MONASH University

CONFIDENTIAL

Physicochemical and metabolic evaluation of four compounds

MMV_PBPK_OSDD

Report #: CDCO_MMV_PBPK_OSDD_19_001

9 August 2019

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.

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A. Experimental Methods

a) Calculated physicochemical parameters using ChemAxon JChem software

A range of physicochemical properties evaluating drug-likeness and likely oral absorption characteristics were calculated using the ChemAxon chemistry cartridge via JChem for Excel software (version 16.4.11). A brief description of each parameter is provided below, along with a suggested ideal range based on research reported in the ADME literature from key industry and academic sources.

MW (< 500): Molecular Weight.

$PSA_{pH\ 7.4}$ (< 140 Å^2): Polar surface area also inversely correlates with membrane permeability.

HBD (< 5) & HBA (< 10): Number of hydrogen bond donors and acceptors gives an indication of the hydrogen bonding capacity, which is inversely related to membrane permeability.

FRB (≤ 10): Number of freely rotating bonds represents the flexibility of a molecule's conformation.

Arom. Rings (< 4): Total number of aromatic and heteroaromatic rings is also related to molecular flexibility.

Fsp^3 (> 0.3): Fraction of sp^3 carbons to total carbons indicates the complexity of a molecule's 3D structure.

cpKa: Ionisation constants impact solubility and permeability. Only physiologically relevant predicted values are provided here (*i.e.* 0 < pKa < 12).

$cLogP/cLogD_{pH}$ (< 5): Partition coefficients reflect the lipophilic character of the neutral structure, while distribution coefficients reflect the partitioning properties of the ionised molecule at a specific pH.

b) Kinetic Solubility Estimation using Nephelometry (Sol_{pH})

Compound in DMSO was spiked into either pH 6.5 phosphate buffer or 0.01 M HCl (approx pH 2.0) with the final DMSO concentration being 1%. After 30 minutes had elapsed, samples were then analysed via Nephelometry to determine a solubility range. See Bevan and Lloyd (2000) Anal Chem, 72:1781-1787.

c) Distribution Coefficient Estimation using Chromatography ($gLogD_{pH}$)

Partition coefficient values (LogD) of the test compounds were estimated at pH 7.4 by correlation of their chromatographic retention properties against the characteristics of a series of standard compounds with known partition coefficient values. The method employed is a gradient HPLC based derivation of the method developed by Lombardo. See Lombardo *et al*. (2001) J Med Chem, 44:2490-2497.

d) *In Vitro* Metabolic Stability

Incubation:

The metabolic stability assay was performed by incubating each test compound in liver microsomes at 37°C and a protein concentration of 0.4 mg/mL. The metabolic reaction was initiated by the addition of an NADPH-regenerating system and quenched at various time points over a 60 minute incubation period by the addition of acetonitrile containing diazepam as internal standard. 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.

The human liver microsomes used in this experiment were supplied by XenoTech, lot # 1410230. The mouse liver microsomes used in this experiment were supplied by XenoTech, lot # 1510256. The rat liver microsomes used in this experiment were supplied by XenoTech, lot # 1510115. Microsomal incubations were performed at a substrate concentration of 1 µM.

Data analysis:

Species scaling factors from Ring *et al*. (2011) J Pharm Sci, 100:4090-4110 were used to convert the *in vitro* CL_{int} (µL/min/mg) to an *in vivo* CL_{int} (mL/min/kg). Hepatic blood clearance and the corresponding hepatic extraction ratio (E_H) were calculated using the well stirred model of hepatic extraction in each species, according to the "*in vitro* $T_{1/2}$" approach described in Obach (1999) Drug Metab. Dispos. 27: 1350-1359. The E_H was then 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. Predicted *in vivo* clearance values have not been corrected for microsomal or plasma protein binding. Species scaling calculations are based on two assumptions: 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*. If significant non-NADPH-mediated degradation is observed in microsome control samples, then assumption (1) is invalid and predicted clearance parameters are therefore not reported.

B. Results

Experimental results are tabulated below.

Table 1: Physicochemical evaluation of four compounds

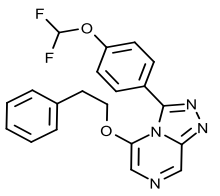
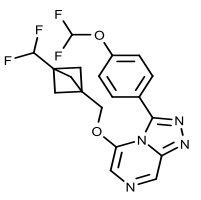
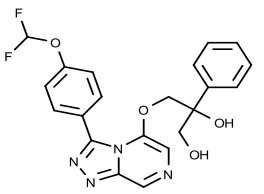
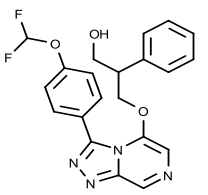
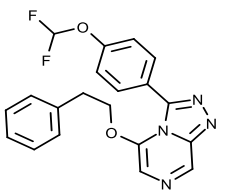
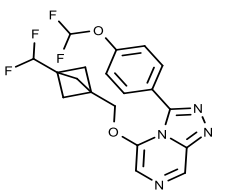
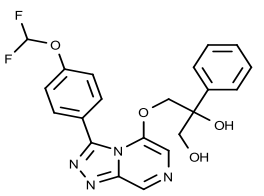
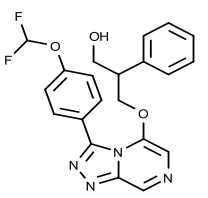
Compound (Batch)	Structure	MW	PSA ($Å^2$)	FRB	HBD	HBA	Arom. Rings	Fsp^3	Predicted pKa (0 - 12 only)	cLogP	cLogD at pH 7.4	gLogD at pH 7.4	Sol $_{2.0}$ (µg/mL)	Sol $_{6.5}$ (µg/mL)
MMV897698 (EGT 92-1)		382.37	61.5	7	0	5	4	0.15	Basic: 1.2 Acidic: none	3.3	3.3	3.7	6.3 - 12.5	6.3 - 12.5
MMV1794642 (EGT 363-1)		408.36	61.5	7	0	5	3	0.42	Basic: 1.2 Acidic: none	2.5	2.5	3.6	12.5 - 25	6.3 - 12.5
MMV1581295 (DGS 81-1)		428.4	102.0	8	2	7	4	0.19	Basic: 1.2 Acidic: none	1.6	1.6	2.3	25 - 50	50 - 100
MMV693155 (EGT 198-4)		412.4	81.8	8	1	6	4	0.19	Basic: 1.2 Acidic: none	2.3	2.3	2.8	12.5 - 25	12.5 - 25

Table 2: Metabolic evaluation of four compounds

Compound (Batch)	Structure	Microsome Species	$T_{1/2}$ (min)	$CL_{int,\ in\ vitro}$ (µL/min/ mg protein)	Predicted $CL_{int,\ in\ vivo}$ (mL/min/kg)	Predicted CL_{blood} (mL/min/kg)	Predicted E_H	Clearance classification
MMV897698 (EGT 92-1)		Human	26	66	55	15	0.72	high
		Mouse	7	262	675	102	0.85	high
		Rat	9	187	321	56	0.83	high
MMV1794642 (EGT 363-1)		Human	190	9	7	5	0.27	low
		Mouse	66	26	68	43	0.36	intermediate
		Rat	152	11	20	15	0.23	low
MMV1581295 (DGS 81-1)		Human	116	15	12	8	0.37	intermediate
		Mouse	41	42	109	57	0.48	intermediate
		Rat	38	45	78	36	0.54	intermediate
MMV693155 (EGT 198-4)		Human	73	24	20	10	0.49	intermediate
		Mouse	14	121	311	87	0.72	high
		Rat	11	160	275	54	0.80	high