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Radioiodinated benzimidazole derivatives as single photon emission computed tomography probes for imaging of β-amyloid plaques in Alzheimer's disease

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Abstract

Five iodinated 2-phenyl-1H-benzo[*d*]imidazole derivatives were synthesized and evaluated as potential probes for β-amyloid (Aβ) plaques. One of the compounds, 4-(6-iodo-1H-benzo[*d*]imidazol-2-yl)-*N*,*N*-dimethylaniline (**12**), showed excellent affinity for $A\beta_{1\text{-}42}$ aggregates (K_i=9.8 nM). Autoradiography with sections of postmortem Alzheimer's disease (AD) brain revealed that a radioiodinated probe [^{125}I]**12**, labeled Aβ plaques selectively with low nonspecific binding. Biodistribution experiments with normal mice injected intravenously with [^{125}I]**12** showed high uptake [4.14 percent injected dose per gram (% ID/g) at 2 min] into and rapid clearance (0.15% ID/g at 60 min) from the brain, which may bring about a good signal-to-noise ratio and therefore achieve highly sensitive detection of Aβ plaques. In addition, [^{125}I]**12** labeled amyloid plaques in vivo in an AD transgenic model. The preliminary results strongly suggest that [^{125}I]**12** bears characteristics suitable for detecting amyloid plaques in vivo. When labeled with ^{123}I, it may be a useful SPECT imaging agent for Aβ plaques in the brain of living AD patients.

Keywords: Alzheimer's disease; β-Amyloid; Single photon emission computed tomography (SPECT); Imaging agent

1. Introduction

Alzheimer's disease (AD) is an age-related, irreversible form of dementia characterized by memory loss, a progressive decline in intellectual ability, language impairment and personality and behavioral changes that eventually interfere with daily life [1]. The accumulation of β-amyloid (Aβ) aggregates (major protein aggregates of senile plaques) in the brain is considered one of the hallmarks of AD [2,3]. Today, the clinical diagnosis of AD is primarily based on history and memory testing, which is often difficult and not accurate, as the early cognitive and behavioral symptoms of AD are difficult to distinguish from normal signs of aging. To facilitate the early diagnosis of this disease, there is an urgent need for the sensitive non-invasive detection of biomarkers for the pathophysiology. Toward achieving this goal, nuclear imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been employed. Radionuclide-labeled agents targeting the Aβ plaques in the brain may greatly facilitate the diagnosis of AD [4,5].

Over recent years, great efforts have been put into developing Aβ imaging agents for PET, including 2-(4′-[^{11}C]methylaminophenyl)-6-hydroxybenzothiazole [6,7], "2-(4'-[^{11}C]methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]PIB)[6,7], 4-*N*-[^{11}C]methylamino-4'-hydroxystilbene ([^{11}C]SB-13)[8,9], [^{11}C]-2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole ([^{11}C]BF-227) [10], [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)-naphthalene-6-yl)ethylidene)malononitrile ([^{18}F]FDDNP)

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[11-13], [^{18}F]-4-(*N*-methylamino)-4'-(2-(2-(2-fluoroethoxy) ethoxy)ethoxy)-stilbene ([^{18}F]BAY94-9172)[14], and [^{18}F] -(*E*)-4-(2-(6-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-*N*-methylaniline ([^{18}F]AV-45)[15,16]2-(4'-[^{11}C] methylaminophenyl)-6-hydroxybenzothiazole [6,7] (Fig. 1). However, the development of imaging agents for SPECT is lagging far behind. Although some groups have reported radioiodinated ligands for Aβ plaques, unfavorable pharmacokinetics in vivo such as low uptake into the brain and a slow washout have prevented further development as imaging agents for SPECT [17–20]. [^{123}I]-6-iodo-2-(4′-dimethylamino-)phenyl-imidazo [1,2] pyridine ([^{123}I]IMPY, Fig. 1) is the first SPECT probe to be tested in humans. The preliminary clinical data showed a poor signal-to-noise ratio, making it difficult to distinguish AD patients, possibly due to high lipophilicity and low stability [21–23].

In an attempt to develop more practical Aβ imaging agents for SPECT with favorable pharmacokinetics in vivo, especially high signal-to-noise ratios, we have screened the structure of 2-phenyl-1H-benzo[*d*]imidazole (BZMZ) which is similar to IMPY. We reasoned that the active hydrogen in the imidazole ring would reduce the lipophilicity of the probes, thus reducing nonspecific binding and enhancing the signal-to-noise ratio. As expected, the calculated log D value of phenylbenzo[*d*]imidazole, 2.69, was lower than that of phenylimidazo[1,2-*a*]pyridine (4.21) (calculated with the Sparc On-Line Calculator). Here, we report the synthesis and biological evaluation of BZMZ derivatives as potential Aβ imaging agents for SPECT.

2. Methods and materials

2.1. General remarks

All the chemicals used were commercial products employed without further purification. The ^{1}H-nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz on JEOL JNM-AL400 NMR spectrometers in CD_3OD solutions at room temperature with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported as δ values relative to the internal TMS. Coupling constants are reported in Hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were acquired with Shimadzu GC-MS-QP2010 Plus (ESI). High-performance liquid chromatography (HPLC) was performed with a Shimadzu system (a LC-10AT pump with a SPD-10A ultraviolet detector, λ=254 nm) using a column of Cosmosil C18 (Nakalai Tesque, 5C_{18}-AR-II, 4.6×150 mm) and acetonitrile/water (0.1% Et_3N) (50/50) as the mobile phase at a flow rate of 1.0 ml/min. Fluorescent observation was performed by microscope (Nikon Eclipse 80i) equipped with a BV-2A filter set (excitation, 400–440 nm; diachronic mirror, 455 nm; long pass filter, 470 nm). All key compounds were proven by this method to show ≥95% purity.

2.2. Chemistry

2.2.1. 4-(6-Bromo-1H-benzo[d]imidazol-2-yl)-N,N-dimethylaniline (1)

A mixture of 4-bromobenzene-1,2-diamine (187 mg, 1.0 mmol), 4-(dimethylamino)benzaldehyde (149 mg, 1.0 mmol), and $Na_2S_2O_5$ (190 mg, 1.0 mmol) dissolved in 8 ml of dimethylformamide (DMF) was heated to reflux for 2 h. Ice water (50 ml) was added, and the precipitate formed was collected by filtration, washed with water and dried under vacuum to obtain 271 mg of **1** (85.8%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, *J*=9.0 Hz, 2H), 7.76 (d, *J*=1.6 Hz, 1H), 7.53 (d, *J*=8.5 Hz, 1H), 7.39 (dd, *J*=8.5, 1.8 Hz, 1H), 6.88 (d, *J*=9.1 Hz, 2H), 3.04 (s, 6H). MS (ESI): *m/z* calcd for $C_{15}H_{14}BrN_3$ 315.04; found 316.00 ($M+H^+$).

2.2.2. 6-Bromo-2-(4-nitrophenyl)-1H-benzo[d]imidazolo (2)

The same reaction described above to prepare **1** was used, and 282 mg of **2** was obtained in a yield of 88.7%. ^{1}H NMR (400 MHz, CD_3OD) δ 8.57 (d, *J*=8.7 Hz, 2H), 7.91 (s, 1H), 7.30 (d, *J*=8.2 Hz, 1H), 7.14 (d, *J*=8.3 Hz, 1H), 6.69 (d, *J*=8.8 Hz, 2H). MS (ESI): *m/z* calcd for $C_{13}H_8BrN_3O_2$ 316.98; found 318.01 ($M+H^+$).

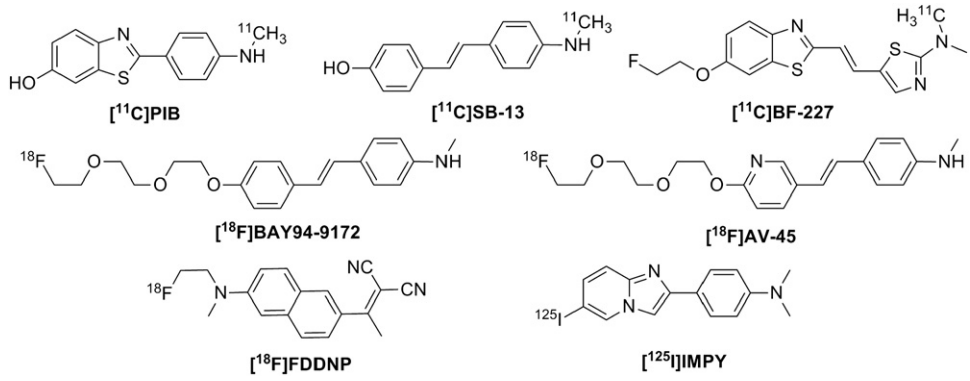

Fig. 1. Chemical structure of Aβ imaging probes in clinical trials.