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Metabolite–P450 Complex Formation by Methylenedioxyphenyl HIV Protease Inhibitors in Rat and Human Liver Microsomes

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ABSTRACT. P450 complex formation and the unusual pharmacokinetics of methylenedioxyphenyl HIV protease inhibitors were examined by *in vitro* studies using human and rat liver microsomes and by *in vivo* oral dosing studies. *In vitro* spectral studies indicated that the formation of a P450 complex having absorbance maxima at 425 and 456 nm was time and concentration dependent; 27–60% of the total P450 was complexed in dexamethasone-induced rat liver microsomes after a 30-min incubation with 100 μM HIV protease inhibitors. Methoxy substitution on the phenyl ring of the methylenedioxyphenyl moiety increased formation of the P450 complex, whereas chlorine substitution markedly decreased the P450 complexation. Kinetic studies on the P450 complex formation indicated that both methoxy and chlorine substitution affected the maximum complex formation rate (V_{max}), while it had little effect on K_m values (~10 μM). This complexation in human liver microsomes was inhibited markedly by an anti-CYP3A1 antibody. Furthermore, the P450 complex formation resulted in a time-dependent loss of CYP3A-catalyzed marker activities (testosterone 2β/6β-hydroxylase) in both rat and human liver microsomes. Collectively, these results point to the involvement of CYP3A isoforms in P450 complexation by methylenedioxyphenyl HIV protease inhibitors. Additionally, after oral administration to rats, one of these HIV protease inhibitors (Compound **I**), which complexed P450 to the greatest extent, showed no elimination over a period of 500 min after administration of the highest dose. It is suggested that formation of a quasi-irreversible metabolite–CYP3A complex with methylenedioxyphenyl HIV protease inhibitors was responsible for the CYP3A-selective time-dependent loss of catalytic function and the unusual dose-dependent pharmacokinetics after oral administration. BIOCHEM PHARMACOL **56**;2:223–230, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. P450; CYP3A; CYP2D; MI complex

Time- and dose-dependent pharmacokinetics, such as an accumulation of drugs during chronic dosing and an increase of the apparent half-life with dose, have been well documented [1–3], and it is recognized that enzyme inactivation is one of the main reasons for such unusual pharmacokinetics. The most important enzyme system catalyzing Phase I metabolism is P450§ system. The mechanism of P450 inactivation generally is classified into two groups: one involves covalent binding of a reactive intermediate to the enzyme protein(s) and/or heme, which leads to irreversible inhibition of catalytic function; the second involves a quasi-irreversible coordination of a reactive intermediate(s) to the P450 [4]. The major classes of chemical entities that form P450 complexes include methylenedioxybenzene derivatives, alkyl amines, macrolide antibiotics, and hydrazines [4]. Methylenedioxyphenyl compounds such as piperonyl butoxide and isosafrole [5, 6] undergo P450-catalyzed oxidation to form intermediates that coordinate tightly to the prosthetic heme iron [7]. This coordination forms a characteristic metabolite-ferrous form of the P450 complex having absorption maxima in the difference spectra at 425 and 455 nm [5, 6, 8]. The metabolite complex is readily dissociated by many lipophilic compounds [9, 10].

During the course of the discovery and development of potent HIV protease inhibitors at Merck, several candidates containing a methylenedioxyphenyl function were synthesized (Fig. 1). The purpose of this study was to examine the potential of these compounds to complex P450 in rat and human liver microsomes. The effect of substitution (on the methylenedioxyphenyl moiety) on P450 complex formation also was examined.

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§ *Abbreviations:* C_{max}, maximum plasma concentration; DX, desamethasone; P450, liver microsomal cytochrome P450; and PB, phenobarbital.

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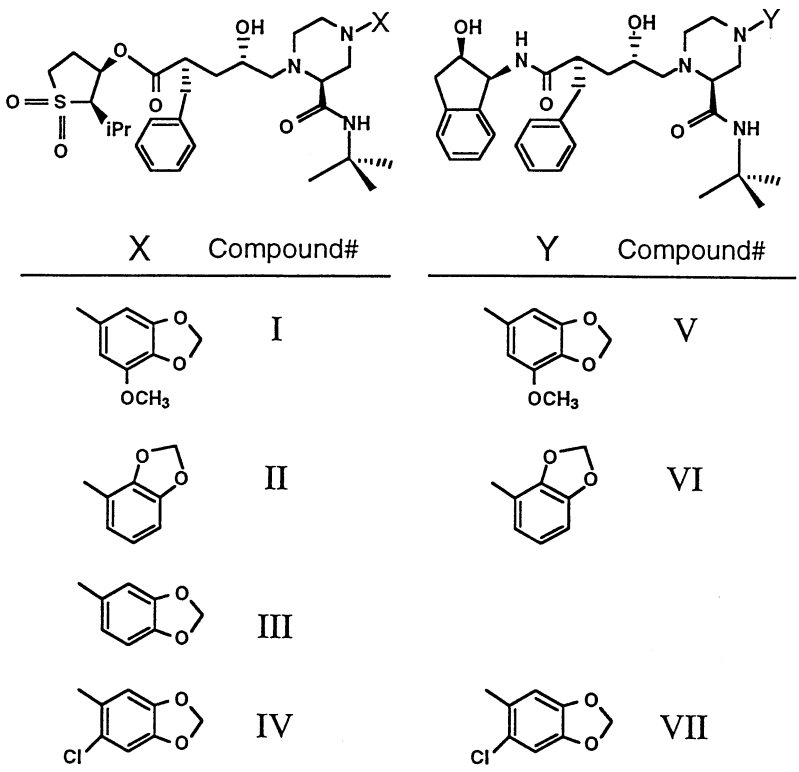

FIG. 1. Methylenedioxyphenyl HIV protease inhibitors.

MATERIALS AND METHODS

Chemicals

HIV protease inhibitors **I–VII** (Fig. 1) were synthesized at Merck Research Laboratories and were assigned code numbers of L-762,658, L-763,473, L-764,307, L-763,474, L-758,978, L-759,895, and L-758,998, respectively. DX, PB, ferricyanide, and testosterone were purchased from the Sigma Chemical Co. Metabolites of hydroxylated testosterone at the 2α-, 2β-, 6β-, 7α-, 16α-, and 16β-positions were obtained from Steraloids. Tolbutamide and its methyl hydroxylated metabolite were obtained from Research Biochemical International. (±)-Bufuralol and its 1′-hydroxylated metabolite were obtained from the Gentest Corp. All other reagents were of analytical grade.

Animals

Male adult Sprague–Dawley rats weighing 280–400 g were purchased from Taconic Farms. The animals were housed according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and maintained under a 12-hr light/dark cycle with free access to water. All animal protocols were approved by the animal care committee (IACUC). Animals were fed a standard diet from Purina (Rodent Laboratory Chow, Purina Mills, Inc.).

In Vivo *Studies*

For oral dosing studies, all rats were equipped with an indwelling cannula (silicone rubber/polyethylene) implanted in the right jugular vein for blood sampling. After an overnight fast, three groups of rats (N = 4–6) received an oral dose of HIV protease inhibitor **I** (Fig. 1) at 10, 20, and 40 mg/kg as a solution in 0.05 M of citric acid. Blood samples were drawn periodically from the jugular vein at the designated times and centrifuged to obtain plasma. The plasma samples were kept frozen (−20°) until assayed by HPLC.

HPLC Method for the Quantitation of HIV Protease Inhibitor I in Plasma

One hundred twenty-five nanograms of the internal standard (L-763,473) and 5 mL of diethyl ether were added to 0.2 or 0.5 mL of plasma. After shaking the sample for 15 min and centrifuging, the organic layer was transferred to a clean tube where it was evaporated to dryness under N_2. The residue was dissolved in 250 μL of the mobile phase, and 200 μL was injected onto a Zorbax RX-C_8 (4.6 mm × 25 cm) analytical column. The mobile phase was acetonitrile:phosphoric acid (15 mM) (24:76, v/v, adjusted to pH 3.2 with triethylamine), and the flow rate was 1.5 mL/min. The column effluent was monitored by UV absorption at 220 nm.

Human Liver Microsomes and Anti-Rat CYP3A1 Rabbit Polyclonal Antibody

Microsomal fractions from human adult subjects (original liver sample code, age, sex, cause of death) (HHM-059,

50-year-old, female, subarachnoid hemorrhage; HHM-065, 46-year-old, female, subarachnoid hemorrhage; HHM-095, 63-year-old, female, subarachnoid hemorrhage) were obtained from Keystone Skin Bank and equally pooled by volume for experiments. Rabbit polyclonal antibody prepared against rat CYP3A1 was obtained from Human Biologics Inc. The immunoinhibitory potency of CYP3A4-catalyzed activity in human liver microsomes was checked by measuring testosterone 2β- and 6β-hydroxylation activities in the presence of different amounts of antibody. Preliminary studies revealed that anti-rat CYP3A1 antibody showed a strong immunoinhibitory effect on testosterone 2β- and 6β-hydroxylation in human liver microsomes.

Microsomal Preparations

Rats were pretreated with an oral dose of PB (80 mg/kg/day for 4 days) or DX (50 mg/kg/day for 4 days). All rats were killed 24 hr after the last treatment. The livers were excised quickly and perfused with ice-cold 1.15% KCl (w/v). Liver microsomes were prepared by a differential ultracentrifugation method, and the final pellets were resuspended in 0.15 M of Tris–HCl buffer (pH 7.4). Microsomal protein was measured by the method of Lowry *et al.* [11] with bovine serum albumin as the standard.

Formation of Metabolite–P450 Complexes

The formation of a metabolite–P450 complex was measured with liver microsomes obtained from humans as well as PB-treated or DX-treated rats. The reaction mixture contained 0.15 M of Tris–HCl (pH 7.4), 1 mM of EDTA, 10 mM of $MgCl_2$, and 1 mg of microsomal protein in a final volume of 0.5 mL. Various concentrations of HIV protease inhibitors (dissolved in ethanol) were added to the test cuvette, whereas the same volume of ethanol was added to the reference cuvette. After the baseline was corrected, NADPH (1 mM) was added to both cuvettes at 37°, and the difference spectra were recorded from 400 to 500 nm at the designated time point using a DU-640 spectrophotometer (Beckman Instruments). In the antibody study, the human liver microsomes were preincubated with different amounts of antibody at room temperature for 30 min prior to the measurement of metabolite–P450 complexation.

Kinetics of Complex Formation

Samples contained 0.15 M of Tris–HCl (pH 7.4), 1 mM of EDTA, 10 mM of $MgCl_2$, and 1 mg/mL of DX-induced rat liver microsomes. The HIV protease inhibitor (dissolved in ethanol) was added at various concentrations to the test cuvette, while the same volume of ethanol was added to the reference cuvette. After a 5-min preincubation at 37°, the reaction was started by the addition of NADPH (1 mM), and the difference spectra (between 400 and 500 nm) were recorded every 30 sec using a DU-640 spectrophotometer (Beckman Instruments). The initial rate of complex formation typically was obtained from the first four points (2 min). Apparent kinetic constants (K_m and V_{max}) for complex formation were determined using a Michaelis–Menten equation of initial complex formation rate versus initial HIV protease inhibitor concentration with the aid of the nonlinear least-squares fitting program SCIENTIST (MicroMath).

P450 Marker Assays

Testosterone hydroxylations at 2α (or 16α), 7α, and 2β (or 6β) positions were used as functional probes for CYP2C11, 2A1, and 3A2, respectively, in rats [12]. Testosterone 2β (or 6β)-hydroxylation, bufuralol 1′-hydroxylation, and tolbutamide methyl hydroxylation are marker activities for human CYP3A4, 2D6, and 2C9, respectively [12]. Testosterone hydroxylations, bufuralol 1′-hydroxylation, and tolbutamide methyl hydroxylation were measured following the preincubation of a 25-μM concentration of HIV protease inhibitors with rat and human liver microsomes (1 mg of microsomal protein) in the presence of NADPH (1 mM) for the designated time period according to methods described elsewhere [13–15].

RESULTS

Methylenedioxyphenyl derivatives undergo P450-dependent metabolism and form a stable metabolite–intermediate (MI) complex. The complex formation is observed as an absorbance maximum at 455 nm in the ferrous state of P450. Therefore, we examined the interaction of methylenedioxyphenyl HIV protease inhibitors with ferrous P450 by measuring the spectrum at 455 nm. The *in vitro* spectral studies with DX-induced rat liver microsomes showed that the methylenedioxyphenyl HIV protease inhibitors all formed a metabolite–P450 complex having absorption maxima at 425 and 456 nm after a 30-min incubation in the presence of NADPH (Table 1 and curve **a** in Fig. 2). Approximately 75% of the initial P450 gave the reduced CO-P450 spectrum in the presence of dithionite (curve **b** in Fig. 2).

The extent of MI complex formation is known to be concentration and time dependent. Therefore, we examined the time-course of metabolite–P450 complex formation in DX-induced rat liver microsomes (Fig. 3). The complex formed immediately upon incubation, and the absorption reached a plateau within 10 min. Although all of the compounds formed metabolite–P450 complex spectra, the fraction of total P450 complexed differed among compounds (Table 1). A marked difference in the fraction of total P450 complexed after incubation with 100 μM of HIV protease inhibitor for 30 min was observed between methoxy-substituted (compounds **I** and **V**) and chlorine-substituted (compounds **IV** and **VII**) compounds in both series (Table 1 and Fig. 3). Kinetic studies revealed that the initial metabolite–P450 complex formation rate was satu-

TABLE 1. Characteristics of methylenedioxyphenyl HIV protease inhibitor metabolite complexes with P450 in DX-treated rat liver microsomes*

Compound	Peaks (absorbance unit/mg)		Complexed† (nmol/mg)	P450 complexed (%)
	$\Delta A_{425-490}$	$\Delta A_{456-490}$		
I	0.0726 (0.0051)	0.0668 (0.0094)	0.446 (0.063)	58.2 (8.2)
II	0.0546 (0.0120)	0.0547 (0.0109)	0.365 (0.072)	47.6 (9.5)
III	0.0619 (0.0168)	0.0530 (0.0112)	0.353 (0.075)	46.1 (9.8)
IV	0.0247 (0.0093)	0.0467 (0.0099)	0.311 (0.066)	40.6 (8.6)
V	0.0827 (0.0218)	0.0579 (0.0066)	0.386 (0.044)	46.2 (2.4)
VI	0.0564 (0.0028)	0.0545 (0.0067)	0.363 (0.045)	43.6 (3.3)
VII	0.0112 (0.0012)	0.0303 (0.0029)	0.226 (0.032)	27.2 (3.9)

*Data represent the means of three different preparations (± SD).
†Calculation based on an extinction coefficient of 75 mM^{-1} cm^{-1} for $\Delta A_{456-490}$.

rable with apparent K_m values of ~10 μM (Fig. 4 and Table 2) and showed no significant difference between test compounds. The maximum complex formation rate (V_{max}) was calculated with the same extinction coefficient of 75 mM^{-1} cm^{-1} for all HIV protease inhibitor–P450 complexes. The V_{max} values thus obtained, in contrast to K_m values, varied by more than two-fold between **I** and **IV**. The rank order of V_{max} (nmol P450 complexed/min/mg protein) was: **I** (0.162) > **II** (0.116) > **III** (0.0967) > **IV** (0.0746).

Selectivity in the interaction of methylenedioxyphenyl HIV protease inhibitors with P450 was examined by measuring P450 isoform-selective marker activities in microsomes preincubated with the inhibitors. Preincubation of rat liver microsomes with 25 μM HIV protease inhibitors in the presence of an NADPH-generating system impaired testosterone 2β- and 6β-hydroxylase activities in a time-dependent manner (data not shown). This inactivation appeared to be specific to CYP3A-catalyzed metabolism (Fig. 5): at 25 μM, the compounds inhibited control 2β- and 6β-hydroxylase activities by ~75% during a 30-min preincubation, while they had little effect on the other hydroxylations in DX-induced rats. Significant and selective inhibition of CYP3A also was observed in untreated rat liver microsomes (Fig. 6), although the extent of inhibition was much less than in DX-induced microsomes.

Spectral studies demonstrated that Compound **I** formed a metabolite–P450 complex in human liver microsomes similar to that observed in the DX-induced rats (Fig. 7). Furthermore, the preincubation of microsomes with an anti-rat CYP3A1 antibody markedly inhibited the complex formation in a concentration-dependent manner (Fig. 7).

As observed in rat liver microsomes, the preincubation of HIV protease inhibitors with human liver microsomes showed a time-dependent loss of CYP3A4-catalyzed testosterone 6β-hydroxylase activity (Fig. 8); approximately 90% of the activity was inactivated within 10 min by a 25-μM concentration of Compound **I**. Again, the inhibition was specific to CYP3A4, with a minimal effect on the metabolism of CYP2C9- and CYP2D6-marker substrates (Fig. 9).

To examine the effect of *in vitro* P450 complexation on *in vivo* pharmacokinetics, a pharmacokinetic study on

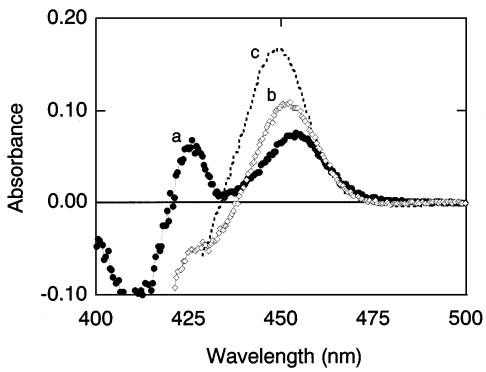

FIG. 2. Metabolite–P450 complex spectrum in DX-treated rat liver microsomes. Spectra (a) was recorded after a 30-min incubation with 100 μM of HIV protease inhibitor I and 1 mM of NADPH in DX-treated rat liver microsomes. Then the reduced CO-difference P450 spectrum (b) was recorded after the sample was bubbled for 1 min with carbon monoxide in the presence of sodium dithionite. Spectrum (c) represents the reduced CO-difference P450 spectrum obtained before incubation with HIV protease inhibitor I.

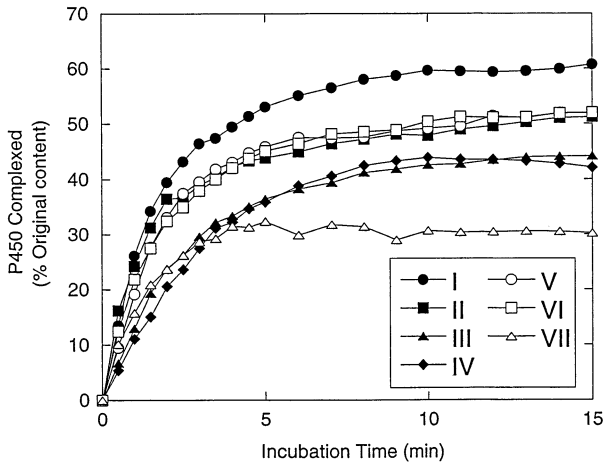

FIG. 3. Time-course of metabolite–P450 complex formation in DX-treated rat liver microsomes. Metabolite–P450 complex formation was calculated from Δ absorbance at 456 and 490 nm in a sample incubated with 1 mM of NADPH and 100 μM of HIV protease inhibitor for the designated time period. Data represent means of three separate experiments.

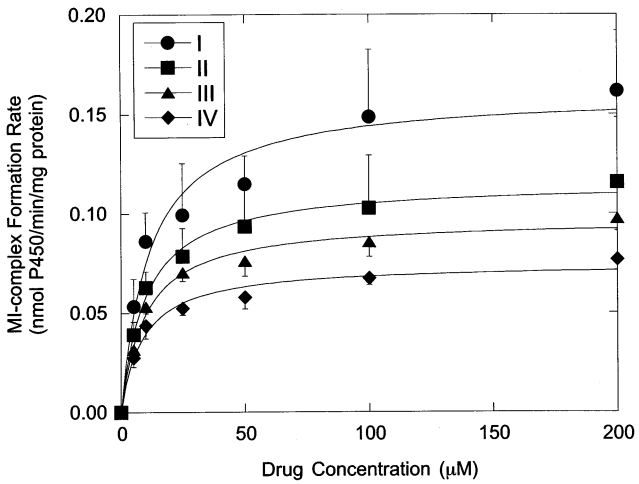

FIG. 4. Kinetics of metabolite–P450 complex formation in DX-treated rat liver microsomes. Kinetics were examined based on the initial rate of metabolite–P450 complex formation (obtained within 120 sec). Curves are the simulated results of the K_m and V_{max} values listed in Table 2. Data represent means (with SD) of three separate experiments.

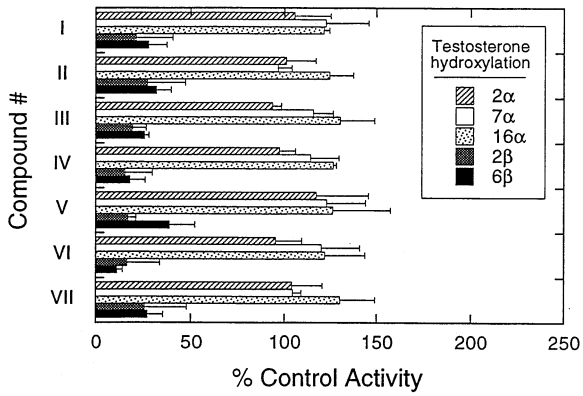

FIG. 5. Specificity of the inactivation of P450 isoforms in DX-treated rat liver microsomes. Values are presented as percent control activity obtained from samples preincubated with NADPH (1 mM) alone and NADPH plus 25 μM of HIV protease inhibitors for 30 min. The values are means (with SD) of three separate experiments. Control values in DX-treated rats were: 0.288 ± 0.066 nmol/min/mg of protein for 2α-hydroxylase activity; 0.165 ± 0.110 nmol/min/mg of protein for 7α-hydroxylase activity; 0.490 ± 0.121 nmol/min/mg of protein for 16α-hydroxylase activity; 0.738 ± 0.125 nmol/min/mg of protein for 2β-hydroxylase activity; and 5.01 ± 0.45 nmol/min/mg of protein for 6β-hydroxylase activity. Significant differences were observed only in 2β/6β-hydroxylase activities of testosterone between samples preincubated with NADPH alone and with NADPH plus HIV protease inhibitors ($P < 0.01$).

Compound **I** was carried out in rats. After Compound **I** was given orally at different doses (10, 20 and 40 mg/kg), the plasma concentration–time profile of **I** was found to be dose-dependent (Fig. 10). Compound **I** was eliminated rapidly with a half-life of ~90 min when given to rats orally at 10 mg/kg. The plasma concentration of Compound **I** declined log-linearly with time at 10 mg/kg p.o., although the apparent half-life increased dramatically with an increase of dose. At the highest oral dose of 40 mg/kg, the plasma concentration remained at the C_{max} for 500 min post-administration.

DISCUSSION

In vitro spectral studies revealed that the methylenedioxyphenyl HIV protease inhibitors formed metabolite–P450 complexes with characteristic spectral absorption maxima at 425 and 456 nm when incubated with human and DX-induced rat liver microsomes in the presence of NADPH. Kinetic studies on the metabolite–P450 complex formation revealed that substitution on the methylenedioxyphenyl moiety played an important role in the maximum complex formation rate (V_{max}), whereas it had little effect on K_m values. Substitution of a methoxy group on the phenyl ring of the methylenedioxyphenyl moiety (Compound **I**) increased both V_{max} and the fraction of total P450 complexed, whereas chlorine substitution (Compound **IV**) decreased both values. Incubations of methylenedioxyphenyl HIV protease inhibitors with rat and human liver microsomes in the presence of NADPH demonstrated that the metabolism of CYP3A marker substrates was impaired selectively in a time-dependent manner. The time-dependent loss of CYP3A-catalyzed metabolic activity most likely was due to the P450 complexation. Furthermore, Compound **I** showed dose-dependent kinetics in rats after oral administration.

TABLE 2. Comparison of the kinetics of metabolite P450 complexation of HIV protease inhibitors in DX-treated rat liver microsomes*

Compound	K_m (μM)	V_{max} (nmol/min/mg protein)
I	12.5 (4.9)	0.162 (0.032)
II	10.1 (2.7)	0.116 (0.020)
III	9.65 (1.42)	0.0967 (0.0069)
IV	9.15 (3.06)	0.0746 (0.0059)

*Data represent the means of three different preparations (± SD).

Methylenedioxyphenyl and alkylamine functions are well-known chemical structures that can be oxidized to form a complex between reactive metabolite(s) and ferrous P450(s) [4, 7, 16]. It has been proposed that a carbene is the reactive intermediate generated from the methylenedioxyphenyl moiety, which coordinates with the prosthetic heme of P450 [17]. Furthermore, the side-chain substituent on the methylenedioxyphenyl moiety is known to be an important determinant of the extent to which a metabolite–P450 complex is formed. Complex formation is reduced when the substituent is an electron-withdrawing group, which destabilizes the carbene-iron bridge, whereas if the substituent is electron-donating or a long-chain alkyl group, complex formation is increased and stabilized [10, 18, 19]. This electron-donating stabilization of the metabolite–P450 complex by the substituent also was observed in the present studies with methylenedioxyphenyl HIV pro-

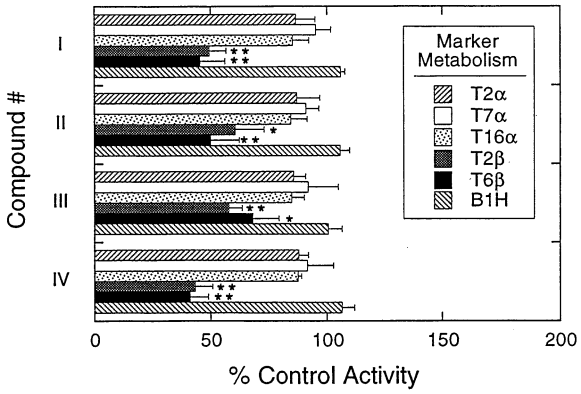

FIG. 6. Specificity of the inactivation of rat P450 isoforms in control rat liver microsomes. Values are presented as percent control activity obtained from samples preincubated with NADPH (1 mM) alone and NADPH plus 25 μM of HIV protease inhibitors for 30 min. Marker activities are abbreviated as follows: T2α, testosterone 2α-hydroxylation; T7α, testosterone 7α-hydroxylation; T16α, testosterone 16α-hydroxylation; T2β, testosterone 2β-hydroxylation; T6β, testosterone 6β-hydroxylation; and B1H, bufuralol 1′-hydroxylation. The values are means (with SD) of three separate experiments. Control values were: 1.49 ± 0.26 nmol/min/mg of protein for 2α-hydroxylase activity; 0.052 ± 0.012 nmol/min/mg of protein for 7α-hydroxylase activity; 2.14 ± 0.36 nmol/min/mg of protein for 16α-hydroxylase activity; 0.064 ± 0.014 nmol/min/mg of protein for 2β-hydroxylase activity; 0.722 ± 0.132 nmol/min/mg of protein for 6β-hydroxylase activity; and 1.24 ± 0.20 nmol/min/mg of protein for bufuralol 1′-hydroxylase activity. Significant differences were observed in 2β/6β-hydroxylase activities of testosterone between samples preincubated with NADPH alone and with NADPH plus HIV protease inhibitors (*$P < 0.05$, **$P < 0.01$).

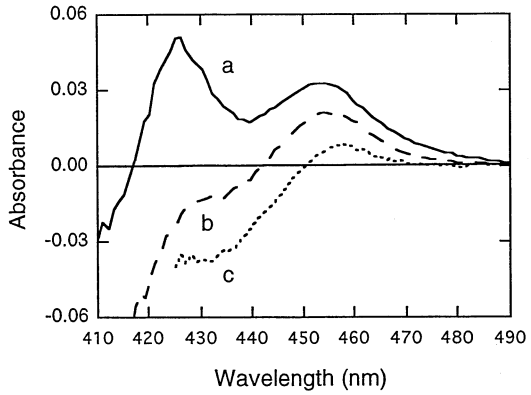

FIG. 7. Inhibitory effect of anti-rat CYP3A1 antibody on metabolite–P450 complex formation of HIV protease inhibitor I in human liver microsomes. Human liver microsomes were preincubated with 0, 0.22, and 0.45 mg of anti-CYP3A1 antibody/mg of microsomal protein for 20 min at room temperature. Then the reaction was started by the addition of 1 mM of NADPH and 100 μM of HIV protease inhibitor I, and carried out for 30 min before the spectra were recorded. Spectra (a), (b), and (c) represent metabolite–P450 complex in samples preincubated with 0, 0.22, and 0.45 mg of anti-rat CYP3A1 antibody/mg of microsomal protein, respectively.

tease inhibitors. While substitution had little effect on the K_m values for complex formation (Table 2), V_{max} values clearly were affected as predicted kinetically: **I** (methoxy-substituted) > **II** and **III** (nonsubstituted) > **IV** (chlorine-substituted). Assuming that the same extinction coefficient (75 mM^{-1} cm^{-1}) is applicable to all MI complexes, these results are consistent with the hypothesis that the mechanism for the methylenedioxyphenyl metabolite–P450 complex formation involves coordination of the metabolically formed carbene function to the prosthetic heme of P450.

P450-isoform selectivity also has been demonstrated in many metabolite–P450 complex formations [4, 16, 20, 21]. The present studies showed that all seven methylenedioxyphenyl HIV protease inhibitors selectively inhibited CYP3A-catalyzed testosterone 6β-hydroxylase activity in both rat and human liver microsomes (Figs. 5, 6 and 8). Metabolite–P450 complexation clearly was observed in the DX-treated rat liver microsomes, whereas it was insignificant and very minor (<5%) in control and PB-treated microsomes, respectively (data not shown). In human liver microsomes, an anti-rat CYP3A1 antibody diminished metabolite–P450 complex formation in an antibody concentration-dependent manner (Fig. 7). Although the possibility of multiple P450 involvement in the MI complex formation cannot be ruled out, these results strongly suggest that a P450 isoform(s) in the CYP3A subfamily is inactivated selectively by metabolite–P450 complex formation with methylenedioxyphenyl HIV protease inhibitors in both species.

Several classes of inhibitors are known to be activated metabolically to a reactive intermediate(s) that, in turn, is irreversibly or quasi-irreversibly bound to the enzyme(s). In our previous studies, an investigational HIV protease inhibitor, L-754,394, which has the same core structure as the Y-series compounds (Fig. 1), but has a furanopyridine instead of methylenedioxyphenyl ring, showed quite similar dose- and time-dependent pharmacokinetics [22] as well as a selective inhibitory effect on CYP3A [13, 22]. *In vitro*

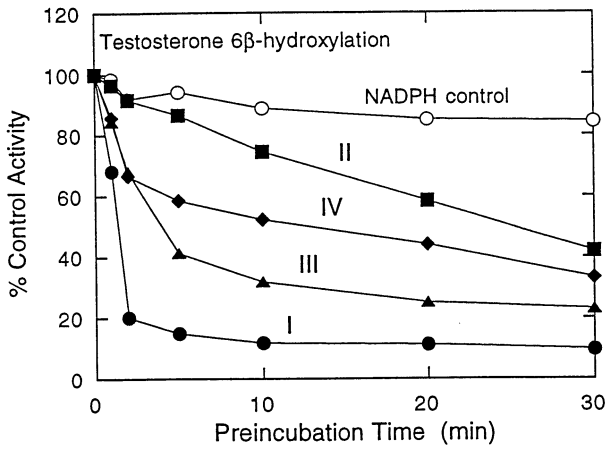

FIG. 8. Time-course of P450 inactivation by HIV protease inhibitors in human liver microsomes. Human liver microsomes were preincubated for the designated time period with 25 μM of HIV protease inhibitors and 1 mM of NADPH. The control value was 1.98 nmol/min/mg of protein. This experiment was performed once.

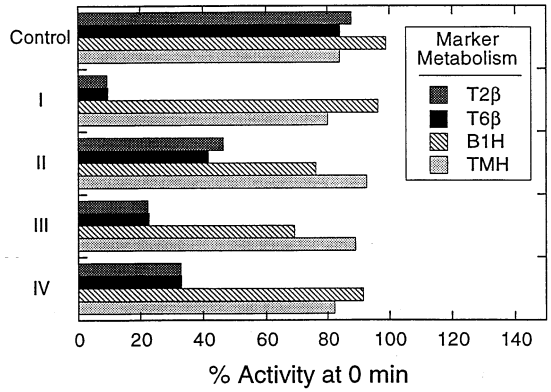

FIG. 9. Selective P450 inactivation by HIV protease inhibitors in human liver microsomes. Human liver microsomes were preincubated for 30 min with 25 μM of HIV protease inhibitors and 1 mM of NADPH. Control values were: 0.17 nmol/min/mg of protein for testosterone 2β-hydroxylation (T2β); 1.61 nmol/min/mg of protein for testosterone 6β-hydroxylation (T6β); 0.191 nmol/min/mg of protein for bufuralol 1′-hydroxylation (B1H); and 0.673 nmol/min/mg of protein for tolbutamide methyl hydroxylation (TMH). This experiment was performed once.

studies revealed that the inhibition was mechanism-based [13], and the formation of the reactive intermediate, a furan epoxide, was proposed [23]. L-754,394 did not show any metabolite–P450 complex formation, and more than 80% of the total P450 was destroyed within 10 min in human liver microsomes [13]. Therefore, different mechanisms (i.e. irreversible P450 destruction versus P450 metabolic complexation) operated with different structures (i.e. furanopyridine versus methylenedioxyphenyl ring) that were responsible for the dose-/time-dependent pharmacokinetics and CYP3A-selective time-dependent *in vitro* inhibition observed with HIV protease inhibitors.

In summary, methylenedioxyphenyl HIV protease inhibitors formed a complex with P450 upon incubation with rat and human liver microsomes in the presence of NADPH. *In vitro* studies demonstrated further that the metabolism of CYP3A marker substrates was impaired selectively during preincubation with NADPH in microsomes. It is suggested that formation of a quasi-irreversible metabolite–CYP3A complex with methylenedioxyphenyl HIV protease inhibitors was responsible for the time-dependent loss of CYP3A catalytic function and the unusual dose-dependent pharmacokinetics after oral administration. The development of methylenedioxyphenyl HIV protease inhibitors has been discontinued because of these unfavorable properties.

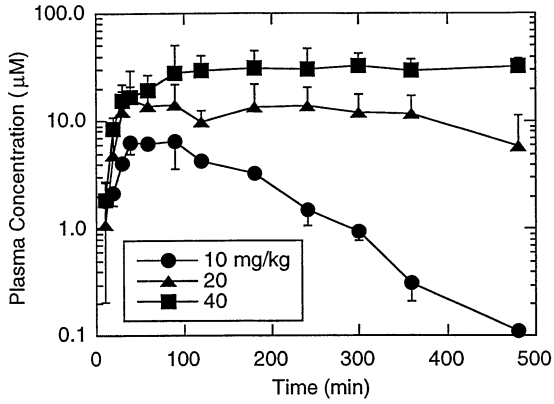

FIG. 10. Plasma concentration versus time profiles of I after different oral doses in rats. Values are means (with SD) of three separate experiments.

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