ABSTRACT:

The disposition of torcetrapib \((-\text{[2R,4S]}\)-4-[(3,5-bis-trifluoromethylbenzyl)-methoxycarbonyl-amino]-2-ethyl-6-trifluoromethyl-3,4-dihydro-2H-quinoline-1-carboxylic acid ethyl ester), a cholesteryl ester transfer protein inhibitor, was studied in rats, monkeys, and mice after oral administration of a single dose of \([^{14}\text{C}]\)torcetrapib. Total mean recoveries of the radiocarbon were 90.9, 93.4, and 86.8% from mice, rats, and monkeys, respectively. Excretion of radioactivity was rapid and nearly complete within 48 h after dosing, with a majority excreted in the feces in all species. Torcetrapib was not detected in the urine and/or bile across species, suggesting that it is primarily cleared by metabolism in these species. More than 28 metabolites were identified in all species and were products of oxidation and conjugation pathways. The primary metabolic pathways of torcetrapib involved hydrolysis of the carbamate ester (M2) and the oxidation of the ethyl moieties. M2 was subsequently metabolized in parallel by oxidative cleavage to novel and unusual quinoline metabolites (M3, M4, M5, M9, and M17), M1 (bis trifluoromethyl benzoic acid), and M28 [3,5-bis(trifluoromethyl)-phenyl-(methoxycarbonyl)methanesulfonic acid]. The structures of several metabolites were established by high-resolution liquid chromatography-mass spectrometry and \(^1\text{H}\) NMR. The major circulating and excretory metabolites in mice, rats and monkeys were species-dependent; however, several common metabolites were observed in more than one species. In addition to parent torcetrapib, M1, M3, and M4 in rats, M4 and M17 in mice, and M3 and M8 in monkeys detected as the major circulating metabolites. A mechanism for the formation of an unusual metabolite M28 has been proposed.

Although low-density lipoprotein-cholesterol (LDL-C)-lowering statin therapies have shown consistent benefit for reduction of the mortality and morbidity associated with coronary artery disease (CHD) (Schaefer and Brousseau, 2000), there still remains a significant number of patients for which these therapies have not prevented events due to cardiovascular disease (Shepherd et al., 1995; Sacks et al., 1996; Downs et al., 1998). In addition to high levels of LDL-C, low levels of high-density lipoprotein-cholesterol (HDL-C) are also a strong and independent risk factor for the incidence of CHD (Bartter et al., 2003). Plasma concentrations of HDL-C are regulated in part by production of nascent HDL particles, primarily by the liver and intestine, and by the actions of a series of plasma enzymes and transfer proteins, including lipoprotein and hepatic lipase, lecithin-cholesterol acyltransferase, and CETP (Bruce et al., 1998). The effects of CETP activity on plasma lipoproteins and atherosclerosis have been examined in animal models and human subjects (Whitlock et al., 1989; Inazu et al., 1990; Marotti et al., 1993; Evans et al., 1994; Gaynor et al., 1994; Teh et al., 1998; Plump et al., 1999). These studies seem to suggest that although total elimination of CETP activity may not necessarily afford a benefit, at least the partial inhibition of CETP appears to provide a reduction in atherosclerosis and CHD risk. Thus, a number of academic and industrial enterprises have pursued a pharmacological inhibitor of CETP to reduce the morbidity and mortality associated with cardiovascular diseases.

Torcetrapib, a CETP inhibitor, was being developed in combination with atorvastatin as a means of reducing cardiovascular risk by increasing levels of HDL-C and reducing levels of LDL-C (Clark et al., 2006). In phase 2 studies, administration of torcetrapib has re-

ABBREVIATIONS: LDL-C, low-density lipoprotein-cholesterol; CHD, coronary artery disease; HDL-C, high-density lipoprotein-cholesterol; CETP, cholesteryl ester transfer protein; torcetrapib, \((-\text{[2R,4S]}\)-4-[(3,5-bis-trifluoromethylbenzyl)-methoxycarbonyl-amino]-2-ethyl-6-trifluoromethyl-3,4-dihydro-2H-quinoline-1-carboxylic acid ethyl ester; SD, Sprague-Dawley; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; \(\beta\)-RAM, radioactive monitor; AUC, area(s) under the curve; BTFMBA, bis-trifluoromethylbenzoic acid; CID, collision-induced dissociation.
sulted in dose-dependent increases in plasma HDL and decreases in plasma LDL concentrations. However, development of torcetrapib was discontinued after an excess in mortality in the active treatment arm of the study (Howes and Kostner, 2007). The underlying cause of this imbalance remains undetermined.

Preclinical pharmacokinetic studies in nonclinical species suggested that torcetrapib is well absorbed and readily distributed throughout the body tissues. Metabolic pathways of drug candidates in animals, used for safety evaluation studies, are required to ensure that the selected animal species are exposed to all major metabolites formed in humans (http://www.fda.gov/cder/guidance/index.htm). Recently, the Food and Drug Administration suggested that additional toxicological testing on metabolites that have higher exposure in humans than preclinical species may be required (http://www.fda.gov/cder/guidance/index.htm). The objectives of the present study were to characterize the disposition of torcetrapib in rats, mice, and monkeys and to identify and quantify its excreta of the present study were to characterize the disposition of torcetrapib-A as described above. Bile was collected from male rat 1 over to 48 h postdose and for 0 to 24 h from male rat 2 and female rats 3 and 4 (the cannulae of rats 2–4 came out after 24 h). The bile samples were stored at −20°C until analysis.

For pharmacokinetic experiments, a third group of jugular vein-cannulated SD rats (n = 3/gender) was administered a single oral 20 mg/kg dose of [14C]torcetrapib-A as described above. Blood (0.6 ml) was collected from each rat via the jugular vein in heparinized tubes at predose, 0.5, 1, 2, 4, 8, 12, 24, and 48 h postdose. A fourth group of animals (n = 2/sex) was dosed for the identification of circulating metabolites. Blood was collected in heparinized tubes by decapitation of one male and one female at 4 and 6 h postdose. Blood samples were centrifuged at 1000g for 10 min to obtain the plasma. Plasma was transferred to clean tubes and stored at −20°C until analysis.

A fifth group of six noncannulated (n = 3/gender) SD rats was administered a single 20 mg/kg oral dose of [14C]torcetrapib-B to determine the fate of bis-trifluorobenzoic acid formed by N-dealkylation of the trifluoromethyl-3,4-dihydro-2H-quinoline moiety of torcetrapib. Urine and feces were collected from each up to 168 h postdose as described earlier. A sixth group of two male and two female rats was administered a single 20 mg/kg oral dose of [14C]torcetrapib-B for identification of circulating metabolites. Blood samples were collected from two rats (one male and one female) at 2 and 8 h postdose. Plasma was separated and stored at −20°C until analysis.

Monkeys. Four (two male and two female) intact and two (male) bile duct-cannulated cynomolgus monkeys (3–4 kg) were housed individually in stainless steel metabolism cages. A single dose of [14C]torcetrapib-A was administered orally at a target dose level of 20 mg/kg and 120 μCi of radio carbon. The dose was formulated in a mixture of olive oil/Creomphor EL/water (40:10:50) at a target concentration of 5 mg/ml. Urine was collected into containers at 0- to 8-, 8- to 24-, and 24-h intervals during the study through 168 h postdose. The feces was collected over 24-h intervals up to 168 h postdose. The total weight of the urine and feces was recorded after each collection. The samples were stored at −20°C until the day of analysis.

For biliary excretion experiments, another group of two male and two female bile duct-cannulated rats was administered a single 20 mg/kg oral dose of [14C]torcetrapib-B as described above. Bile was collected from male rat 1 over to 48 h postdose and for 0 to 24 h from male rat 2 and female rats 3 and 4 (the cannulae of rats 2–4 came out after 24 h). The bile samples were stored at −20°C until analysis.

Materials and Methods

General Chemicals. Commercially obtained chemicals and solvents were of HPLC or analytical grade. Zorbax C-18 HPLC analytical and preparative columns were obtained from Phenomenex (Torrance, CA). Ecolite was obtained from MP Biomedicals, Inc. (Irvine, CA). Carborosorb and Permafluor E scintillation cocktail was obtained from MP Biomedicals, Inc. (Irvine, CA). HPLC-grade acetonitrile, methanol and water, and certified aqueous counting scintillant-grade ammonium acetate and acetic acid were obtained from Thermo Fisher Scientific (Waltham, MA).

Radiolabeled Drug and Reference Compounds. [14C]Torcetrapib-A, labeled at the C-4 position of the trifluoromethyl-3,4-dihydro-2H-quinoline moiety (specific activity, 58 mCi/mmol) and [14C]torcetrapib-B, labeled at the benzylic position of the bis-trifluorophenyl ring (specific activity, 56 mCi/mmol) were synthesized by the radiochemistry group at Pfizer Global Research and Development (Groton, CT) (Fig. 1). Both showed a radiochemical purity of ≥98%, as determined by HPLC using an in-line radioactivity detector and characterized by MS and NMR. The synthetic reference compounds standards, 6-(trifluoromethyl) quinoline-2-carboxylic acid, 6-trifluoromethyl-2-methyl quinoline, 6-(trifluoromethyl) quinolin-2-yl methanol, and 3,5-bis(trifluoromethyl)phenyl-(methoxycarbonyl-amino)methanesulfonic acid, were synthesized at Pfizer Global Research and Development using standard procedures (Damon et al., 2006).

Animals, Dosing, and Sample Collection. Intact, bile duct-, or jugular vein-cannulated SD rats (190–270 g) and mice (25–30 g) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Cynomolgus monkeys (4–6 kg) were from an established in-house colony. Animals were quarantined for a minimum of 3 days prior to treatment and maintained on a 12-h light/dark cycle. The animals were fasted overnight prior to administration of the dose and were fed 6 h after the dose. The animals were provided water ad libitum. All studies were conducted in a research facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All animal studies were approved by the institutional animal care and use committee.

Rats. A group of six noncannulated (n = 3/gender) SD rats was housed individually in stainless steel metabolism cages. A single dose [14C]torcetrapib-A was administered orally at a target dose level of 20 mg/kg and 120 μCi of radio carbon. The dose was formulated in a mixture of olive oil/Creomphor EL/water (40:10:50) at a target concentration of 5 mg/ml. Urine was collected into containers at 0- to 8-, 8- to 24-, and 24-h intervals during the study through 168 h postdose. The feces was collected over 24-h intervals up to 168 h postdose. The total weight of the urine and feces was recorded after each collection. The samples were stored at −20°C until the day of analysis.

For biliary excretion experiments, another group of two male and two female bile duct-cannulated rats was administered a single 20 mg/kg oral dose of [14C]torcetrapib-A as described above. Bile was collected from male rat 1 over to 48 h postdose and for 0 to 24 h from male rat 2 and female rats 3 and 4 (the cannulae of rats 2–4 came out after 24 h). The bile samples were stored at −20°C until analysis.

For pharmacokinetic experiments, a third group of jugular vein-cannulated SD rats (n = 3/gender) was administered a single oral 20 mg/kg dose of [14C]torcetrapib-A as described above. Blood (0.6 ml) was collected from each rat via the jugular vein in heparinized tubes at predose, 0.5, 1, 2, 4, 8, 12, 24, and 48 h postdose. A fourth group of animals (n = 2/sex) was dosed for the identification of circulating metabolites. Blood was collected in heparinized tubes by decapitation of one male and one female at 4 and 6 h postdose. Blood samples were centrifuged at 1000g for 10 min to obtain the plasma. Plasma was transferred to clean tubes and stored at −20°C until analysis.

A fifth group of six noncannulated (n = 3/gender) SD rats was administered a single 20 mg/kg oral dose of [14C]torcetrapib-B to determine the fate of bis-trifluorobenzoic acid formed by N-dealkylation of the trifluoromethyl-3,4-dihydro-2H-quinoline moiety of torcetrapib. Urine and feces were collected from each up to 168 h postdose as described earlier. A sixth group of two male and two female rats was administered a single 20 mg/kg oral dose of [14C]torcetrapib-B for identification of circulating metabolites. Blood samples were collected from two rats (one male and one female) at 2 and 8 h postdose. Plasma was separated and stored at −20°C until analysis.

Monkeys. Four (two male and two female) intact and two (male) bile duct-cannulated cynomolgus monkeys (3–4 kg) were housed individually in stainless steel metabolism cages. A single dose of [14C]torcetrapib-A was administered orally at a target dose level of 60 mg/kg (~150 μCi/animal). The dose was formulated in a mixture of miglyol 812 (84 ml), propylene carbonate (28 ml), and Creomphor EL (28 ml) and Milli-Q (Millipore Corporation, Billerica, MA) water (92.5 ml) at a target concentration of 12 mg/ml on the day of the dose administration. Urine was collected into containers surrounded by dry ice at predose (~12 to 0 h), 0- to 8-, 8- to 24-, and 24-h intervals during the study through 192 h postdose. The feces was collected over predose (~12 to 0) and then over 24-h intervals, up to 192 h postdose. Urine, bile, and feces were collected from bile duct-cannulated monkeys at predose (~12 to 0), 0 to 24, and 24 to 48 h postdose. The total weight of the urine, bile, and feces was recorded after each collection. Blood samples (5 ml) were collected from the vascular access port or via venipuncture of a peripheral vessel in heparinized tubes at predose, 0.5, 1, 2, 4, 8, 12, and 24 h postdose and 24-h intervals through 168 h postdose. Cage debris was collected after each fecal collection, and the cage floors, screens, and pans were rinsed with aqueous ethanol (50%). After the last urine and fecal collection, the cages were washed with aqueous ethanol (50%), and the washes were collected.

*Denotes sites of 14C-label

Mice. A group of nine male and nine female CD-1 mice (25–31 g) was housed in Nalgene metabolism cages (three mice/cage) designed to collect urine and feces. Each animal was administered a single oral 20 mg/kg dose of [14C]torcetrapib-A. The 14C dose was formulated at a target concentration of 2 mg/ml in Cremophor EL/olive oil/water (1:4:5, v/v). Urine and feces were collected from these animals for 7 days at 0 to 2 h, 2 to 4 h, 4 to 8 h, and 8 to 24 h. Each animal was administered four groups of three animals per sex were given a single oral 20 mg/kg dose of [14C]torcetrapib-A. Blood was collected from three animals/gender in heparinized tubes at 2, 4, 6, and 8 h post dose. For pharmacokinetics, eight groups of three animals per sex were administered a single oral dose of 20 mg/kg [14C]torcetrapib-A. Whole blood was collected from three animals/gender in heparinized tubes at 30 min and 1, 2, 4, 8, 12, 24, and 48 h post dose. Plasma was separated from whole blood by centrifugation and stored at −20°C until use.

Determination of Radioactivity. Radioactivity in plasma, urine, bile, and feces was measured by liquid scintillation counting. Aliquots of plasma (25–100 µl, in duplicate), urine (100–500 µl, in triplicate), and bile (100 µl, in triplicate) for each sampling time from all animals were mixed with 15 ml of Ecolite (±) scintillation cocktail and counted in a Wallac 1409 (GE Healthcare, Chalfont St. Giles, UK) or a model LS 6000 (Beckman Coulter, Fullerton, CA) liquid scintillation counter. For determination of radioactivity in feces, the weight of each fecal sample was determined, and the samples were homogenized in deionized water using a probe-type homogenizer. After homogenization, triplicate aliquots (0.1–0.5 g) of each homogenate were transferred into tared cones and pads and air-dried before combustion in an automatic sample oxidizer (PerkinElmer oxidizer 300). Radioactivity in the combustion products was determined by trapping the liberated CO2 in Carbo-sorb followed by liquid scintillation counting using Permafluor E+ as a scintillation cocktail. Combustion efficiency was determined daily, prior to the combustion of study samples, using diluted dose formulation or a carbon-14 standard. The measured radioactivity content in the combusted samples was adjusted using the combustion efficiency values. Samples were analyzed for radioactivity for 2 min (5 or 10 min for plasma). 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. Radioactivity less than twice the background value was considered to be below the limit of determination.

Samples collected prior to dosing were used as controls and counted to obtain a background count rate. Radioactivity in the dose was expressed as 100%, and the radioactivity in urine, bile, and feces at each sampling time was defined as the percentage of dose excerted in the respective matrices at that sampling time. The amount of radioactivity in plasma was expressed as nanogram equivalents of torcetrapib per milliliter and was calculated by using the specific activity of the dose administered.

Extraction of Metabolites from Biological Samples. Urine from 0 to 72 h for both rats and monkeys and 0 to 48 h for mice was pooled proportional to the volumes of urine collected at each time point. The pooled urine accounted for greater than 90% of the excreted radioactivity. The pooled urine samples were then centrifuged (1000g for 10 min), and the aliquots (100 µl) of supernatants were directly injected onto the HPLC column without further purification. An aliquot of bile (0–24 h) was diluted with 4 volumes of acetonitrile, and the precipitated material was removed by centrifugation. The pellet was washed with an additional 1 volume of acetonitrile, and both supernatants were combined. The extraction recovery of the radioactivity in bile was about 85% after extraction. The supernatant was evaporated to dryness under nitrogen in a TurboVap LV evaporator (Caliper Life Sciences, Hopkinton, MA), and the residue was redissolved in 20:80 acetonitrile/10 mM ammonium formate, pH 3.0. An aliquot was injected onto the HPLC column.

The fecal homogenates were pooled from 0 to 48 h for rats and mice and 0 to 72 h for monkeys so that 90% or greater radioactivity was accounted for. From the pooled samples, aliquots (10–12 g) were suspended in 30 ml of acetonitrile. Suspensions were sonicated (30 min.), vortexed, and centrifuged at 1000g for 10 min. After supernatant transfer to clean conical tubes, the residues were further extracted three times with 30 ml of acetonitrile as described above. Aliquots (100 µl) from the pooled extracts were counted in a liquid scintillation counter. The recovery of radioactivity extracted ranged from 85 to 96%. The supernatants were evaporated in a TurboVap LV evaporator at 35°C under nitrogen. The residues obtained were reconstituted in 1 to 2 ml of acetonitrile/H2O, and aliquots (100 µl) were injected onto the HPLC column.

Rat and mouse plasma obtained for metabolite profiling and identification was diluted with 3 volumes of acetonitrile. The mixture was then centrifuged, and the supernatant was transferred to a clean tube. The pellet was extracted with an additional 2 ml of acetonitrile. Both supernatants were combined, and an aliquot was initially counted in a scintillation counter. The extraction recovery of the radioactivity in plasma was >80%. The supernatant was evaporated in a TurboVap at 35°C under nitrogen. The residue was reconstituted in mobile phase (see below) and injected onto the LC/MS/MS.

For monkeys, plasma (3 ml pooled; equal volume of each time point) was diluted with 9 ml of acetonitrile, and the precipitated protein was removed by centrifugation. The pellets were extracted with an additional 2 ml of acetonitrile. The extraction recovery of the radioactivity in plasma was 80 to 88%. The supernatants from the two extractions were combined and concentrated under nitrogen in a TurboVap LV evaporator. The residues were reconstituted in 500 µl of HPLC mobile phase, and aliquots (100 µl) were injected onto the LC/MS/MS column without further sample purification.

Chromatography. The HPLC system consisted of an HP-1100 solvent delivery system, an HP-1100 membrane-degasser, an HP-1100 Autoinjector from Hewlett Packard (Palo Alto, CA), and an IN/US β-RAM radioactivity monitor (IN/US Systems, Tampa, FL). Chromatography was performed on a Zorbax C18 column (5 µm, 4.5 × 150 mm) with a mobile phase containing a mixture of 10 mM ammonium formate, pH 2.0 (solvent A), and acetonitrile (solvent B). For urine and plasma of rats and monkeys, the mobile phase was initially composed of solvent A/solvent B (95:5) and held for 5 min. The mobile phase composition was then linearly programmed to solvent A/solvent B (60:40) over 10 min and then changed to solvent A/solvent B (40:60) over 13 min. A short gradient was programmed to solvent A/solvent B (5:95) over 2 min, and the mobile phase composition was returned to the starting solvent mixture over 5 min. The system was allowed to equilibrate for approximately 15 min before making the next injection. The flow rate was 1.0 ml/min, and the separation was achieved at ambient temperature.

For separation of biliary metabolites, the mobile phase was initially composed of solvent A/solvent B (80:20), held isocratic for 5 min, then ramped to 20:80 over 35 min, followed by another ramp to 5:95 over 10 min. The system was returned to the starting solvent mixture over 2 min and was allowed to equilibrate for 8 min prior to the next injection.

For separation of fecal and plasma metabolites of mice, chromatography was performed on an Agilent C-18 SB column (4.6 × 150 mm, 5 µm; Agilent Technologies, Santa Clara, CA) with a mobile phase containing a mixture of 10 mM ammonium formate, pH 3.0 (solvent A) and acetonitrile (solvent B). The mobile phase was initially composed of solvent A/solvent B (80:20), held for 5 min, and was then linearly programmed to solvent A/solvent B (20:80) over 35 min. A gradient was programmed to solvent A/solvent B (5:95) over 10 min, and the mobile phase composition was returned to the starting solvent mixture over 2 min. The system was allowed to equilibrate for approximately 10 min before making the next injection. The flow rate was 1.0 ml/min, and the separation was achieved at ambient temperature. Due to coeluting peaks in the urine samples using the pH 3.0 formate buffer, urine samples were profiled using the exact conditions as stated above, except that the solvent A consisted of ammonium formate at pH 6.4.

For separation of urinary metabolites of rats dosed with [14C]torcetrapib-B, C-14 labeled at the benzylcyclic position, the mobile phase was initially composed of solvent A/solvent B (90:10) and held for 5 min. The mobile phase composition was then linearly programmed to solvent A/solvent B (70:30) over 5 min and then changed to solvent A/solvent B 30:70 over 20 min. A short gradient was programmed to solvent A/solvent B (15:85) for 5 min and to solvent A/solvent B (10:90) for 5 min. The mobile phase composition was returned to the starting solvent mixture over 5 min. The system was allowed to equilibrate for approximately 15 min before making the next injection. The flow rate was 1.0 ml/min, and the separation was achieved at ambient temperature.

Quantitative Assessment of Metabolites. Quantification of the metabolites was carried out by measuring radioactivity in the individual HPLC-separated peaks using a β-RAM. The β-RAM provided an integrated printout in counts
per minute and percentage of the radiolabeled material and representation. The β-RAM was operated in the homogeneity liquid scintillation counting mode, with addition of 3 ml/min Tru-Count scintillation cocktail to the effluent post-UV detection. For the quantification of plasma metabolites of mice, the HPLC effluent was directed into the flow cell of a β-RAM radioactivity detector. The β-RAM and HPLC apparatus were controlled externally, using an Accurate radioisotope counting (AIM Research, Hockessin, DE) system for low-level radioactivity counting.

LC/MS/MS. LC/MS/MS was conducted with a PE-Sciex API 2000 (PerkinElmerSciex Instruments, Waltham, MA), a Micromass Q-TOF (Waters, MA), or a Finnigan LCQ Ion Trap (Thermo Fisher Scientific) equipped with an electrospray ion source. The effluent from the HPLC column was split, and about 50 μl/min was introduced into the atmospheric ionization source. The remaining effluent was directed to the flow cell of β-RAM. The β-RAM response was recorded as a real-time analog signal by the MS data collection system. This allowed simultaneous real time monitoring of radioactivity and the detection of the total ion chromatogram. All mass spectrometers were, unless mentioned otherwise, operated in the positive ion mode. Data were collected in the Q1 scanning, neutral loss scanning, precursor ion scanning, product ion scanning, multiple reaction monitoring scanning, and data-dependent ion scanning modes, with instrument settings and potentials (e.g., collision energy) adjusted to provide optimal data in each mode. Additional metabolite identification and verification were performed by elemental composition determination using the Micromass Q-TOF. Lock mass for the Q-TOF was achieved by infusing quinidine at 325.1916 Da for accurate mass determination calculations.

LC/MS/NMR. LC/MS/NMR system was consisted of an Agilent 1100 binary pump, a membrane degasser, and Autoinjector (Agilent Technologies), a Bruker BioSpin BSFU-0 column oven (Bruker Daltonics, Billerica, MA), a Bruker BioSpin photodiode array detector, a Bruker BioSpin BNMI interface using a 20:1 split, a Bruker Daltonics Esquire 3000 ion trap MS (Bruker Daltonics, Billerica, MA) equipped with an electrospray source, a Bruker BioSpin BPSU-36 peak storage unit, and a Bruker BioSpin 500 MHz Avance DRX Spectrometer equipped with a 4-mm 1H-13C inverse z-gradient LC flow probe. Proton chemical shifts (δ) are reported in parts per million relative to tetramethylsilane as referenced from the shift of residual protons in MeCN-d3 (1.94 ppm). Chromatography for M4 was performed on a Zorbax C8 Rx column (5 μm, 4.6 × 150 mm), with a mobile phase containing a mixture of 0.1% deuterated acetic acid in D2O (solvent A) and MeCN-d3 (solvent B). The mobile phase, initially composed of solvent A/solvent B (80:20), was then linearly programmed to solvent A/solvent B (60:40) over 40 min. The flow rate was 1.0 ml/min, and the separation was achieved at ambient temperature. Chromatography for M28 was performed on a Develosil C30 column (3 μm, 3.0 × 150 mm), with a mobile phase containing a mixture of 15 mM AcOD-d4 in D2O, pH 5 (solvent A) and MeCN-d3 (solvent B). The mobile phase, initially composed of solvent A/solvent B (80:20), was then linearly programmed to solvent A/solvent B (50:50) over 60 min. The flow rate was 0.5 ml/min, and the separation was achieved at ambient temperature.

Quantitation of Torcetrapib. The plasma samples were analyzed for unchanged torcetrapib using PerkinElmerSciex API 3000 (PerkinElmerSciex Instruments). A 100-μl aliquot of each plasma sample containing torcetrapib was treated with 10 μl of 1 μg/ml internal standard, a structure analog of torcetrapib, and extracted on a Varian C18 (25 mg) microtiter-packed bed (Varian, Inc., Palo Alto, CA). The bed was washed with an acetonitrile/water mixture (1:1), and the analytes were eluted with 200 μl of acetonitrile and injected onto a LC/MS/MS system for analysis. The internal standard and torcetrapib were separated chromatographically using a Keystone C-8 (4.6 × 30 mm) analytical column at ambient temperature. The mobile phase of 80% acetonitrile and 20% 10 mM ammonium acetate was used to separate the internal standard and torcetrapib in the plasma. The total run time for each sample was 3.5 min, with a flow rate of 0.5 ml/min. The retention times of torcetrapib and the internal standard were 1.52 and 1.67 min, respectively. The compounds were detected in a negative ion mode using a turbo ion spray source at m/z 659.2 and 685.2 for torcetrapib and internal standard, respectively. Data collection and integration were done on Sciex Software sample control and Mac Quan (version 1.6), respectively. The ratio of peak area responses of drug relative to internal standard was used to construct a standard curve using a linear least square regression with a 1/x weighting. The dynamic range of the assay was 25 to 1000 ng/ml. The performance of the assay was monitored by inclusion of quality control samples prepared in monkey plasma.

Pharmacokinetic Analysis. Pharmacokinetic parameters were determined using the WinNonlin-Pro version 2.1 program (Pharsight, Mountain View, CA) by noncompartmental analysis. For estimation of the means and pharmacokinetic parameters, concentrations at the 0 h and those <25.0 ng/ml were assumed to be 0 ng/ml. The means were calculated only if greater than 50% of the data were more than the lower limit of quantification.

Results

14C Excretion. Rats. After oral administration of a single 20 mg/kg dose of [14C]torcetrapib-A to SD rats, an overall mean of 93.4% of the total dose was recovered in the urine, feces, and cage wash of the rats over a period of 168 h postdose (Table 1). The mean percentage of dose excreted in male and female rats was 94.8 and 91.9%, respectively. The mean cumulative dose recovered in feces of male and female rats was 59.1 and 52.7%, respectively. The mean cumulative excretion in the urine of male and female rats was 21.3 and 21.6% of the dose (Table 1). There was no gender-related difference in the routes of excretion of torcetrapib radioactivity.

After a single oral administration of [14C]torcetrapib-B, labeled at the benzyl position, an overall mean of 94.4% of the total dose was recovered in the urine, feces, and cage rinse of the rats. The mean cumulative dose recovered in feces of male and female rats was 59.1 and 52.7%, respectively. The mean cumulative excretion in the urine of male and female rats was 21.3 and 21.6% of the dose (Table 1). There was no gender-related difference in the routes of excretion of torcetrapib radioactivity.

Monkeys. After a single oral administration of [14C]torcetrapib-A, a mean of 86.8% of the total dose was recovered in the urine, feces, and cage wash of the monkeys (Table 1). The cumulative dose recovery in females and male monkeys ranged from 31.4 to 59.8% (mean 44.5%). The cumulative excretion in the urine of monkeys ranged from 6.59 to 45.6% (mean 29.2%).

Mice. After a single oral dose of [14C]torcetrapib-A to mice, equal amounts of radioactivity were recovered in urine and feces. Urine accounted for 41.2 and 40.6% of the dose, respectively, in male and female mice (Table 1). Feces accounted for 44.3 and 40.0% of the dose in males and females, respectively (Table 1). Including the radioactivity recovered in the cage wash and carcass, approximately 90.7% of the radioactivity was recovered in CD-1 mice.

Pharmacokinetics. Rats. Mean plasma concentration versus time curves of torcetrapib and total radioactivity after a single 20 mg/kg oral dose of [14C]torcetrapib-A to rats are shown in Fig. 2. The

<table>
<thead>
<tr>
<th>Animal</th>
<th>Urine</th>
<th>Feces</th>
<th>Carcass</th>
<th>Cage Rinse</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats (n = 3)*</td>
<td>21.3</td>
<td>59.1</td>
<td>7.54</td>
<td>6.87</td>
<td>94.8</td>
</tr>
<tr>
<td>Female rats (n = 3)*</td>
<td>21.6</td>
<td>52.7</td>
<td>9.87</td>
<td>7.74</td>
<td>91.9</td>
</tr>
<tr>
<td>Mean*</td>
<td>21.5</td>
<td>55.9</td>
<td>8.77</td>
<td>7.30</td>
<td>93.4</td>
</tr>
<tr>
<td>Male rats (n = 3)**</td>
<td>21.1</td>
<td>62.6</td>
<td>8.29</td>
<td>3.85</td>
<td>95.9</td>
</tr>
<tr>
<td>Female rats (n = 3)**</td>
<td>21.7</td>
<td>56.4</td>
<td>10.3</td>
<td>4.44</td>
<td>92.8</td>
</tr>
<tr>
<td>Mean**</td>
<td>21.4</td>
<td>59.5</td>
<td>9.40</td>
<td>4.14</td>
<td>94.4</td>
</tr>
<tr>
<td>Male Monkeys (n = 2)*</td>
<td>39.0</td>
<td>35.1</td>
<td>N.D.</td>
<td>15.1</td>
<td>89.2</td>
</tr>
<tr>
<td>Female monkeys (n = 2)*</td>
<td>19.3</td>
<td>53.8</td>
<td>N.D.</td>
<td>11.3</td>
<td>84.4</td>
</tr>
<tr>
<td>Mean*</td>
<td>29.2</td>
<td>44.5</td>
<td>N.D.</td>
<td>13.2</td>
<td>86.8</td>
</tr>
<tr>
<td>Male mice (n = 3/cage)*</td>
<td>41.2</td>
<td>44.3</td>
<td>3.05</td>
<td>2.51</td>
<td>91.1</td>
</tr>
<tr>
<td>Female mice (n = 3/cage)*</td>
<td>40.6</td>
<td>40.0</td>
<td>4.86</td>
<td>4.90</td>
<td>90.3</td>
</tr>
<tr>
<td>Mean*</td>
<td>40.9</td>
<td>42.1</td>
<td>3.96</td>
<td>3.91</td>
<td>90.7</td>
</tr>
</tbody>
</table>

N.D., not determined.
* Dosed with [14C]torcetrapib-A.
** Dosed with [14C]torcetrapib-B.
exposure (AUC$_{0 \rightarrow \infty}$) to torcetrapib in female rats was 2.3-fold greater than that in male rats. Similarly, the exposure (AUC$_{0 \rightarrow \infty}$) to total radioactivity in female rats was 1.6-fold greater than that in male rats. The mean $C_{\text{max}}$ of torcetrapib in male and female rats was 1590 and 2220 ng/ml, respectively, and the mean AUC$_{0 \rightarrow \infty}$ of torcetrapib in male and female rats was 6490 and 15,000 ng/h/ml, respectively (Table 2). The mean $C_{\text{max}}$ and AUC$_{0 \rightarrow \infty}$ of total circulating radioactivity in the male rats were 3020 ng Eq/ml and 49,400 ng Eq · h/ml, respectively, and the mean $C_{\text{max}}$ and AUC$_{0 \rightarrow \infty}$ in the female rats were 4150 ng Eq/ml and 80,700 ng Eq/h/ml, respectively (Table 3). Based on AUC values, the majority of circulating radioactivity in rats was attributable to unchanged torcetrapib. The mean $T_{\text{1/2}}$ of torcetrapib and total radioactivity in rats were 2.7 and 6 h, respectively. The mean terminal elimination $t_{1/2}$ of torcetrapib and total radioactivity in rats were 3.5 and 9.6 h, respectively (Tables 2 and 3).

Monkeys. Mean plasma concentration versus time curves of torcetrapib and total radioactivity after a single 60 mg/kg oral dose of [14C]torcetrapib-A to monkeys are shown in Fig. 3. The mean $C_{\text{max}}$ of torcetrapib and the total circulating radioactivity were 893 ng/ml and 10,400 ng/h/ml, respectively (Table 2). The mean $T_{\text{max}}$ of torcetrapib and the total circulating radioactivity was 10.4 (4–24) and 17.6 (4–24) h, respectively. The mean AUC$_{0 \rightarrow \infty}$ of torcetrapib and the total circulating radioactivity were 79,200 and 110,000 ng Eq/h/ml, respectively. AUC$_{0 \rightarrow \infty}$ of total radioactivity in male and female monkeys was 6490 and 15,000 ng/h/ml, respectively (Table 2).

For total radioactivity in male mice, $C_{\text{max}}$ of 9380 ng Eq/ml occurred at 2 h postdose (Table 3), whereas in females, $C_{\text{max}}$ of 9810 ng Eq/ml occurred at 8 h postdose, which was only slightly higher than the concentration at 2 h postdose, which was observed at 9610 ng Eq/ml. The terminal phase $t_{1/2}$ of total radioactivity was estimated as 11.7 and 12.2 h for males and females, respectively. AUC$_{0 \rightarrow \infty}$ of total radioactivity were 79,200 and 110,000 ng Eq/h/ml for males and females, respectively (Table 3). Based on AUC values, the majority of circulating radioactivity was attributable to metabolites.

Metabolic Profiles. Rat urine. A representative HPLC radiochromatogram for urinary metabolites of [14C]torcetrapib-A is shown in Fig. 5. There were no qualitative differences in the urinary metabolic profiles between male and female rats. The mean percentages of urinary metabolites in relation to the administered dose excreted from the feces of male and female rats are presented in Table 4. A total of four radioactive peaks were observed in rat urine. These were identified as M1 (6.41 and 6.37%), M2 (2.0 and 1.87%), and M3 (13.0 and 12.2%) of the dose in male and female rats, respectively.

A representative HPLC radiochromatogram for urinary metabolites of [14C]torcetrapib-B is shown in Fig. 6. Five radioactive peaks were detected in rat urine. Unchanged BTFMBA accounted for 1.5% of the dose excreted in the urine. The glucuronide conjugate of BTFMBA, M26 (a–d), and M28 were the two major peaks in radiochromatogram for urinary metabolites of [14C]torcetrapib-A to monkeys are shown in Fig. 3. The mean $C_{\text{max}}$ of torcetrapib and the total circulating radioactivity were 893 ng/ml and 10,400 ng/h/ml, respectively (Table 2). The mean $T_{\text{max}}$ of torcetrapib and the total circulating radioactivity was 10.4 (4–24) and 17.6 (4–24) h, respectively. The mean AUC$_{0 \rightarrow \infty}$ of torcetrapib and the total circulating radioactivity were 79,200 and 110,000 ng Eq/h/ml, respectively. AUC$_{0 \rightarrow \infty}$ of total radioactivity in male and female monkeys was 6490 and 15,000 ng/h/ml, respectively (Table 2).

For total radioactivity in male mice, $C_{\text{max}}$ of 9380 ng Eq/ml occurred at 2 h postdose (Table 3), whereas in females, $C_{\text{max}}$ of 9810 ng Eq/ml occurred at 8 h postdose, which was only slightly higher than the concentration at 2 h postdose, which was observed at 9610 ng Eq/ml. The terminal phase $t_{1/2}$ of total radioactivity was estimated as 11.7 and 12.2 h for males and females, respectively. AUC$_{0 \rightarrow \infty}$ of total radioactivity were 79,200 and 110,000 ng Eq/h/ml for males and females, respectively (Table 3). Based on AUC values, the majority of circulating radioactivity was attributable to metabolites.

Metabolic Profiles. Rat urine. A representative HPLC radiochromatogram for urinary metabolites of [14C]torcetrapib-A is shown in Fig. 5. There were no qualitative differences in the urinary metabolic profiles between male and female rats. The mean percentages of urinary metabolites in relation to the administered dose excreted from the feces of male and female rats are presented in Table 4. A total of four radioactive peaks were observed in rat urine. These were identified as M5 (6.41 and 6.37%), M4 (11.9 and 9.63%), M21 (0.94 and 1.98%), and M22 (1.98 and 3.75%) of the dose in male and female rats, respectively.

A representative HPLC radiochromatogram for urinary metabolites of [14C]torcetrapib-B is shown in Fig. 6. Five radioactive peaks were detected in rat urine. Unchanged BTFMBA accounted for 1.5% of the dose excreted in the urine. The glucuronide conjugate of BTFMBA, M26 (a–d), and M28 were the two major peaks in radiochromatogram for urinary metabolites of [14C]torcetrapib-A to monkeys are shown in Fig. 3. The mean $C_{\text{max}}$ of torcetrapib and the total circulating radioactivity were 893 ng/ml (336.0–1270 ng/ml) and 6190 ng Eq/ml (3110–12,100 ng Eq/ml), respectively (Table 2). The mean $T_{\text{max}}$ of torcetrapib and the total circulating radioactivity was 10.4 (4–24) and 17.6 (4–24) h, respectively. The mean AUC$_{0 \rightarrow \infty}$ of torcetrapib and the total circulating radioactivity were 79,200 and 110,000 ng Eq/h/ml, respectively. AUC$_{0 \rightarrow \infty}$ of total radioactivity in male and female monkeys was 6490 and 15,000 ng/h/ml, respectively (Table 2).

For total radioactivity in male mice, $C_{\text{max}}$ of 9380 ng Eq/ml occurred at 2 h postdose (Table 3), whereas in females, $C_{\text{max}}$ of 9810 ng Eq/ml occurred at 8 h postdose, which was only slightly higher than the concentration at 2 h postdose, which was observed at 9610 ng Eq/ml. The terminal phase $t_{1/2}$ of total radioactivity was estimated as 11.7 and 12.2 h for males and females, respectively. AUC$_{0 \rightarrow \infty}$ of total radioactivity were 79,200 and 110,000 ng Eq/h/ml for males and females, respectively (Table 3). Based on AUC values, the majority of circulating radioactivity was attributable to metabolites.

Metabolic Profiles. Rat urine. A representative HPLC radiochromatogram for urinary metabolites of [14C]torcetrapib-A is shown in Fig. 5. There were no qualitative differences in the urinary metabolic profiles between male and female rats. The mean percentages of urinary metabolites in relation to the administered dose excreted from the feces of male and female rats are presented in Table 4. A total of four radioactive peaks were observed in rat urine. These were identified as M5 (6.41 and 6.37%), M4 (11.9 and 9.63%), M21 (0.94 and 1.98%), and M22 (1.98 and 3.75%) of the dose in male and female rats, respectively.
chromatogram and accounted for 33.6 and 39.3% of the dose in male and female rats, respectively. Other metabolites identified were M2 (0.69 and 0.63%), M7 (6.5 and 7.12%), M12 (4.82 and 3.60), and M13 (6.75 and 2.03%) of the dose in male and female rats, respectively.

**Rat bile.** A representative radiochromatogram of biliary metabolites of [14C]torcetrapib-A in rats is shown in Fig. 7. Mean percentage of biliary metabolites of in relation to total radioactivity excreted in bile of male and female rats is shown in Table 5. Several peaks were detected in the radiochromatogram of bile from rats. There were no qualitative differences in the biliary metabolic profile of the male and female rats. Approximately 82 and 89% of the radioactivity excreted in bile was identified in males and females, respectively. The remaining radioactivity could not be distinguished from the background. The metabolites identified in the bile of rats were the glucuronide conjugate of quinoline 2-carboxylic acid, M5 (13.5%); quinoline-2-carboxylic acid, M4 (13.6%); glucuronide conjugate of hydroxylated M2 (M8A, 7.9%); M8B (6.1%); M7 (30.1%); and M13 (14.0%).

**Rat plasma.** An HPLC radiochromatogram of circulating metabolites at 4 h postdose of rats dosed with [14C]torcetrapib-A is shown in Fig. 7. The relative percentage of metabolites in relation to total circulating radioactivity at 4 and 6 h postdose is listed in Table 6. Four radioactive peaks were observed in plasma samples from rats. Three peaks were identified as unchanged torcetrapib, M3 and M4. These peaks accounted for 30.1, 24.1, and 31.8% of the circulating radioactivity at 4 h and 38.0, 17.7, and 37.5% of the circulating radioactivity at 6 h after dosing, respectively. The fourth peak eluting at approximately 29 min was not identified due to lack of sample. The HPLC radiochromatogram of circulating metabolites at 6 h postdose of rats dosed with [14C]torcetrapib-B is shown in Fig. 6. Unchanged torcetrapib and metabolite M1 were the only two peaks that were observed in the plasma sample.

**Monkey urine.** A representative HPLC radiochromatogram for urinary metabolites of [14C]torcetrapib-A in monkeys is shown in Fig. 8. The individual and mean percentages of urinary metabolites in relation to the administered dose excreted in urine of male and female monkeys are shown in Table 7. A total of five radioactive peaks were observed in the monkey urine. These were identified as M10 (9.91%), M11A (9.13%), M11B (4.32%), M16 (3.43%), and M23 (1.65%).

**Monkey feces.** A representative HPLC radiochromatogram for metabolites [14C]torcetrapib-A in the monkey feces is shown in Fig. 8. A total of five radioactive peaks were observed in monkey fecal samples. Mean percentage of metabolites in relation to the administered dose excreted in feces of male and female monkeys is shown in Table 7. Torcetrapib constituted the major peak in the radiochromatogram and accounted for 40% of the dose. The other metabolites were identified as M2 (1.38%), M6A (0.59%), M6B (1.23%), and M10 (0.68%).

**Monkey bile.** A representative HPLC-radiochromatogram for biliary metabolites of [14C]torcetrapib-A is presented in Fig. 9. A total of 11 metabolites were identified in bile; however, no parent torcetrapib was detected by β-RAM or LC/MS/MS. The percentage of metabolites with respect to total administered radioactive dose is presented in Table 5. All the identified metabolites constituted <2% of the total dose, excreted in bile.

**Monkey plasma.** The representative HPLC radiochromatogram for metabolites of [14C]torcetrapib in plasma is shown in Fig. 9. The mean percentages of circulating metabolites for male and female monkeys are shown in Table 6. Six radioactive peaks were observed in the monkey plasma. These were identified as M3, M9, M8A, M8B, M10, and M11 and unchanged torcetrapib. In pooled plasma from 0 to 8 h, the mean percentage of circulating metabolites M3 + M9, M8, M10, M11, and torcetrapib was 28.0, 24.2, 15.1, 10.7, and 11.3% of the total circulating radioactivity, respectively. In pooled plasma from
12 to 24 h, the mean percentage of circulating metabolites M3 + M9, M8, M10, M11, and torcetrapib was 38.3, 16.9, 22.1, 8.0, and 14.8% of the total circulating radioactivity, respectively.

**Mouse urine.** A representative HPLC-radiochromatogram of urinary metabolites of $^{[14C]}$torcetrapib-A in mice is shown in Fig. 10. A total of six radioactive peaks were detected in the chromatogram. The mean percentage of metabolites in relation to the administered dose excreted in feces of male and female mice is shown in Table 8. The metabolites were identified as M3 (3.05%), M4 (5.0%), M17 (1.4%), M20 (8.9%), M21 (8.3%), and M22 (3.3%).

**Mouse feces.** A representative HPLC-radiochromatogram of fecal metabolites of $^{[14C]}$torcetrapib-A in mice is shown in Fig. 10. Torcetrapib and a total of three metabolites were detected. Mean percentages of metabolites in relation to the administered dose excreted in feces of male and female mice are shown in Table 8. Torcetrapib constituted the major peak in the radiochromatogram and accounted for 33.9% of the dose. The other metabolites were identified as M2 (1.8%), M6 (2%), and M13 (4.15%).

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Male Rats</th>
<th>Female Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td>Torcetrapib</td>
<td>33.6</td>
<td>33.6</td>
</tr>
<tr>
<td>M2</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>M4</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>M5</td>
<td>6.41</td>
<td>6.41</td>
</tr>
<tr>
<td>M7</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>M12</td>
<td>4.82</td>
<td>4.82</td>
</tr>
<tr>
<td>M13</td>
<td>6.75</td>
<td>6.75</td>
</tr>
<tr>
<td>M21</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>M22</td>
<td>1.98</td>
<td>1.98</td>
</tr>
</tbody>
</table>
**Mouse plasma.** A representative HPLC-radiochromatogram of circulating metabolites of [14C]torcetrapib-A at 2 h postdose from male mice is shown in Fig. 10. A total of four radioactive peaks were detected in the chromatogram. The mean percentages of circulating metabolites for male and female mice are shown in Table 6. The metabolites were identified as unchanged torcetrapib (6.83 and 11.7%), M2 (3.71 and 3.88%), M4 (41.8 and 25.0%), and M11 (40.2 and 47.2%) of the circulating radioactivity in male and female mice, respectively.

**Mass Spectral Fragmentation of Torcetrapib.** Torcetrapib had a retention time of 40 min on the HPLC system. Full-scan MS of torcetrapib produced an intense ion at \( m/z \) 618 [M+NH₄]⁺. The product ion mass spectrum of \( m/z \) 618 gave major fragment ions at \( m/z \) 272 and 254, as shown in Fig. 11. The major fragment ion at \( m/z \) 300 resulted from the loss of the N-(bis-trifluoromethylbenzyl) methylcarbamate moiety from torcetrapib. The fragment ions at \( m/z \) 272 and 254 resulted from loss of the ethylene group and an ethanol moiety, respectively, from \( m/z \) 300. The ion at \( m/z \) 228 resulted from loss of the ethylcarbamoyloxy group from \( m/z \) 300.

**Identification of Metabolites.** The structures of metabolites were elucidated by ion spray LC/MS/MS using a combination of Q1, product ion and multiple reaction monitoring scanning, and accurate mass determination techniques (Kamel and Prakash, 2006; Prakash et al., 2007).
Metabolite M1. Metabolite M1 was detected in the urine, bile, plasma, and feces of rats dosed with \([^{14}C]\)torcetrapib-B. It gave a signal at \(m/z\) 257 in the negative ion mode. The product ion mass spectrum of \(m/z\) 257 gave a major fragment ion at \(m/z\) 213, which was attributed to the loss of the carboxyl group. The mass spectrum and retention time of peak were similar that of the synthetic standard of BTFMBA.

Metabolite M2. Metabolite M2 was detected in all species dosed with \([^{14}C]\)torcetrapib-A. M2 showed a protonated molecule of \(m/z\) 529, 72 Da lower than the parent compound, suggesting a loss of the methyl N-bis-trifluoromethylbenzyl carbamate moiety. The accurate mass (observed 529.1560 versus calculated mass of 529.1538, \(\Delta 4.2\) ppm error) of M2 suggested its empirical formula as \(C_{23}H_{22}N_2O_2F_9\). The CID spectrum of M2 (\(m/z\) 529) produced a major fragment ion at \(m/z\) 228, the same as the parent compound. The mass spectrum and the HPLC retention time of M2 were similar to those of a synthetic standard, methyl 3,5-bis(trifluoromethyl)benzyl-(2-ethyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinoline-4-yl)carbamate.

Metabolite M3. Metabolite M3 was detected in mouse urine and rat and monkey plasma. M3 displayed a protonated molecule of \(m/z\) 212, lower than the parent compound, suggesting that it was a cleaved product of torcetrapib. Accurate mass analysis of protonated ion was determined to be 212.0701 (\(\Delta 1.0\) ppm, theoretical), corresponding to an empirical formula of \(C_{11}H_9NF_3\). The CID spectrum of M3 gave minor fragment ions at \(m/z\) 196, 192, and 143 (Fig. 12). Comparison of the spectrum and retention time of M3 with that of the synthetic standard suggested that M3 was 2-methyl-6-trifluoromethylquinoline. The lack of any other resonance indicated that the bistrifluoromethyl ring and the ethoxy, ethyl, piperidine, and

Metabolite M4. Metabolite M4 was detected in rats and mice. M4 displayed a protonated molecule of \(m/z\) 242, lower than the parent compound, suggesting that it was also a cleaved product of torcetrapib. Accurate mass analysis of protonated ion was determined to be 242.0429 (\(\Delta 1.8\) ppm, theoretical), corresponding to an empirical formula of \(C_{11}H_7NO_2F_3\). The CID spectrum of M4 at \(m/z\) 242 gave fragment ions at \(m/z\) 214, 196, and 176. The ion at \(m/z\) 196 loss of the formic acid (46 Da) from \(m/z\) 242, suggested the presence of a carboxylic acid (Fig. 12). \(^1\)H NMR of M4 contained only five resonances in the entire spectrum, representing five protons in two distinct spin systems (Fig. 13). Three of the five aromatic resonances (8.49 s; 8.35 d; and 8.12 d) were consistent with the presence of the phenyl ring containing one trifluoromethyl group. The two remaining aromatic resonances (8.80 d and 8.30 d) were consistent with the formation of a new aromatic ring.
carbamate moieties were missing. Based on these data, M4 was identified as the quinoline-2-carboxylic acid. The structure was further confirmed by comparison of its HPLC retention time and CID spectrum with those of a synthetic standard.

**Metabolite M5.** Metabolite M5 was detected only in rat urine and bile as a mixture of four isomers. All four isomers displayed a protonated molecule of \( m/z \) 418, 176 Da higher than the metabolite M4, suggesting that all metabolites were glucuronide conjugates of M4 and that the four peaks resulted from the acyl-rearrangement of the glucuronide conjugate. The CID spectra of all the four isomers were very similar. The representative spectrum of M5 at \( m/z \) 418 resulted in fragment ions at \( m/z \) 400, 242, and 196. The fragment ion at \( m/z \) 242 was similar to the molecular ion of quinoline-2-carboxylic acid M4, and the ion at \( m/z \) 196 was a result of the loss of the carboxyl group from M4.

**Metabolite M6.** Metabolite M6 was found only in mouse and monkey feces. M6 displayed a protonated molecular ion at \( m/z \) 545, 16 Da higher than the metabolite M2, suggesting that it was a
hydroxylated metabolite of M2. The exact mass of M6 was determined as 545.1453, corresponding to an empirical formula of C12H11NO4F3 (Δ -6.3 ppm, theoretical). The CID product ion spectrum of M6 gave only one ion at m/z 244. Based on the empirical formula and CID spectrum, M6 was identified as a hydroxy metabolite of M2.

**Metabolite M7.** Metabolite M7 was only detected in the rat feces and bile. M7 displayed a protonated molecule of m/z 559, 30 Da higher than the metabolite M2, suggesting the oxidation of the methyl group to a carboxylic acid. The CID spectrum of M7 at m/z 559 gave major fragment ions at m/z 258 and 198, which were resulted from the loss of the methyl N-bis-trifluoromethylbenzyl carbamate moiety and a subsequent loss of acetic acid. Based on these data, M7 was identified as methyl 3,5-bis(trifluoromethyl)benzyl(2-carboxyethyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinolin-4-yl)carbamate.

**Metabolites M8A and M8B.** Metabolites M8A and M8B were found only in bile of rats. Both M8A and M8B displayed a protonated molecule of m/z 721, 192 Da higher than the metabolite M2, suggesting that they both were glucuronide conjugates of the hydroxylated metabolites of M2. A representative CID spectrum of M8A at m/z 721 gave two fragment ions at m/z 420 and 244. The major fragment ion at m/z 420 indicated a loss of the bis-trifluoromethylbenzyl amine moiety, and the ion at m/z 244 indicated a loss of 176 Da from the ion at m/z 420. The exact positions of the hydroxy groups could not be determined from the mass spectra.

**Metabolite M9.** M9 was found only in monkey bile and plasma. M9 displayed a protonated molecule of m/z 418, lower than the parent compound, suggesting that it was a cleaved product of torcetrapib. Accurate mass analysis of protonated ion was determined to be 424.0793 (Δ 1.0 ppm, theoretical), corresponding to an empirical formula of C12H11NO4F3 (Δ 1.0 ppm, theoretical). The CID spectrum of M9 gave fragment ions at m/z 224, 196, 186, and 170. The fragment ion at m/z 224 indicated a loss of water molecule, suggesting that the ethyl side chain was hydroxylated. The fragment ion at m/z 196 was a result of the loss of ethylene moiety from m/z 224. Based on these data, M9 was tentatively identified as the 2-hydroxyethyl-6-trifluoromethylquinoline. The exact location of hydroxyl group in M9 could not be ascertained from these data.

**Metabolite M10.** Metabolite M10 was detected only in monkeys. M10 displayed a protonated molecular ion at m/z 322.0367 in full-scan MS. Elemental analysis of these data gave a molecular formula C12H11NO4F3S, indicating a sulfate conjugation of metabolite M9. The CID spectrum of M10 gave fragment ions at m/z 242 and 224. The fragment ion at m/z 242 suggested a loss of 80 Da from the molecule, and the fragment ion at m/z 224 indicated a loss of 98 Da from ion at m/z 322. Based on these data, M10 was tentatively identified as a sulfate conjugate of hydroxyethyl-6-trifluoromethylquinoline (M9).

**Metabolites M11A and M11B.** Metabolites M11A and M11B were detected in bile and plasma of monkeys. Both metabolites displayed a protonated molecule of m/z 418, 176 Da higher than the metabolites M4 or M9, suggesting that both metabolites were glucuronide conjugates of M4 and/or M9. The CID spectra of M11A and M11B were identical and showed major fragment ions at m/z 242 and 224. The accurate mass of the fragment ion at m/z 242 was similar to metabolite M9. These data suggested that both the metabolites were glucuronide conjugates of hydroxyethyl-6-trifluoromethylquinoline (M9).

**Metabolite M12.** Metabolite M12 was detected only in rat feces and bile. M12 showed a protonated molecule of m/z 617, 16 Da higher than the parent compound. The CID product ion spectrum at m/z 617 showed fragment ions at m/z 316 and 270. The ion at m/z 617 suggested hydroxylation of torcetrapib. The fragment ions at m/z 316 and 270 resulted from loss of the methyl N-bis-trifluoromethylbenzyl carbamate moiety and subsequent loss of ethanol. The position of the hydroxy group could not be determined from the mass spectrum.

**Metabolite M13.** Metabolite M13 was detected in both rat and mouse. M13 showed a protonated molecular ion at m/z 631.1516 (30 Da higher than the parent drug). Elemental analysis of these data gave a molecular formula C26H24N2O6F9 (Δ 3.9 ppm, theoretical), suggesting the oxidation of the methyl group to a carboxylic acid. M13 showed prominent fragment ions at m/z 330, 270, and 198. The fragment ions at m/z 330 and 270 resulted from the loss of the methyl N-bis-trifluoromethylbenzyl carbamate moiety and subsequent loss of an acetic acid. The minor fragment ion at m/z 198 resulted from loss of the ethylcarbamoyloxy group from m/z 270. Based on these data, M13 was identified as 4-[bis-trifluoromethylbenzyl)-methoxycar-
boryl-amino]-2-carboxyethyl-6-trifluoromethyl-3,4-dihydro-2H-quinoline-1-carboxylic acid ethyl ester.

Metabolite M15. Metabolite M15 was detected only in monkey bile. M15 displayed a protonated molecular ion at m/z 258, 16 Da higher than M4 and M9, suggesting that it was a hydroxylated metabolite of either M4 or M9. Accurate mass determination provided molecular formula to be equivalent to C_{11}H_{9}NO_{2}F_{3}. CID product ion spectrum of M15 produced fragment ions at m/z 240, 212, 211, and 143. Based on this information, M15 was tentatively identified as hydroxyl trifluoromethyquinolinylethanol.

Metabolite M16. Metabolite M16 was detected only in monkey urine. M16 displayed a protonated molecular ion at m/z 434, 176 Da higher than M15, suggesting that it was a glucuronide conjugate of M15. Based on these data, M16 was identified as a glucuronide conjugate of hydroxyl trifluoromethyquinolinylethanol (M15).

Metabolite M17. Metabolite M17 was found only in mouse urine and plasma. M17 showed a protonated molecular ion at m/z 228. Accurate mass analysis of protonated ion determined to be 228.0636, corresponding to an empirical formula of C_{11}H_{9}NO_{2}F_{3} (±0.1 ppm, theoretical). The CID spectrum of M17 showed prominent fragment ions at m/z 210.0554 and 190.0489 (Fig. 14). Comparison of the CID spectrum and retention time of M17 with those of the synthetic standard suggested that M17 was 6-trifluoromethyquinolinylethanol.

Metabolite M18. Metabolite M18 was detected only in monkey bile. Its full-scan MS displayed a protonated molecular ion at m/z 659. The CID product ion spectrum of m/z 659 gave major fragment ions at m/z 483 and 465 and minor fragment ions at m/z 238, 141, and 113. Fragment ion at m/z 483 was due to loss of the glucuronide from the parent ion, and additional loss of a water molecule gave fragment ion at m/z 465. Fragment ion at m/z 238 resulted by cleavage of the bis-trifluoromethybenzyl moiety and subsequent losses of the glucuronide and water at the trifluoromethyl quinoline portion of the molecule. Additionally, accurate mass measurements of M18 gave an elemental composition of C_{27}H_{24}N_{2}O_{7}F_{9}. Based on these data, M18 was tentatively identified as a glucuronide conjugate of 4-(3,5-bis-trifluoromethyl-benzylamino)-2-hydroxyethyl-6-trifluoromethyl-quinoline.

Metabolite M19. Metabolite M19 was detected in monkey bile. Its full-scan MS showed a protonated molecular ion at m/z 717. The CID product ion spectrum of m/z 717 gave fragment ions at m/z 541, 523, 283, 254, and 227. Fragment ion at m/z 541, loss of 176 Da from the parent ion, suggested that it was a glucuronide conjugate. Fragment ion at m/z 523 was due to the subsequent loss of a water molecule from ion at m/z 541. Product ion at m/z 283 resulted by cleavage of the bis-trifluoromethybenzyl moiety and subsequent losses of the glucuronide and CH_{3}O (31 Da), and ion at m/z 265 was an additional loss of water from ion at m/z 283. The accurate mass measurements of M19 gave an elemental composition of C_{29}H_{26}N_{2}O_{9}F_{9}. Based on these data, M19 was tentatively identified as a glucuronide conjugate of 4-(3,5-bis-trifluoromethyl-benzyl-methoxycarbonyl-amino)-2-hydroxyethyl-6-trifluoromethyl-quinoline.

Metabolite M20. Metabolite M20 was found only in mouse urine. M20 showed a protonated molecular ion at m/z 349, 107 Da higher than M4, suggesting conjugation of M4 with taurine. The accurate mass of MH' (349.0471) proposed the empirical formula of C_{13}H_{10}N_{2}O_{3}F_{3} (±0.02 ppm, theoretical). MS/MS spectrum of M20 showed prominent fragment ions at m/z 311, 242, 224, 214, and 196. Based on these data, M20 was tentatively identified as the taurine conjugate of M4.

Metabolite M21. Metabolite M21 was found in mouse urine. Its protonated molecular ion at m/z 299, 57 Da higher than the metabolite M4, suggested that M21 was a glycine conjugate of M4. The accurate mass of MH' (299.0636) proposed the empirical formula of C_{13}H_{10}N_{2}O_{3}F_{3} (±2.6 ppm, theoretical). MS/MS spectrum of M21...
showed prominent fragments at \( m/z \) 253, 224, 214, and 196. The fragment ions at \( m/z \) 253 and 224 resulted from loss of the formic acid and glycine moiety from \( m/z \) 299, respectively. The ion at \( m/z \) 196 was characteristic of the quinoline-2-carboxylic acid (M4). Based on these data, the structure of M21 was identified as glycine conjugate of M4.

**Metabolite M22.** Metabolite M22 was found only in mouse urine. It displayed a protonated molecular ion at \( m/z \) 284, 42 Da higher than the metabolite M4, suggesting that M21 was a conjugate of M4. The accurate mass of MH\(^+\) (284.0652) proposed the empirical formula of C\(_{12}\)H\(_9\)N\(_3\)O\(_2\)F\(_3\) (\( \Delta -0.2 \) ppm, theoretical), which corresponded to a urea conjugate of M4. MS/MS spectrum of M22 gave prominent fragments at \( m/z \) 224, 214, and 196 (Fig. 14). M22 rapidly loses NH\(_3\) in the ion source to form an ion at \( m/z \) 267, with an empirical formula of C\(_{12}\)H\(_9\)N\(_3\)O\(_2\)F\(_3\). The other fragment ions at \( m/z \) 224 resulted from loss of the urea moiety from \( m/z \) 299. Based on these data, M22 was identified as the urea conjugate of M4.

**Metabolite M26.** Metabolite M26 was detected in the urine of rats dosed with \([^{14}\text{C}]\)torcetrapib as a mixture of four isomers. All four isomers displayed a deprotonated molecule, \([\text{M-H}]^-\), of \( m/z \) 380 in the negative ion mode. The product ion mass spectrum of \( m/z \) 380 gave a major fragment ion at \( m/z \) 81, which was attributed to the sulfonate moiety and minor fragment ions at \( m/z \) 348, 284, and 240. The fragment ion at \( m/z \) 348 and 284 indicated a loss of the methylamine moiety and a sulfonate moiety from \( m/z \) 380, and the ion at \( m/z \) 240 suggested the loss of the methylcarbamamoyl and sulfonate moieties from \( m/z \) 380. These data suggested that the metabolite was bisulfite adduct of methyl carbamate. The high-resolution mass spectrum of M28 suggested the empirical formula of C\(_{11}\)H\(_9\)F\(_6\)NO\(_5\)S. \(^1\text{H} NMR of M28 suggested the loss of the methylcarbamoyloxy and sulfonate moieties resulting in \( m/z \) 224, 214, and 196. The fragment ions at \( m/z \) 81 indicated a loss of the formic acid moiety and a sulfonate moiety from \( m/z \) 380. These data suggested that the metabolite was bisulfite adduct of methyl carbamate. The high-resolution mass spectrum of M28 contained two aromatic resonances (3H total), suggesting that the bis(trifluoromethyl)phenyl ring was unsubstituted. The methyl resonance at 83.61 ppm was indicative of no change to the methyl carbamate moiety, and the methine proton at 85.70 ppm was indicative of the sulfite substitution at the benzyl position (Fig. 15). Based on these data, M28 was identified as 3,5-bis(trifluoromethyl)phenyl) (methoxycarbonylaminomethanesulfonic acid. The structure of M28 was consistent with heteronuclear multiple-bond correlation spectroscopy correlations and was unequivocally confirmed by comparison of its HPLC retention time and mass spectrum with those of the synthetic standard.

**Discussion**

We report here the metabolic fate and disposition of torcetrapib in rats, monkeys, and mice, the animal species used for safety toxicology and carcinogenicity studies. \([^{14}\text{C}]\)torcetrapib, labeled at the trifluoromethyl-3,4-dihydro-2H-quinoline moiety, was administered orally to rats, monkeys, and mice. Additionally, \([^{14}\text{C}]\)torcetrapib labeled at the benzylic carbon of the bis-trifluorophenyl ring was administered to SD rats to determine the fate of bis-trifluoromethylbenzoic acid formed by N-dealkylation of the tetrahydroquinoline moiety of torcetrapib. The use of the C-14 label at two different positions not only facilitated the tracing of metabolites that were formed through oxidative cleavage of torcetrapib but also aided in their identification. The administered radioactive dose was quantitatively recovered in all species (rat, 93.4/94.4%; monkey, 86.8%; and mouse, 90.7%). Excre-
tion of the radioactivity was rapid and nearly complete within 48 h after dosing. In the rats and monkeys, the majority of dose was excreted in feces, whereas in the mice, the dose was recovered equally in urine and feces. In the separate studies using bile duct-cannulated rats and monkeys, <10% % of the administered radioactivity was recovered in bile. This suggested that a major portion of dose excreted in the feces of rats and monkeys was mainly due to unabsorbed dose. There were no discernible gender differences in the excretion pattern of radioactivity in these species after oral administration of [14C]torcetrapib-A. However, pharmacokinetics of torcetrapib and total radioactivity displayed distinct gender-related differences in male and female rats and mice but not in monkeys. The exposure (Cmax and AUC) of torcetrapib and total radioactivity was higher in females compared with males. In addition, the t1/2 values of total radioactivity and unchanged torcetrapib were similar in monkeys, but the t1/2 of total radioactivity was 2.7- and 8-fold greater than the t1/2 of the parent compound in rats and mice, respectively, suggesting that one or more of the metabolites circulated for a longer time in these species. The gender-related differences in the pharmacokinetics of xenobiotics, especially for rats, have been well known and can be the result of the differences in hormone levels, plasma protein binding, and/or expression level of drug-metabolizing enzymes (Tanaka et al., 1991a,b; Prakash and Soliman, 1997).

The urine and/or bile radiochromatograms from rats, monkeys, and mice indicate that torcetrapib is extensively metabolized before excretion because no unchanged drug was detected in urine or bile. The major portion of administered radioactivity was excreted in urine and bile as the products of oxidation and conjugation pathways. There were no gender-related qualitative differences in the profile of metabolites. However, there were notable species-related qualitative and quantitative differences in the metabolic profiles. More than 28 metabolites were identified in all species by ion spray LC/MS/MS (Kamel and Prakash, 2006; Prakash et al., 2007). A proposed scheme for the biotransformation pathways of torcetrapib in rats, monkeys, and mice is shown in Fig. 16. The primary metabolic pathway in all species involved hydrolysis of the ethyl carbamate moiety to form metabolite M2, which then underwent oxidation at the ethyl group with subsequent glucuronidation and/or aromatization of the tetrahydroquinoline moiety. The other major metabolites were due to multiple oxidation and conjugation of the trifluoromethyl-3,4-dihydro-2H-quinoline moiety.

**Fig. 16.** Proposed biotransformation pathways of [14C]torcetrapib in rats, monkeys, and mice.
The major circulating and excretory metabolites in rats, monkeys, and mice were species-dependent; however, several common metabolites were observed in more than one species (e.g., M1, M3, M4, M8A, M8B, M11, and M13). The major components of drug-related material in rat excreta were identified as M4 (10.8%), M5 (6.39%), M7 (6.81%), M12 (4.1%), and M13 (4.39%). The metabolic profile in male and female rats was qualitatively similar. However, the metabolite M13, the acid derivative of torcetrapib, was 3-fold higher in the feces of male rats than that of females. Two major circulating metabolites, M3 and M4, were detected at 4 and 6 h postdose in the male and female rats. The percentage of the circulating metabolite M4 was similar to that of parent at both time points, whereas the M3/torce-trapib ratio was 0.8 and 0.46 at 4 and 6 h postdose, respectively. Studies with [14C]torcetrapib, with the label at the benzylic position, indicated that BTFMBA (M1) was the major metabolite in rats and accounted for 94% of the circulating radioactivity, although only 3.5% of the total dose was excreted as M1. Glucuronidation was the primary route of clearance of M1 and represented 13.3% of the total dose in rats. In addition, an unusual and novel metabolite, a bisulfite adduct of methyl carbamate (M28), was also detected in the urine and accounted for 8.67% of the total dose.

The major oxidative metabolites in monkeys were due to multiple oxidations to form 2-hydroxyethyl-6-trifluoromethylquinoline metabolite (M9). It was further metabolized to form major metabolites, M10 (sulfate conjugate), M11 (glucuronide conjugate), and M16 (a glucuronide conjugate of its hydroxyl metabolite, M15). The metabolite M10, the sulfate conjugate, was 3.5-fold higher in the excreta of males than that of females. Two major circulating metabolites were identified as M8, M9, and M10 in both male and female monkeys.

In addition to unchanged torcetrapib, a total of nine metabolites were identified in mice. Of all drug-related materials, unchanged torcetrapib in feces comprised the greatest abundance, at 36.3% and 31.4% of dose in males and females, respectively. Metabolites M20 (taurine conjugate) and M21 (glycine conjugate), both in urine, were present at 8.9 and 8.3% of the dose, respectively. All other excretory metabolites were present at less than 6% of dose and included M2, M3, M4, M6, M13, M17, and M22. The major circulating metabolites in mice include M4 and M11, which ranged from 25 to 47% of total radioactivity. Torcetrapib comprised 6.8 and 11.7% of radioactivity in males and females, respectively.

In this study, several novel and unusual quinoline metabolites (M3, M4, M5, M9, M17, and M22), and a sulfite conjugate, M28, formed by multiple pathways (N-dealkylation, oxidation, aromatization, and sulfation), were identified. The structures of two metabolites, M4 and M28, were elucidated by accurate mass measurements and LC/NMR. M4 was detected only in rats dosed with [14C]torcetrapib labeled at the tetrahydroquinoline ring, suggesting loss of the bis-(trifluoromethyl)phenyl ring of torcetrapib. One of the spin systems (two doublets coupled to each other and a singlet) in the 1H NMR of M4 was consistent with the presence of the phenyl ring containing one trifluoromethyl group. The other spin system (two doublets coupled to each other) indicated the aromatization of the tetrahydroquinoline of torcetrapib. The aromatization of the N-substituted tetrahydroquinoline and tetrahydropyridines has been reported and catalyzed by P450s, monoamine oxidases, horseradish peroxidase, and myeloperoxidase (Shaffer et al., 2001; Dalvie and O’Connell, 2004; Gu et al., 2006; Obach and Dalvie, 2006). The mechanism and the enzyme(s) involved for the formation of M4 are not known at this time.

On the other hand, sulfonic acid conjugate metabolite M28 was detected only in rats dosed with [14C]torcetrapib labeled at the benzylic carbon of the bis-trifluoromethylphenyl ring, suggesting loss of the tetrahydroquinoline ring of torcetrapib. The presence of proton resonances at 8.08 ppm (2H) and 7.93 ppm (1H) in the 1H NMR spectrum of M28 was consistent with the aromatic ring that contains two trifluoromethyl groups. The methine resonance at 85.70 ppm suggested that the substitution of a sulfonate group had occurred at the benzylic position. The proton resonance at 83.61 ppm (3H) and its corresponding carbon resonance at 54 ppm were consistent with the methyl group of the carbamate moiety. The mechanism for the formation of M28 is under investigation and will be reported separately. These types of sulfonic acid metabolites are rare but have been reported and are formed by either degradation of cysteine and glutathione conjugates or direct addition of sulfite ion (Yoshino et al., 1993; Chen et al., 2003; He et al., 2003). Therefore, the formation of M28 can be speculated by initial formation of the methyl bis(trifluoromethyl)-benzylidene-carbamate followed by conjugation with glutathione, subsequent cleavage of the C-S bond (catalyzed by C-S lyase), and oxidation of SH to sulfite as reported by Yoshino et al. (1993) (Fig. 17). The similar electrophilic metabolite and GSH con-
jugate of capsaicin have been reported in laboratory animals (Reilly and Yost, 2006).

In summary, torcetrapib was primarily cleared by metabolism in the nonclinical species. The primary metabolic pathway of torcetrapib involved hydrolysis of the carbamate ester (M2) and the oxidation of the ethyl moieties. M2 was further metabolized by oxidative cleavage to novel and unusual quinoline metabolites and 3, 5-bistrifluoromethyl benzoic acid (M1). The major circulating and excretory metabolites in mice, rats, and monkeys were species-dependent; however, several common metabolites were observed in more than one species.

Acknowledgments. We thank Klaas Schildknecht, Roger Ruggeri, and Gregory Dolnikowski for providing radiolabeled torcetrapib and synthetic metabolite standards, Jian Lin and Beth Obach for technical assistance, and David Plowchalk and Scott Obach for helpful discussions.

References
E-mail: chandra.prakash@biogenidec.com

Address correspondence to: Dr. Chandra Prakash, Drug Metabolism and Pharmacokinetics, Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142.
E-mail: chandra.prakash@biogenidec.com


IN VIVO METABOLISM OF TORCETRAPIB IN PRECLINICAL SPECIES

Downloaded from dmd.aspetjournals.org at ASPET Journals on June 15, 2017