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CARBONIC ANHYDRASE ACTIVATORS: SYNTHESIS OF HIGH AFFINITY ISOZYMES I, II AND IV ACTIVATORS, DERIVATIVES OF 4-(4-TOSYLUREIDO-AMINO ACYL)ETHYL-1*H*-IMIDAZOLE (HISTAMINE DERIVATIVES)*

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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 4-tosylureido amino acids/dipeptides (ts-AA) in the presence of carbodiimides, afforded after deprotection of the imidazole moiety, a series of compounds with the general formula ts-AA-Hst (ts = 4-$MeC_6H_4SO_2NHCO$). Some structurally related dipeptide derivatives with the general formula ts-AA1-AA2-Hst, were also prepared, by in a similar way to the amino acyl compounds mentioned above. The new derivatives were examined 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 1–10 nanomolar range for the best compounds. hCA II was on the other hand activatable with affinities around 20–50 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.

Keywords: Carbonic anhydrase; Histamine; Amino acid; Dipeptide; Tosyl isocyanate; Tosylurea; Enzyme activators; Proton shuttling

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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] epilepsy,[3] gastric and duodenal ulcers,[4] or acid-base disequilibria[5] among others. In contrast to inhibitors, activators of this enzyme (for which at least 14 different isozymes have been isolated in higher vertebrates)[6] have been 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[7] and phenylalanine (in this case a tertiary complex, in which azide is also bound to the Zn(II) ion)[8] have been reported by this group. Furthermore, few other QSAR[9] or synthetic chemistry[10] 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 considerable number of patients.[11] 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.[7,10]

The lead molecule considered by us for obtaining tighter binding CA activators was histamine **1** itself. As seen from the X-ray co-ordinates from 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 does not experience any contact with the enzyme, but is extended 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.[7] Thus, it appeared of interest to derivatize the lead at its 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 Whiteside's[12] and our groups[7,13] for the design of tight-binding, isozyme-specific sulfonamide CA inhibitors. Moreover, recently we[1] have reported some sulfonylated 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

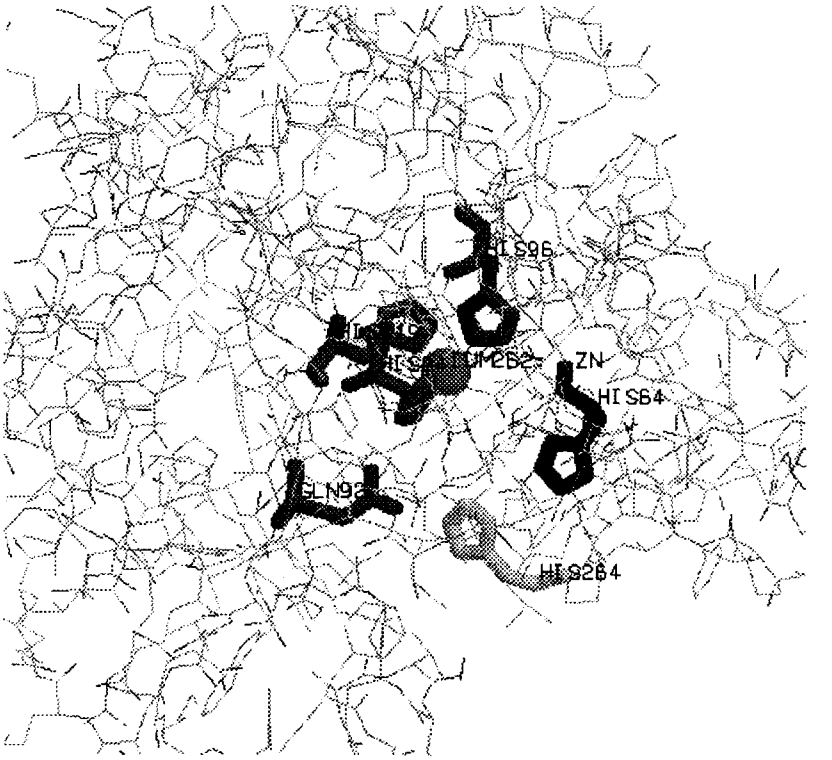


FIGURE 1 hCA II – histamine adduct: the Zn(II) ion (central sphere) and its three histidine ligands (His 94, His 96 and His 119) are shown at the center of the active site, whereas histamine (numbered as His 264) is situated at the entrance in it, between residues His 64 and Gln 92. The figure was generated from the X-ray coordinates of the hCA II–histamine adduct reported by this group,[7] with the program RasMol for Windows 2.6. The coordinates of this structure are deposited in the Brookhaven Protein Database (PDB entry 4TST).

types of moieties that might be attached at the aliphatic end of the molecule, and tosylureido-amino acyl groups appeared of interest, due to the possible interactions of these highly polarized groups with amino acid residues at the edge of the active site, which presumably would lead to increased stabilities of the enzyme–activator adducts.

In this paper we report the synthesis of a series of 4-tosylureido-amino acyl/dipeptidyl (ts-AA) histamine (Hst) derivatives possessing the general formula tsAA-Hst, obtained by reaction of appropriately protected[1,14] histamine with tosylureido amino acids/dipeptides, in the presence of carbodiimide. 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.

MATERIALS AND METHODS

Melting points were determined with a heating plate microscope and are not corrected. IR spectra were obtained in KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer, whereas ^{1}H-NMR spectra were obtained 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 $\pm 0.4\%$ of the theoretical values. Preparative HPLC was done using C_{18} reversed-phase Bondapack or Dynamax-60A (25×250 mm) columns.

Compounds used in synthesis (histamine, natural and unnatural amino acids, tritylsulfenyl chloride, tetrabromophthalic anhydride, hydrazine, etc) were commercially available compounds (from Sigma, Acros or Aldrich). The tosylureido-amino acid/dipeptide derivatives were prepared as described previously,[15] by the reaction of 4-tosylisocyanate (Acros) with amino acids/dipeptides (from Sigma or Aldrich). Acetonitrile, acetone, dioxane (Merck) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in an anhydrous condition.

Human CA I and CA 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.*[16] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[17] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[18] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of $49\,mM^{-1}\cdot cm^{-1}$ for CA I and $54\,mM^{-1}\cdot cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[19,20] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[21]

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.[22] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied

between $2 \cdot 10^{-2}$ M and $1 \cdot 10^{-6}$ M, working at 25°C. A molar absorption coefficient ε of 18,400 $M^{-1} \cdot cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis under the conditions of the experiments (pH 7.40), as reported in the literature.[22] 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. [7]. 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[23] and higher concentrations had to be used for the measurements).

Preparation of *N*-1-tritylsulfenyl-histamine 3

An amount of 5.55 g (50 mM) of histamine 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 to the deprotection step. Hydrazinolysis was effected by dissolving the above mentioned precipitate in 200 mL of ethanol and then addition of 15 mL of hydrazinium hydroxide followed by stirring for 5 h at room temperature. The solvent was then evaporated, a small excess of 2 N HCl solution was added and the precipitated tetrabromophthalhydrazide was filtered and discarded. The solution containing **3** was brought to pH 7 with solid $NaHCO_3$, reduced to a small volume by *in vacuo* evaporation of the solvent, and the precipitated **3** was then recrystallized from ethanol (overall yield of 80% based on histamine) to give tan crystals, m.p. 177–8°C ^{1}H-NMR (300 MHz, DMSO-d_6), 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). 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 Tosylureido Amino Acids/Dipeptides ts-AA

An amount of 20 mM of amino acid/dipeptide was suspended/dissolved in 50 mL of anhydrous acetone or acetonitrile, and the stoichiometric amount of 4-toluenesulfonyl isocyanate (TsNCO) was added in one portion, with energetic stirring and eventual cooling of the reaction mixture. The mixture was then stirred for 1–2 h at 4°C, the solvent was evaporated *in vacuo* and the product purified either by recrystallization from water–ethanol (1 : 1, v/v), or by preparative HPLC (in the case of tsGlyGly; tsHis; tsVal; tsTrp and tsPhe, when the tosylureido amino acid/dipeptide contained variable amounts of unreacted amino acid and tosylamide). Conditions were: C_{18} reversed-phase Bondapack or Dynamax-60A (25 × 250 mm) columns; 90% acetonitrile/8% ethanol/2% water, 30 mL/min. Remarkably, the reaction of *L*-Lys monohydrochloride or *L*-Arg monohydrochloride with TsNCO in the conditions mentioned above led to the formation of only one very pure product, i.e., the α-tosylureido amino acid, without derivatization of the ε-amino moiety in the case of Lys, or the guanidino one in the case of Arg. This is probably due to the fact that H^+ acts in this case as a very good side chain protecting group for these two amino acids. This has been further confirmed by the synthesis of α-tsLys and α-tsArg from the appropriately protected amino acid derivatives (*N*-ε-acetyl-*L*-Lys and ω-*N*-tritylsulfenyl-*L*-Arg) and TsNCO, followed by deprotection under standard conditions (data not shown).

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 4-tosylureido 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 5% 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 *N*-1 imidazole moiety in the following way. The crude **4** was 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 tritylsulfenyl chloride formed during the deprotection step extracted with 2×50 mL of Et_2O. The aqueous phase was evaporated *in vacuo* to a small volume when, generally, compounds **A1–A24** precipitated on allowing the mixture to stand at 4°C overnight. The pure compounds were obtained after recrystallization from ethanol–water (1 : 1, v/v). In some cases, preparative HPLC was done (C_{18} reversed-phase Bondapack or Dynamax-60A (25×250 mm) columns; 90% acetonitrile/8% methanol/2% water, 30 mL/min) in order to obtain the pure title derivatives.

4-[β-(4-Toluenesulfonylureido-glycylamido)-ethyl]-1H-imidazole **A1** as white crystals, m.p. 271–3°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1287 (amide III), 1375 (SO_2^{as}), 1579 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.63 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$, Hst CONHC$\underline{H}_2$), 3.67 (s, 2H, C$\underline{H}_2$ of Gly), 7.34 (m, 1H, imidazole CH), 7.65 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.99 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.21 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.1 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 40.8 (s, $\underline{C}H_2$ of Gly), 122.4 (s, C-4 of Hst), 130.9 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (s, NH$\underline{C}$ONH), 134.8 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.1 (CONH). Found: C, 49.13; H, 6.30; N, 23.91. $C_{15}H_{19}N_5O_4S$ requires: C, 48.91; H, 6.48; N, 24.02%.

4-[β-(4-Toluenesulfonylureido-alanylamido)-ethyl]-1H-imidazole **A2** as white crystals, m.p. 254–5°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1285 (amide III), 1375 (SO_2^{as}), 1574 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.84 (d, $^3J_{HH} = 6.5$, 3H, CHC$\underline{H}_3$ of Ala), 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.64 (s, 3H, $C\underline{H}_3C_6H_4$), 3.01 (t, 2H, $J = 7.0$, Hst CONHC$\underline{H}_2$), 3.98 (q, 1H, C$\underline{H}$ of Ala), 7.34 (m, 1H, imidazole CH), 7.65 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.99 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.21 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 22.1 (s, CH$\underline{C}$H$_3$ of Ala), 26.1 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 34.5 (s, $\underline{C}$HCH$_3$ of Ala), 122.4 (s, C-4 of Hst), 130.9 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.8 (s, NH$\underline{C}$ONH), 134.8 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.9 (CONH). Found: C, 50.71; H, 5.42; N, 18.35. $C_{16}H_{21}N_5O_4S$ requires: C, 50.65; H, 5.58; N, 18.46%.

4-[β-(4-Toluenesulfonylureido-β-alanylamido)-ethyl]-1H-imidazole **A3** as white crystals, m.p. 270–1°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1284

(amide III), 1375 (SO_2^{as}), 1575 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.60 (s, 3H, $C\underline{H}_3C_6H_4$), 2.73 (t, $^3J_{HH} = 6.6$, 1H, $CH_2C\underline{H}_2CO$ of β-Ala), 2.99 (t, 2H, $J = 7.0$, Hst CONHCH_2), 3.27 (t, $^3J_{HH} = 6.7$, 1H, $CH_2C\underline{H}_2CO$ of β-Ala), 3.43 (t, $^3J_{HH} = 6.3$, 2H, $C\underline{H}_2CH_2CO$ of β-Ala), 7.34 (m, 1H, imidazole CH), 7.62 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.00 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.28 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d$_6$), δ, ppm: 26.1 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.5 (s, $NH\underline{C}H_2CH_2$ of β-Ala), 37.9 (s, $\underline{C}H_2$ of Hst), 40.9 (s, $CH_2\underline{C}H_2CO$ of β-Ala), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.3 (s, NH$\underline{C}$ONH), 134.8 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 144.8 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.8 (CONH). Found: C, 50.44; H, 5.50; N, 18.27. $C_{16}H_{21}N_5O_4S$ requires: C, 50.65; H, 5.58; N, 18.46%.

4-[β-(4-Toluenesulfonylureido-n-butyramido)-ethyl]-1H-imidazole **A4** as white crystals, m.p. 235–7°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1287 (amide III), 1375 (SO_2^{as}), 1579 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 2.49 (t, 2H, $J = 7.0$ Hz, Hst CH_2), 2.60 (s, 3H, $C\underline{H}_3C_6H_4$), 2.75 (t, $^3J_{HH} = 6.5$, 1H, $(CH_2)_2C\underline{H}_2CO$ of GABA), 2.99 (t, 2H, $J = 7.0$ Hz, Hst CONHCH_2), 3.27 (t, $^3J_{HH} = 6.7$, 1H, $(CH_2)_2C\underline{H}_2CO$ of GABA), 3.31–3.56 (m, 4H, $(C\underline{H}_2)_2CH_2CO$ of GABA), 7.36 (m, 1H, imidazole CH), 7.62 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.00 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.25 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d$_6$), δ, ppm: 26.1 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.3 (s, $NH\underline{C}H_2CH_2$ of GABA), 37.9 (s, $\underline{C}H_2$ of Hst), 40.9 (s, $CH_2CH_2\underline{C}H_2CO$ of GABA), 41.3 (s, $CH_2\underline{C}H_2CH_2CO$ of GABA), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.3 (s, NH$\underline{C}$ONH), 134.8 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 144.8 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.5 (CONH). Found: C, 51.96; H, 5.74; N, 17.54. $C_{17}H_{23}N_5O_4S$ requires: C, 51.89; H, 5.89; N, 17.80%.

4-[[β-(4-Toluenesulfonylureido-glycyl)glycylamido]-ethyl]-1H-imidazole **A5** as white crystals, m.p. 212–3°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1289 (amide III), 1375 (SO_2^{as}), 1584 (amide II), 1715 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 2.49 (m, 6H, $J = 7.0$, Hst $CH_2 + 2C\underline{H}_2$ of GlyGly), 2.73 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$, Hst CONHCH_2), 7.34 (m, 1H, imidazole CH), 7.75 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.09 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.23 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d$_6$), δ, ppm: 26.0 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 35.8

(s, $\underline{C}H_2$ of GlyGly), 37.9 (s, $\underline{C}H_2$ of Hst), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (s, NH$\underline{C}$ONH), 134.7 (s, C-5 of Hst), 134.9 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.1 (CONH); 172.5 (CONH). Found: C, 48.52; H, 5.12; N, 19.76. $C_{17}H_{22}N_6O_5S$ requires: C, 48.33; H, 5.25; N, 19.89%.

4-[β-(4-Toluenesulfonylureido-valylamido)-ethyl]-1H-imidazole **A6** as white crystals, m.p. 250–2°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1280 (amide III), 1375 (SO_2^{as}), 1573 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.11 (d, $^3J_{HH} = 6.7$, 6H, CH(C$\underline{H}_3$)$_2$ of Val), 2.29–2.50 (m, 1H, C$\underline{H}$(CH$_3$)$_2$ of Val), 2.54 (t, 2H, $J = 7.0$, Hst CH_2), 2.70 (s, 3H, C$\underline{H}_3C_6H_4$), 3.01 (t, 2H, $J = 7.0$, Hst CONHC$\underline{H}_2$), 3.75 (d, $^3J_{HH} = 4.3$, 1H, NHC$\underline{H}$CH of Val), 7.34 (m, 1H, imidazole CH), 7.72 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.04 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.21 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 22.3 (s, CH(C$\underline{H}_3$)$_2$ of Val), 26.1 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 34.0 (s, $\underline{C}$H(CH$_3$)$_2$ of Val), 37.9 (s, $\underline{C}H_2$ of Hst), 64.4 (s, NH$\underline{C}$HCH of Val), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.3 (s, NH$\underline{C}$ONH), 134.7 (s, C-5 of Hst), 134.9 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.9 (CONH). Found: C, 53.15; H, 5.94; N, 17.02. $C_{18}H_{25}N_5O_4S$ requires: C, 53.06; H, 6.18; N, 17.19%.

4-[β-(4-Toluenesulfonylureido-leucylamido)-ethyl]-1H-imidazole **A7** as white crystals, m.p. 215–6°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1280 (amide III), 1375 (SO_2^{as}), 1575 (amide II), 1713 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.43 (d, $^3J_{HH} = 6.7$, 6H, CH(C$\underline{H}_3$)$_2$ of Leu), 1.55 (m, 2H, CH_2 of Leu), 2.54 (t, 2H, $J = 7.0$, Hst CH_2), 2.70 (s, 3H, C$\underline{H}_3C_6H_4$), 3.01 (t, 2H, $J = 7.0$, Hst CONHC$\underline{H}_2$), 3.70 (m, 1H, NHC$\underline{H}$CH$_2$ of Leu), 4.23 (m, 1H, CHCH$_2$C$\underline{H}$(CH$_3$)$_2$ of Leu), 7.34 (m, 1H, imidazole CH), 7.72 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.04 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.29 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 21.7 (s, CH(C$\underline{H}_3$)$_2$ of Leu), 26.3 (s, $\underline{C}H_3C_6H_4$), 31.9 (s, $\underline{C}H_2$ of Leu), 33.3 (s, $\underline{C}H_2$ of Hst), 34.0 (s, $\underline{C}$H(CH$_3$)$_2$ of Leu), 37.9 (s, $\underline{C}H_2$ of Hst), 56.2 (s, NH$\underline{C}$HCH$_2$ of Leu), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.7 (s, NH$\underline{C}$ONH), 134.6 (s, C-5 of Hst), 134.9 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.1 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.5 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.6 (CONH). Found: C, 53.98; H, 6.55; N, 16.50. $C_{19}H_{27}N_5O_4S$ requires: C, 54.14; H, 6.46; N, 16.61%.

4-[β-(4-Toluenesulfonylureido-isoleucylamido)-ethyl]-1H-imidazole **A8** as white crystals, m.p. 194–6°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1283

(amide III), 1375 (SO_2^{as}), 1575 (amide II), 1715 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.15 (d, $^3J_{HH} = 6.5$, 3H, C$\underline{H}_3$ 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), 2.70 (s, 3H, C$\underline{H}_3C_6H_4$), 3.01 (t, 2H, $J = 7.0$, Hst CONHCH_2), 3.22 (m, 1H, EtC$\underline{H}$(Me)- of Ile), 3.75 (m, 1H, NHC$\underline{H}$CH of Ile), 7.36 (m, 1H, imidazole CH), 7.71 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.06 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$); 8.26 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH); 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 21.9 (s, CH$\underline{C}H_3$ of Ile), 22.5 (s, $\underline{C}H_3$-CH_2 of Ile), 26.3 (s, $\underline{C}H_3C_6H_4$), 31.4 (s, $\underline{C}H_2$ of Ile), 33.3 (s, $\underline{C}H_2$ of Hst), 34.0 (s, $\underline{C}H(CH_3)_2$ of Leu), 37.9 (s, $\underline{C}H_2$ of Hst), 46.4 (s, Et$\underline{C}$H(Me)- of Ile), 55.0 (s, NH$\underline{C}$HCH$_2$ of Ile), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of CH_3-C_6H_4), 132.8 (s, NH$\underline{C}$ONH), 134.7 (s, C-5 of Hst), 134.9 (s, $\underline{C}_{ortho}$ of CH_3-C_6H_4), 137.3 (s, C-2 of Hst), 145.1 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.5 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 167.6 (CONH). Found: C, 54.11; H, 6.50; N, 16.39. $C_{19}H_{27}N_5O_4S$ requires: C, 54.14; H, 6.46; N, 16.61%.

4-[β-(4-Toluesulfonylureido-asparaginylamido)-ethyl]-1H-imidazole **A9** as white crystals, m.p. 241–2°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1287 and 1295 (amide III), 1375 (SO_2^{as}), 1572 (amide II), 1710 and 1723 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.54 (t, 2H, $J = 7.0$, Hst CH_2), 2.59 (d, 2H, $^3J_{HH} = 6.3$, C$\underline{H}_2$ of Asn), 2.63 (s, 3H, C$\underline{H}_3C_6H_4$), 3.01 (t, 2H, $J = 7.0$, Hst CONHCH_2), 4.55 (m, 1H, NHC$\underline{H}$CH$_2$ of Asn), 7.33 (m, 1H, imidazole CH), 7.65 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.94 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.10–8.30 (br s, 5H, $CONH_2$ + CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.0 (s, $\underline{C}H_3C_6H_4$), 30.1 (s, CH$\underline{C}$H$_2$ of Asn), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 70.4 (s, $\underline{C}$HCH$_2$ of Asn), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 134.1 (s, NH$\underline{C}$ONH), 134.7 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.5 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 177.0 (CONH), 178.9 (s, $\underline{C}ONH_2$ of Asn). Found: C, 48.43; H, 5.16; N, 19.58. $C_{17}H_{22}N_6O_5S$ requires: C, 48.33; H, 5.25; N, 19.89%.

4-[β-(4-Toluenesulfonylureido-glutaminylamido)-ethyl]-1H-imidazole **A10** as white crystals, m.p. 238–9°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1285 and 1293 (amide III), 1375 (SO_2^{as}), 1575 (amide II), 1710 and 1724 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.54 (t, 2H, $J = 7.0$, Hst CH_2), 2.55–2.63 (m, 2H, C$\underline{H}_2$ of Gln), 2.61 (s, 3H, C$\underline{H}_3C_6H_4$), 3.01 (t, 2H, $J = 7.0$, Hst CONHCH_2), 3.05–3.34 (m, 2H, C$\underline{H}_2$ of Gln), 4.61 (m, 1H, NHC$\underline{H}$CH$_2$ of Gln), 7.34 (m, 1H, imidazole CH), 7.64 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.94 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$); 8.10–8.33 (br s,

5H, $CONH_2 + CONH + NHCONH$), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.1 (s, $\underline{C}H_3C_6H_4$), 30.5 (s, $CH\underline{C}H_2CH_2$ of Gln), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 44.7 (s, $CHCH_2\underline{C}H_2$ of Gln), 72.6 (s, $\underline{C}HCH_2CH_2$ of Gln), 122.4 (s, C-4 of Hst), 130.9 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 133.1 (s, $NH\underline{C}ONH$), 134.8 (s, C-5 of Hst), 135.1 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.5 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 177.2 (CONH), 179.5 (s, $CH_2\underline{C}ONH_2$ of Gln). Found: C, 49.30; H, 5.55; N, 19.18. $C_{18}H_{24}N_6O_5S$ requires: C, 49.53; H, 5.54; N, 19.25%.

4-[β-(4-Toluenesulfonylureido-arginylamido)-ethyl]-1H-imidazole **A11** as white crystals, m.p. 276–8°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1286 (amide III), 1375 (SO_2^{as}), 1584 (amide II), 1712 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.70–2.00 (m, 2H, $CHCH_2C\underline{H}_2$ of Arg), 2.49 (t, 2H, $J = 7.0$ Hz, Hst CH_2), 2.50–2.58 (m, 2H, $CHC\underline{H}_2CH_2$ of Arg), 2.63 (s, 3H, $C\underline{H}_3C_6H_4$), 2.75 (t, $^3J_{HH} = 6.5$, 1H, $(CH_2)_2C\underline{H}_2CO$ of Arg), 2.99 (t, 2H, $J = 7.0$ Hz, Hst $CONHC\underline{H}_2$), 3.30–3.45 (m, 2H, $CH_2C\underline{H}_2NH$ of Arg), 3.45–3.60 (m, 1H, $CH_2C\underline{H}(NH)CO$ of Arg), 7.36 (m, 1H, imidazole CH), 7.64 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.98 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.25 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.0 (s, $\underline{C}H_3C_6H_4$), 29.7 (s, $CH_2\underline{C}H_2CH_2$ of Arg), 33.3 (s, $\underline{C}H_2$ of Hst), 35.4 (s, $CHCH_2\underline{C}H_2$ of Arg), 37.9 (s, $\underline{C}H_2$ of Hst), 45.7 (s, $CH_2\underline{C}H_2NH$ of Arg), 59.8 (s, $CH_2\underline{C}H(NH)CO_2H$ of Arg), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 131.5 (s, $NH\underline{C}ONH$), 134.6 (s, C-5 of Hst), 134.9 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 144.8 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 161.5 (s, $NH\underline{C}(=NH)NH_2$ of Arg), 170.8 (CONH). Found: C, 49.12; H, 5.81; N, 21.69. $C_{19}H_{28}N_8O_4S$ requires: C, 49.26; H, 5.59; N, 21.97%.

4-[β-[α(4-Toluenesulfonylureido-lysylamido)]-ethyl]-1H-imidazole **A12** as white crystals, m.p. 212–4°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1284 (amide III), 1375 (SO_2^{as}), 1579 (amide II), 1711 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.66–2.20 (m, 6H, $CH(C\underline{H}_2)_3CH_2$ of Lys), 2.49 (t, 2H, $J = 7.0$ Hz, Hst CH_2), 2.61 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$ Hz, Hst $CONHC\underline{H}_2$), 3.13 (t, $^3J_{HH} = 6.7$, 2H, $CH_2C\underline{H}_2NH_2$ of Lys), 3.84 (t, $^3J_{HH} = 6.7$, 1H, $CH_2C\underline{H}(NH)CO$ of Lys), 7.36 (m, 1H, imidazole CH), 7.63 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.98 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.26 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.0 (s, $\underline{C}H_3C_6H_4$), 26.6 (s, $H_2NCH_2(\underline{C}H_2)_3$ of Lys), 31.4 (s, $H_2NCH_2(\underline{C}H_2)_3$ of Lys), 33.3 (s, $\underline{C}H_2$ of Hst), 34.8 (s, $H_2NCH_2(\underline{C}H_2)_3$ of Lys), 37.9 (s, $\underline{C}H_2$

of Hst), 43.8 (s, $H_2N\underline{C}H_2(CH_2)_3$ of Lys), 58.8 (s, $CH_2\underline{C}H(NH)CO$ of Lys), 122.4 (s, C-4 of Hst), 130.8 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (s, $NH\underline{C}ONH$), 134.8 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 144.9 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 171.2 (CONH). Found: C, 52.17; H, 6.70; N, 19.10. $C_{19}H_{28}N_6O_4S$ requires: C, 52.28; H, 6.47; N, 19.25%.

4-[β-(4-Toluenesulfonylureido-histidylamido)-ethyl]-1H-imidazole **A13** as white crystals, m.p. 266–8°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1283 (amide III), 1375 (SO_2^{as}) 1585 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.63 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$, Hst $CONHC\underline{H}_2$), 3.32–3.46 (m, 2H, $CHC\underline{H}_2$ of His), 4.01–4.08 (m, 1H, $C\underline{H}CH_2$ of His), 7.34 (m, 1H, imidazole CH of Hst), 7.46 (s, 1H, $C\underline{H}$-5 of His), 7.55 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.89 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.21 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.51 (s, 1H, $C\underline{H}$-2 of His), 8.80 (s, 2H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.1 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 40.9 (s, $\underline{C}H_2$ of His), 58.6 (s, $CH_2\underline{C}H$ of His), 122.2 (s, C-4 of His), 122.4 (s, C-4 of Hst), 130.9 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (s, $NH\underline{C}ONH$), 134.1 (s, C-5 of His), 134.6 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.2 (s, C-2 of Hst), 137.5 (s, C-2 of His), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of CH_3-C_6H_4), 167.1 (CONH). Found: C, 51.21; H, 5.12; N, 22.00. $C_{19}H_{23}N_7O_4S$ requires: C, 51.23; H, 5.20; N, 22.01%.

4-[β-(4-Toluenesulfonylureido-phenylglycylamido)-ethyl]-1H-imidazole **A14** as white crystals, m.p. 235–7°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1284 (amide III), 1375 (SO_2^{as}), 1580 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.63 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$, Hst $CONHC\underline{H}_2$), 4.08 (m, 1H, $PhC\underline{H}$ of Phg), 7.29–7.58 (m, 8H, $\underline{H}_{ortho}$ of $CH_3C_6H_4 + \underline{H}_{arom}$ of Phg + imidazole CH of Hst), 7.95 (d, $^3J_{HH} = 8.2$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.20 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.2 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 59.9 (s, $Ph\underline{C}H$ of Phg), 122.4 (s, C-4 of Hst), 132.3 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.7 (s, $NH\underline{C}ONH$), 133.9 (s, $\underline{C}_{meta}$ of Phg), 134.5 (s, $\underline{C}_{ortho}$ of Phg), 134.8 (s, C-5 of Hst), 135.1 (s, $\underline{C}_{ortho}$ of CH_3-C_6H_4), 137.3 (s, C-2 of Hst), 141.5 (s, $\underline{C}_{ipso}$ of Phe), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 166.9 (CONH). Found: C, 57.15; H, 5.22; N, 15.70. $C_{21}H_{23}N_5O_4S$ requires: C, 57.13; H, 5.25; N, 15.86%.

4-[β-(4-Toluenesulfonylureido-phenylalanylamido)-ethyl]-1H-imidazole **A15** as white crystals, m.p. 232–3°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}),

1286 (amide III), 1375 (SO_2^{as}), 1581 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2), 2.65 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J=7.0$, Hst CONHC$\underline{H}_2$), 3.10–3.55 (m, 2H, $C\underline{H}_2CH$ of Phe), 4.08 (dd, $^3J_{HH}=5.0$, $^3J_{HH}=7.8$, 1H, $CH_2C\underline{H}$ of Phe), 7.29–7.58 (m, 8H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$ and $\underline{H}_{arom}$ of Phe + imidazole CH of Hst), 7.95 (d, $^3J_{HH}=8.2$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.21 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.2 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 41.7 (s, $\underline{C}H_2CH$ of Phe), 59.3 (s, $CH_2\underline{C}H$ of Phe), 122.4 (s, C-4 of Hst), 132.3 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.7 (s, NH$\underline{C}$ONH), 133.8 (s, $\underline{C}_{meta}$ of Phe), 134.4 (s, $\underline{C}_{ortho}$ of Phe), 134.8 (s, C-5 of Hst), 135.1 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 141.5 (s, $\underline{C}_{ipso}$ of Phe), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 166.9 (CONH). Found: C, 58.20; H, 5.66; N, 15.14. $C_{22}H_{25}N_5O_4S$ requires: C, 58.01; H, 5.53; N, 15.37%.

4-[β-(4-Toluenesulfonylureido-tryptophanylamido)-ethyl]-1H-imidazole **A16** as white crystals, m.p. 190–1°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1280 (amide III), 1375 (SO_2^{as}), 1581 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2), 2.62 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J=7.0$, Hst CONHC$\underline{H}_2$), 3.44 (dd, $^3J_{HH}=9.0$, $^2J_{HH}=14.6$, 1H, $C\underline{H}_2CH$ of Trp), 3.65 (dd, $^3J_{HH}=4.1$, $^2J_{HH}=15.0$, 1H, $C\underline{H}_2CH$ of Trp), 4.14 (dd, $^3J_{HH}=4.3$, $^3J_{HH}=8.0$, 1H, $CH_2C\underline{H}$ of Trp), 7.22–7.82 (m, 8H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$ + $\underline{H}_{arom}$ of Trp + 1H, imidazole CH of Hst), 7.92 (d, $^3J_{HH}=8.2$, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$), 8.21 (br s, 3H, CONH + NHCONH), 8.35 (s, 1H, imidazole CH), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.0 (s, $\underline{C}H_3C_6H_4$), 31.6 (s, $\underline{C}H_2CH$ of Trp), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 58.8 (s, $CH_2\underline{C}H$ of Trp), 113.9 (s, $\underline{C}$-2 of Trp), 116.9 (s, $\underline{C}$-7 of Trp), 122.4 (s, C-4 of Hst), 123.4 (s, $\underline{C}$-5 of Trp), 124.3 (s, $\underline{C}$-6 of Trp), 126.8 (s, $\underline{C}$-4 of Trp), 129.0 (s, $\underline{C}$-1 of Trp), 130.9 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.1 (s, $\underline{C}$-8 of Trp), 132.5 (s, NH$\underline{C}$ONH), 134.7 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 141.2 (s, $\underline{C}$-3 of Trp), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 (s, $\underline{C}_{para}$ of CH_3-C_6H_4), 168.2 (CONH). Found: C, 58.23; H, 5.41; N, 16.76. $C_{24}H_{26}N_6O_4S$ requires: C, 58.29; H, 5.30; N, 16.99%.

4-[β-(4-Toluenesulfonylureido-prolylamido)-ethyl)]-1H-imidazole **A17** m.p. 270–3°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1287 (amide III), 1375 (SO_2^{as}), 1589 (amide II), 1718 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.18–1.38 (m, 1H, $\underline{H}$CH of Pro), 1.55–1.65 (m, 1H, HC$\underline{H}$), 1.70–1.85 (m, 2H, CH_2 of Pro), 2.49 (t, 2H, $J=7.0$, Hst CH_2), 2.62 (s, 3H, $C\underline{H}_3C_6H_4$), 2.99 (t, 2H, $J=7.0$, Hst CONHC$\underline{H}_2$), 3.16–3.30 (m, 2H, CH_2N of Pro), 3.75–3.80 (m, 1H, C$\underline{H}$CO of Pro), 7.34 (m, 1H, H-5 of Hst), 7.42

(d, $^3J_{HH} = 8.3$, 2H, H_{ortho} of tosyl), 7.95 (d, $^3J_{HH} = 8.3$, 2H, $\underline{H}_{meta}$of tosyl), 8.21 (br s, 2H, CONH + Pro-NCONH), 8.35 (s, 1H, H-2 of Hst), 8.80 (s, 1H, imidazole NH), ^{13}C-NMR (DMSO-d_6), δ, ppm: 15.6 (s, CH_2 of Pro), 21.3 (s, CH_2 of Pro), 26.7 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 46.9 (s, CH_2N of Pro), 64.5 (s, $\underline{C}HCO$ of Pro), 122.4 (s, C-4 of Hst), 132.1 (s, NH$\underline{C}$ON), 132.4 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 134.8 (s, C-5 of Hst), 135.1 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 170.3 (s, Pro-$\underline{C}$ONH). Found: C, 53.60; H, 5.61; N, 17.23. $C_{18}H_{23}N_5O_4S$ requires: C, 53.32; H, 5.72; N, 17.27%.

4-[β-(4-Toluenesulfonylureido-pipecolylamido)-ethyl]-1H-imidazole **A18** m.p. 236–8°C; IR (KBr), cm^{-1}: 1150(SO_2^{sym}), 1284 (amide III), 1375 (SO_2^{as}), 1589 (amide II), 1712 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.19–1.43 (m, 1H, $\underline{H}$CH of Pip); 1.50–1.65 (m, 1H, HC$\underline{H}$), 1.69–1.87 (m, 4H, $2CH_2$ of Pip), 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.62 (s, 3H, $C\underline{H}_3C_6H_4$), 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$\underline{H}$CO of Pip), 7.33 (m, 1H, H-5 of Hst), 7.42 (d, $^3J_{HH} = 8.3$, 2H, H_{ortho} of tosyl), 7.95 (d, $^3J_{HH} = 8.3$, 2H, $\underline{H}_{meta}$of tosyl), 8.20 (br s, 2H, CONH + Pip-NCONH), 8.35 (s, 1H, H-2 of Hst), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 15.6 (s, CH_2 of Pip), 18.0 (s, CH_2 of Pip), 21.3 (s, CH_2 of Pip), 26.5 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 46.4 (s, CH_2N of Pip), 64.9 (s, $\underline{C}HCO$ of Pip), 122.4 (s, C-4 of Hst), 132.0 (s, NH$\underline{C}$ON), 132.4 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 134.8 (s, C-5 of Hst), 135.1 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 170.0 (s, Pip-$\underline{C}$ONH). Found: C, 54.63; H, 5.89; N, 16.60. $C_{19}H_{25}N_5O_4S$ requires: C, 54.40; H, 6.01; N, 16.69%.

4-[β-(4-Toluenesulfonylureido-nipecotylamido)-ethyl]-1H-imidazole **A19** m.p. 239–41°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1282 (amide III), 1375 (SO_2^{as}), 1593 (amide II), 1710 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.24–1.67 (m, 4H, CH_2CH_2 of Nip), 2.49 (t, 2H, $J = 7.0$, Hst CH_2), 2.62 (s, 3H, $C\underline{H}_3C_6H_4$), 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$\underline{H}$CO of Nip), 7.33 (m, 1H, H-5 of Hst), 7.42 (d, $^3J_{HH} = 8.1$, 2H, H_{ortho} of tosyl), 7.95 (d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$of tosyl), 8.24 (br s, 2H, CONH + Nip-NCONH), 8.35 (s, 1H, H-2 of Hst), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 16.3 (s, CH_2 of Nip), 21.0 (s, CH_2 of Nip), 26.5 (s, $\underline{C}H_3C_6H_4$), 33.3 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 46.4 (s, CH_2N of Nip), 47.5 (s, NCH_2 of Nip), 53.6 (s, $\underline{C}HCO$ of Nip), 122.4 (s, C-4 of Hst), 132.0 (s, NH$\underline{C}$ON), 132.4 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 134.8 (s, C-5 of Hst), 135.1 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{ipso}$ of

$CH_3C_6H_4$), 170.0 (s, Nip-CONH). Found: C, 54.27; H, 5.94; N, 16.52. $C_{19}H_{25}N_5O_4S$ requires: C, 54.40; H, 6.01; N, 16.69%.

4-[β-(4-Toluenesulfonylureido-isonipecotylamido)-ethyl]-1H-imidazole **A20** m.p. 275–6°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1278 (amide III), 1375 (SO_2^{as}), 1591 (amide II), 1711 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.90–2.33 (m, 8H, 2 CH_2CH_2 of Inp), 2.49 (t, 2H, $J=7.0$, Hst CH_2); 2.62 (s, 3H, $CH_3C_6H_4$), 2.99 (t, 2H, $J=7.0$, Hst CONHCH_2), 3.13–3.39 (m, 1H, CHCO of Inp), 7.33 (m, 1H, H-5 of Hst), 7.42 (d, $^3J_{HH}=8.3$, 2H, H_{ortho} of tosyl), 7.95 (d, $^3J_{HH}=8.3$, 2H, H_{meta} of tosyl), 8.27 (br s, 2H, CONH + Inp-NCONH), 8.35 (s, 1H, H-2 of Hst), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 21.6 (s, CH_2 of Inp); 26.5 (s, CH_3-C_6H_4), 33.3 (s, CH_2 of Hst), 37.9 (s, CH_2 of Hst), 47.0 (s, NCH_2 of Inp), 53.2 (s, CHCO of Inp), 122.4 (s, C-4 of Hst), 132.0 (s, NHCON), 132.4 (s, C_{meta} of $CH_3C_6H_4$), 134.8 (s, C-5 of Hst), 135.1 (s, C_{ortho} of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, C_{para} of $CH_3C_6H_4$), 148.4 (s, C_{ipso} of CH_3-C_6H_4), 170.6 (s, Inp-CONH). Found: C, 54.60; H, 6.12; N, 16.45. $C_{19}H_{25}N_5O_4S$ requires: C, 54.40; H, 6.01; N, 16.69%.

4-[β-(4-Toluenesulfonylureido-glycyl-histidylamido)-ethyl]-1H-imidazole **A21** m.p. 221–3°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1287 (amide III), 1375 (SO_2^{as}), 1592 (amide II), 1715 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2), 2.64 (s, 3H, $CH_3C_6H_4$), 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, $CHCH_2$ of His), 7.34 (s, 2H, CH-5 of His + Hst), 7.53 (d, $^3J_{HH}=8.1$, 2H, H_{ortho} of $CH_3C_6H_4$), 7.87 (d, $^3J_{HH}=8.1$, 2H, H_{meta} of $CH_3C_6H_4$), 8.29 (br s, 4H, 2CONH + NHCONH), 8.35 (s, 2H, CH-2 of His + Hst), 8.80 (s, 2H, imidazole NH from His and Hst); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.1 (s, $CH_3C_6H_4$), 33.3 (s, CH_2 of Hst), 37.9 (s, CH_2 of Hst), 40.5 (s, CH_2 of Gly), 59.6 (s, $CHCH_2$ of His), 122.2 (s, C-4 of His), 122.4 (s, C-4 of Hst), 130.7 (s, C_{meta} of $CH_3C_6H_4$), 132.0 (s, C-5 of His), 132.1 (s, NHCONH), 134.2 (s, C_{ortho} of $CH_3C_6H_4$), 134.8 (s, C-5 of Hst), 137.2 (s, C-2 of His), 137.4 (s, C-2 of Hst), 139.1 (s, C_{para} of $CH_3C_6H_4$), 145.6 (s, C_{ipso} of $CH_3C_6H_4$), 175.0 (s, CH_2CO of Gly), 176.4 (s, CONH of His). Found: C, 50.21; H, 5.32; N, 22.18. $C_{21}H_{26}N_8O_5S$ requires: C, 50.19; H, 5.21; N, 22.30%.

4-[β-(4-Toluenesulfonylureido-β-alanyl-histidylamido)-ethyl]-1H-imidazole **A22** m.p. 244–5°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1288 (amide III), 1375 (SO_2^{as}), 1592 (amide II), 1715 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.49 (t, 2H, $J=7.0$, Hst CH_2), 2.63 (s, 3H, $CH_3C_6H_4$), 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,

CHCH$_2$ of His), 7.33 (s, 2H, CH-5 of His + Hst), 7.56 (d, $^3J_{HH} = 8.1$, 2H, H$_{ortho}$ of $CH_3C_6H_4$), 7.87 (d, $^3J_{HH} = 8.1$, 2H, H$_{meta}$ of $CH_3C_6H_4$), 8.29 (br s, 4H, 2CONH + NHCONH), 8.35 (s, 2H, CH-2 of His + Hst), 8.80 (s, 2H, imidazole NH from His and Hst); ^{13}C-NMR (DMSO-d$_6$), δ, ppm: 25.8 (s, $CH_3C_6H_4$), 33.3 (s, CH$_2$ of His), 33.4 (s, CH$_2$ of Hst), 37.4 (s, NHCH$_2$CH$_2$ of β-Ala), 37.9 (s, CH$_2$ of Hst), 40.8 (s, CH$_2$CH$_2$CO of β-Ala), 59.6 (s, CHCH$_2$ of His), 122.2 (s, C-4 of His), 122.5 (s, C-4 of Hst), 130.6 (s, C$_{meta}$ of $CH_3C_6H_4$), 132.3 (s, NHCONH), 134.2 (s, C-5 of His), 134.8 (s, C-5 of Hst), 135.2 (s, C$_{ortho}$ of $CH_3C_6H_4$), 137.2 (s, C-2 of His), 137.5 (s, C-2 of Hst), 145.6 (s, C$_{para}$ of $CH_3C_6H_4$), 149.1 (s, C$_{ipso}$ of $CH_3C_6H_4$), 175.6 (s, CH$_2$CO of β-Ala), 176.7 (s, CONH of His). Found: C, 51.26; H, 5.70; N, 21.43, $C_{22}H_{28}N_8O_5S$ requires: C, 51.15; H, 5.46; N, 21.69%.

4-[β-(4-Toluenesulfonylureido-phenylalanyl-prolylamido)-ethyl]-1H-imidazole **A23** m.p. 239–41°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1289 (amide III), 1375 (SO_2^{as}), 1590 (amide II), 1717 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 1.19–1.40 (m, 1H, HCH of Pro), 1.55–1.69 (m, 1H, HCH), 1.75–1.85 (m, 2H, CH$_2$ of Pro), 2.49 (t, 2H, $J = 7.0$, Hst CH$_2$), 2.65 (s, 3H, $CH_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$, Hst CONHCH_2), 3.10–3.56 (m, 2H, CH$_2$CH of Phe), 3.20–3.35 (m, 2H, CH$_2$N of Pro), 3.75–3.80 (m, 1H, CHCO of Pro), 4.12 (dd, $^3J_{HH} = 5.0$, $^3J_{HH} = 7.8$; 1H, CH$_2$CH of Phe), 7.29–7.58 (m, 8H, H$_{ortho}$ of tosyl + H$_{arom}$ of Phe + H-5 of Hst), 7.95 (d, $^3J_{HH} = 8.3$, 2H, H$_{meta}$ of tosyl), 8.29 (br s, 4H, 2CONH + NHCONH), 8.34 (s, 1H, CH-2 of Hst), 8.80 (s, 1H, imidazole NH), ^{13}C-NMR (DMSO-d$_6$), δ, ppm: 15.5 (s, CH$_2$ of Pro), 21.5 (s, CH$_2$ of Pro), 26.9 (s, $CH_3C_6H_4$), 33.5 (s, CH$_2$ of Hst), 37.9 (s, CH$_2$ of Hst), 41.6 (s, CH$_2$CH of Phe), 46.9 (s, CH$_2$N of Pro), 59.5 (s, CHCH$_2$ of Phe), 64.5 (s, CHCO of Pro), 122.4 (s, C-4 of Hst), 130.2 (s, C$_{para}$ of Phe), 132.1 (s, NHCONH), 132.5 (s, C$_{meta}$ of $CH_3C_6H_4$), 133.9 (s, C$_{meta}$ of Phe), 134.7 (s, C$_{ortho}$ of Phe), 134.6 (s, C-5 of Hst), 135.0 (s, C$_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 141.5 (s, C$_{ipso}$ of Phe), 145.3 (s, C$_{para}$ of $CH_3C_6H_4$), 148.4 (s, C$_{ipso}$ of $CH_3C_6H_4$), 172.3 (s, Phe-CONH), 174.0 (Pro-CONH). Found: C, 58.73; H, 5.59; N, 15.14. $C_{27}H_{32}N_6O_5S$ requires:C, 58.68; H, 5.84; N, 15.21%.

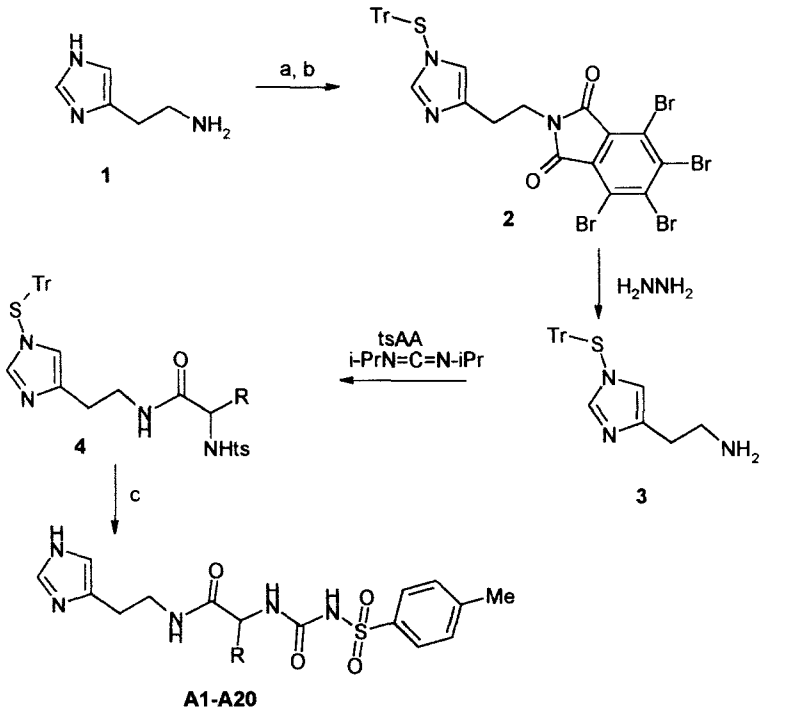
4-[β-(4-Toluenesulfonylureido-prolyl-glycylamido)-ethyl]-1H-imidazole **A24** m.p. 239–40°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1289 (amide III), 1375 (SO_2^{as}), 1590 (amide II), 1717 (amide I), 3060 (NH); ^{1}H–NMR (DMSO–d$_6$), δ, ppm: 1.18–1.38 (m, 1H, HCH of Pro), 1.55–1.65 (m, 1H, HCH), 1.70–1.85 (m, 2H, CH$_2$ of Pro), 2.49 (t, 2H, $J = 7.0$, Hst CH$_2$), 2.63 (s, 3H, $CH_3C_6H_4$), 2.99 (t, 2H, $J = 7.0$, Hst CONHCH_2), 3.16–3.30 (m, 2H, CH$_2$N of Pro), 3.67 (s, 2H, CH$_2$ of Gly), 3.75–3.80 (m, 1H, CHCO of Pro), 7.34 (m, 1H, H-5 of Hst), 7.65 (d, $^3J_{HH} = 8.1$, 2H, H$_{ortho}$ of tosyl), 7.99

(d, $^3J_{HH} = 8.1$, 2H, $\underline{H}_{meta}$ of tosyl), 8.23 (br s, 3H, 2CONH + Pro-NCONH), 8.35 (s, 1H, H-2 of Hst), 8.80 (s, 1H, imidazole NH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 15.6 (s, CH_2 of Pro), 21.3 (s, CH_2 of Pro), 26.1 (s, $\underline{C}H_3C_6H_4$), 33.5 (s, $\underline{C}H_2$ of Hst), 37.9 (s, $\underline{C}H_2$ of Hst), 40.8 (s, $\underline{C}H_2$ of Gly), 46.4 (s, CH_2N of Pro), 64.7 (s, $\underline{C}HCO$ of Pro), 122.4 (s, C-4 of Hst), 132.1 (s, $NH\underline{C}ONH$), 132.4 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 134.7 (s, C-5 of Hst), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 137.3 (s, C-2 of Hst), 145.0 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 148.4 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 175.6 (s, Pro-$\underline{C}ONH$), 176.3 (s, $\underline{C}ONH$ of Gly). Found: C, 51.78; H, 5.77; N, 18.06. $C_{20}H_{26}N_6O_5S$ requires: C, 51.94; H, 5.67; N, 18.17%.

RESULTS AND DISCUSSION

The study of CA activators has been delayed by a controversial report from Silverman's laboratory[24] of hCA II activation by histidine, followed by its subsequent retraction,[25] and consideration that the activation initially observed was due to chelation of trace Cu(II) ions (which inhibit CA) 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,[26] and this issue of CA activation has not been thereafter investigated by other researchers. Only recently, by the report of the first X-ray crystallographic data[7,8] of adducts of histamine and phenylalanine with hCA II, has this topic received again the attention it deserves, and some types of high affinity activators have been investigated mainly by this[7–13] and Chegwidden's[27] groups.

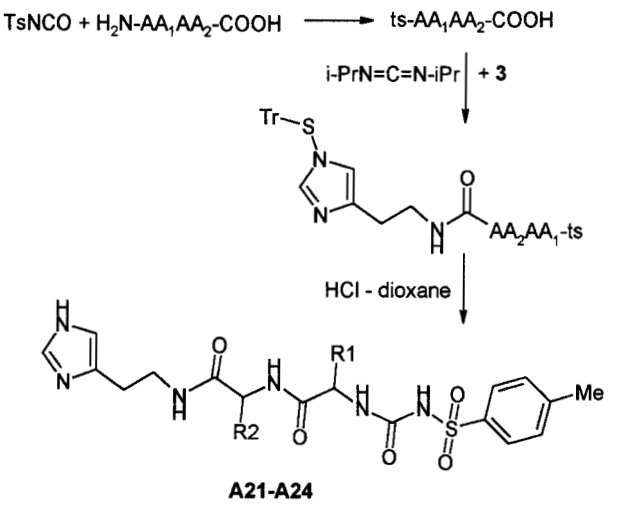
The key intermediate for obtaining novel types of activators reported in this paper, *N*-1-tritylsulfenyl histamine **3** was obtained by standard procedures, involving the initial protection of the primary amine moiety by means of phthalimide derivatives, followed by protection of the imidazole NH moiety with tritylsulfenyl chloride, and hydrazinolysis of the phthalimido moiety under mild conditions (Scheme 1). The overall yield of the three steps was good (around 80%) and the purification procedures quite simple. The approach shown here would thus indicate that the tritylsulfenyl moiety might be a good protecting group for the side chains of "difficult" amino acids such as histidine and arginine, for (solid phase) peptide synthesis (Supuran, C.T., unpublished results).[28b] Subsequent reaction of the key intermediate **3** with tosylureido-amino acid/dipeptide derivatives[15,28] in the presence of carbodiimides[29] afforded a series of *N*-tritylsulfenylated compounds **4**, which were deprotected under standard conditions (dioxane–HCl), leading to the desired



SCHEME 1 Synthesis of compounds **A1–A20**. Reagents: a – tetrabromophthalic anhydride; b – tritylsulfenyl chloride; c – 4 M HCl–dioxane.

derivatives **A1–A20**. Similarly prepared were some dipeptide derivatives of histamine, **A21–A24**, as outlined in Scheme 2. 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).

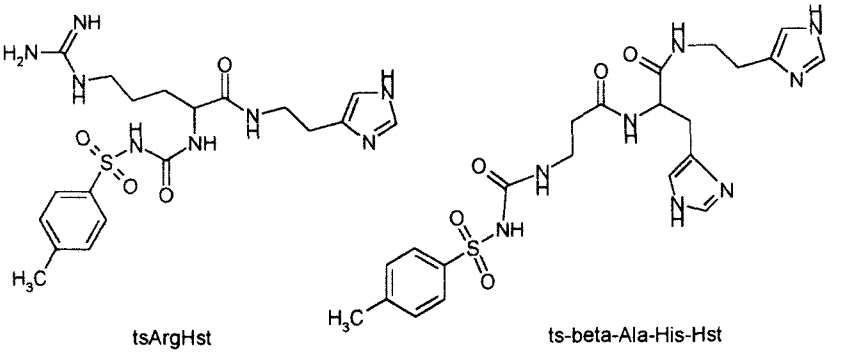
The data of Table I show significant differences between the investigated isozymes in their behavior towards both "classical" activators, such as histamine **1**, as well as the new class of activators synthesized in the present work. Thus, histamine **1** is a potent hCA I activator, and a relatively weak hCA II activator, whereas isozyme bCA IV possesses an intermediate behavior. The most interesting finding of the present study is high susceptibility of the cytosolic isozyme, hCA II to activation by some of the histamine derivatives of types **A1–A24**, as compared to the lead molecule (compounds with activation constants in the 0.02–0.06 μM were frequently obtained). Moreover, the highly abundant and most prone to activation (by histamine) isozyme hCA I was also susceptible to activation by the new derivatives reported



SCHEME 2 Synthesis of compounds **A21**–**A24**.

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 turnover 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) the slightly less active 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 which were more active than the β-Ala derivative (**A3**), which in turn was more active than the Ala or Gly derivatives (**A2** and **A1**) and, (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 1–20 nM range against hCA I and bCA IV, and 20–60 nM range against hCA II. Probably the many heteroatoms present in the tosylureido-dipeptidyl moieties confer to the obtained compounds "adhesive" properties, i.e., they are able to participate in many interactions with amino acid residues from the active site, assuring thus the formation of very stable E–A (enzyme–activator) adducts.

A special mention should also be made regarding compounds such as **A11**, **A12**, **A13**, **A21** or **A22**, which due to the fact that they possess a second moiety able to shuttle protons, in addition to the parent histamine one



tsArgHst
A11

ts-beta-Ala-His-Hst
A22

(the guanidino-; ϵ-amino- or imidazolic ring of histidine, respectively), behave as very effective CA activators against all three isozymes investigated here.

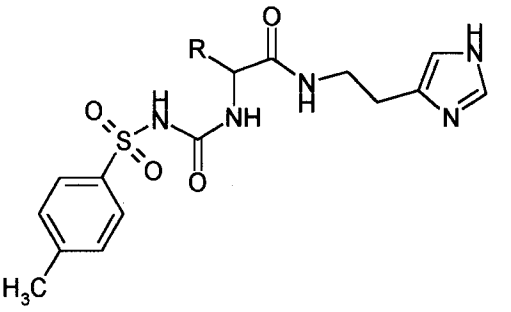
Similarly to all CA activators reported up to now, the compounds obtained in the present work presumably intervene in the catalytic cycle of the enzyme, leading to the formation of CA–activator complexes (similarly to the enzyme–inhibitor adducts, but without substitution of the metal bound solvent molecule), in which the activator bound within the active site facilitates proton transfer processes (which represent the rate-limiting step in catalysis).[7–13] The driving force of this effect might be the fact that intramolecular reactions are more rapid than intermolecular ones. Thus, in the presence of activators (symbolized as "A"), the rate-limiting step is described by equation (1) below:[7–13]

$$EZn^{2+}\text{–}OH_2 + A \Leftrightarrow [EZn^{2+}\text{–}OH_2 - A] \Leftrightarrow [EZn^{2+}\text{–}HO^- - AH^+] \Leftrightarrow EZn^{2+}\text{–}OH^- + AH^+ \quad (1)$$

enzyme–activator complexes

Obviously, compounds of the types reported here possess the imidazole moiety which can participate in the proton transfer processes between the active site and the environment (similarly to histamine **1**) but due to the presence of the tosylureido amino acyl/dipeptidyl tails also present in their molecule, they can bind more effectively to the enzyme, allowing thus for more efficient activation processes as compared to **1**. Indeed, the active site edge of all three CA isozymes investigated by us contain a high proportion of polar amino acid residues which might interfere with polar groups such as $MeC_6H_4SO_2NHCO$-amino acyl. In fact such amino acid residues might explain the different catalytic properties of the diverse isozymes, as well as

TABLE I CA isozymes I, II and IV activation with histamine **1** (Hst) the 4-tosylureido-aminoacyl derivatives ts-AA-Hst, **A1–A20**, and the 4-tosylureido-dipeptide derivatives **A21–A24**



No.	*X*	*AA/AA*$_1$*AA*$_2$	K_A* (μM)			*Yield*
			hCA I[a]	*hCA II*[a]	*bCA IV*[b]	
1	histamine	—	2	125	41	—
A1	—	Gly	0.24	12	1.9	48
A2	—	*L*-Ala	0.22	13	3.0	52
A3	—	*β*-Ala	0.20	11	1.5	67
A4	—	GABA	0.16	11	1.3	69
A5	—	GlyGly	0.15	8	1.3	71
A6	—	*L*-Val	0.18	7	5.4	49
A7	—	*L*-Leu	0.17	7	5.0	48
A8	—	*L*-Ile	0.21	9	5.0	50
A9	—	*L*-Asn	0.18	8	4.4	81
A10	—	*L*-Gln	0.14	6	2.7	89
A11	—	*L*-Arg	0.03	1.3	0.5	75
A12	—	*L*-Lys	0.05	1.5	0.4	62
A13	—	*L*-His	0.03	0.8	0.5	84
A14	—	*L*-Phg[c]	0.09	9	3.0	90
A15	—	*L*-Phe	0.08	7	2.2	87
A16	—	*L*-Trp	0.24	12	5.0	92
A17	—	*L*-Pro	0.21	10	3.0	65
A18	—	*L*-Pip[d]	0.18	10	3.0	78
A19	—	*D,L*-Nip[e]	0.18	8	3.1	75
A20	—	*D,L*-Inp[f]	0.15	7	3.2	77
A21		*L*-GlyHis	0.002	0.04	0.02	43
A22	—	*L*-*β*-AlaHis	0.001	0.02	0.008	54
A23		*L*-PhePro	0.005	0.06	0.009	55
A24		*L*-ProGly	0.007	0.05	0.01	52

A1–A20: ts-AA-Hst. **A21–A24**: ts-AA$_1$-AA$_2$-Hst.

*Mean from at least three determinations by the esterase method.[22] Standard error was in the range of 5–10%;

[a]Human cloned isozyme;

[b]Purified from bovine lung microsomes;[21] ts = 4-MeC$_6$H$_4$SO$_2$NHCO

[c]Phg = phenylglycine;

[d]Pip = pipecolic acid (piperidine-2-carboxylic acid);

[e]Nip = nipecotic acid (piperidine-3-carboxylic acid);

[f]Inp = isonipecotic acid (piperidine-4-carboxylic acid).

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)[7,8] which could easily participate in hydrogen bond formation (as well as other types of interactions) with the histamine derivatives reported here. This might explain the greater efficiency of the compounds reported in the present work in activating this isozyme, as compared to histamine, which is a relatively weak hCA II activator.

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