Published in final edited form as:

Drug Metab Pharmacokinet. 2013 ; 28(4): 362–371.

In vitro Analysis and Quantitative Prediction of Efavirenz Inhibition of Eight Cytochrome P450 (CYP) Enzymes: Major Effects on CYPs 2B6, 2C8, 2C9 and 2C19

Cong Xu and Zeruesenay Desta

Indiana University School of Medicine, Indianapolis, IN, USA

Summary

In order to quantitatively predict drug interactions associated with efavirenz-based anti-HIV therapy, we evaluated reversible and time-dependent inhibitions of efavirenz on eight cytochrome P450 (CYP) enzymes *in vitro*. Present study showed that efavirenz was a potent competitive inhibitor of CYP2B6 in HLMs (average $K_i = 1.68 \mu\text{M}$) and expressed CYP2B6 ($K_i = 1.38 \mu\text{M}$). Moderate inhibition of CYP2C8 by efavirenz was observed in pooled HLMs ($K_i = 4.78 \mu\text{M}$) and HLMs with CYP2C8*3/*3 genotype ($K_i = 4.80 \mu\text{M}$). Efavirenz was a moderate inhibitor of CYP2C9 ($K_i = 19.46 \mu\text{M}$) and CYP2C19 ($K_i = 21.31 \mu\text{M}$); and a weak inhibitor of CYP3A ($K_i = 40.33 \mu\text{M}$). No appreciable inhibition was observed on CYP1A2, CYP2A6 or CYP2D6. No time-dependent inhibition of the CYPs by efavirenz was observed in this study. Quantitative predictions showed that single dose of efavirenz may substantially slow the elimination of drugs predominately cleared by CYP2B6, CYP2C19 or by both enzymes and may also lower the area under the plasma concentration time curve (AUC) of active metabolites of some pro-drugs (e.g. clopidogrel and proguanil) by up to 30%. Depending on substrates, chronic administration of efavirenz may increase the AUC of CYP2C8 and CYP2C9 substrates about 3.5 ~ 4.4-fold and 1.7 ~ 2.0-fold at steady state.

Keywords

Antiretroviral agents; cytochrome P450; reversible inhibition; time-dependent inhibition; drug-drug interaction; quantitative prediction

Introduction

Efavirenz, a potent non-nucleoside reverse transcriptase inhibitor (NNRTI), remains preferred component of highly active antiretroviral therapy (HAART) for treatment naïve patients despite an emergence of second generation of NNRTIs and new classes of antiretroviral agents (Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents.

Corresponding author: Zeruesenay Desta, Ph.D Associate Professor of Medicine, Pharmacology and Toxicology Department of Medicine/Division of Clinical Pharmacology Indiana University School of Medicine 1001 West 10th Street, WD Myers Bldg., W7123, Indianapolis, IN 46202, USA zdesta@iupui.edu; **Telephone:** (317) 630 8860; **Fax:** (317) 630 8185.

Part of this study was presented at the American Society Clinical Pharmacology and Therapeutics 112th Annual Meeting, Dallas Texas, March 2-5th, 2011 and was published in abstract form in Clinical Pharmacology and Therapeutics 89 (Supplement 1): S15.

Department of Health and Human Services, March 27, 2012). It is the only antiretroviral agent currently on the market that has been combined with other two nucleoside/nucleotide reverse transcriptase inhibitors in a single tablet and administered once-daily, which significantly improve adherence ¹⁾. However, its propensity for clinically important drug-drug interactions is high. Efavirenz is always used in combination therapy and in concert with drugs directed at the treatment of opportunistic infections, cancers and other HIV-related co-morbidities. The alterations in pharmacokinetics of co-administered drugs by efavirenz lead to either lack of efficiency or adverse drug reactions of the victim drug ¹⁾. In order to predict and avoid adverse drug interactions with efavirenz-based therapy, it is important to identify mechanisms underlying those drug interactions.

Efavirenz is known to alter the pharmacokinetics of a long list of co-administered drugs [Product Information of Efavirenz (Sustiva), Bristol-Myers Squibb Company, June 2012], probably by modulating the activities of multiple drug metabolizing enzymes and/or drug transporters. Efavirenz is a substrate of cytochrome P450s (CYPs) and UDP-glucuronosyltransferases (UGTs). It is predominantly cleared mainly by CYP2B6-mediated 8-hydroxylation, with small contribution from other CYPs (e.g., CYP2A6, CYP3A and CYP1A2) ²⁻³⁾. Two minor pathways, efavirenz 7-hydroxylation and N-glucuronidation, are predominantly catalyzed by CYP2A6 ³⁾ and UGT2B7 ⁴⁾, respectively. Efavirenz, through activation of constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR), enhances the expression of multiple enzymes regulated by these nuclear receptors, including CYP2B6, CYP2C19 and CYP3A ⁵⁻⁶⁾. Therefore, many drug interactions associated with efavirenz at steady state including decreased exposures of methadone ⁷⁾, statins ⁸⁾, omeprazole ⁹⁾, voriconazole ¹⁰⁾, proguanil ¹¹⁾ and etravirine ¹²⁾, and protease inhibitors ¹³⁾ can be primarily explained by the inductive effect of efavirenz. Furthermore, efavirenz enhances its own metabolism (auto-induction) upon repeated administration compared to a single dose ¹⁴⁾, probably through induction of CYP2B6 and other enzymes involved in its metabolism ²⁻⁴⁾. Besides induction, there is *in vitro* evidence that efavirenz may directly inhibit the activities of certain CYPs ¹⁵⁻¹⁸⁾. Indeed, scattered clinical cases of adverse drug interactions, e.g., with amodiaquine ¹⁹⁾, warfarin ²⁰⁾ and phenytoin ²¹⁾ suggest that efavirenz may alter the pharmacokinetics of co-administered drugs through inhibition of CYPs.

A comprehensive inhibitory analyses that encompass all major drug-metabolizing CYPs are important because: a) not all pharmacokinetic drug interactions involving efavirenz can be explained by the known inductive effect of efavirenz and by the CYPs studied so far; and b) the *in vitro* studies describing inhibition of CYPs by efavirenz provide only qualitative information, without generating *in vitro* inhibition parameters that will allow quantitative prediction of *in vivo* condition and without taking the contribution of time-dependent inactivation into account. In addition, the net effect on the pharmacokinetics of co-administered drugs seems to depend on its varied potencies of inhibition and induction on individual CYP isoform. In order to better predict *in vivo* drug-drug interactions associated with such mixed mechanisms, it is necessary to simultaneously take reversible inhibition, time-dependent inhibition, and induction into account.

The purpose of present study was to systematically evaluate the *in vitro* inhibitory potency of efavirenz on eight major human CYP isoforms and determine the mechanisms involved.

For those isoforms that were inhibited in pilot experiments, inhibition constants (K_i values) were estimated with which the extent of *in vivo* drug interactions was quantitatively predicted.

Materials and Methods

Chemicals

Efavirenz, 8-hydroxyefavirenz, 7-hydroxycoumarin, bupropion, 4-hydroxybupropion, desethylamodiaquine, S-mephenytoin, 4-hydroxymephenytoin, R-omeprazole, R-hydroxyomeprazole and ritonavir were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Acetaminophen, chloroquine, coumarin, dextromethorphan, dextrorphan, desmethyldiazepam, 8-methoxypsoralen, phenacetin, tolbutamide, 4-hydroxytolbutamide, testosterone, 6- β hydroxytestosterone, glucose-6-phosphate, NADP and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). Amodiaquine and levallorphan were purchased from the United States Pharmacopeia (Rockville, MD). All the other chemicals were of high performance liquid chromatography (HPLC) grade.

Microsomal preparations

Pooled human liver microsomes (HLMs) from 24 individual donors, HLMs with *CYP2C8**3/*3 genotype, and other HLMs were obtained from BD Biosciences (Woburn, MA). Human CYP2B6 and CYP2C8 expressed in baculovirus infected insect cells with oxidoreductase and without co-expression of cytochrome (Cyt) b5 were obtained from BD Biosciences. All microsomal preparations were stored at -80°C until analysis.

General incubation conditions

Using incubation conditions specific to each isoform that were linear for time, substrate and protein concentrations, isoform selective substrate probes were incubated in duplicate at 37°C with HLMs (or with expressed CYP when required), 200 mM sodium phosphate reaction buffer (pH 7.4) and NADPH-regenerating system (1.3 mM NADP, 3.3 mM glucose-6-phosphate, 3.3 mM MgCl_2 , and 1 $\mu\text{l/ml}$ glucose-6-phosphate dehydrogenase) in the absence or presence of varying concentrations of efavirenz (or 8-hydroxyefavirenz). The test inhibitors were dissolved and diluted in methanol to required concentrations and methanol was removed by drying in speed vacuum before the addition of the incubation components. The following HLMs concentrations were used: 1 mg protein/ml for CYP1A2, CYP2C9, CYP2C19 and CYP2D6; 0.5 mg protein/ml for CYP2A6, 0.25 mg protein/ml for CYP2B6 and CYP3A; and 0.1 mg/ml for CYP2C8 incubations. Inhibition constants (K_i values) were determined in expressed CYP2C8 (26 pmol) and CYP2B6 (5 pmol).

Enzyme activity assays

The inhibitory effects of efavirenz on the activities of different CYP isoforms were studied in HLMs, expressed CYP2B6 and CYP2C8 using the following selective reaction probes: phenacetin O-deethylation (CYP1A2); coumarin 7-hydroxylation (CYP2A6); bupropion 4-hydroxylation (CYP2B6); amodiaquine N-desethylation (CYP2C8); tolbutamide 4-hydroxylation (CYP2C9); S-mephenytoin 4-hydroxylation/R-omeprazole 5-hydroxylation

(CYP2C19); dextromethorphan O-demethylation (CYP2D6); and testosterone 6- β hydroxylation (CYP3A). The methods to measure the activity of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2D6 and CYP3A in the absence or presence of the test inhibitors were adopted from previous studies²²⁻²³. LC/MS/MS assays were developed for quantification of bupropion 4-hydroxylation (CYP2B6) as described elsewhere²⁴. The MS/MS system was an API2000 MS/MS triple quadrupole system (Applied Biosystems, Foster City, CA) equipped with a turbo ion spray and was coupled with a Shimadzu (Columbia, MD) HPLC system consisting of an LC-20AB pump and SIL-20A HT autosampler (Applied Biosystems/MDS Sciex, Foster City, CA). In Brief, bupropion, 4-hydroxybupropion, and the internal standard (nevirapine) were separated using Zorbax SB-C18 column (100 $\times$ 2.00 mm, 3- μ m particle size), a Luna C18 guard column (30 $\times$ 4.6 mm, 5 μ m), and an isocratic mobile phase that consisted of 75% formic acid (0.1% in H₂O) and 25% acetonitrile (flow rate, 0.3 ml/min). 4-Hydroxybupropion and nevirapine were detected using multiple reactions monitoring at a m/z of 256.1/238.0 and 267.2/224.4 in positive ion mode, respectively. R-omeprazole 5-hydroxylation (CYP2C19) was assayed as described previously²⁵. The MS/MS system was the same as that for bupropion hydroxylation assay above. R-Hydroxyomeprazole, and the internal standard (R-lansoprazole) were separated by Chiral-AGP (150 $\times$ 4.60 mm; 5 μ m; Phenomenex). A gradient elution profile was used: initial mobile phase, 95% of 20 mM ammonium acetate (adjusted to pH 6.5) and 5% of acetonitrile (v/v); and the secondary mobile phase consisted of 90% of acetonitrile and 10% of 20 mM ammonium acetate (adjusted to pH 6.5) (v/v). The secondary mobile phase was increased from 0% to 40% linearly between 0 and 8 min; the initial mobile phase conditions were resumed after 9 min and remained constant for an additional 6 min, allowing the column to equilibrate. The selected reaction-monitoring transitions of the precursor ions to selected product ions were m/z 362.13/214.10 for R-5-hydroxyomeprazole and m/z 370.25/252.30 for the internal standard (R-lansoprazole).

Kinetic analysis

Kinetic analysis was performed for each substrate probe reaction before initiation of the inhibition experiments, and the data generated were used as a guide for selection of the appropriate concentrations of the substrate probes in the subsequent inhibition experiments. Thus, the kinetic parameters for the metabolism of each probe substrate were determined by incubating a range of different concentrations of the substrate (without the inhibitor) at 37°C in duplicate with HLMs (or expressed enzymes) and the NADPH-generating system. Phenacetin (5 to 1000 μ M), coumarin (0.1 to 50 μ M), bupropion (1 to 1000 μ M), amodiaquine (0.1 to 100 μ M), tolbutamide (5 to 500M), S-mephenytoin (5 to 100 μ M), R-omeprazole (1 to 200 μ M), dextromethorphan (1 to 200 μ M), and testosterone (1 to 200 μ M) were used. Formation rates of metabolite versus the substrate concentrations were fit to appropriate enzyme kinetic equations to estimate the apparent kinetic parameters.

Screening of inhibition on multiple CYPs

A single isoform-specific substrate concentration at about the respective K_m value (50 μ M phenacetin, 10 μ M coumarin, 50 μ M bupropion, 25 μ M amodiaquine, 150 μ M tolbutamide, 50 μ M S-mephenytoin, 25 μ M R-omeprazole, 10 μ M dextromethorphan, and 10 μ M testosterone) was incubated at 37°C in duplicate with HLMs and the NADPH-generating

system in the absence or the presence of efavirenz concentrations at 10 and 50 μM , which covered the range of average steady-state C_{max} receiving 600mg once daily [Product Information of Efavirenz (Sustiva), Bristol-Myers Squibb Company, June 2012]. Processing of the incubation mixture and HPLC analysis of the metabolites formed were performed as described above.

Positive control experiments were run in parallel by incubating each probe substrate at 37°C in duplicate with HLMs and the NADPH-generating system in the absence (control) and the presence of the following isoform-specific inhibitors: furafylline (20 μM ; specific for CYP1A2), pilocarpine (50 μM ; specific for CYP2A6), thioTEPA (50 μM ; specific for CYP2B6), ticlopidine (5 μM ; specific for CYP2B6), quercetin (10 μM ; specific for CYP2C8), sulfaphenazole (25 μM ; specific for CYP2C9), ticlopidine (5 μM ; specific for CYP2C19), quinidine (1 μM ; specific for CYP2D6), and ketoconazole (1 μM ; specific for CYP3A). The substrate probes and concentrations that were used in the screening experiments (see above) were used for these positive control experiments. Formation rates of the metabolites in the presence of the isoform-specific inhibitor were compared with that for controls in which the inhibitor was replaced with vehicle.

Determination of inhibition constants (K_i values)

In pilot experiments, efavirenz showed inhibition on the activities of CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A (by >20% at 50 μM); inhibition on the other CYPs (CYP1A2, 2A6, and 2D6) was minimal. Therefore, Dixon plots for the inhibition of CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A were determined by incubating multiple concentrations of the respective substrate probe in the presence and absence of multiple concentrations of efavirenz with HLMs and cofactors. K_i values in expressed enzymes were determined for two CYP isoforms that showed potent inhibition in pooled HLMs with $K_i < 10\mu\text{M}$, i.e. CYP2B6 and CYP2C8. The following isoform-specific probe substrate concentrations were used: 25 to 75 μM bupropion for CYP2B6; 10 to 100 μM amodiaquine for CYP2C8; 50 to 250 μM tolbutamide for CYP2C9; 15 to 75 μM S-mephenytoin for CYP2C19 and 5 to 50 μM testosterone for CYP3A. The concentrations of efavirenz used were 0 to 100 μM . The inhibition data obtained from the pilot experiments were used as a guide to generate appropriate probe substrate and test inhibitor concentrations for the determination of the K_i values for each isoform.

Assessment of time-dependent inhibition

Efavirenz was reported to be a time-dependent inhibitor of expressed CYP2B6, with a K_I value of 30 μM ¹⁸. Therefore, time-dependent inhibition was tested on eight major CYPs except for CYP2B6 using pooled HLMs. Efavirenz (50 μM) was pre-incubated in duplicate with HLMs and 200 mM sodium phosphate reaction buffer (pH 7.4) (without or with the NADPH-generating system) in the absence of a substrate probe for 0, 5, 10, 15 and 30 min at 37°C. The preincubation reaction was started by adding the NADPH-generating system. Controls were pre-incubated for 0 min without efavirenz and without the NADPH generating system. The total volume of the preincubation mixture was 600 μl . After 0, 5, 10, 15 and 30 min of preincubation, 50 μl of preincubation mixture was added to a glass tube containing 950 μl mixture that consisted of a substrate (final concentration corresponding to

the $V_{\max}$), phosphate reaction buffer and NADPH-generating system. The mixture was further incubated for the time specific for each assay. The reaction was stopped and processed as described above for the co-incubation experiments.

Data analysis

Apparent kinetic constants (K_m , $V_{\max}$) were estimated by fitting formation rates of metabolites versus substrate concentrations to simple single-site Michealis-Menten equation by nonlinear regression analysis using SigmaPlot 11.0 (Systat Software Inc., Richmond, CA). To calculate K_i values, the inhibition data were fit to different models of enzyme inhibition (competitive, noncompetitive, and uncompetitive) by nonlinear least-squares regression analysis with the Prism Version 5.0 software (GraphPad software Inc., San Diego, CA, USA). The final model for each data set was selected on the basis of visual inspection of Lineweaver-Burk, Dixon, and Eadie-Hofstee plots, as well as the size of the sum of squares of residuals, the Akaike information criterion, and Schwartz criterion values.

Prediction of *in vivo* drug interactions

Predictions of *in vivo* drug interaction potential of efavirenz were made using the following equations, which has been described previously ²⁶⁾ :

$$\frac{AUC_1}{AUC} = \frac{1}{\frac{f_m}{1 + \frac{[I]}{K_i}} + (1 - f_m)} \quad (1)$$

where: $\frac{AUC_1}{AUC}$ is the ratio of the AUC of substrate after inhibition to the AUC of the uninhibited substrate; f_m is the fraction of substrate metabolized by the inhibited CYP pathway. The utilities of four different values for *in vivo* inhibitor concentrations, which are the total systemic $C_{\max}$, free systemic $C_{\max}$, total hepatic inlet $C_{\max}$ estimated after oral administration and free hepatic inlet $C_{\max}$ in the prediction of drug interactions have been compared before and estimation using free hepatic inlet $C_{\max}$ yielded the most accurate predictions of the magnitude of drug interactions ²⁶⁾. But efavirenz is highly protein bound with fraction unbound in plasma estimated to be 0.029 ²⁷⁾. In order to avoid underestimation of potential risk for drug interaction, total hepatic inlet $C_{\max}$ ($C_{\text{hep,inlet}}$) of efavirenz were used in present study (equation 2), which also showed a reasonably good prediction in the previous study ²⁶⁾.

$$C_{\text{hep,inlet}} = C_{\max} + \frac{K_a F_a D}{Q_h} \quad (2)$$

Where: $C_{\max}$ is maximum plasma concentration; K_a is the absorption rate constant, which is estimated to be 0.3 h^{-1} ²⁸⁾; F_a is the fraction of the inhibitor passing through the intestine unchanged; D is the administered therapeutic dose (600 mg/day); and Q_h is hepatic blood flow (87 L/h) ²⁹⁾. Values of F_a can be estimated from oral bioavailability and hepatic extraction. Since both values are not available for efavirenz, a value of unity for F_a was assumed as the most cautious possibility ²⁹⁻³⁰⁾. There is no evidence that efavirenz enhances the activities of CYP2C8 and CYP2C9 *in vivo*. Therefore, $C_{\max}$ of efavirenz at steady state was used ($9.2 \sim 16.6 \mu\text{M}$) to predict the AUC changes of CYP2C8 and CYP2C9 substrates

[Product Information of Efavirenz (Sustiva), Bristol-Myers Squibb Company, June 2012]. However, efavirenz has been shown to enhance the activities of CYP2B6, CYP2C19 and CYP3A upon multiple dosing, suggesting inhibition effect may be masked by inductive effect of efavirenz in a time-dependent manner. Therefore, predicting AUC changes of CYP2B6, CYP2C19 and CYP3A substrates was estimated using C_{max} (4.6 ~ 8.4 μ M) obtained after the administration of a single 600 mg oral dose of efavirenz to 20 healthy volunteers (unpublished data). Specifically, we focused on substrates that exhibit narrow therapeutic range and thus initiation of efavirenz to patients stabilized on these drugs may increase the risk of adverse effects (methadone) or failure of therapy (clopidogrel and proguanil). Of note, clopidogrel and proguanil are pro-drugs that require conversion to pharmacologically active metabolites primarily by CYP2C19³¹⁻³³). For these prodrugs that require conversion by CYP2C19 to active metabolites, the following equation published previously³⁴) was slightly modified to predict the extent of inhibition of bioactivation:

$$\frac{AUC_m}{AUC_p} = \frac{f_{u,p} \times Cl_f}{f_{u,m} \times Cl_m} \quad (3)$$

where $\frac{AUC_m}{AUC_p}$ is the ratio of the AUC of metabolite to the AUC of parent compound. The $f_{u,p}$ and $f_{u,m}$ are the plasma fraction unbound of the parent and metabolite. Cl_f and Cl_m are the formation and elimination clearance of metabolite.

The majority of clopidogrel is metabolized by an esterase³⁵) and relative importance of CYP2C19 for the overall elimination of proguanil is much lower than it is for the formation of cycloguanil³²). Assuming that efavirenz does not affect the elimination of metabolite and the change in the AUC of parent is negligible, the equation 4 can be derived from equation 1 and 3 as:

$$\frac{AUC_m}{AUC_{m,I}} = \frac{Cl_f}{Cl_{f,I}} = \frac{1}{\frac{f_m}{1 + \frac{[I]}{K_i}} + (1 - f_m)} \quad (4)$$

where $\frac{AUC_m}{AUC_{m,I}}$ is the ratio of the uninhibited AUC of metabolite to the AUC of metabolite with inhibition. $\frac{Cl_f}{Cl_{f,I}}$ is the ratio of the uninhibited formation clearance to the formation clearance after inhibition. f_m for clopidogrel and proguanil is defined as the fraction of active metabolites formed by CYP2C19. Since f_m for proguanil is not available in the literature, it was estimated using a pharmacogenetic method modified from a recent study for CYP2D6 substrate³⁶). The original method was based on the observation that the ratio of AUC in poor metabolizer (PM), AUC^{PM} , to the AUC in extensive metabolizer (EM), AUC^{EM} . Assuming that genetic polymorphisms do not affect the elimination of metabolite and has limited effects on the AUC of the parent compound, the value of f_m for proguanil metabolized to cycloguanil by CYP2C19 was estimated from a clinical study³²) using the following equation:

$$\frac{AUC_m^{EM}}{AUC_m^{PM}} = \frac{1}{1 - f_m^{EM}} \quad (5)$$

where $\frac{AUC_m^{EM}}{AUC_m^{PM}}$ is the ratio of metabolite AUC in poor metabolizer (PM) to the metabolite AUC in extensive metabolizer (EM).

Results

Screening for inhibition of multiple CYPs by efavirenz

The inhibitory effect of efavirenz at 10 and 50 μ M on the activities of eight CYP isoforms in pooled HLMs is shown in Figure 1. Efavirenz was a potent inhibitor of CYP2B6 (by 90% at 10 and 50 μ M). It also showed inhibition of CYP2C8, CYP2C9 and CYP2C19 by >20% at 10 μ M and by >50% at 50 μ M. Efavirenz only showed weak inhibition on the activity of CYP3A (testosterone β -hydroxylation) by 10% at 10 μ M and by 40% at 50 μ M.

The activity of CYP2C19 was assessed using two substrates (R-omeprazole and S-mephenytoin) since substrate-dependent effect on CYP2C19 inhibition profile was observed previously³⁷. In the present study, efavirenz inhibited CYP2C19 activity by 35% and 70% at 10 and 50 μ M, when S-mephenytoin was used as a substrate (Figure 1), but its effect on CYP2C19-mediated R-omeprazole 5-hydroxylation was marginal (by 10% at 50 μ M efavirenz) (data not shown). This result is consistent with a previous report that S-mephenytoin is more sensitive to CYP2C19 inhibition than R-omeprazole³⁷. Therefore, S-mephenytoin was used as a substrate of CYP2C19 in subsequent inhibition experiment.

We have *in vivo* evidence that efavirenz reduces CYP1A2 activity, as measured by caffeine metabolism: compared to a single efavirenz dose (600 mg orally), pretreatment with efavirenz (600 mg/day for 17 days) significantly decreased 5-7hrs concentration ratio of paraxanthine/caffeine (from 0.52±0.28 single dose to 0.31±0.18 multiple dose; P<0.0001)³⁸. However, the present *in vitro* data derived from pooled HLMs shown in Figure 1 did not indicate that efavirenz inhibits CYP1A2 activity (Figure 1). Therefore, we tested whether the major metabolite of efavirenz, 8-hydroxyefavirenz, contributes to inhibition of CYP1A2 and showed that 8-hydroxyefavirenz inhibited CYP1A2 by ~20% up to 10 μ M (Figure 2), which suggests that other alternative mechanisms should account for the reduced CYP1A2 activity that we observed *in vivo*.

The inhibitory effect of efavirenz on the activity of CYP2A6 and CYP2D6 was negligible (less than 10% at both efavirenz concentrations) (Figure 1).

Estimation of K_i values

In order to obtain quantitative prediction of magnitude of drug interaction *in vivo*, further experiments were performed to determine the K_i values for the inhibition of CYP2B6, 2C8, 2C9 and 2C19 by efavirenz. Although relatively weak inhibition of CYP3A was observed by efavirenz, K_i value was determined in pooled HLMs because a previous study reported that the value of IC₅₀ is around 20 μ M using midazolam as a substrate¹⁶.

Of all the CYPs tested, CYP2B6 was the most sensitive to efavirenz inhibition (Table 1). Visual inspection of the Dixon plot and further analysis of the parameters of the enzyme inhibition models suggested that the inhibition data fit well to a competitive type of inhibition. The K_i values estimated by using a nonlinear regression model for competitive

enzyme inhibition of CYP2B6-catalyzed bupropion 4-hydroxylation in pooled HLMs and CMV negative HLMs were $2.96 \pm 0.67 \mu\text{M}$ and $0.39 \pm 0.10 \mu\text{M}$, respectively. K_i value determined in expressed CYP2B6 was $1.38 \pm 0.09 \mu\text{M}$. Representative Dixon plots for the inhibition of CYP2B6 in CMV negative HLMs and expressed CYP2B6 are shown in Figure 3A and 3B, respectively.

Inhibition of CYP2C8 by efavirenz was determined in two HLMs and expressed CYP2C8. As shown in Table 1, efavirenz showed potent competitive inhibition of CYP2C8 activity in pooled HLMs ($K_i = 4.78 \pm 2.24 \mu\text{M}$). The second HLMs was obtained from human liver tissues with the CYP2C8*3/*3 genotype and the K_i value ($4.80 \pm 0.35 \mu\text{M}$) derived from this HLM was not different from that derived from pooled HLMs (Table 1). Efavirenz exhibited similar competitive inhibition in expressed CYP2C8 with an estimated K_i value of $6.05 \pm 2.86 \mu\text{M}$ (Table 1). In Figure 4, Dixon plots for the inhibition of CYP2C8-catalyzed N-desethylation of amodiaquine by efavirenz in pooled HLMs (Figure 4A), HLMs with CYP2C8*3/*3 genotype (Figure 4B) and expressed CYP2C8 (Figure 4C) are shown.

Efavirenz was found to be a moderate inhibitor of CYP2C9 ($K_i = 19.46 \pm 2.78 \mu\text{M}$; Table 1 and Figure 5) and CYP2C19 ($21.31 \pm 2.57 \mu\text{M}$; Table 1 and Figure 6), and a weak inhibitor of CYP3A ($K_i = 40.33 \pm 0.33 \mu\text{M}$; Table 1 and Figure 7).

Time-dependent inactivation

As shown in Figure 8, efavirenz preincubation for 30 min only marginally inhibited the activity of those CYPs tested. Shorter preincubation times (5-15 min) were also tested, but did not show any indication of time-dependent inactivation.

Quantitative prediction of *in vivo* drug interactions

The predicted ratios of $\frac{AUC_I}{AUC}$ for each substrate co-administered with a single dose or multiple doses of efavirenz are listed in Table 2. Compared to control (without efavirenz), a single 600 mg oral dose of efavirenz was predicted to result in ~3-fold changes in the exposure of methadone (CYP2B6 substrate). Also, based on the inhibition data generated using S-mephenytoin hydroxylation as a marker of CYP2C19, we predicted lower AUCs of active metabolites of clopidogrel and proguanil (by 17% ~ 29% and 29% ~ 33% respectively), and higher omeprazole AUC (by 1.4- to 1.6-fold) in extensive metabolizer of CYP2C19. However, when data generated using R-omeprazole 5-hydroxylation is used, no inhibition could be predicted *in vivo*. Based on our *in vitro* data, a single dose of efavirenz is unlikely to alter the pharmacokinetics of CYP3A substrates.

After multiple doses of efavirenz, the AUC of CYP2C8 and CYP2C9 substrates was predicted to be ~3.5- to 4.4-fold higher (CYP2C8 substrate: amodiaquine) and 1.7- to 2.0-fold higher (CYP2C9 substrates: phenytoin and S-warfarin) compared to controls (without efavirenz).

Discussion

The primary aim of this study is to characterize the inhibition constant (K_i) of efavirenz in CYPs that showed inhibition and quantitatively predict its inhibition effect on the AUC of

clinically important co-administered drugs. Previous *in vitro* studies only provided qualitative information (IC_{50} values). In the present study, we have shown that efavirenz is a potent competitive inhibitor of CYP2B6 (average K_i = 1.68 μ M in HLMs and K_i = 1.38 μ M in expressed CYP2B6) and CYP2C8 (K_i = 4.78 μ M in pooled HLMs and K_i = 4.80 μ M in HLMs with *CYP2C8*3/*3* genotype). In pooled HLMs, efavirenz showed moderate inhibition of CYP2C9 (K_i = 19.46 μ M) CYP2C19 (K_i = 21.31 μ M), and a weak inhibitor of CYP3A (K_i = 40.33 μ M). Inhibition of CYP1A2, CYP2A6 and CYP2D6 by efavirenz was marginal. No time-dependent inactivation of the CYP isoforms tested was observed. Based on the *in vitro* to *in vivo* quantitative prediction, efavirenz is expected to : a) increase the AUC of methadone (CYP2B6 substrate) by 2.9- to 3.1-fold, omeprazole by 1.4- to 1.6-fold (CYP2C19 substrate), and may also lower the AUC of active metabolites of some pro-drugs (e.g. clopidogrel and proguanil) by up to 30% during initiation of efavirenz-based anti-HIV therapy; and b) increase the AUC of amodiaquine by ~3-fold (CYP2C8 substrate) and phenytoin and warfarin by 1.7- to 2.0-fold (CYP2C9 substrates) during a single dose or multiple doses of efavirenz. Our data suggest that efavirenz may increase the risk for adverse effects by increasing the exposure of the parent drug or reduce efficacy by diminishing the formation of pharmacologically active metabolites from prodrugs.

Of the CYPs tested, CYP2B6 was most sensitive to efavirenz inhibition with K_i value of ~1.7 μ M in HLMs and ~1.38 μ M in expressed CYP2B6. Although the ability of efavirenz to inhibit CYP2B6 was previously reported ¹⁷⁻¹⁸⁾, the present data provide key information that allowed *in vivo* quantitative prediction of the magnitude of interaction. The high inhibition potency of efavirenz in our study is worth commenting. Efavirenz has a higher binding affinity to CYP2B6 with K_m values of 13 ~ 20 μ M ²⁻³⁾ than bupropion with K_m values of 90 ~ 130 μ M ³⁹⁻⁴⁰⁾. Thus, it is plausible that the high inhibition potency of efavirenz on bupropion hydroxylation could be due to the fact that efavirenz has higher binding affinity to CYP2B6 than bupropion. Similar mechanism contributing to high inhibition potency has been reported for CYP2D6 ⁴¹⁾. To put the *in vitro* inhibition data on CYP2B6 into perspective, it is important to point out that efavirenz enhances its own metabolism upon multiple doses preferentially through CAR-mediated induction of CYP2B6 ⁴²⁾. Efavirenz also enhances the metabolism of co-administered CYP2B6 substrates, including methadone ^{7, 43)} and bupropion ⁴⁴⁾. Considering the high inhibition potency of efavirenz, a substantial increase in AUC of CYP2B6 substrates and potentially the risk to adverse effects may be expected, when efavirenz-based therapy is initiated in patients who are stabilized on CYP2B6 substrates. We predicted approximately 2.9- to 3.1-fold increase in methadone AUC, when a single 600 mg oral dose of efavirenz is co-administered. However, during chronic administration, inhibition of CYP2B6 by efavirenz appears to be masked by its marked induction and the net effect becomes induction.

Our study demonstrates that efavirenz inhibits CYP2C8-mediated amodiaquine desethylation with K_i values of 4.78 and 6.05 μ M in pooled HLMs and expressed CYP2C8 respectively, which broadly concurs with an IC_{50} of 4 μ M reported in expressed CYPs ¹⁵⁾. The inhibition potency of efavirenz in HLMs with the *CYP2C8*3/*3* genotype, the most frequent and functionally relevant variant in Caucasians ⁴⁵⁾, was not different from that observed in pooled HLMs. The possibility of substrate-dependent interaction cannot be fully

excluded⁴⁶⁾, but our data suggest that the *CYP2C8*3* allele does not seem to alter susceptibility to efavirenz inhibition. We expect ~3-fold higher AUC of amodiaquine and probably other substrates such as chloroquine, certain anti-diabetics, montelukast and rosiglitazone⁴⁷⁻⁴⁸⁾, when co-administered with efavirenz. A clinical study that was designed to evaluate drug interactions between anti-malarials and efavirenz-based anti-HIV therapy was prematurely discontinued after the first two subjects developed hepatotoxicity¹⁹⁾. A 2.2- to 4-fold increase in amodiaquine AUC was also noted¹⁹⁾ and it is highly likely that this interaction occurred through inhibition of CYP2C8, as predicted from our *in vitro* data.

Our data showed that efavirenz inhibits CYP2C9 activity ($K_i = 19.46 \mu\text{M}$) with 1.7- to 2-fold predicted increase in AUC of drugs mainly cleared by CYP2C9, consistent with an *in vitro* study reporting an IC_{50} value of $\sim 15 \mu\text{M}$ ¹⁶⁾. This enzyme is involved in the metabolism of more than 100 currently used drugs, including drugs with narrow therapeutic range, e.g., oral anticoagulants, oral hypoglycemic agents and phenytoin⁴⁹⁾. Therefore, co-administration of efavirenz may likely increase the risks to adverse effects of these drugs, which is supported by clinical cases of inhibition drug interactions of efavirenz with the CYP2C9 substrate phenytoin²¹⁾ and warfarin²⁰⁾.

We found that the extent of CYP2C19 inhibition by efavirenz was substrate-dependent: modest inhibition of S-mephenytoin 4-hydroxylation ($K_i = 21.31 \mu\text{M}$) and marginal inhibition of R-omeprazole 5-hydroxylation consistent with a previous study reporting that R-omeprazole is less sensitive to CYP2C19 inhibitors than S-mephenytoin³⁷⁾. The clinical relevance of efavirenz inhibition on CYP2C19 and the mechanism of substrate-dependent inhibition remain unclear, but this interaction may be important for prodrugs that require bioactivation by CYP2C19. For example, at a single dose or acute dosing, efavirenz may inhibit the formation of active metabolite of clopidogrel by CYP2C19 and to some extent by CYP2B6 and CYP2C9⁵⁰⁾. Provided that efavirenz is a potent inhibitor of CYP2B6 and a moderate inhibitor of CYP2C19 and CYP2C9 (present data), it is likely that administration of efavirenz to patients stabilized on clopidogrel may result in substantially reduced formation of active metabolite and lack of efficacy. At steady state, efavirenz may be a mixed inhibitor and inducer of CYP2C19⁹⁾, although induction appears to predominate as shown with the enhanced metabolism of CYP2C19 substrates including voriconazole¹⁰⁾, omeprazole⁹⁾, etravirine¹²⁾. Contradictory effects were observed in another CYP2C19 substrate proguanil, with decreased proguanil exposure co-administered with 600 mg/day efavirenz for 1 month¹¹⁾ and increased proguanil exposure co-administered with 400 mg/day efavirenz for 9 days⁵¹⁾. This discrepancy may be due to differences in dose regimens, duration of treatment and study populations.

Efavirenz-mediated *in vivo* inhibition of CYP3A seems unlikely given the high K_i value ($K_i = \sim 40 \mu\text{M}$) observed in the present study. Using triazolam as a substrate, another study reported lower IC_{50} values ($17\text{--}20 \mu\text{M}$)¹⁶⁾, but the significance of *in vivo* inhibition of CYP3A, if any, is likely marginal. Efavirenz, through activation of PXR/CAR, induces CYP3A *in vitro*⁵⁻⁶⁾ and *in vivo*^{9, 52)}. Hence, efavirenz enhances the elimination of many CYP3A substrates, including protease inhibitors, statins⁸⁾, calcium channel blockers and anti-fungals [Product Information of Efavirenz (Sustiva), Bristol-Myers Squibb Company,

June 2012]. Therefore, induction drug interactions between efavirenz and CYP3A substrates appear to predominate at steady-state.

CYP1A2, CYP2A6 and UGT2B7 all have been shown to be involved in efavirenz metabolism^{2, 4)}. Efavirenz is not only a substrate but also a moderate inhibitor of UGT2B7⁴⁾, while no reversible inhibition or time-dependent inhibition by efavirenz was observed for CYP1A2 and CYP2A6 in the present study. The drug interaction with CYP2A6 substrates mediated by efavirenz inhibition seems very unlikely, but the possibility of efavirenz inhibiting the metabolism of CYP1A2 substrates can't be excluded, because the preliminary results from our laboratory showed that efavirenz reduces CYP1A2 activity as measured by caffeine metabolism *in vivo*.

The use of efavirenz is made difficult by high interindividual variability in its disposition and by often unpredictable and complex drug interactions. The extent of drug interactions with efavirenz varies greatly among individuals, and interpatient differences in efavirenz exposure contribute to this variability. Efavirenz exposure is governed by complex factors: efavirenz is mainly cleared by CYP2B6, with some contribution from accessory pathways catalyzed by enzymes that include CYP2A6, CYP1A2, CYP3A and UGT2B7^{2-4, 53-55)}; efavirenz activates CAR and PXR, induces CYP2B6 (and other drug disposition genes) and autoinduces its own metabolism⁴²⁾ upon repeated administration; and efavirenz is a potent inhibitor of CYP2B6 with no effect on CYP2A6 and CYP1A2 (present study) and a moderate inhibitor of UGT2B7 *in vitro*⁴⁾. These complex inhibition/induction processes and genetic variations of CYP2B6 would likely contribute to variable efavirenz exposure and drug interactions. The net effect of efavirenz on drug interactions (induction versus inhibition) *in vivo* is likely to be dependent on: duration of efavirenz administration (acute versus chronic); genetic and nongenetic factors; and the potency with which efavirenz induces or inhibits drug metabolizing enzymes. Although predicting the extent and direction of drug interactions with efavirenz *in vivo* might be difficult for the individual patient, general comments could be made based on our data and the literature. During initiation of efavirenz-based therapy, it is expected that efavirenz reduces the elimination of CYP2B6, CYP2C8, CYP2C9 and CYP2C19 substrates. Mixed induction/inhibition occurs upon repeated administration of efavirenz, but the net effect of efavirenz appears to be induction for CYP2B6 and CYP2C19, while inhibition appears to dominate for CYP2C8 and CYP2C9. Induction appears the main mechanism for efavirenz interactions involving CYP3A. Together, efavirenz's complex interaction with enzymes involved in its own metabolism and the metabolism of co-administered drugs may contribute to the large interindividual variability of efavirenz exposure and unpredictable drug interactions associated with it.

Acknowledgments

The project described here was supported by the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD [GM078501, GM078501-04S1, and 2R56GM067308-09A1]

Abbreviations

P450	cytochrome P450
HLMs	human liver microsomes
Cyt b5	cytochrome b5
HPLC	high performance liquid chromatography
LC/MS/MS	liquid chromatography/tandem mass spectrometry
EFV	efavirenz
8-OHEFV	8-hydroxyefavirenz
BUP	bupropion
SNP	single nucleotide polymorphism
K_m	Michaelis-Menten constant
V_{max}	maximum enzyme velocity
AUC	area under the plasma concentration-time curve
EM	extensive metabolizer
PM	poor metabolizer

Reference

- Deeks ED, Perry CM. Efavirenz/emtricitabine/tenofovir disoproxil fumarate single-tablet regimen (Atripla(R)): a review of its use in the management of HIV infection. *Drugs*. 2010; 70:2315–2338. [PubMed: 21080746]
- Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J. Pharmacol. Exp. Ther.* 2003; 306:287–300. [PubMed: 12676886]
- Ogburn ET, Jones DR, Masters AR, Xu C, Guo Y, Desta Z. Efavirenz primary and secondary metabolism in vitro and in vivo: identification of novel metabolic pathways and cytochrome P450 2A6 as the principal catalyst of efavirenz 7-hydroxylation. *Drug Metab. Dispos.* 2010; 38:1218–1229. [PubMed: 20335270]
- Belanger AS, Caron P, Harvey M, Zimmerman PA, Mehlotra RK, Guillemette C. Glucuronidation of the antiretroviral drug efavirenz by UGT2B7 and an in vitro investigation of drug-drug interaction with zidovudine. *Drug Metab. Dispos.* 2009; 37:1793–1796.
- Hariparsad N, Nallani SC, Sane RS, Buckley DJ, Buckley AR, Desai PB. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. *J. Clin. Pharmacol.* 2004; 44:1273–1281. [PubMed: 15496645]
- Faucette SR, Zhang TC, Moore R, Sueyoshi T, Omiecinski CJ, LeCluyse EL, Negishi M, Wang H. Relative activation of human pregnane X receptor versus constitutive androstane receptor defines distinct classes of CYP2B6 and CYP3A4 inducers. *J. Pharmacol. Exp. Ther.* 2007; 320:72–80. [PubMed: 17041008]
- Clarke SM, Mulcahy FM, Tjia J, Reynolds HE, Gibbons SE, Barry MG, Back DJ. The pharmacokinetics of methadone in HIV-positive patients receiving the nonnucleoside reverse transcriptase inhibitor efavirenz. *Br. J. Clin. Pharmacol.* 2001; 51:213–217. [PubMed: 11298066]
- Gerber JG, Rosenkranz SL, Fichtenbaum CJ, Vega JM, Yang A, Alston BL, Brobst SW, Segal Y, Aberg JA. Effect of efavirenz on the pharmacokinetics of simvastatin, atorvastatin, and pravastatin:

- results of AIDS Clinical Trials Group 5108 Study. *J. Acquir. Immune. Defic. Syndr.* 2005; 39:307–312. [PubMed: 15980690]
9. Michaud V, Ogburn E, Thong N, Aregbe AO, Quigg TC, Flockhart DA, Desta Z. Induction of CYP2C19 and CYP3A Activity Following Repeated Administration of Efavirenz in Healthy Volunteers. *Clin. Pharmacol. Ther.* 2012; 91:475–482. [PubMed: 22318618]
10. Liu P, Foster G, LaBadie RR, Gutierrez MJ, Sharma A. Pharmacokinetic interaction between voriconazole and efavirenz at steady state in healthy male subjects. *J. Clin. Pharmacol.* 2008; 48:73–84. [PubMed: 18025525]
11. van Luin M, Van der Ende ME, Richter C, Visser M, Faraj D, Van der Ven A, Gelinck L, Kroon F, Wit FW, Van Schaik RH, Kuks PF, Burger DM. Lower atovaquone/proguanil concentrations in patients taking efavirenz, lopinavir/ritonavir or atazanavir/ritonavir. *AIDS.* 2010; 24:1223–1226. [PubMed: 20299957]
12. Boffito M, Jackson A, Lamorde M, Back D, Watson V, Taylor J, Waters L, Asboe D, Gazzard B, Pozniak A. Pharmacokinetics and safety of efavirenz administered once or twice daily after 2 weeks treatment with efavirenz in healthy volunteers. *J. Acquir. Immune. Defic. Syndr.* 2009; 52:222–227. [PubMed: 19620877]
13. Staszewski S, Morales-Ramirez J, Tashima KT, Rachlis A, Skiest D, Stanford J, Stryker R, Johnson P, Labriola DF, Farina D, Manion DJ, Ruiz NM. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team. *N. Engl. J. Med.* 1999; 341:1865–1873.
14. Zhu M, Kaul S, Nandy P, Grasela DM, Pfister M. Model-based approach to characterize efavirenz autoinduction and concurrent enzyme induction with carbamazepine. *Antimicrob. Agents Chemother.* 2009; 53:2346–2353. [PubMed: 19223624]
15. Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL. Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. *Clin. Pharmacol. Ther.* 2007; 82:197–203. [PubMed: 17361129]
16. von Moltke LL, Greenblatt DJ, Granda BW, Giancarlo GM, Duan SX, Daily JP, Harmatz JS, Shader RI. Inhibition of human cytochrome P450 isoforms by nonnucleoside reverse transcriptase inhibitors. *J. Clin. Pharmacol.* 2001; 41:85–91. [PubMed: 11225565]
17. Hesse LM, von Moltke LL, Shader RI, Greenblatt DJ. Ritonavir, efavirenz, and nelfinavir inhibit CYP2B6 activity in vitro: potential drug interactions with bupropion. *Drug Metab. Dispos.* 2001; 29:100–102. [PubMed: 11159797]
18. Bumpus NN, Kent UM, Hollenberg PF. Metabolism of efavirenz and 8-hydroxyefavirenz by P450 2B6 leads to inactivation by two distinct mechanisms. *J. Pharmacol. Exp. Ther.* 2006; 318:345–351. [PubMed: 16611850]
19. German P, Greenhouse B, Coates C, Dorsey G, Rosenthal PJ, Charlebois E, Lindegardh N, Havlir D, Aweeka FT. Hepatotoxicity due to a drug interaction between amodiaquine plus artesunate and efavirenz. *Clin. Infect. Dis.* 2007; 44:889–891. [PubMed: 17304470]
20. Bonora S, Lanzafame M, D'Avolio A, Trentini L, Lattuada E, Concia E, Di Perri G. Drug interactions between warfarin and efavirenz or lopinavir-ritonavir in clinical treatment. *Clin. Infect. Dis.* 2008; 46:146–147. [PubMed: 18171231]
21. Robertson SM, Penzak SR, Lane J, Pau AK, Mican JM. A potentially significant interaction between efavirenz and phenytoin: a case report and review of the literature. *Clin. Infect. Dis.* 2005; 41:e15–18. [PubMed: 15983905]
22. Jeong S, Nguyen PD, Desta Z. Comprehensive in vitro analysis of voriconazole inhibition of eight cytochrome P450 (CYP) enzymes: major effect on CYPs 2B6, 2C9, 2C19, and 3A. *Antimicrob. Agents Chemother.* 2009; 53:541–551. [PubMed: 19029318]
23. Zhao XJ, Jones DR, Wang YH, Grimm SW, Hall SD. Reversible and irreversible inhibition of CYP3A enzymes by tamoxifen and metabolites. *Xenobiotica.* 2002; 32:863–878. [PubMed: 12419016]
24. Xu C, Ogburn ET, Guo Y, Desta Z. Effects of the CYP2B6*6 Allele on Catalytic Properties and Inhibition of CYP2B6 in vitro: Implication for the Mechanism of Reduced Efavirenz Metabolism and Other CYP2B6 Substrates in vivo. *Drug Metab. Dispos.* 2012; 40:717–725. [PubMed: 22232427]

25. Lu WJ, Xu C, Pei Z, Mayhoub AS, Cushman M, Flockhart DA. The tamoxifen metabolite norendoxifen is a potent and selective inhibitor of aromatase (CYP19) and a potential lead compound for novel therapeutic agents. *Breast Cancer Res. Treat.* 2011; 133:99–109. [PubMed: 21814747]
26. Obach RS, Walsky RL, Venkatakrishnan K, Gaman EA, Houston JB, Tremaine LM. The utility of in vitro cytochrome P450 inhibition data in the prediction of drug-drug interactions. *J. Pharmacol. Exp. Ther.* 2006; 316:336–348.
27. Shou M, Hayashi M, Pan Y, Xu Y, Morrissey K, Xu L, Skiles GL. Modeling, prediction, and in vitro in vivo correlation of CYP3A4 induction. *Drug Metab. Dispos.* 2008; 36:2355–2370. [PubMed: 18669588]
28. Csajka C, Marzolini C, Fattinger K, Decosterd LA, Fellay J, Telenti A, Biollaz J, Buclin T. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. *Clin. Pharmacol. Ther.* 2003; 73:20–30. [PubMed: 12545140]
29. Walsky RL, Astuccio AV, Obach RS. Evaluation of 227 drugs for in vitro inhibition of cytochrome P450 2B6. *J. Clin. Pharmacol.* 2006; 46:1426–1438. [PubMed: 17101742]
30. Obach RS, Walsky RL, Venkatakrishnan K, Houston JB, Tremaine LM. In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: qualitative relationships, quantitative predictions, and the rank-order approach. *Clin. Pharmacol. Ther.* 2005; 78:582–592. [PubMed: 16338274]
31. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, Aiach M, Lechat P, Gaussem P. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood.* 2006; 108:2244–2247. [PubMed: 16772608]
32. Jeppesen U, Rasmussen BB, Brosen K. Fluvoxamine inhibits the CYP2C19-catalyzed bioactivation of chloroguanide. *Clin. Pharmacol. Ther.* 1997; 62:279–286. [PubMed: 9333103]
33. Carrington HC, Crowther AF, Davey DG, Levi AA, Rose FL. A metabolite of paludrine with high antimalarial activity. *Nature.* 1951; 168:1080. [PubMed: 14910643]
34. Lutz JD, Isoherranen N. Prediction of relative in vivo metabolite exposure from in vitro data using two model drugs: dextromethorphan and omeprazole. *Drug Metab. Dispos.* 2012; 40:159–168. [PubMed: 22010218]
35. Lins R, Broekhuysen J, Necciari J, Deroubaix X. Pharmacokinetic profile of 14C-labeled clopidogrel. *Semin. Thromb. Hemost.* 1999; 25(Suppl 2):29–33. [PubMed: 10440420]
36. Tod M, Goutelle S, Gagnieu MC. Genotype-Based Quantitative Prediction of Drug Exposure for Drugs Metabolized by CYP2D6. *Clin. Pharmacol. Ther.* 2011; 90:582–587. [PubMed: 21866098]
37. Foti RS, Wahlstrom JL. CYP2C19 inhibition: the impact of substrate probe selection on in vitro inhibition profiles. *Drug Metab. Dispos.* 2008; 36:523–528. [PubMed: 18048485]
38. Metzger, IF.; Lu, JB.; Thong, N.; Desta, Z. Abstract accepted for presentation at the Annual Meeting of American Society for Clinical Pharmacology and Therapeutics. Indianapolis; Indiana, USA: Mar 6-9. 2013 The antiretroviral drug efavirenz inhibits CYP1A2 Activity in Healthy Volunteers..
39. Faucette SR, Hawke RL, Lecluyse EL, Shord SS, Yan B, Laethem RM, Lindley CM. Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metab. Dispos.* 2000; 28:1222–1230. [PubMed: 10997944]
40. 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. *Drug Metab. Dispos.* 2000; 28:1176–1183. [PubMed: 10997936]
41. Vandenbrink BM, Foti RS, Rock DA, Wienkers LC, Wahlstrom JL. Prediction of CYP2D6 Drug Interactions from In Vitro Data: Evidence for Substrate-Dependent Inhibition. *Drug Metab. Dispos.* 2012; 40:47–53. [PubMed: 21976621]
42. Oswald S, Meyer zu Schwabedissen HE, Nassif A, Modess C, Desta Z, Ogburn ET, Mostertz J, Keiser M, Jia J, Hubeny A, Ulrich A, Runge D, Marinova M, Lutjohann D, Kroemer HK, Siegmund W. Impact of efavirenz on intestinal metabolism and transport: insights from an interaction study with ezetimibe in healthy volunteers. *Clin. Pharmacol. Ther.* 2012; 91:506–513. [PubMed: 22297387]

43. Kharasch ED, Whittington D, Ensign D, Hoffer C, Bedynek PS, Campbell S, Stubbert K, Crafford A, London A, Kim T. Mechanism of efavirenz influence on methadone pharmacokinetics and pharmacodynamics. *Clin. Pharmacol. Ther.* 2012; 91:673–684. [PubMed: 22398970]
44. Robertson SM, Maldarelli F, Natarajan V, Formentini E, Alfaro RM, Penzak SR. Efavirenz induces CYP2B6-mediated hydroxylation of bupropion in healthy subjects. *J. Acquir. Immune. Defic. Syndr.* 2008; 49:513–519. [PubMed: 18989234]
45. Daily EB, Aquilante CL. Cytochrome P450 2C8 pharmacogenetics: a review of clinical studies. *Pharmacogenomics.* 2009; 10:1489–1510. [PubMed: 19761371]
46. Kaspera R, Naraharisetti SB, Evangelista EA, Marcianti KD, Psaty BM, Totah RA. Drug metabolism by CYP2C8.3 is determined by substrate dependent interactions with cytochrome P450 reductase and cytochrome b5. *Biochem. Pharmacol.* 2011; 82:681–691. [PubMed: 21726541]
47. Lai XS, Yang LP, Li XT, Liu JP, Zhou ZW, Zhou SF. Human CYP2C8: structure, substrate specificity, inhibitor selectivity, inducers and polymorphisms. *Curr. Drug Metab.* 2009; 10:1009–1047. [PubMed: 20214592]
48. Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin. Pharmacol. Ther.* 2005; 77:341–352.
49. Rettie AE, Jones JP. Clinical and toxicological relevance of CYP2C9: drug-drug interactions and pharmacogenetics. *Annu. Rev. Pharmacol. Toxicol.* 2005; 45:477–494. [PubMed: 15822186]
50. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab. Dispos.* 2010; 38:92–99. [PubMed: 19812348]
51. Soyinka JO, Onyeji CO. Alteration of pharmacokinetics of proguanil in healthy volunteers following concurrent administration of efavirenz. *Eur. J. Pharm. Sci.* 2010; 39:213–218. [PubMed: 19961932]
52. Mouly S, Lown KS, Kornhauser D, Joseph JL, Fiske WD, Benedek IH, Watkins PB. Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clin. Pharmacol. Ther.* 2002; 72:1–9. [PubMed: 12151999]
53. Cho DY, Ogburn ET, Jones D, Desta Z. Contribution of N-glucuronidation to efavirenz elimination in vivo in the basal and rifampin-induced metabolism of efavirenz. *Antimicrob. Agents. Chemother.* 2011; 55:1504–1509. [PubMed: 21282425]
54. di Iulio J, Fayet A, Arab-Alameddine M, Rotger M, Lubomirov R, Cavassini M, Furrer H, Gunthard HF, Colombo S, Csajka C, Eap CB, Decosterd LA, Telenti A. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. *Pharmacogenet. Genomics.* 2009; 19:300–309. [PubMed: 19238117]
55. Kwara A, Lartey M, Sagoe KW, Kenu E, Court MH. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS.* 2009; 23:2101–2106. [PubMed: 19779319]
56. Li XQ, Bjorkman A, Andersson TB, Ridderstrom M, Masimirembwa CM. Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. *J. Pharmacol. Exp. Ther.* 2002; 300:399–407. [PubMed: 11805197]
57. 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. *Eur. J. Clin. Pharmacol.* 2001; 57:31–36. [PubMed: 11372587]
58. Boulenc X, Djebli N, Shi J, Perrin L, Brian W, Van Horn R, Hurbin F. Effects of Omeprazole and Genetic Polymorphism of CYP2C19 on the Clopidogrel Active Metabolite. *Drug Metab. Dispos.* 2012; 40:187–197. [PubMed: 22004687]

Figure 1. Inhibitory effects of efavirenz on human CYP activities in pooled HLMs

A substrate probe of each specific CYP isoform at a single concentration was incubated with pooled HLMs and cofactors in the absence (control) or the presence of efavirenz (10 and 50 μ M) for times and with protein concentrations that were linear for the respective reaction described in detail in Material and Methods. The specific concentrations of each probe used are illustrated in Materials and Methods. Each point represented the average of duplicate incubations. EFV, efavirenz.

Figure 2. Inhibition of CYP1A2 by 8-hydroxyefavirenz in pooled HLMs

Phenacetin (50 μM) was incubated with pooled HLMs (1 mg/ml) and cofactors in the absence (control) or the presence of 8-hydroxyefavirenz (0.5 to 10 μM) at 37°C for 30 min. Each point represented the average of duplicate incubations. 8OHEFV, 8-hydroxyefavirenz.

Figure 3. Dixon plots for the inhibition of bupropion 4-hydroxylation by efavirenz in CMV Negative HLMs (A) and expressed CYP2B6 (B)

Bupropion (25 to 75 μM) was incubated with CMV negative HLMs (0.25 mg/ml) or expressed CYP2B6 (5 pmol) and cofactors at 37°C for 15 min with or without efavirenz (0-1 μM). Each point represented the average of duplicate incubations.

Figure 4. Dixon plots for the inhibition of amodiaquine desethylation by efavirenz in pooled HLMs (A), HLMs with CYP2C8*3/*3 genotype (B) and expressed CYP2C8 (C)
 Amodiaquine (10 to 100 μ M) was incubated with pooled HLMs (0.1 mg/ml) or HLMs with CYP2C8*3/*3 genotype (0.1 mg/ml) and cofactors at 37°C for 15 min with or without efavirenz (0-50 μ M). Expressed CYP2C8 (26 pmol) was used in the inhibition study. Each point represented the average of duplicate incubations.

Figure 5. Dixon plot for the inhibition of tolbutamide 4-hydroxylation by efavirenz in pooled HLMs

Tolbutamide (50 to 250 μM) was incubated with pooled HLMs (1 mg/ml) and cofactors at 37°C for 15 min with or without efavirenz (0-100 μM).

Figure 6. Dixon plot for the inhibition of S-mephenytoin 4-hydroxylation by efavirenz in pooled HLMs

S-mephenytoin (15 to 75 μM) was incubated with pooled HLMs (1 mg/ml) and cofactors at 37°C for 15 min with or without efavirenz (0-100 μM).

Figure 7. Dixon plot for the inhibition of testosterone β -hydroxylation by efavirenz in pooled HLMs

Testosterone (5 to 50 μM) was incubated with pooled HLMs (0.25 mg/ml) and cofactors at 37°C for 15 min with or without efavirenz (0-50 μM).

Figure 8. Time-dependent inhibition of CYP isoforms by efavirenz in pooled HLMs
Efavirenz (50 μ M) was preincubated in duplicate with HLMs and phosphate reaction buffer (pH 7.4) (without or with the NADPH-generating system) in the absence of a substrate probe for 0, 5, 10, 15 and 30 min at 37°C. Controls were preincubated for 0 min without efavirenz and without the NADPH generating system. Protein concentrations and the specific concentrations of each probe used are illustrated in Materials and Methods. Each point represents the average of duplicate incubations.

Table 1

K_i values of efavirenz for the inhibition of CYPs in HLMs and expressed CYPs.

CYP Isoform	Substrate	Systems	K_i value (μ M) (inhibition model)
CYP2B6	Bupropion	pooled HLMs	2.96 ± 0.67 (competitive)
		CMV negative HLMs	0.39 ± 0.10 (competitive)
		expressed CYP2B6	1.38 ± 0.09 (competitive)
CYP2C8	Amodiaquine	pooled HLMs	4.78 ± 2.24 (competitive)
		HLMs with CYP2C8*3/*3	4.80 ± 0.35 (competitive)
		expressed CYP2C8	6.05 ± 2.86 (competitive)
CYP2C9	Tolbutamide	pooled HLMs	19.46 ± 2.78 (non-competitive)
CYP2C19	S-mephenytoin	pooled HLMs	21.31 ± 2.57 (competitive)
CYP3A	Testosterone	pooled HLMs	40.33 ± 0.33 (competitive)

Table 2

Prediction of changes in AUC of CYP2B6, 2C9 and 2C19 substrates *in vivo* by efavirenz.

	f_m	Predicted AUC Ratio	Reported AUC Ratio
Methadone	0.75 ²⁷⁾	2.9 -3.1	N.A.
Amodiaquine	0.93 ⁵⁶⁾	3.5 -4.4	2.15 —4.02 ¹⁹⁾
Phenytoin	0.90 ⁵⁷⁾	1.7 -2.0	N.A.
S-Warfarin	0.91 ²⁶⁾	1.7 -2.0	N.A.
Omeprazole	0.87 ²⁶⁾	1.4 -1.6	N.A.
Proguanil ^a	0.84	29% ~33%	
Clopidogrel ^a	0.56-0.64 ⁵⁸⁾	17% ~29%	N.A.

Plasma concentrations of efavirenz after a single dose were used to predict its effect on AUC change of methadone, omeprazole and the active metabolites of proguanil and clopidogrel. Plasma concentrations upon multiple doses were used for predicting AUC change of amodiaquine, phenytoin and S-warfarin.

^a, The value of percentage change in the AUC of active metabolite (file) was predicted. N.A.: not available