Title: Metabolism and Disposition of Cabozantinib in Healthy Male Volunteers and Pharmacologic Characterization of Its Major Metabolites

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Running Title: Biotransformation of Cabozantinib in Healthy Volunteers

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Abbreviations: ABC, ATP-binding cassette; ACN, acetonitrile; AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; BDC, bile duct-cannulated; BDI, bile duct-intact; BMI, body mass index; BSEP, bile salt export pump; CHO, Chinese hamster ovary; CID, collision-induced dissociation; CR, column recovery; CYP, cytochrome P450; DDI, drug-drug interaction; ER, extraction recovery; ESI, electrospray ionization; FA, formic acid; FBE, freebase equivalent; GLP, Good Laboratory Practice; HBSS, Hank’s Balanced
Salt Solution; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LSC, liquid scintillation counter; MATE, multidrug and toxin extrusions organic cation antiporter; MET, hepatocyte growth factor receptor; MetOH, methanol; MRP2, multidrug resistance protein 2; MTC, medullary thyroid cancer; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OR, overall recovery; PEG400, polyethylene glycol 400; pFA, parafluoroaniline; P-gp, P-glycoprotein; PopPK, population pharmacokinetics; RET, rearranged during transfection receptor; RFD, radio flow-through detector; RR, reconstitution recovery; RTK, receptor tyrosine kinase; SD, Sprague Dawley; SLC, solute carrier; t1/2, half-life; T_max, time to peak plasma concentration; VEGFR, vascular endothelial growth factor receptor.
Abstract

Metabolism and excretion of cabozantinib, an oral inhibitor of receptor tyrosine kinases, was studied in 8 healthy male volunteers after a single oral dose of 175-mg cabozantinib L-malate containing $^{14}$C-cabozantinib (100 µCi/subject). Total mean radioactivity recovery within 48 days was 81.09%; radioactivity was eliminated in feces (53.79%) and urine (27.29%). Cabozantinib was extensively metabolized with seventeen individual metabolites identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in plasma, urine, and feces. Relative plasma radioactivity exposures (analyte AUC$_{0-t}$/total AUC$_{0-t}$ for cabozantinib+major metabolites) were 27.2%, 25.2%, 32.3%, 7%, and 6% for cabozantinib and major metabolites monohydroxy sulfate (EXEL-1646), 6-desmethyl amide cleavage product sulfate (EXEL-1644), N-oxide (EXEL-5162), and amide cleavage product (EXEL-5366), respectively. Comparable relative plasma exposures determined by LC-MS/MS analysis were 32.4%, 13.8%, 45.9%, 4.9%, and 3.1%, respectively. These major metabolites each possess in vitro inhibition potencies ≤1/10$^\text{th}$ of parent cabozantinib against the targeted kinases MET, RET, and VEGFR2/KDR. In an in vitro cytochrome P450 (CYP) panel, cabozantinib and EXEL-1644 both inhibited most potently CYP2C8 ($K_{i_{app}}$ = 4.6 and 1.1 µM, respectively). In an in vitro drug transporter panel, cabozantinib inhibited most potently MATE1 and MATE2-K ($IC_{50}$ = 5.94 and 3.12 µM, respectively) and was a MRP2 substrate; EXEL-1644 inhibited most potently OAT1, OAT3, OATP1B1, MATE1, and OATP1B3 ($IC_{50}$ = 4.3, 4.3, 6.1, 16.7, and 20.6 µM, respectively) and was a substrate of MRP2, OAT3, OATP1B1, OATP1B3, and possibly P-gp. Therefore, cabozantinib appears to be the primary pharmacologically active circulating analyte, while both cabozantinib and EXEL-1644 may represent potential for drug-drug interactions.
Introduction

Cabozantinib is a small molecule inhibitor of multiple receptor tyrosine kinases (RTKs), including MET (hepatocyte growth factor receptor), VEGFR (vascular endothelial growth factor receptor), and RET (rearranged during transfection), which are implicated in tumor growth, angiogenesis, and metastatic progression of cancer (Yakes et al, 2011; Bentzien et al, 2013). Cabozantinib has been approved in the United States and European Union for the treatment of subjects with progressive metastatic medullary thyroid cancer (MTC). Cabozantinib is currently under clinical investigation in other cancers including renal cell and hepatocellular.

The plasma pharmacokinetics of cabozantinib has been characterized in subjects with solid tumors (Kurzrock et al, 2011). After repeat daily dosing, cabozantinib showed a long terminal half-life (91.3 ±33.3 hours), accumulated four- to five-fold compared to Day 1, and reached apparent steady-state plasma levels by Day 15. A population pharmacokinetic (PopPK) analysis of cabozantinib was performed using data collected from 289 patients with solid tumors (including MTC) following oral administration of a daily 175-mg salt weight (140 mg freebase equivalent [FBE] weight) dose (CDER, 2012a). The PopPK analysis indicated that cabozantinib has a predicted effective half-life ($t_{1/2}$) of approximately 55 hours, an oral volume of distribution of approximately 349 L, and an oral clearance at steady-state estimated to be 4.4 L/hour.

Cabozantinib is highly protein bound in human plasma ($\geq 99.7\%$).

The present study was conducted to characterize the metabolism, elimination, and pharmacokinetics of cabozantinib and its biotransformation products in healthy male volunteers who received a single oral dose of 175 mg cabozantinib L-malate (140-mg FBE) containing $^{14}$C-cabozantinib (100 µCi/subject). Individual metabolites present at higher relative plasma
exposures (eg, ≥25% of the parent drug) were also subject to additional toxicological and/or pharmacological evaluations, including potential for drug-drug interactions (DDIs), consistent with current regulatory authority guidances (EMA, 2012; FDA, 2008, 2012).
Materials and Methods

Chemicals and Reference Compounds. Cabozantinib was synthesized by Piramal Healthcare (Aurora, Ontario, Canada). $^{14}$C-cabozantinib freebase (specific activity 119.6 µCi/mg; radiochemical purity >99%) was synthesized by Aptuit, Inc (Kansas City, MO). The metabolite synthetic standards 2-[1-((4-[(6,7 dimethoxyquinolin-4yl)oxy]phenyl) carbamoyl)cyclopropane carboxamido-5-fluorophenyl hydrogen sulfate] (EXEL-1646), 4-(4-1-[4 fluorophenyl carbamoyl] cyclopropanecarboxamido)-6,7-dimethoxyquinoline 1-oxide) (EXEL-5162), 1-[[4-[(6,7 dimethoxyquinolin-4-yl)oxy]phenyl]carbamoylcyclopropanecarboxylic acid] (EXEL-5366), and 1-[[4-[(7-methoxy-6-(sulfooxy)quinolin-4-yl)phenyl]carbamoylcyclopropanecarboxylic acid] (EXEL-1644) were synthesized by Exelixis, Inc (South San Francisco, CA). The chemical structure of cabozantinib freebase (XL184) with location of the radiolabel and the structures of the 4 metabolite synthetic standards are illustrated in Fig. 1. The $^{13}$C₆-cabozantinib internal standard was synthesized by Bristol-Myers Squib (New Brunswick, NJ). All commercially available reagents were either analytical or high-performance liquid chromatography (HPLC) grade.

Clinical Study Design and Subjects. The clinical phase of the study was performed at Celerion (Lincoln, NE). Study protocols, amendments, and informed consent documents were approved by an independent institutional review board. The study adhered to the principles outlined in “Guideline for Good Clinical Practice” International Conference on Harmonization E6 Tripartite Guideline (January 1996), and was conducted in compliance with the Declaration of Helsinki. All subjects provided informed consent before any study-related procedure. Study authorization was granted by the Nebraska Department of Health and Human Services, Division of Public
Health, Office of Radiological Health, who reviewed the study protocol, the institutional review board approval letter, and the dosimetry report prior to study initiation.

Eight healthy male adult volunteers (age range: 19 to 55 years) with body mass index (BMI) ≥18 and ≤33.0 kg/m² were enrolled in this open-labeled, single dose study. Subjects had acceptable medical histories, electrocardiograms, vital signs, and physical examinations; no history of alcohol abuse and negative urine drug/alcohol testing at screening and check-in; had not used any tobacco- or nicotine-containing products within 3 months prior to screening; had clinical laboratory profiles within reference range for the test laboratory; and had not used any over-the-counter, nonprescription preparations (including H2 blockers, proton pump inhibitors, or herbal supplements) within 7 days prior to check-in, or any prescription medications or products (including any drugs known to induce or inhibit CYP isozymes) within 28 days prior to check-in through discharge from the study. During the study, 2 g per day or less of acetaminophen, 3.2 g per day or less of ibuprofen, 8 mg per day or less of loperamide, 60 mL per day or less of magnesium hydroxide, and 80 mL per day or less of liquid antacid could be administered orally at the discretion of the investigator. When permitted on dosing days, the allowed medications were not to be taken until at least 4 hours after dosing.

Subjects fasted overnight before dosing through 4 hours postdose. Subjects received a single calculated oral dose intended to contain a total of 175 mg of cabozantinib L-malate (140 mg FBE) containing 100 µCi of ¹⁴C. Total radioactivity was determined using a calibrated liquid scintillation counter (LSC) for the dosing solution prior to administration and was counted in triplicate. These values could vary by up to 10% to account for normal, acceptable measurement variability. A single dose oral solution was administered from the scintillation vial at Hour 0 on Day 1 by the clinic staff. Following dosing, the scintillation vial was rinsed 3 times with room
temperature distilled water and the rinsate was administered to the subject. Residual radioactivity was determined by LSC for each dosing vial. The total volume of liquid administered including radiolabelled study drug, rinses, and water given for dosing (in addition to the rinses) was the same for each subject.

Sample Collection and Aliquotting. Blood samples for plasma analysis of total radioactivity (10 mL each) and for liquid chromatography-tandem mass spectrometry (LC-MS/MS) bioanalysis of cabozantinib free base and/or metabolite concentrations (3 mL each) were collected in appropriately labeled, K$_2$-EDTA vacutainer tubes predose and at 0.5, 1, 2, 3, 4, 5, 8, 14, 24, 72, 168, 336, 504, and 648 hours postdose; additional samples for analysis of total radioactivity only were collected at 48, 120, 144, 240, and 408 hours postdose. The tubes were chilled on wet ice or in a refrigerator prior to collection. Immediately after blood collection, blood tubes were inverted several times and then kept on wet ice until centrifuged. Within 30 minutes of blood collection, samples were separated by centrifugation for 10 minutes (1000-1200 g) at ~4°C. The resultant plasma was aliquoted into approximately equal volumes into two appropriately labeled polypropylene tubes (one primary and one back-up sample) and stored (within 1 hour of the blood sampling time) at -70°C or lower until shipment. Blood samples (4 mL) for whole blood analysis of total radioactivity to determine the percentage of radioactivity associated with erythrocytes were collected predose and at 1, 2, 3, 8, 14, 24, and 72 hours postdose. The whole blood samples were aliquoted into approximately equal volumes into two appropriately labeled polypropylene tubes (one primary and one back-up sample) and stored (within 1 hour of the blood sampling time) at -20°C or lower until shipment. Urine samples were collected at predose, at 0-8 and 8-24 hours postdose, and thereafter at 24-hour intervals. Feces were collected predose and at 24-hour intervals. The fecal output from each time interval was
homogenized and the total weight recorded. Urine and fecal collections stopped following the conclusion of the Day 49 scheduled events regardless of percentage of total radioactive dose recovered; one subject withdrew after 37 days, and all other subjects remained in the study through Day 49. Two aliquots each of urine (~30 mL each) and fecal (~30 g each) samples collected from each time interval were transferred to two appropriately labeled polypropylene tubes (one primary and one back-up). The resultant urine and fecal samples were stored at -70°C or lower until shipment. The primary sample of all plasma, urine, and fecal homogenate samples designated for LC-MS/MS bioanalysis were shipped frozen on dry ice to QPS, LLC (Newark, DE).

**Determinations of Radioactivity.** Total radioactivity in whole blood, plasma, urine, and feces was determined using a LSC (2900 TR; Packard Instruments, Meridian, CT) at the Celerion Scintillation Laboratory (Lincoln, NE). All analyses were conducted using calibrated LSCs and oxidizers. Urine and plasma were analyzed by direct counting of sample aliquots in vials containing liquid scintillation cocktail (Ultima Gold XR). The aliquot volume for plasma was 0.5 mL. The initial aliquot volume for urine was 1 mL. However, beginning at the 504-hour interval, the sample size was increased to 5 mL to increase measurement sensitivity for $^{14}$C. In addition, beginning at the 504-hour interval, 24-hour urine samples were again pooled over a 72-hour interval to increase measurement sensitivity. Fecal samples were homogenized and oxidized prior to counting. Fecal samples were initially pooled over a 24-hour interval. Similar to urine, beginning at the 528-hour interval, 24-hour pooled fecal samples were again pooled over a 72-hour interval to increase measurement sensitivity. The homogenates were prepared with an approximate 1:4 dilution in water. For small samples, sufficient water was added to ensure a homogenate volume of approximately 200 mL. The initial aliquot mass for fecal homogenates
was 0.5 g. However, beginning at the 504-hour interval, the sample size was increased to 1 g to increase measurement sensitivity for $^{14}$C. Homogenate aliquots were dried, oxidized, and measured for $^{14}$C content using LSC. The aliquot volume for whole blood was 0.5 g. Whole blood aliquots were dried, oxidized, and measured for $^{14}$C content using LSC.

**Metabolite Profiling and Identification.** Two plasma samples from Subject 1 (4 and 72 hours postdose) were used for initial extraction and recovery determination. The total radioactivity for each plasma sample was defined as 100%. After thawing under a biological hood, two 0.5 mL aliquots of each plasma sample were added to 3 volumes (1.5 mL) of methanol (MeOH): acetonitrile (ACN) (20:80, v/v) and vortexed (5 min). The mixtures were centrifuged at 2000 rpm for 10 minutes, and the supernatants were transferred to clean tubes. The pellets were extracted with two additional 3 volumes of MeOH:ACN (20:80, v/v). These mixtures were then centrifuged, and the supernatants were combined. Aliquots were analyzed by a 2900 TR LSC. The plasma extraction recovery ($ER_p$) was calculated as follows: $ER_p (%) = (\text{DPM in supernatant}/\text{DPM in plasma sample}) \times 100$. The supernatants from the extraction were evaporated to dryness under a stream of nitrogen in an ambient water bath. The residues were then reconstituted in 0.35 – 0.5 mL of MeOH:ACN:water (10:20:70, v/v/v). The reconstituted samples were centrifuged at 15,000 rpm for 10 minutes, and aliquots were analyzed by LSC for reconstitution recovery ($RR_p$) and calculated as follows: $RR_p (%) = (\text{DPM in reconstitution solution}/\text{DPM in supernatant}) \times 100$. The mean extraction recoveries of radioactivity from 4 and 72 hour plasma samples were 98.43% and 94.99%, respectively. After drying down and reconstitution into MeOH:ACN solution, the reconstitution recoveries were 92.73% and 85.90%, respectively. The overall recoveries ($OR_p = ER_p \times RR_p / 100\%)$ were 91.27% and 81.60%, respectively.
Pooled urine samples from Subject 2 (0-72, 168-192, and 312-336 hours postdose) were lyophilized in triplicate (each 4 mL), and the residues were reconstituted in 1 mL of water:ACN:formic acid (FA) (80:20:0.1, v/v/v). The radioactivity in pooled urine and reconstituted solution was counted using LSC, and the reconstitution recovery calculated (94.7%). Urine centrifugation recoveries determined using 0-8, 24-48, 72-96, and 120-144 hours postdose samples from Subject 1 ranged between 102% and 104%.

To evaluate the extraction recovery of fecal samples, two fecal homogenate samples from Subject 1 (24-48 hours and 144-168 hours postdose) were thawed under a biological hood. Approximately 5.5-6 g fecal homogenate was accurately weighed out for the extraction. Fifteen mL ACN:MeOH (80:20) was added to the fecal homogenates. The mixtures were vortexed for 3 minutes and centrifuged at 3000 rpm for 10 minutes. The supernatants were transferred to clean tubes. The extraction procedure was repeated two more times. The supernatants from all three extractions were combined. The radioactivity in the combined supernatants was determined by LSC. The extraction recovery for fecal samples (ERf) was calculated using the following formula: \( ER_f \ (%) = (DPM \ in \ supernatant/DPM \ in \ fecal \ homogenate) \times 100. \) The supernatant was concentrated under a nitrogen stream at ambient temperature, and the residues reconstituted in MeOH:ACN:water (10:20:70). Aliquots of reconstitution solution were counted using LSC for reconstitution recovery (RRf), calculated as follows: \( RR_f \ (%) = (DPM \ in \ reconstitution \ solution/DPM \ in \ supernatant) \times 100. \) Overall recovery (ORf %) was calculated as follows: \( = ER_f \ (%) \times RR_f \ (%) / 100. \) For pooled fecal samples from 0-48 hours postdose, the \( ER_f, RR_c, \) and \( OR_f \) values were 98.48%, 88.80%, and 87.37%, respectively. For pooled fecal samples of 120-168 hours postdose, \( ER_f, RR_c, \) and \( OR_f \) values were 85.85%, 87.69%, and 75.24%, respectively.
HPLC column recovery (CR) was carried out to demonstrate that all radioactive components were effectively eluted from the column using HPLC Method 1. Aliquots of urine samples (Subject 1, 24-48 hours postdose) were injected onto the HPLC system with or without a column, and the eluents from 0 to 30 minutes were collected into clean 50-mL centrifuge tubes. The weights of eluent from each injection were obtained after collection, and duplicate aliquots (1 mL) were counted using LSC. The average value of the counts was used to calculate the total radioactivity contained in the collected eluent with or without a column installed. The CR value for the urine samples was calculated as follows: \( CR_u(\%) = \left( \frac{\text{DPM in eluent with column}}{\text{DPM in eluent without column}} \right) \times 100 \). The radioactivity recovery from HPLC column for urine was 97.05%. HPLC Method 2 was used for pooled plasma only, and the column recovery was not performed due to limited sample volume available.

Metabolite profiling, identification, and radio-quantitation was performed using individual plasma samples (0.5, 1, 2, 3, 4, 5, 8, 14, 24, 72, 168, and 336 hours postdose), and 3 pooled urine samples (0-72 hours, 168-192 hours, and 312-336 hours postdose) and 3 pooled fecal samples (0-72, 168-192, and 312-336 h postdose) from six individual subjects. Plasma samples (1.0 – 2 mL depending on the volume available and radioactivity level of the samples) and fecal samples were extracted employing the same methods as described above. The plasma sample supernatants were evaporated to dryness under a stream of nitrogen in an ambient water bath, and the residues were reconstituted in 0.35 – 0.5 mL of MeOH:ACN:water (10:20:70, v/v/v). Each pooled urine sample was lyophilized, and the residue reconstituted in water:ACN:FA (80:20:0.1, v/v/v). The fecal sample supernatants were dried under a nitrogen stream, and the residues were also reconstituted in water:ACN:FA (80:20:0.1, v/v/v). The reconstituted plasma, urine, and fecal samples were centrifuged at 15,000 rpm for 10 minutes. Aliquots of the
supernatants were injected onto the HPLC system for analysis. LC-MS/MS analysis coupled with a radio flow-through detector (RFD) was used for metabolite profiling and identification for samples with sufficient levels of radioactivity.

The system for metabolite profiling and identification (LC-MS/MS/RFD) consisted of an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland), a Surveyor HPLC pump (Thermo Electron, Waltham, MA), a Thermo Finnigan LTQ linear ion trap mass spectrometer (Thermo Electron), and a β-RAM Model 3 RFD (LabLogic, Brandon, FL). Data from mass spectrometry and RFD were processed by Xcalibur (Thermo Fisher Scientific, Waltham, MA) and Laura Lite 3 (LabLogic Systems, Brandon, FL) software, respectively. The HPLC eluent was split between the RFD and mass spectrometer with a ratio of 3 to 1.

Two HPLC methods were used to separate cabozantinib and its metabolites. HPLC Method 1 was used for the analysis of pooled urine and fecal samples and individual plasma samples from different time points. HPLC Method 2 was used for pooled plasma samples generated by Hamilton pooling across time points 0-168 hours postdose to separate metabolites that were co-eluted with metabolite EXEL-1646 (Hamilton et al, 1981). HPLC separation was performed using a Synergi Max RP, 4.6 × 250 mm, 4-μm column (Phenomenex, Torrance, CA) for Method 1 and an Xbridge phenyl, 4.6 × 150 mm, 3.5-μm column (Waters, Milford, MA) for Method 2. The mobile phases for Method 1 were as follows: A, 0.1% FA in water; B, 0.1% FA in ACN; B was cycled from 20% to 95% to 20% over 34 min at a flow rate of 800μL/min. The mobile phases for Method 2 were as follows: A, 0.1% FA in water; B, 0.15% FA in ACN; B was cycled from 20% to 95% to 20% over 55 min at a flow rate of 800μL/min. Mass spectrometry conditions for both methods were as follows: sheath gas, 50 units; auxiliary gas, 20 units; sweep gas, 10 units; ion spray voltage, 5 kV (Method 1: 5 kB for electrospray ionization [ESI]+; 4.3 kV
for ESI-); capillary temperature, 300°C; capillary voltage, 22 V (Method 1: 22 V for ESI+; -9 V for ESI-); tube lens voltage, 80 V (Method 1: 80 V for ESI+; -96 V for ESI-); and ionization mode, ESI+ (Method 1: ESI+ and ESI-).

The HPLC-MS system for high-resolution MS consisted of a Paradigm MS4B HPLC (Michrom Bioresources, Auburn, CA) and a LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific). Chromatographic conditions and the ion source parameters were the same as HPLC Method 1 for the LTQ system. Data were acquired with a resolution of 30000 in centroid mode.

An HPLC/TopCount NXT (PerkinElmer) system was used for the radio-quantitation of plasma samples. The system consisted of an HTC PAL autosampler, two HPLC pumps (Shimadzu, Chicago, IL), and a Foxy Jr. Fraction Collector (Isco, Lincoln, NE). HPLC fractions collected in a LumaPlate 96-well plate were dried using an EZ-2 plus Personal Evaporator (Genevac, Valley Cottage, NY), and the dried samples were counted by TopCount NXT Microplate Scintillation & Luminescence Counter (PerkinElmer). Data were processed using ProFSA (PerkinElmer) software. The HPLC methods were the same as described above.

Metabolites that represented greater than 5% of the total radioactivity or 5% of total AUC in the matrix were identified according to the following process. Mass spectra (MS, MS/MS, and MS/MS/MS) of cabozantinib and its metabolite reference standards were acquired on an ion trap mass spectrometer. Major fragment patterns were proposed. Identification of these metabolites was confirmed by matching mass spectra (MS and MS/MS) and retention times with authentic reference standards. For other unknown metabolites, molecular ions were searched on LC/MS chromatograms operating in full scan positive as well as negative ionization modes at the same retention times as those found on LC-radiochromatogram. Product ion mass spectra and high
resolution mass spectra were then acquired for the corresponding molecular ions. Putative metabolite structures were proposed based on the analysis of their mass fragment patterns.

**Quantification of Cabozantinib and Its Metabolites in Human Plasma.** *Cabozantinib, EXEL-5366, EXEL-5162, EXEL-1646, and parafluoroaniline (pFA).* Plasma concentrations of cabozantinib, EXEL-5366, EXEL-5162, and EXEL-1646 were determined using a validated LC-MS/MS assay at QPS, LLC. Briefly, 50 µL aliquots of K<sub>2</sub>EDTA plasma samples and prepared standard controls were transferred to 96-well plates and protein precipitated with 300 µL MeOH:ACN (20:80, v/v). After vortex-mixing and centrifugation, 30 µL of the supernatant was transferred to 96-well plates containing 270 µL of ACN:water:FA (50:50:0.1, v/v/v). After vortex-mixing and centrifugation, samples were analyzed via LC-MS/MS as described above. This method was validated with acceptable accuracy and precision for the following concentration ranges: 1-500 ng/mL for cabozantinib, EXEL-5366, and EXEL-5162; and 4-400 ng/mL for EXEL-1646. A separate LC-MS/MS bioanalytical method was validated for pFA (linear range: 2-800 ng/mL) at Exelixis, Inc (South San Francisco, CA).

**EXEL-1644.** Plasma concentrations of EXEL-1644 were determined using a qualified LC-MS/MS assay at QPS, LLC. Briefly, 50 µL aliquots of K<sub>2</sub>EDTA plasma samples and prepared standard controls were transferred to 96-well plates and protein precipitated with 300 µL of ACN containing an internal standard. After vortex-mixing and centrifugation, 100 µL of the supernatant was transferred to 96-well plates along with 100 µL of water. After vortex-mixing and centrifugation, samples were analyzed via LC-MS/MS as described above. This method was qualified for the concentration range 2-500 ng/mL.
Pharmacokinetic Analysis. Plasma concentration-time data were analyzed by a noncompartmental pharmacokinetic method using WinNonlin Professional version 5.2 (Pharsight, Mountain View, CA). C\text{max} and T\text{max} were directly determined from the observed blood/plasma concentrations data. AUC\text{0-24}, AUC\text{0-72}, AUC\text{0-\tau}, and AUC\text{0-inf} were calculated using the linear trapezoidal method. Estimates of t\text{1/2} were calculated using Equation 1, where the value of the terminal-phase disposition rate constant (k\text{el}) of the apparent phase was determined by a noncompartmental analysis using WinNonlin.:

\[ t_{1/2} = \frac{0.693}{k_{el}} \]  

Eq. 1

The AUC\text{0-inf} was computed using Equation 2, where \(C_t\) was the last measurable concentration:

\[ AUC_{0-inf} = AUC_{0-\tau} + \frac{C_t}{k_{el}} \]  

Eq. 2

Distribution to Red Blood Cells. To determine the percentage of radioactivity associated with erythrocytes in whole blood over time, the following was calculated: the amount of radioactivity in plasma versus whole blood, adjusted for hematocrit, at the specific time points of comparison (% radioactivity in whole blood associated with erythrocytes =Xe/Xb=1-[Cp \times (1-Hct)/Cb]), where Xe and Xb stands for amount of radioactivity in erythrocytes or whole blood, respectively, Cp is the amount of radioactivity in plasma, Cb is the amount of radioactivity in blood, and Hct is the hematocrit value (hematocrit values for Days -1, 2, and 4 were averaged for use in this calculation).
Nonclinical Safety Evaluations of EXEL-1644. All animal experiments were performed in facilities accredited by AALAC according to protocols approved by IACUCs. A 2-week repeat-dose toxicity study of EXEL-1644 in rats was conducted at Covance Laboratories (Madison, WI) in compliance with GLP regulations. Groups (10/sex/group) of Hsd:Sprague Dawley (SD) rats (Harlan Laboratories, Inc., Indianapolis, IN) received a subcutaneous injection daily of vehicle formulation only (ethanol [EtOH]:polyethylene glycol 400 [PEG400]:water, 5:45:50, v/v/v) or EXEL-1644 (in vehicle) at dose levels of 3, 10, or 30 mg/kg/day for 2 weeks. Animals were dosed via subcutaneous injection into two dose sites/dose at a volume of 10 mL/kg (Days 1 through 7 of the dosing phase) or 5 mL/kg (Days 9 through 15 of the dosing phase).

Reversibility, persistence, or delayed occurrence of any effects after a 4-week recovery was evaluated in recovery cohorts (5/sex/group) receiving vehicle alone or EXEL-1644 (30 mg/kg/day). Toxicokinetic cohorts receiving vehicle alone (3/sex/group) or EXEL-1644 (9/sex/group) were included to assess systemic exposure to test article. Male and female rats were group-housed (up to five animals/sex/cage) in polycarbonate cages with hardwood chip bedding. Animals were individually housed for study-related procedures or behavioral or health reasons. Control group rats were housed on separate racks from animals given the test article. Animals were provided water ad libitum and offered certified Rodent Diet #2016C (Harlan Laboratories, Inc.) ad libitum unless fasted for study procedures. Environmental controls were set to maintain the following animal room conditions: temperature range of 20 to 26°C, relative humidity range of 30% to 70%, 10 or greater air changes/hour, and a 12-hour light/12-hour dark cycle. The light/dark cycle was interrupted for study-related activities. Assessment of toxicity was based on mortality, clinical observations, irritation scoring, food consumption, body weights, ophthalmic examination, and clinical and anatomic pathology. Toxicokinetic plasma
samples were measured using a validated LC-MS/MS method for EXEL-1644. Pharmacokinetic analysis of plasma drug concentration data was performed using WinNonlin Professional 5.2.

**Pharmacokinetics of 14C-Cabozantinib-Derived Radioactivity in Bile Duct-Intact (BDI) and Bile Duct-Cannulated (BDC) Dogs.** The pharmacokinetics of 14C-cabozantinib-derived radioactivity and the excretion profiles of radioactivity were examined in male bile duct-intact (BDI) and bile duct-cannulated (BDC) beagle dogs following a single 100-mg/kg oral gavage dose (5 mL/kg) of cabozantinib L-malate salt containing 14C-cabozantinib (50-μCi/kg) formulated in EtOH: PEG400:water (5:45:50, v/v/v) vehicle. This study was conducted by Covance Laboratories in accordance with the Wisconsin Department of Health Services, Radiation Protection Section. Six male purebred beagle dogs from Covance Research Products (Cumberland, VA) were acclimated to study conditions for approximately 3 weeks prior to dose administration. Animals were acclimated to the jacket and tether system for bile collection prior to bile duct-cannulation surgery. At dosing, the animals weighed 10.4 to 12.0 kg and were 12 to 13 months of age. All animals were housed in individual, stainless steel cages during acclimation. During the test period, animals were housed as appropriate for sample collection: animals designated for excreta collection were housed in individual stainless steel metabolism cages designed for the separation and collection of urine and feces; animals designated for collection of excreta and bile were housed in individual stainless steel metabolism cages designed for the separation and collection of urine, feces, and bile (using a tether and swivel system). Certified Canine Diet #2027C (Harlan) was provided ad libitum, except as specified for clinical pathology, bile duct-cannulation, or under dosing procedures. Water was provided fresh daily ad libitum. Environmental controls for the animal room were set to maintain a temperature of 20 to 26°C, a relative humidity of 50 ± 20%, and a 12-hour light/12-hour dark cycle. As
necessary, the 12-hour dark cycle was interrupted to accommodate study procedures. Animals underwent bile duct cannulation surgery according to Covance standard operating procedures or study specific procedures. The animals were fasted overnight before surgery and were allowed at least 10 days to recover from the surgical procedures prior to dose administration. An anesthetic regimen consisting of an intravenous sedative for induction and appropriate inhalation anesthetic for maintenance was used. Using sterile surgical procedures, the bile duct was cannulated to allow collection of bile, and a second cannula was placed into the duodenum to allow infusion of a bile salts replacement solution or other fluids, as required. Antibiotics, analgesics, and intravenous fluids were administered as deemed appropriate by a staff veterinarian. Each animal was fitted with a jacket and tether to conduct the catheters to the outside of the cage. Beginning the day of surgery through approximately 3 days post-surgery, a solution of Lactated Ringer's solution was administered via the duodenal cannula at a rate of 3.0 mL/kg/hour to prevent dehydration. Beginning on approximately the fourth post-surgical day, an appropriate amount of a mixed bile salts replacement solution was infused via the duodenal cannula continuously at a rate of approximately 1 mL/kg/hour. The bile salts solution was prepared by adding approximately 18.0 g/L of cholic acid, 9 g/L of sodium chloride, and 1.3 g/L of sodium bicarbonate to purified water. Blood (approximately 5 mL) was collected from a jugular vein into tubes containing K$_2$EDTA anticoagulant predose and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours postdose. Samples were maintained on wet ice or in a chilled cryorack until aliquoted and centrifuged to obtain plasma. Urine, bile, and feces were collected at specified intervals through 168 hours postdose. Urine was collected in plastic containers surrounded by dry ice at 0-12 and 12-24 hours postdose and at 24-hour intervals through 168 hours postdose. The weight of each sample was recorded. Feces were collected at 24-hour
intervals through 168 hours postdose at room temperature and transferred to plastic containers and stored at approximately -70°C. The weight of each sample was recorded. Bile was collected in plastic containers surrounded by dry ice at 0-4, 4-8, and 8-24 hours postdose and at 24-hour intervals through 168 hours postdose from BDC dogs. The weight of each sample was recorded. Concentrations of radioactivity in whole blood, plasma, urine, bile, and feces (after combustion) were determined by LSC. Selected samples of plasma, urine, bile, and fecal homogenates were pooled and profiled for metabolites of cabozantinib using LC-MS/MS methodology similar to that described previously.

**Pharmacokinetics of ¹⁴C-Cabozantinib-Derived Radioactivity in BDI and BDC Rats.** The pharmacokinetics of ¹⁴C-cabozantinib-derived radioactivity and the excretion profiles of radioactivity were also examined in male BDI and BDC SD rats following a single oral gavage dose (10 mL/kg) of approximately 50-mg/kg cabozantinib L-malate (750-µCi/kg ¹⁴C-cabozantinib) formulated in EtOH:PEG400:H₂O (5:45:50, v/v/v vehicle). This study was conducted by Covance Laboratories in accordance with the Wisconsin Department of Health Services, Radiation Protection Section. Nine male jugular vein-cannulated and BDC Sprague Dawley rats (Hsd:SD) were obtained from Harlan Laboratories (Madison, WI). At dosing, the animals weighed 291 to 326 g and were 10 to 11 weeks of age. All animals were housed in individual, suspended, stainless steel, wire mesh cages during acclimation, and in individual Nalgene cages designed for the collection of bile and for the separation and collection of urine and feces. Certified Rodent Diet #2016CM (Harlan) or Certified Rodent Diet #2016C (Harlan) were provided ad libitum except during dosing procedures. Water was provided ad libitum. Environmental controls for the animal room were set to maintain a temperature of 20 to 26°C, a relative humidity of 50 ± 20%, and a 12-hour light/12-hour dark cycle. As necessary, the 12-hour
dark cycle was interrupted to accommodate study procedures. Animals were placed in the jackets for bile collection. Starting the day prior to dose administration, animals were connected to the bile collection tether system and a solution of taurocholic acid (2.3 mg/mL in 0.9% saline) was infused via the distal (duodenal) cannula at a rate of 0.9 mL/hour until the time of sacrifice. Blood (approximately 0.4 mL) was collected from a jugular vein cannula via syringe and transferred into tubes containing K$_2$EDTA anticoagulant at 0.5, 1, 2, 4, 8, 24, 48, 72, 96, and 120 hours postdose from all animals. Samples were maintained on wet ice or stored at approximately 5°C until aliquoted and centrifuged to obtain plasma. Urine was collected in plastic containers surrounded by dry ice predose, at 0-4, 4-8, and 8-24 hours postdose, and at 24-hour intervals through 120 hours postdose. The weight of each sample was recorded. Feces were collected in plastic containers surrounded by dry ice predose and at 24-hour intervals through 120 hours postdose. The weight of each sample was recorded. Bile was collected in plastic containers surrounded by dry ice predose, at 0-2, 2-4, 4-6, 6-8, and 8-24 hours postdose, and at 24-hour intervals through 120 hours postdose. The weight of each sample was recorded. Concentrations of radioactivity in whole blood, plasma, urine, bile, and feces (after combustion) were determined by LSC. Selected samples of plasma, urine, bile, and fecal homogenates were pooled and profiled for metabolites of cabozantinib using LC-MS/MS methodology as described previously.

**EXEL-1644 Plasma Protein Binding.** An equilibrium dialysis method was used to measure EXEL-1644 plasma protein binding. The protein binding of EXEL-1644 was determined in rat and human plasma at five targeted concentrations (125, 200, 250, 350, and 500 μM) using LC-MS/MS bioanalytical methods. The percentage of bound test compound was calculated from the following equation: % Bound = \([ (C_{ISS} - 2C_{DS,C2fub}) / C_{ISS} ] \times 100\) where C$_{ISS}$ is the concentration.
of the incubated standard plasma sample at the time of the measurement, $C_{DS-C2}$ is the compound concentration in the basolateral (buffer) chamber at the time of the measurement, and $f_{ub}$ is a correction factor representing the percentage of free EXEL-1644 that is bound nonspecifically to the equilibrium dialysis device. The mean percentages of EXEL-1644 bound to rat (99.729 to 99.966%) and human plasma proteins (99.950 to 99.996%) were concentration-dependent, with percentage bound decreasing as the EXEL-1644 concentration increased.

**Deconjugation of EXEL-1644 and EXEL-1646 by Sulfatase In Vitro.** Two sulfate metabolites (EXEL-1644 and EXEL-1646) were incubated with sulfatase enzyme for determination of their respective deconjugated monohydroxy products (EXEL-5526 and EXEL-1744). The sulfate metabolites (1 and 10 μM) were incubated with sulfatase enzyme (0.5 and 1 mg/mL) for 8 hours. Positive control incubations were performed with 10 μM 7-hydroxy coumarin sulfate in the presence of the sulfatase isoenzyme for 8 hours. All incubations were performed in triplicate; samples were extracted and analyzed by LC-MS/MS for determination of deconjugated products. Incubation with the sulfatase enzyme was shown to result in the deconjugation (hydrolysis) of the two sulfate metabolites EXEL-1644 and EXEL-1646 to the corresponding hydroxy precursors EXEL-1744 and EXEL-5526, respectively. Deconjugation of sulfonated metabolites EXEL-1644 and EXEL-1646 by sulfatase enzyme did not result in the formation of unchanged cabozantinib. Hydrolysis of EXEL-1644 was nearly complete for each of the incubation conditions with only 0.7% to 6% remaining after 8 hours. EXEL-1646 was hydrolyzed more slowly; under the same conditions, and 45% to 97% remained after 8 hours.

**In Vitro Pharmacologic Activity Characterization.** Cabozantinib and its major metabolites were tested in vitro against a panel of receptor tyrosine kinases (AXL, KIT, EGFR, FLT1, FLT3, FLT4, KDR, MET, PDGFRβ, RET, Ron, and TIE2) and serine/threonine kinases (Aurora-A and
Aurora-B) that are implicated in the promotion of tumor growth, tumor metastasis, and angiogenesis. Radiometric protein kinase assays that measured the inhibitory effects of cabozantinib, EXEL-1646, EXEL-1644, EXEL-5162 (RET only), and EXEL-5366 (RET only) on the catalytic incorporation of $^{33}$P-$\gamma$-ATP into kinase substrates (KinaseProfiler™) were performed by Millipore/Eurofins Pharma Discovery (Dundee, UK). Each compound was tested at 9 concentrations at half-log dilutions (with vehicle control wells) in order to estimate IC$_{50}$ values (IC$_{50}$Profiler™) for individual kinases. Inhibition of tyrosine kinases KIT, FLT1 and KDR by EXEL-5162 and EXEL-5366 were performed by Exelixis, Inc. using AlphaScreen™ (Perkin Elmer) technology (a proximity assay method employing microparticles). Singlet oxygen derived from a donor bead following laser excitation results in chemiluminescence when in proximity (100 Å) to an acceptor bead due to biomolecular interactions. For the assay described below, donor beads (coated with streptavidin), acceptor beads (coated with PY100 anti-phosphotyrosine antibody), and substrate (biotinylated poly(Glu,Tyr) 4:1) were obtained from Perkin Elmer. Substrate phosphorylation was measured after addition of donor/acceptor beads by chemiluminescence following donor-acceptor bead complex formation. Test compounds (evaluated at ten different concentrations), ATP (3-5 µM at the approximate K$_m$ for the respective kinase), substrate (3 nM), and kinase (0.05 nM - 0.5 nM) were added to assay buffer (20 mM TrisHCl, pH 7.5; 10 mM MgCl$_2$; 3 mM MnCl$_2$; 1 mM DTT; 0.01% Triton) to a volume of 20 µL in a 384-well white, medium binding microtiter plate (Greiner). Reaction mixtures were incubated for 1 hour at ambient temperature. Reactions were quenched by addition of 10 µL of 15-30 mg/mL AlphaScreen bead suspension containing 75 mM Hepes, pH 7.4, 300 mM NaCl, 120 mM EDTA, 0.3% BSA, and 0.03% Tween-20. After a 2- to 16-hour incubation at ambient temperature, plates were read using an AlphaQuest reader (Perkin Elmer). Inhibition of tyrosine
kinases Aurora-B, EGFR, FLT3, MET, and PDGFRβ by EXEL-5162 and EXEL-5366 were performed by Exelixis, Inc. using a luciferase-coupled chemiluminescence assay. Kinase activity is measured as the percentage of ATP consumed following the kinase reaction using luciferase-luciferin-coupled chemiluminescence. Reactions were conducted in 384-well white, medium-binding microtiter plates. Kinase reactions were initiated by combining test compounds (evaluated at 10 different concentrations), ATP (0.5-5 μM at the approximate K_m for the respective kinase), and kinase (0.02 nM - 10 nM) in assay buffer (EGFR and PDGFRβ: 20 mM TrisHCl, pH 7.5; 10 mM MgCl_2; 3 mM MnCl_2; 1 mM DTT; 0.01% Triton; MET: 20 mM TrisHCl, pH 7.5; 10 mM MgCl_2; 0.02% Triton X-100; 1 mM DTT; 2 mM MnCl_2; FLT3: 20 mM TrisHCl, pH 7.5; 10 mM MgCl_2; 3 mM MnCl_2; 1 mM DTT; 0.1% BSA; 0.03% Chaps; 25 mM glycerolphosphate) in a total 20 μL volume. The reaction mixture was incubated at ambient temperature for 120 min (MET), 180 min (EGFR and PDGFRβ), or 210 min (EGFR). Following the kinase reaction, a 20 μL aliquot of luciferase-luciferin mix was added, and the chemiluminescence signal was measured using a Victor^2 plate reader (Perkin Elmer). The luciferase-luciferin mix contained 50 mM HEPES, pH 7.8, 67 mM oxalic acid (pH 7.8), 5 mM DTT, 0.4% Triton X-100, 0.25 mg/mL coenzyme A, 63 μM AMP, 28 μg/mL luciferin, and 40,000 units/mL luciferase. Total ATP consumption was limited to 25%-45%. The IC_{50} values based on AlphaScreen and luciferase-coupled chemiluminescence assays were calculated by nonlinear regression analysis using the following four-parameter equation: \( Y = \text{min} + (\text{max} - \text{min}) / [1 + (X/\text{IC}_{50})^N] \), where Y is the observed signal, X is the inhibitor concentration, min is the background signal in the absence of enzyme (0% enzyme activity), max is the signal in the absence of inhibitor (100% enzyme activity), IC_{50} is the inhibitor concentration at 50% enzyme inhibition, and N represents the empirical Hill slope as a measure of cooperativity. The IC_{50}
values determined for AlphaScreen and luciferase-coupled chemiluminescence assays generally correlated well with those determined by radiometric assays.

Off-target in vitro pharmacologic specificity covering a broad range of approximately 75 receptors, ion channels, transporters, enzymes, and second messengers, was conducted by Cerep SA (Poitiers, France). Binding of target-specific radioligands was measured by scintillation counting in the presence and absence of cabozantinib, EXEL-1644, or EXEL-1646 (1 µM duplicate wells each) in a 96-well assay. Target-specific reference compounds evaluated concurrently demonstrated IC_{50} values consistent with historical laboratory results, thereby establishing assay sensitivity. Results are expressed as a percent of control specific binding (ie, measured specific binding/control specific binding × 100) and as a percent inhibition of control specific binding (ie, 100 – [measured specific binding/control specific binding × 100]) obtained in the presence of the test compounds. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH) were determined by nonlinear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting (ie, 
\[ Y = D + \frac{A - D}{1 + (C/IC_{50})^{nH}} \]
where Y = specific binding, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, and nH = slope factor.

**In Vitro CYP Interaction Analyses.** The direct and metabolism-dependent inhibition potential of EXEL-1644 on CYP enzyme activities phenacetin O-deethylase (CYP1A2), bupropion hydroxylase (CYP2B6), amodiaquine N-deethylase (CYP2C8), diclofenac 4′-hydroxylase (CYP2C9), S-mephenytoin 4′-hydroxylase (CYP2C19), bufuralol 1′-hydroxylase (CYP2D6), testosterone 6β-hydroxylase (CYP3A4/5), and midazolam 1′-hydroxylase (CYP3A4/5) was
conducted at Covance Laboratories using pooled human hepatic microsomes. Incubation mixtures (0.5 mL final volume) performed in 96-well plates contained microsomal protein and a CYP isozyme-selective substrate in a 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA (assay buffer). Microsomal protein was incubated at 37°C for at least 5 minutes in the presence of substrate and EXEL-1644 (0.03 to 100 μM) prior to the addition of warmed (37°C) NADPH to initiate the reaction. The final concentration of organic solvent in incubations was ≤1% (v/v). Incubations were terminated by the addition of 100 μL of an internal standard solution and vortex mixing. Protein was removed by centrifugation at 1582 × g for 5 minutes at 4°C, and supernatants were transferred to a separate plate and stored at 4°C prior to LC-MS/MS analysis. The analyte for each activity assay was quantitated by comparison to a linear curve of authentic standard prepared using human microsomal protein. All sample and control incubations were performed in triplicate. All analytical standards and quality control samples were performed in duplicate. The extent of inhibition was assessed by comparing activities from control (vehicle only) and treated (containing test article) microsomal incubations. IC₅₀ values were determined from unweighted data and a 3-parameter sigmoidal curve using Microsoft Excel. The inhibition constant (Kiₐₚ) and type of inhibition for EXEL-1644 were determined for amodiaquine N-deethylase (CYP2C8) and diclofenac 4′-hydroxylase (CYP2C9) activities. Kinetic calculations were performed using SigmaPlot for Windows with Enzyme Kinetics Module or GraFit. Kinetic constants (Kₘₐₚ, Vₘₐₓ, and Kiₐₚ) were determined using nonlinear regression analysis according to Michaelis-Menten kinetics. Data were compared for fit with competitive, noncompetitive, uncompetitive, and mixed inhibition models. For metabolism-dependent inhibition, incubations containing concentrated (10×) microsomal protein were preincubated with EXEL-1644 (1, 10, and 100 μM) in the absence and presence of NADPH.
during the initial 30-minute incubation prior to dilution and addition of an isozyme-selective substrate; control incubations were conducted in the absence of NADPH.

**In Vitro Drug Transporter Interaction Analyses.** Cabozantinib and EXEL-1644 were evaluated as substrates and/or inhibitors of human solute carrier (SLC) transporters organic anion transporter (OAT) 1, OAT3, organic cation transporter (OCT) 1, OCT2, organic anion transporting polypeptide (OATP) 1B1, OATP1B3, and multidrug and toxin extrusions organic cation antiporters (MATE1 and MATE2-K), and of human ATP-binding cassette (ABC) transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), multidrug resistance protein 2 (MRP2), and bile salt export pump (BSEP). Drug transporter substrate and/or inhibition assays with cabozantinib were performed at Covance Laboratories using stably transfected Chinese hamster ovary (CHO) cells (OAT1, OAT3, OCT1, OCT2, OATP1B1, and OATP1B3), Caco-2 cell monolayers (P-gp and BCRP), transfected insect cell Sf9 membrane vesicles (BSEP and MRP2), and at Optivia (Menlo Park, CA) using transfected MDCK-II cell monolayers (MATE1 and MATE2-K). Drug transporter substrate and/or inhibition assays with EXEL-1644 were performed at Optivia (Menlo Park, CA) using with transfected MDCK-II cell monolayers (OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, and MATE2-K), MDCK-MDR1 cell monolayers (P-gp), transfected insect cell Sf9 membrane vesicles (BSEP and MRP2), and Caco-2 cell monolayers (BCRP). Test system transporter activities were confirmed using a positive control substrate or inhibitor for the individual drug transporter. Percent transporter inhibition was calculated as: 100-[100×(transporter mediated uptake) with inhibitor/(transporter mediated uptake) without inhibitor]. IC\textsubscript{50} values were calculated as \( V=V_0/1+([I]/IC_{50})^n \), where \( V_0 \) is the mean transporter-mediated flux in the absence of the test article, \( V \) is the transporter-mediated flux in the presence of the test article throughout the concentration range tested, \( [I] \) is
the inhibitor concentration, IC₅₀ represents the value at which transport is inhibited by 50%, and
n is a Hill coefficient.

Assessment of cabozantinib as a substrate or inhibitor of SLC transporters. ¹⁴C-cabozantinib (1
and 10 μM final concentration) was added to 500 μL of pre-warmed (37°C) dosing solution
(Hank’s Balanced Salt Solution [HBSS]) with 10 mM HEPES, pH 7.4) in 12-well plates
containing transfected CHO cells (seeded and grown to 100% confluency). Uptake of ¹⁴C-
cabozantinib by each uptake transporter and the vector control was measured at 5, 15, and 30
minutes, in the absence and presence of a known inhibitor for each uptake transporter (200 μM
probenecid for OAT1 and OAT3, 256 μM quinidine for OCT1 and OCT2, and 10 μM
cyclosporine A for OATP1B1 and OATP1B3). The incubations were conducted at 37°C and
terminated by quickly and completely removing the dosing solution by aspiration and rinsing the
cells twice with 1 mL of ice-cold HBSS with 10 mM HEPES (pH 7.4). The cells were then lysed
by incubation with 0.5 or 1 mL of 1 N sodium hydroxide for at least 10 minutes. The cell lysate
from each well was analyzed by LSC to determine the amount of radioactivity. All incubations
were performed in at least triplicate. Uptake of the known substrate by each uptake transporter
(OAT1: 1 μM ¹⁴C-para-aminohippurate; OAT3: 1 μM ³H-estrone-3-sulfate; OCT1: 1 μM ¹⁴C-
tetraethylammonium; OCT2: 1 μM ¹⁴C-tetraethylammonium; OATP1B1: 0.5 μM ³H-estradiol-
17β-D-glucuronide; OATP1B3: 1 μM ³H-cholecystokinin octapeptide) and the vector control in
the absence and presence of 0.002% PS-80 was performed as a positive control. For transporter
inhibition assays, uptake of the known substrate by each transporter was conducted in the
presence and absence of a known inhibitor or cabozantinib (5 and 15 μM) according to the
uptake incubation procedure. Uptake of the known substrate by the vector control in the absence
and presence of 0.002% PS-80 was performed as a control. Inhibition of MATE1 and MATE2-K
transporters by cabozantinib (0.1-20 μM) was performed with MDCK-II cell monolayers using
the SLC transporter inhibition assay procedures described below for EXEL-1644.

Assessment of EXEL-1644 as a substrate or inhibitor of SLC transporters. Assays were
conducted using MDCK-II cell monolayers grown on a permeable PCF membrane (0.4 μ) in a
Costar 96-well culture plate system (Corning) maintained at 37°C and 5% CO₂. Transport
experiments were run in triplicate wells containing identical cells expressing the transporter of
interest or control cells lacking the transporter (to correct for non-transporter mediated test article
transport). HPBSS (37°C) was added to the apical (30 μL) and basal (150 μL) compartments.
For transporter substrate studies, the following was added to the basal compartments of
individual wells: EXEL-1644 (1.0, 3.0 and 10 μM final concentrations) alone, 3.0 μM EXEL-
1644 in combination with a reference inhibitor (OCT1 and OACT2: 100 μM quinidine; OAT1
and OAT3: 100 μM probenecid; OATP1P1 and OATP1B3: 100 μM rifampicin; MATE1 and
MATE2-K: 10 μM cimetidine), or positive control substrate probes for individual transporters
(OCT1: 10 μM ³H-1-methyl-4-phenylpyridinium; OCT2: 10 μM ¹⁴C-metformin; OAT1: 2 μM
³H-aminohippurate; OAT3: 10 μM ³H-aminohippurate; OATP1B1: 2 μM ³H-estradiol-17β-D-
glucuronide; OATP1B3: 2 μM ³H-cholecystokinin-8; MATE1 and MATE2-K: 10 μM ³H-
metformin). For transporter inhibition studies, the basal compartment contained the reference
inhibitor and positive control substrate or EXEL-1644 (1-250 μM). Plates were placed on an
orbital shaker for 5 min at 37°C, and then each well was washed four times with cold PBS
followed by addition of 60 μL of ACN:water (50:50). Plates were again placed on an orbital
shaker for 15 min. Aliquots were taken for analysis of EXEL-1644 (LC-MS/MS) or probe
substrates (LSC).
Assessment of cabozantinib as a substrate or inhibitor of ABC transporters P-gp and BCRP.

Experiments were conducted using Caco-2 cell monolayer cultures grown on Costar Transwell® polycarbonate membrane inserts (pore size 0.4 μ). The apparent permeability in both the apical-to-basolateral (A to B) direction and basolateral-to-apical (B to A) direction was determined to calculate the efflux ratio (ER) for cabozantinib as follows: ER = P_B to A / P_A to B, where P_B to A is the apparent permeability (cm/sec) from the basolateral to apical side, and P_A to B is the apparent permeability (cm/sec) from the apical to basolateral side. For substrate evaluations, ¹⁴C-cabozantinib (1 and 10 μM) was added to 250 μL pre-warmed (37°C) dosing solution in the apical donor compartment; for inhibitor assays, ¹⁴C-cabozantinib (5 and 50 μM) was added to 700 μL pre-warmed dosing solution in the basolateral compartment. For transporter substrate evaluations, the apparent permeability of ¹⁴C-cabozantinib was determined in the presence of vehicle and known inhibitor (2 μM zosuquidar [P-gp], 1 μM Ko143 [BCRP]) and negative inhibitor controls (2 μM zosuquidar [BCRP], 1 μM Ko143 [P-gp]) for 1, 2, 3, and 4 hours. For each transporter inhibitor evaluation, the apparent permeability of known substrate (1 μM ³H-digoxin [P-gp], 0.1 μM ³H-estrone-3-sulfate [BCRP]) was determined in the presence of vehicle, known inhibitor, and cabozantinib for 1 hour. The apparent permeability of each known substrate was also assessed in the presence of vehicle and each known inhibitor in the absence and presence of 0.002% PS-80 as a control for both transporter substrate and inhibitor evaluations. At the end of the incubation period, the total volume of the donor and receiver compartments was removed to determine the amount of radioactivity. All incubations were performed in triplicate. Samples were stored at approximately -20°C until analysis by LSC. Trans-epithelial electrical resistance and the apparent permeability of mannitol were used to confirm the membrane integrity of the monolayers.
Assessment of EXEL-1644 as a substrate or inhibitor of ABC transporters P-gp and BCRP.

Experiments were performed using similar P-gp and BCRP assay conditions as described above for the cabozantinib assays, with the following exceptions: MDCK-MDR1 cell monolayers were used for P-gp transport studies; for transporter substrate assays EXEL-1644 (1.0, 3.0, and 10 μM) was tested alone or at 3.0 μM with reference inhibitors verapamil (100 μM) for P-gp and chrysin (100 μM) for BCRP; for transporter inhibition assays reference probe substrates for P-gp (100 nM ³H-digoxin) and BCRP (25 nM ³H-genistein) were tested alone or in combination with EXEL-1644 (1-250 μM); incubation times were 15 min for BCRP and 120 min for P-gp; and analysis of EXEL-1644 was by LC-MS/MS.

Assessment of cabozantinib as a substrate or inhibitor of ABC transporters MRP2 and BSEP.

Evaluation of cabozantinib as a MRP2 substrate utilized measurement of MRP2 ATPase activity (BD Gentest) in incubations containing MRP2 membranes, 0.002% PS-80, and cabozantinib (2 and 10 μM) or reference substrate probenecid (1000 μM). Control incubations were conducted in the presence or absence of 0.002% PS-80. ATP-dependent uptake activity was calculated as (Activity_{ATP} – Activity_{AMP}), where Activity_{ATP} is the uptake activity with ATP (pmol/min/mg protein) and Activity_{AMP} is the uptake activity with AMP (pmol/min/mg protein). Assessment of cabozantinib as an inhibitor of MRP2 uptake was measured by determining uptake of ³H-leukotriene C4 (0.1 μM) by MRP2 membranes in the presence of cabozantinib (5 and 50 μM) and 0.002% PS-80 using the MRP Vesicle Assay Kit (BD Gentest). MK-571 (100 μM) was used as the reference MRP2 inhibitor. Control incubations were conducted in the absence and presence of PS-80. All incubations were conducted in triplicate. Assessment of cabozantinib as a BSEP substrate was determined by measuring uptake of ¹⁴C-cabozantinib (2 and 10 μM) into BSEP membranes using the BSEP Vesicle Assay Kit (BD Gentest). Uptake of 1 μM ³H-
taurocholate (taurocholic acid, TCA) by BSEP membranes was performed as a positive control in the absence and presence of 0.002% PS-80. Assessment of cabozantinib as an inhibitor of BSEP was determined by uptake of 1 μM ³H-TCA conducted in the presence of cabozantinib (5 and 15 μM) and reference inhibitor bosentan (200 μM) in the absence and presence of 0.002% PS-80. Aliquots were analyzed by LSC.

**Assessment of EXEL-1644 as a substrate or inhibitor of ABC transporters MRP2 and BSEP.**

Assays were performed under similar experimental conditions as described above for MRP2 and BSEP assays with cabozantinib, with the following exceptions: for evaluations of EXEL-1644 as a substrate of either transporter, ATPase activity was measured in the presence of EXEL-1644 (1.0, 3.0, and 10) alone or at 3.0 μM in the presence of reference inhibitor rifampicin (300 μM); for evaluations of EXEL-1644 as a transporter inhibitor, reference substrates for BSEP (1 μM ³H-taurocholic acid) and MRP2 (50 μM ³H-estradiol-17β –D-glucuronide) were tested alone and in the presence of EXEL-1644 (1-250 μM); incubation times were 5 min for MRP2 and 15 min for BSEP; and PS-80 was omitted from the incubations.
Results

Demographic, Safety, and Tolerability Data. Eight male subjects (five white and one each American Indian, Asian, and African American) were enrolled and completed the study. Their mean age was 34.3 years (range: 22.0-54.0), and their mean BMI was 25.5 kg/m² (range: 19.8-29.6). No subject was withdrawn early from the study or experienced emesis. There were no deaths, other serious adverse events, discontinuations due to adverse events, or other significant adverse events reported during the study. Six subjects (75%) reported a total of 36 treatment-emergent adverse events, the majority of which were mild in severity. The exception was one event of treatment-related dizziness, which was moderate in severity. Most treatment-emergent adverse events (31/36, 86%) resolved within 1 to 3 days. Apart from the preferred terms “headache” and “flatulence,” which were both reported in three (37.5%) subjects, all other preferred terms were reported in only one subject each. Five subjects (62.5%) reported treatment-emergent adverse events that were assessed as related to the study treatment. There were no clinically significant changes from baseline in any laboratory values.

Mass Balance and Blood-to-Plasma Profiles. The mean cumulative recovery of total radioactivity (as percentage of dose) in urine and feces after a single oral dose of 140-mg cabozantinib FBE containing 100 µCi \(^{14}\text{C}\)-cabozantinib to 8 healthy male volunteers is represented graphically in Fig. 2. Mean (±SD) recovery of total radioactivity through 48 days postdose was 81.09 ± 1.56%; approximately two-thirds of total mean recovered radioactivity was eliminated in feces (53.79 ± 4.52%) and the remaining approximate one-third in urine (27.29 ± 4.65%). Less than 1% of total mean radioactivity was recovered in feces and urine after Day 28 postdose.
The mean concentration-time profiles of total radioactivity in plasma and whole blood and of parent cabozantinib in plasma (determined by LC-MS/MS) are shown in Fig. 3. Peak (mean C_{max}) radioactivity in plasma (2000 ngEq/mL) and whole blood (1200 ngEq/mL), and peak cabozantinib plasma concentration (1147 ng/mL) was achieved at approximately 2 hours postdose. The mean percentage total radioactivity concentration present in erythrocytes relative to whole blood (range: 0.174 ± 4.51% to 12.3 ± 3.71%) through 72 hours postdose indicates that radioactivity was present primarily in plasma and not markedly associated with red blood cells. At approximately 24 hours postdose, a second peak was evident for total plasma radioactivity (1514 ngEq/mL) and was larger than a corresponding second peak for parent cabozantinib (598 ng/mL), suggesting significant metabolism and delayed absorption or enterohepatic recirculation of parent cabozantinib.

**Metabolite Profiling.** Metabolites of cabozantinib present in plasma, urine, and feces after a single oral dose of 140-mg cabozantinib FBE containing 100 µCi ^14C-cabozantinib were identified using LC-MS/MS/RFD and high-resolution MS. Proposed metabolite structures were based on LC-MS/MS fragmentation patterns and, in the case of major metabolites EXEL-1646, EXEL-5162, EXEL-5366, and EXEL-1644, confirmed using authentic metabolite standards. Structures of the 17 identified metabolites and a proposed scheme for the biotransformation pathways of cabozantinib in humans are shown in Fig. 4.

Cabozantinib may undergo hydroxylation on the fluorophenyl moiety to form M16, which further undergoes sulfation to form M9 (EXEL-1646; cabozantinib monohydroxy sulfate), or M13, an isomer of M16. Oxidation of cabozantinib at a position not on the fluorophenyl moiety results in the formation of M17. Oxidation at the nitrogen of the quinoline moiety results in the formation of M19 (EXEL-5162; cabozantinib N-oxide). Hydrolysis at the amide bond of
cabozantinib results in M7 (EXEL-5366; cabozantinib amide cleavage product), which is further metabolized to form sulfate conjugate M2a (EXEL-1644; cabozantinib 6-desmethyl amide cleavage product sulfate), methylated metabolite M10, or oxidative product M14. Metabolites M5 and M6 arise from oxidative cleavage of a C-O bond followed by glucuronidation and sulfation, respectively. O-demethylation results in the formation of M15, which undergoes glucuronidation to form M3 and M8.

**Structural Characterization of Cabozantinib and its Metabolites.** The retention time of cabozantinib was 19.1 min and had a protonated molecular ion at \( m/z \) 502. The collision-induced dissociation (CID) product ion spectrum of \( m/z \) 502 (Fig. 5) showed prominent fragment ions at \( m/z \) 391, which was formed by the cleavage of amide C-N bond adjacent to the fluorophenyl group, and at \( m/z \) 323 and 297, which were formed by the cleavage of C-C and C-N bonds from another amide group.

The retention times of M2a and M2b were 10.0 and 10.7 min, respectively. The deprotonated molecular ion of M2a and M2b was at \( m/z \) 473. Figure 6 shows that the CID product ion spectrum of \( m/z \) 473 displayed prominent ions at \( m/z \) 393 formed by the loss of \( \text{SO}_3 \) (80 Da), indicative of a sulfate conjugate, and at \( m/z \) 429 (loss of \( \text{CO}_2 \), 44 Da) and 349 (loss of \( \text{SO}_3 \) and \( \text{CO}_2 \), 124 Da). Metabolite M2a was assigned as cabozantinib 6-desmethyl amide cleavage product sulfate (EXEL-1644) by comparison with authentic reference standard. M2b was assigned as cabozantinib 7-desmethyl amide cleavage product sulfate.

The retention time of M5 was 13.0 min and had a deprotonated molecular ion at \( m/z \) 489. The accurate mass of the deprotonated molecular ion of M5 was 489.13092, which matched the theoretical mass (489.13148 Da, mass difference, -1.14 ppm) for the glucuronide conjugate of
desquinolinyl cabozantinib having molecular formula $\text{C}_{23}\text{H}_{22}\text{FO}_9\text{N}_2$. The CID product ion spectrum of $m/z$ 489 showed prominent ions at $m/z$ 313, which was formed by the loss of a glucuronic acid moiety (176 Da), and $m/z$ 175, which was the glucuronic acid moiety. Further fragmentation of $m/z$ 313 by CID produced fragment ions at $m/z$ 134, 176, 178, and 202. Based on MS data, M5 was identified as desquinolinyl cabozantinib glucuronide.

The retention time of M6 was 13.6 min and had a deprotonated molecular ion at $m/z$ 393. The accurate mass of the deprotonated molecular ion of M6 was 393.05624, which matched the theoretical mass (393.05621 Da, mass difference, 0.08 ppm) for the sulfate conjugate of desquinolinyl cabozantinib having molecular formula $\text{C}_{17}\text{H}_{14}\text{FO}_6\text{N}_2$. The CID product ion spectrum of $m/z$ 393 showed a prominent fragment ion at $m/z$ 313, which was formed by the loss of $\text{SO}_3$ (80 Da). The CID product ion spectrum of $m/z$ 313 was the same as M5. Based on MS data, M6 was identified as desquinolinyl cabozantinib sulfate.

M7 with retention time of 13.4 min had a protonated molecular ion at $m/z$ 409. Its CID product ion spectrum (Fig. 7) displayed fragment ions at $m/z$ 391 (loss of $\text{H}_2\text{O}$, 18 Da), 365 (loss of $\text{CO}_2$, 44 Da), and 297 (cleavage of amide C-N bond). The retention time and mass spectra of M7 were the same as authentic standard of cabozantinib amide cleavage product (EXEL-5366).

M10 with retention time of 24.1 min had a protonated molecular ion at $m/z$ 423, 14 Da more than M7, suggesting that it is a methylation product of M7. The CID product ion spectrum of $m/z$ 423 showed prominent ions at $m/z$ 391, 323, and 297, which were the same fragment ions from cabozantinib. Based on MS data, M10 was assigned as methyl ester of M7.

M14 at 17.2 min had a deprotonated molecular ion at $m/z$ 423, 16 Da more than that of M7, suggesting that it is an oxidation product of M7. The CID production spectrum of $m/z$ 423
showed prominent ions at m/z 379 and 311, which were 16 Da more than the two corresponding fragments at m/z 363 and 295 from M7 in negative ESI mode. M14 was assigned as oxidation product of M7 with oxidation on the substituted quinolone moiety.

The retention time of M15 was 17.1 min and had a protonated molecular ion at m/z 488, 14 Da less than that of cabozantinib, suggesting that it is a demethylation product of cabozantinib. The CID product ion spectrum of m/z 488 showed prominent ions at m/z 377, 309, and 283, which were 14 Da less than the three corresponding fragment ions at m/z 391, 323, and 297 from cabozantinib. Based on MS data, M15 was identified as a desmethyl cabozantinib.

The retention times of M3 and M8 were 15.9 and 19.4 min, respectively, and had protonated molecular ions at m/z 664, 176 Da more than M15. The CID product ion spectra of m/z 664 from the two isomers were the same and showed a predominant fragment ion at m/z 488, which was formed by the loss of glucuronic acid moiety (176 Da). The CID product ion spectra of m/z 488 from M3 and M8 were the same as M15. Therefore, M3 and M8 were assigned as glucuronide conjugates of M15.

M9 with retention time of 15.3 min had the same retention time and mass spectra as authentic standard of cabozantinib monohydroxy sulfate (EXEL-1646). The protonated molecular ion of M9 was m/z 598; however, fragment ion m/z 518 formed from the loss of SO₃ was observed on the full scan mass spectrum due to in-source fragmentation under the experimental conditions. Figure 8 shows the CID product ion spectrum of m/z 518 from M9. The m/z 500 fragment was formed from m/z 518 by the loss of H₂O (18 Da); m/z 518 was not observed as it was fully fragmented under experimental conditions. The CID product ion spectrum of m/z 518 showed prominent ions at m/z 391, 323, and 297, which were the same fragment ions from cabozantinib.
M9 was identified as cabozantinib monohydroxy sulfate (with sulfate on the meta- position of fluorophenyl moiety) by comparison with the authentic reference standard.

The retention time of M16 was 17.7 min and had a protonated molecular ion at m/z 518, 16 Da more than cabozantinib, indicative of addition of one oxygen atom to cabozantinib. The CID product ion spectrum of M16 was the same as the CID product ion spectrum of m/z 518 from M9. Therefore, M16 was assigned as monohydroxy cabozantinib (EXEL-5526) with hydroxylation on the meta- position of fluorophenyl moiety.

The retention time of M13 was 16.5 min and had a protonated molecular ion at m/z 518, 16 Da more than cabozantinib, indicative of addition of one oxygen atom to cabozantinib. The CID product ion spectrum of M13 was similar to M16 and showed prominent ions at m/z 391, 323, and 297. M13 was an isomer of M16 and assigned as monohydroxy cabozantinib with hydroxylation on the ortho- position of fluorophenyl moiety.

Metabolite M12 had a retention time of 16.0 min. The protonated molecular ion m/z 598 was not observed on MS due to in-source fragmentation; instead, its fragment ion at m/z 518 was observed. The CID product ion spectrum of m/z 518 was the same as M13 and showed prominent ions at m/z 391, 323, and 297. Based on MS data, M12 was identified as a sulfate of M13.

M17, M18, and M19 have retention times of approximately 18.4, 20.5, and 23.3, respectively; and their protonated molecular ions were at m/z 518, 16 Da more than cabozantinib, indicative of the addition of one oxygen atom to cabozantinib. The CID product ion spectra of M17 and M18 displayed similar fragment ions at m/z 407, 339, and 313, which were 16 Da more than the three corresponding fragment ions at m/z 391, 323, and 297 from cabozantinib. These fragment ions
suggested that the oxidation occurred on the substituted quinoline moiety. M17 and M18 were assigned as monohydroxy cabozantinib with hydroxylation on the substituted quinoline moiety. The CID product ion spectrum of M19 (Fig. 9) displayed prominent fragment ions at \( m/z \) 407, 339, and 313, which were the same as metabolite M17 and M18. It also displayed fragment ions at \( m/z \) 390, 322, and 296, which were 17 Da less than \( m/z \) 407, 339, and 313. The retention time, molecular ion, and CID product ion spectrum of M19 were the same as the authentic standard of cabozantinib \( N \)-oxide (EXEL-5162). Therefore, M19 was identified as cabozantinib \( N \)-oxide.

**Radiochromatography.** Representative radiochromatograms show the major circulating \( ^{14}\text{C} \)-drug-related structures detected in human plasma at 8 hours (Fig 10A) and 24 hours (Fig 10B) postdose as parent cabozantinib and metabolites M9 (EXEL-1646) and M19 (EXEL-5162). However, by 72 hours postdose, metabolite M2a (EXEL-1644) was observed as the major circulating metabolite (Fig. 10C). Metabolite M7 (EXEL-5366) and an unidentified metabolite (P5) were minor metabolites; a mass spectrum of metabolite P5 could not be obtained due to low concentrations.

Hamilton pooled plasma samples from 6 subjects were prepared to measure the contribution of the individual major circulating \( ^{14}\text{C} \)-drug-related structures relative to total plasma radioactivity exposure. The individual plasma exposure (AUC\(_{0-168h}\)) value (mean ±SD) for cabozantinib (27.2 ± 6.9% of total plasma radioactivity) indicates that parent drug underwent extensive metabolism. Sulfate-conjugated metabolites EXEL-1646 (25.2 ± 3.9%) and EXEL-1644 (32.3 ± 10%) were the two predominant circulating metabolites, whereas nonconjugated metabolites EXEL-5162 and EXEL-5366 were present at much lower exposures (6.5 ±5.6% and 8.7 ± 4.7%, respectively, of total radioactivity). The contribution of a co-eluting peak (M8) to the EXEL-5366 exposure estimate was shown to be minimal.
Cabozantinib and its metabolites were measured by radiochromatography in pooled urine and fecal samples collected over 3 time periods (0-72, 144-192, and 288-336 hours) postdose. The major metabolites present in urine were M5 (dequinolinyl cabozantinib glucuronide), M6 (dequinolinyl cabozantinib sulfate), and M7 (EXEL-5366); no parent cabozantinib was detected in urine samples. The major components in feces were cabozantinib and metabolites M11 (structure unidentified), M15 (dimethyl cabozantinib), and M16 (cabozantinib oxidation B).

**Pharmacokinetics.** Cabozantinib and metabolites M7 (EXEL-5366), M19 (EXEL-5162), M9 (EXEL-1646), and pFA were measured in plasma samples from 6 healthy male subjects following a single oral administration of 140-mg cabozantinib FBE containing $^{14}$C-cabozantinib (100 $\mu$Ci) using a validated LC-MS/MS method; plasma concentrations of metabolite M2a (EXEL-1644) were measured using a qualified (non-validated) LC-MS/MS method. Plasma concentration profiles for cabozantinib and metabolites through 648 hours postdose are presented in Fig. 11. A summary of plasma pharmacokinetic parameters for cabozantinib and metabolites EXEL-5366, EXEL-5162, EXEL-1646, and EXEL-1644 is presented in Table 1. Plasma pFA concentrations were below the lower limit of quantitation (2 ng/mL) at all time points for all subjects.

Cabozantinib was rapidly absorbed after oral administration (mean $T_{\text{max}}$ approximately 1.49 hours) and slowly eliminated (102-hour mean estimated $t_{1/2}$). Metabolites EXEL-5366, EXEL-5162, and EXEL-1646 had peak plasma concentrations by 24 hours postdose and were also slowly eliminated ($t_{1/2}$ range: 86.0-91.8 hours). Plasma concentrations of EXEL-1644, the main circulating metabolite, increased steadily until 168 hours postdose and were followed by a slow elimination phase through 648 hours postdose. The terminal $t_{1/2}$ for EXEL-1644 could not be accurately calculated because the terminal elimination phase was not adequately characterized.
(ie, >40% of the AUC\textsubscript{0-inf} value was extrapolated). Mean exposure ratios for parent cabozantinib and each metabolite (EXEL-5366, EXEL-5162, EXEL-1646, and EXEL-1644) relative to total exposure (AUC\textsubscript{0-t} [each analyte]/AUC\textsubscript{0-t} [parent+4 measured metabolites]) were 32.4%, 3.09%, 4.90%, 13.8%, and 45.9%, respectively.

**Pharmacologic Activity Characterization of Cabozantinib and Its Major Metabolites.**

Cabozantinib and metabolites EXEL-5366 (amide cleavage product), EXEL-5162 (N-oxide), EXEL-1646 (monohydroxy sulfate), and EXEL-1644 (6-desmethyl amide cleavage product sulfate) were profiled in in vitro biochemical kinase inhibition assays. Cabozantinib showed generally broader and more potent kinase inhibition than did its metabolites (Table 2). Estimated IC\textsubscript{50} values for primary kinase targets MET, RET, and VEGFR2/KDR were markedly lower for parent cabozantinib (2 nM, 8 nM, and 14 nM, respectively) than for metabolites EXEL-1646 (199 nM, 234 nM, and 308 nM, respectively) and EXEL-5162 (190 nM, >1000 nM, and 140 nM, respectively). The metabolites EXEL-5366 and EXEL-1644 each showed minimal potency in this in vitro kinase inhibition panel (estimated IC\textsubscript{50} values for all kinases generally >1000 nM).

Cabozantinib and its two major metabolites EXEL-1646 and EXEL-1644 also showed minimal non-target specificity in a CEREP panel of 74 pharmacologic receptors, transporters, and enzymes; in this assay panel, cabozantinib had only one IC\textsubscript{50} value <1 µM (ie, adenosine A3 receptor IC\textsubscript{50} = 0.9 µM), whereas IC\textsubscript{50} values for EXEL-1646 and EXEL-1644 all exceeded 1 µM.

**Cabozantinib and EXEL-1644 CYP Isozyme Inhibition.** CYP isozyme inhibition kinetics determined using human hepatic microsomal incubations for EXEL-1644, the metabolite with the highest plasma exposure, are presented in Table 3; CYP inhibition data for parent cabozantinib are provided for reference (Nguyen et al, 2015). EXEL-1644 and cabozantinib are
both more potent direct inhibitors of CYP2C8 (Ki = 1.1 µM and 4.6 µM, respectively) than of CYP2C9 (Ki = 32.5 µM and 10.4 µM, respectively). EXEL-1644 showed no direct inhibition of the other CYPs evaluated (ie, IC₅₀ values were >100 µM for CYP1A2, CYP2B6, CYP2C19, CYP2D6, and CYP3A4/5 [both midazolam 1'-hydroxylase and testosterone 6β-hydroxylase]). In contrast, cabozantinib is a direct inhibitor of CYP2B6, CYP2C19, and CYP 3A4/5 (midazolam 1'-hydroxylase) with IC₅₀ values of 10.1, 40, and 272 µM, respectively; however, cabozantinib did not inhibit CYP1A2, CYP2D6, or CYP 3A4/5 (testosterone 6β-hydroxylase) with IC₅₀ values for each isozyme >100 µM. Neither cabozantinib nor EXEL-1644 was shown to be a metabolism-dependent inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5.

**Cabozantinib and EXEL-1644 Drug Transporter Interactions.** Results from the in vitro evaluation of cabozantinib and metabolite EXEL-1644 as potential substrates or inhibitors of drug transporters are presented in Table 4. Cabozantinib appears to be a substrate for transporter MRP2 only, whereas EXEL-1644 appears to be a substrate for several drug transporters (ie, OAT3, OATP1B1, OATP1B3, BCRP, and MRP2). Assay data were inconclusive as to whether EXEL-1644 is a P-gp substrate. Cabozantinib demonstrated inhibition of MATE1 and MATE2-K (estimated IC₅₀ values of 5.94 and 3.12 µM, respectively), but no marked inhibition of BCRP, BSEP, MRP2, and P-gp, (ie, IC₅₀ values >50 µM), or of OAT1, OAT3, OCT1, OATP1B1, and OATP1B3 (ie, IC₅₀ values >15 µM). EXEL-1644 demonstrated most potent inhibition of OAT1, OAT3, and OATP1B1 (IC₅₀ range: 4.3-6.1 µM), less potent inhibition of BSEP, MRP2, OATP1B3, MATE1, and MATE2-K (IC₅₀ range: 16.7-78.5 µM), and no marked inhibition of BCRP, P-gp, OCT1, and OCT2 (ie, IC₅₀ values exceeded the assay incubation solubility limit of 250 µM).
14C-Cabozantinib Elimination Pathways in Rats and Dogs. The elimination pathways of cabozantinib were characterized in BDI and BDC rats and dogs administered a single oral dose of 14C-cabozantinib. BDI (n=3) and BDC (n=6) rats received a single dose via gavage (5 mL/kg) of cabozantinib (50 mg/kg) containing 14C-cabozantinib (750 µCi/kg); BDI (n=3) and BDC (n=3) dogs received a single dose via gavage (5 mL/kg) of cabozantinib (100 mg/kg) containing 14C-cabozantinib (25 µCi/kg). The mean total (±SD) recoveries of radioactivity in feces, urine, and bile in rats (through 120 hours postdose) and dogs (through 168 hours postdose) are summarized in Table 5. The majority of the radioactivity was recovered in feces from BDI and BDC rats and dogs. Renal elimination was minimal, with a mean total of approximately 2% of the administered radioactivity recovered in urine from BDI rats and dogs. Mean recoveries of approximately 29% and 15% of the administered dose in bile from BDC rats and dogs, respectively, suggest that hepato-biliary excretion plays a predominant role in the elimination of absorbed 14C-cabozantinib-related radioactivity in both species. Unchanged 14C-cabozantinib was the major radioactive component detected in plasma and feces from rats and dogs; however, it was present at low levels in bile from both species. Unchanged 14C-cabozantinib accounted for only 0.2% of the radioactive dose in dog bile. The majority of the radioactive peaks in bile from both species were metabolites. Dog bile contained both non-conjugated and conjugated (methyl, cysteine, glucuronide, and sulfate) metabolites, all present at low levels (each <5% of the administered radioactive dose). A radiochromatographic peak associated with EXEL-1644 was not detected in plasma or bile samples from rats or dogs.

Toxicity Evaluation of EXEL-1644 in Rats. A 2-week repeat-dose toxicity study was conducted in rats to characterize the toxicity associated with EXEL-1644, the main circulating metabolite of cabozantinib in humans but non-detectable in plasma in rats and dogs administered
"14C-cabozantinib. Daily subcutaneous injections of EXEL-1644 at dose levels up to 30 mg/kg/day for 2 weeks to male and female Hsd:SD rats resulted in no mortality and no EXEL-1644-related adverse clinical observations, alterations of body weight or food consumption, ophthalmic changes, clinical pathology test results, organ weight changes, or microscopic findings consistent with systemic toxicity. Adverse effects without any consistent relationship to test article or dose level were limited to the dosing sites, with macroscopic findings (e.g., discoloration, scab, sore, and/or thickening) and correlative microscopic changes in subcutaneous injection sites (scapular and lumbar), including epidermal hyperplasia, ulceration, and exudate; dermal fibrosis; subcutis hemorrhage, necrosis, chronic inflammation, and fibrosis; and/or adnexal atrophy. Thus, the no-observable-adverse-effect-level for this study was considered to be 30 mg/kg/day, corresponding to mean C_max values of 104,000 ng/mL (approximately 220 µM) in males and 99,300 ng/mL (approximately 210 µM) in females on Day 15 of the dosing phase."
Discussion

The metabolism and excretion of cabozantinib and its metabolites was studied in 8 healthy male volunteers who received a single oral dose of 140-mg cabozantinib FBE containing 100 μCi $^{14}$C-cabozantinib. The mean recovery of total radioactivity was 81.09%, with radioactivity eliminated in feces (53.79%) and urine (27.29%). Total radioactivity eliminated primarily in feces has also been reported in mass balance studies of anticancer RTK inhibitors gefinitinb and erlotinib (Mckillop et al, 2004; Ling et al, 2006). Sample collection in our study was extended to 48 days postdose to maximize total $^{14}$C-cabozantinib-related radioactivity recovery; however, <1% of total mean radioactivity was ultimately recovered in feces and urine after Day 28 postdose. The total recovery of <90% of administered $^{14}$C-cabozantinib radioactive dose is consistent with reports for mass balance studies of other anticancer drugs with long plasma elimination $t_{1/2}$ (Bemer et al, 2006).

In the present study, the mean elimination $t_{1/2}$ measured for total radioactivity in plasma (269 hours) was longer than the plasma $t_{1/2}$ of cabozantinib (~102 hours); this observation may reflect radioactivity elimination associated with a metabolite with a longer plasma $t_{1/2}$ than cabozantinib. Sulfate conjugate EXEL-1644, the predominant circulating metabolite with a plasma exposure exceeding that of parent cabozantinib (46% vs 32% of total plasma exposure [AUC$_{0-t}$] to parent+4 major metabolites based on LC-MS/MS analysis), has an estimated plasma $t_{1/2} >$4-fold longer than cabozantinib. The high plasma exposure and long plasma $t_{1/2}$ for EXEL-1644 may reflect a slow intrinsic clearance and correlates with its high binding affinity for human plasma proteins (>99.99%) at clinically-relevant plasma concentrations.
The coincident second peaks observed in the plasma-time profiles for total radioactivity and for cabozantinib (measured by LC-MS/MS) at approximately 24 hours postdose following $^{14}\text{C}$-cabozantinib administration suggests that cabozantinib undergoes either enterohepatic recirculation or delayed intestinal absorption. Enterohepatic recirculation (rather than delayed absorption) would better account for the relatively high levels of $^{14}\text{C}$-cabozantinib present in feces at 1 week postdose, constituting up to 42% of the total radioactivity eliminated in feces from individual subjects over the 144-192 hour postdose collection, when measurable total radioactivity was still being eliminated in feces (mean 18.5% [individual range: 9-40%] of total 0-1080 hour postdose radioactivity eliminated in feces). Sulfate conjugate EXEL-1646 (M9), the only human metabolite present at high plasma concentrations and detected in feces with a structure capable of possible biotransformation back to parent cabozantinib, would not appear to be a likely source of enterohepatically recirculated cabozantinib. In vitro studies demonstrated that EXEL-1646 in the presence of sulfatase enzyme is converted back only to its nonconjugated monohydroxy precursor (M16; detected in feces only) and not to parent cabozantinib.

Studies in rats and dogs suggest that enterohepatic recirculation does not play a marked role in plasma pharmacokinetics of cabozantinib in either species. Although hepato-biliary excretion does appear to be the predominant elimination pathway of absorbed $^{14}\text{C}$-cabozantinib-related radioactivity in BDC rats and dogs, bile from both species contained only low levels of unchanged parent drug. Metabolism of cabozantinib appears to be markedly different in humans than in rats and dogs. Major metabolites represented $>65\%$ of total drug-related plasma exposure in healthy volunteers administered $^{14}\text{C}$-cabozantinib. In comparison, unchanged parent drug constitutes $>89\%$ of total drug-related plasma exposure in rats and dogs administered $^{14}\text{C}$-cabozantinib (CDER, 2012b); the major metabolites found in human plasma are also present at
low or negligible (EXEL-1644) plasma concentrations in both species based on LC-MS analysis (unpublished data). Thus, rat and dog models do not appear appropriate to evaluate the contribution of enterohepatic recirculation on cabozantinib plasma pharmacokinetics in humans.

Seventeen metabolites of cabozantinib were structurally identified based on analysis of plasma, fecal, and urine samples using LC-MS/MS/RFD. Major metabolites present in plasma with structures confirmed using authentic reference standards include sulfate conjugates M2a (EXEL-1644) and M9 (EXEL-1646), N-oxide M19 (EXEL-5162), and amide cleavage product M7 (EXEL-5366). Fecal samples contained cabozantinib, major metabolites EXEL-5366, EXEL-5162, and EXEL-1646, and multiple oxidative products including M13, M15, M16, M17, M18, and M19. Cabozantinib was not detected in urine, although metabolites dequinolinyl glucuronide conjugate M5, dequinolinyl sulfate conjugate M6, oxidative products M19 and M14, and amide bond hydrolysis product M7 were present. Extensive metabolism of cabozantinib in humans is also indicated by the approximately 3-fold greater plasma exposure (mean \( \text{AUC}_{0-t} \pm \text{SD} \)) for total radioactivity (259,000 ± 42,700 h·ngEq/mL) relative to that of parent cabozantinib determined by LC-MS/MS (67,200 ± 6,880 h·ng/mL). In addition, mean plasma exposure ratios for parent cabozantinib and major metabolites EXEL-5366, EXEL-5162, EXEL-1646, and EXEL-1644 relative to total drug-associated exposure (\( \text{AUC}_{0-t} \) [each analyte]/\( \text{AUC}_{0-t} \) [parent+4 measured metabolites]) determined by radioquantitation (27.2%, 8.7%, 6.5%, 25.2%, and 32.3%, respectively) generally correlated well with those determined by LC-MS/MS methods using authentic standards (32.4%, 3.09%, 4.90%, 13.8%, and 45.9%, respectively). Thus, the combined plasma exposure (\( \text{AUC}_{0-t} \)) associated with these four major metabolites (~67% of total) far exceeds that of parent cabozantinib in humans.
Cabozantinib appears to be the major pharmacologically active analyte, compared with its major metabolites, based on its relative plasma exposure (AUC₀₋₄/AUC₀₋₄ total) and on-target kinase inhibition potency. Compared to parent cabozantinib, metabolites EXEL-1646, EXEL-5162, and EXEL-5366 have 40-90% lower plasma exposures and at most 1/10⁰th of parent inhibition potency against on-target receptor kinases MET, RET, and VEGFR2/KDR. Whereas plasma exposure to EXEL-1644 is approximately 40% higher than parent, it possesses maximally 1/70⁰th of parent inhibition potency against these on-target kinases. Metabolite EXEL-1644 was present at low plasma exposures following cabozantinib dosing in rats and dogs, the nonclinical species used to evaluate cabozantinib toxicity. Therefore, additional evaluations were performed to better characterize the toxicologic properties of EXEL-1644. No systemic tissue toxicity resulted when EXEL-1644 was administered subcutaneously to rats for 2 weeks at >20-fold clinically relevant plasma exposures.

Drug interactions are an ongoing concern associated with clinical use of FDA-approved anticancer RTK inhibitors (for a recent review, see Thomas-Schoemann et al, 2013). Cabozantinib and EXEL-1644 both appear to represent minimal clinical risk of a DDI by inhibition of CYP isozymes. Cabozantinib and EXEL-1644 each most potently inhibited CYP2C8 (I/Ki = approximately 1.0 and 7.5, respectively); however, no effect was observed on the plasma pharmacokinetics of CYP2C8 probe substrate rosiglitazone at clinically relevant steady-state plasma exposures of cabozantinib and metabolite EXEL-1644 in subjects with solid tumors (Nguyen et al, 2015). Thus cabozantinib and EXEL-1644 would not be considered potential inhibitors of metabolism in vivo for substrates of CYP2C8 and other CYP isozymes with lower in vitro I/Ki values. The clinical risk of a drug transporter DDI is less clear. Cabozantinib and EXEL-1644 both have high plasma protein binding yielding low estimated free
fraction concentrations at clinically relevant steady-state plasma concentrations (approximately 13.8 nM and 0.8 nM, respectively, at 140 mg cabozantinib FBE daily). Per EMA and International Transporter Consortium decision tree criteria, neither compound reaches the threshold for conducting a clinical DDI transporter study based on drug transporter IC50 values and free fraction concentrations (EMA, 2012; Giacomini et al, 2010). In contrast, the FDA DDI Guidance (2012) utilizes a total (bound and free) steady-state drug Cmax value in its P-gp decision tree criteria (approximately 4.4 µM and 8.2 µM for cabozantinib and EXEL-1644, respectively; see Table 3 footnote c). An in vivo DDI study with a P-gp substrate would be recommended based on [I]/IC50 value (≥0.1) using the P-gp IC50 for cabozantinib determined previously in MDCK-MDR1 cells (IC50 = 7.0 µM; CDER, 2012a). The enhanced cytotoxicity of P-gp substrate anticancer drugs in human hepatoma HepG2 cell lines exposed to cabozantinib has also been associated with cabozantinib-mediated inhibition of P-gp efflux transporter activity (Xiang et al, 2014). Current drug labelling for cabozantinib (COMETRIQ) cautions against use of co-administered substrates of P-gp (COMETRIQ™ US Prescribing Information, 2012). The IC50 values for cabozantinib and EXEL-1644 for the other drug transporters evaluated do not reach FDA decision tree thresholds for conducting in vivo DDI studies. Cabozantinib and EXEL-1644 were both shown to be drug transporter substrates; thus, their plasma pharmacokinetics may also be affected by drugs that inhibit these transporters.

In summary, a mean of 81.09% of 14C-cabozantinib (100 µCi/subject) administered orally to 8 healthy male volunteers was recovered by 48 days postdose, and was eliminated primarily in feces (53.79%) and the remainder in urine (27.29%). No parent cabozantinib was detected in urine. Seventeen metabolites of cabozantinib were identified in feces, urine, and plasma matrices. Four major metabolites of cabozantinib, constituting approximately 70% of total
circulating drug exposure, were identified in plasma by quantitative radiochromatography and LC-MS/MS analyses. Sulfate conjugate EXEL-1644 was the main metabolite identified in plasma (representing 45.9% of total plasma drug exposure); metabolites EXEL-1646, EXEL-5162, and EXEL-5366 were present at lower relative plasma exposures (13.8%, 4.9%, and 3.1%, respectively). Parent cabozantinib appears to be the major pharmacologically active circulating analyte, based on its relative plasma exposure (32.4% of total plasma drug exposure) and generally broader and >10-fold greater on-target kinase inhibition potency compared with its major metabolites. Predominant circulating metabolite EXEL-1644 shows minimal on-target receptor kinase inhibition potency and appears to represent minimal risk of a clinically meaningful DDI by inhibition of CYP isozymes or drug transporters.
Acknowledgments

Becky Norquist provided medical writing support for this manuscript. Wentao Zhang was responsible for the conduct and interpretation for kinase inhibition experiments performed at Exelixis, Inc using AlphaScreen™ and luciferase-coupled chemiluminescence assay for EXEL-5162 and EXEL-5366.

Authorship Contributions

Participated in research design: Lacy, Hsu, Miles, Wang, Nguyen

Conducted experiments: Lacy, Aftab, Hsu, Wang, Nguyen

Contributed new reagents or analytic tools: Hsu, Wang

Performed data analysis: Lacy, Hsu, Miles, Wang, Nguyen

Wrote or contributed to the writing of the manuscript: Lacy, Hsu, Wang, Nguyen
References


Center for Drug Evaluation and Research (CDER) (2012b) Nonclinical pharmacology and toxicology review[s] for cabozantinib [COMETRIQ]. Available at: www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203756Orig1s000PharmR.pdf.


Footnotes
Funded by Exelixis, Inc.

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Figure Legends

Fig. 1. Chemical structure of $^{14}$C-cabozantinib (* indicates the position of $^{14}$C-radiolabel and $^{13}$C$_6$-label of internal standard) and four synthetic metabolites.

Fig. 2. Mean (±SD) cumulative excretion of total radioactivity (as percentage of dose) in urine and feces after a single oral dose of 140-mg cabozantinib free base equivalents (100 µCi $^{14}$C-cabozantinib) to healthy male volunteers (n=8).

Fig. 3. Mean (±SD) total radioactivity in blood and plasma and cabozantinib (XL184) plasma concentrations (measured by LC-MS/MS) vs. time (top [A]: 0-648 hours postdose; bottom [B]: 0-120 hours postdose) following a single oral dose of 140-mg cabozantinib free base equivalents (100 µCi $^{14}$C-cabozantinib) to healthy male volunteers (n=8).

Fig. 4. Proposed metabolic scheme for the biotransformation of $^{14}$C-cabozantinib in humans.

Fig. 5. The collision-induced dissociation (CID) product ion spectrum of m/z 502 from cabozantinib (XL184)

Fig. 6. The collision-induced dissociation (CID) product ion spectrum of m/z 473 for EXEL-1644 (metabolite M2a; cabozantinib 6-desmethyl amide cleavage product sulfate)

Fig. 7. The collision-induced dissociation (CID) product ion spectrum of m/z 409 for EXEL-5366 (metabolite M7; cabozantinib amide cleavage product)
Fig. 8. The collision-induced dissociation (CID) product ion spectrum of m/z 518 for EXEL-1646 (metabolite M9; cabozantinib monohydroxy sulfate)

Fig. 9. The collision-induced dissociation (CID) product ion spectrum of m/z 518 for EXEL-5162 (metabolite M19; cabozantinib N-oxide)

Fig. 10. Representative radiochromatograms of cabozantinib and metabolites in plasma at 8 [A], 24 [B], and 336 [C] hours after a single oral dose of 140-mg cabozantinib free base equivalents (100 µCi 14C-cabozantinib).

Fig. 11. Mean (±SD) plasma concentrations of cabozantinib (XL184) and 4 major metabolites following a single oral dose of 140-mg cabozantinib free base equivalents to healthy male volunteers (n=6) measured by LC-MS/MS (top [A]: 0-648 hours postdose; bottom [B]: 0-120 hours postdose).
TABLE 1. Summary (mean ± SD) of plasma pharmacokinetic parameters for cabozantinib and selected metabolites using LC-MS/MS following a single oral dose of 140-mg cabozantinib free base equivalents to healthy male volunteers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cabozantinib</th>
<th>EXEL-5366</th>
<th>EXEL-5162</th>
<th>EXEL-1646</th>
<th>EXEL-1644</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, ng/mL</td>
<td>1250 ± 238</td>
<td>52.9 ± 17.3</td>
<td>118 ± 33.7</td>
<td>236 ± 66.7</td>
<td>230 ± 91.2</td>
</tr>
<tr>
<td>$T_{\text{max}}$, h$^a$</td>
<td>1.49 (1.00, 3.00)</td>
<td>18.99 (5.00, 24.10)</td>
<td>13.50 (2.00, 24.30)</td>
<td>24.00 (3.00, 48.00)</td>
<td>168.00 (71.97, 240.00)</td>
</tr>
<tr>
<td>AUC$_{0-24}$, h·ng/mL</td>
<td>14300 ± 2600</td>
<td>1080 ± 341</td>
<td>2030 ± 682</td>
<td>3970 ± 1350</td>
<td>951 ± 377</td>
</tr>
<tr>
<td>AUC$_{0-72}$, h·ng/mL</td>
<td>35000 ± 6770</td>
<td>3120 ± 976</td>
<td>5610 ± 1940</td>
<td>12600 ± 4180</td>
<td>7530 ± 3200</td>
</tr>
<tr>
<td>AUC$_{0-t}$, h·ng/mL</td>
<td>67200 ± 6880</td>
<td>6540 ± 1680</td>
<td>10100 ± 3210</td>
<td>28900 ± 10700</td>
<td>99500 ± 34500</td>
</tr>
<tr>
<td>Ratio, %$^b$</td>
<td>NA</td>
<td>9.93 ± 3.20</td>
<td>15.0 ± 3.80</td>
<td>42.9 ± 14.4</td>
<td>150 ± 51.5</td>
</tr>
<tr>
<td>Ratio, %$^c$</td>
<td>32.4 ± 6.07</td>
<td>3.09 ± 0.689</td>
<td>4.90 ± 2.01</td>
<td>13.8 ± 5.63</td>
<td>45.9 ± 11.2</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$, h·ng/mL</td>
<td>68000 ± 6910</td>
<td>6770 ± 1700</td>
<td>10300 ± 3170</td>
<td>29500 ± 10600</td>
<td>NC$^d$</td>
</tr>
<tr>
<td>$k_{\text{el}}$, 1/h</td>
<td>0.00712 ± 0.00176</td>
<td>0.00807 ± 0.00218</td>
<td>0.00846 ± 0.00256</td>
<td>0.00859 ± 0.0022</td>
<td>NC$^d$</td>
</tr>
<tr>
<td>$t_{1/2}$, h</td>
<td>102 ± 23.3</td>
<td>91.8 ± 25.4</td>
<td>89.2 ± 29.2</td>
<td>86.0 ± 24.3</td>
<td>NC$^d$</td>
</tr>
</tbody>
</table>
NA, not applicable; NC, not calculated.

\(^a\) Median (range); \(^b\) ratio of \(\text{AUC}_{0-t}\) (metabolite)/\(\text{AUC}_{0-t}\) (parent); \(^c\) ratio of \(\text{AUC}_{0-t}\) (each analyte)/\(\text{AUC}_{0-t}\) (parent + 4 measured metabolites). \(^d\) value could not be accurately calculated based on incomplete characterization of terminal elimination phase (>40% extrapolation of \(\text{AUC}_{0-\inf}\)).
TABLE 2. Estimated IC₅₀ values (nM) for selected kinases for cabozantinib and metabolites EXEL-1646, EXEL-5162, EXEL-5366, and EXEL-1644

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Cabozantinib</th>
<th>EXEL-1646</th>
<th>EXEL-5162</th>
<th>EXEL-5366</th>
<th>EXEL-1644</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurora-A</td>
<td>381</td>
<td>152</td>
<td>ND</td>
<td>ND</td>
<td>1000</td>
</tr>
<tr>
<td>Aurora-B</td>
<td>23</td>
<td>52</td>
<td>&gt;3600</td>
<td>&gt;3600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>AXL</td>
<td>8</td>
<td>87</td>
<td>ND</td>
<td>ND</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>KIT</td>
<td>752</td>
<td>&gt;1,000</td>
<td>&gt;3600</td>
<td>&gt;3600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>EGFR</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>&gt;3600</td>
<td>&gt;3600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>FLT1</td>
<td>13</td>
<td>&gt;1,000</td>
<td>&gt;3600</td>
<td>&gt;3600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>FLT3</td>
<td>21</td>
<td>155</td>
<td>530</td>
<td>&gt;3600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>FLT4</td>
<td>3</td>
<td>175</td>
<td>ND</td>
<td>ND</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>KDR</td>
<td>14</td>
<td>308</td>
<td>140</td>
<td>&gt;10000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>MET</td>
<td>2</td>
<td>199</td>
<td>190</td>
<td>5000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>575</td>
<td>&gt;1,000</td>
<td>&gt;3600</td>
<td>&gt;3600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>RET</td>
<td>8</td>
<td>234</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>RON</td>
<td>46</td>
<td>&gt;1,000</td>
<td>ND</td>
<td>ND</td>
<td>&lt;1000⁺</td>
</tr>
<tr>
<td>TIE2</td>
<td>13</td>
<td>60</td>
<td>ND</td>
<td>ND</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

ND, not determined.

⁺ Residual activity 25% at 1 µM.
**TABLE 3.** Calculated kinetic and inhibition parameters of CYP isozyme activities by cabozantinib and metabolite EXEL-1644 in human hepatic microsomal incubations

<table>
<thead>
<tr>
<th>Activity Assay</th>
<th>Substrate a</th>
<th>Inhibition Model</th>
<th>IC₅₀ (µM)</th>
<th>Kiₚₚ (µM)</th>
<th>[I]/Kiₚₚ b, c</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8</td>
<td>Amodiaquine N-deethylase</td>
<td>Cabozantinib</td>
<td>Noncompetitive</td>
<td>3.8</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>EXEL-1644</td>
<td>Mixed-nonlinear</td>
<td>5.6</td>
<td>1.1</td>
<td>7.5</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac 4’-hydroxylase</td>
<td>Cabozantinib</td>
<td>Mixed</td>
<td>40</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>EXEL-1644</td>
<td>Mixed</td>
<td>31</td>
<td>32.5</td>
<td>0.2</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin 4’-hydroxylase</td>
<td>Cabozantinib</td>
<td>Mixed</td>
<td>59</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>EXEL-1644</td>
<td>ND</td>
<td>&gt;100</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam 1’-hydroxylase</td>
<td>Cabozantinib</td>
<td>Competitive</td>
<td>272</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>EXEL-1644</td>
<td>ND</td>
<td>&gt;100</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Kiₚₚ, apparent inhibition constant; ND, not determined.


b [I] = 4.4 µM (2220 ng/mL) steady-state plasma cabozantinib Cₘₐₓ at 140 mg cabozantinib daily in subjects with solid tumors (Kurzrock et al, 2011).
[I] = 8.2 µM (3920 ng/mL) is the estimated steady-state plasma EXEL-1644 C<sub>max</sub> based on a 4.1 µM (1960 ng/mL) EXEL-1644 plasma concentration determined at ≥100 mg cabozantinib daily in subjects with solid tumors at Day 22 (unpublished data) when plasma concentrations would be approximately half steady-state based on a conservative estimated EXEL-1644 half-life value (~500 hours).
TABLE 4. In vitro evaluation of cabozantinib and EXEL-1644 as substrates and inhibitors of selected drug transporters

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cabozantinib</th>
<th>EXEL-1644</th>
<th>Cabozantinib&lt;sup&gt;a,c&lt;/sup&gt;</th>
<th>EXEL-1644&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCRP</td>
<td>–</td>
<td>+</td>
<td>&gt;50</td>
<td>&gt;250</td>
</tr>
<tr>
<td>BSEP</td>
<td>–</td>
<td>–</td>
<td>&gt;50</td>
<td>50</td>
</tr>
<tr>
<td>MRP2</td>
<td>+</td>
<td>+</td>
<td>&gt;50</td>
<td>78.5</td>
</tr>
<tr>
<td>P-gp</td>
<td>–</td>
<td>+/-</td>
<td>&gt;50</td>
<td>&gt;250</td>
</tr>
<tr>
<td>OAT1</td>
<td>–</td>
<td>–</td>
<td>&gt;15</td>
<td>4.3</td>
</tr>
<tr>
<td>OAT3</td>
<td>–</td>
<td>+</td>
<td>&gt;15</td>
<td>4.3</td>
</tr>
<tr>
<td>OCT1</td>
<td>–</td>
<td>–</td>
<td>&gt;15</td>
<td>&gt;250</td>
</tr>
<tr>
<td>OCT2</td>
<td>–</td>
<td>–</td>
<td>&gt;15</td>
<td>&gt;250</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>–</td>
<td>+</td>
<td>&gt;15</td>
<td>6.1</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>–</td>
<td>+</td>
<td>&gt;15</td>
<td>20.6</td>
</tr>
<tr>
<td>MATE1</td>
<td>ND</td>
<td>ND</td>
<td>5.94</td>
<td>16.7</td>
</tr>
<tr>
<td>MATE2K</td>
<td>ND</td>
<td>ND</td>
<td>3.12</td>
<td>65.3</td>
</tr>
</tbody>
</table>

ND, not determined.

<sup>a</sup> Concentrations tested: 5 and 50 µM (BCRP, BSEP, MRP2, P-gp); 5 and 15 µM (OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3); <sup>b</sup> Concentration range tested: 1-250 µM; <sup>c</sup> Approximate limit of solubility in assay medium: 20 µM cabozantinib, 250 µM EXEL-1644.
TABLE 5. Mean total (±SD) recovery of radioactivity in feces, urine and bile from bile duct-intact (BDI) and bile duct-cannulated (BDC) rats and dogs administered a single oral dose of $^{14}$C-cabozantinib

<table>
<thead>
<tr>
<th>Species</th>
<th>Groupa</th>
<th>% Total Recovery</th>
<th>% Dose in Feces</th>
<th>% Dose in Urine</th>
<th>% Dose in Bile</th>
<th>% Dose in Cage</th>
<th>Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>BDI</td>
<td>92.5 ± 1.30</td>
<td>84.6 ± 4.65</td>
<td>2.44 ± 0.261</td>
<td>NA</td>
<td>0.880 ± 0.203</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BDC</td>
<td>91.6 ± 1.89</td>
<td>45.8 ± 6.95</td>
<td>7.44 ± 4.63</td>
<td>29.1 ± 5.48</td>
<td>1.69 ± 1.06</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>BDI</td>
<td>92.6 ± 1.08</td>
<td>86.2 ± 4.37</td>
<td>1.88 ± 1.39</td>
<td>NA</td>
<td>1.91 ± 1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BDC</td>
<td>91.7 ± 4.29</td>
<td>73.3 ± 4.96</td>
<td>0.679 ± 0.264</td>
<td>14.5 ± 7.71</td>
<td>1.10 ± 1.28</td>
<td></td>
</tr>
</tbody>
</table>

NA, not applicable.

a BDI (n=3) and BDC (n=6) rats received a single dose via gavage (5 mL/kg) of cabozantinib (50 mg/kg) containing $^{14}$C-cabozantinib (750 µCi/kg); BDI (n=3) and BDC (n=3) dogs received a single dose via gavage (5 mL/kg) of cabozantinib (100 mg/kg) containing $^{14}$C-cabozantinib (25 µCi/kg); data reflect mean (±SD) radioactivity recoveries in matrices through 120 hours (rats) and 168 hours (dogs) postdose.
Figure 1

[Chemical structures of Cabozantinib (XL184), M9, M19, M7, and M2a]

M9: XL184 Monohydroxy Sulfate (EXEL-04621646; EXEL-1646)

M19: XL184 N-Oxide (EXEL-04625162; EXEL-5162)

M7: XL184 Amide Cleavage Product (EXEL-04615366; EXEL536)

M2a: XL184 6-Desmethyl Amide Cleavage Product Sulfate (EXEL-04621644; EXEL-1644)
Figure 2
Figure 3

A 0-648 hours

B 0-120 hours

Radioactivity in Plasma
Radioactivity in Whole Blood
XL184 (by LC/MS/MS method)
Figure 4

GA: glucuronic acid moiety
* M2b, XL184 7-desmethyl amide cleavage product sulfate, is an isomer of M2a.
Figure 6

201202230Shamanmet #772-789  RT: 11.41-11.65  AV: S  NL: 2.26E2
F: ITMS - c ESI Full ms2 473.00@cid30.00 [130.00-600.00]
Figure 7
Figure 8

The figure shows a mass spectrum with the following key points:

- RT: 15.19-15.48
- AV: 11
- NL: 2.08E5
- F: ITMS + c ESI Full ms2 518.00@25.00 [140.00-600.00]

The mass spectrum peaks are at:
- m/z 391.2
- m/z 323.2
- m/z 297.2
- m/z 347.2
- m/z 250.3

The compounds represented in the spectrum are indicated by chemical structures.
Figure 9
Figure 10

A 8 hours

B 24 hours

C 336 hours
Figure 11

A

0-648 hours

Concentration (ng/mL)

Time (Hour)

0 100 200 300 400 500 600 700

0 100 1000 10000

XL184
EXEL-5366
EXEL-5162
EXEL-1646
EXEL-1644

B

0-120 hours

Concentration (ng/mL)

Time (Hour)

0 20 40 60 80 100 120

0 10 100

XL184
EXEL-5366
EXEL-5162
EXEL-1646
EXEL-1644