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Carbonic Anhydrase Activators: Amino Acyl/Dipeptidyl Histamine Derivatives Bind with High Affinity to Isozymes I, II and IV and Act as Efficient Activators†

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Abstract—Reaction of histamine (Hst) with tetrabromophthalic anhydride and protection of its imidazole moiety with tritylsulfenyl chloride, followed by hydrazinolysis, afforded *N*-1-tritylsulfenyl-histamine, a key intermediate which was further derivatized at its aminoethyl moiety. Reaction of the key intermediate with *N*-Boc-amino acids/dipeptides (Boc-AA) in the presence of carbodiimides afforded, after deprotection of the imidazolic and amino moieties, a series of compounds with the general formula AA-Hst (AA = amino acyl; dipeptidyl). The new derivatives were assayed as activators of three carbonic anhydrase (CA) isozymes, hCA I, hCA II (cytosolic forms) and bCA IV (membrane-bound form). Efficient activation was observed against all three isozymes, but especially against hCA I and bCA IV, with affinities in the nanomolar range for the best compounds. hCA II was, on the other hand, activatable with affinities around 10–20 nM. This new class of CA activators might lead to the development of drugs/diagnostic agents for the CA deficiency syndrome, a genetic disease of bone, brain and kidneys.

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) inhibitors of the unsubstituted sulfonamide type, RSO_2NH_2, are widely used drugs for the treatment or prevention of a variety of diseases, such as: glaucoma,[2,3] epilepsy,[4] gastric and duodenal ulcers,[5] or acid–base disequilibria.[6] In contrast to inhibitors, activators of this enzyme (for which at least 14 different isozymes have been isolated up to now in higher vertebrates)[7] were much less investigated. Only recently the X-ray crystallographic structures of the first adducts of the physiologically relevant isozyme II (hCA II) with the activators histamine (Hst)[8] and phenylalanine (in this case a tertiary complex, in which azide is also bound to the Zn(II) ion)[9] have been reported by this group. Furthermore, few other quantitative structure–activity relationship (QSAR)[10–12] or synthetic chemistry[13,14] studies have been reported in the field of CA activators, although some of these compounds might be used in the treatment of the CA deficiency syndrome, a genetic disease of bone, brain and kidney affecting a large number of patients.[15] In this condition, a certain CA isozyme gene (generally CA II, I or IV) is either not expressed, or its protein product is unstable due to deleterious mutations, and the corresponding CA isozyme is absent in the blood, kidney or lung of such patients. No pharmacologically specific treatment for this condition is available up to now. CA activators are also important for understanding the CA catalytic and inhibition mechanisms.[8–12]

The lead molecule considered by us for obtaining tighter binding CA activators was histamine **1** itself. As seen from the X-ray coordinates with which Figure 1 was generated, the activator molecule is bound at the entrance of the hCA II active site cavity, where it is anchored by hydrogen bonds to amino acid side-chains and to water molecules. Such hydrogen bonds involve only the nitrogen atoms of the imidazole moiety, whereas the terminal aliphatic amino group is not experiencing any contact with the enzyme, but is extending away from the cavity into the solvent. On the other hand, the Nδ1 and Nε2 atoms of the histidine imidazole ring are engaged in hydrogen bonds with the side-chains of Asn 62, His 64, Gln 92 and with Wat152.[8] Thus, it appeared of interest to derivatize the lead at its

Key words: Carbonic anhydrase; histamine; amino acid; dipeptide; enzyme activators; proton shuttle.

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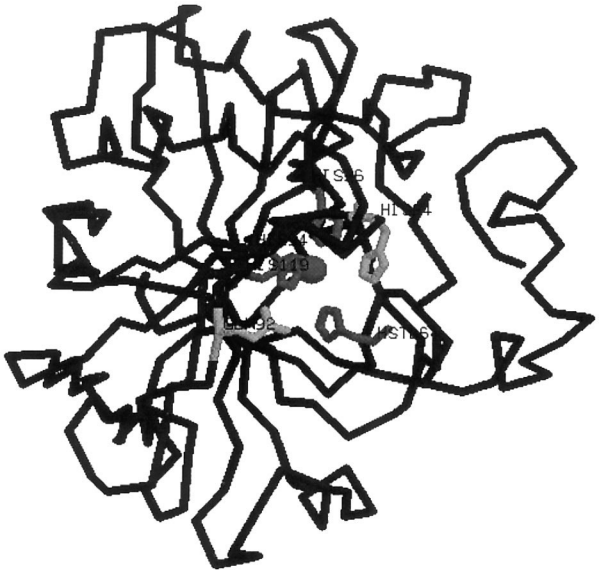


Figure 1. hCA II–histamine adduct: the Zn(II) ion (central sphere) and its three histidine ligands (His 94, His 96 and His 119) are shown at the center of the active site, whereas histamine (numbered as Hst 264) is situated at the entrance in it, between residues His 64 and Gln 92. The figure was generated from the X-ray coordinates of the hCA II–histamine adduct reported by this group,[8] with the program RasMol for Windows 2.6. The coordinates of this structure are deposited in the Brookhaven Protein Database (PDB entry 4TST).

aliphatic NH_2 moiety, just in order to exploit the energy of binding of such modified groups with amino acid residues at the edge of the active site. This approach has been successfully used both by Whitesides'[16,17] and our groups[8,18,19] for the design of tight-binding, isozyme-specific sulfonamide CA inhibitors. Moreover, recently we[1] have reported some tosylureido-amino acyl derivatives of histamine (at the aliphatic amino group) possessing high affinities for the three CA isozymes mentioned above. Thus, it appeared of interest to explore other types of moieties that might be attached at the aliphatic end of the molecule, and amino acyl groups appeared of great interest, due to the possible interactions of these highly polarized moieties with amino acid residues at the edge of the active site (which presumably would lead to increased stabilities of the enzyme–activator adducts), as well as the presence of a second proton shuttle moiety (the free amino group) which might influence positively the rate-limiting step of the catalytic cycle, which is a proton transfer process from the active site cavity to the reaction medium.[8,9]

In this paper, we report the synthesis of a series of amino acyl/dipeptidyl histamine derivatives possessing the general formula AA-Hst, obtained by reaction of appropriately protected[1,20] Hst with Boc-protected amino acids/dipeptides, in the presence of carbodiimides, followed by removal of the two protecting groups. The new compounds were assayed as activators of three CA isozymes, hCA I, hCA II and bCA IV (h = human, b = bovine isozyme), and generally showed very good activities. SAR in this series of derivatives is also discussed.

Results

Synthesis

Compounds reported in the present work were prepared starting from *N*-1-tritylsulfenyl-histamine **3** (obtained as shown in Scheme 1) and Boc-protected amino acids/dipeptides, in the presence of carbodiimide derivatives. Treatment of the intermediates **4** with TFA, and removal of the tritylsulfenyl moiety with HCl–dioxane, afforded the new compounds **A1**–**A24**, of the type AA-Hst or AA1-AA2-Hst, respectively.

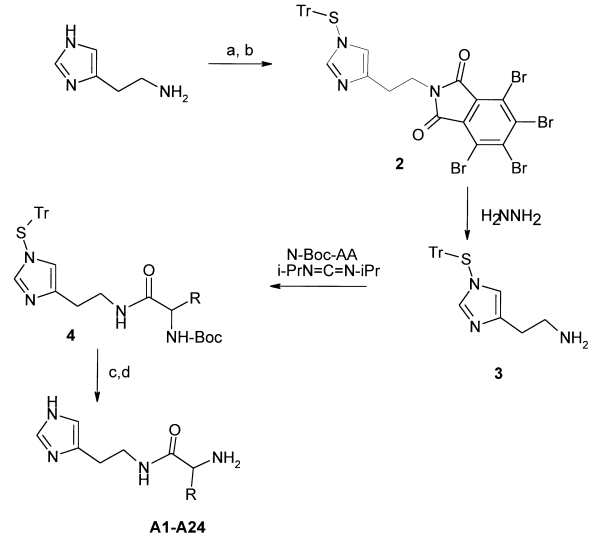
CA activation study

Activation of three CA isozymes, hCA I, hCA II and bCA IV (h = human; b = bovine isozymes), with Hst and the new compounds **A1**–**A24** is shown in Table 1.

Detailed activation curves with Hst and some of the new derivatives are shown in Figure 2.

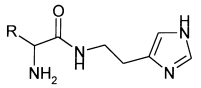
Discussion

The study of CA activators has been overlooked since a report of hCA II activation by His,[21] followed by its subsequent retraction,[22] and consideration that the activation initially observed was due to chelation of trace Cu(II) ions (which inhibit CA), eventually present in the enzyme preparation, by EDTA added in the buffer. Obviously, the experimental protocols of Silverman's laboratory presented many unresolved problems, as explained by us in a previous work,[23] and this issue of CA activation has not thereafter been re-investigated by other researchers. Only recently, by the report of the first X-ray crystallographic data[8,9] of adducts of Hst and phenylalanine with hCA II, has this topic again received deserved attention, and some types of high affinity activators investigated mainly by this[8–19] and Chegwidden's groups.[24]



Scheme 1. Reagents: (a) tetrabromophthalic anhydride; (b) tritylsulfenyl chloride; (c) TFA; (d) 4 M HCl–dioxane.

Table 1. CA isozymes I, II and IV activation with histamine (Hst) **1** and the aminoacyl/dipeptidyl derivatives AA-Hst, **A1**–**A24**. The activation constant K_A is defined similarly to the inhibition constant K_I, as the equilibrium constant for the reaction: E + A↔E–A (where E, enzyme; A, activator; E–A, enzyme–activator complex)[8]



A1-A24 (AA-Hst)

No.	AA	K_A[a] (μM)			Yield
		hCA I[b]	hCA II[b]	bCA IV[c]	
1	—	2	125	41	—
A1	Gly	0.21	10	1.5	54
A2	L-Ala	0.20	11	2.3	50
A3	β-Ala	0.20	9	1.2	60
A4	GABA	0.15	7	1.0	67
A5	GlyGly	0.13	7	1.1	45
A6	L-Val	0.18	6	5.1	62
A7	L-Leu	0.18	7	4.6	39
A8	L-Ile	0.20	7	4.1	55
A9	L-Asn	0.16	6	4.0	78
A10	L-Gln	0.14	5	2.1	80
A11	L-Arg	0.02	0.3	0.3	53
A12	L-Lys	0.03	0.4	0.3	61
A13	L-His	0.01	0.4	0.2	70
A14	L-Phg[d]	0.07	7	2.3	84
A15	L-Phe	0.06	5	2.2	83
A16	L-Trp	0.20	10	4.1	95
A17	L-Pro	0.16	8	3.1	66
A18	L-Pip[e]	0.12	6	2.1	42
A19	D,L-Nip[f]	0.13	5	2.1	49
A20	D,L-Inp[g]	0.11	5	1.2	51
A21	L-GlyHis	0.001	0.02	0.01	35
A22	L-β-AlaHis	0.001	0.01	0.003	61
A23	L-PhePro	0.003	0.03	0.005	39
A24	L-ProGly	0.004	0.03	0.008	30

[a] Mean from at least three determinations by the esterase method.[34] Standard error was in the range of 5–10%.
[b] Human cloned isozyme.
[c] Purified from bovine lung microsomes.[33]
[d] Phg = phenylglycine.
[e] Pip = pipecolic acid (piperidine-2-carboxylic acid).
[f] Nip = nipecotic acid (piperidine-3-carboxylic acid).
[g] Inp = isonipecotic acid (piperidine-4-carboxylic acid).

The key intermediate for obtaining novel types of activators reported in this paper, *N*-1-tritylsulfenyl-Hst **3**, was obtained by non-exceptional procedures, involving the initial protection of the primary amine moiety by means of phthalimide derivatives, followed by protection of the imidazolic NH moiety with tritylsulfenyl chloride, and hydrazinolysis of the phthalimido moiety in mild conditions (Scheme 1). The overall yield of the three steps was good (around 80%) and the purification procedures quite simple. Subsequent reaction of the key intermediate **3** with Boc-amino acid/dipeptide derivatives[21,25,26] in the presence of carbodiimides[27] afforded a series of *N*-tritylsulfenylated compounds **4**, which were deprotected in standard conditions, leading to the desired derivatives **A1**–**A24**. All the new compounds reported here have been characterized by IR, ^{1}H and ^{13}C NMR spectroscopy, as well as elemental analysis (±0.4% of the theoretical data, calculated for the proposed formulas).

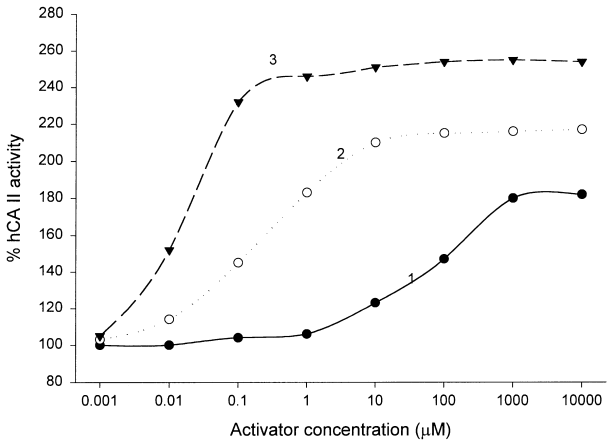
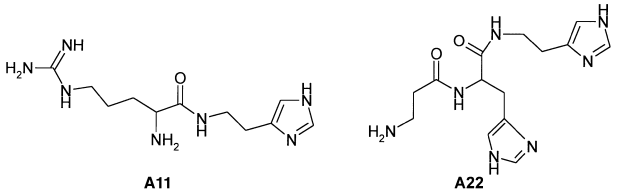


Figure 2. hCA II activation (0.012 μM) with histamine (curve 1), compound **A11** (curve 2) and compound **A22** (curve 3) in the concentration range of 1 nM–10 mM, for the hydrolysis of 4-nitrophenyl acetate. Control CA activity in the absence of activator was taken as 100%. Substrate concentration was 2.5 mM, 10 mM Tris buffer, pH 7.40, at 25°C and ionic strength of 0.1 (K_2SO_4).

The data of Table 1 show significant differences between the investigated isozymes in their behavior towards both 'classical' activators, such as Hst **1**, as well as the new class of activators synthesized in the present work. Thus, Hst **1** is a potent hCA I activator, and a relatively weak hCA II activator, whereas isozyme bCA IV possesses an intermediate behavior. The most interesting finding of the present study is represented by the high susceptibility of the cytosolic isozyme, hCA II, to be activated by some of the derivatives of Hst of types **A1**–**A24**, as compared to the lead molecule (compounds with activation constants in the 0.01–0.03 μM range were frequently obtained). Moreover, the highly abundant and most prone to activation (by Hst) isozyme hCA I was also susceptible to activation by the new derivatives reported here (with constants in the nanomolar range for the most active derivatives), but differences of activity are not so pronounced as compared to the situation for the rapid isozyme hCA II. bCA IV, on the other hand, had an intermediate behavior towards the new class of activators, with activation constants in the 0.008–0.02 μM range for the most active compounds. Efficient CA activators were: (i) derivatives of basic amino acids (Arg, Lys, His), such as **A11**–**A13**, as well as the phenylglycine and phenylalanine derivatives **A14** and **A15**; (ii) slightly less active were the compounds derived from Pro, Pip, Nip, Inp, Asn, Gln, as well as the hydrophobic amino acid derivatives (Val, Leu, Ile, Trp); (iii) GlyGly (**A5**) and GABA (**A4**) derivatives were more active than the β-Ala derivative (**A3**), which in turn was more active than the Ala or Gly derivatives (**A2** and **A1**); (iv) the best activators in this series were those derived from dipeptides such as Gly-His, β-Ala-His (carnosine), Phe-Pro or Pro-Gly. These compounds possessed activation constants in the 1–5 nM range against hCA I and bCA IV, and 10–30 nM range against hCA II. Probably the many heteroatoms present in these dipeptidyl moieties confer to the obtained compounds a 'sticky' nature, i.e. they are able to participate in many interactions with amino acid

residues from the active site, thus assuring the formation of very stable enzyme–activator (E–A) adducts.



A11 A22

A special mention should also be made regarding compounds such as **A11**, **A12**, **A13**, **A21** or **A22**, that possess multiple moieties able to shuttle protons, in addition to the Hst imidazolic one (the α-, β-, or γ-NH_2, plus the guanidino-, ε-amino- or imidazolic ring NH moieties, respectively), and behave as very effective CA activators against all three isozymes investigated here.

Typical activation curves with some of the best activators reported here (such as **A11**, **A22**) and Hst as standard, for hCA II catalyzed 4-nitrophenyl acetate hydrolysis, are shown in Figure 2. It is obvious from the data shown in this figure that Hst starts to activate hCA II significantly only at concentrations higher than 50 μM, then reaching a plateau of around 180% of the control activity at concentrations of 1 mM or higher. In contrast, the new activators show a much more pronounced activation at lower concentrations (0.01–1 μM), the above-mentioned plateau is reached at lower activator concentrations, and the level of hCA II activity enhancement is much higher (220–260%) as compared to the corresponding histamine data.

Similarly to all CA activators reported up to now, the compounds obtained in the present work presumably intervene in the catalytic cycle of the enzyme, leading to the formation of CA–activator complexes (similarly to the enzyme–inhibitor adducts, but without substitution of the metal bound solvent molecule), in which the activator bound within the active site facilitates proton transfer processes (which represent the rate-limiting step in catalysis).[8–19] The driving force of this effect is probably the fact that intramolecular reactions are more rapid than intermolecular ones. Thus, in the presence of activators (symbolized as 'A'), the rate-limiting step in the CA catalytic cycle is described by eq (1) below:[8–19]

$$\begin{aligned} &EZn^{2+}-OH_2 + A \Longleftrightarrow \\ &\left[EZn^{2+}-OH_2-A\right] \Longleftrightarrow \underbrace{\left[EZn^{2+}-HO^- - AH^+\right]}_{\text{enzyme-activator complexes}} \\ &\Longleftrightarrow EZn^{2+}-OH^- + AH^+. \end{aligned} \quad (1)$$

Compounds of the types reported here possess the imidazolic Hst moiety which can participate in the proton transfer processes between the active site and the environment (similarly to the lead, Hst **1**), but due to the presence of the amino acyl/dipeptidyl tails also present in their molecule, they can bind more effectively to the enzyme, thus allowing for much more efficient activation processes as compared to **1**. Furthermore, these tails can also participate in efficient proton transfer processes due to the presence of other groups with pK_a values in the range of 4.0–9.0 in their molecule, thus assuring alternative proton transfer pathways. Indeed, the active site edges of all three CA isozymes investigated by us contain a high proportion of polar amino acid residues which might interfere with amino acyl/dipeptidyl groups by means of a multitude of interaction types. In fact such amino acid residues also explain the different catalytic properties of the diverse isozymes, as well as their diverse susceptibility to be inhibited/activated by modulators of activity.[7,8] For instance, the entrance of the active site of isozyme hCA II contains a cluster of six histidine residues (His 3, His 4, His 10, His 15, His 17 and His 64), some of which possess different conformations (as shown by X-ray crystallography)[8,9] which could easily participate in hydrogen bond formation (as well as other types of interactions) with the Hst derivatives reported here. This might in fact explain the greater efficiency of the compounds reported in the present work in activating this isozyme, as compared to Hst, which is a relatively weak hCA II activator, and might lead to the development of pharmacological agents from this class of compounds. Activators are also critical for understanding the CA catalytic mechanism as well as for explaining the great differences in activity between the many CA isozymes,[8] some of which have an unknown physiological function for the moment.

Experimental

Melting points (mp) were determined with a heating plate microscope and are not corrected; IR spectra were obtained in KBr pellets with a Perkin–Elmer 16PC FT-IR spectrometer and ^{1}H NMR spectra with a Varian 300CXP apparatus in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and were ±0.4% of the theoretical values. Preparative high performance liquid chromatography (HPLC) was done on C_{18} reversed-phase μ-Bondapack or Dynamax-60A (25×250 mm) columns with a Beckmann HPLC-3069 instrument.

Compounds used in synthesis (Hst, natural and non-natural Boc-protected amino acids/dipeptides, tritylsulfenyl chloride, tetrabromophthalic anhydride, hydrazine, TFA, etc.) were commercially available compounds (from Sigma, Acros or Aldrich) or were prepared by standard procedures (from Boc-On and the corresponding amino acids/dipeptides). Acetonitrile, acetone, dioxane (E. Merck) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

hCA I and hCA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Forsman et al.[28] (the two plasmids were a gift from Professor Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[29] and enzymes were purified by affinity chromatography

according to the method of Khalifah et al.[30] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for hCA I and 54 mM^{-1} cm^{-1} for hCA II, respectively, based on $M_r = 28.85$ kDa for hCA I, and 29.30 kDa for hCA II, respectively.[31,32] bCA IV was isolated and purified to homogeneity from bovine lung microsomes as described by Maren et al. and its concentration has been determined by titration with ethoxzolamide.[33]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[34] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25°C. A molar absorption coefficient ε of 18,400 M^{-1} cm^{-1} was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40), as reported in the literature.[34] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each activator concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of activator (1 mM) were prepared in distilled–deionized water with 10–20% (v/v) DMSO and dilutions up to 0.01 nM were done thereafter with distilled–deionized water. Activator and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–A complex. The activator constant K_A was determined as described in ref 8. Control experiments done in the presence of 10 mM EDTA added in the assay buffer did not influence in any way the activation data reported in this paper, proving once again that the CA activation is not due to chelation of adventitious metal ions possibly present in the enzyme preparations. Enzyme concentrations were 3.2 nM for hCA II, 9 nM for hCA I and 16 nM for bCA IV (this isozyme has a decreased esterase activity[35] and higher concentrations had to be used for the measurements).

Preparation of *N*-1-tritylsulfenyl-histamine 3. Amounts of 5.55 g (50 mM) of Hst and 23.15 g (50 mM) of tetrabromophthalic anhydride were suspended in 300 mL of dry toluene and refluxed under Dean–Stark conditions until water was separated (generally 2–3 h). The solvent was evaporated in vacuo, the crude product dissolved in 150 mL of anhydrous acetonitrile and treated with 15.5 g (50 mM) of tritylsulfenyl chloride and 6.95 mL (50 mM) of triethylamine. The mixture was stirred at room temperature for 3 h (TLC control), then the solvent was evaporated and the crude product **2** stirred with 100 mL of water and ice. The tan precipitate obtained was filtered, dried and used directly in the deprotection step. Hydrazinolysis was effected by dissolving the above mentioned precipitate in 200 mL of ethanol and addition of 15 mL of hydrazinium hydroxide, followed by stirring for 5 h at room temperature. Then the solvent was evaporated, a small excess of 2 N HCl solution was added, the precipitated tetrabromophthalhydrazide was filtered and discarded, whereas the solution containing **3** was brought to pH 7 with solid $NaHCO_3$, brought to a small volume by in vacuo evaporation of the solvent, and the precipitated **3** was then recrystallized from ethanol (yield of 80%, based on Hst, after the three steps described above). Tan crystals, mp 177–178°C; ^{1}H NMR (300 MHz, TFA), δ, ppm: 2.47 (t, 2H, $J = 7.0$ Hz, CH_2); 2.96 (q, 2H, $J = 6.2$, 12.5 Hz, H_2NCH_2); 4.23 (m, 2H, NH_2); 7.10–7.30 (m, 15H, trityl); 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH). Anal. found: C, 75.12; H, 5.83; N, 10.88%; $C_{24}H_{23}N_3S$ requires: C, 74.77; H, 6.01; N, 10.90%.

General procedure for the preparation of compounds A1–A24. An amount of 10 mM *N*-1-tritylsulfenyl-histamine **3** was dissolved in 50 mL of anhydrous acetonitrile and then treated with a solution obtained from 10 mM of *N*-Boc-amino acid/dipeptide (10 mM) dissolved in 10 mL of the same solvent, followed by 10 mM of liquid diisopropyl-carbodiimide (or EDCI·HCl + Et_3N) and 10 mM of 1-hydroxybenzotriazole in anhydrous MeCN as solvent. The reaction mixture was stirred at 4°C for 3–9 h (TLC control). The solvent was evaporated in vacuo and the residue taken up in ethyl acetate (50 mL), poured into a 5% solution of sodium bicarbonate (50 mL) and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and filtered, and the solvent removed in vacuo. In many cases the compounds of type **4** precipitated, were filtered, dried and deprotected at the α-amino- and *N*-1 imidazolic moieties in the following way. The crude **4** was dissolved in 20 mL of CH_2Cl_2, treated with 4.8 mL of trifluoroacetic acid (TFA) and stirred for 10 min at 0°C. The solvent was evaporated in vacuo and the syrup obtained dissolved in 20 mL of dioxane and treated with 25 mL of a 4 M HCl solution in dioxane, followed by heating at 40°C for 6–8 h (TLC control). The solvent was then evaporated under reduced pressure, the residue taken up in 50 mL of a 5% solution of sodium bicarbonate and the trityl sulfenyl chloride formed during the deprotection step extracted in 2×50 mL of Et_2O. The water phase was evaporated in vacuo to a small volume, when generally crude compounds **A1**–**A24** precipitated by letting the mixture stand at 4°C overnight. The pure compounds **A1**–**A24** were obtained by means of preparative HPLC (C_{18} reversed-phase μ-Bondapack or Dynamax-60A (25×250 mm) columns; 90% acetonitrile/8% methanol/2% water, 30 mL/min).

4-[β-(Glycylamido)-ethyl]-1*H*-imidazole A1. White crystals, mp 279–281°C (dec.); IR (KBr), cm^{-1}: 1287 (amide III), 1579 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, $J = 7.0$, Hst CH_2); 2.99 (t, 2H, $J = 7.0$, Hst $CONHCH_2$); 3.67 (s, 2H, CH_2 of Gly); 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH_2 of Hst); 37.9 (s, CH_2 of Hst); 40.8 (s, CH_2 of Gly); 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.1 (CONH). Anal. found: C, 49.78; H, 7.20; N, 33.24%; $C_7H_{12}N_4O$ requires: C, 49.99; H, 7.19; N, 33.31%.

4-[β-(α-Alanylamido)-ethyl]-1*H*-imidazole A2. White crystals, mp 266–267°C; IR (KBr), cm^{-1}: 1285 (amide

III), 1574 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.84 (d, $^3J_{HH}=6.5$, 3H, CHCH_3 of Ala), 2.49 (t, 2H, $J=7.0$, Hst CH_2); 3.01 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.98 (q, 1H, CH of Ala); 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 22.1 (s, CHCH$_3$ of Ala); 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 34.5 (s, CHCH$_3$ of Ala); 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.9 (CONH). Anal. found: C, 52.71; H, 7.42; N, 30.39%; $C_8H_{14}N_4O$ requires: C, 52.73; H, 7.74; N, 30.75%.

4-[β-(β-Alanylamido)-ethyl]-1*H*-imidazole A3. White crystals, mp 278–279°C (dec.); IR (KBr), cm^{-1}: 1284 (amide III), 1575 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.73 (t, $^3J_{HH}=6.6$, 1H, CH$_2$CH_2CO of β-Ala); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.27 (t, $^3J_{HH}=6.7$, 1H, CH$_2$CH_2CO of β-Ala), 3.43 (t, $^3J_{HH}=6.3$, 2H, CH_2-CH$_2$CO of β-Ala), 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH$_2$ of Hst); 37.5 (s, NHCH$_2$CH$_2$ of β-Ala), 37.9 (s, CH$_2$ of Hst); 40.9 (s, CH$_2$$CH_2$CO of β-Ala), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.8 (CONH). Anal. found: C, 52.90; H, 7.68; N, 30.51%; $C_8H_{14}N_4O$ requires: C, 52.73; H, 7.74; N, 30.75%.

4-[β-(γ-Aminobutyramido)-ethyl]-1*H*-imidazole A4. White crystals, mp 256–257°C (dec.); IR (KBr), cm^{-1}: 1287 (amide III), 1579 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.75 (t, $^3J_{HH}=6.5$, 1H, (CH$_2$)$_2$CH_2CO of GABA); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.27 (t, $^3J_{HH}=6.7$, 1H, (CH$_2$)$_2$CH_2CO of GABA), 3.31–3.56 (m, 4H, (CH_2)$_2$CH$_2$CO of GABA), 7.36 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH$_2$ of Hst); 37.3 (s, NHCH$_2$CH$_2$ of GABA), 37.9 (s, CH$_2$ of Hst); 40.9 (s, CH$_2$CH$_2$$CH_2$CO of GABA), 41.3 (s, CH$_2$$CH_2CH_2$CO of GABA), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.5 (CONH). Anal. found: C, 54.96; H, 8.24; N, 28.54%; $C_9H_{16}N_4O$ requires: C, 55.08; H, 8.22; N, 28.55%.

4-[[β-(Glycyl)glycylamido]-ethyl]-1*H*-imidazole A5. White crystals, mp 229–230°C; IR (KBr), cm^{-1}: 1290 (amide III), 1584 (amide II), 1715 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (m, 6H, $J=7.0$, Hst CH_2 + 2CH_2 of GlyGly); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH$_2$ of Hst); 35.8 (s, CH$_2$ of GlyGly); 37.9 (s, CH$_2$ of Hst); 122.4 (s, C-4 of Hst); 134.7 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.1 (CONH); 172.5 (CONH). Anal. found: C, 48.10; H, 6.62; N, 30.97%; $C_9H_{15}N_5O_2$ requires: C, 47.99; H, 6.71; N, 31.09%.

4-[β-(Valylamido)-ethyl]-1*H*-imidazole A6. White crystals, mp 256–258°C; IR (KBr), cm^{-1}: 1280 (amide III), 1573 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.11 (d, $^3J_{HH}=6.7$, 6H, CH(CH_3)$_2$ of Val), 2.29–2.50 (m, 1H, CH(CH$_3$)$_2$ of Val), 2.54 (t, 2H, $J=7.0$, Hst CH_2); 3.01 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.75 (d, $^3J_{HH}=4.3$, 1H, NHCHCH of Val), 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 22.3 (s, CH(CH$_3$)$_2$ of Val), 33.3 (s, CH$_2$ of Hst); 34.0 (s, CH(CH$_3$)$_2$ of Val), 37.9 (s, CH$_2$ of Hst); 64.4 (s, NHCHCH of Val), 122.4 (s, C-4 of Hst); 134.7 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.9 (CONH). Anal. found: C, 57.15; H, 8.94; N, 26.53%; $C_{10}H_{18}N_4O$ requires: C, 57.12; H, 8.63; N, 26.64%.

4-[β-(Leucylamido)-ethyl]-1*H*-imidazole A7. White crystals, mp 251–253°C; IR (KBr), cm^{-1}: 1280 (amide III), 1575 (amide II), 1713 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.43 (d, $^3J_{HH}=6.7$, 6H, CH(CH_3)$_2$ of Leu), 1.55 (m, 2H, CH_2 of Leu); 2.54 (t, 2H, $J=7.0$, Hst CH_2); 3.01 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.70 (m, 1H, NHCHCH$_2$ of Leu), 4.23 (m, 1H, CHCH$_2$-CH(CH$_3$)$_2$ of Leu), 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 21.7 (s, CH(CH$_3$)$_2$ of Leu), 31.9 (s, CH$_2$ of Leu); 33.3 (s, CH$_2$ of Hst); 34.0 (s, CH(CH$_3$)$_2$ of Leu), 37.9 (s, CH$_2$ of Hst); 56.2 (s, NHCHCH$_2$ of Leu), 122.4 (s, C-4 of Hst); 134.6 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.6 (CONH). Anal. found: C, 58.98; H, 8.64; N, 24.70%; $C_{11}H_{20}N_4O$ requires: C, 58.90; H, 8.99; N, 24.98%.

4-[β-(Isoleucylamido)-ethyl]-1*H*-imidazole A8. White crystals, mp 221–223°C; IR (KBr), cm^{-1}: 1280 (amide III), 1575 (amide II), 1715 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.15 (d, $^3J_{HH}=6.5$, 3H, CH_3 of Ile), 1.21 (t, 3H, $^3J_{HH}=6.7$, CH_3 of Et moiety of Ile); 1.54 (m, 2H, CH_2 of Ile); 2.54 (t, 2H, $J=7.0$, Hst CH_2); 3.01 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.22 (m, 1H, EtCH(Me)– of Ile); 3.75 (m, 1H, NHCHCH of Ile), 7.36 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 21.9 (s, CHCH$_3$ of Ile), 22.5 (s, CH$_3$CH$_2$ of Ile); 31.4 (s, CH$_2$ of Ile); 33.3 (s, CH$_2$ of Hst); 34.0 (s, CH(CH$_3$)$_2$ of Leu), 37.9 (s, CH$_2$ of Hst); 46.4 (s, EtCH(Me)– of Ile), 55.0 (s, NHCHCH2 of Ile), 122.4 (s, C-4 of Hst); 134.7 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 167.6 (CONH). Anal. found: C, 59.15; H, 8.75; N, 24.83%; $C_{11}H_{20}N_4O$ requires: C, 58.90; H, 8.99; N, 24.98%.

4-[β-(Asparaginylamido)-ethyl]-1*H*-imidazole A9. White crystals, mp 254–255°C; IR (KBr), cm^{-1}: 1280 and 1295 (amide III), 1572 (amide II), 1710 and 1723 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.54 (t, 2H, $J=7.0$, Hst CH_2); 2.59 (d, 2H, $^3J_{HH}=6.3$, CH_2 of Asn), 3.01 (t, 2H, $J=7.0$, Hst CONHCH_2); 4.55 (m, 1H, NHCHCH$_2$ of Asn), 7.33 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 30.1 (s, CHCH$_2$ of Asn), 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 70.4 (s, CHCH$_2$ of Asn), 122.4 (s, C-4 of Hst); 134.7 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 177.0 (CONH); 178.9 (s, CONH$_2$ of Asn). Anal. found: C, 48.12; H, 6.86; N, 31.05%; $C_9H_{15}N_5O_2$ requires: C, 47.99; H, 6.71; N, 31.09%.

4-[β-(Glutaminylamido)-ethyl]-1*H*-imidazole A10. White crystals, mp 259–260°C; IR (KBr), cm^{-1}: 1280 and 1293 (amide III), 1575 (amide II), 1710 and 1724 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.54 (t, 2H,

J = 7.0, Hst CH_2); 2.55–2.63 (m, 2H, CH_2 of Gln), 3.01 (t, 2H, J = 7.0, Hst CONHCH_2); 3.05–3.34 (m, 2H, CH_2 of Gln), 4.61 (m, 1H, NHCHCH$_2$ of Gln), 7.34 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 30.5 (s, CHCH$_2$CH$_2$ of Gln), 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 44.7 (s, CHCH$_2$$CH_2$ of Gln), 72.6 (s, CHCH$_2$CH$_2$ of Gln), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 177.2 (CONH); 179.5 (s, CH$_2$$CONH_2$ of Gln). Anal. found: C, 49.98; H, 7.35; N, 29.18%; $C_{10}H_{17}N_5O_2$ requires: C, 50.20; H, 7.16; N, 29.27%.

4-[β-(Arginylamido)-ethyl]-1*H*-imidazole A11. White crystals, mp 277–279°C (dec.); IR (KBr), cm^{-1}: 1285 (amide III), 1584 (amide II), 1712 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.70–2.00 (m, 2H, CHCH$_2$-CH_2 of Arg), 2.49 (t, 2H, J = 7.0 Hz, Hst CH_2); 2.50–2.58 (m, 2H, CHCH_2CH$_2$ of Arg), 2.75 (t, $^3J_{HH}$ = 6.5, 1H, (CH$_2$)$_2$CH_2CO of Arg); 2.99 (t, 2H, J = 7.0 Hz, Hst CONHCH_2); 3.30–3.45 (m, 2H, CH$_2$CH_2NH of Arg), 3.45–3.60 (m, 1H, CH$_2$CH(NH)CO of Arg), 7.36 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 29.7 (s, CH$_2$$CH_2CH_2$ of Arg), 33.3 (s, CH$_2$ of Hst); 35.4 (s, CHCH$_2$$CH_2$ of Arg), 37.9 (s, CH$_2$ of Hst); 45.7 (s, CH$_2$$CH_2$NH of Arg), 59.8 (s, CH$_2$$C$H(NH)CO$_2$H of Arg), 122.4 (s, C-4 of Hst); 134.6 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 161.5 (s, NHC(=NH)NH$_2$ of Arg), 170.8 (CONH). Anal. found: C, 49.19; H, 7.81; N, 36.69%; $C_{11}H_{21}N_7O$ requires: C, 49.42; H, 7.92; N, 36.68%.

4-[β-[α-(Lysylamido)]-ethyl]-1*H*-imidazole A12. White crystals, mp 223–224°C; IR (KBr), cm^{-1}: 1281 (amide III), 1579 (amide II), 1711 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.66–2.20 (m, 6H, CH(CH_2)$_3$CH$_2$ of Lys), 2.49 (t, 2H, J = 7.0 Hz, Hst CH_2); 2.99 (t, 2H, J = 7.0 Hz, Hst CONHCH_2); 3.13 (t, $^3J_{HH}$ = 6.7, 2H, CH$_2$CH_2NH$_2$ of Lys), 3.84 (t, $^3J_{HH}$ = 6.7, 1H, CH$_2$CH-(NH)CO of Lys), 7.36 (m, 1H, imidazole CH); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 26.6 (s, H$_2$NCH$_2$(CH$_2$)$_3$ of Lys), 31.4 (s, H$_2$NCH$_2$(CH$_2$)$_3$ of Lys), 33.3 (s, CH$_2$ of Hst); 34.8 (s, H$_2$NCH$_2$(CH$_2$)$_3$ of Lys), 37.9 (s, CH$_2$ of Hst); 43.8 (s, H$_2$NCH$_2$(CH$_2$)$_3$ of Lys), 58.8 (s, CH$_2$CH(NH)CO of Lys), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 171.2 (CONH). Anal. found: C, 55.17; H, 8.70; N, 29.13%; $C_{11}H_{21}N_5O$ requires: C, 55.21; H, 8.84; N, 29.26%.

4-[β-(Histidylamido)-ethyl]-1*H*-imidazole A13. White crystals, mp 270–272°C (dec.); IR (KBr), cm^{-1}: 1282 (amide III), 1585 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, J = 7.0, Hst CH_2); 2.99 (t, 2H, J = 7.0, Hst CONHCH_2); 3.32–3.46 (m, 2H, CHCH_2 of His), 4.01–4.08 (m, 1H, CHCH$_2$ of His), 7.34 (m, 1H, imidazole CH of Hst); 7.46 (s, 1H, CH-5 of His), 8.35 (s, 1H, imidazole CH); 8.51 (s, 1H, CH-2 of His); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 40.9 (s, CH$_2$ of His); 58.6 (s, CH$_2$$C$H of His), 122.2 (s, C-4 of His); 122.4 (s, C-4 of Hst); 134.1 (s, C-5 of His); 134.6 (s, C-5 of Hst); 137.2 (s, C-2 of Hst); 137.5 (s, C-2 of His); 167.1 (CONH). Anal. found: C, 53.21; H, 6.18; N, 33.79%; $C_{11}H_{16}N_6O$ requires: C, 53.21; H, 6.50; N, 33.85%.

4-[β-(Phenylglycylamido)-ethyl]-1*H*-imidazole A14. White crystals, mp 256–257°C (dec.); IR (KBr), cm^{-1}: 1284 (amide III), 1580 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, J = 7.0, Hst CH_2); 2.99 (t, 2H, J = 7.0, Hst CONHCH_2); 4.08 (m, 1H, PhCH of Phg), 7.29–7.58 (m, 6H, H_{arom} of Phe + imidazole CH of Hst); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 59.9 (s, PhCH of Phg), 122.4 (s, C-4 of Hst); 133.9 (s, C_{meta} of Phg), 134.5 (s, C_{ortho} of Phg), 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 141.5 (s, C_{ipso} of Phe), 166.9 (CONH). Anal. found: C, 64.15; H, 6.28; N, 22.70%; $C_{13}H_{16}N_4O$ requires: C, 63.92; H, 6.60; N, 22.93%.

4-[β-(Phenylalanylamido)-ethyl]-1*H*-imidazole A15. White crystals, mp 239–241°C (dec.); IR (KBr), cm^{-1}: 1280 (amide III), 1581 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, J = 7.0, Hst CH_2); 2.99 (t, 2H, J = 7.0, Hst CONHCH_2); 3.10–3.55 (m, 2H, CH_2CH of Phe), 4.08 (dd, $^3J_{HH}$ = 5.0, $^3J_{HH}$ = 7.8, 1H, CH$_2$CH of Phe), 7.29–7.58 (m, 6H, H_{arom} of Phe + imidazole CH of Hst); 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 41.7 (s, CH$_2$CH of Phe), 59.3 (s, CH$_2$$C$H of Phe), 122.4 (s, C-4 of Hst); 133.8 (s, C_{meta} of Phe), 134.4 (s, C_{ortho} of Phe), 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 141.5 (s, C_{ipso} of Phe), 166.9 (CONH). Anal. found: C, 65.21; H, 7.13; N, 21.54%; $C_{14}H_{18}N_4O$ requires: C, 65.09; H, 7.02; N, 21.69%.

4-[β-(Tryptophanylamido)-ethyl]-1*H*-imidazole A16. White crystals, mp 208–210°C; IR (KBr), cm^{-1}: 1280 (amide III), 1581 (amide II), 1710 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 2.49 (t, 2H, J = 7.0, Hst CH_2); 2.99 (t, 2H, J = 7.0, Hst CONHCH_2); 3.44 (dd, $^3J_{HH}$ = 9.0, $^2J_{HH}$ = 14.6, 1H, CH_2CH of Trp), 3.65 (dd, $^3J_{HH}$ = 4.1, $^2J_{HH}$ = 15.0, 1H, CH_2CH of Trp), 4.14 (dd, $^3J_{HH}$ = 4.3, $^3J_{HH}$ = 8.0, 1H, CH$_2$CH of Trp), 7.22–7.82 (m, 6H, H_{arom} of Trp + 1H, imidazole CH of Hst), 8.35 (s, 1H, imidazole CH); ^{13}C NMR (TFA), δ, ppm: 31.6 (s, CH$_2$CH of Trp), 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 58.8 (s, CH$_2$$C$H of Trp), 113.9 (s, C-2 of Trp), 116.9 (s, C-7 of Trp), 122.4 (s, C-4 of Hst); 123.4 (s, C-5 of Trp), 124.3 (s, C-6 of Trp), 126.8 (s, C-4 of Trp), 129.0 (s, C-1 of Trp), 132.1 (s, C-8 of Trp), 134.7 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 141.2 (s, C-3 of Trp), 168.2 (CONH). Anal. found: C, 64.56; H, 6.41; N, 23.47%; $C_{16}H_{19}N_5O$ requires: C, 64.63; H, 6.44; N, 23.55%.

4-[β-(Prolylamido)-ethyl]-1*H*-imidazole A17. Mp 276–278°C (dec.); IR (KBr), cm^{-1}: 1286 (amide III), 1589 (amide II), 1718 (amide I), 3060 (NH); ^{1}H NMR (TFA), δ, ppm: 1.18–1.38 (m, 1H, HCH of Pro); 1.55–1.65 (m, 1H, HCH); 1.70–1.85 (m, 2H, CH$_2$ of Pro); 2.49 (t, 2H, J = 7.0, Hst CH_2); 2.99 (t, 2H, J = 7.0, Hst CONHCH_2); 3.16–3.30 (m, 2H, CH$_2$N of Pro); 3.75–3.80 (m, 1H, CHCO of Pro), 7.34 (m, 1H, H-5 of Hst); 8.35 (s, 1H, H-2 of Hst); ^{13}C NMR (TFA), δ, ppm: 15.6 (s, CH$_2$ of Pro); 21.3 (s, CH$_2$ of Pro); 33.3 (s, CH$_2$ of Hst); 37.9 (s, CH$_2$ of Hst); 46.9 (s, CH$_2$N of Pro); 64.5 (s, CHCO of Pro), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 170.3 (s, Pro-CONH). Anal. found: C,

57.60; H, 7.61; N, 26.82% $C_{10}H_{16}N_4O$ requires: C, 57.67; H, 7.74; N, 26.90%.

4-[β-(Pipecolylamido)-ethyl]-1*H*-imidazole A18. Mp 237–238°C; IR (KBr), cm^{-1}: 1284 (amide III), 1589 (amide II), 1712 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 1.19–1.43 (m, 1H, *H*CH of Pip); 1.50–1.65 (m, 1H, HC*H*); 1.69–1.87 (m, 4H, $2CH_2$ of Pip); 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.16–3.34 (m, 2H, CH_2N of Pip); 3.70–3.83 (m, 1H, C*H*CO of Pip), 7.33 (m, 1H, H-5 of Hst); 8.35 (s, 1H, H-2 of Hst); ^{13}C NMR (TFA), δ, ppm: 15.6 (s, CH_2 of Pip); 18.0 (s, CH_2 of Pip); 21.3 (s, *C*H_2 of Pip); 33.3 (s, *C*H_2 of Hst); 37.9 (s, *C*H_2 of Hst); 46.4 (s, CH_2N of Pip); 64.9 (s, *C*HCO of Pip), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 170.0 (s, Pip-*C*ONH). Anal. found: C, 59.63; H, 7.89; N, 25.16%; $C_{11}H_{18}N_4O$ requires: C, 59.44; H, 8.16; N, 25.20%.

4-[β-(Nipecotylamido)-ethyl]-1*H*-imidazole A19. Mp 244–245°C; IR (KBr), cm^{-1}: 1280 (amide III), 1593 (amide II), 1710 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 1.24–1.67 (m, 4H, CH_2CH_2 of Nip); 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.16–3.34 (m, 4H, CH_2NCH_2 of Nip); 3.72–3.88 (m, 1H, C*H*CO of Nip), 7.33 (m, 1H, H-5 of Hst); 8.35 (s, 1H, H-2 of Hst); ^{13}C NMR (TFA), δ, ppm: 16.3 (s, CH_2 of Nip); 21.0 (s, CH_2 of Nip); 33.3 (s, *C*H_2 of Hst); 37.9 (s, *C*H_2 of Hst); 46.4 (s, CH_2N of Nip); 47.5 (s, NCH_2 of Nip); 53.6 (s, *C*HCO of Nip), 122.4 (s, C-4 of Hst); 34.8 (s, C-5 of Hst); 135.1 (s, C_{ortho} of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst); 170.0 (s, Nip-*C*ONH). Anal. found: C, 59.40; H, 8.05; N, 25.11%; $C_{11}H_{18}N_4O$ requires: C, 59.44; H, 8.16; N, 25.20%.

4-[β-(Isonipecotylamido)-ethyl]-1*H*-imidazole A20. Mp 270–271°C (dec.); IR (KBr), cm^{-1}: 1277 (amide III), 1591 (amide II), 1711 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 1.90–2.33 (m, 8H, 2 CH_2CH_2 of Inp); 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.13–3.39 (m, 1H, C*H*CO of Inp), 7.33 (m, 1H, H-5 of Hst); 8.35 (s, 1H, H-2 of Hst); ^{13}C NMR (TFA), δ, ppm: 21.6 (s, CH_2 of Inp); 33.3 (s, *C*H_2 of Hst); 37.9 (s, *C*H_2 of Hst); 47.0 (s, NCH_2 of Inp); 53.2 (s, *C*HCO of Inp), 122.4 (s, C-4 of Hst); 134.8 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 170.6 (s, Inp-*C*ONH). Anal. found: C, 59.28; H, 8.15; N, 25.00%; $C_{11}H_{18}N_4O$ requires: C, 59.44; H, 8.16; N, 25.20%.

4-[β-(Glycyl-histidylamido)-ethyl]-1*H*-imidazole A21. Mp 220–221°C; IR (KBr), cm^{-1}: 1288 (amide III), 1592 (amide II), 1715 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.67 (s, 2H, CH_2 of Gly), 3.35–3.46 (m, 2H, CHCH_2 of His), 4.59–4.67 (m, 1H, C*H*CH_2 of His), 7.34 (s, 2H, C*H*-5 of His + Hst), 8.35 (s, 2H, C*H*-2 of His + Hst); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, *C*H_2 of Hst); 37.9 (s, *C*H_2 of Hst); 40.5 (s, *C*H_2 of Gly), 59.6 (s, *C*HCH$_2$ of His), 122.2 (s, *C*-4 of His), 122.4 (s, *C*-4 of Hst); 132.0 (s, *C*-5 of His), 134.8 (s, *C*-5 of Hst); 137.2 (s, *C*-2 of His), 137.4 (s, C-2 of Hst); 175.0 (s, CH_2CO of Gly), 176.4 (s, *C*ONH of His). Anal. found: C, 51.21; H, 6.32; N, 32.10%; $C_{13}H_{19}N_7O_2$ requires: C, 51.14; H, 6.27; N, 32.11%.

4-[β-(β-Alanyl-histidylamido)-ethyl]-1*H*-imidazole A22. Mp 240–243°C; IR (KBr), cm^{-1}: 1288 (amide III), 1592 (amide II), 1715 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 2.79–2.88 (m, 2H, CH_2 of β-Ala), 3.11–3.26 (m, 2H, CH_2 of β-Ala), 3.34–3.45 (m, 2H, CHCH_2 of His), 4.57–4.63 (m, 1H, C*H*CH_2 of His), 7.33 (s, 2H, C*H*-5 of His + Hst), 8.35 (s, 2H, C*H*-2 of His + Hst); ^{13}C NMR (TFA), δ, ppm: 33.3 (s, *C*H_2 of His), 33.4 (s, *C*H_2 of Hst); 37.4 (s, NH*C*H_2CH_2 of β-Ala), 37.9 (s, *C*H_2 of Hst); 40.8 (s, CH_2CH_2CO of β-Ala), 59.6 (s, *C*HCH$_2$ of His), 122.2 (s, *C*-4 of His), 122.5 (s, C-4 of Hst); 134.2 (s, *C*-5 of His), 134.8 (s, C-5 of Hst); 137.2 (s, *C*-2 of His), 137.5 (s, C-2 of Hst); 175.6 (s, CH_2CO of β-Ala), 176.7 (s, *C*ONH of His). Anal. found: C, 52.44; H, 6.70; N, 30.53%; $C_{14}H_{21}N_7O_2$ requires: C, 52.65; H, 6.63; N, 30.70%.

4-[β-(Phenylalanyl-prolylamido)-ethyl]-1*H*-imidazole A23. Mp 233–234°C (dec.); IR (KBr), cm^{-1}: 1289 (amide III), 1590 (amide II), 1717 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 1.19–1.40 (m, 1H, *H*CH of Pro); 1.55–1.69 (m, 1H, HC*H*); 1.75–1.85 (m, 2H, CH_2 of Pro); 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.10–3.56 (m, 2H, CH_2CH of Phe), 3.20–3.35 (m, 2H, CH_2N of Pro); 3.75–3.80 (m, 1H, C*H*CO of Pro), 4.12 (dd, $^3J_{HH}=5.0$, $^3J_{HH}=7.8$, 1H, CH_2CH of Phe), 7.29–7.58 (m, 6H, H_{arom} of Phe + H-5 of Hst); 8.34 (s, 1H, C*H*-2 of Hst); ^{13}C NMR (TFA), δ, ppm: 15.5 (s, CH_2 of Pro); 21.5 (s, CH_2 of Pro); 33.5 (s, *C*H_2 of Hst); 37.9 (s, *C*H_2 of Hst); 41.6 (s, *C*H_2CH of Phe), 46.9 (s, CH_2N of Pro); 59.5 (s, *C*HCH$_2$ of Phe), 64.5 (s, *C*HCO of Pro), 122.4 (s, C-4 of Hst); 130.2 (s, C_{para} of Phe), 133.9 (s, C_{meta} of Phe), 134.7 (s, C_{ortho} of Phe); 134.6 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 141.5 (s, C_{ipso} of Phe), 172.3 (s, Phe-*C*ONH); 174.0 (Pro-*C*ONH). Anal. found: C, 64.35; H, 7.15; N, 19.58%; $C_{19}H_{25}N_5O_2$ requires: C, 64.20; H, 7.09; N, 19.70%.

4-[β-(Prolyl-glycylamido)-ethyl]-1*H*-imidazole A24. Mp 235–237°C; IR (KBr), cm^{-1}: 1289 (amide III), 1590 (amide II), 1717 (amide I), 3060 (NH); ^1H NMR (TFA), δ, ppm: 1.18–1.38 (m, 1H, *H*CH of Pro); 1.55–1.65 (m, 1H, HC*H*); 1.70–1.85 (m, 2H, CH_2 of Pro); 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2); 3.16–3.30 (m, 2H, CH_2N of Pro); 3.67 (s, 2H, CH_2 of Gly), 3.75–3.80 (m, 1H, C*H*CO of Pro), 7.34 (m, 1H, H-5 of Hst); 8.35 (s, 1H, H-2 of Hst); ^{13}C NMR (TFA), δ, ppm: 15.6 (s, CH_2 of Pro); 21.3 (s, CH_2 of Pro); 33.5 (s, *C*H_2 of Hst); 37.9 (s, *C*H_2 of Hst); 40.8 (s, *C*H_2 of Gly), 46.4 (s, CH_2N of Pro); 64.7 (s, *C*HCO of Pro), 122.4 (s, C-4 of Hst); 134.7 (s, C-5 of Hst); 137.3 (s, C-2 of Hst); 175.6 (s, Pro-*C*ONH); 176.3 (s, *C*ONH of Gly). Anal. found: C, 54.28; H, 7.27; N, 26.09%; $C_{12}H_{19}N_5O_2$ requires: C, 54.33; H, 7.22; N, 26.40%.

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References and Notes

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