Supplemental Information

Title: Characterization of ADME Properties of [^{14}C]Asunaprevir (BMS-650032) in Humans

Authors: Jiachang Gong, Timothy Eley, Bing He, Vinod Arora, Thomas Philip, Hao Jiang, John Easter, W. Griffith Humphreys, Ramaswamy A. Iyer, and Wenying Li

Pharmaceutical Candidate Optimization (J.G., W.L., W.G.H., R.A.I.), Exploratory Clinical and Translational Research (B.H., T.E.), Analytical and Bioanalytical Development (H.J.), Bristol-Myers Squibb, Princeton, New Jersey, Princeton, NJ, and Chemical Synthesis (J.E.), Bristol-Myers Squibb, Wallingford, CT.

Materials and methods

Incubation of [^{14}C]Asunaprevir with Liver Microsomes. P450-mediated metabolism of [^{14}C]asunaprevir was carried out at 37 °C in a shaking water bath. The incubation mixtures consisted of [^{14}C]asunaprevir (10 μM , 58 nCi/mL), liver microsomes from mice, rats, dogs, monkeys or humans (1.0 mg protein/mL), NADPH (1.0 mM), MgCl_2 (0.5 mM) and potassium phosphate buffer (100 mM, pH 7.4) with a final volume of 0.5 mL. Negative control experiments were conducted under similar conditions but in the absence of NADPH. For the glutathione (GSH) trapping experiments, GSH (5 mM) was added to the incubation mixtures with other ingredients unchanged. The mixtures were allowed to react for 1 hour and were quenched with the addition of 0.5 mL cold acetonitrile. The samples were vortex mixed and centrifuged for 10 minutes at 14000 rpm. Portions (200 μL) of the supernatant were taken for LC/MS profiling and metabolite identification.

Incubation of [^{14}C]Asunaprevir with Hepatocytes. Cryopreserved hepatocytes were thawed as recommended by the manufacturer and the cell suspensions were transferred to 50 mL Falcon centrifuge tubes containing 25 mL pre-warmed K-H buffer. Additional K-H buffer was added to each tube to bring the volume to 40 mL. The diluted suspensions were centrifuged at 50 g for 5 minutes and the supernatants were removed with a Pasteur pipette. The cell pellets were re-suspended in 3 to 5 mL cold K-H buffer. The viability of the hepatocytes was in the range of 66-75%, evaluated with a trypan blue exclusion assay and a hemocytometer.

Incubations were carried out at 37 °C for 4 hours in an NAPCO 6000 incubator (VMR, WestChester, PA) which was maintained under an oxygen/carbon dioxide (95:5) atmosphere. Each incubation contained [^{14}C]asunaprevir (10 μM) and ~2 million cells with a final volume of 1 mL. The reaction was stopped with the addition of 1 mL ice-cold acetonitrile. The samples were centrifuged at 14000 rpm for 10 minutes. Portions (200 μL) of the supernatant were taken for LC/MS profiling and metabolite identification.

Dosing and Sample Collection. All animal studies were conducted under the standards recommended by the Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee.

The [^{14}C]asunaprevir dosing solution was prepared in polyethylene glycol 400 (PEG400)/d- α -tocopherol polyethylene glycol 1000 succinate (TPGS) (60:40, v/v). Animals were administered single oral doses of [^{14}C]asunaprevir at dose levels that were well-tolerated in toxicological studies. Blood samples from animals were collected into tubes containing K_2EDTA . All blood samples were placed on ice immediately and were centrifuged within 30 minutes after collection for 15 minutes at approximately 1000 g to separate plasma.

Intact Mouse Study. The mass-balance of radioactivity and metabolism of [^{14}C]asunaprevir were investigated in intact male rasH2 mice (Charles River Laboratories, Wilmington, MA). Following a fasting period of approximately 5.5 hours, [^{14}C]asunaprevir was administered orally by gavage to 30 male rasH2 mice in Group 1 and 9 male rasH2 mice in Group 2 at a target dose level of 100 mg/kg (200 $\mu\text{Ci/kg}$). The animals were fed Certified Rodent Diet 5002 (PMI Nutrition International, Inc.) and were provided fresh, filtered tap water except when fasted. Terminal blood samples were collected from animals in Group 1 (5 mice/time point) at 1, 4, 8, 12, 24, and 48 hours postdose. Mice in Group 2 were housed 3 per cage in metabolism cages and urine and feces were collected before dosing and over intervals through 168 hours postdose. Cage rinses were performed after each collection interval and a cage wash was performed at the end of study period.

Intact Rat Study. The mass-balance of radioactivity and metabolism of [^{14}C]asunaprevir were investigated in intact male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). The

study consisted of 1 group of 27 male Sprague-Dawley rats (Group 1) and 1 group of 3 male Sprague-Dawley rats (Group 2). Following a fasting period of approximately 8 hours, [¹⁴C]asunaprevir was administered orally by gavage to each animal (excluding the animals designated for predose collections in Group 1) at a target dose level of 80 mg/kg (100 µCi/kg). The animals were fed Certified Rodent Diet 5002 (PMI Nutrition International, Inc.) and were provided fresh, filtered tap water except when fasted. Blood samples were collected from animals in Group 1 (3 animals/time point) prior to dosing and at 1, 2, 4, 8, 12, 24, 48, and 96 hours postdose into tubes containing K₃EDTA. Animals in group 2 were individually housed in metabolism cages and urine and feces were collected predose and over 24 hour intervals through 168 hours after dosing. Cage rinses were performed after each collection interval and a cage wash was performed at the end of study period.

Intact Dog Study: The mass-balance of radioactivity and metabolism of [¹⁴C]asunaprevir were investigated in intact male beagle dogs (Charles River Laboratories, Wilmington, MA). Following a fasting period of approximately 13 hours, [¹⁴C] asunaprevir was administered orally by gavage to 3 male beagle dogs at a target dose level of 50 mg/kg (20 µCi/kg). Blood samples for biotransformation profiling were collected from a peripheral vessel of each animal prior to dosing and at 1, 4, 8, 24, and 48 hours postdose into tubes containing K₃EDTA. Urine and feces were collected predose and over 24 hour intervals through 168 hours after dosing. Cage debris was collected daily after each postdose excreta collection.

BDC Rat Study. The excretion of radioactivity and metabolism of [¹⁴C]asunaprevir were investigated in BDC male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). BDC rats (n = 3) were individually housed in metabolism cages and were freely mobile during the entirety of the study with the exception of brief manual restraint for oral dosing. Each animal was allowed to recover for approximately 48 hours after surgery and following a fasting period of approximately 15 hours, was administered a single oral dose of [¹⁴C]asunaprevir by gavage at a target dose level of 80 mg/kg (100 µCi/kg). The animals were fed Certified Rodent Diet 5002 (PMI Nutrition International, Inc.) and were provided fresh, filtered tap water except when fasted. Blood samples were collected from each animal prior to dosing and at 1, 4, 12, 24, and 48 hours postdose. Bile, urine, and feces were collected prior to dosing and over intervals through 48 hours postdose. A cage rinse was performed after each postdose excreta collection through 24 hours postdose. During the period of sample collection, bile salts were replenished by infusing control bile collected in the day before dose administration at a rate of 1 mL/min.

BDC Monkey Study. The excretion of radioactivity and metabolism of [¹⁴C]asunaprevir were investigated in BDC male cynomolgus monkeys (Metabolism and Pharmacokinetics Technical Support Unit, Bristol Myers Squibb, Princeton, NJ). BDC monkeys (n = 3, weighing approximately 4.5-5.8 kg) were individually housed in metabolism cages and were freely mobile during the entirety of the study with the exception of brief manual restraint for oral dosing. The animals were fed Certified Primate Diet 5048 (PMI Nutrition International, Inc.) and were provided fresh, filtered tap water except when fasted. Following a fasting period of approximately 15 hours, the animals received single oral doses of [¹⁴C]asunaprevir administered by gavage at a target dose level of 100 mg/kg (40 µCi/kg). Blood samples for biotransformation analysis were obtained from each animal prior to dosing and at 1, 2, 4, 8, 24, 48 and 72 hours postdose. Bile, urine and feces were collected at predose, and consecutive intervals up to 72 hours postdose. During the period of sample collection, a bile salt replacement solution (18 mg/mL cholic acid and 1.3 mg/mL sodium bicarbonate in saline, pH 7.6) was administered to the animals via the distal flushing catheter.

Mass Spectrometry for Metabolite Identification. Mass spectrometry analysis was performed on a Thermo-Finnigan LTQ ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA) interfaced with an Agilent 1100 HPLC system. The ESI source was set in positive ion mode and analytical conditions were set as follows: voltage, 4 kV; current, 10 µA; and capillary temperature, 275 °C. MS data were collected for a mass range of 250-1000 amu, and MS/MS data were acquired with the following

parameters: isolation width, 1.5 amu; collision energy, 35 eV; and activation time, 30 ms. The nitrogen flow rate, spray current and voltages were adjusted to give maximum sensitivity. MS and MS/MS analysis were performed to identify the drug related compounds.

The Rationale for Structural Assignment:

Under positive ESI, asunaprevir had a protonated molecule at m/z 748 with product ions at m/z 692, m/z 648 and m/z 535. The mass spectral fragmentation pattern for asunaprevir, illustrated in Table 6, revealed the loss of the *tert*-butyl group to give a product ion at m/z 692 and loss of the *tert*-butoxycarbonyl (BOC) group resulting in a product ion at m/z 648. Similar fragmentation pattern was observed in the metabolites of asunaprevir, which was utilized in the structure assignment.

Metabolite M1 had a protonated molecule at m/z 1071, 307 Da higher than that of M3, a characteristic of GSH adducts. Major mass spectral fragments included a daughter ion at m/z 971 resulting from the loss of the BOC group, and m/z 764 resulting from the loss of GSH moiety. Another fragment ion was observed at m/z 551 which was generated through the cleavage of amide bond, suggesting that GSH was localized near the sulfonamide group.

Metabolite M2 appeared at m/z 535, loss of 213 Da compared to the parent. The MS² spectra showed major product ions at m/z 479 (loss of *tert*-butyl group) and m/z 435 (loss of BOC). Daughter ion at m/z 210 represented an intact isoquinoline methoxy group in the metabolite. Therefore, the structure of M2 was tentatively assigned as shown in Figure 4.

Metabolite M3, M5 and M7 had a protonated molecule at m/z 764 under positive full MS scans, 16 Da higher than that of asunaprevir. Therefore M3, M5 and M7 were tentatively assigned as mono-hydroxylated metabolites of asunaprevir. M5 and M7 had nearly identical MS² spectra with the major daughter ion at m/z 664 generated through the cleavage of BOC, 16 Da higher than the daughter ion of the parent. On the other hand, the fragment ion (m/z 551) of M5 and M7 through the cleavage of amide was identical to that of parent suggesting that the oxidation occurred at the central part of the molecular as shown in Figure 6. The MS² spectrum of M3 was similar to those of M5 and M7, except that the daughter ion (m/z 535) after the cleavage of the amide bond was 16 Da less than that of M5 and M7, suggesting M3 was generated through the oxidation on the sulfonamide portion of the molecule.

Metabolites M4, M21, M22 and M24 had a protonated molecule at m/z 780 under positive full MS scans, 32 Da higher than that of asunaprevir, a characteristic of di-hydroxylated metabolites of asunaprevir. The MS² spectra of the metabolites were nearly identical where the major daughter ions either through the loss BOC (m/z 680) or through the cleavage of the amide group (m/z 567) were 32 Da higher than that of asunaprevir. This suggests that both oxidation occurred at the central portion of the molecule.

Metabolite M6 produced a molecular ion at m/z 734 with a mass of 14 Da less than parent, implying a demethylation metabolite. The MS² spectra showed major product ions at m/z 678 (loss of *tert*-butyl group), m/z 634 (loss of BOC) and m/z 521. Further confirmation of the metabolite M6 structure came from co-elution and LC/MS/MS comparison with the chemically synthesized reference standard.

Metabolite M8 had a protonated molecule at m/z 645 under positive ESI. The MS² spectra showed major product ions at m/z 589 (loss of *tert*-butyl group), m/z 545 (loss of BOC) and m/z 436 (loss of isoquinoline moiety). M8 was tentatively identified as the product through the sulfonamide.

Mass spectral analysis of M9, under positive ESI, resulted in a protonated molecule at m/z 557, consistent with an ether cleavage product. The MS² fragmentation generated a daughter ion at m/z 457 resulting from the loss of BOC, and m/z 344 through the cleavage of the amide. Moreover, the retention time and mass spectra data were identical to those of the chemically synthesized reference standard.

Metabolite M11, M19 and M20 had protonated molecular ions at m/z 661, 16 Da higher than that of M8. Therefore, M11, M19 and M20 were tentatively identified as mono-hydroxylated metabolites of M8. M19 and M20 had similar MS² spectra with major fragments at m/z 643 (loss of water), m/z 605 (loss of *tert*-butyl) and m/z 561 (loss of BOC), suggesting that no structure modification occurred on the BOC moiety. However, the fragment of M19 after the cleavage of proline amide was 16 da higher than that of M20, and the fragment after the cleavage of the amide bond was 16 Da less than that of M20, indicating that M19 was likely formed through the oxidation of t-butyl group adjacent to the proline and M20 was formed through the oxidation near the cyclopropyle. M8 had daughter ions at m/z 589 and m/z 545, through the loss of t-butyl and the BOC, respectively, suggesting that the oxidation occurred on the BOC moiety.

Metabolite M12, M25 and M26 had protonated molecular ions at m/z 573, 16 Da higher than that of M9. Therefore, M11, M19 and M20 were tentatively identified as mono-hydroxylated metabolites of M9. The MS² spectra of the metabolites were nearly identical, which displayed daughter ions at m/z 360 (cleavage of the amide bond), m/z 517 (loss of *tert*-butyl) and m/z 473 (loss of the BOC), suggesting the oxidation occurred on the central portion of the molecule.

The definite structure of M12 was characterized with NMR analysis. The absence of protons in the aromatic region is consistent with loss of the chloro-methoxyisoquinoline moiety. ¹H NMR suggested that protons on the olefin have shifted upfield significantly (δ 5.1 to δ 3.9, and δ 5.3 to δ 3.6) compared with the parent, while other protons had no obvious shift. In addition, the carbon atoms on the olefin shifted upfield by 55ppm from 117.5 to 62.5 ppm and by 73.6 ppm from 133.2 to 59.6 ppm, respectively, on ¹³C NMR. These data pointed to the formation of an epoxide at the terminal olefin position of M12.

Metabolite M15 had a protonated molecular ion at m/z 565 under positive full MS scans. The MS² spectra displayed fragment ions at m/z 509 (loss of t-butyl), m/z 465 (loss of the BOC) and m/z 356 (loss of isoquinoline moiety). M15 was tentatively identified a carboxyl acid metabolite as shown in Figure 4.

Table S1. Relative distribution of metabolites in incubations of [¹⁴C]asunaprevir and liver microsomes (LM) and hepatocytes (Hep) from mouse, rat, dog, monkey and human.^a

Metabolite ^a	% Distribution of Radioactivity ^b									
	Mouse		Rat		Dog		Monkey		Human	
	LM	Hep	LM	Hep	LM	Hep	LM	Hep	LM	Hep
Asunaprevir	78.8	76.7	79.7	63.3	85.1	81.4	44.0	65.5	75.9	69.1
M1	nd	0.4	nd	2.6	nd	MS	nd	1.5	nd	0.9
M2	1.5	1.5	1.4	MS	1.6	1.7	3.2	2.0	2.0	0.7
M3	MS	MS	0.9	1.4	MS	0.3	2.9	1.5	2.1	0.3
M4	MS	MS	0.8	1.5	nd	MS	5.1	0.8	1.9	MS
M5	MS	nd	MS	MS	nd	nd	MS	1.0	MS	nd
M6	4.6	3.1	1.3	2.4	3.1	4.8	5.7	1.9	2.3	10.0
M7	3.1	2.3	2.6	2.8	1.6	1.0	8.3	4.2	4.7	1.4
M8	1.8	nd	0.2	0.3	MS	nd	1.1	0.4	MS	nd
M9	2.1	2.6	1.0	5.4	1.2	1.3	3.4	3.0	1.0	3.5
M11	1.8	MS	0.8	0.2	0.2	0.9	1.5	1.1	1.4	0.6
M10	nd	nd	nd	MS	nd	MS	nd	nd	nd	nd
M12	0.1	0.5	0.4	2.4	MS	0.8	3.5	1.7	0.8	1.7
M15	1.1	1.2	MS	1.4	MS	1.1	1.4	1.2	0.9	0.9
Total^c	94.9	88.3	89.1	83.7	92.8	93.3	80.1	85.8	93.0	89.1

Abbreviations: nd: not detected; P: parent compound; M: metabolite; MS: only detected by mass spectrometry.

^a Structures of metabolites are presented in Table 3. Metabolites were identified by mass spectrometry and by comparison of retention times to chemically synthesized reference standards.

^b Radioactive peaks are reported as a percentage of total radioactivity eluted from the column after background subtraction.

^c The total sample radioactivity (sum of radioactive peaks) was less than 100% due to the presence of small unidentified radioactive peaks. Each of these peaks represented < 0.2% of the total sample radioactivity.

Table S2. Percent of radioactive dose recovery in intact mice, intact and BDC rats, intact dogs and BDC monkeys after oral administration of [¹⁴C]asunaprevir.

Species	No. of Subjects	Dose	Collection Interval (h)	Percentage of Radioactive Dose			
				Urine	Bile	Feces	Total ^b
Intact Mouse	9	100 mg/kg	0 – 168	1.4 ± 0.8	N.A. ^a	87.9 ± 6.7	94.33 ± 5.47
Intact Rat	3	80 mg/kg	0 – 168	0.3 ± 0.2	N.A.	86.9 ± 11.5	91.20 ± 7.77
BDC Rat	3	80 mg/kg	0 – 48	1.0 ± 0.8	25.5 ± 1.7	43.0 ± 10.0	89.76 ± 9.36
Intact Dog	3	50 mg/kg	0 – 168	0.4 ± 0.2	N.A.	77.1 ± 13.8	97.14 ± 1.22
BDC Monkey	3	100 mg/kg	0 – 72	0.4 ± 0.2	26.3 ± 14.7	55.6 ± 12.3	82.30 ± 6.0

a. N.A., not applicable

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

Table S3. Metabolite exposures (AUC) in plasma from mice, rats and dogs following oral doses of [¹⁴C]asunaprevir ^a

Metabolite ID	Metabolite AUC in plasma (µg asunaprevir equiv./L*h)		
	Mouse plasma AUC _{0-8 h}	Rat plasma AUC _{0-8 h}	Dog plasma AUC _{0-8 h}
TRA	1.15×10^5	1.04×10^5	4.55×10^4
Asunaprevir	6.57×10^4	7.96×10^4	3.34×10^4
M3	5.14×10^2	9.62×10^2	4.03×10^3
M4	- ^b	9.63×10^2	- ^b
M7	7.63×10^2	7.21×10^2	2.13×10^2
M8	1.45×10^2	9.97×10^1	7.95×10^2
M9	7.95×10^2	- ^b	1.67×10^2
M12	3.37×10^2	- ^b	- ^b
M15	4.24×10^2	- ^b	2.24×10^2
M20	1.06×10^3	4.62×10^2	6.54×10^2
M25	4.28×10^2	1.83×10^2	- ^b

a. AUC values in mice, rats and dogs are estimated from concentration data based on TRA concentration of plasma and percentage distribution of metabolites in plasma.

b. AUC was not calculated due to low concentration of metabolites.

Table S4. Relative distribution of metabolites in plasma from mice, rats, dogs and BDC monkeys after oral administration of [¹⁴C]asunaprevir.^a

Metabolite ID ^b	% Distribution of Radioactivity in plasma ^c									
	Mouse			Rat			Dog			BDC Monkey
	1 h	4 h	8 h	1h	4 h	8 h	1 h	4 h	8 h	2 h
P	70.8	53.3	43.0	74.9	90.8	79.0	74.2	72.4	74.3	16.0
M3	1.6	1.9	1.2	1.0	0.8	2.3	0.5	0.6	5.4	2.8
M4	MS	MS	MS	0.7	0.2	0.3	ND	ND	ND	1.8
M5	0.6	0.8	MS	ND	ND	ND	MS	1.0	MS	MS
M7	2.5	5.1	MS	0.7	0.7	1.2	0.6	0.8	MS	2.6
M8	0.4	0.5	0.8	0.2	0.2	ND	ND	ND	ND	3.7
M9	1.5	2.9	9.0	0.9	ND	ND	0.3	0.4	0.5	10.2
M12	1.3	1.0	0.8	MS	MS	MS	MS	MS	0.4	3.2
M15	1.3	1.5	1.4	MS	MS	0.3	MS	0.7	2.0	9.6
M19	ND	ND	ND	0.5	MS	MS	ND	ND	ND	ND
M20	2.6	4.8	2.2	0.3	0.5	0.7	1.0	1.7	2.2	2.3
M21	1.1	1.3	0.9	ND	ND	ND	ND	ND	ND	ND
M22	0.9	1.8	0.8	0.3	MS	MS	0.4	0.2	ND	ND
M24	ND	ND	ND	1.0	0.7	1.9	0.5	0.7	1.2	ND
M25	1.5	1.3	1.6	0.3	0.4	MS	ND	ND	ND	4.3
Total	86.1	76.2	61.7	80.8	94.3	85.7	77.5	78.5	86.0	56.5

- Additional Information: The structures of metabolites were determined based on their LC/MS fragmentation pattern, comparison of HPLC retention times and MS fragmentation patterns. Abbreviations: ND= not detected by radioactivity; ms=detected by mass-spectrometry
- Structure of metabolites are presented in Table 6.
- Radioactive peaks are reported as a percentage of total radioactivity eluted from the column after background subtraction

Table S5. Relative distribution of metabolites in urine, feces and bile from Mouse, Rat, Dog and monkey after oral administration of [¹⁴C]asunaprevir.^a

Metabolite ^b	% Distribution of Radioactivity in Urine and Feces Samples ^c (% dose)					
	Urine	Feces (0-168 h)			Bile (0-48 h for rat and 0-72 h for monkey)	
	Mouse	Mouse	Rat	Dog	BDC Rat	BDC Monkey
P	37.0 (0.5)	40.0 (35.1)	41.6 (36.2)	54.6 (42.1)	71.8 (18.3)	11.8 (3.1)
M1	ND	ND	ND	1.0 (0.8)	ND	ND
M3	1.9 (0.0)	1.3 (1.1)	2.1 (1.8)	3.0 (2.3)	0.5 (0.1)	2.0 (0.5)
M4	ND	0.9 (0.8)	0.4 (0.3)	ND	0.4 (0.1)	7.8 (2.0)
M5	ND	1.3 (1.1)	1.3 (1.1)	2.1 (1.6)	1.0 (0.3)	0.7 (0.2)
M7	5.3 (0.1)	6.4 (5.6)	4.2 (3.7)	1.5 (1.2)	2.9 (0.8)	3.9 (1.0)
M8	ND	0.9 (0.8)	2.5 (2.2)	1.0 (0.8)	MS	3.1 (0.8)
M9	12.7 (0.2)	9.5 (8.3)	8.1 (7.0)	3.0 (2.3)	1.8 (0.5)	8.9 (2.3)
M11	ND	2.4 (2.1)	0.3 (0.2)	MS	MS	MS
M12	6.9 (0.1)	4.9 (4.3)	3.2 (2.8)	1.6 (1.2)	1.8 (0.5)	6.4 (1.7)
M15	ND	1.5 (1.3)	2.7 (2.3)	0.9 (0.7)	0.6 (0.2)	3.7 (1.0)
M19	ND	4.7 (4.2)	3.7 (3.2)	0.6 (0.5)	0.9 (0.2)	0.5 (0.1)
M20	ND	2.0 (1.8)	2.7 (2.3)	1.5 (1.2)	0.7 (0.2)	1.5 (0.4)
M21	ND	0.4 (0.3)	0.5 (0.4)	0.8 (0.6)	0.3 (0.1)	0.6 (0.2)
M22	3.2 (0.0)	1.6 (1.4)	1.2 (1.0)	1.5 (1.2)	0.7 (0.2)	5.9 (1.5)
M24	ND	3.0 (2.6)	2.4 (2.1)	1.7 (1.3)	MS	MS
M25	ND	2.3 (2.0)	2.4 (2.1)	ND	MS	10.6 (2.8)
M26	ND	0.5 (0.4)	0.9 (0.8)	ND	ND	0.5 (0.1)
Total	67.0 (1.0)	83.6 (73.5)	80.2 (69.7)	74.8 (57.6)	83.4 (21.3)	67.9 (17.8)

- Additional Information: The structures of metabolites were determined based on their LC/MS fragmentation pattern, comparison of HPLC retention times and MS fragmentation patterns. Abbreviations: ND= not detected by radioactivity; ms=detected by mass-spectrometry
- Structure of metabolites are presented in Table 6.
- Radioactive peaks are reported as a percentage of total radioactivity eluted from the column after background subtraction

Table S6. HPLC- MRM peak area ratios of metabolites relative to parent and estimated AUC_{0-12h} values

Metabolites	Treatment A (single dose)		Treatment D (multiple doses)		% of metabolite to the total exposures in treatment D
	Metabolite/Parent Area Ratio	AUC _{0-12h} (µg/L·h)	Metabolite/Parent Area Ratio	AUC _{0-12h} ^b (µg/L·h)	
BMS-650032	-	207	-	529	47.2
M4	0.5	10.9	1.21	67.5	6.0
M3	1.02	10.9	3.76	103	9.2
M12	0.17	5.65	0.83	70.5	6.3
M9	0.08	17.3	0.14	77.5	6.9
M8	0.09	6.02	0.1	17.1	1.5
M15	0.02	7.53	0.08	77.0	6.9
M20	0.14	4.53	0.56	46.3	4.1
M7	0.91	11.7	1.84	60.4	5.4
M25	0.12	16.2	0.21	72.4	6.5

Figure S1. Metabolite profiles of [^{14}C]asunaprevir (10 μM) incubated with liver microsomes and hepatocytes from mouse, rat, dog, monkey, and human.

Figure S2. Metabolite profiles of pooled plasma from (A) mice (1 h); (B) rats (1 h); (C) dogs (1 h) and (D) BDC monkeys after an oral administration of [^{14}C]asunaprevir. The profiles are background-subtracted reconstructed radiochromatograms of 15-s fractions collected from a HPLC run.

Figure S3. Metabolite profiles of pooled feces from (A) mice; (B) rats and (C) dogs after an oral administration of [^{14}C]asunaprevir. The profiles are background-subtracted reconstructed radiochromatograms of 15-s fractions collected from a HPLC run.

Figure S4. Metabolite profiles of pooled bile from (A) BDC rats and (B) BDC monkeys after an oral administration of [^{14}C]asunaprevir. The profiles are background-subtracted reconstructed radiochromatograms of 15-s fractions collected from a HPLC run.