CTD 第 2 部

2.6　非臨床試験の概要文及び概要表

2.6.4　薬物動態試験の概要文

MSD 株式会社

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LIST OF ABBREVIATIONS, ACRONYMS AND DEFINITION OF TERMS

Abbreviation/Acronym	Definition
3TC	Lamivudine
ADME	Absorption, Distribution, Metabolism, Excretion
AhR	Arylhydrocarbon Receptor
AME	Absorption, Metabolism, Excretion
AUC	Area Under Curve
BAR	Bioanalytical Reports
BCRP	Breast Cancer Resistance Protein
BID	Twice per Day
BSEP	Bile Salt Export Pump
CAR	Constitutive Androstane Receptor
CCK-8	Cholecystokinin-8
CL_{int}	Intrinsic Clearance
CL_p	Plasma Clearance
C_{max}	Maximal Concentration
CYP	Cytochrome P450
DMSO	Dimethyl Sulfoxide
DOR	Doravirine
$E_2$17βG	Estradiol 17β-Glucuronide
EDTA	Ethylenediaminetetraacetic Acid
EM	Exposure Multiples
F%	Bioavailability
FDC	Fixed-Dose Combination
GD	Gestation Day
GLP	Good Laboratory Practice
HEK	Human Embryonic Kidney
HPMCAS	Hydroxypropyl Methylcellulose Acetate Succinate
HPC-DOSS	Hydroxypropylcellulose-SL (10% w:v) and Dioctyl Sulfosuccinate Sodium Salt (5% w:v) in Water
HRMS	High Resolution Mass Spectrometry
IC_{50}	Half-Maximal Inhibitory Concentration
IV	Intravenous
K_m	Substrate Concentration at Half Maximum Velocity
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LD	Lactation Day
LE	Long Evans
LLC-PK1	Lilly Laboratory Cell-Porcine Renal Epithelial Cells
LLOQ	Lower Limit of Quantification
LSC	Liquid Scintillation Counting
mAb	Monoclonal Antibody
MATE	Multidrug and Toxin Extrusion Protein
MC	Methylcellulose
MDCK-II	Madin-Darby Canine Kidney-II

MDR1	Multidrug Resistance Protein 1
mRNA	Messenger Ribonucleic Acid
MRP4	Multidrug Resistance-Associated Protein 4
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, Reduced
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
P_{app}	Apparent Permeability Coefficient
PEG-400	Polyethylene Glycol-400
P-gp	P-glycoprotein
PK	Pharmacokinetic
P.O.	Per os (by mouth)
PXR	Pregnane X Receptor
QWBA	Quantitative Whole-Body Autoradiography
rCYP	Recombinant CYP
SBP	Standard Bioanalytical Procedures
SD	Standard Deviation
SEM	Standard Error of the Mean
$T_{½}$	Half-Life
TA	Trace Amount
TCA	Taurocholic Acid
TDF	Tenofovir Disoproxil Fumarate
TEA	Tetraethylammonium
T_{max}	Time at Maximum Concentration
UDPGA	Uridine 5'-Diphosphoglucuronic Acid
UGT	UDP-Glucuronosyl Transferase
Vd_{ss}	Distribution Volume at Steady State
V_{max}	Maximum Reaction Velocity
VR	Validation Reports
WH	Wistar Hannover

1 BRIEF SUMMARY

1.1 Doravirine

Doravirine (DOR), also known as MK-1439, is an inhibitor of the HIV-1 reverse transcriptase for the treatment of HIV-1 infection. It is intended for administration with other anti-retroviral drugs or as part of a fixed-dose combination (FDC) regimen with lamivudine (3TC, 300 mg) and tenofovir disoproxil fumarate (TDF, 300 mg). The FDC, also known as MK-1439A is referred to as DOR/3TC/TDF in this submission. The absorption and disposition of DOR were studied in mice, rats, rabbits, and dogs, the species used in toxicology studies, and human. Protein binding and metabolism in preclinical species as well as humans are discussed for purposes of interspecies comparison between nonclinical models and humans. In addition, in vitro studies using human liver preparations, recombinant enzymes or cells transfected with human transporters were conducted to assess metabolic pathways and potential for drug interactions.

Tissue distribution, metabolism and excretion studies were conducted using [^{3}H]- or [^{14}C]-labeled DOR [Figure 2.6.4: 1]. To overcome solubility issues, all studies requiring intravenous (IV) administration were conducted with solutions of DOR in 1:3:1 dimethyl sulfoxide (DMSO):polyethylene glycol (PEG)-400:deionized water. Studies requiring oral administration were conducted using an enabled formulation that consisted of [redacted] drug on a hydroxypropyl methylcellulose acetate succinate (HPMCAS) polymer at [redacted]% drug load suspended in acidified 0.5% methylcellulose in deionized water. In vivo studies that required oral administration of radiolabeled DOR were conducted by solubilizing the drug in PEG-400 or 10% polysorbate 80. In support of chronic toxicology studies, several formulations were evaluated to maximize exposures of DOR at high doses (50 mg/kg or higher) in rats and dogs.

The pharmacokinetics of DOR in preclinical safety species were characterized by low plasma clearance (CL_p, 0.44 to 6. 05 mL/min/kg) and moderate steady state volume of distribution (Vd_{ss}, 0.9 to 2.7 L/kg), with half-life ($t_{½}$) values of 2.7, 6.4, 9, and 21.7 hr in mice, rats, rabbits, and dogs, respectively. DOR, administered in an enabled formulation, had moderate oral bioavailability (39-47%) in all four species. DOR displayed good permeability in vitro (P_{app}=25x10^{-6} cm/sec in LLC-PK1 cells), and this, in conjunction with its low clearance across preclinical species and humans, and less than dose proportional increases in exposure with dose, suggest that solubility-limited absorption is the major determinant of bioavailability.

Quantitative whole-body autoradiography (QWBA) studies conducted with [^{14}C]DOR in rats indicated that radioactivity distributed well across tissues, except the brain, where penetration was limited. The highest concentrations of radioactivity were observed in the alimentary canal, liver, and kidney, due to transit of the dose through the gastrointestinal tract and/or involvement in elimination. Doravirine did not display affinity for melanin. In gestating rats and rabbits, DOR was able to cross the placenta. Doravirine had moderate binding to plasma proteins from preclinical species and humans (unbound fraction approximately 0.24 across species) and did not distribute preferentially to blood cells.

Doravirine was eliminated primarily by oxidative metabolism in preclinical species and humans. Excretion of unchanged drug in urine was not a major pathway of elimination (2.6-to 19% of the dose in preclinical species compared to 6% in humans), while Phase II metabolism, including glucuronide and glutathione conjugation, was minor. In studies with bile duct-cannulated rats and dogs, biliary excretion of unchanged drug was not significant (5% of the dose or lower). In humans, although not determined, biliary excretion is not anticipated to be a significant elimination pathway based on data from the absorption, metabolism and excretion (AME) study, where the calculated systemic bioavailability was consistent with the dose recovered in feces as unchanged DOR. The major metabolic pathway in preclinical species and humans involved formation of M9 [Figure 2.6.4: 1], an oxidative metabolite resulting from CYP-mediated oxidation. In all species, except rat, in which M9 underwent significant glucuronidation, the majority of M9 was eliminated without further modification, predominantly in urine. At pharmacologically relevant DOR exposures, M9 was the major metabolite circulating in mice and humans but not rats, rabbits, or dogs, where M9 was present in trace amounts or not detected. Studies conducted to assess exposure of M9 in pivotal safety assessment studies indicated that exposures of M9 in rat and dog plasma after chronic DOR administration at the NOAEL doses of 450 and 1000 mg/kg/day were within two-fold of the exposures achieved in humans at the clinical dose of 100 mg DOR. The unbound fraction of M9 in rat and dog plasma was approximately 2.2 and 2.8-fold higher, respectively, than in human plasma, so that the unbound exposures to M9 in the chronic safety studies and humans at the clinical dose were approximately similar.

The structure of M9 [Figure 2.6.4: 1] was determined by NMR analysis to be a product of oxidation and subsequent re-arrangement of the triazolinone ring in DOR. Additional characterization of M9 indicated that it did not inhibit the wild-type HIV-1 reverse transcriptase or the most common mutants K103N, V106A, and Y181C (IC_{50} >8.4 μM) and displayed no off-target activity against a wide range of endogenous pharmacological targets.

Doravirine did not undergo appreciable metabolism in rat, dog, or human hepatocytes. In liver microsome preparations, supplementation with NADPH resulted in slow metabolism for all three species and M9 was the most abundant metabolite relative to other metabolites, which were observed only in trace amounts. Doravirine did not undergo glucuronidation in rat, dog, or human liver microsomes supplemented with UDPGA. Similar to DOR, M9 was not appreciably metabolized in dog and human hepatocytes. However, in rat hepatocytes, it was converted to a glucuronide (M7). These results, in conjunction with the observations that M7 was the most abundant metabolite in rat excreta, indicated that, as in the other species studied, formation of M9 is the primary route of metabolism in rat, even though M9 was not the most abundant metabolite.

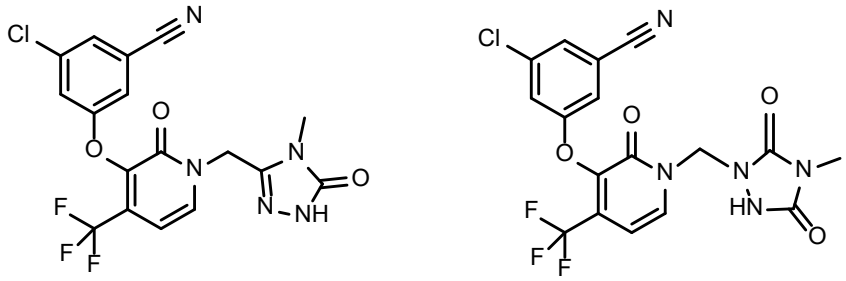
In vitro studies indicated that the metabolism of DOR in humans is catalyzed primarily by CYP3A enzymes. Recombinant CYP3A4 and CYP3A5 were the only enzymes capable of metabolizing DOR, and consistent with observations in the human AME study, the major metabolite generated by these enzymes was M9. In addition, anti-CYP3A inhibitory antibodies completely inhibited the formation of M9 by human liver microsomes. Kinetic studies with recombinant enzymes indicated a larger contribution of CYP3A4 to the metabolism of DOR because of its higher catalytic efficiency and relative abundance compared to CYP3A5. Based on the in vitro data, drug interactions are anticipated when

Doravirine is co-administered with CYP3A inducers or inhibitors, and results from clinical drug interaction studies conducted with CYP3A inhibitors and inducers are consistent with these data. Doravirine was also a substrate of human P-glycoprotein (P-gp) in vitro, and this characteristic is likely to limit penetration of DOR in brain at the low unbound concentrations (lower than1 µM) in plasma associated with the clinical dose of 100 mg, as observed in the tissue distribution study. However, P-gp is not anticipated to play a significant role in limiting oral absorption of DOR at the projected concentrations in the intestinal lumen, as the compound demonstrated good permeability in vitro. Furthermore, the minor role of renal excretion and the expectation (based on preclinical and human metabolism data) of minimal biliary excretion of unchanged drug, suggest that P-gp does not play a major role in the elimination of DOR.

Doravirine has low potential to cause drug interactions mediated via drug metabolizing enzymes or drug transporters as the mean maximal unbound concentrations (C_{max}) achieved at the clinical dose are not expected to exceed 1 µM. In vitro studies indicated that DOR was not an inhibitor of major CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) or UGT1A1 at concentrations up to 100 µM, and was not an inducer of CYP1A2 or 2B6. Doravirine caused a small increase in CYP3A4 mRNA at 10 and 20 µM with no increase in activity, suggesting a potential for weak induction. This was in line with results from a drug interaction study with midazolam, where multiple once-daily administration of 120 mg doses of DOR did not have a meaningful effect on the exposure of midazolam.

In addition, DOR was tested as an inhibitor of transporters in vitro; BSEP (no inhibition up to 50 µM), BCRP (IC_{50} 51 µM), P-gp (no inhibition up to 100 µM), OATP1B1 (IC_{50} 39 µM), OATP1B3 (IC_{50} 31 µM), OAT1 (IC_{50} above 75 µM), OAT3 (IC_{50} 16 µM), OCT2 (IC_{50} 67 µM), and MATE1 and MATE2K (IC_{50} above 50 µM). Based on the high IC_{50} values relative to an anticipated mean plasma unbound C_{max} lower than 1 µM, no clinically relevant interactions with substrates of these transporters are expected. Co-administration of DOR with atorvastatin (OATP1B1 substrate), TDF (Tenofovir, the prodrug hydrolysis product and major elimination product, is a substrate for OAT1 and OAT3), 3TC and metformin (OCT2 and MATE1/2K substrates) and dolutegravir (BCRP substrate) did not result in clinically meaningful effects on the pharmacokinetics of those drugs [Sec. 2.7.2.2.3]

Figure 2.6.4: 1 Structure of DOR (A) and M9 (B)



(A) (B)

1.2 Doravirine/Lamivudine/Tenofovir Disoproxil Fumarate

No preclinical pharmacokinetic studies were conducted with DOR/3TC/TDF. Absorption, distribution, metabolism and excretion (ADME) data for lamivudine and TDF were obtained from available regulatory approval documentation as well as prescribing information and scientific literature. A summary of relevant data for 3TC and TDF is provided below.

1.3 Lamivudine

Lamivudine (3TC) is a nucleoside analog that requires conversion to a triphosphate by intracellular kinases for activity. It had good (76-90%) oral bioavailability in preclinical species and it was excreted in urine by glomerular filtration and active tubular secretion. Metabolism plays a minor role in the elimination of 3TC in rats. In dogs, elimination of 3TC occurs via metabolism and renal excretion. Based on available clinical and preclinical data, 3TC is not likely to perpetrate drug interactions via CYP enzymes or transporters. In vitro data demonstrated that 3TC is a substrate of OCT2 and its pharmacokinetics may be affected by inhibitors of organic cation transporters in kidney. This interaction, however, appears to be of little clinical significance. Observed interactions with trimethoprim and zidovudine that resulted in increases in plasma exposures of 3TC of approximately 40% were considered not clinically relevant [Ref. 4.3: 04P95N], [Ref. 4.3: 04664N], [Ref. 4.3: 03RRT8], [Ref. 4.3: 03TKG7], [Ref. 4.3: 03RL6N], [Ref. 4.3: 03W0QN], [Ref. 4.3: 03W0QP], [Ref. 4.3: 03W0QQ], [Ref. 4.3: 03X0YB].

1.4 Tenofovir Disoproxil Fumarate

Tenofovir disoproxil fumarate (TDF) is a prodrug of the nucleotide analog tenofovir , which requires conversion to a diphosphate for antiviral activity. TDF is readily converted to tenofovir presystemically in preclinical species and humans and tenofovir is the major species circulating in plasma and the major product of elimination in rats, dogs, and humans following administration of TDF. Tenofovir is primarily excreted via glomerular filtration with a small contribution from active tubular secretion. In vitro, tenofovir is a substrate of OAT1, OAT3, and MRP4. The co-administration of products containing TDF with some protease inhibitors and hepatitis C antivirals has been cautioned as increased levels of tenofovir in plasma have been observed. Due to the association of tenofovir with renal toxicity, monitoring of renal function is recommended when co-administering TDF with drugs that impair renal function. [Ref. 4.3: 04P95P], [Ref. 4.3: 04N7D7], [Ref. 4.3: 0438FY], [Ref. 4.3: 0438G4], [Ref. 4.3: 043B7S], [Ref. 4.3: 043B7Z].

The pharmacokinetic and drug interaction profiles for the individual components of the FDC indicated that drug interactions between the individual components are not likely. Significant changes in the pharmacokinetics of the FDC components are only anticipated for DOR when the combination is co-administered with CYP3A inducers or inhibitors. Additionally, co-administration with drugs that impair renal function is cautioned as with other products containing TDF.

2 METHODS OF ANALYSIS

Bioanalytical methods using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) were used for the analysis of DOR in plasma, urine, muscle, and milk samples collected during preclinical studies. Sample analysis for GLP studies was conducted in compliance with GLP procedures. The bioanalytical methods used in support of GLP studies for the analysis of mouse, rat, rabbit, and dog, plasma, and rat milk were validated in accordance with regulatory guidances in effect at the time the studies were conducted. The lower limit of quantification was 12 ng/mL (0.0282 µM) and the upper limit of quantification was up to 4900 ng/mL (11.5 µM). In mouse plasma an LC-MS/MS assay with a lower limit of quantification around 4.00 ng/mL (0.00940 µM) and an upper limit of quantitation around 4800 ng/mL (11.3 µM) was also validated.

In some instances, a bioanalytical method, validated according to laboratory procedures for exploratory work, was used according to the study purpose to quantify DOR in rabbit plasma, rat urine, and rat muscle. These assays had a concentration range of 12 to 4900 ng/mL in rabbit plasma, 25 (0.0564 µM) to 10,000 ng/mL (23.5 µM) in rat urine, and 400 ng/mL (0.940 µM) to 200,000 ng/mL (470 µM) in rat muscle.

The accuracy and precision of the assays in the various matrices are presented in [Sec. 2.6.5.2]. The LC-MS/MS methods used for the quantitation of DOR in various studies are summarized in [Table 2.6.4: 1] and are described in the Standard Bioanalytical Procedures (SBP), Validation Reports (VR), and Bioanalytical Reports (BAR) included in Module 4 of this submission.

For metabolism and excretion studies, total radioactivity in plasma, blood, urine, and bile was determined by liquid scintillation counting (LSC). Metabolites in plasma, bile, and urine were separated using a reverse-phase HPLC column with a mobile phase consisting of 0.1% formic acid in water and acetonitrile. DOR and its metabolites were detected by high-resolution mass spectrometry (HRMS) and on-line radiometric detection. The proposed identity of the metabolites was based on exact mass or MS/MS fragmentation patterns. Plasma protein binding was determined by equilibrium dialysis with quantification by LSC or LC-MS/MS. The structure of M9 was elucidated by nuclear magnetic resonance (NMR) and confirmed by comparison to an authentic standard.

Table 2.6.4: 1 Bioanalytical Methods and Assay Details

Study No.	Species/Strain	GLP	LC-MS/MS Method Details
TT #[redacted]-6010	Mouse/ rasH2 Wild Type Mice (Hybrid)	Yes	Assay Matrix: Mouse plasma Sample processing: Protein Precipitation Assay Range: 3.96 to 4750 ng/mL Validation Method: SBP 406 V5
TT #[redacted]-6013	Mouse/ CD1	Yes	Assay Matrix: Mouse plasma Sample processing: Protein Precipitation Assay Range: 3.96 to 4750 ng/mL Validation Method: SBP 406 V5
TT #[redacted]-6005	Mouse/ rasH2 transgenic Mice	Yes	Assay Matrix: Mouse plasma Sample processing: Protein Precipitation Assay Range: 3.98 to 4780 ng/mL Validation Method: SBP 406 V6

Study No.	Species/Strain	GLP	LC-MS/MS Method Details
TT #[redacted]-6029	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V2
TT #[redacted]-6040	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V3
TT #[redacted]-6019	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4780 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-9011	Rat/ Long Evans	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4780 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-0019	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.2 to 4750 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-6029	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL and 12.2 to 4750 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-6034	Rat/ Wistar Han	No	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL Validation Method: SBP 406 V4 Assay Matrix: Rat muscle Sample processing: Protein Precipitation Assay Range: 400 to 200000 ng/mL Validation Method: SBP 406 V8
TT #[redacted]-6035	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.2 to 4750 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-1033	Rat/ Wistar Han	No	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-1095	Rat/ Wistar Han	No	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V4 Assay Matrix: Rat urine (supernatant and sediment) Sample processing: Protein Precipitation Assay Range: 24.7 to 9650 ng/mL Validation Method: SBP 406 V12 (supernatant) and V13 (sediment)
TT #[redacted]-6016	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-6017	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL Validation Method: SBP 406 V4

Study No.	Species/Strain	GLP	LC-MS/MS Method Details
TT #[redacted]-1065	Rat/ Wistar Han	No	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4800 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-7040	Rat/ Wistar Han	Yes	Assay Matrix: Rat maternal plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V3
TT #[redacted]-7130	Rat/ Wistar Han	Yes	Assay Matrix: Rat maternal plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4800 ng/mL Validation Method: SBP 406 V4 Assay Matrix: Rat milk Sample processing: Protein Precipitation Assay Range: 12.3 to 4800 ng/mL Validation Method: SBP 406 V15
TT #[redacted]-7005	Rat/ Wistar Han	No	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-7140	Rat/ Wistar Han	Yes	Assay Matrix: Rat plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4850 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-7206	Rabbit/ Dutch-Belted	No	Assay Matrix: Rabbit plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V2
TT #[redacted]-7207	Rabbit/ Dutch-Belted	No	Assay Matrix: Rabbit plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V2
TT #[redacted]-7277	Rabbit/ Dutch-Belted	No	Assay Matrix: Rabbit plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4780 ng/mL Validation Method: SBP 406 V3
TT #[redacted]-7050	Rabbit/ Dutch-Belted	Yes	Assay Matrix: Rabbit maternal plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4780 ng/mL Validation Method: SBP 406 V3
TT #[redacted]-7080	Rabbit/ Dutch-Belted	Yes	Assay Matrix: Rabbit maternal plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4800 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-6030	Dog/ Beagle	Yes	Assay Matrix: Dog plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V2
TT #[redacted]-6039	Dog/ Beagle	Yes	Assay Matrix: Dog plasma Sample processing: Protein Precipitation Assay Range: 12.4 to 4830 ng/mL Validation Method: SBP 406 V3
TT #[redacted]-6018	Dog/ Beagle	Yes	Assay Matrix: Dog plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4780 ng/mL and 12.3 to 4800 ng/mL Validation Method: SBP 406 V3

Study No.	Species/Strain	GLP	LC-MS/MS Method Details
TT #[redacted]-1061	Dog/ Beagle	No	Assay Matrix: Dog plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL, 12.3 to 4800 ng/mL, 12.4 to 4830 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-1081	Dog/ Beagle	No	Assay Matrix: Dog plasma Sample processing: Protein Precipitation Assay Range: 12.1 to 4730 ng/mL, 12.3 to 4800 ng/mL, 12.4 to 4830 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-1106	Dog/ Beagle	No	Assay Matrix: Dog plasma Sample processing: Protein Precipitation Assay Range: 12.3 to 4800 ng/mL, 12.4 to 4830 ng/mL Validation Method: SBP 406 V4
TT #[redacted]-1064	Dog/ Beagle	No	Assay Matrix: Dog plasma Sample processing: Protein Precipitation 12.3 to 4800 ng/mL, 12.4 to 4850 ng/mL Validation Method: SBP 406 V4 and V16

Abbreviations: LC-MS/MS=Liquid chromatography-tandem mass spectrometry; GLP=Good Laboratory Practices; SBP=Standard Bioanalytical Procedure; V=version

3 ABSORPTION

The pharmacokinetics of DOR were evaluated in mice, rats, rabbits, and dogs following intravenous (IV) administration of a bolus dose (1 or 2 mg/kg) as a solution in dimethylsulfoxide (DMSO):polyethyleneglycol 400 (PEG-400):deionized water (1:3:1 v/v/v). Oral administration was conducted using [redacted] DOR [redacted] over a HPMCAS polymer at a drug load of [redacted]% and suspended in 0.5% methylcellulose acidified with 5 mM HCl.

The pharmacokinetic properties of DOR were similar across species, with plasma concentrations decaying in a monoexponential manner, low plasma clearance (CL_p) and moderate volume of distribution at steady state (Vd_{ss}). Moderate oral bioavailability was observed across species. Absorption is expected to be limited by solubility rather than permeability [Sec. 2.6.4.7.2]. The use of [redacted] drug (also referred to as [redacted]), with HPMCAS as [redacted], enabled [redacted] of the [redacted] drug, which has low but better solubility than the [redacted] form ([redacted] vs. [redacted] to [redacted] µg/mL) [Sec. 2.7.1.1.2].

First-pass effect is not expected to play a significant role in limiting the bioavailability of DOR in preclinical species due to its low intrinsic clearance. A summary of the pharmacokinetic properties of DOR in preclinical species is presented in [Table 2.6.4: 2] and plasma profiles are shown in [Figure 2.6.4: 2] to [Figure 2.6.4: 5].

Table 2.6.4: 2 IV and P.O. Pharmacokinetic Parameters of DOR in Male CD-1 Mice, Male Wistar-Hannover Rats, Female Dutch-Belted Rabbits, and Male Beagle Dogs

I. Intravenous (IV)

Species	Dose (mg/kg)	AUC_{0-x}[a] (μM•hr)	$AUC_{0-\infty}$ (μM•hr)	CL_p (mL/min/kg)	Vd_{ss} (L/kg)	$t_{½}$ (hr)
Mouse[b]	1	6.01	6.33	6.05	0.99	2.7
Rat[c]	2	34.4 ± 15.7	37.3 ± 17.2	2.6 ± 1.6	1.4 ± 0.8	6.4 ± 1.5
Rabbit[c]	1	9.75 ± 1.21	9.98 ± 1.26	4.0 ± 0.50	2.67 ± 0.38	9.03 ± 0.51
Dog[c]	1	79.3 ± 6.4	88.9 ± 8.7	0.44 ± 0.04	0.9 ± 0.05	21.7 ± 2.1

II. Oral (P.O.)[d]

Species	Dose (mg/kg)	AUC_{0-x}[e] (μM•hr)	$AUC_{0-\infty}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)	F[f] (%)
Mouse[b]	5	12.43	12.45	2.07	1.0	39
Rat[c]	5	42.3 ± 15.8	43.9 ± 17.0	4.1 ± 1.1	3.3 ± 1.2	46 ± 18
Rabbit[c]	5	18.87 ± 6.73	20.53 ± 8.67	1.78 ± 0.53	3.5 ± 2.78	41 ± 13
Dog[c]	5	176 ± 44	205 ± 47	5.7 ± 1.5	0.8 ± 0.3	47 ± 14

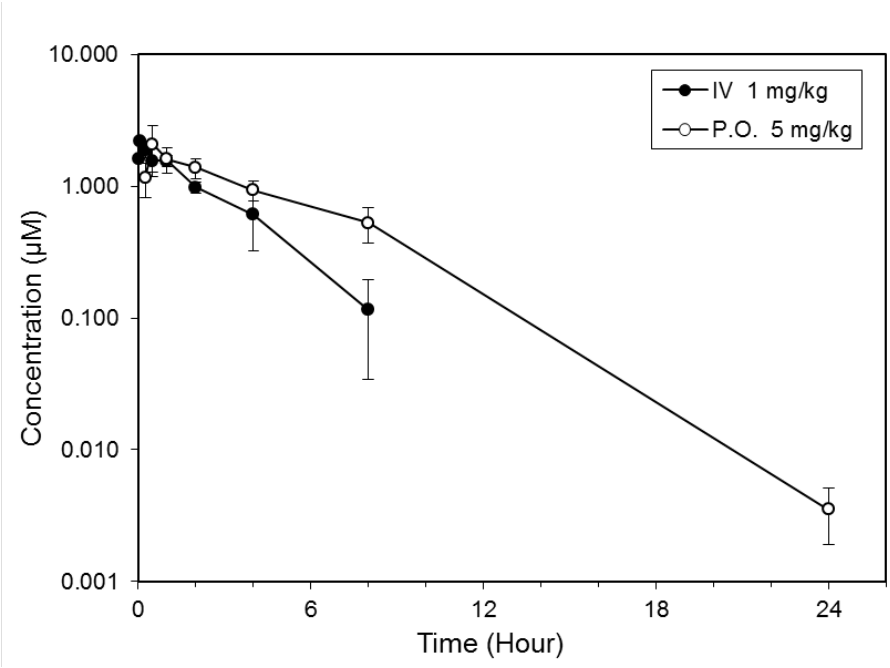
[a] x=8, 24, 48, and 72 hr for mouse, rat, rabbit, and dog, respectively.
[b] PK parameters in mouse determined by sparse sampling across 15 mice for IV and 12 mice for oral (n=3 per time point).
[c] Mean ± SD (n=3).
[d] Oral doses administered to all species as a suspension of drug [redacted] on HPMCAS polymer at a drug load of [redacted]% and suspended in 0.5% aqueous methylcellulose acidified with 5 mM HCl.
[e] x=24, 24, 48, and 72 hr for mouse, rat, rabbit, and dog, respectively.
[f] Bioavailability (F%) in mouse was determined using the mean $AUC_{(0-\infty)}$ value at 5 mg/kg P.O. relative to the mean $AUC_{(0-\infty)}$ at 1 mg/kg. For rat, F% was calculated using the individual $AUC_{(0-\infty)}$ value at 5 mg/kg P.O. relative to the mean $AUC_{(0-\infty)}$ at 2 mg/kg IV. For rabbit and dog, F% was calculated in a crossover fashion using the $AUC_{(0-\infty)}$ value at 5 mg/kg P.O. relative to the $AUC_{(0-\infty)}$ at 1 mg/kg IV for each animal.

[Sec. 2.6.5.3.1].

3.1 Mice

Following intravenous administration to male CD-1 male mice, concentrations of DOR in plasma decreased in a monoexponential manner [Figure 2.6.4: 2]. Pharmacokinetic analysis indicated that plasma clearance was low (6.05 mL/min/kg) and the estimated blood clearance was 9 mL/min/kg. DOR had a moderate volume of distribution in mice (0.99 L/kg) and a half-life of 2.7 hr. Oral administration of 5 mg/kg DOR in the [redacted] formulation resulted in rapid absorption, with a T_{max} of 1 hr and a bioavailability of 39% [Table 2.6.4: 2].

Figure 2.6.4: 2 Plasma Concentration-Time Profiles of DOR Following IV and P.O. Administration to Male CD-1 Mice

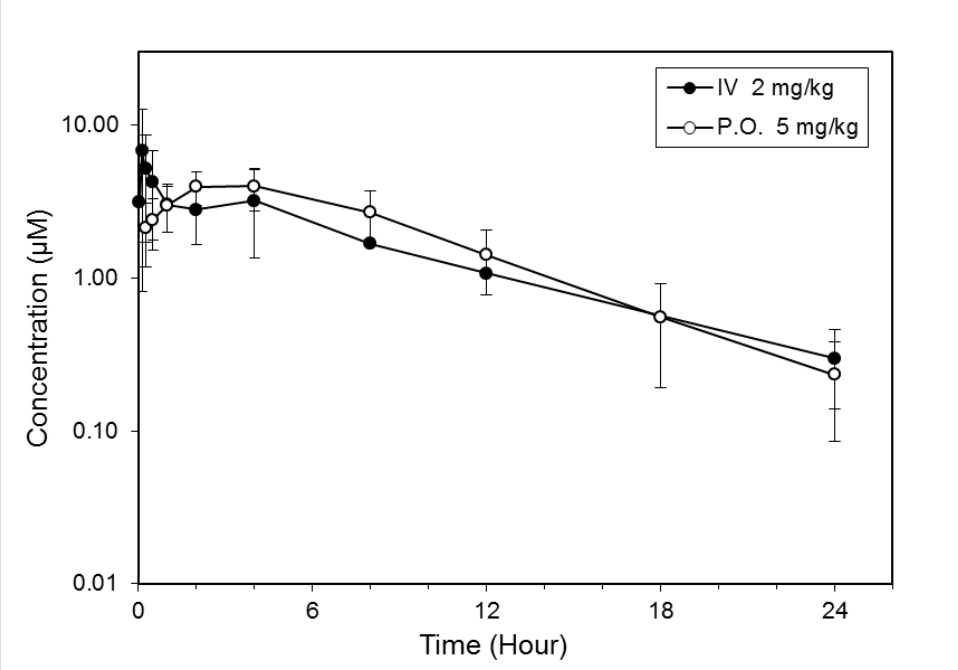


Mean ± SD (N=3 per time point).
[Sec. 2.6.5.3.1].

3.2 Rats

Mean DOR plasma concentrations following IV and oral administration to Wistar-Hannover male rats are depicted in [Figure 2.6.4: 3]. The IV pharmacokinetic parameters of DOR were comparable to those observed in mice, such that the CLp was low (2.6 mL/min/kg) and the volume of distribution was moderate (1.4 L/kg). As the blood-plasma distribution ratio was approximately 1 [Table 2.6.4: 9], blood clearance is similar to CLp. The half-life was 6.4 hr. Oral administration of 5 mg/kg DOR in the [redacted] formulation resulted in a bioavailability of 46%. The T_{max} in this species was 3.3 hr indicating somewhat prolonged absorption [Table 2.6.4: 2].

Figure 2.6.4: 3 Plasma Concentration-Time Profiles of DOR Following IV and P.O. Administration to Male Wistar-Hannover Rats



Mean ± SD (N=3).
[Sec. 2.6.5.3.1].

As indicated above, the bioavailability of DOR was anticipated to be solubility-limited. As a consequence, exposures were not expected to be dose-proportional and additional evaluation was conducted to determine the exposures achieved with several formulations considered appropriate for GLP safety studies. The performance of [redacted] vs. [redacted] drug in various vehicles is shown in [Table 2.6.4: 3]. Less-than proportional increases in exposure were observed for [redacted] and ball milled DOR in 10% polysorbate 80. The exposures ($AUC_{0\text{-}24hr}$) of ball-milled DOR in 10% polysorbate 80 at 10 mg/kg, were lower than exposures achieved with [redacted] DOR at 5 mg/kg (37.6 vs. 42.3 µM) [Table 2.6.4: 2] [Table 2.6.4: 3].

Exposures at 100 mg/kg were less than dose-proportional for all the formulations tested but were generally higher when DOR was administered in the [redacted] ([redacted]) form. The use of [redacted] forms at particle sizes of 2 to 5 µm (suspended in 10% polysorbate 80 or emulsifying lipids) or 0.2 µm (in hydroxypropylcellulose-dextrose) did not have a significant impact on exposure. Despite this improvement, administration of 100 mg/kg [redacted] DOR resulted only in a three-fold increase in AUC relative to 5 mg/kg. Oral BID dosing did not result in higher exposures in comparison to a single administration [Sec. 2.6.5.3.3].

For chronic safety studies in rats, the maximal feasible dose of [redacted] DOR (450 mg/kg) was used. Exposures following chronic administration were two-fold higher (279 µM•hr) [Sec. 2.6.6.9] than those observed at 100 mg/kg in the single dose studies described here,

consistent with the expected under-proportionality and minimal accumulation based on the $T_{½}$ of DOR in rats.

Table 2.6.4: 3 Comparison of the Performance of Multiple Formulations on the Pharmacokinetic Parameters for DOR Administered Orally to Male Wistar-Hannover Rats

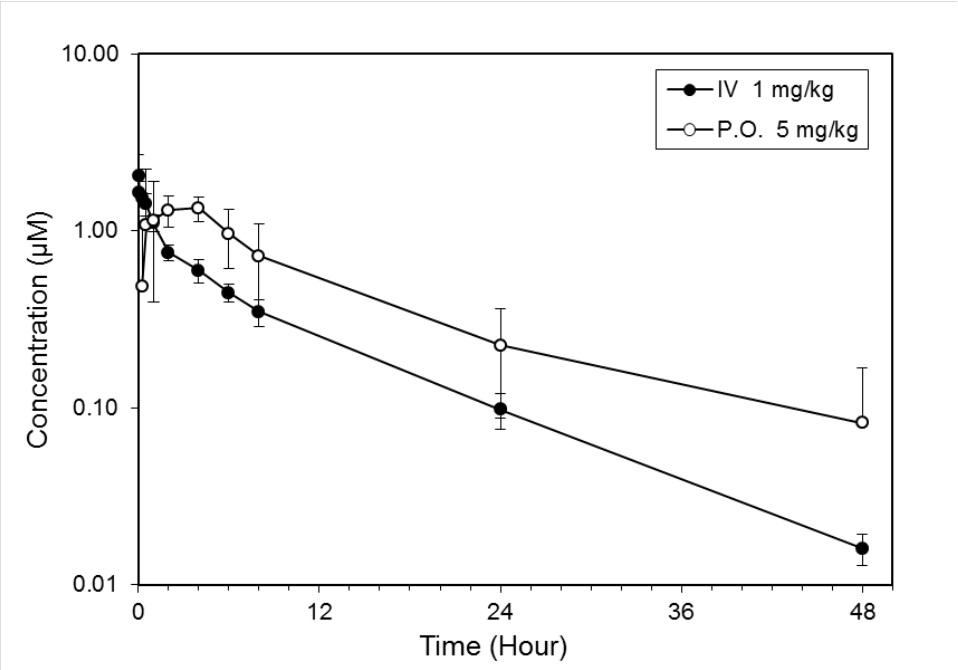
Form	Vehicle	Dose (mg/kg)	C_{max} (µM)	AUC_{0-24h} (µM•hr)
[redacted]	10% Polysorbate 80	1	1.2	7.3
		100	2.7	30.4
Ball Milled	10% Polysorbate 80	1	0.8	5.8
		10	3.4	37.6
		100	9.9	102
[redacted]	Emulsifying lipids[a]	100	4.9	74.1
Nanosuspension	HPC-dextrose in water[b]	50, BID	6.2	74.2
		100	4.5	60.0
[redacted][c]	0.5% MC[d]	50, BID	9.4 ± 0.6	134 ± 62
		100	8.83	136

Data represent mean (N=2) or mean ± SD (N=3).
[a] Emulsifying lipids = labrasol (79%) : capryol 90 (13%): labrafil M 1944CS (8%).
[b] HPC = hydroxypropylcellulose-SL (0.25% w:v) and dextrose (5% w:v) in water.
[c] [redacted] with HPMCAS at a [redacted]% drug load.
[d] MC = methylcellulose acidified with 10 mM citrate.
[Sec. 2.6.5.3.3]

3.3 Rabbits

The pharmacokinetic properties of DOR were evaluated in female Dutch-Belted rabbits. Overall, the pharmacokinetics of DOR were similar to those observed in rodents. Plasma concentrations decreased in a mono-exponential manner [Figure 2.6.4: 4] and CLp was low (4 mL/min/kg). Blood clearance should be similar to CLp as DOR distributes well between blood cells and plasma [Table 2.6.4: 9]. The volume of distribution of DOR was also moderate in this species (2.67 L/kg) and the half-life was 9 hr. Administration of 5 mg/kg [redacted] DOR by the oral route resulted in a bioavailability of 41%. The T_{max} was 3.5 hr suggesting somewhat prolonged absorption [Table 2.6.4: 2].

Figure 2.6.4: 4 Plasma Concentration-Time Profiles of DOR Following IV and P.O. Administration to Female Dutch-Belted Rabbits

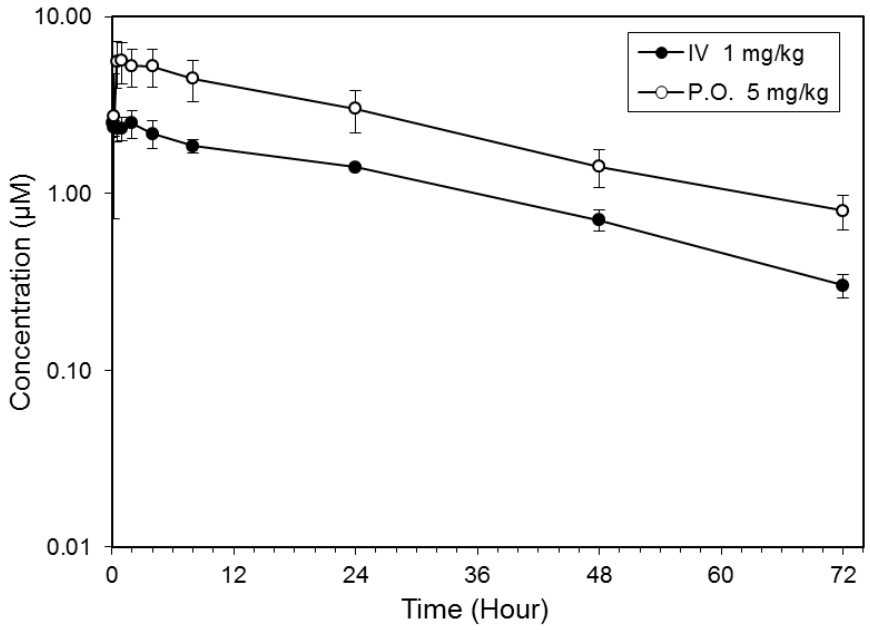


Mean ± SD (N=3).
[Sec. 2.6.5.3.2].

3.4 Dogs

Mean DOR plasma concentrations following IV and oral administration to male Beagle dogs are shown in [Figure 2.6.4: 5]. Consistent with the observed pharmacokinetics in mice, rats and rabbits, concentrations of DOR in plasma decreased in a monoexponential manner following IV administration of 1 mg/kg. The plasma clearance was very low (0.44 mL/min/kg), the volume of distribution was moderate (0.9 L/kg), and the half-life in this species was long (21.74 hr). As the blood-to-plasma distribution ratio in this species was close to 1, blood clearance is expected to be equal to CLp. Oral administration of 5 mg/kg [redacted] DOR, resulted in a bioavailability of 47%. Absorption was rapid, with a T_{max} of 0.8 hr [Table 2.6.4: 2].

Figure 2.6.4: 5 Plasma Concentration-Time Profiles of DOR Following IV and P.O. Administration to Male Beagle Dogs



Mean ± SD (N=3).
[Sec. 2.6.5.3.2].

The exposure of DOR in conventional formulations was evaluated in dogs at high doses for the purpose of assessment for long term oral administration in safety studies [Table 2.6.4: 4]. Jet milled DOR in 10% polysorbate 80 achieved approximately 2-fold higher exposures at 1000 mg/kg in comparison to a suspension of nano-milled material at 450 mg/kg. Due to the longer half-life of DOR observed in this species (21.7 hr) [Table 2.6.4: 2], it was expected that accumulation would be observed upon multiple daily administration resulting in adequate exposure and therefore, the jet-milled formulation was selected for long term studies as it could provide better margins in safety studies. Consistent with this, data from chronic studies, where jet-milled DOR suspended in 10% polysorbate 80 was administered to dogs at 1000 mg/kg, resulted in average AUC exposures of 673 µM•hr [Sec. 2.6.6.9].

Table 2.6.4: 4 Comparison of Pharmacokinetic Parameters for DOR Administered Orally to Beagle Dogs

Form	Vehicle	Dose (mg/kg)	C_{max} (µM)	AUC_{0-24hr} (µM•hr)
Jet Milled	10% Polysorbate 80	1000	13.6	283
Nanosuspension	HPC-DOSS	450	7.3	147

Data represent averages from two animals per group.
[a] HPC-DOSS = hydroxypropylcellulose-SL (10% w:v) and dioctyl sulfosuccinate sodium salt (5% w:v) in water.
[Sec. 2.6.5.3.3]

4 DISTRIBUTION

4.1 Tissue Distribution in Rats

The tissue distribution of DOR was studied using QWBA conducted in Wistar-Hannover (WH) albino and Long-Evans (LE) partially pigmented rats. A solution of [^{14}C]-labeled DOR in 10% Polysorbate 80 was administered via oral gavage at 5 mg/kg. The radioactivity distributed to a wide range of tissues. Tissue concentrations of radioactivity in albino WH rats were generally similar to those in partially-pigmented LE rats. Blood concentrations were maximal at 2 hr in both strains and corresponded to 0.758 µg equiv/g and 0.738 µg equiv/g, respectively. Similarly, the highest tissue concentrations were observed at 2 hr post-dose for the majority of tissues in both strains. The similarity between tissue concentrations in WH and LE rats, including concentrations in pigmented tissues, (e.g. eye, uvea, and skin), suggested no specific association of DOR or its metabolites with melanin. The highest overall concentrations were observed in the contents of the alimentary canal, bile, and urine, consistent with movement through the alimentary canal and the major routes of elimination. Most tissues, except central nervous system tissues, eye lens, and bone had concentrations that were greater than the concentration in blood throughout the study.

The last measurable concentration in blood was at 24 hr for WH rats (0.084 µg equiv/g) and at 8 hr for LE rats (0.277 µg equiv/g).

Elimination of radioactivity was complete in most tissues (including brain and spinal cord) by 48 hr post-dose (WH rats) or 168 hr (LE rats). In the liver of WH rats, a low concentration, just above the lower limit of quantification (LLOQ), was observed at 168 hr post-dose (last time point) [Sec. 2.6.5.4.1] to [Sec. 2.6.5.4.4].

4.2 Placental Transfer in Rats and Rabbits

Placental transfer of DOR was investigated in pregnant WH rats following daily oral administration of suspensions of [redacted] DOR at 5 or 450 mg/kg on Gestation Day (GD) 6 through 20 [Sec. 2.6.5.4.5]. Maternal and fetal blood samples were collected at 2 and 24 hr after the last dose on GD 20, and drug concentrations were measured in plasma. All rats survived until scheduled study termination. As shown in [Table 2.6.4: 5], the ratios of fetal to maternal plasma concentration of DOR ranged from 0.48 to 0.52 at the two time points in the two dose groups studied. These results indicate that DOR can cross the placenta in rats.

Table 2.6.4: 5 Mean Plasma Concentrations of DOR on Gestation Day 20 in Rats Following Daily Oral Dosing

Dose (mg/kg/day)	Time (hr)	Plasma Concentration, µM[a]		Ratio[b] (Fetal/Maternal)
		Maternal	Fetal	
5	2	4.50 ± 0.31	2.13 ± 0.12	0.48 ± 0.02
	24	0.63 ± 0.09	0.30 ± 0.03	0.49 ± 0.02
450	2	27.8 ± 1.17	14.3 ± 0.63	0.52 ± 0.03
	24	2.87 ± 0.64	1.47 ± 0.41	0.49 ± 0.03

[a] Mean ± SEM (standard error of the mean) (n=4 animals per time point).
[b] Values are the mean ± SEM of the individual fetal/maternal plasma ratios.
[Sec. 2.6.5.4.5].

Placental transfer of DOR also was investigated in pregnant Dutch-Belted rabbits by measuring concentrations in maternal and fetal plasma on Gestation Day (GD) 20. An oral dose of DOR at 300 mg/kg was administered once a day on GD 7 through 20. Maternal and fetal blood samples were collected at 4 and 24 hr after the last dose on GD 20. All animals survived until scheduled study termination. As shown in [Table 2.6.4: 6], the ratios of fetal to maternal plasma concentration of DOR ranged from 0.36 to 0.40 at the two time points studied. These data demonstrate that, similar to rats, DOR is able to cross rabbit placenta.

Table 2.6.4: 6 Mean Plasma Concentrations of DOR on Gestation Day 20 in Rabbits Following Daily Oral Dosing

Dose (mg/kg/day)	Time (hr)	Plasma Concentration, µM [a]		Ratio[b] (Fetal/Maternal)
		Maternal	Fetal	
300	4	35.3 ± 1.30	12.8 ± 0.815	0.363 ± 0.011
	24	10.9 ± 1.17	4.35 ± 0.674	0.404 ± 0.059

[a] Mean ± SEM (standard error of the mean) (n=4 animals per time point).
[b] Values are the mean ± SEM of the individual fetal/ maternal plasma ratios.
[Sec. 2.6.5.4.6].

4.3 In Vitro Plasma Protein Binding

The binding of DOR and its major circulating metabolite, M9, to plasma proteins was determined using equilibrium dialysis. Binding of DOR (0.1 to 5 µM) to plasma proteins was moderate in all species tested, mouse, rat, rabbit, dog, and human. There were no significant differences in binding of DOR across species and no dependence on concentration within a range that reflects clinical exposures [Table 2.6.4: 7].

The protein binding of M9 in rat, dog, and human plasma was moderate and did not vary between 0.1 and 1 µM M9. In dog, the extent of binding was comparable to DOR binding. In rat, binding was within 2-fold of that for DOR; however, in human plasma, the unbound fraction of M9 was 3-fold lower than that of DOR. Furthermore, in comparison to the unbound fraction in human plasma, the unbound fraction of M9 in rat and dog plasma was approximately 2.2- and 2.8-fold larger, respectively [Table 2.6.4: 8].

Table 2.6.4: 7 In Vitro Protein Binding of DOR in Plasma from Mice, Rats, Rabbits, Dogs, and Humans

Compound	Species	Fraction Unbound[a]			
		0.1 µM	1.0 µM	3.0 µM	5.0 µM
DOR	Mouse	0.242 ± 0.025	0.238 ± 0.027	0.251 ± 0.020	0.262 ± 0.021
	Rat	0.349 ± 0.005	0.324 ± 0.012	0.282 ± 0.021	0.272 ± 0.014
	Rabbit	0.221 ± 0.012	0.240 ± 0.013	0.256 ± 0.029	0.279 ± 0.011
	Dog	0.241 ± 0.005	0.258 ± 0.003	0.187 ± 0.019	0.192 ± 0.009
	Human	0.257 ± 0.003	0.241 ± 0.009	0.233 ± 0.039	0.251 ± 0.032

[a] Values represent mean ± SD of 3-6 replicates.
[Sec. 2.6.5.4.7].

Table 2.6.4: 8 In Vitro Protein Binding of Metabolite M9 in Plasma from Rats, Dogs, and Humans

Compound	Species	Fraction Unbound[a]	
		0.1 µM	1.0 µM
M9	Rat	0.18 ± 0.02	0.20 ± 0.01
	Dog	0.24 ± 0.01	0.25 ± 0.01
	Human	0.08 ± 0.003	0.09 ± 0.004

[a] Values represent mean ± SD of 3-6 replicates.
[Sec. 2.6.5.4.7].

4.4 In Vitro Blood-to-Plasma Partitioning

The partitioning of DOR (1 to 10 µM) into blood cells was determined in incubations with fresh, heparinized whole blood. The equilibrium blood-to-plasma concentration ratio was independent of the DOR concentration and was approximately 1 across species, except in mouse, for which the ratio varied from 0.65 to 0.71 [Table 2.6.4: 9]. These results indicate no preferential distribution of DOR into blood cells. Therefore, blood and plasma clearance are similar, with the exception of mouse, for which blood clearance is slightly higher than plasma clearance.

The blood-to-plasma partitioning ratio for metabolite M9 was in general lower across species compared to DOR, with values of 0.7 to 0.9 in rat, dog, and human [Table 2.6.4: 9].

Table 2.6.4: 9 In Vitro Blood-to-Plasma Concentration Ratio of DOR and its Metabolite M9 in Preclinical Species and Humans

Compound	Species	0.1 µM	1.0 µM	10 µM
DOR	Mouse	0.65 ± 0.02	0.66 ± 0.02	0.71 ± 0.01
	Rat	1.1 ± 0.1	1.1 ± 0.1	0.92 ± 0.09
	Rabbit	0.97 ± 0.06	1.06 ± 0.02	1.05 ± 0.02
	Dog	0.98 ± 0.07	0.90 ± 0.03	1.0 ± 0.1
	Human	0.92 ± 0.08	0.95 ± 0.07	1.06 ± 0.03
M9	Rat	0.76 ± 0.06	0.70 ± 0.03	ND
	Dog	0.92 ± 0.01	0.82 ± 0.01	ND
	Human	0.66 ± 0.02	0.69 ± 0.08	ND

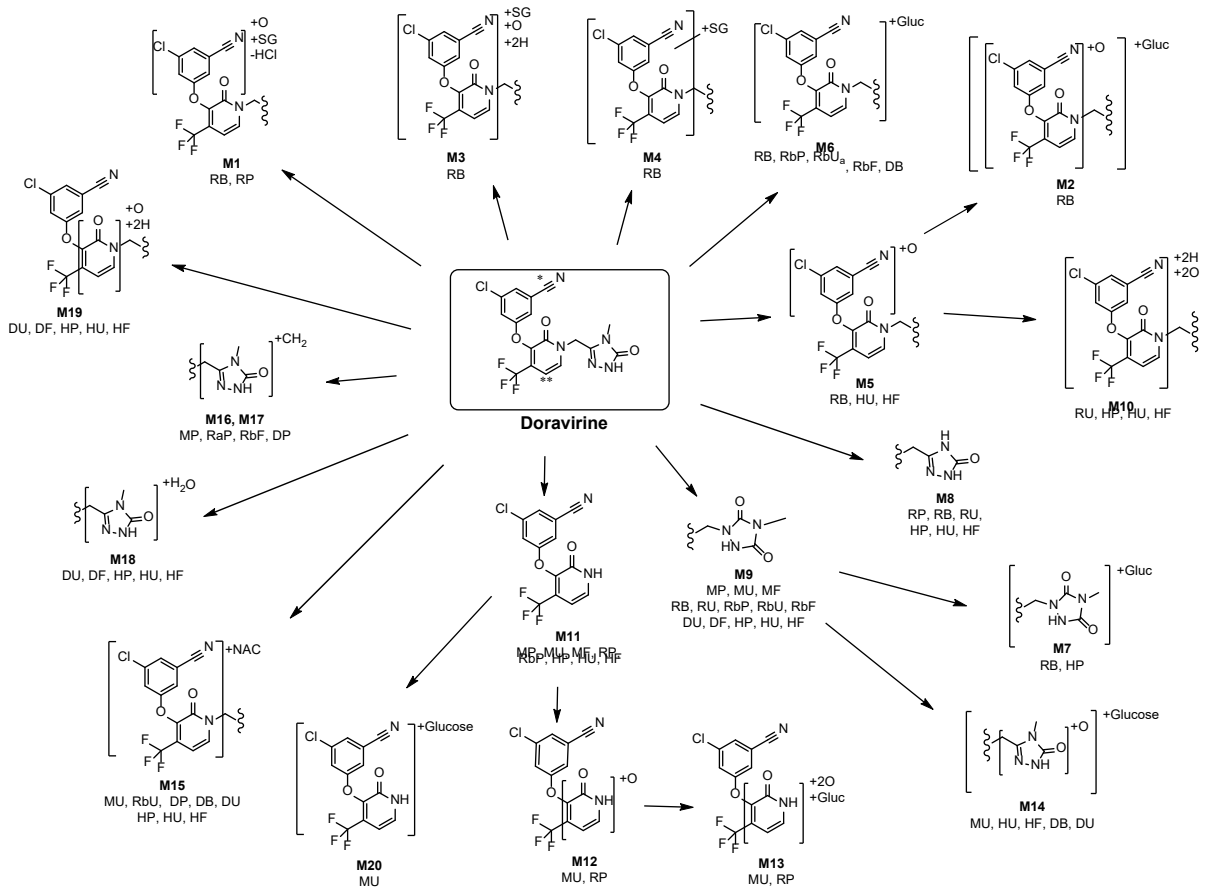
Values represent mean ±SD of three replicates.
ND = Not determined.
[Sec. 2.6.5.4.8].

5 METABOLISM

The in vivo metabolism of DOR was investigated in intact male CD-1 mice, intact female Dutch-Belted rabbits, bile duct-cannulated male Wistar-Hannover rats, and male Beagle dogs to which radiolabeled DOR (either [^{3}H] or [^{14}C]) was administered as a solution in 10% polysorbate 80 or PEG-400. The metabolism of DOR in humans was evaluated in healthy subjects (P008) [Sec. 2.7.2.2.1.3] and a summary is included below. The proposed structures of the metabolites identified in excreta and plasma from preclinical species and humans [Figure 2.6.4: 6] were based on HRMS analysis and for M9 in particular, on NMR spectroscopy.

Elimination of DOR in preclinical species and humans was mediated primarily via metabolism. Oxidation was the major metabolism pathway in all species, except dog, in which conjugation was also significant. Conjugation pathways (e.g. glucuronidation and glutathione conjugation) were very minor pathways of elimination in mice, rats, rabbits, and humans. Renal excretion of unchanged DOR was not a major pathway of elimination in preclinical species or humans. In clinical studies, M9 was identified as the major metabolite circulating in plasma. Assessment of M9 levels in chronic safety studies in rats and dogs in comparison to levels in humans indicated that the M9 levels were adequately covered.

Figure 2.6.4: 6 Proposed Structures of DOR Metabolites in Mice, Rats, Rabbits, Dogs, and Humans



* Indicates the position of ^{14}C and ** indicates the position of ^{3}H.

[a] Two metabolites identified in rabbit urine as doravirine glucuronides appeared to be different from M6 and were designated as M6a and M6b to differentiate from M6 observed in other species.

MP, RP, RbP, DP, HP=mouse, rat, rabbit, dog, human plasma; RB, DB=rat, dog bile; MU, RU, RbU, DU, HU=mouse, rat, rabbit, dog, human urine; MF, RF, RbF, DF, HF=mouse, rat, rabbit, dog, human feces. Gluc=glucuronide, NAC=*N*-Acetylcysteine, SG=glutathione
[Sec. 2.6.5.5.5].

5.1 In Vivo Metabolism in Mice

[^{14}C]Doravirine was administered to CD-1 male mice as a solution in Polysorbate 80. A single dose of 5 mg/kg was administered orally and metabolites were characterized in plasma and excreta. In agreement with the pharmacokinetic data, DOR was well-absorbed with this formulation. The major route of elimination in this species was metabolism. The major metabolite identified in mouse urine and feces was M9, which represented 31.6% of the administered dose (20 and 11.6% excreted in urine and feces, respectively). Additional metabolites eliminated in urine were secondary products of the N-dealkylated metabolite M11 including a secondary oxidation product (M12) and a glucuronide (M20) [Figure 2.6.4: 6]. The N-dealkylated metabolite M11 was only observed in trace amounts. Approximately 23.7% of the administered dose was recovered in feces as unchanged,

presumably unabsorbed, DOR within 24 hr. Only 2.6% of the administered dose was eliminated in urine as unchanged DOR [Sec. 2.6.5.5.1].

In plasma, M9 was identified as the major circulating metabolite and at levels comparable to those of DOR at the 2 time points with quantifiable levels of radioactivity (1 and 6 hr). The N-dealkylated metabolite M11 and methylated DOR (M17) were observed in trace amounts [Figure 2.6.4: 7] [Table 2.6.4: 11].

5.2 In Vivo Metabolism in Rats

The metabolism of [^{3}H]DOR was characterized in male, bile duct-cannulated Wistar-Hannover rats following oral administration of a solution of 5 mg/kg [^{3}H]DOR in PEG-400.

Metabolism was extensive and the major route of elimination in this species. The majority of the metabolites corresponded to secondary oxidation products or conjugates of oxidative metabolites [Figure 2.6.4: 6]. The majority of the metabolites were excreted in bile. The major metabolite in rat, M7, accounted for 19.5% of the administered dose and was excreted predominantly in bile. M7 was confirmed to be the glucuronide of M9 in co-elution experiments with a glucuronide of M9 generated in rat hepatocytes from chemically-synthesized M9. Metabolite M2, another glucuronide conjugate of an oxidative metabolite of DOR was also observed in bile and accounted for 6.1% of the dose. A de-methylated product was also abundant in bile (M8, 5.7% of the dose). Unlike M2 and M7, which were excreted in bile only, M8 was also excreted in urine (1.9% of the dose). Other metabolites detected in bile and/or urine included primary (M5, M9) and secondary (M3, M10) oxidative products. A glucuronide of DOR (M6) and various glutathione conjugates (M1, M3, and M4) were excreted in bile. The metabolite M9, which was the major metabolite in mice, was a relatively minor metabolite in rats, accounting for 2.6 and 1.4% of the dose excreted in bile and urine, respectively. Thus, these results suggest that formation of M9 is a significant metabolic pathway in rats but further glucuronidation of M9 to form M7, rather than direct excretion, is predominant in this species.

Excretion of unchanged DOR was an alternate route of elimination. Approximately 18.5% and 4.7% of the radioactive dose was recovered in urine and bile, respectively, as unchanged DOR [Table 2.6.4: 11]. Although profiling of fecal extracts was not conducted in this study, only 13% of the radioactive dose was recovered in this matrix and part of it is anticipated to correspond to unabsorbed DOR.

In a separate study in intact rats that were administered 10 mg/kg [^{3}H]DOR in 10% polysorbate 80, plasma samples were collected at 2, 4, 6, and 24 hr. Doravirine was the major circulating drug-related component. There were no major circulating metabolites. Some metabolites, including M1, M8, M11, M12, and M13, were observed in trace amounts. The metabolite M9 was not observed in this study [Figure 2.6.4: 7] [Table 2.6.4: 11].

5.3 In Vivo Metabolism in Rabbits

The metabolism of [^{14}C]DOR was characterized in female Dutch-Belted rabbits following oral administration of a single 5 mg/kg dose formulated in 10% polysorbate 80. In

agreement with the bioavailability of DOR observed in pharmacokinetic studies (41%), the majority of the radioactivity (60.7%) was recovered as unchanged DOR in feces, presumably as unabsorbed drug, while 28.5% of the radioactivity was recovered in the urine. The oxidative metabolite M9 was the major metabolite in rabbit urine, accounting for 19.5% of the dose. Additional metabolites eliminated in urine included three glucuronides of DOR (M6, M6a, M6b), and an *N*-acetyl-cysteine conjugate of DOR (M15). Excretion of unchanged DOR in urine was minor (6.9% of the radioactive dose). Therefore, metabolism appears to be the major route of elimination of DOR in rabbits, and oxidation, predominantly via formation of M9, was the predominant metabolic pathway.

The major circulating species in plasma was DOR. Several metabolites, including the DOR glucuronide M6, the oxidative metabolite M9,the N-dealkylated metabolite M11, and the methylated metabolite M17 were detected, albeit in trace amounts [Figure 2.6.4: 7] [Table 2.6.4: 11].

5.4 In Vivo Metabolism in Dogs

Following oral administration of a 5 mg/kg dose of [^{3}H]DOR in 10% polysorbate 80 to bile duct-cannulated, male, Beagle dogs, approximately 73.6% of the dose was recovered within 72 hr, consistent with the DOR long half-life (21.7 hr) in this species. The metabolite M9 was present in significant amounts in both urine and feces (8 and 4.8% of the administered dose, respectively). Other significant metabolites, recovered predominantly in bile, but also observed in urine, were a glucose conjugate of an oxidative metabolite (M14, 6.5, and 0.7% of the dose in bile and urine, respectively) and an N-acetylcysteine conjugate of DOR (M15, 6.3, and 2.1% of the dose in bile and urine, respectively). Unchanged DOR was recovered in urine (19% of the dose) and in feces, presumably as unabsorbed dose (24.5% of the dose). Excretion of DOR in bile was limited (0.6% of the administered dose). In conclusion, elimination of DOR in dogs was mediated via metabolism (predominantly oxidative) and renal excretion of unchanged DOR. Biliary excretion of unchanged DOR was not a significant route of elimination in this species.

Doravirine was the major circulating species identified in plasma. No metabolites were present in significant amounts in plasma from dogs. The N-acetyl-cysteine conjugate (M15), and two methylation products (M16 and M17) were present in plasma but in trace amounts. The metabolite M9 was not detected in plasma from this study [Figure 2.6.4: 7] [Table 2.6.4: 11].

5.5 In Vivo Metabolism in Humans

The absorption, metabolism, and excretion (AME) of DOR were studied following administration of a target oral dose of 350 mg [^{14}C]DOR sodium pentahydrate salt (~200 µCi, equivalent to 277 mg free acid DOR) as drug in capsule to six healthy male subjects. Feces and urine were collected up to 8 days. Recovery was determined by measuring the radioactivity in feces and urine using liquid scintillation counting. Urine and feces samples were also analyzed for metabolites using HPLC coupled to HRMS and radiometric detection. Recovery was complete (~100%) within 8 days [Sec. 2.7.2.2.1.3]. Measurement of DOR plasma levels by LC-MS/MS [Sec 2.7.2.2.1.3] (P008) and comparison

to IV exposure data [Sec. 2.7.1.2.1.1.2] indicated low bioavailability (16%) for the formulation used in this study which when corrected for hepatic extraction (1% of the absorbed dose, using a CL of 62 mL/min [Sec. 2.7.1.2.1.1.2] and blood flow of 1500 mL/min), resulted in approximately 17% of the dose absorbed. Consistent with this, DOR was the major component in feces, accounting for 84.1% of the administered dose, indicating that the majority of the radioactivity in feces corresponded to unabsorbed DOR.

Unlike the compressed tablet, which had good bioavailability (~64%) [Sec. 2.7.2.3.1.1.4], only a small fraction of the dose was absorbed in the AME study. Therefore, the predominant mechanism of elimination, based on the absorbed, rather than the administered dose, was metabolism, primarily via oxidation to M9. The metabolite M9 was the major metabolite excreted in urine (6.7% of the administered dose, 39.4% of the absorbed dose) and in feces (2.7% of the administered dose, 15.9% of the absorbed dose). Minor metabolites, each representing 1.2% or less of the recovered dose, included two *N*-dealkylated products (M8 and M11), other oxidative products (M5, M10, M18, and M19), and conjugates of oxidative products (M14, M15) [Table 2.6.4: 10]. These metabolites were all observed in preclinical species [Table 2.6.4: 11].

Renal excretion of unchanged DOR was low (2.2% of the radioactive dose or 12.9% of the absorbed dose). The low systemic bioavailability (16%) observed in this study, likely due to poor solubility, in conjunction with the significant recovery of unchanged DOR in feces (84.1% of the administered dose), suggested that biliary excretion is unlikely to be a significant route of elimination for DOR in humans. This is similar to data obtained from bile duct-cannulated rats and dogs, for which biliary excretion was not a significant pathway of elimination.

Doravirine was the major circulating component, accounting for 75% of the total drug-related material in plasma [Figure 2.6.4: 6]. The major metabolite in plasma was the oxidative product M9 (12.9%). Minor metabolites such as M8, M10, M11, M15, M18, M19, and the glucuronide conjugate M7 previously identified in rat excreta, were also identified in plasma by radiometric detection or LC-HRMS.

All of the metabolites detected in the excreta and in circulation have also been observed in mice, rats, rabbits or dogs [Table 2.6.4: 11].

Table 2.6.4: 10 Relative Amounts of DOR and its Major Metabolites in Excreta from Humans After Oral Administration of 350 mg [^{14}C]DOR Sodium Pentahydrate

Component	Relative Amount as % of Total Dose		Relative Amount as % of Absorbed[a] Dose
	Feces	Urine	
DOR	84.1	2.2	12.9[b]
M8	Trace	0.2	1.2
M9	2.7	6.7	55.3[c]
M10	Trace	0.1	0.6
M18	Trace	0.2	1.2

[a] Calculated as follows: Relative amount as % of absorbed dose=[Relative amount as % of total dose]*100/ [Absorbed dose]. Where absorbed dose was estimated as 17% based on systemic bioavailability of 16% and anticipated liver extraction of 1% and no gut extraction).
[b] Estimated using radioactivity in urine only. The amount in feces was determined to be unabsorbed dose.
[c] Calculated after adding % in feces (2.7%) and urine (6.7%).
Metabolites M5, M11, M14, M15, and M19 were also detected in feces and/or urine by LC/MS but levels in the radioachromatogram were too low to be quantifiable; therefore were not included here.
[Sec. 2.6.5.5.1]

Additional studies were conducted using plasma samples obtained from healthy subjects who received 240 mg DOR once daily administered as [redacted] drug in oral compressed tablets in P001 [Sec. 2.7.2.2.1.1]. Plasma samples obtained throughout day 1 and day 10 were pooled in an AUC-proportional manner and following HRMS analysis, the relative amounts of drug-derived components (i.e. DOR and its metabolites) were estimated. Results from these studies indicated that M9 was present in amounts that were approximately 5.6% and 6.7% of the total drug-related components on day 1 and day 10, respectively, while DOR levels were 85.4% and 79.4%, on day 1 and day 10, respectively [Sec. 2.6.5.5.2]. These results indicated good agreement in the levels of M9 observed in the AME study and those observed following multiple once daily administration with the DOR tablet despite the different formulations used in these trials [Sec. 2.6.5.5.2].

Table 2.6.4: 11 The Presence of DOR and their Metabolites in Plasma and/or Excreta From Nonclinical Species and in Humans Following P.O. Administration of [^{14}C] or [^{3}H]DOR

Species	Mouse			Rat				Rabbit			Dog				Human		
Biological matrix	P	U	F	P	B	U	F	P	U	F	P	B	U	F	P	U	F
DOR	~50	2.6	23.7	~100	4.7	18.5	NA	~100	6.9	60.7	~100	0.6	19	24.5	75	2.2	84.1
Unknown	-	4.7	-	-	5.7	-	NA	-	-	-	-	-	-	-	-	-	-
M1	-	-	-	TA	3.3	-	NA	-	-	-	-	-	-	-	-	-	-
M2	-	-	-	-	6.1	-	NA	-	-	-	-	-	-	-	-	-	-
M3	-	-	-	-	2.8	-	NA	-	-	-	-	-	-	-	-	-	-
M4	-	-	-	-	1.8	-	NA	-	-	-	-	-	-	-	-	-	-
M5	-	-	-	-	2.2	-	NA	-	-	-	-	-	-	-	-	TA	TA
M6	-	-	-	-	2.3	-	NA	TA	TA	TA	-	0.9	-	-	-	-	-
M6a	-	-	-	-	-	-	NA	-	TA	-	-	-	-	-	-	-	-
M6b	-	-	-	-	-	-	NA	-	TA	-	-	-	-	-	-	-	-
M7[a]	-	-	-	-	19.5	-	NA	-	-	-	-	-	-	-	2.8[b]	-	-
M8	-	-	-	TA	5.7	1.9	NA	-	-	-	-	-	-	-	TA	0.2	TA
M9	~50	20	11.6	-	2.6	1.4	NA	TA	19.5	TA	-	-	8	4.8	12.9	6.7	2.7
M10	-	-	-	-	-	1.2	NA	-	-	-	-	-	-	-	TA	0.1	TA
M11	TA	TA	TA	TA	-	-	NA	TA	-	-	-	-	-	-	TA	TA	TA
M12	-	2.3	-	TA	-	-	NA	-	-	-	-	-	-	-	-	-	-
M13	-	0.9	-	TA	-	-	NA	-	-	-	-	-	-	-	-	-	-
M14	-	TA	-	-	-	-	NA	-	-	-	-	6.5	0.7	-	-	TA	TA
M15	-	TA	-	-	-	-	NA	-	TA	-	TA	6.3	2.1	-	[b]	TA	TA
M16	-	-	-	-	-	-	NA	-	-	-	TA	-	-	-	-	-	-
M17	TA	-	-	-	-	-	NA	TA	-	TA	TA	-	-	-	-	-	-
M18	-	-	-	-	-	-	NA	-	-	-	-	-	TA	TA	TA	0.2	TA
M19	-	-	-	-	-	-	NA	-	-	-	-	-	TA	TA	TA	TA	TA
M20	-	2.2	-	-	-	-	NA	-	-	-	-	-	-	-	-	-	-

TA: Trace amount. Detected by LC/MS but not observed in radiometric profile: - Not Detected: NA: Not available
The abundance of the metabolites in excreta was based on their abundance in radiochromatography profiles relative to the dose recovered in each matrix. The abundance of the metabolites in plasma was calculated relative to the total radioactivity in the chromatogram.
[a] Studies conducted in rat hepatocytes with authentic standard of M9 confirmed that M7 is the glucuronide of M9.
[b] Co-eluted with M7, % radioactivity includes both M7 and M15.
[Sec. 2.6.5.5.1]

Figure 2.6.4: 7 Representative Radiochromatograms of Plasma from Mice, Rats, Rabbits, Dogs, and Humans Following Oral Administration of [^{3}H]- or [^{14}C]DOR

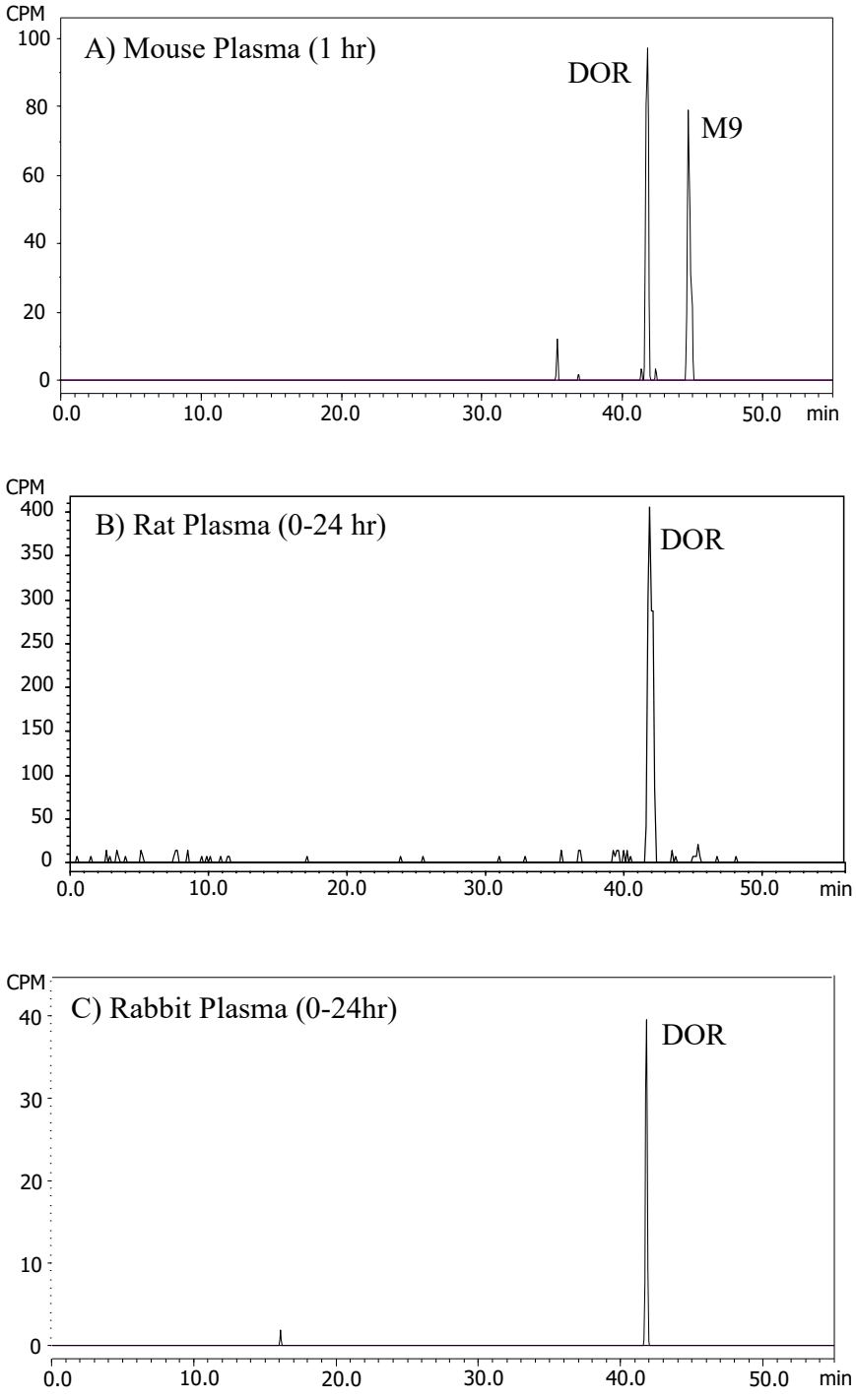
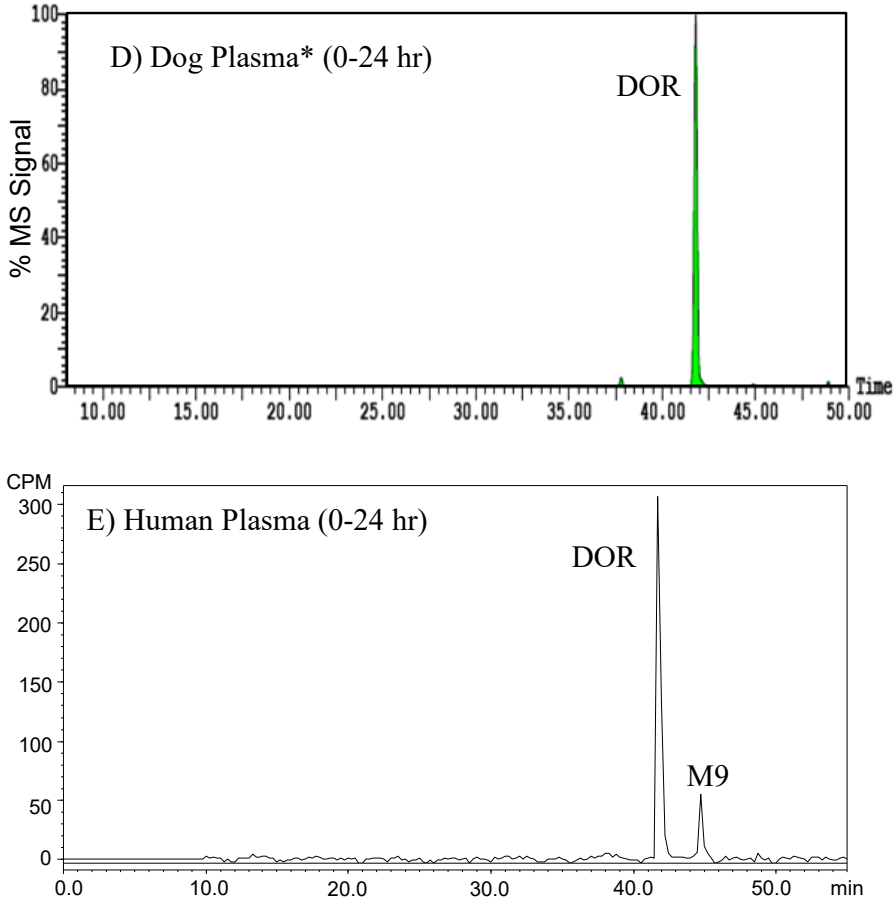


Figure 2.6.4: 7 Representative Radiochromatograms of Plasma from Mice, Rats, Rabbits, Dogs, and Humans Following Oral Administration of [^{3}H]- or [^{14}C]DOR (Cont.)



Mice and rabbits were dosed with 5 mg/kg [^{14}C]doravirine in 10% polysorbate 80. Rats and dogs were dosed with [^{3}H]doravirine in 10% polysorbate 80 at 10 and 5 mg/kg, respectively. Healthy human subjects received 350 mg [^{14}C]doravirine sodium salt in drug-filled capsules.
* MS signal depicted due to insufficient radioactivity.
[Sec. 2.6.5.5.1]

5.6 Assessment of M9 in Plasma from Rat and Dog Chronic Safety Studies

Exploratory analyses were performed to assess levels of the M9 metabolite in plasma from rats or dogs in the chronic safety studies relative to levels of M9 in clinical samples. Plasma samples collected on day 81 and 87 from the rat and dog chronic safety studies, respectively, were pooled in an $AUC_{(0\text{-}24hr)}$ proportional manner. A similar AUC-pooled sample prepared from plasma from healthy subjects that had received daily doses of 240 mg DOR for 10 days and for which the presence of M9 had been confirmed (see above, [Sec. 2.6.4.5.5]) was used as reference. Comparison of the M9 peak area in rat and dog plasma to that in the human plasma sample enabled the estimation of the concentration of M9 in the safety samples relative to its concentration in the clinical sample without quantifying it. Plasma samples were mixed with control plasma from the other species to minimize matrix effect. The data

indicated that after chronic administration of DOR at the NOAEL doses (450 and 1000 mg/kg/day for rats and dogs, respectively), M9 was present in rat and dog plasma at levels that were respectively 52 and 51% relative to levels observed in plasma from healthy subjects who received multiple once daily 240 mg doses of DOR [Table 2.6.4: 12]. Furthermore, the unbound fraction of M9 in rat and dog plasma was respectively 2.2- and 2.8-fold higher than in human plasma [Table 2.6.4: 8], so that unbound concentrations in the safety species, were approximately similar to the unbound concentrations in human plasma after administration of multiple once-daily administration of 240 mg DOR. At the therapeutic dose (100 mg), the AUC of DOR [Sec. 2.7.2.1.3] is expected to be approximately 40% lower than the AUC at 240 mg. These results indicated that the exposures of M9 in plasma from patients administered the clinical dose (100 mg once a day) were adequately covered in the chronic safety studies.

Table 2.6.4: 12 Exposures of M9 in Plasma from Rats and Dogs Relative to Exposures in Human Plasma after 10 Daily Doses of 240 mg DOR Following Chronic Administration of DOR

Species	Dose (mg/kg/day)	Gender	M9 estimated EM	Average EM[a]
Rat	450	Female	0.6	0.52 (1.16)
		Male	0.43	
Dog	1000	Female	0.48	0.51 (1.44)
		Male	0.54	

EM=exposure multiples relative to exposure in healthy subjects after 10 daily doses of 240 mg DOR compressed tablets

[a] Numbers in parenthesis are the EMs corrected by differences in unbound fraction in plasma of rats vs. humans (2.2) or dog vs humans (2.8) according to [Table 2.6.4: 8].

[Sec. 2.6.5.5.3]

5.7 In Vitro Metabolism in Rats, Dogs and Humans

The in vitro metabolic turnover of DOR (10 µM) in liver microsomes was very low. Very small amounts of the oxidative products M9 and M10 were generated in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) in human liver microsomes. Only M9 was observed in dog liver microsomes while several metabolites (M5, M8, M9, and M10) were observed in rat liver microsomes. Relative to other metabolites, M9 was the most abundant metabolite in dog and human liver microsomes. No glucuronidation products were detected in incubations of DOR with liver microsomes in the presence of uridine 5'-diphospho-glucuronic acid (UDPGA). Also, no metabolites were detected with suspensions of hepatocytes from rats, dogs, or humans [Sec. 2.6.5.5.4].

5.8 Characterization of M9

The metabolism of M9, the major circulating metabolite in humans, was assessed in rat, dog, and human hepatocytes. Similar to DOR, M9 was not metabolized in dog or human hepatocytes following a 2-hr incubation. Turnover in rat hepatocytes was low, with the glucuronide M7 as the major metabolite. These results are in agreement with in vivo data which indicated that further metabolism of M9 is significant only in rats, where M7 is the major metabolite in excreta, while in dogs and humans M9 is readily excreted, predominantly

in urine, without significant further metabolism. In humans, M7 was only observed in plasma but in small amounts relative to M9 [Table 2.6.4: 11].

Further characterization of M9 was conducted following isolation from urine obtained from the AME study, and purification. Nuclear Magnetic Resonance (NMR) spectroscopy revealed differences in the chemical shift of the protons of the triazolinone methyl on M9 which suggested that oxidative metabolism may have occurred at the quaternary carbon adjacent to the methylene group on the triazolinone ring. Additional NMR experiments provided data that could only be consistent with the structure proposed in [Figure 2.6.4: 1] whereby M9 was formed by an initial oxidative step followed by an intramolecular rearrangement. The structure of M9 was confirmed by chemical synthesis of the proposed structure and demonstrated that the synthetic compound co-eluted with the metabolite isolated from human urine and had essentially identical NMR spectra. Synthetic M9 was tested in a multiple-round HIV-1 infection assay (ViKinG) against wild type and 3 major mutants (K103N, V106A, and Y181C) of the HIV-1 virus at concentrations up to 8.4 µM. No significant antiviral activity was observed. In addition, M9 (10 µM) did not have off-target activity against a panel of 115 cellular targets [Sec. 2.6.5.5.5].

6 EXCRETION

The excretion of radioactivity following oral administration of ^{3}H- or ^{14}C-labeled DOR to mice, rats, rabbits, and dogs is summarized in [Table 2.6.4: 13]. In these studies, DOR was administered as a solution and the absorption of radioactivity in mice, rats, and dogs was higher compared to the absorption of DOR administered as a suspension of the [redacted] drug in the pharmacokinetic studies [Table 2.6.4: 2].

6.1 Excretion of Radioactivity in Mice, Rats, Rabbits, and Dogs

In CD-1 mice dosed with 5 mg/kg of [^{14}C]DOR, approximately 70.1% of the administered dose was recovered over a 72-hr period. Excretion was similar between urine (34.8% including cage wash) and feces (35.3%).

Following a 5 mg/kg oral dose of [^{3}H]DOR to bile-duct cannulated rats (n=3), the average total radioactivity recovered in the excreta represented 93.4% of the dose. Over a 72-hr collection period, 56.7% of the dose was excreted in bile, 23.8% in urine (including cage wash) and 13.0% in feces. The majority of the radioactivity was recovered within the first 24 hr.

In Dutch-Belted rabbits dosed with 5 mg/kg of [^{14}C]DOR, approximately 89.2% of the administered dose was recovered over a 72-hr period. The majority of the dose was excreted in feces (60.7%) [Table 2.6.4: 13], as DOR [Table 2.6.4: 11], assuming no biliary excretion in this species, this is consistent with a bioavailability of 41% in this species. Approximately 28.5% of the dose was excreted in urine (including cage wash).

Following a 5 mg/kg oral dose of [^{3}H]DOR to bile duct-cannulated (n=2) dogs, the average total radioactivity recovered in the excreta over a 72-hr collection period represented 73.6% of the dose. Approximately 14.3% of the dose was excreted in bile, 30.1% in urine

(including cage wash), and 29.3% in feces. A third dog, for which a loose cannula was found at the 2 hr bile collection, was excluded from any further analysis.

Table 2.6.4: 13 Recovery of a Radioactive Oral Dose of DOR in Mice, Rats, Rabbits, and Dogs

Species	Dose Recovery (%)			
	Bile	Urine[a]	Feces	Total
CD-1 mice[b,c]	N/A	32.7	35.3	70.1
Wistar-Hannover Rats (BDC)[d]	56.7	22.9	13.0	93.4
Dutch-Belted Rabbits[b]	N/A	26.4	60.7	89.2
Beagle Dogs (BDC)[e]	14.3	29.7	29.3	73.6

All animal studies conducted in males, except for studies in rabbits, which were conducted in females.
[a] Includes amounts recovered from cage rinses.
[b] Administered at 5 mg/kg as a solution of [^{14}C]DOR in 10% polysorbate 80.
[c] Sample collection was terminal at each time point.
[d] Administered at 5 mg/kg as a solution of [^{3}H]DOR in PEG-400.
[e] Administered at 5 mg/kg as a solution of [^{3}H]DOR in 10% polysorbate 80.
Values represent means of 3 for rats and rabbits and 2 for dogs. The data in mice were obtained from one reading per collection interval of samples pooled from 3 animals.
BDC=Bile duct-cannulated; N/A=Not available, studies were conducted in intact animals.

[Sec. 2.6.5.6.1]

6.2 Excretion in Milk of Rats

The excretion of DOR into the milk of lactating rats was investigated by measuring concentrations in maternal plasma and milk on Lactational Day (LD) 14 following daily oral administration of DOR at 5 or 450 mg/kg/day from Gestational Day (GD) 6 to LD 14. Maternal whole blood samples from animals were collected from the jugular vein on Lactation Day (LD) 14, at 2 hr post-dose into EDTA-treated tubes, and processed for plasma analysis. Pups were removed approximately 2 hr prior to milk collection and a minimum of 0.5 mL of milk per female was collected over a period of approximately 10 minutes into scintillation vials and transferred into microtiter tubes. Following the 5 and 450 mg/kg/day doses, concentrations of DOR in milk were about 1.47- and 1.32-fold higher than the maternal plasma concentrations, respectively. Thus, the results demonstrated excretion of circulating DOR into the milk of lactating rats [Sec. 2.6.5.6.2].

7 DRUG INTERACTIONS

7.1 Drug Metabolizing Enzymes Involved in the Elimination of Doravirine

Incubation of DOR (10 µM) with recombinant human CYP isoforms, namely CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9*1, 2C9*2, 2C9*3, 2C18, 2C19, 2D6*1, 2E1, 2J2, 3A4, 3A5, and 3A7, demonstrated that oxidation of DOR was carried out primarily by CYP3A4 and CYP3A5 [Sec. 2.6.5.7.1].

To confirm the involvement of CYP3A in the metabolism of DOR, studies using an inhibitory anti-CYP3A monoclonal antibody (mAb) were conducted. Incubation of DOR (10 µM) with either human liver microsomes or recombinant CYP3A4 (rCYP3A4) and rCYP3A5 enzymes in the presence of anti-CYP3A mAb demonstrated near complete

inhibition of oxidative metabolism. Based on these data, it was concluded that the oxidative metabolism of DOR was mainly catalyzed by CYP3A4 and CYP3A5 [Sec. 2.6.5.7.2].

Further kinetic studies aimed at determining the relative contribution of CYP3A4 and CYP3A5 in the metabolism of DOR were conducted using rCYP3A4 and rCYP3A5. The enzyme kinetics for rCYP3A4 and rCYP3A5 were determined under linear rate conditions. Michaelis-Menten analysis of the velocity vs. substrate concentrations enabled estimation of K_m and V_{max} for each enzyme. Eadie-Hofstee analysis suggested the presence of two binding sites and provided estimation of $K_{m1,app}$, $V_{max1,app}$, $K_{m2,app}$, and $V_{max2,app}$. These values were subsequently corrected to account for binding to microsomes and CL_{int} values were calculated for the Michaelis-Menten fit as well as for the high affinity, low capacity phase (K_{m1} and V_{max1}) of the Eadie-Hofstee plots. Based on the one-site and two-site binding models, the ranges of CL_{int} values for rCYP3A4 and rCYP3A5 were determined to be 0.110-0.142 and 0.0048-0.0049 µL/min/pmol CYP, respectively. These results indicated an approximate 20-fold higher catalytic efficiency for CYP3A4 relative to CYP3A5. This finding, added to the predominant expression of CYP3A4 in intestine and liver suggested that CYP3A4 is the major enzyme responsible for the elimination of DOR in humans [Sec. 2.6.5.7.2].

These findings, in addition to in vivo metabolism data, indicate that the major elimination pathway of DOR is CYP3A4-mediated metabolism. In agreement with these results, clinical studies demonstrated that DOR is subject to drug interactions with CYP3A inhibitors and inducers. In interaction studies with ketoconazole and ritonavir, the AUC of DOR increased approximately 3-fold and in drug interaction studies with the inducers rifampin and rifabutin, the AUC of DOR decreased by 90 and 50%, respectively [Sec. 2.7.2.2.3.1].

7.2 Drug Transporters Involved in the Disposition of Doravirine

The passive permeability of DOR was evaluated in LLC-PK1 cells. The mean apparent permeability coefficient (P_{app}) for 0.1 µM DOR was 25 x 10^{-6} cm/sec, and did not change significantly at 0.5 and 1 µM, indicating good passive permeability. The P_{app} for the positive control verapamil was 40.1 x 10^{-6} cm/sec in this assay [Sec. 2.6.5.7.4]. Due to its good passive permeability, transporters are not expected to play a significant role in the disposition of DOR.

Efflux of Doravirine by BCRP and P-gp

Doravirine was evaluated as a substrate of the human Breast Cancer Resistance Protein (BCRP) in BCRP-transfected MDCK-II cells. The P_{app} of DOR in control MDCK-II cells was 10.4 to 11.5 × 10^{-6} cm/s at 0.1 to 1 µM while the positive control prazosin had a P_{app} of 22.7 × 10^{-6} cm/s. The ratio of basolateral-to-apical/apical-to-basolateral (B-A/A-B) transport was 1.1 in control cells and 1.9 in BCRP-expressing cells. The B-A/A-B ratio in the BCRP-expressing cells was not decreased in the presence of the BCRP inhibitor Ko143. In the same experiment, the BA/AB ratio for [^{3}H]prasozin was 4.7 in BCRP-transfected cells and decreased to 1.4 in the presence of Ko143, indicating the functionality of the assay. Thus, results from these studies indicated that DOR is not a substrate for BCRP [Sec. 2.6.5.7.3].

Doravirine was evaluated as a substrate of human Multidrug Resistance protein 1 (MDR1), or P-gp in MDR1-transfected LLC-PK1 cells. Over a range of concentrations (0.1, 0.5, and 1 µM), the B-A/A-B ratio for transport in P-gp transfected cells was 3.5 to 4.7, compared to 0.9 to 1 in non-transfected cells indicating that DOR is a substrate of P-gp [Sec. 2.6.5.7.4]. Drug interactions with P-gp inhibitors are not anticipated to be significant due to the high permeability of DOR.

Uptake by OATP1B1 and OATP1B3

Doravirine was evaluated as a substrate of the Organic Anion Transporting Polypeptide (OATP)1B1 and OATP1B3 in MDCKII cells stably expressing OATP1B1 or OATP1B3. Rapid, time-dependent uptake of DOR into control cells was observed, likely as a result of good passive permeability. A small increase in uptake into OATP1B1, but not into OATP1B3, expressing cells, relative to control cells, was observed. This uptake was not inhibited by the OATP1B1 and 1B3 inhibitor bromosulphophthalein (BSP, 100 µM). Similar studies conducted in HEK-293 cells demonstrated that uptake into OATP1B1- or OATP1B3-expressing cells was similar to that in control cells, indicating that DOR was not a substrate of either transporter. In the same studies, uptake of the OATP1B1 substrate estradiol 17β-glucuronide ($E_2$17βG), and the OATP1B3 substrate cholecystokinin-8 (CCK8), was effectively inhibited by BSP [Sec. 2.6.5.7.5] [Sec. 2.6.5.7.6].

Follow-up studies in cryopreserved human hepatocytes demonstrated uptake of DOR to be reduced by a maximum of 1.4-fold in the presence of a cocktail of transporter inhibitors (10 µM cyclosporin A, 10 µM rifamycin SV, 100 µM rifampin and 50 µM quinidine) designed to fully inhibit OATP1B1, OATP1B3, NTCP, and OCT1, as compared to in the absence of inhibitors. In the same studies, the uptake of 1 µM [^{3}H] $E_2$17βG, 5 nM [^{3}H] CCK8, 1 µM [^{3}H] taurocholic acid (TCA) and 1 µM [^{14}C] tetraethylammonium (TEA), known substrates of OATP1B1, OATP1B3, NTCP (Na^+-taurocholate cotransporting polypeptide) and OCT1, respectively, was reduced by 4.0 to 12.5-fold in the presence of the cocktail, compared to in the absence. As observed in the OATP1B1 and 1B3 transfected cells, good passive permeability appeared to be the major driver for the uptake of DOR in hepatocytes, with a weak transporter mediated component. Results from these studies indicate that OATP1B1 and OATP1B3 are likely not involved in the hepatic uptake of DOR [Sec. 2.6.5.7.7].

7.3 Inhibition of Drug Metabolizing Enzymes and Transporters by Doravirine

7.3.1 Inhibition of CYP Enzymes

The reversible inhibitory effects of DOR on human liver microsomal activity for CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 were evaluated. The IC_{50} values [Table 2.6.4: 14] were more than 100-fold above the unbound C_{max} (less than1 µM) at the therapeutic dose, indicating that inhibition of these isoforms in vivo is not likely.

Table 2.6.4: 14 Effect of DOR on Cytochrome P450 Marker Enzyme Activities in Pooled Human Liver Microsomes

CYP	DOR IC_{50}
1A2	>100 (0%)
2B6	>100 (2.1 ± 3.4%)
2C8	>100 (17 ± 6.3%)
2C9	>100 (28 ± 2.6%)
2C19	>100 (46 ± 1.9%)
2D6	>100 (7.4 ± 1.2%)
3A4[a]	>100 (0%)
3A4[b]	>100 (35 ± 0.52%)

The values in parentheses represent the percent inhibition (mean ± SD) observed at 100 µM DOR.
[a] Midazolam as probe substrate.
[b] Testosterone as probe substrate.
[Sec. 2.6.5.7.8.1].

Furthermore, DOR did not cause time-dependent inhibition of CYP3A4 activity at 10 and 50 µM, so that measured rates of enzyme inactivation were not different from that observed in the solvent control. In the same experiment, incubation with the time-dependent inhibitor mifepristone resulted in significant decrease in enzyme activity [Sec. 2.6.5.7.8.2].

7.3.2 Inhibition of UGT1A1

In experiments with UDPGA-supplemented liver microsomes, the UGT1A1-catalyzed glucuronidation of 17β-estradiol was evaluated in the presence of DOR at concentrations up to 100 µM. Under these conditions, DOR was not an inhibitor of UGT1A1. In the same experiments, nicardipine, a known inhibitor of UGT1A1 inhibited the glucuronidation of 17β-estradiol with an IC_{50} of 3.2 ± 0.27 µM [Sec. 2.6.5.7.8.3].

7.3.3 Inhibition of Drug Transporters

BCRP, P-gp, and BSEP

Doravirine's maximal concentrations at the clinical dose of 100 mg are anticipated to be below 4 µM, with a corresponding unbound concentration below 1 µM. The in vitro IC_{50} value (51 µM) obtained in experiments with transfected cells expressing BCRP was more than 50-fold above DOR free concentrations in plasma [Table 2.6.4: 15] [Sec. 2.6.5.7.9.1]. Therefore, systemic drug interactions via this transporter are unlikely. However, DOR could inhibit the intestinal efflux of substrates of this transporter and increase their exposure. The theoretical gut lumen concentrations of DOR (400 µg/mL or 940 µM, assuming complete dissolution of the 100 mg dose in 250 mL of intestinal fluid) at the therapeutic dose exceed the in vitro IC_{50}. However, the solubility of DOR is limited. In vitro, the solubility of DOR was [redacted] µg/mL ([redacted] µM) [Sec. 2.7.1.1.2.1], so that actual concentrations in the gut lumen are unlikely to exceed this value. In clinical studies, co-administration with 200 mg DOR resulted in a 30% increase in the exposure of the BCRP substrate dolutegravir. This effect was not clinically meaningful [Sec. 2.7.2.2.3.2.3].

Doravirine did not inhibit P-gp up to 100 µM [Table 2.6.4: 15] [Sec. 2.6.5.7.9.2], and it is not anticipated to alter the pharmacokinetics of P-gp substrates via systemic interactions.

Inhibition of intestinal P-gp was not adequately tested if, as indicated above, gut lumen concentrations of DOR approach the estimated aqueous solubility of [redacted] µM. Attempts to test DOR inhibition of P-gp at 300 µM in vitro resulted in 8.7% inhibition; however, DOR precipitated in the assay media. Thus, although inhibition of gut P-gp cannot be excluded, it is likely to be limited by DOR's solubility and be of no clinical relevance.

Doravirine did not inhibit BSEP at concentrations up to 50 µM in vitro [Table 2.6.4: 15] [Sec. 2.6.5.7.9.3]. Thus, it is unlikely to perpetrate interactions at the clinical dose via inhibition of this transporter.

OATP1B1 and 1B3

The inhibitory effect of DOR at concentrations up to 75 µM on the uptake of the OATP1B1 substrate pitavastatin was evaluated in MDCK-II cells stably transfected with OATP1B1. Results indicated that DOR inhibited OATP1B1 with an IC_{50} value of 39 µM [Table 2.6.4: 15] [Sec. 2.6.5.7.9.4] [Sec. 2.6.5.7.9.5]. In the same experiment, 10 µM cyclosporin effectively inhibited the uptake of pitavastatin. Based on a bioavailability of approximately 64%, a mean unbound C_{max} lower than 1 µM and an unbound hepatic inlet concentration of approximately 3 µM, DOR is unlikely to inhibit OATP1B1 in vivo. The hepatic inlet unbound C_{max} after an oral 100 mg dose was estimated assuming that the fraction absorbed was 0.67, the rate of absorption was 1.4 hr^{-1}, and the C_{max} was 2.3 µM [Sec. 2.7.2.3.1.1], with a hepatic blood flow of 90 L/hr and unbound fraction of 0.24. These results indicate low risk for DOR to perpetrate drug interactions via this transporter. In a clinical study, DOR did not have a clinically meaningful effect on the pharmacokinetics of atorvastatin, an OATP1B1 substrate [Sec 2.7.2.2.3.2.4].

In experiments conducted in MDCK-II cells stably transfected with OATP1B3, DOR inhibited the uptake of 0.1 µM [^{3}H]BSP with an IC_{50} value of 31 µM. In the same experiments, 10 µM cyclosporin effectively inhibited the uptake of [^{3}H]BSP [Table 2.6.4: 15]. As is the case for OATP1B1, inhibition of OATP1B3 by DOR is not anticipated to be of clinical relevance.

OAT1, OAT3, and OCT2

Based on the inhibition data obtained for OAT1, OAT3, and OCT2 [Table 2.6.4: 15] [Sec. 2.6.5.7.9.6] to [Sec. 2.6.5.7.9.8], the in vitro IC_{50} values obtained in experiments with transfected cells expressing the aforementioned transporters are respectively, at least 75-, 16- and 67-fold above DOR unbound concentrations in plasma. Therefore, systemic drug interactions via these transporters are unlikely. In clinical studies, DOR co-administration with TDF did not affect the pharmacokinetics of tenofovir, an OAT1/3 substrate. In addition, the pharmacokinetics of metformin, an OCT substrate, were not affected by co-administration with DOR [Sec. 2.7.2.2.3.2.5].

MATE1 and MATE2K

The inhibitory effect of DOR on the uptake of 5 µM [^{14}C]metformin was evaluated in CHO-K1 cells transfected with MATE1 and in MDCK-II cells transfected with MATE2K. At 50 µM, the highest concentration tested, 28 and 39% inhibition was observed for MATE1

and MATE2K, respectively [Table 2.6.4: 15] [Sec. 2.6.5.7.9.9] [Sec. 2.6.5.7.9.10]. Thus, although the IC_{50} could not be accurately estimated, it is expected to be above 50 µM. This weak inhibition, added to an unbound C_{max} at therapeutic concentrations likely below 1 µM, indicates that the risk for interaction of DOR with substrates of these transporters in very low. In agreement with this, DOR co-administration with metformin did not alter the pharmacokinetics of metformin.

Table 2.6.4: 15 Effect of DOR on the Activity of Human Uptake and Efflux Transporters

Transporter	IC_{50} (µM)	Maximum Concentration Tested (µM)
BCRP	51 ± 4	75
P-gp	> 100 (0%)	100
BSEP	> 50 (0%)	50
OATP1B1	39 ± 2	75
OATP1B3	31 ± 4	75
OAT1	> 75 (13%)	75
OAT3	16 ± 0.7	75
OCT2	67 ± 9	75
MATE1	> 50 (28%)	50
MATE2	> 50 (39%)	50

IC_{50} values are expressed as means ± SD.
Values in parenthesis represent the % inhibition at the maximal concentration tested when the IC_{50} was above it.
[Sec. 2.6.5.7.9]

7.4 Induction of Human Cytochrome P450 Enzymes

The potential for DOR to up-regulate the activity of drug metabolizing enzymes and transporters via ligand-activated nuclear receptors, including the Arylhydrocarbon receptor (AhR), the Constitutive Androstane Receptor (CAR) and the Pregnane X Receptor, was evaluated in cryopreserved plated human hepatocytes from 3 donors. The hepatocytes were treated for 48 hours with vehicle control, DOR (0.1 to 20 µM), or the positive control inducers omeprazole (50 µM), phenobarbital or rifampicin (10 µM) for AhR, CAR, and PXR, respectively. The activation of these receptors was determined by concentration dependent increases in messenger RNA (mRNA) and activity of CYP1A2, 2B6, and 3A4 by monitoring phenacetin O-demethylation, bupropion hydroxylation and testosterone 6β-hydroxylation, respectively.

Results indicated that CYP1A2 mRNA and enzyme activity was not altered significantly by DOR at concentrations of up to 20 µM. The positive control omeprazole induced CYP1A2 mRNA by 21.3- to 32.1-fold and enzyme activity by 8.9- to 22-fold across 3 hepatocyte lots [Sec. 2.6.5.7.10.1].

Doravirine did not induce CYP2B6 mRNA or enzyme activity while the positive control phenobarbital induced mRNA by 14- to 18.5-fold and enzyme activity by 7.2- to 13.7-fold across 3 hepatocyte lots [Sec. 2.6.5.7.10.2].

Doravirine did not induce CYP3A4 mRNA or enzyme activity at 0.1-5 µM. An increase in CYP3A4 mRNA was observed at 10 µM (1.2- to 4.0-fold) and 20 µM (1.4- to 4.2-fold). This effect was small (3.1 to 20.3% across hepatocytes lots, relative to the positive control

rifampicin at 10 µM) and had no corresponding effect on CYP3A4 enzyme activity, indicating that the change in mRNA expression was not large enough to effect significant changes in protein expression [Sec. 2.6.5.7.10.3]. In agreement with these results, 14 daily doses of 120 mg DOR in healthy subjects did not have a clinically meaningful effect on the pharmacokinetics of the CYP3A substrate midazolam [Sec. 2.7.2.2.3.2.1].

Results from these experiments indicated that DOR is not likely to cause drug interactions via activation of AhR, CAR, or PXR.

8 OTHER PHARMACOKINETIC STUDIES

No additional pharmacokinetic studies were conducted.

9 DISCUSSION AND CONCLUSIONS

Doravirine had a low clearance in all preclinical species studied (mouse, rat, rabbit, and dog) with a moderate volume of distribution. Bioavailability in these species was moderate (39 to 47%) and in vitro data indicated that absorption was not limited by permeability. The low clearance of DOR indicated that first-pass effect was minimal and therefore not limiting bioavailability. Doravirine is a low solubility drug; however, the use of an enabled formulation of the [redacted] drug minimized the impact of poor solubility on its bioavailability at pharmacologically relevant doses and afforded better exposures in rats in comparison to formulations of the [redacted] drug. In dogs, a suspension of jet-milled DOR provided adequate exposures upon chronic administration.

Doravirine binding to plasma proteins was similar across preclinical species and human and did not change within the concentration range expected at the therapeutic dose of 100 mg (0.1 to 5 µM). Consistent with its good permeability, DOR distributed freely into blood cells and tissues. However, the distribution of DOR into the central nervous system was limited. Doravirine was a substrate for P-gp in vitro and at the clinical dose, access to the brain may be limited, at least in part, by P-gp efflux.

The elimination of DOR in preclinical species and humans occurred primarily via CYP-mediated oxidation. Renal excretion of unchanged drug was not a major pathway of elimination. Elimination via conjugation pathways was significant in dogs but in general minor in other preclinical species and humans. All the metabolites observed in humans were also observed in preclinical species.

The oxidative metabolite M9 was the major component in excreta from preclinical species and humans. Doravirine was the major drug-related component in circulation and M9 was observed circulating in significant amounts in mouse (approximately 50% of total radioactivity) and human (12.9% of total radioactivity) plasma, but it was present in trace amounts in rabbit plasma and not detected in rat or dog plasma at pharmacologically relevant doses (5 mg/kg). Follow up analysis of plasma from rats and dogs administered DOR chronically at the NOAEL doses (450 and 1000 mg/kg/day, respectively), demonstrated that exposure of M9 in both species was within two-fold of that observed in plasma from healthy subjects that received multiple 240 mg once-daily doses of DOR. Furthermore, the unbound fraction of M9 was 2.2- and 2.8-fold higher in rat and dog plasma compared to human

plasma, so that unbound concentrations at the NOAEL doses were approximately comparable to the unbound concentrations in humans following repeated administration of daily 240 mg doses to healthy volunteers. Thus, the safety of M9 at the therapeutic dose (100 mg) was adequately characterized in chronic safety studies.

In vitro, the metabolism of DOR, primarily via formation of M9, was mediated by CYP3A4, with minor contribution from CYP3A5, so that DOR is anticipated to be a victim of drug interaction when co-administered with inhibitors or inducers of CYP3A enzymes. This is consistent with results from clinical trials with CYP3A inhibitors ritonavir and ketoconazole, and multiple doses of CYP3A inducers, rifampin and rifabutin, which demonstrated an effect of these drugs on the exposure of DOR, where the co-administration with inhibitors caused increases in AUC of approximately 3-fold and the inducers rifampin and rifabutin caused decreases in AUC of 90% and 50%, respectively [Sec. 2.7.2.2.3.1.2] to [Sec. 2.7.2.2.3.1.5].

Doravirine was a substrate for P-gp but an effect of P-gp inhibitors on the pharmacokinetics of DOR is unlikely to be clinically meaningful. Based on preclinical and clinical data, the role of P-gp on the systemic clearance of DOR is anticipated to be minor. At the absorption level, clinical drug interactions with P-gp inhibitors may be small due to the good passive permeability of DOR. This was confirmed in a single dose interaction study with rifampin, a P-gp inhibitor, where the C_{max} of DOR increased only by 40% [Sec. 2.7.2.2.3.1.4]. Similarly, DOR C_{max} increased by only 25-30% after co-administration with ritonavir [Sec 2.7.2.2.3.1.2] or ketoconazole [Sec. 2.7.2.2.3.1.3], which inhibit P-gp as well as CYP3A.

Doravirine was not a substrate for the liver uptake transporters OATP1B1 and OATP1B3 or the efflux transporter BCRP. Thus, effects of inhibitors of these transporters on the pharmacokinetics of DOR are unlikely.

Doravirine was not an inhibitor of the major CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) or UGT1A1. Therefore, it is not expected to be a perpetrator of drug interactions via inhibition of these enzymes. In addition, DOR was not an inducer of CYP1A2 or 2B6 at clinically relevant concentrations. Weak induction of CYP3A4 mRNA was observed in vitro at DOR concentrations of 10 and 20 µM. However, in clinical studies, co-administration of DOR with midazolam, a sensitive CYP3A4 substrate, had no meaningful impact on the pharmacokinetics of midazolam [Sec. 2.7.2.2.3.2.1]. Similarly, DOR was a weak inhibitor of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, BCRP, MATE1, and MATE2K in vitro, so that no clinically meaningful interactions with substrates of these transporters are anticipated at therapeutic doses. In clinical interaction trials, DOR did not elicit clinically meaningful changes on the pharmacokinetics of dolutegravir (BCRP substrate), atorvastatin (OATP1B1 substrate), tenofovir (OAT1 and OAT3 substrate), 3TC and metformin (OCT2, MATE1, and MATE2K substrates) [Sec. 2.7.2.2.3.1.8] and [Sec. 2.7.2.2.3.2]. Doravirine did not inhibit BSEP at concentrations up to 50 µM in vitro. In addition, DOR was not an inhibitor of P-gp at 100 µM in vitro and is therefore unlikely to perpetrate systemic drug interactions on substrates of this transporter. DOR could not be tested in vitro to assess P-gp inhibition at concentrations relevant to exposures in the gut. However, due to its limited solubility, DOR is not likely to achieve concentrations in the gut more than two-fold above 100 µM, the in vitro concentration at which no inhibition was observed. Therefore, although potential for

interactions with substrates of P-gp at the absorption level are possible, they are unlikely to be clinically relevant.

In summary, DOR was not a perpetrator of clinically meaningful drug interactions but its pharmacokinetic profile was affected by CYP3A inhibitors and inducers, as demonstrated in clinical interaction studies with ketoconazole, ritonavir, rifampin, and rifabutin.

Lamivudine (3TC) has been used in the clinic as part of a 3-drug regimen and demonstrated good oral bioavailability and short half-life in rats and dogs. It distributed quickly and widely across tissues in rats and had low binding to human plasma proteins. Renal excretion, primarily via glomerular filtration and active tubular secretion was the major route of elimination of 3TC in rats while the elimination in dog was balanced between renal excretion and metabolism. In vitro data, as well as clinical experience indicated low potential for 3TC to perpetrate pharmacokinetic drug interactions. Inducers and inhibitors of CYP enzymes did not alter the pharmacokinetics of 3TC. 3TC was a substrate of OCT2, MATE1, and MATE2K in vitro and inhibitors of these transporters may increase the plasma exposures of 3TC but this effect is unlikely to have clinical significance. Clinical experience indicates that 3TC does not perpetrate significant drug interactions with major HIV drugs eliminated via metabolism or renal excretion, so that interactions with TDF or DOR are unlikely.

The prodrug TDF improved the oral bioavailability of tenofovir in preclinical species and humans. Following oral administration of TDF, efficient presystemic hydrolysis resulted in minimal plasma exposures of TDF, with tenofovir as the major circulating drug-related component in preclinical species and humans. Excretion of tenofovir in urine occurs by glomerular filtration with some involvement of active tubular secretion. In vitro data and clinical experience indicated that TDF is unlikely to cause clinically relevant pharmacokinetic drug interactions via CYP enzymes and transporters.

Based on their ADME properties, drug interactions are not anticipated between the components in DOR/3TC/TDF. Clinical data indicated no effect of co-administration on the pharmacokinetics of the individual components in DOR/3TC/TDF [Sec. 2.7.2.3.1.2] and [Sec. 2.7.2.3.1.3].

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2.6　非臨床試験の概要文及び概要表

2.6.5　薬物動態試験概要表

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Sec. 2.6.5.1 Pharmacokinetics: Overview

Test Article: Doravirine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Analytical Methods and Assay Validation				
SBP 406 Verison 1	Rat	NA	MSD	[Ref. 4.2.2.1: 049748]
SBP 406 Verions 16	Dog	NA	MSD	[Ref. 4.2.2.1: 04B53V]
VR 406 GLP Dog Version 1	Dog	NA	MSD	[Ref. 4.2.2.1: 04CFGX]
VR 406 GLP Rat Version 1	Rat	NA	MSD	[Ref. 4.2.2.1: 04CFHM]
Absorption after single dose				
Pharmacokinetics	Mouse	IV, P.O.	MRL	[Ref. 4.2.2.2: PK011MK1439]
Pharmacokinetics	Rat	IV	MRL	[Ref. 4.2.2.2: PK001MK1439]
Pharmacokinetics	Rabbit	IV, P.O.	MRL	[Ref. 4.2.2.2: PK011MK1439]
Pharmacokinetics	Dog	IV, P.O.	MRL	[Ref. 4.2.2.2: PK001MK1439]
Pharmacokinetics	Rat, Dog	P.O.	MRL	[Ref. 4.2.2.2: PK020MK1439]
Distribution				
Single-Dose Tissue Distribution	Rat	P.O.	[redacted]	[Ref. 4.2.2.3: PK005MK1439]
Placental Transfer[a]	Rat	P.O.	MRL	[Ref. 4.2.2.3: TT[redacted]7130FIN]
Placental Transfer[a]	Rabbit	P.O.	MRL	[Ref. 4.2.2.3: TT[redacted]7080FIN]
Reversible Plasma Protein Binding	Mouse, Rat, Rabbit, Dog, and Human	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439] [Ref. 4.2.2.3: PK012MK1439] [Ref. 4.2.2.4: PK015MK1439] [Ref. 4.2.2.3: PK016MK1439]
Blood-to-Plasma Partitioning	Mouse, Rat, Rabbit, Dog, and Human	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439] [Ref. 4.2.2.3: PK012MK1439] [Ref. 4.2.2.4: PK015MK1439]
Metabolism				
Metabolites in Urine, Feces, and Plasma	Mouse	P.O.	MRL	[Ref. 4.2.2.4: PK013MK1439]

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Metabolites in Urine, Bile, and Plasma	Rat	P.O.	MRL	[Ref. 4.2.2.4: PK002MK1439]
Metabolites in Urine, Feces, and Plasma	Rabbit	P.O.	MRL	[Ref. 4.2.2.4: PK013MK1439]
Metabolites in Urine, Feces, Bile, and Plasma	Dog	P.O.	MRL	[Ref. 4.2.2.4: PK004MK1439]
Metabolites in Urine, Feces, and Plasma	Human	P.O.	MRL	[Ref. 4.2.2.4: PK006MK1439] [Ref. 4.2.2.4: PK008MK1439]
Semi-Quantitative Analysis of M9 in Plasma	Rat, Dog, and Human	P.O.	MRL	[Ref. 4.2.2.4: PK019MK1439]
Metabolism in Liver Microsomes and Hepatocytes	Rat, Dog, and Human	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439]
Excretion				
Mass Balance (Urine, Feces)	Mouse	P.O.	MRL	[Ref. 4.2.2.4: PK013MK1439]
Mass Balance (Urine, Bile, Feces)	Rat	P.O.	MRL	[Ref. 4.2.2.4: PK002MK1439]
Mass Balance (Urine, Feces)	Rabbit	P.O.	MRL	[Ref. 4.2.2.4: PK013MK1439]
Mass Balance (Urine, Feces, Bile)	Dog	P.O.	MRL	[Ref. 4.2.2.4: PK004MK1439]
Excretion into Milk[a]	Rat	P.O.	MRL	[Ref. 4.2.2.3: TI[redacted]7130FIN]
Pharmacokinetic Drug Interactions				
Human CYP Phenotyping	Human Liver Microsomes	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439] [Ref. 4.2.2.4: PK017MK1439]
BCRP Transport	MDCKII Cells	In vitro	MRL	[Ref. 4.2.2.6: PK014MK1439]
P-gp Transport	LLC-MDR1	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439]
OATP1B1- and OATP1B3-Mediated Transport	MDCKII Cells, HEK293 Cells	In vitro	MRL	[Ref. 4.2.2.6: PK007MK1439] [Ref. 4.2.2.6: PK018MK1439]
Uptake in Human Hepatocytes	Human Hepatocytes	In vitro	MRL	[Ref. 4.2.2.6: PK018MK1439]
Inhibition of Cytochromes P450	Human Liver Microsomes	In vitro	MRL,[redacted]	[Ref. 4.2.2.3: PK003MK1439]
Time-Dependent Inhibition of CYP3A4 Activity	Human Liver Microsomes	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439]

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Inhibition of UGT1A1	Human Liver Microsomes	In vitro	MRL	[Ref. 4.2.2.6: PK009MK1439]
BCRP Inhibition	Sf9 Cell Membrane Vesicles	In vitro	MRL	[Ref. 4.2.2.6: PK007MK1439]
P-gp Inhibition	LLC-MDR1 Cells	In vitro	MRL	[Ref. 4.2.2.3: PK003MK1439]
BSEP Inhibition	Sf9 Cell Membrane Vesicles	In vitro	MRL	[Ref. 4.2.2.6: PK018MK1439]
OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 Inhibition	MDCKIICells, CHO-K1 Cells	In vitro	MRL	[Ref. 4.2.2.6: PK007MK1439]
MATE1 and MATE2K Inhibition	CHO-K1 Cells	In vitro	MRL	[Ref. 4.2.2.6: PK018MK1439]
CYP1A2, CYP2B6, and CYP3A4 Induction in Human Hepatocytes	Human Hepatocytes	In vitro	MRL, [redacted]	[Ref. 4.2.2.3: PK003MK1439] [Ref. 4.2.2.6: PK010MK1439]

[a] Report contains a GLP Compliance Statement.
MSD: Merck Sharp & Dohme
NA: Not applicable
MRL: Merck Research Laboratories.
[redacted]

Sec. 2.6.5.2 Analytical Methods and Validation Reports

Test Article: Doravirine

Validation Report Number	VR 406 GLP Mouse Version 1	VR 406 GLP Mouse Addendum 1 Version 1	VR 406 GLP Mouse Addendum 2 Version 1	VR 406 GLP Mouse Addendum 3 Version 1
Validation Method Number	SBP406 V4	SBP 406 V5 [a]	SBP 406 V5 [a]	SBP 406 V5[a]
Activity	Full validation in mouse plasma	Partial validation in mouse plasma – change LLOQ	Incurred sample reanalysis (Study: TT#[redacted]-6010)	Working solution stability (64 days at 4°C)
Species	Mouse	Mouse	Mouse	Mouse
Analyte	Doravirine	Doravirine	Doravirine	Doravirine
Matrix	Plasma	Plasma	Plasma	Plasma
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	12.3 - 4780	3.96 - 4750	3.96 - 4750	3.96 - 4750
Standard Inter-day Accuracy Range (%Bias)	-3 to 2	-5 to 6[b]	-	-
Standard Inter-day Precision Range (% CV)	1 to 6	-	-	-
QC Nominal Concentration (ng/mL)	31.9, 1990, 3980	11.9, 297, 3960	-	-
QC Inter-day Accuracy Range (%Bias)	-1 to 1	-2 to 6[b]	-	-
QC Inter-day Precision Range (%CV)	2 to 5	2 to 7[b]	-	-
Matrix LTS (at -70°C)	15 Days	98 Days	-	-
Dilution Integrity QC (ng/mL) (Dilution)	28200 (1/200)	3960 (1/5)	-	-

Additional Information:

- Abbreviations: CV = Coefficient of variation; GLP = Good Laboratory Practice; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; LLOQ = Lower limit of quantitation; LTS = Long-term stability; QC = Quality control; SBP = Standard bioanalytical procedure; ULOQ = Upper limit of quantitation; V = version; - = Data not applicable or not available.
- [a] SBP 406 V5 was updated to SBP 406 V6 to correct the purpose section and no method changes were made.
- [b] Intra-day accuracy or precision range reported.

Sec. 2.6.5.2 Analytical Validation Reports (Cont.)

Test Article: Doravirine

Validation Report Number	VR 406 GLP Rat Version 1[a]	VR 406 GLP Rat Addendum 1 Version 1	VR 406 GLP Rat Addendum 2 Version 1	VR 406 GLP Rat Addendum 3 Version 1	VR 406 GLP Maternal Rat Version 1	VR 406 GLP Maternal Rat Addendum 1 Version 1
Validation Method Number	SBP 406 V1[b], V2	SBP 406 V2	SBP406 V4	SBP406 V4	SBP 406 V3	SBP 406 V3
Activity	Partial validation in rat plasma	Incurred sample reanalysis (Study: TT#[redacted]-6029)	Extraction protected versus non protected from light	Long-term matrix stability	Partial validation in Maternal Rat Plasma	Long-term matrix stability
Species	Rat	Rat	Rat	Rat	Maternal Rat	Maternal Rat
Analyte	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine
Matrix	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	12.4 - 4850	12.4 - 4830	12.2 - 4750	12.2 - 4750	12.4 - 4830	12.4 - 4830
Standard Inter-day Accuracy Range (%Bias)	-1 to 2[c]	-	-	-	-4 to 2	-
Standard Inter-day Precision Range (% CV)	1 to 11[c]	-	-	-	1 to 7	-
QC Nominal Concentration (ng/mL)	29.5, 1480,3690 29.3, 1470,3660	-	31.7, 1980, 3960	-	29.1, 1450, 3630	-
QC Inter-day Accuracy Range (%Bias)	-4 to 2[c] -2 to 0[c]	-	-1 to 0[c,d] -3 to -1[c,e]	-	0 to 3	-
QC Inter-day Precision Range (%CV)	1 to 4[c] 3 to 5[c]	-	1 to 3[c,d] 1 to 2[c,e]	-	2 to 4	-
Matrix LTS (at -70°C)	29 Days	-	-	1200 Days	13 Days	47 Days
Dilution Integrity QC (ng/mL) (Dilution)	47200 (1/200)	-	-	-	48800 (1/200)	-

Additional Information:

- Abbreviations: CV = Coefficient of variation; GLP = Good Laboratory Practice; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; LLOQ = Lower limit of quantitation; LTS = Long-term stability; QC = Quality control; ULOQ = Upper limit of quantitation; V = version; - = Data not applicable or not available.
- [a] Validation report number: VR 406 GLP Rat Version 1[Ref. 4.2.2.1: 04CFHM].
- [b] Validation method number: SBP 406 V1.[Ref. 4.2.2.1: 049748].
- [c] Intra-day accuracy and precision range reported.
- [d] Not protected from light.
- [e] Protected from light.

Sec. 2.6.5.2 Analytical Validation Reports (Cont.)

Test Article: Doravirine

Validation Report Number	VR 406 GLP Maternal Rat Addendum 2	VR 406 GLP Fetal Rat Plasma	VR 406 GLP Fetal Rat Plasma Addendum 1	VR 406 GLP Rat Milk	VR 406 GLP Rat Milk Addendum 1	VR 406 GLP Rat Milk Addendum 2
Validation Method Number	SBP 406 V4	SBP 406 V4	SBP 406 V 4	SBP 406 V14, V15	SBP 406 V15	SBP 406 V15
Activity	Incurred sample reanalysis (Study: TT#[redacted]-7130)	Partial validation in fetal rat plasma	Incurred sample reanalysis (Study: TT#[redacted]-7130)	Full validation in rat milk	Long-term matrix stability	Incurred sample reanalysis (Study: TT#[redacted]-7130)
Species	Maternal Rat	Fetal Rat	Fetal Rat	Rat	Rat	Rat
Analyte	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine
Matrix	Plasma	Plasma	Plasma	Milk	Milk	Milk
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	12.3 - 4800	12.3 - 4800	12.3 - 4800	12.3 - 4800	12.3 - 4800	12.3 - 4800
Standard Inter-day Accuracy Range (%Bias)	-	-	-	-1 to 1	-	-
Standard Inter-day Precision Range (% CV)	-	-	-	1 to 3	-	-
QC Nominal Concentration (ng/mL)	-	30.3, 505, 4040	-	30.3, 505, 4040	-	-
QC Inter-day Accuracy Range (%Bias)	-	-7 to -5[a]	-	-6 to -2	-	-
QC Inter-day Precision Range (%CV)	-	1 to 5[a]	-	1 to 5	-	-
Matrix LTS (at -70°C)	-	-	-	7 Days	22 Days	-
Dilution Integrity QC (ng/mL) (Dilution)	-	-	-	23800 (1/10, 1/100)	-	-

Additional Information:

- Abbreviations: CV = Coefficient of variation; GLP = Good Laboratory Practice; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; LLOQ = Lower limit of quantitation; LTS = Long-term stability; QC = Quality control; ULOQ = Upper limit of quantitation; V = version; - = Data not applicable or not available.
- [a] Intra-day accuracy and precision range reported.

Sec. 2.6.5.2 Analytical Validation Reports (Cont.)

Test Article: Doravirine

Validation Report Number	VR 406 Non GLP Rat Urine Version 1	VR 406 Non GLP Rat Urine Addendum 1 version 1	VR 406 Non GLP Urine Sediment Version 1	VR 406 Non GLP Rat Muscle Version 1
Validation Method Number	SBP 406 V10[a]	SBP 406 V10[a]	SBP406 V11[b]	SBP 406 V7, V8
Activity	Non GLP validation in rat urine	Non GLP Long-term matrix Stability	Non GLP validation in rat urine sediment	Non GLP validation in rat muscle homogenate
Species	Rat	Rat	Rat	Rat
Analyte	Doravirine	Doravirine	Doravirine	Doravirine
Matrix	Urine	Urine	Urine Sediment	Muscle
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	24.7 - 9650	24.7 - 9650	24.7 - 9650	400 - 200000
Standard Inter-day Accuracy Range (%Bias)	-6 to 4[c]	-	-2 to 6[c]	-10 to 4[c]
Standard Inter-day Precision Range (% CV)	-	-	-	-
QC Nominal Concentration (ng/mL)	60.3, 1010, 8040	-	60.3, 1010, 8040	1590, 159000
QC Inter-day Accuracy Range (%Bias)	-11 to 2[c]	-	-1 to 0[c]	-2 to 0[c]
QC Inter-day Precision Range (%CV)	1 to 2[c]	-	1 to 3[c]	1 to 7[c]
Matrix LTS (at -70°C)	5 Days	53 Days	5 Days	-
Dilution Integrity QC (ng/mL) (Dilution)	56300 (1/200)	-	56300 (1/200)	-

Additional Information:

- Abbreviations: CV = Coefficient of variation; GLP = Good Laboratory Practice; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; LLOQ = Lower limit of quantitation; LTS = Long-term stability; QC = Quality control; ULOQ = Upper limit of quantitation; V = version; - = Data not applicable or not available.
- [a] SBP 406 V10 was updated to SBP 406 V12 to correct typographical errors. No method changes were made.
- [b] SBP 406 V11 was updated to SBP 406 V13 to correct typographical errors. No method changes were made.
- [c] Intra-day accuracy or precision range reported.

Sec. 2.6.5.2 Analytical Validation Reports (Cont.)

Test Article: Doravirine

Validation Report Number	VR 406 Non GLP Rabbit Version 1[a]	VR 406 GLP Maternal Rabbit Version 1	VR 406 GLP Maternal Rabbit Addendum 1 Version 1	VR 406 GLP Maternal Rabbit Addendum 2 Version 1	VR 406 GLP Fetal Rabbit Plasma
Validation Method Number	SBP 406 V2	SBP 406 V3	SBP 406 V3	SBP 406 V3	SBP 406 V4
Activity	Non GLP validation in rabbit plasma	Partial validation in maternal rabbit plasma	Long-term matrix stability	Incurred sample reanalysis (Study: TT#[redacted]-7050)	Incurred sample reanalysis(Study: TT#[redacted]-7080)
Species	Rabbit	Maternal Rabbit	Maternal Rabbit	Maternal Rabbit	Fetal Rabbit
Analyte	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine
Matrix	Plasma	Plasma	Plasma	Plasma	Plasma
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	12.4 - 4830	12.4 - 4830	12.4 - 4830	12.3 - 4780	12.3 - 4800
Standard Inter-day Accuracy Range (%Bias)	-3 to 7[b]	-5 to 4	-	-	-
Standard Inter-day Precision Range (% CV)	-	2 to 8	-	-	-
QC Nominal Concentration (ng/mL)	29.3, 1470, 3660	29.1, 1450, 3630	-	-	-
QC Inter-day Accuracy Range (%Bias)	-3 to 6[b]	-1 to 2	-	-	-
QC Inter-day Precision Range (%CV)	1 to 5[b]	2 to 6	-	-	-
Matrix LTS (at -70°C)	-	13 days	180 days	-	-
Dilution Integrity QC (ng/mL) (Dilution)	-	48800 (1/200)	-	-	-

Additional Information:

- Abbreviations: CV = Coefficient of variation; GLP = Good Laboratory Practice; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; LLOQ = Lower limit of quantitation; LTS = Long-term stability; QC = Quality control; ULOQ = Upper limit of quantitation; V = version; - = Data not applicable or not available.
- [a] VR 406 Non GLP Rabbit Version 1demonstrated stability after 1 freeze/thaw cycle. VR 406 Non GLP Rabbit Addendum 1 version 1 demonstrated stability after 2 freeze/thaw cycles.
- [b] Intra-day accuracy or precision range reported.

Sec. 2.6.5.2 Analytical Validation Reports (Cont.)

Test Article: Doravirine

Validation Report Number	VR 406 GLP Dog Version 1[a]	VR 406 GLP Dog Addendum 1 Version 1	VR 406 GLP Dog Addendum 2 Version 1	VR 406 GLP Dog Addendum 3 Version 2	VR 406 GLP Dog Addendum 4 Version 1	VR 406 Non GLP Dog Plasma Version 1
Validation Method Number	SBP 406 V1[b] and SBP 406 V2	SBP 406 V2	SBP 406 V2	SBP 406 V3	SBP 406 V4	SBP 406 V16[e]
Activity	Full validation in dog plasma	Incurred sample reanalysis (Study: TT#[redacted]-6030)	Working Solution Stability Assessments (105 days at 4°C)	Cross-validation of standard material, stock preparation protected and not protected from light	Long-term matrix stability SBP updated with new template and wash for carryover	Robotic method transfer (from Tecan to Hamilton)
Species	Dog	Dog	Dog	Dog	Dog	Dog
Analyte	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine	Doravirine
Matrix	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	12.4 - 4850	12.4 - 4830	_	12.3 - 4780	12.1 - 4370	12.4 to 4850
Standard Inter-day Accuracy Range (%Bias)	-1 to 1	-	-	-3 to 2[c]	-	-
Standard Inter-day Precision Range (% CV)	2 to 6	-	-	0 to 7[c]	-	-
QC Nominal Concentration (ng/mL)	29.5, 1480, 3690	29.3, 1470, 3660	29.5, 1480, 3690	29.1, 1450, 3630 30.0, 1500, 3750	-	30.2, 505, 4020
QC Inter-day Accuracy Range (%Bias)	-2 to 3	-	-	-3 to 1[c] -5 to 0[d]	-	-2 to 0
QC Inter-day Precision Range (%CV)	2 to 4	-	-	2 to 7[c] 2 to 6[d]	-	1 to 7
Matrix LTS (at -70°C)	23 Days	-	-	-	1555 Days	-
Dilution Integrity QC (ng/mL) (Dilution)	47200 (1/200)	-	-	-	-	4020 (1/2, 1,10) 27800 (1/100)

Additional Information:

- Abbreviations: CV = Coefficient of variation; GLP = Good Laboratory Practice; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; LLOQ = Lower limit of quantitation; LTS = Long-term stability; QC = Quality control; SIL = Stable labeled internal standard; ULOQ = Upper limit of quantitation; V = version; - = Data not applicable or not available.
- [a] Validation report: VR 406 GLP Dog Version 1.[Ref. 4.2.2.1: 04CFGX].
- [b] Validation method number: SBP 406 V1.[Ref. 4.2.2.1: 049748].
- [c] Intra-day accuracy and precision range reported (preparation with [redacted]-000V weigh not protected from light).
- [d] Intra-day accuracy and precision range reported (preparation with [redacted] -005F weigh protected from light).
- [e] Validation method number: SBP 406 V16 [Ref. 4.2.2.1: 04B53V]. Publication record for SBP 406 versions 1 to 16 is included in method.

Sec. 2.6.5.3 Absorption

Sec. 2.6.5.3.1 IV and P.O. Pharmacokinetic Parameters of Doravirine in Male CD-1 Mice and Wistar Hannover Rats

Test Article: Doravirine

Study Number	[Ref. 4.2.2.2: PK011MK1439]	[Ref. 4.2.2.2: PK011MK1439]	[Ref. 4.2.2.2: PK001MK1439]	[Ref. 4.2.2.2: PK001MK1439]
Species / Strain	Mouse/CD-1	Mouse/CD-1	Rat/Wistar Hannover	Rat/Wistar Hannover
Gender / Number of animals	M/15 (3/time point)	M/12 (3/time point)	M/3	M/3
Feeding condition	Fasted	Fasted	Fasted	Fasted
Vehicle/Formulation	20% DMSO/60% PEG400/20% Water/Solution	0.5% Methyl cellulose with 5mM HCl/Suspension	20% DMSO/60% PEG400/20% Water/Solution	0.5% Methyl cellulose with 5 mM HCl/Suspension
Method of administration	IV	P.O.	IV	P.O.
Dose (mg/kg)	1	5	2	5
Sample	Plasma	Plasma	Plasma	Plasma
Analyte	Doravirine	Doravirine	Doravirine	Doravirine
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters:				
Total CL_p (mL/min/kg)	6.05	NA	2.6 ± 1.6	NA
Vd_{ss} (L/kg)	0.99	NA	1.4 ± 0.8	NA
$T_{1/2}$ (hr)	2.7	NA	6.4 ± 1.5	NA
$AUC_{0-\infty}$ (µM•hr)	6.33	12.45	37.3 ± 17.2	43.9 ± 17.0
C_{max} (µM)	NA	2.07	NA	4.1 ± 1.1
T_{max} (hr)	NA	1.0	NA	3.3 ± 1.2
Bioavailability (%)	NA	39.0	NA	46 ± 18

Additional Information:

- DMSO = dimethylsulfoxide; HCl = Hydrochloric acid; NA = Not applicable.
- Values are shown as mean or mean ± standard deviation. Bioavailability in mice was calculated using the mean dose-normalized AUC values after oral vs. IV administration. Bioavailability in rats was calculated using the individual dose-normalized oral AUC values vs. mean IV AUC.
- Plasma was obtained from serial bleeding of 3 animals for rats and by sequential orbital and terminal bleeding for mice, so that 2 sampling times were taken from each animal.
- [redacted] doravirine on hydroxypropyl methylcellulose acetate succinate-LG (HPMCAS)-LG polymer at a drug load of [redacted]% (w/w) was used for the oral suspension formulations.

Sec. 2.6.5.3.2 IV and P.O. Pharmacokinetic Parameters of Doravirine in Female Dutch-Belted Rabbits and Male Beagle Dogs

Test Article: Doravirine

Study Number	[Ref. 4.2.2.2: PK011MK1439]	[Ref. 4.2.2.2: PK011MK1439]	[Ref. 4.2.2.2: PK001MK1439]	[Ref. 4.2.2.2: PK001MK1439]
Species / Strain	Rabbit/Dutch Belted	Rabbit/Dutch Belted	Dog/Beagle	Dog/Beagle
Gender / Number of animals	F/3	F/3	M/3	M/3
Feeding condition	Fasted	Fasted	Fasted	Fasted
Vehicle/Formulation	20% DMSO/60% PEG400/20%Water/Solution	0.5% Methyl cellulose/ 5mM HCL/Suspension	20% DMSO/60% PEG400/20%Water/Solution	0.5% Methyl cellulose/ 5mM HCL/Suspension
Method of administration	IV	P.O.	IV	P.O.
Dose (mg/kg)	1	5	1	5
Sample	Plasma	Plasma	Plasma	Plasma
Analyte	Doravirine	Doravirine	Doravirine	Doravirine
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Mean ± SD):				
Total CL_p (mL/min/kg)	4.0 ± 0.50	NA	0.44 ± 0.04	NA
Vd_{ss} (L/kg)	2.67 ± 0.38	NA	0.9 ± 0.05	NA
$T_{1/2}$ (hr)	9.03 ± 0.51	NA	21.7 ± 2.1	NA
$AUC_{0-\infty}$ (µM•hr)	9.98 ± 1.26	20.53 ± 8.67	88.9 ± 8.73	205 ± 47
C_{max} (µM)	NA	1.78 ± 0.53	NA	5.7 ± 1.5
T_{max} (hr)	NA	3.5 ± 2.78	NA	0.8 ± 0.3
Bioavailability (%)	NA	41 ± 13	NA	47 ± 14

Additional Information:

- Abbreviations: SD = Standard Deviation; NA = Not Applicable.
- [redacted] doravirine on HPMCAS-LG polymer at a drug load of [redacted]% (w/w) was used for the oral suspension formulations.
- Bioavailability (F%) in dogs and rabbits were calculated in a crossover fashion using the $AUC_{0-\infty}$ value at 5 mg/kg P.O. relative to the $AUC_{0-\infty}$ at 1 mg/kg IV for each animal.

Sec. 2.6.5.3.3 Comparison of Pharmacokinetic Parameters of Doravirine in Male Wistar Hannover Rats and Male Beagle Dogs Following Oral Administration of Various Formulations

Test Article: Doravirine

Study Number		[Ref. 4.2.2.2: PK020MK1439]					
Species / Strain		Rat/Wistar Hannover, Dog/Beagle					
Gender / Number of animals		M/2 or 3					
Method of administration		PO					
Sample		Plasma					
Analyte		Doravirine					
Assay		LC-MS/MS					
Species	**Form**	**Vehicle**	**Dose (mg/kg)**	**Cmax (µM)**	**Tmax (hr)**	**$AUC_{0\text{-}24h}$ (µM•hr)**	**$nAUC_{0\text{-}24\ hr}$[f] (µM•hr)**
Rat	[redacted]	10% Polysorbate 80	1	1.2	2.0	7.3	7.3
		10% Polysorbate 80	100	2.7	4.0	30.4	0.30
	Ball Milled	10% Polysorbate 80	1	0.8	2.0	5.8	5.8
		10% Polysorbate 80	10	3.4	2.0	37.6	3.76
		10% Polysorbate 80	100	9.9	2.0	102	1.02
	[redacted]	Emulsifying lipids[a]	100	4.9	8.0	74.1	0.74
	Nanosuspension	HPC-dextrose in water[b]	50, BID	6.2	10	74.2	1.48
		HPC-dextrose in water[b]	100	4.5	6.0	60.0	0.60
	[redacted][c]	0.5% MC[d] + 10 mM citrate	50, BID	9.4 ± 0.6	7.3 ± 3.1	134 ± 62	2.68
		0.5% MC[d] + 10 mM citrate	100	8.83	6	136	1.36
Dog	Jet Milled	10% Polysorbate 80	1000	13.6	4.0	283	0.28
	Nanosuspension	HPC-DOSS in water[e]	450	7.3	6.0	147	0.33

Additional Information:

- Data represent mean values of n=2 or mean values of n=3 ± standard deviation.
- BID dosing at 50 mg/kg per dose for a total daily dose of 100 mg/kg.
- Ball milled, nanosuspension and jet milled formulations were prepared using [redacted] doravirine. The nanosuspension and jet-milled doravirine formulations had mean particle sizes of 0.2 µm and 5.2 µm, respectively. Ball milled formulations have typical particle size of 20 µm. The [redacted] formulation was prepared with [redacted] doravirine stabilized in [redacted] form with a polymer, in this case, HPMCAS.
- [a] Emulsifying lipids: Labrasol (79%) Capryol 90 (13%) and Labrafil M 1944 CS (8%).
- [b] HPC = hydroxypropylcellulose-SL (0.25% w:v) and dextrose (5% w:v) in water.
- [c] [redacted] doravirine, at a [redacted]% drug load with HPMCAS.
- [d] MC = methylcellulose.
- [e] HPC-DOSS = hydroxypropylcellulose-SL (10% w:v) and dioctyl sulfosuccinate sodium salt (5% w:v) in water.
- [f] nAUC = normalized AUC was calculated by dividing the $AUC_{0\text{-}24\ hr}$ by the dose.

Sec. 2.6.5.4 Distribution

Sec. 2.6.5.4.1 Concentrations of Radioactivity in Tissues of Male Wistar Hannover (Albino) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg

Test Article: Doravirine

Study Number		[Ref. 4.2.2.3: PK005MK1439]								
Species		Wistar Hannover Rats								
Gender / Number of animals		Male / 9 (1 per time point)								
Feeding condition		Fasted								
Vehicle/Formulation		10% polysorbate 80 in water/Solution								
Method of administration		Oral gavage								
Dose (mg/kg/µCi/kg)		5/100								
Radionuclide		^{14}C								
Specific activity (µCi/mg)		20.33								
Analyte		Radioactivity								
Assay		Quantitative whole body autoradiography, Liquid scintillation counting (plasma)								
Sampling times		0.5, 1, 2, 4, 8, 12, 24, 48, and 168 hr								
Tissue Type	**Tissue**	**Concentration (µg equiv/g)**								
		Rat # 1 0.5 hr	**Rat # 2 1 hr**	**Rat # 3 2 hr**	**Rat # 4 4 hr**	**Rat # 5 8 hr**	**Rat # 6 12 hr**	**Rat # 7 24 hr**	**Rat # 8 48 hr**	**Rat # 9 168 hr**
Vascular/ Lymphatic	Aorta	0.984	2.216	2.805	1.476	1.063	0.715	0.302	BLQ	BLQ
	Blood (cardiac)	0.462	0.817	0.932	0.604	0.296	0.203	0.084	BLQ	BLQ
	Bone Marrow	1.216	1.387	2.031	0.979	0.537	0.399	0.161	BLQ	BLQ
	Lymph Node	1.052	1.454	2.104	1.308	0.706	0.347	0.095	BLQ	BLQ
	Plasma	0.875	1.048	1.415	0.829	0.538	0.367	0.102	0.014	BLQ
	Spleen	1.599	1.462	2.554	1.545	0.771	0.480	0.171	BLQ	BLQ
	Thymus	0.693	1.440	1.954	1.229	0.669	0.330	0.119	BLQ	BLQ
Excretory/ Metabolic	Bile (in duct)	4.829	17.817	31.828	15.162	9.068	10.458	2.536	BLQ	BLQ
	Kidney (cortex)	4.094	4.674	6.879	4.013	1.895	1.408	0.392	0.034	BLQ
	Kidney (medulla)	2.093	2.658	3.917	1.933	1.114	0.789	0.186	0.024	BLQ
	Liver	7.388	9.022	10.155	5.350	3.616	2.526	0.654	0.184	0.019
	Urinary Bladder	2.331	0.880	1.803	0.887	3.113	NI	0.111	BLQ	BLQ
	Urinary Bladder (content)	1.411	1.849	6.929	6.492	0.359	0.439	1.009	BLQ	BLQ

Sec. 2.6.5.4.1 Concentrations of Radioactivity in Tissues of Male Wistar Hannover (Albino) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Concentration (µg equiv/g)								
		Rat # 1 0.5 hr	Rat # 2 1 hr	Rat # 3 2 hr	Rat # 4 4 hr	Rat # 5 8 hr	Rat # 6 12 hr	Rat # 7 24 hr	Rat # 8 48 hr	Rat # 9 168 hr
Central Nervous System	Brain (cerebellum)	0.056	0.065	0.103	0.071	0.067	0.047	0.033	BLQ	BLQ
	Brain (cerebrum)	0.018	0.034	0.101	0.072	0.078	0.042	0.022	BLQ	BLQ
	Brain (medulla)	0.067	0.079	0.115	0.095	0.070	0.053	0.019	BLQ	BLQ
	Brain (olfactory lobes)	0.057	0.029	0.095	0.061	0.085	0.027	0.031	BLQ	BLQ
	Choroid Plexus	0.547	1.157	1.312	1.323	0.699	0.309	BLQ	BLQ	BLQ
	Spinal Cord	0.049	0.053	0.117	0.069	0.09	0.058	0.026	BLQ	BLQ
Endocrine	Adrenal gland	3.813	5.091	7.107	3.635	2.019	1.472	0.512	BLQ	BLQ
	Pituitary Gland	1.915	2.512	2.775	1.622	1.154	0.574	0.310	BLQ	BLQ
	Thyroid	2.054	2.368	3.696	BLQ	1.330	0.697	0.268	BLQ	BLQ
Secretory	Exorbital Lacrimal Gland	1.720	2.477	4.217	2.746	1.294	0.853	0.322	BLQ	BLQ
	Intraorbital Lacrimal Gland	1.486	2.860	4.340	2.896	1.328	0.894	NI	BLQ	BLQ
	Harderian Gland	1.359	2.607	6.466	3.569	1.495	1.124	0.386	BLQ	BLQ
	Mammary Gland Region	0.199	0.431	NI	0.031	BLQ	BLQ	0.036	BLQ	BLQ
	Pancreas	2.916	3.321	5.146	2.629	1.334	0.841	0.345	0.027	BLQ
	Preputial Gland	1.397	2.632	4.218	3.179	2.244	2.325	1.747	0.621	BLQ
	Salivary Gland	2.306	3.175	3.705	2.321	1.137	0.705	0.226	BLQ	BLQ
Fatty	Adipose (brown)	2.176	2.130	4.766	2.451	1.424	0.594	0.222	BLQ	BLQ
	Adipose (white)	0.417	0.513	0.447	0.411	0.163	0.153	0.021	BLQ	BLQ
Dermal	Skin (non-pigmented)	0.411	0.572	1.115	0.701	0.457	0.337	0.077	BLQ	BLQ
Reproductive	Epididymis	0.328	0.959	1.684	1.198	0.890	0.534	0.173	BLQ	BLQ
	Prostate Gland	0.756	1.482	2.767	1.404	0.774	0.446	0.093	BLQ	BLQ
	Seminal Vesicles	0.099	0.217	0.642	0.550	0.390	0.205	0.091	BLQ	BLQ
	Testis	0.067	0.184	0.356	0.442	0.419	0.287	0.096	0.021	BLQ
Skeletal/ Muscular	Bone	0.053	0.096	0.134	0.077	0.041	0.022	BLQ	BLQ	BLQ
	Diaphragm	1.099	1.951	2.856	1.675	0.950	0.575	0.193	BLQ	BLQ
	Heart	1.843	3.400	4.127	2.079	1.220	0.712	0.259	BLQ	BLQ
	Skeletal Muscle	0.555	1.035	2.059	1.365	0.660	0.413	0.167	BLQ	BLQ

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Sec. 2.6.5.4.1 Concentrations of Radioactivity in Tissues of Male Wistar Hannover (Albino) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Concentration (µg equiv/g)								
		Rat # 1 0.5 hr	Rat # 2 1 hr	Rat # 3 2 hr	Rat # 4 4 hr	Rat # 5 8 hr	Rat # 6 12 hr	Rat # 7 24 hr	Rat # 8 48 hr	Rat # 9 168 hr
Respiratory	Lung	1.293	1.518	2.649	1.300	0.735	0.468	0.144	BLQ	BLQ
	Nasal Turbinates	0.187	0.452	0.491	0.313	0.219	0.159	0.062	BLQ	BLQ
Alimentary Canal	Cecum	1.816	1.170	3.259	1.309	4.875	3.379	0.692	0.031	BLQ
	Cecum (contents)	0.025	1.969	2.013	6.783	130.653	62.453	9.343	0.585	BLQ
	Colon	1.051	1.333	4.221	1.568	0.938	0.472	0.687	0.029	BLQ
	Colon (contents)	0.017	BLQ	0.110	2.820	14.379	126.823	9.902	1.086	BLQ
	Esophagus	0.708	1.386	1.946	0.930	0.606	1.193	0.114	BLQ	BLQ
	Oral Mucosa	0.623	0.956	1.404	0.832	0.403	0.205	0.140	BLQ	BLQ
	Small Intestine	1.824	5.871	10.301	8.666	1.784	1.096	1.687	0.030	BLQ
	Small Intestine (contents)	93.097	72.591	40.374	40.607	25.079	21.647	5.925	0.324	BLQ
	Stomach (gastric mucosa)	5.159	3.189	4.625	3.094	1.228	0.873	0.355	0.019	BLQ
	Stomach (contents)	523.613[a]	543.816[a]	790.366[a]	138.778	0.070	0.029	5.820	0.073	BLQ
Ocular	Eye (lens)	BLQ	BLQ	0.030	BLQ	0.071	0.045	0.046	BLQ	BLQ
	Eye (uvea)	0.192	1.275	1.446	0.958	0.531	0.396	0.051	BLQ	BLQ

Additional Information:

- Abbreviations: BLQ = Value is below the LLOQ; NI = Tissue not identified on sections.
- Lower Limit of Quantification (LLOQ) = 0.017 µg DOR equivalents/g tissue. Upper Limit of Quantification (ULOQ) = 264.8 µg DOR equivalent/g tissue.
- [a] Above the upper limit of quantification; value is believed to be reliable due to the linearity of phosphor imaging.

Sec. 2.6.5.4.2 Tissue to Plasma Ratios of Radioactivity in Male Wistar Hannover (Albino) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK005MK1439]
Species	Wistar Hannover Rats
Gender / Number of animals	Male / 9 (1 per time point)
Feeding condition	Fasted
Vehicle/Formulation	10% polysorbate 80 in water/Solution
Method of administration	Oral gavage
Dose (mg/kg/µCi/kg)	5/100
Radionuclide	^{14}C
Specific activity (µCi/mg)	20.33
Analyte	Radioactivity
Assay	Quantitative whole body autoradiography, Liquid scintillation counting
Sampling times	0.5, 1, 2, 4, 8, 12, 24, 48, and 168 hr

Tissue Type	Tissue	Tissue to Plasma Ratio								
		Rat # 1 0.5 hr	**Rat # 2 1 hr**	**Rat # 3 2 hr**	**Rat # 4 4 hr**	**Rat # 5 8 hr**	**Rat # 6 12 hr**	**Rat # 7 24 hr**	**Rat # 8 48 hr**	**Rat # 9 168 hr**
Vascular/ Lymphatic	Aorta	1.12	2.11	1.98	1.78	1.98	1.95	2.96	ND	ND
	Blood (cardiac)	0.53	0.78	0.66	0.73	0.55	0.55	0.82	ND	ND
	Bone Marrow	1.39	1.32	1.44	1.18	1.00	1.09	1.58	ND	ND
	Lymph Node	1.20	1.39	1.49	1.58	1.31	0.95	0.93	ND	ND
	Spleen	1.83	1.40	1.80	1.86	1.43	1.31	1.68	ND	ND
	Thymus	0.79	1.37	1.38	1.48	1.24	0.90	1.17	ND	ND
Excretory/ Metabolic	Bile (in duct)	5.52	17.00	22.49	18.29	16.86	28.50	24.86	ND	ND
	Kidney (cortex)	4.68	4.46	4.86	4.84	3.52	3.84	3.84	2.43	ND
	Kidney (medulla)	2.39	2.54	2.77	2.33	2.07	2.15	1.82	1.71	ND
	Liver	8.44	8.61	7.18	6.45	6.72	6.88	6.41	13.14	ND
	Urinary Bladder	2.66	0.84	1.27	1.07	5.79	ND	1.09	ND	ND
	Urinary Bladder (content)	1.61	1.76	4.90	7.83	0.67	1.20	9.89	ND	ND

Sec. 2.6.5.4.2 Tissue to Plasma Ratios of Radioactivity in Male Wistar Hannover (Albino) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Tissue to Plasma Ratio								
		Rat # 1 0.5 hr	Rat # 2 1 hr	Rat # 3 2 hr	Rat # 4 4 hr	Rat # 5 8 hr	Rat # 6 12 hr	Rat # 7 24 hr	Rat # 8 48 hr	Rat # 9 168 hr
Central Nervous System	Brain (cerebellum)	0.06	0.06	0.07	0.09	0.12	0.13	0.32	ND	ND
	Brain (cerebrum)	0.02	0.03	0.07	0.09	0.14	0.11	0.22	ND	ND
	Brain (medulla)	0.08	0.08	0.08	0.11	0.13	0.14	0.19	ND	ND
	Brain (olfactory lobes)	0.07	0.03	0.07	0.07	0.16	0.07	0.30	ND	ND
	Choroid Plexus	0.63	1.10	0.93	1.60	1.30	0.84	ND	ND	ND
	Spinal Cord	0.06	0.05	0.08	0.08	0.17	0.16	0.25	ND	ND
Endocrine	Adrenal gland	4.36	4.86	5.02	4.38	3.75	4.01	5.02	ND	ND
	Pituitary Gland	2.19	2.40	1.96	1.96	2.14	1.56	3.04	ND	ND
	Thyroid	2.35	2.26	2.61	ND	2.47	1.90	2.63	ND	ND
Secretory	Exorbital Lacrimal Gland	1.97	2.36	2.98	3.31	2.41	2.32	3.16	ND	ND
	Intraorbital Lacrimal Gland	1.70	2.73	3.07	3.49	2.47	2.44	ND	ND	ND
	Harderian Gland	1.55	2.49	4.57	4.31	2.78	3.06	3.78	ND	ND
	Mammary Gland Region	0.23	0.41	ND	0.04	ND	ND	0.35	ND	ND
	Pancreas	3.33	3.17	3.64	3.17	2.48	2.29	3.38	1.93	ND
	Preputial Gland	1.60	2.51	2.98	3.83	4.17	6.34	17.13	44.36	ND
	Salivary Gland	2.64	3.03	2.62	2.80	2.11	1.92	2.22	ND	ND
Fatty	Adipose (brown)	2.49	2.03	3.37	2.96	2.65	1.62	2.18	ND	ND
	Adipose (white)	0.48	0.49	0.32	0.50	0.30	0.42	0.21	ND	ND
Dermal	Skin (non-pigmented)	0.47	0.55	0.79	0.85	0.85	0.92	0.75	ND	ND
Reproductive	Epididymis	0.37	0.92	1.19	1.45	1.65	1.46	1.70	ND	ND
	Prostate Gland	0.86	1.41	1.96	1.69	1.44	1.22	0.91	ND	ND
	Seminal Vesicles	0.11	0.21	0.45	0.66	0.72	0.56	0.89	ND	ND
	Testis	0.08	0.18	0.25	0.53	0.78	0.78	0.94	1.50	ND
Skeletal/ Muscular	Bone	0.06	0.09	0.09	0.09	0.08	0.06	ND	ND	ND
	Diaphragm	1.26	1.86	2.02	2.02	1.77	1.57	1.89	ND	ND
	Heart	2.11	3.24	2.92	2.51	2.27	1.94	2.54	ND	ND
	Skeletal Muscle	0.63	0.99	1.46	1.65	1.23	1.13	1.64	ND	ND

Sec. 2.6.5.4.2 Tissue to Plasma Ratios of Radioactivity in Male Wistar Hannover (Albino) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Tissue to Plasma Ratio								
		Rat # 1 0.5 hr	Rat # 2 1 hr	Rat # 3 2 hr	Rat # 4 4 hr	Rat # 5 8 hr	Rat # 6 12 hr	Rat # 7 24 hr	Rat # 8 48 hr	Rat # 9 168 hr
Respiratory	Lung	1.48	1.45	1.87	1.57	1.37	1.28	1.41	ND	ND
	Nasal Turbinates	0.21	0.43	0.35	0.38	0.41	0.43	0.61	ND	ND
Alimentary Canal	Cecum	2.08	1.12	2.30	1.58	9.06	9.21	6.78	2.21	ND
	Cecum (contents)	0.03	1.88	1.42	8.18	242.85	170.17	91.60	41.79	ND
	Colon	1.20	1.27	2.98	1.89	1.74	1.29	6.74	2.07	ND
	Colon (contents)	0.02	ND	0.08	3.40	26.73	345.57	97.08	77.57	ND
	Esophagus	0.81	1.32	1.38	1.12	1.13	3.25	1.12	ND	ND
	Oral Mucosa	0.71	0.91	0.99	1.00	0.75	0.56	1.37	ND	ND
	Small Intestine	2.08	5.60	7.28	10.45	3.32	2.99	16.54	2.14	ND
	Small Intestine (contents)	106.40	69.27	28.53	48.98	46.62	58.98	58.09	23.14	ND
	Stomach (gastric mucosa)	5.90	3.04	3.27	3.73	2.28	2.38	3.48	1.36	ND
	Stomach (contents)	598.41[a]	518.91[a]	558.56[a]	167.40	0.13	0.08	57.06	5.21	ND
Ocular	Eye (lens)	ND	ND	0.02	ND	0.13	0.12	0.45	ND	ND
	Eye (uvea)	0.22	1.22	1.02	1.16	0.99	1.08	0.50	ND	ND

Additional Information:

- Abbreviations: ND = Not determined due to tissue levels being below the limit of quantification.
- [a] Tissue concentration was above upper limit of quantification; value is believed to be reliable due to the linearity of phosphor imaging.

Sec. 2.6.5.4.3 Concentrations of Radioactivity in Tissues of Male Long-Evans (Partially-Pigmented) Rats After a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg

Test Article: Doravirine

Study Number		[Ref. 4.2.2.3: PK005MK1439]				
Species		Long Evans Rats				
Gender / Number of animals		Male / 9 (1 per time point)				
Feeding condition		Fasted				
Vehicle/Formulation		10% polysorbate 80 in water/Solution				
Method of administration		Oral gavage				
Dose (mg/kg/µCi/kg)		5/100				
Radionuclide / Specific activity (µCi/mg)		^{14}C / 20.33				
Analyte		Radioactivity				
Assay		Quantitative whole body autoradiography, Liquid scintillation counting (plasma)				
Sampling times		2, 8, 24, 168, and 504 hr				
Tissue Type	**Tissue**	**Concentration (µg equiv/g)**				
		Rat # 10 2 hr	**Rat # 11 8 hr**	**Rat # 12 24 hr**	**Rat # 13 168 hr**	**Rat # 14 504 hr**
Vascular/ Lymphatic	Aorta	2.536	0.927	BLQ	BLQ	BLQ
	Blood (cardiac)	0.894	0.277	BLQ	BLQ	BLQ
	Bone Marrow	1.278	0.335	0.032	BLQ	BLQ
	Lymph Node	1.784	0.573	0.03	BLQ	BLQ
	Plasma	1.083	0.503	0.095	BLQ	BLQ
	Spleen	1.522	0.471	0.033	BLQ	BLQ
	Thymus	1.8	0.405	BLQ	BLQ	BLQ
Excretory/ Metabolic	Bile (in duct)	23.456	NI	2.094	0.019	BLQ
	Kidney (cortex)	3.939	1.604	0.167	BLQ	BLQ
	Kidney (medulla)	2.34	0.796	0.116	BLQ	BLQ
	Liver	8.023	2.68	0.419	0.026	BLQ
	Urinary Bladder	1.297	0.484	0.043	BLQ	BLQ
	Urinary Bladder (content)	5.358	5.019	0.159	BLQ	BLQ
Central Nervous System	Brain (cerebellum)	0.105	0.061	BLQ	BLQ	BLQ
	Brain (cerebrum)	0.126	0.034	BLQ	BLQ	BLQ
	Brain (medulla)	0.131	0.068	BLQ	BLQ	BLQ
	Brain (olfactory lobes)	0.074	0.054	0.017	BLQ	BLQ
	Choroid Plexus	1.561	0.434	BLQ	BLQ	BLQ
	Spinal Cord	0.115	0.077	BLQ	BLQ	BLQ

Sec. 2.6.5.4.3 Concentrations of Radioactivity in Tissues of Male Long-Evans (Partially-Pigmented) Rats After a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Concentration (µg equiv/g)				
		Rat # 10 2 hr	Rat # 11 8 hr	Rat # 12 24 hr	Rat # 13 168 hr	Rat # 14 504 hr
Endocrine	Adrenal gland	4.894	1.258	0.166	BLQ	BLQ
	Pituitary Gland	2.676	0.648	0.078	BLQ	BLQ
	Thyroid	3.221	1.079	BLQ	BLQ	BLQ
Secretory	Exorbital Lacrimal Gland	3.698	0.872	0.063	BLQ	BLQ
	Intraorbital Lacrimal Gland	4.410	0.685	0.097	BLQ	BLQ
	Harderian Gland	3.838	1.074	0.132	BLQ	BLQ
	Mammary Gland Region	0.353	0.111	BLQ	BLQ	BLQ
	Pancreas	3.086	1.154	0.095	BLQ	BLQ
	Preputial Gland	3.349	1.794	1.000	BLQ	BLQ
	Salivary Gland	3.760	0.911	0.067	BLQ	BLQ
Fatty	Adipose (brown)	2.888	0.845	0.052	BLQ	BLQ
	Adipose (white)	0.349	0.102	BLQ	BLQ	BLQ
Dermal	Skin (non-pigmented)	0.858	0.309	0.105	BLQ	BLQ
	Skin (pigmented)	0.910	0.261	0.182	BLQ	BLQ
Reproductive	Epididymis	1.745	0.615	0.029	BLQ	BLQ
	Prostate Gland	0.874	0.358	BLQ	BLQ	BLQ
	Seminal Vesicles	0.655	0.252	BLQ	BLQ	BLQ
	Testis	0.526	0.362	0.021	BLQ	BLQ
Skeletal/ Muscular	Bone	0.107	0.035	BLQ	BLQ	BLQ
	Diaphragm	2.724	0.795	0.036	BLQ	BLQ
	Heart	3.238	0.995	0.068	BLQ	BLQ
	Skeletal Muscle	1.454	0.407	0.024	BLQ	BLQ
Respiratory	Lung	1.807	0.542	0.046	BLQ	BLQ
	Nasal Turbinates	0.688	0.266	0.037	BLQ	BLQ

Sec. 2.6.5.4.3 Concentrations of Radioactivity in Tissues of Male Long-Evans (Partially-Pigmented) Rats After a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Concentration (µg equiv/g)				
		Rat # 10 2 hr	Rat # 11 8 hr	Rat # 12 24 hr	Rat # 13 168 hr	Rat # 14 504 hr
Alimentary Canal	Cecum	1.471	5.444	0.289	BLQ	BLQ
	Cecum (contents)	9.028	174.249	8.862	BLQ	BLQ
	Colon	2.048	1.827	1.252	BLQ	BLQ
	Colon (contents)	0.235	206.798	8.133	BLQ	BLQ
	Esophagus	1.377	0.406	0.085	BLQ	BLQ
	Oral Mucosa	1.331	0.359	0.062	BLQ	BLQ
	Small Intestine	2.241	1.977	0.066	BLQ	BLQ
	Small Intestine (contents)	17.184	29.123	1.788	BLQ	BLQ
	Stomach (gastric mucosa)	1.181	1.180	0.076	BLQ	BLQ
	Stomach (contents)	45.014	0.814	BLQ	BLQ	BLQ
Ocular	Eye (lens)	0.023	0.059	BLQ	BLQ	BLQ
	Eye (uvea)	2.056	1.015	BLQ	BLQ	BLQ

Additional Information:

- Abbreviations: BLQ = Value is below the lower limit of quantification (LLOQ); ULOQ = Upper limit of quantification.
- LLOQ = 0.017 µg DOR equivalent /g tissue.
- ULOQ = 264.8 µg DOR equivalent /g tissue.

Sec. 2.6.5.4.4 Tissue to Plasma Ratios of Radioactivity in Male Long-Evans (Partially-Pigmented) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg

Test Article: Doravirine

Study Number		[Ref. 4.2.2.3: PK005MK1439]				
Species		Long Evans Rats				
Gender / Number of animals		Male / 9 (1 per time point)				
Feeding condition		Fasted				
Vehicle/Formulation		10% polysorbate 80 in water/Solution				
Method of administration		Oral gavage				
Dose (mg/kg/µCi/kg)		5/100				
Radionuclide / Specific activity (µCi/mg)		^{14}C / 20.33				
Analyte		Radioactivity				
Assay		Quantitative whole body autoradiography, Liquid scintillation counting (plasma)				
Sampling times		2, 8, 24, 168, and 504 hr				
Tissue Type	**Tissue**	**Tissue to Plasma Ratio**				
		Rat # 10 2 hr	**Rat # 11 8 hr**	**Rat # 12 24 hr**	**Rat # 13 168 hr**	**Rat # 14 504 hr**
Vascular/ Lymphatic	Aorta	2.34	1.84	ND	ND	ND
	Blood (cardiac)	0.83	0.55	ND	ND	ND
	Bone Marrow	1.18	0.67	0.34	ND	ND
	Lymph Node	1.65	1.14	0.32	ND	ND
	Spleen	1.41	0.94	0.35	ND	ND
	Thymus	1.66	0.81	ND	ND	ND
Excretory/ Metabolic	Bile (in duct)	21.66	ND	22.04	ND	ND
	Kidney (cortex)	3.64	3.19	1.76	ND	ND
	Kidney (medulla)	2.16	1.58	1.22	ND	ND
	Liver	7.41	5.33	4.41	ND	ND
	Urinary Bladder	1.20	0.96	0.45	ND	ND
	Urinary Bladder (content)	4.95	9.98	1.67	ND	ND
Central Nervous System	Brain (cerebellum)	0.10	0.12	ND	ND	ND
	Brain (cerebrum)	0.12	0.07	ND	ND	ND
	Brain (medulla)	0.12	0.14	ND	ND	ND
	Brain (olfactory lobes)	0.07	0.11	0.18	ND	ND
	Choroid Plexus	1.44	0.86	ND	ND	ND

Sec. 2.6.5.4.4 Tissue to Plasma Ratios of Radioactivity in Male Long-Evans (Partially-Pigmented) Rats after a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Tissue to Plasma Ratio				
		Rat # 10 2 hr	Rat # 11 8 hr	Rat # 12 24 hr	Rat # 13 168 hr	Rat # 14 504 hr
Central Nervous System (Cont.)	Spinal Cord	0.11	0.15	ND	ND	ND
Endocrine	Adrenal gland	4.52	2.50	1.75	ND	ND
	Pituitary Gland	2.47	1.29	0.82	ND	ND
	Thyroid	2.97	2.15	ND	ND	ND
Secretory	Exorbital Lacrimal Gland	3.41	1.73	0.66	ND	ND
	Intraorbital Lacrimal Gland	4.07	1.36	1.02	ND	ND
	Harderian Gland	3.54	2.14	1.39	ND	ND
	Mammary Gland Region	0.33	0.22	ND	ND	ND
	Pancreas	2.85	2.29	1.00	ND	ND
	Preputial Gland	3.09	3.57	10.53	ND	ND
	Salivary Gland	3.47	1.81	0.71	ND	ND
Fatty	Adipose (brown)	2.67	1.68	0.55	ND	ND
	Adipose (white)	0.32	0.20	ND	ND	ND
Dermal	Skin (non-pigmented)	0.79	0.61	1.11	ND	ND
	Skin (pigmented)	0.84	0.52	1.92	ND	ND
Reproductive	Epididymis	1.61	1.22	0.31	ND	ND
	Prostate Gland	0.81	0.71	ND	ND	ND
	Seminal Vesicles	0.60	0.50	ND	ND	ND
	Testis	0.49	0.72	0.22	ND	ND
Skeletal/ Muscular	Bone	0.1	0.07	ND	ND	ND
	Diaphragm	2.52	1.58	0.38	ND	ND
	Heart	2.99	1.98	0.72	ND	ND
	Skeletal Muscle	1.34	0.81	0.25	ND	ND
Respiratory	Lung	1.67	1.08	0.48	ND	ND
	Nasal Turbinates	0.64	0.53	0.39	ND	ND

Sec. 2.6.5.4.4 Tissue to Plasma Ratios of Radioactivity in Male Long-Evans (Partially-Pigmented) Rats After a Single Oral Gavage Dose of [^{14}C]Doravirine at 5 mg/kg (Cont.)

Test Article: Doravirine

Tissue Type	Tissue	Tissue to Plasma Ratio				
		Rat # 10 2 hr	**Rat # 11 8 hr**	**Rat # 12 24 hr**	**Rat # 13 168 hr**	**Rat # 14 504 hr**
Alimentary Canal	Cecum	1.36	10.82	3.04	ND	ND
	Cecum (contents)	8.34	346.42	93.28	ND	ND
	Colon	1.89	3.63	13.18	ND	ND
	Colon (contents)	0.22	411.13	85.61	ND	ND
	Esophagus	1.27	0.81	0.89	ND	ND
	Oral Mucosa	1.23	0.71	0.65	ND	ND
	Small Intestine	2.07	3.93	0.69	ND	ND
	Small Intestine (contents)	15.87	57.90	18.82	ND	ND
	Stomach (gastric mucosa)	1.09	2.35	0.80	ND	ND
	Stomach (contents)	41.56	1.62	ND	ND	ND
Ocular	Eye (lens)	0.02	0.12	ND	ND	ND
	Eye (uvea)	1.90	2.02	ND	ND	ND

Additional Information:
ND = Not determined due to insufficient data.

Sec. 2.6.5.4.5 Maternal and Fetal Plasma Doravirine Concentrations in Rats Following Dosing of Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: TT[redacted]7130FIN]	
Species / Strain	Rat / Wistar Hannover	
Gestation day / Number of animals	Day 20/ 8 per dose	
Vehicle/Formulation	0.5% (w/v) methylcellulose with 5 mM hydrochloric acid in deionized water / Suspension	
Method of administration	Oral gavage	
Analyte	Doravirine	
Assay	LC-MS/MS	
Dose (mg/kg/day)	5	450
Analyte	Doravirine	Doravirine
Gestation day	Day 20	Day 20
Concentration (µM)[a]:		
Maternal plasma		
2 hr (n=4)	4.50 ± 0.312	27.8 ± 1.17
24 hr(n=4)	0.626 ± 0.0901	2.87 ± 0.638
Fetal plasma		
2 hr	2.13 ± 0.120	14.3 ± 0.629
24 hr	0.300 ± 0.0299	1.47 ± 0.405
Ratio[b] (Fetal/Maternal)		
2 hr	0.475 ± 0.0182	0.517 ± 0.0249
24 hr	0.488 ± 0.0178	0.495 ± 0.0323

Additional Information:

- [a] Mean ± SEM (standard error of the mean).
- [b] Mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.
- [redacted] DOR on hydroxypropyl methylcellulose acetate succinate (HPMCAS-LG) polymer at a drug load of [redacted]% (w/w) was used for the oral suspension formulations.
- Doravirine dosing: from Gestation Day 6 to Gestation Day 20.
- Doravirine was orally administered to pregnant rats from gestation days (GD) 6 through 20 at doses of 5 or 450 mg/kg/day of the [redacted] form of doravirine in vehicle (n=8 rats/dose), and doravirine was observed in the maternal and fetal plasma at 2 and 24 hr postdose on GD 20. All rats survived until scheduled study termination.

Sec. 2.6.5.4.6 Maternal and Fetal Plasma Doravirine Concentrations in Rabbits Following Dosing of Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: TT[redacted]7080FIN]
Species / Strain	Rabbit / Dutch Belted
Gestation day / Number of animals	Day 20/ 8 per dose
Vehicle/Formulation	0.5% (w/v) methylcellulose with 5 mM hydrochloric acid in deionized water /Suspension
Method of administration	Oral gavage
Analyte	Doravirine
Assay	LC-MS/MS
Dose (mg/kg/day)	300
Analyte	Doravirine
Gestation day	Day 20
Concentration (µM)[a]	
Maternal plasma	
4 hr (n=4)	35.3 ± 1.30
24 hr (n=4)	10.9 ± 1.17
Fetal plasma	
4 hr	12.8 ± 0.815
24 hr	4.35 ± 0.674
Ratio[b] (Fetal/Maternal)	
4 hr	0.363 ± 0.0107
24 hr	0.404 ± 0.0594

Additional Information:

- [a] Mean ± SEM (standard error of the mean).
- [b] Mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.
- [redacted] of doravirine in HPMCAS-LG polymer.
- Doravirine dosing: from Gestation Day 6 to Gestation Day 20.
- All animals survived until scheduled termination.

Sec. 2.6.5.4.7 Reversible Binding to Plasma Proteins

Test Articles: Doravirine, Doravirine Metabolite M9

Study Number	[Ref. 4.2.2.3: PK003MK1439] [Ref. 4.2.2.3: PK012MK1439] [Ref. 4.2.2.4: PK015MK1439] [Ref. 4.2.2.3: PK016MK1439]				
Study system	In vitro				
Species/Matrix	Mouse, rat, rabbit, dog, human/plasma				
Method	Equilibrium dialysis. Plasma samples containing the target concentrations of DOR or M9 (3to 6 replicates per concentration) were incubated at 37°C in 5% CO_2, for at least 5 hr in dialysis plates to ensure that equilibrium between plasma and buffer chamber was achieved. Aliquots were then taken from each chamber and analyzed.				
Analyte	Radioactivity (^{3}H), Doravirine or M9				
Assays	LSC or LC-MS/MS				
Compound	**Species**	**Unbound Fraction in Plasma**			
		0.1 µM	**1.0 µM**	**3.0 µM**	**5.0 µM**
Doravirine	Mouse	0.242 ± 0.025	0.238 ± 0.027	0.251 ± 0.020	0.262 ± 0.021
	Rat	0.349 ± 0.005	0.324 ± 0.012	0.282 ± 0.021	0.272 ± 0.014
	Rabbit	0.221 ± 0.012	0.240 ± 0.013	0.256 ± 0.029	0.279 ± 0.011
	Dog	0.241 ± 0.005	0.258 ± 0.003	0.187 ± 0.019	0.192 ± 0.009
	Human	0.257 ± 0.003	0.241 ± 0.009	0.233 ± 0.039	0.251 ± 0.032
M9	Rat	0.18 ± 0.016	0.20 ± 0.006	ND	ND
	Dog	0.24 ± 0.007	0.25 ± 0.009	ND	ND
	Human	0.082 ± 0.003	0.088 ± 0.004	ND	ND

Additional Information:

- Abbreviations: LSC -Liquid scintillation counting; ND = Not determined.
- Unbound fraction was determined using the following equation: $Unbound\ fraction = \frac{Concentration\ in\ buffer\ chamber}{Concentration\ in\ plasma\ chamber}$
- Values represent mean of at least three replicate measurements ± standard deviation (n=3).
- LSC was used to measure binding for 0.1 and 1 µM [^{3}H]doravirine in rat, dog, and human plasma. LC-MS/MS was used for all other plasma protein binding assays.

Sec. 2.6.5.4.8 Blood-to-Plasma Partitioning

Test Articles: Doravirine, Doravirine Metabolite M9

Study Number	[Ref. 4.2.2.3: PK003MK1439] [Ref. 4.2.2.3: PK012MK1439] [Ref. 4.2.2.4: PK015MK1439]			
Study system	In vitro			
Species/Matrix	Mouse, rat, rabbit, dog, human/plasma, blood			
Analyte	Radioactivity (^{3}H and ^{14}C)			
Method	The partitioning of DOR into blood cells was determined in incubations with fresh, heparinized whole blood. Three replicates of fresh blood containing the target concentrations of DOR or M9 were incubated at 37°C for 30 min. The samples were then centrifuged at 3000 or 4300 rpm and aliquots of plasma were used for analysis. Radioactivity in these aliquots was compared against radioactivity in aliquots of plasma that were treated with DOR or M9 and used as surrogate of whole blood.			
Assays	LSC			
Compound	**Species**	**Concentration**		
		0.1 µM	**1 µM**	**10 µM**
Doravirine	Mouse	0.65 ± 0.02	0.66 ± 0.02	0.71 ± 0.01
	Rat	1.1 ± 0.1	1.1 ± 0.1	0.92 ± 0.09
	Rabbit	0.97 ± 0.06	1.06 ± 0.02	1.05 ± 0.02
	Dog	0.98 ± 0.07	0.90 ± 0.03	1.0 ± 0.1
	Human	0.92 ± 0.08	0.95 ± 0.07	1.06 ± 0.03
M9	Rat	0.76 ± 0.06	0.70 ± 0.03	ND
	Dog	0.92 ± 0.01	0.82 ± 0.01	ND
	Human	0.66 ± 0.02	0.69 ± 0.08	ND

Additional Information:

- Abbreviations: LSC = Liquid scintillation counting; ND = Not determined.
- The blood –to-plasma distribution ratio was determined according to the equation : $\frac{b}{p} ratio = \frac{Radioactivity\ in\ blood\ surrogate}{radioactivity\ in\ harvested\ plasma}$
- [^{3}H]Doravirine was used for studies with rat, dog, and human blood. [^{14}C]Doravirine was used for studies with mouse and rabbit blood.
- The blood to plasma ratios were obtained using an average of 3 measurements of blood concentration and an average of 3 measurements of plasma concentration.

Sec. 2.6.5.5 Metabolism

Sec. 2.6.5.5.1 The Presence of Doravirine and its Metabolites in Plasma and Excreta From Nonclinical Species and in Humans Following P.O. Administration of [^{14}C] or [^{3}H]Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.4: PK013MK1439]		[Ref. 4.2.2.4: PK002MK1439]		[Ref. 4.2.2.4: PK013MK1439]	[Ref. 4.2.2.4: PK004MK1439	[Ref. 4.2.2.4: PK006MK1439]
Species	Mouse		Rat		Rabbit	Dog	Human
Gender/ Number of Animals	M/3	M/6	M/3	M/3	F/3	M/2	M/6
Feeding Conditions	Fasted	Fasted	Fasted	Fasted	Fasted	Fasted	Fasted
Vehicle/ Formulation	10% PS80/ Solution	10% PS80/ Solution	PEG400/ Solution	10% PS80/ Solution	10% PS80/Solution	10% PS80/Solution	Sodium salt/ HPMC capsules
Method of Administration	P.O.	P.O.	P.O.	P.O.	P.O.	P.O.	P.O.
Dose	5 mg/kg	5 mg/kg	5 mg/kg	10 mg/kg	5mg/kg	5 mg/kg	350 mg
Radionuclide	^{14}C	^{14}C	^{3}H	^{3}H	^{14}C	^{3}H	^{14}C
Specific activity, µCi/mg	19.97	19.97	81.714	6.86	19.95	4.17	0.72
Biological samples	Urine, Feces	Plasma	Bile, Urine	Plasma	Urine, Feces, Plasma	Urine, Bile, Feces, Plasma	Urine, Feces, Plasma
Analytes	Doravirine and metabolites						
Sample Collection and Preparation Methods	Urine samples were pooled proportionally across time points for each animal/subject and equal aliquots from these pools were further pooled across subjects for metabolite profiling. Fecal samples were homogenized with deionized water, pooled proportionally across time points and single animal pools were combined across animals for analysis. Blood samples were collected terminally (rat and mouse) or serially (rabbit and dog) at intervals up to 24, 48, or 72 hr. Plasma was harvested by centrifugation and pooled either in AUC-proportionally or at each time point across animals in the study. Briefly, samples were precipitated with acetonitrile, centrifuged and aliquots of the supernatants were analyzed.						
Assays	LC-MS/MS with on-line radiometric detection or off-line radiometric analysis following fractionation of LC column eluents.						

Sec. 2.6.5.5.1 The Presence of Doravirine and its Metabolites in Plasma and/or Excreta From Nonclinical Species and in Humans Following P.O. Administration of [^{14}C] or [^{3}H]Doravirine (Cont.)

Test Article: Doravirine

Species	Mouse				Rat				Rabbit				Dog				Human			
Biological matrix	**P**	**B**	**U**	**F**	**P**	**B**	**U**	**F**	**P**	**B**	**U**	**F**	**P**	**B**	**U**	**F**	**P**	**B**	**U**	**F**
DOR	~50	NS	2.6	23.7	~100	4.7	18.5	NA	~100	NS	6.9	60.7	~100	0.6	19	24.5	75	NS	2.2	84.1
Unknown	-	NS	4.7	-	-	5.7	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M1	-	NS	-	-	TA	3.3	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M2	-	NS	-	-	-	6.1	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M3	-	NS	-	-	-	2.8	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M4	-	NS	-	-	-	1.8	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M5	-	NS	-	-	-	2.2	-	NA	-	NS	-	-	-	-	-	-	-	NS	TA	TA
M6	-	NS	-	-	-	2.3	-	NA	TA	NS	TA	TA	-	0.9	-	-	-	NS	-	-
M6a	-	NS	-	-	-	-	-	NA	-	NS	TA	-	-	-	-	-	-	NS	-	-
M6b	-	NS	-	-	-	-	-	NA	-	NS	TA	-	-	-	-	-	-	NS	-	-
M7[a]	-	NS	-	-	-	19.5	-	NA	-	NS	-	-	-	-	-	-	2.8[b]	NS	-	-
M8	-	NS	-	-	TA	5.7	1.9	NA	-	NS	-	-	-	-	-	-	TA	NS	0.2	TA
M9	~50	NS	20	11.6	-	2.6	1.4	NA	TA	NS	19.5	TA	-	-	8	4.8	12.9	NS	6.7	2.7
M10	-	NS	-	-	-	-	1.2	NA	-	NS	-	-	-	-	-	-	TA	NS	0.1	TA
M11	TA	NS	TA	TA	TA	-	-	NA	TA	NS	-	-	-	-	-	-	TA	NS	TA	TA
M12	-	NS	2.3	-	TA	-	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M13	-	NS	0.9	-	TA	-	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-
M14	-	NS	TA	-	-	-	-	NA	-	NS	-	-	-	6.5	0.7	-	-	NS	TA	TA
M15	-	NS	TA	-	-	-	-	NA	-	NS	TA	-	TA	6.3	2.1	-	[b]	NS	TA	TA
M16	-	NS		-	-	-	-	NA	-	NS	-	-	TA	-	-	-	-	NS	-	-
M17	TA	NS	-	-	-	-	-	NA	TA	NS	-	TA	TA	-	-	-	-	NS	-	-
M18	-	NS	-	-	-	-	-	NA	-	NS	-	-	-	-	TA	TA	TA	NS	0.2	TA
M19	-	NS	-	-	-	-	-	NA	-	NS	-	-	-	-	TA	TA	TA	NS	TA	TA
M20	-	NS	2.2	-	-	-	-	NA	-	NS	-	-	-	-	-	-	-	NS	-	-

Additional Information:

- Abbreviations: PS80= Polysorbate 80; P = plasma; B = bile; U = urine; F = feces; NS = Not studied due to sample not collected; MA = Matrix collected but not analyzed; TA = Trace amount, detected by LC/MS but not observed in radiomatric profile; - = Not detected.
- Numbers represent percentages relative to the administered dose (B, U, F) or percentages relative to the total radioactivity in the sample (P).
- [a] Studies conducted in rat hepatocytes with authentic standard of M9 confirmed that M7 is derived from M9.
- [b] Coeluted with M7, % radioactivity includes both M7 and M15.

Sec. 2.6.5.5.2 Semi-Quantitative Analysis of Doravirine and Metabolites from Human Plasma (Study 001 at 240 mg) Pooled from 0 to 24 hr

Test Article: Doravirine

Study Number	[Ref. 4.2.2.4: PK008MK1439]				
Species	Human				
Type of study	Metabolite profiling in plasma				
Gender/Number of subjects	M/6				
Methods	Aliquots of plasma samples were obtained on day 1 and day 10 from subjects administered 240 mg doravirine tablets once daily (P001). The samples were combined in an AUC-proportional manner for each subject and each sampling day. Equal volumes from each of the 6 AUC pools were combined for each dosing day into a single sample so that two final pooled samples were obtained. Each sample pool was then processed by protein precipitation and centrifuged. Aliquots of the supernatant were analyzed				
Biological samples:	Plasma				
Analytes	Doravirine and metabolites				
Assays	LC-MS/MS				
	RT	Day 1		Day 10	
	(min)	PAR	%total[a]	PAR	%total[a]
MK-1439	41.8	2.62	85.4	3.61	79.4
M9 (+O)	44.72	0.17	5.6	0.31	6.7
M16 (+CH2)	44.88	0.14	4.5	0.24	5.3
M17 (+CH2)	48.91	0.05	1.8	0.13	2.8
M7 (+O+gluc)	37.26	0.01	0.2	0.01	0.2
M15 (+NAC)	37.83	0.08	2.5	0.13	2.8
M11 (dealk)	45.14	ND	ND	0.06	1.4
M10 (+O2+H2)	37	ND	ND	0.03	0.7
M13 (dealk+O+gluc)	28.93	ND	ND	0.02	0.5

Additional Information:

- Abbreviations: ND = Not detected; PAR = Peak area ratio of analyte to internal standard; RT = Retention Time
- [a] %total = (metabolite PAR*100)/(combined PAR for all drug-related components). Assumes that ionization efficiency of parent and all metabolites are similar, therefore amounts are approximate.

Sec. 2.6.5.5.3 Semi-Quantitative Analysis of Doravirine Metabolite M9 in Rat, Dog, and Human Plasma

Test Article: Doravirine Metabolite M9

Study Number	[Ref. 4.2.2.4: PK019MK1439]				
Type of Study	Exposure multiples of doravirine metabolite M9 in rat and dog plasma vs. human plasma				
Species/Strain	Rat/Wistar Hannover, Dog/Beagle, Human				
Methods	Male and female rat plasma obtained on day 87 from the 30 and 450 mg/kg/day dose groups (TT#[redacted]-6019, SD 87), and male and female dog plasma obtained on day 88 from the 10 and 1000 mg/kg/day dose groups (TT#[redacted]-6018, SD 88) were separately pooled proportionally to the area-under-the-concentration curve (AUC) over a 24 hr period. Aliquots of the pooled rat and dog plasma samples were matrix matched by combining each with an equal volume of control human plasma. Human plasma from healthy volunteers dosed for 10 days with 240 mg doravirine were pooled in an AUC proportional manner over a 24 hr period. Aliquots of the pooled human sample were matrix matched by combining with an equal volume of either rat or dog control plasma. The rat, dog and human pooled plasma samples were treated with acentonitrile:methanol (9:1) and supernatants analyzed by UPLC-HRMS.				
Analyte	Doravirine metabolite M9				
Assay	UPLC-HRMS				
Species	Doravirine Dose	Gender	M9 PAR[a]	M9 estimated EM[b]	Average EM
Rat vs. Human					
Human	240 mg/day	Male	5.3	1	
Rat TT#[redacted]-6019 (Study Day 87)	30 mg/kg/day	Female	0.4	0.08	0.11
		Male	0.7	0.13	
	450 mg/kg/day	Female	3.2	0.6	0.52
		Male	2.3	0.43	
Dog vs. Human					
Human	240 mg/day	Male	4.8	1.0	
Dog TT#[redacted]-6018 (Study Day 88)	10 mg/kg/day	Female	1.2	0.25	0.27
		Male	1.4	0.29	
	1000 mg/kg/day	Female	2.3	0.48	0.51
		Male	2.6	0.54	

Additional Information:

- [a] $PAR = Peak\ area\ ratio = \frac{Peak\ Area\ M9}{Peak\ Area\ IS}$ [b] $EM = Exposure\ multiple = \frac{PAR\ M9\ in\ Chron\ Tox\ Sample}{PAR\ M9\ in\ \text{human}}$
- All samples were pooled from 0 to 24 hr in an AUC-proportional manner so that PAR for M9 was proportional to its $AUC_{(0\text{-}24hr)}$.

Sec. 2.6.5.5.4 Metabolites of [^{3}H]Doravirine Detected in Liver Microsomes

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]		
Study system	In vitro		
Species/Matrix	Rat, dog, and human/liver microsomes and hepatocytes		
Method for Liver microsomes	[^{3}H]Doravirine (10 µM) was incubated with liver microsomes (LM) at 37°C for 75 min in 0.1 M potassium phosphate buffer (pH 7.4) containing 2 mg/mL LM protein, and 1 mM NADPH. The reactions were terminated with acetonitrile. Aliquots of the supernatant were analyzed following centrifugation to remove precipitated protein.		
Method for Hepatocytes	[^{3}H]Doravirine (10 µM) was incubated with hepatocytes in suspension at 37°C for 2 hr with 1×10^6 cells/mL in Krebs-Henseleit buffer in the presence of 95% O_2 / 5% CO_2. The reactions were terminated with acetonitrile. Aliquots of the supernatant were analyzed following centrifugation to remove precipitated protein and cell debris.		
Analyte	Radioactivity (^{3}H)		
Assays	LC-MS/MS with radiometric detection.		
Metabolite	**RLM**	**DLM**	**HLM**
Doravirine	X	X	X
M5	X	-	-
M8	X	-	-
M9	X	X	X
M10	X	-	X

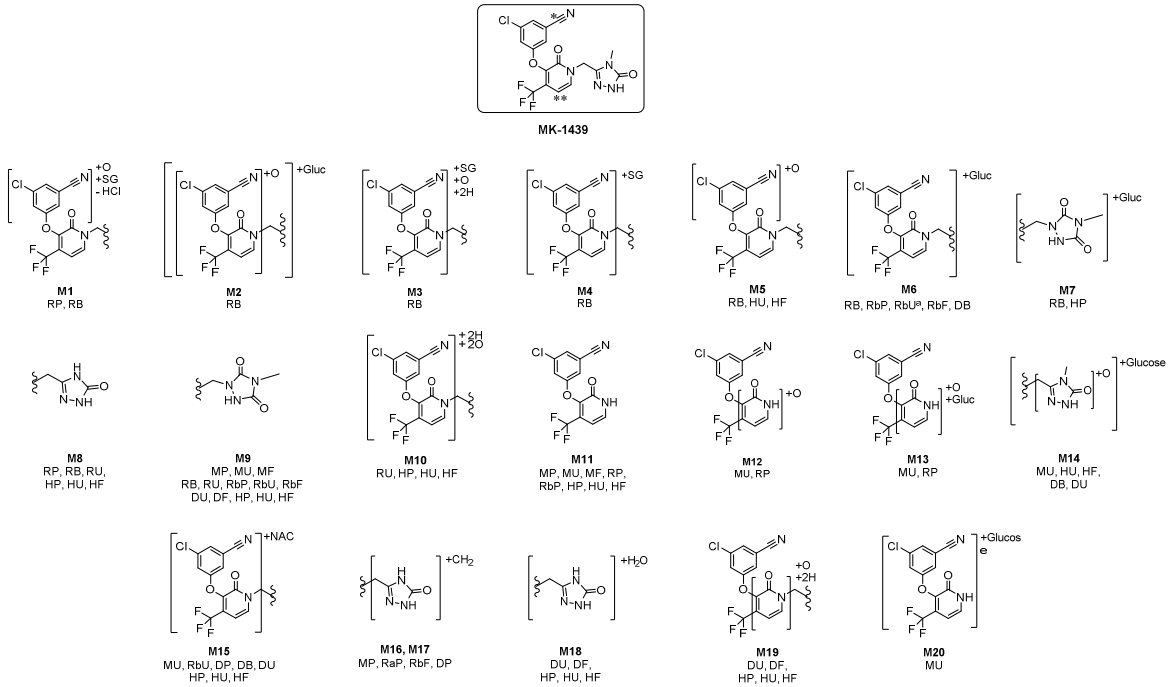
Additional Information:

- Abbreviations: RLM, DLM, HLM = rat, dog, human liver microsomes; X= Detected by LC/MS and radiometric detection; - = Not detected.
- No metabolites of [^{3}H]doravirine were detected in hepatocytes or in HLM in the presence of uridine diphosphate glucuronic acid (UDPGA) as a cofactor, indicating that doravirine is not a substrate for glucuronosyl transferases (UGT) in vitro.

Sec. 2.6.5.5.5 Proposed Metabolic Pathways

Test Article: Doravirine

Study Number	[Ref. 4.2.2.4: PK002MK1439] [Ref. 4.2.2.4: PK004MK1439] [Ref. 4.2.2.4: PK006MK1439] [Ref. 4.2.2.4: PK013MK1439]



Additional Information:

- Abbreviations: MP, RP, RbP, DP, HP = mouse, rat, rabbit, dog, human plasma; RB, DB = rat, dog bile; MU, RU, RbU, DU, HU = mouse, rat, rabbit, dog, human urine; MF, RF, RbF, DF, HF = mouse, rat, rabbit, dog, human feces; SG = Glutathione; NAC = *N*-acetylcysteine; Gluc = Glucuronide.
- * Indicates the position of ^{14}C and ** indicates the position of ^{3}H.
- [a] Two metabolites identified in rabbit urine as doravirine glucuronides appeared to be different from M6 and were designated as M6a and M6b to differentiate from M6 observed in other species.
- M9 was purified from human urine and its structure elucidated by nuclear magnetic resonance (NMR). The structure was confirmed by comparing to compound synthesized chemically. Synthetic M9 was tested in a multiple-round HIV-1 infection assay (ViKinG) against wild type and 3 major mutants (K103N, V106A, and Y181C) of the HIV-1 virus at concentrations up to 8.4 µM. No significant antiviral activity was observed. In addition, M9 (10 µM) did not have activity against a panel of 115 cellular targets [Ref. 4.2.2.4: PK015MK1439].

Sec. 2.6.5.6 Excretion

Sec. 2.6.5.6.1 Excretion of Total Radioactivity Following a P.O. Dose of [^{14}C]Doravirine to Mice, Rats, Rabbits, and Dogs

Test Article: Doravirine

Study Number		[Ref. 4.2.2.4: PK013MK1439]	[Ref. 4.2.2.4: PK002MK1439]	[Ref. 4.2.2.4: PK013MK1439]	[Ref. 4.2.2.4: PK004MK1439]
Species/Strain		Mouse/CD-1	Rat (BDC)/WH	Rabbit/Dutch Belted	Dog (BDC)/Beagle
Gender/Number of animals		M/3	M/3	F/3	M/2
Feeding condition		Fasted	Fasted	Fasted	Fasted
Vehicle/Formulation		10% polysorbate 80/Solution	PEG400/Solution	10% polysorbate 80/Solution	10% polysorbate 80/Solution
Method of administration		P.O.	P.O.	P.O.	P.O.
Dose (mg/kg)		5	5	5	5
Radionuclide		^{14}C	^{3}H	^{14}C	^{3}H
Specific activity (µCi/mg)		19.97	81.714	19.95	4.17
Analyte/Assay		Total radioactivity/Liquid scintillation counting			
Matrix	**Collection Time**	**% of Dose Recovered (Mean or Mean ± SD)**			
		Mouse	**Rat**	**Rabbit**	**Dog**
Urine	0-24 hr	31.7	18.7 ± 1.4	17.7 ± 2.0	10.9
	24-48 hr	0.8	3.6 ± 1.3	7.3 ± 1.4	11.4
	48-72 hr	0.2	0.5 ± 0.2	1.4 ± 1.3	7.5
	Sub-total	**32.7**	**22.9 ± 1.4**	**26.4 ± 2.7**	**29.7**
Feces	0-24 hr	34.4	6.2 ± 6.3	45.3 ± 6.8	10.5
	24-48 hr	0.7	5.9 ± 0.3	12.8 ± 0.4	19.2
	48-72 hr	0.2	0.9 ± 0.6	2.7 ± 0.3	4.9
	Sub-total	**35.3**	**13.0 ± 6.5**	**60.7 ± 7.1**	**29.3**
Bile	0-2 hr	ND	0.8 ± 0.7	ND	0.3
	2-4 hr	ND	14.2 ± 4.2	ND	0.9
	4-6 hr	ND	7.9 ± 1.2	ND	
	6-24 hr	ND	26.7 ± 4.7	ND	5.6
	24-48 hr	ND	6.4 ± 2.6	ND	4.8
	48-72 hr	ND	0.6 ± 0.2	ND	2.8
	Sub-total	**ND**	**56.7 ± 1.6**	**ND**	**14.3**
Total[a]		**70.1**	**93.4 ± 7.6**	**89.2 ± 8.5**	**73.6**

Additional Information:

- Abbreviations: BDC = Bile duct-cannulated; ND = Not determined; SD = Standard deviation of 3 animals for the rat and rabbit studies.
- For the mouse study, three animals were dosed and excreta collected but samples were pooled across animals per collection interval for processing and analysis. For the dog study, three animals were included in the study but one was excluded from the analysis due to a blocked cannula so that numbers represent average of 2 animals.
- [a] Total includes cage radioactivity in cage wash.

Sec. 2.6.5.6.2 Excretion in Nursing Rats (Excretion Into Milk)

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: TT[redacted]7130FIN]	
Species /Strain	Rat / Wistar Hannover (nursing)	
Lactation day/Number of animals	Day 14/ 4 animals per dose	
Vehicle/Formulation	0.5% (w/v) methylcellulose with 5 mM hydrochloric acid in deionized water / [redacted] of doravirine	
Method of administration	P.O.	
Dose (mg/kg/day)	5 and 450 mg/kg/day	
Method of sample collection	Maternal blood samples (~ 0.35 mL) were collected from the jugular vein into EDTA-treated tubes. As soon as possible after blood collection, each female was injected with oxytocin (1 unit IM). Approximately 5 minutes later, milk samples (~ 0.5 mL) were collected by aspiration over a 10-minute period.	
Analyte	Doravirine	
Assay	LC-MS/MS	
Dose of doravirine (mg/kg/day)	5	450
Lactation day	Day 14	Day 14
Maternal Plasma Conc. (µM)[a]		
2 hour	3.50 ± 0.174	42.7 ± 3.52
Maternal Milk Conc. (µM)[a]		
2 hour	5.13 ± 0.223	56.6 ± 5.09
Maternal Milk/Plasma Ratio[b]		
2 hour	1.47 ± 0.0637	1.32 ± 0.0281

Additional Information:

- [a] Mean ± SEM (standard of the mean) calculated using all individual plasma concentrations.
- [b] Values are the mean ± SEM of the individual milk concentration divided by the corresponding maternal plasma concentration.
- Doravirine dosing: from Gestation Day 6 to Lactation Day 14.
- All rats survived until scheduled study termination.

Sec. 2.6.5.7 Drug Interactions

Sec. 2.6.5.7.1 Human CYP Phenotyping

Sec. 2.6.5.7.1.1 Oxidative Metabolism of Doravirine in the Presence of Recombinant Human CYPs (rCYPs)

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]
Types of Studies	rCYP Phenotyping
Methods	[^{3}H]Doravirine (10 µM) was incubated with microsomes containing the recombinant human CYP (rCYP) isoforms indicated below (200 pmol protein/mL protein) for 75 min at 37°C in 0.1 M potassium phosphate buffer (pH 7.4) and 1 mM NADPH. Negative control samples contained insect cell microsomes not expressing CYP enzymes. Reactions were terminated by the addition of an equal volume of acetonitrile and samples were then vortex mixed and centrifuged. Aliquots of the supernatant were subjected to analysis.
Analyte	[^{3}H]Doravirine
Assay	HPLC-MS with on-line radiometric detection.
Results:	

Metabolite	**rCYPs**																
	1A1	**1A2**	**1B1**	**2A6**	**2B6**	**2C8**	**2C9*1**	**2C9*2**	**2C9*3**	**2C18**	**2C19**	**2D6*1**	**2E1**	**2J2**	**3A4**	**3A5**	**3A7**
M9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
M10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-

Additional Information:
(-) = Not detected.
(+) = Detected in the mass spectrometry and radioactivity chromatogram.

Sec. 2.6.5.7.1.2 Oxidative Metabolism of Doravirine in the Presence of Inhibitory Anti-CYP Monoclonal Antibodies

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]
Types of Studies	Recombinant CYP (rCYP) Immunoinhibition
Methods	[^{3}H]Doravirine (10 μM) or testosterone (50 μM) were preincubated with either human liver microsomes (2.0 mg/mL) or CYP3A4 microsomes (200 pmol CYP/mL) with an inhibitory anti-CYP3A4/5 monoclonal antibody (mAb) in 100 mM potassium phosphate buffer (pH 7.4), 1 mM MgCl2. Control incubations were conducted in the presence of serum. The solutions were then warmed to 37°C and the reactions were initiated by the addition of NADPH (1 mM) and continued for 75 min. Reactions were terminated by the addition of an equal volume of acetonitrile. Samples were then vortex-mixed, centrifuged, and the resulting supernatants were subjected to HPLC-MS radiochromatographic analysis.
Analyte	[^{3}H]Doravirine
Assay	HPLC-MS radiometric analysis

Results:

Substrate / Enzyme System	**% Substrate Remaining (t=75min)**		
	-NADPH / -mAB	**+NADPH / +mAB**	**+NADPH / –mAB**
Testosterone / HLM	100%	96%	1%
Testosterone / CYP3A4 microsomes	100%	95%	4%
[^{3}H]Doravirine / HLM	100%	100%	88%
[^{3}H]Doravirine / CYP3A4 microsomes	100%	99%	49%

Additional Information:

- HLM = Human liver microsomes.
- Testosterone was extensively metabolized in both HLM and CYP3A4 microsomes in the presence of NADPH, and its metabolism was inhibited by the anti-CYP3A mAb, confirming the functionality of the assay.

Sec. 2.6.5.7.2 Kinetic Characterization of CYP3A4 and CYP3A5-Catalyzed Oxidation of Doravirine

Sec. 2.6.5.7.2.1 Unbound Fraction in Recombinant CYP3A4 and CYP3A5

Test Article: Doravirine

Study Number	[Ref. 4.2.2.4: PK017MK1439]			
Type of Study	Determination of unbound fraction in rCYP3A4 and rCYP3A5 incubations			
Methods	The unbound fraction of doravirine in enzyme kinetics incubations was determined by ultracentrifugation where doravirine (0.5 and 5 µM) was incubated with either recombinant CYP3A4 (rCYP3A4) or rCYP3A5 at 37°C in 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM $MgCl_2$. After 30 minutes, aliquots were removed from each incubation to be used as reference controls, and the remainder was subjected to ultracentrifugation at 182,000 × g for 1 hr. Aliquots of the resulting supernatants were removed and all samples were quenched by the addition of an equal volume of acetonitrile. The resulting supernatants were analyzed by LC-MS/MS.			
Analyte	Doravirine			
Assay	LC-MS/MS			
Results:				
Unbound Fraction in rhCYP3A4 and rhCYP3A5 Incubations				
Substrate	**rCYP3A4** (25 pmol CYP/mL)		**rCYP3A5** (100 pmol CYP/mL)	
Doravirine	0.5 µM	5 µM	0.5 µM	5 µM
	0.90 ± 0.01	0.93 ± 0.01	0.40 ± 0.02	0.43 ± 0.03

Additional Information:
Data represent mean ± standard deviation for n=3 replicates.

Sec. 2.6.5.7.2.2 Kinetic Parameters for Recombinant CYP3A4- and CYP3A5-Catalyzed Oxidation of Doravirine to Metabolite M9

Test Article: Doravirine

Study Number	[Ref. 4.2.2.4: PK017MK1439]		
Type of Study	Enzyme kinetics study		
Methods	Enzyme kinetics studies were conducted in a 96-well plate format using a Hamilton Star Plus automated liquid handler to initiate and terminate the reactions. Increasing concentrations of Doravirine were incubated with insect cell microsomes containing recombinant human CYP3A4 (rCYP3A4 + oxidoreductase (OR) + cytochrome b5 (b5) or 3A5 (rCYP3A5+ OR + b5) at 37°C in 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM $MgCl_2$. Reactions were initiated by the addition of NADPH and terminated by the addition of an equal volume of acetonitrile. Aliquots were then analyzed by LC-MS/MS.		
Analyte	M9 (Metabolite of Doravirine)		
Assay	LC-MS/MS		
Assay Conditions			
CYP isoform	**Final Doravirine conc. (µM)**	**rhCYP conc. (pmol/mL)**	**Incubation Time (min)**
rhCYP3A4	0.3, 0.45, 0.7, 1.0, 1.5, 2.5, 4.0, 6.5, 10, 17.5, 50	25	15
rhCYP3A5		100	

Kinetic Parameters		
	rhCYP3A4	**rhCYP3A5**
One-site binding model		
K_m ± SE (µM)	20.9 ± 0.8	31.1 ± 3.3
V_{max} ± SE (pmol/min/pmol CYP)	2.3 ± 0.04	0.15 ± 0.01
$CL_{int,u}$ (µL/min/pmol CYP)	0.11	0.0048
Two-site binding model		
K_{m_1} ± SE (µM)	3.6 ± 1.6	5.4 ± 1.5
V_{max_1} ± SE (pmol/min/pmol CYP)	0.51 ± 0.20	0.026 ± 0.007
K_{m_2} ± SE (µM)	17.6 ± 1.9	34.2 ± 3.5
V_{max_2} ± SE (pmol/min/pmol CYP)	2.1 ± 0.1	0.15 ± 0.01
$CL_{int,u}$ (µL/min/pmol CYP)	0.142	0.0049

Additional Information:

- The rate of formation of M9 was linear with respect to time and enzyme concentration.
- Data represent mean ± SE from global fit of n=3 replicates per substrate concentration.
- One-site binding model: $K_{m,app}$ and V_{max} were calculated using the Michaelis Menten equation: $v = {Vmax * S}/{(Km, app + S)}$, where V_{max} is the maximal velocity, v is the velocity of the reaction and $K_{m, app}$ is the apparent Michaelis-Menten constant, corresponding to the doravirine concentration that achieves half the V_{max} for the formation of M9.
- The K_m was calculated by correcting $K_{m,app}$ for non-specific binding to rCYP3A4 and rCYP3A5 in the incubations using the equation: $K_m = K_{m.app} * f_{u,inc}$, where $f_{u,inc}$ is the unbound fraction of doravirine in each respective assay.
- Two-site binding model: $K_{m1,app}$, V_{max1}, $K_{m2,app}$, and V_{max2} were calculated using the Eadie-Hofstee equation: $v = V_{max} - K_{m,app}(\frac{v}{S})$ for each phase of the biphasic plot obtained by plotting v vs. v/S. K_{m1} and K_{m2} were corrected for non-specific binding to rCYP3A4 and rCYP3A5 as described above.

Sec. 2.6.5.7.3 Evaluation of Doravirine as a Substrate of Human BCRP

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK014MK1439]		
Type of Study	In vitro transport in cells expressing human breast cancer resistance protein (BCRP)		
Methods	Bi-directional transport of [^{3}H]doravirine (0.1 to 1 µM) was measured across monolayers of Madin-Darby canine kidney (MDCKII) and MDCKII cells stably expressing human BCRP (MDCKII-BCRP) at 37°C, in the absence and presence of the BCRP inhibitor Ko143 (2 µM). The P_{app} reported is the average of the P_{app} for transport from A to B and P_{app} for transport from B to A values. The B-A/A-B ratio was calculated by dividing the P_{app} from B to A by the P_{app} from A to B. Bi-directional transport of [^{3}H]prazosin (5 µM) was measured in parallel as a positive control.		
Analytes	[^{3}H]Doravirine, [^{3}H]Prazosin		
Compound	**MDCKII-BCRP BA/AB Ratio**	**MDCKII BA/AB Ratio**	**MDCKII Papp (x10^{-6} cm/s)**
5 µM [^{3}H]Prazosin	4.7	1	22.7
5 µM [^{3}H]Prazosin + 2 µM Ko143	1.4	1.2	25.4
0.1 µM [^{3}H]Doravirine	1.9	1.1	10.9
0.1 µM [^{3}H]Doravirine + 2 µM Ko143	2.3	1.7	10.6
1 µM [^{3}H]Doravirine	1.7	1.1	10.4
1 µM [^{3}H]Doravirine + 2 µM Ko143	2.1	1.3	11.5

Additional Information:
The experiment was performed in triplicate. A BA/AB ratio > 3 is indicative of active transport. The P_{app} BA/AB ratios for 5 µM [^{3}H]prazosin, a BCRP substrate, in the presence and absence of the BCRP inhibitor, Ko143, confirmed the functionality of the assay. Doravirine was not a substrate of BCRP.

Sec. 2.6.5.7.4 Evaluation of Doravirine as a Substrate of Human MDR1 (P-glycoprotein, P-gp) and In Vitro Passive Permeability

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]
Type of Study	In vitro transport in cells expressing human P-glycoprotein
Methods	Bi-directional transport of doravirine (0.1 to 1 µM) was measured across monolayers of porcine renal epithelial cells (LLC-PK1) and LLC-PK1 cells stably expressing human MDR1 (LLC-MDR1) at 37°C. The Papp reported is the average of the P_{app} for transport from A to B and P_{app} for transport from B to A. The B-A/A-B ratio was calculated by dividing the P_{app} from B to A by the P_{app} from A to B. Bi-directional transport of verapamil (1 µM) was measured in parallel as a positive control.
Analytes	Doravirine, Verapamil
Assay	LC-MS/MS

Compound (µM)	**P_{app} BA/P_{app} AB Ratio**		**P_{app} x 10^{-6} (cm/s)**
	LLC-MDR1 (human)	**LLC-PK1**	**LLC-PK1**
Doravirine (0.1)	4.7	0.9	25
Doravirine (0.5)	3.5	0.9	24.7
Doravirine (1)	4.3	1.0	28.2
Verapamil (1)	4.5	1.3	40.1

Additional Information:
The experiment was performed in triplicate. A BA/AB ratio > 3 is indicative of active transport. The P_{app} BA/AB ratio for 1 µM verapamil a P-gp substrate, confirmed the functionality of the assay. Doravirine is a P-gp substrate.

Sec. 2.6.5.7.5 Evaluation of Doravirine as a Substrate of OATP1B1

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439] [Ref. 4.2.2.6: PK018MK1439]
Type of Study	In vitro uptake in cells expressing the organic anion transporting polypeptide 1B1 (OATP1B1)
Methods	Uptake of [^{3}H]doravirine (1 µM) was measured in the absence and presence of the OATP1B1 inhibitor sulfobromophthalein (BSP; 100 µM) in human embryonic kidney (HEK293) cells, HEK293 cells transiently transfected with OATP1B1 (HEK293-OATP1B1), Madin-Darby canine kidney (MDCKII) cells, and MDCKII cells stably expressing human OATP1B1 (MDCKII-OATP1B1). Incubations in HEK293 and HEK293-OATP1B1 were conducted in the absence and presence of 0.1% BSA. Cells were incubated at 37°C for the indicated time, and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed 3-4 times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting. The uptake of [^{3}H]estradiol-17β-glucuronide ([^{3}H]$E_2$17βG; 1 µM) was measured in parallel as the positive control.
Analytes	[^{3}H]Doravirine, [^{3}H]$E_2$17βG
Assay	Liquid scintillation counting

Uptake of [^{3}H]Doravirine into MDCKII Cells Stably Transfected with OATP1B1

Substrate	Inhibitor	Time (min)	MDCKII (pmol/10^6 cells) Mean ± SEM	MDCKII-OATP1B1 (pmol/10^6 cells) Mean ± SEM
[^{3}H]$E_2$17βG	-	10	0.220 ± 0.018	2.055 ± 0.198
	BSP	10	0.223 ± 0.012	0.536 ± 0.100
[^{3}H]Doravirine	-	0	2.776 ± 0.372	3.663 ± 0.198
	-	1	5.383 ± 0.041	8.213 ± 0.175
	-	3	6.301 ± 0.262	8.899 ± 0.328
	-	5	6.486 ± 0.376	9.231 ± 0.298
	-	10	7.797 ± 0.543	10.958 ± 0.422
	BSP	10	7.962 ± 0.368	12.100 ± 0.513

Sec. 2.6.5.7.5 Evaluation of Doravirine as a Substrate of OATP1B1 (Cont.)

Test Article: Doravirine

Uptake of [^{3}H]Doravirine in HEK293 and HEK293-OATP1B1 Cells

Substrate	Inhibitor	Time (min)	HEK293 (pmol/10^6 cells/min)	HEK293-OATP1B1 (pmol/10^6 cells/min)
			Mean ± SEM	Mean ± SEM
[^{3}H]$E_2$17βG	-	5	0.897 ± 0.065	45.672 ± 2.849
	BSP	5	0.802 ± 0.015	0.751 ± 0.044
[^{3}H]Doravirine	-	0	3.701 ± 0.136	3.954 ± 0.160
	-	1	14.669 ± 0.374	12.450 ± 0.167
	-	2	16.244 ± 0.679	13.587 ± 0.200
	--	5	19.831 ± 0.347	15.400 ± 0.351
	BSP	5	19.799 ± 0.481	15.157 ± 0.249

Uptake of [^{3}H]Doravirine in HEK293 and HEK293-OATP1B1 Cells in Presence of 0.1% BSA

Substrate	Inhibitor	Time (min)	HEK293 (pmol/10^6 cells/min)	HEK293-OATP1B1 (pmol/10^6 cells/min)
			Mean ± SEM	Mean ± SEM
[^{3}H]$E_2$17βG	-	5	0.476 ± 0.011	62.802 ± 0.946
	BSP	5	0.647 ± 0.220	0.768 ± 0.060
[^{3}H]Doravirine	-	0	3.779 ± 0.111	5.176 ± 0.123
	-	1	14.091 ± 0.475	14.072 ± 0.339
	-	2	16.778 ± 0.534	17.208 ± 0.349
	-	5	18.671 ± 0.494	18.507 ± 0.075
	BSP	5	19.354 ± 0.388	18.327 ± 0.371

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- Test compounds are considered to be a substrate of OATP1B1 when uptake of the compound is time-dependent and greater than 1.5-fold higher in OATP1B1 transfected cells compared to either MDCKII or HEK293 parental cells, at the minimum of two time points. Based on these criteria, it was concluded that doravirine is not an OATP1B1 substrate. BSP (100 μM) inhibited OATP1B1-mediated [^{3}H]$E_2$17βG uptake, confirming the functionality of the assay.

Sec. 2.6.5.7.6 Evaluation of Doravirine as a Substrate of OATP1B3

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK018MK1439]
Type of Study	In vitro uptake in cells expressing the organic anion transporting polypeptide 1B3 (OATP1B3)
Methods	Uptake of [^{3}H]doravirine (1 µM) was measured in the absence and presence of the OATP1B3 inhibitor sulfobromophthalein (BSP; 100 µM) in human embryonic kidney (HEK293) cells, HEK293 cells transiently transfected with OATP1B3 (HEK293-OATP1B3), Madin-Darby canine kidney (MDCKII) cells, and MDCKII cells stably expressing human OATP1B3(MDCKII-OATP1B3). Incubations in HEK293 and HEK293-OATP1B3 were conducted in the absence and presence of 0.1% BSA. Cells were incubated at 37°C for the indicated time, and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed 3 to 4 times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting. The uptake of [^{3}H]cholecystokinin octapeptide ([^{3}H]CCK8; 5 nM) was measured in parallel as the positive control.
Analytes	[^{3}H]Doravirine, [^{3}H]CCK8
Assay	Liquid scintillation counting

Uptake of [^{3}H]Doravirine in MDCKII and MDCKII-OATP1B3 Cells

Substrate	Inhibitor	Time (min)	MDCKII (pmol/10^6 cells/min)	MDCKII-OATP1B3 (pmol/10^6 cells/min)
			Mean ± SEM	Mean ± SEM
[^{3}H]CCK8	-	5	0.0010 ± 0.0001	0.0032 ± 0.0002
	BSP	5	0.0015 ± 0.0002	0.0013 ± 0.0001
[^{3}H]Doravirine	-	0	1.558 ± 0.107	1.275 ± 0.006
	-	1	6.931 ± 0.148	6.903 ± 0.094
	-	2	9.107 ± 0.159	9.152 ± 0.066
	-	5	11.424 ± 0.265	11.868 ± 0.108
	BSP	5	12.861 ± 0.244	13.898 ± 0.050

Sec. 2.6.5.7.6 Evaluation of Doravirine as a Substrate of OATP1B3 (Cont.)

Test Article: Doravirine

Uptake of [^{3}H]Doravirine in HEK293 and HEK293-OATP1B3 Cells

Substrate	Inhibitor	Time (min)	HEK293 (pmol/10^6 cells/min)	HEK293-OATP1B3 (pmol/10^6 cells/min)
			Mean ± SEM	Mean ± SEM
[^{3}H]CCK8	-	5	0.0024 ± 0.0002	0.1070 ± 0.0018
	BSP	5	0.0020 ± 0.0001	0.0018 ± 0.0002
[^{3}H]Doravirine	-	0	4.146 ± 0.089	5.078 ± 0.169
	-	1	12.261 ± 0.175	13.116 ± 0.170
	-	2	15.080 ± 0.012	15.994 ± 0.615
	--	5	18.390 ± 0.416	18.689 ± 0.200
	BSP	5	16.706 ± 0.482	17.018 ± 0.394

Uptake of [^{3}H]Doravirine in HEK293 and HEK293-OATP1B3 Cells in Presence of 0.1% BSA

Substrate	Inhibitor	Time (min)	HEK293 (pmol/10^6 cells/min)	HEK293-OATP1B3 (pmol/10^6 cells/min)
			Mean ± SEM	**Mean ± SEM**
[^{3}H]CCK8	-	5	0.0019 ± 0.0001	0.1375 ± 0.0039
	BSP	5	0.0020 ± 0.0001	0.0037 ± 0.0001
[^{3}H] Doravirine	-	0	3.779 ± 0.111	4.642 ± 0.153
	-	1	14.091 ± 0.475	16.049 ± 0.217
	-	2	16.778 ± 0.534	18.713 ± 0.354
	--	5	18.671 ± 0.494	21.077 ± 0.321
	BSP	5	19.354 ± 0.388	20.496 ± 0.347

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- Test compounds are considered to be a substrate of OATP1B3 when uptake of the compound is time-dependent and greater than 1.5-fold higher in OATP1B3-transfected cells, compared to either HEK293 or MDCKII parental cells, at the minimum of two time points. Based on these criteria, it was concluded that doravirine is not an OATP1B3 substrate. BSP (100 μM) inhibited OATP1B3-mediated [^{3}H]CCK8 uptake in MDCKII-OATP1B3 and HEK293-OATP1B3 cells, confirming the functionality of the assay.

Sec. 2.6.5.7.7 Uptake of Doravirine into Cryopreserved Human Hepatocytes in Suspension

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK018MK1439]		
Type of Study	In vitro uptake in human hepatocytes		
Methods	Uptake of [^{3}H]doravirine (1 µM) or positive control substrates [[^{3}H]estradiol-17β-glucuronide (^{3}H]E217βG; 1 µM), [^{3}H]cholecystokinin octapeptide ([^{3}H]CCK8; 5 nM), [^{3}H]taurocholic acid ([^{3}H]TCA; 1 µM), and [^{3}H]tetraethylammonium chloride ([^{3}H]TEA; 1 µM)] was measured in cryopreserved human hepatocytes in the absence or presence of a transporter inhibitor cocktail containing cyclosporin A (10 µM), rifamycin SV (10 µM), rifampin (100 µM) and quinidine (50 µM). Cells were incubated at 37°C for the indicated time, and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed three times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.		
Analytes	[^{3}H]Doravirine, [^{3}H]$E_2$17βG, [^{3}H]CCK8, [^{3}H]TCA, [^{3}H]TEA		
Assay	Liquid scintillation counting		
Substrate	**Time**	**Without Inhibitors (pmol/10^6 cells/min)**	**With Inhibitors (pmol/10^6 cells/min)**
		Mean ± SEM	Mean ± SEM
[^{3}H]E217βG	5	37.718 ± 2.152	7.522 ± 0.277
[^{3}H]CCK-8	5	0.0225 ± 0.0005	0.0056 ± 0.0003
[^{3}H]TCA	5	169.636 ± 3.246	13.531 ± 0.734
[^{14}C]TEA	5	8.712 ± 0.092	2.203 ± 0.111
[^{3}H]Doravirine	0	17.360 ± 0.594	15.984 ± 0.543
	1	65.728 ± 2.411	52.642 ± 3.315
	2	80.126 ± 1.811	61.091 ± 0.511
	5	91.870 ± 2.463	67.055 ± 1.682

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- Transporters are considered to contribute to the hepatic uptake of the test compound if uptake into hepatocytes is time-dependent, and greater than 1.5-fold higher in the absence of cocktail transport inhibitors, compared to in the presence of inhibitors, at the minimum of two time points. Based on these criteria, it was concluded that transporters do not contribute to the hepatic uptake of doravirine.

Sec. 2.6.5.7.8 Inhibition of Drug Metabolizing Enzymes

Sec. 2.6.5.7.8.1 Inhibition of Human Cytochrome P450 Enzymes by Doravirine (Reversible Inhibition)

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]		
Type of Study	Reversible Inhibition of cytochrome P450 (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) by doravirine		
Methods	The incubation mixtures (0.2 mL final volume) contained microsomal protein (0.25 mg/mL), the appropriate CYP probe substrate and doravirine (0.05 to 100 µM) in 100 mM potassium phosphate buffer (pH 7.4) with 3.14 mM MgCl2. The reaction was initiated with an NADPH-regenerating system, and was allowed to proceed for 3 to 20 min (37°C in a shaking water bath). The reactions were terminated with the addition of methanol or 50% acetonitrile/2.5% formic acid/0.1% methanol (v/v/v, in water) containing an appropriate internal standard and the samples were vortexed and centrifuged. The supernatants were analyzed by LC-MS/MS.		
Analytes	Acetaminophen (CYP1A2), (±)-Hydroxybupropion (CYP2B6), N-Desethylamodiaquine (CYP2C8), 4′-Hydroxydiclofenac (CYP2C9), (±)-4-Hydroxymephenytoin (CYP2C19), Dextrorphan (CYP2D6), α-Hydroxymidazolam (CYP3A4), 6β-Hydroxytestosterone (CYP3A4)		
Assay	LC-MS/MS		
CYP	**Reaction**	**Control Inhibitor (IC_{50}, µM)[a]**	**Doravirine (IC_{50}, µM)[a, b]**
1A2	Phenacetin *O*-Deethylation	α-Naphthoflavone (0.0074)	>100 (0%)
2B6	Bupropion Hydroxylation	Ticlopidine (0.74)	>100 (2.1 ± 3.4%)
2C8	Amodiaquine N-Deethylation	Montelukast (0.23)	>100 (17 ± 6.3%)
2C9	Diclofenac 4′-Hydroxylation	Sulfaphenazole (0.74)	>100 (28 ± 2.6%)
2C19	*S*-Mephenytoin 4′-Hydroxylation	Benzylnirvanol (0.20)	>100 (46 ± 1.9%)
2D6	Dextromethorphan *O*-Demethylation	Quinidine (0.083)	>100 (7.4 ± 1.2%)
3A4	Midazolam 1′-Hydroxylation	Ketoconazole (0.027)	>100 (0%)
3A4	Testosterone 6β-Hydroxylation	Ketoconazole (0.022)	>100 (35 ± 0.52%)

Additional Information:

- [a] The IC_{50} is defined as the inhibitor concentration that yields 50% of the mean control activity.
- [b] The values in parentheses represent the percent inhibition (mean ± standard deviation) observed at 100 µM.

Sec. 2.6.5.7.8.2 Time-Dependent Inhibition of Testosterone 6ß-Hydroxylation by Doravirine in Human Liver Microsomes

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]
Type of Study	Time-dependent inhibition of human liver CYP3A4 (testosterone 6ß-hydroxylase activity).
Methods:	Pooled human liver microsomes (1 mg/mL) were preincubated at 37°C with 10or 50 µM of doravirine in 100 mM potassium phosphate buffer (pH 7.4) with 1 mM EDTA, 6 mM MgCl2, and an NADPH-regenerating system for a duration ranging from 5 to 45 min. At the end of the incubation, the incubation mixtures were diluted 10-fold with the same buffer containing 250 µM testosterone and an NADPH-regenerating system. The incubation was continued for an additional 10 min to monitor the extent of testosterone 6β-hydroxylation. The first order rate constants (k_{obs}) for inactivation at 10 and 50 µM were calculated from the negative slope of the lines by using linear regression analysis of the natural logarithm of the remaining activity as a function of time.
Analyte	6β-Hydroxytestosterone
Assay	LC-MS/MS
Compound	**Rate Constant (min^{-1})**
Solvent (Negative control)	< 0.01
Mifepristone (Positive control, 10 µM)	0.084
Doravirine (10 µM)	< 0.01
Doravirine (50 µM)	< 0.01

Additional Information:
Result: Doravirine (10 and 50 µM) did not cause time-dependent inhibition of CYP3A4.

Sec. 2.6.5.7.8.3 Inhibition of UGT1A1 by Doravirine in Human Liver Microsomes

Test Article: Doravirine

Study Number		[Ref. 4.2.2.6: PK009MK1439]
Type of Study		In vitro inhibition of UGT1A1
Methods:		Pooled human liver microsomes (0.5 mg/mL) were incubated with 20 µM estradiol, 5 mM UDPGA, 9 mM MgCl2 and 25 µg/mL alamethicin in 200 µL of 81 mM HEPES buffer, pH 7.0 at 37°C for 20 min. Estradiol glucuronidation was quantified in the absence and presence of increasing concentrations of either doravirine (0.78 to 100 µM) or nicardipine (0.078 to 10 µM) a known inhibitor of UGT1A1. Ice cold methanol was added at the end of the incubation to precipitate the microsomal protein. Samples were centrifuged, and the supernatant was transferred to new tubes for analysis of estradiol 3-glucuronide by LC-MS/MS
Analyte		Estradiol 3-glucuronide
Assay		LC-MS/MS
Substrate	**Inhibitor**	**IC_{50}**
Estradiol 3-glucuronide	Nicardipine	3.2 ± 0.27 µM
Estradiol 3-glucuronide	Doravirine	IC_{50} > 100 µM

Additional Information:
Result: Doravirine (0.78 to 100 µM) did not inhibit estradiol 3-glucuronidation in human liver microsomes (IC_{50} >100 µM).

Sec. 2.6.5.7.9 Inhibition of Transporters

Sec. 2.6.5.7.9.1 Inhibition of BCRP by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439]
Type of Study	In vitro inhibition of human breast cancer resistance protein (BCRP)
Methods	The ATP-dependent uptake of [^{3}H]methotrexate ([^{3}H]MTX; 10 µM) was measured in membrane vesicles containing human BCRP in the absence or presence of doravirine (0 to 75 µM) or the positive control inhibitor Ko143 (5 µM). Uptake was initiated by the addition of either ATP (5 mM) or AMP (5 mM) as negative control in the presence of an ATP-regenerating system, at 37°C for 5 minutes. Uptake was stopped by the addition of ice cold stop buffer, and vesicles were transferred to a glass fiber filter plate under vacuum and washed six times with ice-cold stop buffer. The radioactivity in the dried filters was determined by liquid scintillation counting.
Analyte	[^{3}H]MTX
Assay	Liquid scintillation counting

Substrate	Inhibitor	Inhibitor concentration (µM)	AMP (pmol/mg vesicles/min) Mean ± SEM	ATP (pmol/mg vesicles/min) Mean ± SEM	% Control Mean ± SEM
[^{3}H]MTX	-	0	10.056 ± 2.092	126.138 ± 2.337	
[^{3}H]MTX	Ko143	5	11.429 ± 0.153	17.808 ± 1.262	
[^{3}H]MTX	Doravirine	0	10.056 ± 2.092	126.138 ± 2.337	100.0 ± 1.6
		3	10.676 ± 1.400	109.615 ± 3.677	85.2 ± 2.0
		10	9.280 ± 0.441	97.168 ± 6.184	75.7 ± 3.1
		25	12.138 ± 0.682	87.289 ± 6.879	64.7 ± 3.4
		35	12.891 ± 1.820	80.356 ± 1.926	58.1 ± 1.3
		50	8.815 ± 0.595	70.190 ± 4.565	52.9 ± 2.3
		75	8.727 ± 0.559	64.387 ± 2.517	47.9 ± 1.3

Additional Information:

- Abbreviations: SEM = standard error of the mean from 3 replicates. AMP = Adenosine monophosphate; ATP = Adenosine triphosphate.
- ATP-dependent (BCRP-mediated) [^{3}H]MTX uptake was calculated by subtracting the uptake of [^{3}H]MTX in the absence of ATP (AMP present in the incubations) from that in the presence, and data were then normalized to % control, where uptake in the absence of test compound was 100%. Inhibition by the positive control inhibitor Ko143 confirmed the functionality of the assay.

Result: Doravirine inhibited BCRP-mediated transport of methotrexate with an IC_{50} value = 51 ± 4 µM.

Sec. 2.6.5.7.9.2 Inhibition of MDR1 (P-glycoprotein) by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]		
Type of Study	In vitro inhibition of P-glycoprotein		
Methods	Bi-directional transport of [^{3}H]digoxin (0.1 µM) was measured across monolayers of porcine renal epithelial cells (LLC-PK1) and LLC-PK1 cells stably expressing human MDR1 (LLC-MDR1) at 37°C in the absence or presence of doravirine (0.3 to 300 µM) or the positive control inhibitor cyclosporine A (CsA; 10 µM). Percent control was calculated by dividing net [^{3}H]digoxin transport ((%Transport B-A) – (%Transport A-B)) in the presence of inhibitor by the net [^{3}H]digoxin transport for the no-inhibitor control.		
Analyte	[^{3}H]Digoxin		
Assay	Liquid scintillation counting		
Doravirine	**Digoxin B-A/A-B Ratio**	**% Net**	
Concentration (µM)	**LLC-MDR1**	**Transport**	**% of Control**
No Inhibitor	11.3	4.68	100
0.3	14.7	5.72	122.3
1	17.9	5.86	125.3
3	17.5	6.30	134.7
10	12.9	5.86	125.3
30	12.3	5.28	113.0
100	13.6	4.54	97.1
300	15.1	4.27	91.3[a]
Positive Control Inhibitor (10 µM CsA)	1.5		

Additional Information:

- The experiment was performed in triplicate. The P_{app} BA/AB ratios for 1 µM [^{3}H]digoxin, in the presence and absence of the P-gp inhibitor CsA confirmed the functionality of the assay.
- The BA/AB ratio for digoxin in control LLC-PK1 cells was 2.
- [a] Doravirine precipitated at 300 µM.

Result: Doravirine (0.3 to 300 µM) had no significant effect on P-glycoprotein-mediated transport of digoxin (IC_{50} >300 µM).

Sec. 2.6.5.7.9.3 Inhibition of BSEP by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK018MK1439]
Type of Study	In vitro inhibition of the human bile salt export pump (BSEP)
Methods	The ATP-dependent uptake of [^{3}H]taurocholic acid ([^{3}H]TCA; 1 µM) was measured in membrane vesicles containing human BSEP in the absence or presence of doravirine (0 to 50 µM) or the positive control inhibitor atorvastatin (ATV; 100 µM). Uptake was initiated by the addition of either ATP (5 mM) or AMP (as a negative control, 5 mM), in the presence of an ATP-regenerating system, at 37°C for 5 minutes. Uptake was stopped by the addition of ice cold stop buffer, and vesicles were transferred to a glass fiber filter plate under vacuum and washed six times with ice-cold stop buffer. The radioactivity in the dried filters was determined by liquid scintillation counting.
Analyte	[^{3}H]TCA
Assay	Liquid scintillation counting

Substrate	Inhibitor	Inhibitor concentration (µM)	AMP (pmol/mg vesicles/min) Mean ± SEM	ATP (pmol/mg vesicles/min) Mean ± SEM	% Control Mean ± SEM
[^{3}H]TCA	-	0	3.578 ± 0.417	40.913 ± 3.986	
[^{3}H]TCA	ATV	100	2.454 ± 0.247	7.109 ± 0.822	
[^{3}H]TCA	Doravirine	0	3.578 ± 0.417	40.913 ± 3.986	100.0 ± 6.2
		1	3.786 ± 0.685	40.604 ± 4.777	98.6 ± 7.5
		5	2.892 ± 0.360	40.139 ± 1.303	99.8 ±2.1
		10	3.320 ± 0.673	39.285 ± 1.906	96.3 ± 3.1
		20	2.581 ± 0.284	39.095 ± 1.944	97.8 ± 3.0
		35	3.562 ± 0.140	40.731 ± 3.265	99.6 ± 5.1
		50	3.183 ± 0.039	39.651 ± 1.946	97.7 ± 3.0

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- BSEP-mediated [^{3}H]TCA uptake was calculated by subtracting the uptake of [^{3}H]TCA in the presence of AMP from that in the presence of ATP, and data were normalized to % control, where uptake in the absence of test compound was 100%. Inhibition by the positive control inhibitor ATV confirmed the functionality of the assay.

Result: Doravirine (1 to 50 µM) had no effect on BSEP-mediated transport of taurocholic acid (IC_{50} >50 µM).

Sec. 2.6.5.7.9.4 Inhibition of OATP1B1 by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439]				
Type of Study	In vitro inhibition of the human organic anion transporting polypeptide 1B1 (OATP1B1)				
Methods	Uptake of [^{3}H]pitavastatin (0.1 µM) was measured in Madin-Darby canine kidney (MDCKII) and MDCKII cells stably expressing human OATP1B1(MDCKII-OATP1B1)) cells in the absence or presence of doravirine (0 to 75 µM) or the control inhibitor cyclosporine A (CsA; 10 µM). Cells were incubated at 37°C for 5 minutes and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed 4 times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.				
Analyte	[^{3}H]Pitavastatin				
Assay	Liquid scintillation counting				
Substrate	**Inhibitor**	**Inhibitor concentration (µM)**	**MDCKII (pmol/10^6 cells/min)**	**MDCKII-OATP1B1 (pmol/10^6 cells/min)**	**% Control**
			Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**
[^{3}H]PTV	-	0	0.044 ± 0.0031	0.257 ± 0.0074	
[^{3}H]PTV	CsA	10	0.051 ± 0.0050	0.083 ± 0.0099	
[^{3}H]PTV	Doravirine	0	0.044 ± 0.0031	0.257 ± 0.0074	100.0 ± 3.8
		3	0.036 ± 0.0021	0.246 ± 0.0036	98.6 ± 1.9
		10	0.039 ± 0.0025	0.204 ± 0.0059	77.5 ± 3.0
		25	0.041 ± 0.0034	0.170 ± 0.0020	60.6 ± 1.9
		35	0.043 ± 0.0012	0.164 ± 0.0048	56.8 ± 2.3
		50	0.048 ± 0.0020	0.144 ± 0.0052	45.1 ± 2.6
		75	0.048 ± 0.0007	0.111 ± 0.0031	29.6 ± 1.5

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- OATP1B1-mediated [^{3}H]pitavastatin uptake was calculated by subtracting the uptake of [^{3}H]pitavastatin into MDCKII cells from that observed in MDCKII-OATP1B1 cells and data were normalized to % control, where uptake in the absence of test compound was 100%. Inhibition by the positive control inhibitor CsA confirmed the functionality of the assay.

Result: Doravirine inhibited OATP1B1-mediated transport of pitavastatin with IC_{50} = 39 ± 2 µM.

Sec. 2.6.5.7.9.5 Inhibition of OATP1B3 by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439]
Type of Study	In vitro inhibition of organic anion transporting polypeptide 1B3 (OATP1B3)
Methods	Uptake of [^{3}H]sulfobromophthalein ([^{3}H]BSP; 0.1 µM) was measured in Madin-Darby canine kidney (MDCKII) and MDCKII cells stably expressing human OATP1B3(MDCKII-OATP1B3) cells in the absence or presence of doravirine (0 to 75 µM) or the control inhibitor cyclosporine A (CsA; 10 µM). Cells were incubated at 37°C for 5 minutes and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed 4 times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.
Analyte	[^{3}H]BSP
Assay	Liquid scintillation counting

Substrate	**Inhibitor**	**Inhibitor concentration (µM)**	**MDCKII (pmol/10^6 cells/min) Mean ± SEM**	**MDCKII-OATP1B3 (pmol/10^6 cells/min) Mean ± SEM**	**% Control Mean ± SEM**
[^{3}H]BSP	-	0	0.297 ± 0.049	2.178 ± 0.182	
[^{3}H]BSP	CsA	10	0.281 ± 0.047	0.380 ± 0.025	
[^{3}H]BSP	Doravirine	0	0.297 ± 0.049	2.178 ± 0.182	100.0 ± 10.0
		3	0.331 ± 0.052	1.913 ± 0.142	84.1 ± 8.0
		10	0.34 ± 0.060	1.753 ± 0.036	75.1 ± 3.7
		25	0.286 ± 0.034	1.444 ± 0.175	61.6 ± 9.5
		35	0.293 ± 0.038	1.205 ± 0.098	48.5 ± 5.6
		50	0.294 ± 0.047	0.905 ± 0.019	32.5 ± 2.7
		75	0.286 ± 0.045	0.829 ± 0.005	28.9 ± 2.4

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- OATP1B3-mediated [^{3}H]BSP uptake was calculated by subtracting the uptake of [^{3}H]BSP into MDCKII cells from that observed in MDCKII-OATP1B3 cells and data were normalized to % control, where uptake in the absence of test compound was 100%. Inhibition by the positive control inhibitor CsA confirmed the functionality of the assay.

Result: Doravirine inhibited OATP1B3-mediated transport of BSP with IC_{50} = 31 ± 4 µM.

Sec. 2.6.5.7.9.6 Inhibition of OAT1 by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439]
Type of Study	In vitro inhibition of the human organic anion transporter 1 (OAT1)
Methods	Uptake of [^{3}H]cidofovir ([^{3}H]CDF; 1 µM) was measured in Madin-Darby canine kidney (MDCKII) cells and MDCKII cells stably expressing human OAT1 (MDCKII-OAT1) in the absence or presence of doravirine (0 to 75 µM) or the positive control inhibitor probenecid (1 mM). Cells were incubated at 37°C for 5 minutes and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed four times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.
Analyte	[^{3}H]Cidofovir
Assay	Liquid scintillation counting

Substrate	Inhibitor	Inhibitor concentration (µM)	MDCKII (pmol/10^6 cells/min)	MDCKII-OAT1 (pmol/10^6 cells/min)	% Control
			Mean ± SEM	Mean ± SEM	Mean ± SEM
[^{3}H]CDF	-	0	0.011 ± 0.003	4.032 ± 0.017	
[^{3}H]CDF	Probenecid	1000	0.015 ± 0.004	0.332 ± 0.300	
[^{3}H]CDF	Doravirine	0	0.011 ± 0.003	4.032 ± 0.017	100.0 ± 0.2
		3	0.007 ± 0.002	4.226 ± 0.097	104.9 ± 1.4
		10	0.008 ± 0.001	3.959 ± 0.036	98.2 ± 0.5
		25	0.010 ± 0.002	4.097 ± 0.057	101.6 ± 0.8
		35	0.013 ± 0.003	4.319 ± 0.264	107.1 ± 3.8
		50	0.013 ± 0.002	3.871 ± 0.083	95.9 ± 1.2
		75	0.009 ± 0.001	3.500 ± 0.044	86.8 ± 0.6

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- OAT1-mediated [^{3}H]cidofovir uptake was calculated by subtracting the uptake of [^{3}H]Cidofovir into MDCKII cells from that observed in MDCKII-OAT1 cells and data were normalized to % control, where uptake in the absence of doravirine was 100%. Inhibition by the positive control inhibitor probenecid confirmed the functionality of the assay.

Result: Doravirine (3-75 µM) had no significant effect on OAT1-mediated transport of cidofovir (IC_{50} >75 µM).

Sec. 2.6.5.7.9.7 Inhibition of OAT3 by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439]
Type of Study	In vitro inhibition of the human organic anion transporter 3 (OAT3)
Methods	Uptake of [^{3}H]estrone sulfate ([^{3}H]ES; 1 µM) was measured in Madin-Darby canine kidney (MDCKII) cells and MDCKII cells stably expressing human OAT3 (MDCKII-OAT3) in the absence or presence of doravirine (0 to 75 µM) or the positive control inhibitor probenecid (1 mM). Cells were incubated at 37°C for 5 minutes and uptake was stopped by the addition of ice-cold PBS. Cells were harvested by centrifugation at 4°C and then washed four times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.
Analyte	[^{3}H]ES
Assay	Liquid scintillation counting

Substrate	Inhibitor	Inhibitor concentration (µM)	MDCKII (pmol/10^6 cells/min)	MDCKII-OAT3 (pmol/10^6 cells/min)	% Control
			Mean ± SEM	Mean ± SEM	Mean ± SEM
[^{3}H]ES	-	0	0.092 ± 0.005	6.477 ± 0.358	
[^{3}H]ES	Probenecid	1000	0.115 ± 0.014	0.551 ± 0.374	
[^{3}H]ES	Doravirine	0	0.092 ± 0.005	6.477 ± 0.358	100.0 ± 3.2
		3	0.103 ± 0.004	5.598 ± 0.449	86.1 ± 4.1
		10	0.092 ± 0.009	3.872 ± 0.251	59.2 ± 2.3
		25	0.095 ± 0.011	2.571 ± 0.127	38.8 ± 1.1
		35	0.101 ± 0.009	2.186 ± 0.085	32.7 ± 0.8
		50	0.088 ± 0.006	1.599 ± 0.115	23.7 ± 1.0
		75	0.093 ± 0.007	1.330 ± 0.125	19.4 ± 1.1

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- OAT3-mediated [^{3}H]ES uptake was calculated by subtracting the uptake of [^{3}H]ES into MDCKII cells from that observed in MDCKII-OAT3 cells and data were normalized to % control, where uptake in the absence of doravirine was 100%. Inhibition by the positive control inhibitor probenecid confirmed the functionality of the assay.

Result: Doravirine inhibited OAT3-mediated transport of estrone sulfate with IC_{50} = 16.0 ± 0.7 µM.

Sec. 2.6.5.7.9.8 Inhibition of OCT2 by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK007MK1439]
Type of Study	In vitro inhibition of the human organic cation transporter 2 (OCT2)
Methods	Uptake of [^{14}C]metformin ([^{14}C]MTF[;10 µM) was measured in Chinese hamster ovary (CHO-K1) and CHO-K1 cells stably expressing human OCT2 (CHO-K1-OCT2) cells in the absence or presence of doravirine (0 to 75 µM) or the positive control inhibitor quinidine (100 µM). Cells were incubated at 37°C for 5 minutes and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed four times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.
Analyte	[^{14}C]MTF
Assay	Liquid scintillation counting

Substrate	**Inhibitor**	**Inhibitor concentration (µM)**	**CHO-K1 (pmol/10^6 cells/min)**	**CHO-K1-OCT2 (pmol/10^6 cells/min)**	**% Control**
			Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**
[^{14}C]MTF	-	0	0.321 ± 0.023	1.096 ± 0.043	
[^{14}C]MTF	QD	100	0.143 ± 0.005	0.174 ± 0.004	
[^{14}C]MTF	Doravirine	0	0.321 ± 0.023	1.096 ± 0.043	100.0 ± 6.3
		3	0.262 ± 0.025	1.125 ± 0.012	111.4 ± 3.5
		10	0.309 ± 0.019	1.085 ± 0.069	100.2 ± 9.3
		25	0.350 ± 0.025	0.960 ± 0.049	78.8 ± 7.1
		35	0.335 ± 0.033	0.773 ± 0.041	56.5 ± 6.7
		50	0.312 ± 0.028	0.757 ± 0.053	57.4 ± 7.7
		75	0.305 ± 0.035	0.631 ± 0.032	42.0 ± 6.2

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- OCT2-mediated [^{14}C]MTF uptake was calculated by subtracting the uptake of [^{14}C]MTF into CHO-K1 cells from that observed in CHO-K1-OCT2 cells and data were normalized to % control, where uptake in the absence of doravirine was 100%. Inhibition by the positive control inhibitor quinidine confirmed the functionality of the assay.

Result: Doravirine inhibited OCT2-mediated transport of metformin with IC_{50} = 67 ± 9 µM.

Sec. 2.6.5.7.9.9 Inhibition of MATE1 by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK018MK1439]
Type of Study	In vitro inhibition of the human transporter multidrug and toxin extrusion protein 1 (MATE1)
Methods	Uptake of [^{14}C]metformin ([^{14}C]MTF; 5 µM) was measured in Chinese hamster ovary (CHO-K1) and CHO-K1 cells stably expressing human MATE1 (CHO-K1-MATE1) cells in the absence or presence of doravirine (0 to 50 µM) or the positive control inhibitor quinidine (100 µM). Cells were incubated at 37°C for 10 minutes and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed three times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.
Analyte	[^{14}C]MTF
Assay	Liquid scintillation counting

Substrate	Inhibitor	Inhibitor concentration (µM)	CHO-K1 (pmol/10^6 cells/min)	CHO-K1-MATE1 (pmol/10^6 cells/min)	% Control
			Mean ± SEM	Mean ± SEM	Mean ± SEM
[^{14}C]MTF	-	0	0.062 ± 0.002	0.985 ± 0.061	
[^{14}C]MTF	Quinidine	100	0.040 ± 0.001	0.111 ± 0.008	
[^{14}C]MTF	Doravirine	0	0.062 ± 0.002	0.985 ± 0.061	100.0 ± 6.6
		1	0.069 ± 0.007	1.045 ± 0.061	105.7 ± 6.7
		5	0.072 ± 0.003	1.027 ± 0.050	103.5 ± 5.4
		10	0.067 ± 0.006	0.989 ± 0.050	99.9 ± 5.5
		20	0.075 ± 0.008	0.965 ± 0.055	96.4 ± 6.0
		35	0.076 ± 0.004	0.824 ± 0.031	81.0 ± 3.3
		50	0.071 ± 0.002	0.732 ± 0.017	71.6 ±1.8

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- MATE1-mediated [^{14}C]MTF uptake was calculated by subtracting the uptake of [^{14}C]MTF into CHO-K1 cells from that in CHO-K1-MATE1 cells and data were normalized to % control, where uptake in the absence of test compound was 100%. Inhibition by the positive control inhibitor quinidine confirmed the functionality of the assay.

Result: Doravirine (1-50 µM) had minimal effect on MATE1-mediated transport of metformin (IC_{50} >50 µM).

Sec. 2.6.5.7.9.10 Inhibition of MATE2K by Doravirine

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK018MK1439]
Type of Study	In vitro inhibition of the human transporter multidrug and toxin extrusion protein 2K (MATE2K)
Methods	Uptake of [^{14}C]metformin ([^{14}C]MTF; 5 µM) was measured in Madin-Darby canine kidney (MDCKII) and MDCKII cells stably expressing human MATE2K (MDCKII-MATE2K) cells in the absence or presence of doravirine (0 to 50 µM) or the positive control inhibitor pyrimethamine (5 µM). Cells were incubated at 37°C for 5 minutes and uptake was stopped by the addition of ice cold PBS. Cells were harvested by centrifugation at 4°C and then washed three times with PBS at 4°C. The radioactivity in the cell pellets was determined by liquid scintillation counting.
Analyte	[^{14}C]MTF
Assay	Liquid scintillation counting

Substrate	Inhibitor	Inhibitor concentration (µM)	MDCKII (pmol/10^6 cells/min)	MDCKII-MATE2K (pmol/10^6 cells/min)	% Control
			Mean ± SEM	Mean ± SEM	Mean ± SEM
[^{14}C]MTF	-	0	0.106 ± 0.005	0.627 ± 0.009	
[^{14}C]MTF	Pyrimethamine	5	0.102 ± 0.007	0.147 ± 0.012	
[^{14}C]MTF	Doravirine	0	0.106 ± 0.005	0.627 ± 0.009	100.0 ± 2.0
		1	0.096 ± 0.010	0.571 ± 0.045	91.2 ± 8.8
		5	0.087 ± 0.006	0.542 ± 0.050	87.3 ± 9.7
		10	0.095 ± 0.010	0.547 ± 0.055	86.8 ± 10.7
		20	0.086 ± 0.002	0.508 ± 0.037	81.0 ± 7.1
		35	0.101 ± 0.003	0.467 ± 0.040	70.2 ± 7.8
		50	0.105 ± 0.008	0.421 ± 0.025	60.7 ± 5.0

Additional Information:

- SEM = standard error of the mean from 3 replicates.
- MATE2K-mediated [^{14}C]metformin uptake was calculated by subtracting the uptake of [^{14}C]metformin into MDCKII cells from that in MDCKII-MATE2K cells and data were normalized to % control, where uptake in the absence of test compound was 100%. Inhibition by the positive control inhibitor PYR confirmed the functionality of the assay.

Result: Doravirine (1-50 µM) had had minimal effect on MATE2K-mediated transport of metformin (IC_{50} >50 µM).

Sec. 2.6.5.7.10 Induction of CYP1A2, CYP3A4 and CYP2B6 in Human Hepatocytes

Sec. 2.6.5.7.10.1 The Effect of Doravirine on CYP1A2 mRNA and Enzyme Activity in Cryopreserved Human Hepatocytes

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]
Type of Study:	CYP1A2 induction (To evaluate the potential of doravirine to induce CYP1A2 mRNA and activity in human hepatocytes)
Methods:	Commercially available cryopreserved human hepatocytes prepared from 3 single donors were thawed, plated, and cultured for 24 hours in plating medium prior to initiation of the study. The hepatocytes were treated for 48 hours with vehicle control 0.1% (v/v) DMSO, doravirine (0.1 to 20 µM), or the positive control inducer, omeprazole (50 µM) prepared in serum-free incubation medium with all solutions being replaced with fresh solutions at 24 hours. At the end of the 48 hour incubation, whole cell-based CYP1A2 enzyme activity was determined using HPLC-MS/MS, and total RNA was isolated for quantitative PCR analysis of CYP1A2 mRNA expression. Changes in the measured responses following treatment with test compounds were reported as fold changes (response to test compound relative to vehicle control) and as percent relative to the positive control.
Analyte:	CYP1A2 mRNA and O-Demethylated phenacetin
Assay:	RT-PCR or LC-MS/MS.

Treatment	µM	hCYP1A2 mRNA		Phenacetin *O*-Demethylation		hCYP1A2 mRNA		Phenacetin *O*-Demethylation		hCYP1A2 mRNA		Phenacetin *O*-Demethylation	
		Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]
OM	50	31.3	100	22	100	32.1	100	8.9	100	21.3	100	13	100
Doravirine	0.1	1	nr	1.1	0.7	1.2	0.6	1.1	1.7	0.9	nr	1.2	1.6
	0.5	1.1	0.3	1.1	0.3	0.8	nr	1.2	2.6	0.7	nr	1	nr
	1	1.1	0.3	1.1	0.6	0.9	nr	1.1	0.7	1.1	0.4	1.2	1.5
	5	1	0	1.1	0.2	1	0.1	1.0	0.2	0.8	nr	1.1	0.8
	10	1.3	1	1.2	1.1	2.6	5.3	1.4	5.6	1.1	0.4	1.4	3.4
	20	1.2	0.8	1	nr	1.9	3	1.8	9.7	1	nr	1.1	0.9

Additional Information:
nr = not reported; response was less than vehicle
Fold – represents the mean fold change of treated samples compared to vehicle control samples.
[b] % PC – represents the percent of induction relative to positive control rifampicin (10 µM) corrected for vehicle control.

Sec. 2.6.5.7.10.2 The Effect of Doravirine on CYP2B6 mRNA and Enzyme Activity in Cryopreserved Human Hepatocytes

Test Article: Doravirine

Study Number	[Ref. 4.2.2.6: PK010MK1439]												
Type of Study:	CYP2B6 induction (To evaluate the potential of doravirine to induce CYP2B6 mRNA and activity in human hepatocytes.)												
Methods	Commercially available cryopreserved human hepatocytes prepared from 3 single donors were thawed, plated, and cultured for 24 hours in plating medium prior to initiation of the study. The hepatocytes were treated for 48 hours with vehicle control 0.1% (v/v) DMSO, doravirine (0.1 to 20 µM), or the positive control inducer phenobarbital (1000 µM) prepared in serum-free incubation medium with all solutions being replaced with fresh solutions at 24 hours. At the end of the 48 hour incubation, whole cell-based CYP2B6 enzyme activity was determined using HPLC-MS/MS, and total RNA was isolated for quantitative PCR analysis of CYP2B6 mRNA expression. Changes in the measured responses following treatment with test compounds were reported as fold changes (response to test compound relative to vehicle control) and as percent positive control.												
Analyte	Hydroxybupropion												
Assay	RT-PCR or LC-MS/MS.												
Treatment	**µM**	**hCYP2B6 mRNA**		**Bupropion hydroxylation**		**hCYP2B6 mRNA**		**Bupropion hydroxylation**		**hCYP2B6 mRNA**		**Bupropion hydroxylation**	
		Fold[a]	**% of PC[b]**	**Fold[a]**	**% of PC[b]**	**Fold[a]**	**% of PC[b]**	**Fold[a]**	**% of PC[b]**	**Fold[a]**	**% of PC[b]**	**Fold[a]**	**% of PC[b]**
PB	1000	15.7	100.0	7.3	100.0	18.5	100.0	7.2	100.0	14.0	100.0	13.7	100.0
Doravirine	0.1	1.0	Nr	0.7	nr	1.4	2.2	1.0	nr	0.7	nr	0.8	nr
	0.5	1.1	0.7	0.7	nr	1.4	2.2	1.0	nr	0.9	nr	0.9	nr
	1	1.3	1.7	0.7	nr	1.3	1.6	1.0	nr	0.8	nr	0.8	nr
	5	1.5	3.3	0.9	nr	1.5	2.8	1.0	nr	1.1	0.6	0.9	nr
	10[c]	1.7	4.5	0.9	nr	1.8	4.5	1.1	1.2	1.1	1.1	1.0	nr
	20[c]	1.9	6.1	1.0	1.4	1.5	2.8	1.1	1.9	1.2	1.2	0.8	nr

Additional Information:
nr = not reported since response was less than vehicle control.
[a] Fold – represents the mean fold change of treated samples (test article) compared to DMSO vehicle control samples (n=3), or the mean fold change of positive control compared to PBS vehicle control samples (n=3).
[b] % PC – represents the percent of induction relative to positive control phenobarbital (1000 µM) corrected for vehicle control.
[c] [redacted] particles were observed in wells treated with 10 and 20 µM doravirine after 48-hour treatment period.

Sec. 2.6.5.7.10.3 The Effect of Doravirine on CYP3A4 mRNA and Enzyme Activity in Cryopreserved Human Hepatocytes

Test Article: Doravirine

Study Number	[Ref. 4.2.2.3: PK003MK1439]
Type of Study	CYP3A4 induction (To evaluate the potential of doravirine to induce CYP3A4 mRNA and activity in human hepatocytes.)
Methods	Commercially available cryopreserved human hepatocytes prepared from 3 single donors were thawed, plated, and cultured for 24 hours in plating medium prior to initiation of the study. The hepatocytes were treated for 48 hours with vehicle control 0.1% (v/v) DMSO, doravirine (0.1 to 20 µM), or the positive control inducer rifampicin (10 µM) prepared in serum-free incubation medium with all solutions being replaced with fresh solutions at 24 hours. At the end of the 48 hour incubation, whole cell-based CYP3A4, enzyme activity was determined using HPLC-MS/MS, and total RNA was isolated for quantitative PCR analysis of CYP3A4 mRNA expression. Changes in the measured responses following treatment with test compounds were reported as fold changes (response to test compound relative to vehicle control) and as percent relative to the positive control.
Analyte	6β-Hydroxytestosterone
Assay	RT-PCR or LC-MS/MS.

Treatment	µM	hCYP3A4 mRNA		Testosterone 6β-Hydroxylation		hCYP3A4 mRNA		Testosterone 6β-Hydroxylation		hCYP3A4 mRNA		Testosterone 6β-Hydroxylation	
		Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]
RIF	10	8.8	100	5.9	100	16.8	100	9	100	18.2	100	19.7	100
Doravirine	0.1	0.5	nr	0.8	nr	1.5	3.4	1	nr	1.8	4.6	1.2	1.2
	0.5	0.7	nr	0.9	nr	0.8	nr	1	nr	1.7	4.1	1.1	0.8
	1	0.7	nr	0.9	nr	0.9	nr	1	0.3	1.9	5.1	0.9	nr
	5	0.8	nr	1	nr	1.6	3.7	1	0.3	2.7	9.8	1.1	0.3
	10	1.2	3.1	1	nr	3.7	17.2	1.1	0.8	4	17.6	1.1	0.3
	20	1.4	4.7	1	nr	4.2	20.3	1	nr	3.8	16.5	0.9	nr

Additional Information:
nr – not reported; response was less than vehicle.
[a] Fold – represents the mean fold change of treated samples compared to vehicle control samples.
[b] %PC – represents the percent of induction relative to positive control rifampicin (10 µM) corrected for vehicle control.

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