# CHAPTER 5

## Article:

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# The inhibition of monoamine oxidase by phenformin and pentamidine

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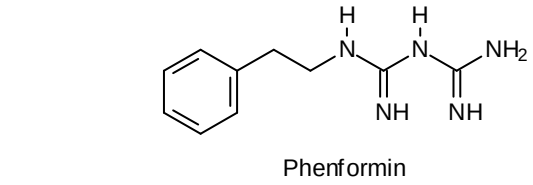
**Abstract**

A computational study has suggested that phenformin, an oral hypoglycaemic drug, may bind to the active sites of the monoamine oxidase (MAO) A and B enzymes. The present study therefore investigates the MAO inhibitory properties of phenformin. Pentamidine, a structurally related diamidine compound, has previously been reported to be a MAO inhibitor and was included in this study as a reference compound. Using recombinant human MAO-A and MAO-B, this study finds that phenformin acts as a moderately potent MAO-A selective inhibitor with an $IC_{50}$ value of 41 µM. Pentamidine, on the other hand, potently inhibits both MAO-A and MAO-B with $IC_{50}$ values of 0.61 µM and 0.22 µM, respectively. An examination of the recoveries of the enzymatic activities after dilution and dialysis of the enzyme-inhibitor complexes shows that both compounds interact reversibly with the MAO enzymes. A kinetic analysis suggests that pentamidine acts as a competitive inhibitor with estimated $K_i$ values of 0.41 µM and 0.22 µM for the inhibition of MAO-A and MAO-B, respectively. Phenformin also exhibited a competitive mode of MAO-A inhibition with an estimated $K_i$ value of 65 µM. This study concludes that biguanide and amidine functional groups are most likely important structural features for the inhibition of the MAOs by phenformin and pentamidine, and compounds containing these and closely related functional groups should be considered as potential MAO inhibitors. Furthermore, the biguanide and amidine functional groups may act as useful moieties in the future design of MAO inhibitors.

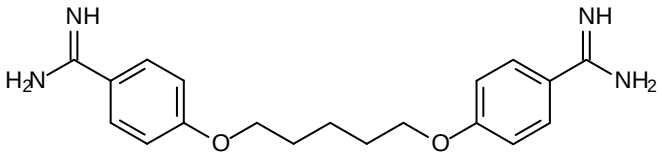
*Keywords*: biguanides, amidines, guanidine, phenformin, pentamidine, inhibition, monoamine oxidase

## Introduction

Guanidine containing compounds have had a large impact on medicinal chemistry, with pharmacological effects ranging from antimicrobial, antifungal, antiviral and antidiabetic to neurotoxic [1–3]. Several guanidine containing compounds, such as the antihypertensive drugs, guanadrel and guanethidine, have found therapeutic applications [1,4]. The guanidine functional group is also found in naturally occurring compounds such as the amino-acid, *L*-arginine. *L*-arginine is metabolized by the nitric oxide synthases (NOS) to yield *L*-citruline and nitric oxide (NO) in a two-step reaction [5]. NO is an important physiological mediator that is involved in a large number of signalling processes. Another class of guanidine derivatives is the biguanides, phenformin and metformin, which are used as oral hypoglycaemic drugs in the treatment of type II diabetes mellitus (Fig. 1) [6]. Phenformin was withdrawn from the US market in 1977 due to an increased incidence of fatal lactic acidosis associated with its use, but it is still available in other countries [6,7]. The biguanides act by sensitizing peripheral tissues to insulin and by inhibiting hepatic gluconeogenesis [8]. The biguanides are also activators of AMP-activated protein kinase (AMPK), and phenformin is used to examine the role of AMPK in cell signalling pathways [9,10]. There has been a renewed interest in phenformin as an antineoplastic drug since it has been shown that phenformin exerts antitumor activity in a variety of animal models [11]. In addition, phenformin and metformin may also enhance the antitumor effects of established chemotherapeutic agents [12,13].




Phenformin




Pentamidine

**Fig. 1**. The structures of phenformin and pentamidine.

Using a computational approach, a virtual library of drugs was recently screened for compounds that may bind to the active sites of the monoamine oxidase (MAO) A and B enzymes (unpublished results). The results of these modelling studies suggested that the

drug, phenformin, may bind to the MAOs. Based on this, the current study aims to examine the potential MAO-A and MAO-B inhibitory properties of phenformin. For this purpose, the recombinant human MAO enzymes will be employed. Earlier studies reported that a structurally related diamidine compound, pentamidine (an antibiotic in the treatment of *Pneumocystis jirovecii* pneumonia and West African trypanosomiasis [14]), is a potent inhibitor of rabbit and rat liver MAO [15,16]. Interestingly, pentamidine was found to inhibit rat liver MAO non-competitively and irreversibly [16]. For comparison, the MAO inhibitory properties of pentamidine will be re-examined in this study, with particular emphasis on the reversibility of inhibition. This account will thus investigate, for the first time, the MAO inhibitory properties of pentamidine using the human enzymes. This study will furthermore investigate the selectivity of inhibition of the MAO-A and MAO-B isoforms by pentamidine.

The MAO enzymes are of therapeutic and toxicological importance since they metabolize dietary amines, amine neurotransmitters and amine containing drugs [17]. MAO inhibitors are considered to be useful therapeutic agents: MAO-A inhibitors increase the central levels of serotonin and norepinephrine, and are employed in the therapy of depression [18], while MAO-B inhibitors elevate the central levels of both endogenous dopamine and dopamine derived from exogenously administered levodopa, and are thus used in the treatment of Parkinson's disease [19,20]. Besides central effects, MAO inhibitors also exert peripheral pharmacological actions. When MAO-A inhibitors are combined with tyramine-containing foods, a potentially fatal hypertensive reaction, the "cheese-reaction", may occur [21]. MAO-A catalyzes the first-pass metabolism of tyramine and other sympathomimetic dietary amines and thus prevents them from reaching the systemic circulation. The irreversible inhibition of intestinal MAO-A allows these vasopressor amines to enter the systemic circulation, which in turn may lead to a hypertensive crisis. It should be noted that reversible MAO-A inhibitors, in general, do not precipitate the "cheese-reaction" [22]. A second serious adverse effect of MAO-A inhibition is serotonin toxicity, which is caused by the combination of MAO-A inhibitors and serotonin-releasing agents or selective serotonin reuptake inhibitors [23,24]. Serotonin toxicity is potentially fatal and is the result of excessive extracellular concentrations of serotonin in the central nervous system. This occurs due to the concurrent inhibition of the MAO-A-catalyzed degradation of serotonin, the inhibition of central serotonin reuptake or the stimulation of serotonin release.

Based on the discussion above, this study examined the human MAO-A and MAO-B inhibitory properties of phenformin and pentamidine in an attempt to evaluate whether the therapeutic and toxicological effects of MAO inhibition should be considered when using these drugs in humans. Even though pentamidine is a known MAO inhibitor, its potencies

towards the human MAOs have not been established. In addition, it is not known which MAO isoform is inhibited by pentamidine. This study may also give an indication if the structures of phenformin and pentamidine may serve as leads for the future design of MAO inhibitors.

## Materials and Methods

### *Materials and instrumentation*

Fluorescence spectrophotometry was conducted with a Varian® Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/ml), kynuramine dihydrobromide, phenformin hydrochloride, pentamidine isethionate, pargyline hydrochloride, (R)-deprenyl hydrochloride and toloxatone were obtained from Sigma–Aldrich®. Lazabemide was synthesized according to the patented method [25]. For the recovery of enzyme activity after the dilution and dialysis experiments, the Kruskal-Wallis test with Dunn's post hoc test was used to determine if statistical differences exist between the means of the residual enzyme rates recorded before and after dilution and dialysis. A p value $< 0.05$ is judged as being statistical significantly different. These analyses were carried out with the Prism 5 software package (GraphPad).

### *$IC_{50}$ determinations*

The recombinant human enzymes were employed to determine the $IC_{50}$ values for the inhibition of MAO-A and MAO-B [26]. The enzymatic reactions were carried out to a final volume of 500 µl and contained the MAO-A/B mixed substrate kynuramine (45 µM for MAO-A and 30 µM for MAO-B) and different concentrations of the test inhibitor (1–1000 µM for phenformin and 0.003–100 µM for pentamidine). Potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) was used as reaction buffer and stock solutions of the test inhibitors were prepared in DMSO and were added to the reactions to yield a final concentration of 4% DMSO. The reactions were initiated with the addition of MAO-A or MAO-B (0.0075 mg protein/ml), incubated for 20 min at 37 ºC and terminated by the addition of 400 µl NaOH (2 N). To each reaction, 1000 µl water was added, and the concentrations of 4-hydroxyquinoline, the MAO-generated oxidation product of kynuramine, were subsequently measured by fluorescence spectrophotometry ($\lambda_{ex}$ = 310 nm; $\lambda_{em}$ = 400 nm) [27]. For this purpose linear calibration curves (4-hydroxyquinoline: 0.047–1.56 µM) were constructed. The MAO catalytic rates were calculated and fitted to the one site competition model incorporated into the Prism software package (GraphPad). The $IC_{50}$ values were determined in triplicate from the resulting sigmoidal concentration–inhibition curves and are expressed as mean ± SD.

*Recovery of enzyme activity after dilution*

Phenformin [$IC_{50}$(MAO-A) = 41 μM], pentamidine [$IC_{50}$(MAO-A) = 0.61 μM] or pargyline [$IC_{50}$(MAO-A) = 13 μM] at concentrations equal to 10 × $IC_{50}$ and 100 × $IC_{50}$ for the inhibition of MAO-A were preincubated with recombinant human MAO-A (0.75 mg/ml) for 30 min at 37 ºC [28]. Potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) served as reaction buffer in all instances and DMSO (4%) was added as co-solvent to all preincubations. Pentamidine [$IC_{50}$(MAO-B) = 0.22 μM] or (R)-deprenyl [$IC_{50}$(MAO-B) = 0.079 μM] [26] were similarly preincubated with recombinant human MAO-B (0.75 mg/ml) at concentrations equal to 10 × $IC_{50}$ and 100 × $IC_{50}$. Control incubations of MAO-A and MAO-B were conducted in the absence of inhibitor. The reactions were diluted 100-fold with the addition of kynuramine (45 μM and 30 μM for MAO-A and MAO-B, respectively) to yield final concentrations of the inhibitors equal to 0.1 × $IC_{50}$ and 1 × $IC_{50}$. This yielded final concentrations of MAO-A and MAO-B of 0.0075 mg/ml. The reactions were incubated for a further 20 min at 37 °C, terminated and the residual rates of 4-hydroxyquinoline formation were measured as described above. These reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean ± SD.

*Dialysis*

For the dialysis of mixtures containing MAO and the test inhibitors, Slide-A-Lyzer® dialysis cassettes (Thermo Scientific) with a molecular weight cut-off of 10 000 and a sample volume capacity of 0.5–3 ml were used. The MAO enzymes (0.03 mg/ml) and phenformin (only MAO-A) or pentamidine (MAO-A and MAO-B), at concentrations equal to 4 × $IC_{50}$ for the inhibition of the MAO enzymes, were preincubated for 15 min at 37 °C. Potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose to final volume of 0.8 ml was used as reaction medium, and DMSO (4%) was added as co-solvent. As controls, MAO-A and MAO-B were similarly preincubated in the absence of inhibitor (negative control) and presence of the irreversible inhibitors, pargyline and (R)-deprenyl, respectively (positive controls). The concentrations of pargyline [$IC_{50}$(MAO-A) = 13 μM] [28] and (R)-deprenyl [$IC_{50}$(MAO-B) = 0.079 μM] [26] employed were equal to 4 × $IC_{50}$ for the inhibition of the MAO enzymes. The reactions (0.8 ml) were subsequently dialyzed at 4 °C in 80 mL of outer buffer (100 mM potassium phosphate, pH 7.4, 5% sucrose). The outer buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. At 24 h after dialysis was started the reactions were diluted twofold with the addition of kynuramine and the residual MAO activities were measured as described above. The final concentration of kynuramine in these reactions was 50 μM while the final inhibitor concentrations were equal to 2 × $IC_{50}$.

Potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) served as reaction buffer for these reactions. Undialyzed mixtures of the MAOs with phenformin and pentamidine were maintained at 4 °C over the same time period. These reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean ± SD.

*The construction of Lineweaver–Burk plots*

Sets consisting of five Lineweaver–Burk plots (1/V vs. 1/[S]) were constructed to evaluate the modes of MAO-A and MAO-B inhibition by phenformin and pentamidine. This kinetic analysis was also used to measure $K_i$ values for the binding of these drugs to the MAO enzymes. For each set of Lineweaver–Burk plots, the first plot was constructed in the absence of inhibitor, while the remaining four plots were constructed in the presence of different concentrations of phenformin and pentamidine. The concentrations of phenformin employed were ½ × $IC_{50}$, ¾ × $IC_{50}$, 1 × $IC_{50}$ and 1¼ × $IC_{50}$ for the inhibition of MAO-A. The concentrations of pentamidine employed were ¼ × $IC_{50}$, ½ × $IC_{50}$, ¾ × $IC_{50}$ and 1¼ × $IC_{50}$ for the inhibition of the MAO enzymes. Kynuramine at eight different concentrations (15–250 μM) served as substrate and the concentrations of recombinant human MAO-A and MAO-B employed were 0.015 mg/ml. The rates of formation of the MAO-generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry as described above. Linear regression analysis was performed using Prism 5 [29]. $K_i$ values were estimated from the x-axis intercept ($–K_i$) of a replot of the slopes of the Lineweaver–Burk plots versus inhibitor concentration.

**Results**

*$IC_{50}$ values*

As mentioned in the introduction, the inhibitory properties of phenformin and pentamidine were investigated using recombinant human MAO-A and MAO-B. To measure the activities of the MAO enzymes, the MAO-A/B mixed substrate, kynuramine, was used as substrate. The MAO enzymes oxidize kynuramine to yield 4-hydroxyquinoline, as end-product. While kynuramine is a non-fluorescent compound, 4-hydroxyquinoline fluoresces ($\lambda_{ex}$ = 310 nm; $\lambda_{em}$ = 400 nm) in alkaline media and can thus be measured by fluorescence spectrophotometry [27]. At the concentrations and conditions used in this study, phenformin and pentamidine do not fluoresce or significantly suppress fluorescence. When high

concentrations of phenformin (1000 µM) and pentamidine (100 µM) were added to samples containing 4-hydroxyquinoline, the fluorescence intensities did not change relative to the intensities of the samples recorded in the absence the test inhibitors. The $IC_{50}$ values for the inhibition of MAO-A and MAO-B by phenformin and pentamidine were estimated employing sigmoidal concentration–inhibition curves. These curves are given in Fig. 2 and Fig. 3 and show that phenformin inhibits human MAO-A with an $IC_{50}$ value of 40.5 ± 4.36 µM. In contrast, phenformin does not inhibit human MAO-B, even at a maximal tested concentration of 100 µM. Pentamidine was found to be a potent inhibitor of both human MAO-A and MAO-B with $IC_{50}$ values of 0.607 ± 0.010 µM and 0.220 ± 0.046 µM, respectively. Based on the $IC_{50}$ values, pentamidine is therefore a 2.8-fold more selective inhibitor of MAO-B compared to the MAO-A isoform. For comparison, the reversible MAO-A inhibitor, tolaxatone, is reported to inhibit MAO-A with an $IC_{50}$ value of 3.92 µM under identical conditions while the reference MAO-B inhibitor, lazabemide, is reported to inhibit MAO-B with an $IC_{50}$ value of an 0.091 µM under identical conditions [30]. Phenformin is thus a tenfold weaker MAO-A inhibitor than toloxatone, while pentamidine is a sixfold more potent MAO-A inhibitor than toloxatone. Even though pentamidine exhibits a 2.4-fold weaker MAO-B inhibitory potency than lazabemide, its $IC_{50}$ value for the inhibition of MAO-B is in the nanomolar range. Based on these inhibitory values, it may be concluded that phenformin is a moderately potent MAO-A inhibitor while pentamidine is a potent inhibitor of both MAO isoforms.

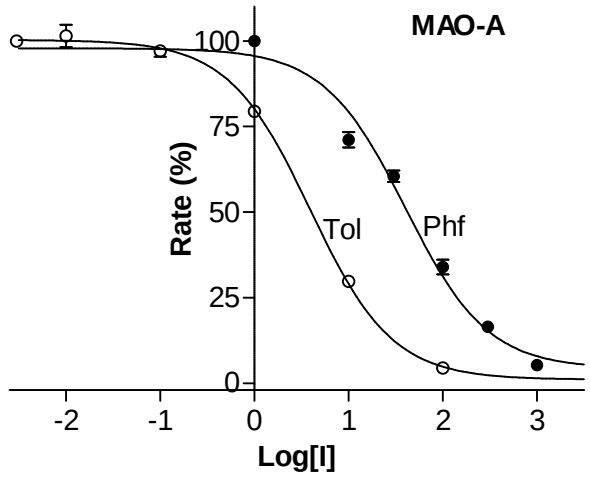



**Fig. 2.** The sigmoidal concentration-inhibition curve (filled circles) for the inhibition of human MAO-A by phenformin (Phf). For comparison, the sigmoidal concentration-inhibition curve (open circles) for the inhibition of MAO-A by toloxatone (Tol) is also provided [30].

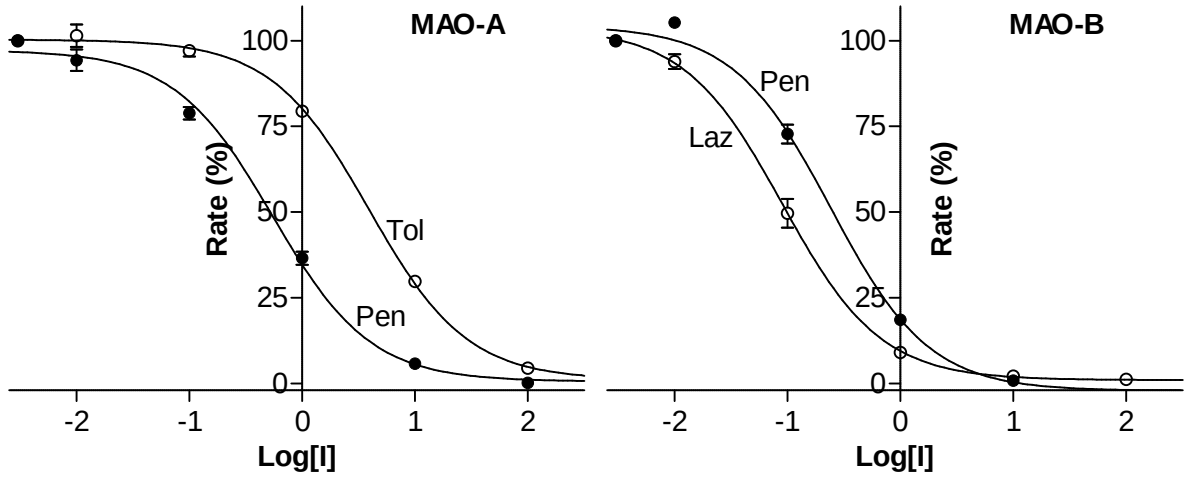



**Fig. 3.** The sigmoidal concentration-inhibition curves (filled circles) for the inhibition of human MAO-A and MAO-B by pentamidine (Pen). For comparison, the sigmoidal concentration-inhibition curves (open circles) for the inhibition of MAO-A by toloxatone (Tol) and for the inhibition of MAO-B by lazabemide (Laz) are also provided [30].

*Reversibility of inhibition*

The reversibility of MAO-A and MAO-B inhibition by phenformin and pentamidine was examined by evaluating the recoveries of the MAO catalytic activities after dilution of the enzyme-inhibitor complexes. MAO-A was preincubated with phenformin and pentamidine, while MAO-B was preincubated with pentamidine at concentrations of 10 × $IC_{50}$ and 100 × $IC_{50}$ for the inhibition of the MAO enzymes by these inhibitors. The incubations were subsequently diluted 100-fold to yield concentrations of the test inhibitors equal to 0.1 × $IC_{50}$ and 1 × $IC_{50}$, and the residual MAO catalytic activities were measured. The results of these studies are given in Fig. 4 and Fig. 5 and show that after diluting the MAO-A-phenformin mixtures to concentrations equal to 0.1 × $IC_{50}$ and 1 × $IC_{50}$, the MAO-A activities were recovered to levels of 95% and 56% of the control value, respectively. Similarly, after diluting the MAO-A-pentamidine mixtures to concentrations equal to 0.1 × $IC_{50}$ and 1 × $IC_{50}$, the MAO-A activities were recovered to levels of 98% and 67% of the control value, respectively. The results also document that after dilution of the MAO-B-pentamidine mixtures to concentrations equal to 0.1 × $IC_{50}$ and 1 × $IC_{50}$, the MAO-B activities were recovered to levels of 99% and 51% of the control value, respectively. These results suggest that the inhibition of MAO-A by phenformin, and MAO-A and MAO-B by pentamidine, are reversible. For reversible inhibition, it is expected that after dilution of the enzyme-inhibitor mixtures to inhibitor concentrations of 0.1 × $IC_{50}$ and 1 × $IC_{50}$, enzyme activity would recover to values of

approximately 90% and 50%, respectively. For irreversible inhibition, dilution of enzyme-inhibitor mixtures would not restore enzyme activity. This behaviour was apparent after similar experiments were carried out with the irreversible MAO inhibitors pargyline and (R)-deprenyl. Pargyline and (R)-deprenyl, at concentrations equal to 10 × $IC_{50}$, were preincubated with MAO-A and MAO-B, respectively, and the resulting enzyme-inhibitor complexes were diluted 100-fold to yield inhibitor concentrations of 0.1 × $IC_{50}$. As shown in Fig. 4 and Fig. 5, after dilution the enzyme activities did not recover and were in the range of only 3.4–6.8% of the control values recorded in absence of inhibitor.

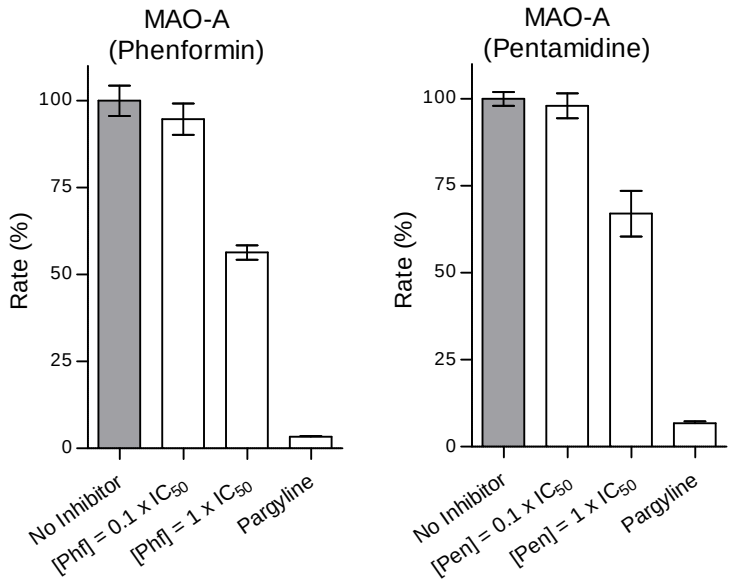



**Fig. 4.** Reversibility of inhibition of MAO-A by phenformin (Phf) and pentamidine (Pen). MAO-A was preincubated with phenformin or pentamidine at 10 × $IC_{50}$ and 100 × $IC_{50}$ for 30 min and then diluted to 0.1 × $IC_{50}$ and 1 × $IC_{50}$, respectively. The residual enzyme activities were subsequently measured. For comparison, MAO-A was also preincubated with the irreversible inhibitor, pargyline, at 10 × $IC_{50}$ and then diluted to 0.1 × $IC_{50}$ and the residual enzyme activities were subsequently measured. *Statistical significantly different from the mean of no inhibitor.

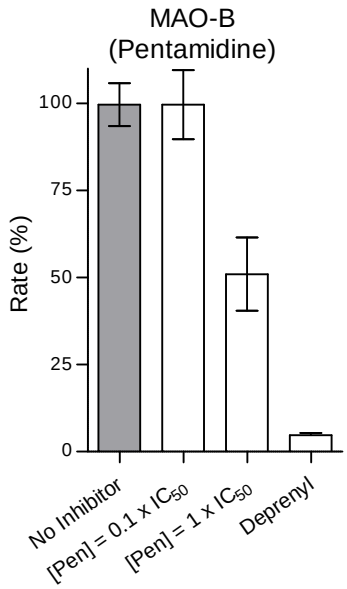



**Fig. 5.** Reversibility of inhibition of MAO-B by pentamidine (Pen). MAO-B was preincubated with pentamidine at 10 × $IC_{50}$ and 100 × $IC_{50}$ for 30 min and then diluted to 0.1 × $IC_{50}$ and 1 × $IC_{50}$, respectively. The residual enzyme activities were subsequently measured. For comparison, MAO-B was also preincubated with the irreversible inhibitor, (R)-deprenyl, at 10 × $IC_{50}$ and then diluted to 0.1 × $IC_{50}$ and the residual enzyme activities were subsequently measured.

The reversibility of MAO inhibition by phenformin and pentamidine was further investigated by measuring the recoveries of enzyme activities after dialysis of enzyme-inhibitor mixtures [22]. The MAO enzymes and phenformin or pentamidine, at concentrations equal to 4 × $IC_{50}$, were preincubated for a period of 15 min and subsequently dialyzed for 24 h. The results are given in Fig. 6 and show that MAO-A inhibition by phenformin is completely reversed after 24 h of dialysis with the MAO-A activity recovering to a level of 110% of the control value (recorded in the absence of inhibitor). In contrast, the MAO-A activity of undialyzed mixtures of the enzymes with phenformin is 36% of the control value. This behaviour suggests that phenformin acts as a reversible MAO-A inhibitor. The results given in Fig. 7 show that MAO-A and MAO-B inhibition by pentamidine are also completely reversed after 24 h of dialysis, with the catalytic activities of MAO-A and MAO-B recovering to levels of 107% and 125% of the control values, respectively. The MAO-A and MAO-B catalytic activities of undialyzed mixtures of the enzymes with pentamidine are both 29% of the control values. These data suggests that pentamidine is a reversible inhibitor of both MAO-A and MAO-B. For comparison, after similar preincubation and dialysis of mixtures of MAO-A and MAO-B with the irreversible inhibitors, pargyline and (R)-deprenyl, respectively, the enzyme activities are

not recovered. After dialysis of MAO-A–pargyline and MAO-B–(R)-deprenyl mixtures, the residual enzyme activities are recovered to levels of only 1.5% and 2.2%, respectively, of the control values.

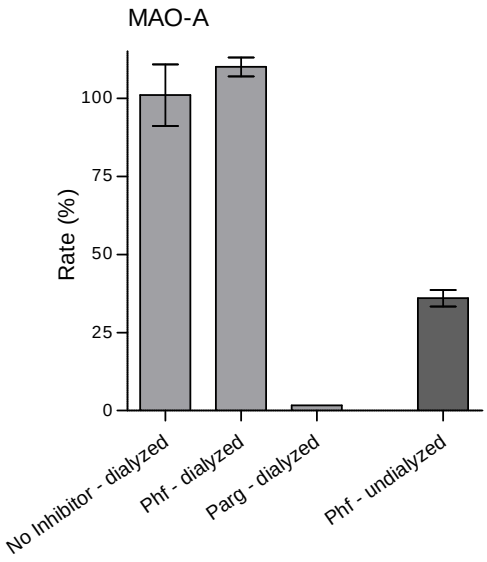



**Fig. 6.** Reversibility of inhibition of MAO-A by phenformin. MAO-A and phenformin, at an inhibitor concentration of 4 × $IC_{50}$, were preincubated together, dialyzed and the residual MAO-A activity was subsequently measured (Phf–dialyzed). For comparison, MAO-A was similarly preincubated in the absence (No inhibitor–dialyzed) and presence of the irreversible inhibitor pargyline (Parg–dialyzed) and dialyzed. The residual MAO-A activities of undialyzed mixtures (Phf–undialyzed) of MAO-A with phenformin is also shown. *Statistical significantly different from the mean of Phf-dialyzed.

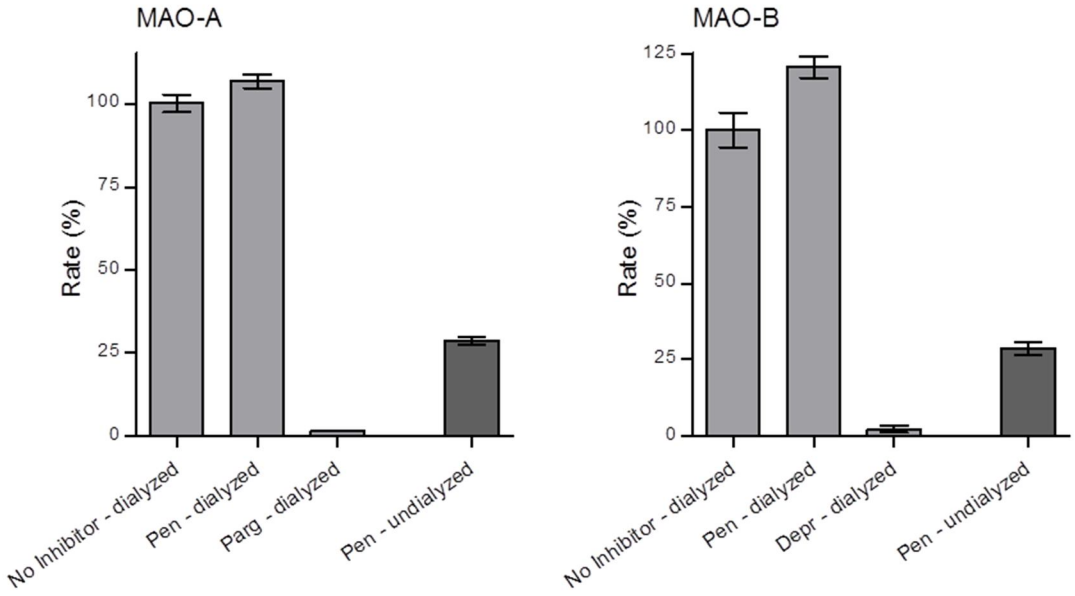



**Fig. 7.** Reversibility of inhibition of MAO-A and MAO-B by pentamidine. The MAO enzymes and pentamidine, at an inhibitor concentration of 4 × $IC_{50}$, were preincubated together, dialyzed and the residual MAO activities were subsequently measured (Pen–dialyzed). For comparison, MAO-A and MAO-B were similarly preincubated in the absence (No inhibitor–dialyzed) and presence of the irreversible inhibitors, pargyline (Parg–dialyzed) and (R)-deprenyl (Depr–dialyzed), respectively, and dialyzed. The residual MAO activities of undialyzed mixtures (Pen–undialyzed) of the MAOs with pentamidine are also shown. *Statistical significantly different from the mean of Pen-dialyzed.

*Mode of inhibition*

The modes of MAO inhibition by phenformin and pentamidine were further investigated. For this purpose, sets of Lineweaver–Burk plots were constructed. The MAO catalytic activities were recorded at eight substrate concentrations (15–250 μM) in the absence and presence of four different concentrations of the test inhibitors. The plots obtained for pentamidine are given in Fig. 8 and demonstrate that for the inhibition of both MAO-A and MAO-B, the Lineweaver–Burk plots are linear and intersect at a single point. These data suggest that pentamidine most likely interacts competitively and therefore reversibly with the human MAO enzymes. These findings are in contrast to literature which reports that pentamidine acts as an irreversible inhibitor of rat MAO [15,16]. Graphing the slopes of the Lineweaver–Burk plots versus the inhibitor concentrations, allows for the estimation of $K_i$ values for the inhibition of the MAO isoforms. For the inhibition of MAO-A and MAO-B by pentamidine, $K_i$ values of 0.41 μM and 0.224 μM, respectively, are estimated. The plots obtained for

phenformin are given in Fig. 9 and similarly show that this drug most likely interacts competitively with human MAO-A. For the inhibition of MAO-A, a $K_i$ value of 65 µM is estimated.

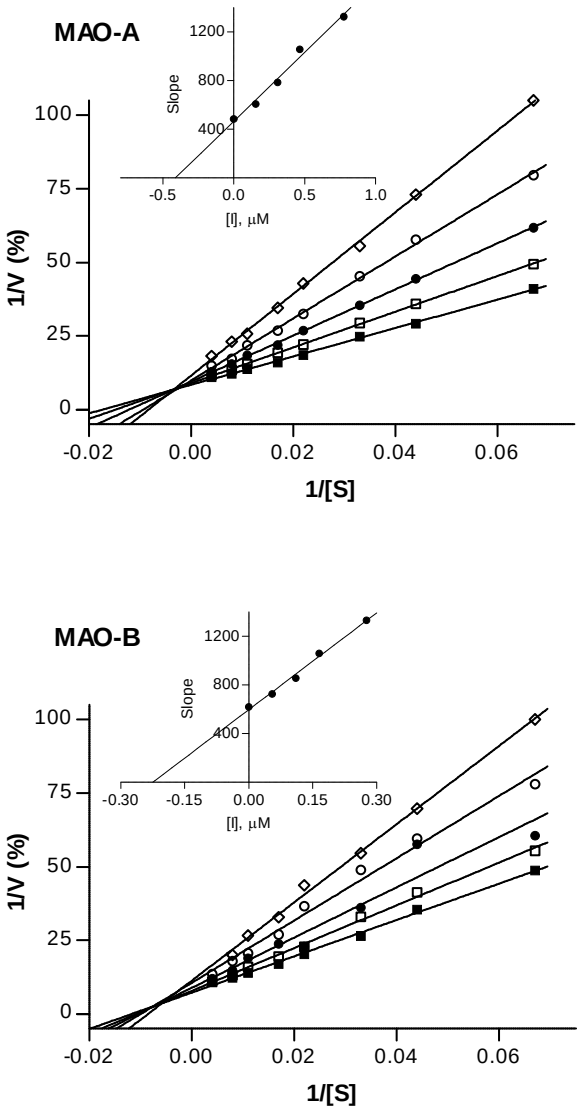



**Fig. 8.** Lineweaver-Burk plots of human MAO-A and MAO-B activities in the absence (filled squares) and presence of various concentrations of pentamidine. For these studies the concentrations of pentamidine employed were ¼ × $IC_{50}$, ½ × $IC_{50}$, ¾ × $IC_{50}$ and 1¼ × $IC_{50}$. The insets are the graphs of the slopes of the Lineweaver-Burk plots versus inhibitor concentration.

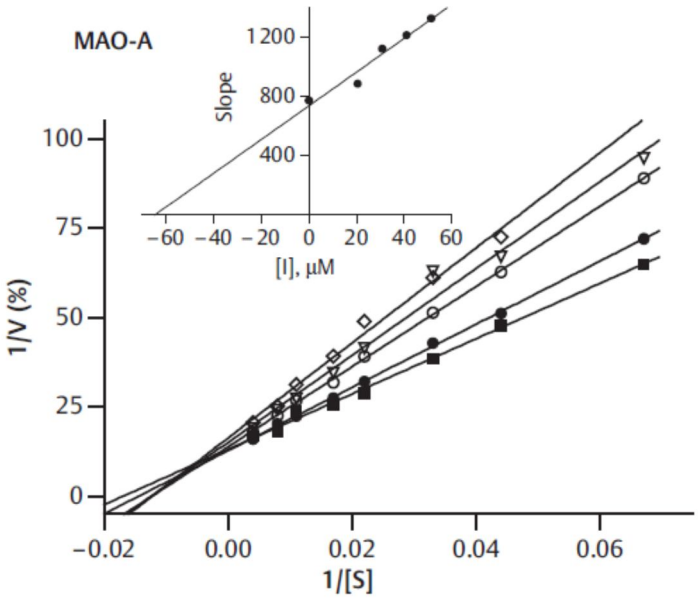



**Fig. 9.** Lineweaver-Burk plots of human MAO-A activities in the absence (filled squares) and presence of various concentrations of phenformin. For these studies the concentrations of phenformin employed were ½ × $IC_{50}$, ¾ × $IC_{50}$, 1 × $IC_{50}$ and 1¼ × $IC_{50}$. The inset is a graph of the slopes of the Lineweaver-Burk plots versus inhibitor concentration.

**Discussion**

This study set out to determine whether the biguanide, phenformin, acts as an inhibitor of the human MAOs. The structurally related diamidine compound, pentamidine, was included in this study since it has been reported to act as a potent MAO inhibitor. As mentioned in the Introduction, even though pentamidine is a known MAO inhibitor [15,16], its potencies towards the human MAOs have not been measured. In particular, it has not been established which MAO isoform is inhibited by pentamidine. Based on the report that pentamidine is an irreversible MAO inhibitor, the reversibility of human MAO inhibition by pentamidine was examined.

The results document that phenformin is a moderately potent MAO-A selective inhibitor with an $IC_{50}$ value of 40.5 µM. It was also shown that phenformin interacts reversibly with MAO-A. To evaluate the probability that phenformin may act as a physiological significant MAO-A inhibitor, the plasma levels attained in humans may be considered. Following a single oral dose of 50 mg phenformin hydrochloride to 8 human subjects, plasma levels of 0.33–0.74 µM are obtained [31]. Considering that the $IC_{50}$ value for the inhibition of MAO-A is at least 54-fold higher than the plasma levels obtained by phenformin, a pharmacological relevant interaction between MAO-A and phenformin is unlikely. It also does not seem likely that

phenformin would accumulate in tissues where MAO-A are found to levels above its $IC_{50}$ value.

In contrast to phenformin, pentamidine was found to be a potent MAO-A and MAO-B inhibitor with $K_i$ values of 0.41 μM and 0.224 μM, respectively. Pentamidine is a reversible and competitive inhibitor of both human MAO isoforms. This finding is in contrast to literature, which reports that pentamidine acts as an irreversible inhibitor of rabbit and rat liver MAO [15,16]. An explanation for these different findings is not readily apparent. To evaluate the probability that pentamidine may inhibit MAO-A and MAO-B in the clinical setting, the plasma levels attained in humans should be considered. Following intramuscular injection of pentamidine isethionate at a dose of 4 mg/kg in humans, plasma levels of 0.51–2.36 μM are attained [14]. The maximal plasma concentration (2.36 μM) is well above the $K_i$ values for the inhibition of MAO-A and MAO-B, and assuming similar concentrations are attained in tissues, inhibition of the MAOs in such tissues are possible. This is of significance since pentamidine may inhibit the metabolic breakdown of tyramine and other sympathomimetic dietary amines by the MAO-A present in the gut wall and vascular endothelial cells [21]. Pentamidine may thus, in theory, lead to tyramine-associated hypertensive crisis when combined with tyramine-containing foods. It should however be noted that, in contrast to irreversible MAO-A inhibitors, reversible inhibitors are in general not associated with hypertensive crisis [22]. For example, the reversible MAO-A inhibitor toloxatone, does not elicit tyramine-associated adverse effects when combined with a dose of tyramine consistent with normal food intake [32]. Even though pentamidine inhibits MAO-A, the potentiation of tyramine-induced side effects may be unlikely since this drug acts as a reversible MAO-A inhibitor. The MAO-A inhibitory properties of pentamidine may also be a significance since MAO-A inhibitors may lead to serotonin toxicity when combined with serotonin-releasing agents or selective serotonin reuptake inhibitors [23,24]. Since pentamidine does not appear to penetrate the central nervous system to a large degree, this compound is not expected to inhibit MAO-A in the brain and thus is not expected precipitate serotonin toxicity when used in conjunction with 5-hydroxytryptaminergic agents [14].

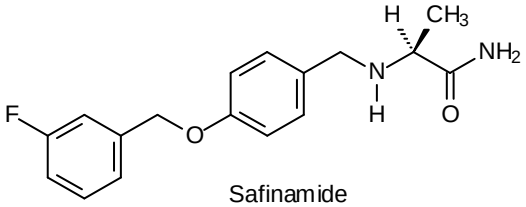
The inhibition of MAO-B by pentamidine may also be of pharmacological significance. One of the principle functions of MAO-B is to protect neurons from stimulation by the false neurotransmitter, β-phenylethylamine [19,33]. β-Phenylethylamine is metabolized to a large extent by brain microvessel MAO-B, which limits its entry into the brain [34]. The central levels of β-phenylethylamine, normally present in only trace amounts, may be enhanced several thousand-fold by the administration of MAO-B inhibitors. It may thus be expected that pentamidine may enhance the central levels of β-phenylethylamine by blocking its MAO-

B-catalyzed metabolism. Since β-phenylethylamine is both a releaser of dopamine as well as an inhibitor of active dopamine uptake [35], the blocking of its metabolism results in an increase in striatal extracellular dopamine levels. Pentamidine also may elevate central dopamine levels by inhibiting the MAO-A and MAO-B mediated catabolism of dopamine in the brain. As mentioned above, pentamidine, however, does not appear to penetrate the central nervous system to a large degree. Based on the excellent safety profile of clinically used MAO-B inhibitors, the MAO-B inhibitory properties of pentamidine should not add to its toxicological profile [36].

To evaluate the contributions of the biguanide and amidine functional groups of phenformin and pentamidine, respectively, to the binding affinities of these drugs for the MAOs, the architectures of the active sites should be considered. The MAO-A and MAO-B active sites are reported to be largely hydrophobic with a polar space in front of the FAD cofactor, the space where the aminyl groups of amine substrates are thought to bind [37]. Polar functional groups of inhibitors are reported to also occupy this space where several hydrogen bond interactions with waters and amino acid residues are possible. For example, the crystal structure of MAO-B in complex with safinamide shows that the propanamidyl moiety binds in proximity to the FAD where the amide group is engaged in hydrogen bonding (Fig. 10) [38]. It is therefore likely that the biguanide and amidine functional groups of phenformin and pentamidine, also bind in proximity to the FAD and thus stabilize the inhibitor via polar interactions in this region. The observation that pentamidine is a more potent MAO-B inhibitor than an MAO-A inhibitor may be explained by the reports that the MAO-B active site, in general, accommodates larger inhibitors better than the MAO-A active site. The side chain of Ile-199 in MAO-B may rotate from the active site cavity to allow for the binding of larger inhibitor such as pentamidine. In the MAO-A active site the residue corresponding to Ile-199 in MAO-B is Phe-208. The increased size of the Phe aromatic ring (compared to the side chain of Ile) prevents it from rotating into an alternative conformation, a structural feature which may hinder larger inhibitors from binding to MAO-A [39,40]. Reasons for the relatively weak MAO inhibitory properties of phenformin are not clear. Phenformin was, however, found to be a MAO-A selective inhibitor. While the reason for this behaviour is unclear, a possible explanation may be that relatively small structures, such as phenformin, are better accommodated by the MAO-A active site than MAO-B. In general, larger extended structures are MAO-B selective inhibitors [39].

This analysis suggests that compounds containing the amidine functional group, and to a lesser extent the biguanide functional group, should be considered as potential MAO inhibitors. Furthermore, the biguanide and amidine functional groups may act as useful

moieties in the future design of MAO inhibitors. As mentioned in the introduction, MAO inhibitors are useful therapeutic agents for the treatment of depression and Parkinson's disease. The potential MAO-A inhibitory properties of compounds containing the amidine functional group are also of interest from a toxicological point of view since MAO-A inhibitors are associated with serious adverse reactions, such as the "cheese-reaction" and serotonin toxicity, when combined with certain foods and drugs. It is, however, important to evaluate the reversibility of MAO-A inhibition of potential MAO inhibitors. As mentioned in the introduction, reversible MAO-A inhibitors are less likely to precipitate the "cheese-reaction" compared to irreversible inhibitors [22].



Safinamide

**Fig. 10**. The structure of safinamide.

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**Conflict of interest**

The authors declare that they have no conflicts of interest to disclose.

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