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

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INTRODUCTION

SPL026 (*N,N*-dimethyltryptamine, DMT, fumarate) is a short-acting serotonergic psychedelic currently in clinical development for the treatment of major depressive disorder. Its behavioural profile and efficacy is associated with its complex receptor pharmacology and rapid metabolism by monoamine oxidase A (MAO-A). Here we present data on the preclinical characterisation of deuterated (d-)DMT analogues, which are hypothesized to prolong the duration of subjective experience via inhibition of metabolism. These effects may offer clinical advantage through optionality of more practical dosing routes and/or providing prolonged exposure over therapeutic concentrations for the treatment of specific psychiatric disorders.

In vitro pharmacokinetics

Human hepatocytes

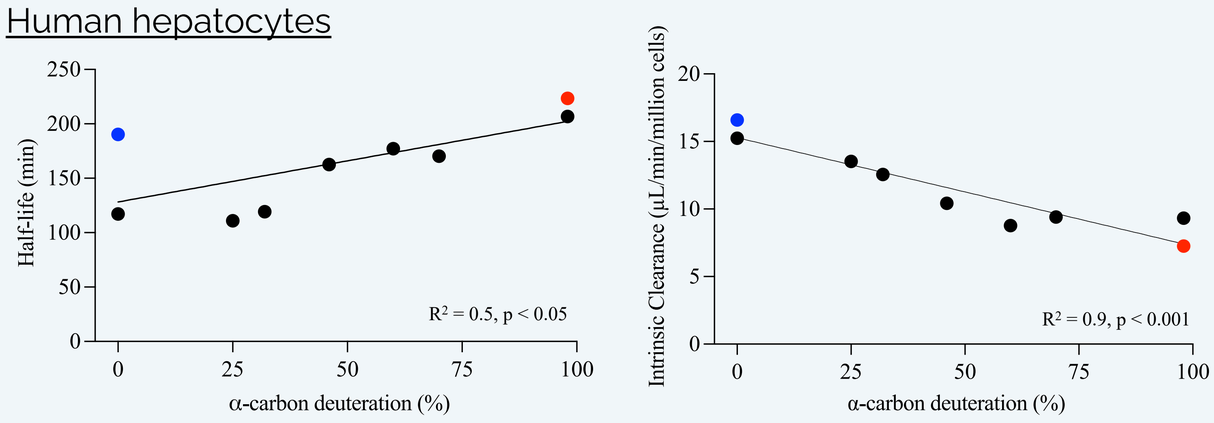
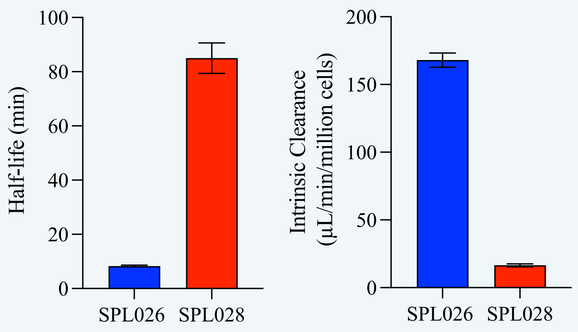


Figure 1. The metabolic stability of d-DMT analogues at the α-carbon and methyl groups in human hepatocytes. Increasing deuteration at the α-carbon correlates with increased metabolic stability

Human mitochondrial fraction



SPL028 (α,α-bisdeutero-*N,N*-dimethyltryptamine, D_2DMT) was selected for further preclinical testing

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 ($p<0.001$)

In vitro pharmacodynamics

In vitro receptor profiling

Table 1. Receptor binding affinities and enzyme inhibition for SPL028 and SPL026 (0.003-10 µM). Data shown from 6 serotonergic receptors with high affinity and MAO-A enzyme

Receptor/ enzyme (Ligand)	**SPL028**		**SPL026**	
	IC50 (µM) [nH]	**Ki (µM)**	**IC50 (µM) [nH]**	**Ki (µM)**
5-HT$_{2A}$ (0.5 nM [3H] Ketanserin)	0.15 [0.78]	0.04	0.22 [0.84]	0.06
5-HT$_7$ (5.50 nM [3H] LSD)	0.12 [0.91]	0.07	0.09 [0.67]	0.05
5-HT$_{1A}$ (1.50 nM [3H] 8-OH-DPAT)	0.18 [0.97]	0.10	0.19 [1.26]	0.11
5-HT$_{2C}$ (1.0 nM [3H] Mesulergine)	0.53 [1.38]	0.28	0.39 [0.94]	0.20
5-HT$_{2B}$ (2.0 nM [3H] Mesulergine)	0.48 [1.08]	0.35	0.41 [0.98]	0.30
5-HT$_6$ (1.50 nM [3H] LSD)	1.21 [0.84]	0.56	1.08 [0.77]	0.50
MAO-A (-)	1.18 [-]	-	1.5 [-]	-

SPL028 and SPL026 have equivalent *in vitro* receptor profile across 92 receptors tested

In vivo pharmacokinetics

Intravenous dosing

Individual animals' PK profiles following IV administration were highly variable. Inconsistent differences between SPL028 and SPL026 profile over the dose range 0.6–6 mg/kg IV however, cassette dosing of SPL026 and SPL028(viii) IV found no differences in exposure and significant difference in half-life ($p<0.05$)

Intramuscular dosing

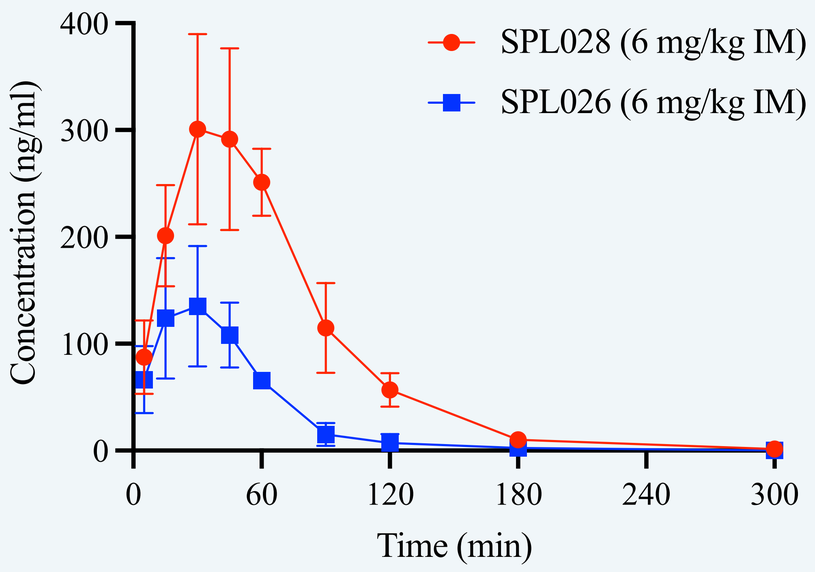
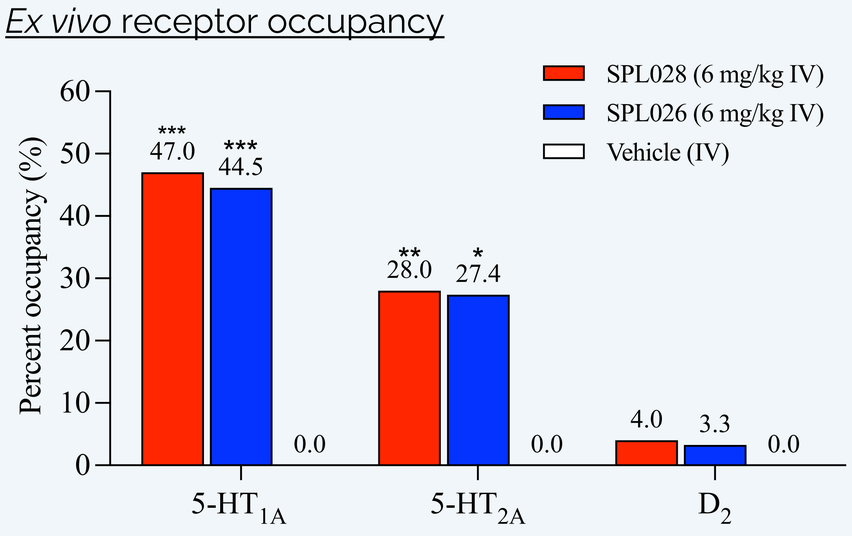


Figure 3. The PK profile of SPL028 vs SPL026 following IM administration in male and female Sprague Dawley rats (n=3/sex/group), demonstrating increased SPL028 exposure and half-life

In vivo pharmacodynamics

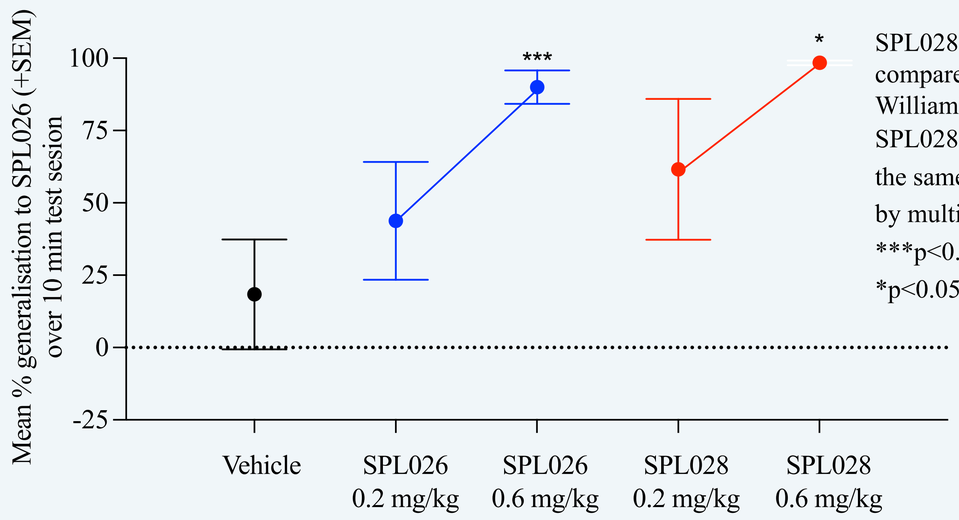
Ex vivo receptor occupancy



SPL028 and SPL026 were compared to vehicle by William's tests.
SPL028 was compared to the same dose of SPL026 by multiple t test.
***$p<0.001$ vs vehicle,
*$p<0.05$ vs vehicle

Figure 4. Ex vivo receptor occupancy of SPL028 vs SPL026 at (on-target) rat hippocampal 5-HT$_{1A}$, rat cortical 5-HT$_{2A}$; and (off-target) striatal D_2 receptors in Sprague Dawley rats (n=5/group). No differences in occupancy between SPL028 and SPL026

Drug discrimination



Test period (min)	5	5	5	5	5
No. animals tested	8	8	8	8	8
No. animals disrupted	1	1	1	0	5

SPL028 and SPL026 were compared to vehicle by William's tests.
SPL028 was compared to the same dose of SPL026 by multiple t test.
***$p<0.001$ vs vehicle,
*$p<0.05$ vs vehicle

Figure 5. Effect on overall % generalisation response of IV doses of SPL026 and SPL028 at a dose-test interval of 5 min post dose in female lister hooded rats trained to discriminate SPL026 (3 mg/kg IP) from vehicle. SPL028 broadly generalized to SPL026 cue when tested at 5 min after dosing. There was a near identical dose-dependent effect with SPL026 and SPL028, with full generalization at the higher dose 0.6 mg/kg. This effect was lost with both compounds at the later timepoints, 30 min onwards

CONCLUSIONS

- Deuterium substitution at the α-carbon of DMT was demonstrated to correlate with increased metabolic stability. The molecule with the greatest degree of deuteration at the α-carbon (D2-DMT, SPL028) was selected to undergo further preclinical testing
- Reduced clearance and increased half-life of SPL028 *in vivo* resulting in a prolonged PK profile and increased exposure when compared to SPL026 following IM administration
- The *in vitro* and *ex vivo* receptor binding profile of SPL028 was comparable to SPL026, with the highest affinity at serotonergic receptors
- No impact on behavioural pharmacology assessed by SPL028 generalisation to SPL026 cue

Progress in Clinical studies:

Phase I studies to investigate the safety, tolerability and PK of SPL026 (ClinicalTrials.gov Id: NCT05644093) and SPL028 (EudraCT No: 2022-002618-17) administered via IV and IM began in Q1 2023

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Disclosures: Meghan Good is an employee and option holder of Small Pharma Ltd