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Inhibition of Pig Liver and *Zea mays* L. Polyamine Oxidase: A Comparative Study

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Polyamine oxidase (PAO) is involved in polyamine metabolism and production of hydrogen peroxide in animal and plants, thus representing a key system in development and programmed cell death. In the present study, the inhibitory effect of amiloride, *p*-aminobenzamidine, clonidine, 4′,6-diamidino-2-phenyl-indole (DAPI), gabexate mesylate, guazatine, and *N,N′-bis*(2,3-butadienyl)-1,4-butane-diamine (MDL72527) on the catalytic activity of pig liver and *Zea mays* L. PAO, *Lens culinaris* L. and *Pisum sativum* L. and swine kidney copper amine oxidase, bovine trypsin, as well as neuronal constitutive nitric oxide synthase (NOS-I) was investigated. Moreover, agmatine and N^3-prenylagmatine (G3) were observed to inhibit pig liver and *Zea mays* L. PAO, bovine trypsin, and NOS-I action, but were substrates for *Lens culinaris* L., *Pisum sativum* L. and swine kidney copper amine oxidase. Guazatine and G3 inhibited selectively *Zea mays* L. PAO with K_i values of 7.5×10^{-9} M and 1.5×10^{-8} M, respectively (at pH 6.5 and 25.0 °C). As a whole, the data reported here represent examples of enzyme cross-inhibition, and appear to be relevant in view of the use of cationic L-arginine-and imidazole-based compounds as drugs.

Keywords: Pig liver polyamine oxidase, *Zea mays* L. Polyamine oxidase, *Lens culinaris* L. copper amine oxidase, *Pisum sativum* L. copper amine oxidase, Swine kidney copper amine oxidase, Bovine trypsin, Neuronal constitutive nitric oxide synthase (NOS-I), Agmatine, Amiloride, *p*-aminobenzamidine, Clonidine, 4′,6-Diamidino-2-phenylindole, Gabexate mesylate, Guazatine, N^3-prenylagmatine, *N,N′*-bis(2,3-Butadienyl)-1, 4-butane-diamine, Enzyme inhibition

Abbreviations: PAO, polyamine oxidase; NOS-I, neuronal constitutive nitric oxide synthase; DAPI, 4′,6-diamidino-2-phenylindole; G3, N^3-prenylagmatine; MDL72527, *N,N′*-bis(2,3-butadienyl)-1,4-butane-diamine

INTRODUCTION

Spermidine and spermine are polyamine molecules, essential for cell proliferation and differentiation and are well known to bind DNA.[1,2] Moreover, some proteins such as the insulin receptor, protein kinase CK2, certain potassium channels and NMDA receptors possess a specific polyamine binding site, possibly having a regulatory function.[3–5] Growing evidence arises

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indicating that polyamine catabolism in plants is associated to physiological events, such as lignification, cell wall stiffening and cell defence.[6–7] Polyamine levels are controlled by a major regulatory system, which includes pathways for polyamine biosynthesis and degradation as well as for their transport across the cell membrane.[2,8]

Polyamine oxidase (PAO) catalyses the oxidation of spermidine and spermine at the secondary amino groups. The exact nature of the reduction products depends on the source of the enzyme. Vertebrate and invertebrate PAO transforms spermidine and spermine (and more efficiently their N^1-acetyl derivatives) into putrescine (1,4-diaminobutane) and spermidine, respectively, plus 3-amino-propionaldehyde. PAOs with similar characteristics occurs in fungi.[2] Conversely, bacterial, protozoa, and plant PAOs catalyses the conversion of spermidine and spermine to 4-aminobutyraldehyde and 3-aminopropyl-4-aminobutyraldehyde, respectively, plus 1,3-diaminopropane.[2] Despite these differences, PAOs from different taxa essentially share similar molecular properties.[9] PAO is a monomeric soluble protein with a molecular mass of about 53 kDa showing a non-covalently bound FAD molecule as cofactor.[9] The *Zea mays* L. enzyme represents the first PAO of known primary and tertiary structures[6,10] and the overall topology of the *Zea mays* L. PAO resembles that of other flavoproteins and reveals a remarkable U-shaped catalytic tunnel.[10]

PAO is a key enzyme in the polyamine catabolic pathway. Among others, the interest in polyamine metabolism has increased with the discovery that some polyamine analogues show antitumoral effect(s) on a number of cell lines.[11] Although the precise mechanism of the cytotoxic activity is not known, it has been noticed that the accumulation of polyamine analogues leads to DNA fragmentation and cell death by apoptosis.[12] However, some polyamine analogues, such as N^{11}-ethyl-N^{11}-[(cyclopropyl)methyl]-4,8-diazaundecane, may induce programmed cell death by increasing polyamine catabolism and consequent production of toxic H_2O_2.[13] Recently, it has been reported that the PAO inhibitor MDL72527 selectively induces apoptosis of transformed haematopoietic cells through lysosomotropic effects.[14,15] Moreover, the polyamine interconversion pathway has an important role in the increase of putrescine levels after traumatic brain injury. In this connection PAO inhibitors (e.g., MDL72527) block the polyamine interconversion pathway and may be neuroprotective against edema formation and necrotic cavitation.[16] Therefore, progress in the study of these compounds is a promising route for the design of new drugs.[1] Moreover, it has been suggested that PAO is involved in programmed cell death in plants as well.[7]

On the basis of the PAO recognition properties,[10] cationic L-arginine- and imidazole-based compounds, such as drugs,[17] by resembling the cationic substrates may inhibit enzyme activity. Therefore, the effect of agmatine, amiloride, *p*-aminobenzamidine, clonidine, 4′,6-diamidino-2-phenylindole (DAPI), gabexate mesylate, guazatine, N^3-prenylagmatine (G3), and *N,N′-bis*(2,3-butadienyl)-1,4-butane-diamine (MDL72527) on the catalytic properties of pig liver and *Zea mays* L. PAO, plant and mammalian copper amine oxidases, bovine trypsin, as well as neuronal constitutive nitric oxide synthase (NOS-I) has been investigated and reported here. Generally, guazatine and MDL72527 represent useful model compounds for the development of PAO modulators.

MATERIALS AND METHODS

Materials

Pig liver PAO was prepared according to Tsukada *et al.*[18] *Zea mays* L. PAO was purified as detailed elsewhere.[19] *Pisum sativum* L. copper amine oxidase was purified according to McGuirl *et al.*[20] Swine kidney copper amine oxidase was kindly provided by Prof. B. Mondovì

(Department of Biochemical Sciences 'A. Rossi Fanelli', University of Rome 'La Sapienza', Rome, Italy). NOS-I was prepared from mouse brain, as previously reported.[21] Bovine trypsin, N^1-acetylspermine, agmatine, amiloride, *p*-aminobenzamidine, *N*-α-carbobenzoxy-L-arginine *p*-nitroanilide, clonidine, 4′-6′-diamidino-2-phenyl-indole (DAPI), Hepes, putrescine, and spermidine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Gabexate mesylate was purchased from Lepetit S.P.A. (Milano, Italy), guazatine was obtained from Rhone-Poulenc Agro Italia and [^{3}H]L-arginine was obtained from NEN™ Life Science Products (Boston, MA, USA). N^3-Prenylagmatine (G3), an hypotensive agent isolated from *Verbesina caracasana*,[22] was converted into its methane sulfonic acid derivative[23] and then used. *N,N′-Bis*(2,3-butadienyl)-1,4-butane-diamine (MDL72527) was a generous gift from Dr. M. De Agazio (Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma-Montelibretti). All the other products were obtained from Merck AG (Darmstadt, Germany). All chemicals were of analytical grade and were used without further purification.

Assays

The oxidation of N^1-acetylspermine catalysed by pig liver PAO and of spermidine catalysed by *Zea mays* L. PAO,[7] the oxidation of agmatine catalysed by swine kidney copper amine oxidase,[24] the oxidation of putrescine catalysed by copper amino oxidase from *Pisum sativum* L.,[25] the bovine trypsin catalysed hydrolysis of *N*-α-carbobenzoxy-L-arginine *p*-nitroanilide[26] as well as the conversion L-arginine to L-citrulline and NO catalysed by NOS-I[27] were investigated in the absence and presence of amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, guazatine, and MDL72527. Moreover, the inhibitory effect of agmatine and G3 on pig liver and *Zea mays* L. PAO, bovine trypsin and NOS-I action was investigated.

In the enzyme assay, the *Zea mays* L. PAO as well as the *Pisum sativum* L. and swine kidney copper amine oxidase concentration ranged between $2.0–5.0 \times 10^{-9}$ M, the bovine trypsin concentration was 1.0×10^{-6} M, and the NOS-I concentration ranged between $0.2–1.0 \times 10^{-6}$ M (i.e., $[E] < [S]$ and $[E] < [I]$, where [E], [S] and [I] indicate the enzyme, the substrate, and the inhibitor concentration). Furthermore, the substrate (i.e., N^1-acetylspermine, agmatine, G3, putrescine, spermidine, *N*-α-carbobenzoxy-L-arginine *p*-nitroanilide and [^{3}H]L-arginine) concentration ranged between $0.1 \times K_m < [S] < 10 \times K_m$ (where K_m is the Michaelis constant). Moreover, the inhibitor (i.e., agmatine, amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, guazatine, G3 and MDL72527) concentration ranged between $0.1 \times K_i < [I] < 10 \times K_i$ (where K_i is the apparent dissociation equilibrium constant for the formation of the reversible enzyme: inhibitor complex).

In a typical experiment: 20 μL of a buffered enzyme solution (i.e., *Zea mays* L. PAO) were added to 1.0 mL of a buffered solution containing the substrate (i.e., spermidine) and/or the inhibitor (e.g., guazatine). Under all the experimental conditions, the initial velocity for the enzyme catalysed conversion of the substrate was unaffected by the enzyme/inhibitor incubation time, preceding substrate addition, and by the enzyme/substrate incubation time, preceding inhibitor addition. In fact, the enzyme/inhibitor/substrate equilibration time was very short, being complete within the mixing time (e.g., 15 s).

Values of K_i for the pig liver and *Zea mays* L. PAO competitive inhibition by agmatine, amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, guazatine, G3 and MDL72527 were determined from the linear dependence of the K_m^{app}/K_m^0 ratio on the inhibitor concentration ([I]), according to Equation (1):

$$K_m^{app}/K_m^0 = K_i^{-1} \cdot [I] + 1 \qquad (1)$$

where K_m^{app} is the apparent Michaelis constant determined at a fixed inhibitor concentration and K_m^0 is the intrinsic Michaelis constant determined in the absence of the inhibitor.[28] Also values of K_i for the *Pisum sativum* L. and swine kidney copper amine oxidase competitive inhibition by guazatine and MDL72527, for the bovine trypsin competitive inactivation by agmatine, amiloride, clonidine, guazatine, G3 and MDL72527, as well as for NOS-I competitive inhibition by guazatine, G3 and MDL72527 were determined, according to Equation (1).[28] The effect of DAPI on NOS-I activity was not determined since DAPI induced NADPH precipitation.

Values of K_m^0 and K_m^{app} (see Equation (1)) for the N^1-acetylspermine oxidation by pig liver PAO, for the spermidine oxidation by *Zea mays* L. PAO, for the agmatine, putrescine and G3 oxidation by *Pisum sativum* L. and swine kidney copper amine oxidase, for the bovine trypsin catalysed hydrolysis of *N*-α-carbobenzoxy-L-arginine *p*-nitroanilide, and for the [^{3}H]L-arginine to [^{3}H]L-ornitine conversion by NOS-I were determined from the dependence of the initial velocity on the substrate concentration, according to the Michaelis-Menten equation, in the absence and presence of the inhibitor, respectively, at fixed [E], [E] < [S] and [E] < [I].[28] Values of K_m^0 for spermidine oxidation by *Zea mays* L. PAO was 2.3×10^{-5} M,[19] for N^1-acetylspermine oxidation by pig liver PAO was 2.0×10^{-6} M,[18] for agmatine oxidation by *Pisum sativum* L. was 4.0×10^{-4} M (present study), for agmatine, putrescine and G3 oxidation by swine kidney copper amine oxidase was 1.0×10^{-5} M,[25] 1.0×10^{-5} M[25] and 5.4×10^{-5} M (present study), respectively, for the bovine trypsin catalysed hydrolysis of *N*-α-carbobenzoxy-L-arginine *p*-nitroanilide was 1.3×10^{-4} M,[26] and for the [^{3}H]L-arginine to [^{3}H]L-ornitine conversion by NOS-I was 4.0×10^{-6} M.[29] As expected for a simple competitive inhibition system,[28] values of V_{max} were unaffected by the inhibitor, and values of K_i were independent of the substrate, within the experimental error (±5%). All data were obtained between pH 6.5 and 8.0 (1.0×10^{-1} M and 1.0×10^{-2} M Hepes and phosphate buffers), and between 25.0 °C and 37.0 °C. The experimental details are given in Table I.

RESULTS AND DISCUSSION

Agmatine, amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, guazatine, G3, and MDL72527 (see Figure 1) competitively inhibited spermidine oxidation catalysed by *Zea mays* L. PAO (see Figure 2 and Table I). On the other hand, only agmatine, gabexate mesylate, guazatine, and G3 inhibited competitively N^1-acetylspermine oxidation catalysed by pig liver PAO (see Table I). As already reported,[30] MDL72527 inhibited pig liver PAO irreversibly. The affinity of agmatine, DAPI, gabexate mesylate, guazatine, G3, and MDL72527 for *Zea mays* L. PAO was higher than that observed for amiloride, *p*-aminobenzamidine, and clonidine (see Table I). Note that guazatine and G3 selectively inactivated *Zea mays* L. PAO (see Table I).

As shown in Table I, amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, and guazatine competitively inactivated plant and swine kidney copper amine oxidases, bovine trypsin and NOS-I. Moreover, G3 was a substrate for swine kidney copper amine oxidase, without affecting bovine trypsin and NOS-I action (see Table I). On the other hand, MDL72527 inhibited plant copper amine oxidase and NOS-I without influencing swine kidney copper amine oxidase and bovine trypsin (see Table I). It should be noted that agmatine was a substrate for *Pisum sativum* L. (present study) and swine kidney copper amine oxidase,[25] inhibited competitively NOS-I, without influencing bovine trypsin (see Table I).

It has been reported that, amiloride inhibited human placental copper amine oxidase, the human urinary plasminogen activator, human and dog tryptase, as well as bovine trypsin,

TABLE I Values of K_i for agmatine, amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, guazatine, G3 and MDL72527 binding to pig liver PAO, *Zea mais* L. PAO, plant copper amine oxidase, swine kidney copper amine oxidase, bovine trypsin and NOS-I

Inhibitor	Pig liver PAO	*Zea mais* L. PAO[b]	Plant copper amine oxidase	Swine kidney copper amine oxidase	Bovine trypsin	NOS-I
Agmatine	$K_i = 7.0 \times 10^{-6}$ M	$K_i = 3.0 \times 10^{-6}$ M	–[c]	–[c]	$K_i > 10^{-2}$ M[g]	$K_i = 6.6 \times 10^{-4}$ M[m]
Amiloride	$K_i > 10^{-2}$ M	$K_i = 1.9 \times 10^{-3}$ M	$K_i = 4.1 \times 10^{-4}$ M[d,e]	$K_i = 1.0 \times 10^{-5}$ M[h]	$K_i = 8.0 \times 10^{-6}$ M[g]	$K_i = 4.5 \times 10^{-4}$ M[n]
p-Aminobenzamidine	$K_i > 10^{-2}$ M	$K_i = 8.0 \times 10^{-4}$ M	$K_i = 6.0 \times 10^{-4}$ M[d,e]	$K_i = 2.5 \times 10^{-4}$ M[e]	$K_i = 6.1 \times 10^{-6}$ M[j]	$K_i = 1.2 \times 10^{-4}$ M[o]
Clonidine	$K_i > 10^{-2}$ M	$K_i = 1.5 \times 10^{-4}$ M	$K_i = 5.0 \times 10^{-4}$ M[d,e]	$K_i = 9.0 \times 10^{-4}$ M[h]	$K_i => 10^{-2}$ M[g]	$K_i = 1.5 \times 10^{-4}$ M[p]
DAPI	$K_i > 10^{-2}$ M	$K_i = 1.3 \times 10^{-5}$ M	$K_i = 1.9 \times 10^{-5}$ M[d,e]	$K_i = 1.3 \times 10^{-5}$ M[e]	$K_i = 4.7 \times 10^{-6}$ M[k]	–[q]
Gabexate mesylate	$K_i = 1.2 \times 10^{-5}$ M	$K_i = 5.0 \times 10^{-5}$ M	$K_i = 2.5 \times 10^{-4}$ M[d,e]	$K_i = 2.7 \times 10^{-5}$ M[h]	$K_i = 2.6 \times 10^{-6}$ M[l]	$K_i = 1.0 \times 10^{-4}$ M[r]
Guazatine	$K_i = 8.0 \times 10^{-6}$M	$K_i = 7.5 \times 10^{-9}$ M	$K_i = 1.2 \times 10^{-5}$ M[f,g]	$K_i = 1.5 \times 10^{-7}$ M[g]	$K_i = 6.5 \times 10^{-5}$ M[g]	$K_i = 5.0 \times 10^{-5}$ M[s]
G3	$K_i = 5.7 \times 10^{-5}$ M	$K_i = 1.5 \times 10^{-8}$ M	–[c]	–[c]	$K_i > 10^{-2}$ M[g]	$K_i => 10^{-2}$ M[s]
MDL72527	$K_i = 9.0 \times 10^{-8}$ M[a]	$K_i = 5.5 \times 10^{-7}$ M	$K_i = 3.0 \times 10^{-4}$ M[f,g]	$K_i > 10^{-2}$ M[g]	$K_i > 10^{-2}$ M[g]	$K_i = 6.0 \times 10^{-4}$ M[s]

[a]pH 9.0 (borate buffer) and 30.0 °C. [b]pH 6.5 (2.0×10^{-1} M phosphate buffer) and 25.0 °C. Present study. [c]Agmatine and G3 behave as substrates for plant copper amine oxidase and swine kidney copper amine oxidase. Present study. [d]Copper amine oxidase from *Lens culinaris* L. [e]pH 7.0 (1.0×10^{-1} M Hepes or phosphate buffer) and 25.0 °C. [f]Copper amine oxidase from *Pisum sativum* L. [g]pH 7.0 (1.0×10^{-1} M phosphate buffer) and 25.0 °C. Present study. [h]pH 7.0 (1.0×10^{-1} M Hepes or phosphate buffer) and 25.0 °C. [j]pH 7.4 (2.0×10^{-2} M phosphate buffer, 1.0×10^{-1} M NaCl) and 25.0 °C. [k]pH 6.2 (1.0×10^{-1} M phosphate buffer) and 30.0 °C. [l]pH 6.8 (1.0×10^{-1} M Tris buffer) and 20.0 °C. [m]pH 7.8 (5.0×10^{-2} M Tris buffer) and 37.0 °C. [n]pH 7.5 (5.0×10^{-2} M Hepes buffer) and 37.0 °C. [o]pH 7.5 (5.0×10^{-2} M Hepes buffer) and 37.0 °C. [p]pH 7.5 (5.0×10^{-2} M Hepes buffer) and 37.0 °C. [q]DAPI induces NADPH precipitation, and the K_i value for NOS-I inhibition was not determined. Present study. [r]pH 7.4 (1.0×10^{-1} M Hepes buffer) and 37.0 °C. [s]pH 7.5 (5.0×10^{-2} M Hepes buffer) and 37.0 °C. Present study.

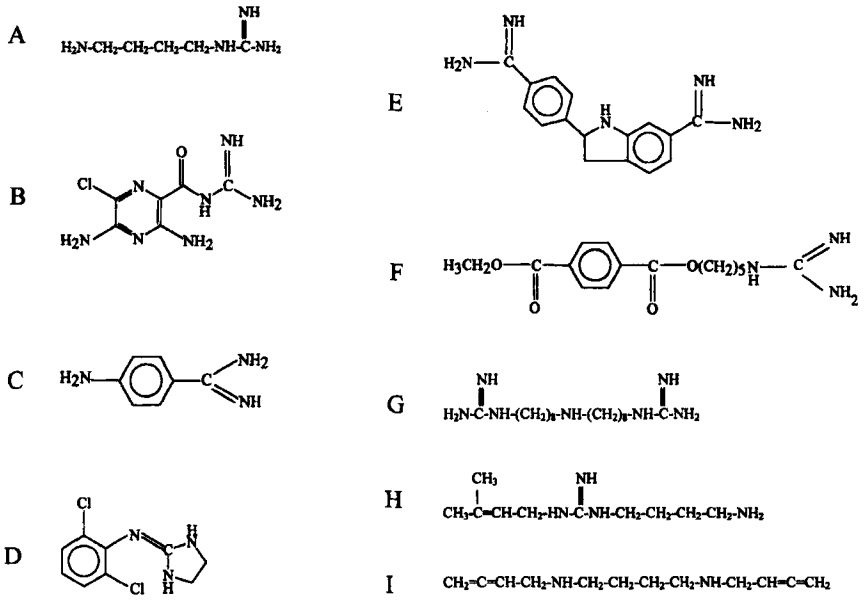

FIGURE 1 Chemical structures of agmatine (A), amiloride (B), *p*-aminobenzamidine (C), clonidine (D), DAPI (E), gabexate mesylate (F), guazatine (G), G3 (H) and MDL72527 (I).

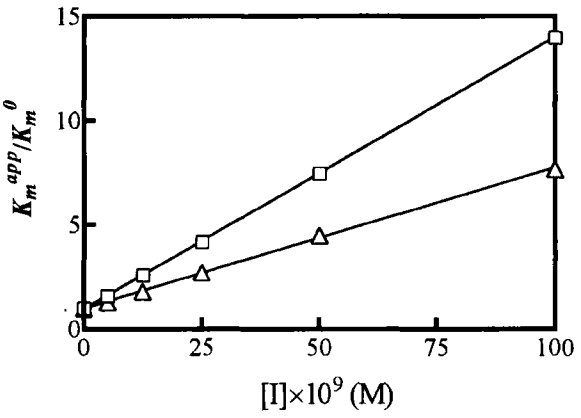

FIGURE 2 Effect of guazatine (□) and N^3-prenylagmatine (G3; Δ) concentration on the K_m^{app}/K_m^0 ratio for the competitive inhibition of *Zea mays* L. PAO catalysed oxidation of spermidine. The continuous lines were calculated according to Equation (1) with the K_i values given in Table I. All data were obtained at pH 6.5 (2.0×10^{-1} M phosphate buffer), and 25.0 °C. For further details, see text and Table I.

without influencing the human tissue plasminogen activator, human plasmin, human thrombin and human plasma kallikrein action.[31–33] However, human urinary kallikrein inhibition by amiloride is a controversial issue, and the enzyme inactivation mode has been reported to be non-competitive.[31] Moreover, DAPI inactivated competitively S-adenosyl-L-methionine decarboxylase,[34] and gabexate mesylate inhibited several serine proteinases (e.g., bovine thrombin, bovine factor Xa, human plasmin and the human urinary plasminogen activator), without affecting human urinary and porcine pancreatic kallikrein activities.[35,36]

Inspection of the three-dimensional structures of *Zea mays* L. PAO[10] (see Figure 3), copper amine oxidase from *E. coli* [37,38] and from *Pisum sativum* L.,[39] bovine trypsin[40,41] and NOS-I,[42] as well as the enzyme-inhibitor/substrate binding mode (see Table I) allowed the following conclusions.

The three-dimensional structure of *Zea mays* L. PAO is characterised by a U-shaped tunnel[10] (see Figure 3). The walls of the catalytic tunnel are decorated by carbonyl and carboxylate oxygen atoms, which take part in the binding and recognition of the polyamine positively charged substrate. The narrow width of the tunnel makes it complementary to linear ligands as reflected

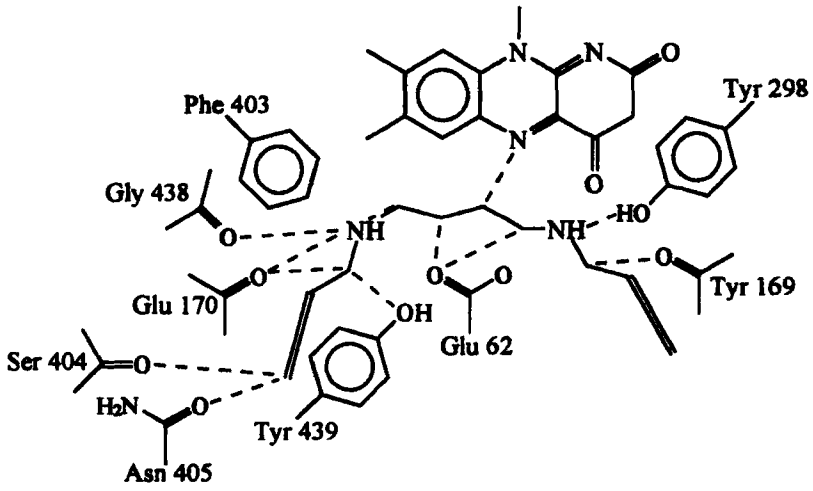

FIGURE 3 Schematic representation of the active centre of *Zea mays* L. PAO in the presence of MDL72527. The inhibitor is in the centre of the picture. Dashed lines indicate the enzyme: MDL72527 hydrogen bonds.

by values of K_i, the most potent inhibitors showing unbranched aliphatic chains, such as agmatine, guazatine, G3 and MDL72527. Conversely, bulkier molecules such as amiloride, *p*-aminobenzamidine, clonidine, DAPI, and gabexate mesylate exhibited a lower affinity (see Table I). At present, mammalian PAO primary structure is not known and information on its three-dimensional structure is lacking. Therefore, *Zea mays* L. PAO may represent a predictive model for the interaction of pharmacologically active compounds with mammalian PAO.

Competitive inactivation of copper amine oxidases by amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, and guazatine may reflect the non-covalent reversible electrostatic interaction(s) between the inhibitor and the enzyme 2,4,5-trihydroxyphenyl-alanine quinone-Asp catalytic diad.[24,25] The same recognition mechanism may be envisaged also for the *P. sativum* L.:MDL72527 complex formation and for substrate (e.g., agmatine and G3) binding. Moreover, agmatine and clonidine derivatives, amiloride,*p*-aminobenzamidine, gabexate mesylate, guazatine and related compounds may bind reversibly to the primary specificity subsite of trypsin-like serine proteinases, forming a salt bridge with the invariant Asp189 residue.[31,35,36,41,43,44] Furthermore, agmatine, amiloride,*p*-aminobenzamidine, clonidine, gabexate mesylate, guazatine, and MDL72527 may bind to the heme distal pocket of NOS-I by interacting with the Glu171 residue, as observed for aminoguanidine association.[42]

Finally, PAO, copper amine oxidase, nitric oxide synthase, as well as trypsin-like serine proteinase cross-modulation may also occur *in vivo*. Systemic therapy with blockers of copper amine oxidase, the main histamine degrading enzyme, may lead to significant increase of histamine-induced hypotensive effects, allergic reaction and food intolerance.[45] Thus, some amiloride antihypertensive effects may be related to the increased levels of histamine and/or agmatine (described as a clonidine-displacing compound), following copper amine oxidase inhibition.[33] A significant extent of the pharmacological activity of clonidine, one of the most widespread antihypertensive drug exerting an agonistic effect on α_2-adrenergic receptors,[17] may be due to the inhibition of copper amine oxidase. Similarly, guazatine and G3 showed hypotensive effects.[46,47] Moreover, gabexate mesylate, a drug widely used for protease

inhibition,[17] has been shown to induce allergic reactions and anaphylaxis.[48,49] However, gabexate mesylate has been used as an ointment for preventing inflammation, erosion and ulceration of the skin and mucosae.[50] On the other hand, the decreased levels of NO, as a consequence of nitric oxide synthase inhibition, might induce some clinically-adverse amiloride reactions, such as an unexpected reduced antihypertensive effect. Moreover, the inhibition of the human urinary plasminogen activator-catalysed activation of plasminogen by amiloride[31] might induce unexpected intravascular coagulation phenomena. Accordingly, L-arginine- and imidazole-based modulators of copper amine oxidase used as drugs, may affect trypsin-like serine proteinases and NOS-I action.

As a whole, agmatine, amiloride, *p*-aminobenzamidine, clonidine, DAPI, gabexate mesylate, guazatine, G3, and MDL72527 modulated the activity of pig liver and *Zea mays* L. PAO, copper amine oxidase, nitric oxide synthase as well as trypsin-like serine proteinase. Moreover, these compounds might affect, among others, arginase, L-arginine-glycine transaminase, kyotorphine synthase and L-arginine decarboxylase, all acting on L-arginine based compounds.[51] Therefore, the use of some of these compounds as drugs, modulating (un)related function(s), should be under careful control. Moreover, the knowledge of selective inhibition mechanisms of the L-arginine and imidazole related compounds here investigated may be a tool in designing drugs for pharmacologically active recombinant enzymes.

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