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PET ligands [^{18}F]LSN3316612 and [^{11}C]LSN3316612 quantify O-linked- β -N-acetyl-glucosamine hydrolase in the brain

Shuiyu Lu^{1,§}, Mohammad B. Haskali^{1,§,†}, Kevin M. Ruley², Nicolas J.-F. Dreyfus³, Susan L. DuBois², Soumen Paul^{1,‡}, Jieh-San Liow¹, Cheryl L. Morse¹, Aneta Kowalski¹, Robert L. Gladding¹, Jeremy Gilmore³, Adrian J. Mogg³, S. Michelle Morin², Peter J. Lindsay-Scott³, J. Craig Ruble², Nancy A. Kant², Sergey Shcherbinin², Vanessa N. Barth², Michael P. Johnson², Maria Cuadrado⁴, Enrique Jambrina⁴, Andrew J. Mannes⁵, Hugh N. Nuthall³, Sami S. Zoghbi¹, Cynthia D. Jesudason², Robert B. Innis¹, Victor W. Pike^{1,*}

¹Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Building 10, Room B3C346, 10 Center Drive, Bethesda, Maryland 20892-1003, USA.

²Eli Lilly & Co, Indianapolis, Indiana 46285, USA.

³Eli Lilly & Co, Windlesham, GU20 6PH, UK.

⁴Lilly, S. A. Avenida de la Industria 30, 28108-Alcobendas, Madrid, Spain.

⁵Department of Perioperative Medicine, Clinical Center, National Institutes of Health, 10 Center Drive, Bethesda, Maryland 20892-1510, USA.

Abstract

We aimed to develop effective radioligands for quantifying brain O-linked- β -N-acetyl-glucosamine (O-GlcNAc) hydrolase (OGA) using positron emission tomography in living subjects as tools for evaluating drug target engagement. Post-translational modifications of tau, a biomarker of Alzheimer's disease, by O-GlcNAc through the enzyme pair OGA and O-GlcNAc transferase (OGT) are inversely related to the amounts of its insoluble hyper-phosphorylated form. Increase in tau O-GlcNAcylation by OGA inhibition is believed to reduce tau aggregation. LSN3316612, a highly selective and potent OGA ligand ($IC_{50} = 1.9$ nM), emerged as a lead ligand after in silico analysis and in vitro evaluations. [^3H]LSN3316612 imaged and quantified OGA in postmortem brains of rat, monkey, and human. The presence of fluorine and carbonyl functionality in LSN3316612 enabled labeling with positron-emitting fluorine-18 or carbon-11.

*Corresponding author. pikev@mail.nih.gov.

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§These authors contributed equally to this work.

†Present address: The Centre for Molecular Imaging and Translational Research Laboratory, The Peter MacCallum Cancer Centre, Melbourne, Victoria 3000, Australia.

‡Present address: Molecular Imaging Core, University of Virginia, Charlottesville, Virginia 22908, USA.

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Both [^{18}F]LSN3316612 and [^{11}C]LSN3316612 bound reversibly to OGA in vivo and such binding was blocked by pharmacological doses of thiamet G, an OGA inhibitor of different chemotype, in monkeys. [^{18}F]LSN3316612 entered healthy human brain avidly (~ 4 SUV) without radiodefluorination or adverse effect from other radiometabolites, as evidenced by stable brain V_T values by 110-min of scanning. Overall, [^{18}F]LSN3316612 is preferred over [^{11}C]LSN3316612 for future human studies, whereas either may be an effective PET radioligand for quantifying brain OGA in rodent and monkey.

One Sentence Summary:

Two radioligands, [^{18}F]LSN3316612 and [^{11}C]LSN3316612, image and quantify brain *O*-linked- β -*N*-acetyl-glucosamine hydrolase in vivo.

INTRODUCTION

Neurofibrillary tangles, neuropil threads, and dystrophic neurites contain hyper-phosphorylated, insoluble tau protein and are a hallmark of Alzheimer's disease (AD) and other tauopathies (1). The magnitude and location of pathological tau correlates closely with the symptoms and severity of disease (2). Taken together, there is very strong evidence supporting the aim of reducing the accumulation and spread of misfolded insoluble tau as a key strategy for treating Alzheimer's disease and related tauopathies (3). Intracellular proteins can be modified by the single-sugar *O*-linked- β -*N*-acetyl-glucosamine (*O*-GlcNAc) and this modification is regulated by the action of two enzymes, *O*-GlcNAc transferase (OGT) and *O*-GlcNAc hydrolase (*O*-GlcNAcase, OGA) (4-6). Inhibition of OGA leads to increase in protein *O*-GlcNAc expression through the continued action of the OGT enzyme. Tau may undergo many post-translational modifications, but tau *O*-GlcNAc modification is inversely related to the amounts of hyper-phosphorylated tau. Increasing tau *O*-GlcNAc modification through OGA inhibition is believed to reduce tau aggregation into the insoluble, hyper-phosphorylated form (7-11). Because of the presence of *O*-GlcNAc on many intracellular proteins, OGA is also linked to other diseases, such as Parkinson's and Huntington's diseases (12), type 2 diabetes (13), pathogenic effects of stress in cardiac tissues (14), and cancer (15).

Positron emission tomography (PET) with suitable radioligands may quantify the distribution of proteins of interest through the measurement of radioactivity distribution in living subjects. Such radioligands may reveal how these distributions are changed between healthy and diseased states. They may also assist in the development and evaluation of effective therapies (16-20). Key to exploiting the utility of PET is the availability of effective radioligands. The development of PET radioligands for imaging proteins in brain is especially challenging because radioligand design has to satisfy a wide array of chemical, biochemical, and pharmacological requirements (21, 22). Although some tau PET radioligands (23-25) in clinical development might allow quantification of the concentration of tau upon treatment by an OGA inhibitor, a selective PET radioligand for OGA would be useful for determining target engagement during both preclinical and clinical drug development. OGA itself may become a useful biomarker for early disease investigation and/or intervention. Thiamet G is an OGA inhibitor that blocks hyper-phosphorylation

of tau in vivo (26) and three compounds with the same pyranothiazole core have been labeled with the positron-emitter carbon-11 ($t_{1/2} = 20.4$ min) (27). MK-8553 has been labeled with the positron-emitter fluorine-18 ($t_{1/2} = 109.8$ min) for preclinical evaluation and human dosimetry studies (28). However, the structures and/or radioligand properties of these compounds have not been fully disclosed (29).

Here we report our interdisciplinary efforts from synthetic chemistry, pharmacology, radiochemistry, and PET preclinical and clinical evaluations for the discovery and development of two piperidyl-thiazole radioligands, [^{18}F]LSN3316612 and [^{11}C]LSN3316612. These radioligands enabled in vivo quantification of OGA in brain with PET and may be useful for drug development.

RESULTS

Selection of lead compounds

OGA ligands were designed and synthesized based on their in silico tracer-like properties, such as suitable molecular weight (< 500 Da), low hydrogen bond donor (HBD) number (< 3), moderate lipophilicity ($\log D_{7.4} 2-3$), and absence of efflux transporter liability. Prospective tracers were further profiled ex vivo by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (30) in rat to determine brain uptake, kinetics, and specific binding. LSN3316612 emerged as a lead candidate because it has low molecular weight (MW 364 Da), only one HBD, moderate lipophilicity ($\log P = 3.05$, $\log D_{7.4} = 2.90$), and absence of substrate behavior for efflux pumps, such as multidrug resistance protein 1 (MRP1, ABCC1) or breast cancer resistant protein (BCRP, ABCG2) (Table 1). The IC_{50} value for LSN3316612 was determined to be 1.9 ± 0.8 nM ($n = 3$). The IC_{50} value for thiamet G was 1.2 ± 0.3 nM ($n = 3$) in the same assay. LSN3316612 has an amido carbonyl group and an aryl fluoro substituent that may be labeled potentially with carbon-11 or fluorine-18, respectively. Based on these favorable initial assessments, we chose LSN3316612 for further in vitro and ex vivo evaluation and development of prospective PET radioligands.

Synthesis of LNS3316612 and radioligand precursors

Ligand LNS3316612 was synthesized in three steps from commercially available starting materials (fig. S1). Thus, treatment of (2*S*,4*S*)-piperidinol (compound 1) (31) with 2,6-difluoropyridine under basic condition furnished *tert*-butyl (2*S*,4*S*)-4-((6-fluoropyridin-2-yl)oxy)-2-methylpiperidine-1-carboxylate (compound 2) in 76% yield (fig. S2). Exposure of compound 2 to trifluoroacetic acid in dichloromethane and subsequent concentration of the reaction mixture gave a crude amine salt, which was then coupled with *N*-(5-formylthiazol-2-yl)acetamide (compound 3) (32) under the action of sodium triacetoxyborohydride. This reductive amination protocol delivered the target molecule LSN3316612 in 75% yield over two steps (fig. S3). Further purification with supercritical fluid chromatography followed by trituration in isopropanol allowed LSN3316612 to be isolated as a crystalline solid in multigram quantities with $>98\%$ purity and 99.7% enantiomeric excess (ee). Reductive amination of compound 2 with *tert*-butyl (5-formylthiazol-2-yl)carbamate (compound 5) (33) gave *tert*-butyl (5-(((2*S*,4*S*)-4-((6-

fluoropyridin-2-yl)oxy)-2-methylpiperidin-1-yl)methyl)thiazol-2-yl)carbamate (compound 6) in 41% yield (fig. S4). Deprotection of compound 6 with trifluoroacetic acid in dichloromethane afforded the amine precursor 5-(((2*S*,4*S*)-4-((6-fluoropyridin-2-yl)oxy)-2-methylpiperidin-1-yl)methyl)thiazol-2-amine (precursor 7) in quantitative yield (fig. S5). Treatment of (2*S*,4*R*)-piperidinol mesylate (compound 8) (34) with 6-nitropyridin-2-ol furnished the corresponding ether *tert*-butyl (2*S*,4*S*)-2-methyl-4-((6-nitropyridin-2-yl)oxy)piperidine-1-carboxylate (compound 9) with inversion of stereochemistry in 56% yield (fig. S6). The nitro precursor *N*-(5-(((2*S*,4*S*)-2-methyl-4-((6-nitropyridin-2-yl)oxy)piperidin-1-yl)methyl)thiazol-2-yl)acetamide (precursor 10) was obtained in 46% yield following coupling with compound 3 and reductive amination (fig. S7).

[³H]LSN3316612 binds with high affinity to native tissues

An important aspect of PET radioligand development is to consider variations in enzyme affinities ($1/K_D$) and expression concentrations (B_{max}) across species in relevant native tissues. To perform these in vitro evaluations, we prepared [³H]LSN3316612 (fig. S8). Saturation binding experiments were performed using cortical, striatal, hippocampal, and cerebellar homogenates from rat, cynomolgus monkey, and human as native tissues (fig. S9). K_D values ranged from 0.7 to 4.8 nM with some variability observed in B_{max} across species (Table 2). The highest B_{max} value was observed in rat cortex (46 nM). B_{max} values were high in striatum (> 12 nM) across species. B_{max} values were generally low (< 7 nM) in cerebellar tissues.

Regional visualization of [³H]LSN3316612 binding in monkey and human brain with autoradiography

The binding of [³H]LSN3316612 to brain slices from rhesus monkey (Fig. 1A) and human (Fig. 1B) was also examined with autoradiography. In rhesus monkey, the highest specific binding was observed in the hypothalamus, hippocampus, and the nucleus of solitary tract (11.4, 10.3, and 8.3 nCi/mg, respectively). Moderate, about equivalent binding was observed in cortical (5.3 vs. 4.1), striatal (5.9 vs. 3.7), and cerebellar regions (4.1 vs. 3.0) of both rhesus monkey and human (table S1). In both species there was minimal non-displaced binding remaining after blockade by thiamet G at 20 μ M.

Binding study in postmortem Alzheimer's brain

Binding studies with [³H]LSN3316612 in AD and control cortical tissue homogenates were performed to investigate binding potential (B_{max}/K_D) from postmortem human brain (fig. S10). Binding potential showed an increase (~ 40%) in postmortem AD brain over control (3.7 vs. 2.6, $P = 0.19$, $n = 9$).

LC-MS/MS tracer evaluation in rats

Intravenous administration of a low dose of LSN3316612 and subsequent analysis with an in vivo/ex-vivo LC-MS/MS method (30) showed rapid brain uptake followed by moderately slow washout (Fig. 2A). Uptake in frontal cortex was 4.0 standardized uptake value (SUV) and 1.9 SUV at 5 min and 90 min after injection, respectively. The peak tracer uptake in frontal cortex (4.0 SUV) was higher than in cerebellum (1.9 SUV). The latter declined to 0.6

SUV at 90 min after injection. These uptakes could be blocked in a dose-dependent manner by orally administered thiamet G (Fig. 2B). At 30 mg/kg p.o. dose, 80% of the uptake was blocked, whereas at 100 mg/kg, 94% of the uptake was blocked.

Radiochemistry

Radiochemistry was performed in lead-shielded hot-cells for protection of personnel. [^{11}C]LSN3316612 was synthesized through $\text{Pd}(\text{PPh}_3)_4$ -mediated ^{11}C -carbonyl insertion between iodomethane and precursor 7 in two steps (Fig. 3). Radiochemically pure (> 98%) doses of [^{11}C]LSN3316612 were obtained in $3.5 \pm 1.3\%$ (0.4 to 0.9 GBq) yield from starting cyclotron-produced [^{11}C]carbon dioxide and with molar activity of 74 ± 39 GBq/ μmol ($n = 4$) at 50 min after radionuclide production. The pH was adjusted with aq. NaHCO_3 (50 μL , 8.4% USP aqueous solution) from 3.0 to 6.5.

Sterile and formulated [^{18}F]LSN3316612 was produced by nucleophilic substitution of the nitro group from precursor 10 in 90 min with anhydrous [^{18}F]fluoride ion (Fig. 3). [^{18}F]LSN3316612 was obtained in $35 \pm 9\%$ (0.3 to 2.2 GBq) yield with 98.9% radiochemical purity and molar activity of 76 ± 25 GBq/ μmol ($n = 37$) at the end of radiosynthesis. Radiochemical identity was confirmed by observation of co-mobility with authentic LSN3316612 on radio-high performance liquid chromatography (radio-HPLC) and also by LC-MS of the carrier ($[M+H]^+$, observed: $m/z = 365.0$; calculated 365.1). HPLC analysis showed that both formulated [^{11}C]LSN3316612 and [^{18}F]LSN3316612 were > 97% radiochemically unchanged after 2 h at room temperature (RT).

Lipophilicity ($\log D_{7.4}$) and stability measurements

Lipophilicity influences critical imaging characteristics, such as plasma free fraction (f_p), brain penetration, non-specific binding, and susceptibility to metabolism (21, 35-37). Generally, a moderate lipophilicity is desirable in PET radioligands intended to image brain proteins. The $\log D_{7.4}$ of [^{11}C]LSN3316612 was found to be 2.96 ± 0.01 ($n = 6$) by a technique (38) based on distribution of the radioligand between 1-octanol and sodium phosphate buffer (0.15 M; pH 7.4). The $\log D_{7.4}$ of [^{18}F]LSN3316612 was 3.04 ± 0.01 ($n = 5$). Both values are in line with $\log D_{7.4}$ of LSN3316612 at 2.90. [^{11}C]LSN3316612 and [^{18}F]LSN3316612 were stable in phosphate buffer for at least 2.5 h at RT ($98.7 \pm 0.1\%$, $n = 2$ and $100.0 \pm 0.1\%$, $n = 2$, respectively). [^{11}C]LSN3316612 was stable for at least 30 min at RT in human plasma (100%) and in brain homogenates of rat (98.4%), monkey (98.2%), and human (99.2%).

PET imaging of [^{11}C]LSN3316612 and [^{18}F]LSN3316612 in rhesus monkey brain

Intravenous administration of [^{11}C]LSN3316612 into a rhesus monkey at baseline resulted in good brain radioactivity uptake (Fig. 4A). Regional radioactivity uptakes and summed PET images (Fig. 4B, top row) reflected OGA enzyme distribution seen in autoradiography (Fig. 1A). All brain regions, such as frontal cortex, striatum, hippocampus, and thalamus showed continuous accumulation of radioactivity. Cerebellum showed slow wash-out of radioactivity after peaking at around 45 min. In an enzyme preblocking experiment, the same monkey was given thiamet G (10 mg/kg) intravenously 45 min before the injection of [^{11}C]LSN3316612. Whole brain activity decreased rapidly and regional time-activity

curves were almost indistinguishable, showing a fast and smooth decline from a higher peak radioactivity (~ 2.8 SUV). This higher peak value is possibly attributable to a greater availability of radioligand in plasma because of block of peripheral OGA enzyme. Summed images showed that the distribution of radioactivity across brain became low and uniform (Fig. 4B, bottom row), indicating that a high amount of brain radioactivity in the baseline experiment was due to specific binding to OGA enzyme.

Intravenous administration of [^{18}F]LSN3316612 into another rhesus monkey at baseline produced very similar brain distribution to those with [^{11}C]LSN3316612, albeit with somewhat higher brain radioactivity uptake, less noise, and better resolution (Fig. 4C). In an enzyme preblocking experiment, the same monkey was given thiamet G (10 mg/kg) intravenously 45 min before the injection of [^{18}F]LSN3316612. Again, whole brain activity decreased rapidly and all regional time-activity curves became indistinguishable, with fast and smooth declines from a higher peak radioactivity (~ 4.8 SUV). PET images showed that there was no radioactivity uptake in the skull, indicating that no radiodefluorination had occurred (Fig. 4D). These results validated the dose dependency of the preblocking effects observed in the LC-MS/MS study of rats injected with low doses of LSN3316612 (Fig. 2B).

Emergence of radiometabolites in monkey plasma

30 min after intravenous administration of [^{11}C]LSN3316612 into a monkey, five radiometabolites were detected in plasma ([^{11}C]A–[^{11}C]E, fig. S11A). The radiometabolites were all less lipophilic than [^{11}C]LSN3316612 except [^{11}C]E, as judged by their shorter retention times in reversed-phase HPLC. [^{11}C]Acetic acid ([^{11}C]C) was a major radiometabolite; its lower lipophilicity relative to [^{11}C]LSN3316612 and high degree of ionization at physiological pH restricts its brain entry to contaminate receptor-specific signal. Other radiometabolites were not identified. [^{11}C]A and [^{11}C]E were almost negligible. The percentage of radioactivity in plasma represented by unchanged [^{11}C]LSN3316612 decreased to about 60% within the initial 30 min scan interval, and thereafter slowly decreased to $\sim 50\%$ at the end of the scan (90 min). Under the preblocking condition, unchanged [^{11}C]LSN3316612 showed a steady decrease from $\sim 60\%$ at 30 min to 35% at 90 min (fig. S11B). [^{11}C]LSN3316612 was not substantially metabolized in monkey brain homogenates in vitro. [^{18}F]LSN3316612 also produced five metabolites less lipophilic than [^{18}F]LSN3316612 in plasma (fig. S11, C and D).

Two-tissue compartmental modeling of PET monkey data

Two-tissue compartmental modeling with a radiometabolite-corrected arterial input function showed that the total volumes of distribution (V_T) for [^{11}C]LSN3316612 in enzyme-rich frontal cortex and striatum were 25.8 and 25.7 mL/cm³, respectively. V_T in cerebellum was 10.4, indicating some substantial presence of OGA in this brain region. After preblocking with thiamet G (10 mg/kg), V_T in all regions decreased to about 1.6 mL/cm³, representing greater than 90% reduction. The signal is therefore specific to the presence of OGA. For baseline scans, the two-tissue compartmental model showed that V_T values are within 10% of the terminal value by 60 min of imaging in several brain regions.

Two-tissue compartmental modeling with arterial input function showed that the V_T for [^{18}F]LSN3316612 in enzyme-rich frontal cortex and striatum, respectively, was 27.3 and 28.5 mL/cm³. V_T in cerebellum was 13.8 mL/cm³. After preblocking with thiamet G (10 mg/kg), V_T in all regions decreased by more than 90%. For baseline scans, the two-tissue compartmental model showed that V_T values are within 10% of the terminal value by 60–80 min of imaging in brain regions such as frontal cortex, thalamus, and cerebellum.

The stable V_T values again indicate a lack of accumulation of radiometabolites in these brain regions, consistent with the observations that all radiometabolites in plasma were less lipophilic than the parent radioligands. Therefore, [^{11}C]LSN3316612 and [^{18}F]LSN3316612 binding to OGA in the brain can be quantified from about 80 min of PET imaging data along with the measured arterial input function.

PET imaging of [^{18}F]LSN3316612 in human brain

[^{18}F]LSN3316612 was evaluated in eight healthy human subjects (table S2). After intravenous injection of [^{18}F]LSN3316612, good overall brain radioactivity uptake and distribution were observed (Fig. 5A). Time-activity curves were similar for striatum and cerebellum and peaked at about 4 SUV by 25 min before a steady slow decline. The time-activity curve of hippocampus displayed a somewhat lower peak and flatter profile. Regional radioactivity uptakes and parametric PET images (Fig. 5B) correlated well with OGA enzyme distribution observed in autoradiography (Fig. 1B). No radiodefluorination was observed, as evidenced by absence of radioactivity in skull.

Unchanged [^{18}F]LSN3316612 represented > 95% of plasma radioactivity 3 min after injection, which decreased to 20–30% 3 h after injection (Fig. 5C). At least five unresolved radiometabolites appeared in plasma but they all eluted earlier than [^{18}F]LSN3316612 in reversed-phase HPLC, indicating they are of lower lipophilicity. Two-tissue compartmental modeling with arterial input function showed V_T values were 14.1, 12.2, 10.7, and 9.8 mL/cm³ for amygdala, hippocampus, thalamus, and cerebellum, respectively (table S3). Although lower than those observed in monkey (39), these values are in the same rank order and are well above V_T under blocking condition (V_{ND}), indicating a high amount of specific binding to OGA (assuming V_{ND} is similar between monkey and human). Regional mean V_T in 8 subjects reached 90% of terminal 3-h values by 110 min (Fig. 5D). Regions with high V_T (hippocampus) were slower to reach stable V_T than regions with lower V_T (cerebellum), consistent with the notion that higher enzyme density regions will take longer to reach equilibrium. As in monkey, stable V_T measures were attainable, indicating absence of troublesome influence from radiometabolites.

Plasma free fraction (f_p) measurement

At baseline, the plasma free fraction (f_p) of [^{11}C]LSN3316612 for rhesus monkey was $9.87 \pm 0.00\%$ ($n = 3$). Under the pre-blocked condition, f_p was slightly lower at $8.38 \pm 0.17\%$ ($n = 3$). In the same monkey at baseline, the f_p of [^{18}F]LSN3316612 was $6.70 \pm 0.36\%$ ($n = 3$) and under the pre-blocked condition was slightly lower at $5.36 \pm 0.15\%$ ($n = 3$). These f_p values are in the range expected for a radioligand with a measured $\log D_{7.4}$ value of 2.96 (37). f_p in human plasma was $4.36 \pm 0.91\%$ ($n = 8$) for the eight human subjects under

evaluation. These f_p values are readily measurable with good accuracy and are consistent with the ability of [^{11}C]LSN3316612 and [^{18}F]LSN3316612 to readily enter brain from plasma.

DISCUSSION

The power of PET as a molecular imaging modality in large part derives from the range of effective radiotracers that are available. The discovery and development of PET radioligands for imaging proteins in brain has special considerations (21, 22). A primary consideration is the regional target protein density ($B_{\max}$), a measure of the amount of imaging target expressed in normal human brain and whether the density is expected to increase or decrease in one or more regions during the progression of a disease. A candidate radioligand must satisfy a wide array of chemical, biochemical, and pharmacological requirements, including: (i) high radioligand affinity represented by K_d , K_i , or IC_{50} – radioligands for imaging brain targets must usually have high affinity in the nM or sub-nM range; (ii) high binding potential (BP , BP_{ND}) – ideally, binding potential expressed as $B_{\max}/K_d$ should be ≥ 5 for accurate quantification; (iii) selectivity for target binding – an ideal PET radioligand should bind only to the target protein and show no appreciable affinity for off-target sites within the same brain tissue location; (iv) ability to pass the blood-brain barrier (BBB) – molecular weight < 500 Da, lipophilicity ($\log D_{7.4}$ of 2–3), absence of charge, low HBD capacity (< 3), ionization ability (pK_a of most basic site < 9.5) and topological polar surface area ($TPSA < 90 \text{ \AA}^2$), favorable BBB penetration; (v) absence of efflux transporter substrate behavior – radioligands should not be a substrate for the most common efflux pumps, such as P-glycoprotein (P-gp; MDR1/mdr-1a; ABCB1), breast cancer resistant protein (BCRP; ABCG2), and multidrug resistance associated protein (MRP1; ABCC1); (vi) adequate and measurable free fraction in plasma – PET quantification is based on the assumption that only radioligand that is free in plasma may cross the BBB for binding in brain; (vii) low nonspecific binding (or low non-displaceable binding) – excessive nonspecific binding masks any specific binding and so can render a radioligand of little or no utility; (viii) lack of accumulation of radiometabolites in brain or skull – for ^{18}F -labeled radioligands, avoiding radiodefluorination is essential; and finally (ix), amenability to labeling with carbon-11 or fluorine-18. Armed with data from in vitro screening, we were able to proceed with radiolabeling and evaluation of the labeled LSN3316612 in animal models and humans with PET.

[^{11}C]Carbon monoxide has shown tremendous utility for labeling organic compounds in carbonyl positions (40, 41). Aryl carboxamides may be labeled through transition-metal mediated [^{11}C]carbonyl insertion reactions between aryl halides and secondary amines (42–45). Such reactions generally tolerate wide functionality and may be carried out rapidly in high yields. The aryl-Pd complexes are stable enough (45) to be isolated, purified, and stored before use for the synthesis of [^{11}C]amides, even for some structurally demanding molecular targets. Iodomethane is inherently less reactive than more commonly used iodoarenes for carbon monoxide insertion reactions. Nevertheless, sufficient radiolabeled product has been produced from iodomethane (46).

For the synthesis of [^{11}C]LSN3316612, a modified Synthia platform (47, 48), incorporating a module for generating [^{11}C]carbon monoxide from cyclotron-produced [^{11}C]carbon dioxide and a micro-autoclave (49), was used for automated radiochemistry in a lead-shielded hot-cell. Although a wide range of palladium reagents has been shown to be effective for [^{11}C]carbonyl insertion reactions, only $\text{Pd}(\text{PPh}_3)_4$ was used in this study. $\text{Pd}(\text{PPh}_3)_4$ is slightly air and light sensitive. [^{11}C]Carbon monoxide insertion efficiency decreased over several days when conducted with the same source and amount of $\text{Pd}(\text{PPh}_3)_4$, despite storage of the reagent in a nitrogen-protected glove-box and adopting the practice of only withdrawing the $\text{Pd}(\text{PPh}_3)_4$ within 1 h before synthesis. [^{11}C]Carbon monoxide insertion efficiency could be restored by increasing the quantity of $\text{Pd}(\text{PPh}_3)_4$ used by 20 to 30 mole% from the same batch within a 2- to 3-week period. Tetrahydrofuran (THF) was the preferred solvent because of its easy removal before aminolysis. THF must be fresh and anhydrous to achieve optimal insertion and minimal formation of [^{11}C]acetic acid byproduct. The other reagents could be handled in open air. The efficiency of the aminolysis step depends on the order of reagent addition (50). When a solution of precursor 7 and tributylamine in THF was preloaded into the 5-mL glass vial and the [^{11}C]acetyl-Pd complex solution was then transferred at a lower temperature ($< 80^\circ\text{C}$), aminolysis went smoothly upon complete evaporation of THF. The inefficiency of the molecular sieves for trapping and release of the starting cyclotron-produced [^{11}C]carbon dioxide contributed to a low overall yield of [^{11}C]LSN3316612.

The radiosynthesis of [^{18}F]LSN3316612 was based on the displacement of a nitro group in an ortho position of a pyridinyl ring with cyclotron-produced [^{18}F]fluoride ion (51), followed by HPLC separation. Treatment of the nitro precursor 10 with K 2.2.2-potassium [^{18}F]fluoride ion complex in dimethyl sulfoxide (DMSO) at 115°C for 20 min gave [^{18}F]LSN3316612 in 47% isolated yield before formulation. The subsequent steps of dilution, concentration on a C_{18} solid phase extraction (SPE) cartridge, elution, and sterile filtration decreased the final yield to 27%. About 8% of the radioactivity was adsorbed on the sterile filter. Most of the remaining radioactivity loss was due to low trapping efficiency on the C_{18} SPE cartridge. This procedure was optimized to produce sufficient quantities of [^{18}F]LSN3316612 for clinical PET studies under Current Good Manufacturing Practice (CGMP) conditions.

Initial evaluation in rhesus monkey showed that both [^{11}C]LSN3316612 and [^{18}F]LSN3316612 met criteria for being effective PET radioligands. They are both stable when formulated for intravenous injection. Both radioligands showed high brain uptake with a high proportion of specific binding to OGA. Radiometabolites from each radioligand did not interfere with quantification as evidenced by stable V_T . Clinical evaluation of [^{18}F]LSN3316612 clearly demonstrated its suitability for further studies in human subjects. Longer half-life and lack of radiodefluorination in vivo gives [^{18}F]LSN3316612 a moderate advantage over [^{11}C]LSN3316612 for acceptance by more researchers and clinicians. Moreover, an ^{18}F -labeled tracer from a single producer can be transported to research and clinical facilities where a cyclotron is unavailable.

Detailed evaluations in OGA gene knock-out mice (for target selectivity, metabolism), additional monkey (39) (dose dependence, pharmacokinetics), dosimetry (radiation

safety) and test/retest evaluation in healthy human subjects are needed to progress [^{18}F]LSN3316612 to full clinical applications. With overall scores for pharmacokinetic performance, suitability for CGMP production, accessibility to the wider clinical and research communities, [^{18}F]LSN3316612 emerges as the radioligand of choice for further studies of OGA with PET. The finding of a trend for increased enzyme expression in postmortem AD over control brain with [^3H]LSN3316612 is biologically and pharmacologically interesting and deserves more investigation with [^{18}F]LSN3316612 in PET studies in vivo. The definite presence of enzyme in AD brain shows that the target is indeed available for PET imaging.

The low overall yield for the synthesis of [^{11}C]LSN3316612 is a limitation for its use, unless further optimized. Carbon-11 labeled radioligands are useful because the short half-life can allow two imaging experiments in one subject, one at baseline and one with drug challenge, within a single day. Our initial study with [^{18}F]LSN3316612 was limited to eight healthy human subjects. Although the current study demonstrates the suitability of [^{18}F]LSN3316612 for drug occupancy studies for OGA inhibitor in healthy human subjects, future studies with larger numbers of healthy subjects and recruitment of patients will ascertain the links between OGA expression, disease state, and disease progression. Further studies in human subjects are required to assess whether there may be major unexpected influences on radioligand behavior and utility arising from, for example, variations in subject genotype or metabolic behavior.

MATERIALS AND METHODS

Study design

OGA is a potential biomarker and therapeutic target for tauopathy in neurodegenerative diseases such as Alzheimer's. The objective of this study was to discover and develop an efficacious and selective radioligand primarily for studying OGA drug target engagement for post-translational modifications of tau with PET in human brain. Owing to the ubiquity of OGA in the human body, a PET radioligand would also be applicable to elucidating disease pathophysiology and assisting drug development for diseases other than AD. A prerequisite to the development of useful PET radioligands targeting the OGA enzyme for neuroscience translational research is consideration of (i) regional density of the target; (ii) radioligand affinity; (iii) selectivity for the target, (iv) ability to cross the BBB; (v) efflux transporter action; (vi) adequate and measurable plasma free fraction; (vii) lack of contamination of PET measurement with radiometabolites; (viii) amenability to quantification; and (ix) choice of radioisotopes and labeling sites. We assessed the suitability of the candidate chemical entity LSN3316612 to meet each of these requirements, through in vitro and ex vivo evaluations. After labeling of LSN3316612 with a positron-emitter, either carbon-11 or fluorine-18, the two radioligands were evaluated in monkeys (one for each radioligand). [^{18}F]LSN3316612 was then progressed to evaluation in eight healthy human subjects. The results provided pre-clinical and clinical data which may be successfully translatable to further studies in human subjects.

All in vitro, and ex vivo studies were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) under protocols approved by the Animal

Care and Use Committee of Eli Lilly and Company. Ex vivo studies in rodents and PET imaging experiments in monkeys were performed in accordance with the Guide for Care and Use of Laboratory Animals (52) and were approved by the National Institute of Mental Health Animal Care and Use Committee. PET imaging of healthy human subjects was approved by the National Institutes of Health Institutional Review Board (IRB, #16-M-0105, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03632226) Identifier: [NCT03632226](https://clinicaltrials.gov/ct2/show/study/NCT03632226)) and Radiation Safety Committee (RSC, #2571). Informed consent was obtained before imaging from each subject. As an exploratory investigation for PET radioligand development, we recruited eight healthy human subjects to test the efficacy and variability of the radioligand. No power calculation of sample size was performed and blind analysis was not applicable.

Syntheses of compounds 2 to 10

Syntheses and characterization of compounds 2 to 10 are detailed in Supplementary Materials.

In vitro enzyme assays and saturation binding

Experimental details of in vitro enzyme assays and saturation binding are described in Supplementary Materials.

[³H]LSN3316612 Autoradiography

Tissue sections (20 µm) from fresh frozen rhesus monkey brain (male, Covance Research Products) or human brain (male, Analytical Biological Services) were collected onto gelatin/chrome alum coated slides and stored at –80 °C until use. For autoradiography, the sections were thawed and pre-incubated at RT in assay phosphate buffered saline (PBS, Hyclone SH30258.01) for 15 min to remove endogenous receptor ligands. Sections were subsequently incubated for 60 min on ice in assay buffer containing [³H]LSN3316612 (5 nM) with or without non-radioactive thiamet G (20 µM) for determination of nonspecific binding. The sections were then rinsed twice by immersion in ice-cold assay buffer for 90 s, followed by a final rinse in ice-cold purified water, and then dried under a stream of warm air. The dried tissue sections were exposed to Fuji BAS-TR2025 phosphor imaging plates along with calibrated radioactive standards (American Radiolabeled Chemicals, Inc. ARC-0123). The phosphor imaging plates were scanned with a BAS-5000 imager (Fuji) and autoradiogram analysis was performed with a computer-assisted image analyzer (MCID7.0; Imaging Research Inc.).

Ex vivo rat evaluation by LC-MS/MS analysis

For intravenous administration at a dose of 10 µg/kg into the rat lateral tail vein, LSN3316612 was formulated in (2-hydroxypropyl)-β-cyclodextrin solution (25%) at a dose volume of 0.5 mL/kg with sonication in a water bath for 30 min. To determine the best post-tracer survival interval, a time-course of tracer concentration was analyzed. Rats (*n* = 3–4) were sacrificed 5, 40, and 90 min after injection of LSN3316612 by cervical dislocation. Trunk blood was obtained, and brains were quickly removed. The whole blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes and placed on wet ice for later centrifugation. Brains were rinsed in distilled water. Frontal cortex and cerebellum

were dissected, placed in centrifuge tubes, and weighed. At the study end, four volumes (w/v) of acetonitrile containing 0.1% formic acid were added to each tube. The tissues were homogenized using an ultrasonic dismembrator probe and centrifuged for 20 min at 15,000 rpm. One volume of supernatant was added to four volumes of water in an autosampler vial and vortexed. An aliquot (5 μ L) was injected onto an HPLC (1260 Infinity; Agilent) using an autosampler (1290 Infinity; Agilent) for the course study. An C_{18} column (part number 971700-902; Agilent) and mobile phase gradient of 10% to 90% acetonitrile/water (0.1% formic acid) with a run time of 3.2 min was used to achieve separation. A triple quadrupole mass spectrometer (Agilent 6400) operating in multiple reaction monitoring (MRM) mode monitoring ions 365.1/113 (for LSN3316612) was used for detection. Calibration curves for all studies were generated by adding known quantities of analytes to naïve brain tissue, then extracting and analyzing them as stated above. Frontal cortex, cerebellar and plasma tracer concentrations were measured. The standardized uptake value (SUV) was calculated by taking the ratio of the measured tracer concentrations in brain region divided by the injected tracer dose.

Rat OGA occupancy of thiamet G

Thiamet G (53) was administered orally in HEC (hydroxyethyl cellulose 1%/Tween 80 0.25%/Antifoam 0.05% in water) vehicle at a volume of 3 mL/kg. The same procedures were also applied to evaluate the occupancy of thiamet G after oral gavage in Harlan Sprague Dawley rats (200–300 g in weight). The doses of thiamet G included 1, 3, 10, 30, 100 mg/kg and were administered 2 h before tracer injection. The animals were sacrificed at 40 min after injection of LSN3316612. A similar analytical protocol was used to determine the concentration of LSN3316612 with HPLC (Shimadzu) and an API 4000 MS/MS (Applied Biosystems) in the MRM mode monitoring ions 365.1/211, as well as an Xbridge C_{18} column (3.5 μ m, 2.1 mm $\times$ 50 mm; Waters). The amount of tracer was measured in each cortical sample.

Radiochemistry

Production of [11 C]LSN3316612.—No-carrier-added (NCA) [11 C]carbon dioxide was produced via the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction in a target containing 1% oxygen in nitrogen, initially pressurized to 1551 kPa. This target was bombarded with a beam (45 μ A) of protons (16.5 MeV) from a PETtrace cyclotron (GE Medical Systems). Irradiations were conducted for 40 min for radioligand production and typically provided about 110 GBq of [11 C]carbon dioxide. Shorter irradiations were used to produce lower amounts of [11 C]carbon dioxide for initial radiochemistry experiments. At the end of irradiation, radioactivity was released to a trap containing molecular sieve (13X; 80/100 mesh, 300 mg) at RT, which was then swept with helium at 80 mL/min to remove residual oxygen.

[11 C]Carbon dioxide was released from the molecular sieve trap at 280 $^{\circ}\text{C}$ in a stream of helium (10 mL/min) for concentration in a cryotrap containing silica (10 mg) cooled in liquid nitrogen. This trap was heated with a halogen lamp (150 W) to release [11 C]carbon dioxide into helium (10 mL/min) for conversion into [11 C]carbon monoxide by passage through a heated (875 $^{\circ}\text{C}$) quartz tube (22 cm length; 0.7 cm internal diameter, i.d.) packed with molybdenum wire (99.97%, 0.05 mm diameter; Strem Chemicals). The effluent was

passed through ascarite to strip-off unconverted [^{11}C]carbon dioxide and the [^{11}C]carbon monoxide was then trapped in a second silica cryotrap at $-196\text{ }^{\circ}\text{C}$ in liquid nitrogen. By heating this trap with a halogen lamp, [^{11}C]carbon monoxide was released in helium for transfer to an autoclave containing $\text{Pd}(\text{PPh}_3)_4$ ($1.1\text{ }\mu\text{mol}$) and iodomethane ($24.1\text{ }\mu\text{mol}$) in THF ($80\text{ }\mu\text{L}$). The autoclave was then pressurized to $2.4 \times 10^3\text{ kPa}$ with THF by a HPLC solvent pump (SSI Isocratic; Scantech Lab) and heated at $130\text{ }^{\circ}\text{C}$ for 3 min. The mixture was then flushed out of the autoclave into a 5-mL glass vial (Alltech; Sigma Aldrich) that had been loaded with a solution of the precursor 7 ($1.4\text{ }\mu\text{mol}$) and Bu_3N ($6.3\text{ }\mu\text{mol}$) in THF ($50\text{ }\mu\text{L}$). The vial was heated from 68 to $95\text{ }^{\circ}\text{C}$ while THF was evaporated off to dryness under N_2 gas purge (200 mL/min , 3 min) through a vent needle. Radioactive residue was re-dissolved in a trifluoroacetic acid (TFA) solution in MeCN (2% v/v, $400\text{ }\mu\text{L}$), diluted with water (2.5 mL). The aqueous mixture was injected onto an Xterra C_{18} column ($10\text{ }\mu\text{m}$, $7.8 \times 300\text{ mm}$; Waters) eluted at 6 mL/min , initially with (MeCN: 0.05% TFA ($\text{pH} = 2.5$, 18:82 v/v) for 17 min, and then with the MeCN increased linearly to 90% over 1 min for 8 min. Absorbance was monitored at 270 nm (System Gold 166; Beckman Coulter Inc.) while radioactivity was monitored with a pin-diode detector (Bioscan Inc.) (fig. S12). The fraction eluting between 14.0 and 16.0 min was collected and concentrated under vacuum at $80\text{ }^{\circ}\text{C}$ for 1 min to remove solvents. The radioactive residue was reconstituted in saline (9 mL), ethanol (1 mL), and aq. NaHCO_3 (8.4%, $50\text{ }\mu\text{L}$) and sterilized by filtration through a $0.22\text{ }\mu\text{m}$ sterile filter (Millipore-MP; Waters). An aliquot ($\sim 60\text{ }\mu\text{L}$) of the formulated product was analyzed with radio-HPLC on a Luna C_{18} column ($10\text{ }\mu\text{m}$, $250 \times 4.6\text{ mm}$; Phenomenex) to obtain radiochemical purity, chemical purity, and molar activity (fig. S13). The column was eluted at 2 mL/min with MeCN:20 mM aq. ammonium formate (50:50, v/v) with eluate monitored for absorbance at 270 nm (System Gold 166 detector) and for radioactivity with a photomultiplier tube (PMT) detector (Bioscan Inc.). The retention time of [^{11}C]LSN3316612 was 5.5 min. The absorbance response had been pre-calibrated with respect to mass of LSN3316612 in the injectate, to permit calculation of molar activity.

Production of [^{18}F]LSN3316612.—NCA [^{18}F]fluoride ion was produced with the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ reaction by irradiating ^{18}O -enriched water (95 atom %; 1.8 mL) with a beam of protons (14.1 MeV ; $20\text{--}25\text{ }\mu\text{A}$, 90 min) from a PETtrace cyclotron (GE Medical Systems). [^{18}F]LSN3316612 was prepared in an automated TRACERlab $\text{FX}_{\text{F-N}}$ radiosynthesis apparatus (GE Healthcare). A solution composed of K2.2.2 (5.0 mg in $90\text{ }\mu\text{L}$ MeCN) and K_2CO_3 (0.5 mg in $10\text{ }\mu\text{L}$ H_2O) was added to the [^{18}F]fluoride ion in [^{18}O]water (7.4 GBq) from the cyclotron target, and dried by two additions and evaporations of acetonitrile (2 mL each) under nitrogen at $65\text{--}88\text{ }^{\circ}\text{C}$. Nitro precursor 10 ($1.6 \pm 0.1\text{ mg}$) in DMSO (0.6 mL) was added to the dried [^{18}F]fluoride ion complex, and heated to $115\text{ }^{\circ}\text{C}$ for 20 min. The mixture was then transferred to water (2 mL). [^{18}F]LSN3316612 was then separated with HPLC on a Luna C_{18} column ($10\text{ }\mu\text{m}$; $250 \times 10\text{ mm}$; Phenomenex) eluted with MeCN-0.01M ammonium formate solution (30:70 v/v) at 6 mL/min (fig. S14). The fraction containing [^{18}F]LSN3316612 (R_t 34–36 min) was collected, diluted with water (70 mL), and passed through a pre-conditioned C_{18} SPE cartridge. The cartridge was then washed with water (5 mL), and eluted with ethanol (1 mL) into a product collection vial. Saline (9 mL) was then passed through the C_{18} SPE cartridge into the product collection vial to afford fully formulated [^{18}F]LSN3316612 solution. Finally, this solution was passed through a sterile

filter (0.22 μm pore size; Millex-MP) into a sterile dose-vial (10 mL; Hospira) to afford sterile [^{18}F]LSN3316612 solution ready for injection. An aliquot of the formulated sample was analyzed with HPLC on a Luna C₁₈ column (10 μm ; 250 $\times$ 4.6 mm; Phenomenex) eluted with MeCN-0.01 M ammonium formate solution (50:50 v/v) at 2 mL/min (fig. S15) and with LC-MS of radioligand carrier. The retention time of [^{18}F]LSN3316612 was 4.1 min. Electrospray ionization mass spectrometry (ESI-MS): calculated for C₁₇H₂₂FN₄O₂S m/z [$M+H$]⁺ 365.1, found m/z 365.2. Molar activity of [^{18}F]LSN3316612 at the end of radiosynthesis was also determined with radio HPLC analysis with absorbance response at 270 nm calibrated for mass of LSN3316612. For production of [^{18}F]LSN3316612 under CGMP conditions, the amount of nitro precursor 10 was reduced (0.95 ± 0.5 mg). An XBridge C₁₈ column (5 μm ; 250 $\times$ 10 mm; Waters) eluted with MeCN-0.1M ammonium formate solution (25:75 v/v) initially at 4 mL/min, and then 7 mL/min over 3 min was used for radioligand purification. The varied flow rate and gradient were selected to reduce the associated column back pressure. The fraction containing [^{18}F]LSN3316612 (R_t 30.5–32.5 min) was collected for formulation.

Lipophilicity measurements and stability in aqueous buffer

The distribution coefficients ($\log D_{7.4}$) of [^{11}C]LSN3316612 or [^{18}F]LSN3316612 between 1-octanol and sodium phosphate buffer (0.15 M, pH 7.4) were determined using a technique we have described previously (38). For the purpose of measuring the degree of instability, formulated [^{11}C]LSN3316612 solution was placed in sodium phosphate buffer (0.15 M; pH 7.4) for the duration of a $\log D_{7.4}$ determination, and reanalyzed by radio-HPLC on an X-Terra C₁₈ column (10 μm ; 7.8 $\times$ 300 mm; Waters) eluted with MeOH:H₂O:Et₃N (75:25:0.1 by vol.) at 3.5 mL/min. [^{18}F]LSN3316612 was analyzed with radio-HPLC on the same X-Terra column, but with a mobile phase of MeOH:aqueous ammonium formate (10 mM) (72.5:27.5, by vol.) at 4.0 mL/min. For the $\log D_{7.4}$ determination, the percentage of radioactivity represented by unchanged [^{11}C]LSN3316612 or [^{18}F]LSN3316612 in the aqueous buffer at the end of extraction with 1-octanol was determined with radio-HPLC.

Stability of radioligands in brain homogenate of rat, monkey and human

Brain tissues that had been stored at -70°C were thawed on the day of the experiment to make brain homogenate samples with 1:10 dilution in PBS. Formulated [^{11}C]LSN3316612 or [^{18}F]LSN3316612 solution (~ 185 kBq; 1.0 μL) was incubated with rat, monkey, or human brain homogenate (500 μL) in phosphate-buffered saline (pH 7.4) for 30 min at RT, and then analyzed with reversed phase HPLC to measure change in radiochemical purity.

Analysis of radiometabolites in monkey blood and plasma

Experiments were performed to verify the stability of [^{11}C]LSN3316612 in whole monkey blood and plasma to ensure that no appreciable degradation of [^{11}C]LSN3316612 would occur between blood sampling from monkey and analytical processing. Thus, formulated [^{11}C]LSN3316612 solution (~ 23 μL ; < 35 kBq) was incubated with monkey whole blood (200 μL) and monkey plasma (200 μL) at RT for at least 30 min. After incubation, the radioactive whole blood sample (200 μL) was mixed with distilled water (300 μL) for 30 s to lyse blood cells. Samples of the lysed cells (450 μL) and of the incubated radioactive plasma were each added to MeCN (720 μL) for deproteinization. The samples were then

centrifuged (10,000 g) and the clear supernatant liquids were analyzed with radio-HPLC. Radioactivity in the precipitates were counted in a γ -counter to allow calculation of the extraction efficiency. The extraction efficiencies of all samples were high at $91.5 \pm 7.4\%$ ($n = 6$). The percentage of unchanged radioligand in the analyte, as determined by radio-HPLC, was divided by its radiochemical purity to give the stabilities in whole blood and plasma.

For the analysis of [^{11}C]LSN3316612 and radiometabolites in plasma, arterial blood samples were collected at different times after intravenous injection of [^{11}C]LSN3316612 into a male rhesus monkey (54). Plasma was separated and then analyzed with radio-HPLC on an X-Terra C₁₈ column (10 μm , 7.8×300 mm; Waters) eluted at 4.0 mL/min with MeOH:H₂O:Et₃N (82.5:17.5:0.1 by vol.). Full recoveries of radioactivity from these HPLC analyses were routinely verified. Radioactivity concentrations in different blood components were expressed as SUV, defined as $[(\% \text{ injected activity}/\text{cm}^3) \times \text{body weight (g)}]/100$. Metabolites analysis for [^{18}F]LSN3316612 was carried out in similar manner but with modifications in HPLC mobile phases of MeOH:aqueous ammonium formate (10 mM) (72.5:27.5, by vol.) at 4.0 mL/min.

Plasma free fraction determination

The monkey plasma free fraction (f_p) of [^{11}C]LSN3316612 or [^{18}F]LSN3316612 was measured by ultrafiltration through membrane filters (Centrifree; Millipore), as previously described (55).

PET imaging and quantification

PET monkey scans.—A rhesus monkey (male, 9.8 kg, age 19) was anesthetized with ketamine (10 mg/kg, i.m.) and then maintained in anesthesia with 1.5% isoflurane and 98.4% O₂ while undergoing two PET scan sessions with [^{11}C]LSN3316612. Thus, at baseline, [^{11}C]LSN3316612 (163 MBq; 86 GBq/ μmol) was injected intravenously as a bolus. On the same day about 3 h later, thiamet G (10 mg/kg) was administered intravenously at 45 min before [^{11}C]LSN3316612 (218 MBq; 43 GBq/ μmol). Another rhesus monkey (male, 12.2 kg, age 14) was imaged with [^{18}F]LSN3316612 in a similar way. At baseline, [^{18}F]LSN3316612 (207 MBq; 39 GBq/ μmol) was injected intravenously as a bolus. Under enzyme-preblocked condition, thiamet G (10 mg/kg) was administered intravenously at 45 min before [^{18}F]LSN3316612 (251 MBq; 29 GBq/ μmol) in the same monkey two weeks later.

In each experiment, PET images of brain were acquired with a microPET Focus 220 scanner (Siemens Medical Solution) for 120 min with scan durations ranging from 30 s to 5 min. The position of the head was fixed using a stereotaxic frame. Electrocardiogram, body temperature, heart and respiration rates were monitored throughout the experiment. Arterial blood samples were drawn for radiometabolite analysis and determination of a radiometabolite-corrected arterial input function.

Images were reconstructed using Fourier rebinning plus two-dimensional filtered back-projection. PET images were co-registered to a standardized monkey magnetic resonance imaging (MRI) template using Statistical Parametric Mapping - SPM5 (Wellcome Trust

Centre). A set of 34 predefined brain regions of interest from the template were then applied to the co-registered PET image to obtain regional decay-corrected time-activity curves. All PET images were corrected for attenuation and scatter. Cerebellar gray matter, which is not included in the template, was delineated semiautomatically using isocontour regions of interest. Uptake of radioactivity in each region of interest was expressed as SUV.

PET human scans.—Eight healthy volunteers participated in brain PET scans (age 35 ± 9 y; weight 75 ± 18 kg, table S2). All subjects were free of medical or psychiatric illness, as determined by medical history, physical examination, electrocardiogram, urinalysis including drug screening and laboratory blood test. Injected [^{18}F]LSN3316612 activity was 189 ± 3 MBq, with molar activity at the time of injection of 54.6 ± 1.32 GBq/ μmol , and an associated mass dose 0.051 ± 0.019 nmol/kg. All healthy subjects underwent brain PET scans for 180 min, using a Siemens mCT scanner (Siemens Medical Solution) with concurrent arterial blood sampling. A computed tomography (CT) scan was acquired before radioligand injection for attenuation correction. Arterial blood sampling was performed continuously using an automatic blood sampler (Veenstra PBS-101, Commerce) for the first 10 min with three interruptions for manual samples, followed by all discrete manual samples thereafter. Radiometabolite analysis (54) was performed on all manual samples as described above.

For each subject, a frame-based motion correction was applied to the dynamic PET image. Individual frames were aligned to the mean image created by averaging all frames of the dynamic scan. For spatial normalization, mean image of the dynamic scan was co-registered to an MRI template in the MNI (Montreal Neurological Institute) space. The transformation information was then used to reorient all frames of the dynamic scan into the MNI space. Regional time-activity curves were obtained by applying a set of predefined regions of interest in the MNI space (Automated Anatomical Labeling - AAL template) (56) to the spatially normalized images. Brain uptake was expressed as SUV.

PET data analysis.—Total distribution volumes (V_T) (57) were estimated for different regions by two tissue compartmental modeling with PET brain time-activity curves and the measured radiometabolite-corrected arterial input function. Parametric images were processed by Logan graphical analysis (58). The temporal stabilities of V_T in cerebellum and other regions in the baseline and preblocking experiments were assessed by estimating V_T from progressively time-truncated data sets in 10-min increments. The PET data analysis was performed using PMOD (PMOD Technologies Ltd.).

Statistical analysis

All data were expressed as means $\pm$ SEM, as indicated in the figure or table legends. Statistical analysis was completed with GraphPad Prism 8.3.0. *P*-value in fig. S10 was obtained with non-paired, one-sided and unequal variance *t*-test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Autoradiography of $[^3\text{H}]\text{LSN3316612}$ binding to brain tissue.

$[^3\text{H}]\text{LSN3316612}$ (5 nM) binding to OGA (left columns) or non-displaced bindings after thiamet G blocking (20 μM , right columns) in brain sections of (A) monkey ($n = 1$) and (B) human ($n = 1$). Brain regions are Cb = cerebellum; Cd = caudate nucleus; Hp = hippocampus; Hy = hypothalamus; Ns = substantia nigra; PFC = prefrontal cortex; Pu = putamen; and St = striatum.

Fig. 2. Ex vivo distribution of LSN3316612 in rat brain.

(A) Tissue distribution and plasma concentrations of LSN3316612 over time. (B) Thiamet G blockade of LSN3316612 receptor occupancy in rats. LSN3316612 (10 μ g/kg) was administered to rats intravenously. Data are mean $\pm$ SEM from $n = 3-4$ male rats per treatment group.

Fig. 3. Radiosyntheses of $[^{11}\text{C}]\text{LSN3316612}$ and $[^{18}\text{F}]\text{LSN3316612}$.

^{11}C -Labeling is by palladium-mediated $[^{11}\text{C}]$ carbon monoxide insertion and $[^{18}\text{F}]$ -labeling by aromatic nucleophilic substitution with $[^{18}\text{F}]$ fluoride ion. Red indicates that ^{11}C and ^{18}F are radioactive.

Fig. 4. PET imaging of $[^{11}\text{C}]\text{LSN3316612}$ and $[^{18}\text{F}]\text{LSN3316612}$ in monkey.

(A) Whole brain time-activity curves in a rhesus monkey administered $[^{11}\text{C}]\text{LSN3316612}$ at baseline and after preblocking of OGA with thiamet G (10 mg/kg, i.v.). (B) Summed brain PET images (0–120 min) after injection of $[^{11}\text{C}]\text{LSN3316612}$ at baseline (top row) and after preblocking of OGA with thiamet G (10 mg/kg, i.v.) (bottom row). (C) Whole brain time-activity curves in a rhesus monkey administered $[^{18}\text{F}]\text{LSN3316612}$ at baseline and after preblocking of OGA with thiamet G (10 mg/kg, i.v.). (D) Summed brain PET images (0–120 min) after injection of $[^{18}\text{F}]\text{LSN3316612}$ at baseline (top row) and after preblocking of OGA with thiamet G (10 mg/kg, i.v.) (bottom row). Images in (B, D) are: left, coronal scans; middle, sagittal scans; right, transaxial scans.

Fig. 5. PET imaging of $[^{18}\text{F}]\text{LSN3316612}$ in human subjects.

(A) Representative brain region PET time-activity curves in a healthy human subject ($n = 8$) administered $[^{18}\text{F}]\text{LSN3316612}$ at baseline. (B) Representative derived parametric (V_T) human brain images (0–180 min) from the injection of $[^{18}\text{F}]\text{LSN3316612}$ at baseline (bottom row) and the corresponding MRI images (top row) (left: coronal scans; middle: sagittal scans; right, transaxial scans). (C) Unchanged $[^{18}\text{F}]\text{LSN3316612}$ in whole blood and in plasma after intravenous injection of $[^{18}\text{F}]\text{LSN3316612}$ into a human subject. The plasma curve represents the radiometabolite-corrected arterial input function. (D) Normalized regional distribution volumes (V_T) as a function of duration of image acquisition after $[^{18}\text{F}]\text{LSN3316612}$ injection at baseline in human subjects. V_T was normalized to values at 180 min.

Table 1.

Properties of LSN3316612.

Property	Optimal tracer space	LSN3316612
OGA Enzyme IC_{50} (nM)	<10	1.9 ± 0.8
Measured brain free fraction	>0.01	0.11 (rat)
MW (Da)	<500	364.44
HBD	<3	1
pK_a	<9.5	10.57
$TPSA$ ($\text{\AA}^2$)	<90	66.3 ^a
$\log P(\log D_{7.4})$	2-3	3.05 (2.90)
MRP1 efflux ratio	<3	0.85
BCRP efflux ratio	<3	1.00

^aCalculated with ChemDraw Professional 16.0.

Table 2.

Summary of [^3H]LSN3316612 binding affinity (K_d) and OGA concentration (B_{max}) values in native tissue.

Tissue	K_d (nM)	B_{max} (nM)
Rat cortex	3.6 ± 0.8	45.9 ± 3.4
Rat striatum	3.3 ± 2.9	32.0 ± 9.6
Rat hippocampus	4.8 ± 3.7	32.6 ± 9.8
Rat cerebellum	1.3 ± 0.4	6.6 ± 0.6
Cynomolgus cortex	1.9 ± 1.0	7.4 ± 1.1
Cynomolgus striatum	3.3 ± 1.9	12.7 ± 2.5
Cynomolgus hippocampus	3.4 ± 0.7	8.5 ± 0.6
Cynomolgus cerebellum	2.1 ± 1.3	4.8 ± 0.9
Human cortex	2.2 ± 0.4	16.5 ± 1.0
Human striatum	1.6 ± 0.4	36.7 ± 2.6
Human hippocampus	2.6 ± 0.7	9.8 ± 0.8
Human cerebellum	0.7 ± 1.5	1.7 ± 0.9

Mean $\pm$ SEM for $n = 3$.