Effects of cytochrome P450 inhibitors on peroxidase activity

Marketa Martinkova, Bozena Kubickova, Marie Stiborova

Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic

Correspondence to: Prof. RNDr. Marie Stiborova, DSc.
Department of Biochemistry, Faculty of Science, Charles University in Prague, Albertov 2030, 128 40 Prague 2, Czech Republic.
tel: +420-221951285; fax: +420-221951283; e-mail: stiborov@natur.cuni.cz

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Abstract

OBJECTIVES: Of several enzymes metabolizing xenobiotics, cytochrome P450 (CYP) and peroxidase enzymes seem to be most important. One of the major challenges in studies investigating metabolism of xenobiotics is to resolve which of these two groups of enzymes is predominant to metabolize individual xenobiotic compounds. Utilization of selective inhibitors of CYP and peroxidase enzymes might be a useful tool to identify the contribution of these enzymes to metabolism of xenobiotics in samples, where both types of enzymes are present. The aim of this study was to investigate specificities of several known CYP inhibitors to these enzymes; whether they inhibit only the CYP enzymes and do not inhibit peroxidases. **METHODS:** Since the oxidation of *o*-anisidine catalyzed by a model peroxidase used, horseradish peroxidase (HRP), is a two-substrate reaction, the inhibition potential of tested chemicals was studied with respect to both peroxidase substrates, *o*-anisidine and hydrogen peroxide. Initial velocities of *o*-anisidine oxidation by HRP under various conditions were determined spectrophotometrically. **RESULTS:** The CYP inhibitors metyrapone, troleandomycine, disulfiram, sulfaphenazole, quinidine and 1-aminobenzotriazole do not inhibit *o*-anisidine oxidation catalyzed by HRP. In contrast, ketoconazole, diethyldithiocarbamate, ellipticine, α-naphtoflavone, proadifen SKF525A, piperonylbutoxide, were found to inhibit not only the CYPs, but also the HRP-mediated oxidation of *o*-anisidine. Interestingly, α-naphtoflavone inhibits oxidation of *o*-anisidine by HRP with respect to H_2O_2, but not with respect to *o*-anisidine. Diethyldithiocarbamate is the most potent peroxidase inhibitor of *o*-anisidine oxidation with Ki with respect to *o*-anisidine of 10 μM and K_i with respect to H_2O_2 of 60 μM, being even the better peroxidase inhibitor than the classical "peroxidase inhibitor" – propyl gallate (Ki with respect to *o*-anisidine of 60 μM and K_i with respect to H_2O_2 of 750 μM). **CONCLUSIONS:** The results of the present study demonstrate that 1-aminobenzotriazole, a potent inhibitor of various CYP enzymes, seems to be the best candidate suitable for utilization in studies evaluating participation of CYP enzymes in metabolism of xenobiotics in various complex biological materials containing both CYP and peroxidase enzymes. Moreover, precaution to prevent misinterpretation of results is necessary in cases when proadifen SKF525A, piperonylbutoxide, diethyldithiocarbamate, ketoconazole, α-naphtoflavone and ellipticine are used in similar studies (as CYP inhibitors in various complex biological materials containing both CYP and peroxidase enzymes), since these chemicals can except of CYP enzymes inhibit also peroxidase-mediated reactions.

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ORIGINAL ARTICLE

Abbreviations:

CYP	- cytochrome P450
HRP	- horseradish peroxidase
Ki	- Inhibition constant
Km	- Michaelis constant
UV	- ultraviolet
VIS	- visible
Vmax	- maximum reaction rate

INTRODUCTION

Cytochromes P450 (CYP) (EC 1.14.14.1) and peroxidases (EC 1.11.1.7) are two distinctive families of hemoproteins that play an important role in the metabolism of a wide variety of xenobiotics such as drugs and other foreign compounds, i.e., carcinogens and endogenous substances (Guengerich & Shimada 1991; Gonzalez & Gelboin 1992; Stiborova *et al.* 2000; Miksanova *et al.* 2001; Naiman *et al.* 2010). These enzymes are ubiquitous among prokaryotic and eukaryotic organisms including plant and animal kingdom (Miksanova *et al.* 2001; Stiborova *et al.* 2003)

Functions of both types of the enzymes in metabolism of xenobiotics in organisms as well as their contribution to such metabolism are the subject of extensive investigation (Guengerich & Shimada 1991, Gonzalez & Gelboin 1992; Rendic & DiCarlo 1997; Stiborova *et al.* 2000; Miksanova *et al.* 2001). It should be however mentioned that their contribution to metabolism of xenobiotics in a very complex biological material containing both CYPs and peroxidases is difficult to be resolved. One of the useful tools to identify participation of individual types of enzymes in metabolism of xenobiotics is employing the selective inhibitors of these enzymes. Nevertheless, whereas inhibitors of CYP enzymes have already been identified (Guengerich & Shimada 1991; Bourrie *et al.* 1996; Rendic & DiCarlo 1997), there is essentially no information about their effects on peroxidases. In addition, selective inhibitors of peroxidases are not known. Only a few of the compounds such as cyanide, azide and propyl gallate have been utilized as the "peroxidase inhibitors" (Grintsevich *et al.* 2000), but again nothing is known about their specificities to inhibit only peroxidases. Namely, minimum information exists whether these compounds can inhibit also other heme enzymes, including CYPs (Yang & Strickhart 1974).

Recently, some inhibitors of CYPs have been reported not to be selective enough to inhibit CYPs, exhibiting an inhibition potential also to several other enzymes metabolizing xenobiotics (Izumi *et al.* 1994). Moreover, the majority of CYP inhibitors has not been ever tested for their potential to inhibit peroxidase reaction. Besides the CYP inhibitors selective to individual CYP enzymes such as CYP3A4, 1A, 2C, 2A6, 2D6 and 2E1, the enzymes that are expressed at high levels in the major organ responsible for metabolism of xenobiotics – in the human liver, there are also compounds capable of inhibiting a majority of various individual CYP enzymes (Emoto *et al.* 2003; Kluwe & Hook 1981). Here, we investigated whether both groups of CYP inhibitors, (i) unspecific inhibitors, inhibiting the various CYP enzymes (so called general CYP inhibitors) (1-aminobenzotriazole, proadifen SKF525A and piperonylbutoxide) and (ii) inhibitors of individual CYP enzymes (diethyldithiocarbamate, disulfiram, ketoconazole, methyrapone, α-naphtoflavone, sulfaphenazole, troleandomycine, quinidine, ellipticine) (Scheme 1) can influence the peroxidase activity.

In this report, we investigated the effects of twelve CYP inhibitors with different structures (1-aminobenzotriazole, piperonylbutoxide and proadifen SKF525A, which inhibit various CYP isoforms; α-naphtoflavone, an inhibitor of CYP1A; sulfaphenazole, which inhibits CYP2C; quinidine, which inhibits CYP2D; diethyldithiocarbamate, which inhibits CYP2E1 and CYP2A6; disulfiram, which inhibits CYP2E1; ellipticine, which inhibits CYP1A and 3A; ketoconazole, metyrapone and troleandomycine, which inhibit CYP3A) (Kluwe and Hook 1981, Bourrie *et al.* 1996, Rendic & DiCarlo 1997, Emoto *et al.* 2003, Aimova & Stiborova 2005) and another compound that is used as an inhibitor of peroxidase, propyl gallate (Reddan *et al.* 2003) (Scheme 1) on peroxidase activity. Propyl gallate, being a representative of classical "peroxidase inhibitor", was also utilized in this study, in order to compare its inhibition potential on the peroxidase reaction with that of other tested compounds. Horseradish peroxidase (HRP) was used as a model peroxidase and oxidation of its substrate, *o*-anisidine (Stiborova *et al.* 2002) (Scheme 2), as a model reaction catalyzed by HRP. The aim of this study was to evaluate whether the well known CYP inhibitors do not influence the peroxidase mediated reactions and, hence, to re-evaluate their CYP selectivity.

MATERIAL AND METHODS

Chemicals and enzymes

Chemicals were obtained from the following sources: horseradish peroxidase type VIA, α-naphthoflavone, ketoconazole, diethyldithiocarbamate, sulfaphenazole, troleandomycine, quinidine, disulfiram, methyrapone, 1-aminobenzotriazole, proadifen SKF525A and propyl gallate from Sigma Chemical Co. (St. Louis, MO, USA); 2-methoxyaniline (*o*-anisidine), ellipticine, dimethyl sulfoxide, piperonylbutoxide from Fluka Chemie AG (Buchs, Switzerland). All chemicals were reagent grade purity or better.

Incubation and analysis of the enzyme reaction

The reaction mixtures consisted of 0.1 μg HRP, 0.03–0.6 mM H_2O_2, 0.07–0.75 mM *o*-anisidine in the final volume of 1.0 ml of 0.1 M phosphate buffer, pH 6.5. The reaction was started by addition of the H_2O_2 and the absorbance at 460 nm was followed for the initial 30 seconds on a Hewlett Packard 8453 UV spectrophotomer. An increase in absorbace at 460 nm clearly

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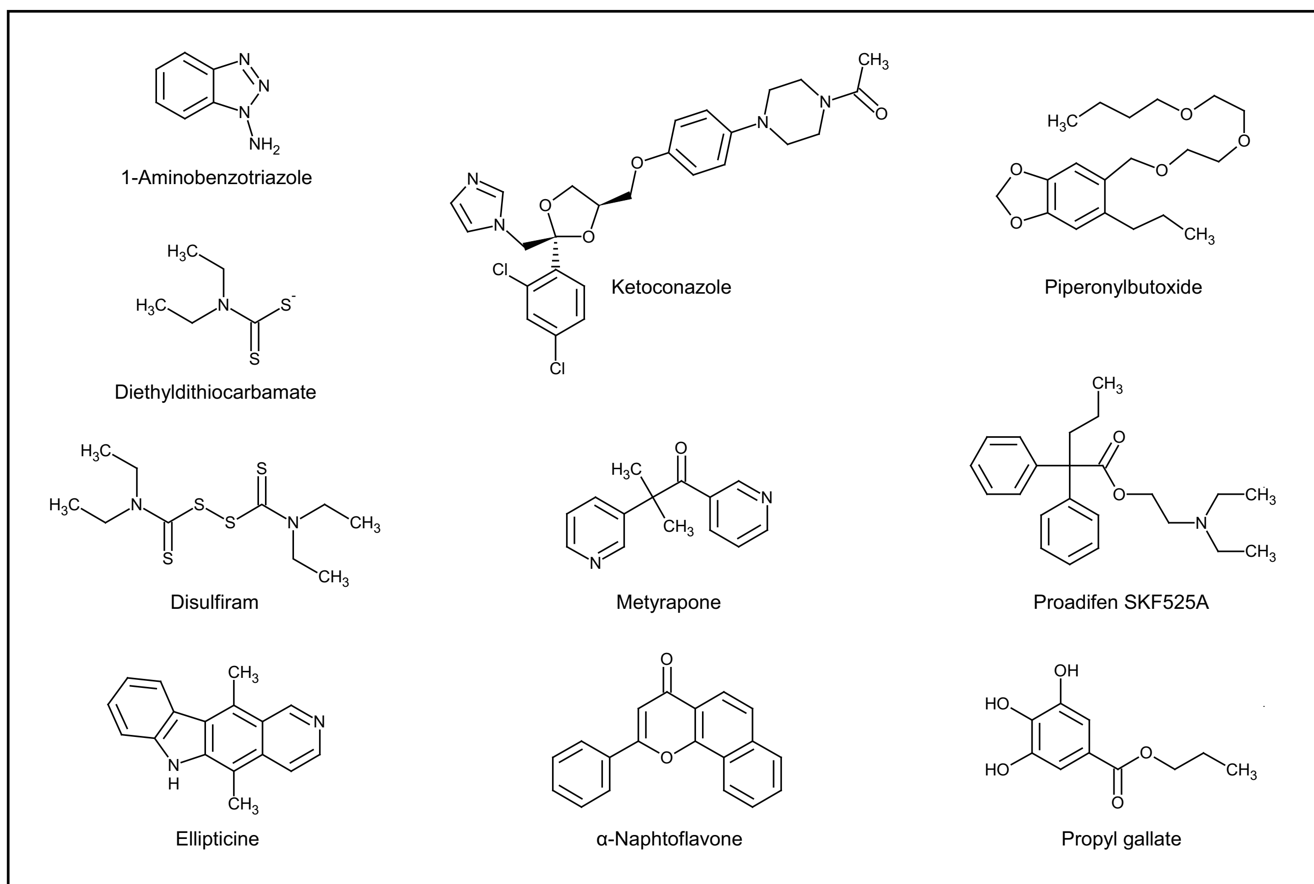
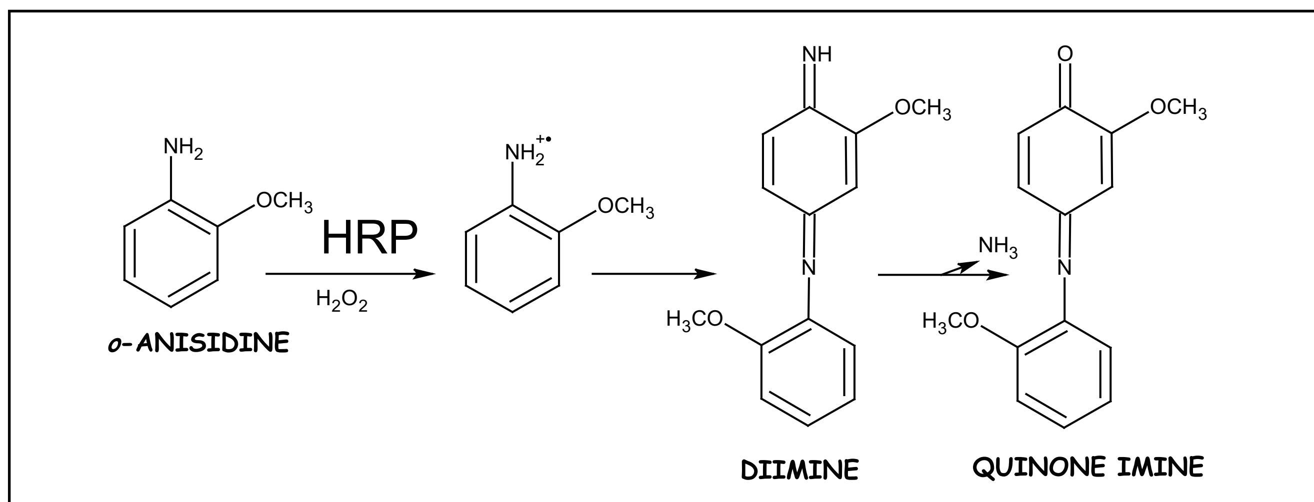
reflects the initial formation of diimine of *o*-anisidine (λ_{max}=460 nm) caused specifically by the peroxidase reaction (Scheme 2) (Stiborova *et al.* 2002).

Inhibition studies

In order to test the inhibition potential of the above mentioned compounds on the reactions catalyzed by HRP, inhibition experiments were performed as reported previously (Miksanova *et al.* 2001) with slight modifications. The compounds tested in the study were dissolved in 10.0 µl of methanol (sulfaphenazole, troleandomycine, propyl gallate), ethanol (quinidine), dimethyl sulfoxide (metyrapone, disulfiram, α-naphtoflavone, ellipticine) or buffer (diethyldithiocarbamate) and added to the incubation mixtures, composition of which was the same as described above (see



Scheme 1. Chemical structure of compounds tested for their potential to inhibit peroxidase reaction.



Scheme 2. Proposed metabolic pathways for *o*-anisidine oxidation by HRP.

chapter Incubation and analysis of the enzyme reaction) to yield final concentrations of 0.01-10 mM. An equal volume of methanol, ethanol, dimethyl sulfoxide or buffer alone was added to the control incubations (0 mM inhibitor). The reaction was started by addition of the H_2O_2 and the absorbance at 460 nm was followed for the initial 30 seconds on a Hewlett Packard 8453 UV spectrophotomer. All experiments were performed at least three times and are shown in the Table 1 as the mean values. The oxidation of *o*-anisidine catalyzed by HRP is the two-substrate reaction obeying the ping-pong mechanism (Stiborova *et al.* 2002). Therefore, the inhibition potential was studied with respect to both substrates separately. The inhibition study with respect to *o*-anisidine was performed under excess of H_2O_2 (0.6 mM) and the inhibition study with respect to H_2O_2 was performed under excess of *o*-anisidine (0.75 mM). The inhibition constants were obtained from the Dixon plots ([I] versus 1/v). Kinetic analyses were carried out using the non-linear least-squares method (Origin v7, OriginLab).

Tab. 1. Inhibition constants K_i of chemicals for *o*-anisidine oxidation catalyzed by HRP.

Compound	$K_i^{o\text{-anisdine}}$	$K_i^{H_2O_2}$
1-Aminobenzotriazole	NO INHIBITION	
Disulfiram	NO INHIBITION	
Metyrapone	NO INHIBITION	
Quinidine	NO INHIBITION	
Sulfaphenazole	NO INHIBITION	
Troleandomycine	NO INHIBITION	
α-Naphtoflavone	NO INHIBITION	0.01mM competitive
Proadifen SKF525A	3.00 mM competitive	NO INHIBITION
Piperonylbutoxide	0.38 mM competitive	NO INHIBITION
Ketoconazole	0.13 mM competitive	NO INHIBITION
Propyl gallate	0.06 mM competitive	0.75 mM competitive
Ellipticine	0.03 mM competitive	0.35 mM competitive
Diethyldithiocarbamate	0.01 mM competitive	0.06 mM competitive

Experimental conditions are described in Material and methods, *o*-anisidine (0.07 – 0.75 mM), H_2O_2 (0.03 - 0.6 mM) and 0.1 μg HRP were present in the incubation. The inhibition study with respect to *o*-anisidine was performed under excess of H_2O_2 (0.6 mM) and the inhibition study with respect to H_2O_2 was performed under excess of *o*-anisidine (0.75 mM). Values in the table are averages of three determinations.

RESULTS

Since oxidation of *o*-anisidine catalyzed by HRP is the two-substrate reaction (Stiborova *et al.* 2002), the inhibition potential of the tested compounds was studied with respect to both substrates.

Of the 13 compounds (Scheme 1) tested for their potential to inhibit oxidation of *o*-anisidine by HRP, 1-aminobenzotriazole, which inhibit various CYP enzymes; disulfiram, which inhibits CYP2E1; metyrapone, which inhibit CYP3A; quinidine, which inhibits CYP2D; sulfaphenazole, which inhibits CYP2C and troleandomycine, a CYP3A inhibitor, do not inhibit *o*-anisidine oxidation catalyzed by HRP. In contrast to these results, α-naphtoflavone, an inhibitor of CYP1A; ketoconazole, an inhibitor of CYP3A; proadifen SKF525A and piperonylbutoxide, which inhibit various CYP enzymes, ellipticine, an inhibitor of CYP1A and 3A, propyl gallate, a radical scavenger used as "peroxidase inhibitor" (Reddan *et al.* 2003) and diethyldithiocarbamate, which inhibit CYP2E1 and CYP2A6, inhibit the model peroxidase reaction, but with different efficiencies (Table 1, Figures 1-4).

The values of inhibition constants of these compounds to inhibit oxidation of *o*-anisidine ranged between 0.01 to 3 mM (Table 1). The values of inhibition constants with respect to *o*-anisidine were usually more than five times lower than the values of inhibition constants with respect to H_2O_2. Ketoconazole, proadifen SKF525A and piperonylbutoxide exhibited either very weak or no measurable efficiency to inhibit the model peroxidase reaction with respect to H_2O_2. Therefore, it was impossible to determine the values of their inhibition constants; no inhibition constants with respect to H_2O_2 could be determined for ketoconazole, proadifen SKF525A and piperonylbutoxide (Table 1). Different situation was, however, observed with α-naphtoflavone. α-Naphtoflavone did not inhibit HRP with respect to *o*-anisidne, but the inhibition with respect to H_2O_2 was measurable (Table 1, Figure 3).

Ellipticine, propyl gallate and diethyldithiocarbamate exhibited classical competitive inhibition of HRP with respect to *o*-anisidine oxidation. The values of their inhibition constants with respect to H_2O_2 were significantly lower than the values of inhibition constants with respect to *o*-anisidine (Table 1). It was seen from the absorption spectra measured during the inhibition of *o*-anisidine oxidation with ellipticine and propyl gallate that new peaks with characteristic wavelengths different from those of the original HRP substrate (*o*-anisidine) and/or *o*-anisidine metabolites were visible (data not shown). They might originate from oxidation of ellipticine and/or propyl gallate by HRP. Therefore, these compounds seem to be the substrates of HRP, as it has already been found for ellipticine (Stiborova *et al.* 2007, 2010, 2011). However, in the case of adding the diethyldithiocarbamate to the reaction mixture, no new peaks corresponding to its metabolite were observed.

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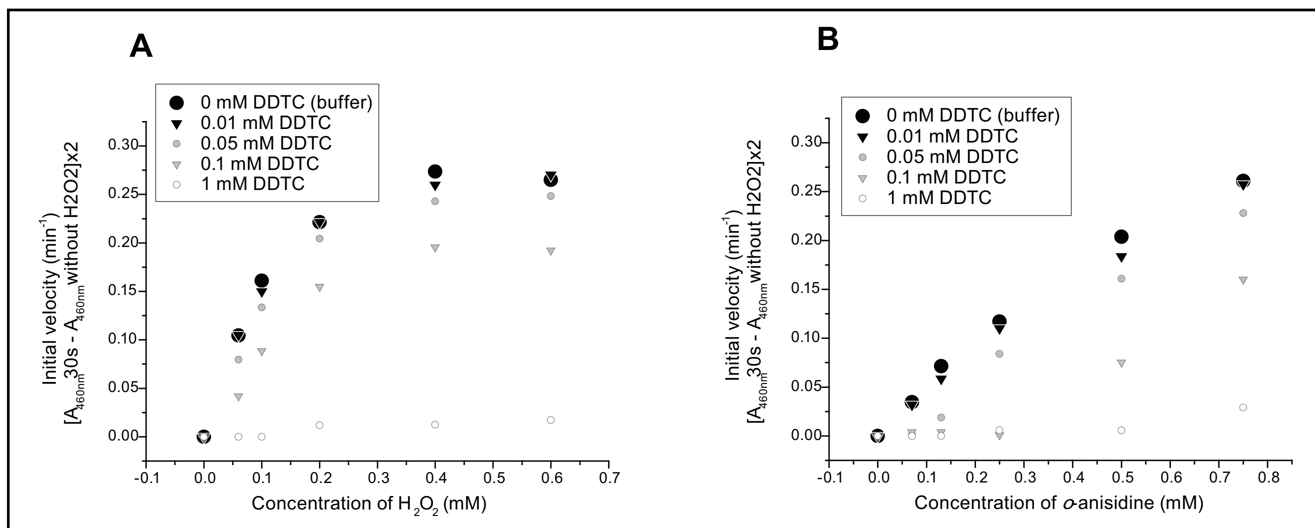

Fig. 1. Initial velocities of *o*-anisidine oxidation by HRP to diimine in excess of *o*-anisidine (0.75 mM) (A) and in excess of H_2O_2 (0.6 mM) (B) either in the absence or in the presence of different concentrations of diethyldithicarbamate. Experimental conditions are described in Material and methods. DDTC, diethyldithiocarbamate.

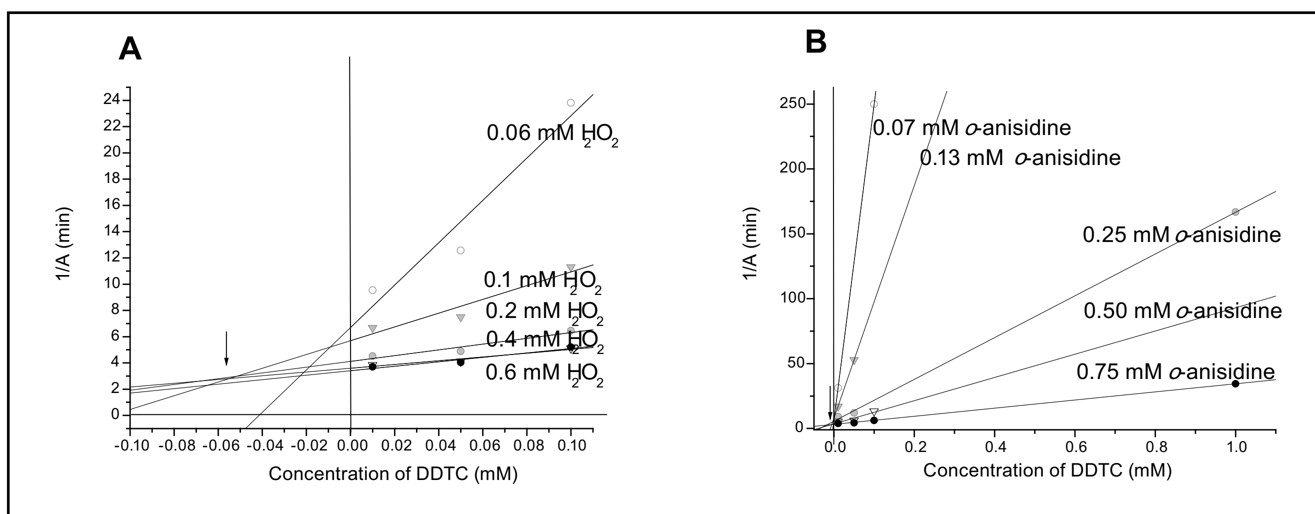

Fig. 2. Dixon plots of initial velocities of *o*-anisidine oxidation catalyzed by HRP *versus* concentrations of diethyldithicarbamate in the excess of *o*-anisidine (0.75 mM) (A) and in the excess of H_2O_2 (0.6 mM) (B). Experimental conditions are described in Material and methods. DDTC, diethyldithiocarbamate.

Of the tested compounds, diethyldithiocarbamate was the strongest peroxidase inhibitor having values of the inhibition constants of $K_i^{o\text{-anisidine}}$ equaled to 10 μM and K_i^{H2O2} equaled to 60 μM**,** followed by ellipticine and propyl gallate (Table 1, Figures 1 and 2). Interestingly, the value of the inhibition constant of α-naphtoflavone with respect to H_2O_2 was estimated to be 10 μM. However, α-naphtoflavone did not show any inhibition of *o*-anisidine oxidation with respect to *o*-anisidine (Table 1, Figure 3).

DISCUSSION

The results of the present study show that both inhibitors of individual CYP enzymes such as metyrapone, troleandomycine, disulfiram, sulfaphenazole and quinidine (Bourrie *et al.* 1996) and 1-aminobenzotriazole, which is considered to be a general inhibitor of CYPs (Emoto *et al.* 2003), do not inhibit *o*-anisidine oxidation catalyzed by HRP (Table 1). Therefore, these compounds proved their high selectivity only to CYP. In the case of sulfaphenazole, one could speculate that the aromatic amino group in its molecule (Scheme 1) could be a target of the peroxidase reaction, because chemicals containing aromatic amino groups are known to be the excellent peroxidase substrates (Stiborova *et al.* 2000, 2002). However, we proved that sulfaphenazole does not inhibit peroxidase-mediated oxidation of *o*-anisidine. Therefore it is probably not oxidized by peroxidase or the rate of its oxidation is several orders of magnitude lower than the rate of oxidation of *o*-anisidine.

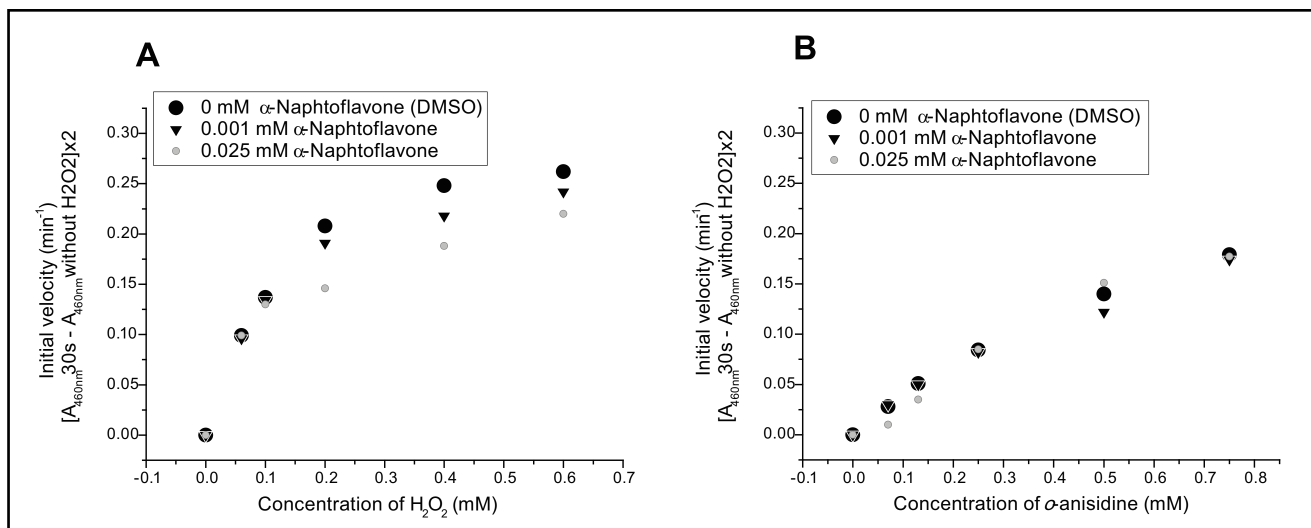

Fig. 3. Initial velocities of *o*-anisidine oxidation by HRP to diimine in excess of *o*-anisidine (0.75 mM) (A) and in excess of H_2O_2 (0.6 mM) (B) either in the absence or in the presence of different concentrations of α-naphtoflavone. Experimental conditions are described in Material and methods. DMSO, dimethyl sulfoxide

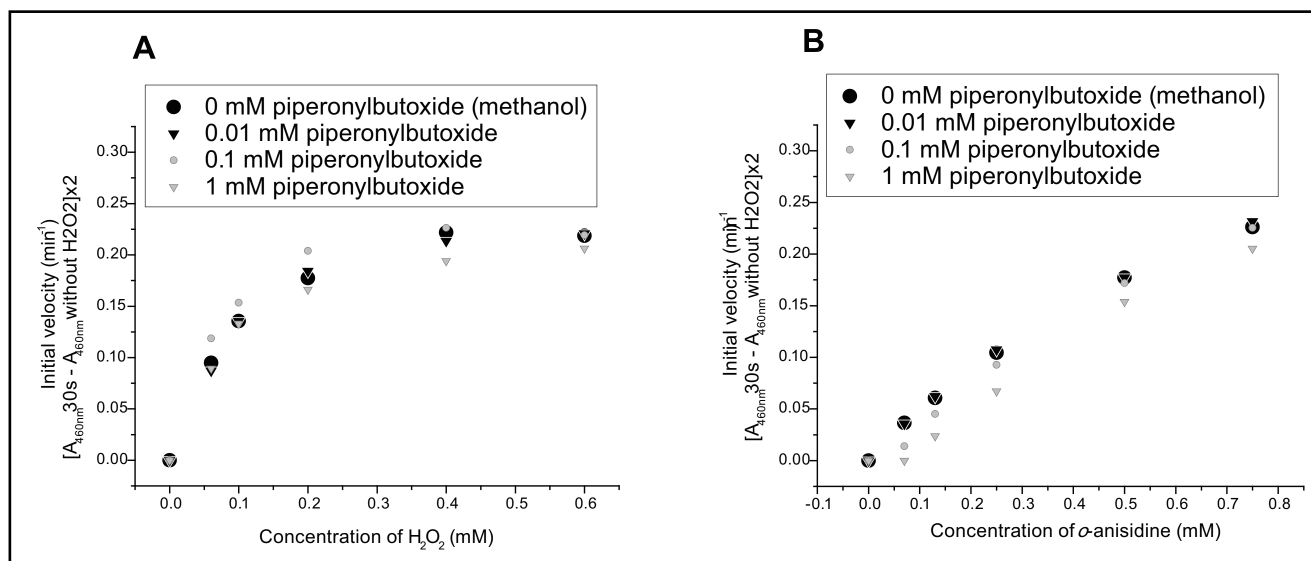

Fig. 4. Initial velocities of *o*-anisidine oxidation by HRP to diimine in excess of *o*-anisidine (0.75 mM) (A) and in excess of H_2O_2 (0.6 mM) (B) either in the absence or in the presence of different concentrations of piperonylbutoxide. Experimental conditions are described in Material and methods.

α-Naphtoflavone, an inhibitor of CYP1A, ketoconazole, an inhibitor of CYP3A, proadifen SKF525A and piperonylbutoxide, which inhibit various CYP isoforms inhibit *o*-anisidine oxidation by HRP, but only to a certain extent (Table 1). These four chemicals inhibited the reaction either with respect to *o*-anisidine (ketoconazole, proadifen SKF525A and piperonylbutoxide) or with respect to H_2O_2 (α-naphtoflavone). The reason, why the peroxidase inhibition by α-naphtoflavone with respect to *o*-anisidine was not found, might be the chemical structure of this compound. Whereas no binding of this compound to the HRP active centre probably occurs, the excess of H_2O_2 present in the reaction mixture might cause destroying the enzyme protein structure and therefore its enzymatic activity. Likewise, other chemicals with a low inhibition potential for HRP (ketoconazole, proadifen SKF525A and piperonylbutoxide) seem to be the compounds with much lower binding affinities to HRP (if any), compared with *o*-anisidine.

Another CYP inhibitor, diethyldithiocarbamate, known as an inhibitor of CYP2E1 and 2A6, was a strong inhibitor of *o*-anisidine oxidation by HRP. Of the compounds tested in this study, this compound was even the strongest peroxidase inhibitor with $K_i^{o\text{-anisidine}}$ of 10 µM and $K_i^{H_2O_2}$ of 60 µM (Table 1, Figure 1 and Figure 2). Diethyldithiocarbamate has not been proven to be a true inhibitor of HRP or its real substrate, but it

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is known that interacts directly with H_2O_2. Hydrogen peroxide oxidizes diethyldithiocarbamate, leading to the formation of disulfiram, a dimer of it having a disulfide group (Bottu 1989). Moreover, we found that disufiram did not inhibit *o*-anisidine oxidation catalyzed by HRP, however, its *in vivo* metabolite, diethyldithiocarbamate (Cobby *et al.* 1977) causes a strong decrease in its oxidation. Mankhetkorn *et al.* (1994) found that no radical intermediate was observed in this process. In the presence of an excess of any of the reagents, the hydrated form of disulfiram transforms into different products corresponding to the fixation of oxygen by sulfur atoms or replacement of C = S group by ketone function, in the presence of an excess of hydrogen peroxide (Mankhetkorn *et al.* 1994). In addition, diethyldithiocarbamate can reduce the radicals generated during the oxidation of *o*-anisidine by HRP (Stiborová *et al.* 2002) back to the initial compound, *o*-anisidine.

It should be noted that some of the CYP inhibitors tested in this work had already been analyzed for their efficiencies to inhibit peroxidases. For example, ketoconazole has already been reported to inhibit lactoperoxidase with IC_{50} of 0.2 mM (Comby *et al.* 1994), while piperonylbutoxide has showed no effects on activity of another peroxidase, manganese peroxidase (Ning *et al.* 2010). Likewise, diethyldithiocarbamate has already been shown that it can inhibit myeloperoxidase (Izumi *et al.* 1994) and tobacco cell peroxidases (as a noncompetitive inhibitor) (Plewa *et al.* 1991). However, the exact mechanisms of inhibition of peroxidases by diethyldithiocarbamate have not been evaluated as yet. Inhibition constants of diethyldithiocarbamate for HRP have been determined in this work for the first time.

Besides the classical inhibitors of CYPs, we also examined the inhibition potential of propyl gallate and ellipticine to inhibit HRP activity. Both these chemicals were excellent inhibitors of *o*-anisidine oxidation catalyzed by HRP. Ellipticine is a known substrate of HRP (Stiborova *et al.* 2007), competing with *o*-anisidine for oxidation by HRP, thereby inhibiting this reaction. Indeed, it has already been shown that ellipticine is oxidized by HRP, lactoperoxidase and cyclooxygenase I to ellipticine dimer (Stiborova *et al.* 2007). Propyl gallate is known as a peroxidase inhibitor and also as a potent radical scavenger (Reddan *et al.* 2003; Tayama & Nakagawa 2001). This chemical might probably be a peroxidase substrate, but it was also suggested that it interacts directly with H_2O_2 and decomposes it (Reddan *et al.* 2003; Tayama & Nakagawa 2001). Peroxidase-catalyzed oxidation of 2,2'-azino-di-(3-ethyl-2,3-dihydrobenzthiazoline-6-sulfonate) was inhibited by propyl gallate, values of the inhibition constant equaled to 62 μM (Naumchik *et al.* 2005). Such results are in good agreement with our findings.

Taken together the results found with ellipticine, propyl gallate and diethyldithiocarbamate, it should be emphasized that they are potent peroxidase inhibitors. These compounds are either substrates of the enzyme, can decompose the second enzyme substrate, hydrogen peroxide, or might reduce the radical intermediates generated during oxidation of *o*-anisidine by HRP back to the initial compound. However, such inhibitions seem not to be specific only for this enzyme. Ellipticine is also a substrate and inhibitor of other enzymes metabolizing xenobiotics such as CYPs (Stiborova *et al.* 2007, 2011), propyl gallate is a general radical scavenger (Reddan *et al.* 2003) and diethyldithicarbamate also inhibits superoxidedismutase (Lushchak *et al.* 2005), CYPs and xanthine oxidase (Kober *et al.* 2003).

To conclude, the results of our study demonstrate that 1-aminobenzotriazole, a potent general CYP inhibitor (Emoto *et al.* 2003), does not influence the peroxidase activity measured with *o*-anisidine as a substrate. Therefore, 1-aminobenzotriazole seems to be the best candidate suitable for utilization in studies evaluating participation of CYP and peroxidase enzymes in metabolism of xenobiotics in various complex biological materials. Also disulfiram, metyrapone, quinidine, sulfaphenazole, and troleandomycine, can be utilized as selective inhibitors of individual CYP enzymes without risk that they can influence the peroxidase activity. However, precaution is necessary in the cases when proadifen SKF525A, piperonylbutoxide, diethyldithiocarbamate, ketoconazole, α-naphtoflavone and ellipticine would be used in such studies, since these chemicals can except of CYP enzymes inhibit also peroxidase-mediated reactions.

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Potential Conflicts of Interest: *None disclosed.*

REFERENCES

1 Aimova D and Stiborova M (2005). Antitumor drug ellipticine inhibits the activities of rat hepatic cytochromes P450. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. **149**: 437-440.
2 Bottu G (1989). The effect of quenchers on the chemiluminescence of luminol and lucigenin. J Biolumin Chemilumin. **3**: 59-65.
3 Bourrie M, Meunier V, Berger Y and Fabre G (1996). Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. J Pharmacol Exp Ther. **277**: 321-332.
4 Cobby J, Mayersohn M and Selliah S (1977). The rapid reduction of disulfiram in blood and plasma. J. Pharmacol. Exp. Ther. **202**: 724–731.
5 Comby F, Lagorce JF, Buxeraud J and Raby C (1994). Antithyroid action of ketoconazole: in-vitro studies and rat in-vivo studies. J Pharm Pharmacol. **46**: 50-53.
6 Emoto C, Murase S, Sawada Y, Jones BC and Iwasaki K (2003). In vitro inhibitory effect of 1-aminobenzotriazole on drug oxidations catalyzed by human cytochrome P450 enzymes: a comparison with SKF-525A and ketoconazole. Drug Metab Pharmacokinet. **18**: 287-295.

7 Gonzalez FJ and Gelboin HV (1992). Human cytochromes P450: evolution and cDNA-directed expression. Environ Health Perspect. **98**: 81–85.
8 Grintsevich EE, Senchuk VV, Puchkaev AV and Metelitza DI (2000). Propyl gallate inhibition of iodide and tetramethylbenzidine oxidation catalyzed by human thyroid peroxidase. Biochemistry. **65**: 924-32.
9 Guengerich FP and Shimada T (1991). Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol. **4**: 391–407.
10 Izumi Y, Ogino K, Murata T, Kobayashi H and Hobara T (1994). Effects of diethyldithiocarbamate on activating mechanisms of neutrophils. Pharmacol Toxicol. **74**: 280-286.
11 Kluwe WM and Hook JB (1981). Potentiation of acute chloroform nephrotoxicity by the glutathione depletor diethyl maleate and protection by the microsomal enzyme inhibitor piperonyl butoxide. Toxicol Appl Pharmacol. **59**: 457-466.
12 Kober T, Konig I, Weber M and Kojda G (2003). Diethyldithiocarbamate inhibits the catalytic activity of xanthine oxidase. FEBS Lett. **551**: 99-103.
13 Lushchak V, Semchyshyn H, Lushchak O and Mandryk S (2005). Diethyldithiocarbamate inhibits in vivo Cu,Zn-superoxide dismutase and perturbs free radical processes in the yeast Saccharomyces cerevisiae cells. Biochem Biophys Res Commun. **338**: 1739-1744.
14 Mankhetkorn S, Abedinzadeh Z and Houee-Levin C (1994). Antioxidant action of sodium diethyldithiocarbamate: reaction with hydrogen peroxide and superoxide radical. Free Radic Biol Med. **17**: 517-527.
15 Miksanova M, Hudecek J, Paca J and Stiborova M (2001). To the mechanism of peroxidase-mediated degradation of a recalcitrant dye Remazol Brilliant Blue R. Collect Czech Chem Commun. **66**: 663-675.
16 Naiman K, Frei E and Stiborova M (2010). Identification of rat cytochromes P450 metabolizing N-(2-methoxyphenyl)hydroxylamine, a human metabolite of the environmental pollutants and carcinogens o-anisidine and o-nitroanisole. Neuroendocrinol Lett. **31** (Suppl. 2): 36-45.
17 Naumchik IV, Karaseva EI and Metelitsa DI (2005). Inhibition of peroxidase-catalyzed oxidation of chromogenic substrates by propyl gallate and its polydisulfide. Prikl Biokhim Mikrobiol. **41**: 376-382.
18 Ning D, Wang H and Ding C, Lu H (2010). Novel evidence of cytochrome P450-catalyzed oxidation of phenanthrene in Phanerochaete chrysosporium under ligninolytic conditions. Biodegradation. **21**: 889-901.
19 Plewa MJ, Smith SR and Wagner ED (1991). Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. Mutat Res. **247**: 57-64.
20 Reddan JR, Giblin FJ, Sevilla M, Padgaonkar V, Dziedzic DC, Leverenz VR, et al. (2003). Propyl gallate is a superoxide dismutase mimic and protects cultured lens epithelial cells from H_2O_2 insult. Exp Eye Res. **76** :49-59.
21 Rendic S and DiCarlo FJ (1997). Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab Rev. **29**: 413–480.
22 Stiborova M, Miksanova M, Martinek V and Frei E (2000). Heme peroxidases: structure, function, mechanism and involvement in activation of carcinogens. a review. Collect Czech Chem Commun. **65**: 297-325.
23 Stiborova M, Miksanova M, Havlicek V, Schmeiser HH and Frei E (2002). Mechanism of peroxidase-mediated oxidation of carcinogenic o-anisidine and its binding to DNA. Mutat Res. **500**: 49-66.
24 Stiborova M, Sucha V, Miksanova M, Paca J Jr and Paca J (2003). Hydroxylation of phenol to catechol by Candida tropicalis: Involvement of cytochrome P450. Gen Physiol Biophys. **22**: 167-179.
25 Stiborova M, Poljakova J, Ryslava H, Dracinsky M, Eckschlager T and Frei E (2007). Mammalian peroxidases activate anticancer drug ellipticine to intermediates forming deoxyguanosine adducts in DNA identical to those found in vivo and generated from 12-hydroxyellipticine and 13-hydroxyellipticine. Int J Cancer. **120**: 243-251.
26 Stiborova M, Moserova M, Mrazova B, Kotrbova V and Frei E (2010). Role of cytochromes P450 and peroxidases in metabolism of the anticancer drug ellipticine: additional evidence of their contribution to ellipticine activation in rat liver, lung and kidney. Neuroendocrinol Lett. **31** (Suppl. 2): 26-35.
27 Stiborova M, Rupertova M and Frei E (2011). Cytochrome P450- and peroxidase-mediated oxidation of anticancer alkaloid ellipticine dictates its anti-tumor efficiency. Biochim Biophys Acta. **1814**: 175-185.
28 Tayama S and Nakagawa Y (2001). Cytogenetic effects of propyl gallate in CHO-K1 cells. Mut Res. **498**: 117–127.
29 Yang CS and Strickhart FS (1974). Inhibition of hepatic mixed function oxidase activity by propyl gallate. Biochem Pharmacol. **23**: 3129-3138.

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