Fluorinated and iodinated (*Z*)-2-(4-(2-fluoroethoxy)benzylidene)-5-iodobenzofuran-3(2*H*)-one

[^{18}F/^{125}I]3

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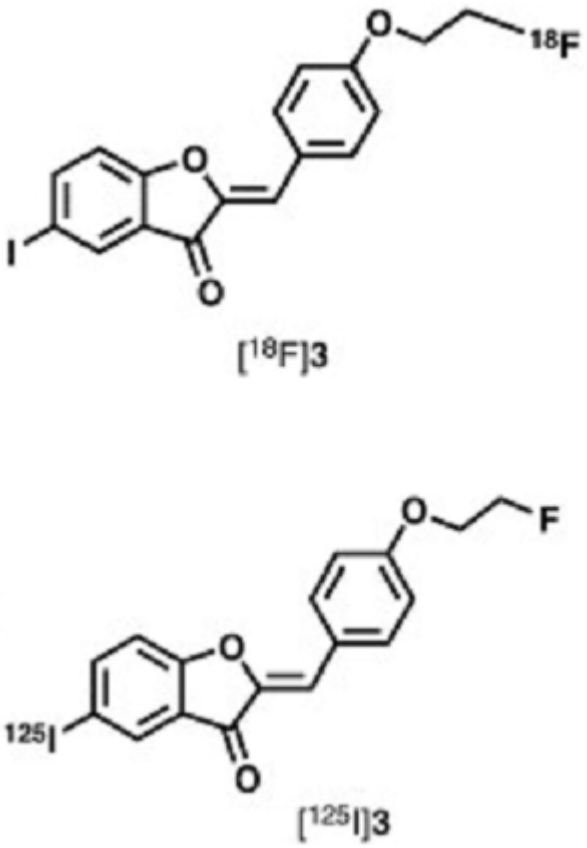
Chemical name:	Fluorinated and iodinated (*Z*)-2-(4-(2-fluoroethoxy)benzylidene)-5-iodobenzofuran-3(2*H*)-one
Abbreviated name:	[^{125}I]3, [^{18}F]3
Synonym:	
Agent Category:	Compounds
Target:	β-amyloid (Aβ)
Target Category:	Accepters
Method of detection:	Single-photon emission computed tomography (SPECT), positron emission tomography (PET)
Source of signal / contrast:	^{125}I, ^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents • Humans



Structures of [^{125}I]3 and [^{18}F]3 by Watanabe et al. (1).

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Background

[PubMed]

Fluorinated and iodinated (*Z*)-2-(4-(2-fluoroethoxy)benzylidene)-5-iodobenzofuran-3(2*H*)-one (compound 3), abbreviated as [^{18}F]3 and [^{125}I]3, respectively, is an aurone derivative synthesized by Watanabe et al. for single-photon emission computed tomography (SPECT) and positron emission tomography (PET) of Alzheimer's disease (AD) by targeting β-amyloid (Aβ) plaques (1).

AD is characterized in pathology by the presence of extracellular Aβ plaques, intraneuronal neurofibrillary tangles, and neuronal loss in the cerebral cortex (2, 3). Of them, Aβ deposit is the earliest neuropathological marker and is relatively specific to AD and closely related disorders. Aβ plaques are composed of abnormal paired helical filaments 5–10 nm in size. These filaments are largely made of insoluble Aβ peptides that are 40 or 42 amino acids in length (4).

In recent years, molecular imaging by targeting the extracellular Aβ has been intensively investigated in attempts to detect early AD, assess Aβ content *in vivo*, determine the timing of anti-plaque therapy, and evaluate the therapeutic efficacy (4). Radiolabeled Aβ40 peptides were tested first, but they showed poor penetration ability to cross the blood–brain barrier (BBB) (4). Based on the fact that Aβ can be specifically stained *in vitro* with dyes of Congo red, chrysamine G, and thioflavin-T, an effort was made to develop imaging agents with these dyes. This effort, however, was in general unsuccessful because the bulky ionic groups of heteroatoms in these dyes prevent them from crossing the BBB (2). Importantly, a large class of derivatives (e.g., aminonaphthalenes, benzothiazoles, stilbenes, and imidazopyridines) was synthesized with these dyes as templates (4). Clinical and preclinical studies have shown that these derivatives not only possess a high binding affinity with Aβ plaques as their parent compounds, but also exhibit good penetration ability through the BBB and rapid washout from brain.

Ono et al. first synthesized a class of radioiodinated flavone derivatives that present a high binding affinity with Aβ plaques and good penetration ability through the BBB (5). However, these flavone derivatives display poor clearance from the brain, which leads to a high brain background. The investigators then explored another class of flavonoids with aurone as the core structure (6, 7). Aurone is a heterocyclic chemical compound that contains a benzofuran element associated with a benzylidene linked in position 2 and a chalcone-like group being closed into a five-member ring. The aurone derivatives possess a nucleophilic group (NH_2, NHMe, or NMe_2) at the 4' position and a radioiodine at the 5

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position. Although these aurone derivatives exhibit a strong binding affinity with Aβ (inhibition constant (K_i) = 1.2–6.8 nM), high penetration ability through the BBB (1.9% –4.6% injected dose per gram tissue (ID/g) at 2 min), and a fast washout from the brain (0.3%–0.5% ID/g at 30 min), the pharmacokinetics of these compounds are less favorable for brain imaging than the pharmacokinetics of the agent [^{123}I]IMPY (6-iodo-2-(4'-dimethylamino)phenyl-imidazo[1,2]pyridine), which is the only SPECT agent to be tested in humans to date (1, 8, 9). The investigators also modified the flavone and aurone derivatives by pegylating them with 1–3 units of ethylene glycol at the 4' position or by conjugating them with the chelating agent bis-amino-bis-thiol (BAT) (7). Favorable pharmacokinetics for brain imaging was observed for the pegylated derivatives ([^{18}F]8(a–c)) but not for the BAT-chelated derivatives ([^{99m}Tc]BAT-FL and [^{99m}Tc]BAT-AR) (6, 7).

This series of chapters summarizes the data obtained with flavone and aurone derivatives, including [^{125}I]15, [^{125}I]9, [^{125}I]14, [^{125}I]16, [^{125}I]17, [^{99m}Tc]BAT-FL, [^{99m}Tc]BAT-AR, [^{18}F]8(a-c), [^{125}I]3, and [^{18}F]3 (1, 6-8). This chapter presents the data obtained with [^{125}I]3 and [^{18}F]3 (1).

Related Resource Links:

- Amyloid-targeted imaging agents in MICAD
- Amyloid-targeted imaging clinical trials in ClinicalTrials.gov
- Structures and other information of amyloid peptides in PubChem
- Alzheimer's disease articles in Online Mendelian Inheritance in Man

Synthesis

[PubMed]

Compound 3 was prepared by the reaction of 5-iodobenzofuran-3(2*H*)-one with 4-(2-fluoroethoxy)benzaldehyde in the presence of Al_2O_3 in chloroform at room temperature (yield = 27%) (1). Its tributyltin derivative ((*Z*)-2-(4-(2-fluoroethoxy)benzylidene)-5-(tributylstannyl)benzofuran-3(2*H*)-one) was prepared using a halogen-to-tributyltin exchange reaction catalyzed by Pd(0) (yield = 32.2%). The tributyltin derivative was used for radioiodination to prepare [^{125}I]3. To prepare [^{18}F]3, 5-iodobenzofuran-3(2*H*)-one was first reacted with 4-(2-hydroxyethoxy)benzaldehyde in the presence of Al_2O_3 in chloroform at room temperature to form (*Z*)-2-(4-(2-hydroxyethoxy)benzylidene)-5-iodobenzofuran-3(2*H*)-one (compound 5; yield = 48%). The free OH groups of compound 5 were then converted into a tosylate (Ts) by reacting with TsCl in the presence of pyridine to produce (*Z*)-2-(4-(2-tosyloxyethoxy)benzylidene)-5-iodobenzofuran-3(2*H*)-one (compound 6), which was used as the precursor to prepare [^{18}F]3. The radiochemical identities of [^{125}I]3 and [^{18}F]3 were verified with high-performance liquid chromatography (HPLC).

The radiochemical yields of both [^{125}I]3 and [^{18}F]3 were >23%, and their radiochemical purities were >95% after purification with HPLC. [^{125}I]IMPY was also prepared as a competing radioligand using the standard iododestannylation reaction. [^{125}I]IMPY had a

radiochemical purity of >95%. The theoretical specific activities of [^{125}I]3 and [^{125}I]IMPY were anticipated to be similar to that of ^{125}I (81.4 TBq/mmol (2,200 Ci/mmol)) with no-carrier-added preparation. No data for measured specific activities of [^{125}I]3, [^{18}F]3, and [^{125}I]IMPY were reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding affinity of compound 3 was measured with Aβ(1–42) aggregates; [^{125}I]IMPY was used as the competing radioligand (1). The measurement showed a K_i value of 6.81 nM. The octanol/buffer partition coefficient (log *P*) of [^{125}I]3 was measured to be 2.45 ± 0.04. The log *P* value of [^{18}F]3 was not reported.

The binding of unradiolabeled compound 3 with mouse Aβ plaques was confirmed in brain sections of double transgenic AD mice (*Tg2576*) with fluorescent staining (1). Amyloid plaques were clearly stained with compound 3, and the labeling pattern was consistent with that observed with thioflavin S staining. These results suggest that compound 3 can specifically bind with Aβ plaques in the mouse brain.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{125}I]3 and [^{18}F]3 was tested in normal mice (n = 5–6 mice/time point for each agent) (1). The initial brain uptake of [^{125}I]3 was 2.34% ID/g at 2 min, and the agent was then rapidly eliminated (0.19% ID/g at 60 min after injection). [^{18}F]3 showed an initial uptake of 3.66% ID/g at 2 min and rapid clearance from brain, with 1.75% ID/g at 60 min after injection. Although [^{125}I]3 and [^{18}F]3 displayed some difference in brain uptake, these results suggest that [^{125}I]3 and [^{18}F]3 may function as a PET/SPECT probes for detecting Aβ plaques in the AD brain.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

Postmortem brain tissues from an autopsy-confirmed case of AD and a control subject were used to analyze the binding of [^{125}I]3 with human Aβ plaques in brain sections with autoradiography (1). Autoradiographic images showed high levels of radioactivity in the brain sections, and the hot spots of [^{125}I]3 were consistent with those of *in vitro* immunohistochemical staining in the same brain sections. Normal human brain displayed no remarkable accumulation of [^{125}I]3. These results demonstrate the feasibility of using [^{125}I]3 as a SPECT probe for detecting Aβ plaques in the brains of AD patients. No data were reported for the binding of [^{18}F]3 with human Aβ plaques.

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