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Original article

Protease inhibitors – Part 3. Synthesis of non-basic thrombin inhibitors incorporating pyridinium-sulfanilylguanidine moieties at the P1 site

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Abstract – Using benzamidine and sulfaguanidine as lead molecules, three series of derivatives have been prepared by reaction of sulfaguanidine with pyrylium salts, with the pyridinium derivatives of glycine and with the pyridinium derivatives of β-alanine, respectively. The new compounds were assayed as inhibitors of two serine proteases, thrombin and trypsin. The study showed that in contrast to the leads, possessing K_I's around 100–300 nM against thrombin, and 1 200–1 500 nM against trypsin, respectively, the new derivatives showed inhibition constants in the range of 15–50 nM against thrombin, whereas their affinity for trypsin remained relatively low. Derivatives of β-alanine were more active than the corresponding Gly derivatives, which in turn were more inhibitory than the pyridinium derivatives of sulfaguanidine possessing the same substitution pattern at the pyridinium ring. Thus, the present study proposes two novel approaches for the preparation of high affinity, specific thrombin inhibitors: a novel S1 anchoring moiety in the already large family of arginine/amidine-based inhibitors, i.e., the $SO_2N{=}C(NH_2)_2$ group, and novel non-peptidomimetic scaffolds obtained by incorporating alkyl-/aryl-substituted-pyridinium moieties in the hydrophobic binding site(s). The first one is important for obtaining bioavailable thrombin inhibitors, devoid of the high basicity of the commonly used arginine/amidine-based inhibitors, whereas the second one may lead to improved water solubility of such compounds due to facilitated salt formation as well as increased stability at hydrolysis (in vivo). © 1999 Éditions scientifiques et médicales Elsevier SAS

thrombin / trypsin / sulfaguanidine / pyridinium salts / pyridinium amino acid / non-basic thrombin inhibitor

1. Introduction

Thrombin (EC 3.4.21.5) has become an important target for drug design in recent years, in the search for low molecular-weight potent and selective inhibitors with applications as diagnostic and therapeutic agents for the increasingly common thrombotic diseases [1–8]. Although a large number of potent active site-directed thrombin inhibitors, such as peptide aldehydes [9, 10], boronates [11], benzamidine- [2, 3, 12, 13] or arginine/guanidine-derived [14] inhibitors are reported, none of them meet all the criteria needed for an ideal antithrombotic drug [2, 15]. Thus, the largest majority of the presently available low-molecular weight inhibitors, such as argatroban (MQPA) **1** [16], inogatran **2** [8], NAPAP **3** [17], 4-TAPAP **4** or its 3-amidino-isomer, 3-TAPAP **5** [2, 17] (*figure 1*), are poorly bioavailable, either due to their high basicity, connected with the presence of guanidino-/amidino moieties in their molecule, or are not absorbable orally, or are rapidly eliminated from the circulation, mainly due to their peptidic nature. Although recently some non-basic S1 anchoring groups have been incorporated in the molecules of some thrombin inhibitors [3, 7, 18], the presence of guanidino-/benzamidino moieties in such compounds is critical, since it is by means of the interaction of these highly polar groups with Asp 189, the central amino acid residue from the specificity pocket, that the enzyme-inhibitor adduct is initially formed (obviously, a lot of other secondary interactions are responsible for the formation of high affinity adducts between thrombin and its inhibitors) [3–5, 12–14]. In order to exploit the intrinsically high affinity of guanidino-/benzamidino-containing inhibitors for the thrombin active site, but also to avoid undesired properties connected with their too high basicity, we propose here a novel approach for designing tight-binding such inhibitors, by using sulfanilylguanidino moieties as an-

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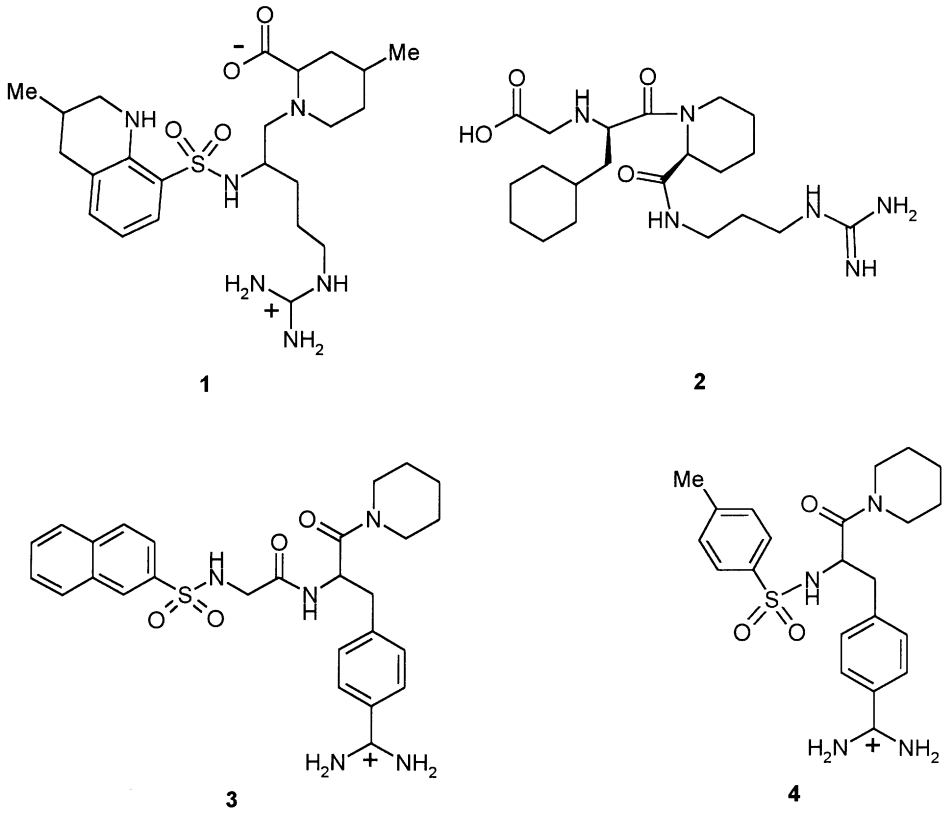


Figure 1. Structures of serine protease inhibitors **1–4**.

choring groups to the S1 specificity pocket. Obviously, the presence of the SO_2 group in the neighbourhood of the guanidino moiety strongly reduces the basicity of the latter, presumably without precluding to the binding of inhibitors within the enzyme active site.

In this paper we report the preparation and serine protease inhibitory properties (against human thrombin and human trypsin) of three series of compounds obtained by reaction of sulfaguanidine with pyrylium salts, with the pyridinium derivatives of glycine (prepared from Gly and pyrylium salts) and with the pyridinium derivatives of β-alanine (obtained from β-Ala and pyrylium salts), respectively. From the point of view of their thrombin inhibitory properties, as well as that of their specificity for thrombin over trypsin, some of our compounds showed inhibition constants of the same order of magnitude as those of the clinically used compounds, argatroban (MQPA) **1** [16], and inogatran **2** [8], in the 15–50 nM range against thrombin, whereas maintaining a much lower trypsin affinity (inhibition constants around 1 200–1 500 nM) as compared to the above-mentioned clinically used derivatives.

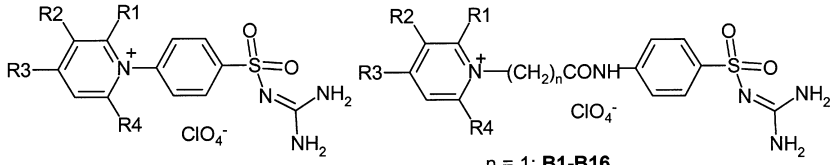
2. Chemistry

Compounds prepared by reaction of di-, tri- or tetrasubstituted pyrylium salts with sulfaguanidine, of types **A1**–**A16**, as well as the corresponding Gly derivatives of types **B1**–**B16** and β-Ala derivatives **C1–C16** are shown in *table I*.

Non-exceptional synthetic procedures have been used for the reactions of pyrylium salts with nucleophiles (for the preparation of compounds **A**, **B**, **C** (**1–16**) as well as the pyridinium amino acid intermediates **10** and **11**) [19, 20], whereas for attaching the pyridinium-amino acyl moieties, the condensation reactions in the presence of carbodiimide derivatives has been used, as outlined in *figure 2* [21, 22].

Sulfanilylguanidine **7** was reacted with di-, tri- or tetrasubstituted pyrylium salts **6** leading to the pyridinium

Table I. Pyridinium-sulfanilylguanidines (**A1–A16)**, pyridinium-methylcarboxamido- (**B1–B16**) and pyridinium-ethylcarboxamido-sulfanilylguanidines (**C1–C16**) prepared in the present study, with their inhibition data against human thrombin and human trypsin.



A1-A16

Compound	n	R^1	R^2	R^3	R^4	K_I[a]	
						Thrombin	Trypsin (nM)
A1	–	Me	H	Me	Me	83 ± 5	1 290 ± 80
A2	–	i-Pr	H	Me	Me	76 ± 6	1 180 ± 90
A3	–	i-Pr	H	Me	i-Pr	102 ± 5	1 440 ± 105
A4	–	Me	H	Ph	Me	41 ± 2	1 120 ± 75
A5	–	Et	H	Ph	Et	37 ± 3	1 100 ± 62
A6	–	n-Pr	H	Ph	n-Pr	54 ± 7	1 165 ± 63
A7	–	i-Pr	H	Ph	i-Pr	48 ± 5	1 200 ± 85
A8	–	Me	H	Ph	Ph	32 ± 2	1 265 ± 102
A9	–	Et	H	Ph	Ph	30 ± 4	1 240 ± 77
A10	–	n-Pr	H	Ph	Ph	36 ± 5	1 260 ± 80
A11	–	i-Pr	H	Ph	Ph	34 ± 2	1 210 ± 104
A12	–	n-Bu	H	Ph	Ph	60 ± 5	1 340 ± 120
A13	–	t-Bu	H	Ph	Ph	33 ± 3	1 170 ± 96
A14	–	Ph	H	Ph	Ph	54 ± 4	1 950 ± 130
A15	–	Ph	H	H	Ph	58 ± 6	1 950 ± 140
A16	–	Me	Me	Me	Me	79 ± 6	1 300 ± 90
B1	1	Me	H	Me	Me	75 ± 5	1 210 ± 91
B2	1	i-Pr	H	Me	Me	62 ± 4	1 120 ± 60
B3	1	i-Pr	H	Me	i-Pr	80 ± 7	1 320 ± 95
B4	1	Me	H	Ph	Me	34 ± 3	1 100 ± 72
B5	1	Et	H	Ph	Et	30 ± 3	1 020 ± 60
B6	1	n-Pr	H	Ph	n-Pr	50 ± 4	1 150 ± 45
B7	1	i-Pr	H	Ph	i-Pr	43 ± 5	1 175 ± 75
B8	1	Me	H	Ph	Ph	21 ± 2	1 250 ± 88
B9	1	Et	H	Ph	Ph	17 ± 1	1 200 ± 105
B10	1	n-Pr	H	Ph	Ph	23 ± 2	1 210 ± 65
B11	1	i-Pr	H	Ph	Ph	22 ± 2	1 175 ± 90
B12	1	n-Bu	H	Ph	Ph	50 ± 5	1 210 ± 60
B13	1	t-Bu	H	Ph	Ph	25 ± 3	1 100 ± 55
B14	1	Ph	H	Ph	Ph	50 ± 4	1 900 ± 60
B15	1	Ph	H	H	Ph	57 ± 5	1 905 ± 65
B16	1	Me	Me	Me	Me	73 ± 6	1 265 ± 90
C1	2	Me	H	Me	Me	69 ± 4	1 140 ± 87
C2	2	i-Pr	H	Me	Me	47 ± 4	1 100 ± 100
C3	2	i-Pr	H	Me	i-Pr	71 ± 5	1 290 ± 97
C4	2	Me	H	Ph	Me	29 ± 2	1 055 ± 73
C5	2	Et	H	Ph	Et	26 ± 2	1 010 ± 55
C6	2	n-Pr	H	Ph	n-Pr	47 ± 5	1 105 ± 102
C7	2	i-Pr	H	Ph	i-Pr	41 ± 4	1 100 ± 79
C8	2	Me	H	Ph	Ph	18 ± 2	1 210 ± 75
C9	2	Et	H	Ph	Ph	15 ± 1	1 165 ± 50
C10	2	n-Pr	H	Ph	Ph	21 ± 2	1 200 ± 60
C11	2	i-Pr	H	Ph	Ph	20 ± 1	1 155 ± 70
C12	2	n-Bu	H	Ph	Ph	47 ± 5	1 200 ± 85
C13	2	t-Bu	H	Ph	Ph	19 ± 2	1 130 ± 50
C14	2	Ph	H	Ph	Ph	42 ± 5	1 380 ± 45
C15	2	Ph	H	H	Ph	50 ± 3	1 520 ± 50
C16	2	Me	Me	Me	Me	70 ± 5	1 210 ± 40

[a]K_I values were obtained from Dixon plots using a linear regression program [26], from at least three different assays.

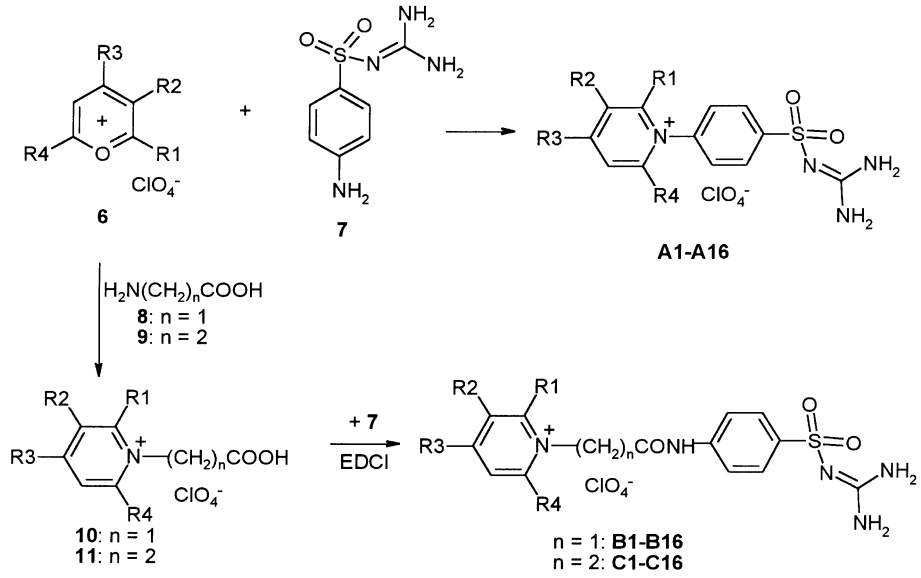


Figure 2. Synthesis of derivatives **A1**–**A16**, **B1**–**B16** and **C1**–**C16**.

derivatives **A1–A16**. Alternatively, reaction of pyrylium salts with Gly or β-Ala afforded the pyridinium amino acid derivatives **10** and **11**, which were coupled with **7** in the presence of EDCI or diisopropylcarbodiimide as condensing agents, leading to compounds **B1–B16**, and **C1–C16**, respectively.

3. Pharmacology

Inhibition data against two serine proteases, human thrombin and human trypsin are shown in *table I*. The chromogenic substrate Chromozym TH (Ts-Gly-Pro-Arg-*p*-nitroanilide) was used in the assay, with the spectrophotometric method of Lottenberg et al. [23]. Inhibition data with the standard serine protease inhibitors **1–3** are also provided for comparison in *table II*.

pK_a values for the guanidino/amidino and sulfonamido moieties for some of the thrombin inhibitors reported here, as well as standard compounds, are shown in *table III*.

4. Results and discussion

The lead molecule for obtaining novel types of thrombin inhibitors considered by us was benzamidine **12**, one of the simplest of such compounds, which possesses an inhibition constant K_I = 300 nM against human thrombin; moreover, the X-ray crystallographic structure for the complex of benzamidine with this enzyme has recently been reported (PDB entry: 1DWB) [24]. From the X-ray data it was observed that the amidino moiety of the inhibitor is anchored to the S1 specificity pocket of the enzyme, interacting electrostatically and by means of hydrogen bonds with Asp 189. Several other van der Waals contacts between the inhibitor molecule and the enzyme were also evidenced [24]. Obviously, benzamidine is a weak thrombin inhibitor, since the binding energy is only gained due to the strong electrostatic interaction of the carboxylate of Asp 189 and the positively charged amidino moiety. On the other hand, as already mentioned in the introductory section, the amidino moiety possesses too high a basicity for allowing the formation of bioavailable enzyme inhibitors, and it thus

Table II. Inhibition data of two serine proteases with standard inhibitors **1–3** and **12**, and sulfaguanidine **7**.

Compound		K_I (nM)[a]	
		Thrombin	Trypsin
1	Argatroban[b]	19 ± 2	–
2	Inogatran	15 ± 1	540 ± 11
3	NAPAP	6.5 ± 0.05	690 ± 24
7	Sulfaguanidine	95 ± 4	1 350 ± 120
12	Benzamidine	300 ± 5	450 ± 6

[a]K_I values were obtained from Dixon plots using a linear regression program [26], from at least three different assays. [b]From ref. [5].

Table III. pK_a data of serine protease inhibitors **1–3, 7, A9, B9** and **C9**.

Compound		pK_a [a]	
		Guanidino/amidino moiety	SO_2NH moiety
1	Argatroban[b]	12.5	–
2	Inogatran[b]	12.3	–
3	NAPAP	12.6	–
7	Sulfaguanidine	8.4	7.0
A9		8.1	7.0
B9		8.3	7.1
C9		8.5	7.1

[a]pK_a values were determined in 30% Et-OH/water (v/v) as described in the Experimental section. [b]From ref. [8].

appeared of great interest to elaborate on non-basic variants of this interesting serine protease anchoring group. The sulfonylguanidino moiety appeared as an attractive candidate for such a purpose, since the presence of the SO_2 moiety in the neighbourhood of the strong base, guanidine, should drastically weaken its basicity. Such modified anchoring groups should not presumably interfere with the binding of the inhibitor to the enzyme, since the hydrogen-bonding donor/acceptor properties, as well as the possibility to interact electrostatically with the enzyme for the compounds incorporating them, should not differ too much from those of the classical amidino-/guanidino-based inhibitors of types **1–5** or **12**. The sulfonyl-guanidines possess a large number of possible tautomeric forms, and this factor might also be a critical one for the binding of such a compound to thrombin. Thus, in previous work [25] we have shown that arylsulfonyl guanidines, including sulfaguanidine **7**, possess moderate but specific thrombin inhibitory properties. Moreover, by means of AM1 and MOPAC calculations it was demonstrated that the tautomer of type **13A** of benzenesulfonylguanidine is much more stable than the tautomer **13B** (*figure 3*), a situation that seems to be important for binding to the enzyme [25]. Thus, we presume that the same is true for the pyridinium-based compounds reported here, i.e., that the symmetrical tautomers of type **13A** are more stable than the corresponding non-symmetrical tautomers of type **13B**. It is obvious from the above data that the symmetrical nature of the favoured tautomer should enable stronger interactions with the carboxylate moiety of Asp 189 and presumably, the formation of high affinity E-I adducts.

Thus, three series of pyridinium containing sulfanilylguanidines **A1**–**A16, B1**–**B16** and **C1**–**C16** were prepared in order to test the above-mentioned hypothesis (*table I*). These compounds were obtained by reactions of

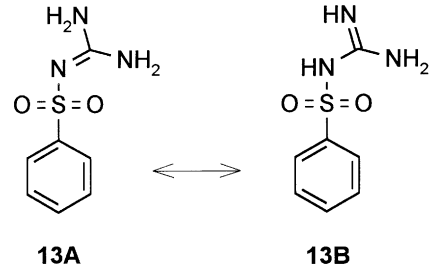


Figure 3. Tautomeric forms of compound **13**.

pyrylium salts with sulfaguanidine, or alternatively, by condensation of sulfaguanidine with the pyridinium derivatives of glycine or β-alanine, obtained from the two mentioned amino acids and pyrylium salts, by the original procedure of Balaban's group [27–32].

The following should be noted regarding the serine protease inhibition data of *tables I* and *II* with the new compounds and standard inhibitors: (i) the pyridinium derivatives **A**, **B**, **C** (1–16) reported here generally behave as stronger thrombin inhibitors as compared to the lead molecule from which they were derived, i.e., benzamidine **12** and sulfaguanidine **7**. At the same time, their affinity for trypsin is relatively low, which constitutes a positive feature for the putative clinical use of such compounds; (ii) in the three subseries of investigated compounds, thrombin inhibitory properties increased from the pyridinium derivatives of sulfaguanidine **A (1–16)** to the corresponding pyridinium-Gly-derivatives **B (1–16)**, with the pyridinium-β-Ala derivatives **C(1-16)** behaving as the most active inhibitors in the whole series of reported compounds (obviously, this discussion takes into account the same substitution pattern at the pyridinium ring for compounds in the three investigated subseries); (iii) the nature of R1–R4 groups substituting the pyridinium ring was critical for the biological activity of the obtained compounds, similarly to the situation evidenced for the carbonic anhydrase sulfonamide inhibitors reported previously [19, 20]. Thus, tri- or tetraalkylpyridinium- as well as 2,6-di- or 2,4,6-triphenylpyridinium moieties were generally less effective than 2-alkyl-4,6-diphenyl-pyridinium groups in inducing strong thrombin inhibitory properties to the compounds incorporating them. Practically, the most active derivatives in all three subseries were those containing 2-alkyl-4,6-diphenyl-pyridinium moieties, such as 2-methyl-; 2-ethyl-; 2-*iso*-propyl- or 2-*tert*-butyl-4,6-diphenyl-pyridinium groups. Replacing the 2-alkyl group mentioned above with a bulky phenyl one (such as in compounds **A14**, **B14** or **C14**) or with a longer aliphatic

chain (*n*-butyl, such as in **A12**, **B12** or **C12**) led to a drastic reduction of the thrombin inhibitory effects of the corresponding compounds. On the other hand, compounds possessing 2,6-dialkyl-4-phenyl-pyridinium moieties in their molecule (such as **A**, **B**, **C** (**4** and **5**)) possessed a behaviour intermediate between the strong inhibitors of the type **A**, **B**, **C (8**, **9**, **11** and **13)** and the relatively weak inhibitors of type **A**, **B**, **C (1–3** and **14–16)**. Anyhow, the best substitution for inducing strong thrombin inhibitory properties was that incorporating the 2-ethyl-4,6-diphenylpyridinium moiety in the molecules of the new derivatives. Some of the compounds containing this substitution pattern, such as **B9** and **C9** (but also the structurally-related compounds **B8**, **B10**, **B11**, **C8**, **C10** and **C11**) showed thrombin inhibitory properties of the same order of magnitude as the clinically used derivatives argatroban **1** and inogatran **2**, although they are less effective as compared to the very potent inhibitor NAPAP (*table II*). A special mention should be made regarding the fact that the new compounds reported here possess a much lower affinity for trypsin as compared to the standard inhibitors **1–3**, which constitutes a highly desirable feature for a compound to be developed for clinical use.

pK_a values for the amidino/guanidino as well as sulfonamido moieties of some of the newly synthesized serine protease inhibitors and standard compounds such as inogatran, argatroban and NAPAP (*table III*) prove that the approach proposed here for reducing the basicity of such an enzyme inhibitor is a successful one. Thus, unlike the highly basic guanidines/amidines of type **1–3** (pK_a's around 12.3–12.6), sulfaguanidine **7** and its derivatives reported here (such as compounds **A9**, **B9** or **C9**) have pK_a values of the guanidino moiety around 8.1–8.5, being at least 10^4 times less basic than the previously mentioned derivatives. Furthermore, due to the presence of the sulfonyl moiety in their molecules, these compounds also possess a weakly acidic character, with another ionization step around the pK_a value of 7, due to the loss of the SO_2NH proton. These features should positively influence the pharmacological profile of a thrombin inhibitor of the type described here.

The strong thrombin inhibitory properties of some compounds reported in this study might be explained by taking into account the X-ray crystallographic structure of the enzyme as well as those of some of its adducts with guanidine/amidine-based inhibitors [4, 5, 33, 34]. Thus, it was shown that effective binding is achieved when a proline, a pipecolic acid or a similarly non-hydrophilic moiety is present in the P2 position, which allows favourable interactions with the enzyme S2 cavity, comprising among others, amino acid residues Trp 60D and Tyr 60A as well as when hydrophobic (generally aromatic: Ph, Ts; naphthyl) groups are present at P3, which allow strong interactions with the S3 site, comprising residues Leu 99; Trp 215 and Ile 174 among others [4, 5, 33, 34]. Some moieties present in the compounds prepared by us might possess just the required structural elements for the formation of high affinity adducts with thrombin. For example, for the strongest inhibitor reported in this paper, **C9** (K_I = 15 nM against thrombin), the CH_2CH_2CO moiety might interact with the S2 cavity, whereas the two phenyls substituting the pyridinium moiety probably bind within the aryl binding site (S3). Obviously, the sulfonylguanidino moiety of all these inhibitors probably fills the S1 specificity pocket, interacting with Asp 189, as discussed earlier. But another aspect might be important for explaining the relatively high affinity of this entire class of inhibitors for thrombin. Thus, around the entrance of the specificity pocket of this enzyme, ten negatively-charged amino acid residues are clustered [4, 5, 33, 34]. Some of these (such as Asp 189 and Glu 192) are directly involved in the substrate/inhibitor recognition process, whereas some others might be crucial for driving or stabilizing the inhibitor within the active site [4]. In the case of cationic inhibitors, such as the compounds reported in the present paper, the presence of such a cluster of ten negatively-charged residues at the entrance of the active site might be extremely favourable for obtaining strong E-I adducts, due to the possibility of salt bridge formation between the cationic moiety of the inhibitor and the anionic groups of the enzyme. Associated with precise geometric requirements for binding to the S2 and S3 sites (mentioned above) our approach led to high affinity, less basic thrombin inhibitors.

5. Conclusion

Three series of cationic sulfaguanidine derivatives have been prepared by reaction of sulfaguanidine with di-, tri- or tetrasubstituted pyrylium salts bearing alkyl, aryl or a combination of the two moieties in their molecule, and with the corresponding Gly-pyridinium and β-Ala pyridinium derivatives, respectively. Qualitative SAR proved that the best activity for inhibiting thrombin was obtained for compounds bearing 2-alkyl-4,6-diaryl- pyridinium moieties, and that the β-Ala derivatives were more active than the corresponding Gly derivatives, which in turn were more active than the corresponding pyridinium-sulfaguanidines. The obtained compounds generally possessed a low affinity for trypsin, which might be considered a positive feature for the putative pharmacological development of such a throm-

bin inhibitor. Thus, our study proposes two novel approaches for the preparation of high affinity, specific thrombin inhibitors: 1) a novel S1 anchoring moiety of the arginine/amidine type, i.e., the $SO_2N=C(NH_2)_2$ group; and 2) novel non-peptidomimetic scaffolds obtained by incorporating alkyl-/aryl-substituted-pyridinium moieties in the hydrophobic binding site(s). The first approach is important for obtaining bioavailable thrombin inhibitors devoid of the high basicity of the commonly used arginine/amidine-based inhibitors, with some of the new derivatives proving to be 10^4 times less basic than the standard compounds in clinical use. The second one may lead to improved water solubility of such derivatives due to facilitated salt formation as well as increased in vivo stability at hydrolysis.

6. Experimental

6.1. Chemistry

Melting points: heating plate microscope (not corrected); IR spectra: KBr pellets, 400–4 000 cm^{-1} Perkin-Elmer 16PC FTIR spectrometer; ^{1}H-NMR spectra: Varian 300CXP apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard); Elemental analysis (± 0.4% of the theoretical values, calculated for the proposed formulas, data not shown): Carlo Erba Instrument CHNS Elemental Analyzer, Model 1106. All reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm precoated silica gel plates (E. Merck). Preparative HPLC was performed on a Dynamax-60A column (25 × 250 mm), with a Beckman EM-1760 instrument. The detection wavelength was 254 nm. Sulfaguanidine, triethylamine, carbodiimides, and amino acids used in the syntheses were commercially available compounds (from Sigma, Acros or Aldrich). Pyrylium salts were prepared as described in the literature [30–32]. Acetonitrile, acetone, dioxane, ethyl acetate (E. Merck, Darmstadt, Germany) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions. Inogatran was from Astra Hassle (Molndal, Sweden). Benzamidine, NAPAP, human thrombin, human trypsin and Chromozym TH were from Sigma Chem. Co. (St Louis, MO, USA).

6.1.1. General procedure for the preparation of compounds **A** (**1–16**)

Method A: an amount of 0.21 g (1 mM) of sulfaguanidine **7** and the stoichiometric amount of pyrylium salt **6** and 140 μL of triethylamine (1 mM) were dissolved/suspended in 20 mL of absolute methanol. The mixture was refluxed for 30 min, then 0.45 mL of glacial acetic acid were added and refluxation was continued for another 2 h. The cold mixture was treated with 100–200 mL of diethyl ether for the precipitation of the pyridinium salts **A1–A16** which were recrystallized from water with 2–5% perchloric acid.

Method B: an amount of 0.60 g (2.9 mM) of sulfaguanidine **7** and 2.9 mM of pyrylium salt **6** were suspended in 5 mL of anhydrous methanol and poured into a stirred mixture of 14.5 mM of triethylamine and 5.8 mM of acetic anhydride. After 5 min of stirring, another 10 mL of methanol were added to the reaction mixture, which was heated to reflux for 15 min. Then 14.5 mM of acetic acid was added and heating was continued for 2–5 h. The role of the acetic anhydride is to react with the water formed during the condensation reaction between the pyrylium salt and the aromatic amine, in order to shift the equilibrium towards the formation of the pyridinium salts of type **A1–A16**. In the case of sulfaguanidine, this procedure is the only one which gave acceptable yields in pyridinium salts possessing 2-methyl groups. The precipitated pyridinium salts obtained were then purified by treatment with concentrated ammonia solution (which also converts the eventually unreacted pyrylium salt to the corresponding pyridine which is soluble in acidic medium), reprecipitation with perchloric acid and recrystallization from water with 2–5% $HClO_4$.

6.1.1.1. 1-N-(4-Guanidinosulfonyl-phenyl)-2,4,6-trimethylpyridinium perchlorate **A1**

White crystals, m.p. 273–275 °C (yield of 34%); IR (KBr), cm^{-1}: 625, 740, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 040, 3 245, 3 335; ^{1}H-NMR (TFA), δ ppm: 2.56 (s, 6H, 2,6-$(Me)_2$); 2.81 (s, 3H, 4-Me); 7.35–7.85 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 8.10 (s, 2H, ArH, 3,5-H from pyridinium). Anal. $C_{15}H_{19}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.2. 1-N-(4-Guanidinosulfonyl-phenyl)-2-iso-propyl-4,6-dimethylpyridinium perchlorate **A2**

Pale yellow crystals, m.p. 255–256 °C (yield of 51%); IR (KBr), cm^{-1}: 625, 680, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 020, 3 235; ^{1}H-NMR (TFA), δ ppm: 1.50 (d, 6H, 2Me from *i*-Pr); 2.70 (s, 3H, 6-Me); 2.83 (s, 3H, 4-Me); 3.48 (heptet, 1H, CH from *i*-Pr); 7.25–8.45 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 7.98 (s, 2H, ArH, 3,5-H from pyridinium). Anal. $C_{17}H_{23}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.3. 1-N-(4-Guanidinosulfonyl-phenyl)-2,6-di-iso-propyl-4-methylpyridinium perchlorate **A3**

Tan crystals, m.p. 201–202 °C (yield of 76%); IR (KBr), cm^{-1}: 625, 685, 820, 1 100, 1 175, 1 290, 1 345,

1 580, 1 675, 3 030, 3 250; ^{1}H-NMR (TFA), δ ppm: 1.51 (d, 12H, 4Me from 2 *i*-Pr); 2.80 (s, 3H, 4-Me); 3.42 (heptet, 2H, 2CH from 2 *i*-Pr); 7.31–8.51 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 8.05 (s, 2H, ArH, 3,5-H from pyridinium). Anal. $C_{19}H_{27}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.4. 1-N-(4-Guanidinosulfonyl-phenyl)-2,6-dimethyl-4-phenylpyridinium perchlorate **A4**

White crystals, m.p. 280–281 °C (yield of 50%); IR (KBr), cm^{-1}: 625, 690, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 030, 3 260, 3 330; ^{1}H-NMR (TFA), δ ppm: 2.58 (s, 6H, 2,6-$(Me)_2$); 8.10–9.12 (m, 11H, ArH from 1,4-phenylene, pyridinium and 4-Ph). Anal. $C_{20}H_{21}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.5. 1-N-(4-Guanidinosulfonyl-phenyl)-2,6-diethyl-4-phenylpyridinium perchlorate **A5**

Yellow crystals, m.p. 263–265 °C (yield of 37%); IR (KBr), cm^{-1}: 625, 765, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 040, 3 270, 3 360; ^{1}H-NMR (TFA), δ ppm: 1.43 (t, 6H, 2 Me from ethyl); 2.82 (q, 4H, 2 CH_2 from Et); 7.68–8.87 (m, 11H, ArH from 1,4-phenylene, pyridinium and 4-Ph). Anal. $C_{22}H_{25}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.6. 1-N-(4-Guanidinosulfonyl-phenyl)-2,6-di-n-propyl-4-phenylpyridinium perchlorate **A6**

Yellowish crystals, m.p. 215–216 °C (yield of 61%); IR (KBr), cm^{-1}: 625, 775, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 060, 3 220, 3 315; ^{1}H-NMR (TFA), δ ppm: 1.01 (t, 6H, 2 Me from propyl); 1.70 (sextet, 4H, 2CH_2 (β) from *n*-Pr); 2.80 (t, 4H, 2 CH_2 (α) from *n*-Pr); 7.55–8.78 (m, 11H, ArH from 1,4-phenylene, pyridinium and 4-Ph). Anal. $C_{24}H_{29}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.7. 1-N-(4-Guanidinosulfonyl-phenyl)-2,6-di-iso-propyl-4-phenylpyridinium perchlorate **A7**

White crystals, m.p. 198–199 °C (yield of 24%); IR (KBr), cm^{-1}: 625, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 060, 3 270, 3 315; ^{1}H-NMR (TFA), δ ppm: 1.45 (d, 12H, 4 Me from *i*-Pr); 2.95 (heptet, 2H, 2 CH from *i*-Pr); 7.92–8.97 (m, 11H, ArH from 1,4-phenylene, pyridinium and 4-Ph). Anal. $C_{24}H_{29}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.8. 1-N-(4-Guanidinosulfonyl-phenyl)-2-methyl-4,6-diphenylpyridinium perchlorate **A8**

White crystals, m.p. 262–263 °C (yield of 30%); IR (KBr), cm^{-1}: 625, 770, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 040, 3 245, 3 350; ^{1}H-NMR (TFA), δ ppm: 2.72 (s, 3H, 2-Me); 7.55–8.73 (m, 16H, ArH from 1,4-phenylene, pyridinium and 4,6-Ph_2). Anal. $C_{25}H_{23}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.9. 1-N-(4-Guanidinosulfonyl-phenyl)-2-ethyl-4,6-diphenylpyridinium perchlorate **A9**

White-yellow crystals, m.p. 233–234 °C (yield of 39%); IR (KBr), cm^{-1}: 625, 700, 770, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 040, 3 250, 3 350; ^{1}H-NMR (TFA), δ ppm: 1.50 (t, 3H, Me from ethyl); 2.97 (q, 2H, CH_2); 7.40–8.57 (m, 16H, ArH from 1,4-phenylene, pyridinium and 4,6-Ph_2). Anal. $C_{26}H_{25}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.10. 1-N-(4-Guanidinosulfonyl-phenyl)-2-n-propyl-4,6-diphenylpyridinium perchlorate **A10**

White crystals, m.p. 243–244 °C (yield of 36%); IR (KBr), cm^{-1}: 625, 700, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 030, 3 270, 3 350; ^{1}H-NMR (TFA), δ ppm: 1.05 (t, 3H, Me from propyl); 1.93 (sextet, 2H, β-CH_2 from *n*-Pr); 2.93 (t, 2H, α-CH_2 from *n*-Pr); 7.38–8.53 (m, 16H, ArH from 1,4-phenylene, pyridinium and 4,6-Ph_2). Anal. $C_{27}H_{27}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.11. 1-N-(4-Guanidinosulfonyl-phenyl)-2-iso-propyl-4,6-diphenylpyridinium perchlorate **A11**

White crystals, m.p. 183–184 °C (yield of 25%); IR (KBr), cm^{-1}: 625, 700, 770, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 040, 3 250, 3 360; ^{1}H-NMR (TFA), δ ppm: 1.52 (d, 6H, 2 Me from *i*-propyl); 2.52–3.25 (m, 1H, CH from *i*-Pr); 7.33–8.60 (m, 16H, ArH from 1,4-phenylene, pyridinium and 4,6-Ph_2). Anal. $C_{27}H_{27}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.12. 1-N-(4-Guanidinosulfonyl-phenyl)-2-n-butyl-4,6-diphenylpyridinium perchlorate **A12**

White crystals, m.p. 244–245 °C (yield of 72%); IR (KBr), cm^{-1}: 625, 710, 770, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 060, 3 260, 3 345; ^{1}H-NMR (TFA), δ ppm: 0.90 (t, 3H, Me from butyl); 1.10–2.15 (m, 4H, CH_3-*CH_2*-*CH_2*-CH_2 from *n*-Bu); 2.97 (t, 2H, α-CH_2 from *n*-Bu); 7.25–8.52 (m, 16H, ArH from 1,4-phenylene, pyridinium and 4,6-Ph_2). Anal. $C_{28}H_{29}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.13. 1-N-(4-Guanidinosulfonyl-phenylmethyl)-2-tert-butyl-4,6-diphenylpyridinium perchlorate **A13**

White crystals, m.p. 197–198 °C (yield of 62%); IR (KBr), cm^{-1}: 625, 765, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 060, 3 270; ^{1}H-NMR (TFA), δ ppm: 1.90 (s, 9H, *t*-Bu); 6.83–8.83 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{28}H_{29}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.14. 1-N-(4-Guanidinosulfonyl-phenyl)-2,4,6-triphenylpyridinium perchlorate **A14**

Yellow crystals, m.p. 234–235 °C (yield of 80%); IR (KBr), cm^{-1}: 625, 700, 770, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 030, 3 260, 3 350; ^{1}H-NMR (TFA), δ ppm: 7.47–8.63 (m, 21H, ArH from 1,4-phenylene, pyridinium and 2,4,6-Ph_3). Anal. $C_{30}H_{25}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.15. 1-N-(4-Guanidinosulfonyl-phenyl)-2,6-diphenylpyridinium perchlorate **A15**

Yellow-orange crystals, m.p. 250–252 °C (yield of 36%); IR (KBr), cm^{-1}: 625, 705, 765, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 050, 3 260; ^{1}H-NMR (TFA), δ ppm: 6.71–8.40 (m, 17H, ArH from 1,4-phenylene, 2,6-Ph_2 and 3,4,5-H from pyridinium). Anal. $C_{24}H_{20}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.1.16. 1-N-(4-Guanidinosulfonyl-phenyl)-2,3,4,6-tetramethylpyridinium perchlorate **A16**

White crystals, m.p. 256–257 °C (yield of 28%); IR (KBr), cm^{-1}: 625, 750, 1 100, 1 175, 1 290, 1 345, 1 580, 1 675, 3 040, 3 245, 3 330; ^{1}H-NMR (TFA), δ ppm: 2.45 (s, 3H, 3-Me); 2.50 (s, 3H, 4-Me); 2.55 (s, 3H, 6-Me); 2.75 (s, 3H, 2-Me); 8.03–9.17 (m, 5H, ArH from 1,4-phenylene and pyridinium 5-H). Anal. $C_{16}H_{21}N_4O_2S^+$ ClO_4^- (C, H, N, S).

6.1.2. General procedure for the preparation of derivatives **10** *and* **11**

An amount of 10 mM of amino acid (Gly or β-Ala) was suspended/dissolved in 50 mL of anhydrous acetonitrile and the stoichiometric amount (10 mM) of pyrylium salt **6** and triethyl amine (10 mM, 1.47 mL) were added. The reaction mixture was heated at reflux for 4 h, then 2.5 mL of glacial acetic acid were added and refluxation was continued for another 2 h. The obtained reaction mixture was treated as described above (Method A), in order to obtain the pure intermediates **10** and **11** (recrystallized from water with 2–5% perchloric acid).

6.1.3.General procedure for the preparation of compounds **B** *and* **C** (**1–16**)

An amount of 1 mM of pyridinium-amino acid derivative **10** or **11** was dissolved/suspended in 25 mL of anhydrous acetonitrile or acetone, and then treated with 210 mg (1 mM) of sulfaguanidine **7** and 190 mg (1 mM) of EDCI. HCl or di-isopropyl-carbodiimide. The reaction mixture was magnetically stirred at room temperature for 15 min, then 30 μL (2 mM) of triethylamine were added and stirring was continued for 16 h at 4 °C. The solvent was evaporated in vacuo and the residue taken up in ethyl acetate (5 mL), poured into a 5% solution of sodium bicarbonate (5 mL) and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and filtered, and the solvent removed in vacuo. Preparative HPLC (Dynamax-60A column (25 × 250 mm); 90% acetonitrile/8% methanol/2% water; flow rate of 30 mL/min) afforded the pure compounds **B** and **C (1–16)** as colourless solids.

6.1.3.1. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,4,6-trimethylpyridinium perchlorate **B1**

White-tan crystals, m.p. 280–282 °C (yield of 80%); IR (KBr), cm^{-1}: 625, 680, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 030, 3 250; ^{1}H-NMR (TFA), δ ppm: 2.70 (s, 3H, 4-Me); 2.85 (s, 6H, 2,6-$(Me)_2$); 4.12 (s, 2H, Gly CH_2); 7.13–8.41 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 8.00 (s, 2H, ArH, 3,5-H from pyridinium). Anal. $C_{17}H_{21}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.2. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-iso-propyl-4,6-dimethylpyridinium perchlorate **B2**

Light orange crystals, m.p. 205–207 °C (yield of 64%); IR (KBr), cm^{-1}: 625, 680, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 020, 3 235; ^{1}H-NMR (TFA), δ ppm: 1.50 (d, 6H, 2Me from *i*-Pr); 2.80 (s, 3H, 6-Me); 2.90 (s, 3H, 4-Me); 3.48 (heptet, 1H, CH from *i*-Pr); 4.12 (s, 2H, Gly CH_2); 7.25–8.43 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 7.98 (s, 2H, ArH, 3,5-H from pyridinium). Anal. $C_{19}H_{25}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.3. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,6-di-iso-propyl-4-methylpyridiniumperchlorate **B3**

Tan crystals, m.p. 202–203 °C (yield of 75%); IR (KBr), cm^{-1}: 625, 820, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 030, 3 250; ^{1}H-NMR (TFA), δ ppm: 1.51 (d, 12H, 4Me from 2 *i*-Pr); 2.83 (s, 3H, 4-Me); 3.42 (heptet, 2H, 2CH from 2 *i*-Pr); 4.12 (s, 2H, CH_2); 7.31–8.51 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 8.03 (s, 2H, ArH, 3,5-H from pyridinium). Anal. $C_{21}H_{29}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.4. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,6-dimethyl-4-phenylpyridinium perchlorate **B4**

Orange-red crystals, m.p. 240–241 °C (yield of 69%); IR (KBr), cm^{-1}: 625, 765, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 050, 3 265; ^{1}H-NMR (TFA), δ ppm: 3.00 (s, 6H, 2,6-$(Me)_2$); 4.12 (s, 2H, CH_2); 7.21–8.51 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{22}H_{23}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.5. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,6-diethyl-4-phenylpyridinium perchlorate **B5**

Tan crystals, m.p. 223–224 °C (yield of 53%); IR (KBr), cm^{-1}: 625, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 060, 3 230; ^{1}H-NMR (TFA), δ ppm: 1.55 (t, 6H, 2 Me from Et); 3.30 (q, 4H, 2 CH_2 from Et); 4.12 (s, 2H, N^+ -CH_2); 7.08–8.63 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{24}H_{27}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.6. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,6-di-n-propyl-4-phenylpyridinium perchlorate **B6**

Tan crystals, m.p. 218–219 °C (yield of 55%); IR (KBr), cm^{-1}: 625, 775, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 060, 3 240; ^{1}H-NMR (TFA), δ ppm: 1.15 (t, 6H, 2 Me from Pr); 1.90 (sextet, 4H, 2 CH_2 from Pr); 3.18 (t, 4H, 2 CH_2 from Pr); 4.12 (s, 2H, N^+-CH_2); 7.10–8.50 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{26}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.7. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,6-di-iso-propyl-4-phenylpyridiniumperchlorate **B7**

Tan crystals, m.p. 210–213 °C (yield of 79%); IR (KBr), cm^{-1}: 625, 775, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 060, 3 240; ^{1}H-NMR (TFA), δ ppm: 1.55 (d, 12H, 4 Me from *i*-Pr); 3.53 (heptet, 2H, 2 CH from *i*-Pr); 4.13 (s, 2H, N^+-CH_2); 7.23–8.65 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{26}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.8. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-methyl-4,6-diphenylpyridinium perchlorate **B8**

Yellow crystals, m.p. 254–255 °C (yield of 51%); IR (KBr), cm^{-1}: 625, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 050, 3 250; ^{1}H-NMR (TFA), δ ppm: 3.00 (s, 3H, 2-Me); 4.12 (s, 2H, CH_2); 7.08–8.58 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{27}H_{25}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.9. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-ethyl-4,6-diphenylpyridinium perchlorate **B9**

White crystals, m.p. 221–223 °C (yield of 84%); IR (KBr), cm^{-1}: 625, 705, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 050, 3 250; ^{1}H-NMR (TFA), δ ppm: 1.60 (t, 3H, Me from Et); 3.27 (q, 2H, CH_2 from Et); 4.12 (s, 2H, N^+-CH_2); 7.08–8.60 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{28}H_{27}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.10. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-n-propyl-4,6-diphenylpyridinium perchlorate **B10**

White-yellowish crystals, m.p. 200–201 °C (yield of 53%); IR (KBr), cm^{-1}: 625, 685, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 080, 3 250; ^{1}H-NMR (TFA), δ ppm: 1.18 (t, 3H, Me from Pr); 2.10 (sextet, 2H, CH_2 from *n*-Pr); 3.20 (t, 2H, CH_2 from *n*-Pr); 4.12 (s, 2H, N^+-CH_2); 7.08–8.63 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{29}H_{29}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.11. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-iso-propyl-4,6-diphenylpyridinium perchlorate **B11**

Tan crystals, m.p. 187–188 °C (yield of 62%); IR (KBr), cm^{-1}: 625, 710, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 070, 3 250; ^{1}H-NMR (TFA), δ ppm: 1.55 (d, 6H, 2 Me from *i*-Pr); 3.55 (heptet, 1H, CH from *i*-Pr); 4.10 (s, 2H, N^+-CH_2); 7.08–8.63 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{29}H_{29}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.12. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-n-butyl-4,6-diphenylpyridinium perchlorate **B12**

Tan crystals, m.p. 198–199 °C (yield of 43%); IR (KBr), cm^{-1}: 625, 690, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 080, 3 250; ^{1}H-NMR (TFA), δ ppm: 0.93 (t, 3H, Me from *n*-Bu); 1.55 (sextet, 2H, CH_2 from *n*-Bu); 2.05 (quintet, 2H, CH_2 from *n*-Bu); 3.17 (t, 2H, CH_2 from *n*-Bu); 4.12 (s, 2H, N^+-CH_2); 7.08–8.58 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{30}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.13. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2-tert-butyl-4,6-diphenylpyridinium perchlorate **B13**

White crystals, m.p. 201–203 °C (yield of 54%); IR (KBr), cm^{-1}: 625, 705, 765, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 060, 3 270; ^{1}H-NMR (TFA), δ ppm: 1.90 (s, 9H, *t*-Bu); 4.22 (s, 2H, CH_2); 6.83–8.83 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{30}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.14. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,4,6-triphenylpyridinium perchlorate **B14**

Orange crystals, m.p. 234–235 °C (yield of 70%); IR (KBr), cm^{-1}: 625, 705, 770, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 050, 3 270; ^{1}H-NMR (TFA), δ ppm: 4.09 (s, 2H, CH_2); 6.70–8.56 (m, 21H, ArH from 1,4-phenylene, 2,4,6-Ph_3 and 3,5-H from pyridinium). Anal. $C_{32}H_{27}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.15. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,6-diphenylpyridinium perchlorate **B15**

Yellow-orange crystals, m.p. 204–206 °C (yield of 40%); IR (KBr), cm^{-1}: 625, 705, 765, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 050, 3 260; ^{1}H-NMR (TFA), δ ppm: 4.13 (s, 2H, CH_2); 6.71–8.40 (m, 17H, ArH from 1,4-phenylene, 2,6-Ph_2 and 3,4,5-H from pyridinium). Anal. $C_{26}H_{22}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.16. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylmethyl]-2,3,4,6-tetramethylpyridinium perchlorate **B16**

White-tan crystals, m.p. 253–255 °C (yield of 65%); IR (KBr), cm^{-1}: 625, 800, 1 100, 1 175, 1 290, 1 345, 1 535, 1 580, 1 640, 1 675, 3 030, 3 305; ^{1}H-NMR (TFA), δ ppm: 2.60 (s, 3H, 4-Me); 2.77 (s, 3H, 3-Me); 2.87 (s, 6H, 2,6-$(Me)_2$); 4.12 (s, 2H, CH_2); 7.21–8.50 (m, AA´BB´, 4H, ArH from 1,4-phenylene); 7.90 (s, 1H, ArH, 5-H from pyridinium). Anal. $C_{18}H_{23}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.17. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,4,6-trimethylpyridinium perchlorate **C1**

White crystals, m.p. 266–267 °C (yield of 84%); IR (KBr), cm^{-1}: 625, 680, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 060, 3 250, 3 330; ^{1}H-NMR (TFA), δ ppm: 2.66 (s, 3H, 4-Me); 2.88 (s, 6H, 2,6-$(Me)_2$); 3.12 (t, 2H, CH_2); 4.05 (t, 2H, CH_2); 7.47–8.38 (m, 6H, ArH from 1,4-phenylene and 3,5-H from pyridinium). Anal. $C_{18}H_{23}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.18. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-iso-propyl-4,6-dimethylpyridinium perchlorate **C2**

White crystals, m.p. 244–245 °C (yield of 83%); IR (KBr), cm^{-1}: 625, 685, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 040, 3 255, 3 380; ^{1}H-NMR (TFA), δ ppm: 1.47 (d, 6H, 2Me from *i*-Pr); 2.68 (s, 3H, 4-Me); 2.90 (s, 3H, 6-Me); 3.10–3.75 (m, 3H, CH from *i*-Pr + CH_2); 4.03 (t, 2H, CH_2); 7.33–8.35 (m, 6H, ArH from 1,4-phenylene and 3,5-H from pyridinium). Anal. $C_{20}H_{26}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.19. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,6-di-iso-propyl-4-methylpyridinium perchlorate **C3**

White crystals, m.p. 250–251 °C (yield of 76%); IR (KBr), cm^{-1}: 625, 685, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 040, 3 235, 3 410; ^{1}H-NMR (TFA), δ ppm: 1.48 (d, 12H, 4Me from 2 *i*-Pr); 2.70 (s, 3H, 4-Me); 3.15–3.79 (m, 4H, 2CH from 2 *i*-Pr + CH_2); 4.02 (t, 2H, CH_2); 7.33–8.27 (m, 6H, ArH from 1,4-phenylene and 3,5-H from pyridinium). Anal. $C_{22}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.20. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,6-dimethyl-4-phenylpyridinium perchlorate **C4**

White crystals, m.p. 221–223 °C (yield of 72%); IR (KBr), cm^{-1}: 625, 690, 780, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 050, 3 280; ^{1}H-NMR (TFA), δ ppm: 3.08 (s, 6H, 2,6-$(Me)_2$); 3.15 (t, 2H, CH_2); 4.03 (t, 2H, CH_2); 7.55–8.37 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{23}H_{25}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.21. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,6-diethyl-4-phenylpyridinium perchlorate **C5**

White crystals, m.p. 227–229 °C (yield of 80%); IR (KBr), cm^{-1}: 625, 700, 780, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 060, 3 240, 3 335; ^{1}H-NMR (TFA), δ ppm: 1.67 (t, 6H, 2 Me from Et); 3.15–3.80 (m, 6H, 2 CH_2 from Et + CH_2 from ethylene bridge); 4.07 (t, 2H, CH_2 from ethylene bridge); 7.57–8.50 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{25}H_{29}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.22. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,6-di-n-propyl-4-phenylpyridinium perchlorate **C6**

White crystals, m.p. 212–214 °C (yield of 63%); IR (KBr), cm^{-1}: 625, 685, 775, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 050, 3 255, 3 335; ^{1}H-NMR (TFA), δ ppm: 1.23 (t, 6H, 2 Me from Pr); 2.03 (q, 4H, 2 CH_2 from Pr); 3.07–3.75 (m, 6H, 2 CH_2 from Pr + CH_2 from ethylene bridge); 4.05 (t, 2H, CH_2 from ethylene bridge); 7.55–8.43 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{27}H_{33}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.23. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,6-di-iso-propyl-4-phenylpyridinium perchlorate **C7**

White crystals, m.p. 241–243 °C (yield of 69%); IR (KBr), cm^{-1}: 625, 685, 765, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 060, 3 270, 3 350; ^{1}H-NMR

(TFA), δ ppm: 1.60 (d, 12H, 4 Me from *i*-Pr); 3.10–3.83 (m, 4H, 2 CH from *i*-Pr + CH_2 from ethylene bridge); 4.13 (t, 2H, CH_2 from ethylene bridge); 7.47–8.43 (m, 11H, ArH from 1,4-phenylene, 4-Ph and 3,5-H from pyridinium). Anal. $C_{27}H_{33}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.24. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-methyl-4,6-diphenylpyridinium perchlorate **C8**

White crystals, m.p. 233–234 °C (yield of 77%); IR (KBr), cm^{-1}: 625, 675, 775, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 050, 3 245, 3 435; ^{1}H-NMR (TFA), δ ppm: 3.03–3.39 (m, 5H, 2-Me + CH_2 from ethylene bridge); 4.06 (t, 2H, CH_2 from ethylene bridge); 7.05–8.45 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{28}H_{27}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.25. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-ethyl-4,6-diphenylpyridinium perchlorate **C9**

White crystals, m.p. 229–230 °C (yield of 54%); IR (KBr), cm^{-1}: 625, 685, 750, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 050, 3 220, 3 390; ^{1}H-NMR (TFA), δ ppm: 1.72 (t, 3H, Me from Et); 2.90–3.78 (m, 4H, CH_2 from Et + CH_2 from ethylene bridge); 4.08 (t, 2H, CH_2 from ethylene bridge); 6.88–8.47 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{29}H_{29}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.26. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-n-propyl-4,6-diphenylpyridinium perchlorate **C10**

White crystals, m.p. 235–236 °C (yield of 59%); IR (KBr), cm^{-1}: 625, 705, 775, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 080, 3 255, 3 340; ^{1}H-NMR (TFA), δ ppm: 1.32 (t, 3H, Me from Pr); 2.17 (sextet, 2H, CH_2 from *n*-Pr); 2.82–3.66 (m, 4H, CH_2 from *n*-Pr + CH_2 from ethylene bridge); 4.09 (t, 2H, CH_2 from ethylene bridge); 6.83–8.43 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{30}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.27. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-iso-propyl-4,6-diphenylpyridinium perchlorate **C11**

White crystals, m.p. 234–235 °C (yield of 73%); IR (KBr), cm^{-1}: 625, 700, 765, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 070, 3 250, 3 350; ^{1}H-NMR (TFA), δ ppm: 1.70 (d, 6H, 2 Me from *i*-Pr); 3.15 (t, 2H, CH_2 from ethylenic bridge); 3.50–4.03 (m, 1H, CH from *i*-Pr); 4.11 (t, 2H, CH_2 from ethylenic bridge); 6.95–8.53 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{30}H_{31}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.28. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-n-butyl-4,6-diphenylpyridinium perchlorate **C12**

White crystals, m.p. 207–208 °C (yield of 78%); IR (KBr), cm^{-1}: 625, 685, 7650, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 080, 3 255, 3 330; ^{1}H-NMR (TFA), δ ppm: 1.15 (t, 3H, Me from *n*-Bu); 1.38–2.45 (m, 4H, 2 CH_2 from *n*-Bu); 3.00–3.68 (m, 4H, CH_2 from *n*-Bu + CH_2 from ethylenic bridge); 4.10 (t, 2H, CH_2 from ethylenic bridge); 7.02–8.43 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{31}H_{33}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.29. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2-tert-butyl-4,6-diphenylpyridinium perchlorate **C13**

White crystals, m.p. 220–222 °C (yield of 69%); IR (KBr), cm^{-1}: 625, 700, 765, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 060, 3 250, 3 370; ^{1}H-NMR (TFA), δ ppm: 1.92 (s, 9H, *t*-Bu); 3.14 (t, 2H, CH_2); 4.10 (t, 2H, CH_2 from ethylene bridge); 6.90–8.77 (m, 16H, ArH from 1,4-phenylene, 4,6-Ph_2 and 3,5-H from pyridinium). Anal. $C_{31}H_{33}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.30. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,4,6-triphenylpyridinium perchlorate **C14**

Yellow crystals, m.p. 213–214 °C (yield of 82%); IR (KBr), cm^{-1}: 625, 680, 770, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 050, 3 260, 3 335; ^{1}H-NMR (TFA), δ ppm: 3.12 (t, 2H, CH_2 from ethylene bridge); 4.05 (t, 2H, CH_2 from ethylene bridge); 6.57–8.40 (m, 21H, ArH from 1,4-phenylene, 2,4,6-Ph_3 and 3,5-H from pyridinium). Anal. $C_{33}H_{29}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.31. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,6-diphenylpyridinium perchlorate **C15**

Yellow crystals, m.p. 212–214 °C (yield of 16%); IR (KBr), cm^{-1}: 625, 700, 760, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 050, 3 240, 3 325; ^{1}H-NMR (TFA), δ ppm: 3.07 (t, 2H, CH_2); 4.13 (t, 2H, CH_2 from ethylene bridge); 6.55–8.50 (m, 17H, ArH from 1,4-phenylene, 2,6-Ph_2 and 3,4,5-H from pyridinium). Anal. $C_{27}H_{24}N_4O_3S^+$ ClO_4^- (C, H, N, S).

6.1.3.32. 1-N-[(4-Guanidinosulfonyl-phenyl)aminocarbonylethyl]-2,3,4,6-tetramethylpyridinium perchlorate **C16**

White crystals, m.p. 210–211 °C (yield of 51%); IR (KBr), cm^{-1}: 625, 680, 1 100, 1 175, 1 285, 1 345, 1 540, 1 580, 1 645, 1 675, 3 030, 3 245, 3 325; ^{1}H-NMR

(TFA), δ ppm: 2.52 (s, 3H, 3-Me); 2.62 (s, 3H, 4-Me); 2.83 (s, 3H, 6-Me); 2.92 (s, 3H, 2-Me); 3.13 (t, 2H, CH_2); 4.07 (t, 2H, CH_2); 7.61–8.55 (m, 5H, ArH from 1,4-phenylene + 5-H from pyridinium). Anal. $C_{19}H_{25}N_4O_3S^+ ClO_4^-$ (C, H, N, S).

6.2. Pharmacology

6.2.1. Enzyme assays: K_I determinations

Human thrombin and human trypsin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); their concentrations were determined from the absorbance at 280 nm and the extinction coefficients furnished by the supplier. The activity of such preparations was in the range of 2 500–3 000 NIH units/mg. The potency of standard and newly obtained inhibitors was determined from the inhibition of the enzymatic (amidolytic) activity of these serine proteases, at 21 °C, using Ts-Gly-Pro-Arg-*p*NA (Chromozym TH) from Sigma as substrate, by the method of Lottenberg et al. [23]. The substrate was reconstituted as a 4 mM stock in ultrapure water and brought to pH 4 with hydrochloric acid. Substrate concentrations were determined from absorbance at the isosbestic wavelength for the peptide-*p*-nitroanilide/*p*-nitroaniline mixtures. Extinction coefficients of 8 270 $L.mol^{-1}.cm^{-1}$ in the used buffer (0.01 M Hepes, 0.01 M Tris, 0.1 M NaCl, 0.1% polyethylene glycol 6000; pH 7.80) were employed. The rate of *p*-nitroanilide hydrolysis was determined from the change in absorbance at 405 nm using an extinction coefficient for *p*-nitroaniline of 9 920 $L.mol^{-1}.cm^{-1}$ for the above-mentioned reaction buffer. Measurements were made using a Cary 3 spectrophotometer interfaced with a PC. Initial velocities were thus estimated using the direct linear plot-based procedure as reported by Lottenberg et al. [23]. K_I's were then determined according to Dixon, using a linear regression program [26]. The K_I values determined are the means of at least three determinations.

6.2.2. pK_a determination

The half neutralization point was measured by titrating the organic acids/bases with 0.05 N NaOH and 0.05 N HCl in EtOH/water (30%, v/v), using a glass electrode, as described by Bell and Roblin [35] for the structurally-related antibacterial sulfonamides.

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References

[1] Shafer J.A., Curr. Opin. Chem. Biol. 2 (1998) 458–465.
[2] Stürzebecher J., Meier J., J. Enzyme Inhib. 9 (1995) 1–2.
[3] Stürzebecher J., Prasa D., Hauptmann J., Vieweg H., Wikstrom P., J. Med. Chem. 40 (1997) 3091–3099.
[4] Pavone V., De Simone G., Nastri F., Galdiero S., Staiano N., Lombardi A., Pedone C., Biol. Chem. 379 (1998) 987–1006.
[5] Engh R.A., Brandstetter H., Sucher G., Eichinger A., Baumann U., Bode W. et al., Structure 4 (1996) 1353–1362.
[6] Babine R.E., Bender S.L., Chem. Rev. 97 (1997) 1359–1472.
[7] Salemme F.R., Spurlino J., Bone R., Structure 5 (1997) 319–324.
[8] Eriksson U.G., Renberg L., Bredberg U., Teger-Nilsson A.C., Regardh C.G., Biopharm. Drug Dispos. 19 (1998) 55–64.
[9] Bajusz S., Szell E., Bagdy D., Barbas E., Horvath G., Dioszegi M. et al., J. Med. Chem. 33 (1990) 1729–1735.
[10] Krishnan R., Zhang E., Hakansson K., Arni R.K., Tulinsky A., Lim-Wilby M.S. et al., Biochemistry 37 (1998) 12094–13103.
[11] Claeson G., Philipp M., Agner E., Scully M.F., Metternich R., Kakkar V.V., Desoyza T., Niu L.H., Biochem. J. 290 (1993) 309–312.
[12] Stürzebecher J., Prasa D., Bretschneider E., Bode W., Bauer M., Brandstetter H., Wikstrom P., Vieweg H., New developments in the field of benzamidine-derived thrombin inhibitors, in: Muller-Berghaus G., Madlener K., Blomback M., ten Cate J.W. (Eds.), DIC – Pathogenesis, Diagnosis, and Therapy of Disseminated Intravascular Fibrin Formation, Excerpta Medica, Amsterdam, London, New York, Tokyo, 1993, pp. 183–190.
[13] Lumma W.C., Witherup K.M., Tucker T.J., Brady S.F., Sisko J.T., Naylor-Olsen A.M. et al., J. Med. Chem. 41 (1998) 1011–1013.
[14] Semple J.E., Rowley D.C., Brunck T.K., Ha-Uong T., Minami N.K., Owens T.D. et al., J. Med. Chem. 39 (1996) 4531–4536.
[15] Sixma J.J., de Groot P.G., Thromb. Res. 68 (1992) 507–512.
[16] Okamoto S., Kinjo K., Hijikata A., Kikumoto R., Tamao Y., Ohkubo K., Tonomura S., J. Med. Chem. 23 (1980) 827–830.
[17] Stürzebecher J., Markwardt F., Voigt B., Wagner G., Walsmann P., Thromb. Res. 29 (1983) 635–642.
[18] Groutas W.C., Kuang R., Venkataraman R., Epp J.B., Ruan S., Prakash O., Biochemistry 36 (1997) 4739–4750.
[19] Supuran C.T., Manole G., Dinculescu A., Schiketanz A., Gheorghiu M.D., Puscas I., Balaban A.T., J. Pharm. Sci. 81 (1992) 716–719.
[20] Supuran C.T., Scozzafava A., Ilies M.A., Iorga B., Cristea T., Chiraleu F., Banciu M.D., Eur. J. Med. Chem. 33 (1998) 577–594.
[21] Anderson G.W., Zimmerman J.E., Callahan F.M., J. Am. Chem. Soc. 85 (1963) 3039.
[22] Sheehan J.C., Ledis S.L., J. Am. Chem. Soc. 95 (1973) 875–879.
[23] Lottenberg R., Christensen U., Jackson C.M., Coleman P.L., Meth. Enzymol. 80 (1981) 341–361.
[24] Banner D.W., Hadvary P., J. Biol. Chem. 266 (1991) 20085–20093.
[25] Supuran C.T., Scozzafava A., Briganti F., Clare B.W., J. Med. Chem. in press.
[26] Hemker H.C., Willems G.M., Beguin S.A., Thromb. Haemostas. 56 (1986) 9–17.
[27] Balaban A.T., Toma C., Tetrahedron Suppl. 7 (1966) 1–7.
[28] Dinculescu A., Balaban A.T., Rev. Roum. Chim. 25 (1980) 1505–1528.
[29] Supuran C.T., Banciu M.D., Balaban A.T., Rev. Roum. Chim. 38 (1993) 199–205.
[30] Balaban A.T., Dinculescu A., Dorofeenko G.N., Fischer G.W., Koblik A.V., Mezheritskii V.V., Schroth W., Pyrylium salts: synthe-

ses, reactions and physical properties, in: Katritzky A.R. (Ed.), Advances in Heterocyclic Chemistry, Academic Press, New York, 1982, pp. 8–360.

[31] Supuran C.T., Pop E., Dinculescu A., Heterocycles 37 (1994) 667–671.

[32] Neidlein R., Witerzens P., Monats. Chem. 106 (1975) 643–648.

[33] Bode W., Turk D., Karshikov A., Protein Sci. 1 (1992) 426–471.

[34] Stubbs M.T., Bode W., Thromb. Res. 69 (1993) 1–58.

[35] Bell P.H., Roblin R.O., J. Am. Chem. Soc. 64 (1942) 2905–2917.