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Metabolism of eight compounds in human and mouse liver microsomes

MMV_OSDD

Report #: CDCO_MMV_OSDD_13_005

3 December, 2013

Quality Statement:

This non-GLP study was conducted using established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Centre for Drug Candidate Optimisation, Monash University. This report accurately reflects the raw data obtained during the performance of this study.

The results described represent part of an on-going lead optimisation/drug discovery program. The study has been conducted to provide preliminary information on the *in vitro* metabolic characteristics of the candidate compound(s) under standard incubation conditions. A complete kinetic analysis has not been conducted.

Project Leader:	Susan Charman, PhD
Project Coordinator:	Karen White, PhD
Section Leader:	Francis Chiu, PhD

Study contributors:

Analytical/Metabolism:	Eileen Ryan, PhD and Jenna McLaren

Study number:	MMV_OSDD_13_006

www.cdco.monash.org

Centre for Drug Candidate Optimisation
Monash Institute of Pharmaceutical Sciences
Monash University (Parkville campus)
381 Royal Parade, Parkville,VIC 3052, Australia

MONASH University
Institute of Pharmaceutical Sciences

A. Study Objective

To determine the *in vitro* metabolic stability of MMV668957, MMV668958, MMV669304, MMV670438, MMV670944, MMV671651, MMV672723 and MMV672727 using human and mouse liver microsomes as a preliminary indication of the likely *in vivo* metabolic clearance.

B. Experimental Methods

Incubation methods:

The metabolic stability assay was performed by incubating each test compound (1 µM) with human and mouse liver microsomes (Xenotech, Lot# 1210057 and 1210302, respectively) at 37°C and 0.4 mg/mL protein concentration. The metabolic reaction was initiated by the addition of an NADPH-regenerating system (i.e. NADPH is the cofactor required for CYP450-mediated metabolism) and quenched at various time points over the 60 minute incubation period by the addition of acetonitrile. Control samples (containing no NADPH) were included (and quenched at 2, 30 and 60 minutes) to monitor for potential degradation in the absence of cofactor. Samples were analysed by UPLC-MS (Waters/Micromass Xevo G2 QTOF) under positive electrospray ionisation and MS spectral data acquired in a mass range of 80 to 1200 Daltons.

Notes: A metabolite screen was not included, however, since data was acquired using MS^E mode, which allows for the simultaneous acquisition of low and high collision energy MS spectra, a post-hoc metabolite search may be conducted at a later date if warranted. In addition, potential for primary glucuronidation was not assessed in this study.

Calculations:

Test compound concentration versus time data were fitted to an exponential decay function to determine the first-order rate constant for substrate depletion. In cases where clear deviation from first-order kinetics was evident, only the initial linear portion of the profile was utilised to determine the degradation rate constant (k). Each substrate depletion rate constant was then used to calculate: [1] a degradation half-life, [2] an in vitro intrinsic clearance value (CL_{int}, in vitro); [3] a predicted in vivo hepatic intrinsic clearance value (CL_{int}); [4] a predicted in vivo blood clearance value (CL_{blood}); and [5] a predicted in vivo hepatic extraction ratio (EH).

[1] $$t_{1/2} = \frac{\ln(2)}{k}$$

[2] $$CL_{int,\ in\ vitro} = \frac{k}{\text{microsomal protein content (0.4 mg protein/mL)}}$$

[3]* $$CL_{int} = CL_{int,\ in\ vitro} \times \frac{\text{liver mass (g)}}{\text{body weight (kg)}} \times \frac{\text{mg microsomal protein}}{\text{g liver mass}}$$

[4]* $$CL_{blood} = \frac{Q \times CL_{int}}{Q + CL_{int}}$$

[5]* $$E_H = \frac{CL_{blood}}{Q} = \frac{CL_{int}}{Q + CL_{int}}$$

* The following scaling parameters were assumed in the above calculations:

Species	Liver mass (g liver/kg body weight)	Microsomal protein (mg/g liver mass)	Hepatic blood flow (Q) (mL/min/kg body weight)
Human [a]	25.7	32	20.7
Mouse [a]	54.9	47	120

[a] Ring et al. (2011) *Journal of Pharmaceutical Sciences*, 100:4090-4110.

Predictions of *in vivo* hepatic extraction ratios:

The microsome-predicted hepatic extraction ratios (E_H) obtained based on the relative rates of test compound degradation *in vitro*, were used to classify compounds as low (< 0.3), intermediate (0.3 – 0.7), high (0.7 – 0.95) or very high (> 0.95) extraction compounds. The assumptions underlying these classifications are stated below.

NOTE:
Calculations of intrinsic clearance are based on the "*in vitro* $T_{1/2}$ method" (Obach, 1999, *Drug Metab. Dispos*. 27: 1350-1359), which assumes:

1) The substrate concentration employed is well below the apparent K_M for substrate turnover; and,
2) There is no significant product inhibition, nor is there any mechanism-based inactivation of enzyme.

The use of hepatic microsomes in the prediction of the *in vivo* hepatic extraction ratio has two further inherent assumptions (Obach, 1999) which:

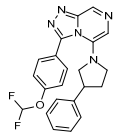
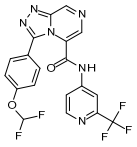
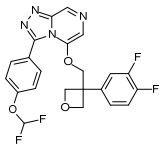
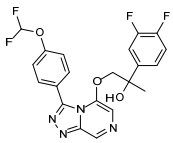
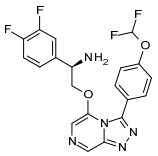
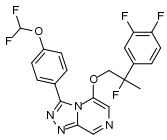
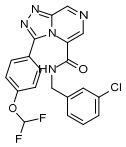
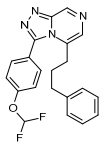
1) NADPH-dependent oxidative metabolism predominates over other metabolic routes (i.e. direct conjugative metabolism, reduction, hydrolysis, etc.); and,
2) Rates of metabolism and enzyme activities *in vitro* are truly reflective of those that exist *in vivo*.

Data should be considered within these terms of reference.

C. Results and Discussion

- The eight compounds in this study exhibited intermediate to high rates of degradation in human and mouse liver microsomes and metabolic stability parameters were broadly comparable between species (Table 1). Based on the predicted *in vitro* clearance values these compounds would be expected to be subject to moderate to rapid hepatic clearance *in vivo.*
- There was no measurable degradation in control incubations in either species for any of compounds, suggesting that there was no major non-cofactor dependent metabolism contributing to the overall rates of metabolism.

Table 1: Metabolic stability parameters for eight compounds based on NADPH-dependent degradation profiles in human and mouse liver microsomes.

Compound (Batch #)	Compound Structure	Species	Degradation half-life (min)	*In vitro* CL_{int} (µL/min/mg protein)	Microsome-Predicted E_H
MMV668957 (PCCBTAK-0074)		Human	87	20	0.44
		Mouse	26	67	0.59
MMV670944 (PCCBTAK-0275)		Human	84	21	0.45
		Mouse	81	21	0.31
MMV670438 (PCCBTAK-0234)		Human	53	33	0.56
		Mouse	14	120	0.72
MMV672723 (PCCBTAK-0367)		Human	47	37	0.59
		Mouse	18	94	0.67
MMV671651 (PCCBTAK-0284)		Human	42	41	0.62
		Mouse	23	77	0.62
MMV672727 (PCCBTAK-0371)		Human	31	55	0.69
		Mouse	6	273	0.85
MMV668958 (PCCBTAK-0075)		Human	11	154	0.86
		Mouse	6	274	0.85
MMV669304 (PCCBTAK-0127)		Human [a]	2	961	0.97
		Mouse [a]	2	847	0.95

[a] No measurable concentrations of MMV669304 were detected past 5 minutes, therefore degradation parameters were estimated using the initial two time points (i.e. 2 and 5 minutes) only and hence values reported are approximate.