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**Inhibitory Effects of Selected Antituberculosis Drugs on Common Human Hepatic  
Cytochrome P450 and UDP-glucuronosyltransferase Enzymes ‡§\*\***

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**DMD # 76034**

The running title: **Inhibitory Effects of Anti-TB Drugs on Human CYPs and UGTs**

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ABBREVIATIONS: APAP, acetaminophen; CYP, cytochrome P450; DDI, drug-drug interactions; DMSO, dimethyl sulfoxide; FDA, U.S. Food and Drug Administration; HLM, human liver microsome;  $K_i$ , the inhibition constant for the inhibitor; MEOH, methanol;  $MgCl_2$ , magnesium chloride; NADP, nicotinamide adenine dinucleotide phosphate; TB, tuberculosis; UDPGA, uridine 5'-diphosphoglucuronic acid; UGT, UDP-glucuronosyltransferase; WHO, World Health Organization

## DMD # 76034

### ABSTRACT

The comorbidities of tuberculosis and diseases such as HIV require long-term treatment with multiple medications. Despite substantial *in vitro* and *in vivo* information on effects of rifampicin and isoniazid on human CYPs, there is limited published data regarding the inhibitory effects of other anti-TB drugs on human CYPs and UGTs. The inhibitory effects of 5 first-line anti-TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and rifabutin), and the newly approved bedaquiline, were evaluated for 6 common human hepatic UGT enzymes (UGT1A1, 1A4, 1A6, 1A9, 2B7 and 2B15) *in vitro* using HLMs. Pyrazinamide, ethambutol, rifabutin and bedaquiline were also studied for their inhibitory effects on 8 of the most common human CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A). Rifabutin inhibited multiple CYPs to varying degrees *in vitro*, but with all IC<sub>50</sub> values exceeding 25 μM. Rifabutin and rifampicin also inhibited several human UGTs including UGT1A4. The K<sub>i</sub> value for rifabutin on human hepatic UGT1A4 was 2 μM. Finally, the 6 anti-TB drugs produced minimal inhibition of acetaminophen glucuronidation *in vitro*. Overall, the findings do not raise major concerns regarding metabolic inhibition of human hepatic CYPs and UGTs by the tested anti-TB drugs.

## DMD # 76034

### Introduction

Tuberculosis is one of the leading causes of morbidity and mortality worldwide. The World Health Organization estimated that in 2015 there were an estimated 10.4 million incident TB cases, and 1.4 million deaths from TB, and an additional 0.4 million deaths associated with co-infection with HIV (WHO, 2016). The comorbidity of TB and other diseases requires treatment with multiple medications. Understanding of potential drug-drug interactions (DDIs) is of importance in planning safe and effective combination therapies.

Isoniazid, rifampicin (or rifampin), pyrazinamide, ethambutol, rifabutin, and rifapentine are the principal first-line anti-TB drugs to treat drug-susceptible tuberculosis (Zumla et al., 2013).

Bedaquiline is a novel anti-mycobacterial agent which was approved by FDA in 2012 to treat multidrug resistant tuberculosis (Worley et al., 2014). Among those, rifampicin is a potent inducer of CYPs and UGTs, as well as the P-glycoprotein transport system both *in vitro* (Rae et al., 2001; Soars et al., 2004) and clinically (Baciewicz et al., 2013). Rifampicin is reported also to be an inhibitor of some human CYPs *in vitro* (Kajosaari et al., 2005), but its overall effect is enzymatic induction, reducing systemic concentrations of many drugs (Ochs et al., 1981).

Compared to rifampicin, rifabutin has less potency as a CYP3A inducer and is used as a substitute for rifampicin in patients receiving protease inhibitor and integrase inhibitor-based antiretroviral therapy (Zumla et al., 2013; Baciewicz et al., 2013; WHO, 2010). Isoniazid is known as an inhibitor of many human CYPs *in vitro* (Wen et al., 2002; Polasek et al., 2004) and clinically (Ochs et al., 1981; Ochs et al., 1983).

## DMD # 76034

Both the inductive effects of rifampicin and inhibitory effects of isoniazid on human CYPs have been extensively reported *in vitro* and *in vivo*. However, the data of their effects on human UGTs is limited. Furthermore, the information on other anti-TB drugs is also limited. In this work, inhibitory effects of isoniazid and rifampicin on human hepatic UGTs were studied; and inhibitory properties of the selected anti-TB drugs, including pyrazinamide, ethambutol, rifabutin, and bedaquiline were also studied *in vitro* with human hepatic CYP and UGT enzymes. Acetaminophen is widely used as an analgesic and antipyretic agent. Since APAP glucuronidation is the pathway responsible for converting two-thirds of a dose of APAP into non-toxic glucuronide conjugates, we also evaluated the inhibitory effect of the anti-TB drugs on acetaminophen glucuronidation.

## Materials and Methods

Chemicals and solvents were purchased from Sigma-Aldrich Corp (St. Louis, MO) and Fisher Scientific (Pittsburg, PA). Isoniazid [Synonym: 4-Pyridinecarboxylic acid hydrazide], rifampin [Synonym: rifampicin, or 3-(4-Methylpiperazinyliminomethyl)rifamycin SV], pyrazinamide, ethambutol hydrochloride [Synonym: 2,2'-(1,2-Ethanediyldiimino)bis-1-butanol dihydrochloride], and rifabutin [Synonym: Mycobutin] were purchased from Sigma-Aldrich Corp (St. Louis, MO). Bedaquiline [a mixture of diastereomers, Synonym: 6-Bromo- $\alpha$ -[2-(dimethylamino)ethyl]-2-methoxy- $\alpha$ -1-naphthalenyl- $\beta$ -phenyl-3-quinolineethanol] was purchased from Toronto Research Chemicals Inc. (North York, Canada). Water was purified with a Milli-Q system (Millipore Corporation, Milford, MA).

## DMD # 76034

Liver samples from individual human donors with no known liver disease were provided by the International Institute for the Advancement of Medicine (Exton, PA), the Liver Tissue Procurement and Distribution System, University of Minnesota (Minneapolis, MN, USA), or the National Disease research Interchange (Philadelphia, PA, USA). HLMs were prepared as previously described (Greenblatt et al., 2011; von Moltke et al., 1993a). Fifty-three individual liver microsomal preparations were combined to make a batch of pooled HLMs, by mixing an equal amount of protein from each HLM.

**Inhibition Studies on CYP-mediated Oxidation Using HLMs.** Previously published incubation procedures using HLMs (Greenblatt et al., 2011; von Moltke et al., 2001; Sonnichsen et al., 1995; Giancarlo et al., 2001; Hesse et al., 2000) were used with modifications. Briefly, appropriate substrates and positive controls (Table 1) were added to incubation tubes. The anti-TB drugs were individually added in a series of concentrations to separate incubation tubes. Isoniazid, rifampicin, pyrazinamide, and ethambutol were at concentrations of 0, 10, 60, 100, 200, 400, 600 and 1000  $\mu\text{M}$ ; rifabutin was at concentrations of 0, 10, 60, 100, 200, 400, and 600  $\mu\text{M}$ , except for CYP2C9 and 2D6 with an extra concentration of 1000  $\mu\text{M}$ ; and bedaquiline was at concentrations of 0, 0.78, 1.56, 3.13, 6.25, 12.5, 20 and 25  $\mu\text{M}$ . The solvent (methanol) was evaporated to dryness at 40°C under mild vacuum conditions. Due to their poor solubility in methanol, propofol (the UGT1A9 substrate) and bedaquiline were prepared in DMSO and added directly to incubation tubes (1% DMSO v/v). Methanol at 1% (v/v) in the final incubation mixture was added to reconstitute the anti-TB compounds (except for bedaquiline) after dryness. The incubation mixtures for CYP-mediated oxidation contained 50 mM phosphate buffer (pH 7.5), 5 mM  $\text{MgCl}_2$ , 0.5 mM NADP, isocitrate and an isocitric dehydrogenase regenerating

## DMD # 76034

system, and appropriate amounts of the pooled HLMs. The anti-TB drugs were preincubated with HLMs (without the index substrates) for 20 minutes at 37°C, and then followed by another timed incubation with the substrates (250µL). All incubations were performed in duplicate. Initial tests for detecting IC<sub>50</sub> shifts were carried out by comparing incubations with 20 minutes' preincubation to incubations without preincubation. 100 µL of acetonitrile (or acidified acetonitrile adjusted with 85% H<sub>3</sub>PO<sub>4</sub> for CYP2B6 and 2C9) with internal standards was used to stop the reactions. After centrifugation, the supernatant was transferred to HPLC vials for HPLC-UV or HPLC-fluorescence analysis.

**Inhibition Studies on Glucuronidation Using HLMs.** Previously described incubation procedures were used with modifications (von Moltke et al., 1993b; Court, 2010; Court, 2005). The incubation mixtures for the glucuronidation studies were prepared with 50 mM phosphate buffer (pH 7.5), 5 mM MgCl<sub>2</sub>, alamethicin (50 µg per mg protein), and appropriate amounts of the pooled HLMs. The mixtures were kept on ice for 5 minutes before use. UDPGA was freshly prepared separately in the phosphate buffer. The reactions were initiated by addition of the UDPGA solution (a final concentration of 10 mM) in the incubation mixtures (100uL). All incubations were performed in duplicate. The incubations were conducted without preincubation except for those with β-estradiol (UGT1A1), trifluoperazine (UGT1A4), and APAP, for which the incubations with 20 minutes' preincubation were also conducted. The reactions were stopped by adding 40 µL of acetonitrile (or acidified acetonitrile adjusted with 85% H<sub>3</sub>PO<sub>4</sub> for UGT2B7 and APAP glucuronidation) with internal standards to the incubation mixtures. After centrifugation, the supernatant was transferred to HPLC vials for HPLC-UV analysis.

## DMD # 76034

**K<sub>i</sub> Value for Reversible Enzymatic Inhibition.** Inhibition of UGT1A4 by rifabutin was observed with an IC<sub>50</sub> value of 11 μM, which is low enough to trigger a DDI concern. As there was no IC<sub>50</sub> shift with and without preincubation, the experimental design for reversible enzymatic inhibition (Greenblatt et al., 2011) was applied to determine the K<sub>i</sub> value for rifabutin versus human UGT1A4 using pooled HLMs. Varying concentrations of the index substrate (trifluoperazine) at 0, 2, 5, 10, 20, 34.2, 72.4, 144.9 and 336.6 μM were incubated at 37°C with pooled HLMs in presence of varying concentrations of the inhibitor (rifabutin), at 0, 1.25, 5, 10, 30, and 60 μM respectively. Probenecid at 2.4 mM was used as the positive inhibitory control. After 30 minutes' incubation, the reactions were stopped with 40 μL of acetonitrile (in the incubation mixtures of 100 μL) with the internal standard (phenacetin). After centrifugation, the supernatant was transferred to HPLC vials for HPLC-UV analysis.

## Analytical Methods

Previously described methods, with modifications were used for analysis of the *in vitro* samples in this study (von Moltke et al., 2001; Court, 2005). The HPLC conditions and detection methods are summarized (Supplemental Table 1). APAP glucuronide generated from the *in vitro* incubations was analyzed using the previously described method, with modifications (Zhao et al., 2015). Briefly, the HPLC analysis was carried out using a Hydro-RP column (4 μm, 250x4.6 mm, Synergi Hydro-RP, Phenomenex, Torrance, CA), with a flow rate of 1.2 mL/min. The injection volume was 30 μL, and the UV detection wavelength was 254 nm. A multistep gradient was started at 96.5% mobile phase A (20 mM potassium phosphate buffer, pH 2.2) and 3.5% mobile phase B (methanol) for 5 minutes, increased to 16% B during the next 5 minutes, and reached 20% B at 15 minutes, then to 40% B at 30 minutes, followed by a 9 minutes'



## DMD # 76034

isocratic run at 100% mobile phase *C* (50% H<sub>2</sub>O, 50% methanol), followed by another 10 minutes' isocratic run at 3.5% *B*. The integration and quantitation were done with the software Chemstation (Agilent, Santa Clara, California).

## Data Analysis

**IC<sub>50</sub> Values.** IC<sub>50</sub> values were determined using nonlinear regression as described previously (Greenblatt et al., 2011; von Moltke et al., 2001). Sigmaplot 11.0 was applied for the nonlinear regression procedure. Briefly, the relationships between the formation of the metabolites of the substrates and the inhibitory concentrations of the tested anti-TB drugs were analyzed by nonlinear regression fitting using Equation 1. The IC<sub>50</sub> values were then generated from the IC values using Equation 2 in order to take into consideration the possibility of incomplete inhibition

$$R = 100 \left( 1 - \frac{E_{\max} [I]^b}{[I]^b + IC^b} \right) \quad \text{Equation 1}$$

$$IC_{50} = \frac{IC}{(2E_{\max} - 1)^{1/b}} \quad \text{Equation 2}$$

*R* is the formation rate of the metabolite of interest, expressed as a percentage fraction of the control reaction velocity with no inhibitor; *E*<sub>max</sub>, the maximum degree of inhibition; [*I*], the concentration of the anti-TB drugs; *b*, an exponent; *IC*, the inhibitor concentration producing an *R* value of 50% of (100-*E*<sub>max</sub>), as determined from the nonlinear regression procedure; *IC*<sub>50</sub>, the concentration of the tested anti-TB drugs producing 50% inhibition compared to the inhibitor-free control value, as calculated from the *IC* value using Equation 2.

## DMD # 76034

**$K_i$  Value for Reversible Enzymatic Inhibition.** The  $K_i$  value for rifabutin on human UGT1A4 was determined by nonlinear regression using the reversible inhibition model of full competitive inhibition (Greenblatt et al, 2011) on Sigmaplot 13.0. Significant substrate inhibition of trifluoperazine was observed at the concentration of 336.6  $\mu\text{M}$  in our study, as reported previously (Uchaipichat et al., 2006). Thus, the reversible model of full competitive inhibition was fitted using concentrations of trifluoperazine up to 144.9  $\mu\text{M}$ .

## Results

**$\text{IC}_{50}$  Values for Rifabutin on Human Hepatic CYPs.** Rifabutin inhibited human CYP3A, 2B6, 2D6, 1A2, 2C8 and 2C9 to varying degrees *in vitro* using the pooled HLMs (Table 2, Fig. 1). At the highest tested concentration (600  $\mu\text{M}$ ), no inhibition of human CYP2E1 or 2C19 was observed *in vitro* with rifabutin (Fig. 1).

**$\text{IC}_{50}$  Values for Rifabutin on Human Hepatic UGTs, and  $K_i$  Value for Rifabutin on UGT1A4.** Rifabutin inhibited UGT1A1, 1A4 and 2B15 to varying degrees (Table 2, Fig. 2 and 3), and partially inhibited human UGT1A9 and 2B7 (Table 2, Fig. 2) at a high concentration of 600  $\mu\text{M}$ . The  $\text{IC}_{50}$  values for rifabutin on human hepatic UGT1A4 were 10.8 and 11.3  $\mu\text{M}$  respectively, for the incubations with and without preincubation. The  $K_i$  value for rifabutin on UGT1A4 using trifluoperazine as the index substrate was 2  $\mu\text{M}$ , with the pattern of inhibition consistent with reversible competitive inhibition (Fig. 4).

## **DMD # 76034**

**IC<sub>50</sub> Values for Rifampicin and Isoniazid on Human Hepatic UGTs.** Rifampicin had inhibitory effects on UGT1A1, 1A4 and 2B15 with varying IC<sub>50</sub> values (Fig. 3); partial inhibition of human UGT1A6 was observed at the highest tested concentration (1000 μM) (Fig. 2). No inhibitory effects of isoniazid were observed up to the highest tested concentration (1000 μM) (Fig. 2).

**Inhibitory Effects of Pyrazinamide, Ethambutol and Bedaquiline on Human Hepatic CYPs and UGTs.** Up to the highest tested concentration (1000 μM), no significant inhibitory effects of pyrazinamide or ethambutol were observed on the 8 screened CYPs (Table 2) or 6 UGTs (Fig. 2). At the highest tested concentration (25 μM), bedaquiline partially inhibited human hepatic CYP3A, 2B6, 2C8, 2C19 and 2D6 at varied levels, but inhibition did not exceed 50% of the control metabolite formation rate (Fig. 2).

**Inhibitory Effects of Anti-TB Drugs on APAP Glucuronidation.** The IC<sub>50</sub> values for inhibition of APAP glucuronidation by rifabutin, with or without 20 minutes' preincubation, were 237 μM and 422 μM respectively, and 860 μM and 397 μM respectively for rifampicin. Isoniazid, pyrazinamide, ethambutol, and bedaquiline produced minimal inhibition of APAP glucuronidation (Table 2, Fig. 5). The positive control (probenecid at 0.5 mM) produced approximately 50% inhibition of APAP glucuronidation.

## **Discussion**

Rifabutin inhibited human CYP3A, 2B6, 2C8, 2D6, 1A2, 2C9, UGT1A1, 2B15, UGT1A9 and 2B7 in HLMs, with varying inhibitory potency. However, most of those inhibitory effects

## DMD # 76034

observed *in vitro* are not likely to be of clinical importance, since the IC<sub>50</sub> values were much higher than the reported maximum clinical plasma concentration of rifabutin of approximately 1.1 μM (Peloquin, 2002; Skinner, 1989).

Rifabutin inhibition of UGT1A4 ( $K_i = 2\mu\text{M}$  in pooled HLM) is potentially clinically relevant. Based on FDA guidance (CDER, 2012), an approximate estimation of the anticipated clinical DDI was calculated using the ratio of  $[I]/K_i$  where  $[I]$  is the maximum *in vivo* plasma concentration of rifabutin (1.1 μM) (Skinner, 1989) and  $K_i$  was 2 μM in this estimation. The ratio of 0.55 indicates a possibility that rifabutin may increase the systemic exposure of some drugs which are metabolized mainly by human UGT1A4. On the other hand, it has been widely reported that rifabutin induces human CYPs and UGTs (Baciewicz et al., 2013). Thus the prediction of the overall drug-drug interaction of rifabutin needs to consider both its inhibitory and possible inductive properties.

Human UGT1A1 is the principal metabolizing enzyme for several anti-HIV drugs such as raltegravir (Kassahun et al., 2007) and dolutegravir (Castellino et al., 2013). The IC<sub>50</sub> values for rifabutin and rifampicin on UGT1A1 were approximately 35 μM and 70 μM respectively (Fig. 3). However, the inhibitory effects of rifabutin and rifampicin are not of major clinical importance, as their overall effects show predominantly inductive properties. In clinical studies, rifampicin significantly decreased the systemic exposure of dolutegravir (Dooley et al., 2013). Co-administration of rifabutin, on the other hand, did not alter the pharmacokinetics of raltegravir (Brainard et al., 2011) or dolutegravir (Dooley et al., 2013).

## DMD # 76034

The  $IC_{50}$  values for rifabutin on CYP1A2 demonstrated a leftward shift (smaller  $IC_{50}$ ) between the incubations without and with preincubation, consistent with time-dependent inhibition (Fig. 1). We did not determine the rate constant for inactivation in this study. Nevertheless, to our knowledge, no clinically meaningful DDIs due to inhibition of CYP1A2 by rifabutin have been reported.

The lack of significant inhibition of UGTs by isoniazid is reassuring for the use of isoniazid for latent TB treatment in HIV-infected patient receiving integrase strand transfer inhibitors (INSTIs)-based antiretroviral therapy. Isoniazid for 6 or 9 months is one of the preferred regimens for the treatment of latent TB (Getahun et al, 2015). The INSTIs such as dolutegravir, raltegravir or elvitegravir that are primarily metabolized by UGTs are essential components of preferred first-line antiretroviral therapy for HIV infection (Günthard et al., 2016). The findings in this *in vitro* study suggest that no dose adjustment of the INSTIs is necessary when co-administered with isoniazid, although *in vivo* studies may be needed to confirm this.

Incomplete inhibition was observed for a number of the CYP and UGT isoforms in this study. This might be explained by the participation of multiple isoforms in a given biotransformation pathway, particularly if the substrate is not specific for the target enzyme isoform of interest. The incomplete inhibition in Figure 5B and 5C could be explained by this. However, the explanation for incomplete inhibition in Figure 3E is not clear, since the substrate trifluoperazine is reported to be highly specific for UGT1A4 (Uchaipichat et al., 2006). In addition, the incomplete inhibition was only observed with rifampicin but not with rifabutin. In any case, the calculated  $IC_{50}$  values correctly represent the inhibitor concentration that reduces

## DMD # 76034

the metabolite formation to 50% of the inhibitor-free control value (fixed at 100%). In the scenario of incomplete inhibition ( $E_{\max}$  less than 1.0 in Equation 1), the true  $IC_{50}$  is calculated from IC using Equation 2.

Since high concentrations of the anti-TB drugs were used in the incubations, 1% methanol was introduced to improve solubility. Bedaquiline was prepared in DMSO for better solubility and added directly to the incubation mixtures. Because methanol and DMSO may themselves inhibit metabolic activities of CYPs and UGTs, inhibitor-free controls were included using the same concentrations of these solvents to control for any solvent effects that might occur.

Acetaminophen is a common over-the-counter analgesic and antipyretic. The CYP mediated oxidation pathway produces the toxic intermediate *N*-acetyl-*p*-benzoquinone imine (Miner and Kissinger, 1979), known to be responsible for acetaminophen hepatotoxicity. Parallel glucuronidation and sulfation of APAP are the major metabolizing pathways for generation of non-toxic metabolite conjugates. Several UGTs, including UGT1A1, 1A6, 1A9 and 2B15 are involved in APAP glucuronidation. (Court et al., 2001; Court and Greenblatt, 2000; Krishnaswamy et al., 2005; Mutlib et al., 2006). None of the selected anti-TB drugs significantly inhibited glucuronidation of APAP *in vitro* in this study (Fig. 5)..

In conclusion, this study provides data on the inhibitory effects of anti-TB drugs on common CYPs and UGTs using HLMs *in vitro*. Rifabutin and rifampicin showed inhibitory properties to varying degrees. The findings for the other tested anti-TB drugs do not raise new concerns about clinical DDIs involving inhibition of hepatic CYPs and UGTs.

## **DMD # 76034**

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### **Authorship Contributions**

*Participated in research design:* Greenblatt, Kwara and Cao

*Conducted experiments:* Cao

*Contributed new reagents or analytic tools:* Greenblatt, and Kwara

*Performed data analysis:* Cao, Greenblatt and Kwara

*Wrote or contributed to the writing of the manuscript:* Cao, Greenblatt, and Kwara

## DMD # 76034

### References

- Baciewicz AM, Chrisman CR, Finch CK, and Self TH. (2013) Update on rifampin, rifabutin, and rifapentine drug interactions. *Curr Med Res Opin* **29**:1-12.
- Brainard DM, Kassahun K, Wenning LA, Petry AS, Liu C, Lunceford J, Hariparsad N, Eisenhandler R, Norcross A, DeNoia EP, Stone JA, Wagner JA, and Iwamoto M. (2011) Lack of a clinically meaningful pharmacokinetic effect of rifabutin on raltegravir: in vitro/in vivo correlation. *J Clin Pharmacol* **51**:943-50.
- Castellino S, Moss L, Wagner D, Borland J, Song I, Chen S, Lou Y, Min SS, Goljer I, Culp A, Piscitelli SC, and Savina PM. (2013) Metabolism, excretion, and mass balance of the HIV-1 integrase inhibitor dolutegravir in humans. *Antimicrob Agents Chemother* **57**:3536-3546.
- Court MH. (2010) Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system. *Drug Metab Rev* **42**:209-224.
- Court MH. (2005) Isoform-selective probe substrates for in vitro studies of human UDP-glucuronosyltransferases. *Methods Enzymol* **400**:104-116.
- Court MH and Greenblatt DJ. (2000) Molecular genetic basis for deficient acetaminophen glucuronidation by cats: UGT1A6 is a pseudogene, and evidence for reduced diversity of expressed hepatic UGT1A isoforms. *Pharmacogenetics* **10**:355-369.
- Court MH, Duan SX, von Moltke LL, Greenblatt DJ, Patten CJ, Miners JO, and Mackenzie PI. (2001) Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. *J Pharmacol Exp Ther* **299**:998-1006.



**DMD # 76034**

- Dooley KE, Sayre P, Borland J, Purdy E, Chen S, Song I, Peppercorn A, Everts S, Piscitelli S, Flexner C. (2013) Safety, Tolerability, and Pharmacokinetics of the HIV Integrase Inhibitor Dolutegravir Given Twice Daily With Rifampin or Once Daily With Rifabutin: Results of a Phase 1 Study Among Healthy Subjects. *JAIDS* **62**:21-27
- Getahun H, Chaisson RE, Raviglione M. (2015) Latent Mycobacterium tuberculosis Infection. *N Engl J Med.* **373**(12):1179-80
- Giancarlo GM, Venkatakrishnan K, Granda BW, von Moltke LL, Greenblatt DJ. Relative contributions of CYP2C9 and 2C19 to phenytoin 4-hydroxylation in vitro: inhibition by sulfaphenazole, omeprazole, and ticlopidine. (2001) *Eur J Clin Pharmacol.* **57**:31-6.
- Günthard HF, Saag MS, Benson CA, del Rio C, Eron JJ, Gallant JE, Hoy JF, Mugavero MJ, Sax PE, Thompson MA, Gandhi RT, Landovitz RJ, Smith DM, Jacobsen DM, Volberding PA. (2016) Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults: 2016 Recommendations of the International Antiviral Society-USA Panel. *JAMA.* **316**(2):191-210
- Greenblatt DJ, Zhao Y, Venkatakrishnan K, Duan SX, Harmatz JS, Parent SJ, Court MH, and von Moltke LL. (2011) Mechanism of cytochrome P450-3A inhibition by ketoconazole. *J Pharm Pharmacol* **63**:214-221.
- Hesse LM, Venkatakrishnan K, Court MH, von Moltke LL, Duan SX, Shader RI, Greenblatt DJ. CYP2B6 mediates the in vitro hydroxylation of bupropion: potential drug interactions with other antidepressants. (2000) *Drug Metab Dispos.* **28**:1176-83.
- Kajosaari LI, Laitila J, Neuvonen PJ, and Backman JT. (2005) Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin. *Basic Clin Pharmacol Toxicol* **97**:249-256.

**DMD # 76034**

Kassahun K, McIntosh I, Cui D, Hreniuk D, Merschman S, Lasseter K, Azrolan N, Iwamoto M,

Wagner JA, and Wenning LA. (2007) Metabolism and disposition in humans of raltegravir (MK-0518), an anti-AIDS drug targeting the human immunodeficiency virus 1 integrase enzyme. *Drug Metab Dispos* **35**:1657-1663.

Krishnaswamy S, Hao Q, Al-Rohaimi A, Hesse LM, von Moltke LL, Greenblatt DJ, and Court

MH. (2005) UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: II. Functional impact of the three most common nonsynonymous UGT1A6 polymorphisms (S7A, T181A, and R184S). *J Pharmacol Exp Ther* **313**:1340-1346.

Miner DJ and Kissinger PT. (1979) Evidence for the involvement of N-acetyl-p- quinoneimine in

acetaminophen metabolism. *Biochem Pharmacol* **28**:3285-3290.

Mutlib AE, Goosen TC, Bauman JN, Williams JA, Kulkarni S, and Kostrubsky S. (2006)

Kinetics of acetaminophen glucuronidation by UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential implications in acetaminophen-induced hepatotoxicity. *Chem Res Toxicol* **19**:701-709.

Ochs HR, Greenblatt DJ, Roberts GM and Dengler HJ. (1981) Diazepam interaction with

antituberculosis drugs. *Clin Pharmacol and Therap* **29**:671-678.

Ochs HR, Greenblatt DJ and Knüchel M. (1983) Differential effect of isoniazid on triazolam

oxidation and oxazepam conjugation. *Br J Clin Pharmacol* **16**:743-746

Peloquin CA. (2002) Therapeutic drug monitoring in the treatment of tuberculosis. *Drugs*

**62**:2169-2183.

Polasek TM, Elliot DJ, Lewis BC, and Miners JO. (2004) Mechanism-based inactivation of

human cytochrome P4502C8 by drugs in vitro. *J Pharmacol Exp Ther* **311**:996-1007.

**DMD # 76034**

- Rae JM, Johnson MD, Lippman ME, and Flockhart DA. (2001) Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. *J Pharmacol Exp Ther* **299**:849-857.
- Skinner MH, Hsieh M, Torseth J, Pauloin D, Bhatia G, Harkonen S, Merigan TC, and Blaschke TF. (1989) Pharmacokinetics of rifabutin. *Antimicrob Agents Chemother.* **33**(8): 1237–1241.
- Soars MG, Petullo DM, Eckstein JA, Kasper SC, and Wrighton SA. (2004) An assessment of UDP-glucuronosyltransferase induction using primary human hepatocytes. *Drug Metab Dispos* **32**:140-148.
- Sonnichsen DS, Liu Q, Schuetz E, Schuetz J, Pappo A and Relling M. (1995) Variability in human cytochrome P450 Paclitaxel metabolism. *J Pharmacol Exp Ther* **275**:566-575
- U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). (2012) Guidance for Industry Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (DRAFT GUIDANCE).
- Uchaipichat V, Mackenzie PI, Elliot DJ, and Miners JO. (2006) Selectivity of substrate (trifluoperazine) and inhibitor (amitriptyline, androsterone, canrenoic acid, hecogenin, phenylbutazone, quinidine, quinine, and sulfinpyrazone) "probes" for human UDP-glucuronosyltransferases. *Drug Metab Dispos* **34**:449-456.
- von Moltke LL, Greenblatt DJ, Harmatz JS, and Shader RI. (1993a) Alprazolam metabolism in vitro: studies of human, monkey, mouse, and rat liver microsomes. *Pharmacology* **47**:268-276.

**DMD # 76034**

- von Moltke LL, Manis M, Harmatz JS, Poorman R, and Greenblatt DJ. (1993b) Inhibition of acetaminophen and lorazepam glucuronidation *in vitro* by probenecid. *Biopharma and Drug Dispos* **14**:119-130.
- von Moltke LL, Greenblatt DJ, Giancarlo GM, Granda BW, Harmatz JS, and Shader RI. (2001) Escitalopram (S-citalopram) and its metabolites in vitro: cytochromes mediating biotransformation, inhibitory effects, and comparison to R-citalopram. *Drug Metab Dispos* **29**:1102-1109.
- Wen X, Wang JS, Neuvonen PJ, and Backman JT. (2002) Isoniazid is a mechanism-based inhibitor of cytochrome P450 1A2, 2A6, 2C19 and 3A4 isoforms in human liver microsomes. *Eur J Clin Pharmacol* **57**:799-804.
- Worley MV, and Estrada SJ. (2014) Bedaquiline: A novel antitubercular agent for the treatment of multidrug-resistant tuberculosis. *Pharmacotherapy* **34**:1187-1197
- World Health Organization (WHO). (2016) Global Tuberculosis Report 2016.
- World Health Organization (WHO). (2010) Guidelines for treatment of tuberculosis.
- Zhao Y, Harmatz JS, Epstein CR, Nakagawa Y, Kurosaki C, Nakamura T, Kadota T, Giesing D, Court MH and Greenblatt DJ. (2015) Favipiravir inhibits acetaminophen sulfate formation but minimally affects systemic pharmacokinetics of acetaminophen. *Br J Clin Pharmacol* **80**:1076-1085
- Zumla A, Nahid P, and Cole ST. (2013) Advances in the development of new tuberculosis drugs and treatment regimens. *Nat Rev Drug Discov* **12**:388-404.

## **DMD # 76034**

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### **Footnotes**

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## DMD # 76034

### Legends for Figures

**Fig. 1** *In vitro* inhibitory effects of rifabutin on human hepatic CYPs. (A) CYP1A2, (B) CYP3A, (C) CYP2B6, (D) CYP2C8, (E) CYP2C9, (F) CYP2C19, (G) CYP2D6 and (H) CYP2E1. The incubations were with preincubation (closed circle) and without preincubation (open circle). Data points represent the means  $\pm$  standard errors (SEM) of each concentration of rifabutin that was tested in duplicate.  $IC_{50}$  values were determined by non-linear regression and summarized in Table 2.

**Fig. 2** *In vitro* inhibitory effects of the selected anti-TB drugs on human hepatic UGTs. (A) UGT1A1, (B) UGT1A4, (C) UGT1A6, (D) UGT1A9, (E) UGT2B7, and (F) UGT2B15. Two concentrations of each compound were tested. z: Control without inhibition (black: 0  $\mu$ M); a: Pyrazinamide (black:100  $\mu$ M, gray:1000  $\mu$ M); b: Ethambutol (black:100  $\mu$ M, gray:1000  $\mu$ M); c: Rifabutin (black:100  $\mu$ M, gray:600  $\mu$ M); d\*: Bedaquiline (in (A) and (B), black: 26  $\mu$ M, gray: 52  $\mu$ M); d: Bedaquiline (black:12.5  $\mu$ M, gray: 25  $\mu$ M); e: Rifampicin (black:100  $\mu$ M, gray:1000  $\mu$ M); f: Isoniazid (black:100  $\mu$ M, gray:1000  $\mu$ M)

**Fig. 3** *In vitro* inhibitory effects of rifabutin and rifampicin on human hepatic UGTs. (A) Rifabutin with UGT1A1; the concentrations of rifabutin are 0, 10, 60, 100, 200, 400 and 600  $\mu$ M. (B) Rifabutin with UGT1A4; the concentrations of rifabutin are 0, 5, 10, 60, 100, 200 and 400  $\mu$ M. (C) Rifabutin with UGT2B15; the concentrations of rifabutin are 0, 5, 10, 60, 100, 200 and 400  $\mu$ M. (D) Rifampicin with UGT1A1; the concentrations of rifampicin are 0, 10, 60, 100, 200, 400, 600 and 1000  $\mu$ M. (E) Rifampicin with UGT1A4; the concentrations of rifampicin are 0, 5, 10, 60, 100, 200, 400, 600 and 1000  $\mu$ M. (F) Rifampicin with UGT2B15; the concentrations

## DMD # 76034

of rifampicin are 0, 10, 60, 100, 200, 400, 600 and 1000  $\mu\text{M}$ . The incubations were with preincubation (closed circle) and without preincubation (open circle). Data points represent the means  $\pm$  standard errors (SEM) of each drug concentration that was tested in duplicate.  $\text{IC}_{50}$  values were determined by non-linear regression and summarized in Table 2.

**Fig. 4** Rates of formation of trifluoperazine glucuronide in presence of inhibitory rifabutin (Rif) in a series of concentrations (0, 1.25, 5, 10, 30 and 60  $\mu\text{M}$ ). The concentrations of trifluoperazine are at 2, 5, 10, 20, 34.2, 72.4, and 144.9  $\mu\text{M}$ . The  $K_i$  value for rifabutin on human hepatic UGT1A4 using trifluoperazine as the index substrate is 2  $\mu\text{M}$  ( $K_m = 77.6 \mu\text{M}$ ,  $V_{\max} = 3.6$ ). Data points represent the means  $\pm$  standard errors (SEM) of duplicate.

**Fig. 5** (A) *In vitro* inhibitory effects of the tested anti-TB drugs on APAP glucuronidation using HLMs. Two concentrations of each compound were tested. z: Control without inhibitor (black: 0  $\mu\text{M}$ ); p: Probenecid as the positive control (0.5mM); a: Pyrazinamide (black:100  $\mu\text{M}$ , gray:1000  $\mu\text{M}$ ); b: Ethambutol (black:100  $\mu\text{M}$ , gray:1000  $\mu\text{M}$ ); c\*: Rifabutin (black:80  $\mu\text{M}$ , gray:600  $\mu\text{M}$ ); d\*: Bedaquiline (black:26  $\mu\text{M}$ , gray:52  $\mu\text{M}$ ); e\*: Rifampicin (black:80  $\mu\text{M}$ , gray:1000  $\mu\text{M}$ ); f: Isoniazid (black:100  $\mu\text{M}$ , gray:1000  $\mu\text{M}$ ). (B) *In vitro* inhibitory effects of rifabutin on APAP glucuronidation. The concentrations of rifabutin are 0, 8, 47.7, 79.6, 159.1, 318.3, and 477.4  $\mu\text{M}$ . (C) *In vitro* inhibitory effects of rifampicin on APAP glucuronidation. The concentrations of rifampicin are 0, 8, 47.7, 79.6, 159.1, 318.3, 477.4 and 795.7  $\mu\text{M}$  in incubations with preincubation (closed circle) and 0, 8, 47.7, 79.6, 159.1, 318.3, and 477.4  $\mu\text{M}$  in incubations without preincubation (open circle). Data points represent the means  $\pm$  standard

## **DMD # 76034**

errors (SEM) of each drug concentration that was tested in duplicate.  $IC_{50}$  values were determined by non-linear regression and summarized in Table 2.



**Table 1** *In vitro* systems using HLMs for evaluating inhibitory activities of the selected anti-TB drugs on human CYPs and UGTs

<i>Enzyme Isoform</i>	<i>Substrate (Conc.)</i>	<i>Internal standard</i>	<i>Metabolite assayed</i>	<i>Inhibitor</i>	<i>Protein Conc. (µg/mL)</i>	<i>Incubation time (min)</i>
CYP1A2	Phenacetin (100uM)	2-acetaminophenol	Acetaminophen	α-Naphthoflavone	250	20
CYP3A	Triazolam (250uM)	Phenacetin	α-Hydroxytriazolam	Ketoconazole	250	20
CYP2B6	Bupropion (80uM)	2-acetaminophenol	Hydroxybupropion	Clopidogrel	333	20
CYP2C8	Taxol (25uM)	Phenacetin	6-Hydroxytaxol	Quercetin	300	20
CYP2C9	Flurbiprofen (5uM)	Naproxen	4'-Hydroxyflurbiprofen	Sulfaphenazole	250	20
CYP2C19	S-mephenytoin (25uM)	Phenacetin	4'-Hydroxymephenytoin	Ticlopidine	500	40
CYP2D6	Dextromethorphan (25uM)	Pronethalol	Dextrophan	Quinidine	250	20
CYP2E1	Chlorzoxazone (50uM)	Phenacetin	6-Hydroxychlorzoxazone	Diethyldithiocarbamate	250	20
UGT1A1	β-Estradiol (100uM)	Phenacetin	Estradiol-3-glucuronide	Probenecid	500	30
UGT1A4	Trifluoperazine (200uM)	Phenacetin	Trifluoperazine-glucuronide	Probenecid	250	30
UGT1A6	Serotonin (4 mM)	Phenacetin	Serotonin-glucuronide	Probenecid	50	30
UGT1A9	Propofol (100uM)	Phenacetin	Propofol-glucuronide	Niflumic acid	250	30
UGT2B7	3'-azidothymidine (AZT) (500uM)	3-acetaminophenol	AZT-glucuronide	Probenecid	500	120
UGT2B15	Oxazepam (100uM)	Phenacetin	S-oxazepam-glucuronide	Niflumic acid	500	120
APAP Glucuronidation	Acetaminophen (0.6mM)	3-acetaminophenol	APAP-glucuronide	Probenecid	500	120

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**DMD # 76034**

**Table 2** IC<sub>50</sub> values of the selected anti-TB drugs on common human hepatic CYPs and UGTs

	<i>Bedaquiline</i>		<i>Rifabutin</i>		<i>Pyrazinamide</i>		<i>Ethambutol</i>		<i>Rifampicin</i>		<i>Isoniazid</i>	
	W <sup>a</sup> μM	W/O <sup>b</sup> μM	W μM	W/O μM	W μM	W/O μM	W μM	W/O μM	W μM	W/O μM	W μM	W/O μM
CYP1A2	NC <sup>c</sup>	NC	NC	NC	NC	NC	NC	NC	-	-	-	-
CYP3A	NC	NC	<b>27.9</b>	<b>31.5</b>	NC	NC	NC	NC	-	-	-	-
CYP2B6	NC	NC	<b>120.8</b>	<b>165.3</b>	NC	NC	NC	NC	-	-	-	-
CYP2C8	NC	NC	<b>64.0</b>	<b>62.6</b>	NC <sup>f</sup>	NC	NC <sup>g</sup>	NC	-	-	-	-
CYP2C9	NC	NC	<b>75</b>	<b>150.9</b>	NC	NC	NC	NC	-	-	-	-
CYP2C19	NC	NC	NC	NC	NC	NC	NC	NC	-	-	-	-
CYP2D6	NC	NC	<b>147.7</b>	<b>166.5</b>	NC	NC	NC	NC	-	-	-	-
CYP2E1	NC	NC	NC	NC	NC	NC	NC	NC	-	-	-	-
UGT1A1	NC	NC	<b>35</b>	<b>44</b>	NC	NC	NC	NC	<b>70</b>	<b>63</b>	NC	NC
UGT1A4	- <sup>d</sup>	NC	<b>10.8</b>	<b>11.3</b>	-	NC	-	NC	-	<b>230</b>	-	NC
UGT1A6	-	NC	-	NC	-	NC	-	NC	-	<sup>h</sup>	-	NC
UGT1A9	-	NC	-	NC	-	NC	-	NC	-	NC	-	NC
UGT2B7	-	NC	-	<sup>e</sup>	-	NC	-	NC	-	NC	-	NC
UGT2B15	-	NC	-	<b>81.3</b>	-	NC	-	NC	-	<b>357</b>	-	NC
APAP-Glucuronidation	NC	NC	<b>237.2</b>	<b>422.2</b>	NC	NC	NC	NC	<b>860</b>	<b>545</b>	NC	NC

<sup>a</sup>: Incubations with preincubation; <sup>b</sup>: Incubations without preincubation; <sup>c</sup>: Not calculated (No IC<sub>50</sub> values were obtained due to less than 50% inhibition at the highest tested concentrations: 1000 μM for rifampicin , pyrazinamide, ethambutol, and isoniazid; 600 μM for rifabutin, and 25 μM for bedaquiline); <sup>d</sup>: not tested; <sup>e</sup>: 57% inhibition at 600 μM; <sup>f</sup>: 41% inhibition at 1000 μM; <sup>g</sup>: 37% inhibition at 1000 μM; <sup>h</sup>: 58% inhibition at 1000 μM

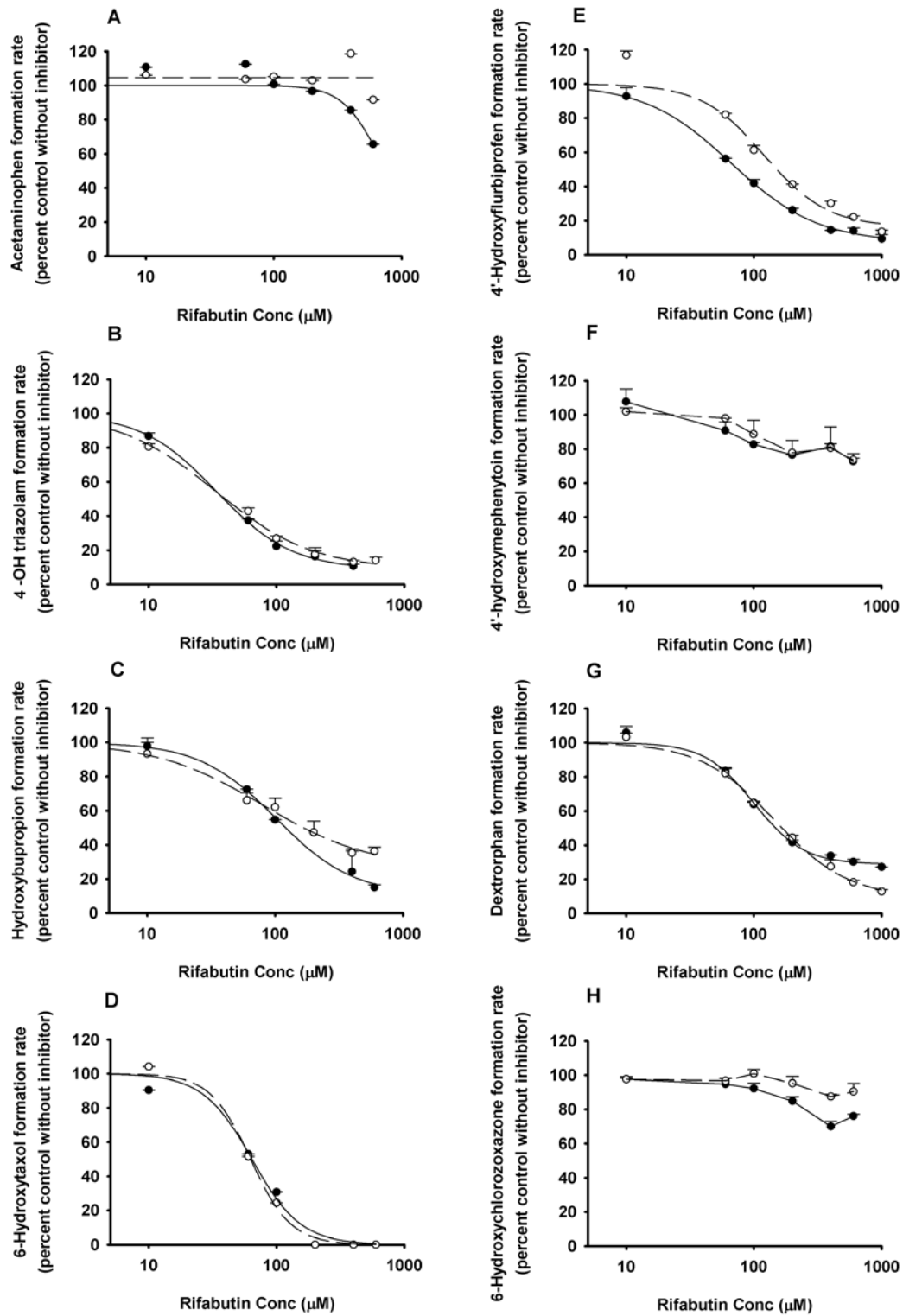


Figure 1

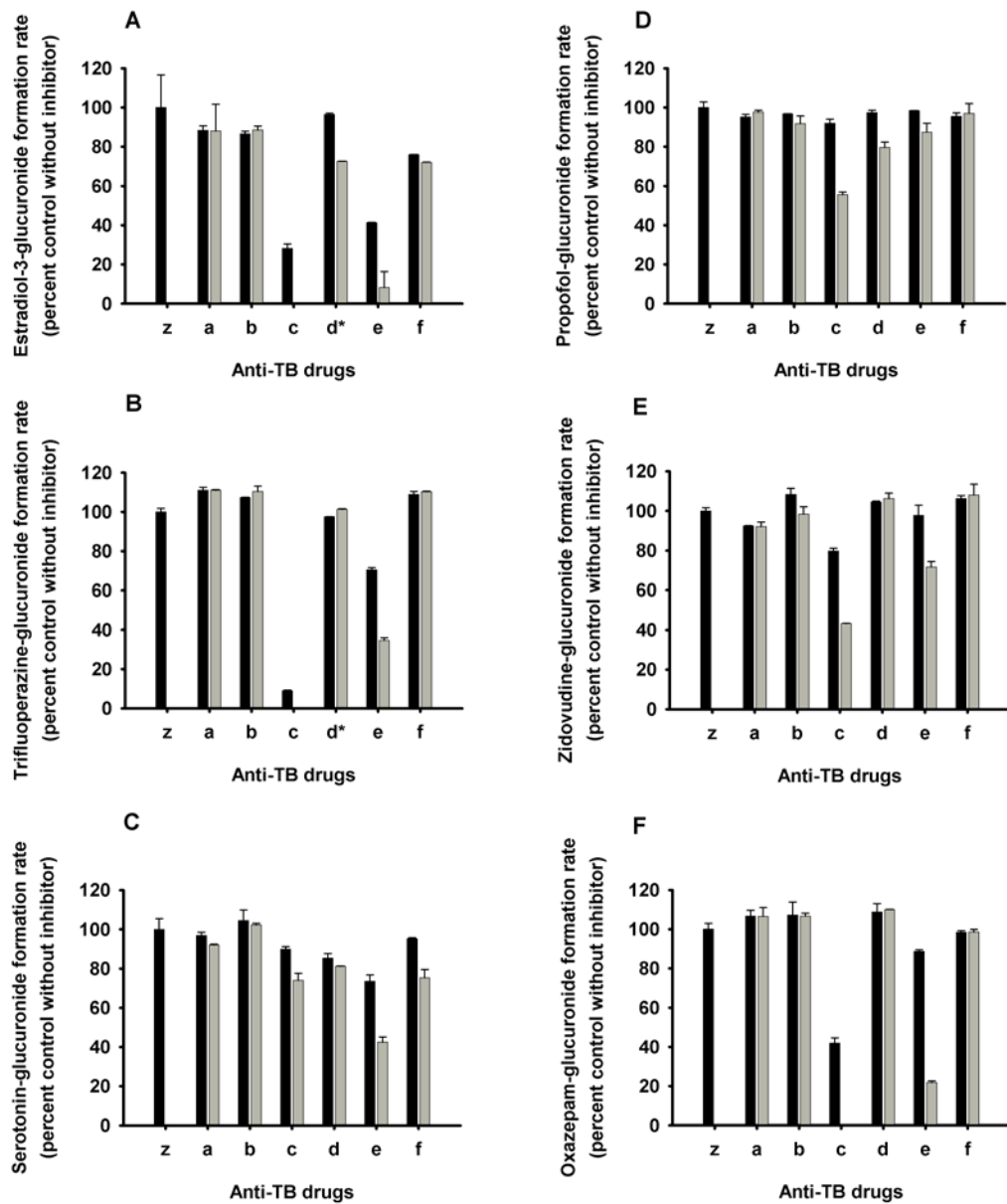


Figure 2

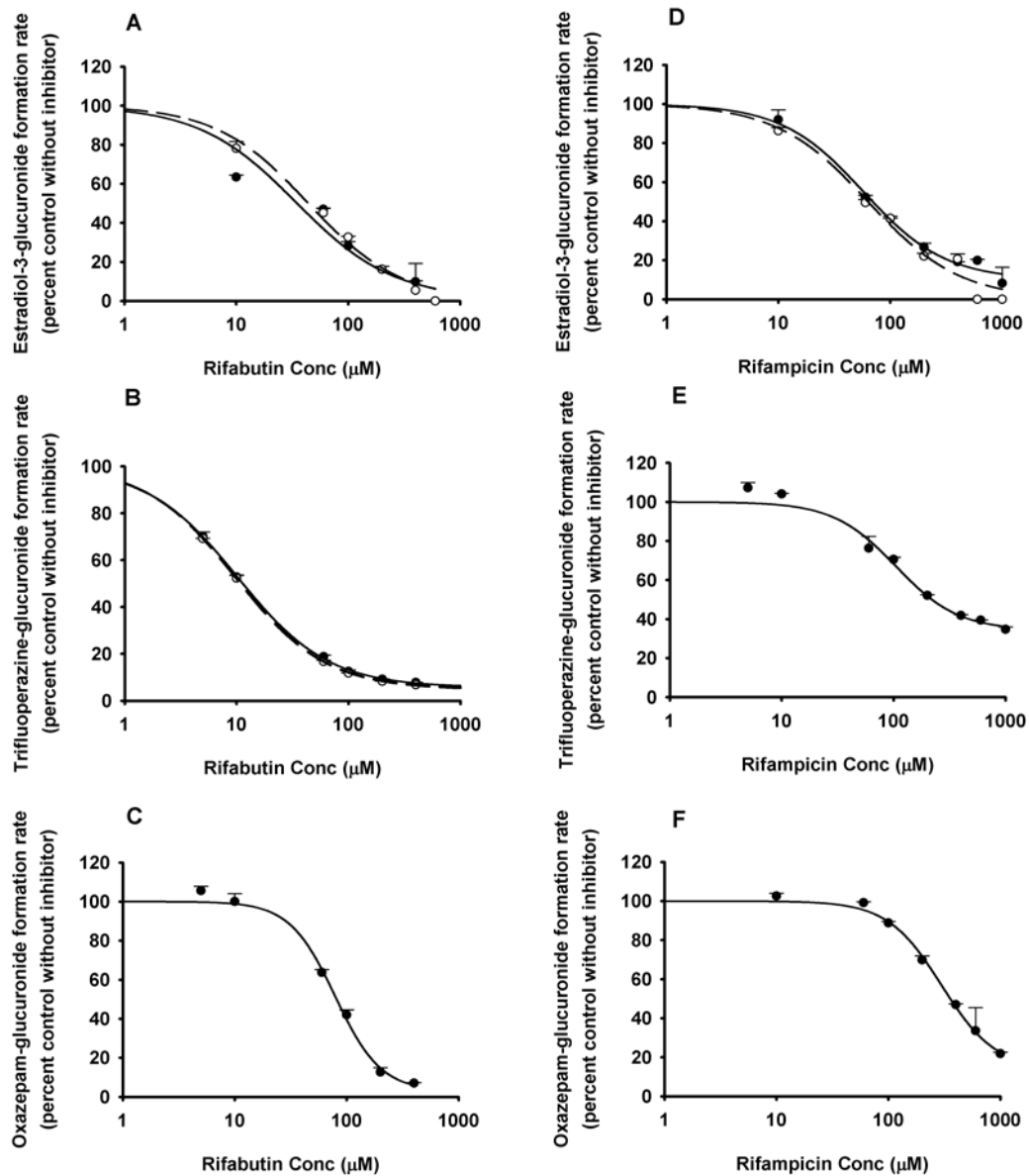


Figure 3

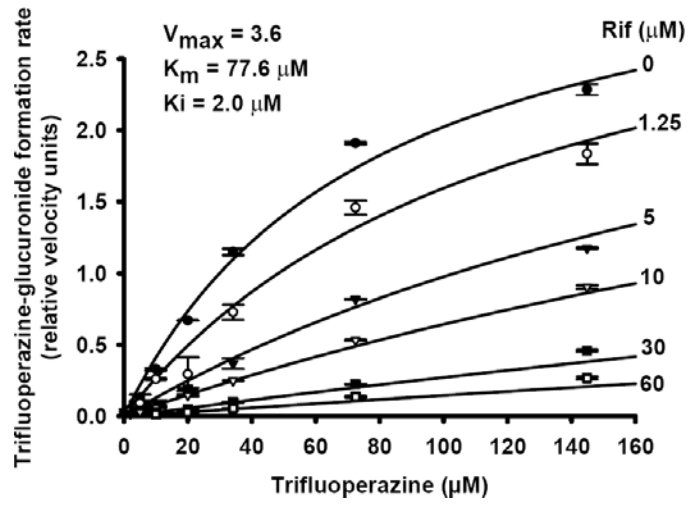


Figure 4

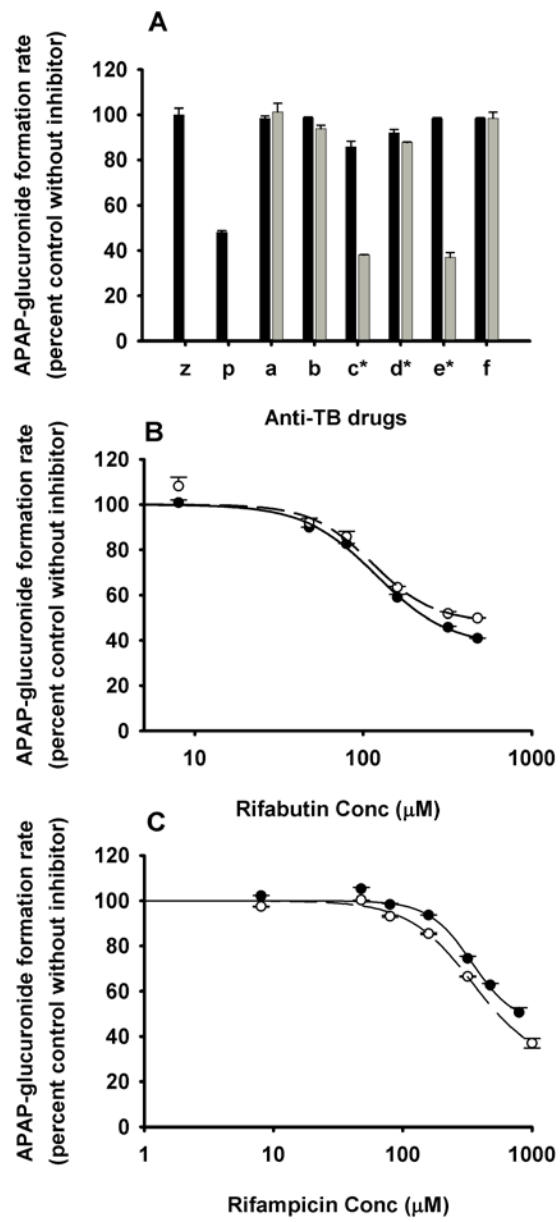


Figure 5

Information for the supplemental table:

**Title: Inhibitory Effects of Selected Antituberculosis Drugs on Common Human Hepatic Cytochrome P450 and UDP-glucuronosyltransferase enzymes**

Authors: Lei Cao, David J Greenblatt, Awewura Kwara

Journal: Drug Metab Dispos

**Supplemental Table 1** Analytical methods used in this study

<i>Index reactions</i>	<i>Metabolite assayed</i>	<i>Column</i>	<i>Mobile phase</i>	<i>HPLC conditions</i>	<i>Detection</i>
CYP1A2	Acetaminophen	a <sup>a</sup>	50mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN, 88:12)	isocratic separation	UV 254nm
CYP3A	$\alpha$ -Hydroxytriazolam	a	10mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN:MEOH, 67.5:22.5:10)	isocratic separation	UV 220nm
CYP2B6	Hydroxybupropion	b <sup>b</sup>	50mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN, 85:15); pH=3	isocratic separation	UV 214nm
CYP2C8	6-Hydroxytaxol	c <sup>c</sup>	H <sub>2</sub> O:MEOH, 40.5:59.5	isocratic separation	UV 230nm
CYP2C9	4'-Hydroxyflurbiprofen	a	20mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN, 65:35); pH=2.5	isocratic separation	fluorescence excitation 260nm; emission 320nm
CYP2C19	4'-Hydroxymephenytoin	b	50mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN, 80:20)	isocratic separation	UV 204nm
CYP2D6	Dextrorphan	b	50mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN, 75:25); pH=6	isocratic separation	fluorescence excitation 280nm; emission 310nm



CYP2E1	6-Hydroxychlorozoxazone	a	50mM potassium phosphate buffer in mixture (H <sub>2</sub> O:ACN, 75:25)	isocratic separation	UV 295nm
UGT1A1	Estradiol-3-glucuronide	d <sup>d</sup>	Mobile phase A: 20mM potassium phosphate buffer, pH=4.5; Mobile phase B: MEOH	Court, 2005	UV 280nm
UGT1A4	Trifluoperazine-glucuronide	d	Mobile phase A: 0.1% TFA in H <sub>2</sub> O; Mobile phase B: MEOH	Court, 2005	UV 254nm
UGT1A6	Serotonin-glucuronide	d	Mobile phase A: 20mM potassium phosphate buffer, pH=4.5; Mobile phase B: MEOH	Court, 2005	UV 270nm
UGT1A9	Propofol-glucuronide	a	Mobile phase A: 20mM potassium phosphate buffer, pH=4.5; Mobile phase B: MEOH	Court, 2005	UV 214nm
UGT2B7	AZT-glucuronide	d	Mobile phase A: 20mM potassium phosphate buffer, pH=2.2; Mobile phase B: MEOH	Court, 2005	UV 266nm
UGT2B15	S-oxazepam-glucuronide	d	Mobile phase A: 20mM potassium phosphate buffer, pH=4.5; Mobile phase B: MEOH	Court, 2005	UV 214nm
APAP Glucuronidation <sup>e</sup>	APAP-glucuronide	d	Mobile phase A: 20mM potassium phosphate buffer, pH=2.2; Mobile phase B: MEOH	Zhao, 2015	UV 254nm
APAP sulfation	APAP-sulfate	d	Mobile phase A: 20mM potassium phosphate buffer, pH=2.2; Mobile phase B: MEOH	Zhao, 2015	UV 254nm

<sup>a</sup>: C<sub>18</sub> reversed phase 150 x 3.9 mm Nova-pak (Waters); <sup>b</sup>: C<sub>18</sub> reversed phase 300 x 3.9 mm uBondapak (Waters); <sup>c</sup>: ODS reversed phase 250 x 4.6mm (Alltech); <sup>d</sup>: Synergi 4μm Hydro-RP 80A 250 x 4.6mm (Phenomenex); <sup>e</sup> APAP glucuronidation involves several UGT isoforms, such as UGT1A1, 1A6, 1A9 and 2B15.