Influence of Structural Variations in Peptidomimetic 4-Amidinophenylalanine-Derived Thrombin Inhibitors on Plasma Clearance and Biliary Excretion in Rats

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Purpose. Systemic and hepato-biliary clearance of peptidomimetic thrombin inhibitors of the 4-amidinophenylalanine amide-type, derived from NAPAP (Nα-[2-naphthylsulfonyl-glycyl]-4-amidinophenylalanine-piperidide) by substituting Gly in P2 for natural and unnatural amino acids or by varying the C- and N-terminal moieties, resp., were investigated.

Methods. Concentrations of the compounds administered as intravenous bolus injection at a dose of 1 mg/kg to bile duct-cannulated rats were determined in plasma and bile samples collected over 4 hours using reversed-phase HPLC.

Results. NAPAP and the derivatives with additional charged groups are comparatively hydrophilic compounds. For NAPAP and most of the derivatives the biliary clearance accounted for a high percentage of the rapid systemic plasma clearance. Derivatives **2a-c** with a second basic group in P2 position showed lower systemic and biliary clearance compared to NAPAP, whereas their cumulative biliary excretion after a period of 120 min was less affected. Bis-benzamidine derivatives **4a** and **5** with the second amidino group in the N-terminal moiety had the lowest biliary clearance. Additional carboxylic groups reduced the systemic and biliary clearance only as free amidinophenylalanine carboxyl in **3a** and **5**. No influence compared to NAPAP was observed for **2d** with a free carboxyl group in P2 position.

Conclusions. The weak correlation of the log P values of the compounds with the clearance parameters indicates the influence of structural variations, especially of charged groups, in this series of compounds rather than overall lipophilicity on hepato-biliary elimination mediated by hepatocellular transporters.

KEY WORDS: thrombin inhibitors; structures; charged groups; systemic clearance; biliary clearance.

INTRODUCTION

In recent years, the development of small molecule inhibitors of the key blood coagulation enzyme, thrombin, has led to a number of highly potent and selective inhibitors (1,2,3,4). They are effective as anticoagulants and have a potential for use as antithrombotic agents in various clinical settings. The pharmacodynamics of thrombin inhibitors are closely related to their pharmacokinetics: Circulating blood is the site of action for inhibitors of coagulation enzymes, the blood/plasma level of a thrombin inhibitor is strongly correlated with the extent and duration of anticoagulant and antithrombotic effects. Clearance from blood is, therefore, the dominant pharmacokinetic determinant for these anticoagulant/antithrombotic drugs.

"First generation" tripeptide-type or peptidomimetic thrombin inhibitors are structurally characterized by a peptide backbone and by stronlgy positive guanidino or amidino groups in the P1 position. Like numerous peptidomimetic inhibitors of other proteinases, these thrombin inhibitors—as a group of structurally related compounds—are rapidly eliminated, primarily by the hepato-biliary route and are orally bioavailable to a low percentage only (1,2,5,6).

NAPAP (Nα-[2-naphthylsulfonyl-glycyl]-4-amidinophenylalanine-piperidide), is a highly potent and selective 4-amidinophenylalanine amide-type thrombin inhibitor (7). It has served as one of the prototype thrombin inhibitors and lead for thrombin inhibitor development. NAPAP is rapidly cleared from blood. In rabbits, *in situ* elimination of the liver from circulation increased the half-life and drastically reduced the plasma clearance of NAPAP (8). The extensive hepato-biliary elimination of NAPAP showed saturation kinetics, obviously not caused by limited hepatic uptake, but rather by saturable biliary excretion in rabbits and rats (9). A derivative with Pro instead of Gly in the 4-amidinophenylalanine Nα-side chain had somewhat longer half-life and lower biliary excretion in rabbits (10). The thrombin inhibitors CRC 220, structurally similar to NAPAP, and napsagatran have similar pharmacokinetic characteristics, i.e., high hepato-biliary clearance in various species.

There is greater structural diversity in newly synthesized inhibitors in which weakly basic or neutral P1 moieties and hydrophobic P3 moieties have been introduced. Current design strategies are aimed at optimizing lead structures not only with regard to potency and selectivity but also with regard to pharmacokinetics (clearance, oral bioavailability). Detailed studies on structure-pharmacokinetics relationships in this field, however, have not yet been published.

The aim of the present study is the elucidation of the influence on plasma clearance and biliary excretion in rats of structural variations, including introduction of charged groups, of the 4-amidinophenylalanine amide-type thrombin inhibitor, NAPAP.

MATERIALS AND METHODS

Materials

The chemical structures of the thrombin inhibitors studied are given in Fig. 1. The compounds were synthesized as described elsewhere (compounds **1-3b** and **4b:** (11) and compounds **4b** and **5:** (12)). Compounds **4b** and **5** were kindly provided by Drs. L. Moroder and B. Gabriel, Max-Planck-Institute of Biochemistry, Martinsried, Germany. All chemicals used were of analytical or HPLC grade.

Determination of Inhibition Constants

Inhibition constants K_i for bovine thrombin were determined as described elsewhere (13).

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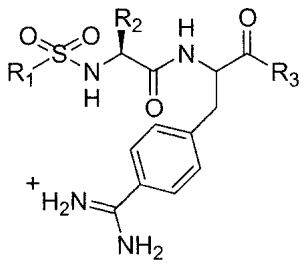

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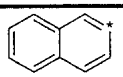
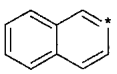
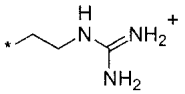
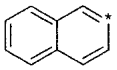
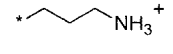
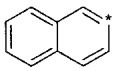
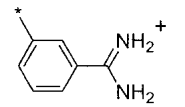
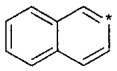
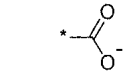
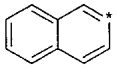
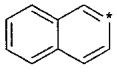
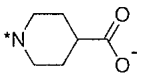
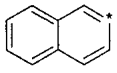
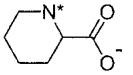
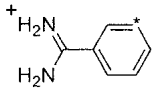
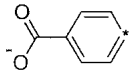
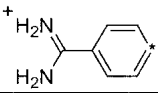
1		H	
2a			
2b			
2c			
2d			
3a		H	O^-
3b		H	
3c		H	
4a		H	
4b		H	
5		H	O^-

Fig. 1. Chemical structures of the compounds studied (★ indicates the position of bonding).

Determination of Octanol/Water Partition Coefficients

Octanol/water partition of the compounds was determined by the shake-flask method; briefly by dissolving them in octanol-saturated phosphate buffer (0.16 mol/l, pH 7.0) at a concentration of 20 μmol/l, adding equal volumes of buffer-saturated n-octanol, agitating for 30 min at room temperature and finally separating the buffer phase, after transferring the samples to appropriate tubes, by centrifugation. The final concentrations in the buffer phase were measured spectrophotometrically (between 205 and 235 nm; in most cases the first main absorption peak of the benzene ring was measured) and referred to standard curves obtained from the buffer phase and graded dilutions prior to mixing with n-octanol. The values presented are the means from 4 determinations.

Animals and Experimental Design

Female Wistar rats, 240–320 g body weight (Charles River-Wiga, Sulzfeld, Germany) were used. The animals were kept under conventional conditions with free access to standard diet and tap water. The animal experiments were in accordance with the "Principles of Laboratory Animal Care". Anesthesia was performed with ethylurethane (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 duodenal loop was mobilized and the bile duct cannulated near the ostium using a gauge 23 G needle connected to a short plastic tube (0.5 mm I.D.).

Blood samples were withdrawn at 2, 5, 15, 30, 45 min, and 1, 1.5, 2, 2.5, 3, 3.5, and 4 h after administration. Blood was taken into 3.80% sodium citrate solution (1/10, v/v); citrated plasma was obtained by centrifugation at 1200 *g* for 10 min. The blood sample volume withdrawn was replaced by injecting 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 one hour and in 30 min-fractions later on. Bile volume was determined gravimetrically. The duration of the experiment was limited to 4 h.

Sample Analyses

The concentrations of the compounds in plasma and bile were determined by HPLC. Briefly, samples were pretreated with conditioned Chromabond C18 cartridges (Macherey-Nagel, Düren, Germany), analyzed on a Nucleosil 7 C18 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 containing a naphthylsulfonyl moiety were quantified by fluorescence (λ_{exc} 232 nm, λ_{em} 343 nm), the others by UV detection (225–230 nm). Under the HPLC conditions used enantiomers were not separated, so that racemic compounds with one chiral center showed a single peak.

Bile samples were diluted into the calibration range with the mobile phase. Quantification was done by referring to calibration standards (addition of the respective compound to blank plasma and bile). The detection limit of the assay for NAPAP was 2 ng/ml for plasma.

Pharmacokinetic Analysis

Plasma concentration-time data were analyzed as follows: Systemic plasma clearance was calculated from plasma AUC (biexponential or triexponential fit of the plasma concentration-time curve data and integration of the fitted curve). AUC was truncated to 240 min and not extrapolated to time infinity in order not to lead to erroneous values for compounds with plasma levels scattering in the terminal elimination phase for various reasons (e.g., for analytical reasons: different detection limits of the individual compounds studied and sometimes low and varying plasma concentrations).

Biliary excretion rates, referred to unit time and unit body weight were calculated for the bile fractions.

Biliary clearance was calculated as $Cl_b = v \times c_b / c_p$, where v is bile flow (ml/min) and c_b and c_p are the concentrations (μg/ml) in bile and plasma, respectively, for the the individual samples in the period from 5 to 60 min after administration. In this period bile flow was rather stable. Concentrations in plasma corresponding to the middle of the respective bile-collecting period were interpolated for calculation of biliary clearance. Finally, biliary clearance was calculated as mean from the individual time point data (the first 5 min bile fraction was left unconsidered). Clearance values were referred to unit body weight.

The data presented are means with standard deviations. Statistical significance ($P \leq 0.05$) was calculated by two-sided *t* test or ANOVA.

RESULTS

Structural variation of NAPAP (**1**) led to derivatives with varying affinities to thrombin. Table I shows the K_i values of **1** and of the derivatives studied in vivo. Substituting P2 Gly for basic or acidic amino acids was of minor influence on the inhibitory potency, whereas charged groups at the N- or C-terminus drastically reduced the affinity of the derivatives to thrombin. The octanol-water partition coefficients showed that the compounds are rather hydrophilic with several derivatives being more hydrophilic than **1.**

In rats, the compounds were injected intravenously at a dose of 1 mg/kg as bolus (about 15 s duration) after a 30 min equilibration period following bile duct-cannulation.

Figure 2 shows the time courses of the plasma concentrations, biliary excretion rates, and biliary clearances for **1** and two representative derivatives, **2c** and **3a**, of the series studied; in addition, bile flow is shown. The different patterns of the time courses of plasma level, biliary excretion rate, and biliary clearance, resp., are obvious. The terminal phases of the plasma level courses, however, appeared to show similar slopes after about 60–90 min.

Racemic NAPAP (**1**) showed a rapid decline of the plasma level indicating high clearance; biliary clearance accounted for the major fraction of systemic clearance. Systemic plasma clearance of NAPAP approached systemic liver blood flow (estimated at about 60–70 ml/min × kg). During the first 15 min period after administration, about two third of that amount of drug excreted cumulatively within 120 min had already appeared in bile. Biliary clearance, calculated for de-

Table I. Structural Characteristics and Thrombin Inhibitory Potency of NAPAP and Derivatives

No.	Amino acid configuration P1	P2	Inhibition constant K_i(μmol/l)	Partition coeffient log P, pH 7.0
1	D,L		0.006	–0.64
1*	D		0.002	–0.60
2a	D	L	0.011	–1.26
2b	D	L	0.006	–2.13
2c	D	L	0.059	–0.80
2d	D,L	L	0.005	–0.19
3a	D,L		31	–0.59
3b	D,L		2.0	–0.68
3c	D,L		1.4	–0.42
4a	D,L		2.1	–2.00
4b	D,L		9.4	–1.70
5	D,L		>1000	–1.88

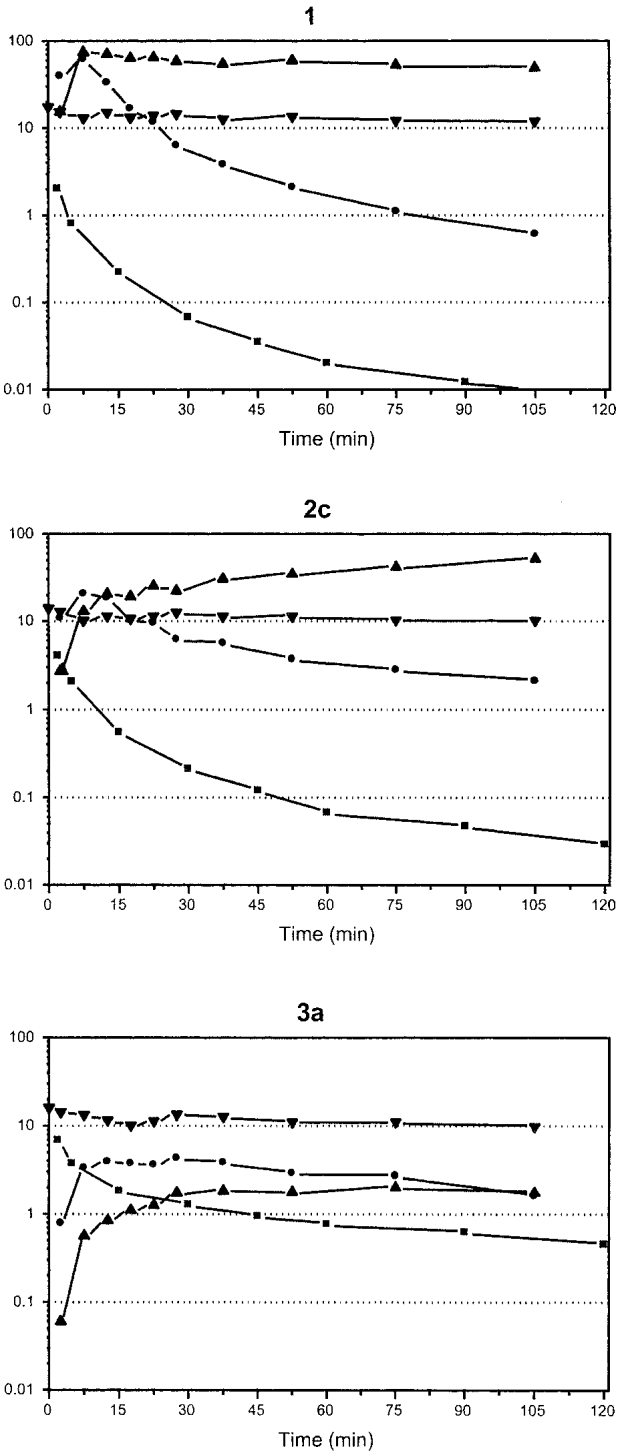


Fig. 2. Time courses of plasma levels (μg/ml; ■), biliary excretion rates (nmole/min × kg; ●) and biliary clearances (ml/min × kg; ▲) of NAPAP (**1**) and two representative derivatives (**2c**, **3a**) after intravenous injection (1 mg/kg) in rats. Bile flow (μl/min; ▼) is shown additionally. Values are mean values from three experiments each (SD omitted because of legibility of figure).

fined time points, showed a time course with an increase over 5–10 min and nearly plateau values after 15 min. After a maximum in the first bile fractions, concentrations and excretion rates declined exponentially, parallel to the decline in plasma levels.

The D-enantiomer of NAPAP (**1***), the inhibitory active enantiomer, had elimination parameters not significantly different from those of racemic NAPAP.

The calculated parameters systemic clearance, biliary clearance and cumulative biliary excretion (for two selected periods) are summarized in Table II. The higher the systemic clearance of a given derivative the higher was the amount excreted with bile in the first bile fractions over 15 min (see Fig. 3).

Derivatives with basic natural or unnatural amino acid (**2a**: Arg; **2b**: Lys; **2c**: 3-amidino-phenylalanine) in P2 showed significantly lower systemic and biliary clearances than **1**. The time courses of biliary clearance indicated kinetics of the hepatic uptake and biliary secretion processes, resp., differing from those of **1**. The bililary clearance value slightly but steadily increased during the 120-min period instead of reaching a plateau as observed for most of the other compounds (see Fig. 2). In contrast, introduction of the acidic amino acid Asp in P2 (**2d**) did not change the elimination pattern compared to **1**.

Derivatives with acidic or basic substituents in positions other than P2 showed quite different characteristics: When the carboxylic group was in the C-terminal part of the molecule (pipecolic or iso-nipecotic acid instead of piperidine: **3b** and **3c**) systemic clearance was significantly reduced compared to **1**, whereas biliary clearance was reduced to a lower extent and cumulative biliary excretion remained unchanged. The same was true of **4b** with a carboxylic group in the N-terminal part. For these three derivatives hepato-biliary clearance was still the dominant elimination route. The most pronounced differences in the characteristics of the derivatives containing a carboxylic group showed the C-terminal unsubstituted 4-amidinophenylalanine (**3a**); systemic clearance was one order of magnitude lower than for **1**, biliary clearance was further reduced, also cumulative biliary excretion was lower. Figure 3 illustrates the marked differences in the time course of biliary excretion of **3a** compared to **1**.

The derivative with an additional amidino moiety, introduced by 4-amidinobenzene (**4a**) instead of naphthalene in the N-terminal moiety, had a significantly lower plasma clearance; biliary clearance and cumulative biliary excretion were drastically reduced compared to **1**. The combination of an additional C-terminal amidino group and an acidic group in **5** brought about the most marked change in the elimination characteristics, biliary clearance and cumulative biliary excretion were lowest for this derivative.

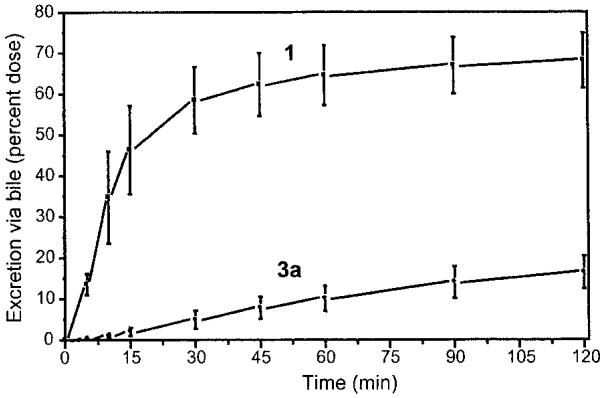


Fig. 3. Cumulative biliary excretion of the 4-amidinophenylalanine amide derivative NAPAP (**1**) and the corresponding 4-amidinophenylalanine derivative (**3a**) after intravenous injection (1 mg/kg) in rats. Data are mean ± SD from three experiments each.

Correlations between biliary clearance and cumulative biliary excretion of NAPAP and the derivatives were weak. Systemic clearance showed a better correlation with cumulative biliary excretion over the first 15 min ($r = 0.7679$, $p = 0.00354$) compared to the data for 120 min ($r = 0.5987$, $p = 0.05165$).

Figure 4 shows the correlation between the octanol/water partition coefficients of the derivatives and the biliary clearance.

DISCUSSION

The present study on derivatives of the thrombin inhibitor NAPAP, with parts of the molecule altered by introducing substituents with charged groups but leaving the arylsulfonyl-

Table II. Elimination Characteristics of NAPAP and Derivatives Administered Intravenously in Rats (mean ± SD, n = 3 each)

No.	Systemic clearance (ml/min×kg)	Biliary clearance (ml/min×kg)	Cumulative biliary (% dose in 15 min)	Excretion (% dose in 120 min)
1	60.4 ± 14.9	58.1 ± 15.4	46.3 ± 10.7	68.1 ± 6.7
1*	54.3 ± 14.8	75.6 ± 17.4	46.3 ± 22.0	68.6 ± 27.3
2a	29.9 ± 18.0	20.2 ± 5.9[a]	23.8 ± 5.8[a]	44.4 ± 11.5[a]
2b	27.6 ± 9.7[a]	16.1 ± 9.9[a]	16.3 ± 6.2[a]	35.4 ± 6.5[a]
2c	24.1 ± 6.8[a]	23.6 ± 1.2[a]	22.1 ± 4.1[a]	58.7 ± 21.9
2d	60.6 ± 27.4	55.9 ± 16.5	49.1 ± 18.1	69.2 ± 13.2
3a	7.3 ± 1.4[a]	1.4 ± 0.6[a]	2.0 ± 0.9[a]	16.4 ± 4.0[a]
3b	19.0 ± 1.2[a]	24.9 ± 6.8[a]	42.8 ± 16.5	76.0 ± 20.4
3c	35.6 ± 6.4	41.3 ± 7.7	51.1 ± 1.3	74.8 ± 4.7
4a	11.8 ± 1.1[a]	0.3 ± 0.1[a]	0.8 ± 0.3[a]	1.6 ± 1.0[a]
4b	15.4 ± 0.4[a]	18.5 ± 4.7[a]	26.1 ± 0.5[a]	74.7 ± 5.0
5	4.5 ± 1.2[a]	0.1[b]	0.2[b]	2.7[b]

[a] $P < 0.05$.
[b] Data from two animals only.

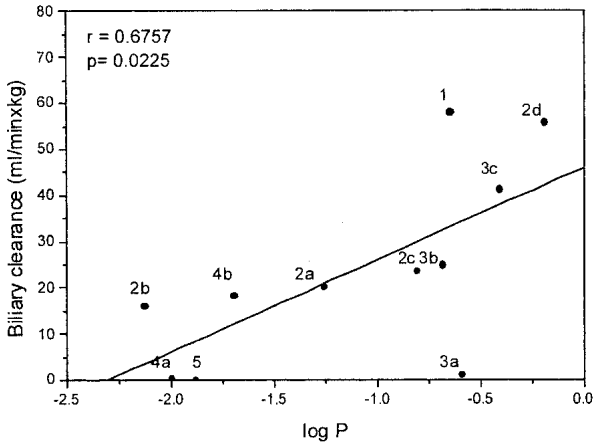


Fig. 4. Relationship between lipophilicity (log P) and biliary clearance of NAPAP and derivatives in rats.

aminoacylated 4-amidinophenylalanine moiety unaltered, is to our knowledge the first aimed at elucidating systematically structural features important for the elimination characteristics of a series of pseudopeptide thrombin inhibitors. With the main elimination route characterised and the respective organ clearance contributing to systemic clearance to a high degree, as in the case of NAPAP and structurally related thrombin inhibitors undergoing hepato-biliary elimination, the data on systemic and biliary clearance indicate the influence of structural variations in the derivatives.

The pharmacokinetics of many small-molecule, peptide-type or peptidomimetic proteinase inhibitors are characterized by fast clearance with predominant hepato-biliary excretion in various species. Especially, tripeptide and peptidomimetic thrombin inhibitors derived from arginine, benzamidine or amidinopiperidine share this feature (8,9,14,15, 16,17,18,19). In the search for peptidomimetic thrombin inhibitors introduction of a carboxylate group into the highly basic molecules had been sometimes useful for improvement of characteristics, e.g. tolerability and pharmacokinetics (1). The potent thrombin inhibitors argatroban, napsagatran, inogatran, melagatran, and CRC 220 bear a carboxylic group. The clearance of the NAPAP-analogue, CRC 220, containing such a carboxylic group (Gly substituted for Asp), however, was as fast as that of NAPAP in rats (14). The extensive hepatocellular uptake of CRC 220 is mediated by the multispecific organic anion transporter polypeptide (Oatp1) (20). Noteworthy, the uptake into rat hepatocytes of CRC 220 was decreased by NAPAP and other 4-amidinophenylalanine-derivatives; a derivative of CRC 220 lacking the basic amidino group competed highly effectively with uptake, whereas a derivative having a less hydrophobic Nα-substituent was less effective (20). Also for further thrombin inhibitors with a carboxylic group it is evident that this group *per se* not necessarily alters the elimination characteristics. Argatroban, an arginine amide, the first thrombin inhibitor approved for clinical use, has a rather short half-life in various species and is hepatically metabolized and biliary excreted (21,22). The elimination of argatroban was not affected by a CYP3A inhibitor, obviously, rather the hepatic uptake process governs its pharmacokinetics (23). In patients with hepatic insufficiency, systemic clearance was reduced by 75% (24). Napsagatran, an amidinopiperidine derivative, had a systemic clearance equal to NAPAP in rats with predominantly non-renal clearance (19,25). The biliary clearance in various species was close to liver blood flow and hepatic uptake was estimated faster than biliary excretion (19).

In our study in rats, the individual time courses of biliary excretion rates and fractional biliary clearances of the derivatives of NAPAP with additional charged groups varied markedly between the compounds. The cumulative biliary excretion of most derivatives was similar to NAPAP over 120 min, even for derivatives whose systemic clearance was reduced by about 50 to 60%.

Biliary clearance is accounting for almost all of the systemic clearance of NAPAP. Substitution of Gly in P2 for neutral aliphatic, heterocyclic or aromatic amino acids (Ser, His, Thr, Phe) did not have a marked influence on systemic and biliary clearance (11). The derivatives with a carboxylic group - irrespective of the position in the molecule (**2d**, **3b**, **3c**, **4b**) - showed mainly unchanged cumulative excretion and a biliary clearance accounting for almost 100% of systemic clearance except **3a**, for which biliary clearance accounts for 20% of systemic clearance only. The latter represents the only derivative with a carboxylic group showing reduced cumulative biliary excretion besides **5** with the carboxylic group in the same position but having a different N-terminal part (see below). For comparison, an amidinophenylalanine-derived thrombin inhibitor, UK-156406, with a carboxylic group in the amide moiety, structurally similar to **3b** and **3c,** was reported to have reduced hepatic clearance (1,6).

Biliary clearance is also accounting for all (**2c**) or a high degree (**2a**, **2b**) of the systemic clearance of the derivatives with a basic substituent in P2. So, one might conclude from a structural point of view that the side chain, either neutral, basic or acidic, of the P2 amino acid of NAPAP-type thrombin inhibitors is of minor significance for the hepato-biliary elimination processes. However, a basic substituent in greater distance to the P1 amidino group (replacement of the N-terminal β-naphthyl by a positively charged amidinophenyl moiety in **4a**) brings about a dramatic reduction of the ratio biliary clearance/systemic clearance. When this type of derivative had also a free carboxylic group at the C-terminus (**5**)—lacking the piperidine moiety—clearance was further reduced. With the latter two derivatives biliary clearance accounts for only 2% of systemic clearance. The systemic clearance of **5** was in the same range as that of the derivative with the respective carboxylic group but still containing the neutral β-naphthyl moiety (**3a**), whereas the biliary clearance of **5** was one order of magnitude lower. The reason for the very low degree of cumulative biliary excretion of the dibasic derivatives **4a** and **5** might be extensive sequestration in hepatocyte organelles followed by slow excretion into bile as it has been shown for a number of bulky amphiphilic basic compounds (26).

In the present study, bile flow was a sensitive indicator of hepato-biliary function in general, transient variations in bile flow in individual experiments translated into corresponding variations of the biliary excretion rates and, hence, of the fractional biliary clearance values.

One may assume that the basolateral, multispecific transporter oatp1 and/or other transporters of this family, known to transport anionic, neutral as well as cationic molecules, are responsible for the uptake into hepatocytes of the compounds studied. In addition, various canalicular transporters such as

mdr 1a/1b, mrp 2 or BSEP of which a potential role in the excretion of amphiphilic compounds have been reported, albeit not established yet for the thrombin inhibitors studied here, might be involved in the secretion of NAPAP and the other dibasic or zwitterionic derivatives. Hence, the differences in the biliary excretion rates and cumulative excretion seen might also be consequences of the various transport kinetics at the canalicular side of the hepatocyte. Evidently, the correlation between cumulative biliary excretion and systemic clearance was rather weak. On the other hand, there was a strong linear correlation between the biliary clearance of the NAPAP-derivatives and the systemic clearance.

As regards the physico-chemical characteristics, the highly basic thrombin inhibitors of the amidinophenylalanine-type are hydrophilic compounds. The range of the log P values of the derivatives studied was –0.17 to –2.13 with **1** having a log P of –0.64. Overall lipophilicity of **1** is not markedly influenced by a carboxylic group (**2d**, **3a**, **3b**, **3c**) in the molecule, probably owing to intra- or intermolecular interactions of this group with the strongly basic amidino group, fully ionized at physiological pH. Such an interaction seems less likely for **4b** with the carboxylic group in greater distance and showing a lower log P value than **1**. A second basic group in all cases brings about log P values lower than for **1**. The experimentally determined logP values should have more clearly shown the influence of ionisable groups on overall lipophilicity than calculated values which often inadequately account for steric or conformational effects resulting from intramolecular interactions. The logP values of our series of NAPAP derivatives showed comparatively weak correlations with systemic clearance, cumulative biliary excretion and biliary clearance with only the latter being statistically significant.

In several investigations, lipophilicity had been found an important factor for various endogenous and exogenous compounds to be eliminated via the hepato-biliary route. This was clearly shown for the hepatic clearance in rats of a homologous series of barbiturates (27). In rabbits, hepatic clearance of lipophilic basic drugs was correlated to the apparent octanol-water partition coefficient (28). In the isolated rat liver, initial extraction ratio and apparent hepatic clearance of a series of cationic amphiphilic aminosteroids showed a similar degree of correlation with lipophilicity over a range of log P values from –2.48 to 0.69 (26). In this series, the lowest clearance values were found for bisquaternary compounds. The relationship between lipophilicity and biliary clearance for these cationic drugs was sigmoideal (29). In the same model, a strong correlation between lipophilicity and first-pass hepatic extraction was also found for tetrapeptides with different charges and lipophilicity (30).

It remains to be further evaluated for the individual derivatives of NAPAP showing reduced systemic clearance and biliary excretion, resp., whether interrelated processes such as plasma protein binding and whole body distribution, that can largely influence excretion rate, may have also a major impact on the hepatic involvement in overall clearance. Alternatively, structure-dependent variations in V_{max}- and K_m-values for the interaction of the inhibitors under study with membrane transporters for uptake and secretion in the excretory organs may explain the observed differences in the clearance patterns.

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