Metabolism and Disposition of [14C]Brivanib Alaninate after Oral Administration to Rats, Monkeys, and Humans

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ABSTRACT:

Brivanib [(R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2,4][1,2,4]triazin-6-yloxy]-1-methylethyl ester, BMS-582664], is currently under development as an oral agent for the treatment of cancer. Results from clinical studies conducted in patients with hepatocellular carcinoma and colorectal cancer have shown that brivanib was a prominent circulating component. All the metabolites that humans were exposed to were also present in toxicological species. On the basis of metabolite exposure and activity against VEGF and FGF receptors of the prominent human circulating metabolites, only brivanib is expected to contribute to the pharmacological effects in humans. Unchanged brivanib was not detected in urine or bile samples, suggesting that metabolic clearance was the primary route of elimination. The primary metabolic pathways were oxidative and conjugative metabolism of brivanib.

Introduction

Angiogenesis, a biological process of recruiting endothelial cells to form new blood vessels (Klagsbrun and Moses, 1999; Carmeliet, 2000), is crucial for the development, progression, and metastasis of most tumor types (Folkman, 1995; Ellis and Fidler, 1996; Klagsbrun and Moses, 1999; Carmeliet and Jain, 2000). Vascular endothelial growth factor receptor 2 (VEGFR-2), fibroblast growth factor receptor 1 (FGFR-1), and their isoforms are among the most important regulators for these processes (Ferrara, 2002; Ferrara et al., 2003; Klebl and Müller, 2005). Inhibition of VEGFR-2 and FGFR-1 pathways inhibits tumor progression (Ellis and Fidler, 1996; Gerwins et al., 1999; Tassi and Wellstein, 2006).

Brivanib [(R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2,4][1,2,4]triazin-6-yloxy)propan-2-ol, BMS-540215] is a potent and selective dual inhibitor of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) signaling pathways. Its alanine prodrug, brivanib alaninate [(R)-2-aminopropionic acid 2-[4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy]-1-methylethyl ester, BMS-582664], is currently under development as an oral agent for the treatment of cancer. This study describes the in vivo biotransformation of brivanib after a single oral dose of [14C]brivanib alaninate to intact rats, bile duct-cannulated (BDC) rats, intact monkeys, BDC monkeys, and humans. Fecal excretion was the primary route of elimination of drug-derived radioactivity in animals and humans. In BDC rats and monkeys, the majority of radioactivity was excreted in bile. Brivanib alaninate was rapidly and completely converted via hydrolysis to brivanib in vivo. The area under the curve from zero to infinity of brivanib accounted for 14.2 to 54.3% of circulating radioactivity in plasma in animals and humans, suggesting that metabolites contributed significantly to the total drug-related radioactivity. In plasma from animals and humans, brivanib was a prominent circulating component. All the metabolites that humans were exposed to were also present in toxicological species. On the basis of metabolite exposure and activity against VEGF and FGF receptors of the prominent human circulating metabolites, only brivanib is expected to contribute to the pharmacological effects in humans. Unchanged brivanib was not detected in urine or bile samples, suggesting that metabolic clearance was the primary route of elimination. The primary metabolic pathways were oxidative and conjugative metabolism of brivanib.

ABBREVIATIONS: VEGFR, vascular endothelial growth factor receptor; FGF, fibroblast growth factor receptor; BMS-540215, [(R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2,4][1,2,4]triazin-6-yloxy)propan-2-ol; BMS-582664, (1RS)-2-aminopropionic acid 2-[4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy]-1-methylethyl ester; ADME, absorption, disposition, metabolism, and excretion; BDC, bile duct-cannulated; GI, gastrointestinal; LC, liquid chromatography; MS, mass spectroscopy; HLM, human liver microsomes; UDPG, UDP-glucuronic acid trisodium salt; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; MS/MS, tandem mass spectrometry; LSC, liquid scintillation counting; TRA, total radioactivity; ESI, electrospray ionization; MRM, multiple reaction monitoring; 1-D, one-dimensional; 2-D, two-dimensional; HMBC, heteronuclear multiple-bond correlation spectroscopy; AUC, area under the concentration-time curve.
has been effective in cancer treatment with a manageable safety profile.

Previous studies indicated that brivanib alaninate was rapidly and efficiently hydrolyzed to brivanib, both in vitro and in vivo (Marathe et al., 2009). When incubated with hepatocytes and liver S9 fractions from mouse, rat, dog, monkey, and human, brivanib alaninate was rapidly hydrolyzed with a half-life of less than 10 min. In addition, brivanib alaninate underwent hydrolysis to brivanib in serum and plasma from animals and humans. After oral doses of brivanib alaninate to mouse, rat, dog, and monkey, when plasma was collected under conditions to stabilize brivanib alaninate, no circulating prodrug was detected, suggesting that the prodrug hydrolysis occurred presystemically.

Absorption, distribution, metabolism, and excretion (ADME) studies have become an integral part of drug development. Data generated from ADME studies address a number of questions that are critical to development of a drug. On the basis of metabolite structures identified in human plasma, urine, and feces/bile, the primary metabolic pathways of a compound can be determined. In practice, ADME studies are often conducted in both humans and preclinical species used in long-term safety evaluation to confirm that human circulating metabolites are also present in toxicology species. For drugs that undergo conjugative metabolism, ADME studies in bile-duct-cannulated (BDC) animals are necessary, because conjugative metabolites tend to be hydrolyzed during their passage through the gastrointestinal (GI) tract, resulting in the underestimation of the role of this pathway in the overall metabolic clearance of a new chemical entity. This article describes the in vivo disposition and biotransformation of brivanib in intact rats, intact monkeys, BDC rats, BDC monkeys, and humans after single oral doses of [14C]brivanib alaninate.

Materials and Methods

Materials. [14C]Brivanib alaninate (Fig. 1) (13.1 μCi/mg, radiochemical purity 98.5%) was supplied by the radiochemistry group of the Department of Chemical Synthesis, Bristol-Myers Squibb Research and Development (Princeton, NJ). Nonradiolabeled brivanib alaninate, brivanib (Fig. 1), stable labeled [14C]brivanib alaninate (Fig. 1), and reference standards, 4-(4-fluoro-2-methyl-1H-indol-5-yl-oxy)-5-methylpyrrolo[1,2,4]triazin-6-ol (M9) and 1-(4-(4-fluoro-2-methyl-1H-indol-5-yl-oxy)-5-methyl-pyrrolo[1,2,4]triazin-6-yl-oxy)propan-2-one (M31), were supplied by Bristol-Myers Squibb Research and Development. Materials used for the pharmacological activity determination of (R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yl-oxy)-5-methylpyrrolo[1,2-\(\text{f}\)][1,2,4]triazin-6-yl-oxy)propan-2-ol (M7), (R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yl-oxy)-5-methylpyrrolo[1,2-\(\text{f}\)][1,2,4]triazin-6-yl-oxy)propan-2-yl hydrogen sulfate (M25), and (R)-4-fluoro-5-(6-(2-hydroxypropoxy)-5-methylpyrrolo[1,2-\(\text{f}\)][1,2,4]triazin-4-yl-oxy)-1H-indole-2-carboxylic acid (M26) were synthesized in-house.

Dosing and Sample Collection. All animal studies were conducted under the standards recommended by the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and were approved by the institutional animal care and use committee. All human patients provided written consent before participation in the study, and the protocol for the human study was approved by the institutional review board and radiation safety committee at the investigational site.

The [14C]brivanib alaninate dosing solution was prepared in 50 mM sodium citrate buffer (pH 3.5), with a concentration of 1 mg/ml for animals and 40 mg/ml for humans. Rats and monkeys were administered single oral doses of [14C]brivanib alaninate at dose levels that were well tolerated in toxicological studies. Blood samples from animals were collected into tubes containing K2EDTA. In the human study, tubes containing K2EDTA as anticoagulant and glycine buffer (pH 3.5) as a stabilizer for brivanib alaninate were used for collecting blood. All blood samples were placed on ice immediately and were centrifuged within 30 min after collection for 15 min at approximately 1000 g to separate plasma.

Intact rat study. The mass balance and pharmacokinetics of radioactivity and metabolism of brivanib were investigated in intact male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) after administration of [14C]brivanib alaninate. Eighteen rats, weighing approximately 250 to 314 g, were placed into three groups. Each animal received a single oral dose of [14C]brivanib alaninate administered by gavage at a target dose level of 100 mg/kg (100 μCi/kg). Animals were fasted overnight before dosing. Approximately 4 h after dosing, animals were fed Cervified Rodent Diet 5002 (PMI Nutrition International, Inc., Henderson, CO) and were given unlimited access to water. Terminal blood samples (maximum volume) for biotransformation profiling were collected from animals in group 1 (n = 12, 3 animals/time point) at 1, 2, 6, and 12 h after dosing. Animals in group 2 (n = 3) were individually housed in metabolism cages for the separate collection of urine and feces. The excreta were collected before dosing and over 24-h intervals through 168 h after dosing. Cage rinses were performed after each collection interval, and a cage wash was performed at the end of the study period. Serial blood samples for pharmacokinetic analysis of radioactivity were collected from the animals in group 3 (n = 3) via the tail vein before dosing and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after dosing.

Intact monkey study. The mass balance and pharmacokinetics of radioactivity and metabolism of brivanib were investigated in intact male cynomolgus monkeys (Charles River Laboratories, Inc.) after administration of [14C]brivanib alaninate. The monkeys (n = 3), weighing approximately 4.2 to 6.3 kg, were individually housed in metabolism cages. Animals were fasted overnight before dosing. Approximately 4 h after dosing, animals were fed Certified Primate Diet 5048 (PMI Nutrition International, Inc.) and were given unlimited access to water. Each animal, restrained during dose administration, received a single oral dose of [14C]brivanib alaninate administered by gavage at a target dose level of 10 mg/kg (20 μCi/kg). Serial blood samples were collected from each monkey via the vascular access port before dosing and at 1, 2, 4, 6, 12, 24, 48, and 72 h after dosing. Urine and feces were collected from each monkey before dosing and over 24-h intervals through 168 h postdose. Cage rinses were performed after each collection interval, and a cage wash was performed at the end of the study period.

BDC rat study. The excretion of radioactivity into bile, feces, and urine and metabolism of brivanib were investigated in BDC male Sprague-Dawley rats (Technical Support Unit, Bristol-Myers Squibb) after administration of [14C]brivanib alaninate. BDC rats (n = 3, weighing approximately 255–290 g) were individually housed in metabolism cages and were freely mobile during
the entire study with the exception of brief manual restraint for oral dosing. Each animal was allowed to recover for approximately 48 h after surgery and was administered a single oral dose of \(^{14}C\) brivanib alanine administered by gavage at a target dose level of 100 mg/kg (100 μCi/kg). Animals were fasted overnight before dosing. Approximately 4 h after dosing, animals were fedCertified Rodent Diet 5002 (PMI Nutrition International, Inc.) and were given unlimited access to water. During the period of sample collection, bile salts were replenished by infusing control bile collected in the day before dose administration at a rate of 1 ml/min. Bile was collected into containers that were surrounded by dry ice before dosing and in intervals from 0 to 3, 3 to 6, 6 to 12, 12 to 24, and 24 to 48 h after dosing. Urine and feces were collected before dosing and over 12-h intervals through 48 h postdose.

**BDC monkey study.** The excretion of radioactivity into bile, feces, and urine and metabolism of brivanib were investigated in BDC male cynomolgus monkeys (Charles River Laboratories, Inc.) after administration of \(^{14}C\) brivanib alanine. BDC monkeys (n = 2, weighing approximately 5–6 kg) were individually housed in metabolism cages and were freely mobile during the entire study with the exception of brief manual restraint for oral dosing. Each animal received a single oral dose of \(^{14}C\) brivanib alanine administered by gavage at a target dose level of 10 mg/kg (20 μCi/kg). Animals were fasted overnight before dosing. Approximately 4 h after dosing, animals were fed Certified Primate Diet 5048 (PMI Nutrition International, Inc.). During the period of sample collection, a bile salt replacement solution (18 mg/ml cholic acid and 1.3 mg/ml sodium bicarbonate in saline, pH 7.6) was administered to the animals via the distal flushing catheter with the volume of the replacement solution being approximately 23 ml/kg per day. Bile was collected into containers that were surrounded by dry ice before dosing and from 0 to 4, 4 to 8, 8 to 24, 24 to 48, and 48 to 72 h after dosing. Urine and feces were collected before dosing and over 24-h intervals through 72 h postdose. Blood samples were collected from each monkey via the vascular access port before dosing and at 1, 2, 4, 6, 12, 24, and 48 h after dosing.

**Human study.** A group of four patients with advanced or metastatic solid tumors were each administered a single dose of 800 mg of \(^{14}C\) brivanib alanine oral solution containing 100 μCi of total radioactivity. Blood samples were collected into tubes containing K3EDTA and glycine buffer (pH 3.5) from each patient before dosing and 1, 2, 4, 8, 12, 24, 96, and 168 h after dosing. Urine and feces were collected from each patient over intervals through 288 h postdose. Further details of this study were reported by Mekhail et al. (2010).

**Preparation of Biological Samples for Metabolite Profiling.** Plasma samples were segregated by collection time and pooled by combining equal volumes of plasma at a given time point. Pooled plasma (1–5 ml) was extracted with 3 volumes of methanol-acetonitrile (50:50, v/v). The mixtures were vortex-mixed, sonicated for 5 min, and centrifuged at 4000 g for 10 min. A portion of supernatant (50–80 μl) was injected into the LC-MS/MS system for biotransformation profiling and mass spectral analysis. Human urine samples were prepared by combining 10% by volume of each collection over 0 to 168 h (rat and monkey urine) or 0 to 288 h (human urine) interval. Pooled rat urine was centrifuged at 14,000 g for 15 min. A portion (50–80 μl) of supernatant was injected into the LC-MS/MS system without further treatment for biotransformation profiling and mass spectral analysis. Urine from monkeys and humans had a low amount of radioactivity. Pooled urine samples (20 ml) were evaporated to dryness under a stream of nitrogen for concentration. The residue was suspended in 2 ml of methanol-acetonitrile-water (1:1:1, v/v/v). The mixtures were vortex-mixed, sonicated for 5 min, and centrifuged at 4000 g for 15 min. The supernatant was collected, and the remaining pellet was extracted twice with 2 ml of methanol-acetonitrile-water (1:1:1, v/v/v). The supernatants were combined and evaporated to dryness under nitrogen. The residue was suspended in 0.5 ml of methanol-water (1:1, v/v) and centrifuged at 14,000 g for 10 min. A portion of supernatant (50–80 μl) was injected into the LC-MS system for biotransformation profiling and mass spectral analysis.

**Analysis of Brivanib in Plasma.** The plasma concentration of brivanib was quantified with an achiral LC-MS/MS method. Stable isotope-labeled brivanib (Fig. 1) was used as an internal standard. The internal standard stock solution was prepared in H2O-MeOH (80:20, v/v). An aliquot of plasma (0.1 ml) was mixed with internal standard (50 μl, 500 ng/ml). The mixture was loaded on a 96-well-solid-phase extraction plate (Oasis HLB μElation; Waters, Milford, MA) and was eluted with 100 μl of methanol. The extracts were mixed with 100 μl of water and 20 μl of the mixture was injected into the LC-MS/MS system for analysis. The concentrations of brivanib in human plasma were determined with an achiral LC-MS/MS method that was described by Mekhail et al. (2010).

**Analysis of Brivanib in Plasma.** The plasma concentration of brivanib was quantified with an achiral LC-MS/MS method. Stable isotope-labeled brivanib (1:1:1, v/v/v). The supernatants were combined and evaporated to dryness under a stream of nitrogen. The residue was suspended in 2 ml of methanol-acetonitrile-water (1:1:1, v/v/v). The combined supernatant was evaporated to dryness under a stream of nitrogen. The residue was suspended in 0.5 ml of methanol-water (1:1, v/v) and centrifuged at 14,000 g for 10 min. A portion of supernatant (50–80 μl) was injected into the LC-MS system for biotransformation profiling and mass spectral analysis.

**Radiochromatographic Analysis of Metabolites.** Biotransformation profiling was performed on a Shimadzu HPCL system equipped with two pumps (model LC-10AT), an autoinjector (SIL-10AD), and a diode array detector (SPC-M10A). Chromatographic separation was achieved with a Zorbax SB C18 HPLC column (2.1 × 150 mm, 5 μm; Agilent Technologies, Palo Alto, CA). A gradient of two solvent systems, A and B, was used for HPLC profiling. Solvent A consisted of 0.1% formic acid in water, and solvent B was acetonitrile. The gradient started at 10% B for 1 min, increased linearly to 90% over 14 min, was held at 90% B for 2 min, and returned to the initial condition over 1 min. The HPLC flow rate was 0.3 ml/min. The QTRAP mass spectrometer was equipped with a TurboSon Spray source and was operated in positive electrospray ionization (ESI) mode. Nitrogen was used as the nebulizer and auxiliary gas. The dwell times for multiple reaction monitoring (MRM) analysis were 50 to 70 ms, and the interscan pause time for all MRM analysis was 5 ms. The desolvation temperature was 300°C, and the source temperature was 150°C. Detection of brivanib and internal standard was achieved through MRM. The individual selected reaction monitoring transitions were m/z 371.2 → m/z 313.2 for brivanib and m/z 376.2 → m/z 318.2 for internal standard. The limit of quantitation in plasma from both animal species was 10 ng/ml.
Acetonitrile (25 ml) was added after 4 h to quench the reaction. The mixture was centrifuged at 4000 rpm for 20 min, and the resulting supernatant was collected and concentrated to ~2 ml under nitrogen. Metabolite M28 was isolated with a preparative Shimadzu HPLC system. Chromatographic separation was achieved with a preparative Synergi Polar-RP HPLC column. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The flow rate was 10 ml/min. The mobile phase composition started at 10% B and increased to 90% B over the course of 30 min, followed by a column wash with 90% B for 5 min. Fractions containing M28 were collected and dried under nitrogen.

**NMR Sample Preparation and Analysis.** Brivanib and metabolites M7, M25, M26, and M28 were dissolved in DMSO-d$_4$, and analyzed with a Bruker Avance 700 MHz NMR spectrometer equipped with a 5-mm TXI CryoProbe. $^1$H NMR spectra were recorded at 30°C using a single pulse or a 1-D nuclear overhauser enhancement spectroscopy presaturation sequence for suppression of the DMSO and water peaks. The chemical shifts are reported in parts per million and referenced to the DMSO peak that was calibrated to tetramethylsilane at 0 ppm. $^1$C chemical shifts were deduced from 2-D correlation spectroscopy, total correlation spectroscopy, heteronuclear single quantum coherence spectroscopy, and heteronuclear multiple-bond correlation spectroscopy (HMB) experiments.

**In Vitro Pharmacological Activities of M7, M25, and M26.** In vitro pharmacological activities toward the inhibition of VEGFR-2 and FGFR-1 kinases were determined using the synthetic standards of M7, M25, and M26. Recombinant proteins containing the cytoplasmic domains of the kinases were used in the assay. Stock solutions of the test compounds were prepared in DMSO, and the final concentrations of the test compounds were in the range of 0 to 45 μM. Assay conditions and data analysis are described in published articles (Borzillieri et al., 2005; Bhide et al., 2006).

**Results**

**Excretion of Radioactive Dose.** The mean percent recovery of radioactivity in the urine and feces from intact rats and monkeys, and in the urine, bile, and feces from BDC rats and BDC monkeys, after a single oral administration of [14C]brivanib alaninate, is summarized in Table 1. Recovery of radioactivity in humans after oral administration of [14C]brivanib alaninate has been reported previously (Meikhal et al., 2010) and also is included in Table 1 for comparison. The majority of the radioactive dose administered to the intact animals and humans was excreted in feces. The mean recovery of radioactivity in feces represented approximately 83.6, 76.3, and 81.5% of the dose in rats, monkeys, and humans, respectively. In animals with bile collection, the majority of the radioactivity was excreted in bile. The mean recovery of radioactivity in bile accounted for approximately 78.4% (0–48 h) and 47.7% (0–72 h) of the dose in BDC rats and BDC monkeys, respectively. A portion of radioactivity was recovered in feces, representing approximately 3.3 and 26.4% of the dose in BDC rats and monkeys, respectively. Approximately 10% of radioactivity was excreted in the urine from intact animals and humans. Total recovery of radioactivity in intact animals and humans ranged from approximately 89.8 to 93.7% across species.

**Plasma Concentration-Time Profiles.** The concentration-time profiles of brivanib and TRA in plasma from intact rats and monkeys are shown in Fig. 2, and the mean pharmacokinetic parameters of brivanib and TRA are summarized in Table 2. In both rats and monkeys, levels of brivanib in plasma were significantly lower than plasma TRA. The AUC$_{0-\infty}$ of brivanib in plasma represented 14.2 and 54.3% of that of TRA in rats and monkeys, respectively, with the remaining radioactivity presumably contributed by the metabolites. In
human plasma, radioactivity contributed by brivanib was approximately 23% of TRA (Mekhail et al., 2010).

**Metabolite Profiles in Plasma.** The recovery of radioactivity in pooled plasma from animals and humans after extraction was greater than 90%. Representative plasma radiochromatograms are shown in Fig. 3, and the distribution of metabolites is summarized in Table 3. Brivanib alaninate was not detected in plasma from any species presumably because of rapid presystemic hydrolysis (Marathe et al., 2009). In rat plasma, brivanib accounted for 22.0% of the plasma radioactivity at 1 h. In plasma from monkeys and humans, brivanib was the most abundant drug-related component, representing 70.7 and 72.4%, respectively, of the plasma radioactivity at 1 h. Metabolites identified in plasma analyzed at later time points (2 and 6 h for rat, 2, 4, 6, and 12 h for monkey, and 2, 4, 8, and 12 h for human) were similar to the 1-h samples. Plasma samples collected beyond these time points did not contain sufficient radioactivity to generate metabolite profiles.

Overall, 11 circulating metabolites were identified in humans and animals. In rat plasma, M6, a sulfate conjugate of M9, an O-dealkylated metabolite, and M32, a carboxylic acid derivative of M6, were the most prominent metabolites. Together, these metabolites represented approximately 50% of plasma radioactivity at 1 h. Other circulating metabolites observed in rats were M9, M2 (a glucuronide conjugate of M9), M25 (a sulfate conjugate of brivanib), M30 (a carboxylic acid metabolite of brivanib), and M19 (a sulfate conjugate of M7, a hydroxyl metabolite of brivanib). The prominent metabolites in monkey plasma were M26 (a carboxylic acid metabolite of brivanib), M28 (a glucuronide conjugate of brivanib), M6, and M25. Metabolites M19 and M32 were minor circulating metabolites in monkeys. Prominent circulating metabolites in humans were M6 and M26, which together accounted for 15.6% of plasma radioactivity at 1 h. Metabolites M25 and M32 were not observed in humans at 1 h but became prominent in plasma collected at later time points and accounted for 10.8 and 24.3% of the plasma radioactivity at 12 h, respectively.

To assess the systemic exposures of metabolites in animals and humans, the AUC values of a few circulating metabolites were estimated on the basis of their fraction of total radioactivity of the HPLC profiles at selected time points in plasma (Table 4). These metabolites included M6, M19, M25, M26, and M32, which were prominent metabolites in human plasma. As a comparison, AUC values of brivanib and TRA in plasma at the same time point were determined. For the majority of the metabolites, except M32, AUC values were less than 10% of the TRA.

**Metabolite Profiles in Urine.** In all species, urinary excretion was a minor pathway for elimination of drug-related materials. The distribution of drug-related components in urine is summarized in Table 5, and representative radiochromatograms are shown in Supplemental Fig. S1. Radioactive peaks corresponding to brivanib or brivanib alaninate were not observed in urine from any species. In rat urine, the most prominent metabolites were M6 and M32, which together represented 82.0% of the radioactivity in urine. The most abundant radioactive peak in monkey urine was M19, along with small amounts of M2, M32, and M26. Metabolite M19 represented 47.1% of radioactivity excreted in monkey urine. Prominent radioactive peaks in human urine were M19, M32, and M33 (a sulfate conjugate of M26), which together accounted for 76.3% of the radioactivity excreted in urine or 9.3% of the dose.

**Metabolite Profiles in Feces.** Drug-related materials in feces were extracted into methanol-water with the recovery of radioactivity ranging from 85 to 95%. HPLC radiochromatograms of the methanol extracts of the pooled fecal samples from rats, monkeys, and humans after oral administration of [14C]brivanib alaninate are shown in Fig. 4. The distribution of metabolites is shown in Table 5. Unchanged brivanib comprised 1.8, 6.5, and 7.5% of the radioactive dose in rat, monkey, and human, respectively. Metabolites M9 and M26 were among the most abundant drug-related components in rat feces, together representing 55.6% of the radioactive dose. Metabolites M19, M33, M6, and M25 were also observed in rat feces with amounts ranging from 1 to 6% of the

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**TABLE 1**

Percentage of radioactive dose recovery in rats, monkeys, and humans after oral administration of [14C]brivanib alaninate

<table>
<thead>
<tr>
<th>Species</th>
<th>No. Subjects</th>
<th>Dose</th>
<th>Collection Interval</th>
<th>%Radioactive Dose</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Rat</td>
<td>3</td>
<td>100 mg/kg</td>
<td>0–168</td>
<td>9.46 ± 1.81</td>
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<tr>
<td>BDC rat</td>
<td>3</td>
<td>100 mg/kg</td>
<td>0–48</td>
<td>7.33 ± 1.51</td>
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<tr>
<td>Intact rat</td>
<td>3</td>
<td>10 mg/kg</td>
<td>0–168</td>
<td>10.30 ± 0.39</td>
</tr>
<tr>
<td>BDC monkey</td>
<td>2</td>
<td>10 mg/kg</td>
<td>0–72</td>
<td>3.56 (2.95, 4.16)</td>
</tr>
<tr>
<td>Human</td>
<td>4</td>
<td>800 mg</td>
<td>0–288</td>
<td>12.18 ± 4.36</td>
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</tbody>
</table>

N.A., not applicable.

* Recovery of radioactivity in cage wash and cage wipe was included in the total recovery for animal studies.

* Only two animals were dosed in the study. S.D. (CV) was not available.

* Radioactive dose recovery in human was reported in a separate manuscript and is included here for comparison (Mekhail et al., 2010).
administered dose. The most prominent metabolites in monkey feces were M25 and M26, which together represented 57.2% of the administered dose. Other prominent metabolites in monkey feces included M19, M33, and M9. Prominent metabolites excreted in human feces were M19, M25, M26, and M33, representing 8.3, 26.2, 7.4, and 26.6% of the administered dose, respectively.

**Metabolite Profiles in Bile.** HPLC radiochromatograms of the pooled bile samples from rats and monkeys are shown in Fig. 4, and the distribution of the metabolites is shown in Table 5. Brivanib was not observed in bile from either species. A large portion of radioactivity excreted in rat bile was attributed to the O-dealkylated metabolite M9 and its secondary metabolites (M2 and M6), which together accounted for 81.4% of the radioactivity in rat bile or 63.6% of the radioactive dose. Metabolites resulting from both oxidative and conjugative metabolism were observed in monkey bile. The prominent metabolites in monkey bile were M25, M26, and M19, which together accounted for 36.0% of the radioactive dose.

### Table 2: Mean pharmacokinetic parameters of brivanib and plasma TRA in rats, monkeys, and humans after oral administration of [14C]brivanib alaninate

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Analyte</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; (µg · h/ml)</th>
<th>AUC % of Brivanib to TRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>100 mg/kg</td>
<td>Brivanib</td>
<td>6.4 ± 0.2</td>
<td>1</td>
<td>35.5 ± 3.6</td>
<td>14.2</td>
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<tr>
<td></td>
<td></td>
<td>TRA</td>
<td>35.2 ± 10.4</td>
<td>2</td>
<td>250 ± 9</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>10 mg/kg</td>
<td>Brivanib</td>
<td>6.2 ± 0.2</td>
<td>2</td>
<td>49.0 ± 4.5</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRA</td>
<td>9.7 ± 0.6</td>
<td>2</td>
<td>90.3 ± 13.7</td>
<td></td>
</tr>
<tr>
<td>Human&lt;sup&gt;a&lt;/sup&gt;</td>
<td>800 mg</td>
<td>Brivanib</td>
<td>6.1 ± 0.9</td>
<td>1</td>
<td>45.9 ± 4.1</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRA</td>
<td>9.4 ± 0.4</td>
<td>1</td>
<td>203.8 ± 40.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The concentration of brivanib was determined with an achiral LC-MS/MS method. TRA in rat and monkey plasma was determined by LSC. TRA in human plasma was determined by accelerator mass spectrometry (Mekhail et al., 2010).

<sup>b</sup> The pharmacokinetic parameters of brivanib and plasma TRA in humans were reported in a separate article and are included here for comparison (Mekhail et al., 2010).

Definitive structure characterization with NMR analysis was performed on a number of metabolites including M7, M25, M26, and M28. Metabolites M25 and M26 were isolated from monkey bile with a preparative HPLC system. Levels of M7 and M28 in vivo were not sufficient for NMR characterization. Therefore, large-scale incubations of brivanib in human liver microsomes in the presence of UDPGA or NADPH were performed to obtain sufficient quantities of these metabolites for NMR analysis. LC-MS/MS analysis demonstrated that the isolated metabolites had the same retention time, m/z, and MS/MS fragmentation patterns as those formed in vivo, indicating that they were structurally identical. Isolated metabolites were characterized with 1-D and 2-D NMR methods (HMBC, heteronuclear multiple quantum coherence spectroscopy, and total correlation spectroscopy). A 1H NMR spectrum of brivanib was also obtained and was used as a reference to determine sites of modification on the structures of the metabolites.

**Brivanib.** Under positive ESI, brivanib had a protonated molecule at m/z 371 with product ions at m/z 353 and m/z 313. The mass spectral fragmentation pattern for brivanib revealed cleavage of the hydroxyl group, resulting in the product ion at m/z 353, and cleavage of the side chain, resulting in the product ion of m/z 313 (Table 6). Further cleavage of m/z 313 generated a fragment ion at m/z 164.

**Metabolite M2.** M2 had a protonated molecule at m/z 489, 176 Da higher than that of M9. Major mass spectral fragments included a daughter ion at m/z 313, resulting from the loss of the glucuronide moiety. Further fragmentation of m/z 313 resulted in a mass spectrum identical to that of M9. M2 was thus tentatively identified as a glucuronide of M9.

**Metabolite M6.** M6 had a protonated molecule at m/z 393, 80 Da higher than that of M9. The major MS/MS fragmentation pattern, shown in Table 6, was similar to that of M2. Therefore, M6 was tentatively assigned as a sulfate conjugate of M9.

**Metabolite M9.** Mass spectral analysis of M9, under positive ESI, resulted in a protonated molecule at m/z 313, consistent with an O-dealkylated metabolite. The MS/MS fragmentation generated a daughter ion at m/z 149, resulting from the loss of an indole moiety.

**Identification of Metabolites.** LC-MS/MS analysis was the primary tool to generate the metabolite profiles and elucidate the structures of the metabolites. Structures were proposed on the basis of molecular ions, MS/MS fragmentation patterns, and comparison of HPLC retention times with those of the reference standards. A list of the metabolites observed in vivo, along with the MS spectrum data of each metabolite, is shown in Table 6. The proposed structures are illustrated in Fig. 5. The rationale for structural assignment is described below.

- **Brivanib.** Under positive ESI, brivanib had a protonated molecule at m/z 371 with product ions at m/z 353 and m/z 313. The mass spectral fragmentation pattern for brivanib revealed cleavage of the hydroxyl group, resulting in the product ion at m/z 353, and cleavage of the side chain, resulting in the product ion of m/z 313 (Table 6). Further cleavage of m/z 313 generated a fragment ion at m/z 164.

- **Metabolite M2.** M2 had a protonated molecule at m/z 489, 176 Da higher than that of M9. Major mass spectral fragments included a daughter ion at m/z 313, resulting from the loss of the glucuronide moiety. Further fragmentation of m/z 313 resulted in a mass spectrum identical to that of M9. M2 was thus tentatively identified as a glucuronide of M9.

- **Metabolite M6.** M6 had a protonated molecule at m/z 393, 80 Da higher than that of M9. The major MS/MS fragmentation pattern, shown in Table 6, was similar to that of M2. Therefore, M6 was tentatively assigned as a sulfate conjugate of M9.

- **Metabolite M9.** Mass spectral analysis of M9, under positive ESI, resulted in a protonated molecule at m/z 313, consistent with an O-dealkylated metabolite. The MS/MS fragmentation generated a daughter ion at m/z 149, resulting from the loss of an indole moiety.
and m/z 164 through the loss of a hydroxyl indole. Moreover, the retention time and mass spectral data were identical to those of the chemically synthesized reference standard.

Metabolite M7. M7 showed a molecular ion at m/z 387, 16 Da higher than that of brivanib, suggesting a monohydroxylated metabolite. MS/MS analysis of m/z 387, shown in Table 6, revealed a fragment of m/z 329, resulting from the loss of the side chain. This fragment was 16 Da higher than that from brivanib, indicating that structure modification occurred on the ring portion of the molecule. Definitive structure characterization of M7 using 1-D and 2-D NMR spectroscopy was conducted using the material isolated from a large-scale incubation of brivanib in HLM in the presence of NADPH. A comparison of the 1H and 13C chemical shifts of M7 and brivanib revealed no changes for any atoms except the carbons and protons on position 11 and 12 (Table 7). Protons H12 shifted downfield by 2.20 ppm compared to those of brivanib, suggesting a hydroxylated metabolite of M7. Major MS/MS fragments included a daughter ion at m/z 329, resulting from the loss of a sulfate group. Further fragmentation of m/z 329 (M5) resulted in a mass spectrum similar to that of M9. M13 was thus tentatively identified as a sulfate conjugate of M13, as illustrated in Fig. 5.

Metabolite M17. M17 had a protonated molecule at m/z 409, 80 Da higher than that of M13. Major MS/MS fragments included a daughter ion at m/z 329, resulting from the loss of a sulfate group. Further fragmentation of m/z 329 (M5) resulted in a mass spectrum similar to that of M7. M19 was thus tentatively identified as a sulfate conjugate of M7 in Fig. 5.

Metabolite M25. M25 had a protonated molecule at m/z 451, 80 Da higher than that of M13. Major MS/MS fragments included a daughter ion at m/z 371, resulting from the loss of sulfate. Further fragmentation of m/z 371 (M3) resulted in a mass spectrum similar to that of M7. M25 was thus tentatively identified as a sulfamate conjugate of M7.
from loss of the sulfate group. The sulfate could be conjugated either on the hydroxyl group on the side chain of brivanib or on the nitrogen atom on the indole group. Thus, the definitive structure of M25 could not be determined on the basis of MS data. A sufficient quantity of M25 was isolated from monkey bile and analyzed by NMR. The key NMR data for M25 are summarized in Table 7. Comparison of the $^1$H and $^{13}$C chemical shifts of M25 and brivanib revealed no change for any atoms except the carbons and protons on positions 1, 2, and 3 (Table 7). Protons $H_1$, $H_3a$, $H_3b$, and $H_2$ shifted downfield by 0.10, 0.12, 0.26, and 0.48 ppm, respectively. Likewise, carbon atoms on the side chain had more significant shifts than those on the aromatic rings. $C_1$ and $C_3$ shifted upfield by 1.9 and 2.6 ppm, respectively, whereas $C_2$ shifted downfield by 5.4 ppm. These data suggested that conjugation had occurred at the secondary hydroxyl group of the brivanib alkyl side chain.

**Metabolite M26.** M26 had a protonated molecule at $m/z$ 401, 30 Da higher than that of brivanib, suggesting a carboxylic acid metabolite. MS/MS analysis of $m/z$ 401 yielded a major fragment ion at $m/z$ 383, resulting from the loss of water, and $m/z$ 343, resulting from the loss of the side chain. To determine the structure of M26, it was isolated from monkey bile for NMR analysis, and the results are summarized in Table 7. NMR analysis revealed the loss of proton signal, as well as heteronuclear multiple quantum coherence spectroscopy signal, of the methyl group on the indol moiety. Chemical shifts of all protons

<table>
<thead>
<tr>
<th>Metabolite ID</th>
<th>%Dose</th>
<th>Urine&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Feces&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bile&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>M2</td>
<td>MS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M6</td>
<td>2.7</td>
<td>0.7</td>
<td>N.D.</td>
<td>4.6</td>
</tr>
<tr>
<td>M9</td>
<td>MS</td>
<td>N.D.</td>
<td>N.D.</td>
<td>42.8</td>
</tr>
<tr>
<td>M13</td>
<td>0.4</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.5</td>
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<tr>
<td>M17</td>
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<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M19</td>
<td>MS</td>
<td>4.9</td>
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<td>4.8</td>
</tr>
<tr>
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<td>0.8</td>
<td>MS</td>
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<tr>
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<td>0.4</td>
<td>12.8</td>
</tr>
<tr>
<td>M28</td>
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<td>0.3</td>
<td>1.1</td>
<td>N.D.</td>
</tr>
<tr>
<td>M30</td>
<td>MS</td>
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<td>N.D.</td>
<td>1.8</td>
</tr>
<tr>
<td>M31</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M32</td>
<td>5.1</td>
<td>3.3</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M33</td>
<td>N.D.</td>
<td>MS</td>
<td>2.4</td>
<td>5.7</td>
</tr>
<tr>
<td>M34</td>
<td>MS</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Brivanib</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.8</td>
</tr>
</tbody>
</table>

N.D., not detected.

<sup>a</sup> Samples were profiled with an achiral HPLC method. Brivanib is reported as a total of brivanib and its enantiomer.

<sup>b</sup> MS indicates that metabolite was only observed by mass spectrometry.

![FIG. 4. Metabolite profiles of (A) pooled bile from BDC rats, (B) pooled feces from intact rats, (C) pooled bile from BDC monkeys, (D) pooled feces from intact monkeys, and (E) pooled feces from humans, after oral administration of $[^{14}$C]brivanib alaninate. Samples were profiled with an achiral HPLC method. The profiles are background-subtracted reconstructed radiochromatograms of 15-s fractions collected from a HPLC run. Arrows show the potential conversion of conjugative metabolites to their corresponding unconjugated precursor in their passage through the GI tract. The M prefix is omitted from the metabolite labels.](image-url)
was further confirmed by a 2-D NMR analysis in which a long range proposed structure illustrated in Table 7. The structural assignment shifted downfield by 7.7 ppm. These changes are consistent with a side chain were shifted downfield (0.08–0.43 ppm), and C 2 was 13C chemical shift of the atoms on the aromatic rings. Protons on the except H8,H 9, and H10 were within 0.01 ppm deviation compared J the coupling constant (\(J_{HF} = 7.8\) Hz) of the anomeric proton is characteristic of a\(\beta\)-glucuronide (Casati et al., 2009).

**Metabolite M30.** M30 showed a protonated molecule at \(m/z\) 371. The fragmentation pattern of \(m/z\) 371 was similar to that of brivanib, indicating that the structural modification occurred on the side chain rather than on the aromatic rings. This metabolite eluted approximately 5 min earlier than brivanib, suggesting that the metabolite was more polar than brivanib. A proposed structure of M30 is shown in Fig. 5.

**Metabolite M31.** Mass spectral analysis of M31, under positive ESI, resulted in an ion peak at \(m/z\) 369, consistent with a ketone metabolite. The MS/MS fragments included a daughter ion of \(m/z\) 313, resulting from the loss of side chain. The retention time and mass spectral data were identical to those of a chemically synthesized reference standard.

**Metabolite M32.** M32 had a protonated molecule at \(m/z\) 423, 80 Da higher than that of M34. Major MS/MS fragments included a daughter ion of \(m/z\) 343, resulting from the loss of sulfate, which indicated that M32 could be a sulfate conjugate of M34.

**Metabolite M33.** M33 had a protonated molecule at \(m/z\) 481, 80 Da higher than that of M26. Major MS/MS fragments included a daughter ion of \(m/z\) 401, resulting from the loss of sulfate, which indicated that M33 could be a sulfate conjugate of M26.

**Metabolite M34.** Mass spectral analysis of M34, under positive ESI, resulted in an ion peak at \(m/z\) 343, 30 Da higher than that of M9. The MS/MS fragments included a major daughter ion at \(m/z\) 325, resulting from the loss of a water molecule. The data supported the proposal that M34 was a carboxyl acid metabolite of M9.

**In vitro pharmacological activity.** Metabolites M7, M25, and M26 were tested for their activity toward the inhibition of VEGFR-2 and FGFR-1 human receptor tyrosine kinases. As a comparison, the phar-
macological activity of brivanib was also evaluated under the same conditions. Results, summarized in Table 8, indicated that M25 displayed potency similar to that of brivanib against VEGFR-2 and FGFR-1 with an IC$_{50}$ of 28 and 26 nM, respectively. M7 was found to moderately inhibit VEGFR-2 and FGFR-1 with an IC$_{50}$ of 78 and 760 nM, respectively. M26 did not inhibit the kinase activity of either enzyme to any significant extent.

**Discussion**

This study describes the biotransformation and disposition of brivanib in rats, monkeys, and humans after a single oral administration of [14C]brivanib alaninate. Rats and monkeys were the species used in long-term toxicity studies of brivanib. Brivanib was well tolerated in patients with advanced cancer at a dose level of 800 mg, with mild adverse events, including fatigue, nausea, diarrhea, and constipation (Mekhail et al., 2010). No drug-related adverse events were observed in rats and monkeys in the current study after a single oral dose of [14C]brivanib alaninate.

Brivanib alaninate is an amino acid ester prodrug of brivanib. Use of the prodrug to improve solubility and bioavailability in preclinical species is common during drug discovery (Beaumont et al., 2003). Although this approach offers great advantages in improving drug absorption, it poses challenges in the assessment of the systemic exposure of the prodrug because it can be hydrolyzed ex vivo during
sample collection, storage, and processing. It has been demonstrated that brivanib alaninate is cleaved to brivanib in rodent and nonrodent plasma, a reaction catalyzed by various esterases (Marathe et al., 2009). To ensure the accurate representation of the systemic exposure of drug-related components in humans, blood samples were collected into acidic glycine buffer, a condition that stabilizes brivanib alaninate in human blood through the inhibition of esterase activity (Fung et al., 2010). Further analysis revealed that the prodrug was indeed absent in plasma samples, in which, in addition to unchanged brivanib, numerous metabolites were also present. Prolonged declines of plasma concentrations of TRA and brivanib were parallel in rats and monkeys within 24 h after dosing, indicating that the total concentration of metabolites did not seem to accumulate in plasma. From the previously published data, the decline of TRA seems to be slower than that of brivanib in humans over the longer period of time after dosing, suggesting that certain metabolites have slower clearance than the parent drug (Mekhail et al., 2010). On the basis of the radiochromatographic profile of human plasma at the latest time point (12 h; Table 3), we suspect that the slowly cleared metabolite could be M32. Previous analysis of the structure-activity relationship has shown that M32 is not expected to be pharmacologically active (Bhide et al., 2006). Multiple oxidative and conjugative metabolites were observed in plasma from animals and humans. All metabolites observed in human plasma were detected in plasma from at least one of the toxicological species. In all species, AUC estimates were determined using the latest time point; however, in certain species, the metabolites were not observed, and thus AUC was assumed to be the same as in plasma from animals.

### TABLE 8

IC\textsubscript{50} for inhibition of VEGFR-2 and FGFR-1 receptors by M7, M25, M26, and brivanib

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC\textsubscript{50} a(M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brivanib</td>
<td>26</td>
</tr>
<tr>
<td>M7</td>
<td>78</td>
</tr>
<tr>
<td>M25</td>
<td>28</td>
</tr>
<tr>
<td>M26</td>
<td>&gt;30,000</td>
</tr>
</tbody>
</table>

a IC\textsubscript{50} Values were determined according to the procedures described previously (Borzilleri et al., 2005).

Metabolite profiles of plasma provided a direct comparison of the systemic exposures of drug-related components in animals and humans at well tolerated dose levels. Plasma pharmacokinetic profiles of brivanib and TRA suggested that metabolites contributed significantly to plasma TRA. This finding is consistent with the metabolite profiles of plasma samples, in which, in addition to unchanged brivanib, numerous metabolites were also present. Prolonged declines of plasma concentrations of TRA and brivanib were parallel in rats and monkeys within 24 h after dosing, indicating that the total concentration of metabolites did not seem to accumulate in plasma. From the previously published data, the decline of TRA seems to be slower than that of brivanib in humans over the longer period of time after dosing, suggesting that certain metabolites have slower clearance than the parent drug (Mekhail et al., 2010). On the basis of the radiochromatographic profile of human plasma at the latest time point (12 h; Table 3), we suspect that the slowly cleared metabolite could be M32. Previous analysis of the structure-activity relationship has shown that M32 is not expected to be pharmacologically active (Bhide et al., 2006). Multiple oxidative and conjugative metabolites were observed in plasma from animals and humans. All metabolites observed in human plasma were detected in plasma from at least one of the toxicological species. In all species, AUC estimates were determined using the latest time point; however, in certain species, the metabolites were not observed, and thus AUC was assumed to be the same as in plasma from animals.
plasma concentrations and because none of the metabolites have significantly higher pharmacological activity than brivanib. The pharmacological activities of a few selected metabolites, namely, M7, M25, and M26, were determined in the current study. Information regarding activity against VEGFR-2 and FGFR-1 of these metabolites, together with the structure-activity relationship data previously reported (Bhide et al., 2006), suggested that modification of the alkyl side chain should have little influence on the inhibition potency against VEGFR-2 and FGFR-1 kinases. M25, the sulfate conjugate of brivanib, consistently showed potency similar to that of brivanib, and M9, a metabolite without alkyl side chain, exhibited potent kinase inhibitory activity (Bhide et al., 2006). On the other hand, replacement of the methyl group on the indole with a hydroxymethyl or carboxyl group should significantly diminish inhibition potency. Thus, M7 and M26, with a hydroxymethyl and carboxyl group on the indole, respectively, were less potent than brivanib. Given that M25 (equi-potent to brivanib) was less than 6% of the AUC of TRA (Table 4) and the other circulating components were significantly less potent or inactive, it is unlikely that metabolites contribute significantly to the target-related pharmacology.

Recovery of radioactivity was nearly complete after 168 h postdose for animals and after 288 h postdose for humans. Excretory profiles of radioactivity in the urine, bile, and feces suggested that biliary excretion followed by fecal elimination was the major route of elimination of drug-derived radioactivity. Metabolite profiles in urine, feces, and bile were qualitatively similar across species. Unchanged brivanib was not detected in urine or bile from rats and monkeys, indicating that the parent drug underwent extensive metabolism before excretion. A small portion of unchanged brivanib was observed in feces from animals and humans, which could be due to incomplete absorption and due to the hydrolysis of the conjugative metabolites in the GI tract. The percentage of brivanib absorbed in humans, estimated by excluding the recovery of unchanged brivanib in feces from total recovery of radioactivity, was at least 86.2%, suggesting that the compound is well absorbed in humans.

The role of conjugative metabolism in drug disposition and elimination is well understood. The percentage of conjugative metabolites in excreta is often underestimated in intact animal studies because sulfate and glucuronide conjugates tend to be hydrolyzed in the GI tract to their parent structure (Hazenberg et al., 1988; Roberts et al., 2002). To understand the role of conjugative metabolism, biotransformation studies of brivanib were conducted in BDC rats and monkeys. LC-MS/MS and radiochromatographic analysis indicated that the sulfate-conjugated metabolites (M19 and M25) were the most prominent metabolites in monkey bile. Despite the potential for hydrolysis, these two metabolites, together with another sulfate-conjugated metabolite, M33, were among the most prominent metabolites in human feces. In addition, a significant portion of radioactivity in rat bile was attributed to the sulfate and glucuronide conjugates (M6 and M2) of M9. These data suggest that conjugative metabolism plays a significant role in the disposition and elimination of brivanib.

A comparison of the metabolite profiles of feces and bile from rats demonstrated that the conjugative metabolites of brivanib were hydrolyzed in the GI tract. Thus, the two most abundant metabolites (M2 and M6) in bile became minor metabolites in feces presumably due to hydrolysis, whereas their precursor (M9) became the predominant radioactive component in rat feces. Likewise, a comparison of radiochromatograms of bile and feces from monkeys revealed that a significant portion of M19 was hydrolyzed and further oxidized to M26 in the monkey GI.

The proposed pathway for biotransformation of brivanib is shown in Fig. 5. Primary pathways responsible for the biotransformation of brivanib in animals and humans include O-dealkylation to form M9, oxidation to the hydroxylated metabolite M7, and sulfation to form M25. M7 was a minor metabolite in rat and monkey and was not detected in human. However, when brivanib was incubated with human liver microsomes in the presence of NADPH, M7 was the most abundant metabolite. In vitro studies indicated that M7 was further metabolized to M26, M19, and M33 (data not shown). Although quantitatively different, metabolites of brivanib across species were qualitatively similar. Brivanib underwent metabolic chiral inversion to its enantiomer with the plasma concentration of the enantiomer less than 3% of brivanib in human. Detailed in vitro and in vivo metabolic chiral inversion of brivanib will be described in a separate manuscript. The enzymes responsible for the oxidation and sulfation of brivanib in humans are presumably cytochrome P450 enzymes and sulfotransferases, respectively (Marathe et al., 2009). The involvement of multiple enzymes in the metabolism of brivanib should potentially minimize drug-drug interactions.

In summary, the biotransformation and disposition of brivanib in animals and humans are described here. Drug-derived radioactivity was mainly eliminated through biliary excretion followed by fecal elimination. The prodrug was rapidly and completely hydrolyzed to brivanib, the active moiety, and the latter underwent extensive metabolism before excretion. The primary pathways for biotransformation of brivanib were O-dealkylation, methyl oxidation, and sulfation. Systemic exposure of brivanib and metabolites was qualitatively similar across species. All human circulating metabolites were present in plasma from rats or monkeys and were at sufficient levels to ensure that the toxicity studies in these species gave a full representation of potential toxicity issues with brivanib as well as brivanib metabolites. In addition, all metabolites seen in human plasma were either at very low levels relative to that of brivanib or were expected to be pharmacologically inactive with respect to target related pharmacology and thus not contribute to the efficacy seen with the compound.

Acknowledgments

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Authorship Contributions

Participated in research design: Gong, Gan, Masson, Williams, and Iyer. Conducted experiments: Gong. Contributed new reagents or analytic tools: Allentood, Lago, and Tran. Performed data analysis: Gong, Caceres-Cortes, Christopher, Arora, Pursley, and Iyer.

Wrote or contributed to the writing of the manuscript: Gong, Christopher, Masson, and Iyer.

References


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