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**EFFECT OF CYTOCHROMES P450 CHEMICAL INHIBITORS AND
MONOCLONAL ANTIBODIES ON HUMAN LIVER MICROSOMAL
ESTERASE ACTIVITY**

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Running Title: CYP selective chemical inhibitors and monoclonal antibodies of human
esterase activity

Number of text pages: **26**

Number of Tables: **2**

Number of Figures: **3**

Number of References: **45**

Number of words in Abstract: **250**

Number of words in Introduction: **528**

Number of words in Discussion: **1213**

Abbreviations: CYP, cytochrome P450; mAbs, monoclonal antibodies; FD, fluorescein diacetate; IC₅₀, concentration of drug required for 50% inhibition; β -NADPH, β -nicotinamide adenine dinucleotide 2'-phosphate reduced; K_m, Michaelis-Menten constant; V_i, initial velocity; [S], substrate concentration; max, maximum inhibition; min, minimum inhibition; X, inhibitor concentration; n, unitless number; CES, carboxylesterase; DDC, Diethyldithiocarbamate

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ABSTRACT

Selective and non-selective cytochromes P450 (CYP) chemical inhibitors and monoclonal antibodies (mAbs) are routinely used to determine the contribution of CYP enzymes involved in the biotransformation of a drug. A fluorometric assay has been established using fluorescein diacetate (FD) as a model substrate to determine the effect of some commonly used CYP inhibitors and mAbs on human liver microsomal esterase activity. Of those inhibitors studied, only alpha-naphthoflavone, clotrimazole, ketoconazole, miconazole, nicardipine, and verapamil significantly inhibited human liver microsomal esterase activity with apparent IC_{50} values of 18.0 μ M, 20.5 μ M, 6.5 μ M, 15.0 μ M, 19.4 μ M, and 5.4 μ M, respectively. All of these showed $\geq 20\%$ inhibition of human liver microsomal esterase activity at concentrations typically used for CYP reaction phenotyping studies, with clotrimazole, miconazole, nicardipine, and verapamil showing $>60\%$ inhibition. Unlike the chemical inhibitors, no inhibition of human liver microsomal esterase activity was observed in the presence of mAb to CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4. These results suggest that CYP chemical inhibitors are capable of inhibiting human liver microsomal esterase activity and should not be used to assess the role of CYP enzymes in the biotransformation of esters. The lack of inhibition of human liver microsomal esterase activity by CYP-specific monoclonal antibodies suggests that they may be used to assess the role of CYP enzymes in the biotransformation of esters. Additional experiments to assess the contribution of oxidative enzymes in the metabolism of esters may include incubations in the presence and absence of β -nicotinamide adenine dinucleotide 2'-phosphate reduced (β -NADPH).

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Cytochromes P450 (CYPs) are phase I biotransformation enzymes. They are widely distributed among tissues with high concentrations in liver endoplasmic reticulum, kidney, lung, nasal passages, and gut (Parkinson, 1996; Ortiz de Montellano, 1999). Human liver microsomes contain more than 15 different CYP isoenzymes, including CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7, that have the potential to biotransform xenobiotics and/or endogenous substrates (Parkinson, 1996; Madan et al., 2002). Due to broad substrate specificity of CYP enzymes, it is possible for more than one enzyme to be involved in the metabolism of a single compound. It is also possible for one CYP enzyme to catalyze two or more metabolic pathways for the same drug. In vitro methods have been established to determine which CYP isoform(s) is (are) involved in the metabolism of a specific drug. This process, also referred to as CYP reaction phenotyping, integrates data obtained from native human liver microsomes, intact cell models, recombinant CYPs, and inhibition studies with CYP selective chemical inhibitors and specific antibodies (Parkinson, 1996; Rodrigues, 1999). Reaction phenotyping studies can establish whether a particular CYP enzyme is involved in the biotransformation of a drug, as well as to what extent the CYP enzyme contributes to the metabolism of that drug. Once it has been determined, by in vitro methods, which CYP enzyme(s) is (are) involved in the metabolism of a specific drug, then clinical studies can be designed to address potential issues such as drug-drug interactions and genetic polymorphisms, which could affect pharmacokinetics, efficacy, or toxicity.

While CYP enzymes play a prominent role in drug metabolism, other enzymes, such as esterases, can also be important. Mammalian carboxylesterases are located in the

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endoplasmic reticulum and cytosol of many tissues where they hydrolyze ester- and amide-containing chemicals and drugs to their respective free acids. They contribute to the detoxification or the metabolic activation of exogenous compounds such as drugs, environmental toxicants and carcinogens as well as endogenous compounds (Sato and Hosakawa, 1998; Sato et al., 2002). In general, the types of esterases involved in the metabolism of exogenous compounds include carboxylesterases, arylesterases and cholinesterases, whereas endogenous compounds are mainly hydrolyzed by lipases, acylesterases and acetylcholinesterases (Williams, 1985). However, the metabolism of ester-containing compounds may not be exclusively catalyzed by hydrolase enzymes. This was shown by Guengerich and colleagues (1987; 1988) who demonstrated the CYP catalyzed oxidative ester cleavage of 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylic acid diethyl ester. The product, 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylic acid monoethyl ester, was formed in the presence of substrate, enzymatically active microsomes and an NADPH generating system. The formation of these products was observed with five different CYP enzymes, however the extent of formation varied with the enzyme (Guengerich, 1987; Guengerich et al., 1988).

It has recently been observed in our lab that several CYP chemical inhibitors have the potential to inhibit liver microsomal esterase activity *in vitro*. This may result in a misinterpretation of the contribution of CYP enzymes in the biotransformation of ester-containing compounds. This paper characterizes the effect of selective and non-selective CYP chemical inhibitors and monoclonal antibodies on human liver microsomal esterases and provides a means to assess the role of CYP enzymes in the biotransformation of esters.

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MATERIALS AND METHODS

Materials. Fluorescein diacetate was purchased from Acros Organics (Morris Plains, NJ). Clotrimazole and fluconazole were purchased from MP Biomedicals (Irvine, CA). (S)-mephenytoin was purchased from Ultrafine (Manchester, England). Fluvoxamine maleate was purchased from Tocris (Ellisville, MO). Bufuralol, 1'-hydroxybufuralol, 4-hydroxydiclofenac, and 4'-hydroxymephenytoin were purchased from Gentest (Woburn, MA). Dextromethrophan was purchased from Research Biochemicals International (Natick, MA). Pooled human liver microsomes were purchased from either Xenotech LLC (Kansas City, KS) or BD Biosciences (Woburn, MA). CYP mAbs were produced in hybridoma cells obtained through immunization of mice with the microsomal fraction from Sf21 cells expressing either human CYP1A2, 2C8, 2C9, 2C19, 2D6 or 3A4. All CYP mAbs used were generated in-house (Merck & Co., West Point, PA; Mei et al., 1999; Mei et al., 2000). All other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO), Fisher Scientific International Inc. (Hampton, NH), or Aldrich Chemical Co. (Milwaukee, WI).

FD (fluorescein diacetate) Fluorometric Assay. FD is a model fluorogenic substrate for measuring esterase activity. In the presence of microsomal esterases, FD is hydrolyzed to the fluorescent product fluorescein through a two-step process, referred to as fluorochromasia (Rotman and Papermaster, 1965; Dive et al., 1987) (Fig. 1). Fluorescein formation was measured using a fluorometer (SPECTRAMax® Gemini plate reader; Molecular Devices Corp, Sunnyvale, CA) at excitation and emission set at 490 nM and 520 nM, respectively.

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Determination of the K_m for FD Hydrolysis by Human Liver Microsomes.

Experiments were conducted in 96-well flat bottom plates in a volume of 0.2 mL, containing 0.198 mL potassium phosphate buffer (0.1 M, pH 7.4) and 1 μ L pooled human liver microsomes (5 μ g/mL microsomal protein). The plates were pre-incubated at 37°C in a fluorometer for 3 minutes. Reactions were initiated by the addition of 1 μ L FD (0.78-100 μ M final conc.), and the reaction product (fluorescein) was measured for 10 minutes at 9 second intervals. Control incubations also were performed in the absence of protein, and FD. The percent solvent from the FD stock solution did not exceed 0.5% in the incubations. The K_m range was estimated from both a Lineweaver-Burke plot ($1/V_i$ vs. $1/[S]$) of the FD hydrolysis data, and a Michaelis-Menten plot (V_i vs. $[S]$), where V_i was determined from the linear portion of the product formation vs. time curve, and S represented the substrate concentration.

Initial Screen for Inhibition of Human Liver Microsomal Esterase Activity.

Pooled human liver microsomes were incubated with FD, at a concentration close to the apparent K_m (30 μ M), in the presence and absence of CYP chemical inhibitors. Initial incubations were performed in 96-well flat bottom plates containing 0.198 mL potassium phosphate buffer (0.1 M, pH 7.4), 1 μ L CYP chemical inhibitor (100 μ M final concentration), and 1 μ L pooled human liver microsomes (5 μ g/mL microsomal protein). The plates were analyzed using the FD assay as described above. The percent solvent from FD and the inhibitors did not exceed 0.5% in the incubations. The % inhibition was estimated comparing the velocities of the fluorescein product formation in the presence and absence of inhibitor.

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Determination of IC₅₀ Values for CYP Specific Inhibitors. If, from the initial screen, a specific inhibitor inhibited the hydrolysis of FD, then studies were conducted to characterize the apparent IC₅₀. Incubations were performed in 96-well flat bottom plates containing 0.198 mL potassium phosphate buffer (0.1 M, pH 7.4), 1 µL CYP chemical inhibitor (0.024-200 µM final concentration), and 1 µL pooled human liver microsomes (5 µg/mL microsomal protein). The plates were analyzed in the FD assay as described above. The percent solvent from FD and the inhibitors did not exceed 0.5% in the incubations. The percent activity remaining of FD hydrolysis was plotted against the range of inhibitor concentrations on a semi-log scale. The IC₅₀ value was determined by fitting the equation; $[(\text{max}-\text{min})/(1+(X/\text{IC}_{50})^n)+\text{min}]$ to the percent activity remaining versus the log of the inhibitor concentration data (Sai et al., 2000).

CYP Specific Monoclonal Antibody Incubations in Human Liver Microsomes. Incubations with pooled human liver microsomes were conducted to determine the inhibitory potential of specific mAbs against CYP1A2, 2C8, 2C9, 2C19, CYP2D6, CYP3A4 or control IgG on human liver microsomal esterase activity. A 0.5 mg protein concentration of ascites containing the inhibitory mAb, or control IgG, was added to 0.180 mL of potassium phosphate buffer (0.1 M, pH 7.4) containing 1 mg/mL of liver microsomes (Mei et al., 1999). The mixture was vortexed and pre-incubated for 15 minutes on ice (5 minutes at room temperature for the mAb to CYP2D6). A 5 µg/mL microsomal protein aliquot of the pre-incubation mixture was then removed and assayed by the FD esterase activity assay as described above. The percent solvent from FD did not exceed 0.5% in the incubations. A separate 0.25 mg/mL microsomal protein aliquot of the pre-incubation mixture was assayed for CYP activity.

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CYP Activity. Incubation mixtures, to evaluate the activity of CYP following incubation with the mAbs, contained 50 μ L human liver microsomal protein from the pre-incubation (0.25 mg/mL), potassium phosphate buffer (0.1 M, pH 7.4) and the appropriate CYP marker substrate (phenacetin (100 μ M) for CYP1A2, taxol (100 μ M) for CYP2C8, diclofenac (100 μ M) for CYP2C9, (S)-mephenytoin (400 μ M) for CYP2C19, dextromethorphan (50 μ M) for CYP2D6, or testosterone (250 μ M) for CYP3A4) in a 0.2 mL final volume. The reaction was initiated with 1 mM NADPH following a 3 minute pre-incubation at 37 °C in a shaking water bath and was allowed to proceed for 10 to 30 minutes depending on the CYP tested. The reactions were terminated with the addition of two volumes of acetonitrile containing the appropriate internal standard, and the samples were vortexed and centrifuged (3000 rpm for 10 minutes). The supernatant was diluted with an equal volume of 0.1% formic acid in water and analyzed by LC-MS/MS. The samples were analyzed using a Perkin Elmer HPLC system coupled with a Sciex API 2000 triple quadrupole mass spectrometer. Percent of control enzyme activity values were obtained by comparison of samples in the presence of each mAb versus control IgG. Conditions for LC-MS/MS analysis of phenacetin, taxol, diclofenac, mephenytoin, and testosterone were described by Lu et al. (2003). CYP2D6 activity was measured by dextromethorphan *O*-demethylation. Dextromethorphan and its metabolite (dextrorphan) were separated on a BDS Hypersil C8 column (5 μ m particle; 2.0 x 50 mm; Thermo Electron Corporation, Waltham, MA) with a mobile phase consisting of solvent A (90:10 water:methanol with 0.05% formic acid in water) and solvent B (10:90 water:methanol with 0.05% formic acid in water). Samples were eluted with a 2.0 minute linear gradient from a 0% to 50% solvent B (flow rate at 1.5 mL/min). Metabolite and internal standard

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were identified using an APCI ion source in the positive ion mode (dextrorphan m/z 258.1 (MH^+) \rightarrow 157.1 (collision energy 53 V, dwell time 150 ms); levallorphan (IS), m/z 284.1 (MH^+) \rightarrow 199.1 (collision energy 38 V, dwell time 150 ms).

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RESULTS

K_m Determination. An FD fluorometric assay was used to examine the inhibition of human liver microsomal esterases by compounds typically used for CYP reaction phenotyping. Initial incubations were used to establish conditions in which there was linear product formation with respect to protein concentration and time (data not shown). Subsequent studies were conducted using the linear product formation conditions (5 $\mu\text{g/mL}$ protein, 10 minutes, 37 $^{\circ}\text{C}$). Based on the linear regression analysis of the Lineweaver-Burke plot of FD hydrolysis in human liver microsomes, the apparent K_m was estimated to be (28.8 μM) (Fig. 2). Subsequent incubations were conducted using an FD concentration close to the apparent K_m (30 μM). Fit of the data to the Michaelis-Menten equation yielded results similar to what was observed from the Lineweaver-Burke plot. Incubations conducted in the absence of liver microsomal protein suggested that the non-enzymatic hydrolysis of FD was negligible under the experimental conditions.

CYP Chemical Inhibitor Incubations with Pooled Human Liver Microsomes.

Of the CYP inhibitors studied at 100 μM (Table 1), only alpha-naphthoflavone, clotrimazole, ketoconazole, miconazole, nicardipine and verapamil inhibited human liver microsomal esterase to any appreciable extent. Further characterization of the inhibition potency of alpha-naphthoflavone, clotrimazole, ketoconazole, miconazole, nicardipine and verapamil on human liver microsomal esterase activity yielded apparent IC_{50} values of 18.0 μM , 20.5 μM , 6.5 μM , 15.0 μM , 19.4 μM and 5.4 μM , respectively (Table 1 and Fig. 3). Alpha-naphthoflavone and ketoconazole IC_{50} values were > 3-fold higher than concentrations typically used for CYP reaction phenotyping studies. Conversely, the IC_{50}

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values for clotrimazole, miconazole, nifedipine and verapamil were approximately 3 to 20-fold lower than concentrations typically used for CYP reaction phenotyping studies.

CYP Monoclonal Antibody Incubations with Pooled Human Liver Microsomes. Studies were conducted with mAbs to CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 at concentrations used for reaction phenotyping studies. No inhibition of human liver microsomal esterase activity was observed for any of the mAbs relative to control incubations in the presence of control IgG. The mAbs did show $\geq 70\%$ inhibition of their respective CYP activity, compared to control IgG, consistent with previously observed data (unpublished; Table 2).

DISCUSSION

Esterases are phase I enzymes that play an important role in drug metabolism. Esterases hydrolyze a significant number of structurally diverse drugs and other xenobiotics. Similar to other phase I enzymes, alterations in the activity of esterases, such as induction and polymorphisms, can have clinical implications. Esterase activity can be influenced by a variety of compounds enzymatically and at the transcriptional expression level. Various laboratories have demonstrated the inducibility of microsomal esterase activity by known CYP inducers (Kaur and Ali, 1983; Ashour et al., 1987; Hosokawa et al., 1988). Recently, Zhu et al. (2000) studied rat and human hepatocytes to examine the mechanism of carboxylesterase induction by CYP inducers including dexamethasone. Based on their findings, they proposed that regulation of carboxylesterase gene expression by dexamethasone was due to an alteration in transcriptional rate and/or mRNA stability. The glucocorticoid receptor (GR) and the pregnane X receptor (PXR)

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are known to mediate dexamethasone induction, with the GR requiring nanomolar levels and PXR requiring micromolar levels for activation. Zhu et al. (2000) suggested that the dexamethasone induction of hCE-1 and hCE-2 (the two major human liver isozymes) was mediated by PXR due to the observation of hCE-1 and hCE-2 induction only when dexamethasone was at micromolar levels.

In addition to the liver, the expression of hCE-1 and hCE-2 has been observed in small intestine, colon, testis, kidney, spleen, and heart (Satoh et al., 2002). Both hCE-1 and hCE-2 are important for systemic clearance of esters from blood through the liver. In many instances they catalyze the metabolism of the same substrates with different efficiencies, presumably due to the affinity of different structural features within the active site (Satoh et al., 2002). Recent studies of esterases have provided evidence for multiple forms of the enzymes. Sequences of newly identified carboxylesterase isozymes showing high homology with human liver carboxylesterases also showed high substrate specificity similarity. This led to the proposal of Satoh and Hosakawa (1998) to classify carboxylesterases into four families, CES1, CES2, CES3 and CES4. The CES1 family includes the major forms of carboxylesterase isozymes and has been divided into subfamilies (i.e. CES1A) and sub-subfamilies (i.e. CES1A1). CES1A1 includes the major form of human carboxylesterases. CES1B includes esterases which catalyze long-chain acyl-CoA hydrolysis and CES1C are secretory-type esterases (Satoh and Hosakawa, 1998; Satoh et al., 2002). hCE-1 and hCE-2 belong to the CES1 and CES2 classes of carboxylesterases, respectively. As well, more general esterase classification systems have been proposed, based on substrate and inhibitor specificity or interactions with organophosphorous insecticides (Williams, 1985; McCracken et al., 1993).

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The role of esterases in the metabolism of compounds is becoming increasingly important as, during the discovery of new drugs, candidate molecules are being synthesized which may contain an ester function. However, due to the non-selective substrate and inhibition specificity of esterases, phenotyping of specific esterases involved in the metabolism of a given compound is difficult.

Esterases have been reported to exhibit clinically relevant polymorphisms which can lead to variable clearance of drugs. Cholinesterase, or butyrylcholinesterase, is abundant in human plasma and serum and hydrolyzes the muscle relaxant succinylcholine to succinylmonocholine and choline (Lockridge, 1990; Daly, 1993). Succinylcholine was introduced for use in 1951 having a quick onset of action resulting in complete paralysis, and a rapid recovery free of toxic side-effects. In some patients however, paralysis lasted for hours, instead of minutes, which often required life-saving intervention. For detailed discussion of the biochemistry and pharmacogenomics of cholinesterase, the authors refer the reader to Lockridge (1990); Darvesh et al. (2003); and Kalow (2004).

Another esterase identified as having genetic variants is paraoxonase-1 (PON1). PON1 is a serum enzyme that catalyzes the hydrolysis of organophosphate esters, carbamates and aromatic carboxylic acid esters. For detailed discussion of the pharmacogenomics and catalytic efficiency of PON1, refer to Costa et al. (2003).

Since it has been shown that CYP enzymes, in addition to esterases, catalyze the cleavage of ester-containing compounds, it becomes important to understand the involvement of these different enzyme families in the metabolism of a drug candidate. The determination of which enzyme family is involved may have an impact on the

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development of a drug candidate, such as potential for drug-drug interactions and the potential for polymorphic disposition. For many pharmaceutical drug programs, routine screening of compounds occurs in the discovery stage, and it is possible to generate large numbers of esters with α -hydrogens which may undergo CYP mediated cleavage. The present study demonstrated the potential of CYP selective and non-selective inhibitors to inhibit human liver microsomal esterase activity.

FD has been used by a number of laboratories as a model substrate for esterases, including those bound to microsomes. In Bort et al. (1996) a 10 μ M concentration of FD was used to measure esterase activity of sera and liver fractions including S9, cytosol and microsomes. Breeuwer et al. (1995) stated that FD is hydrolyzed by intracellular esterases following transport into cells. They also stated that apparently most cells, whether mammalian, yeast or bacteria, could hydrolyze FD. The authors of this paper have experimentally tested for the formation of the fluorescein product of fluorescein diacetate hydrolysis and only observed this to occur at any reasonable rate in the presence of the human liver microsomes. This provides evidence that fluorescein diacetate is a model substrate for liver microsomal esterases. The number of esterases for which it is a substrate, and their identities, however, is unknown at this time.

Using FD to measure human liver microsomal esterase activity, alpha-naphthoflavone, clotrimazole ketoconazole, miconazole, nifedipine, and verapamil significantly inhibited the formation of the fluorescein product. The apparent IC_{50} values of esterase activity for alpha-naphthoflavone and ketoconazole were 18 μ M and 6.5 μ M, respectively (Fig. 3). These values were > 3-fold higher than the concentrations typically used for CYP reaction phenotyping studies. As such, it can be expected that

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significant esterase inhibition will occur. At concentrations typically used for reaction phenotyping, clotrimazole, miconazole, nicardipine and verapamil could be expected to inhibit human liver microsomal esterase by > 20% (data not shown). In contrast, mAbs to CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 showed no inhibition of human liver microsomal esterase activity at levels which almost completely ($\geq 70\%$) inhibited their respective CYP enzyme.

Much attention is directed towards the CYP metabolism of drugs and as a result, many other biotransformation enzymes, such as esterases, often get overlooked. Esterases however, are responsible for the metabolism of a number of endogenous and exogenous compounds. They can be induced, inhibited, and are subject to genetic polymorphisms, which can have clinical implications for the development of a drug. The results from the current study suggest that CYP chemical inhibitors should not be used to assess the role of CYP enzymes in the biotransformation of esters. Their potential to inhibit human liver microsomal esterase activity may result in an overestimation of the contribution of CYP enzymes in the metabolism of esters leading to a misinterpretation of potential drug-drug interactions. In contrast, CYP mAbs may be a useful tool to determine the contribution of CYP enzymes on the metabolism of esters as they were shown to have no effect on human liver microsomal esterase activity. Additional experiments to assess the contribution of oxidative enzymes in the metabolism of esters may include incubations in the presence and absence of β -nicotinamide adenine dinucleotide 2'-phosphate reduced (β -NADPH).

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ACKNOWLEDGMENTS

We gratefully acknowledge Drs. Qin Mei, Magang Shou, Ed Carlini and Mr. Yuhlin Fang for the use of their CYP mAbs.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. *Hydrolysis of fluorescein diacetate.*

Figure 2. *Lineweaver-Burke plot of fluorescein diacetate (FD) hydrolysis by human liver microsomes (5 $\mu\text{g}/\text{mL}$ protein) with an apparent K_m of 28.8 μM . A Michaelis-Menten plot in inset. Incubations conducted in the absence of liver microsomal protein suggested that the non-enzymatic hydrolysis of FD was negligible under the experimental conditions.*

Figure 3. *Representative inhibition profiles of human liver microsomal esterase activity by CYP chemical inhibitors. Alpha-naphthoflavone (—●—), and ketoconazole (···○···), with apparent IC_{50} values of 18.0 μM and 6.5 μM , respectively.*

TABLE 1

Compounds assayed for inhibition of human liver microsomal esterase activity

Inhibitor Name	CYP subtype	CYP K_i (μM)	Concentration Typically Used for CYP Reaction Phenotyping (μM)	Apparent IC_{50} (μM) of Esterase Activity	References
α -Naphthoflavone	CYP1A2	0.01	5	18	Zhang et al., 2002; Rodrigues, 1999
Clotrimazole	general	---	50	20.5	Rodrigues et al., 1994
Coumarin	CYP2A6	0.3	20	N.D.	Rodrigues, 1999
DDC *	CYP2E1	4	50	N.D.	Rodrigues, 1999
Disulfiram	CYP2E1	---	100	>100	Hazai et al., 2002
Erythromycin	CYP3A4	39	100	N.D.	Zhao and Ishizaki, 1999; Chang et al., 1999
Fluconazole	CYP3A4	11	30	N.D. (<10% inhibition)	Gibbs et al., 1999
Fluoxetine	CYP2D6	0.33	1	>100	Polasek et al., 2004; Hemeryck et al., 2000
Fluvoxamine	CYP1A2	---	1	>100	Wang and DeVane, 2003
Furafylline *	CYP1A2	$K_i=0.6$	20	N.D.	Rodrigues, 1999; Oda et al., 2001
Ketoconazole	CYP3A4	0.02	1	6.5	Gibbs et al., 1999; Wang and DeVane, 2003
S-mephenytoin	CYP2C19	175	400	N.D.	Zhang et al., 2002; Harris et al., 1994
Methoxychlor	CYP2B6	----	25	>100	Stresser and Kupfer, 1999
8-methoxypsoralen *	CYP2A6	1	10	N.D.	Rodrigues, 1999; Hazai et al., 2002
4-methylpyrazole	CYP2E1	4	50	N.D.	Rodrigues, 1999
Miconazole	CYP3A4	---	50	15	Harris et al., 1994
Nicardipine	CYP3A4	---	100	19.3	Chauret et al., 1999
Omeprazole	CYP2C19	4.5	10	>100	Zhao and Ishizaki, 1999; Warrington et al., 2000
Pilocarpine	CYP2A6	4	100	N.D.	Zhang et al., 2002
Quercetin	CYP2C8	1	20	>100	Prueksaritanont et al., 2003
Quinidine	CYP2D6	0.07	5	N.D.	Rodrigues, 1999; Oda et al., 2001
Sulfaphenazole	CYP2C9	0.3	10	N.D.	Zhang et al., 2002; Warrington et al., 2000
Sulfapyrazole	CYP2C9	---	100	>100	Draper et al., 1997
Tamoxifen	CYP2C8	0.2	5	69	Polasek et al., 2004; Zhou et al., 2004
Tranlycypromine	CYP2A6	0.04	1	N.D.	Wang and DeVane, 2003; Draper et al., 1997
Trimethoprim	CYP2C8	----	100	N.D. (<10% inhibition)	Wang and DeVane, 2003
Troleandomycin *	CYP3A4	7.7	50	N.D. (<10% inhibition)	Zhao and Ishizaki, 1999; Ma et al., 2000
Verapamil	CYP3A4	20	100	5.4	Ma et al., 2000; Yamamoto et al., 2004

N.D. = not detected

--- = no literature reference

* = mechanism based inhibitors characterized by K_i , k , and k_{inact}

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TABLE 2

*Mean (\pm S.D.) percent inhibition of CYP and esterase activities in human liver
microsomes following incubations with CYP mAbs.*

CYP Monoclonal Antibody (mAb)	% Inhibition of CYP Activity	% Inhibition of Esterase Activity
CYP1A2	72 \pm 4%	-4 \pm 27%
CYP2C8	85 \pm 1%	-17 \pm 14%
CYP2C9	79 \pm 1%	-17 \pm 17%
CYP2C19	88 \pm 2%	-1 \pm 10%
CYP2D6	79 \pm 5%	-1 \pm 19%
CYP3A4	97 \pm 0.2%	-1 \pm 13%

Figure 1

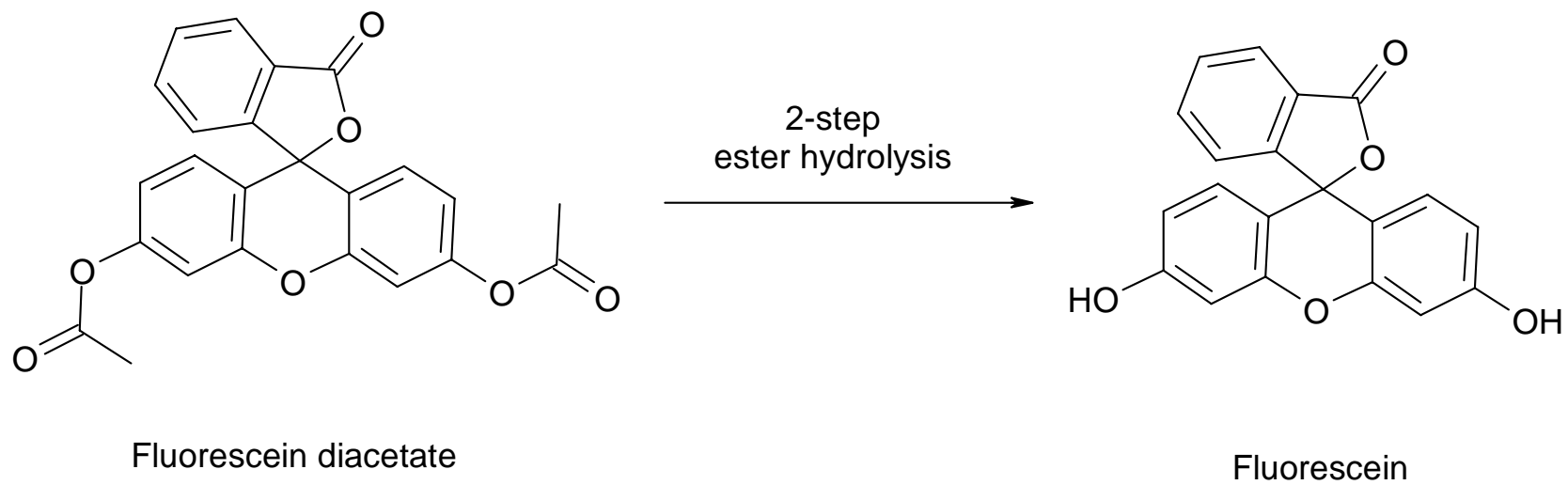


Figure 2

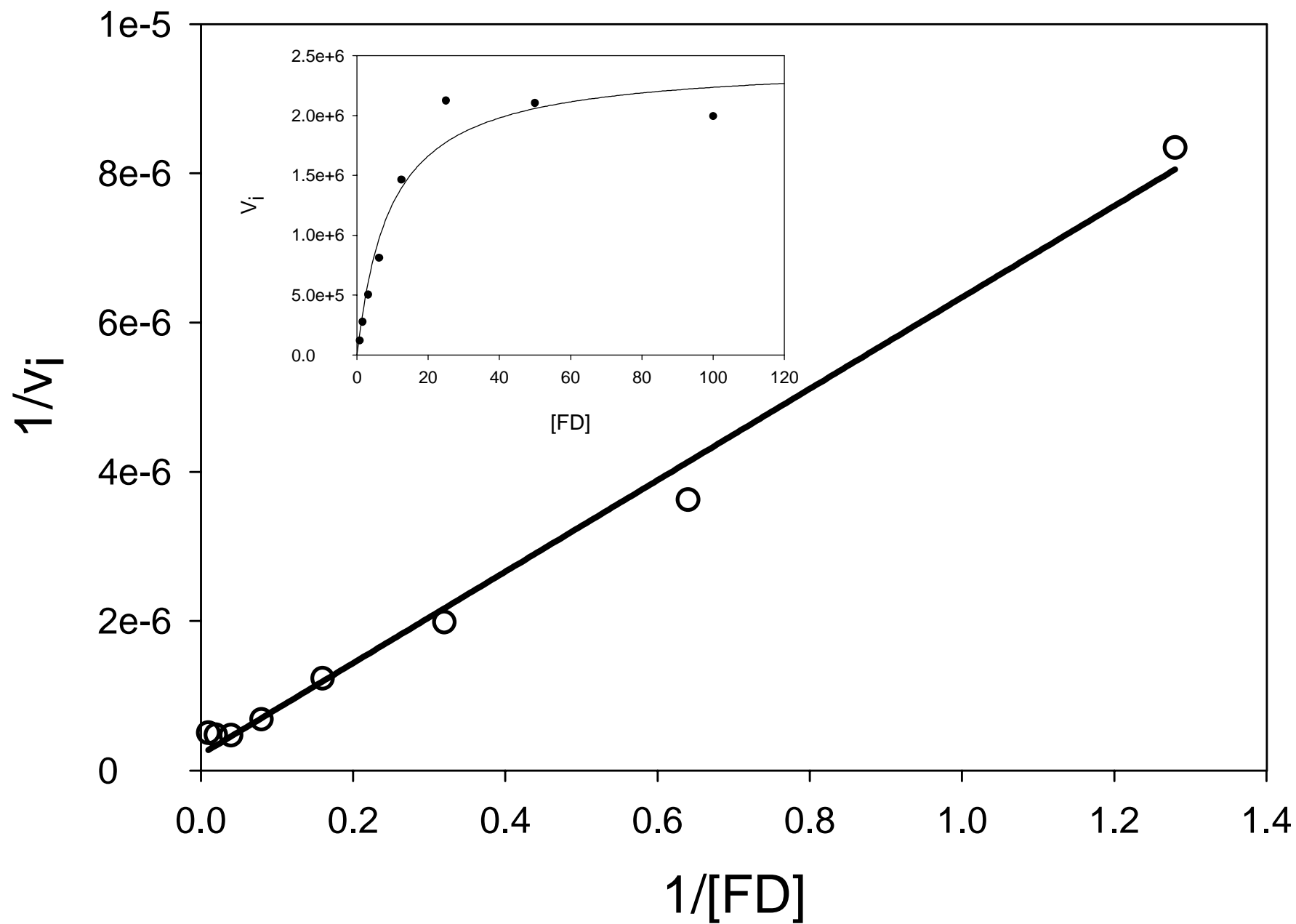


Figure 3

