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Discovery of boronic acid-based fluorescent probes targeting amyloid-beta plaques in Alzheimer's disease

Seung-Jin Jung [a], Jun Young Lee [a], Tae Ho Kim [c], Dong-Eun Lee [a], Jongho Jeon [a,d], Seung Dae Yang [a], Min Goo Hur [a], Jung-Joon Min [b], Yong Dae Park [a,b,*]

[a] Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Republic of Korea
[b] Department of Nuclear Medicine, Chonnam National University Hwasun Hospital, Hwasun, Republic of Korea
[c] Department of Chemistry and Research Institute of Natural Science, Gyeongsang National University, 501 Jinju Daero, Jinju 660-701, Republic of Korea
[d] Department of Radiation Biotechnology and Applied Radioisotope Science, Korea University of Science and Technology, Daejeon 34113, Republic of Korea

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ABSTRACT

A boronic acid-based fluorescent probe was developed for diagnosis of amyloid-β (Aβ) plaques from Alzheimer's disease (AD). Probe **4c**, which included boronic acid as a functional group, exhibited a significant increase (64.37-fold, $F_{A\beta}/F_0$) in fluorescence intensity as a response to Aβ aggregates, with a blue shift (105 nm) in the maximum emission wavelength. We found that boronic acid as a functional group improved the binding affinity (K_D value = 0.79 ± 0.05 μM for **4c**) for Aβ aggregates and confirmed that **4c** selectively stained Aβ plaques in brain sections from APP/PS1 mice. Ex vivo fluorescence imaging using mice (normal and APP/PS1) also revealed that **4c** was able to penetrate the blood–brain barrier (BBB) and to stain Aβ plaques in the brain. From these results, we believe that **4c** will be useful as a fluorescent probe in preclinical research related to AD. Furthermore, we believe that our results with boronic acid also provide valuable information for the development of a probe for Aβ plaques.

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive decline, irreversible memory loss, disorientation, and language impairment.[1,2] One of the pathological hallmarks is the presence of amyloid-β (Aβ) plaques in the brains of AD patients. Aβ plaques are central in the molecular pathogenesis and form oligomers, fibrils, and plaques. Biomarkers to monitor Aβ aggregation are important for further detailed studies of the disease mechanisms and drug development. Aβ is misfolded into a cross β-sheet structure and thereby binds to dyes, such as Congo red and Thioflavin T.[3,4]

A number of imaging techniques, such as magnetic resonance imaging (MRI)[5], positron emission tomography (PET),[6,7] and single-photon emission computed tomography (SPECT),[8] have been developed for the specific imaging of Aβ plaques. Although imaging techniques employing these probes have produced promising results in vitro, ex vivo, and in vivo, using these imaging methods with probes carries the disadvantages of poor spatial and temporal resolution, low sensitivity, exposure to radioactivity, and short-lived isotopes.[9] Additionally, the handling of these probes requires stringent safety regulations due to their radioactivity.

Fluorescent probes have attracted interest in the labelling and imaging of Aβ plaques because these probes are relatively safe, low cost and user friendly and have real-time and multiplexing capabilities. Appropriate fluorescent probes for Aβ plaques should have the following properties: (a) specificity for Aβ plaques, (b) sufficient binding affinity to Aβ peptides, (c) an emission wavelength above 650 nm to minimize background fluorescence from brain tissue, (d) appropriate lipophilicity (log*P* value between 1 and 3) to rapidly cross the blood–brain barrier (BBB), (e) a significant change in fluorescence upon binding to Aβ plaques, and (f) straightforward synthesis.[10] Although fluorescent probes are unfavorable for in vivo imaging on humans and animals except mice due to their limited penetration depth, they are a useful tool for preclinical evaluation.[11] Therefore, fluorescent probes, such as Congo red, NIAD-4, BODIPY 7, and CRANAD-2, have been continuously developed for the detection of Aβ plaques and have subsequently been reported.[12–15]

Recently, we developed and reported fluorescent chalcone-mimic probes for the detection of Aβ plaques that rapidly bind to Aβ plaques upon intravenous injection in mice.[16] Using chalcone-mimic probes, we found that their clearance from the brain occurs much more quickly than expected because of their relatively low binding affinity to Aβ plaques.

* Corresponding author. Tel.: +82 63 570 3574; fax: +82 63 570 3549.
E-mail address: ydpark@kaeri.re.kr (Y.D. Park).

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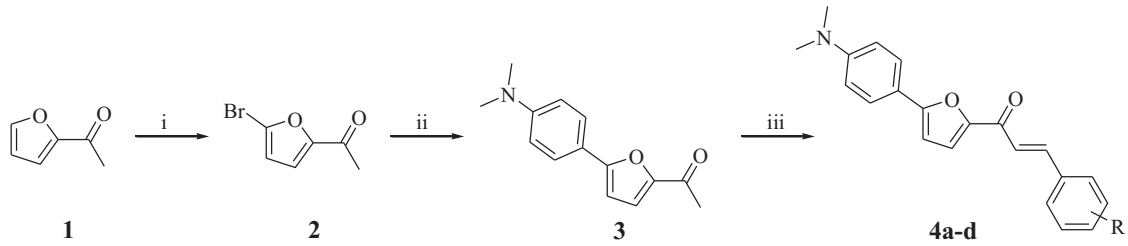

Scheme 1. Reaction scheme for synthesis of the optical probes. Reagents and conditions: (i) DMF (dimethylformamide), NBS (*N*-bromosuccinimide), room temperature, 24 h; (ii) DME (1,2-dimethoxyethane), 2.0 M aqueous Na_2CO_3, $Pd(PPh_3)_4$, 4-(dimethylamino) phenylboronic acid; and (iii) DMF, NaOH, aldehyde derivatives, room temperature, 8 h.

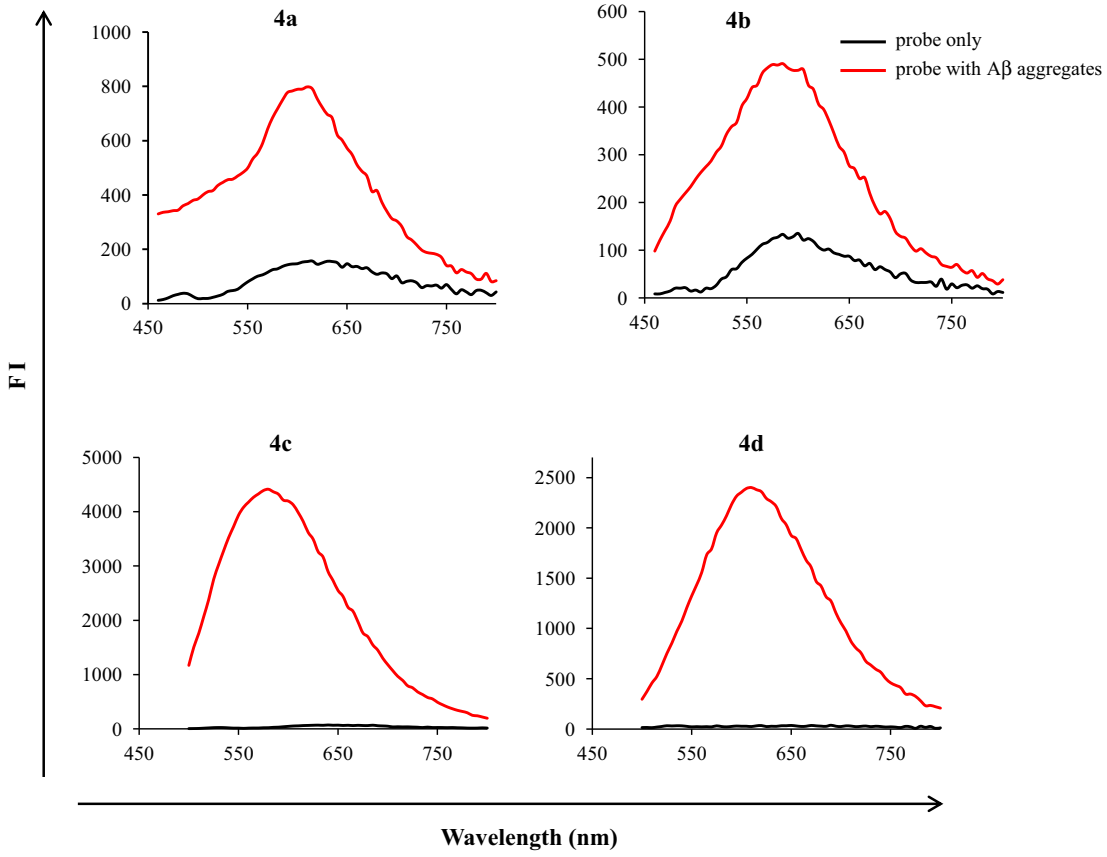

Figure 1. Emission spectra of probes upon responses with and without Aβ aggregates.

Here, we describe the discovery and further optimization of chalcone-mimic probes. In this process, we developed boronic acid-based probes and found that boronic acid was effective at binding to Aβ plaques. In recent years, boronic acid compounds have attracted much attention. Many of these compounds have been used in the fields of medicinal chemistry, such as fluorescent reporter[17], enzyme inhibition, cancer therapy, and antibody mimic development.[18] As a Lewis acid, boronic acid can form a covalent bond with Lewis bases, such as alcohol, amine, thiol.[19] We envisioned that the boronic acid analogue might form stronger bonds with amino acid residues of Aβ plaques to enhance its affinity. These compounds have also been used in research related to AD.[20] In this study, we report the optical and biological properties of fluorescent probes with boronic acid for the diagnosis of Aβ plaques from AD.

Probe synthesis is outlined in Scheme 1. The formation of backbone **3** was synthesized by the Suzuki coupling reaction of 4-(dimethylamino) phenylboronic acid with compound **2**. Probes (**4a**, **4b**, **4c**, and **4d**) were successfully prepared by the condensation of **3** and aldehyde derivatives in the presence of NaOH (53.4%, 48.4%, 37.9%, and 32.6% yields, respectively). The X-ray crystal structure of **4d** is depicted in Figure S5.

First, we evaluated the fluorescent properties, including the excitation and emission wavelengths, of the synthesized probes