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# A COMPARISON OF 2-PHENYL-2-(1-PIPERIDINYL)PROPANE (PPP), THIOTEPA CLOPIDOGREL, AND TICLOPIDINE AS SELECTIVE INACTIVATORS OF HUMAN CYTOCHROME P450 2B6

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PPP, 2-phenyl-2-(1-piperidinyl)propane; thioTEPA, 1,1',1"-phosphinothioylidynetris-aziridine,

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## ABSTRACT

The use of selective chemical inhibitors of human cytochrome P450 enzymes represents a powerful method by which the relative contributions of various human P450 enzymes to the metabolism of drugs can be determined. However, the identification of CYP2B6 in the metabolism of drugs has been more challenging due to the lack of a well-established inhibitor of this enzyme. In this report, we describe the selectivity of 2-phenyl-2-(1-piperidinyl)propane (PPP) as an inactivator of CYP2B6, and compare this selectivity vs other CYP2B6 inactivators: thioTEPA, clopidogrel, and ticlopidine. Values of $K_I$ and $k_{inact}$ for PPP were 5.6 μM and 0.13/min for bupropion hydroxylase catalyzed by pooled human liver microsomes, and values for thioTEPA were similar (4.8 μM and 0.20/min, respectively). Intrinsic inactivation capability was considerably greater for clopidogrel due to a greater $k_{inact}$ value (1.9/min). Ticlopidine was potent with $K_I$ and $k_{inact}$ values of 0.32 μM and 0.43/min, respectively. The selectivity of these four agents for CYP2B6 was determined by testing their effects on other human P450 enzyme activities using conditions that yield ~90% inactivation of CYP2B6 activity. The results showed that preincubation of human liver microsomes with PPP at 30 μM for 30 min provided more selective inhibition for CYP2B6 than thioTEPA, clopidogrel, and ticlopidine. Furthermore, the use of clopidogrel is complicated by the observation that this agent is not stable in the presence of human liver microsomes, even without addition of NADPH. Therefore, PPP can serve as a selective chemical inactivator of CYP2B6 and be used to define the role of CYP2B6 in the metabolism of drugs.

## INTRODUCTION

Cytochrome P450 enzymes are the most important enzymes in drug metabolism and responsible for clearance of a majority of drugs. Five of these enzymes, CYP1A2, 2C9, 2C19, 2D6, and 3A4, have been considered as important drug metabolizing P450 enzymes in humans, and it has been proposed that these enzymes should be routinely examined as potential contributors to the metabolism of new drugs using in vitro approaches (Bjornsson, et al, 2003). Well-characterized, selective chemical inhibitors of these five enzymes have been described and applied throughout the past several years in examinations of the metabolism of drugs and other xenobiotics (Madan, et al., 2002). The most frequently used chemical inhibitors are furafylline (CYP1A2 inactivator), sulfaphenazole (CYP2C9 reversible inhibitor), S-mephenytoin (CYP2C19 competitive substrate), quinidine (CYP2D6 reversible inhibitor), and ketoconazole (CYP3A reversible inhibitor), although there are others that have also been successfully applied.

However, recently, some other human P450 enzymes have been demonstrated to play a major role in the metabolism of some drugs. Of the human P450 enzymes, CYP2B6 has recently emerged as one of increasing importance. CYP2B6 has been shown to be involved in the metabolism of several drugs including efavirenz, bupropion, cyclophosphamide, ifosphamide, and sertraline (Chang, et al., 1993; Roy, et al., 1999; Granvil, et al., 1999; Kobayashi, et al., 1999; Faucette, et al., 2000; Hesse, et al., 2000; Huang, et al., 2000; Rae, et al, 2002; Ward, et al., 2003; Turpienen, et al., 2005; Obach, et al., 2005). However, robust, well-characterized inhibitors of CYP2B6 that are selective for this enzyme vs other human P450 enzymes have not been thoroughly evaluated. An early report on CYP2B6 described orphenadrine as a potential CYP2B6

inhibitor, however the selectivity is not high (Guo, et al, 1997). More recent work has described potent mechanism-based inactivation of CYP2B6 by clopidogrel (Richter, et al., 2004), ticlopidine (Richter, et al., 2004; Turpeinen, et al., 2004), and thioTEPA (Turpeinen, et al., 2004; Harleton, et al., 2004; Richter, et al., 2005). In studies designed to gain a better understanding of the biochemistry of CYP2B6, Hollenberg and coworkers identified an analogue of phencyclidine, 2-phenyl-2-(1-piperidinyl)propane (PPP; Figure 1), as a mechanism-based inactivator of human CYP2B6 (Chun, et al., 2000).

The potential for PPP to be used as a tool in identifying a role for CYP2B6 in the metabolism of drugs has not been explored. This requires an examination of the potential for PPP to not affect other human P450 enzymes at inhibitor concentrations that would provide 90% or more inhibition of CYP2B6. In this report, the selectivity of PPP, thioTEPA, clopidogrel, and ticlopidine as CYP2B6 inactivators has been examined in an attempt to determine which, if any, would be a superior CYP2B6 reaction phenotyping tool and to define experimental conditions under which these agents could be used in cytochrome P450 reaction phenotyping experiments.

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## MATERIALS AND METHODS

Materials. CYP substrates, metabolite standards, and internal standards were obtained as previously described (Walsky and Obach, 2004). Ticlopidine, and thioTEPA were obtained from Sigma Chemical Co. (St Louis, MO). Clopidogrel was obtained from Sequoia Research Products (Oxford, UK). PPP was synthesized under contract by Syncom BV (Groningen, Netherlands). Human liver microsomes pooled from 53 donors were obtained under contract from Gentest, Inc. (Woburn, MA).

Reversible Inhibition of CYP2B6. Specific aspects of the incubation conditions (e.g. protein concentration, incubation time, reaction termination solvent, etc) were previously reported (Walsky and Obach, 2004). The Michaelis constant for bupropion hydroxylase was 82 ± 1 μM for pooled liver microsomes. In general, human liver microsomes (0.05 mg/ml) were mixed with buffer (100 mM $KH_2PO_4$, pH 7.4), $MgCl_2$ (3.3 mM), and substrate (at $K_M$), then warmed to 37°C in a 96-well temperature controlled heater block. Aliquots of this mixture (0.18 ml) were delivered to each well of a 96-well polypropylene PCR plate maintained at 37°C followed by addition of the inhibitor or control solvent (mixture of water and $CH_3CN$) as applicable. The inhibitors, standards and QCs were prepared as 100X stock solutions and 2 μl aliquots dispensed to the appropriate incubations maintaining solvent concentrations at 1% (v/v) or less. Incubations were commenced with the addition of NADPH stock (assay concentration = 1.3 mM) to a final incubation volume of 0.2 ml and maintained at 37°C for 10 min. Incubations were terminated by acidification upon addition of 0.02 ml termination solvent ($H_2O/CH_3CN$/HCOOH; 92:5:3) containing [$^2H_6$]hydroxybupropion as an internal

standard, followed by filtration through a 45 μ filter and analysis by HPLC-MS/MS as previously described (Walsky and Obach, 2004).

Reversible Inhibition of Other Human Cytochrome P450 Enzymes. Reversible inhibition assays for CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A were conducted according to described methods (Walsky and Obach, 2004). Substrate concentrations used were equal to $K_M$ for each reaction.

Time Dependent Inhibition ($K_I/k_{inact}$) CYP2B6 Assay. Specific aspects of the $K_I/k_{inact}$ methodology employed have been previously reported (Obach et al., 2007). Preincubations consisted of the following conditions: pooled human liver microsomes (0.5 mg/ml), $MgCl_2$ (3.3 mM), in 100 mM potassium phosphate buffer, pH 7.4. Microsomes, buffer, and inhibitor were mixed, prewarmed at 37°C for 5 min followed by initiation of inactivation by addition of NADPH (1.3 mM). After preincubation periods, aliquots of 20 μl were removed and added to a mixture of bupropion (800 μM, 10X $K_M$) and NADPH (1.3 mM), in 100 mM potassium phosphate buffer, pH 7.4, followed by incubation at 37°C for 12 min. Incubations were terminated by acidification upon addition of 0.02 ml termination solvent ($H_2O/CH_3CN/HCOOH$; 92:5:3) containing [$^2H_6$]hydroxybupropion as an internal standard followed by filtration and analysis by LC/MS/MS.

Time Dependent Inhibition ($K_I/k_{inact}$) CYP2B6 Assay (Clopidogrel). To reduce an observed instability of clopidogrel in the presence of human liver microsomes, a modified experimental procedure was followed. Preincubations consisted of the following conditions: $MgCl_2$ (3.3 mM), NADPH (1.3 mM) in 100 mM potassium phosphate buffer, pH 7.4. Buffer and inhibitor were mixed and prewarmed at 37°C,

preincubations were initiated by the addition of pooled human liver microsomes (0.5 mg/ml). After preincubation periods, aliquots of 20 µl were removed and added to a mixture of bupropion (800 µM, 10X $K_M$) and NADPH (1.3 mM), in 100 mM potassium phosphate buffer, pH 7.4, followed by incubation at 37°C for 12 min. Incubations were terminated by acidification upon addition of 0.02 ml termination solvent ($H_2O/CH_3CN/HCOOH$; 92:5:3) containing [$^2H_6$]hydroxybupropion as an internal standard followed by filtration and analysis by LC/MS/MS.

Determination of Selectivity for CYP2B6 Inactivation and Effect of Protein Concentration. After determination of inactivation kinetic parameters for CYP2B6, inactivators were tested for selectivity among other CYP enzymes using a concentration and incubation time that yielded 90% inactivation of CYP2B6. PPP and thioTEPA (30 µM; 30 min), ticlopidine and clopidogrel (3 µM; 10 min), were incubated with NADPH and human liver microsomes at protein concentrations that were 10-fold greater than those required for the specific P450 assays. After this inactivation incubation, the mixtures were diluted 10-fold into the activity assays for CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A according to described methods (Walsky and Obach, 2004). Substrate concentrations used were equal to $K_M$ for each reaction.

For CYP2B6 inactivation, the effect of microsomal protein concentration in the inactivation incubation was tested. PPP (30 µM), thioTEPA (30 µM), ticlopidine (3 µM), and clopidogrel (3 µM) were incubated with liver microsomes (0.5 to 10 mg/ml) for 10 min (ticlopidine and clopidogrel) or 30 min (PPP and thioTEPA). After this, an aliquot of the mixture was withdrawn and diluted 10-fold into the CYP2B6 activity incubation as described above. Additionally, because we had observed clopidogrel to be unstable when

in the presence of liver microsomes even in the absence of NADPH, this compound was also tested as above, following a 20 min incubation with liver microsomes.

Data Analysis. Standard curve fitting was accomplished with QuanLynx (v4.1) software (Micromass, Beverly, MA). Data were fit to quadratic curves using $1/x^2$ weighting. Assay run acceptance was defined by the accuracy and precision of independently prepared quality control samples at three concentrations. Substrate saturation curves and inhibition data were analyzed using the Enzyme Kinetics module of SigmaPlot v8.0 (SPSS, Chicago, IL). Best fit models were selected on the basis of the Aikake Information Criterion.

Data for $IC_{50}$ determinations were fit to the following equation:

$$\%\,\text{Control Activity} = 100 \bullet \left( A - \left( \frac{B \bullet I}{I + IC_{50}} \right) \right)$$

in which I is the inhibitor concentration, the $IC_{50}$ represents the inflection point, and the value of 1-(A-B) is the maximum percentage inhibition observed at an infinite inhibitor concentration. Determination of inactivation kinetic parameters were determined using non-linear regression of the data to the formula:

$$k_{inact,obs} = k_{inact,obs,[I]=0} + \frac{k_{inact} \bullet [I]}{K_I + [I]}$$

in which [I] refers to the concentration of inactivator in the inactivation preincubation, $k_{inact,obs}$ is the negative value of the slope of the natural logarithm of the percent activity remaining vs inactivation incubation time at various [I], $k_{inact,obs,[I]=0}$ is the apparent inactivation rate constant measured in the absence of inactivator, $k_{inact}$ is the theoretical maximum inactivation rate constant at $[I]=\infty$, and $K_I$ is the inactivator concentration yielding $k_{inact,obs}$ at the sum of $k_{inact,obs,[I]=0}$ and 0.5 times $k_{inact}$.

## RESULTS

Determination of Reversible Inhibition. PPP, thioTEPA, clopidogrel, and ticlopidine were first tested for reversible inhibition of cytochrome P450 activities in pooled human liver microsomes. For PPP, the $IC_{50}$ for CYP2B6 bupropion hydroxylase activity was 5.1 μM, while the next most potently inhibited CYP activity was CYP2D6 ($IC_{50}$ = 74 μM) yielding a 15-fold split (Table 1). Other CYP activities were not significantly inhibited at PPP concentrations as high as 300 μM. Inhibition data are shown in Figure 2. Clopidogrel most potently inhibited CYP2B6 with an $IC_{50}$ of 0.046 μM and a selectivity of 86X (to CYP2C19). ThioTEPA yielded comparable data to PPP for CYP2B6 inhibition and the next most potently inhibited activity was CYP3A catalyzed testosterone 6β-hydroxylase activity (12X fold-split). Ticlopidine showed potent inhibition of CYP2B6 ($IC_{50}$ = 0.21 μM) however it also potently inhibited CYP2C19 activity ($IC_{50}$ = 0.85 μM).

Kinetics of Mechanism-Based Inactivation. Since these compounds have been previously shown to be mechanism-based inactivators of CYP2B6 (Chun et al., 2000; Richter, et al., 2004, 2005), the potential for them to inactivate human P450 enzymes was examined. Inactivation of CYP2B6 was observed when these compounds were incubated with human liver microsomes in the presence of NADPH prior to the addition of bupropion as the substrate. This increase in inhibition was not observed when NADPH was not included, consistent with mechanism-based inactivation. Inactivation experiments were done to yield values for $K_I$ and $k_{inact}$ for CYP2B6 (Figure 3). Clopidogrel was the inactivator with the greatest intrinsic activity ($k_{inact}/K_I$ = 1400

ml/min/μmol) followed by ticlopidine ($k_{inact}/K_I$ = 1300 ml/min/μmol), thioTEPA ($k_{inact}/K_I$ = 42 ml/min/μmol), and PPP ($k_{inact}/K_I$ = 23 ml/min/μmol). The inactivation data for clopidogrel appeared biphasic (data not shown) and only the initial rapid inactivation phase data points occurring at less than 1.5 min were utilized. The reason for this is not known, but one potential explanation is offered (see below).

Determination of P450 Selectivity. In order to determine which of these four compounds would be suitable probe inhibitors for P450 reaction phenotyping protocols, they were tested for their effects on multiple human P450 activities under conditions that yield a ≥90% reduction in CYP2B6 activity. Results are shown in Figure 4. Preincubation of human liver microsomes with 30 μM PPP in the presence of NADPH for 30 min followed by 10X dilution into marker substrate incubations yields 90% inhibition of CYP2B6, while other P450 activities were not inhibited (inhibition of ≤ 10%) (Figure 4), demonstrating that this concentration of PPP would be adequately selective for assessing the role of CYP2B6 in human liver microsomal metabolism of substrates. With a 30 min preincubation, 30 μM thioTEPA demonstrated inactivation of other CYP enzymes besides CYP2B6 (consistent with previous observation in this laboratory; Obach, et al., 2007). Specifically, the extent of inactivation of CYP3A and CYP2A6 by thioTEPA under conditions that provide 90% inactivation of CYP2B6 would not permit its use as a selective reaction phenotyping probe. Despite the apparent lack of selectivity of ticlopidine for CYP2B6 under reversible inhibition conditions (see above), when this agent is preincubated with human liver microsomes for 10 min at 3 μM followed by a 10-fold dilution prior to assessment of P450 activities, >90% inactivation of CYP2B6 is obtained with little effect on the other P450 enzymes. When tested as an inactivator (at 3

μM for 10 min), clopidogrel demonstrated selectivity for CYP2B6. However, clopidogrel possesses other challenges with its use as a reaction phenotyping probe, as discussed below.

Cytochrome P450 reaction phenotyping for various compounds can require different protein concentrations in the incubations in order to provide adequate activity for the reaction under examination. Thus, it must be known if the potency of selective P450 probe inhibitors can be affected by different protein concentrations, which would require adjustment of the concentration of the inhibitor. Preincubation of PPP, thioTEPA, clopidogrel, and ticlopidine in the presence of NADPH with human liver microsomal concentrations ranging from 0.5 to 10 mg/ml for 10 min (clopidogrel and ticlopidine) or 30 min (PPP and thioTEPA), demonstrated that CYP2B6 inactivation is reduced as the protein concentration increases (Figure 5). The effect was substantial for clopidogrel. It was noted that when clopidogrel was mixed with liver microsomes for 20 min prior to the addition of NADPH, inactivation was not observed. Incubation of clopidogrel with pooled human liver microsomes at 0.5 mg/ml at 37$^{o}$C in the absence of NADPH led to a marked decline in clopidogrel concentrations such that less than 10% remained after 15 min (Figure 5B). This is consistent with the previous observation of hydrolysis of this compound in liver microsomes (Tang, et al, 2006). Thus, the use of this agent as a CYP2B6 reaction phenotyping tool is burdened with the added complexity of instability of the reagent during incubation.

## DISCUSSION

Cytochrome P450 reaction phenotyping has become a commonplace exercise in drug development and discovery, and the practice has evolved to such an extent that such data are expected in the registration dossiers for new drugs that are metabolized by P450 enzymes. The information is included in drug package insert data used to make clinical dosing decisions to avoid/minimize drug-drug interactions. Thus it is of utmost importance that the tools used in these studies are adequately selective so as to avoid erroneous conclusions regarding the contribution of individual P450 enzymes to drug clearance. Three approaches have been commonly applied in P450 reaction phenotyping: (a) the use of selective chemical inhibitors or inhibitory antibodies of the metabolism of the drug in human liver microsomes, (b) determination of the metabolism of the drug in heterologously expressed recombinant human P450 enzyme systems, and (c) correlation of the metabolism of the drug in a panel of liver microsome samples from individual donors to well-established marker activities for individual P450 enzymes. The relative merits of each of these approaches have been described in detail in a consensus white paper (Bjornsson, et al., 2003) and will not be reiterated here, but of note in this consensus is the emphasis of the use of inhibition data (using selective chemical inhibitors or inhibitory antibodies) as the approach that is most reliable and always recommended. In order to successfully accomplish P450 reaction phenotyping through the use of chemical inhibitors, it is imperative that these inhibitors be suitably selective for individual P450 enzymes. Selectivity has been well established for inhibitors for several of the P450 enzymes that have been the focus of investigation for years.

However, for P450 enzymes that have not received as much attention until recently (such as CYP2B6), selective inhibition tools are not as well established.

In this study, the selectivity of four compounds as potential reaction phenotyping tools for human CYP2B6 has been compared. The four compounds chosen, PPP, thioTEPA, ticlopidine, and clopidogrel, were selected for the present study because each had been recently described as a mechanism-based inactivator of CYP2B6 (Richter, et al., 2004, 2005; Chun, et al., 2000). The use of mechanism-based inactivators as P450 reaction phenotyping tools offers the potential advantages of being able to dilute the inactivator considerably when measuring the metabolism of the compound of interest as well as permitting the option of preparing pools of inactivated microsomes that can be well characterized and stored frozen for use at a later date.

Under a reversible inhibition experimental protocol, PPP, thioTEPA, and clopidogrel appeared to possess the greatest selectivity for CYP2B6, although the potency toward the next most potently inhibited enzyme for PPP and thioTEPA (CYP2D6 and CYP3A, respectively) was great enough to make the selectivity marginal. Great care would be needed to use PPP and thioTEPA in this manner for CYP2B6 reaction phenotyping to avoid 'spillover' into the next most potently inhibited enzyme. Ticlopidine did not offer adequate selectivity for CYP2B6 when tested in a reversible inhibition protocol; concentrations required to effectively inhibit CYP2B6 would also partially inhibit CYP2C19 and CYP2D6. Under a reversible inhibition protocol, clopidogrel appeared to possess adequate selectivity for CYP2B6, however this agent was observed to be considerably unstable upon incubation with human liver microsomes which would confound efforts to define its potency.

Under an experimental protocol in which these agents were used as irreversible inactivators of CYP2B6, followed by determination of selectivity for CYP2B6, PPP and ticlopidine yielded improved selectivity while the selectivity of thioTEPA was diminished to such an extent to render it inadequate for P450 reaction phenotyping. PPP appeared to be slightly more selective than ticlopidine and clopidogrel, despite these latter two compounds possessing greater intrinsic inactivation capability for CYP2B6. Additionally, the potency of these inactivators can be altered if the microsomal protein concentration during the inactivation incubation is elevated. Decreases in inhibitory potency with increasing microsome concentration have been observed for other agents (e.g. fluoxetine for CYP2D6 or montelukast for CYP2C8; Margolis and Obach, 2003; Walsky, et al., 2005), and this has been attributed to increased non-specific binding of the inhibitor to phospholipid membranes effectively decreasing the concentration available to the enzyme. Whether this is responsible for the effect of protein concentration on PPP and ticlopidine has not been determined. It was observed that incubation of clopidogrel with liver microsomes led to a marked decline in concentration of the inactivator. This occurred even in the absence of NADPH, indicating that this instability is not due to P450 catalyzed metabolism. Clopidogrel possesses a methyl ester moiety, which has been shown to be subject to enzymatic hydrolysis in liver microsomes (Tang, et al., 2006), and it is possible that the resulting carboxylic acid does not inactivate CYP2B6. Additionally, other kinetic anomalies were observed for clopidogrel. Inactivation kinetics were particularly difficult to obtain as inactivation showed a biphasic rate over time with an extremely rapid initial phase lasting less than 1 min, followed by a considerably decreased inactivation rate. A plot of the apparent inactivation rate constant

vs. inactivator concentration that included clopidogrel concentrations in excess of 1.1 μM, showed a decrease in inactivation. Clearly, further mechanistic characterization of the inactivation of CYP2B6 by clopidogrel is needed to attempt to understand these observations. Nevertheless, this kinetic behavior, whatever the reason, makes the use of clopidogrel as a CYP2B6 inactivator for reaction phenotyping less appealing because slight changes in experimental conditions would confound obtaining reproducible and reliable results.

It is important to note that this investigation describes the use of these agents as in vitro tools only; use as probe inhibitors in vivo requires other experimentation. Because of its inherent toxicity as an anticancer agent, thioTEPA would be inappropriate for use as a CYP2B6 inhibitor in healthy clinical study subjects, and additionally, the in vitro data suggest that it could also have problems with a lack of selectivity. PPP, while shown to be useful for in vitro reaction phenotyping studies, cannot be used in clinical studies at this time because it is not a substance approved for administration to humans. Clopidogrel and ticlopidine are clinical agents used to prevent the formation of blood clots and can therefore, with some care, be used in clinical pharmacokinetic drug-drug interaction studies in healthy human study subjects. In fact, both of these agents have been shown to alter the metabolism of bupropion in vivo (Turpienen, et al., 2005), albeit the magnitude of the interaction was not great because CYP2B6 catalyzed hydroxylation only partially contributes to the total metabolic clearance of bupropion (Welch, et al., 1987). As the number of drugs shown to have a considerable portion of their clearance catalyzed by CYP2B6 (e.g. efavirenz), or their pharmacological activity dependent on

CYP2B6 catalyzed metabolism (e.g. cyclophosphamide) increases, more clinical experimentation into the potential for drug interactions based on CYP2B6 will be needed.

In conclusion, the utility of PPP as a CYP2B6 selective inactivator that can be useful in P450 reaction phenotyping studies has been shown. Conditions under which this agent is selective for this enzyme have been described. A comparison has been made for PPP, clopidogrel, ticlopidine, and thioTEPA, and the conclusion is that PPP can serve as a selective probe inhibitor of CYP2B6 in human liver microsomes under either irreversible or reversible inhibition protocols, ticlopidine and clopidogrel could be used as a selective CYP2B6 inactivator when great care is used in study design aspects to ensure selectivity and stability, and that thioTEPA, while an inactivator of CYP2B6, lacks adequate enzyme selectivity for routine use.

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FIGURE LEGENDS

Figure 1. Structures the inactivators examined in this report.

Figure 2. $IC_{50}$ curves for inhibition of human cytochrome P450 activities by PPP (panel A), thioTEPA (panel B), clopidogrel (panel C), and ticlopidine (panel D). Symbols are: CYP1A2 (●), CYP2A6 (■), CYP2B6 (◆), CYP2C8 (⬣), CYP2C9 (▲), CYP2C19 (▼), CYP2D6 (○), CYP2E1 (◘), CYP3A4 midazolam 1'-hydroxylase (◇) and testosterone 6β-hydroxylase (△) activities.

Figure 3. Relationship between inactivator concentration and apparent inactivation rate of CYP2B6 for PPP (panel A), thioTEPA (panel B), clopidogrel (panel C), and ticlopidine (panel D). Note in panel C the points at [clopidogrel] > 2 μM were not included in the determination of $k_{inact}/K_I$.

Figure 4. Comparison of the effects of PPP (panel A), thioTEPA (panel B), clopidogrel (panel C), and ticlopidine (panel D) on CYP2B6 and other human P450 activities. Concentrations and preincubation times used were: PPP: 30 μM and 30 min, thioTEPA: 30 μM and 30 min, clopidogrel: 3 μM and 10 min, ticlopidine: 3 μM and 10 min. The designation CYP3A(M) and CYP3A(T) refers to CYP3A catalyzed midazolam 1'-hydroxylase and testosterone 6β-hydroxylase activities, respectively.

Figure 5. Relationship between the amount of inactivation of CYP2B6 by PPP, thioTEPA, ticlopidine, and clopidogrel and the concentration of human liver microsomal protein in the inactivation incubation (A) and stability of clopidogrel (3 μM) in human liver microsomes (0.5 mg/ml) in the absence of NADPH (B). Concentrations and preincubation times used were: PPP at 30 μM and 30 min (●), thioTEPA at 30 μM and 30 min (○), ticlopidine at 3 μM and 10 min, (■), clopidogrel at 3 μM and 10 min (▼),

and clopidogrel at 3 μM and 10 min following incubation for 20 min in microsomes without NADPH (△). Binding to liver microsomes in the absence of NADPH was determined at a protein concentration of 5.0 mg/ml. Percent bound values were 22, 97, and 97 for PPP, ticlopidine, and clopidogrel, respectively. Binding for thioTEPA was negligible.

Table 1. Reversible Inhibitory $IC_{50}$ (+/- SE) Values for PPP, ThioTEPA, Clopidogrel, and Ticlopidine for Several Human Cytochrome P450 Activities in Pooled Human Liver Microsomes.

| | | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| Enzyme | Activity | PPP | ThioTEPA | Clopidogrel | Ticlopidine |
| CYP1A2 | Phenacetin O-Deethylase | >300 | >300 | 4.4 ± 0.1 | 12 ± 2 |
| CYP2A6 | Coumarin 7-Hydroxylase | >300 | 240 ± 30 | >300 | >300 |
| CYP2B6 | Bupropion Hydroxylase | 5.1 ± 0.1 | 8.3 ± 1.3 | 0.046 ± 0.01 | 0.21 ± 0.02 |
| CYP2C8 | Amodiaquine N-Deethylase | >300 | >300 | 18 ± 2 | 100 ± 30 |
| CYP2C9 | Diclofenac 4'-Hydroxylase | >300 | >300 | 21 ± 4 | 51 ± 19 |
| CYP2C19 | S-Mephenytoin 4'-Hydroxylase | >300 | >300 | 3.7 ± 0.5 | 0.85 ± 0.09 |
| CYP2D6 | Dextromethorphan O-Demethylase | 74 ± 16 | >300 | 200 ± 30 | 3.3 ± 0.2 |
| CYP2E1 | Chlorzoxazone 6-Hydroxylase | >300 | >300 | >300 | >300 |
| CYP3A | Felodipine Oxidase | >300 | >300 | 66 ± 13 | >300 |
| CYP3A | Midazolam 1'-Hydroxylase | >300 | 230 ± 60 | 34 ± 2 | >300 |
| CYP3A | Testosterone 6β-Hydroxylase | >300 | 100 ± 40 | 23 ± 5 | 25 ± 4 |
| Selectivity for CYP2B6 | | 15 | 12 | 86 | 4 |

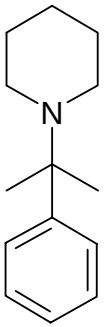
Table 2. Inactivation Kinetic Constants for PPP, ThioTEPA, Clopidogrel, and Ticlopidine for CYP2B6 Catalyzed Bupropion Hydroxylase in Pooled Human Liver Microsomes.[a]

| Parameter | PPP | ThioTEPA | Clopidogrel[b] | Ticlopidine |
|---|---|---|---|---|
| $K_I$ (μM) | 5.6 ±1.2 | 4.8 ±1.0 | 1.4 ±0.1 | 0.32 ±0.04 |
| $k_{inact}$ ($min^{-1}$) | 0.13 ±0.01 | 0.20 ±0.01 | 1.9 ±0.1 | 0.43 ±0.02 |
| $k_{inact}/K_I$ (ml/min/μmol) | 23 | 42 | 1400 | 1300 |

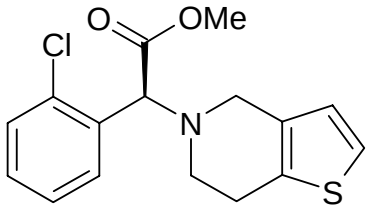
[a]Values represent the mean +/- SE.

[b]Kinetic parameters for clopidogrel were estimated using data obtained at [clopidogrel] < 2 μM, excluding the decrease in $k_{inact,app}$ observed at high concentrations, and should be considered as estimates.

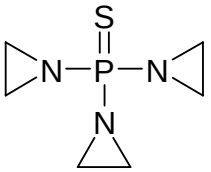
Figure 1



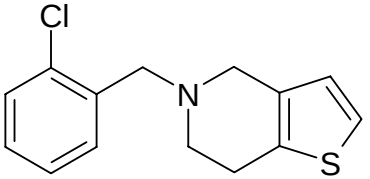

PPP
(2-phenyl-2-(1-piperdinyl)propane)




Clopidogrel




ThioTEPA
(triethylenethiophosphoramide)




Ticlopidine

Figure 2

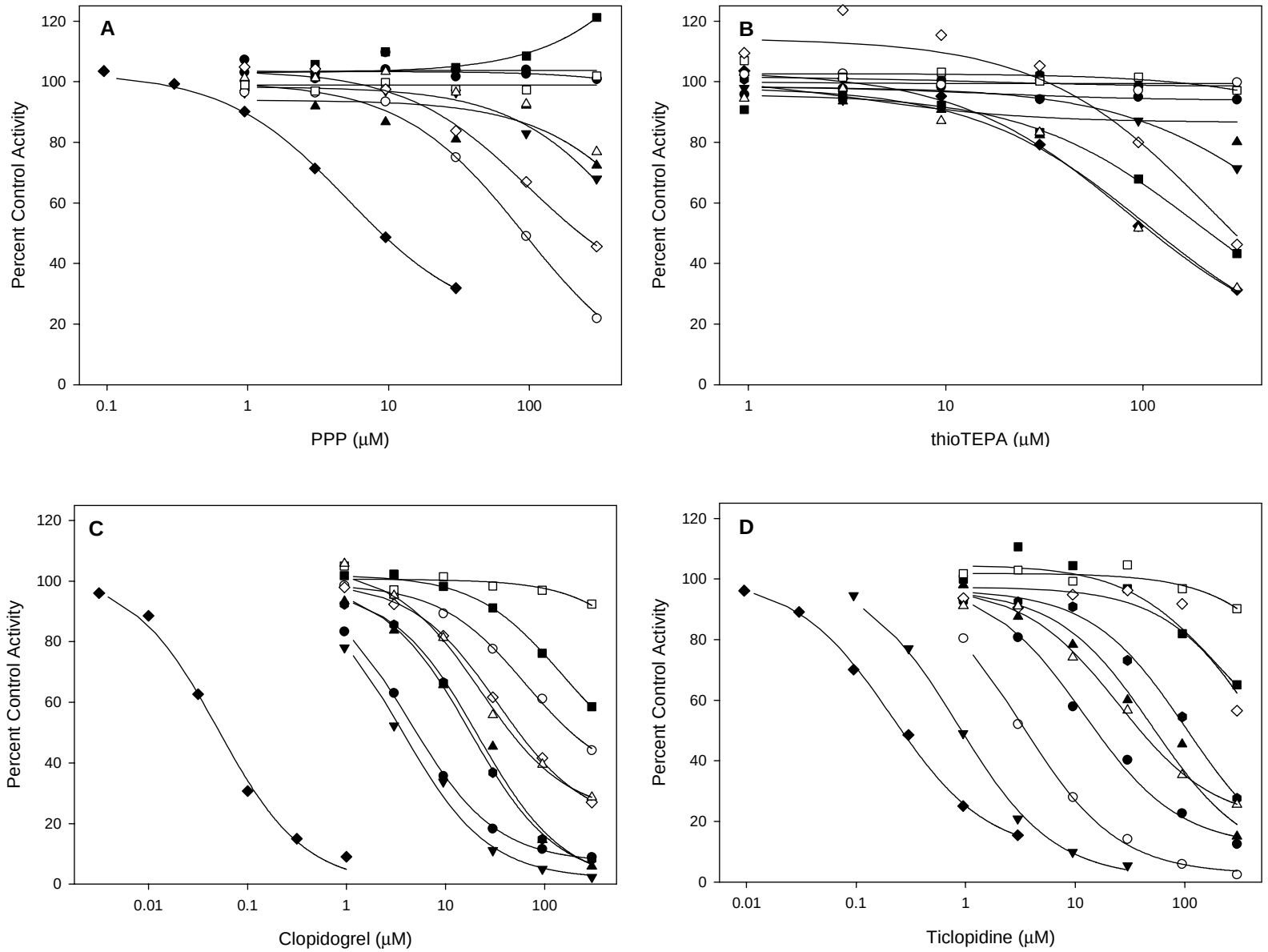
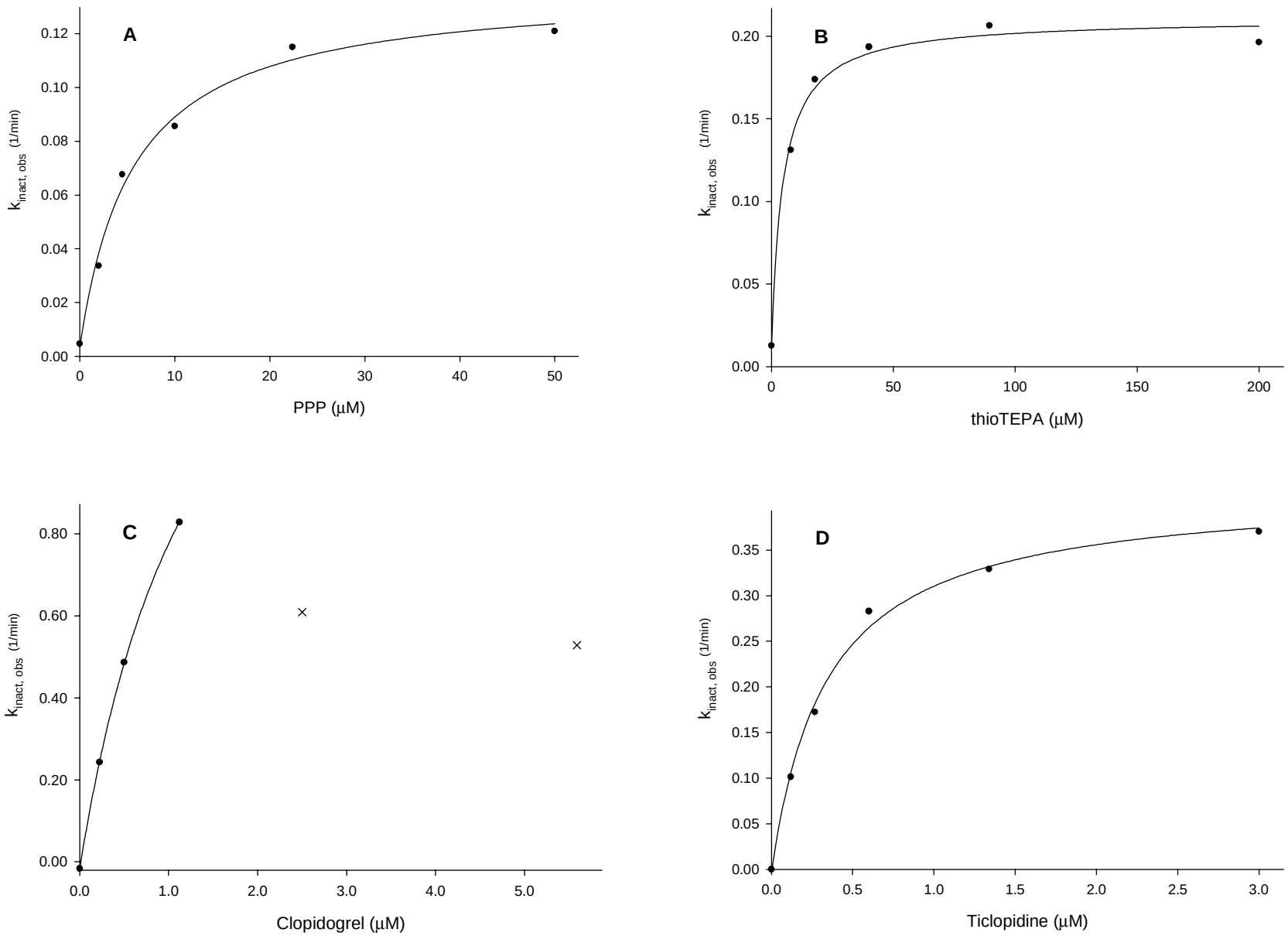


Figure 3



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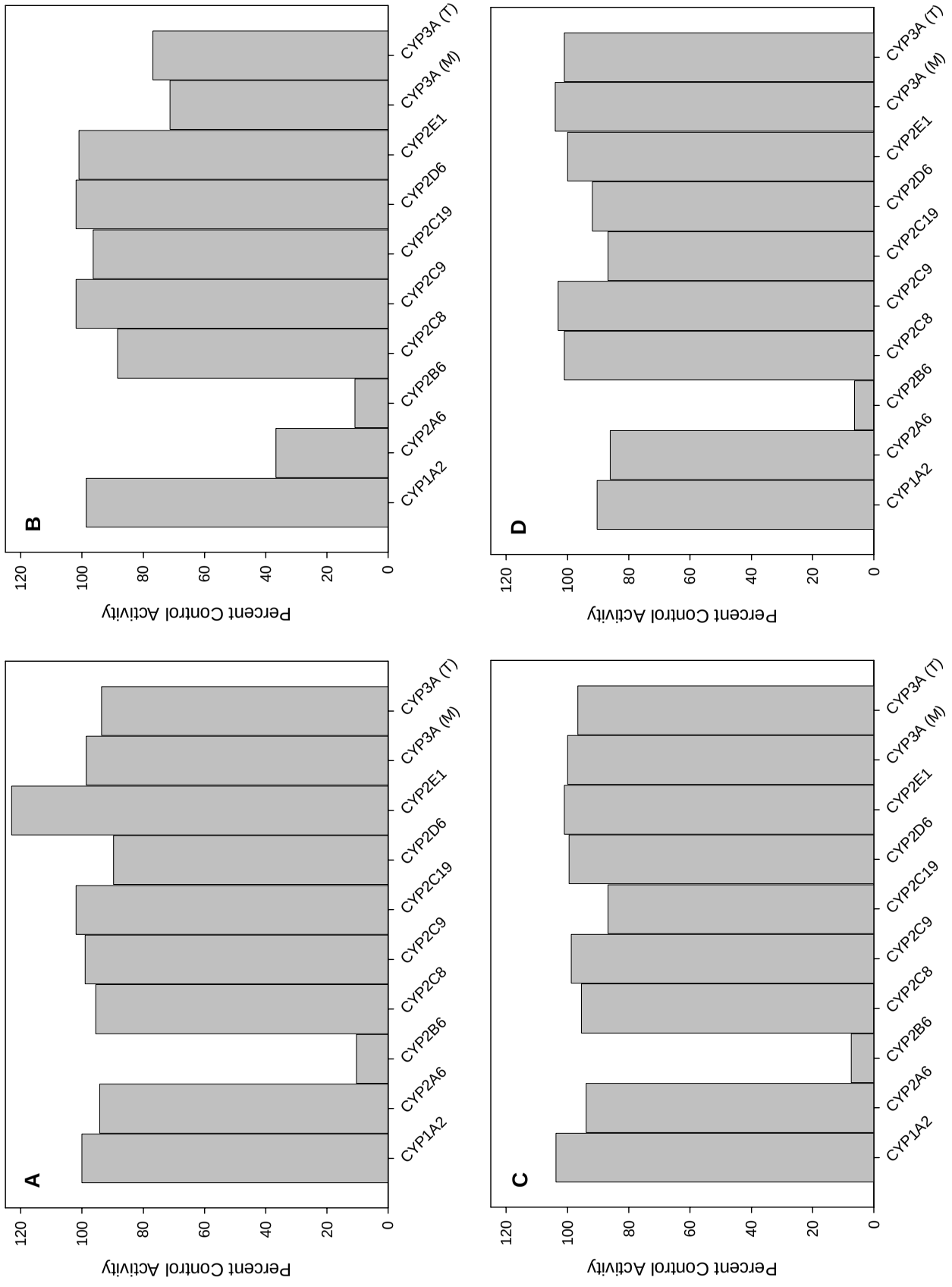


Figure 4

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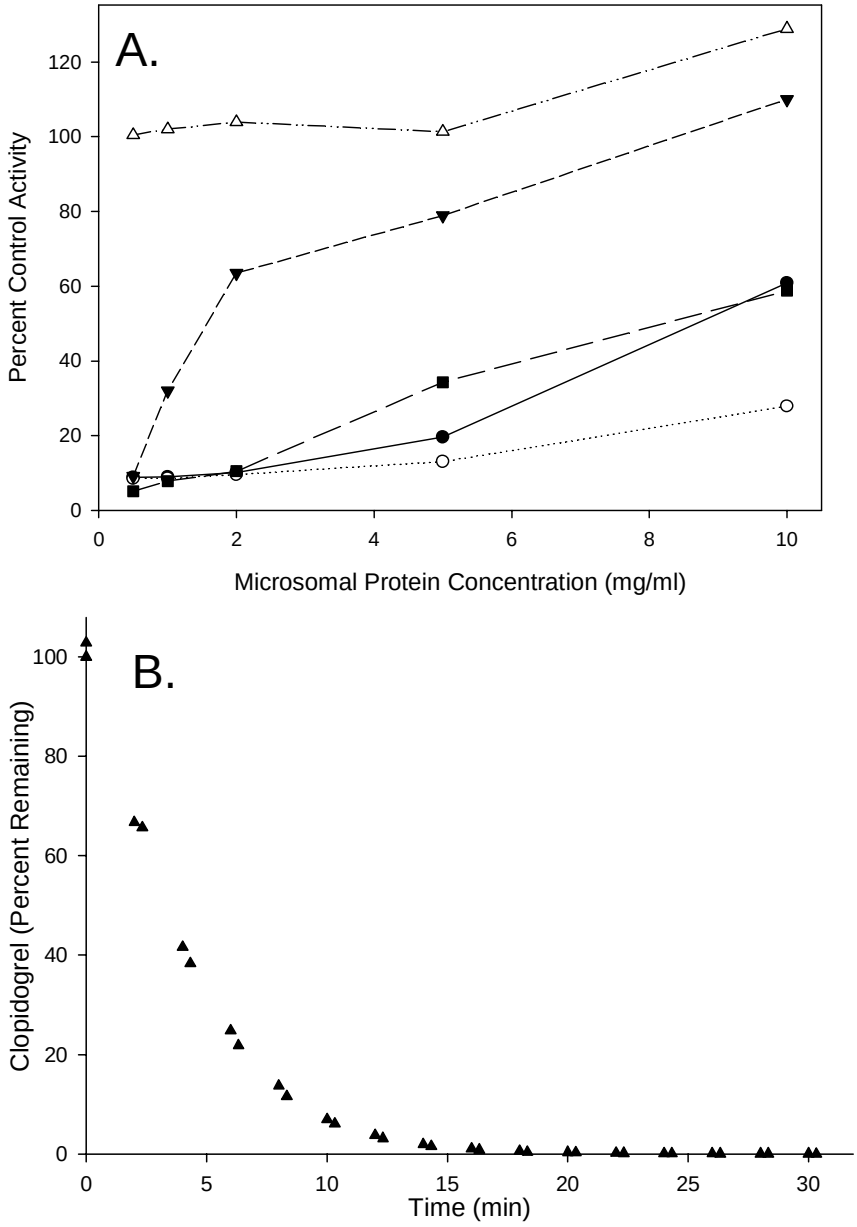


Figure 5