RADIOSYNTHESIS AND EVALUATION OF POTENTIAL b-AMYLOID IMAGING RADIOTRACERS FOR PET

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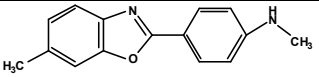
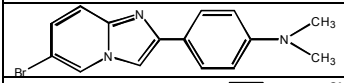
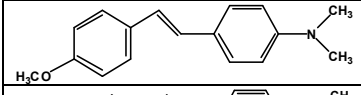
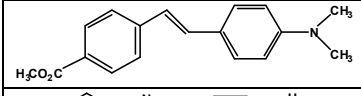
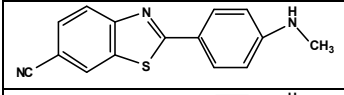
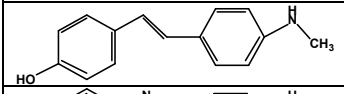
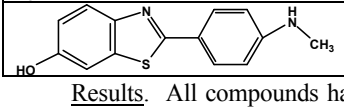
Keywords: PET, Alzheimers, Carbon-11, loop method, transgenic mice

We report here the radiosynthesis, in vitro, and in vivo characterisation of novel -amyloid binding radiotracers and compare them to the recently reported [^{11}C]-6-OH-BTA-1, currently being tested in Alzheimer's patients [1]. The compounds in the Table below were radiolabelled with ^{11}C and their binding affinity for A 1-40 aggregates measured. They were evaluated using in vitro and ex vivo autoradiography in transgenic mice expressing ß-amyloid protein.

Structure	Affinity Ki, nM	cLog P Measured Log P	Tg mice Ex vivo	Autorad in vitro
	37	3.70 4.21 (Wilson)	No Specific Binding	Yes, requires xylene wash
	10	4.653 3.47 (Wilson)	Pending	Yes, no Xylene wash
	1	4.97 3.85 (Wilson)	Specific Binding	Not done
	1	4.98 3.15 (Wilson)	No Specific Binding	Yes, requires xylene wash
	14	3.43 3.95 (Wilson)	No Specific Binding	Yes, no xylene wash
	6	2.98 3.17 (Wilson)	Specific Binding	Yes, no xylene wash
	7	3.99 2.0 (Mathis)	Specific Binding	Yes, no xylene wash

Results. All compounds had nM affinity for A 1-40 aggregates and imaged amyloid plaques using in vitro autoradiography of transgenic mice brain slices, albeit after aggressive tissue washing in many cases. Ex vivo biodistribution studies demonstrated that all ligands readily entered the brain. In most cases no differentiation in brain uptake between Tg and control wild-type mice was observed. Only the two least lipophilic tracers, (E)-4-methylamino-4'-hydroxystilbene and 6-OH-BTA-1 both showed preferential uptake in cortical regions (plaque rich) compared with cerebellum (plaque poor). Both of these radiotracers also displayed similar promising in vitro autoradiography, requiring much less aggressive washing of brain slices to reveal specific plaque binding.

In addition to the previously reported [^{11}C]-6-OH-BTA-1, [^{11}C]-(E)-4-methylamino-4'-hydroxystilbene has promise as a ligand to image ß-amyloid protein in Alzheimer's patients using PET. Human imaging trials comparing the two radiotracers are currently underway.

Reference; (1) Engler, H. et al. Neurobiol. Aging, 23, S429 (2002).

DEVELOPMENT OF ^{18}F-LABELLED THIOFLAVIN-T ANALOGUES AS AMYLOID PLAQUE IMAGING AGENTS

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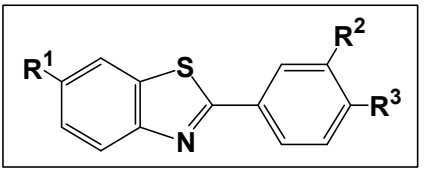
Keywords: F-18, amyloid, Alzheimer's disease

We recently evaluated a series of lipophilic, ^{11}C-labelled thioflavin-T analogues as amyloid plaque imaging agents for PET (e.g., 1). One of these radioligands, [N-methyl-^{11}C]2-(4'-methylamino-phenyl)-6-hydroxy-benzothiazole, has been used successfully to image amyloid deposition in the brains of Alzheimer's disease (AD) subjects (2). Our more recent efforts have focused on ^{18}F-labelled thioflavin-T analogues for the same purposes, permitting the incorporation of the longer-lived radiolabel for extended imaging studies and potential off-site distribution.

Fluorinated analogues were synthesized, radiolabelled with high specific activity ^{18}F, and their in vitro and in vivo properties as potential amyloid imaging agents were evaluated. Some representative examples of the fluorinated analogues are shown below. The binding affinities of the analogues for synthetic beta-amyloid fibrils [A (1-40)] varied from 2 nM to 80 nM, with compound **9** exhibiting the highest affinity and compound **10** the lowest. Binding assays using postmortem AD brain tissue homogenates provided similar results. The log(Poct-water) values varied from 1.1 to 3.5, spanning the optimum range for good brain entry. Brain uptake in normal mice following i.v. tail-vein injection varied from 6 to 14 %ID/g at 2 min and 1 to 4 %ID/g at 60 min, indicating excellent brain uptake of the compounds and good clearance of non-specifically bound and free tracer from brain tissue. Brain uptake and clearance results in normal baboon brain were similar to those in mice. Analyses of plasma samples indicated rapid metabolism, with ~80% parent compound at 2 min and <5% parent at 60 min post injection for all compounds, and the absence of radiolabelled lipophilic metabolites for all compounds. Femoral bone uptake in mice at 60 min varied from 0.3 to 5 %ID/g, with **5** lowest and **1** highest. Most of the radioligands were labelled with high specific activity [^{18}F]fluoride using conventional methods (e.g., ^{18}F-for-tosyloxy or ^{18}F-for-trimethylammonium triflate substitutions) except for compounds **9-11,** which were synthesized by ^{18}F-for-Cl substitution employing an *ortho*-nitro activating group (3) that was subsequently rapidly reduced to the amine using sodium borohydride. The combination of relatively high affinity for amyloid and high brain uptake and good clearance in normal control mice and baboon brain provides several promising ^{18}F-labelled amyloid imaging agents for PET studies of amyloid in AD.

Compd	R^1	R^2	R^3
1	OEtF	H	NH_2
2	OEtF	H	NHMe
3	H	H	NHPrF
4	OH	H	NHEtF
5	OH	H	NHPrF
6	OH	H	NHBuF
7	OH	H	NMeEtF
8	OH	H	NMePrF
9	OMe	F	NH_2
10	OH	F	NH_2
11	OH	F	NHMe
12	OH	H	NHBZL-4"-F



1. Mathis CA et al., *Bioorg Med Chem Lett* 2002; 12:295-298.
2. Engler H et al., *Neurobiology of Aging* 2002; 23:S149.
3. Karramkam M et al., *J Label Compd Radiopharm* 2002; 45:1103-1113.

NIDA52189 AND NIDA522131: PROMISING NEW RADIOLIGANDS FOR IMAGING EXTRATHALAMIC HIGH AFFINITY NICOTINIC RECEPTORS WITH PET

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Keywords: Brain, nicotinic receptor, PET, F-18, radioligand, Rhesus monkey

Cerebral nicotinic acetylcholine receptors containing -2 subunits (nAChRs) with high affinity for nicotine have been defined as " 4 2-like" nicotinic receptors. Non-invasive PET imaging of these receptors in the cerebral cortex and striatum could be extremely valuable for elucidating the role of nAChRs in the pathogenesis of Alzheimer's and Parkinson's diseases and potentially in their early diagnosis. Research focused on developing PET radioligands for nAChRs has identified 2-[^{18}F]F-A-85380 (2-[^{18}F]FA) (K_d *ca.* 130 pM at 37 °C). This radioligand allows visualization of brain regions having high levels of nAChRs in humans. Although 2-[^{18}F]FA is suitable for quantifying thalamic nAChRs *in vivo* (BP *ca.* 2), its BP values in regions containing lower receptor densities than in the thalamus (e.g., cortex, striatum) are less than 1. Therefore, 2-[^{18}F]FA will be useful for assaying nAChRs in the brains of smokers, as the nAChR densities are likely to be twice those in non-smokers, but use of this radioligand for quantifying nAChRs in extrathalamic regions in non-smokers, and particularly in patients with neurodegenerative diseases characterised by a substantial loss of nAChRs, will be more difficult.

The goal of the current project was to develop radioligands suitable for quantitative analysis of PET data in brain regions with low to moderate densities of nAChRs. For this purpose, a series of high affinity derivatives of A-85380 was developed (see Zhang et al., this volume), and several ligands from this series were radiofluorinated and evaluated in the Rhesus monkey using PET. The distribution patterns of radioactivity in monkey brain for radiolabeled 6-chloro-3-((2-(S)-azetidinyl)methoxy)-5-(2-[^{18}F]fluoropyridin-5-yl)pyridine ([^{18}F]NIDA52189) and 6-chloro-3-((2-(S)-azetidinyl)methoxy)-5-(2-[^{18}F]fluoropyridin-4-yl)pyridine (([^{18}F]NIDA522131) were similar to that for 2-[^{18}F]FA and corresponded to the distribution of nAChRs. Pretreatment with the nicotinic agonist cytisine (1 mg/kg, s.c.) dramatically reduced the accumulation of radioactivity in regions having moderate to high densities of nAChRs, resulting in images in which the distribution of radioactivity was nearly random.

The BP values for [^{18}F]NIDA52189 and [^{18}F]NIDA522131 were 2.5 - 3.0 times those for 2-[^{18}F]FA. Consistent with their increased affinity for nAChRs (K_d 9 and 7 pM at 37 °C, respectively), [^{18}F]NIDA52189 and [^{18}F]NIDA522131 exhibited increased specific volumes of distribution that were *ca*. 17 times that of 2-[^{18}F]FA. The values of non-displaceable volume of distribution for the new ligands were also increased (*ca.* 6 that of 2-[^{18}F]FA) because they were more lipophilic than 2-[^{18}F]FA. Estimated BP values in monkey brain were *ca* 5.0 and 6.8 in thalamus, *ca*. 1.0 and 1.4 in cortex and *ca*. 0.9 and 1.1 in striatum for [^{18}F]NIDA52189 and [^{18}F]NIDA522131, respectively. As the BP values for 2-[^{18}F]FA in human brain regions were 20 – 50 % higher than those in Rhesus monkey, BP values for the new radioligands in midbrain, pons, striatum and some parts of cortex are expected to be above 1.5 in humans.

The greater lipophilicities of the new ligands increased their binding with plasma proteins (*ca*. 4 times greater than 2-[^{18}F]FA; 82% and 74% for [^{18}F]NIDA52189 and [^{18}F]NIDA522131 vs. 20% for 2-[^{18}F]FA) The portions of radioactivity in the blood plasma at 6 h after administration corresponding to non-metabolised ligand were 50%, 29% and 25% of total plasma radioactivity for [^{18}F]NIDA52189, [^{18}F]NIDA522131 and 2-[^{18}F]FA, respectively. Preliminary studies in mice demonstrated that the toxicity of NIDA52189, NIDA522131 and 2FA were similar.

Taken together these results suggest that [^{18}F]NIDA52189 and [^{18}F]NIDA522131 are promising ligands for the studies of extrathalamic nAChRs in human brain.

PET RADIOLIGANDS WITH POTENTIAL FOR IMAGING EXTRATHALAMIC HIGH AFFINITY NICOTINIC ACETYLCHOLINE RECEPTOR (NACHR)

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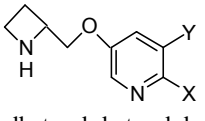
Keywords: Radiosynthesis, nicotinic ligands, QSAR, nicotinic receptor, PET, F-18, radiotracer

PET imaging of nAChRs containing beta-2 subunits in the human brain is now possible with 2-[^{18}F]F-A-85380 (2-[^{18}F]FA). However, due to insufficient binding potential (BP) in extrathalamic brain regions, 2-[^{18}F]FA may be suitable for quantitative imaging of nAChRs only in the thalamus, the brain region having the highest receptor density.

In order to develop more advanced ligands that are suitable for quantitative analysis of PET data in the regions with lower density of nAChRs such as cortex and striatum, a series of sixteen halogenated heteryl derivatives of A-85380 (Fig. 1) exhibiting *in vitro* affinities in the low picomolar range and low lipophilicities (log*D*) was prepared. The synthesis and the structure–activity relationships for the compounds in the series will be presented.



where: Y = vinylheteryl, heteryl, halogenated heteryl
X = H, F, Cl, Br

Figure 1: Novel series of nAChR ligands

Four ligands of this series (Fig 2) were radiolabeled with ^{18}F. The radiochemical yield of all radiolabeled compounds was 5-30%, and their specific radioactivity ranged from 4500 to 29000 mCi/ mol.

In vivo evaluation of these radioligands demonstrated that the BP values (see S. Chefer et al., this meeting) for [^{18}F]NIDA52189 (K_i = 5.8 pM; log*D* = –0.8) and [^{18}F]NIDA522131 (K_i = 5.8 pM; log*D* = –0.9) in Rhesus monkey brain were 2.5-3.0 - fold higher compared with those for 2-[^{18}F]FA. These findings suggest that [^{18}F]NIDA52189 and [^{18}F]NIDA522131 may be useful for quantitative imaging of extrathalamic nAChRs.

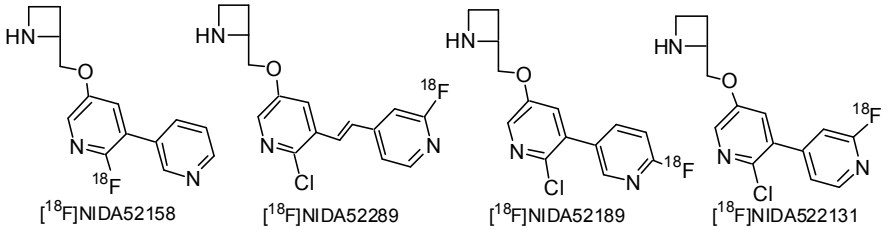


Figure 2: Radiolabeled tracers

FLUORINE-18 LABELLED *N*-FLUOROETHYL PIPERIDINEMETHANOL ESTERS FOR CEREBRAL ACHE AND BCHE MAPPING BY PET

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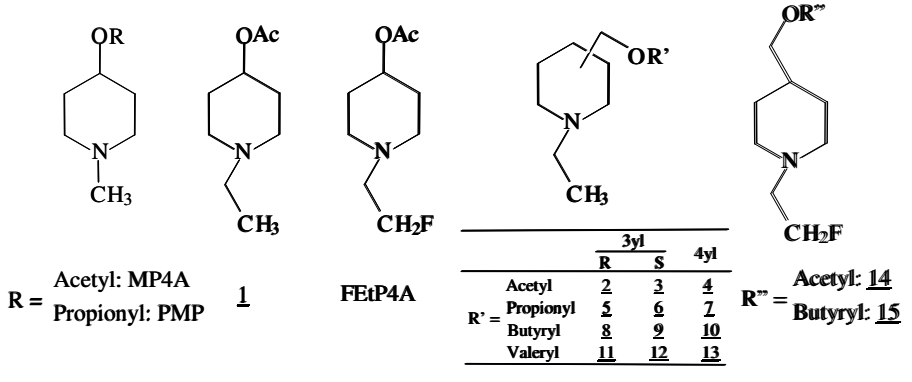
Keywords: acetylcholinesterase, butyrylcholinesterase, fluorine-18

A decrease in acetylcholinesterase (AChE) activity and an increase in butyrylcholinesterase (BChE) activity are simultaneously occurred in the brain of patients with Alzheimer disease. Recently, *N*-[^{18}F]fluoroethylpiperidin-4-yl acetate ([^{18}F]FEtP4A) has been developed as a candidate tracer for AChE mapping by positron emission tomography (PET). When compared with ^{11}C-labeled *N*-methylpiperidin-4-yl acetate (MP4A) and propionate (PMP) that have suitable hydrolysis rate and high AChE specificity for clinical use, this compound showed much lower hydrolysis rate, 1 / 20 of MP4A and 1 / 5 of PMP. In a search for *N*-[^{18}F]fluoroethyl tracer with hydrolysis rate similar to those of MP4A and PMP, a series of *N*-[^{14}C]ethylpiperidine-methanol esters including optical isomers (2-13) were synthesized and evaluated because cholinesterases hydrolyse primary alcohol esters faster than secondary ones. Then, some compounds were labelled with fluorine-18 for further evaluation.

N-[^{14}C]ethyl and *N*-[^{18}F]fluoroethyl labelling were performed with [^{14}C]ethyl iodide (55 Ci / mol) and [^{18}F]fluoroethyl bromide (20 Ci / μmol), respectively. The radiochemical purities of labelled compounds were more than 98%. To understand the effect of fluorination on the enzymatic hydrolysis of the esters in the brain tissue, hydrolysis rates of [^{18}F]FEtP4A and *N*-[^{14}C]ethylpiperidin-4-yl acetate (1) was measured in rat cerebral cortical homogenate. *N*-ethyl compound 1 showed similar hydrolysis rate (0.15 min^{-1}g^{-1}) to that of *N*-fluoroethyl compound, FEtP4A (0.07). The hydrolysis rates of *N*-[^{14}C]ethyl primary esters (2-13) by pure human AChE and BChE were measured. Among them, the acetyl and propionyl esters (2-7) were hydrolysed more rapidly than butyryl and valeryl esters by AChE. The observed rates of 2-7 (0.30-0.85 min^{-1}Unit^{-1}mL^{-1}) were comparable to that of MP4A (0.50). Of the acetyl and the propionyl esters, compound 4 demonstrated extremely higher AChE selectivity (8.90 of the AChE / BChE ratio) than others (< 0.5). These results implied that *N*-fluoroethyl derivative of 4 would be the most promising AChE tracer. Indeed, *N*-[^{18}F]fluoroethyl piperidin-4-yl methyl acetate (14) showed comparable hydrolysis rate and AChE specificity (1.67 min^{-1}g^{-1}, 86%) to that of MP4A (1.73 min^{-1}g^{-1}, 96%) in rat cerebral cortical homogenate. From similar consideration about BChE, *N*-[^{18}F]fluoroethyl piperidin-4-yl methyl butyrate 15 may be suitable for measurement of BChE activity by PET.



R = Acetyl: MP4A, Propionyl: PMP — 1 — FEtP4A

R' =	3yl R	3yl S	4yl
Acetyl	2	3	4
Propionyl	5	6	7
Butyryl	8	9	10
Valeryl	11	12	13

R''' = Acetyl: 14, Butyryl: 15