Preclinical Pharmacokinetics of a HepDirect Prodrug of a Novel Phosphonate-Containing Thyroid Hormone Receptor Agonist

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List of Nonstandard Abbreviations:
CMC, carboxymethylcellulose
GI, gastrointestinal
PEG-400, polyethyleneglycol 400
PG, propylene glycol
MB07344, 3,5-dimethyl-4-(4'-hydroxy-3'-isopropylbenzyl)]phenoxylmethylphosphonic acid
MB07811, cis-(S)-4-{3-chlorophenyl)-2-oxo-2-lambda-5-[1,3,2]dioxaphosphinan-2-ylmethoxy-2,6-dimethylbenzyl}-2-isopropyl-1-phenol
QWBA, quantitative whole body autoradiography
TR, thyroid hormone receptor

ABSTRACT

The prodrug (MB07811) of a novel phosphonate-containing thyroid hormone receptor (TR) agonist (MB07344) is the first application of the HepDirect[1] liver-targeting approach to a non-nucleotide agent. The disposition of MB07811 was characterized in rat, dog, and monkey to assess its liver-specificity which is essential in limiting the extrahepatic side effects associated with this class of lipid lowering agents. MB07811 was converted to MB07344 in liver microsomes from all species tested (CL_{int} 1.23 – 145.4 µL/min/mg). The plasma clearance and volume of distribution of MB07811 matched or exceeded 1 L/h/kg and 3 L/kg, respectively. Although absorption of prodrug was good, its absolute oral bioavailability as measured systemically was low (3-10%), an indication of an extensive hepatic first pass effect. This effect was confirmed by comparison of systemic exposure levels of MB07811 following portal and jugular vein administration to rats, which demonstrated a hepatic extraction ratio of >0.6 with liver CYP3A-mediated conversion to MB07344 a major component. The main route of elimination of MB07811 and MB07344 was biliary, with no evidence for enterohepatic recirculation of MB07344. Similar metabolic profiles of MB07811 were obtained in liver microsomes across the species tested. Tissue distribution and whole body autoradiography confirmed that liver is the major target organ of MB07811 and that conversion to MB07344 was high in the liver relative to other tissues. Hepatic first pass extraction and metabolism of MB07811, coupled with likely selective distribution of MB07811-derived MB07344, led to a high degree of liver targeting of MB07344.

INTRODUCTION

The thyroid hormones (T_3 and T_4) are important modulators of lipid homeostasis, thermogenesis, and metabolic rate (Yen, 2001). The effects of the thyroid hormones are widespread and arise from their binding to specific nuclear receptors found in most cell types and tissues (Hulbert, 2000). There are four main isoforms of thyroid hormone receptors (TR), TRα1, TRα2, TRβ1, TRβ2, that are expressed differentially in tissues (Lazar, 1993). The lipid lowering activity of thyroid hormones and related analogues as a treatment for hypercholesterolemia and obesity has been demonstrated in animals as well as in man (Krotkieswki, 2000). But to date, the development of TR agonists as safe and effective agents to treat hypercholesterolemia and obesity has been hampered mainly by dose-limiting cardiovascular toxicities resulting in an insufficient safety window (Underwood et al., 1986). Because beneficial effects such as lowering of plasma cholesterol are mediated primarily by activation of the TRβ isoform in liver (Forrest et al., 1996) and effects on cardiac function are mediated by TRα in heart (Wikstrom et al., 1998), one approach pursued to improve the therapeutic index of TR agonists has focused on TRβ-selective agents (Ye et al., 2003). This strategy, however, does not circumvent other potentially deleterious effects of TRβ activation in extrahepatic tissues, among which are thyroid hormone axis suppression, muscle wasting, and bone loss (Brenta et al., 2007).

The design of a synthetic TR agonist that specifically targets the liver, the major site of cholesterol metabolism, and reduces or eliminates the deleterious cardiac and other extrahepatic side effects should in theory result in an effective therapeutic. In an effort to reach this goal, a TRß-selective, phosphonate-containing agonist was designed, MB07344 (K_i = 35 and 3 nM at TRα and TRβ, respectively) (Erion et al., 2007). Because

phosphonates are negatively charged at physiological pH, MB07344 was predicted to have

a low volume of distribution and to distribute preferentially to liver, potentially due to

efficient uptake by hepatic organic anion transporters (Erion et al., 2005a). To enhance

liver-targeting of the pharmacological effects of this novel agent, MB07811, the

HepDirect[1] prodrug of MB07344, was synthesized (Figure 1).

The HepDirect approach enables the delivery of certain phosphate and

phosphonate drugs to the liver with high specificity (Erion, 2006; Hecker et al., 2007).

By masking the phosphate or phosphonate groups, HepDirect prodrugs are predicted to

increase the absorption of the parent compound by increasing its permeability across the

gut membrane (Erion et al., 2004). HepDirect prodrugs are then specifically activated by

CYP3A, a P450 enzyme that is localized predominantly in the liver, releasing the active

metabolite, or active metabolite precursor, and a byproduct that is neutralized by

conjugation with glutathione (Erion et al., 2005b), which is present at mM concentrations

in liver. Formation of the byproduct is not associated with hepatocellular or other

toxicity (Erion et al., 2005b). To date, HepDirect prodrugs of PMEA, i.e., pradefovir

(MB06866Q), an oral hepatitis B virus (HBV) antiviral drug (Lin et al., 2005), and of

cytarabine 5'-monophosphate, i.e., MB07133, an agent delivered by IV infusion for the

treatment of hepatocellular carcinoma (HCC), have reached clinical status (Boyer et al.,

2005). Pradefovir and MB07133 are prodrugs of mononucleotide analogues and target

their respective therapeutically active metabolites (i.e., nucleoside analogue 5'-

triphosphates) to the liver in animal models (Erion et al., 2005b). MB07811 is the first

non-nucleotide HepDirect prodrug to be evaluated in humans.

Preclinical proof of concept for MB07811 was obtained in multiple studies in normal rodents and rodent models of hyperlipidemia (Erion et al., 2007). The pharmacological targeting of MB07811-derived MB07344 is perhaps best illustrated by an evaluation of the relative effects of MB07811 and T3 treatment of rats on the expression of TR-responsive genes in various tissues. As shown in Table 1, acute treatment with MB07811 resulted in marked changes in expression of TR-responsive genes in liver but relatively minor changes in the other tissues evaluated. This is in contrast to T3 treatment, which resulted in marked changes in gene expression in all tissues evaluated. In repeat dose studies in the rat, doses of MB07811 up to 50 mg/kg/day (~125-fold the ED_{50} for cholesterol lowering) did not affect heart rate or contractile function (Erion et al., 2007). In contrast, T3 treatment led to marked increases in heart rate and left ventricular pressure at doses <7-fold higher than the ED_{50} for cholesterol lowering (0.012 mg/kg). These and other studies allowed the conclusion that MB07811 has a significantly improved therapeutic index relative to the natural TR ligand, T3.

Preclinical pharmacokinetic properties of MB07344 and MB07811 were evaluated in the rat, dog, and monkey, species that have served as models for evaluating cholesterol lowering. Studies described here assessed the liver first pass effect and distribution of MB07344 to test the concept that administration of its HepDirect prodrug leads to liver targeting of the active metabolite.

METHODS

Materials. All protocols involving animal experimentation were reviewed and approved by the respective IACUC (Institution Animal Care and Use Committee) of each test facility. Unless otherwise specified, studies were performed at Metabasis Therapeutics, La Jolla, CA. In-life procedures were in compliance with the Guide for the Care and Use of Laboratory Animals, National Research Council, Institute of Laboratory Animal Resources, 1996. The HepDirect prodrug MB07811, its active metabolite MB07344, and MB06588, the glutathione adduct of the byproduct formed after conversion of MB07811, were prepared at Metabasis Therapeutics, Inc. (Figure 1). Radiolabeled [bridge-^{14}C]MB07811, [5'-^{3}H]MB07811, and [5'-^{3}H]MB07344 were synthesized at Moravek Biochemicals (Brea, CA). Animal and human liver microsomes were purchase from In Vitro Technologies (Baltimore, MD). Microsomes prepared from baculovirus-infected insect cell lines containing wild type baculovirus (empty vector), recombinant human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2D6, CYP3A4, and CYP3A5 (Supersomes™) were purchased from BD Gentest Corp. (Woburn, MA). Chemicals were reagent grade or better. Plasma and tissue awaiting analysis were stored frozen at -20ºC.

In Vitro Studies

Kinetics of conversion of MB07811 to MB07344 in animal and human liver microsomes and effect of ketoconazole. The conversion of MB07811 to MB07344 in pooled male and female human, cynomolgus monkey, beagle dog, Sprague Dawley (SD) rat, New Zealand White (NZW) rabbit, and ICR/CD1 mouse liver microsome preparations was determined

by quantification of MB06588, a known byproduct of the reaction, and/or MB07344 by

reverse phase high performance liquid chromatography (HPLC). After verifying the

linearity of the reactions, substrate saturation kinetics were performed at a 1 mg/mL

microsomal protein concentration and a range of MB07811 concentrations (12.5, 25, 50,

100 and 200 µM) in duplicate reaction mixtures (5 min, 37°C). Reactions were

quenched with methanol (60%, v:v). The Enzyme Kinetics module of Sigma Plot®

software (version 9.01; Systat Software, Inc.; Point Richmond, CA) was used to

determine V_{max} and K_m kinetic values by fitting the kinetic data to the Henri-Michaelis-

Menten equation. Sigma Plot® was used to generate double-reciprocal data plots. CL_{int}

values were calculated by dividing V_{max} values by K_m values. Statistical significance was

determined by the unpaired Student's t-test using the GraphPad InStat software (version

3.01 for Windows 95/NT; GraphPad Software Inc.; San Diego, CA), and p values less

than 0.05 were considered statistically significant. V_{max}, K_m and CL_{int} values are reported

as mean values and the standard error of the mean (SEM). To determine whether the

conversion of MB07811 to MB07344 is mediated by CYP3A4, the metabolism of

MB07811 was assessed in human liver microsomes in the presence of a specific CYP3A4

inhibitor, ketoconazole (0.01, 1, and 100 µM).

Metabolism of MB07811 in liver microsomes. Tritium-labeled [5'-^{3}H]-MB07811 was

incubated in nicotinamide adenine dinucleotide phosphate- (NADPH-) and glutathione-

fortified, mixed pools of liver S9 or microsomes (2 mg/mL) from male and female SD

rats, male beagle dogs, male cynomolgus monkeys, or male humans for 0 to 1 h at 37°C.

The reactions were terminated by addition of 1.5 volumes of methanol. Following

mixing and centrifugation of the extract to remove the protein, the supernatants were

analyzed by on-line radiography-coupled HPLC. Interspecies differences were

qualitatively assessed by visual comparison of the radiochromatograms.

CYP phenotyping. MB07811 (5 µM) was incubated in triplicate with microsomes

prepared from baculovirus-infected insect cell lines containing recombinant human CYP

enzymes (25 pmol) for 0, 15, and 30 min, including CYP1A1, CYP1A2, CYP2A6,

CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP3A4, and CYP3A5.

Since the protein concentration per pmol enzyme activity varied among the recombinant

enzymes, the protein concentration per incubation was normalized by making up the

difference with microsomal protein from wild-type baculovirus-infected insect cells

(control cells). The reactions were started by addition of NADPH solution (1 mM) and

terminated by addition of ice-cold acetonitrile. Incubations with microsomes prepared

from control cells served as a negative control. Conversion of MB07811 to MB07344

was monitored by LC-MS. The activity (nmoles of MB07344 per min per mg of

microsomal protein) was normalized for estimated mean expression levels of each CYP

enzyme in the liver (Harper and Brassil, 2008; Dennison et al., 2007; Drahushuk et al.,

1998).

Animal Studies

Pharmacokinetics of MB07811 in rats. Three groups (n = 5/group) of instrumented,

male, SD rats (250-300 g, 6-8 weeks old) were dosed intravenously at the carotid artery

with MB07811 at 3 mg/kg in 100% propylene glycol (PG) or orally with MB07811 at 3

or 10 mg/kg in 100% polyethylene glycol (PEG)-400. Animals were fasted for 3 h prior

to oral administration and were refed 1 h after dosing. Animals had free access to food

prior to and during the IV evaluation. Blood samples were collected from the jugular vein at pre-dose and at 5, 20, and 40 min and 1, 1.5, 2, 3, 5, 8, 12, and 24 h following IV administration of MB07811 or at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h following oral administration of MB07811. Plasma was prepared from blood samples by centrifugation.

First pass effect of MB07811 in the rat. Instrumented male SD rats (230-250 g; 6-8 weeks old, n = 7-8) were administered a 2-min IV infusion of 1 mg/kg of MB07811 as a solution in PG via either the jugular or portal vein. Blood samples (30-100 µL) were collected from the carotid artery at pre-specified times (pre-dose, 0.5, 1, 2, 4, 8, 12, and 24 h post-dose), heparinized, and centrifuged to obtain plasma. Plasma samples were extracted with methanol, and the resulting supernatants were analyzed for MB07811 and MB07344 by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The area under the curve AUC of the plasma concentration-time profile of MB07811 and MB07344 from the portal vein (AUC_{pv}) and jugular vein (AUC_{jv}) were determined by noncompartmental analysis. Hepatic extraction (E_H) of MB07811 was determined from the equation $E_H = 1 - (AUC_{pv} / AUC_{jv})$ (Ward et al., 2001).   The fraction (f_h) of the i.v. administered drug metabolized in the liver was calculated from AUCs of the MB07344 metabolite after PV and IV administration of MB07811, as the AUC_{jv} of MB07344 divided by the AUC_{pv} of MB07344 (Kumar et al., 1999).

Assessment of enterohepatic recirculation of MB07344 in the rat. Non-circulating bile-duct cannulated (n=3) or naïve rats were administered an IV bolus administration of 10 mg/kg of MB07344. Blood samples from the jugular vein were taken 2.5, 5, 10, 20 min and 1, 2, 3, 4, 6, 10 and 24 h post dose. Plasma concentrations of MB07344 were evaluated by LC-MS/MS.

Mass balance and tissue distribution in the rat. In the mass balance study, urine (plus cage wash) and feces were collected over a 96-h interval following IV bolus administration (via the tail vein) of 2 mg/kg and oral administration (by gavage) of 5 mg/kg of ^{14}C-radiolabeled MB07811 to male SD rats (n=3-4). The urine and cage wash samples were analyzed for radioactivity by liquid scintillation counting without dilution. The fecal samples were extracted with 50% (v:v) acetonitrile in water, decolorized with sodium hypochlorite at 60ºC, and then analyzed for radioactivity by liquid scintillation counting. In the tissue distribution study, male SD rats (~250 g, ~7 weeks of age) were gavaged with 5 mg/kg of ^{14}C-radiolabeled MB07811 (1% carboxymethylcellulose suspension, 210 µCi/kg). Sets of rats (n = 3-4/time point) were sacrificed at 3, 8, 24, and 96 h after dose administration, and the following tissues were harvested: cervical lymph nodes, thyroid gland, testes, epididymal fat, urinary bladder, prostate, spleen, pancreas, stomach, mesenteric lymph nodes, small intestine, large intestine, liver, adrenal glands, kidneys, thymus, heart, lungs, bone marrow, quadriceps muscle, eyes, brain, pituitary gland, skin, blood, plasma, and bone (femur). For the stomach, small intestine and large intestine, the luminal contents and a wash were also collected. The tissues were rinsed in water, dissolved by the addition of Soluene-350, decolorized as necessary, and then analyzed for radioactive content by liquid scintillation counting. Liquid samples (washes, bone marrow, blood, and plasma) were decolorized and analyzed directly by liquid scintillation counting. Separate samples of liver, kidney, epididymal fat, small and large intestine samples collected at 3 hr post dose were homogenized in 60% acetonitrile for metabolite identification by HPLC radioactivity analysis as described below.

Quantitative whole body autoradiography (QWBA) in the rat. SD rats were administered 5 mg/kg of [^{14}C]MB07811 (100 µCi per animal). At 1, 2, 4, 8, 12, 18, 24, and 96 h post administration, the animals were euthanized and the intact carcass was frozen in hexane-dry ice bath. The frozen carcass was embedded in 2% CMC. Cryosectioning (50 µm-thick slices) of the carcass was performed and the sections were dried. Digital imaging of the radioactivity was accomplished by phosphor imaging or by direct imaging. Tissue concentrations of radioactivity were determined for the following tissues and/or contents: adipose (brown and white), adrenal gland (cortex and medulla), bile (in duct), blood, bone, bone marrow, brain (cerebrum, cerebellum, medulla), cecum and contents, epididymis, eye (uvea and lens), Harderian gland, heart, kidney cortex and medulla, large intestine (and contents), liver, lung, lymph node, pancreas, pituitary gland, prostate gland, salivary gland, seminal vesicles, skeletal muscle, skin (pigmented and non-pigmented), stomach (gastric mucosa and contents), small intestine (and contents), spleen, spinal cord, testis, thymus, thyroid and urinary bladder (and contents).

Pharmacokinetics of MB07811 in dogs. A total of 3 male and 3 female adult beagle dogs were dosed IV with MB07811 in 100% PEG-400 at 3 mg/kg in the fed state or orally with MB07811 at 3 mg/kg in 0.5% CMC/1% Lutrol F68 in the fasted or fed state at MPI Research (Mattawan, MI). Blood samples (heparin) were taken prior to, and at 5, 15, and 30 min and 1, 2, 4, 6, 8, 12 and 24 h following IV administration and prior to and at 0.5, 1, 2, 4, 6, 8, 12, 16 and 24 h following oral administration. Plasma was prepared from blood samples by centrifugation.

Pharmacokinetics of MB07811 in monkeys. A total of 12 adult (2-9 years old) cynomolgus monkeys [six males (5.3-6.4 kg) and six females (2.3-3.7 kg)] were

randomized into 3 groups of 2 males and 2 females. Animals were fasted for 2 h and

each group dosed orally with MB07811 in PEG-400 at 3, 10, or 30 mg/kg at MPI

Research. Food was returned 1 h following MB07811 administration. In a subsequent

study, one of the groups was dosed IV with MB07811 in PEG-400 at 3 mg/kg. Animals

had free access to food prior to and during the IV evaluation. Blood samples were taken

prior to and at 0.5, 1, 2, 4, 8, 12, and 24 h following oral administration and prior to and

at 2, 15, and 30 min and 1, 1.5, 2, 3, 5, 8, 12 and 24 h following IV administration.

Plasma was prepared by centrifugation.

Tissue distribution in the monkey. Three male cynomolgus monkeys were administered 3

mg/kg of [^{14}C]MB07811 (100 µCi per animal). One monkey was euthanized at each time

of 4, 12, and 24 h post dose with urine collection from each animal up to its termination

time. Tissue concentrations of radioactivity were determined for the following tissues:

adipose, adrenal glands, blood, heart, kidney, liver, pituitary gland, skeletal muscle,

spleen, and thymus. Urine and tissue samples were processed and measured for

radioactivity as described for rats.

Analytical and Pharmacokinetic Analyses

LC-MS/MS analysis of MB07811 and MB07344. Plasma and bile samples were analyzed

using an LC-MS/MS (Applied Biosystems, API 4000) equipped with an Agilent 1100

binary pump and a LEAP injector. Plasma and bile samples were extracted with 1.5

volumes of methanol and then centrifuged to remove the precipitate. A ten microliter

sample of the supernatant was injected onto a Luna C8 column (5 µm, 2 x 50 mm,

Phenomenex) fitted with a SecurityGuard C18 guard column (5 µm, 4.0 x 3.0 mm,

Phenomenex) and eluted with a gradient from mobile phase A [20 mM N,N-dimethylhexylamine (DMHA) and 10 mM propionic acid in 20% methanol] to B (20 mM DMHA and 10 mM propionic acid in 80% methanol) at a flow rate of 0.3 mL/min (0 min, 60% B; 0-1 min, 60-100 % B; 1-6 min, 100 % B; 6-6.1 min, 100- 60 % B; 6.1-9 min, 60 % B). The injector temperature was set at 10°C. Elution times for MB07344 and MB07811 were approximately 4.1 and 5.6 min, respectively. MB07811 and MB07344 were detected using the MS/MS mode (513/63.1 for MB07811 and 363.3/63.1 for MB07344) and quantified by comparison of peak areas to standard curves obtained by spiking known concentrations of the analytes to blank plasma or bile. Linearity was observed from 10 to 3000 ng/mL and calibration curves for MB07811 and MB07344 were generated. The limit of quantitation (LOQ) for both MB07344 and MB07811 was 10 ng/mL. The analytical method met acceptable %CV of <20% at the lowest standard (typically 10 ng/mL) and <15% for higher standard concentrations.

HPLC-UV analysis of MB06588. HPLC-UV analysis of MB06588 was performed on an Agilent 1100 series instrument (Agilent Technologies Inc.; Palo Alto, CA). Chromatographic separation was accomplished with an Ultrasphere™ C18 column (5 µm; 4.6 x 250 mm; Catalog # 235329; Beckman Coulter Inc.; Fullerton, CA) equipped with an Econosphere™ C18 guard column (5µm, 4.6 x 7.5 mm Catalog # 96121, W. R. Grace & Co., Columbia, MD). The column was equilibrated with 10% acetonitrile in 20 mM potassium phosphate pH 6.2 and eluted with a linear gradient of 10-60% acetonitrile over 10 min at a flow rate of 1.0 mL/min and at a column temperature of 40°C. The UV absorbance was monitored at 245 nm. The retention time of MB06588 was

approximately 6.3 min. MB06588 was quantified by comparison to single or multiple

point standards diluted in the appropriate matrix.

HPLC radioactivity analysis. The analysis of the S9, microsomal and cellular extracts

was performed on a Hewlett Packard HP1050 HPLC (Palo Alto, CA). The mobile phase

was degassed by helium sparging. The samples were analyzed on a Beckman

Ultrasphere C18 column (5 µm, 4.6 x 250 mm; Cat #235329; Alltech, Deerfield, IL)

equipped with an All-Guard cartridge containing an Econosphere C18 (5µm, 4.6 x 7.5

mm) guard column (Cat #96121; Alltech, Deerfield, IL). The column was equilibrated

with 20% acetonitrile and 80% 20 mM potassium phosphate pH 6.2 and eluted with a

linear gradient of 20-80% acetonitrile over 20 min at a flow rate of 1.5 mL/min. The

column temperature was ambient (~22°C) and not controlled. The UV absorbance was

monitored at 280 nm and radioactivity was monitored by a Radiometric Series A-100

detector (open channel; Packard, Madison, CT) connected through an Agilent 35900E

interface. The ratio of liquid scintillant Ultima-FLO^{TM} M or Ultima-FLO^{TM} AP to HPLC

eluent was 3 to 1. The retention times of MB07344 and MB07811 were approximately

8.4 and 20.4 min, respectively. The retention time of MB06588 was approximately 4 min

in the UV chromatograms. ^{3}H-MB07811 and ^{3}H-MB07344 were used to confirm the

identification of the prodrug and active metabolite, respectively, in the incubation

mixtures.

Pharmacokinetic analysis. The temporal profile of drug concentrations in plasma were

analyzed by non-compartmental methods using WinNonLin version 1.1 (Scientific

Consulting, Inc., Cary, NC) as defined by Gibaldi and Perrier (1982). Unless otherwise

noted, the AUC of drug concentration in plasma was determined by trapezoidal

summation to the last measurable time point. When calculating mean drug

concentrations, values below LOQ were treated as zero. Consequently reported means

may be below LOQ. The absolute oral bioavailability of MB07811 was estimated by

comparison of the AUC values of the plasma profile of MB07811 following oral dosing

of prodrug with the AUC values of MB07811 following IV administration of prodrug in

each individual monkey (Kwan, 1997). Absorption as measured by relative oral

bioavailability of MB07811 was determined by dividing the plasma AUC of MB07344

after oral dosing of MB07811 by the plasma AUC of MB07344 following IV

administration of the prodrug. The metabolic ratio is defined as the ratio of the active

metabolite (MB07344) to prodrug (MB07811) and was calculated by dividing the plasma

molar AUC_{0-24h} of MB07344 with that of MB07811.

RESULTS

Kinetics of conversion of MB07811 to MB07344 in animal and human liver microsomes.

The conversion of MB07811 to MB07344 was linear with respect to time and protein

concentration in microsome preparations from all six species. The V_{max} values ranged

from 0.16 (male rabbit) to 2.74 (male rat) nmoles/mg/min (Table 2). The K_m values

ranged from 18.8 (male rat) to 127.0 (female rabbit) µM. For the male human, monkey,

dog, rat, rabbit, and mouse liver microsome preparations, the CL_{int} values for MB07811

were 4.9, 34.3, 15.3, 145.4, 1.2, and 4.2 µL/min/mg, respectively. For the female human,

monkey, dog, rat, rabbit, and mouse liver microsome preparations, the CL_{int} values were

9.8, 23.7, 10.6, 3.6, 5.0, and 6.8 µL/min/mg, respectively. Ketoconazole at 0.1, 1, and

100 µM inhibited the conversion of MB07811 to MB07344 in human liver microsomes

by 10, 73, and 100%, respectively.

Metabolism of MB07811 in liver microsomes. The rate of metabolism of MB07811 in

liver microsomes, as measured by its apparent rate of disappearance of the prodrug peak

over time, was highest in the male SD rat liver S9 and microsomes and lowest in the male

human (Figure 2). The general pattern of the profile of the major metabolites was similar

between the species: MB07344 and an unknown metabolite, eluting approximately 4 min

later, were observed in all species, and both increased over time. In addition, an

unresolved peak of radioactivity at the solvent front increased over time in all species.

The glutathione adduct byproduct MB06588 was observed in most of the UV traces of

samples from all species. One minor metabolite peak eluting approximately 2 min prior

to the MB07344 peak was detected in human and monkey samples and, to a lesser extent,

dog samples. This peak was not observed in either male or female rat samples.

MB07811 was poorly converted to MB07344 in female SD rat liver S9 and microsomes.

CYP reaction phenotyping.   The contribution of individual P450 enzymes in the

conversion of MB07811 to MB07344 was assessed in a panel of 11 human recombinant

CYP enzymes (Figure 3). Linearity of product formation with incubation time was

demonstrated for each of the CYP enzymes. The results indicated that CYP1A1,

CYP2C8, CYP3A4, and CYP3A5 catalyzed the formation of MB07344 with highest

activity attributed to CYP3A4. An evaluation of the intrinsic clearance of MB07811 in

each of the CYP enzymes would be required to fully determine the relative importance of

each of the individual P450 enzymes to the overall oxidative metabolism of MB07811

(Harper and Brassil, 2008).

Pharmacokinetics of MB07811 following IV bolus administration. The plasma

concentration-time profile of MB07811 and MB07344 in the rat, dog and monkey are

shown in Figure 4. The pharmacokinetic parameters are summarized in Table 3. The

metabolic ratio after IV dosing was highest in the rat and monkey (~2) and lowest in the

dog (~0.1). Following an IV administration of 3 mg/kg, the plasma C_{max} values of the

active metabolite MB07344 were 0.09, 0.01, and 0.63 µg/mL in the rat, dog, and

monkey, respectively.

Pharmacokinetics of MB07811 following oral administration. Metabolic ratios of 9, 7,

and 24 were observed following oral administration of MB07811 to rats, dogs, and

monkeys, respectively. These ratios are considerably higher (7- to 24-fold) than the

corresponding values seen after IV dosing, an indication of a large first pass clearance of

the prodrug in the liver. The absolute oral bioavailability of MB07811 was below 10% in

the rat, dog, and monkey (Figure 4; Table 3). But uptake of MB07811 from the gut was good based on the extent of absorption of >20% across the species evaluated (Table 3). Plasma C_{max} values of MB07344 of at least 18 ng/mL were observed following administration of 10 mg/kg of MB07811 to the rat, dog, and monkey.

First pass effect of MB07811 in the rat. For the rats dosed IV via either the portal or jugular vein, the mean (±s.d.) plasma AUC_{last} values of systemic MB07811 were 0.14 ± 0.04 and 0.37 ± 0.12 mg·h/L, respectively (Figure 5). The corresponding mean plasma AUC values of systemic MB07344 were 0.45 ± 0.18 and 0.54 ± 0.30 mg·h/L. Based on these values, the hepatic extraction ratio (E_H) for MB07811 was approximately 0.61. The fraction of the IV administered prodrug metabolized in the liver (f_H) was 1.20.

Assessment of enterohepatic recirculation of MB07344 in the rat. The plasma concentration-time profile of MB07344 following IV bolus administration of MB07344 to non-circulating bile-duct cannulated (n = 3) or naïve rats is shown in Figure 6. The plasma AUC of MB07344 was observed to be similar, an indication that MB07344 was not subject to enterohepatic recycling.

Mass balance and tissue distribution in the rat. Following IV bolus administration of [14C]MB07811 in the mass balance study, the mean percents of 14C-radioactivity excreted in the urine (including washes) and feces were 3% and 95%, respectively, with a total recovery of radioactivity of 98% after 96 h (Table 4). Extraction recoveries of radioactivity were >80% across species evaluated. After oral administration, the mean percents of 14C-radioactivity excreted in the urine (including washes) and feces were 2% and 93%, respectively, with a total recovery of radioactivity of 95% after 96 h. Following oral administration of [14C]MB07811 in the tissue distribution study, the mean

percents of total radioactivity remaining in the rat (expressed as a % of the total dose given) were 72%, 7%, and <1% at 3, 24, and 96 h, respectively. The total content and concentration of radiolabel in the liver were at minimum 10-fold higher than all other tissues, except the gastrointestinal (GI) tract throughout the 96-hour time period (Table 5). The levels of radioactivity found in the pituitary gland and bone marrow were negligible, and there was no significant accumulation of radioactivity in the muscle, heart, brain, epididymal fat, or kidney. After 96 hours less than 0.4 % of the radiolabel administered remained in the animals. Of the tissues evaluated in the rat, the liver contained the highest percentage of MB07344 (53%) based on total tissue radioactivity. On the other hand, less than 20% of the radioactivity in the intestines was MB07344.

Quantitative whole body autoradiography in the rat. Most of the radioactivity was associated with the GI tract, its content and the liver following oral administration of [^{14}C]MB07811 (Figure 7). Specifically, 1 h following administration, most radioactivity was measured in the small intestinal content, stomach content, and liver. In 8 out of 23 tissues/organs where radioactivity was observed, levels were higher than the blood level. At 2, 4, 8, 12 and 18 h post-dose, most of the radioactivity continued to be found in the liver and gastrointestinal tract plus contents. By 96 h post-dose, most of the radioactivity was eliminated from the animals, but residual radioactivity was still observed in the liver.

Tissue distribution in the monkey. Of the tissues measured, most of the radioactivity was found in the liver of monkeys administered an oral dose of [^{14}C]MB07811 (Table 6). No accumulation of radioactivity was detected in the heart, kidney, spleen, adipose, skeletal muscle, or pituitary gland.

DISCUSSION

MB07811, a HepDirect prodrug of a phosphonate-containing TR agonist
MB07344, is absorbed intact and converted in the liver to the active metabolite by
CYP3A, a P450 enzyme that is expressed predominantly in liver (Erion et al., 2007).
Liver selectivity is achieved as a result of the liver-selective conversion of MB07811 as
well as the limited tissue distribution of MB07344 that is postulated to arise from the
phosphonic acid, its high negative charge, and the inability of these compounds to
distribute into most tissues. Because exposure of TR agonists to extrahepatic tissues
could potentially lead to safety issues such as tachycardia, bone loss, muscle wasting, and
thyroid hormone axis suppression, demonstration of hepatic extraction and liver targeting
were critical elements in the preclinical pharmacokinetic assessment of MB07811 and
MB07344.

The expected general pharmacokinetic properties of HepDirect liver-targeted
prodrugs are: (1) high plasma clearance relative to hepatic blood flow; (2) high volume
of distribution relative body water volume; and (3) good absorption (>20%) from the gut
despite (4) poor absolute oral bioavailability (<20%) as assessed by systemic circulation
exposure (Erion et al., 2004). The compounds of this novel class of prodrugs are
intended to be susceptible to hepatic first pass effects as they are designed to be readily
converted to their active metabolites or active metabolite precursors by liver CYP3A.
The hepatic extraction ratio of MB07811 of 0.61 in rat is in close agreement to the 0.55
value obtained by a comparing systemic and portal vein concentrations of MB07811
following oral administration (Erion et al., 2007). Moderate plasma clearance relative to
hepatic blood flow was observed with MB07811with a systemic clearance in the rat and

dog estimated in excess of 70% of liver blood flow and estimated at 40% of liver blood flow in the monkey (Davies and Morris, 1993). Consistent with a moderate to high hepatic first pass, the absolute oral bioavailability of plasma MB07811 was <10% in all three species evaluated. The plasma clearance of MB07811 in the rat exceeded hepatic blood flow may be attributed to extrahepatic disposition or metabolism.

Although the absolute oral bioavailability of intact MB07811 is low across the species tested, the absorption of the active agent MB07344 from the gut was significantly enhanced when delivered as the HepDirect prodrug. The plasma AUC values of MB07344 generated following IV administration of MB07811 via jugular (systemic) and portal veins are similar (i.e., $f_h \sim 1$), an indication of negligible extrahepatic metabolism (Kumar et al., 1999) of MB07811. Therefore, the extent of absorption of MB07344 was estimated by comparing dose-normalized plasma AUC of MB07344 following oral and IV administration of MB07811. Based on this measurement, extents of absorption of 29%, 50%, and 32% are observed in rats, dogs, and monkeys, respectively. These values are a considerable improvement over the oral bioavailability of MB07344 (i.e., <10% in the rat) when administered as the free phosphonate (Erion et al., 2007).

Another pharmacokinetic outcome of liver sequestration of HepDirect agents is that the volume of distribution of these prodrugs is extremely high. The volume of distribution of MB07811 was observed to be several-fold higher than the volume of body water in animals. As evident from the tissue distribution and QWBA studies, the apparent large volume of distribution is primarily due to the concentration of MB07811 and its metabolites in the liver as opposed to even distribution of the highly permeable prodrug throughout the body. In contrast, the volume of distribution of MB07344, a

charged anion at physiological pH values, is relatively low when delivered by IV bolus administration. Although the volume of distribution of MB07344 is below blood volume, significant partitioning of the free phosphonate to the liver was observed suggesting involvement of hepatic organic anion transporters (Miyazaki et al., 2004). Some isoforms of organic anion transporters (e.g., OAT2) are predominantly expressed in the liver (Mizuno et al., 2003; Zhou and You, 2007). Liver targeting of MB07344 is achieved by a combination of low extra-hepatic tissue distribution and high liver uptake.

Mass balance studies following IV bolus and oral administration of [^{14}C]MB07811 to rats demonstrated that the major route of elimination is biliary since the majority of radioactivity was found in the feces independent of route of administration. These results are in agreement with biliary excretion studies of MB07344 that indicated quantitative recovery of MB07344 in the bile following IV administration to rats (Erion et al., 2007). Biliary excretion is also likely to be a dominant clearance mechanism for MB07811 in monkeys because the renal clearance was low (<7% of dose) as it had been in lower species. Based on the tissue distribution study, more than 90% of the radioactivity was eliminated from rats after 24 h, an observation consistent with the results of a mass balance study in which 92% of the radioactivity was recovered after a similar time period. Consistent with the QWBA results, this finding indicated only residual radioactivity remaining in the liver at 24 h post dose. The results from both the tissue distribution and QWBA studies confirm the utility of the HepDirect approach for liver targeting. In both rat and monkey tissue distribution studies following oral administration of [^{14}C]MB07811, the highest concentration of radioactivity in non-

gastrointestinal tract tissue was found in liver, where the major metabolite (i.e., >50% of radioactivity in the rat) was MB07344.

High metabolic ratios of MB07344 to MB07811 observed following oral administration to animals indicate conversion of the prodrug to active metabolite occurs *in vivo* and this was further confirmed in the kinetics studies. MB07811 was readily converted to MB07344 in liver microsome preparations from human and all three animal species tested. The CL_{int} values in liver microsome preparations from males were highest in rat, followed by monkey, dog, and human. The largest difference in CL_{int} values between the sexes were observed in rat (~41-fold lower in females vs. males) probably since total CYP3A activity is lower in female rats due to lack of the expression of the CYP3A2 isoform found in male rats (Lin et al., 2003). Differences in CL_{int} values between the sexes were within ~2-fold in the case of human, monkey, and dog liver microsome preparations. The CL_{int} values of MB07811 in male rat and dog liver microsomes were several-fold higher than the corresponding CL_{int} values of pradefovir which had demonstrated efficacy in both man and animals (Tillman, 2007). In human liver microsomes, the CL_{int} values of MB07811 exceeded that of pradefovir (Lin et al., 2005). Based on the dose-dependent inhibition of the conversion of MB07811 to MB07344 in human liver microsomes by ketoconazole, a specific inhibitor of CYP3A4 (Turan et al., 2001), CYP3A4 is a major isoform responsible for the conversion of MB07811 although a contribution from other CYP isozymes is possible as inhibition was incomplete at 1 μM ketoconazole. A major role of CYP3A4 was confirmed by monitoring the conversion of MB07811 to MB07344 by a panel of recombinant P450 enzymes. Minor contributions to MB07811 conversion to MB07344 by other hepatic

CYP enzymes (CYP2C8 and CYP3A5) and, to an even lesser extent, by a non-hepatic

P450 enzyme (CYP1A1) were also observed. A contribution of CYP1A1 is likely

minimal based on the liver selectivity observed in the tissue distribution studies.

The metabolic profile of [^{3}H]MB07811 after incubation with liver microsomes

was similar among humans and animals. The major metabolites observed by

radiography-coupled HPLC following incubation of MB07811 in human and animal liver

S9 and microsomes included the active metabolite MB07344, a known byproduct

MB06588 (see Figure 1), an unidentified product with a retention time between

MB07344 and MB07811, and an unresolved peak of radioactivity eluting at the solvent

front. An additional minor metabolite (<5% of total radioactivity) was observed in

human, monkey, and, to a lesser extent, in dog liver microsomes but not in rat

preparations. The metabolic profile of MB07811 in human liver microsomes was most

similar to that of monkey liver. Current studies are underway to identify the unknown

metabolites and will be reported elsewhere.

In summary, oral absorption of the TR agonist MB07344 is significantly

improved as the HepDirect prodrug MB07811. Once in the liver, the prodrug is

converted to the active and major metabolite MB07344 by action of CYP3A resulting in

a hepatic first pass extraction of MB07811 of over 60%. Both MB07811 and MB07344

are eliminated in the bile as well as some being released from the liver into the general

circulation and ultimately returning to the liver to be cleared. Consistent with its low oral

bioavailability, MB07344 eliminated in the gut is not reabsorbed nor enterohepatically

recirculated.   Based on the low volume of distribution of MB07344 and confirmed by

radiolabeled tissue distribution studies, the circulating TR agonist is not expected to be

significantly taken up by extrahepatic tissues such as heart, pituitary, thyroid glands, bone

or muscle thereby reducing its potential to adversely affect heart rate, contraction and

conductance, thyroid hormone production and metabolism, or to cause bone loss or

muscle wasting. Like other phosphonates, circulating MB07344 is likely taken into the

liver via organic anion transporters due to its negative charge. This property coupled

with the characteristics of the HepDirect prodrug leads to enhanced liver targeting and

thereby the significant improvement of the therapeutic index observed in preclinical

proof-of-concept studies (Erion et al. 2007).

ACKNOWLEDGMENTS

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FOOTNOTES

[1] HepDirect is a registered trademark of Metabasis Therapeutics, Inc.

LEGENDS FOR FIGURES

Figure 1. Sequence of conversion of HepDirect MB07811 to the active metabolite

MB07344 releasing glutathione (GSH) byproduct MB06588.

Figure 2. Representative radiochromatograms of ^{3}H-MB07811 and its products in male

(A) rat, (B) dog, (C) monkey, and (D) human liver microsomes at 0 (dashed line) and

after 30 min (solid line) of incubation.

Figure 3. Mean (±s.d.) formation of MB07344 from MB07811 by a panel of

recombinant human P450 enzymes (n=3). Metabolite formation was measured following

a 30 min incubation of MB07811 (5 μM) with baculovirus-insect cell microsomes

containing expressed human P450 enzymes (25 pmol). The activity was normalized for

estimated expression levels of each CYP enzyme in the liver and is expressed as nmoles

of MB07344 generated per min per mg of microsomal protein.

Figure 4. Mean (±s.d.) plasma concentration-time profiles of MB07811 (●) and

MB07344 (▼) following single oral and IV bolus administration of 10 mg/kg of

MB07811 to male SD rats (n=5) (oral, A; IV, D), beagle dogs (n=6) (oral, B; IV, E), and

cynomolgus monkeys (n=4) (oral, C; IV, F).

Figure 5. Mean (±SEM) carotid artery plasma concentrations of (A) MB07811 and (B)

MB07344 following jugular (●) or portal (○) vein administration of 1 mg/kg of

MB07811 to male Sprague Dawley rats (n=7-8).

Figure 6. Mean (±s.d.) plasma concentration-time profile of MB07344 following IV

bolus administration of MB07344 to naïve and non-circulating bile-duct cannulated rats

(n=3).

Figure 7. Typical whole body autoradiograms (renal, adrenal and mid-line plane, left to right) obtained at 2 (Rat 1002) and 4 h (Rat 1003) following oral dosing of a 5 mg/kg dose of [^{14}C]MB07811. Incremental values indicate relative radioactivity concentrations.

Table 1. Relative mRNA levels in tissues of male Sprague Dawley rats (n=6/group) treated with either MB07811 or T3 at single doses in 10-fold excess of the ED_{50} of the cholesterol lowering response in cholesterol-fed rats (4 and 0.12 mg/kg, respectively). The values represent the fold change at the time point at which a maximal response was obtained with T3 treatment (3, 8 and 24 hr time points were evaluated). See reference (Erion et al., 2007) for detailed methods.

Tissue/organ	Target gene	Maximal Response Time (h)	Fold change relative to control	
			T3	MB07811
Liver	Malic enzyme	24	6.1 ± 0.6	4.0 ± 0.6
Liver	SREBP-1c	24	0.37 ± 0.05	0.39 ± 0.06
Heart	MHCß	24	0.08 ± 0.05	0.64 ± 0.14
Heart	Deiodinase 1	24	11.2 ± 3.7	1.58 ± 0.50
Pituitary	TSHß	24	0.16 ± 0.09	1.0 ± 0.23
Pituitary	Deiodinase 1	24	1.89 ± 0.184	0.98 ± 0.09
Muscle	UCP3	24	9.5 ± 2.0	1.5 ± 0.39
Spleen	Deiodinase 1	3	1.88 ± 0.46	0.47 ± 0.05
Kidney	Deiodinase 1	24	2.01 ± 0.26	0.76 ± 0.05

Table 2.   Summary of the kinetics of MB07811 metabolism in animal and human liver microsome preparations. Metabolism was measured by the quantification of the derivatized byproduct MB06588 as described in the Methods. Values are expressed as mean ± SEM. Number of individual microsome sample pooled = n. * Statistical significant difference between sexes, $p < 0.5$ by the unpaired Student's t test.

Species	Sex	n	V_{max} (nmol/min/mg)	K_m (μM)	CL_{int} (μL/min/mg)
Human	Male	10	0.25 ± 0.03	49.7 ± 18.6	4.9 ± 2.0
Human	Female	10	0.32 ± 0.03	32.5 ± 10.4	9.8 ± 3.3
Cynomolgus Monkey	Male	4	1.87 ± 0.30	54.5 ± 22.2	34.3 ± 15.0
Cynomolgus Monkey	Female	7	1.23 ± 0.42	52.0 ± 46.3	23.7 ± 22.6
Beagle Dog	Male	4	0.47 ± 0.02*	30.5 ± 4.2	15.3 ± 2.3
Beagle Dog	Female	5	0.98 ± 0.08	92.4 ± 16.0	10.6 ± 2.0
New Zealand White Rabbit	Male	3	0.16 ± 0.01*	127.0 ± 22.9	1.23 ± 0.25
New Zealand White Rabbit	Female	3	0.48 ± 0.04	95.1 ± 16.1	5.03 ± 0.94
Sprague Dawley Rat	Male	40	2.74 ± 0.12*	18.8 ± 3.1	145.4 ± 24.5*
Sprague Dawley Rat	Female	40	0.12 ± 0.01	32.3 ± 5.9	3.58 ± 0.69
ICR/CD-1 Mouse	Male	400	0.15 ± 0.01*	36.4 ± 6.5	4.23 ± 0.80
ICR/CD-1 Mouse	Female	400	0.34 ± 0.01	49.6 ± 4.4	6.81 ± 0.65

Table 3. Pharmacokinetic parameters (mean ± s.d.) of MB07811 following IV and oral administration of 3 and 10 mg/kg, respectively, to rats, dogs, and monkeys.

Analyte	Parameter	Unit	Rat (n=5)		Dog (n=6)		Monkey (n=4)	
			IV	oral	IV	oral	IV	oral
MB07811	AUC_{last}	mg·h/L	0.26 ± 0.04	0.026 ± 0.011	2.53 ± 0.88	0.066 ± 0.033	3.01 ± 0.41	0.085 ± 0.017
	C_{max}	µg/mL	0.60 ± 0.22	0.012 ± 0.008	2.09 ± 0.98	0.015 ± 0.008	7.32 ± 3.61	0.018 ± 0.009
	T_{max}	h	0.0 ± 0.0	2.1 ± 1.0	0.0 ± 0.0	4.0 ± 6.0	0.0 ± 0.0	1.5 ± 0.6
	$t_{½}$	h	1.23 ± 0.15	0.95 ± 0.35	4.4 ± 3.8	5.0 ± 2.6	4.07 ± 0.73	4.32 ± 1.99
	CL	L/hr/kg	11.55 ± 1.93	NA	1.27 ± 0.39	NA	1.00 ± 0.12	NA
	V_{ss}	L/kg	14.82 ± 3.99	NA	3.38 ± 1.20	NA	2.76 ± 0.81	NA
	MRT	h	1.20 ± 0.20	2.49 ± 0.72	2.3 ± 0.8	6.5 ± 3.7	2.77 ± 0.74	6.89 ± 2.86
	F (absolute)	%	NA	5-10	NA	2.8 ± 1.7	NA	2.8 ± 0.6
MB07344	AUC_{last}	mg·h/L	0.34 ± 0.06	0.135 ± 0.035	0.16 ± 0.06	0.42 ± 0.16	4.60 ± 1.64	1.45 ± 0.64
	C_{max}	µg/mL	0.092 ± 0.017	0.018 ± 0.003	0.014 ± 0.006	0.056 ± 0.031	0.63 ± 0.19	0.18 ± 0.12
	T_{max}	h	0.60 ± 0.15	3.6 ± 1.1	17.6 ± 9.2	5.7 ± 5.3	0.88 ± 0.25	3.00 ± 1.15
	$t_{½}$	h	8.82 ± 2.38	9.25 ± 6.24	ND	10.2 ± 4.2	5.70 ± 1.23	6.52 ± 1.45
	F (relative)	%	NA	20-39	NA	~50†	NA	31.8 ± 11.5

NA = Not Applicable
ND= Not Determined
† Based on AUC to infinity

Table 4. Recovery of radioactivity (% of dose) in mass balance of radiolabel following IV bolus and oral administration of [^{14}C]MB07811 to male SD rats (n=4).

Recoveries of radioactivity (% of Dose)				
IV	0-24h	0-48 h	0-72 h	0-96 h
Urine + wash	2.8 ± 0.7	3.1 ± 0.7	3.2 ± 0.8	3.4 ± 0.8
Feces	89.2 ± 10.2	93.6 ± 9.8	94.8 ± 9.4	94.9 ± 9.4
Total	92.0 ± 10.8	96.6 ± 10.4	98.1 ± 10.1	98.2 ± 10.1

Oral	0-24h	0-48 h	0-72 h	0-96 h
Urine + wash	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Feces	83.0 ± 5.9	92.8 ± 9.5	93.2 ± 9.6	93.3 ± 9.6
Total	84.5 ± 5.8	94.4 ± 9.4	95.0 ± 9.6	95.1 ± 9.6

Table 5. Tissue and fluid concentrations of ^{14}C-MB07811 and metabolites in the rat. Mean (± s.d.) concentrations of radiolabel in specific tissues and fluids following oral administration of 5 mg/kg of ^{14}C-MB07811 to male SD rats.

Tissue	3 Hour (nmol/g)	24 Hour (nmol/g)	96 Hour (nmol/g)
lymph(c)	0.12 ± 0.18	0.03 ± 0.00	0.03 ± 0.02
thyroid	0.20 ± 0.13	0.03 ± 0.02	0.01 ± 0.00
testes	0.03 ± 0.02	0.04 ± 0.02	0.00 ± 0.00
fat	0.15 ± 0.10	0.55 ± 0.96	0.01 ± 0.00
bladder	0.18 ± 0.12	0.05 ± 0.02	0.01 ± 0.00
prostate	0.12 ± 0.09	0.03 ± 0.02	0.01 ± 0.00
spleen	2.57 ± 4.81	0.01 ± 0.00	0.00 ± 0.00
pancreas	0.25 ± 0.11	0.25 ± 0.05	0.09 ± 0.04
stomach	76.96 ± 48.75	0.22 ± 0.09	0.02 ± 0.01
stom. C&W	24.52 ± 10.88	0.06 ± 0.02	0.01 ± 0.00
lymph(m)	16.61 ± 17.57	0.05 ± 0.01	0.03 ± 0.01
small int.	28.56 ± 22.73	0.40 ± 0.22	0.01 ± 0.00
sma. C&W	39.69 ± 30.16	0.20 ± 0.03	0.01 ± 0.00
large int.	1.23 ± 1.12	0.64 ± 0.21	0.01 ± 0.00
larg. C&W	4.68 ± 2.85	2.50 ± 0.89	0.01 ± 0.00
liver	6.67 ± 3.54	3.67 ± 0.64	0.49 ± 0.23
adrenal	0.77 ± 0.46	0.09 ± 0.02	0.04 ± 0.01
kidneys	0.48 ± 0.37	0.14 ± 0.01	0.02 ± 0.00
thymus	0.09 ± 0.05	0.01 ± 0.00	0.01 ± 0.00
heart	0.36 ± 0.36	0.05 ± 0.01	0.03 ± 0.00
lungs	0.31 ± 0.21	0.04 ± 0.00	0.03 ± 0.00
marrow	0.06 ± 0.09	0.01 ± 0.00	0.01 ± 0.00
muscle	0.07 ± 0.05	0.01 ± 0.00	0.01 ± 0.00
eyes	0.04 ± 0.02	0.01 ± 0.00	0.00 ± 0.00
brain	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
pituitary	0.09 ± 0.12	0.02 ± 0.00	0.01 ± 0.00
skin	0.08 ± 0.05	0.15 ± 0.10	0.01 ± 0.00
blood	0.12 ± 0.07	0.05 ± 0.01	0.02 ± 0.00
plasma	0.15 ± 0.10	0.05 ± 0.01	0.01 ± 0.00
bone	0.14 ± 0.14	0.02 ± 0.01	0.01 ± 0.00

Table 6. Tissue and fluid concentrations of ^{14}C-MB07811 and metabolites in the monkey. Concentrations of radiolabel in specific tissues and fluids following oral administration of 5 mg/kg of ^{14}C-MB07811 to male cynomolgus monkeys. Other than blood and plasma, tissues were collected from Monkeys 1001, 1002, and 1003 at 4, 12, and 24 h post-dose, respectively

Tissue	Timepoint	Monkey 1 (4 h)	Monkey 2 (12 h)	Monkey 3 (24 h)
BLOOD	4 h	360	272	425
BLOOD	12 h	N.S.	92	136
BLOOD	24 h	N.S.	N.S.	67
PLASMA	4 h	640	479	773
PLASMA	12 h	N.S.	165	241
PLASMA	24 h	N.S.	N.S.	120
ADIPOSE	4, 12, 24 hr	136	68	31
ADRENAL GLANDS	4, 12, 24 hr	248	90	75
ADIPOSE	4, 12, 24 hr	117	77	34
HEART	4, 12, 24 hr	205	<1	<1
KIDNEYS	4, 12, 24 hr	1707	304	198
LIVER	4, 12, 24 hr	4594	2768	2095
PITUITARY	4, 12, 24 hr	202	55	27
SKELETAL MUSC(PECT)	4, 12, 24 hr	80	<1	<1
SKELETAL MUSC(THIGH)	4, 12, 24 hr	83	<1	<1
SPLEEN	4, 12, 24 hr	156	<1	<1
THYMUS	4, 12, 24 hr	N.C.	258	129

N.C. = Not Calculated   N.S. = No Sample

Figure 1

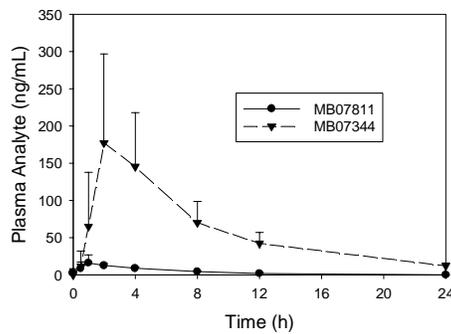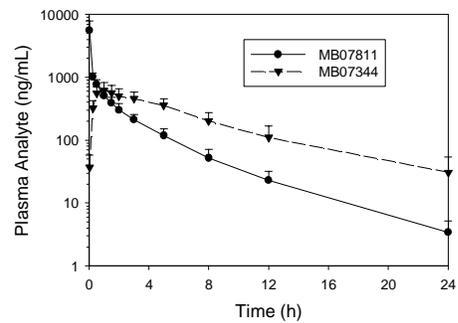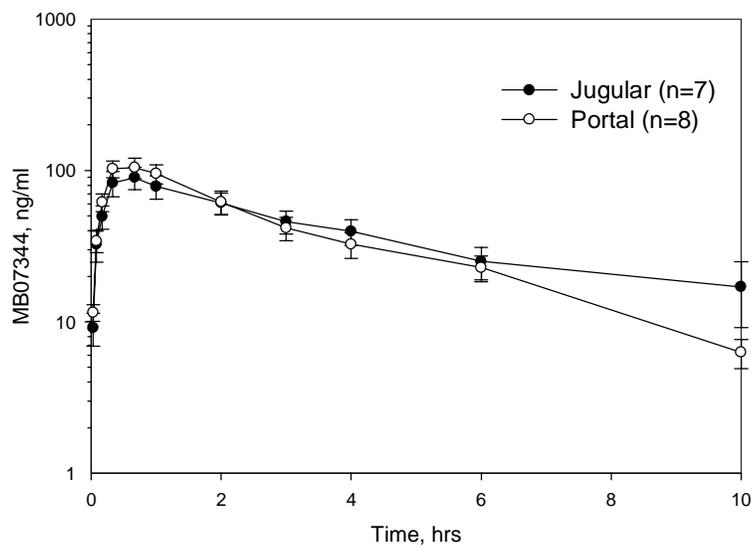MB07811

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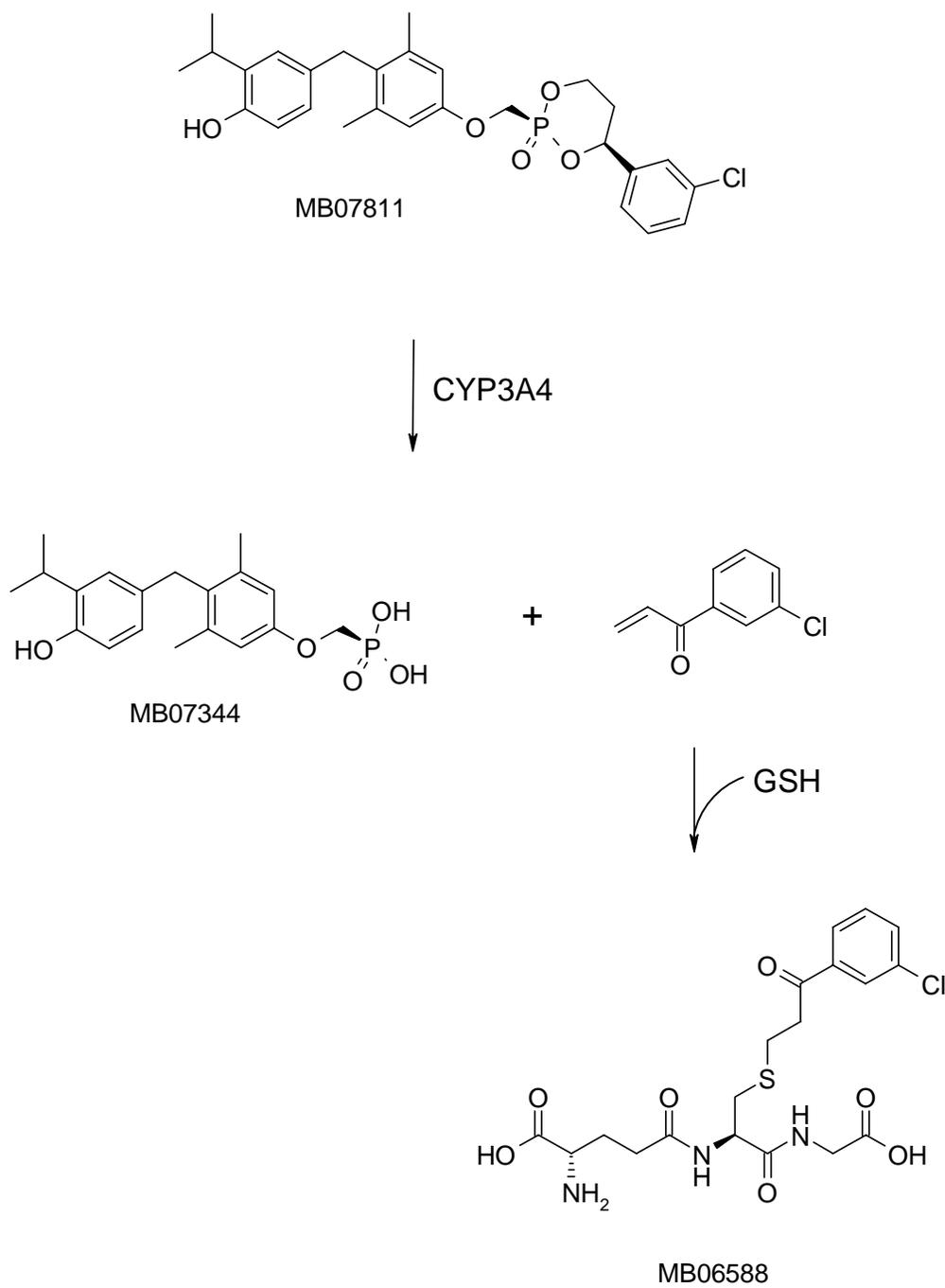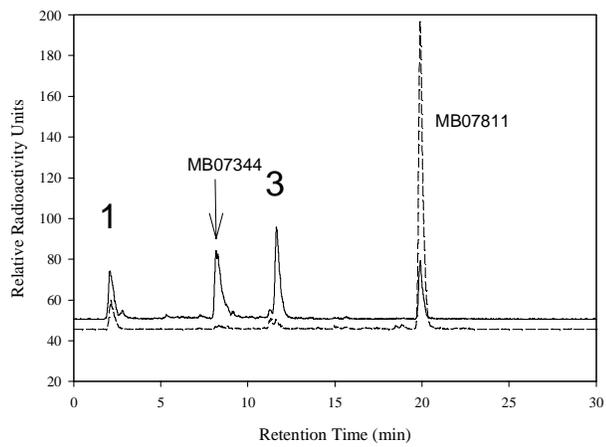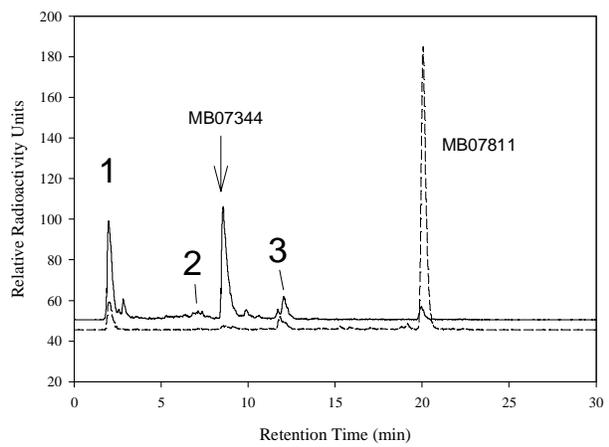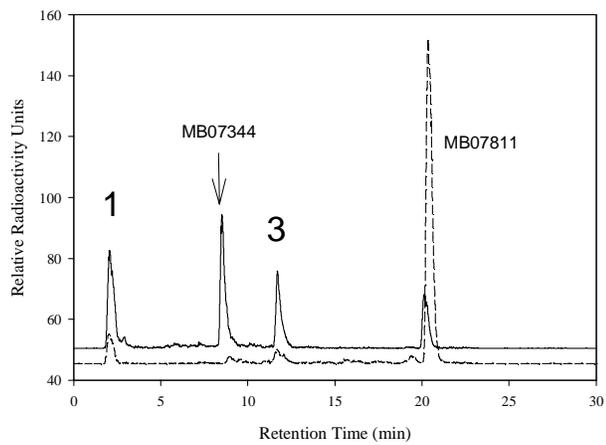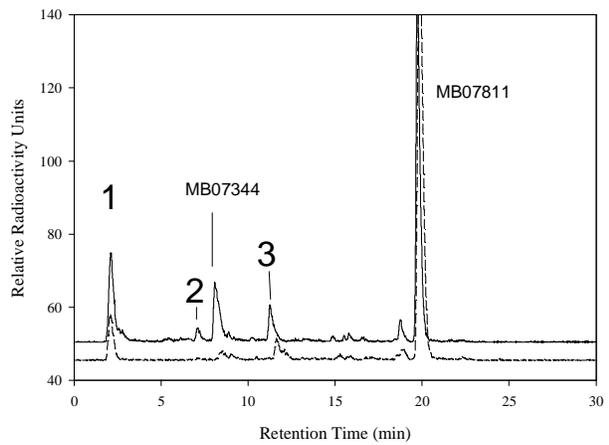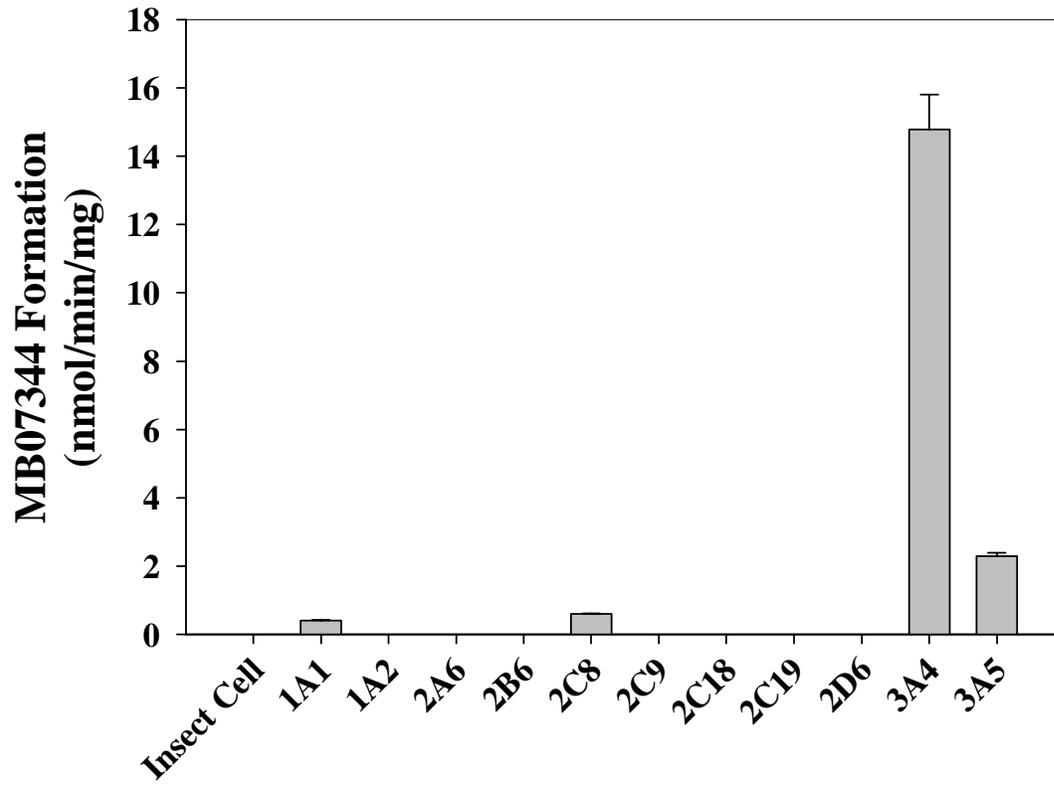
Figure 2

Figure 3

Figure 4

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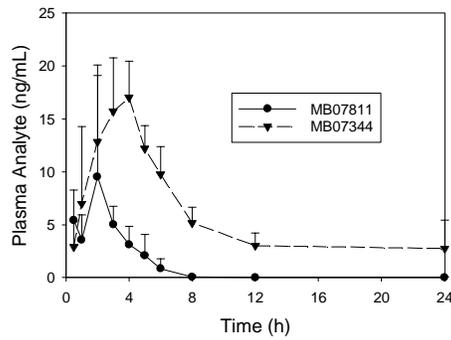
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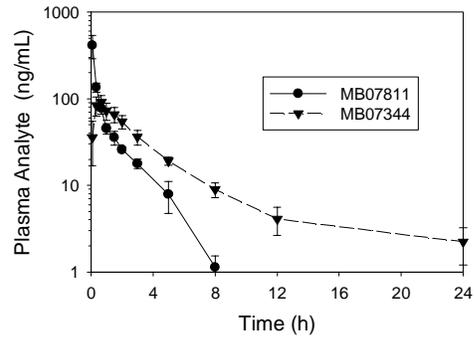
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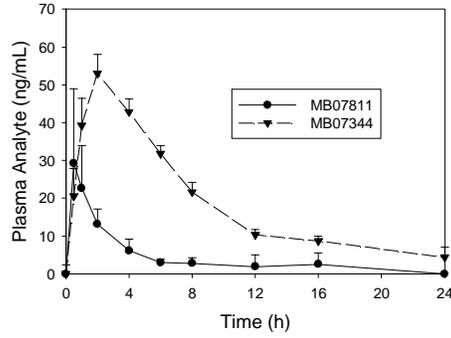
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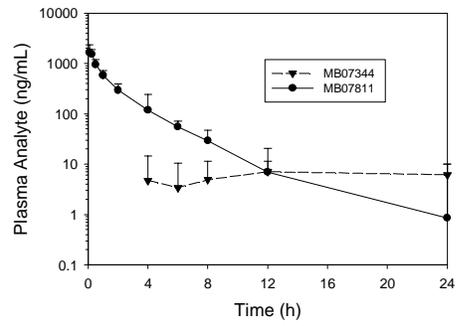
C

F

Figure 5

A

B

Figure 6

Figure 7

Rat 1002 (2 h)

Study AA 38748, 1002 (2 h)

Rat 1003 (4 h)

Study AA 38748, 1003 (4 h)