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

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Abbreviations: rivoglitazone,
(RS)-5-[(6-Methoxy-1-methyl-1H-benzimidazol-2-yl)methoxy]benzyl)-1,3-thiazolidine-2,4-dione monohydrochloride); TZD, thiazolidinedione; PPARγ, peroxisome
proliferator-activated receptor gamma; IS, internal standard; HP-β-CD, Hydroxypropyl-β-cyclodextrin; HPLC, high-performance liquid chromatography; i.v., intravenous; BDC, bile duct-cannulated; LC/MS/MS, liquid chromatography/tandem mass spectrometry; ESI, electrospray ionization; MRM, multiple reaction monitoring; radio-HPLC, radioactivity detection high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; TFA, trifluoroacetic acid; SAM, S-adenosyl-L-methionine; Q-Tof, quadrupole time-of-flight; CID, collision-induced dissociation; MaxiPost,

(3S)-(+)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indole-2-one; bucolome, 5-n-butyl-1-cyclohexyl-2,4,6-trioxoperpyrimidine;
MK-0767,

(±)-5-[(2,4-dioxothiazolidin-5-yl)methyl]-2-methoxy-N-[(4-trifluoromethyl)phenyl]methyl]benzamide; nirvanol, 5-ethyl-5-phenylhydantoin; bucolome, 5-n-butyl-1-cyclohexyl-2,4,6-trioxoperpyrimidine; MRL-A,

(±)-5-[(2,4-dioxothiazolidin-5-yl)methyl]-2-methoxy-N-[(4-trifluoromethoxy)phenyl]methyl]benzamide.
Abstract

The pharmacokinetics, metabolism, and excretion of rivoglitazone ((RS)-5-{4-[(6-Methoxy-1-methyl-1H-benzimidazol-2-yl)methoxy]benzyl}-1,3-thiazolidine-2,4-dione monohydrochloride), a novel thiazolidinedione (TZD) peroxisome proliferator-activated receptor gamma selective agonist, were evaluated in male F344/DuCrICrj rats and cynomolgus monkeys. The total body clearance and volume of distribution of rivoglitazone were low in both animals (0.329–0.333 ml/min/kg and 0.125–0.131 l/kg for rats, 0.310–0.371 ml/min/kg and 0.138–0.166 l/kg for monkeys), and the plasma half-life was 4.55–4.84 h for rats and 6.21–6.79 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, while that was excreted in urine and feces with the same ratio in monkeys. Since excreted rivoglitazone in urine and bile was low, metabolism is estimated to be mainly contributed to total body clearance. The structures of 20 metabolites (M1–M20) were identified, and five 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 non-enzymatically hydrolyzed to TZD ring-opened N-glucuronide (M9), and the amount of these metabolites in monkeys was larger than that in rats. In plasma, rivolitazone 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 minor metabolites in
monkeys.
Introduction

Rivoglitazone, (RS)-5-{4-[(6-Methoxy-1-methyl-1H-benimidazol-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 gamma (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 (T2DM) and showed a safety profile consistent with the specific side effects (eg., 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, HbA1c, and hemodilution 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 \[^{14}\text{C}]\text{rivoglitazone} were performed, and the contribution of metabolic pathways in vivo was evaluated. In addition, the quantitative profiles of plasma metabolites were evaluated to support the safety of metabolites in the light of the Safety Testing of Drug Metabolites (FDA, 2008).
Materials and Methods

Materials.

Rivoglitazone, internal standards (IS; R-121171, R-132064, R-252121, R-252122, and R-395374; Fig. 1), synthetic standards of metabolite (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-didemethyl rivoglitazone (M16), N-demethyl rivoglitazone (M17), TZD ring 5-hydroxyl 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 30.0–35.4 µCi/mg and the radiochemical purity was 98.2–99.6%. 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/DuCrI/Crlj 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 experimental conditions before use at least 5 days for rats and 53 days for monkeys. Food and waters were supplied ad libitum throughout the acclimatization and experimental period; however, prior to administration, the animals were fasted overnight and fed at 8 h post-dose for rats and at least 6 h post-dose 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 5 parallel groups (0.1 and 0.5 mg/kg for intravenous (i.v.) administration, 0.1, 0.5, and 1 mg/kg for oral administration, 153–175 g, n = 4 for each group) for the pharmacokinetic study and 1 mg/kg oral dosing (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: pre-dose and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose for i.v. dose; pre-dose and 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose for oral dose. Plasma was obtained by centrifugation at 4°C and stored at −80°C until analysis.

**Mass Balance Study in Rats.**
[\textsuperscript{14}C]\textit{Rivoglitazone} (1 mg/kg, 30.0 \(\mu\text{Ci/kg}\)) was administrated intravenously via the tail vain (190–195 g, \(n = 4\)) or by oral gavage (174–179 g, \(n = 4\)) to rats. After dosing, each rat was housed individually in glass metabolic cages (SUGIYAMA-GEN Co., Ltd., Tokyo, Japan), and urine and feces were collected in a water bath for 168 h at 0–24, 24–48, 48–72, 72–120, and 120–168 h post-dose.

\textit{Mass Balance Study in Bile Duct-Cannulated (BDC) 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 by placing a ligature around the tube to prevent dislocation under anesthesia with ethyl ether inhalation. The tube was exteriorized at the back of the neck. After recovery from the anesthesia, the dosing solution at a dose of 0.5 mg/kg (17.7 \(\mu\text{Ci/kg}\)) of [\textsuperscript{14}C]rivoglitazone was administrated intravenously via the tail vein (209–220 g, \(n = 3\)) or by oral gavage (213–217 g, \(n = 3\)). The animals were individually accommodated in metabolic cages, urine and bile were collected at 0–8, 8–24, 24–48 h, and feces was collected at 0–24 and 24–48 h under ice-cold conditions.

\textit{Sample Collection for Qualitative and Quantitative Metabolite Profiling in Rats.}

For qualitative plasma profiles, blood was collected via the abdominal aorta at 6 h after oral dosing of [\textsuperscript{14}C]rivoglitazone (5 mg/kg, 150 \(\mu\text{Ci/kg}\)) to rats (177–195 g, \(n = 10\)), and the plasma was obtained by centrifugation at 4\(^\circ\text{C}\). Urine was collected for the periods of 0–8, 8–24, and 24–48 h post-dose after oral dosing of [\textsuperscript{14}C]rivoglitazone (1 mg/kg, 34.6 \(\mu\text{Ci/kg}\)) to rats (148–150 g, \(n = 4\)). Bile was collected for the periods of 0–8, 8–24, and 24–48 h post-dose after oral dosing of [\textsuperscript{14}C]rivoglitazone (1 mg/kg,
34.6 μCi/kg) to BDC rats (149–153 g, n = 4). Feces was collected for the periods of 0–24 and 24–48 h post-dose after oral dosing of [14C]rivoglitazone (1 mg/kg, 35.4 μCi/kg) to 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 oral, 0.0986 mg/kg i.v., 0.493 mg/kg oral, 0.493 mg/kg i.v., 0.986 mg/kg oral, and 0.986 mg/kg i.v. Blood (1 ml) was collected from the femoral vein with a heparinized syringe at pre-dose, 0.083, 0.25, 0.5, 1, 2, 3, 5, 7, and 24 h post-dose for i.v. dosing and at pre-dose, 0.25, 0.5, 1, 2, 3, 5, 7, and 24 h post-dose for oral dosing. For the metabolite profiling study, monkeys (3.65–5.25 kg, n = 4) were dosed orally at 1 mg/kg and blood was collected at pre-dose, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose. Plasma was obtained by centrifugation at 4°C and stored at −20°C or −80°C until analysis.

**Mass Balance Study in Monkeys.**

Monkeys (5.1–6.9 kg, n = 3) were given i.v. 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 post-dose. 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 crural saphenous vein or the cephalic vein of the forearm in the test tube containing
heparin sodium at 6 h post-dose after oral dosing. Plasma was obtained by centrifugation at 4°C and stored at −20°C.

**Sample Collection for Qualitative and Quantitative 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 0–8, 8–24 and 24–72 h (n = 3) and the bile in 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 0–24 and 24–72 h post-dose. 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/tandem mass spectrometry (LC/MS/MS). 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 internal standard (500 ng/ml of 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 Separation Module; Waters, Milford,
MA) 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 398→176 and 404→182 for rivoglitazone and the internal standard, respectively.

For the metabolite profiling the study, the plasma sample (50 μl) including study samples or blank sample (for standards, single blank, double blank, and quality controls) was mixed with 150 μl of assay solution including IS (R-121171 for rivoglitazone and M19; R-132064 for M5, M7, M9, M11, and M13; R-252121 for M10 and M12; R-252122 for M17, M18, and M20; R-395374 for M6, M15, and M16), 50 μl of 1% formic acid in acetonitrile, and 150 μl of water.

After centrifugation at 4°C, the supernatant was filtrated using a Captiva 96-well filtration plate (pore size: 0.45 μm; Varian Inc., Palo Alto, CA) and was mixed with purified water (150 μl), and then preliminary added to a 96-well collection plate. Aliquot (2 μl) was injected onto an LC-MS/MS system. A MDS Sciex API 5000 mass spectrometer (Applied Biosystems, Foster City, CA) connected to the ACQUITY UPLC system (Waters, Milford, MA) was used as the LC-MS/MS system. Analyte detection was achieved with ESI in the positive ion mode using MRM of transitions of m/z 403→176 for M5 and M7, m/z 402→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, respectively.
The MRM of transitions for IS was \( m/z \ 404 \rightarrow 182 \) for R-121171, \( m/z \ 469 \rightarrow 389 \) for R-132064 and \( m/z \ 389 \rightarrow 167 \) for R-252121 and R-252122, respectively. 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 the following gradient elution with mobile phase A (methanol/water/ammonium acetate; 950:50:0.385, v/v/w) and mobile phase B (methanol/water/ammonium acetate/formic acid; 50:950:0.385:1, v/v/w/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 0.6 min, to 70% B at 1.7 min, hold 1.7 min, to 90% B at 3.5 min, hold 1 min, and back to 40% B at 4.6 min, hold 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), urine (100 μl–1 ml), and the cage wash sample (1 ml) were mixed with tissue solubilizer (1–2 ml, NCS-II, GE Healthcare Japan Corporation (Tokyo, Japan)) and a scintillation cocktail (10 ml, HIONIC-FLUOR; PerkinElmer Life and Analytical Sciences, Boston, MA or Clear-sol I; Nacarai Tesque, Inc., Kyoto, Japan), and then analyzed by a liquid scintillation analyzer (TRI-CARB 2250CA; PerkinElmer Life and Analytical Sciences, Boston, MA). 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), 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, NCS-II) and a scintillation cocktail (10 ml, HIONIC-FLUOR), and then analyzed by 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) was 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, Boston, MA), mixed with a scintillation cocktail (9 ml, PERMAFLUOR E+; PerkinElmer Life and Analytical Sciences), and then analyzed by 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, Nacarai Tesque, Inc.), 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 (Radio-HPLC) and Structure Analysis of Metabolites.**

The plasma samples collected at 6 h post-dose 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 the aliquot was analyzed by radio-HPLC, liquid chromatography/mass spectrometry (LC/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 also 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 were pooled from each subject 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 Rikakiki 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 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, Waltham, MA) or an L-2000 HPLC system (Hitachi, Ltd.) 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 held at 90% solvent B for 5 min. The column effluent was monitored using an L-4000 UV detector (UV at 290 nm; Hitachi, Ltd.) and a radioactivity detector with a 3 ml/min flow rate for the Ultima-Flo M liquid scintillator (PerkinElmer Life and Analytical Sciences).

The composition of each metabolite in urine, bile, and feces was calculated as the ratio of the radioactive peak area of each metabolite to the sum of the radioactive peak area over the run time (40 min) using Flo-One for Windows. The lower limit of detection for the measurement of the metabolites was set based on 2-fold of the background peak height. The excretion of metabolites in the urine, bile, and feces was calculated by multiplying the radioactivity in samples by the composition of the metabolites in the samples, and then dividing by 100. An aliquot of the sample was subjected to radioactivity measurement. The recovery of radioactivity in the sample was calculated by the concentration of radioactivity in each sample multiplied by the weight of the total sample divided by the radioactivity in the analysis sample, which was applied to the pretreatment. The concentrated samples were injected onto radio-HPLC, and the elute from radio-HPLC system was collected and weighed. Aliquots of 10 ml of the elute were transferred into a vial and were weighed. Aliquots of 50 μl of the samples were mixed with 10 ml of Ultima-Flo M liquid scintillator. Aliquots of 10 ml of Ultima-Flo M were transferred to a vial and were used as the background sample. The radioactivity in the elute sample was divided by the sampling weight and was multiplied by the total weight of the eluate. This value
was taken as the total radioactivity injected. The HPLC column recovery was calculated as the percentage of the total radioactivity in the eluate to the total radioactivity injected.

The HPLC column recovery in urine (rat: 100.2%, monkey: 104.8%), bile (rat: 101.5%, monkey: 99.6%), and feces (rat: 97.9–106.4%, monkey: 97.8–98.8%) was calculated. The recovery of radioactivity in the extract of feces was 88.9–90.9%.

Aliquots of the samples were injected onto radio-HPLC. The area percentage (%) of each radioactive peak was calculated. The radioactive peaks were identified by comparing the \( t_R \) of the radioactive peaks and the UV \( t_R \) of the synthetic standards.

**LC/MS and LC/MS/MS for Structure Analysis of Metabolites.**

The conditions described below were used for the LC/MS and LC/MS/MS of the rivoglitazone metabolites. These analyses were performed using a quadrupole time-of-flight (Q-Tof) mass spectrometer (Waters, Manchester, UK) with an L-7000 HPLC system (Hitachi, Ltd.) consisting of an intelligent pump (model L-7100), a column oven (model L-7300), a chromato-integrator (model D-7500), and a UV detector (model L-7400S). The LC/MS was conducted using ESI in the positive ion mode. The capillary voltage and cone voltage were set at 3300 V and 45 V, respectively. The source temperature and desolvation gas temperature were 120°C and 300°C, respectively. The mass range from \( m/z \) 50 to 1000 was acquired with an integration time of 1 s. The LC/MS/MS was performed using a collision energy of 20 eV and xenon as the collision gas. Chromatographic separation was performed on a YMC-Pack ODS-A column (1.5 × 150 mm, 5 µm; YMC Co., Ltd.); the column temperature was maintained at 30°C and the UV detection was at 290 nm. The
The mobile phase consisted of water containing 0.01% TFA (solvent A) and acetonitrile containing 0.01% TFA (solvent B). 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 held at 90% solvent B for 5 min. The flow rate was set at 0.1 ml/min, and the elution flow from HPLC was introduced into the Q-Tof mass spectrometer ionization source through an ESI interface.

**Whole Blood-to-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 (C_B) and 100 μl of plasma (C_P), which was obtained after centrifugation at 15,000 rpm of 400 μl blood for 2 min at 4°C, was measured. The blood-to-plasma concentration ratio (R_B) was calculated by C_B/C_P.

**Pharmacokinetic Analysis.**

Pharmacokinetic parameters were calculated using a non-compartmental model with computer software WinNonlin Professional (ver. 4.0.1., Pharsight Corp.). The area under the plasma concentration versus the time curve up to the last quantifiable time (AUC_0-t) was calculated by the linear trapezoidal rule. The AUC up to infinity (AUC_0-inf) was calculated by the following formula:

\[
AUC_{0\text{-inf}} = AUC_{0\text{-t}} + \frac{C_{last}}{\lambda_z}
\]

where, C_{last} and \( \lambda_z \) are the plasma concentration at the last quantifiable time and the elimination rate constant associated with the terminal phase, respectively. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained...
from the observed data. The terminal elimination half-life ($t_{1/2}$) was calculated by \( \ln(2)/\lambda_z \). The total body clearance (CL_tot) and oral clearance (CL_{tot}/F) were calculated as the ratio of dose to AUC$_{0\text{-}inf}$ for i.v. and oral dosing, respectively. The volume of distribution at steady-state ($V_{ss}$) was calculated as \( \text{dose} \cdot \text{AUMC}/(\text{AUC}_{0\text{-}inf})^2 \), where AUMC is the area under the first moment of the plasma concentration-time curve. Oral bioavailability ($F_{oral}$) for each dose was calculated as \( \frac{\text{AUC}_{0\text{-}inf} \text{ for oral dose}}{\text{AUC}_{0\text{-}inf} \text{ for i.v. dose}} \) at the same dose. For rats, the AUC$_{0\text{-}inf}$ for i.v. dose was used as the mean values, and for monkeys, AUC$_{0\text{-}inf}$ for oral dose and AUC$_{0\text{-}inf}$ for i.v. dose were used for the individual monkey.
Results

**Pharmacokinetics.**

Pharmacokinetic parameters after i.v. and oral administration of rivoglitazone to rats and monkeys are summarized in Table 1 and Table 2, respectively. For i.v. 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 CL\textsubscript{tot} and V\textsubscript{ss} were similar and were low values in both species: 0.329–0.333 ml/min/kg and 0.125–0.131 l/kg for rats and 0.310–0.371 ml/min/kg and 0.138–0.166 l/kg for monkeys. The mean t\textsubscript{1/2} in rats was 4.55–4.84 h and that in monkeys was 6.21–6.79 h. For oral dosing, AUC\textsubscript{0–t}, AUC\textsubscript{0–inf}, and C\textsubscript{max} 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 T\textsubscript{max} was 1.13–3.25 h for rats and 3.00–4.25 h for monkeys. The mean F\textsubscript{oral} was high: >95.0% in rats and >76.1% in monkeys. The t\textsubscript{1/2} for oral dosing was similar to that for intravenous dosing: 4.35–4.98 h for rats and 7.34–8.33 h for monkeys. The mean CL\textsubscript{tot}/F exhibited similar values to CL\textsubscript{tot}: 0.304–0.354 ml/min/kg for rats and 0.410–0.485 ml/min/kg for monkeys. The mean R\textsubscript{B} of rivoglitazone was 0.593–0.671 in rats and 0.710–0.810 in monkeys, respectively.

**Excretion of Radioactivity.**

Excretion of radioactivity after i.v. and oral administration of [\textsuperscript{14}C]rivoglitazone to rats and monkeys is summarized in Table 3. After i.v. and oral administration of [\textsuperscript{14}C]rivoglitazone (1 mg/kg) to rats, the mean of 25.6% and 75.4% of i.v. dose and mean of 18.3% and 82.0% of oral dose were recovered in urine and feces, up to 168 h (Table 3), respectively. Almost all of the radioactivity was excreted within 48 h, and
the mean was 99.7% of i.v. dose and 99.1% of oral dose.

In bile BDC rats, the mean biliary excretion up to 48 h was 55.4% for i.v. 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 i.v. dosing and 81.1% of dose for oral dosing. In addition, the mean urinary and fecal excretion in BDC rats were 18.4% and 7.0% for i.v. dosing and 19.9% and 19.1% for oral dosing, respectively.

After i.v. and oral administration of [14C]rivoglitazone (1 mg/kg) to monkeys, the mean was 47.4% and 38.4% of i.v. dose, and 36.7% and 53.0% of 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–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 Metabolites 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 (CID) 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 to 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 $m/z$ 464, which was 80 Da higher than that of M12. The LC/MS/MS spectrum of the ion [M + H]+ at $m/z$ 464 and the proposed fragmentation scheme of M11 are shown in Fig. 5. Product ions at $m/z$ 147, 162, 242, 268, and 384 were obtained. Furthermore, the LC/MS/MS spectrum and HPLC retention time of M11 were identical to those of the synthetic standard. Based on these results, M11 was identified as an O-demethyl-O-sulfate.

The positive ion LC/MS spectrum of M4 showed a protonated molecule [M + H]+ at $m/z$ 560, which was 176 Da higher than that of M12. The LC/MS/MS spectrum of the ion [M + 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 [M + H]+ at $m/z$ 560. The loss of 176 Da corresponded to the glucuronic acid moiety. The product ion at $m/z$ 338 formed via the loss of 222 Da from the ion [M + H]+ at $m/z$ 560 suggested that the 6-methoxy-1-methyl-benzimidazole moiety was demethylated and subsequently glucuronidated. Based on these results, M4 was proposed to be an O-demethyl-O-glucuronide.

The positive ion LC/MS spectrum of M2 showed a protonated molecule [M + H]+ at $m/z$ 548. The LC/MS/MS spectrum of the ion [M + 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 [M + H]+ at $m/z$ 548. The loss of 176 Da corresponded to the glucuronic acid moiety. The product ion at
m/z 338 formed via the loss of 210 Da from the ion [M + H]+ at m/z 548 suggested that the 6-methoxy-1-methyl-benzimidazol 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 spectrum of M13 showed a protonated molecule [M + H]+ at m/z 574, which was 176 Da higher than that of rivoglitazone. The LC/MS/MS spectrum of the ion [M + 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 N-glucuronide.

The positive ion LC/MS spectrum of M9 showed a protonated molecule [M + H]+ at m/z 592, which was 18 Da higher than that of M13. The LC/MS/MS spectrum of the ion [M + 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. Based on these results, M9 was identified as TZD ring-opened N-glucuronide, a hydrolyzed metabolite of M13.

The positive ion LC/MS spectrum of M14 showed a protonated molecule [M + H]+ at m/z 578, which was 14 Da lower than that of M9. The LC/MS/MS spectrum of the ion [M + 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 \([M + H]^+\) at \( m/z \) 578 indicated that the 6-methoxy-1-methyl-benzimidazole moiety was demethylated. The product ion at \( m/z \) 402 was formed via the loss of 176 Da from the ion \([M + H]^+\) 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 \([M + H]^+\) at \( m/z \) 578. Based on 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 \([M + H]^+\) at \( m/z \) 387. The LC/MS/MS spectrum of the ion \([M + H]^+\) 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 \([M + H]^+\) at \( m/z \) 403, which was 16 Da higher than that of M19. The LC/MS/MS spectrum of the ion \([M + H]^+\) 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 spectrum of M7 showed a protonated molecule \([M + H]^+\) at \( m/z \) 403, which was identical to that of M5. The LC/MS/MS spectrum of the ion \([M + H]^+\) 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–S30. The elucidated structures of these metabolites were further identified by comparing of 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 have 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 \( \text{AUC}_{0-t} \) 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 h 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 main metabolites and M11, M16, and M19 were detected as minor metabolites (0.25–0.64%). The mean t½ of the main metabolite M17 (19.8 h) was about 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 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 of M11 (10.0%), M6 (3.9%), M7 (1.7%), M10 (1.0%), and M2 (0.7%) were excreted. Other 12 unknown peaks (total recovery of 3.1% of dose) were also detected. In feces, unchanged rivoglitazone of 23.7% of dose was excreted and the major metabolites of M12 (25.6%), M8 (8.7%), M19 (3.0%), and more than 28 unknown minor peaks (total recovery of 13.8%) were detected. In bile, unchanged rivoglitazone was not observed and the major metabolites of 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%) were excreted. In addition, 8 unknown peaks (total recovery of 2.1% of dose) were detected.

In feces after i.v. dosing to BDC rats, unchanged rivoglitazone (2.3% of dose), M12 (2.0%), M8 (0.6%), M19 (0.1%), and other unknowns (2.0%) were detected. For oral dosing, an 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 $[^{14}\text{C}]\text{rivoglitazone}$ (1 mg/kg) are shown in Table 7. In urine, 0.3% of dose of unchanged rivoglitazone was detected and the main metabolites of M11+M12 (8.1%), M5+M6 (4.8%), M7+M15 (4.1%), M16 (3.8%), M4 (2.6%), M13 (1.4%), M10 (1.3%), M2 (1.2%), and M18 (0.4%) were detected. Other minor 14 unknown peaks were observed (total recovery of 7.4%). In feces, 23.5% of dose of unchanged rivoglitazone was excreted and the major metabolites of M12 (18.0%), M9 (6.6%), M8 (2.9%), M18 (1.7%), M19 (0.8%), and other minor unknown peaks (>38 peaks) were detected.
Discussion

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

In rats, the radioactivity after i.v. and oral dosing of \[^{14}C\]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 routes of urinary and fecal routes with the same ratio, which was slower than 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 i.v. dosing and 19% for oral dosing. Since the unchanged rivoglitazone was found in feces, the contribution of intestinal excretion was estimated in rats. In monkeys, the unchanged rivoglitazone of 23.5% was observed in feces after oral dosing (1 mg/kg). Since the mean \(F_{\text{oral}}\) at 0.986 mg/kg dose was 76.1%, the unchanged rivoglitazone in feces may be due to the unabsorbed dose. While it is the main metabolite in monkey bile, \(N\)-glucuronide (M13), was not detected in feces.
M13 was de-conjugated to rivoglitazone after incubation in monkey fecal homogenate (data not shown). Therefore, some fraction of unchanged rivoglitazone in feces may be derived from de-conjugation via microflora. Since the mass balance study in BDC monkey 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 estimated 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 estimated 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 the TZD ring. In particular, we have found the M13 and 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 have already found the N-glucuronides and TZD ring-opened N-glucuronides of rosiglitazone (Uchiyama et al., 2010a) and pioglitazone (Uchiyama et al., 2010b) 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, \(N\)-glucuronides of MaxiPost (Zhang et al., 2005), nirvanol (Maguire et al., 1982), and bucolome (Mohri et al., 1985) for \(N\)-glucuronidation of cyclic amide compounds, and that of PNU-107859 for \(N\)-glucuronidation of 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 about 0.7 h) in buffer solution (pH 7.4) during incubation at 37°C (data not shown). After \(N\)-glucuronidation of the TZD ring, the 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, become easily attacked by 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 the S-methylation in the presence of S-adenosyl-L-methionine (SAM) in the 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., 2010a), pioglitazone (Baughman et al., 2005, Uchiyama et al., 2010b), and the TZD derivatives MK-0767 (Karanam et al., 2004; Liu et al., 2004; Kochansky et al., 2006) and 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 M10, which are estimated to form via the TZD ring-opened mercapto amide (intermediate I in Fig. 8). The synthetic standard of intermediate I was immediately metabolized to TZD ring-opened methylmercapto amide (M20) by S-methylation in the presence of SAM in the human liver microsomes or cytosol (data not shown). In this condition, we have observed traces of intermediate II, but the contribution of this pathway to form intermediate II will be very low. The oxidative TZD ring opening and subsequent pathways via the generation of reactive intermediates have been reported for some toxicological concerns (Kassahun et al., 2001; Tettey et al., 2001; Smith, 2003; He et al., 2004; Baughman et al., 2005; Alvarez-Sanchez et al., 2006). On the other hand, for the new ring-opening reaction via N-glucuronidation of rivoglitazone, further studies are needed to investigate the toxicological evaluation.

The metabolites related to the O-demethylation pathway were estimated to be M1, M2, M3, M4, M8, M11, M12, and M14; those to the TZD ring-opening pathway were M6, M10, M15 and M20; those to the N-glucuronidation pathway were estimated to be M5, M7, M9, M13, M15, and M19; those to the N-demethylation pathway were estimated to be M16 and M17; and that to the hydroxylation was M18, respectively. From these assignments, the contribution of metabolic pathways in rats was estimated to be 45.0% for O-demethylation, 4.9% for TZD ring opening, 4.7% for N-glucuronidation based on the urine and feces data; and 43.1% for O-demethylation, 6.4% for TZD ring opening, and 3.3% for N-glucuronidation based on the urine and bile data, respectively. In monkeys, the contribution of metabolic pathways was estimated to be 32.8% for O-demethylation, 12.9% for N-glucuronidation, 3.8% for N-demethylation, 2.1% for TZD ring hydroxylation, 1.3% for TZD ring-opening based
on the urine and feces data. Thus, O-demethylation was evaluated to be the main metabolic pathway in both animals, but N-demethylation and TZD ring hydroxylation were observed only in monkeys. The amounts of N-glucuronide formed in monkeys were also higher than those in rats. In general, N-glucuronidation activity to nitrogen-containing aromatic heterocycles, such as rivoglitazone in monkeys has been reported to be higher than that in rats (Chiu and Huskey, 1998). In addition, since there were many minor metabolites that were observed in urine, feces, and bile, rivoglitazone is thought to be extensively metabolized and excreted.

Rivoglitazone was the main component in plasma for both species, but there were species differences in metabolite plasma profiles. In rat plasma, M11 was observed as the main metabolite, which was more than 10% of molar basis parent AUC₀₋ᵣ (23.4%), and the other metabolites were less than 1%. On the other hand, in monkey plasma, there were no metabolites more than 10% of parent AUC₀₋ᵣ, but M17, M13, M12, and M9 exhibited >1% of parent AUC₀₋ᵣ. In particular, AUC₀₋ᵣ of M11, which was the main metabolite based on urine and feces data, was low (0.64% of parent AUC₀₋ᵣ) in monkeys. The t₁/₂ of M11 and M17, which was the major metabolite in rats and monkey, respectively, was longer than that for rivoglitazone, suggesting that the rate limiting step is a disposition of the metabolite (Smith, 1999). In addition, since the pharmacological activity based on PPARγ activation activity of M11 and M17 were low compared to rivoglitazone (data not shown), rivoglitazone is thought to be mainly contributed to efficacy.

In summary, the pharmacokinetics, metabolism, and disposition of rivoglitazone were investigated in rats and monkeys. The pharmacokinetics of rivoglitazone is characterized by low clearance, low volume of distribution, and a 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, Okazaki, Iwabuchi, and Izumi.

Conducted experiments: Uchiyama, Tsuruta, Abe, Takahashi, Koda, and Izumi.

Contributed new reagents or analytic tools: Oguchi.

Performed data analysis: Uchiyama, Iwabuchi, and Izumi.

Wrote or contributed to the writing of the manuscript: Uchiyama and Izumi.

Other: not applicable.
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Footnotes

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**Figure Legends**

Fig. 1. Chemical structure of $[^{14}\text{C}]$rivoglitazone and internal standards: R-121171, R-132064, R-252121, R-252122, and R-395374.

Fig. 2. Representative radiochromatograms of the metabolites in rat plasma, urine, bile, and feces after a single oral administration of $[^{14}\text{C}]$rivoglitazone at a dose of 1 or 5 mg/kg.

Fig. 3. Representative radiochromatograms of the metabolites in monkey plasma, urine, bile, and feces after a single oral administration of $[^{14}\text{C}]$rivoglitazone at a dose of 1 mg/kg.

Fig. 4. LC/MS/MS spectrum and the proposed fragmentation scheme of rivoglitazone.

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.

Fig. 7. Plasma profiles of rivoglitazone and its metabolites after oral administration of rivoglitazone (1 mg/kg) to rats and monkeys. Each point represents the mean of four animals.

Fig. 8. Proposed in vivo metabolic pathways of rivoglitazone in rats and monkeys.
Table 1  Pharmacokinetic parameters of rivoglitazone after i.v. administration to rats and monkeys

Values represent mean ± S.D.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC_{0-t} (μg·h/ml)</th>
<th>AUC_{0-inf} (μg·h/ml)</th>
<th>CL_{tot} (ml/min/kg)</th>
<th>V_{ss} (l/kg)</th>
<th>t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.1 (n = 3)</td>
<td>4.53 ± 0.07</td>
<td>4.67 ± 0.07</td>
<td>0.329 ± 0.005</td>
<td>0.125 ± 0.002</td>
<td>4.55 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.5 (n = 4)</td>
<td>22.3 ± 1.0</td>
<td>22.9 ± 1.0</td>
<td>0.333 ± 0.014</td>
<td>0.131 ± 0.006</td>
<td>4.84 ± 0.31</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.0986 (n = 4)</td>
<td>3.80 ± 1.64</td>
<td>5.00 ± 1.21</td>
<td>0.312 ± 0.071</td>
<td>0.166 ± 0.022</td>
<td>6.38 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>0.493 (n = 4)</td>
<td>21.7 ± 7.6</td>
<td>23.5 ± 9.0</td>
<td>0.371 ± 0.179</td>
<td>0.155 ± 0.024</td>
<td>6.21 ± 1.46</td>
</tr>
<tr>
<td></td>
<td>0.986 (n = 4)</td>
<td>51.7 ± 18.6</td>
<td>57.2 ± 22.0</td>
<td>0.310 ± 0.166</td>
<td>0.138 ± 0.019</td>
<td>6.79 ± 1.74</td>
</tr>
</tbody>
</table>
Table 2  Pharmacokinetic parameters of rivoglitazone after oral administration to rats and monkeys

Values represent mean ± S.D.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose mg/kg</th>
<th>AUC$_{0-t}$ μg·h/ml</th>
<th>AUC$_{0-inf}$ μg·h/ml</th>
<th>C$_{max}$ μg/ml</th>
<th>T$_{max}$ h</th>
<th>t$_{1/2}$ h</th>
<th>CL$_{tot}$/F ml/min/kg</th>
<th>F$_{oral}$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.1 (n = 4)</td>
<td>4.34 ± 0.90</td>
<td>4.45 ± 0.91</td>
<td>0.409 ± 0.096</td>
<td>4.35 ± 0.36</td>
<td>0.354 ± 0.071</td>
<td>98.1 ± 20.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 (n = 4)</td>
<td>21.0 ± 0.4</td>
<td>21.8 ± 0.3</td>
<td>2.09 ± 0.22</td>
<td>1.13 ± 0.63</td>
<td>4.98 ± 0.54</td>
<td>0.352 ± 0.004</td>
<td>95.0 ± 11.0</td>
</tr>
<tr>
<td></td>
<td>1 (n = 4)</td>
<td>48.5 ± 5.0</td>
<td>49.8 ± 4.9</td>
<td>4.57 ± 0.76</td>
<td>2.25 ± 1.26</td>
<td>4.50 ± 0.37</td>
<td>0.304 ± 0.030</td>
<td>N.A.</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.0986 (n = 4)</td>
<td>3.44 ± 1.50</td>
<td>4.47 ± 2.19</td>
<td>0.242 ± 0.125</td>
<td>3.00 ± 2.71</td>
<td>8.33 ± 1.83</td>
<td>0.432 ± 0.293</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>0.493 (n = 2)</td>
<td>17.2 ± 6.6</td>
<td>17.7 ± 6.5</td>
<td>1.33 ± 0.67</td>
<td>4.25 ± 2.22</td>
<td>7.56 ± 2.46</td>
<td>0.485 ± 0.433</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>0.986 (n = 3)</td>
<td>35.8 ± 11.7</td>
<td>43.1 ± 17.8</td>
<td>2.93 ± 0.85</td>
<td>3.50 ± 2.38</td>
<td>7.34 ± 2.37</td>
<td>0.410 ± 0.222</td>
<td>76.1 ± 6.1</td>
</tr>
</tbody>
</table>

N.A., not applicable.
Table 3  Excretion of radioactivity after i.v. and oral administration of 
$[^{14}C]$rivoglitazone to rats and monkeys

Values represent mean ± S.D.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose/Route</th>
<th>Sampling time</th>
<th>Recovery of Radioactivity (% of Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–48 h</td>
<td>Urine</td>
</tr>
<tr>
<td>Rat</td>
<td>1 mg/kg, i.v. (n = 4)</td>
<td>25.5 ± 1.3</td>
<td>74.2 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>1 mg/kg, oral (n = 4)</td>
<td>18.0 ± 1.2</td>
<td>81.1 ± 0.6</td>
</tr>
<tr>
<td>BDC Rat</td>
<td>0.5 mg/kg, i.v. (n = 3)</td>
<td>18.4 ± 7.7</td>
<td>7.0 ± 4.1</td>
</tr>
<tr>
<td>Monkey</td>
<td>1 mg/kg, i.v. (n = 3)</td>
<td>39.4 ± 6.3</td>
<td>10.3 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>1 mg/kg, oral (n = 3)</td>
<td>29.7 ± 4.7</td>
<td>24.6 ± 19.8</td>
</tr>
</tbody>
</table>

N.A., not applicable.
Table 4  Characterization of rivoglitazone metabolites in rats and monkeys after a single oral administration of [14C]rivoglitazone

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Retention Time (min)</th>
<th>[M+H]+</th>
<th>Identity</th>
<th>Product Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rivoglitazone</td>
<td>23.0 - 23.2</td>
<td>398</td>
<td>Rivoglitazone</td>
<td>161, 176 (B), 282, 327</td>
</tr>
<tr>
<td>M1</td>
<td>9.9</td>
<td>468</td>
<td>O-demethyl-O-sulfate-TZD ring-opened methyl sulfoxide amide</td>
<td>147, 162 (B), 242, 281, 307, 324, 361</td>
</tr>
<tr>
<td>M2</td>
<td>12.9 - 13.2</td>
<td>548</td>
<td>O-demethyl-O-glucuronide-TZD ring-opened methylmercapto amide</td>
<td>147, 162, 267, 280, 338, 372 (B)</td>
</tr>
<tr>
<td>M3</td>
<td>13.8</td>
<td>484</td>
<td>O-demethyl-O-sulfate-TZD ring-opened methyl sulfone amide</td>
<td>147, 162 (B), 242, 281, 324, 404</td>
</tr>
<tr>
<td>M4</td>
<td>15.1</td>
<td>560</td>
<td>O-demethyl-O-glucuronide</td>
<td>147, 162, 268, 338, 384 (B)</td>
</tr>
<tr>
<td>M5</td>
<td>15.8 - 15.9</td>
<td>403</td>
<td>TZD ring-opened methyl sulfoxide carboxylic acid</td>
<td>161, 176 (B), 295, 339</td>
</tr>
<tr>
<td>M6</td>
<td>16.1 - 16.5</td>
<td>402</td>
<td>TZD ring-opened methyl sulfoxide amide</td>
<td>161, 176 (B), 295, 321</td>
</tr>
<tr>
<td>M7</td>
<td>16.8 - 17.2</td>
<td>403</td>
<td>TZD ring-opened methyl sulfoxide carboxylic acid</td>
<td>161, 176 (B), 295, 339</td>
</tr>
<tr>
<td>M8</td>
<td>16.9 - 17.0</td>
<td>372</td>
<td>O-demethyl-TZD ring-opened methylmercapto amide</td>
<td>147, 162 (B), 267, 268, 280, 281</td>
</tr>
<tr>
<td>M9</td>
<td>17.2 - 17.6</td>
<td>592</td>
<td>TZD ring-opened N-glucuronide</td>
<td>161, 176 (B), 282, 327, 373, 399, 416, 458</td>
</tr>
<tr>
<td>M10</td>
<td>18.1 - 18.5</td>
<td>418</td>
<td>TZD ring-opened methyl sulfone amide</td>
<td>161, 176 (B), 295, 321, 338</td>
</tr>
<tr>
<td>M11</td>
<td>19.0 - 19.1</td>
<td>464</td>
<td>O-demethyl-O-sulfate</td>
<td>147, 162 (B), 242, 268, 384</td>
</tr>
<tr>
<td>M12</td>
<td>19.0 - 19.2</td>
<td>384</td>
<td>O-demethyl rivoglitazone</td>
<td>147, 162 (B), 268, 313</td>
</tr>
<tr>
<td>M13</td>
<td>19.8 - 19.9</td>
<td>574</td>
<td>N-glucuronide</td>
<td>161, 176 (B), 282, 398</td>
</tr>
<tr>
<td>M14</td>
<td>13.9</td>
<td>578</td>
<td>O-demethyl-TZD ring-opened N-glucuronide</td>
<td>147, 162 (B), 313, 359, 385, 402, 444</td>
</tr>
<tr>
<td>M15</td>
<td>16.8 - 17.2</td>
<td>492</td>
<td>TZD ring-opened S-cysteine conjugate</td>
<td>161, 176 (B), 295, 340</td>
</tr>
<tr>
<td>M16</td>
<td>17.8</td>
<td>370</td>
<td>O,N-didemethyl rivoglitazone</td>
<td>148 (B), 254</td>
</tr>
<tr>
<td>M17</td>
<td>21.5</td>
<td>384</td>
<td>N-demethyl rivoglitazone</td>
<td>147, 162 (B), 268, 313</td>
</tr>
<tr>
<td>M18</td>
<td>21.3 - 21.5</td>
<td>414</td>
<td>TZD ring 5-hydroxy rivoglitazone</td>
<td>161, 176 (B), 309, 354</td>
</tr>
<tr>
<td>M19</td>
<td>24.1</td>
<td>387</td>
<td>TZD ring-opened methylmercapto carboxylic acid</td>
<td>161, 176 (B), 282, 295</td>
</tr>
<tr>
<td>M20</td>
<td>20.9</td>
<td>386</td>
<td>TZD ring-opened methylmercapto amide</td>
<td>161, 176 (B), 281, 282, 294, 295</td>
</tr>
</tbody>
</table>

B, base peak.
Table 5  AUC$_{0-t}$ of rivoglitazone and its metabolites after oral dosing of rivoglitazone (1 mg/kg) to rats and monkeys

Values represent mean ($n = 4$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC$_{0-t}$</td>
<td>Ratio of Molar Basis</td>
</tr>
<tr>
<td></td>
<td>ng·h/ml</td>
<td>%</td>
</tr>
<tr>
<td>Rivoglitazone</td>
<td>40500</td>
<td>100</td>
</tr>
<tr>
<td>M5</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<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>
<td>9.85</td>
<td>0.02</td>
</tr>
<tr>
<td>M11</td>
<td>11000</td>
<td>23.4</td>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>M20</td>
<td>2.71</td>
<td>0.01</td>
</tr>
</tbody>
</table>

N.A., not applicable.

$^a$t represents the last quantifiable time for each analyte.
Table 6  Qualitative metabolite profiles (% of dose) in urine, feces, and bile after oral administration of \([14\text{C}]\text{rivoglitazone (1 mg/kg)}\) to rats

Values represent mean \((n = 4)\).

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Rivoglitazone</td>
<td>N.D.</td>
<td>23.7</td>
<td>N.D.</td>
<td>23.7</td>
<td>N.D.</td>
</tr>
<tr>
<td>M1</td>
<td>N.D.</td>
<td>N.D.</td>
<td>3.8</td>
<td>N.D.</td>
<td>3.8</td>
</tr>
<tr>
<td>M2</td>
<td>0.7</td>
<td>N.D.</td>
<td>2.8</td>
<td>0.7</td>
<td>3.5</td>
</tr>
<tr>
<td>M3</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.3</td>
<td>N.D.</td>
<td>1.3</td>
</tr>
<tr>
<td>M4</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.5</td>
<td>N.D.</td>
<td>1.5</td>
</tr>
<tr>
<td>M5</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.6</td>
<td>N.D.</td>
<td>0.6</td>
</tr>
<tr>
<td>M6</td>
<td>3.9</td>
<td>N.D.</td>
<td>0.6</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>M7</td>
<td>1.7</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>M8</td>
<td>N.D.</td>
<td>8.7</td>
<td>N.D.</td>
<td>8.7</td>
<td>N.D.</td>
</tr>
<tr>
<td>M7, M8, M9</td>
<td>N.D.</td>
<td>N.D.</td>
<td>9.2</td>
<td>N.D.</td>
<td>9.2</td>
</tr>
<tr>
<td>M11</td>
<td>10.0</td>
<td>N.D.</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>M12</td>
<td>25.6</td>
<td>N.D.</td>
<td>25.6</td>
<td>N.D.</td>
<td>33.0</td>
</tr>
<tr>
<td>M13</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.0</td>
<td>N.D.</td>
<td>1.0</td>
</tr>
<tr>
<td>M19</td>
<td>N.D.</td>
<td>3.0</td>
<td>N.D.</td>
<td>3.0</td>
<td>N.D.</td>
</tr>
<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>5.2</td>
</tr>
</tbody>
</table>

N.D., not detected.
Table 7  Qualitative metabolite profiles (% of dose) in urine and feces after oral administration of [14C]rivoglitazone (1 mg/kg) to monkeys

Values represent mean ($n = 3$ for urine and $n = 4$ for feces).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urine (0–72 h)</th>
<th>Feces (0–72 h)</th>
<th>Urine + Feces (0–72 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rivoglitazone</td>
<td>0.3</td>
<td>23.5</td>
<td>23.8</td>
</tr>
<tr>
<td>M2</td>
<td>1.2</td>
<td>N.D.</td>
<td>1.2</td>
</tr>
<tr>
<td>M4</td>
<td>2.6</td>
<td>N.D.</td>
<td>2.6</td>
</tr>
<tr>
<td>M5, M6</td>
<td>4.8</td>
<td>N.D.</td>
<td>4.8</td>
</tr>
<tr>
<td>M7, M15</td>
<td>4.1</td>
<td>N.D.</td>
<td>4.1</td>
</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>1.3</td>
</tr>
<tr>
<td>M11, M12</td>
<td>8.1</td>
<td>N.D.</td>
<td>8.1</td>
</tr>
<tr>
<td>M12</td>
<td>N.D.</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>M13</td>
<td>1.4</td>
<td>N.D.</td>
<td>1.4</td>
</tr>
<tr>
<td>M16</td>
<td>3.8</td>
<td>N.D.</td>
<td>3.8</td>
</tr>
<tr>
<td>M18</td>
<td>0.4</td>
<td>1.7</td>
<td>2.1</td>
</tr>
<tr>
<td>M19</td>
<td>N.D.</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Unknown Metabolites</td>
<td>7.4 (14 peaks)</td>
<td>11.0 (&gt;38 peaks)</td>
<td>18.4</td>
</tr>
<tr>
<td>Total</td>
<td>35.4</td>
<td>64.5</td>
<td>99.9</td>
</tr>
</tbody>
</table>

N.D., not detected.
Fig. 1

![Chemical structures]

Rivoglitazone

* indicates position of $^{14}$C label

R-121171

R-252121

R-132064

R-252122

R-395374

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Fig. 2

Rat plasma (6 h)

Rat urine (0 - 48 h)

Rat bile (0 - 48 h)

Rat feces (0 - 24 h)
Fig. 3

Radioactivity (cpm)

Time (min)

monkey plasma
(6 h)

Rivoglitazone

monkey urine
(0 - 72 h)

M16
M15
M10
M11
M12
M13
M18
M20
M6
M7
M4
M5
Rivoglitazone

monkey bile
(8 h)

M7, M15
M11
M13
M14
M2
M5
M9
M17

monkey feces
(0 - 24 h)

M18
Rivoglitazone

M9
M8
M12
M19
Fig. 4

[Chemical structure and mass spectrum diagram]

- Relative intensity (%)
- m/z scale
- Peaks at 161, 176, 282, 327, 398 [M+H]⁺
Fig. 5
Fig. 6
Fig. 7

Rat

Monkey

Plasma concentration (ng/ml)

Time (h)

- Rivoglitazone
- M5
- M6
- M7
- M9
- M10
- M11
- M12
- M13
- M15
- M16
- M17
- M18
- M19
- M20
Supplemental Data

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

Minoru Uchiyama, Haruo Iwabuchi, Fujiko Tsuruta, Koji Abe, Makoto Takahashi, Hiroko Koda, Minoru Oguchi, Osamu Okazaki, and Takashi Izumi.

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-butyl 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,
Synthesis of M3. M3 was synthesized as shown in Fig. S2.

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) hexahy drate, CH₂Cl₂, rt, 27 h, 50°C, 4 h; (b) KOH, H₂O,
Synthesis of M11 and M12. M11 and M12 were synthesized as shown in Fig. S7.

**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.

**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.**

![Synthetic scheme of M16](image)

**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.**

![Synthetic scheme of M17](image)

**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%.
**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) monoperophthalic 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
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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]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 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]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 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]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 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]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 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]^+; ^1H NMR (400 MHz, DMSO-d$_6$) δ 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]^+; \(^1\)H NMR (400 MHz, DMSO-d6) 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]^+; \(^1\)H NMR (400 MHz, DMSO-d6) 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]^+; \(^1\)H NMR (400 MHz, DMSO-d6) 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), 6.82 (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]^+; \(^1\)H NMR (400 MHz, DMSO-d6) 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.
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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.