Inactivation of Cytochrome P450 (P450) 3A4 but not P450 3A5 by OSI-930, a Thiophene-Containing Anticancer Drug^[S]

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Received April 23, 2010; accepted November 10, 2010

ABSTRACT:

An investigational anticancer agent that contains a thiophene moiety, 3-[(quinolin-4-ylmethyl)-amino]-*N*-[4-trifluoromethoxy]phenyl]thiophene-2-carboxamide (OSI-930), was tested to investigate its ability to modulate the activities of several cytochrome P450 enzymes. Results showed that OSI-930 inactivated purified, recombinant cytochrome P450 (P450) 3A4 in the reconstituted system in a mechanism-based manner. The inactivation was dependent on cytochrome *b*₅ and required NADPH. Catalase did not protect against the inactivation. No inactivation was observed in studies with human 2B6, 2D6, or 3A5 either in the presence or in the absence of *b*₅. The inactivation of 3A4 by OSI-930 was time- and concentration-dependent. The inactivation of the 7-benzoyloxy-4-(trifluoromethyl)coumarin catalytic activity of 3A4 was characterized by a *K*_i of 24 μ M and a *k*_{inact} of 0.04 min⁻¹. This *K*_i is significantly greater than the clinical OSI-930 *C*_{max} of 1.7 μ M at the maximum tolerated dose, indicating that clinical drug interactions of OSI-930 via this pathway are not likely. Spectral analysis of the inactivated protein indicated that the decrease in the reduced CO spectrum at 450 nm was comparable to the amount of inactivation,

thereby suggesting that the inactivation was primarily due to modification of the heme. High-pressure liquid chromatography (HPLC) analysis with detection at 400 nm showed a loss of heme comparable to the activity loss, but a modified heme was not detected. This result suggests either that the heme must have been modified enough so as not to be observed in a HPLC chromatograph or, possibly, that it was destroyed. The partition ratio for the inactivation of P450 3A4 was approximately 23, suggesting that this P450 3A4-mediated pathway occurs with approximately 4% frequency during the metabolism of OSI-930. Modeling studies on the binding of OSI-930 to the active site of the P450 3A4 indicated that OSI-930 would be oriented properly in the active site for oxidation of the thiophene sulfur to give the sulfoxide, which has previously been shown to be a significant metabolite of OSI-930. Because OSI-930 is an inactivator of P450 3A4 but does not exhibit any effect on P450 3A5 activity under the same conditions, it may be an appropriate probe for exploring unique aspects of these two very similar P450s.

Introduction

Cytochrome P450 monooxygenases catalyze diverse oxidations including hydroxylations of aliphatic and aromatic carbons, epoxidations of olefins, *N*-dealkylations of amines, and *O*-dealkylations of ethers by activation of molecular oxygen (Guengerich, 2001). Constituting the oxidation category of xenobiotic biotransformation, the P450s thus facilitate the elimination of drugs and toxins by acting as mixed-function oxidases and thereby contribute to the clearance of more than 70% of drugs cleared by metabolism.

Many drugs contain thiophene rings (Dalvie et al., 2002) and, of note, thiophene compounds have been reported to be activated to electrophilic intermediates by cytochrome P450-mediated oxidation.

This work was supported in part by the National Institutes of Health National Cancer Institute [Grant CA16954].

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.110.034074.

[S] The online version of this article (available at <http://dmd.aspetjournals.org>) contains supplemental material.

The resulting sulfoxides can then be covalently modified by glutathione and other thiol-containing compounds. Several publications describe evidence for the formation of thiophene sulfoxides as a primary intermediate in the oxidative metabolism of two thiophene derivatives (Mansuy et al., 1991; Dansette et al., 1992; Valadon et al., 1996; Treiber et al., 1997). These sulfoxides react rapidly with various nucleophiles by a Michael-type addition at position 5 of the thiophene ring; reactions with nucleophilic residues of proteins result in covalent binding to proteins (Valadon et al., 1996).

After formation in vivo, these alkylating agents meet one of several fates: 1) they covalently bind to the active site of the enzyme in which they were formed; 2) they degrade by various mechanisms including hydrolysis; or 3) they are released from the enzyme, after which alkylation of another biological nucleophile, such as GSH or another protein, occurs.

3-[(Quinolin-4-ylmethyl)-amino]-*N*-[4-trifluoromethoxy]phenyl]thiophene-2-carboxamide (OSI-930), shown in Fig. 1, is an investigational anticancer agent in clinical development that contains a thiophene moiety (Petti et al., 2005; Garton et al., 2006). OSI-930 is a novel selective

ABBREVIATIONS: OSI-930, 3-[(quinolin-4-ylmethyl)-amino]-*N*-[4-trifluoromethoxy]phenyl]thiophene-2-carboxamide; P450, cytochrome P450; *K*_i, the concentration of inactivator required to give the half-maximal rate of inactivation; *k*_{inact}, the maximal rate constant for inactivation at a saturating concentration of the inactivator; BFC, 7-benzoyloxy-4-trifluoromethyl(coumarin); HPLC, high-pressure liquid chromatography.

FIG. 1. The chemical structure of OSI-930.

inhibitor of Kit and kinase insert domain receptor tyrosine kinases with antitumor activity in mouse xenograft models. The P450-mediated bio-transformation of the thiophene moiety in OSI-930 to a sulfoxide can result in a covalent reaction with thiols such as GSH (Medower et al., 2008).

When the P450 substrate is catalytically activated to a reactive intermediate, this transient molecule may react with available nucleophilic residues from the enzyme, thereby resulting in the inactivation of the P450. Abrogation of P450 pathways of drug metabolism by clinical agents is a general concern because the resulting elevated exposures to other coadministered drugs may cause toxicities. The effects of P450 inactivation on the pharmacokinetics of coadministered drugs or on the inactivator itself depend on complex factors involving the molecular entities, the kinetics of inactivation (K_i and k_{inact}), the partition ratio, the zero-order synthesis rate of new enzyme, multiple pathways of metabolism (competing pathways), dose, or exposure, and specific patient characteristics. Herein, we report the determination of P450 3A4 inactivation efficiency parameters and describe the apparent site of adduction. It is noteworthy that this same reaction does not occur within the P450 3A5 active site, nor does this reaction occur without cytochrome b_5 .

Materials and Methods

Chemicals. NADPH, catalase, GSH, L- α -dilauroyl-phosphocholine, L- α -dioleoyl-*sn*-glycero-3-phosphocholine, and L- α -phosphatidylserine were purchased from Sigma-Aldrich (St. Louis, MO). 7-Benzyloxy-4-trifluoromethyl-(coumarin) (BFC) was obtained from BD Biosciences (San Jose, CA). 7-Ethoxy-4-(trifluoromethyl)coumarin and 7-hydroxy-4-(trifluoromethyl)coumarin were obtained from Invitrogen (Carlsbad, CA) and Indofine Chemicals (Hillsborough, NJ), respectively. OSI-930 was provided by OSI Pharmaceuticals, Inc. (Boulder, Co). All other chemicals and solvents were of the highest purity available from commercial sources.

Purification of Enzymes. The plasmids for P450 3A4, 3A5, 2B6, and 2D6 were expressed as His-tagged proteins in *Escherichia coli* TOPP3 cells (Domanski et al., 2001; Lin et al., 2005). Cytochrome b_5 was purified from liver microsomes of phenobarbital-treated Long-Evans rats. The purification procedures for all the P450s, cytochrome b_5 , and NADPH-cytochrome P450 reductase (reductase) were described previously (Lin et al., 2005).

Enzyme Assay and Inactivation. The primary reaction mixture contained 60 μ g of a mixture of L- α -dilauroyl-phosphocholine, L- α -dioleoyl-*sn*-glycero-3-phosphocholine, and L- α -phosphatidylserine (1:1:1), along with 1 nmol of P450, 2 nmol of reductase, 1 nmol of b_5 , 100 units of catalase, and 2 mM GSH in 1 ml of 100 mM potassium phosphate buffer (pH 7.7). The reconstituted system was incubated at room temperature for 30 min and then kept on ice until used for the experiments. For the studies on the concentration- and time-dependent inactivation of P450s by OSI-930, the reactions were initiated by adding 1 mM NADPH to the primary reaction mixture containing various concentrations of OSI-930 at 37°C. At the time points indicated, 10- μ l aliquots

were transferred into 990 μ l of a secondary reaction mixture containing 50 μ M BFC for 3A4 and 3A5 or 100 μ M 7-ethoxy-4-(trifluoromethyl)coumarin for 2B6 and 2D6 in 200 mM potassium phosphate buffer (pH 7.7) and 200 μ M NADPH. Incubations were performed for 15 min, and the reactions were terminated by the addition of 300 μ l of acetonitrile. The formation of the 7-hydroxy-4-(trifluoromethyl)coumarin product was determined by fluorescence measurement (excitation 409 nm and emission 530 nm) using an RF-5301PC spectrofluorophotometer (Shimadzu, Kyoto, Japan).

Partition Ratio. OSI-930 at concentrations ranging from 2.5 to 300 μ M was added to the primary reaction mixture containing the reconstituted system with 1 μ M P450 3A4. The reaction mixtures were initiated by the addition of 1 mM NADPH and incubated at 37°C for 1 h, allowing the inactivation to go to completion (Silverman, 1996). Aliquots were removed and assayed for residual BFC activity as described above.

Spectral Analysis. After incubation of the primary reaction mixture containing P450 3A4 in the reconstituted system with 100 μ M OSI-930 in the control (–NADPH) or inactivated samples (+NADPH) at 37°C for 30 min, the reduced CO difference spectra of aliquots of the control and inactivated samples containing 0.2 nmol of P450 were determined by scanning from 400 to 500 nm on a UV-2501PC spectrophotometer (Shimadzu), and the P450 content was determined as described by Omura and Sato (1964).

HPLC Separation of the Components of the Reconstituted System and Analysis of Heme Content. An HPLC system with a Waters 600E system controller was used to investigate the loss of native heme and the formation of heme adducts. Control and inactivated samples (100 pmol of P450), prepared as described for the spectral analysis studies, were analyzed using a C4 reverse-phase column (5 μ m, 4.6 $\times$ 250 mm, 300 Å; Phenomenex, Torrance, CA). The solvent system consisted of solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.05% trifluoroacetic acid in acetonitrile). The column was eluted with a linear gradient from 30 to 80% B over 40 min at a flow rate of 1 ml/min. The eluant was monitored using a model 996 diode array detector (Millipore Corporation, Billerica, MA) with detection at 220 nm for proteins and 400 nm for heme.

Docking OSI-930 into the P450 3A4 Crystal Structure and the P450 3A5 Homology Model. The OSI-930 ligand was docked into the active sites of P450s 3A4 and 3A5 to investigate the structural determinants responsible for the mechanism-based inactivation of P450 3A4 using energy-based docking software (AutoDock, version 4.0) (Morris et al., 1996). The coordinates of P450 3A4 were obtained from the Protein Data Bank (1TQN), whereas the coordinates of the lowest energy conformation of the OSI compound were obtained using ChemBioOffice 2000 (CambridgeSoft Corporation, Cambridge, MA). The flexible OSI-930 ligand was docked into the rigid P450 3A4 with the Lamarckian Genetic Algorithm approach of AutoDock 4.0 with the following parameters: mutation rate = 0.02; crossover = 0.80; local search frequency = 0.06; and maximal number of generations = 2.7×10^4 .

Because the crystal structure of P450 3A5 is not available, a homology model of P450 3A5 was constructed on the basis of the crystal structure of P450 3A4 (1TQN) using Modeller 9v8 software (Sali and Blundell, 1993). Twenty-seven amino acid residues were truncated from the N terminus of P450 3A5 to match the number of the amino acid residues of P450 3A4 present in the crystal structure of 1TQN. The best homology model was selected on the basis of the DOPE scores generated by Modeller 9v8 and the LGscores obtained at the ProQ server (<http://www.sbc.su.se>). This model was further optimized to remove bad contacts and clashes by energy minimization in explicit water for 50,000 steps with the steepest descent algorithm using Gromacs 4.0 (Lindahl et al., 2001). The OSI-930 ligand was docked into the active site of the P450 3A5 homology model using docking parameters identical to those described for P450 3A4.

Results

Inactivation of P450 3A4 by OSI-930. The inactivation of P450 3A4 in the reconstituted system by OSI-930 showed an absolute requirement for NADPH (data not shown) and was markedly enhanced by cytochrome b_5 (Fig. 2). In the absence of cytochrome b_5 , there was a relatively small loss of activity comparable to that normally seen in negative controls. Moreover, the addition of catalase did not protect against inactivation either in the absence or presence of b_5 .

FIG. 2. The requirement for cytochrome b_5 for the inactivation of P450 3A4 by OSI-930. The P450 3A4 reconstituted system was incubated with 100 μM OSI-930 in the presence (●) or in the absence (■) of cytochrome b_5 . Aliquots were removed at the times indicated and assayed for residual BFC debenzoylation activity as described under *Materials and Methods*. The rate constants for the inactivation in the presence and absence of b_5 were 0.015 and 0.003 min^{-1} , respectively.

No inactivation was observed in studies with human P450 2B6, 2D6, or 3A5, either in the presence or in the absence of cytochrome b_5 (data not shown). Because P450 3A4 was the only P450 tested that showed inactivation by OSI-930, all subsequent studies were performed only with human 3A4 in the presence of cytochrome b_5 .

As shown in Fig. 3A, the inactivation of P450 3A4 by OSI-930 was time- and concentration-dependent. Linear regression analysis of the time course data was used to estimate the initial rate constants (k_{obs}) for the inactivation of 3A4 by OSI-930 at various concentrations of OSI-930. From the double reciprocal plot shown in Fig. 3B of the values for k_{obs} versus the concentration of OSI-930, the K_1 and k_{inact} for the inactivation of the 3A4-mediated catalysis of BFC biotransformation by OSI-930 were determined to be 24 μM and 0.04 min^{-1} , respectively.

Reduced CO Difference Spectra. To determine whether the protein or the heme was modified by reaction with the OSI-930 metabolite, studies were performed in which P450 3A4 was incubated with

100 μM OSI-930 at 37°C for 30 min, resulting in approximately 75% inactivation, and then the reduced CO spectrum was determined. As can be seen in Fig. 4, there was a significant decrease in the spectrally detectable reduced CO spectrum at 450 nm of approximately 70% compared with a control sample incubated in the absence of NADPH that had exhibited essentially no inactivation. This decrease is comparable to the amount of inactivation. Figure 4 also shows the spectra that were originally recorded with the absorbance at 490 nm set at 0; however, the spectrum of the inactivated P450 has been offset for clarity. The critical measurement is the difference in the absorbance between 450 and 490 nm.

HPLC Analysis of Heme Content. Because the spectral results suggested that the inactivation was primarily, if not solely, due to heme modification, HPLC analysis was performed on the inactivated protein with monitoring at 400 nm to look for modified heme. After incubation of the 3A4 reconstituted system with 100 μM OSI-930 at 37°C for 30 min, heme, reductase, b_5 , and 3A4 were separated by HPLC under acidic conditions. The elution profiles monitored at 400 nm, as displayed in Fig. 5, show that the native (unmodified) heme for both the control and the inactivated samples eluted at approximately 18 min. In the inactivated sample there was no evidence for a modified heme eluting with a different retention time. The area of the heme peak for the inactivated sample was approximately 40% of that of the control sample, which is comparable to the amount of active protein remaining in this experiment. These results further support the fact that the inactivation was due primarily to heme modification and that the heme must have been modified enough (possibly destroyed) so that it could no longer be observed by HPLC with detection at 400 nm. Studies aimed to demonstrate possible labeling of P450 3A4 by OSI-930 using liquid chromatography-mass spectrometry were not attempted because the inactivation appeared to be almost solely due to heme modification, and, in addition, the P450 3A4 protein did not appear to ionize well in the mass spectrometer. Studies on the reversibility of the inactivation were not performed because inactivation due to heme destruction is not reversible.

Partition Ratio for the Inactivation of 3A4 by OSI-930. To determine the partition ratio for the inactivation of P450 3A4 by

FIG. 3. Time and concentration dependence for the mechanism-based inactivation of P450 3A4 by OSI-930. The reconstituted system containing 3A4 was incubated with 0 (○), 10 (●), 20 (▽), 40 (△), 60 (□), and 100 (■) μM OSI-930. Aliquots were removed at the times indicated and assayed for residual BFC debenzoylation activity as described under *Materials and Methods*. A, linear regression analysis of time- and concentration-dependent inactivation to estimate the k_{obs} . B, double reciprocal plot of k_{obs} as a function of the OSI-930 concentration to determine K_1 and k_{inact} . The data shown represent the average of three separate experiments done in duplicate that did not differ by more than 10% either within or between experiments.

FIG. 4. Effect of OSI-930 inactivation on the reduced CO difference spectrum of P450 3A4. The reduced CO difference spectra of P450 3A4 in the reconstituted system incubated with 100 μ M OSI-930 in the absence (solid line) or presence (dashed line) of NADPH for 30 min were measured as described under *Materials and Methods*.

OSI-930, the P450 3A4 was incubated in the reconstituted system with various concentrations of OSI-930 for 1 h so that the inactivation could reach completion. The percentage of activity remaining was plotted as a function of the molar ratio of OSI-930 to P450, as shown in Fig. 6. The partition ratio was estimated from the intercept of the linear regression line obtained from lower ratios of OSI-930 to P450, with the straight line derived from higher ratios of OSI-930 to P450 as

described previously (Silverman, 1996). With use of this method, a partition ratio of approximately 23 was determined, suggesting that OSI-930 partitions toward the inactivation of P450 3A4 in approximately 4% of the turnover events.

Binding of the OSI-930 in the Active Sites of P450 3A4 and 3A5.

In attempts to better understand the binding of the OSI-930 in the active site of the 3A4 and identify potential sites for metabolism, modeling of the OSI-930 molecule to the 3A4 substrate binding site was performed. The results of modeling studies for P450 3A4 are displayed in Fig. 7A, which shows the conformation of the OSI-930 that mostly likely leads to the formation of sulfoxide. It can be seen that the distance from the heme iron to the thiophene sulfur of the OSI-930 is only 3.0 Å, and the OSI-930 is within hydrogen bonding distances with several amino acid residues. In particular, two of the three fluorine atoms of the trifluoromethyl group of the OSI-930 are within 3.0 Å of the side chains of Ser312 and Gln484, and the trifluoromethoxy oxygen is 2.4 Å from the amide hydrogen of Leu483. Moreover, the Arg212 may form hydrogen bonds with the two nitrogen atoms of the OSI-930. These hydrogen bonds may provide additional energy for the binding of OSI-930 in the active site of P450 3A4.

In contrast, the OSI-930 is bound to the active site of P450 3A5 in a different conformation as shown in Fig. 7B. Most notably, the hydrogen bonds between OSI-930 and the protein are absent in P450 3A5, which contributes to the reduction of the binding energy (ΔG) by ~ 2 kcal/mol based on the docking results (data not shown), and the thiophene sulfur is swung further away from the heme iron, which gives an Fe-S distance of 3.3 Å and positions the thiophene sulfur atom not directly above the heme iron, but at a $\sim 33^\circ$ angle (see Supplemental Materials, Fig. 1S). These changes are not in favor of the oxidation of the thiophene sulfur by P450 3A5. Comparison of the OSI-930 binding between P450 3A4 and P450 3A5 led us to conclude that Arg212 may play an important role in determining the binding

FIG. 5. HPLC elution profiles of the P450 3A4 reconstituted system after incubation with 100 μ M OSI-930 in the absence (top panel) or presence (bottom panel) of NADPH for 30 min. The eluates were monitored at 400 nm for heme. The experimental procedures are described under *Materials and Methods*.

FIG. 6. Determination of the partition ratio for the inactivation of P450 3A4. Samples were incubated with various concentrations of OSI-930 for 1 h in the presence of NADPH until the inactivation reaction was essentially complete. The percentage of catalytic activity remaining was then determined as a function of the molar ratio of OSI-930 to P450. The partition ratio was estimated from the intercept of the linear regression line from the lower ratios of OSI-930 to 3A4 and the straight line obtained from higher ratios of OSI-930 to 3A4.

orientation of OSI-930 in P450 3A4. Not only is Arg212 hydrogen bonded to OSI-930, but also the extended side chain of Arg212 allows the trifluoromethoxy group to interact with Ser312, Leu483, and Gln484. In P450 3A5, a lysine residue is substituted for Arg212. This substitution abolishes the potential hydrogen bonding with the OSI-930 and prevents the trifluoromethoxy group from interacting with Ser312, Leu483, and Gln484.

Discussion

Our results from molecular modeling demonstrate that OSI-930 may bind to the active site of P450 3A4 in such a conformation that would lead to oxidation of the thiophene sulfur to give the sulfoxide, which has previously been demonstrated to be a significant pathway for metabolism (Medower et al., 2008). We hypothesize that Arg212 may play an important role in the thiophene sulfur oxidation not only because it forms hydrogen bonds with the OSI-930 ligand but also because the extended side chain of Arg212 allows the trifluoromethoxy group to interact with Ser312, Leu483, and Gln484 to form additional hydrogen bonds to enhance this catalytically relevant conformation.

Unlike benzothiophene *S*-oxides, thiophene *S*-oxides are a very reactive species. Although few of them have been described thus far (Dansette et al., 1990, 1991; Mansuy, 1997; Bonierbale et al., 1999), recent results show that oxidation of thiophene leads to adducts from a Michael-type addition of a thiol-containing nucleophilic reagent to a thiophene *S*-oxide intermediate (Mansuy et al., 1991).

Drug-mediated inactivation of P450, particularly P450 3A4, can result in pharmacokinetic interactions with coadministered drugs, thus reducing clearance and elevating exposure to the codosed drug that is metabolized by this same form of P450. The magnitude of this pharmacokinetic interaction depends on many factors, including the efficiency of the inactivation process. With the “efficiency” of enzyme-catalyzed reactions characterized by the k_{cat}/K_m ratio, approximately 10 of the recently reviewed inactivation reactions exhibit distinctly high k_{inact}/K_I ratios of $>0.15 \mu\text{M}^{-1} \cdot \text{min}^{-1}$ (Johnson, 2008). Among these 10 very efficient inactivators, however, 7 exhibit no known interaction. Therefore, despite this high inactivation efficiency, 70% of these inactivators do not manifest any known clinically relevant drug interaction. This incongruity highlights the fact that the estimation or prediction of drug interactions requires a com-

FIG. 7. Molecular modeling showing the binding of OSI-930 in the active sites of P450 3A4 (A) and P450 3A5 (B). OSI-930 was docked to the crystal structure of P450 3A4 (Protein Data Bank 1TQN) and to the homology model of P450 3A5 using Autodock 4.0. The homology model of P450 3A5 was constructed on the template of P450 3A4 (1TQN) using Modeller 9v8 as described under *Materials and Methods*. A, the lowest energy conformation of OSI-930 poised for oxidation of the thiophene sulfur. OSI-930 is shown in stick, and carbon, nitrogen, and hydrogen atoms are colored in cyan, blue, and white, respectively. The heme is shown in red stick and the I-helix is shown in yellow ribbon. The distance is 3.0 Å from the heme iron to the sulfur atom of the OSI-930. B, the docked conformation of OSI-930 in P450 3A5 that shows the shortest Fe-S distance. The coloring scheme is the same as in A.

prehensive and quantitative assessment of metabolism. The effects of P450 inactivation on the pharmacokinetics of coadministered drugs or on the inactivator itself depend on complex factors involving the molecular entities, the kinetics of inactivation (K_I and k_{inact}), the partition ratio, the zero-order synthesis rate of new enzyme, multiple pathways of metabolism (competing pathways), the dose or exposure, and specific patient characteristics. Indeed, OSI-930 has multiple pathways of biotransformation that include P450 2D6, P450 2B6, and P450 2A6. Although the OSI-930 inactivation kinetics is sufficient for effective inactivation *in vitro*, the efficiency is still much less than that of many marketed drugs (Johnson, 2008) with a k_{inact}/K_I of only

TABLE 1

Effect of OSI-930 on the BFC catalytic activity remaining in CYP3A4 and CYP3A5

Percentage of activity remaining relative to the control without NADPH (as 100%) in the primary reaction as described under *Materials and Methods*.

Time	P450 3A4	P450 3A5
	%	%
0 min	100	100
10 min	71	113
20 min	50	109
30 min	39	98

$0.0017 \mu\text{M}^{-1} \cdot \text{min}^{-1}$. Furthermore, the partition ratio for the inactivation event versus the released metabolite products is approximately 23, showing that, on average, approximately 23 catalytic turnover events leading to product formation occur before one inactivation event. Most importantly, the absence of a potential clinical drug interaction is probably due to the comparatively low systemic concentrations of OSI-930 relative to the K_I (Michaelis constant of inactivation) -1.7 versus $24 \mu\text{M}$.

These studies demonstrate that OSI-930 is able to act as a time-dependent inhibitor of human P450 3A4 with a K_I of $24 \mu\text{M}$, a k_{inact} of 0.04 min^{-1} , and a partition ratio of approximately 23. The k_{inact}/K_I , a measure of the efficiency of a compound to act as a time-dependent inhibitor, is $0.0017 \mu\text{M}^{-1} \cdot \text{min}^{-1}$. Thus, it is significantly less efficient as a time-dependent inhibitor of P450 3A4 than several widely used drugs and food components, including tamoxifen ($0.02 \mu\text{M}^{-1} \cdot \text{min}^{-1}$), 17α -ethynylestradiol ($0.04 \mu\text{M}^{-1} \cdot \text{min}^{-1}$), ritonavir ($1.35 \mu\text{M}^{-1} \cdot \text{min}^{-1}$), mifepristone ($0.02 \mu\text{M}^{-1} \cdot \text{min}^{-1}$), and bergamottin ($0.04 \mu\text{M}^{-1} \cdot \text{min}^{-1}$), the major component in grapefruit juice responsible for time-based inhibition (Johnson, 2008). The inactivation is primarily due to heme modification rather than to protein modification and the inactivation requires cytochrome b_5 .

It is particularly interesting to note that this P450 3A4 inactivator exhibits no effect on P450 3A5 activity under the same conditions, even in the presence of cytochrome b_5 (Table 1). This unique result suggests that OSI-930 can provide the valuable and novel opportunity both to distinguish the relative clearance roles of these two structurally similar enzymes and to explore unique aspects of their very tolerant and overlapping substrate-binding active sites.

Acknowledgments

We thank Professor Adriane Stewart for editorial review of the article.

Authorship Contributions

Participated in research design: Lin, Hollenberg, and Johnson.

Conducted experiments: Lin, Zhang, and Medower.

Contributed new reagents or analytic tools: Johnson.

Wrote or contributed to the writing of the manuscript: Lin, Zhang, Hollenberg, Medower, and Johnson.

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