In vitro characterisation of SPL028: a deuterated derivative of *N,N*-dimethyltryptamine, developing a treatment for mental health disorders

Zelah Joel[1], Meghan Good[1], Ellen James[1], Tiffanie Benway[1], Peter Rands[1], Marie Layzell[1], Carol Routledge[1]
[1]Small Pharma, London, UK

INTRODUCTION

SPL026 (N,N-dimethyltryptamine (DMT) fumarate) is a short-acting psychedelic currently in clinical trials to assess its potential, alongside therapy, as a treatment of Major Depressive Disorder (MDD). Here we present the *in vitro* characterisation of a series of novel deuterated compounds, designed to retain the primary receptor pharmacology of SPL026 while extending the pharmacokinetic (PK) and thus pharmacodynamic (PD) properties. It is hypothesised that prolonging the subjective experience may enhance therapeutic potential.

In vitro pharmacokinetics

Deuteration increases metabolic stability in human hepatocytes

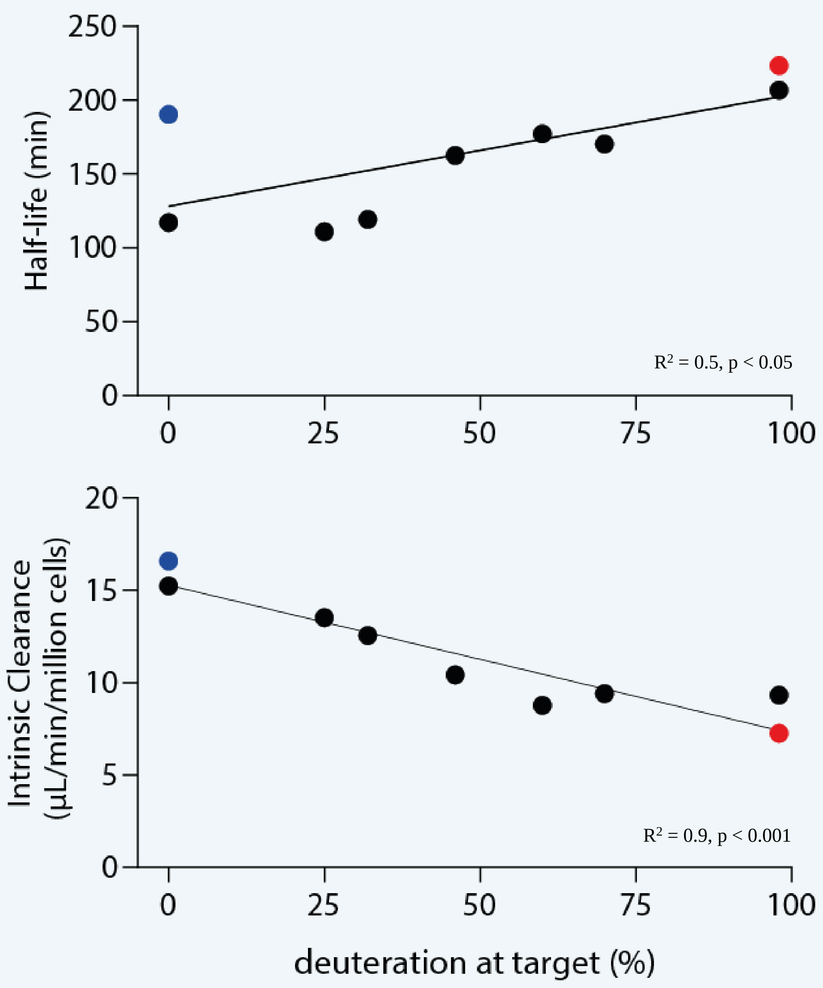

Figure 1. A series of DMT analogues with varying levels of deuteration were created. It was shown that the degree of deuteration at the target carbon correlates with increased metabolic stability in human hepatocytes. Linear regression showed that the degree of deuteration was found to significantly predict an increase in half-life ($F = 6.3\ 1,7$; $p = 0.04$) and decrease in clearance ($F = 51.04$; $1,7$; $p = 0.0002$). Blue: SPL026; red: SPL028.

SPL028 (bisdeutero-N,N-dimethyltryptamine, D2DMT) was selected for further testing

Significant increases in metabolic stability in monoamine oxidase A (MAO-A) enriched fractions

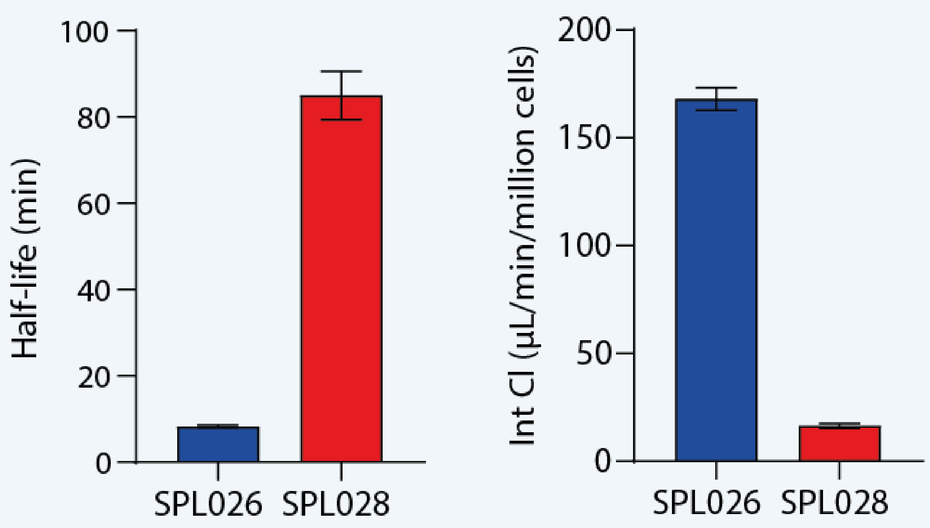

Figure 2. The metabolic stability of SPL028 vs SPL026 in human mitochondrial fraction significantly increased compared to SPL026: half-life ($p<0.001$); intrinsic clearance ($p<0.001$)

Physicochemical properties are not affected by deuteration

LogD7.4	SPL026	0.15
	SPL028	0.11

The mean blood/plasma ratio for SPL028 was slightly lower than SPL026, although both compounds demonstrated drug distribution into the blood cells.

mean blood/plasma	SPL026	1.53
	SPL028	1.34

Plasma protein binding in human plasma was comparable.

Plasma protein binding	SPL026	67.7% unbound
	SPL028	69.6% unbound

In vitro pharmacodynamics

Deuteration does not affect receptor binding

Table 1. Determined IC50 and ki for SPL026 and SPL028 at 18 receptors/enzymes. Data determined using a concentration range of 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µM. Fold difference in SPL028 IC50/Ki relative to SPL026 is shown. Comparison of SPL026 and SPL028 at the tested receptors were considered to be equivalent. Non-significant binding (<50% inhibition) at the highest test item (SPL026 and SPL028) concentration of 10 µM was observed at the Sigma σ1 receptor (43.6% SPL028; 38.8% SPL026) and SERT (39.7% SPL028; 40.3% SPL026).

	SPL026		SPL028		Fold difference in SPL028 IC50/Ki, relative to SPL026
Receptor/ enzyme (Ligand)	**IC50 (µM) [nH]**	**Ki (µM)**	**IC50 (µM) [nH]**	**Ki (µM)**	
5-HT1A (1.50 nM [3H] 8-OH-DPAT)	0.19 [1.26]	0.11	0.18 [0.97]	0.1	1.1
5-HT1B (1.0 nM [3H] GR125743)	1.99 [0.74]	1.52	2.42 [0.76]	1.84	0.8
5-HT2A (0.5 nM [3H] Ketanserin)	0.22 [0.84]	0.06	0.15 [0.78]	0.04	1.5
5-HT2B (2.0 nM [3H] Mesulergine)	0.41 [0.98]	0.3	0.48 [1.08]	0.35	0.9
5-HT2C (1.0 nM [3H] Mesulergine)	0.39 [0.94]	0.2	0.53 [1.38]	0.28	0.7
5-HT5A (1.70 nM [3H] LSD)	3.32 [1.01]	1.71	3.32 [0.91]	1.71	1.0
5-HT6 (1.50 nM [3H] LSD)	1.08 [0.77]	0.5	1.21 [0.84]	0.56	0.9
5-HT7 (5.50 nM [3H LSD)	0.09 [0.67]	0.05	0.12 [0.91]	0.07	0.7
α1A (0.6 nM [3H] Prazosin)	0.99 [1]	0.48	0.58 [0.75]	0.28	1.7
α1B (0.2 nM [3H] Prazosin)	0.93 [0.94]	0.37	0.67 [0.94]	0.26	1.4
α1D (0.6 nM [3H] Prazosin)	3.26 [0.73]	1.6	3.07 [0.85]	1.51	1.1
α2A (1.50 nM [3H] Rauwolscine)	3.03 [1]	1.52	2.22 [0.76]	1.11	1.4
α2B (2.50 nM [3H] Rauwolscine)	1.67 [0.97]	0.76	1.58 [1.09]	0.72	1.1
α2C (0.5 nM [3H] Rauwolscine)	6.1 [1.03]	2.71	9.47 [0.56]	4.21	0.6
H1 (1.20 nM [3H] Pyrilamine)	0.34 [0.63]	0.16	0.52 [1.09]	0.25	0.6
I2, central (2.0 nM [3H] Idazoxan)	2.6 [0.99]	1.73	1.93 [0.87]	1.29	1.3
nAChα3β4 (0.05 nM [125I] Epibatidine)	4.4 [0.9]	3.18	4.2 [0.79]	3.03	1.0
MAO-A (-)	1.5 [-]	-	1.18 [-]	-	1.3

CONCLUSIONS

The acute psychedelic effects of DMT are short-lasting due to its rapid metabolism. Modification of DMT using deuteration demonstrated improved metabolic stability. The bisdeuterated DMT compound (SPL028) was selected as the optimal analogue, showing the greatest improvement in *in vitro* metabolic stability, attributed to inhibited oxidative deamination. There were no marked changes in physicochemical properties to suggest an impact on drug promiscuity, exemplified by the minimal influence on in vitro binding affinities.

Progress in Clinical studies:
Phase I study to investigate the safety, tolerability and PK of SPL028 (EudraCT No.: 2022-002618-17) administered via IV and IM began in Q1 2023

Small Pharma

Disclosures: Zelah Joel is an employee and option holder of Small Pharma Ltd