CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 22-291

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Promacta (Eltromobag)

Date: October 8, 2008
To: File for NDA #22-291
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined the labeling and the pharmacology/toxicology supporting review provided by Dr. Chopra. The nonclinical information presented in the label is supported by the review. I concur with the conclusions that Promacta may be approved and that no additional pharmacology/toxicology studies are necessary.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

John Leighton
10/8/2008 12:46:59 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-291
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/19/07
PRODUCT:	Promacta (Eltromobag©)
INTENDED CLINICAL POPULATION:	Previously Treated Chronic idiopathic thrombocytopenic purpura patients
SPONSOR:	GlaxoSmithKline, Inc.
DOCUMENTS REVIEWED:	Electronic submission (eCTD)
REVIEW DIVISION:	Division of Medical Imaging and Hematology Drug Products (HFD-160)
PHARM/TOX REVIEWER:	Yash M. Chopra, M.D., Ph.D.
PHARM/TOX SUPERVISOR:	Adebayo Laniyonu, Ph.D.
DIVISION DIRECTOR:	Rafel D. Rieves, M.D.
PROJECT MANAGER:	Hyon-Zu Lee, D.Pharm.

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
NDA 22-291 is recommended for approval from P/T perspective.

B. Recommendation for nonclinical studies
None

C. Recommendations on labeling

8.1 Pregnancy
Pregnancy Category C
There are no adequate and well-controlled studies of eltrombopag use in pregnancy. In animal reproduction and developmental toxicity studies, there was evidence of embryolethality and reduced fetal weights at maternally toxic doses. PROMACTA should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Pregnancy Registry: A pregnancy registry has been established to collect information about the effects of Promacta during pregnancy. Physicians are encouraged to register pregnant patients,

In an early embryonic development study, female rats received eltrombopag at doses of 0.8, 2 and 7 times the human clinical exposure (based on AUC). Increased pre- and post-implantation loss and reduced fetal weight were observed at the highest dose which also caused maternal toxicity.

In an embryofetal development study, pregnant rats received eltrombopag at doses of 0.8, 2, and 7 times the human clinical exposure (based on AUC). Decreased fetal weights and a slight increase in the presence of cervical ribs were observed at the highest dose which also caused maternal toxicity. However, no evidence of major structural malformations was observed. In an embryofetal development study in pregnant rabbits treated with oral eltrombopag doses of 0.1, 0.3, and 0.6 times the human clinical exposure (based on AUC) no evidence of fetotoxicity, embryolethality, or teratogenicity was observed.

In a pre- and post-natal developmental toxicity study in pregnant rats (F0), no adverse effects on maternal reproductive function or on the development of the offspring (F1) were observed at doses up to 2 times the human clinical exposure (based on AUC), Eltrombopag was detected in the plasma of offspring (F1). The plasma concentrations in pups increased with dose (0.8 and 2 times the human clinical exposure based on AUC) following administration of drug to the F0 dams.

8.3 Nursing Mothers

It is not known whether eltrombopag is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing

infants from PROMACTA, a decision should be made whether to discontinue nursing or to discontinue PROMACTA taking into account the importance of PROMACTA to the mother and the known benefits of nursing.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Eltrombopag does not stimulate platelet production in rats, mice, or dogs because of unique TPO receptor specificity. Data from these animals do not fully model effects in humans. Eltrombopag was not carcinogenic in mice at doses up to 75 mg/kg/day or in rats at doses up to 40 mg/kg/day (exposures up to 4 and 5 times the human clinical exposure based on AUC, respectively).

Eltrombopag was not mutagenic or clastogenic in a bacterial mutation assay or in 2 in vivo assays in rats (micronucleus and unscheduled DNA synthesis, 11 times the human clinical exposure based on C_{max}). In the in vitro mouse lymphoma assay, eltrombopag was marginally positive (<3-fold increase in mutation frequency).

Eltrombopag did not affect female fertility in rats at doses up to 20 mg/kg/day (2 times the human clinical exposure based on AUC). Eltrombopag did not affect male fertility in rats at doses up to 40 mg/kg/day, the highest dose tested (5 times the human clinical exposure based on AUC).

13.2 Animal Pharmacology/Toxicology

Eltrombopag is phototoxic and photoclastogenic in vitro. In vitro photoclastogenic effects were observed only at cytotoxic drug concentrations (≥15 mcg/mL) and at UV light exposure intensity (30 MED, minimal erythematous dose). No evidence of in vitro photoclastogenicity was observed at higher drug concentrations (up to 58.4 mcg/mL) and UV light exposure of 15 MED. There was no evidence of in vivo cutaneous phototoxicity in mice, photo-ocular toxicity in rats or photo-ocular toxicity in mice at exposures up to 11, 6, and 7 times the human clinical exposure based on AUC, respectively.

Treatment-related cataracts were detected in rodents in a dose- and time-dependent manner. At ≥7 times the human clinical exposure based on AUC, cataracts were observed in mice after 6 weeks and in rats after 28 weeks of dosing. At ≥5 times the human clinical exposure based on AUC, cataracts were observed in mice after 13 weeks and in rats after 39 weeks of dosing. Cataracts were not observed in dogs after 52 weeks of dosing (3 times the human clinical

exposure based on AUC). The clinical relevance of these findings is unknown *[see Warnings and Precautions (5.7)]*.

Renal tubular toxicity was observed in studies up to 14 days in duration in mice and rats at exposures that were generally associated with morbidity and mortality. Tubular toxicity was also observed in a 2-year oral carcinogenicity study in mice at doses of 25, 75, and 150 mg/kg/day. The exposure at the lowest dose was 1.4 times the human clinical exposure based on AUC. No similar effects were observed after 13 weeks at exposures greater than those associated with renal changes in the 2-year study, suggesting that this effect is both dose- and time-dependent. Renal tubular toxicity was not observed in rats in a 2-year carcinogenicity study or in dogs after 52 weeks at exposures 5 and 3 times the human clinical exposure based on AUC, respectively.

Eltrombopag produced hepatocellular hypertrophy in mice (7 times the human clinical exposure based on AUC), rats (5 times the human clinical exposure based on AUC), rabbits (1.4 times the human clinical exposure based on AUC), and dogs (4 times the human clinical exposure based on AUC) and hepatocellular vacuolation in rats (2 times the human clinical exposure based on AUC).

13.3 Reproductive and Developmental Toxicology

Eltrombopag was administered orally to pregnant rats in an embryofetal development study at 10, 20, or 60 mg/kg/day (0.8, 2, and 7 times the human clinical exposure, respectively, based on AUC). Decreases in maternal body weight gain and food consumption occurred in the 60 mg/kg/day dose group. At this maternally toxic dose, male and female fetal weights were significantly reduced (6% to 7%) and there was a slight increase in the presence of cervical ribs, a fetal variation.

In an embryofetal development study in mated female rabbits, eltrombopag was administered orally at 30, 80, or 150 mg/kg/day (0.1, 0.3, and 0.6 times the human clinical exposure, respectively, based on AUC). There was no evidence of fetotoxicity, embryolethality, or teratogenicity at any dose.

In a pre- and post-natal developmental toxicity study in pregnant rats (F0), no adverse effects on maternal reproductive function or on the development of the offspring (F1) were observed at doses up to 2 times the human clinical exposure (based on AUC), Eltrombopag was detected in the plasma of offspring (F1). The plasma concentrations in pups increased with dose (0.8 and 2 times the human clinical exposure based on AUC) following administration of drug to the F0 dams.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacologic activity

Eltrombopag (SB-497115) is an orally active compound that acts as a selective STAT activator and induces proliferation and differentiation of megakaryocytes from bone marrow progenitor cells. It selectively increases platelet production in chimpanzee and humans as the structural transmembrane domain of TPO-R of these species contained His499 essential for binding. In an in vitro study on hERG currents from human embryonic kidney (HEK-293) cells stably transfected with hERG-1 cDNA, the tail currents were inhibited in a concentration-dependent manner (IC_{50}: 0.69 μM). No change in prolongation of action potential in dog purkinji fiber was seen but eltrombopag caused a decrease in the upstroke amplitude, maximum rate of depolarization and action potential durations. In conscious dogs, no effects on arterial blood pressure, heart rate or electrocardiographic (ECG) parameters were seen at doses up to 30 mg/kg [12 times maximum human dose (MHD) based on body surface area (BSA)].

Absorption, Distribution, Metabolism and Excretion (ADME):

The ADME studies conducted in mouse, rat, rabbit, dog and monkey demonstrated that orally administered SB-497115-X (sodium salt) in a solution formulation was absorbed in a linear manner. In rats, the bioavailability of SB-497115-X (disodium salt) in different oral formulations (solution, suspension and capsule) was 26%, 41% and 6%, respectively. The half-life ($t_{1/2}$) of orally administered compound was 7.7 hr in monkeys and not estimated by oral routes in rats or dogs. The plasma protein binding was up to 94% in mouse, rat, dog, monkey and human. In male and female rats, up to 88 and 90.6% was excreted in feces. In bile duct cannulated male rats the fecal excretion was 65.7%. In male and female mice, 72.8 and 76.6% of compound was excreted in urine. In bile duct-cannulated (BDC) male mice, 64.0% was excreted in feces and 4.26% in urine. About 64.8 and 63.7% of the orally administered compound was excreted in feces in male and female rabbits. Metabolism was chiefly through conjugation with glucuronic acid in rat, dog, monkey and human hepatocytes. The qualitative metabolic profile across the species was similar to humans and no human specific metabolite was detected. SB-497115-X was shown to inhibit human cytochrome P450 enzymes and significant inhibition of CYP1A2 and CYP2C9 activity was seen (IC_{50} = 3.5 and 9.3 uM, respectively).

Single dose Toxicity Study:

A single oral dose of 100 mg/kg in dogs produced emesis, diarrhea, decreased activity and reduction in body weight and the peak plasma concentration was seen after 1 to 4 hr of an oral dose.

Repeat dose Toxicity Studies:

a. Mouse: SB-497115 at the oral gavage doses of 0, 30, 100 and 300 mg/kg/day (6/sex/group) for 2 weeks produced similar plasma concentrations in male and female mice. 300 mg/kg/day dose was lethal and 100 mg/kg/day well tolerated. The hepatocellular hypertrophy and renal atrophy/degeneration in animals suggested the liver and kidneys were the target organs of toxicity. A second 14-day tolerability study conducted at 150 and 200 mg/kg/day SB-497115 doses showed that both doses were tolerated and plasma concentrations were similar in both sexes. In a 13-week study, oral doses of 10, 60 and 100 mg/kg/day SB-497115 for 13 weeks produced a dose related increase in plasma concentration and no toxicity was seen in study animals. The NOEL was 100 mg/kg/day (5 times MHD based on AUC). The target organs of toxicity were not identified.

b. Rat: SB-497115 at 40 mg/kg/day for 2 weeks in rats caused hepatocellular vacuolation but no adverse effects were seen in animals of 10 and 20 mg/kg/day treatment groups, NOEL was 20 mg/kg/day (2 times MHD based on AUC). In another 2-week oral study, SB-497115 at 0, 20 and 40 mg/kg/day produced treatment related changes of hepatocellular vacuolation in the 40 mg/kg/day group (6 times MHD based on AUC). The liver was the target organ of toxicity in both of these studies. In a 28-week chronic toxicity study, 3, 10, 30 and 60 mg/kg/day SB-497115 produced dose-related increase in plasma concentrations. There were no apparent sex differences in plasma concentration (Cmax) or systemic exposure (AUC). The systemic exposure was 2 to 3-fold higher after repeated dosing suggesting accumulation of the drug after repeated dosing. The decreased activity, irregular respiration, and increase in absolute and relative reticulocytes were seen in the 60 mg/kg/day animals and males were more sensitive than females. The liver, kidneys, blood and lymphoid tissues of spleen, lymph nodes and thymus were target organs of toxicity. A dose of 30 mg/kg/day (5 times MHD based on AUC) was the highest tolerable dose.

In a 2-week toxicity study in juvenile rats, SB-497115 at oral doses of 1 to 15 mg/kg/day produced similar treatment-related exposure in both sexes. Lymph node hemorrhage and myeloid hypercellularity was seen in animals of the 15 mg/kg/day group. The target organs of toxicity were lymphocytes and bone marrow. In a 28 day study in juvenile rats, a slight reduction in RBCs, hemoglobin and hematocrit values, increase in reticulocytes counts and decrease in serum cholesterol and triglycerides was noted in 40 mg/kg/day group.

c. Rabbit: In 13-day dose ranging study in female rabbits, SB-497115 from oral doses of 80, 150, 200 and 300 mg/kg/day produced a linear plasma increase with no treatment related effects. Doses $\geq$200 mg/kg/day were lethal. A linear increase in the plasma concentration was seen in animals and the histopathological effects of hepatocellular hypertrophy and erosion of stomach were seen in 80, 150 or 200 mg/kg/day treatment groups. Based on this, the identified target organ of toxicity was liver in both sexes and, the additional target organ of toxicity in males was stomach. A dose of 150 mg/kg/day (0.4x MHD based on AUC) was a 'no adverse effect dose (NOAEL)'. In 13-day iv dose ranging study in non-pregnant rabbits, 6 and 12 mg/kg/day were administered in 2 groups of non-pregnant rabbits, a dose related plasma concentrations of 2.79 and 31.8 ug.h/ml (AUC 0-24hr) were reached in animals.

d. Dog: The toxicity of SB-497115 was investigated in dogs at 0, 3, 10 or 30 mg/kg/day doses for 14 days and a dose related plasma concentrations was seen in animals. The exposure was about 18.3 and 13.6 folds on day 1 and day 13 in animals of 30 mg/kg/day treatment group. The hepatic enzymes and reticulocytes were increased in 30 mg/kg/day treatment group animals and, an NOEL was 10 mg/kg/day (1.2 times MHD based on AUC). The chronic 52-week toxicity study in dogs was conducted at 0, 3, 10 or 30 mg/kg/day (0.4-3 times MHD based on AUC) in gelatin capsules. The plasma exposure in male and female dogs were similar and the AUC at 30 mg/kg/day dose was approximately 3, 2 and 3 folds higher on week 4, 13 and 26 than on day 1 suggesting accumulation of the drug after repeated dosing. A linear increase of alkaline phosphatase by 1.7 and 1.9 folds in males and, 1.7 and 2.3 folds in females and a dose related hepatocellular vacuolation was seen in 10 and 30 mg/kg/day treatment groups. Based on this, liver was the target organ of toxicity and NOAEL was 10 mg/kg/day (1.2 times MHD based on AUC).

Carcinogenicity Studies:

Mouse: In a 104-week mouse carcinogenicity study, oral doses of 0, 25, 75, 150/115 and 300 mg/kg/day SB-497115 (as recommended by Ex-CAC) were administered. The doses of 300 and 150/115 mg/kg/day were lethal. Because of overt mortality at 300 mg/kg/day, the group was terminated. Treatment-related decrease in survival was seen in females at all doses, yet survival among females of 25 and 75 mg/kg/day groups were adequate to assess carcinogenicity. SB-497115 did not induce neoplastic changes or tumors in mice at doses up to 75 mg/kg/day (4 times MHD based on AUC).

Rat: The oral gavage 104-week carcinogenicity study in rats was conducted at 0, 10, 20 and 40 mg/kg/day doses (recommended by Ex-CAC). Dose proportional plasma concentrations were achieved from the early period to the late period of the study. The survival among treatment and control group animals was similar. A reduction in the incidences of malignant adenoma in animals (7/22, 2/26 and 1/23 males and, 5/35, 6/22 and 1/17 females) of 0, 20 and 40 mg/kg/day treatment groups was reported. The significance of this observation was not discussed. SB-497115 did not induce neoplastic changes or tumor of any class, benign or malignant in study rats at doses up to 40 mg/kg/day (5 times MHD based on AUC).

Reproduction Toxicology:

Fertility and early embryonic developmental toxicity study in female rats done at oral gavage doses of 0, 10, 20 and 60 mg/kg/day (0.8, 2 and 7x MHD based on AUC) showed that SB-497115 was embryo- and feto- toxic and increased pre and post implantation losses in treated females of 60 mg/kg/day group. The adverse effects were not seen in animals of 10 and 20 mg/kg/day. The highest tolerable dose for fertility and reproductive performance in females was 20 mg/kg/day (2 times MHD based on AUC).

Fertility and reproductive performance toxicity study in male rats was conducted at oral gavage doses of 0, 10, 20 and 40 mg/kg/day SB-497115. The males treated for 14 days before mating with untreated females till mating was confirmed (42 to 46 doses) showed treatment related reduction in body weight gain associated with reduced food consumption, increase in mean testes weight without an effect on mating and fertility. The fetal growth or external morphology of the fetuses of females sired by the treated males was similar to fetuses of control group females. The no-observed adverse effect dose was 40 mg/kg/day (5 times MHD based on AUC).

Embryo-fetal developmental toxicity studies were conducted in pregnant rats and rabbits. In rats, SB-497115-GR was administered at 10, 20 and 60 mg/kg/day (0.8-7x MHD based on AUC) from post coitum day 6 to 17. There were no treatment-related teratogenic effects in the study. In rabbit, SB497115 was administered at 0, 30, 80 and 150 mg/kg/day doses from day 7 to 19 postcoitum. The dose of 150 mg/kg/day was lethal and was reduced to 90 mg/kg/day. The minimal adverse effect of reduced stools and body weight retardation was seen in 80 mg/kg/day treatment group animals. SB-497115-GR was non-teratogenic and 80 mg/kg/day (0.3x MHD based on AUC) was the highest tolerable dose in pregnant rabbits.

In a pre- and post-natal oral toxicity study in pregnant female rats, SB-497115 was given at 10, 20 or 60 mg/kg/day (0.8-7x MHD based on AUC) starting from day 6 postcoitum to postpartum. A significant body weight loss, severe decrease of food consumption, decreased activity, bleeding, vaginal bleeding, pale appearance, diarrhea, brown watery feces and ptosis and death in high dose group females was reported. The high dose group was terminated before completion of study due to increased lethality. At 10 and 20 mg/kg/day (2 times MHD based on AUC), no prenatal or postnatal fetal abnormality was seen among fetuses of treated dams.

Mutagenicity:

SB-497115 was not mutagenic or clastogenic in a bacterial mutation assay or in 2 in vivo assays in rats (rat micronucleus and unscheduled DNA synthesis, 11 times the human clinical exposure based on C_{max}). In the in vitro mouse lymphoma assay (with and without metabolic activation), SB-497115 was marginally positive (<3-fold increase in mutation frequency).

B. Nonclinical safety issues relevant to clinical use

None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

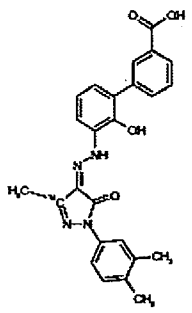
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-291
Review number: 001
Sequence number/date/type of submission: 000
Information to sponsor: Yes ( ) No (X)
Manufacturer for drug substance: SmithKline Beecham d/b/a/ Glaxo-Smith-Kline, King of Prussia, PA 19406
Reviewer name: Yash M. Chopra, M.D., Ph.D.

Division name: Division of Medical Imaging & Hematology Drug Products
HFD #: 160
Review completion date: September 21, 2008

Drug:

Trade name: (Eltromobag/PROMACTA©) Tablets
Generic name: SB-497115, SB-497115- GR, SB-497115-X
Code name: None
Chemical name: 3'-{N'-[1-(3,4-Dimethyl-phenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-2'-hydroxybiphenyl-3-carboxylic acid, bis-monoethanolamine salt.
CAS registry number: 496775-62-3.
Molecular formula/molecular weight: $C_{25}H_{22}N_4O_4 . 2 (C_2H_7NO)/564.65$.
Structure:



Pyrazole ring (original)
(SB-497115-AAA)

Relevant INDs/NDAs/DMFs: IND 63,293

Drug class: Thrombopoietin receptor (TPO-R) agonist

Intended clinical population: Previously treated patients with chronic idiopathic thrombocytopenic purpura (ITP).

Clinical formulation: Tablets

Table 1 Composition of Eltrombopag Tablets, 25 mg and 50 mg

Tablet Strength (mg free acid/tablet):	25 mg	50 mg		
Component	Quantity (mg/tablet)		Function	Reference to Standard
Eltrombopag olamine,			Active	GlaxoSmithKline[2]
Mannitol				USP
Microcrystalline cellulose				NF
Povidone				USP
				USP
Sodium starch glycolate				NF
Magnesium stearate[4]				NF
Film-coating components				NF
				USP
			Film coat	Supplier
Blue			Film coat	Supplier
Total tablet weight	364	364		

b(4)

Route of administration: Oral
Recommended Dose: The recommended starting dose of eltrombopag is 50 mg/day for up to six weeks. The dosage can be increased if the platelet count does not rise above 50Gi/L within 3 weeks.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

PRECLINICAL STUDIES AND TESTING LABORATORIES

Type of Study	Study #	Drug Batch #	Review Page#
I. Pharmacology	--	--	16
II. ABSORPTION, DISTRIBUTION, METABILISM AND EXCRETION.			
Investigation of Intravenous Pharmacokinetics and Oral Bioavailability of SB-497115 in Conscious Rat	#SB497115/RSD-101TJP/1/D100891 and 100891_1)	NL44560-165A1 – Free acid NL44560-168A1 – Disod. salt	34

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2. Preliminary Investigation of Intravenous Pharmacokinetics and Oral Bioavailability of SB-497115 in Conscious Male Dog	#SB497115/RSD-101TJNP/1/D100843 and 100843_2 #SB497115/RSD	NL44560-165A1 – Free acid NL44560-168A1 – Disod. salt KD45207-130A1 (free acid, suspension and solution	36
3. Preliminary Pharmacokinetic Evaluation of Free acid and Ethanolamine Salt [redacted] ormulations of SB-497115 Following Oral administration to Male Dog	RSD101TJR/1/D101233	KD45207-166A1 (free acid, suspension and solution	37
4. Preliminary Investigations of IV PK Parameters and Oral Bioavailability of SB-497115 in the Male Monkey	#SB497115/RSD-101TJL/2/D100817	NL44560-165A1	38
5. a. Preliminary Investigating of Protein Binding to Rat, dog, Monkey and Mouse Plasma Protein by Equilibrium Dialysis and also to determine the blood to plasma Ratio of SB-497115 in Each Species	#SB497115/RSD-101TJM/1/D100821)	DE45559-036A1 (Note book code)	39
b. „	#101 TJK/1; D1001005	„	40
6. Quantitative Tissue Distribution of Drug Related Material Using Whole Body Autoradiography Following 10 mg/kg [^{14}C-SB-497115 to Male Long Evans Rats	# CD2003/00515/00; PK-897; RSD-101W6L/1	#SL201723-003A1	40
7. In Vitro Investigation of the potential of SB-497115-GR to inhibit P-glycoprotein mediated Transport in MDCKII cells Heterogygously expressing Human P-Glycoprotein	# CD2003/00637/00; 03DMM017	GF120918A U1637/133/1 – Radioactive F033082 – Non-radioactive	44
8. In Vitro Investigation of Transport via Human P-Glycoprotein and the Passive membrane Permeability of ^{14}C-SB-497115-GR in MDCKII cells.	# CD2003/01046/00; 03DMM077	R1039/137/1 - Radioactive	45
METABOLISM:			
9. In Vitro Investigation of	#C002003/00721/00;	--	45

b(4)

Human and Rat PXR activation	03DMM042		
10. In Vitro Effects on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Female rats following oral Administration of 0, 100, 300 and 1000 mg/kg/day SB-497115 for 4 days	#497115/RSD-101PK2/1; CMS 33045B1/6146-232	--	47
11. The Effect on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female rats following oral Administration of 0, 3, 10 and 40 mg/kg/day SB-497115 for 14 days	#SB-497115/RSD-101TKB/1; CMS 39792B/7274-151	--	48
12. The Effect on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female Dogs following oral Administration of 0, 3, 10 and 40 mg/kg/day SB-497115 for 14 days.	SB-497115/RSD-101XHX/1; CMS 39792A/7274-150)		49
13. An in Vitro Evaluation of the Inhibitory Potential of SB-497115 on Human Cytochrome P450 Enzymes	#CD2003/00990/00/7274-290	F033083/USO200061	50
14. Elimination of Radioactivity following single oral (10 mg/kg) Administration of ^{14}C-SB-497115 to Male and Female rats intact and Bile-Duct-Cannulated Rats	#SB-497115/RSD-101TKF/2; CMS 39120B	#SL201723-003A1–Radio NW 100477-003	51
15. [^{14}C]SB-497115 (10 mg/kg) to Male and Female Intact and Male Bile Duct-Cannulated Mice	CD2007/00070/00; 717-318	Batch No. 1 (Code CFQ14930)]	52
EXCRETION:			
16. Elimination of Radioactivity following single oral (10 mg/kg) Administration of ^{14}C-SB-497115 to Male and Female	#SB-497115/RSD-101TKD/1; CMS 39120A)	SL201723006A1 – Radio NW100477-003A2 – Non radio	54

intact and Bile-Duct-Cannulated Dogs			
17. Preliminary In Vitro Investigating of Metabolism of ^{14}C-SB-497115 in Rat, dog, Cynomolgus Monkey and Man	#SB497115/RSD-101TJJ/1/D100903	SL45241-199A1 - Radio	55
18. Metabolism of ^{14}C-SB-497115 in Rat	#CD-2004/00068/00/D102031	F033082	57
19. Metabolism of SB-497115 in Rabbits	#CD2004/00830/00/03D MM055	F033082	62
20. Metabolism of SB-497115 in Beagle Dogs	#CD2003/00649/00/D102057		67
III. TOXICOLOGY			
Acute Toxicity Studies:			
Oral:			
a. Dog	RSD-101S5D1/D01013	RC43490-167A1	74
b. Rat	RSD-101V81/1	"	
SUBACUTE, SUBCHRONIC AND CHRONIC TOXICITY STUDIES:			
RATS:			
14-Day Oral	CD2003/00252/00	F033082	75
14-Day Oral with 4-Week Reversibility	CD2003/00327/00	F033082	78
28-week Oral	CD2003/00332/00; 274-374	F033082	81
SB-497115-GR: Juvenile Oral (Gavage Once Daily) 28-day General Toxicity Study in the Male and Female Rats	#CD2005/00661/00; Project No. 900710/GSK Reference No. G05081	F074359007	85
SB-497115-GR: Juvenile Oral (Gavage Once Daily) 28-day General Toxicity Study in the Male and Female Rats	#CD2006/0065/100		88
MOUSE:			
14-Day oral	CD2003/00476/00	F033082	89
2-Week oral Gavage Tolerability & Toxicokinetic study	CD2004/00836/00	TPO-E-01C	92
13-Week Oral Gavage	CD2004/00627/00	TPO-E-01C	94
DOGS			
14-Day Oral	RSD-101V3C/1	20456-037	97
52-Week oral Capsule	RD2003-01148/00	201633-001	100
RABBITS			104

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7-Day Oral	CD2003/00854/00; #G03186	F033082	104
III. MUTAGENICITY:			
1.Ames Test in Salmonella Typhimurium and Escherichia Coli	RSD-101KTS/1; G01565	KD 45207-166A1	109
2.In Vitro Mammalian Cell Mutation Test at Thymidine (HGPRT Locus) in mouse lymphoma L15178Y cells	RSD-101KFT/1, G01566	KD45207-166A1 — b(4)	110
3. Aminoethanolamine: Mutation assay with L5178Y Mouse Lymphoma Cells at TK Locus	WD2002/00089/00; V23485	10K0191, 99.9% GlaxoSmithKline, Hertfordshire	112
4.SB-497115-GR Ehanolamine salt: Mutation Test at Thymidine (HGPRT Locus) in mouse lymphoma L15178Y cells	WD-2003/00167/00/GLP #V23910	F033082; 96.1% GlaxoSmithKline, Hertfordshire	113
5. In Vivo Micronucleus Induction Test in Rat Bone Marrow	WD-2003/01250/00; R23395	US0200061/F033082; 96.1% — b(4)	115
6. Study title: Unscheduled DNA synthesis (UDS) in Rat Liver using in Vitro and in Vivo Procedure.	#WD2003/01251/00; R23610/V23610		116
CARCINOGENICITY STUDIES:			
104-Week oral gavage mouse	#7274-666/GSK#CD2006/00751/02	TPO-E-01C; 07/31/2006; TPO-E-07	117
104-Week oral gavage rat	#7274-638/GSKG04281/ #CD2007/00923/00	#TPO-E-02C — F076633 (99.0%); and F083255 (99.8%) b(4)	126
REPRODUCTIVE TOXICITY:			
Oral Female Segment I. Fertility & Early Development Study in Rats	CD2003/00612/00	F033082	136
Oral Male Segment I. Fertility & Early Development Study in Rats	CD2005/00236/00	F074359007	139
Rat Segment II. Embryo-Fetal Development	CD2003/00938/00	F033082	143
Rabbit Segment II. Embryo-Fetal Development	CD2003/01136/00/GSK #G03322	F033082	147
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2.6.2 PHARMACOLOGY & TOXICOLOGY REVIEW

2.6.2.1 Brief summary

SB-497115-GR bis-ethanolamine salt is an orally active thrombopoietin receptor agonist indicated for the treatment of immune thrombocytopenic pupura (ITP). The platelet proliferative activity of Eltrombopag was demonstrated in chimpanzees and, on human TPO-R preparations. It did not show the proliferative activity in any other species. The toxicology studies with the compound were conducted in rats, mice and dogs and were of 4 weeks or longer duration. In these species, the platelet proliferative activity of Eltrombopag was not observed. However, adverse effects seen in these animals were liver and kidney toxicity and, the development of cataract.

2.6.2.2 PRIMARY PHARMACODYNAMICS

Thrombopoietin (Tpo) an endogenous megakaryocyte growth factor and development factor, produces proliferation and differentiation of megakaryocyte progenitor cells and increases the release of platelets. SB-497115-GR a selective Tpo agonist produced an increase number of platelets/megakaryocyte by promoting the increase in the number and size of platelets and stimulates both signal transducer and activator of transcription (STAT) based interferon regulatory factor-1 (IRF-1) promoter (EC_{50} 0.27 μM) and glycoprotein IIb (gpIIb), a megakaryocyte-specific promoter (EC_{50} 0.1 μM) in vitro. It up-regulates the expression of certain early response genes associated with megakaryocyte proliferation and TPO activation. The expression of the genes by the compound was similar to thromopoietin. The effect on ADP-induced platelet aggregation was insignificant.

Mechanism of action:

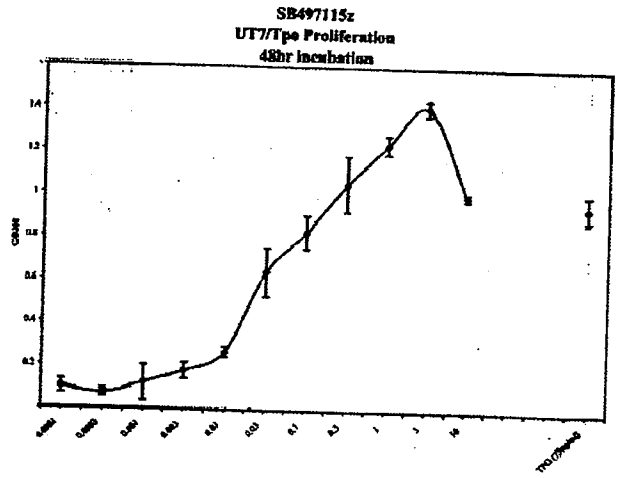
SB-497115-GR acts by increasing the production and systemic release of platelets, by inducing the proliferation and differentiation of Tpo dependent cell line (UT7-TPO) in bone marrow progenitor cells. It stimulated both signal transducer and activator of transcription (STAT) based interferon regulatory factor-1 (IRF-1) promoter and glycoprotein IIb (gpIIb), a megakaryocyte-specific promoter in vitro and up-regulates the expression of certain early response genes associated with megakaryocyte proliferation and TPO activation . The kinetics and expression of the genes with the compound was similar to TPO. It was a selective TPO-R agonist and lacked activity in assays in which other cytokines were active. Its proliferative and anti-apoptotic effects on N2C-TPO cells were additive with TPO showed the activity on same receptors. In vitro, eltrombopag treated washed platelets showed phosphorylation of STAT-1, -3 and -5 with the induction of anti-phospho-specific antibodies but phosphorylation of protein kinase B (AKT) was not seen. In contrast, eltrombopag inhibits proliferation of leukemia cell lines and liver cancer cells and did not effect ADP, thrombin receptor activator peptide or collagen induced stimulation.

Drug activity related to proposed indication:

1. **Proliferation and differential of Megakaryocte cell Lines and Bone Marrow Progenitor cells:** (GRS029/SB497115)

CD34+ early bone marrow cells when incubated with SB-497115 for 10 days from a concentration of 0.0001 to 10 uM was seen to induce a concentration dependent proliferation of thrombopoietin (Tpo)-dependent cell line. The UTT-TPO cell proliferation as measured by incorporation of BrdU was observed in a concentration dependent manner and ED_{50} was 30 nM. The maximum activity was noted at 100 ng/ml. In human bone marrow cell line proliferation test (containing endogenous optimum levels of TpoR and having the property of differential conversion of normal cells into CD41+ cells), cell proliferation was increased by 50% with 0.3 uM SB-497115 and greater than 30% produced by 100 ng/ml TPO. SB-497115 was about 1.8 times greater cell proliferation and differentiation activity than Tp.

Figure 2.2. Proliferation of UT7-TPO cells in Response to SB-497115 Treatment



2. **Effects on Thrombopoietin Signaling Pathways:** (GRS048/01)

In an vitro reporter gene assay, IL3-starved overnight (in starving media containing 30 uM $ZnCl_2$ - BAF3-TPO (BAF3-3B5) cells stably- transfected with human Tpo receptor and IRF-1 promotor linked to luciferase were mixed with compound or 75 ng/ml Tpo for 15 to 120 min to determine the early response on gene expression and activation of gpIIb promotor. SB-497115 increased lucerifase production in a concentration dependent manner with EC_{50} = 100 nM and showed megakarocyte specific promotor activity. The STAT5 activation in UT7-TPO cells was 95%, similar to TPO and the activity peaked at 10 min and reduced by 60 min. SB-49117115 was observed to increase the expression of certain genes and Tpo activation (Fos, ERG-1 and thyroid-like receptors 3) and these

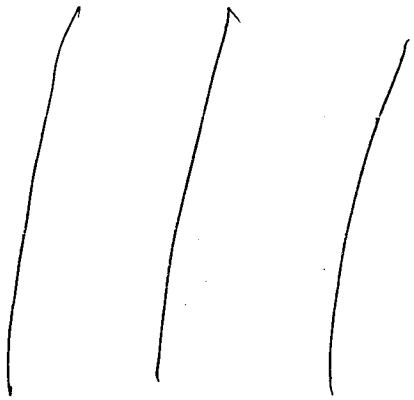
were associated with proliferation and Tpo activation. The activity was selective for TpoR as 3-folds concentration of the compound did not produce proliferation of other systems using G-CSF, Epo, IL-3, Interferon-α or Interferon-γ. The addition of human serum albumin (44 mg/ml) at physiological levels induced 2 folds decrease in SB-497115 activity (EC_{50}= 270 nM).

The transduction of SB497115 related activity was studied in TPO pathway experiment (JAK/STST) of MAPK signal transduction pathway. UTT-TPO was incubated overnight with SB-497115 (up to 70 uM TPO or 100 ng/ml) for 3 to 30 min in Western blot experiment using phospho-p44/42 MAPK, 44/42 MAPK, p44/42 MAPK plus antiserum 55STS (antibodies for immunoblotings. STAT activity and p42/44 MAPK of SB-497115 was similar to TPO. SB-497115 treatment increased TPO expression of certain early gene and proliferation of cells. SB-497115 and TPO caused an induction of Fos mRNA, mERG1 mRNA in first 30 min and up to 120 min, respectively. Thyroid like receptors were also seen at 60 to 120 min of treatment.

In the study, the potential change in the activity of SB-497115 when given in conjunction with TPO was determined. TPO up to the concentration of 100 ng/ml did not potentiate the activity of 0.001 to 100 uM SB 497115. However, the observed effects were of additive nature. Thus TPO did not produce synergism with the compound.

The species specificity was studied in an in vitro gene assay (study #UH2004/0060/1) using BAF3-TPO (BAF3-3B5) cells stably- transfected with human Tpo receptor. SB 497115 from 0.3 to 30 uM was shown to have selectivity for human and chimpanzee platelets Tpo receptor on their surface over a wide range of concentrations. The treatment with 100 ng/ml Tpo activated JAK/STAT pathway in platelets of cynomolgus macaques, cat, mouse, pig, ferret, cotton rat and tupaia (tree shrew). SB 497115 resulted in the activation of STATs in human and chimpanzee platelets only. The specificity of SB 497115 for these two species was due to a lack of STAT activation in EMSA platelets assay. The data is shown below in sponsor's scanned figure 2.4.

Figure 2.4. STAT Activation Determined by Electrophoretic Mobility Shift Assay Using Platelets from Various Species



b(4)

Eltrombopag has activity only on human and chimpanzee TPO-R and the transmembrane domain of TPO-R of these species contains particularly His499 which was a necessary factor for eltrombopag/TPO-R interaction.

4. **Effect on Platelets Activation** (UH2003/00030/00): In sub-maximally stimulated platelets, neither 1 to 1.5 uM ADP nor 0.01 to 10 uM SB-497115 potentiate the platelet aggregation whereas 150 ng/ml TPO concentration was shown to potentiate the platelets aggregation. Thus, SB-497115GR did not increase CD62P (P-selectin) expression on human platelets. The platelet aggregation induced by 3 uM ADP, 2 ug/ml collagen or 20 uM thrombin receptor activating peptide (TRAP) was also not inhibited/affected by 1 to 10 uM SB-497115.

5. **Effect on Platelets Production:** (Study # UH2003/00031/01) Using Ba/F3 TpoR cells (murine B lymphocyte cell line transfected with human TPO receptor) or UT7/Tpo cells (a human megakaryoblastic cell line) the cell proliferation activity of 0.001 to 30 uM SB-497115 was studied in the presence of 1.4 or 11.25 mg/ml albumin. The rate of proliferation was measured by the addition of 10 uCi/ml methyl ^{3}H-thymidine. In the presence of 1, 11 and 44 mg/ml HAS, a dose related decrease in the proliferation activity was seen with an EC_{50} of 1250 nM. The estimated EC_{50} was 2750 nM. In UT7/Tpo cells proliferation assay, the addition of α-acid glycoprotein (AAG) did not affect the SB-49711GR activity.

6. **Effect on Human Serum albumin**: SB-497115 binds up to 99% with plasma proteins and 99.4 to 99.8% to serum albumin columns. The addition of 1.4 or 11.5 mg/ml HSA produced a decrease in the activity which was similar to the media containing 5% FBS. However, the addition of the compound in the presence of 1 mg/ml HSA caused an increased proliferation of UT7/TPO cells (IC_{50}= 1250 nM) and HAS concentration at the physiological concentrations produced a 2 fold decrease (EC_{50}= 2750 nM). The addition of alpha acid glycoprotein did not affect the potency of SB-495117.

Table 2.3. HAS Effects on SB-497115-GR Potency in vitro

Assay Format	Endogenous BSA [1] (mg/mL)	HSA (mg/mL)	SB-497115 EC_{50} (nM)
BaF3/hTpoR (RPMI/5% FBS media)	0.9		60
	0.9	1.4	160
	0.9	11.25	220
UT7/TPO (SFEM media)		1	1250
		11	1750
		44	2750

Key:
1. Endogenous BSA levels in the 5% fetal bovine serum were calculated based on the manufacturer's certificate of analysis.

7. **Oral Dose Safety, Pharmacology and Pharmacokinetics Study in Female Chimpanzees:** (study # CD2004/00D02055

This GLP study was conducted from May 17, 2002 to September 9, 2004 to determine the safety, pharmacology and pharmacokinetics of a single and multiple oral doses of SB-497115.

Five female chimpanzees (28 to 38 kg) 7 to 8 years old were administered a single doses of 0 (vehicle), 0.3, 1, 3 or 10 mg/kg (Cycle 1; 1/group). In cycle 2, the compound was administered at the doses of 0, 0.1, 0.3, 1 or 3 mg/kg/day for 5 days (Cycle 2; 1 animal/group) and, 5 daily doses of 0 (2/group) or 10 mg/kg (3/group) SB-497115-GR. The dose selection was based on the results of other species (as no study in chimpanzee was available), i.e., a 10 day oral dose range toxicity study in rats [GSK Document No. RD2001/01419/00] and a 4-day oral dose range toxicity study in dogs [GSK Document No. SB-497115/RSD-101T3T/1]. Plasma drug concentrations were determined on blood samples collected on day 1 and 5 of each dosing cycle, Day 3 of Cycle 3, and approximately 3 to 10 days after the last dose of each dosing cycle. Chimpanzees were returned to the stock colony following study completion.

Five days treatment of SB-497115-GR produced an increase in platelet counts from 1.3 to 2.4 times at the selected doses after about 7 days of the last dose. The reticulated platelets counts were increased in treated chimpanzees as shown in the sponsor's table below.

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TABULATED SUMMARY

SB-497115-GR: Oral Dose Safety, Pharmacology and Pharmacokinetic Study in Female Chimpanzees

Species/ Strain	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg)	Number of Animals/Sex	Noteworthy Findings	GSK Document No./ Study No.
			CYCLE 1			
Chimpanzee	Oral gavage/ (2% HPMC/ 0.2% SLS suspension)	1 day	0 0.3 1 3 10	1 1 1 1 1	None	CD2004/00054/00 D02055
			CYCLE 2			
Chimpanzee	Oral gavage/ (2% HPMC/ 0.2% SLS suspension)	5 days	0 0.1 0.3 1 3	1 1 1 1 1	None	CD2004/00054/00 D02055
			CYCLE 3			
Chimpanzee	Oral gavage/ (2% HPMC/ 0.2% SLS suspension))	5 days	0 10	2 3	Increases in platelet counts consistent with the pharmacologic activity of SB-497115-GR.	CD2004/00054/00 D02055

a. Doses are expressed in terms of pure active moiety.

Treatment related non-proportional plasma concentrations were seen as shown below. A decrease in the plasma concentration was seen in high dose treated animals.

Table 3g. Pharmacokinetics: Absorption After a Single Dose Test Article: Eltrombopag (bis-monoethanolamine salt)

Location in CTD: m4.2.1.1
Report No.: CD2004/00054/00 Study No.: D02055

Species:	Chimpanzee			
Gender (M/F)/Number of Animals:	1F	1F	1F	1F
Feeding Condition:	Unknown	Unknown	Unknown	Unknown
Vehicle/Formulation:	2% HPMC with 0.2% SLS	2% HPMC with 0.2% SLS	2% HPMC with 0.2% SLS	2% HPMC with 0.2% SLS
Method of Administration:	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)
Salt Form:	Bis-monoethanolamine salt	Bis-monoethanolamine salt	Bis-monoethanolamine salt	Bis-monoethanolamine salt
Dose (mg/kg):	0.3	1	3	10
Sample:	Plasma	Plasma	Plasma	Plasma
Analyte:	SB-497115	SB-497115	SB-497115	SB-497115
Assay:	Negative-ion LC/MS/MS	Negative-ion LC/MS/MS	Negative-ion LC/MS/MS	Negative-ion LC/MS/MS
PK Parameters:				
t_{max} (h)	6.63	24.42	6.60	24.43
C_{max} (µg/mL)	0.110	0.284	2.081	0.525
AUC_{0-t} (µg.h/mL)	1.42	6.27	19.3	12.1

Key:
LC/MS/MS = Liquid chromatography with tandem mass spectroscopy.
HPMC = Hydroxypropylmethylcellulose.
SLS = Sodium lauryl sulfate.
SB-497115 = Eltrombopag (parent compound).

2.6.2.3 Secondary Pharmacodynamics

1. Study on the effect of RC43490-167A1 in Various Receptor Binding and Enzyme Assays.

b(4)

Study # SB-497115-/RSD-101V7X1; —— study # 882158/UA 2003/00039/00

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Conducting laboratory and location: [redacted] b(4)

Methods

The affinity of SB-497115 on various receptors and on different enzyme systems (activity) was assessed in a radioligand binding assays. The binding was studied on the following receptors as tabulated below in a vitro system and enzyme assays were also performed. Following the incubation of the membranes or cells in suspension with radioactive ligand as shown in the following table, the mixture were rapidly filtered, centrifuged and bound radioactivity measured using scintillating counter.

2.1 Binding assays

The assays were performed using the following general procedures

Receptor	Origin	Reference compound	Bibliography
α_{1A}	rat salivary glands	WB 4101	Michel et al. (1989)
α_{1B}	rat liver	spiperone	Michel et al. (1989)
α_{2A}	HT 29 cells	yohimbine	Bylund et al. (1988)
α_{2B}	NG 108-15 cells	yohimbine	Bylund et al. (1988)
	human recombinant (Sf9 cells)	yohimbine	Devedjian et al. (1994)
	human recombinant (Sf9 cells)	ICI 118551	Smith & Teitler (1999)
β_3	rat adipose tissue	cyanopindolol	Sillence et al. (1993)
	human recombinant (Hela cells)	saralasin	Tsuzuki et al. (1994)
CGRP *(h)*	SK-N-MC cells	hCGRPα	Muff et al. (1992)
CB_1 *(h)*	human recombinant (HEK 293 cells)	WIN 55212-2	Matsuda et al. (1990)
CB_2 *(h)*	human recombinant (HEK 293 cells)	WIN 55212-2	Munro et al. (1993)
D3 *(h)*	human recombinant (CHO cells)	(+)butaclamol	Mackenzie et al. (1994)
D4.4 *(h)*	human recombinant (CHO cells)	clozapine	Van Tol et al. (1992)
	human recombinant (CHO cells)	endothelin-3	Buchan et al. (1994)
$GABA_A$	rat cerebral cortex	muscimol	Snodgrass (1978)
AMPA	rat cerebral cortex	L-glutamate	Murphy et al. (1987)
Kainate	rat cerebral cortex	kainic acid	Monaghan & Cotman (1982)
NMDA	rat cerebral cortex	CGS 19755	Sills et al. (1991)
I_2 (central)	rat cerebral cortex	idazoxan	Brown et al. (1990)
LTB_4 *(h)* (BLT)	U-937 cells	LTB_4	Winkler et al. (1988)
N (neuronal) (α-BGTX-insensitive)	rat cerebral cortex	nicotine	Pabreza et al. (1991)
5-HT_{1D}	bovine caudate	serotonin	Heuring & Peroutka (1987)
5-HT_{2C} *(h)*	human recombinant (CHO cells)	mesulergine	Bonhaus et al. (1995)
5-HT_4	guinea-pig striatum	serotonin	Grossman et al. (1993)

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2.2 Enzyme assays

The assays were performed using the following general procedures :

Assay	Origin	Reference compound	Bibliography
COX_1 *(h)*	human platelets	diclofenac	Miralpeix et al. (1997)
COX_2 *(h)* (isol. enz.)	HUV-EC-C cells	NS 398	Miralpeix et al. (1997)
Phosphodiesterase I	bovine brain	8-methoxy-IBMX	Nicholson et al. (1989)
Phosphodiesterase II *(h)*	differentiated U-937 cells	EHNA	Torphy et al. (1992)
Phosphodiesterase III *(h)*	human platelets	milrinone	Weishaar et al. (1986)
Phosphodiesterase IV *(h)*	U-937 cells	rolipram	Torphy et al. (1992)
Phosphodiesterase V *(h)*	human platelets	dipyridamole	Weishaar et al. (1986)

Receptor	Origin	Reference compound	Bibliography
Estrogen α *(h)*	human recombinant (Sf9 cells)	17-β-estradiol	Parker et al. (2000)
Estrogen β *(h)*	human recombinant (Hi5 cells)	17-β-estradiol	Parker et al. (2000)
Progesterone *(h)*	MCF-7 cells (cytosol)	R 5020	Eckert & Katzenellenbogen (1982)
Testosterone	rat prostate (cytosol)	mibolerone	Schilling & Liao (1984)
Ca^{2+} channel (L, DHP site)	rat cerebral cortex	nitrendipine	Lee et al. (1984)
Na^+ channel (site 1)	rat cerebral cortex	tetrodotoxin	Catterall et al. (1979)

The experimental conditions are summarized below :

Receptor	Ligand	Conc.	Nonspecific	Incubation
α_{1A}	[^{3}H]prazosin	0.06 nM	phentolamine (10 µM)	60 min./22°C
α_{1B}	[^{3}H]prazosin	0.05 nM	phentolamine (10 µM)	60 min./22°C
α_{2A}	[^{3}H]RX 821002	0.7 nM	(-)epinephrine (100 µM)	30 min./22°C
α_{2B}	[^{3}H]RX 821002	2.5 nM	(-)epinephrine (100 µM)	25 min./22°C
α_{2C} *(h)*	[^{3}H]RX 821002	5 nM	(-)epinephrine (100 µM)	60 min./22°C
β_2 *(h)*	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	30 min./22°C
β_3	[^{125}I]CYP (+ 1 µM (-)propranolol)	0.3 nM	(-)propranolol (1 mM)	90 min./37°C
AT_2 *(h)*	[^{125}I]CGP 421 I2A	0.05 nM	angiotensin II (1µM)	180 min./37°C
CGRP *(h)*	[^{125}I]hCGRPα	0.04 nM	hCGRPα (1 µM)	60 min./22°C
CB_1 *(h)*	[^{3}H]WIN 55212-2	2 nM	WIN 55212-2 (10 µM)	90 min./30°C
CB_2 *(h)*	[^{3}H]WIN 55212-2	0.8 nM	WIN 55212-2 (5 µM)	40 min./30°C
D3 *(h)*	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C
D4.4 *(h)*	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C
ET_B *(h)*	[^{125}I]endothelin-1	0.03 nM	endothelin-1 (0.1 µM)	120 min./37°C
$GABA_A$	[^{3}H]muscimol	5 nM	muscimol (10 µM)	10 min./4°C
AMPA	[^{3}H]AMPA	8 nM	L-glutamate (1 mM)	60 min./4°C
Kainate	[^{3}H]kainic acid	5 nM	L-glutamate (1 mM)	60 min./4°C
NMDA	[^{3}H]CGP 39653	5 nM	L-glutamate (100 µM)	60 min./4°C
I_2 (central)	[^{3}H]idazoxan	2 nM	cirazoline (10 µM)	30 min./22°C
LTB_4 *(h)* (BLT)	[^{3}H]LTB_4	0.2 nM	LTB_4 (0.2 µM)	40 min./22°C
N (neuronal) (α-BGTX-insensitive)	[^{3}H]cytisine	1.5 nM	nicotine (10 µM)	75 min./4°C
$5\text{-}HT_{1D}$	[^{3}H]serotonin	2 nM	serotonin (10 µM)	30 min./22°C

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Results:

SB-497115 was selective thrombopoietin receptor agonist but also had shown an activity on estrogen receptors.

Table I

Effects of RC43490-167A1 on the specific radioligand binding to the receptors studied and IC_{50} values for the reference compounds

Receptors	RC43490-167A1	Reference compounds		
	1 µM		IC_{50} (nM)	*(nH)*
α_{1A}	-	WB 4101	0.48	*(1.4)*
α_{1B}	11	spiperone	1.1	*(1.0)*
α_{2A}	-	yohimbine	3.9	*(1.1)*
α_{2B}	38	yohimbine	18	*(0.7)*
α_{2C} *(h)*	-	yohimbine	7.1	*(2.0)*
β_2 *(h)*		ICI 118551	2.8	*(1.3)*
β_3	10	cyanopindolol	230	*(1.0)*
AT_2 *(h)*	-	saralasin	0.58	*(1.1)*
CGRP *(h)*		hCGRPα	0.51	*(1.1)*
CB_1 *(h)*	-	WIN 55212-2	24	*(0.9)*
CB_2 *(h)*	20	WIN 55212-2	4.8	*(1.2)*
D3 *(h)*		(+)butaclamol	11	*(1.1)*
D4.4 *(h)*	-	clozapine	175	*(1.2)*
ET_B *(h)*	-	endothelin-3	0.16	*(1.1)*
$GABA_A$	18	muscimol	13	*(1.0)*
AMPA	-	L-glutamate	560	*(0.9)*
Kainate	-	kainic acid	32	*(0.9)*
NMDA	-	CGS 19755	439	*(0.8)*
I_2 (central)	88	idazoxan	5.9	*(1.1)*
LTB_4 *(h)* (BLT)	-	LTB_4	1.4	*(1.2)*
N (neuronal) (α-BGTX-insensitive)	13	nicotine	7.9	*(0.9)*
$5\text{-}HT_{1D}$	12	serotonin	3.7	*(1.0)*
$5\text{-}HT_{2C}$ *(h)*	-	mesulergine	2.8	*(1.4)*
$5\text{-}HT_4$	20	serotonin	187	*(0.8)*
Estrogen α *(h)*	85	17-β-estradiol	3.5	*(1.0)*
Estrogen β *(h)*	33	17-β-estradiol	30	*(1.2)*
Progesterone *(h)*		R 5020	5.9	*(1.1)*
Testosterone	-	mibolerone	4.4	*(1.0)*
Ca^{2+} channel (L. DHP site)	12	nitrendipine	2.3	*(1.4)*
Na^+ channel (site 1)		tetrodotoxin	13	*(1.0)*

For RC43490-167A1, the results are expressed as a percent inhibition of control specific binding (mean values ; n = 2).
The symbol - indicates an inhibition of less than 10%.

Table 2

Effects of RC43490-167A1 on the studied enzyme activities and IC_{50} values for the reference compounds

Enzymes	RC43490-167A1 1 µM	Reference compounds	IC_{50} (µM)	(nH)
COX_1 *(h)*	+	diclofenac	0.030	*(0.9)*
COX_2 *(h)* (isol. enz.)	-	NS 398	7.9	*(0.8)*
Phosphodiesterase I	10	8-methoxy-IBMX	1.7	*(1.1)*
Phosphodiesterase II *(h)*	17	EHNA	0.91	*(0.8)*
Phosphodiesterase III *(h)*	11	milrinone	0.24	*(0.8)*
Phosphodiesterase IV *(h)*	-	rolipram	0.27	*(0.6)*
Phosphodiesterase V *(h)*	20	dipyridamole	0.47	*(1.5)*
Elastase *(h)*	-	3', 4'dichloroisocoumarin	0.72	*(1.2)*
Protein kinase C	19	staurosporine	0.059	*(1.2)*
EGF-tyrosine kinase	-	PD 153035	0.0055	*(1.7)*
ATPase (Na^+/K^+)	-	ouabain	0.35	*(1.2)*

For RC43490-167A1, the results are expressed as a percent inhibition of control activity (mean values ; n = 2).
The symbol - indicates an inhibition of less than 10%.
The symbol + indicates a stimulation higher than twice the control activity.

SB-497115 at 1 uM concentration showed insignificant effect, i.e., less than 20% inhibition in the enzyme assay system but its effect on COX1 (h) enzyme was two times the control group.

Determination of the Affinity of SB-497115 for Various Receptors: (—— Study #882161)
In a radioligand assay for the estimation of the affinity of SB-497115 for various receptors, autofluorescence of two estrogen receptors was seen at a concentration of SB-497115 more than 1 uM in estrogen receptor assays. The reference compounds used were yohimbine, idazoxan and 17 β-estradiol. The IC_{50} were 15500, 1710, 344 and 1940 nM for α2B, I2 (central), estrogen α (h) and estrogen β (h) receptors.

Both pre-clinical and clinical studies suggest eltrombopag also inhibits organic anion transporting polypeptide OATP1B1. In a clinical study in healthy volunteers, concurrent eltrombopag and rosuvastatin treatment resulted in a 2.03-fold increase in plasma rosuvastatin Cmax and 55% increase in AUC(0-∞). Pre-clinical studies using expressed human transporters suggest that eltrombopag is not a substrate for P-glycoprotein (Pgp) or OATP1B1.

In pre-clinical studies, eltrombopag was reported to be an inhibitor of UGT1A9, UGT1A3, UGT1A1, UGT2B15, UGT1A6, UGT2B7, and UGT1A4. Based on in vitro data, eltrombopag inhibits UGT1A9 with the greatest potency. The effect of this inhibition was not studied clinically by the sponsor.

2.6.2.4 SAFETY PHARMACOLOGY

Neurological effects:

1. **Single Oral dose Neurobehavioral Study in Male Rats.**

Study no.: RD2002/00693/00; R41084/1807-018
Conducting laboratory and location: / b(4)
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Batch #: F033082
Methods

Doses: 3, 10 or 40 mg/kg in 2% hydroxypropylmethylcellulose plus 0.2% sodium lauryl sulfate in 10 ml/kg volume. The doses selected were the same as used in 14-day oral toxicity study in rats b(4)

Species/strain: –.CD(SD)IGS BR VAF/Plus male rats with mean body weight of 403.2 to 413.2 g

Route, formulation, volume, and infusion rate: Oral gavage

Functional observational battery of tests were performed 24 hr prior and 2 hr post dosing periods since peak of plasma concentration was seen after 2hr post-dosing. The parameters evaluated included behavioral tests, autonomic functions, reactivity, excitability, gait, sensorimotor coordination and forelimb and hind limb grip strength and (2) clinical observations and body weight. The animals were observed twice daily and for the changes in general appearance once daily. The body weight recorded weekly and also on day of conduct of behavioral tests. In the functional observation behavior evaluation, the parameters evaluated were lacrimation, salivation, palpebral closure, eye closure and eye prominence, pupillary reaction to light, piloerection, respiratory changes and urination and defection, visual, tactile, pain and sensorimotor stimuli, reactions in open field chamber. All rats were sacrificed 24 hr after the 4th functional observational battery tests for gross lesions evaluation.

Results:

The number of rears in 10 mg/kg treated animals was significantly ($p<0.05$) different from the control animals but not in 40 mg/kg treated animals so not considered treatment related. It did not affect the tail pinching, air righting, hind limb grip and the pupil closure responses (response to light). The response periods were not different in treated and control group animals. SB-497115 up to 40 mg/kg (5 times MHD based on BSA) dose failed to elicit any change in the neurobehavioral parameters of the rats.

Cardiovascular effects:

2. **In Vitro Effects on Human Ether-a-go-go (hERG) Tail Current Recorded from Stably Transfected HEK-293 cells.**

Reviewer: Chopra NDA No. 22-291

Study #: FD2004/00272/00; V25512

Testing Laboratory: GlaxoSmithKline, Welwyn, Hertforshire (UK).
GLP & QAU Requirements: A statement of compliance with UK GLP and ICH S7A safety Pharmacology regulations was submitted.
Batch #: TPO-E-02C

Methods: The study was undertaken to assess the effects of the compound on hERG tail current on delayed potassium current (Ikr) recorded from stably transfected with hERG (cloned human ether-go-go gene) cDNA using whole cell-patch clamp technique in HEK293 cells. The potential of the compound for the inhibition of hERG-expressed currents representing rapid delayed rectifier potassium currents (I_{kr}) and, the current density of the inward cell currents and transient outward potassium currents (capacitance-pA/pF) were calculated at the maximal soluble concentration of 21.73 uM (9.615 ug/ml) of the compound. SB497115-GR solutions from the concentration of 0.006519, 0.02173, 0.06519, 0.2173, 0.6519, 2.173 and 21.73 uM (0.003, 0.010, 0.029, 0.096, 0.288, 0.961 was used. The substance was equilibrated for about 10 min during which time the voltage was recorded on continuous basis. The percent inhibition as the percent of control tail current was compared. The positive control used in the study was E-4031 (4′-[[1-[2-(6-methyl-2-pyridyl) ethyl]-4-piperidyl]carbonyl] methanesulfonanilide), a class III anti arrhythmic agent. The study included SB497115-GR, n= 4-5 cell/concentration: 1% water, n=4 cells and E-4031, n=2 treatment groups.

Results: SB497115-GR was found to inhibit hERG channel tail current in a concentration-dependent manner. The IC_{25}, IC_{50} and IC_{75} values for the inhibition of hERG tail currents were estimated to be 0.09, 0.69 and 5.13 µM, respectively (equivalent to 0.04, 0.31 and 2.27 µg/ml active moiety, respectively). Positive control E-4031 (100 nM) caused 94% inhibition of hERG tail current confirming validity of the hERG assay. The results are summarized in the table below.

Concentration (uM)	Concentration (ug/ml)	% Inhibition of hERG tail current (% of vehicle control)
0.006519	0.003	0
0.02173	0.010	6.2
0.06519	0.029	19.3
0.2173	0.096	39.6
0.6519	0.288	54.4
2.173	0.961	70.2
21.73	9.615	72.4

3. **Effects on Action Potential in Dog Isolated Cardiac Purkinji Fibers.**

Study #: DGES1092, GSK Studt#V23848

Testing Laboratory: [redacted] b(4)

Reviewer: Chopra NDA No. 22-291

Dates of Start and Completion of Study: June 12, 2002 and August 14, 2002.

GLP & QAU Requirements: A statement of compliance with UK GLP, 1999 and OECD GLP which are acceptable to the FDA was submitted.

Batch #: F033082

Methods: The study was to observe the electrophysiological effects of the compound on action potential duration parameters of APD_{90}, APD_{50} (repolarization phases), amplitude (APA), maximal upstroke velocity (Vmax) and resting membrane potential (RMP). The inhibition of one or more of the potassium current (Ikr/Iks and IKI) contributes to the increase of QT interval. At least 2 free running Purkinje fiber preparations per dog was prepared and only one of these were used for the evaluation of the compound. The vehicle effects were measured on a different preparation. The 4 fibers were exposed to each concentration of the compound and 4 to the vehicle for 30 min after the fiber was stabilized. The reference substance, dl sotalol (50 uM) was used. The action potential parameters for any fiber preparation during its control baseline were recorded and these were action potential duration at 30, 60 and 90% (APD30, APD60 and APD 90), maximum rate of depolarization (MRD), upstroke amplitude (UA) and resting membrane potential (RMP). SB497115 was used at 10 and 25 uM in single simulated dog Purkinje fiber (1 and 0.2 Hz stimulation frequency of 2 to 5 min for 0.5 to 2 ms duration square wave stimuli. For early after depolarization – bradycardia, the stabilized Purkinje fibers (-60mV) were stimulated from 0.5 Hz for 2 to 5min then increased the frequency to 1 Hz and stabilized and the test was initiated at 0.5 Hz to 3 Hz.

Results: SB-497115-GR did not show an increase on RMP and APD30. A slight and dose related insignificant decrease was seen at 10 and 25 uM. The MRD was decreased from 10 to 21% at 25 uM SB-497115-GR (1 Hz) and the vehicle did not exert any effect in the test system. The change of frequency from 1 to 3 Hz produced a decrease which was similar to vehicle treated (5.6 VS 9.2% in vehicle). SB-497115-GR did not show any significant activity on APD_{90}, APD_{60} and UA ($p<0.05$ to 0.01 vs. control) at 3 and 5 mV (both $p<0.05$). The decrease in stimulation frequency to 0.5 Hz did not cause any decrease in UA. The action potential data is shown below in the sponsor's table 1 (vol 3:3, pp 27).

Table 5 Effect of SB-497115-GR or Vehicle on Action Potential Parameters (0.5 Hz)

Action Potential Parameter	SB-497115-GR Baseline	SB-497115-GR 10 uM	SB-497115-GR 25 uM
RMP (mV)	-88.7 ± 1.4	-87.9 ± 1.5	-87.8 ± 1.1
UA (mV)	119.9 ± 0.6	115.9 ± 2.1	117.5 ± 1.8
MRD (V/s)	629.6 ± 65.3	564.2 ± 46.1	558.3 ± 43.9
APD_{30} (ms)	46.0 ± 19.8	42.9 ± 19.1	38.5 ± 19.7
APD_{60} (ms)	335.8 ± 54.5	287.9 ± 49.9	278.4 ± 44.9
APD_{90} (ms)	401.2 ± 55.7	357.4 ± 47.3	344.6 ± 43.3

Action Potential Parameter	DMSO Baseline	DMSO 1%	DMSO 1%
RMP (mV)	-88.7 ± 0.5	-88.2 ± 1.1	-88.6 ± 1.0
UA (mV)	111.9 ± 4.7	111.9 ± 5.9	112.8 ± 6.5
MRD (V/s)	508.7 ± 29.8	527.6 ± 17.0	566.4 ± 70.5
APD_{30} (ms)	72.2 ± 16.0	81.2 ± 28.4	62.3 ± 11.4
APD_{60} (ms)	317.2 ± 23.0	308.4 ± 20.9	319.2 ± 24.9
APD_{90} (ms)	398.5 ± 40.6	396.9 ± 43.3	406.9 ± 45.3

Data are mean ± s.e. mean of n = 4 preparations. For abbreviations see text.

The action potential parameters of Purkinji fibers were not affected by up to 25 uM SB-497115-GR. The positive control dl-sotalol produced a significant ($P<0.05$) increase in APD60 and APD90 as the APD60 was 262.5 and 414.5 ms in control and 50 uM sotalol treated fibres. APD90 was 332.9 and 507.4 ms in control and 50 uM sotalol treated fibres. SB-497115-GR did not produce the increase in the rate of contractions and early after-depolarization at 0.5, 1 or 3 Hz stimulation.

TABLE: Effect of SB-497115, vehicle or Sotalol on MRD (V/s) at stimulation frequencies of 1 and 3 Hz

	Maximum Rate of Depolarization (V/s)		
Stimulation Frequency (Hz)	Vehicle (1% DMSO)	SB-497115-GR (25 uM)	50 uM Sotalol
1	633.1 ± 28.3	565.1 ± 50.1	578.8 ±30.7
3	572.6 ± 29.3	534.7 ± 56.9	--
% Difference Between MRD at 1 and 3 Hz	-9.2 ± 4.9	-5.6 ± 3.6	--

No significant effect was noted on the action potential of cardiac sodium channels (underlying MRD) of dog isolated cardiac purkinje fibers.

4. **Single Oral Dose Cardiovascular Study in Dogs**

Study no.: SB497115-GR/RSD-101TT9/1; G02046
Conducting laboratory and location: b(4)

GLP compliance: A statement of compliance was enclosed
QA report: yes (X) no ( )
Batch #: F033082
Methods

Doses: 0, 3, 10 or 30 mg/kg in capsules
Species/strain: Male Beagle Dogs 2 to 4 years old and weighing 10 to 12 kg
Route, formulation, volume, and infusion rate: Oral capsules
Methods: Four male conscious un-restrained dogs were administered 0, 3, 10 or 30 mg/kg SB-497115 by capsules on separate days, with 7 days between each dose according to Latin square cross-over design. The cardiovascular effects including EKG parameters were monitored continuously from approximately 2 hr prior to dosing to approximately 48 hr post dosing.

Blood pressure, EKG parameters like QT and QTc were calculated using SAS System for Windows. Unique study design or methodology (if any): The QTC was selected by $QTc = QT + a(e^{b70} - e^{bRR})$ using the SAS System for Window programs.

Results:
There were no treatment related changes in the conscious animals treated up to a dose of 30 mg/kg SB-497115. The mean blood pressure, systolic or diastolic blood pressure, heart rate were not significantly altered. Similar changes in these parameters were seen in vehicle control treated animals. The data of the study indicated the compound did not produce significant changes in cardiovascular parameters of the dog in the selected doses.

The oral dose of up to 30 mg/kg (12 times MHD based on BSA) SB497115 did not produce an effect on RR, QT, QTc intervals of the conscious animals and this was identified as the NOEL for the study.

5. **Pharmacodynamics and Pharmacokinetics of a single and multiple doses in Chimpanzee.**

Study no.: CD2004/000054/00; D02155/762-PT-O
Conducting laboratory and location: b(4)

Date of study initiation & completion: May 17, 2002 and September 9, 2004
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )

Batch #: F033082
Methods

Doses: 0, 1, 3, or 10 mg/kg (cycle 1: 1/group) in 2% hydroxy-propylmethyl cellulose plus 0.2% sodium lauryl sulfate in 1 ml/kg;
(ii) 0, 0.1, 0.3, 1 or 3 mg/kg (cycle 2: 1/group) or
(iii) 10 mg/kg (group 3)

Species/strain: Male chimpanzees 7 to 8 years old and weighing 28 to 38 kg

Route, formulation, volume, and infusion rate: Oral gavage

Toxicokinetics samples: On day 1, the blood samples were collected at 0, 0.5, 1, 2, 6, 24, 48 hr post treatment and, pretreatment samples on day 5, 10 and 15 from each of the animals of cycle 1. Trough samples on day 35 and 39 (during treatment) and 44 (5 days after treatment) of cycle 2 and, on day 98, 100 and102 (treatment period) of cycle 3 were collected. The blood samples on day 98 and 102 were collected after 1 hr postdosing.

Unique study design or methodology: The doses were selected based on 10 day oral dose ranging in rats (GSK document # RD2001/01419/00) and, 4-day oral dose ranging toxicity study in dogs (GSK document #SB497115/RSD-101T3T/1).
The body weight, in life observations, hematology/blood chemistry, reticulated platelets in cycle 3 animals.

Results:
No treatment related adverse clinical observations were seen in the animals included in cycle 1 and 2. The blood chemistry and hematology parameters of the treated animals in these cycles were not affected by the treatment. In cycle 3, an insignificant increase in the platelet counts from 335.4+79.9 to 540+337.5X10^3 and reticulated platelet counts (from 42528 to 64071.4/cmm) on day 4 (with big ±SD) was noted after 5 days treatment.

A single dose of 0.3 to 10 mg/kg SB497115-GR produced a non-dose proportional increase in plasma concentration. On day 1, the AUC values were 1.42, 6.27, 19.3 and 12.1 ug.hr/ml SB-497115 and the peaks were seen after 5, 25 and 5 hr after the administration of the compound (the time were approximated from the graphs of the study). In cycle 2, the 24-hr trough samples were collected. The TK data of samples collected on day 98 and 102 was estimated. In cycle 3 animals, 1 hr post dosing mean plasma concentration were 0.087 and 0.894 ug/ml on day 1 and 5 (day 98 and 102), respectively. The compound was detected at the concentration of 0.218 ug/ml in 3 day post treatment samples indicating the residual drug was retained in post treatment samples.

6. **Pulmonary effects: Single Oral Dose Respiratory Study in Rats**

Study no.: (SB-497115/RSD-101TW2/1; G02045)
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.
Date of study initiation & Completion: April 26, 2002 and August 22, 2002
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )

Batch #: F033082
Methods

Doses: 0, 3, 10, or 40 mg/kg in 2% hydroxypropylmethylcellulose plus 0.2% sodium lauryl sulfate in 10 ml/kg volume. The highest selected dose produced liver and spleen toxicity in 10-day toxicity study in rats.

Species/strain: — CD(SD)IGS BR VAF/Plus male rats of 13 weeks of age and with mean body weight of 366 to 398 g b(4)

Route, formulation, volume, and infusion rate: Oral gavage

Unique study design or methodology (if any): The 4 male conscious rats were treated with 3, 10 and 40 mg/kg SB497115-GR in a volume of 10 ml/kg according to Latin square cross-over design. One rat was replaced with another rat on day 22 because of apparent loss of the telemetry signals. The dose schedule is shown below in the table (sponsor's table of the study SB-497115/RSD-101TW2/1):

Animal Identification Number	Dosing Schedule (3, 10 or 40 mg/kg SB-497115 or vehicle)*			
	Day 1	Day 8	Day 15	Day 22
R02M-6042	vehicle	3	10	40
R02M-6043**	10	vehicle	40	3
R02M-6044	40	10	3	vehicle
R02M-6045	3	40	vehicle	10

* Dose is expressed as mg/kg of parent compound and the first day of dosing is designated as Day 1.
** Rat R02M-6046 replaced rat R02M-6043 on day 22 of dosing.

Ventilatory functions including tidal volume, respiratory rate, minute volume, total pulmonary resistance were measured 1 to 4 hr (peak dose time) and 24 hr (post-dose and reversibility) postdosing.

Results:

SB-497115 from 3 to 40 mg/kg did not produce any change in the ventilatory functions/tidal volume, respiratory rate, and minute volume. Based on this, the NOEL for respiratory functions was identified as 40 mg/kg (5 times MHD based on BSA).

7. **Renal effects**: were not studied by sponsor.

8. **Gastrointestinal effects**: were not evaluated by sponsor.

9. **Abuse liability**: Not applicable

Other: None

2.6.2.5. PHARMACODYNAMIC DRUG INTERACTIONS.

No study was submitted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

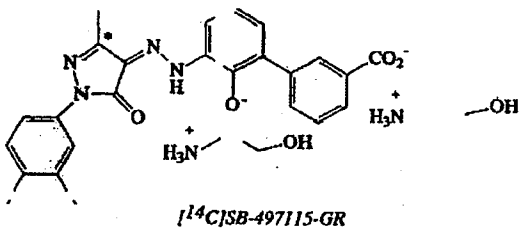
2.6.4.1 BRIEF SUMMARY

The ADME studies conducted in mouse, rat, rabbit, dog and monkey demonstrated that orally administered SB-497115-X (sodium salt) in a solution formulation was absorbed in a linear manner. In rats, the bioavailability of SB-497115-X (disodium salt) in different oral formulations (solution, suspension and capsule) was 26%, 41% and 6%, respectively. The half-life ($t_{1/2}$) of orally administered compound was 7.7 hr in monkeys and not estimated by oral routes in rats or dogs. The plasma protein binding was up to 94% in mouse, rat, dog, monkey and human. In male and female rats, up to 88 and 90.6% was excreted in feces. In bile duct cannulated male rats the fecal excretion was 65.7%. In male and female mice, 72.8 and 76.6% of compound was excreted in urine. In bile duct-cannulated (BDC) male mice, 64.0% was excreted in feces and 4.26% in urine. About 64.8 and 63.7% of the orally administered compound was excreted in feces in male and female rabbits. Metabolism was chiefly through conjugation with glucuronic acid in rat, dog, monkey and human hepatocytes. The qualitative metabolic profile across the species was similar to humans and no human specific metabolite was detected. SB-497115-X was shown to inhibit human cytochrome P450 enzymes and significant inhibition of CYP1A2 and CYP2C9 activity was seen (IC_{50} = 3.5 and 9.3 uM, respectively).

2.6.4.2 METHODS OF ANALYSIS

The compound with the following radioactive site (s) was used in the distribution and metabolism studies of the present submission:

Reviewer: Chopra NDA No. 22-291



[^{14}C]SB-497115-GR

2.6.4.3 **ABSORPTION**

1. **Investigation of Intravenous Pharmacokinetics and Oral Bioavailability of SB-497115 in Conscious Rat**: (Study #SB497115/RSD-101TJP/1/D100891 and 100891_1)

Methods and Materials: The study was conducted for the estimation of the pharmacokinetic parameters and oral bioavailability of the compound in 3 different systems, i.e., solution, suspension and capsules formulations, in a cross-over design manner. Three rats with implanted intravenous cannula were administered 0.935 mg/kg SB-497115 as a solution (in 10% PEG 400 in isotonic saline, pH 8.0-8.5) in 60 min infusion on day 1. On day 2, each of the rats was orally administered 6.05 mg/kg of the compound by gavage (solution was prepared in 10% PEG 400, pH 8.0). On day 3, the rats were administered 4.45 mg/kg SB-497115 via oral gavage in 1% methylcellulose suspension and on day 4, 5.44 mg/kg compound was administered in capsule. The solutions were administered in rats in the final concentrations of 0.223 mg/ml, 0.276 and 0.277 mg/ml SB-497115. The blood samples were collected at 0, 15, 30, 60, 65, 75, 90, 120, 180, 240, 360, 480, 600, 1440, 1800 and 2880 min after iv dose and, 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 1440, 1800 and 2880 min after oral dosing estimating the plasma concentrations of the compound by LC/MS//MS method (sensitivity = 10.0 to 2500 ng/ml of the parent compound).

Results: The plasma concentrations ($AUC_{0\text{-}infin}$) were 2119, 3730, 4161 and 651 ug.ml/min respectively after the intravenous infusion, oral solution, oral suspension and oral capsule. The corresponding doses of the compound were 0.935, 6.05, 4.45 and 5.44 mg/kg of the compound. The clearance after intravenous infusion was of 0.45 ml/min/kg with the $T_{1/2}$ of 12hr, mean volume of distribution of 0.196 l/kg and the residence time of 7.4 hr. The estimated bioavailability was 26.1, 41 and 5.3% respectively after oral solution, oral suspension and oral capsule, respectively. The PK data of the compound by these routes of administration is shown in the following table (table 5 of the sponsor, vol 3.4, pp 22 of RSD-101TJP/1).

Table 5 Mean Pharmacokinetic Parameters of SB-497115 in the Rat

Parameter	Intravenous	Oral Solution	Oral Suspension[a]	Oral Capsule[b]
Dose (mg/kg)	0.935 ± 0.03	6.05 ± 0.16	4.45 ± 0.14	5.44 ± 0.29
CL_p (mL/min/kg)	0.45 ± 0.08	c	c	c
AUC (0-inf) (min•ug/mL)	2119 ± 441	3730 ± 2167	4161 ± 1025	651 ± 237
% Extrap AUC (0-inf)	2.14 ± 0.98	4.75 ± 3.27	13.1 ± 15.8	5.64 ± 3.28
AUC (last) (min•ug/mL)	2071 ± 408	3552 ± 2036	3509 ± 142	615 ± 225
Vss (L/kg)	0.196 ± 0.01	c	c	c
MRT (min)	444 ± 94.3	760 ± 198	1658 ± 1952	741 ± 264
$T_{1/2}$ (min)	708 ± 65.4	c	c	c
Cmax (ug/mL)	12.6 ± 1.55	10.1 ± 6.55	19.0 ± 0.61	3.15 ± 1.51
Observed Tmax (min)	61.7 ± 2.89	100 ± 17.3	60.0 ± 0.00	155 ± 178
F (%)	c	26.1 ± 12.5	41.1 ± 1.60	5.28 ± 1.68

Values are the mean ± SD of n = 3 animals.

[a] Suspension of disodium salt in 1% aqueous methylcellulose; particle size analysis indicated D_{50} of 9.07 um.

[b] Solid disodium salt enclosed in a gelatin capsule.

[c] Not calculated for this route of administration.

2. Preliminary Investigation of Intravenous Pharmacokinetics and Oral Bioavailability of SB-497115 in Conscious Male Dog: (Study #SB497115/RSD-101TJNP/1/D100843 and 100843_2)
Methods and Materials: The study was conducted to estimate the pharmacokinetic parameters and oral bioavailability of 3 different oral preparations of the compound, i.e., solution, suspension and capsules formulations (free acid, disodium salt, free acid and free acid) in a cross-over design manner. Three male beagle dogs with implanted intravenous cannula in cephalic vein for blood samples were administered 1.44 mg/kg SB-497115 solution (in 10% PEG 400 in isotonic saline, pH 8.0-8.5) in 60 min infusion on day 1. On day 2, each of the dogs was orally administered 3.52 mg/kg of disodium salt of the compound by gavage (suspension was prepared in 1% methylcellulose). On day 3, each of the dogs was orally administered 3.60 mg/kg of free acid of the compound by gavage (suspension was prepared in 1% methylcellulose) and on day 4, each of the dogs was orally administered 5.52 mg/kg of free acid in capsules (containing [redacted] On day 5, 1.44 mg/kg SB-497115 solution (in 10% PEG 400 in isotonic saline, pH 8.0-8.5) was administered in each of the dogs as a 60 min infusion after placing a cannula in saphenous vein. The blood samples collected at 0, 15, 30, 45, 60, 65, 75, 90, 120, 180, 240, 360, 480, 600, 1440, 1800 2880 and 4320 min after iv infusion dose and, 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 1440, 1800, 2880 and 4320 min after oral dosing for the determining the plasma concentrations of the compound by LC/MS/MS method and oral bioavailability.

Results: The plasma concentrations ($AUC_{0\text{-}infin}$) were 3734, 7683, 8591, 1474 and 1070 ug.ml/min respectively after the intravenous infusion, oral free acid solution, oral disodium salt suspension, free acid suspension and oral free acid capsule. The corresponding doses of the compound were 1.44, 3.60, 3.52, 3.45 and 5.25 mg/kg of the compound. The clearance after intravenous infusion was of

0.44 ml/min/kg with the $T_{1/2}$ of 13.9hr, mean volume of distribution of 0.47 l/kg and the residence time of 17.93 hr. The estimated bioavailability was 83.0, 91.7, 15.8 and 7.09% respectively after the oral free acid solution, oral disodium salt suspension, free acid suspension and oral free acid capsule. The summary PK data of the compound by these routes of administration has been shown in the following table (table 6 of the sponsor, vol 3.4, pp 23 of RSD-101TJN/1):

Table 6 Mean Pharmacokinetic Parameters of SB-497115 in the Dog

Parameter	Intravenous Infusion[a]	Oral free acid Solution	Oral di-Na Salt Suspension	Oral free acid Suspension	Oral free acid Capsule
Dose (mg/kg)	1.44 ± 0.003	3.60 ± 0.03	3.52 ± 0.07	3.45 ± 0.06	5.25 ± 0.49
CL_p (mL/min/kg)	0.44 ± 0.17	b	b	b	b
AUC (0-inf) (min•ug/mL)	3734 ± 1747	7683 ± 3463	8591 ± 5262	1474 ± 1000	1070 ± 983
% Extrap AUC (0-inf)	2.48 ± 0.63	4.04 ± 1.49	2.28 ± 0.25	2.03 ± 1.28	3.52 ± 0.85
AUC (last) (min•ug/mL)	3639 ± 1695	7380 ± 3357	8388 ± 5117	1437 ± 956	1027 ± 935
Vss (L/kg)	0.47 ± 0.17	b	b	b	b
MRT (min)	1076 ± 122	1281 ± 221	1195 ± 108	1011 ± 228	999 ± 335
$T_{1/2}$ (min)	834 ± 56.9	b	b	b	b
Cmax (ug/mL)	7.47 ± 3.06	8.27 ± 2.45	9.67 ± 3.97	1.51 ± 0.81	1.76 ± 1.46
Observed Tmax (min)	b	81 ± 86.6	60.0 ± 0.00	150 ± 52.0	60 ± 30
F (%)	b	83.0 ± 5.48	91.7 ± 25.9	15.8 ± 4.73	7.09 ± 4.04

Values are the mean ± SD of n = 3 animals.

[a] Due to unexpected low dose concentration, the IV leg was repeated. The data for the initial IV leg were not reported here, but can be found in the study file.

[b] Not calculated or reported for this route of administration.

3. Preliminary Pharmacokinetic Evaluation of Free acid and Ethanolamine Salt — — Formulations of SB-497115 following Oral Administration to Male Dog. (Study #SB497115/RSD-101TJR/1/D101233) b(4)

Methods and Materials: The study was conducted to compare the oral pharmacokinetic parameters of free acid and ethanolamine salt — formulations. The compound was administered in 2 different — preparations — free acid — and, — ethanolamine salt —, oral capsule preparations in a cross over design. Four male fasted beagle dogs with implanted intravenous cannula in cephalic vein for blood samples were administered 5.0 mg/kg SB-497115 (as parent compound). On day 2, each of the dogs was orally administered 5.0 mg/kg of — ethanolamine salt — of the compound in capsule. The blood samples collected at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 1440, 1800, 2880 and 4320 min after dosing for determining the plasma concentrations of the compound by LC/MS//MS method and using non-compartmental analysis. b(4)

Results: The plasma concentrations ($AUC_{0\text{-infin}}$) were 45.3 and 102.7 ug.hr/min respectively after — free acid —) and ethanolamine salt —) administered in capsules, respectively. The corresponding AUC values of the same doses of the compound were 2.3 times greater after ethanolamine salt than free acid capsules. The summary PK data of the compound by b(4)

these routes of administration is shown in the following table (table 3 of the sponsor, vol 3.4, pp 19 of RSD-101TJR/1):

Summary Table of Mean (+SD) Pharmacokinetic Parameters for SB-407115 in Male Beagle dogs Following 5 mg/kg Oral Administration

Formulations	Cmax (ug/ml)	Tmax (min)	$AUC_{(0\text{-}inf)}$ (µg/hr/ml)	AUC Ratio II/I
[redacted] Free Acid [redacted] as capsule I	2978 + 420	143 + 83	2719 + 1517	--
[redacted] ethanolamine salt [redacted] as capsule II	8191 + 2610	83 + 15	6160 + 1682	2.93 + 1.92

b(4)

4. Preliminary Investigations of i.v. PK Parameters and Oral Bioavailability of SB-497115 in the Male Monkey: (Study #SB497115/RSD-101TJL/2/D100817)

Methods and Materials: The study was conducted to estimate the pharmacokinetic parameters and oral bioavailability of the compound in 4 male Cynomolgus monkeys. On day 1, 0.90 mg/kg SB-497115 (batch #NL44560-165A1; 2.0 umol/kg) was administered as an i.v. infusion (solution in 10% PEG 400 and isotonic saline, pH 8.0-8.5 plus 0.37% DMSO). On day 2, each of the monkeys was administered 4.54 mg/kg of the compound by oral gavage (solution in 10% PEG 400 and isotonic saline, pH 8.0-8.5 plus 0.37% DMSO). On day 3, these animals were administered 4.38 mg/kg (10 umol/key) of disodium salt suspension (in 1% methylcellulose; batch #NL44560-168A1) by gavage. The blood samples collected at 0, 15, 30, 45, 90, 120, 180, 240, 360, 480, 600, 1440, 1800 2880 and 4320 min after dosing. The plasma concentrations were analyzed by LC/MS/MS analysis employing positive ion Turbo IonSpray ionization and oral bioavailability was calculated.

Results: The plasma concentrations ($AUC_{0\text{-}infin}$) were 0.28, 1.19 and 1.4 ug.hr/min respectively after intravenous, oral solution and oral suspension, respectively. The clearance rate of intravenously administered compound was low, 3.34 ml/min/kg with terminal half-life of 7.70 hr of intravenously administered compound. The half-life of the orally administered compound was not calculated. The peak plasma concentration (Cmax) of oral solution and oral suspension was similar to the intravenous preparation, i.e., 1.98, 1.94 and 1.81 ug/ml. PK data summary by these routes of administration were shown in table 3 of the sponsor of study #RSD-101TJR/1):

Parameter	Intravenous	Oral Solution	Oral Suspension[a]
Dose (mg/kg)	0.90 ± 0.01	4.54 ± 0.04	4.38 ± 0.04
CL_p (mL/min/kg)	3.34 ± 0.55	b	b
AUC (0-inf) (min•mg/mL)	0.28 ± 0.05	1.19 ± 0.15	1.40 ± 0.16
% Extrap AUC (0-inf)	4.54 ± 4.18	3.40 ± 0.67	3.69 ± 1.15
AUC last (min•mg/mL)	0.26 ± 0.05	1.15 ± 0.14	1.35 ± 0.17
Vss (L/kg)	1.39 ± 0.37	b	b
MRT (min)	424 ± 135	998 ± 56.0	969 ± 104
$T_{1/2}$ (min)	462 ± 153	b	b
Cmax (ug/mL)	1.98 ± 0.23	1.94 ± 0.45	1.81 ± 0.47
Observed Tmax (min)	37.5 ± 19.4	255 ± 76.0	173 ± 126
F (%)	b	88.7 ± 21.1	107.6 ± 26.8

Values are the mean ± SD of n = 4 animals.

[a] Suspension of disodi salt in 1% aqueous methylcelhulose, particle size analysis indicated D_{50} (b)(4)
c

[b] Not calculated for this route of administration.

2.6.4.4 **DISTRIBUTION**

In Vitro:

5. **Preliminary Investigating of Protein Binding to Rat, dog, Monkey and Mouse Plasma Protein by Equilibrium Dialysis and also to determine the blood to plasma Ratio of SB-497115 in Each Species.** (Study #SB497115/RSD-101TJM/1/D100821)

Methods and Materials: The study was conducted to study the binding of the compound to rat, dog, monkey and mouse plasma proteins by equilibrium dialysis. The human, beagle dog and Cynomolgus monkey blood samples were collected by peripheral venipuncture and pooled heparinized samples were used. The mouse and rat plasma samples were purchased. The dialysates were incubated at 37°C for 6 hr. The plasma and buffer samples were assayed by LC/MS/MS analysis; LLQ = 10 ng/ml). The percent bound to plasma was calculated by the formula:
Fb = 100[cdon-Crec)(Vdon/Vinit)/((Cdon-Crec)Vdon-Vinit)+Crec))]
Crec and Cdon in the formula are the concentrations on the receiver and donor sides of the cell, respectively. Vinit and Vdon are the initial and the recovered volumes from the donor side of the cell. The percent of the recovery was calculated and the binding to the membrane was calculated.

Results:

The initial mean concentrations of the compound was >1558 ng/ml with the minimum protein biding was >99%. In mouse, the actual initial concentration was 187 ng/ml; the minimum binding was 93.7%. The plasma protein binding and recovery of SB-497115 is shown in sponsor's table in vol 3.4, pp15 of study #SB497115/RSD-101TJM/1/D100821):

Table of Plasma Protein Binding and Recovery (+SD) Following Equilibrium Dialysis

Species	Mean Plasma Concentration (ng/ml)	Fb %	Recovery (%)
Mouse	187	93.7 ± 0.3	89.6 ± 4.7
Rat	1558	99.2 ± 0.1	80.4 ± 9.6
Dog	3073	99.6 ± 0.1	87.0 ± 16.9
Monkey		99.7 ± 0.0	102± 12.4

Values are the mean of the 3 determinations each assessed in triplicate. No Quantifiable concentrations detected in receiver samples after dialysis, LLQ of the assay was 10 ng/ml.

b. In another preliminary study, the protein binding and blood to plasma ratio of the compound in species of animals was determined. Following equilibrium dialysis, the free drug in the receiving chamber was below the quantitation indicating that the most of the compound at 2000 and 6000 ng/ml concentrations was bound to plasma proteins (the data shown in the table of the study #SB497115/RSD-101TJK/1)

Summary Table of Fraction Bound Drug (Fb) to Plasma Protein and % Blood to Plasma Partitioning

Species	Target Blood/Plasma Concentration (ng/ml)	Fraction Bound to Plasma Proteins (%)	Drug in Plasma (%)
Human	2000	99.4	75.0 +7.3.
	6000	99.8	71.8 + 6.8
Rat	2000	99.4	89.0 + 5.2
	6000	99.8	55.1 + 2.4
Dog	2000	99.1	ND*
	6000	99.1	66.6 + 35.8
Monkey	2000	99.0	79.2 + 6.1
	6000	99.7	73.4 +4.0

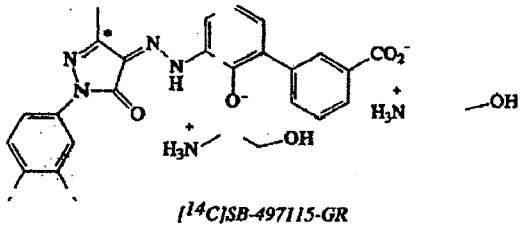
Values are the mean of the 3 determinations each assessed in triplicate. No Quantifiable concentrations detected in receiver samples after dialysis, LLQ of the assay was 10 ng/ml, *= ND not determined..

In Vivo:

6. **Quantitative Tissue Distribution of Drug Related Material Using Whole Body Autoradiography Following 10 mg/kg [^{14}C-SB-497115 to Male Long Evans Rats:** (Study # CD2003/00515/00; PK-897; RSD-101W6L/1)

Methods and Materials: The study was conducted by [redacted] In this study, the tissue distribution of 10 mg/kg [^{14}C]-SB-497115 (bis monoethanolamine salt, batch #SL201723-003A1: 96.8% pure] was determined in 6 Long Evans pigmented male rats weighing between 203 to 212 g. The structural radioactive sites are shown as astrick (*) in the following figure: b(4)

Reviewer: Chopra NDA No. 22-291

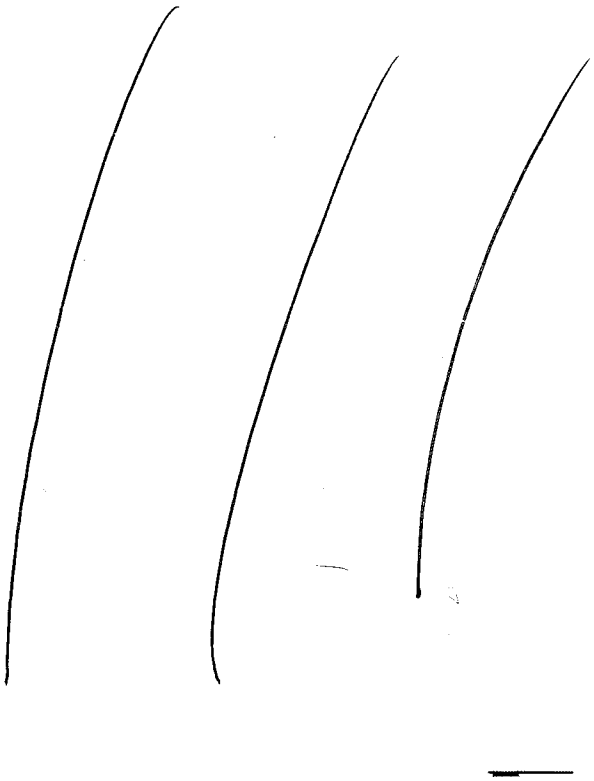


[^{14}C]SB-497115-GR

One rat/time point was killed, i.e., 4, 4, 24 hr and, day 2, 7 and 35 post dose. Each of the rat was embedded in aqueous 2% carboxymethylcellulose wax for quantitative whole body radiography. The selected tissues were exposed to phosphorimaging screen and the radioactivity of the selected tissues was quantified from whole body autoradiograms using imaging analysis. The final solution vehicle was 2% hydroxypropyl methylcellulose with 0.2% sodium lauryl sulfate in water. The quantitation of radioactivity was done by determining the nCi/g of the lowest standard by image analysis and value converted to ug/g of the specific activity of the free acid form of the compound.

Results: The orally administered radioactivity with the compound was widely distributed in male pigmented rats. The maximum amount of radioactivity was seen after 4 hr of administration thereafter in decreased amounts at 8, 24 hr and, day 2. The radioactivity was below the quantitation limits after 2 days of the administration of the compound. At 4-hr observation period, the radio activity counts were (in descending order): small intestine contents, bile, liver, stomach contents, esophageal contents, adrenal cortex, kidney, cecum, small intestines, bulbo-urethral gland, renal medulla, lung, myocardium, salivary gland, preputial glands, brown fat, thyroid, pituitary gland, choroid plexus, diaphragm, testes and prostate as shown in sponsor's scanned radiograph and Table below:

Figure 1 Whole-body autoradiogram of the radioactivity distribution in a male pigmented rat at 4 h after a single oral administration of [^{14}C]SB-497115-GR at a target dose of 10 mg free acid/kg.



b(4)

The whole body radioactivity distribution in pigmented rat tissues is shown below:

Table 2 Concentrations of radioactivity in the tissues of male pigmented rats after a single oral administration of [^{14}C]SB-497115-GR at a target dose of 10 mg free acid/kg.

			Radioactivity Concentration (ug equiv/g)					
Tissue Type	Tissue	Time Point / Animal No.	4 h 1-4	8 h 1-8	24 h 1-24	2 day 1-48	7 day 1-168	35 day 1-840
Vascular/ lymphatic	Blood							
	Lymph nodes							
Excretory/ metabolic	Bile							
	Kidney							
	Liver							
	Renal cortex							
	Renal medulla							
	Urine							
Central nervous system	Cerebellum							
	Cerebrum							
	Choroid plexus							
	Medulla							
	Pineal gland							
	Spinal cord							
Endocrine	Adrenal gland							
	Pituitary gland							
	Thymus							
	Thyroid							
Secretory	Exorbital lacrimal gland							
	Harderian gland							
	Intra-orbital lacrimal gland							
	Salivary gland (submaxillary)							
Fatty	Fat (abdominal)							
	Fat (brown)							
	Fat (subcutaneous)							
Dermal	Skin (non-pigmented)							
	Skin (pigmented)							
Gonads	Bulbo-urethral gland							
	Epididymis							
	Preputial gland							
	Prostate							
	Seminal vesicle							
	Testes							
Muscular	Diaphragm							
	Muscle							
	Myocardium							
Unclassified	Bone							
	Bone marrow							
	Incisor pulp							
	Lung							
	Nasal turbinates							
	Pancreas							
	Spleen							
	Trachea							
	Urinary bladder							

b(4)

Table 2 cont. Concentrations of radioactivity in the tissues of male pigmented rats after a single oral administration of [^{14}C]SB-497115-GR at a target dose of 10 mg free acid/kg.

			Radioactivity Concentration (ug equiv/g)					
		Time Point	4 h	8 h	24 h	2 day	7 day	35 day
Tissue Type	Tissue	Animal No.	1-4	1-8	1-24	1-48	1-168	1-840
Alimentary canal	Cecum							
	Cecum contents							
	Esophageal contents							
	Esophagus							
	Gastric mucosa							
	Large intestinal contents							
	Large intestine							
	Small intestinal contents							
	Small intestine							
	Stomach							
	Stomach contents							
Ocular	Eye							
	Ciliary body/processes							
	Cornea							
	Iris							
	Retina/choroid							

BLQ indicates below limit of quantification (<0.089)
NR indicates not represented.

b(4)

7. In Vitro Investigation of the potential of SB-497115-GR to inhibit P-glycoprotein mediated Transport in MDCKII cells Heterozygously expressing Human P-Glycoprotein: (Study # CD2003/00637/00; 03DMM017)

Methods and Materials: The study was conducted to observe the P-glycoprotein mediated transport of the probe substrate digioxin. The polarized Madin-Darby canine kidney MDCKII-MDRR1 cells heterozygously expressing human glycoprotein (characterized to express glycoprotein) were used. The cell monolayers were used for the transport studies. The transepithelial electrical resistance (TEER) was measured as monolayer cell integrity. The effect of 0, 0.1, 1.0, 3.0, 10.0 30.0, 50, 75 and 100 uM SB497115 on glycoprotein-mediated transport of digioxin was assessed as a bilateral to apical [B→A] transport. The difference of transport with and without digioxin was determined and compared with a potent inhibitor of P-glycoprotein , GF120918A at the concentration of 2 uM. The integrity of the cell monolayer was evaluated by measuring the fluorescence using a ——— fluorescence microplate counter with a soft ware at λ_{ex} 430 nm and λ_{em} 538. The ^{3}H-digioxin transport rate as pmol transported/cm2/h and permeability coefficient for passive membrane transport of lucifer yellow was determined. The mass balance was defined as the percent ratio of the total measured concentration at t90 in donor and receiver cell to initial measured concentration at t10 in the donor working solution.

b(4)

Results:

The radioactivity of digioxin was 99.5% with no single impurity accounting for — of the detected radioactivity. The transport rate was decreased significantly by 2.0 uM GF120918 but the mass balance was not affected by the control drug. SB-497115 from 0.1 uM to 100 uM concentrations did not decrease the transport rate at the selected concentrations rather a slight non-concentration dependent increase was observed (see sponsor table 1, #CD2003/00637/00; pp 13). No inhibition was observed by the selected SB-497115 concentrations.

b(4)

Table 1 **Effect of SB-497115 on Human P-glycoprotein Mediated Transport of 30 nM [^{3}H]-digoxin using MDCKII-MDR1 Cells**

Treatment	Transport Rate (pmoles/cm²/h) ± SD	Transport Rate (% control) ± SD	Mass Balance (%) ± SD
Digoxin only (control)	1.29 ± 0.175	100	82.3 ± 0.83
Digoxin + 2.0 µM GF120918	0.25 ± 0.04	19.5 ± 3.02	81.4 ± 1.15
Digoxin + 0.1 µM SB-497115	1.11 ± 0.257	86.4 ± 19.9	90.6 ± 1.25
Digoxin + 1.0 µM SB-497115	1.38 ± 0.058	107 ± 4.51	92.6 ± 2.19
Digoxin + 3.0 µM SB-497115	1.56 ± 0.128	121 ± 9.91	96.5 ± 3.19
Digoxin +10.0 µM SB-497115[1]	1.38	106	96.9
Digoxin + 30.0 µM SB-497115	1.74 ± 0.176	135 ± 13.6	99.0 ± 2.91
Digoxin + 50.0 µM SB-497115	1.74 ± 0.304	135 ± 23.5	97.7 ± 2.11
Digoxin + 75.0 µM SB-497115	1.47 ± 0.155	114 ± 11.9	96.3 ± 1.76
Digoxin + 100 µM SB-497115	1.51 ± 0.20	116 ± 15.3	94.2 ± 2.39

Data are the mean of values (± SD) obtained from three wells.
1. 1. Data are the mean of values obtained from two wells.

8. In Vitro Investigation of Transport via Human P-Glycoprotein and the Passive membrane Permeability of ^{14}C-SB-497115-GR in MDCKII cells: (Study # CD2003/01046/00; 03DMM077)

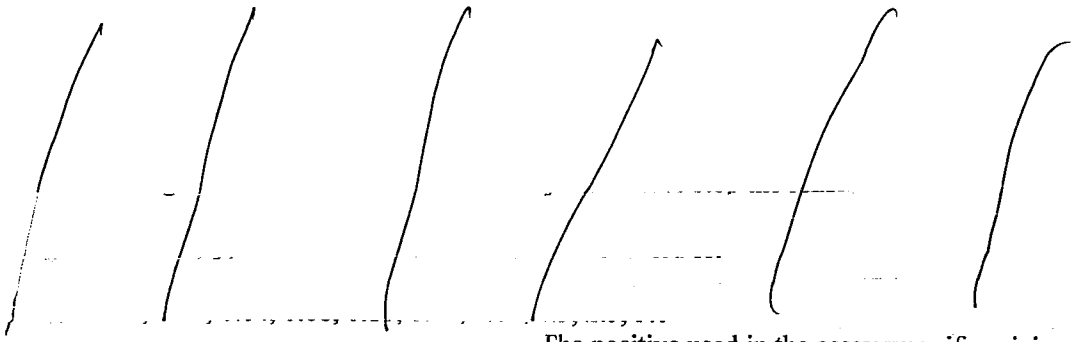
In this test, the transport of the compound across confluent monolayers of Madin Darby canine kidney type II cells over expressing the human MDR1 gene that expresses P-human glycoprotein were used in the presence and absence of Pgp inhibitor GF120918. The transport of ^{14}C-SB-497115 (purity 94.8% pure) at the target concentration of 3 uM in 2 directions (apical to basolateral [A→B] and basolateral to apical [B→A] was used in the presence and absence of GF120918A. Amprenavir (radioactive and non radioactive) was used as used as a positive control in the study. The apical efflux with the test compound and positive control were 1.0 and 28.0, respectively. The results indicated that the test was performed in the optimal test conditions and SB-497115-GR at 7 uM did not affect the passive permeability across membrane barrier and the compound was not P-gp substrate.

9. In Vitro Investigation of Human and Rat PXR activation:
(Study #C002003/00721/00; 03DMM042)

Reviewer: Chopra NDA No. 22-291

The study was undertaken to determine the effect of the compound on the activation of human or rat nuclear hormone receptor (PXR) activation using human hepatoma cell (HuH) line transiently transfected with full length human or rat PXR.

Methods and Materials:



b(4)

The positive used in the assay was rifampicin (human PXR) or pregnenolone 16-α-carbonitrile (PCN for rat PXR) and the activation of positive control measured in 4 wells and the dose-response curve of the compound formed. On day 4, the solution of the wells was assayed for SPAP activity and the cells were assayed for β-glactosidase. The activation of PXR by the compound as the percent and as folds determined. The EC_{50} (half maximal effective concentration) for positive control was determined. Activation of PXR by SB-497115 was expressed as percent of negative control.

Results:
The maximum response for rifampicin was 119% with an EC_{50} of 3.0 uM. The maximum response to rat PXR positive control (PCN) was 3%. The response was not statistical or treatment related. The compound was stated to have no significant effect on human or rat nuclear receptors. The compound was a weak activator of human PXR and did not activate the rat PXR. The lack of activation of PXR was indicative of insignificant effect on the induction of CYP3A.

Table 1 *In Vitro* Inhibition of CYP450 Enzymes in Human Liver Microsomes by SB-497115

CYP450 Enzyme	Activity Measured	Metabolism-Independent IC_{50} (μM)[a]	Metabolism-Dependent[b]
CYP1A2	Phenacetin O-deethylation	>100	No effect
CYP2A6	Coumarin 7-hydroxylation	>100	No effect
CYP2C8	Paclitaxel 6α-hydroxylation	24.8	No effect
CYP2C9	Diclofenac 4'-hydroxylation	20.2	No effect
CYP2C19	S-Mephenytoin 4'-hydroxylation	>100	No effect
CYP2D6	Bufuralol 1'-hydroxylation	>100	No effect
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	No effect
CYP3A4/5	Lovastatin 6β-hydroxylation	>100	No effect
CYP3A4/5	Midazolam 1'-hydroxylation	>100	No effect
CYP3A4/5	Nifedipine oxidation	>100	No effect
CYP3A4/5	Testosterone 6β-hydroxylation	>100	No effect
CYP4A9/11	Lauric acid 12-hydroxylation	>100	No effect

Note: Values were calculated using the average data obtained from duplicates for each incubation condition. The IC_{50} values were calculated using Microsoft Excel and SigmaPlot. Since no notable inhibition was observed for any CYP450 enzyme activity, Ki was not determined.

a: Evaluated using a pooled microsomal sample.

b: Evaluated using one pooled and two individual microsomal samples (one containing low and one containing high enzyme activity).

Metabolism

10. **In Vitro Effects on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Female rats following oral Administration of 0, 100, 300 and 1000 mg/kg/day SB-497115 for 4 days:** (Study #497115/RSD-101PK2/1; CMS 33045B1/6146-232)

The study was conducted in accordance with GLP by [redacted] to determine the effect of the compound on hepatic P-4550 microsomal enzymes (CYP1A2, CYP2B, CYP2E, CYP3A and CYP4A) in female rats after 4 days of treatment with SB-497115. b(4)

Methods and Materials:
Animals: Eleven female rats were divided in to 4 groups and were treated with 0, 100, 300 and 1000 mg/kg/day for 4 days. The liver of each of these animals was separated at necropsy and processed in liquid nitrogen and stored at -60°C till total protein cytochrome P450 content and enzymes activities test was performed. About 28 to 30% liver sample was suspended in Tris buffer containing sucrose (pH 7.0) and homogenized. Supernatant was separated by centrifugation, and the pellet was homogenized and microsomal suspended in phosphate buffer and homogenized and aliquots dispensed in vials and stored at –70°C. The total cytochrome P450 concentration in microsomal fractions was determined at 400 to 500 nm. The total cytochrome activity was calculated using the absorbance difference between 450 and 490 nm and the molar extinction coefficient of 104 $mM^{-1}cm^{-1}$. The ethoxyresorufin O-deethylase activity (EROD), testosterone hydroxylase and lauric acid 11-hydroxylase activities in the microsome preparations were determined. The radiolabeled testosterone metabolites were measured by HPLC analysis and the retention times of the metabolites recorded.

Results:

The total cytochrome P450 protein and total cytochrome P450 liver activity were increased in a dose-related manner. The cytochrome P450 protein was increased by 131, 166 and 249% and liver cytochrome P450 activity was increased by 125, 134 and 264% in rats included in 0, 100, 300 and 1000 mg/kg/day SB-497115 treatment groups.

The ethoxyresorufin O-deethylase liver activities (EROD) were affected insignificantly, i.e., 104, 74 and 101%. No significant activities were seen total protein and P450 contents as shown in the following table (extracted from sponsor's table 1, 2 and 3).

Dose Levels	Protein Content	Total Cytochrome P450 (nmol/mg)	Total Cytochrome P450 (nmol/g liver)
0	19.5	0.430	8.42
100	18.6 (95.%)	0.565 (131%)	10.5 (125%)
300	15.9(82%)	0.715 (166%)	11.3 (134%)

1000	20.7(106%)	1.07 (249%)	22.2 (264%)

The lauric acid 11-hydroxylase and lauric acid 12-hydroxylase activities were also not affected by SB-497115 administration in rats. SB-497115 up to 1000 mg/kg/day for 4 days did not alter the microsomal enzyme activities.

11. **The Effect on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female rats following oral Administration of 0, 3, 10 and 40 mg/kg/day SB-497115 for 14 days**: (Study #SB-497115/RSD-101TKB/1; CMS 39792B/7274-151)

The study was conducted at _____ in accordance with GLP to determine the effect of 0, 3, 10 and 40 mg/kg/day SB-497115 treatment in male and female rats for 14 days on hepatic microsomal total cytochrome P450 concentrations and activities. The liver enzyme analysis was done at _____ b(4)

Methods and Materials:
Four groups of rats (3/sex/group) were treated with the oral doses of 0, 3, 10 and 40 mg/kg/day for 14 days. After 24 hr of the administration of the last dose, the liver of each of these animals was separated at necropsy and processed in liquid nitrogen and stored at -60°C till total protein cytochrome P450 content and enzymes activities was performed. 28 to 30% of the liver sample was suspended in Tris buffer containing sucrose (pH 7.0) and homogenized. Supernatant was separated by centrifugation and the pellet was used to estimate the total cytochrome P450 enzymes concentrations. The activities, ethoxyresorufin O-deethylase activity (EROD), testosterone hydroxylase and lauric acid 11-hydroxylase activities in the microsome preparations determined. The radio labeled testosterone metabolites were also measured by the similar HPLC analysis.

Results:

The total cytochrome P450 protein and total cytochrome P450 as per liver weight were increased only slightly which was of no clinical relevance. The cytochrome P450 protein was increased by 117, 112 and 109% in males and 118, 122 and 100% in females of 3, 10 and 40 mg/kg/day treatment groups. The liver cytochrome P450 protein contents were increased slightly by 116, 118 and 125% in males and, 108, 107 and 120% in female rats included in 3, 10 and 40 mg/kg/day SB-497115 treatment groups. The increase of total cytochrome P450 protein was small and similar in both male and female rats. These were not of clinical/statistical significance. The effect of the compound on total protein and P450 contents are shown in the following table (extracted from sponsor's vol 3:6 # SB-497115/RSD-101TKB/1, pp 24-25, table 1 and 2).

Dose Levels (mg/kg/day)	Protein Content	Total Cytochrome P450 (nmol/mg)	Total Cytochrome P450 (nmol/g liver)
Males			
0	18.8	0.618	11.8

3	22.1 (117%)	0.719 (116%)	15.9 (134%)
10	21.1 (112%)	0.727 (118%)	15.3 (129%)
40	20.6 (106%)	0.771 (125%)	15.9 (134%)
Females			
0	13.3	0.549	7.28
3	15.7 (118%)	0.593 (108%)	9.37 (129%)
10	16.3 (122%)	0.586 (107%)	9.54 (131%)
40	13.4 (100%)	0.0.66 (120%)	8.77 (121%)

The ethoxyresorufin O-deethylase liver activity (EROD) was increased in an insignificant amounts, i.e., 139, 121 and 148% of the controls in males and, 111, 111 and 97% of the controls in females. The lauric acid 11-hydroxylase activity and lauric acid 12-hydroxylase activities were also not affected by SB-497115 administration in rats.

SB-497115 up to 40 mg/kg/day for 4 days did not alter the microsomal enzyme activities.

12. **The Effect on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female Dogs following oral Administration of 0, 3, 10 and 40 mg/kg/day SB-497115 for 14 days:** (Study #SB-497115/RSD-101XHX/1; CMS 39792A/7274-150)

Methods and Materials:

b(4)

The study conducted in accordance with GLP by _____ was to evaluate the effects of the compound on hepatic microsomal total cytochrome P450 contents and activities of CYP1A, CYP3A, CYP2B, CYP2E and CYP4A. The activities of these enzymes were measured as the activities of ethoxyresorufin O-deethylase activity (EROD), testosterone 6β-hydroxylase, testosterone 16β-hydroxylase and lauric acid 11-hydroxylase activities in the microsome preparations. Four groups of dogs (3/sex/group) were treated with the oral doses of 0, 3, 10 and 30 mg/kg/day for 14 days. After 24 hr of the administration of the last dose, the liver of each of these animals was separated at necropsy and processed in liquid nitrogen and stored at -60°C till total protein cytochrome P450 content and enzymes activities was performed.
Results:

The total cytochrome P450 protein and total cytochrome P450 as per liver weight were increased only slightly which was of no clinical relevance. The cytochrome P450 protein content was 96, 95 and 126% in males and 114, 116 and 141% in females of 3, 10 and 30 mg/kg/day treatment groups. The total cytochrome P450 liver was increased slightly in high dose treatment group dogs by 107, 108 and 150% in males and, 121, 136 and 181% in female dogs included in 3, 10 and 30 mg/kg/day SB-497115 treatment groups. The slight increase of total cytochrome P450 protein was similar in male and females. These were not of any clinical significance.

The ethoxyresorufin O-deethylase (EROD) liver production was reduced in an insignificant amount, i.e., 86, 75 and 93% of the controls in males and, 58, 93 and 143% of the controls in females. The

effect of the compound on total protein and P450 contents are shown in the following table (prepared from data of sponsor's table in the submission).

Dose Levels	Protein Content	Total Cytochrome P450 (nmol/mg)	Total Cytochrome P450 (nmol/g liver)
Males			
0	20.3	0.554	11.2
3	22.7 (111%)	0.529 (96%)	12.0 (107%)
10	23.1 (113%)	0.523 (95%)	12.1 (108%)
30	24.3 (119%)	0.695 (126%)	16.8 (150%)
Females			
0	20.4	0.507	10.3
3	21 4 (105%)	0.577 (114%)	12.4 (121%)
10	23.9 (117%)	0.587 (116%)	14.0 (136%)
30	25.5 (125%)	0.717 (141%)	18.6 (181%)

The lauric acid 11-hydroxylase activity and lauric acid 12-hydroxylase activities were also affected in insignificant manner by SB-497115 administration in dogs. SB-497115 up to 30 mg/kg/day for 14 days did not alter the microsomal enzyme activities in dogs.

13. **An in Vitro Evaluation of the Inhibitory Potential of SB-497115 on Human Cytochrome P450 Enzymes:** (Study #CD2003/00990/00/7274-290)

Methods and Materials:

The study was conducted in accordance with Japanese GLP by [redacted] b(4)
, to evaluate if the compound had inhibitory effects on the major hepatic microsomal total cytochrome P450 enzymes. The activities of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A9/11 were measured in the presence and absence of the compound. The IC50 values for each enzyme activity was measured at the target concentrations of specific probe (equal to Km) and the test was repeated in the presence of 0, 0.1, 0.3, 1, 3, 10 30 and 100 uM SB-497115. The probe substrate was added and the inhibitory activities of the human CYP450 enzymes in the metabolism-independent medium in the presence of SB-497115 were determined initially. In the second part of the assay, SB-497115 was evaluated as a metabolism-dependent inhibitor of CYP450 enzymes by using human liver microsomes (pooled and microsomes of 2 individuals). The concentration of SB-497115 causing 30% inhibition of the enzyme activity was selected. The inhibitory activity of CYP450 enzymes was determined and if the 50% inhibition was not achieved at the highest concentration, IC_{50} was as >100 uM.

Results:

SB-497115 showed an inhibition of CYP2C8 and CYP2C9 with an IC_{50}s of 24.8 and 20.2 uM. It did not show any effect on the other microsomal enzyme systems in metabolism dependent medium and the IC50 of the enzymes were >100uM. Similarly, the IC_{50} was not established in metabolism-

dependent inhibition medium. The results are shown in the following table of the sponsor (vol 3.5, pp 22 of CD2003/00990/00):

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Table 1 *In Vitro* **Inhibition of CYP450 Enzymes in Human Liver Microsomes by SB-497115**

CYP450 Enzyme	Activity Measured	Metabolism-Independent: IC_{50} (μM)[a]	Metabolism-Dependent[b]
CYP1A2	Phenacetin O-deethylation	>100	No effect
CYP2A6	Coumarin 7-hydroxylation	>100	No effect
CYP2C8	Paclitaxel 6α-hydroxylation	24.8	No effect
CYP2C9	Diclofenac 4'-hydroxylation	20.2	No effect
CYP2C19	S-Mephenytoin 4'-hydroxylation	>100	No effect
CYP2D6	Bufuralol 1'-hydroxylation	>100	No effect
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	No effect
CYP3A4/5	Lovastatin 6β-hydroxylation	>100	No effect
CYP3A4/5	Midazolam 1'-hydroxylation	>100	No effect
CYP3A4/5	Nifedipine oxidation	>100	No effect
CYP3A4/5	Testosterone 6β-hydroxylation	>100	No effect
CYP4A9/11	Lauric acid 12-hydroxylation	>100	No effect

Note: Values were calculated using the average data obtained from duplicates for each incubation condition. The IC_{50} values were calculated using Microsoft Excel and SigmaPlot. Since no notable inhibition was observed for any CYP450 enzyme activity, Ki was not determined.

a: Evaluated using a pooled microsomal sample.

b: Evaluated using one pooled and two individual microsomal samples (one containing low and one containing high enzyme activity).

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14. **Preliminary in Vitro Investigating of Metabolism of ^{14}C-SB-497115 in Rat, dog, Cynomolgus Monkey and Man:** (Study #SB497115/RSD-101TJJ/1/D100903)

Methods and Materials: The study was conducted at the Sponsor's facility to study the preliminary metabolism of ^{14}C-SB-497115 (lot # SL45241-199A1, sp. Activity 16.9 mCi/mmol, purity 96.4%) in rat, dog, monkey and human liver hepatocytes in an vitro system. The rat ——— , human, beagle dog and cynolmolgus monkey hepatocytes were freshly prepared for the study. The cells were suspended and pellets after centrifugation was again suspended. The hepatocytes with optimal viability (determined in the test) were used in the study. The solution at the concentration of 10 or 50 uM was used in the study. After incubation of the hepatocytes with SB497115 for 0, 6 and 24 hr, the reaction was stopped and cells scrapped. These cells were incubated with 6-ethoxycoumarin for 0, 0.5, 1, 1.5, 2, 4 and 6 hr. The recovery of radioactivity was also determined. The radio metabolite profile was determined by on line radio detection using ——— radiodetector and HPLC.

b(4)

b(4)

Results:

Reviewer: Chopra NDA No. 22-291

The mean radioactivity recovery was 85.1, 65.1, 82.3 and 70.0% for the human, rat, dog and monkey hepatocytes, respectively. The low recovery of the compound from the hepatocytes was due to the chelation of SB-497115 to hepatocytes as an EDTA extraction technique increased the recovery up to 102.5%. On HPLC separation, 9, 8, 9 and 9 peaks were detected in human, dog, rat and monkey hepatocytes, respectively. These peaks were identified as peak A to N with the retention time as shown below (sponsor's table 1 of Study #SB497115/RSD-101TJJ, page 20).

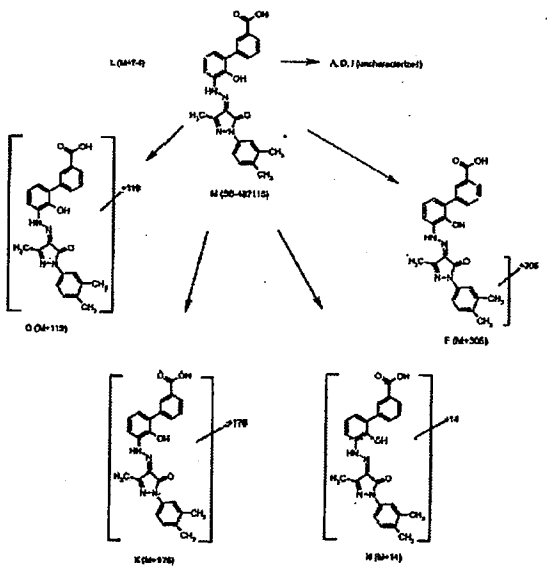
Table 1 Summary of the radio-HPLC and LC/MS analyses of hepatocytes incubated with [^{14}C]SB-497115

Peak ID	Molecular ion[1]	Inference/ Structure	Human #1 10 uM 24 h	Dog 50 uM 24 h	Rat 50 uM 24 h	Monkey 50 uM 24 h
A[2]	unknown	unknown	2.4	ND	ND	ND
B	unknown	unknown	ND	ND	17.4	17.5
C	unknown	unknown	ND	ND	18.4	18.4
D	unknown	unknown	22.6	23.4	23.0	ND
E	unknown	unknown	ND	ND	ND	24.3
F	748	+305	24.4	25.3	25.1	25.1
G	562	+119	26.0	26.2	25.5	25.5
H	473	+30	ND	ND	ND	26.3
I	unknown	unknown	27.2	27.1	ND	ND
J	459	+16	ND	29.4	28.4	ND
K[3]	619	+176	30.2	30.4	30.1	30.1
L	517	+74	32.6	33.3	ND	ND
M	443	SB-497115	34.2	34.6	34.1	34.1
N	457	+14	37.2	ND	37.1	37.1

Retention times for peaks from radio-HPLC analyses; ND = Peak not detected by radio-HPLC or LC/MS.
1. $[M+H]^+$
2. Metabolite A was observed in rat, monkey and dog hepatocytes when incubated with 10 uM of SB-497115 for 24 hour.
3. The peak contained at least three isomers of M+176, and coeluted with at least one M+30 metabolite. The M+176 metabolites were prominent components based on MS response.

The metabolites detected were quantitatively different in the different species as seen in the above table. Based on the finding that all the human hepatocytes preparations formed the similar metabolites, the metabolic profile of the compound was independent of its concentration. The route of metabolism was predominantly by conjugation with cysteine (metabolite G) and glucuronide (metabolite K). The metabolite F was identified as glutathione conjugation and formation of M+14 product (metabolite N) were considered as minor metabolites. The proposed metabolic pathway as proposed by sponsor is shown below:

Proposed metabolic scheme for SB-497115 in human hepatocyte incubates



The metabolic profile of SB-497115 in rat, dog and monkey hepatocytes was qualitatively similar to human. A hydroxylation product (metabolite B and C in rat and monkey and metabolite E and H in monkey) were also detected. Metabolite H was seen only in monkey hepatocyte preparation. Thus conjugation and glucuronidation were the major routes of metabolism in human hepatocytes and no specific human metabolite was detected.

15. Metabolism of ^{14}C-SB-497115 in Rat: (Study #CD-2004/00068/00/D102031)

Methods and Materials: The study was conducted to identify and quantify the major metabolites of SB-497115 in the pooled urine, bile, feces, liver and plasma samples of intact and bile duct cannulated rats. The metabolites were analyzed by HPLC and structural characterization was conducted on selected samples by mass spectrometry (HPLC-MS and HPLC-MS-MS). The bile metabolites were structurally identified by NMR. The animals were treated at the oral dose of 10 mg/kg ^{14}C-SB-497115 (a NOEL in a 14-day toxicity study in rats (CD2003/00252/00). The study was conducted in 3 intact rats and 3 bile duct cannulated rats. The plasma, and dose formulations were stored at –70oC and all other biological samples (urine and feces) were stored at –20oC. The samples were pooled by weight ratio based on percent of dose excreted in each collection of matrix. Liver and plasma samples were pooled as shown in the following table (sponsor's table on p 11 of the study.

Species	Route	Status	Animal no.	Time period (h) over which samples were pooled		
				Urine	Bile	Feces
Rat	Oral	Intact[1]	1	0-48	NS	0-72
			2	0-48	NS	0-72
			3	0-24	NS	0-72
Rat	Oral	BDC	1	0-12	0-72	0-48
			2	0-12	0-72	0-48
			3	0-12	0-72	0-48

NS= No sample
1. The same pooling scheme was used for both males and females

The samples were analyzed in triplicate and aliquots of fecal and liver samples were weighed and allowed to dry overnight. Combustaid (200 ul) was added in the samples and combusted and the product analyzed by LSC. The liver and plasma samples were also pooled, combusted and analyzed by LSC to determine the liver radioactivity. The bile and fecal matter were pooled, combusted and analyzed by HPLC. The fecal extract was analyzed by off-line radio-HPLC. Each time, the control ^{14}C-SB-497115 was used to spike the system. The radio-metabolite profile was determined by appropriate aliquots of plasma, fecal etc samples by radio labeled HPLC. The metabolites were isolated by the procedures described above in method section of the study.

Results:

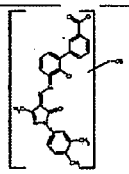
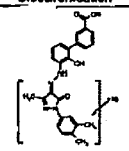
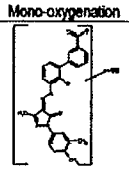
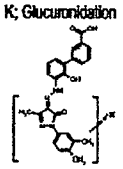
The orally administered SB-497115 was excreted in urine and feces in 6.6 and 88.0% in male and 4.9 and 90.6% in female treated with 10 mg/kg. The secretion in bile in BDC animals was 42.6% and excretion was 8.9 and 40.2% in urine and feces. The metabolites were detected and identified by their retention time and structures confirmed by NMR. The metabolites were designated by alphabetic letters. The mean radioactivity recovery from pooled plasma samples in female rats was 96, 89 and 88% at 4, 24 and 48 hr, respectively. The trichloracetic acid and 1:4 (v:v) diethyl ether:methanol resulted in additional recovery of 3, 9 and 2% plasma radioactivity at 4, 24 and 48 hr, respectively giving the total recovery of 100, 100 and 96%.

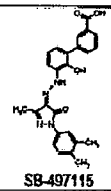
Plasma:
At 4-hr post dosing, the unchanged compound was main peak 89 and 76% in male and female animals. The 4 metabolites (D, J, and K/O) in male and, 4 metabolites (D, U, K/O and AD) in female rats were identified. Metabolite O and K eluted together in 2:1 ratio, i.e., 6 and 2% of the total activity in the male and female animals (1.7 and 0.5 ug/g). Metabolite K was identified as a glucuronide, metabolite O was an oxidation product, metabolite J was a mono-oxygenation (0.4 ug/g in the male rat and not detected in females), Metabolite U (2% or 0.7 ug/g of plasma protein a glucuronide of SB497115) was detected in plasma. Metabolite AD accounted for approximately 13% of the plasma radioactivity, i.e., 3.6 ug/g in female). Metabolite D accounted for 1% (0.3 ug/g) of plasma radioactivity in both sexes. Metabolites A and AD are not identified.

After 24 hr dosing, the levels of the unchanged compound radioactivity changed to 42 and 58% in male and female animals. This amounts to 1.1 ug equivalents/g for both sexes. Metabolite K/O was present in 33 and 22% of the plasma activity in male and female animals (0.1 and 0.2 ug

equivalents/g). The metabolite K/O, AD and unchanged compound was reduced further and 16 and 10% of the plasma activity in male and female animals (0.1 ug equivalents/g of each). The structures of the metabolites are shown in the table below:

Table 1 **Quantification of Radio-Metabolites in Pooled Plasma Extracts Following a Single Oral Administration of [^{14}C]-SB-497115-GR to Intact Rats at a Nominal Dose Level of 10 mg/kg**

Peak ID	Metabolite Structure	Male			Female		
		4 h	24 h	48 h	4 h	24 h	48 h
		% Plasma Radioactivity[1] (*µg equivalent SB-497115/g*)[2]					
D	Unknown	0.83 *(0.22)*	ND *(ND)*	ND *(ND)*	1.15 *(0.32)*	ND *(ND)*	ND *(ND)*
U	 Glucuronidation	ND *(ND)*	ND *(ND)*	ND *(ND)*	2.42 *(0.66)*	ND *(ND)*	ND *(ND)*
J	 Mono-oxygenation	1.48 *(0.39)*	ND *(ND)*	ND *(ND)*	ND *(ND)*	ND *(ND)*	ND *(ND)*
K/O[3]	 K; Glucuronidation  O; Oxidation	6.32 *(1.68)*	32.96 *(0.84)*	15.86 *(0.14)*	1.92 *(0.53)*	21.47 *(0.40)*	NQ *(NQ)*

Peak ID	Metabolite Structure	Male 4 h	Male 24 h	Male 48 h	Female 4 h	Female 24 h	Female 48 h
		% Plasma Radioactivity[1] (µg equivalent SB-497115/g)[2]					
M	 SB-497115	88.79 (23.66)	41.80 (1.07)	58.68 (0.50)	76.71 (20.79)	57.66 (1.07)	78.17 (0.27)
AD	Unknown	ND (ND)	5.53 (0.14)	8.47 (0.08)	13.05 (3.58)	8.36 (0.16)	NQ (NQ)
Total [1,2]		97.4 (26.0)	89.3 (2.1)	84.0 (0.7)	94.2 (25.8)	67.4 (1.6)	78.2 (0.3)
Concentration [3]		27.1	2.3	0.8	27.4	1.7	0.3
% Overall recovery [4]		101.7	89.8	95.6	99.8	91.7	90.8

NQ - Non-quantifiable, ND - Not detected, NA – Not applicable
* Metabolites K and O co-eluted in approximately 2:1 ratio (based on MS response)
1. Percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery
2. Expressed as µg equivalents per g of plasma.
3. Total radioactivity concentration in plasma (data from excretion study).
4. Recovery of radioactivity following ethyl alcohol:acetonitrile:EDTA extraction, evaporation and re-constitution of plasma samples

Bile:

The radioactivity from the rat bile samples in 0-72 hr samples was equated to be 41% of the compound administered which was 97% of the compound excreted in the bile. The unchanged SB-497115 (M) was the predominant component in the pooled bile and was approximately 21% of the total biliary radioactivity.
The amount of metabolite K/O and K were 24 and 11% of biliary radioactivity (10 and 4.7% of the dose). The bile metabolite K and O were identified as glucuronide and oxidation products. Metabolite F, a glutathione conjugate was present in bile and 11 other metabolites (X, Y, B, C, AA, AB, D, Z, R, S and T) were isolated from bile duct cannulated rats. The chief metabolites were glucuronidation metabolite K and O (oxidation metabolites in 23.9% of the radioactivity) and glutathione conjugation metabolite F (in 11.3% of the radioactivity). The other minor metabolites of the unidentified structures were (% of the metabolite present in bile): C (5.2%), D (4.9%), B (3.7%), T (2.4%), Z (2.0), R (1.9%), Y (1.6 %), X (1.2%), AA (1.2%), AB (0.6%) and unknown S (1.3%).

Feces:

The mean recovery of radioactivity in feces was 50.2 and 53.3% in male and female rats and the % mean dose excreted in feces was 88.0 and 90.6% in male and female rats. In bile duct cannulated rats, 65.7% of the radioactivity was excreted in feces and 63.3% was the unchanged compound. The other metabolites detected in feces were metabolite D, U, J, K/O and unchanged substance (M).

Peak ID	Bile			
	Male	Female	BDC	
D	4.2	3.1	2.3	4.9
U	6.2	2.1	ND	3.8
J	NQ	1.1	NQ	1.9
K/O	1.6	1.1	NQ	23.9
M (Unchanged compd.)	38.2	45.9	63.3	20.8
Total	50.2	53.3	65.7	89.1

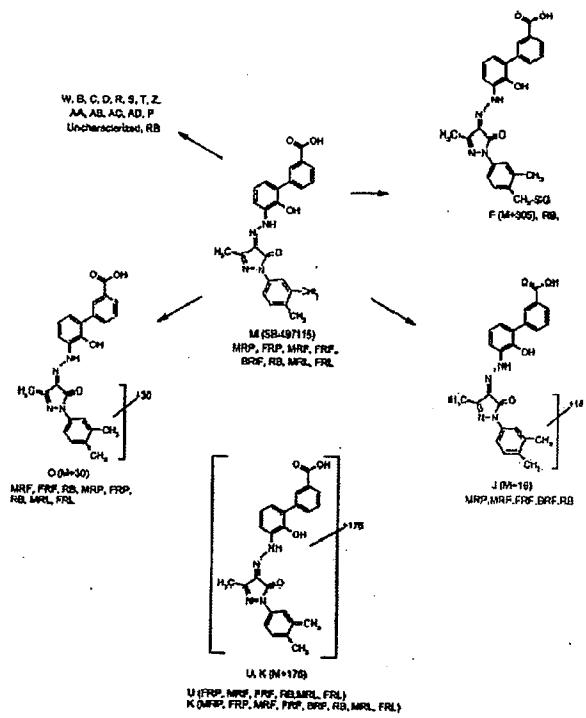
The unchanged compound was still the major component in male and female animals, which was 59 and 78% of the plasma radioactivity (0.5 and 0.3 ug equivalents/g). On HPLC separation, 9, 8, 9 and 9 peaks were detected in human, dog, rat and monkey hepatocytes, respectively. These peaks were identified as peak A to W with the retention time as shown in the table 1 (sponsor's table of Study #SB497115/RSD-101TJJ, page 20).

The metabolites detected were quantitatively different in the different species as seen in the above table. Based on the finding that all the human hepatocytes preparations formed similar metabolites, the concentrations of the compound will not affect the metabolic profile. The route of metabolism was predominantly by conjugation and with cysteine (metabolite G) and glucuronide (metabolite K) were detected. The other metabolite F was identified as glutathione conjugation (metabolite F) and formation of M+14 product (metabolite N) were considered as minor metabolites. The sponsor's proposed metabolic pathway is shown below, figure 1, of the study CD2004/00068/00/D102031.

Liver Extracts: At 48 hr, the radio metabolites in extracts of liver pooled homogenates after a single oral dose of 10 mg/kg in intact rats were glucuronide and oxidized metabolites K/O, an unknown metabolite P, metabolite M (unchanged compound) and another unknown compound AD in 28.71, NQ, 35.6% in males and, in 25.34, 3.28, 30.01% in female, respectively.

Sponsor putative metabolic pathway of the compound is shown below:

Figure 1 Putative Metabolic Scheme for SB-497115 in the Rat



MRP = male rat plasma; FRP = female rat plasma; MRF = male rat feces; FRF = female rat feces; BRF = BDC rat feces; RB = rat bile, MRL = male rat liver, FRL = female rat liver

16. **Metabolism of SB-497115 in Rabbits:** (Study# CD2004/00830/00/03DMM055)

Methods and Materials: The study was conducted at the Sponsor's facility from August 23, 2003 to January 4, 2004 to identify and quantify the major metabolites of SB-497115 in the pooled urine, bile, feces, liver and plasma samples of intact and bile duct cannulated rabbits. The metabolites were analyzed by HPLC and structural characterization was conducted on selected samples by mass spectrometry (HPLC-MS and HPLC-MS-MS). The bile metabolites were structurally identified by NMR. The animals _____ were treated at the oral dose of 10 mg/kg ^{14}C-SB-497115. The study was conducted in 3 intact rats and 1 bile duct cannulated animals. The urine samples from intact rabbit were collected from 0-48 hr as shown in the following sponsor's table: b(4)

Species	Route	Status	Animal #	Time Period (h) for pooled Samples

Rabbit	Oral	Intact Male/female	1 2	0-48/0-12,24-48 0-12, 24-48/0-24	0-48/0-48 0-48/0/48
Rabbit	Oral	BDC	1	NS	NS

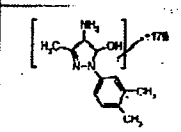
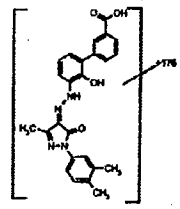
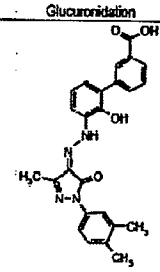
The samples were weighed and analyzed in triplicate and, the aliquots of fecal and liver extracts were allowed to dry overnight. Combust aid (200 ul) was added in the samples and combusted and the product analyzed by LSC. The plasma samples were pooled, combusted and analyzed by LSC to determine the liver radioactivity. The bile (0-4 hr sample), urine and fecal matter (0-24 hr sample) were pooled, combusted, extracted with 10 volumes of 3:1 dimethylformamide (DMF):0.1M ammonium sulfate at room temperature and extract was centrifuged and the extract was used to find the radioactivity. The fecal extract was analyzed by off-line radio-HPLC. The radio-metabolite profile was determined by appropriate aliquots of plasma, fecal etc samples by radio labeled HPLC. The metabolites were isolated by the procedures described above in method section of the study.

Results:

The plasma radioactivity of the orally administered 10 mg/kg SB-497115 in male rabbit was 79, 57 and 38% at 4, 24 and 48 hr sampling period. The plasma radioactivity of female rabbit was 68, 48 and 23% at 4, 24 and 48 hr sampling period. An additional 4 and 0% radioactivity in male and, 8 and 7% radioactivity in female was seen in 4 and 24 hr sample but no radioactivity was seen in samples collected at 48 hr sampling time. The excretion in bile of cannulated animals was based on the data of the 1 animal, as the bile in the other animal could not be obtained. The metabolites in male and female were similar and these were identified by their retention time.

Plasma:
At 4-hr post dosing, the main peak was of unchanged compound and consisted of 10 and 14% in male and female animals. The broad peak of AE (identified as pair cleavage glucuronide products) 38 and 26% in male and female. The metabolite K (glucuronide of the parent compound) 9 and 14% of male and female rabbits. Metabolite A of unknown identity was seen as a chief product at 24 hr sampling in 11 and 15% in male and female animals. Metabolite K was in an insignificant amount at this time. The structures of the metabolites are shown in the table below:

Table 1 **Quantification of Radio-metabolites in Extracts of Pooled Plasma following a Single Oral Administration of [^{14}C]SB-497115-GR to Intact Rabbits at a Nominal Dose Level of 10 mg/kg**

Peak ID	Proposed Metabolite Structure	Male			Female		
		4 h	24 h	48 h	4 h	24 h	48 h
		% Plasma Radioactivity[1] (ug equivalent SB-497115/g)[2]					
A	Unknown	NQ (NQ)	10.82 (0.01)	NA (NA)	NQ (NQ)	15.05 (0.03)	NA (NA)
AE[3]	 Cleavage, reduction and glucuronidation	37.80 (0.13)	ND (ND)	NA (NA)	25.48 (0.16)	ND (ND)	NA (NA)
K	 Glucuronidation	8.53 (0.03)	NQ (NQ)	NA (NA)	13.65 (0.10)	NQ (NQ)	NA (NA)
M	 SB-497115	9.63 (0.03)	ND (ND)	NA (NA)	13.81 (0.10)	ND (ND)	NA (NA)
Total[1,2]		55.96 (0.19)	10.82 (0.01)	NA (NA)	52.94 (0.38)	15.05 (0.03)	NA (NA)
Concentration[3]		0.33	0.11	0.04	0.72	0.21	0.09
% Overall recovery[4]		83.8	56.0	38.2	64.8	40.9	22.7

Bile:

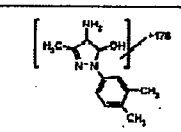
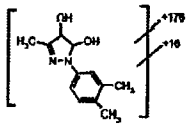
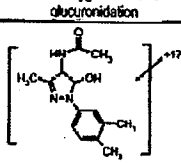
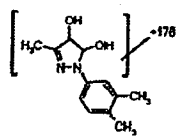
Due to the difficulty in collecting the bile sample from 1 of the 2 animals, the data was scanty and not authentic. The metabolites characterized in the sample were metabolite J, K and O. Metabolite J was mono-oxidized product while other 2 compounds were glucuronides of SB-497115.

Feces:

The mean recovery of radioactivity in feces was about 105 and 96% in male and female rabbits and the % dose excreted in feces was 41 and 32% in male and female animals. The minor metabolites were J and K metabolites (mono-oxygenation and glucuronide product). Metabolite U (another

glucuronide) was also detected in 5 and 4% of the fecal radioactivity. Metabolite N, a mono-oxygenation metabolite (conversion of methyl group to aldehyde) was also detected. A1 and A2 metabolites eluting together were not identified. The structures of the metabolites and unchanged substance are shown in the sponsor's table 2 in vol 3.5, pp 27-29):

Table 2 **Quantification of Radio-metabolites in Pooled Urine and Fecal Extracts following a Single Oral Administration of [^{14}C]SB-497115-GR to Intact Rabbits at a Nominal Dose Level of 10 mg/kg**

Peak ID	Metabolite Structure	Urine		Feces	
		Male	Female	Male	Female
		Mean % Sample Radioactivity[1] (% *Administered Dose*)[5]			
A	Unknown	NA*	NA*	0.38 (0.24)	0.87 (0.50)
A1	Unknown	NA*	NA*	2.59 (1.68)	1.28 (0.73)
AE	 Cleavage, reduction and glucuronidation	40.62 (8.18)	38.53 (8.74)	ND (ND)	ND (ND)
AF	 Cleavage, reduction, mono-oxygenation and glucuronidation	2.97 (0.59)	5.56 (0.96)	ND (ND)	ND (ND)
AG	 Cleavage, reduction, acetylation and glucuronidation	4.50 (0.87)	4.17 (0.71)	ND (ND)	ND (ND)
AH	 Cleavage, reduction and	19.40 (3.51)	10.79 (1.85)	ND (ND)	ND (ND)

Peak ID	Proposed Metabolite Structure	Male			Female		
		4 h	24 h	48 h	4 h	24 h	48 h
		% Plasma Radioactivity[1] (*µg equivalent SB-497115/g*)[2]					

NQ - Non-quantifiable. ND - Not detected. NA - Not available

1. Percent radioactivity recovered under each peak, corrected by the overall sample preperation recovery
2. Expressed as µg equivalents per g of plasma.
3. Total radioactivity concentration expressed as µg equivalents per g of plasma (data from excretion
4. study).
5. % Recovery of radioactivity following ethyl alcohol:acetonitrile:EDTA extraction, evaporation and re-constitution of plasma samples

* AE is the mixture of two cloeluting glucuronide conjugates of a cleavage product of SB-497115.

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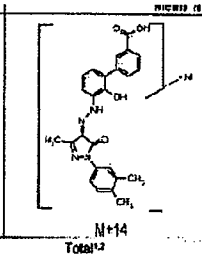
	Metab. in Sample (Radioactivity - (% Administered Dose))				
N**	 M+14	ND (ND)	ND (ND)	0.82 (0.53)	ND (NQ)
Total[1,2]		67.10 (13.14)	60.05 (10.25)	55.55 (35.99)	41.85 (28.75)
% Dose Profiled[3]		19.22	17.98	64.81	63.73
% Dose Excreted[4]		19.29	17.89	64.81	63.73
% Overall Recovery[5]		105.4	102.7	72.6	60.8

NA - Not available. NQ - Non-quantifiable. ND - Not detected.

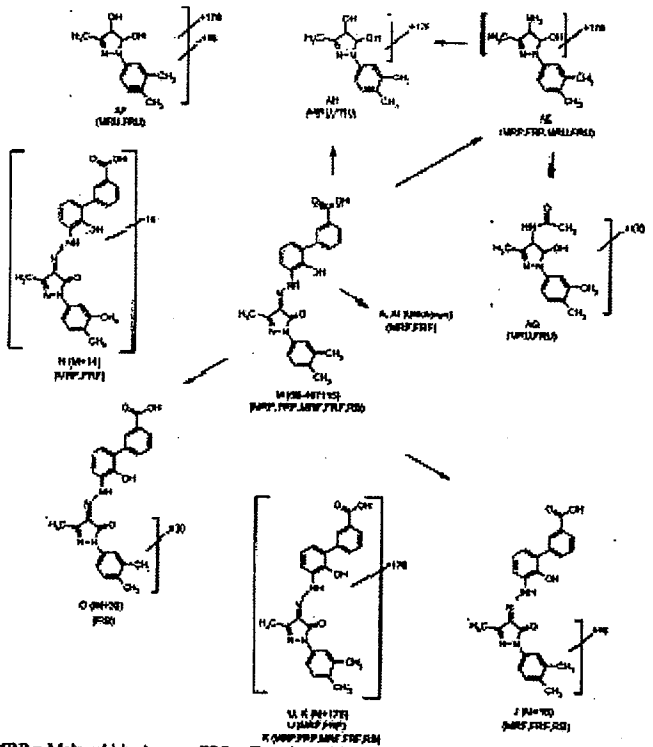
1. % Radioactivity recovered under each peak, corrected for centrifugation and overall sample preparation recovery for urine and feces, respectively.
2. % Dose recovered under each peak, corrected for centrifugation and overall sample preparation recovery for urine and feces, respectively.
3. % Mean dose in sample being profiled.
4. %Total dose excreted via urine or feces (data from GlaxoSmithKline Document Number CD2004/00158/00).
5. % Recovery of radioactivity following centrifugation and solvent extraction for urine and feces, respectively.

* Metabolites A and AI were unknown metabolites observed in urine chromatographed when with HPLC method #1 Their occurrences could not be confirmed with HPLC method #2 under which the identified urine metabolites were quantitated. Therefore, no attempt was made to quantitate these two unknown metabolites in urine.

** Metabolites U and N were assigned based on comparison in retention time with the metabolites characterized in a previous study (GlaxoSmithKline Document Number CD2003/00649/00).

The proposed metabolic pathway in rabbit is shown in the following figure (taken from sponsor's submission (vol 3.5, pp 30):

Figure 1 Metabolic Scheme for SB-497115 in the Rabbit



MRP = Male rabbit plasma; FRP = Female rabbit plasma; MRF = Male rabbit feces; FRF = Female rabbit feces; MRU = Male rabbit urine; FRU = Female rabbit urine; RB = Rabbit bile

17. Metabolism of SB-497115 in Beagle Dogs: (Study #CD2003/00649/00/D102057)

Methods: The study was conducted to identify and quantify the major metabolites of SB-497115 in the pooled urine, plasma, bile and feces samples of intact and bile duct cannulated male Beagle dogs. The metabolites were analyzed by HPLC and structural characterization was conducted on selected samples by mass spectrometry (HPLC-MS and HPLC-MS-MS). The animals were treated at the single oral dose of 10 mg/kg ^{14}C-SB-497115 (the NOEL in 14-day oral toxicity study in dogs). The study was conducted in 3 intact dogs and 3 bile duct cannulated animals. The fecal samples from intact and bile duct cannulated animals were collected from 0-48 hr and, also 0-72 hr post dosing in intact animals. The bile samples collected for 0-48 hr were pooled. Pooling of bile and feces was done as shown in the following sponsor's table:

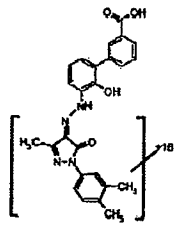
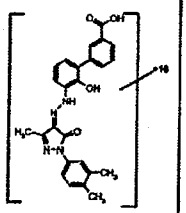
Species	Route	Status	Animal #	Time Period (h) for pooled Samples	
				Bile	Feces
Dog	Oral	Intact Male/female	1 2 3	NS NS NS	0-48/0-72 0-48/0-72 0-48/0-72
Dog	Oral	BDC	1	0-24 0-24 0-24	0-48 0-48 0-48

The samples were weighed and analyzed in triplicate and, the aliquots of fecal and liver extracts were allowed to dry overnight. Combust aid (200 ul) was added in the pooled samples and combusted and the product analyzed by LSC for the liver and fecal radioactivity. The fecal extract was analyzed by off-line radio-HPLC. The radio-metabolite profile was determined by appropriate aliquots of plasma and fecal samples. The metabolites were isolated by the procedures described earlier in the method section of rat study.

Results:

Plasma:
The total recovery of radioactivity from the male plasma samples were 84.5, 54.8 and 62.3% at 4, 24 and 48 hr sampling period and 84.0, 79.4 and 66.2% in female dogs. The plasma radioactivity from ethanol:acetonitrile:EDTA extraction (overall recovery) was 94, 69.7 and 80.3% in male dog and, 93.3, 87.3 and 80.2% in female dogs at 4, 24 and 48 hr sampling period. The unchanged compound (M) accounted for 79.7 and 83.1% in male and female dogs and the other minor were metabolite J, V, U and N. The identified structures and amounts of the metabolites in the animals had been shown in the following table:

Table 1 **Quantification of Radio-Metabolites in Plasma Extracts Following a Single Oral Administration of [^{14}C]SB-497115-GR to Intact Dogs at a Nominal Dose Level of 10 mg/kg**

Peak ID	Proposed Metabolite Structure	Male			Female		
		4 h	24 h	48 h	4 h	24 h	48 h
		% Plasma Radioactivity[1] (*µg equivalent SB-497115/g*)[2]					
U	M+176 Glucuronide of SB-497115	1.2 *(0.1)*	0.7 *(NQ)*	ND *(ND)*	ND *(ND)*	ND *(ND)*	ND *(ND)*
J	 Mono-oxygenated SB-497115	2.6 *(0.2)*	3.2 *(0.1)*	ND *(ND)*	0.4 *(0.1)*	ND *(ND)*	ND *(ND)*
V	 Mono-oxygenated SB-497115	2.4 *(0.2)*	3.9 *(0.1)*	ND *(ND)*	0.5 *(0.1)*	NQ *(NQ)*	2.2 *(0.1)*

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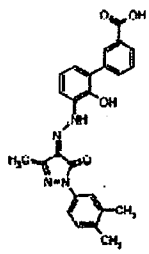
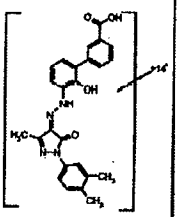
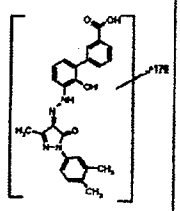
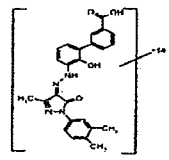
Peak ID	Proposed Metabolite Structure	Male			Female		
		4 h	24 h	48 h	4 h	24 h	48 h
		% Plasma Radioactivity[1] (*µg equivalent SB-497115/g*)[2]					
M	 SB-497115	79.7 (5.9)	52.1 (1.8)	62.3 (0.7)	83.1 (7.3)	79.4 (4.7)	64.7 (1.2)
N	 M+14	0.7 (0.1)	0.4 (NQ)	ND (ND)	ND (ND)	ND (ND)	ND (ND)
Total[1,2]		84.5 (6.3)	54.8 (1.8)	62.3 (0.7)	84.0 (7.4)	79.4 (4.7)	66.2 (1.3)
Concentration[3]		7.0	2.5	1.0	8.4	5.2	1.6
% Overall recovery[4]		94.0	69.7	80.3	93.3	87.3	80.2

NQ - Non-quantifiable. ND - Not detected.
1. Percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery
2. Expressed as µg equivalents per g of plasma.
3. Total radioactivity concentration in plasma (data from excretion study).
4. % Recovery of radioactivity following ethyl alcohol:acetonitrile:EDTA extraction, evaporation and re-constitution of plasma samples

Bile:

The mean recovery of the radioactivity as unchanged compound (M) was 51.8% in bile. Other metabolites like R (unknown structure), U (M+176; a glucuronide), O (oxidation compound) and C (unknown) were identified in 0-24 hr bile sample. The structures and amounts of these metabolites in the animals have been shown in the following table (Table 2 on pp 23-25 of CD2003/00649/00):

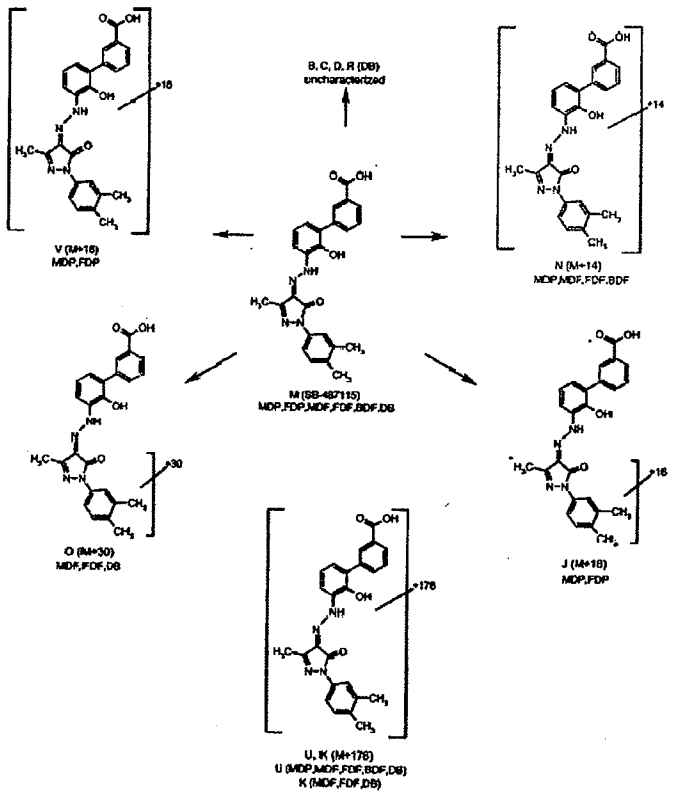
Table 2 **Quantification of Radio-Metabolites in Pooled Bile and Feces Extracts Following a Single Oral Administration of [^{14}C]SB-497115-GR to Intact and Bile Duct Cannulated Dogs at a Nominal Dose Level of 10 mg/kg**

		Feces			Bile
Peak ID	Metabolite Structure	Male	Female	BDC	
	Mean % Samples Radioactivity[1] (% *Administered Dose*)[2]				
B	Unknown	ND *(ND)*	ND *(ND)*	ND *(ND)*	NQ *(NQ)*
C	Unknown	ND *(ND)*	ND *(ND)*	ND *(ND)*	4.2 *(0.1)*
D	Unknown	ND *(ND)*	ND *(ND)*	ND *(ND)*	NQ *(NQ)*
U	M+176 Glucuronide of SB-497115	1.2 *(0.9)*	2.2 *(2.0)*	3.0 *(2.8)*	2.5 *(0.2)*
R	Unknown	ND *(ND)*	ND *(ND)*	ND *(ND)*	3.8 *(0.3)*
K	 Glucuronide of SB-497115	3.8 *(3.1)*	2.6 *(2.4)*	ND *(ND)*	7.5 *(0.4)*
N	 M+14	0.6 *(0.6)*	0.7 *(0.7)*	1.1 *(1.0)*	ND (ND)
Total [1,2]		60.0 *(51.4)*	56.0 *(51.5)*	72.0 *(64.4)*	71.0 *(4.1)*
% Mean Dose [3]		59.8	64.4	68.7	5.1
% Dose Excreted [4]		91.6	96.5	89.9	6.7
% Overall Recovery [5]		69.9	70.1	76.8	99.8

NQ - Non-quantifiable. ND - Not detected.
1. % Percent radioactivity recovered under each peak, corrected for centrifugation and overall sample preparation recovery for bile and feces respectively
2. % Dose recovered under each peak, corrected for centrifugation and overall sample preparation recovery for bile and feces, respectively
3. % Mean dose in sample being profiled (data from excretion study).
4. %Total dose excreted via bile or feces (data from excretion study).
5. % Recovery of radioactivity following centrifugation and solvent extraction for bile and feces, respectively.

Feces:
The mean recovery of radioactivity in feces was about 95, 97 and 98% for intact male, female and bile duct cannulated dogs. The unchanged compound (M) was in the amount of 53.3 and 49.2% in male and female dogs. The minor metabolites were U, J, O, N and K metabolites (mono-oxygenation and glucuronide product). Their retention times were 24.9, 29.7, 30.0, 37.9 and 29.1 min respectively. The structures of the metabolites and unchanged compounds are shown in the above table of the sponsor. The proposed metabolic pathway of the compound is shown below in Figure 1, pp 26.

Figure 1 Putative Metabolic Scheme for SB-497115 in the Dog



MDP = Male dog plasma; FDP = Female dog plasma; MDF = Male dog feces; FDF = Female dog feces; BDF = BDC dog feces; DB = Dog bile

2.6.4.6 Excretion

18. Elimination of Radioactivity following single oral (10 mg/kg) Administration of ^{14}C-SB-497115 to Male and Female rats intact and Bile-Duct-Cannulated Rats: (Study #SB-497115/RSD-101TKF/2; CMS 39120B)

Methods and Materials:

The study was conducted by [redacted] to determine the rate and extent of radioactivity elimination in feces and urine in 12 male and female rats and in bile, urine and feces of 3 bile-duct cannulated rats after a single dose of 10 mg/kg ^{14}C-SB-497115 (solution with mean concentration of 21.2 uCi/g, provided 1.01 mg/ml SB-497115). A group of 3/sex intact (group 1) animals were used for the determination of 0-12 hr and 12-24 hr and at 24 hr intervals for 168 hr post dosing. The feces samples were collected at 24 hr intervals for 168 hr. The 3 males of bile duct cannulated group (group 2) were used and for the determination of 0-12 hr and 12-24 hr and at 24 hr intervals for 168 hr post dosing done and mixed in the solution. At the termination, blood, gastrointestinal tract and remaining carcasses were also collected from intact and bile duct cannulated rats for radioanalysis.

b(4)

Results:

The total dose of SB-497115 (purity 97.6%) was 10.1 and 10.4 mg/kg in intact and bile duct cannulated rats, respectively. In intact rats, the excretion was 88.0 and 6.6% in male and, 90.6 and 4.93% in females and, in bile duct cannulated rats, about total mean radioactivity excretion was 40.2 and 8.86 in feces and urine. The other minor radioactivity from the cage and cannula rinses and GI tract are shown in the following sponsor's table. The total excretion was 96.0, 96.4 and 94.5% in the intact male, female and bile cannulated rats, respectively. The difference in bile cannulated rats was the excretion of 42.6% compound in bile.

Table 3 Total mean recovery of radioactivity following a single oral administration of [^{14}C]SB-497115-GR (10 mg/kg) to male and female intact rats and male bile duct-cannulated rats.

Matrix	Percent of Administered Dose Group Intact Males	Intact Females	BDC Males
Feces	88.0 ± 1.78	90.6 ± 0.81	40.2 ± 4.58
Urine	6.60 ± 2.69	4.93 ± 0.82	8.86 ± 1.83
Bile		-	42.6 ± 4.80
Cage Rinse	0.37 ± 0.15	0.20 ± 0.02	0.43 ± 0.06
Cage Wash	0.07 ± 0.09	0.01 ± 0.00	0.12 ± 0.04
Cage Wipe	0.08 ± 0.11	0.01 ± 0.01	0.16 ± 0.08
Bile Cannula			0.00 ± 0.01
Jacket Rinse		-	0.00 ± 0.01
GI Tract	0.90 ± 0.13	0.65 ± 0.18	0.04 ± 0.02
Residual Carcass	0.79 ± 0.11	0.55 ± 0.14	2.02 ± 0.17
Total	96.0 ± 1.33	96.4 ± 0.12	94.5 ± .25

Note: Values are the mean ± standard deviation (n=3)
- Not determined

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The blood to plasma radioactivity concentration ratios were similar in both sexes up to 48 hr, i.e., 0.560, 0.552 and 0.544 in males and, 0.546, 00.553 and 0.594 in females showing minimal binding to blood cells. The liver concentrations were highest at 4 hr and declined significantly at 48 hr. The liver to blood concentration was uniformly increased during the study period of 48 hr indicating rapid hepatic clearance. But the concentration liver:blood ratio was higher in females than in male intact rats and these were 5.96, 16.7 and 21.6 in males and, 6.88, 22.4 and 37.1 in females.

19. Elimination of Radioactivity Following a Single Oral Administration of [14C]SB-497115 (10 mg/kg) to Male and Female Intact and Male Bile Duct-Cannulated Mice: (Report No.: 7717-318; CD2007/00070/00)

Methods and Materials: The study was conducted at the [redacted] to identify and quantify the major metabolites of SB-497115 in the pooled urine, bile, feces, liver and plasma samples of intact and bile duct cannulated CD-1 mice. The metabolites were analyzed by HPLC and structural characterization was conducted on selected samples by mass spectrometry (HPLC-MS and HPLC-MS-MS). The bile metabolites were structurally identified by NMR. The animals [redacted] were treated at the oral dose of 10 mg/kg ^{14}C-SB-497115. The study was conducted in 3 intact rats and 1 bile duct cannulated animals. The urine samples from intact rabbit were collected from 0-48 hr in male bile duct cannulated mice.

b(4)

Results:
In intact male mice, the peak blood and plasma concentrations were 4.68 µg/g and 9.33 µg eq.of radioactivity at 1 h post-dose (the first collection time point). The concentration of radioactivity in liver (36.0 µg equiv/g) was also highest at 1 h post-dose. In intact female mice, similar highest blood (4.06 µg equiv/g) and plasma (7.96 µg equiv/g) concentrations of radioactivity were also observed at 1 h. By 96 h, the concentrations in blood and plasma were below 0.02 µg equiv/g. The concentration of radioactivity was highest in liver (33.3 µg equiv/g) at peak concentration time. For male and female

intact mice, ratios of the concentration of radioactivity in blood to plasma ranged from 0.501 to 0.902 and were increasing through 96 h post-dose,
indicating low bound radioactivity with blood cells. Ratios of concentration of radioactivity in liver to blood ranged from 5.87 to 8.88 in male mice and 8.03 to 12.7 in female mice from 1 h through 48 h post-dose.

A single oral dose of 10 mg/kg [^{14}C]SB-497115 (10 mg/kg) in intact mice was excreted in feces (72.8% and 76.6% of the dose in males and females). Urinary excretion was ≤15% of the dose. In male bile duct-cannulated mice, biliary secretion accounted for a mean of 21.1% of the administered dose. Fecal and urinary excretion of radioactivity accounted for about 64.0% and 4.26% of the dose, respectively. The recoveries in bile and urine showed that 25.4% of the administered radioactive dose was absorbed. In intact males and females, about 91.9% and 93.3% of the radioactivity respectively was recovered. About 92.5% of the radioactivity was recovered from bile duct-cannulated males. In blood and plasma concentrations declined with time and were below 0.02 µg equiv/g by 96 h in both male and female mice.

20. Elimination of Radioactivity following single oral (10 mg/kg) Administration of ^{14}C-SB-497115 to Male and Female intact and Bile-Duct-Cannulated Dogs: (Study #SB-497115/RSD-101TKD/1; CMS 39120A)

Methods and Materials:

The study was conducted by [redacted] to determine the rate and extent of radioactivity elimination in feces and urine in 3/sex intact fasted dogs and in 3 bile duct cannulated dogs after a single oral dose of 10 mg/kg ^{14}C-SB-497115 in capsule. The urine and feces samples from intact animals were collected at 12 hr before, 0-12hr (urine only), 12-24 hr (urine only) and at 24 hr intervals up to 168 hr. The cage rinse was performed every 24 hr for 144 hr. The blood samples collected via jugular vein at 4, 24, 48 and 168 hr for the estimation of plasma concentration. The urine, bile and feces were collected from 3 males of bile duct cannulated group (group 2) 12 hr prior, 12-24 hr and at 24 hr intervals through 168 hr post dosing. The radioactivity in blood, urine and feces samples was estimated. The food consumption, general observations were also made during the study. b(4)

Results:

The total dose of SB-497115 (purity 97.6%) was 10 mg/kg in intact and bile duct cannulated dogs. In intact rats, the radioactivity excretion was 91.6 and 1.45% in male and, 96.5 and 1.80% in females and, in bile duct cannulated dogs, about 89.9 and 0.34% of the total mean radioactivity was excreted in feces and urine. The excretion of the radioactivity was about 6.66% in the cannulated dogs. The radioactivity extracted from cage pan, cage wash etc., was shown in the following table of the sponsor. The total excretion was 94.0, 98.8 and 97.5% in the intact male, female and bile cannulated dogs, respectively.

Table 2 Total mean recovery of radioactivity following an oral administration of [^{14}C]SB-497115-GR (10 mg/kg) to male and female intact dogs and male bile duct-cannulated dogs.

Matrix	Percent of Administered Dose		
	Group		
	Intact Males	Intact Females	BDC Males
Feces	91.6 ± 5.81	96.5 ± 1.19	89.9 ± 7.26
Urine	1.45 ± 0.49	1.80 ± 0.52	0.34 ± 0.10
Bile	-	-	6.66 ± 4.68
Cage Pan/screen Rinse	0.22 ± 0.21	0.10 ± 0.06	0.06 ± 0.01
Cage Debris	0.32 ± 0.46	0.20 ± 0.23	0.49 ± 0.33
Cage Wash	0.03 ± 0.03	0.02 ± 0.03	0.02 ± 0.01
Cage Wipe	0.40 ± 0.52	0.07 ± 0.05	0.08 ± 0.04
Bile Cannula	-	-	0.00[a]
Jacket Rinse			0.00[a]
Total	94.0 ± 4.20	98.8 ± 0.32	97.5 ± 2.17

Note: Values are the mean ± standard deviation (N=3)
BLQ < 2 times background dpm per sample aliquot
- : Not Applicable
a: At least one individual value was BLQ; therefore the SD was not reported.

2.6.4.7 PHARMACOKINETIC DRUG INTERACTIONS

In vitro, eltrombopag was not a substrate of human organic anion transporting polypeptide (OATP1B1) The compound was shown to be an inhibitor of OATP1B1 (IC_{50} value of 2.7 μM), suggesting that it could show interaction with the substrates of this hepatic uptake transporter as shown below:

	Uptake rate (pmoles/min/mg protein)							
	Absence of Rifamycin (10 µM)				Presence of Rifamycin (10 µM)			
	CHO-OATP1B1 cells		CHO-WT cells		CHO-OATP1B1 cells		CHO-WT cells	
Test Compound	37°C	0°C	37°C	0°C	37°C	0°C	37°C	0°C
[^{3}H]Estradiol 17β-D-glucuronide	0.783	0.19	0.0164	0.0093	0.0232	0.021	0.0118	0.0081
Alternate labeled [^{14}C]eltrombopag (3 µM)	430	217	282	168	456	247	298	201
Alternate labeled [^{14}C]eltrombopag (30 µM)	2569	887	1789	705	2692	910	1751	675
Alternate labeled [^{14}C]eltrombopag (100 µM)	4848	1760	3411	1228	4660	1614	3223	1319

	Uptake rate (pmoles/min/mg protein)							
	Absence of Cyclosporine A (3 µM)				Presence of Cyclosporine A (3 µM)			
	CHO-OATP1B1 cells		CHO-WT cells		CHO-OATP1B1 cells		CHO-WT cells	
Test Compound	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
[^{3}H]Estradiol 17β-D-glucuronide	0.664	0.929	0.0227	0.0174	0.0189	0.0408	0.0192	0.054
Alternate labeled [^{14}C]eltrombopag (3 µM)	511	459	406	259	578	534	529	327
Alternate labeled [^{14}C]eltrombopag (30 µM)	3064	2480	2691	1650	1609	1743	1552	1148
Alternate labeled [^{14}C]eltrombopag (100 µM)	6698	5009	5378	3262	5437	4300	4336	2962

Key: Data are the mean from 3 wells. CHO= Chinese hamster ovary.
OATP1B1 = Organic anion transporting polypeptide 1B1. WT = Wild type.

In a clinical study, the interaction of eltrombopag with rosuvastatin was seen and the increased plasma rosuvastatin C_{max} was increased by 2-fold and AUC by 55% by eletromobag.

In vitro studies demonstrated that eltrombopag is neither an inhibitor nor a substrate of human P-glycoprotein (Pgp). The eltrombopag did not interact with Pgp substrates or its inhibitors .

No specific nonclinical pharmacodynamic drug interaction studies were conducted by sponsor.

OTHER PHARMACOKINETIC STUDIES

None.

2.6.4.9 Discussion and Conclusions

The bioavailability of SB-497115 was greater in solution formulations than capsule formulation in the mouse, rat, rabbit, dog and monkey. SB-497115-X (sodium salt) was absorbed in a linear manner in male dog and 83.0 and 91.7% was bioavailable after 3.52 and 3.6 mg/kg oral dose as a solution and suspension administration, while the bioavailability in capsule form was 7%. The orally administered compound attained plasma concentration in 3 to 6 hr in monkeys with a variable bioavailability from 89% (solution) to 108% (suspension), low plasma clearance and the volume of distribution of about 2 times the total water weight were seen. The half life (t1/2) in dogs and monkeys was 13.9 (IV and not known by oral route) and 7.7 (oral) hr, respectively. It binds with blood plasma proteins rather than to cells and, up to 94% of the compound binds to mouse, rat, dog, monkey and human plasma proteins. It did not form a good substrate for glycoproteins. The metabolic profile of SB-497115 in rat, dog and monkey hepatocytes was qualitatively similar to human. Conjugation and glucuronidation were the major routes of metabolism in rat, dog, monkey and human hepatocytes and no specific human metabolites were detected. SB-497115 was seen to inhibit human cytochrome P450 enzymes and it significantly inhibited CYP1A2 and 2C9 activity with IC50 = 3.5 and 9.3 uM, respectively. SB-497115 was excreted mostly in feces up to 88 to

90.6% in male and female rats, 40.2 and 89% in feces and urine of bile duct cannulated rats and, 64.8 and 63.7% in male and female rabbits. The urinary excretion was 6.6 and 4.9% in rats, 19.2 and 18.1% in male and female rabbits and in 0.27% in bile duct cannulated rabbits.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Single-dose toxicity: The sponsor conducted single dose exposure study in rats and maximum tolerated oral dose toxicity studies in dogs. In rat exposure study, the compound attained the peak plasma concentration in between 1 to 4 hr. The plasma concentrations were increased from 1 to 3 times when the compound was mixed in 1% methylcellulose. At a single dose of 100 mg/kg in dogs emesis, diarrhea, decreased activity and reduction in body weight were reported.

Repeat-dose toxicity: Repeat-dose toxicity studies were conducted in mice, rats, rabbits and dogs. In mice, SB-497115 at the oral gavage doses of 0, 30, 100 and 300 mg/kg/day (6/sex/group) for 2 weeks produced similar plasma concentrations in male and female mice. 300 mg/kg/day dose was lethal and 100 mg/kg/day well tolerated. The hepatocellular hypertrophy and renal atrophy/degeneration in animals suggested the liver and kidneys were the target organs of toxicity. In a 13-week study, oral doses of 10, 60 and 100 mg/kg/day SB-497115 for 13 weeks produced a dose related increase in plasma concentration and no toxicity was seen in study animals. The NOAEL was 100 mg/kg/day (5 times MHD based on AUC). The target organs of toxicity were not identified.

In rats, SB-497115 at 40 mg/kg/day for 2 weeks caused hepatocellular vacuolation but no adverse effects were seen in animals of 10 and 20 mg/kg/day treatment groups, NOAEL was 20 mg/kg/day (2 times MHD based on AUC). In another 2-week oral study, SB-497115 at 0, 20 and 40 mg/kg/day produced treatment related changes of hepatocellular vacuolation in the 40 mg/kg/day group (6 times MHD based on AUC). The liver was the target organ of toxicity in both of these studies. In a 28-week chronic toxicity study in rats, 3, 10, 30 and 60 mg/kg/day SB-497115 produced dose-related increase in plasma concentrations. There were no apparent sex differences in plasma concentration (Cmax) or systemic exposure (AUC). The systemic exposure was 2 to 3-fold higher after repeated dosing suggesting accumulation of the drug after repeated dosing. The decreased activity, irregular respiration, and increase in absolute and relative reticulocytes were seen in the 60 mg/kg/day animals and males were more sensitive than females. The liver, kidneys, blood and lymphoid tissues of spleen, lymph nodes and thymus were target organs of toxicity. A dose of 30 mg/kg/day (5 times MHD based on AUC) was the highest tolerable dose.

In a 2-week toxicity study in juvenile rats, SB-497115 at oral doses of 1 to 15 mg/kg/day produced similar treatment-related exposure in both sexes. Lymph node hemorrhage and myeloid hypercellularity was seen in animals of the 15 mg/kg/day group. The target organs of toxicity were lymphocytes and bone marrow. In a 28 day study in juvenile rats, a slight reduction in RBCs,

hemoglobin and hematocrit values, increase in reticulocytes counts and decrease in serum cholesterol and triglycerides was noted in 40 mg/kg/day group.

In a 13-day dose ranging study in female rabbits, SB-497115 from oral doses of 80, 150, 200 and 300 mg/kg/day produced a linear plasma increase with no treatment related effects. Doses ≥200 mg/kg/day were lethal. Hepatocellular hypertrophy and erosion of stomach were seen in 80, 150 or 200 mg/kg/day treatment groups. Based on this, the identified target organ of toxicity was liver in both sexes and, the additional target organ of toxicity in males was stomach.

In dogs, toxicity of SB-497115 was investigated at 0, 3, 10 or 30 mg/kg/day doses after 14 days of repeat administration. The hepatic enzymes and reticulocytes were increased in 30 mg/kg/day treatment group animals and, an NOAEL was 10 mg/kg/day (1.2 times MHD based on AUC). The chronic 52-week toxicity study in dogs was conducted at 0, 3, 10 or 30 mg/kg/day (0.4-3 times MHD based on AUC). The plasma exposure in male and female dogs were similar and the AUC at 30 mg/kg/day dose was approximately 3, 2 and 3 folds higher on week 4, 13 and 26 than on day 1 suggesting accumulation of the drug after repeated dosing. A linear increase of alkaline phosphatase by 1.7 and 1.9 folds in males and, 1.7 and 2.3 folds in females and a dose related hepatocellular vacuolation was seen in 10 and 30 mg/kg/day treatment groups. Based on this, liver was the target organ of toxicity and NOAEL was 10 mg/kg/day (1.2 times MHD based on AUC).

2.6.6.2 Single-dose toxicity :

Acute Toxicity Studies:

1. **Acute Oral Dose Systemic Exposure in Rats:** (Study #SB497115/RSD-101V81/1; D01013)

Materials & Methods: It is a GLP study and conducted at GlaxoSmithKline, King of Prussia, PA. from January 24, 2001 to August 13, 2002.
Strain of Animals:
Rats: SD male rats of 11 weeks of age (weights not given)

Three groups of male rats (2/group) were randomly administered a single oral gavage dose of 40 mg/kg SB-497119; batch # RC43490-167A1 in 1.0% aqueous methylcellulose plus 1 molar NaOH (vehicle A), 1.0% aqueous methylcellulose with 2% sodium laurly sulfate (vehicle B) or 1.0% aqueous methylcellulose with 10% Encapsin (vehicle C) to determine the systemic exposure in these 3 different solvents. The clinical signs, mortality and body weight were recorded for 4 hr post-dose. The plasma concentration was determined in plasma samples collected at 0 (pre dose), 0.5, 1, 1.5, 2 and 4 hr post treatment. At 4 hr of the treatment, the animals were returned.

Results:
None of the animals died and the sponsor did not describe the change in the clinical signs.

The peak plasma concentration was seen after 1 to 1.5 hr of the compound administered in either vehicle A or C. The average peak concentrations (n=2) in rats were 129945.5 and 2477.3 ng/ml, respectively after SB-497115 administered in vehicle A and C. The peak average concentration of 2791.5 ng/ml was seen after 4 hr of the administration of compound in vehicle B. The peak concentration of the compound was similar in rats treated with a solution prepared in vehicle B and C. The peak of the compound in vehicle A (1.0% aqueous methylcellulose plus 1 molar NaOH) was 52.4 and 46.5 folds greater when administered in or than the compound administered in 1.0% aqueous methylcellulose with 2% sodium laurly sulfate (vehicle B) and vehicle C.

2.6.6.3 Repeat-dose toxicity

Study title: 14-Day Oral Dose Toxicity Study in Mice

Key study findings: SB-497115 attained a linear and dose proportional plasma concentration at 30 and 100 mg/kg/day doses and the concentrations were similar in both sexes. The hypertrophy and vacuolation and mixed cell inflammation in lung muscle, hepatocellular centrilobular vacuolation and hepatocellular coagulative necrosis, cortical renal tubular necrosis and regenerative necrosis of medullary tubule and coagulative necrosis of zona fasciculata of adrenal were seen in animals of 100 mg/kg/day. The no effect dose was 100 mg/kg/day (7x MHD based on AUC) and the highest tolerable dose is between 100 and 300 mg/kg/day. The liver, adrenal, kidney and lungs were the identified target organs of toxicity.
Study no.: CD2003/00476/00 (protocol #D03076)
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.
Date of study initiation & Completion: May 14, 2003 and November 10, 2003
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: F033082 with 75.3% pure salt

Reviewer: Chopra NDA No. 22-291

Methods

Doses: 0, 30, 100 or 300 mg/kg/day in a solution of 2% hydroxypropyl- methylcellulose plus 0.2% sodium lauryl sulfate
Species/strain: CD-1 mice
Number/sex/group or time point (main study): 6/sex in control and treatment groups. On day 15, 10 females of group 1 to 3 were necropsied and on day 43, 10 females in group 1 and 3 were terminated.
Route, formulation, volume, and infusion rate: Oral gavage, (vol = 10 ml/kg)
Groups used for toxicokinetics or recovery: 24/sex/treatment group, the animals were discarded on day 15
Age: 11 weeks old
Weight: Mean weight of 34.5 to 35.1 g (male) and 27.7 to 28.4 g (female)
Sampling times: On day 14, blood samples of mice included in TK groups (n=3/sex/group) were collected prior to dosing, 0.5, 1, 2, 4, 8, 12 and 24 hr post dosing

Observation and Times:

Clinical signs and mortality: Once daily during randomization and treatment until necropsied of group 1 to 7. Detailed examination done on day 14 (main study group)
Body weights: Once prior to treatment, once every day after treatment and terminally. Recovery group animals were weighed weekly following day 15.
Food consumption: Once every week of treatment in main study group animals.
Hematology: Blood samples were not collected from main study groups animals.
Clinical chemistry: Blood samples (from main study groups animals were not collected.
TK: Blood samples (0.25 to 0.4 ml) from TK group animals (3/sex/group) on day 14.
Urinalysis: The urine samples from main study group were not collected
Gross pathology: On day 15, all main study group animals were killed and the organs separated were adrenal, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eye/optic nerve, Harderian glands, heart, hind limb, ileum, jejunum, kidneys, liver, lung, lymph nodes, lesions, mammary glands, ovaries, pancreas, parathyroids, pituitary, rectum, rib, salivary glands, seminal vesicles, skin, skull, spinal cord, spleen, stomach, testes, thymus, trachea, urinary bladder, uterus, vagina and prostate. The tissues of all main groups of animals were examined macroscopically.
Organ weights (specify organs weighed if not in histopathology table): Adrenal, brain, kidneys, liver, heart and thymus.

Histopathology: The microscopic examination of all the tissues belonging to main study groups (1 to 4) animals was performed.

Results:

Reviewer: Chopra NDA No. 22-291

Mortality: Eleven animals of the main study groups died during the study. These were 1, 1, 1 and 4 males and, 0, 0, 1 and 3 females belonging to 0, 30, 100 and 300 mg/kg/day treatment groups, respectively.

Clinical signs: The highest dose of 300 mg/kg/day produced treatment related hypoactivity, hypothermia, hunched posture, unkempt appearance and, only few feces were seen in animals included in 100 and 300 mg/kg/day treatment groups. The animals which died during the study showed these symptoms before death. The animals in 30 mg/kg/day showed no treatment related effects.

Body weights and Food consumption: No treatment related changes.

Hematology & Clinical chemistry: These parameters were not studied during the study.

Urinalysis: Urine samples were not collected during the study.

Gross pathology: No treatment related adverse effects were reported in animals belonging up to 100 mg/kg/day treatment groups. The animals which died during the study showed pale to greenish colored liver in 3 females and small thymus in 1 male of 300 mg/kg/day treatment group.

Organ weights: A dose related increase ($p = 0.05$) in the absolute weights of liver was noted. The liver weights were not reported, 1.89, 2.12 and 2.4 g in males and, 1.42, 1.60, 1.74 and 2.09 g in females included in 0, 30, 100 and 300 mg/kg/day treatment groups. Sponsor did not provide records for the organ weights of brain, heart, kidneys, adrenal, ovaries and thymus of the study animals.

Histopathology: Hypertrophy and vacuolation in the smooth lung muscle was noted in 2 males and 5 females of 300 mg/kg/day treatment group. Mixed cell suppurative inflammation in lung was observed in 0, 1, 1 and 2 males and 0, 2, 1 and 2 females, hepatocellular centrilobular vacuolation was noted in 3 and 3 males and 0 and 2 females of 100 and 300 mg/kg/day treatment groups. The microscopic lesions in animals of 300 mg/kg/day treatment group included hepatocellular coagulative necrosis in 3 males and 6 females. Cortical tubular necrosis and regenerative necrosis of medullary tubule were in 1 and 2 males and 1 and 4 females of 100 and 300 mg/kg/day treatment groups. The coagulative necrosis of zona fasciculata of adrenal was in 1, 1, 1 and 2 males and, in 0, 0, 1 and 4 females of the study groups.

Toxicokinetics:

A linear dose proportional increase in the plasma concentration was observed in animals included in 30 and 100 mg/kg/day treatment groups as shown below in the table. The plasma concentrations of animals of 300 mg/kg/day treatment group were not recorded. The TK data of males and females were similar as shown below in the table.

	Cmax (ug/ml)		AUC (0-24hr) (ug.hr/ml)	
	M	F	M	F
30	30.5	44.1	290	308
100	97.5	134.1	770	1274

In summary, SB-497115 attained a linear and dose proportional plasma concentration at 30 and 100 mg/kg/day doses and the concentrations were similar in both sexes. The hypertrophy and vacuolation and mixed cell inflammation in lung muscle, hepatocellular centrilobular vacuolation and hepatocellular coagulative necrosis, cortical renal tubular necrosis and regenerative necrosis of medullary tubule and coagulative necrosis of zona fasciculata of adrenal were seen in animals of 100 mg/kg/day. The no effect dose was 100 mg/kg/day (7 times MHD based on AUC) and the highest tolerable dose should be between 100 and 300 mg/kg/day. The identified target organs of toxicity were liver, adrenal, kidney and lungs.

Study title: 2-Week oral Gavage Tolerability and Toxicokinetic Study in Mice

Key study findings: SB-497115 attained a dose proportional plasma concentration at 150 and 200 mg/kg/day doses and the plasma concentration of males and females was not different. None of study animals died during the study

b(4)

Study no.: CD2004/00836/00 (protocol #D04138)
Conducting laboratory and location: [redacted]
Date of study initiation & Completion: June 14, 2004 and August 31, 2004
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: TPO-E-01C (Sponsor considered that the new drug batch might not be associated with the adverse effects seen in the previous study # CD2003/00476/00)

Methods

Doses: 0, 150 or 200 mg/kg/day in a solution of 2% hydroxypropylmethyl cellulose plus 0.2% sodium lauryl sulfate.
Species/strain: CD-1 mice

Number/sex/group or time point (main study): 6/sex in control and treatment groups. On day 15, all animals of the study groups were necropsied.

Route, formulation, volume: Oral gavage, (vol = 10 ml/kg)

Groups used for toxicokinetics or recovery: 18/sex/treatment group, the animals were discarded on day 15

Age: 6 weeks old

Weight: Mean weight of 27.9 to 34.0 g (male) and 20.4 to 28.4 g (female)

Sampling times: On day 14, blood samples of non-fasted mice (n=3/sex/group) were collected prior to dosing and 2, 4, 8 and 24 hr post dosing

Observation and Times:

Clinical signs and mortality: Once daily during randomization and treatment until necropsied of group 1 to 7 (main study group)
Body weights: Once prior to treatment, once every day after treatment and terminally. Recovery group animals were weighed weekly following day 15.
Food consumption: Once every day during week –1.
Hematology: Blood samples from main study group's animals were not collected.
Clinical chemistry: Blood samples (from main study groups animals were not collected.
TK: Blood samples (0.25 to 0.4 ml) from TK group animals (3/sex/group) on day 14.
Urinalysis: The urine samples from main study group were not collected
Gross pathology: Not done.

Histopathology: Not done.

Results:

Mortality: None of the animals of the main and toxicokinetic study groups died during the study.

Clinical signs: The quantity of feces in the treated animals was reduced, and hunched posture and unkempt appearance were seen in animals included in 150 and 200 mg/kg/day treatment groups.

Body weights and Food consumption: No remarkable differences.

Hematology & Clinical chemistry: These parameters were not studied during the study.

Urinalysis: Urine samples were not collected during the study.

Gross pathology: No data was submitted.

Organ weights: The organ weights were not recorded.

Histopathology: The microscopic evaluation was not included in the study.

Toxicokinetics:

A linear dose proportional increase in the plasma concentration was observed in 4.08 to 4.12 hr in animals of 150 and 200 mg/kg/day treatment groups as shown below in the table. The TK data of males and females were similar as shown below in the table.

	Cmax	AUC (0-24hr)	Tmax (hr)