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Amine oxidases of the quinoproteins family: Their implication in the metabolic oxidation of xenobiotics

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► **To cite this version:**

Martine Largeron. Amine oxidases of the quinoproteins family: Their implication in the metabolic oxidation of xenobiotics. *Annales Pharmaceutiques Françaises*, 2011, 69 (1), pp.53-61. <10.1016/j.pharma.2010.10.002>. <hal-02384856>

HAL Id: hal-02384856

<https://hal.science/hal-02384856v1>

Submitted on 5 Feb 2021

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HAL Authorization

Manuscript Number: PHARMA-D-10-00031R1

Title: Amine oxidases of the quinoproteins family : their implication in the metabolic oxidation of xenobiotics

Article Type: Séance thématique / Thematic session

Keywords: Copper amine oxidase; oxidative metabolism; xenobiotics; toxicity; inhibitor; enzyme mimic.

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Manuscript Region of Origin: FRANCE

Abstract: Copper amine oxidases (CuAOs) are ubiquitous enzymes which play a vital role in the physiology and pathology of mammals in controlling the metabolism of various primary monoamines, diamines and polyamines of endogenous or xenobiotic origin. CuAOs, which belong to the quinoproteins family, possess two cofactors: tightly bound CuII and a quinone residue which catalyzes the oxidative deamination of primary amines with concomitant production of aldehyde, ammonia and hydrogen peroxide through a « ping pong » mechanism. Interest in human enzymes of the CuAOs class has increased in recent years driven by the discovery that the human vascular adhesion protein-1 (VAP-1), which regulates leucocyte trafficking and glucose transport, is a CuAO enzyme. The activities of CuAOs are increased in various human disorders, such as diabetes, Alzheimer's disease and many inflammation-associated diseases leading to the overproduction of toxic metabolites, especially hydrogen peroxide and aldehyde compounds. As most consequences are pathological, effective and selective inhibitors of CuAOs should be of great interest as therapeutic agents. Nevertheless, the utilization of CuAOs to generate enzymatic toxic products into cancer cells for selective in situ killing deserves to be considered in cancer therapy. This paper briefly highlights recent progress in the study of physiological, pathological and molecular aspects of CuAOs in mammals. Furthermore, a small molecule that mimics the metabolic activity of CuAOs toward endogenous and exogenous amines is described because it could be used as a surrogate of enzymes for a preliminary screening of potential inhibitors of CuAO enzymes.

Suggested Reviewers:

Opposed Reviewers:

Abstract

Copper amine oxidases (CuAOs) are ubiquitous enzymes which play a vital role in the physiology and pathology of mammals in controlling the metabolism of various primary monoamines, diamines and polyamines of endogenous or xenobiotic origin. CuAOs, which belong to the quinoproteins family, possess two cofactors: tightly bound Cu^{II} and a quinone residue which catalyzes the oxidative deamination of primary amines with concomitant production of aldehyde, ammonia and hydrogen peroxide through a « ping pong » mechanism. Interest in human enzymes of the CuAOs class has increased in recent years driven by the discovery that the human vascular adhesion protein-1 (VAP-1), which regulates leucocyte trafficking and glucose transport, is a CuAO enzyme. The activities of CuAOs are increased in various human disorders, such as diabetes, Alzheimer's disease and many inflammation-associated diseases leading to the overproduction of toxic metabolites, especially hydrogen peroxide and aldehyde compounds. As most consequences are pathological, effective and selective inhibitors of CuAOs should be of great interest as therapeutic agents. Nevertheless, the utilization of CuAOs to generate enzymatic toxic products into cancer cells for selective in situ killing deserves to be considered in cancer therapy. This paper briefly highlights recent progress in the study of physiological, pathological and molecular aspects of CuAOs in mammals. Furthermore, a small molecule that mimics the metabolic activity of CuAOs toward endogenous and exogenous amines is described because it could be used as a surrogate of enzymes for a preliminary screening of potential inhibitors of CuAO enzymes.

1 **Keywords**
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4 Copper amine oxidase; oxidative metabolism; xenobiotics; toxicity; inhibitor;
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6 enzyme mimic.
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11 **Abbreviations**
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13 CuAOs, copper amine oxidases; VAP-1, vascular adhesion protein-1; AOs, amine
14 oxidases; MAOs, monoamine oxidases; SSAOs, semicarbazide-sensitive amine
15 oxidases; PAOs, polyamine oxidases; FAD, flavine adenine dinucleotide; DAOs,
16 diamine oxidases; LOs, lysyl oxidases; TPQ, topaquinone; LTQ, lysine
17 tyrosylquinone, ROS, reactive oxygen species; PrAOs, primary amine oxidases;
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31 **Résumé**
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34 Les enzymes amine-oxydase à cuivre (CuAOs) sont des enzymes ubiquitaires qui
35 jouent un rôle essentiel chez les mammifères, tant au plan physiologique que
36 pathologique, puisqu'elles contrôlent le métabolisme de diverses monoamines
37 primaires, diamines et polyamines, d'origine endogène ou exogène. Les CuAOs, qui
38 appartiennent à la famille des quinoprotéines, possèdent deux cofacteurs : un ion
39 cuivrique et un résidu quinonique qui catalyse la désamination oxydante des amines
40 primaires avec production concomitante d'aldéhydes, ammoniac et peroxyde
41 d'hydrogène au travers d'un mécanisme « ping pong ». Récemment, un intérêt
42 particulier a été porté à la famille des CuAOs humaines depuis la découverte de leur
43 identité avec la protéine d'adhésion vasculaire humaine (VAP-1), qui joue un rôle
44 régulateur dans la circulation des leucocytes et l'assimilation du glucose. Dans de
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1 nombreuses pathologies tels que le diabète, la maladie d'Alzheimer et les maladies
2 inflammatoires, l'activité des CuAOs est accrue et conduit à une surproduction de
3 métabolites toxiques, en particulier de peroxyde d'hydrogène et d'aldéhydes.
4
5 Comme la plupart des conséquences sont pathologiques, la recherche d'inhibiteurs
6 efficaces et sélectifs de CuAOs présente un intérêt en thérapeutique. Néanmoins,
7 l'utilisation des CuAOs pour générer des produits toxiques, peroxyde d'hydrogène
8 et aldéhydes, à l'intérieur des cellules cancéreuses, ouvre de nouvelles perspectives
9 en thérapie anticancéreuse. Cet article décrit brièvement les progrès récemment
10 réalisés dans la connaissance de la physiologie, de la pathologie et des mécanismes
11 moléculaires des CuAOs chez les mammifères. De plus, une petite molécule qui
12 mime l'activité métabolique des CuAOs envers les amines primaires, endogènes et
13 exogènes, est présentée dans la mesure où elle pourrait être utilisée pour sélectionner
14 de nouveaux inhibiteurs d'enzymes CuAOs.
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34 **Mots Clés**

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36 Amine oxydase à cuivre; métabolisme oxydatif; xénobiotiques; toxicité; inhibiteur;
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38 mime d'enzyme.
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1 **Introduction**

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4 The implication of amine oxidases (AOs) in the oxidative metabolism of xenobiotic
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6 amines in humans has been largely neglected in the past [1], exclusive of studies
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8 concerning the metabolism of xenobiotics by mitochondrial monoamine oxidases
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10 (MAOs) A and B, since these enzymes have been considered for a long time as
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12 targets for inhibition [2]. Interestingly, when MAO A and MAO B activities were
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14 completely blocked by an irreversible inhibitor, an enzymatic activity able to
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16 selectively oxidize primary amines remained in mammalian tissues [3-5]. The
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18 sensitivity of these novel enzymes to inhibition by semicarbazide led to refer them
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20 as semicarbazide-sensitive amine oxidases (SSAOs). Then, the superfamily of AOs
21
22 was divided into two main categories: one class, which encompasses MAOs (A and
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24 B) (EC 1.4.3.4) and polyamine oxidases (PAOs), is characterized by the presence of
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26 flavine adenine dinucleotide (FAD) as the redox cofactor (Fig. 1) [6,7]. The second
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28 class is represented by CuAOs enzymes which possess tightly bound Cu^{II} and a
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30 quinone residue as the redox cofactor [8,9]. This class, which belongs to the
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32 quinoproteins family, not only includes SSAOs, which are located in plasma
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34 membranes of various tissues and in blood plasma, but also intracellular diamine
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36 oxidases (DAOs) and lysyl oxidases (LOs). CuAOs (EC 1.4.3.6) use as redox
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38 cofactor the tyrosine-derived 2,4,5-trihydroxyphenylalanine quinone, also named
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40 topaquinone (TPQ) [10], at the exception of LOs (EC 1.4.3.13) whose active site
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42 has been identified as lysine tyrosylquinone (LTQ) (Fig. 1) [11].
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51 CuAOs are ubiquitous enzymes isolated from mammals, many higher-order
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53 plants, fungi, yeasts and bacteria. Interest in human enzymes of the CuAOs class has
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55 increased in recent years driven by the discovery that the human vascular adhesion
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1 protein-1 (VAP-1), which regulates leucocyte trafficking and glucose transport,
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3 belongs to the CuAOs family [12].
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6 This paper briefly highlights the progress recently achieved in understanding the
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8 involvement of mammalian CuAOs in the metabolic oxidation of xenobiotics,
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10 suggesting that the physiological role of CuAOs may be more diverse than expected.
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12 As most consequences are pathological, it is also highly desirable to obtain
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14 inhibitors with selectivity toward CuAOs over MAOs. In this respect, a potent orally
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16 active and selective inhibitor of CuAOs activity (LJP 1586), which is currently
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18 under investigation as a potential anti-inflammatory agent is presented. Furthermore,
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20 we describe a small molecule that mimics the metabolic activity of CuAOs toward
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22 endogenous and xenobiotic primary amines and that could be used as a surrogate of
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24 enzymes for a preliminary screening of potential inhibitors of human enzymes.
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32 **Amine substrates of mammalian CuAOs**

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35 CuAOs metabolize various primary monoamines, diamines and polyamines of
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37 endogenous or xenobiotic origin. However, some specificity is observed among the
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39 enzymes of this group [13]. For example, SSAOs catalyze the oxidative deamination
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41 of methylamine resulting from the metabolism of adrenaline and nicotine by MAO,
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43 and also present in tobacco smoke. Aminoacetone, a threonine and glycine
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45 catabolite, is also a specific substrate for SSAOs. DAOs preferably metabolize
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47 short-chain diamines such as histamine present in fish, cheese or alcoholic drinks,
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49 together with putrescine and cadaverine, both ubiquitous in food and also resulting
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51 from enzymatic decarboxylation of ornithine and S-adenosyl-methionine, while LOs
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53 oxidatively deaminates the ϵ -amino group of peptidyl-L-lysyl-peptide residues of
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1 collagen. Although CuAOs markedly differ from MAOs, they share some overlap in
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3 substrate specificity. For example, the non physiological benzylamine, which has
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5 been commonly used as a model of primary amine substrate, is a good substrate for
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7 both CuAOs and MAOs. Similarly, dopamine neurotransmitter, which can also be
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9 found in fruits and some vegetables, as well as tyramine greatly present in cheese,
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11 chicken liver and alcoholic drinks, are substrates for SSAOs, DAOs and MAOs.
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15 To date, the involvement of CuAOs in the in vivo metabolism of marketed drug
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17 amines has received little attention [14]. Nevertheless, the loss of the amino group
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19 upon incubation of amlodipine, an antihypertensive agent, in dog and human
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21 plasma, is compatible with the implication of SSAO in amlodipine metabolism
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23 (Scheme 1) [15,16]. Similarly, the cytoprotective drug WR 1065 [2-(3-
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25 aminopropylamino)ethanethiol)], administered as the thiophosphate derivative
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27 amifostine and utilized to protect tissues against the damaging effects of
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29 radiotherapy and chemotherapy, is converted by CuAOs to an aldehyde which
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31 spontaneously decomposes into cysteamine and acrolein (Scheme 2) [14, 17].
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40 **Recent changes in nomenclature of CuAOs (2009)**

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42 The term SSAO obviously is a confusing name because DAOs and LOs are also
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44 inhibited by semicarbazide, and it has often been used to refer specifically to AOs
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46 that are active toward primary amines. However, this has never been universally
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48 accepted and there are several publications on SSAOs that actually refer to DAOs
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50 [13, 18]. To solve these problems, the Nomenclature Committee of the International
51
52 Union of Biochemistry and Molecular Biology (IUBMB) has recently proposed to
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54 reclassify CuAOs (EC 1.4.3.6) as primary amine oxidases (PrAOs) (EC 1.4.3.21),
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1 the enzymes oxidizing primary monoamines with little or no activity toward
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3 diamines, and as diamine oxidases (DAOs) (EC 1.4.3.22), the enzymes oxidizing
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5 diamines such as histamine and also some primary monoamines. Both enzymes are
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7 inactive toward secondary and tertiary amines. These changes are now available at
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9 <http://www.enzyme-database.org>.
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15 **Metabolic role of CuAOs**

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18 The role of CuAOs is to regulate levels of endogenous and xenobiotic primary
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20 mono- and polyamines, by catalyzing their oxidative deamination with the
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22 concomitant production of hydrogen peroxide, ammonia and aldehyde (Scheme 3).
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24 Each of these products is potentially harmful, since hydrogen peroxide can act as a
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26 source of reactive oxygen species (ROS) produced through the Fenton reaction,
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28 while ammonia and aldehyde products are known to be toxic in a number of systems
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30 [19-22]. Activities of **plasma** CuAOs are mostly increased in various human
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32 disorders, including type 1 and type 2 diabetes, congestive heart failure,
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34 atherosclerosis, liver cirrhosis, Alzheimer's disease and many inflammation-
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36 associated diseases leading to the overproduction of toxic metabolites especially
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38 hydrogen peroxide and aldehydes [23].
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48 **Diabetes, Alzheimer's disease and CuAOs**

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51 PrAOs catalyze the oxidative deamination of various endogenous substrates to
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53 produce aldehydes capable of inducing protein cross-linkage. Especially, the
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55 oxidative metabolism of methylamine and aminoacetone by PrAOs generate
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57 formaldehyde (Scheme 4, equation a) and methylglyoxal (Scheme 4, equation b)
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1 respectively as reactive metabolites which contribute to the formation of advanced-
2 glycation end products (AGEs), a phenomenon associated with vascular
3 complications of diabetes [24]. These aldehydes are also capable of inducing protein
4 cross-linking. Especially, they are able of enhancing the formation of β -amyloid
5 misfolding oligomers and protofibrils, but also of increasing the size of the
6 aggregates, two phenomena closely associated with Alzheimer's disease [25-28].
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10 11 12 13 14 15 16 17 18 **Inflammatory diseases and CuAOs** 19

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21 The fact that PrAO/VAP-1 exerts both enzymatic and adhesion activities, and the
22 strong correlation between its upregulation in many inflammatory conditions, make
23 it an interesting therapeutic target. This interdependence of the adhesive and
24 enzymatic functions of PrAO/VAP-1 provides the opportunity to develop enzyme
25 inhibitors where the anti-inflammatory effects potentially would result from both
26 blocking leucocytes migration and reducing the production of reactive metabolites.
27
28 Furthermore, it would be highly desirable to obtain inhibitors with notable
29 selectivity toward CuAOs over mitochondrial flavoproteins MAO A and MAO B. In
30 this respect, a potent and selective inhibitor of PrAO activity (LJP 1207), that
31 displayed anti-inflammatory activity in models of stroke, colitis and multiple
32 sclerosis, was described. However, LJP 1207 was a hydrazine derivative and this
33 class of compounds has potential for toxicity under prolonged administration [29-
34 31]. To avoid this problem, a structurally modified analog [LJP 1586, Z-3-fluoro-2-
35 (4-methoxybenzylallylamine)] with an amine functionality, is currently under
36 investigation as a potential anti-inflammatory agent [32]. As shown in Table 1, LJP
37 1586 exhibits good specificity for inhibition of PrAO over both copper-dependent
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1 DAO and the FAD-dependent MAO A and MAO B. In addition, this orally active
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3 small molecule has demonstrated a good therapeutic window in adsorption,
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5 distribution, metabolism, excretion and toxicological studies performed to date.
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10 **Cancer and CuAOs**

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12 The natural polyamines such as spermidine or spermine, formed from the
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14 decarboxylation products of ornithine and S-adenosyl-methionine, and ubiquitous in
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16 food, are also substrates for CuAO enzymes [33]. In cancerous cells, they are
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18 present at elevated levels as compared to normal tissues, because of enhanced
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20 putrescine synthesis from ornithine by ornithine decarboxylase, but also due to
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22 increased uptake of polyamines. Consequently, by delivering CuAOs [especially,
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24 bovine serum amine oxidase (BSAO)] into cancerous cells, the cytotoxic products of
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26 polyamines oxidation, hydrogen peroxide and aldehydes, could be produced in situ
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28 for selective killing of the same cells. For example, the oxidative deamination of
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30 spermidine produces an unstable aldehyde which spontaneously decomposes into
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32 the highly toxic acrolein and putrescine products [34] (Scheme 5). Interestingly,
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34 results obtained from the spermine oxidation by BSAO indicated that both hydrogen
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36 peroxide and acrolein were responsible for most of the observed cytotoxic effects on
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38 drug-sensitive and drug-resistant colon adenocarcinoma cells [35]. As drug-resistant
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40 cells were much more sensitive to the toxic enzymatic metabolites than the wild
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42 types, the utilization of CuAOs in anticancer therapy has been recently suggested as
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44 a promising strategy to overcome multidrug resistance of cancer [36-39].
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Catalytic mechanism of CuAOs

Through the utilization of synthetic models of TPQ and LTQ cofactors and using benzylamine as the model substrate [40-44], it has been established that TPQ catalyzes the conversion of a primary amine into an aldehyde through a ping pong mechanism (Scheme 6) [45-47]. The reductive half reaction (ping), which involves amine oxidation into aldehyde, results in the reduction of TPQ_{ox} into an aminoquinol form TPQ_{red}. A general consensus has been reached on the mechanism for this first half-reaction. The oxidative half reaction (pong) corresponds to the reduction of dioxygen to hydrogen peroxide, with concomitant formation of TPQ_{imq}. The final step under single turnover conditions is the hydrolysis of TPQ_{imq} to regenerate TPQ_{ox} [45]. However, the rate of hydrolysis under single turnover conditions is too slow to be involved under the catalytic turnover conditions [48] and most likely TPQ_{imq} reacts directly with amine substrate through a transimination reaction leading to the Schiff base product bypassing TPQ_{ox}.

Although there is no question regarding the crucial role of Cu^{II} ion in the biogenesis of TPQ from the autocatalytic post-translational oxidation of a tyrosine residue [9,49,50], contrastingly, there is no consensus as to whether Cu^{II} plays a redox role in catalysis, in other words whether the semiquinone TPQ_{sq} is on the reaction pathway [51-57]. The role of Cu^{II} in the oxidative half reaction has been partially clarified by studying copper-free and metal-substituted forms of CuAOs [58-60]. While the evidence from bacteria and plant amine oxidases supports transient reduction of copper, with TPQ_{sq} being the species that preferentially reacts with dioxygen (see brackets in scheme 6) [59,61,62], in the oxidative half reaction of mammalian amine oxidases, the electron transfer occurs directly between the

1 aminoquinol form TPQ_{red} and dioxygen, without change in the redox state of copper
2
3 and without TPQ_{sq} on the reaction pathway [60]. Nevertheless, copper could play a
4
5 role in orienting the TPQ ring correctly in the active site.
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8 9 10 **A small molecule that replicates the metabolic activity of CuAOs**

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12 A few years ago, we described a small molecule **1_{ox}** (Scheme 7) which behaves as an
13
14 effective biomimetic catalyst for the oxidation of primary aliphatic monoamines,
15
16 under metal- free conditions [63]. Note the structural analogy of **1_{ox}** with the active
17
18 form TPQ_{imq} (Scheme 6). As **1_{ox}** is an unstable *o*-iminoquinone species, it is
19
20 generated in situ through an electrochemical oxidation process. The catalytic cycle
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22 produced the reduced catalyst **1_{red}** and *N*-alkylidenealkylamine as the product of
23
24 amine oxidation. This process is the analog of the ping pong mechanism above
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26 described for the CuAO enzymes, but *N*-alkylidenealkylamine was evidenced
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28 instead of aldehyde, because methanol was used as the solvent in place of aqueous
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30 solution. Consequently, no hydrolysis into aldehyde took place during the catalytic
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32 process.
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40 This biomimetic electrocatalytic system displayed two features that are most
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42 often associated with enzymatic systems: (a) the reaction was enhanced through the
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44 participation of 1-acetyl and 2-hydroxyl substituents as they prevented the
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46 competing formation of Michael adducts; (b) the presence of the active 2-hydroxyl
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48 group, which was engaged in an intramolecular hydrogen bond with the imine
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50 nitrogen to form a highly reactive Schiff base cyclic transition state **1'_{ox}**, constituted
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52 a prerequisite to the development of the catalytic process (Scheme 7) [64].
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1 Using diverse endogenous and xenobiotic primary amines, we found that **1_{ox}**
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3 presented the chemoselectivity observed for the CuAOs enzymes, that is high
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5 reactivity with unbranched primary amines and with the primary amino group of
6
7 diamines and polyamines (Table 2). Overall, the electrocatalytic system **1_{ox}** mimics
8
9 not only the metabolic activity of PrAO enzymes, as high catalytic performances
10
11 have been observed with primary monoamines (benzylamine, aminoacetone,
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13 propylamine and methylamine) and the terminal primary amino group of
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15 spermidine, but also that of DAOs as shown by the data obtained with putrescine,
16
17 and to a lesser extent histamine [65]. Contrary to FAD-dependent amine oxidases,
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19 no activity was observed with secondary and tertiary amines (Table 3). Finally, a
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21 last question emerges whether selective CuAOs inhibitors such as LJP 1586 can also
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23 prevent the activity of the electrocatalyst **1_{ox}**. This study is currently envisioned in
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25 our laboratory because, in the affirmative, this small molecule might be used for a
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27 preliminary screening of potential inhibitors of CuAO enzymes.
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Conclusions

Interest in CuAOs has greatly increased in recent years, especially since the discovery that vascular adhesion protein 1 belongs to the CuAOs family. Thus, it has been established that CuAOs play a vital role in the physiology and pathology of mammals in controlling the metabolism of various primary monoamines, diamines and polyamines of endogenous or xenobiotic origin. As most consequences are pathological, effective inhibitors of CuAOs are of current interest because of their desired applications as therapeutic agents. We can now expect clinical study results that show whether control of CuAOs activity may serve as a new tool for treating various diseases. In this respect, the same toxic metabolites, formaldehyde and methylglyoxal, derived from CuAOs-mediated deamination of methylamine and aminoacetone respectively, cause proteins to aggregate and to crosslink, modifications which originate Alzheimer's disease and diabetes. This is consistent with recent studies providing strong evidence that Alzheimer's disease represents a form of diabetes that selectively involves the brain, hence the designation of Alzheimer's disease as "type 3 diabetes" [66,67]. Finally, CuAOs may be a potential target for the treatment of Alzheimer's disease and diabetes and no doubt the future of CuAOs research appears very promising.

Conflict of interest

None.

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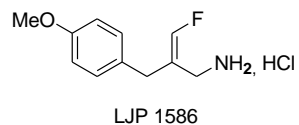
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3 **Table 1** Selective inhibition of LJP 1586 for PrAOs
4 *Inhibition sélective de LJP 1586 pour les PrAOs*
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Human Enzyme	UC PrAO	CHO PrAO	DAO	MAO A	MAO B
IC ₅₀ (μM)	0.027	0.043	96	98	2.2

20 UC : umbilical cord

21 CHO : lysates of human PrAO-expressing CHO cells
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Table 2 I_{ox} -mediated oxidation of CuAOs mono- and polyamine substrates
Oxydation de mono- et polyamines substrats des CuAOs catalysée par I_{ox}

Amine substrate	Catalytic efficiency (%) ^a
benzylamine	100
methylamine	50 ^b
propylamine	70
aminoacetone	94
histamine	20 ^c
putrescine	88 ^c
spermidine	94 ^c

(I_{red}) = 0.4 mM, (amine substrate) = 20 mM, MeOH, rt, Pt anode (E = + 0.6 V vs SCE). ^a imine was isolated by converting to the 2,4-dinitrophenylhydrazone obtained after workup of the oxidized solution with 2,4-dinitrophenylhydrazine under aqueous acidic conditions. ^b Volatile reaction products were partly lost during the anodic electrolysis. ^c The Pt anode was replaced by a Hg anode (E = 0.0 V vs SCE) because polyamines spontaneously attached to the Pt electrode surface.

Table 3 Substrate specificity for amine oxidase enzymes in comparison with mimic **1_{ox}**
Comparaison de la spécificité de substrats des enzymes amine oxydases à celle du mime 1_{ox}

Substrate	MAO A and B FAD	PAO FAD	CuAO TPQ	Mimic 1_{ox}
Primary amine (unbranched)	×	–	×	×
Secondary amine	×	–	–	–
Tertiary amine	×	–	–	–
Diamine	–	–	×	×
Polyamine (<i>primary amino group</i>)	–	–	×	×
Polyamine (<i>secondary amino group</i>)	–	×	–	–

(×) catalytic activity; (–) no catalytic activity

1 **Figure and Scheme Captions**
2
3

4 **Figure 1.** Chemical structures of flavine adenine dinucleotide (FAD), topaquinone
5 (TPQ) and lysine tyrosylquinone (LTQ) redox cofactors.
6

7 *Structure chimique des cofacteurs redox, flavine adenine dinucléotide (FAD),
8 topaquine (TPQ) et lysine tyrosylquinone (LTQ).*
9
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12
13 **Scheme 1.** Postulated metabolism of the antihypertensive agent amlodipine by
14 SSAOs: the conversion of the amine function present in the amino side chain into an
15 aldehyde group is compatible with the implication of SSAOs in amlodipine
16 metabolism.
17
18

19 *Métabolisme supposé de l'antihypertenseur amlodipine par les SSAOs : la
20 conversion de la fonction amine présente sur la chaîne latérale aminée, en
21 groupement aldéhyde, est compatible avec l'implication des SSAOs dans le
22 métabolisme de l'amlodipine.*
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29 **Scheme 2.** Postulated metabolism of the cytoprotective drug amifostine (WR 1065).
30 The primary amine function of WR 1065 is converted by CuAOs to an aldehyde
31 which spontaneously decomposes into cysteamine and acrolein.
32
33

34 *Métabolisme supposé du médicament cytoprotecteur amifostine (WR 1065). La
35 fonction amine primaire du WR 1065 est convertie par les CuAOs en aldéhyde qui
36 se décompose spontanément en cystéamine et acroléine.*
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42 **Scheme 3.** Metabolic role of CuAOs : they regulate levels of endogenous and
43 xenobiotic primary mono- and polyamines, by catalyzing their oxidative
44 deamination with the concomitant production of hydrogen peroxide (H₂O₂),
45 ammonia (NH₃) and aldehyde (CHO). Furthermore, H₂O₂ can act as a source of
46 hydroxide radicals (HO[•]) produced through the Fenton reaction.
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50 *Rôle métabolique des CuAOs : elles régulent les niveaux des mono- et polyamines
51 primaires, endogènes et xénobiotiques, en catalysant leur désamination oxydante
52 avec production concomitante de peroxyde d'hydrogène (H₂O₂), ammoniac (NH₃) et
53*
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1 aldéhyde (CHO). De plus, H_2O_2 peut agir comme source de radicaux hydroxyle
2
3 ($HO\cdot$) produits via la réaction de Fenton.
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7 **Scheme 4.** oxidative metabolism of methylamine into formaldehyde (a) and
8
9 aminoacetone into methylglyoxal (b) by PrAOs

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11 *Métabolisme oxydant de la méthylamine en formaldéhyde (a) et de l'aminocétone*
12
13 *en méthylglyoxal (b) par les PrAOs*
14

15
16 **Scheme 5.** oxidative metabolism of spermidine by PrAOs : the oxidative
17
18 deamination of spermidine produces an unstable aldehyde which spontaneously
19
20 decomposes into the highly toxic acrolein and putrescine products.

21
22 *Métabolisme oxydant de la spermidine par les PrAOs : la désamination oxydante de*
23
24 *la spermidine produit un aldéhyde instable qui se décompose spontanément en*
25
26 *acroléine, hautement toxique, et en putrescine.*
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29 **Scheme 6.** Proposed reaction mechanisms of CuAOs [9]: The reductive half reaction
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31 (ping), which involves amine oxidation into aldehyde, results in the reduction of
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33 TPQ_{ox} into an aminoquinol form TPQ_{red} . The oxidative half reaction (pong)
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35 corresponds to the reduction of dioxygen to hydrogen peroxide, with concomitant
36
37 formation of TPQ_{imq} . Most likely TPQ_{imq} reacts directly with amine substrate
38
39 through a transimination reaction leading to the Schiff base product bypassing
40
41 TPQ_{ox} .
42
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46 *Mécanisme réactionnel proposé pour les CuAOs [9] : La demi-réaction de*
47
48 *réduction (ping), qui implique l'oxydation de l'amine en aldéhyde, entraîne la*
49
50 *réduction de TPQ_{ox} en une forme aminoquinol TPQ_{red} . La demi-réaction*
51
52 *d'oxydation (pong) correspond à la réduction de l'oxygène moléculaire en peroxyde*
53
54 *d'hydrogène, avec formation simultanée de TPQ_{imq} . Très vraisemblablement,*
55
56 *TPQ_{imq} réagit directement avec l'amine substrat selon une réaction de*
57
58 *transimination pour donner la base de Schiff produit sans repasser par TPQ_{ox} .*
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1 **Scheme 7.** I_{ox} -mediated catalytic oxidation of primary aliphatic amines into imines: the
2
3 catalyst I_{ox} is generated in situ through an electrochemical oxidation at a platinum
4
5 anode, in methanol. The catalytic cycle produces the reduced catalyst I_{red} and *N*-
6
7 alkylidenealkylamine as the product of amine oxidation.
8
9

10 *Oxydation d'amines aliphatiques primaires en imines catalysée par I_{ox} : le*
11 *catalyseur I_{ox} est généré in situ par oxydation électrochimique, à une anode de*
12 *platine, dans le méthanol. Le cycle catalytique produit la forme réduite du*
13 *catalyseur I_{red} et une *N*-alkylidènealkylamine comme produit d'oxydation de*
14 *l'amine.*
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Figure 1

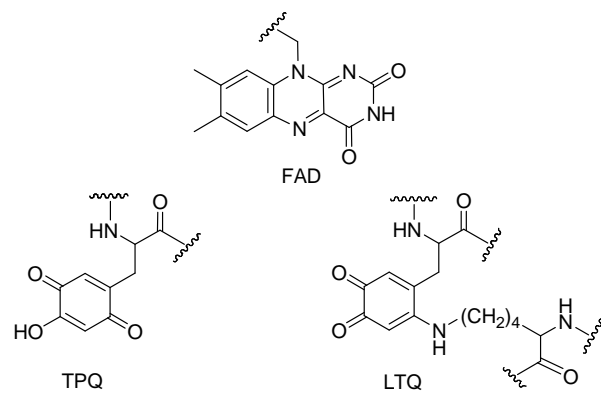
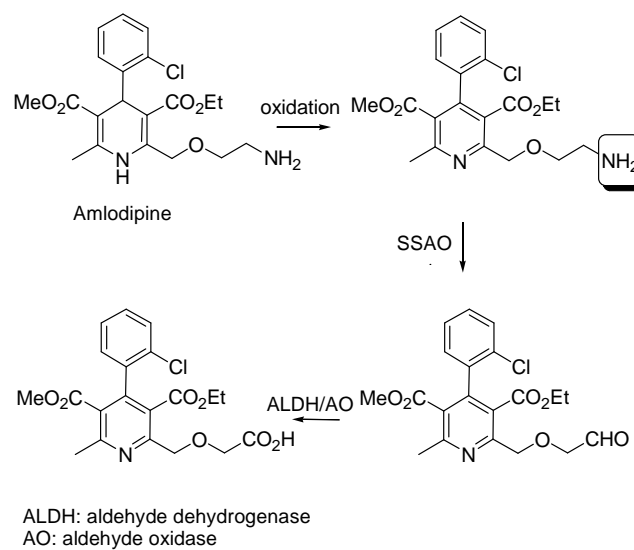


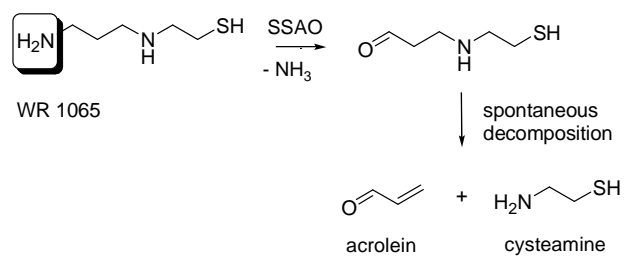
Figure 1

Scheme 1



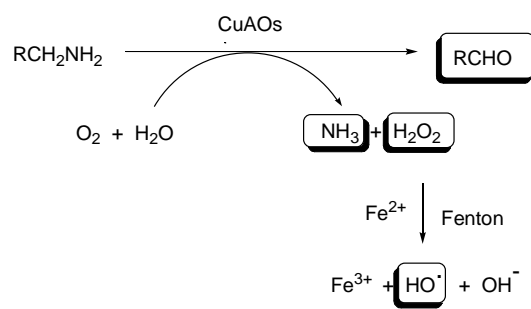
Scheme 1

Scheme 2



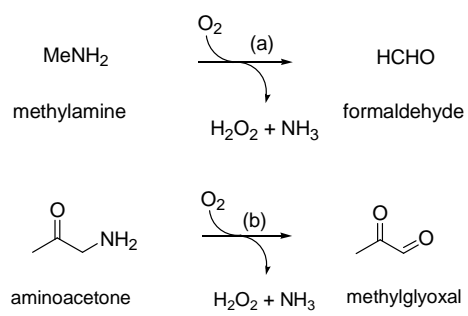
Scheme 2

Scheme 3



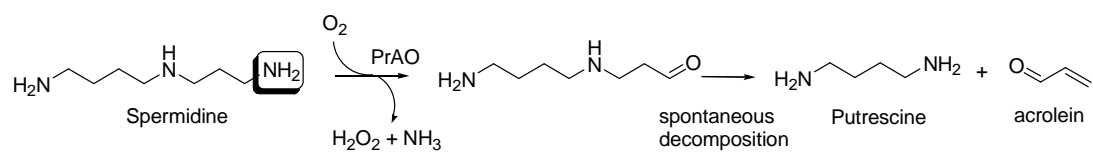
Scheme 3

Scheme 4

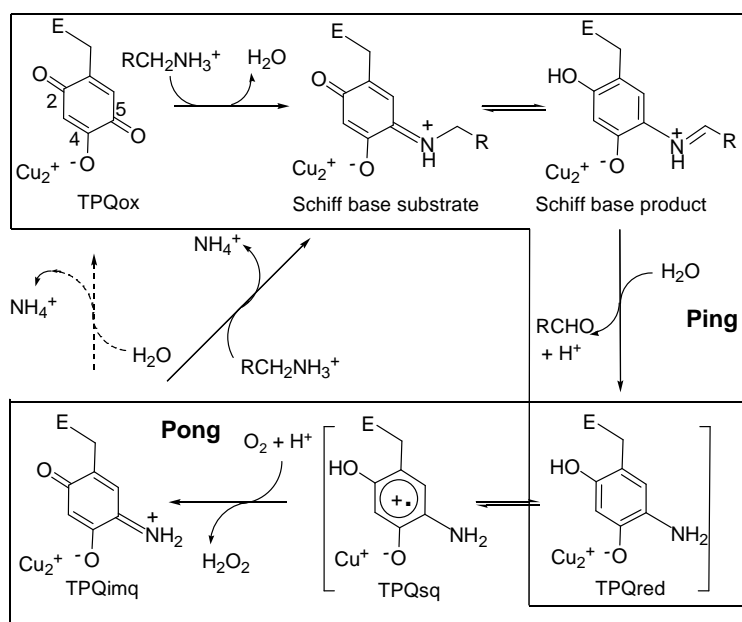


Scheme 4

Scheme 5



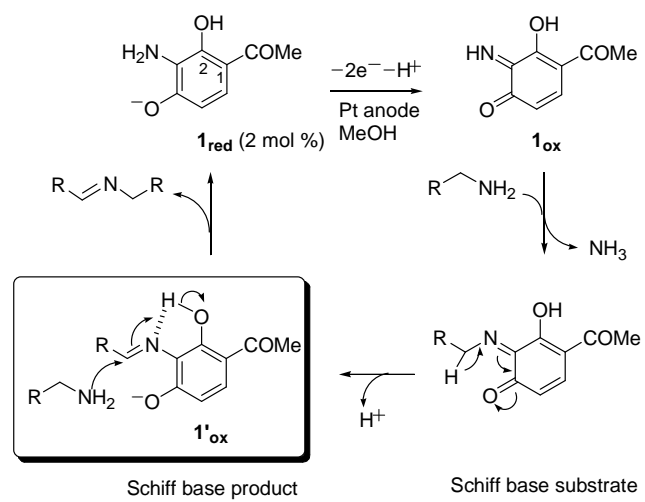
Scheme 5



sq: semiquinone; imq: iminoquinone

Scheme 6

Scheme 7



Scheme 7

Response to reviewer 1

Modifications are indicated in yellow in the manuscript

Point 1. Detailed descriptions of figure and schemes are now included.

Point 2. Physiological observations that the activities of CuAOs are increased in some human diseases have been made **in plasma**. So, plasma is now indicated in the manuscript page 8.

Réponse aux commentaires du rapporteur 2

Les modifications sont indiquées en jaune dans le manuscrit

Point 1. Les abréviations demandées ont été insérées.

Point 2. Le schéma 1 a été corrigé

Point 3. La légende du tableau a été modifiée comme suit :

Selective inhibition of LJP 1586 for PrAOs

Inhibition sélective de LJP 1586 pour les PrAOs

Point 4. Les références 50, 51, 60 et 67 ont été corrigées.