Pharmacokinetics, Metabolism, and Disposition of Rivoglitazone, a Novel Peroxisome Proliferator-Activated Receptor γ Agonist, in Rats and Monkeys

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ABSTRACT:
The pharmacokinetics, metabolism, and excretion of rivoglitazone [(RS)-5-{[6-methoxy-1-methyl-1H-benazimidazol-2-yl][methoxy]benzyl}-1,3-thiazolidine-2,4-dione monohydrochloride], a novel thiazolidinedione (TZD) peroxisome proliferator-activated receptor γ selective agonist, were evaluated in male F344/DuCrj rats and cynomolgus monkeys. The total body clearance and volume of distribution of rivoglitazone were low in both animals (0.329-0.333 ml per min/kg and 0.125-0.131 l/kg for rats and 0.310-0.371 ml per min/kg and 0.138-0.166 l/kg for monkeys), and the plasma half-life was 4.55 to 4.84 h for rats and 6.21 to 6.79 h for monkeys. The oral bioavailability was high (>95% in rats and >76.1% in monkeys), and the exposure increased dose proportionally. After administration of [14C]rivoglitazone, radioactivity was mainly excreted in feces in rats, whereas radioactivity was excreted in urine and feces with the same ratio in monkeys. Because excreted rivoglitazone in urine and bile was low, metabolism was predicted to be the main contributor to total body clearance. The structures of 20 metabolites (M1-M20) were identified, and 5 initial metabolic pathways were proposed: O-demethylation, TZD ring opening, N-glucuronidation, N-demethylation, and TZD ring hydroxylation. O-Demethylation was the main metabolic pathway in both animals, but N-demethylation and TZD ring hydroxylation were observed only in monkeys. N-Glucuronide (M13) was nonenzymatically hydrolyzed to TZD ring-opened N-glucuronide (M9), and the amount of these metabolites in monkeys was larger than that in rats. In plasma, rivoglitazone was observed as the main component in both animals, and O-demethyl-O-sulfate (M11) was observed as the major metabolite in rats but as many metabolites in monkeys.

Introduction
Rivoglitazone, (RS)-5-{[6-methoxy-1-methyl-1H-benazimidazol-2-yl][methoxy]benzyl}-1,3-thiazolidine-2,4-dione monohydrochloride (Fig. 1), is a novel thiazolidinedione (TZD) that selectively activates the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (Kanda et al., 2009). PPARγ regulates the expression of genes involved in glucose and lipid metabolism, and the TZD-containing drugs such as pioglitazone and rosiglitazone are thought to exert their antidiabetic effects via the activation of PPARγ (Diamant and Heine, 2003; Semple et al., 2006).

In phase II dose-ranging trials of 6 and 26 weeks in duration, rivoglitazone treatment improved glycemic control in participants with type 2 diabetes mellitus and showed a safety profile consistent with the specific side effects (e.g., weight gain, edema, and hemodilution) observed in clinical development of the currently marketed TZD-containing drugs (Rohatagi et al., 2008). Rivoglitazone has linear pharmacokinetics in a dose range intended for future use, a half-life consistent with once-daily dosing, and a very low renal clearance in humans. In pharmacokinetic-pharmacodynamic analysis based on modified indirect-response models, the changes in fasting plasma glucose, hemoglobin A1C, and hemodialysis are well characterized as a function of rivoglitazone plasma concentrations (Rohatagi et al., 2008).

The present studies were performed to characterize the pharmacokinetics, metabolism, and disposition of rivoglitazone in rats and monkeys, which were used in the safety and pharmacology assessment. In particular, qualitative and quantitative profiles of metabolites in urine, bile, and feces after oral administration of [14C]rivoglitazone were obtained, and the contribution of metabolic pathways in vivo was evaluated. In addition, the quantitative profiles of plasma metabolites
Analytical or high-performance liquid chromatography (HPLC) grade. All other reagents and solvents used were commercially available and either of Rivoglitazone and [14C]rivoglitazone were dissolved in the 20% (w/v) HP-Cyclodextrin (HP-CD) solution.

Materials and Methods

**Materials.** Rivoglitazone, internal standards (ISs) (R-121171, R-132064, R-252121, R-252122, and R-395374; Fig. 1), synthetic standards of metabolites (O-demethyl-O-sulfate-TZD ring-opened methyl sulfoxide amide (M1), O-demethyl-O-sulfate-TZD ring-opened methyl sulfone amide (M3), TZD ring-opened methyl sulfoxide carboxylic acid (M5), TZD ring-opened methyl sulfoxide amide (M6), TZD ring-opened methyl sulfoxide carboxylic acid (M7), O-demethyl-TZD ring-opened methylmercapto amide (M8), TZD ring-opened N-glucuronide (M9), TZD ring-opened methyl sulfone amide (M10), O-demethyl-O-sulfate (M11), O-demethyl rivoglitazone (M12), N-glucuronide (M13), TZD ring-opened S-cysteine conjugate (M15), O,N-dimethyl rivoglitazone (M16), N-demethyl rivoglitazone (M17), TZD ring 5-hydroxy rivoglitazone (M18), TZD ring-opened methylmercapto carboxylic acid (M19), and TZD ring-opened methylmercapto amide (M20) were synthesized by Daiichi Sankyo Co., Ltd. or Daiichi Sankyo RD Associe Co., Ltd. (Tokyo, Japan). [14C]Rivoglitazone was synthesized by GE Healthcare (Little Chalfont, Buckinghamshire, UK). The specific radioactivity was 30.0 to 35.4 Ci/mg, and the radiochemical purity was 98.2 to 99.6%. Hydroxypropyl-β-cyclodextrin (HP-β-CD) was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). All other reagents and solvents used were commercially available and either of analytical or high-performance liquid chromatography (HPLC) grade.

**Animal Studies.** All animal studies were conducted with approval in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. and Mitsubishi Chemical Medience Corporation (Ibaraki, Japan). We used male F344/DuCrlCrlj rats, which were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and male cynomolgus monkeys, which were purchased from CLEA Japan, Inc. (Tokyo, Japan), Japan Laboratory Animals, Inc. (Tokyo, Japan), and Celeste Corporation (Tokyo, Japan). All animals were acclimated to the experimental conditions before use at least 5 days for rats and 53 days for monkeys. Food and water were supplied ad libitum throughout the acclimatization and experimental period; however, before administration, the animals were fasted over-night and fed at 8 h postdose for rats and at least 6 h postdose for monkeys. Rivoglitazone and [14C]rivoglitazone were dissolved in the 20% (w/v) HP-β-CD solution.

Pharmacokinetic and Plasma Metabolite Profiling Studies in Rats. Rivoglitazone was administered intravenously via the tail vein and by oral gavage to five parallel groups (0.1 and 0.5 mg/kg for intravenous administration and 0.1, 0.5, and 1 mg/kg for oral administration, 153–175 g, n = 4 for each group) for the pharmacokinetic study and by oral dosing (1 mg/kg, 158–162 g, n = 4) for the plasma metabolite profiling study. Approximately 200 μl of blood was collected from the femoral vein with a heparinized syringe at the following times under diethyl ether anesthesia: predose and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose for the intravenous dose; and predose and 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose for the oral dose. Plasma was obtained by centrifugation at 4°C and stored at −80°C until analysis.

Mass Balance Study in Rats. [14C]Rivoglitazone (1 mg/kg, 30.0 μCi/kg) was administrated intravenously via the tail vein (190–195 g, n = 4) or by oral gavage (174–179 g, n = 4) to rats. After dosing, each rat was housed individually in a glass metabolic cage (Sugiyama-gen Co., Ltd., Tokyo, Japan), and urine and feces were collected in a water bath for 168 h at 0 to 24, 24 to 48, 48 to 72, 72 to 120, and 120 to 168 h postdose.

Mass Balance Study in Bile Duct-Cannulated Rats. Rats were subjected to cannulation with a flexible polyethylene tube (PE-10; Nippon Becton Dickinson Company, Ltd., Tokyo, Japan) into the common bile duct and fixed in the abdominal aorta at 6 h after oral dosing of [14C]rivoglitazone (5 mg/kg, 150 μCi/kg) to rats (177–195 g, n = 10), and the plasma was obtained by centrifugation at 4°C. Urine was collected for the periods of 0 to 8, 8 to 24, and 24 to 48 h postdose after oral dosing of [14C]rivoglitazone (1 mg/kg, 34.6 μCi/kg) to bile duct-cannulated (BDC) rats (149–153 g, n = 4). Feces was collected for the periods of 0 to 24 and 24 to 48 h postdose after oral dosing of [14C]rivoglitazone (1 mg/kg, 34.6 μCi/kg) to bile duct-cannulated (BDC) rats (149–153 g, n = 4).
dosing of [14C]rivoglitazone (1 mg/kg, 35.4 Ci/g/kg) rats (179–187 g, n = 4). All samples were stored at −80°C until analysis.

Pharmacokinetic and Plasma Metabolite Profiling Study in Monkeys.

The pharmacokinetic studies were conducted with 2-week washout periods between doses. The monkeys (3.40–5.25 kg, n = 4) were dosed intravenously via a saphenous vein and orally via the nose using flexible tubing with a 2-ml water rinse in the following dosing order: 0.0986 mg/kg p.o., 0.0986 mg/kg i.v., 0.493 mg/kg p.o., 0.493 mg/kg i.v., 0.986 mg/kg p.o., and 0.986 mg/kg i.v. Blood (1 ml) was collected from the femoral vein with a heparinized syringe at predose and 0.25, 0.5, 1, 2, 3, 5, 7, and 24 h postdose for intravenous dosing and at predose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose. Plasma was obtained by centrifugation at 4°C and stored at −20 or −80°C until analysis.

Mass Balance Study in Monkeys.

Monkeys (5.1–6.9 kg, n = 3) were given intravenous or oral doses of [14C]rivoglitazone (1 mg/kg, 30 µCi/kg) and housed individually in stainless steel metabolic cages. The urine and feces were collected at 8 (urine only), 24, 48, 72, 96, 120, 144, and 168 h postdose. The cage was washed with ethanol/water (1:1, v/v) after the last collection of urine (168 h). The blood for qualitative metabolite profiles was collected from the cranial saphenous vein or the cephalic vein of the forearm in the test tube containing heparin sodium at 6 h postdose after oral dosing. Plasma was obtained by centrifugation at 4°C and stored at −20°C.

Sample Collection for Quantitative and Qualitative Metabolite Profiling in Monkeys.

[14C] Rivoglitazone (1 mg/kg, 34.6 µCi/kg) was orally administered to monkeys (3.5–4.3 kg), and urine at 0 to 8, 8 to 24, and 24 to 72 h (n = 3) and bile in the gallbladder at 8 h (n = 2) were collected. For sampling of feces, [14C] rivoglitazone (1 mg/kg, 35.4 µCi/kg) was orally administered to male cynomolgus monkeys (3.28–3.85 kg, n = 4), and the feces were collected for the periods of 0 to 24 and 24 to 72 h postdose. All samples were stored at −80°C until analysis.

Sample Analysis. Quantitative analysis of plasma rivoglitazone and its metabolites. Plasma concentrations of rivoglitazone and its metabolites in rats and monkeys were determined by liquid chromatography (LC)-tandem mass spectrometry (MSMS). For the pharmacokinetic study, the plasma sample (100 µl) including study samples or control plasma (for standard and quality controls) was mixed with 100 µl of internal standard (500 ng/ml R-121171 in ethyl acetate) and 100 µl of ethyl acetate for study samples or standard or quality control samples in ethyl acetate and ethyl acetate (3 ml). Then, it was shaken for 10 min at room temperature and centrifuged at 4°C. The organic solvent layer was transferred to another tube and evaporated by nitrogen stream, and the residue was dissolved in a 1-ml mobile phase for HPLC (described below) and then analyzed by LC-MS/MS. A Quattro II mass spectrometer (Waters, Manchester, UK) connected to an Alliance 2690 Separations Module (Waters) was used as the LC-MS/MS system. An Inertsil ODS-3 column (2.1 × 150 mm, 2 µm; GL Science Inc., Tokyo, Japan) was used as the chromatography column, and a mixture of acetonitrile/water/acetic acid (80:20:0.2, v/v/v) was used as the mobile phase at a flow rate of 0.2 ml/min. Analyte detection was achieved with electrospray ionization (ESI) in the positive ion mode using multiple reaction monitoring (MRM) of transitions unique to each compound: m/z 592 → 176 for M6, m/z 592 → 161 for M9, m/z 418 → 176 for M10, m/z 464 → 384 for M11, m/z 384 → 162 for M12, m/z 574 → 161 for M13, m/z 492 → 176 for M15, m/z 370 → 148 for M16, m/z 384 → 162 for M17, m/z 414 → 176 for M18, m/z 387 → 176 for M19, and m/z 386 → 176 for M20. The MRM of transitions for the IS was m/z 404 → 182 for R-121171, m/z 469 → 389 for R-132064, and m/z 389 → 167 for R-252121 and R-252122. The sample was separated on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm; Waters) and maintained at 40°C with gradient elution using mobile phase A (methanol/water/ammonium acetate, 950:0.385: v/v/v) and mobile phase B (methanol/water/ammonium acetate/formic acid, 50:50:0.385: v/v/v). The elution was achieved using a linear gradient: 0 min at 40% of mobile phase B, hold for 0.9 min, to 45% B at 1 min, hold for 0.6 min, to 70% B at 1.7 min, hold for 1.7 min, to 90% B at 3.5 min, hold for 1 min, and back to 40% B at 4.6 min, hold for 2.4 min. The flow rate was 0.4 ml/min except for 0.6 min from 3.5 to 4.5 min.

Radioactivity analysis. Aliquots of plasma (100 µl), bile (20–50 µl), and urine (100 µl–1 ml) and the cage wash sample (1 ml) were mixed with tissue solubilizer (1–2 ml of NCS-II; GE Healthcare Japan Corporation, Tokyo, Japan) and a scintillation cocktail (10 ml of Hionic-Fluor; PerkinElmer Life and Analytical Sciences, Waltham, MA, or Clear-sol I; Nacalai Tesque, Kyoto, Japan), and then analyzed with a liquid scintillation analyzer (Tri-Carb 2250CA; PerkinElmer Life and Analytical Sciences). Aliquots of blood (100 µl) were mixed with NCS-II (1 ml) and hydrogen peroxide (300 µl; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and allowed to stand overnight at room temperature. Radioactivity was measured using a liquid scintillation analyzer.

For mass balance studies in rats, feces were soaked in water (25 ml) and refrigerated overnight, and methanol (30 ml) was subsequently added and homogenized. Aliquots of homogenate (100–500 µl) were mixed with a tissue solubilizer (1–2 ml of NCS-II) and a scintillation cocktail (10 ml of Hionic-Fluor) and then were analyzed with a liquid scintillation analyzer. For mass balance studies in BDC rats and fecal metabolite profiling studies in rats and monkeys, the fecal sample was mixed with water of approximately 9-fold volumes of the sample weight and then homogenized. The fecal homogenate (500 µl) placed on a combust pad and weighed and then combusted with a sample oxidizer. The resulting 14CO2 was absorbed in Carbo-Sorb E (6 ml; PerkinElmer Life and Analytical Sciences), mixed with a scintillation cocktail (9 ml, Permafluor E+; PerkinElmer Life and Analytical Sciences), and then analyzed with the liquid scintillation analyzer.

For mass balance studies in monkeys, after fecal samples were lyophilized, the total weight of the samples was measured and powdered using a mixer. The sample was weighed out into a vial containing tissue solubilizer (1 ml; Nacalai Tesque), subjected to mechanical solubilization (Biomerit instrument; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) for 20 min, mixed with a scintillation cocktail (15 ml), and then analyzed by the liquid scintillation analyzer. For mass balance studies in rats, the inside of cages was washed with water, and the radioactivity in the washing solutions was added to the recoveries of urine.

Sample Preparation for Qualitative Metabolite Profiling by Radioactivity Detection High-Performance Liquid Chromatography and Structure Analysis of Metabolites. The plasma samples collected at 6 h postdose were pooled from each animal. The plasma sample (2 ml) was extracted with acetonitrile (4 ml) and centrifuged at 4°C. The supernatant was saved and the pellet was re-extracted with acetonitrile (4 ml) and centrifuged at 4°C. The supernatants were combined and evaporated to dryness under a nitrogen stream. The residue was dissolved in acetonitrile/water (2:8, v/v), and an aliquot was analyzed by radio-HPLC, LC-mass spectrometry (MS), and LC-MS/MS for structure analysis of the metabolites.

The urine samples were pooled from each animal on a percent weight basis. The pooled urine sample (8 ml) was lyophilized. The residue was dissolved in acetonitrile/water (2:8, v/v), and the aliquot was analyzed by radio-HPLC, LC-MS, and LC-MS/MS for structure analysis of the metabolites. The remaining urine sample was used for the purification of the metabolites (several metabolites) by HPLC. The purified metabolites were also analyzed by LC-MS and LC-MS/MS.

The bile samples from each subject were pooled on a percent weight basis. The pooled bile sample (8 ml) was centrifuged at 4°C. An aliquot of the supernatant was analyzed by radio-HPLC, LC-MS, and LC-MS/MS for structure analysis of the metabolites. The remaining bile sample was also used for...
the purification of the metabolites (several metabolites) by HPLC. The purified metabolites were analyzed by LC-MS and LC-MS/MS.

The fecal homogenates samples were pooled from each animal on a percent weight basis. The fecal homogenate sample (2 ml) was extracted with acetonitrile (4 ml) and centrifuged at 4°C. The supernatant was saved, and the pellet was re-extracted with acetonitrile (4 ml) and centrifuged at 4°C. The supernatants were combined and evaporated to dryness under a nitrogen stream. The residue was dissolved in acetonitrile/water (2:8, v/v), and the aliquot was analyzed by radio-HPLC, LC-MS, and LC-MS/MS for structure analysis of the metabolites.

Sample Preparation for Quantitative Metabolite Profiling. Bile and urine samples. A portion (1 ml) of the urine and bile samples was concentrated by a centrifugal evaporator (CVE-2000; Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and its supernatant was analyzed by radio-HPLC.

Fecal samples. The fecal homogenate (1 ml) was weighed and mixed with acetonitrile (2-fold volumes). The mixture was centrifuged at 4°C, and then the resulting supernatant was collected. After the precipitate was added with acetonitrile (same volume), sonicated for 5 min, and mixed, it was extracted twice as described above. The extracts were combined and weighed. An aliquot of the extract (100 μl) was subjected to radioactivity measurement by the liquid scintillation analyzer. The residual extract was evaporated under a nitrogen stream at 40°C, and the resulting residue was dissolved in acetonitrile/water (3:7, v/v; 500 μl). The solution was centrifuged at 4°C, and the supernatant was analyzed by radio-HPLC.

Qualitative and Quantitative Metabolite Profiling by Radio-HPLC. Metabolites in plasma, urine, bile, and feces were analyzed by radio-HPLC. Radiochromatographic analysis of samples was performed on an L-6000 HPLC system (Hitachi, Ltd., Tokyo, Japan) combined with a Radiomatic 525TR radioactivity detector (PerkinElmer Life and Analytical Sciences) or an L-2000 HPLC system (Hitachi, Ltd.) at 4°C combined with a Radiomatic 625TR radioactivity detector. Chromatographic separation was performed on a YMC-Pack ODS-A column (6.0 × 150 mm, 5 μm; YMC Co., Ltd., Kyoto, Japan) at ambient temperature. The mobile phase, consisting of water containing 0.01% trifluoroacetic acid (TFA) (solvent A) and acetonitrile containing 0.01% TFA (solvent B), was delivered at a flow rate of 1 ml/min. The gradient started at 12% solvent B, increased linearly to 60% solvent B for 30 min, increased linearly to 90% solvent B for 5 min, and then was held at 90% solvent B for 5 min. The flow rate was set at 0.1 ml/min, and the elution flow from the HPLC system was introduced into the quadrupole time-of-flight mass spectrometer ionization source through an ESI interface.

Whole Blood/Plasma Concentration Ratio of Rivoglitazone. To 1.98 ml of rat and monkey blood, 20 μl of [14C]rivoglitazone in methanol solution was added (final concentrations of 0.1, 1, and 5 μg/ml) and incubated at 37°C for 1 h. The radioactivity of 100 μl of blood (C₀) and 100 μl of plasma (Cₚ), which was obtained after centrifugation at 15,000 rpm of 400 μl of blood for 2 min at 4°C, was measured. The blood/plasma concentration ratio (Rₚ) was calculated by Cₚ/C₀.

Pharmacokinetic Analysis. Pharmacokinetic parameters were calculated using a noncompartmental model with computer software WinNonlin Professional (version 4.0.1; Pharsight, Mountain View, CA). The area under the plasma concentration versus the time curve up to the last quantifiable time (AUC₀–inf) was calculated by the linear trapezoidal rule. The AUC up to infinity (AUC₀–inf) was calculated by the following formula:

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\text{AUC}_{0–\text{inf}} = \text{AUC}_{0–\text{t}} + C_{\text{t}}A_{\text{t}}
\]

where Cₜ and λₜ are the plasma concentration at the last quantifiable time and the elimination rate constant associated with the terminal phase, respectively. The maximum plasma concentration (Cmax) and the time to reach Cmax (Tmax) were obtained from the observed data. The terminal elimination half-life (t½) was calculated by ln(2)/λ. The total body clearance (CLtot/F) and oral clearance (CLint/F) were calculated as the ratio of dose to AUC₀–inf for intravenous and oral dosing, respectively. The volume of distribution at steady-state (Vₛ) was calculated as dose × AUMC/(AUC₀–inf)², where AUMC is the area under the first moment of the plasma concentration-time curve. Oral bioavailability (Foral) for each dose was calculated as (AUC₀–inf/oral dose)/(AUC₀–inf for intravenous dose) at the same dose. For rats, the AUC₀–inf for the intravenous dose was used as the mean value, and for monkeys, the AUC₀–inf for the oral dose and AUC₀–inf for the intravenous dose were used for the individual monkey.

Results

Pharmacokinetics. Pharmacokinetic parameters after intravenous and oral administration of rivoglitazone to rats and monkeys are summarized in Tables 1 and 2, respectively. For intravenous dosing, the AUC exhibited a dose-proportional increase in rats (0.1 and 0.5 mg/kg) and in monkeys (from 0.0986 to 0.986 mg/kg). The CLint and Vₛ were similar and were low values in both species: 0.329 to 0.333 ml per min/kg and 0.125 to 0.131 l/kg for rats and 0.310 to 0.371 ml per min/kg and 0.138 to 0.166 l/kg for monkeys. The mean t½ in rats was 4.55 to 4.84 h and that in monkeys was 6.21 to 6.79 h. For oral dosing, AUC₀–inf, AUC₀–int, and Cmax exhibited a dose-proportional increase in rats (from 0.1 to 1 mg/kg) and in monkeys (from 0.0986 to 0.986 mg/kg). The mean Tₘₚ was 1.13 to 3.25 h for rats and 3.00...
to 4.25 h for monkeys. The mean $F_{\text{oral}}$ was high: >95.0% in rats and >76.1% in monkeys. The $t_{1/2}$ for oral dosing was similar to that for intravenous dosing: 4.35 to 4.98 h for rats and 7.34 to 8.33 h for monkeys. The mean $CL_{\text{oral}}$ exhibited similar values to those for $CL_{\text{rat}}$: 0.304 to 0.354 ml per min/kg for rats and 0.410 to 0.485 ml per min/kg for monkeys. The mean $R_{98}$ of rivoglitazone was 0.593 to 0.671 in rats and 0.710 to 0.810 in monkeys, respectively.

**Excretion of Radioactivity.** Excretion of radioactivity after intravenous and oral administration of [14C]rivoglitazone to rats and monkeys is summarized in Table 3. After intravenous and oral administration of [14C]rivoglitazone (1 mg/kg) to rats, the mean was 25.6 and 36.7 and 53.0% of the oral dose, which was recovered in urine and feces up to 168 h (Table 3), respectively. Almost all the radioactivity was excreted within 48 h, and the mean was 99.7% of the intravenous dose and 99.1% of the oral dose.

In BDC rats, the mean biliary excretion up to 48 h was 55.4% for intravenous dosing (0.5 mg/kg) and 37.2% for oral dosing (0.5 mg/kg), which was lower than the expected values based on the fecal recovery of radioactivity up to 48 h in intact rats: 74.2% of dose for intravenous dosing and 81.1% of dose for oral dosing. In addition, the mean urinary and fecal excretion in BDC rats was 18.4 and 7.0% for intravenous dosing and 19.9 and 19.1% for oral dosing, respectively. After intravenous and oral administration of [14C]rivoglitazone (1 mg/kg) to monkeys, the mean was 47.4 and 38.4% of the intravenous dose, and 63.7 and 53.0% of the oral dose, which was recovered in urine and feces up to 168 h, respectively.

**Qualitative Metabolic Profiles of [14C]Rivoglitazone in Rats and Monkeys.** Representative radiochromatograms of the metabolites in rat plasma, urine, bile, and feces after a single oral administration of [14C]rivoglitazone are shown in Fig. 2. Representative radiochromatograms of the metabolites in monkey plasma, urine, bile, and feces after a single oral administration of [14C]rivoglitazone are shown in Fig. 3. Chromatographic analysis and further LC-MS revealed 20 metabolite peaks in rat and monkey plasma, urine, bile, and feces. These metabolite peaks were designated as M1 to M20. Major components were elucidated as follows: rivoglitazone and M11 in rat plasma, M6 and M11 in rat urine, M11 in rat bile, rivoglitazone and M12 in rat feces, rivoglitazone in monkey plasma, M11 in monkey urine, M11 and M13 in monkey bile, and rivoglitazone and M12 in monkey feces.

**Structure Analysis of Metabolites by LC-MS/MS.** The structures of the 20 metabolites were elucidated by LC-MS/MS (Table 4). The elucidated structures of 17 metabolites were further identified by comparing their mass spectra and retention times on HPLC with those of synthetic standards (Supplemental Figs. S1–S12).

**Structure Analysis of Rivoglitazone for Metabolite Analysis.** The positive ion LC-MS spectrum of rivoglitazone showed a protonated molecule [M + H]$^+$ at $m/z$ 398. The LC-MS/MS spectrum of rivoglitazone, which was obtained by collision-induced dissociation of the ion [M + H]$^+$ at $m/z$ 398, and the proposed fragmentation scheme are shown in Fig. 4. Product ions at $m/z$ 161, 176, 282, and 327 were obtained. Among the product ions, two were characteristic of substructures of rivoglitazone. The most intense product ion at $m/z$ 176 was formed via the loss of 222 Da from the ion [M + H]$^+$ at $m/z$ 398. The loss of 222 Da corresponded to the TZD ring-substituted p-methylphenoxy moiety. The product ion at $m/z$ 282 formed via the loss of the TZD ring from the ion [M + H]$^+$ at $m/z$ 398 was useful in elucidating the location of the metabolism.

**Structure Analysis of M12, M11, M4, and M2.** The positive ion LC-MS spectrum of M12 showed a protonated molecule [M + H]$^+$ at $m/z$ 384, which was 14 Da lower than that of rivoglitazone. The LC-MS/MS spectrum of the ion [M + H]$^+$ at $m/z$ 384 and the proposed fragmentation scheme of M12 are shown in Supplemental Fig. S13. Product ions at $m/z$ 147, 162, 268, and 313 were obtained. Furthermore, the LC-MS/MS spectrum and HPLC retention time of M12 were identical to those of the synthetic standard. Based on these results, M12 was identified as an O-demethyl rivoglitazone.

The positive ion LC-MS spectrum of M11 showed a protonated molecule [M + H]$^+$ at $m/z$ 384, which was 14 Da lower than that of rivoglitazone. The LC-MS/MS spectrum of the ion [M + H]$^+$ at $m/z$ 384 and the proposed fragmentation scheme of M12 are shown in Supplemental Fig. S13. Product ions at $m/z$ 147, 162, 268, and 313 were obtained. Furthermore, the LC-MS/MS spectrum and HPLC retention time of M12 were identical to those of the synthetic standard. Based on these results, M12 was identified as an O-demethyl rivoglitazone.
The positive ion LC-MS spectrum of M4 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 560\), which was 176 Da higher than that of M12. The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 560\) and the proposed fragmentation scheme of M4 are shown in Supplemental Fig. S14. Product ions at \(m/z\ 147, 162, 268, 338\), and 384 were obtained. The most intense product ion at \(m/z\ 384\) was formed via the loss of 176 Da from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 560\). The loss of 176 Da corresponded to the glucuronic acid moiety. The product ion at \(m/z\ 338\) was formed via the loss of 222 Da from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 560\). The loss of 176 Da suggested that the 6-methoxy-1-methyl-benzimidazolide moiety was demethylated and subsequently glucuronidated. On the basis of these results, M4 was proposed to be an O-demethyl-O-glucuronide.

The positive ion LC-MS spectrum of M2 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 548\). The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 548\) and the proposed fragmentation scheme of M2 are shown in Supplemental Fig. S15. Product ions at \(m/z\ 147, 162, 267, 280, 338\), and 372 were obtained. The most intense product ion at \(m/z\ 372\) was formed via the loss of 176 Da from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 548\). The loss of 176 Da corresponded to the glucuronic acid moiety. The product ion at \(m/z\ 338\) was formed via the loss of 210 Da from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 548\) suggested that the 6-methoxy-1-methyl-benzimidazolide moiety was demethylated and subsequently glucuronidated. Based on these results, M2 was proposed to be an O-demethyl-O-glucuronide-TZD ring-opened methylmercapto amide.

**Structure Analysis of M13, M9, and M14.** The positive ion LC-MS/MS spectrum of M13 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 574\), which was 176 Da higher than that of rivoglitazone. The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 574\) and the proposed fragmentation scheme of M13 are shown in Supplemental Fig. S16. Product ions at \(m/z\ 161, 176, 282\), and 398 were obtained. Furthermore, the LC-MS/MS spectrum and HPLC retention time of M13 were identical to those of the synthetic standard. Based on these results, M13 was identified as a TZD ring-opened N-glucuronide.

The positive ion LC-MS/MS spectrum of M9 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 592\), which was 18 Da higher than that of M13. The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 592\) and the proposed fragmentation scheme of M9 are shown in Fig. 6. The product ions at \(m/z\ 161, 176, 282, 327, 373, 399, 416\), and 458 were obtained. Furthermore, the LC-MS/MS spectrum and HPLC retention time of M9 were identical to those of the synthetic standard. On the basis of these results, M9 was identified as TZD ring-opened N-glucuronidate, a hydroxylated metabolite of M13.

The positive ion LC-MS/MS spectrum of M14 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 578\), which was 14 Da lower than that of M9. The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 578\) and the proposed fragmentation scheme of M14 are shown in Supplemental Fig. S17. Product ions at \(m/z\ 147, 162, 313, 359, 385, 402\), and 444 were obtained. The most intense product ion at \(m/z\ 162\) formed via the loss of 416 Da from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 578\) indicated that the 6-methoxy-1-methyl-benzimidazolide moiety was demethylated. The product ion at \(m/z\ 402\) was formed via the loss of 176 Da from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 578\). The loss of 176 Da corresponded to the glucuronic acid moiety. The product ion at \(m/z\ 313\) was formed via the elimination of the carbamoyl glucuronic acid moiety and the carboxylic acid group from the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 578\). On the basis of these results, M14 was proposed to be an O-demethyl-TZD ring-opened N-glucuronide.

**Structure Analysis of M19, M5, and M7.** The positive ion LC-MS spectrum of M19 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 387\). The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 387\) and the proposed fragmentation scheme of M19 are shown in Supplemental Fig. S18. Product ions at \(m/z\ 161, 176, 282\), and 295 were obtained. Furthermore, the LC-MS/MS spectrum and HPLC retention time of M19 were identical to those of the synthetic standard. Based on these results, M19 was identified as a TZD ring-opened methylmercapto carboxylic acid.

The positive ion LC-MS spectrum of M5 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 403\), which was 16 Da higher than that of M19. The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 403\) and the proposed fragmentation scheme of M5 are shown in Supplemental Fig. S19. Product ions at \(m/z\ 161, 176, 295\), and 339 were obtained. The positive ion LC-MS/MS spectrum of M7 showed a protonated molecule \([\text{M} + \text{H}]^+\) at \(m/z\ 403\), which was identical to that of M5. The LC-MS/MS spectrum of the ion \([\text{M} + \text{H}]^+\) at \(m/z\ 403\) and the proposed fragmentation scheme of M7 are shown in Supplemental Fig. S20. Similar to M5, product ions at \(m/z\ 161, 176, 295\), and 339 from M7 were obtained. Furthermore, the LC-MS/MS spectra and HPLC retention times of M5 and M7 were identical to those of the synthetic standards, respectively. Based on these results, M5 and M7 were identified as TZD ring-opened methyl sulfoxide carboxylic acids, a pair of diastereoisomers for each R- or S-isomer of sulfoxide.

**Structure Analysis of M1, M3, M6, M8, M10, M15, M16, M17, M18, and M20.** The structures of the metabolites M1, M3, M6, M8, M10, M15, M16, M17, M18, and M20 were elucidated by LC-MS/MS (Table 4). The LC-MS/MS spectra and the proposed fragmentation schemes of these metabolites are shown in Supplemental Figs. S21 to S30. The elucidated structures of these metabolites were further identified by comparing their mass spectra and retention times on HPLC with those of synthetic standards (Supplemental Figs. S1–S12).
Quantitative Plasma Metabolite Profiles in Rats and Monkeys.

To evaluate the quantitative plasma metabolite profiles, we determined the plasma concentrations of 14 metabolites (M5, M6, M7, M9, M10, M11, M12, M13, M15, M16, M17, M18, M19, and M20) using their synthetic standards by LC-MS/MS. The plasma profiles after oral administration of rivoglitazone (1 mg/kg) to rats and monkeys are shown in Fig. 7, and the AUC values are summarized in Table 5.

In rat plasma, M11 was observed as a major metabolite (ratio of molar basis AUC\(_{0-t}\) to rivoglitazone: 23.4%) and M12, M6, M18, M17, M10, M19, and M20 were observed as minor metabolites (0.01–0.72%). The mean \(t_{1/2}\) of M11 and M12 was 12.4 and 10 h, respectively, which was longer than that of rivoglitazone (4.4 h in this study).

In monkey plasma, M17 (7.01%), M13 (2.15%), M9 (1.53%), and M12 (1.22%) were observed as major metabolites and M11, M16, and M19 were detected as minor metabolites (0.25–0.64%). The mean \(t_{1/2}\) of metabolite M17 (19.8 h) was approximately 2 times longer than that of rivoglitazone (9.27 h in this study).

Quantitative Metabolite Profiles of Urine, Feces, and Bile in Rats.

The quantitative metabolite profiles in urine, feces, and bile up

![Representative radiochromatograms of the metabolites in rat plasma, urine, bile, and feces after a single oral administration of \([^{14}C]\)rivoglitazone at a dose of 1 or 5 mg/kg.](image-url)
to 48 h after oral administration of [14C]rivoglitazone (1 mg/kg) are summarized in Table 6. In urine, unchanged rivoglitazone was not detected, and the major metabolites excreted were M11 (10.0%), M6 (3.9%), M7 (1.7%), M10 (1.0%), and M2 (0.7%). An additional 12 unknown peaks (total recovery of 3.1% of dose) were also detected. In feces, unchanged rivoglitazone (23.7% of dose) was excreted, and the major metabolites were M12 (25.6%), M8 (8.7%), and M19 (3.0%). In addition more than 28 unknown minor peaks (total recovery of 13.8%) were detected. In bile, unchanged rivoglitazone was not observed and the major metabolites excreted were M11 (23.0%), M1 (3.8%), M2 (2.8%), M4 (1.5%), M3 (1.3%), M10 (1.0%), M13 (1.0%), M5 (0.6%), M6 (0.6%), and a mixture of M7, M8, and M9 (9.2%). In addition, 8 unknown peaks (total recovery of 2.1% of dose) were detected.

In feces after intravenous dosing to BDC rats, unchanged rivoglitazone (2.3% of dose), M12 (2.0%), M8 (0.6%), M19 (0.1%), and other unknown peaks (2.0%) were detected. For oral dosing, unchanged rivoglitazone (12.6%), M12 (3.6%), M8 (0.8%), M19 (0.3%), and other unknown peaks (1.7%) were detected in feces.
Quantitative Metabolite Profile of Urine and Feces in Monkeys.

The quantitative metabolite profiles in urine and feces up to 72 h after oral dosing of [14C]rivoglitazone (1 mg/kg) are shown in Table 7. In urine, 0.3% of dose of unchanged rivoglitazone was detected, and the major metabolites detected were M11 (8.1%), M5 (4.8%), M7 (3.8%), M4 (2.6%), M13 (1.4%), M10 (1.3%), M2 (1.2%), and M18 (0.4%). An additional 14 minor unknown peaks were observed (total recovery of 7.4%). In feces, 23.5% of dose of unchanged rivoglitazone was excreted, and the major metabolites detected were M12 (18.0%), M9 (6.6%), M8 (2.9%), M18 (1.7%), and M19 (0.8%) in addition to other minor unknown peaks (38 peaks).

Discussion

After oral administration of [14C]rivoglitazone, unchanged rivoglitazone was not excreted in rat urine and bile, and a small amount (0.3% of dose) of rivoglitazone was excreted in monkey urine. Therefore, metabolic clearance is predicted to mainly contribute to CL\textsubscript{tot} of rivoglitazone. Blood clearance, calculated by CL\textsubscript{tot}/R\textsubscript{B} of rivoglitazone in rats and monkeys, was 0.520 to 0.526 and 0.417 to 0.499 ml per min/kg, respectively, which was very low compared with the hepatic blood flow (55.2 ml per min/kg for rats and 43.6 ml per min/kg for monkeys) (Davies and Morris, 1993). These low values suggest that hepatic first-pass metabolism will be ignored. In fact, Foral exhibited high values: 95% in rats and 90% (0.0986 and 0.493 mg/kg) and 76% (0.986 mg/kg) in monkeys (Table 2). The V\textsubscript{ss} in rats (0.125–0.131 l/kg) and monkeys (0.138–0.166 l/kg) was relatively low and comparable to that in the extracellular fluid [0.2 l/kg (Panyam and Patil, 2008)].

In rats, the radioactivity after intravenous and oral dosing of [14C]rivoglitazone was mainly excreted via the fecal route, which finished within 48 h. On the other hand, in monkeys, the radioactivity...
was excreted in both urinary and fecal routes with the same ratio, which was slower than that in rats (90% for 168 h). The biliary excretion in BDC rats was lower than the fecal excretion in intact rats, and the fecal excretion in BDC rats was 7% for intravenous dosing and 19% for oral dosing. Because unchanged rivoglitazone was found in feces, the contribution of intestinal excretion was estimated in rats. In monkeys, unchanged rivoglitazone (23.5%) was observed in feces after oral dosing (1 mg/kg). Because the mean $F_{oral}$ at 0.986 mg/kg dose was 76.1%, unchanged rivoglitazone in feces may be due to the unabsorbed dose. Although it is the main metabolite in monkey bile, N-glucuronide (M13), was not detected in feces. M13 was deconjugated to rivoglitazone after incubation in monkey fecal homogenate (data not shown). Therefore, some fraction of unchanged rivoglitazone in feces may be derived from deconjugation via microflora.

Because a mass balance study in BDC monkeys was not conducted, the contribution of intestinal excretion in monkeys is unknown.

In this study, the chemical structures of 20 metabolites of rivoglitazone were identified. From the elucidation of metabolite structures, the main metabolic pathways of rivoglitazone were proposed to be five routes: O-demethylation, TZD ring opening, N-glucuronidation, N-demethylation, and TZD ring hydroxylation (Fig. 8). The main metabolic site of rivoglitazone was determined to be the TZD ring and 6-methoxy-1-methyl-benzimidazole moiety. On the other hand, the main metabolic sites of other TZD-containing drugs, such as rosiglitazone (Bolton et al., 1996; Baldwin et al., 1999; Cox et al., 2000; Hop et al., 2002) and pioglitazone (Krieter et al., 1994; Kiyota et al., 1997; Maeshiba et al., 1997; Shen et al., 2003), have been reported to be the oxidation, glucuronidation, or sulfation of the other moiety except for

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**Fig. 5.** LC-MS/MS spectrum and the proposed fragmentation scheme of M11.

**Fig. 6.** LC-MS/MS spectrum and the proposed fragmentation scheme of M9.
the TZD ring. In particular, we found M13 and the TZD ring-opened N-glucuronide (M9). Furthermore, M13 seems to be sequentially hydrolyzed to M9 and further metabolized via TZD ring-opened mercapto carboxylic acid (intermediate II) to TZD ring-opened methyl sulfoxide carboxylic acids (M5 and M7) as shown in Fig. 8.

We previously found the N-glucuronides and TZD ring-opened N-glucuronides of rosiglitazone (Uchiyama et al., 2010b) and pioglitazone (Uchiyama et al., 2010a) as minor novel metabolites in vitro using human, rat, and monkey hepatocytes. Thus, the N-glucuronide and sequential TZD ring-opened N-glucuronide are thought to be common metabolites for TZD-containing drugs, but this report is also the first report on them in vivo. As examples of N-glucuronidation to analogs related to TZDs, the N-glucuronides of (3S)-(+)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indole-2-one (MaxiPost) (Zhang et al., 2005), 5-ethyl-5-phenylhydantoin (nirvanol) (Maguire et al., 1982), and 5-n-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (bucolome) (Mohri et al., 1985) for N-glucuronidation of cyclic amide compounds and that of PNU-107859 for N-glucuronidation of the cyclic thioamide compound (Kuo et al., 1999) have been reported. For all cases, N-glucuronides were found to be major metabolites and were stable.

From the in vitro study using M13 (synthetic standard), the non-enzymatic hydrolysis of M13 to M9 was observed (half-life of approximately 0.7 h) in buffer solution (pH 7.4) during incubation at 37°C (data not shown). After N-glucuronidation of the TZD ring, formation of hydrogen bonding between the hydroxyl group at the 2-position of the glucuronic acid moiety and the carbonyl group at the 4-position of the TZD ring is likely to be proposed. As a result, the TZD ring may increase electrophilicity and become easily attacked by the nucleophile and hydrolyzed. In addition, M9 was speculated to be transformed to M19 via TZD ring-opened mercapto carboxylic acid (intermediate II in Fig. 8), because the synthetic standard of intermediate I was immediately metabolized to TZD ring-opened methylmercapto carboxylic acid (M19) by S-methylation in the presence of S-adenosyl-L-methionine in human liver microsomes or cytosol (data not shown).

This TZD ring-opening pathway is distinct from the known oxidative TZD ring-opening pathway of other TZD-containing drugs, such as troglitazone (Kassahun et al., 2001), rosiglitazone (Uchiyama et al., 2010b), and pioglitazone (Baughman et al., 2005; Uchiyama et al., 2010a) and the TZD derivatives (±)-5-[2,4-dioxothiazolidin-5-yl)methyl]-2-methoxy-N-[[(4-trifluoromethyl)phenyl]methyl]benzamide (MK-0767) (Karanam et al., 2004; Liu et al., 2004; Kochansky et al., 2006) and (±)-5-[2,4-dioxothiazolidin-5-yl)methyl]-2-methoxy-N-[(4-trifluoromethyl)phenyl]methyl]benzamide (MRL-A) (Reddy et al., 2005). We have also identified some metabolites related to the oxidative TZD ring-opening pathways of rivoglitazone: M20, M6, and

<table>
<thead>
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<th>Compound</th>
<th>Rat</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0–t</td>
<td>Ratio of Molar Basis AUC0–t of Rivoglitazone</td>
</tr>
<tr>
<td>Rivoglitazone</td>
<td>40.500</td>
<td>100</td>
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<tr>
<td>M5</td>
<td>N.A.</td>
<td>N.A.</td>
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<tr>
<td>M6</td>
<td>33.9</td>
<td>0.08</td>
</tr>
<tr>
<td>M7</td>
<td>0.356 (n = 3)</td>
<td>0.00</td>
</tr>
<tr>
<td>M9</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M10</td>
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<tr>
<td>M11</td>
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<tr>
<td>M12</td>
<td>280</td>
<td>0.72</td>
</tr>
<tr>
<td>M13</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M15</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M16</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>M17</td>
<td>9.59</td>
<td>0.02</td>
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<tr>
<td>M18</td>
<td>11.4</td>
<td>0.03</td>
</tr>
<tr>
<td>M19</td>
<td>3.29</td>
<td>0.01</td>
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<tr>
<td>M20</td>
<td>2.71</td>
<td>0.01</td>
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N.A., not applicable.

* t represents the last quantifiable time for each analyte.
M10, which are estimated to form via the TZD ring-opened mercaptoamide (intermediate I in Fig. 8). The synthetic standard of intermediate I was immediately metabolized to TZD ring-opened methylmercaptoamide (intermediate I in Fig. 8). The synthetic standard of intermediate I was immediately metabolized to TZD ring-opened methylmercaptoamide (intermediate I in Fig. 8).

### Table 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urine (0–48 h)</th>
<th>Feces (0–48 h)</th>
<th>Bile (0–48 h)</th>
<th>Urine + Feces (0–48 h)</th>
<th>Urine + Bile (0–48 h)</th>
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<tr>
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<td>23.7</td>
<td>N.D.</td>
</tr>
<tr>
<td>M1</td>
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<td>N.D.</td>
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<tr>
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<td>0.7</td>
<td>3.5</td>
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<tr>
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</tr>
<tr>
<td>M4</td>
<td>N.D.</td>
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<td>N.D.</td>
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<tr>
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<tr>
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<td>3.9</td>
<td>4.5</td>
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<tr>
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<td></td>
</tr>
<tr>
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<td>N.D.</td>
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</tr>
<tr>
<td>M9, M12, M13</td>
<td>8.1</td>
<td>N.D.</td>
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<td></td>
</tr>
<tr>
<td>M10</td>
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<td>1.0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>M11</td>
<td>10.0</td>
<td>23.0</td>
<td>10.0</td>
<td></td>
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</tr>
<tr>
<td>M12</td>
<td>N.D.</td>
<td>25.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M13</td>
<td>N.D.</td>
<td>1.0</td>
<td>N.D.</td>
<td></td>
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<tr>
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<td>3.0</td>
<td></td>
<td></td>
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<tr>
<td>Unknown metabolites</td>
<td>3.1 (12 peaks)</td>
<td>13.8 (&gt;28 peaks)</td>
<td>2.1 (8 peaks)</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20.4</td>
<td>74.8</td>
<td>46.9</td>
<td>95.2</td>
<td>67.3</td>
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N.D., not detected.

### Table 7

<table>
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<tr>
<th>Compound</th>
<th>Urine (0–72 h)</th>
<th>Feces (0–72 h)</th>
<th>Urine + Feces (0–72 h)</th>
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<td>Rivoglitazone</td>
<td>0.3</td>
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<td>23.8</td>
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<tr>
<td>M2</td>
<td>1.2</td>
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</tr>
<tr>
<td>M4</td>
<td>2.6</td>
<td>N.D.</td>
<td>2.6</td>
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<tr>
<td>M5, M6</td>
<td>4.8</td>
<td>N.D.</td>
<td></td>
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<tr>
<td>M7, M15</td>
<td>4.1</td>
<td>N.D.</td>
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</tr>
<tr>
<td>M8</td>
<td>N.D.</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>M9</td>
<td>N.D.</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>M10</td>
<td>1.3</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>M11, M12</td>
<td>8.1</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>M13</td>
<td>N.D.</td>
<td>18.0</td>
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<tr>
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<td>N.D.</td>
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<td>N.D.</td>
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<tr>
<td>M18</td>
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<tr>
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<td>N.D.</td>
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<td>11.0 (&gt;38 peaks)</td>
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</tr>
<tr>
<td>Total</td>
<td>35.4</td>
<td>64.5</td>
<td>99.9</td>
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N.D., not detected.
RIVOGLITAZONE PHARMACOKINETICS, METABOLISM, AND DISPOSITION

Fig. 8. Proposed in vivo metabolic pathways of rivoglitazone in rats and monkeys.

ume of distribution, and high oral bioavailability in both species. Rivoglitazone was metabolized via multiple metabolic pathways involving oxidation and N-glucuronidation and finally was excreted as many metabolites in urine and feces via biliary excretion.

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Authorship Contributions

Participated in research design: Uchiyama, Iwabuchi, Okazaki, and Izumi.
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References


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Supplemental Data

Pharmacokinetics, metabolism, and disposition of rivoglitazone, a novel peroxisome proliferator-activated receptor γ agonist, in rats and monkeys

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Drug Metabolism and Disposition
Synthesis of M1 and M8. M1 and M8 were synthesized as shown in Fig. S1.

Fig. S1. Synthetic scheme of M1 and M8. Reagents and conditions: (a) Boc₂O, NaH, THF, rt, 0.5 h, 83%; (b) NaH, DMF, rt, 0.5 h, then MeI, rt, 10 h, 89%; (c) BnOH, NaH, DMF, rt, 1 h, 83%; (d) H₂ / 10% Pd-C, MeOH, dioxane, quant.; (e) KOH, EtOH, H₂O, 75°C, 4.5 h; (f) 4N HCl / dioxane, EtOH, rt, 2 days, 59% (2 steps); (g) MeI, Et₃N, THF, rt, 1.5 h, 89%; (h) tert-butyli bromoacetate, Cs₂CO₃, MeCN, rt, 4.5 h; (i) 4N HCl / dioxane, EtOH, rt, 12 h, 69% (2 steps); (j) diethyl cyanophosphonate, Et₃N, THF, rt, 2 days, 52%; (k) 4N HCl / dioxane, EtOH, rt, 12 h, 66%; (l) KOH, H₂O, DMF, rt, 2 days, 48%; (m) ClCO₂Et, Et₃N, rt, 1.5 h, then NH₄OH, rt, 12 h, 50%; (n) mCPBA, HCO₂H, MeCN,
−10°C, 0.5 h, 51%; (o) ClSO₃H, pyridine, MeCN, rt, 14 h, 50%.

**Synthesis of M3.** M3 was synthesized as shown in **Fig. S2.**

![Synthetic scheme of M3](image)

**Fig. S2.** Synthetic scheme of M3. Reagents and conditions: (a) NaNO₂, 47% HBr, H₂O, MeOH, DMF, rt, 0.5 h, then n-butyl acrylate, CuBr, rt, 20 h; (b) MeSNa, THF, DMF, 2°C, 24 h; (c) Ac₂O, Et₃N, THF, rt, 20 h; (d) mCPBA, CH₂Cl₂, 2°C, 3 days, rt, 24 h, 14% (4 steps); (e) KOH, H₂O, MeOH, rt, 15 h, 92%; (f) CDI, DMF, rt, 3 h, then 7 N NH₃ / MeOH, rt, 15 h, 86%; (g) ClSO₃H, pyridine, rt, 1 h, 59%.
Synthesis of M5 and M7. M5 and M7 were synthesized as shown in Fig. S3.

Fig. S3. Synthetic scheme of M5 and M7. Reagents and conditions: (a) MeONa / MeOH, DMF, 60°C, 20 h, 91%; (b) Boc₂O, Et₃N, DMF, 55°C, 24 h, 28%; (c) NaH, DMF, rt, 20 min, then MeI, rt, 17 h, 81%; (d) H₂ / 7.5% Pd-C, MeOH, rt, 3 h, 99%; (e) 4-nitrophenoxyacetic acid, diethyl cyanophosphonate, Et₃N, THF, −10°C, 2 days; (f) 4N HCl / dioxane, 60°C, 16 h, 74% (2 steps); (g) H₂ / 7.5% Pd-C, MeOH, rt, 4 h, 83%; (h) NaNO₂, 48% HBr, H₂O, MeOH, DMF, rt, 2 h, then tert-butyl acrylate, CuBr, rt, 19 h, 45°C, 8 h, 28%; (i) MeSNa, THF, −10°C, 15 h; (j) Mg bis(monoperoxyphthalate) hexahydrate, rt, 24 h, 18% (2 steps); (k) KOH, H₂O, MeOH, rt, 5 days, 56%.
**Synthesis of M6.** M6 was synthesized as shown in Fig. S4.

**Fig. S4.** Synthetic scheme of M6. Reagents and conditions: (a) NaNO₂, 48% HBr, H₂O, MeOH, rt, 20 h, then acrylamide monomer, CuBr, rt, 3 days, 55°C, 24 h, 48%; (b) MeSNa, DMF, −10°C, 24 h, 54%; (c) KOH, H₂O, MeOH, rt, 4 h, 88%; (d) CDI, DMF, rt, 3 h, then 26, 2°C–rt, 12 h; (e) 4N HCl / dioxane, 50°C, 10 h, 52% (2 steps); (f) mCPBA, DMA, CH₂Cl₂, −78°C, 1.5 h, 37%.
Synthesis of M9 and M13. M9 and M13 were synthesized as shown in Fig. S5.

Fig. S5. Synthetic scheme of M9 and M13. Reagents and conditions: (a) acetobromo-α-D-glucuronic acid methyl ester, Cs2CO3, MeCN, 70°C, 3 h; (b) allyl alcohol, 4N HCl / dioxane, rt, 2 days, 65% (2 steps); (c) tetrakis(triphenylphosphine)palladium, morpholine, CH2Cl2, rt, 2 h; (d) AcONa, MeCN, H2O, rt, 6 days, 26%.

Synthesis of M10. M10 was synthesized as shown in Fig. S6.

Fig. S6. Synthetic scheme of M10. Reagents and conditions: (a) Mg bis(monoper oxyphthalate) hexahydrate, CH2Cl2, rt, 27 h, 50°C, 4 h; (b) KOH, H2O,
MeOH, rt, 2 days, 33% (2 steps); (c) CDI, DMF, rt, 3 h, then NH₄OH, rt, 17 h, 84%.

**Synthesis of M11 and M12.** M11 and M12 were synthesized as shown in Fig. S7.

![Synthetic scheme of M11 and M12](image)

**Fig. S7.** Synthetic scheme of M11 and M12. Reagents and conditions: (a) 5, diethyl cyanophosphonate, Et₃N, THF, rt, 14 h; (b) 4N HCl / dioxane, rt, 14 h, 98% (2 steps); (c) ClSO₃H, Pyridine, MeCN, reflux, 2 h, 21%.

**Synthesis of M15.** M15 was synthesized as shown in Fig. S8.

![Synthetic scheme of M15](image)

**Fig. S8.** Synthetic scheme of M15. Reagents and conditions: (a) AcSK, DMF,
−78°C–rt, 3 h, 98%; (b) triphenylphosphine, morpholine, MeOH, rt, 15 h, 50°C, 6 h, 70%; (c) benzophenone imine, CH₂Cl₂, rt, 3 days, 94%; (d) dithioerithritol, Et₃N, CHCl₃, rt, 16 h, quant.; (e) NBS, NaHCO₃, dioxane, rt, 10 h, 39% (2 steps); (f) 4N HCl / dioxane, 45°C, 5 h, 35%.

**Synthesis of M16.** M16 was synthesized as shown in [Fig. S9](#).

![Fig. S9](image.png)

*Fig. S9.* Synthetic scheme of M16. Reagents and conditions: (a) BnOH, NaH, DMF, 60°C, 4 days, 32%; (b) H₂ / 10% Pd-C, MeOH, rt, 5 h, 92%; (c) ClCO₂-i-Bu, Et₃N, THF, DMF, rt, 24 h; (d) 4N HCl / dioxane, 60°C, 15 h, 15% (2 steps).

**Synthesis of M17.** M17 was synthesized as shown in [Fig. S10](#).

![Fig. S10](image.png)

*Fig. S10.* Synthetic scheme of M17. Reagents and conditions: (a) H₂ / 10% Pd-C, toluene, MeOH, rt, 4 h; (b) diethyl cyanophosphonate, Et₃N, THF, DMF, −10°C, 1.5 h, 62% (2 steps); (c) trifluoroacetic acid, 65°C, 2 days, 50%.
Supplemental Data
DMD #36194

Synthesis of M18. M18 was synthesized as shown in Fig. S11.

![Synthetic scheme of M18](image)

Fig. S11. Synthetic scheme of M18. Reagents and conditions: (a) TIPSOTf, DBU, CH₂Cl₂, −5°C–rt, 1 h, 62%; (b) monoperxyphthalic acid magnesium salt, CH₂Cl₂, MeOH, −5°C, 21%.

Synthesis of M19 and M20. M19 and M20 were synthesized as shown in Fig. S12.

![Synthetic scheme of M19 and M20](image)

Fig. S12. Synthetic scheme of M19 and M20. Reagents and conditions: (a) NaH, DMF, −10°C, 30 min, then tert-butyl bromoacetate, rt, 5 days, 87%; (b) H₂ / 7.5% Pd-C, MeOH, rt, 10 h, quant.; (c) NaNO₂, 48% HBr, H₂O, MeOH, rt, 30 min, then n-butyl
Supplemental Data
DMD #36194

acrylate, CuBr, rt, 3 days, 16%; (d) MeSNa, DMF, rt, 15 h, 63%; (e) 4N HCl / dioxane, 50°C, 5 h, 96%; (f) CDI, DMF, rt 6 h, then 26, rt, 5 days, 60%; (g) 4N HCl / dioxane, 60°C, 15 h, 70%; (h) KOH, H2O, MeOH, rt, 3 days, 92%; (i) ClCO2i-Bu, Et3N, rt, 1 h, then 7 N NH3 / MeOH, rt, 3 days, 53%.

MS and NMR data of synthetic standards.

M1. MS m/z 468 [M + H]⁺; ¹H NMR (400 MHz, DMSO-d6) δ (2.56 (s), 2.60 (s), 3H), 2.9–3.15 (2H, m), 3.6–3.7 (1H, m), 3.96 (3H, s), 5.64 (2H, s), 7.11 (2H, d, J = 8.7 Hz), 7.2–7.25 (2H, m), 7.4–7.45 (1H, m), 7.56 (1H, s), 7.7–7.75 (2H, m).

M3. MS m/z 484 [M + H]⁺; ¹H NMR (400 MHz, DMSO-d6) δ 3.05 (3H, s), 3.1–3.25 (2H, m), 3.96 (3H, s), 4.07 (1H, dd, J = 3.4 and 11.4 Hz), 5.65 (2H, s), 7.11 (2H, d, J = 8.8 Hz), 7.23 (2H, d, J = 8.8 Hz), 7.35–7.45 (2H, m), 7.65–7.75 (4H, m).

M5. MS m/z 403 [M + H]⁺; ¹H NMR (400 MHz, DMSO-d6) δ 2.66 (3H, s), 2.95–3.1 (2H, m), 3.81 (3H, s), 3.82 (3H, s), 3.85 (1H, dd, J = 4.3 and 10.2 Hz), 5.32 (2H, s), 6.83 (1H, dd, J = 2.3 and 8.7 Hz), 7.05 (2H, d, J = 8.8 Hz), 7.12 (1H, d, J = 2.3 Hz), 7.20 (2H, d, J = 8.8 Hz), 7.51 (1H, dd, J = 0.4 and 8.7 Hz), 13.2 (1H, br s).

M6. MS m/z 402 [M + H]⁺; ¹H NMR (400 MHz, DMSO-d6) δ 2.57 (3H, d, J = 14.5 Hz), 2.9–3.1 (2H, m), 3.6–3.7 (1H, m), 3.81 (3H, s), 3.82 (3H, s), 5.32 (2H, s), 6.83 (1H, dd, J = 2.4 and 8.8 Hz), 7.04 (2H, d, J = 8.9 Hz), 7.1–7.2 (3H, m), 7.51 (1H, d, J = 8.8 Hz), 7.54 (2H, m).
M7. MS $m/z$ 403 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.64 (3H, s), 2.95–3.1 (2H, m), 3.81 (3H, s), 3.82 (3H, s), 3.85 (1H, dd, $J$ = 5.4 and 9.9 Hz), 5.32 (2H, s), 6.83 (1H, dd, $J$ = 2.4 and 8.8 Hz), 7.05 (2H, d, $J$ = 8.8 Hz), 7.12 (1H, d, $J$ = 2.2 Hz), 7.20 (2H, d, $J$ = 8.8 Hz), 7.51 (1H, dd, $J$ = 0.4 and 8.8 Hz), 13.1 (1H, br s).

M8. MS $m/z$ 372 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.7–2.8 (1H, m), (2.73 (s), 2.89 (s), 3H), 3.02 (1H, dd, $J$ = 9.2 and 13.7 Hz), 3.3–3.4 (1H, m), 3.73 (3H, s), 5.29 (2H, s), 6.72 (1H, d, $J$ = 8.8 Hz), 6.83 (1H, s), 6.90 (1H, s), 7.01 (2H, d, $J$ = 8.7 Hz), 7.14 (2H, d, $J$ = 8.7 Hz), 7.34 (1H, s), 7.42 (1H, d, $J$ = 8.6 Hz), 9.37 (1H, s).

M9. MS $m/z$ 592 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.91 (1H, dd, $J$ = 6.5 and 13.8 Hz), 3.05–3.3 (4H, m), 3.61 (1H, d, $J$ = 9.5 Hz), 3.81 (3H, s), 3.82 (3H, s), 4.1–4.15 (1H, m), 4.7–4.8 (1H, m), 5.09 (1H, d, $J$ = 5.8 Hz), 5.15 (1H, d, $J$ = 3.9 Hz), 5.31 (2H, s), 6.8–6.85 (1H, m), 7.03 (2H, d, $J$ = 8.9 Hz), 7.13 (2H, d, $J$ = 8.9 Hz), 7.1–7.2 (1H, m), 7.51 (1H, d, $J$ = 8.9 Hz), 8.99 (1H, d, $J$ = 8.4 Hz), 12.7 (2H, br s).

M10. MS $m/z$ 418 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 3.04 (3H, s), 3.05–3.25 (2H, m), 3.81 (3H, s), 3.82 (3H, s), 4.03 (1H, dd, $J$ = 3.4 and 11.7 Hz), 5.32 (2H, s), 6.82 (1H, dd, $J$ = 2.4 and 8.8 Hz), 7.05 (2H, d, $J$ = 8.8 Hz), 7.51 (1H, dd, $J$ = 0.4 and 8.8 Hz), 7.35–7.7 (1H, m).

M11. MS $m/z$ 464 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 3.11 (1H, dd, $J$ = 8.9 and 14.3 Hz), 3.34 (1H, dd, $J$ = 4.4 and 14.7 Hz), 3.96 (3H, s), 4.90 (1H, dd, $J$ = 4.4 and 8.8 Hz), 5.66 (2H, s), 7.13 (2H, d, $J$ = 8.8 Hz), 7.26 (2H, d, $J$ = 8.8 Hz), 7.42 (1H,
dd, $J = 1.8$ and 9.1 Hz), 7.7–7.75 (2H, m), 12.02 (1H, s).

**M12.** MS $m/z$ 384 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.11 (1H, dd, $J = 8.8$ and 14.1 Hz), 3.34 (1H, dd, $J = 4.4$ and 14.1 Hz), 3.92 (3H, s), 4.90 (1H, dd, $J = 4.4$ and 8.8 Hz), 5.64 (2H, s), 7.09 (1H, dd, $J = 2.1$ and 8.8 Hz), 7.14 (2H, d, $J = 8.8$ Hz), 7.19 (1H, d, $J = 2.1$ Hz), 7.25 (2H, d, $J = 8.8$ Hz), 7.63 (1H, d, $J = 8.8$ Hz), 10.25 (1H, br s), 12.04 (1H, s).

**M13.** MS $m/z$ 574 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.9–3.1 (1H, m), 3.2–3.5 (3H, m), 3.66 (1H, t, $J = 9.7$ Hz), 3.80 (3H, s), 3.82 (3H, s), 4.0–4.3 (1H, m), 4.7–5.1 (2H, m), 4.7–5.5 (3H, m), 5.33 (2H, s), 6.83 (1H, dd, $J = 2.4$ and 8.8 Hz), 7.0–7.1 (2H, m), 7.12 (1H, d, $J = 2.4$ Hz), 7.15–7.25 (2H, m), 7.52 (1H, d, $J = 8.8$ Hz).

**M15.** MS $m/z$ 492 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.8–3.1 (3H, m), 3.3–3.35 (1H, m), 3.5–3.6 (1H, m), 3.7–3.8 (1H, m), 3.81 (3H, s), 3.82 (3H, s), 5.31 (2H, s), 6.82 (1H, dd, $J = 2.4$ and 8.8 Hz), 7.02 (2H, dd, $J = 1.7$ and 8.6 Hz), 7.12 (1H, d, $J = 2.4$ Hz), 7.18 (2H, d, $J = 8.6$ Hz), 7.51 (1H, d, $J = 8.8$ Hz).

**M16.** MS $m/z$ 370 [M + H]$^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.06 (1H, dd, $J = 9.1$ and 14.1 Hz), 3.31 (1H, dd, $J = 4.4$ and 14.1 Hz), 4.87 (1H, dd, $J = 4.4$ and 9.1 Hz), 5.20 (2H, s), 6.66 (1H, dd, $J = 2.3$ and 8.6 Hz), 6.84 (1H, s), 7.02 (2H, d, $J = 8.8$ Hz), 7.18 (2H, d, $J = 8.8$ Hz), 7.34 (1H, d, $J = 8.6$ Hz), 9.10 (1H, br s), 12.05 (1H, br s), 12.23 (1H, br s).
M17. MS m/z 384 [M + H]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 3.06 (1H, dd, $J = 9.1$ and 14.2 Hz), 3.31 (1H, dd, $J = 4.3$ and 14.2 Hz), 3.78 (3H, s), 4.87 (1H, dd, $J = 4.3$ and 9.1 Hz), 5.23 (2H, s), 6.81 (1H, d, $J = 8.8$ Hz), 6.9–7.3 (1H, br s), 7.02 (2H, d, $J = 8.8$ Hz), 7.19 (2H, d, $J = 8.8$ Hz), 7.44 (1H, br s), 12.01 (1H, br s), 12.47 (1H, br s), 12.23 (1H, br s).

M18. MS m/z 414 [M + H]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 3.1–3.3 (2H, m), 3.81 (3H, s), 3.82 (3H, s), 5.32 (2H, s), 6.83 (1H, dd, $J = 2.4$ and 8.8 Hz), 7.04 (2H, d, $J = 8.7$ Hz), 7.12 (1H, d, $J = 2.4$ Hz), 7.21 (2H, d, $J = 8.7$ Hz), 7.52 (1H, d, $J = 8.8$ Hz), 7.81 (1H, s), 11.96 (1H, s).

M19. MS m/z 387 [M + H]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 2.10 (3H, s), 2.79 (1H, dd, $J = 6.4$ and 13.8 Hz), 3.01 (1H, dd, $J = 9.1$ and 13.8 Hz), 3.41 (1H, dd, $J = 6.5$ and 9.1 Hz), 3.81 (3H, s), 3.82 (3H, s), 5.32 (2H, s), 6.83 (1H, dd, $J = 2.4$ and 8.8 Hz), 7.02 (2H, d, $J = 8.8$ Hz), 7.12 (1H, d, $J = 2.2$ Hz), 7.18 (2H, d, $J = 8.8$ Hz), 7.51 (1H, dd, $J = 0.4$ and 8.8 Hz), 12.51 (1H, br s).

M20. MS m/z 386 [M + H]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 2.07 (3H, s), 2.72 (1H, dd, $J = 6.1$ and 13.8 Hz), 3.02 (1H, dd, $J = 9.1$ and 13.8 Hz), 3.3–3.35 (1H, m), 3.81 (3H, s), 3.82 (3H, s), 5.31 (2H, s), 6.82 (1H, dd, $J = 2.4$ and 8.8 Hz), 6.89 (1H, s), 7.01 (2H, d, $J = 8.8$ Hz), 7.11 (1H, d, $J = 2.2$ Hz), 7.14 (2H, d, $J = 8.8$ Hz), 7.32 (1H, d, $J = 1.9$ Hz), 7.51 (1H, dd, $J = 0.4$ and 8.8 Hz).
Structure analysis of metabolites.

Fig. S13. LC/MS/MS spectrum and the proposed fragmentation scheme of M12.

Fig. S14. LC/MS/MS spectrum and the proposed fragmentation scheme of M4.
Fig. S15. LC/MS/MS spectrum and the proposed fragmentation scheme of M2.

Fig. S16. LC/MS/MS spectrum and the proposed fragmentation scheme of M13.
Fig. S17. LC/MS/MS spectrum and the proposed fragmentation scheme of M14.

Fig. S18. LC/MS/MS spectrum and the proposed fragmentation scheme of M19.
Fig. S19. LC/MS/MS spectrum and the proposed fragmentation scheme of M5.

Fig. S20. LC/MS/MS spectrum and the proposed fragmentation scheme of M7.
Fig. S21. LC/MS/MS spectrum and the proposed fragmentation scheme of M1.

Fig. S22. LC/MS/MS spectrum and the proposed fragmentation scheme of M3.
Supplemental Data
DMD #36194

Fig. S23. LC/MS/MS spectrum and the proposed fragmentation scheme of M6.

Fig. S24. LC/MS/MS spectrum and the proposed fragmentation scheme of M8.
Fig. S25. LC/MS/MS spectrum and the proposed fragmentation scheme of M10.

Fig. S26. LC/MS/MS spectrum and the proposed fragmentation scheme of M15.
Fig. S27. LC/MS/MS spectrum and the proposed fragmentation scheme of M16.

Fig. S28. LC/MS/MS spectrum and the proposed fragmentation scheme of M17.
Fig. S29. LC/MS/MS spectrum and the proposed fragmentation scheme of M18.

Fig. S30. LC/MS/MS spectrum and the proposed fragmentation scheme of M20.