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Development and evaluation of [^{18}F]Flotaza for Aβ plaque imaging in postmortem human Alzheimer's disease brain

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Abstract

Positron emission tomographic (PET) studies of amyloid β (Aβ) accumulation in Alzheimer's disease (AD) have shown clinical utility. The aim of this study was to develop and evaluate the effectiveness of a new fluorine-18 radiotracer [^{18}F]Flotaza (2-{2-[2-[^{18}F]fluoroethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene), for Aβ plaque imaging. Nucleophilic [^{18}F]fluoride was used in a one-step radiosynthesis for [^{18}F]flotaza. Using post mortem human AD brain tissues consisting of anterior cingulate (AC) and corpus callosum (CC), binding affinity of Flotaza, Ki = 1.68 nM for human Aβ plaques and weak (>10^{-5} M) for Tau protein. Radiosynthesis of [^{18}F] Flotaza was very efficient in high radiochemical yields (>25%) with specific activities >74 GBq/μmol. Brain slices from all AD subjects were positively immunostained with anti-Aβ. Ratio of [^{18}F]Flotaza in gray matter AC to white matter CC was >100 in all the 6 subjects. Very little white matter binding was seen. [^{18}F]Flotaza binding in AC strongly correlated with anti-Aβ immunostains. [^{18}F]Flotaza is therefore a suitable fluorine-18 PET radiotracer for PET imaging studies of human Aβ plaques.

Keywords

[^{18}F]Flotaza; Human Aβ plaques; Alzheimer's disease; PET imaging

Introduction

Successful clinical research studies using [^{11}C]PIB for diagnostic evaluation of amyloid β (Aβ) plaques in Alzheimer's disease (AD) patients and therapeutic efficacy assessment of drugs for AD has accelerated the development and translation of positron emission tomography (PET) radiotracers for clinical use.[1] Substantial studies on the accumulation of Aβ plaques in the brains of patients with AD have been carried out in the Alzheimer's disease neuroimaging initiative.[2] further supporting previous pathological findings (Braak et al., 2011).[3]

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The diagnostic value of imaging Aβ plaques accelerated the development of fluorine-18 labeled longer half-life radiotracers for easier translation to clinical use. [^{18}F]Florbetapir (Fig. 1; **2**) was the first fluorine-18 agent approved for clinical use in AD.[4] This was followed by [^{18}F]florbetaben (Fig. 1; **3**) and [^{18}F]flutemetamol (Fig. 1; **4**). Although the fluorine-18 offers advantages of the longer half-life, high white matter binding of these radiotracers resulting in low standard uptake values (SUV) in the cortex of AD patients compared to normal subjects has been an issue. Thus, an agent that can provide a significantly higher SUV in the AD cortex may be an improvement towards clinical value. There has been continued interest in the development of Aβ plaque imaging agents.[5] With increasing efforts to find treatments and cure for AD, there is much research into imaging plaques essential to the diagnosis and clinical management of AD.[6]

We previously reported [^{11}C]TAZA as a radiotacer for Aβ plaques (Fig. 1; **5**).[7] The following unique properties of [^{11}C]TAZA were observed: (1). Increased signal to noise ratio. In postmortem human AD brain sections, [^{11}C]TAZA exhibited higher binding to the Aβ-plaques compared to [^{11}C]PIB. The increased signal to noise ratio may increase sensitivity of detection of Aβ-plaques in PET studies. (2). Decreased white matter binding. A lower amount of white matter binding was seen with [^{11}C]TAZA due to the heteroatoms in the “azo” functionality. This improved difference between grey matter and white matter may improve earlier delineation of AD, MCI and normal controls.

In order to obtain a fluorine-18 analog of [^{11}C]TAZA, two options included incorporation of fluiorine-18 on the aromatic ring or use the polyethylene glycol 3 (PEG3) strategy used for florbetaben and florbetapir. Because of structural similarity of TAZA backbone with the stilbene derivatives incorporation of PEG3 would be an appropriate strategy. Thus, 2-{2-[2-Fluoroethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene (Flotaza; Fig. 1; **6)** was considered an appropriate fluorinated analog of TAZA, which may potentially bind to Aβ plaques with high affinity.

Synthesis of Flotaza is shown in Fig. 2 starting with 4-hydroxy-4′-dimethylaminoazobenzene (**7**). Pegylated alcohol, **8** was successfully prepared by reacting **7** with bromo-PEG3-alcohol in moderate yields.[8] Reaction of the alcohol **8** with diethylamino sulfur trifluoride (DAST) led to a complex mixture, which may have been due to the high reactivity of DAST. Therefore, the alcohol was first converted to the corresponding tosylate **9**,[9] followed by nucleophilic fluoride displacement using tetrabutylammonium fluoride.[10] This reaction was cleaner and provided Flotaza **10**, in good yields. Overall, the synthesis of Flotaza and tosylate precursor was simplified compared to that of florbetaben and florbetapir because of the *N*,*N*-dimethyl functionality not requiring protection and deprotection.

Using our previously reported methods, in vitro binding affinity of Flotaza in human AD brain slices using [^{3}H]PIB for Aβ plaques[11,12] and [^{125}I]IPPI for Tau[13,14] were carried out. The affinity of Flotaza was marginally weaker than TAZA for Aβ plaques (Ki = 1.68 nM for Flotaza versus 0.54 nM for TAZA), suggesting that fluoropegylation is well tolerated in the TAZA backbone. Flotaza did not have any significant effect on the binding of [^{125}I]IPPI confirming weak Tau binding (Fig. 3). Thus, Flotaza is a selective Aβ plaque agent.

Compared to the two other fluoropegylated derivatives, florbetaben and florbetapir, Flotaza has comparable, good affinity.[7] This is indicative of the good prospects of [^{18}F]Flotaza of serving as an imaging agent for Aβ plaques. Additionally, the diaza functionality in Flotaza provides unique flexibility in binding to the Aβ plaques. This was observed in our reported results of increased [^{11}C]TAZA binding to human Aβ plaques compared to [^{11}C]PIB and [^{11}C]Dalene.[7]

Similar to our previously reported alkyl tosylates used for nucleophilic fluorine-18 labeling,[15] The tosylate precursor, **9** served as an excellent precursor for one-step nucleophilic fluorine-18 radiolabeling. Radiosynthesis of [^{18}F]Flotaza was efficiently carried out and purified on reverse-phase HPLC.[16] RadioTLC confirmed radiochemical purity of >95% for [^{18}F]Flotaza (Fig. 4) and was obtained in amounts of 370–740 MBq in specific activities generally >37 TBq/mmol.[16] [^{18}F]Flotaza was found to be stable in 10% ethanolic saline solution for in vitro studies.

Radiosynthesis of [^{18}F]Flotaza is simpler because it can be accomplished in a single step without the need for a deprotection step, which is the case with the other fluorine-18 labeled Aβ plaque radiotracers shown in Fig. 1. The precursor tosylate is easier to make from commercially available reagents and these reagents, including [^{18}F]Flotaza are not propreitory, unlike the other fluorine-18 radiotracers shown in Fig. 1. Thus, this should enable easier access to an excellent fluorine-18 PET radiotracer for Aβ plaque imaging studies.

Well characterized brain samples were obtained from Banner Health Research Institute.[17] Brain slices from six AD subjects included anterior cingulate (gray matter, GM) and corpus callosum (white matter, WM) as shown for one subject in Fig. 5A. The AD brain sections of the six AD subjects were further confirmed to contain Aβ plaques in the GM regions by immunostaining with anti-Aβ Biolegend (known to stain Aβ1–16) as shown in Fig. 5B.[18]

Extensive binding of [^{18}F]Flotaza was seen in the grey matter regions of all the AD subjects.[19] Figure-5C shows brain slice of one subject with binding of [^{18}F]Flotaza in the anterior cingulate, while white matter had very little binding. This grey matter binding was significantly reduced when the brain sections were treated with PIB. Figure-5E shows [^{18}F] Flotaza binding through the cortical layers, showing greater binding in the outer layers. Similarly, high levels of binding in the gray matter were seen in all the six subjects (Fig. 5D) and was consistent with immunostaining in adjacent sections. White matter binding was very small across all the subjects and ratios between gray matter and white matter was found to be >100 in all the subjects. It must be noted that in these experiments, after [^{18}F]Flotaza binding, the slices were washed with 50% alcohol in PBS buffer. The white matter binding increased significantly when the slices were washed only with PBS buffer.

The high degree of binding of [^{18}F]Flotaza in AD brain slices is similar to our reported studies with [^{11}C]TAZA.[7] However the ratio of gray matter to white matter was significantly higher for [^{18}F]Flotaza compared to [^{11}C]TAZA. The GM/WM ratios of [^{11}C]TAZA ranged between 20 and 30 in hippocampal AD brain sections. Similarly, [^{11}C]PIB showed lower GM/WM ratios in the hippocampal brain sections, compared to [^{11}C]TAZA. Thus, “AZA”

functionality renders unique properties to the molecule yielding higher binding to Aβ plaques. Fluoropegylation is known to reduce lipophilicity of molecules and gives additional advantage to [^{18}F]Flotaza compared to [^{11}C]TAZA. Molecular modeling analysis of the binding of TAZA and Flotaza revealed very similar binding energies to preferred sites on the Aβ amyloid fibrils.

The olefin analog of the [^{11}C]TAZA, [^{11}C]Dalene exhibited highest amount of white matter binding.[7] Since [^{11}C]Dalene is a close fluoropegylated structural analog of [^{18}F]florbetaben, our results suggest that [^{18}F]Flotaza, which is a fluoropegylated analog of [^{11}C]TAZA is likely to yield higher GM/WM ratios compared to [^{18}F]florbetaben. The most significant structural difference between [^{18}F]Flotaza and [^{18}F] florbetaben is the presence of the "AZA" functionality replacing the olefin (Fig. 1).

Previous studies with "AZA" group containing PDB derivatives suggested that the "benzothiazole moiety" present in the PDB derivatives may be contributing to their affinity to Tau.[20] Using [^{125}I]IPPI labeled brain slices, flotaza did not have any significant effect on [^{125}I]IPPI binding thus suggesting poor affinities of Flotaza for Tau (Fig. 3).

Binding profile of [^{18}F]Flotaza to Aβ plaques and [^{125}I]IPPI to Tau on adjacent brain slices containing anterior cingulate and corpus callosum of the same subject were compared (Fig. 6). Immunostaining of adjacent slices confirmed the presence of Aβ plaques (Fig. 6A and C) and Tau (Fig. 6D and F). Both, [^{18}F]Flotaza (Fig. 6B) and [^{125}I]IPPI (Fig. 6E) bound to anterior cingulate extensively in adjacent slices, and was consistent throughout the gray matter regions. This binding is consistent with the immunostaining of the two biomarkers and supports the usefulness of [^{18}F]Flotaza in the diagnostic use of Aβ plaques in AD.

Our previous PET studies with [^{11}C]TAZA in rats demonstrated good in vivo brain permeability.[7] PEGylation is a suitable approach to enhance targeted drug delivery by extending circulating times, reducing lipophilicity and altering metabolic clearance of the drugs. Thus, compared to [^{11}C]TAZA, we anticipate [^{18}F]Flotaza may be more brain permeable. Greater clearance of [^{18}F]Flotaza from nonspecific white matter regions due to the presence of the fluoropegylated side chain may be expected. This increased clearance from nonspecific binding regions is likely to result in greater target-to-nontarget ratios.

In summary [^{18}F]Flotaza is a new PET radiotracer for imaging Aβ plaques in the human brain. Although in vitro ratios do not directly translate to in vivo measures, the findings reported here indicate that [^{18}F]Flotaza may be expected to give a significantly higher target to nontarget ratios in PET studies. Our goal is to complement metabolic studies in transgenic mice models of AD[21] and Parkinson's disease[22] with imaging of disaggregated proteins. Thus, we plan to carry out [^{18}F] Flotaza PET imaging studies in transgenic 5XFAD mice to examine the suitability of [^{18}F]Flotaza in imaging mice Aβ plaques. If Aβ plaque imaging in the 5XFAD transgenic mice is successful with [^{18}F]Flotaza, it will then enable evaluation of therapeutic drugs designed to lower Aβ plaque burden in the transgenic mice models.[23]

Acknowledgements

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8. Synthesis: All solvents used were provided by Fisher Scientific. For QC chemical purity, a Gilson HPLC system with UV detector set at 350 nm was used with 4.6 × 250 mm C18 Econosil reverse-phase analytical column was used. A Semi-preparative HPLC column 100 × 250 mm 10 μm Econosil C18 reverse-phase was supplied by Grace Discovery Corp. for purification. Millex-FG sterile pyrogen free 0.20 μm filters and Corning sterile filters were used as vent cartridges. Syringes were supplied by BD. The standard 2-(4′-methylaminophenyl)-6-hydroxybenzothiazole (PIB) were obtained from ABX, Germany. Analytical thin-layer chromatography (TLC) was used to monitor reactions (Baker-flex, Phillipsburg, NJ, USA). Electrospray mass spectra were obtained from a Model 7250 mass spectrometer (Micromass LCT). Proton NMR spectra were recorded on a Bruker OMEGA 500-MHz spectrometer. (2-{2-[2-Hydroxyethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene) **8**:To a solution of 4-hydroxy-4′-dimethylaminoazobenzene (241 mg; 1 mmol; Aurum Pharmtech Inc., New Jersey) in 10 mL tetrahydrofuran (THF) was reacted with bromo-PEG3-alcohol (213 mg; 1 mmol; 1Click Chemistry Inc., New Jersey) in the presence of potassium *tert*-butoxide (123 mg; 1.1 mmol) at 90 °C for 24 h. The reaction mixture was diluted with water and extracted with dichloromethane (CH_2Cl_2). This extract was purified on preparative TLC (1:1 hexane–ethyl acetate) to provide pure **8** (145 mg; 39% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 7.86–7.82 (m, 4H), 7.02–7.0 (d, 2H), 6.80–6.78 (d, 2H), 4.22–4.20 (t, 2H), 3.91–3.89 (t, 2H), 3.77–3.67 (m, 6H), 3.64–3.61 (t, 2H), 3.08 (s, 6H, $N(CH_3)_2$), 1.65 (br, 1H). Mass spectra (*m*/*z*, %): 374 (10%, $[M+H]^+$), 396 (100%, $[M+Na]^+$)$\mathbf{8}_{22}\mathbf{8}^1{}_{332}{}^{++}$.
9. -{2-[2-Tosylethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene) **9**: To a solution of 2-{2-[2-Hydroxyethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene (19.6 mg; 53 μmol) in 2 mL CH_2Cl_2 was reacted with toluenesulfonyl chloride (16 mg; 84 μmol) in the presence of pyridine (40 μL) at room temperature for 24 h. The reaction mixture was diluted with water and extracted with dichloromethane. This extract was purified on preparative TLC (1:1 hexane–ethyl acetate) to provide pure **9** 2 mg, 42 μmol). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 7.87–7.79 (m, 6H), 7.32–7.33 (d, 2H), 7.0–6.98 (d, 2H), 6.79–6.77 (d, 2H), 4.19–4.16 (dt, 2H), 3.87–3.85 (t, 2H), 3.72–3.70 (t,

2H), 3.69–3.68 (m, 6H), 3.08 (s, 6H, $N(CH_3)_2$), 2.43 (s, 3H, tosyl-CH_3). Mass spectra (*m*/*z*, %): 528 (55%, $[M + H]^+$), 550 (100%, $[M + Na]^+$)$\mathbf{9}_{22}\mathbf{9}^{1}{}_{3323}{}^{++}$.

10. (2-{2-[2-Fluoroethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene), FLOTAZA **10**: To a solution of 2-{2-[2-tosyloxyethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene (10 mg; 19 μmol) in 1 mL THF was reacted with tetrabutylammonium fluoride (0.1 mL of IM solution in THF) at 65 °C for 24 h. The reaction mixture was diluted with water and extracted with dichloromethane. This extract was purified on preparative TLC (1:1 hexane–ethyl acetate) to provide pure **10** (5 mg; 13 μmol). ^{1}H NMR (CDCl3, 500 MHz) δ ppm: 7.90–7.84 (m, 4H), 7.02–7.0 (d, 2H), 6.80–6.79 (d, 2H), 4.62–4.54 (dt, 2H, CH_2F; J_{HF}=46 Hz, $J_{2,3}$=15 Hz), 4.23–4.21 (t, 2H), 3.92–3.90 (t, 2H), 3.80–3.73 (m, 6H), 3.64–3.62 (m, 2H), 3.08 (s, 6H, $N(CH_3)_2$). ^{19}F NMR δ ppm: 222.9 (CH_2F). Mass spectra (*m*/*z*, %): 376 (85%, $[M + H]^+$), 398 (100%, $[M + Na]^+$)$\mathbf{1010}^{1}{}_{2HF2,332}{}^{19}{}_{2}{}^{++}$.
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12. In Vitro Binding Affinity: [^{3}H]PIB for Aβ: Human AD brain tissue (Banner Health Research Institute, Sun City, Arizona) were sectioned (10 μm thickness) on a Leica 1850 Cryostat and collected on Fisher slides. The slides contained 3 to 4 brain sections each were placed in separate glass chambers (six slides per chamber) were preincubated in PBS buffer for 15 min. The preincubation buffer was discarded and then to the chambers, [^{3}H]PIB in 10% ethanol PBS buffer pH 7.4 (60 mL; 74 kBq/mL), was added and the chambers were incubated at 25 °C for 1 hr.[11] Nonspecific binding was measured in separate chambers in the presence of 10 μM PIB. The slices were then washed with cold 10% alcohol PBS buffer (2×3 mins), cold deionized water 1 min, respectively. The brain sections were air dried, exposed overnight on a phosphor film, and then placed on the Phosphor Autoradiographic Imaging System/Cyclone Storage Phosphor System (Packard Instruments Co). Regions of interest (ROIs) were drawn on the slices and the extent of binding of [^{3}H]PIB was measured in digital light units, DLU/mm^2 using the OptiQuant acquisition and analysis program (Packard Instruments Co)^{331132}.
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14. I]IPPI for Tau: Human AD brain tissue containing anterior cingulate and corpus callosum from 6 AD subjects were preincubated in PBS buffer for 15 min. The slides contained 3 to 4 brain sections each were placed in separate glass chambers (six slides per chamber) were preincubated in PBS buffer for 15 min. The preincubation buffer was discarded and then to the chambers, I]IPPI in 10% ethanol PBS buffer pH 7.4 (60 mL; 3.7 kBq/mL), was added and the chambers were incubated at 25 °C for 1.25 hr. Nonspecific binding was measured in separate chambers in the presence of 10 μM MK-6240. The slices were then washed with cold PBS buffer, 50% ethanolic PBS buffer twice, PBS buffer and cold water for 2,1,1,2,1 min, respectively. The brain sections were air dried, exposed overnight on a phosphor film, and read as described above.
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16. Radiosynthesis: Fluorine-18 radioactivity were counted in a Capintec CRC-15R dose calibrator while low level counting was carried out in a Capintec Caprac-R well-counter. The radiosynthesis of [^{18}F]flotaza was conducted in the chemistry process control unit (CPCU). Hydrogen [^{18}F]fluoride in $H_2{}^{18}O$ from PETNET was passed through light QMA sep-pak (Waters Corp.), preconditioned with 3 mL of potassium carbonate, 140 mg/mL, followed by 3 mL of anhydrous acetonitrile. The trapped [^{18}F]-fluoride in QMA was eluted with 1 mL of Kryptofix 2.2.2 (Aldrich)/potassium carbonate solution (stock solution of 360 mg Kryptofix and 75 mg potassium carbonate in 24 mL acetonitrile and 1 mL water) and transferred to the reaction vessel in the CPCU. The SYNTH1 program was used for the synthesis that involved an initial drying step of the [^{18}F]fluoride, Kryptofix 2.2.2., and K_2CO_3 mixture at 120°C for 10 min. The [^{18}F] solution was further dried with acetonitrile (2 mL) at 120 °C for 7 min. Dried [^{18}F] reaction mixture was treated with the tosylate precursor ((2-{2-[2-Tosylethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene) **10** (1 mg) in acetonitrile (0.5 mL). This solution was heated at 96 °C for 30 min and cooled. The crude product was transferred out of CPCU using methanol (5 mL). The methanol containing [^{18}F]flotaza was evaporated *in vacuo*. Semipreparative HPLC

purification was performed using an Alltech C18 column (10 μm, 25×10 mm) and UV detector (254 nm), mobile phase: 60% acetonitrile-40% 0.1% aqueous triethylamine, 2.5 mL/min, r.t. = 18 min. [^{18}F]Flotaza **6** was made in modest yields (10% decay corrected) in specific activities of 74 GBq/μmol. The collected fraction was taken to near dryness *in vacuo*. The final formulation was carried out using approx. 5 mL of saline (0.9% NaCl INJ) followed by filtration through a membrane filter (0.22 μm) into a sterile dose vial[18181818]$_{23}$[1818]**10**[1818]**6**.

17. Human postmortem brain tissue samples were obtained from Banner Sun Health Research Institute, Sun City, AZ brain tissue repository for in vitro experiments. Age and gender matched AD brain and cognitively normal (CN) brain tissue samples selected for end-stage pathology (Braak & Braak stage of VI). Chunks of frozen tissue were dissected for immunohistochemical and autoradiographic techniques, as well as for biochemical experiments. Human postmortem brain slices were obtained on a Leica 1850 cryotome. Fluorine-18 autoradiographic studies were carried out by exposing tissue samples on storage phosphor screens (Perkin Elmer Multisensitive, Medium MS). The apposed phosphor screens were read and analyzed by OptiQuant acquisition and analysis program of the Cyclone Storage Phosphor System (Packard Instruments Co., Boston, MA). Postmortem human brain studies were approved by the Institutional Biosafety Committee of University of California, Irvine.
18. Neighboring slices were immunostained with DAKO polyclonal antibody to total Tau which detects all 6 six isoforms of Tau, dilution 1: 3000, A0024 (Agilent, CA, USA) using reported protocols. For Aβ plaques, slices from all subjects were immunostained with anti-Aβ Biolegend 803015 (Biolegend, CA, USA) which is reactive to amino acid residue 1–16 of β-amyloid. Pictures were taken on a Trinocular microscope from AmScope, Inc.
19. Postmortem [^{18}F]Flotaza Human Brain Autoradiography: [^{18}F]Flotaza were used for autoradiographic studies. Human anterior cingulate sections, containing corpus callosum (10 μm thick) were placed in a glass chamber and preincubated in PBS buffer for 10 min. The brain sections were placed in a glass chamber and incubated with [^{18}F]Flotaza (approximately 740 kBq/cc) in 40% EtOH at 37 °C for 1 hr. The slices were then washed with cold millipore water, 70%−90%−70% EtOH, water for 2,1,1,1,1 min, respectively. Nonspecific binding was measured in the presence of 10 μM PIB. The brain sections were air dried, exposed overnight on a phosphor film, and then placed on the Phosphor Autoradiographic Imaging System (Packard Instruments Co). Regions of interest (ROIs) were drawn on the slices and the extent of binding of ^{11}C-PIB was measured with DLU/mm^2 using the OptiQuant acquisition and analysis program (Packard Instruments Co). Neighboring slices were immunostained with 4G8 antibody using modifications of reported methods (Braak et al., 2011). Slides were warmed to room temperature and washed in TBS (Tris buffered saline, pH 7.5), followed by antigen retrival in 70% formic acid for 15 min. After endogenous peroxidase quenching, sections were stained with biotinylated anti-Aβ antibody 4G8 (Covance, Princeton, NJ) at 1:800 dilution followed by incubation according to manufacturer instructions (Vector Labs, Burlingame, CA). Pictures were taken on Olympus BX61 microscope[181818112].
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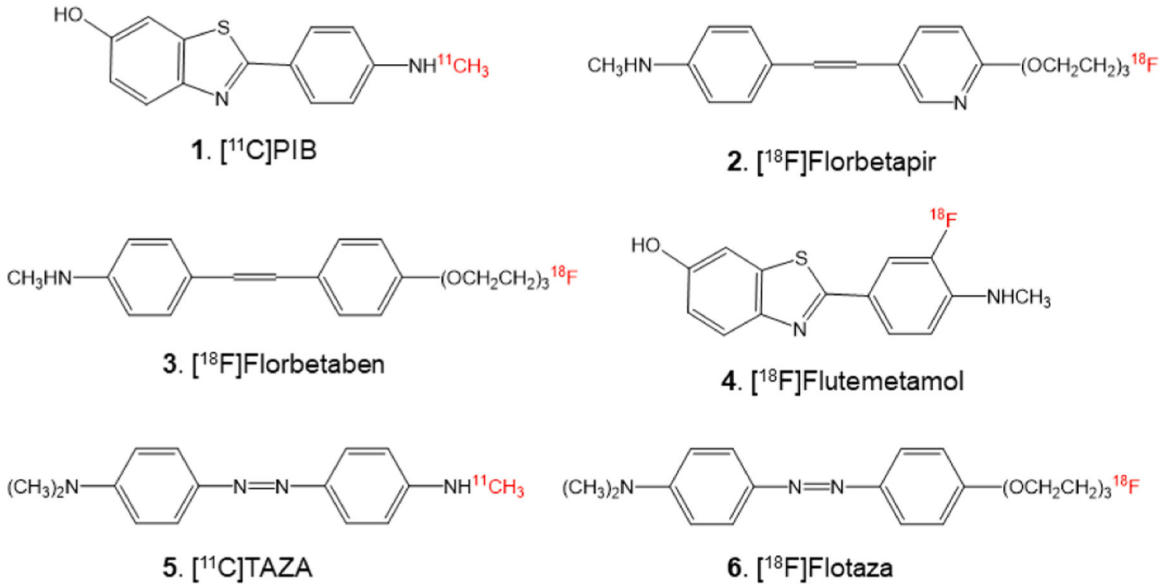

Fig. 1.

Chemical structures of select Aβ plaque binding PET radiotracers: Carbon-11 labeled: [^{11}C]PIB and [^{11}C]TAZA; Fluorine-18 labeled: [^{18}F]Florbetapir; [^{18}F] Florbetaben; [^{18}F]Flutmetamol; [^{18}F]Flotaza.

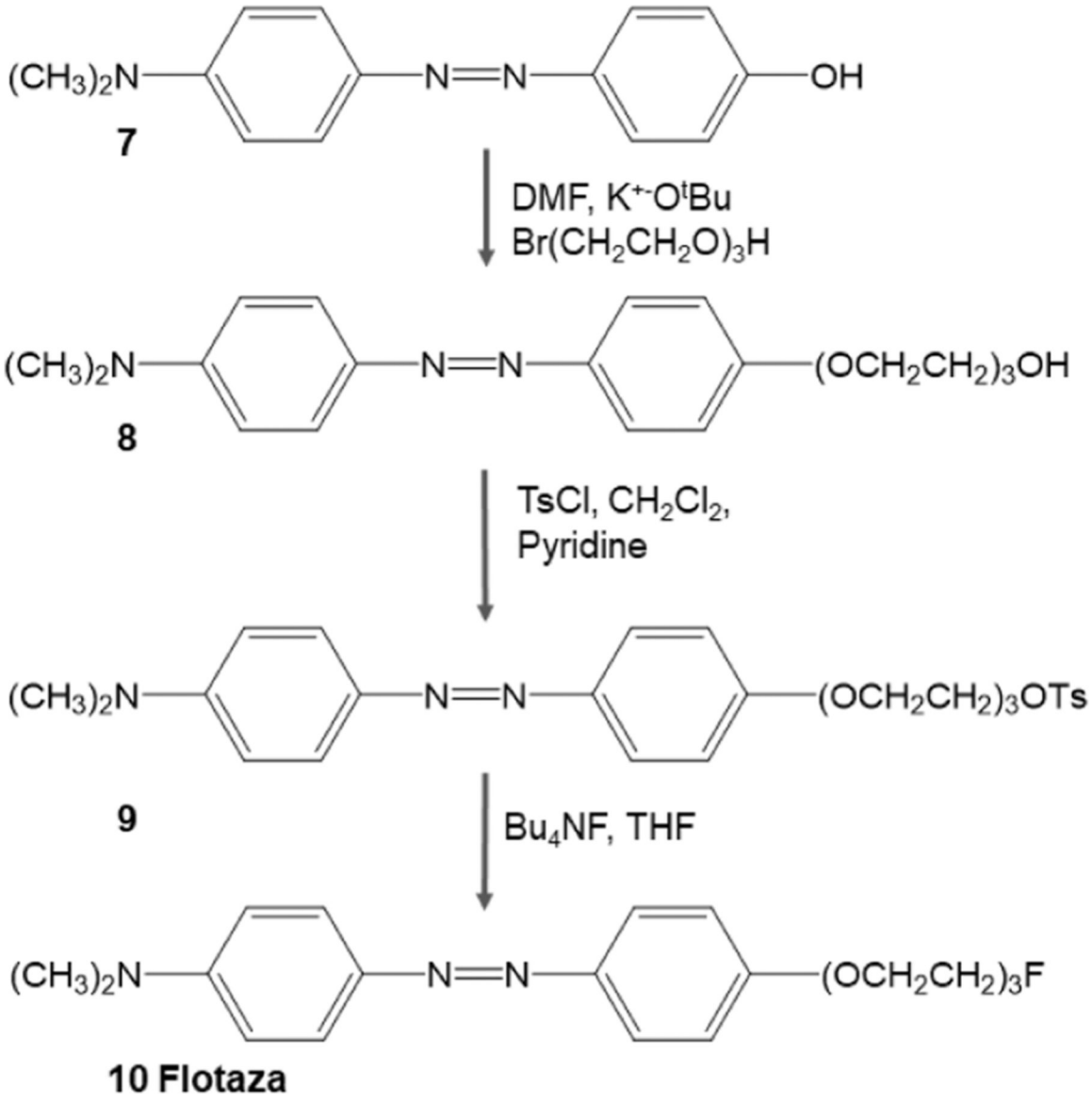

Fig. 2.

Synthesis scheme of Flotaza: 4-hydroxy-4′-dimethylaminoazobenzene (**7**) was reacted with bromo-PEG3-alcohol ($Br(CH_2CH_2O)_3H$) in dimethylformamide (DMF) in the presence of potassium *tert*-butoxide (K^+O^tBu). Tosylate **9** was obtained by reacting toluenesulfonyl chloride (TsCl) with 2-{2-[2-Hydroxyethoxy]ethoxy}ethoxy)-4′-*N*,*N*-dimethylaminoazobenzene (**8**) in dichloromethane (CH_2Cl_2). Flotaza (**10**) was prepared by reaction of tosylate **9** with tetrabutylammonium fluoride (Bu_4NF) in tetrahydrofuran (THF).

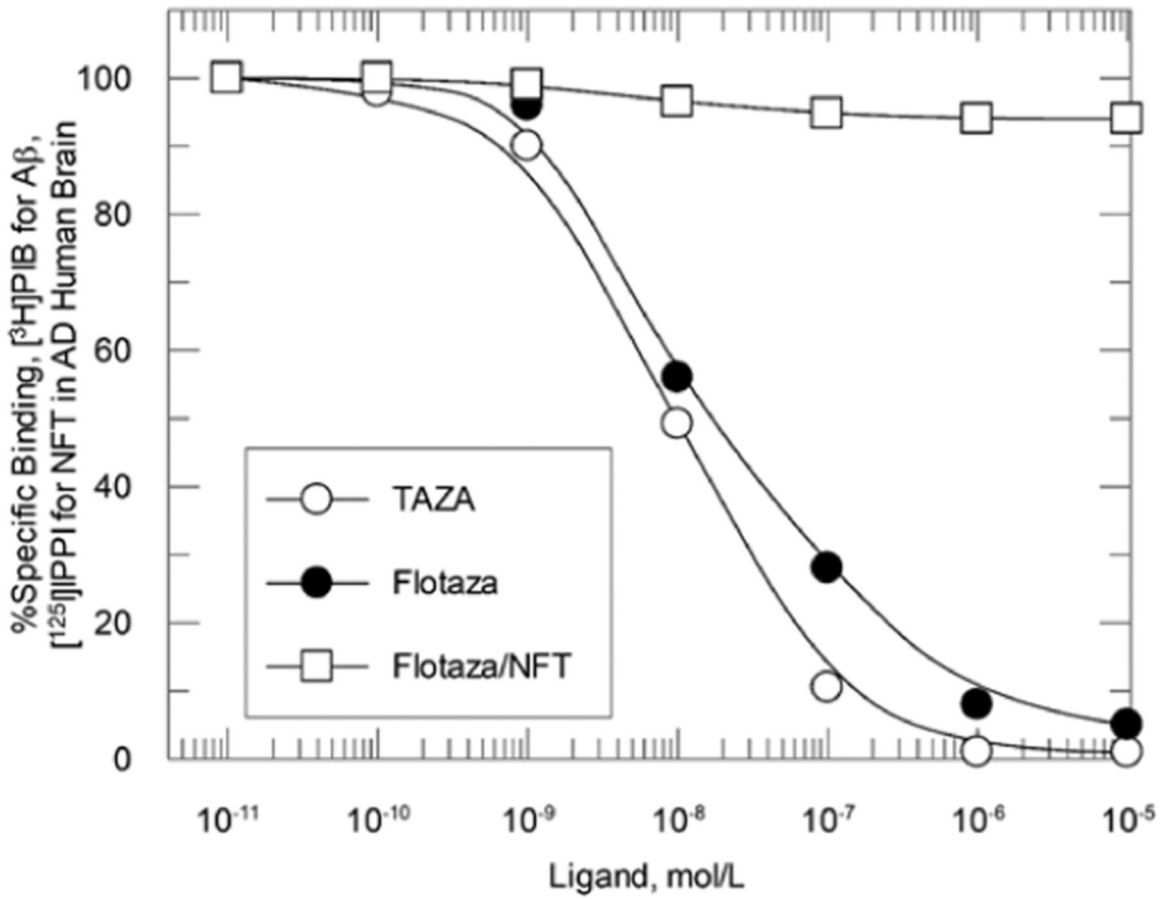

Fig. 3.

Binding Affinity of Flotaza: Human postmortem AD brain sections (10 μm) were radiolabeled with [^{3}H]PIB for Aβ plaques or [^{125}I]IPPI for NFT for competition assay with Flotaza. Flotaza Ki = 1.68 nM for Aβ plaques and >10 μM for NFT.

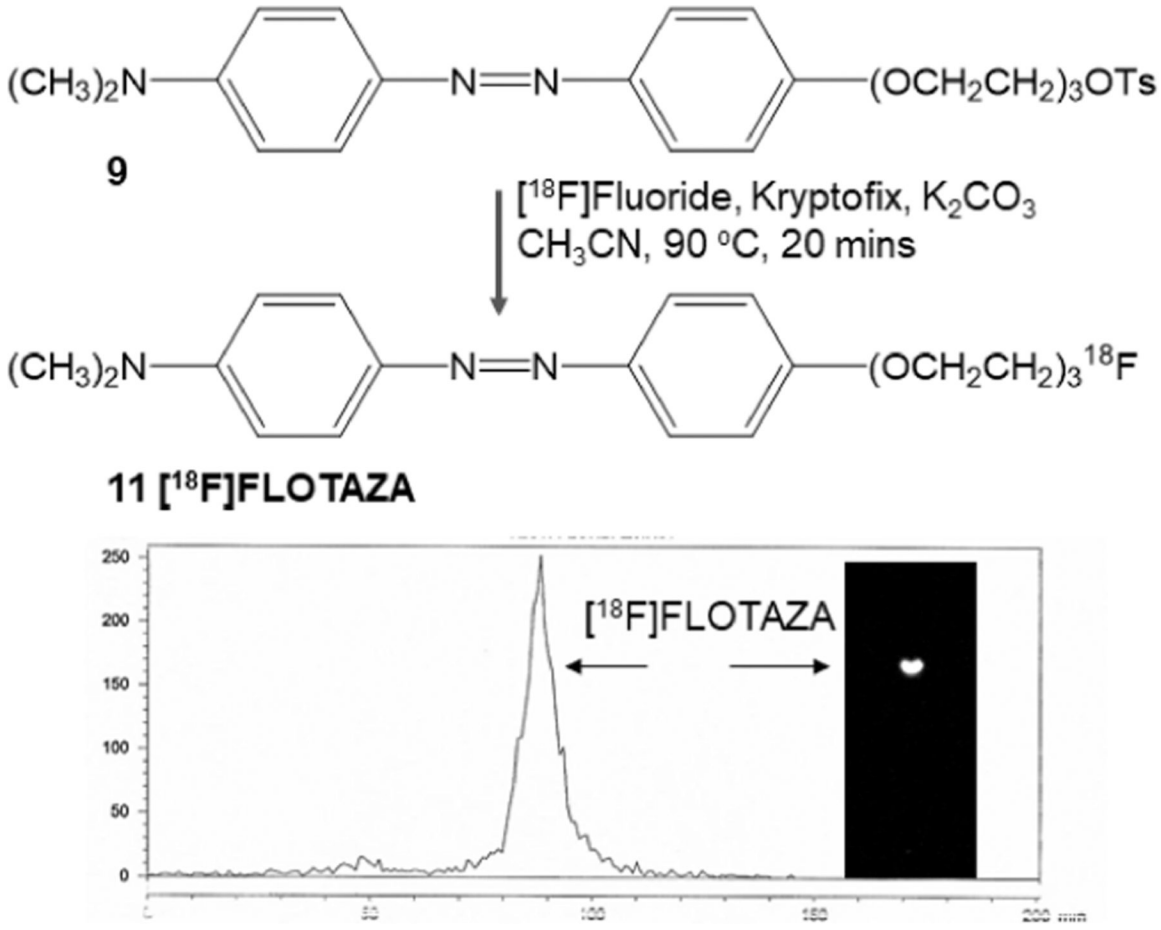

Fig. 4.

One-step radiosynthesis of [^{18}F]Flotaza: Tosylate (**9**) reacted with [^{18}F]fluoride, Kryptofix and potassium carbonate (K_2CO_3) in acetonitrile (CH_3CN) to provide [^{18}F]Flotaza (**11**). Thin layer radio-chromatograph of purified [^{18}F]Flotaza confirming radiochemical purity.

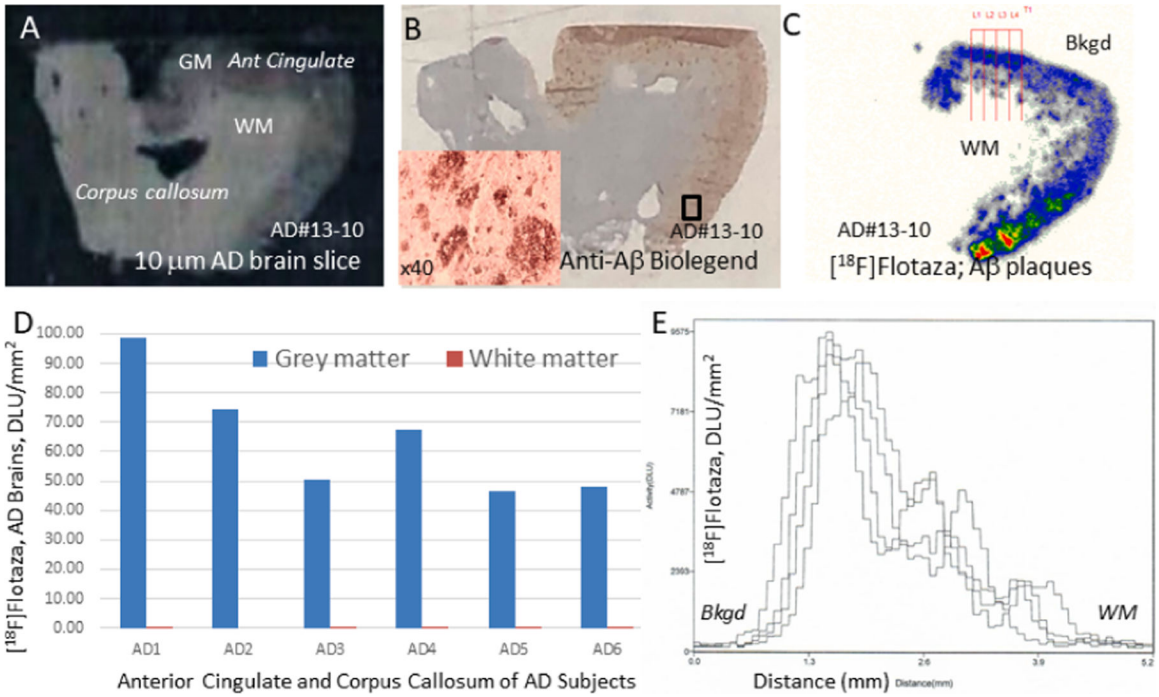

Fig. 5.

Postmortem human brain autoradiography [^{18}F]Flotaza in AD subjects: A. AD brain slice showing gray matter (GM), anterior cingulate and white matter (WM) corpus callosum; B. Anti-Aβ immunostained adjacent section showing presence of Aβ plaques (inset at × 40 magnification); C. [^{18}F]Flotaza binding in the gray matter regions in adjacent slices, consistent with the presence of Aβ plaques; D. High levels of [^{18}F]Flotaza binding in gray matter in six AD subjects with very little white matter binding; E. A 5 mm long plot through cortex (red lines shown in C) showing high amounts of [^{18}F]Flotaza in the outer layers of the cortex, with almost background levels in white matter.

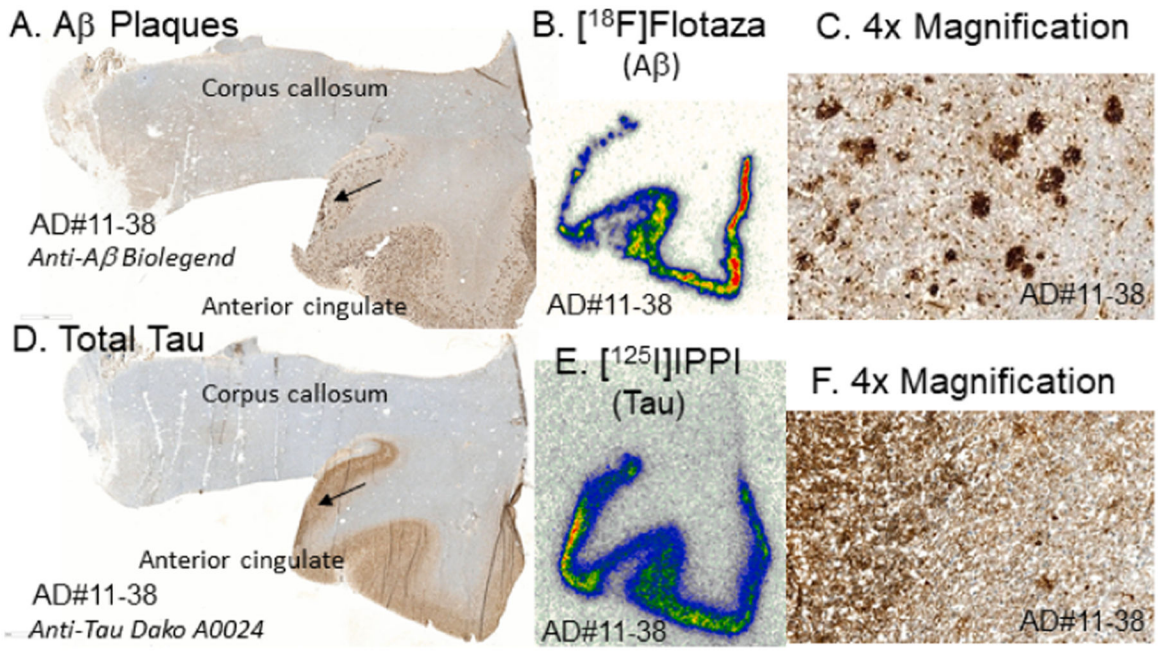

Fig. 6.

[^{18}F]Flotaza for Aβ plaques and [^{125}I]IPPI NFT in same AD subject: A. Anti-Aβ immunostained section showing presence of Aβ plaques (×4 magnification, C); B. [^{18}F]Flotaza binding in the anterior cingulate in adjacent slices, consistent with the presence of Aβ plaques; D. Anti-Tau immunostained section showing presence of total Tau protein (×4 magnification, F); E. [^{125}I]IPPI binding in the anterior cingulate in adjacent slices, consistent with the presence of NFT.