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Novel styrylbenzene derivatives for detecting amyloid deposits

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ABSTRACT

Background: Various styrylbenzene compounds were synthesized and evaluated as mainly Aβ amyloid sensors. These compounds, however, cannot be used for detecting amyloid deposition in peripheral nerves because of the inherent sensitivity of the compounds. These compounds often generate false positives especially in the basement membrane of blood vessels in histochemical studies. To overcome these problems, we must first synthesize other styryl compounds for detecting amyloid fibrils in tissues.
Methods: A wide variety of symmetrical and unsymmetrical styrylbenzene derivatives were synthesized and then these compounds were used to detect amyloid fibrils in autopsy and biopsy samples from patients with various systemic and localized forms of amyloidosis such as familial amyloidotic polyneuropathy (FAP), senile systemic amyloidosis (SSA), amyloid A (AA) amyloidosis, localized AL amyloidosis, and Alzheimer's disease.
Results: 1-Methoxy-2,5-bis-styrylbenzene and 2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-2,5-bis-styrylbenzene (EEEFSB) detected amyloid fibrils in both in vitro and in vivo histopathological studies. 1-Methoxy-2,5-bis-styrylbenzene also showed a high strength of fluorescence with amyloid deposition in peripheral nerves in a patient with FAP.
Conclusions: 1-Methoxy-2,5-bis-styrylbenzene and EEEFSB may prove a useful tool for diagnosing amyloidosis, not only in a histochemical study but also in whole body amyloid positron emission tomography (PET) imaging.

1. Introduction

Amyloidosis is a group of diseases in which a protein, called amyloid, builds up in the organs and tissues. The buildup may happen in a single organ (localized) or throughout the body (systemically) [1,2]. Although 27 different amyloidgenic proteins have been identified, the mechanism of amyloid formation has been clarified in several types of amyloidosis [3].

As it is not easy to predict the presence of these amyloid deposits on the basis of patients' clinical manifestations, the most important step in the diagnosis of amyloidosis is detection of amyloid deposits in tissues. Of several histopathological methods utilizing stains, Congo red staining is the most common method for detecting amyloid deposits in tissues [4]. Unfortunately, Congo red-stained histochemical specimens are not always easily interpreted. In addition, it is sometimes difficult to make a diagnosis using only the biopsy samples because the pattern of amyloid deposition in the body varies depending on the stage or type of amyloidosis [5]. Moreover, biopsy for diagnostic purposes cannot usually be performed in patients with localized amyloidosis of the brain such as in Alzheimer's disease (AD). Thus, the amyloid positron emission tomography (PET) scan has had a substantial impact on the diagnosis of amyloidosis. ^{11}C-Pittsburgh compound B (^{11}C-PiB) (Fig. 1) has played an especially critical role in this field, contributing to the increasing understanding of disease development and the cognitively normal older population [6–8]. However, the use of PiB is restricted because the compound is labeled ^{11}C. Although the recent developments of ^{18}F-labeled radioligands such as BF-227 or florbetapir, which are derivatives of thioflavin-T, promise to facilitate the accessibility of amyloid imaging [9–11], the usefulness of these compounds is limited. Especially, a positive florbetapir scan does not establish a diagnosis of AD or other cognitive disorder. Moreover, safety and effectiveness of florbetapir have not been established for predicting development of dementia or other neurologic condition and monitoring responses to therapies.

We previously developed a fluorescent probe, (trans,trans)-1-bromo-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB) (Fig. 1), for in vivo detection of amyloid deposits in patients with

Abbreviations: TTR, transthyretin; FAP, familial amyloidotic polyneuropathy; SSA, senile systemic amyloidosis; AD, Alzheimer's disease; PET, positron emission tomography.

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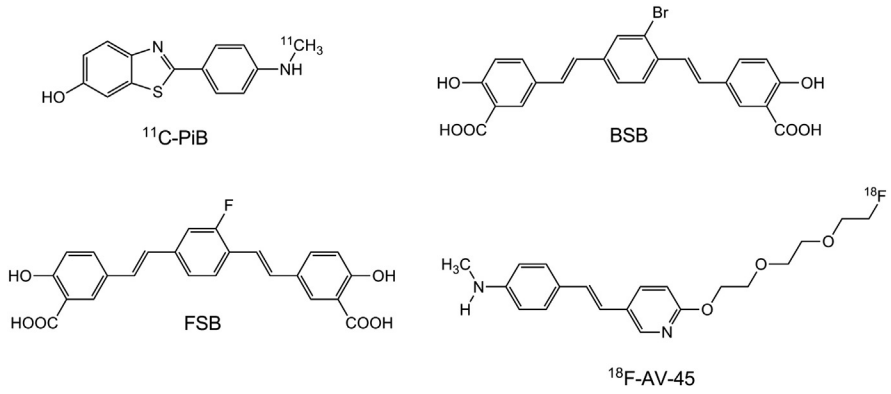

Fig. 1. Chemical structure of ^{11}C-PiB, BSB, FSB and ^{18}F-AV-45.

various systemic and localized forms of amyloidosis such as familial amyloidotic polyneuropathy (FAP), amyloid A (AA) amyloidosis, light chain (AL) amyloidosis, dialysis-related amyloidosis, AD and familial prion disease [12]. On the heels of BSB, (trans,trans)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB) (Fig. 1) was also synthesized and utilized for ^{19}F MRI detection of amyloid plaques in the brain [13,14]. As styrylbenzene compounds such as BSB and FSB are useful dyes for detecting amyloid deposition, various styrylbenzene compounds were synthesized and evaluated as mainly Aβ amyloid sensors [15–26]. These compounds, however, cannot be used for detecting amyloid deposition in peripheral nerves because of the inherent sensitivity of the compounds. Moreover, these compounds often generate false positives especially in the basement membrane of blood vessels in histochemical studies. To overcome these problems, we must first synthesize other styryl compounds for detecting amyloid fibrils in tissues.

2. Materials and methods

2.1. Chemicals

Benzaldehyde, 3-formylbenzoic acid, 4-nitrobenzaldehyde, *N*-Boc-5-methoxyindole-2- boronic acid, thianaphthalene-3-boronic acid and 3-quinoline boronic acid were purchased from Aldrich. 2,5-Dimethylanisole, *N*-bromosuccinimide, carbon tetrachloride, triethylphosphate, sodium methoxide methanol solution, 5-formylsalicylic acid, benzyl chloride, sodium hydride, tin chloride, sodium borohydride, paraformaldehyde, sodium cyanoborohydride and palladium (II) acetate were purchased from Wako Pure Chemical Industries, Ltd. Benzoyl peroxide, diethyl (4-iodobenzyl)-phosphonate and 2-naphthalene boronic acid were purchased from Tokyo Chemical Industry, Co. Ltd. All other chemicals and solvents were of analytical reagent grade.

2.2. Instruments

^{1}H-NMR spectra were obtained on a Varian UNITY plus spectrometer at 500 MHz. FAB MS spectra were obtained on a JEOL JMS HX-110A (JEOL).

2.3. Design and synthesis of styrylbenzene derivatives

^{18}F-AV-45 (florbetapir) (Fig. 1) and C-11 Pittsburgh compound (^{11}C-PiB) are π-conjugated molecules. BSB and FSB are also π-conjugated molecules. Styrylbenzene derivatives having various functional groups were designed and synthesized. First, we would like to determine which symmetrical or unsymmetrical styrylbenzenes are better to detect amyloid fibrils. As shown in Scheme 1, various symmetrical styrylbenzenes were synthesized. In synthesizing unsymmetrical styrylbenzenes, we selected naphthalene, indole, benzothiophene and quinoline (Scheme 2) as heterocyclic compounds having π-conjugation structure. Synthetic routes of Compounds 1–9 and Compounds 10–17 are shown in Schemes 1 and 2, respectively. In the molecular design of symmetrical styrylbenzene derivatives, the structure of Compound 3 does not contain the hydroxyl group and carboxyl group found in BSB. Compounds 4 and 5 are much more hydrophobic than BSB (Fig. 1). Stankoff et al. reported that Compound 7 crosses the blood–brain barrier and binds to myelin [27]. The methylation of the amino group in styrylbenzene derivatives displayed a high binding affinity for Aβ amyloid. On the basis of these results, Compounds 8 and 9 were designed. Derivatives containing naphthalene, indole, benzothiophene and quinoline have been developed as PET probes for detecting Aβ amyloid plaques. Therefore, styrylbenzene derivatives containing these heterocyclic compounds may also be capable of detecting other amyloids including Aβ amyloid. We are very interested in the detection of several types of systemic amyloids using unsymmetrical styrylbenzene derivatives Compounds 14–17.

Compounds 3–7 were synthesized in a manner similar to that of a reported procedure for FSB [13]. A Wittig–Horner reaction by phosphate ester and aldehyde was useful for the production of the styrylbenzene moieties. The reduction of the nitro group in Compound 6 and the methylation of the amino group in Compound 7 produced Compounds 8 and 9, respectively. Compounds 10–13 were synthesized by Suzuki–Miyaura coupling and the Wittig–Horner reaction. The detailed procedures and spectroscopic characterization for all compounds were described in Supplementary data.

2.4. Histopathological studies

For the histopathological examinations, biopsy and autopsy samples from 7 patients with FAP (4 men and 3 women; average age 43.9 ± 3.3 years old; 5 FAP ATTR V30M and 1 FAP ATTR Y114C), 2 patients with SSA (1 man and 1 woman; 73 and 76 y), 3 patients with localized AL amyloidosis (2 men; 56 and 72 y), and 5 patients with AD were included in the study. All FAP and SSA patients had been definitively diagnosed on the basis of genetic and histochemical tests. Rabbit anti-human TTR antibody, rabbit anti-human kappa, lambda light chain antibodies, and rabbit anti-human amyloid A component antibody (Dako) were used for the immunohistochemical studies for diagnosis.

2.5. Histochemical analysis

Sections, 10 μm thick, were cut from paraffin-embedded blocks of tissue and then stained with alkaline Congo red. Congo red reactivity was confirmed under polarized light. For our compound staining, sections were immersed in each compound (0.1 mg/ml) in 50% EtOH for 30 min. Sections were quickly differentiated in saturated Li_2CO_3 and rinsed with 50% EtOH before examination by fluorescence microscopy [28]. After staining with the compounds, we measured the density