Synthesis and Preliminary Biological Evaluation of Novel α-Synuclein PET Probes

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Abstract

Abstract Abnormally aggregated α-synuclein constitutes the principal pathological hallmark of Parkinson's disease, and the development of α-synuclein PET imaging agents can facilitate early diagnosis and treatment of this disorder. This study designed and synthesized a novel α-synuclein probe, 18F-YM (2-((3-fluoro-18F-benzyl)thio)-6-(3-fluoropropoxy)benzothiazole), employing a copper-mediated radiofluorination labeling strategy, and evaluated the in vivo performance of this novel probe through PET imaging in A53T mice and normal mice. The study demonstrated that the synthesis time for 18F-YM was approximately 60 minutes, the uncorrected yield exceeded 10%, the specific activity was 8.5 GBq/ mol, and the radiochemical purity was greater than 95%. Small-animal PET imaging results revealed that the benzothiazole compound 18F-YM accumulated in the brains of α-synuclein-expressing A53T mice, with uptake values significantly higher than the corresponding values in normal mice. Quantitative analysis showed that at 30 minutes post-injection, the brain uptake values in A53T mice and normal mice were 2.35$\pm$0.06$\pm$$0.15 %ID/g, respectively. In vitro and in vivo autoradiography and pathological analysis confirmed that 18F-YM could identify aggregated α-synuclein in brain regions such as the thalamus, substantia nigra, and striatum of A53T mice. In vivo biodistribution studies demonstrated that 18F-YM was rapidly cleared from the brains of normal mice. This indicates that 18F-YM exhibits low non-specific binding in the brain, which is conducive to obtaining images with good contrast. Preclinical studies preliminarily indicate that the benzothiazole compound, 18F-YM, possesses favorable imaging performance and may serve as a potential α-synuclein PET probe.

Full Text

Synthesis and Preliminary Biological Evaluation of a Novel PET Tracer for α-Synuclein Imaging

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Abstract

Background: Accumulation of α-synuclein is a major hallmark of Parkinson's disease. The development of PET tracers to visualize aggregated α-synuclein is useful for early diagnosis and treatment of Parkinson's disease.

Purpose: We prepared and labeled a small molecule compound based on benzothiazole scaffolds, 2-((3-fluorobenzyl)thio)-6-(3-[fluorine-18]propoxy)benzo[d]thiazole, denoted as ^{18}F-YM, using Cu(II)-mediated radiofluorination methods. The imaging properties of the tracer were primarily evaluated through PET imaging in A53T mice and normal mice. Additionally, the imaging properties of the probe were investigated through biodistribution experiments as well as ex vivo autoradiography and pathological analysis.

Methods: Through chemical synthesis, compounds Sn-YM and ^{19}F-YM were obtained. Compound Sn-YM was labeled with ^{18}F using the organotin fluoride method, and the resulting product ^{18}F-YM was verified by high performance liquid chromatography. The in vitro stability and octanol–water partition coefficient of ^{18}F-YM were determined. Finally, small animal microPET imaging was used to assess the affinity of ^{18}F-YM for α-synuclein, and autoradiography, pathological analysis, and biodistribution were used to validate the results of small animal microPET imaging.

Results: The ^{18}F-labeled small molecule compound was prepared in nearly 1 hour and obtained with an undecayed yield greater than 10% and radiochemical purity greater than 95%. In vivo PET imaging revealed that significantly more radioactivity was detected in the brain of A53T mice than in normal mice after administration of ^{18}F-YM. Quantitative analysis showed that the uptake values in the brain of A53T mice and normal mice were 2.35$\pm$0.06$\pm$0.15 %ID/g, respectively. Furthermore, ex vivo autoradiography and histological examination confirmed the detection of aggregated α-synuclein in the thalamus, substantia nigra, and striatum using ^{18}F-YM. A biodistribution study in normal mice found that ^{18}F-YM was quickly cleared from the brain, indicating low non-specific binding of ^{18}F-YM in the brain, which allowed for obtaining good contrast images.

Conclusion: The preclinical study demonstrated that the benzothiazole analog ^{18}F-YM possesses preferable imaging properties and may be a new candidate for α-synuclein PET imaging.

Keywords: α-synuclein, Parkinson's disease, PET imaging

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder in the elderly, with incidence showing a trend toward younger populations. It is estimated that by 2030, the number of PD patients in China will reach nearly 5 million, accounting for half of the global patient total [1-3]. Abnormal aggregation and fibrillation of α-synuclein constitute the core pathological feature of PD [4-6]. α-synuclein is a soluble protein composed of 140 amino acids located in presynaptic nerve terminals that, under physiological conditions, controls synaptic vesicle transport and regulates dopamine synthesis. Under induction by viral or genetic factors, α-synuclein misfolds, and the resulting oligomers aggregate into insoluble fibrous proteins in neurons, leading to selective functional defects in synaptic vesicles, reduced release of neurotransmitters such as dopamine, and ultimately dopaminergic neuron death. As the disease progresses, the content of abnormally aggregated α-synuclein gradually increases, and its distribution gradually spreads to the cortex. Studies have confirmed that inhibiting abnormal α-synuclein aggregation using antibodies or small molecules is an effective strategy for treating PD. Therefore, α-synuclein serves as a key biomarker for the diagnosis and treatment of Parkinson's disease [7-8].

Currently, clinical diagnosis of PD primarily relies on enzyme-linked immunosorbent assay to measure total α-synuclein content in cerebrospinal fluid for early diagnosis. While this method has moderate sensitivity (61-94%), its specificity is low (25-64%). Moreover, lumbar puncture sampling of cerebrospinal fluid is invasive and can cause side effects such as intracranial hypotension syndrome, cerebral herniation, and postoperative infection. Peripheral blood detection, though convenient to perform, has sensitivity and specificity of only 70% and 50%, respectively, and most (>99%) of α-synuclein in plasma remains within red blood cells, which may interfere with results [9-10].

Positron emission tomography (PET) is the most mature molecular imaging technology in clinical application, offering advantages including non-invasiveness, real-time imaging, trace-level detection, excellent spatial resolution, high sensitivity, and quantitative analysis capability. PET has been widely used in research on neuropsychiatric diseases such as PD, Alzheimer's disease, and schizophrenia. Specific probes are essential prerequisites for PET to contribute to precision medicine. Using specific PET probes to detect α-synuclein expression levels in the living human brain will facilitate early diagnosis of PD and evaluation of candidate therapeutic drug efficacy [11-12].

Benzothiazole derivative thioflavin T is the most commonly used fluorescent dye for α-synuclein in post-mortem brain tissue [13]. Various ^{18}F-labeled benzothiazole small molecule compounds have been developed for α-synuclein PET imaging. ^{18}F-BF227 was the first α-synuclein PET probe; in vitro experiments showed high affinity for α-synuclein, but no significant difference in brain uptake between PD model mice and normal mice [14]. ^{18}F-2FBox showed approximately 4-fold higher in vitro affinity than ^{18}F-BF227, yet still could not detect α-synuclein expression in PD model mouse brains [15]. ^{18}F-C05-05 could detect pre-implanted α-synuclein in monkey striatum, but also showed high non-specific binding signals in brain white matter regions [16]. ^{18}F-F0502B can recognize protein aggregates in macaque brains pre-injected with α-synuclein; however, its uptake value in non-primate animal brains is moderate, and its imaging performance requires further evaluation [17,18].

In addition to dye analogs such as thioflavin T, radiolabeled small molecule inhibitors have also been used to detect α-synuclein aggregation. For example, anle138b can bind to proteins in PD animal models and prevent α-synuclein aggregation [19-21]. Clinical trials have shown that anle138b has favorable safety, tolerability, and pharmacokinetic properties. High uptake of anle138b analog ^{11}C-MODAG-001 was observed in brain regions pre-injected with α-synuclein fibrils in rats and pigs. Although this probe does not have high selectivity for α-synuclein, the study suggests that small molecule inhibitors could serve as lead compounds for α-synuclein PET probes [22-23].

It has also been reported that small molecule inhibitors containing thioether linkers can significantly reduce pathological α-synuclein aggregation [24-26]. Therefore, benzothiazole-modified thioether compounds may be candidate PET probes targeting α-synuclein. In view of this, we prepared a benzothiazole small molecule compound, 2-((3-fluoro[^{18}F]benzyl)thio)-6-(3-fluoropropoxy)benzothiazole, abbreviated as ^{18}F-YM, and performed ^{18}F labeling. The biological properties of the probe were preliminarily evaluated using small animal PET imaging combined with biodistribution experiments, ex vivo autoradiography, and pathological analysis.

1.1 General Methods

All reagents were commercial products used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian MR spectrometer. Mass spectrometry was performed using a Waters ZQ2000 mass spectrometer. The labeled product was purified by semi-preparative high performance liquid chromatography (HPLC) (Waters) equipped with a Waters 2487 dual-wavelength absorbance detector and a Radiomatic 610TR flow scintillation analyzer (PerkinElmer). The stationary phase was a semi-preparative C18 HPLC column (5 m, 250$×$10 mm, Phenomenex). The mobile phase was changed from 60% solvent A (0.1% trifluoroacetic acid in water) and 40% solvent B

(0.1% trifluoroacetic acid in acetonitrile) at 0 minutes to 5% solvent A and 95% solvent B at 10 minutes. The flow rate was 5 mL/min.

Analytical HPLC (Waters) equipped with a Waters 2487 dual-wavelength absorbance detector and a Radiomatic 610TR flow scintillation analyzer (PerkinElmer) was used for quality control of the labeled product and cold compound. The stationary phase was an analytical C18 HPLC column (5 m, 250$×$4.6 mm, Phenomenex). The mobile phase was changed from 60% solvent A (0.1% trifluoroacetic acid in water) and 40% solvent B (0.1% trifluoroacetic acid in acetonitrile) at 0 minutes to 5% solvent A and 95% solvent B at 10 minutes. The flow rate was 1 mL/min.

1.2 Animals

C57BL/6J mice and PDGF-hα-Synuclein A53T (referred to as A53T mice) were purchased from Cavens Laboratory Animal Co. and Beijing HFK Bioscience Co., respectively. Mice were routinely raised until 6 months of age for PET imaging studies. All animal experiments were approved by the Animal Welfare Committee of Jiangsu Institute of Nuclear Medicine and complied with national legal regulations.

1.3 Chemical Synthesis

The synthetic route for the compounds is shown in [Figure 1: see original paper].

2-bromobenzothiazol-6-ol (1): 2-bromo-6-methoxybenzothiazole (25 mmol) was dissolved in 40 mL dichloromethane, then boron tribromide (100 mmol) was slowly added dropwise under argon in an ice bath and the mixture was reacted overnight at room temperature. The mixture was added dropwise to ice water, and after filtration, a white solid was obtained (5.2 g, 91.2%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.39 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 8.8, 2.5 Hz, 1H). MS(ESI) m/z 230 $[M+H]^+$.

2-bromo-6-(3-fluoropropoxy)benzothiazole (2): To a solution of 2-bromobenzothiazol-6-ol (10 mmol) in DMF (30 mL) was added sodium carbonate (30 mmol), and the mixture was reacted at room temperature for 10 minutes. 1-fluoro-3-iodopropane (15 mmol) was added dropwise. The mixture was reacted overnight at 100°C. After cooling, the mixture was extracted with ethyl acetate. The combined organic layers were dried with anhydrous sodium sulfate. After solvent removal, a white solid was obtained (2.35 g, 81.3%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 (d, J = 8.9 Hz, 1H), 7.71 (d, J = 2.6 Hz, 1H), 7.13 (dd, J = 8.9, 2.6 Hz, 1H), 4.66 (t, J = 5.9 Hz, 1H), 4.58 (t, J = 5.9 Hz, 1H), 4.13 (t, J = 6.3 Hz, 2H), 2.14 (dp, J = 25.7, 6.1 Hz, 2H). MS(ESI) m/z 290 $[M+H]^+$.

6-(3-fluoropropoxy)benzothiazole-2-thiol (3): At room temperature, sodium hydrosulfide (4 mmol) was added to a solution of 2-bromo-6-(3-fluoropropoxy)benzothiazole (2 mmol) in DMF/H_2O (10.0 mL/5.0 mL). The

mixture was stirred at 60°C for 1 hour. The mixture was extracted with ethyl acetate and water. The organic layer was washed again with brine and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography with hexane/ethyl acetate to obtain a white solid (0.42 g, 87.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.62 (s, 1H), 7.36 (d, J = 2.5 Hz, 1H), 7.21 (d, J = 8.9 Hz, 1H), 7.00 (dd, J = 8.8, 2.5 Hz, 1H), 4.66 (t, J = 5.9 Hz, 1H), 4.54 (t, J = 5.9 Hz, 1H), 4.06 (t, J = 6.3 Hz, 2H), 2.10 (dp, J = 25.8, 6.1 Hz, 2H). MS(ESI) m/z 244 $[M+H]^+$.

2-((3-fluorobenzyl)thio)-6-(3-fluoropropoxy)benzothiazole (^{19}F-YM): 6-(3-fluoropropoxy)benzothiazole-2-thiol (1.0 mmol) was dissolved in 15 mL DMF. Sodium hydroxide (3.0 mmol) and 1-(bromomethyl)-3-fluorobenzene (1.0 mmol) were added sequentially. After stirring at room temperature for 5 hours, the pH of the mixture was adjusted to 9-10 with saturated sodium carbonate solution. The reaction mixture was extracted with ethyl acetate and water. The organic layer was washed with brine and dried with anhydrous sodium sulfate. After solvent removal, a colorless oily liquid was obtained (322 mg, 61.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (d, J = 8.9 Hz, 1H), 7.63 (d, J = 2.6 Hz, 1H), 7.43–7.29 (m, 3H), 7.15–7.05 (m, 2H), 4.68 (t, J = 5.9 Hz, 1H), 4.62 (s, 2H), 4.56 (t, J = 5.9 Hz, 1H), 4.11 (t, J = 6.3 Hz, 2H), 2.12 (dp, J = 25.8, 6.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.67, 162.86, 161.25, 156.35, 147.55, 140.27, 140.19, 136.67, 130.97, 130.89, 125.64, 125.61, 122.24, 116.37, 116.15, 116.02, 114.95, 114.75, 106.13, 82.08, 80.47, 64.66, 64.61, 36.52, 36.50, 30.27, 30.07. MS(ESI) m/z 352 $[M+H]^+$.

6-(3-fluoropropoxy)-2-((3-iodobenzyl)thio)benzothiazole (4): In a round-bottom flask, 6-(3-fluoropropoxy)benzothiazole-2-thiol (1.0 mmol), sodium hydroxide (3.0 mmol), and 1-(bromomethyl)-3-iodobenzene (1.0 mmol) were dissolved in 15 mL DMF. The mixture was refluxed at 85°C for 2 hours. After extraction with ethyl acetate and water, the organic layer was collected. The solvent was removed under reduced pressure to obtain a colorless oily liquid (337 mg, 59.1%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 (d, J = 1.7 Hz, 1H), 7.77 (d, J = 8.9 Hz, 1H), 7.65–7.61 (m, 2H), 7.50 (dt, J = 7.8, 1.3 Hz, 1H), 7.13 (t, J = 7.8 Hz, 1H), 7.08 (dd, J = 8.9, 2.6 Hz, 1H), 4.66 (t, J = 5.9 Hz, 1H), 4.57 (d, J = 10.6 Hz, 3H), 4.12 (t, J = 6.3 Hz, 2H), 2.13 (dp, J = 25.8, 6.1 Hz, 2H). MS(ESI) m/z 460 $[M+H]^+$.

6-(3-fluoropropoxy)-2-((3-(tributylstannyl)benzyl)thio)benzothiazole (Sn-YM): In a flask, 6-(3-fluoropropoxy)-2-((3-iodobenzyl)thio)benzothiazole (0.4 mmol), tetrakis(triphenylphosphine)palladium (0.024 mmol), palladium acetate (0.024 mmol), lithium chloride (0.606 mmol), hexabutylditin (0.40 mmol), and toluene (40 mL) were added. The mixture was refluxed at 110°C for 6 hours. After filtration and removal of solvent under vacuum, the residue was purified by silica gel chromatography. After removal of solvent under reduced pressure, a colorless oily liquid was obtained (122 mg, 49.8%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.75 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 2.5 Hz,

1H), 7.52 (s, 1H), 7.37 (d, J = 7.4 Hz, 1H), 7.29 (dt, J = 14.3, 7.0 Hz, 2H), 7.07 (dd, J = 8.9, 2.6 Hz, 1H), 4.65 (t, J = 5.9 Hz, 1H), 4.57 (d, J = 3.8 Hz, 3H), 4.11 (t, J = 6.2 Hz, 2H), 2.12 (dp, J = 24.9, 6.1 Hz, 2H), 1.50–1.39 (m, 6H), 1.22 (h, J = 7.3 Hz, 6H), 1.03–0.92 (m, 6H), 0.79 (t, J = 7.3 Hz, 9H). MS(ESI) m/z 624 $[M+H]^+$.

1.4 ^{18}F Labeling

^{18}F-YM was prepared according to literature methods [27] ([Figure 1: see original paper]). Briefly, ^{18}F ion was adsorbed on a QMA cartridge and eluted into a reaction vial using eluent (500 L of 20 mg/mL potassium triflate aqueous solution + 50 L of 1 mg/mL potassium carbonate aqueous solution). One milliliter of ultra-dry acetonitrile was added, and water was removed by azeotropic distillation at 110°C, followed by addition of 1.5 mL ultra-dry acetonitrile for a second azeotropic distillation. Three milligrams of labeling precursor (Sn-YM) dissolved in 0.6 mL ultra-dry N,N-dimethylacetamide (DMA) was injected into the reaction vial, followed by addition of 100 L of 1 M pyridine and 100 L of 0.2 M copper(II) triflate DMA solution. After mixing uniformly, the reaction was carried out at 140°C for 20 minutes. After cooling to room temperature, the product was purified using semi-preparative HPLC. The collected fraction was diluted with water, enriched by a C18 solid-phase extraction cartridge, eluted with ethanol, diluted with saline, and filtered through a sterile membrane to obtain the product ^{18}F-YM. A sample was taken to determine the radiochemical purity and specific activity of the labeled product by HPLC.

1.5 In Vitro Stability Study

Three point seven MBq of ^{18}F-YM solution was incubated with mouse plasma and PBS at 37°C for 2 hours. The radiochemical purity of ^{18}F-YM in mouse plasma and PBS was determined using methods reported in the literature [28].

1.6 Determination of Oil-Water Partition Coefficient

The lipophilicity (log P) of the labeled product was determined using methods reported in the literature [29]. Three hundred seventy KBq of the ^{18}F-labeled compound was added to a test tube containing 0.8 mL n-octanol and 0.8 mL PBS (pH = 7.4). After stirring for 20 seconds, the mixture was centrifuged at 2000g for 2 minutes to separate the two layers. Samples from the n-octanol layer (0.1 mL) and PBS layer (0.5 mL) were collected and measured for radioactivity using a gamma counter (PerkinElmer). The log P value was calculated based on the radioactivity ratio between the n-octanol and PBS layers. Then, 0.5 mL of the n-octanol layer was added to 0.3 mL n-octanol and mixed with 0.8 mL PBS. This equilibrium process was repeated 5 times until a constant log P value was obtained.

1.7 In Vitro Affinity Assay

Surface plasmon resonance (SPR) was used to test the affinity between α-synuclein protein and both thioflavin T and the cold compound ^{19}F-YM. The experimental method is briefly described as follows: α-synuclein protein solution was prepared at 50 µg/mL using sodium acetate buffer at pH 4.0. The α-synuclein protein was coupled to a CM5 chip (Cytiva) using an amine coupling kit, and uncoupled sites were blocked with ethanolamine. The blank channel was directly blocked with ethanolamine.

Thioflavin T and ^{19}F-YM DMSO aqueous solutions were prepared at concentrations ranging from 0.39 µM to 50 µM. These solutions were sequentially loaded onto the chip coupled with α-synuclein protein at a flow rate of 30 µL/min for 120 seconds. Dissociation was performed at 30 µL/min for 120 seconds. The affinity between compounds and α-synuclein protein was measured using a Biacore T100 SPR instrument (Cytiva).

1.8 PET Imaging

Ten-month-old C57BL/6J mice and A53T mice were anesthetized with isoflurane and placed in an Inveon Micro PET scanner (Siemens). After tail vein injection of 3.7 MBq ^{18}F-YM, dynamic scanning was performed for 30 minutes (10\$×1$minute, 5×2minutes$, 2×\$5 minutes, total 17 frames). Images were reconstructed using a 3D-OSEM algorithm. Regions of interest (ROIs) were drawn, and imaging data were processed [28].

1.9 Autoradiography

Autoradiography was performed using methods reported in the literature [30]. Immediately after PET imaging, mice were sacrificed and brains were dissected. Frozen sections with a thickness of 20 m were prepared at -80°C and placed on a phosphor imaging plate. The plate was then read using a Cyclone reader (USA). Quantitative analysis was performed using OptiQuant software.

1.10 Pathological Analysis

After radioactive decay, mouse brain slices were subjected to immunohistochemical staining using methods reported in the literature [29]. Briefly, α-synuclein polyclonal antibody (Proteintech Group, Inc) was incubated with mouse brain tissue slices at 4°C for 24 hours, followed by washing with PBS. Polymer IgG (Gene Tech) was then applied as a secondary antibody for 2 hours. Slices were observed using a fluorescence microscope (Olympus X81, Japan), and images were analyzed using ImageJ software (NIH Image).

1.11 Biodistribution Study

Eight-week-old normal C57BL/6J mice were sacrificed at 5, 15, 30, and 60 minutes after tail vein injection of 3.7 MBq ^{18}F-YM and dissected. Major organs

were weighed, and radioactivity in tissues was measured using a γ-counter. Results are expressed as %ID/g.

1.12 Statistical Analysis

Quantitative data are expressed as mean ± standard deviation. One-way ANOVA and t-tests were used to compare means. P values below 0.05 were considered statistically significant.

Results

2.1 Chemical Synthesis

The cold standard compound ^{19}F-YM was obtained in 61.1% yield. HPLC showed its purity to be greater than 90%.

2.2 Radiochemistry

The radiolabeled small molecule compound ^{18}F-YM was successfully obtained with an uncorrected yield of 13.25$±$1.16%. The total synthesis time was approximately 60 minutes. The specific activity of the tracer was 8.5 GBq/ mol, and the radiochemical purity was greater than 95% ([Figure 2: see original paper]).

2.3 In Vitro Stability Study

In vitro studies demonstrated that ^{18}F-YM has good stability; after incubation in PBS or mouse plasma at 37°C for 2 hours, no free $^{18}F^-$ was detected ([Figure 2: see original paper]).

2.4 Oil-Water Partition Coefficient

Generally, PET probes for the nervous system should have appropriate lipophilicity (log P = 1-3) to cross the blood-brain barrier (BBB) and enter the brain [31]. To investigate the lipophilicity of the probe, we determined its oil-water partition coefficient. The log P value of ^{18}F-YM was 2.80$±$0.06, which falls within the appropriate lipophilicity range, suggesting that ^{18}F-YM can cross the BBB to enter the brain and bind to target proteins.

2.5 In Vitro Affinity Assay

After data fitting and analysis, the affinity constants of α-synuclein protein for thioflavin T and ^{19}F-YM were 14.5 μM and 14.2 μM, respectively. This indicates that ^{19}F-YM has similar α-synuclein protein affinity to thioflavin T, a fluorescent dye commonly used in post-mortem examinations.

2.6 PET Imaging

The accuracy of PET imaging depends heavily on the specificity of probe binding to target proteins. Although in vitro affinity assays suggested that ^{19}F-YM may have relatively low affinity, in vitro studies may not accurately reflect in vivo compound behavior. For example, in vivo PET imaging showed that integrin-targeting peptides FBA-PGDLAVLA and FBA-KLDLHTLE, with IC_{50} values of 1 M and 15 M respectively, both accumulated in human melanoma xenografts after ^{18}F labeling, ranking second and fourth among 43 candidate peptides (26 of which had IC_{50} values less than 100 nM) [32]. Therefore, we used in vivo PET imaging to more accurately evaluate the imaging performance of the ^{18}F-labeled α-synuclein candidate probe in mouse models.

The A53T mouse model used in this study is currently the primary model for researching α-synuclein-related neurological diseases such as PD. At 6 months, A53T mice spontaneously express human α-synuclein A53T mutation. Similar to human Parkinson's disease, Lewy bodies composed of α-synuclein are highly expressed in brain regions such as the striatum, substantia nigra, and thalamus of A53T mice [33].

PET imaging showed that within 30 minutes of tail vein injection, brain uptake of ^{18}F-YM in A53T mice was nearly double that in normal mice. Representative PET images and brain radioactivity-time curves for normal (C57BL/6J) mice and A53T mice are shown in [Figure 3: see original paper]. After probe injection, the tracer rapidly entered the brain. At 2 minutes post-injection, whole-brain uptake values in A53T mice and normal mice were 5.40$±0.26±$0.25 %ID/g, respectively. The study also found that radioactivity in A53T mouse brains was significantly higher than in corresponding normal mice after tail vein injection of ^{18}F-YM. ROI analysis showed that at 30 minutes post-injection, brain uptake in A53T mice was 2.35$±0.06±$0.15 %ID/g). Notably, the brain uptake ratio between A53T mice and normal mice at 30 minutes post-injection was 1.7, which is significantly higher than the corresponding value for another benzothiazole analog, ^{18}F-C05-05 ((E)-1-^{18}F-3-((2-(4-(6-(methylamino)pyridin-3-yl)but-3-en-1-ynyl)benzothiazol-6-yl)oxy)propanol) (approximately 1.1), suggesting that the probe's targeting binding capability may be superior to ^{18}F-C05-05.

At 30 minutes post-injection, uptake values in the heart, liver, and kidneys of A53T mice were 1.85$±0.12±1.31±1.35±0.12±1.41±$0.76 %ID/g, suggesting that the probe is primarily metabolized through the liver and kidneys ([Figure 4: see original paper]).

2.7 Autoradiography and Pathological Analysis

Autoradiography of ^{18}F-YM in brains of A53T mice and normal mice is shown in [Figure 5: see original paper]. High radioactive uptake was detected in brain regions including the thalamus, substantia nigra, and striatum of A53T mice. Pathological analysis confirmed the presence of large amounts of abnormally aggregated α-synuclein in these brain regions. In contrast, no high radioactive

uptake was detected in the thalamus, substantia nigra, or striatum of normal mice, and no α-synuclein expression was observed in corresponding brain regions at the same time ([Figure 5: see original paper]), consistent with reported results [33]. Autoradiography and pathological analysis results confirmed that ^{18}F-YM binds highly to α-synuclein.

2.8 Biodistribution Study

Biodistribution data for ^{18}F-YM in normal mice are shown in . The results showed that ^{18}F-YM initially accumulated in mouse brain (2.34$±0.41±$0.83 %ID/g at 60 minutes post-injection. The moderate brain uptake of ^{18}F-YM may be related to the compound's appropriate lipophilicity. Over time, ^{18}F-YM was gradually cleared from the brain. Notably, the brain uptake ratio at 5 minutes and 60 minutes post-injection was nearly 5, which is significantly higher than corresponding values for other reported α-synuclein candidate PET tracers such as chalcone analog ^{18}F-FHCL-1 [34] (approximately 1.4) and bisquinoline analog ^{18}F-BQ-2 [35] (approximately 1.1). This indicates that ^{18}F-YM has low non-specific binding in the brain, enabling acquisition of images with good contrast.

At 30 and 60 minutes post-injection, probe uptake in the liver and kidneys was 3.46$±0.37±0.28±0.58±$0.17 %ID/g, respectively. This indicates that ^{18}F-YM is primarily excreted through the renal and hepatobiliary systems. Similar to other reported α-synuclein probes, radioactivity in the liver was higher than in the kidneys at 30 minutes post-injection, which may be related to the probe's lipophilicity. These data further demonstrate that ^{18}F-YM has favorable pharmacokinetic properties in vivo, providing strong support for subsequent studies.

Preclinical studies have preliminarily shown that ^{18}F-YM exhibits good α-synuclein imaging characteristics in rodents, including high targeting specificity, low non-specific uptake, and favorable pharmacokinetics. Further structural optimization of this compound may accelerate the development of specific α-synuclein PET probes.

Author Contributions

Ce Wang: experimental design, chemical synthesis, animal experiments, data analysis, and manuscript writing. Donghui Pan: experimental data analysis. Siyi Tan: animal experiments and immunohistochemistry. Lizhen Wang: experimental data organization. Xinyu Wang: guidance on experimental data analysis. Junjie Yan: chemical synthesis design and guidance. Chongyang Chen: guidance on animal experiments. Lianghua Zou and Yuping Xu: overall experimental design guidance and manuscript revision.

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Note: Figure translations are in progress. See original paper for figures.