CYP2C8- and CYP3A-Mediated C-Demethylation of (3-[(4-tert-Butylbenzyl)-(pyridine-3-sulfonyl)-amino]-methyl-phenoxy)-acetic Acid (CP-533,536), an EP2 Receptor-Selective Prostaglandin E2 Agonist: Characterization of Metabolites by High-Resolution Liquid Chromatography-Tandem Mass Spectrometry and Liquid Chromatography/Mass Spectrometry-Nuclear Magnetic Resonance

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Received June 13, 2008; accepted July 22, 2008

ABSTRACT: CP-533,536, (3-[(4-tert-butyl-benzyl)-(pyridine-3-sulfonyl)-amino]-methyl-phenoxy)-acetic acid (1), an EP2 receptor-selective prostaglandin E2 agonist, is being developed to aid in the healing of bone fractures. To support the development of this program, in vitro metabolism of 1 was investigated in human liver microsomes and major recombinant human cytochrome P450 (P450) isoforms. 1 was metabolized in vitro by at least three recombinant human P450s: CYP3A4, CYP3A5, and CYP2C8. The turnover of 1 was NADPH-dependent and was completely inhibited by ketoconazole and quercetin in the CYP3A4/5 and CYP2C8 incubations, respectively. The major metabolic pathways were caused by oxidation of the tert-butyl moiety to form the \( \omega \)-hydroxy metabolite (M4), oxidation of the pyridine moiety, and/or \( \text{N} \)-dealkylation of the methylphenoxy acetic acid moiety. The alcohol metabolite M4 was further oxidized to the corresponding carboxylic acid M3. In addition to these pathways, three unusual metabolites (M22, M23, and M26) resulting from C-demethylation of the tert-butyl group were identified using high-resolution liquid chromatography/tandem mass spectrometry and liquid chromatography/mass spectrometry/NMR. The C-demethylated metabolites were not detected on incubation of carboxylic acid metabolite M3 with either human liver microsomes or CYP3A/2C8 isoforms, suggesting that these metabolites were not derived from decarboxylation of M3. A possible mechanism for C-demethylation may involve the oxidation of M4 to form an aldehyde metabolite (M24), followed by P450-mediated deformylation, to give an unstable carbon-centered radical and formic acid. The carbon-centered radical intermediate then undergoes either oxygen rebound to form an alcohol metabolite M23 or hydrogen abstraction leading to an olefin metabolite M26.
major human metabolites of a drug candidate early in its development is useful to enable the judicious selection of animal species used for safety evaluation and to ensure that the selected animal species are exposed to all the major metabolites formed in humans (Baillie et al., 2002; http://www.fda.gov/cder/guidance/index.htm). Human unique metabolites otherwise may need to be tested in animals in additional studies (http://www.fda.gov/cder/guidance/index.htm). The metabolic profiles of a drug candidate are determined definitively in preclinical species and humans following its radiolabel administration. Studies with radiolabeled drug in humans are conducted at a later stage of drug development because of high attrition during phase I and phase II clinical trials (Caldwell, 1996). In vitro studies using animal and human hepatocellular and subcellular fractions and/or recombinant enzymes are generally used to predict metabolites in vivo (Bachmann and Ghosh, 2001; Testino and Patonay, 2003; Baranczewski et al., 2006). In vivo studies in rats have shown that 1 is extensively metabolized. The major oxidative pathway in rats was a result of the oxidation of the tert-butyl side chain to form the ω-hydroxy metabolite, which was further oxidized to form the ω-carboxy metabolite or conjugated via sulfation to form a sulfate conjugate. Other metabolites were caused by N-oxidation of the pyridine ring and aromatic hydroxylation, and conjugation with glucuronic acid (Johnson and Prakash, 2005). The objective of the present study was to characterize the in vitro metabolites of 1 in human liver microsomes and major recombinant human cytochrome P450 (P450) isoforms. The metabolites were characterized by high-resolution liquid chromatography/tandem mass spectrometry (LC/MS/MS) and LC/MS/NMR and by comparisons of their retention times on high-performance liquid chromatography (HPLC), and MS spectra with those of the synthetic standards.

**Materials and Methods**

**General Chemicals.** Commercially obtained chemicals and solvents were of HPLC or analytical grade. YMC AQ (C-18) and YMC basic column analytical and preparative columns were obtained from YMC, Inc. (Wilmington, NC). Phenomenex Luna C8 (2) column was obtained from Phenomenex (Torrance, CA). Ecolite (+) scintillation mixture was obtained from ICN (Irvine, CA). HPLC grade acetonitrile, methanol, water, certified ACS grade ammonium acetate, and acetic acid were obtained from J. T. Baker (Phillipsburg, NJ). CYP3A4/3A5 and CYP2C8 specific inhibitors, ketoconazole and quercetin, were obtained from Sigma-Aldrich Chemicals (St. Louis, MO).

Human liver samples were obtained from organ donors (both male and female between the ages of 18–65 years) and purchased from Tissue Transformation Technology (Edison, NJ) on an approval from ethics committee of the vendor. Liver and microsomal samples were stored at −70°C until used. Human liver microsomes were prepared and characterized for P450 isoforms at Pfizer Global Research and Development (Groton, CT) using standard procedures (Prakash et al., 2000). Human liver mix was prepared by mixing liver microsomes from several donors to represent the P450 in normal humans. Recombinant human P450 isoforms (CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5) were purchased from Gentest (Bedford, MA). The microsomal protein was assayed using BCA assay kit (Pierce, Rockford, IL), and its P450 contents were determined by the method described previously (Omura and Sato, 1964).

**Radiolabeled Drug and Reference Compounds.** [14C]1, specific activity 4.36 mCi/mmol (Fig. 1), was synthesized by the Radiosynthesis Group at Pfizer Global Research and Development. It showed a radiochemical purity of ≥98%, as determined by radio-HPLC. The synthetic reference standards,
ω-carboxy- (M3) and ω-hydroxy- (M4), were synthesized by the Medicinal Chemistry Group at Pfizer Global Research and Development.

**Human Liver Microsomes and Recombinant Human P450 Isoform Incubations.** Before use, human liver microsomes and recombinant P450 isoforms were thawed on ice and reconstituted in 100 mM potassium phosphate buffer, pH 7.4. [14C]I (10–20 μM) was preincubated with microsomes (0.5 mg/ml protein microsomes) or recombinant P450s (20–60 pmol/ml) for 3 min at 37°C in a shaking water bath. The incubation was initiated with the addition of 100 μl of cofactor (1.1 mM NADPH, 10 mM MgCl2) per 1 ml of incubation mixture. After 30 min, the incubations were terminated by the addition of 100 μl of cold acetonitrile and ascorbic acid (50 μl of a 20 mM stock solution in water).

**Large-Scale Microsomal Incubations.** Compound 1 was incubated with recombinant human P450 isoforms CYP3A4, CYP3A5, and CYP2C8 to generate metabolites in sufficient quantity for LC/MS/NMR study (50-ml total incubation). The quantities of expressed protein in each incubation were approximately 1 mg of protein/ml of incubation. Before use, microsomes were thawed on ice and reconstituted using 100 mM potassium phosphate, pH 7.4, containing I (50 μM). Samples were preincubated for 3 min with recombinant P450s at 37°C in a shaking water bath. Incubations were initiated with the addition of 100 μl of cofactor (1.1 mM NADPH, 10 mM MgCl2) per milliliter of incubation mixture. Sixty minutes following the addition of NADPH cofactor, the samples were acidified with 500 μl of acetic acid.

**Extraction of Metabolites from in Vitro Matrices.** Incubations were stopped by the addition of an equal volume of cold acetonitrile, sonicated, and centrifuged at 3000 rpm for 10 min. The supernatants were removed and evaporated to dryness in a nitrogen Turbo Vap LV evaporator (Caliper Life Sciences Corporate, Hopkinton, MA). Residues were reconstituted in 200 μl of 50:50 acetonitrile/water, and aliquots (50–90 μl) were injected onto the HPLC system for analysis. The incubation mixture from large incubations was extracted with an equal volume of methyl-tert-butyl ether. The organic solvent was separated and evaporated to dryness in a nitrogen Turbo Vap LV evaporator (Caliper Life Sciences Corporate). The residue was reconstituted in 300 μl of 50:50 acetonitrile/water, and an aliquot (100 μl) was injected into the HPLC system for analysis.

**Quantitative Assessment.** Quantification of metabolites was carried out by measuring the radioactivity in the individual peaks that were separated on HPLC using β-RAM (IN/US, Tampa, FL). The β-RAM provided an integrated printout in counts per minute and the percentage of the radiolabeled material, as well as the peak representation. The β-RAM was operated in the homogeneous liquid scintillation counting mode with the addition of 3 ml/min TruCount scintillation mixture (IN/US) to the effluent post-UV detection.

**HPLC.** The HPLC system consisted of an HP-1050 solvent delivery system, an HP-1050 membrane degasser, an HP-1050 autoinjector (Hewlett Packard, Palo Alto, CA), a Thermo Separations (Thermo Electron Corporation, Billerica, MA) operated in the positive ion mode using electrospray ionization. A make-up flow of 125 ml/min was used to dilute the eluent to the mass spectrometer. The majority of the LC effluent was directed to a Bruker DRX 500-MHz NMR system (Bruker Daltonics, Inc., Billerica, MA) equipped with a 4-mm H-1/C-13 inverse x-gradient LC flow probe. Metabolite peaks were captured in the NMR probe using the loop storage method. 1H spectra were obtained on the LC peaks of interest using 1D nuclear Overhauser effect spectroscopy double-preasurelent of solvent NMR resonances. All the chemical shifts are reported in parts per million downfield from tetramethylsilane as referenced from the shift of residual protons in MeCN-d4 at 81.94 ppm.

**Results**

Representative HPLC radiochromatograms obtained from the analysis of human liver microsomes and recombinant human CYP3A4, CYP3A5, and CYP2C8 incubations of 1 are shown in Fig. 2. The incubation of 1 with HLM and CYP3A4/A5 and CYP2C8 in the presence of the NADPH-regenerating system yielded 12 metabolites. The turnover of 1 was greatest in CYP3A4, CYP3A5, and CYP2C8. Other isoforms (CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1) exhibited less than 2% turnover based on radioisotope detection (not shown). Metabolites were quantitated by in-line radioactive counting, and relative percentages are presented in Table 1.

The structures of metabolites were characterized and assigned based on their high-resolution MS, product ion mass spectra, and 1H NMR. Metabolites M2, M3, M4, M5, and M11 were further confirmed by comparison with synthetic standards and/or metabolites observed in rats in vivo (Johnson and Prakash, 2005). The protonated...
FIG. 2. HPLC-radiochromatograms of metabolites of 1 in human liver microsomes CYP2C8, CYP3A4, and CYP3A5.

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molecular and characteristic product ions of I and its metabolites, along with HPLC retention times, are summarized in Table 2. The protonated molecular ion (MH)^+ of I was observed at m/z 469, with MS^2 spectrum of this ion giving rise to prominent fragment ions at m/z 423, 413, 326, 165, and 147. The product ion at m/z 423 occurred from the loss of the formic acid from the molecule. The loss of the tert-butyl group resulted in the ion at m/z 413. The ion at m/z 326 resulted from the loss of a sulfonyle phynide moiety. The ion at m/z 165 was caused by a carbocation at the methyl phenoxaycetic acid moiety, and the ion at m/z 147 was the result of a tert-butyl tropylion.

Metabolite M2 had a retention time of ~14.0 min on HPLC and showed an MH^+ at m/z 501 (32 Da higher than I). It showed prominent fragment ions at m/z 483, 342, 324, 165, and 145 (Table 2). The ion at m/z 483 occurred from the loss of water from the molecule. The ion at m/z 342, a loss of the modified sulfonyle pyridine moiety, suggested the addition of an oxygen atom to the tert-butyl moiety. The fragment at m/z 324 resulted from a loss of water from the ion at m/z 342. The ion at m/z 165, similar to that of the parent compound, was caused by the methyl phenoxaycetic acid moiety. The ion at m/z 145, a loss of water molecule from the modified tert-butyl benzyl moiety, indicated the presence of an alcohol group. Based on these data, M2 was tentatively identified as the dihydroxy metabolite.

Metabolite M3 had a retention time of ~15.5 min on HPLC and showed an MH^+ at m/z 485 (16 Da higher than I). Its CID product ion spectrum showed prominent fragment ions at m/z 467, 342, 355, 324, 165, and 145 (Table 2). HPLC retention time and MS^2 spectrum of M4 were similar to those of a synthetic standard. Based on these data, M4 was identified as 3-[(4-(2-hydroxy-1,1-dimethyl-ethyl)-benzyl)-(pyridine-3-sulfonamido)-methyl]-phenoxy-acetic acid.

Metabolite M4 had a retention time of ~15.5 min on HPLC and showed an MH^+ at m/z 485 (16 Da higher than I). The CID product ion spectrum of M4 showed prominent fragment ions at m/z 439, 326, 165, and 147 (Table 2). The ions at m/z 326, 147, and 165 were all similar to those seen in the CID spectrum of the parent compound, suggesting that the tert-butylbenzyl and the methylphenoxacycetic acid moieties were unmodified. Treatment of M4 with aqueous TiCl3 resulted in an increase of the relative area of I in the chromatogram.

The MS^2 spectrum of M11 showed fragments at m/z 289, 146, and 131 (Table 2). The ions at m/z 131 and 146 resulted from the modified tert-butylbenzyl and tert-butylbenzyl amine moieties with the concomitant loss of the formic acid. Based on these data, M11 was tentatively identified as 2-methyl-2-[(4-(pyridine-3-sulfonylamino)-methyl)-phenyl]-propionic acid.

Metabolite M19 had a retention time of ~16.6 min on HPLC and showed an MH^+ at m/z 321 (148 Da lower than the parent compound). The MS^2 spectrum of M19 showed prominent fragment ions at m/z 321, 265, 162, and 147 (Table 2). The ions at m/z 162 and 147 were similar to those of the parent compound, suggesting that the phenyl tert-butylbenzyl moiety was unchanged and the molecule had undergone N-dealkylation. The ion at m/z 265 resulted from the loss of the tert-butyl moiety, suggesting that an oxidation had occurred on the pyridine moiety. Based on these data, M19 was tentatively identified as pyridine-N-oxide-3-sulfonic acid-4-tert-butyl-benzamidine.

Metabolite M20 had a retention time of 19.3 min on HPLC and showed an MH^+ at m/z 305 (164 Da lower than the parent compound). The CID product ion spectrum of M20 showed prominent fragment ions at m/z 162 and 147 (Table 2). These ions were similar to those observed in the CID product ion spectrum of the parent compound, suggesting that the tert-butylbenzyl moiety was unchanged and I had undergone N-dealkylation. Based on these data,
M20 was tentatively identified as pyridine-3-sulfonic acid-4-tert-butyl-benzylamide.

Metabolite M21 displayed an MH$^+$ at m/z 321 and had a retention time of ~14.3 min on HPLC. The MS$^2$ spectrum of M21 showed prominent ions at m/z 178, 160, 145, 133, and 78 (Table 2). The ion at m/z 178 resulted from cleavage of the sulfonamide bond. A loss of water from the ion at m/z 178 generated the fragment at m/z 160. The ion at m/z 145, 2 Da lower than that of the parent compound (m/z 147), suggested that the hydroxylation had occurred on the tert-butyl moiety. The ion at m/z 78 was the result of charge stabilization on the pyridine ring. Based on these data, the structure of M21 was tentatively identified as pyridine-3-sulfonic acid-4-(2-hydroxy-1,1-dimethyl-ethyl)-benzyl amide.

Metabolite M22 had a retention time of ~13.1 min on HPLC and displayed an MH$^+$ at m/z 487 (18 Da higher than the parent compound). The MS$^2$ spectrum of M22 showed prominent fragment ions at m/z 469, 451, 423, 395, 335, 326, 165, and 147 (Fig. 3; Table 2). The ion at m/z 165, similar to that of parent compound, indicated that
the methylphenoxy acetic acid moiety was unchanged. The ions at \( m/z \) 469 and 451, two consecutive losses of 18 Da from the protonated molecular ion, suggested that two molecules of water had been lost. Accurate mass analysis of protonated ion was determined to be 487.1592, corresponding to an empirical formula of \( \text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_7\text{S} \).

\( ^1\text{H} \) spectrum of M22 gave resonance at 1.36 (s, 3H, \( \text{CH}_3 \)), 3.50 (s, \( 2\text{H}, \text{CH}_2\text{OH} \)). All the other resonances were substantially similar to parent compound (Fig. 4). Based on these data, M22 was tentatively identified as (3-\{[4-(1,2-dihydroxy-1-methyl-ethyl)-benzyl]-pyridine-3-sulfonyl]-amino]-methyl\}-phenoxy)-acetic acid.

Metabolite M23 had a retention time of \( \sim 15 \) min on HPLC and showed an \( \text{MH}^+ \) at \( m/z \) 471 (2 Da higher than 1). The MS\(^2 \) spectrum of M23 showed prominent fragment ions at \( m/z \) 453, 335, 310, 165, and 131 (Fig. 3; Table 2). The ion at \( m/z \) 453, a loss of 18 Da from the protonated molecular ion, suggested that a molecule of water had been lost. The ions at \( m/z \) 310 and 131 (16 Da lower than those observed in the parent compound) suggested that a methyl group was replaced by a hydroxyl group, which was then lost during fragmentation. Accurate mass analysis of protonated ion was determined to be 471.1596, corresponding to an empirical formula of \( \text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_6\text{S} \).
\(^1\)H NMR spectrum of M23 gave resonance at \(\delta\) (ppm) 1.39 (s, 6H, \(2\text{CH}_3\)). All the other resonances were substantially similar to parent compound (Fig. 4). Based on these data, M23 was tentatively identified as (3-[[4-(1-hydroxy-1-methyl-ethyl)-benzyl]-(pyridine-3-sulfonyl)-amino]-methyl]-phenoxy)-acetic acid.

Metabolite M24 had a retention time of \(~17.1\) min on HPLC and showed an MH\(^+\) at \(m/z\) 483 (14 Da higher than the parent compound). The CID product ion spectrum of M24 showed prominent fragment ions at \(m/z\) 483, 437, 409, 340, 295, 266, 165, and 161 (Fig. 5; Table 2). The ions at \(m/z\) 437, 340, and 161 were 14 Da higher than those observed in the spectrum of the parent compound, suggesting the addition of an oxygen atom with a concomitant loss of two hydrogen atoms. Accurate mass analysis of protonated ion was determined to be 483.1592, corresponding to an empirical formula of \(C_{25}H_{27}N_2O_6S\).

\(^1\)H NMR spectrum of M24 gave resonance at \(\delta\) (ppm) 1.33 (s, 6H, \(2\text{CH}_3\)) and 9.34 (s, 1H, \(\text{CHO}\)). All the other resonances were substantially similar to parent compound (Fig. 6). Based on these data, M24 was identified as (3-[[4-(1,1-dimethyl-2-oxo-ethyl)-benzyl]-(pyridine-3-sulfonyl)-amino]-methyl]-phenoxy)-acetic acid.

Metabolite M26 had a retention time of \(~18.3\) min on HPLC and an MH\(^+\) ion at \(m/z\) 453 (16 Da lower than the parent compound). The CID product ion spectrum of M26 showed prominent fragment ions at
The ion at $m/z$ 165 was similar to that observed in the parent drug, suggesting that the methyl phenoxyacetic acid moiety was unchanged. The ions at $m/z$ 407, 310, and 131 were 16 Da lower than the ions observed in the spectrum of parent drug, suggesting that 16 mass units were lost from the tert-butyl moiety. Accurate mass analysis of protonated ion was determined to be 453.1506, corresponding to an empirical formula of $C_{24}H_{25}N_2O_5S$. 

**Discussion**

The predictive power of in vitro studies using animal and human subcellular fractions and/or recombinant enzymes has advanced considerably because of an ever increasing understanding of the relationships between in vitro and in vivo drug metabolism and disposition. In vitro systems are routinely used to investigate the metabolite profile of drug candidates and to design clinical drug-drug interactions studies. We here report the in vitro metabolism of a (tert-butyl-benzyl)pyridyl...
sulfonamide analog, an EP2-receptor agonist, 1 in human liver microsomes and recombinant human P450 isoforms. 1 is metabolized in vitro by at least three recombinant human P450 isoforms: CYP3A4, CYP3A5, and CYP2C8. The turnover of 1 was NADPH-dependent and was completely inhibited with the use of the isoform-specific inhibitors ketoconazole and quercetin in incubations of 1 with CYP3A4/5 and CYP2C8, respectively (not shown). Twelve metabolites were identified. The postulated metabolic pathways of 1 in human liver microsomes and recombinant human P450 isoforms CYP3A4, CYP3A5, and CYP2C8 are shown in Fig. 1. The metabolites were primarily characterized by the LC/MS/MS method, which is a method of choice for the high-throughput and rapid structural characterization of metabolites from biological fluids (Kamel and Prakash, 2006; Prakash et al., 2007b). Interpretation of CID mass spectra of metabolites was able to define the oxidative possibilities when they occur in positions characterized by distinct product ions. The structures of metabolites were further supported by high-resolution LC/MS and LC/1H NMR.

The major oxidative primary pathways were the result of the oxidation of the tert-butyl side chain to form the ω-hydroxy metabolite M4, N-oxidation of the pyridine moiety, and/or N-dealkylation of the methylphenoxyacetic acid moiety. M4 was further oxidized to the ω-carboxy metabolite M3. N-Oxidation of the pyridine moiety formed metabolite M5. The protonated molecular ion of M5 at \( m/z \) 485 was indicative of the addition of an oxygen atom. The prominent fragment ions at \( m/z \) 147, 165, and 326 suggested that the oxidation had occurred at the pyridine ring. Reduction of M5 with aqueous TiCl3 provided the evidence that M5 was an N-oxide (Kulanthaivel et al., 2004; Johnson and Prakash, 2005). Metabolite M20 was identified by N-dealkylation of the methylphenoxy acetic acid moiety, a common pathway for N-substituted alkyamines (Prakash et al., 1997; Parkinson, 2001). Other metabolites (M11, M19, and M21) were formed by combination of these primary pathways. Metabolites M2, M3, M4, M5, and M11 were also observed in rats in vivo (Johnson and Prakash, 2005).

Two unusual metabolites (M23 and M26) formed by C-demethylation of the tert-butyl group were identified as (3-[[4-(1-hydroxy-1-methyl-ethyl)-benzyl]-(pyridine-3-sulfonyl)-amino]-methyl]-phenoxy)-acetic acid (M23) and (3-[[4-isopropenyl-benzyl]-(pyridine-3-sulfonyl)-amino]-methyl]-phenoxy)-acetic acid (M26). The protonated molecular ion of M23 at \( m/z \) 471 was indicative of the loss of a methyl group and the subsequent addition of an oxygen atom. The fragment ion at \( m/z \) 453, a loss of 18 Da (H2O) from the protonated molecular ion, was indicative of the presence of an alcohol group. The ions at \( m/z \) 310 and 131 suggested that one of the methyl group was replaced by an oxygen atom. Further, the resonance at δ1.33 ppm in the \(^1\)H NMR of M23 corresponds to two methyl groups, indicating that one of the methyl groups has been removed or altered. All the other resonances are substantially similar to parent compound.

The MH\(^+\) of M24 at \( m/z \) 453 suggested that the molecule had undergone demethylation and dehydrogenation. The prominent ions at \( m/z \) 407, 310, and 131 were 16 Da lower than the ions observed in the spectrum of parent drug, indicating that the C-demethylation and dehydrogenation had occurred at the tert-butyl moiety. \(^1\)H NMR of M26 displayed two new singlet resonances (one proton each) at 5.06 and 5.34 ppm, which were consistent with a terminal olefin group. The aromatic resonances in the spectrum are substantially similar to the parent compound. In addition, there was a shift in the UV-visible spectrum from 220/263 nm in the parent spectrum to 250 nm in the metabolite M26, indicating a change in the chromophore.

Metabolite M22 was identified as (3-[[4-(1,2-dihydroxy-1-methyl-ethyl)-benzyl]-(pyridine-3-sulfonyl)-amino]-methyl]-phenoxy)-acetic acid and formed by further oxidation of M23. \(^1\)H NMR of M22 showed a resonance at δ1.36 ppm corresponding to only one methyl group, indicating that two of the methyl groups have been altered or removed. Also, an additional two-proton singlet resonance at 6.35 ppm was consistent with a hydroxyl group on a methylene group.

Metabolite M24 was identified as (3-[[4-(1,1-dimethyl-2-oxo-ethyl)-benzyl]-(pyridine-3-sulfonyl)-amino]-methyl]-phenoxy)-acetic acid, an aldehyde intermediate in the formation of the ω-carboxy metabolite acid. The NMR spectrum of M24 showed a resonance at δ1.33 ppm corresponding to two methyl groups, indicating that one of the methyl groups has been removed or altered. Also, an additional...
one-proton singlet resonance at δ9.34 ppm was consistent with an aldehyde.

The novel findings in this study were the characterization of several C-demethylated metabolites: M22, M23, and M26. N-, O-, and S-demethylation are frequently observed in the metabolism of xenobiotics, but C-demethylation is not a common biotransformation reaction (Prakash et al., 1997; Parkinson, 2001). Both alcohol and carboxylic acid metabolites have been observed for drugs containing the tert-butyl side chain such as terfenadine and finasteride (Rodrigues et al., 1995; Carlin et al., 1997; Prakash et al., 2007a), but the C-demethylated metabolites have never been reported for these drugs. However, it has been reported that a carboxylic acid was an intermediate in the C-demethylation of N-tert-butylnorcholylcizine and 4-α-methyl-5a-cholest-7-en-3β-ol (Miller and Gaylor, 1970; Kamm and Szuna, 1973), and this carboxylic acid is decarboxylated by a microsomal, cyanide-sensitive enzyme (Gaylor and Mason, 1968). Recently, C-demethylation of the tert-butyl group of a novel dipeptidyl peptide-4 inhibitor, LC15-0133, has been reported in rat liver microsomes (Yoo et al., 2008). It was further revealed that the C-demethylated metabolite of LC15-0133 was formed by nonenzymatic decarboxylation of the carboxyl metabolite. However, incubation of the carboxyl metabolite M3 with human liver microsomes or CYP3A4/3A5 and CYP2C8 did not produce any C-demethylated metabolite (M23 or M26), suggesting that these metabolites are formed by an alternate mechanism. A possible mechanism for C-demethylation of I may occur by deamination of the aldehyde metabolite (M24) by a stepwise radical process with an intermediate carbon radical that can form the alcohol metabolite M23 by oxygen rebound or the olefin metabolite M26 by hydrogen abstraction (Fig. 7). P450-mediated deamination of xenobiotics to form olefins has been reported (Roberts et al., 1991; Vaz, 2001).

CYP3A is the most abundant human hepatic P450 and is clinically very important because it has been shown to metabolize approximately 50% of commonly administered drugs. In adults, CYP3A4 and CYP3A5 are predominant among the four known isoforms (CYP3A4, CYP3A5, CYP3A7, and CYP3A43) in the liver and intestine. Most of the CYP3A4 substrates are also metabolized by CYP3A5 (Williams et al., 2002). However, some differences have been reported in enzymatic properties of CYP3A4 and CYP3A5, including substrate specificity and inhibition (Khojasteh-Bakht et al., 2003; Emoto and Iwasaki, 2006). In the current study, it was found that N-oxide (MS) and its N-dealkylated metabolite (M19) were formed only by CYP3A4 but not CYP3A5. The identification of these in vitro metabolic pathways of I will aid in understanding its metabolism in humans in vivo and in designing clinical drug-drug interaction studies.

Acknowledgments. We thank Dr. Kathleen Zandi and Sandra Miller for providing radiolabeled CP-533,536. We also thank Dr. Alfin Vaz for critical evaluation of the manuscript.

References


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