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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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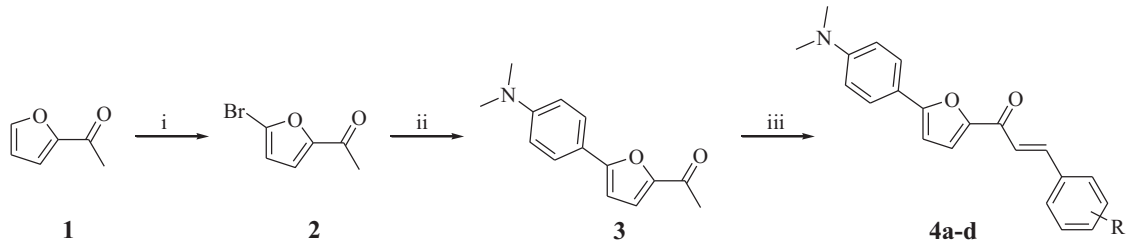

Scheme 1. Reaction scheme for synthesis of the optical probes. Reagents and conditions: (i) DMF (dimethylformamide), NBS (*N*-bromosuccinimide), room temperature, 24 h; (ii) DME (1,2-dimethoxyethane), 2.0 M aqueous Na_2CO_3, $Pd(PPh_3)_4$, 4-(dimethylamino) phenylboronic acid; and (iii) DMF, NaOH, aldehyde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

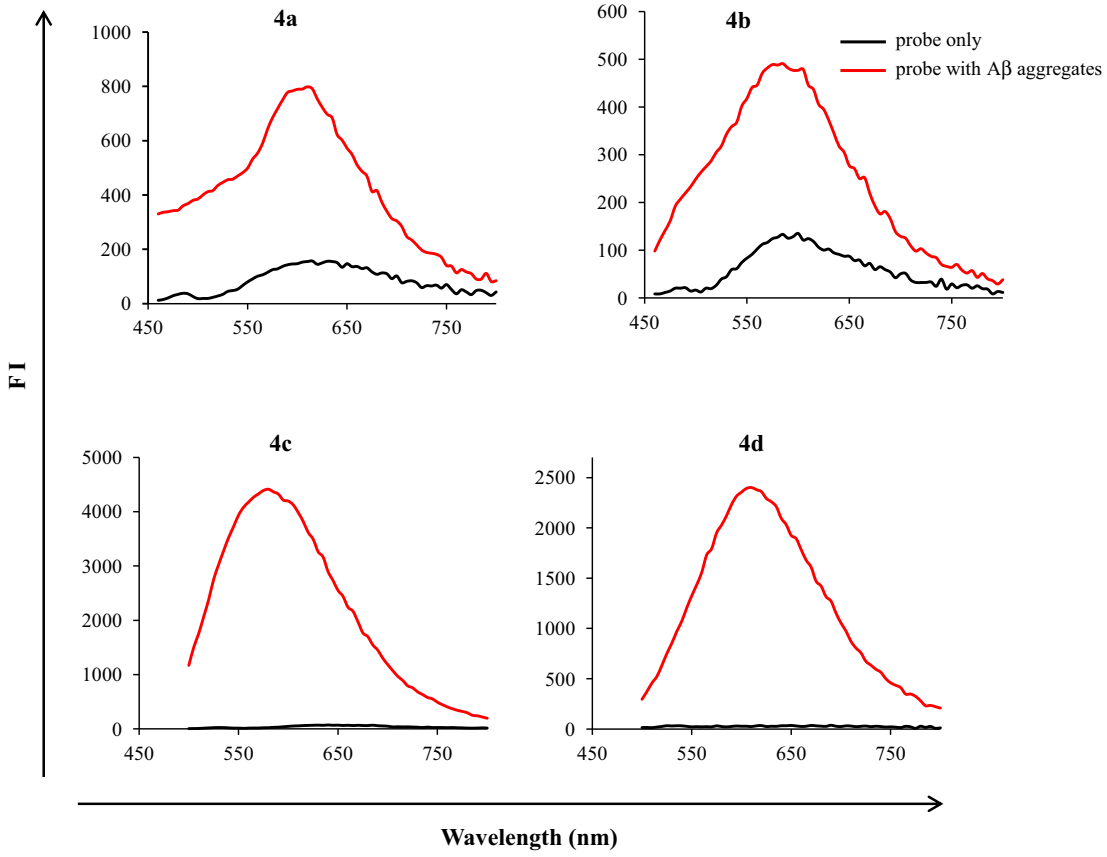

Figure 1. Emission spectra of probes upon responses with and without Aβ aggregates.

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

Table 1
Structures, fluorescence profiles, and K_D values of probes with Aβ aggregates

Probe	R	λ_{ex} (nm)	λ_{em} (nm)	Fold increase[a] ($F_{Aβ}/F_0$)	K_D (mean ± SD)[b] (μM)
4a	4-N$(CH_3)_2$	415	610	6.66	–
4b	4-N$(CH_2CH_3)_2$	415	610	3.69	–
4c	4-B$(OH)_2$	445	685	64.37	0.79 ± 0.05
4d	3-B$(OH)_2$	450	685	70.66	0.90 ± 0.02

[a] Fold-change values were calculated using the fluorescence emission intensities at λ_{em} of probes (10 μM compound and 20 μM Aβ42 aggregates were used for the measurements).
[b] The K_D values are expressed as the means ± S.D. of values obtained from three independent replicates ($N = 3$).

in PBS (phosphate-buffered saline, 0.1 M, pH = 7.4). There were large Stokes shifts of 195 nm for **4a** and **4b** ($\lambda_{max(ex)} = 415$ nm, $\lambda_{max(em)} = 610$), 240 nm ($\lambda_{max(ex)} = 445$ nm, $\lambda_{max(em)} = 685$) for **4c**, and 235 nm ($\lambda_{max(ex)} = 450$ nm, $\lambda_{max(em)} = 685$ nm) for **4d** (Fig. S1). The large Stokes shifts easily separated the excitation and emission wavelengths, which could also be applied to the fluorescent probes with great advantage.

Next, the synthesized probes were tested with the synthetic Aβ42 aggregates for their fluorescence responses. Probes **4a** and **4b** did not contain boronic acid and thus did not show changes in fluorescent response with the Aβ42 aggregates. In contrast, probes **4c** and **4d**, which included boronic acid, exhibited blue shifts of 105 nm (from 685 to 580 nm) for **4c** and 75 nm (685–610 nm) for **4d** on the maximum emission wavelength, with a remarkable increase in fluorescence intensity (64.4- and 70.6-fold, respectively) in response to Aβ42 aggregates (Fig. 1). Blue shifts in the fluorescence emission spectra and significant increases in fluorescence intensity upon binding with Aβ aggregates are important properties for good fluorescent probes for the detection of Aβ aggregates.[21] Additionally, to quantitatively evaluate the binding affinities of the two boronic acid probes for Aβ aggregates, a saturation binding assay was conducted. Probes **4c** and **4d** exhibited strong binding affinities (K_D values = 0.79 ± 0.05 μM and 0.90 ± 0.02 μM, respectively) (Fig. S2), and the binding affinities for the Aβ aggregates were higher than that of a previously reported fluorescent probe (K_D value = 1.59 ± 0.13 μM).[16] All of the values of the fluorescent properties of the probes are shown in Table 1. These results indicated that boronic acid improved the binding affinity for the Aβ plaques and that the probes (**4c** and **4d**) with boronic acid specifically stained Aβ plaques in the brain. However, the position of boronic acid did not have a significant influence on the properties of the Aβ aggregates. This finding might be due to the fact that boronic acids are Lewis acids and isosteres of carboxylic acid. Boronic acid pK_a values are ~9–10; therefore, at physiological pH, boronic acids remain unionized.[22] Thus, the electron-deficient boronic acid moiety could increase the binding affinity by forming a hydrogen bond between its OH group and a histidine residue of the Aβ peptides.

Based on the above results, we selected probes **4c** and **4d** with boronic acid as fluorescent probes to stain Aβ plaques, and additional experiments were conducted using these probes. In vitro pathological fluorescent staining of Aβ plaques in slices of brain tissue from double-transgenic mice (APP/PS1) was performed to evaluate the affinity of the boronic acid probes (**4c** and

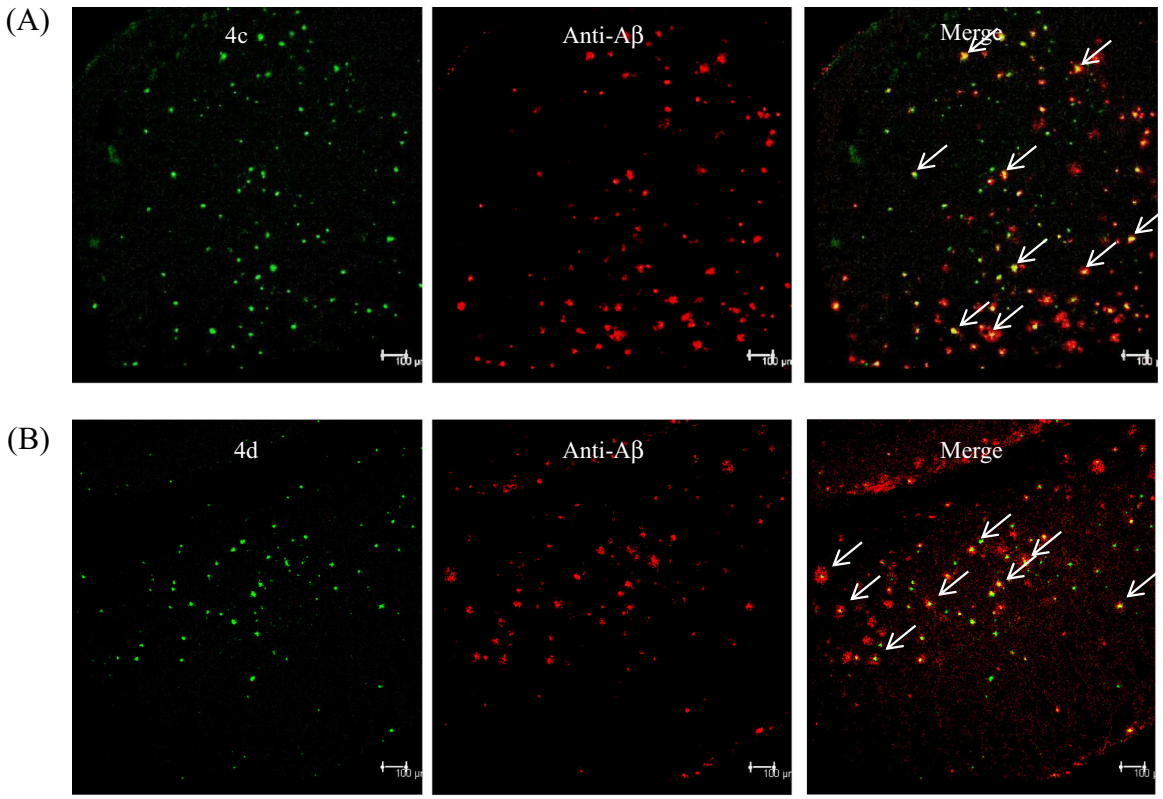

Figure 2. Confocal images of histological double-staining in transgenic mice (APP/PS1) brain sections. Aβ plaques stained with anti-Aβ (middle, red), stained with probes **4c** (A) and **4d** (B) (left, green), and merged with anti-Aβ and probes (right, orange). The spots stained by anti-Aβ and probes were cologically matched (white arrow).

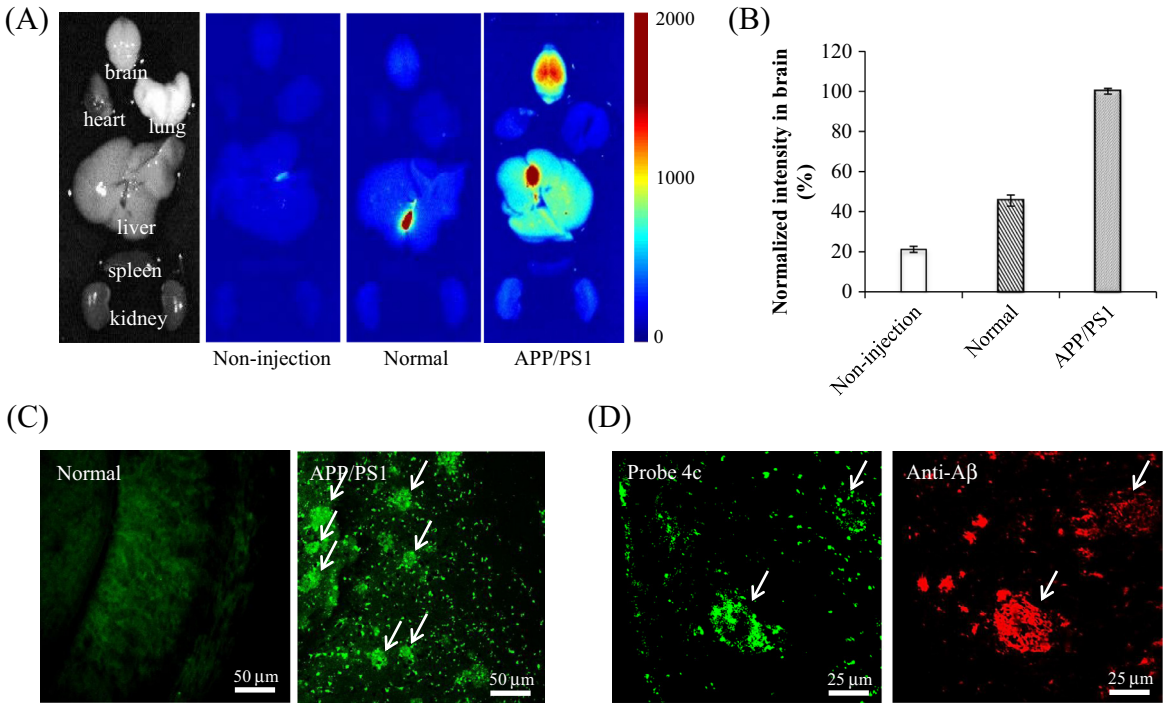

Figure 3. Ex vivo fluorescent images of mouse organs. (A) Fluorescent images of organs removed from APP/PS1 and normal mice 30 min after intravenous injection of probe **4c**. The fluorescent images were obtained using an orange filter at an excitation of 415 nm using software provided with the MAESTRO™ 2.4 fluorescence imaging system. (B) Comparison of the fluorescence intensities in the brain from non-injection, injected-normal, and injected-APP/PS1 mice. (C) Fluorescent images of brain sections from normal and APP/PS1 mice 30 min after intravenous injection of probe **4c** (Aβ plaques are indicated with white arrows). (D) Fluorescent images of Aβ plaques (white arrow) bound with probe **4c** and anti-Aβ. The excitation wavelengths for probe **4c** and anti-Aβ were 455 and 550 nm, respectively. Fluorescent images were obtained using a laser confocal scanning microscope.

4d). As shown in Fig. 2, specific staining of plaques was observed in brain slices of APP/PS1 mice treated with probes **4c** and **4d**. The presence and distribution of Aβ plaques were confirmed by staining with anti-Aβ; the plaques colocalized with the staining results with our probes. From probes **4c** and **4d**, we selected **4c** (due to its better binding affinity compared to **4d**) for additional in vivo experiments using mice. Probe **4c** exhibited a log*P* value = 2.97 and met the requirement to penetrate the BBB. It has been reported that BBB penetration is dependent on lipophilicity, and a moderate log*P* value is between 1 and 3.[23]

We conducted an ex vivo experiment in APP/PS1 and normal mice using probe **4c** to further confirm binding of the probe to Aβ plaques in vivo. Mice were injected with 5 mg/kg of **4c** through the tail vein, and whole organs were removed 30 min after injection and scanned using a fluorescence imaging system (MAESTRO™ 2.4 software). Fluorescent images of ex vivo organs from normal mice showed that large amounts of probe **4c** initially accumulated in the brain and liver. The maximum uptake values were observed 10 min after injection (Fig. S3). The observed signals in both organs then quickly decreased over the time; the fluorescent signals from other organs displayed similar trends. The results indicated that most of the injected probe was rapidly excreted from the body without significant retention of the probe in the brain region. However, fluorescent signals in the brains of APP/PS1 mice were optically more abundant than those of normal mice. Additionally, there were approximately two-fold differences in the regions of interest (ROIs) of brains from both the APP/PS1 and the normal mice (Fig. 3A and B). These results demonstrated show that probe **4c** showed slow clearance kinetics from the brains of APP/PS1 mice than those of normal mice due to abundance of Aβ plaques in the brain. Additionally, the fluorescent signals from the brains of APP/PS1 and normal mice exhibited different emission spectra (Fig. S4) those were consistent with the in vitro data. These results further confirmed that **4c** penetrated the BBB and specifically stained Aβ plaques. The livers from APP/PS1 mice also exhibited higher fluorescent signals than those of normal mice because the accumulated probe **4c** in the brains of APP/PS1 mice underwent slower excretion via the hepatobiliary pathway (Fig. 3A). Additionally, we checked whether **4c** selectively stained the Aβ plaques in the brain sections from the APP/PS1 mice 30 min after intravenous injection with **4c**. Weak fluorescent signals were observed from the brains of normal mice (Fig. 3C), while large amounts of Aβ plaques were confirmed in the cortex region of the brain from APP/PS1 mice (Fig. 3C); these Aβ plaques were also cologically stained with probe **4c** in the brain section restrained with anti-Aβ (Fig. 3D). These results supported the specific binding of probe **4c** with the Aβ plaques in the brain.

In conclusion, we developed a fluorescent probe, **4c**, with boronic acid for the detection of Aβ plaques from AD. We found that boronic acid favorably affected the binding affinity for Aβ plaques. Probe **4c** satisfied the requirements of a fluorescent probe, such as the fluorescent properties, good binding affinity, specificity for Aβ plaques, and reasonable lipophilicity in both in vitro and in vivo experiments. We believe that probe **4c** with boronic acid will be useful as a fluorescent probe in preclinical experiments related to AD. Our result with boronic acid also provides valuable information for the development of probes for Aβ plaques.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.042.

References and notes

1. Ono, M.; Haratake, M.; Mori, H.; Nakayama, M. *Bioorg. Med. Chem.* **2007**, *15*, 6802.
2. Minati, L.; Edginton, T.; Bruzzone, M. G.; Giaccone, G. *Am. J. Alzheimers Dis. Other Demen.* **2009**, *24*, 95.
3. Glenner, G. G. *N. Engl. J. Med.* **1980**, *302*, 1333.
4. Glenner, G. G. *N. Engl. J. Med.* **1980**, *302*, 1283.
5. Higuchi, M.; Iwata, N.; Matsuba, Y.; Sato, K.; Sasamoto, K.; Saido, T. C. *Nat. Neurosci.* **2005**, *8*, 527.
6. Johnson, A. E.; Jeppsson, F.; Sandell, J.; Wensbo, D.; Neelissen, J. A.; Jureus, A.; Strom, P.; Norman, H.; Farde, L.; Svensson, S. P. *J. Neurochem.* **2009**, *108*, 1177.
7. Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D. P.; Bergstrom, M.; Savitcheva, I.; Huang, G. F.; Estrada, S.; Ausen, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Langstrom, B. *Ann. Neurol.* **2004**, *55*, 306.
8. Newberg, A. B.; Wintering, N. A.; Plossl, K.; Hochold, J.; Stabin, M. G.; Watson, M.; Skovronsky, D.; Clark, C. M.; Kung, M. P.; Kung, H. F. *J. Nucl. Med.* **2006**, *47*, 748.
9. Liu, K.; Guo, T. L.; Chojnacki, J.; Lee, H. G.; Wang, X.; Siedlak, S. L.; Rao, W.; Zhu, X.; Zhang, S. *ACS Chem. Neurosci.* **2012**, *3*, 141.
10. Ran, C.; Xu, X.; Raymond, S. B.; Ferrara, B. J.; Neal, K.; Bacskai, B. J.; Medarova, Z.; Moore, A. *J. Am. Chem. Soc.* **2009**, *131*, 15257.
11. Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620.
12. Hintersteiner, M.; Enz, A.; Frey, P.; Jaton, A. L.; Kinzy, W.; Kneuer, R.; Neumann, U.; Rudin, M.; Staufenbiel, M.; Stoeckli, M.; Wiederhold, K. H.; Gremlich, H. U. *Nat. Biotechnol.* **2005**, *23*, 577.
13. Li, Q.; Lee, J. S.; Ha, C.; Park, C. B.; Yang, G.; Gan, W. B.; Chang, Y. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 6331.
14. Nesterov, E. E.; Skoch, J.; Hyman, B. T.; Klunk, W. E.; Bacskai, B. J.; Swager, T. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 5452.
15. Raymond, S. B.; Skoch, J.; Hills, I. D.; Nesterov, E. E.; Swager, T. M.; Bacskai, B. J. *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, S93.
16. Jung, S. J.; Park, S. H.; Lee, E. J.; Park, J. H.; Kong, Y. B.; Rho, J. K.; Hur, M. G.; Yang, S. D.; Park, Y. D. *Arch. Pharm. Res.* **2015**, *38*, 1992.
17. Kotoku, N.; Guo, X. H.; Arai, M.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4152.
18. Yang, W.; Gao, X.; Wang, B. *Med. Res. Rev.* **2003**, *23*, 346.
19. Wu, Z.; Li, M.; Fang, H.; Wang, B. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7179.
20. Jimenez-Aligaga, K.; Bermejo-Bescos, P.; Martin-Aragon, S.; Csaky, A. G. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 426.
21. Hong, M. C.; Kim, Y. K.; Choi, J. Y.; Yang, S. Q.; Rhee, H.; Ryu, Y. H.; Choi, T. H.; Cheon, G. J.; An, G. I.; Kim, H. Y.; Kim, Y.; Kim, D. J.; Lee, J. S.; Chang, Y. T.; Lee, K. C. *Bioorg. Med. Chem.* **2010**, *18*, 7724.
22. Kumar, S. K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N. E.; Khan, S. R. *J. Med. Chem.* **2003**, *46*, 2813.
23. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2012**, *46*, 3.