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# Synthesis and biological evaluation of indole-chalcone derivatives as β-amyloid imaging probe

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ABSTRACT

A series of chaclone derivatives containing an indole moiety were evaluated in competitive binding assays with $A\beta_{1-42}$ aggregates versus [$^{125}$I]IMPY. The affinity of these compounds ranged from 4.46 to >1008 nM, depending on the substitution on the phenyl ring. Fluorescent staining in vitro showed that one compound with a *N,N*-dimethylamino group intensely stained Aβ plaques within brain sections of AD transgenic mice. The radioiodinated probe [$^{125}$I]-(*E*)-3-(1*H*-indol-5-yl)-1-(4-iodophenyl)prop-2-en-1-one, [$^{125}$I]**4**, was prepared and autoradiography in sections of brain tissue from an animal model of AD showed that it labeled Aβ plaques specifically. However, experiments with normal mice indicated that [$^{125}$I]**4** exhibited a low uptake into the brain in vivo (0.41% ID/g at 2 min). Additional chemical modifications of this indole-chalcone structure may lead to more useful imaging agents for detecting β-amyloid plaques in the brains of AD patients.



Alzheimer's disease (AD) is a progressive and fatal brain disease which accounts for the majority of dementia cases. Although the cause and progression of AD are not well understood, research indicates that the disease is associated with β-amyloid plaques (Aβ) and neurofibrillary tangles (NFTs). The amyloid cascade hypothesis indicates that the deposition of amyloid plaques constitutes a central and probably early event in the pathogenesis of AD.[1–3] Therefore, a positron emission tomography (PET) or single photon emission computed tomography (SPECT) tracer agent specifically targeting Aβ plaques would provide an important tool for the early and non-invasive diagnosis of this disease.

During the last decade, a number of Aβ plaque-specific imaging agents for PET or SPECT have been reported. Among them, 2-(4′-[$^{11}$C]methylaminophenyl)-6-hydroxybenzothiazole ([$^{11}$C]PIB),[4,5] 4-*N*-[$^{11}$C]methylamino-4′-hydroxystilbene ([$^{11}$C]SB-13),[6,7] [$^{18}$F]-4-(*N*-methylamino)-4′-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-stilbene ([$^{18}$F]BAY94-9172)[8] and [$^{123}$I]-6-iodo-2-(4′-dimethylamino)-phenyl-imidazo[1,2]pyridine ([$^{123}$I]IMPY)[9,10] have been tested clinically and demonstrated potential for imaging Aβ plaques in vivo. Recently, a styryl pyridine derivative, [$^{18}$F]-(*E*)-4-(2-(6-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-*N*-methylaniline ([$^{18}$F]AV-45), has shown promise as a probe in living human brain tissue in phase II and III clinical trials.[11,12] These probes are derived from Congo Red or thioflavin-T, and few other core structures for imaging Aβ have been found.

In the search for more useful candidates for Aβ imaging probes, we found iodinated and fluorinated chalcones to function as a new scaffold. The chalcone derivatives showed strong binding to Aβ aggregates and high brain penetration, but also slow clearance from the brain, resulting in low background-to-noise ratios.[13–15]

To develop more useful probes based on the chalcone structure, we decided to further extend our research using the new chaclone core, which possesses an indole ring (Fig. 1). The indole structure is often used as a fused ring of *N*-methyl- or *N,N*-dimethylamino-groups, and expected to maintain the affinity for Aβ aggregates.[16] In the present study, we synthesized 10 new indole-chalcone derivatives and evaluated their utility as Aβ imaging probes. To our knowledge, this is the first time indole-chalcone derivatives have been proposed as Aβ imaging probes for detecting AD.

The procedures used to produce the indole-chalcone derivatives are outlined in Scheme 1. The key step in the synthesis was the base-catalyzed Claisen condensation reaction. Compounds **1**–**10** were obtained from substituted acetophenones and 1*H*-indole-5-carbaldehyde in the presence of a basic catalyst (28% $CH_3ONa$) in ethanol at room temperature (yield, 5.8–59.8%). The tributyltin precursor **11** was prepared from the corresponding bromo compound in a bromo to tributyltin exchange reaction catalyzed by $(PPh_3)_4Pd$ (yield 18.4%).

The affinity of these indole-chalcone ligands (**1**–**10**) for $A\beta_{1-42}$ aggregates was tested with a competition binding assay using [$^{125}$I]IMPY as the competing radioligand. IMPY was also screened using the same system for comparison. As shown in Table 1,

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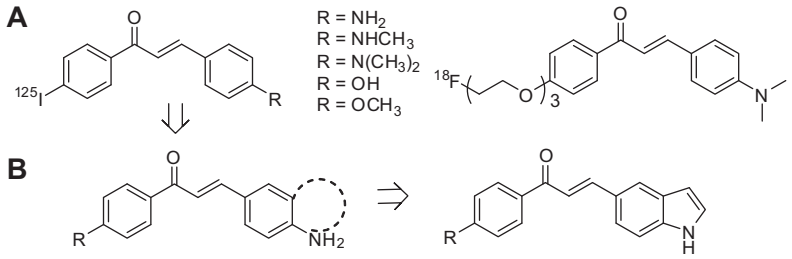
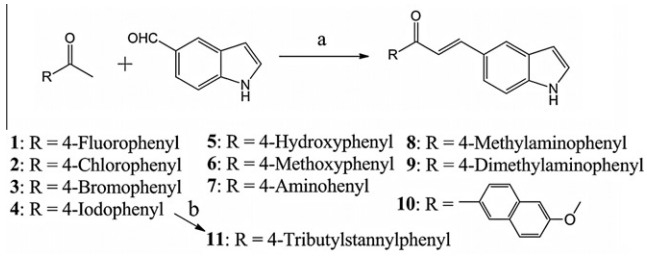
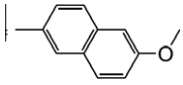


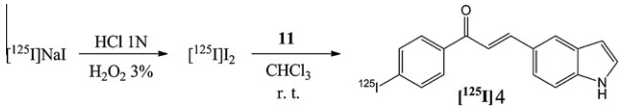

**Figure 1.** (A) Radiolabeled chalcone derivatives as β-amyloid imaging probes; (B) The ring-fusing indole-chalcone derivatives designed based on the chalcone structure.




**Scheme 1.** Reagents and conditions: (a) ethanol, NaOMe (1 M in MeOH), r.t.; (b) $(Bu_3Sn)_2$, $(PPh_3)_4Pd$, toluene.

**Table 1**
Inhibition constants ($K_i$) for binding to $A\beta_{1-42}$ aggregates versus [$^{125}$I]IMPY

| Compound | R | $K_i$ (nM) |
|---|---|---|
| **1** | 4-Fluorophenyl | 35.06 ± 6.21 |
| **2** | 4-Chorophenyl | 8.43 ± 2.13 |
| **3** | 4-Bromophenyl | 8.96 ± 0.92 |
| **4** | 4-Iodophenyl | 8.22 ± 1.46 |
| **5** | 4-Hydroxyphenyl | >360 |
| **6** | 4-Methoxyphenyl | 8.52 ± 2.15 |
| **7** | 4-Aminophenyl | >1008 |
| **8** | 4-Methylaminophenyl | 51.09 ± 7.71 |
| **9** | 4-Dimethylaminophenyl | 5.17 ± 0.32 |
| **10** |  | 4.46 ± 0.87 |
| DMIC-14 | — | 1.97 ± 0.26 |
| IMPY | — | 10.5 ± 1.0 |




**Scheme 2.** Radiolabeling of **4**.

binding was dependent on the substitution patterns of the phenyl ring. Compared with the fluorinated ligand **1**, halogenation at the *para*-position (Cl, Br or I) on the phenyl ring enhanced the binding affinity (**2**, **3** and **4**). Replacing the *para*-halogen with a hydroxy group decreased the affinity (**5**) dramatically, while methylation of the hydroxy group restored it (**6**). Binding was totally abolished when an amino group was placed at the *para*-position. However, if gradually increased with the methylation of the amino group, as reflected by the following order: *N*,*N*-dimethylated derivative (**9**) > *N*-monomethylated derivative (**8**) ≫ primary amino derivative (**7**). Enlargement of the aromatic conjugation system also increased the affinity as shown by **11** with an enlarged naphthalene ring. Also, compared with IMPY, the iodinated compound **4** had a lower $K_i$ value. Interestingly, all of these indole-chalcone ligands showed competition with [$^{125}$I]IMPY for binding to $A\beta_{1-42}$ aggregates. This means that the derivatives and IMPY share the same binding pocket on Aβ fibers. Previously, our research indicated chaclones to have a binding site on Aβ aggregates different from that of thioflavin-T and Congo Red, because neither of them could inhibit the binding of a radioiodinated chaclone to Aβ aggregates.[13] Initially, we considered that enlargement of the aromatic conjugation system (phenyl ring changed to a indole ring) may alter the binding. So we chose a chaclone compound, (*E*)-3-(4-(dimethylamino)phenyl)-1-(4-iodophenyl)prop-2-en-1-one (DMIC), for comparison. To our surprise, DMIC inhibited the binding of [$^{125}$I]IMPY in a dose-dependent manner with high affinity for $A\beta_{1-42}$ aggregates ($K_i$ = 1.97 nM). These findings suggested chaclone and IMPY to share the same binding site (thioflavin-T) on Aβ aggregates or at least have overlapping binding sites.

Due to the encouraging results obtained for the iodinated ligand **4**, further biological evaluations were carried out with an [$^{125}$I]-labeled probe. [$^{125}$I]**4** was prepared from the corresponding tributyltin precursor through an iododestannylation reaction in two steps. HPLC-purified [$^{125}$I]**4** showed greater than 95% purity with 21.1% yield. The specific activity of the no-carrier-added preparation was comparable to that of Na$^{125}$I, 2200 Ci/mmol. Finally, the identity of [$^{125}$I]**4** was verified by a comparison of retention time with the nonradioactive compound **4**(Scheme 2) (see Supplementary data).

To confirm the specific binding of these indole-chalcone derivatives to Aβ plaques, we performed fluorescent staining and auto-

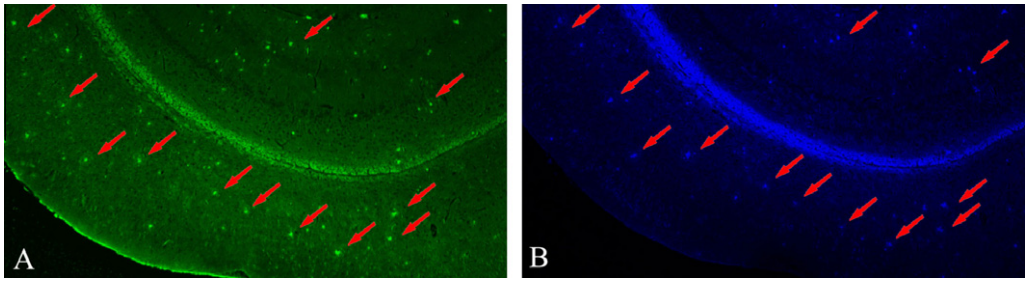



**Figure 2.** Fluorescence staining of **9** (A) in a section of brain tissue from a Tg-C57 (APP/PS1) mouse (6 μm thick) with a filter set for GFP. The labeled plaques were confirmed by staining of the adjacent section with thioflavin-S (B) using a filter set for DAPI.

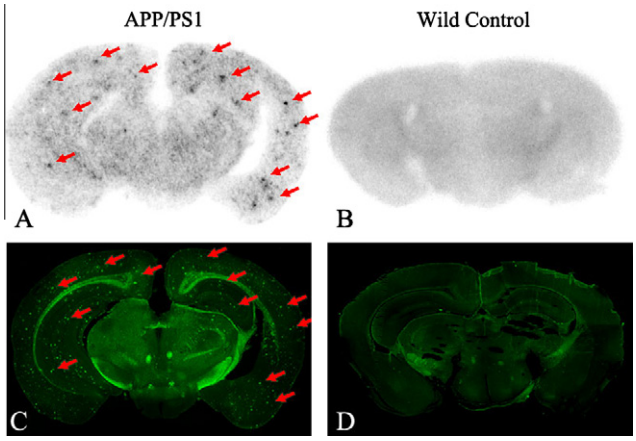



**Figure 3.** In vitro labeling of brain sections from Tg-C57(APP/PS1) mice (6 μm thick) and wild-type controls by autoradiography. [$^{125}$I]**4** labeled the Aβ plaques in the cortex of the brain (A), while the control case was clearly void of any notable Aβ labeling (B). The same sections were also stained with thioflavin-S (C, D) and the distribution of Aβ plaques was consistent with the results of autoradiography (red arrows).

**Table 2**
Biodistribution in normal ddY mice after iv injection of [$^{125}$I]**4**[a]

| Organ | 2 min | 15 min | 30 min | 60 min | 120 min |
|---|---|---|---|---|---|
| Blood | 8.85 ± 0.48 | 5.70 ± 0.24 | 4.79 ± 0.53 | 4.15 ± 0.51 | 3.72 ± 0.77 |
| Brain | 0.41 ± 0.02 | 0.29 ± 0.02 | 0.20 ± 0.03 | 0.21 ± 0.05 | 0.13 ± 0.02 |
| Heart | 3.27 ± 0.31 | 2.27 ± 0.25 | 1.72 ± 0.10 | 1.61 ± 0.21 | 1.33 ± 0.26 |
| Liver | 2.69 ± 0.22 | 2.21 ± 0.10 | 1.91 ± 0.22 | 1.48 ± 0.25 | 1.43 ± 0.42 |
| Spleen | 3.02 ± 0.48 | 2.96 ± 0.84 | 2.40 ± 0.16 | 2.27 ± 0.34 | 1.89 ± 0.31 |
| Lung | 7.33 ± 0.71 | 4.86 ± 0.17 | 4.20 ± 0.27 | 3.40 ± 0.59 | 3.19 ± 0.63 |
| Kidney | 7.30 ± 1.08 | 4.03 ± 0.12 | 3.26 ± 0.07 | 3.11 ± 0.33 | 2.78 ± 0.19 |
| Stomach[b] | 2.01 ± 0.37 | 1.07 ± 0.28 | 0.85 ± 0.10 | 0.96 ± 0.34 | 1.19 ± 0.52 |
| Intestine | 1.36 ± 0.27 | 2.01 ± 0.43 | 2.32 ± 0.53 | 1.72 ± 0.43 | 2.13 ± 0.56 |

[a] Expressed as % injected dose per gram. Average for five mice ± standard deviation.
[b] Expressed as % injected dose per organ.

radiography in vitro with sections of brain tissue from transgenic mice (C57, APP/PS1, 12 months). As shown in Figure 2 **9** with a *N*,*N*-dimethyl amino group clearly stained Aβ plaques with low background levels (Fig. 2A), the staining pattern being consistent with that obtained with thioflavin-S in adjacent sections (Fig. 2B). In addition, [$^{125}$I]**4** labeled Aβ plaques with minimal background levels (Fig. 3A). The control case was clearly void of any notable Aβ labeling (Fig. 3B). The same sections were also stained with thioflavin-S and the location of Aβ plaques was consistent with the results of autoradiography (Fig. 3C, red arrow).

Biodistribution experiments with ddY normal mice showed that [$^{125}$I]**4** initially accumulated in the blood, lungs and kidneys. The proportion taken up into the brain was 0.41% ID/g at 2 min postinjection, and radioactivity was slowly washed out from the brain at 30 min (0.20% ID/g) (Table 2). Under the experimental conditions, [$^{125}$I]**4** displayed a lower partition coefficient (log $D$ = 2.03 ± 0.10) which may explain the low brain uptake.

In conclusion, we have successfully designed and synthesized a series of indole-chalcone derivatives, whose affinity for Aβ aggregates depends on the substitution at the phenyl ring. We also found that the derivatives likely bind to Aβ aggregates at the thioflavin-T site. A radioiodinated ligand, [$^{125}$I]**4**, showed specific labeling of Aβ plaques in sections of brain tissue from an animal model of AD. However, [$^{125}$I]**4** displayed a relatively low initial uptake into the brain. Further chemical modifications of the indole-chalcone structure may lead to a useful β-amyloid imaging agent.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.045.

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