Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Inorganic Biochemistry 95 (2003) 141–148
JOURNAL OF Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Effect of cadmium and zinc ethanolamine complexes on rat brain monoamine oxidase-B activity in vitro

Nadia Z. Shaban[a], Alaa E. Ali[b], Mamdouh S. Masoud[c,*]

[a]Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt
[b]Physics and Chemistry Department, Faculty of Education 'Damanhour', Alexandria University, Alexandria, Egypt
[c]Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

Received 13 December 2002; received in revised form 2 March 2003; accepted 6 March 2003

Abstract

Monoamine oxidase-B (MAO-B) from rat brain was inhibited strongly by the prepared cadmium and zinc ethanolamine complexes obtained from their sulphate and chloride salts. The inhibition of MAO-B by these complexes was time-dependent and fully reversible after dilution and sedimentation. In vitro, the cadmium ethanolamine complexes were more potent at inhibiting MAO-B than the zinc complexes. The inhibitory effect of these complexes follow the order: TEA>DEA>MEA, due to the alkyl residues and steric effect properties. The inhibition of MAO-B by cadmium and zinc ethanolamine complexes was a noncompetitive type. The K_i values were calculated. The influence of the complexes on the activity of MAO-B was rather evaluated. It decreased the MAO-B activity. The IC_{50} values of the two potent cadmium and zinc triethanolamine complexes on MAO-B were evaluated indicating that the complexes were tightly binding, but reversible inhibitors for MAO-B. In general, these systems may be used for preventing some neurodegenerative diseases.

Keywords: Cadmium; Zinc ethanolamine; Rat brain; Monoamine oxidase-B activity

1. Introduction

Amine oxidases are widely distributed in nature. They are found in microorganisms (bacteria and fungi) and in higher organisms like mammals including humans [1]. Monoamine oxidase (amine=oxygen oxidoreductase (deaminating) flavine-containing, EC 1.4.3.4) plays an essential role in the oxidative deamination of biogenic and food-derived amines, both in the central nervous system and in peripheral tissues [2]. In addition to their roles in the regulation of neurotransmitters levels, these enzymes also catalyze the oxidation of xenobiotic amines [3] including dietary tyramine [4]. The products of the oxidative deamination of these amines are NH_3, aldehydes and H_2O_2, agents with established or potential toxicity. MAO exists in two functional isoenzyme forms (MAO-A and MAO-B). The two forms are encoded by different genes [5,6] and have different substrate and inhibitor specificities [7]. MAO-A preferentially oxidizes serotonin (5-hydroxytryptamine) and is irreversibly inhibited by low concentrations of clorgyline, while MAO-B preferentially oxidizes phenylethyamine and benzylamine and it is reversibly inactivated by low concentrations of pargyline and deprenyl. Dopamine, tyramine and tryptamine are common substrates for both MAO forms [7]. MAO-B consists of 520 amino acids [8]. The enzyme has a FAD covalently linked to a cysteine residue –397, through an 8α (*S*-cysteinyl-riboflavine) [9].

MAO inhibitors can be classified according to their selectivity for the two forms of the enzyme. The first generation of MAO inhibitors with drugs such as tranylcypromine has no selective inhibitory potency towards MAO-A and MAO-B, whereas the second or new generation of inhibitors involves selective inhibitors of MAO-A such as moclobemide and MAO-B such as L-deprenyl. MAO-B inhibitors are used as an adjunct to the dopamine precursor L-DOPA in therapy of Parkinson's disease [10]. In addition, several other brain diseases have been discussed as potential indication for MAO inhibitors [11,12].

*Corresponding author. Tel.: +20-2-01234-31922; fax: +20-2-0339-11794.
E-mail address: drmsmasoud@yahoo.com (M.S. Masoud).

0162-0134/03/$ – see front matter
doi:10.1016/S0162-0134(03)00096-5

One of these is epilepsy (common brain disorder characterized by spontaneous recurrent seizures).

Masoud and co-workers [13–23] published a series of papers about the chemistry and the structure of mono-, di- and triethanolamine complexes. Our aim in the future is to study the biological effect of these complexes and the plan begins in this paper by effect of cadmium and zinc ethanolamine complexes on rat brain monoamine oxidase-B activity.

The aim was to study the inhibition ability of MAO-B enzyme towards Zn- and Cd-ethanolamine complexes under optimum experimental conditions. The ability for Michaelis–Menten model and the type of inhibition are outlined.

2. Experimental

2.1. Preparation of brain MAO

This was done as described previously [6]. Briefly, rats ($n=12$) were decapitated and allowed to bleed. The brains were removed as quickly as possible, placed on a filter paper and their weights determined. All subsequent procedures were performed at 0–4 °C. The brains were rinsed thoroughly in cold saline (0.9% NaCl), then homogenized in 4 vol (w/v) of 0.25 M sucrose, 0.1 M sodium phosphate buffer (pH 7.4) in a Teflon glass homogenizer.

The homogenates were centrifuged at 600×g for 10 min. The supernatant fraction was divided into 3-ml portions in small screw-cap vials and kept frozen for later assaying of MAO.

2.2. Synthesis of the solid ethanolamine complexes

The metal–ethanolamine complexes were prepared by mixing the molar amount of the metal salts (Zn^{II}, Cd^{II} as chloride or sulphate) dissolved in 10 ml water with the calculated amount of the ligand saturated with ethanol. The mixture was refluxed for about 5 min. The complexes were precipitated and were filtered, then washed several timed with a mixture of EtOH-H_2O and dried in a desiccator over anhydrous $CaCl_2$.

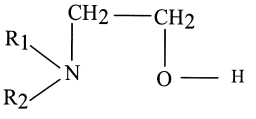
The metal ion contents were determined by complexmetric titration procedures [24]. The halogen content was determined by titration with standard $Hg(NO_3)_2$ solution using diphenyl carbazone indicator [25]. The sulphate content was determined gravimetrically as $BaSO_4$ [25]. The analytical data of the prepared complexes are collected in Table 1. The complexes under investigation are identified typically to that prepared before and were found to have the same octahedral geometrical structure [23] as shown in Fig. 1.

2.3. Kinetic assay of MAO-B

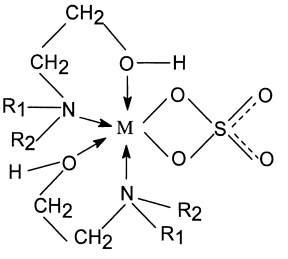
All assays were carried out in triplicate at 30±0.1 °C in

Table 1
The analytical data of the prepared complexes

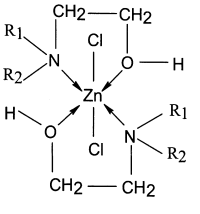
No.	Complex	% Found (% calculated)		
		M	Cl	SO_4
1	$[Cd(MEA)_2SO_4]$	34.3 (34.4)	–	29.0 (29.0)
2	$[Cd(DEA)_2SO_4]$	26.8 (26.8)	–	22.9 (22.9)
3	$[Cd(TEA)_2SO_4]$	22.1 (22.2)	–	18.9 (18.9)
4	$[Zn(MEA)_2SO_4]$	23.0 (23.0)	–	33.8 (33.8)
5	$[Zn(MEA)_2Cl_2]$	25.2 (25.3)	27.5 (27.4)	–
6	$[Zn(DEA)_2Cl_2]$	18.8 (18.8)	20.4 (20.4)	–
7	$[Zn(TEA)_2Cl_2]$	15.0 (15.0)	16.3 (16.3)	–



$R_1 = H$	; $R_2 = H$	Monoethanolamine	(MEA)
$R_1 = H$	; $R_2 = CH_2CH_2OH_2$	Diethanolamine	(DEA)
$R_1 = CH_2CH_2OH$	; $R_2 = CH_2CH_2OH$	Triethanolamine	(TEA)



No	R_1	R_2	M	Complex
1	H	H	Cd	$[Cd(MEA)_2SO_4]$
2	H	CH_2CH_2OH	Cd	$[Cd(DEA)_2SO_4]$
3	CH_2CH_2OH	CH_2CH_2OH	Cd	$[Cd(TEA)_2SO_4]$
4	H	H	Zn	$[Zn(MEA)_2SO_4]$



No	R_1	R_2	M	Complex
5	H	H	Zn	$[Zn(MEA)_2Cl_2]$
6	H	CH_2CH_2OH	Zn	$[Zn(DEA)_2Cl_2]$
7	CH_2CH_2OH	CH_2CH_2OH	Zn	$[Zn(TEA)_2Cl_2]$

Fig. 1. The structure of the mono-, di- and triethanolamine and their complexes.

0.1 M sodium phosphate buffer, pH 7.4. Results are mean values±standard deviation of the mean. MAO-B activity was measured using benzylamine as substrate [26] as follows: Mitochondrial protein (100 μl, 600 μg protein) in 0.1 M sodium phosphate buffer, pH 7.4 (total assay volume, 3 ml) was incubated in cuvette (pathlength 10 mm) in Ultraspec 1000 UV/visible Spectrophotometer (Pharmacia Biotech). The reaction was started by addition of benzylamine (25 μl of 1 mM solution in water) and the progress of the reaction (formation of benzaldehyde) was monitored at 250 nm. Initial velocities as ΔA/min were measured from the time scanning of the reactions at 250 nm, ε (M^{-1} cm^{-1}) 12 500. The maximum velocity was expressed as μmol/mg protein per min.

2.4. Inhibition of MAO-B basal activities and determination of enzyme kinetic parameters

Inhibition curves for different complexes were assessed against MAO-B activities. The reaction mixture containing the enzyme and various complexes concentrations (1.7–33.4 μM) was stabilized for 2 min at 30 °C, then the complex inhibitory effect was determined by measuring the activity spectrophotometrically for 10 min. Control samples, in which the enzyme was replaced by identical volume of buffer. The remaining MAO-B activities were expressed as percentages of control basal activity and plotted as a function of the complex concentrations. For each complex the IC_{50} value, defined as the complex concentration necessary to give 50% enzyme inhibition was calculated by non-linear regression by the use of Grafit programme [27]. Steady state kinetic constants (K_{max}, Michaelis constant and $V_{max,}$ maximum velocity) were determined from studies of the effects of substrate concentration on the initial velocity of MAO-B activity in the absence and in the presence of various ethanolamine complexes.

Lineweaver–Burk plots [28] were used to determine the kinetic parameters of MAO-B. K_i value was determined by Lineweaver–Burk plot.

Reversibility tests: the reversibility or irreversibility of MAO-B inhibition was examined by two methods. The first sedimentation test [29] was carried out by incubation of mitochondrial preparation sample with each complex at a sufficient concentration to cause more than 70% inhibition for 30 min at 30 °C. The mitochondria was then sedimented by centrifugation at 26 000×g for 45 min and the pellet was washed twice by resuspension and centrifugation with 0.1 M sodium phosphate buffer before it was finally resuspended in that buffer and assayed. Control samples of mitochondria were carried through an identical procedure, the complex (inhibitor) solution being replaced by an equal volume of distilled water. Total recovery of the activity after washing procedure was taken as an indication that the inhibition was reversible. The above results were confirmed by the second method dilution test [29] that was carried out by preincubation of mitochondrial preparation with several inhibitor concentrations in a concentrated mixture. MAO activities were measured after dilution (12.5-fold) of the mixture and compared with inhibition of these complexes of the same final concentrations of enzyme and complexes without preincubation.

2.5. Protein assay

Protein was assayed in the preparation as reported using bovine serum albumin as a standard [30].

3. Results and discussion

The inhibitory effect of Cd^{II} and Zn^{II} ethanolamine complexes on the MAO-B activity in the rat brain homogenate was dependent on the preincubation period (at 30 °C) (Fig. 2). The inhibition sequence follows the order: Cd^{II} complexes>Zn^{II} complexes where TEA→DEA→MEA complexes. This may be attributed to the alkyl group property. The inhibitory effects of the tested complexes were non-linear concentration dependent (Fig. 3). Furthermore, the increasing of the inhibition upon the preincubation of the enzyme with the complexes, inhibitors (maximal after 60 min of preincubation), suggested that the inhibition of MAO-B by these complexes could be due to complex formation rather than the enzyme oxidation. The increasing in the inhibitory potency after preincubation is in agreement with the earlier results [29,31] and might suggest a covalent interaction between the enzyme and the complexes.

3.1. Reversibility of MAO-B inhibition by Cd- and Zn-ethanolamine complexes in rat brain homogenate

The 17 μM Cd complexes, **1**, **2** and **3**, $[Cd(MEA)_2SO_4]$, $[Cd(DEA)_2SO_4]$, $[Cd(TEA)_2SO_4]$, and 17 μM Zn complexes, **4**, **5**, **6** and **7**, $[Zn(MEA)_2SO_4]$, $[Zn(MEA)_2Cl_2]$, $[Zn(DEA)_2Cl_2]$ and $[Zn(TEA)_2Cl_2]$ reduced the MAO activity by 82, 86, 90, 81, 78, 82 and 85%, respectively. A 12.5-fold dilution of the samples and by the sedimentation test results in all cases in a total recovery of MAO activity indicating the reversible nature of the complexes as inhibitors (Table 2). The data are in harmony to that obtained from studying the effect of time on the activity of the enzyme in presence of the Cd- and Zn-ethanolamine complexes.

The degree of inhibition of MAO activity as a function of 1.7–33.3 μM concentrations was determined after the preincubation period of 2 min between the enzyme and the different ethanolamine complexes, before the addition of the substrate (8.4 μM) (Fig. 3). The values of IC_{50} are recorded in Table 3. As shown from Table 3, Cd complexes exhibited more MAO-B inhibition comparable to those of Zn complexes. The IC_{50} values of $[Cd(MEA)_2SO_4]$, $[Cd(DEA)_2SO_4]$ and $[Cd(TEA)_2SO_4]$ complexes were 9.8,

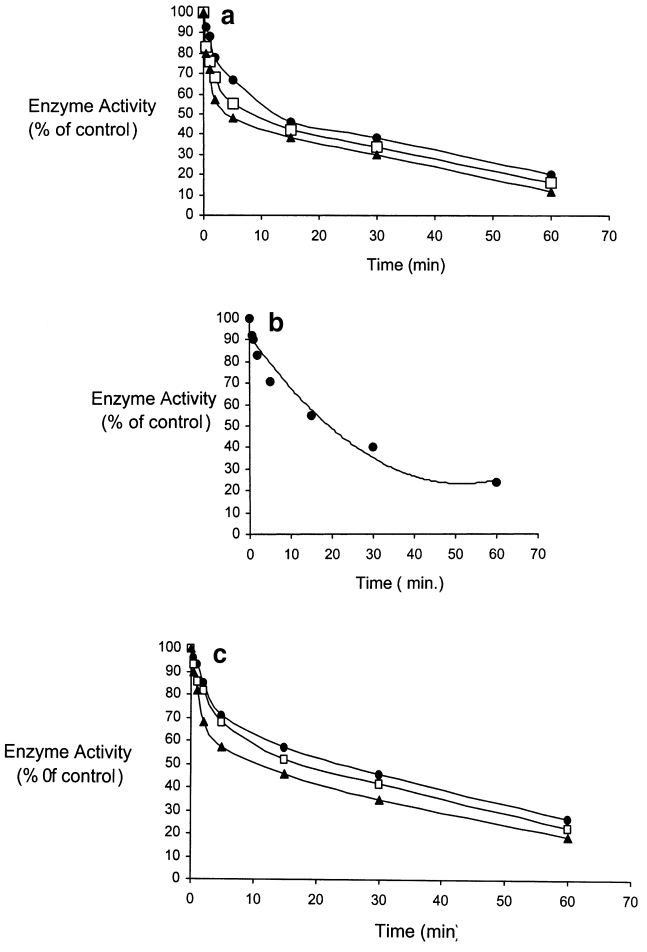


Fig. 2. Time course of the inhibition of rat brain MAO-B by Cd- and Zn-ethanolamine complexes. Rat brain mitochondria (600 μg protein/assay) were incubated with 3.4 μM Cd- (panel a, complexes 1 (●), 2 (□), 3 (▲)) and 3.4 μM Zn- (panel b complex 4 (●); panel c, complexes 5 (●), 6 (□), 7 (▲)) complexes for different period. After the preincubation period, MAO-B activity was determined as described in Section 2 using 8.3 μM benzylamine as specific substrate and compared to control samples that had been incubated for the same time in the absence of inhibitor. Each point represents the mean of triplicate experiments.

7.0 and 5.2 μM, respectively. This indicated that the hydrogen substitution on the amino group of the ligands in the complexes gave rise to MAO-B inhibition. However this substitutions decreased the value of IC_{50} (Table 3). Thus, increasing the hydroxyl groups in the ligands are most likely essential for these bioactivities. This fact is also reached from studying the IC_{50} of Zn complexes, Table 3. This conclusion is in agreement with the reported data where stilbenoids inhibit MAO [32]. However, glucosylation of the stilbenoids could diminish the activity against MAO.

Substitution of chloride with sulphate in the Zn–MEA complex, leads to increase the MAO inhibition. This may be due to the multi-oxygen atoms of the sulphate group that may interact closely with the enzyme. The low IC_{50} values for the inhibition of MAO-B by ethanolamine complexes as inhibitors places them into the category of

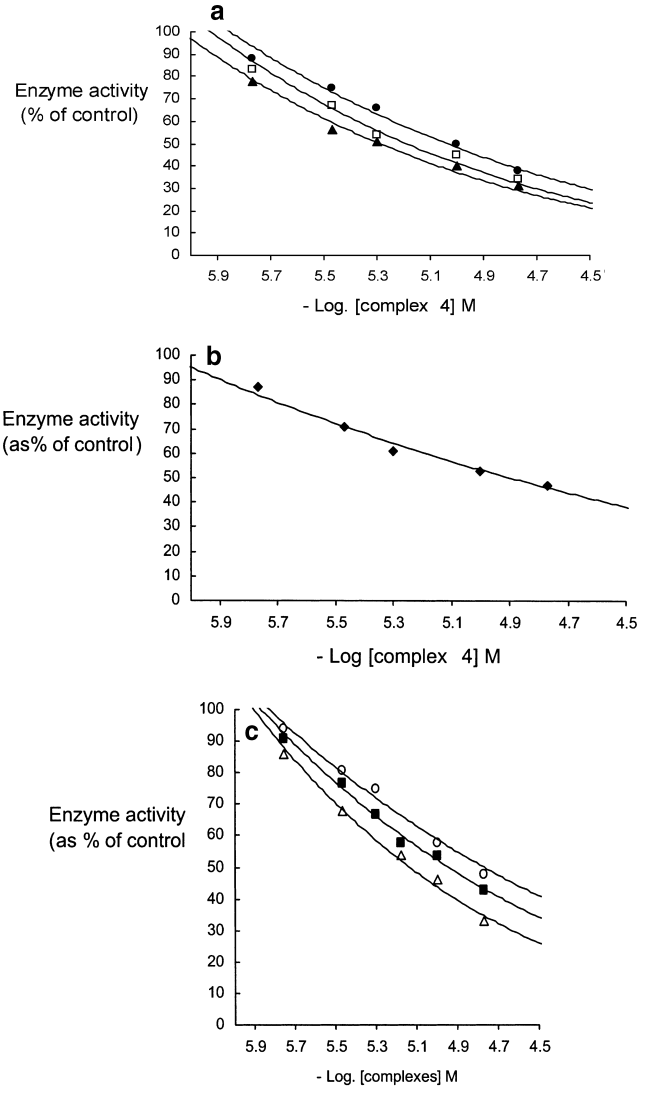


Fig. 3. The effect of Cd- and Zn-ethanolamine complexes on the deamination of benzylamine by MAO in rat brain mitochondria. Rat brain mitochondria (600 μg protein/assay) were incubated with Cd (panel a, complexes 1 (●), 2 (□), 3 (▲)) and Zn (panel b complex 4 (◆); panel c, complexes 5 (○), 6 (■), 7 (△)) complexes in the concentration range 1.7–33.4 μM for 2 min at 30 °C prior to the addition of benzylamine (8.4 μM) to assay for activity.

Table 2
Effect of dialysis and sedimentation on the inhibition of MAO-B by Cd- and Zn ethanolamine complexes in catalyzing the oxidation of benzylamine

Complex no.	MAO-B activity (% of control)		
	Before dialysis or sedimentation	After dialysis	After sedimentation
1	18	96	94
2	14	93	93
3	10	92	91
4	19	97	96
5	22	99	98
6	18	98	97
7	15	96	96

Table 3
The values of IC_{50} for MAO-B inhibition by Cd- and Zn-ethanolamine complexes

Complex no.	IC_{50} (μM)
1	9.8
2	7.0
3	5.20
4	12.58
5	19.98
6	12.40
7	7.50

''tight-binding'' inhibitors [33]. In such cases, the observed rate of enzyme inhibition is time dependent. However, at low enzyme and inhibitor concentrations, the second-order rate of the enzyme-inhibitor complex formation can be relatively slow [34].

3.2. Inhibition type

The MAO-B activities were measured within a range of 1.14–48.4 μM substrate concentration. Plotting the velocities of the reactions against substrate concentrations gave rectangular hyperbola best fitted to all studied ethanolamine complexes (Fig. 4). The Lineweaver–Burk plot ($1/v$ versus $1/[S]$); is a characteristic pattern of non-competitive type inhibition [28] (Fig. 5). The kinetic constants were calculated from a double reciprocal plot. The K_{max}, V_{max}, K_i and %I values are given in Table 4. In general, the V_{max} values are decreased for the complexes compared to that of the control. The data indicated that these complexes bind to some sites other than the substrate-binding site, i.e., the substrate and complexes bind reversibly, randomly and independently at different sites. This indicated that the complexes, inhibitors, I, bind to enzyme, E, and to the enzyme–substrate complex, ES, resulting in EI and ESI species. So, the ESI complex is catalytically inactive [28]. Therefore, a non-competitive inhibitor acts by decreasing the turnover number rather than by diminishing the proportion of enzyme molecules that are bound to substrate. The apparent K_i values for MAO enzyme towards benzylamine indicated that both $[Cd(TEA)_2SO_4]$ and $[Zn(TEA)_2Cl_2]$ are more potent inhibitors. These data are in agreement with IC_{50} values. We concluded that Cd- and Zn-ethanolamine complexes may have different effects on flavine adenine dinucleotide (FAD)-dependent mitochondrial enzymes at the level of the essential cofactor. Such findings are in harmony with the reported data that Cu(II) appears to exert an inhibitory effect on both NAD and FAD-dependent enzymes [35]. Zn- and Cd-ethanolamine complexes are as potent as beloxatone [36] and cimoxatone [37], and more potent than the other reversible MAO inhibitors including harmaline [38], brofaromine [31], toloxatone BW 1370 U 87 [39], maclobemide [40], RS 8359 [41] and SR 9519 [42].

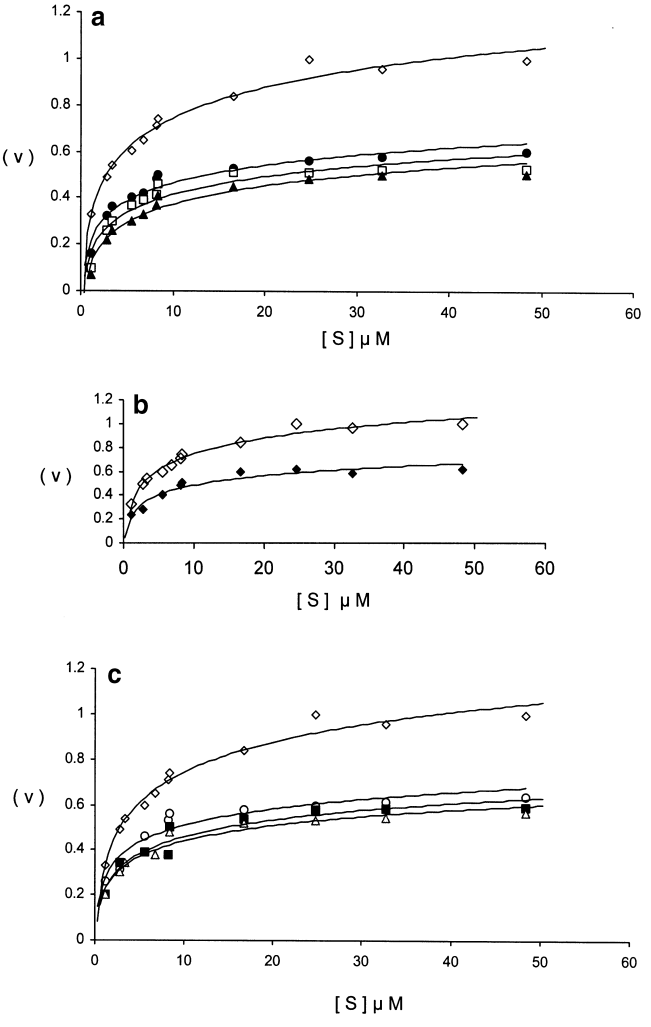


Fig. 4. Plots of v against [S] in the absence (◇) and presence of Cd (panel a, complexes 1 (●), 2 (□), 3 (▲)) and Zn (panel b complex 4 (◆); panel c, complexes 5 (○), 6 (■), 7 (△)) ethanolamine complexes (v = μmol/mg protein per min).

3.3. Effect of temperature and pH

The variation of MAO activity catalyzing the oxidative deamination of benzylamine over the temperature rang 10–70 °C in the presence and in the absence of $[Cd(TEA)_2SO_4]$ and $[Zn(TEA)_2Cl_2]$ complexes were investigated. MAO was incubated at the given temperature and then assayed. The optimum temperature in the absence and in the presence of the two complexes was 30 °C (Fig. 6). At high temperature, the tested enzyme velocities followed the same pattern in the absence and in the presence of the ethanolamine complexes, Fig. 6. So, it seams likely that MAO inhibition by these complexes does not induce a change in its heat stability.

A similar study of the enzyme over the pH range of 4–9 was made in the absence and in the presence of these two complexes. The general bell shape of the curve was the same in the two cases (Fig. 7). The decline in activity

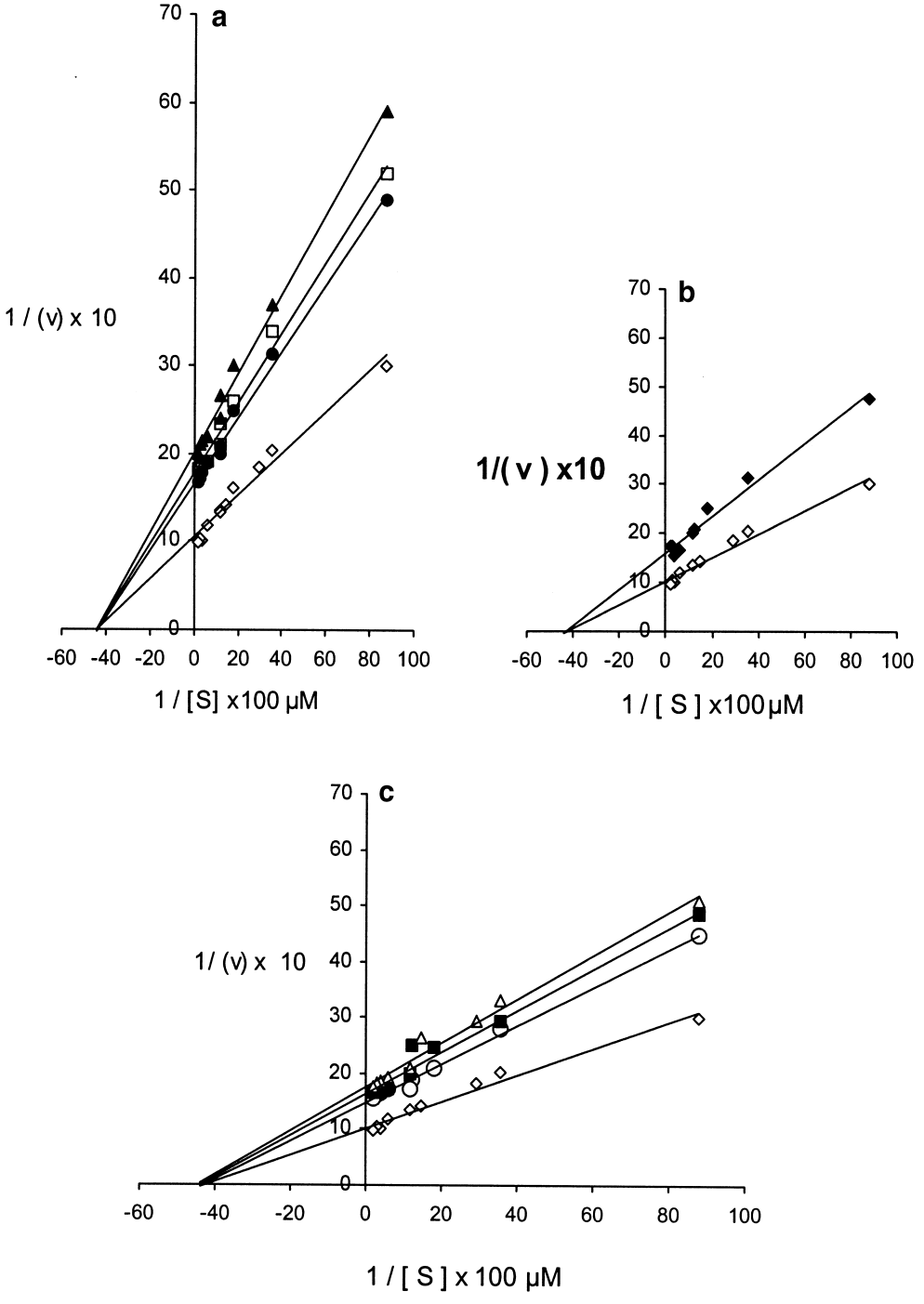


Fig. 5. Lineweaver–Burk plot analysis of MAO-B inhibition by Cd (panel a, control (◇), complexes 1 (●), 2 (○), 3 (▲)) and Zn (panel b complex 4 (▲); panel c complexes 5 (○), 6 (■), 7 (△)) ethanolamine complexes. Rat brain mitochondria (600 μg protein/assay) were preincubated with 3.4 μM Cd and 3.4 μM Zn ethanolamine complexes for 2 min at 30 °C prior to the addition of benzylamine (the concentrations ranging from 1.14 to 48.4 μM). Each point represents the mean of triplicate determinations.

between pH 7.4 and 6 and between pH 7.4 and 8 can be ascribed to the effect of pH on ionizable groups of the active site of the substrate. The decline in activity above pH 8 and below pH 5 can be ascribed to irreversible denaturation of the enzyme (Fig. 7).

3.4. Therapeutic outlook

Considered globally, the IC_{50} values reported here are relatively strong ones especially for $[Cd(TEA)_2SO_4]$ and $[Zn(TEA)_2Cl_2]$ complexes. The IC_{50} values in the range of

Table 4
Kinetic parameters of rat brain MAO-B inhibition by Cd- and Zn-ethanolamine complexes

Kinetic parameter	Control	Complex no.						
		1	2	3	4	5	6	7
K_{max} (μM)	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
V_{max}	1.0	0.63	0.59	0.51	0.63	0.67	0.61	0.57
K_i (μM)		5.63	4.83	3.56	5.63	6.76	5.20	4.48
I (%)		37.5	41	48.7	37.5	33.3	39	42.8

K_{max} and V_{max} values were determined as described in Section 2. V_{max} is expressed as μmol/mg protein per min. The parameters are calculated according to the equation:

$$\frac{1}{V_{maxi}} = \frac{1 + ([I]/K_i)}{V_{max}}$$

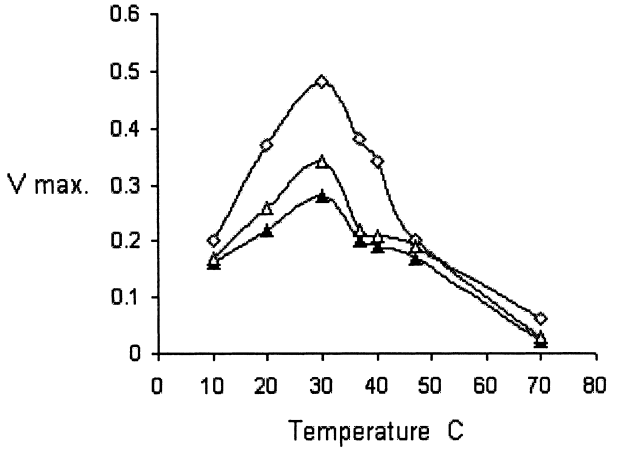


Fig. 6. Heat stability of rat brain MAO-B. Rat brain mitochondria was heated at different temperature (10–70 °C) for 10 min and V_{max} was determined under standard conditions in the absence (◇) or presence of 3.4 μM Cd complex 3 (▲) and 3.4 μM Zn complex 7 (△).

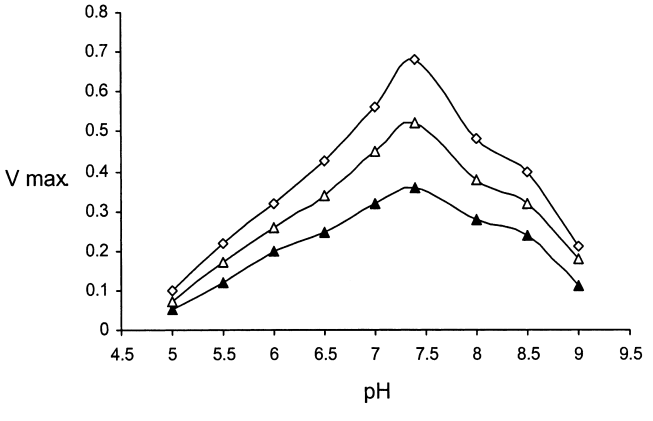


Fig. 7. Effect of pH on V_{max} of rat brain MAO-B in the absence (◇) and presence of 3.4 μM Cd (3, ▲) and 3.4 μM Zn (7, △) complexes. The reaction mixtures contained 600 μg protein/assay, 8.4 μM benzylamine as substrate. Each point represents the mean of triplicate experiments.

0.1–10 μM are usually necessary for compounds to be effective in vivo inhibitors of toxication reactions leading to chemical carcinogenesis or toxicity [43].

References

[1] B. Schilling, K. Lerch, Biochim. Biophys. Acta 1243 (1995) 529–537.
[2] W. Lóscher, H. Lehmann, H. Teschendorf, M. Traut, G. Gross, J. Pharmacol. Exp. Ther. 288 (1999) 984–992.
[3] M. Strolin, K.F. Tipton, J. Neural Transm. Suppl. 52 (1998) 149–171.
[4] N. Hauptmann, J. Grimsby, J. Shih, C. Cadenas, Arch. Biochem. Biophys. 335 (1996) 295–304.
[5] M.D. Berry, A.V. Juorio, L.A. Paterson, Prog. Neurobiol. 42 (1994) 375–391.
[6] J.S. Fowler, N.D. Volkow, G.J. Wang, N. Pappas, J. Logan, C. Shea, D. Alexoff, R.R. MacGregor, D.J. Schlyer, I. Zezulkova, A.P. Walf, Proc. Natl. Acad. Sci. USA 93 (1996) 14065–14069.
[7] I. Rebrin, R.M. Geha, K. Chen, J.C. Shih, J. Biol. Chem. 276 (2001) 29499–29506.
[8] A.W. Bach, N.C. Lan, D.L. Johnson, C.W. Abell, M.K. Bembenek, S.W. Kwan, P.H. Seeburg, J.C. Shih, Proc. Natl. Acad. Sci. USA 85 (1988) 4934–4938.
[9] H.F. Wu, K. Chen, J.C. Shih, Mol. Pharmacol. 43 (1993) 888–893.
[10] D.L. Murphy, P.B. Mitchell, W.Z. Potter, Novel pharmacological approches to the treatment of depression, in: F.E. Bloom, D.Y. Kupfer (Eds.), Psychopharmacology. The Fourth Generation of Progress, Raven Press, New York, 1995, pp. 1143–1153.
[11] P.H. Yu, Gen. Pharmacol. 25 (1994) 1527–1539.
[12] T. Palomo, R. Beninger, T. Archer, in: Dopamine Disease States, CYM, Madrid, 1996, pp. 569–605.
[13] M.S. Masoud, T.M. Salem, R.M. Issa, Egypt. J. Chem. 17 (1974) 415–423.
[14] M.S. Masoud, T.M. Salem, R.M. Issa, Indian J. Chem. 15A (1977) 721–723.
[15] M.S. Masoud, A.F. Abd-El Momeim, Bulgarian Chem. Soc. XIV (1981) 399–407.
[16] S. Hedewy, S.K. Hoffmann, M.S. Masoud, J. Goslar, Spectrosc. Lett. 19 (1986) 917–928.
[17] M.S. Masoud, M.A. Al-Dessouky, J. Iraqi Chem. Soc. 12 (1987) 85–92.
[18] M.S. Masoud, S.M. El-Rabie, I.M. Abed, A.E. Ali, Lebanese Sci. Res. Rep. 2 (1997) 578–594.
[19] M.S. Masoud, A.M. Hafez, A.E. Ali, Spectrosc. Lett. 31 (1998) 901–911.

[20] M.S. Masoud, H.A. Motaweh, A.E. Ali, Ind. J. Chem. 40A (2001) 733–737.
[21] M.S. Masoud, A.M. Hafez, M.S. Ramadan, A.E. Ali, J. Saudi Chem. Soc. 6 (2002) 341–352.
[22] M.S. Masoud, A.M. Hafez, M.S. Ramadan, A.E. Ali, J. Serb. Chem. Soc. 67 (2002) 833–842.
[23] M.S. Masoud, S.A. Abu El Enein, I.M. Abed, A.E. Ali, J. Coord. Chem. 55 (2002) 153–178.
[24] G. Schwartzenbach, in: Complexmetric Titration, Methuen, London, 1967.
[25] A.I. Vogel, in: A Text Book of Quantitative Inorganic Analysis, Longmann, London, 1978.
[26] C.W. Tabour, H. Tabour, S.M. Rosenthal, J. Biol. Chem. 208 (1954) 645–661.
[27] R.J. Leatherbarrow, Grafit version 2.0, Erithacus Software Ltd., Stains, UK, 1990.
[28] H. Lineweaver, D. Burk, J. Am. Chem. Soc. 56 (1934) 658–666.
[29] M.C. Anderson, P.C. Waldmeier, K.F. Tipton, Biochem. Pharmacol. 41 (1991) 1871–1877.
[30] T.S. Ohnishi, J.K. Parr, J. Anal. Biochem. 86 (1978) 193–200.
[31] P.C. Waldmeier, A.E. Feler, K.F. Tipton, Eur. J. Pharmacol. 94 (1983) 73–83.
[32] C.X. Zhou, L.D. Kong, W.C. Ye, C.H. Cheng, R.X. Tan, Plant Med. 67 (2001) 158–161.
[33] M. Dixon, W.C. Webb, in: Enzymes, 3rd Edition, Longmann, London, 1979, pp. 361–368.
[34] K.F. Tipton, C.J. Folwer, in: K. Tipton, P. Dostert, M. Strolin (Eds.), MAO and Diseases, Academic Press, London, 1984, pp. 27–40.
[35] P. Heron, K. Cousins, C. Boyd, S. Daya, Life Sci. 23 (2001) 1575–1583.
[36] O. Curet, G. Damoiseau, N. Aubin, N. Sontag, V. Rovel, F.X. Jarreau, J. Pharmacol. Exp. Ther. 277 (1996) 253–264.
[37] C.J. Fowler, M. Stroline, J. Neurochem. 40 (1983) 510–513.
[38] W.P. Burkard, R. Kettler, Biochem. Pharmacol. 126 (1977) 1303–1306.
[39] H.L. White, P.W. Scates, Drug Dev. Res. 25 (1992) 191–199.
[40] M. Da Parada, R. Kettler, H.H. Keller, W.P. Burkard, D. Muggli, W.E. Haffely, J. Pharmacol. Exp. Ther. 248 (1989) 400–414.
[41] T. Yokoyama, T. Karube, N. Iwata, J. Pharm. Pharmacol. 41 (1989) 32–36.
[42] J.P. Kan, R. Steinberg, J. Leclercq, P. Worms, K. Biziere, J. Neurochem. 50 (1988) 1137–1144.
[43] S. Beyeler, B. Testa, D. Perrissoud, Biochem. Pharmacol. 37 (1988) 1971–1979.