7.5

	Inhibition constants Ki [μM][11]				Inhibition constants Ki [μM][11]		
	thrombin	trypsin	plasmin		thrombin	trypsin	plasmin
(*R*)-**11a**	0.2	>500	>500	**13a**	7	>500	>500
(S)-**11a**	300	>500	>500	**13b**	0.7	>500	>500
(*R*)-**11b**	2	>500	>500	**13c**	0.3	>500	>500
(*R*)-**11c**	10	>500	>500	**14**	0.07	>500	>500
12[13]	0.006	0.69	30				

The terminal phenyl group was varied in the same way as that of the NAPAP-like series **11a-c**. The rank order of potency of **13a-c** turned out to be the inverse of that of the corresponding NAPAP-like compounds (Table 2). This suggested that the distal pocket of thrombin is approached differently by the two series. It was evident from the solid state structure of the (*R*)-**11a** / thrombin complex that in **11a** the sulfonamide group

functions as a hydrogen bond donor to Gly216 of thrombin. Given the observed discrepancy in the rank order, we speculated that the sulfonamide groups of the **13** series may not have the same function. To test this hypothesis, we prepared the *N*-alkyl derivative **14**. The superior potency of **14** compared to **13c** indicated that the sulfonamide may not be the hydrogen bond donor as anticipated in our initial model. This was confirmed by the crystal structure of the **13c** / thrombin complex.[15]

Although the 4-aminopyridine moiety seems to contribute relatively little to the binding energy, it clearly contributes to the selectivity of these new thrombin inhibitors. A superposition of the X-ray crystal structures of the NAPAP / trypsin[18] and **13c** / thrombin complexes (Figure 1) highlights the unique features of the binding geometry of the 4-aminopyridine moiety in the selectivity pocket: The aromatic group extends deeper into the pocket compared to benzamidine. This region at the bottom of the specificity pocket is more hydrophobic in thrombin than in trypsin due the different side chains at position 190. In trypsin (and also in plasmin), Ser190-Oγ would contact the 2-carbon atom of the pyridine ring, while in thrombin the methyl group of Ala190 provides a more favorable environment. It is conceivable that this difference between the two enzymes governs the selectivity.

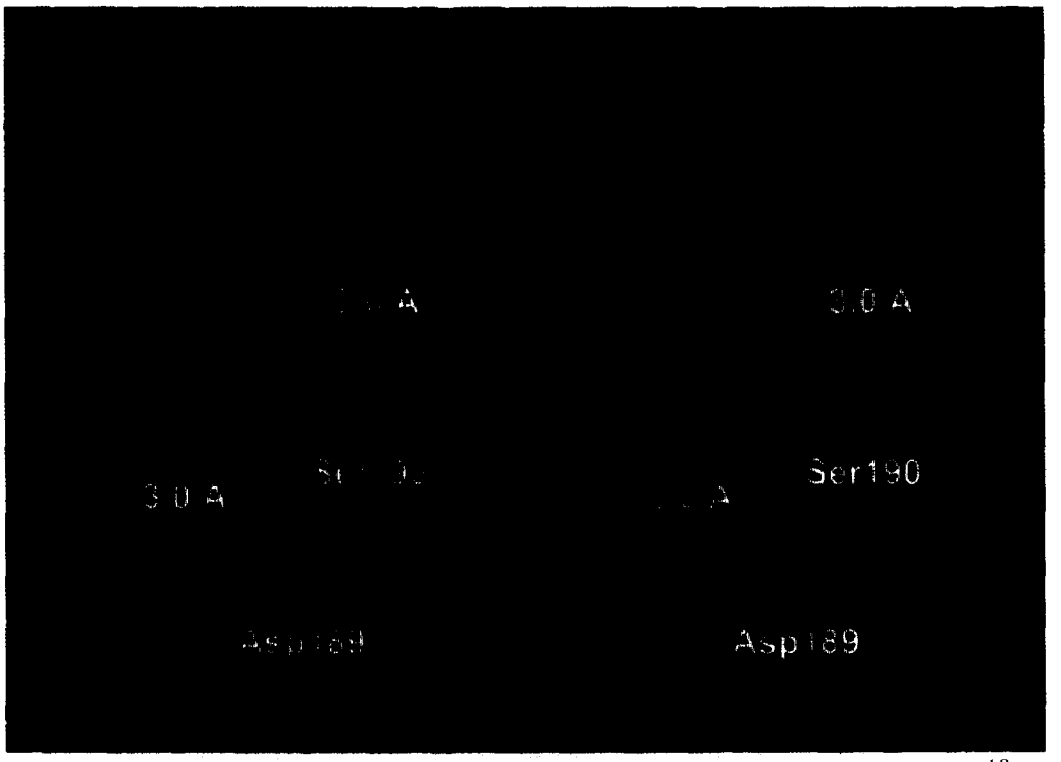


Figure 1. Stereo drawing of the superposition of the selectivity binding moieties of NAPAP in trypsin[18] (thin stick model) and **13c** in thrombin (thick stick model)

Conclusion

Our objective was to discover low basicity thrombin inhibitors in a screening program and to improve the potency of such a compound to nanomolar inhibition constants. The goal was achieved with the discovery of the 4-aminopyridine derivative **1** and its subsequent conversion to **14**. The binding energy of **1** was increased 40 fold despite the fact that the 4-aminopyridine moiety contributes only negligibly to the total binding energy. The diaryl sulfonamide **14** constitutes a novel type of thrombin inhibitor which is non-peptidic, has a low basicity P1 residue, and is not hydrogen bonded to Gly216.

References and Notes

1. Stone, S. R. *Trends Cardiovasc. Med.* **1995**, *5*, 134. Verstraete, M.; Zoldhely, P. *Drugs* **1995**, *49*, 856; Das, J.; Kimball, S. D. *Bioorganic. Med. Chem.*, **1995**, *3*, 999.
2. Bode, W.; Mayr, I.; Baumann, U.; Huber, R.; Stone, S. R.; Hofsteenge, J. *EMBO J.* **1989**, *8*, 3467.
3. Kimball, S. D. *Curr. Pharm. Design* **1995**, *1*, 441.
4. Wiley, M. R.; Chirgadze, N. Y.; Clawson, D. K.; Craft, T. J.; Gifford-Moore, D. S.; Jones, N. D.; Olkowski, J. L.; Weir, L. C.; Smith, G. F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2387.
5. For a recent review on the design principles for orally bioavailable drugs see: Navia, M. A.; Chaturvedi, P. R. *Drug Discovery Today* **1996**, *1*, 179.
6. The benzamidine derivative NAPAP (**12**) has Ki = 6 nM, while its benzylamine analog has Ki = 19 μM: Stürzebecher, J.; Horn, H.; Walsmann, P.; Voigt, B.; Markwardt, F.; Wagner, G. *Pharmazie* **1988**, *43*, 782. The boro-arginine thrombin inhibitor DuP 714 has Ki = 0.04, while its boro-lysine derivative has Ki = 0.24: Cacciola, J.; Fevig, J. M.; Alexander, R. S.; Brittelli, D. R.; Kettner, C. A.; Knabb, R. M.; Weber, P. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 301.
7. For a recent review on the methods of structure based drug design see: Böhm, H.-J.; Klebe, G. *Angew. Chem.* **1996**, *108*, 2750; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2588.
8. Brandstetter, H.; Turk, D.; Hoeffken, W.; Grosse, D.; Stürzebecher, J.; Martin, P. D.; Edwards, B. F. P.; Bode, W. *J. Mol. Biol.* **1992**, *226*, 1085.
9. Dega-Szafran, Z.; Kania, A.; Nowak-Wydra, B.; Szafran, M. *J. Mol. Struct.* **1994**, *322*, 223.
10. Numbering of thrombin residues is based on that for chymotrypsinogen,[2] and the subsite nomenclature of Banner et al. is used: Banner, D. W.; Hadváry, P. *J. Biol. Chem.* **1991**, *266*, 20085.
11. The inhibition constants were determined as described in: von der Saal, W.; Engh, R. A.; Eichinger, A.; Gabriel, B.; Kucznierz, R.; Sauer, J. *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 73.
12. The Ki values of benzamidine are taken from Markwardt, F.; Landmann, H.; Walsmann, P. *Eur. J. Biochem.* **1968**, *6*, 502.
13. Stürzebecher, J.; Markwardt, F.; Walsmann, P.; Voigt, B.; Wagner, G. *Pharmazie* **1987**, *42*, 114.
14. Roberts, S. M.; Suschitzky, H. *J. Chem. Soc. C* **1968**, 2844.
15. 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.
16. Lorand, L.; Rule, N. G. *Nature (London)* **1961**, *190*, 722.
17. Wiley, M. R.; Chirgadze, N. Y.; Clawson, D. K.; Craft, T. J.; Gifford-Moore, D. S.; Jones, N. D.; Olkowski, J. L.; Schacht, A. L.; Smith, G. F.; Weir, L. C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2835.
18. PDB ID code 1PPC: Bode, W.; Turk, D.; Stüzebecher, J. *Eur. J. Biochem.* **1990**, *193*, 175.

(*Received in Belgium* 18 *February* 1997; *accepted* 15 *April* 1997)