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# Analytical and Pharmacological Characterization of 1-(Furan-2-Carbonyl)-LSD (1F-LSD) and Comparison With 1-(Thiophene-2-Carbonyl)-LSD (1T-LSD)

Simon D. Brandt<sup>1</sup>  | Pierce V. Kavanagh<sup>2</sup>  | Sarah Gare<sup>3</sup>  | Simon P. Elliott<sup>4,5</sup>  | Alexander Stratford<sup>6</sup> | Adam L. Halberstadt<sup>7,8,9</sup> 

<sup>1</sup>School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK | <sup>2</sup>Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James Hospital, Dublin 8, Ireland | <sup>3</sup>Department of Chemistry, School of Physical Sciences, University of Liverpool, Liverpool, UK | <sup>4</sup>Elliott Forensic Consulting, Birmingham, UK | <sup>5</sup>Department of Analytical, Environmental and Forensic Sciences, King's College London, London, UK | <sup>6</sup>Synex Synthetics BV, Maastricht, The Netherlands | <sup>7</sup>Department of Psychiatry, University of California San Diego, La Jolla, USA | <sup>8</sup>Center for Psychedelic Research, University of California San Diego, La Jolla, USA | <sup>9</sup>Research Service, VA San Diego Healthcare System, San Diego, USA

**Correspondence:** Adam L. Halberstadt ([ahalberstadt@health.ucsd.edu](mailto:ahalberstadt@health.ucsd.edu))

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## ABSTRACT

The classical psychedelic drug (+)-lysergic acid diethylamide (LSD) continues to attract considerable multidisciplinary interest, and over the last eight decades, many derivatives and analogs of LSD have been synthesized. One site on the ergoline scaffold of LSD that has been frequently modified is the  $N^1$ -position, with the  $N^1$ -acylated LSD derivative 1-acetyl-LSD (1A-LSD, ALD-52) being one of the earliest examples. In more recent years, several other alkylcarbonyl- and cycloalkylcarbonyl-substituted LSD derivatives have been evaluated, including several distributed as research chemicals. Although  $N^1$ -substitution is detrimental for the activity of LSD at the 5-HT<sub>2A</sub> receptor (the primary site of action of psychedelic drugs),  $N^1$ -acylated LSD derivatives are rapidly hydrolyzed in vivo and are believed to act as prodrugs for LSD. Recently, 1-(thiophene-2-carbonyl)-LSD (1T-LSD, SYN-L-021) was detected as a new recreational drug, signaling a move towards  $N^1$ -acyl groups with an aromatic character. The present study was conducted to investigate the analytical profile and pharmacology of 1-(2-furoyl)-lysergic acid diethylamide (1F-LSD, SYN-L-005), a novel analog of 1T-LSD. The binding of 1F-LSD to the 5-HT<sub>2A</sub> receptor and other monoamine sites was assessed using radioligand binding. Furthermore, the in vivo activities of 1F-LSD and 1T-LSD were assessed in C57BL/6J mice by comparing their biotransformation to LSD and effects on the head-twitch response (HTR), a 5-HT<sub>2A</sub>-mediated behavior. Both 1F-LSD and 1T-LSD induced the HTR in mice and were hydrolyzed to LSD after in vivo administration, indicating that both substances exhibit LSD-like properties and may serve as prodrugs for LSD.

## 1 | Introduction

Classical psychedelic drugs such as (+)-lysergic acid diethylamide (LSD) and psilocybin have been the subject of considerable

interest in recent years due to their therapeutic potential for treating psychiatric disorders and their status as popular recreational drugs. The psychedelic effects of these molecules are believed to be largely mediated by activation of the 5-HT<sub>2A</sub>

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receptor in the brain [1]. Although clinical trials are being conducted with LSD to test its efficacy against disorders such as depression, substance abuse, and chronic pain, new analogs and derivatives of LSD are also being synthesized and explored [2]. The indole nitrogen moiety in the ergoline scaffold is amenable to substitution with acyl and other groups, making this position a frequent target for modification. During the 1950s and 1960s, *N*<sup>1</sup>-methyl-LSD (MLD-41) and *N*<sup>1</sup>-acetyl-LSD (ALD-52) were synthesized and found to act as psychedelic drugs in humans [3–7]. More recently, numerous novel *N*<sup>1</sup>-alkylcarbonyl- and *N*<sup>1</sup>-cycloalkylcarbonyl-substituted LSD derivatives have appeared as new psychoactive substances (NPS) and have been distributed online [8–17]. Notable examples include *N*<sup>1</sup>-propanoyl-LSD (1P-LSD) and *N*<sup>1</sup>-butanoyl-LSD (1B-LSD) [8, 10]. These and other *N*<sup>1</sup>-acyl-LSD derivatives are hydrolyzed after *in vivo* administration and are believed to serve as prodrugs for LSD [18, 19]. When LSD binds to the 5-HT<sub>2A</sub> receptor, the indole *N*<sup>1</sup>hydrogen interacts with S242<sup>5,46</sup> in the binding pocket [20]. Because the interaction is required for receptor activation, *N*<sup>1</sup>-substitution reduces the affinity of LSD for the 5-HT<sub>2A</sub> receptor by an order of magnitude and significantly reduces its agonist efficacy [19]. *N*<sup>1</sup>-Acyl LSD derivatives therefore have limited ability to activate the 5-HT<sub>2A</sub> receptor directly and their rapid hydrolysis to LSD likely accounts for their psychedelic activity *in vivo* [18].

Recently, 1-(thiophene-2-carbonyl)-LSD (1T-LSD, SYN-L-021, Figure 1) was detected in recreational drug samples from Japan and Germany, signifying a transition of NPS toward new *N*<sup>1</sup>-acyl LSD derivatives containing an aromatic ring system. Pharmacological data about this substance are lacking, although it has been described in the patent literature [2].

The aim of the present study was to close the information gap regarding 1T-LSD and compare its pharmacological properties with those of the closely related analog 1-(furan-2-carbonyl)-LSD (1F-LSD, SYN-L-005; Figure 1). Receptor binding studies were conducted to assess the affinity of 1F-LSD for the 5-HT<sub>2A</sub> receptor and 21 other monoaminergic receptors. Experiments compared the effect of 1T-LSD and 1F-LSD on the head-twitch response (HTR), a rapid rotational head shaking induced by psychedelic drugs, in male C57BL/6J mice. The HTR is mediated by 5-HT<sub>2A</sub> receptor activation and serves as a validated rodent behavioral proxy for the psychedelic effects induced by LSD-like drugs in humans [8, 10, 13, 17, 19]. To test the hypothesis that 1T-LSD and 1F-LSD may serve as prodrugs, additional experiments evaluated whether those molecules are hydrolyzed to LSD after administration to mice. It is not known whether 1F-LSD is currently available as a NPS, but key analytical details

are also included to aid its detection in scientific research and in forensic cases.

## 2 | Experimental

### 2.1 | Materials

All chemicals and solvents were of analytical or HPLC grade and were obtained from Aldrich (Dorset, UK). 1F-LSD hemitartrate (2:1) and 1T-LSD hemitartrate (2:1) were provided by Synex Synthetics BV (Maastricht, The Netherlands). It is worth noting that the code 1F-LSD has occasionally been used to describe 1-formyl-LSD [21–23] although it is unclear whether this substance was unambiguously identified. For the purpose of the present investigation, 1F-LSD denotes 1-(furan-2-carbonyl)-LSD.

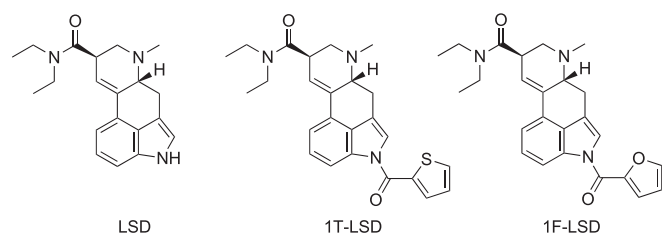
### 2.2 | Instrumentation

#### 2.2.1 | Gas Chromatography-Electron Ionization Mass Spectrometry (GC-EI-MS)

Electron ionization mass spectra were recorded on an Agilent 5977A MSD detector (Agilent, Cheadle, UK). Temperature settings were as follows: transfer line 275 °C, source 230 °C, and quadrupole 150 °C. The mass spectrometer settings were as follows: solvent delay 3 min; EI mode, 70 eV, and range *m/z* 28–500. Chromatographic analysis was carried out using an Agilent 7890A system (Agilent, Cheadle, UK). The carrier gas was helium at a flow rate of 1 mL/min. The injection temperature was 275 °C. Separations were performed on a 30 m × 0.25 mm (0.25 μm film thickness) Agilent HP-5MS column. The column temperature was programmed as follows: 100 °C held for 1 min, then heated at 20 °C/min to 310 °C and held constant for 22.5 min (total run time 34 min). A 1 μL solution of 1F-LSD tartrate in acetonitrile (2 mg/mL) was injected for analysis (split: 1:25).

#### 2.2.2 | High Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (UHPLC-QTOF-MS/MS)

UHPLC-ESI-QTOF-MS was performed on a QTOF (Agilent 6540, Cheadle, UK) instrument coupled with a 1290 Infinity II UPLC from Agilent Technologies (Cheadle, UK). Chromatographic separation was achieved on an EC C18 Poroshell 120 column (50 mm × 2.1 mm, 1.9 μm particle size) from Agilent Technologies. Mobile phase A (0.1% v/v formic acid in water and B was 0.1% v/v formic acid in acetonitrile). The elution profile was programmed as follows: T<sub>min</sub>/A:B (70:30); T<sub>6</sub>/10:90; T<sub>8</sub>/10:90; flow rate: 0.2 mL/min; column oven was at 30 °C. The injection volume was 0.5 μL and 0.25 μL for MS/MS and MS respectively. Agilent MassHunter version B.08:00 was used for acquisition and analysis. The QTOF was operated in positive electrospray ionization mode, acquiring spectra in the range *m/z* 50–1000 (acquisition rate 1.15 spectra/s). Acquisition was performed in full scan/AutoMS/MS mode at four fixed collision energies (10–40 eV). The drying gas temperature was at 300 °C with a flow rate of N<sub>2</sub> at 8.0 L/min. The nebulizer gas pressure was 35 psi. Nitrogen was used as the collision gas. The



**FIGURE 1** | Structures of (+)-lysergic acid diethylamide (LSD), 1-(thiophene-2-carbonyl)-LSD (1T-LSD, SYN-L-021), and 1-(furan-2-carbonyl)-LSD (1F-LSD, SYN-L-005).

voltage for the capillary was 3500 V, nozzle voltage was 1000 V and the fragmentor voltage was a 100 V. Mass calibration was performed using G1969-85000 ESI-L low concentration tuning mix for dual ESI Jet stream source. The reference masses used to internally calibrate the QTOF were purine and HP-0921 ( $m/z$  121.0509 and  $m/z$  922.0098 (Agilent Technologies).

### 2.2.3 | High Performance Liquid Chromatography Diode Array Detection

A Dionex 3000 Ultimate liquid chromatography system coupled to a UV diode array detector (Thermo Fisher, St. Albans, UK) was used with a Phenomenex Synergi Fusion column (150 mm  $\times$  2 mm, 4  $\mu$ m) protected by a 4 mm  $\times$  3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Macclesfield, UK). The mobile phases were 70% acetonitrile with 25 mM of triethylammonium phosphate buffer (TEAP) (B) and aqueous TEAP (25 mM) buffer (A). The gradient elution commenced with 4% B and ramped to 70% B over 15 min and then held for 3 min, resulting in a total acquisition time of 18 min at a flow rate of 0.6 mL/min. The diode array detection window was set at 200–595 nm (collection rate 2 Hz).

### 2.2.4 | Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra ( $^1\text{H}$  at 600 MHz;  $^{13}\text{C}$  at 150 MHz) of the powdered sample (10 mg, 0.75 mL DMSO- $d_6$ s) were recorded using a Bruker AVANCE III 600 MHz spectrometer (Bruker UK Ltd, Coventry, UK). Experiments were carried out at 298 K with a 5 mm PA BBO probe with z-gradient. Spectra were referenced to residual solvent and assignments were supported by both 1D and 2D experiments.

## 2.3 | Animal Pharmacology

Male C57BL/6J mice (6–8 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed up to four per cage with a reversed light-cycle (lights on at 1900 h, off at 0700 h). Food and water were provided *ad libitum*, except during behavioral testing. Testing was conducted between 1000 and 1830 h. All animal experiments were carried out in accordance with NIH guidelines and were approved by the UCSD animal care committee. The HTR was assessed using a head-mounted magnet and a magnetometer detection coil [24]. Mice were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a two-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between experiments to avoid carryover effects. In Experiment 1, mice were injected IP (5 mL/kg injection volume) with vehicle (saline) or 1F-LSD. In Experiment 2, mice were injected IP with vehicle (water containing 12% dimethylsulfoxide) or 1T-LSD. After drug treatment, mice were immediately placed in a glass cylinder surrounded by a magnetometer coil and head movement was recorded continuously for 30 min. Coil voltage was low-pass filtered (2-kHz cutoff frequency), amplified, digitized (20-kHz

sampling rate, 16-bit ADC resolution), and saved to disk using a Powerlab 8/35 data acquisition system with LabChart software ver. 8.1.16 (ADInstruments, Colorado Springs, CO, USA). To detect head twitches, events in the recordings were transformed to scalograms, deep features were extracted using the deep convolutional neural network ResNet-50, and then the images were classified using a support vector machine (SVM) [25]. Total head twitch counts were analyzed using a one-way ANOVA. HTR counts were also binned in 5-min blocks and analyzed using a two-way ANOVA (drug  $\times$  time). *Post-hoc* comparisons were made using Dunnett's test. Significance was demonstrated by surpassing an  $\alpha$ -level of 0.05.  $\text{ED}_{50}$  values and 95% confidence intervals were calculated using non-linear regression.

## 2.4 | Assessment of the Metabolism of 1T-LSD and 1F-LSD to LSD

### 2.4.1 | Sample Collection

Male C57BL/6J mice were injected IP with 1 mg/kg of 1T-LSD or 1F-LSD ( $n = 3$  mice/compound, six total). A DMSO stock solution was prepared immediately before the experiment. The injection volume was 5 mL/kg. Thirty minutes later, the mice were anesthetized with isoflurane and sacrificed by decapitation. Trunk blood was collected in tubes coated with  $\text{K}_2\text{EDTA}$ . Within 30 min of collection, the blood was centrifuged (2000 rpm) for 12 min at 4  $^\circ\text{C}$ , and then plasma was collected in 50  $\mu\text{L}$  aliquots, flash frozen with dry ice, and stored at  $-80^\circ\text{C}$ .

### 2.4.2 | Sample Preparation by Solid-Phase Extraction

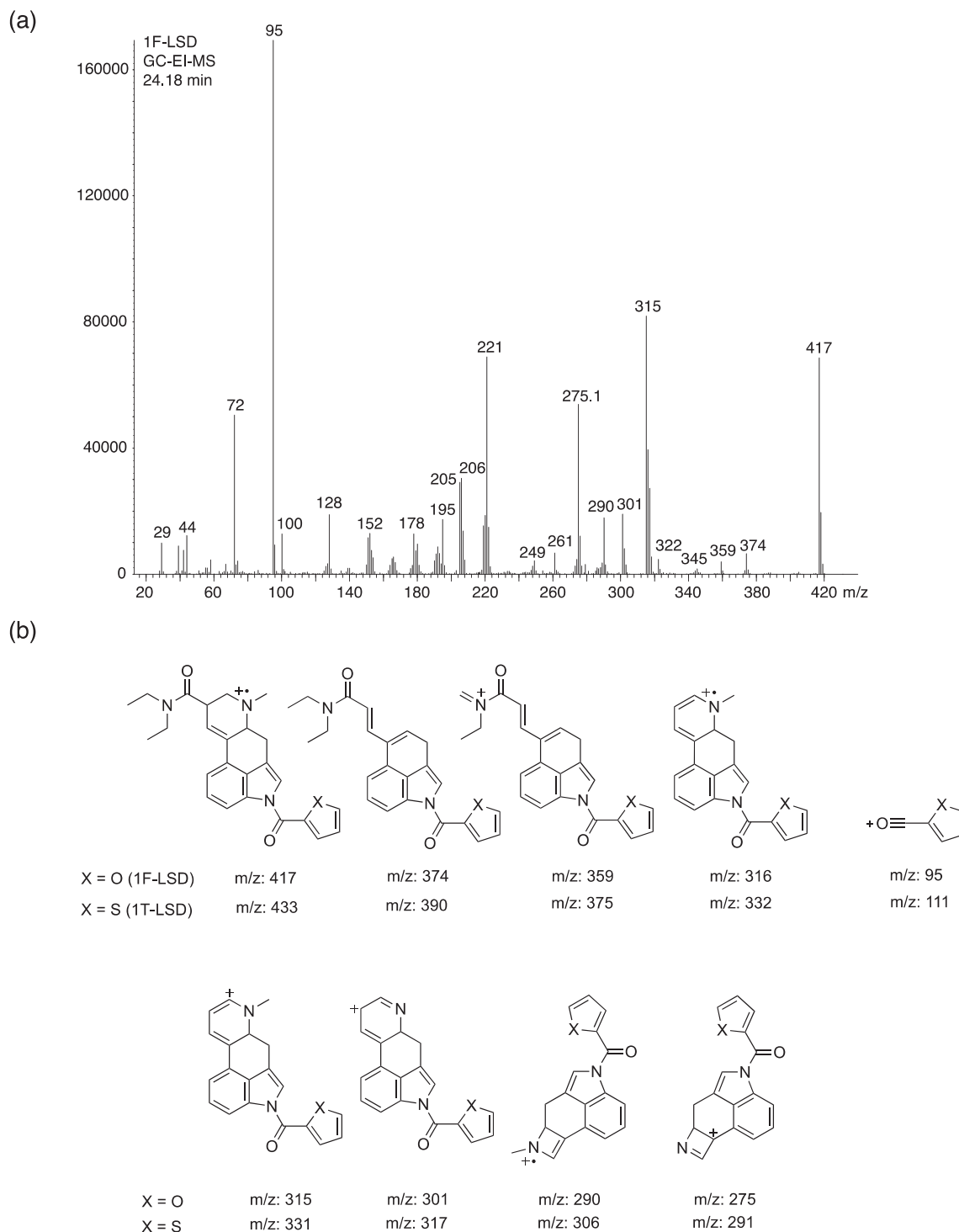
Sample preparation was performed as described previously with minor modifications [19, 26]. Ten microliters of methanolic LSD- $d_3$  (as internal standard, final plasma concentration 5 ng/mL) were added to 0.1 mL of plasma, diluted with 2.9 mL of purified water, mixed for 15 s on a rotary shaker, and loaded on a HXC cartridge (130 mg, 3 mL) previously conditioned with 1 mL of methanol and 1 mL of purified water. After extraction, the cartridge was washed with 1 mL of purified water, 1 mL of 0.01 M aqueous hydrochloric acid, and 2 mL of methanol. Reduced pressure was applied until the cartridge was dry and the analytes were eluted with 1 mL of a freshly prepared mixture of methanol-aqueous ammonia (98:2, v/v) into a reaction tube. The eluate was evaporated to dryness under a stream of nitrogen at 70  $^\circ\text{C}$  and the residue was dissolved in 25  $\mu\text{L}$  of a mixture of 10 mM aqueous ammonium formate-acetonitrile (1:1, v/v) containing 0.1% formic acid. The LSD plasma concentration was determined using an LC-ion trap MS apparatus and an LC-high-resolution MS/MS apparatus by calculating the mean value of both analyses.

### 2.4.3 | LC-Ion Trap MS Apparatus for LSD Quantification

As previously described [19], samples were analyzed using a ThermoFisher Scientific (TF, Dreieich, Germany) LXQ linear ion trap MS, coupled to a TF Accela ultra high performance

LC (UHPLC) system consisting of a degasser, a quaternary pump, and an autosampler. Gradient elution was performed on a TF Hypersil GOLD C18 column (100 mm × 2.1 mm inner diameter, 1.9 μm particle size). The mobile phase consisted of 10 mM aqueous ammonium formate plus 0.1% formic acid (pH 3.4, eluent A) and acetonitrile plus 0.1% formic acid (eluent B). The flow rate was set to 0.5 mL/min and the following gradient was used: 0–2.0 min 2% B, 2.0–4.0 min to 80% B, 4.0–6.0 min hold 80% B, 6.0–6.5 min to 90% B, 6.5–7.0 min hold 90% B, 7.0–10.0 min hold 80% B, 10.0–17.0 min hold 2% B.

Analyses were performed in a targeted acquisition mode with an inclusion list, where MS2 spectra of given precursor ions (LSD and LSD-d3) were recorded. The injection volume was 10 μL each. The MS was equipped with a heated electrospray ionization II (HESI-II) source, other conditions were as follows: positive ionization mode; sheath gas, nitrogen at flow rate of 34 arbitrary units (AU); auxiliary gas, nitrogen at flow rate of 11 AU; vaporizer temperature, 250 °C; source voltage, 3.00 kV; ion transfer capillary temperature, 300 °C; capillary voltage, 38 V; tube lens voltage, 110 V; automatic gain control

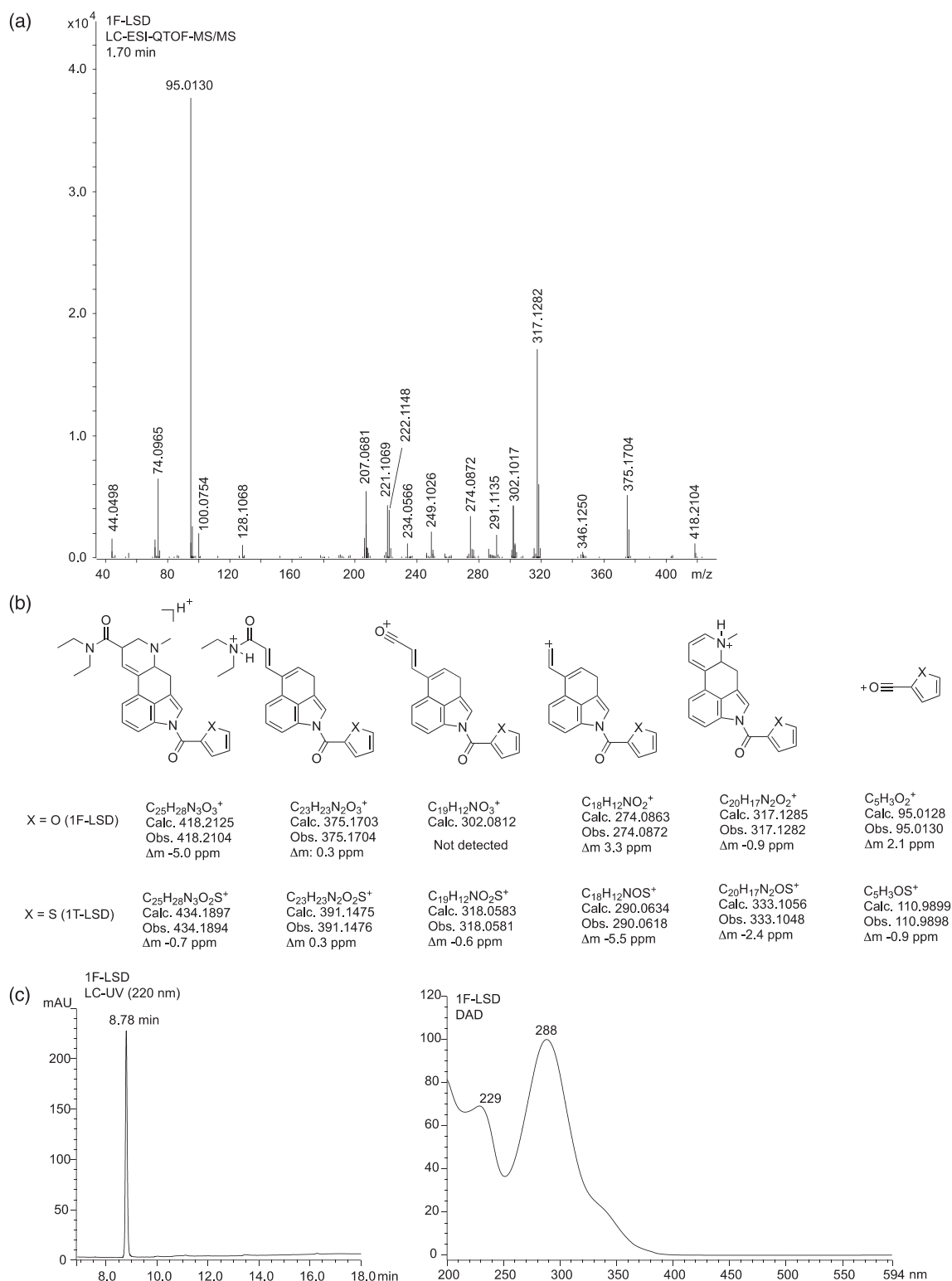


**FIGURE 2** | (a) Electron ionization mass spectrum recorded for 1F-LSD. (b) Proposed, generalized key ions reflecting the presence of the N<sup>1</sup>-acyl group. The mass spectrum for the sulfur analog 1T-LSD can be found as Supporting Information for comparison.

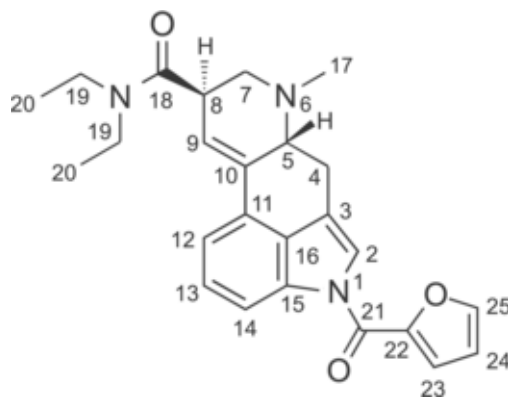
(AGC) target, 5000 ions for MS2; data type, centroid; normalized collision energy, 35.0; wideband activation, enabled; isolation width,  $m/z$  1.5. TF Xcalibur Qual Browser software version 2.0.7 was used for data evaluation and LSD concentration was determined comparing the peak areas of LSD and LSD- $d_3$  within the same run.

## 2.5 | Binding Studies

A screening at 22 receptor binding sites was performed by the NIMH Psychoactive Drug Screening Program (NIMH PDSP). 1F-LSD was tested at  $10\mu\text{M}$  in competition assays against radioactive probe compounds; each primary binding assay was



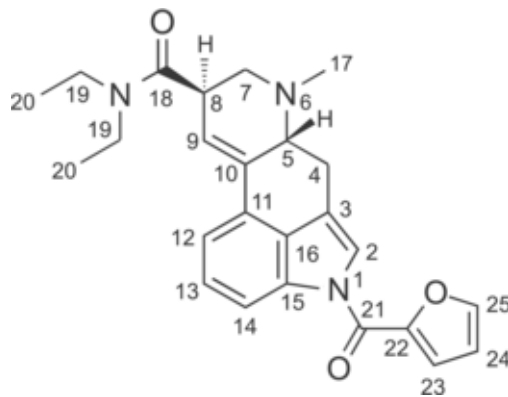
**FIGURE 3** | (a) Electrospray ionization QTOF tandem mass spectrum of 1F-LSD. (b) Proposed, generalized key ions reflecting the presence of the  $N^1$ -acyl group. The mass spectrum for the sulfur analog 1T-LSD can be found as Supporting Information for comparison. (c) HPLC-UV-DAD data recorded for 1F-LSD.

**TABLE 1** |  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for 1F-LSD hemitartrate (2:1) in  $\text{DMSO-}d_6$  at 600/150 MHz.

No.	$^{13}\text{C}$ [ $\delta/\text{ppm}$ ]	$^1\text{H}$ [ $\delta/\text{ppm}$ ]
2	120.71 or 120.70	7.88 (d, $J=1.7\text{Hz}$ , 1 H)
3	117.13	—
4	26.12	2.49–2.45 (m, 1 H) * Overlapping with H-17 (3 H) and solvent 3.56 (dd, $J=15.4, 5.4\text{Hz}$ , 1 H)
5	61.76	3.13–3.05 (m, 1 H)
6	—	—
7	55.34	3.01 (dd, $J=11.0, 4.9\text{Hz}$ , 1 H) 2.61 (t, $J=10.8\text{Hz}$ , 1 H)
8	38.95	3.86–3.79 (m, 1 H)
9	122.10	6.37 (s, 1 H)
10	133.38	—
11	128.01	—
12	117.26	7.41 (d, $J=7.5\text{Hz}$ , 1 H)
13	126.09	7.35 (t, $J=7.8\text{Hz}$ , 1 H)
14	114.94	8.03 (d, $J=8.0\text{Hz}$ , 1H)
15	134.00	—
16	127.45	—
17	43.14	2.48 (s, 3 H) * Overlapping with solvent and partially overlapping with H-4 (1 H)
18	170.39	—
19	41.58	3.45 (q, $J=7.0\text{Hz}$ , 2 H)
19	39.72	3.32 (AB qq, $J=13.0, 6.9\text{Hz}$ , 2 H)
20	14.84	1.19 (t, $J=7.1\text{Hz}$ , 3 H)
20	13.07	1.06 (t, $J=7.0\text{Hz}$ , 3 H)
21	156.18	—
22	145.83	—
23	120.71 or 120.70	7.57 (d, $J=3.6\text{Hz}$ , 1 H)
24	112.66	6.84 (dd, $J=3.6, 1.7\text{Hz}$ , 1 H)
25	147.89	8.13 (m, 1 H)

(Continues)

TABLE 1 | (Continued)



No.	$^{13}\text{C}$ [ $\delta$ /ppm]	$^1\text{H}$ [ $\delta$ /ppm]
TA <sup>a</sup>	173.29	—
TA <sup>a</sup>	71.98	4.23 (s, ~1.3 H)

<sup>a</sup>TA: Tartaric acid.

performed in quadruplicate. Sites exhibiting > 50% inhibition at 10  $\mu\text{M}$  were tested in secondary assays at the identified receptor using 11 concentrations of 1F-LSD, measured in triplicate, to generate competition binding isotherms.  $K_i$  values were obtained from best-fit  $\text{IC}_{50}$  values (derived from nonlinear regression of the binding isotherms) using the Cheng-Prusoff equation [27]. (Cheng and Prusoff, 1973). The experimental protocols are available from the NIMH PDSP website [28].

### 3 | Results and Discussion

The electron ionization (EI) mass spectrum of 1F-LSD is presented in Figure 2a followed by some proposed key fragment ions associated with the  $\text{N}^1$ -acyl group, which were based on suggested pathways reported previously (Figure 2b) [8, 10, 13, 15–17, 29–31]. Other ions and ion clusters typically detected in EI mass spectra of many lysergamides have been described abundantly, so only some key ions specifically relevant for 1F-LSD in relation to its 1-(thiophene-2-carbonyl) counterpart 1T-LSD (EI mass spectrum in Supporting Information) are shown. The EI mass spectrum of 1T-LSD reported in this study is consistent with those reported earlier [32, 33]. As shown in Figure 2b, the selected key ions recorded in the mass spectra showed a mass shift of 16 Da that reflected the mass difference between the oxygen and sulfur atoms located in the acyl groups. The oxonium ion at  $m/z$  111 observed in the EI mass spectrum of 1T-LSD was also reported for 1-(2-thiophenecarbonyl)-6-allyl-nor-lysergic acid diethylamide (1T-AL-LAD) [34]. However, one example reflecting independence from the substituent at the  $\text{N}^6$ -position recorded in the spectrum of 1T-LSD and 1T-AL-LAD appeared to be observable at  $m/z$  291. The furanoyl counterpart detected in the mass spectrum of 1F-LSD might have been the  $m/z$  275 species (Figure 2a,b).

Analysis by GC-MS also resulted in the detection of two additional peaks that appeared to be artificially induced since they were not detectable under LC-MS conditions. The identity of these degradants could not be solved with certainty but mass

spectral considerations led to the hypothesis that they might have reflected  $\text{N}^6$ -demethylation and ring-opening leading to a secondary amine (Supporting Information).

Figure 3a depicts the electrospray ionization QTOF tandem mass spectrum of 1F-LSD. Similar to the examples shown in Figure 2, some key product ions suggested to reflect the presence of the  $\text{N}^1$ -acyl group are shown in Figure 3. The principles of their formation proposed formation were based on related mechanisms reported before [8, 10, 13, 15–17, 29–31]. The QTOF tandem mass spectrum of 1T-LSD is shown as Supporting Information and was essentially comparable with a high-resolution spectrum reported in the literature [32]. As shown in the Supporting Information, the chromatographic separation between 1F- and 1T-LSD was straightforward when using the chosen UHPLC-QTOF-MS method.

The LC-DAD-UV data recorded for 1F-LSD is shown in Figure 3c. The full scan UV spectrum showed two maxima at 229 and 288 nm, which were slightly lower compared to the spectrum reported for 1T-LSD (235 and 293 nm) [33], possibly reflecting the two different heteroatoms. When exploring an alternative HPLC-DAD method (Supporting Information), the slight differences in their two UV spectra were also noted. The nuclear magnetic resonance (NMR) spectroscopy data for 1F-LSD are shown in Table 1 (full spectra shown as Supporting Information). Assignments were aided by 2-dimensional experiments. NMR spectra recorded for 1T-LSD have been reported previously [32, 33]. For comparison, the proton and DEPTQ spectra recorded for 1T-LSD were included as Supporting Information. However, it has to be noted that the analysis of this sample (obtained separately) revealed the detection of imidazole.

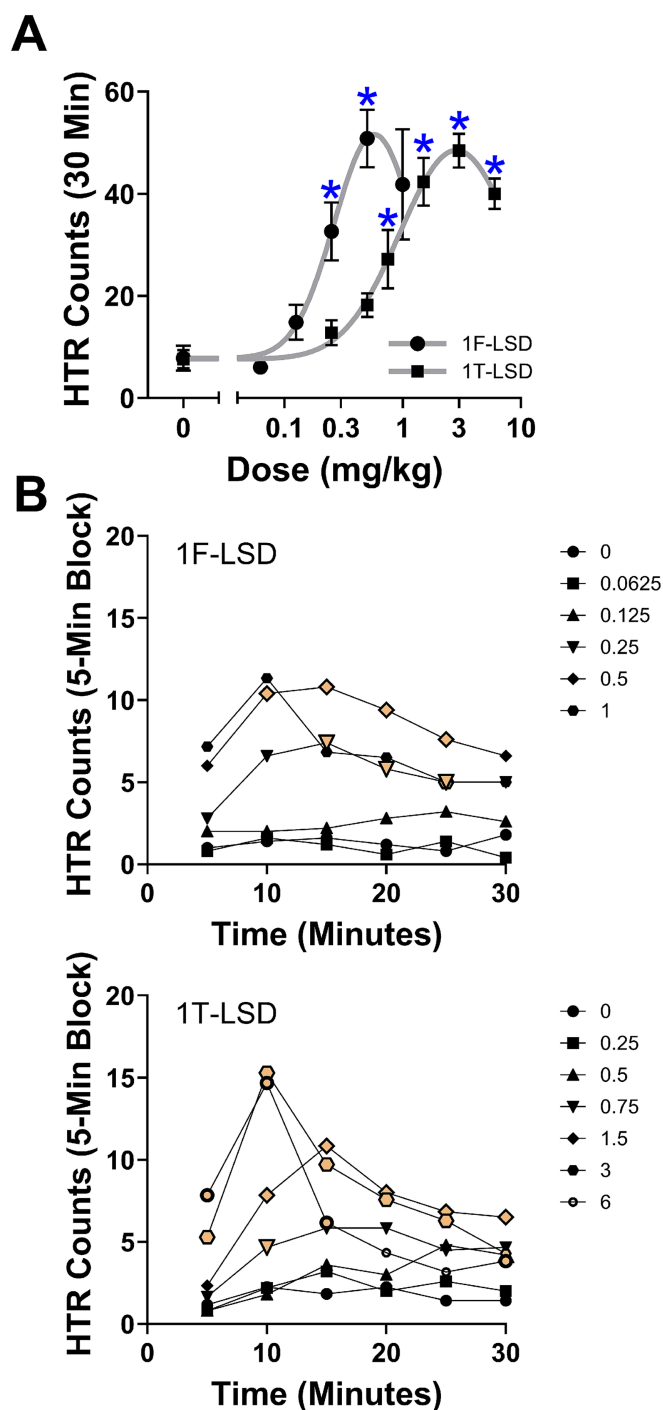
#### 3.1 | Head Twitch Response (HTR)

Both 1T-LSD ( $F_{6,40} = 25.41$ ,  $p < 0.0001$ ) and 1F-LSD ( $W_{5,10.5} = 15.15$ ,  $p = 0.0002$ ) produced dose-dependent

increases in HTR counts over baseline levels (Figure 4a). The median effective dose (ED<sub>50</sub>) of 1F-LSD was 229.5 μg/kg (95% CI 160.4–328.5), whereas 1T-LSD was less potent and induced head twitches with an ED<sub>50</sub> of 780.4 μg/kg (95% CI 594.9–975.3). Given their molecular weights, the ED<sub>50</sub> values are equivalent to 466 nmol/kg (1F-LSD) and 1.534 μmol/kg (1T-LSD). 1F-LSD thus has a threefold higher potency, which is a significant difference based on an extra-sum-of-squares *F*-test ( $F_{1,28} = 9.825$ ,  $p = 0.004$ ). When tested under similar

experimental conditions, LSD induces the HTR in C57BL/6J mice with an ED<sub>50</sub> = 132.8 nmol/kg [24], making it about 3× as potent as 1F-LSD and 10× as potent as 1T-LSD. The potency of 1F-LSD is roughly equivalent to that of the *N*<sup>1</sup>-acyl analogs 1P-LSD (ED<sub>50</sub> = 349.6 nmol/kg), 1V-LSD (ED<sub>50</sub> = 373 nmol/kg), and 1cP-LSD (ED<sub>50</sub> = 430 nmol/kg).

When tested in the HTR assay, the maximal response induced by LSD and many *N*<sup>1</sup>-acyl derivatives occurs 5–10 min



**FIGURE 4** | (a) Effects of 1F-LSD and 1T-LSD on the head twitch response. Data are presented as group means  $\pm$  SEM for the entire 30-min test session; \* $p < 0.05$ , significant difference from vehicle control group (Dunnett's test). (b) Time course of the head twitch responses induced by both test drugs. Data are presented as group means during 5-min time blocks. The time blocks where there were significant differences from the vehicle control group are identified using colored symbols,  $p < 0.05$  (Dunnett's test).

after IP dosing. A similar time-course was apparent when the HTR data for 1T-LSD and 1F-LSD were binned in 5-min blocks (Figure 4b; 1T-LSD Drug×Time:  $F_{30,200} = 8.752$ ,  $p < 0.0001$ ; 1F-LSD Drug×Time:  $F_{25,125} = 2.548$ ,  $p = 0.0004$ ). For both compounds, the response to maximally active doses peaked during the 5–10 min time block, whereas the response induced by lower doses peaked slightly later, during the 10–15 min time block.

### 3.2 | Biotransformation

To assess whether 1T-LSD and 1F-LSD are hydrolyzed to LSD *in vivo*, biotransformation studies were conducted in male C57BL/6J mice. Each compound was administered at an IP dose of 1 mg/kg, plasma samples were collected 30 min later, and then the concentration of LSD was quantified using LSD- $d_3$  as an internal standard. After treatment with 1T-LSD and 1F-LSD, the plasma samples contained high concentrations of LSD ( $150.0 \pm 25.2$  ng/mL [mean  $\pm$  SEM] in mice treated with 1T-LSD and  $100.3 \pm 5.5$  ng/mL in mice treated with 1F-LSD). A discrepancy was noticed between the potency of 1F-LSD and 1T-LSD in the HTR paradigm and the plasma levels of

LSD. One complicating factor is that, in addition to functioning as prodrugs,  $N^1$ -acyl derivatives of LSD can also act as 5-HT<sub>2A</sub> antagonists. Although the conversion of 1T-LSD to LSD appeared to be more efficient than that of 1F-LSD, if 1T-LSD exhibits greater potency at the 5-HT<sub>2A</sub> receptor compared to 1F-LSD, it may induce a more substantial level of 5-HT<sub>2A</sub> blockade, thereby diminishing its potency in the HTR assay. Additionally, it remains uncertain whether the sulfur heteroatom in 1T-LSD serves as a site of biotransformation. The sulfur atom in a thiophene ring may theoretically undergo oxidation, resulting in a sulfoxide metabolite, and there is also the possibility of thiophene ring opening. Both of these biotransformation pathways could potentially produce relatively potent 5-HT<sub>2A</sub> antagonists, which may contribute to the diminished HTR activity observed with 1T-LSD. However, more in-depth investigations would be warranted.

### 3.3 | Receptor Binding

LSD is a pharmacologically diverse drug and has submicromolar affinity for most monoaminergic receptor subtypes. As

**TABLE 2** | Receptor binding data for 1F-LSD.

Receptor	Species <sup>a</sup>	Radioligand	$K_i$ (nM) $\pm$ SEM <sup>b,c</sup>
5-HT <sub>1A</sub>	Human	[ <sup>3</sup> H]8-OH-DPAT	385 $\pm$ 116 (3)
5-HT <sub>1B</sub>	Human	[ <sup>3</sup> H]GR125743	1455 $\pm$ 374 (3)
5-HT <sub>1D</sub>	Human	[ <sup>3</sup> H]GR125743	1146 $\pm$ 154 (3)
5-HT <sub>2A</sub>	Human	[ <sup>3</sup> H]ketanserin	225 $\pm$ 36 (4)
5-HT <sub>2B</sub>	Human	[ <sup>3</sup> H]LSD	9.8 $\pm$ 2.7 (3)
5-HT <sub>2C</sub>	Human	[ <sup>3</sup> H]mesulergine	125 $\pm$ 14 (3)
$\alpha_{1A}$	Human	[ <sup>3</sup> H]prazosin	> 10,000 <sup>c</sup>
$\alpha_{1B}$	Human	[ <sup>3</sup> H]prazosin	> 10,000 <sup>c</sup>
$\alpha_{1D}$	Human	[ <sup>3</sup> H]prazosin	> 10,000 <sup>c</sup>
$\alpha_{2B}$	Human	[ <sup>3</sup> H]rauwolscine	> 10,000 <sup>c</sup>
$\beta_1$	Human (heart)	[ <sup>125</sup> I]pindolol	> 10,000 <sup>c</sup>
$\beta_2$	Human	[ <sup>3</sup> H]CGP12177	> 10,000 <sup>c</sup>
$\beta_3$	Human	[ <sup>3</sup> H]CGP12177	> 10,000 <sup>c</sup>
D <sub>1</sub>	Human	[ <sup>3</sup> H]SCH23390	1986 $\pm$ 390 (3)
D <sub>2</sub>	Human	[ <sup>3</sup> H] <i>N</i> -methylspiperone	> 10,000 <sup>c</sup>
D <sub>3</sub>	Human	[ <sup>3</sup> H] <i>N</i> -methylspiperone	1429 $\pm$ 504 (3)
D <sub>4</sub>	Human	[ <sup>3</sup> H] <i>N</i> -methylspiperone	2271 $\pm$ 418 (3)
D <sub>5</sub>	Human	[ <sup>3</sup> H]SCH23390	> 10,000 <sup>c</sup>
H <sub>1</sub>	Human	[ <sup>3</sup> H]pyrilamine	> 10,000 <sup>c</sup>
H <sub>2</sub>	Human	[ <sup>3</sup> H]tiotidine	1027 $\pm$ 347 (3)
H <sub>3</sub>	Guinea pig	[ <sup>3</sup> H] $\alpha$ -methylhistamine	> 10,000 <sup>c</sup>
H <sub>4</sub>	Human	[ <sup>3</sup> H]histamine	> 10,000 <sup>c</sup>

<sup>a</sup>Cloned receptors were used unless noted otherwise.

<sup>b</sup>The number of independent determinations (performed in triplicate) is indicated in parentheses.

<sup>c</sup>Values of > 10,000 nM are listed when there was < 50% displacement at 10  $\mu$ M in the primary or secondary binding assay.

previously reported [19],  $N^1$ -butanoyl substitution reduces the affinity of LSD for most monoamine receptors by 10–100-fold. Receptor binding data for 1F-LSD at 22 monoaminergic sites are shown in Table 2. The  $N^1$ -(furan-2-carbonyl) group in 1F-LSD has a similar effect on its receptor interactions. While LSD has high nanomolar affinity for the 5-HT<sub>2A</sub> receptor labeled with [<sup>3</sup>H]ketanserin ( $K_i = 14.7$  nM [19]), the affinity of 1F-LSD for 5-HT<sub>2A</sub> ( $K_i = 225$  nM) is 15-fold lower. Likewise, compared to LSD ( $K_i = 9.5$  nM [19]), 1F-LSD has 40-fold lower affinity ( $K_i = 385$  nM) for the 5-HT<sub>1A</sub> receptor. 1F-LSD has even lower (micromolar) affinity for 5-HT<sub>1B</sub> ( $K_i = 1455$  nM), 5-HT<sub>1D</sub> ( $K_i = 1146$  nM), D<sub>1</sub> ( $K_i = 1978$  nM), D<sub>3</sub> ( $K_i = 1429$  nM), and D<sub>4</sub> ( $K_i = 2171$  nM) receptors. In addition, while LSD has moderate to high affinity for  $\alpha_1$ -adrenergic and dopamine D<sub>2</sub> and D<sub>5</sub> receptors, 1F-LSD lacks appreciable affinity for those sites (10  $\mu$ M 1F-LSD produced <50% displacement of radioligand binding). Although the  $N^1$ -(furan-2-carbonyl) group in 1F-LSD is generally detrimental for receptor binding, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> sites are exceptions. 1F-LSD binds to the 5-HT<sub>2B</sub> receptor with  $K_i = 9.8$  nM, which is very similar to the affinity of 1B-LSD ( $K_i = 3.5$  nM; [19]) and LSD ( $K_i = 3.7$  nM [35]). There was only a threefold difference in the affinity of LSD ( $K_i = 45.3$  nM [19]; and 1F-LSD ( $K_i = 125$  nM) for the 5-HT<sub>2C</sub> receptor.

## 4 | Conclusion

These data add to the accumulating knowledge base about newly emerging lysergamide NPS. Both 1T-LSD and 1F-LSD induced head twitches in mice, indicating these substances are capable of activating the 5-HT<sub>2A</sub> receptor *in vivo* and would likely act as psychedelic drugs in humans, similar to LSD. Both compounds are hydrolyzed to LSD after administration to mice, which likely explains why they are capable of inducing the HTR in mice despite containing an  $N^1$ -substituent. These data may inform further multidisciplinary investigations into these and other lysergamides that may be developed or appear in the future. The HTR data confirmed that 1F-LSD and its thiophene analog 1T-LSD exhibited LSD-like behavioral activity *in vivo*, suggesting that it might act as a serotonergic hallucinogen in humans. Further studies are warranted to shed further light on their pharmacodynamic and pharmacokinetic properties and their potential for abuse.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Additional data are available as Supporting Information.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section.