DRUG-DRUG INTERACTIONS OF ANTI-INFECTIVE DRUGS: UTILITY OF FLUORESCENCE CYP INHIBITION ASSAYS IN DRUG DISCOVERY

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SUMMARY

Drug interactions are often a result of induction or inhibition of cytochrome P450 (CYP) enzymes by co-administered drugs. A high throughput fluorescence assay using cDNA-expressed human CYP isozymes and fluorogenic substrates has been reported for the study of CYP inhibition. We used this assay to evaluate CYP inhibition profiles of 21 marketed anti-infective drugs. We found that six of the eight potent inhibitors identified in this screen (IC_{50} <10 μM against at least one CYP isozyme) correlated with significant drug-drug interactions in the clinic. In contrast, the intermediate and weak inhibitors (IC_{50} >10 μM) did not indicate clinically significant drug interactions. Furthermore, we observed that results obtained in the fluorescence assay correlated with conventional, well-established, low throughput methods that utilize human liver microsomes. These data suggest that

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in the early stages of drug discovery, the fluorescence assay for CYP inhibition could be used in conjunction with a human liver microsomal assay to identify new chemical entities with a potential for drug-drug interactions.

KEY WORDS

drug-drug interactions, anti-infective drugs, CYP inhibition, clinical significance

INTRODUCTION

With an increased reliance on multi-drug therapy and prescription of novel drug classes, drug-drug interactions are being reported fairly frequently. The severity of drug interactions can range from slight alterations in plasma level of co-administered drugs to life-threatening toxicities or sub-optimal clinical outcomes /1-3/. Drugs identified to perpetrate interactions find restricted use. In the event of severe toxicity, the interacting drug may be withdrawn from the market /4,5/. Thus, identification, resolution and prevention of drug interactions are critical for safe and efficacious therapy /6-9/. In addition, the regulatory authorities require that the metabolism of an investigational new drug must be defined during the development phase and its interactions with other drugs should also be explored as part of an adequate assessment of safety and efficacy /9,10/.

The clinical consequences of drug interactions with anti-infectives go beyond the risk of increased toxicity. The presence of an interacting drug in a multi-drug anti-infective regimen can have detrimental effects on therapeutic efficacy /11-13/. Drug interactions that decrease the exposure of the anti-infective drug lead to sub-optimal therapy causing treatment failures. Moreover, sub-therapeutic levels of anti-infectives also lead to a rapid development of resistance. Several clinical reports have indicated that the difference between clinical cure and treatment failure is dependent on the achievement of pharmacokinetic and pharmacodynamic (PK/PD) breakpoints through an appropriate dosing regimen /14-17/.

Drug interactions are often the result of induction or inhibition of cytochrome P450 (CYP) enzymes by co-administered drugs. Since

inhibition of CYP isozymes is more common, CYP inhibition assays are used to identify drug-drug interaction potential with new chemical entities. Traditionally, human or rat liver microsome-based assays have been used to monitor inhibition of CYP isozymes. Since microsomes contain an array of different drug-metabolizing enzymes, substrates that are highly specific towards individual isozymes must be used /18,19/. Moreover, microsomal methods are laborious and require chromatographic separation of the substrate from the metabolites. This makes these assays impractical for use in early drug discovery cascades that demand a short turn-around time. To meet such demands, high throughput screens, such as the fluorescence assay /20/, the cocktail incubation assay by LC-MS-MS /21/, and the radiometric assay /22/, have been reported. The fluorescence assay using cDNA-expressed human CYP isozymes /20,23/ obviates the need for extraction and separation steps and thus provides the highest throughput over all the assays. Several reports have confirmed that with the exception of CYP3A4, the inhibition of individual cDNA-expressed CYP isozymes correlates with the inhibition seen in human microsomes /22,24-26/. We used the fluorescence CYP inhibition assay to study the inhibitory potential of 21 anti-infective drugs against five major drug metabolizing CYPs. Drugs that inhibited CYP2C9 were confirmed in the human liver microsomal assay monitoring diclofenac 4′-hydroxylation. Furthermore, we observed that potent CYP inhibitors with IC_{50} less than 10 μM are often involved in clinically significant drug interactions. This suggests that the fluorescence based CYP inhibition screen can provide a preliminary evaluation of the drug interaction potential in the early stages of drug discovery.

MATERIALS AND METHODS

Amphotericin, ampicillin, azidothymidine, cefaclor, chloramphenicol, ciprofloxacin, clindamycin, clotrimazole, ethambutol, isoniazid, metronidazole, piperacillin, pyrazinamide, roxithromycin, streptomycin, sulfaphenazole, sulfasalazine, tobramycin, trimethoprim, troleandomycin and vancomycin were purchased from Sigma Chemicals, St. Louis, MO, USA. Quinidine sulfate was purchased from Research Biochemicals Inc., MA, USA. Insect cell CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 SUPERSOMES™ and

3-[2-(N,N-diethyl-N-methyl-ammonium)ethyl]-7-methoxy-4-methyl-coumarin (AMMC), were purchased from BD-Gentest™, Woburn, MA, USA. The cytochrome P450 content, protein content, cytochrome C reductase activity and specific activity of each batch of isozymes was provided as a product insert from the manufacturer. Human liver microsomes were purchased from XenoTech LLC, Kansas City, KS, USA. Glucose-6-phosphate, 7-hydroxy-3-trifluoro-coumarin (7-HFC), 7-methoxy-3-trifluoro-coumarin (7-MFC), magnesium chloride hexahydrate, β-NADP, potassium phosphate dibasic, potassium phosphate monobasic, and Trizma® base were purchased from Sigma Chemicals, St. Louis, MO, USA. NADPH was purchased from SuSkanda Organics Inc., Ishaquah, USA. Acetonitrile (HPLC grade) was purchased from Spectrochem, Mumbai, India. Acetic acid, hydrochloric acid, methanol (HPLC grade) and perchloric acid were purchased from S-D-Fine-Chem Ltd, Mumbai, India. Trifluoroacetic acid was purchased from Applied Biosystems, Foster City, CA, USA. Flat-bottom Costar® microtiter plates (Greiner Labortechnik, Frickenhausen, Germany) were used for incubations of recombinant CYP isozymes. Microtiter plates were incubated in a LEAD Instruments Incubator-25 (Bangalore, India). Incubations of human liver microsomes were performed in Eppendorf® tubes (Hamburg, Germany) using a Precision reciprocal shaking bath, Model 25 (Chicago, USA). An electronic 12-channel (50 or 300 µl) Finnpipette® (Helsinki, Finland) was used for dispensing solutions in the microtiter plate assay. Fluorescence measurements were made in a Wallac Victor 1420 multilabel HTS reader (Perkin Elmer, Boston, USA). IC_{50} was calculated using GraphPad Prism 3.0 (GraphPad Software Inc.). The HPLC system used for monitoring diclofenac and its metabolite consisted of a Class VP series Shimadzu HPLC (Shimadzu, Japan), equipped with a DGU-14A online degasser, LC-10AT binary pumps, SIL-10AD autosampler, SCL-10A system controller and SPD-10A UV-Vis detector. Chromatographic separation was achieved using a reverse phase Kromasil 100 column (C18 5 µm, 250 x 4.6 mm; Flexit-Jour Laboratories, Pune, India) at 25°C.

Preparation of stock solutions and serial dilutions

Ampicillin, azidothymidine, cefaclor, clindamycin and isoniazid were dissolved in water. Ethambutol, metronidazole, piperacillin, pyrazinamide, streptomycin, tobramycin and vancomycin were

dissolved in 0.5 M phosphate buffer, pH 7.4. Chloramphenicol, clotrimazole, roxithromycin, sulfaphenazole, trimethoprim and quinidine were dissolved in acetonitrile (AcN). Amphotericin B was dissolved in a solution of AcN:DMSO (2:3, v/v). Ciprofloxacin was prepared in a solution of AcN:phosphate buffer (1:4, v/v) and 0.4% trifluoroacetic acid. Sulfasalazine was dissolved in a solution of AcN:DMSO (94:6, v/v). Eight concentrations of each anti-infective drug were made by three-fold dilutions of an initial stock of approximately 100 μM. Diclofenac sodium was dissolved in 0.1 M Tris buffer, pH 7.5.

Inhibition of CYP isozymes by anti-infective drugs

A high-throughput CYP inhibition screen based on cDNA expressed isozymes and fluorogenic substrates was chosen to evaluate CYP inhibition potential of anti-infective drugs. This assay is based on CYP catalyzed O-dealkylation reactions, which generate an easily detectable fluorescent product. The IC_{50} for each anti-infective drug was determined using the protocol specified in the BD Gentest™ Technical Bulletin (http//:www.gentest.com) /27/. Briefly, the inhibition potential of each drug was evaluated against CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 isozymes. The CYP substrates used in the assays for each isozyme were 3-cyano-7-ethoxycoumarin (CEC) for CYP1A2 and CYP2C19, 7-methoxy-4-trifluoromethylcoumarin (7-MFC) for CYP2C9, 3-[2-N,N-diethyl-N-methylammonium)ethyl]-7-methoxy-4-methylcoumarin (AMMC) for CYP2D6, and 7-benzyloxyresorufin (BzRes) for CYP3A4. These substrates were maintained at the apparent Km for each isozyme. The assay was validated with positive controls: furafylline (CYP1A2), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6) and ketoconazole (CYP3A4). Drugs that inhibited CYP2C9 were confirmed in a secondary screen monitoring diclofenac metabolism in human liver microsomes. Since amphotericin B and sulfasalazine were dissolved in a high concentration of organic solvent, serial dilutions of each solvent were included as a control.

Inhibition of diclofenac-4′-hydroxylation by anti-infective drugs in human liver microsomes

Incubations were carried out in duplicate in a 0.5 ml reaction volume containing human liver microsomes (0.1 mg protein/ml),

diclofenac (3 μM) and an NADPH generating system consisting of $NADP^+$ (1.3 mM), glucose-6-phosphate (3.3 mM) and glucose-6-phosphate dehydrogenase (0.4 U/ml) in 0.1 M Tris buffer, pH 7.5. Serial dilutions of the anti-infective drug were pre-incubated with cofactors and buffer for 5 minutes at 37°C in a water bath. Reaction was initiated by the addition of human liver microsomes. After incubation at 37°C for 15 minutes the reaction was stopped by addition of 200 μl acetonitrile. The tubes were cooled on ice for 5 minutes, vortexed for 15 minutes and centrifuged at 14,000 rpm for 10 minutes to precipitate proteins. Diclofenac and 4′-hydroxydiclofenac were separated on a reverse phase column. The column was eluted at a flow rate of 1 ml/min using two mobile phases: A (30% acetonitrile, 70% water, 1 mM perchloric acid) and B (methanol). The elution conditions were 30% B increasing with a linear gradient to 100% B over 17 minutes. Diclofenac and 4′-hydroxydiclofenac were detected and quantified from the processed samples at 280 nm using Shimadzu Class VP series software by comparison of the absorbance against a standard curve for diclofenac and 4′-hydroxydiclofenac.

Data analysis

The IC_{50} of the positive controls and the test compounds were determined by semi-log plot of the percent inhibition versus concentration of inhibitor using Graph-Pad Prism. The percent inhibition at a particular inhibitor concentration was calculated as shown in equation [1] by relating activity to that in the control incubation without inhibitor.

$$\%\_inhibition = 100 - \frac{activity}{control\_activity} \cdot 100 \qquad \text{Equation [1]}$$

Non-linear regression analysis with an equation for sigmoidal dose-response was used to fit a curve to the plotted data. This equation is solved for 50% inhibition to obtain the IC_{50}. The potencies of the inhibitors were rated as potent (IC_{50} <10 μM), intermediate (IC_{50} = 10-100 μM) and weak (>20% inhibition at ~100 μM).

RESULTS

Inhibition of CYP isozymes by anti-infective drugs

The IC_{50} values for the positive controls were in close agreement with the values reported in the Gentest Technical Bulletin /27/, as shown in Table 1. The IC_{50} and the spectrum of CYP inhibition produced by each anti-infective drug on the five isozymes are reported in Tables 2 and 3. Of the 21 drugs tested, eight drugs (amphotericin, chloramphenicol, clotrimazole, isoniazid, sulfaphenazole, roxithromycin, sulfasalazine, and troleandomycin) were potent inhibitors (IC_{50} <10 μM), three drugs were intermediate inhibitors, and the remaining ten drugs were either weak or non-inhibitors of CYP isozymes. The pattern of isozyme inhibition produced by the anti-infective drugs was variable. While several drugs inhibited CYP3A4, and some inhibited CYP2C19, very few drugs inhibited CYP1A2, CYP2C9 and CYP2D6 (Table 4). When solvent controls were run, the AcN:DMSO (2:3 v/v, resulting in final concentration of 1.2% AcN and 1.8% DMSO) cocktail produced an IC_{50} within two-fold of that observed in the presence of amphotericin B. However, the cocktail used to dissolve sulfasalazine (AcN:DMSO 94:6 v/v, resulting in a final concentration of 2.8% AcN and 0.18% of DMSO) did not produce inhibition.

Human liver microsome assay for confirmation of CYP2C9 inhibition

Drugs that produced greater than 20% inhibition of CYP2C9 in the fluorescence assay (Tables 2 and 3) were tested for their inhibitory activity in the diclofenac 4′-hydroxylation assay. As shown in Table 5, only sulfaphenazole, clotrimazole and sulfasalazine inhibited diclofenac 4′-hydroxylation in human liver microsomes with IC_{50} values of 0.59 μM, 0.37 μM and 8.7 μM, respectively (Table 5). The IC_{50} values obtained in the human liver microsomal assay were within 2-fold of the IC_{50} obtained in the fluorescent assay. Amphotericin B, cefachlor, chloramphenicol, ciprofloxacin and isoniazid did not inhibit diclofenac metabolism when tested at a maximum concentration of around 100 μM.

TABLE 1

Comparison of IC_{50} of positive controls for each CYP isozyme against the reported value from the BD-Gentest Technical Bulletin /27/

CYP isozyme	Positive control	IC_{50} in the fluorescence assay (μM)	Reported IC_{50} (μM)
CYP1A2	Furafylline	1.65 ± 0.12 (n=6)	1.3
CYP2C9	Sulfaphenazole	0.36 ± 0.08 (n=15)	0.27
CYP2C19	Tranylcypromine	0.86 ± 0.15 (n=6)	0.75
CYP2D6	Quinidine	0.008 ± 0.002 (n = 21)	0.014
CYP3A4	Ketoconazole	0.13 ± 0.01 (n = 7)	0.11

Clinical significance of drug interactions with anti-infective drugs

Drug interactions were reported for all drugs that produced potent inhibition of CYP isozymes, with the exception of amphotericin B. As presented in Table 6, the interactions reported included variations in exposure, clearance or half-life of drugs co-administered with anti-infective agents screened in this study. No reports of clinically significant drug interactions were found for anti-infectives that produced intermediate or weak inhibition of CYP isozymes, with the exception of ciprofloxacin.

DISCUSSION

Amphotericin B, chloramphenicol, clotrimazole, isoniazid, roxithromycin, sulfaphenazole, sulfasalazine and troleandomycin were identified as potent inhibitors of CYP isozymes in the fluorescence assay (Table 4). With the exception of amphotericin B and sulfasalazine, all drugs produced clinically significant drug interactions, mainly via inhibition of drug metabolizing CYPs (Table 6). The apparent inhibition observed with amphotericin B was due to the high concentration of DMSO, a solvent known to inhibit CYP isozymes quite dramatically. This was further confirmed with the results of the human liver microsome CYP2C9 assay wherein amphotericin B did not produce inhibition. The lack of clinical interaction with sulfasalazine could probably be attributed to its metabolism in the gastrointestinal tract by intestinal microflora /28/. Furthermore, our results indicate that the CYP isozyme inhibition profile observed was similar to that elucidated using other techniques.

We classified cefaclor, clindamycin, and trimethoprim as intermediate or weak inhibitors of CYP isozymes in the fluorescence assay (Table 4). These drugs were not reported to produce pharmacokinetic alterations of co-administered drugs in the clinic. Ciprofloxacin was the only case in which the inhibitory response to CYP2C9 and CYP2D6 was weak *in vitro* but was reported to be significant *in vivo*. Ciprofloxacin is known to alter the pharmacokinetics of metoprolol or clozapine, theophylline and caffeine, which are substrates for CYP1A2 or CYP2D6 /29-31/. However, drug interactions with ciprofloxacin were previously shown to be non-predictive by CYP inhibition studies /32/. Ampicillin, azidothymidine, ethambutol,

TABLE 2

Inhibitory effects of antibacterial drugs on CYP1A2, CYP2C9 and CYP2C19 measured in the fluorescence inhibition assay

Compound		CYP1A2			CYP2C9			CYP2C19		
		IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)	IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)	IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)
1	Amphotericin	2.9			2.1			NA	-	-
2	Ampicillin		101	NI		106	NI		106	NI
3	Azidothymidine		100	NI		112	NI		60	21%
4	Cefaclor		101	8%		96	41%		99	NI
5	Chloramphenicol		108	NI		97	37%	4.42		
6	Ciprofloxacin		106	NI		111	22%		90	15%
7	Clindamycin		80	5%		96	NI	68.8		
8	Clotrimazole		98	NI	0.25			0.22		
9	Ethambutol		98	NI		129	12%		73	NI
10	Isoniazid		116	10%		110	47%	2.29		
11	Metronidazole		106	NI		112	12%		230	28%

Compound		CYP1A2			CYP2C9			CYP2C19		
		IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)	IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)	IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)
12	**Piperacillin**		100	17%		98	17%		90	31%
13	**Pyrazinamide**		108	10%		102	10%		260	42%
14	**Roxithromycin**		118	12%		95	12%		50	42%
15	**Streptomycin**		101	NI		98	NI			
16	**Sulfaphenazole**	NA	-	-	0.35				79	29%
17	**Sulfasalazine**	25.1			15.9			21		
18	**Tobramycin**		98	NI		98	NI		70	NI
19	**Trimethoprim**		94	18%		94	18%		94	NI
20	**Troleandomycin**		97	NI		97	NI		97	NI
21	**Vancomycin**		98	17%		97	17%		97	NI

[a] Percent inhibition at the maximum concentration tested.
NI = no inhibition was observed at the maximum concentration tested.
NA= data not available.

TABLE 3

Inhibitory effects of antibacterial drugs on CYP2D6 and CYP3A4 measured in the fluorescence inhibition assay

Compound		CYP2D6			CYP3A4		
		IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)	IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)
1	Amphotericin	31.6			54.3		
2	Ampicillin		106	NI		101	NI
3	Azidothymidine		112	NI		100	10%
4	Cefaclor		96	NI	93.5		
5	Chloramphenicol		97	23%		108	20%
6	Ciprofloxacin		111	31%		105	10%
7	Clindamycin		95	NI	49.2		
8	Clotrimazole	10.5			0.97		
9	Ethambutol		129	NI		98	NI
10	Isoniazid		110	47%	3.80		
11	Metronidazole		112	NI		105	18%
12	Piperacillin		98	NI		100	15%

Compound		CYP2D6			CYP3A4		
		IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)	IC_{50} (μM)	Max. test conc. (μM)	Inhibition [a] (%)
13	**Pyrazinamide**		102	NI		108	NI
14	**Roxithromycin**		95	21%	1.95		
15	**Streptomycin**		98	NI		101	NI
16	**Sulfaphenazole**		107	NI	NA	-	-
17	**Sulfasalazine**		90	35%	5.30		
18	**Tobramycin**		98	NI		98	NI
19	**Trimethoprim**		94	NI	59.2		
20	**Troleandomycin**		97	NI	0.97		
21	**Vancomycin**		97	NI		98	NI

[a] Percent inhibition at the maximum concentration tested.
NI = no inhibition was observed at the maximum concentration tested.
NA= data not available.

TABLE 4

Analysis of CYP inhibition potential as measured from the fluorescence and human liver microsomal assay and comparison with the reported clinical significance of CYP inhibition

Compound		In vitro fluorescence CYP inhibition assay					HLM assay		Clinical reports[a]		
		CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP2C9	Reported drug interactions[a]	Clinical significance[a]	Enzyme involved[a]
1	Amphotericin B	Potent	Potent	ND	Int.	Int.	NI	No		
2	Ampicillin	NI	NI	NI	NI	NI	NA	No		
3	Azidothymidine	NI	NI	NI	NI	NI	NA	No		
4	Cefaclor	NI	Weak	NI	NI	Int.	NI	No		
5	Chloramphenicol	NI	Weak	Potent	Weak	NI	NI	Yes	Yes	CYP2C9
6	Ciprofloxacin	NI	Weak	NI	Weak	NI	NI	Yes	Yes	CYP1A2
7	Clindamycin	NI	NI	Int	NI	Int.	NA	No		
8	Clotrimazole	NI	Potent	Potent	Int.	Potent	Potent	Yes	Yes	CYP3A4
9	Ethambutol	NI	NI	NI	NI	NI	NA	No		
10	Isoniazid	NI	Weak	Potent	Weak	Potent	NI	Yes	Yes	CYP3A4
11	Metronidazole	NI	NI	NI	NI	NI	NA	No		CYP2C9 CYP2C19
12	Piperacillin	NI	NI	Weak	NI	NI	NA	No		

Compound		In vitro fluorescence CYP inhibition assay					HLM assay	Clinical reports[a]		
		CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP2C9	Reported drug interactions	Clinical significance[a]	Enzyme involved[a]
13	Pyrazinamide	NI	NI	NI	NI	NI	NA	No		
14	Roxithromycin	NI	NI	Weak	Weak	Potent	NA	Yes	Yes	CYP3A4
15	Streptomycin	NI	NI	NI	NI	NI	NA	No		
16	Sulfiphenazole	NA	Potent	Weak	NI	NA	Potent	Yes	Yes	CYP2C9
17	Sulfasalazine	Int.	Int.	Int.	Weak	Potent	Potent	No		
18	Tobramycin	NI	NI	NI	NI	NI	NA	No		
19	Trimethoprim	NI	NI	NI	NI	Int.	NA	No		
20	Troleandomycin	NI	NI	NI	NI	Potent	NA	Yes	Yes	CYP3A4
21	Vancomycin	NI	NI	NI	NI	NI	NA	No		

Inhibitors are classified as potent (IC_{50} <10 μM), intermediate (Int. IC_{50} = 10-100 μM) and weak (>20% inhibition at ~100 μM).
NI = not inhibitory (<20% inhibition at ~100 μM).
NA = data not available.
ND = not done.
[a] References are provided in Table 6.

TABLE 5

Correlation of IC_{50} values obtained in the MTP and HLM assay for selected drugs identified as CYP2C9 inhibitors

Compound	CYP2C9 IC_{50} in the fluorescence assay	CYP2C9 IC_{50} in the HLM assay	Fold difference[a]
Sulfaphenazole	0.35 µM	0.59 µM	+1.68
Clotrimazole	0.25 µM	0.37 µM	+1.48
Sulfasalazine	15.9 µM	8.7µM	-1.83
Amphotericin	2.1 µM	Not inhibitory at 101 µM	NA

[a] A positive value indicates that the IC_{50} is greater in the HLM assay than in the MTP assay.

metronidazole, piperacillin, pyrazinamide, streptomycin, tobramycin, and vancomycin did not inhibit any of the five CYP isozymes in the fluorescence assay. Of these drugs, only metronidazole is reported to inhibit CYP2C9 and CYP2C19 /33/. In healthy volunteers, metronidazole reduces the clearance of phenytoin by 15%. This interaction is attributed to the inhibition of CYP2C9 and is not clinically significant /34/.

CONCLUSION

Using a set of 21 anti-infective drugs we have shown that the *in vitro* fluorescent assay using recombinant human isozymes can predict metabolism based clinical interactions for most anti-infectives studied. An IC_{50} cut-off at 10 μM in this assay seems to be indicative of a drug interaction with a potential clinical liability. Furthermore, the assay can identify the pattern of isozyme inhibition reliably and provide a faster read-out in comparison to microsomal assays or *in vivo* inhibition studies. By using the fluorescence assay as a primary screen and the human liver microsome inhibition assay as the secondary screen, it is possible to distinguish false positives such as those arising from high organic solvent concentrations, or from inherent fluorescence or fluorescence quenching properties of the drugs. However, one must be cautious of commonly encountered uncertainties and sources of bias and error in extrapolation of these results to predict *in vivo* drug interactions. Such factors include non-specific microsomal binding, solvent effects on enzyme activities, and uncertainties in determining enzyme-available drug concentrations. In addition, the dose and route of administration, atypical multi-site kinetics of drug metabolizing enzymes, and concurrent induction and inhibition, can impact the clinical relevance of drug interactions. Thus, the overall pharmacokinetics of the drug, the roles of other drug metabolizing enzymes, active transporter systems, gut flora, and the presence of metabolites with an inhibitory potential, need to be evaluated. Until then the information from the fluorescence based *in vitro* CYP inhibition assays can only be used as an indication of potential drug interaction liability of new chemical entities.

TABLE 6

Reported drug interactions, mechanism of drug interaction and clinical significance of each interaction

Interacting drug	Drug affected	CYP450(s) involved	Observed change	Clinical significance	Reference
Chloramphenicol	Tolbutamide	CYP2C9	Increased C_p	No	/35/
	Phenytoin	CYP2C19	Decreased CL	Yes	/36/
Ciprofloxacin	Caffeine	CYP1A2	Increased AUC	No (?)	/29/
	Clozapine	CYP1A2	Increased C_p	NE	/37/
	Metoprolol	CYP2D6	Increased AUC	No	/31/
	Theophylline	CYP1A2	Decreased CL	Yes	/30/
Clotrimazole	Tacrolimus	CYP3A4	Increased C_p	Yes	/38/
Isoniazid	Carbamazepine	CYP3A4	Decreased CL	Yes	/39/
	Phenytoin	CYP2C19, CYP2C9	Increased C_p	Yes	/40 41/
	Triazolam	CYP3A4, CYP2C19	Decreased CL	No	/42/
Metronidazole	Phenytoin	CYP2C19, CYP2C9	Increased $t_{½}$	NE (?)	/34/
	Warfarin	CYP2C9	Increased C_p	NE (?)	/33/

Interacting drug	Drug affected	CYP450(s) involved	Observed change	Clinical significance	Reference
Sulfaphenazole	Phenytoin	CYP2C19, CYP2C9	Increased C_p	Yes (?)	/43,44/
	Tolbutamide	CYP2C9	Increased $t_{½}$	Yes	/45/
Troleandomycin	Alfentanil	CYP3A4	Decreased CL	Yes (?)	/46/
	Carbamazepine	CYP3A4	Intoxication	Yes	/47/
	Imipramine	CYP3A4	Decreased CL	No	/48/
	Theophylline	CYP1A2	Decreased CL	NE (?)	/49/
	Triazolam	CYP3A4	Increased AUC	Yes	/50/
Roxithromycin	Midazolam	CYP3A4	Increased AUC and $t_{½}$	No (?)	/51/

CL = clearance; C_p = concentration in plasma; AUC = area under the curve; $t_{½}$ = half life; NE = not evaluated.

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