Preclinical Metabolism and Pharmacokinetics of SB1317 (TG02), a Potent CDK/JAK2/FLT3 inhibitor

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Abstract

The preclinical ADME of SB1317 (TG02), a potent CDK/JAK2 inhibitor, was characterized. SB1317 was soluble, highly permeable in Caco-2 cells, and showed >99% binding to plasma from mice, dog and humans. It was metabolically stable in human and dog liver microsomes relative to mouse and rat. SB1317 was mainly metabolized by CYP3A4 and CY1A2 in vitro. SB1317 did not inhibit any of the major human CYPs in vitro except CYP2D6 (IC$_{50}$=1 uM). SB1317 did not significantly induce CYP1A and CYP3A4 in human hepatocytes in vitro. The metabolic profiles in liver microsomes from preclinical species were qualitatively similar to humans. In pharmacokinetic studies SB1317 showed moderate to high systemic clearance (relative to liver blood flow), high volume of distribution (>0.6 L/kg), oral bioavailability of 24%, ~ 4 and 37% in mice, rats and dogs, respectively; and extensive tissue distribution in mice. The favorable ADME of SB1317 supported its preclinical development as a drug candidate.

Key words: ADME, CYP450, Metabolism, Pharmacokinetics, SB1317/TG02
INTRODUCTION

Cyclin dependent kinases (CDKs) are serine/threonine protein kinases which play a significant role in cell division, apoptosis, and differentiation [1]. The Janus kinases (JAK1, 2 and 3) along with stimulator and activators of transcription (STAT) are central in signal transduction pathways of cytokines which control hematopoiesis and immune system function [2]. Aberrant expressions of CDKs and JAKs are implicated in a variety of cancers like solid tumors, myeloproliferative neoplasms and lymphomas. Inhibition of CDKs and JAKs provide a novel strategy for the treatment of cancer. SB1317 is a novel multikinase inhibitor, with potent activity on CDK/JAK2/FLT3 that is being investigated for the treatment of both solid and hematologic cancers in the clinic [3]. In this article, we present the data characterizing the preclinical ADME of SB1317.

MATERIALS AND METHODS

Chemical and Reagents

Human liver microsomes (HLM) (Lot No. 452161), dog liver microsomes (DLM) (Lot No. 452601) and mouse liver microsomes (MLM) (Lot No. 452701) were purchased from BD Gentest (NJ, USA). Rat liver microsomes (RLM) were prepared in-house. The cDNA expressed human cytochrome P450 enzymes (Bactosomes™) were purchased from Cypex Ltd (Dundee, UK). Human hepatocytes were obtained from the UK human tissue bank. The Caco-2 permeability assay was carried out at Absorpti systems (USA). The blank human (Lot No. IR05-053), dog (Lot No.52560603) and mouse plasma (Lot No. 30159695) were purchased from iDNA Biotechnology Pte Ltd (Singapore). SB1317/TG02(16E)-14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (chemical structure 1) was synthesized in the medicinal chemistry department at S*BIO Pte Ltd (Singapore). Ethoxyresorufin, tolbutamide, mephenytoin, dextromethorphan, midazolam, α-naphthoflavone, sulphaphenazole, tranylepyromine, quinidine, ketoconazole, dimethylsulfoxide (DMSO), potassium dihydrogen phosphate and potassium hydrogen phosphate were purchased form Sigma-Aldrich (Singapore). Nicotinamide adenine dinucleotide phosphate reduced [NADPH] regeneration system A and B were purchased form BD Gentest (USA). Formic acid was purchased from Merck (Germany). Acetonitrile and methanol were of highest analytical grade.

Caco-2 bi-directional permeability assay

Caco-2 cells were obtained from American Type Culture Collection (ATCC) and used between 40-60 passage numbers. The cells were seeded on Millipore Multiscreen Caco-2 plates at a density of 1 x 10^5 cells/cm² and cultured for 20 days using Dulbecco’s Modified Eagle’s Medium (DMEM) to form monolayers. The DMEM media was changed once in every 2-3 days. Hanks Balanced Salt Solution (HBSS) pH 7.4 buffer with 25 mM HEPES and 4.45 mM glucose at 37°C was used as the medium in the permeability studies. The monolayers were rinsed with HBSS on both apical and basolateral side, twice and incubated with HBSS for 40 min to stabilize physiological parameters. HBSS was removed from the apical compartment and replaced with SB1317 (5 µM) in HBSS. The apical compartment inserts are then placed into basolateral compartment containing fresh HBSS. For basolateral to apical permeability, the HBSS in the
basolateral compartment was replaced by HBSS containing SB1317 and the apical inserts had HBSS. Incubations were carried out in an atmosphere of 5% CO₂, relative humidity of 95%, at 37°C for 120 min. At the end of incubation, both the apical and basolateral samples were diluted and analyzed by LC/MS/MS. The integrity of the monolayers was checked by monitoring lucifer yellow permeation using fluorimetric analysis. The permeability was expressed as apparent permeability (P_app) calculated using the following equation P_app = (dQ/dt / C₀ x A), where dQ/dt is the rate of permeation of SB1317 across the cells, C₀ is the concentration in donor compartment at time zero, and A is the area of the cell monolayer. The efflux ratio was estimated as the ratio of apparent permeability in basolateral to apical (B→A) to that of apical to basolateral (A→B).

Plasma protein binding (PPB)

The PPB of SB1317 in mouse, dog and human plasma was determined using equilibrium dialysis. The method comprised of a dialyzer cell containing two chambers of 250 µL volume each, separated by a semi permeable membrane. Plasma containing SB1317 at 1 µg/mL concentration was aliquoted in one of the chambers and the other chamber was filled with phosphate buffer saline (PBS) pH 7.4. The assembly was incubated for 4 hours at 37°C in a water bath. At the end of incubation, an aliquot of plasma and PBS were extracted using methyltertiarybutyl ether. The samples were vortexed for 30 min, centrifuged for 10 min at 13000 rpm at 4°C. The supernatant was dried in a SpeedVac at 43°C for 35 min. The dried samples were reconstituted with methanol and milliQ water mixture (6:4) and analyzed by LC/MS/MS. The %PPB of SB1317 was determined using the following equation
% PPB = % total drug - % free drug.

Microsomal stability

The reaction mixture consisted of 0.1 M potassium phosphate buffer (pH 7.4), liver microsomes (1.0 mg/mL), NADPH regenerating mix (1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, 3.3 mM MgCl₂, 0.4 U/ml of glucose-6-phosphate dehydrogenase, 50 µM sodium citrate) and SB1317 (5 µM; DMSO <0.1%) in a total volume of 100 µL. The incubation mixture was prewarmed for 2 min before the addition of SB1317. Reactions were terminated by adding 75 µL reaction mix to 100 µL of chilled acetonitrile-DMSO mixture (8:2) at time 0, 5, 15, 30, 45 and 60 min. The samples were centrifuged at 2000 rpm for 15 min at 4°C. The supernatants were analyzed by LC/MS/MS. Control samples were made by omitting NADPH regenerating mix from reaction mixture. Metabolic stability was determined by plotting the natural logarithm of % SB1317 remaining versus time and k (the first order rate constant) was estimated from the log-linear portion of the curve. The half-life (t₁/₂) was estimated using the equation t₁/₂ = 0.693/k. Microsomal intrinsic clearance (CLₘᵢᵣ) was estimated using the equation CLₘᵢᵣ = k x volume of incubation (mL) /mg microsomal protein. The hepatic intrinsic clearance (CLₑᵣᵣ) of was estimated using the equation
\[
CLₑᵣᵣ = CLₘᵢᵣ' x (45 mg microsomal protein concentration / g of liver weight) x (g liver weight / kg body weight)
\]

Hepatic plasma clearance (CL_p) was obtained by using the well-stirred model [4] using the equation
\[
CL_p = (Q_H * f_u * CLₘᵢᵣ)/(Q_H + \{[f_u/C_B/CP]* CLₘᵣ,u\})
\]

Where, Q_H=liver blood flow [LBF] [5]; f_u=fraction unbound in plasma; CLₘᵣ,u = unbound intrinsic microsomal clearance; C_B/CP=Blood to plasma ratio.
The fraction unbound in microsomes was assumed to be equal to \( f_u \), and \( C_B/C_P \) was assumed to be 1.

**Cytochrome P450 (CYP450) phenotyping**

The cDNA expressed human CYP450 enzymes co-expressed with human CYP450 reductase (Bactosomes\textsuperscript{TM}) were used. The incubations consisted of Bactosomes\textsuperscript{TM} (final CYP450 concentration CYP1A2 100 pmol/mL, CYP2C9 25 pmol/mL, CYP2C19 100 pmol/mL, CYP2D6 50 pmol/mL and CYP3A4 25 pmol/mL), 0.1 M phosphate buffer pH 7.4 and SB1317 (final concentration 5µM; DMSO 0.25%) and NADPH (final concentration 1mM). The total volume of the reaction mixture was 25 µL. SB1317 was incubated with each isofrom (CYP1A2, 2C9, 2C19, 2D6 and 3A4) for 0, 5, 15, 30 and 45 min. At the end of incubations, reactions were terminated by the addition of 50 µL of methanol containing internal standard (IS). The samples were centrifuged at 2500 rpm for 20 min at 4°C and the supernatants were analyzed by LC/MS/MS. The positive controls, specific substrates for each isofrom, were used as control compounds. The natural logarithm of peak area ratio (SB1317/IS) was plotted against time, and \( k \) (First order rate constant) was estimated from the linear portion of the curve. The \( t_{1/2} \) was determined using the equation \( t_{1/2} = 0.693/k \).

**CYP450 inhibition in human liver microsomes**

Five major CYP450 isoforms CYP1A, CYP3A4, CYP2C9, CYP2C19 and CYP2D6 were screened in this assay. Reactions were performed by incubating SB1317 (0.05, 0.25, 0.5, 2.5, 5 and 25 µM; DMSO 0.35%) with HLM (0.25 mg/mL for CYP1A and CYP3A4, 0.5 mg/mL for CYP2C19 and CYP2D6 and 1 mg/mL for CYP2C9) in 0.1M phosphate buffer, NADPH (1mM) and selective probe substrates of individual isofrom at 37°C. Ethoxyresorufin (0.5 µM), midazolam (2.5 µM), tolbutamide (120 µM), mephenytoin (25 µM) and dextromethorphan (5 µM) were used as probe substrates of CYP1A, CYP3A4, CYP2C9, CYP2C19 and CYP2D6, respectively. The incubation times were 5 min for CYP1A and 3A4, 30 min for CYP2D6 and 60 min for CYP 2C9 and CYP2C19. Following incubations, the CYP1A reaction was terminated and the formation of metabolite resorufin was measured by fluorescence (excitation wavelength 535 nm and emission wavelength 595 nm). For CYP3A4, CYP2C9, CYP2C19 and CYP2D6 incubations, the reactions were terminated with methanol containing IS. The samples were centrifuged and the supernatants were analyzed for the formation of metabolites (1-hydroxymidazolam (CYP3A4), 4-hydroxytolbutamide (CYP2C9), 4-hydroxymephenytoin (CYP2C19) and dextrorphan (CYP2D6)) by LC/MS/MS. The selective inhibitors \( \alpha \)-naphthoflavone (CYP1A), ketoconazole (CYP3A4), sulphaphenazole (CYP2C9), tranylcypromine (CYP2C19) and quinidine (CYP2D6) were screened alongside SB1317 as positive controls. A decrease in the formation of metabolites compared to vehicle control (100%) was used to estimate % inhibition, and the IC\textsubscript{50} was estimated from concentration-response curves using the sigmoid \( I_{max} \) model.

**CYP450 induction**

Freshly isolated human hepatocytes were seeded in a 24-well plate at a density of 0.15 x 10\(^6\) cells/cm\(^2\) in Williams E media. The hepatocytes were incubated at 37°C, 95% humidity, 5% CO\(_2\), for 48 hours. At the end of 48 hours, hepatocytes were treated with SB1317 at varying concentrations (0.1, 1, 10 µM) or with dexamethasone (50 µM), rifampicin (10 µM) and omeprazole (50 µM). The final concentration of the solvent (DMSO) was 0.1%. The treatment
continued for 72 hours, with daily renewal of the medium plus SB1317 or reference controls. Negative controls were 0.1% DMSO in media. After 72 hours of incubation, the hepatocytes were incubated with probe substrates of CYP1A (ethoxyresorufin, 20 µM) and CYP3A4 (midazolam, 20 µM) for 60 and 30 min, respectively. At the end of incubation, the media was mixed with equal volume of methanol and the samples were centrifuged and the supernatant was analyzed for the formation of metabolites, resorufin (CYP1A) and 1-hydroxymidazolam (CYP3A4). Resorufin was measured by fluorescence (excitation wavelength 535 nm and emission wavelength 595 nm) and 1-hydroxymidazolam was detected using LC/MS/MS. Both the metabolites were quantified using a calibration curve.

**Metabolite identification**

SB1317 was incubated with human, mouse, rat and dog liver microsomes (1.0 mg/mL) individually, at three different concentrations (5, 10 and 50 µM). The incubation mixture consisted of 0.1M phosphate buffer, liver microsomes, NADPH regeneration mix and SB1317 at varying concentrations, in a volume of 1.0 mL. Reactions were initiated by the addition of SB1317 and incubating the samples at 37°C. Samples containing MLM and RLM were incubated for 0, 5, 15, 30 and 60 min and samples with HLM and DLM were incubated for 0, 30, 60 and 120 min. A blank sample without SB1317 in the reaction mix was made to detect any interference caused by the matrix. A negative control without NADPH regeneration mix was prepared to check the stability of SB1317 during incubation. At each time point, 100 µL of the reaction mixture was quenched using 200 µL of chilled acetonitrile and DMSO mixture (8:2) and centrifuged at 13200 rpm for 15 min at 4°C and the supernatants were analyzed using LC/MS/MS.

A QTRAP 3200 LC/MS/MS (Applied Biosystems) triple quadrupole mass spectrometer was used for the analysis. The metabolites were separated on an Agilent 1100 series HPLC system using Agilent Zorbax-Eclipse XDB-C18, 150 x 4.6 mm (id), 5 microns column. The mobile phase consisted of 0.1% formic acid in milliQ water (A) and 0.1% formic acid in acetonitrile (B). The flow of the mobile phase was maintained in a gradient mode at 1.0 mL/min flow rate with 100% A at 0 min; 85% A at 3.1 min; 45%A at 20 min, which was maintained till 27 min. At 27.01 min % A was adjusted back to 100% and maintained till 30 min. The mass spectrometer was tuned in positive mode with scan rate of 1000 amu/s. The mass parameters comprised of declustering potential of 61 V, entrance potential 8.5 V, collision energy 30 V and ion spray voltage of 5500 V. The curtain gas (nitrogen flow) was maintained at 10 psi, temperature at 500°C, collisionally activated dissociation (CAD) at medium and ion spray gas 1 and gas 2 at 60 and 65 psi, respectively. The interface heater was on during the analysis. Analyst software 1.4.2 version was used for processing and analyzing the data.

**Animal studies**

All the studies were conducted as per Institutional Animal Care and Use Committee (IACUC) guidelines. A) Pharmacokinetics (PK): Female BALB/c nude mice of age 10-12 weeks (17-20 g), male Wistar rats of age 6-8 weeks (~225-250 g) and male Beagle dogs of age 1-2 years and weighing ~ 12-14 kg were used. For rat and dog studies, food was withheld for a period of 4-12 h prior to dosing and returned to all the animals 4 hr post dosing and water was supplied ad libitum. The doses of SB1317 were as follows: mice - 5 mg/kg intravenous (i.v.) and 75 mg/kg oral (p.o.); rat- 2 mg/kg (i.v.) and 10 mg/kg (p.o.); and in dogs - 1 mg/kg (i.v.) and 5 mg/kg (p.o.). The formulations used in i.v. and p.o. studies were 5% dimethylacetamide (DMA)
+ 90% sterile water and 0.5% methylcellulose (MC) + 0.1% tween 80, respectively. Blood samples were collected at pre-determined time points via cardiac puncture (mice), superior vena cava (rats) and foreleg vein (dogs) over a period of 24 h in eppendorf tubes containing K3EDTA as anticoagulant. The blood samples were centrifuged at 3000 rpm for 10 min, plasma was collected and stored at -80°C until analysis. B) Tissue distribution study: From the oral PK study in mice at 75 mg/kg, liver, lungs, kidney, heart and brain tissues were collected, rinsed in PBS, dried and stored in vials at -80°C until analysis.

Bioanalytical method

SB1317 was extracted from plasma and tissue homogenate samples using liquid-liquid extraction procedure. An aliquot of 50 µL (mice and rat), 50 uL of tissue homogenate, or 100 µL (dog) plasma was spiked with 10 µL of 125 ng/mL of carbamazepine (IS). The samples were extracted using 1.5 mL of methyltertiarybutylether solvent, vortexed for 30 min and centrifuged at 13200 rpm for 10 min at 4°C. The supernatant was dried under gentle stream of nitrogen. The concentrated samples were reconstituted with methanol and milliQ water (6:4) and analyzed by LC/MS/MS. A Micromass triple quadrupole mass spectrometer (MS) equipped with electrospray ionization and coupled with Alliance HT2795 (Waters) HPLC system was used for the analysis. Both, SB1317 and the internal standard were separated using Phenomenex Luna, C18(2) (2.5mm x 50 mm ; 5 µm i.d.) column. The mobile phase consisted of 0.1% formic acid in milliQ water (A) and methanol (B). The mobile phase was delivered in an isocratic mode (A: B:: 40: 60), at a constant rate of 0.3 mL/min. The run time was 5 min. The MS was tuned in positive ion mode (ESI+). The samples were introduced into the interface through the heated nebulizer source maintained at 120°C. Nitrogen was used as the nebulizer and desolvation gas, which was set at 50 L/h and 500 L/h, respectively. Argon was used as the collision gas. The other mass parameters were ion spray voltage 3.0 kV; cone voltage 40 V (SB1317) and 35 V (IS); ion source temperature 130°C; entrance potential - 3 V; collision energy 22 eV (SB1317) and 20 eV (IS); collision cell exit potential – 1 V. The dwell time was maintained at 0.5 sec for both the analytes. The spectrometer was operated in multiple reaction monitoring (MRM) mode and the analytes were detected by selecting specific transition of precursor ion to fragment for each analyte. The transition pairs of SB1317 and IS were selected as m/z 373 → m/z 342 and m/z 237 →m/z 194, respectively.

Data Analysis

The pharmacokinetic parameters clearance (CL), volume of distribution at steady state (Vss), area under the plasma concentration time curve from time zero to time of last measured concentration or infinity (AUC0-∞), maximum concentration (Cmax), time of Cmax (tmax), half-life (t1/2) and elimination rate constant (ked) were estimated using non-compartmental analysis in WinNonlin version 5.1 (Pharsight, Mountain View, CA). The oral bioavailability (F) of SB1317 was calculated using the following equation F (%) = [AUC0-∞,p.o/AUC0-∞,i.v ]x [Dosei.v./Dosep.o]x100. The ratio of AUCtissue to AUCplasma was estimated to assess the extent of accumulation of SB1317 in tissues.
RESULTS

The *in vitro* ADME properties of SB1317 are summarized in table 1. The plasma protein binding (PPB) was uniformly high across species. SB1317 showed high permeability and low efflux in Caco-2 cells. In microsomal stability studies, SB1317 was rapidly metabolized in mouse and rat liver microsomes, and was relatively more stable in dog and human microsomes. The scaled hepatic plasma clearance was ~ 70% of LBF in mice and rats, and ~ 50% of LBF in dog and humans. In CYP phenotyping studies, SB1317 was primarily cleared by CYP1A2 and 3A4. SB1317 did not inhibit any of the major CYP enzymes except CYP2D6. SB1317 did not significantly induce human CYP3A4 and 1A *in vitro* (table 2), where the fold induction of both the enzymes was <40% of that of the corresponding positive controls.

The CYP450 mediated metabolism of SB1317 (*m/z* 373) in microsomal incubations showed the formation of several metabolites (figure 1, Supplementary figures 1, 2, 3 and 4; table 3). An *N*-demethylated product (*m/z* 359) appeared to be the major metabolite of SB1317 (figure 1). There were four metabolites M2, M5, M6 and M8 with *m/z* 389 formed due to mono-oxidation. However, metabolite M8 (*m/z* 389) was only observed in mouse and human liver microsomes In addition biotransformation of SB1317 also led to metabolites M3 (Oxidation followed by *N*-demethylation, *m/z* 375), M4 (Oxidation followed by dehydration, *m/z* 371) and M7 (Di-oxidation, *m/z* 405). Overall, most of the metabolites formed in human liver microsomes were also observed in mice, rat and dog liver microsomes. Based on the fragmentation pattern observed with different metabolite ions, a metabolic pathway for SB1317 as depicted in figure 2 is proposed.

The PK profiles of SB1317 in mice, rats and dogs are shown in figure 3(A, B and C) and PK parameters are summarized in table 4. Following intravenous (i.v.) dosing, SB1317 displayed multi-exponential first order kinetics in mice, rats and dogs. The systemic plasma clearance (CL) was >100%, 72% and 61% of the corresponding LBF of mice, rat and dog, respectively [5]. The volume of distribution at steady state (Vss) of SB1317 was high (>0.6 L/kg) in mice, rat and dog, suggesting extensive distribution to tissues. Following oral administration, SB1317 showed rapid absorption (tmax ranged between 0.08 and 1 h) followed by multi-exponential disposition in mice, rat and dog. The extrapolated AUC was < 20% of the AUC0-∞ in the i.v. and oral studies in all the three species (table 4). The oral bioavailability (F) of SB1317 was 24 and 37% in mice and dog, respectively and was poor in rat (~4%). The results from the tissue distribution study in mice are shown in figure 3D and in table 5. SB1317 showed rapid distribution to tissues and was eliminated at similar rates from the tissues when compared to plasma. Liver and lungs showed the highest levels relative to plasma, followed by kidney with the lowest levels in heart and brain tissues.

DISCUSSION

SB1317 is a low molecular weight (MW 372.5) compound, with moderately high LogP (4.1), basic in nature (pK_a=9.95), with an aqueous solubility of ~72 µg/mL (193 µM) (Williams et al., 2011 manuscript submitted to *J. Med. Chem*). The high permeability of SB1317, along with the low efflux ratio in Caco-2 cells, suggests high intestinal absorption in humans. Its high PPB could be attributed its hydrophobic and basic nature. Basic compounds are known to bind to α1-acid glycoprotein [6]. The low to moderate stability of SB1317 in liver microsomal fractions show that metabolism could be a clearance mechanism *in vivo*. The scaled hepatic plasma
clearance from the microsomal stability studies was in reasonable agreement with the observed systemic plasma clearance (compare table 1 and 4). This was further substantiated by the formation of metabolites in in vitro metabolism studies, where the major metabolite was the demethylated product, in addition to the oxidation products. The metabolic profiles in mouse, rat and dog were similar to that of in humans, indicating that they could be the appropriate preclinical species for toxicology studies. Since F was low in rats, mouse was recommended as the murine species for toxicology studies. SB1317 was primarily cleared by CYP3A4 and 1A2 in vitro suggesting that these two enzymes may play a significant role in clearance of the compound in humans. Radiolabelled mass balance studies need to be done to assess the main clearance and elimination mechanisms for SB1317. Since the fold induction of human CYP3A4 and 1A by SB1317 in hepatocytes was <40% of the corresponding positive controls, it did not show the potential to induce these enzymes in humans, as per the PhRMA guidance document [7]. The inhibition of CYP2D6 by SB1317 suggests that it may display DDI with drugs cleared by CYP2D6 in humans. However, the pharmacologically active concentrations of SB1317 observed in preclinical pharmacological models were ~10 fold lower [3] than its IC50 on CYP2D6 suggesting that the chance of DDI in humans is low. The PK profile of SB1317 in mice at 75 mg/kg supported the excellent efficacy observed in preclinical studies at similar and lower doses, where a clear PK/PD correlation was observed between plasma concentrations and target biomarker inhibition resulting in inhibition of tumor regression [3]. SB1317 showed high plasma CL in mice and rats (72-100% of LBF). Despite the high CL, SB1317 showed an oral bioavailability of 24% in mice. This may be due to saturation of the metabolic enzymes in the intestine and liver leading to higher bioavailability or due to relatively lower contribution of metabolism to overall clearance. The latter is a stronger possibility since the scaled plasma CL in mice was lesser than the observed systemic clearance in mice. The poor and moderate F in rats and dog, respectively, was consistent with their plasma CL. The high Vss observed in the preclinical species could be explained by the hydrophobic and basic nature of the compound. Basic drugs tend to bind to the negatively charged phospholipids of the cell membranes and resulting in high tissue binding [6]. The scaled human plasma CL (0.6 L/h/kg) from microsomal data suggested low hepatic extraction, and therefore SB1317 is expected to show good oral bioavailability in humans. This needs to be verified when clinical PK data become available. Pacritinib, a potent JAK2 inhibitor, belonging to the same chemical class as SB1317, with approximately similar physicochemical and ADME properties, showed excellent oral exposures in humans [8]. The high tissue levels in mice were consistent with its high Vss. In preclinical pharmacological studies in xenograft mice models, SB1317 showed 10-16 fold higher levels in tumors and was consistent with its high efficacy [3].

In summary, the favorable ADME properties of SB1317 supported its progression to preclinical and clinical development. It is currently undergoing Phase 1 clinical trials in multiple myeloma patients (http://clinicaltrials.gov; ClinicalTrials.gov Identifier NCT01204164).

LIST OF ABBREVIATIONS
**Abbreviations:** AAG: Alpha 1 acid glycoprotein, AUC$_{0-t}$: Area under the plasma concentration-time curve from time zero to the last measured non-zero concentration, AUC$_{0-\infty}$: Area under the plasma concentration-time curve from time zero to infinity, B/P: Blood to plasma ratio, $C_{\text{max}}$: Peak concentration in plasma, CL: Systemic clearance, CL/F: Oral clearance, DDI: Drug-Drug Interaction, EMS: Enhanced Mass Spectrum, F: Oral bioavailability, $f_u$: Fraction of unbound drug in plasma, $f_{u,\text{mic}}$: Fraction of unbound drug in microsomal incubations, LBF: Liver Blood Flow, LLOQ: Lower Limit of Quantitation, PPB: Plasma Protein Binding, TIC: Total Ion Current, $t_{1/2}$: half-life, $V_{\text{ss}}$: Volume of distribution at steady state, $V/F$: Apparent volume of distribution, XIC: Extracted ion chromatogram

**ACKNOWLEDGEMENTS**

**REFERENCES**


Figure 1: TIC-EMS chromatogram of SB1317 and its metabolites in A) Human liver microsomes; B) Dog liver microsomes; C) Rat liver microsomes and D) Mice liver microsomal incubations.
Figure 2: Proposed metabolic pathway of SB1317 and its metabolites formed in microsomal incubations
Figure 3: Mean plasma concentration time profiles of SB1317 following single oral and intravenous doses in A) BALB/c nude mice; B) Wistar rats; C) Beagle dogs; D) In different tissues of BALB/c nude mice. Error bars represent the S.E.M.
CHEMICAL STRUCTURE 1: Chemical structure of SB1317/TG02 ((16E)-14-Methyl-20-oxa5,7,14,26tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa1(25),2(26),3,5,8(27),9,11,16,21,23-decaene
### TABLES

Table 1: *In vitro* ADME properties of SB1317

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<thead>
<tr>
<th>In vitro PPB</th>
<th>% bound*</th>
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<tbody>
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<td>Mouse</td>
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<tr>
<td>Dog</td>
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<tr>
<td>Human</td>
<td>99.9 ± 0.0</td>
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<th>Caco-2 bi-directional permeability</th>
<th>$P_{app} \times 10^{-6}$ cm/s**</th>
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<td>A→B</td>
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<td>B→A</td>
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<td>Efflux Ratio ($P_{app,B→A}/P_{app,A→B}$)</td>
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<th>$CL_{int}$ [µL/min/mg] (CLp L/h/kg)**</th>
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<td>Mouse</td>
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<thead>
<tr>
<th>In vitro CYP inhibition</th>
<th>IC$_{50}$ (µM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>&gt;25</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>&gt;25</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>&gt;25</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

*Mean ± SD; **Estimated from mean values

# 70-100% of parent remained at the end of the assay
Table 2: Fold induction of human CYP3A4 and 1A by SB1317 in human hepatocytes *in vitro*

<table>
<thead>
<tr>
<th>Compound (µM)</th>
<th>CYP3A4</th>
<th>Fold induction relative to positive control (%)</th>
<th>CYP1A</th>
<th>Fold induction relative to positive control &amp; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB1317 (0.1)</td>
<td>0.82 ± 0.09</td>
<td>15$^#$; 23$^s$</td>
<td>0.82 ± 0.02$^*$</td>
<td>5.0</td>
</tr>
<tr>
<td>SB1317 (1.0)</td>
<td>0.03 ± 0.01$^{**}$</td>
<td>0.5$^#$;0.9$^s$</td>
<td>1.2 ± 0.02$^*$</td>
<td>6.7</td>
</tr>
<tr>
<td>SB1317 (10.0)</td>
<td>0.005 ± 0.002$^{**}$</td>
<td>0.09$^#$;0.14$^s$</td>
<td>1.2 ± 0.03$^{**}$</td>
<td>6.7</td>
</tr>
<tr>
<td>Rifampicin (10.0)</td>
<td>5.6 ± 0.2$^{**}$</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dexamethasone (50.0)</td>
<td>3.5 ± 0.2$^{**}$</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Omeprazole (50.0)</td>
<td>NA</td>
<td>NA</td>
<td>17.8 ± 2.3$^{**}$</td>
<td>100</td>
</tr>
</tbody>
</table>

$^p < 0.05$: significantly different from 0.1% DMSO control  
$^{**} p < 0.01$: significantly different from 0.1% DMSO control  
$^\#$ Rifampicin  
$^s$ Dexamethasone  
& Omeprazole  
NA: Not Applicable
Table 3: Metabolites of SB1317 formed in human, dog, rat and mouse liver microsomes based on LC/MS/MS analysis

<table>
<thead>
<tr>
<th>Peak</th>
<th>Description</th>
<th>m/z</th>
<th>HLM</th>
<th>DLM</th>
<th>RLM</th>
<th>MLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Parent</td>
<td>373</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M1</td>
<td>N-demethylation</td>
<td>359</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M2</td>
<td>Oxidation</td>
<td>389</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M3</td>
<td>Oxidation + N-demethylation</td>
<td>375</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M4</td>
<td>Oxidation + dehydration</td>
<td>371</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M5</td>
<td>Oxidation</td>
<td>389</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M6</td>
<td>Oxidation</td>
<td>389</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M7</td>
<td>Di-oxidation</td>
<td>405</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>M8</td>
<td>Oxidation</td>
<td>389</td>
<td>√</td>
<td>ND</td>
<td>ND</td>
<td>√</td>
</tr>
</tbody>
</table>

ND: Not detected
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mouse</th>
<th>Rat (n=3)*</th>
<th>Dog (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>i.v.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>5.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cl(L/h/kg)</td>
<td>6.6</td>
<td>2.4 ± 1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>V&lt;sub&gt;ss&lt;/sub&gt;(L/kg)</td>
<td>23</td>
<td>1.80 ± 1.2</td>
<td>3.1</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;(h)</td>
<td>4.6</td>
<td>1.4 ± 0.31</td>
<td>2.9</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;(ng.h/mL)</td>
<td>732</td>
<td>897 ± 308</td>
<td>819</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;(ng.h/mL)</td>
<td>752</td>
<td>916 ± 309</td>
<td>900</td>
</tr>
</tbody>
</table>

**oral**

| Dose (mg/kg)    | 75    | 10         | 5         |
| t<sub>max</sub>(h) | 0.5    | 0.08 ± 0.0 | 1.0       |
| C<sub>max</sub>(ng/mL) | 1029   | 62 ± 25    | 493       |
| t<sub>1/2</sub>(h) | 6.1    | NE         | 2.9       |
| AUC<sub>0-t</sub>(ng.h/mL) | 2523   | 172 ± 38   | 1659      |
| AUC<sub>0-∞</sub>(ng.h/mL) | 2700   | NE         | 1663      |
| Cl/F(L/h/kg)    | 28    | NE         | 3.0       |
| V/F(L/kg)       | 245   | NE         | 12.6      |
| F (%)           | 24    | 3.8*       | 37        |

* Mean ± SD
* Estimated using mean AUC values
NE: Not Estimated as the terminal phase was flat
Table 5: Pharmacokinetic parameters for SB1317 in plasma and different tissues following a single oral dose of 75 mg/kg in BALB/c nude mice.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/g * or ng/ml)</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; (ng.h/g * or ng.h/ml)</th>
<th>Tissue to Plasma ratio (AUC&lt;sub&gt;0-∞,tissue&lt;/sub&gt; / AUC&lt;sub&gt;0-∞,plasma&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>21095</td>
<td>38918</td>
<td>15.4</td>
</tr>
<tr>
<td>Lung</td>
<td>13618</td>
<td>34751</td>
<td>13.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>5789</td>
<td>17187</td>
<td>6.8</td>
</tr>
<tr>
<td>Heart</td>
<td>1513</td>
<td>7137</td>
<td>2.8</td>
</tr>
<tr>
<td>Brain</td>
<td>2121</td>
<td>6052</td>
<td>2.4</td>
</tr>
<tr>
<td>Plasma</td>
<td>1029</td>
<td>2523</td>
<td>-</td>
</tr>
</tbody>
</table>

* For tissues
Supplementary figure 1: EMS spectra of SB1317 and its metabolites in human liver microsomes with mass ion of A) \( m/z \) 373; B) \( m/z \) 359; C) \( m/z \) 389; D) \( m/z \) 375; E) \( m/z \) 371; F) \( m/z \) 389; G) \( m/z \) 389; H) \( m/z \) 405 and I) \( m/z \) 389.
Supplementary figure 2: EMS spectra of SB1317 and its metabolites in dog liver microsomes with mass ion of A) m/z 373; B) m/z 359; C) m/z 389; D) m/z 375; E) m/z 371; F) m/z 389; G) m/z 389 and H) m/z 405.
Supplementary figure 3: EMS spectra of SB1317 and its metabolites in rat liver microsomes with mass ion of A) m/z 373; B) m/z 359; C) m/z 389; D) m/z 375; E) m/z 371; F) m/z 389; G) m/z 389 and H) m/z 405
Supplementary figure 4: EMS spectra of SB1317 and its metabolites in mice liver microsomes with mass ion of A) m/z 373; B) m/z 359; C) m/z 389; D) m/z 375; E) m/z 371; F) m/z 389; G) m/z 389; H) m/z 405 and I) m/z 389.