

Monoamine Oxidase Inhibition In the Light of New Structural Data

M. Reyes-Parada^{1,*}, A. Fierro², P. Iturriaga-Vásquez³ and B.K. Cassels^{3,4}

¹ Department of Neuroscience, Faculty of Medical Sciences, University of Santiago, Chile

² Faculty of Chemistry and Biology, University of Santiago, Chile

³ Millennium Institute for Advanced Studies in Cell Biology and Biotechnology

⁴ Department of Chemistry, Faculty of Sciences, University of Chile

Abstract: The recent description of the crystal structures of rat MAO-A and human MAO-B provides an unprecedented framework to elucidate the mechanisms underlying the selective interactions between these proteins and their ligands. The analysis of previous and emerging data, in the light of the structural similarities and differences between both isozymes, allows a better understanding of the requirements that determine the affinity and selectivity of substrates and inhibitors. This augurs a new impulse for the rational design of potent and selective MAO inhibitors with therapeutic potential.

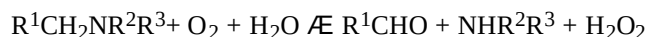
Keywords: Monoamine oxidase, MAO inhibitors, structure-activity relationships, crystal structures.

This is an exciting time for scientists working on monoamine oxidase. The recent description of the crystal structures of the two isoforms of the enzyme provides a nurturing framework to elucidate the mechanisms underlying the selective interactions between these proteins and their ligands, to probe the catalytic mechanism and to gain a better understanding of the pharmacophoric requirements necessary for the rational design of potent and selective enzyme inhibitors with therapeutic potential.

Several reviews concerning different aspects of monoamine oxidase research have appeared during the last two years [1-10 to name a few]. Specifically, the structural aspects of the membrane binding and the active site (inhibitor/substrate binding) domains of human monoamine oxidase type B, and the local environment of the FAD binding site and its effects on function, have been extensively reviewed with reference to the recently described crystal structures of the free enzyme and its complexes with several inhibitors [11-14]. These aspects are therefore summarily discussed here. The attention of the present paper is focused on a review of the structural similarities and differences between the two enzyme isoforms and the consequences that these might have for function and drug design.

GENERAL BACKGROUND

Monoamine oxidase (monoamine oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4; MAO) exists in two isoforms termed MAO-A and MAO-B. Both isozymes are outer mitochondrial membrane-bound flavoproteins, with the FAD cofactor covalently bound to the protein. Both catalyse the oxidative deamination of primary amines as well as some secondary and tertiary amines according to the overall reaction



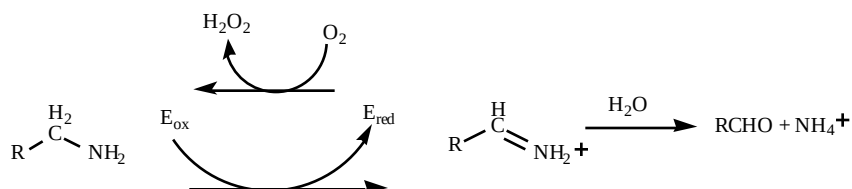
The metabolic reaction involves the generation of an imine intermediate and the reduction of the flavin cofactor, which is reoxidised by molecular oxygen producing hydrogen peroxide. The imine intermediate is hydrolysed, in a non-enzymatic process, generating ammonia and the corresponding aldehyde (Scheme 1 exemplifies this for a primary amine).

Physiologically, MAOs oxidize biogenic neurotransmitters such as dopamine (**1**), noradrenaline (**2**), serotonin (5-HT, **3**) and b-phenethylamine (PEA, **4**), and also dietary and xenobiotic amines such as tyramine (**5**), benzylamine (**6**) and the parkinsonism-producing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **7**) [1,15-17].

Due to the role of MAOs in the metabolism of monoamine neurotransmitters, MAO inhibitors are useful tools in the treatment of diverse neuropsychiatric and neurological disorders. In particular, selective and reversible MAO-A inhibitors are used as effective antidepressant and anti-anxiety drugs, whilst selective MAO-B inhibitors are used in the treatment of Parkinson's disease [18-21]. Selective inhibitors of each isoform are currently under clinical investigation for their use as antidepressants and for the treatment of Parkinson's and Alzheimer's diseases and related disorders [22-26].

Although both isoforms have similar catalytic activities, they differ in their molecular genetics, substrate preference, inhibitor selectivity and tissue distribution. MAO-A and MAO-B are encoded by separate genes situated on the X chromosome [27,28] and have identical intron-exon organization, suggesting that both proteins are derived from duplication of a common ancestral gene [29]. Amino acid sequences of both enzymes from several species including human [30,31] and rat [32,33] have been elucidated by cDNA cloning. These studies have shown that both proteins have molecular weights of ~60 kDa and that the isoforms from the same species show about 70 % identity. 85-88 % identity is observed between the same isoforms from human and rat [see 34 for a recent review].

*Address correspondence to this author at the Department of Neuroscience, Faculty of Medical Sciences, University of Santiago, Alameda 3363, Santiago, Chile; E-mail: mreyes@usach.cl



Scheme 1.

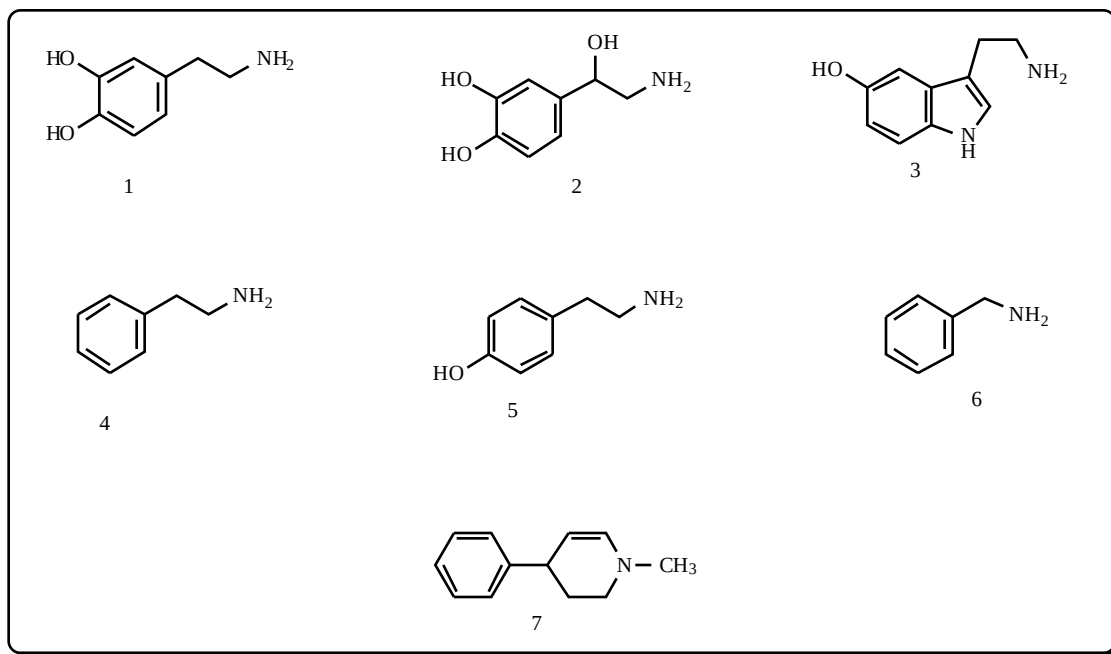
MAO-A preferentially metabolises 5-HT and is irreversibly inhibited by nanomolar concentrations of clorgyline (**8**) [35], whereas MAO-B preferentially catalyses the oxidative deamination of PEA and benzylamine and is irreversibly inhibited by nanomolar concentrations of *l*-deprenyl (**9**). Dopamine and tyramine are non-selective substrates of both isoforms. MAO-A-deficient humans [37] and mice [38] exhibit elevated brain levels of 5-HT and noradrenaline and MAO-A KO mice have a phenotype characterized by increased aggressive behaviour. Increased levels of PEA but not 5-HT, noradrenaline or dopamine are observed in MAO-B KO mice, and these animals are resistant to the Parkinsonism induced by MPTP [39]. Both isozymes are present in most mammalian tissues, with the human placenta (MAO-A) and platelets (MAO-B) being two of the few tissues that have been shown to express predominantly one form of the enzyme [20,40]. In the CNS, catecholaminergic neurons contain predominantly MAO-A, whereas serotonergic neurons express MAO-B [41,42]. This rather unexpected neuronal distribution (MAO-B in 5-HT containing neurons), has been suggested to play a role in protecting these cells from foreign monoamines which may act as false neurotransmitters [20,41]. The specific expression of MAO-B in 5-HT neurons and its proposed role, has also been implicated as one of the important mechanisms underlying the serotonergic neurotoxicity induced – presumably via H_2O_2 generation – by the commonly abused drug methylenedioxymethamphetamine (MDMA, also known as Ecstasy) [43,44].

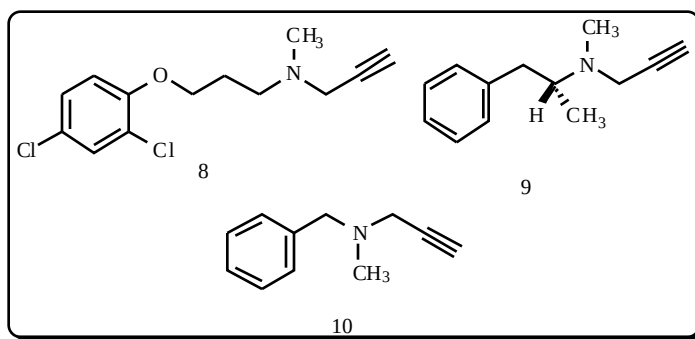
GENERAL FEATURES OF RAT MAO-A AND HUMAN MAO-B AS REVEALED BY THEIR CRYSTAL STRUCTURES

High level expression of MAO in *Saccharomyces cerevisiae* [45] or *Pichia pastoris* [46] provided the systems that yield the large quantities of homogeneous and functional enzyme required for detailed structural studies.

The crystal structure of human MAO-B, both free and covalently complexed with pargyline (**10**; PDB code 1GOS) was originally described at 3 Å resolution [47]. The enzyme crystallized as a dimer in two different crystal forms (orthorhombic and triclinic), which show a significant monomer-monomer interaction area (~ 15 % of the accessible monomer surface), suggesting that the dimeric assembly may also occur in its *in vivo* membrane environment. Improved crystal quality allowing resolutions up to 1.6 Å have been reported by the same authors [48,49] for human MAO-B forming complexes with several structurally diverse, reversible and irreversible inhibitors (PDB codes 1OJA, 1OJB, 1OJC, 1OJD, 1OJ9, 1S2Q, 1S2Y, 1S3E, and 1S3B).

More recently, the crystal structure of rat MAO-A, in complex with clorgyline (PDB code 1O5W), has been determined at 3.2 Å resolution [50]. Although the enzyme packed in a tetrameric formation in the crystal (a dimer of dimers), this form was disregarded as the biologically relevant structure, since the membrane-binding C termini of one dimer extended in a different direction from those in





the other dimer. As in human MAO-B, an extensive monomer-monomer interaction area (~ 11 % of the accessible monomer surface) was observed in each dimer of rat MAO-A. Thus, considering the similarities in the overall folding between crystal dimeric assemblies of human MAO-B and rat MAO-A, it has been suggested that the latter also forms a dimeric structure *in vivo*. Fig. (1) shows a schematic representation of the MAO-A and MAO-B monomers.

THE MEMBRANE BINDING REGION

MAO-A and MAO-B are tightly bound to the outer mitochondrial membrane. Different reports had either shown [51,52] or suggested [53] that the C-terminal tail of MAO was essential for attachment of the protein to the membrane. Crystal structures have confirmed that the globular portion of each monomer in both isozymes is attached to a C-terminal hydrophobic α -helix which is presumably inserted into the membrane in an orientation almost perpendicular to the lipid bilayer. In addition, the dimer structure predicts no interactions between the helices from each monomer.

Remarkably, Ma *et al.* [50] were able to crystallize MAO-A monomers showing helical C-terminal structures consisting of five or five and half turns in which only the last five to six amino acid residues were not visible in the electron density map. The length of these α -helices (~ 30 Å), which is consistent with a protein segment almost completely traversing a lipid bilayer, and the hydrophilic characteristics of some of the missing amino acid residues, strongly suggest that the C-terminal assembly observed in the crystal structure of rat MAO-A is the same as that present *in vivo*, and that MAO-A is a transmembrane protein. Although a similar arrangement for the C-terminal helix is predicted in the case of human MAO-B, the fact that the last 20 amino acid residues appear disordered in the crystal structure, has so far precluded any certainty as to whether this isoform also has a transmembrane domain. Indeed, an alternative membrane anchoring model in which the last residues, rather than traversing the lipid bilayer, may turn back to position the C-terminal on the same side of the outer membrane surface where the main body of the MAO-B dimer is located, has been proposed [11].

From the analysis of the crystal structures, several other hydrophobic and electrostatic interactions have been

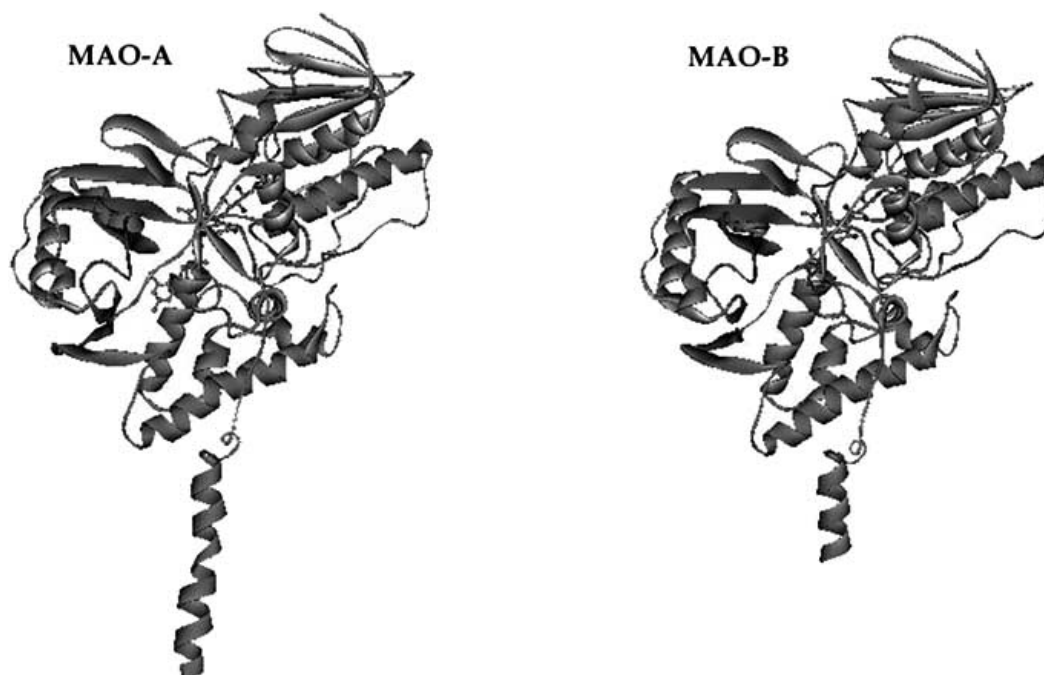


Fig. (1). Schematic representation of the MAO-A and MAO-B monomers in complex with clorgyline (1O5W) and isatin (1OJA) respectively. The FAD cofactor and the inhibitors are shown as ball-and-stick figures.

proposed to be implicated in MAO-membrane binding. This is in agreement with data showing that truncation of a significant part or complete deletion of the C-terminal produced analogues of the enzyme that still retained affinity for membranes [51-53]. Indeed, in the case of rat MAO-A, a peptide sequence preceding the C-terminal helix (residues 489-493) is predicted to be helically buried in the membrane [50]. In human MAO-B, a longer sequence of eight amino acids (residues 481- 488) which includes the same residues discussed above for MAO-A, has been regarded as being involved in the protein-membrane interaction, but current data do not determine if this peptide may actually be inserted into the membrane [11,47].

In spite of the subtle differences discussed, it is clear that both MAO-A and MAO-B interact significantly with the mitochondrial membrane. The attraction of protonated substrates to the negatively charged membrane surface and the consequent increase in their local concentration near the catalytic site has been proposed as one of the possible roles of this interaction [11,14].

THE FAD BINDING DOMAIN

It has been known for several years that FAD is covalently bound to MAO via a thioether linkage between the 8a-methyl group of the cofactor and Cys406 or Cys397 in MAO-A and MAO-B respectively [54,55].

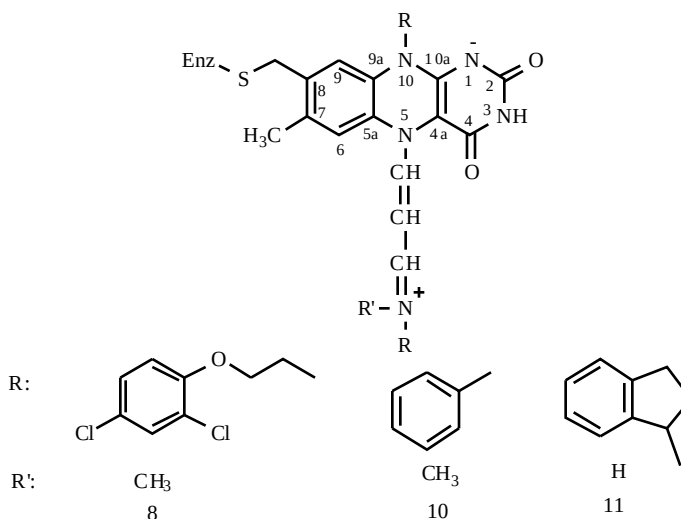
Crystallographic data confirmed this information, revealing that the FAD binding domain is buried inside the molecules with the active site situated on the *re* side of the flavin ring, and showed that there is an extensive array of hydrophobic and hydrogen bonding interactions between MAO-A or MAO-B and the cofactor. As expected, the conserved character of the amino acid residues that were originally described to interact with all the structural elements of FAD in MAO-B, determines that the nature of these interactions is very similar in MAO-A as well. This information also strongly supports the notion that covalent flavin linkage [56-58] and non-covalent FAD interactions [45,59,60] play a critical role in the stabilization of enzyme

structure [See 13 for a recent review]. In this sense, the interaction established between Lys296 of MAO-B (Lys305 in MAO-A) and the isoalloxazine ring of the cofactor deserves mention. This amino acid residue is hydrogen-bonded to N5 of the flavin ring through a water molecule [12,48,49], constituting a Lys-H₂O-flavin N5 substructure which has also been found in some other flavoenzyme oxidases, and which therefore is thought to be a structural motif of these enzymes. Although the resolution of the MAO-A crystal structure does not allow the presence of a water molecule bridging Lys305 with the N5 atom of the flavin to be definitely established, the conserved character of the FAD-enzyme interactions suggests that this feature might also be present in MAO-A. Interestingly, site-directed mutagenesis studies have shown that substitution of Lys305 or Lys296 by an alanine, in human MAO-A and -B respectively, resulted in the complete loss of catalytic activity [61].

Another striking feature of FAD seen in both enzyme crystal structures is that the isoalloxazine ring is in a bent conformation, differing from the planar structure observed for this aromatic system in solution. It should be noted that the dihedral angle formed by the median planes of the benzene and the pyrimidinedione rings of the flavin appears to be slightly less in MAO-A than in MAO-B. As discussed by Edmondson *et al.* [13,14] for MAO-B and Trickey *et al.* [62] for trimethylamine dehydrogenase (an FMN-dependent dehydrogenase that also shows a high degree of butterfly bending on its flavin cofactor), this bent structure of the flavin ring might facilitate the catalysis and the formation of adducts at either the N(5) or the C(4a) positions of the isoalloxazine ring, such as those observed with clorgyline for MAO-A and pargyline or tranlylcypromine, among other irreversible inhibitors, for MAO-B [48].

THE SUBSTRATE/INHIBITOR BINDING SITE

Crystallographic data have revealed that *N*-propargyl-substituted irreversible inhibitors bind covalently to either MAO-A (clorgyline) or MAO-B (pargyline, rasagiline (**11**) and three of its analogues) *via* a linkage between the



Scheme 2.

terminal carbon atom of the propargyl moiety and the N5 atom of FAD (scheme 2).

In all of these enzyme-inhibitor complexes, the longer axis of the inhibitor molecule is oriented perpendicular to the longer axis of the isoalloxazine ring. Indeed, in all cases but one, MAO-B crystal structures obtained in the presence of reversible or irreversible inhibitors have shown that the ligands bind with their aromatic moieties perpendicular to the flavin. The single exception is the adduct formed by tranlylcypromine (**12**), in which the phenyl ring of the inhibitor lies parallel to the isoalloxazine ring, possibly allowing a π stacking interaction between these two systems. It is noteworthy that in this case the adduct involves C4a instead of N5. Also, a glutamine active site residue (Gln206) has to move ~ 1 Å away from the conformation observed with other ligands in order to allow this relative ring orientation. Unlike the situation found with MAO-B, in the case of MAO-A the aromatic ring of clorgyline is not perpendicular to the flavin ring. The larger size of clorgyline, which may constrain its aromatic ring far away from the isoalloxazine ring in MAO-A, and/or the existence of hydrogen bonds between the two chlorine atoms and Cys323 and Thr336, might account for this observed orientation. Therefore, the possibility that the aromatic ring of a small inhibitor of MAO-A shares the same binding pattern as that of MAO-B inhibitors can not be excluded.

According to the structural data from these enzyme-inhibitor complexes, the substrate/inhibitor binding sites of rat MAO-A and human MAO-B display several

common characteristics. Both can be described as a pocket lined by the isoalloxazine ring and several aliphatic and aromatic amino acid residues (Fig. 2), which provide the highly hydrophobic environment predicted from modelling [63,64] and several QSAR studies [see for instance 65-67]. In addition, a critical role of Tyr444, Tyr407, Gln215 and Ile180 of MAO-A (Tyr435, Tyr398, Gln206 and Leu171 in MAO-B) in the orientation and stabilization of the substrate/inhibitor binding can be inferred from the crystal structures of both proteins.

In the case of MAO-B, it has been shown that the substrate binding site is actually a cavity (a flat entity of 420 Å³ in volume, termed the “substrate cavity”), which can be distinguished, in some cases, from another hydrophobic entity (290 Å³ in volume, termed the “entrance cavity”) situated closer to the protein surface [14,47,48]. It is believed that the entrance cavity functions as a passageway for the diffusion of substrates and inhibitors into the catalytic site. Both cavities can either be physically separated or fused into one single entity in the presence of different inhibitors. It has been demonstrated that the Ile199 side-chain can act as a “gate” opening or closing the connection between the two cavities by modifying its conformation. Thus, when relatively small inhibitors such as isatin (**13**), pargyline or tranlylcypromine are bound to the active site of MAO-B, the two cavities appear separated, and the Ile199 side-chain is in a “closed” conformation (Fig. 2 shows this conformational state). In contrast, this residue adopts an “open” conformation when bulkier ligands such as 1,4-diphenyl-2-butene (**14**) or lauryldimethylamine *N*-oxide (**15**) are bound, allowing

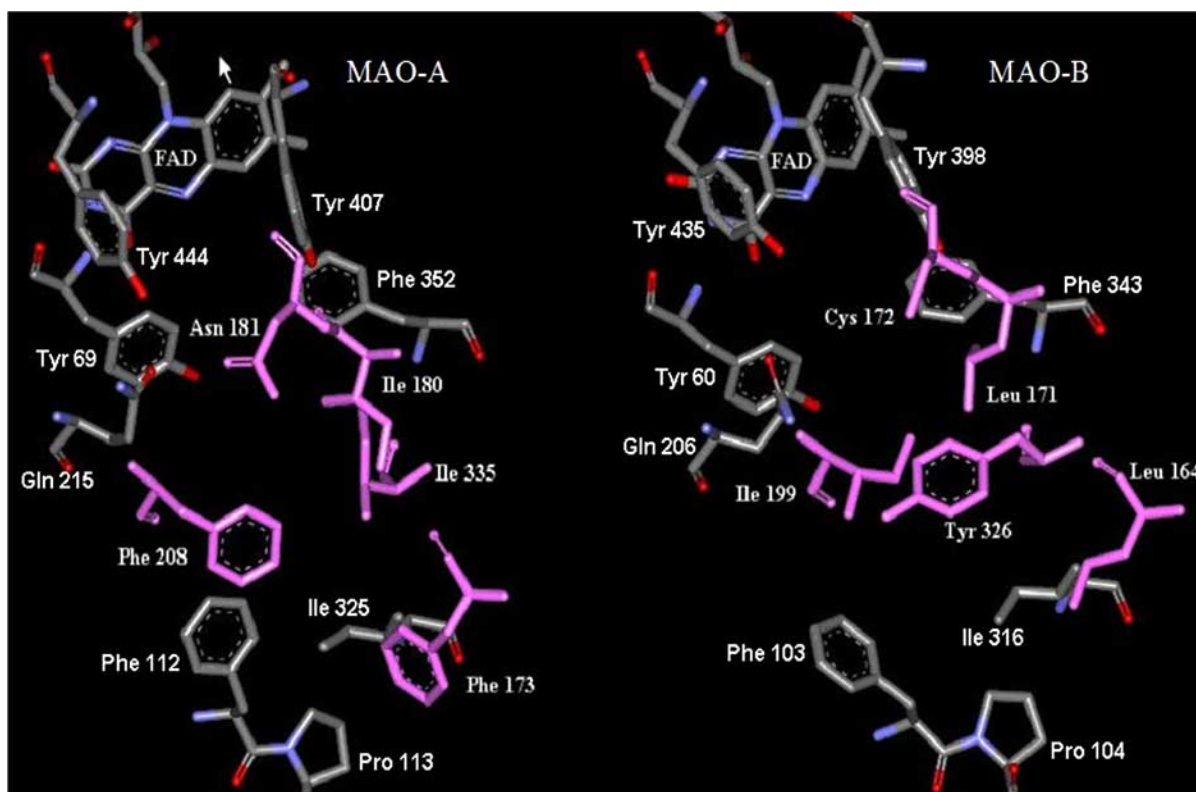
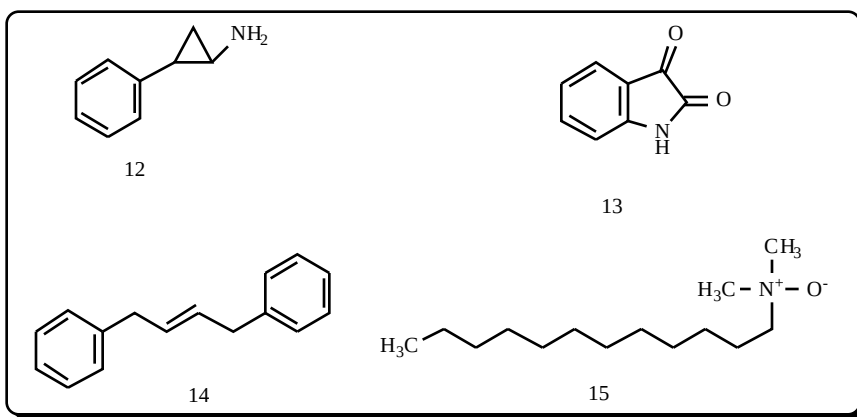


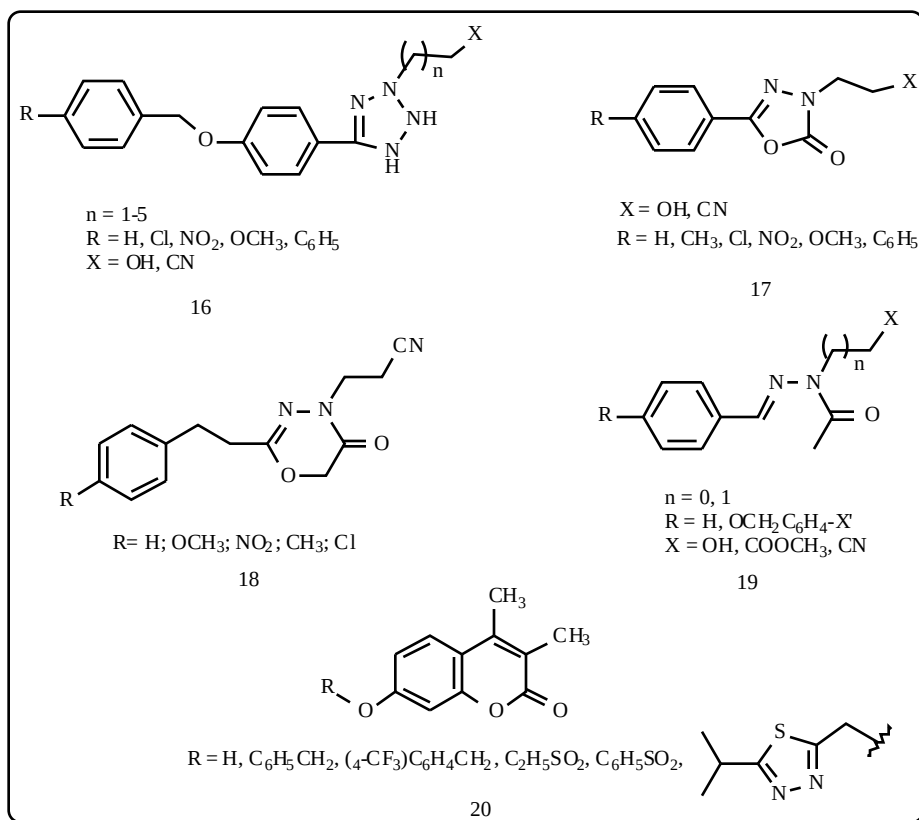
Fig. (2). Substrate/Inhibitor binding sites of MAO-A (left) and MAO-B (right). Non-conserved amino acid residues are shown in purple. To simplify the comparison, some residues are not shown.

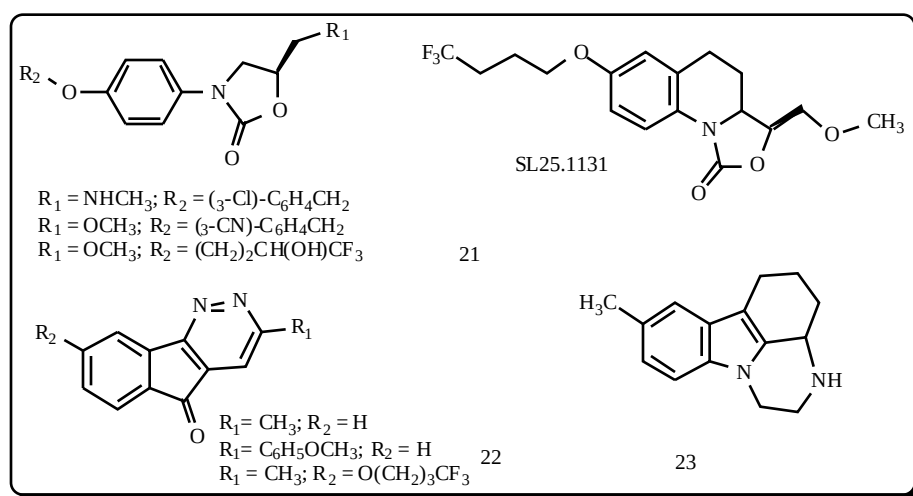


these compounds to span both cavities, which now may be seen as a single larger binding domain.

The extension of ligands through both cavities had been predicted (although without mentioning any change in the conformation of Ile199), by a flexible docking study performed with several tetrazole (**16**), oxadiazolone (**17**), oxadiazinone (**18**), aryl-*N*-acylhydrazide (**19**) and coumarin (**20**) derivatives, most of them reversible and selective MAO-B inhibitors [68]. It appears from this work that, in theory, optimal binding ought to implicate some parts of these molecules traversing the space that defines the separation between both cavities. It should be noted, however, that extension of a ligand into both cavities is not an absolute requirement for an “open” conformation, since rasagiline and some of its derivatives, which fit snugly into the substrate cavity, are able to interact with Ile199 in a way that forces this residue to adopt an open conformation [49].

Such an arrangement is not observed in the MAO-A-clorgyline crystal structure, which seems to delimit a larger binding site in this isoform, in agreement with SAR studies [See 69,70 for reviews]. However, it can be noted that in MAO-A there is an array of two aromatic amino acids (Phe208 and Phe 177) situated in close proximity to an aliphatic residue that might adopt two different conformations (Ile335). This arrangement is at a similar distance from the flavin ring as the corresponding Ile199, Phe168 and Tyr326 triad observed in MAO-B at the juncture of the substrate and entrance cavities. Thus, the displacement of Ile335 in MAO-A might determine an open or closed state of the substrate cavity much as Ile199 does in the case of MAO-B. It may be envisaged that clorgyline, because of its size, hydrophobic and/or electrostatic characteristics, interacts with Ile335 of MAO-A in a way that forces this residue to be in an “open” conformation, much as the MAO-B cavity-spanning





ligands do with Ile199. Further studies, in which MAO-A crystallizes with smaller inhibitors, are necessary to confirm whether Ile335 can act as “gate” residue similar to Ile199 in MAO-B. In this sense, due to its nonselective character, pargyline seems to be a good candidate to attempt this analysis.

Assuming the existence of separate entrance and substrate cavities in MAO-A, the volume of the latter should presumably be similar to that determined for MAO-B. It is worth pointing out that this is quite precisely what has been estimated from the dimensions of the largest rigid isatin and pirlindole analogues able to inhibit MAO-A [71,72]. If the dynamic substrate cavity systems demonstrated for MAO-B were also present in MAO-A, the exchanged locations of the aromatic and aliphatic non-conserved residues (Phe208/Ile199 and Ile335/Tyr326, in MAO-A and -B respectively) would determine a “gate” operating in opposite directions depending on the isoform considered. This difference might be implicated in the affinity and selective recognition towards substrate and inhibitors, as has been shown by site-directed mutagenesis studies in which reciprocal interchanges of some of these residues generate mutants with altered substrate/inhibitor specificities and affinities [73,74].

The predominantly hydrophobic entrance cavity (MAO-B) and putative channel (MAO-A) preceding the ligand binding pocket appear to be very similar, as might be expected from the high degree of sequence identity of both proteins (Fig. 2). In fact, both differ only in that Leu164 and Thr314 of MAO-B are replaced by Phe173 and Cys323, respectively, in MAO-A. A study in which all the cysteine residues of both isoforms were mutated systematically for serine gave no indication that such an aminoacid in the entrance region might be important in determining specificity [75]. However, chimeric constructs showed that the sequence from residue 152 to 366 in human MAO-B (and 161 to 375 in human MAO-A) contains the residue(s) responsible for selective ligand recognition [76]. Although it has been shown that Tyr326 and Ile335 play an important role in isoform selectivity [73], no attention has been paid to the possible relevance of Leu164 and Phe173, located at the rear of the entrance cavity of MAO-B or the corresponding region of MAO-A. The fact that some large inhibitors extend into this part of

the MAO-B molecule has been mentioned as a possible basis for drug design [48,49,68], and the Leu/Phe dichotomy might be exploited in a rational search for new selective compounds.

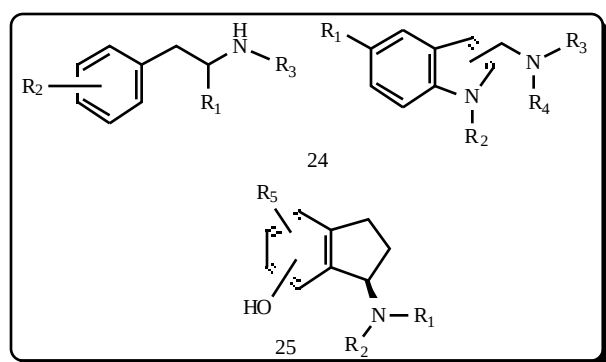
The active sites, i.e. where the oxidation of the substrate is carried out, are very similar in both MAO-A and MAO-B as revealed by diffraction data. Two conserved tyrosine residues (Tyr407, Tyr 444 and Tyr398, Tyr435 in MAO-A and -B respectively), whose aromatic rings face each other, are located almost perpendicular to the isoalloxazine ring defining an “aromatic cage”, that is believed to be critical for the alignment of the substrate amino group in front of the N5-C4a motif of the flavin ring (Fig 2). [12]. A similar aromatic cage has been revealed in the crystal structure of polyamine oxidase [77]. Site-directed mutagenesis studies have also demonstrated the importance of the presence of these aromatic residues near the isoalloxazine ring for the catalytic activity either in MAO-A or MAO-B, since inactive mutants were obtained when any of these tyrosines was replaced by a serine [61], while decreased but still significant activity was observed when either tyrosine was mutated to phenylalanine [61,78]. In addition, high-resolution X-ray data have demonstrated the existence of two ordered and conserved water molecules between Tyr398 and Tyr435 in MAO-B [49], the presence of which had also been predicted as a protein-ligand complex stabilizing feature by the docking study mentioned above [68]. Taken together, these results strongly suggest that the cage formed by aromatic residues and the flavin ring is the recognition site for the substrate amino group in several flavoenzymes including MAO-A and MAO-B. Furthermore, either through favourable polar or H-bond interactions, this site should be important to allocate the relatively hydrophilic groups of several classes of MAO-A or MAO-B inhibitors, which therefore should prevent the catalytic cycle, as had been predicted by several SAR, QSAR and docking studies [65,68,79-81].

MODE OF LIGAND BINDING

Many SAR studies on MAO inhibition have been carried out using unrelated families of compounds [69,70]. Coumarin [67], aryloxazolidinones (21) [79,82], indenopyridazines (22) [66], indoloquinoline (23) [72],

arylalkylamine (**24**) [83,84], and aminoindan (**25**) [85] derivatives have been the subject of particularly extensive analyses.

In some cases electronic or electrostatic properties have been claimed as of paramount importance, while in others lipophilic or steric features have been identified as major determinants of inhibitory potency. However, the inherent structural homogeneity of these sets of molecules suggests that in each case some particular aspect of their interactions with the enzyme may have been overlooked, and that the preferences identified may therefore be biased by the choice of compounds. In the light of our present knowledge of the structures of the active sites of both isoforms of the enzyme and the routes giving access to their ligands, it seems likely that a subtle interplay of a variety of factors determines the potencies and selectivities of the wide array of inhibitors studied to date.



A model assuming that the aromatic moiety of MAO ligands stacks parallel to the flavin cofactor [69] is no longer tenable in view of the available crystallographic studies. In order to approach the catalytic site, substrates and inhibitors must fit into a narrow space ($\sim 7\text{\AA}$ width) lined by Gln206 and Leu171, Tyr 398 and Tyr 435 in MAO-B (Gln215 and Ile180, Tyr 407 and Tyr 444 in MAO-A), which obliges the aromatic rings to adopt a perpendicular orientation with regard to the long axis of the flavin ring. The narrowness of the active sites is in good agreement with that predicted by SAR and QSAR studies, which had highlighted that inhibitors have certain “thickness” limitations and that more planar molecules are more potent than their less planar congeners [64,67,71,86]. The perpendicular orientation of the aromatic moieties of MAO ligands in the active site would allow π stacking interactions with the tyrosine residues, which in turn might explain several correlations which were rationalized assuming parallel stacking with the isoalloxazine. Moreover, (possibly water-mediated) hydrogen bonds between the polar substituents of the tyrosine and glutamine residues and nitrogen or oxygen functionalities of substrates or inhibitors could further stabilise the positioning of these ligands. The likelihood of these geometrical constraints and all the functional interactions mentioned is supported by several docking studies in MAO-B [68,80,87-89] and by *in silico* superimposition of a number of inhibitors into the active site of MAO-A as defined by clorgyline [50]. Nevertheless, as mentioned above, tranlylcypromine is able to interact with the glutamine residue in MAO-B, forcing it to move $\sim 1\text{\AA}$ from its original position, and eventually resides with its

aromatic ring in an almost parallel orientation. This observation highlights the plasticity of this binding site. The non-selective character of the inhibitory properties of tranlylcypromine, suggests that a similar conformational change could be observed in MAO-A. In fact, conformational changes of the binding site have been invoked to explain shifts in the UV and CD spectra of MAO-A upon interaction with different inhibitors [86,90,91].

The importance of hydrophobicity in several series of inhibitors of both MAO isoenzymes has been well established. Thus, since most of the amino acid residues in the rear of the substrate binding cavity and in the entrance cavity or channel are hydrophobic, relevant interactions with longer inhibitor molecules should occur at these sites. Relatively few inhibitor molecules, however, are long enough to occupy much of the entrance. Therefore, exploration of flexible molecules that might reach deeply into this region is still an attractive approach for drug design.

Regarding selectivity of substrates and inhibitors for MAO-A or -B, it is reasonable to assume that this is determined at two levels, namely by access to the substrate binding site and/or by differential interactions with specific residues once the molecule has entered. Thus, mutational studies have shown that the interchange of nonconserved amino acids in the binding site modifies the ligand selectivity of the isoenzymes [73,74]. Any explanations based on the crystal structures, however, should be handled with caution, as mutation of specific residues in the rat and human MAO-A have not always resulted in identical functional changes [74,92]. Likewise, the conclusions extracted from docking studies at the substrate binding site are limited not only by the crudeness of the algorithms used and by the fact that human MAO-B models have sometimes been tested using biological results obtained with the rat enzyme, or vice versa, but also by the lack of dynamic studies on the trajectory of ligands from the outside of the protein to their final interaction locus.

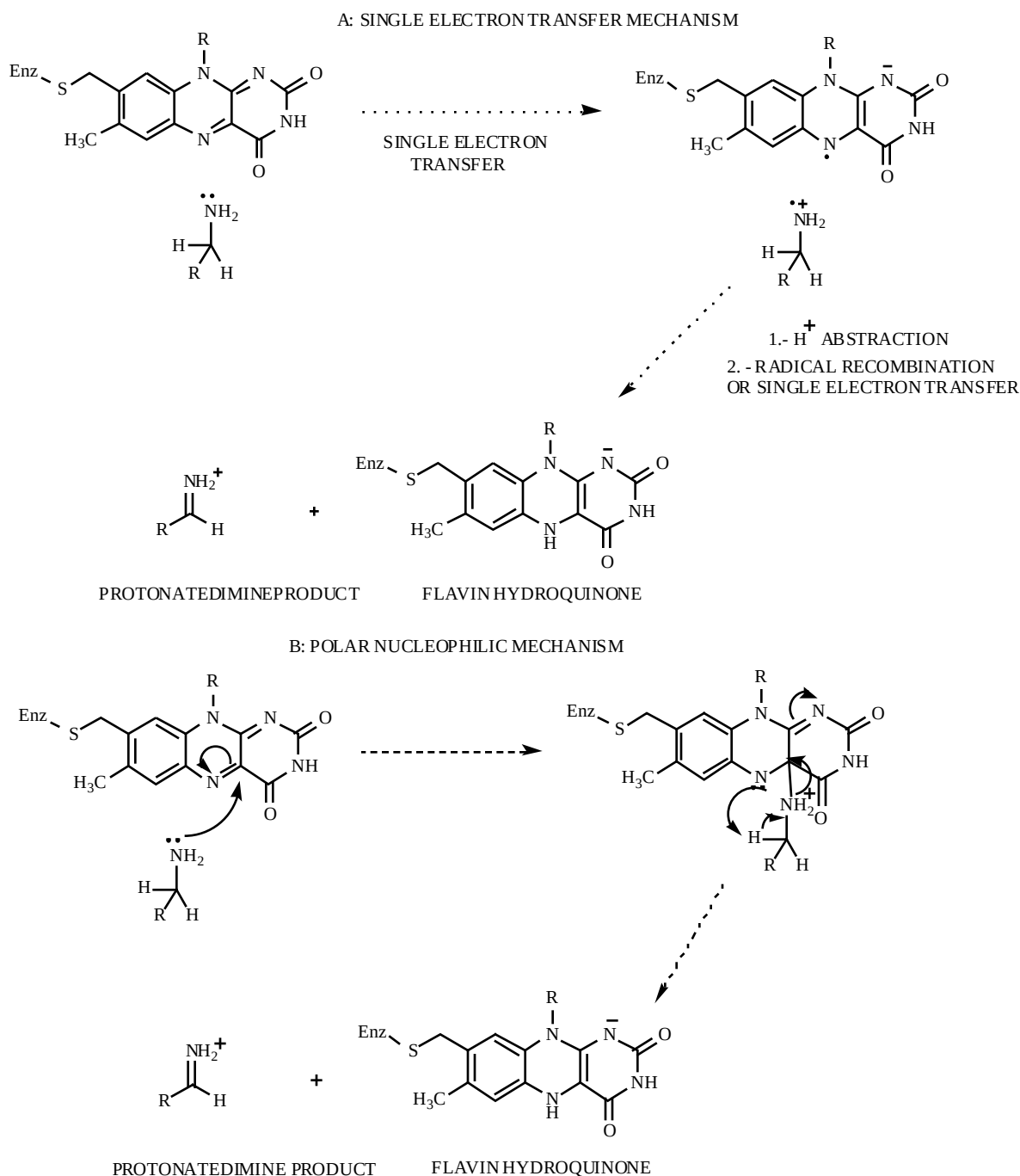
CATALYSIS

As intimated by Tipton and colleagues [1], there are certainties and uncertainties regarding the mechanism underlying the substrate oxidation reaction. It has been demonstrated, using a benzylamine derivative, that catalysis involves a 1:1 stoichiometry between substrate and MAO-A [93], and the same is true in the case of inhibitors [90,93; structural data]. In addition, data obtained from QSAR and kinetic studies for benzylamine and several of its derivatives have shown that the α -C-H bond cleavage is the rate-limiting step for both MAO isozymes [93,94]. This step almost certainly involves proton abstraction, as shown in the latter study by a Hammett analysis of the substituent effects. The stereospecific abstraction of the pro-R-hydrogen has been demonstrated with several substrates [95,96]. Interestingly, the introduction of a methyl group at the α -methylene of substrates such as benzylamine or phenethylamine generates chiral α -methylbenzylamine or amphetamine derivatives, both of whose isomers inhibit MAOs, regardless of whether the pro-R- or the pro-S-hydrogen is the substituted atom [97-99].

Two models (Scheme 3) for the MAO-catalysed oxidation of amines are currently debated. In general terms, the single electron transfer (SET) mechanism [100,101] proposes that oxidation of the substrate would involve an initial one-electron transfer from the lone pair of the amine substrate to the FAD to yield an aminium cation radical and the flavin semiquinone. Proton abstraction, the formation of a carbon-centred radical and a subsequent second electron transfer would follow to yield the iminium species and the reduced flavin. The polar nucleophilic mechanism [14,93], proposes a concerted pathway in which the substrate amino group would react nucleophilically with the flavin C4a. The concomitant

increase in the basic character of the FAD N5 would allow this atom to abstract the proton from the substrate's α -carbon to yield the imine and the reduced flavin.

Although the new structural results do not make either catalytic mechanism obvious, and a great deal of data support both options, the current balance of evidence seems to favour the latter proposal [14]. In this sense, a recent theoretical study on the interaction of lumiflavin with several *b*-carboline (which are MAO-A inhibitors) indicates that protonation at N5, in particular, strongly increases the electrophilicity of C4a [102]. Therefore, the role of the essential Lys296 (in MAO-B) or 305 (in MAO-A) may be rationalized not only as a structural but also as



Scheme 3.

a mechanistic necessity if it is viewed as a proton source, via an intermediate, conserved, hydrogen-bonded water molecule. In spite of these considerations, it is clear that further studies are necessary to definitely decide this issue.

CONCLUDING REMARKS

Recent results have shown that there is ample scope for the development of highly potent MAO inhibitors. Knowledge of the crystal structures of both isoenzymes augurs a new impulse to this rapidly moving field. Solid-state structural studies, however, only provide a snapshot of one of the many conformations available to proteins, even when complexed with high-affinity ligands. Consequently, experimental and theoretical approaches that give adequate consideration to dynamic aspects are an important requisite in order to better understand the physiological functioning of these enzymes and to place drug design on a more solid basis.

ACKNOWLEDGEMENTS

The authors thank Drs. Pilar Rivas and Gerald Zapata for their valuable theoretical insights into isoalloxazine chemistry and MAO structure. The support of FONDECYT grant 1000776 and DICYT-USACH is gratefully acknowledged.

LIST OF ABBREVIATIONS

MAO	= Monoamine Oxidase
FAD	= Flavin-Adenine Dinucleotide
5-HT	= Serotonin
PEA	= <i>b</i> -Phenethylamine
MPTP	= 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
CNS	= Central Nervous System
MDMA	= Methylenedioxymethamphetamine
PDB	= Protein Data Bank
FMN	= Flavin Mononucleotide
SAR	= Structure-Activity Relationships
QSAR	= Quantitative Structure-Activity Relationships

REFERENCES

- Tipton, K.F.; Boyce, S.; O'Sullivan, J.; Davey, G.P.; Healy, J. *Curr. Med. Chem.*, **2004**, *11*, 1965-1982.
- Shih, J.C. *Neurotoxicol.*, **2004**, *25*, 21-30.
- Youdim, M.B.H.; Weinstock, M. *Neurotoxicol.*, **2004**, *25*, 243-250.
- Léonard, N.; Lambert, C.; Depiereux, E.; Wouters, J. *Neurotoxicol.*, **2004**, *25*, 47-61.
- Wouters, J. *Mini Rev. Med. Chem.*, **2003**, *3*, 501-510.
- Magyar, K.; Pálfi, M.; Tábi, T.; Kalász, H.; Szende, B.; Szökő, E. *Curr. Med. Chem.*, **2004**, *11*, 2017-2031.
- Holt, A.; Berry, M.D.; Boulton, A.A. *Neurotoxicol.*, **2004**, *25*, 251-266.
- Castagnoli, K.; Murugesan, T. *Neurotoxicol.*, **2004**, *25*, 279-291.
- Oreland, L.; Hallman, J.; Damberg, M. *Curr. Med. Chem.*, **2004**, *11*, 2007-2016.
- Ramsay, R.R.; Gravestock, M.B. *Mini Rev. Med. Chem.*, **2003**, *3*, 129-36.
- Binda, C.; Hubálek, F.; Edmondson, D.E.; Mattevi, A. *FEBS lett.*, **2004**, *564*, 225-228.
- Binda, C.; Mattevi, A.; Edmondson, D.E. *J. Biol. Chem.*, **2002**, *277*, 23973-23976.
- Edmondson, D.E.; Binda, C.; Mattevi, A. *Neurotoxicol.*, **2004**, *25*, 63-72.
- Edmondson, D.E.; Mattevi, A.; Binda, C.; Li, M.; Hubálek, F. *Curr. Med. Chem.*, **2004**, *11*, 1983-1993.
- Shih, J.C.; Chen, K.; Ridd, M. *Ann. Rev. Neurosci.*, **1999**, *22*, 197-217.
- Tipton, K.F.; Strolin-Benedetti, M. In *Enzyme systems that metabolise drugs and other xenobiotics*; Ioannides C., Ed.; John Wiley & Sons, Chichester, UK, **2001**; pp. 95-146.
- Singer, T.P.; Ramsay, R.R. *FASEB J.*, **1995**, *9*, 605-610.
- Strolin-Benedetti, M.; Dostert, P. *Adv. Drug Res.*, **1992**, *23*, 65-125.
- Tetrad, J.W.; Langston, J.W. *Science*, **1989**, *245*, 519-522.
- Cesura, A.M.; Pletscher, A. *Prog. Drug Res.*, **1992**, *38*, 171-297.
- Yamada, M.; Yasuhara, H. *Neurotoxicol.*, **2004**, *25*, 215-221.
- Bymaster, F.P.; McNamara, R.K.; Tran, P.V. *Expert Opin. Investig. Drugs*, **2003**, *12*, 531-543.
- Parkinson Study Group. *Arch. Neurol.*, **2004**, *61*, 561-566.
- Youdim, M.B.H.; Weinstock, M. *Cell Mol. Neurobiol.*, **2001**, *21*, 555-573.
- Thomas, T. *Neurobiol. Aging*, **2000**, *21*, 343-348.
- Weinstock, M.; Gorodetsky, E.; Poltyrev, T.; Gross, A.; Sagi, Y.; Youdim, M. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2003**, *27*, 555-561.
- Kochersperger, L.M.; Parker, E.L.; Siciliano, M.; Darlington, G.J.; Denney, R.M. *J. Neurosci. Res.*, **1986**, *16*, 601-616.
- Lan, N.C.; Heinzmann, C.; Gal, A.; Klisak, I.; Orth, U.; Lai, E.; Grimsby, J.; Sparkes, R.S.; Mohandas, T.; Shih, J.C. *Genomics*, **1989**, *4*, 552-559.
- Grimsby, J.; Chen, K.; Wang, L.-J.; Lan, N.C.; Shih, J.C. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 3637-3641.
- Bach, A.W.J.; Lan, N.C.; Jhonson, D.L.; Abell, C.W.; Bemkenek, M.E.; Kwan, S.-W.; Seeburg, P.H.; Shih, J.C. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 4934-4938.
- Hsu, Y.P.; Weyler, W.; Chen, S.; Sims, K.B.; Rinehart, W.B.; Utterback, M.C.; Powell, J.F. *J. Neurochem.*, **1988**, *51*, 1321-1324.
- Ito, A.; Kuwahara, T.; Inadome, S.; Sagara, Y. *Biochem. Biophys. Res. Commun.*, **1988**, *157*, 970-976.
- Kwan, S.W.; Abell, C.W. *Comp. Biochem. Physiol.*, **1992**, *102B*, 143-147.
- Nagatsu, T. *Neurotoxicol.*, **2004**, *25*, 11-20.
- Johnston, J.P. *Biochem. Pharmacol.*, **1968**, *17*, 1285-1287.
- Knoll, J.; Magyar, K. *Adv. Biochem. Psychopharmacol.*, **1972**, *5*, 393-408.
- Brunner, H.G.; Nelen, M.; Breakfield, X.O.; Ropers, H.H.; Van Oost, B.A. *Science*, **1993**, *262*, 578-580.
- Cases, O.; Seif, I.; Grimsby, J.; Gaspar, P.; Chen, K.; Pourmin, S.; Muller, U.; Aguet, M.; Babinet, C.; Shih, J.C.; De Maeyer, E. *Science*, **1995**, *268*, 1763-1766.
- Grimsby, J.; Toth, M.; Chen, K.; Kumazawa, T.; Klaidman, L.; Adams, J.D.; Karoum, F.; Gal, J.; Shih, J.C. *Nat. Genet.*, **1997**, *17*, 206-210.
- Billett, E.E. *Neurotoxicol.*, **2004**, *25*, 139-148.
- Westlund, K.N.; Denney, R.M.; Rose, R.M.; Abell, C.W. *Neuroscience*, **1988**, *25*, 439-456.
- Luque, J.M.; Kwan, S.W.; Abell, C.W.; Da Prada, M.; Richards, J.G. *J. Comp. Neurol.*, **1995**, *363*, 665-680.
- Sprague, J.E.; Everman, S.L.; Nichols, D.E. *Neurotoxicol.*, **1998**, *19*, 427-442.
- Gudelsky, G.A.; Yamamoto, B.K. *Methods Mol. Med.*, **2003**, *79*, 55-73.
- Ma, J.; Ito, A. *J. Biochem.*, **2002**, *131*, 107-111.
- Newton-Vinson, P.; Hubálek, F.; Edmondson, D.E. *Prot. Exp. Purif.*, **2000**, *20*, 334-345.
- Binda, C.; Newton-Vinson, P.; Hubálek, F.; Edmondson, D.E.; Mattevi, A. *Nat. Struct. Biol.*, **2002**, *9*, 22-26.
- Binda, C.; Li, M.; Hubálek, F.; Restelli, N.; Edmondson, D.E.; Mattevi, A. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 9750-9755.

- [49] Binda, C.; Hubálek, F.; Li, M.; Herzig, Y.; Sterling, J.; Edmondson, D.E.; Mattevi, A. *J. Med. Chem.*, **2004**, 47, 1767-1774.
- [50] Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. *J. Mol. Biol.*, **2004**, 338, 103-114.
- [51] Mitoma, J.-Y.; Ito, A. *J. Biochem.*, **1992**, 111, 20-24.
- [52] Rebrin, I.; Geha, R. M.; Chen, K.; Shih, J. C. *J. Biol. Chem.*, **2001**, 276, 29499-29506.
- [53] Weyler, W. *J. Neural Transm. Suppl.*, **1994**, 41, 3-15.
- [54] Kearney, E.B.; Salach, J.I.; Walker, W.H.; Seng, R.L.; Kenney, W.; Zeszotek, E.; Singer, T.P. *Eur. J. Biochem.*, **1971**, 24, 321-327.
- [55] Nagy, J.; Salach, J.I. *Arch. Biochem. Biophys.*, **1981**, 208, 388-394.
- [56] Nandigama, R.K.; Edmondson, D.E. *J. Biol. Chem.*, **2000**, 275, 20527-20532.
- [57] Miller, J.R.; Edmondson, D.E. *J. Biol. Chem.*, **1999**, 274, 23515-23525.
- [58] Hiro, I.; Tsugeno, Y.; Hirashiki, I.; Ogata, F.; Ito, A. *J. Biochem.*, **1996**, 120, 759-765.
- [59] Zhou, B.P.; Wu, B.; Kwan, S.-W.; Abell, C.W. *J. Biol. Chem.*, **1998**, 273, 14862-14868.
- [60] Kirksey, T.J.; Kwan, S.-W.; Abell, C.W. *Biochemistry*, **1998**, 37, 12360-12366.
- [61] Geha, R.M.; Chen, K.; Wouters, J.; Ooms, F.; Shih, J.C. *J. Biol. Chem.*, **2002**, 277, 17209-17216.
- [62] Trickey, P.; Basran, J.; Lian, L.-Y.; Chen, Z.-W.; Barton, J.D.; Sutcliffe, M.J.; Scrutton, N.S.; Mathews, F.S. *Biochemistry*, **2000**, 39, 7678-7688.
- [63] Veselovsky, A.V.; Medvedev, A.E.; Tikhonova, O.V.; Skvortsov, V.S.; Ivanov, A.S. *Biochemistry (Moscow)*, **2000**, 65, 910-916.
- [64] Veselovsky, A.V.; Medvedev, A.E.; Ivanov, A.S.; Medvedev, A.E. *Neurotoxicol.*, **2004**, 25, 37-46.
- [65] Wouters, J.; Ooms, F.; Jegham, S.; Koenig, J.J.; George, P.; Durant, F. *Eur. J. Med. Chem.*, **1997**, 32, 721-730.
- [66] Altomare, C.; Cellamare, S.; Summo, L.; Catto, M.; Carotti, A.; Thull, U.; Carrupt, P.-A.; Testa, B.; Stoeckli-Evans, H. *J. Med. Chem.*, **1998**, 41, 3812-3820.
- [67] Gnerre, C.; Catto, M.; Leonetti, F.; Weber, P.; Carrupt, P.-A.; Altomare, C.; Carotti, A.; Testa, B. *J. Med. Chem.*, **2000**, 43, 4747-4758.
- [68] Carrieri, A.; Carotti, A.; Barreca, M.L.; Altomare, C. *J. Comp.-Aid. Mol. Des.*, **2002**, 16, 769-778.
- [69] Wouters, J. *Curr. Med. Chem.*, **1998**, 5, 137-162.
- [70] Kalgutkar, A.S.; Dalvie, D.K.; Castagnoli, Jr. N.; Taylor, T.J. *Chem. Res. Toxicol.*, **2001**, 14, 1139-1162.
- [71] Medvedev, A.; Ivanov, A.S.; Kamyshanskaya, N.S.; Kinkel, A.Z.; Moskvitina, T.A.; Gorkin, V.Z.; Li, N.Y.; Marshakov, V.Yu. *Biochem. Mol. Biol. Int.*, **1995**, 36, 113-122.
- [72] Medvedev, A.E.; Veselovsky, A.V.; Shvedov, V.I.; Tikhonova, O.V.; Moskvitina, T.A.; Fedotova, O.A.; Axenova, L.N.; Kamyshanskaya, N.S.; Kinkel, A.Z.; Ivanov, A.Z. *J. Chem. Inf. Comput. Sci.*, **1998**, 38, 1137-1144.
- [73] Geha, R. M.; Rebrin, I.; Chen, K.; Shih, J. C. *J. Biol. Chem.*, **2001**, 276, 9877-9882.
- [74] Tsugeno, Y.; Ito, A. *J. Biol. Chem.*, **1997**, 272, 14033-14036.
- [75] Wu, H.F.; Chen, K.; Shih, J.C. *Mol. Pharmacol.*, **1993**, 43, 888-893.
- [76] Grimsby, J.; Zentner, M.; Shih, J.C. *Life Sci.*, **1996**, 58, 777-787.
- [77] Binda, C.; Coda, A.; Angelini, R.; Federico, R.; Ascenzi, P.; Mattevi, A. *Structure Fold Des.*, **1999**, 7, 265-276.
- [78] Nandigama, R.K.; Miller, J.R.; Edmondson, D.E. *Biochemistry*, **2001**, 40, 14839-14846.
- [79] Wouters, J.; Moureau, F.; Evrard, G.; Koenig, J.-J.; Jeghan, S.; George, P.; Durant, F. *Bioorg. Med. Chem.*, **1999**, 7, 1683-1693.
- [80] Manna, F.; Chimenti, F.; Bolasco, A.; Secci, D.; Bizzarri, B.; Befani, O.; Turini, P.; Mondovi, B.; Alcaro, S.; Tafi, A. *Bioorg. Med. Chem. Lett.*, **2002**, 12, 3629-3633.
- [81] Chimenti, F.; Bolasco, A.; Manna, F.; Secci, D.; Chimenti, P.; Befani, O.; Turini, P.; Giovannini, V.; Mondovi, B.; Cirilli, R.; La Torre, F. *J. Med. Chem.*, **2004**, 47, 2071-2074.
- [82] Mai, A.; Artico, M.; Esposito, M.; Ragno, R.; Sbardella, G.; Massa, S. *Il Farmaco*, **2003**, 58, 231-241.
- [83] Morón, J.A.; Campillo, M.; Perez, V.; Unzeta, M.; Pardo, L. *J. Med. Chem.*, **2000**, 43, 1684-1691.
- [84] Vallejos, G.; Rezende, M.C.; Cassels, B.K. *J. Comput.-Aid. Mol. Des.*, **2002**, 16, 95-103.
- [85] Sterling, J.; Herzig, Y.; Goren, T.; Finkelstein, N.; Lerner, D.; Goldenberg, W.; Miskolczi, I.; Molnar, S.; Rantal, F.; Tamas, T.; Toth, G.; Zagyva, A.; Zekany, A.; Finberg, J.; Lavian, G.; Gross, A.; Friedman, R.; Razin, M.; Huang, W.; Kraiss, B.; Chorev, M.; Youdim, M.B.; Weinstock, M. *J. Med. Chem.*, **2002**, 45, 5260-5279.
- [86] Hynson, R.M.G.; Wouters, J.; Ramsay, R.R. *Biochem. Pharmacol.*, **2003**, 65, 1867-1874.
- [87] Ooms, F.; Frédéric, R.; Durant, F.; Petzer, J.P.; Castagnoli Jr. N.; Van der Schyf, C.J.; Wouters, J. *Bioorg. Med. Chem. Lett.*, **2003**, 13, 69-73.
- [88] Severina, I.S.; Axenova, L.N.; Veselovsky, A.V.; Pyatakova, N.V.; Buneeva, O.A.; Ivanov, A.S.; Medvedev, A.E. *Biochemistry (Moscow)*, **2003**, 68, 1048-1054.
- [89] Osorio-Olivares, M.; Rezende, M.C.; Sepúlveda-Boza, S.; Cassels, B.K.; Fierro, A. *Bioorg. Med. Chem.*, **2004**, 12, 4055-4066.
- [90] Ramsay, R.R.; Hunter, D.J.B. *Biochem. Biophys. Acta*, **2002**, 1601, 178-184.
- [91] Hynson, R.M.G.; Kelly, S.M.; Price, N.C.; Ramsay, R.R. *Biochem. Biophys. Acta*, **2004**, 1672, 60-66.
- [92] Geha, R.M.; Chen, K.; Shih, J.C. *J. Neurochem.*, **2000**, 75, 1304-1309.
- [93] Miller, J.R.; Edmondson, D.E. *Biochemistry*, **1999**, 38, 13670-13683.
- [94] Walker, M.C.; Edmondson, D.E. *Biochemistry*, **1994**, 33, 7088-7098.
- [95] Yu, P.H.; Davis, A.B. *Int. J. Biochem.*, **1988**, 20, 1197-1201.
- [96] Yu, P.H. *Biochem. Cell. Biol.*, **1988**, 66, 853-861.
- [97] Arai, Y.; Toyoshima, Y.; Kinemuchi, H. *Jpn. J. Pharmacol.*, **1986**, 41, 191-197.
- [98] Edmondson, D.E.; Bhattacharya, A.K.; Xu, J. *Biochem. Biophys. Acta*, **2000**, 1479, 52-58.
- [99] Hurtado-Gúzman, C.; Fierro, A.; Iturriaga-Vásquez, P.; Sepúlveda-Boza, S.; Cassels, B.K.; Reyes-Parada, M. *J. Enz. Inhib. Med. Chem.*, **2003**, 18, 339-347.
- [100] Silverman, R.B.; Hoffman, S.J.; Catus, W.B. *J. Am. Chem. Soc.*, **1980**, 102, 7126-7128.
- [101] Silverman, R.B. *Acc. Chem. Res.*, **1995**, 28, 335-342.
- [102] Rivas, P.; Zapata-Torres, G.; Melin, J.; Contreras, R. *Tetrahedron*, **2004**, 60, 4189-4196.