

binding to NFTs, our fluorescence studies obtained at high tracer concentrations—10,000-fold higher than the concentrations typically achieved during a PET scan—demonstrated inconsistent THK523 staining of A β plaques. THK523 stained the dense core of some A β plaques in the frontal cortex of AD sections but did not stain dense A β plaques in the hippocampus (Figure 1, right panel). It is noteworthy that variable staining of NFTs at high concentrations of PiB has also been reported [52]. In addition to previous reports of *in vitro* studies [29,30], several lines of evidence support the notion that THK523 selectively binds to PHF-tau and does not bind to A β *in vivo*: (1) Cortical THK523 retention is significantly higher in AD; (2) THK523 retention follows the known distribution of PHF-tau in the AD brain; (3) PiB and THK523 show different brain regional distribution patterns; (4) hippocampal THK523 retention significantly correlated with cognitive parameters, but hippocampal PiB retention did not; and (5) hippocampal THK523 retention significantly correlated with hippocampal volume, but hippocampal PiB retention did not [32].

The selectivity of THK523 for tau over other β -sheet aggregated proteins was further demonstrated by fluorescence microscopy studies showing the absence of THK523 fluorescence in brain sections exhibiting immunolabelled α -synuclein-containing Lewy bodies (Figure 5, right panel).

The PSP patient showed neither ¹⁸F-THK523 nor ¹⁸F-florbetaben retention in the brain, suggesting the absence not only of A β plaques but also of tau deposits. Neuropathological examination of the brain confirmed the absence of A β plaques; however, typical tau lesions were present in different brain regions that were not stained by THK523. Given the ultrastructural diversity of tau aggregates, the information derived from these THK523 studies is highly valuable for the future design of tau imaging ligands.

Conclusion

In the present study, we have demonstrated that THK523 selectively binds to PHF-tau with negligible binding to PSP, CBD and PiD tau aggregates, as well as to A β and α -synuclein aggregates. The results of this study also show that novel tracers that bind to non-PHF tau aggregates are needed.

Abbreviations

AD: Alzheimer's disease; A β : Amyloid- β ; CBD: Corticobasal degeneration; CDR: Clinical Dementia Rating Scale; CDR-SOB: Clinical Dementia Rating Scale—Sum of Boxes; CSF: Cerebrospinal fluid; FTLD: Frontotemporal lobar degeneration; GM: Grey matter; MMSE: Mini Mental State Examination; NFT: Neurofibrillary tangle; PET: Positron emission tomography; PiB: Pittsburgh compound B; PiD: Pick's disease; PSP: Progressive supranuclear palsy; ROI: Region of interest; SF: Straight filament; SUV: Standardised uptake value; TF: Twisted filament.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

VLV, MTF-T, KY, NO and CLM designed the experiments. SF, RSM, RH, KY, YK and NO designed and manufactured THK523. LT and IB planned and conducted the human brain section immunostaining experiments. CAM planned and conducted the pathological characterisation of human brain samples. VL and CCR planned and coordinated human PET studies. MTF-T and VLV drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Fairlie Hinton and Geoff Pavey from the Victorian Brain Bank Network (VBBN) for sourcing and preparing the human brain tissue (HREC University of Melbourne 9414748). The VBBN is supported by The Florey Institute of Neuroscience and Mental Health, The Alfred and the Victorian Forensic Institute of Medicine, and it is funded by Australia's National Health & Medical Research Council (NHMRC) and Parkinson's Victoria. The research was supported in part by Alzheimer's Drug Discovery Foundation Research Grant 20101208 AFTD and NHMRC project grant 1044361.

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Received: 11 July 2013 Accepted: 13 February 2014

Published: 26 February 2014

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doi:10.1186/alzrt240

Cite this article as: Fodero-Tavoletti et al.: Assessing THK523 selectivity for tau deposits in Alzheimer's disease and non-Alzheimer's disease tauopathies. *Alzheimer's Research & Therapy* 2014 **6**:11.

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Synthesis and preliminary evaluation of 2-arylhydroxyquinoline derivatives for tau imaging

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Alzheimer's disease (AD) is the most common cause of dementia. Senile plaques, consisting of β -amyloid, and neurofibrillary tangles (NFTs), composed of tau protein, are representative pathological hallmarks of AD. It is believed that the accumulation of NFTs precedes the onset of clinical symptoms of AD and correlates with the progression of memory dysfunction. Thus, the use of noninvasive detection techniques including radiolabeled probes and positron emission tomography (PET) will facilitate early diagnosis or staging of AD. In this study, we synthesized and evaluated novel hydroxylated 2-arylquinoline derivatives as tau imaging PET probes. The binding affinities of compounds for tau were evaluated by fluorescent staining of the AD hippocampal section and a competitive binding assay using [¹⁸F]THK-523. THK-951 showed high binding affinity for tau pathology in an AD brain section and K18 Δ 280K fibrils ($K_i = 20.7$ nM); thus, we radiosynthesized a ¹¹C-labeled THK-951 and further studied its potential as a tau PET probe. The [¹¹C]THK-951 demonstrated excellent kinetics in a normal mouse brain (3.23% ID/g at 2 min postinjection and 0.15% ID/g at 30 min postinjection) and showed the labeling of NFTs in an AD brain section by autoradiography assay. These findings indicate the availability of [¹¹C]THK-951 for in vivo PET imaging of tau pathology in AD.

Keywords: Alzheimer's disease; tau; positron emission tomography; imaging

Introduction

Alzheimer's disease (AD) is a typical neurodegenerative disorder associated with memory impairment, disorientation, and language disorder. Because AD shows complex and diverse clinical symptoms, which overlap with other dementias, its definitive diagnosis is reliant on postmortem examination rather than clinical diagnosis. Neuronal atrophy and deposition of senile plaques (SPs) and neurofibrillary tangles (NFTs) in the brain represent the pathological hallmarks of AD.^{1–3} Consequently, noninvasive imaging techniques to measure these pathological changes can be indispensable to the differential diagnosis of AD.

According to amyloid hypothesis, the AD pathogenic mechanism starts with the abnormal accumulation of SPs consisting of β -amyloid aggregation in the brain.⁴ With a view to presymptomatic diagnosis, investigation into the detection of lesions using positron emission tomography (PET) or single photon emission computed tomography has been promoted in this decade, and many radiolabeled ligands were developed ([¹⁸F]FDDNP,⁵ [¹¹C]PIB,⁶ [¹⁸F]AV-45,⁷ [¹¹C]BF-227,⁸ and [¹²³I]/¹²⁵I]IMPY⁹).¹⁰ These ligands have been successful for the detection of SPs in living human brains and were useful for confirming the correlation between PET images and autopsy results.^{11–13}

Although β -amyloid PET imaging studies have high sensitivity and can detect SPs before clinical symptoms appear, they also showed that the association between $A\beta$ signals and acuteness of symptom was limited.^{4,14} Therefore, to estimate AD severity

from pathological changes, other quantitatively detectable biomarkers are desired.

Tau proteins, which associate with the stabilization of microtubules, are abnormally phosphorylated and form paired helical filaments (PHFs) in the AD patient's brain.^{15,16} PHFs finally assemble into NFTs, neuropil threads, and dystrophic neurites. Neurofibrillary lesions appear in certain brain areas before the onset of dementia, and autopsy studies indicate a higher association between tau pathology levels and cognitive dysfunction relative to $A\beta$ pathology.^{17–20} Thus, quantitative imaging of tau burden may serve to not only monitor the progression of neurodegeneration but also evaluate the therapeutic effect of longitudinal tau-targeted AD treatments.

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Recently, several tau ligands for PET or single photon emission computed tomography imaging have been reported.^{21,22} Kolb and colleagues have reported ¹⁸F-labeled tau tracers, [¹⁸F]T807 and [¹⁸F]T808, and these tracers showed promising results in both *in vitro* and *in vivo* studies.^{23–25} We have developed 2-arylquinoline derivatives with a preference to bind tau lesions rather than A β as previously reported.^{26,27} Through the structural optimization, we recently reported ¹⁸F-labeled derivatives [¹⁸F]THK-5105 and [¹⁸F]THK-5117.²⁸ They appear to have more preferable properties as a PET tau imaging radiotracer compared with [¹⁸F]THK-523.²⁷ The preliminary clinical studies using these tracers are currently in progress.

Lipophilic ligands display higher nonspecific binding; therefore, lower lipophilicity is important for ideal brain kinetics as brain imaging PET tracers. Low nonspecific binding contributes to high signal-to-background ratio and facilitates the visualization of lower burden of tau pathology. The aim of the present study was to develop a ¹¹C-labeled tau probe with fast clearance from nonspecific regions. We developed novel hydroxylated quinoline derivatives (Figure 1) with lower lipophilicity and evaluated their potential as tau probes for PET.

Experimental

Synthesis of 2-arylquinoline derivatives

Methods for the synthesis and characterization data of 2-arylquinoline derivatives (shown in Schemes 1 and 2) are described in detail in the Supporting Information.

Radiolabeling of [¹¹C]THK-951

[¹¹C]Methyl iodide was prepared from [¹¹C]carbon dioxide produced with a Cypris HM12 cyclotron (Sumitomo Heavy Industries Inc., Tokyo, Japan) by a catalytic iodination reaction via [¹¹C]methane (Mel MicroLab, GE Healthcare, Waukesha, WI, USA). [¹¹C]Methyl triflate ([¹¹C]MeOTf) used for radiolabeling was prepared from [¹¹C]methyl iodide by passing through a silver triflate-graphpac (Sigma-Aldrich, St Louis, USA) gas chromatography column heated at 200 °C. The compound **15** indicated in Scheme 2 was used as a radiolabeling precursor. The precursor (1.0 mg) and tBuOK (1.0 mg) were dissolved in dimethyl sulfoxide (DMSO) (400 μ L). [¹¹C]MeOTf was bubbled through the solution, followed by heating at 110 °C for 1 min. Then, 2N HCl (1 mL) was added to the solution, and it was heated at an additional 10 min at the same temperature. The solution was neutralized with 4N NaOH (0.5 mL), and the crude mixture was purified with semi-preparative HPLC using an Inertsil® ODS-3 (GL Sciences Inc., Tokyo, Japan) eluted with acetonitrile/20 mM NaH₂PO₄ (pH 4.8) (40/60, flow rate: 5.0 mL/min). The fraction containing [¹¹C]THK-951 was mixed with water (20 mL) and 25% ascorbic acid (0.5 mL) and passed through an activated Sep-Pak tC18 Plus (Waters, Milford, MA, USA). The Sep-Pak cartridge was washed with water, and then, [¹¹C]THK-951 was eluted with ethanol. The ethanol solution was used for the *in vitro* autoradiography (ARG) assay. For the *ex vivo* biodistribution study, ethanol was removed by evaporation, and the product was solubilized into saline with polysorbate 80 (<0.1%).

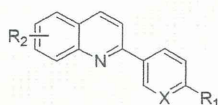


Figure 1. Chemical structure of hydroxyquinoline derivatives. Compounds in this study include the following: X = CH, N; R₁ = NH₂, NHCH₃; R₂ = 6-OH, 7-OH.

Measurement of logP values

The octanol/water partition coefficients of the tested compounds were estimated by HPLC method of the OECD (Organisation for Economic Co-operation and Development) guideline²⁹ according to the slightly modified procedures we recently reported.²⁸

In vitro fluorescent binding assay with tau fibrils

Preparation of K18 Δ 280K-tau fibrils

Recombinant K18 Δ 280K-tau was custom generated by Life Technologies Japan Ltd. (Tokyo, Japan). K18 Δ 280K-tau fibrils were prepared as described previously.^{27,30} Briefly, K18 Δ 280K-tau solution was diluted with phosphate buffered saline (pH 7.4) to a final concentration of 20 μ M. The solution was incubated at 37 °C for a day with agitation (1000 rpm). Fibril formation was confirmed by thioflavin-S fluorescence spectroscopy.

In vitro fluorescence binding assay

Synthetic K18 Δ 280K-tau fibrils (200 nM) were incubated with 3 μ M (THK-951 and THK-5272) or 5 μ M (THK-953 and THK-5273) compounds at room temperature for 1 h. The fluorescence intensity was measured at an excitation wavelength appropriate for each compound. The incubations were carried out on Nunc 96 MicroWell™ black plates (Nalge Nunc Int., Rochester, NY, USA), using 200 μ L of phosphate buffer saline (pH 7.4) as the reaction mixture. All experiments were conducted in triplicate.

In vitro neuropathological staining using brain sections

The staining properties of the tested compounds were examined using postmortem brain tissues from an autopsy-confirmed case of AD (82-year-old woman). The experiments were performed in accordance with the regulations of the ethics committee of Tohoku University. A series of 8 μ m thick sections from paraffin-embedded blocks of hippocampus were used for staining. According to the method reported previously,^{28,31} the sections were immunostained with anti-tau antibody (AT8; Innogenetics, Ghent, Belgium). Stained images were examined using an Olympus (Tokyo, Japan) BX51 microscope equipped with blue-violet (BP, 400–440; DM, 455; BA, 475) and blue filters (BP, 460–490; DM, 500; BA, 520IF).

Competitive binding assay with [¹⁸F]THK-523

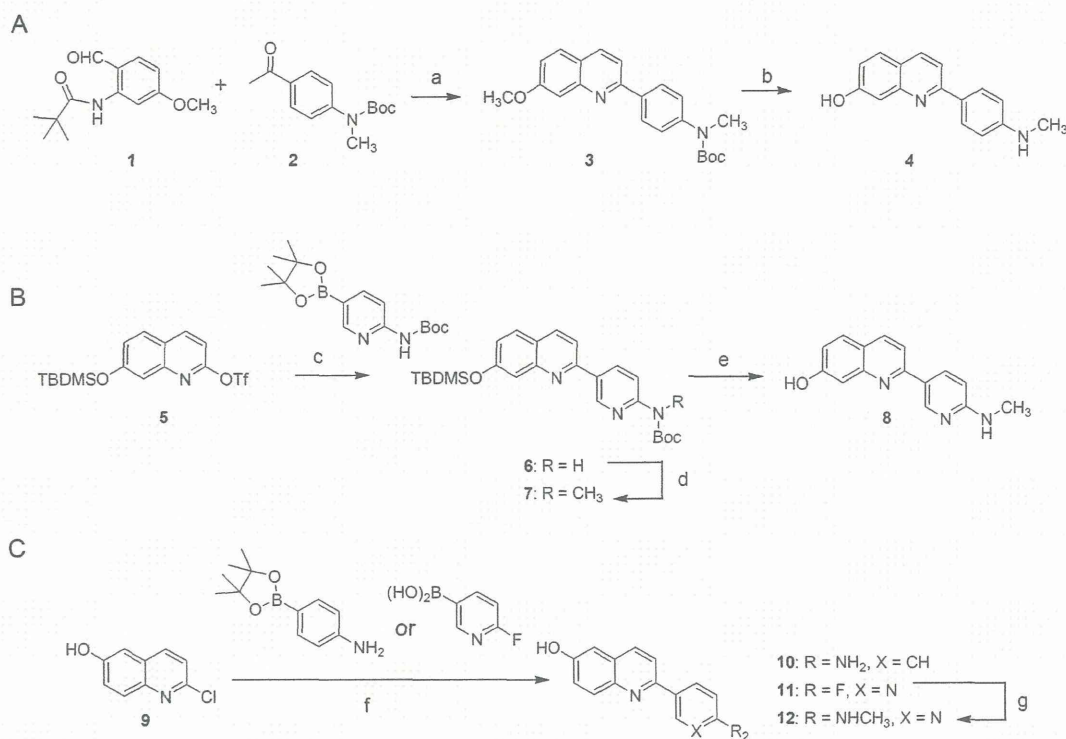
[¹⁸F]THK-523 was synthesized as described previously.²⁷ The product was formulated with DMSO. The specific activity of [¹⁸F]THK-523 was 121.7 GBq/ μ mol (end of synthesis), and the radiochemical purity was >99%. Competitive binding assay was performed as described previously²⁸ with [¹⁸F]THK-523 (final concentration: 4.1 nM) used as a radioligand of the assay.

Autoradiography of [¹¹C]THK-951

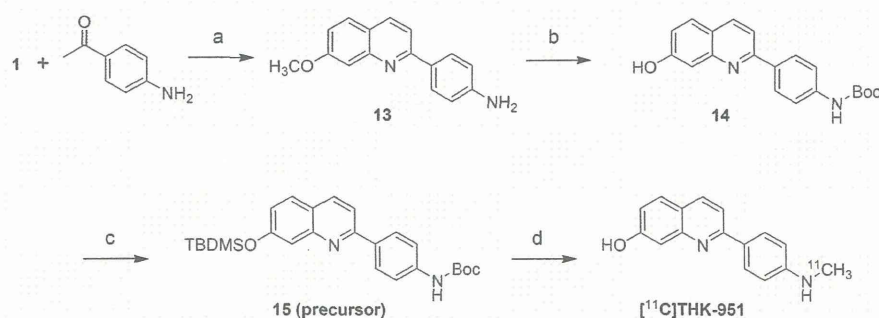
The hippocampal brain sections from a case of AD (82-year-old woman) were used for *in vitro* ARG. Deparaffinized sections were treated with a saline solution of [¹¹C]THK-951 (25% ethanol, 0.4 MBq/mL) or [¹⁸F]THK-523 (25% DMSO, 0.6 MBq/mL) for 10 min at room temperature. The sections were washed briefly with H₂O and 50% ethanol. After drying, the labeled sections were exposed to a BAS-III imaging plate (Fuji Film, Tokyo, Japan) for 3 h ([¹¹C]THK-951) or overnight ([¹⁸F]THK-523). Autoradiographic images were obtained using the BAS-5000 phosphor imaging instrument (Fuji Film). Adjacent brain sections were immunostained with anti-tau antibody (AT8; Innogenetics) and anti-A β antibody (6F/3D; Dako, Glostrup, Denmark).

Ex vivo biodistribution assay of [¹¹C]THK-951 in normal mice

Ex vivo biodistribution assay was performed as described previously.²⁸ Briefly, [¹¹C]THK-951 (saline solution: 1.8–1.9 MBq/200 μ L) was administered to male ICR mice (average weight: 29 g) via the tail vein. The mice were anesthetized with isoflurane and sacrificed by decapitation at 2, 10, 30, 60, and 90 min postinjection. The brain, blood, and other organs were excised.



Scheme 1. Synthesis of hydroxyquinoline derivatives (refer to Supporting Information). A: Synthesis of THK-951. (a) KHMOS, THF. (b) BBr_3 , CH_2Cl_2 . B: Synthesis of THK-953. (c) Na_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, $\text{DME}/\text{H}_2\text{O}$. (d) CH_3 , NaH , DMF . (e) 1. TBAF, THF, 2. TFA, CH_2Cl_2 . C: Synthesis of THK-5272 and THK-5273. (f) Na_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, $\text{DME}/\text{H}_2\text{O}/\text{EtOH}$. (g) NH_2CH_3 , MeOH .



Scheme 2. Synthesis of the precursor (refer to Supporting Information) and radiosynthesis of $[^{11}\text{C}]\text{THK-951}$. (a) 1. KHMOS, THF, 2. concentrated HCl, EtOH . (b) 1. BBr_3 , CH_2Cl_2 , 2. $(\text{Boc})_2\text{O}$, NaHCO_3 , $\text{THF}/\text{H}_2\text{O}$, 3. aqueous KOH, MeOH . (c) TBDMSCl, imidazole, DMF . (d) 1. $[^{11}\text{C}]\text{CH}_3\text{OTf}$, tBuOK , DMSO , 2. 2 N HCl.

After the organs were weighed, their radioactivity was counted with an automatic γ -counter. The percentage injected dose per gram (%ID/g) was calculated by comparing tissue counts to tissue weight. Each % ID/g value is an average of four separate experiments.

Results and discussion

Pittsburgh compound B, a representative β -amyloid probe for PET, is a 6-hydroxy substituted derivative of 2-arylbenzothiazole. Introduction of a hydroxyl group resulted in low lipophilicity of the probe and rapid clearance of free radioactivity from the brain.⁶ These characteristics are essential features for brain imaging probes because nonspecific signals interfere with quantitative analysis of PET images. Therefore, we focused on hydroxyl substituted BF-158 derivatives as tau imaging

probe candidates expecting they would provide appropriate brain kinetics.

The synthetic procedures of THK-951, THK-953, THK-5272, and THK-5273 are shown in Scheme 1 (details of the synthesis are described in the Supporting Information). THK-951 is a 2-phenylquinolin-7-ol derivative synthesized through a condensation reaction of two phenyl rings (Scheme 1A). The cyclocondensation of benzaldehyde (1) and acetophenone (2) in the presence of potassium *bis*(trimethylsilyl)amide gave 3. THK-951 (4) was obtained by the deprotection of 3 by boron tribromide.

The THK-953 synthesis was outlined in Scheme 1B. 6 was obtained by Suzuki coupling between 7-hydroxyl-protected quinoline triflate and pyridine boronic acid pinacol ester. After the *N*-methylation (7), deprotection of the TBDMS and Boc groups by treating with TBAF and TFA, respectively, gave THK-953 (8).

The 2-chloroquinolin-6-ol (**9**), which was prepared from quinolin-2,6-diol by selective chlorination, was used for the synthesis of the 6-hydroxy substituted 2-arylquinoline derivatives **10** (THK-5272) and **11** via Suzuki coupling reaction (Scheme 1C). THK-5273 (**12**) was obtained by a nucleophilic substitution reaction with methylamine from **11**.

To estimate the blood–brain barrier permeability of hydroxyquinoline derivatives, logP values were measured by an HPLC method and found to range from 0.5 to 1.3 (Table 1). These data indicated that the hydroxyquinoline derivatives have sufficient lipophilicity to penetrate the blood–brain barrier.²⁷

To evaluate the tau fibril binding characteristics of the synthesized hydroxyquinoline derivatives, we carried out fluorescence analyses (Figure 2). Fluorescence of the dye thioflavin-T or thioflavin-S increases when they bind to amyloid fibrils including fibrils from recombinant tau constructs.^{30,32} Similar to these dyes, BF-158 derivatives show enhanced fluorescence intensities upon binding to a cross β -sheet structure. The results from the fluorescence binding assays using recombinant K18 Δ 280K-tau fibrils indicated that the fluorescence enhancement of THK-951 and THK-5272 was observed in the presence of tau fibrils (Figure 2A, G). In contrast, the pyridine-containing derivatives, THK-953 and THK-5273, showed little change in fluorescence intensity or, interestingly, slightly weakening in the presence of tau fibrils (Figure 2D, J). The elaborate verification of the cause of this fluorescence change was not examined in the present study, but the possibility of a quenching effect caused by the binding conformation of the compounds is presumed.

Subsequently, fluorescent staining using AD hippocampal sections was performed to examine the in vitro binding affinity of the tested compounds for tau pathology in AD tissues. THK-951 clearly stained NFTs and neuropil threads in the hippocampal section of an AD brain under a blue filter (Figure 2B, C). THK-5272 also stained NFTs but with a lower contrast image compared with THK-951 (Figure 2E, F). The neuropathological staining with BF-170, which is a dehydroxylated derivative of THK-5272, showed high affinity for NFTs rather than SPs²⁶; thus, a contribution from the substituted position of the hydroxyl group to the affinity for NFTs was indicated. THK-953 (Figure 2H, I) and THK-5273 (Figure 2K, L) are highly fluorescent compounds, but very weak images of tau pathology were observed in this study. These results correlated with the results from the fluorescence binding assay using recombinant tau fibrils and indicated that the quinoline-pyridine derivatives bind very little to regions of tau pathology or have low binding affinity easily removable by a brief wash.

For further comparison of the binding affinity of hydroxyquinoline derivatives for recombinant tau fibrils, we determined their K_i values

in competition with [¹⁸F]THK-523, a tau imaging probe for PET. The K_i values of THK-951, THK-953, THK-5272, and THK-5273 were 20.7, 110.4, 36.1, and 30.4, respectively (Table 1). THK-951 had the highest affinity for tau fibrils from the hydroxyquinoline derivatives tested. In contrast with the image obtained for its fluorescent staining, THK-5273 showed comparable affinity with THK-5272. The weak staining of THK-5273 was probably caused by its high sensitivity to the washing technique used or the absence of a fluorescent hyperchromic effect compared with THK-5272.

In this study, the quinoline derivatives with higher lipophilicities had lower K_i values. For $A\beta$ ligands, it is considered that the lipophilicity of the ligand correlates with the degree of nonspecific binding but is not correlated with its binding affinity for $A\beta$ fibrils.^{6,33} It is possible that the lipophilicity of a compound has a considerable effect on its binding affinity for the cross β -sheet structures of tau; however, more structure–activity related studies are needed to understand this further.

Following the results of the fluorescence assays and competitive binding assay, we selected THK-951 for additional studies using a radiolabeled ligand, with consideration of the availability of THK-951 for a tau PET probe. Radiolabeling of THK-951 was performed using an *N*-Boc and *O*-TBDMS protected precursor (**15**) and [¹¹C]MeOTf (Scheme 2). [¹¹C]THK-951 was obtained in yields of 39% on average with radiochemical purity greater than 99% after HPLC purification. The average specific activity of [¹¹C]THK-951 was 83.2 GBq/ μ mol.

The poor brain kinetics of a PET tracer can harm the quantitative performance of a PET study, so high blood–brain barrier permeability and rapid clearance from the brain are essential for brain imaging agents. To evaluate the brain kinetics of [¹¹C]THK-951, we carried out an ex vivo biodistribution study using normal mice (Table 2). [¹¹C]THK-951 showed rapid brain uptake ($3.23 \pm 0.27\%$ ID/g at 2 min) and smooth washout from the normal brain ($0.11 \pm 0.01\%$ ID/g at 60 min), resulting to a high 2-min-to-60 min ratio (29.4) of brain uptake of [¹¹C]THK-951. This uptake ratio in a normal mouse brain is superior to that of [¹⁸F]THK-523 (1.86), [¹⁸F]THK-5105 (9.20), or [¹⁸F]THK-5117 (23.1),²⁸ indicating that our structural optimization worked as intended. Compared with these ¹⁸F-labeled tracers, the binding affinity of [¹¹C]THK-951 for tau aggregates is slightly lower, but the superiority in brain kinetics could make up for its weakness in binding affinity. It is known that the concentrations of tau aggregates are lower than those of $A\beta$ in an AD brain²²; therefore, a higher elimination efficiency of the free radiotracer from the brain would be more important for tau probes than for $A\beta$ probes. The fast clearance of [¹¹C]THK-951 from a normal mouse brain is likely caused by its low lipophilicity. The uptake in the kidney was highest at 2 min postinjection (19.8 % ID/g) followed by a fast clearance. The uptake in the liver showed a slow clearance after 10 min postinjection, while the radioactivity in the small intestine increased with time, indicating the biliary excretion of radioactive agents.

Finally, as an assessment of the binding affinity of [¹¹C]THK-951 for the regions of tau pathology, ARG analysis using AD hippocampal sections was performed. The ARG image showed high accumulation of [¹¹C]THK-951 in the CA1 region of the AD hippocampus (Figure 3A). Distribution of the tracer signal of [¹¹C]THK-951 correlated well with that of [¹⁸F]THK-523 (Figure 3B) and tau immunostaining (Figure 3C). On the other hand, the distribution of the tracer signal was different from the immunostained $A\beta$ pathology, which showed spotty distribution in that region (Figure 3D).

Table 1. Lipophilicity (logP) and binding affinity (K_i values) of hydroxyquinoline derivatives to recombinant tau fibrils

Compound name	X	R ₁	R ₂	logP*	K_i (nM)
THK-951	C	NHCH ₃	7-OH	1.28	20.7
THK-953	N	NHCH ₃	7-OH	0.56	110.4
THK-5272	C	NH ₂	6-OH	0.61	36.1
THK-5273	N	NHCH ₃	6-OH	0.90	30.4

*logP values were determined by HPLC method.

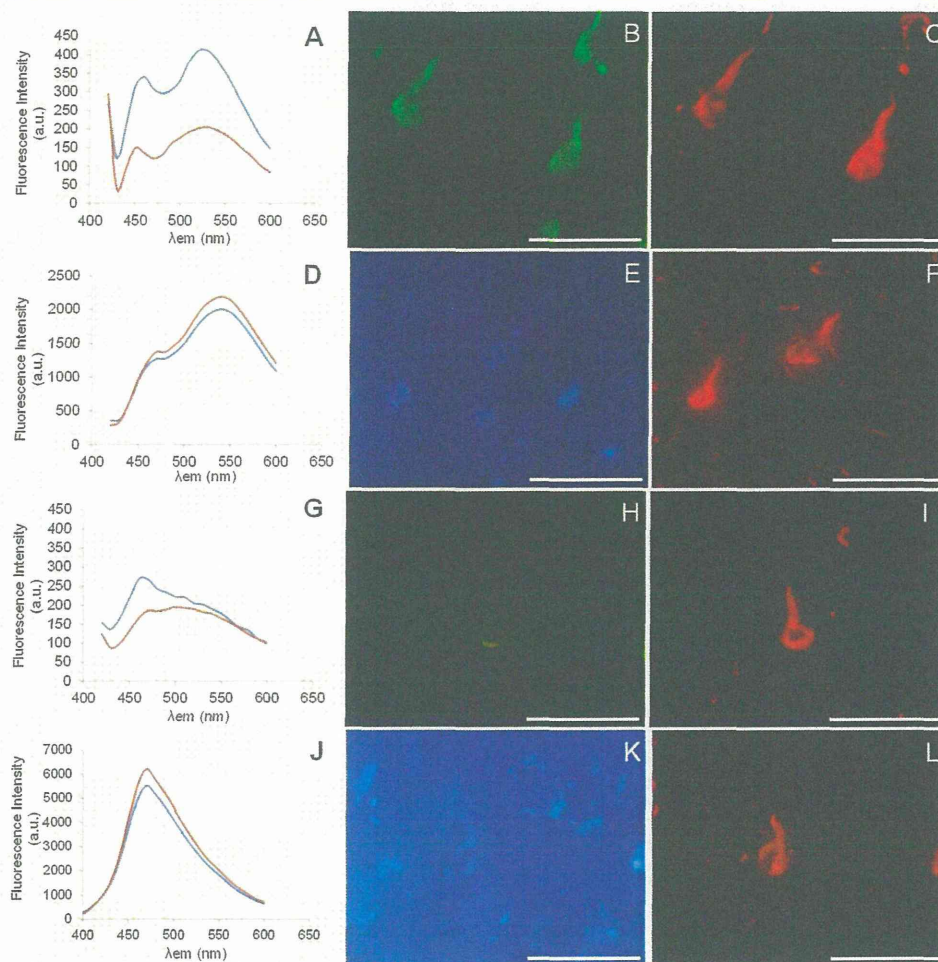


Figure 2. Fluorescence spectral analyses and neuropathological staining of THK-951 (A–C), THK-953 (D–F), THK-5272 (G–I), and THK-5273 (J–L). (A, D, G, and J) Fluorescence spectra of THK-951 (A, $\lambda_{\text{ex}} = 390$ nm), THK-953 (D, $\lambda_{\text{ex}} = 390$ nm), THK-5272 (G, $\lambda_{\text{ex}} = 390$ nm), and THK-5273 (J, $\lambda_{\text{ex}} = 360$ nm) in the presence or absence of K18 Δ 280K-tau fibrils (K18 Δ 280K-tau: blue lines; control (ligands only): red lines). (B, C, E, F, H, I, K, and L) Double staining with hydroxyquinoline derivatives and anti-pTau antibody (AT8). (B, E, H, and K) Fluorescent staining of hippocampal sections from cases of Alzheimer’s disease (THK-951: B; THK-953: E; THK-5272: H; THK-5273: K). (C, F, I, and L) Immunostaining by AT8. Scale bars: 50 μm . This figure is available in color online at wileyonlinelibrary.com/journal/jlcr

Table 2. Biodistribution of [^{11}C]THK-951 in ICR mice

Tissue	2 min	10 min	30 min	60 min	90 min
Blood	2.65 (0.46)	1.20 (0.48)	0.20 (0.04)	0.12 (0.01)	0.11 (0.02)
Brain	3.23 (0.27)	0.81 (0.13)	0.15 (0.05)	0.11 (0.01)	0.06 (0.01)
Liver	10.14 (2.66)	10.00 (1.79)	1.99 (0.45)	1.10 (0.23)	0.85 (0.27)
Kidney	19.79 (6.77)	4.10 (0.34)	0.96 (0.43)	0.49 (0.11)	0.35 (0.09)
Small intestine	4.72 (1.44)	12.22 (3.10)	24.91 (3.17)	29.33 (2.23)	24.64 (1.83)

Data are expressed as mean of % ID/g (Standard Deviation) ($n = 4$).

These results indicated that [^{11}C]THK-951 was promising in its ability to bind regions of tau pathology in the AD brain.

In vivo tau imaging will allow the accurate measurement of the distribution of tau pathology in the AD brain. The combined

assessments of tau burden and other biomarkers of AD (e.g., $A\beta$ burden, brain metabolism, and clinical findings) are needed to understand the accurate pathogenic mechanism or time course of AD.⁴ One of the advantages of developing ^{11}C -labeled tau probes is that tau probes labeled with this short-half-life radionuclide will facilitate two PET studies in the same day by using other PET probes such as [^{18}F]FDG or $A\beta$ PET after the tau imaging.

To develop probes with ideal brain kinetics, we introduced a hydroxyl group at the 6 or 7 position of quinoline. Lipophilicity has considerable influences on not only tracer kinetics but also binding affinity; therefore, lipophilicity needs to be optimized for tau tracers. In this study, the properties of [^{11}C]THK-951 ($\log P = 1.28$) were balanced between achieving high binding affinity for tau fibrils and blood–brain permeability with a fast clearance from mouse brain. We plan to evaluate other derivatives having different hydroxylated positions or alkyl amino groups to obtain additional structure–activity relationship information.

A competitive binding assay using a radiolabeled ligand may ensure the examination of tau binding ligands, which do not seem to have binding affinity for tau pathology in a fluorescence

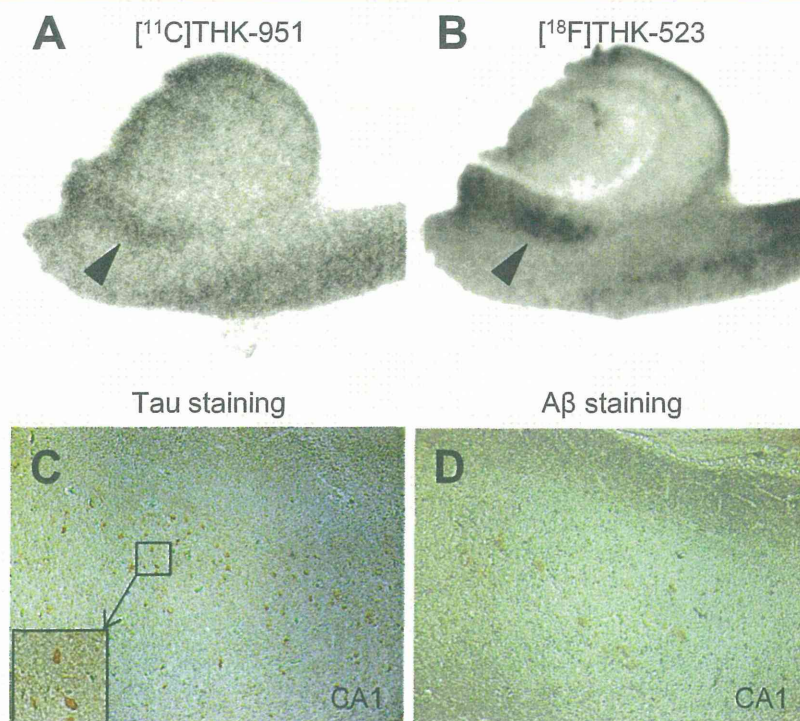


Figure 3. Characterization of in vitro binding of [^{11}C]THK-951 to tau pathology by autoradiography and immunohistochemistry. [^{11}C]THK-951 showed high accumulation in the hippocampal CA1 region (A, filled arrowhead). Similarly, [^{18}F]THK-523, a well-characterized tau probe, also highly accumulated in the CA1 region (B, filled arrowhead). Immunostaining of adjacent sections demonstrated that numerous tau immunopositive neurofibrillary tangles were observed in CA1 region (C). By contrast, $\text{A}\beta$ staining showed weak immunoreactivity in CA1 region (D).

binding assay using AD brain sections. On the other hand, some researchers indicated that the PHFs constructed from tau fragments do not fully conform to the structure of PHFs from an AD brain.^{22,32} Thus, these in vitro binding assay results should be considered carefully, and comparison with the results from future clinical studies would facilitate our selection of an evaluation method.

Conclusion

We synthesized four hydroxyquinoline derivatives and performed biological evaluations to estimate their potential as tau imaging probes. From these results, we selected THK-951 for additional studies and successfully radiosynthesized [^{11}C]THK-951. [^{11}C]THK-951 showed ideal brain kinetics in normal mice, and its binding affinity for regions of tau pathology was confirmed by an in vitro ARG assay using AD hippocampal sections. Further evaluation of THK-951 and structurally optimized derivatives will facilitate our understanding about AD tau pathology in the living body.

Acknowledgements

This study was supported by the Industrial Technology Research Grant Program of the NEDO (09E51025a) in Japan, the Health and Labour Sciences Research Grants from the Ministry of Health, Labour, and Welfare of Japan, Grant-in-Aid for Scientific Research (B) (23390297), and the 'Japan Advanced Molecular Imaging Program (J-AMP)' of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Conflict of Interest

This study was supported by the research fund from GE Healthcare.

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Supporting information

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