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Brain-Penetrant LSD1 Inhibitors Can Block Memory Consolidation

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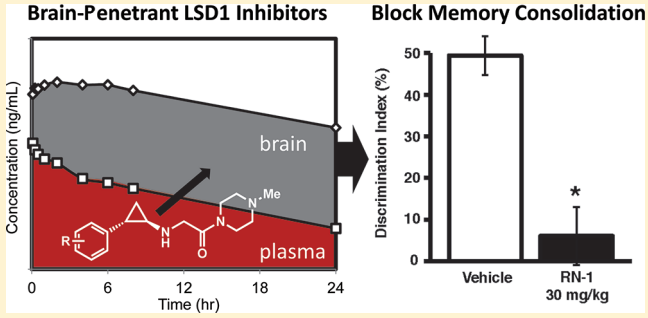
Supporting Information

ABSTRACT: Modulation of histone modifications in the brain may represent a new mechanism for brain disorder therapy. Post-translational modifications of histones regulate gene expression, affecting major cellular processes such as proliferation, differentiation, and function. An important enzyme involved in one of these histone modifications is lysine specific demethylase 1 (LSD1). This enzyme is flavin-dependent and exhibits homology to amine oxidases. Parnate (2-phenylcyclopropylamine (2-PCPA); tranylcypromine) is a potent inhibitor of monoamine oxidases, and derivatives of 2-PCPA have been used for development of selective LSD1 inhibitors based on the ability to form covalent adducts with flavin adenine dinucleotide (FAD). Here we report the synthesis and in vitro characterization of LSD1 inhibitors that bond covalently to FAD. The two most potent and selective inhibitors were used to demonstrate brain penetration when administered systemically to rodents. First, radiosynthesis of a positron-emitting analogue was used to obtain preliminary biodistribution data and whole brain time−activity curves. Second, we demonstrate that this series of LSD1 inhibitors is capable of producing a cognitive effect in a mouse model. By using a memory formation paradigm, novel object recognition, we show that LSD1 inhibition can abolish long-term memory formation without affecting short-term memory, providing further evidence for the importance of reversible histone methylation in the function of the nervous system.



KEYWORDS: *LSD1, mechanism-based inhibitors, histone demethylase, epigenetics, brain*

Chromatin modification is not only crucial to cell differentiation and function, but also mammalian development and behavior, including learning and memory.[1−3] Post-translational modifications of histones such as phosphorylation, acetylation, and methylation are proposed elements of a "histone code" transmitted to the cellular machinery to produce a specific gene regulatory outcome.[4,5] Epigenetic dysfunction is a common factor in disorders of synaptic plasticity and cognition including neurodegenerative disorders, depression, and anxiety. Among these modifications, lysine methylation at various sites of histone leads to transcriptional activation or silencing.[6] Within the past few years, a number of histone demethylases have been discovered including the flavin adenine dinucleotide (FAD)-dependent lysine-specific demethylase 1 (LSD1)[7−9] and LSD2.[10] LSD1 removes methyl groups from mono- and dimethylated lysine 4 or 9 of H3 histone tails. This occurs via an imine intermediate that undergoes hydrolysis.[7,11] The action of LSD1 can serve as either a repressor or activator.[12,13] Jumonji C domain proteins are required for removal of trimethylated H3K4 or H3K9, and LSD1 does not demethylate trimethylated lysine consistent with the oxidation mechanism.[14,15] LSD1 does not act as a free-functioning enzyme in vivo but rather as part of a complex with histone deacetylase (HDAC)1/2, CtBP, CoREST, BHC80, SANT, and

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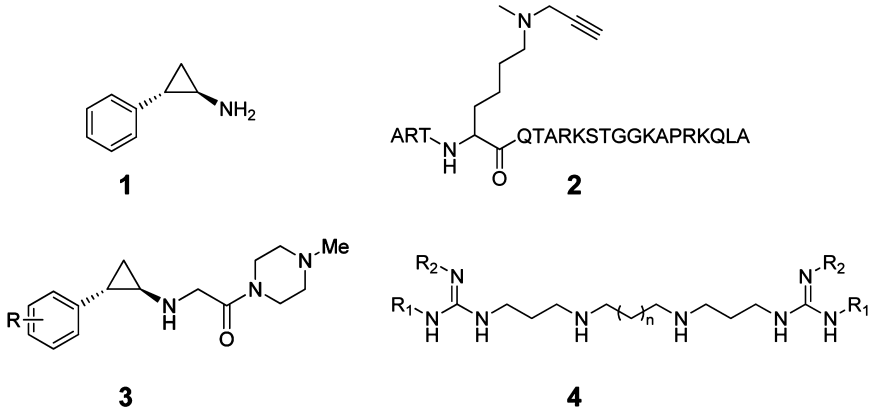

Figure 1. Known classes of LSD1 inhibitors: Parnate (**1**), peptide with a propargyl lysine structure (**2**), alkylated Parnate derivatives (**3**), and polyamine derivatives (**4**).

PHD domains.[16−19] In fact, LSD1 has been found to be a core component of a number of transcriptional repressor complexes that participate in a stepwise process involving HDAC1/2-mediated deacetylation of H3K9Ac where deacetylation is proposed to precede the binding of CoREST, which is followed by LSD1-mediated H3K4Me$_{1/2}$ demethylation and binding of BHC80 subunits to H3K4.[9] It is because of this relationship to HDACs that chemical inhibitors of LSD1 have been postulated to synergize with the antitumor properties of HDAC inhibitors.[20]

LSD1 is up-regulated in various cancers,[21,22] including glioblastoma,[23] neuroblastoma,[24] and retinoblastoma.[25] Interest in LSD1 in a neurological context stems from the observation that monoamine oxidase (MAO) inhibitors such as Parnate, chlorgyline, and pargyline inhibit LSD1, albeit with low potency and selectivity (Figure 1). Parnate has been used in the treatment of depression, Parkinson's disease, as well as neurodegenerative conditions, which may highlight additional clinical applications of selective LSD1 inhibitors.

LSD1 Inhibitor Development. Although there are only a few reports of selective LSD1 inhbitors, three general classes have already been described:[26] (1) Propargyl lysine-4 H3 tail peptide analogues were reported based on the *N*-methylpropargyl functionality and Parnate.[11,27,28] (2) Bis-guanidine (polyamine) compounds have been shown to inhibit LSD1 noncompetitively between 1.0 and 2.5 μM.[29,30] (3) Parnate (PCPA)-derivatives have been described that interact with FAD to form stable covalent FAD-adducts.[31] Binda et al. demonstrated that 2-PCPAs can show modest selectivity between LSD1 and LSD2.[32] Gooden et al. reported a facile synthetic route to substituted 2-PCPAs and studied inhibitory activity toward LSD1 and MAO A/B. They show that these inhibitors are more potent and selective than Parnate.[33] An enantioselective synthesis of PCPA analogues and 4-bromo-PCPA (K_i = 3.7 μM) was accomplished. These derivatives were more potent than Parnate in both enzymatic assays and a human LNCaP prostate cancer cell line.[34] Guibourt et al.[35] reported the synthesis of *N*-alkylated Parnate derivatives and biological studies of their inhibitory activities with LSD1, MAO-A, and MAO-B. This approach employs Parnate as a chemical scaffold for the design of novel LSD1 inhibitors and biological studies of their inhibitory activities with LSD1, MAO-A, and MAO-B. Our studies build on this previous work. Herein we synthesize inhibitors of LSD1, evaluate their potency, selectivity, and ability to penetrate the central nervous system (CNS), and provide an initial characterization of the consequence of LSD1 inhibition in a behavioral model assessing memory formation in mice. Our study is first to report dynamic positron emission tomography (PET) analysis of LSD1 inhibitors in the rodent brain. This will eventually facilitate a more detailed understanding of physiological and biological aspects following drug treatment. These studies represent the first steps toward our long-term goal of developing brain-penetrant LSD1 inhibitors for investigating the role of LSD1-mediated demethylation in the nervous system and the first step toward tools for epigenetic imaging of the density of LSD1 in the rodent and human brain.

RESULTS AND DISCUSSION

Mechanism-based irreversible enzyme inhibitors often exhibit high target selectivity and can, in certain cases, provide some clinical advantages over reversible inhibitors.[36] The most potent LSD1 inhibitors described to date are structural analogues of the irreversible inhibitor Parnate; we thus surmised that these compounds would most likely generate robust histone methylation changes in vivo. In turn, these histone methylation changes could lead to alterations of cell function, brain function, and perhaps behavior. Thus, we began our synthesis efforts by preparing derivatives of Parnate like those found in the recent patent literature.[35] Selectivity of these compounds for LSD1 over MAO was paramount given that MAO is highly expressed in the brain (our target tissue) and MAO and LSD1 are homologous in the amine oxidase domain (17.6% identity).[37] Parnate itself actually exhibits limited selectivity for human MAOs versus LSD1, with selectivity of 2.4- and 16-fold higher for MAO A and MAO B, respectively.[37] In situ hybridization assays detecting *Lsd1* mRNA expression in adult mouse brain indicate that LSD1 is likely enriched in the hippocampus and cerebellum with perhaps lower expression in the cortex.[38,39] Thus, we set out to produce brain-penetrant LSD1 inhibitors with selectivity over MAO greater than 100-fold. In doing this, we maintained a position that would allow us to radiolabel each inhibitor with carbon-11 or fluorine-18 so that we could eventually study pharmacokinetics and binding in vivo.

Synthesis of LSD1 Inhibitors. Many methods are available for the synthesis of Parnate derivatives (discussed above). Our synthesis efforts relied on two of these methods.[35,40] First, the chemical synthesis of RN-1 was accomplished by the published procedure.[35] Our general

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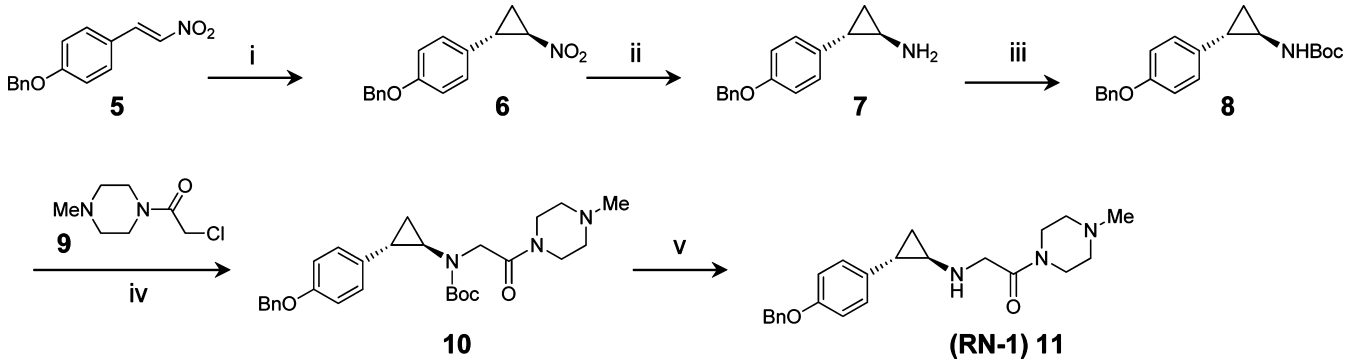
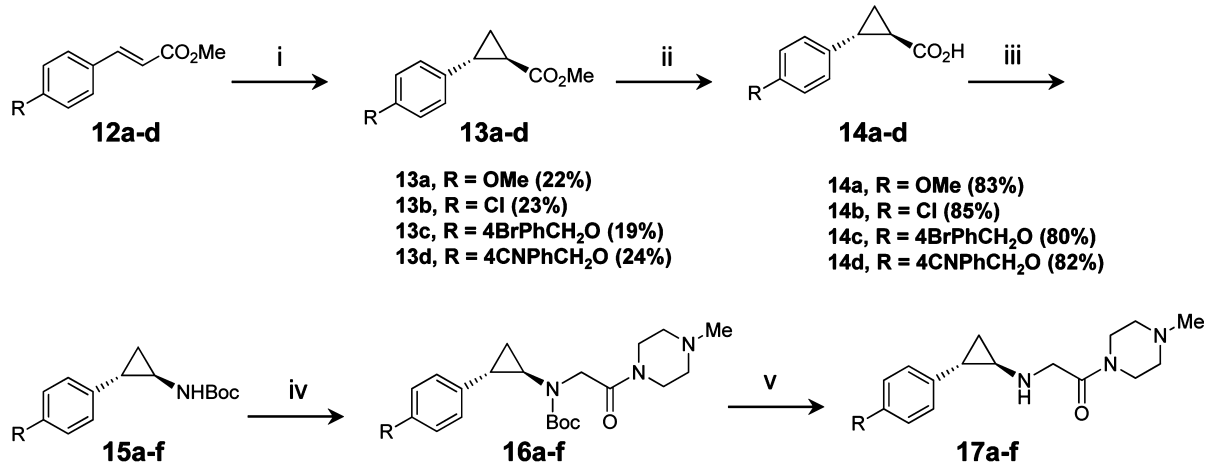
Scheme 1. [a]



[a]Reagents and conditions: (i) $Me_3S(O)I$, *t*-BuOK, DMSO, rt, 2 h, 26%; (ii) Zn/HCl, *i*-PrOH, rt, 17 h, 27%; (iii) Boc_2O, Et_3N, THF, rt, 3 h, 72%; (iv) **9**, NaH/DMF, 0 °C-rt, 1 h, 45%; (v) Et_2O-HCl/Et_2O, rt, 5 h, 70%.

Scheme 2. [a]



[a]Reagents and conditions: (i) $Me_3S(O)I$, *t*-BuOK, DMSO, rt, 4 h; (ii) aq. K_2CO_3, MeOH, reflux, 3 h; (iii) DPPA, Et_3N, *t*-BuOH/toluene, reflux, overnight; (iv) **9**, NaH/DMF, 0 °C to rt, 1 h, 45%; (v) Et_2O-HCl/Et_2O, rt, 5 h, 70%. *__15e__ and __15f__ were prepared from 4-bromo Parnate and Parnate respectively.

strategy was to produce a panel of *trans*-2-arylcyclopropylamines from commercially available 4-substituted nitrostyrenes (Scheme 1). For example, reaction of nitrostyrene **5** with the Corey–Chaykovsky reagent ($Me_3S(O)I$) in DMSO gave the cyclopropyl nitro compound **6**, which was reduced into an amine using zinc and HCl. The amine **7** was then Boc-protected, affording intermediate **8**. Alkylation of the carbamate derivative **8** in the presence of NaH and DMF gave **10**. Deprotection of the Boc-group by ethereal HCl solution at room temperature provided the amine (**11**, **RN-1**) as a water-soluble HCl salt.

The syntheses of other derivatives by this method were plagued by difficulties during reduction of the nitro group using many reaction conditions. Thus, to avoid this reduction step we used the method of Ueda et al. [40] for the synthesis of the rest of the RN-series (Scheme 2). In this method, commercially available cinnamic acid or cinnamate ester derivatives were used as starting materials and the general route followed these steps: cyclopropanation, hydrolysis and Curtius rearrangement. The overall yields of this sequence were moderate (20–30%) but far better than the previous method that required the nitro reduction.

To increase the divergency of our synthesis scheme, we prepared the bromo-derivative **15e** for use in palladium-catalyzed cross-coupling reactions. For example, we used Suzuki coupling of **15e** with commercially available boronic acids to give pyridyl analogues **18a,b** (Scheme 3). Using these methods, we have prepared 10 final compounds reported herein; however, additional efforts are ongoing to increase the diversity of this inhibitor panel.

LSD1 Inhibition Assays. With potential inhibitors in hand, we turned our efforts to assessing their potency and selectivity for LSD1. Various biochemical assays to determine LSD1 inhibition in vitro with recombinant enyzmes have been

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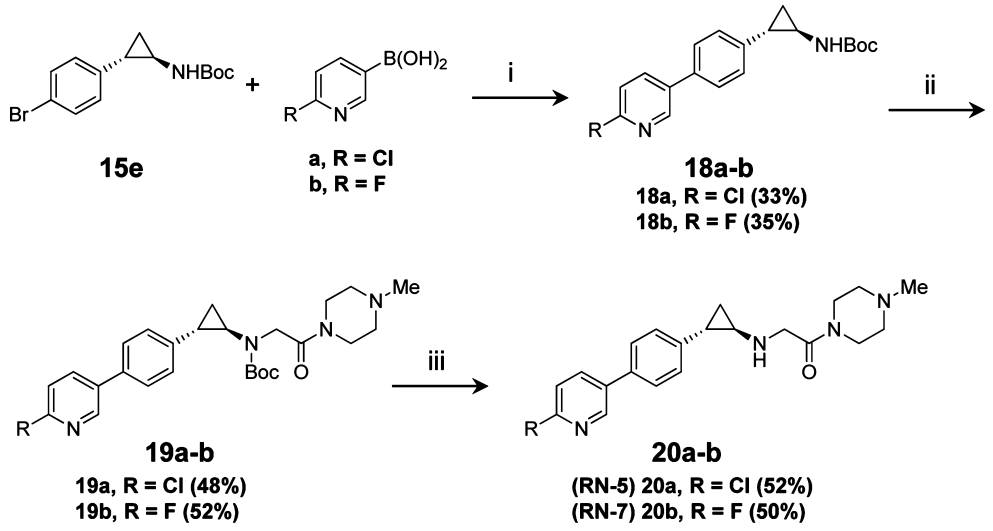
Scheme 3. [a]



[a]Reagents and conditions: (i) **a**/**b**, $Pd(PPh_3)_4$, K_2CO_3, CH_3CN:H_2O (4:1), N_2 atm, reflux, 4 h; (ii) **9**, NaH/DMF, 0 °C to rt, 4 h; (iii) Et_2O-HCl/Et_2O, rt, 5 h.

reported;[41−43] however, no single method has become the standard assay for comparing inhibitors between research groups. Because of this, we chose to assess LSD1 inhibition using three biochemical assay formats to provide orthogonal validation of assay results and to help inform future studies aiming to optimize LSD1 inhibitors in a streamlined fashion: a horseradish peroxidase (HRP)-coupled assay, a time-resolved fluorescence energy transfer (TR-FRET) assay, and a label free, direct mass spectrometry (MS) assay (see the Supporting Information for assay validation).[44]

For LSD1, the HRP-coupled assay has been the method of choice due to its low cost, convenience, and robustness to support SAR efforts. In the HRP-coupled assay, a dimethylated H3K4 substrate is demethylated by recombinant human LSD1 producing H_2O_2. The peroxide that is formed can be detected and quantified through a reaction with ADHP (10-acetyl-3,7-dihydroxyphenoxazine). The reaction occurs in the presence of HRP and produces the fluorescent oxidation product. The intensity of fluorescence from the oxidation product is directly proportional to the LSD1 enzyme activity. Therefore, when LSD1 activity decreases by inhibition, the fluorescent signal will also decrease. Only a minimal change in background fluorescence was observed upon leaving out of the $H3K4Me_2$ peptide. As a source of LSD1, we used a truncated form of recombinant human LSD1 (Δ1−157) purified from *E. coli*, based upon the published studies of Forneris et al.[44]

In our hands, this HRP-coupled assay for measuring LSD1 was robust, reproducible, and highly suitable for high-throughput screening, but due to the multiple components involved in the detection steps has at times given false positives and false negatives through the interaction of small molecules with peroxide, HRP, or ADPH. For this assay (and the others), we used Parnate as a positive control of inhibition. Parnate exhibited an IC_{50} of >100 μM. This value is within the range of IC_{50}'s previously reported by others using similar assay formats (32−271 μM).[32,43,45] Since inhibitors were added to the aqueous reaction as DMSO solutions, we verified that there was no effect of DMSO in the range of 0−5% v/v. As seen in Table 1, the most potent compounds were **RN-1** and **RN-7** with IC_{50}s between 30 and 70 nM. For each compound, the assay was repeated at least 4 times to determine reproducibility. Individual assays were variable in the 2−3 fold range likely because the inhibitors show time-dependent increases in potency (likely due to the irreversible mechanism). The IC_{50}'s reported in Table 1 were collected during one single parallel experiment (including Parnate) so that direct comparisons can be made. In all the assays, preincubation time (inhibitor + enzyme) was 10 min and substrate reaction time was 20 min. We determined in control experiments that the enzyme was stable for this length of time but we observed degradation of function in experiments where LSD1 was incubated longer than 1 h at room temperature.

Table 1. LSD1, MAO A, and MAO B Inhibition in Vitro

	LSD1 assays IC_{50} (μM)			MAO assays IC_{50} (μM)	
	HRP	TR-FRET	MS	MAO A	MAO B
RN-1	0.07	0.01	0.02	0.51	2.785
RN-5	0.20	0.10		2.00	
RN-7	0.031	0.003	0.007	12.88	10.85
RN-11	0.60	0.20	0.65	0.10	
RN-21	0.034	0.002	0.015	3.69	11.41
RN-22	0.047	0.30	8.37	1.20	23.45
RN-23	2.0	0.72	0.37	0.15	
RN-24	0.047	0.004	0.019	0.51	
RN-27	0.055	0.009	0.033	1.50	1.274
Parnate	>100	>100	>100	0.48	4.881

Results from the HRP-coupled LSD1 assays were directly validated by assessing demethylation of the $H3K4Me_2$ peptide substrate using label-free, high throughput mass spectroscopy (RapidFire MS) detection.[46,47] The LSD1 demethylation reaction was performed under identical assay conditions to those used for the HRP-coupled assay, and reactions were quenched by the addition of formic acid. Detection of the $H3K4Me_1$ and $H3K4Me_0$ products were accomplished on an Agilent RF300 mass spectrometry system with RapidFire chromatography in line with a triple stage quadrapole mass spectrometer. Using our assay conditions, $H3K4Me_1$ was the major product of the demethylation reaction. Substrate

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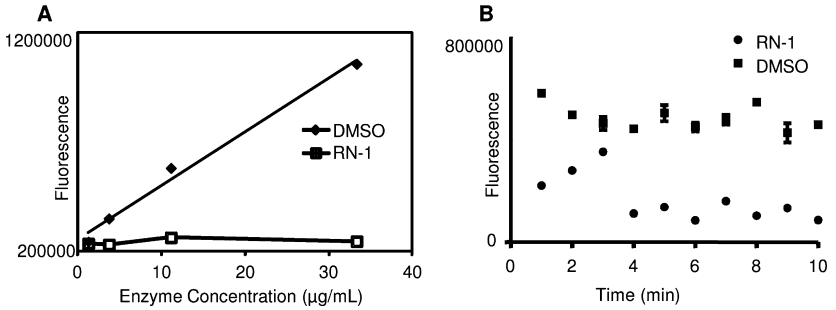
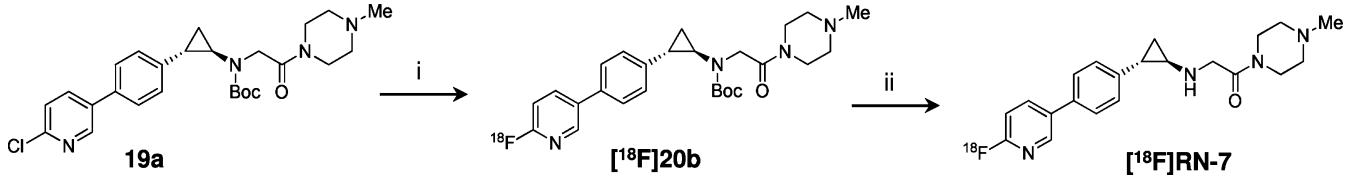


Figure 2. Covalent inhibition assays. (A) LSD1 enzyme was incubated with the inhibitor RN-1 and then diluted to determine if inhibitor dissociation occurs. DMSO was used as the control in this reaction. RN-1 inhibited the enzyme at high dilutions indicating that inhibition was not reversible. (B) Representative progress curves for LSD1 activity in the presence of varying concentrations of RN-1. LSD1 enzyme was incubated with the inhibitor RN-1 and then serially diluted at 1 min time intervals to determine if the inhibitor would dissociate. DMSO was used as the control in this reaction. RN-1 can be diluted and enzyme activity partially restored up to 4 min after initial incubation. After this time, LSD1 remains fully inhibited even after a 1:100 dilution.

Scheme 4. [a]



[a]Reagents and conditions: (i) [^{18}F]fluoride, K_2CO_3/K_{222}, DMSO, 140 °C, 10 min; (ii) TFA, 90 °C, 10 min.

conversion values were used to calculate the IC_{50} values for each test compound.

Since RapidFire MS analysis requires dedicated and specialized instrumentation, we also turned our attention to further validate the compounds using a TR-FRET assay for LSD1, which is designed to detect the H3K4Me$_1$ product. TR-FRET assays are simple to perform, and for us it offered a quick way of in house compound testing using a multilabel plate reader.[43] Recently, Yu et al.[48] developed a similar assay into a high-throughput assay for identifying inhibitors of LSD1 and JMJD2C histone lysine demethylases. Because the TR-FRET assay has a more direct method of assessing the peptide methylation state, it was less variable than the HRP-coupled assay. Overall, the TR-FRET and RapidFire MS LSD1 assays, which both measure conversions to the H3K4Me$_1$ product, were in good agreement and indicated that the LSD1 inhibitors RN-1, -7, -21, -24, and -27 were highly potent, more so than indicated in the HRP-coupled assay.

LSD1 versus MAO Selectivity. To determine the selectivity of the RN-series, we also used a commercially available MAO-Glo assay and determined inhibition for both MAO-A and MAO-B. This was accomplished according to the manufacturer's guidelines with the exception that we miniaturized each assay to 1/4th the volume. The RN-series of LSD1 inhibitors were moderately potent against MAO-A and MAO-B with IC_{50} in the 0.5−13 μM range. With the caveats described above, this placed our selectivity at between 6- and 400-fold for LSD1 over the MAOs (using the more conservative estimates of potencies from the HRP-coupled assay). Using the MAO-Glo assays, the inhibition of MAO-A was greater than MAO-B for our RN-series of compounds. Although we did not counter screen for activity against LSD2, previous reports have demonstrated that 2-PCPA derivatives can inhibit LSD2 as well.[32] The lead candidates from our small panel of compounds, both in terms of potency for LSD1 and selectivity over MAO, were RN-7 and RN-1. To the best of our knowledge, these are two of the most potent and selective LSD1 inhibitors described to date.

LSD1 Inhibition Kinetics. To verify the mode of inhibition and kinetic profile of our novel LSD1 inhibitors, we used two dilution experiments with RN-1. This allowed us to examine the reversibility of binding using our standard kinetic analysis assays. In the first experiment (Figure 2), a solution of the LSD1 enzyme was incubated for 10 min with inhibitor (RN-1) or DMSO (as a control). Serial dilutions were performed to promote dissociation of the inhibitor and then substrate turnover for each solution was determined. RN-1 fully inhibits LSD1 and dilution does not have a significant effect on inhibition activity. This contrasts with experimental results with noncovalent inhibitors, like the bis-guanidine compounds previously reported, where LSD1 inhibition can be diluted out indicating reversibility of binding. In a second experiment, we examined the effect of inhibitor incubation time in parallel with dilution. LSD1 was incubated with two equivalents of RN-1 in reaction buffer. An equal volume of DMSO was added to a separate stock of same amount of LSD1 as a control. At 1 min intervals, each preincubated enzyme solution was diluted by 100-fold using buffer. A significant dissociation is expected if the inhibitor is noncovalent. LSD1 remains fully inhibited even after a 1:100 dilution; this suggests that RN-1 inhibits LSD1 through a nondissociable, likely covalent, mechanism as expected for a Parnate analogue.[37]

Biodistribution and Blood-Brain Barrier Penetration. To evaluate whether the RN-series of compounds, in general, exhibits blood-brain barrier (BBB) penetration, we radiolabeled **19a** with F-18. To prepare a labeling precursor, the 4-bromo substituted Parnate derivative **15e** was used as an intermediate. Coupling of **15e** with 2-chloropyridyl-5-boronic acid in the presence of palladium catalyst afforded the product (**18a**) in good yield. Alkylation of the carbamate derivative **18a** with **9**

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gave **19a**. The Boc-group containing chloropyridyl derivative was used as the precursor for the radiolabeling after we determined that the free amine at this position limited radiochemical yield and promoted degradation under the labeling conditions. The synthesis of [^{18}F]RN-7 was achieved by fluorination of the chloro moiety of the precursor in DMSO at 140 °C for 10 min using standard kryptofix/carbonate conditions (Scheme 4). The radiolabeled product was separated from the reaction mixture by reversed-phase column chromatography and the eluent was treated with TFA for 10 min to give [^{18}F]RN-7, which could be easily formulated in an ethanol/water mixture using solid-phase extraction. The chemical identity of [^{18}F]RN-7 was confirmed by coinjection with a sample of standard RN-7 on an analytical HPLC and by monitoring radioactivity on TLC (see the Supporting Information). The average time required for the [^{18}F]-labeling, purification, deprotection, and reformulation was 70 min from the end-of-bombardment (EOB).

With material in hand, we first examined the brain/blood ratio of radioactivity at early time points. These studies indicate that [^{18}F]RN-7 exhibits good brain penetration and retention (Figure 3), with the blood to plasma ratio > 1 for [^{18}F]RN-7.

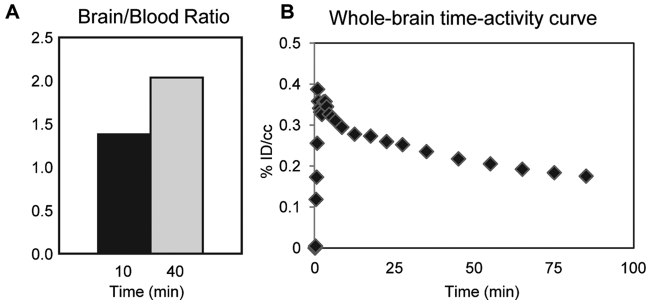

Figure 3. Brain penetration. (A) Brain to blood ratio calculated from biodistribution data. (B) Whole-brain region of interest was used to generate time−activity curve (TAC) for [^{18}F]RN-7.

We are currently exploring whether all members of the RN-series have equal BBB penetration by labeling the piperazine methyl group with carbon-11. Biodistribution experiments with [^{18}F]RN-7 indicated normal excretion with some uptake in the lungs and a minimal amount of defluorination occurring as observed by uptake in bone (see the Supporting Information). Initial studies have indicated that uptake is not saturable, but additional experiments are needed to evaluate the potential of the RN-series as selective PET radiotracers for LSD1 inhibitors.

Pharmacokinetics and Brain Penetration of RN-1. In parallel with PET imaging and biodistribution of [^{18}F]RN-7, we evaluated the brain PK of RN-1 by LC-MS/MS. Brain and plasma concentration−time data (ng/mL) for RN-1 was determined following intraperitoneal administration of RN-1 (10 mg/kg). Plasma and brain concentrations declined exponentially with T_{max} of 0.08 and 2.0 h, respectively. After intraperitoneal administration of RN-1, concentrations were detectable up to 24 h post dose in both plasma and brain tissues. The brain/plasma exposure ratio was found to be 88.9 (Table 2). Plasma and brain concentrations of RN-1 over time are presented in Figure 4. Based on these data, we prioritized RN-1 for evaluation in behaving animals.

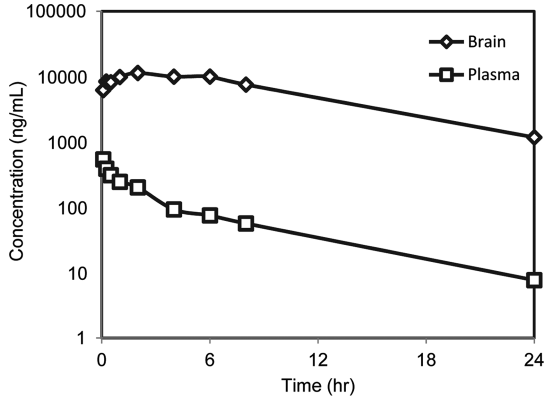

Figure 4. Mean plasma and brain concentration−time profiles of RN-1 following a single intraperitoneal administration to C57BL/6 male mice, 10 mg/kg (n = 3) × (9 time points).

Preliminary Evaluation of RN-1 in Behaving Animals. It has been demonstrated that chromatin modification is a critical mechanism by which chromatin structure is modified to activate or silence transcription required for long-term memory.[49] Although histone methylation has been implicated in the regulation of gene expression underlying memory formation,[50] no study to date has examined the specific histone demethylases involved.

To begin to understand the role of LSD1 in long-term memory formation, we examined the effect of the LSD1 inhibitor RN-1 on novel object recognition (NOR). Given that systemic administration of RN-1 inhibits LSD1 throughout the brain rather than in a local brain region, we utilized the NOR task because multiple cortical brain regions have been shown to be critical for this type of long-term memory.[51,52] During training, mice were placed in an arena with two identical objects for a 10 min session, which we have previously demonstrated will result in long-term memory formation.[53,54] Immediately following training, mice were administered RN-1 or vehicle and then returned to the same arena 24 h later, this time with one familiar object and one novel object (Figure 5B). In contrast to the vehicle treated mice, RN-1 treated mice exhibited no significant long-term memory for the familiar object (vehicle: 49.43 ± 4.64, n = 10; RN-1: 6.07 ± 6.97, n = 10; Student's t test, t_{18} = 5.18, p < 0.0001). To examine whether short-term memory was affected by LSD1 inhibition, we trained a different group of mice with two identical objects and tested 90 min later for object recognition memory (Figure 5C). Mice treated with RN-1 exhibited discrimination for the novel object that was not significantly different from that of vehicle treated mice (vehicle: 30.48 ± 4.32, n = 7; RN-1: 31.21 ± 5.08, n = 7; Student's t test,

Table 2. Pharmacokinetic Parameters of RN-1 Following a Single Intraperitoneal Administration (10 mg/kg) in male C57BL/6 Mice (n = 27, n = 3 × 9 Time Points)

compd	route	matrix	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{INF} (hr*ng/mL)	brain/plasma exposure ratio	brain homogenate binding
RN-1	i.p.	plasma	0.08	541.7	1661.2	1723	88.9	95.5 ± 0.3 bound (n = 3)
		brain	2.00	11 390.5	147 682.4	157 624		

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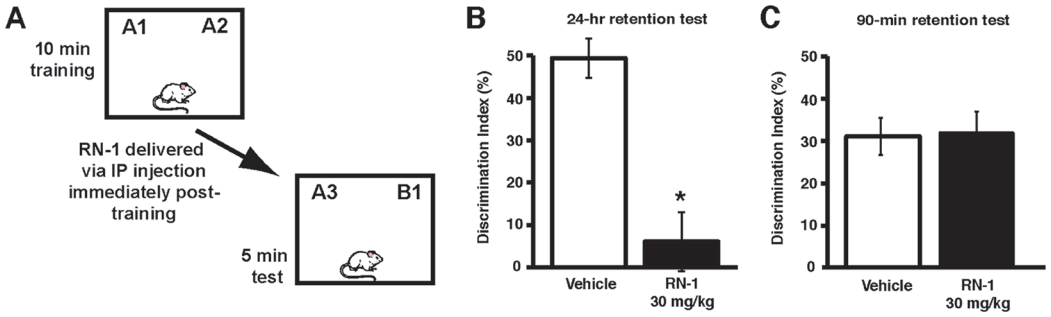

Figure 5. LSD1 inhibition blocks long-term memory formation. (A) Schematic diagram of novel object recognition (NOR) task. (B) Mice treated with the LSD1 inhibitor (n = 10) immediately following NOR training exhibit a significant 24 h long-term memory deficit ($p < 0.05$) compared with vehicle treated mice (n = 10). (C) LSD1 inhibitor treated mice (n = 7) exhibit normal 90 min short-term memory for a familiar object as compared with vehicle treated mice (n = 7).

t_{18} = 0.11, p = 0.91). Together, these results indicate that the LSD1 inhibitor RN-1 significantly impairs long-term memory, but not short-term memory. Administration of RN-1 directly to the brain (i.c.v.) will be needed to demonstrate that long-term memory impairment is due to a brain-specific effect and not changes occurring peripherally. This is important to determine given that monoamine oxidase inhibitors are known to interact with pancreatic islets and perturb insulin production,[55] and glucocorticoid receptors in the hippocampus are involved in memory consolidation.[56] Furthermore, it will be important to determine the histone methylation sites regulated by LSD1 and how methylation of those sites contributes to gene expression in the service of memory formation both in the NOR paradigm as well as other behavioral paradigms. Future experiments are required to determine the role of LSD1 in different forms of long-term memory. The NOR task has the several limitations and clearly at this point we are unable to identify the key brain regions in which LSD1 activity is exerting its effects. However, we are currently working to elucidate the mechanisms (in and out of the brain) that may contribute to RN-1-mediated blockade of memory consolidation.

Detailed procedures for synthesis, enzyme assays, and in vivo assessment of LSD1 inhibitors are located in the Supporting Information.

■ CONCLUSION

Novel LSD1 inhibitors were synthesized and characterized in vitro. We found the use of a panel of three orthogonal LSD1 biochemical assays, which were well correlated overall, helped reach a consensus of the potency of the inhibitors and eliminate artifacts that may be observed with any one assay. We demonstrated that this series of compounds is potent for LSD1, selective for LSD1 over the monoamine oxidases (A and B), and appears to exhibit good brain penetration when administered systemically. Our study is the first to demonstrate that LSD1 may be an essential positive regulator of long-term memory formation. Systemic treatment with a potent LSD1 inhibitor resulted in significantly impaired long-term memory, leaving short-term memory intact. Our results suggest that LSD1 may not have a role in post-translational modification mechanisms involved in short-term memory, but rather may have an essential role in histone demethylation to facilitate gene expression required for long-term memory consolidation. Future studies are needed to identify the molecular targets of LSD1 and how they are linked to the regulation of transcription required for memory formation. It is known that crosstalk between histone modifications give rise to specific modification patterns that regulate transcription. For example increased methylation of histone H3 lysine 9 correlates with decreased acetylation of histone H3 lysine 14.[1] Additional studies are also needed to investigate the effect of LSD1 inhibition on histone methylation and how those changes in methylation are linked to histone acetylation. Beyond these studies, the role of LSD1 in other forms of memory, other behaviors, and the consequence at the level of short- and long-term neuroplasticity remains unexplored but of great interest. Finally, we are working on other modes of LSD1 inhibition that may be reversible and alter LSD1 through mechanisms other than competitive inhibition of substrates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures, characterization data, biological evaluation of the inhibitors, radiolabeling and biodistribution data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

J.M.H. and S.J.H. conceived and directed the project. R.N. performed all chemical syntheses. D.P., E.L.R., R.N., and S.N. were responsible for assay development and testing. S.M.C., J.M.H. and R.N. performed radiosynthesis. E.L.R. and I.T.H. performed LSD1 inhibition kinetics experiments. M.A.W. and M.M. performed and analyzed the behavioral assays (NOR). R.N. and J.M.H. drafted the manuscript with contributions from D.P., E.L.R., M.A.W. and M.M. All authors read and agreed on the final manuscript.

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Notes

The authors declare no competing financial interest.

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