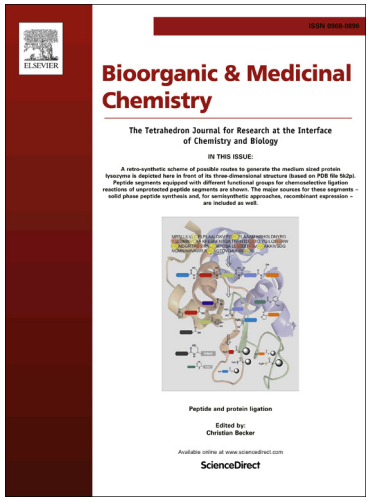
Accepted Manuscript



Synthesis and biological evaluation of novel radioiodinated benzimidazole derivatives for imaging α-synuclein aggregates

Hiroyuki Watanabe, Taisuke Ariyoshi, Akihiko Ozaki, Masafumi Ihara, Masahiro Ono, Hideo Saji

PII: S0968-0896(17)31522-5
DOI: https://doi.org/10.1016/j.bmc.2017.10.010
Reference: BMC 14015

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 26 July 2017
Revised Date: 10 October 2017
Accepted Date: 13 October 2017

Please cite this article as: Watanabe, H., Ariyoshi, T., Ozaki, A., Ihara, M., Ono, M., Saji, H., Synthesis and biological evaluation of novel radioiodinated benzimidazole derivatives for imaging α-synuclein aggregates, *Bioorganic & Medicinal Chemistry* (2017), doi: https://doi.org/10.1016/j.bmc.2017.10.010

Synthesis and biological evaluation of novel radioiodinated benzimidazole derivatives for imaging α-synuclein aggregates

Hiroyuki Watanabe[1], Taisuke Ariyoshi[1], Akihiko Ozaki[2], Masafumi Ihara[3], Masahiro Ono[*,1], Hideo Saji[1]

[1]Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan.

[2]Department of Neurology, Osaka Saiseikai Nakatsu Hospital, 2-10-39 Shibata, Kita-ku, Osaka 530-0012, Japan.

[3]Department of Stroke and Cerebrovascular Diseases, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita-shi, Osaka 565-8565, Japan.

*To whom correspondence should be addressed. Phone: +81-75-753-4608, Fax: +81-75-753-4568, e-mail: ono@pharm.kyoto-u.ac.jp for M. Ono.

Abstract

α-Synuclein (α-syn) aggregates are commonly found in the brains of patients with Parkinson's disease (PD), dementia with Lewy bodies (DLB), and some other diseases. Therefore, in vivo imaging of α-syn aggregates would aid in drug development, early diagnosis, and monitoring of disease status. In order to develop imaging probes targeting α-syn aggregates, we synthesized and evaluated three novel radioiodinated benzoimidazole (BI) derivatives for selective imaging of α-syn aggregates. In binding experiments, BI-2 exhibited the highest selective binding affinity for α-syn aggregates among the BI derivatives. In addition, BI-2 clearly stained Lewy bodies in PD brain sections, but did not label senile plaques deposited in AD brain sections. However, in the biodistribution study using normal mice, [^{125}I]BI-2 did not demonstrate high brain uptake (0.56%ID/g at 2-min post-injection). Further structural modifications of the BI derivatives are needed, but the BI scaffold may be an attractive candidate for developing α-syn imaging probes.

Keywords; α-synuclein, imaging probe, Parkinson's disease, Lewy body

1. Introduction

α-Synuclein (α-syn), a 140-amino acid presynaptic protein, is the major fibrillary component of Lewy bodies, which are commonly found in the brains of patients with Parkinson's disease (PD), dementia with Lewy bodies (DLB), Parkinson's disease dementia (PDD), and multiple system atrophy (MSA).[1] PD is the most common movement disorder, and DLB is the second most common form of neurodegenerative dementia in elderly people.[2, 3] However, there are no effective methods for early diagnosis or basic remedy for these diseases. In addition, α-syn plays an important role in the pathogenesis of the diseases because α-syn aggregates form early in the disease and spread with time in the brain.[4] Therefore, in vivo imaging of α-syn aggregates would aid in drug development, early diagnosis, and monitoring of disease status.[5, 6]

Nuclear medical techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), are valuable tools for in vivo imaging because of their high sensitivity and quantitative capability in deep tissues. As β-amyloid (Aβ) and tau aggregates are the neuropathological hallmark of Alzheimer's disease (AD), a number of PET and SPECT probes targeting these aggregates have been

developed and utilized for the diagnosis of AD.[7-10] Notably, some PET probes for in vivo imaging of Aβ aggregates, such as flutemetamol, florbetaben, and florbetapir, were approved by the FDA. On the other hand, there have been no reports on the development of clinically useful PET and SPECT probes to detect α-syn aggregates in vivo.

Recently, a few α-syn imaging probes that can be used only for in vitro evaluation have been reported (Figure 1). Phenothiazine derivatives, including SIL5, SIL23, and SIL26, were reported as the first candidate probes for selective imaging of α-syn aggregates.[11, 12] SIL23 exhibited the moderate binding affinity for α-syn aggregates (K_d = 148 nM), but the selectivity for α-syn aggregates against Aβ and tau aggregates was insufficient (2.5- and 1.6- fold, respectively). In addition, the biodistribution and pharmacokinetics of [^{125}I]SIL23 were not evaluated. SIL5 and SIL26 also showed binding affinity for α-syn aggregates (66 and 16 nM, respectively). The selectivity of SIL5 for α-syn aggregates against Aβ and tau aggregates was low (1.7- and 2.1- fold, respectively), but SIL26 showed moderate selectivity for these aggregates (6.6- and 8.1-fold selectivity versus Aβ and tau aggregates). In a biodistribution study, [^{11}C]SIL5 and

[^{18}F]SIL26 were able to cross the blood-brain barrier. Therefore, further structural optimization may lead to development of useful α-syn imaging probes. 3-(Benzylidine)indolin-2-one derivatives were reported as second generation lead compounds for development of α-syn selective imaging probes.[13] Among them, **46a** showed high affinity for α-syn aggregates (K_i = 2 nM) and favorable selectivity against Aβ and tau aggregates (70- and 40-fold, respectively). ^{18}F labeling of **46a** succeeded, but the brain uptake of [^{18}F]**46a** was not evaluated. More recently, we also developed radioiodinated diphenyl (IDP) derivatives as novel ligands for α-syn aggregates.[14] IDP-4 exhibited high affinity for α-syn aggregates (K_d = 5.4 nM), but the selectivity of IDP-4 against Aβ aggregates was not high (4.3-fold). In addition, [^{125}I]IDP-4 showed low brain uptake (0.45%ID/g at 2-min postinjection in mice). Due to such situations, the development of useful α-syn imaging probes is strongly desired.

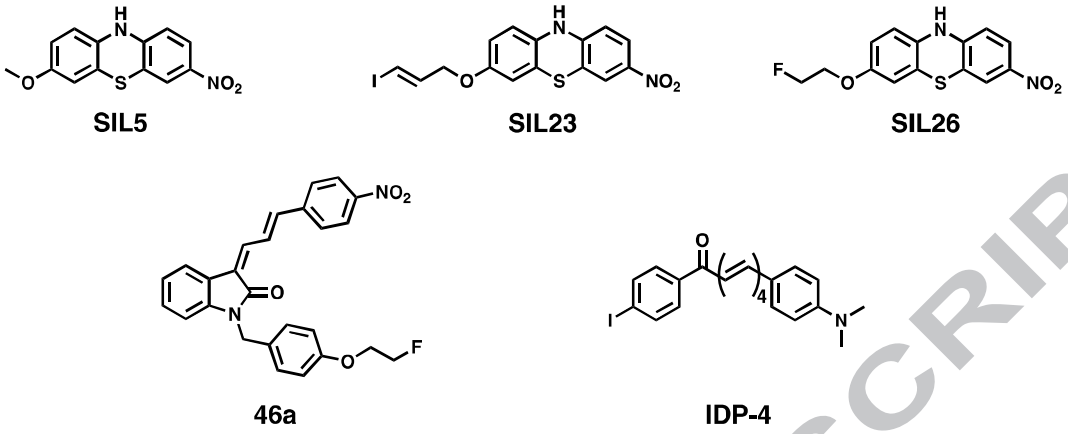


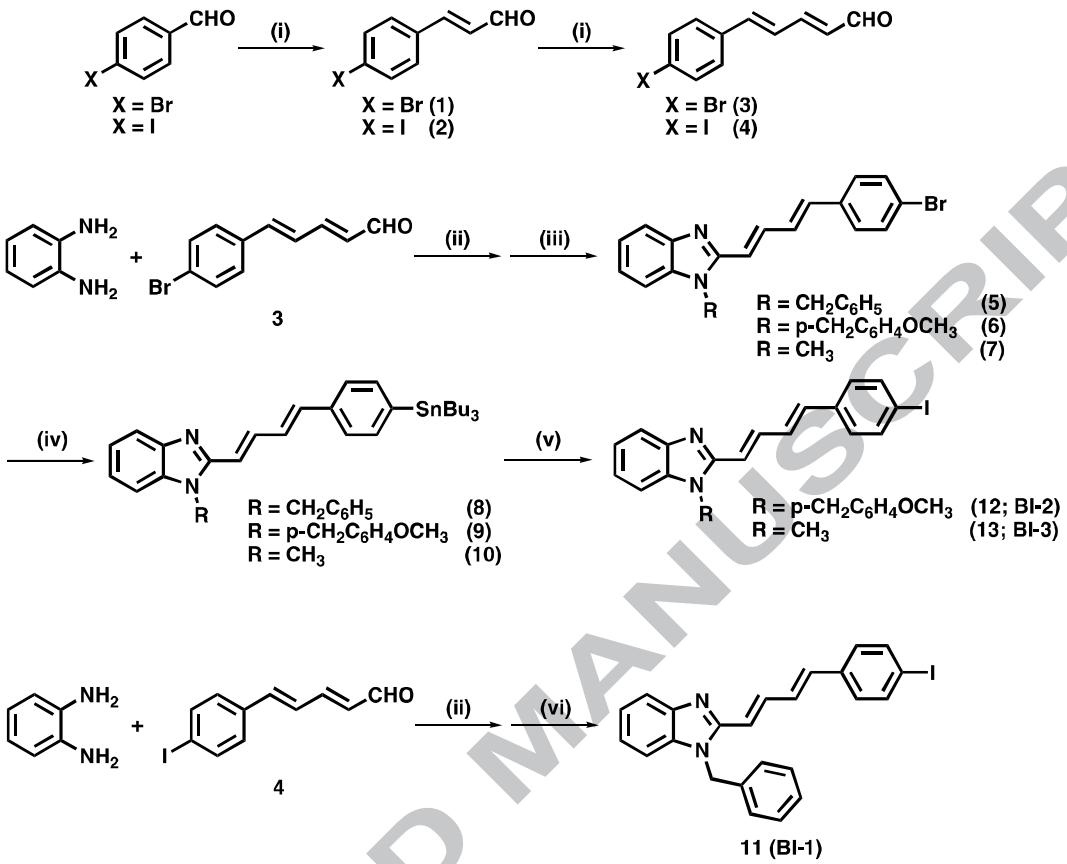
Figure 1. Chemical structures of previously reported α-syn imaging probes.

In this study, we searched for new scaffolds that could selectively detect α-syn aggregates. As a recent study found that styryl benzimidazole could bind to α-syn aggregates in vitro, we selected the benzoimidazole (BI) scaffold as the backbone of the α-syn imaging probes developed in this study.[15] Another recent report demonstrated that the presence of the diene moiety is important for the affinity for α-syn aggregates.[13, 16] In addition, bulky substitution groups, such as benzyl groups, enhanced the selectivity of α-syn versus Aβ.[14] Based on these findings, we designed and synthesized three novel BI derivatives and evaluated their potential as probes for in vivo imaging of α-syn aggregates.

2. Results and discussion

2.1. Chemistry

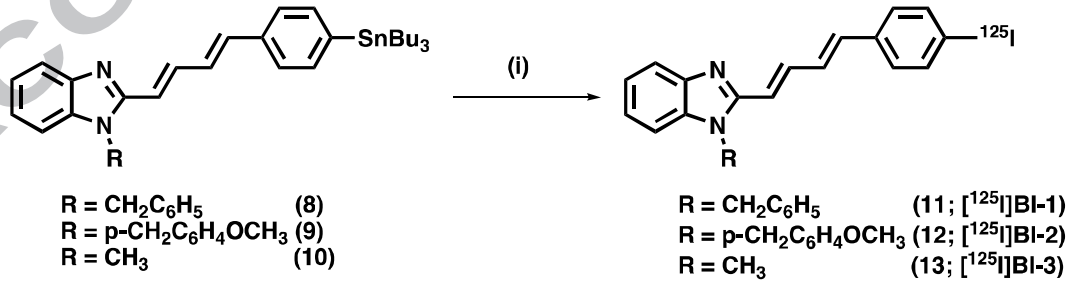
As shown in Scheme 1, compounds **3** and **4** were prepared according to methods reported previously.[17] The BI scaffold was obtained by an intermolecular cyclization reaction between 1,2-phenylenediamine with **3** or **4** using $Na_2S_2O_5$ as an oxidant.[18] Following the reaction, (bromomethyl)benzene, 1-(chloromethyl)-4-methoxybenzene, or iodomethane was added to obtain **5**, **6**, **7**, and **11**. The tributyltin derivatives (**8**, **9**, and **10**) were prepared from the corresponding bromo compounds using the exchange reaction of bromine by tributyltin catalyzed by Pd(0). These tributyltin compounds were also used as the starting materials for radioiodination in the preparation of the corresponding ^{125}I-labeled derivatives. We could not obtain enough of **8** that is used as a precursor for the synthesis of **11**. Therefore, the synthetic route of **11** was different from that of **12** and **13**.



Scheme 1 Synthetic route for BI derivatives. Reagents and conditions: (i) 1) (1, 3-dioxolan-2-yl)methyl-triphenylphosphonium bromide, 18-crown-6, NaH, THF, rt; 2) 20% HCl, THF, rt; (ii) $Na_2S_2O_5$, DMF, 105°C; (iii) 1) NaH, DMF, 0°C; 2) (bromomethyl)benzene, 1-(chloromethyl)-4-methoxybenzene, or iodomethane, DMF, rt; (iv) $(SnBu_3)_2$, $(Ph_3P)_4Pd$, dioxane, Et_3N, 95°C; (v) I_2, $CHCl_3$, rt; (vi) 1) NaH, DMF, 0°C; 2) (bromomethyl)benzene, DMF, rt.

2.2. Radiolabeling

^{125}I-labeled BI derivatives were obtained by an iododestannylation reaction using [^{125}I]NaI and hydrogen peroxide as the oxidants under acidic conditions (Scheme 2). After the reaction was completed, the radioiodinated compounds were purified by HPLC, as detailed in the experimental section. The no-carrier-added derivatives were expected to be produced as a final product with a theoretical specific activity of 81.4 TBq/mmol, similar to that of ^{125}I. The radiochemical identity of the radioiodinated ligands was confirmed by co-injection with non-radioiodinated compounds from their HPLC profiles (Table S1). [^{125}I]BI-1, [^{125}I]BI-2, and [^{125}I]BI-3 were obtained in a radiochemical yield of 37.5, 52.0, and 81.0%, respectively, with a radiochemical purity of >99%.



Scheme 2. Radiosynthesis of [^{125}I]BI derivatives. Reagents and conditions: (i) [^{125}I]NaI,

3% H_2O_2, 1 N HCl, rt.

2.3. Binding assay using recombinant α-syn and $A\beta_{1-42}$ aggregates

It is well known that α-syn aggregates often colocalize with Aβ aggregates in the PDD and DLB brain, thus α-syn imaging probes need to have selective binding affinity for α-syn aggregates versus Aβ aggregates.[10, 19] After confirming that the fluorescence intensity of BI derivatives increased in the presence of α-syn and $A\beta_{1-42}$ aggregates, we performed saturation binding assays to calculate the apparent binding dissociation constant (K_d) of these aggregates. Table 1 summarizes the K_d values of the BI derivatives for α-syn and $A\beta_{1-42}$ aggregates. The K_d value of BI-1, BI-2, and BI-3 for α-syn aggregates was 485, 99.5, and 874 nM, respectively. For $A\beta_{1-42}$ aggregates, BI-1, BI-2, and BI-3 had K_d values of 179, 727, and 271 nM, respectively. The selectivity for α-syn aggregates versus $A\beta_{1-42}$ aggregates (K_d ratio of $A\beta_{1-42}$/α-syn) was 0.37, 7.31, and 0.31 for BI-1, BI-2, and BI-3, respectively. These results suggested that the substituent of the BI scaffold affected the affinity and selectivity for α-syn aggregates. Among the BI derivatives, BI-2 exhibited the highest selective binding affinity for α-syn aggregates.

The selectivity of BI-2 for α-syn aggregates versus Aβ aggregates was higher than or comparable with that of IDP derivatives and phenothiazine derivatives, such as SIL5, SIL23, and SIL26, but lower than some 3-(benzylidine)indolin-2-one derivatives, including **46a**,[12-14] indicating that BI-2 has the potential to selectivity detect α-syn aggregates.

Table 1. K_d values of BI derivatives for recombinant α-syn and $A\beta_{1-42}$ aggregates

Compounds	K_d (nM)[a]	
	α-syn	$A\beta_{1-42}$
BI-1	485 ± 160	179 ± 59.1
BI-2	99.5 ± 20.8	727 ± 227
BI-3	874 ± 169	271 ± 67.3

[a]Values are the mean ± standard error of the mean for three independent experiments.

2.3. Fluorescent staining of PD and AD brain sections

To confirm the selective binding affinity for Lewy bodies, we carried out

fluorescent staining experiments of PD and AD brain sections using BI-2. Several fluorescent spots were observed in the brain section of a PD patient (Figure 2A). When the same section was immunostained with an antibody against α-syn, the fluorescent spots obtained with BI-2 corresponded with the results of immunohistochemical staining (Figure 2B). In the immunohistochemical staining of AD brain sections with anti-Aβ antibody, we observed extensive accumulation of Aβ aggregates, but marked fluorescence derived from BI-2 was not observed (Figure 2C and 2D). These results suggested that BI-2 selectively bound α-syn aggregates deposited in PD brain sections in vitro.

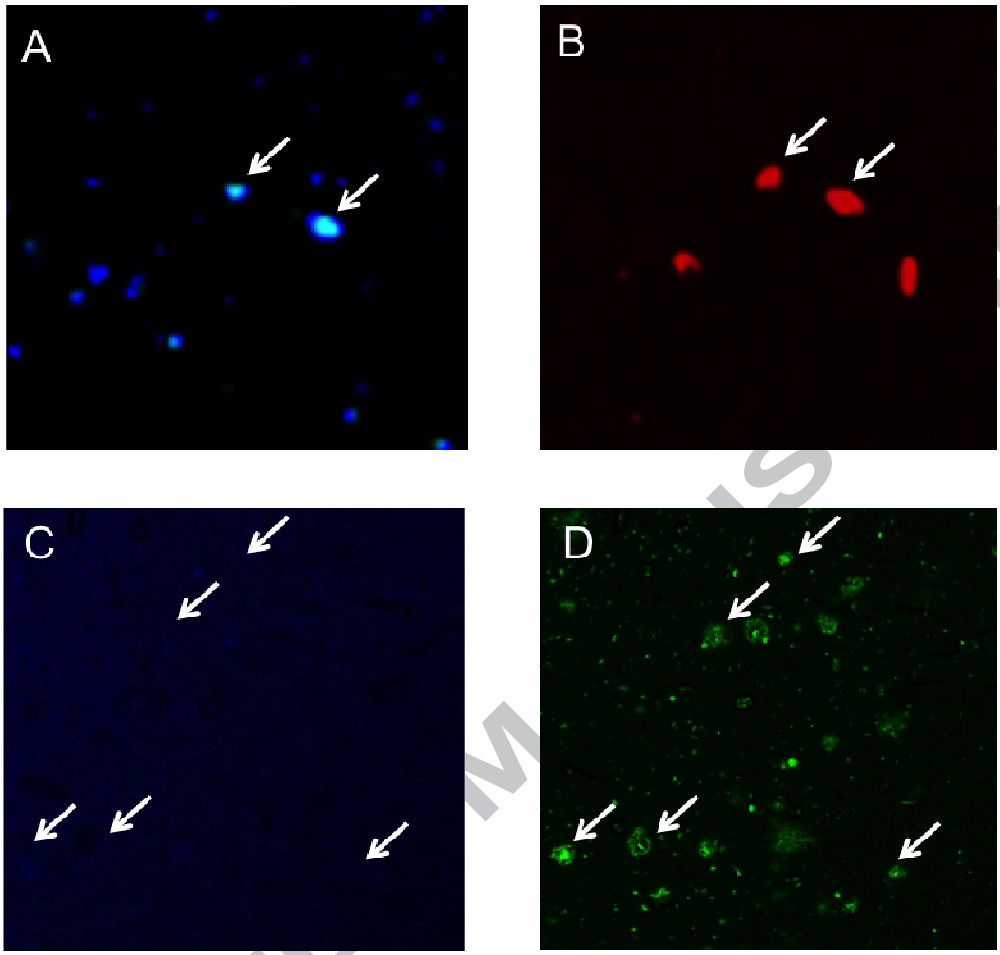


Figure 2. Fluorescence staining of BI-2 in midbrain sections from a PD patient (A) and cortical sections from an AD patient (C). Immunohistochemical staining with fluorescent antibody against α-syn (B) and Aβ (D).

2.4. In vivo biodistribution in normal mice

To evaluate the brain uptake and washout of [^{125}I]BI derivatives, we performed biodistribution experiments using normal mice. The results of the biodistribution study

are shown in Table S2 in Supplementary Information. [^{125}I]BI-3 exhibited moderate uptake (1.89%ID/g) at 2-min post-injection and clearance from brain over time (1.0 and 0.53%ID/g at 30- and 60-min post-injection, respectively). In contrast, [^{125}I]BI-1 and [^{125}I]BI-2 displayed low initial uptake at 2-min post-injection (0.52 and 0.56%ID/g respectively) and retention in the brain (Figure 3). The bulky substitution groups of [^{125}I]BI-1 and [^{125}I]BI-2 may have reduced penetration of the blood-brain barrier. In general, the criteria for useful Aβ and tau imaging probes are high initial brain uptake (>4% ID/g at 2-min post-injection in mice) and rapid clearance from normal brains (<1% ID/g at 30-min post-injection in mice).[20, 21] Useful imaging probes targeting α-syn aggregates have not been reported, but the criteria for α-syn imaging probes may be the same as those for probes targeting Aβ and tau aggregates. Considering brain uptake, the BI derivatives do not meet the criteria for clinical use. To develop useful α-syn imaging probes, additional structural changes are needed to improve the brain uptake of the compounds without impairing selective affinity for α-syn aggregates.

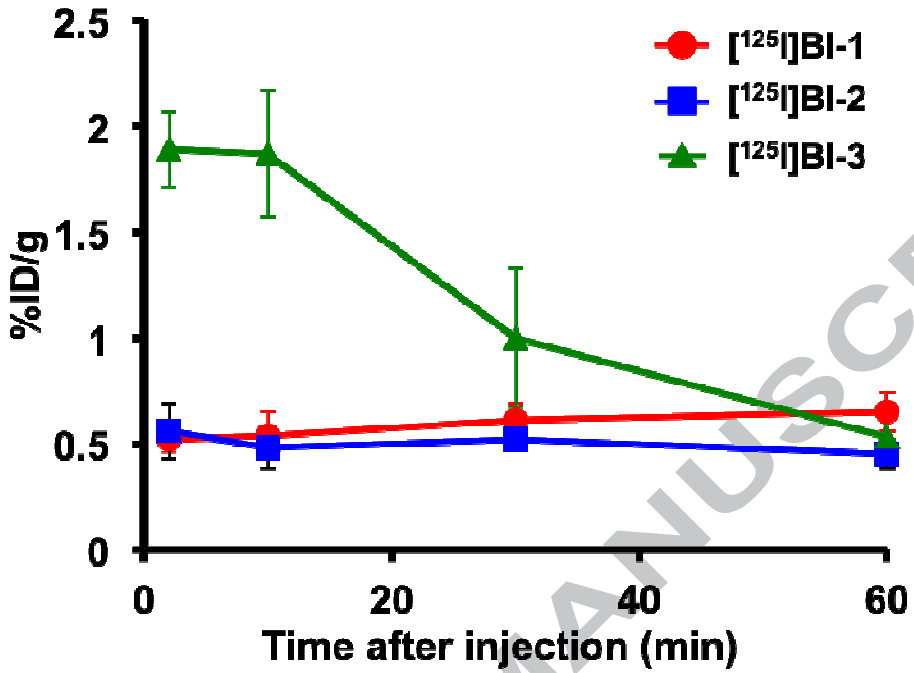


Figure 3. Comparative brain uptake of radioactivity after intravenous injection of [^{125}I]BI derivatives.

3. Conclusion

In conclusion, we designed and synthesized three novel radioiodinated BI derivatives for imaging α-syn aggregates in the brain. Among the derivatives tested in this study, [^{125}I]BI-2 exhibited the highest selective binding affinity for α-syn aggregates. In addition, BI-2 selectively stained Lewy bodies in the brain sections, but the brain uptake and clarance of [^{125}I]BI-2 did not meet the criteria for the in vivo imaging of α-syn aggregates in the brain. Taken together, further structural

modifications of the BI derivatives may lead to useful α-syn imaging probes.

4. Experimental Part

Materials and Methods

All reagents were commercial products and used without further purification unless indicated otherwise. Smart Flash EPCLC W-Prep 2XY (Yamazen Corp., Osaka, Japan) was used for silica gel chromatography. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL JNM-ECS400 (JEOL, Tokyo, Japan), and chemical shifts were recorded in δ (ppm) relative to TMS as an internal standard. Coupling constants are reported in Hertz. Multiplicity was defined as singlet (s), doublet (d), or multiplet (m). Mass spectra were obtained on a SHIMADZU LCMS-2020 EV (SHIMADZU, Kyoto, Japan) and JEOL JMS-SX 102A QQ. HPLC was performed with the Shimadzu system (LC-20AD pump with an SPD-20A UV detector, $\lambda = 254$ nm) using a Cosmosil C_{18} column (Nacalai Tesque, Kyoto, Japan, $5C_{18}$-AR-II, 4.6 × 150 mm) and CH_3CN/H_2O as the mobile phase at a flow rate of 1.0 mL/min. All key compounds were proven by this method to be 95% pure.

4.1. Chemistry

4.1.1. (*E*)-4-(2-Bromovinyl)benzaldehyde (1)

To a stirred solution of 4-bromobenzaldehyde (2000 mg, 10.81 mmol), 18-crown-6 (25 mg) and (1,3-dioxolan-2-ylmethyl)-triphenylphosphoniumbromide (7000 mg, 16.3 mmol) in THF (90 mL) under N_2, NaH (1038 mg, 86.5 mmol) was added in one portion. After 2-h stirring at room temperature, the reaction mixture was quenched with water and extracted with EtOAc. After evaporation of EtOAc, the residue was dissolved in the mixture of THF (90 mL) and 20% hydrochloric acid, and stirred for 2 h at room temperature. After the mixture was turned basic with 2 N NaOHaq and aqueous saturated solution of $NaHCO_3$, the organic solvent was evaporated, and the mixture extracted with EtOAc. The combined EtOAc extracts were washed with brine and dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel chromatography (EtOAc/hexane = 17/83) to give **2** (1568 mg, 68.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 7.6 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 16.0 Hz, 1H), 6.71 (dd, J_1 = 8.0 Hz, J_2 = 16.8

Hz, 1H). MS (ESI) *m/z* 211 and 213 [MH^+].

4.1.2. (*E*)-4-(2-Iodovinyl)benzaldehyde (2)

The same reaction as described above to prepare **1** was used and 1784 mg of **2** was obtained in a 78.7% yield from 4-iodobenzaldehyde. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 6.72 (dd, J_1 = 7.6 Hz, J_2 = 16.0 Hz, 1H). MS (ESI) *m/z* 258.9 [MH^+].

4.1.3. 4-((1*E*,3*E*)-4-Bromobuta-1,3-dien-1-yl)benzaldehyde (3)

The same reaction as described above to prepare **1** was used and 659 mg of **3** was obtained in a 37.4% yield from **1**.^{1}H NMR (400 MHz, DMSO-d_6) δ9.60 (d, J = 8.4 Hz, 1H), 7.57-7.63 (m, 4H), 7.50 (dd, J_1 = 10.4 Hz, J_2 = 15.2 Hz, 1H), 7.31 (dd, J_1 = 10.8 Hz, J_2 = 15.6 Hz, 1H), 7.17 (d, J = 15.2 Hz, 1H), 6.30 (dd, J_1 = 8.0 Hz, J_2 = 16.8 Hz, 1H). MS (ESI) *m/z* 237 and 239 [MH^+].

4.1.4. 4-((1*E*,3*E*)-4-Iodobuta-1,3-dien-1-yl)benzaldehyde (4)

The same reaction as described above to prepare **1** was used and 1192 mg of **4** was obtained in a 61.9% yield from **2**. ^{1}H NMR (400 MHz, CD_3OD) δ9.57 (d, J = 7.6 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.46 (dd, J_1 = 10.8 Hz, J_2 = 15.2 Hz, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.21 (dd, J_1 = 12.0 Hz, J_2 = 15.2 Hz, 1H), 7.09 (d, J = 16.0 Hz, 1H), 6.30 (dd, J_1 = 8.0 Hz, J_2 = 16.8 Hz, 1H). MS (ESI) m/z 284.9 [MH^+].

4.1.5.

1-Benzyl-2-((1*E*,3*E*)-4-(4-bromophenyl)buta-1,3-dien-1-yl)-1H-benzo[d]imidazole (5)

A mixture of 1,2-phenylenediamine (108 mg, 1.0 mmol), **3** (237 mg, 1.0 mmol), and $Na_2S_2O_5$ (190 mg, 1.0 mmol) in DMF (5 mL) was stirred at 105˚C under N_2 for 2.5 h. After the mixture was cooled to 0˚C, NaH (48 mg, 2.0 mmol) was added and stirred for 30 min at 0˚C. Then, (bromomethyl)benzene (342 mg, 2.0 mmol) was added and stirred at room temperature for 18 h. The solution was quenched with water and extracted with EtOAc, The combined EtOAc extracts were washed with brine and dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel

chromatography (EtOAc/hexane = 26/74) to give **5** (79.4 mg, 19.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ7.60-7.64 (m, 2H), 7.55-7.57 (m, 3H), 7.49 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.25-7.27 (m, 1H), 7.21-7.23 (m, 1H), 7.19-7.20 (m, 2H), 7.14 (d, J = 7.2 Hz, 2H), 7.06 (d, J = 15.2 Hz, 1H), 6.95 (d, J = 15.2 Hz, 1H), 5.61 (s, 2H). MS (ESI) *m/z* 415 and 417 [MH$^+$].

4.1.6.

2-((1*E*,3*E*)-4-(Bromophenyl)buta-1,3-dien-1-yl)-1-(4-methoxybenzyl)-1H-benzo[d]imidazole (6)

The same reaction as described above to prepare **5** was used with 1-(chloromethyl)-4-methoxybenzene as a substitute for (bromomethyl)benzene and 215 mg of **6** was obtained in a 24.2% yield from **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57-7.63 (m, 4H), 7.51 (d, J = 8.0 Hz, 2H), 7.19-7.29 (m, 4H), 7.14 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 14.0 Hz, 1H), 6.96 (d, J = 15.6 Hz, 1H), 6.88 (d, J = 8.0 Hz, 2H), 5.53 (s, 2H), 3.69 (s, 3H). MS (ESI) *m/z* 445 and 447 [MH$^+$].

4.1.7.

2-((1*E*,3*E*)-4-(Bromophenyl)buta-1,3-dien-1-yl)-1-methyl-1H-benzo[d]imidazole (7)

The same reaction as described above to prepare **5** was used with iodomethane as a substitute for (bromomethyl)benzene and 237 mg of **7** was obtained in a 35.0% yield from **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (d, J = 11.6 Hz, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 6.8 Hz, 1H), 7.51-7.55 (m, 3H), 7.27-7.31 (m, 1H), 7.22 (dd, J_1 = 6.4 Hz, J_2 = 13.2 Hz, 2H), 7.03 (d, J = 15.2 Hz, 1H), 6.97 (d, J = 15.6 Hz, 1H), 3.86 (s, 3H). MS (ESI) *m/z* 339 and 341 [MH^+].

4.1.8.

1-Benzyl-2-((1*E*,3*E*)-4-(4-(tributhylstinnyl)phenyl)buta-1,3-dien-1-yl)-1H-benzo[d]imidazole (8)

A mixture of **5** (75 g, 0.18 mol), $(SnBu_3)_2$ (314 g, 0.54 mol), and $(Ph_3P)_4Pd$ (42 mg, 0.036 mmol) in a mixed solvent (15 mL, 2:1 dioxane/Et_3N mixture) was stirred at 95˚C for 1.5 h. The solvent was removed, and the residue was purified by silica gel chromatography (EtOAc/hexane = 1/3) to give 13.7 mg of **8** (13.7%). ^{1}H NMR (400

MHz, CD_3OD) δ 7.61-7.67 (m, 2H), 7.41-7.44 (m, 5H), 7.24-7.30 (m, 5H), 7.12 (d, J = 8.4 Hz, 3H), 6.88 (d, J = 15.2 Hz, 1H), 6.85 (d, J = 15.2 Hz, 1H), 5.56 (s, 2H), 0.87-1.58 (m, 27H). MS (ESI) *m/z* 627 [MH^+].

4.1.9.

1-(4-Methoxybenzyl)-2-((1*E*,3*E*)-4-(4-(tributhylstinnyl)phenyl)buta-1,3-dien-1-yl)-1H-benzo[d]imidazole (9)

The same reaction as described above to prepare **8** was used and 39 mg of **9** was obtained in a 13.7% yield from **6**. ^1H NMR (400 MHz, CD_3OD) δ 7.61-7.67 (m, 2H), 7.43-7.48 (m, 5H), 7.21-7.28 (m, 2H), 7.16 (d, J = 8.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 6.0 Hz, 1H), 6.86 (d, J = 8.4 Hz, 3H), 5.50 (s, 2H), 3.73 (s, 3H), 0.85-1.61 (m, 27H). MS (ESI) *m/z* 657 [MH^+].

4.1.10.

1-Methyl-2-((1*E*,3*E*)-4-(4-(tributhylstinnyl)phenyl)buta-1,3-dien-1-yl)-1H-benzo[d]imidazole (10)

The same reaction as described above to prepare **8** was used and 68 mg of **10** was obtained in a 21 % yield from **10**. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.56-7.64 (m, 2H), 7.42-7.54 (m, 5H), 7.12-7.27 (m, 2H), 7.00 (d, J = 15.2 Hz, 1H), 6.95 (d, J = 16.0 Hz, 1H), 3.86 (s, 3H), 0.84-1.56 (m, 3H). MS (ESI) *m/z* 551 [MH^+].

4.1.11.

1-Benzyl-2-((1*E*,3*E*)-4-(4-iodophenyl)buta-1,3-dien-1-yl)-1H-benzo[d]imidazole (11; BI-1)

The same reaction as described above to prepare **5** was used and 186 mg of **11** was obtained in a 20.1% yield from **4**. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.74 (d, J = 8.4 Hz, 2H), 7.61-7.63 (m, 2H), 7.55 (dd, J_1 = 3.6 Hz, J_2 = 6.0 Hz, 1H), 7.31-7.36 (m, 4H), 7.27 (d, J = 7.2 Hz, 1H), 7.24 (d, J = 4.4 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 7.20 (d, J = 3.6 Hz, 1H), 7.15 (d, J = 7.2 Hz, 2H), 7.07 (d, J = 14.8 Hz, 1H), 6.93 (d, J = 15.6 Hz, 1H) 5.61(s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ150.6, 143.0, 137.6, 137.3 136.6, 136.2, 135.5, 135.2, 129.1, 128.8, 128.7, 127.6, 126.6, 122.43, 122.38, 118.7, 118.3, 110.5, 94.4, 45.7. HRMS (FAB) *m/z* calcd for $C_{18}H_{16}N_2I$ 387.0358; found 387.0361.

4.1.12.

2-((1*E*,3*E*)-4-(Iodophenyl)buta-1,3-dien-1-yl)-1-(4-methoxybenzyl)-1H-benzo[d]imidazole (12; BI-2)

A solution of **9** (60 mg, 0.092 mmol) and I_2 (34.7 mg, 0.137 mmol) in $CHCl_3$ (10 mL) was stirred for 30 min at room temperature. The reaction mixture was quenched with aqueous saturated $NaHSO_3$ and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with brine and dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel chromatography (EtOAc/hexane = 1:2) to give 32 mg of **12** (71%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.66 (d, J = 8.4 Hz, 2H), 7.57-7.63 (m, 2H), 7.42 (d, J = 8.0 Hz, 1H), 7.20 (m, 4H), 7.13 (d, J = 15.6 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 15.6 Hz, 1H), 6.84 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.0 Hz, 1H), 5.46 (s, 2H), 3.71 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.6, 150.5, 143.1, 137.6, 136.5, 136.2, 135.4, 135.1, 129.2, 129.1, 128.7, 128.1, 122.34, 122.30, 118.7, 118.5, 114.1, 110.5, 94.4, 55.1, 45.3. HRMS (FAB) *m/z* calcd for $C_{25}H_{22}N_2OI$ 493.0777; found 493.0780.

4.1.13.

2-((1*E*,3*E*)-4-(Iodophenyl)buta-1,3-dien-1-yl)-1-methyl-1H-benzo[d]imidazole (13; BI-3)

The same reaction as described above to prepare **12** was used and 20 mg of **13** was obtained in a 94% yield from **10**. ^{1}H NMR (400 MHz, DMSO-d_6) δ7.76 (d, J = 8.8 Hz, 2H), 7.53-7.63 (m, 3H), 7.37 (d, J = 8.4 Hz, 2H), 7.19-7.31 (m, 3H), 7.03 (d, J = 15.6 Hz, 1H), 6.94 (d, J = 15.6 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ150.7, 142.9, 137.6, 136.2, 136.1, 136.0, 134.9, 129.2, 128.7, 122.1, 118.6, 118.5, 110.1, 94.3, 29.5. HRMS (FAB) *m/z* calcd for $C_{24}H_{20}N_2I$ 463.0671; found 463.0675.

4.2. Radiolabeling

The radioiodinated compounds [^{125}I]**11** ([^{125}I]BI-1), [^{125}I]**12** ([^{125}I]BI-2), and [^{125}I]**13** ([^{125}I]BI-3) were prepared from the corresponding tributyltin derivatives by iododestannylation. Briefly, to initiate the reaction, 50 µL of H_2O_2 (3%) was added to a mixture of a tributyltin derivative (100 µg/100 µL EtOH), [^{125}I]NaI (3.7-14.8 MBq,

specific activity 81.4 TBq/mmol), and 50 μL of 1 N HCl in a sealed vial. The reaction was allowed to proceed at room temperature for 5 min and terminated by the addition of saturated $NaHSO_3$ aq. (100 μL). After neutralization with sodium hydrogen carbonate and extraction with EtOAc, the extract was dried by passing through an anhydrous Na_2SO_4 column. The solution was blown dry with a stream of nitrogen gas. The radioiodinated ligand was purified by HPLC on a Cosmosil C_{18} column with an isocratic elution using CH_3CN/H_2O (7/3) as the solvent, at a flow rate of 1.0 mL/min.

4.3. In vitro binding assay using α-syn and $A\beta_{1-42}$ aggregates

α-Syn and $A\beta_{1-42}$ were purchased from rPeptide (Bogart, GA, USA) and the Peptides Institute, Inc. (Osaka, Japan), respectively. α-Syn aggregates were prepared by incubating recombinant α-syn monomers (1.67 mg/mL in 50 mM Tris-HCl, 250 mM NaCl, pH 7.48) at 37˚C for 6 days with stirring at 1000 rpm. $A\beta_{1-42}$ aggregates were prepared by incubating the $A\beta_{1-42}$ peptides (0.25 mg/mL in PBS, pH 7.4) at 37˚C for 2 days with gentle and constant shaking. The formation of the α-Syn or $A\beta_{1-42}$ aggregates was confirmed with the measurement of the fluorescence intensity of thioflavin-S. The

reaction mixtures containing BI-1, BI-2, or BI-3 (final conc. 0-20 μM) and α-syn or $A\beta_{1\text{-}42}$ aggregates (final conc. 2.2 μM) were incubated at room temperature for 30 min. Then, fluorescence intensities were recorded using Infinite M200PRO (TECAN, Männedorf, Switzerland) and K_d binding curves were generated by GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

4.4. Fluorescence staining of human PD and AD brain sections

The experiments involving human tissues were performed in accordance with relevant guidelines and regulations, and were approved by the ethics committee of Osaka Seiseikai Nakatsu Hospital and the National Cerebral and Cardiovascular Center. Informed consent for the experiments using post-mortem brain tissues was obtained from all subjects in this study-antemortem. Postmortem brain tissues from autopsy-confirmed cases of PD (female, 69 years old) were obtained from Osaka Seiseikai Nakatsu Hospital and those of AD (male, 76 years old) were obtained from the National Cerebral and Cardiovascular Center. Eight- and six- micrometer-thick serial sections of paraffin-embedded blocks for PD and six- for AD, respectively, were used

for staining. The sections were subjected to two 15-min incubations in xylene, two 1-min incubations in 100% EtOH, one 1-min incubation in 90% EtOH, and one 1-min incubation in 70% EtOH, followed by two 2.5-min washes in water. The sections were incubated with BI-2 (200 μM, 50% EtOH) for 15 min. The sections were observed with a fluorescence microscope (Eclipse 80i, Nikon, Tokyo, Japan) equipped with a U-2A filter set and multispectral imaging system (Nuance FX, Caliper Life Sciences Inc., Hopkinton, MA, USA).

4.5. Immunohistochemical staining of α-syn in human PD brain sections

After fluorescence staining, the midbrain sections were activated by 90% formic acid and incubated with anti-phosphorylated α-syn primary antibody (pSyn#64, Wako) at 4˚C for 3 h. After three 5-min incubations in PBS-Tween 20, they were incubated with a fluorescent secondary antibody labeled with Alexa Fluor 647 at 4˚C for 1 h. After three 5-min incubations in PBS-Tween 20, the sections were observed with a fluorescence microscope (FSX100, Olympus Corp., Tokyo, Japan) equipped with a U-DM-CY5-3 filter set.

4.6. Immunohistochemical staining of Aβ in human AD brain sections

After fluorescence staining, the brain sections were activated by 90% formic acid and incubated with $A\beta_{1-42}$ (BC05, Wako) primary antibody at room temperature for 1 h. After three 5-min incubations in PBS containing 0.02% Tween 20 (PBS-Tween20), they were incubated with fluorescent secondary antibody labeled with Alexa Fluor 488 at room temperature for 1 h. After three 5-min incubations in PBS-Tween 20, the sections were observed with a fluorescence microscope (FSX100) equipped with a U-MWIBA3 filter set.

4.7. *In vivo* biodistribution in normal mice

The experiments with animals were conducted in accordance with our institutional guidelines and approved by Kyoto University. A saline solution (100 μL, 20 kBq) of each [^{125}I]BI derivative containing EtOH (10 μl) was injected directly into the tail vein of ddY mice (5 weeks old, male). The mice were sacrificed at 2, 10, 30, and 60 min post-injection. The organs of interest were removed and weighed, and

radioactivity was measured with a γ counter (Wizard 1470, PerkinElmer).

Acknowledgements

The study was supported by JSPS KAKENHI Grant Number (17H04260, 17H05092), Japan Research Foundation for Clinical Pharmacology, Takeda Science Foundation, and the Mochida Memorial Foundation for Medical Pharmaceutical Research.

Supporting Information

The retention time of the radioiodinated compounds and cold congeners and the results of biodistribution experiments for all organs.

References

1. Irwin DJ, Lee VM, Trojanowski JQ. Parkinson's disease dementia: convergence of α-synuclein, tau and amyloid-β pathologies. *Nat Rev Neurosci.* 2013;14:626-636.

2. Mueller C, Ballard C, Corbett A, Aarsland D. The prognosis of dementia with Lewy bodies. *Lancet Neurol.* 2017;16:390-398.

3. Surmeier DJ, Obeso JA, Halliday GM. Selective neuronal vulnerability in Parkinson disease. *Nat Rev Neurosci.* 2017;18:101-113.

4. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* 2003;24:197-211.

5. Shah M, Seibyl J, Cartier A, Bhatt R, Catafau AM. Molecular imaging insights into neurodegeneration: focus on α-synuclein radiotracers. *J Nucl Med.* 2014;55:1397-1400.

6. Eberling JL, Dave KD, Frasier MA. α-synuclein imaging: a critical need for Parkinson's disease research. *J Parkinsons Dis.* 2013;3:565-567.

7. Hall B, Mak E, Cervenka S, Aigbirhio FI, Rowe JB, O'Brien JT. In vivo tau PET imaging in dementia: Pathophysiology, radiotracer quantification, and a systematic review of clinical findings. *Ageing Res Rev.* 2017;36:50-63.

8. Herholz K, Ebmeier K. Clinical amyloid imaging in Alzheimer's disease. *Lancet Neurol.* 2011;10:667-670.

9. Ono M, Saji H. Recent advances in molecular imaging probes for β-amyloid plaques. *Med. Chem. Commun.* 2015;6:391-402.

10. Villemagne VL, Fodero-Tavoletti MT, Masters CL, Rowe CC. Tau imaging: early progress and future directions. *Lancet Neurol.* 2015;14:114-124.

11. Zhang X, Jin H, Padakanti PK, Li J, Yang H, Fan J, Mach RH, Kotzbauer P, Tu Z. Radiosynthesis and in Vivo Evaluation of Two PET Radioligands for Imaging α-Synuclein. *Appl Sci.* 2014;4:66-78.

12. Bagchi DP, Yu L, Perlmutter JS, Xu J, Mach RH, Tu Z, Kotzbauer PT. Binding of the radioligand SIL23 to α-synuclein fibrils in Parkinson disease brain tissue establishes feasibility and screening approaches for developing a Parkinson disease imaging agent. *PLoS One.* 2013;8:e55031.

13. Chu W, Zhou D, Gaba V, Liu J, Li S, Peng X, Xu J, Dhavale D, Bagchi DP, d'Avignon A, Shakerdge NB, Bacskai BJ, Tu Z, Kotzbauer PT, Mach RH. Design, Synthesis, and Characterization of 3-(Benzylidene)indolin-2-one Derivatives as Ligands for α-Synuclein Fibrils. *J Med Chem.* 2015;58:6002-6017.

14. Ono M, Doi Y, Watanabe H, Ihara M, Ozaki A, Saji H. Structure–activity relationships of radioiodinated diphenyl derivatives with different conjugated double bonds as ligands for α-synuclein aggregates. *RSC Adv.* 2016;6:44305-44312.

15. Ribeiro Morais G, Vicente Miranda H, Santos IC, Santos I, Outeiro TF, Paulo A. Synthesis and in vitro evaluation of fluorinated styryl benzazoles as amyloid-probes. *Bioorg Med Chem.* 2011;19:7698-7710.

16. Neal KL, Shakerdge NB, Hou SS, Klunk WE, Mathis CA, Nesterov EE, Swager TM, McLean PJ, Bacskai BJ. Development and screening of contrast agents for in vivo imaging of Parkinson's disease. *Mol Imaging Biol.* 2013;15:585-595.

17. Plazuk D, Janowska I, Klys A, Hameed A, Zakrzewski J. A convenient synthesis of conjugated omega-arylpolyenals via Wittig reaction with (1,3-dioxan-2-yl-methyl)triphenylphosphonium bromide/sodium hydride. *Synth*

Commun. 2003;33:381-385.

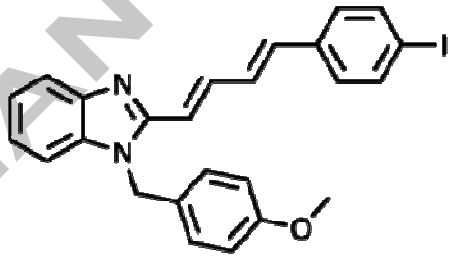
18. Matsumura K, Ono M, Kitada A, Watanabe H, Yoshimura M, Iikuni S, Kimura H, Okamoto Y, Ihara M, Saji H. Structure-Activity Relationship Study of Heterocyclic Phenylethenyl and Pyridinylethenyl Derivatives as Tau-Imaging Agents That Selectively Detect Neurofibrillary Tangles in Alzheimer's Disease Brains. *J Med Chem.* 2015;58:7241-7257.

19. Kotzbauer PT, Cairns NJ, Campbell MC, Willis AW, Racette BA, Tabbal SD, Perlmutter JS. Pathologic Accumulation of α-Synuclein and Aβ in Parkinson Disease Patients With Dementia. *Arch Neurol.* 2012;69:1326.

20. Mason NS, Mathis CA, Klunk WE. Positron emission tomography radioligands for in vivo imaging of Aβ plaques. *J Labelled Comp Radiopharm.* 2013;56:89-95.

21. Okamura N, Harada R, Furumoto S, Arai H, Yanai K, Kudo Y. Tau PET imaging in Alzheimer's disease. *Curr Neurol Neurosci Rep.* 2014;14:500.

Graphical abstract



BI-2

	α-synuclein	β-amyloid
K_d (nM)	99.5	727