

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Similarities Between the Binding Sites of Monoamine Oxidase (MAO) from Different Species — Is Zebrafish a Useful Model for the Discovery of Novel MAO Inhibitors?

Angelica Fierro, Alejandro Montecinos,
Cristobal Gómez-Molina, Gabriel Núñez,
Milagros Aldeco, Dale E. Edmondson,
Marcelo Vilches-Herrera, Susan Lühr,
Patricio Iturriaga-Vásquez and Miguel Reyes-Parada

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/35874>

1. Introduction

Zebrafish (*Danio rerio*) is an animal model that is attracting increasing interest in pharmacology and toxicology. The relatively ease with which large numbers of individuals can be obtained and their inexpensive maintenance makes zebrafish a particularly suitable tool for drug discovery. Thus, in recent years diverse compounds have been assayed both in larval and adult specimens and changes of behavioral patterns, for instance, have been related to anxiolytic, addictive or cognitive effects. In this context, the molecular characterization of drug targets in zebrafish, comparing them to their mammalian counterparts, arises as a subject of paramount importance.

Monoamine oxidase (MAO) is the main catabolic enzyme of monoamine neurotransmitters and the primary target of several clinically relevant antidepressant and antiparkinsonian drugs. In mammals, it exists in two isoforms termed MAO-A and MAO-B, which share a number of structural and mechanistic features, but differ in genetic origin, tissue localization and inhibitor selectivity. High-resolution structures of MAOs from rat and human have

been reported during the last decade, allowing detailed comparison of their overall structures and respective active sites. On the other hand, a few studies have shown that zebrafish contains a single MAO gene and that enzyme activity is due to a single form (zMAO) which resembles, but is distinct from, both mammalian MAO-A and MAO-B. No three-dimensional structural data exist thus far for zMAO. Sequence comparison of the putative substrate binding site of zMAO with those of human MAO isoforms suggests that the fish enzyme resembles mammalian MAO-A more than MAO-B. Nevertheless, biochemical studies have shown that zMAO exhibits such unique behavior toward MAO-A and -B substrates and inhibitors, that the results of studies using zebrafish MAO function, either as a disease model or for drug screening, should be considered with caution.

Functional and evolutionary relationships between proteins can be reliably inferred by comparison of their sequences, structures or binding sites. From a drug-discovery perspective, the study of binding site similarities (and differences) can be particularly insightful since it aids the design of selective or non-selective ligands and the detection of off-targets. In addition, knowledge of ligand-binding site similarity could increase our understanding of divergent and convergent evolution and the origin of proteins, even in those cases where no obvious sequence or structural similarity exists. In recent years, a number of algorithms have been developed for the identification and comparison of ligand-binding sites. Even though each method has its own merits and limitations, the performance of these computational tools is continuously improving. Advances in this field, associated with the increasing availability of structural data and reliable homology models of thousands to millions of protein molecules, provide an unprecedented framework to investigate the mechanisms underlying the molecular interactions between these proteins and their ligands, as well as to evaluate the similarities between the binding sites of related and unrelated proteins

On the basis of the foregoing, the first section of this chapter provides an overview on: a) the relevance of zebrafish as an animal model of increasing interest in pharmacology; b) the impact that MAO crystal structures and molecular simulation approaches have had on the development of novel MAO inhibitors, as well as comparative structural and functional information about zMAO and its mammalian counterparts; c) recent developments in computational methods to evaluate similarities between ligand-binding sites, emphasizing their usefulness for the rational design of multitarget (promiscuous) drugs.

The second part of the chapter describes unpublished results regarding a further characterization of zMAO activity and its comparison with MAOs from mammals. Specific topics in this section include: a) the construction of homology models of zMAO, built using human MAO-A and -B crystal structures as templates; b) a three-dimensional analysis of the binding site similarities between MAOs from different species using a statistical algorithm; c) a functional evaluation of zMAO activity in the presence of a small series of reversible and selective MAO-A and -B inhibitors.

2. Zebrafish as a model in pharmacology, monoamine oxidase and computational methods to evaluate binding site similarities: An overview

2.1. Zebrafish as an animal model in pharmacology and neurobehavioral studies

In order to understand complex behaviors observed in nature, scientists have always tried to develop models that could be used and tested under controlled conditions in the laboratory. In the last 30 years a new animal model, zebrafish (*Danio rerio*), has emerged as a powerful tool mostly for studying developmental biology. The scientific potential of zebrafish was originally assessed by George Streisinger (Streisinger et al., 1981). This work was the starting point for rapid progress in molecular and genetic analysis of zebrafish neurodevelopment, which allowed the construction of many genetic mutants and the identification of several genes that affect different brain functions such as learning and memory (Norton & Bally-Cuif, 2010). During the last decade zebrafish has also become an attractive model for behavioral and drug discovery studies, particularly those related to actions in the central nervous systems (Chakraborty & Hsu, 2009; King, 2009; Rubinstein, 2006; Zon & Peterson, 2005).

Zebrafish develop rapidly and almost all organs are developed at 7 days post-fertilization. Their fecundity makes it easy to obtain large numbers of individuals for experimentation, which are relatively inexpensive to maintain. In addition, they can absorb chemical substances from their tank water, and their genome has been almost fully sequenced, which makes genetic manipulation more accessible. These characteristics have stimulated the use of zebrafish in medicinal chemistry to assay the effects of different compounds in whole animals (Goldsmith, 2004; Kaufman & White, 2009). Another attractive characteristic of zebrafish is its potential for use in *in vivo* high-throughput screening assays. Consequently, a number of studies which take advantage of this possibility have been reported recently (Kokel et al., 2010; Kokel & Peterson, 2011; Rihel et al., 2010; Zon & Peterson, 2005).

Zebrafish exhibit many social characteristics that can be assimilated to those observed in mammals. They recognize each other by sight and odor (Tebbich et al., 2002) and display an interesting social learning (Reader et al., 2003). This teleost also shows a characteristic aggressive behavior (Payne, 1998), a pheromone-mediated danger alarm (Suboski, 1988; Suboski et al., 1990), cognitive and adaptive behaviors such as habituation (Miklosi et al., 1997; Miklosi & Andrew 1999), spatial navigation abilities and Pavlovian conditioning (Hollis, 1999). These features make this species a valuable tool for either the development or the adaptation of behavioral paradigms. Thus, behavioral protocols such as an aquatic version of the T-maze, which is used for studies of discrimination, reinforcement and memory in rodents, had been used to assess color discrimination in zebrafish (Colwill et al., 2005). Another interesting model is the aquatic version of conditioned place preference (CPP), where the fish can be exposed to different stimuli in two separate compartments and is then allowed to freely explore the apparatus without partition (Darland & Dowling, 2001). A further paradigm, the novel tank diving test, has been used by different research groups (Bencan & Levin 2008; Bencan et al., 2009; Egan et al., 2009; Levin et al., 2007) as a model for anxiety. It is

conceptually similar to the rodent open field test, because it takes advantage of the instinctive behavior of both zebrafish and rats to seek refuge when exposed to an unfamiliar environment (Levin et al., 2007). In the case of the novel tank diving test, the fish dives to the bottom of the tank and remains there until it presumably feels safe enough to explore the rest of the tank and gradually starts to explore the upper zone (Egan et al., 2009). Similar observations can be made in an open field test for rodents, where initially they spend a lot of time near the walls, which is considered as an indication of an anxious state. The time spent by the zebrafish in the lower or upper part of the tank, as well as erratic movements, have been established as anxiety indices (Egan et al., 2009). It is considered that the zebrafish is anxious when it shows a longer latency to enter the upper part of the tank, or when the time spent at the top is reduced. Conversely, when an anxiolytic drug is administered, animals spend much more time in the upper portion of the tank. Figure 1 illustrates this response by showing the typical traces of motor activity observed for control animals (left) and for animals exposed to nicotine (right), which has been reported to have anxiolytic properties in this paradigm (Levin et al., 2007).

Based on these findings, the potential of zebrafish for neurobehavioral studies is increasingly recognized (Bencan & Levin, 2008; Eddins et al., 2010). Thus, this animal has been used as a model in studies of memory (Levin & Chen, 2006), anxiety (Bencan et al., 2009; Levin et al., 2007), reinforcement properties of drugs of abuse (Ninkovic & Bally-Cuif, 2006), neuroprotection of dopaminergic neurons (McKinley et al., 2005), and movement disorders (Flinn et al., 2008).

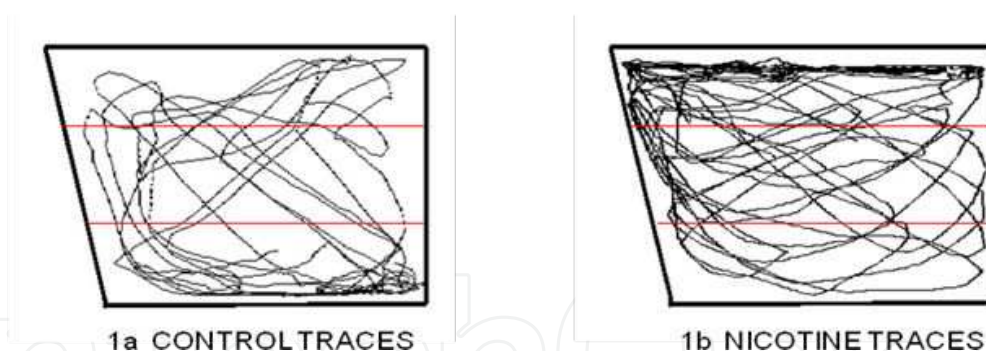


Figure 1. Representative traces of characteristic behavior of control-saline- (left) and nicotine- (right) treated zebrafish. Traces were recorded during 5 min in a glass trapezoidal test tank (22.9 cm long at the bottom, 27.9 cm long at the top, 15.2 cm high, 6.4 cm wide), filled with 1.5 L of artificial sea water. Nicotine was administered 5 min before the test. All other experimental conditions were as previously published (Levin et al., 2007).

A final word of caution should be said regarding the apparent usefulness of zebrafish as a research tool. One critical aspect to be considered when using animal models to understand a specific behavior is its validity. Mammals such as rats and mice have been widely used as models to study several functions since, among other characteristics, many brain regions and their neurotransmitter systems are well characterized. Thus, even though genome and the genetic pathways controlling signal transduction and development appear to be highly conserved between zebrafish and humans (Postlethwait et al., 2000), further validation of

this model is needed, particularly if human systems or conditions are the final aims to be addressed.

2.1.1. Monoamine oxidase: general characteristics and the impact of crystal structures on the understanding of enzyme function and inhibition

Monoamine oxidase (monoamine oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4; MAO) is a key enzyme in the inactivation of neurotransmitters such as serotonin, dopamine and noradrenaline. In mammals it exists in two isoforms termed MAO-A and MAO-B which have molecular weights of ~60 kDa. Both proteins are outer mitochondrial membrane-bound flavoproteins, with the FAD cofactor covalently bound to the enzyme. MAO-A and MAO-B are encoded by separate genes (Kochersperger et al., 1986; Lan et al., 1989) and the isoforms from the same species show about 70% sequence identity, whereas 85-88% identity is observed between the same isoforms from human and rat (Nagatsu, 2004). Both neurological and psychiatric diseases have been related to MAO dysfunction. Consequently, the search for inhibitors of each isoform has lasted decades. Currently, selective inhibitors of MAO-A are used clinically as antidepressants and anxiolytics, while MAO-B inhibitors are used to reduce the progression of Parkinson's disease and of symptoms associated with Alzheimer's disease (Youdim et al., 2006).

In 2002, Binda and colleagues (Binda et al., 2002) published a groundbreaking article showing the high-resolution structure of human MAO-B in complex with the irreversible inhibitor pargyline. Subsequent structures of this enzyme (Binda et al., 2003, 2004), as well as that of rat MAO-A (Ma et al., 2004), and more recently human MAO-A (De Colibus et al., 2005; Son et al., 2008), have allowed a detailed comparison of the overall structures of both isoforms, and new insights regarding their active sites (Edmondson et al., 2007, 2009; Reyes-Parada et al., 2005). Based on these findings, the substrate/inhibitor binding site of both isozymes can be described as a pocket lined by the isoalloxazine ring of the flavin cofactor and several aliphatic and aromatic residues (in the second part, close ups of this binding site are depicted in Figures 5 and 8). In particular, two conserved tyrosine residues (Y407, Y444 and Y398, Y435 in MAO-A and -B, respectively), whose aromatic rings face each other, are located almost perpendicularly to the isoalloxazine ring defining an "aromatic cage". This conformational arrangement provides a path to guide the substrate amine towards the reactive positions on the flavin ring and therefore seems to be essential for catalytic activity. In addition, a critical role of residues G215 and I180 of MAO-A (G206 and L171 being the corresponding residues in MAO-B) in the orientation and stabilization of the substrate/inhibitor binding can be inferred from the X-ray diffraction data. In MAO-B, the substrate/inhibitor binding site is a cavity (~400 Å³, termed the "substrate cavity") which can be distinguished, in some cases, from another hydrophobic pocket (~300 Å³, termed the "entrance cavity") located closer to the protein surface. It has been demonstrated that the I199 side-chain can act as a "gate" opening or closing the connection between the two cavities by modifying its conformation (Binda et al., 2003). In contrast, the MAO-A binding site consists of a single cavity (De Colibus et al., 2005; Ma et al., 2004). It should be noted that, although residues lining the binding site of human and rat MAO-A are identical, the human MAO-A cavity is larger

(~550 Å³) than that in rat MAO-A (~450 Å³). Remarkably, an exchanged location of aromatic and aliphatic nonconserved residues in the active sites of MAO-A and MAO-B (F208/I199 and I335/Y326, respectively) has been implicated in the affinity and selective recognition of substrates and inhibitors, and provides a molecular basis for the development of specific reversible inhibitors of each isoform (Edmondson et al., 2009).

The availability of the aforementioned crystal structures has made an enormous impact on our knowledge about the function and regulation of the enzyme and has also allowed a quicker pace in the rational design of novel MAO inhibitors. Different theoretical approaches and computational methods have been used since, to explore **how**, **where** and **why** some interactions are central in MAO-ligand complexes. For instance, quantum mechanics calculations have been used to obtain insights about the mechanism by which amines are oxidized by MAO (Erdem & Büyükmeneke, 2011), whereas molecular dynamics simulations have been recently employed to study specific interactions involved in the access of reversible MAO inhibitors to their binding site (Allen & Bevan 2011). In addition, a number of studies describing potent and selective inhibitors have been reported during the last decade and in most of them molecular simulation approaches have been used to rationalize and/or to predict the functional interactions between the proteins and their inhibitors. Figure 2 illustrates this situation by showing the progression of published articles about MAO in which computational methodologies were used.

It should be pointed out however, that crystal structures only provide a snapshot of one of the many conformations available to proteins. Therefore theoretical (and experimental) approaches, adequately considering dynamic aspects, will grow in importance in order to better understand the physiological functioning of these enzymes.

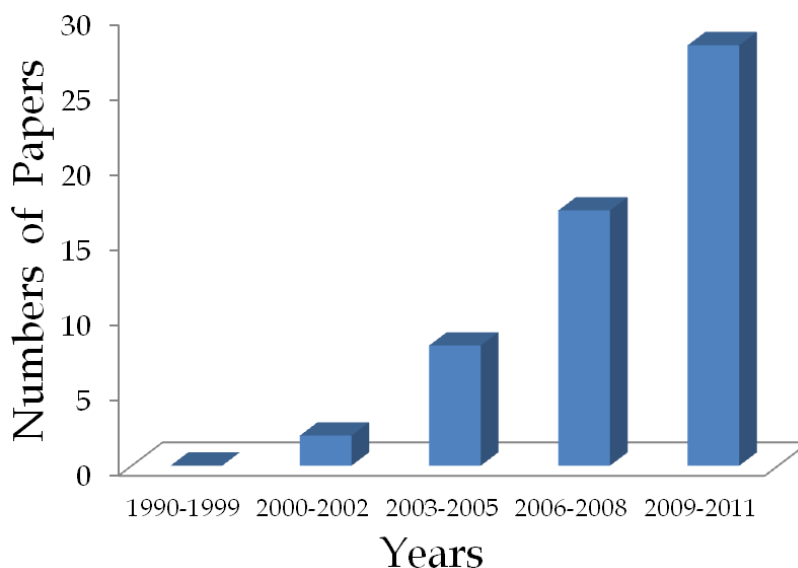


Figure 2. Progression of research articles involving docking studies on MAO before and after (2002) the first three-dimensional structure of MAO was deposited in the Protein Data Bank. Data from PubMed. "MAO" and "docking" were used as keywords.

2.1.2. Comparative functional and structural information about zebrafish MAO and its mammalian counterparts

Unlike mammals, zebrafish have only one MAO gene (Anichtchik et al., 2006; Setini et al., 2005). This gene is located in chromosome 9 and exhibits an identical intron-exon organization as compared to mammals, which suggests a common ancestral gene (Anichtchik et al., 2006; Panula et al., 2010). Sequencing studies have shown that zebrafish MAO (zMAO) contains 522 amino acids and has a molecular weight of about 59 kDa (Setini et al., 2005), which is very similar to that found in mammalian MAO-A and MAO-B. zMAO displays about 70% identity with human MAO-A or -B, and its predicted secondary structure indicates that the flavin-binding-, the substrate- and the membrane-binding- domains, which are typical in other MAOs, should also be present in the fish enzyme. Indeed, a recent study (Arslan & Edmondson, 2010) has demonstrated that (like the mammalian isoforms), zMAO is also a mitochondrial enzyme, presumably bound to the outer membrane, and that the flavin cofactor is covalently bound to the protein via an α -thioether linkage likely established with C406. Beyond its overall identity, the amino acid sequence of the presumed zMAO binding domain shows ~67% and ~83% identity with the corresponding binding sites of human MAO-B and MAO-A respectively (Panula et al., 2010). Interestingly, some residues that have been shown to be critical for inhibitor and substrate selectivity in human MAOs such as the pairs F208/I335 (in MAO-A) and I199/Y326 (in MAO-B), are identical or conservatively replaced in zMAO (F200/L327) as compared with MAO-A.

Regarding functional studies, recent data obtained using *para*-substituted benzylamine analogs as substrates suggest that, as in mammalian MAOs, α -C-H bond cleavage is the rate-limiting step in zMAO catalysis (Aldeco et al., 2011). Furthermore, a variety of substrates and inhibitors have been tested against zMAO. Preferential substrates of both MAO-A (e.g. serotonin) and MAO-B (e.g. phenethylamine, benzylamine, MPTP) as well as non-selective substrates such as tyramine, dopamine or kynuramine, have been shown to be deaminated, although with different catalytic efficiency, by zMAO (Aldeco et al., 2011; Anichtchik et al., 2006; Arslan & Edmondson, 2010; Sallinen et al., 2009; Setini et al., 2005). In addition, irreversible selective inhibitors such as clorgyline (MAO-A) or deprenyl (MAO-B) exhibit similar inhibitory profiles toward zMAO (Anichtchik et al., 2006; Arslan & Edmondson, 2010; Setini et al., 2005). Interestingly, the *in vivo* administration of deprenyl to zebrafish increases serotonin levels about 10-fold while levels of dopamine remain unchanged (Sallinen et al., 2009). These data indicate that zMAO is essential for serotonin metabolism in zebrafish, but also underline the distinctive character of this enzyme since in rodents dopamine concentrations are increased after deprenyl treatment, whereas serotonin levels remain unchanged. Structurally diverse reversible MAO inhibitors such as harmane, tetrindole, methylene blue, amphetamine, 8-(3-chlorostyryl)-caffeine, 1,4-diphenyl-1,3-butadiene, farnesol, safinamide or zonisamide display a wide range of inhibitory potencies, from nM to μ M to no effect, against zMAO (Aldeco et al., 2011; Binda et al., 2011). Remarkably, methylene blue is the most potent zMAO inhibitor tested thus far, exhibiting a K_i value of 4 nM.

Based on sequence similarity, substrate preference and inhibitor sensitivity, it has been consistently suggested that the functional properties of zMAO resemble more strongly those of

MAO-A than those of MAO-B. Nevertheless, virtually all articles published so far recognize that, although some overlapping properties can be detected, zMAO also shows characteristics of its own that distinguish it from its mammalian counterparts.

2.2. Recent developments in computational methods to evaluate similarities between ligand-binding sites

The concept of protein binding-site similarity and the development of methods to evaluate it are receiving much attention. This is viewed as a step forward in protein classification, as compared with classical sequence-based approaches, since it should allow proteins with low sequence similarity but high similarity at their binding sites to be related (Milletti & Vulpetti, 2010). On the contrary, as will be analyzed below, this approach can also detect subtle differences between highly homologous proteins, and therefore be useful to determine the suitability of non-human proteins as models for drug design aimed to the treatment of human conditions.

One of the newest applications of the study of binding site similarities is polypharmacology. Thus, the classical idea that selective drugs acting on a single target related to one disease will have maximal efficacy has been challenged by increasing evidence showing that most clinically effective drugs bind to several targets, even if these targets are not originally related to the disease (Keiser et al., 2009; Schrattenholz & Soskić 2008). Even though this pharmacological promiscuity may be seen as a negative property, primarily related with the incidence of side effects, recent observations increasingly indicate that multitarget compounds might have better profiles regarding both efficacy and side effects, since they would be acting on a pharmacological network, where several nodes underlie the physiopathology of the disease (Apsel et al., 2008; Hopkins 2008). Thus, the concept of polypharmacology has motivated several groups to find new drug-target associations, based on the idea that a given compound can interact simultaneously with two or more relevant targets if they have similar binding sites. It should be stressed that these associations are pursued considering that two proteins could share a ligand even if they are structurally or functionally very different (Kahraman et al., 2007).

One aspect that has critically fueled this field is the increasing availability of 3D protein structures in public databases (almost 75.000), which allows us to explore the complexity of protein-ligand interactions. This exploration has yielded important insights in order to obtain a good characterization of the binding sites and has confirmed the notion that protein-ligand binding depends not only on shape complementarity but also on complementary physicochemical features (Henrich et al., 2010).

Several algorithms have been developed to compare binding sites of different proteins. In most of them, two main steps are present: the creation of a database that requires the calculation of fingerprints describing each binding site and a pocket screening that requires multiple similarity alignments between the query pocket and the database. These applications are used as a strategy to assess specific issues, such as off-target identification for drug repurposing (Cleves & Jain, 2006; Keiser et al., 2009; Moriaud et al., 2011), functional classification of unknown proteins (Kinnings & Jackson, 2009; Russell et al., 1998), drug discovery by

sequence analysis (Xie et al., 2009), detection of evolutionary relationships (Xie & Bourne, 2008) and polypharmacology predictions (Milletti & Vulpetti, 2010; Pérez-Nueno & Ritchie, 2011). The main step before finding similarity between two or more binding sites is their characterization. Several methodologies have been proposed with this purpose: geometrics approaches, which mainly analyze cavities through the exploration of the solvent-accessible protein surface (Weisel et al., 2007); energetics approaches, which use van der Waals and electrostatic energies to define cavities (Laurie & Jackson, 2005); structure and sequence comparison approaches, which use the information of known binding sites to compare and define unknown cavities through the analysis of sequence and structural similarity (Brylinski & Skolnick, 2009); and approaches involving the dynamics of protein structures, which use dynamics simulations to include the natural flexibility of proteins and possible allosteric modifications of binding sites (Landon et al., 2008). Although the determination of similarities between binding sites could seem a simple mathematical method, several approaches have been developed using different characteristics. For example, the Isocleft algorithm measures the similarity by initially defining a cleft in any protein to be compared. These clefts are determined by a set of overlapping spheres that are represented by the van der Waals radii of atoms in the binding sites. Finally each cleft is viewed like a graph and the similarity is measured by finding the largest common subgraph (Najmanovich et al., 2008). The SitesBase algorithm uses a triangular geometric determination of binding sites establishing the cutoff at 5 Å. Similarity is measured by an atom–atom score which finds the largest possible matching constellation (similar atom types with a similar spatial orientation) (Gold & Jackson, 2006). The ProFunc server uses sequence and structural information to find similarities between binding sites. This process includes a phylogenetic component that is used for the identification of homologous proteins (Laskowski et al., 2005). The Sumo algorithm flags each functional group as a node in a graph. Then the similarity is measured through a strategy that does not necessarily find the maximal common subgraph between a pair of binding sites (Jambon et al., 2003). The FLAP algorithm utilizes GRID methodology to calculate the energy of interaction between a molecular probe and the binding sites. These interactions, which include van der Waals and electrostatic terms, are then compared through a geometric approach (Baroni et al., 2007). In another recently developed algorithm (Hoffmann et al., 2010) the binding sites are represented as a set of atoms in the 3D space described by 3D vectors. Initially the algorithm calculates the similarity between two binding sites comparing vectors that only consider the atom coordinates, although different additional parameters such as atom type and charges could be included in the algorithm. The Pocket-Match algorithm involves three basic steps: a) each binding site is represented as a sort list of distances between three selected points in every amino acid present at one specific distance from the ligand, b) the two sets of sorted distances are aligned and c) finally the similarity percentage is calculated (Yeturu & Chandra, 2008).

Although most algorithms used to measure the similarities between binding sites have shown high performance when the comparison involves related proteins, doubtful results are obtained when the proteins are not related. In these cases it is very important to select the best algorithm taking into account some critical issues: a ligand may change its orientation in different binding sites; some protein-ligand conformations may have a favorable

binding energy, but natural allosteric regulations (not always considered) might not favor such conformations; protein structures from databases could have been determined in different conformational states (active, inactive, closed, open, etc.); finally, it is also very important to consider the solvent and ion concentrations in every system.

Beyond these considerations, the continuous increase in both the number of protein structures and computational power, augurs the development of ever more accurate similarity searching tools, which likely will allow not only better results in virtual screening programs but also a novel view on the evolution of structure and function of proteins.

3. MAO from different species: a biochemical evaluation and a theoretical analysis using molecular simulation and a biostatistical algorithm

As mentioned, even though amino acids lining the zMAO binding site exhibit a high level of identity with those of rat and human MAOs, a few studies have shown that the fish's enzyme shows unexpected sensitivities for known specific substrates and inhibitors. Since zebrafish has been proposed as a model that could be useful for the identification of novel MAO inhibitors (Kokel et al., 2010), we further characterized zMAO using three different approaches. First, we determined the inhibitory potency of a small series of compounds which have been previously evaluated against rat and human MAOs. Then, we built homology models of zMAO based on the crystal structures of human MAO-A or MAO-B and performed docking experiments with a drug selected from the biochemical evaluations. Finally, we used the recently described algorithm PocketMatch (Yeturu & Chandra, 2008) to explore similarities and differences between MAO isoforms from human, rat and zebrafish.

3.1. Biochemical evaluation

3.1.1. Methods

4-Methylthioamphetamine (MTA), 2-naphthylisopropylamine (NIPA), (6-methoxy-2-naphthyl)isopropylamine (MeONIPA), all as hydrochloride salts, 2-(4'-butoxyphenyl)thiomorpholine (BTI), 2-(4'-benzyloxyphenyl)thiomorpholine (ZTI), both as oxalate salts, as well as 2-(4'-butoxyphenyl)thiomorpholin-5-one (BTO) and 2-(4'-benzyloxyphenyl)thiomorpholin-5-one (ZTO) were synthesised following published methods (Hurtado-Guzmán et al., 2003; Lühr et al., 2010; Vilches-Herrera et al., 2009). The expression and purification of zMAO in *Pichia pastoris* was performed as previously described (Arslan & Edmondson, 2010). Enzyme kinetic studies were done spectrophotometrically in 50 mM potassium phosphate buffer (pH = 7.4), 0.5% (w/v) reduced Triton X-100 with kynuramine as substrate. The spectrophotometer used was a Perkin-Elmer Lambda-2 UV-Vis at 25 °C.

3.1.2. Results and discussion

Figure 3 shows the chemical structures of the inhibitors evaluated.

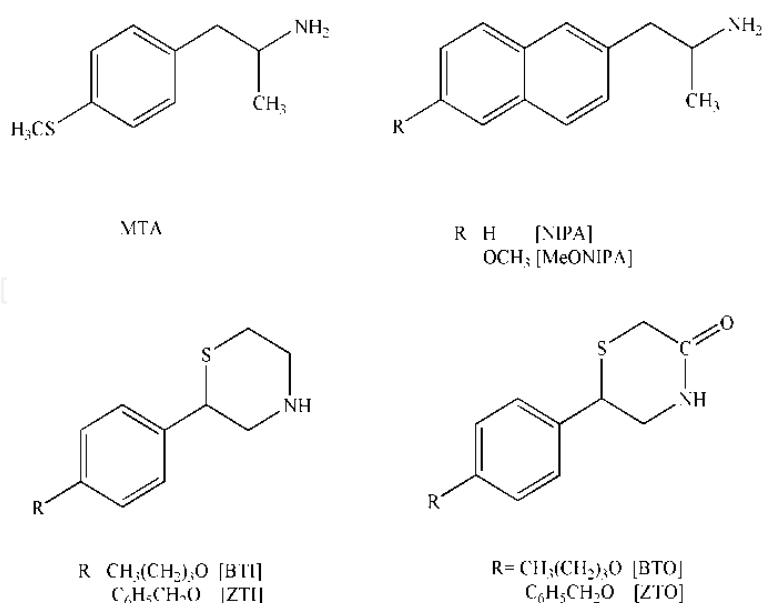


Figure 3. Chemical structures of the compounds used in the biochemical evaluation

Table 1 summarizes the effects of these compounds upon zMAO and also includes, for comparative purposes, the reported values of their inhibitory activities against MAO-A and -B from human and rat (Fierro et al., 2007; Hurtado-Guzmán et al., 2003; Lühr et al., 2010; Vilches-Herrera et al., 2009).

Compound	K_i (μ M)				
	zMAO	hMAO-A	rMAO-A	hMAO-B	rMAO-B
MTA ^a	NE	0.13 ± 0.02	0.25 ± 0.02	NE	NE
NIPA ^b	17.7 ± 2.6	0.48 ± 0.31	0.42 ± 0.04	>100	>100
MeONIPA ^b	4.8 ± 0.4	0.24 ± 0.02	0.18 ± 0.05	5.1 ± 0.4	16.3 ± 7.8
BTO ^c	NE	10.0 ± 0.3	50.9 ± 6.1	0.46 ± 0.18	0.16 ± 0.01
ZTO ^c	NE	>100	27.5 ± 4.6	0.048 ± 0.03	0.074 ± 0.003
BTI ^c	30.4 ± 3.8	2.5 ± 0.2	14.1 ± 1.2	0.068 ± 0.05	0.27 ± 0.02
ZTI ^c	NE	>100	19.0 ± 0.4	0.038 ± 0.003	0.13 ± 0.01

Table 1. zMAO inhibitory properties of known selective mammalian MAO inhibitors. Comparative data for human and rat MAO inhibition are from: ^aHurtado-Guzmán et al., 2003; ^bVilches-Herrera et al 2009; ^cLühr et al, 2010. NE: No effect

The amphetamine derivative MTA, which is a potent and selective inhibitor of rat and human MAO-A (Fierro et al., 2007; Hurtado-Guzmán et al., 2003), showed no significant effect upon zMAO activity. Similarly, the 2-arylthiomorpholine analogue ZTI, and the 2-arylthiomorpholin-5-one derivatives BTO and ZTO, which are highly selective MAO-B inhibitors

(Lühr et al., 2010), did not inhibit the fish's enzyme. In contrast, naphthylisopropylamine derivatives NIPA and MeONIPA, which are selective inhibitors of MAO-A (Vilches-Herrera et al., 2009), as well as the 2-arylthiomorpholine derivative BTI which selectively inhibits MAO-B (Lühr et al., 2010), exhibited zMAO inhibitory properties with K_i values in the micromolar range. MeONIPA was the most potent compound of the series evaluated, showing a K_i value (4.8 μM) very similar to that found against human MAO-B (5.1 μM). These results agree with a notion that can be inferred from previous data (Aldeco et al., 2011; Anichtchik et al., 2006), indicating that effects on zMAO cannot be straightforwardly used to predict an effect upon either MAO-A or MAO-B. In addition, these data suggest that the zMAO binding site is significantly different from those of both MAO-A and MAO-B from mammals.

3.2. Homology models of zMAO and molecular docking

3.2.1. Modeling methods

Since neither the MAO-A nor MAO-B structure can be chosen *a priori* as a better template for modeling zMAO, we decided to build two different models using each isoform of human MAO as templates. The MAO-A (Protein Data Bank, PDB code: 2BXS) and MAO-B (PDB code: 2BYB) crystal structures at 3.15 Å and 2.2 Å resolution respectively (De Colibus et al., 2005) were employed. The amino acid sequence and crystal structure of each protein were extracted from the National Center for Biotechnology Information (NCBI) and PDB databases. Sequence alignments were prepared separately. Models were built using standard parameters and the outcomes were ranked on the basis of the internal scoring function of the program MODELLER9v6 (Sali & Blundell, 1993). The best model obtained in each case (using MAO-A or MAO-B as template) was submitted to the H++ server (Gordon et al., 2005; <http://biophysics.cs.vt.edu/H++>) to compute pK_a values of ionizable groups and to add missing hydrogen atoms according to the specified pH of the environment. Each structure selected was inserted into a POPC membrane, TIP3 solvated and ions were added creating an overall neutral system simulating approximately 0.2 M NaCl. The ions were equally distributed in a water box. The final system was subjected to a molecular dynamics (MD) simulation for 5 ns using NAMD 2.6 (Phillips et al., 2005). The NPT ensemble was used to perform MD calculations. Periodic boundary conditions were applied to the system in the three coordinate directions. A pressure of 1 atm was used and temperature was kept at 310 K. The simulation time was sufficient to obtain an equilibrated system (RMSD < 2 Å). Stereochemical and energy quality of the homology models were evaluated using the PROCHECK server (Wiederstein & Sippl 2007) and Procheck (Laskowski et al., 1993).

3.2.2. Docking methods

Dockings of (S)-MeONIPA in the zMAO models, as well as in the human MAO-A and MAO-B structures were done using the AutoDock 4.0 suite (Morris et al., 1998). MeONIPA was selected for this study since it was the most potent zMAO inhibitor of the series evaluated and because it also inhibited both human MAO-A and MAO-B at low concentrations. The choice of the (S)-isomer for MeONIPA docking experiments was done on the basis that

(S)-amphetamine derivatives (which are always dextrorotatory) are usually the eutomers at MAO (Hurtado-Guzmán et al., 2003). All other docking conditions were as previously reported (Fierro et al., 2007; Vilches-Herrera et al., 2009). Briefly, the grid maps were calculated using the autogrid4 option and were centered on the putative ligand-binding site. The volumes chosen for the grid maps were made up of $40 \times 40 \times 40$ points, with a grid-point spacing of 0.375 Å. The autotors option was used to define the rotating bond in the ligand. The docked compound complexes were built using the lowest docked-energy binding positions. MeONIPA was built using Gaussian03 (Frisch et al., 2004) and the partial charges were corrected using ESP methodology.

3.2.3. Results and discussion

Figure 4 depicts the global zMAO models obtained using human MAO-A (left) and human MAO-B (right) as templates. As expected, the overall structure of zMAO was similar to those of the human enzymes. The presumed ligand binding site appears lined by a series of hydrophobic residues and the isoalloxazine ring of the flavin cofactor (top inset Fig. 4). Amino acids forming the binding site of zMAO and human MAO-A and -B are shown in insets of Figure 4.

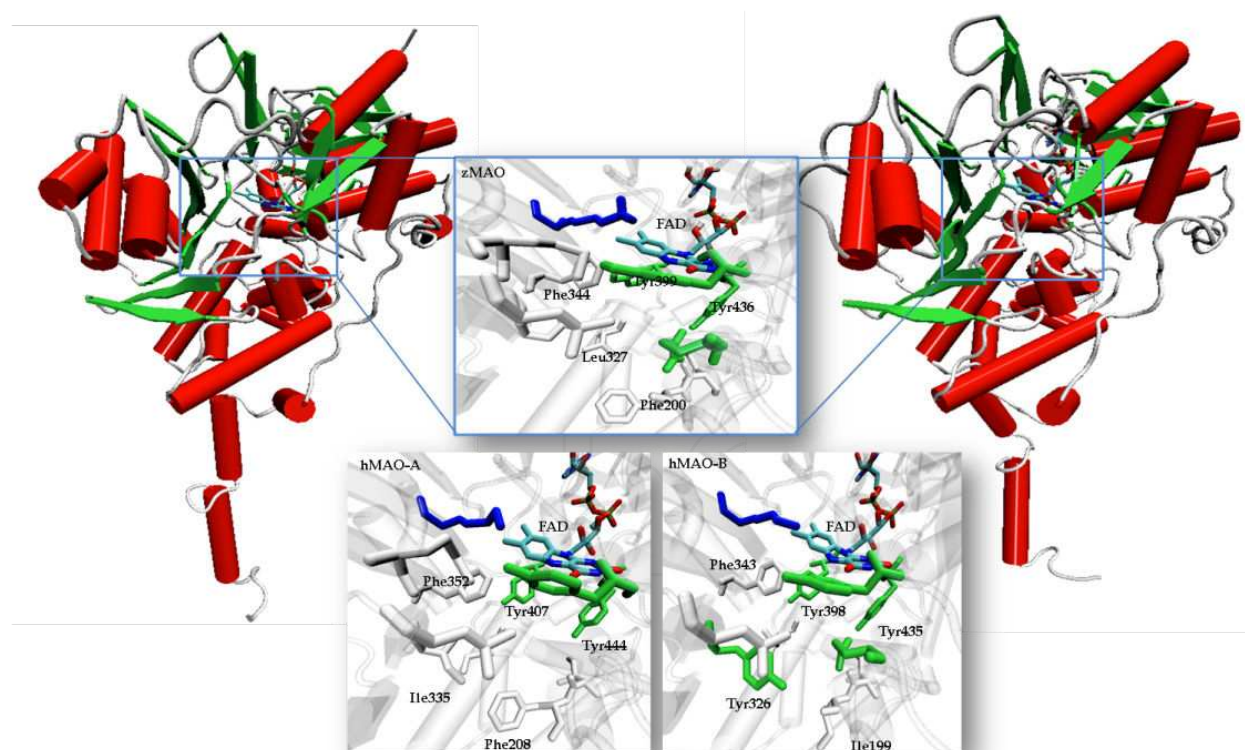


Figure 4. Cartoons of zMAO models obtained using human MAO-A (left) or human MAO-B (right). Insets show the main amino acids of the active sites of zMAO (top), human MAO-A (left) and human MAO-B (right). Amino acids in white, green or blue indicate apolar, polar or positively charged residues respectively.

As shown in Figure 5, docking experiments revealed that in both zMAO models, MeONIPA exhibits a binding mode where the aromatic ring is oriented almost perpendicularly to the isoalloxazine ring of FAD, with the methoxyl group pointing to the binding site entrance, whereas the aminopropyl chain points toward the isoalloxazine ring and appears positioned close to two tyrosine residues which, together with the isoalloxazine ring, form the so-called aromatic cage (Figs. 5 A and 5B). Interestingly, docking of MeONIPA in both human MAO-A and MAO-B, yielded binding modes where the inhibitor molecule adopted an almost opposite orientation to those observed in zMAO models. Thus, the most energetically favorable conformations of MeONIPA were those in which the amino group points away from the flavin ring, whereas the methoxyl group is located between the corresponding tyrosine residues (Figs. 5 C and 5D). These results suggest that the different inhibitory potencies of MeONIPA (and likely other inhibitors) toward zebrafish and human MAOs, might be attributed to the differential binding modes exhibited by the drug. Similar conclusions attempting to explain why MAO inhibitors show differential inhibition properties upon MAO from different species have been reached in previous studies (Fierro et al., 2007; Nandingham et al., 2002). Moreover, our findings suggest that, even in the cases where similar potencies are detected, the mechanism of enzyme inhibition for a given drug might be different in zebrafish and human MAOs.

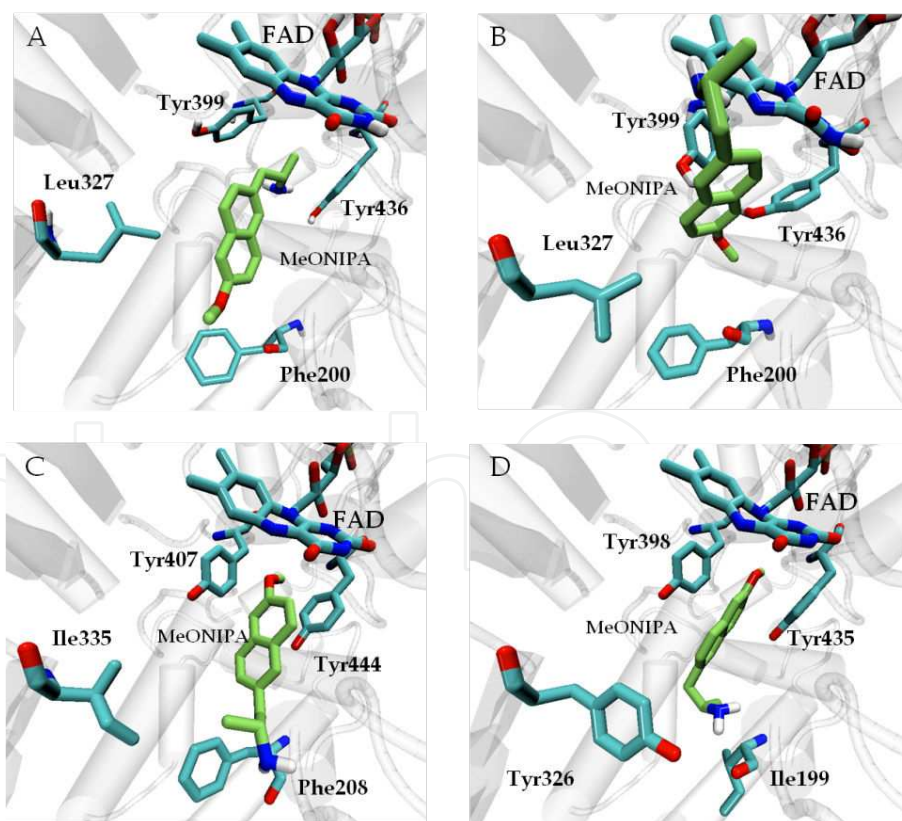


Figure 5. Comparison of the binding modes of MeONIPA into zMAO (A and B), human MAO-A (C) and human MAO-B (D) active sites. Figures 5 A and 5B show the docking poses of MeONIPA into zMAO models obtained using human MAO-A and human MAO-B respectively. Main active site amino acid residues and FAD are rendered as stick models.

3.3. Similarities between the binding sites of MAO from different species.

3.3.1. Protein structures employed

The structures of human and rat MAO-A co-crystallized with clorgyline (PDB codes: 2BXS and 1O5W respectively) and human MAO-B co-crystallized with *l*-deprenyl (PDB code: 2BYB) were employed. Furthermore, structures of zMAO models and human MAO-A and MAO-B obtained after docking of MeONIPA (see previous section), were used in additional comparisons.

3.3.2. Binding site comparison methods

The PocketMatch algorithm was selected for this study due to its relatively low computational complexity and high performance. All aspects involved in binding site comparisons followed the procedure published in the original article describing the algorithm (Yeturu & Chandra, 2008). Briefly, each binding site was considered as that determined by the residues for which one or more atoms surround either a crystallographic or a docked ligand at a given distance (4 Å by default; in some cases distances from 3 Å to 10 Å from the ligand were considered; see following section). Each residue was classified into one of 5 groups, taken into account its chemical properties. Then, each residue was represented as a set of three points corresponding to the coordinates of the C-Alpha, the C-Beta and the Centroid Atom of the side chain. Distances between every three points of each residue in the binding sites were measured. All distances computed were sorted in ascending order and stored in sets of distances organized by type of pairs of points and type of pairs of tags. The sorted and organized distances were aligned and compared using a threshold of 0.5 Å, which was established considering the natural dynamics of biological systems. The similarity between sites, referred to as the PMScore, was measured by scoring the alignment of the pair of sites under comparison. Thus, the PMScore represents the percentage of the number of “matches” calculated over the maximal number of distances computed for each binding site. A PMScore of 0.5 (50 %) or higher was considered as indicative of similarity between binding sites.

3.3.3. Results and discussion

Initially, we compared human and rat MAO-A. The amino acid sequence in the active sites of both proteins is identical, and therefore we expected to find a high degree of similarity. Surprisingly, a PMScore value of 0.27 was obtained after comparing the residues located at 4 Å from the ligand (clorgyline in both proteins), which is the PocketMatch default condition. It should be considered that PMScores > 0.5 are indicative of binding site similarity, whereas values below 0.5 indicate lack of similarity. It should also be noted that, as shown in the original report by Yeturu & Chandra (2008), a distance of 4 Å from the ligand was clearly suitable to find similarities between a series of structurally related and unrelated proteins. Therefore, it was rather intriguing that such a low PMScore should be obtained, suggesting the existence of relevant differences between rat and human MAO-A binding sites, most likely in the form in which residues in close proximity to the ligand are arranged. Such a conformational difference has been revealed by the crystal structures of both proteins,

which show that the cavity-shaping loop 210–216 and specifically residues Gln215 and Glu216 are differentially oriented in human and rat MAO-A (De Colibus et al., 2005). This differential arrangement determines a larger volume of the active site of human MAO-A (550 \AA^3) as compared to that of rat MAO-A (450 \AA^3). Thus, our results confirm that rat and human MAOs are not as similar as could be inferred from the analysis of their amino acid sequences, and highlight the sensitivity of PocketMatch to determine subtle differences between highly related proteins.

Despite these considerations, we developed a script that allows the automatic evaluation of PMScores considering distances from 3 \AA to 10 \AA from the ligand, with the hope that such an analysis could yield further information regarding the similarity of the binding sites of MAOs. Thus, we were able to build “similarity profiles”, which graphically show at what distance from the ligand (if any) the binding sites begin to be similar. Figure 6 shows the similarity profile after comparing rat and human MAO-A.

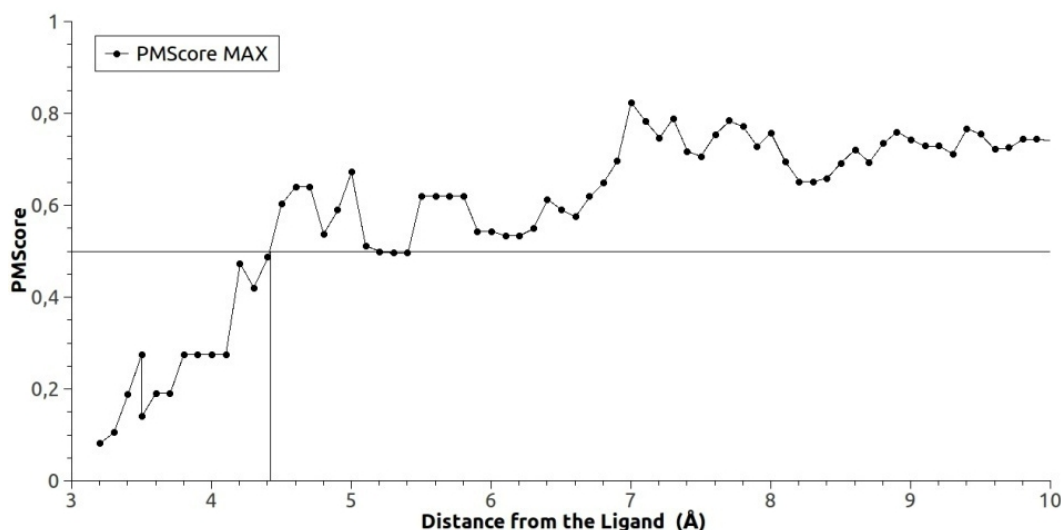


Figure 6. Similarity profile between rat and human MAO-A, both co-crystallized with clorgyline, as calculated using PocketMatch. The horizontal black line indicates PMScore = 0.5. The vertical black line indicates the distance from the ligand where the PMScore begins to be consistently greater than 0.5. Each point corresponds to the PMScore.

As can be seen, PMScores greater than 0.5 appeared at 4.5 \AA and were consistently observed at longer distances from the ligand. Since most amino acids located at 4.5 \AA from the ligand line the binding site (see Figure 8A and 8B), these results indicate that, beyond the shape differences revealed by crystal structures and detected by PocketMatch, the binding sites of MAO-A from rat and human are quite similar.

In contrast, when binding sites of human MAO-A and MAO-B were compared, PMScores indicating similarity (> 0.5) were only found at distances higher than 6.4 \AA from the ligand (Fig. 7).

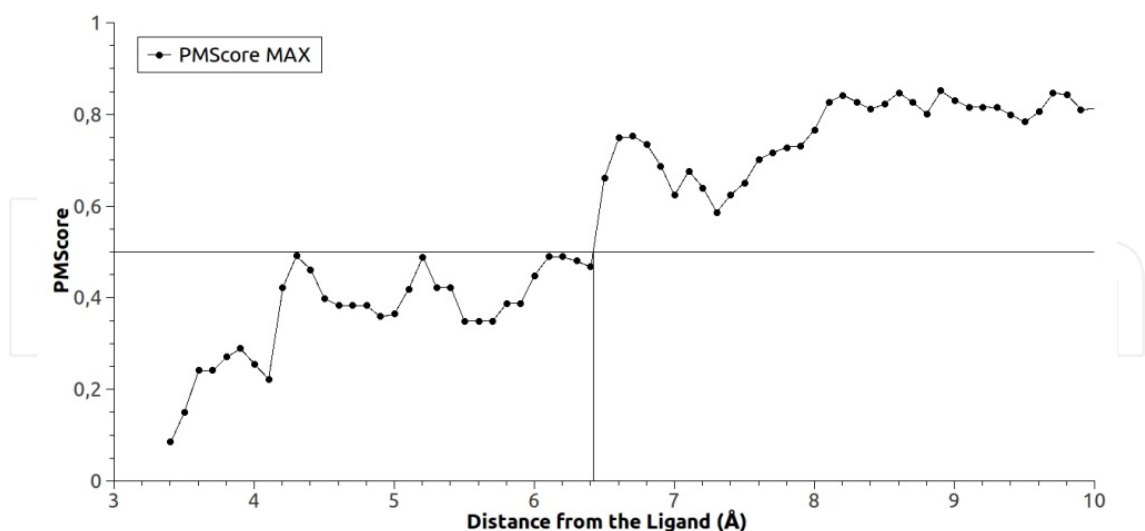


Figure 7. Similarity profile between human MAO-A (co-crystallized with clorgyline) and human MAO-B (co-crystallized with deprenyl), as calculated using PocketMatch. The horizontal black line indicates PMScore = 0.5. The vertical black line indicates the distance from the ligand where the PMScore begins to be consistently greater than 0.5. Each point corresponds to the PMScore.

As shown in Figures 8C and 8D, at a distance of 6.4 Å from the ligand, several amino acids considered in the similarity determination are located outside the binding site.

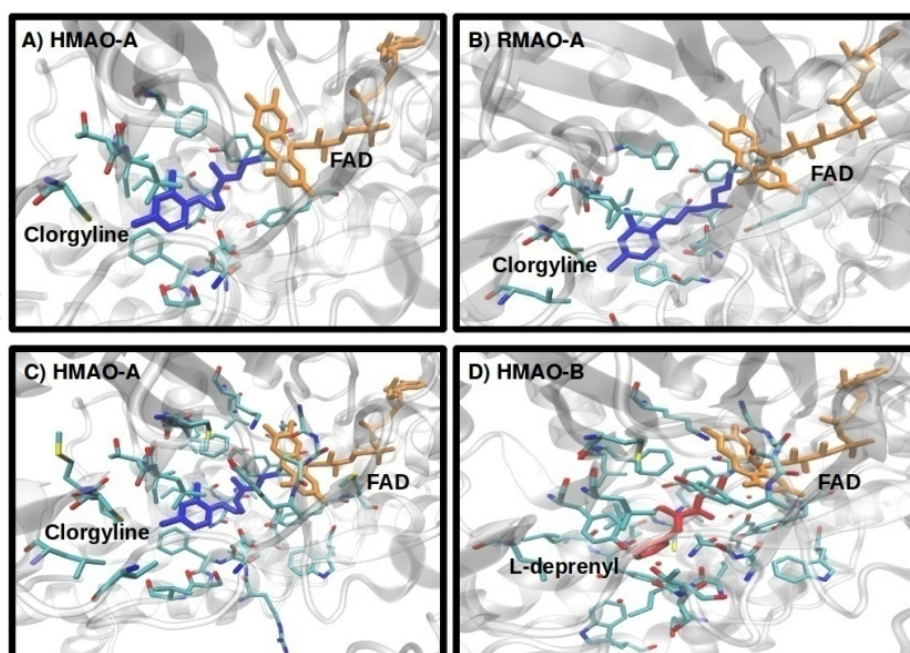


Figure 8. Binding site residues surrounding the inhibitors clorgyline (blue) and deprenyl (pink) bound to human MAO-A (HMAO-A), rat MAO-A (RMAO-A) or human MAO-B (HMAO-B). Figures 8A and 8B show the residues located at 4.5 Å from the ligand, while figures 8C and 8D show the residues located at 6.5 Å from the ligand

Therefore, the similarity profile shown in Figure 7 indicates that human MAO-A and MAO-B binding sites are less similar than those of rat and human MAO-A. It also shows that, although showing differences at their binding sites, human MAO-A and MAO-B exhibit a high degree of global structural similarity (all PMScores obtained at distances longer than 6.5 Å were well over 0.5). Though both findings might be considered obvious from the analysis of each protein sequence and function, they confirm the suitability of PocketMatch to find and predict such characteristics, an aspect that could be particularly useful when comparing proteins from which less functional information is available. In addition, our results suggest that in some cases the determination of similarity profiles can be more informative than point comparisons.

Figures 9 and 10 show the similarity profiles after comparing the homology models of zMAO with those of human MAO-A and MAO-B, respectively. As mentioned, in all cases, MeONIPA docked in each MAO structure was used as ligand.

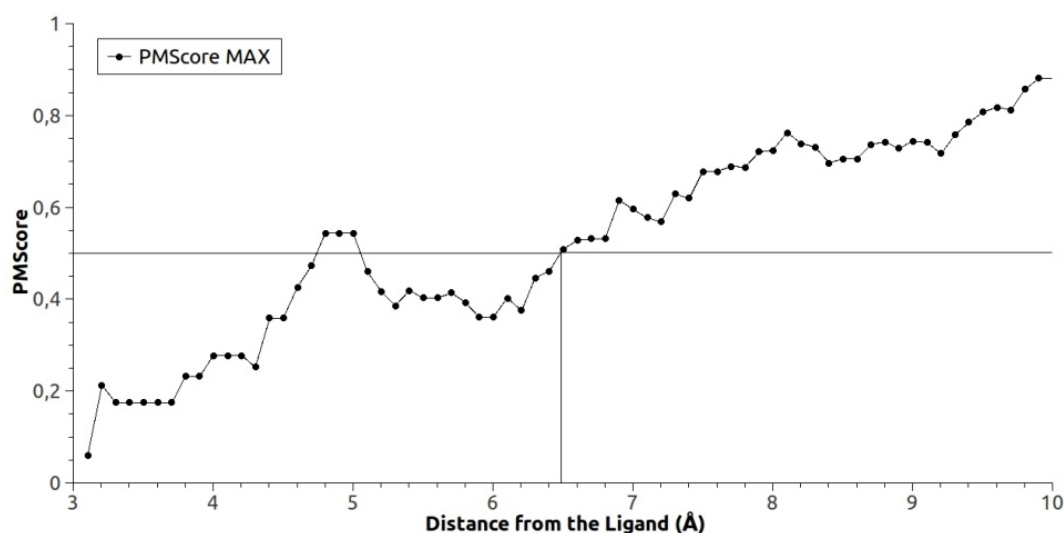


Figure 9. Similarity profile between zMAO (in this case the model corresponds to that based on human MAO-A) and human MAO-A, as calculated using PocketMatch. In both proteins, docked MeONIPA was used as ligand. The horizontal black line indicates PMScore = 0.5. The vertical black line indicates the distance from the ligand where the PMScore begins to be consistently greater than 0.5. Each point corresponds to the PMScore.

As shown in Figures 9 and 10, PMScores indicative of similarity between the binding sites of zMAO and human MAO-A or MAO-B (i.e., PMScore > 0.5) were consistently seen at distances higher than 6 Å from the ligand. It should be noted that comparable values were obtained even though the zMAO model was built using either human MAO-A or MAO-B as templates, and regardless of which human enzyme was used for the comparison. These results suggest that the zMAO binding site is as different from those of both human isoforms as the binding site of MAO-A differs from that of MAO-B. In addition, the similarity profiles of zMAO against both human proteins indicate that global structural similarity is found across these species, while the main differences are found at their binding sites. Since, to perform the similarity determination, PocketMatch considers both the shape and the chemi-

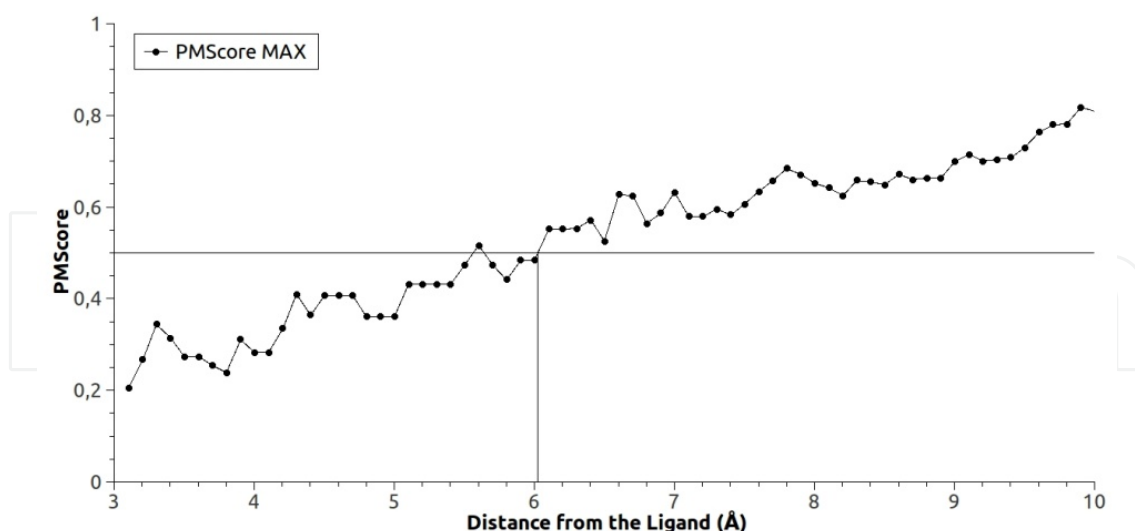


Figure 10. Similarity profile between zMAO (in this case the model corresponds to that based on human MAO-B) and human MAO-B, as calculated using PocketMatch. In both proteins, docked MeONIPA was used as ligand. The horizontal black line indicates PMScore = 0.5. The vertical black line indicates the distance from the ligand where the PMScore begins to be consistently greater than 0.5. Each point corresponds to the PMScore.

cal nature of the residues forming the site (Yeturu & Chandra, 2008), these two factors are likely involved in the differences detected between the MAO isoforms. Considering the sequence identity between zebrafish and human enzymes, one may predict that conformational differences are more important when comparing zMAO and human MAO-A, while the chemical features of the residues are more relevant to the differences between zMAO and human MAO-B. Nevertheless, further analyses are necessary to determine the relative contribution of each aspect to the differences found.

4. Conclusion

In summary, results from biochemical evaluation, molecular simulation and similarity detection studies presented here add novel evidence to the notion that even though zMAO exhibits some functional and structural properties overlapping those of MAO-A and -B, the zebrafish protein behaves quite distinctively from its mammalian counterparts. Therefore, although still an attractive model for drug discovery, in our opinion zebrafish is not a useful model for the identification of novel MAO inhibitors aimed for use in humans.

Acknowledgements

We thank Dr. K. Yeturu and Prof. N. Chandra for their valuable comments regarding Pocket Match results and functioning. We also thank Prof. Bruce K. Cassels for critical reading of the

manuscript. This work was funded by MSI Grant P05/001-F, PBCT grant PDA-23 to AF and FONDECYT Grants 110-85002 to AF, 110-0542 to PI-V and 109-0037 to MR-P. D.E.E. acknowledges research support from the National Institutes of Health GM 29433

Author details

Angelica Fierro^{1,2}, Alejandro Montecinos¹, Cristobal Gómez-Molina³, Gabriel Núñez⁴, Milagros Aldeco⁵, Dale E. Edmondson⁵, Marcelo Vilches-Herrera³, Susan Lühr^{2,3}, Patricio Iturriaga-Vásquez¹ and Miguel Reyes-Parada^{2,6*}

*Address all correspondence to: miguel.reyes@usach.cl

1 Faculty of Chemistry and Biology, University of Santiago de Chile, Chile

2 Millennium Institute for Cell Dynamics and Biotechnology, Chile

3 Faculty of Sciences, University of Chile, Chile

4 PhD Program in Biotechnology, University of Santiago de Chile, Chile

5 Department of Biochemistry and Chemistry, Emory University, USA

6 Faculty of Medical Sciences, University of Santiago de Chile, Chile

References

- [1] Aldeco, M, Arslan, B. K, & Edmondson, D. E. (2011). Catalytic and inhibitor binding properties of zebrafish monoamine oxidase (zMAO): comparisons with human MAO A and MAO B. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, , 159, 78-83.
- [2] Allen, W. J, & Bevan, D. R. (2011). Steered molecular dynamics simulations reveal important mechanisms in reversible monoamine oxidase B inhibition. *Biochemistry*, , 50, 6441-6454.
- [3] Anichtchik, O, Sallinen, V, Peitsaro, N, & Panula, P. (2006). Distinct structure and activity of monoamine oxidase in the brain of zebrafish (*Danio rerio*). *Journal of Comparative Neurology*, , 498, 593-610.
- [4] Arslan, B. K, & Edmondson, D. E. (2010). Expression of zebrafish (*Danio rerio*) monoamine oxidase (MAO) in *Pichia pastoris*: purification and comparison with human MAO A and MAO B. *Protein Expression and Purification*, , 70, 290-297.

- [5] Apsel, B, Blair, J. A, González, B, Nazif, T. M, Feldman, M. E, Aizenstein, B, Hoffman, R, Williams, R. L, Shokat, K. M, & Knight, Z. A. (2008). Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. *Nature Chemical Biology*, , 4, 691-699.
- [6] Baroni, M, Cruciani, G, Sciabola, S, Perruccio, F, & Mason, J. S. (2007). A common reference framework for analyzing/comparing proteins and ligands. Fingerprints for Ligands and Proteins (FLAP): theory and application. *Journal of Chemical Information and Modeling*, , 47, 279-294.
- [7] Bencan, Z, & Levin, E. D. (2008). The role of $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in the nicotine-induced anxiolytic effect in Zebrafish. *Physiology & Behavior*, , 95, 408-412.
- [8] Bencan, Z, Sledge, D, & Levin, E. D. (2009). Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacology Biochemistry and Behavior*, , 4, 75-80.
- [9] Binda, C, Newton-vinson, P, Hubálek, F, Edmondson, D. E, & Mattevi, A. (2002). Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nature Structural Biology*, , 9, 22-26.
- [10] Binda, C, Li, M, Hubálek, F, Restelli, N, Edmondson, D. E, & Mattevi, A. (2003). Insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high-resolution crystal structures. *Proceedings of the National Academy of Sciences USA*, , 100, 9759.
- [11] Binda, C, Hubálek, F, Li, M, Herzig, Y, Sterling, J, Edmondson, D. E, & Mattevi, A. (2004). Crystal structures of monoamine oxidase B in complex with four inhibitors of the *N*-propargylamino-indan class. *Journal of Medicinal Chemistry*, , 47, 1767-1774.
- [12] Binda, C, Aldeco, M, Mattevi, A, & Edmondson, D. E. (2011). Interactions of monoamine oxidases with the antiepileptic drug zonisamide: specificity of inhibition and structure of the human monoamine oxidase B complex. *Journal of Medicinal Chemistry*, , 54, 909-912.
- [13] Brylinski, M, & Skolnick, J. (2009). FINDSITE: a threading-based approach to ligand homology modeling. *PLoS Computational Biology*, , 5, e1000405.
- [14] Chakraborty, C, & Hsu, C. H. (2009). Zebrafish: a complete animal model for in vivo drug discovery and development. *Current Drug Metabolism*, , 10, 116-124.
- [15] Cleves, A. E, & Jain, A. N. Robust ligand-based modeling of the biological targets of known drugs. *Journal of Medicinal Chemistry*, , 49, 2921-2938.
- [16] Colwill, R. M, Raymond, M. P, Ferreira, L, & Escudero, H. (2005). Visual discrimination learning in zebrafish (*Danio rerio*). *Behavioral Processes*, , 70, 19-31.

- [17] Darland, T, & Dowling, J. E. (2001). Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proceedings of the National Academy of Sciences USA.*, , 98, 11691-11696.
- [18] De Colibus, L, Li, M, Binda, C, Lustig, A, Edmondson, D. E, & Mattevi, A. (2005). Three-dimensional structure of human monoamine oxidase A (MAO A): Relation to the structures of rat MAO A and human MAO B. *Proceedings of the National Academy of Sciences USA.*, , 102, 12684-12689.
- [19] Eddins, D, Cerutti, D, Williams, P, Linney, E, & Levin, E. D. (2010). Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: Comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. *Neurotoxicology and Teratology*, , 2, 99-108.
- [20] Edmondson, D. E, Binda, C, & Mattevi, A. (2007). Structural insights into the mechanism of amine oxidation by monoamine oxidases A and B. *Archives of Biochemistry and Biophysics*, , 464
- [21] Edmondson, D. E, Binda, C, Wang, J, Upadhyay, A. K, & Mattevi, A. (2009). Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Biochemistry*, , 48, 4220-4230.
- [22] Egan, R. J, Bergner, C. L, Hart, P. C, Cachat, J. M, Canavello, P. R, Elegante, M. F, Elkhayat, S. I, Bartels, B. K, Tien, A. K, Tien, D. H, Mohnot, S, Beeson, E, Glasgow, E, Amri, H, Zukowska, Z, & Kalueff, A. V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, , 205, 38-44.
- [23] Erdem, S. S, & Büyükmeneke, B. (2011). Computational investigation on the structure-activity relationship of the biradical mechanism for monoamine oxidase. *Journal of Neural Transmission*, , 118, 1021-1029.
- [24] Flinn, L, Bretaude, S, Lo, C, Ingham, P. W, & Bandmann, O. (2008). Zebrafish as a new animal model for movement disorders. *Journal of Neurochemistry*, , 106, 1991-1997.
- [25] Fierro, A, Osorio-olivares, M, Cassels, B. K, Edmondson, D. E, Sepúlveda-boza, S, & Reyes-parada, M. (2007). Human and rat monoamine oxidase-A are differentially inhibited by (S)-4-alkylthioamphetamine derivatives: insights from molecular modeling studies. *Bioorganic and Medicinal Chemistry*, , 15, 5198-56206.
- [26] Frisch, M. J, Trucks, G. W, Schlegel, H. B, Scuseria, G. E, Robb, M. A, Cheeseman, J. R, et al. (2004). Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford CT.
- [27] Gold, N. D, & Jackson, R. M. (2006). SitesBase: a database for structure-based protein-ligand binding site comparisons. *Nucleic Acids Research, Database issue*, , 34, D231-D234.
- [28] Goldsmith, P. (2004). Zebrafish as a pharmacological tool: the how, why and when. *Current Opinion in Pharmacology*, , 4, 504-512.

- [29] Gordon, J. C, Myers, J. B, Folta, T, Shoja, V, Heath, L. S, & Onufriev, A. (2005). H++: a server for estimating pKas and adding missing hydrogens to macromolecules. *Nucleic Acids Research*, Web Server issue), , 33, W368-W371.
- [30] Henrich, S, Salo-ahen, O. M, Huang, B, Rippmann, F. F, Cruciani, G, & Wade, R. C. (2010). Computational approaches to identifying and characterizing protein binding sites for ligand design. *Journal of Molecular Recognition*, , 23, 209-219.
- [31] Hollis, K. L. (1999). The role of learning in the aggressive and reproductive behavior of blue gouramis, *Trichogaster trichopterus*. *Environmental Biology of Fishes*, , 54, 355-369.
- [32] Hoffmann, B, Zaslavskiy, M, Vert, J. P, & Stoven, V. (2010). A new protein binding pocket similarity measure based on comparison of clouds of atoms in 3D: application to ligand prediction. *BMC Bioinformatics*, , 11, 99.
- [33] Hopkins, A. L. (2008). Network pharmacology: the next paradigm in drug discovery. *Nature Chemical Biology*, , 4, 682-690.
- [34] Hurtado-guzmán, C, Fierro, A, Iturriaga-vásquez, P, Sepúlveda-boza, S, Cassels, B. K, & Reyes-parada, M. (2003). Monoamine oxidase inhibitory properties of optical isomers and N-substituted derivatives of 4-methylthioamphetamine. *Journal of Enzyme Inhibition and Medicinal Chemistry*, , 18, 339-347.
- [35] Jambon, M, Imberty, A, Deléage, G, & Geourjon, C. (2003). A new bioinformatic approach to detect common 3D sites in protein structures. *Proteins*, , 52, 137-145.
- [36] Kahraman, A, Morris, R. J, Laskowski, R. A, & Thornton, J. M. (2007). Shape variation in protein binding pockets and their ligands. *Journal of Molecular Biology*, , 368, 283-301.
- [37] Kaufman, C. K, & White, R. M. (2009). Chemical genetic screening in the zebrafish embryo. *Nature Protocols*, , 4, 1422-1432.
- [38] Keiser, M. J, Setola, V, Irwin, J. J, Laggner, C, Abbas, A. I, Hufeisen, S. J, Jensen, N. H, Kuijter, M. B, Matos, R. C, Tran, T. B, Whaley, R, Glennon, R. A, Hert, J, Thomas, K. L, Edwards, D. D, Shoichet, B. K, & Roth, B. L. (2009). Predicting new molecular targets for known drugs. *Nature*, , 462, 175-181.
- [39] Kinnings, S. L, & Jackson, R. M. (2009). Binding site similarity analysis for the functional classification of the protein kinase family. *Journal of Chemical Information and Modeling*, , 49, 318-329.
- [40] King, A. (2009). Researchers find their Nemo. *Cell*, , 139, 843-846.
- [41] Kochersperger, L. M, Parker, E. L, Siciliano, M, Darlington, G. J, & Denney, R. M. (1986). Assignment of genes for human monoamine oxidases A and B to the X chromosome. *Journal of Neuroscience Research*, , 16, 601-616.
- [42] Kokel, D, Bryan, J, Laggner, C, White, R, Cheung, C. Y, Mateus, R, Healey, D, Kim, S, Werdich, A. A, Haggarty, S. J, Macrae, C. A, Shoichet, B, & Peterson, R. T. (2010).

Rapid behavior-based identification of neuroactive small molecules in the zebrafish. *Nature Chemical Biology*, , 6, 231-237.

- [43] Kokel, D, & Peterson, R. T. (2011). Using the zebrafish photomotor response for psychotropic drug screening. *Methods in Cell Biology*, , 105, 517-524.
- [44] Lan, N. C, Chen, C. H, & Shih, J. C. (1989). Expression of functional human monoamine oxidase A and B cDNAs in mammalian cells. *Journal of Neurochemistry*, , 52, 1652-1654.
- [45] Landon, M. R, Amaro, R. E, Baron, R, Ngan, C. H, Ozonoff, D, Mccammon, J. A, & Vajda, S. (2008). Novel druggable hot spots in avian influenza neuraminidase H5N1 revealed by computational solvent mapping of a reduced and representative receptor ensemble. *Chemical Biology & Drug Design*, , 71, 106-116.
- [46] Laskowski, R. A. MacArthur, MW.; Moss, DS. & Thornton, JM. ((1993). PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, , 26, 283-291.
- [47] Laskowski, R. A, Watson, J. D, & Thornton, J. M. (2005). ProFunc: a server for predicting protein function from 3D structure. *Nucleic Acids Research*, Web Server issue), , 33, W89-W93.
- [48] Laurie, A. T, & Jackson, R. M. an energy-based method for the prediction of protein-ligand binding sites. *Bioinformatics*, , 21, 1908-1916.
- [49] Levin, E. D, & Chen, E. (2006). Nicotinic involvement in memory function in zebrafish. *Neurotoxicology Teratology*, , 6, 731-735.
- [50] Levin, E. D, Bencan, Z, & Cerutti, D. T. (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology and Behavior*, , 90, 54-58.
- [51] Lühr, S, Vilches-herrera, M, Fierro, A, Ramsay, R. R, Edmondson, D. E, Reyes-parada, M, Cassels, B. K, & Iturriaga-vásquez, P. (2010). Arylthiomorpholine derivatives as potent and selective monoamine oxidase B inhibitors. *Bioorganic & Medicinal Chemistry*, , 18, 1388-1395.
- [52] Ma, J, Masato, Y, Yamashita, E, Nakagawa, A, Ito, A, & Tsukihara, T. (2004). Structure of rat monoamine oxidase and its specific recognitions for substrates and inhibitors. *Journal of Molecular Biology*, , 338, 103-114.
- [53] Mckinley, E. T, Baranowski, T. C, Blavo, D. O, Cato, C, Doan, T. N, & Rubinstein, A. L. (2005). Neuroprotection of MPTP-induced toxicity in Zebrafish dopaminergic neurons. *Molecular Brain Research*, , 141, 128-137.
- [54] Miklosi, A, Andrew, R. J, & Savage, H. (1997). Behavioural lateralisation of the tetrapod type in the zebrafish (*Brachydanio rerio*). *Physiology & Behavior*, , 63, 127-135.
- [55] Miklosi, A, & Andrew, R. J. (1999). Right eye use associated with decision to bite in zebrafish. *Behavioural Brain Research*, , 105, 199-205.

- [56] Milletti, F, & Vulpetti, A. (2010). Predicting polypharmacology by binding site similarity: from kinases to the protein universe. *Journal of Chemical Information and Modeling*, , 50, 1418-1431.
- [57] Moriaud, F, Richard, S. B, Adcock, S. A, Chanas-martin, L, & Surgand, J. S. Ben Jeloul, M. & Delfaud, F. ((2011). Identify drug repurposing candidates by mining the protein data bank. *Briefings in Bioinformatics*,, 12, 336-240.
- [58] Morris, G. M, Goodsell, D. S, Halliday, R. S, Huey, R, Hart, W. E, Belew, R. K, & Olson, A. J. (1998). Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *Journal of Computational Chemistry*, , 19, 1639-1662.
- [59] Nagatsu, T. (2004). Progress in monoamine oxidase (MAO) research in relation to genetic engineering. *Neurotoxicology*, , 25, 11-20.
- [60] Najmanovich, R, Kurbatova, N, & Thornton, J. (2008). Detection of 3D atomic similarities and their use in the discrimination of small molecule protein-binding sites. *Bioinformatics*, , 24, i105-i111.
- [61] Nandigama, R. K, Newton-vinson, P, & Edmondson, D. E. (2002). Phentermine inhibition of recombinant human liver monoamine oxidases A and B. *Biochemical Pharmacology*, , 63, 865-869.
- [62] Norton, W, & Bally-cuif, L. (2010). Adult zebrafish as a model organism for behavioural genetics. *BMC Neuroscience*, , 11, 90.
- [63] Ninkovic, J, & Bally-cuif, L. (2006). The zebrafish as a model system for assessing the reinforcing properties of drug abuse. *Methods*, , 39, 262-274.
- [64] Panula, P, Chen, Y. C, Priyadarshini, M, Kudo, H, Semenova, S, Sundvik, M, & Sallinen, V. (2010). The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. *Neurobiology of Disease*, , 40, 46-57.
- [65] PayneRJH. ((1998). Gradually escalating fights and displays: The cumulative assessment model. *Animal Behavior*, , 56, 651-662.
- [66] Pérez-nueno, V. I, & Ritchie, D. W. (2011). Using consensus-shape clustering to identify promiscuous ligands and protein targets and to choose the right query for shape-based virtual screening. *Journal of Chemical Information and Modeling*, , 51, 1233-1248.
- [67] Phillips, J. C, Braun, R, Wang, W, Gumbart, J, Tajkhorshid, E, Villa, E, Chipot, C, Skeel, R. D, Kalé, L, & Schulten, K. (2005). Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry*, , 26, 1781-1802.
- [68] Postlethwait, J. H, Woods, I. G, Ngo-hazelett, P, Yan, Y. L, Kelly, P. D, Chu, F, Huang, H, Hill-force, A, & Talbot, W. S. (2000). Zebrafish comparative genomics and the origins of vertebrate chromosomes. *Genome Research*, 2000, , 10, 1890-1902.

- [69] Reader, S. M, Kendal, J. R, & Laland, K. N. (2003). Social learning of foraging sites and escape routes in wild Trinidadian guppies. *Animal Behavior*, , 66, 729-739.
- [70] Reyes-parada, M, Fierro, A, Iturriaga-vásquez, P, & Cassels, B. K. (2005). Monoamine oxidase inhibition in the light of new structural data. *Current Enzyme Inhibition*, , 1
- [71] Rihel, J, Prober, D. A, Arvanites, A, Lam, K, Zimmerman, S, Jang, S, Haggarty, S. J, Kokel, D, Rubin, L. L, Peterson, R. T, & Schier, A. F. (2010). Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*, , 327, 348-351.
- [72] Rubinstein, A. L. (2006). Zebrafish assays for drug toxicity screening. *Expert Opinion on Drug Metabolism & Toxicology*, , 2, 231-240.
- [73] Russell, R. B, Sasieni, P. D, & Sternberg, M. J. (1998). Supersites within superfolds. Binding site similarity in the absence of homology. *Journal of Molecular Biology*, , 282, 903-918.
- [74] Sali, A, & Blundell, T. L. (1993). Comparative protein modeling by satisfaction of spatial restraints. *Journal of Molecular Biology*, , 234, 779-815.
- [75] Sallinen, V, Sundvik, M, Reenilä, I, Peitsaro, N, Khrustalyov, D, Anichtchik, O, Tolei-kyte, G, Kaslin, J, & Panula, P. (2009). Hyperserotonergic phenotype after monoamine oxidase inhibition in larval zebrafish. *Journal of Neurochemistry*, , 109
- [76] Schrattenholz, A, & Soskic, V. (2008). What does systems biology mean for drug development? *Current Medicinal Chemistry*, , 15, 1520-1528.
- [77] Setini, A, Pierucci, F, Senatori, O, & Nicotra, A. (2005). Molecular characterization of monoamine oxidase in zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, , 140, 153-161.
- [78] Son, S. Y, Ma, J, Kondou, Y, Yoshimura, M, Yamashita, E, & Tsukihara, T. (2008). Structure of human monoamine oxidase A at 2.2-Å resolution: the control of opening the entry for substrates/inhibitors. *Proceedings of the National Academy of Sciences USA*, , 105, 5739-5744.
- [79] Streisinger, G, Walker, C, Dower, N, Knauber, D, & Singer, F. (1981). Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature*, , 291, 293-296.
- [80] Suboski, M. D. (1988). Acquisition and social communication of stimulus recognition by fish. *Behavioral Processes*, , 16, 213-244.
- [81] Suboski, M. D, Bain, S, Carty, A. E, & Mcquoid, L. M. (1990). Alarm reaction in acquisition and social transmission of simulated predator recognition by zebra danio fish. *Journal of Comparative Psychology*, , 104, 101-112.
- [82] Tebbich, S, Bshary, R, & Grutter, A. S. (2002). Cleaner fish *Labroides dimidiatus* recognise familiar clients. *Animal Cognition*, , 5, 139-145.

- [83] Vilches-herrera, M, Miranda-sepúlveda, J, Rebolledo-fuentes, M, Fierro, A, Lühr, S, Iturriaga-vasquez, P, Cassels, B. K, & Reyes-parada, M. (2009). Naphthylisopropylamine and N-benzylamphetamine derivatives as monoamine oxidase inhibitors. *Bioorganic and Medicinal Chemistry*, , 17, 2452-2460.
- [84] Weisel, M, Proschak, E, & Schneider, G. (2007). PocketPicker: analysis of ligand binding-sites with shape descriptors. *Chemistry Central Journal*, , 1, 7.
- [85] Wiederstein, M, & Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Research, Web Server issue*), , 35, W407-W410.
- [86] Xie, L, & Bourne, P. E. (2008). Detecting evolutionary relationships across existing fold space, using sequence order-independent profile-profile alignments. *Proceedings of the National Academy of Sciences USA.*, , 105, 5441-5446.
- [87] Xie, L, Xie, L, & Bourne, P. E. (2009). A unified statistical model to support local sequence order independent similarity searching for ligand-binding sites and its application to genome-based drug discovery. *Bioinformatics*, , 25, i305-i312.
- [88] Yeturu, K, & Chandra, N. (2008). PocketMatch: a new algorithm to compare binding sites in protein structures. *BMC Bioinformatics*, , 9, 543.
- [89] YoudimMBH.; Edmondson, D. & Tipton, KF. ((2006). The therapeutic potential of monoamine oxidase inhibitors. *Nature Reviews Neuroscience*, , 7, 295.
- [90] Zon, L. I, & Peterson, R. T. (2005). In vivo drug discovery in the zebrafish. *Nature Reviews Drug Discovery*, , 4, 35-44.

