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Letter

Discovery and *In Vitro* Characterization of SPL028: Deuterated *N,N*-Dimethyltryptamine

Marie Layzell,* Peter Rands, Meghan Good, Zelah Joel, Rick Cousins, Tiffanie Benway, Ellen James, and Carol Routledge

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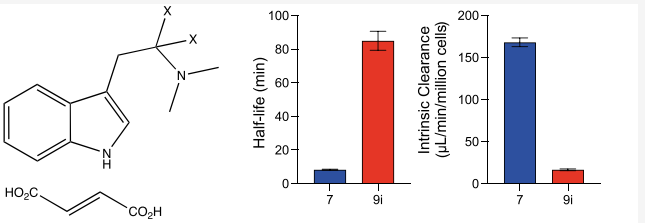
ABSTRACT: The psychedelic *N,N*- dimethyltryptamine (DMT) is in clinical development for the treatment of major depressive disorder. However, when administered via intravenous infusion, its effects are short-lived due to rapid clearance. Here we describe the synthesis of deuterated analogues of DMT with the aim of prolonging the half-life and decreasing the clearance rate while maintaining similar pharmacological effects. The molecule with the greatest degree of deuteration at the α-carbon (N,N-D_2-dimethyltryptamine, D_2-DMT) demonstrated the longest half-life and intrinsic clearance in hepatocyte mitochondrial fractions when compared with DMT. The *in vitro* receptor binding profile of D_2-DMT was comparable to that of DMT, with the highest affinity at the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors. D_2-DMT was therefore the preferred candidate to consider for further evaluation.



7 X = H, SPL026: *N,N*-dimethyltryptamine (DMT) fumarate
9i X = D, SPL028: D_2-*N,N*-dimethyltryptamine (D_2-DMT) fumarate
Prolonged clearance and half-life and comparable receptor binding profile of 9i compared to 7

KEYWORDS: *Psychedelic, N,N-Dimethyltryptamine, Deuterium kinetic isotope effect, Mental health, Depression*

Psychedelic compounds present a novel approach to the treatment of many mental health disorders with a number of clinical studies demonstrating potential efficacy of psychedelic assisted therapy in patients with major depressive disorder (MDD) and treatment-resistant depression.[1−3]

N,N-Dimethyltryptamine (DMT) **1** is an endogenous indole alkaloid compound that is currently in clinical development to investigate its efficacy in the treatment of MDD (ClinicalTrials.gov Identifier: NCT04673383; NCT05553691). **1** is capable of producing profound psychedelic effects[4] owing to its modulation of multiple neurotransmitter receptors[5,6] and consequential modification of signal diversity and functional connectivity.[7] The primary psychological and potentially therapeutic effects of **1** are associated with its activity at serotonin receptors;[8−10] in particular, 5-HT_{2A},[8,11−13] 5-HT_{1A},[11−13] and 5-HT_{2C} receptor agonism.[12,14−16] Modified signaling via the metabotropic glutamate receptor ($mGluR_2$)[17] and sigma-1 receptors[18] have also been suggested to contribute to the complex receptor pharmacology of **1**. These pharmacological effects can induce a unique and profound subjective experience which when complemented by psychological therapy, can facilitate emotional processing, the disruption of rigid thought patterns and the promotion of new ways of thinking.[19,20]

1 is not orally active as it is rapidly metabolized, chiefly by monoamine oxidase A (MAO-A).[21] Moreover, after intravenous (IV) administration of **1** in healthy subjects and patients with MDD, psychological effects are apparent almost immediately, subside within a matter of minutes (∼20−30 min), and are dose-dependent.[3,16] It has been hypothesized that a more complete psychedelic experience could be achieved if peak plasma and, therefore, brain concentrations of **1** are maintained over a longer period.[22] Prolonging the acute pharmacological effects and/or the duration of the psychedelic experience is anticipated to increase cognitive flexibility,[7] offering enhanced potential for therapeutic benefits. In addition, it is hypothesized that extending the duration of the patient's psychedelic journey could be beneficial for more severe depression and, potentially, for treating other psychiatric indications. Studies exploring the pharmacokinetics (PK), pharmacodynamics (PD) and efficacy of extended DMT injection regimens are ongoing.

Chemical modification of drug molecules by deuteration represents an alternative approach to extending their PK profile.[23] Deuterium (D) is a hydrogen isotope that contains one additional neutron to the most common isotope of hydrogen, protium (from here on referred to as hydrogen [H]).[24] Deuterium is the simplest bioisostere for hydrogen with a slightly smaller molar volume, lower lipophilicity (Δlog

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P_{oct} = −0.006), and a shorter C−D bond than the C−H bond.[25] In organic compounds, the physical properties remain primarily unchanged when hydrogen is replaced by deuterium, thus retaining the same pharmacological profile of the parent molecule.[26,27] Substitution of hydrogen with deuterium increases the bond strength. The rates of cleavage of C−H, N−H, and O−H bonds are 7.0, 8.5, and 10.6 times faster than the rates of cleavage for deuterated analogues.[28] Deuterium replacement of hydrogen in an oxidatively labile carbon−hydrogen bond, when the cleavage of this bond is involved in the rate-determining step, can afford a kinetic isotope effect (KIE) by virtue of the difference in bond strength.[29−31] If a KIE is realized in the modified compound, then the altered metabolic fate will change and potentially improve the efficacy, safety, and/or tolerability of the active substance.[32−36]

The KIE bestows a significant difference on the *in vivo* PK profile and behavioral effects of $\alpha,\alpha,\beta,\beta$-tetradeuterated DMT ($D_4$-DMT) as compared with **1**, thought to be mediated by an inhibition of MAO-mediated metabolism and transport dynamics.[37−39] D_4-DMT was found to have a shorter time to onset and potentiation of behavior-disrupting effects when compared to equivalent doses of **1** however, no kinetic data were reported to quantify the deuterium KIE.[39]

In vitro studies of trace amines and their deuterated analogues including the $\alpha\alpha$-d_2 and $\beta\beta$-d_2 positions of **1** found that deuteration of the α-carbon reduced deamination, indicating the α-position as a rate-limiting step of enzymatic deamination, whereas deuteration of the β-carbon was shown to cause a slight enhancement in deamination.[40] These observations suggest that modification of **1** with appropriate deuterium substitution of the α-hydrogen atoms of the indole-ethylamine group would have a direct effect on the enzymatic cleavage involved in the formation of indole-3-acetic acid (IAA) **4**.

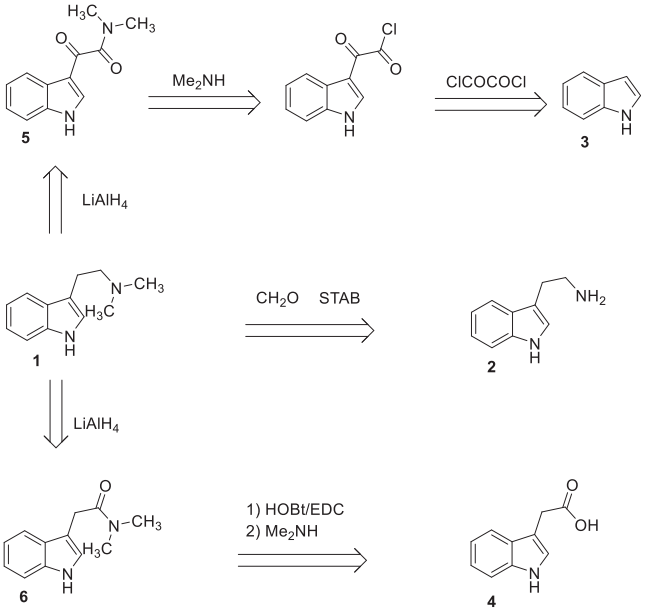
The synthesis of *N,N*-D_2-dimethyltryptamine (D_2-DMT) **8i**, chemical name (IUPAC) [2-(1*H*-indol-3-yl)(1,1-D2) ethyl]-dimethylamine, has been reported in the literature; however, no biological or metabolic data have been published.[41]

We expect to find improved metabolic stability and altered *in vivo* exposure of the *N,N*-D_2-dimethyltryptamine analogue blends (**8i**−**8vi**) and in *N,N*-D_8-dimethyltryptamine (D_8-DMT, **13**). It is unlikely that deuterium substitution would have a direct effect on the rate of N-oxide formation; however, it may be possible that secondary isotope effects are present depending on the mechanism of the N-oxide formation, and so a deuterium KIE may still be possible.

The purpose of the studies presented here was to characterize the synthesis and *in vitro* activity of a series of novel deuterated DMT compounds designed to retain the primary receptor pharmacology of DMT while extending the PK and thus pharmacodynamic (PD) properties of DMT. The ultimate aim of the modifications is to increase the duration of psychedelic experience elicited by these compounds and explore their therapeutic efficacy for the treatment of mental health disorders. These compounds have previously been discussed[43] and are disclosed in patents WO 2020/245133 and WO 2022/117359.

Design and Synthesis of the DMT Derivatives. ^{1}H NMR and HPLC data for the novel compounds can be found in the Supporting Information. The first described synthesis of **1** converted tryptamine **2** via alkylation with methyl iodide.[42] The synthetic approaches to deliver **1** have been reviewed,[44] while the synthesis and characterization of **1** hemifumarate for human clinical trials have been reported.[45] Three potential routes were considered, as illustrated in the retrosynthesis shown in Scheme 1. To support planned activities and

Scheme 1. Retrosynthetic Design for DMT



preclinical and clinical development, a scaled-up preparation of **1** was required. The preferred synthetic route details (Scheme 2) and characterization are provided in the Experimental Section in the Supporting Information.

A one-step conversion of tryptamine **2** using formaldehyde and sodium triacetoxyborohydride (STAB) chemistry was discarded due to the genotoxicity risk of the reagents. An initial comparison of routes (5 g) starting with indole **3** or **4** indicated little difference between the routes in terms of output yield and purity compared to previously reported yields.[45] However, the route starting with **4** was favored as it starts from the inexpensive and readily available starting material auxin, has a shorter overall route (isolation of intermediate not required), uses low toxicity reagents (1-ethyl-3-carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) versus oxalyl chloride), and required less $LiAlH_4$ in the second step (0.9 equiv versus 2.0 equiv) when compared to the double reduction of β-keto-amide **5**.

After a slurry purification step with hot *tert*-butyl methyl ether (TBME) to remove impurities from the coupling procedure, the conversion of **4** (267 g) to the *N,N*-d **6** using EDC and dimethylamine (Scheme 2) afforded a high yield (90%), with a solid product of high purity (98.5% by HPLC). It should be noted that a semibatch addition of EDC hydrochloride and dimethylamine was used to help to control the reaction, with relatively small exotherms of 5−10 °C observed thus avoiding the need for excessive cooling in both cases: EDC.HCl (337.5 g, 1.760 mol) was charged portion-wise over 5 min at 16−22 °C, and stirred for 2 h at ambient temperature before 2 M dimethylamine in THF (1100 mL, 2.200 mol) was charged dropwise over 20 min at 20−30 °C. For the second step, initial processing on multiple (10 g) reactions indicated a minimum of 0.9 equiv of $LiAlH_4$ was required to achieve reaction completion and that warming the reaction to 60 °C also aided reaction completion without the

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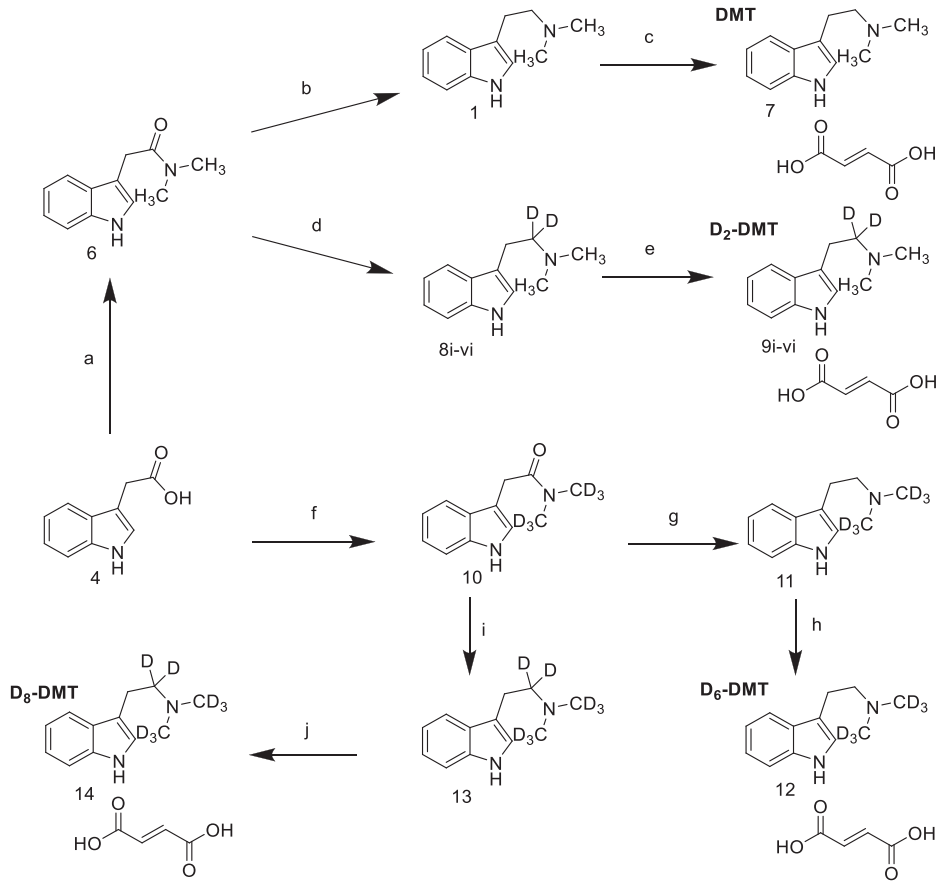
Scheme 2. Synthesis of Deuterium and Non-deuterium Containing *N,N*-Dimethyltryptamine Compounds[a]



[a]Reagents and conditions: (a) (i) DCM/HOBt/EDC (ii) 2 M Me_2NH in THF, 90%. (b) $LiAlH_4$, THF, 92%. (c) EtOH, fumaric acid, 78%. (d) LiAlH4/LiAlD4 THF. (e) EtOH, fumaric acid, 52−68%. (f) (i) DCM/HOBt/EDC (ii) Me2NH.HCl, DIPEA, THF, 58%. (g) LiAlH4, THF, 97%. (h) EtOH, fumaric acid, 87%. (i) LiAlD4, THF, 97%. (j) EtOH, fumaric acid, 81%.

Table 1. Levels of Deuteration in Compound 1 Afforded by Varying the Ratio of $LiAlH_4/LiAlD_4$ in the Reduction of Amide 6[a]

				Deuteration % *Mwt freebase*								
				D_0	D_1	D_2	D_4	D_5	D_6	D_7	D_8	
	$LiAlH_4$:$LiAlD_4$ ratio	output stage 3 (yield)	purity by HPLC (%)	188	189	190	192	193	194	195	196	Mwt
SPL028i (**9i**)	0:1	5.3 g (65%)	99.3	0.7	2.7	96.6						306.32
SPL028ii (**9ii**)	1:1	5.7 g (63%)	99.9	30.0	48.3	21.7						305.28
SPL028iii (**9iii**)	1:2	4.2 g (52%)	99.9	16.5	46.8	36.8						305.74
SPL028iv (**9iv**)	1:3	5.6 g (68%)	99.8	9.3	41.5	49.2						305.75
SPL028v (**9v**)	2:1	4.2 g (52%)	99.8	47.5	41.3	11.2						304.98
SPL028vi (**9vi**)	3:1	5.0 g (62%)	99.4	57.4	35.3	7.4						305.03
SPL028vii (**12**)	1:0	5.0 g (49%)	99.9				<0.01	1.2	98.8			310.38
SPL028viii (**14**)	0:1	4.6 g (46%)	99.9						0.1	3.2	96.7	312.39

[a]Percentage ratios of deuteration measured by LCMS. All purity by NMR was >95%. Mwt, Molecular weight.

need for extended stir-out times. Trial reactions found a reverse quench of the complete reaction mixture into 25% Rochelle's solution (aqueous) that allowed for good control of exotherms and off-gassing as well as no significant impurity formation. The optimized workup procedure avoids the use of dichloromethane extraction, which can lead to the formation of small quantities of undesired byproduct *N*-chloromethyl-*N,N*-dimethyltryptamine chloride.[46] The optimized reduction of intermediate **6** (282.5 g) with LiAlH4 (0.9 equiv) in tetrahydrofuran (THF) afforded **1** in 92% yield with 95% purity by HPLC. A mini-salt screen using solutions of **1** (0.25−2 M in ethanol) of five different acids (maleic, fumaric, hydrochloric, tartaric and citric) in four solvents (isopropanol [IPA], THF, isopropyl acetate and acetonitrile) indicated the fumarate salt to be the best candidate with successful salt formations in all four solvents. Further investigation showed that IPA and ethanol were the most suitable candidates for single-solvent salt formations. The results from these indicated that an ethanol-based salt formation in 10−15 volumes of solvent would allow for a good recovery of ∼80% as well as significant purging of impurities (input purity, ∼95%; output purity, 99.9%). Solubility of the fumarate salt of **1** in ethanol at

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temperatures above 65 °C also allowed for a suitable polish filtration window required for Good Manufacturing Practice (GMP) processing (ICH Q7 − Good Manufacturing Practice for Active Pharmaceutical Ingredients). With an input of 248.2 g of free base **1**, and applying the optimized conditions, we afforded fumarate salt **7** (1:1 ratio) in 78% yield with 99.9% purity by HPLC as a crystalline solid (XRPD: Pattern A).

To generate a series of differentially deuterated DMT compounds, a modification of the amide **6** reduction chemistry was developed (Table 1). As sourcing $LiAlD_4$ as a solution in THF proved troublesome, the reaction conditions were modified to use solid $LiAlH_4/LiAlD_4$ mixtures. This proved successful, although it required an excess of $LiAlH_4/LiAlD_4$ using 1.8 equiv instead of the 0.9 equiv used in the optimized synthesis of nondeuterated **1** described above. The previously reported synthesis of D_2-DMT **8** also reduced amide **6** but required 2eq of $LiAlD_4$.[41] By varying ratios of solid $LiAlH_4$ and $LiAlD_4$ as set out in Table 1, the six preparations afforded deuterated products of **8i−vi** as mixtures. D_2-DMT compounds **8i−vi** ($C_{12}H_{14}D_2N_2$) with varying levels of deuteration at the α-carbon to the nitrogen of the tertiary amine, as measured by MS. All final products **9i-vi** were obtained as fumarate salts ($C_{16}H_{18}D_2N_2O_4$) in >99% purity as measured by HPLC in overall yields ranging from 52% to 68%.

The impact of additional deuteration in structure **1** on metabolic stability was explored by extending the described synthetic strategy to afford previously unreported preparative methodology for D_6-DMT **11** ($C_{12}H_{10}D_6N_2$) and D_8-DMT **13** ($C_{12}H_8D_8N_2$). The conversion of acid **4** to amide **10** was achieved using the HOBt/EDC coupling methodology developed previously except for the addition of 3 equiv of *N,N*-diisopropylthylamine (DIPEA) as the D_6-dimethylamine hydrochloride salt. The reaction proceeded with a clean conversion to the desired product, but the reaction stalled with 28% of the starting material **4** remaining. Further additions of DIPEA did not affect the reaction profile. Following reaction workup and chromatography, **10** was afforded in 58% yield. Amide **10** was smoothly reduced to D_6-DMT compound **11** in 97% yield prior to conversion to fumarate salt **12** ($C_{16}H_{14}D_6N_2O_4$) in 87% yield. MS analysis found the following deuterium content: D_0 = not detected; D_1 = 0.01%; D_5 = 1.2%; D_6 = 98.8%. Similarly, amide **10** was smoothly reduced to D_8-DMT compound **13** in 97% yield prior to conversion to fumarate salt **14** ($C_{16}H_{12}D_8N_2O_4$) in 81% yield. MS analysis found the following deuterium content: D_0, not detected; D_6 = 0.1%; D_7 = 3.2%; D_8 = 96.7%.

Hepatocyte and Mitochondrial Fraction Clearance of Deuterated Compounds. The *in vitro* whole cell hepatocyte and mitochondrial fraction metabolic stability of each deuterated DMT analogue (**9i−9vi**, **12**, **14**) and DMT (**7**) investigates the extent of KIE caused by deuteration at the α-carbon and *N*-methyl substituents and is summarized below. Sumatriptan, benzylamine, serotonin, diltiazem and diclofenac were included in each test batch as controls and demonstrated suitable clearance compared to historical data. There was an increase in *in vitro* stability for each DMT analogue with deuterium substitution at the α-carbon position in the human hepatocyte fraction when compared to DMT. A simple linear regression showed that the degree of deuteration at the α-carbon was found to significantly predict an increase in half-life (R^2 adjusted; F = 6.9; 1,7; p = 0.03) and a decrease in clearance (F = 51.04; 1,7; p = 0.0002) (Figure 1).

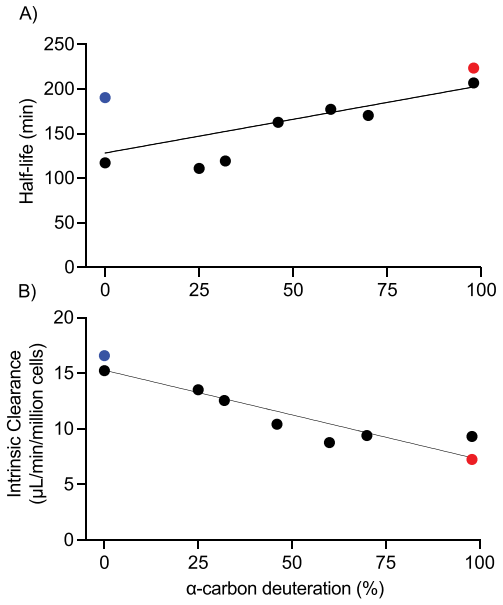

Figure 1. Effect of DMT α-carbon deuteration on the *in vitro* (A) half-life and (B) intrinsic clearance in human hepatocytes. DMT (**7**) is shown in blue, and 96.6% D_2-DMT (**9i**) is shown in red. *N,N*-D_6-dimethyltryptamine, D_6-DMT (**12**) (*i.e.*, no α-carbon deuteration), is the first black point, and *N,N*-D_8-dimethyltryptamine, D_8-DMT (**14**), is the last black point. The black points between these extremes are the other *N,N*-D_2-dimethyltryptamine analogue blends (**9ii−9vi**).

9i (96.6% D_2-DMT, formulated as a fumarate salt) demonstrated the greatest effect on half-life (223.4 min) and clearance (7.3 μL/min/million cells) compared with parent **7** (DMT fumarate) (half-life, 190.4 min and clearance, 16.6 μL/min/million cells). Compound **14** exhibited a similar effect on half-life (206.9 min) and clearance (9.3 μL/min/million cells) on the *in vitro* stability of D_2-DMT in human hepatocytes. The D_6-DMT analogue **12** demonstrated a somewhat reduced half-life (117.2 min) but similar intrinsic clearance to the parent **7** (15.2 μL/min/million cells), indicating that deuteration of the α-carbon has a greater effect on metabolic stability compared to *N*-methyl substituents alone. Therefore, compound **9i** was selected to undergo further *in vitro* characterization.

Human liver mitochondrial fractions contain a high proportion of MAO enzymes as well as aldehyde dehydrogenases and other xenobiotic-metabolizing enzymes[47] and thereby provide a useful tool to measure the clearance of MAO substrates such as DMT.[21] Separate independent two sample *t* tests were performed to compare the mean clearance rate and mean half-life of **9i** and **7**. There was a significant difference in clearance rate between **9i** (mean = 16.5, standard deviation [SD] = 2.2) and **7** (mean = 168.0, SD = 10.5); t(6) = 28.2, p < 0.001 (Figure 2); and a significant difference in half-life between **9i** (mean = 85.1, SD = 11.3) and **7** (mean = 8.3, SD = 0.5); t(6) = 13.6, p < 0.001.

Physicochemical Properties. The physicochemical properties of a drug following deuterium substitution are largely unchanged and so binding to biological targets are unaltered.[25,26] However, the extent of the KIE in physiological systems can impact the clearance and distribution of a deuterated compound.

The distribution coefficient log $D_{7.4}$ determines the distribution ratio between the lipid and aqueous phases in a solution of pH 7.4, *i.e.*, the pH of blood. The log $D_{7.4}$ values of

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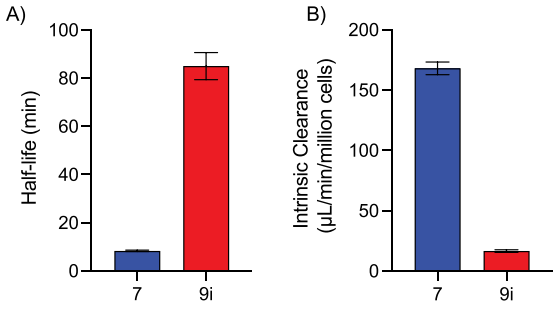

Figure 2. (A) Half-life and (B) intrinsic clearance of **9i** and **7** in human liver mitochondrial fractions.

9i and **7** were conducted in order to determine the lipophilicity of **9i** relative to **7**. Mean log $D_{7.4}$ (n = 2) value was 0.11 for **9i** and 0.15 for **7**, demonstrating that lipophilicity is low for both DMT and D_2-DMT. Mean log $D_{7.4}$ values for ketoconazole (3.56), propranolol (1.26), and verapamil (2.67) positive controls were within normal control range. These results indicate that **9i**, similar to **7**, will have poor membrane permeability and low nonspecific binding which was further supported by plasma protein binding data.

9i is largely unbound to proteins in human plasma (mean ratio free concentration:total concentration [fu] = 0.70) [mean recovery = 98%] and has similar plasma protein binding to **7** (human fu = 0.68 (mean recovery = 98%). The mean fu (mean recovery) of sumatriptan, warfarin and verapamil (control drugs) were 0.68 (96%), 0.05 (98%) and <0.010 (105%) respectively. These data indicate that 70% of **9i** remains unbound and is available in the plasma for diffusion and metabolism.

The mean blood/plasma ratio of **9i** is 1.34 in human blood (n = 2) revealing a lower distribution of **9i** to erythrocyte blood fraction relative to plasma when compared to **7** (1.53 n = 2). Chloroquine (4.93), diclofenac (0.63), and verapamil (0.78) (control drugs) blood/plasma ratio data passed acceptance criteria.

Receptor Binding Profile of 9i. As expected, the *in vitro* receptor binding profile for **9i** was comparable to that of **7**. Assay results are presented as the percent inhibition of radioligand specific binding or enzyme activity. Both compounds demonstrated >50% inhibition of specific binding or activity at a concentration of 10 μM (6 μM free base) at 16 receptors and MAO-A enzyme (Table 2; full *in vitro* characterization is presented in Table S1).

As expected from the published literature, inhibition > 90% with **9i** was observed at the 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors.[8−10] Additionally, a high percentage of inhibition was observed at the 5-HT_{1B}, 5-HT_{2B}, 5-HT_{5A}, 5-HT_6, and 5-HT_7 receptors, which have previously been proposed as targets for **7**.[12] This is of interest as preclinical and clinical studies suggest an agonist role for some of these receptors in the therapeutic effects of a number of antidepressants.[48] Notably, >50% inhibition with **9i** was observed at all α-adrenergic receptors and also at H_1 receptors and $nACh_{\alpha3\beta4}$ receptors. It has been suggested that interactions at multiple types of receptors may underpin the psychedelic effects of **7**,[49] but which receptors are critical for mediating the psychedelic experience remains unclear.

Notably, at σ_1 receptors, inhibition with **9i** at 10 μM (freebase) was 45%. However, as literature emphasizes, the effect of sigma binding of **7** may also be important to the

Table 2. Mean Percent Inhibition in Enzyme and Agonist/Antagonist Radioligand Assay Using 10 μM[a] 9i and 7

receptor	ligand	9i percent inhibition	7 percent inhibition
5-HT_{1A}	1.50 nM [^{3}H] 8-OH-DPAT	97.6	96.0
5-HT_{2B}	1.20 nM [3H] lysergic acid diethylamide (LSD)	96.5	95.5
5-HT_7	5.50 nM [^{3}H] lysergic acid diethylamide (LSD)	95.5	90.4
5-HT_{2A}	0.5 nM [^{3}H] ketanserin	93.9	89.9
5-HT_{2C}	1.0 nM [^{3}H] mesulergine	93.3	93.7
H_1	1.20 nM [3H] pyrilamine	91.4	88.8
α_{1B}	0.2 nM [^{3}H] prazosin	87.2	85.8
5-HT_6	1.50 nM [^{3}H] lysergic acid diethylamide (LSD)	86.2	86.1
α_{1A}	0.6 nM [^{3}H] prazosin	85.1	85.1
MAO-A		84.3	85.3
α_{2B}	2.50 nM [^{3}H] rauwolscine	79.8	81.1
5-HT_{1B}	1.0 nM [^{3}H] GR125743	75.5	73.1
5-HT_{5A}	1.70 nM [^{3}H] lysergic acid diethylamide (LSD)	70.2	72.7
α_{2A}	1.50 nM [^{3}H] rauwolscine	69.1	67.1
$nACh_{\alpha3\beta4}$	0.05 nM [^{125}I] epibatidine	63.5	64.6
α_{1D}	0.6 nM [^{3}H] prazosin	63.2	54.1
α_{2C}	0.5 nM [^{3}H] rauwolscine	57.6	58.4

[a]Concentration given as fumarate salt form.

therapeutic potential of DMT,[18] and to this end, the binding of **9i** to sigma receptors was tested at a range of concentrations. In line with previously published data on DMT, neither **7** nor **9i** showed significant inhibition of [^{3}H] pentazocine (15.0 nM) binding at sigma receptors at ≤10 μM. The concentrations used to report sigma-1 binding affinity in previously published data likely fall outside of biologically relevant concentrations.[15] Inhibition of appropriate ligands of >50% was not observed for any of the ion channels tested or enzymes other than MAO-A.

Receptor binding affinities with **9i** were highest for 5-HT_7, 5-HT_{2A}, and 5-HT_{1A} (Table 3), and profiles for **9i** and **7** support published data for DMT. Keiser et al. reported strongest binding affinities for DMT at 5-HT_{1D} (K_i = 0.039 μM), 5-HT_{2A} (K_i = 0.128 μM), 5-HT_{1B} (K_i = 0.129 μM), 5-HT_{1A} (K_i = 0.183 μM), 5-HT_{2B} (K_i = 0.039 μM), 5-HT_7 (K_i = 0.204 μM).[12] **9i** and **7** were shown to have a greater percent inhibition of specific binding for 5-HT_7 and 5-HT_{2A} and a lower binding affinity for 5-HT_{1B} compared to published data for DMT. 5-HT_{1D} receptor binding was not assessed in the present study due to a lack of assay availability.

It is well-established that DMT can induce profound psychedelic effects and, based on this, has the potential to improve well-being in healthy subjects and therapeutic efficacy in patients.[3,16,50] However, the acute psychedelic effects of DMT are short-lasting due to its rapid metabolism and high clearance rate. In the study presented here, DMT has been modified using deuteration with the aim of modifying the PK profile of the resulting DMT analogues. The D_2-DMT compound **9i** was selected as the optimal analogue, this compound showed improved *in vitro* metabolic stability attributed to inhibited oxidative deamination. Such modifications to the metabolic profile are anticipated to persist *in vivo* (Good et al., manuscript in preparation), resulting in prolonged drug exposure and pharmacodynamic effects. There were no marked changes in physicochemical properties

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Table 3. Receptor Binding Affinities and Enzyme Inhibition for 9i and 7

		9i			7		
receptor/enzyme	ligand	IC_{50} (μM)	K_i (μM)	nH	IC_{50} (μM)	K_i (μM)	nH
5-HT_{1A}	1.50 nM [^{3}H] 8-OH-DPAT	0.19	0.11	1.26	0.18	0.10	0.97
5-HT_{1B}	1.0 nM [^{3}H] GR125743	1.99	1.52	0.74	2.42	1.84	0.76
5-HT_{2A}	0.5 nM [^{3}H] ketanserin	0.22	0.06	0.84	0.15	0.04	0.78
5-HT_{2B}	2.0 nM [^{3}H] mesulergine	0.41	0.30	0.98	0.48	0.35	1.08
5-HT_{2C}	1.0 nM [^{3}H] mesulergine	0.39	0.20	0.94	0.53	0.28	1.38
5-HT_{5A}	1.70 nM [^{3}H] lysergic acid diethylamide (LSD)	3.32	1.71	1.01	3.32	1.71	0.91
5-HT_6	1.50 nM [^{3}H] lysergic acid diethylamide (LSD)	1.08	0.50	0.77	1.21	0.56	0.84
5-HT_7	5.50 nM [^{3}H] lysergic acid diethylamide (LSD)	0.09	0.05	0.67	0.12	0.07	0.91
α_{1A}	0.6 nM [^{3}H] prazosin	0.99	0.48	1.00	0.58	0.28	0.75
α_{1B}	0.2 nM [^{3}H] prazosin	0.93	0.37	0.94	0.67	0.26	0.94
α_{1D}	0.6 nM [^{3}H] prazosin	3.26	1.60	0.73	3.07	1.51	0.85
α_{2A}	1.50 nM [^{3}H] rauwolscine	3.03	1.52	1.00	2.22	1.11	0.76
α_{2B}	2.50 nM [^{3}H] rauwolscine	1.67	0.76	0.97	1.58	0.72	1.09
α_{2C}	0.5 nM [^{3}H] rauwolscine	6.10	2.71	1.03	9.47	4.21	0.56
H_1	1.20 nM [3H] pyrilamine	0.34	0.16	0.63	0.52	0.25	1.09
I_2, central	2.0 nM [3H] idazoxan	2.60	1.73	0.99	1.93	1.29	0.87
$nACh_{\alpha3\beta4}$	0.05 nM [^{125}I] epibatidine	4.40	3.18	0.90	4.20	3.03	0.79
MAO-A		1.50			1.18		

to suggest an impact on drug promiscuity, exemplified by the minimal influence on *in vitro* binding affinities observed when comparing **9i** and parent compound **7** (highest for all 5-HT receptors and α receptors tested; confirming previous published studies). Blood/plasma ratio for **9i** was slightly lower than that for **7**, although both compounds demonstrated drug distribution into the blood cells. This property should be considered when interpreting pharmacokinetic data.

Further assessments will determine whether the similarities in pharmacological profiles of **9i** and **7** are reflected in preclinical *in vivo* studies (Good et al., in preparation) and, ultimately, in the safety, tolerability, and efficacy when administered to healthy subjects and patients in future clinical trials. The safety, tolerability, PK, and psychedelic effects of **7** (as SPL026) have been evaluated in psychedelic-naïve healthy subjects and in patients with major depressive disorder (where therapeutic efficacy was the primary end point) in a Phase I/IIa clinical trial (NCT04673383). A Phase I clinical study comparing the safety, tolerability, PK, and PD of **9i** (as SPL028) administered by the IV *vs* intramuscular route is planned.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00143.

Molecular formula strings (CSV)

Chemistry, experimental information; *in vitro* studies, experimental information; ^{1}H NMR data for test compounds **7**, **9i**–**9vi**, **12**, and **14**; HPLC data for test compounds **7**, **9i**–**9vi**, **12**, and **14**; mean *in vitro* percent inhibition with 10 μM **9i**; *in vitro* receptor, transporter, and ion channel binding assays; *in vitro* enzyme inhibition assays (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Marie Layzell − *Small Pharma., London EC1Y 8RT, U.K.*; orcid.org/0009-0004-7317-9766; Phone: (+44) 7813 878018; Email: marie.layzell@smallpharma.com

Authors

Peter Rands − *Small Pharma., London EC1Y 8RT, U.K.*

Meghan Good − *Small Pharma., London EC1Y 8RT, U.K.*; orcid.org/0000-0002-3829-0723

Zelah Joel − *Small Pharma., London EC1Y 8RT, U.K.*

Rick Cousins − *Cinnabar Consulting Ltd., Clifton, Beds SG17 5QT, U.K.*

Tiffanie Benway − *Small Pharma., London EC1Y 8RT, U.K.*

Ellen James − *Small Pharma., London EC1Y 8RT, U.K.*

Carol Routledge − *Small Pharma., London EC1Y 8RT, U.K.*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.3c00143

Author Contributions

The manuscript was written through contributions of all the authors.

Notes

The authors declare the following competing financial interest(s): M.L., P.R., M.G., Z.J., T.B., E.J. and C.R. are all currently paid employees of Small Pharma and have owned stock in the company, R.C. is a paid independent consultant engaged by Small Pharma.

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■ ABBREVIATIONS

ACE, angiotensin converting enzyme; DCM, dichloromethane; DMT, *N*-dimethyltryptamine; COC, cyclo-oxygenase; DIPEA, *N*,*N*-diisopropylthylamine; DMSO, dimethyl sulfoxide; EDC,

1-ethyl-3-carbodiimide; GMP, good manufacturing practice; HEPES, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; IAA, indole-3-acetic acid; IPA, isopropanol; IV, intravenous; KIE, kinetic isotope effect; LC/MS, liquid chromatography/mass spectrometry; MAO, monoamine oxidase; MDD, major depressive disorder; mGluR, metabotropic glutamate receptor; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; PD, pharmacodynamics; PK, pharmacokinetics; RED, Rapid Equilibrium Dialysis; STAB, sodium triacetoxyborohydride; TBME, *tert*-butyl methyl ether; THF, tetrahydrofuran

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