

# Prioritization of Clinical Drug Interaction Studies Using *In Vitro* Cytochrome P450 Data: Proposed Refinement and Expansion of the “Rank Order” Approach

A. David Rodrigues\*

Bristol-Myers Squibb, Princeton, New Jersey 08543, USA

**Abstract:** For any new chemical entity (NCE), *in vitro* inhibition constants ( $K_i$ ) for the different human cytochrome P450 (CYP) forms can be ranked (lowest to highest). The CYP form with the lowest *in vitro*  $K_i$  is evaluated first clinically, employing a suitable probe drug like midazolam (CYP3A4), theophylline (CYP1A2), (*S*)-warfarin (CYP2C9) and desipramine (CYP2D6), and the NCE is classified as a “none”, “weak”, “moderate”, or “strong” inhibitor. In turn, the classification governs the next steps. A two stage strategy, *in vitro* ranking followed by classification, has the potential to enable decision making within an industrial and regulatory setting. With additional refinement and validation, the approach could be applied to mechanism-based inhibitors, inducers and substrates of CYPs also.

**Key Words:** Cytochrome P450, classification, interactions, inhibition, *in vitro*, strategy.

## INTRODUCTION

Inhibition of different human cytochrome P450 (CYP) enzymes can be studied *in vitro* using human liver microsomes and a battery of CYP form-selective substrates [1-4]. In each case, the new chemical entity (NCE) is evaluated in parallel with the appropriate positive controls and inhibition parameters such as  $IC_{50}$  (concentration of inhibitor leading to 50% decrease in activity),  $K_i$  (inhibition constant for reversible inhibition),  $k_{inact}$  (maximal rate of enzyme inactivation in the presence of a mechanism-based inhibitor) and  $K_i$  (inhibitor concentration supporting half maximal rate of inactivation in the presence of a mechanism-based inhibitor) are generated. As a result, most pharmaceutical companies now regularly include such *in vitro* data in their submissions to regulatory agencies [1]. In turn, agencies have acknowledged the usefulness of this information, which can be used to assess whether or not an *in vivo* drug-drug interaction is likely to occur, and if further *in vivo* clinical studies of drug interactions are necessary [2,5-7].

Despite progress *in vitro*, and increased success of *retrospective in vitro-in vivo* correlations, it is accepted widely that quantitative (*prospective*) predictions are difficult and can be confounded by numerous factors [8-14]. Therefore, many investigators have avoided the extrapolation of *in vitro* data, and have chosen increasingly to use it as a “guide” only. In this instance, one considers the concentration of the inhibitor *in vivo* ( $[I]$ ), relates it to the *in vitro*  $K_i$ , and assesses the interaction as “likely” ( $[I]/K_i > 1.0$ ), “possible” ( $[I]/K_i = 0.1$  to  $1.0$ ), or “remote” ( $[I]/K_i < 0.1$ ) [1]. Unfortunately, having taken great care to generate high quality *in vitro* data, one is forced to estimate the inhibitor concentration at the enzyme. Such estimates are often erroneous in the absence of clinical drug interaction data [8, 9, 11, 13]. Alternatively, it is possible to simply rank the *in vitro*  $K_i$ s in order of potency

(lowest  $K_i$  to highest  $K_i$ ) [15]. The CYP form with the lowest  $K_i$  is evaluated first clinically, so that the *in vitro* data enable the prioritization of clinical studies only. Such an approach is gaining greater acceptance, as sponsors try to facilitate decision making.

At the same time, PhRMA (The Pharmaceutical Research and Manufacturers of America) has proposed a clinically-based classification system for inhibitors of CYP3A4. Inhibitors are classified based on their impact on midazolam pharmacokinetics. Specifically, the increase in the area under the plasma concentration-time curve (AUC) for oral midazolam reported as a geometric mean ratio or “GMR” (versus placebo). The NCE is classified as “weak” (AUC GMR  $> 1.25$  to  $< 2.0$ ), “moderate” (AUC GMR  $\geq 2.0$  to  $4.9$ ), or “strong” (AUC GMR  $> 5.0$ ) inhibitor [1]. A “none inhibitor” classification is assigned when the 90% confidence interval about the GMR falls entirely within with range 0.8 to 1.25. Recently, the US Food and Drug Administration has expanded the classification system to include other forms of CYP [16].

For brevity, the present discussion will focus on four CYP forms only (CYP3A4, CYP2D6, CYP1A2, and CYP2C9). It is proposed that the clinical classification system can be combined with the rank order approach and, with additional refinement, the combined strategy can form the basis of a roadmap that enables decision making within an industrial and regulatory setting. Although the discussion is focused on reversible inhibitors, mechanism-based inhibitors, substrates and inducers of CYP are discussed briefly.

## CHOOSING THE APPROPRIATE CYP PROBE DRUGS

In order for a classification system to work, the appropriate probe drugs have to be chosen for each CYP form. The choice of probe will depend upon a number of factors such as safety, the ease of analysis (e.g., parent drug in plasma), pharmacokinetics and sensitivity to the effects of CYP inhibitors. Sensitivity will depend on the fraction metabolized by the inhibited CYP in question ( $f_{m,CYP}$ ), which is the prod-

\*Address correspondence to this author at the Bristol-Myers Squibb, Pharmaceutical Research Institute, Mailstop F14-04, P.O. Box 4000, Princeton, NJ 08543, USA; Tel: (609) 252-7813; Fax: (609) 252-7156; E-mail: david.rodrigues@bms.com

uct of  $f_m$  and  $f_{CYP}$  [10,17-20,21]. The former represents the fraction metabolized by all CYPs and the latter is the fraction of total CYP-dependent metabolism catalyzed by the individual (target) CYP. Pharmacokinetic models indicate that the most appropriate oral CYP probes are well absorbed, extensively metabolized by the target CYP ( $f_{m,CYP} \geq 0.7$ ; <5% of the dose recovered unchanged or as non-CYP metabolites), exhibit linear pharmacokinetics, and provide the best dynamic range with respect to changes in parent AUC. Such a dynamic range allows one to resolve “weak” from “moderate” and “moderate” from “strong” inhibitors [10,17-20,21]. In this regard, drugs such as midazolam (CYP3A4), (S)-warfarin (CYP2C9), theophylline (CYP1A2), and desipramine (CYP2D6) can serve as clinical probes (Table 1). *In vitro* reaction phenotype data show that these drugs are metabolized by one major CYP form, which has been corroborated clinically with potent inhibitors [17-20]. For some of the drugs (e.g., (S)-warfarin and desipramine) their CYP selectivity has been confirmed with genotyped subjects [21]. More importantly, these same CYP form-selective substrates can be used with human liver microsomes. This allows better integration of *in vitro*  $K_i$  data and clinical data.

As described in Table 1, ketoconazole and mibefradil can be classified as strong inhibitors of CYP3A4 based on their effect on midazolam AUC, while fluconazole and diltiazem are moderate inhibitors. Fentanyl and roxithromycin do not impact midazolam AUC and are both classified as weak inhibitors [1]. Similarly, with theophylline as probe, zafirlukast is classified as a strong inhibitor of CYP1A2 [22], zileuton and fluvoxamine as moderate inhibitors [23, 24], and diltiazem as a weak inhibitor of the enzyme [25]. Employing desipramine as probe, both quinidine and fluoxetine are classified as strong inhibitors of CYP2D6 [26, 27]. Duloxetine and cimetidine are classified as moderate and weak inhibitors of CYP2D6, respectively [28, 29]. Likewise, miconazole (strong), fluconazole (moderate), fluvastatin and metronidazole (weak) are differentially classified as inhibitors of CYP2C9 using (S)-warfarin [19, 30-32].

## ILLUSTRATION OF STRATEGY (CYP INHIBITION)

For the sake of discussion, a hypothetical example is shown in Fig. (1). The NCE in question has been evaluated as an inhibitor of CYP3A4, CYP2C9, CYP2D6 and CYP1A2 *in vitro* using human liver microsomes (CYP form-selective substrates) and the  $K_i$  values have been determined. Time-dependent (mechanism-based) inhibition (MBI) has been ruled out. In addition, the compound is not an inducer of CYPs in human primary hepatocytes, so it is anticipated that induction will not confound interpretation of the clinical data. All *in vitro* assays have been performed under linear conditions (time of incubation and protein concentration) using robust and sensitive analytical methods. Such methods allow one to use a low concentration of microsomal protein (e.g., < 0.1 mg/mL) and reduce (differential) non-specific binding across different CYP assays. This is important because one needs to minimize the impact of non-specific binding on the ranking of  $K_i$ s.

In the example, the compound is an inhibitor of CYP3A4 ( $K_i = 1 \mu\text{M}$ ), CYP2C9 ( $K_i = 10 \mu\text{M}$ ), CYP1A2 and CYP2D6 ( $K_i > 50 \mu\text{M}$ ). At this moment, the  $K_i$ s are ranked in order of potency (CYP3A4 > CYP2C9 > CYP1A2 = CYP2D6) and this governs the first clinical drug interaction study. If  $K_i$ s are not available, then the  $IC_{50}$  for each CYP is determined at a fixed concentration of substrate (e.g.,  $\sim K_m$ ) and the values are also ranked in order of potency. In all cases, no attempt is made to predict the  $[I]/K_i$  ratio for each CYP. Because the *in vitro* potency is highest for CYP3A4, an oral midazolam study is conducted first with the appropriate dose of the NCE. Dose selection would be based on existing clinical (multiple dose) data.

Upon completion of the midazolam study, the compound is classified as a “none”, “weak”, “moderate” or “strong” inhibitor of CYP3A4 (Table 1). If the NCE is classified as a “strong” inhibitor then its development would have to be terminated, or evaluated critically in terms of medical need (Fig. 1). A decision not to terminate would mean that the

**Table 1. Classification of Weak, Moderate and Strong Inhibitors of CYP**

Probe Drug (Target CYP)	$f_{m,CYP}^a$	AUC GMR <sup>b</sup>		
		> 1.25 to < 2.0 (“Weak”)	≥ 2.0 to 4.9 (“Moderate”)	≥ 5.0 (“Strong”)
Midazolam (CYP3A4)	≥ 0.7	Fentanyl (1.4) Roxithromycin (1.5)	Fluconazole (3.0) Diltiazem (3.7)	Ketoconazole (8.7) Mibefradil (8.9)
Theophylline (CYP1A2)	≥ 0.7	Diltiazem (1.3)	Zileuton (2.0) Fluvoxamine (3.3)	Zafirlukast (7.0)
Desipramine (CYP2D6)	≥ 0.7	Cimetidine (1.6)	Duloxetine (2.9)	Quinidine (6.7) Fluoxetine (10)
(S)-Warfarin (CYP2C9)	≥ 0.7	Fluvastatin (1.3) Metronidazole (1.6)	Fluconazole (2.0)	Miconazole (5.0)

<sup>a</sup>Fraction (probe drug) metabolized by the target CYP;  $f_{m,CYP}$  is the product of the fraction metabolized by all CYPs ( $f_m$ ) and the fraction of total CYP-dependent metabolism catalyzed by the individual (target) CYP ( $f_{CYP}$ ). For the four probe drugs described, only a small fraction of the dose is recovered unchanged (<5%) and metabolism via CYPs is extensive ( $f_m \sim 1.0$ ).

<sup>b</sup>Area under the plasma concentration-time curve (AUC) geometric mean ratio (GMR) for the probe drug in the presence of inhibitor drug (versus placebo). Example inhibitor drugs have been taken from the literature and are shown (GMR in parentheses) [1, 19, 22-28, 30-32]. Inhibitors are considered to have “no effect” on the CYP in question if the 90% confidence intervals about the GMR fall entirely within the range 0.8 to 1.25.

CYP with the next highest  $K_i$  (CYP2C9) is evaluated clinically. With the data from the two clinical drug interaction studies it would be possible to determine if potent inhibition is limited to CYP3A4 only. Classification of the NCE as a “weak” or “none” inhibitor of CYP3A4 would preclude additional CYP probe studies, because the “worst case”  $K_i$  had been evaluated and midazolam is a sensitive CYP3A4 probe. Classification as a “moderate” inhibitor of CYP3A4 might also warrant a follow up study with (S)-warfarin.

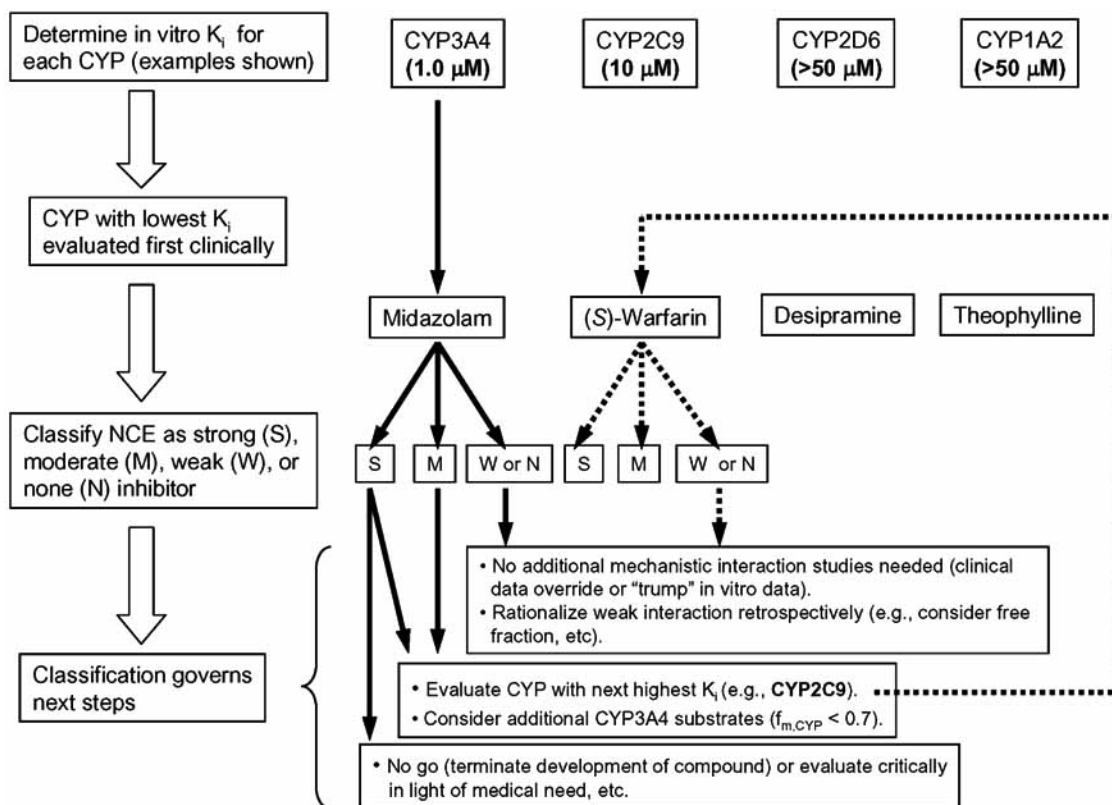
In turn, the effect of the compound on the AUC of (S)-warfarin governs the next steps (Fig. 1). For example, a desipramine or theophylline study would be conducted if the compound was a strong or moderate inhibitor of CYP2C9. Obviously, the development of any new chemical entity classified as a strong inhibitor of CYP3A4, CYP2C9 and CYP2D6 would have to be questioned. On the other hand, if the NCE is classified as a none or weak inhibitor of CYP2C9, then there would be no need to assess additional CYP forms clinically.

The approach described above can be applied to NCEs with different *in vitro* profiles. For example, if the different  $K_i$  values are similar (within 2-fold), and the CYPs cannot be resolved, then any one of the four CYPs can be evaluated first clinically. Because quantitative predictions are difficult, it may be prudent also to conduct at least one CYP probe

study for an NCE that is a weak reversible inhibitor (e.g.,  $K_i > 50 \mu\text{M}$ ;  $\text{IC}_{50} > 100 \mu\text{M}$ ). In this instance, an oral midazolam study can be conducted as a default, because this covers inhibition of both gut and liver CYP3A4. NCEs that exhibit relatively weak reversible inhibition of CYPs *in vitro* should be evaluated for MBI also. If MBI is observed, then the different CYP forms can be ranked (highest to lowest) on the basis of their  $k_{\text{inact}}/K_i$  ratio. By analogy with *in vitro*  $K_i$  ranking, it follows that the CYP form with the highest  $k_{\text{inact}}/K_i$  ratio is evaluated first clinically (Fig. 1).

**WHAT ABOUT CYP SUBSTRATES ?**

In the case of a substrate, the contribution of each individual CYP to total CYP-dependent metabolism ( $f_{\text{CYP}}$ ) can be determined *in vitro* using standard reaction phenotyping approaches [1, 21]. Therefore, the  $f_{\text{CYP}}$  values can be ranked (highest to lowest) and the CYP form with the highest  $f_{\text{CYP}}$  is evaluated first clinically. For example, if CYP3A4 contributes greatly to total CYP-dependent metabolism *in vitro* ( $f_{\text{CYP3A4}} > 0.6$ ), then a clinical study with ketoconazole is conducted first. Ketoconazole is a potent CYP3A4 inhibitor and has been used clinically [1, 33, 34]. Here one would report the result as an increase in AUC (GMR versus placebo) also. The NCE would be classified as a “none substrate” if the 90% confidence interval about the GMR fell entirely within with range 0.8 to 1.25. The contribution of CYP3A4



**Fig. (1).** Evaluation of a new chemical entity (NCE) as a human CYP inhibitor. *In vitro*  $K_i$ s for the different CYP forms are ranked in order of potency. The CYP with the lowest  $K_i$  is evaluated first (CYP3A4 shown) and a clinical study with the appropriate probe (midazolam) is conducted. The NCE is classified as a “none”, “weak”, “moderate” or “strong” inhibitor. This classification governs the next steps. A similar decision tree is possible for mechanism-based inhibitors of different CYP forms. In this instance, the CYP forms can be ranked in order of  $k_{\text{inact}}/K_i$  ratio (highest to lowest) and the one with the highest ratio is evaluated first clinically.

towards the overall clearance of the NCE would be classified as “low” (e.g., AUC GMR  $\geq 1.25$  to  $< 2.0$ ), “intermediate” (e.g., AUC GMR  $> 2.0$  to  $4.9$ ), or “extensive” (e.g., AUC GMR  $> 5.0$ ) and a decision tree similar to that described in Fig. (1) could be set up. Additional factors, such as the NCE’s therapeutic index, would be taken into account during the decision making process.

Clinical studies with inhibitors of other CYPs, like CYP2D6 (paroxetine) or CYP2C9 (fluconazole), can be conducted also if the corresponding  $f_{CYP}$  values are highest *in vitro* [33]. For CYPs like CYP2C19, CYP2C9, CYP2D6, CYP3A5, and CYP2B6, one can also dose the NCE in question to subjects that have been genotyped for different alleles, or phenotyped as “poor metabolizers” or “extensive metabolizers” [21].

In reality, the two step strategy described above can be implemented in one of three ways. The first involves a follow up clinical study and classification of the compound “at risk” in the absence of any human data related to  $f_m$ . Although this enables rapid classification of the NCE, it is possible that in some instances one ends up with discordant data. For example, if  $f_{CYP3A4}$  is high *in vitro* ( $> 0.6$ ), and the compound is classified as a none CYP3A4 substrate, then one would have to assess the contribution of additional CYPs, excretion of unchanged parent drug (renal and biliary), and other metabolic (non-CYP) pathways involving conjugation or non-microsomal oxidations (see below).

The second approach involves initial estimates of  $f_m$ , based on data from a single dose (non-radiolabel) Phase I study; it is possible to profile urine and look for unchanged parent drug following a non-radiolabel dose. If the majority of the dose (e.g.,  $> 80\%$ ) is recovered unchanged in urine, then the inhibition of CYP would not be clinically relevant when renal function is normal. In this instance, the *in vitro* reaction phenotype data would have to be assessed in light of a low  $f_m$  ( $< 0.2$ ) prior to any clinical drug interaction study.

Alternatively, a human radiolabel study would be conducted early in a clinical program and the results integrated with *in vitro* reaction phenotype data before conducting any clinical study with a CYP inhibitor. This would enable more accurate estimates of  $f_m$  if the majority of the radiolabel (as parent, CYP and non-CYP metabolites) was recovered in the urine. If a considerable fraction of the dose is recovered in the feces, poor absorption of parent and biliary secretion of drug-related material would complicate the interpretation of fecal metabolic profiles and estimates of  $f_m$ . This would warrant the harvesting and profiling of human bile.

#### WHAT ABOUT CYP INDUCERS ?

Induction of human CYPs can be assessed *in vitro* with primary cultures of hepatocytes [1, 35]. Therefore, it is possible to obtain a dose response curve for each inducible CYP (e.g., CYP1A2, CYP3A4 and CYP2B6), fit the data, and determine  $EC_{50}$  (concentration of NCE that gives 50% of the maximal response) and the  $E_{max}$  (maximal response) [35]. The  $E_{max}/EC_{50}$  ratio for each CYP is then ranked (highest to lowest), and the CYP with the highest ratio is evaluated first clinically. For example, if the ratio is highest for CYP3A4 then one would conduct a follow up clinical study with oral

midazolam. By analogy with inhibitors (Fig. 1), the classification of the NCE as a “none inducer” (AUC GMR 0.8 to 1.25), “weak inducer” (e.g., AUC GMR 0.8 to 0.6), “moderate inducer” (e.g., AUC GMR  $< 0.6$  to 0.4), or “strong inducer” (e.g., AUC GMR  $< 0.4$ ) of CYP3A4 would determine the next steps if the induction of additional CYP forms was observed *in vitro*.

#### CONCLUSIONS

A relatively simple strategy has been proposed that combines ranking of *in vitro*  $K_i$ s with a clinically-based classification system for CYP inhibitors. The latter employs commonly used and widely accepted probe drugs, like midazolam (CYP3A4), theophylline (CYP1A2), (S)-warfarin, (CYP2C9) and desipramine (CYP2D6), and can be expanded to include additional CYP forms if needed (e.g., CYP2C8, CYP2B6 and CYP2C19). Within an industrial and regulatory setting, the combined strategy has the potential to enable decision making once *in vitro*  $K_i$  data become available. With additional refinements and validation, the approach could be expanded to include mechanism-based inhibitors, inducers and substrates of the different CYPs.

For such a strategy to be implemented, some challenges remain. For example, when reporting AUC as a GMR, what if the 90% confidence intervals fall either side of a classification boundary (e.g., “weak” versus “moderate” or “moderate” versus “strong”) ? How would this impact the classification of a compound ? Additionally, the list of preferred clinical probes would have to be shortened considerably [1], so that there was only one preferred probe for each form of CYP and a more standardized study protocol (e.g., dose of probe drug, NCE dose regimen, subject group size). In the long run, such standardization would greatly simplify on-line drug interaction databases, it would facilitate the comparison of different drugs, and enable *in vitro-in vivo* correlations with greater numbers of compounds.

There is no doubt that progress with CYP inhibitors, inducers and substrates is needed, so that the strategy can be expanded to include newer probe drugs for non-CYP enzymes and drug transporters. Given the rapid progress in these areas, it is already apparent that selective clinical probes are needed as follow up to *in vitro* studies [36-42].

#### REFERENCES

- [1] Bjornsson, T. D.; Callaghan, J. T.; Einolf, H. J.; Fischer, V.; Gan, L.; Grimm, S.; Kao, J.; King, S. P.; Miwa, G.; Ni, L.; Kumar, K.; McLeod, J.; Obach, R. S.; Roberts, S.; Roe, A.; Shah, A.; Snikeris, F.; Sullivan, J. T.; Tweedie, D.; Vega, J.M.; Walsh, J.; Wrighton, S. A. *Drug Metab. Dispos.* **2003**, *31*, 815.
- [2] Tucker, G. T.; Houston, J. B.; Huang, S. M. *Br. J. Clin. Pharmacol.* **2001**, *52*, 107.
- [3] Wrighton, S. A.; Ring, B. J.; Vanden Branden, M. *Toxicol. Pathol.* **1995**, *23*, 199.
- [4] Rodrigues, A. D.; Lin, J. H. *Curr. Opin. Chem. Biol.* **2001**, *5*, 396.
- [5] Davit, B.; Reynolds, K.; Yuan, R.; Ajayi, F.; Conner, D.; Fadiran, E.; Gillespie, B.; Sahajwalla, C.; Huang, S. M.; Lesko, L. J. *J. Clin. Pharmacol.* **1999**, *39*(9), 899.
- [6] Yuan, R.; Parmelee, T.; Balian, J. D.; Uppoor, R. S.; Ajayi, F.; Burnett, A.; Lesko, L. J.; Marroum, P. *Clin. Pharmacol. Ther.* **1999**, *66*, 9.
- [7] Huang, S. M.; Lesko, L. J.; Williams, R. L. *J. Clin. Pharmacol.* **1999**, *39*, 1006.
- [8] Lin, J. H. *Curr. Drug Metab.* **2000**, *1*, 305.

- [9] Grime, K.; Riley, R. J. *Curr. Drug Metab.* **2006**, *7*, 251.
- [10] Ito, K.; Iwatsubo, T.; Kanamitsu, S.; Ueda, K.; Suzuki, H.; Sugiyama, Y. *Pharmacol. Rev.* **1998**, *50*, 387.
- [11] Pelkonen, O.; Raunio, H. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 49.
- [12] Blanchard, N.; Richert, L.; Coassolo, P.; Lave, T. *Curr. Drug Metab.* **2004**, *5*, 147.
- [13] Wienkers, L. C. *Eur. J. Pharmaceu. Sci.* **2002**, *15*, 239.
- [14] Bachmann, K. A. *Curr. Drug Metab.* **2006**, *7*, 1.
- [15] Obach, R.S.; Walsky, R. L.; Venkatakrisnan, K.; Houston, J. B.; Tremaine, L. M. *Clin. Pharmacol. Ther.* **2005**, *78*, 582.
- [16] <http://www.fda.gov/cder/drug/drugInteractions/tableSubstrates.htm#classInhibit>
- [17] Ito, K.; Hallifax, D.; Obach, R. S.; Houston, J. B. *Drug Metab. Dispos.* **2005**, *33*, 837.
- [18] Galetin, A.; Burt, H.; Gibbons, L.; Houston, J. B. *Drug Metab. Dispos.* **2006**, *34*, 166.
- [19] Kunze, K. L.; Trager, W. F. *Drug Metab. Dispos.* **1996**, *24*, 429.
- [20] Yao, C.; Kunze, K. L.; Kharasch, E. D.; Wang, Y.; Trager, W.F.; Ragueneau, I.; Levy, R. H. *Clin. Pharmacol. Ther.* **2001**, *70*, 415.
- [21] Rodrigues, A. D.; Rushmore, T. H. *Curr. Drug Metab.* **2002**, *3*, 289.
- [22] Katial, R. K.; Stelzle, R. C.; Bonner, M. W.; Marino, M.; Cantilena, L. R.; Smith, L. J. *Arch. Intern. Med.* **1998**, *158*, 1713.
- [23] Granneman G. R.; Braeckman R. A.; Locke C. S.; Cavanaugh J. H.; Dube L. M.; Awni W. M. *Clin. Pharmacokinet.* **1995**, *2*, 77.
- [24] Rasmussen, B. B.; Jeppesen, U.; Gaist, D.; Brosen, K. *Ther. Drug Monit.* **1997**, *19*, 56.
- [25] Soto, J.; Sacristan, J. A.; Alsar, M. J. *Ther. Drug Monit.* **1994**, *16*, 49.
- [26] Brosen, K.; Gram, L. F. *Eur. J. Clin. Pharmacol.* **1989**, *37*, 155.
- [27] Bergstrom, R. F.; Peyton, A. L.; Lemberger, L. *Clin. Pharmacol. Ther.* **1992**, *51*, 239.
- [28] Skinner, M. H.; Kuan H. Y.; Pan, A.; Sathirakul, K.; Knadler, M. P.; Gonzales, C. R.; Yeo, K. P.; Reddy, S.; Lim, M.; Ayan-Oshodi, M.; Wise, S. D. *Clin. Pharmacol. Ther.* **2003**, *73*, 170.
- [29] Steiner, E.; Spina, E. *Clin. Pharmacol. Ther.* **1987**, *42*, 278.
- [30] O'Reilly, R. A.; Goulart, D. A.; Kunze, K. L.; Neal, J.; Gibaldi, M.; Eddy, A. C.; Trager, W. F. *Pharmacol. Ther.* **1992**, *6*, 656.
- [31] Kim, M. J.; Nafziger, A. N.; Kashuba, A. D.; Kirchheiner, J.; Bauer, S.; Gaedigk, A.; Bertino, J.S. *Eur. J. Clin. Pharmacol.* **2006**, *62*, 431.
- [32] O'Reilly, R.A. *N. Engl. J. Med.* **1976**, *295*, 354.
- [33] Ouellet, D.; Bramson, C.; Roman, D.; Remmers, A.E.; Randinitis, E.; Milton, A.; Gardner, M. *Br. J. Clin. Pharmacol.* **2006**, published on line at <http://www.blackwell-synergy.com/toc/bcp/0/0>.
- [34] Chung, E.; Nafziger, A. N.; Kazierad, D. J.; Bertino, J. S. *Clin. Pharmacol. Ther.* **2006**, *79*, 350.
- [35] Persson, K. P.; Ekehed, S.; Otter, C.; Lutz, E. S.; McPheat, J.; Masimirembwa, C. M.; Andersson, T. B. *Pharm. Res.* **2006**, *23*, 56.
- [36] Shitara, Y.; Sato, H.; Sugiyama, Y. *Ann. Rev. Pharmacol. Toxicol.* **2005**, *45*, 689.
- [37] Mizuno, N.; Niwa, T.; Yotsumoto, Y. *Pharmacol. Rev.* **2003**, *55*, 425.
- [38] Kusuhara, H.; Sugiyama, Y. In *Drug-Drug Interactions* Rodrigues A.D., Ed.; Marcel Dekker, New York, **2002**, pp. 123-188.
- [39] Zhang, L.; Strong, J. M.; Qiu, W.; Lesko, L. J.; Huang, S. M. *Mol. Pharmaceut.* **2006**, *3*, 62.
- [40] Court, M. H. *Methods Enzymol.* **2005**, *400*, 104.
- [41] Baranczewski, P.; Kallin, A.; Andersson, A.; Hagigi, S.; Aberg, M.; Postlind, H.; Mankowitz, L. *Assay Drug Dev. Technol.* **2004**, *2*, 345.
- [42] Uchaipichat, V.; Mackenzie, P. I.; Elliot, D. J.; Miners, J. O. *Drug Metab. Dispos.* **2006**, *34*, 449.