PHARMACOKINETICS, METABOLISM AND EXCRETION OF ANACETRAPIB, A
NOVEL INHIBITOR OF THE CHOLESTERYL ESTER TRANSFER PROTEIN
(CETP), IN RATS AND Rhesus Monkeys

Eugene Y. Tan¹, Georgy Hartmann, Qing Chen, Antonio Pereira, Scott Bradley², George
Doss, Andy Shiqiang Zhang, Jonathan Z. Ho, Matthew P. Braun, Dennis C. Dean³, Wei
Tang and Sanjeev Kumar

Departments of Drug Metabolism and Pharmacokinetics (E.Y.T, G.H, J.N, Q.C, A.P,
S.B., D.C.D, W.T and S.K), Medicinal Chemistry (G.D) and Labeled Compound
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Reprint requests and inquiries should be directed to:

Dr. Sanjeev Kumar, PhD
Department of Drug Metabolism and Pharmacokinetics
Merck Research Laboratories
126 E. Lincoln Avenue, RY80-141, Rahway, NJ 07065
Phone: (732) 594 0261
Fax: (732) 594 2382
email: sanjeev_kumar@merck.com

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Abbreviations: IV, intravenous administration; P.O., oral administration; LC/MS/MS, liquid chromatography-tandem mass spectrometry; AUC, area under the plasma concentration-time curve; ADME, absorption, distribution, metabolism and excretion; CETP, cholesteryl ester transfer protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
Abstract

The pharmacokinetics and metabolism of anacetrapib (MK-0859), a novel CETP inhibitor, were examined in rats and rhesus monkeys. Anacetrapib exhibited a low clearance in both species and a moderate oral bioavailability of ~38% in rats and ~13% in monkeys. The area under the plasma concentration-time curve in both species increased in a less than dose-proportional manner over an oral dose range of 1 to 500 mg/kg. Following oral administration of [14C]anacetrapib at 10 mg/kg, ~80 and 90% of the radioactive dose were recovered over 48 h post-dose from rats and monkeys, respectively. The majority of the administered radioactive dose was excreted unchanged in feces in both species. Biliary excretion of radioactivity accounted for ~15%, and urinary excretion for less than 2% of the dose. Thirteen metabolites, resulting from oxidative and secondary glucuronic acid conjugation, were identified in rat and monkey bile. The main metabolic pathways consisted of O-demethylation (M1) and hydroxylation on the biphenyl moiety (M2), and hydroxylation on the isopropyl side chain (M3); these hydroxylations were followed by O-glucuronidation of these metabolites. A glutathione adduct (M9), an olefin metabolite (M10), and a propionic acid metabolite (M11) also were identified. In addition to parent anacetrapib, M1, M2 and M3 metabolites were detected in rat, but not in monkey, plasma. Overall, it appears that anacetrapib exhibits a low-to-moderate degree of absorption following oral dosing and majority of the absorbed dose is eliminated via oxidation to a series of hydroxylated metabolites which undergo conjugation with glucuronic acid before excretion into bile.
Introduction

A significant amount of effort is being put into developing therapeutics that are capable of lowering low-density lipoprotein (LDL) levels because increased circulating levels of LDL have been demonstrated to increase risk for cardiovascular disease and associated clinical sequale (Kannel et al., 1979; Castelli et al., 1983; Stamler et al., 1988). Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) remain the cornerstone of LDL lowering therapy and have been demonstrated to reduce cardiovascular risk in humans (Shepherd et al., 1995). However, there remains a high incidence of residual cardiovascular events even after aggressive treatment with LDL lowering drugs (Bays and Stein, 2003). Recently, it has been shown that, in addition to LDL, high-density lipoprotein (HDL) cholesterol is an independent factor that may modulate the risk of cardiovascular disease. Epidemiological evidence suggest that plasma HDL cholesterol levels are inversely correlated with atherosclerosis and cardiovascular risk (Asztalos et al., 2004; Cobain et al., 2007). However, there are no clinical outcome data to support this hypothesis at the present time. One class of dyslipidaemia agents that affect HDL cholesterol levels are the inhibitors of cholesteryl ester transfer protein (CETP) (Hesler et al., 1987; Linsel-Nitschke and Tall, 2005). CETP is a plasma glycoprotein that regulates the exchange of cholesteryl esters and triglycerides between HDL and LDL cholesterol (Dullart et al., 1991; Tall et al., 1993), and therefore, it has recently become an attractive molecular target for the treatment of dyslipidemia. Preliminary evidence from dalcetrapib (JTT-705, R1658, Roche) and torcetrapib (Pfizer) indicate that CETP inhibition resulted in rapid accumulation of cholesterol in HDL particles and, hence,
caused an elevation of plasma HDL cholesterol concentration in humans (de Grooth et al., 2002; Clark et al., 2004; Dullaart et al., 2007). It has been widely believed until recently that, through inhibition of CETP and elevation of HDL cholesterol, the formation of atherosclerotic plaques can be significantly reduced, thus improving the long-term clinical outcomes in cardiovascular disease. However, unfavorable cardioavascular outcomes data from long-term treatment with torcetrapib have recently raised doubts as to whether CETP inhibitors will ultimately provide clinical benefit (Tall, 2007); although it appears that cardiovascular outcomes from treatment with torcetrapib may have been confounded by its adverse blood pressure raising effects in humans (Howes and Kostner, 2007; Forrest et al., 2008).

Anacetrapib (MK-0859 or [4S,5R]-5-[3,5-bis(trifluoromethyl)phenyl]-3-[[4'-fluoro-2'-methoxy-5'-{(propan-2-y1)-4-(trifluoromethyl)[1,1'-biphenyl]-2-yl}methyl]-4-methyl-1,3-oxazolidin-2-one; Merck & Co, Whitehouse Station, NJ, USA; Figure 1) is a selective inhibitor of CETP and is undergoing development as an oral drug for the treatment of primary hypercholesterolaemia and mixed hyperlipidaemia. It is a potent CETP inhibitor with IC$_{50}$ values of 16 and 29 nM on the CETP-mediated transfer of cholesteryl esters and triglycerides, respectively (unpublished Merck data). In phase I and II clinical trials, anacetrapib raised HDL cholesterol levels by ~130% and reduced LDL cholesterol levels by ~40% with no associated major adverse events (Krishna et al., 2007; Krishna et al., 2008; Bloomfield et al., 2009). In addition, it showed no treatment-related effect on blood pressure in a 24 hours ambulatory blood pressure clinical trial (Krishna et al., 2007), suggesting that the blood pressure raising effects with torcetrapib are likely molecule-, and not target-, specific and clinical benefit of CETP inhibitors in
Atherosclerotic disease needs to be further assessed. In this regard, the safety, tolerability, and favorable pharmacokinetic and pharmacodynamic properties of anacetrapib have recently been assessed in a Phase-I clinical study (Krishna et al., 2008).

In this study, we investigated the pharmacokinetics, metabolism and excretion of anacetrapib in Sprague-Dawley rats and rhesus monkeys, the two species that were used for the preclinical toxicological evaluation of this agent. Identification of excretory and circulating metabolites of novel small molecule pharmaceuticals in preclinical toxicology species is an integral part of their development program in order to ascertain that toxicity of all human metabolites of these agents is adequately characterized in preclinical studies (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065014.htm). In cases where disproportionate human metabolites are observed relative to the preclinical toxicology species, the regulatory agencies recommend appropriate testing of these metabolites in separate preclinical toxicology studies. The present studies were conducted following oral administration of [14C]anacetrapib to rats and monkeys and metabolite profiling of radioactivity in plasma and excreta. Metabolites of anacetrapib were identified by means of LC/MS/MS. Where possible, metabolites were purified by preparative LC and the structures were confirmed by 1H NMR and/or via comparison of HPLC retention time and mass spectral fragmentation with synthetic standards.
Materials and Methods

Drugs, Standards and Reagents

Anacetrapib (MK-0859; Figure 1) and synthetic standards of metabolites M1, M2, M3, and M10 were synthesized by Medicinal Chemistry and Process Research at Merck Research Laboratories (MRL, Rahway, NJ). Free base [Isopropyl-1-^{14}C]anacetrapib and [Methyl-O-^{13}CD_{3}]anacetrapib were synthesized by the Labeled Compound Synthesis group (MRL, Rahway, NJ). The radiochemical purity was >98% and the specific activity was >89 μCi/mg. Liver microsomes from monkeys were prepared at MRL, Rahway, NJ. All other reagents were obtained from commercial sources with an appropriate quality.

Pharmacokinetic Studies

All experiments were performed according to procedures approved by the Merck Research Laboratories Institutional Animal Care and Use Committee.

Rats. Adult male Sprague-Dawley rats, weighing ~ 280 to 330 g, were fasted overnight prior to the study. The animals were allowed access to food 4 hr postdose, but water was provided ad libitum. For intravenous (IV) administration, four rats received a dose of anacetrapib (dosing volume 2.5 mL/kg) via bolus injection through a catheter previously implanted into the femoral vein, followed by a 300 μL saline flush. The IV dose of anacetrapib was formulated in polyethylene glycol 300:water (7:3, v/v) at a concentration of 0.2 mg/ml. For oral (P.O.) administration, four rats received a dose via oral gavage
(dosing volume 2 mL/kg). These P.O. doses of 5, 50, 100, and 500 mg/kg were formulated in imwitor:tween (1:1, w/w) at concentrations of 2.5, 25, 50, and 250 mg/ml, respectively. Blood samples (~0.3 mL) were collected into EDTA-containing tubes at the following time points: predose, 0.083 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 h postdose. Blood samples were stored on ice, and plasma was prepared by centrifugation. Plasma was transferred to a 96-well plate and stored at –70°C until analysis.

Monkeys. For pharmacokinetic studies in monkeys, three adult male rhesus macaques (Macaca mulatta), weighing ~6 to 8 kg, were fasted overnight but allowed free access to water and transferred to restraint chairs the following morning. Indwelling catheters were placed by percutaneous venipuncture into the saphenous veins for collection of blood. Animals were fed and allowed access to water 4 hr post-dose. For intravenous administration of 0.1 mg/kg anacetrapib, three monkeys received a dose at 0.5 mL/kg via bolus injection through an intravenous catheter, followed by a 2.5 mL saline flush. This intravenous dose of anacetrapib was formulated in polyethylene glycol 300:water (7:3, v/v) at a concentration of 0.2 mg/ml. For oral administration, three monkeys received a dose at 2 mL/kg via nasogastric intubation and gavage, followed by a 3.5 mL saline flush. These oral doses of 1, 30, and 500 mg/kg were formulated in Imwitor:Tween (1:1, w/w) at concentrations of 0.5, 15, and 250 mg/ml, respectively. Blood samples (1 mL) were collected into EDTA-containing Vacutainer® tubes at the following time points: predose, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 hr postdose. Blood samples were stored on ice, and plasma was prepared by centrifugation within 30 min. Plasma was transferred to a 96-well plate and stored at –70°C until quantitative analysis.
Quantitative Analysis of Anacetrapib in Plasma

Concentrations of anacetrapib in rat and monkey plasma were determined by LC/MS/MS following protein precipitation. Calibration curves were generated using triplicate sets of standards. These standard samples were prepared by spiking 50 µL of control plasma with 10 µL of stock solutions containing 10, 25, 50, 250, 500, 1000, and 2500 ng anacetrapib/mL of acetonitrile:water (20:80, v/v). This yielded calibration standards effectively containing 2, 5, 10, 50, 100, 200, and 500 ng analyte/mL plasma. The monkey plasma samples obtained postdose were treated as follows. From most sample vials, 50 µL plasma were removed for analysis. Samples were diluted with blank plasma when they were expected to contain levels of analyte exceeding the upper limit of quantification. To all 50 µL aliquots of standard and postdose plasma samples, 10 µL of 100 ng/mL [D₃,¹³C]anacetrapib in acetonitrile:water (20:80, v/v) were added as internal standard (IS). This step was followed by protein precipitation with 200 µL of acetonitrile. Sample mixtures were vortex-mixed for 10 minutes, and 600 µL of acetonitrile added to each. After centrifuging for 15 min at 1600 g and 10°C, the resulting supernatants were harvested by a Tomtec Quadra 96 Model 320, and dried at 25°C under a nitrogen stream in a Zymark TurboVap 96 evaporator. The residues were reconstituted in 200 µL of mobile phase, vortex-mixed, centrifuged for 15 min at 1600 g and 10°C, and analyzed by LC/MS/MS.

The LC/MS/MS system was comprised of a Leap Technologies HTS-PAL autosampler, two Shimadzu LC-10ADvp pumps, an SCL-10Avp system controller, a Type W 6-port Valco switching valve, and a Sciex API 4000 mass spectrometer. Chromatographic
separation of the analytes was achieved on an Aquasil C18 column (2.1 x 50 mm, 5 μm particle size, ThermoElectron Corp) using isocratic elution with 80% acetonitrile and 20% water containing 10 mM ammonium formate and 0.1% formic acid. Total run time for a single injection was 3.0 min. Mass spectrometric detection of the analytes was accomplished using the Turbo Ionspray interface operated in the positive ionization mode. Analyte response was measured by multiple reaction monitoring (MRM) of selective mass transitions for each compound. The transitions of the protonated precursor ions to the selected product ions were \( m/z \ 638 \rightarrow m/z \ 283 \) for anacetrapib and \( m/z \ 642 \rightarrow m/z \ 287 \) for \([D_3,^{13}C]\)anacetrapib.

Data were acquired and processed using the Sciex Analyst 1.3.1 software. Calibration curves were generated by plotting the peak area ratios of analyte to IS as a function of the nominal concentrations of the standard samples. A line of best fit was generated from the curve points by linear regression with a weighting factor of \( 1/x^2 \) and concentrations of anacetrapib were interpolated from this calibration curve. The lower limit of quantitation (LLOQ) for this assay was 2 ng/mL (0.00314 μM) for anacetrapib.

**Calculation of Pharmacokinetic Parameters**

Pharmacokinetic parameters were calculated by established non-compartmental methods. The area under the plasma concentration versus time curve (AUC) was determined using the Watson® LIMS software (version 6.2.0.02), with linear trapezoidal interpolation in the ascending slope and linear-log linear trapezoidal interpolation in the descending slope. The portion of the AUC from the last measurable concentration to infinity was estimated
from the equation, \( \frac{C_t}{k_{el}} \), where \( C_t \) represents the last measurable concentration and \( k_{el} \) is the elimination rate constant. The latter was determined from the concentration versus time curve by linear regression at the terminal phase of the semi-logarithmic plot.

**In Vitro Reversible Plasma Protein Binding**

\(^{14}\text{C}\)Anacetrapib in ethanol (2.5 µL) was added to rat or monkey plasma (497.5 µL) to achieve final concentrations of 1, 5, and 20 µM. Equilibrium dialysis of plasma samples was performed for 6 h against isotonic phosphate buffer (pH 7.4, 1.8 g/L \( \text{KH}_2\text{PO}_4 \), 7.6 g/L \( \text{Na}_2\text{HPO}_4 \), and 3.9 g/L \( \text{NaCl} \)) at 37°C in 1 mL acrylic dialysis cells (Scienceware, NJ). The plasma and buffer were separated by a membrane (Sigma, MO) that had a molecular weight cutoff of 12.4 kDa. Aliquots (100 µL) of post-dialysis plasma and phosphate buffer were measured for radioactivity by a liquid scintillation counter (Beckman LS6500 or Packard 1900TR). Estimation of the unbound fraction of anacetrapib was based on the ratio of radioactivity in the buffer over that in plasma samples. The limit of quantification using liquid scintillation counting (~2-fold above background radioactivity) in this assay was ~5 nM.

**In Vitro Blood-to-Plasma Partition Ratio**

\(^{14}\text{C}\)Anacetrapib in ethanol (2.5 µL) was added to rat or monkey blood and plasma (497.5 µL each) to achieve final concentrations of 1, 5, and 20 µM. The resulting blood and plasma samples (0.5 mL each) were incubated at 37°C for 30 min. Blood samples
then were subjected to centrifugation at 3,000 rpm (Beckman GS6R) for 10 min to obtain plasma. Aliquots (0.1 mL) of plasma samples were measured for radioactivity by a scintillation counter (Beckman LS6500). The blood-to-plasma partition ratio was estimated by comparison of the radioactivity in plasma prepared from the treated blood samples with that in control plasma.

**Excretion of [14C]Anacetrapib in Bile Duct-Cannulated Animals**

**Rats.** Four bile duct-cannulated male Sprague-Dawley rats (~0.4 kg) were dosed P.O. with 10 mg/kg [14C]anacetrapib via oral bolus gavage. The dose, with a final drug concentration of 5 mg/mL, was formulated in imwitor:tween (1:1, w/w) and contained 85 μCi/mL of radioactivity.

Cumulative bile and urine samples were collected into tared bottles during the time intervals from 0 to 4, 4 to 8, 8 to 24, and 24 to 48 h post-dosing. Feces were collected at 24-h intervals for 2 days. Terminal blood samples (~3 mL) were collected into EDTA-containing tubes at 48 h after P.O. dosing. Plasma was prepared by centrifugation of the blood sample and was stored at -20°C until analysis.

**Monkeys.** Three bile duct-cannulated male rhesus monkeys (9.7-10.7 kg) were orally dosed at 10 mg/kg [14C]anacetrapib via nasogastric intubation and oral gavage, followed by a 3.5 mL saline flush. These oral dose was formulated in imwitor 742:tween (1:1, w/w) at a drug concentration of 10 mg/mL and 16 μCi/mL of radioactivity. Bile was collected into tared bottles during the time intervals from 0 to 4, 4 to 8, 8 to 24, 24 to 48,
48 to 72, and 72 to 96 h following drug administration. Urine was collected in a similar manner from 0 to 8, 8 to 24, 24 to 48, 48 to 72, and 72 to 96 h after dosing. Feces were collected at 24-hr intervals for 4 days. Pre-dose bile, urine and plasma samples also were collected. Blood (2 mL) was collected into heparinized vacutainer tubes at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, 72 and 96 h postdose, and plasma was prepared by centrifugation. All samples were stored at -20°C until analysis.

Sample Processing and Radiometric Analysis. Aliquots of bile or urine from a single animal were pooled proportionally based on excrete volumes over 0-48 h. Bile or urine samples for LC/MS analysis were prepared by mixing equal volumes of the corresponding pooled single animal excreta samples. Aliquots (1 mL) of pooled feces homogenate from each animal over 0-48 h were extracted twice by adding 5 mL of acetonitrile, followed by centrifugation and separation of supernatants which were then evaporated to dryness under N₂. The residues were reconstituted in 0.3 mL of 75% aqueous acetonitrile (v/v) for LC/MS/MS analysis. The recovery of radioactivity was approximately 80%. Plasma was pooled from each animal over 0 to 48 h. Aliquot (1 mL) of the pooled samples were mixed with 5 mL of acetonitrile by vortexing. The mixture was centrifuged, and the resulting supernatants were separated and evaporated to dryness under N₂. The residues were reconstituted in 0.3 mL of 75% aqueous acetonitrile (v/v) for LC/MS/MS analysis. The extraction efficiency of radioactivity from plasma samples was approximately 70%.

Aliquots of bile (50 μL) or urine (100 μL) were mixed with 6 mL of ScintiSafe Gel scintillation cocktail (Fisher Scientific), and the radioactivity was measured by a
Beckman Liquid Scintillation Counter (Model LS6500). Total radioactivity in feces was determined by combustion of fecal homogenates (feces:water ratio of 1:2, w/v) with Packard Oxidizer (Model 307, Downers Grove, IL) and scintillation counting.

**Excretion Mass Balance of $[^{14}C]$Anacetrapib in Monkeys**

Excretion mass balance of $[^{14}C]$anacetrapib in monkeys following a single oral dose was determined at Charles River Laboratories (Preclinical Services, MA). This study was conducted in compliance with the Food and Drug Administration Good Laboratory Practice (GLP) Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations, Part 58 and were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. In addition, this study was inspected by the Charles River Laboratories Quality Assurance Unit to assure conformity with the Good Laboratory Practice (GLP) regulations promulgated by the United States Food and Drug Administration. $[^{14}C]$Anacetrapib in imwitor 742:tween 80 (1:1, w:w) was administered orally by gavage to each animal at a target dose of 5 mg/kg (75 μCi/kg) in a dose volume of 2 mL/kg. Body weights of animals that were used in dose calculations were determined on the day of dosing. The dosing tubes were flushed with 6 mL of water immediately after dosing, removed from the animal, and then retained for extraction of residual radioactivity. The amount of dose formulation administered to each animal was determined from predose and postdose syringe weights. Animals were fasted for approximately 10 to 11 hours prior to dosing. Food was returned to the animals after collection of the 4-hour postdose blood sample. Blood samples (6 mL/time point) were
collected before dosing and at 2, 4, 6, 8, 24, and 48 hours after dosing. Blood samples were collected from an indwelling venous catheter and vascular access port or by venipuncture of a femoral vein into tubes containing dipotassium EDTA and were mixed by inversion of tubes. Urine and feces were collected separately from 0-8 hours and 8-24 hours postdose, and then over 24-hour intervals through 672 hours postdose. Urine was collected into containers, which were surrounded by dry ice, and feces were collected at room temperature. Cage debris (mostly composed of residual food and hair) was collected daily after each postdose excreta collection. After collection of the cage debris, the floor, screen, and pan of each cage were rinsed and the rinse was collected into the cage debris container. After the final collections at 672 hours postdose, the cages were washed thoroughly with 50% (v/v) reagent alcohol, and the wash was collected into a separate container for each cage. The cages were wiped with gauze pads, and the gauze pads were placed into a separate container for each cage. All sample weights were recorded. Blood samples were kept chilled on wet ice before centrifugation to obtain plasma samples. All other samples were stored at -20 ± 5°C, unless processed or analyzed immediately after collection.

Sample Analysis. Triplicate weighed aliquots (approximately 0.2-0.4 g each) of fecal, cage debris and cage wash homogenate were placed into cones and pads, dried for at least 4 hours at ambient temperature, combusted, and then analyzed by LSC. These samples were combusted by Oxidizer Model 307 (PerkinElmer Inc., Boston, Massachusetts). The resulting [14C]CO2 was trapped in 9 mL of Carbo-Sorb E (PerkinElmer Inc.) and mixed with 9 mL of Permafluor E+ (PerkinElmer Inc.) scintillation fluid. The combustion
efficiency was determined for each combustion session. Plasma and urine samples were mixed with 15 mL of Emulsifier-Safe (PerkinElmer Inc.) scintillation fluid. All samples were analyzed for total radioactivity by liquid scintillation counting (LSC) for 2 minutes in a Model LS 6000 or LS 6500 liquid scintillation counter (Beckman Instruments Inc., Fullerton, California). Scintillation counter data were automatically corrected for counting efficiency using an external standardization technique and an instrument-stored quench curve generated from a series of sealed quench standards. A value of zero was assigned to samples where radioactivity (dpm) was less than or equal to twice the background dpm; for other samples background radioactivity was subtracted before calculating concentrations of radioactivity and percentages of the radioactive dose recovered. Amounts of radioactivity in combusted experimental samples were adjusted for the efficiency of the combustion system, which was determined from oxidized and nonoxidized standards counted along with the experimental samples. The anacetrapib-equivalent concentration of radioactivity in a sample was calculated by dividing the concentration of radioactivity in the sample by the specific activity and were expressed as ng equivalents/g. The total radioactivity recovered in a matrix was expressed as a percentage of the actual administered radioactive dose. Statistical analyses were limited to the calculation of the mean and standard deviation, as appropriate.

Qualitative Analysis with LC/MS/MS, Radioflow Detector, and NMR

The LC/MS/MS system consisted of a Shimadzu SIL-10ADvp autosampler, a Shimadzu SCL-10Avp system controller, two Shimadzu LC-10ADvp pumps (Columbia, MD),
Packard Flow Scintillation Analyzer (500TR Series, Meriden, CT), and a ThermoFinnigan LCQ XPluss ion trap mass spectrometer (San Jose, CA). The mass spectrometer was operated with an electrospray interface in either positive or negative ion detection mode, and data were acquired using Xcalibur software (version 1.2). The MS spectra were recorded in a data- and list-dependent mode. A Zorbax RX-C8 HPLC column (4.6 x 250 mm; 4 μm from Waters Corporation, MA) was used for chromatographic separation. The mobile phases A and B were 5% aqueous acetonitrile (v/v) and 95% aqueous acetonitrile (v/v), respectively, both containing 0.1% formic acid and 1 mM ammonium acetate. The gradient program began at 5% B for first 3 min and was increased linearly to 35% B in 2 min, to 42% B in 43 min, to 75% B in 17 min, and finally to 100% B in 5 min, followed by a hold at 100% B for 5 min. The flow rate was 1.5 mL/min, and the eluate was diverted in a 5:1 ratio to the Packard Flow Scintillation Analyzer and the LCQ mass spectrometer, respectively. Scintillation cocktail (Packard Ultima Flo-M) was pumped at a rate of 3.8 mL/min to the radiometric detector.

NMR spectra of anacetrapib and its metabolites were recorded on a Varian Inova 600 MHz NMR spectrometer.
Results

All animals were observed frequently during the course of the studies and appeared to be healthy, with no overt adverse signs.

Pharmacokinetics of Anacetrapib

Rats. The pharmacokinetic parameters of anacetrapib in rats following IV and P.O. administration are presented in Tables 1 and 2, and the mean plasma concentration versus time curves are shown in Figure 2. Following an IV dose of 0.5 mg/kg, the mean values for systemic plasma clearance, steady-state volume of distribution and terminal half-life were 2.3 mL/min/kg, 1.1 L/kg, and 12 h, respectively. Following P.O. dosing at 5 mg/kg, the bioavailability of anacetrapib was 38%. Exposures (area under the plasma concentration-time curve, AUC) increased in a less than dose-proportional manner from 23 μM·h at 5 mg/kg to 362 μM·h at 500 mg/kg. In this dose range, the peak plasma level (C_max) ranged from 5 to 26 µM, and the time to reach peak plasma level (T_max) ranged from 3 to 4.5 h.

Monkeys. A summary of the pharmacokinetic parameters of anacetrapib in monkeys following an IV dose of 0.1 mg/kg and oral doses of 1, 30 and 500 mg/kg is presented in Tables 1 and 2, and the mean plasma concentration versus time curves are shown in Figure 3. The mean values for systemic plasma clearance, steady state volume of distribution, and half-life were 0.11 mL/min/kg, 0.31 L/kg, and 34 hr, respectively. Following P.O. dosing at 1 mg/kg, the bioavailability of anacetrapib was 11%. Similar to
rats, the mean plasma AUC values increased in a less than dose-proportional manner from 26 µM·hr at 1 mg/kg to 1500 µM·hr at 500 mg/kg. The T<sub>max</sub> values ranged from 4 to 5 h, and the C<sub>max</sub> values ranged from 2 to 205 µM at these doses.

*In Vitro* Reversible Plasma Protein Binding and Blood-to-Plasma Partition Ratio

The unbound fraction of anacetrapib in rat and monkey plasma was less than 0.5% over a concentration range of 1 to 20 µM. The average blood-to-plasma partition ratios of anacetrapib over the same concentration range were 0.6 in the rat and 0.7 in the monkey.

**Excretion of [14C]anacetrapib in bile duct-cannulated animals**

*Rats.* The excretion of [14C]anacetrapib-associated radioactivity was examined in bile duct-cannulated male Sprague-Dawley rats, and the data are summarized in Table 3. Following oral dosing of [14C]anacetrapib at 10 mg/kg, ~96% of the administered radioactive dose was recovered over a period of 48 h, out of which 15% was recovered in bile, 1% in urine and 80% in feces. The intact parent compound excreted in bile accounted for ~1% of the dose, but it was not detected in urine. Based on a high recovery of the intact parent compound (63% of dose) in feces which likely represents unabsorbed drug, the extent of oral absorption of [14C]anacetrapib appears to be low-to-moderate at best.

*Monkeys.* Following oral dosing of [14C]anacetrapib to bile duct-cannulated male monkeys at 10 mg/kg, nearly 100% of the dose was recovered over a period of 96 h. Similar to rats, 13% of the dose was recovered in bile, 1% in urine and a much larger proportion, 90%, in feces. A large amount of the intact parent compound (74% of
radioactivity) was recovered in feces, suggesting that, similar to rats, the extent of [14C]anacetrapib oral absorption was low in monkeys as well.

**Excretion Mass Balance of [14C]Anacetrapib in Monkeys**

In the excretion mass balance study in monkeys, the excretion of [14C]anacetrapib-associated radioactivity was monitored for a total 28 days. Total recoveries of radioactivity for all matrices are summarized in Table 4. Fecal excretion was the major route of elimination of drug-related radioactivity. Mean recoveries of the radioactive dose through 672 hours postdose were 75% in feces and 1.2% in urine. The majority of the fecal radioactivity was excreted within 48 hours after dosing, but elimination of radioactivity in feces continued through 672 hours postdose. Cage residues contained an average total of 3.6% of the dosed radioactivity. The mean total recovery of the radioactive dose from all matrices collected through 672 hours postdose was 79.6%.

**In Vivo Metabolite Profiles**

*Rats.* Representative HPLC chromatograms for metabolites of [14C]anacetrapib in rat plasma, bile and feces are shown in Figure 4. The contributions of various biliary and fecal metabolites to the overall elimination of anacetrapib dose are shown in Table 5. In rat urine, only 1-2% of the administered radioactive dose was recovered and the radioactivity levels were too low for meaningful analysis.

*Rat plasma.* The profile from rat plasma samples pooled over 48 h post-dose showed four radioactive peaks that were identified as anacetrapib and the oxidative metabolites M1, M2 and M3. The parent drug was the major radioactive component, accounting for
~38% of the total radioactivity. The metabolites M1, M2 and M3 accounted for ~17%, ~14% and ~31% of the radioactivity, respectively.

**Rat feces.** The metabolite profile from pooled rat feces was similar to that from rat plasma in that parent anacetrapib, M1, M2 and M3 were detected. In feces, parent drug accounted for 63% of the total radioactive dose. M1, M2 and M3 accounted for 4.8%, 2.7% and 5.6% of the dose, respectively.

**Rat bile.** Approximately 81% of the radioactivity excreted in bile could be identified. As shown in the representative HPLC radiochromatogram (Figure 4), anacetrapib was extensively metabolized in the rat, giving rise to at least nine metabolites in rat bile. The intact parent compound excreted over 48 h accounted 4.7% of the radioactivity excreted in bile which, in turn, corresponds to 0.7% of the total administered radioactive dose. All identified metabolites represented ~11% of the dose. As was shown by LC/MS/MS analysis (see also section "Metabolite Identification"), biliary metabolites resulted from O-demethylation on the biphenyl moiety (M1) and hydroxylation on the isopropyl side chain (M3), and subsequent glucuronidation on the aromatic and aliphatic hydroxyl groups (M4, M5, M6, M7, M8). In addition, a glutathione adduct (M9) and an olefinic metabolite (M10) were detected. The three most abundant metabolites in rat bile were M4, M5 and M7 and these accounted for 1.7%, 3.5% and 3.3% of the radioactive dose, respectively. The quantities of all other metabolites were below 1% of the dose in each case.

**Monkeys.** Representative HPLC chromatograms for metabolites of [14C]anacetrapib in monkey plasma, bile and feces are shown in Figure 5. The mean percentages of biliary
and fecal metabolites relative to the administered dose are shown in Table 5. Similar to rats, only 1–2% of the administered radioactive dose was recovered in monkey urine and radioactivity concentrations in urine were too low for meaningful analysis.

**Monkey plasma.** Based on radiometric data, intact parent drug was the only circulating entity in monkey plasma. The presence of trace levels of metabolites, similar to those seen in rat plasma (M1, M2, M3), cannot be completely ruled out; limitations in sample volume and the sensitivity of the radiometric assay did not allow us to obtain more conclusive metabolite data.

**Monkey feces.** Parent anacetrapib was the major radioactive component in monkey feces, accounting for 74% of the administered dose. Radiochromatographic analysis of the feces (Figure 5) also showed minor amounts of M1, M2 and M3 which accounted for 2.8%, 2.7% and 5.0% of the radioactive dose, respectively.

**Monkey bile.** In monkey bile, approximately 80% of the total excreted radioactivity could be identified. Unchanged parent drug was not detected, suggesting that absorbed anacetrapib likely underwent complete metabolic turnover in monkey liver before excretion. The vast majority of the radioactivity excreted in monkey bile could be attributed to metabolites resulting from oxidative metabolism (O-demethylation, aromatic and aliphatic hydroxylation) and subsequent conjugation with glucuronic acid. One metabolite (M11) resulted from oxidation of the isopropyl side chain to a propionic acid derivative. Out of the nine metabolites identified, the most abundant were the glucuronic acid conjugates, M4 and M12, which accounted for 1.1% and 2.8% of the administered radioactive dose, respectively. The other glucuronic acid conjugates (M5, M6, M7, M8,
M13), as well as the propionic acid metabolite M11, each accounted for less than 1% of the dose.

**Mass Spectral Fragmentation of Anacetrapib**

Full-scan MS of anacetrapib showed a molecular ion at \( m/z \) 638 \([M + H]^+\). As shown in Figure 6A, the CID product ion spectrum of \( m/z \) 638 produced characteristic fragment ions at \( m/z \) 325, 314 and 283. The \( m/z \) values of fragment ions indicated that fragmentation of the parent molecule occurs through cleavage of the bond between the nitrogen of the oxazolidinone and the methylene carbon linkage to the biphenyl moiety, generating fragment ions at \( m/z \) 325 and \( m/z \) 314. High-resolution MS analysis of the most abundant fragment at \( m/z \) 283 (data not shown) indicated an elemental formula of \([C_{15}H_{11}F_4O]^+\), suggesting that it was derived from the 4'-fluoro-5'-isopropyl-2'-methoxy-4-(trifluoromethyl)biphenyl moiety of the molecule.

**Identification of Metabolites**

To determine structures of metabolites, LC/MS/MS with radiometric detection was employed. Where possible, metabolites were isolated and purified by means of semi-preparative HPLC for structure elucidation by \(^1\)H NMR. The CID fragmentation and \(^1\)H-NMR spectra for anacetrapib are presented in Figure 6. Similarly, fragmentation spectra for M1, M2 and M3 (the three metabolites that circulate in rat and human plasma) are presented in Figure 7 for illustrative purposes. The mass spectral fragmentation and \(^1\)H-NMR data on all of the remaining metabolites are summarized in Tables 6 and 7, respectively. In addition, chemical structures of a number of metabolites (M1, M2, M3,
M4, M6, M8, M10, M12) were confirmed by comparing their chromatographic retention times and mass spectral fragmentation to those of the corresponding synthetic standards. A proposed scheme of metabolic pathways of anacetrapib in rats and monkeys is presented in Figure 8.

Metabolite M1. In negative ion mode, M1 gave a molecular ion at \( m/z \) 622, corresponding to a loss of 16 mass units relative to parent anacetrapib in positive ion mode suggesting that this was the O-demethylated derivative. The CID spectrum of \( m/z \) 622 showed two major fragments at \( m/z \) 578 and 312 (Figure 7A) which were presumably formed via the loss of elements of CO\(_2\) from the oxazolidinone ring and by cleavage between the oxazolidinone ring and the methylene linkage to the biphenyl, respectively.

Metabolites M2 and M3. Both M2 and M3 gave molecular ions at \( m/z \) 638 in negative ion mode indicating a net addition of 2 Da to anacetrapib; this presumably arose from concurrent O-demethylation and mono-hydroxylation. Comparison of the CID product ion spectra of the two metabolites showed that M3 generated a fragment ion at \( m/z \) 620 due to loss of water, which was not the case for M2 (Figures 7B and 7C). From this finding and from additional fragment ions at \( m/z \) 325 (M2) and \( m/z \) 312 (M3), it was concluded that for M2, hydroxylation had occurred on the biphenyl moiety, whereas M3 likely incorporated a hydroxyl group on the isopropyl side chain. The proposed structures were further confirmed through comparing the chromatographic retention times and mass spectral fragmentation of synthetic standards of the putative metabolites with those of M2 and M3.
Metabolite M4. CID mass spectral analysis of M4 (molecular ion at $m/z$ 798 in negative mode) indicated loss of the glucuronic acid, giving rise to a fragment ion at $m/z$ 622 corresponding to the O-desmethylated analog M1 (Table 6). The NMR spectra confirmed M4 to be the glucuronic acid conjugate of the O-desmethyl analog, as the methoxy signal (near 3.7 ppm in the parent spectrum) was noticeably absent (Table 7). Subsequent glucuronidation on the phenolic hydroxyl was also confirmed by the presence of the glucuronic acid signals. The chemical shift of the anomeric proton (near 5.0 ppm) is consistent with a phenolic $O$-glucuronide structure (Table 7).

Metabolite M5. M5 was the most abundant metabolite in rat bile, and was also present in large quantities in monkey bile. M5 (molecular ion at $m/z$ 814 in negative ion mode) exhibited an addition of +16 amu relative to M4. In the CID spectrum, a fragment ion at $m/z$ 638 indicated loss of the glucuronic acid moiety (Table 6). The MS$^3$ CID fragment spectrum of $m/z$ 638 (not shown) did not demonstrate loss of a water molecule and was in fact identical to the fragmentation spectrum of M2. These data suggest that the additional hydroxylation in M5 had occurred on the aromatic moiety and that M2 likely represented the aglycone portion of the M5 structure; the exact site of the glucuronic acid conjugation at either of the two hydroxy moieties of the catechol was, however, was not identified.

Metabolite M6. Mass spectral analysis of M6 (molecular ion at $m/z$ 828 in negative mode) indicated a change in molecular weight of 192 amu (+16+176 amu) relative to the parent. In the CID spectrum, the loss of a glucuronic acid gave a fragment ion at $m/z$ 652.
The presence of the methoxy group was confirmed by the peak near 3.7 ppm in the NMR spectrum (Table 7). Hydroxylation at one of the isopropyl methyls was readily apparent from the loss of one methyl signal (near 1.1 ppm in the parent spectrum) and the appearance of a new set of signals consistent with a CH$_2$OH moiety (near 3.6 and 4.0 ppm). Glucuronidation was confirmed by the presence of the glucuronic acid signals. The location of the glucuronic acid moiety was determined to be on the newly introduced hydroxyl group, as the chemical shift of the anomeric proton (near 4.2 ppm) is consistent with an aliphatic O-glucuronide structure.

**Metabolite M7.** M7 (molecular ion at $m/z$ 830 in negative mode) demonstrated the addition of +32 amu relative to M4. In the CID spectrum, the loss of a glucuronic acid moiety gave a fragment ion at $m/z$ 654 (Table 6). As with M6, hydroxylation at one of the isopropyl carbons was evident by the appearance of –CH$_2$OH proton signals in the $^1$H-NMR spectrum (Table 7). In addition, hydroxylation of the fluorinated aromatic ring was apparent from the loss of one of the corresponding proton signals. Based on a comparison of the measured $^1$H-$^19$F coupling constant (7.6 Hz) to those in the parent spectrum ($^4J_{HF} = 8.8$ Hz vs. $^3J_{HF} = 12.5$ Hz), the peak near 6.8 ppm was assigned as the proton *meta* to the fluorine, indicating hydroxylation had occurred at the carbon in between the fluorine and the existing hydroxyl (Table 7). The slight upfield shift of this proton signal from the parent spectrum (near 7.1 ppm) is consistent with the addition of a hydroxyl group at its *para* position. Glucuronidation was confirmed by the presence of the glucuronic acid signals, but the location of the glucuronide could not be precisely determined. The aliphatic hydroxyl can be excluded because the chemical shift of the
anomeric proton (near 4.8 ppm) is too far downfield to be an aliphatic glucuronide (cf. 4.2 ppm for M6); thus, the glucuronic acid moiety is likely attached to one of the aromatic hydroxyls.

**Metabolite M8.** In the HPLC radiochromatogram (Figure 5), M8 eluted in two separate radioactive peaks, M8A and M8B, with a 2-minute difference in retention times. The Q1 full scan and CID fragment ion spectra of the two peaks were found to be identical. M8A and M8B demonstrated the addition of +32 amu relative to M4, and a molecular ion at \( m/z \) 830 was seen in negative ion mode. The CID spectra for both analytes showed fragment ions at \( m/z \) 654 (loss of glucuronic acid) and \( m/z \) 636 (loss of glucuronic acid and water) (Table 6). The NMR spectra of both metabolites indicated that hydroxylation had occurred at one of the isopropyl methyls and at the benzylic carbon for the same reasons outlined in the description of metabolites M6 and M12, respectively (Table 7). The similarity observed for all the physical data suggests M8A and M8B were diastereomers, differing only in the stereochemistry of the benzylic carbon.

**Metabolite M9.** M9 was found only in rat bile. The mass spectrum of M9 showed a molecular ion at \( m/z \) 945 in positive mode, reflecting a net addition of 307 amu to parent anacetrapib which can be rationalized as demethylation (-14Da), hydroxylation (+16 Da) and addition of glutathione (+305 Da). The CID spectrum displayed fragment ions with characteristic masses of 816 and 638 (Table 6), indicating the loss of pyroglutamate (129 amu) and glutathione (307 amu); the loss of the entire glutathionyl moiety under CID indicates that it is likely attached to an aliphatic carbon. The most likely structure of M9
shown in Table 6 is consistent with the above fragmentation spectrum and can be rationalized by the addition of glutathione to a putative electrophilic \( p \)-quinone methide intermediate.

**Metabolite M10.** M10 was also found in trace amounts only in rat bile. The molecular ion \((m/z 796\) in negative ion mode) in the full scan showed a mass difference of -2 amu relative to the mass of M4, the glucuronic acid conjugate of the \( O \)-demethyl metabolite M1, indicating either dehydrogenation on an aliphatic moiety (isopropyl side chain), or loss of water from an aliphatic hydroxyl group through in-source CID. The NMR spectra of M10 contained no signals indicative of hydroxylation. Instead, one of the isopropyl methyl signals had shifted from 1.2 ppm to 2.1 ppm, and the other signal was missing entirely. A new signal corresponding to two protons appeared near 5.2 ppm. Such signals are consistent with the conversion of the isopropyl group to a propene moiety (Table 7).

**Metabolite M11.** The metabolites M11, M12 and M13 were found only in monkey bile. The MS full scan in negative ion mode detected a molecular ion at \( m/z 666\), corresponding to the net addition 30 amu to the mass of the parent drug. The fragment ions in the CID spectrum, with characteristic loss of 44 amu (corresponding to elements of \( CO_2 \)), suggested the presence of a carboxylic acid group on the isopropyl side chain of the biphenyl moiety (Table 6).

**Metabolite M12.** M12 was found to be the most abundant metabolite in monkey bile, accounting for approximately 3% of the administered dose. M12 (molecular ion at \( m/z \)
814) exhibited the addition of +16 amu relative to M4. In the CID spectrum, fragment ions at m/z 638 and 620 indicated sequential losses of the glucuronic acid and water. Furthermore, the loss of the isopropyl methine proton signal (seen in the parent ¹H NMR spectra near 3.2 ppm) indicated the formation of the tertiary alcohol from hydroxylation at the benzylic carbon (Table 7). Chemical shift of the anomeric proton of the glucuronic acid moiety indicates conjugation on the biphenyl ring.

**Metabolite M13.** The molecular ion signal at m/z 846 in negative ion mode indicated a mass change of +48+176 relative to the O-demethylated metabolite M1. The fragment ions in the CID spectrum suggested that three hydroxylations and a conjugation with glucuronic acid had occurred on the biphenyl moiety of the molecule (Table 6).
Discussion

The objective of this study was to examine the pharmacokinetics and disposition of anacetrapib in rats and monkeys, the two species that are being used for the preclinical toxicological evaluation of this drug candidate.

The pharmacokinetics of anacetrapib were characterized by a low systemic plasma clearance (2.3 and 0.1 mL/min/kg in the rat and monkey, respectively) and a long half-life ranging from 12 h to 34 h. The oral bioavailability of anacetrapib was low to moderate (13% in monkeys, 38% in rats) and the increase in systemic exposure was less than dose-proportional in both species; this was likely related to low aqueous solubility of the drug that leads to dissolution limited absorption after oral administration.

In excretion studies using bile duct-cannulated rats and monkeys, it was shown that a almost complete recovery of drug-related radioactivity was achieved over a time period of 48 h in rats or 96 h in monkeys after an oral dose, with the majority of the radioactive dose excreted in feces. An additional study monitoring the excretion of radioactivity over 28 days in rhesus monkeys led to the same conclusion, although in this latter study the total radioactivity recovery was slightly lower (~80%) than that obtained in bile duct-cannulated monkeys (~100%). The reason for the somewhat lower apparent excretion recovery in intact monekys relative to bile-duct cannulated animals is not clear.

The in vivo metabolic profile of anacetrapib appeared to be consistent across species. In both the rat and the monkey, oxidative metabolism was predominant and involved O-
demethylation and multiple hydroxylations on the biphenyl moiety; in contrast, the oxazolidinone and the 3,5-bis(trifluoromethyl)phenyl moieties of the molecule were metabolically stable. The major metabolites in rat bile were products of $O$-demethylation (M1) and aromatic hydroxylation (M2), which subsequently underwent glucuronidation on the catechol hydroxyl groups (M5, M7) before excretion into bile. In monkey bile, the most abundant metabolite (M12) was a product of $O$-demethylation followed by glucuronidation on the resulting phenolic hydroxyl group and concomitant hydroxylation on the isopropyl side chain. Furthermore, the metabolites M1, M2 and M3 were found to circulate in rat plasma at significant levels relative to the parent. Their presence in monkey plasma seems likely, but could not be conclusively proven. The fact that these three metabolites also appeared in the feces of bile duct-cannulated rats and monkeys would suggest that they were either excreted into the gut lumen by mechanisms other than biliary excretion (e.g., intestinal excretion) or formed by the metabolic activity of intestinal bacteria.

Additional (minor) metabolic pathways present included dehydrogenation of the isopropyl side chain to yield an olefin metabolite (detected as glucuronic acid conjugate M10 in rats), or its oxidation to a propionic acid derivative (M11, in monkeys). Furthermore, trace amounts of a glutathione adduct (M9) were detected in the rat which is likely formed via capture of a quinone methide intermediate at the biphenyl moiety by glutathione. The structures of the majority of metabolites identified in rats and monkeys indicate that the $O$-demethylation of anacetrapib to form M1 is the core first step in the biotransformation of this drug in both species and several of the other metabolites
represent secondary and tertiary products of further oxidation of M1 and their conjugation with glucuronic acid.

As discussed in the companion manuscript on the metabolism of anacetrapib in humans (Kumar et al., 2009), the only metabolites detected in biological matrices (plasma and feces) from healthy human volunteers given a single 150 mg oral dose of \[^{14}\text{C}]\)anacetrapib were M1, M2 and M3. All of these metabolites were present at much lower levels compared to the parent in human plasma (<14% of total plasma radioactivity). Furthermore, similar to animals, majority of the administered \[^{14}\text{C}]\)anacetrapib-associated radioactivity (91%) was excreted in feces in humans as the intact parent drug which likely reflects unabsorbed drug. Metabolites (M1, M2 and M3) were the only other drug-related entities detected in feces, with each accounting for <5% of the administered dose. Given that M1, M2 and M3 were formed in both rats and monkeys, and were found to circulate in rat plasma at levels in excess of what is observed in humans, we conclude that these metabolites have been adequately qualified in the preclinical safety studies of anacetrapib (Guidance for Industry on Safety Testing of Drug Metabolites, U.S. Department of Health and Human Services, Center for Drug Evaluation and Research, Food and Drug Administration, Feb 2008; http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065014.htm).

Data on animal and human disposition and metabolism of torcetrapib (Pfizer), another CETP inhibitor which progressed into late clinical development before it was
discontinued as a result of unfavorable cardiovascular outcomes, have also recently been published (Dalvie et al., 2008; Prakash et al., 2008). A comparison of the ADME profile for anacetrapib and torcetrapib reveals significant differences between the disposition and metabolism of these two CETP inhibitors. The two drugs appear strikingly different in their routes of elimination and their metabolic profiles. The findings of this study indicate that anacetrapib is primarily excreted in feces as unchanged drug in both rats and monkeys, with minimal excretion of the radioactive dose in urine. By comparison, the elimination of torcetrapib-associated radioactivity is more balanced in both rats and monkeys, with both urinary and fecal excretion of metabolites playing an important role in elimination of drug-related material (Prakash et al., 2008). This difference in routes of excretion of drug-related material between the two agents appears related to differences in their metabolic pathways. The metabolism of anacetrapib involves oxidative O-demethylation and hydroxylation at multiple sites on the biphenyl and isopropyl moieties as shown in Figure 8; these oxidative metabolites are then conjugated with glucuronic acid before excretion into bile/feces. Furthermore, the plasma radioactivity profiles following [14C]anacetrapib administration in both rats and monkeys are dominated by the parent drug; smaller amounts of three oxidative metabolites M1, M2 and M3 circulate in rat plasma whereas metabolites do not appear to account for significant amounts of drug-related material in monkey plasma. Torcetrapib exhibits a complex metabolic fate with as many as 28 primary and secondary metabolites identified in preclinical species (Prakash et al., 2008). A key feature of the metabolism of torcetrapib appears to be related to oxidative cleavage in its core structure between the bistrifluoromethybenzyl and dihydroquinoline moieties which results in the formation of low molecular weight
metabolites including various further oxidized and/or conjugated derivatives of 2-methyl-6-trifluomethyl quinoline and bistrifluoromethylbenzoic acid from the two halves of the parent compound. These low molecular weight metabolites are more suited to excretion via the renal pathway and appear to account for the observed differences in routes of excretion of drug-related material between torcetrapib and anacetrapib. In contrast, the overall structure of anacetrapib appears to stay largely intact following metabolism and the metabolites have a relatively high molecular weight (>600 Da) which is more suited for excretion via the biliary/fecal pathway. Furthermore, in contrast to anacetrapib, the plasma radioactivity profile following [14C]torcetrapib administration is dominated by metabolites in both rats and monkeys. In particular, plasma exposure to low molecular weight metabolites of torcetrapib including 2-methyl-6-trofluormethyl quinole and bistrifluoromethylbenzoic acid and their secondary oxidized and/or conjugated derivatives is much greater relative to the parent drug (Prakash et al., 2008). The relevance of these abundant low molecular weight circulating metabolites of torcetrapib to its toxicological profile remains to be determined.

In conclusion, pharmacokinetics and metabolism of [14C]anacetrapib were investigated in rats and monkeys. The majority of the administered radioactivity was excreted via the fecal route and was comprised largely of the unabsorbed parent drug, along with small amounts of oxidative metabolites. The metabolic pathways identified included O-demethylation to M1 which was likely further hydroxylated at the biphenyl and isopropyl moieties to form a number of other oxidative metabolites; many of these metabolites were conjugated with glucuronic acid before excretion into bile. Collective data on the
disposition of anacetrapib in rats and monkeys indicate that anacetrapib likely exhibits a low-to-moderate degree of oral absorption across both species and a major pathway of elimination of the absorbed drug is through oxidative metabolism followed by excretion of metabolites (and their conjugates) via the biliary/fecal route.
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Footnotes

1 Current affiliation: Department of Clinical Pharmacokinetics, Novartis, East Hanover, NJ.

2 Current affiliation: Lilly Research Laboratories, Indianapolis, IN 46285

3 Current affiliation: Drug Metabolism and Pharmacokinetics, Vertex Pharmaceuticals, Cambridge, MA 02139
Legends for Figures

Figure 1. Structure of [14C]anacetrapib (MK-0859). * denotes the position of the 14C label.

Figure 2. Mean plasma concentration-time curves of anacetrapib in rats following intravenous and oral doses. Anacetrapib was administered intravenously in PEG300:water (7:3, v/v) or orally in Imwitor:Tween (1:1, w/w) to overnight-fasted male Sprague-Dawley rats. Plasma concentrations of anacetrapib were determined by LC/MS/MS assay, and mean ± standard deviation for n = 3 to 4 animals are shown.

Figure 3. Mean plasma concentration-time curves of anacetrapib in monkeys following intravenous and oral doses. Anacetrapib was administered intravenously in PEG300:water (7:3, v/v) or orally in Imwitor:Tween (1:1, w/w) to overnight-fasted male Rhesus monkeys. Plasma concentrations of anacetrapib were determined by LC/MS/MS assay, and mean ± standard deviation for n = 3 animals are shown.

Figure 4. HPLC-radiochromatographic profiles of anacetrapib and metabolites in 48 hr pooled samples of plasma, bile and feces of rats after a 10 mg/kg oral dose of [14C]anacetrapib.

Figure 5. HPLC-radiochromatographic profiles of anacetrapib and metabolites in plasma, bile and feces of monkeys after a 10 mg/kg oral dose of [14C]anacetrapib.
**Figure 6.** CID product ion spectrum (A) and $^1$H NMR spectrum (B) of anacetrapib.

**Figure 7.** CID product ion spectrum of M1 (A), M2 (B) and M3 (C).

**Figure 8.** Proposed metabolic pathways of anacetrapib in rats and monkeys. * denotes the position of the $^{14}$C radiolabel. "Glu" and "SG" indicate glucuronic acid and glutathione, respectively. For sake of simplicity, only one pathway out of the several possible for the formation of M7, M8, M10, M12, M13 is depicted.
Table 1. Pharmacokinetic parameters\(^a\) for anacetrapib in rats and monkeys after intravenous administration.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC(^b)(_{(0-\infty)}) (µM·h)</th>
<th>CL (mL/min/kg)</th>
<th>V(_{dss}) (L/kg)</th>
<th>t(_{1/2}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.5</td>
<td>5.9 ± 1.5</td>
<td>2.3 ± 0.6</td>
<td>1.1 ± 0.6</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.1</td>
<td>24 ± 6.7</td>
<td>0.1 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>34 ± 5</td>
</tr>
</tbody>
</table>

\(^a\) CL, plasma clearance; V\(_{dss}\), volume of distribution at steady-state; t\(_{1/2}\), terminal phase half-life.

The IV doses were formulated in PEG300:water (70:30, v/v).

\(^b\) Percentage AUC extrapolated for rats and monkeys were 3 and 37%, respectively.
Table 2. Pharmacokinetic parameters $^a$ for anacetrapib in rats and monkeys after oral administration.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC$^b_{(0-\infty)}$ (µM.h)</th>
<th>C$_{max}$ (µM)</th>
<th>T$_{max}$ (h)</th>
<th>F$_{oral}^c$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>5</td>
<td>$23 \pm 2$</td>
<td>$5 \pm 1$</td>
<td>$3 \pm 1$</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>$82 \pm 29$</td>
<td>$11 \pm 2$</td>
<td>$3 \pm 1$</td>
<td>ND$^d$</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>$183 \pm 38$</td>
<td>$26 \pm 4$</td>
<td>$4 \pm 2$</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>$362 \pm 134$</td>
<td>$21 \pm 3$</td>
<td>$5 \pm 3$</td>
<td>ND</td>
</tr>
<tr>
<td>Monkey</td>
<td>1</td>
<td>$26 \pm 3$</td>
<td>$2 \pm 1$</td>
<td>$5 \pm 1$</td>
<td>11</td>
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<tr>
<td></td>
<td>30</td>
<td>$365 \pm 104$</td>
<td>$42 \pm 22$</td>
<td>$4 \pm 0$</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>$1500 \pm 581$</td>
<td>$205 \pm 151$</td>
<td>$5 \pm 1$</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$ C$_{max}$, observed peak plasma concentration following oral dosing; T$_{max}$, time to reach the C$_{max}$; F, bioavailability. The formulation was Imwitor 742:Tween 80 (1:1, w/w).

$^b$ Percentage AUC extrapolated ranged from 0.4 to 22%.

$^c$ Bioavailability for rats and monkeys was determined by a relative comparison of the mean AUC values associated with the IV and the lowest PO doses.

$^d$ ND, not determined.
Table 3. Excretion of [¹⁴C] radioactivity in bile duct-cannulated rats (n = 4) and monkeys (n = 3) following oral administration of 10 mg/kg [¹⁴C]anacetrapib. (nd = not determined)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Rat</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile</td>
<td>Urine</td>
</tr>
<tr>
<td>0 to 24</td>
<td>10 ± 2.6</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>24 to 48</td>
<td>4.3 ± 2.3</td>
<td>0.1 ± 0</td>
</tr>
<tr>
<td>48 to 96</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Total</td>
<td>15 ± 2.8</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>
Table 4. Recovery of radioactivity through 672 hours following 5 mg/kg and 75 µCi/kg oral dose of [14C]anacetrapib to male Rhesus monkeys (n=4).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Time (h)</th>
<th>Recovery of Radioactivity (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>0-672</td>
<td>1.2</td>
</tr>
<tr>
<td>Feces</td>
<td>0-672</td>
<td>74.7</td>
</tr>
<tr>
<td>Cage Debris</td>
<td>0-672</td>
<td>3.4</td>
</tr>
<tr>
<td>Cage Wash</td>
<td>672</td>
<td>0.1</td>
</tr>
<tr>
<td>Cage Wipe</td>
<td>672</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>0-672</td>
<td>79.6</td>
</tr>
</tbody>
</table>
**Table 5.** Relative abundance of biliary and fecal metabolites of anacetrapib in rat and monkey after oral administration of 10 mg/kg $[^{14}C]$anacetrapib.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Percentage of Radioactivity Dose (%)</th>
<th>Rat</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bile</td>
<td>Feces</td>
</tr>
<tr>
<td>Anacetrapib</td>
<td></td>
<td>0.7</td>
<td>63</td>
</tr>
<tr>
<td>M1</td>
<td></td>
<td>0.2</td>
<td>4.8</td>
</tr>
<tr>
<td>M2</td>
<td></td>
<td>nd $^b$</td>
<td>2.7</td>
</tr>
<tr>
<td>M3</td>
<td></td>
<td>0.7</td>
<td>5.6</td>
</tr>
<tr>
<td>M4</td>
<td></td>
<td>1.7</td>
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<td>M5</td>
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<td>3.5</td>
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</tr>
<tr>
<td>M6</td>
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</tr>
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<td>M7</td>
<td></td>
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</tr>
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</tr>
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<td>0.2</td>
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</tr>
<tr>
<td>M11</td>
<td></td>
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</tr>
<tr>
<td>M13</td>
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</tbody>
</table>

$^a$ Calculation was based on dose recovery during the first 48 h period. Approximately 1-2% of the dose was detected in urine, and the radioactivity in urine was too low for meaningful analysis.

$^b$ not detected.
Table 6. Summary of key mass-spectral data for anacetrapib and its metabolites in the rat and monkey. "Glu" represents "glucuronic acid".

<table>
<thead>
<tr>
<th>Parent or Metabolite</th>
<th>Chemical Structure and Proposed Fragmentation Scheme</th>
<th>Molecular ion [M-H]- (m/z)</th>
<th>Molecular ion [M+H]+ (m/z)</th>
<th>Characteristic CID Fragment Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>-</td>
<td>638</td>
<td>325, 314, 283</td>
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<tr>
<td>M1</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>622</td>
<td>-</td>
<td>578, 312</td>
</tr>
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<td>-</td>
<td>594, 325</td>
</tr>
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<td>-</td>
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<td>-</td>
<td>622</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
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<td></td>
</tr>
<tr>
<td><strong>M5</strong></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>814</td>
<td>-</td>
<td></td>
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<tr>
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<td></td>
<td></td>
<td>638, 594, 325</td>
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<tr>
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<td>828</td>
<td>-</td>
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<tr>
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<td></td>
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<td>652</td>
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<td>830</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td><strong>M8</strong></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>830</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td>654, 636</td>
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<tr>
<td><strong>M9(^a)</strong></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>-</td>
<td>945</td>
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<tr>
<td></td>
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<td></td>
<td>816, 798, 638, 308</td>
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</tr>
<tr>
<td><strong>M10</strong></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>796</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>620</td>
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</table>
The regiochemistry of hydroxylation and glutathione addition shown is tentative and is based only on mass spectral fragmentation and on the collective knowledge of the overall metabolic routes of anacetrapib.
Table 7. Proton NMR chemical shifts of Anacetrapib and metabolites $^{a,b}$.

<table>
<thead>
<tr>
<th>Position</th>
<th>Parent</th>
<th>M4</th>
<th>M6</th>
<th>M7</th>
<th>M8</th>
<th>M10</th>
<th>M12</th>
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<td>7.661</td>
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<td>7.036</td>
<td>6.831</td>
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<td>7.059</td>
<td>7.104</td>
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<td>e*</td>
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<td>7.093</td>
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<td>7.934</td>
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<td>5.046</td>
<td>5.040</td>
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</tr>
</tbody>
</table>

$^a$ Chemical shifts are in ppm, measured at 600 MHz in CD$_3$CN:D$_2$O, 10:1.

$^b$ Rotamers (atropisomers) are indicated by asterisks (*) whenever resolved.

Nonequivalent CH$_2$ protons are indicated by primes (').
Figure 1
Figure 2

Plasma Concentration (µM) vs. Time (h)

- IV 0.5mpk
- PO 5mpk
- PO 50mpk
- PO 100mpk
- PO 500mpk

[Graph showing the plasma concentration over time for different dose forms of the drug]
Figure 3

[Graph showing plasma concentration over time for different dosing methods: IV 0.1mpk, PO 1mpk, PO 30mpk, PO 500mpk.]

Time (h)

Plasma Concentration (µM)

- IV 0.1mpk
- PO 1mpk
- PO 30mpk
- PO 500mpk
Figure 5

Radiocactivity (dpm)

Plasma

Anacostatib

Bile

Time (min)

Feces

M1 M2 M3 M4 M5 M6 M7 M8 M11 M12 M13
Figure 6A

MH\(^+\) m/z 638
Figure 7A

A)
Figure 7B

B)

- $M-H^- \text{ m/z } 638$
- Chemical structure with peaks at:
  - 281.0
  - 282.0
  - 305.1
  - 312.0
  - 325.1
  - 326.1
  - 594.1
  - 595.2

Relative Abundance

m/z

0 20 40 60 80 100

200 250 300 350 400 450 500 550 600
Figure 7C
Figure 8

M11 → Anacetrapib → M6

M9 → M4

M1 → M2 → M4

M5 → M1 → M2

3 O + Glu → M10

M7 → M10

M8 → M12