DISPOSITION AND METABOLISM OF

\[^{14}C\]SB-649868 AN OREXIN 1 and 2 RECEPTOR

ANTAGONIST IN HUMANS

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RUNNING TITLE

a) Disposition and metabolism of $[^{14}\text{C}]\text{SB-649868}$ in humans

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d) Abbreviations used are:

AUC = area under the plasma concentration-time curve
BQL = below quantification limit
$C_{\text{max}}$ = maximal plasma concentration
CL = systemic plasma clearance
CI = confidence interval
F= Bioavailability
HPLC = high performance liquid chromatography
HPLC-MS/MS = high performance liquid chromatography tandem mass spectrometry
QC = quality control
Q-TOF = quadrupole/ time-of-flight
NMR = nuclear magnetic resonance spectroscopy
SB-649868 = N-[[2S]-1-[[5-(4-fluorophenyl)-2-methyl-4-thiazolyl]carbonyl]-2-piperidinyl]methyl]-4-benzofurancarboxamide
t_{\text{max}} = \text{time to reach } C_{\text{max}}

t_{1/2} = \text{half life}

WMA = \text{World Medical Association}
ABSTRACT

SB-649868 is a novel orexin 1 and 2 receptor antagonist under development for insomnia treatment. The disposition of [14C]SB-649868 was determined in eight healthy male subjects using an open label study design after a single oral dose of 30 mg. Blood, urine and feces were collected at frequent intervals after dosing and samples were analyzed by High Performance Liquid Chromatography/Mass Spectrometry coupled with off-line radiodetection for metabolite profiling and characterization. Nuclear magnetic resonance spectroscopy was also employed to further characterize certain metabolites. Elimination of drug-related material was almost complete over a 9 day period, occurring principally via the feces (79%), whereas urinary excretion accounted only for 12% of total radioactivity. Mean apparent half life (t1/2) of plasma radioactivity was notably longer (39.3 h), with respect to that of unchanged SB-649868 (4.8 h), suggesting the presence of more slowly cleared metabolites. SB-649868 and an unusual hemiaminal metabolite, M98 (GSK2329163), resulting from oxidation of the benzofuran ring and subsequent rearrangement, were the principal circulating components in plasma extracts. Two additional minor metabolites were also observed, a benzofuran ring opened carboxylic acid M25 (GSK2329158) and an amine metabolite (M8). SB-649868 was extensively metabolized and only negligible amounts were excreted unchanged. The principal route of metabolism was via oxidation of the benzofuran ring with the resultant M25 being the principal metabolite in excreta, representing at least 12% of the administered dose across urine and feces.
INTRODUCTION

SB-649868, N-[(2S)-1-[[5-(4-fluorophenyl)-2-methyl-4-thiazolyl]carbonyl]-2-piperidinyl]methyl]-4-benzofurancarboxamide, is a potent and selective orally active orexin 1 and orexin 2 (OX1/OX2) receptor antagonist under development for insomnia treatment. A large body of evidence supports a role for orexin in the control of arousal and sleep/wake regulation [Lin et al., 1999; Chemilli et al., 1999; Kilduff and Peyron, 2000].

Animal studies have shown that SB-649868 has a hypnotic activity similar to that of zolpidem, but that it can be differentiated from benzodiazepine-like agents acting at the GABAA receptor complex, in terms of hypnotic profile and side effect potential (Gerrard et al., manuscript in preparation).

Single oral doses up to a maximum dose of 80 mg and repeat doses up to a maximum dose of 30 mg have been administered to healthy volunteers (Bettica et al., submitted). After single dose, SB-649868 C\textsubscript{max} and AUC increased approximately proportionally over the 10-60 mg dose range with terminal phase half-life in the 2.2-7.4 h range. SB-649868 steady-state exposure was dose- and time-dependent and generally slightly higher than following single-dose administration.

The excretion, metabolism and pharmacokinetics of SB-649868 in rat, dog and mouse have been studied (unpublished data). Elimination of SB-649868 in rat and mouse was largely by metabolism, with metabolites secreted primarily in the bile and/or feces. Elimination of SB-649898 in the Beagle dog appeared to be largely as unchanged parent compound via the feces. The elimination half-life was short in rat and dog (t\textsubscript{1/2}<1h).

The purpose of this work was to characterize the disposition and metabolism of [\textsuperscript{14}C] SB-649868 after a single oral administration to healthy male volunteers.
MATERIALS AND METHODS

Chemicals. [14C]SB-649868, SB-649868, [2H4]SB-649868 (deuterated in the monofluoro ring) and two synthetic metabolites (provided as a semi-crude reaction mixture), M25 (coded as GSK2329158) and M98 (coded as GSK2329163), were all supplied by Synthetic Chemistry, GlaxoSmithKline Research and Development, Stevenage (UK). These materials were used as chromatographic, mass spectrometric and/or nuclear magnetic resonance spectroscopy standards during the study. Commercially obtained chemicals and solvents were of high-performance liquid chromatography (HPLC) or analytical grade. Liquid scintillation cocktails were obtained from PerkinElmer LAS (UK) Limited (Beaconsfield, Bucks, UK).

SB-649868 Formulation. [14C]SB-649868 (2.37 μCi /mg, radiochemical purity 99.8%) was provided as powder in the bottle, to be reconstituted into a solution prior to dosing via a hard gelatine capsule (00 size). [14C]SB-649868 (604 mg, 1.43 mCi) was weighed into a new glass vial. Propylene glycol monocaprylate (14.2 g) was added to the vial and the vial sonicated for 80 min and stability of the dose subsequently confirmed. A portion of the solution (0.737 g, equivalent to 30 mg of [14C]SB-649868) was weighed into pre-weighed capsules.

Study Design and Subjects. The clinical study (GSK study number OXS109139) was performed at Charles River Laboratories Clinical Services (Edinburgh, UK) in accordance with Good Clinical Practice and the principles of the WMA of the Declaration of Tokyo (2004). The protocol was reviewed and approved, as appropriate, by the Edinburgh Independent Ethics Committee for Medical Research and the Medicine and Healthcare products Regulatory Agency (MHRA). The proposed radioactive dose was approved by the Administration of Radioactive
Substances Advisory Committee (ARSAC). Written consent was obtained from all subjects before any protocol-specific procedures.

The study was an open-label, single session study. Eight healthy male subjects (Caucasian), between 30 and 55 years of age, body weight >50 Kg and body mass index between 18.5 and 29.9 kg/m² were selected from a panel of volunteers. They were determined healthy by a responsible physician based on medical evaluation. Subjects had normal levels of FSH, LH and testosterone at screening and with no history of drug or alcohol abuse, and were on no other medication at the time of the study, with no prescribed medication within 14 days of the study commencing.

**Study Procedure.** All subjects received a single oral administration of 30 mg (approximately 70 µCi) of [¹⁴ C]SB-649868 in a capsule dose swallowed with 160 ml of water. After oral administration, blood samples (7 ml) for total radioactivity and parent drug analysis were collected in potassium EDTA tubes at pre-dose, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9,12, 18, 24, 32, 48, 72 and 96 h post-dose; additional samples (30 ml) were collected from each subject at 3, 12, 24, and 48 h post-dose for metabolite analysis and an additional 20 ml were collected from one single subject as pre-dose sample. Blood samples were mixed, immediately chilled on crushed ice, and centrifuged for 10 min at 1500 g at approximately 4°C to obtain plasma. Total radioactivity was measured using triplicate aliquots of plasma (0.25 ml); the remaining plasma was stored at -80°C before the assay for SB-649868 or metabolite profiling by HPLC.

Urine samples were collected pre-dose and between 0 to 6 h, 6 to 12 h and 12 to 24 h after drug administration and then at 24 h intervals until 216 h post-dose. The urine samples collected at each time point from each subject were combined and the pH and weight recorded. A single sub-sample (50 ml) was removed from the
bulk sample for metabolite profiling and identification and stored at -20°C. Total radioactivity was measured using triplicate aliquots (ca. 1 ml) of urine.

Feces samples were collected quantitatively pre-dose and at 24 h intervals until 216 h post-dose. Feces from each collection interval were mixed, weighed and homogenized 1:1 with water and the total homogenate weight recorded. Triplicate aliquots (0.3 g) of feces homogenates were used for radioactivity determination; a sub-sample (30 g) was stored at -20°C for metabolite profiling and identification.

**Assay of Total Radioactivity.** Triplicate aliquots of urine (1 ml) and plasma (0.25 ml) were mixed with Aquasafe 500 Plus scintillation fluid (10 ml, Zinsser Analytic, Maidenhead, UK) for Liquid Scintillation Counting (LSC), together with blank and spiked samples. Plasma samples had an additional 1 ml of water added. Triplicate aliquots of feces homogenates (0.3 g) were weighed into Combustocones containing Combustopads for oxidation using a Packard Tri-Carb 307 automatic sample oxidizer (PerkinElmer LAS (UK) Ltd.). The $^{14}$CO$_2$ generated was collected by absorption in CarboSorb (8 ml) to which Permafluor was added. Radioactivity was quantified using a liquid scintillation counter (PerkinElmer LAS (UK) Ltd.) with automatic quench correction using an external standard method (Botta et al., 1985). Prior to calculation of individual results a background count rate was determined and subtracted from each sample count rate.

**SB-649868 Quantification.** Plasma concentrations of SB-649868 were quantified using a validated analytical method based on protein precipitation with acetonitrile, followed by HPLC tandem mass spectrometry (HPLC-MS/MS) analysis. SB-649868 was extracted from 50 μl of human plasma sample by protein precipitation using acetonitrile (200 μl) containing $^{[2]$H$_4]$SB-649868 (at a concentration of 0.5 μg/ml) as an internal standard. After vortex mixing, the deproteinized samples were centrifuged
for 15 min at approximately 3000g. The supernatant was analyzed by HPLC-MS/MS using a TurbolonSpray™ interface and multiple reaction monitoring. The chromatography made use of a 50 x 2.1 mm i.d. Hypersil- Keystone BetaMax Neutral C18 (5 μ) column (column temperature set at 40°C) and an isocratic elution at a flow rate of 0.8 ml/min. The isocratic mobile phase composition was 0.1% formic acid:acetonitrile, 50:50 (v/v). The mass spectrometer used was a IONICs EP10 triple quadrupole (Applied Biosystems, Concord, Ontario, Canada) operated in the positive-ion mode. The temperature of the probe was maintained at 480°C with a curtain gas setting of 6 and collision gas setting of 4.

The concentration of SB-649868 in plasma samples was back calculated from calibration plots of the analyte peak area ratio to that of the internal standard versus the nominal concentration, constructed with a duplicate set of calibration standards prepared at known concentration of SB-649868 in human plasma. A weighted 1/x² linear regression was applied in each case over the range 5 to 5000 ng/ml. Quality control (QC) samples, prepared at three different analyte concentrations and stored with study samples, were analyzed with each batch of samples against separately prepared calibration standards. QC samples and calibration standards were prepared using independently prepared stock solutions of SB-649868 reference materials. For the analysis to be acceptable, no more than one third of the QC results were to deviate from the nominal concentration by more than 15%, and at least 50% of the results from each QC concentration were to be within 15% of nominal.

**Quantification and Profiling of Metabolites in Plasma, Urine and Feces.**

Plasma samples from each time point for each volunteer were analyzed separately. Representative samples of urine and feces were pooled by total weight ratio for each volunteer, in order to obtain a pool containing 90% or greater of the radioactivity
excreted via that route. Urine was generally pooled over a 0 – 72 h collection period for each volunteer, whereas for feces, typical pooling schedule incorporated 24 – 72 h collection, however in two of the volunteers, feces collected up to the 120 h collection was incorporated in the pool.

After thawing, aliquots of plasma samples were taken for LSC prior to solvent extraction. Samples were extracted by vortex-mixing aliquots (1 to 4 ml) with five volumes (5 to 20 ml) of methanol. The extracts were then centrifuged at 3,300 g for 10 min at 4°C. The supernatant was removed and the process repeated two additional times. The supernatants were combined, evaporated to dryness and reconstituted in methanol:water, 50:50 (v/v, 0.5 ml) prior to radio-HPLC analysis. The residual pellet was digested in 1 M sodium hydroxide and the total digest was split into further aliquots, each of which was subsequently diluted with scintillation fluid (15 ml) before being analyzed by LSC to determine actual recovery in the extract.

Fecal homogenates (approximately 2 g) were extracted by mixing with 10 ml of methanol. After centrifugation, the supernatant was removed and the process was repeated two additional times. The residual pellets were dried in a drying cabinet before being oxidised to determine the extent of any unextracted radioactivity in the pellets. An aliquot (500 µl) of the initial methanol extract (which contained the majority of the radioactivity) was diluted with 500 µl of distilled water and the entire sample subjected to radio-HPLC or HPLC-MS^n analysis.

No pre-treatment of any pooled urine samples was undertaken prior to radio-HPLC or HPLC-MS^n analysis.

All spiked control and blank control samples were subjected to the same methods of pre-treatment as detailed for the test samples.
**HPLC method**: The chromatographic instrument for radio-HPLC analysis consisted of a Jasco Intelligent binary pump PU1580, autosampler and column oven (30°C) (Jasco Model AS 1555 and Jasco model CO160, respectively) using a Waters Symmetry Shield RP8, 25 cm x 4.6 mm, 5 μm with a Phenomenex C8 security guard column (Phenomenex Inc, Macclesfield, Cheshire, UK). The chromatographic instrument for HPLC-MS\(^n\) analysis consisted of a HP 1100 gradient pump, autosampler (CTC Analysis LC PAL) using a Waters Symmetry Shield RP8, 25 cm x 4.6 mm, 5 μm with a Phenomenex C8 security guard column.

The mobile phase consisted of 45 mM ammonium acetate pH 3.8 (with formic acid) (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. A complex gradient system was utilised which included three phases of linear increases each followed by an isocratic period. Initial conditions were set at 5% B. The first phase included a two step linear increase; initially to 25% B after 5 min and then up to 36% B after 20 min, followed by an isocratic period until 24 min. The second phase included a further two step linear increase; initially to 42% B after 30 min and then up to 55% B after 35 min, followed by a second isocratic period until 40 min. The third phase of the gradient was a linear increase to 95% B by 45 min, with these conditions being maintained for a further 5 min.

HPLC column recoveries were determined on selected samples by collecting the total HPLC column eluate for the appropriate run and assaying the radioactivity to assess recovery of injected radioactivity. Full recoveries of radioactivity were obtained from the HPLC eluate collected.

Radio-HPLC data were captured off-line (Bruin et al., 2006) with chromatographic fractions collected using a Gilson 222XL fraction collector into 96 deep-well microtitre LUMAPLATES™ containing yttrium silicate solid scintillant (Perkin Elmer LAS (UK))
Radioactivity determination was performed by scintillation counting using a Packard Topcount NXT counter (Perkin Elmer LAS (UK) Ltd).

Metabolites of interest were isolated by preparative HPLC (Agilent HP 1100 pumps) with a Symmetry shield RP8, 25 cm x 4.6 mm, 5 µm. The mobile phase consisted of 45 mM ammonium acetate in D$_2$O (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. The fractions were submitted directly for NMR analysis.

**Structural Identification of Metabolites.** Structural characterization was performed on selected samples by HPLC-MS$^n$ using triple quadrupole Quattro Micro (Waters MS Technologies, Manchester, UK.). Electrospray ionization, in the positive ion mode, was used. The HPLC flow was split (1:3) between mass spectrometer and waste or fraction collector.

Metabolites were identified based on charged molecular ions and their collision-induced dissociation fragmentation (Oliveira and Watson, 2000). Authentic standards, when available, were used to compare chromatographic retention times and fragmentation patterns. Supporting data from preclinical studies were also used in the assignment of metabolite structures. For many metabolites, confirmation of the structure has been obtained by $^1$H- NMR experiments using a Bruker Avance 600 spectrometer (Bruker, Rheinstetten, Germany) equipped with an inverse 5-mm TCI Cryo-Probe (Bruker) ($^1$H/$^{13}$C/$^{15}$N) operating at 600.40 MHz under the control of Topspin 2.1. $^1$H-NMR spectra were acquired using a standard NOESYPRESAT pulse sequence with spoil gradients for solvent suppression with time-shared double presaturation of the water and acetonitrile frequencies. In these experiments, typically 128 transients were acquired into 48 K data points over a spectral width of 12019 Hz (20 ppm) with an interscan delay of 3 s giving a pulse repetition time of 5 s. Acquisition time was extended up to 1k transients for some metabolites to improve
signal to noise. Fully characterized metabolites were designated by the letter M followed by a number; where a synthetic standard was available, a GSK code number was assigned.

**Pharmacokinetic analysis.** Actual blood collection time and the actual dose administered were used for all pharmacokinetic calculations. Standard noncompartmental methods were used to derive pharmacokinetic parameters using WinNonlin (version 5.01 Pharsight corporation, Mountain View, CA). Maximum plasma concentration (C\text{max}) and time of C\text{max} (t\text{max}) were taken directly from the pharmacokinetic concentration-time data. Where possible, the terminal plasma elimination rate constant (λz) was estimated from log-linear regression analysis of the terminal phase of the plasma concentration time profile. The numbers of points included in the terminal phase was determined by visual inspection of the semi-log plots of the plasma concentration time profiles. The associated t_{1/2} was calculated as ln2/ λz. Values of AUC\text{0-1} and AUC\text{ 0-inf} were calculated by a combination of linear and logarithmic trapezoidal methods. The linear trapezoidal method was used for all incremental trapezoids arising from increasing concentrations and the logarithmic trapezoidal method was used for those arising from decreasing concentrations. Values of CL/F were calculated as Dose/AUC\text{0-inf}. 
RESULTS

Mass Balance of Total Radioactivity. The actual oral dose of [14C]SB-649868 administered ranged from 29.6 to 30.2 mg (69.8 to 71.9 μCi, respectively). The cumulative excretion of total radioactivity following single oral administration of [14C]SB-649868 to healthy male subjects at a target dose of 30 mg was 91.1%, details of which are shown in Table 1.

Following oral administration, total radioactivity was eliminated primarily in the feces, accounting for a mean of 79.1% of the administered dose by 216 h post-dose. Total radioactivity recovered in urine accounted for a mean of 12.0% of the administered dose by the end of collection period. A mean recovery of 81.2% was reached by 96 h post-dose, with the remaining radioactivity recovered between urine and feces up to 216 h post-dose.

Pharmacokinetics. A summary of the plasma pharmacokinetic parameters for SB-649868 and total radioactivity following oral administration is presented in Table 2. Median plasma concentrations of SB-649868 and total radioactivity following oral administration are shown in Fig. 2.

In plasma, concentrations of total drug-related material (radioactivity) were measurable in all subjects between 0.5 and 96 h post-dose (last blood sampling time). Mean plasma levels of total radioactivity reached a peak of 1.66 μg equiv /ml by 4 h post-dose, declining thereafter in a biphasic manner with an inflection point about 18 to 24 h, and were still clearly detectable (0.108 μg equiv/ml) in the final plasma sample taken at 96 h post-dose. The mean apparent t1/2 of total plasma radioactivity was 39.3 h.

SB-649868 was measurable in plasma from all subjects between 0.5 and 24 h post-dose, with mean Cmax (1.2 μg/ml) occurring at 4 h post-dose. Concentrations of SB-
649868 declined thereafter with a t\(_{1/2}\) ranging between 4 and 6 h, and by 96 h post-dose, SB-649868 concentrations were below the limit of quantification in all subjects.

**Metabolic profiles.** *Plasma.* The mean recovery of radioactive material following extraction of human plasma samples was initially high but decreased at later time-points, from ca. 97% at 3 h to 34% at 48 h post-dose. Fig. 3 shows three representative radiochromatogram profiles at 3 h, 12 h and 24 h post-dose. Quantification of drug-related material in plasma extracts after oral administration at selected time-points is summarized in Table 3.

At each of the four time-points examined, metabolite profiles were qualitatively similar with unchanged SB-649868 being the principal component at 3 h (68%) and 12 h (33%) and decreasing to 10% of plasma radioactivity at 24 h post-dose. Another notable radiolabeled component observed at each time point was a hemiaminal metabolite M98 (GSK2329163), which accounted for between 6% and 19% of plasma radioactivity across the four time-points examined and was the predominant component in plasma extracts at 24 h and 48 h post-dose. A benzofuran ring opened carboxylic acid M25 (GSK2329158) and an amine metabolite (M8) were also detected at each of the time-points, representing up to 7% and 3% of plasma radioactivity, respectively. A ratio of the relative exposures of each metabolite can be determined using the percentage of total radioactivity for each component at each of the four time-points (to calculate an approximate AUC\(_{0-48h}\)). This shows M98, M25 and M8 accounting for around 11%, 2% and 1% of the total radioactivity, with parent compound approximating to 26% of the total. Peak assignments accounted for between 18% and 75% of the plasma radioactivity across the four time-points examined. Proposed structures and supporting spectral data are shown in Table 4.
**Urine.** Fig. 4a shows a representative radiochromatogram of urine. The major radiolabeled metabolites detected in human urine following oral administration were GSK2329158 (M25), its glucuronide conjugate (M41) and a benzofuran dihydrodiol or ring opened carboxylic acid glucuronide (M95), which accounted for ca. 13%, 14% and 11% of urinary radioactivity, respectively (each less than 2% of the administered dose, respectively). Other metabolites detected included M8 and a hydroxylated benzofuran component (M86), each representing 5 - 6% of urinary radioactivity, respectively (<1% of the administered dose, respectively). All other identified urinary radiometabolites accounted for 0.3% or less of the dose, and SB-649868 was not quantifiable. Proposed structures and supporting spectral data are shown in Table 4.

**Feces.** Radio-HPLC analysis of fecal extracts after oral administration of [14C]SB-649868 showed many radiometabolites. A representative radiochromatogram of fecal extracts is shown in Fig. 4b.

Mean recovery of radioactive material in feces following solvent extraction was 67%. Radio-HPLC analysis of human fecal extracts revealed a complex pattern of radiolabeled peaks with GSK2329158 (M25) being the major radiolabeled component in fecal extracts (mean of ca. 14% of the fecal radioactivity and 10% of the administered dose). Other noteworthy metabolites included benzofuran dihydrodiols (M20/M21) which together accounted for 9% of fecal radioactivity (6% of the dose) and oxygenated benzofuran dihydrodiol or ring opened carboxylic acid metabolites (M99, M100, M102), which in total represented 10% of the fecal radioactivity (7% of the administered dose). The hemiaminal, M98 (GSK2329163), which co-eluted with a hydrated metabolite (M101), together represented ~4% of the fecal radioactivity (~3% of the administered dose). All other identified fecal radiometabolites accounted for...
2% or less of the dose, with SB-649868 present in negligible amounts. Proposed structures and supporting spectral data are shown in Table 4.
DISCUSSION

Following a single oral dose of [14C]SB-649868 at 30 mg (approximately 70 μCi) to eight male healthy subjects, the compound was safe and well tolerated with the most frequent adverse event being somnolence, that was expected and is considered related to the pharmacology of SB-649868.

Total radioactivity was eliminated primarily in feces (approximately 79%), with urinary excretion of total radioactivity accounting for 12% of the dose after the 9 day collection period. Mean recovery of total radioactivity was > 91% over a 9-day period and can be considered complete (Roffey et al., 2007), with 81% excreted within the first 4 days, with fecal elimination largely occurring between 24 and 72 h. Post 96 h, the remaining radioactivity was subsequently eliminated more slowly and was still detectable in fecal samples at the end of the continuous collection period (9 days), although clearance of SB-649868 from plasma was almost complete within 24 h of dosing.

The mean t½ of SB-649868 was short (4 to 6 h), whilst apparent half life of total radioactivity was much longer (about 40 h), suggesting the presence of metabolites that are either formed at later time-points or are much more slowly cleared from plasma (or both), as further evidenced by the ratio of systemic exposure (AUC and Cmax) of SB-649868 plasma concentrations to total radioactivity of approximately 22% and 72%, respectively.

SB-649868 represented the principal component in extracts of human plasma up to 12 h after dose administration. An unusual hemiaminal metabolite, M98 (GSK2329163), was the only other drug-related material observed in plasma extracts at >10% drug-related material, and was more notable relative to parent compound at later time-points. Two additional minor metabolites were also observed in human
plasma extracts: a ring-opened carboxylic acid M25 (GSK2329158) and an amine (M8) resulting from cleavage of the molecule. The relative exposures of each metabolite (AUC<sub>0-48h</sub> using percentage of total radioactivity values from plasma profiles) showed M98, M25 and M8 accounting for approximately 11%, 2% and 1% of the total radioactivity. The characterization of M98 structure in human plasma posed a significant challenge.

M98 freely lost water, and was first detected as its dehydrated isoquinolinone product (M94). Nonetheless, on careful handling, sufficient intact material could be preserved and compared against synthetic material by NMR (Fig. 5). M98 showed loss of benzofuran resonances and the addition of an aliphatic three spin system (ABX). Long range proton-carbon correlations ruled out a plausible lactol structure, unequivocally proving M98 to be the hemiaminal. Mechanism of formation is tentatively suggested as nucleophilic attack of the amide moiety on an aldehyde intermediate (Bayer and Maier, 2004). Based on the structure of metabolites a putative simplified metabolic scheme is shown in Fig. 6. The first step in the formation of both M98 and M25 was inferred to be the oxidation of the benzofuran moiety via the formation of a potentially reactive epoxide intermediate (Guengerich, 2002; Kalgutkar et al., 2005). Oxidative ring opening of furan containing moieties through formation of an epoxide intermediate, mediated by CYP450 has been previously reported (Dalvie et al., 2002), supporting the hypothesis of the mechanism of formation of M25. In addition, the formation of M25 can be postulated both via M86, the hydroxylated benzofuran metabolite, and via an aldehyde and the action of aldehyde dehydrogenase (Kobayashi et al., 1987). Moreover, in vitro data confirmed that oxidative metabolism of SB-649868 was occurring largely via the benzofuran moiety. Experiments conducted utilizing Bactosomes (derived from E.coli) containing
individually overexpressed human CYP450 enzymes (1A2, 2C8, 2C9, 2C19, 2D6 and 3A4) and incubations with human liver microsomes, in the presence and absence of selective inhibitors (azamulin was used as a selective inhibitor of CYP3A4), confirmed metabolism was primarily due to CYP3A4. The predominant metabolite of SB-649868 in these incubations was identified as M86, along with M25. Following initial oxidation activating the furan ring, competing processes are possible, intermolecular hydrolysis to the acid (M25) versus intramolecular condensation to the hemiaminal, M98. Other notable routes of metabolism of SB-649868 in vitro were oxidation to form benzofuran dihydrodiol isomers (M20 and M21) all of these biotransformations appearing to be catalyzed primarily by CYP3A4. Interestingly, in-vitro formation of M86 could be attenuated by the addition of glutathione, with subsequent preferential formation of the glutathione adduct being observed. The formation of M98 was not, however, observed with either human liver microsomes or recombinant enzymes. Subsequent work with rat and mouse Aroclor 1254-induced liver S9 did show the formation of M98, along with other drug-related components, which suggests that cytosolic enzymes may contribute to the formation of M98.

All the human metabolites observed in plasma were also seen in preclinical species. Both GSK2329163 (M98) and GSK2329158 (M25) were observed in plasma from preclinical species, after single and repeat dose administration, at levels lower (rat and mouse) or equivalent (dog) to human, with SB-649868 the major observed component in plasma in all preclinical species evaluated. Subsequent analysis of both metabolites (using a validated bioanalytical method) following single and repeat dosing of SB-649868 (once daily) to humans showed similar accumulation of GSK2329163 (M98) to that seen with parent compound (approximately 1.5-fold, Bettica et al), whereas GSK2329158 (M25) showed around 2- to 3-fold accumulation.
The result is GSK2329163 has systemic exposures around 10% of parent compound, whilst GSK2329158 has comparable exposure to that of SB-649868 upon repeat dose (unpublished data). Both metabolites were pharmacologically tested, in-vitro, versus OX1 and OX2 receptors and both have lower affinities against each receptor, and therefore are not anticipated to augment the pharmacological activity of SB-649868.

SB-649868 was extensively metabolized in human and parent compound was negligible in both urine and feces. As expected from in-vitro data, metabolism of SB-649868 in humans occurred predominantly via oxidations of the benzofuran moiety, some of which are likely to proceed via the formation of a potentially reactive epoxide intermediate. The principal drug-related component observed in excreta was M25 (GSK2329158), which accounted for a mean of 12% of the administered dose. M86 was observed but only as a minor urinary component, and there was an absence of glutathione or glycylcysteine conjugates. This suggests that either formation of M25 predominate in-vivo or M86 (or its precursor) is rapidly degrading or is further metabolized, or potentially binds to protein. Although M98 was notable in plasma, it was a more minor route of elimination (~3% of dose), indicating formation may be limited with its presence in plasma suggestive of a low volume of distribution. Nonetheless, M98 together with benzofuran dihydrodiols (M20/M21), oxygenated benzofuran dihydrodiol or ring opened carboxylic acid metabolites (M99, M100, M102), and a glucuronide of an oxygenated benzofuran dihydrodiol or ring opened carboxylic acid (M41), accounted for a mean of ca. 20% of the administered dose, and further underline oxidation of the benzofuran as the predominant route of metabolism in humans. This is consistent with preclinical species, despite the
absence of any glutathione or glycylcysteine conjugates observed preclinically, most notably within the bile of rats.

Low recovery of radioactive material from feces (and later plasma samples), together with protracted elimination of total drug-related material, would also be consistent with metabolic activation to a reactive species and subsequent binding to endogenous protein material (Zhang et al., 2003). Although no attempt was made to establish whether the binding to plasma proteins was covalent in nature, there is evidence indicating covalent binding of drug-related material to microsomal protein (based on SDS-Page electrophoresis) following in-vitro incubations (unpublished data). The persistence of low levels of drug-related material in numerous tissues in rats beyond 10 days post-dosing of [14C]SB-649868 is also consistent with this inference. The formation of reactive metabolites has been linked to certain toxicities, e.g. hepatotoxicity, which may involve modification of proteins and/or direct cell damage, either of which could induce an immune-mediated response, although proper elucidation of this relationship remains unclear (Uetrecht, 2008). Furthermore, the proposed low therapeutic dose of SB-649868 (20 mg) reduces the risk of toxicity associated with the presence of a reactive pathway, since the daily dose appears to be a key underlying factor in drug toxicity (Lammert et al., 2008; Kalgutkar and Didiuk, 2009).

Following oral administration of [14C]SB-649868 to humans a mean of ca. 37% of the administered dose was assigned structures, which represented between 59% and 65% of the radioactivity in either pooled (urine) or extracted (feces) samples. The remaining unaccounted radioactivity can be ascribed to several areas where losses were incurred: - a balance excretion recovery of ~91% of the administered dose (at 216 h); low recovery during fecal extractions (33% of fecal radioactivity was
unrecovered); numerous components that individually represented 1% or less of the dose or could not be distinguished above background radioactivity; samples containing insufficient radioactivity to warrant pooling and quantitative analysis (typically represented up to 7% of the dose).

In conclusion, following oral administration of [14C]SB-649868 to humans, drug-related material is cleared almost exclusively via metabolism, predominantly via oxidation of the benzofuran moiety, with metabolites excreted primarily in feces, the most notable of which was GSK2329158 (M25). One major circulating metabolite was identified as GSK2329163 (M98) which accounted for >10% of circulating drug-related material, although this metabolite was a minor route of elimination. Despite the complex metabolism of SB-649868 a good understanding of clearance routes in humans was obtained.
ACKNOWLEDGEMENTS

We thank Peter Szeto for synthesizing SB-649868 and its metabolites, GSK2329158 and GSK2329163, and for his collaboration in the characterization of GSK2329163 structure. We also thank Glynn Williams for the synthesis of [14C]SB-649868, Lindsay McGregor, Christopher Irvine and Janet Dickson from Charles River, Richard Snell for HPLC/MS quantification of SB-649868, Steve Plested for the radiometabolite profiling, and the entire SB-649868 Clinical Pharmacology team for the design, conduct, and analysis of the clinical portions of the study. We also thank Gordon Dear and Andy Ayrton for their helpful discussion and Maxine Taylor for provision of the human enzymology data.
AUTHORSHIP CONTRIBUTION

Participated in research design: Zamuner, Bettica

Conducted experiments: Nash, Thomas, Wright

Performed data analysis: Nash, Thomas, Wright, Zamuner

Wrote or contributed to the writing of the manuscript: Renzulli, Nash, Wright, Thomas, Zamuner, Pellegatti, Bettica, Boyle

Other: Bettica (Medical monitor)
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Kalgutkar AS and Didiuk MT (2009) Structural alerts, reactive metabolites, and protein covalent binding: how reliable are these attributes as predictors of drug toxicity? *Chemistry and Biodiversity* **6**: 2115-37.


LEGENDS FOR FIGURES

Figure 1:
Structure of [14C]SB-649868

Figure 2:
Median SB-649868 concentrations and total radioactivity levels (semi-logarithmic scale) in plasma after oral administration of [14C]SB-649868 at the target dose of 30 mg.

Figure 3:
Representative radiochromatograms of human plasma at various time-points after oral administration of [14C]SB-649868 at the target dose of 30 mg.

Figure 4:
Representative radiochromatograms of human (a) urine and (b) feces after oral administration of [14C]SB-649868 at the target dose of 30 mg

Figure 5:
Assigned 1H NMR of hemiaminal metabolite (M98, GSK2329163)

Figure 6:
Putative simplified metabolic scheme for SB-649868 in humans
### TABLE 1

Mean (S.D.) cumulative total radioactivity excreted through urine and feces (% of dose) after single oral administration of [\(^{14}\text{C}\)]SB-649868 at a target dose of 30 mg to eight healthy volunteers

<table>
<thead>
<tr>
<th>Time elapsed from dose (h)</th>
<th>N</th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>8</td>
<td>2.3 (0.5)</td>
<td>-</td>
<td>2.3 (0.5)</td>
</tr>
<tr>
<td>0-12</td>
<td>8</td>
<td>5.9 (1.1)</td>
<td>-</td>
<td>5.9 (1.1)</td>
</tr>
<tr>
<td>0-24</td>
<td>8</td>
<td>8.9 (1.5)</td>
<td>1.9 (3.1)</td>
<td>10.8 (2.4)</td>
</tr>
<tr>
<td>0-48</td>
<td>8</td>
<td>10.7 (1.8)</td>
<td>36.8 (23.5)</td>
<td>47.5 (22.8)</td>
</tr>
<tr>
<td>0-72</td>
<td>8</td>
<td>11.3 (1.9)</td>
<td>60.0 (19.2)</td>
<td>71.3 (18.5)</td>
</tr>
<tr>
<td>0-96</td>
<td>8</td>
<td>11.6 (1.9)</td>
<td>69.6 (10.9)</td>
<td>81.2 (9.9)</td>
</tr>
<tr>
<td>0-120</td>
<td>8</td>
<td>11.7 (1.9)</td>
<td>75.6 (3.7)</td>
<td>87.3 (2.4)</td>
</tr>
<tr>
<td>0-144</td>
<td>8</td>
<td>11.9 (1.9)</td>
<td>76.8 (3.5)</td>
<td>88.7 (2.3)</td>
</tr>
<tr>
<td>0-168</td>
<td>8</td>
<td>11.9 (2.0)</td>
<td>77.7 (3.3)</td>
<td>89.7 (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-------</td>
<td>------</td>
<td>---</td>
</tr>
<tr>
<td>0-192</td>
<td>8</td>
<td>12.0 (2.0)</td>
<td>78.5 (3.2)</td>
<td>90.5 (2.2)</td>
</tr>
<tr>
<td>0-216</td>
<td>8</td>
<td>12.0 (2.0)</td>
<td>79.1 (3.0)</td>
<td>91.1 (2.2)</td>
</tr>
</tbody>
</table>
### TABLE 2

*Geometric Mean (95% CI) SB-649868 and total radioactivity plasma pharmacokinetic parameters after single oral administration of [14C]SB-649868 at a target dose of 30 mg*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SB-649868</th>
<th>Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (μg.h/mL) $^a$</td>
<td>8.30 (5.90, 11.7)</td>
<td>37.3 (31.0, 44.8)</td>
</tr>
<tr>
<td>AUC$_{(0-\infty)}$ (μg.h/mL) $^a$</td>
<td>8.20</td>
<td>31.0</td>
</tr>
<tr>
<td>C$_{max}$ (μg/mL) $^a$</td>
<td>1.20 (1.00, 1.50)</td>
<td>1.66 (1.40, 2.00)</td>
</tr>
<tr>
<td>t$_{max}$ (h) $^b$</td>
<td>4.00 (3.00-4.00)</td>
<td>4.00 (3.00, 6.00)</td>
</tr>
<tr>
<td>t$_{1/2}$ (h)</td>
<td>4.80</td>
<td>39.3</td>
</tr>
<tr>
<td>CL/F (mL/h)</td>
<td>3610 (2570, 5070)</td>
<td>-</td>
</tr>
</tbody>
</table>
Concentration units for total radioactivity are μg-eq/mL.

Median

Values in parentheses represent the range
TABLE 3

Mean (n=8) percentage of radioactivity of SB-649868 and its metabolites in human plasma after single oral administration of [14C]SB-649868 at a target dose of 30 mg

<table>
<thead>
<tr>
<th>Radioactive Component</th>
<th>Mean % of plasma radioactivity</th>
<th>Oral administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
<td>12 h</td>
</tr>
<tr>
<td>SB-649868</td>
<td>67.7</td>
<td>33.2</td>
</tr>
<tr>
<td>M8</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>GSK2329163 (M98)</td>
<td>6.0</td>
<td>16.8</td>
</tr>
<tr>
<td>GSK2329158 (M25)</td>
<td>BQL</td>
<td>6.7</td>
</tr>
</tbody>
</table>

a Observed metabolite radioactivity was determined by 96-well fraction collection with scintillation counting for 5 minutes after a chromatographic separation was performed by HPLC. Where the sample preparation step (centrifugation, extraction or reconstitution) resulted in some loss of radioactive material, if the recovery was <85%, the chromatogram data have been multiplied by the percentage recovered to calculate % sample radioactivity and % dose or µg equivalent figures. Mean (n=7 for 3 and 12 h; n=8 for other time points) percentage of radioactivity per time point does not equal 100% since only distinct radioactive peaks were assigned values and a few minor metabolites were not reported.

BQL = below quantification limit, set to 1% plasma radioactivity
TABLE 4

Relevant metabolites of SB-649868 in human plasma, urine and feces after single oral administration of [14C]SB-649868 at a target dose of 30 mg

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Proposed Structure</th>
<th>Parent Ion [M+H]+ (m/z)</th>
<th>[M+H]+ ion and MS-MS Product ions</th>
<th>1H-NMR (600MHz, 1:1 ACN:D2O) (Where Available)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>[Image of structure]</td>
<td>478 (P)</td>
<td>478, [M+H]+ 460, 317, 316, 242, 241, 220, 145, 98</td>
<td>7.83 (d, 1H), 7.68 (dd, 1H), 7.56 (dd, 1H), 7.37 (dd, 2H), 7.37 (t, 1H), 7.11 (d, 1H), 7.04 (dd, 2H), 5.00 (m, 1H), 3.71 (dd, 1H), 3.31 (dd, 1H), 3.25 (dd, 1H), 2.97 (td, 1H), 2.17 (s, 3H), 1.72 (dd, 1H), 1.61 (dm, 1H), 1.55 (tm, 1H), 1.47 m (2H), 0.83, m (1H) Signals doubled due to rotamers, major sub-spectrum quoted.</td>
</tr>
<tr>
<td>PL</td>
<td>[Image of structure]</td>
<td>334 (P-144)</td>
<td>334 [M+H]+ 317, 316, 220, 98</td>
<td>7.39 (dd, 2H), 6.98 (t, 2H), 2.70 (s, 3H) Other resonances obscured</td>
</tr>
</tbody>
</table>

PL: plasma
UR (BQL): urine
FE (BQL): feces

SB-649868

Amine (M8)

NMR conditions: 600 MHz, 1:1 ACN:D2O (Where Available)
<table>
<thead>
<tr>
<th>Matrix (mean % dose)</th>
<th>Proposed Structure</th>
<th>Parent Ion $[\text{M+H}]^+$ (m/z)</th>
<th>$[\text{M+H}]^+$ ion and MS-MS Product ions</th>
<th>$^1$H-NMR (600MHz, 1:1 ACN:D2O) (Where Available)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UR (0.3)</td>
<td><img src="image" alt="Proposed Structure" /></td>
<td>512 (P+34)</td>
<td>512 $[\text{M+H}]^+$, 478, 317, 257, 239, 222</td>
<td>No NMR</td>
</tr>
<tr>
<td>FE (6.2)</td>
<td>Benzofuran dihydrodiol (M20, M21)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table has not been copyedited and formatted. The final version may differ from this version.
<table>
<thead>
<tr>
<th>Matrix (mean % dose)</th>
<th>Proposed Structure</th>
<th>Parent Ion [M+H]+ (m/z)</th>
<th>[M+H]+ ion and MS-MS Product ions</th>
<th>1H-NMR (600MHz, 1:1 ACN:D2O) (Where Available)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>Benzofuran ring-opened carboxylic acid (GSK2329158 - M25)</td>
<td>512 (P+2+16+16)</td>
<td>512 [M+H]+ 494, 334, 317, 316, 257, 220</td>
<td>7.46 (dd, 2H), 7.42 (dd, 2H), 7.22 (t, 1H), 7.20 (t, 1H), 7.16 (dd, 2H), 7.05 (dd, 1H), 7.01 (dd, 2H), 7.01 (dd, 1H), 7.00 (dd, 1H), 6.97 (dd, 1H), 4.98 (m, 1H), 4.44 (dm, 1H), 3.86 (m, 1H), 3.71 (d, 1H), 3.70 (dd, 1H), 3.69 (dd, 1H), 3.65 (d, 1H), 3.64 (s, 2H), 3.54 (dd, 1H), 3.32 (dm, 1H), 3.23 (dd, 1H), 3.17 (td, 1H), 2.95 (td, 1H), 2.67 (s, 3H), 2.42 (s, 3H), 1.7-1.8 (m, 2H), 1.4-1.7 (m, 6H), 1.2-1.4 (m, 2H), 0.95 (m, 1H), 0.86 (m, 1H) 1:1 Rotameric sub-spectra observed, both quoted.</td>
</tr>
<tr>
<td>UR (1.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FE (10.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FE (1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix (mean % dose)</td>
<td>Proposed Structure</td>
<td>Parent Ion ([\text{M+H}]^+) (m/z)</td>
<td>([\text{M+H}]^+) ion and MS-MS Product ions</td>
<td>(^1\text{H}-\text{NMR (600MHz, 1:1 ACN:D2O) (Where Available)})</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------</td>
<td>-------------------------------</td>
<td>---------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>UR (1.6)</td>
<td><img src="image" alt="Benzofuran ring opened carboxylic acid glucuronide (M41)" /></td>
<td>688 (P+176+16+16+2)</td>
<td>688 ([\text{M+H}]^+) 670, 626, 512, 468, 450, 355, 275</td>
<td>No NMR</td>
</tr>
<tr>
<td>UR (0.6)</td>
<td><img src="image" alt="Benzofuran hydroxy (M86)" /></td>
<td>494 (P+16)</td>
<td>494 ([\text{M+H}]^+) 476, 317, 316, 258, 257, 220, 98</td>
<td>7.58 (dd, 2H), 7.37 (dd, 2H), 6.56 (s, 1H), 4.98 (m, 1H) Other resonances obscured 13C: Benzofuran 3-carbon 101.2ppm</td>
</tr>
<tr>
<td>FE (0.7)</td>
<td><img src="image" alt="Oxygenated (M90)" /></td>
<td>494 (P+16)</td>
<td>494 ([\text{M+H}]^+) 333, 220</td>
<td>No NMR</td>
</tr>
<tr>
<td>Matrix</td>
<td>Proposed Structure</td>
<td>Parent Ion [M+H]^+ (m/z)</td>
<td>[M+H]^+ ion and MS-MS Product ions</td>
<td>'H-NMR (600MHz, 1:1 ACN:D2O) (Where Available)</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------</td>
<td>--------------------------</td>
<td>----------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>UR (BQL)</td>
<td><img src="image" alt="Dehydrated hemiaminal" /></td>
<td>478 (P+0)</td>
<td>478 [M+H]^+ 317, 249, 242, 241, 220, 162</td>
<td>7.62 (dd, 1H), 7.36 (t, 1H), 7.29 (dd, 2H), 7.14 (dd, 1H), 7.08 (dd, 2H), 6.96 (d, 1H), 6.83 (d, 1H), 4.46 (dm, 1H), 4.25 (dd, 1H), 4.09 (m, 1H), 3.80 (dd, 1H), 3.12 (dd, 1H), 2.02 (s, 3H), 1.64 (dm, 1H), 1.50 (qm, 1H), 1.43 (dm, 1H), 1.42 (dm, 1H), 1.26 (m, 1H), 0.56 (m, 1H)</td>
</tr>
<tr>
<td>FE (0.7)</td>
<td><img src="image" alt="Dehydrated hemiaminal" /></td>
<td>688 (P+176+16+16+2)</td>
<td>688 [M+H]^+ 293, 276</td>
<td>No NMR</td>
</tr>
</tbody>
</table>

Dehydrated hemiaminal (M94)

Benzofuran dihydrodiol or ring opened carboxylic acid glucuronide (M95)
<table>
<thead>
<tr>
<th>Matrix</th>
<th>Proposed Structure</th>
<th>Parent Ion</th>
<th>[M+H]^+ ion and MS-MS Product ions</th>
<th>'H-NMR (600 MHz, 1:1 ACN:D2O) (Where Available)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td><img src="image.png" alt="Proposed Structure PL" /></td>
<td>496 (P+18)</td>
<td>496 [M+H]^+ MS-MS of [M-H_2O+H]^+ ion : 478, 317, 285, 249, 241, 220</td>
<td>7.40 (dd, 2H), 7.32 (dd, 1H), 7.20 (t, 1H), 7.19 (dd, 2H), 7.05 (dd, 1H), 4.93 (dd, 1H), 4.43 (dm, 1H), 3.95 (m, 1H), 3.57 (dd, 1H), 3.37 (dd, 1H), 3.22 (dd, 1H), 3.10 (dd, 1H), 2.92 (td, 1H), 2.02 (s, 3H), 1.69 (dm, 1H), 1.43 (qm, 1H), 1.34 (dm, 1H), 1.28 (dm, 1H), 1.18 (m, 1H), 0.25 (m, 1H)</td>
</tr>
<tr>
<td>UR (0.2)</td>
<td>![Proposed Structure UR (0.2)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FE (2.6)</td>
<td>![Proposed Structure FE (2.6)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td><img src="image.png" alt="Proposed Structure PL" /></td>
<td>528 (P+16+34)</td>
<td>528 [M+H]^+ 510, 492, 333, 332, 299, 257, 236</td>
<td>No NMR</td>
</tr>
<tr>
<td>UR (0.3)</td>
<td>![Proposed Structure UR (0.3)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FE (3.6)</td>
<td>![Proposed Structure FE (3.6)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix (mean % dose)</td>
<td>Proposed Structure</td>
<td>Parent Ion [M+H]+ (m/z)</td>
<td>[M+H]+ ion and MS-MS Product ions</td>
<td>¹H-NMR (600MHz, 1:1 ACN:D2O) (Where Available)</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------------</td>
<td>--------------------------</td>
<td>----------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>FE (1.3)</td>
<td><img src="image1" alt="Structure" /></td>
<td>528 (P+16+34)</td>
<td>528 [M+H]+ 333, 332 MS-MS of [M-H₂O+H]+ 528 333, 332, 317, 315, 220</td>
<td>No NMR</td>
</tr>
<tr>
<td>FE (2.6')</td>
<td><img src="image2" alt="Structure" /></td>
<td>496 (P+18)</td>
<td>496 [M+H]+ 478 MS-MS of [M-H₂O+H]+ 478 317, 241, 220</td>
<td>No NMR</td>
</tr>
<tr>
<td>FE (2.4)</td>
<td><img src="image3" alt="Structure" /></td>
<td>528 (P+16+34)</td>
<td>528 [M+H]+ 510 MS-MS of [M-H₂O+H]+ 510 333, 249, 241, 220</td>
<td>No NMR</td>
</tr>
</tbody>
</table>

PL = plasma, UR = urine and FE = feces
BQL – Below limit of quantification
1- M98 and M101 co-elute during HPLC analysis.
figure 1
Figure 2

Plasma concentrations (ng or ng equivalents/mL)

Time (post-dose in hrs)

- SB-649868
- Total Radioactivity
Figure 3
Figure 6

* = circulating components; minor metabolites not included

M32

M8*

M99, M100, M102

M20 and M21

M86

M25* (GSK2329158)

M41

M95

P* (SB-649668)

M98* (GSK2329163)

M94*