Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3015–3018

Synthesis and evaluation of two ^{18}F-labeled imidazo[1,2-*a*]pyridine analogues as potential agents for imaging β-amyloid in Alzheimer's disease

Fanxing Zeng,[a] Jeanine A. Southerland,[b] Ronald J. Voll,[a] John R. Votaw,[a] Larry Williams,[a] Brian J. Ciliax,[b] Allan I. Levey[b] and Mark M. Goodman[a,*]

[a]*Department of Radiology, Division of Radiological Sciences, Emory University, Atlanta, GA 30322, USA*
[b]*Center for Neurodegenerative Disease and Department of Neurology, Emory University, Atlanta, GA 30322, USA*

Received 12 December 2005; revised 21 February 2006; accepted 21 February 2006
Available online 29 March 2006

Abstract—Two new fluorinated imidazo[1,2-*a*]pyridine derivatives, 6-(2′-fluoroethyl)-2-(4′-dimethylamino)phenylimidazo[1,2-*a*]pyridine (FEPIP) and 6-(3′-fluoropropyl)-2-(4′-dimethylamino)phenylimidazo[1,2-*a*]pyridine (FPPIP), were synthesized. The binding affinity for FEPIP and FPPIP to amyloid plaques in human AD cortical tissues was determined. Radiolabeling, in vitro film autoradiography, and micro-PET study were performed with [^{18}F]FPPIP to determine its utility as a radioligand for amyloid plaque imaging in the brain of AD patients.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder among the elderly. Neuritic plaques containing β-amyloid (Aβ) peptides and neurofibrillary tangles in postmortem brain are the two pathological hallmarks characteristic of AD and provide the basis for the definitive, albeit postmortem, diagnosis of AD.[1,2] While there are no definitive treatments available to affect a cure of AD, much recent interest has been given to the development of anti-amyloid therapies aimed at halting and reversing amyloid formation and deposition. Therefore, development of amyloid-specific imaging agents could be potentially useful for early diagnosis and further neuropathogenesis studies of AD. Amyloid-imaging tracers could also facilitate the evaluation of the efficacy of anti-amyloid therapies currently under intense development.

Efforts to develop radiolabeled Aβ imaging agents for both positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have generated a few lead compounds, including [^{11}C]PIB,[3,4] [^{11}C]SB-13,[5,6] [^{18}F]FDDNP,[7,8] [^{123}I]IBOX,[9] and [^{123}I]IMPY.[10,11] Other structurally similar compounds have been reported for targeting Aβ plaques.[12–14] [^{123}I]IMPY ([^{123}I]6-iodo-2-(4′-*N*,*N*′-dimethylamino)phenylimidazo[1,2-*a*]pyridine) has recently been reported as a potential SPECT agent for imaging Aβ plaques with high binding affinity for preformed synthetic Aβ 40 aggregates (K_i = 15 nM) and human AD cortical homogenates (K_d = 5.3 nM) and desirable pharmacokinetics in normal mouse brain (2.9% initial dose/brain at 2 min and 0.2% initial dose/brain at 60 min).

As part of an effort to develop ^{18}F-labeled tracers for PET imaging of Aβ plaques, we developed two fluorinated analogues of IMPY, in which its iodo group is replaced with fluoroethyl (FEPIP) or fluoropropyl (FPPIP). We report here the synthesis and in vitro affinities of FEPIP and FPPIP, and the radiolabeling and evaluation of [^{18}F]FPPIP as in vivo PET imaging agent for Aβ plaques. (Fig. 1).

The syntheses of unlabeled FEPIP and FPPIP are shown in Scheme 1. IMPY (**1**) was prepared from condensation reaction between commercially available 2-amino-5-iodopyridine and 2-bromo-4′-dimethylaminoacetophenone[15] in the presence of a mild base such as sodium bicarbonate.[11] Palladium catalyzed coupling of **1** with tributyl(vinyl)tin produced the alkene **2** in

Keywords: Fluorine-18; β amyloid; Alzheimer's disease; PET imaging.
* Corresponding author. Tel.: +1 404 727 9366; fax: +1 404 727 3488; e-mail: mgoodma@emory.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.02.055

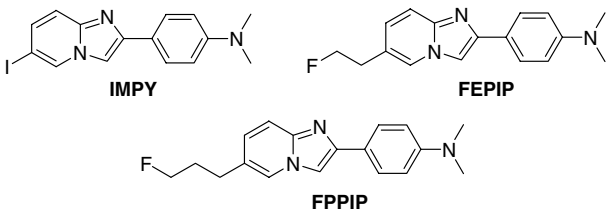


Figure 1. Structures of IMPY and its two fluorinated analogues.

90% yield. The following hydroboration–oxidation reaction of **2** gave the hydroxyethyl compound **3** in 86% yield, which was then converted to FEPIP by reaction with DAST. FPPIP was prepared in good yield by a similar method.

The radiolabeling of [^{18}F]FPPIP was accomplished by treatment of tosylate precursor with anhydrous [^{18}F]KF and K_{222} in acetonitrile for 10 min at 90 °C (Scheme 2). The entire procedure required approximately 105 min from the end of bombardment. [^{18}F]FPPIP was prepared in an average 51% decay-corrected yield. Analytical HPLC demonstrated that the radiolabeled product was over 98% radiochemically pure, and the specific activity of the product was 1.7–2.3 Ci/μmol at time of injection.

In vitro binding affinities of FEPIP and FPPIP to β-amyloid were determined via the binding competition with [^{125}I]IMPY using human AD cortical tissues by quantitative autoradiography.[16] The results shown in Table 1 demonstrate that IMPY and PIB displayed high binding affinity with K_i = 10.3 and 7.8 nM, respectively, which is comparable to the previously reported inhibition constant (K_i = 15 and 4.7 nM) obtained in a preformed Aβ 40 aggregate model system. FPPIP competed well with [^{125}I]IMPY, showing a moderate affinity (K_i = 48.3 nM). Decreasing length of the side chain by a $-CH_2$ group reduced the binding affinity significantly (K_i for FEPIP was 177 nM). Distribution coefficients of FEPIP and FPPIP were measured between 1-octanol and phosphate buffer at pH 7.4,[17] and log *P* values are given in Table 1. FEPIP and FPPIP display moderate lipophilicity and the log *P* values are in the optimal range (1–3) for compounds expected to enter the brain readily.[18]

Table 1. The log *P* and K_i values of IMPY derivatives for binding human AD cortical tissues

Ligand	Log *P*	K_i (nM)
IMPY	3.58	10.3
PIB	1.30[3]	7.8
FEPIP	2.42	177
FPPIP	2.84	48.3

Upon the basis of its higher binding affinity to Aβ plaques, [^{18}F]FPPIP plaque labeling was evaluated by in vitro film autoradiography as shown in Figures 2 and 3. Specific binding of [^{18}F]FPPIP to amyloid plaques in sections from postmortem AD brains was

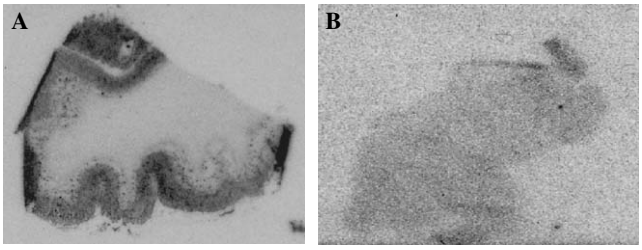
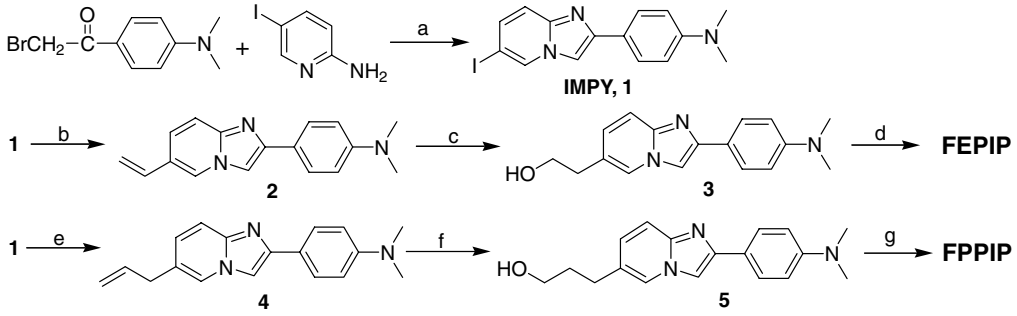
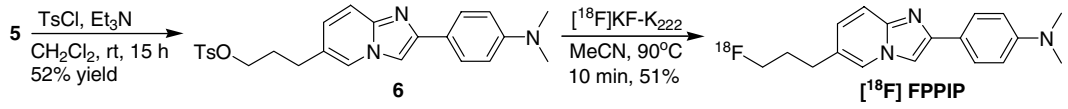


Figure 2. In vitro autoradiographic detection of Aβ amyloid deposits with [^{18}F]FPPIP in postmortem brain tissue sections of frontal lobe from an AD patient and an age-matched control. (A) AD tissue + [^{18}F]FPPIP. (B) Control tissue + [^{18}F]FPPIP.



Scheme 1. Syntheses of FEPIP and FPPIP. Reagents and conditions: (a) EtOH, reflux, 2 h; $NaHCO_3$, reflux, 5 h, 51%; (b) $Bu_3SnCH{=}CH_2$, 1,4-dioxane, Et_3N, $(PPh_3)_4Pd$, 100 °C, 20 h, 90%; (c) 9-BBN, THF, rt, 24 h; 3 N NaOH, 30% H_2O_2, rt, 5 h, 86%; (d) DAST, CH_2Cl_2, −78 °C to 23 °C, 2 h, 32%; (e) $Bu_3SnCH_2CH{=}CH_2$, toluene, $(PPh_3)_4Pd$, 100 °C, 20 h, 58%; (f) 9-BBN, THF, rt, 24 h; 3 N NaOH, 30% H_2O_2, rt, 5 h, 88%; (g) DAST, CH_2Cl_2, −78 °C to 23 °C, 2 h, 44%.



Scheme 2. Radiosynthesis of [^{18}F]FPPIP.

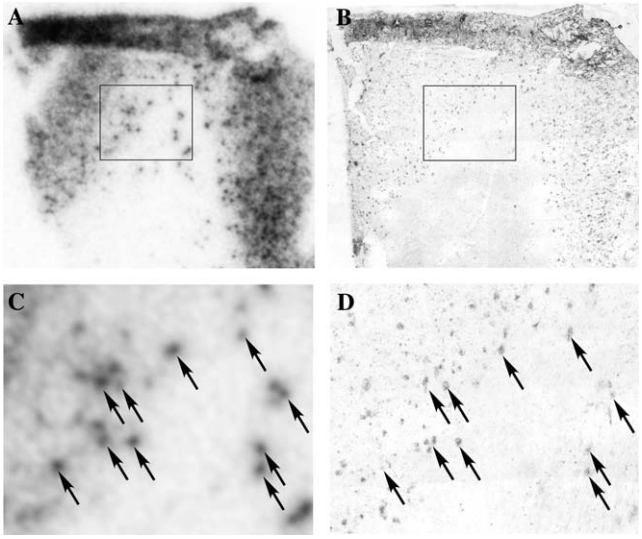


Figure 3. Comparison between plaques visualized by [^{18}F]FPPIP autoradiography and Aβ immunohistochemistry. (A) Film autoradiogram of [^{18}F]FPPIP displays selective in vitro binding to Aβ plaques in AD brain sections. (B) The same labeled plaques are confirmed by immunohistochemistry with 4G8. (C) and (D) Images of those regions demarcated by the rectangles in (A) and (B) were magnified, respectively, and arrows demarcate the same distribution of plaques.

clearly observed in cortical gray matter, but not in the white matter, and the specific binding was eliminated in the AD specimen with the pretreatment with nonradioactive FPPIP (data not shown). The control brain sections showed no specific binding, correlating well with the absence of amyloid plaques in these brains. To confirm the [^{18}F]FPPIP-labeled plaques at a microscopic level and to determine the specificity of [^{18}F]FPPIP binding for Aβ plaques, AD brain sections labeled with [^{18}F]FPPIP were subsequently immunostained with a monoclonal antibody (4G8, Signet, MA) specific for β-amyloid proteins.[19] Clearly, [^{18}F]FPPIP labeled all of the plaques detected by 4G8 on AD brain sections and a good correlation with Aβ plaques' labeling was demonstrated (marked with arrowheads).

The micro-PET imaging study of [^{18}F]FPPIP to assess brain penetration of the radiolabeled derivatives was performed in a rhesus monkey that presumably had no amyloid deposits in its brain. Thus, this experiment reflects brain entry and clearance from normal brain tissue. The time–activity curves shown in Figure 4 indicate that [^{18}F]FPPIP easily penetrates the blood–brain barrier after intravenous injection, with peak value (SUV) of 1.6–2.7 at 9 min. Relatively fast nonspecific binding clearance was observed with the radioactivity ratios of peak—to -105 min 2.6, 1.9, and 2.0 in cerebellum, frontal cortex, and subcortical white matter devoid of specific binding sites. No bone uptake of radioactivity was observed in the skull after the intravenous administration of [^{18}F]FPPIP to the monkey, indicative of low in vivo defluorination.

In summary, two new Aβ plaque ligands, FEPIP and FPPIP, have been synthesized and biologically evaluated. [^{18}F]FPPIP is a promising candidate as PET

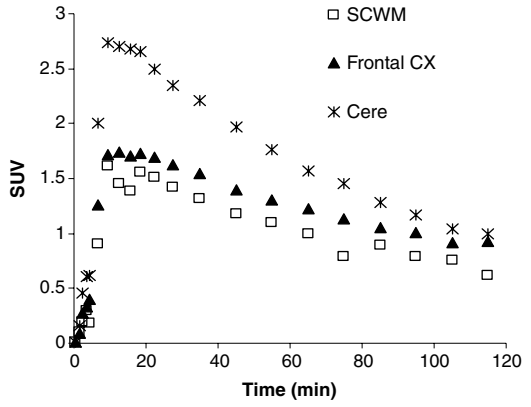


Figure 4. Time–activity curves of brain regions for [^{18}F]FPPIP in a rhesus monkey.

imaging agent for Aβ, based on its favorable pharmacokinetics in a rhesus monkey and its high specific labeling of Aβ plaques in vitro. However, more work is required to refine this structure in order to increase binding affinity.

References and notes

1. Klunk, W. E.; Abraham, D. J. *Psychol. Dev.* **1988**, *6*, 121.
2. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
3. Mathis, C. A.; Wang, Y.; Holt, D. P.; Huang, G.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740.
4. Wang, Y.; Klunk, W. E.; Debnath, M. L.; Huang, G. F.; Holt, D. P.; Shao, L.; Mathis, C. A. *J. Mol. Neurosci.* **2004**, *24*, 55.
5. Verhoeff, N. P.; Wilson, A. A.; Takeshita, S.; Trop, L.; Hussey, D.; Singh, K.; Kung, H. F.; Kung, M.-P.; Houle, S. *Am. J. Geriatr. Psychiatr.* **2004**, *12*, 584.
6. Ono, M.; Wilson, A.; Nobrega, J.; Westaway, D.; Verhoeff, P.; Zhuang, Z.-P.; Kung, M.-P.; Kung, H. F. *Nucl. Med. Biol.* **2003**, *30*, 565.
7. Agdeppa, E. D.; Kepe, V.; Liu, J.; Flores-Torres, S.; Satyamurthy, N.; Petric, A.; Cole, G. M.; Small, G. W.; Huang, S. C.; Barrio, J. R. *J. Neurosci.* **2001**, *21*, RC189.
8. Nordberg, A. *Lancet Neurol.* **2004**, *3*, 519.
9. Zhuang, Z.-P.; Kung, M. P.; Hou, C.; Plossl, K.; Skovronsky, D. M.; Gur, T.; Trojanowski, J. Q.; Lee, V. M.; Kung, H. F. *Nucl. Med. Biol.* **2001**, *28*, 887.
10. Kung, M. P.; Hou, C.; Zhuang, Z.-P.; Zhang, B.; Skovronsky, D. M.; Gur, T.; Lee, V. M.-Y.; Trojanowski, J. Q.; Kung, H. F. *Brain Res.* **2002**, *956*, 202.
11. Zhuang, Z. P.; Kung, M-P.; Wilson, A.; lee, C. W.; Plossl, K.; Hou, C.; Holtzman, D. M.; Kung, H. F. *J. Med. Chem.* **2003**, *46*, 237.
12. Cai, L.; Chin, F. T.; Pike, V. W.; Toyama, H.; Liow, J.-S.; Zoghbi, S. S.; Modell, K.; Briard, E.; Shetty, U. H., et al. *J. Med. Chem.* **2004**, *47*, 2208.
13. Lee, C.-W.; Kung, M.-P.; Hou, C.; Kung, H. F. *Nucl. Med. Biol.* **2003**, *30*, 573.
14. Zhang, W.; Oya, S.; Kung, M.-P.; Hou, C.; Maier, D. L.; Kung, H. F. *J. Med. Chem.* **2005**, *48*, 5980.
15. Diwu, Z.; Beachdel, C.; Klaubert, D. H. *Tetrahedron Lett.* **1998**, *39*, 4987.
16. For the quantitative autoradiography, we modified the binding protocol used by Kung et al. See: (a) Kung, M. P.; Hou, C.; Zhuang, Z.-P.; Skovronsky, D.; Kung, H. F. *Brain Res.* **2004**, *1025*, 98; and performed calibrated

densitometry as described by Penney et al; (b) Penney, J. B.; Pan, H. S. Jr.; Young, A. B.; Frey, K. A.; Dauth, G. W. *Science* **1981**, *214*, 1036; using [I-125] calibrated [C-14]standards. Miller, J. A.; Zahniser, N. R. *Neurosci. Lett.* **1987**, *81*, 345; and Baskin, D. G.; Wimpy, T. H. *Neurosci. Lett.* **1989**, *104*, 171.

17. Wilson, A. A.; Houle, S. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 1277.
18. Dishino, D. D.; Welch, M. J.; Kilbourn, M. R.; Raichle, M. E. *J. Nucl. Med.* **1983**, *24*, 1030.
19. Arai, T.; Akiyama, H.; Ikeda, K.; Kondo, H.; Mori, H. *Brain Res.* **1999**, *823*, 202.