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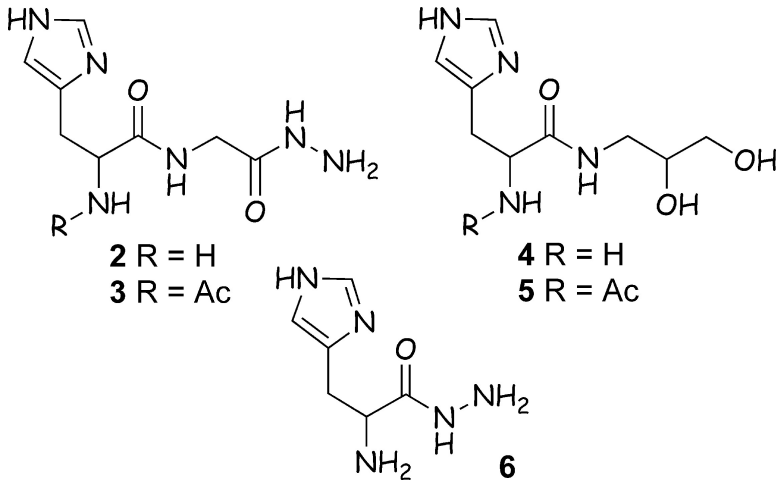

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Synthesis and Evaluation of Neuroprotective α,β-Unsaturated Aldehyde Scavenger Histidyl-Containing Analogues of Carnosine

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The synthesis, scavenging activity, and cytoprotective profiles of histidyl-containing carnosine analogues bearing hydrazide or 1,2-diol moieties is reported. Some compounds have demonstrated higher aldehyde-sequestering efficiency than carnosine and were also efficient in protecting SH-SY5Y neuroblastoma cells and rat hippocampal neurons from 4-hydroxy-*trans*-2,3-nonenal (HNE)-mediated death. The cytoprotective efficacy of these compounds suggests their potential use as therapeutic agents for disorders that involve excessive membrane lipids peroxidation and HNE-mediated neuronal toxicity.

Introduction

Reactive compounds that are byproducts of oxidative attack on membranes can amplify damage to proteins and nucleic acids and may mediate oxidative stress-induced cell death. As an example, numerous α,β-unsaturated aldehydes formed upon oxidation of unsaturated lipids, such as malondialdehyde (MDA, existing under physiological conditions as its unsaturated enol tautomer), 4-hydroxyalkenals, and 2-alkenals (e.g. acrolein and crotonaldehyde), are reactive species of particular interest for their direct or indirect influence in human pathologies.[1,2] Among these, acrolein and 4-hydroxy-*trans*-2,3-nonenal (HNE) have been extensively studied for their involvement in the pathogenesis of diseases such as atherosclerosis, diabetes, and neurodegenerative disorders.[3,4] As bifunctional electrophiles, these species readily alkylate electron-dense centers in both DNA and proteins. Furthermore, after the first reaction step, the second electrophilic center of unsaturated aldehydes may undergo further nucleophilic additions, producing intra- and intermolecular cross-linking of macromolecules.[5−7] These lipid peroxidation products appear to contribute to the etiology of a number of chronic pathologies, including neurodegenerative conditions (e.g. Alzheimer's, Parkinson's, and Huntington's diseases), chronic inflammatory diseases (e.g. rheumatoid arthritis), alcoholic liver disease, cardiovascular disorders (atherosclerosis, stroke), diabetic complications (vasculopathy, nephropathy), and cataract.[8−14]

Pharmacological efforts to attenuate oxidative injury in degenerative diseases have typically focused on drugs with antioxidant properties. Such approaches provide a "first line of defense" against free radicals but do not target secondary products of oxidative stress. A complemetary strategy involves the identification of low molecular weight drugs bearing nucleophilic centers (e.g. primary amine groups) that exhibit high reactivity toward endogenous aldehydes and, acting as "aldehyde scavengers", spare cellular constituents and slow the disease's progression. Carnosine and carnosine-related peptides are promising candidates for this new therapeutic approach. Carnosine (β-alanyl-L-hystidine **1**, Figure 1) is a dipeptide commonly present in mammalian tissues and in particular in skeletal muscle and central nervous system cells; it is responsible for a variety of activities related to the detoxification of free radical species and the byproducts of membrane lipid peroxidation,[15] but recent studies have shown that this small molecule also has membrane-protecting activity, proton buffering capacity, forms complexes with transition metals, and regulates macrophage function.[16] Despite the large literature describing carnosine activities, the research of new and more potent scavengers for HNE and other α,β-unsaturated aldehydes has been minimal such that very few carnosine analogues have been synthesized and tested. Among them *N*-acetylcarnosine, a prodrug of carnosine, is the only significant

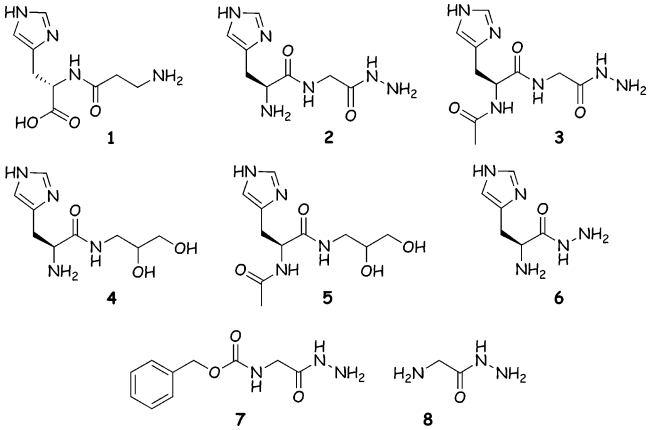

Figure 1. Tested compounds: β-alanyl-L-histidine (carnosine, **1**), L-histidylglycyl hydrazide (**2**), *N*-acetyl-L-hystidylglycyl hydrazide (**3**), 2-amino-*N*-(2,3-dihydroxypropyl)-3-(1*H*-imidazol-4-yl)propionamide (**4**), 2-acetylamino-*N*-(2,3-dihydroxypropyl)-3-(1*H*-imidazol-4-yl)propionamide (**5**), L-histidyl hydrazide (**6**), Z-L-histidyl hydrazide (**7**), glycyl hydrazide (**8**).

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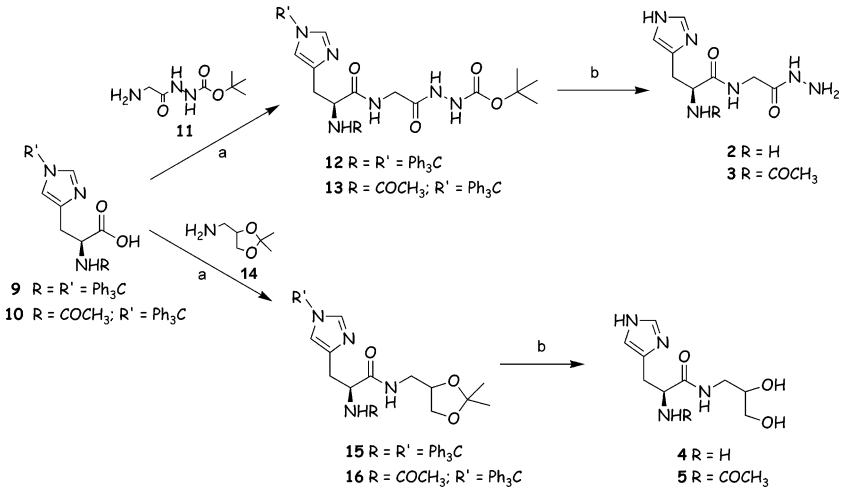
Scheme 1[a]



[a] Reagents: (a) TBTU, HOBt, diisopropylethylamine, r.t., 12 h.; (b) (1) TFA/triisopropylsilane/H_2O 95:4:1, 2 h.; (2) 3 M HCl in dioxane, r.t., 1 h.

example that has been developed and commercialized for pharmaceutical purposes (anticataract eyedrops). Other aldehyde-sequestering drugs have been recently studied by Burcham and colleagues.[17]

We report here the synthesis and the neuroprotective activity studies on new histidyl-containing molecular entities that combine the imidazole ring and the L-stereochemistry of histidine with aldehyde-reactive moieties able to increase the scavenging efficiency against α,β-unsaturated aldehydes. In particular, we considered two nucleophilic moieties known to react promptly with carbonyl species, 1,2-diols and hydrazides. 1,2-Diols are known to react with aldehydes forming very stable cyclic acetals in an acid-catalyzed reaction, driven by the excess of diol and/or by the stability of the cyclic adduct. Hydrazides are also very reactive nucleophiles, and their use in aldehyde scavenging has been applied to solid-phase organic synthesis, where resin-bound sulfonyl hydrazides are used for the removal of excess carbonyls from solutions.[18]

Results

Chemistry. To investigate the effect of modified carnosine analogues on α,β-unsaturated aldehydes we considered the series of molecules depicted in Figure 1. Compounds **2**−**5** were synthesized in our laboratories according to Scheme 1 and bear, in addition to the imidazole ring of L-histidine, an 1,2-diol or hydrazide moiety as an aldehyde-reactive center. The hydrazides **6**−**8** were commercially available.

Scavenging Activity Assay. To test the ability of the synthesized compounds to bind to α,β-unsaturated aldehydes, we incubated in sealed vials each derivative with *trans*-2-nonenal (1:20 ratio) at 40 °C in 5 mM phosphate buffer, pH 7.4. At designated intervals (0, 1, 2, 3, 4, 5, and 24 h), 40 μL aliquots were sampled and injected into an HPLC system (Phenomenex Jupiter C18 column, eluent 0.1 M phosphate buffer pH 7.4, 15% methanol, isocratic gradient, flux 1.5 mL/min). The area of peaks corresponding to each compound were integrated, and the residual amount at each time interval was calculated according to the formula:

$$\text{compound residual concentration } (\%) = (A^0/A^T) \times 100$$

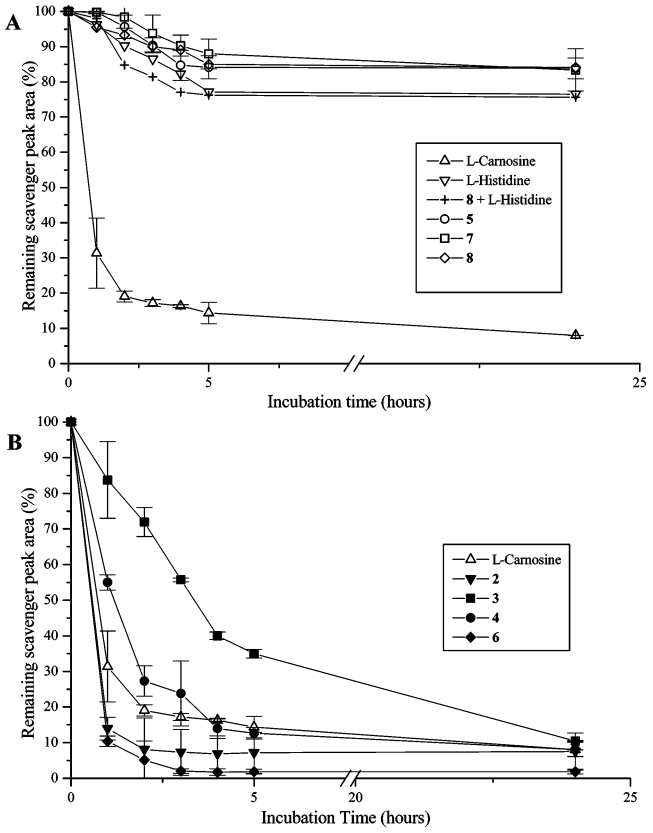


Figure 2. Changes in peak area of carnosine and carnosine analogues during 24 h of incubation of scavenger (1.4 mM) with *trans*-2-nonenal (28 mM; scavenger/aldehyde ratio = 1:20) in 0.12 M KCl, 5 mM phosphate buffer (pH 7.4) at 40 °C. Data represent means ± SD of duplicate analyses.

where A^0 is the peak area at time 0 and A^T is the peak area at each sampling time.

The results obtained were plotted in a graph (Figure 2A,B) together with the data obtained from incubation of commercially available carnosine, histidine, histidyl hydrazide, glycyl hydrazide, and Z-glycyl hydrazide.

In Vitro Toxicity Tests. Cultures of SH-SY5Y neuroblastoma cells and primary rat hippocampal neurons were exposed to increasing concentrations of compounds **2**, **4**, and **6**, and cell survival during a 24 h

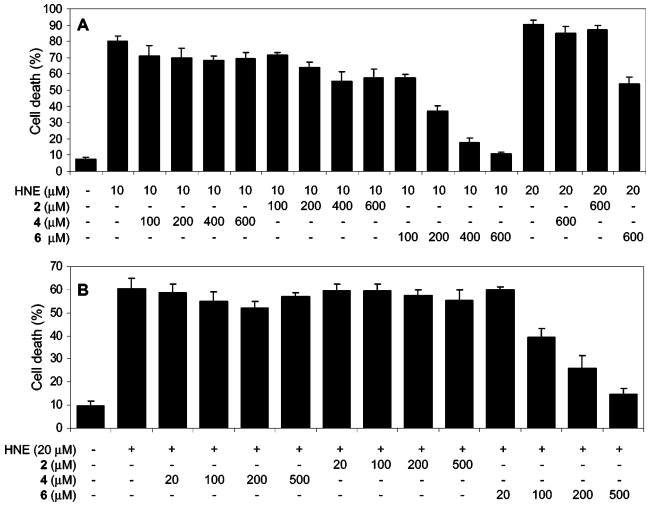


Figure 3. Compound **6** protects neural cells against HNE-induced death. Cultured SH-SY5Y cells (A) or primary hippocampal neurons (B) were pretreated for 1 h with the indicated concentrations of carnosine analogues and were then exposed for 24 h to the indicated concentrations of HNE. Cell survival was quantified and values are the mean ± SD values ($n = 3$).

exposure period was determined. The survival of SH-SY5Y cells and hippocampal neurons was not significantly affected by any of the three analogues at any of the concentrations tested (up to 1 mM).

Protection from HNE-Induced Cell Death. Cultured SH-SY5Y cells and rat hippocampal neurons were pretreated with the carnosine analogues and then exposed to HNE at concentrations of 10−20 μM. In cultures exposed to HNE alone 60−80% of the SH-SY5Y cells and hippocampal neurons died during a 24 h period of exposure (Figure 3A,B).

Compounds **2** and **4** had no significant effect on the number of neurons killed by HNE. In contrast, there was a highly significant decrease in the death of SH-SY5Y cells and hippocampal neurons in cultures pretreated with compound **6**. The cytoprotective effect of **6** was concentration-dependent in the range of 100−600 μM with 400−600 μM compound **6** affording nearly complete protection against HNE-induced cell death.

At equimolar concentrations (500 μM), **6** significantly protected hippocampal neurons against death induced by HNE, whereas carnosine and homocarnosine had no significant effect (Figure 4A). Carnosine, homocarnosine, and compound **6** did not protect hippocampal neurons from being killed by staurosporine (a kinase inhibitor that induces apoptosis) or calcium ionophore A23187 (Figure 4B,C), two insults that kill neurons by a mechanism not involving membrane lipid peroxidation and HNE production.

In a similar experiment, conducted by Teufel and coworkers with SH-SY5Y cells, carnosine (3 mM) reversed the cytotoxic effect of malondialdehyde (MDA, 3 mM) protecting 50% of control-transfected cells.[19]

Discussion

The mechanism of carnosine−α,β-unsaturated aldehyde adduct formation is based, according to the recent papers by Liu[15] and Aldini,[20] on a two-step reaction where, after initial Schiff-base formation between the aldehyde and a nucleophile (primary amine), a rapid intramolecular Michael-type addition between the histidine nitrogen and the double bond occurred. As the formation of the Schiff base is the rate-determining step, we decided to substitute the primary amine of β-alanine with different nucleophiles to produce carnosine analogues that form more stable adducts with aldehydes. We focused on 1,2-diols, which react with carbonyls yielding cyclic acetals, and hydrazides, that are among the strongest aldehyde-sequestering moieties. We also placed the histidine residue at the C-terminus instead of the N-terminus, in the attempt to avoid recognition by the specific enzyme carnosinase. In preliminary tests performed using *trans*-2-nonenal as model (scavenger/aldehyde ratio 1:20) we found that all the synthesized compounds formed stable adducts with the α,β-unsaturated aldehyde. In particular, the hydrazides **2** (H-HisGly-$NHNH_2$) and **6** (H-His-$NHNH_2$) reacted almost completely with *trans*-2-nonenal, with only 10% remaining after 1 h as compared with 30% of carnosine. The 1,2-diol derivative **4** showed a slower reactivity (55% unreacted after 1 h, 28% after 2 h) but eventually matched carnosine after 4 h. Surprisingly compounds **3** and **5** (*N*-acetyl derivatives of **2** and **4**, respectively) were far less reactive, and histidine, in agreement with the literature data, remained largely unmodified. Z-Glycyl hydrazide (**7**) and glycyl hydrazide (**8**) were also far less reactive than carnosine, as expected for compounds lacking the histidyl moiety. The results show that the combined presence of the histidine residue and the hydrazide moiety contribute to the high scavenging activity of **2** and **6**. The hydrazide group alone is not sufficient, as demonstrated by the low reactivity of the glycine derivatives **7** and **8**. Moreover, the physical

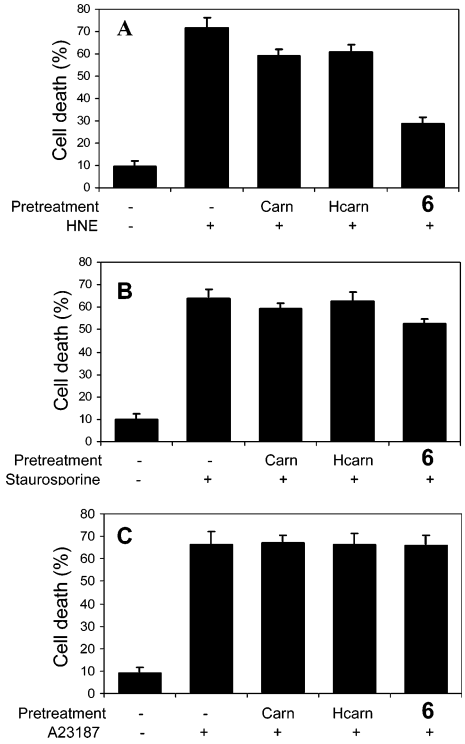


Figure 4. Compound **6** selectively protects neurons against HNE-induced death. Primary cultured rat hippocampal neurons were incubated with 500 μM of compound **6**, L-Carnosine (Carn) or homocarnosine (Hcarn) for 1 h prior to a 24 h exposure to 20 μM HNE (A), 100 nM staurosporine (B), or 0.3 μM A23187. Cell survival was quantified and values are the mean ± SD values ($n = 4$).

mixture of L-histidine and **8** behave as the glycyl hydrazide alone, indicating that the imidazole moiety does not act as catalyst in the reaction, and that the combined presence of the imidazole ring and the nucleophile moiety in the same molecule is paramount for the scavenging activity. The reason for the low reactivity of their acetylated derivatives **3** and **5** still remains unclear, but the fact that compound **8** is almost unreactive, while **6** is among the strongest scavengers of this series, clearly indicate that the imidazole nitrogen, and not the histidine primary amine, is involved in the intramolecular cyclization, after initial aldehyde reaction with the hydrazide moiety. The low reactivity of the acetyl derivatives **3** and **5** is in agreement with literature data for the N^{α}-acetylated form of carnosine: as the acetyl group can be removed in vivo by specific deacetylating enzymes, these compounds could be considered, like *N*-acetylcarnosine, as inactive prodrugs. The three compounds that showed the highest aldehyde scavenging activity (**2**, **4**, and **6**) were chosen for a series of cell culture experiments on neuroblastoma cells and primary rat hippocampal neurons, to assess their ability to protect cells from HNE toxicity. First, the intrinsic toxicities of the compounds were evaluated. The results indicated that at concentrations as high as 1 mM all the compounds were nontoxic. Cytoprotection activity tests toward SH-SY5Y cells indicated that **2** and **4** have a small cytoprotective effect in the presence of 10 and 20 μM concentrations of HNE, while **6** strongly reversed the cytotoxic activity of HNE, resulting in total cell protection at concentrations of 600 μM. Analogous results were obtained when rat hippocampal neurons were incubated with 20 μM HNE in absence and in the presence of compounds **2**, **4**, and **6**, with 500 μM compound **6** still almost completely reversing the cytotoxic effect of HNE. The poor cytoprotective activities of **2** and **4**, after the encouraging results from the *trans*-2-nonenal scavenging experiments, could be ascribed to enzymatic degradation that would release histidine and glycyl hydrazide from **2**, and histidine and 2,3-dihydroxypropylamine from **4**. Our future work will involve the synthesis of new pseudopeptide analogues of **2** and **4** to test their resistance to dipeptidases and their in vitro activity. Other experiments with known cytotoxic compounds, staurosporine and calcium ionophore A23187, showed that **6** has a slight cytoprotective effect against staurosporine, compared with carnosine and homocarnosine, but no effect against A23187. These results confirm the specific activity of the carnosine analogues, which exert their cytoprotective action by targeting membrane lipid peroxidation products such as HNE.

Compound **6** was also compared to carnosine and homocarnosine for cytoprotection of rat hippocampal neurons treated with 20 μM HNE; the superior activity of **6** compared to carnosine and homocarnosine clearly indicates this compound as a candidate for further in vivo tests in animal models of neurodegenerative disorders such as stroke, Alzheimer's, and Parkinson's diseases, where oxidative stress-induced production of HNE may be at least one cause of neuron death.[21−23]

Conclusions

The synthesis of new carnosine analogues with hydrazide or 1,2-diol moieties able to selectively bind to α,β-unsaturated aldehydes with high efficiency has been performed in our laboratories, and the compounds have been tested against *trans*-2-nonenal in preliminary activity tests. Both hydrazides **2** and **6** were more efficient than carnosine, while their acetyl derivatives **3** and **5** were far less active, suggesting a possible role as prodrugs. The efficacy of compound **6** was evidenced in two sets of experiments, showing that it is virtually nontoxic at concentrations as high as 1 mM, but has a strong cytoprotective activity toward SH-SY5Y neuroblastoma cells and rat hippocampal neurons treated with HNE. As HNE is involved in several neurodegenerative diseases, as well as in other pathologies such as cardiovascular disease and cataract, this compound will be further evaluated as a drug candidate for the scavenging of α,β-unsaturated aldehydes.

Experimental Section

Materials and Methods. Amino acids and coupling reagents were from Chem-Impex International and Novabiochem, Carnosine and homocarnosine were from Fluka, and staurosporine and A23187 were purchased from Calbiochem. All other reagents and solvents were purchased from Sigma-Aldrich, J. T. Baker, and Carlo Erba. ESI-MS spectra were acquired with a Navigator LC-MS spectrometer (ThermoQuest Finnigan) equipped with quadrupolar mass analyzer, using external standards for calibration. Samples were prepared by dissolving the compound (10^{-5} M) in acetonitrile/water 1:1 mixture with 1% acetic acid. ^{1}H NMR spectra were registered with a Bruker Advance DRX 400 spectrometer. Chemical shifts (δ) are given in parts per million (ppm) using solvent ($CDCl_3$ or DMSO-d_6) as internal standard. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated Polygram Sil G/UV 254 from Macherey-Nagel) and visualized with UV lamp (254 nm) or iodine vapors.

Synthesis of *N'*-{2-[2-(Triphenylmethylamino)-3-(1-triphenylmethyl-1*H*-imidazol-4-yl)propionylamino]-acetyl}hydrazinecarboxylic Acid *tert*-Butyl Ester [Trt-His(Trt)-Gly-NHNH-Boc] (12). Trt-His(Trt)-OH **9** (1 g, 1.56 mmol), *N'*-(2-aminoacetyl)hydrazincarboxylic acid *tert*-butyl ester **11** (0.44 g, 2.34 mmol), TBTU (0.55 g, 1,72 mmol), HOBt (0.26 g, 1.72 mmol), and DIEA (0.53 mL, 3.12 mmol) were dissolved in a mixture of chloroform (20 mL) and DMF (2 mL) under anhydrous conditions and argon atmosphere. After being stirred 24 h at room temperature, the reaction mixture was diluted with 50 mL of ethyl acetate and extracted with 14% aq $KHSO_4$ (4 × 25 mL), 5% aq $NaHCO_3$ (4 × 25 mL), and brine (50 mL). The organic phase was evaporated under vacuum yielding 1.25 g (99%) of thick oil. TLC (ethyl acetate/methanol 9:1) $R_f = 0.9$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 9H, BOC), 2.75 (s, 2H, $-CH_2$- *Gly*), 3.00 (dd, 1H, H_β His), 3.55 (t, 1H, H_α His), 3.80 (dd, 1H, H_β His), 6.35 (s, 1H, C_4 His), 7.00−7.40 (m, 30H, Trt), 7.80 (m, 2H, C_2 His + CON*H*). MS (ESI): *m/e* 811.4211 [$(M + H)^+$, calcd for $C_{51}H_{51}N_6O_4$ 811.3972].

Synthesis of 2-Amino-*N*-hydrazinocarbonylmethyl-3-(1*H*-imidazol-4-yl)propionamide [H-His-Gly-NHNH$_2$·HCl] (2). The removal of trityl and Boc protecting groups was accomplished by dissolving 100 mg (0.12 mmol) of **12** in a mixture of TFA/triisopropylsilane/H_2O 95:4:1 under stirring for 2 h. The solvent was then removed under vacuum, and the residue was diluted with 2 mL of 3.5 M HCl in dioxane and 0.5 mL of methanol. After evaporation of solvent in vacuo, the residue was dissolved in ethyl acetate and crystallized by addition of diethyl ether, yielding 25 mg (92.6%) of a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (m, 2H, H_β His), 3.90 (d, 2H, CH_2-*Gly*), 4.30 (s, 1H, H_α His), 7.50 (s, 1H, C_4 His), 8.70 (s, 2H, NH_2), 9.10 (s, 1H, C_2 His), 9.20 (s, 1H, N*H*CO), 11.25 (s, 1H, NH). MS (ESI): *m/e* 227.1318 [$(M + H)^+$, calculated for $C_8H_{15}N_6O_2$ 227.1256].

Synthesis of *N′*-{2-[2-Acetylamino-3-(1-triphenylmethyl-1*H*-imidazol-4-yl)propionylamino]acetyl}hydrazinecarboxylic Acid *tert*-Butyl Ester [Ac-His(Trt)-Gly-NHNH-Boc] (13). Ac-His(Trt)-OH **10** (0.7 g, 1.6 mmol), *N′*-(2-aminoacetyl)hydrazincarboxylic acid *tert*-butyl ester **11** (0.45 g, 2.4 mmol), TBTU (0.56 g, 1.76 mmol), HOBt (0.27 g, 1.76 mmol), and DIEA (0.54 mL, 3.2 mmol) were dissolved in a mixture of chloroform (20 mL) and DMF (2 mL) under anhydrous conditions and argon atmosphere. After being stirred 24 h at room temperature, the reaction mixture was diluted with 50 mL of ethyl acetate and extracted with 14% aq $KHSO_4$ (4 × 25 mL), 5% aq $NaHCO_3$ (4 × 25 mL), and brine (50 mL). The organic phase was evaporated under vacuum yielding 0.44 g (45%) of thick oil. TLC (ethyl acetate/methanol 9:1) $R_f = 0.4$ (single spot). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 9H, Boc), 1.90 (s, 3H, CH_3CO), 3.10 (d, 2H, CH_2-*Gly*), 3.90 (d, 1H, Hβ His), 4.08 (dd, 1H, Hβ His), 4.70 (m, 1H, Hα His), 6.70 (s, 1H, C_4 His), 7.10 (m, 6H, Trt), 7.30 (m, 9H, Trt), 7.50 (d, 1H, C_2 His), 7.70 (m, 1H, NH), 7.80 (t, 1H, NH). MS (ESI): *m/e* 611.3201 [(M + H)$^+$ calculated for $C_{34}H_{39}N_6O_5$ 611.2982].

Synthesis of 2-Acetylamino-*N*-hydrazinocarbonylmethyl-3-(1*H*-imidazol-4-yl)propionamide [Ac-His-Gly-NHNH$_2$] (3). Compound **13** (0.44 g, 0.72 mmol) was dissolved in a mixture of TFA/triisopropylsilane/H_2O 95:4:1 (3.5 mL) and kept under vigorous agitation for 2 h. The solvent was then removed under vacuum, and the residue was diluted with 10 mL of 3.5 M HCl in dioxane and 0.5 mL of methanol. After evaporation of solvent in vacuo, the residue was dissolved in ethyl acetate and crystallized by addition of diethyl ether, yielding 0.16 g (84.2%) of a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.90 (s, 3H, CH_3CO-), 2.90 (dd, 1H, Hβ His), 3.10 (dd, 1H, Hβ His), 3.80 (d, 2H, $-CH_2$- *Gly*), 4.60 (m, 1H, Hα His), 7.30 (s, 1H, C_4 His), 8.40 (d, 1H, NH), 8.50 (t, 1H, NH), 9.0 (s, 1H, C_2 His), 11,0 (s, 1H). MS (ESI): *m/e* 269.1491 [(M + H)$^+$ calculated for $C_{10}H_{17}N_6O_3$ 269.1362].

Synthesis of *N*-(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)-2-(triphenylmethylamino)-3-(1-triphenylmethyl-1*H*-imidazol-4-yl)propionamide (15). Trt-His(Trt)-OH **9** (0.85 g, 1.33 mmol), TBTU (0.47 g, 1.46 mmol), and HOBt (0.197 g, 1.76 mmol) were dissolved in 10 mL of anhydrous DMF under argon atmosphere. A solution of 0.35 mL (2.66 mmol) of 2,2-dimethyl-1,3-dioxolane-4-methanamine **14** and 0.45 mL (2.66 mmol) of DIEA in 5 mL of DMF was added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 12 h, and then it was diluted with 50 mL of ethyl acetate and extracted with 5% aq $KHSO_4$ (4 × 25 mL), 10% aq $NaHCO_3$ (4 × 25 mL), and brine (50 mL). The organic phase was dried with Na_2SO_4 and evaporated under vacuum. The resulting oil was dissolved with ethyl acetate; addition of petroleum ether induced crystallization of the title compound, which was filtered and dried yielding 0.55 g (55%) of a crystalline solid. TLC ($CHCl_3$) $R_f = 0.2$ (single spot). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.48 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 3.19 (m, 2H, NHCH_2), 3.79 (dd, 2H, Hβ His), 4.52−4.70 (m, 4H, CH_2O + Hα His + CONH), 4.90 (m, 1H, CHO), 6.54 (s, 1H, C_4 His), 7.00−7.20 (m, 31H, Trt + C_2 His). MS (ESI): *m/e* 753.3826 [(M + H)$^+$ calculated for $C_{50}H_{49}N_4O_3$ 753.3805].

Synthesis of 2-Acetylamino-*N*-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-3-(1-triphenylmethyl-1*H*-imidazol-4-yl)-propionamide (16). Ac-His(Trt)-OH **10** (1 g, 2.27 mmol), TBTU (0.8 g, 2.49 mmol), and HOBt (0.34 g, 2.49 mmol) were dissolved in 10 mL of anhydrous DMF under argon atmosphere. A solution of 0.54 mL (4.22 mmol) of 2,2-dimethyl-1,3-dioxolane-4-methanamine **14** and 0.77 mL (4.54 mmol) of DIEA in 5 mL of DMF was added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 12 h, and then it was diluted with 50 mL of ethyl acetate and extracted with 5% aq $KHSO_4$ (4 × 25 mL), 10% aq $NaHCO_3$ (4 × 25 mL), and brine (50 mL). The organic phase was dried with Na_2SO_4 and evaporated under vacuum. The resulting oil was dissolved with ethyl acetate; addition of petroleum ether induced crystallization of the title compound, which was filtered and dried yielding 0.72 g (57%) of a crystalline solid. TLC (ethyl acetate/methanol 8:2) $R_f = 0.56$ (single spot). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.45 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.10−3.25 (m, 2H, NHCH_2), 3.50 (m, 2H, Hβ His), 3.70 (m, 1H, Hα His), 4.20 (m, 2H, CH_2O), 4.85 (m, 1H, CHO), 6.80 (s, 1H, C_4 His), 7.30−7.50 (m, 16H, Trt + C_2 His). MS (ESI): *m/e* 553.2818 [(M + H)$^+$ calculated for $C_{33}H_{37}N_4O_4$ 553.2815].

Synthesis of 2-Amino-*N*-(2,3-dihydroxypropyl)-3-(1*H*-imidazol-4-yl)propionamide (4). *N*-(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)-2-(tritylamino)-3-(1-trityl-1*H*-imidazol-4-yl)propionamide (**15**) (0.50 g, 0.66 mmol) was dissolved in a mixture of TFA/triisopropylsilane/H_2O 95:4:1 (3.5 mL) and kept under vigorous agitation for 2 h. The solvent was then removed under vacuum, and the residue was diluted with 10 mL of 3.5 M HCl in dioxane and 0.5 mL of methanol. After evaporation of solvent in vacuo, the residue was dissolved in ethyl acetate and crystallized by addition of diethyl ether, yielding 0.16 g (53%) of a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.10−3.17 (m, 2H, NHCH_2), 3.50 (m, 2H, Hβ His), 3.80 (m, 1H, Hα His), 4.25 (m, 2H, CH_2OH), 4.65 (bs, 1H, CONH), 4.86 (m, 1H, CHOH), 6.75 (s, 1H, C_4 His), 7.15 (s, 1H, C_2 His). MS (ESI): *m/e* 229.1454 [(M + H)$^+$ calculated for $C_9H_{17}N_4O_3$ 229.1301].

Synthesis of 2-Acetylamino-*N*-(2,3-dihydroxypropyl)-3-(1*H*-imidazol-4-yl)propionamide (5). 16 (0.70 g, 1.26 mmol) was dissolved in a mixture of TFA/triisopropylsilane/H_2O 95:4:1 (3.5 mL) and kept under vigorous agitation for 2 h. The solvent was then removed under vacuum, and the residue was diluted with 10 mL of 3.5 M HCl in dioxane and 0.5 mL of methanol. After evaporation of solvent in vacuo, the residue was dissolved in ethyl acetate and crystallized by addition of diethyl ether, yielding 0.35 g (57%) of a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, CH_3), 3.15−3.25 (m, 2H, NHCH_2), 3.51 (m, 2H, Hβ His), 3.75 (m, 1H, Hα His), 4.05−4.35 (m, 2H, CH_2OH), 4.92 (m, 1H, CHOH), 6.85 (s, 1H, C_4 His), 7.20 (s, 1H, C_2 His). MS (ESI): *m/e* 271.1541 [(M + H)$^+$ calculated for $C_{11}H_{19}N_4O_4$ 271.1406].

Supporting Information Available: Protocols for biological assays, purity data for the synthesized compounds, and toxicity tests graphics are available free of charge via the Internet at http://pubs.acs.org.

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