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ORIGINAL ARTICLE

Aminooxy analog of histamine is an efficient inhibitor of mammalian L-histidine decarboxylase: combined in silico and experimental evidence

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Abstract Histamine plays highlighted roles in the development of many common, emergent and rare diseases. In mammals, histamine is formed by decarboxylation of L-histidine, which is catalyzed by pyridoxal-5′-phosphate (PLP) dependent histidine decarboxylase (HDC, EC 4.1.1.22). The limited availability and stability of the protein have delayed the characterization of its structure–function relationships. Our previous knowledge on mammalian HDC, derived from both in silico and experimental approaches, indicates that an effective competitive inhibitor should be capable to form an "external aldimine-like structure" and have an imidazole group, or its proper mimetic, which provides additional affinity of PLP-inhibitor adduct to the HDC active center. This is confirmed using HEK-293 cells transfected to express human HDC and the aminooxy analog of histidine, 4(5)-aminooxymethylimidazole (*O*-IMHA, $IC_{50} \approx 2 \times 10^{-7}$ M) capable to form a PLP–inhibitor complex (oxime) in the enzyme active center. Taking advantage of the availability of the human HDC X-ray structure, we have also determined the potential interactions that could stabilize this oxime in the active site of mammalian HDC.

Keywords Histidine decarboxylase · Substrate analogs · Histamine · Drug discovery

Abbreviations

AEA	Aminooxy-2-aminoethane
APA	1-Aminooxy-3-aminopropane
α-FMH	α-Fluoromethyl L-histidine
GST	Glutathione-*S*-transferase
HDC	Histidine decarboxylase
rHDC	Rat histidine decarboxylase
hHDC	Human histidine decarboxylase
HME	L-Histidine methyl ester
ODC	Ornithine decarboxylase
O-IMHA	4(5) Aminooxymethylimidazole
PLP	Pyridoxal 5′-phosphate
VS	Virtual screening
VSDMPI	Virtual screening data management platform

Introduction

Biogenic amines are small molecules mainly involved in signal transmission, so they have a broad spectrum of activities in vivo. Histamine is a key mediator in many physiological processes such as the immune response, neurotransmission, secretion of gastric acid and cell proliferation (Ohtsu and Watanabe 2003; Haas et al. 2008), as

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well as of several pathologies derived from the dysfunction of these processes. Thus, histamine plays highlighted roles in the development of inflammatory, neurological and cancerous diseases, and its probable implication in more than 20 rare diseases has also been addressed (Pino-Ángeles et al. 2012). At present, the regulation of the effects exerted by histamine is achieved using drugs modulating the activity of the four histamine G-protein-coupled receptors, namely H_1 to H_4 (Akdis and Simons 2006; Thurmond et al. 2008). However, this approach encounters certain disadvantages such as lack of specificity for an exclusive receptor or differences in binding affinities and signaling properties that eventually may lead to undesirable side effects. Besides, this strategy does not diminish the high levels of histamine produced and released during abnormal inflammatory conditions, for instance allergy, asthma or the chronic rare disease mastocytosis.

In eukaryotes histamine is formed as the result of the pyridoxal-5′-phosphate (PLP) dependent decarboxylation of L-histidine. This reaction is catalyzed by L-histidine decarboxylase (HDC, EC 4.1.1.22), which is active as a homodimer. It shares a common catalytic mechanism with other PLP-dependent decarboxylases (Fig. 1 step a–c; Olmo et al. 2002; Eliot and Kirsch 2004; Moya-García et al. 2008). The reported L-histidine K_m values range from 0.11 to 0.5 mM (Tagushi et al. 1984; Tanase et al. 1985; Mamune-Sato et al. 1990; Engel et al. 1996). As occurs in other PLP-dependent enzymes, a movement of a "flexible loop" takes place after the external aldimine formation (Fig. 1 step b) that gives rise to a closed conformation of the homodimer (Rodríguez-Caso et al. 2003a; Fleming et al. 2004a; Pino-Ángeles et al. 2010).

HDC is expressed in a few cell types including distinct immune cells, histaminergic neurons and enterochromaffin-like cells, hence its amount in a healthy organism is very low (Moya-García et al. 2005). Furthermore, overproduction of newly nascent histamine has been proven to exert deleterious effects on its producing cells, so the selection of transfected/transgenic models is difficult (Abrighach et al. 2010). Among these, mast cells are the largest producers of histamine and they store it in intracellular granules, together with other biogenic amines, and immune mediators (Ringvall et al. 2008). Mammalian HDC is an extremely labile protein due to the presence of degradation motifs in its sequence and it is a known substrate for proteasome 26S, calpain and caspase-9 (Viguera et al. 1994; Rodríguez-Agudo et al. 2000; Olmo et al. 2002;

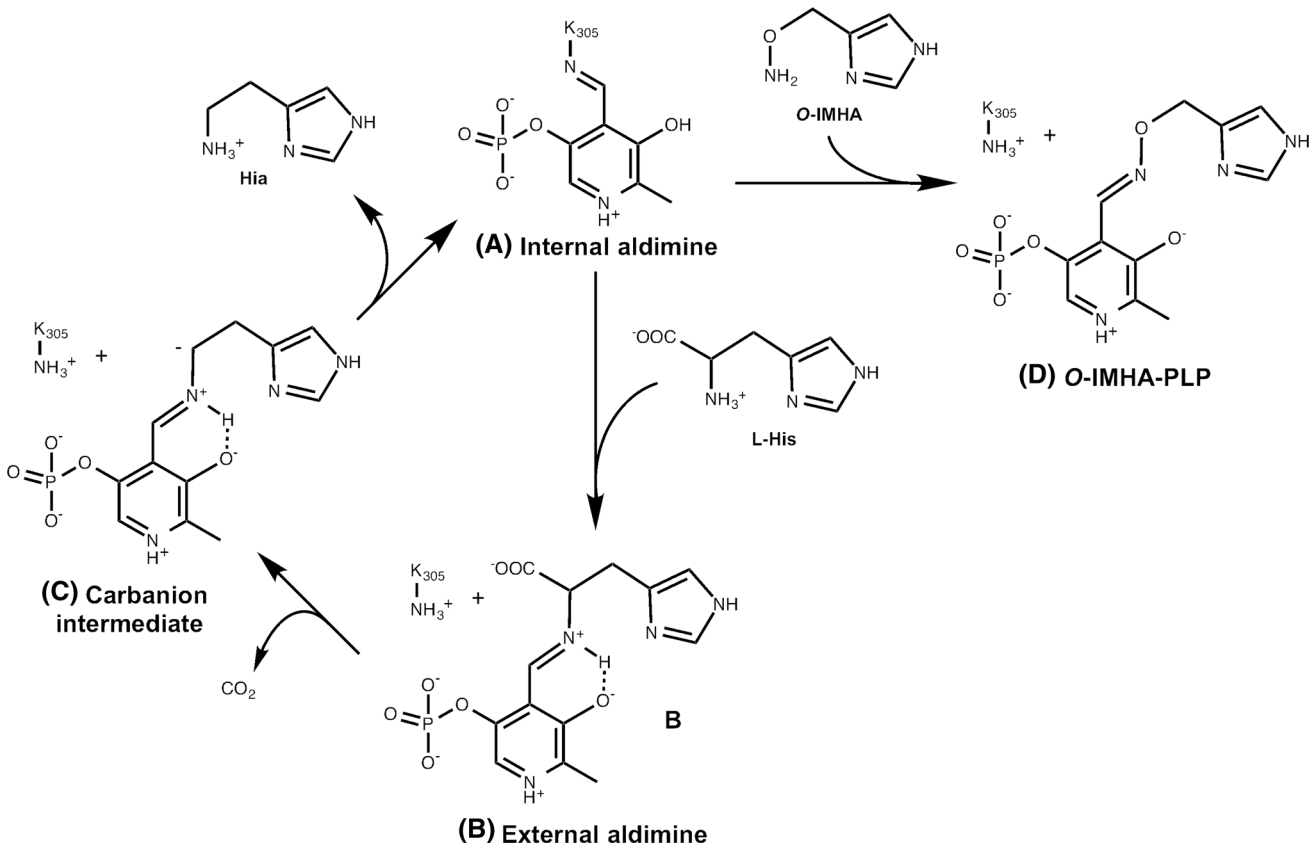


Fig. 1 Mechanism of L-histidine decarboxylation (intermediates *a–c*) and the reaction of internal aldimine with 4(5)-aminooxymethylimidazole (*O*-IMHA) to form the *O*-IMHA–PLP oxime (pathway *d*). K305 is the lysine residue forming the Schiff base with PLP

Furuta et al. 2007). Besides, the conserved flexible loop has a trypsin-like sequence motif (Matsuda et al. 2004; Pino-Ángeles et al. 2010). Therefore, experimental work with histamine-producing cell cultures is not an easy task.

Among a few known inhibitors of HDC, L-histidine methyl ester (HME) and α-fluoromethyl L-histidine (αFMH) have been extensively used for many years (DeGraw et al. 1977; Hayashi et al. 1986). HME binds in the active site and forms a Michaelis complex that cannot undergo decarboxylation, whereas αFMH is described as a suicide inhibitor (Rodríguez-Caso et al. 2003a). *N*-(5′-phosphopyridoxyl)amino acids are stable mimetics of the external aldimine and they are effective inhibitors not only of HDC, but also of other PLP-dependent enzymes (Khomutov et al. 1971; Heller et al. 1975; Wu et al. 2008; Müller et al. 2009). Epigallocatechin 3-gallate (EGCG), a major component of green tea, is also able to inhibit HDC (Rodríguez-Caso et al. 2003b; Nitta et al. 2007; Melgarejo et al. 2010); however, EGCG lacks specificity for mammalian HDC since it also inhibits the PLP-dependent aromatic amino acid decarboxylase (DDC) (Bertoldi et al. 2001; Ruiz-Perez et al. 2012).

High-throughput screening (HTS) techniques are one of the driving forces of drug discovery research. Experimental HTS approaches are enough demanding in terms of time and economical resources, but their computational counterpart, known as virtual screening (VS), has gained a lot of attention in the last decades due to the development and accessibility to more powerful calculation systems, a growing number of enzyme structures and public availability of large databases of drug-like compounds (Mitchell and Matsumoto 2011). For example, the satisfactory results of VS and lead discovery experiments on the structure of pig DDC have provided new structural guidelines for designing compounds that may be used to discover new inhibitors of this enzyme (Daidone et al. 2012).

The first structural model of rat HDC (rHDC) was obtained by comparative modeling (Rodríguez-Caso et al. 2003a) and it has been extensively evaluated to reproduce previously published experimental data (Moya-García et al. 2008). Recently, the structure of the active human histidine decarboxylase (hHDC) complexed with HME was solved by X-ray diffraction (Komori et al. 2012). These data open a possibility to undertake new structure-based drug design and VS experiments.

In the present work, by combining previous knowledge about HDC and results obtained from a VS experiment on this enzyme, we hypothesized that the formation of an external aldimine-like structure(s), being stabilized by a properly positioned imidazole group as an anchor, may result in effective inhibition of mammalian HDC. This hypothesis is validated experimentally using 4(5)-aminooxymethylimidazole (*O*-IMHA), which forms an oxime of HDC and inhibits the enzyme with $IC_{50} \approx 2 \times 10^{-7}$ M under our experimental conditions. Taking advantage of the structural information about hHDC, we have also determined favorable conformations of the *O*-IMHA–PLP oxime in the enzyme binding site.

Materials and methods

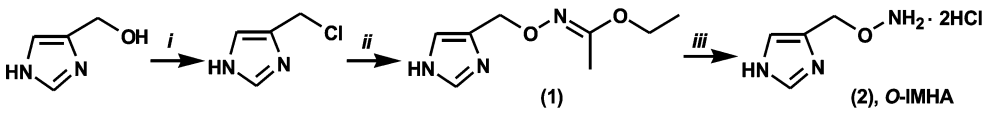
Chemistry

L-Histidine, pyridoxal-5′-phosphate (PLP), isopropylthiogalactoside (IPTG) and 4(5)-Hydroxymethylimidazole hydrochloride were purchased from Sigma-Aldrich. L-[U-^{14}C]-histidine (300 mCi/mmol) was obtained from Amersham Radiochemicals. Ethyl *N*-hydroxyacetimidate (Sigma-Aldrich) was distilled before use, bp 62 °C/12 mmHg. Ethyl alcohol (99.5 %, Merck) was refluxed over 2,4-dinitrophenyl hydrazine and distilled to get rid of acetaldehyde. 4(5)-Chloromethylimidazole hydrochloride was prepared by the known method from 4(5)-hydroxymethylimidazole hydrochloride (Ruoff and Scott 1950). 1-Aminooxy-2-aminoethane dihydrochloride (AEA) and 1-Aminooxy-3-aminopropane dihydrochloride (APA) were prepared as described earlier (Khomutov et al. 1996). (*S*)-2-azido-3-[4(5)-imidazolyl)propionic acid ("histazide", B23) and (*S*)-3-[4(5)-imidazolyl]-2-aminopropion-(2′-thiazolyl)amide ("histidine thiazolamide", E19) were kindly synthesized and provided by Dr. Francisco Sarabia (University of Málaga, Spain).

Purification of flash chromatography was performed on Kieselgel (40–63 μm, Merck), eluting with 1,4-dioxane–25 % NH_4OH, 100:1 mixture. Thin layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} plates, elution systems are indicated in the text. Compounds on TLC with free aminooxy group were detected as fluorescing oximes of PLP solution and imidazole-containing derivatives—using Pauly's reagent (Tabor 1957).

Melting points were determined in open capillary tubes with a "Manual Mel-Temp" apparatus (Barnstead International) and have not been corrected. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 500 DRX spectrometer (Germany) using tetramethylsilane (TMS) in $CDCl_3$ or sodium 3-(trimethylsilyl)-1-propanesulfonate (TSP) in D_2O as internal standards. Chemical shifts are given in ppm, and *J* values are given in Hz.

For synthesis of 4(5)-[[(l′-Ethoxyethylidene)aminooxy]methyl]-imidazole (compound 1 in Scheme 1), a solution of 0.65 g (4.25 mmol) 4(5)-chloromethylimidazole hydrochloride in EtOH (5 ml) was added dropwise with stirring at +4 °C to a solution of 0.49 g (4.75 mmol) ethyl *N*-hydroxyacetimidate in 1.0 M EtONa/EtOH (9.0 ml) and was left for 16 h at this temperature. The reaction mixture



Scheme 1 Synthesis of 4(5)-aminooxymethylimidazole (*O*-IMHA). *i* $PCl_5/CHCl_3/\Delta$; *ii* $CH_3C(OC_2H_5)$=NOH/EtONa/EtOH; *iii* HCl/H_2O. *1*: 4(5)-[[(l′-ethoxyethylidene)aminooxy]methyl]-imidazole; *2*: 4(5)-aminooxymethylimidazole dihydrochloride

was neutralized with AcOH, concentrated in vacuo, the residue was dissolved in EtOAc (10 ml) and washed subsequently with 1 M $NaHCO_3$ (2 × 3 ml), H_2O (2 ml) and brine (4 ml). Solvent was evaporated in vacuo, crude compound 1 was purified by column chromatography on a silica gel (65 g). Then, it was concentrated in vacuo and the residue was dried over P_2O_5 at 1 mmHg which afforded 0.45 g (56 %) of compound 1 as a vaxi oil. R_f 0.55 (1,4-dioxane–25 % NH_4OH, 95:5). ^{1}H NMR ($CDCl_3$): δ 7.61 (1H, s); 7.03 (1H, s); 4.90 (2H, s); 4.01 (2H, q, *J* 7.2); 1.89 (3H, s); 1.25 (3H, t, *J* 7.2). ^{13}C NMR ($CDCl_3$): δ 163.19, 135.24, 133.28, 120.76, 67.36, 62.40, 14.48, 13.89.

The histidine analog 4(5)-aminooxymethylimidazole dihydrochloride (*O*-IMHA or compound 2 (Scheme 1) was synthesized by the following procedure: A volume of 0.6 ml of 37 % HCl was added to a solution of 0.43 g (2.3 mmol) of compound 1 (Scheme 1) in aldehyde-free EtOH (5 ml). After 15 min at 20 °C, the reaction mixture was evaporated to dryness in vacuo. The residue was co-evaporated with EtOH (2 × 5 ml), treated with cold EtOH, filtered off and dried in vacuo over P_2O_5/KOH that afforded 0.28 g of crude compound 2. Then it was dissolved in H_2O (2 ml), the cloudy solution was centrifuged, supernatant was concentrated in vacuo and the residue was crystallized from EtOH to give after drying in vacuo over P_2O_5/KOH 0.19 g (45 %) of cubic crystals of target *O*-IMHA, mp 192–193 °C, dec. R_f 0.27 (1,4-dioxane–25 % NH_4OH, 9:1). ^{1}H NMR (D_2O): δ 8.78 (1H, s); 7.69 (1H, s); 5.18 (2H, s); ^{13}C NMR (D_2O): δ 138.16, 128.29, 123.24, 68.14. Calculated, %: C 25.82; H 4.88; N 22.59. $C_4H_9Cl_2N_3O$. Found, %: C 25.85; H 4.83; N 22.50.

In vitro expression and purification of recombinant hHDC

Primary translation product of mammalian HDC (74 kDa) needs to be processed by cleavage of its C-terminus to become catalytically competent (Fleming et al. 2004b). Fragment 1-512 of the hHDC primary translation product corresponds to a polypeptide able to conform one of the most active versions of mammalian HDC (Fleming et al. 2004b; Komori et al. 2012). The 1-512 hHDC fragment (1/512) was obtained by reverse transcription-PCR as described elsewhere (Abrighach et al. 2010). A recombinant construct to express a glutathione-*S*-transferase (GST)-1/512 fusion protein was generated by cloning in frame into the BamHI/EcoRI sites of the pGEX-6P1 vector (GE Healthcare). The GST-1/512 fusion sequence inserted in the recombinant vector (pGEX6P-HDC1/512) was checked by DNA sequencing (Secugen, Madrid, Spain, http://www.secugen.es). *Escherichia coli* BL21 (DE3) cells, transformed with the pGEX6P-HDC1/512 vector, were grown until reaching an OD_{600} of 0.4–0.8, and then they were induced with 0.02 mM isopropyl β-D-thiogalactoside (IPTG) for 20 h at 15 °C. The cells were lysed by sonication in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1 mM PLP, 150 mM NaCl, 5 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA). Glutathione-*S*-transferase-tagged protein was isolated by batch purification using Glutathione-Sepharose (GE Healthcare) and the GST tag was removed by means of PreScission protease (GE Healthcare), as advised by the manufacturer. Briefly, after binding GST fusion protein to Glutathione-Sepharose beads, the fusion protein–bound matrix was washed with 10 bed volumes of Cleavage Buffer (50 mM Tris–HCl pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT). For each ml of washed Glutathione-Sepharose bed volume, 40 µl (80 units) of PreScission Protease was mixed with 960 µl of Cleavage Buffer at 5 °C. The suspensions were gently resuspended and incubated at 4 °C for 15–17 h. Eluates were collected by centrifugation (500*g*, 5 min). Under our assay conditions (see below), the specific activity of the purified protein was estimated to be 50.59 ± 8.28 µmol CO_2/h mg protein.

Assay of HDC activity

HDC activity was determined by measuring the release of $^{14}CO_2$ from L-[U-^{14}C]-histidine, as reported previously (Fajardo et al. 2001). Briefly, the assay mixture contains 100 mM potassium phosphate pH 6.8, 0.2 mM DTT, 20 µM PLP (or 10 µM PLP, when indicated) and 10 mg/ml polyethylene glycol (PEG)-300. Assay mixtures were pre-incubated (10 min, room temperature) with the inhibitors at the different concentrations before adding the substrate. The final concentration of L-histidine was 40 µM. The reaction was allowed to proceed for 1 h at 37 °C in the absence (control, 100 % activity) or the presence of the histidine analogs at the concentrations indicated in the Figs. 3 and 4, and then stopped by the addition of 100 µl of 80 % (v/v) perchloric acid. Released $^{14}CO_2$ was trapped with Whatman 3MM paper folds impregnated with barium

hydroxide, which were then measured for radioactivity using a Beckman LS 6500 scintillation counter. Results are expressed as percentage of control.

Virtual screening and docking

A VS experiment was carried out using the rHDC structure and a set of approximate 200 substrate analogs in the platform VSDMIP (Gil-Redondo et al. 2009). For the docking of the *O*-IMHA–PLP oxime, we used the program Chimera 1.5.3 (Pettersen et al. 2004) to obtain the input files for both the ligand and receptor. First, the 3D structure of the *O*-IMHA–PLP was generated in the SMILES online translator service from the NIH (http://cactus.nci.nih.gov/translate). Then, hydrogen atoms were added, and AM1-BCC atomic charges were calculated. The structure of hHDC was downloaded from the Protein Data Base (PDB; ID 41EO), and the hydrogen atoms and atomic charges were added. The binding site pocket was limited by a set of 32 overlapping spheres using the program sphgen in DOCK6.5 (Moustakas et al. 2006). Afterwards, a box of side 5 Å was built around the spheres. DOCK6.5 uses a grid-based energy scoring function, so a grid was calculated in the space inside the box; the distance between grid points was set as default (0.3 Å). An anchor-and-grow docking protocol was followed to simulate ligand flexibility, so the pyridine ring and phosphate group in PLP were kept rigid (anchor); the *O*-IMHA was then oriented in the binding site adopting different conformations based on the structure of the pocket. A maximum of 3,000 orientations were calculated and the top 50 were visually inspected.

Molecular dynamic simulations

To overcome the rigidity imposed in the docking calculations, the complexes formed by hHDC and *O*-IMHA were further refined by means of molecular dynamic (MD) simulations. We used the programs in the Amber MD software package for setting up and running the simulations (Case et al. 2008). The parameters for *O*-IMHA were obtained with Antechamber and the General Amber Force Field (Wang et al. 2004). The coordinates and topology files of the protein–ligand complex were generated using the module LEaP. The complexes were solvated in a 12 Å edge truncated octahedron of TIP3P waters and neutralized with counter ions. Long-range electrostatic interactions were calculated with the Particle Mesh Ewald method (Darden et al. 1993). The simulations were carried out with the program sander, and the protocol has been described in detail in a previous publication (Pino-Ángeles et al. 2010). Briefly, energy minimization (EM) is divided in three subsequent steps: minimization of hydrogen atoms only, minimization of the water molecules and ions, and minimization of the whole system. Each EM comprised 50 steps using the steepest descent method and 950 steps using conjugate gradients algorithm. The system was heated to 300 K and equilibrated for 100 ps with Cα restraints. These restraints began with an initial value of 20 kcal/mol/Å^2 and decreased by 5 kcal/mol/Å^2 in five steps (20 ps each) to the unrestrained state. Finally, the production phase was run for 2 ns at 300 K, with the program sander and using the Amber ff03. The analysis of the trajectories was performed with the program ptraj (Case et al. 2008) and the results were visualized with both Pymol and VMD (Humphrey et al. 1996; Schrodinger 2010).

Statistical analysis

Student's unpaired samples *t* test (two tailed) was performed using GraphPad Prism version 5.0b for Mac (GraphPad Software, San Diego, CA, USA; http://www.graphpad.com).

Results and discussion

Many common, emergent and rare diseases are related to the products of both homolog L-amino acid decarboxylases, HDC and DDC, as summarized by several recent reviews (Cellini et al. 2012; Pino-Ángeles et al. 2012; Panula and Nuutinen 2013; Leggio et al. 2013). These facts highlight the necessity for new strategies for pharmacological intervention in biogenic-amine metabolism (Leggio et al. 2013; Sánchez-Jiménez et al. 2013). For many years, a lot of efforts were made in the discovery and optimization of specific ligands for biogenic-amine receptors. Nevertheless, this strategy has found some difficulties, as those derived from the structural and functional similarities among receptors and their ability to form dimers or higher order oligomers, which may alter ligand binding affinity and signaling properties (Ellenbroek 2013). Consequently, the discovery of new modulators of the synthesis of these amines is proposed as a potential cooperative strategy against the wide diversity of biogenic-amine related diseases (neurologic, inflammatory, neuroinflammatory and gastrointestinal diseases, alterations of bone turnover and fertility, and different cancer types) (Wu et al. 2011; Cellini et al. 2012).

Previous structural studies demonstrated that HDC has a more restricted substrate specificity than its homolog DDC (Olmo et al. 2002; Ruiz-Perez et al. 2012; Daidone et al. 2012, and references within). These observations are in agreement with the results obtained in a VS experiment with about 200 L-histidine analogs. The estimated binding energies for these compounds decrease when there is a chemical substitution in the amino group, and abruptly

increase when the imidazole ring is removed (results not shown). In this work, we have tested the activity of two promising candidates of our VS, histazide (B23) and histidine thiazolamide (E19) (Fig. 2a). However, none of them was able to inhibit hHDC at concentrations in the order of 10^{-5} M (Fig. 3a). Competitive inhibitors induce an increase in Michaelis–Menten constant (K_m) but not in maximum velocity (V_{max}). Therefore, the inhibition caused by a histidine analog should be better detected at concentrations $\leq K_m$ value, as it was our case (see "Materials and methods" section). Consequently, our experimental results do not support their being effective hHDC inhibitors.

The results of VS showed that chemical modification of the imidazole ring resulted in increased binding energies. Hence, non-substituted imidazole group may be essential for productive binding. This observation is in agreement with previous experimental evidence on testing transition state analogs mimicking external aldimine, as mammalian HDC inhibitors (Wu et al. 2008, 2011).

Another approach to mimic the external aldimine is to use substrate/product-like *O*-substituted hydroxylamines that will react with the internal aldimine to form an oxime (PLP-inhibitor) at the enzyme active site. All efficient and specific (IC_{50} 10^{-6}–10^{-9} M) hydroxylamine-containing inhibitors of PLP-dependent ornithine decarboxylase (ODC; Khomutov et al. 1985; Poulin et al. 1989; Stanek et al. 1992; Milovica et al. 2001; Das Gupta et al. 2005; Singh et al. 2007); L-glutamic acid decarboxylase (GAD; Sashchenko et al. 1968); aspartate aminotransferase (Delbaere et al. 1989); 1-aminocyclopropane-1-carboxylate synthase (Capitani et al. 2003); and γ-cystathionase (Gabibov et al. 1996) are structurally similar either to the enzyme substrate or resemble the product of the enzymic reaction. Such a similarity ensures high affinity of the inhibitor to the substrate binding site, the side chain of the

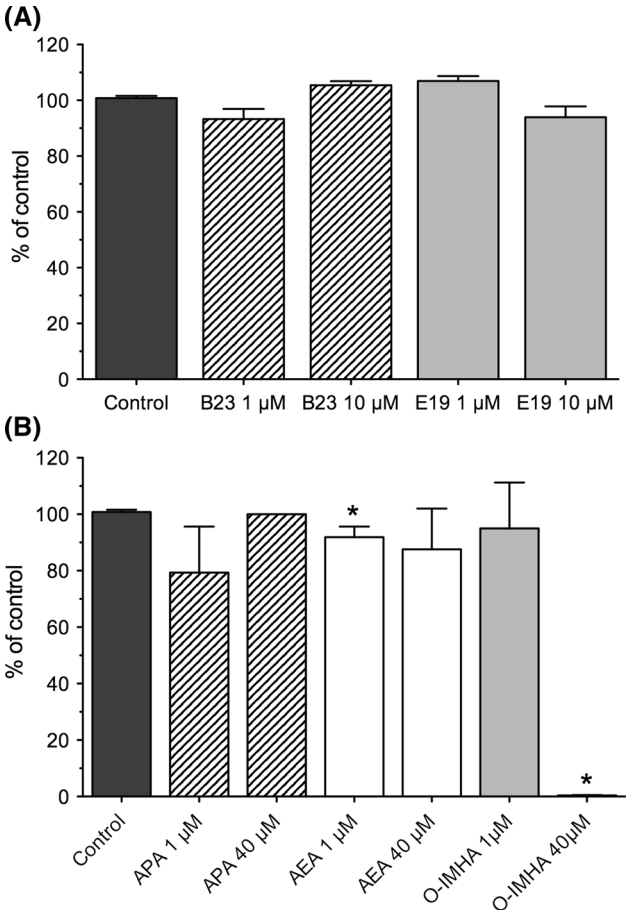


Fig. 3 Effect of substrate analogs on hHDC activity. Results are normalized with the HDC activity in the absence of substrate analogs (control) and represent mean ± SEM of at least three independent experiments. **a** Effect of "histazide", B23 (*hatched bars*) and "histidine thiazolamide", E19 (*grey bars*) at 1 and 10 µM. **b** Effect of the aminooxy derivatives: APA (*hatched bars*), AEA (*white bars*), and *O*-IMHA (*grey bars*) at 1 and 40 µM. $^*P < 0.01$ compared with control by Student's unpaired sample *t* test (two tailed)

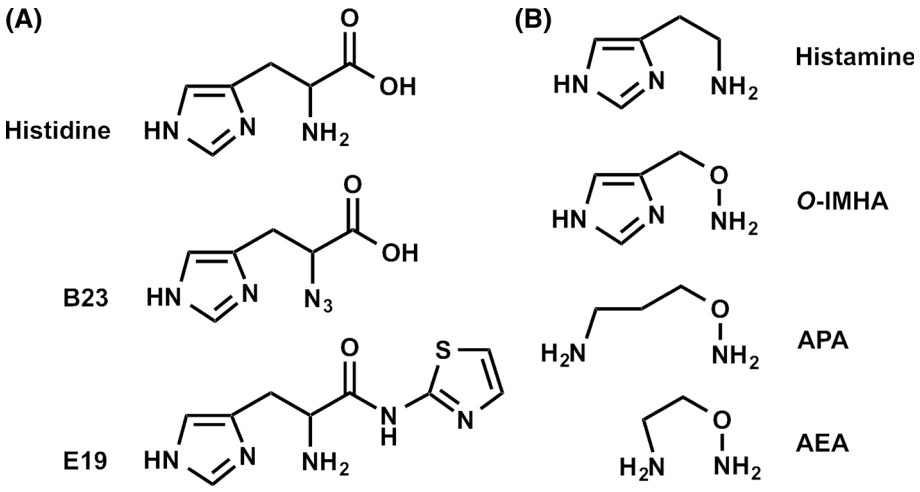


Fig. 2 Chemical structure of the substrate analogs used. **a** Derivates of L-histidine: (*S*)-2-azido-3-[4(5)-imidazolyl) propionic acid ("histazide", B23) and (*S*)-3-[4(5)-imidazolyl]-2-aminopropion-(2′-thiazolyl)amide ("histidine thiazolamide", E19) and **b** aminooxy analogs of histamine: 1-aminooxy-2-aminoethane (AEA), 1-aminooxy-3-aminopropane (APA), and 4(5)-aminooxymethylimidazole (*O*-IMHA)

inhibitor plays anchoring functions, while the properly positioned aminooxy group forms the oxime with PLP. Structural similarity of these oximes to the external aldimine was confirmed with the X-ray studies of the enzyme–inhibitor complexes of aspartate aminotransferase (Markovic-Housley et al. 1996), gamma-aminobutyric acid transaminase (Liu et al. 2004), and also 1-aminocyclopropane-1-carboxylate synthase (Capitani et al. 2003). In the case of the ODC–1-aminooxy-3-aminopropane (APA) complex, the proper binding of the inhibitor to the active center of the enzyme promotes both the cleavage of the C=N-double bond of the internal aldimine and the rotation of the pyridine ring plane. However, the aminooxy group of APA remains "frozen" at the distance of ~3 Å from the carbonyl group of PLP (Dufe et al. 2007).

Therefore, to obtain an effective inhibitor of HDC, earlier unknown 4(5)-aminooxymethylimidazole (*O*-IMHA) was synthesized. For comparison, we used 1-aminooxy-3-aminopropane (APA), and 1-aminooxy-2-aminoethane (AEA). These two latter compounds could be considered as aminooxy-analogs of histamine but lacking the imidazole group at different extents (Fig. 2b).

Among known methods for the preparation of functionally substituted hydroxylamine esters (Zeeh and Metzger 1971), in the present work we used ethyl *N*-hydroxyacetimidate as *N*-protected hydroxylamine derivative to synthesize 4(5)-aminooxymethylimidazole (*O*-IMHA). This hydroxylamine derivative is easily alkylated with alkyl halides producing high yields. Besides, the ethoxyethylidene protection group can be removed with practically quantitative yield by treating with equimolar amounts of strong acid and water for a few minutes at room temperature (Khomutov et al. 1996, and references within), that allows us to deprotect the aminooxy group, even in the presence of *N*-Boc-protected amino groups (Simonian et al. 2006). Recently, we demonstrated that functionally substituted methanesulfonates efficiently alkylate ethyl *N*-hydroxyacetimidate (Khomutov et al. 2010). In some cases, this method may be considered as a good alternative to the preparation of *O*-substituted hydroxylamines from alcohols using *N*-hydroxyphthalimide in the Mitsunobu reaction (Maillard et al. 2005). Nevertheless, to prepare target 4(5)-aminooxymethylimidazole (*O*-IMHA) we used alkylation of ethyl *N*-hydroxyacetimidate with 4(5)-chloromethylimidazole, which was synthesized from commercially available 4(5)-hydroxymethylimidazole (Scheme 1). Intermediate ethoxyethylidene derivative (compound 1 in Scheme 1) was purified by flash chromatography on silica gel and subsequent deprotection of aminooxy group afforded target *O*-IMHA dihydrochloride with 18 % overall yield, as calculated for starting 4(5)-hydroxymethylimidazole.

As depicted in Fig. 1 (step d), the *O*-IMHA oxime is similar to the external aldimine—first intermediate of L-histidine decarboxylation—of HDC. *O*-IMHA was the only aminooxy compound that inhibited (by more than 99 %) the enzyme activity when it was assayed at 40 μM; i.e., at the same concentration as L-histidine in the assay mixture. On the contrary, no significant decrease in the hHDC activity was detected under the same conditions either with APA or AEA (Fig. 3b). APA and AEA may be formally considered as "open" analogs of *O*-IMHA, since the distances between the aminooxy group of *O*-IMHA and the nitrogen atoms in the imidazole ring are about the same as between the aminooxy group of APA/AEA and their terminal amino groups. As depicted in Fig. 4, *O*-IMHA at 10 μM concentration is able to reduce hHDC activity by more than 80 % at any of the assayed PLP concentrations. The imidazole group of L-histidine has p*K*a 6.0 and anchoring of the substrate in the binding site is achieved by a system of hydrogen bonds and hydrophobic interactions (Moya-García et al. 2008), which are also established as in the case of *O*-IMHA. However, protonated amino groups of APA/AEA cannot participate in such types of interactions and this explains the great differences in the activities of *O*-IMHA and APA/AEA.

The efficiency of *O*-IMHA inhibition depends on the concentration of free PLP in the reaction mixture. The IC_{50} value decreases by one order of magnitude (from ≈2 to 0.2 μM) when the cofactor concentration is reduced by half (Fig. 4). This is unlikely to occur due to the competitive reaction of the inhibitor with the free coenzyme. The carbonyl group of PLP forms an internal aldimine with the ε-amino group of a conserved lysine in the HDC active center (Fig. 1a), and it is known that the reactivity of the protonated Schiff base toward semicarbazides, hydrazines and *O*-substituted hydroxylamines is much higher when

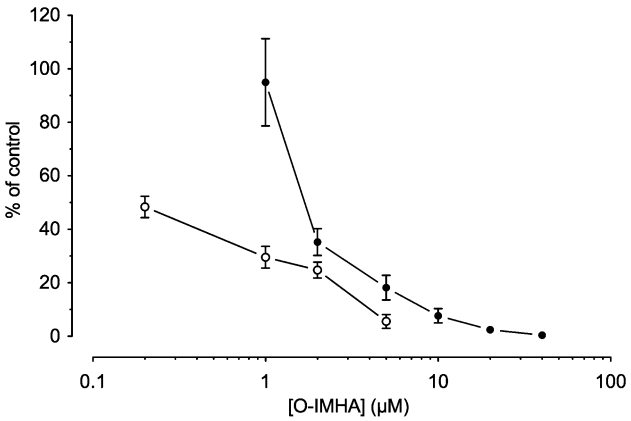


Fig. 4 Inhibition of hHDC with 4(5)-aminooxymethylimidazole (*O*-IMHA) as a function of PLP concentration in the reaction mixture. Final PLP concentration in the assay was 20 μM (*filled circles*) or 10 μM PLP (*open circles*). Results are normalized with respect to the HDC activity in the absence of *O*-IMHA (control) and represent mean ± SEM of at least three independent experiments

compared to the reactivity of the free carbonyl group of PLP (Cordes and Jencks 1962a, b; Dirksen and Dawson 2008). The difference in the inhibitory potency of *O*-IMHA at 10 and 20 μM of PLP is more likely to be related to the reversible character of binding of *O*-IMHA–PLP oxime to *apo*-HDC. This gives rise to an equilibrium, which is shifted to catalytically active *holo*-HDC at higher PLP concentrations. Therefore, *O*-IMHA can be considered as a pseudoirreversible inhibitor.

We have studied the interactions and stability of two putative conformations of the *O*-IMHA–PLP complex. In the X-ray structure of hHDC, HME binds to PLP through its *si* face. This orientation brings the imidazole ring of HME to the vicinity of S354b (in the opposite monomer), which seems to play a key role in regulating of the substrate specificity of hHDC (Komori et al. 2012). In a previous theoretical study carried out on the model of rHDC, we concluded that the decarboxylation step occurs when L-histidine binds to the cofactor through its *re* face (Moya-García et al. 2008).

To check the feasibility of these two different configurations of the *O*-IMHA–PLP oxime, we have chosen two complexes out of our docking results, each of them resembling these two different orientations of the ligand toward the cofactor, and we have studied their stability by means of MD simulations. Conformation A (Fig. 5a) resembles the one adopted by HME in the X-ray structure of hHDC, so the imidazole ring is oriented toward the *si* face of PLP. The phosphate group of PLP is involved in a hydrogen bonding network with the residues T149, S151, N302 and S354b, which remains stable throughout the whole simulation time. This interacting network is quite similar to that observed in the hHDC crystal structure. Nitrogen atom of imidazole ring of *O*-IMHA can form a stable hydrogen bond with S304. The key residue for ligand specificity, S354b, locates within van der Waals distance of the side chain of *O*-IMHA, as it occurs for the imidazole ring of HME according to hHDC X-ray structure. On the contrary, in conformation B (Fig. 5b), the orientation of the side chain of the ligand points toward the *re* face of PLP, resembling the binding mode of L-histidine in the rHDC model. The phosphate group of PLP is also stabilized by multiple hydrogen bonds with V150, S151 and S354b. The imidazole ring may form a hydrogen bond with R447; however, due to its orientation, it is located more than 9 Å apart from S354b. We have not observed the existence of a direct hydrogen bond between nitrogen atom in the pyridine ring of PLP and D273 in any of the two conformations, although this interaction has been reported in the crystal structure of hHDC. Nevertheless, this bond is not completely stable and it even disappears through time, as observed in a short MD simulation of hHDC X-ray structure in complex with HME (data not shown). The results indicate that in both conformations, *O*-IMHA makes stable interactions with several residues in the active site. Nevertheless, in conformation A, the imidazole ring makes a stable interaction with S354b, which has been pointed out as a key residue for substrate specificity in hHDC (Komori et al. 2012). Hence, our results suggest that *O*-IMHA could adopt this conformation.

The precise position of the native substrates in both mammalian HDC and DDC is still not completely clear. For both of them, it has been deduced that the most probable conformation of the substrate would be those in which their side chains locate toward the *re* face of the cofactor (Matsuda et al. 2004; Moya-García et al. 2008). However, the crystal structures of both enzymes showed substrate analogs to bind PLP through its *si* face (Burkhard et al. 2001; Komori et al. 2012). Further research efforts will be necessary to clarify the precise conformation of the natural substrate in the active site of these enzymes and its differences with those of substrate analogs in case they exist.

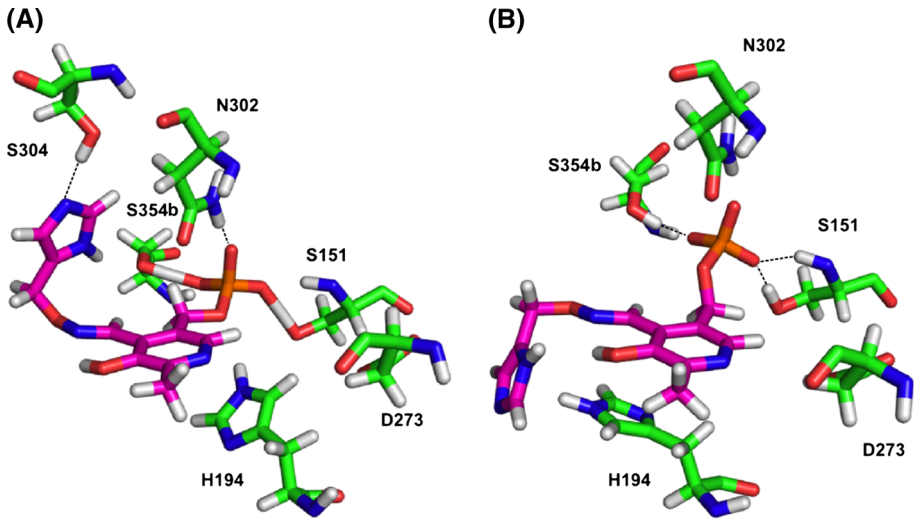


Fig. 5 Average structures of the two conformations of the *O*-IMHA–PLP in complex with hHDC. **a** Conformation resembling HME-PLP in the hHDC crystal structure. **b** Conformation resembling His-PLP in the rHDC model. Both external aldimines are depicted in *pink sticks*, whereas the amino acids in the active site of hHDC are depicted in *green sticks*. *Dashed lines* indicate probable hydrogen bonds between the oxime and the protein (color figure online)

PLP-dependent enzymes perform a lot of different chemical transformations of amino acids under a common catalytic mechanism, based on the versatility of their cofactor. Substrate analogs and mechanism-based intermediates are an important source of inhibitors and modulators of the activity of these enzymes (Eliot and Kirsch 2004). The potent inhibitor of HDC αFMH was reported over 30 years ago; besides, most of the current efforts to regulate the effects of histamine have not been associated with the inhibition of HDC, but to the modulation of its membrane receptors. Recently, the inhibitory potential of external aldimine mimetics has been proposed, and a promising inhibitor was found from an initial set of nine derivatives (Wu et al. 2008, 2011). We used high-throughput VS to find novel HDC inhibitors among the ZINC database and a smaller library of substrate analogs, using our validated model of rHDC (Moya-García et al. 2009). From VS experiments, the importance of the imidazole ring in the analog structure was outlined, as indicated by the huge penalty in the energy binding values when it was modified. After negative experimental results with amino group-substituted analogs, we substituted the amino group by its surrogate (aminooxy group) (Fig. 2) being capable to form stable PLP–analog complexes. The validity of this hypothesis was tested with a series of aminooxy derivatives and proved as correct by results obtained with *O*-IMHA, which forms a stable oxime, has an imidazole moiety and inhibited hHDC at submicromolar concentrations. The *O*-IMHA–PLP oxime seems to be stabilized by a net of hydrogen bonds. In addition, despite the structural similarity between histamine and *O*-IMHA, it is very unlikely that *O*-IMHA would effectively bind to histamine receptors since the p*K*a of the aminooxy group of *O*-IMHA is only about 4.5 and the receptors have high complementarily to protonated histamine. Therefore, *O*-IMHA may be considered as an effective and specific inhibitor of HDC that could be the initial step (a lead compound) for the development of a new type of mammalian HDC modulators with interest in Biomedicine, taking account of the multiple human diseases related to histamine.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Abrighach H, Fajardo I, Sánchez-Jiménez F, Urdiales JL (2010) Exploring polyamine regulation by nascent histamine in a human-transfected cell model. Amino Acids 38:561–573

Akdis CA, Simons FE (2006) Histamine receptors are hot in immunopharmacology. Eur J Pharmacol 533:69–76

Bertoldi M, Gonsalvi M, Voltattorni CB (2001) Green tea polyphenols: novel irreversible inhibitors of dopa decarboxylase. Biochem Biophys Res Commun 284:90–93

Burkhard P, Dominici P, Borri-Voltattorni C, Jansonius JN, Malashkevich VN (2001) Structural insight into Parkinson's disease treatment from drug-inhibited DOPA decarboxylase. Nat Struct Biol 8:963–967

Capitani G, Eliot AC, Gut H, Khomutov RM, Kirsch JF, Grutter MG (2003) Structure of 1-aminocyclopropane-1-carboxylate synthase in complex with an amino-oxy analogue of the substrate: implications for substrate binding. Biochim Biophys Acta 1647:55–60

Case DA, Darden TA, Cheatham TE, Simmerling CL, Wang J et al (2008) AMBER 10. University of California, San Francisco

Cellini B, Montioli R, Oppici E, Voltattorni CB (2012) Biochemical and computational approaches to improve the clinical treatment of dopa decarboxylase-related diseases: an overview. Open Biochem J 6:131–138

Cordes EH, Jencks WP (1962a) Nucleophilic catalysis of semicarbazone formation by anilines. J Am Chem Soc 84:826–831

Cordes EH, Jencks WP (1962b) Semicarbazone formation from pyridoxal, pyridoxal phosphate, and their Schiff bases. Biochemistry 1:773–778

Daidone F, Montioli R, Paiardini A, Cellini B, Macchiarulo A, Giardina G, Bossa F, Borri Voltattorni C (2012) Identification by virtual screening and in vitro testing of human dopa decarboxylase inhibitors. PLoS One 7:e31610

Darden T, York D, Pedersen L (1993) Particle mesh Ewald: an N [center-dot] log(N) method for Ewald sums in large systems. J Chem Phys 98:10089–10092

Das Gupta R, Krause-Ihle T, Bergmann B, Muller IB, Khomutov AR, Muller S, Walter RD, Luersen K (2005) 3-Aminooxy-1-aminopropane and derivatives have an antiproliferative effect on cultured *Plasmodium falciparum* by decreasing intracellular polyamine concentrations. Antimicrob Agents Chemother 49:2857–2864

DeGraw JI, Engstrom J, Ellis M, Johnson HL (1977) Potential histidine decarboxylase inhibitors 1. Alpha- and beta-substituted histidine analogues. J Med Chem 20:1671–1674

Delbaere LT, Kallen J, Markovic-Housley Z, Khomutov AR, Khomutov RM, Karpeisky MY, Jansonius JN (1989) Complexes of aspartate aminotransferase with hydroxylamine derivatives: spectral studies in solution and in the crystalline state. Biochimie 71:449–459

Dirksen A, Dawson PE (2008) Rapid oxime and hydrazone ligations with aromatic aldehydes for biomolecular labeling. Bioconj Chem 19:2543–2548

Dufe VT, Ingner D, Heby O, Khomutov AR, Persson L, Al-Karadaghi S (2007) A structural insight into the inhibition of human and *Leishmania donovani* ornithine decarboxylases by 1-amino-oxy-3-aminopropane. Biochem J 405:261–268

Eliot AC, Kirsch JF (2004) Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu Rev Biochem 73:383–415

Ellenbroek BA (2013) Histamine H3 receptors. The complex interaction with dopamine and its implications for addiction. Br J Pharmacol. doi:10.1111/bph.12221 (in press)

Engel N, Olmo MT, Coleman CS, Medina MA, Pegg AE, Sánchez-Jiménez F (1996) Experimental evidence for structure-activity features in common between mammalian histidine decarboxylase and ornithine decarboxylase. Biochem J 320:365–368

Fajardo I, Urdiales JL, Medina MA, Sánchez-Jiménez F (2001) Effects of phorbol ester and dexamethasone treatment on histidine decarboxylase and ornithine decarboxylase in basophilic cells. Biochem Pharmacol 61:1101–1106

Fleming JV, Sánchez-Jiménez F, Moya-García AA, Langlois MR, Wang TC (2004a) Mapping of catalytically important residues in the rat L-histidine decarboxylase enzyme using bioinformatic and site-directed mutagenesis approaches. Biochem J 379:253–261

Fleming JV, Fajardo I, Langlois MR, Sánchez-Jiménez F, Wang TC (2004b) The carboxyl-terminus of rat L-histidine decarboxylase (HDC) specifically inhibits enzymatic activity and disrupts PLP-dependent interactions with L-histidine substrate analogues. Biochem J 381:769–778

Furuta K, Nakayama K, Sugimoto Y, Ichikawa A, Tanaka S (2007) Activation of histidine decarboxylase through post-translational cleavage by caspase-9 in a mouse mastocytoma P-815. J Biol Chem 282:13438–13446

Gabibov AG, Shuster AM, Khomutov AR, Kostina MB, Khurs EN, Goriachenkova EV, Khomutov RM (1996) Cystathionase: catalytic activity of products of expression of cDNA fragments. Specific inhibition of native enzyme and fusion-protein by substrate-like *O*-substituted hydroxylamine. Dokl Akad Nauk 350:405–407

Gil-Redondo R, Estrada J, Morreale A, Herranz F, Sancho J, Ortiz AR (2009) VSDMIP: virtual screening data management on an integrated platform. J Comput Aided Mol Des 23:171–184

Haas HL, Sergeeva OA, Selbach O (2008) Histamine in the nervous system. Physiol Rev 88:1183–1241

Hayashi H, Tanase S, Snell EE (1986) Pyridoxal 5′-phosphate-dependent histidine decarboxylase. Inactivation by alpha-fluoromethylhistidine and comparative sequences at the inhibitor- and coenzyme-binding sites. J Biol Chem 261:11003–11009

Heller JS, Canellakis ES, Bussolotti DL, Coward JK (1975) Stable multisubstrate adducts as enzyme inhibitors. Potent inhibition of ornithine decarboxylase by *N*-(5′-phosphopyridoxyl)-ornithine. Biochim Biophys Acta 403:197–207

Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14:33–38

Khomutov RM, Dixon HB, Vdovina LV, Kirpichnikov MP, Morozov YV, Severin ES, Khurs EN (1971) *N*-(5′-phosphopyridoxyl)glutamic acid and *N*-(5′-phosphopyridoxyl)-2-oxopyrrolidine-5-carboxylic acid and their action on the apoenzyme of aspartate aminotransferase. Biochem J 124:99–106

Khomutov RM, Denisova GF, Khomutov AR, Belostotskaia KM, Shlosman RB (1985) Aminooxypropylamine—an effective inhibitor of ornithine decarboxylase in vitro and in vivo. Bioorg Khim 11:1574–1576

Khomutov AR, Vepsäläinen JJ, Shvetsov AS, Hyvönen T, Keinänen TA, Pustobaev VN, Eloranta TO, Khomutov RM (1996) Synthesis of hydroxylamine analogues of polyamines. Tetrahedron 52:13751–13766

Khomutov MA, Mandal S, Weisell J, Saxena N, Simonian AR, Vepsalainen J, Madhubala R, Kochetkov SN (2010) Novel convenient synthesis of biologically active esters of hydroxylamine. Amino Acids 38:509–517

Komori H, Nitta Y, Ueno H, Higuchi Y (2012) Structural study reveals that Ser-354 determines substrate specificity on human histidine decarboxylase. J Biol Chem 287:29175–29183

Leggio GM, Salomone S, Bucolo C, Platania C, Micale V, Caraci F, Drago F (2013) Dopamine D_3 receptor as a new pharmacological target for the treatment of depression. Eur J Pharmacol. doi:10.1016/j.ejphar.2013.07.022 (in press)

Liu W, Peterson PE, Carter RJ, Zhou X, Langston JA, Fisher AJ, Toney MD (2004) Crystal structures of unbound and aminooxyacetate-bound *Escherichia coli* gamma-aminobutyrate aminotransferase. Biochemistry 43:10896–10905

Maillard LT, Benohoud M, Durand P, Badet B (2005) A new supported reagent for the parallel synthesis of primary and secondary *O*-alkyl hydroxylamines through a base-catalyzed Mitsunobu reaction. J Org Chem 70:6303–6312

Mamune-Sato R, Tanno Y, Maeyama K, Miura Y, Takishima T, Kishi K, Fukuda T, Watanabe T (1990) Histidine decarboxylase in human basophilic leukemia (KU-812-F) cells. Characterization and induction by phorbol myristate acetate. Biochem Pharmacol 40:1125–1129

Markovic-Housley Z, Schirmer T, Hohenester E, Khomutov AR, Khomutov RM, Karpeisky MY, Sandmeier E, Christen P, Jansonius JN (1996) Crystal structures and solution studies of oxime adducts of mitochondrial aspartate aminotransferase. Eur J Biochem 236:1025–1032

Matsuda N, Hayashi H, Miyatake S, Kuroiwa T, Kagamiyama H (2004) Instability of the apo form of aromatic L-amino acid decarboxylase in vivo and in vitro: implications for the involvement of the flexible loop that covers the active site. J Biochem 135:33–42

Melgarejo E, Medina MA, Sánchez-Jiménez F, Urdiales JL (2010) Targeting of histamine producing cells by EGCG: a green dart against inflammation? J Physiol Biochem 66:265–270

Milovica V, Turchanowa L, Khomutov AR, Khomutov RM, Caspary WF, Stein J (2001) Hydroxylamine-containing inhibitors of polyamine biosynthesis and impairment of colon cancer cell growth. Biochem Pharmacol 61:199–206

Mitchell W, Matsumoto S (2011) Large-scale integrated supercomputing platform for next generation virtual drug discovery. Curr Opin Chem Biol 15:553–559

Moustakas DT, Lang PT, Pegg S, Pettersen E, Kuntz ID, Brooijmans N, Rizzo RC (2006) Development and validation of a modular, extensible docking program: DOCK 5. J Comput Aided Mol Des 20:601–619

Moya-García AA, Medina MA, Sánchez-Jiménez F (2005) Mammalian histidine decarboxylase: from structure to function. BioEssays 27:57–63

Moya-García AA, Ruiz-Pernia J, Marti S, Sánchez-Jiménez F, Tunon I (2008) Analysis of the decarboxylation step in mammalian histidine decarboxylase. A computational study. J Biol Chem 283:12393–12401

Moya-García AA, Pino-Ángeles A, Gil-Redondo R, Morreale A, Sánchez-Jiménez F (2009) Structural features of mammalian histidine decarboxylase reveal the basis for specific inhibition. Br J Pharmacol 157:4–13

Müller IB, Wu F, Bergmann B, Knockel J, Walter RD, Gehring H, Wrenger C (2009) Poisoning pyridoxal 5-phosphate-dependent enzymes: a new strategy to target the malaria parasite *Plasmodium falciparum*. PLoS One 4:e4406

Nitta Y, Kikuzaki H, Ueno H (2007) Food components inhibiting recombinant human histidine decarboxylase activity. J Agric Food Chem 55:299–304

Ohtsu H, Watanabe T (2003) New functions of histamine found in histidine decarboxylase gene knockout mice. Biochem Biophys Res Commun 305:443–447

Olmo MT, Sánchez-Jiménez F, Medina MA, Hayashi H (2002) Spectroscopic analysis of recombinant rat histidine decarboxylase. J Biochem 132:433–439

Panula P, Nuutinen S (2013) The histaminergic network in the brain: basic organization and role in disease. Nat Rev Neurosci 14:472–487

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization

system for exploratory research and analysis. J Comput Chem 25:1605–1612

Pino-Ángeles A, Morreale A, Negri A, Sánchez-Jiménez F, Moya-García AA (2010) Substrate uptake and protein stability relationship in mammalian histidine decarboxylase. Proteins 78:154–161

Pino-Ángeles A, Reyes-Palomares A, Melgarejo E, Sánchez-Jiménez F (2012) Histamine: an undercover agent in multiple rare diseases? J Cell Mol Med 16:1947–1960

Poulin R, Secrist JA 3rd, Pegg AE (1989) Effect of 1-amino-oxy-3-aminopropane on polyamine metabolism and growth of L1210 cells. Biochem J 263:215–221

Ringvall M, Ronnberg E, Wernersson S, Duelli A, Henningsson F, Abrink M, García-Faroldi G, Fajardo I, Pejler G (2008) Serotonin and histamine storage in mast cell secretory granules is dependent on serglycin proteoglycan. J Allerg Clin Immunol 121:1020–1026

Rodríguez-Agudo D, Olmo MT, Sánchez-Jiménez F, Medina MA (2000) Rat histidine decarboxylase is a substrate for m-calpain in vitro. Biochem Biophys Res Commun 271:777–781

Rodríguez-Caso C, Rodríguez-Agudo D, Moya-García AA, Fajardo I, Medina MA, Subramaniam V, Sánchez-Jiménez F (2003a) Local changes in the catalytic site of mammalian histidine decarboxylase can affect its global conformation and stability. Eur J Biochem 270:4376–4387

Rodríguez-Caso C, Rodríguez-Agudo D, Sánchez-Jiménez F, Medina MA (2003b) Green tea epigallocatechin-3-gallate is an inhibitor of mammalian histidine decarboxylase. Cell Mol Life Sci 60:1760–1763

Ruiz-Perez MV, Pino-Ángeles A, Medina MA, Sánchez-Jiménez F, Moya-García AA (2012) Structural perspective on the direct inhibition mechanism of EGCG on mammalian histidine decarboxylase and dopa decarboxylase. J Chem Inf Model 52:113–119

Ruoff PM, Scott RC (1950) Ethers of 4(5)-hydroxymethylimidazole 1,2. J Am Chem Soc 72:4950–4953

Sánchez-Jiménez F, Ruiz-Pérez MV, Urdiales JL, Medina MA (2013) Pharmacological potential of biogenic amine–polyamine interplay beyond neurotransmission. Br J Pharmacol. doi:10.1111/bph.12109 (in press)

Sashchenko LP, Severin ES, Khomutov RM (1968) On the inhibition of L-glutamic acid decarboxylase by derivatives of hydroxylamine and related compounds. Biochemistry (Moscow) 33:142–147

Schrodinger LLC (2010) The PyMOL molecular graphics system, version 1.3r1

Simonian AR, Vespalainen J, Khomutov AR (2006) Aminooxy analogues of spermine and their monoacetyl derivatives. Bioorg Khim 32:643–650

Singh S, Mukherjee A, Khomutov AR, Persson L, Heby O, Chatterjee M, Madhubala R (2007) Antileishmanial effect of 3-aminooxy-1-aminopropane is due to polyamine depletion. Antimicrob Agents Chemother 51:528–534

Stanek J, Frei J, Mett H, Schneider P, Regenass U (1992) 2-Substituted 3-(aminooxy)propanamines as inhibitors of ornithine decarboxylase: synthesis and biological activity. J Med Chem 35:1339–1344

Tabor H (1957) Isolation and determination of histidine and related compounds. Methods Enzymol 3:623–635

Tagushi Y, Watanabe T, Kubota H, Hayashi H, Wada H (1984) Purification of histidine decarboxylase from the liver of fetal rats and its immunochemical and immunohistochemical characterization. J Biol Chem 259:5214–5221

Tanase S, Guirard BM, Snell EE (1985) Purification and properties of a pyridoxal 5′-phosphate dependent histidine decarboxylase from *Morganella morganii* AM-15. J Biol Chem 260:6738–6746

Thurmond RL, Gelfand EW, Dunford PJ (2008) The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov 7:41–53

Viguera E, Trelles O, Urdiales JL, Mates JM, Sánchez-Jiménez F (1994) Mammalian L-amino acid decarboxylases producing 1,4-diamines: analogies among differences. Trends Biochem Sci 19:318–319

Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA (2004) Development and testing of a general amber force field. J Comput Chem 25:1157–1174

Wu F, Yu J, Gehring H (2008) Inhibitory and structural studies of novel coenzyme-substrate analogs of human histidine decarboxylase. Faseb J 22:890–897

Wu F, Christen P, Gehring H (2011) A novel approach to inhibit intracellular vitamin B6-dependent enzymes: proof of principle with human and plasmodium ornithine decarboxylase and human histidine decarboxylase. Faseb J 25:2109–2122

Zeeh B, Metzger H (1971) Methoden zur Herstellung und Umwandlung von Hydroxylaminen, vol X-1. Methoden der Organischen Chemie. Thieme Verlag, Stuttgart