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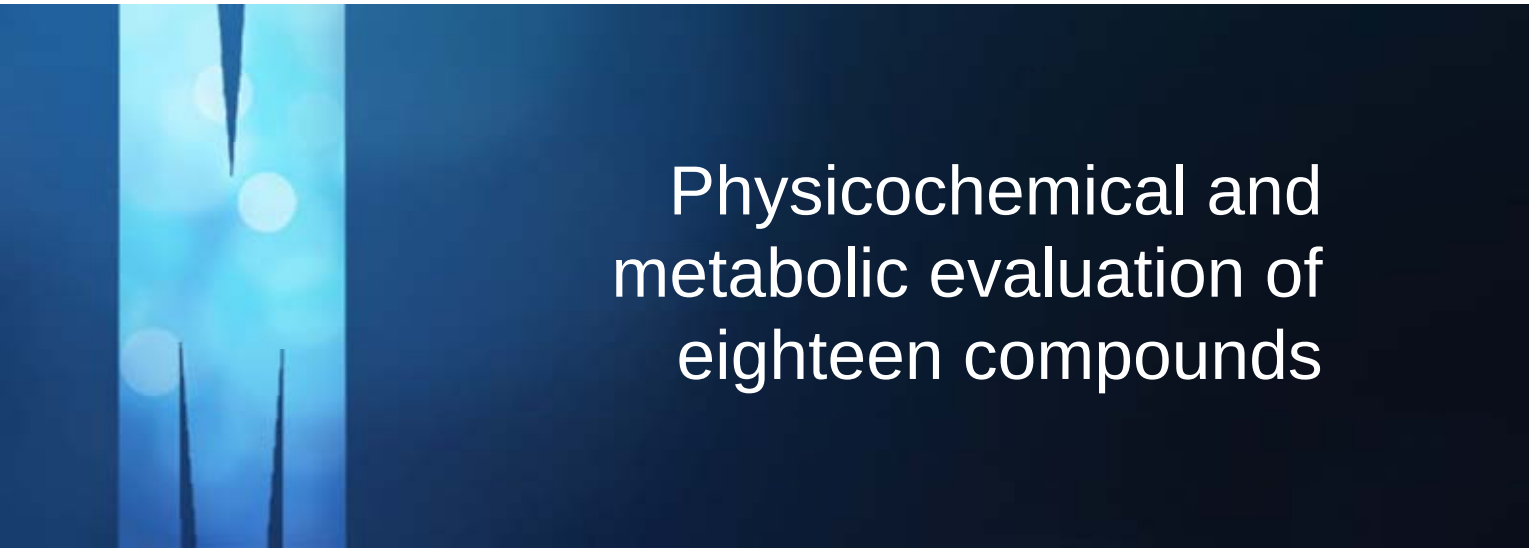

Physicochemical and metabolic evaluation of eighteen compounds

MMV_OSDD

Report #: CDCO_MMV_OSDD_17_001

9 February 2017

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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Study number(s):	MMV_OSDD_17_001

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cdco CENTRE FOR DRUG CANDIDATE OPTIMISATION

A. Experimental Methods

a) Calculated physicochemical parameters using ChemAxon JChem software

A range of physicochemical properties evaluating drug-likness 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 flexibity.

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) Protein Binding Estimation using Chromatography (cPPB)

Protein binding values of the test compounds were estimated by correlation of their chromatographic retention properties on a human albumin column against the characteristics of a series of standard compounds with known protein binding values. The method employed is a gradient HPLC based derivation of the method developed by Valko. See Valko *et al*. (2003) Journal of Pharmaceutical Sciences, 92:2236-2248.

e) *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. Microsomal incubations were performed at a substrate concentration of 1 µM.

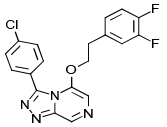
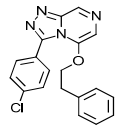
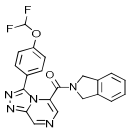
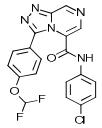
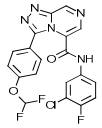
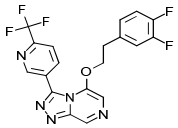
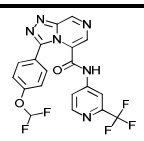
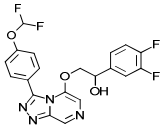
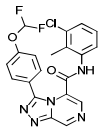
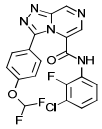
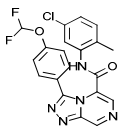
Data analysis:

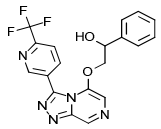
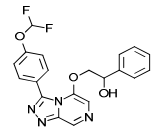
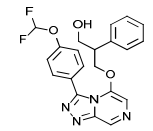
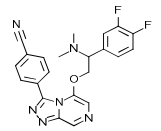
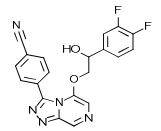
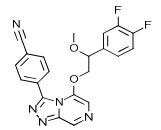
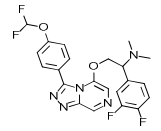
Species scaling factors from Ring *et al*. (2011) J Pharm Sci, 100:4090-4110 were used to convert the *in vitro* CL_{int} (µL/min/mg) to an *in vivo* CL_{int} (mL/min/kg). Hepatic blood clearance and the corresponding hepatic extraction ratio (E_H) were calculated using the well stirred model of hepatic 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 eighteen compounds

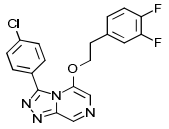
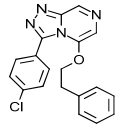
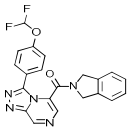
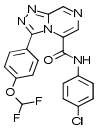
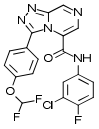
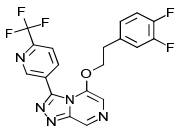
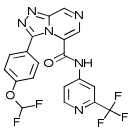
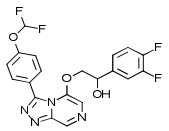
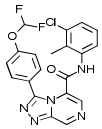
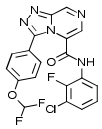
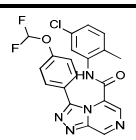
Compound (Batch)	Structure	MW	PSA ($Å^2$)	FRB	HBD	HBA	Arom. Rings	Fsp^3	predicted pKa (0 - 12 only)	cLogP	cLogD at pH 7.4	gLogD at pH 7.4	Sol $_{2.0}$ (µg/mL)	Sol $_{6.5}$ (µg/mL)	cPPB (%)	Notes
MMV639565 (AEW 302-1)		386.79	52.3	5	0	4	4	0.11	Basic: 1.6 Acidic: none	3.4	3.4	3.8	1.6 - 3.1	3.1 - 6.3	95.0	
MMV663915 (EGT 90-1, SSP-4)		350.81	52.3	5	0	4	4	0.11	Basic: 1.6 Acidic: none	3.2	3.2	3.8	6.3 - 12.5	6.3 - 12.5	93.8	
MMV669000 (EGT 111-1)		407.38	72.6	4	0	5	4	0.14	Basic: 1.6 Acidic: none	2.1	2.1	2.8	6.3 - 12.5	12.5 - 25	40.6	
MMV670246 (EGT 95-3)		415.78	81.4	5	1	5	4	0.05	Basic: 1.6 Acidic: none	3.0	3.0	3.1	3.1 - 6.3	1.6 - 3.1	90.3	
MMV670767 (EGT 141-1)		433.78	81.4	5	1	5	4	0.05	Basic: 1.6 Acidic: 11.8	3.1	3.1	3.1	3.1 - 6.3	3.1 - 6.3	92.4	
MMV670936 (AEW 296-1, PCCBTAK-0272)		421.33	65.2	6	0	5	4	0.16	Basic: 1.4 Acidic: none	2.9	2.9	3.4	3.1 - 6.3	3.1 - 6.3	76.9	
MMV670944 (AEW 300-1)		450.33	94.3	6	1	6	4	0.11	Basic: 2.1 Basic: 1.4 Acidic: 11.0	2.4	2.4	2.7	6.3 - 12.5	6.3 - 12.5	73.4	
MMV672687 (EGT 171-1)		434.35	81.8	7	1	6	4	0.15	Basic: 1.7 Acidic: none	2.7	2.7	3.1	12.5 - 25	12.5 - 25	81.5	
MMV675718 (TM 9-2)		429.81	81.4	5	1	5	4	0.10	Basic: 1.6 Acidic: none	3.5	3.5	3.1	12.5 - 25	12.5 - 25	85.8	
MMV675946 (TM 19-2)		433.78	81.4	5	1	5	4	0.05	Basic: 1.6 Acidic: 10.6	3.1	3.1	3.1	3.1 - 6.3	3.1 - 6.3	85.6	
MMV675947 (TM 26-1)		429.81	81.4	5	1	5	4	0.10	Basic: 1.6 Acidic: none	3.5	3.5	3.1	3.1 - 6.3	3.1 - 6.3	87.0	

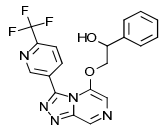
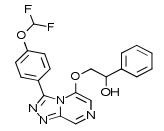
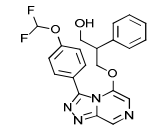
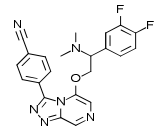
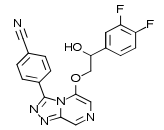
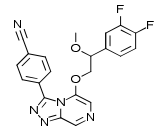
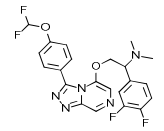
Compound (Batch)	Structure	MW	PSA ($Å^2$)	FRB	HBD	HBA	Arom. Rings	Fsp^3	predicted pKa (0 - 12 only)	cLogP	cLogD at pH 7.4	gLogD at pH 7.4	Sol $_{2.0}$ (µg/mL)	Sol $_{6.5}$ (µg/mL)	cPPB (%)	Notes
MMV688895 (EGT 137-1)		401.35	85.4	6	1	6	4	0.16	Basic: 1.4 Acidic: none	1.7	1.7	2.5	25 - 50	50 - 100	38.1	
MMV688896 (AEW 236-1, EGT 119-2)		398.37	81.8	7	1	6	4	0.15	Basic: 1.7 Acidic: none	2.4	2.4	2.9	25 - 50	25 - 50	68.5	
MMV693155 (AEW 317-1, AEW 294-2)		412.4	81.8	8	1	6	4	0.19	Basic: 1.7 Acidic: none	2.3	2.3	2.8	25 - 50	12.5 - 25	72.0	1
MMV897707 (EGT 145-2)		420.42	80.5	6	0	6	4	0.18	Basic: 7.9 Basic: 1.6 Acidic: none	2.5	1.8	3.0	12.5 - 25	12.5 - 25	67.3	
MMV897708 (AEW 314-1)		393.35	96.3	5	1	6	4	0.10	Basic: 1.6 Acidic: none	1.8	1.8	2.6	25 - 50	25 - 50	56.3	
MMV897709 (AEW 313-1)		407.38	85.3	6	0	6	4	0.14	Basic: 1.6 Acidic: none	2.4	2.4	3.4	12.5 - 25	12.5 - 25	70.3	
MMV897763 (EGT 169-1)		461.42	66.0	8	0	6	4	0.23	Basic: 7.9 Basic: 1.6 Acidic: none	3.4	2.8	3.5	12.5 - 25	6.3 - 12.5	79.1	

Notes

1 - Second chromatographic peak was visible in both the gLogD and the cPPB experiment. The reported values were calculated from the retention time of the major peak (70% by peak area).

Table 2: Metabolic evaluation of eighteen 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	Notes
MMV639565 (AEW 302-1)		Human	53	33	27	12	0.57	intermediate	
		Mouse	9	193	497	97	0.81	high	
MMV663915 (EGT 90-1, SSP-4)		Human	18	94	77	16	0.79	high	
		Mouse	5	319	824	105	0.87	high	
MMV669000 (EGT 111-1)		Human	19	89	74	16	0.78	high	
		Mouse	4	417	1076	108	0.90	high	
MMV670246 (EGT 95-3)		Human	164	11	9	6	0.30	low	
		Mouse	115	15	39	29	0.24	low	
MMV670767 (EGT 141-1)		Human	37	47	39	14	0.65	intermediate	
		Mouse	19	91	234	79	0.66	intermediate	
MMV670936 (AEW 296-1, PCCBTAK-0272)		Human	24	71	59	15	0.74	high	
		Mouse	13	132	340	89	0.74	high	
MMV670944 (AEW 300-1)		Human	95	18	15	9	0.42	intermediate	
		Mouse	73	24	62	41	0.34	intermediate	
MMV672687 (EGT 171-1)		Human	45	38	31	12	0.60	intermediate	
		Mouse	10	170	439	94	0.79	high	
MMV675718 (TM 9-2)		Human	15	114	94	17	0.82	high	
		Mouse	10	166	427	94	0.78	high	
MMV675946 (TM 19-2)		Human	19	90	74	16	0.78	high	
		Mouse	11	160	413	93	0.77	high	
MMV675947 (TM 26-1)		Human	9	201	166	18	0.89	high	
		Mouse	5	360	929	106	0.89	high	

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	Notes
MMV688895 (EGT 137-1)		Human	50	35	29	12	0.58	intermediate	
		Mouse	15	117	302	86	0.72	high	
MMV688896 (AEW 236-1, EGT 119-2)		Human	246	7	6	5	0.22	low	1
		Mouse	6	278	718	103	0.86	high	
MMV693155 (AEW 317-1, AEW 294-2)		Human	35	49	41	14	0.66	intermediate	
		Mouse	5	361	930	106	0.89	high	
MMV897707 (EGT 145-2)		Human	17	104	85	17	0.81	high	
		Mouse	8	213	550	99	0.82	high	
MMV897708 (AEW 314-1)		Human	177	10	8	6	0.28	low	
		Mouse	27	64	166	70	0.58	intermediate	
MMV897709 (AEW 313-1)		Human	37	47	39	13	0.65	intermediate	
		Mouse	11	159	411	93	0.77	high	
MMV897763 (EGT 169-1)		Human	7	264	217	19	0.91	high	
		Mouse	4	478	1233	109	0.91	high	

Notes

1 - This compound exhibited apparent biphasic elimination in mouse liver microsomes. The reported parameters were calculated fitting the initial time points (i.e. 2 to 15 minutes) to a first order degradation profile.