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Rapidly Distinguishing Reversible and Irreversible CYP450 Inhibitors by Using Fluorometric Kinetic Analyses

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SUMMARY

In this study we have evaluated the reliability of a fluorescence-based method used for rapid identification of irreversible CYP inhibitors (mechanism-based inhibitors). This was accomplished by comparing the time-dependence pattern of IC_{50} values from fluorometric kinetic measurements. For irreversible CYP inhibitors, IC_{50} values decreased as incubation proceeded. This was due to progressive inactivation of corresponding enzymes by reactive metabolites generated during the incubation. This change pattern was confirmed using a number of known irreversible CYP inhibitors, including furafylline, midazolam, erythromycin, clarithromycin, oleandomycin, 17α-ethynylestradiol and verapamil. The pattern was different in reversible inhibition, depending upon the compounds tested in the fluorometric kinetic assay. For some compounds, such as clotrimazole, IC_{50} values remained relatively stable, whereas other compounds, such as miconazole, terfenadine and ketoconazole showed a significant increase with incubation time. Monitoring tested compounds by LC-MS/MS during the incubation confirmed that increases of IC_{50} were probably caused by the loss of inhibitors, resulting from either metabolic degradation, or non-specific binding to microsomal proteins.

INTRODUCTION

Multiple drug therapy is a quite common practice in today's medicine. As a result, drug-drug interactions are an increasing concern in drug discovery and development. Such a concern was highlighted by the recent withdrawal of terfenadine (1) and mibefradil (2) from the market due to drug-drug interactions. Because metabolism by cytochrome P450s (CYPs) represents a major route of drug elimination in humans, inhibition of CYP450s is a principal mechanism for metabolism-based drug-drug interactions (3). As part of the effort to reduce attrition rates of new chemical entities (NCEs) and to develop better medicines more quickly in the future, *in vitro* CYP450 inhibition screening has been routinely utilized to evaluate NCEs for potential drug-drug interactions (4). Currently, there are two different approaches widely used for this purpose. A more traditional method (5) measures CYP marker activity in human liver microsomes, using liquid chromatography interfaced with tandem mass spectrometry (LC-MS/MS). A high throughput assay (6,7) determines enzyme activity in a microtiter plate format by using recombinant CYPs and fluorescent substrates.

Inhibition of CYPs can be classified mechanistically into two categories: reversible inhibition and irreversible inhibition (3). In reversible inhibition, compounds directly

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compete at CYP active sites such as the prosthetic heme iron and substrate-binding region of CYP proteins, and enzymatic activity of CYPs can be fully restored both in vitro and in vivo after inhibitors are depleted. As irreversible inhibitors, compounds either inhibit CYP activity by irreversible binding, or they are first converted by the CYP to reactive metabolites that irreversibly bind to the sites within the active center of the enzyme, resulting in the permanent loss of enzymatic activity. Irreversible inhibition of CYPs by reactive metabolites often requires at least one round of CYP-catalyzed reaction; thus it is sometimes referred to as mechanism-based inhibition. In irreversible inhibition, reactive metabolites either form stable complexes with the prosthetic haem of CYP (quasi-irreversible inhibition) or covalently modify CYP proteins (irreversible inhibition) (3). In both cases, enzyme activity cannot be recovered by the removal of inhibitors from the reaction. Unlike reversible inhibition, inactivation of CYP450s by reactive metabolites causes long-term effects on some drug pharmacokinetics, as the inactivated enzyme must be replaced by newly synthesized CYP protein.

Although reversible inhibition accounts for the majority of drug-drug interactions reported in the clinic, irreversible inhibitors are much less favorable and often avoided in drug development. Since they are more likely to fail in the development stage due to either toxicity or drug-drug interaction concerns, it is crucial to identify irreversible inhibitors in the early stage of drug discovery before valuable resources have been devoted to compounds that have less chance of proceeding to the clinic.

Although irreversible inhibition of CYPs often causes more serious problems in drug development, distinguishing irreversible and reversible inhibitors has not been the major focus of current CYP inhibition screening approaches (5-7). So far, irreversible CYP450 inhibitors have often been identified in human liver microsomes by comparing the effect of pre-incubation of test compounds on CYP marker activity (8). This is normally done using either LC or LC-MS/MS to analyze marker metabolites. This laborious and expensive assay limits the ability to handle a high volume of compounds, and has been the major obstacle for routine screening of irreversible inhibitors in drug discovery. In recent years, the application of parallel synthesis and high throughput screening in drug discovery has generated relatively large numbers of drug candidates. The need for a more efficient method to screen for irreversible inhibitors has become obvious. In the present paper, we have fully investigated the feasibility of a fluorometric kinetic assay using recombinant CYP enzymes to rapidly distinguish reversible and irreversible CYP inhibitors. A systemic validation study suggested that this microtiter plate-based method is very reliable and efficient, and can be routinely used for screening large numbers of drug candidates for irreversible CYP inhibition.

MATERIALS AND METHODS

Chemicals

Furafylline, 7-benzyloxy-4-trifloromethylcoumarin (BFC), 3-cyano-7-ethoxycoumarin (CEC) were obtained from Ultrafine Chemicals (Manchester, UK). Terfenadine, miconazole, a-naphathoflavone, ketoconazole, verapamil, diltiazem, desipramine, imipramine, midazolam, clarithromycin, furafylline, orphenadrine, oleandomycin, 17α-ethynylestradiol, miconazole, disopyramide, b-nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma (St. Louis, MO, US). Microsomes containing CYP3A4 and 1A2, derived from baculovirus infected insect cells, were obtained from Gentest Corp. (Woburn, MA, US).

Fluorometric Kinetic CYP Inhibition Assay

The fluorometric kinetic CYP inhibition assays were performed in black wall Costar 96-well plates (Corning Incorporated, Corning, NY). The CYP enzymes were from microsomes containing CYP3A4 and 1A2, derived from baculovirus infected insect cells. All test compounds were dissolved in acetonitrile and tested for fluorescence at the excitation and emission wavelengths for the assay. The compound under test was serially diluted (1:3 dilution) in 50 mM phosphate buffer (pH 7.4, pre-warmed at 37 °C) containing an electron generating system (glucose-6-phosphate, NADP+ and glucose-6-phosphate dehydrogenase). Serial dilutions gave eight concentrations in the assay for calculation of IC_{50} values. The enzymatic reaction was initiated by adding individual CYP enzymes pre-mixed with fluorescent substrates in 50 mM phosphate buffer (pH 7.4). In the final reaction mixtures, the enzyme concentration was 2.5 nM and 5 nM for 1A2 and 3A4 respectively, and substrates used were CEC (5 μM) for 1A2 and BFC (50 μM) for 3A4. Substrate concentrations were chosen to be close to the Km values. Primary studies showed no substrate depletion after 30 min incubations. Instead of using end-point measurements in the method previously described by others (6,7), CYP activity was kinetically measured at intervals of 2 min on an FL600 microplate fluorescence reader (Biotek Instruments, Inc., Winooski, VT). The plate holder of the microplate reader was heated at 37 °C before the reaction was started, and the temperature was held until the end of measurements.

Disappearance of Inhibitors in Microsomes

To examine the disappearance of inhibitors in microsomes, incubations were performed under the same experimental conditions in the CYP inhibition assay as described above. During the incubation, an aliquot of 75 µl reaction mixture was removed at the desired time and quickly chilled on ice. After a brief centrifugation to pellet microsomal proteins, supernants were collected for LC-MS/MS analysis.

LC-MS/MS Analyses

LC-MS/MS analyses were performed on a Micromass (Manchester, UK) Quattro Micro triple quadrupole mass spectrometer interfaced to an Agilent 1100 HPLC system. LC-MS/MS analyses were conducted using an electrospray ionization with positive ion detection. The capillary voltage was 3.1 kV, and the cone voltage was 40V. The source temperature was set at 120 °C, and the desolvation temperature was 300 °C. The collision gas was nitrogen, and the collision energy was set at 28 eV for terfenadine and 22 eV for miconazole respectively. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode. The transitions monitored in MRM were m/z 473→436 for terfenadine and 417→161 for miconazole.

An Agilent Zorbax SB C18 column (2.1 x 50 mm) was used for the chromatographic separation. The starting mobile phase consisted of 70% water (0.5% acetic acid), and the metabolites were eluted using a single gradient of 70% water to 95% acetonitrile over 5 min at a flow rate of 0.3 ml/min. At 5 min, the column was flushed with 95% acetonitrile for 3 min before re-equilibration at initial conditions. During the run, the divert valve was activated to divert the HPLC eluant to waste for the first minute of elution, and then switched to the mass spectrometer for analysis. LC-MS/MS analyses were carried out on 10-µl aliquots from incubations. Data were processed using the Masslynx v3.5 software from Micromass (Manchester, UK).

IC_{50} Calculations

IC_{50} values of both reversible and irreversible inhibitions were calculated by linear extrapolation, using the equation shown below (Technical Manual, Gentest Corp.):

$$IC_{50} = \frac{(50\% - \text{low percentage}) * (\text{high conc.} - \text{low conc.})}{(\text{high percentage} - \text{low percentage})} + \\ + (\text{low conc.})$$

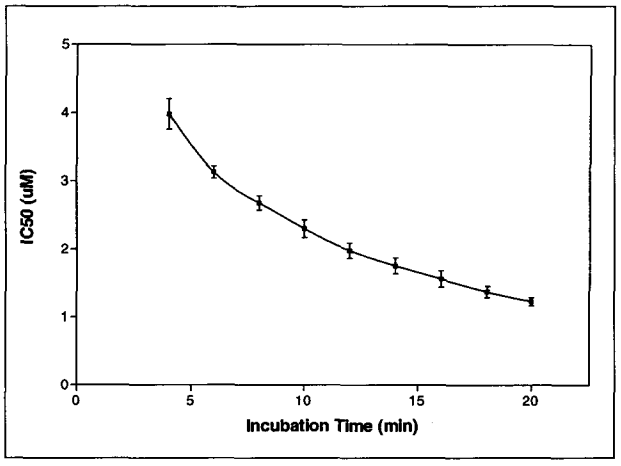


Fig. 1 : IC_{50} Time-dependent pattern of irreversible CYP1A2 inhibitor furafylline

Where,
low percentage = highest percent inhibition less than 50%;
high percentage = lowest percent inhibition greater than 50%;
low concentration = concentration of test compound corresponding to the low percentage inhibition;
high concentration = concentration of test compound corresponding to the high percentage inhibition.

The raw data obtained from fluorometric kinetic assays were transferred to EXCEL. EXCEL functions were created in house to automatically calculate IC_{50} values and their standard deviations, and generate time dependence curves of IC_{50}.

RESULTS

Time Dependence of Irreversible IC_{50}

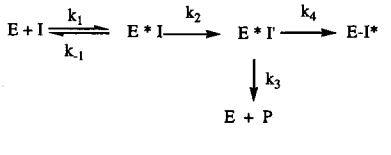
IC_{50} values of test compounds were determined in the fluorescence-based assay using kinetic measurements as described in the Methods section. Fig. 1 shows a representative time dependence curve of furafylline, a well-known irreversible CYP1A2 inhibitor (9). The IC_{50} values of furafylline gradually decreased from 4.0 ± 0.2 µM at 4 min to 1.2 ± 0.1 µM at 20 min of incubation (Fig. 1). In irreversible inhibition, CYP enzymes lost activity progressively when more reactive metabolites were generated during incubation. In the early stage of incubation, more active CYP enzyme existed in the reaction mixture. Therefore, a higher concentration of inhibitor was needed to achieve 50% inhibition. Less active enzyme remained in the later stage of incubation, thus a lower concentration of inhibitor was enough to achieve the same degree of inhibition. Although enzyme activity

may decrease in the process of incubation because of CYP instability, the loss of activity was normally within 15% during an incubation of 30 min. On the other hand, since the effect of CYP instability was the same for both samples (with inhibitor) and controls (no inhibitors); thus, it is expected that instability of CYPs would not cause significant changes in IC_{50} determinations. This rationale was supported by the fact that decreases in IC_{50} with incubation time were compound-related and CYP isoform-specific.

The detection sensitivity of CYP substrates is very critical in kinetic measurements, because the level of fluorescent metabolite was relatively low in the early stage of incubation. As a result, higher errors were usually seen in IC_{50} values in the early stage of incubation. Three replicates were included in the experiments to minimize experimental errors. IC_{50} values of the first two time points were not included in the data analysis because of high experimental errors due to low signal-to-noise ratios.

Potency Considerations

The inhibition potency of a compound is usually measured by the IC_{50} value or inhibition constant (Ki) that reflects the binding affinity of the inhibitor to the CYP enzyme. But IC_{50} values of irreversible inhibitors decreased with incubation time as seen in Fig. 1. Therefore, the initial IC_{50} ($IC_{50,init}$) was introduced in this study as a measurement of inhibition potency for the purpose of comparison. The $IC_{50,init}$ refers to the first reliable IC_{50} measurement (4 min incubation in this study) when the mechanism-based inhibition was still minimal. The IC_{50} value or inhibition constant (Ki) does not seem to be comprehensive measurements of potency of irreversible inhibitors. As shown in Scheme 1, a CYP enzyme catalyzes the conversion of a drug to its reactive or a non-reactive metabolite released as a product. Not only is the IC_{50} value or inhibition constant (Ki) important, but also the ratio of latent inhibitor molecules converted and released as product relative to each turnover leading to enzyme inactivation. According to Scheme 1, a ratio of k3/k4 has been proposed (10) as the partition ratio to measure the



* E , I and E-I* represent CYPs, inhibitor and intermediate-CYP complex respectively.

Table I: Representative results of a list of CYP inhibitors. All compounds were analyzed using fluorometric kinetic measurements as described in the Methods section. Only $IC_{50, init.}$ and IC_{50}, final. were reported to represent results at 4 min and 30 min incubations

Compound (CYP)	$IC_{50, init.}$ (μM)	$IC5_{0, final.}$ (μM)	Relative IC_{50}* (%)
Clarithromycin (3A4)	19.1 ± 1.4	5.6 ± 0.2	29.3**
Erythromycin (3A4)	16.2 ± 0.1	6.1± 0.4	37.0**
Oleandomycin (3A4)	61.7 ± 2.7	14.0 ± 1.4	22.7**
Troleandomycin (3A4)	1.1 ± 0.1	0.3 ± 0.0	27.3**
Ethyinylestradiol (3A4)	43.2 ± 1.6	0.6 ± 0.2	41.0**
Diltiazem (3A4)	10.2 ± 0.6	7.9 ± 0.8	77.5**
a-Naphthoflavone (1A2)	1.2 ± 0.0	1.6 ± 0.1	137.1***
Orphenadrine (3A4)	38.9 ± 5.6	66.8 ± 2.7	171.7***
Impramine (3A4)	80.7± 5.2	90.7 ± 1.2	112.4***
Ketoconazole (3A4)	0.023 ± 0.02	0.044 ± 0.04	191.3***

* The ratio of IC_{50}, final and $IC_{50, init.}$.
irreversible CYP inhibitors; * reversible CYP inhibitors.
Data are presented as mean ± S.D. form three determinations.

efficiency of the inactivation of enzymes. Obviously, this ratio depends upon the reactivity of the active metabolite.

In this study, relative IC_{50} values were calculated using the average of $IC_{50, init}$ as 100%. A different plot was generated to show changes of relative IC_{50} vs. incubation time. As shown in Fig. 2, although midazolam showed a lower $IC_{50, init}$ (3.4 ± 0.6 μM) than verapamil (6.4 ± 0.8 μM), relative IC_{50} values of verapamil decreased more dramatically (20%) than that of midalazom (50%), implying that inactivation of CYP3A4 by verapamil was more efficient than midazolam. Previous studies by others indicated that verapamil is a potent irreversible CYP3A4 inhibitor (11), and the intermediate-CYP3A4 complex generated from midazolam metabolism occurred to a minor extent (12). It seems that changes of relative IC_{50} values vs. incubation time can be used as an effective indicator of partition ratios for the potency ranking purpose, since these ratios are difficult to be determined.

Time Dependence of Reversible IC_{50}

In theory, the IC_{50} value of a reversible inhibitor does not change significantly with incubation time, if the inhibitor strictly follows simple Michaelis-Menten kinetics. As observed in CYP3A4 inhibition by clotrimazole (Fig. 3A), IC_{50} values were relatively stable, and the fluctuation was within the experimental error range. However, IC_{50} values of many compounds showed a significant increase with incubation time. As shown in Fig. 3B, IC_{50} values of

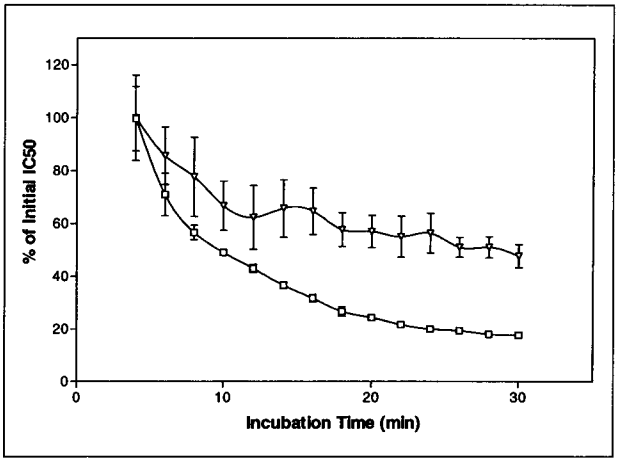


Fig. 2 : Relative IC_{50} of irreversible inhibitors verapamil ( ) and midazolam (▽) vs. incubation time. $IC_{50, init}$. Values are 6.4 ± 0.8 μM and 3.4 ± 0.6 μM for verapamil and midazolam respectively.

reversible inhibitors, miconazole (13) and terfenadine (14) increased approximately 50% and 250% respectively after a 25 min incubation. Analyses of a large number of compounds by fluorometric measurement indicate that approximately 65% of compounds showed an increase in IC_{50} to different degrees with incubation time. These results clearly suggested that the observed increase in IC_{50} with incubation was not assay specific, but was compound related.

Disappearance of Inhibitors

The IC_{50} increase implied a significant decrease in concentrations of inhibitors. To confirm this possibility, the concentration of inhibitors was monitored in the process of incubation. In this experiment, the concentration of inhibitors was chosen to be close to the $IC_{50, init}$ values. As shown in Fig. 4, the concentration of miconazole and terfenadine approximately decreased 25% and 64% respectively after a 30 min-incubation. The data are in agreement with relative IC_{50} results. As shown in Fig. 3B, terfenadine showed more increases in IC_{50} than miconazole.

Unlike other classic enzymes, CYPs, especially CYP3A4, displayed very broad substrate specificities (3). If a test compound is a CYP substrate, extensive metabolism by the CYP can result in a significant decrease in the concentration during the incubation. On the other hand, CYPs used in the assay were not purified enzymes, and concentrations of microsomal proteins were unusually high in reaction mixture. Therefore, non-specific binding to microsomal proteins was significant (15) and could result in a decrease in the free solution concentration of molecules. Metabolism and non-specific protein binding

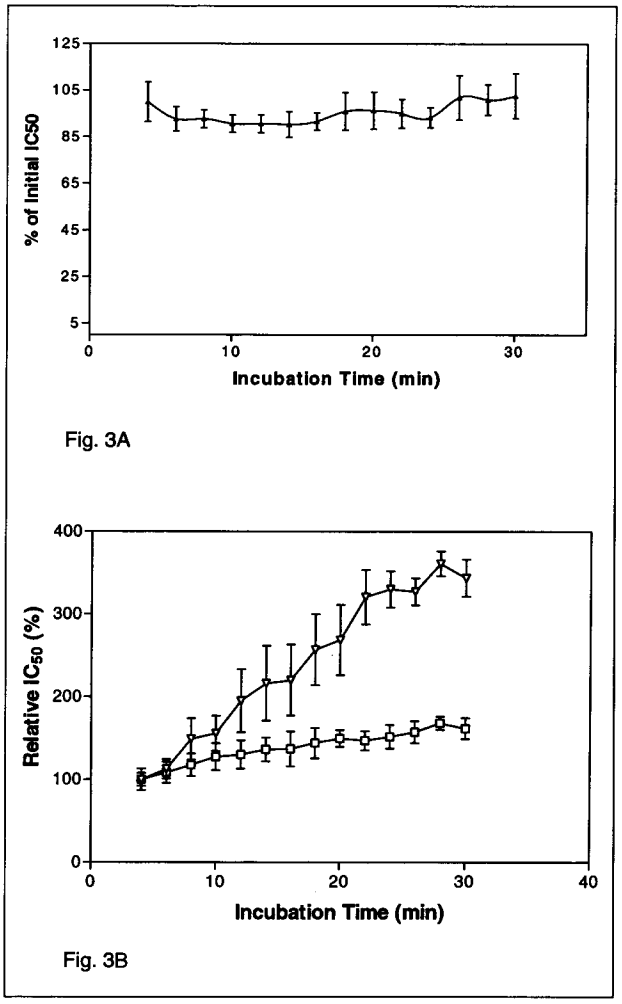


Fig. 3 : Relative IC_{50} of reversible CYP inhibitors, clotrimazole (A), miconazole (B,) and terfenadine (B, ▽) vs. incubation time. $IC_{50, init}$. Values are 11.0 ± 1.1 nM, 0.11 ± 0.01 μM and 0.31 ± 0.04 μM for clotrimazole, miconazole and terfenadine respectively.

exist in all microsomal incubations, but an increase in IC_{50} was observed only in the cases where the concentration change was significant relative to the IC_{50} value.

Validation Studies

A decrease in IC_{50} with incubation time appears to be a good indication of irreversible inhibition. A large number of compounds were used to further validate this approach. Some representative results were shown in Table I. All those irreversible inhibitors showed significant decreases of IC_{50} with incubation time, whereas IC_{50} values of reversible inhibitors either remained stable or increased with incubation. This approach was successfully used to identify, not only potent irreversible CYP3A4 inhibitors such as macrolides (16), but also very weak ones such as

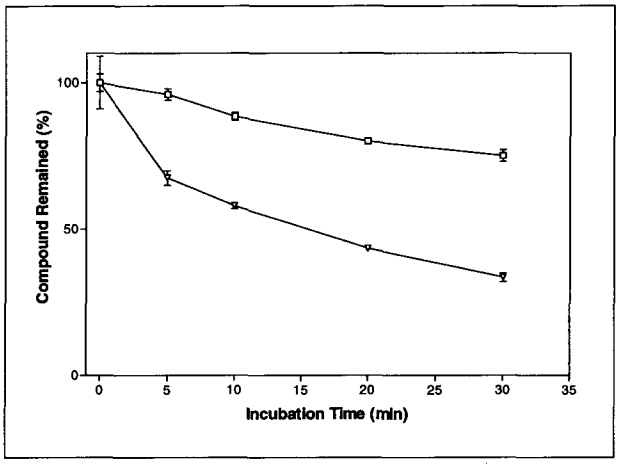


Fig. 4 : Disappearance of terfenadine (V) and miconazole ( ) during the incubation. Incubations were performed as described in the Methods section. Final concentrations of test compounds were 0.1 μM and 0.3 μM for terfenadine and miconazole respectively.

diltiazem (17). These results clearly indicate that the time dependence pattern obtained by fluorometric kinetic measurements can be used to effectively distinguish reversible and irreversible CYP inhibitors. Other irreversible inhibitors specific to CYP2C9, 2C19 and 2D6 were not commercially available, thus were not included in this validation study.

The results presented here clearly suggested that BFC is a reliable substrate for the identification of irreversible CYP3A4 inhibitors. Although three fluorescence substrates were available for CYP3A4, BFC was chosen in this study because of better signal-to-noise ratio. In general, a gradual decrease of 15% in IC_{50} was seen as an indication of irreversible inhibition when experimental errors were taken into account. However, caution should be taken when compounds are analyzed at high concentration ranges (greater than 100 μM), because some non-specific effects on CYPs may occur.

DISCUSSION

The fluorescence-based CYP assay has been widely used for general inhibition evaluations (18, 6), but using the assay for the identification of irreversible inhibitors has not been fully investigated (7). The current study has demonstrated the application of this method for distinguishing irreversible and reversible inhibition by using kinetic measurements. It has been shown that reversible and irreversible CYP inhibitors displayed different IC_{50} time dependence patterns in fluorometric kinetic measurements, and the pattern can be used to unambiguously identify irreversible CYP inhibitors. Validation studies confirmed that this approach could be routinely used for the high throughput screening for irreversible inhibitors in drug discovery.

Because of the availability of irreversible CYP inhibitors, this study was primarily focused on CYP1A2 and 3A4. But this method should be suitable for all other isoforms such as 2D6, 2C9 and 2C19, as long as fluorescent substrates have acceptable sensitivity. Compared to the traditional LC-MS/MS-based method, this approach is more straightforward and does not require expensive instruments; therefore, it is easy to be widely implemented in drug discovery. More importantly, the throughput of this approach is significantly greater. In fact, data from fluorometric kinetic measurements were more informative, compared to that from the end-point measurements in the original HT inhibition assay (6, 7). In a single assay, one can measure the apparent inhibition potency (IC_{50}), distinguish irreversible and reversible CYP inhibitors, and estimate the efficiency of the inactivation and also the reactivity of the active metabolite. Although the data load is relatively high compared to the end-point measurement, this drawback can be easily overcome with a complete integration of data acquisition with IC_{50} calculation using EXCEL. So far, this method has been successively implemented in house to screen for irreversible CYP inhibitors. A screening time of 15 min per compound is practically achievable.

The IC_{50} time dependence revealed in this study also helps explain the inter-laboratory variability of CYP inhibition assays. Inhibition kinetic constants (Ki and IC_{50}) are normally measured by end-point measurements in both the HT inhibition assay and the LC-MS/MS-based method. Large inter-laboratory variability has been reported, even for those well-characterized compounds such as ketoconazole (19, 20) and quinidine (21, 22). Data presented here clearly suggest that incubation time is probably one of important factors contributing to the inter-laboratory variability.

In conclusion, fluorometric kinetic measurements are reliable for the screening of irreversible CYP inhibitors in a high throughput format. This method is complementary to the traditional LC-MS/MS approach used to identify irreversible CYP inhibitors. It can be used as a primary screening approach in the early stage of drug discovery. Detailed studies in human liver microsomes are always necessary to validate the results and determine the in vivo relevance of irreversible inhibition found with recombinant CYP enzymes. Because of the complexity of drug metabolism in vivo, this validation is important when CYP inhibition data become crucial in the lead optimization process.

REFERENCES

1. von Moltke L L; Greenblatt D J; Duan S X; Harmatz J S; Shader R I In vitro prediction of the terfenadine-ketoconazole pharmacokinetic interaction. J. Clin. Pharm. (1994), 34(12), 1222-1227.
2. Griffin J. P. The withdrawal of mibefradil (Posicor). Adverse Drug Reactions and Toxicological Reviews (1998), 17(2-3), 59-60.
3. Lin, J. H.; Lu, A. Y. H.. Inhibition and induction of cytochrome P450 and the clinical implications. Clin. Pharmacokinet. (1998), 35(5), 361-390.
4. Yan Z.; Caldwell G. W. Metabolism profiling, and cytochrome P450 inhibition & induction in drug discovery. Curr. Top. Med. Chem. (2001), 1(5), 403-425.
5. Chu, I.; Favreau, L.; Soares, T.; Lin, C. C.; Nomeir, A. A. Validation of higher-throughput high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry assays to conduct cytochrome P450s CYP2D6 and CYP3A4 enzyme inhibition studies in human liver microsomes. Rapid Comm. in Mass Spectr. (2000), 14(4), 207-214.
6. Crespi, C. L.; Stresser, D. M. Fluorometric screening for metabolism-based drug-drug interactions. J. Pharm. Toxicol. Methods (2001), 44(1), 325-331.
7. Crespi, C. L.; Miller, V. P.; Penman, B. W. Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes P450. Anal. Biochem. (1997), 248(1), 188-190.
8. Palamanda, J. R.; Casciano, C. N.; Norton, L. A.; Clement, R. P.; Favreau, L.; Lin, C-C; Nomeir, A. A. Mechanism-based inactivation of CYP2D6 by 5-fluoro-2-[4-[(2-phenyl-1H-imidazol-5-yl)methyl]-1-piperazineyl] pyrimidine. Drug Metab. Despos. (2001), 29(6), 863-867.
9. Racha, J. K.; Rettie, A. E.; Kunze, K. L. Mechanism-based inactivation of human cytochrome P450 1A2 by furafylline: detection of a 1:1 adduct to protein and evidence for the formation of a novel imidazomethide intermediate. Biochemistry (1998), 37(20), 7407-7419.
10. Silverman, R.B. Mechanism-based Enzyme Inactivation: Chemistry and Enzymology (vol. I) CRC Press, Inc., 1988.
11. Bennett, M.; Prueksaritanont, T.; Lin, J. H. Drug interactions with calcium channel blockers: possible involvement of metabolite-intermediate complexation with CYP3A. Drug Metab Dispos. (2000), 28(2), 125-130.
12. Khan, K. K.; He, Y. Q.; Domanski, T. L.; Halpert, J. R. Midazolam oxidation by cytochrome P450 3A4 and active-site mutants: an evaluation of multiple binding sites and of the metabolic pathway that leads to enzyme inactivation. Mole. Pharm. (2002), 61(3), 495-506.
13. Zhang, W.; Ramamoorthy, Y.; Kilicarslan, T.; Nolte, H.; Tyndale, R. F.; Sellers, E. M. Inhibition of cytochromes P450 by antifungal imidazole derivatives. Drug Metab. Dispos. (2002), 30(3), 314-318.
14. Yun, C. H.; Okerholm, R. A.; Guengerich, F. P. Oxidation of the antihistaminic drug terfenadine in human liver microsomes: role of cytochrome P-450 3A(4) in N-dealkylation and C-hydroxylation. Drug Metab. Dispos. (1993), 21(3), 403-9.
15. Obach, R. Scott. Nonspecific binding to microsomes: impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol. Drug Metab. Dispos. (1997), 25(12), 1359-1369.
16. Lindstrom, T. D.; Hanssen, B. R.; Wrighton, S. A. Cytochrome P-450 complex formation by dirithromycin and other macrolides in rat and human livers. Antimicrob. Agents Chemother. (1993), 37(2), 265-269.
17. Sutton D; Butler A M; Nadin L; Murray M Role of CYP3A4 in human hepatic diltiazem N-demethylation: inhibition of CYP3A4 activity by oxidized diltiazem metabolites. J. Pharm. Exp. Ther. (1997), 282(1), 294-300.
18. Bapiro, T. E.; Egnell, A-C; Hasler, J. A.; Masimirembwa, C. M. Application of higher throughput screening (HTS) inhibition assays to evaluate the interaction of antiparasitic drugs with cytochrome P450S. Drug Metab. Dispos. (2001), 29(1), 30-35.
19. Gibbs, Megan A.; Thummel, Kenneth E.; Shen, Danny D.; Kunze, Kent L. Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: comparison of KI values and impact of CYP3A5 expression. Drug Metab. Dispos. (1999), 27(2), 180-187.
20. Jacobsen, W.; Kirchner, G.; Hallensleben, K.; Mancinelli, L.; Deters, M.; Hackbarth, I.; Benet, L. Z.; Sewing, K-F.; Christians, U. Comparison of cytochrome P-450-dependent metabolism and drug interactions of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors lovastatin and pravastatin in the liver. Drug Metab. Dispos. (1999), 27(2), 173-179.
21. Bachus R; Bickel U; Thomsen T; Roots I; Kewitz H The O-demethylation of the antidementia drug galanthamine is catalysed by cytochrome P450 2D6. Pharmacogenetics (1999), 9(6), 661-668.
22. Bourrie, M.; Meunier, V.; Berger, Y.; Fabre, G. Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. J. Pharmacol. Exp. Ther. (1996), 277(1), 321-32.