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Catalytic and Inhibitor Binding Properties of Zebrafish Monoamine Oxidase (zMAO): Comparisons with human MAO A and MAO B

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Abstract

A comparative investigation of substrate specificity and inhibitor binding properties of recombinant zebrafish (*Danio rerio*) monoamine oxidase (zMAO) with those of recombinant human monoamine oxidases A and B (hMAO A and hMAO B) is presented. zMAO oxidizes the neurotransmitter amines (serotonin, dopamine and tyramine) with k_{cat} values that exceed those of hMAO A or of hMAO B. The enzyme is competitively inhibited by hMAO A selective reversible inhibitors with the exception of d-amphetamine where uncompetitive inhibition is exhibited. The enzyme is unreactive with most MAO B-specific reversible inhibitors with the exception of chlorostyrylcaffeine. zMAO catalyzes the oxidation of *para*-substituted benzylamine analogues exhibiting $^{D}k_{cat}$ and $^{D}(k_{cat}/K_m)$ values ranging from 2–8. Structure-activity correlations show a dependence of log k_{cat} with the electronic factor σ_p with a ρ value of +1.55 ± 0.34; a value close to that for hMAO A but not with MAO B. zMAO differs from hMAO A or hMAO B in benzylamine analogue binding correlations where an electronic effect (ρ = +1.29 ± 0.31) is observed. These data demonstrate zMAO exhibits functional properties similar to hMAO A as well as exhibits its own unique behavior. These results should be useful for studies of MAO function in zebrafish models of human disease states.

Keywords

zebrafish; monoamine oxidase; inhibitor binding; substrate specificity

1 Introduction

The elucidation of the separate gene sequences for human monoamine oxidases (EC 1.4.3.4) A and B (MAO A and MAO B) (Bach, et al. 1988) has provided unequivocal proof for the existence of two isoforms of these membrane-bound flavoenzymes that catalyze the oxidation of amine neurotransmitters. Gene sequences for both forms are available for a number of mammals that are commonly used as animal models for MAO inhibitor development studies (Edmondson, et al. 2009). In contrast, available gene sequence data on

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teleosts show the existence of only a single gene encoding MAO. The initial sequence was published for trout by Shih's group (Chen, et al. 1994). Recently, the gene sequence of zebrafish (*Danio rerio*) has been elucidated (Setini, et al. 2005; Anichtchik, et al. 2006). This organism exhibits a number of characteristics to make it a good animal model system for drug development studies (Kokel et al.,2010; Rihel, et al. 2010) and initial studies have shown MAO to be important in serotonergic biological processes (Sallinen, et al. 2009a; Sallinen, et al. 2009b). A comparative study to determine the functional properties of zMAO is reported here to facilitate further work and to provide a basis for comparison to human MAO's. Previous work from this laboratory has shown the successful expression, purification, and partial characterization of recombinant zMAO (Arslan and Edmondson, 2009). This laboratory has also expressed, purified, and characterized human and rat MAO A and MAO B (Newton-Vinson, et al. 2000; Li, et al. 2002; Upadhyay and Edmondson 2008; Wang and Edmondson, 2009). Therefore, the tools are in place for a detailed comparative functional study of zMAO with those of human MAO A and MAO B.

Previous studies have demonstrated that zMAO exhibits inhibitor binding properties that overlap those of human MAO A and of MAO B (Setini, et al. 2005; Anichtchik, et al. 2006; Sallinen, et al. 2009). The results presented in this manuscript provide a more in-depth approach to verify this suggestion. Investigations of the structure and function of hMAO A and of hMAO B demonstrate the two enzymes differ in active site structures (Binda, et al. 2002; Son, et al. 2008), in inhibitor binding (Youdim,et al. 2006), substrate specificities (Edmondson, et al. 2007), and in analysis of the influence of *para*-substituents of benzylamine analogues on catalysis (Walker and Edmondson 1994; Miller and Edmondson 1999). Crystallographic studies show hMAO B contains a bipartite active site with an entrance cavity (290 $Å^3$) and a substrate cavity (~400 $Å^3$) separated by an Ile199 gate residue (Hubálek, et al. 2005) MAO A contains a monopartite single cavity of ~550 $Å^3$ (DeColibus, et al. 2005) These differences in cavity structures account for specificity of MAO B binding of reversible inhibitors such as 8-(3-chlorostyryl)-caffeine, *trans*-*trans*-farnesol, diphenyl-2-butene and safinamide (Hubálek, et al. 2005; Binda, et al. 2007) . The rates of oxidation of *para*-substituted benzylamine analogues by MAO A exhibit a strong dependence on the electron withdrawing capacity of the *para*-substituent with a Hammett plot exhibiting a ρ value of + 1.89 (Miller and Edmondson 1999) while the rate of MAO B-catalyzed oxidation of this class of substrate analogues exhibits no detectable electronic dependence (Walker and Edmondson 1994, M.Li, PhD Disseration, Emory University). For a detailed discussion of the published differences between the human enzymes, the reader is referred to a recent review article (Edmondson, et al. 2009).

With these demonstrated differences between hMAO A and hMAO B functional behaviors, we report here a comparative investigation of the substrate and inhibitor binding properties of zMAO. The results confirm and extend previous suggestions in the literature that this teleost MAO exhibits functional properties that are more similar to those of MAO A rather than those of MAO B.

2 Materials and Methods

2.1 Materials

Zebrafish (*Danio rerio*) monoamine oxidase was expressed in *Pichia pastoris* and purified as previously described (Arslan and Edmondson, 2009). Reduced Triton X-100, glycerol, HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, isatin, benzylamine, kynuramine, serotonin, 1,4-diphenyl-1,3-butadiene, methylene blue, and 1,4-diphenyl-2-butene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Safinamide was a gift from Newron Pharma (Milan, Italy). 8-(3-Chlorostyryl)-caffeine and *trans*-*trans*-farnesol were gifts from Dr. N. Castagnoli, Department of Chemistry, Virginia Tech. University.

Harmane, pirlindole mesylate and tetrindole mesylate were purchased from TOCRIS Bioscience (Ellisville, MO, USA). All other commercially available reagents were used without further purification. The structures of the MAO A and MAO B specific inhibitors used in the study are shown in Figure 1. All benzylamine analogues used in this study were synthesized in this laboratory as previously described (Walker and Edmondson, 1994;Miller and Edmondson 1999).

2.2 Determination of steady state kinetic parameters

Spectrophotometric enzyme assays were determined using either a Perkin-Elmer Lambda 2 UV-Vis or a Varian Cary 50 UV-Vis spectrophotometer. Assays were conducted at 25 °C in 50 mM potassium phosphate, pH=7.4 buffer containing 0.5% (w/v) reduced Triton X-100 unless otherwise stated. All buffers were equilibrated for 30 min at 25 °C before use. Assays using benzylamine and their α,α-^{2}[H] analogues used the Amplex-red peroxidase coupled assay (Invitrogen) for increased detection sensitivity ($\Delta\varepsilon_{m560}$ = 54,000 $M^{-1}cm^{-1}$). MAO substrates containing phenolic oxygens such as serotonin, dopamine and tyramine were assayed polarographically by measuring the rate of O_2 uptake since they are also substrates for the horseradish peroxidase used in the coupled Amplex Red assay leading to erroneous kinetic results. The rate of oxygen consumption was determined using a Model 782 Oxygen Meter interfaced to a PC (Strathkelvin Instruments Ltd. North Lanarkshire, Scotland). One unit of enzyme activity is defined as the amount of enzyme required to oxidize 1 μmol of substrate in one minute. Inhibition assays for zMAO were performed with kynuramine as substrate. Kinetic measurements at saturated O_2 concentrations required sparging the solutions with O_2. Oxygen concentrations in the reaction buffers were determined polarographically. Enzyme functionality was determined from spectral measurements of the level of enzyme-bound flavin reduction on the anerobic addition of substrate compared to the level of reduction observed on the addition of excess sodium dithionite.

2.3 Data analysis

Steady state kinetic data (k_{cat} and K_m values) were determined from fits to the Michaelis-Menten equation using GraphPad Prism 5.0. Inhibition constants (K_i) were determined from analysis of steady state kinetic values measured with at least 4 inhibitor concentrations using GraphPad Prism 5.0 software. Inhibition data with Methylene Blue were analyzed using methodology for tight binding inhibitors described by Morrison (1968). Values of substituent parameters (σ, the Hammett electronic parameter; and π, the hydrophobicity parameter) were obtained from Hansch (Hansch, et al. 1995) and V_W values (the substituent van der Waals volumes) from Bondi (1964). The values for substrate binding affinities to zMAO (K_d) were calculated from steady state deuterium kinetic isotope effect data as described (Klinman and Matthews, 1985). Apparent K_d values were corrected to reflect the concentration of deprotonated amine in solution at the pH value of the assay since previous studies on mammalian MAO have shown the deprotonated form of the amine substrate is specifically bound (McEwen, et al. 1969; McEwen, et al. 1968). Multi-component statistical analyses of correlations tested were performed using StatView software to determine contributions of various *para*-substituent parameters with kinetic and binding constants. The F value is a statistical term relating the residuals of each point to the fitted line to the residuals of each point to the mean value. F is weighted for the number of variables in the correlation and the number of data points. The higher the F value, the better the correlation. The significance is calculated from the F value and represents the fractional chance that the correlation is meaningless.

3 Results

3.1 Steady State Kinetic Properties of zMAO

To compare the substrate specificities and kinetic properties of zMAO with those of human MAO A and B, a number of commonly used substrates were tested. Since the available kinetic data on the human enzymes have been collected at 25 °C, the same temperature was used in this study for comparative purposes. As shown in Table 1, zMAO oxidizes serotonin (a MAO A-specific substrate) with a k_{cat} value of 187 min^{-1}, which is equivalent to the value exhibited by human MAO A and ~6 times higher than the k_{cat} value for hMAO B; zMAO oxidizes kynuramine and dopamine with k_{cat} values of 75 min^{-1} and 242 min^{-1}, respectively. Thus, zMAO is a more effective catalyst for dopamine oxidation than either hMAO A or hMAO B. The k_{cat} value for zMAO catalyzed oxidation of tyramine (467 min^{-1}) is greater than the values determined for hMAO A (182 min^{-1}) or for hMAO B (343 min^{-1}).

Among the substrates tested, 4-phenylbutylamine and 3-aminomethylpyridine provide interesting distinctions between MAO A and MAO B. 4-Phenylbutylamine functions as a competitive inhibitor for hMAO A (Nandigama and Edmondson 2000) but is a substrate for hMAO B ($k_{cat}/K_m = 5.8 \times 10^6$ $M^{-1}min^{-1}$) and is also found to be a poorer substrate for zMAO ($k_{cat}/K_m = 6.7 \times 10^5$ $M^{-1}min^{-1}$) (Table 1). 3-Aminomethylpyridine is a substrate for hMAO B but not for hMAO A (Li, M, 2006) nor for zMAO. *p*-Carboxybenzylamine is a substrate for zMAO and for hMAO A, but is neither a substrate nor a competitive inhibitor with hMAO B (Table 1). These observations suggest a functional diversity of zMAO in MAO A or MAO B substrate specificities with properties closer to those of hMAOA.

3.2 Binding of reversible MAO A and MAO B inhibitors to zMAO

Inhibition studies further support a “MAO A-like” behavior of zMAO. zMAO catalytic activity is competitively inhibited by a number of MAO A inhibitors (Tables 2 and 3). Of the reversible inhibitors tested, methylene blue is exceptional in that it binds to zMAO with a 4nM K_i value which is 6-fold tighter than observed with hMAO A and ~10^3 tighter than measured with hMAO B. Tetrindole mesylate and pirlindole mesylate are tetracyclic anti-depressants that selectively target and reversibly inhibit the catalytic activity of MAO A (Andreeva, et al. 1992). These indole analogues inhibit zMAO with 6–7 fold weaker affinities than observed with hMAO A under identical conditions. The tricyclic hMAO A inhibitor, harmane, exhibits a similar K_i value with zMAO as it does with hMAO A.

A variety of MAO B-specific inhibitors were tested with zMAO (Table 3). No observable inhibition is observed with a number of MAO B-specific inhibitors that have been shown to occupy both the substrate and entrance cavities of the human enzyme (Binda et al. 2003). 8-(3-Chlorostyryl)-caffeine inhibition of zMAO is the only exception and is the only “dual cavity spanning” MAO B reversible inhibitor observed to bind. The reversible MAO inhibitor *d*-amphetamine (Green and El Hait 1980;Sowa, et al. 2004) functions as an uncompetitive inhibitor of zMAO catalyzed kynuramine oxidation although it competitively inhibits human MAO A (Vintém et al., 2005) and can function either as a competitive or as a mixed inhibitor of human MAO B depending on the substrate (Pearce and Roth, 1985). The sensitivity of zMAO to amphetamine inhibition shows it differs from hMAO A in that inhibition occurs by binding to either E or ES (or EP) forms of zMAO rather than solely to the free enzyme. This behavior then differs with those exhibited by hMAO A and is closer to those exhibited by hMAO B.

3.3 Quantitative structure-activity relationships for zMAO oxidation of para-substituted benzylamine analogues

A major difference in the catalytic properties of human MAO A and MAO B is the influence of *para*-substituents on the rates of oxidation of benzylamine analogues. hMAO A demonstrates a strong electronic contribution (ρ = + 1.89) (Miller and Edmondson, 1999) from *para*-substitution in this class of substrate analogues but no electronic effect is observed with bovine MAO B (Walker and Edmondson 1994) or with human MAO B (Li, 2006) . Given the similarities of zMAO to MAO A, the influence of *para*-substitution on the steady state rates of benzylamine analogue oxidation were determined and the kinetic data are shown in Table 4.

In agreement with published data on hMAO A (Miller and Edmondson, 1999), on bovine MAO B (Walker and Edmondson, 1994), and on human and rat MAO B (Newton-Vinson, et al.,2000;Li, 2006; Upadyhay and Edmondson, 2008), zMAO exhibits deuterium kinetic isotope effects on benzylamine analogue oxidation with $^{D}k_{cat}$ and $^{D}(k_{cat}/K_m)$ values ranging from 2 to 8 (Table 4). These data demonstrate the α-C-H bond cleavage step contributes to the rate limitation in catalysis as is found with MAO A and B from various mammalian sources (Edmondson, 2009). Therefore, substituent effects on rate reflect an influence on the rate of the hydrogen-transfer step in catalysis.

Correlations between k_{cat} and electronic, steric and hydrophobicity parameters of the *para*-substituents were estimated with the electronic parameter (σ) exhibiting the major contribution. Figure 2a shows that zMAO exhibits a linear correlation of log k_{cat} with σ, a property also seen with hMAO A (Miller and Edmondson,1999).

This linear relation is best described by the equation:

$$\log k_{cat} = 1.55\ (\pm 0.34)\ s + 1.1\ (\pm 0.09)\ (F_{1,7}=20; p=0.006)$$

Analysis including additional substituent parameters such as van der Waals volume (V_W) or hydrophobicity (π) did not result in any statistical improvement in the correlation. This equation shows that the rate of turnover increases with increasing electron withdrawing power of the *para*-substituent as shown previously with human MAO A (Miller and Edmondson, 1999) . The calculated ρ value for zMAO (+ 1.55) is close to that (+ 1.89) observed for hMAO A (Miller and Edmondson,1999). These results indicate that the bound benzylamine substrate has a conformation in the substrate binding site that allows transmission of the *para*-substituent electronic effect to the benzyl carbon as found for hMAO A but not in MAO B. These steady state kinetic measurements were performed at air saturation (240 μM O_2) which is approximately twice the K_mO_2 for zMAO (Arslan and Edmondson, 2009). The same correlations are obtained when the k_{cat} values were determined under conditions where [O_2] ~1 mM (not shown); providing additional support that a-C-H bond cleavage is rate-limiting in catalysis.

The corrected binding affinities of deprotonated *para*-substituted benzylamine analogues to the active site of zMAO were analyzed to determine whether any correlations with steric substituent parameters could be observed as found with hMAO A (Miller and Edmondson, 1999). No correlations are observed with either steric or hydrophobicity substituent parameters and binding affinity; however, a reasonable correlation is observed for binding affinity (log K_d) and the substituent electronic parameter (σ) (Figure 2b). This observation differs from the correlations reported for hMAO A and hMAO B where steric parameters dominate. The observed ρ value (+1.3) shows that electron withdrawing groups on the

benzylamine substrate analogue increase the binding affinity to zMAO according to the following relationship:

$$\log K_{d(corr)} = 1.29\,(\pm 0.31)\,\sigma + 5.86\,(\pm 0.08)\;(F_{1,7} = 17.1; p = 0.009)$$

Correlation analysis with two substituent parameters do not improve the statistics although more than seven different examples are required for a more rigorous 2-component analysis. This correlation shows that electron withdrawing groups facilitate benzylamine binding to the active site of zMAO. Since the binding data are already corrected for any substituent effects on benzylamine analogue pK_a values, this correlation indicates that electron withdrawal from the benzenoid ring of the substrate facilitates binding to the active site of zMAO. The structural basis for this effect on binding is currently unknown but suggests the presence of a positively charged residue in the active site of zMAO which is positioned to interact with the aromatic ring of the benzylamine substrate (possibly a π-cation interaction). Despite the high sequence similarity with MAO A in the substrate-binding region, zMAO does exhibit unique properties in its substrate binding site.

4 Discussion

4.1 Functional properties of zMAO

The amino acid sequence of zMAO is ~70% identical with either hMAO A or hMAO B. The sequence of zMAO in the substrate binding domain is identical with hMAO A and ~70% identical with hMAO B. The C-terminal domain of zMAO associated with the membrane-binding transmembrane helix of hMAO A and hMAO B exhibits the lowest identity (30% with hMAO A and 20% with hMAO B). A residue that has been shown to function as a gate separating the bipartite cavity of hMAO B (Ile199) and involved in inhibitor binding specificity (Hubálek et al. 2005) is a Phe in zMAO as it is in hMAO A.

The limited number of publications investigating the functional behavior of zMAO suggests the enzyme exhibits properties of both human MAO A and MAO B, with more similarities to hMAO A (Setini, et al. 2005, Sallinen, et al. 2009a). The finding that deprenyl (an irreversible MAO B inhibitor and clorgyline (an irreversible MAO A inhibitor) inhibit zMAO with similar IC_{50} values (Setini, et al. 2005, Arslon and Edmondson, 2010) documents this unique behavior of zMAO. Deprenyl treatment *in vivo* results in a decrease in serotonin levels indicating zMAO inhibition (Sallinen, et al. 2009) as expected since this acetylenic inhibitor functions irreversibly. The studies reported here for the recombinant form of zMAO provide further validation for the dual functional specificity of zMAO. Since teleosts contain only a single form of MAO, it is reasonable that this MAO would be able to function in both capacities of the mammalian counterparts.

The inhibition profile of zMAO is also further defined in this study. Previous results on hMAO B show the Ile199 gate residue between the entrance and substrate cavities to play an important role in the binding of 8-(3-chlorostyryl)-caffeine, 1,4-diphenyl-2-butene, farnesol, and 1,4- diphenyl-1,3-butadiene (Hubálek, et al. 2005) since none of these inhibitors bind to or inhibit hMAO A. The absence of binding to zMAO by the majority of these MAO B specific inhibitors suggests an active site structure of zMAO closer to that of hMAO A. A detailed understanding of these differences must await the determination of the zMAO structure by x-ray crystallography.

4.2 Mechanistic and structural interpretation of the QSAR data

Previous studies of human MAO A and MAO B substrate binding properties using *para*-substituted benzylamine analogues showed that the two isoforms are influenced differently

by the *para*-substitution (Walker and Edmondson 1994; Miller and Edmondson 1999). In the case of catalytic turnover, hMAO A prefers analogues with strong *para* electron-withdrawing groups while hMAO B catalysis is unaffected by this parameter. Substrate binding to hMAO A is favored by larger *para*-substituents while hMAO B prefers smaller substituents. These properties were originally interpreted to assign a larger binding site to hMAO A and a smaller more hydrophobic binding pocket to hMAO B. The published crystal structures confirm this interpretation (Edmondson, et al. 2007; Son 2008). These correlations also suggest that the conformation of the bound benzylamine substrate determines whether an electronic effect is observed as contributing to enhancement of catalysis in either human isoform [9]. The orientation of the aromatic ring can influence the alignment of the α-C-H with the π-orbitals of the aromatic ring, which allows substituent electronic effects to be transmitted in hMAO A but not in hMAO B where the more restrictive binding site is suggested to preclude a coplanar orientation (Miller and Edmondson, 1999). The steady-state analyses of zMAO with *para-substituted* benzylamines suggest that zMAO catalytic turnover with these substrate analogues is similar to that observed in hMAO A (Figure 2). The a-C-H bond cleavage step appears also to be a main contributor to the rate-determining step in zMAO catalysis. An electronic effect on binding affinity of *para-substituted* benzylamine analogues to zMAO (Figure 2) is unique to zMAO as neither hMAO A nor bovine or hMAO B exhibit such contributions. One interpretation for this behavior in zMAO is the presence of a positively charged residue in a close proximity to the aromatic ring of the substrate where it could stabilize binding *via* a p-cation interaction (Gallivan and Dougherty, 1999). Confirmation of these suggestions requires further structural investigations of zMAO.

5 Conclusions

The studies presented in this paper demonstrate that purified recombinant zMAO exhibits functional properties that are more close to those of hMAO A than to hMAO B. Structure-activity studies suggest a large binding pocket containing a positively charged amino acid residue. Considering that zebrafish contains only one form of MAO, the dual selectivity property of this enzyme for substrate analogues appears reasonable for the organism to degrade biogenic amines. Additional structural and mechanistic work on zMAO should provide new insights into the molecular basis for the differing functional properties of MAO A and of MAO B.

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Abbreviations

hMAO B	human monoamine oxidase B
hMAO A	human monoamine oxidase A
zMAO	zebrafish monoamine oxidase
QSAR	quantitative structure-activity relationships
V_W	van der Waals volume
π	hydrophobicity constant
σ	Hammett electronic substituent constant.

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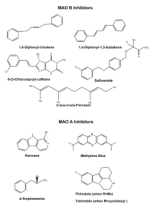

Figure 1.
Structures of reversible MAO inhibitors used in this work.

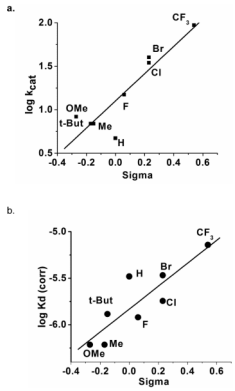

Figure 2.

a. Hammett Plot of the correlation of the logarithm of benzylamine analogue turnover rates (k_{cat} values) of zMAO with the substituent para electronic parameter (σ p). b. Hammett Plot of the correlation of the logarithm of benzylamine analogue Kd values for binding to zMAO with the substituent para electronic parameter (σ_p).

Table 1

Comparison of steady-state kinetic values for zMAO-catalyzed oxidation of amine substrate analogues with those published for hMAO A and hMAO B.

	zMAO		hMAO A		hMAO B	
Substrate	**K_m (μM)**	**k_{cat} (min^{-1})**	**K_m (μM)**	**k_{cat} (min^{-1})**	**K_m (μM)**	**k_{cat} (min^{-1})**
Kynuramine	58 ±5	75 ±2	130 ±10[a]	125 ±8[a]	27 ±2[b]	96 ±11[b]
Serotonin	69 ±11	187 ± 9	295 ±46[a]	182 ±8[a]	2270 ±310[b]	33 ±2[b]
Dopamine	88 ±14	242 ±17	240 ±42[a]	71 ±4[a]	128 ±15[b]	65 ±12[b]
Tyramine	18 ±3	467 ±20	427 ±18[c]	182 ±26[c]	107 ±21	343 ±48
4-Phenylbutylamine	185 ±17	124 ±4	Competitive Inhibitor K_i = 31 ±5 μM[b]		19 ±3	110 ±15
***p*-Carboxybenzylamine**	76 ±8	5.3 ±0.2	540 ±98	2.1 ±0.4	Not a substrate nor a competitive inhibitor	
3-Aminomethylpyridine	Not a Substrate		Competitive Inhibitor[c] K_i=5,200μM		1,255 ±68[c]	223 ±5[c]

See Methods and Materials 1.2.2 and 1.2.3 for experimental details.

[a] Values taken from Li, et al. (2002)

[b] from Nandigama and Edmondson (2000)

[c] Li, (2006).

Table 2

Reversible inhibition constants of zMAO with hMAO A specific inhibitors.[a]

Inhibitor	zMAO	hMAOA	hMAO B
	K_i (μM)		
Harmane	0.13 ±0.02	0.58 ± 0.02[b]	140 ± 47[c]
Tetrindole Mesylate	34.1 ± 2.0	5.3 ± 0.2[b]	No Inhibition
Pirlindole Mesylate	2.4±0.4	0.92 ± 0.04[b]	No Inhibition
Methylene Blue	0.004±0.001	0.027±0.003[d]	1.0 ± 0.05
d-Amphetamine	38.0 ± 5.1	14±0.5[e]	280±10[f]

[a] All inhibition constants are from data that exhibit competitive inhibition with the exception of d-amphetamine where noncompetitive inhibition is observed for zMAO.

[b] Data taken from Wang, et al. (2009).

[c] Taken from Miczek, 2010.

[d] Taken from Ramsay et al. (2007)

[e] Venten, et al.(2005)

[f] Li, (2006). Human MAO B is not inhibited by up to 100 μM concentrations of either indole analogue.

Table 3

Competitive inhibition constants of zMAO with hMAO B specific inhibitors

Inhibitor	zMAO	hMAO B	hMAO A
	K_i (μM)		
8-(3-Chlorostyryl)-caffeine	2.4±0.6	0.27 ± 0.08[a]	No inhibition
1,4-Diphenyl-1,3-Butadiene	No inhibition	7.0 ± 0.2[a]	No inhibition
1,4-Diphenyl-2-Butene	No inhibition	34.5 ± 1.4[a]	No inhibition
Farnesol	No inhibition	2.3 ± 0.4[a]	No inhibition
Safinamide	No inhibition	0.45 ±0.13[b]	365.0 ±18.7[b]

Values are taken from:

[a] Hubálek, et al. (2005)

[b] Binda, et al. (2007)

Table 4

Effect of *para*-substituents on the steady state kinetic properties and deuterium kinetic isotope effects of zMAO oxidation of benzylamine analogues.

Substituent	k_{cat} (H) (min^{-1})	K_m(H) (μM)	k_{cat} (D) (min^{-1})	K_m(D) (μM)	$^{D}k_{cat}$ (min^{-1})	$^{D}(k_{cat}/K_m)$	Calculated K_d (corrected) (μM)
p-MeO	8.3±0.7	55.5±4.1	1.42±0.05	77.1±8.2	5.9±0.7	8.3±0.5	0.61
p-Me	6.9±0.1	67.8±4.1	1.08±0.02	64±4	6.4±0.2	6.0±0.2	0.61
p-H	4.7±0.1	82.2±9.0	2.34±0.20	153±32	2.0±0.2	3.8±0.3	3.3
p-F	14.9±0.3	161±8	2.23±0.05	86±6	6.7±0.2	3.5±0.1	1.2
p-Br	40±3	103±20	4.88±0.17	75±8	8.2±0.7	6.0±0.4	1.8
p-CI	35±1	94±10	5.9±0.17	126±9	5.9±.2	8.0±0.4	3.4
p-CF_3	94±7	115±36	22.5±1.5	140±20	4.2±0.3	5.1±0.4	7.2
p-t-Butyl	6.9±0.2	48±6	3.4±0.2	102±16	2.0±0.1	4.4±0.2	1.3