Comparison of New Tau PET-Tracer Candidates With $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807

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Abstract
Early clinical results of two tau tracers, $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807, have recently been reported. In the present study, the biodistribution, radiometabolite quantification, and competition-binding studies were performed in order to acquire comparative preclinical data as well as to establish the value of T808 and T807 as benchmark compounds for assessment of binding affinities of eight new/other tau tracers. Biodistribution studies in mice showed high brain uptake and fast washout. In vivo radiometabolite analysis using high-performance liquid chromatography showed the presence of polar radiometabolites in plasma and brain. No specific binding of $[^{18}\text{F}]$T808 was found in transgenic mice expressing mutant human P301L tau. In semiquantitative autoradiography studies on human Alzheimer disease slices, we observed more than 50% tau selective blocking of $[^{18}\text{F}]$T808 in the presence of 1 µmol/L of the novel ligands. This study provides a straightforward comparison of the binding affinity and selectivity for tau of the reported radiolabeled tracers BF-158, BF-170, THKS105, lansoprazole, astemizole, and novel tau positron emission tomography ligands against T807 and T808. Therefore, these data are helpful to identify structural requirements for selective interaction with tau and to compare the performance of new highly selective and specific radiolabeled tau tracers.

Keywords
molecular imaging of neurodegenerative diseases, Alzheimer, biomarker, PET imaging

Introduction
In spite of extensive knowledge about epidemiology, histopathological features, and genetics of Alzheimer disease (AD), only symptomatic treatment is available at this moment.¹ Therefore, there is a great need for an efficient treatment that can be initiated in an early phase of the disease. Definite diagnosis can still only be made postmortem on the basis of two pathological hallmarks: senile plaques (SP) and neurofibrillary tangles (NFTs).² Both SP and NFTs have been targeted by positron emission tomography (PET) ligands that can serve as diagnostic biomarkers, as this is a sensitive in vivo method to visualize and quantify AD-specific pathological changes in the living brain.³

Much effort has been invested in the development of PET tracers that specifically bind to amyloid beta sheets (Aβ). While a negative scan substantially limits the chance for...
development of AD and greatly influences clinic decision making, a positive amyloid PET scan is, by itself, not sufficient for a positive diagnosis for AD. In contrast, even at late stages of AD, the burden of NFTs is closely related to the clinical symptoms of AD. This likely implicates that a PET tracer that selectively targets tau aggregates could be a more relevant biomarker for AD conversion and for neurodegenerative tauopathies.

Several aspects make the development of tau imaging agents challenging. Through alternative splicing of the microtubule-associated protein tau gene, six isoforms of tau are expressed in the adult human central nervous system, giving rise to two sets of isoforms: those with three (3R) and those with four (4R) microtubule-binding domains, the latter one being more efficient in stabilizing microtubules. Normally, there is an equal ratio of both isoforms, but under pathological circumstances, several tauopathies express different isoform ratios with diverse morphologies. Due to these structural differences, it may be difficult to develop a tau-specific tracer, with similar affinity for every phenotype. Furthermore, tau is subjected to many posttranslational modifications, which may result in conformational changes in the aggregates, potentially leading to different binding affinities of tau ligands. Additionally, tau tracers need to be highly selective, as in AD, tau aggregates are coexistent with Aβ deposits but occur in concentrations ~5 to 20 times lower than Aβ aggregates. Despite many hurdles, several tau PET imaging agents have already been reported. For six of those agents, 2-(1-(6-((2-

Materials and Methods

General

The precursor and authentic reference material for [18F]T807 and [18F]T808 and the nonradioactive reference material for [18F]THK-5105 were synthesized according to the published procedures. All other chemicals and reagents were purchased from commercial sources and used without further purification. The TLC plates (Macherey-Nagel, Düren, Germany) were developed using mixtures of ethyl acetate and heptane or dichloromethane and methanol as mobile phase. Automated flash column chromatography was performed using silica cartridges (GraceResolv 12 g, Grace, Deerfield, Illinois) on a Reveleris X2 flash system (Grace) equipped with an evaporative light-scattering detector and ultraviolet (UV) detector at 254 nm. H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance II spectrometer (400 MHz, 5 mm probe; Fällanden, Switzerland) using deuterated methanol (MeOD-d4) or deuterated dimethyl sulfoxide (DMSO-d6) as indicated. Chemical shifts are reported in parts per million downfield from tetramethylsilane (δ = 0). Coupling constants are reported in hertz (Hz). Splitting patterns are defined by s (singlet), d (doublet), dd (double doublet), dt (double triplet), t (triplet), q (quartet), or m (multiplet). Exact mass measurements were performed on an ultra-high resolution time-of-flight mass spectrometer (maXis impact LC/MS, Bruker, Bremen, Germany) equipped with an orthogonal electrospray ionization (ESI) interface. Acquisition and processing of data were conducted using HysStar and Compass DataAnalysis (version 3.2; Bruker), respectively. Calculated monoisotopic
mass values were obtained using MarvinSketch (version 6.1.0; ChemAxon http://www.chemaxon.com). High-performance liquid chromatography (HPLC) analysis was performed on a LaChrom Elite HPLC system (Hitachi, Darmstadt, Germany) connected to a UV detector set at 254 nm. For analysis of radiolabeled compounds, the HPLC eluate, after passing through the UV detector, was led over a 3-inch sodium iodide activated with thallium NaI(Tl) scintillation detector connected to a single-channel analyzer (GABI box; Raytest, Straubenhardt, Germany). Data were acquired and analyzed using GINA Star (Raytest) data acquisition systems. Quantification of radioactivity in samples of biodistribution and radiometabolic studies was performed using an automated γ-counter equipped with a 3-inch NaI(Tl) well crystal coupled to a multi-channel analyzer, mounted in a sample changer (Wallac 2480 Wizard 3g; Wallac, Turku, Finland). The values are corrected for background radiation, physical decay, and counter dead time. Alzheimer disease slices of 10 μm thick from the visual cortex of a 68-year-old woman in the latest Braak stage (V-VI) were provided by University Hospitals Leuven (Neurology Department, Leuven, Belgium) after approval from the local ethics committee. Animals were housed in individually ventilated cages in a thermoregulated (≈22°C), humidity-controlled facility under a 12-hour–12-hour light–dark cycle, with access to food and water ad libitum. All animal experiments were conducted according to the Belgian code of practice for the care and the use of animals, after approval from the university animal ethics committee.

Chemistry

2-(4-Nitrophenyl)benzo[d]thiazole (TAU1). Compound was synthesized according to a described procedure. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) (ESI) calculated for C_{13}H_{19}FNS [MH\(^+\)] 320.0434, measured 320.0441; ¹H NMR (400 MHz, MeOD-d4) δ 7.29 (t, 2H, J = 8.7, Ar), 7.44 (t, 2H, J = 7.8, Ar), 7.54 (t, 2H, J = 7.8, Ar), 8.01 (dd, J = 7.9 and 4.0 and 2.0, 2H, Ar), 8.14 (dd, J = 8.6 and 5.5, 2H, Ar).

Methyl 2-(4-nitrophenyl)benzo[d]thiazole-6-carboxylate (TAU2). The compound was synthesized according to a described procedure. LC-HRMS (ESI) calculated for C_{14}H_{19}N_{2}O_{5}S [MH\(^+\)] 266.9991, measured 266.9994; ¹H NMR (400 MHz, DMSO-d6) δ 2.34 (s, 3H, OCH₃), 7.39 (dd, J = 8.8 and 2.0, 1H, Ar), 8.07 (d, J = 2.0, 1H, Ar), 8.17 (d, J = 8.8, 1H, Ar), 8.33 to 8.45 (m, 4H, Ar).

2-[4-(4-Fluorophenyl)-1,3-benzothiazole (TAU3). The compound was synthesized according to T807’s patent application using 4-(benzo[d]thiazol-2-yl)phenylboronic acid and 1-bromo-4-fluorobenzene as starting materials (Figure 1). LC-HRMS (ESI) calculated for C_{19}H_{15}FNS [MH\(^+\)] 306.0747, measured 306.0762; ¹H NMR (400 MHz, DMSO-d6) δ 7.35 (t, J = 8.8, 2H, Ar), 7.48 (t, J = 7.7, 1H, Ar), 7.57 (t, J = 7.7, 1H, Ar), 7.65 to 7.76 (m, 1H, Ar), 7.79 to 7.97 (m, 3H, Ar), 8.09 (d, J = 8.0, 1H, Ar), and 8.14 to 8.25 (m, 3H, Ar).

1-Benzylidene-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (TAU4). The compound was synthesized according to a described procedure. LC-HRMS (ESI) calculated for C_{15}H_{15}N_{2}O [MH\(^+\)] 310.1009, measured 310.1017; ¹H NMR (400 MHz, DMSO-d6) δ 3.78 (s, 3H, OCH₃), 6.96 (d, J = 8.8, 2H, Ar), 7.11 (s, 1H, Phenyl-CH), 7.34 to 7.47 (m, 3H, Ar), 7.64 (d, J = 7.2, 2H, Ar), 7.77 (d, J = 8.8, 2H, Ar), 8.03 (s, 1H, Ar).

2-(4-(4-Methoxyphenyl)thiazol-2-yl)-1-((pyridin-2-yl)methylene)hydrazine (TAU5). The compound was synthesized according to a described procedure. LC-HRMS (ESI) calculated for C_{15}H_{14}N_{2}O [MH\(^+\)] 284.1096, measured 284.1097; ¹H NMR (400 MHz, DMSO-d6) δ 3.78 (s, 3H, OCH₃), 6.97 (d, J = 8.6, 2H, Ar), 7.20 (s, 1H, Phenyl-CH), 7.31 to 7.42 (m, 1H, Ar), 7.74 to 7.90 (m, 4H, Ar), 8.05 (s, 1H, Ar), 8.87 (d, J = 4.7, 1H, Ar).

2-Methoxy-5-{5H-pyrido[4,3-b]indol-7-yl}pyridine (TAU6). The compound was synthesized according to T807’s patent application using 3-bromo-4-nitropyridine and 4-bromophenylboronic acid as starting materials in the first step and 2-methoxypyrido[5,4-b]pyridine-5-boronic acid for the second step (Figure 1). LC-HRMS (ESI) calculated for C_{15}H_{14}N_{2}O [MH\(^+\)] 276.1131, measured 276.1153; ¹H NMR (400 MHz, DMSO-d6) δ 3.90 (s, 3H, OCH₃), 6.93 (d, J = 8.6, 1H, Ar), 7.46 (d, J = 5.6, 1H, Ar), 7.54 (d, J = 8.1, 1H, Ar), 7.75 (s, 1H, Ar), 8.06 to 8.15 (m, 1H, Ar), 8.28 (d, J = 8.1, 1H, Ar), 8.37 to 8.46 (m, 1H, Ar), 8.56 (s, 1H, Ar), 9.33 (s, 1H, Ar).

7-(4-Methoxyphenyl)-5H-pyrido[4,3-b]indole (TAU7). The compound was synthesized according to T807’s patent application using 3-bromo-4-nitropyridine and 4-bromophenylboronic acid as starting material in the first step and 4-methoxyphenylboronic acid for the second step (Figure 1). LC-HRMS (ESI) calculated for C_{16}H_{15}N_{2}O [MH\(^+\)] 275.1179, measured 275.1196; ¹H NMR (400 MHz, DMSO-d6) δ 3.85 (s, 3H, OCH₃), 7.03 (d, J = 8.2, 2H, Ar), 7.48 to 7.58 (m, 2H, Ar), 7.65 (d, J = 8.2, 2H, Ar), 7.71 (s, 1H, Ar), 8.21 (d, J = 8.2, 1H, Ar), 8.37 (d, J = 5.9, 1H, Ar), 9.23 (s, 1H, Ar).

Methyl 5-[5H-pyrido[4,3-b]indol-7-yl]pyridine-2-carboxylate (TAU8). The compound was synthesized according to T807’s patent application using 3-bromo-4-nitropyridine and 4-bromophenylboronic acid as starting materials in the first step and 4-methoxyphenylboronic acid for the second step (Figure 1). LC-HRMS (ESI) calculated for C_{17}H_{16}N_{2}O [MH\(^+\)] 304.1080, measured 304.1088; ¹H NMR (400 MHz, DMSO-d6) δ 3.92 (s, 3H, COOCH₃), 7.40 to 7.14 (m, 1H, Ar), 7.52 (t, J = 8.5, 1H, Ar), 7.72 (dd, J = 8.2 and 1.4, 1H), 7.97 (s, 1H, Ar), 8.17 (d, J = 8.2, 1H, Ar), 8.43 – 8.35 (m, 2H, Ar), 8.46 (d, J = 5.7, 1H, Ar), 9.14 (d, J = 1.8, 1H, Ar), 9.40 (s, 1H, Ar).
N-Methyl-4-(quinolin-2-yl)benzenamine (BF-158). Compound was synthesized according to a described procedure.\textsuperscript{26} LC-HRMS (ESI) calculated for C\textsubscript{16}H\textsubscript{15}N\textsubscript{2} [MH\textsuperscript{+}] = 234.1230, measured 235.1241; \textsuperscript{1}H NMR (400 MHz, MeOD-d\textsubscript{4}) \textdelta 2.86 (s, 3H, N-CH\textsubscript{3}), 6.75 (d, J = 8.3, 2H, Ar), 7.50 (t, J = 7.4, 1H, Ar), 7.71 (t, J = 7.4, 1H), 7.81 to 8.08 (m, 5H, Ar), 8.27 (d, J = 8.6, 1H, Ar).

7-(6-chloropyridin-3-yl)-5H-pyrido[4,3-b]indole (precursor for T807).

**Figure 1.** Synthesis of TAU3, TAU6-8, and the precursor of T807. \textit{i} = Pd(dppe)Cl\textsubscript{2}/Na\textsubscript{2}CO\textsubscript{3}, ACN, 80°C, 16 h, \textit{ii} = Pd(PPh\textsubscript{3})\textsubscript{4}/K\textsubscript{2}CO\textsubscript{3}, dioxane:water (1:1), 90°C, 16 h, \textit{iii} = P(OE\textsubscript{t})\textsubscript{3}, 100°C, 3 h, \textit{iv} = phosphorus pentoxide/methanesulfonic acid (PPMA), 100°C, 1 h.

N-Methyl-4-(quinolin-2-yl)benzenamine (BF-158). Compound was synthesized according to a described procedure.\textsuperscript{26} LC-HRMS (ESI) calculated for C\textsubscript{16}H\textsubscript{15}N\textsubscript{2} [MH\textsuperscript{+}] = 234.1230, measured 235.1241; \textsuperscript{1}H NMR (400 MHz, MeOD-d\textsubscript{4}) \textdelta 2.86 (s, 3H, N-CH\textsubscript{3}), 6.75 (d, J = 8.3, 2H, Ar), 7.50 (t, J = 7.4, 1H, Ar), 7.71 (t, J = 7.4, 1H), 7.81 to 8.08 (m, 5H, Ar), 8.27 (d, J = 8.6, 1H, Ar).

7-(6-chloropyridin-3-yl)-5H-pyrido[4,3-b]indole (precursor for T807).

**Step 1:** A mixture of 3-(4-bromophenyl)-4-nitropyridine\textsuperscript{21} (0.70 g, 2.51 mmol), (4-chloro-3-pyridyl)boronic acid (0.592 g, 3.76 mmol), and potassium carbonate (0.693 g, 5.02 mmol) in 1,4-dioxane (14 mL) and water
(14 mL) was degassed under N₂ flow for 10 minutes. Then, tetrakis(triphenylphosphine)palladium⁰ (0.145 g, 0.125 mmol) was added and the mixture was heated at 140°C for 10 minutes under microwave irradiation. The reaction mixture was diluted with dichloromethane and washed with water. The organic layer was separated, dried (MgSO₄), filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography (silicagel: ethyl acetate in heptane 0/100 to 40/60). The desired fractions were collected and the solvents removed in vacuo to give 2-chloro-5-[4-(4-nitro-3-pyridyl)phenyl]pyridine (0.34 g, 43% yield) as a pale yellow solid that was used as such for the next reaction step.

**Step 2:** A solution of 2-chloro-5-[4-(4-nitro-3-pyridyl)phenyl]pyridine (0.30 g, 0.96 mmol) in triethyl phosphate (3 mL, 17.5 mmol) was heated at 100°C for 3 hours in a sealed tube. After this time, the mixture was cooled to 0°C and the precipitate filtered off and washed with cold diethyl ether. The solid was purified by flash chromatography (silicagel: MeOH/NaH₂O in dichloromethane 0/100 to 04/96). The desired fractions were collected, the solvents were removed in vacuo, and the residue was triturated with diethyl ether to yield 7-(6-chloropyridin-3-yl)-5H-pyrido[4,3-b]indole (0.13 g, 48% yield) as a pale yellow solid (Figure 1). LC-HRMS (ESI) calculated for C₁₄H₁₂ClN₃ [MH]+ 279.0563, measured 279.0567; ¹H NMR (400 MHz, DMSO-d₆) δ 7.51 (dd, J = 5.7 and 0.9, 1H, Ar), 7.64 (dd, J = 8.2 and 1.7, 1H, Ar), 7.65 (d, J = 8.4, 1H, Ar), 7.88 (d, J = 1.1, 1H, Ar), 8.27 (d, J = 8.2 and 2.7, 1H, Ar), 8.36 (d, J = 8.4, 1H, Ar), 8.45 (d, J = 5.5, 1H, Ar), 8.85 (d, J = 2.9, 1H, Ar), 9.39 (s, 1H, Ar), 11.88 (s, 1H, NH).

**Radiolabeling**

2-[4-(2-[¹⁸F]fluoroethyl)piperidin-1-yl]pyrimido[1,2-a]benzimidazole (AV-680, [¹⁸F]T808), 7-[6-[¹⁸F]fluoropyridin-3-yl]-5H-pyrido-[4,3-b]indole (AV-1451, [¹⁸F]T807) and 2-[4-[[¹¹C]methylamino]phenyl]benzothiazol-6-ol ([¹¹C]PiB). Fluorine-18 in the form of fluoride ([¹⁸F]F⁻) was produced by an ¹⁸O(p,n)⁰ reaction in a Cyclone 18/9 cyclotron (Ion Beam Applications, Louvain-la-Neuve, Belgium) by irradiation of 2 mL of 97% enriched ¹⁸O-H₂O (Rotem HYOX18, Rotem Industries, Beer Sheva, Israel) using 18-MeV protons. After irradiation, [¹⁸F]F⁻ was trapped on a SepPak Light Accell plus QMA anion exchange cartridge (CO₂⁻ form, Waters, Milford, Massachusetts) and eluted with a mixture of Kryptofix 2.2.2 (K-222, 27.86 mg) and K₂CO₃ (2.46 mg) dissolved in CH₂CN/H₂O (0.75 mL; 95:5 v/v). After evaporation of the solvent with a stream of helium at 110°C, anhydrous CH₂CN (1 mL) was added, and [¹⁸F]F⁻ was further dried under the same conditions. A solution of the precursor in DMSO (2 mg O-mesyl precursor in 0.6 mL for [¹⁸F]T808 and 2 mg chloro precursor in 0.8 mL for [¹⁸F]T807) was added to the dried [¹⁸F]F⁻–K₂CO₃–K-222 complex, and the mixture was heated at 90°C for 10 minutes (conventional heating) for [¹⁸F]T808 and at 130°C and 50 W for 15 minutes (microwave irradiation) for [¹⁸F]T807. The crude radiolabeling mixture was diluted with 0.6 mL milliQ water (Millipore, Bedford, USA) and purified using reverse-phase HPLC (RP-HPLC) on an XBridge C₁₈ column (5 μm, 4.6 × 150 mm; Waters) eluted with a mixture of 0.05 mol/L NaOAc pH 5.5 and EtOH (80:20 v/v for [¹⁸F]T808 and 78:22 v/v for [¹⁸F]T807) at a flow rate of 1 mL/min and with UV detection at 254 nm. [¹⁸F]T808 and [¹⁸F]T807 eluted at 21 and 16 minutes, respectively. [¹⁸F]T807 was further purified on a SepPak Alumina N Plus Light cartridge (Waters). The purified radiotracer solution was diluted with saline to obtain an ethanol concentration <10%, suitable for intravenous injection. Quality control was performed using RP-HPLC on an XBridge column (C₁₈, 3.5 μm, 3.0 mm × 100 mm; Waters) eluted with a mixture of 0.05 mol/L NaOAc pH 5.5, and CH₃CN (80:20 v/v for [¹⁸F]T808 and 83:17 v/v for [¹⁸F]T807) at a flow rate of 0.8 mL/min. UV detection was performed at 254 nm. [¹⁸F]T808 and [¹⁸F]T807 eluted at 8 and 7 minutes, respectively. [¹¹C]PiB was radiolabeled according to a published procedure.²⁷
which 10 μL was counted in 3 mL LSC (534.6 MBq/mL). The crude mixture was purified using RP-HPLC.

**Biodistribution Studies**

Biodistribution of [18F]T808 and [18F]T807 was studied in healthy male Naval Medical Research Institute (NMRI) mice (body weight: 30-40 g) at 2, 10, 30, and 60 minutes postinjection (PI; n = 4/time point). Mice were anesthetized (2.5% isoflurane in O₂ at 1 L/min flow rate) and injected with about 0.9 MBq of the tracer via a tail vein and killed by decapitation at above-specified time points. Blood and major organs were collected in tared tubes and weighed. Radioactivity in blood, organs, and other body parts was counted using an automated γ-counter. For the calculation of total radioactivity in blood, bone, and muscle, masses were assumed to be, respectively, 7%, 12%, and 40% of the total body mass.

**Radiometabolites**

Plasma radiometabolites. The NMRI mice were anesthetized with isoflurane (2.5% in O₂ at 1 L/min flow rate) and injected with [18F]T808 or [18F]T807 (~3.7 MBq) via a lateral tail vein. They were killed by decapitation at 2, 10, 30, or 60 minutes PI (n = 3/time point). Blood was collected in EDTA-containing tubes (4 mL tubes; BD vacutainer, BD, Franklin Lakes, New Jersey) and stored on ice. Blood was subsequently centrifuged at 2330 × g for 10 minutes to separate the plasma. Plasma (0.5 mL) was isolated and spiked with 10 μg of authentic T808 or T807 and analyzed by HPLC (Chromolith C₁₈, 3.0 × 100 mm; Merck, Darmstadt, Germany) and eluted with gradient mixtures of 0.05 mol/L NaOAc pH 5.5 (A) and CH₃CN (B; 0-4 minutes: isocratic 0% B, 0.5 mL/min; 4-14 minutes: linear gradient 0% B to 90% B, 1 mL/min; 14-17 minutes: isocratic 90% B, 1 mL/min). After passing through a UV detector at 254 nm coupled in series with a storage screen (super-resolution screen; Perkin Elmer, Waltham, Massachusetts). The radioactivity of fractions was counted in a readout system (Perkin Elmer) and analyzed using Optiquant software.

Perfused brain radiometabolites. For each time point, three NMRI mice were injected with [18F]T808 or [18F]T807 (~7.4 MBq). At 10 or 60 minutes PI, the mice were killed by an overdose of pentobarbital (Nembutal; CEVA Santé Animale, Brussels, Belgium; 150 mg/kg intraperitoneally) and perfused with saline via the left ventricle. Brain was isolated and homogenized in CH₃CN (2 mL). After centrifugation at 3000 rpm (1310 × g) for 5 minutes, the supernatant (~1 mL) was collected, diluted with H₂O (1 mL), and filtered through a 0.22-μm filter (Millipore, Bedford, Massachusetts). About 0.5 mL of the filtrate was further diluted with an equal volume of H₂O and spiked with 20 μg authentic T808 or T807 before being analyzed on an analytical XBridge column (C₁₈, 3.5 μm, 3.0 × 100 mm; Waters) and eluted with a mixture of 0.05 mol/L NaOAc pH 5.5 and CH₃CN (80:20 v/v for [18F]T808 and 85:15 v/v for [18F]T807) at a flow rate of 0.8 mL/min. Ultraviolet detection was done at 254 nm. The HPLC eluate was collected as 1 mL fractions, and their radioactivity was counted in an automated γ-counter.

**In Vitro Autoradiography Binding Inhibition Studies**

Air-dried, frozen, 10-μm thick slices of the visual cortex of an patient with AD (68-year old female with Braak stage V-VI) were incubated for 60 minutes with [18F]T808 or [18F]T807 (0.74 MBq/500 μL per section) and subsequently washed with mixtures of phosphate-buffered saline (PBS) and ethanol as described elsewhere. In the case of [11C]PiB, slices were incubated with 0.37 MBq/500 μL per section for 10 minutes. To assess specificity of binding, slices were incubated with tracer in the presence of 1 μmol/L of authentic T808, T807, astemizole, lansoprazole, THK-5105, BF-158, BF-170, TAU1-8, or PiB. After drying, the slices were exposed to a phosphor storage screen (super-resolution screen; Perkin Elmer, Waltham, Massachusetts). Screens were read in a Cyclone Plus system (Perkin Elmer) and analyzed using Optiquant software (Perkin Elmer). Results are expressed as digital light units per square mm (DLU/mm²). Adjacent AD slices were immunostained with anti-tau (T6402) and anti-Aβ antibodies (6F/3D), as described earlier, to correlate with [18F]T808, [18F]T807, or [11C]PiB binding. Slices of the P301L-transgenic mice and their wild-type controls were analyzed in similar manner using...
[18F]T808 (0.74 MBq/500 μL per section) and 1 μmol/L of cold T808.

**PHF Tau and Amyloid Plaques Isolation From Human AD Brain**

Enriched paired helical filaments PHF tau fractions were prepared according to a slightly modified version of the protocol described by Greenberg and Davies28 using human AD brain tissue (occipital cortex with high tau fibril load). Briefly, frozen human AD brain samples (~ 10 g) were homogenized with 10 volumes of cold homogenization buffer (10 mmol/L Tris, 800 mmol/L NaCl, 1 mmol/L ethylene glycol tetracetic acid EGTA, 10% sucrose, pH 7.4) containing PhosSTOP phosphatase and cOmplete EDTA-free protease inhibitor (Roche, Vilvoorde, Belgium) on ice. After centrifugation at 27,000 × g for 20 minutes at 4°C, the supernatant was recovered and 1% (w/v) N-lauroylsarcosine and 1% (v/v) 2-mercaptoethanol were added. The N-lauroylsarcosine/2-mercaptoethanol supernatant was incubated for 2 hours at 37°C while shaking on an orbital shaker. Subsequently, ultracentrifugation at 108,000 × g for 1.5 hours at room temperature enriched PHF tau in the pellet. Supernatant was removed, and the pellet was carefully rinsed twice with a small volume of Tris-buffered saline (TBS; 50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4). Finally, the PHF tau pellet was recovered in TBS and resuspended to ensure sample homogeneity. Small aliquots were stored at −80°C.

Frozen human AD brain samples (10 g—occipital cortex with high amyloid plaques load) were homogenized with a 7-fold volume of cold homogenization buffer (250 mmol/L sucrose, 20 mmol/L Tris base, 1 mmol/L EDTA, 1 mmol/L EGTA, and cOmplete EDTA-free protease inhibitor) on ice. After centrifugation at 27,000 × g for 20 minutes at 4°C, cell debris was removed. Supernatant containing amyloid plaques was aliquoted and stored at −80°C.

**In Vitro Competitive Radioligand Binding Assays**

The competitive radioligand binding assays measure the binding of a radiolabeled reference ligand in the presence of a dose–response concentration range of test compounds T807, T808, lansoprazole, astemizole, PiB, THK5105, TAU1, and TAU4-8 (TAU2-3 were not evaluated due to solubility problems).

Briefly, PHF tau preparations were diluted to 100 μg protein/mL in PBS buffer with 5% ethanol. In a 96-well format, [3H]-T808 (specific activity 1 GBq/μmol) was added at a final concentration of 10 nmol/L to increasing amounts of test compound in the presence of 20 μg protein of PHF tau preparation. Nonspecific binding was defined as the number of counts remaining in the presence of 50 μmol/L thioflavin T (common beta sheet binder). After 2 hours incubation at room temperature, the unbound ligand was removed by filtration of the binding mixtures over GF/B glass filters using a Filtermate 96 harvester instrument (Perkin Elmer, Zaventem, Belgium). The filters were washed three times with PBS buffer containing 20% ethanol. After overnight drying of the filter plate, Microscint O liquid (Perkin Elmer) was added, and the amount of radiolabeled ligand bound to the fibrils was measured by liquid scintillation counting in a Topcount instrument (Packard Instrument, Meriden, Connecticut).

Values for half-maximal inhibitory concentration (IC50) were determined from displacement curves of at least two independent experiments using GraphPad Prism software (GraphPad Software, San Diego, California).

To determine compound binding to the amyloid plaques, a similar assay was put in place but with some minor modifications. Briefly, amyloid preparations were diluted to 150 μg protein/mL in 50 mmol/L Tris with 0.1% bovine serum albumin (BSA) and 5% ethanol. 3H-AV-45 (florbetapir—specific activity 2 GBq/μmol) was added at a final concentration of 10 nmol/L to increasing amounts of test compound in the presence of 30 μg protein of amyloid plaques preparation. Nonspecific binding was determined in the presence of 500 μmol/L thioflavin T. After 150 minutes incubation at room temperature, the binding mixtures were filtered over GF/B glass filters. The filters were washed three times with PBS buffer containing 20% ethanol. Subsequent steps were identical to those described for the PHF tau preps.

**Results**

**Radiolabeling**

[18F]T808 and [18F]T807 were synthesized by a nucleophilic substitution reaction on their corresponding mesyl and chloro precursor with [18F]fluoride in DMSO (Figures 2 and 3). Heating of the precursor solution in the presence of K-222/K2CO3 afforded [18F]T808 and [18F]T807 with an average, decay-corrected, radiochemical yield of 21% and 6%, respectively (relative to radioactivity of [18F]F− in the preparative chromatogram, n = 7). Purification of the crude radiolabeling reaction mixture was done on an isocratic RP-HPLC system using a mixture of acetate buffer and ethanol as mobile phase. Radiochemical purity was examined using HPLC on an analytical C18 column and was more than 99%. Both tracers were obtained within a total synthesis time of 60 minutes and were collected with an average specific radioactivity of 119 GBq/μmol at the end of synthesis (EOS, n = 7) for [18F]T808 and 50 GBq/μmol at EOS (n = 7) for [18F]T807. [11C]PiB had a mean specific activity of 85 GBq/μmol at EOS and a radiochemical purity of ≥99% (n = 2).

**Biodistribution in Normal Mice**

Table 1 shows the percentage of injected dose per gram (%ID/g) of [18F]T808 and [18F]T807 in different organs at 2, 10, 30, and 60 minutes PI. Clearance from blood, defined as the 2 minutes to 60 minutes activity ratio, was slower for [18F]T807 (2.5) than for [18F]T808 (2.7). Both tracers were mainly cleared via the hepatobiliary system into the intestines.
and to a lesser extent via the renal pathway. High initial brain uptake was observed (4.9\%ID/g at 2 minutes PI for $[^{18}\text{F}]T808$ and 7.5\%ID/g at 2 minutes PI for $[^{18}\text{F}]T807$) with fast washout (0.9\%ID/g at 10 minutes PI for $[^{18}\text{F}]T808$ and 2.6\%ID/g at 10 minutes PI for $[^{18}\text{F}]T807$). High 2 to 60 minutes activity ratios for the brain were therefore also found (18.2 for $[^{18}\text{F}]T808$ and 18.3 for $[^{18}\text{F}]T807$). In contrast to $[^{18}\text{F}]T807$, $[^{18}\text{F}]T808$ showed high bone uptake (up to 10\%ID/g at 30 minutes PI) suggesting loss of fluorine 18 from the radioligand in vivo.

**Radiometabolites**

The plasma radiometabolite analysis of $[^{18}\text{F}]T808$ and $[^{18}\text{F}]T807$ in NMRI mice revealed rapid metabolism in vivo.
for $[^{18}\text{F}]$T808 and a relatively slow metabolism for $[^{18}\text{F}]$T807 (Table 2). At 2 minutes PI, only 65\% of the recovered radioactivity of $[^{18}\text{F}]$T808 was still in the form of intact tracer, whereas $[^{18}\text{F}]$T807 remained intact (99\%). At 30 minutes PI, the fraction of intact $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 decreased, respectively, to 29\% and 60\%. All detected radiometabolites were more polar than the intact tracers. The recovery of radioactivity for $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 was high, (respectively, 89\% and 85\% \[n = 12\]).

In brain, the percentage of intact $[^{18}\text{F}]$T808 decreased from 88\% at 10 minutes PI to 34\% at 60 minutes PI, while the fraction of parent $[^{18}\text{F}]$T807 in brain remained close to 100\% at both 10 and 60 minutes PI (Table 2). The detected radiometabolites were more polar than the intact tracers, and no radiometabolites more lipophilic than the intact tracer were detected 60 minutes PI. The recovery of the HPLC column-injected radioactivity for $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 was 85\% and 70\%, respectively \([n = 6]\).

### In vitro Autoradiography Binding Studies

Digital autoradiography with $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 on human AD slices showed binding to tau-rich regions in the 1 to 3 cortical layers and 5 to 6 cortical layers of the visual cortex (Figure 4). Immunohistochemistry with tau and Aβ antibodies, performed on adjacent slices, identified numerous NFTs and neurtic plaque deposits, confirming colocalization of tracer binding with NFTs (Figure 5). To assess the specificity of the tracer binding to these NFTs, blocking studies with several tau-specific ligands and Aβ-specific ligands were performed. An overview of the different compounds used to block (at 1 μmol/L concentration) the binding of $[^{18}\text{F}]$T808, $[^{18}\text{F}]$T807, and $[^{11}\text{C}]$PiB is given in Table 3. These ligands are divided into 3 groups: reported ligands (group 1), newly developed ligands (group 2), and studies with $[^{11}\text{C}]$PiB (group 3). Binding of $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 to NFT-rich regions was reduced with 94\% and 90\% in the presence of 1 μmol/L T807 and T808, respectively. Self-blocking with 1 μmol/L of the cold ligand, on the other hand, resulted in 88\% inhibition for T807 and 89\% for T808. Self-blocking of $[^{11}\text{C}]$PiB resulted in 57\% blocking. More than 70\% reduction in binding of $[^{18}\text{F}]$T808 or $[^{18}\text{F}]$T807 was observed with BF-158, BF-170, THK5105, TAU1, and TAU5-8 at a concentration of 1 μmol/L. With lansoprazole, astemizole, PiB, and TAU2-4 on the other hand, blocking was less pronounced (range 0\%-59\%). No significant inhibition (< 17\%) of $[^{11}\text{C}]$PiB binding was observed with 1 μmol/L T808, T807, and TAU6-8. Finally, $[^{18}\text{F}]$T808 did not show any specific binding to murine tau in the P301L transgenic mouse model (Figure 6).

### In vitro Competitive Radioligand Binding Assays

Table 4 shows the binding assays of $[^{3}\text{H}]$T808 with cold test compounds showing high affinity of reference compounds T807, T808, PiB, and THK-5105 for purified tau but low affinity of lansoprazole and astemizole (pIC<sub>50</sub> equal to or higher than 6.49). Of the newly synthesized compounds, only TAU7 showed high affinity (pIC<sub>50</sub> of 7.46), whereas TAU1, TAU4-6, and TAU8 showed low affinity (pIC<sub>50</sub> < 5). In the competition assays with $[^{3}\text{H}]$AV-45, low affinity was recorded for T808, lansoprazole, and astemizole (pIC<sub>50</sub> < 5). T807 and THK5105 showed specific binding with pIC<sub>50</sub> values of 6.25 and 7.25, respectively. PiB had a pIC<sub>50</sub> of 8.10. In the group of newly synthesized ligands, only TAU7 showed affinity with a pIC<sub>50</sub> of 7.95, while the other TAU compounds had pIC<sub>50</sub> values lower than <5.

### Discussion

One of the aims of this study was to determine some important preclinical properties of $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807, such as brain uptake, washout from brain, and formation of radiometabolites, since these ligands are among the most selective tau tracers with high affinity for NFTs reported in literature to date.\(^5\) In addition, with a clogD<sub>7.4</sub> between 2.0 and 3.5, a polar surface area <80 Å<sup>2</sup>, and a low molecular mass (<500 Da), both compounds show optimal molecular features for a passive transfer across the blood–brain barrier (BBB).\(^{19}\) Although these tracers have already been evaluated in clinical trials,\(^{18,19}\) preclinical data remain essential in the search for new and better $[^{18}\text{F}]-$ and $[^{11}\text{C}]-$labeled imaging agents for tauopathies other than AD. Once we characterized $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 as benchmark compounds, both compounds were used in semiquantitative autoradiography experiments to evaluate and compare binding characteristics of recently reported tau tracers and newly synthesized radioligands.

Although the radiosynthesis of both $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 has been described with relative high specific activities and radiochemical yields,\(^8,11\) we have put low effort in optimization of their labeling process, as this was not required for the type of experiments we performed.

The biodistribution of $[^{18}\text{F}]$T808 and $[^{18}\text{F}]$T807 in normal mice revealed high initial brain uptake with rapid washout after 10 minutes. In contrast to $[^{18}\text{F}]$T807, pronounced accumulation of radioactivity in bone was observed for $[^{18}\text{F}]$T808, likely due to defluorination in vivo.\(^19\) These results were expected based
Figure 4. Human Alzheimer disease (AD) brain slices incubated with $^{[18}F]T808$, $^{[18}F]T807$, and $^{[11}C]PiB$ in the presence of authentic reference ligands and newly developed compounds at 1 mmol/L. Slices with $^{[18}F]T808$ and $^{[18}F]T807$ are from different experiments.

Figure 5. Autoradiography analysis on 10-mm thick slices of the visual cortex of an AD patient (68-year-old female with Braak stage V-VI) with $^{[18}F]T808$ (autoradiogram in the middle). Adjacent slices were immunostained with tau (T6402) on the right and amyloidbeta (Ab: M0872) on the left. Higher magnification on the bottom identifies tau tangles and amyloid plaques (dotted circles). Scale bar: 50 mm.
Table 3. Percentage Blocking of Tracer Binding for $[^{18}F]$T808, $[^{18}F]$T807, and $[^{12}C]$PiB in the Presence of Several Compounds With Affinity for tau or Aβ.

| Group | Compound | nmol/L Tracer | % Block ± SD (n = 3)* |
|-------|----------|---------------|-----------------------|
| 1     | T807     | $[^{18}F]$T807 | 88 ± 0.0              |
|       | T808     | $[^{18}F]$T808 | 89 ± 0.0              |
|       | T807     | $[^{18}F]$T808 | 94 ± 0.0              |
|       | T808     | $[^{18}F]$T808 | 90 ± 10b              |
|       | BF-158   | $[^{18}F]$T808 | 93 ± 0.0              |
|       | BF-170   | $[^{18}F]$T808 | 84 ± 0.1              |
|       | Lansoprazole | $[^{18}F]$T808 | 4 ± 0.5              |
|       | Astemizole | $[^{18}F]$T808 | 22 ± 0.4             |
|       | PiB      | $[^{18}F]$T808 | 19 ± 0.1              |
|       | THKS105  | $[^{18}F]$T808 | 78 ± 0.0              |
| 2     | TAU1     | $[^{18}F]$T808 | 75 ± 0.1              |
|       | TAU2     | $[^{18}F]$T808 | 48 ± 0.0              |
|       | TAU3     | $[^{18}F]$T808 | 29 ± 0.1              |
|       | TAU4     | $[^{18}F]$T808 | 47 ± 0.0              |
|       | TAU5     | $[^{18}F]$T808 | 73 ± 0.0              |
|       | TAU6     | $[^{18}F]$T808 | 94 ± 0.0              |
|       | TAU7     | $[^{18}F]$T808 | 91 ± 0.0              |
|       | TAU8     | $[^{18}F]$T808 | 90 ± 0.0              |
| 3     | PiB      | $[^{11}C]$PiB | 57 ± 0.0              |
|       | T807     | $[^{11}C]$PiB | 11 ± 0.1              |
|       | T808     | $[^{11}C]$PiB | 17 ± 0.0              |
|       | TAU6     | $[^{11}C]$PiB | 0 ± 0.1               |
|       | TAU7     | $[^{11}C]$PiB | 0 ± 0.0               |
|       | TAU8     | $[^{11}C]$PiB | 0 ± 0.1               |

Abbreviations: Aβ, amyloid β; DLU/mm², digital light units per square mm; SD, standard deviation.
* Calculated as (DLU/mm² in the presence of 1 nmol/L blocker) / (DLU/mm² tracer only).

b SD values of three different experiments. Other SD values are on three different slices in one and the same experiment.

on the μ-PET biodistribution data from the original report on $[^{18}F]$T808.8 The μPET data suffer however from limited resolution when compared to an in vivo biodistribution study in which the radioactivity of individual organs is counted after dissection, and thus results of the two approaches are only partly comparable. Ex vivo biodistribution data of $[^{18}F]$T808 and $[^{18}F]$T807 are therefore important as benchmark data in order to compare performance of new potential tau tracers.

We quantified the fraction of radiometabolites of $[^{18}F]$T808 and $[^{18}F]$T807 in plasma and perfused brain of normal mice. In plasma, metabolism was relatively fast for $[^{18}F]$T808, compared to $[^{18}F]$T807. One major polar radiometabolite was detected for each tracer. In view of the observed bone uptake in the biodistribution study, $[^{18}F]$T808’s main radiometabolite is therefore probably $[^{18}F]$Fluoride. This might lead to inaccurate quantification of tracer binding in brain, in view of partial volume effects. Early clinical PET imaging results with $[^{18}F]$T808 showed however no interference with image interpretation at early time points.19 In brain, ideally no radiometabolites should be present, since they may have different brain kinetics and brain distribution than the parent compound and thus complicate tracer modeling and PET quantification. In their original article on preclinical properties of $[^{18}F]$T808, Zhang et al mention the absence of radiometabolites in mouse brain.5 Metabolic stability was however conducted in liver microsomal assays of mice and humans, where rapid metabolism was shown (in the presence of cofactor NADPH) 60 minutes after incubation. Furthermore, while polar metabolites might not always cross the BBB, ex vivo brain metabolite studies should confirm this. We therefore performed ex vivo brain metabolite studies in mice, which showed the presence of two unidentified polar radiometabolites the fraction of which is 66% at 60 minutes PI (see Table 2). Further, in vitro brain radiometabolite analysis is required to determine whether metabolism is peripheral with subsequent passage of the radiometabolite through the BBB and/or whether it occurs within the brain.

In the second part of this study, we performed a semiquantitative autoradiography screening with $[^{18}F]$T808 and $[^{18}F]$T807 as reference tracers, in combination with several tau-specific ligands reported in the literature (group 1 in Table 3) and with newly synthesized ligands containing structural moieties with known affinity for tau, such as benzothiazoles, carbazoles, and phenylthiazolo[3,2-a]pyrimidine compounds (group 2 in Table 3). Similar autoradiography studies were also performed with $[^{11}C]$PiB to examine the selectivity of several compounds for Aβ (group 3 in Table 3). Furthermore, we examined pIC50 values of several compounds in competition binding assays with $[^{1}H]$T808 and $[^{1}H]$AV-45 on purified tau and Aβ from patients with AD (Table 4). These binding studies provide additional, quantitative information on the affinity of several cold compounds for purified human tau and Aβ, but the absence of off-target binding should also be taken into account when comparing the results of autoradiography blocking studies on AD slices. Overall, the screening assays provide valuable information on the nature of structural moieties necessary for binding to tau fibrils and thus can be very helpful in the development of new specific tau-affinity ligands for tau.8,11 The disadvantage of using a particular radioligand in the autoradiography studies, $[^{18}F]$T808 in our case, to screen for new compounds with high affinity for tau is that allosteric binding is overlooked, and only compounds that bind to identical regions of tau are detected. An alternative approach to screen for new tau selective compounds could be the use of fluorescent dyes such as thioflavin or Congo red derivatives. Such compounds emit a stronger fluorescent signal when interacting with beta-sheet aggregates, making quantification challenging. Furthermore, these derivatives show affinity for many different misfolded proteins with beta-sheet structures and are therefore less specific to screen for tau ligands. Thus, using the highly selective and specific tau tracer $[^{18}F]$T808, we specifically screened for compounds with affinity for aggregated tau beta sheets. The in vitro autoradiography and immunohistochemistry studies with human AD brain sections showed preferential binding of $[^{18}F]$T808 and $[^{18}F]$T807 to tau fibrils in the cortical layers of the visual cortex, where large amounts of NFTs and neurit thread deposits are to be expected, based on the literature.30,31 Displacement of $[^{18}F]$T808 by T807 was more pronounced...
than the displacement of \[^{18}\text{F}]\text{T807}\) by T808. SD values of three different experiments with \[^{18}\text{F}]\text{T807},\) displaced by T808, showed however some variability (see Table 3). Self-block, on the other hand, was comparable for both compounds (group 1 in Table 3). Competition blocking studies showed however higher pIC\(_{50}\) values of T808 than T807 for purified tau but higher specificity of T808 compared to T807. These results are in disagreement with the published affinity values of T807 and T808. Be that as it may, Kd affinity values were determined on human AD slices in the literature, while in our competition blocking studies, purified human tau was used.\(^8,11\) Both tracers may, however, be considered as benchmark compounds for tau, as they have high affinity and selectivity for tau fibrils. In the case of AD in which the concentration of tau aggregates in the human brain can be 5 to 20 times lower than that of Aβ aggregates,\(^7\) high affinity combined with a high selectivity for tau is a prerequisite for a good tau PET imaging agent.

In the first group of reference ligands for the semiquantitative autoradiography experiments, authors of the Tohoku university described three tau imaging agents, among others\(^10,13\).
fluorine 18-labeled THK-5105, carbon 11-labeled BF-158, and 
BF-170. All of them are arylquinoline derivatives\textsuperscript{3,13} which in 
our hands were able to reduce the binding of $[^{18}\text{F}]T808$ to NFT-
rich regions in a similar range (up to 93\%, see Table 3). $[^{18}\text{F}]THK5105$, $[^{11}\text{C}]BF-158$, and $[^{11}\text{C}]BF-170$ were all 
reported to have good affinities for tau (or presumed affinities 
as $K_i$ values for BF-158 and BF-170 were \textgreater 5000 nmol/L for A\textsubscript{B} fibrils). Two $K_D$ values (a high and a low affinity site) in 
the nanomolar range were reported for $[^{18}\text{F}]THK5105$, suggesting 
multiple binding sites on tau fibrils.\textsuperscript{13} The introduction of an 
extra methyl group on the structure of BF-170, compared to the 
structure of BF-158 (Figure 7), seems to lower the binding 
affinity for tau (respectively 84\% inhibition instead of 93\%) 
in our autoradiography assay. THK5105 was also evaluated in 
the competition binding assays, where it showed high affinity 
for tau ($pIC_{50}$ of 7.07), but also for A\textsubscript{B} ($pIC_{50}$ of 7.27), thus 
resulting in low selectivity. The latter results are at odds with 
the reported specificity of THK-5105 (25-fold specificity for 
A\textsubscript{B} over tau). The latter result 
was also shown for T808 in the competition binding assays 
(Table 4).

In the second group, newly synthesized ligands were 
included containing scaffolds with presumed affinity for tau 
fibrils\textsuperscript{8,11,37,38} such as benzothiazoles (TAU1-3), carbazoles 
(TAU6-8), and phenylthiazolylhydrazides (TAU4-5). Five of 
eight compounds tested (TAU1 and TAU5-8) were able to 
inhibit the binding of $[^{18}\text{F}]T808$ with more than 50\%, 
three of which even displaced it more than 90\%. The efficacy of 
the latter three compounds (TAU6-8) proves that the pyridine moiety of T807 allows diverse 
substitution without compromising its affinity for PHFs. The 
absence of displacement of $[^{11}\text{C}]\text{PiB}$ binding by these three 
compounds suggests that they also selectively bind to tau (see 
Table 3). A distinct difference was observed between TAU4 
and TAU5. The latter differs from TAU4 by the presence of a 
pyridine ring instead of a phenyl ring, resulting in an extra 26\% 
inhibition (see Table 3). A side chain with a pyridine ring thus 
might be beneficial to interact with NFTs at similar sites as T807 
and T808. The ligands TAU1-3 and PiB share a similar phenyl-
benzothiazole core structure, but only TAU1 inhibited 
$[^{18}\text{F}]T808$ binding to an extent of 75\% (see Table 3). In the

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Figure 7. Chemical structures of reference compounds and newly synthesized compounds.
competition binding assay, only TAU7 showed affinity for tau and Aβ (with a pIC_{20} of 7.07 and 7.27, respectively). Discrepancy between the autoradiography blocking studies and the competition binding assays could have been, as previously mentioned, be explained by the fact that in the competition assays purified tau and Aβ has been used, while in the autoradiography studies both aggregated proteins are in their “natural” environment, as brain slices of the latest Braak stage were used.

[{\textsuperscript{18}}F]T808 was additionally evaluated in tau overexpressing transgenic mice (P301L model). We did not observe any specific binding, in contrast to the experiments using human brain slices. This may be explained by several contributing factors: differences in posttranslational modifications between mice and humans, different composition of tau-isoforms (mice only express 4R isoforms of tau, whereas 3R and 4R are equally expressed in [AD] humans), and ultrastructural differences in cross-beta sheets. Similar \(\mu\)PET results were observed by Xia et al in the P301L transgenic mouse model.\(^\text{11}\) Nonetheless, several tau tracers (THK-523, N-methyl lansoprazole, and PBB3) were reported to bind to tau in transgenic mice models in autoradiography ARX- and \(\mu\)PET studies.\(^\text{10,12,14}\) Thus, the difference in binding affinity of T808 between murine and human tau could also be explained by the fact that NFTs and SPs are subject to an aging process throughout the disease so that the structure of relatively young plaques in mouse models may be quite different from that of the older NFTs and SPs in patients with AD.\(^\text{35}\) Hence, tau tracers may show affinity for specific subfractions of human aggregated tau, as it is the case for \([\textsuperscript{11}}C]\)PBB3, which is able to bind to different tau fractions (3R and/or 4R), namely, tau present not only in AD but also in other tauopathies,\(^\text{14}\) properties which make the development of a more stable and \(\textsuperscript{18}}F\)-labeled compound worth pursuing.

Conclusion

By performing parallel biodistribution, metabolite, and autoradiography studies with \([\textsuperscript{18}}F]T807 and \([\textsuperscript{18}}F]T808, we were able to provide a straightforward comparison of both radioligands and establish them as benchmark compounds for \textit{in vitro} evaluation and comparison of recently reported tau tracers and newly synthesized ligands in semiquantitative autoradiography studies. Apart from T807, only THK5105, BF-158, and BF-170 were able to displace \([\textsuperscript{18}}F]T808 up to 93\%, implying affinity to similar regions as T808 (and T807). Astemizole and lansoprazole, on the other hand, show possible allosteric binding compared to T808 and T807, since their displacement of \([\textsuperscript{18}}F]T808 was negligible. In the group of newly synthesized ligands, bearing scaffolds with known affinity for tau fibrils, three derivatives of T807 (TAU6-8) showed high blocking of \([\textsuperscript{18}}F]T808. Absence of displacement of \([\textsuperscript{11}}C]\)PiB by these three compounds suggests that these compounds also selectively bind to tau. No specific binding of \([\textsuperscript{18}}F]T808 to murine tau in the P301L transgenic mouse model was observed. Differences in affinity between human and murine tau might be explained by interspecies differences between tau fibrils but is more likely due to existence of specific subfractions of human aggregated tau, compared to murine tau. Currently, reasonably good tau markers for AD have been developed in terms of affinity, biodistribution, and selectivity, but development of novel ligands that can target tau aggregates more selectively in different tauopathies, with little or no interaction with Aβ, and that are also able to bind to different tauopathies and transgenic mice models are required.

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