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Synthesis of 2,6-disubstituted benzylamine derivatives as reversible selective inhibitors of copper amine oxidases

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ABSTRACT

In order to obtain substrate-like inhibitors of copper amine oxidases (CAOs), a class of enzymes involved in important cellular processes as well as in crosslinking of elastin and collagen and removal of biogenic primary amines, we synthesized a set of benzylamine derivatives properly substituted at positions 2 and 6 and studied their biological activity towards some members of CAOs.

With benzylamines **6**, **7**, **8** containing linear alkoxy groups we obtained reversible inhibitors of benzylamine oxidase (BAO), very active and selective toward diamine oxidase (DAO), lysyl oxidase (LO) and monoamine oxidase B (MAO B) characterized by a certain toxicity consequent to the crossing of the brain barrier. Poorly toxic, up to very active, reversible inhibitors of BAO, very selective toward DAO, LO and MAO B, were obtained with benzylamines **10**, **11**, **12** containing hydrophilic ω-hydroxyalkoxy groups. With benzylamines **13**, **14**, **15**, containing linear alkyl groups endowed with steric, but not conjugative effects for the absence of properly positioned oxygen atoms, we synthesized moderately active inhibitors of BAO reversible and selective toward DAO, LO and MAO B.

The cross examination of the entire biological data brought us to the conclusion that the bioactive synthesized compounds most likely exert their physiological role of reversible inhibitors in consequence of the formation of a plurality of hydrogen bonds or hydrophobic non-covalent interactions with proper sites in the protein. Accordingly, the reported inhibitors may be considered as a set of research tools for general biological studies and the formation of enzyme complexes useful for X-ray structure determinations aimed at the design of more sophisticated inhibitors to always better modulate the protein activity without important side effects.

1. Introduction

Copper amine oxidases[1] (CAOs, EC 1.4.3.6) are ubiquitous enzymes which control important cellular processes as well as crosslinking of elastin and collagen, by catalyzing the oxidative deamination of primary amines RCH_2NH_2 according to Eq. (1).

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCH{=}O + NH_3 + H_2O_2 \quad (1)$$

Pluridecennial studies and several X-ray structures of copper amine oxidases from bacteria [*Escherichia coli* amine oxidase (ECAO)[2] and *Arthrobacter globiformis* amine oxidase (AGAO)[3]], yeast [*Hansenula polymorpha* amine oxidase (HPAO)[4] and *Pichia pastoris* lysyl oxidase (PPLO)[5]], plants [*Pisum sativum* pea seedling amine oxidase (PSAO)][6] and mammals [*Homo sapiens* diamine oxidase (hDAO)][7] allowed to ascertain that all CAOs are homodimers having one copper ion Cu^{2+} and one quinone cofactor per subunit, and variable percentages of a carbohydrate portion depending on the enzyme source.

For the various typologies of CAOs, excluding lysyl oxidase (LO), the cofactor is 2,4,5-trihydroxyphenylalanine quinone (TPQ) which is connected to the protein through one covalent bond enabling TPQ to assume two conformations, active and inactive, influencing the enzyme reaction mechanism. For LO the cofactor is lysine tyrosylquinone (LTQ)[8] stably connected to the protein through two covalent bonds.

Different typologies of CAOs have 'substrate channels' of different dimensions, from very narrow to very wide, in agreement with the needs of their preferential substrates.

The X-ray structures of CAOs and of several complexes of CAOs with inhibitors of different types, together with the bulk of results from previous studies, allowed to conceive a reasonable pathway for a ping-pong enzyme reaction mechanism corresponding to a sequence of reductive and oxidative half reactions. In the reductive half reaction the substrate amine reacts with TPQ to form a

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quinoneimine (substrate Schiff base) in tautomeric equilibrium with a quinoaldimine (product Schiff base) which hydrolyzes to release aldehyde and to produce the reduced aminoresorcinol form of TPQ (Scheme 1).

In the oxidative half reaction the reduced cofactor in the presence of molecular oxygen is reoxidized to TPQ with releasing of hydrogen peroxide and ammonia (Scheme 2).

Inhibitors of different structure succeed in trapping CAOs in stable complexes through different mechanisms of action mainly disclosed through X-ray structure determinations as cited in the following examples.

The 2-hydrazinopyridine inhibits ECAO by covalently binding at position 5 of the quinone ring of TPQ in a manner similar to a substrate, but the produced hydrazone analogue to a substrate Schiff base, having a nitrogen atom in place of a CH group, prevents any proton abstraction, thus trapping the enzyme in a covalent complex.[9]

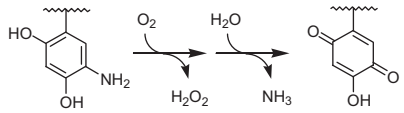
With AGAO the inhibitor 4-(2-naphthyloxy)-2-butyn-1-amine begins to react as a substrate producing aldehyde and transforming the cofactor into the 5-amino form of the reduced TPQ, but such amino group binds covalently to the triple bond present in the aldehydic product giving a stable system which does not allow enzyme regeneration.[10]

Among the two *trans* enantiomers *(1S,2R)*-(+)-*trans*-2-phenylcyclopropylamine [(+)-TCP] and *(1R,2S)*-(−)-*trans*-2-phenylcyclopropylamine [(−)-TCP] only the first one inhibits ECAO. Such enantiomer can place itself into the substrate channel and form the Schiff base at position 5 of TPQ, but the hydrogen on the carbon of the cyclopropyl ring adjacent to the nitrogen linked to TPQ, pointing away from the proton abstraction site on the protein chain, cannot be removed and the enzyme reaction is blocked.[11] Nevertheless, extensive dialysis of the inhibited enzyme removed TCP, restoring the enzyme activity and indicating that binding is reversible.[12]

Berenil [1,3-bis(4′-amidinophenyl) triazene] and Pentamidine [1,5-bis(4-amidinophenoxy) pentane] are excellent inhibitors of hDAO through non-covalent binding.[7] They occupy the narrow asymmetrical cone-shaped cavity of the substrate channel adopting a conformation essentially straight for Berenil and horseshoe-shaped for Pentamidine, blocking the access of substrates to TPQ. Though not covalently bonded to the protein, these substances form a very stable system based on hydrogen bonds and hydrophobic interactions.

Two racemic aryl 2,3-butadienamine analogues, such as 5-phenoxy-2,3-pentadienylamine (POPDA) and 6-phenyl-2,3-hexadienylamine (PHDA), are inhibitors of AGAO.[13] Initially they react with AGAO as substrates giving the corresponding aldehyde and the 5-amino form of the reduced TPQ, but the amino group further reacts with the activated system of the allene aldehydes attacking different carbon atoms either with POPDA or PHDA. In both cases, trapping TPQ in covalent complexes causes the inactivation of the enzyme.

Three hydrazine derivatives, such as benzylhydrazine (BHZ), 4-hydroxybenzylhydrazine (4-OH-BHZ) and phenylhydrazine (PHZ), structural analogues of 2-phenylethylamine, tyramine and benzylamine proved to be the first two good substrates of AGAO and the last poor. The X-ray structures of complexes of these inhibitors with AGAO evidenced the formation of covalent hydrazone



Scheme 2. Oxidative half-reaction of TPQ cofactor.

adducts with TPQ, structural analogous to the substrate Schiff base of TPQ[14] in accordance with the behaviour of 2-hydrazinopyridine towards ECAO (see above). The comparative study of the structures of such adducts with the three different hydrazine derivatives provided relevant structural insights into the substrate specificity of AGAO.

All the above reports prove the current and prominent interest for new inhibitors of CAOs as tools for disclosing the complex biological role of each of these enzymes often endowed with broad substrate specificity adapted to a variety of physiological needs. In view of pharmaceutical use of CAOs inhibitors, especially if irreversible, such peculiar substrate specificity entails severe risk of a complete enzyme inactivation which insidiously can open the way to noxious interferences.[7,15–19]

In this light, reversible inhibitors of CAOs forming rather stable complexes with the protein on the basis of non-covalent interactions give rise to relevant expectations for bioactive molecules able to selectively modulate the protein activity, avoiding the possible risks inherent in irreversible inhibitors.[7,20]

Some of us prepared and studied[21] the first substrate-like, very active, fully reversible inhibitors of different CAOs corresponding to derivatives of 4-aminomethylpyridine with alkoxy (Series 1), alkylthio (Series 2) and alkylamino groups (Series 3) at positions 3 and 5 of the ring, or with alkylamino groups at position 3 (Series 4).

The inhibitory activity of the prepared compounds was tested on different CAOs such as diamine oxidase of porcine kidney (DAO), benzylamine oxidase of porcine serum (BAO), lysyl oxidase of porcine aorta (LO), pea seedling amine oxidase (PSAO), *Hansenula polymorpha* amine oxidase (HPAO), and FAD monoamine oxidases of rat liver (MAO A and MAO B).

Series 1 contained reversible, very active inhibitors of BAO, selective with respect to DAO, LO, PSAO, HPAO, MAO A and MAO B.

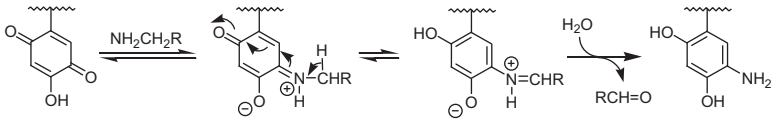
Series 2 contained reversible inhibitors of BAO very selective with respect to DAO and MAO, unexpectedly affording an interesting new type of good substrate of DAO.

Series 3 allowed to suppress the selectivity especially between BAO and DAO, containing reversible inhibitors very active on both enzymes and active on MAO A, MAO B, PSAO and HPAO.

Series 4 contained reversible non-selective inhibitors very active on DAO, BAO and PSAO, and active on HPAO, MAO A and MAO B.

Kinetic experiments on the enzymatic reactions performed with 4-aminomethylpyridine and benzylamine as substrates of DAO and BAO showed an interaction for the pyridine ring stronger than that of benzene ring with both the enzymes.

It is interesting to note that the fully reversible inhibitor 3,5-diethoxy-4-aminomethylpyridine is well absorbed orally in rats with a bioavailability of 51.5% undergoing a rather fast transformation with a blood half-life of 2.0 h.[22]



Scheme 1. Reductive half-reaction of TPQ cofactor.