Pharmacokinetics and Metabolism in Rats, Dogs and Monkeys of the Cathepsin K Inhibitor Odanacatib: Demethylation of a Methylsulfonyl Moiety as a Major Metabolic Pathway

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Running Title: Pharmacokinetics and Metabolism of Odanacatib

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Number of text pages: 23
Number of tables: 5
Number of figures: 8
Number of references: 16
Number of words in Abstract: 243
Number of words in Introduction: 302
Number of words in Discussion: 895

Abbreviations: MK-0822, N-1-(1-cyanocyclopropyl)-4-fluoro-N-{(1S)-2,2,2-trifluoro-1-[4'-(methylsulfonyl)biphenyl-4-yl]ethyl}-L-leucinamide; MTBE, methyl tert-butyl ether; PEG, polyethylene glycol; LSC, liquid scintillation counting; CNS, central nervous system
ABSTRACT

Odanacatib is a potent cathepsin K inhibitor that is being developed as a novel therapy for osteoporosis. The disposition and metabolism of odanacatib were evaluated in rats, dogs and rhesus monkeys after IV and oral administration of [\(^{14}\)C]odanacatib. Odanacatib was characterized by low systemic clearance in all species and by long plasma half-life in monkeys (18 hr) and dogs (64 hr). The oral bioavailability was dependent on the vehicle used and ranged from 18% (monkey) to ~100% (dog) at doses of 1-5 mg/kg, using non-aqueous vehicles. After IV and oral administration to intact rats and monkeys >90% of the dose was recovered mainly in the feces. Studies in bile-duct cannulated animals indicated biliary secretion was the major mode of elimination of radioactivity; odanacatib also underwent some intestinal secretion. In monkeys, odanacatib was almost completely eliminated by metabolism; metabolism also played a major role in the clearance of odanacatib in rats and dogs. The major metabolic pathways were: methyl hydroxylation (formation of M8 and its derivatives), methyl sulfone demethylation (formation of M4 and its derivative M5) and glutathione conjugation (formation of the cyclized cysteinyglycine adduct M6 following addition of glutathione to the nitrile group of odanacatib). The major metabolites in rats (M4, parent-14 Da and M5, oxygenated derivative of M4) were determined to arise from a novel pathway that involved oxidative demethylation of the methyisulfonyl moiety of odanacatib. Overall, odanacatib displayed species-dependent metabolism which at least in part explains the divergent plasma half-life observed.
Introduction

Osteoporosis, a disease in which bone resorption exceeds bone formation, is associated with a high fracture risk. Osteoporosis affects over 10 million individuals in the United States alone and is prevalent in postmenopausal women (Poole and Compston, 2006; US HHS, 2004 Report). Bone resorption involves the acidic dissolution of bone mineral and the proteolytic degradation of bone matrix, primarily of type I collagen. The most abundant collagenase in osteoclasts, the cells responsible for bone resorption, is cathepsin K, a cysteine protease active at acidic pH (Drake et al., 1996; Zhao et al., 2009).

Odanacatib is a cathepsin K inhibitor under development for the treatment of osteoporosis (Gauthier et al., 2008; Stoch et al., 2009). Odanacatib is orally active with an in vitro EC$_{50}$ of 0.2 nM, a selectivity of $\geq$ 300-fold against other known human cathepsins, and a half-life suitable for weekly dosing. Odanacatib prevents loss of bone mineral density (BMD) in ovariectomized rabbits (Pennypacker et al., 2010) and rhesus monkeys (Masarachia et al., submitted to J Bone Miner Res). Histomorphometric analyses in both species suggested that odanacatib treatment preserved normal bone biomechanical properties. In a dose-finding clinical study, odanacatib increased BMD and reduced biochemical markers of bone resorption in postmenopausal women over a period of 3 years (Eisman et al., 2010). Unlike other antiresorptive treatments, odanacatib only transiently inhibited the activity of markers of bone formation, which returned to baseline after approximately two years. The effects on biochemical markers (urinary NTx and serum CTx) were reversible after discontinuation of treatment. There were similar overall rates of adverse experiences between patients treated with odanacatib compared with placebo.
Understanding the metabolism and disposition of a new drug is an important element in its safety and efficacy evaluation. In the present report, the pharmacokinetics, metabolism, and routes of excretion of odanacatib in rats, dogs and monkeys are reported.
Materials and Methods

**Chemicals.** [14C]Odanacatib and authentic standards of metabolites M4, M5, M8 and the hydroxymethylsulfone derivative of odanacatib were synthesized at Merck Research Laboratories (Rahway, NJ and Merck-Frosst, Montreal, Canada; the synthetic methods for M4, M5 and M8 can be found in the supplemental data). To make the hydroxymethylsulfone derivative of odanacatib, the sodium sulfinate salt of the synthetic standard of M4 was reacted with aqueous formaldehyde (37%) and the product, hydroxymethylsulfone odanacatib, was then purified by preparative HPLC using an acidic mobile phase and was isolated as the trifluoroacetic acid salt. Stock solutions of the salt were prepared in methanol for studies conducted with this derivative of odanacatib.

The [14C]odanacatib (specific activity of 146.9 µCi/mg) was synthesized with carbon-14 distributed over the six carbons within the phenyl ring bearing the methylsulfonyl functional group (Figure 1). All other materials were of HPLC or analytical grade.

**Pharmacokinetic and Mass Balance Studies.** All animal studies were performed using protocols approved by the Merck Institutional Animal Care and Use Committee. Studies were conducted in male Sprague-Dawley rats (n=4 per route of administration) and male rhesus monkeys (n=4; IV first and then P.O. after a 1 week washout period) which were given an IV (1 mg/kg) or P.O. (5 mg/kg) dose of [14C]odanacatib. The IV dose was dissolved in DMSO at concentrations of 2.5 mg/mL (for rats) and 10 mg/mL (for monkeys), while the oral dose was dissolved in PEG400 (2.5 mg/mL) and suspended in Imwitor:Tween (1:1) [5 mg/mL] for dosing rats and monkeys, respectively. The average amount of radioactivity administered to each animal was 155.4 ± 9.1 µCi/kg (rat) and 8.2 ± 0.6 µCi/kg (monkey). Animals were fasted
overnight prior to dosing. In rats jugular cannulas were surgically implanted and were used for both administration of the IV dose and collection of blood. In monkeys the IV dose was administered via either the cephalic or saphenous vein and blood collection was via cannulas implanted in either the iliac or femoral artery. Beagle dogs (n=2) were also given an IV (2 mg/kg) and oral (1 mg/kg) dose of non-labeled odanacatib in a non-crossover fashion. The IV dosing solution was formulated in 60% PEG200 in water (2 mg/mL), while the drug was suspended in 10% PEG200 in methyl cellulose for oral dosing (0.2 mg/mL). An additional group of rats (10 mg/kg, n=2) and dogs (5 mg/kg, n=2) were dosed orally using 0.5% methyl cellulose in water as the vehicle. Plasma was prepared by centrifugation immediately after collection of blood samples. Plasma samples were then stored at -20°C until analysis. Urine (rat and monkey) was collected on dry ice, and urine and feces (rat and monkey) samples were stored at –20°C.

Quantitative Analysis of Odanacatib in Plasma. The concentration of odanacatib in rat and monkey plasma was determined by LC-MS/MS in the positive ion mode using the Turbo Ion Spray interface. Odanacatib and the internal standard (MK1) were isolated from plasma (50 μL) by liquid-liquid extraction using MTBE. The extract was evaporated (37°C) to dryness and the residue reconstituted with 40/60 acetonitrile/water containing 0.1 % formic acid (100 μL), and 5 μL aliquots were injected into the LC-MS/MS system. Analysis was performed on a Sciex API 4000 mass spectrometer interfaced to Perkin Elmer 200 Series pump. The column used was Phenomenex Prodigy ODS3 (2x50 mm, 5 μm) and the mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Quantitation was based on selected reaction monitoring of the following precursor/product ion pairs: m/z 526.1→313.2 (odanacatib); m/z 482.2→313.2 (MK1). The lower limit of quantitation was 4 nM and 0.4 nM in rat and monkey
plasma, respectively. The assay linear over the concentration range of 0.4 to 950 nM and the extraction efficiency was 52-64% for both odanacatib and the internal standard. A slightly different method was used for quantitation of odanacatib in dog plasma. An aliquot of plasma (100 µL) containing the internal standard (MK2) was treated with acetonitrile (200 µL) and centrifuged. The supernatant (25 µL) was injected into the LC-MS/MS system and analyzed by positive ion electrospray ionization MS/MS. The lower limit of quantitation was 19 nM.

Calculation of Pharmacokinetic Parameters. Pharmacokinetic parameters were calculated by established noncompartmental methods. The area under the plasma concentration versus time curve (AUC) was determined using Watson® with linear trapezoidal interpolation in the ascending slope and logarithmic trapezoidal interpolation in the descending slope. For rats, the oral bioavailability was calculated by comparing the individual oral AUC to the mean IV AUC. In the case of monkeys, the bioavailability was calculated by comparing each animal's IV and oral AUC. The dog data was obtained using the mean IV and oral AUCs.

Tissue Distribution Study in Rats. The tissue distribution of radioactivity was determined after a single oral dose of [14C]odanacatib to male Long Evans rats (n=6) [conducted at Aptuit, Inc., Kansas City, Missouri]. The drug was formulated as a solution in PEG 400 at a concentration of 150 µCi/5 mg/2 mL/kg of animal. Animals (one animal per time point) were sacrificed at 2, 6, 24, 72, 168 and 672 hr postdose. Blood was collected and plasma was isolated at specified time points (cardiac puncture immediately prior to sacrifice) and the carcasses were frozen for autoradiographic analysis. The distribution of radioactivity was determined by quantitative whole-body autoradiography (QWBA) with storage phosphor imaging plates (radioluminography; thickness of tissue slice was 40 µm). Concentrations of drug-derived radioactivity were determined in blood, plasma, tissues, and bodily fluids to evaluate tissue
distribution and elimination of odanacatib-derived radioactivity. Whole blood and plasma were analyzed for total radioactivity by oxidation and/or liquid scintillation counting. The lower limit of quantitation for QWBA was determined to be 0.96 nCi/g (0.030 µg equiv/g).

Excretion and Metabolism Studies. The in vivo metabolism of odanacatib was evaluated in bile-duct cannulated rats (n=3), rhesus monkeys (n=2) and dogs (n=2) which were administered an IV dose of [14C]odanacatib. The target dose was 1 mg/kg (in DMSO) in a dose volume of 0.4 mL/kg (rat) and 0.1 mL/kg (monkey and dog). The average radioactivity dosed to rats, monkeys and dogs was, respectively, 145.0, 7.8 and 4.3 μCi/kg. Bile and urine samples were collected at various time intervals up to 72 and 96 hr postdose, respectively. Fecal samples were also collected for 96 hr (rat and monkey) and 168 hr (dog). The urine, bile and feces samples were stored at –20°C until analysis.

Preparation of Samples for HPLC-MS/Radiochromatography. Aliquots of urine sample were centrifuged to remove particulate matter and analyzed directly without further purification by HPLC-MS/MS in conjunction with on-line radioactivity detector (RAD). Aliquots of bile were diluted 1:1 with water, centrifuged and analyzed by HPLC-MS/MS-RAD directly. For plasma samples, protein was precipitated with 2 volumes of acetonitrile. The samples were then vortex-mixed and centrifuged, and the supernatant was dried to near dryness on a Turbovap evaporator at 37°C. The residue was reconstituted in 80:20 mobile phase A:B and then analyzed by HPLC-MS/MS-RAD. Feces extracts were prepared by weighing approximately 1 g of aqueous feces homogenate to a vial followed by the addition of acetonitrile (3.0 mL) [recovery of radioactivity was >85%]. The mixture was vortex-mixed and centrifuged, and the resulting supernatant was dried to near dryness on a Turbovap at 37°C. The residue was
dissolved in 250 uL of 80:20 mobile phase A:B and the sample was re-centrifuged to sediment any un-dissolved solid prior to HPLC-MS/MS-RAD analysis.

Radioactivity Measurement. Sample Preparation. Weighed aliquots of bile and urine samples were directly counted in Ultima Gold by liquid scintillation counting (LSC). Similarly, aliquots of plasma were counted directly in Ultima Gold. To each feces sample approximately 3 volumes of water/weight of feces was added and the samples were homogenized using a Omni homogenizer. The total weight of the homogenate was recorded and approximate 0.5 g aliquots were weighed into combustion cones and dried overnight in a laboratory hood. The samples were then combusted using a Packard Model 307 oxidizer. Liquid Scintillation Counting. Samples were counted by LSC on a Perkin Elmer (Packard) TriCarb® 2900 TR liquid scintillation counter using the transformed spectral index of the external standard (tSIE/AEC) as the quench indicating parameter with automatic efficiency correction. For samples other than oxidized feces an Ultima Gold quench curve was used, while for oxidized feces samples a Toluene quench curve was applied.

LC-MS/MS Radiochromatographic Analysis. HPLC analysis was conducted on a Hewlett-Packard HP1100 gradient system interfaced to Thermo Finnigan TSQ Quantum mass spectrometer. Separation was achieved on a Zorbax Rx C18 column (4.6 mm x 25 cm, 5 µm) using a mobile phase consisting of 10 mM aqueous ammonium acetate (solvent A) and 100% acetonitrile (solvent B) at a constant flow rate of 1.0 mL/min. The gradient was as follows: 0-5 min: 20% B, 30 min: 45% B, 40 min: 90% B, 50 min: 90% B. Mass spectral analyses were carried out using electrospray ionization (ESI) in the positive ion mode. Capillary temperature was 350°C and the ESI ionizing voltage was maintained at 5.0 kV for all analyses. Tandem mass spectrometry (MS/MS) was based on collision-induced dissociation (CID) of ions entering the rf-
only octapole region where argon was used as the collision gas at a pressure of 1.5 mtorr. MS and MS/MS experiments used to identify metabolites included: full Q1 scans, precursor ion, neutral loss and product ion scans. HPLC-MS chromatograms were compared to radioactivity profiles using a β-RAM (IN/US) radiochemical detector for HPLC. The HPLC effluent was split between the β-RAM detector and mass spectrometer at a ratio of 8 to 2. The β-RAM was operated in homogeneous liquid scintillation counting mode with a 600 μL flow cell and a scintillation cocktail (Ultima FloM or Tru-Count™) flow rate of 3 mL/min. The % area of each metabolite in the chromatogram was determined and the fraction of dose accounted for by each metabolite was then obtained by multiplying the percentage of radioactivity with the percentage of dose excreted in that sample.

The mechanism of formation of metabolite M4 was studied by performing chromatographic analysis of an authentic sample of the hydroxymethylsulfone derivative of odanacatib under acidic (0.1% formic acid) and pH 7.4 (100 mM phosphate buffer) conditions. To aliquots of a stock solution of hydroxymethylsulfone odanacatib in methanol either initial mobile phase [70:30 mobile phase A:B] (sample A) or phosphate buffer, pH 7.4 (sample B) was added to provide ~10 μM solutions of the compound and the samples were analyzed under the following HPLC conditions using the LC/MS system and conditions described above. The column used was a Zorbax Eclipse XDB C8 column (2.1 mm x 15 cm, 5 μm) and the flow rate was 0.4 mL/min. The mobile phase consisted of 0.1% aqueous formic acid (A) and acetonitrile containing 0.1% formic acid (B) and the gradient was as follows: 0-2 min: 30% B, 12 min: 60% B, 15 min, 80% B, 17 min, 80% B.
Results

**Pharmacokinetics.** The pharmacokinetic parameters of odanacatib in rats, dogs and monkeys following IV and oral administration are shown in Table 1. After an IV dose to rats, the mean values for plasma clearance (CLp), steady-state volume of distribution (Vdss) and terminal half-life (t1/2) were 2.0 mL/min/kg, 1.1 L/kg and 5.8 hr. The corresponding values for dogs were: 0.1 mL/min/kg, 0.7 L/kg and 64 hr. Monkeys displayed a CLp of 6.1 mL/min/kg, a Vdss of 1.6 L/kg and t1/2 of 18 hr. The absolute oral bioavailability was 43, 122 and 18% for rat, dog and monkey, respectively when the dose was prepared in PEG400/200 (rat and dog) and Imwitor/Tween (monkey). The bioavailability was only 8% (rat) and 6% (dog) when odanacatib was dosed as a suspension in 0.5% aqueous methyl cellulose.

**Excretion and Mass Balance.** Absorption and excretion of odanacatib-related radioactivity was studied after oral and IV administration of [14C]odanacatib to rats and rhesus monkeys with collection of excreta from 0 to 96 hr postdose. A recovery of >90 % of the administered dose was observed after both oral and IV administration in both species (Table 2). After both routes of administration the majority of drug related material was recovered in the feces (rat: 88-94 %; monkey: 81-87 %). Excretion studies carried out in bile duct-cannulated animals confirmed that biliary secretion is the primary mode of excretion of radioactivity in rats and monkeys (63% in rats and 71% in monkeys; Table 3). In addition, there was significant excretion of radioactivity into the feces after IV administration to bile duct-cannulated animals (27, 15, and 14% in rats, dogs and monkeys, respectively; Table 3). Renal excretion was a relatively minor pathway (<12% of dose) in all species. Consistent with the long half-life of odanacatib in dogs, excretion of radioactivity was slow. Only 34% of the dose was recovered in
72 hr and bile flow was rerouted to the GI tract after the 72 hr bile collection. An additional 11 day collection resulted in a total recovery of 76%.

**Tissue Distribution in Rats.** Following a single oral dose of [14C]odanacatib (5 mg/kg) to male pigmented rats, radioactivity was widely distributed to tissues by 2 hr postdose, at which time the majority of tissues reached maximum concentrations. The maximum observed plasma and blood concentration (2.3 and 1.4 µg equiv/g, respectively) of odanacatib-related radioactivity were measured at 2 hr postdose and declined thereafter to low levels at 168 hr postdose. With the exception of the ocular, CNS and reproductive tissues, tissue-to-plasma concentration ratios were greater than one in most tissues with measurable concentrations of radioactivity throughout the time course examined. High concentrations of radioactivity were associated with the alimentary canal contents, liver, adrenal and harderian glands. Concentrations of radioactivity in the CNS were low (3% of plasma). The bone-to-plasma concentration ratios were 0.09, 0.11 and 0.16 at 2, 6 and 24 hr postdose, respectively. By 168 hr, elimination was nearly complete, but measurable concentrations of radioactivity remained in four sampled tissues/regions (liver, non-pigmented skin, and small and large intestine contents). At the final sampling time of 672 hr, elimination was essentially complete indicating that odanacatib-related radioactivity is not selectively retained by melanin-containing tissues.

**Metabolite Profiles.** The identities of M4, M5 and M8 (the synthetic standard of M8 was a diastereomeric mixture not separable by the HPLC method used) were established by comparing the HPLC retention times and MS/MS spectra of the metabolites observed in bile/feces/urine with those obtained from authentic standards. The structures of the other metabolites were proposed based on full scan MS and product ion spectra (Table 4 and supplemental data).
Rats. The fraction of the radioactive dose accounted for by metabolites (59.5%) and the parent compound (19%) is shown in Table 5; based on this, the contribution of metabolism and excretion of intact parent to the clearance of an IV dose of odanacatib was estimated to be 76 and 24%, respectively. A representative radiochromatogram obtained from rat bile is shown in Figure 2 and the fraction of the dose accounted for by metabolites is shown in Table 5. The major metabolites in rats were M4 (sulfinic acid), M5 (sulfonic acid), M8 (hydroxylated [methyl] metabolite) and M6 (cyclized cysteinylglycine adduct of odanacatib) [Figure 1]. When a sample of synthetic hydroxymethylsulfone derivative of odanacatib was dissolved in phosphate buffer (pH 7.4) it was shown to decompose to M4 (Figure 4), indicating that hydroxymethylsulfone derivative is the precursor of this metabolite. This decomposition of hydroxymethylsulfone odanacatib upon dissolving in pH 7.4 buffer was instantaneous; on the other hand, it was very stable under acidic (0.1% formic acid) conditions (Figure 3). Other metabolites identified were M1-A and M1-B (MH+=526), M2-A and M2-B (MH+=556), M3 (the glucuronide of M8; β-glucuronidase hydrolysis of M3 gave rise to M8 [data not shown]) and M7 (presumably related to the aldehyde derivative of M8). M1-A and M1-B appear to be isomeric metabolites related to the oxygenated derivatives of M4. The form of these metabolites detected were the dehydrated derivatives (m/z 508). It was shown by manipulating the source temperature that these metabolites underwent MS source fragmentation to the dehydrated products (data not shown). M2-A and M2-B are likely to be the carboxylic acid derivatives of M8. It appears that the HPLC method was able to separate the diastereomers of M1 and M2 (unlike those of M8).

The concentrations of radioactivity and parent compound were determined in plasma of rats after an IV dose and selected plasma samples were profiled by HPLC-
MS/radiochromatography. The plasma concentration vs. time plots of odanacatib and radioactivity (Figure 5) indicate that radioactivity and the parent compound decline in parallel. Representative radiochromatograms obtained from rat plasma (Figures 6) show that the parent compound is the only radioactive peak detected. Consistent with this result, the AUC ratio of parent compound to radioactivity was 98%.

Monkeys. In monkeys, odanacatib underwent extensive metabolism as only trace levels of the parent compound were detected in bile, urine and feces (Figure 2; Table 5). The major metabolites in monkeys were the hydroxylated species M8 and its glucuronide M3 (Figure 2; Table 5). M1 and M7 were also were formed and represented 5.4 and 0.6% of the dose, respectively. In addition, several trace-level metabolites were detected (Table 5). The plasma concentration vs. time profiles of odanacatib and radioactivity are shown in Figure 7. The AUC ratio of parent compound to radioactivity was 54%; however, the parent compound was the only identifiable peak in radiochromatograms obtained from plasma (Figure 8).

Dogs. Because of the slow elimination of the drug in dogs, the fraction of the dose that was profiled was smaller compared to that of rats and monkeys. The metabolites identified in bile and feces were M4, M5 and M6 (Figure 2; Table 5). No metabolites related to the methyl hydroxylation pathway were detectable in dog excreta. The parent compound was also a significant component of the biliary and fecal radioactivity.
Discussion

The pharmacokinetics of odanacatib was evaluated in rats, dogs and monkeys after both IV and oral administration. Odanacatib proved to be a low clearance compound in rats and monkeys (5-20% of hepatic blood flow) and exhibited an extremely low clearance in dogs (<1% of hepatic blood flow). Consistent with the unusually low systemic clearance, the plasma elimination half-life in dogs was long (64 hr; Table 1) despite the modest volume of distribution (0.7 L/kg). The oral bioavailability of odanacatib was limited by the low aqueous solubility of the compound and was, thus, highly dependent on the vehicle used. It also appears that bioavailability is species-dependent with the compound showing complete absorption in the dog when dosed in a non-aqueous vehicle. Using non-aqueous vehicles, the bioavailability was 43, ~100 and 18% in rats, dogs and monkeys, respectively. The bioavailability was only 6% (dog) and 8% (rat) when the dose was suspended in an aqueous vehicle.

In a rat tissue distribution study odanacatib-related radioactivity was shown to undergo extensive tissue distribution, including to the bone; CNS, to which there was little drug distribution, was a notable exception. At the final sampling time of 28 day postdose, elimination was essentially complete indicating that odanacatib-related radioactivity is not selectively retained by any tissue. This contrasts sharply with the tissue distribution behavior of the bisphosphonate class of anti-resorptive osteoporosis drugs which show long bone residence in animals (half-life of 200-1000 days) and humans (half-life of >10 years) (Lin et al., 1991; Usui et al., 1995; Khan et al., 1997).

Odanacatib displayed good mass balance (>90%) following either IV or oral administration in rats and monkeys. After both routes of administration the majority of drug-related material was recovered in the feces (rat: 88-94%; monkey: 81-87%). The source of
most of the fecal radioactivity after IV administration was confirmed to be biliary secretion, indicating that the biliary route is an important mode of excretion of the radioactive dose. Renal excretion was a relatively minor pathway (<12% of dose) in all species. The appearance of a significant fraction of the dose in the feces (27, 15, and 14% in rats, dogs and monkeys, respectively) after IV administration to bile duct-cannulated animals indicates intestinal secretion plays an important role in the elimination of odanacatib and/or its metabolites. Since odanacatib is a good P-glycoprotein substrate in rats, mice and humans (unpublished observation) it is possible that odanacatib (most of the radioactivity in rat feces was composed of odanacatib) is subject to P-glycoprotein-mediated efflux into the intestinal lumen.

In monkeys, odanacatib was almost completely eliminated by metabolism; metabolism also played a major role in the clearance of odanacatib in rats, although excretion of unchanged parent compound was significant (~20% of the dose). In dogs, based on the limited data both metabolism and excretion of the parent compound appear to be important. Odanacatib displayed species-dependent biotransformation, with a different major metabolite in each species. Thus, in monkeys odanacatib is cleared principally via the methyl hydroxylation pathway, while methyl sulfone demethylation is not a significant pathway. On the other hand, the major metabolites in rats result from methyl sulfone demethylation. The hydroxylated metabolite M8 or its derivatives were not detectable in dogs and glutathione conjugation appeared to be a prominent pathway in dogs. Although it was difficult to determine an in vitro intrinsic clearance for any species because of the low turnover in in vitro systems, the absence of the methyl hydroxylation pathway -a major pathway in rats and monkeys- is likely to contribute to the unusually long plasma half-life of odanacatib in dogs.
The major pathway in rats (formation of M4 and M5) likely involves oxidative demethylation of the methyl sulfone moiety of odanacatib. Using a synthetic standard it was shown that the hydroxymethylsulfone derivative of odanacatib was the precursor of the sulfinic acid M4. The hydroxymethylsulfone was completely unstable at physiological pH, but was very stable under acidic conditions. Although the methyl sulfone moiety is a structural feature of many drugs (e.g. Chauret et al., 2001; Dean et al., 2007; Karanam et al., 2007), to date there is no report with respect to this kind of biotransformation and as such this represents a novel oxidative metabolism unprecedented in the literature. The cyclic cysteinylglycine conjugate M6 was detected as metabolite of odanacatib in all three species. This metabolite is presumably formed via reversible addition of glutathione to the nitrile group of odanacatib followed by gamma-glutamyltransferase-mediated removal of the glutamyl residue. The free N-terminus of cysteinylglycine would then allow rearrangement to the stable cyclic form. The synthetic preparation of analogous 5-membered ring 2-thiazolines via condensation of cysteine and other \( \beta \)-mercapto amino acids with nitriles in aqueous carbonate buffer has been previously described (Krimmer et al., 1987).

In summary, the studies described in this manuscript have collectively resulted in a good understanding of the metabolism and pharmacokinetic properties of odanacatib in a number of preclinical species. Odanacatib exhibited reasonable (albeit vehicle-dependent) oral bioavailability, low clearance and long plasma half-life (especially in dogs). The compound is eliminated largely via oxidative metabolism (methyl hydroxylation and methyl sulfone demethylation), although there is excretion of a significant amount of intact parent in rats and dogs. Finally, the disposition and metabolism of odanacatib rats, dogs and monkeys, species that
have been used in the safety evaluation of the compound, were representative of what was observed in humans (unpublished data).
Acknowledgements  We would like to acknowledge Matt Braun, Eric Soli and Nathan Yu (Merck Research Laboratories) for the synthesis of $^{14}$C]odanacatib and the hydroxymethyl sulfone derivative of odanacatib. We also thank Bart Emery (Merck Research Laboratories) for the plasma concentration data, Randall Press (Aptuit, Inc.) for the rat tissue distribution data, and Daniel Koeplinger for assistance with manuscript preparation.
Authorship Contributions

Participated in research design: Kassahun, Black, Nicoll-Griffith, Chauret, Day, and Koeplinger

Conducted experiments: McIntosh, Chauret, Day, and Koeplinger

Contributed new reagents or analytic tools: Chauret

Performed data analysis: Kassahun, McIntosh, Chauret, Day, and Koeplinger

Wrote or contributed to the writing of the manuscript: Kassahun, Black, Nicoll-Griffith, Chauret, Rosenberg, and Koeplinger
References


Legends to Figures

**Figure 1.** Proposed metabolic pathways of odanacatib in rats, dogs and monkeys. *Designates that the C-14 label is distributed over the 6 carbons in this phenyl ring with n=0-6 C-14 per molecule. RB=rat bile, RU=rat urine, RF= rat feces, DB=dog bile, DF=dog feces, MB=monkey bile, MU=monkey urine, MF=monkey feces.

**Figure 2.** Representative radiochromatograms obtained from extracts of bile of rats, dogs and monkeys administered an IV dose of $[^{14}C]$odanacatib.

**Figure 3.** LC-MS/MS data showing the stability of hydroxymethylsulfone odanacatib when the sample was prepared in a solvent containing 0.1% formic acid. The top, middle and bottom chromatograms represent total ion chromatogram, extracted ion chromatogram of m/z 512 and extracted ion chromatogram of m/z 542. The MS/MS spectrum of m/z 542 is shown at the bottom (m/z 542 and 512 represent hydroxymethylsulfone odanacatib and M4, respectively).

**Figure 4.** LC-MS/MS data showing the decomposition of hydroxymethylsulfone odanacatib to M4 when the sample was prepared in pH 7.4 phosphate buffer. The top, middle and bottom chromatograms represent total ion chromatogram, extracted ion chromatogram of m/z 512 and extracted ion chromatogram of m/z 542. The MS/MS spectrum of m/z 512 is shown at the bottom (m/z 542 and 512 represent hydroxymethylsulfone odanacatib and M4, respectively).

**Figure 5.** Mean plasma concentration-time curves of odanacatib and radioactivity following IV (1 mg/kg) administration of $[^{14}C]$odanacatib to rats. Data represent mean ± SD (n=4 animals).

**Figure 6.** A representative radiochromatogram of plasma extract (4 hr) following an IV dose of $[^{14}C]$odanacatib (1 mg/kg) to rats.
Figure 7. Mean plasma concentration-time curves of odanacatib and radioactivity following IV (1 mg/kg) administration of [14C]odanacatib to monkeys. Data represent mean ± SD (n=4 animals).

Figure 8. Radiochromatogram of plasma extract (4 hr pool from all animals) following an IV dose of [14C]odanacatib (1 mg/kg) to monkeys.
### TABLE 1

Pharmacokinetic parameters of odanacatib after IV administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>CLp (mL/min/kg)</th>
<th>Vdss (L/kg)</th>
<th>t1/2 (hr)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µM·hr)</th>
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<tbody>
<tr>
<td>Rat</td>
<td>1</td>
<td>2.0 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>5.8 ± 0.8</td>
<td>17 ± 4.3</td>
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<tr>
<td>Dog</td>
<td>2</td>
<td>0.7</td>
<td>64</td>
<td></td>
<td>520</td>
</tr>
<tr>
<td>Monkey</td>
<td>1</td>
<td>6.1 ± 1.0</td>
<td>1.6 ± 0.1</td>
<td>18 ± 4.3</td>
<td>5.3 ± 0.9</td>
</tr>
</tbody>
</table>

Data represent mean ± SD (n=4) for rat and monkey and a mean of 2 for dog.

Pharmacokinetic parameters of odanacatib after oral administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µM)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µM·hr)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>5</td>
<td>2.2 ± 0.4</td>
<td>1.8 ± 1.5</td>
<td>36 ± 10</td>
<td>43 ± 12</td>
</tr>
<tr>
<td>Dog</td>
<td>1</td>
<td>3.6</td>
<td>8</td>
<td>318</td>
<td>122</td>
</tr>
<tr>
<td>Monkey</td>
<td>5</td>
<td>0.3 ± 0.1</td>
<td>6.0 ± 2.3</td>
<td>4.8 ± 1.8</td>
<td>18 ± 3.8</td>
</tr>
</tbody>
</table>

Data represent mean ± SD (n=4) for rat and monkey and a mean of 2 for dog.

The dose was formulated in 100% PEG400, 60% PEG200 and 100% Imwitor:Tween (1:1) for rat, dog and monkey, respectively.
TABLE 2

Recovery of radioactivity (% of radioactive dose) in urine and feces following oral (5 mg/kg) and IV (1 mg/kg) administration of [14C]odanacatib to rats and monkeys

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route</td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td>Oral</td>
<td>2.9 ± 0.3</td>
<td>88 ± 9.2</td>
</tr>
<tr>
<td>IV</td>
<td>3.7 ± 1.6</td>
<td>94 ± 1.8</td>
</tr>
</tbody>
</table>

Data represent mean ± SD (n=4).
TABLE 3

Excretion of radioactivity (% of dose) in bile duct-cannulated rats, dogs and monkeys after administration of an IV dose (1 mg/kg) of [14C]odanacatib

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Rat&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dog&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Monkey&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile</td>
<td>Feces</td>
<td>Urine</td>
</tr>
<tr>
<td>0-24</td>
<td>41±3.4</td>
<td>9.1±1.7</td>
<td>7.0±1.8</td>
</tr>
<tr>
<td>24-48</td>
<td>17±1.7</td>
<td>11±3.3</td>
<td>2.1±1.3</td>
</tr>
<tr>
<td>48-72</td>
<td>5.3±1.6</td>
<td>4.9±3.2</td>
<td>0.9±0.9</td>
</tr>
<tr>
<td>72-96</td>
<td>NS</td>
<td>2.0±1.3</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>Total</td>
<td>63±2.8</td>
<td>27±7.0</td>
<td>10±3.7</td>
</tr>
<tr>
<td>Total</td>
<td>100±6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD (n=3)

<sup>b</sup> Data is a mean of n=2

<sup>c</sup> Data is a mean of n=2

<sup>d</sup> Because of slow excretion bile was rerouted to the GI tract after the 72 hr bile collection period and feces was collected for another 11 days after which 75% of the dose was recovered.

Rat data represent mean ± SD.

NS = no sample
TABLE 4

The main product ions obtained by performing MS/MS scans on the MH⁺ ions of odanacatib and its metabolites.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MH⁺&gt;MS/MS fragments¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1*</td>
<td>508&gt;299</td>
</tr>
<tr>
<td>M-2</td>
<td>556&gt;538,518,428,313</td>
</tr>
<tr>
<td>M-3</td>
<td>718&gt;542,460,432,313</td>
</tr>
<tr>
<td>M-4</td>
<td>512&gt;494,492,436,385,299,177</td>
</tr>
<tr>
<td>M-5</td>
<td>528&gt;508,452,315,177</td>
</tr>
<tr>
<td>M-6</td>
<td>687&gt;667,611,338</td>
</tr>
<tr>
<td>M-7</td>
<td>540&gt;520,430,313</td>
</tr>
<tr>
<td>M-8</td>
<td>542&gt;522,504,450,432,313</td>
</tr>
<tr>
<td>odanacatib</td>
<td>526&gt;506,450,416,313,177</td>
</tr>
</tbody>
</table>

¹MS/MS spectra available in supplemental data
²508 is not the MH⁺ ion, but (MH-H₂O)⁺
TABLE 5

Percent of radioactive dose accounted for by metabolites in bile, feces and urine following an IV dose (1 mg/kg) to bile duct-cannulated rats, dogs and monkeys

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Rat&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dog&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Monkey&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>3.1±0.8</td>
<td>ND</td>
<td>5.4</td>
</tr>
<tr>
<td>M2</td>
<td>0.1±0.2</td>
<td>ND</td>
<td>Tr</td>
</tr>
<tr>
<td>M3</td>
<td>6.7±1.1</td>
<td>ND</td>
<td>36</td>
</tr>
<tr>
<td>M4</td>
<td>22±4.1</td>
<td>2.2</td>
<td>Tr</td>
</tr>
<tr>
<td>M5</td>
<td>11±3.0</td>
<td>5.5</td>
<td>Tr</td>
</tr>
<tr>
<td>M6</td>
<td>4.6±2.2</td>
<td>8.7</td>
<td>Tr</td>
</tr>
<tr>
<td>M7</td>
<td>1.0±0.5</td>
<td>ND</td>
<td>0.6</td>
</tr>
<tr>
<td>M8</td>
<td>11±3.3</td>
<td>ND</td>
<td>32</td>
</tr>
<tr>
<td>odanacatib</td>
<td>19±7.4</td>
<td>5.7</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

<sup>a</sup> mean ± SD of n=3
<sup>b</sup> Two animals were studied; bile data is from one animal, while only the 24-48 fecal sample from both animals was profiled. Dog urine was not evaluated for metabolites.
<sup>c</sup> mean of n=2
ND = not detected
Tr = Trace
Figure 3

TIC of +Q1: 10 μM hydroxymethylsulfone odanacatib (acidic conditions)

XIC of +Q1: m/z = 512

XIC of +Q1: m/z = 542

MS/MS Spectrum of m/z = 542
Figure 4

TIC of +Q1: 10 μM hydroxymethylsulfone odanacatib (phosphate buffer pH 7.4)

XIC of +Q1: m/z = 512

XIC of +Q1: m/z = 542

MS/MS Spectrum of m/z = 512
Figure 5
Figure 7