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Novel imaging agents for β-amyloid plaque based on the *N*-benzoylindole core

Yang Yang [a], Xin-Hong Duan [a,b], Jun-Yuan Deng [a], Bing Jin [a], Hong-Mei Jia [a], Bo-Li Liu [a,*]

[a] Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, PR China
[b] College of Science, Beijing Forestry University, Beijing, PR China

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ABSTRACT

We report the synthesis and evaluation of a series of *N*-benzoylindole derivatives as novel potential imaging agents for β-amyloid plaques. In vitro binding studies using $A\beta_{1-40}$ aggregates versus [^{125}I]TZDM showed that all these derivatives demonstrated high binding affinities (K_i values ranged from 8.4 to 121.6 nM). Moreover, two radioiodinated compounds [^{125}I]**7** and [^{125}I]**14** were prepared. Autoradiography for [^{125}I]**14** displayed intense and specific labeling of Aβ plaques in the brain sections of AD model mice (C57, APP/PS1) with low background. In vivo biodistribution in normal mice exhibited sufficient initial brain uptake for imaging (2.19% and 2.00% ID/g at 2 min postinjection for [^{125}I]**7** and [^{125}I]**14**, respectively). Although additional modifications are necessary to improve brain uptake and clearance from the brain, the *N*-benzoylindole may be served as a backbone structure to develop novel β-amyloid imaging probes.

Alzheimer's disease (AD), the most common form of dementia in elderly people, is characterized by cognitive impairment and memory loss. Currently, a definite confirmation of AD is attained only by post-mortem histopathology of the brain. It is generally accepted that the accumulation of senile plaques (SPs) and neurofibrillary tangles (NFTs) are two pivotal clinical pathological features of AD, and the formation of amyloid plaques is prior to the onset of clinical symptoms.[1,2] Therefore, noninvasive detection of Aβ plaques in vivo by PET (positron emission tomography) or SPECT (single photon emission computed tomography) would be very useful for early diagnosis of AD.[3,4]

Currently, a lot of radiolabeled ligands derived from Congo Red (CR) or Thioflavin T (ThT) have been developed as Aβ plaques imaging probes. Several PET tracers, such as 2-(4′-[^{11}C]methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]PIB)[5,6], 4-*N*-[^{11}C]methylamino-4′-hydroxystilbene ([^{11}C]SB-13),[7,8] [^{18}F]-4-(*N*-methylamino)-4′-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-stilbene ([^{18}F]BAY94-9172)[9], [^{18}F]-2-(3-fluoro-4-(methylamino) phenyl)benzo[d]thiazol-6-ol ([^{18}F]GE-067)[10], [^{18}F]-(*E*)-4-(2-(6-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-*N*-methylaniline ([^{18}F]AV-45)[11,12] have been evaluated in human studies. However, [^{123}I]-6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2]pyridine ([^{123}I]IMPY)[13–15], the only SPECT tracer tested in human studies, has failed because of its high lipophilicity, in vivo instability and insufficient target-to-background ratio (Fig. 1). In comparison with PET, SPECT is a more widely accessible and cost-effective technique in terms of routine diagnostic use. Therefore, the development of more useful Aβ plaques imaging agents for SPECT has been a critical issue. During the past decade, many SPECT tracers with new backbone structures have been reported as Aβ probes, such as radio-iodinated aza-diphenylacetylenes[16], styrylpyridines[17], flavone derivatives[18,19], aurone derivatives[20,21], chalcones derivatives[22,23], (*E*)-5-styryl-2,2′-bithiophen derivatives[24], as well as ^{99m}Tc labeled chalcone[25], flavone and aurone.[26]

In the search for novel Aβ imaging agents with improved properties, many small-molecule Aβ inhibitors, including nonsteroidal anti-inflammatory drugs (NSAIDs), have been developed with necessary modifications in order to improve the affinities to Aβ plaques and enhance permeability through the BBB.[27] Agdeppa et al. have reported that naproxen and ibuprofen shared the same binding sites of [^{18}F]FDDNP on Aβ fibrils. The K_i values of (S)-naproxen, (R)-ibuprofen, and (S)-ibuprofen were 5.70 ± 1.31 nM, 44.4 ± 17.4 μM and 11.3 ± 5.20 μM, respectively.[28] Indomethacin has been known to inhibit Aβ fibril formation from Aβ at required concentrations, and the anti-aggregation effect of it may due to its interaction with Aβ.[29] In our present study, we selected indomethacin as the lead compound aiming to develop radiotracers with a new core structure to image Aβ plaques. In order to simplify the synthesis process and enhance permeability through the BBB, methyl and CH_2COOH groups were removed from the chemical structure. Furthermore, 'C=C' was introduced to improve the affinities to Aβ plaques (Scheme 1). A series of the resulted *N*-benzoylindole-based compounds were synthesized and their binding affinities for $A\beta_{1-40}$ aggregates were measured. Moreover, two radio-iodinated derivatives were prepared and evaluated as potential SPECT tracers for imaging amyloid plaques in the brain.

* Corresponding author.
E-mail address: liuboli@bnu.edu.cn (B.-L. Liu).

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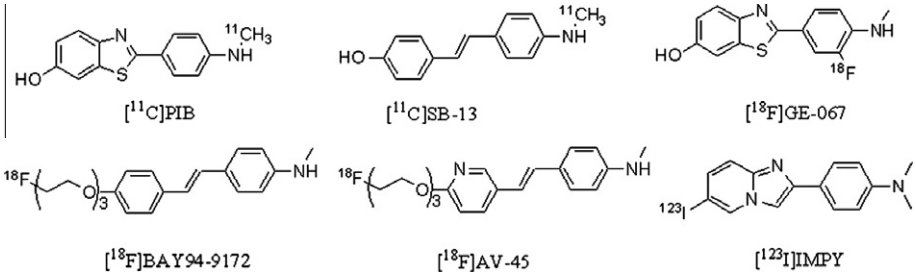
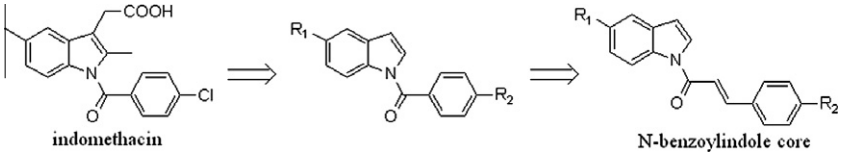


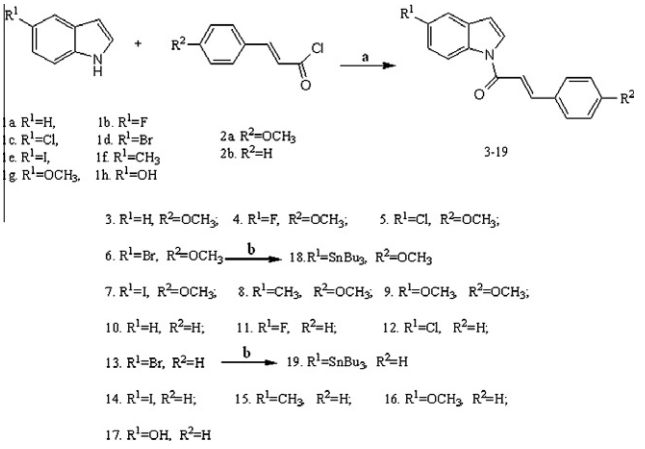
Figure 1. Chemical structure of Aβ imaging probes for clinical study.



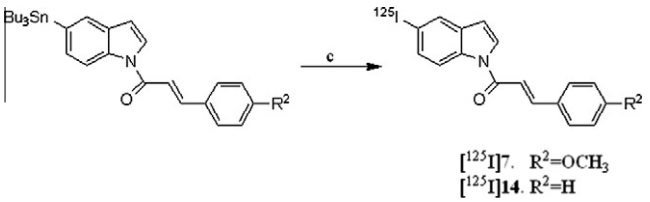
Scheme 1. Design considerations of *N*-benzoylindole-based derivatives.

The synthetic route of *N*-benzoylindole derivatives (**3**–**17**) is outlined in Scheme 2. The key step was the Schotten–Baumann reaction between the suitable 5-substituted indoles and **2**. The tributyltin derivatives (**18**, **19**) were prepared from the bromo-precursors (**6**, **13**) using an exchange reaction catalyzed by Pd(0) with the yields of 15.8% and 22.6%, respectively. [^{125}I]**7** and [^{125}I]**14** were prepared via the iododestannylation reaction using hydrogen peroxide as the oxidant (Scheme 3). The reaction was quenched with saturated $NaHSO_3$ solution. The resulting mixture was purified by HPLC using a reverse-phase column and mobile phase consisting of acetonitrile with a flow rate of 1 mL/min. The radio-ligands were co-injected and co-eluted with the corresponding nonradioactive **7** and **14**. The radiochemical yields of [^{125}I]**7** and [^{125}I]**14** were 57–72% and 50–71%, respectively. The radiochemical purities of [^{125}I]**7** and [^{125}I]**14** were both greater than 98%. The specific activity of the no-carrier-added [^{125}I]NaI was >2200 Ci/mmol at time of delivery. The specific activities of the products were not determined. The log *D* values of [^{125}I]**7** and [^{125}I]**14** were 2.32 ± 0.02 and 2.53 ± 0.04 (measured by a partition between *n*-octanol and pH 7.4 PBS buffer), respectively, which indicate a potential BBB permeability.

The affinities of these *N*-benzoylindole derivatives (**3**–**17**) for $A\beta_{1-40}$ aggregates were determined by competition binding assay using [^{125}I]TZDM as the radiolabeled standard.[30] As shown in Table 1, the binding affinities of these *N*-benzoylindole derivatives for $A\beta_{1-40}$ aggregates varied from 8.4 to 121.6 nM, suggesting that all these compounds share the same binding site with ThT. (*E*)-1-(1*H*-Indol-1-yl)-3-phenylprop-2-en-1-one (**10**) without any substituents showed good affinity (K_i = 15.9 nM). The introduction of methoxy group into the R^2 group in the phenyl-ring effected the affinities slightly (**3**, K_i = 27.3 nM). The introduction of halogen, such as F, Cl, Br, into the R^1 group in the indole-ring resulted in slight difference in affinities (K_i = 32.4, 18.1, 25.4, 32.7, 36.4 and 18.9 nM for compounds **4**, **5**, **6**, **11**, **12** and **13**, respectively), while modification with methoxy or methyl group at the above position resulted to a significant reduction in binding affinities (K_i = 103.3, 60.8, 127.7 and 68.2 nM for compounds **8**, **9**, **15** and **16**, respectively). Since derivatives **7** and **14** with iodine-substituent on the indole-ring displayed the highest binding affinities (K_i = 10.2 and 8.4 nM, respectively), we prepared [^{125}I]**7** and [^{125}I]**14** for further biological studies.



Scheme 2. Reagents and conditions: (a) Et_3N, CH_2Cl_2, reflux; (b) $(Bu_3Sn)_2$, $(Ph_3P)_4Pd$, toluene, reflux.



Scheme 3. Reagents and conditions: (c) [^{125}I]NaI, H_2O_2, HCl, rt.

The radioiodinated probes [^{125}I]**14** was investigated by in vitro autoradiography in the brain sections of a transgenic model mouse (C57, APP/PS1, 12 months). As shown in Figure 2, autoradiographic

Table 1
K_i values of *N*-benzoylindole derivatives on [^{125}I]TZDM binding to the aggregated $A\beta_{1-40}$ peptide in solution

Compound	K_i[a] (nM)	Compound	K_i[a] (nM)
3	27.3 ± 1.3	**11**	32.7 ± 1.2
4	32.4 ± 1.8	**12**	36.4 ± 1.6
5	18.1 ± 2.0	**13**	18.9 ± 1.2
6	25.4 ± 1.6	**14**	8.4 ± 1.4
7	10.2 ± 1.7	**15**	127.7 ± 1.5
8	103.3 ± 1.7	**16**	68.2 ± 1.3
9	60.8 ± 1.2	**17**	21.6 ± 4.6
10	15.9 ± 1.6		

[a] Measured in triplicate with results given as the mean ± SD

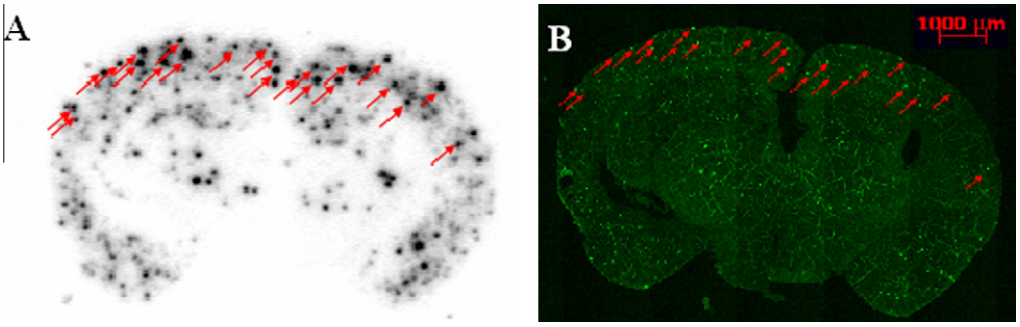


Figure 2. Autoradiography of [^{125}I]**14** in vitro in Tg model mouse (C57-APP/PS1, 12 months old, male) brain sections (A). The presence and distribution of plaques in the sections were confirmed with thioflavin-S (B) (red arrows).

Table 2
Biodistribution in normal mice after iv injection of [^{125}I]**7** and [^{125}I]**14** (% ID/g, avg of 5 mice ± SD) and its partition coefficient (*D*)

Organ	2 min	15 min	30 min	60 min	120 min	240 min
*[^{125}I]**7**(log D = 2.32 ± 0.02)*						
Blood	13.91 ± 1.86	5.82 ± 0.31	4.90 ± 0.44	3.79 ± 0.71	3.12 ± 0.48	2.78 ± 0.35
Brain	2.19 ± 0.16	2.29 ± 0.13	1.60 ± 0.14	1.04 ± 0.17	0.42 ± 0.08	0.21 ± 0.04
Heart	12.64 ± 0.78	3.52 ± 0.26	2.45 ± 0.21	1.53 ± 0.19	1.21 ± 0.45	1.17 ± 0.07
Liver	24.76 ± 3.16	18.05 ± 1.12	14.05 ± 1.09	12.30 ± 1.32	9.30 ± 1.12	7.65 ± 1.12
Spleen	6.00 ± 1.74	8.04 ± 0.45	7.12 ± 2.39	7.16 ± 3.55	6.18 ± 2.39	4.45 ± 1.22
Lung	21.76 ± 4.31	11.13 ± 0.60	9.05 ± 1.44	6.39 ± 1.19	4.31 ± 0.71	2.95 ± 0.57
Kidney	12.01 ± 0.28	9.31 ± 0.38	8.21 ± 0.93	5.71 ± 0.36	4.21 ± 0.66	4.05 ± 0.72
Stomach[a]	0.71 ± 0.18	4.06 ± 0.09	4.92 ± 1.06	5.00 ± 1.27	5.31 ± 1.12	4.14 ± 1.23
Muscle	2.73 ± 0.37	2.62 ± 0.38	1.63 ± 0.32	1.30 ± 0.20	0.65 ± 0.15	0.92 ± 0.18
*[^{125}I]**14**(log D = 2.53 ± 0.04)*						
Blood	14.13 ± 0.44	3.91 ± 0.48	3.75 ± 0.29	2.88 ± 0.92	1.61 ± 0.24	1.07 ± 0.14
Brain	2.00 ± 0.17	1.98 ± 0.13	1.57 ± 0.22	0.77 ± 0.12	0.33 ± 0.04	0.17 ± 0.03
Heart	10.72 ± 0.48	2.90 ± 0.42	2.07 ± 0.11	1.53 ± 0.23	0.99 ± 0.12	0.61 ± 0.16
Liver	15.89 ± 1.38	7.73 ± 0.83	6.48 ± 0.52	4.09 ± 0.85	4.86 ± 1.84	2.23 ± 0.48
Spleen	4.08 ± 0.60	2.44 ± 0.62	1.71 ± 0.23	1.85 ± 1.31	0.63 ± 0.54	0.59 ± 0.12
Lung	14.50 ± 3.50	6.73 ± 0.89	4.88 ± 0.78	2.66 ± 1.00	2.10 ± 0.22	1.27 ± 0.48
Kidney	11.22 ± 1.08	9.68 ± 1.03	10.16 ± 1.12	6.14 ± 0.30	3.54 ± 0.33	2.22 ± 0.72
Stomach[a]	1.09 ± 0.53	1.52 ± 0.21	1.72 ± 0.42	2.08 ± 0.68	2.29 ± 0.67	1.30 ± 0.38
Muscle	3.97 ± 0.32	2.47 ± 0.55	2.06 ± 0.53	1.06 ± 0.16	0.84 ± 0.53	0.19 ± 0.16

[a] Expressed as % ID/organ.

images of [^{125}I]**14** showed excellent labeling of Aβ plaques in the cortex region of the brain sections, and no remarkable accumulation of radioactivity were observed in white matter. The same sections were also stained with thioflavin-S and the localizations of Aβ plaques were in accord with the results of autoradiography. These results demonstrated that [^{125}I]**14** was specific for Aβ plaques, which was consistent with high bind affinity of compound **14** to $A\beta_{1-40}$ aggregates.

Biodistribution experiments were performed in normal mice in order to evaluate the pharmacokinetic properties of these derivatives. [^{125}I]**7** and [^{125}I]**14** were injected intravenously into normal mice for biodistribution studies. As shown in Table 2, the initial brain uptake for [^{125}I]**7** and [^{125}I]**14** were 2.19 and 2.00% ID/g at 2 min postinjection, respectively, indicating a level sufficient for brain imaging probe. But the washout of these probes from the brain in normal mice appears to be relatively slow (1.60% and 1.57% ID/g at 30 min postinjection, respectively), suggesting the high nonspecific binding of these probes. In addition, the initial blood uptake of the two probes was higher (13.91% ID/g for [^{125}I]**7** and 14.13% ID/g for [^{125}I]**14**), which may be due to the relatively high lipophilicity. It has been suggested that the lipophilicity of Aβ imaging agents may play an important role in uptake and washout. Additional structural modifications, such as introducing a hydrophilic group to decrease the lipophilicity, are needed to achieve the fast washout rate from brain and blood for the *N*-benzoylindole-based compounds.

In summary, *N*-benzoylindole-based compounds have been synthesized and evaluated as novel imaging probes for Aβ plaques. They showed high binding affinities with K_i values in the nM range in vitro. Autoradiography for [^{125}I]**14** indicated that it stained Aβ plaques in Tg2576 mouse brain clearly. *N*-benzoylindole-based derivatives penetrated the brain was encouraging. Although additional modifications are necessary to improve the in vivo properties of these derivatives, *N*-benzoylindole may be served as a new backbone structure to develop β-amyloid imaging probes.

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Supplementary data

Supplementary data (procedure for the preparation of new *N*-benzoylindole-based derivatives, in vitro binding studies, in vitro autoradiography using Tg 2576 mouse brain sections and biodistribution studies in normal mice) associated with this article can

be found, in the online version, at doi:10.1016/j.bmcl.2011.06.077.

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