

# The Inhibition of Monoamine Oxidase by Esomeprazole

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## Key words

- monoamine oxidase
- esomeprazole
- inhibition
- competitive
- reversible

## Abstract

Virtual screening of a library of drugs has suggested that esomeprazole, the *S*-enantiomer of omeprazole, may possess binding affinities for the active sites of the monoamine oxidase (MAO) A and B enzymes. Based on this finding, the current study examines the MAO inhibitory properties of esomeprazole. Using recombinant human MAO-A and MAO-B, IC<sub>50</sub> values for the inhibition of these enzymes by esomeprazole were experimentally determined. To examine the reversibility of MAO inhibition by esomeprazole, the recoveries of the enzymatic activities after dilution of the enzyme-inhibitor complexes were evaluated. In addition, reversibility of inhibition was also examined by measuring the recoveries of enzyme activities after dialysis of enzyme-

inhibitor mixtures. Lineweaver-Burk plots were constructed to evaluate the mode of MAO inhibition and to measure K<sub>i</sub> values. The results document that esomeprazole inhibits both MAO-A and MAO-B with IC<sub>50</sub> values of 23 μM and 48 μM, respectively. The interactions of esomeprazole with MAO-A and MAO-B are reversible and most likely competitive with K<sub>i</sub> values for the inhibition of the respective enzymes of 8.99 μM and 31.7 μM. Considering the available pharmacokinetic data and typical therapeutic doses of esomeprazole, these inhibitory potencies are unlikely to be of pharmacological relevance in humans. The MAO inhibitory effects of esomeprazole should however be taken into consideration when using this drug in animal experiments where higher doses are often administered.

## Introduction

Omeprazole, a proton pump (H<sup>+</sup>/K<sup>+</sup>-ATPase) inhibitor, is used clinically to suppress gastric acid secretion. Omeprazole consists of a racemic mixture of its 2 enantiomers, (R)-omeprazole and (S)-omeprazole (esomeprazole) (► Fig. 1). Both enantiomers are absorbed from the gastrointestinal tract and transformed in the acidic compartment of the gastric parietal cells to the achiral sulphonamide, which is the active H<sup>+</sup>/K<sup>+</sup>-ATPase inhibitor. The (R)- and (S)-enantiomers exhibit different pharmacokinetic properties, particularly with regard to their hepatic metabolism [1,2]. Esomeprazole is metabolized to a larger extent by CYP3A4 compared to (R)-omeprazole, which is almost completely metabolized by CYP2C19. In this context, clinical studies have shown that, at equivalent doses, esomeprazole yields higher area under the curve (AUC) values than omeprazole, and as a result a more pronounced inhibitory effect on acid secretion [3]. Accordingly, esomeprazole has been introduced into the clinical market.

Virtual screening of a library of FDA approved drugs has suggested that esomeprazole may possess binding affinities for the active sites of the monoamine oxidase (MAO) A and B enzymes (unpublished results). The MAOs are of pharmacological interest since they catalyze the oxidation of neurotransmitter and dietary amines, ultimately yielding inactive metabolites [4]. Consequently, the MAOs are considered targets for the treatment of various central nervous system (CNS) diseases and inhibitors of these enzymes are in use as therapeutic agents [5]. For example, MAO-A selective inhibitors enhance the central levels of serotonin, norepinephrine and dopamine and are employed in the therapy of depression [6]. MAO-B selective inhibitors are used in the treatment of Parkinson's disease as these drugs may elevate dopamine levels in the basal ganglia of the brain [7]. In Parkinson's disease therapy, MAO-B inhibitors are also thought to enhance the levels of dopamine derived from exogenously administered levodopa, the metabolic precursor of dopamine [8]. In addition,

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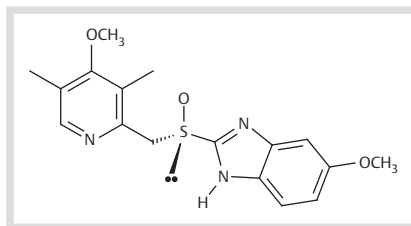
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**Fig. 1** The structure of esomeprazole.

MAO-B inhibitors may also protect against neurodegenerative processes by reducing the levels of potentially neurotoxic aldehydes and  $H_2O_2$ , which are generated as by-products in the MAO catalytic cycle [4].

Besides central effects, MAO inhibitors also exert peripheral pharmacological actions. The most notable of these is a potentially fatal hypertensive reaction which may occur when MAO-A inhibitors are combined with tyramine, which is present in certain foods [9]. In the intestines, tyramine is metabolized by MAO-A, which limits its entry into the systemic circulation. The inhibition of intestinal MAO-A results in excessive amounts of tyramine reaching the circulation, and since tyramine induces the release of norepinephrine from peripheral neurons, this may lead to severe hypertensive crisis [9]. Another important adverse effect of MAO-A inhibitors is serotonin toxicity, a potentially fatal syndrome which develops when 5-hydroxytryptaminergic agents and MAO-A inhibitors are combined [10,11]. Serotonin toxicity is caused by an excessive extracellular serotonin concentration in the CNS and is most often caused by a combination of MAO-A inhibitors, which result in the reduction of the MAO-A-catalyzed degradation of serotonin, with selective serotonin reuptake inhibitors (SSRIs) and serotonin-releasing agents.

Based on the therapeutic applications and potential adverse effects of MAO inhibition, in the present study the MAO-A and -B inhibitory properties of esomeprazole are examined. For this purpose, the MAO inhibitory potencies of esomeprazole were expressed as the corresponding  $IC_{50}$  values. To investigate the reversibility of MAO inhibition by esomeprazole, the recoveries of the enzymatic activities after dilution of the enzyme-inhibitor complexes were evaluated. The reversibility of MAO inhibition was also examined by measuring the recoveries of enzyme activities after dialysis of enzyme-inhibitor mixtures. Lineweaver-Burk plots were constructed to evaluate the mode of MAO inhibition and to measure  $K_i$  values.

## Materials and Methods

### Materials and instrumentation

Fluorescence spectrophotometry was conducted with a Varian® Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant MAO-A or -B (5 mg/ml), kynuramine dihydrobromide, esomeprazole magnesium hydrate and toloxatone were obtained from Sigma-Aldrich®. Lazabemide hydrochloride was synthesized according to the patented method [12].

### $IC_{50}$ determinations

The recombinant human enzymes were employed to determine the  $IC_{50}$  values for the inhibition of MAO-A and MAO-B [13]. The enzymatic reactions were carried out in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) to a final volume of 500  $\mu$ l. The reactions contained the MAO-A/B mixed sub-

strate kynuramine (45  $\mu$ M for MAO-A and 30  $\mu$ M for MAO-B) and different concentrations (0.003–100  $\mu$ M) of the test inhibitor. The concentrations of kynuramine selected for these studies (45  $\mu$ M for MAO-A and 30  $\mu$ M for MAO-B) are similar to the reported  $K_m$  values for the oxidation of kynuramine by the human MAO enzymes [13]. 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 °C and terminated by the addition of 400  $\mu$ l NaOH (2 N). To each reaction, 1 000  $\mu$ l water was added, and the concentrations of 4-hydroxyquinoline, the MAO-catalyzed oxidation product of kynuramine, were subsequently measured by fluorescence spectrophotometry ( $\lambda_{ex}$ =310;  $\lambda_{em}$ =400 nm) [14]. For this purpose linear calibration curves (4-hydroxyquinoline: 0.047–1.56  $\mu$ M) 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  $\pm$  standard deviation (SD).

### Recovery of enzyme activity after dilution

Esomeprazole [ $IC_{50}$ (MAO-A)=23  $\mu$ M] or pargyline [ $IC_{50}$ (MAO-A)=13  $\mu$ M] at concentrations equal to  $10 \times IC_{50}$  (230  $\mu$ M and 130  $\mu$ M for the 2 inhibitors, respectively) for the inhibition of MAO-A were preincubated with recombinant human MAO-A (0.75 mg/ml) for 30 min at 37 °C in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) [15]. Esomeprazole [ $IC_{50}$ (MAO-B)=48  $\mu$ M] or (R)-deprenyl [ $IC_{50}$ (MAO-B)=0.079  $\mu$ M] [13] were similarly preincubated with recombinant human MAO-B (0.75 mg/ml) at concentrations equal to  $10 \times IC_{50}$  (480  $\mu$ M and 0.79  $\mu$ M for the 2 inhibitors, respectively). Control incubations were conducted in the absence of inhibitor, and DMSO (4%) was added as co-solvent to all preincubations. The reactions were diluted 100-fold with the addition of kynuramine to yield final concentrations of the inhibitors equal to  $0.1 \times IC_{50}$ . The final concentration of MAO-A and -B were 0.0075 mg/ml and the concentrations of kynuramine were 45  $\mu$ M and 30  $\mu$ M for MAO-A and -B, respectively. 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  $\pm$  SD.

### Dialysis

The reversibility of the MAO inhibition was also determined by dialysis [16]. For this purpose 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 esomeprazole, at a concentration equal to 4-fold the  $IC_{50}$  values for the inhibition of the respective enzymes, were preincubated for 15 min at 37 °C. These reactions were conducted in potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose to a final volume of 0.8 ml. DMSO (4%) was added as co-solvent to all preincubations. As controls, MAO-A and MAO-B were similarly preincubated in the absence of inhibitor and presence of the irreversible inhibitors, pargyline and (R)-deprenyl, respectively. The concentrations of pargyline [ $IC_{50}$ (MAO-A)=13  $\mu$ M] [15] and (R)-deprenyl [ $IC_{50}$ (MAO-B)=0.079  $\mu$ M] [13] employed were equal to 4-fold the  $IC_{50}$  values for the inhibition of the respective 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 2-fold with the addition of kynuramine (dissolved in potassium phosphate buffer, 100 mM, pH 7.4, made isotonic with KCl) and the residual MAO activities were measured as described above. The final concentration of kynuramine in these reactions was 50  $\mu$ M while the final inhibitor concentrations were equal to 2-fold their  $IC_{50}$  values for the inhibition of the MAOs. For comparison, undialyzed mixtures of the MAOs with esomeprazole 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  $\pm$  SD.

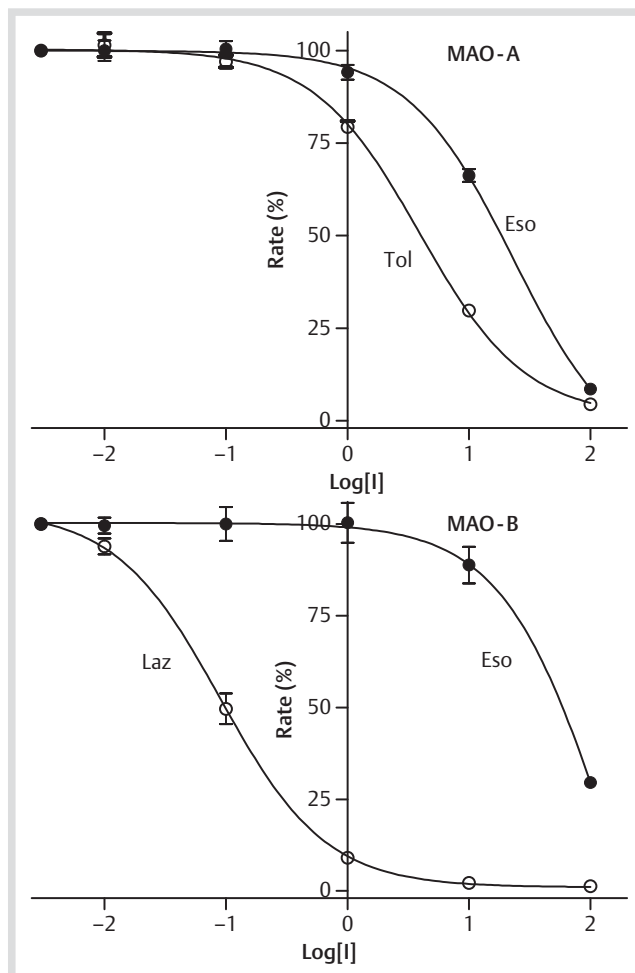
### The construction of Lineweaver-Burk and Dixon plots

A set consisting of 4 Lineweaver-Burk plots ( $1/V$  vs.  $1/[S]$ ) were constructed to evaluate the mode of MAO inhibition and to measure  $K_i$  values. For this purpose, the first plot was constructed in the absence of esomeprazole while the remaining 3 plots were constructed in the presence of different concentrations of esomeprazole. The concentrations of esomeprazole selected for the studies with MAO-A were 5.75  $\mu$ M, 11.5  $\mu$ M and 23  $\mu$ M, while the concentrations selected for the studies with MAO-B were 20  $\mu$ M, 40  $\mu$ M and 80  $\mu$ M. Kynuramine at concentrations of 15–90  $\mu$ M 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 [17].  $K_i$  values were estimated from the x-axis intercept ( $-K_i$ ) of a replot of the slopes of the Lineweaver-Burk plots versus inhibitor concentration. From these data Dixon plots ( $1/V$  vs.  $[I]$ ) were also constructed and, from the intersection points of the Dixon plots,  $K_i$  values may also be estimated [18].

## Results

### IC<sub>50</sub> values

The MAO-A and MAO-B inhibitory properties of esomeprazole were investigated using the commercially available recombinant human enzymes. For the studies with both enzymes, the MAO-A/B mixed substrate, kynuramine, served as substrate. Kynuramine is oxidized by the MAO enzymes 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) and can thus be readily measured by fluorescence spectrophotometry [14]. At the concentrations and conditions used in this study, esomeprazole does not fluoresce. The  $IC_{50}$  values for the inhibition of the MAOs by esomeprazole were estimated from sigmoidal concentration-inhibition curves, which are given in **Fig. 2**. The results show that esomeprazole inhibits human MAO-A with an  $IC_{50}$  value of  $23.2 \pm 1.51$   $\mu$ M. For comparison, the known reversible MAO-A inhibitor, toloxatone, inhibits MAO-A with an  $IC_{50}$  value of  $3.92 \pm 0.015$   $\mu$ M under identical conditions. This value is similar to that (3.26  $\mu$ M) reported in literature [19]. Esomeprazole also acts as an inhibitor of human MAO-B with an  $IC_{50}$  value of  $48.3 \pm 3.08$   $\mu$ M. As positive control, the reversible MAO-B inhibitor, lazabemide, exhibits an  $IC_{50}$  value of  $0.091 \pm 0.015$   $\mu$ M for the inhibition of human MAO-B under identical conditions.



**Fig. 2** The sigmoidal concentration-inhibition curves (filled circles) for the recombinant human MAO-A (top) and MAO-B (bottom) catalyzed oxidation of kynuramine in the presence of various concentrations of esomeprazole (Eso). For comparison, the sigmoidal concentration-inhibition curves (open circles) for the inhibition of MAO-A catalytic activity by toloxatone (Tol) and for the inhibition of MAO-B catalytic activity by lazabemide (Laz) are also provided.

### Reversibility of inhibition

To examine the reversibility of MAO-A and MAO-B inhibition by esomeprazole, the recoveries of the enzymatic activities after dilution of the enzyme-inhibitor mixtures were evaluated. MAO-A and MAO-B were preincubated with esomeprazole at concentrations of  $10 \times IC_{50}$  for the inhibition of the respective enzymes for 30 min and then diluted 100-fold to yield concentrations of  $0.1 \times IC_{50}$ . The results, given in **Fig. 3**, show that after diluting the MAO-esomeprazole mixtures to concentrations equal to  $0.1 \times IC_{50}$ , the MAO-A and MAO-B activities were recovered to levels of 94% and 87% of the control values, respectively. This behaviour is consistent with a reversible interaction of esomeprazole with MAO-A and MAO-B. For reversible inhibition, dilution of the enzyme-inhibitor mixtures to an inhibitor concentration of  $0.1 \times IC_{50}$  is expected to result in approximately 90% recovery in enzyme activity. In contrast, after similar treatment of MAO-A and MAO-B with the irreversible inhibitors pargyline and (R)-deprenyl, respectively, the MAO-A and MAO-B activities were not recovered. Pargyline and (R)-deprenyl, at concentrations of  $10 \times IC_{50}$ , were preincubated with MAO-A and MAO-B, respectively, and the resulting enzyme-inhibitor com-

plexes were diluted 100-fold to yield inhibitor concentrations of  $0.1 \times IC_{50}$ . As shown in **Fig. 3**, after dilution the enzyme activities are only 1.2% and 3.4% of the control values recorded in absence of inhibitor.

The reversibility of MAO-A and MAO-B inhibition by esomeprazole was also investigated by measuring the recoveries of enzyme activities after dialysis of enzyme-inhibitor mixtures [16]. The MAO enzymes and esomeprazole, at a concentration of  $4 \times IC_{50}$ , were preincubated for a period of 15 min and subsequently dialyzed for 24 h. The results, given in **Fig. 4**, show that MAO-A and MAO-B inhibition by esomeprazole is almost completely reversed after 24 h of dialysis with the MAO-A and MAO-B activities recovering to levels of 93% and 88% of the control values (recorded in the absence of inhibitor), respectively. In contrast, the MAO-A and MAO-B activities in undialyzed mixtures of the enzymes with esomeprazole are 24% and 26%, respectively, of the control values. This behaviour is consistent with a reversible interaction between the MAO enzymes and esomeprazole. 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

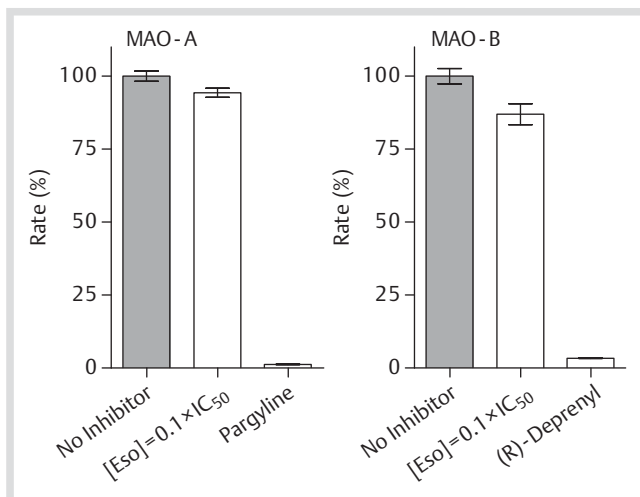
and MAO-B-(R)-deprenyl mixtures, the residual enzyme activities are recovered to levels of only 1.2% and 4.2% of the control values.

### Mode of inhibition

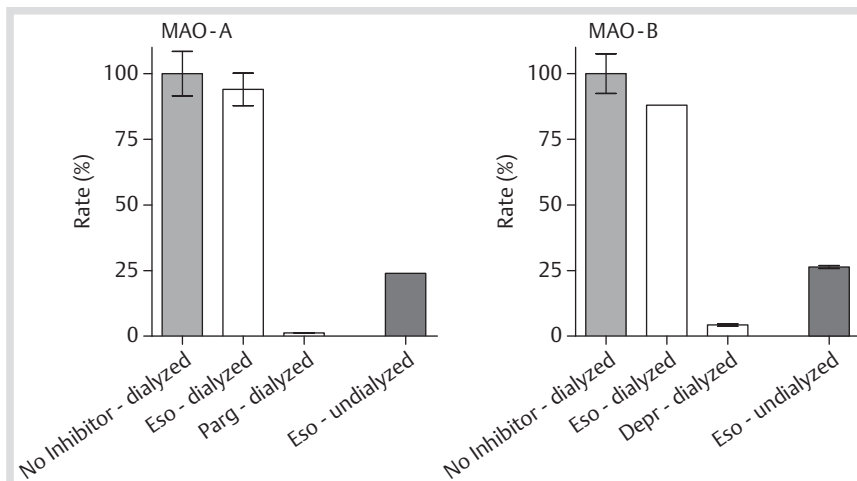
To further examine the interaction of esomeprazole with MAO-A and MAO-B, sets of Lineweaver-Burk plots were constructed. For this purpose the MAO catalytic activities were recorded at 4 substrate concentrations ( $15\text{--}90 \mu\text{M}$ ) in the absence and presence of 3 different concentrations of esomeprazole. These plots are given in **Fig. 5** and show that for the inhibition of both MAO-A and MAO-B, the Lineweaver-Burk plots are linear and intersect at a single point. In addition, the Dixon plots constructed from these data intersect in the second quadrant. From these data it may be concluded that esomeprazole most likely interacts competitively and therefore reversibly with both enzymes. From the replot of the slopes of the Lineweaver-Burk plots vs. the inhibitor concentrations,  $K_i$  values of  $8.99 \mu\text{M}$  and  $31.7 \mu\text{M}$  were calculated for the inhibition of MAO-A and MAO-B, respectively, by esomeprazole. From the intersection points of the Dixon plots, the  $K_i$  values were estimated at  $9.0 \mu\text{M}$  and  $32.0 \mu\text{M}$  for the inhibition of MAO-A and MAO-B, respectively [18]. As expected, these values are similar to those estimated from the Lineweaver-Burk plots.

### Discussion

The MAO enzymes, in particular the MAO-B isoform, are known to exhibit relatively broad inhibitor specificities, and a wide variety of compounds may possess the required structural features for binding to the MAOs. The significance of this is that structures originally designed for activity at other targets are frequently found to also bind to MAO-A and/or MAO-B. Examples of compounds exhibiting this behaviour are (E)-8-(3-chlorostyryl)caffeine (CSC) and pioglitazone [20,21]. Both these compounds are potent MAO-B inhibitors, although they were originally designed for activities at adenosine  $A_{2A}$  receptors and nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), respectively. It is therefore likely that additional drugs exist which bind to the MAOs although intended for action at other molecular targets. The results of this study show that esomeprazole is an example of such a drug. The results document that esomeprazole is a moderately potent inhibitor of the MAOs with approximately 3-fold selectivity for

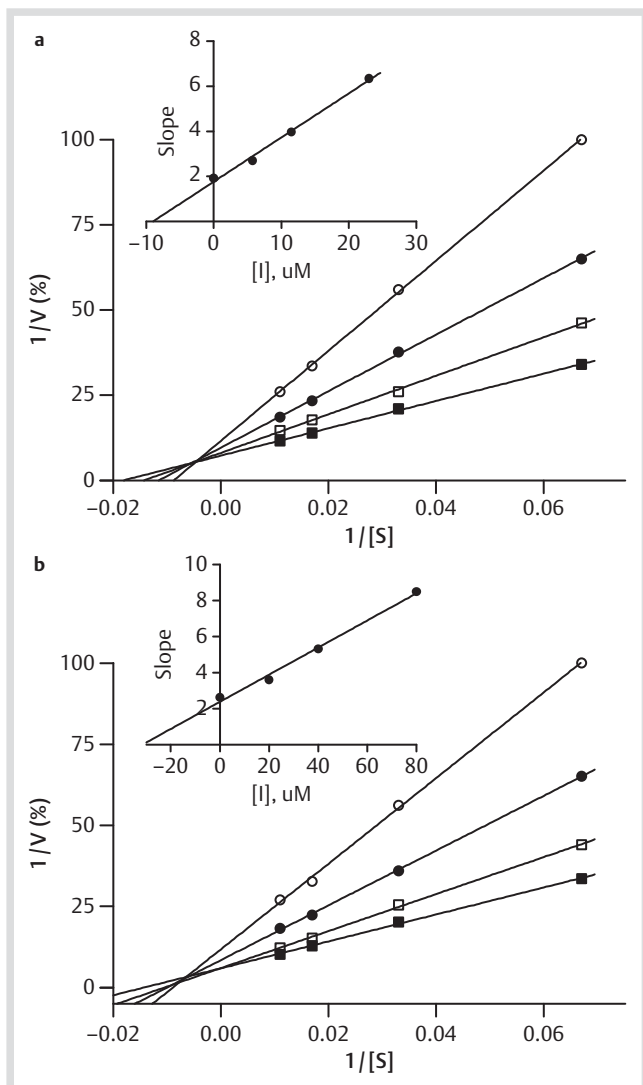


**Fig. 3** Reversibility of inhibition of MAO-A and MAO-B by esomeprazole. MAO-A was preincubated with esomeprazole and pargyline (Panel a), and MAO-B was preincubated with esomeprazole and (R)-deprenyl (Panel b), at  $10 \times IC_{50}$  for 30 min and then diluted to  $0.1 \times IC_{50}$ . The residual enzyme activities were subsequently measured.



**Fig. 4** Reversibility of inhibition of MAO-A (top) and MAO-B (bottom) by esomeprazole. The MAO enzymes and esomeprazole, at a concentration of  $4 \times IC_{50}$ , were preincubated for a period of 15 min, dialyzed for 24 h and the residual enzyme activities were subsequently measured (Eso-dialyzed). For comparison, the MAOs 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. For comparison, the residual MAO activities of undialyzed mixtures (Eso-undialyzed) of the MAOs with esomeprazole are also shown.





**Fig. 5** Lineweaver-Burk plots of recombinant human MAO-A (Panel a) and MAO-B (Panel b) catalytic activities in the absence (filled squares) and presence of various concentrations of esomeprazole. For the studies with MAO-A (Panel a) the concentrations of esomeprazole employed were 5.75  $\mu\text{M}$  (open squares), 11.5  $\mu\text{M}$  (filled circles) and 23  $\mu\text{M}$  (open circles). For the studies with MAO-B (Panel b) the concentrations of esomeprazole employed were: 20  $\mu\text{M}$  (open squares), 40  $\mu\text{M}$  (filled circles) and 80  $\mu\text{M}$  (open circles). The insets are the replots of the slopes of the Lineweaver-Burk plots vs. inhibitor concentration.

the MAO-A isoform (as judged by the  $K_i$  values). Also of significance is the finding that esomeprazole interacts reversibly with the MAO enzymes. To evaluate the probability that esomeprazole may act as a physiological MAO inhibitor, the free-drug concentrations at the molecular targets should be considered. Assuming that the free-drug level of 3-fold the  $K_i$  value of an inhibitor is necessary for >75% enzyme occupancy (for competitive inhibition), significant inhibition would occur [22]. Following a typical dose of 20 mg/day of esomeprazole, a mean  $C_{\text{max}}$  value of 2.55  $\mu\text{M}$  (2.00–3.24  $\mu\text{M}$ ) and mean  $t_{1/2}$  of 1.10 h in the plasma of humans have been reported on day 5 of treatment [3]. Considering that the  $K_i$  values for the inhibition of the MAOs are significantly higher than the  $C_{\text{max}}$  value, and assuming that the concentrations of esomeprazole in the plasma and endothelial compartments are similar, pharmacological relevant interactions between esomeprazole and the MAOs found in the micro-

vessels [23] are improbable. This conclusion is significant since the MAOs play important roles as metabolic barriers in vascular endothelial cells, and inhibition of the MAOs at these compartments may lead pharmacological responses. For example, the MAO-B isoform is thought to protect neurons from stimulation by the false neurotransmitter  $\beta$ -phenylethylamine, which is a trace amine derived from the diet and from the metabolism of phenylalanine [5,24]. This amine is metabolized to a large extent by MAO-B found in brain microvessels, which results in the restriction of its entry into the brain [25]. The modulation of central  $\beta$ -phenylethylamine levels by MAO-B inhibitors may lead to a beneficial effect in Parkinson's disease. Since  $\beta$ -phenylethylamine is both a releaser of dopamine as well as an inhibitor of active dopamine uptake [26], the blocking of its metabolism results in an increase in striatal extracellular dopamine levels, and an antisymptomatic effect in Parkinson's disease. The central levels of  $\beta$ -phenylethylamine, normally present in only trace amounts in the CNS, may be enhanced several 1000-fold by the administration of MAO-B inhibitors. Since the  $K_i$  value for the inhibition of MAO-B is well above  $C_{\text{max}}$  value ( $K_i/C_{\text{max}} = 12$ ), the inhibition of  $\beta$ -phenylethylamine metabolism is improbable and esomeprazole is therefore not expected to significantly enhance central  $\beta$ -phenylethylamine levels.

In contrast to relatively low levels of esomeprazole in the plasma compartment, esomeprazole may reach much higher concentrations in the cytosol of the cells lining the gastrointestinal tract. Following oral administration, the concentrations of esomeprazole may be high at the luminal surface of the intestinal epithelial cells, which, in turn, may lead to a high rate of diffusion into intestinal cells. This may be of significance since intestinal MAO-A catabolizes tyramine, which is found in certain foods. As mentioned in the introduction, tyramine is an indirectly-acting sympathomimetic amine and induces the release of norepinephrine from peripheral neurons, a process which may lead to severe hypertensive crisis [9]. The metabolic breakdown of tyramine by intestinal MAO-A (present in the gut wall) and vascular endothelial cells reduces the amount of this amine that enters the systemic circulation and thus prevents the tyramine-associated adverse effects. In the presence MAO-A inhibition, excessive amounts of tyramine may reach the circulation. Even though esomeprazole concentrations may reach relatively high levels in intestinal epithelial cells, the potentiation of tyramine-induced side effects by esomeprazole is unlikely since this drug acts as a reversible MAO-A inhibitor. In contrast to irreversible MAO-A inhibitors, reversible inhibitors are in general not associated with hypertensive crisis [16]. For example, toloxatone, shown here to be a nearly 6-fold more potent MAO-A inhibitor than esomeprazole, does elicit tyramine-associated adverse effects when combined with a dose of tyramine consistent with normal food intake [27]. The observation that reversible MAO-A inhibitors are unlikely to lead to the potentiation of tyramine-induced side effects is not well understood.

Unfortunately the levels that esomeprazole reaches in the human tissues where MAO-A and MAO-B are present (brain, liver etc.) have not yet been measured. Unless esomeprazole accumulates in these tissues to reach concentrations well above the  $K_i$  values for the inhibition of the MAOs, pharmacological effects of esomeprazole, which are mediated by MAO inhibition in these tissues, are unlikely.

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## Conflict of Interest

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

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