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Return of the lysergamides. Part IV: Analytical and pharmacological characterization of lysergic acid morpholide (LSM-775)

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Complete List of Authors:	Brandt, Simon; School of Pharmacy & Biomolecular Sciences , Liverpool John Moores University Kavanagh, Pierce; St. James Hospital, Department of Pharmacology and Therapeutics Twamley, Brendan; Trinity College Dublin, School of Chemistry Westphal, Dr. Folker; State Bureau of Criminal Investigation Schlesing Holstein, Nacotics/Toxicology Elliott, Simon; Alere Forensics (Forensics Ltd) Wallach, Jason; University of the Sciences, Stratford, Alexander; Synex Synthetics BV Klein, Landon; University of California San Diego, Department of Psychiatry McCorvy, John; University of North Carolina, Department of Pharmacology Nichols, David; University of North Carolina, Chapel Hill, Division of Chemical Biology and Medicinal Chemistry Halberstadt, Adam; University of California San Diego, Department of Psychiatry
Keywords:	New psychoactive substances, LSD, 5-HT_{2A} receptor, Lysergamides, Psychedelics
Abstract:	Lysergic acid diethylamide (LSD) is perhaps one of the best-known psychoactive substances and many structural modifications of this prototypical lysergamide have been investigated. Several lysergamides were recently encountered as "research chemicals" or new psychoactive substances (NPS). Although lysergic acid morpholide (LSM-775) appeared on the NPS market in 2013, there is disagreement in the literature regarding the potency and psychoactive properties of LSM-775 in humans. The present investigation attempts to address the gap of information that exists regarding the analytical profile and pharmacological effects of LSM-775. A powdered sample of LSM-775 was characterized by X-ray crystallography, nuclear magnetic resonance stereoscopy (NMR), gas chromatography mass spectrometry (GC-MS), high mass accuracy electrospray MS/MS, HPLC diode array detection, HPLC quadrupole MS, and GC solid-state infrared analysis. Screening for receptor affinity and functional efficacy revealed that LSM-775 acts as a nonselective agonist at 5-HT1A and 5-HT2A receptors. Head twitch studies were conducted in C57BL/6J mice to determine whether LSM-775 activates 5-HT2A receptors

	and produces hallucinogen-like effects in vivo. LSM-775 did not induce the head twitch response unless 5-HT1A receptors were blocked by pretreatment with the antagonist WAY-100,635 (1 mg/kg, subcutaneous). These findings suggest that 5-HT1A activation by LSM-775 masks its ability to induce the head twitch response, which is potentially consistent with reports in the literature indicating that LSM-775 is only capable of producing weak LSD-like effects in humans.

Return of the lysergamides. Part IV: Analytical and pharmacological characterization of lysergic acid morpholide (LSM-775)

Simon D. Brandt,[a] Pierce V. Kavanagh,[b] Brendan Twamley,[c] Folker Westphal,[d] Simon P. Elliott,[e] Jason Wallach,[f] Alexander Stratford,[g] Landon M. Klein,[h] John D. McCorvy,[i] David E. Nichols,[j] Adam L. Halberstadt[k,*]

[a] *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK*

[b] *Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James Hospital, Dublin 8, Ireland*

[c] *School of Chemistry, Trinity College Dublin, Dublin 2, Ireland*

[d] *State Bureau of Criminal Investigation Schleswig-Holstein, Section Narcotics/Toxicology, Mühlenweg 166, D-24116 Kiel, Germany*

[e] *Alere Forensics (Forensics Ltd), Malvern Hills Science Park, Geraldine Road, WR14 3SZ, UK*

[f] *Department of Pharmaceutical Sciences, Philadelphia College of Pharmacy, University of the Sciences, 600 South 43rd Street, Philadelphia, PA 19104, USA*

[g] *Synex Synthetics BV, Poortweg 4, 2612 PA Delft, The Netherlands*

[h] *Department of Neurosciences, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA*

[i] *Department of Pharmacology, University of North Carolina, Genetic Medicine Building, 120 Mason Farm Road, Chapel Hill, NC 27599-7365, USA*

[i] *Division of Chemical Biology and Medicinal Chemistry, University of North Carolina, Genetic Medicine Building, 120 Mason Farm Road, Chapel Hill, NC 27599, USA*

[k] *Department of Psychiatry, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA*

* Correspondence to: Adam L. Halberstadt, Department of Psychiatry, University of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0804 USA. E-Mail: ahalberstadt@ucsd.edu

Running title: Analytical and pharmacological characterization of LSM-775

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Abstract

Lysergic acid diethylamide (LSD) is perhaps one of the best-known psychoactive substances and many structural modifications of this prototypical lysergamide have been investigated. Several lysergamides were recently encountered as “research chemicals” or new psychoactive substances (NPS). Although lysergic acid morpholide (LSM-775) appeared on the NPS market in 2013, there is disagreement in the literature regarding the potency and psychoactive properties of LSM-775 in humans. The present investigation attempts to address the gap of information that exists regarding the analytical profile and pharmacological effects of LSM-775. A powdered sample of LSM-775 was characterized by X-ray crystallography, nuclear magnetic resonance stereoscopy (NMR), gas chromatography mass spectrometry (GC-MS), high mass accuracy electrospray MS/MS, HPLC diode array detection, HPLC quadrupole MS, and GC solid-state infrared analysis. Screening for receptor affinity and functional efficacy revealed that LSM-775 acts as a nonselective agonist at 5-HT_{1A} and 5-HT_{2A} receptors. Head twitch studies were conducted in C57BL/6J mice to determine whether LSM-775 activates 5-HT_{2A} receptors and produces hallucinogen-like effects *in vivo*. LSM-775 did not induce the head twitch response unless 5-HT_{1A} receptors were blocked by pretreatment with the antagonist WAY-100,635 (1 mg/kg, subcutaneous). These findings suggest that 5-HT_{1A} activation by LSM-775 masks its ability to induce the head twitch response, which is potentially consistent with reports in the literature indicating that LSM-775 is only capable of producing weak LSD-like effects in humans.

Introduction

The hallucinogenic properties of lysergic acid diethylamide (LSD; Figure 1) and other lysergamides have been investigated extensively.[1-7] Although most of the recent research with lysergamides has focused on their pharmacology and structure-activity relationships,[8-11] studies have also assessed potential therapeutic applications.[12,13] LSD is a popular recreational drug and its use has remained relatively stable over the past few decades. Recently, however, other lysergamides have been distributed as "research chemicals" or new psychoactive substances (NPS),[14] including 1-propionyl-LSD (1P-LSD),[15] *N*^{6}-allyl-6-norlysergic acid diethylamide (AL-LAD),[16] (2′*S*,4′*S*)-lysergic acid 2,4-dimethylazetidide (LSZ),[16] *N*^{6}-ethyl-6-norlysergic acid diethylamide (ETH-LAD),[17] and 1-propionyl-ETH-LAD (1P-ETH-LAD).[17] These lysergamides are shown in Figure 1. Lysergic acid morpholide (LSM-775) appeared as an NPS in 2013, presumably based on reports from the late 1950s indicating that it may have LSD-like effects in humans.

The preparation of LSM-775 was first described in the 1950s by Stoll and Hoffmann[18] and several other routes have also been reported.[19-21] There is disagreement in the literature regarding the potency and psychoactive properties of LSM-775 in humans. Goggerty and Dille reported that 75 µg LSM-775 produced approximately the same response as 50 µg LSD in two subjects, although LSM-775 had a shorter duration of action than LSD.[22] In a blinded experiment conducted by Abramson, two subjects administered 150 µg LSM-775 estimated that they had received 25–35 µg LSD, whereas a third subject estimated that they had received a placebo.[23] Isbell *et al*. found that higher doses of LSM-775 (4.5 and 9 µg/kg) produced effects roughly equivalent to a threshold dose of LSD, but with a shorter duration.[24,25] One potential explanation for these discrepant findings is that LSM-775 may be capable of producing only a threshold psychedelic response. However, the methodological weaknesses in early human studies with LSM-775 make interpretation challenging.

The present investigation aimed to fill the gap of information that exists on the analytical profile and pharmacological effects of LSM-775. A powdered sample of LSM-775 was characterized by X-ray crystallography, nuclear magnetic resonance stereoscopy (NMR), gas chromatography mass spectrometry (GC-MS), high mass accuracy electrospray MS/MS, HPLC diode array detection, HPLC quadrupole MS, and GC solid-state infrared analysis.

LSD and other lysergamide hallucinogens act as nonselective serotonin (5-HT) receptor agonists. Although the characteristic effects of serotonergic hallucinogens are thought to be mediated by the 5-HT$_{2A}$ receptor,[26-29] there is evidence indicating that the 5-HT$_{1A}$ receptor may also contribute to or modulate their effects.[4,30] Interactions of LSM-775 with 5-HT$_{1A}$ and 5-HT$_2$ receptor subtypes were assessed using competitive binding and functional assays.

Serotonergic hallucinogens induce the head twitch response (HTR) in rodents due to 5-HT$_{2A}$ receptor activation.[31,32] The HTR is widely used as a behavioral proxy in

rodents for human hallucinogenic effects because it is one of the few behaviors that can reliably distinguish hallucinogenic and non-hallucinogenic 5-HT_{2A} receptor agonists.[33] HTR studies were conducted in C57BL/6J mice to determine whether LSM-775 produces 5-HT_{2A} receptor activation and LSD-like behavioral effects *in vivo*.

Experimental

Materials

All chemicals used were of analytical or HPLC grade and were obtained from Rathburn Chemicals Ltd (Walkerburn, Scotland, UK), Fisher Scientific (Dublin, Ireland) or Aldrich (Dorset, UK). LSM-775 hemitartrate was obtained from Synex Synthetics BV (Delft, The Netherlands). LSM-775 tartrate from Lipomed Inc. (Cambridge, MA, USA) was used in the *in vitro* pharmacological studies. *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100,635) dihydrochloride was donated by Wyeth-Ayerst Research (Princeton, NJ, USA).

Instrumentation

Nuclear magnetic resonance spectroscopy

The hemitartrate sample was prepared in deuterated dimethyl sulfoxide (DMSO-d_6). ^{1}H (600 MHz) and ^{13}C (150 MHz) spectra were recorded on a Bruker AV600 NMR spectrometer using a 5 mm TCI cryoprobe. ^{11}H NMR spectra were referenced to residual solvent at δ = 2.51 ppm and assignments were supported by both 1D and 2D experiments.

Gas chromatography mass spectrometry

A Finnigan TSQ 7000 triple stage quadrupole mass spectrometer coupled to a gas chromatograph (Trace GC Ultra, Thermo Electron, Dreieich, Germany) was used to record electron ionization (EI) mass spectra (70 eV) and a CTC CombiPAL (CTC Analytics, Zwingen, Switzerland) autosampler was employed for sample introduction. The ion source temperature was set at 175°C and the emission current was 200 µA. The scan time was 1 s spanning a scan range between *m/z* 29–*m/z* 600 and samples were injected in splitless mode. Separation was achieved using a fused silica capillary DB-1 column (30 m × 0.25 mm, film thickness 0.25 µm). The temperature program consisted of an initial temperature of 80°C, held for 1 min, followed by a ramp to 310°C at 20 °C/min. The final temperature was held for 23 min. The injector temperature was 250°C. The transfer line temperature was set at 300 °C and the carrier gas was helium in constant flow mode at a flow rate of 1.0 mL/min. Approximately 2 mg were dissolved in 1.5 mL chloroform. For analysis, 1 µL sample solution was injected into the GC-MS system. Retention indices are given as Kovats indices calculated from measurement of a *n*-alkane mixture analyzed with the above mentioned temperature program.

Gas chromatography solid-state infrared analysis

Samples were analyzed using a GC-solid phase-IR-system that consisted of an Agilent GC 7890B (Waldbronn, Germany) with probe sampler Agilent G4567A and a DiscovIR-GC™ (Spectra Analysis, Marlborough, MA, USA). The column eluent was cryogenically accumulated on a spirally rotating ZnSe disk cooled by liquid nitrogen. IR spectra were recorded through the IR-transparent ZnSe disk using a nitrogen-cooled MCT detector. GC parameters: injection in splitless mode with an injection port temperature set at 240°C and a DB-1 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness). The carrier gas was helium with a flow rate of 2.5 mL/min and the oven temperature program was as follows: 80°C for 2 min, ramped to 290°C at 20 °C/min, and held at for 20 min. The transfer line was heated at 280°C. Infrared conditions: oven temperature, restrictor temperature, disc temperature, and Dewar cap temperatures were 280°C, 280°C, -40°C, and 35°C, respectively. The vacuum was 0.2 mTorr, disc speed 3 mm/s, spiral separation was 1 mm, wavelength resolution 4 cm^{-1} and IR range 650–4000 cm^{-1}. Acquisition time was 0.6 s/file with 64 scans/spectrum. Data were processed using GRAMS/AI Ver. 9.1 (Grams Spectroscopy Software Suite, Thermo Fischer Scientific, Dreieich, Germany) followed by implementation of the OMNIC Software, Ver. 7.4.127 (Thermo Electron Corporation, Dreieich, Germany).

High mass accuracy electrospray mass spectrometry

Ultrahigh-performance liquid chromatography quadrupole time-of-flight single and tandem mass spectrometry (UHPLC-QTOF-MS/MS) data were obtained from an Agilent 6540 UHD Accurate-Mass Q-TOF LC-MS system coupled to an Agilent 1290 Infinity UHPLC system (Agilent, Cheshire, UK). Separation was achieved using an Agilent Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 µm) (Agilent, Cheadle, UK). Mobile phases consisted of acetonitrile (1% formic acid) and 1% formic acid in water. The column temperature was set at 40°C (0.6 mL/min) and data were acquired for 5.5 min. The gradient was set at 5–70% acetonitrile over 3.5 min, then increased to 95% acetonitrile in 1 min and held for 0.5 min before returning to 5% acetonitrile in 0.5 min. QTOF-MS data were acquired in positive mode scanning from *m/z* 100–*m/z* 1000 with and without auto MS/MS fragmentation. Ionization was achieved with an Agilent JetStream electrospray source and infused internal reference masses. QTOF-MS parameters: gas temperature 325°C, drying gas 10 L/min and sheath gas temperature 400°C. Internal reference ions at *m/z* 121.05087 and *m/z* 922.00979 were used for calibration purposes.

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 for analysis using a Phenomenex Synergi Fusion column (150 mm × 2mm, 4 µm) that was protected by a 4 mm × 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Cheshire, 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 in 15 min and 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 nm–595 nm (collection rate 2 Hz).

Liquid chromatography electrospray mass spectrometry

Analyses were carried on an Agilent 1100 system using a Restek (Bellefonte, PA, USA) Allure PFPP column (5 µm, 50 × 2.1 mm). The aqueous mobile phase A consisted of 0.1% formic acid, whereas, mobile phase B consisted of 0.1% formic acid in acetonitrile. The total run time was 25 min. The following gradient elution program was used: 0–2 min 2% B, followed by an increase to 60% within 15 min, then up to 80% within 20 min, returning to 2% within 25 min. The Agilent LC-MSD settings were as follows: positive electrospray mode, capillary voltage 3500 V, drying gas (N_2) 12 L/min at 350°C, nebulizer gas (N_2) pressure 50 psi, Scan mode *m/z* 70–500, fragmentor voltage values used for in-source collision-induced dissociation (CID) were 30 V and 150 V, respectively. The sample was dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 10 µg/mL. The injection volume was 1 µL, flow rate was 0.80 mL/min and the column temperature was 30°C.

X-ray crystallography

Data for LSM-775 were collected on a Bruker APEX DUO with Cu Kα radiation (λ = 1.54184 Å) using a MiTeGen micromount and at 100(2) K (Oxford Cobra Cryosystem). Bruker APEX2 software[34] was used to collect and reduce data, determine the space group, solve and refine the structure. Absorption corrections were applied using SADABS.[35,36] All final refinements were performed with XL.[37] All non-hydrogen atoms were refined anisotropically and all donor hydrogen atoms were located and refined with restraints. All other hydrogen atoms were assigned to calculated positions using a riding model. The ethanol solvent molecule C-C bond was restrained (DFIX). Absolute configuration was established by anomalous-dispersion effects in diffraction measurements on the crystal. Cambridge Crystallographic Dater Centre (CCDC) 1486037 contains the supplementary crystallographic data. Crystal data and structure refinement parameters were as follows: $C_{46}H_{60}N_6O_{12}$, M = 889.0, T = 100(2) K, orthorhombic, $P2_12_12_1$, a = 5.9621(2), b = 15.5087(6), c = 47.4811(19) Å, V = 4390.3(3) Å^3, Z = 4, µ (Cu Kα) = 0.807 mm^{-1}, ρ = 1.345 Mg/cm^3, 57701 reflections collected, 8027 independent (R_{int} = 0.0444), $^aR_1$ = 0.0371, wR2 = 0.0979 ($I > 2\sigma(I)$), S = 1.050. CCDC 1486037. $^aR_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Pharmacological Experiments

Head-twitch response

Male C57BL/6J mice (6−8 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed up to four per cage on a reversed light-cycle (lights on at 1900 h, off at 0700 h). Food and water were provided *ad libitum*, except during behavioral testing, which was performed between 1000 h and 1800 h. The head twitch response (HTR) was detected using a head mounted magnet and a

magnetometer coil, as described previously.[32,38] In experiment 1, mice (n = 6/group, 30 total) were treated with vehicle or LSM-775 (0.1, 0.3, 1, or 3 mg/kg). In experiment 2, mice (n = 6/group, 30 total) were pretreated with 1 mg/kg WAY-100,635 20 min prior to treatment with vehicle or LSM-775 (0.1, 0.3, 1, or 3 mg/kg). In experiment 3, mice (n = 5–6/group, 23 total) were pretreated with vehicle or 1 mg/kg WAY-100,635 20 min prior to treatment with vehicle or 1 mg/kg LSM-775. Behavior was monitored for 30 min immediately after treatment with LSM-775. LSM-775 was dissolved in isotonic saline and injected intraperitoneally (IP) (5 mL/kg); WAY-100,635 was dissolved in sterile water and injected subcutaneously (SC) (5 mL/kg). HTR counts were analyzed by one-way or two-way ANOVAs; *post-hoc* comparisons were made using Tukey's studentized range method. Significance was demonstrated by surpassing an α-level of 0.05. ED_{50} values and 95% confidence intervals were calculated using nonlinear regression.

Binding studies

Radioligand binding studies for human 5-HT_{1A} and human 5-HT_{2A} receptors were performed by the NIMH Psychoactive Drug Screening Program (NIMH PDSP). LSM-775 was dissolved in DMSO and primary binding screens were conducted in quadruplicate. Sites exhibiting >50% inhibition at 10 μM were tested in competitive binding assays to determine K_i values. The experimental protocols are available from the NIMH PDSP website.[39]

5-HT_2 receptor functional assays

5-HT_2 functional experiments (measuring G_q-mediated calcium flux) were performed with Flp-In T-REx 293 cells (Invitrogen, Carlsbad, CA, USA) expressing either human 5-HT_{2A}, mouse 5-HT_{2A}, human 5-HT_{2B} or human 5-HT_{2C} INI receptor. The day before the assay, receptor expression was induced with 2 μg/mL tetracycline, and cells were plated into white 384 clear-bottom, tissue culture plates in 40 μL of DMEM containing 1% dialyzed fetal bovine serum (FBS) at a density of approximately 15,000 cells per well. The next day, drug dilutions were diluted in drug buffer (HBSS, 20 mM HEPES, 0.1% BSA, 0.01% ascorbic acid, pH 7.4). Before the assay, media was decanted and replaced with 20 μL per well of drug buffer (HBSS, 20 mM HEPES, pH 7.4) containing Fluo-4 Direct dye (Invitrogen) and incubated for 1 h at 37°C. Plates were allowed to equilibrate to room temperature and calcium flux was measured using a FLIPR^{TETRA} (Molecular Devices, Sunnyvale, CA, USA). Plates were read for fluorescence initially for 10 seconds (1 read per second) to establish a baseline, and then stimulated with drug dilutions or buffer and read for an additional 110 seconds. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. Data were normalized to the maximum peak fold-over-basal fluorescence produced by 5-HT (100%) and baseline fluorescence (0%). Data were analyzed using the sigmoidal dose-response function of GraphPad Prism 5.0.

5-HT_{1A} receptor functional assays

5-HT_{1A} functional experiments (measuring $G_{i/o}$-mediated cAMP inhibition) were performed in HEK293T cells (ATCC, Manassas, VA, USA) co-expressing the

GloSensor cAMP biosensor (Promega, Madison, WI, USA) and the human 5-HT_{1A} receptor. On the day of transfection, HEK cells were washed with PBS, and 10% dialyzed FBS in DMEM was added to cells before addition of calcium phosphate precipitation transfection mix containing a 1:1 ratio of GloSensor-22F and human 5-HT_{1A} receptor DNA. The next day, cells were plated into white 384 clear-bottom, tissue culture plates in 40 µL of DMEM containing 1% dialyzed FBS at a density of approximately 15,000 cells per well. On the day of the assay, drug dilutions prepared for 5-HT_{1A} receptor functional assays were the same drug dilutions used for 5-HT_2 receptor functional assays, where the assays were performed in parallel on the same day. Before the assay, plate media was decanted and replaced with 20 µL per well of drug buffer (HBSS, 20 mM HEPES, pH 7.4) containing GloSensor substrate and allowed to equilibrate for at least 15 min at room temperature. To start the assay, cells were treated with 10 µL per well of drug using a FLIPR (Molecular Devices) and incubated for 15 min. Afterwards, cAMP accumulation was initiated by addition of 0.3 µM isoproterenol (final concentration) and luminescence per well per second was counted 15 min later on a TriLux microbeta plate reader (PerkinElmer, Waltham, MA, USA). Data were normalized to the maximum cAMP inhibition produced by 5-HT (100%) and basal cAMP accumulation induced by isoproterenol (0%). Data were analyzed using the sigmoidal dose-response function of GraphPad Prism 5.0.

Results and Discussion

Analytical characterization

Electron ionization (EI) mass spectra and Kovats indices for LSM-775 are displayed in Figure 2. As shown in the insert of Figure 2A, analysis by GC-MS revealed the detection of a second, minor peak that exhibited a similar mass spectrum, thus, indicating the potential presence of a diastereomer. Whether the presence of the extra peak was artificially induced by GC conditions, as reported previously for lysergic acid 2,4-dimethylazetidide (LSZ)[16] or whether it reflected a contaminant present in the powdered sample could not be established unambiguously. However, as shown below, a second peak was also detected under HPLC single quadrupole MS conditions (HPLC-Q-MS) using single ion monitoring (SIM), which suggested that the diastereomer may have been present in the sample. The EI mass spectrum associated with the major GC peak (Figure 2B, isomer II) reflected the identity of the correctly configured LSM-775. Suggested EI-MS fragmentation pathways for LSM-775 have been provided as Supporting Information, which followed similar principles discussed previously for other lysergamides, such as 1P-LSD, AL-LAD, LSZ, ETH-LAD, and 1P-ETH-LAD.[15-17] Fragments associated with LSM-775 specifically included *m/z* 337 (highly abundant molecular ion), *m/z* 294 (retro-Diels-Alder) and *m/z* 279. Ergoline-related fragments ions and fragment clusters that have also been observed with other lysergamides (e.g. *m/z* 151–*m/z* 154 or *m/z* 178–*m/z* 181)[15-17] were also detected (see the Supporting Information). Figure 3 provides the solid-state infrared (IR) spectrum of the main LSM-775 isomer II following elution from the GC column. A key feature that differentiated LSM-775 from the lysergamides reported previously[15-17] was detected at 1115.6 cm^{-1}, which may reflect the C-O stretch correlated with the morpholine ring.

UHPLC-ESI-QTOF-MS/MS, LC-Q-MS and LC-DAD data collected from LSM-775 are summarized in Figure 4. The proposed identity of the product ions under QTOF-MS/MS conditions are summarized in the Supporting Information section and were based on the principles reported previously for other lysergamides.[15-17] Product ions characteristic for LSM-775, apart from the protonated molecule at *m/z* 338.18660 ($C_{20}H_{24}N_3O_2^+$, 338.18630, Δ = 0.89 ppm), included *m/z* 323.16165 ($C_{19}H_{21}N_3O_2^{\bullet+}$, 323.16283, Δ = -3.65 ppm) and *m/z* 295.14449 ($C_{18}H_{19}N_2O_2^+$, 295.14410, Δ = 1.32 ppm) (see the Supporting Information). A product ion of minor abundance detected at *m/z* 114.05556 may represent the morpholine-4-carbonyl species ($C_5H_8NO_2^+$, 114.05495, Δ = 5.35 ppm). In-source collision-induced dissociation spectra obtained from analysis by HPLC-Q-MS and increasing fragmentor voltages are shown in Figure 4B–4E. The sodiated adduct at *m/z* 360 was also detected. The most abundant product ions were detected at *m/z* 208, *m/z* 223, and *m/z* 295. HPLC-Q-MS analysis in SIM mode also revealed a second peak at 9.039 min (LSM-775 isomer 2) (Figure 4F), although it was not possible to determine whether this represented the 8α-epimer iso-LSM-775. The HPLC-DAD spectrum recorded for LSM-775 (Figure 4G) was essentially similar to that obtained with other lysergamides[15-17] and hence had limited value for differentiation under these conditions.

Table 1 provides a summary of proposed identifications of chemical shifts obtained from analysis by nuclear magnetic resonance spectroscopy (NMR). Assignments were aided by two-dimensional experiments and comparison with lysergamides reported and discussed in detail previously using the same deuterated solvent (d_6-DMSO).[15-17] NMR spectra are provided as Supporting Information. Residual solvent traces, possibly *tert*-butanol and isopropanol,[40] were also detected in the ^1H and ^{13}C NMR spectra (Supporting Information). In the ^{13}C NMR spectrum, the morpholine methylenes were detected as four separate resonances due to restricted rotation associated with the amide bond, although the methylene groups from the amide (C-22) were only separated marginally by 0.18 ppm (see Table 1 and the Supporting Information). The remaining carbon chemical shifts were comparable to the lysergamides reported in earlier studies.[15,16]

The morpholine ring protons could not be sufficiently resolved to determine the corresponding coupling constants or splitting patterns. The HSQC experiment suggested that the broad multiplet between 3.67–3.57 ppm represented overlapping contributions from the four NCH_2 (2 x CH_2-22) and one OCH_2 (1 x CH_2-21) protons. The other two CH_2-21 methylene protons were detected slightly more upfield between 3.53–3.49 ppm and this multiplet overlapped with the resonance from the 4β-proton, also visible in the HSQC experiment. The 4α-H proton overlapped with the N^6-methyl group singlet (CH_3-17), thus giving rise to the multiplet around 2.53–2.49 ppm, which was observed previously with other lysergamides[15,16] (see the Supporting Information). The NMR data also revealed the presence of residual solvents (isopropanol and *tert*-butanol) that may have been used to recrystallize LSM-775 during the manufacturing process (Supporting Information). Some inaccuracies were encountered in the integration of the proton signals due to an

increasing baseline, but these were not subsequently observed when the NMR spectra were recorded again in a different solvent (CD_3OD) (Supporting Information).

Clear colorless needles of LSM-775 obtained by recrystallization with ethanol and water were used to determine the structure at 100 K. The data were solved and refined in the chiral orthorhombic space group $P2_12_12_1$ and the absolute configuration was established. The asymmetric unit consists of two independent protonated cationic molecules of LSM-775 and a dianionic tartrate counter ion, as well as a water and an ethanol molecule (Figure 5A). The two LSM-775 molecules were similar except for a twist between the methylindoloquinoline and morpholine groups that exhibited the same chirality (*R*) at each center (see the Supporting Information for a detailed description of the conformational differences between the two LSM-775 molecules). N11 and N36 on each molecule were protonated and formed hydrogen bonds to the dianionic tartrate molecule, with d(D-A) distances N11-O51 = 2.729(3)Å and N36-$O59^{i4}$ = 2.631(3)Å (symmetry operation i4 = x, y+1, z). The water molecule also forms a hydrogen bond to the tartrate (O61-O60 = 2.831(3)Å), while the ethanol molecule forms a hydrogen bond to the water (O62-O61 = 2.795(4)Å). This solvent/anion unit formed a central scaffold around which three molecules of LSM-775 were arranged: two as discussed above and another that hydrogen bonded *via* the N-H of the five-membered ring in the indoloquinoline (N21-$O26^{i7}$ = 2.859(3)Å; N46-$O53^{i5}$ = 2.908(3)Å; symmetry operation i7 = x-1/2,-y+3/2,-z+2, i5 = -x,y+1/2,-z+3/2) (Figure 5B).

Pharmacology

Interaction of LSM-775 with 5-HT_{1A} and 5-HT_2 receptors

In vitro pharmacological studies were conducted in order to characterize the interaction of LSM-775 with 5-HT_{1A} and 5-HT_2 receptors. The affinity of LSM-775 for recombinant human 5-HT_{1A} and 5-HT_{2A} receptors labeled with [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively, was assessed in competitive binding assays. The K_i values are reported in Table 2. LSM-775 has equivalent affinity for 5-HT_{1A} (K_i = 31.0 ± 7.4 nM) and 5-HT_{2A} (K_i = 29.5 ± 4.1 nM) receptors.

The data from functional studies in cloned receptors are summarized in Table 3. LSM-775 inhibited cAMP accumulation in HEK293 cells expressing the human 5-HT_{1A} receptor and acted as a full agonist, with a 1 nM EC_{50}. The effect of LSM-775 on 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors was assessed using G_q-mediated Ca^{2+} mobilization in HEK293 cells. LSM-775 was a partial agonist at all three 5-HT_2 receptor subtypes. The potency of LSM-775 varied across the three subtypes, with LSM-775 having higher potency and efficacy at human 5-HT_{2A} receptors (EC_{50} = 4.9 nM; E_{max} = 89%) compared to 5-HT_{2B} (EC_{50} = 26 nM; E_{max} = 77%) and 5-HT_{2C} (EC_{50} = 230 nM; E_{max} = 77%) receptors. The effect of LSM-775 on the mouse 5-HT_{2A} receptor was also assessed; LSM-775 had similar efficacies at the human and mouse receptors but activated the human 5-HT_{2A} receptor with almost 4-fold higher potency compared to the mouse receptor.

Head-twitch response

Although there was a main effect of drug treatment ($F(4,25)=5.45$, $p=0.0027$), LSM-775 alone did not significantly increase the HTR rate over baseline levels (Figure 6A). By contrast, as shown in Figure 6B, LSM-775 produced a robust increase in HTR frequency in animals pretreated with 1 mg/kg of the 5-HT_{1A} antagonist WAY-100,635 ($F(4,25)=19.00$, $p<0.0001$). In the presence of WAY-100,635, LSM-775 induced the HTR with an ED_{50} = 0.34 mg/kg (95% CI: 0.24-0.50 mg/kg). Similar to other lysergamides,[16] the response to LSM-775 followed an inverted-U-shaped dose-response function, with the maximal effect occurring at 1 mg/kg.

To confirm that the HTR induced by LSM-775 in the presence of WAY-100,635 is not due to additive effects between the two drugs, the interaction between 1 mg/kg WAY-100,635 and 1 mg/kg LSM-775 was examined in a single experiment. There was a significant interaction between WAY-100,635 and LSM-775 ($F(1,19)=64.22$, $p<0.0001$). Although LSM-775 alone did not significantly increase HTR counts above baseline levels, LSM-775 induced the HTR in mice pretreated with WAY-100,635 ($p<0.0001$, Tukey's test; see Figure 7). There was a main effect of pretreatment with WAY-100,635 ($F(1,19)=79.63$, $p<0.0001$), but WAY-100,635 failed to significantly increase HTR counts (6.3 ± 2.1 (mean ± SEM)) above the level observed in vehicle control mice (4.5 ± 0.8). There was also a main effect of LSM-775 ($F(1,19)=78.45$, $p<0.0001$).

According to several reports, blockade of 5-HT_{1A} receptors with WAY-100,635 or (*S*)-(–)-UH-301 induces the HTR in mice.[41-43] Importantly, however, 5-HT_{1A} antagonists only induce the HTR during the light-phase of the light-dark cycle.[41] The present experiments confirmed that WAY-100,635 does not increase HTR counts when tested in mice during the dark-phase (Figure 7), which is an important finding because any effects of WAY-100,635 on baseline HTR expression would potentially confound interpretation of the interaction with LSM-775.

In summary, LSM-775 does not induce the HTR in mice despite having moderately high affinity for 5-HT_{2A} receptors and acting as an agonist. For comparative purposes LSD binds to cloned human 5-HT_{2A} receptors labeled with [^{3}H]ketanserin with a K_i of 13 nM.[44] Nevertheless, LSM-775 does produce a robust behavioral response in animals pretreated with a 5-HT_{1A} antagonist. In addition to activating 5-HT_{2A} receptors, LSM-775 is also a potent and highly efficacious 5-HT_{1A} receptor agonist. Based on these findings, it appears that 5-HT_{1A} activation by LSM-775 masks its ability to induce the HTR.

There is substantial evidence that 5-HT_{1A} receptor activation can block the HTR and other 5-HT_{2A}-mediated behavioral effects. For example, 5-HT_{1A} receptor agonists such as 8-OH-DPAT, ipsapirone, and buspirone suppress the HTR induced by hallucinogens in rats and mice.[45-47] In monkeys, 8-OH-DPAT and other 5-HT_{1A} agonists have been shown to attenuate the discriminative stimulus effects of the 5-HT_{2A} agonist DOM.[48] Additionally, based on an isobolographic analysis, 5-HT_{1A} and 5-HT_{2A} agonists are believed to produce infra-additive effects on locomotor activity in rats.[49] 5-HT_{1A} and 5-HT_{2A} receptors are also known to produce opposing effects on neuronal excitability[50,51] and body temperature.[52,53] Given the antagonistic

relationship between these two receptors, it is not surprising that 5-HT_{1A} activation by indoleamine hallucinogens would modulate or attenuate their ability to induce the HTR. However, as far as the authors are aware, LSM-775 is the only indoleamine identified to date that fails to elicit the HTR, presumably due to 5-HT_{1A} agonist effects. Indeed, indoleamine hallucinogens such as 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) and LSD induce a robust HTR despite acting as potent 5-HT_{1A} receptor agonists.[30,54]

Although this is one of the first studies to find that a mixed 5-$HT_{1A/2A}$ agonist fails to induce the HTR, similar findings have been reported for certain mixed 5-$HT_{2A/2C}$ agonists. According to a report by Vickers *et al.*,[55] the 5-HT_2 agonist Ro 60-0175 does not produce the HTR in rats unless administered in combination with the selective 5-HT_{2C} antagonist SB-242,084. Similarly, lorcaserin, a 5-HT_2 agonist with moderate selectivity for 5-HT_{2C} versus 5-HT_{2A} receptors, only induces the HTR in the presence of SB-242,084.[56] These findings indicate that 5-HT_{2C} activation can block HTR expression. The present results demonstrate that 5-HT_{1A} activation can produce similar effects on the HTR.

LSM-775 has been distributed by online vendors as an NPS. According to reports on Internet discussion forums and websites, 1 mg LSM-775 mimics the effects produced by low doses of LSD, but also induces considerable nausea and feelings of lethargy and sedation (e.g.[57]). Similarly, when tested in preliminary clinical trials, LSM-775 was only capable of producing a weak psychedelic response, similar to low or threshold doses of LSD. Based on the present results, it is tempting to speculate that the ability of LSM-775 to induce hallucinogenic effects via 5-HT_{2A} activation is limited or counteracted by its effects on 5-HT_{1A} receptors. There is some precedent in the literature for this type of modulatory interaction: pretreatment with the mixed 5-HT_{1A}/β-adrenergic antagonist pindolol reportedly produces a significant enhancement of the subjective response to *N,N*-dimethyltryptamine (DMT) in human volunteers, indicating the psychedelic effects of DMT are blunted by 5-HT_{1A} activation.[58] Additionally, pretreatment with the 5-HT_{1A} agonist buspirone (20 mg p.o.) markedly attenuates the visual effects of psilocybin in human volunteers.[59] Although buspirone failed to completely block the hallucinogenic effects of psilocybin, the limited inhibition is not necessarily surprising because buspirone is a low efficacy 5-HT_{1A} partial agonist.[60] The level of 5-HT_{1A} activation produced by buspirone may not be sufficient to completely counteract the stimulation of 5-HT_{2A} receptors by psilocin (the active metabolite of psilocybin). Another consideration is that psilocin acts as a 5-HT_{1A} agonist.[61] If 5-HT_{1A} activation by psilocin buffers its hallucinogenic effects similar to DMT[58] then competition between psilocin and a weaker partial agonist such as buspirone would limit attenuation of the hallucinogenic response.

One caveat is that WAY-100,635 may only have limited selectivity for 5-HT_{1A} receptors. For example, WAY-100,635 acts as an agonist at dopamine D_4 receptors.[62,63] Although several studies have shown that WAY-100,635 has at least 100-fold selectivity for 5-HT_{1A} vs. D_4 receptors,[62,64,65] another report indicates that the difference in affinities may be closer to 10-fold.[63] WAY-100,635 is capable of producing D_4 receptor-mediated behavioral effects in rats when tested at relatively high doses.[66] Nevertheless, it is difficult to conceptualize how D_4 activation by WAY-

100,635 could be responsible for the behavioral interaction with LSM-775. D_4 receptor agonists such as apomorphine do not induce the HTR;[46,67] in fact, apomorphine actually attenuates the HTR induced by 5-HT_{2A} agonists.[68,69]

WAY-100,635 also has moderately high affinity for α_1 adrenergic receptor subtypes (α_{1A} K_i = 19 nM, α_{1B} K_i = 66 nM, α_{1D} K_i = 4.6 nM),[70] but again this interaction is unlikely to play a role in the present results. Pretreatment with the α_1 agonist cirazoline does not alter the HTR induced by the 5-HT_{2A} agonist DOI.[46] By contrast, the α_1 antagonist prazosin produces significant inhibition of the HTR induced by DOI,[46,71] 5-MeO-DMT,[72,73] and quipazine.[74] Likewise, the α_1 antagonist phenoxybenzamine attenuates the HTR induced by LSD and quipazine.[75] It has not been established whether WAY-100,635 acts as an agonist or an antagonist at α_1 subtypes, but based on the aforementioned findings, it is not likely that activation or blockade of α_1 receptors would enhance the ability of LSM-775 to provoke head twitches.

Conclusion

Although the subjective effects of LSM-775 in humans have not been fully characterized, LSM-775 does not appear to be capable of producing more than a threshold psychedelic response. Regardless of the dose tested, the effects of LSM-775 approximated those induced by low doses of LSD. Based on the results of our *in vitro* and *in vivo* studies, it was concluded that 5-HT_{1A} receptor activation by LSM-775 suppresses its 5-HT_{2A}-mediated behavioral responses. Because the psychedelic effects of serotonergic hallucinogens are mediated by 5-HT_{2A} receptors, one potential explanation for the relative inactivity of LSM-775 in humans is that its effects on the 5-HT_{1A} receptor may also mask its ability to produce hallucinogenic effects. Although there is some precedent in the literature for this type of modulatory interaction, clinical trials are required to determine whether 5-HT_{1A} agonism is responsible for the weak psychedelic response to LSM-775.

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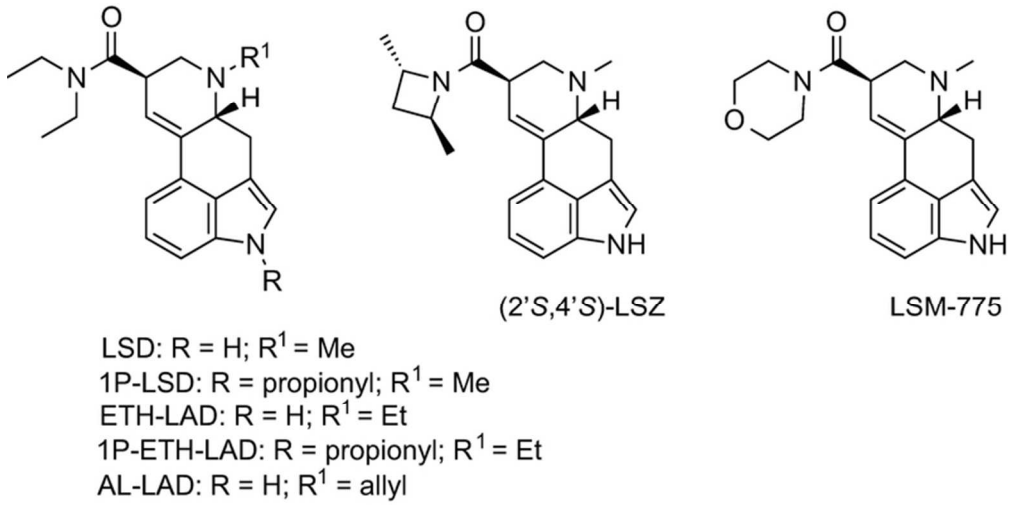


Figure 1. Derivatives of lysergide (LSD) that appeared on the new psychoactive substances market.

67x33mm (300 x 300 DPI)

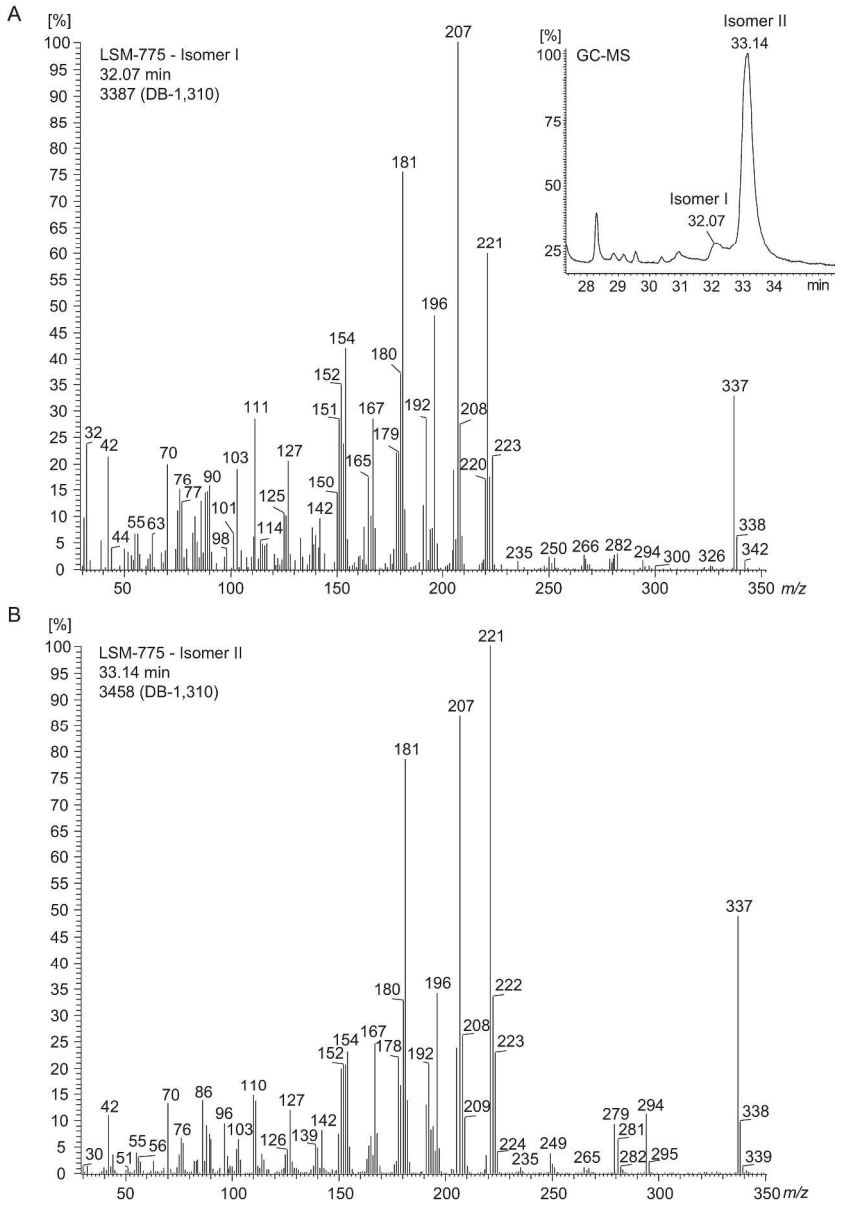
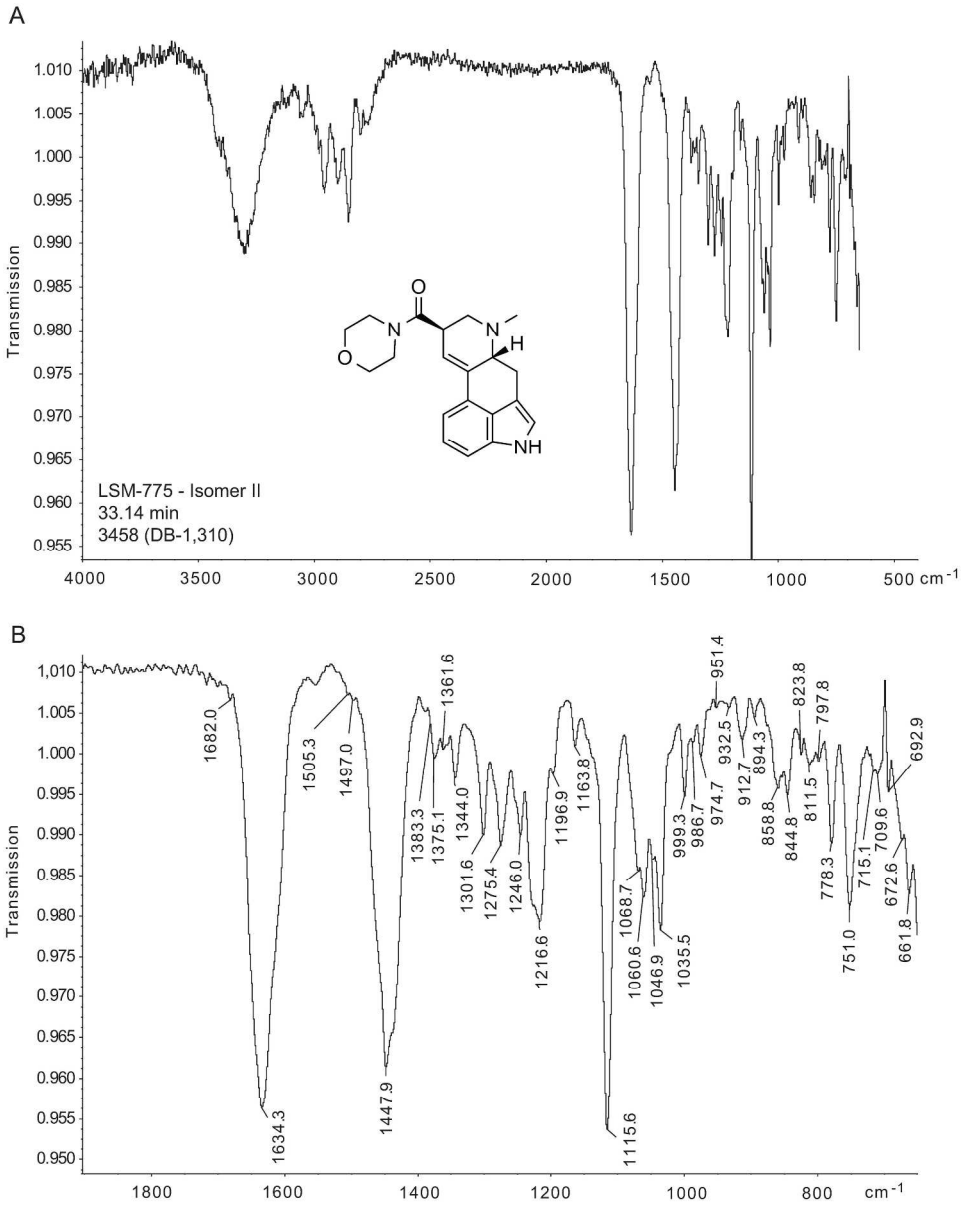


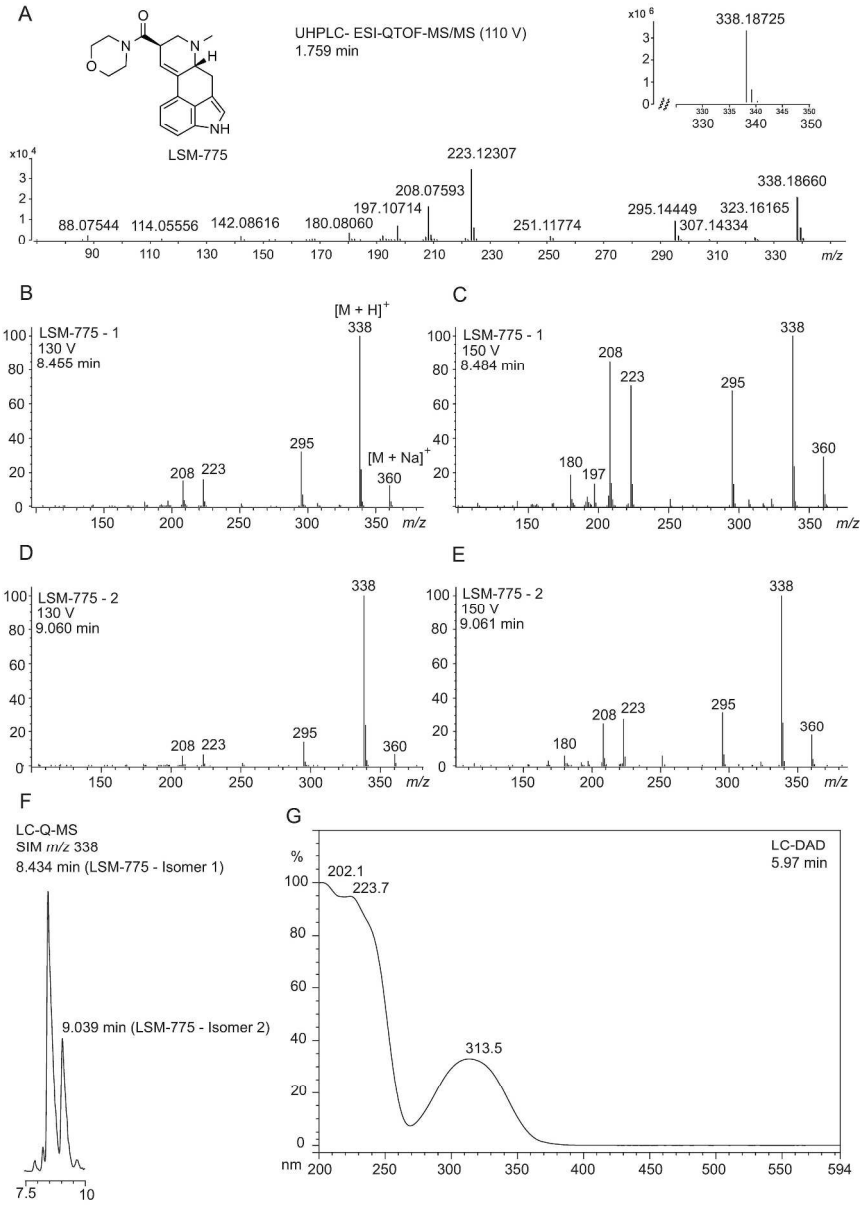
Figure 2. Electron ionization mass spectrum, gas chromatography retention time and Kovats retention index recorded for LSM-775 and its minor isomer.

288x418mm (300 x 300 DPI)



GC-solid state-IR spectrum of LSM-775. Top: entire scan range. Bottom: partial scan range.

253x317mm (300 x 300 DPI)



Quadrupole time of flight tandem mass spectrum (ESI-QTOF-MS/MS) recorded for LSM-775. B–E: in-source CID spectra under single quadrupole mass spectrometry conditions. F: HPLC-Q-MS trace using the m/z value of the protonated molecule for selected ion monitoring (SIM). G: HPLC diode array analysis.

289x405mm (300 x 300 DPI)

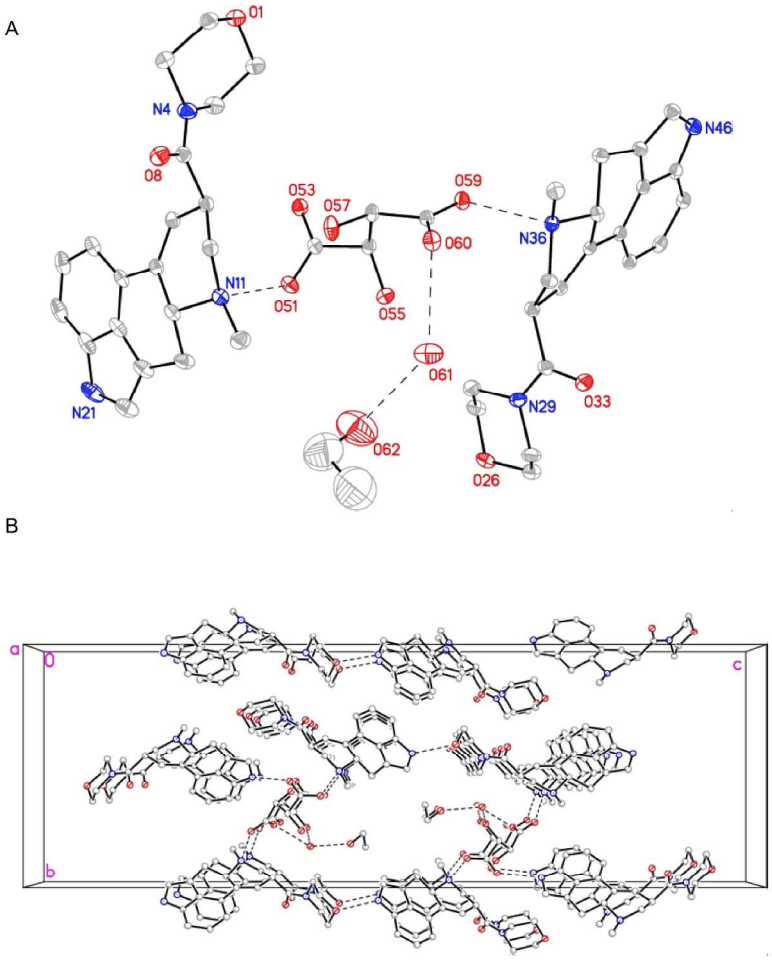


Figure 5. A: Crystal structure data obtained for LSM-775 hemitartrate following recrystallization with ethanol and water. B: Solvent/anion unit formed a central scaffold around which three molecules of LSM-775 were arranged.

290x414mm (300 x 300 DPI)

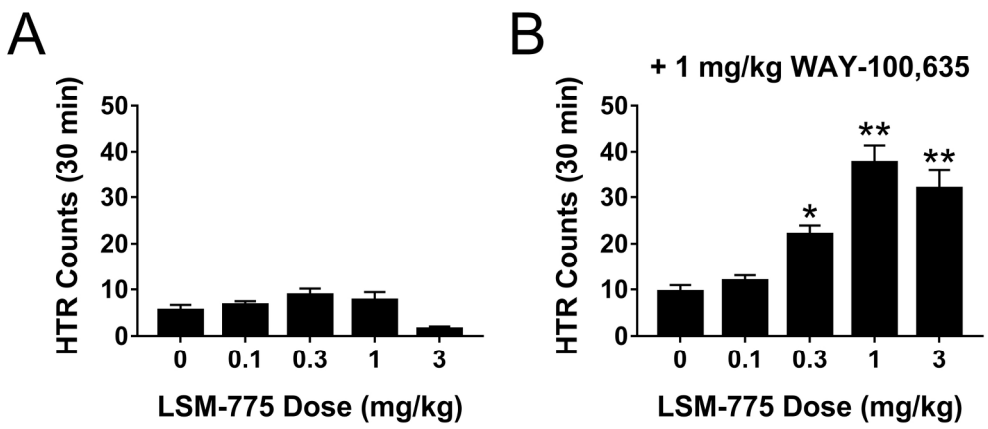


Figure 6. A: LSM-775 did not significantly increase head twitch response (HTR) counts over baseline levels. B: LSM-775 produced a robust increase in HTR counts in mice pretreated with 1 mg/kg of the 5-HT_{1A} antagonist WAY-100,635. Data are presented as group means ± SEM for the entire 30-min test session. *p<0.05, **p<0.01, significant differences from the vehicle control group (Tukey's test).

97x43mm (600 x 600 DPI)

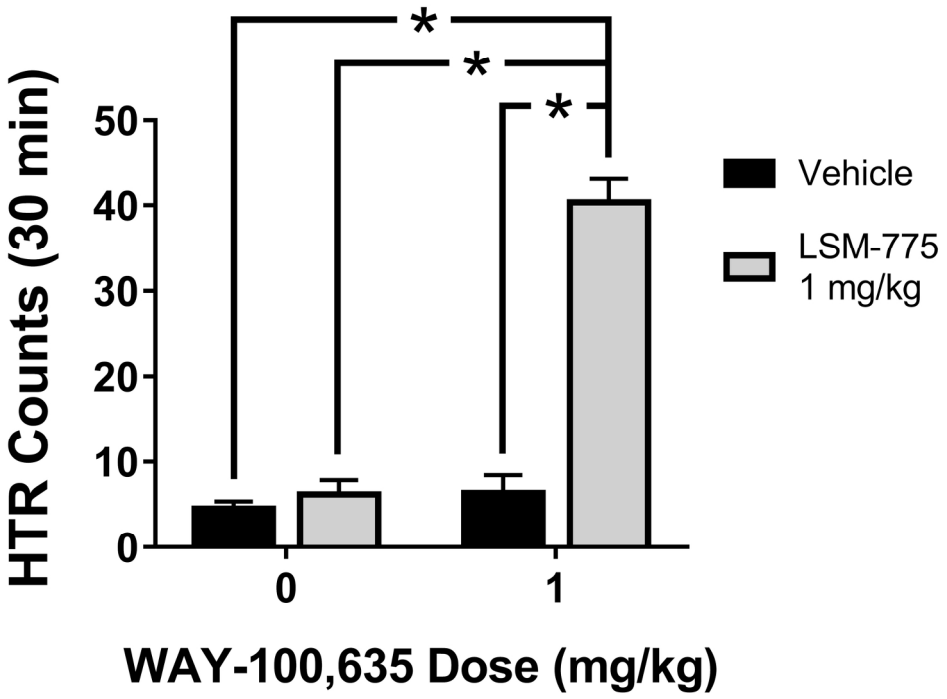
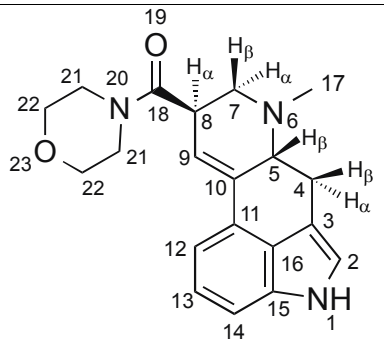


Figure 7. LSM-775 induced the head twitch response (HTR) in mice pretreated with the 5-HT_{1A} antagonist WAY-100,635 but not in mice pretreated with vehicle. Data are presented as group means ± SEM for the entire 30-min test session. *p<0.001, significant differences between groups (Tukey's test).

90x68mm (600 x 600 DPI)

Table 1. ^{1}H and ^{13}C NMR data for LSM-775 hemitartrate in d_6-DMSO at 600 / 150 MHz



No.	^{13}C [δ / ppm]	^{1}H [δ / ppm]
1	–	10.74 (s, 1H)
2	119.39	7.04 (t, *J* = 2.0 Hz,1H) [a]
3	108.53	–
4	26.42	3.51 (dd, *J* = 14.4, 5.4 Hz, 4β-H, 1H) [b] 2.57–2.52 (m, 4α-H, 1H) [c]
5	62.66	3.19–3.12 (m, 5β-H, 1H)
6	–	–
7	54.98	3.05 (dd, *J* = 11.3, 4.8 Hz, 7α-H, 1H) 2.71–2.67 (m, 7β-H, 1H)
8	38.30	3.98–3.94 (m, 8α-H, 1H)
9	119.19	6.30 (s, 1H)
10	134.82	–
11	126.95	–
12	111.19	7.10–7.07 (m, 1H) [d]
13	122.30	7.10–7.07 (t, *J* = 7.2 Hz, 1H) [e]
14	109.94	7.20 (dd, *J* = 7.4, 0.9 Hz, 1H)
15	133.83	–
16	125.77	–
17	43.06	2.54 (s, 3H) [f]
18	169.94	–
19	–	–
20	–	–
21	45.74	3.67–3.57 (m, 2H) [g]
21	41.71	3.53–3.49 (m, 2H) [h]
22	66.30	3.68–3.57 (m, 2H) [i]
22	66.11	3.68–3.57 (m, 2H) [i]
TA [j]	71.95	4.21 (s, 1H)
TA [j]	173.44	–

[a] Part of multiplet between 7.10–7.05 ppm

[b] Overlapping with 1 x 21-CH_2 (2H)

[c] Overlapping with 17-CH_3 (3H)

[d] Overlapping with H-2 and H-13 (2H)

[e] Overlapping with H-2 and H-12 (2H)

[f] Overlapping with 4α-H (1H)

[g] Overlapping with 2 x 22-CH_2 (4H)

[h] Overlapping with 4β-H (1H)

[i] Overlapping with 1 x 21-CH_2 (2H)

[j] TA: tartaric acid

Table 2. Binding affinities of LSM-775 and reference compounds for cloned 5-HT_{1A} and 5-HT_{2A} receptors.

	K_i (nM) [a,b]	
	Human 5-HT_{1A}	Human 5-HT_{2A}
Compound	[^{3}H]8-OH-DPAT	[^{3}H]ketanserin
LSM-775	31.0 ± 7.4 (3)	29.5 ± 4.1 (4)
8-OH-DPAT	0.72 ± 0.10 (3)	ND [c]
Clozapine	ND	11.5 ± 3.3 (4)

[a] Mean ± SEM.
[b] The number of independent determinations are listed in parentheses.
[c] ND: not determined.

Table 3. Functional activity of LSM-775 and 5-HT at selected serotonin receptors.

Receptor	LSM-775		5-HT	
	EC_{50}, nM (pEC_{50} ± SEM)	E_{max} % 5-HT	EC_{50}, nM (pEC_{50} ± SEM)	E_{max} % 5-HT
h5-HT_{1A}	1.03 (8.99 ± 0.03)	98 ± 2	0.26 (9.58 ± 0.03)	100
h5-HT_{2A}	4.9 (8.31 ± 0.07)	89 ± 2	0.35 (9.50 ± 0.04)	100
m5-HT_{2A}	19 (7.72 ± 0.04)	88 ± 1	0.57 (9.24 ± 0.02)	100
h5-HT_{2B}	26 (7.58 ± 0.02)	77 ± 2	0.85 (9.07 ± 0.02)	100
h5-HT_{2C}	230 (6.64 ± 0.03)	77 ± 3	0.20 (9.58 ± 0.03)	100

Data were acquired with HEK293 cells expressing either human 5-HT_{1A} (h5-HT_{1A}), human 5-HT_{2A} (h5-HT_{2A}), mouse 5-HT_{2A} (m5-HT_{2A}), human 5-HT_{2B} (h5-HT_{2B}), or human 5-HT_{2C} INI (h5-HT_{2C}) receptors. Data represent EC_{50} and E_{max} means and standard error of the mean (SEM) from three independent experiments performed in triplicate. E_{max} is defined as percent 5-HT maximum response.