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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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 intravenous and oral administration of [14C]odanacatib. Odanacatib was characterized by low systemic clearance in all species and by a long plasma half-life in monkeys (18 h) and dogs (64 h). The oral bioavailability was dependent on the vehicle used and ranged from 18% (monkey) to 100% (dog) at doses of 1 to 5 mg/kg, using nonaqueous vehicles. After intravenous 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 that 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 derivative, methyl sulfonyl demethylation (formation of M4 and its derivative M5), and glutathione conjugation (formation of the cyclized cysteinylglycine adduct M6 after 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 methylsulfonyl moiety of odanacatib. Overall, odanacatib displayed species-dependent metabolism, which explains, at least in part, 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 more than 10 million individuals in the United States alone and is prevalent in postmenopausal women (Poole and Compston, 2006; US Health and Human Services, 2004). 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 EC50 of 0.2 nM, a selectivity of ≥300-fold against other known human cathepsins, and a half-life suitable for weekly dosing. Odanacatib prevents loss of bone mineral density in ovariectomized rabbits (Pennybacker et al., 2011) and rhesus monkeys (Masarachia et al., 2007). Histomorphometric analyses in both species suggested that odanacatib treatment preserved normal bone biomechanical properties. In a dose-finding clinical study, odanacatib increased bone mineral density and reduced biochemical markers of bone resorption in postmenopausal women over a period of 3 years (Eisman et al., 2011). Unlike other antiresorptive treatments, odanacatib only transiently inhibited the activity of markers of bone formation, which returned to baseline after approximately 2 years. The effects on biochemical markers (urinary N-telopeptide and C-telopeptide cross-linked collagen type I) were reversible after discontinuation of treatment. There were similar overall rates of adverse experiences between patients treated with odanacatib compared with those treated 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, and M8 and the hydroxymethylsulfone derivative of odanacatib were synthesized at Merck Research Laboratories (Rahway, NJ) and Merck-Frosst (Montreal, QC, Canada; the synthesis methods for M4, M5, and M8 can be found at http://dmd.aspetjournals.org). HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS/MS, tandem mass spectrometry; AUC, area under the plasma concentration versus time curve; MS, mass spectrometry; RAD, radioactivity detector; CNS, central nervous system.

ABBREVIATIONS: MK-0822, N-1-(1-cyanocyclopropyl)-4-fluoro-N-[(1S)-2,2,2-trifluoro-1-[4-[(methylsulfonyl) biphenyl-4-yl]ethyl]-L-leucinamide; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS/MS, tandem mass spectrometry; AUC, area under the plasma concentration versus time curve; MS, mass spectrometry; RAD, radioactivity detector; CNS, central nervous system.
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. [14C]Odanacatib (specific activity of 146.9 Ci/mg) was synthesized with 14C distributed over the six carbons within the phenyl ring bearing the methylsulfonyl functional group (Fig. 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; intravenously first and then orally after a 1-week washout period), which were given an intravenous (1 mg/kg) or oral (5 mg/kg) dose of [14C]odanacatib. The intravenous dose was dissolved in dimethyl sulfoxide at concentrations of 2.5 mg/ml (for rats) and 10 mg/ml (for monkeys), and the oral dose was dissolved in PEG400 (2.5 mg/ml) and suspended in Imwitor-Tween 80 (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 before dosing. In rats, jugular cannulas were surgically implanted and were used for both administration of the intravenous dose and collection of blood. In monkeys, the intravenous 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 intravenous (2 mg/kg) and oral (1 mg/kg) dose of nonlabeled odanacatib in a non-crossover fashion. The intravenous dosing solution was formulated in 60% PEG200 in water (2 mg/ml), and 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) was 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.

**FIG. 1.** Proposed metabolic pathways of odanacatib in rats, dogs, and monkeys. *, Designates that the 14C label is distributed over the 6 carbons in this phenyl ring with n = 0 to 6 14C 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.
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 TurboSpray interface. Odanacatib and the internal standard (MK1) were isolated from plasma (50 μl) by liquid-liquid extraction using methyl tert-butyl ether. The extract was evaporated (37°C) to dryness, and the residue was 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 a 200 series pump (PerkinElmer Life and Analytical Sciences, Waltham, MA). The column used was a Prodigy ODS3 (250 mm, 5 μm; Phenomenex, Torrance, CA); 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); and m/z 482.2→313.2 (MK1). The lower limit of quantitation was 4 and 0.4 nM in rat and monkey plasma, respectively. The assay was linear over the concentration range of 0.4 to 950 nM, and the extraction efficiency was 52 to 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 after a single oral dose of [14C]odanacatib to male Long-Evans rats (n = 6) [conducted at Aptuit, Inc., Kansas City, MO]. The drug was formulated as a solution in PEG400 at a concentration of 150 μCi/5 mg/2 ml/kg animal. Animals (one animal per time point) were sacrificed at 2, 6, 24, 72, 168, and 672 h postdose. Blood was collected, plasma was isolated at specified time points (cardiac puncture immediately before sacrifice), and the carcasses were frozen for autoradiographic analysis. The distribution of radioactivity was determined by quantitative whole-body autoradiography 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 quantitative whole-body autoradiography was determined to be 0.96 nCi/g (0.030 μg Eq/g).

Excretion and Metabolism Studies. The in vivo metabolism of odanacatib was evaluated in bile duct-cannulated rats (n = 3), thymus monkeys (n = 2), and dogs (n = 2), which were administered an intravenous dose of [14C]odanacatib. The target dose was 1 mg/kg (in dimethyl sulfoxide) 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 h postdose, respectively. Fecal samples were also collected for 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, MO]. The drug was formulated as a solution in PEG400 at a concentration of 150 μCi/5 mg/2 ml/kg animal. Animals (one animal per time point) were sacrificed at 2, 6, 24, 72, 168, and 672 h postdose. Blood was collected, plasma was isolated at specified time points (cardiac puncture immediately before sacrifice), and the carcasses were frozen for autoradiographic analysis. The distribution of radioactivity was determined by quantitative whole-body autoradiography 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 quantitative whole-body autoradiography was determined to be 0.96 nCi/g (0.030 μg Eq/g).

TABLE 1
Pharmacokinetic parameters of odanacatib after intravenous administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Cmax</th>
<th>tmax</th>
<th>AUC0-∞</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>μM</td>
<td>h</td>
<td>μM·h</td>
</tr>
<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>
</tr>
<tr>
<td>Dog</td>
<td>2</td>
<td>0.1</td>
<td>0.7</td>
<td>64</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>
</tr>
</tbody>
</table>

TABLE 2
Pharmacokinetic parameters of odanacatib after oral administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Cmax</th>
<th>tmax</th>
<th>AUC0-∞</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>μM</td>
<td>h</td>
<td>μM·h</td>
</tr>
<tr>
<td>Rat</td>
<td>5</td>
<td>2.2 ± 0.4</td>
<td>1.8 ± 1.5</td>
<td>36 ± 10</td>
</tr>
<tr>
<td>Dog</td>
<td>1</td>
<td>3.6</td>
<td>8</td>
<td>318</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>
</tr>
</tbody>
</table>

TABLE 3
Recovery of radioactivity (% of radioactive dose) in urine and feces after oral (5 mg/kg) and intravenous (1 mg/kg) administration of [14C]odanacatib to rats and monkeys

<table>
<thead>
<tr>
<th>Route</th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>2.9 ± 0.3</td>
<td>88 ± 9.2</td>
<td>91 ± 9.2</td>
</tr>
<tr>
<td>Intra</td>
<td>3.7 ± 1.6</td>
<td>94 ± 1.8</td>
<td>98 ± 3.2</td>
</tr>
<tr>
<td>Monkey</td>
<td>5.8 ± 2.9</td>
<td>87 ± 6.0</td>
<td>93 ± 3.9</td>
</tr>
<tr>
<td>Intra</td>
<td>11 ± 3.0</td>
<td>81 ± 12</td>
<td>93 ± 12</td>
</tr>
</tbody>
</table>
performed using electrospray ionization in the positive ion mode. Capillary temperature was 350°C and the electrospray ionizing voltage was maintained at 5.0 kV for all analyses. MS/MS was based on collision-induced dissociation of ions entering the radiofrequency-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, precursor ion, neutral loss, and product ion scans. HPLC-MS chromatograms were compared with radioactivity profiles using a β-RAM (INUS Systems, Tampa, FL) radiochemical detector for HPLC. The HPLC effluent was split between the β-RAM detector and mass spectrometer at a ratio of 8:2. 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 percentage area of each metabolite in the chromatogram was determined, and the percentage of the dose accounted for by each metabolite was then obtained by multiplying the percentage of radioactivity by 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 using the LC/MS system and conditions described above under the following HPLC conditions. The column used was a Zorbax Eclipse XDB C8 column (2.1 mm × 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; and 17 min, 80% B.

**Results**

**Pharmacokinetics.** The pharmacokinetic parameters of odanacatib in rats, dogs, and monkeys after intravenous and oral administration are shown in Tables 1 and 2, respectively. After an intravenous 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, 1.1 l/kg, and 5.8 h. The corresponding values for dogs were 0.1 ml/min·kg−1, 0.7 l/kg, and 64 h. Monkeys displayed CLp of 6.1 ml/min·kg−1, Vdss of 1.6 l/kg, and t1/2 of 18 h. The absolute oral bioavailability was 43, 122, and 18% for rat, dog, and monkey, respectively, when the dose was prepared in PEG400 and PEG200 (rat and dog) and Imwitor-Tween 80 (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 were studied after oral and intravenous administration of [14C]odanacatib to rats and rhesus monkeys with collection of excreta from 0 to 96 h postdose. Recovery of >90% of the administered dose was observed after both oral and intravenous administration in both species (Table 3). After both routes of administration, the majority of drug-related material was recovered in the feces (rat, 88–94%; monkey, 81–87%). Excretion studies performed 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 4). In addition, there was significant excretion of radioactivity into the feces after intravenous administration to bile duct-cannulated animals (27, 15, and 14% in rats, dogs, and monkeys, respectively) (Table 4). 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 h, and bile flow was rerouted to the gastrointestinal tract after the 72-h bile collection. An additional 11-day collection resulted in total recovery of 76%.

**Tissue Distribution in Rats.** After a single oral dose of [14C]odanacatib (5 mg/kg) to male pigmented rats, radioactivity was widely distributed to tissues by 2-h postdose, at which time the majority of tissues reached maximum concentrations. The maximum observed plasma and blood concentration (2.3 and 1.4 μg Eq/g, respectively) of odanacatib-related radioactivity were measured at 2-h postdose and declined thereafter to low levels at 168-h postdose. With the exception of the

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**TABLE 4**

<table>
<thead>
<tr>
<th>Time</th>
<th>Bile</th>
<th>Feces</th>
<th>Urine</th>
<th>Bile</th>
<th>Feces</th>
<th>Urine</th>
<th>Bile</th>
<th>Feces</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24 h</td>
<td>41 ± 3.4</td>
<td>9.1 ± 1.7</td>
<td>7.0 ± 1.8</td>
<td>5.6</td>
<td>NS</td>
<td>0.7</td>
<td>69</td>
<td>6.7</td>
<td>8.1</td>
</tr>
<tr>
<td>24–48 h</td>
<td>17 ± 1.7</td>
<td>11 ± 3.3</td>
<td>2.1 ± 1.3</td>
<td>4.9</td>
<td>6.6</td>
<td>0.5</td>
<td>1.9</td>
<td>5.9</td>
<td>0.6</td>
</tr>
<tr>
<td>48–72 h</td>
<td>5.3 ± 1.6</td>
<td>4.9 ± 3.2</td>
<td>0.9 ± 0.9</td>
<td>5.9</td>
<td>8.7</td>
<td>0.6</td>
<td>0.2</td>
<td>1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>72–96 h</td>
<td>NS</td>
<td>2.0 ± 1.3</td>
<td>0.3 ± 0.2</td>
<td>16</td>
<td>15</td>
<td>34a</td>
<td>71</td>
<td>14</td>
<td>94</td>
</tr>
<tr>
<td>Total</td>
<td>63 ± 2.8</td>
<td>27 ± 7.0</td>
<td>10 ± 3.7</td>
<td>NS</td>
<td>NS</td>
<td>0.5</td>
<td>NS</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

NS, no sample.

a Data are the mean ± S.D. (n = 3).

b Data are a mean of n = 2.

c Because of slow excretion, bile was rerouted to the gastrointestinal tract after the 72-h bile collection period, and feces were collected for another 11 days after which 75% of the dose was recovered.

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**TABLE 5**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MH+ &gt; MS/MS Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>5088 &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>

a MS/MS spectra are available in the supplemental data.

b 508 is not the MH+ ion, but (MH3H2O)+.
ocular, central nervous system (CNS), and reproductive tissues, tissue/plasma concentration ratios were greater than 1 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 glands, and harderian glands. Concentrations of radioactivity in the CNS were low (3% of plasma). The bone/plasma concentration ratios were 0.09, 0.11, and 0.16 at 2, 6, and 24 h postdose, respectively. By 168 h, elimination was nearly complete, but measurable concentrations of radioactivity remained in four sampled tissues/regions (liver, nonpigmented skin, and small and large intestine contents). At the final sampling time of 672 h, 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, and urine with those obtained from authentic standards. The structures of the other metabolites were proposed on the basis of full-scan MS and product ion spectra (Table 5 and supplemental data).

### TABLE 6

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Rat#</th>
<th>Dog#</th>
<th>Monkey#</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>3.1 ± 0.8</td>
<td>N.D.</td>
<td>5.4</td>
</tr>
<tr>
<td>M2</td>
<td>0.1 ± 0.2</td>
<td>N.D.</td>
<td>Tr</td>
</tr>
<tr>
<td>M3</td>
<td>6.7 ± 1.1</td>
<td>N.D.</td>
<td>36</td>
</tr>
<tr>
<td>M4</td>
<td>22 ± 4.1</td>
<td>N.D.</td>
<td>2.2</td>
</tr>
<tr>
<td>M5</td>
<td>11 ± 3.0</td>
<td>N.D.</td>
<td>5.5</td>
</tr>
<tr>
<td>M6</td>
<td>4.6 ± 2.2</td>
<td>N.D.</td>
<td>8.7</td>
</tr>
<tr>
<td>M7</td>
<td>1.0 ± 0.5</td>
<td>N.D.</td>
<td>0.6</td>
</tr>
<tr>
<td>M8</td>
<td>11 ± 3.3</td>
<td>N.D.</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>

N.D., not detected; Tr, trace.

# Data are the mean ± S.D. of n = 3.

# Two animals were studied; bile data are from one animal, whereas only the 24- to 48-h fecal sample from both animals was profiled. Dog urine was not evaluated for metabolites.

# Data are mean of n = 2.

Rats. The fraction of the radioactive dose accounted for by metabolites (59.5%) and the parent compound (19%) is shown in Table 6; on the basis of this information, the contribution of metabolism and excretion of intact parent to the clearance of an intravenous dose of odanacatib was estimated to be 76 and 24%, respectively. A representative radiochromatogram obtained from rat bile is shown in Fig. 2, and the fraction of the dose accounted for by metabolites is shown in Table 6. The major metabolites in rats were M4 (sulfonic acid), M5 (sulfonic acid), M8 [hydroxylated (methyl) metabolite], and M6 (cyclic cysteinylglycine adduct of odanacatib) (Fig. 1). When a sample of the synthetic hydroxymethylsulfone derivative of odanacatib was dissolved in phosphate buffer (pH 7.4), it was shown to decompose to M4 (Fig. 3), indicating that the 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, hydroxymethylsulfone odanacatib was very stable under acidic (0.1% formic acid) conditions (Fig. 4). 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 forms 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 intravenous dose, and selected plasma samples were profiled by HPLC-MS/radiochromatography. The plasma concentration versus time plots of odanacatib and radioactivity (Fig. 5) indicate that radioactivity and the parent compound decline in parallel. Representative radiochromatograms obtained from rat plasma (Fig. 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 because only trace levels of the parent compound were
detected in bile, urine, and feces (Fig. 2; Table 6). The major metabolites in monkeys were the hydroxylated species M8 and its glucuronide M3 (Fig. 2; Table 6). M1 and M7 also were formed and represented 5.4 and 0.6% of the dose, respectively. In addition, several trace-level metabolites were detected (Table 6). The plasma concentration versus time profiles of odanacatib and radioactivity are shown in Fig. 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 (Fig. 8).

Dogs. Because of the slow elimination of the drug in dogs, the fraction of the dose that was profiled was smaller compared with that of rats and monkeys. The metabolites identified in bile and feces were M4, M5, and M6 (Fig. 2; Table 6). 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 intravenous 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 h) (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: the compound showed complete absorption in the dog when dosed in a nonaqueous vehicle. Using nonaqueous 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 days postdose, elimination was essentially complete, indicating that odanacatib-related radioactivity is not selectively retained by any tissue. This result contrasts sharply with the tissue distribution behavior of the bisphosphonate...
class of antiresorptive 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%) after either intravenous 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 intravenous 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 intravenous administration to bile duct-cannulated animals indicates that intestinal secretion plays an important role in the elimination of odanacatib and/or its metabolites. Because odanacatib is a good P-glycoprotein substrate in rats, mice, and humans (M. Yamazaki, unpublished observations), it is possible that odanacatib (most of the radioactivity in rat feces was composed of

![Graph showing mean plasma concentration-time curves of odanacatib and radioactivity after intravenous (1 mg/kg) administration of [14C]odanacatib to rats. Data represent the mean ± S.D. (n = 4 animals).](image1)

![Graph showing a representative radiochromatogram of plasma extract (4 h) after an intravenous dose of [14C]odanacatib (1 mg/kg) to rats.](image2)
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, on the basis of 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 primarily via the methyl hydroxylation pathway, whereas 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. 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) probably involves oxidative demethylation of the methyl sulfone moiety of odanacatib. With use of a synthetic standard it was shown that the hydroxymethylsulfone derivative of odanacatib was the precursor of the sulfonic 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, thus, this represents a novel oxidative metabolism not presented previously 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 γ-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 β-mercapto amino acids with nitriles in aqueous carbonate buffer has been described previously (Krimmer et al., 1987).

In summary, the studies described in this article 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 in rats, dogs, and monkeys, species that have been used in the safety evaluation of the compound, were representative of what was observed in humans (K. Kassahun, unpublished data).

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Authorship Contributions
Participants in research design: Kassahun, Black, Nicoll-Griffith, Chauret, Day, and Koeppler.
Conducted experiments: McIntosh, Chauret, Day, and Koeppler.
Contributed new reagents or analytic tools: Chauret.
Performed data analysis: Kassahun, McIntosh, Chauret, Day, and Koeppler.
Wrote or contributed to the writing of the manuscript: Kassahun, Black, Nicoll-Griffith, Chauret, Rosenberg, and Koeppler.

References


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Synthetic standards for M4 and M5 were prepared as described in Scheme 1. Ethyl 3-[(4-bromophenyl)thio]propionate 1 was converted to the corresponding boronate ester using the conditions of Giroux et al. Further coupling with arylbromide 3 provided the functionalized biaryl 4. Oxidation of the sulphide to the corresponding sulfone, followed by treatment with base to cleave the propionate group generated the sulfinic acid M4. Subsequent oxidation to the sulfonic acid provides M5.

A synthetic standard of the M8 metabolite was prepared as described in Scheme 2. Odanacatib was treated with BF3 etherate resulting in elimination of the tertiary fluoride to provide the isobutylene derivative 6. Dihydroxylation with osmium tetroxide provide 7 which was selectively acylated to provide 8. Treatment with DAST re-installed the tertiary fluoride atom. Subsequent cleavage of the acetate provided the desired hydroxyfluoroleucine derivative M8 as a 1.5:1 mixture of diastereomers by 1H NMR.

Scheme 1

Reagents and conditions: (a) bis(pinacolato) diboron, Pd2Cl2dppf, KOAc, DMF; (b) Pd2Cl2dppf, Na2CO3, DMF; (c) Na2WO4·2H2O, nBu4NHSO4, 30% H2O2, EtOAc; (d) NaOEt, THF/EtOH; (e) 30% H2O2, EtOH.
Scheme 2

Reagents and conditions: (a) BF₃·OEt₂, CHCl₃; (b) OsO₄, NMO, Acetone/H₂O; (c) Ac₂O, Et₃N, DMAP, CH₂Cl₂; (d) DAST, CH₂Cl₂; (e) K₂CO₃, MeOH.

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ODANACATIB PARENT DRUG
030902A03 #1427  RT: 36.50  AV: 1  NL: 2.79E6
F: + c Full ms2 526.00@-15.00 [ 150.00-600.00]
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-1
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-2

030903B01 #646  RT: 16.51  AV: 1  NL: 5.22E5
F: + c Full ms2 556.00@-15.00 [ 150.00-600.00]

![Graph showing MS/MS spectra of Odanacatib metabolites with peaks at m/z 313.1, 427.9, 518.0, 538.0, and 555.9.](image)
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-3

030903B01 #637-663 RT: 16.33-16.95 AV: 9 NL: 6.93E4
F: + c Full ms2 718.00@-15.00 [150.00-800.00]
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

HYDROXYMETHYL SULFONE-ODANACATIB
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-4

030903B01 #807-823 RT: 20.64-21.05 AV: 9 NL: 1.33E5
F: + c Full ms2 512.00@-15.00 [ 150.00-600.00]
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-5

030902A03 #827-839 RT: 21.18-21.43 AV: 6 NL: 5.10E4
F: + c Full ms2 528.00@:15.00 [ 150.00-600.00]
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-6

030903B07 #954  RT: 24.40  AV: 1  NL: 1.11E7
F: + c Full ms2 687.00@-25.00 [ 150.00-700.00]
METABOLITE M-7

030902A03 #1207-1226 RT: 30.88-31.34 AV: 7 NL: 9.98E4
F: + c Full ms2 540.00@-15.00 [ 150.00-600.00]

Relative Abundance

m/z

150 200 250 300 350 400 450 500 550 600

312.9 429.9 540.0 520.4
SUPPLEMENTAL DATA - MS/MS SPECTRA OF ODANACATIB METABOLITES

METABOLITE M-8

![MS/MS Spectrum of Odanacatib Metabolite M-8](image-url)