J. Enzyme Inhibition, 2001, Vol. 16, pp. 241–249
Reprints available directly from the publisher
Photocopying permitted by license only

© 2001 OPA (Overseas Publishers Association) N.V.
Published by license under
the Harwood Academic Publishers imprint,
part of Gordon and Breach Publishing,
a member of the Taylor & Francis Group.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

Structure–Activity Relationships of New NAPAP-Analogs

TORSTEN STEINMETZER[a,*], ANDREA SCHWEINITZ[a], SEBASTIAN KÜNZEL[a], PETER WIKSTRÖM[b], JÖRG HAUPTMANN[c] and JÖRG STÜRZEBECHER[c]

[a]*Inst. of Biochemistry & Biophysics, Friedrich Schiller University, Philosophenweg 12, D-07743 Jena, Germany;* [b]*Pentapharm Ltd, Basel, Switzerland;* [c]*Centre for Vascular Biology and Medicine, Friedrich Schiller University, Nordhäuser Str. 78, D-99089 Erfurt, Germany*

(Received 20 September 2000)

Several new analogs of the known thrombin inhibitor NAPAP were synthesized, in which the P2 glycine residue was substituted by natural and unnatural amino acids. The thrombin inhibitory potency was comparable to that of NAPAP. Several of the compounds had inhibition constants lower than 10 nM and a very high selectivity compared to trypsin, factor X_a and plasmin. In addition, analogs were prepared by alkylation of the N^{α}-atom of the 4-amidinophenylalanine in P1 position, which showed a more than 10-fold lower thrombin inhibition. Furthermore, azaglycine was introduced instead of P2 glycine. For most of the inhibitors similar fast elimination rates were seen in rats after intravenous dosing, as found previously for NAPAP. Only some compounds, which contained a second basic group showed a slightly decreased cumulative biliary clearance.

Keywords: Thrombin, Enzyme inhibitors, Anticoagulants, Synthesis, Elimination, NAPAP

INTRODUCTION

The trypsin-like serine protease thrombin is an attractive target for drug design, because of its unique role at the final stage of the blood coagulation cascade and as an activator of platelet aggregation. Increased thrombin activity can lead to thrombotic disorders, such as deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke.[1] In addition to the long known standard therapy for thrombotic diseases, such as oral treatment with warfarin or *i.v.* or *s.c.* injections of heparins, the use of the recombinant 65 amino acid protein hirudin as a direct thrombin inhibitor has been approved recently. Small molecule synthetic thrombin inhibitors could have a potential as effective and selective oral anticoagulants. Despite the existence of several highly potent lead structures with inhibition constants in the lower nanomolar range, there is only limited clinical use of synthetic thrombin inhibitors; at the present time only argatroban is used which requires parenteral application.[2]

In addition to the poor to moderate oral bioavailability of these polar compounds, problems may arise from high plasma protein binding of very hydrophobic compounds or from fast

*Corresponding author. Tel.: +49-3641-508322. Fax: +49-3641-508301.

RIGHTSLINK

elimination of the inhibitors; for several analogs very short half-lives of some minutes have been reported.[1] Another requirement with respect to clinical application is low toxicity and high safety for the protease inhibitors, which requires a high selectivity against the enzymes of the fibrinolytic system, like plasmin, t-PA or u-PA[3] or against the serine protease factor CI of the complement cascade.[4] A poorer safety has been reported for several of the highly potent transition state analog inhibitors, like the peptidyl aldehyde efegatran or the peptidyl trifluoroethoxy methylketone CH 1091. The observed slow binding kinetics of these inhibitors with association rate constants k_{on} smaller than $5 \times 10^6\ M^{-1}s^{-1}$ was found to be associated with steep dose response curves *in vivo*, which may lead to bleeding complications.[5]

One of the known lead structures of direct thrombin inhibitors is NAPAP.[6] Despite a high antithrombin activity with a K_i of 2.1 nM found for the D-enantiomer, fast binding kinetics[7] with a k_{on} of $3 \times 10^7\ M^{-1}s^{-1}$ and a very high thrombin-selectivity, NAPAP has relatively poor performance *in vivo* because it is not orally available and is rapidly eliminated from the circulation. The problems of low oral bioavailability may be overcome in principle by prodrug strategies, especially by masking the strongly basic amidino group, as described recently for the benzamidine-derived thrombin inhibitor melagatran[8] or for the GPIIb/IIIa antagonist Fradafiban.[9]

The main excretion route for various tripeptide and peptidomimetic thrombin inhibitors is via the hepato-biliary system. During our work over the last few years we found a reduced clearance rate for some dibasic inhibitors closely related to the structure of NAPAP;[10] however all of these analogs were relatively weak thrombin inhibitors. A slow elimination with a half-life of more than 60 minutes was described also for the dibasic factor X_a inhibitors DX-9065a[11] and YM-60828.[12]

Therefore, we have synthesized a series of thrombin inhibitors by replacing the glycine residue in NAPAP with several natural and unnatural amino acids containing basic side chains. Some of the thrombin inhibitors described recently, like inogatran or melagatran contain an N-terminal N^{α}(carboxymethyl)-amino acid to improve their pharmacokinetic properties. So we have prepared here an additional NAPAP-analog by alkylation of the N^{α}-nitrogen of the amidinophenylalanine with a carboxymethyl group. Here the inhibitory activities and elimination pattern *in vivo* of these dibasic and alkylated derivatives are compared to NAPAP and some other new monobasic analogs.

MATERIALS AND METHODS

Assays

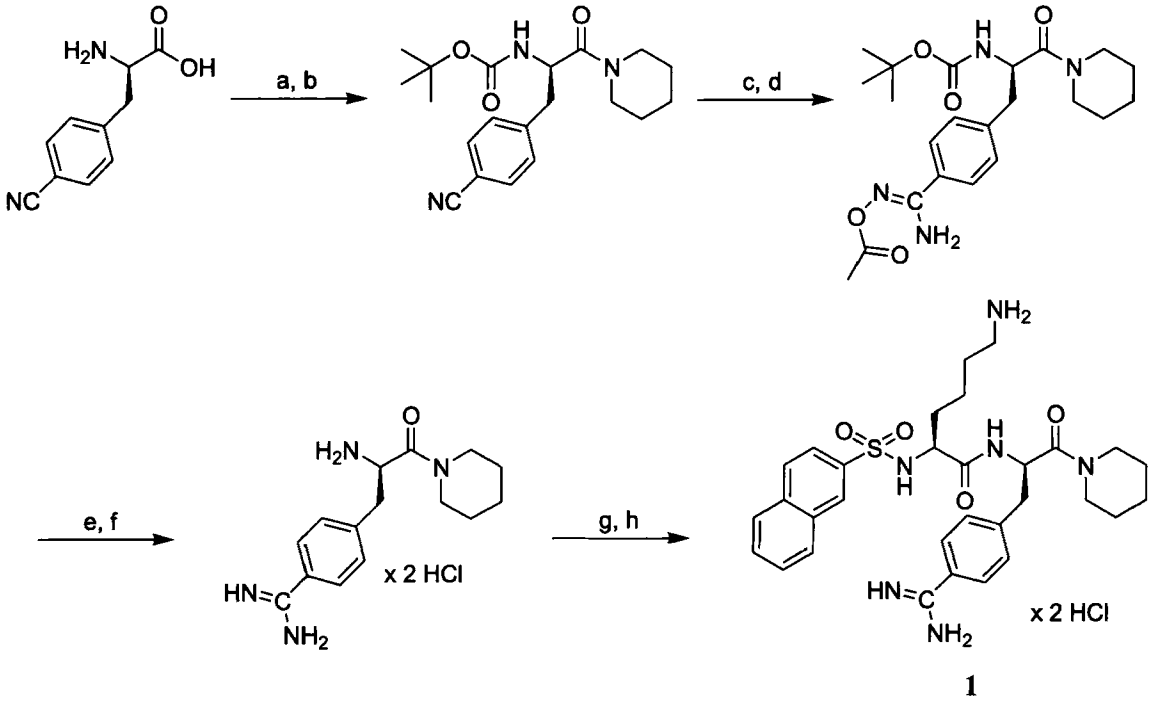
The kinetic assays were performed by the methods described previously.[13]

Chemistry

The inhibitors were prepared with slight modifications of a route published elsewhere[14] as shown in Scheme 1 for the synthesis of inhibitor (**1**). The synthesis starts from D-4-cyanophenylalanine,[15] the conversion of the nitrile function of the intermediate Boc-D-4-cyanophenylalanine-piperidide into the amidine was performed according to the method described by Judkins *et al.*[16] Compared to the direct hydrogenation of the oxamidine the use of the activated *O*-(acetyl) oxamidine leads to a faster conversion to the amidine. The Lys-containing inhibitors (**2**)–(**4**) and the compounds (**5**), (**7**) and (**9**) were synthesized in identical manner using N^{α}-β-naphthylsulfonylated amino acids with Boc-protection in the side chain. β-Nas-Ser-OH and β-Nas-Thr-OH were prepared as described elsewhere[17] and coupled without side chain protection to give inhibitors (**13**) and (**14**). β-Nas-L-3-amidinophenylalanine was obtained from β-Nas-L-3-cyanophenylalanine by the procedure described

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK



SCHEME 1 Synthesis of inhibitor (1). (a) 1.1 eq. Boc_2O in dioxane/H_2O containing NaOH at pH 9, 30 min 0 °C, 3 h rt; (b) formation of mixed anhydride with isobutyl chloroformate and NMM, 10 min −15 °C in THF, treatment with 1.1 eq. of piperidine, 1 h −15 °C, 12 h rt; (c) 1.5 eq $NH_2OH \times HCl$, 1.5 eq. DIEA, reflux in ethanol for 4 h; (d) 2 eq. Ac_2O in acetic acid at rt 1 h; (e) H_2 and Pd/C in acetic acid, 6 h rt; (f) 1 M HCl in acetic acid, 45 min; (g) β-Nas-Lys(Boc)-OH, PyBop, 3 eq. DIEA in DMF, 30 min 0 °C, 1 h rt; (h) 1 M HCl in acetic acid, 45 min.

above,[16] however, the acetate form obtained after final hydrogenation was converted to the HCl salt by treatment with 1 M HCl in acetic acid prior to PyBop coupling to D-4-amidinophenylalanine-piperidide to give (**11**).

During the synthesis of inhibitor (**1**) initial attempts were made to convert the nitrile to the amidine at the final stage of the synthesis using β-Nas-Lys(Cbz)-D-4-cyanophenylalanine-piperidide. However, the treatment with hydroxylamine under conditions used in Scheme 1 resulted in a substantial cleavage of the Lys(Cbz)-D-4-cyanophenylalanine peptide bond, and in addition to the desired oxamidine we obtained significant amounts (approximately 25% based on HPLC analysis at 220 nm) of β-Nas-Lys(Cbz)-hydroxamic acid. This was purified by HPLC and was identified by mass spectrometry (Calc.: m/e 485.16; Found: m/e 484.2 $[M-H]^-$ negative mode, 508.6 $[M+Na]^+$ positive mode) and by the red brown colour found typically for hydroxamic acids, when a 5% $FeCl_3$-solution in 0.5 N HCl was used for visualization of the spots on TLC plates.

The inhibitors (**6**), (**8**) and (**10**) were obtained by final guanylation of the precursors (**5**), (**7**) and (**9**) using pyrazol-1-carboxamidin.[18] The azaglycine derivative (**16**) was synthesized from β-Nas-hydrazide by reaction with 4-nitrophenyl chloroformate, followed by treatment with D-4-amidinophenylalanine-piperidide without purification of the intermediate β-Nas-azaGly-nitrophenylester.[19] Analogs (**17**)–(**19**) were prepared as depicted in Scheme 2 starting from Boc-D/L-4-amidinophenylalanine-piperidide, obtained as described in Scheme 1. The major problem was the coupling of the β-Nas-Gly-OH to the N^{α}-alkylated amidinophenylalanine. All

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK

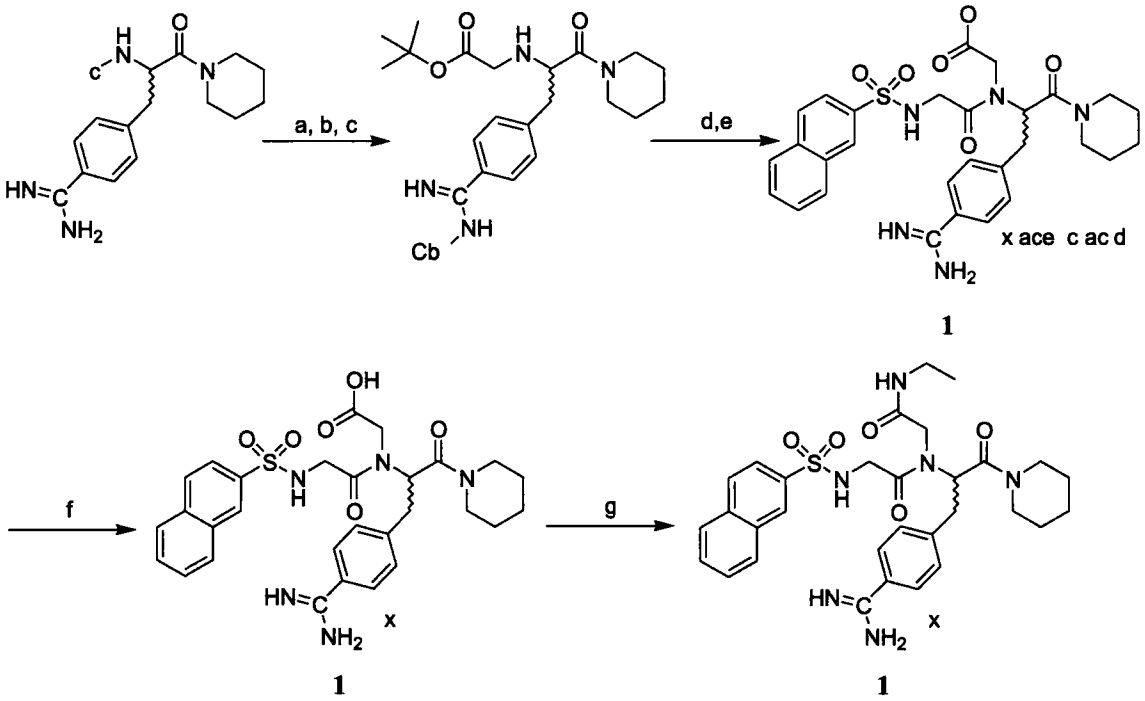
standard procedures, like mixed anhydride, DCC/HOBt, PyBop, or PyBrop coupling gave only very poor yields, the only acceptable method being the attachement using β-Nas-Gly-Cl,[20] however, in this case a large excess of the acid chloride was necessary. A significant amount of a side product was obtained during synthesis of (**19**). Since the mass of this compound was 18 Da smaller than found for (**18**), we assume that a cyclization reaction between the relatively acidic sulphonamide and the activated carboxyl group had occurred, as described for other sulphonamides,[21] which are able to form 6-membered ring structures.

All compounds were finally purified by semi-preparative reversed-phase HPLC to more than 95% purity and characterized by analytical HPLC and mass spectrometry as previously described.[22]

Elimination of compounds in rats

Animals and experimental design

Female Wistar rats, 240–320 g body weight (Charles River-Wiga, Sulzfeld, Germany) were used. Anaesthesia was performed with ethyl-urethane (1.4 g/kg intraperitoneally). The body temperature was kept constant by means of a thermostated infrared lamp. The right carotid artery was exposed and cannulated for drawing blood samples; the left femoral vein was exposed for intravenous injection. After an abdominal midline incision the bile duct was cannulated in a typical manner. Blood samples were withdrawn at 2, 5, 15, 30, 45, 60 min and 1.5, 2, 2.5, 3, 3.5 and 4 h after administration. Blood was taken into sodium citrate (1/10, v/v); citrated plasma was obtained by centrifugation at 1200 g for 10 min. The blood sample volume with-



SCHEME 2 Synthesis of inhibitors (**17**)–(**19**). (a) 1.25 eq. Cbz-Cl in dioxane/H_2O containing 10 eq. NaOH at pH 13–14, 1 h 0°C, 12 h rt; (b) 1 M HCl in acetic acid, 45 min; (c) 1.2 eq. *tert.*butyl-bromoacetate, 1.5 eq Ag_2O in DMF, 12 h rt; (d) 2.5 eq. β-Nas-Gly-Cl, TEA in DMF/CH_2Cl_2, 1 h 0°C, 12 rt; (e) H_2 and Pd/C in methanol/acetic acid, 4 h rt; (f) 90 % TFA, 1 h rt; (g) 1 eq. ethylamine × HCl, PyBop, 3 eq. DIEA in DMF, 30 min 0°C, 1 h rt.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK

drawn was replaced by the corresponding volume of saline. Bile was collected for the first 30 min of the experiment in 5 min-fractions, in 15 min-fractions up to 1 h and in 30 min-fractions thereafter. Bile volume was determined gravimetrically.

Sample analyses

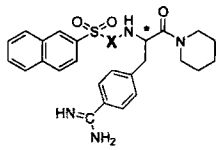
The concentrations of the compounds in plasma and bile were determined by HPLC. Briefly, samples were pretreated with conditioned Chromabond C_{18} cartridges (Macherey-Nagel, Düren, Germany), analyzed on a Nucleosil 7 C_{18} reversed phase column (Macherey-Nagel, Düren, Germany) with acetonitrile (15–30%), water (70–85%), perchloric acid (0.04%) as mobile phase at a flow rate of 1 ml/min. The compounds were quantified by fluorescence detection (λ_{exc} 232 nm, λ_{em} 343 nm) with reference to appropriate calibration standards (addition of the respective compound to blank plasma and bile). Bile samples were diluted into the calibration range with the mobile phase. The detection limit of the assay for NAPAP was 2 ng/ml for plasma.

RESULTS AND DISCUSSION

Dibasic NAPAP analogs

In a first series (Table I) all possible stereoisomers of the Lys-containing NAPAP analog were synthesized with, not surprisingly, compound (**1**) showing the strongest thrombin inhibition. This is in good agreement with results described previously with analogs of the NAPAP-derivative CRC 220 having an N-terminal Mtr-Asp-segment.[23] Therefore, with exception of the racemic (**12**) only amino acids in the L-configuration were used in P2 as Gly substituents for the following compounds. The anti-thrombin activity of (**1**) was slightly lower compared to NAPAP; in contrast a higher selectivity towards trypsin was found.

TABLE I Inhibition of thrombin, trypsin, factor Xa and plasmin by inhibitors of the general formula shown below; K_i values in μM



No.	X	*	Thrombin	Trypsin	Factor Xa	Plasmin
D-NAPAP	Gly	D	0.0021	0.23	20	24
1	Lys	D	0.0061	1.6	130	27
2	D-Lys	D	2.5	12	89	>1000
3	Lys	L	24	>1000	>1000	>1000
4	D-Lys	L	6.2	>1000	>1000	>1000

*Configuration of the 4-amidinophenylalanine.

Examination of the X-ray structure of the NAPAP/thrombin complex (1 ets.pdb)[24] showed, that the C^{α} of the NAPAP glycine has a distance of 4.2 Å from the carboxyl group of the Glu 192 side chain, which is bound to the thrombin residues Gly 216 and Gly 219 by a bridging water molecule. From the X-ray structure of thrombin in complex with a bivalent inhibitor of the hirulog-type,[25] which contains a NAPAP-derived active site-directed segment with a glutamic acid connected by a peptide bond to a linker segment, it was known that the side chain points into the direction of the carboxyl group of Glu 192, making a hydrogen bond between the amide nitrogen of the formed peptide bond to the carboxylate group of Glu 192. Therefore, we performed a stepwise shortening of the aminobutyl side chain of inhibitor (**1**) and obtained analogs (**5**), (**7**) and (**9**) (Table II). The K_i values for thrombin are approximately 5–10 fold higher than those found for NAPAP. This indicates that there is no detectable contribution to binding by an electrostatic interaction as expected, probably also because of the energy loss necessary by removing the bridging water molecule. In several other thrombin/inhibitor complexes it is also shown that the side chain of Glu 192, located at the thrombin surface, is relatively flexible and can point in different directions.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK

TABLE II Inhibition of thrombin, trypsin, factor Xa and plasmin by inhibitors of the general formula, shown in Table I; K_i values in μM

No.	X	*	Thrombin	Trypsin	Factor Xa	Plasmin
5	Orn	D	0.018	5.6	>1000	58
6	Arg	D	0.011	2.8	>1000	22
7	Aeg[a]	D	0.02	7.3	>1000	56
8	Geg[b]	D	0.0068	3.9	>1000	34
9	Amg[c]	D	0.011	1.2	>1000	170
10	Gmg[d]	D	0.0084	2.2	>1000	58
11	3-Adf[e]	D	0.059	6.1	21	4.4
12	D,L-His	D,L	0.02	4.8	66	70

[a]Aeg = 2-aminoethylglycine, [b]Geg = 2-guanidinoethylglycine, [c]Amg = 1-aminomethylglycine, [d]Gmg = 1-guanidinomethylglycine, [e]3-Adf = 3-amidinophenylalanine.

Slightly stronger inhihibitory potency was found, when the amino group was substituted for a guanidino group as in (**6**), (**8**) and (**10**).

NAPAP derivatives with neutral P2 residues

In addition to the replacement of glycine with basic amino acids, we incorporated residues with polar but uncharged side chains, such as serine and threonine, which can act as hydrogen bond donors (Table III). The thrombin inhibitory activity of (**13**) and (**14**) is similar to compound (**8**) and not improved compared to NAPAP; in the case of (**14**) weaker trypsin inhibition was observed. A relatively moderate thrombin inhibition was found for the phenylalanine derivative (**15**). As mentioned above, the side chain of the P2 amino acid is directed into the solvent at the thrombin surface, therefore the hydrophobic benzyl side chain may be unfavourable for binding to the enzyme.

TABLE III Inhibition of thrombin, trypsin, factor Xa and plasmin by inhibitors of the general formula, shown in Table I; K_i values in μM

No.	X	*	Thrombin	Trypsin	Factor Xa	Plasmin
13	Ser	D	0.0075	0.55	88	11
14	Thr	D	0.0052	2.0	>1000	14
15	Phe	D	0.04	4.0	110	27
16	AzaGly	D	0.0039	0.79	49	18

The incorporation of azaamino acids with an NH replacing the α-CH in the peptide backbone is a known standard procedure for peptide modification; this leads to an extension of the area of planarity compared with that of a normal amide linkage and can stabilize the peptide conformation. There are several examples in the literature, in which this exchange resulted in peptidomimetic compounds which had a longer duration of action.[26] The azaglycine derivative (**16**) was the most potent inhibitor in our series with an activity close to that of NAPAP.

NAPAP derivatives with N^{α}-alkylated P1 residue

The search for additional modification sites using the X-ray structure of the NAPAP/thrombin complex identified the N^{α} atom of the 4-amidinophenylalanine as a further starting point for substitution. This amide nitrogen is not involved in any hydrogen bond to thrombin or a water molecule. In addition, as has been described, it can be used for alkylation to form more rigid, cyclic NAPAP analogs.[27] An N-terminal N^{α}(carboxymethyl)-amino acid was incorporated in thrombin inhibitors derived from the D-Phe-Pro-Arg-type, such as inogatran or melagatran, to improve their pharmacokinetic properties. Here we introduced a carboxymethyl group at the N^{α}-atom of the P1-amino acid, analog (**18**) showed a 10-fold higher K_i value than NAPAP and no improved selectivity. The analogous *tert.*butylester (**17**) and the ethylamide (**19**) had a very similar activity profile, which indicates that there is no binding contribution by this alkyl group.

TABLE IV Inhibition of thrombin, trypsin, factor Xa and plasmin by inhibitors (**17–19**), the structures are given in Scheme 2; K_i values in μM

No.	Thrombin	Trypsin	Factor Xa	Plasmin
17	0.050	0.98	16	16
18	0.045	0.47	15	20
19	0.046	0.67	11	56

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

Elimination *in vivo*

A challenge in the development of small synthetic inhibitors of the blood coagulation proteases, the action of which is strictly dependent on their concentration in the blood, is their elimination characteristics. In laboratory animals, racemic D,L-NAPAP is rapidly cleared from the blood by extensive hepato-biliary elimination. Similar data were obtained for the D-enantiomer. Systemic clearance approaches liver blood flow and biliary clearance accounts for a high percentage of systemic clearance of NAPAP (hepatic first pass-effect). High clearance was also described for the closely related CRC220 and for other tripeptide and peptidomimetic thrombin inhibitors, such as argatroban, inogatran or napsagatran.[1]

Derivative (**15**) showed plasma levels *vs* time courses nearly identical to NAPAP. In contrast, for several of the inhibitors with a second basic amino acid, such as Lys, Arg or 3-Adf (**1, 6, 11**) and also for the neutral analogs (**13**), (**14**) and (**16**) somewhat higher plasma levels were found, obviously owing to smaller distribution volumes since elimination half-lives were apparently not changed (Figure 1A and 1B).

Inhibitors (**1–4**), the diastereoisomers of the Lys-derivative of NAPAP, showed similar plasma level time courses (data not shown) but obvious differences in cumulative biliary excretion; highest values were found for the diastereoisomer (**3**) with both amino acids in the L-configuration and lowest values for the diastereoisomer (**2**) with both amino acids in the D-configuration. The differences might arise from a possible stereospecific biliary excretion of these analogs. Figure 2 summarises the biliary excretion of NAPAP and some of the derivatives studied. Cumulative biliary excretion allows an estimation of the extent of hepato-biliary elimination of the compounds.

CONCLUDING REMARKS

Substitution of the P2 glycine in NAPAP for some natural and unnatural amino acids yielded several potent thrombin inhibitors. Although the activity was slightly reduced compared to NAPAP the most active compounds are highly selective thrombin inhibitors with inhibition constants in the low nanomolar range. The highest activities were found for compounds (**16**), (**14**) and (**1**) with azaglycine, threonine, and lysine in the P2 position, respectively. In addition, analogs (**17–19**) were prepared, in which the N^{α}-atom of the P1 residue was alkylated, however, these compounds showed a 10-fold lower potency. As found for NAPAP, most of the other monobasic inhibitors had high systemic

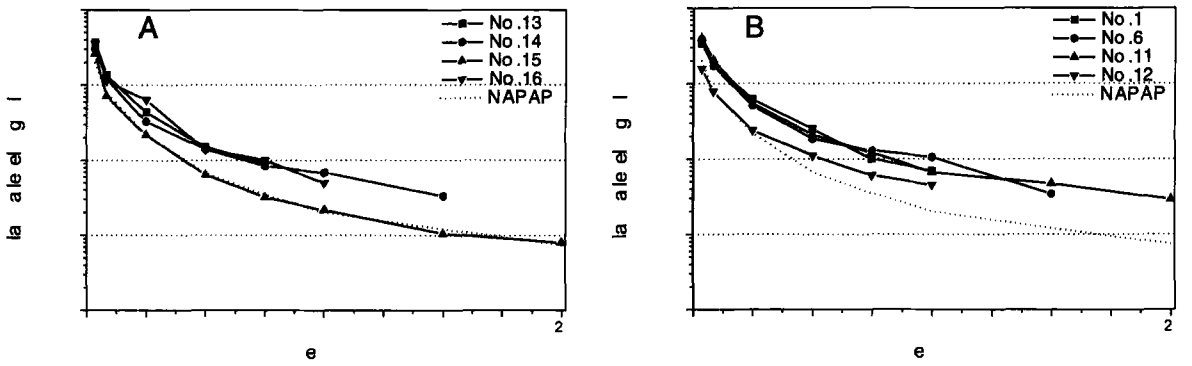


FIGURE 1 Time course of plasma levels in rats after intravenous injection of an inhibitor dose of 1 mg/kg found for NAPAP and inhibitors with neutral (A) and basic P2 amino acids (B).

RIGHTSLINK

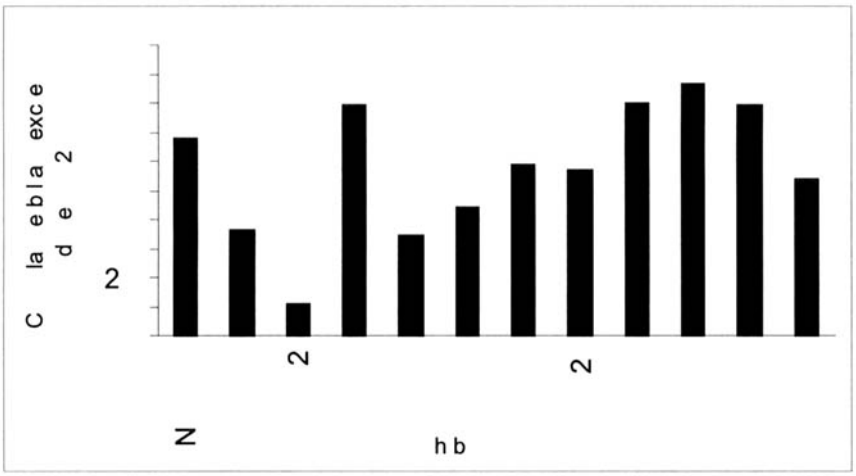


FIGURE 2 Cumulative biliary excretion of selected inhibitors given as a percentage of the dose found in 120 min.

clearance owing primarily to extensive hepatobiliary elimination; in contrast the dibasic inhibitors (**1**), (**6**) and (**11**) showed somewhat reduced plasma clearance with slightly lower biliary excretion.

Acknowledgements

We wish to thank Dr. Lydia Seyfarth for performing the mass spectrometry and Prof. Siegmund Reissmann for his support of this work. In addition we would like to express our thanks to Ute Altmann for excellent technical assistance. Financial aid by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

References

[1] J. Hauptmann and J. Stürzebecher (1999) *Thromb. Res.*, **93**, 203.
[2] R. Pifarre (1997) (Ed.) *New Anticoagulants for the Cardiovascular Patient*, pp. 231–49. Philadelphia: Hanley and Belfus.
[3] D. Gustafsson, T. Antonsson, R. Bylund, U. Eriksson, E. Gyzander, I. Nilsson, M. Elg, C. Mattson, J. Deinum, S. Pehrsson, O. Karlsson, A. Nilsson and H. Sörenson (1998) *Thromb. Haem.*, **79**, 110.
[4] J.M. Fevig, J. Buriak Jr, J. Cacciola, R.S. Alexander, C.A. Kettner, R.M. Knabb, J.R. Pruitt, P.C. Weber and R.R. Wexler (1998) *Bioorgan. Med. Chem. Lett.*, **8**, 301.
[5] M. Elg, D. Gustafsson and J. Deinum (1997) *Thromb. Haem.*, **78**, 1286.
[6] J. Stürzebecher, F. Markwardt, G. Voigt, G. Wagner and P. Walsmann (1983) *Thromb. Res.*, **29**, 635.
[7] C. Taparelli, R. Metternich, C. Ehrhardt, M. Zurini, G. Claeson, M.F. Scully and S.R. Stone (1993) *J. Biol. Chem.*, **268**, 4734.
[8] D. Gustafsson, J.-E. Nystrom, S. Carlsson, U. Bredberg, M. Elg, E. Gyzander, A.-L. Ungell and T. Antonsson (1999) *Blood*, **94**, (Suppl. 1), 26a.
[9] R.C. Carrol (1999) *Current Opinion in Cardiovascular, Pulmonary & Renal Investigational Drugs*, **1**, 104.
[10] B. Gabriel, M.T. Stubbs, A. Bergner, J. Hauptmann, W. Bode, J. Stürzebecher and L. Moroder (1998) *J. Med. Chem.*, **41**, 4240.
[11] T. Hara, A. Yokoyama, H. Ishihara, Y. Yokoyama, T. Nagahara and M. Iwamoto (1994) *Thromb. Haem.*, **71**, 314.
[12] Y. Taniuchi, Y. Sakai, N. Hisamichi, M. Kayama, Y. Mano, K. Sato, F. Hirayama, H. Koshio, Y. Matsumoto and T. Kawasaki (1998) *Thromb. Haem.*, **79**, 543.
[13] J. Stürzebecher, D. Prasa, J. Hauptmann, H. Vieweg and P. Wikström (1997) *J. Med. Chem.*, **40**, 3091.
[14] W. Stüber, R. Koschinsky, C. Kolar, M. Reers, G. Dickneite, D. Hoffmann, J. Czech, K.-H. Diehl and E.-P. Pâgues (1994) In *Peptides, Chemistry and Biology*, Proc. XIII Amer. Peptide Symp., R. Hodges (Ed.), pp. 643–645. Escom; Leiden, NL.
[15] W. Stüber, R. Koschinsky, M. Reers, D. Hoffmann, J. Czech and G. Dickneite (1995) *Peptide Res.*, **8**, 78.
[16] B.D. Judkins, G.D. Allen, T.A. Cook, B. Evans and T.S. Sardharwala (1996) *Synth. Comm.*, **26**, 4351.
[17] R.C. Roemmele and H. Rapoport (1988) *J. Organ. Chem.*, **53**, 2367.
[18] M.S. Bernatowicz, Y. Wu and G.R. Matsueda (1992) *J. Organ. Chem.*, **57**, 2497.
[19] J. Gante and R. Weitzel (1990) *Liebigs Annalen der Chemie*, 349.
[20] G. Wagner, B. Voigt and H. Vieweg (1984) *Pharmazie*, **39**, 226.
[21] T. Lescrinier, C. Pannecouque, J. Rozenski, A. van Aerschot and P. Herdewijn (1995) *Lett. Peptide Sci.*, **2**, 206.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK

[22] T. Steinmetzer, M. Batdorsdhjin, P. Kleinwächter, L. Seyfarth, G. Greiner, S. Reißmann and J. Stürzebecher (1999) *J. Enz. Inhib.*, **14**, 203.
[23] M. Reers, R. Koschinsky, G. Dickneite, D. Hoffmann, J. Czech and W. Stüber (1995) *J. Enz. Inhib.*, **9**, 61.
[24] H. Brandstetter, D. Turk, H.W. Höffken, D. Grosse, J. Stürzebecher, P.D. Martin, B.F. Edwards and W. Bode (1992) *J. Mol. Biol.*, **226**, 1085.
[25] T. Steinmetzer, M. Renatus, S. Künzel, A. Eichinger, W. Bode, P. Wikström, J. Hauptmann and J. Stürzebecher (1999) *Eur. J. Biochem.*, **265**, 598–605.
[26] J. Gante (1988) *Synthesis*, 405.
[27] H. Mack, T. Pfeiffer, W. Hornberger, H.-J. Böhm and H.W. Höffken (1995) *J. Enz. Inhib.*, **9**, 73.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/19/11
For personal use only.

RIGHTSLINK