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Comparison of Different Algorithms for Predicting Clinical Drug-Drug Interactions, Based on the Use of CYP3A4 in Vitro Data; Predictions of Compounds as Precipitants of Interaction

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Investigation of Different Algorithms for Predicting Clinical DDI

Abbreviations

used are: AUC, area under the concentration-time curve; TDI, Time-dependent Inhibition; CYP450, cytochrome P450; HLMs, human liver microsomes.

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Abstract

Cytochrome P4503A4 (CYP3A4) is the most important enzyme in drug metabolism and since it is the most frequent target for pharmacokinetic drug-drug interactions (DDI) it is highly desirable to be able to predict CYP3A4-based DDI from in vitro data. In this study, the prediction of clinical DDI for 30 drugs on the pharmacokinetics of midazolam, a probe substrate for CYP3A4, was done using in vitro inhibition, inactivation, and induction data. Two DDI prediction approaches were employed which account for effects at both the liver and intestine. The first was a model which simultaneously combines reversible inhibition, time-dependent inactivation, and induction data with static estimates of relevant in vivo concentrations of the precipitant drug to provide point estimates of the average magnitude change in midazolam exposure. This model yielded a success rate of 88% in discerning DDI with a mean fold error of 1.74. The second model employed was a computational physiologically-based pharmacokinetic model that uses dynamic estimates of in vivo concentrations of the precipitant drug as well as accounts for interindividual variability among the population (Simcyp^{TM}). This model yielded success rates of 88% and 90% (for 'steady-state' and 'time-based' approaches, respectively) and mean fold errors of 1.59 and 1.47. From these findings it can be concluded that in vivo DDI for CYP3A4 can be predicted from in vitro data, even when more than one biochemical phenomenon occurs simultaneously.

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Introduction

A major focus of the pharmaceutical industry is directed towards early prediction of the likelihood and the magnitude of drug-drug interactions. DDI's involving CYP3A4 are particularly important, as mibefradil, terfenadine, astemizole, cisapride and cerivastatin were all removed from the US market in recent years due, at least in part, to safety issues exacerbated by CYP3A4 DDI. An understanding of the risk for DDI associated with a new chemical entity is a key component of both the drug discovery and development processes. The earlier that risks for DDIs can be identified for new chemical entities under consideration as potential drugs, the greater the probability that this risk can be removed via drug design efforts.

For compounds already in clinical development, in vitro DDI projections can be utilized to prioritize and optimize the design of the appropriate clinical DDI studies. Evaluations of the impact of a new chemical entity (Palmer et al., 2001) on a specific enzyme pathway utilizing probe substrates may be extrapolated to other drugs whose clearances are via the same pathway and to situations where several pathways may be impacted simultaneously**.** Alternatively, NCEs may be evaluated for DDI via interactions with drugs determined to have potential for co-medication and, in this situation, the impacts on multiple pathways may need to be considered for study design optimization. Evaluations built using in vitro probe substrate data and simulated patient populations also enable the scientist to predict the range of magnitude of DDI in individual subjects who may have reduced metabolic capacity.

Numerous methods of predicting these interactions (Galetin et al., 2008), (Ohno et al., 2008), (Brown et al., 2006), (Blanchard et al., 2004), (Ito et al., 2004), (Kanamitsu et al., 2000a) have utilized variety of mathematical models that require static values of precipitant concentrations in the intestine and liver for the prediction of DDI. However, at present, there is no consensus on

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the in vivo precipitant concentration that should be used. For instance, unbound systemic concentration has been widely used for predicting DDI caused by induction and time-dependent inhibition while estimated unbound portal concentration has been the preferred value for reversible inhibition.

One of the difficulties when conducting a DDI risk assessment is how to integrate data from in vitro interaction experiments, such as reversible inhibition studies, time-dependent inhibition studies, and induction studies, into an overarching evaluation of the impact of the co-administration of one compound with another. An example of this is the herbal agent St. John's Wort, where it was demonstrated to be a potent in vitro inhibitor of CYP3A4 (Obach et al., 2000). However, in vivo it was shown to be an inducer of CYP3A4, and this was also seen in in-vitro induction studies (Moore et al., 2000). Projecting potential clinical DDIs from in vitro data has progressed from specific endpoint analysis based on the relationship between the projected therapeutic concentration of the drug and its reversible binding affinity for the particular enzyme of interest (I/K_i) (Kanamitsu et al., 2000a), (Tucker et al., 2001), (Bachmann and Lewis, 2005), (Blanchard et al., 2004) to a comprehensive analysis including the simultaneous evaluation of the potential impact of reversible inhibition, time-dependent inhibition, and induction (Fahmi et al., 2008b).

The idea of a mathematical model for drug-drug interactions, developed from in vitro data, offers a quantitative approach to improving decision making in drug development and discovery. With the Simcyp approach, the combined knowledge of in vitro DDI data, and clinical pharmacokinetics of the drug can be used to simulate various clinical DDI trial scenarios (Einolf et al., 2007), (Jamei et al., 2009), to identify an efficient and effective clinical DDI study

strategy. This eliminates the need for numerous unnecessary clinical DDI studies and accelerates the availability of therapy to patients.

In this study, we have compared two models for predicting DDIs. We have utilized a mathematical model which simultaneously incorporates reversible inhibition, time-dependent inhibition, and induction for both impact on liver and intestines for CYP3A4-based DDI. In addition, we have utilized the Mechanistic Dynamic Model from a population based ADME simulator (Simcyp®).

Methods

Data Source

Clinical midazolam DDI data were collected from the University of Washington Metabolism and Transport drug interaction database (http://www.druginteractioninfo.org/). Thirty drugs involving 50 clinical DDI studies were chosen for this study, based on available data from clinical studies with midazolam. In vitro data reflecting competitive inhibition, time-dependent inhibition and induction of CYP3A4 were collected from the scientific literature (Table 2). In vitro competitive inhibition data as well as time-dependent inhibition data used in this study were from enzyme kinetic data gathered using human liver microsomes. In vitro induction data used were from human cryopreserved hepatocytes system. Of the 30 drugs used in this study, 13 drugs exhibited competitive inhibition with an IC_{50} of less than 10 μM, 12 drugs exhibited time-dependent inactivation, and 11 compounds exhibited induction. Five drugs exhibited all three interaction mechanisms in vitro (troleandomycin, fluoxetine, mibefradil, saquinavir and verapamil).

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CYP3A4 Prediction Mathematical Equation

The equation used to predict the magnitude of DDI (expressed as AUC'/AUC) was previously reported and is shown below, expressed as the ratio of area under the exposure – time curve in the presence (AUC'_{po}) and absence (AUC_{po}) of a pharmacokinetic drug-drug interaction (Fahmi et al., 2008b). This combined mathematical model is based on calculating the net effect of competitive inhibition, inactivation and induction in both the intestine and liver.

$$\frac{AUC'_{po}}{AUC_{po}} = \left(\frac{1}{[A \times B \times C] \times f_m + (1 - f_m)}\right) \times \left(\frac{1}{[X \times Y \times Z] \times (1 - F_G) + F_G}\right) \qquad \text{Net Effect Equation}$$

Where "A" is the term for TDI for the liver $A = \dfrac{k_{deg,H}}{k_{deg,H} + \dfrac{[I]_H \times k_{inact}}{[I]_H + K_I}}$

"B" is the term for Induction for the liver $B = 1 + \dfrac{d \bullet E_{max} \bullet [I]_H}{[I]_H + EC_{50,I}}$

"C" is the term for reversible inhibition in the liver $C = \dfrac{1}{1 + \dfrac{[I]_H}{K_i}}$

"X" is the term for TDI for the intestine $X = \dfrac{k_{deg,G}}{k_{deg,G} + \dfrac{[I]_G \times k_{inact}}{[I]_G + K_I}}$

"Y" is the term for Induction for the intestine $Y = 1 + \dfrac{d \bullet E_{max} \bullet [I]_G}{[I]_G + EC_{50,I}}$

"Z" is the term for reversible inhibition for the intestine $Z = \dfrac{1}{1 + \dfrac{[I]_G}{K_i}}$

$[I]_G$ and $[I]_H$ represent concentrations of inhibitor relevant for the intestine and liver, respectively. For the intestine, an estimate for $[I]_G$ was made using the equation described by (Rostami-Hodjegan et al., 2004) for all calculations. For liver, free systemic C_{max} was used for

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the inactivation (term A) and induction (term B) portions of the expression, while free portal C_{max} was used for the reversible inhibition portion of the expression (term C). The true numerical value for CYP3A4 enzyme degradation rate k_{deg} can make a huge impact on the prediction but it cannot be directly measured in humans in vivo. A wide variety of values of k_{deg} have been published (Thummel and Wilkinson, 1998), (Venkatakrishnan et al., 2003), (McGinnity et al., 2006), (Riley et al., 2007), (Yang et al., 2008), (Galetin et al., 2008). In previous reports, the value used for k_{deg} were derived from modeling the time course of reversal of DDIs caused by induction or inactivation of P450 enzymes in human study subjects. The current most used values for the degradation rates for CYP3A4 (k_{deg}) are 0.00032 min^{-1} based on $t_{1/2}$ = 36 hr and 0.00048 min^{-1} based on $t_{1/2}$ = 24 hr, for the liver and intestine, respectively (Obach et al., 2005; Obach et al., 2007; Fahmi et al., 2008b). E_{max}, EC_{50} and "d" represent the maximum fold induction observed in cultured human hepatocytes, the concentration of inducer associated with half-maximum induction, and a calibration factor "d" (0.3), as described previously (Fahmi et al., 2008b).

F_G has been estimated by several approaches and is well described by Galetin et al., 2008, where the reported value of F_G for midazolam ranges between 0.4 to 0.79 (Ito et al., 2004), (Brown et al., 2006), (Chien et al., 2006). In this study, the value used for fraction of midazolam evading first pass intestinal metabolism (F_G) was 0.57 (Obach et al., 2007), which is closer to the default midazolam value embedded in Simcyp (0.5).

In this study, the value used for the fractional contribution of CYP3A4 to the metabolism of midazolam in the liver (f_m) was 0.93 (Obach et al., 2007) and the value embedded in Simcyp is 0.99.

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Simcyp

Simcyp® Population-Based ADME Simulator (version 7.1) was used to perform time-based and steady-state simulations of clinical drug-drug interaction studies according to the referenced publications, or from Pfizer internal clinical study reports. A northern European Caucasian population was used for the demographics data as provided in the software, and specific study designs were replicated with respect to age range, gender ratio, and number of subjects. Model input parameters for midazolam were used as supplied in the software. Model input parameters for the following precipitant drugs were used as defined in the software; fluconazole, ketoconazole, terbinafine, fluvoxamine, diltiazem, erythromycin, fluoxetine, saquinavir, verapamil and itraconazole. For the other precipitant drugs (roxithromycin, gatifloxicin, simvastatin, azithromycin, clarithromycin, cimetidine, atomoxatine, chlorzoxazone, ranitidine, mibefradil, troleoandomycin, nefazodone, paracoxib, valdecoxib, atorvastatin, carbamazepine, rifampin and conivaptan), input parameters were obtained from various literature sources or estimated (Table 1). The predicted effects of co-administration of the various CYP3A4 precipitants on midazolam exposure were determined using Simcyp, where simulations were carried out using two different approaches, time-based and steady-state. Each drug was simulated with 10 trials and 10 subjects which led to a total of 100 simulations for each combination.

Comparisons of model predictability

The bias of the prediction models was assessed from the geometric mean-fold error (GMFE), which equally weighs over- and under-predictions and the root-mean-square error (RMSE) was calculated to provide a measure of the precision for the predictions:

$$\mathrm{RMSE} = \sqrt{\frac{\sum (\text{predicted DDI - observed DDI})^2}{\text{number of predictions}}}$$

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$$GMFE = 10^{\frac{\sum \left| \log\left(\frac{\text{predicted DDI}}{\text{actual DDI}}\right) \right|}{\text{number of predictions}}}$$

Results

The in vivo drug-drug interaction data gathered from the University of Washington database and utilized in this study are summarized in Table 1. Thirty drugs involving 50 midazolam clinical DDI studies were included in this study. In eight of the clinical studies, midazolam was administered intravenously (flumazenil, parecoxib, atorvastatin, fluconazole, gatifloxicin, ketoconazole, nitrendipine and saquinavir). Two precipitant drugs flumazenil and parecoxib were administered intravenously. Based on clinical outcome, inhibitors were classified based on their magnitude of the interaction according to the FDA Draft Guidance published in September 2006 (Guidance for Industry: Drug interaction studies: study design, data analysis and implications for dosing and labeling http://www.fda.gov/cder/guidance/6695dft.pdf). Two precipitant drugs namely; carbamazepine and rifampin were classified as strong inducers, which precipitated a significant AUC decrease in midazolam exposure. Eight precipitant drugs namely; clarithromycin, conivaptan, itraconazole, ketoconazole, mibefradil, nefazadone, saquinavir and troleandomycin were classified as strong inhibitors, which precipitated an AUC increase in midazolam exposure more than 5-fold. Seven precipitant drugs namely; clarithromycin, conivaptan, diltiazem, erythromycin, fluconazole, saquinavir and troleandomycin were classified as moderate CYP3A4 inhibitors, which precipitated an increase in midazolam AUC of ≥ 2 but <5-fold. Twenty two clinical studies showed weak to no drug-drug interaction, where AUC ratios were 0.8 to 2 in the presence of precipitant drug. Dependent on precipitant dose, as in the cases of clarithromycin and conivaptan, increased dose lead to an increase of the magnitude of clinical DDI, as expected. Also, fluconazole, ketoconazole and saquinavir dosed yielded a

reduced increase in midazolam exposure when midazolam was dosed intravenously compared to orally due to the elimination of first pass intestinal metabolism.

In vitro data reflecting competitive inhibition, time-dependent inactivation and induction of CYP3A4 were collected from the scientific literature (Table 2). Of the 30 drugs used in this study, 13 drugs exhibited competitive inhibition with an IC_{50} of less than 10 μM, 12 drugs exhibited time-dependent inactivation, and 11 compounds exhibited induction. Five drugs exhibited all three interaction mechanisms in vitro (troleandomycin, fluoxetine, mibefradil, saquinavir and verapamil).

To account for all known mechanisms affecting CYP3A4 activity, data from the three possible mechanisms: induction, inactivation, and competitive inhibition were used simultaneously, to make a prediction on the AUC ratio change. Table 3 shows the AUC ratio predictions, by applying the combined model (Fahmi et al., 2008b), as well as the two models of Simcyp.

Although DDI was predicted in all cases with a varying degree of accuracy (Figure 1 and Tables 3&4), no false positive cases were observed. However, a few examples of significant over prediction of the magnitude of DDI were observed with fluoxetine and ketoconazole based on all models, roxithromycin and mibefradil based on Simcyp models, and fluconazole based on the mathematical model. Also a few examples of significant under prediction of DDI were observed with conivaptan (Simcyp) and troleandomycin (mathematical model). The most significant overprediction (138 vs 9) was noted with mibefradil when Simcyp was used under steady state conditions. The clinical pharmacokinetics for midazolam in the clinical midazolam-mibefradil DDI (Veronese et al., 2003) show a different pattern to that predicted by Simcyp in

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that the midazolam concentrations 24 hours post dose are significantly higher using Simcyp prediction than were observed clinically. In contrast, the midazolam levels were still high at 24 hour in the Simcyp time-based simulation. Thus, although the time-based DDI simulation looks superior to that of steady-state, the Simcyp midazolam profile in the presence of mibefradil does not match the clinical profiles. In summary, there are a number of factors which could be contributing to overprediction of the DDI by Simcyp.

The combined mathematical model, steady state and time-bound Simcyp approaches predicted a 'correct' DDI result (interaction or no interaction, defined as a 2-fold change in exposure) in 44, 44, and 45 of the 50 trials, respectively. The corresponding GMFE values were calculated as 1.74, 1.59 and 1.47, for the combined mathematical model, steady-state and time-bound approaches, respectively, as shown in Table 4. Of the trials that had a clinical DDI effect greater than or equal to two fold (n=27), the increase in AUC was predicted within 50% of the actual value in 21, 21, and 24 of the trials for the combined mathematical model, steady-state and time-based approaches, respectively. The corresponding GMFE values were calculated as 1.88, 1.64 and 1.51, for the combined model, steady-state and time-based approaches, respectively. Overall, the combined mathematical model and Simcyp yielded comparable performance in predicting in vivo DDI from in vitro data.

Discussion

Drug-drug interaction caused by the effect of one drug on the clearance of a second is an important consideration in clinical practice. The use of concomitant medications for multiple indications in individual patients is commonplace, especially in elderly patients who have multiple medical problems. In other indications, more than one drug may be indicated to treat multiple inter-related symptoms of a single disease (e.g. psychiatric disorders, cardiovascular

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disease, cancer, etc.). In others, multiple drugs may be needed to treat challenging infections (e.g. HIV, bacterial infections). CYP3A4 is the single most important drug metabolizing enzyme, and is involved in the clearance of over half of clinically used drugs. Effects on CYP3A4 activity are the most frequent mechanisms of DDI, and the pharmacopeia is rife with examples of drugs that inhibit, inactivate, and induce this enzyme. Thus, in the research and development of new pharmacotherapies, the prediction of pharmacokinetic DDI is important, and in vitro approaches for prediction are valuable since they can be used in drug design as well as in selection of candidate compounds for further development with a reduced propensity for causing DDI.

Over the past few years, considerable progress has been made in the development of approaches to predict DDI from in vitro data. For the phenomena of reversible inhibition, irreversible inactivation, and induction, approaches to predict DDI from in vitro data have been developed, but these different mechanisms have been approached separately. For irreversible inactivation, the pioneering work of Hall and colleagues defined relationships between inactivation parameters measured in vitro (k_{inact} and K_I) and in vivo parameters (plasma concentrations, k_{deg}) to predict the magnitude of CYP3A4 DDI, including impact on activities in both liver and intestine (Mayhew et al., 2000; Wang et al., 2004). These concepts were extended to include other P450 enzyme targets (Obach et al., 2007). For reversible inhibition, demonstration of correlation between in vivo DDI and in vitro inhibition potency (K_i) was also demonstrated across a broad panel of P450 inhibitors, importantly showing that estimated portal vein C_{max} was a more accurate parameter to use for $[I]_{in\ vivo}$ than systemic concentrations (Kanamitsu et al., 2000b), (Obach et al., 2006)). Prediction of the magnitude of DDI caused by enzyme inducers is even more challenging since the molecular mechanism is indirect (i.e. the inducer is not binding

with the enzyme itself but acting upon proteins involved in regulation of enzyme transcription). For CYP3A4 inducers, prediction of the magnitude of DDI has been accomplished using a correlative approach using in vitro parameters E_{max} and EC_{50}, (Sinz et al., 2006, Fahmi et al., 2008a). However, in some instances, a compound can affect the activity of a given P450 enzyme by two or three of these mechanisms simultaneously. For example, ritonavir has been demonstrated to be a potent inhibitor and mechanism-based inactivator of CYP3A4 as well as a PXR activator (Zhou, 2008). Prediction of DDI for such an agent is challenging as it is difficult to ascertain which of the phenomena will dominate in the in vivo response, and would require a clinical DDI study to determine what the effect will be. To that end, a "combined model" was proposed for CYP3A4-based DDI for those compounds that exhibit reversible inhibition, inactivation, and induction (Fahmi, et al., 2008b), and it was demonstrated to perform well in the prediction of DDI.

However, most mathematical models provide point estimates of the average DDI, assuming one precipitant concentration and the same in vitro inhibition kinetic parameters effectively assuming the same CYP3A4 enzyme level across the population. Clearly, there is a degree of uncertainty associated with using such data in that the risk to individuals is not evaluated. Although CYP3A4 is the most abundant P450 subfamily in human liver, its level of expression can vary enormously (>10-fold) among individuals. Therefore, using inter individual variability and having the ability to input details of study design in relation to exposure time during interaction studies are important factors in simulating a clinical study. This is where computer simulated program can have an advantage since the concentration used in the model is dynamic and changes with time along with the ability to choose a specific population (poor metabolizer, impaired renal function, etc.) as well as using multiple subjects in the simulation representing

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their variable content of their drug metabolizing enzymes. The approach involves the combination of the concepts used in these aforementioned prediction methods with population pharmacokinetic modeling to provide not only point estimates of DDI magnitudes but also simulation of DDI across different individuals and groups (Einolf et al., 2007); (Rostami-Hodjegan and Tucker, 2007).

The Simcyp steady state approach can potentially show the effect of population variability in DDI and allow direct comparison to the combined mathematical model without accounting for trial design. The Simcyp time-based approach simulations permit concentration changes over time, mimicking actual trials design. Although there is no marked improvement using the time-based Simcyp approach, there is some trend towards better predictions in the parameters shown in Table 4 (vs both the combined model and the steady-state Simcyp model). Indeed, in some instances, this is because of differences in the [I] concentrations used and also in the nature of the input values to the Simcyp models. However, there are differences in the way that the two models handle both reversible and time dependent inhibitors. Einolf (2007) utilized the steady state Simcyp model (referred to as “mechanistic static models” which consider important mechanistic factors such as fractional metabolism (f_m) and the nature of interaction. However, since these models fix the concentrations of substrate at lower level than K_m and fix the level of interacting drug (precipitant) for the a given dose, they cannot account for any trial elements apart from the dose of inhibitor. Thus, dose staggering, variable absorption rate for precipitant drugs, and the effect of volume of distribution on elimination rate and interaction cannot be investigated. The time-based approach “referred to as mechanistic dynamic model" by Einolf et al (2007) assesses the concentration changes over time; facilitating mimicking actual trials design. While the second model assesses the time- and concentration-dependent enzyme

inhibition using unbound systemic plasma concentrations that the liver encounters (i.e. portal vein concentration), the steady state model uses two different "fixed" values of inhibitor concentrations for inhibiting the metabolism during first-pass and subsequent passes; namely highest portal vein concentration following the oral dose of the inhibitor and average steady state concentrations in plasma following multiple oral doses at set intervals. The multiple differences between the model assumptions and their sensitivity (or lack of it) to certain parameters (volume of distribution, accumulation, dosing interval, absorption rate, etc) makes it difficult to expect a uniform pattern regarding the predicted level of DDI when comparing the results from steady state and time-based approach. However, a higher degree of confidence in the model can be obtained by visualisation of the pharmacokinetic profile afforded by the time-based output and comparison with clinical data.

The objective of the present study was to determine the performance of the combined model and the population/simulation model (Simcyp) in the prediction of DDI using midazolam DDI studies reported in the scientific literature as a test set. The population model can be useful in the identification of subgroups at greater risk for clinically relevant interactions. For instance, there is considerable variability in the expression of CYP3A4 and CYP3A5. Those individuals with high expression of CYP3A4 will likely have a greater fraction of clearance of the victim drug via that pathway and thus inhibition may have greater magnitude in these individuals. A total of 50 clinical DDI studies in which midazolam was the affected drug were gathered from the literature with 30 drugs tested as precipitants of these interactions. The interactions ranged from a 16-fold increase caused by ketoconazole to a 96% decrease caused by carbamazepine, with several drugs causing no interaction. The combined model yielded a good success rate for

predicting these DDI, with a mean-fold error of 1.74 and a rate of success (i.e. correctly categorizing a DDI based on a boundary of 2-fold) of 88% (44 of 50 correct) (Table 4). Simcyp V 7.1 offers two simulation approaches referred to as "Steady State" and "Time-Based." From this dataset, it appeared that the time-based approach performed somewhat better, with a mean-fold error of 1.47 vs 1.59 fold. However, the steady-state approach results were skewed by a single outlier prediction (Table 4). Success rates for categorizing DDI as less than or greater than 2-fold was over 88% for both models. The performance of Simcyp in this study was similar to that described by (Einolf et al., 2007) for a different set of DDI studies. Overall, both the combined mathematical model and the Simcyp models performed well in the estimation of CYP3A4-based midazolam DDI from in vitro data, and these would be useful in prospective prediction of CYP3A4 DDI for new compounds.

In the combined model, it was found that the reversible inhibition portion performed the best when the unbound portal vein concentration was used for $[I]_{in\ vivo}$, while for irreversible inactivation and induction, unbound systemic concentration was best (unpublished results). While on the surface this may seem inconsistent, from a physiological perspective it can be rationalized. For reversible inhibition, it is more common for the interaction to be exhibited by an increase in C_{max} (and hence AUC) but not an effect on $t_{1/2}$, particularly, with higher clearance drugs. This indicates that much of the interaction occurs during absorption and hepatic first-pass. After first-pass is complete, the concentrations of inhibitor are diluted to values below these needed to exhibit reversible interaction. The use of estimated unbound portal vein concentrations were also demonstrated to be the most appropriate concentrations in previous work (Obach et al., 2006). For inactivation and induction, systemic concentrations were most appropriate. This also makes sense in that the DDI caused by inactivators and inducers occur on

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C_{max}, AUC, *and* $t_{1/2}$ of the affected drug indicating that the biochemical effect continues to occur after first-pass exposure of the intestine and liver is over. In previous methods in which different values for $[I]_{in\ vivo}$ were considered for inactivation, the free systemic concentration provided the most accurate predictions (Obach et al., 2007) and for induction this was also the case in some investigations (Fahmi et al., 2008a), (Shou et al., 2008) but not others (Sinz et al., 2006). In the Simcyp models, the precise values used for $[I]_{in\ vivo}$ are embedded within the software and the sophistication of the software permits the value for $[I]_{in\ vivo}$ to be dynamic, which is physiologically more realistic.

It should be noted that some of the parameters used by Simcyp and the combined model differ, such as the values for f_m (CYP3A) in the liver. Therefore, some of the performance difference, albeit small, could be due to these different parameters. For example, the combined mathematical model utilizes a hepatic f_m value of 0.93 for midazolam metabolism while Simcyp has an embedded value of 0.99. The sensitivity of prediction of DDI to the f_m parameter, particularly when that value exceeds 0.9, is well-established. In the comparison we have made between Simcyp and the combined mathematical model, our intent was to compare Simcyp as an out-of-the-box application using the parameters embedded in the program. We do not have an explanation for the observation that f_m 0.93 worked with the combined model and 0.99 worked in Simcyp.

Overall, our conclusions are that both the combined mathematical model and computer simulation approaches successfully predict the magnitude of CYP3A4 based DDI, even when the precipitant drug has multiple simultaneous actions (inhibition, inactivation, and/or induction). When the precipitant drug has just one mechanism of action, other more simple approaches can be used, and in these cases the combined model simplifies to algorithms possessing just one of

the interaction terms. Ongoing efforts include the application of this model to DDI for other CYP3A4 cleared drugs besides midazolam as well as application to other P450 enzymes (although in the latter case the number of examples of in vivo DDI caused by multiple mechanisms are far fewer). Results of these investigations will be reported in due course.

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*See supplemental data for additional reference information used for the in vivo data (Table 1).

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Legends for Figures

Figure 1: Predicted versus observed AUC ratios, assuming interaction in the liver and the intestine where prediction is based on the combined mathematical model (A), along with prediction using Simcyp (V7.1) Steady State approach (B) and Time-based approach (C). Solid circles represent compounds showing reversible inhibition in vitro, open circles represent compounds showing reversible inhibition and inactivation in vitro, solid triangle represent compounds showing inhibition and induction, open triangle represent compounds showing all three mechanisms in vitro, open squares represent compounds showing induction only in vitro..

Table 1: Summary of the 48 in vivo clinical studies used in prediction (*observed DDI = ratio of Midazolam AUC in the presence and absence of precipitant

Precipitant	Precipitant Dose	Precipitant Dose Interval	Dose Type	$[I]=C_{sys}$	f_u	Midazolam Dose	Dose Type	*Observed DDI	References
Atomoxetine	60 mg (12 d)	bid	PO	10.6	0.020	5 mg	Oral	1.2	(Sauer et al., 2004)
Atorvastatin	10-40 mg (10 d))	qd	PO	0.023	0.020	0.15 mg/Kg	IV	1.41	(Mc Donnell et al., 2003)
Azithromycin	500 mg (3 d)	qd	PO	0.270	0.120	15 mg	Oral	1.27	(Zimmermann et al., 1996)
Carbamazepine	600-800 mg	bid	PO	34	0.26	15 mg	Oral	0.04	(Backman et al., 1996}
Chlorzoxazone	250 mg	qd	PO	23.6	0.019	5 mg	Oral	1.68	(Palmer et al., 2001)
Cimetidine	400 mg	single dose	PO	3.27	0.790	15 mg	Oral	1.37	(Fee et al., 1987)
Cimetidine	400 mg (1.5 d)	bid	PO	9.92	0.790	15 mg	Oral	1.35	(Salonen et al., 1986)
Cimetidine	800 mg	single dose	PO	9.92	0.790	7.5 mg	Oral	1.50	(Martinez et al., 1999)
Cimetidine	200 mg+400 mg (1.5 d)	qid	PO	3.27	0.790	15 mg	Oral	2.02	(Elliott et al., 1984)
Clarithromycin	250 mg (5 d)	bid	PO	2.40	0.300	15 mg	Oral	3.57	(Yeates et al., 1996)
Clarithromycin	500 mg (7 d)	bid	PO	3.00	0.300	8 mg	Oral	8.40	(Gurley et al., 2006)
Conivaptan	20 mg (5 d)	bid	PO	0.159	0.015	2 mg	Oral	3.49	NDA # 021697
Conivaptan	40 mg (5 d)	bid	PO	0.433	0.016	2 mg	Oral	5.76	NDA # 021697
diltiazem	60 mg (2 d)	tid	PO	0.140	0.220	15 mg	Oral	3.75	(Backman et al., 1994)
Erythromycin	500 mg (5 d)	tid	PO	0.950	0.160	15 mg	Oral	3.81	(Zimmermann, et al. 1996)
Erythromycin	500 mg (7 d)	tid	PO	0.950	0.160	15 mg	Oral	4.42	(Olkkola et al., 1993)
Fluconazole	400 mg	single dose	PO	29.4	0.890	1 mg	IV	1.94	(Kharasch et al., 2005)
Fluconazole	400 mg, 200 mg (5 d)	qd	PO	29.4	0.890	7.5 mg	Oral	3.60	(Olkkola et al., 1996)
Flumazenil	1.75 mg	single dose	IV	0.001	0.600	0.35 mg	IV	0.97	(Rogers et al., 2002)
Fluoxetine	60 mg (5 d) 20 mg (7 d)	qd	PO	0.353	0.050	10 mg	Oral	0.87	(Lam et al., 2003)
Fluvoxamine	50-100 mg (12 d)	bid	PO	0.342	0.230	10 mg	Oral	1.66	(Lam et al., 2003)
Gatifloxicin	400 mg (5 d)	qd	PO	3.82	0.800	1mg	IV	1.08	(Grasela et al., 2000)
Itraconazole	200 mg (4 d)	qd	PO	0.270	0.002	7.5 mg	Oral	10.8	(Olkkola et al., 1994)
Itraconazole	100 mg (4 d)	qd	PO	0.128	0.002	7.5 mg	Oral	5.74	(Ahonen et al., 1995)

Table 1: Summary of the 48 in vivo clinical studies used in prediction (Continued)

Precipitant	Precipitant Dose	Precipitant Dose Interval	Dose Type	[I]=Csys	fu	Midazolam Dose	Dose Type	*Observed DDI	References
Itraconazole	200 mg (6 d)	qd	PO	0.270	0.002	7.5 mg	Oral	6.64	Olkkola et al., 1996
Itraconazole	200 mg (4 d)	qd	PO	0.270	0.002	7.5 mg	Oral	6.16	(Backman et al., 1998)
Ketoconazole	200 mg (1.5 d)	bid	PO	5.42	0.010	2 mg	IV	5.0	(Tsunoda et al., 1999)
Ketoconazole	200 mg (1.5 d)	bid	PO	5.42	0.010	6 mg	Oral	13.6	Tsunoda et al., 1999)
Ketoconazole	200 mg (5 d)	single dose	PO	1.87	0.010	2 mg	Oral	5.2	(McCrea et al., 1999)
Ketoconazole	200 mg (5 d)	single dose	PO	1.87	0.010	2 mg	Oral	6.5	(McCrea, Prueksaritanont et al. 2)
Ketoconazole	200 mg (12 d)	qd	PO	1.88	0.010	10 mg	Oral	8.7	(Lam et al., 2003)
Ketoconazole	200 mg (4 d)	bid	PO	4.76	0.010	75 ug	Oral	6.5	(Eap et al., 2004)
Ketoconazole	400 mg (10 d)	qd	PO	2.82	0.010	5.5 mg	Oral	9.5	(Chung et al., 2006)
Ketoconazole	400 mg (4 d)	qd	PO	2.82	0.010	7.5 mg	Oral	15.9	(Olkkola et al., 1994)
Mibefradil	100 mg	single dose	PO	1.24	0.005	2 mg	Oral	8.9	(Veronese et al., 2003)
Nefazodone	100-200 mg (12 d)	bid	PO	1.73	0.009	10 mg	Oral	5.4	(Lam, Alfaro et al. 2003)
Nitrendipine	20 mg (4 d)	qd	PO	0.041	0.016	20 mg	IV	0.9	(Handel et al., 1988)
Parecoxib	40 mg	single dose	IV	2.53	0.020	0.07 mg/kg	IV	1.1	(Ibrahim et al., 2002)
Ranitidine	150 mg (1.5 d)	bid	PO	1.47	0.850	15 mg	Oral	1.2	(Fee, Collier et al. 1987)
Ranitidine	300 mg	single dose	PO	1.47	0.850	7.5 mg	Oral	1.3	(Martinez et al., 1999)
Ranitidine	150 mg (1.5 d)	bid	PO	1.15	0.850	15 mg	Oral	1.7	(Elliott et al., 1984)
Rifampin	600 mg (5 d)	qd	PO	12.50	0.250	8 mg	Oral	0.1	(Backman et al., 1996)
Roxithomycin	300 mg (6 d)	qd	PO	5.13	0.140	15 mg	Oral	1.5	(Backman et al., 1994)
Saquinavir	1200 mg (5 d)	tid	PO	0.430	0.020	7.5 mg	Oral	5.2	(Palkama et al., 1999)
Saquinavir	1200 mg (5 d)	tid	PO	0.430	0.020	0.05 mg/kg	IV	2.5	Palkama et al., 1999
Simvastatin	80 mg (7 d)	qd	PO	0.026	0.060	2 mg	Oral	1.1	(Prueksaritanont et al., 2000)
Terbinafine	250 mg (4 d)	qd	PO	2.13	0.010	7.5 mg	Oral	0.76	(Ahonen, Olkkola et al. 1995)
Troleandomycin	500 mg (2 d)	bid	PO	1.76	0.038	10 mg	Oral	14.8	(Kharasch et al., 2004)
Valdecoxib	40 mg	bid	PO	2.90	0.020	10 mg	Oral	1.1	Unpublished data
verapamil	80 mg (2 d)	tid	PO	0.480	0.100	15 mg	Oral	2.9	(Backman, Olkkola et al. 1994)

Table 2: CYP3A4 *in-vitro* determined kinetic parameters

	Competitive Inhibition	Time-Dependent Inhibition		Induction		References by order of the in vitro tool
Compound	K_i (uM)	K_I (uM)	k_{inact} (min^{-1})	EC_{50} (uM)	E_{max}	
Atomoxetine	17					Sauer et al., 2004
Atorvastatin	8					Obach et al., 2006
Azithromycin	150	410	0.029			Obach et al., 2006, Venkatakrishnan et al., 2007
carbamazepine	100			55.8	34.3	Fahmi et al., 2008a
Chlorzoxazone	700					Berthou et al., 1995
Cimetidine	115					Obach et al., 2006
Clarithromycin	50	18.9	0.053			Obach et al., 2006, Venkatakrishnan et al., 2007
Conivaptan	0.017	8.06	0.073			NDA # 021697 and unpublished data
diltiazem	30	1.15	0.027			Obach et al., 2006, Venkatakrishnan et al., 2007
Erythromycin	9	13.5	0.041			Obach et al., 2006, Venkatakrishnam et al., 2007
Fluconazole	3.4					Obach et al., 2006
Flumazenil	7.5					Unpublished data
Fluoxetine	8	0.606	0.015	0.54	2.1	Obach et al., 2006, Venkatakrishnan et al., 2007, Fahmi et al., 2008b
Fluvoxamine	21.5					Galetin et al., 2007
Gatifloxicin	150					Obach et al., 2005
Itraconazole	0.005					Obach et al., 2006
Ketoconazole	0.006					Obach et al., 2006
Mibefradil	0.10	2.3	0.400	4.1	6.5	Prueksaritanont et al., 1999, Obach et al., 2006, Fahmi et al., 2008a
Nefazodone	0.45	6.23	0.037			Obach et al., 2007, Unpublished data
Nitrendipine	17			18.2	17.4	Unpublished data
Parecoxib	1000					Unpublished data
Ranitidine	150					Obach et al., 2007
Rifampin	100			0.57	33	Fahmi et al., 2008
Roxithromycin	34	72	0.023			Obach et al., 2007, Polasek et al., 2006
Saquinavir	0.41	0.65	0.260	0.9	34.6	Obach et al., 2007, Ernest et al., 2005, Fahmi et al., 2008
Simvastatin	0.39			25	4	Obach et al., 2007
Terbinafine	150			25	2.3	Obach et al., 2007

Troleandomycin	1.3	2.4	0.032	0.27	15.9	Obach et al., 2007, Fahmi et al., 2008a
Valdecoxib	68			10	2.2	Unpublished data
verapamil	11.50	0.58	0.07	0.16	16.4	Obach et al., 2007, Venkatakrishnam et al., 2007, Fahmi et al., 2008a

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Table 3: Summary of CYP3A4 DDI predictions based on the different models

			Combined Model	Simcyp Steady State Model	Simcyp Time Based Model		
Precipitant	Dose of Precipitant (mg)	Observed DDI	Predicted DDI magnitude	Predicted DDI magnitude	Predicted DDI magnitude	Upper Percentile	Lower Percentile
Drugs Demonstrating Only Reversible Inhibition In Vitro							
Atomoxetine	120	1.0	1.4	1.0	1.0	1.0	1.0
Atorvastatin	25*	1.4	1.0	1.0	1.0	1.0	1.0
Chlorzoxazone	250	1.7	1.1	1.1	1.0	1.0	1.0
Cimetidine	400	1.4	1.7	1.2	1.0	1.0	1.0
Cimetidine	800	1.4	1.7	1.3	1.1	1.1	1.0
Cimetidine	800	1.5	2.2	1.5	1.2	1.4	1.1
Cimetidine	1200	2.0	1.5	1.4	1.1	1.1	1.0
Fluconazole	400*	1.9	7.3	2.5	1.6	2.1	1.2
Fluconazole	200	3.6	11	9.2	2.9	4.4	2.0
Flumazenil	1.75*,**	0.97	1.0	1.0	1.0	1.0	1.0
Fluvoxamine	200	1.7	1.2	1.1	1.0	1.1	1.0
Gatifloxicin	400*	1.1	1.1	1.0	1.1	1.1	1.0
Itraconazole	200	11	3.2	8.2	8.0	18	3.3
Itraconazole	100	5.7	2.5	5.9	4.9	9.5	2.8
Itraconazole	200	6.6	3.2	8.0	3.1	5.8	1.8
Itraconazole	200	6.2	3.2	8.6	7.0	17	2.8
Ketoconazole	400*	5.0	9.2	5.2	3.6	5.6	2.3
Ketoconazole	200	5.2	16	20	13	24	5.8
Ketoconazole	400	14	16	12	8.1	17	3.8
Ketoconazole	200	6.5	16	13	12	22	7.1
Ketoconazole	200	8.7	16	12	10	23	4.8
Ketoconazole	400	6.5	16	20	7.2	13	3.9
Ketoconazole	400	9.5	16	22	8.5	24	3.8
Ketoconazole	400	16	16	21	21	37	9.2
Parecoxib	40*,**	1.1	1.0	1.0	0.90	1.0	0.80
Ranitidine	300	1.2	1.1	1.1	1.1	1.1	1.0
Ranitidine	300	1.3	1.2	1.1	1.0	1.0	1.0
Ranitidine	300	1.7	1.1	1.1	1.0	1.0	1.0
Drugs Demonstrating Reversible Inhibition and Irreversible Inactivation In Vitro							
Azithromycin	500	1.3	1.7	1.7	1.3	1.6	1.2
Clarithromycin	500	3.6	9.0	2.7	4.6	12	2.3
Clarithromycin	1000	8.4	10	4.1	6.2	13	2.5
Conivaptan	40	3.5	2.5	1.9	1.8	2.4	1.4
Conivaptan	80	5.8	3.6	3.1	3.3	6.8	2.1
Diltiazem	180	3.8	4.9	1.6	3.7	7.8	2.1
Erythromycin	1500	3.8	4.0	3.3	5.4	14	2.5

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Erythromycin	1500	4.4	4.0	2.5	3.9	9.6	2.0
Nefazodone	400	5.4	2.4	12	8.7	22	3.0
Roxithromycin	300	1.5	3.0	22	10	27	3.4
Drugs Demonstrating Induction In Vitro							
Carbamazepine	600-800	0.04	0.04	0.02	0.04	0.13	0.01
Rifampin	600	0.05	0.01	0.01	0.03	0.08	0.01
Drugs Demonstrating Reversible Inhibition and Induction In Vitro							
Nitrendipine	20*	0.93	1.00	1.0	1.0	1.0	1.0
Simvastatin	80	1.1	2.1	1.0	1.0	1.1	1.0
Terbinafine	250	0.76	0.89	1.0	1.0	1.0	1.0
Valdecoxib	80	1.1	0.89	1.0	1.0	1.0	1.0
Drugs Demonstrating Reversible Inhibition, Irreversible Inactivation, and Induction In Vitro							
Fluoxetine	40	0.87	3.5	3.2	3.8	9.1	2.1
Mibefradil	100	8.9	8.2	139	14	44	2.5
Saquinavir	3600	2.5	10	3.7	3.9	8.3	1.6
Saquinavir	3600*	5.2	18	13	12	38	3.1
Troleandomycin	1000	15	3.0	15	6.0	10	1.5
Verapamil	240	2.9	7.5	3.1	7.0	16	3.5

*Denotes cases in which midazolam was administered intravenously

**Denotes cases in which the precipitant was administered intravenously

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Table 4. Accuracy of the Combined Mathematical Model and the two Simcyp Models.

	Combined Model	Simcyp Steady-State Model	Simcyp Time-Based Model
All DDI			
GMFE	1.74	1.59*	1.47
RMSE	4.58	5.07*	3.03
Success Rate	88	88	90
DDI > 2X			
GMFE	1.88	1.64**	1.51
RMSE	5.68	5.65**	3.60

*Including one outlier prediction value (mibefradil) yields values for GMFE and RMSE of 1.66 and 19.1, respectively.

**Including one outlier prediction value (mibefradil) yields values for GMFE and RMSE of 1.78 and 25.6, respectively.

Figure 1A

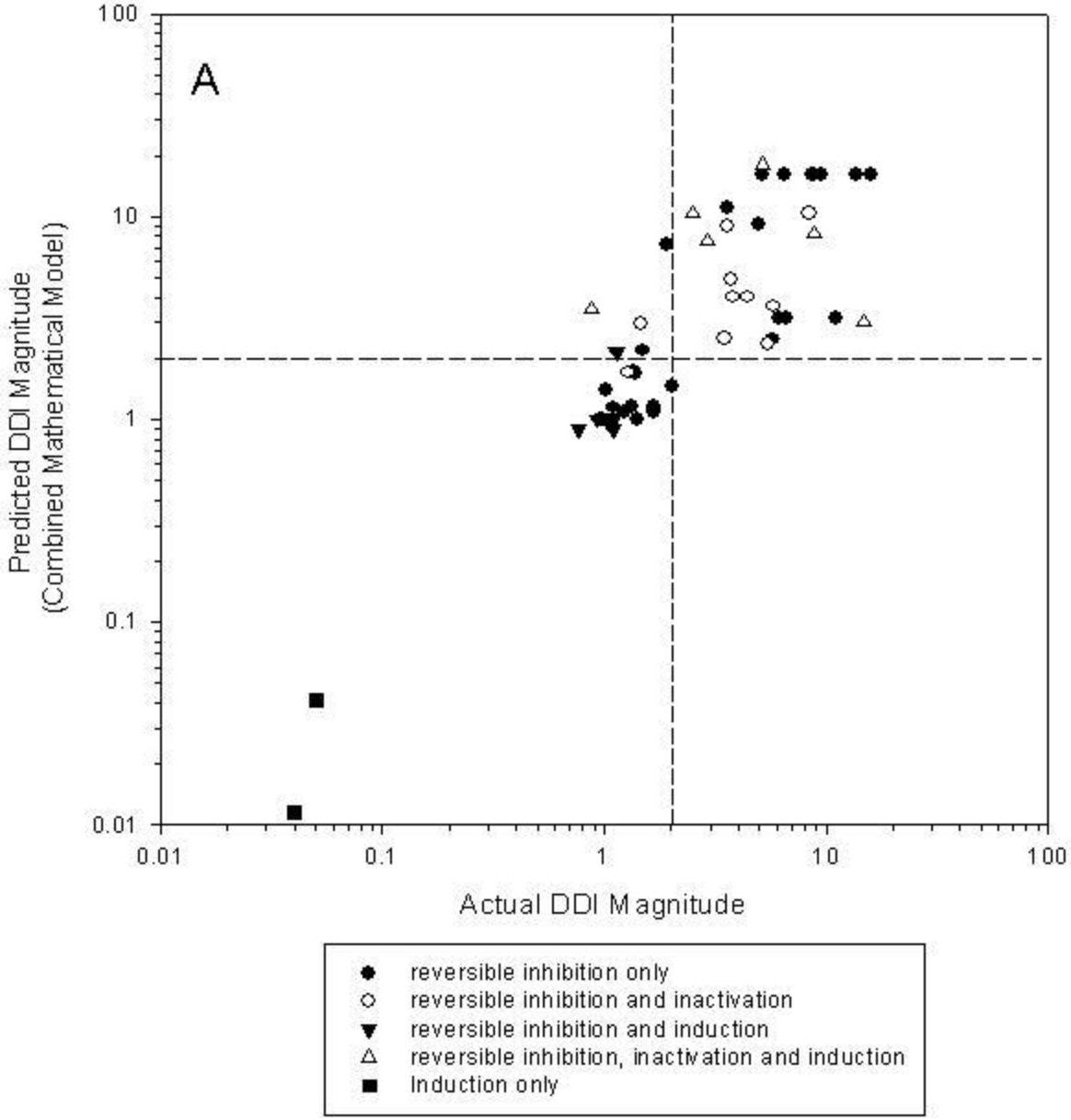

Figure 1B

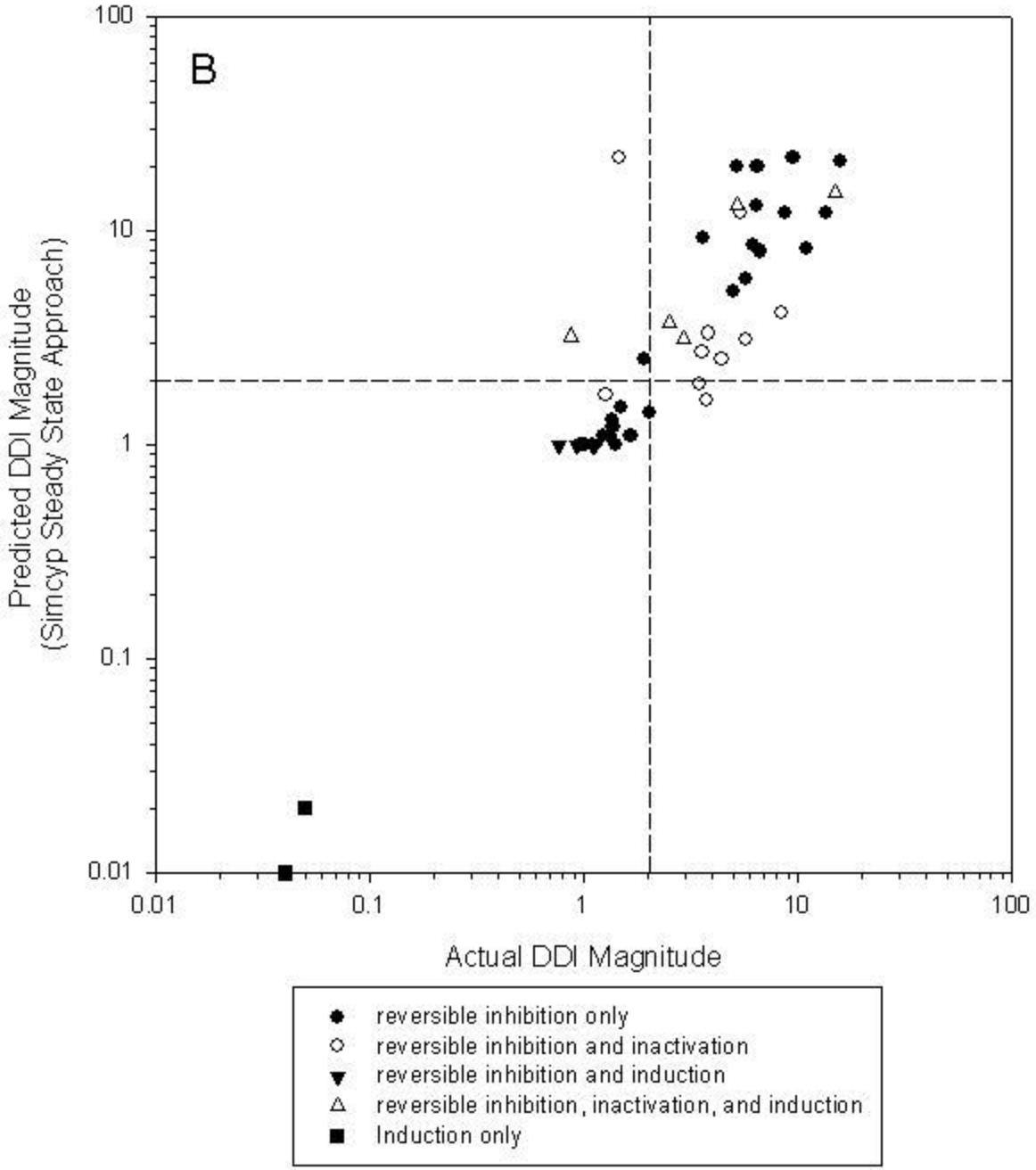

Figure 1C