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Synthesis and evaluation of indolinyl- and indolylphenylacetylenes as PET imaging agents for β-amyloid plaques

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ABSTRACT

Two new phenylacetylene derivatives, 5-((4-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)phenyl)ethynyl)indoline **8** and 5-((4-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)phenyl)ethynyl)-1*H*-indole **14**, targeting β-amyloid (Aβ) plaques have been prepared. In vitro binding carried out in tissue homogenates prepared from postmortem AD brains with [^{125}I]IMPY (6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2-*a*]pyridine) as the radioligand indicated good binding affinities (K_i = 4.0 and 1.5 nM for **8** and **14**, respectively). Brain penetration of the corresponding radiofluorinated ligands, evaluated in the normal mice, showed good initial brain penetration (4.50 and 2.43% ID/g (injected dose/gram) for [^{18}F]**8** and [^{18}F]**14** at 2 min after injection) with moderate to low washout rates from the brain (1.71% ID/g at 2 h and 2.10% ID/g at 3 h, respectively). Autoradiography and homogenate binding studies demonstrated the high specific binding of [^{18}F]**14** to the Aβ plaques; however, [^{18}F]**8** showed low specific binding. These preliminary results identified that indolylphenylacetylene, **14**, may be a good lead for further structural modification to develop a useful Aβ plaque imaging agent.

Alzheimer's disease (AD) is a neurodegenerative disease which currently affects millions of elderly people worldwide. The key pathological features of AD are the formation of senile plaques aggregated by β-amyloid peptide and neurofibrillary tangles piled from phosphorylated tau protein in the brain. Based upon the amyloid cascade hypothesis, formation of Aβ plaques in the brain is the primary influence driving AD pathogenesis.[1–3] Imaging techniques such as positron emission tomography (PET) and single photon emission tomography (SPECT) are potentially useful for diagnosis of AD through imaging Aβ plaques in the brain. Various radiolabeled ligands (including the most well-known agent, [^{11}C]-PIB, *N*-methyl-[^{11}C]2-(4′-methylaminophenyl)-6-hydroxybenzothiazole) had been tested clinically and demonstrated potential usage (Fig. 1).[4–10]

Most of the reported ligands own a common structural feature, they contain a terminal *p*-*N*-methyl- or *p*-*N*,*N*-dimethylaminophenyl-group, and these groups are critical for successful binding affinity.[6,8] In many situations, however, it is speculated that the relatively low metabolic stability of these radioligands was mainly due to a rapid *N*-demethylation in vivo. Until now, efforts have been made to overcome this obstacle by adding an extra neighboring substituent, such as methyl, bromide, chloride, or fluoride group, *ortho*- to the *N*-methylamino-group on the phenyl ring to reduce the in vivo *N*-demethylation while maintaining the desired Aβ plaque binding (Fig. 2 (a)).[11,12] *N*-[^{11}C]-labeled aminophenylbenzothiazoles substituted with fluorine in different positions have been synthesized and evaluated. It was suggested that the substitution pattern of the phenyl ring and the benzothiazole moiety has an influence on slowing down the in vivo metabolism, which in turn has an effect on the brain uptake kinetics.[11] The data suggest that a strategy of reducing in vivo *N*-demethylation may improve the brain kinetics for agents targeting Aβ plaques in the brain.

Recently, the pegylation methodology has been adapted in the design and synthesis of PET Aβ plaque imaging agents in order to adjust the ligands' pharmacokinetic properties.[13,14] We have since reported a series of fluoro-pegylated diphenylacetylene and iodinated aza-diphenylacetylene derivatives as potential PET and SPECT Aβ plaque imaging agents (Fig. 2 (b)).[15,16] Following these successful results, we decided to further extend our search for PET imaging agents using this diphenylacetylene core structure. By fusing the *N*-methylamino group with the phenyl ring, we hope to prevent the probability of in vivo *N*-demethylation. In order to do so, indolinyl and indolyl groups were chosen to replace the *p*-*N*-methyl- or *p*-*N*,*N*-dimethylamino phenyl-group. As such by locking the *N*-methyl group into a heterocyclic indole or indoline system, we

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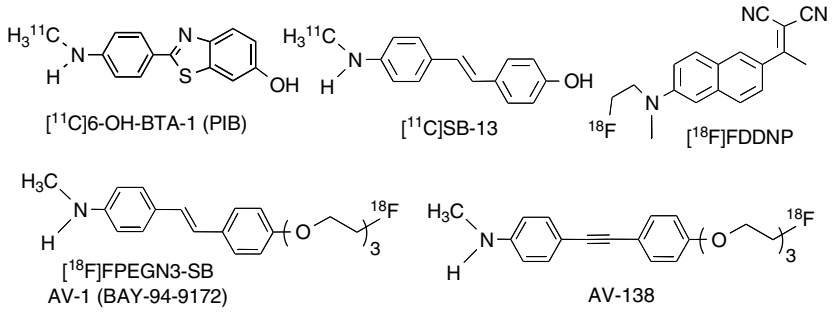


Figure 1. Reported five agents for imaging Aβ plaques in the brain.

hope to effectively prevent the in vivo *N*-demethylation (Fig. 2(c)). We reported herein the synthesis and initial biological evaluation of two phenylacetylene derivatives as improved probes for imaging Aβ plaques in the brain.

The synthetic procedures for these two target molecules are illustrated in Schemes 1 and 2. Copper and palladium co-catalyzed Sonogashira coupling reaction plays a vital role in the assembly of target molecules **8** and **14**. Starting from 5-bromoindoline **1**, the *tert*-butoxycarbonyl (Boc) group protection, microwave heating promoted Sonogashira coupling, and following basic deprotection of trimethylsilyl (TMS) group afforded the intermediate **3**. The Sonogashira reaction was employed a second time to couple intermediates **3** and **4**. This was followed by tosylation, fluorination, and finally *N*-Boc deprotection. Only four steps were needed to achieve the desired product **8** from **3**. For the synthesis of indolylphenylacetylene **14**, a similar synthetic route was adopted with two exceptions: (1) the synthesis of intermediate **11** only requires very mild reaction condition (room temperature, 0.7 h) and (2) a relatively harsh condition (microwave heating to 140 °C) was utilized for the final *N*-Boc deprotection step.

The binding affinities of two non-radioactive ligands **8** and **14** were tested by a competitive binding assay (with [^{125}I]IMPY in postmortem AD brain homogenates).[17] Both new ligands displayed excellent binding affinities; the K_i values are 4.0 ± 0.8 and 1.5 ± 0.3 nM (three determinations were made for each K_i value), respectively.

Encouraged by the binding data observed for these two ligands, we carried out further biological evaluations with the [^{18}F]-labeled probes. Standard nucleophilic substitution reactions of [^{18}F]fluoride with the corresponding tosylate precursors **6** and **17**, in the presence of Kryptofix 222 (K222) as phase transfer catalyst (PTC), were successfully performed.[18] For the product [^{18}F]**8**, however, one extra microwave heating step had been executed for the *N*-Boc deprotection (Scheme 3). The subsequent HPLC purified radioligands, [^{18}F]**8** and [^{18}F]**14**, showed greater than 97% radiochemical purities with 16% and 11% overall yields (decay corrected) and ~670 Ci/mmol and ~1900 Ci/mmol specific activities, respectively. The partition coefficient (PC, commonly measured as log*P*) of two radiofluorinated ligands at pH 7.4 was measured (log*P* = 2.95 and 2.56).[19] The data illustrate that both ligands own suitability as a brain imaging agent.

Two radiofluorinated ligands, [^{18}F]**8** and [^{18}F]**14**, displayed good initial penetrations of the blood–brain barrier with excellent initial brain uptakes (4.50% and 2.43% ID/g at 2 min after tracer injection) in normal mice. However, these two radioligands only displayed moderate to slow washouts with 1.71% ID/g remaining in the brain at 2 h after the tracer injection and 2.10% ID/g at three hours, respectively (Fig. 3). These results show promise; but the rate of

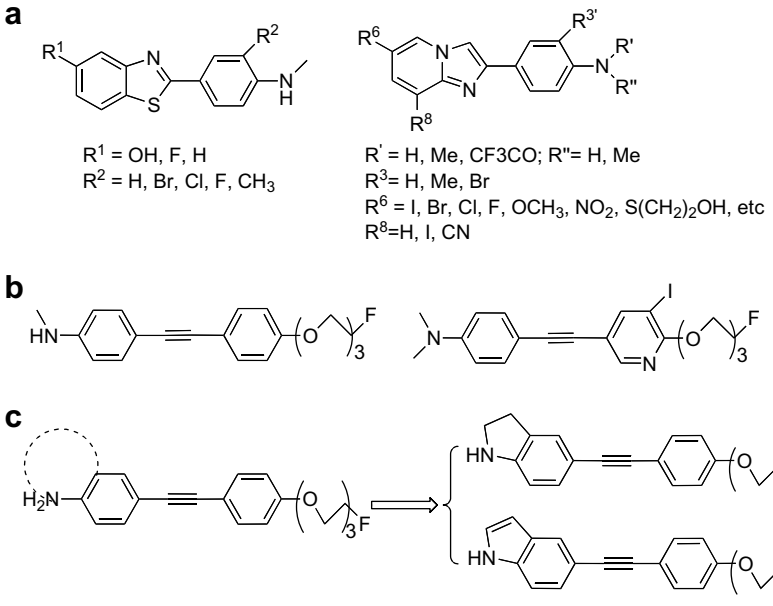
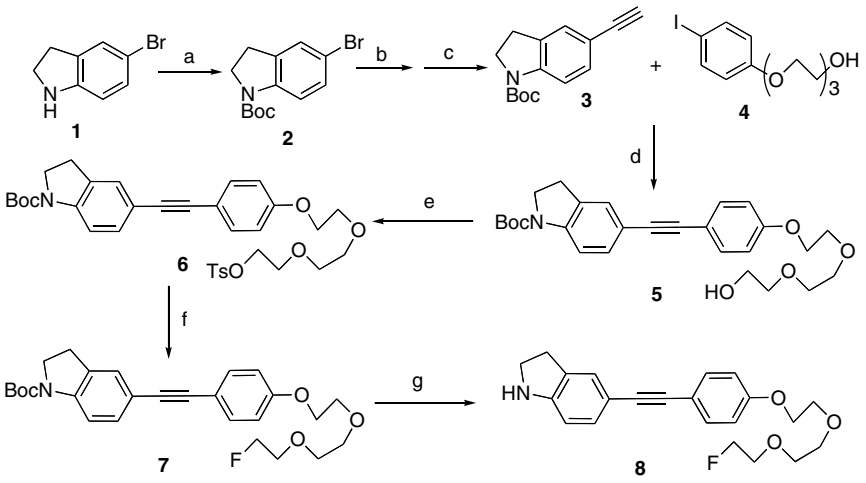
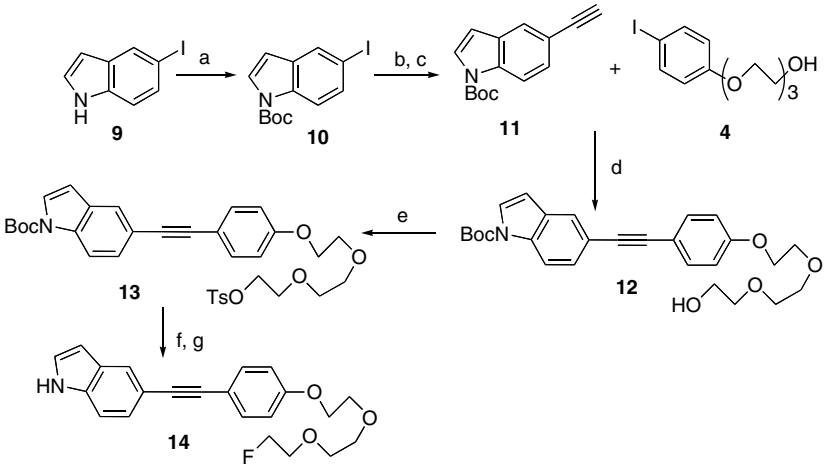


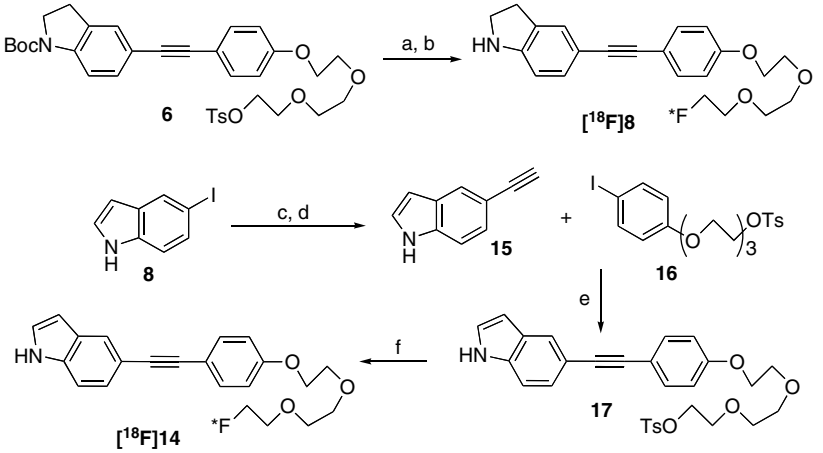
Figure 2. Examples of potential Aβ plaque imaging agents: (a) *N*-methyl group *ortho*-substituted benzothiazole and imidazo[1,2-*a*]pyridine derivatives. (b) Fluoro-pegylated diphenylacetylene and iodinated aza-diphenylacetylene derivatives. (c) Designed ring-fusing targets based on *N*-methylamino phenylacetylene: indolinyl- and indolylphenylacetylenes.



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, THF, rt, 24 h; (b) $Pd(PPh_3)_4$, CuI, trimethylsilylacetylene, diethylamine, DMF, 130 °C, microwave heating, 0.5 h; (c) KOH, MeOH/THF, rt, 0.5 h; (d) $Pd(PPh_3)_4$, CuI, Et_3N, CH_3CN, 0 °C to rt, 1.5 h; (e) TsCl, Et_3N, DMAP, DCM, 0 °C to rt, 2 h; (f) TBAF, THF, 75 °C, 1 h; (g) TMSOTf, 2,6-lutidine, DCM, 0 °C to rt, 1.8 h.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, DMAP, DCM, rt, 1.25 h; (b) $Pd(PPh_3)_4$, CuI, trimethylsilylacetylene, diethylamine, DMF, rt, 0.7 h; (c) KOH, MeOH/THF, rt, 2.5 h; (d) $Pd(PPh_3)_4$, CuI, Et_3N, CH_3CN, 0 °C to rt, 1.0 h; (e) TsCl, Et_3N, DMAP, DCM, 0 °C to rt, 3 h; (f) TBAF, THF, 75 °C, 1.5 h; (g) microwave heating, 140 °C, 0.5 h.



Scheme 3. Reagents and conditions: (a) [^{18}F]KF, K222, K_2CO_3, DMSO, 120 °C, 4.0 min; (b) microwave heating, 150 °C, 2.5 min; (c) $Pd(PPh_3)_4$, CuI, trimethylsilylacetylene, diethylamine, DMF, rt, 0.7 h; (d) KOH, MeOH/THF, rt, 2.5 h; (e) $Pd(PPh_3)_4$, CuI, Et_3N, CH_3CN, 0 °C to rt, 1.0 h; (f) [^{18}F]KF, K222, K_2CO_3, DMSO, 120 °C, 4.0 min.

brain washout is not optimal for an Aβ plaque-targeting imaging agent since a fast washout rate may help generate the better signal-to-noise ratios and therefore may be better for Aβ plaque detection.[20]

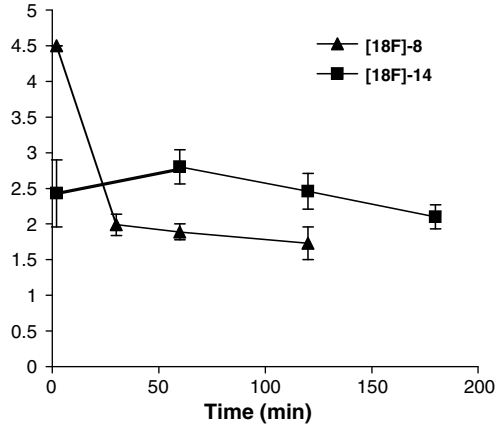


Figure 3. Brain uptake and washout of [^{18}F]**8** and [^{18}F]**14** in normal mice. Data are presented as % ID/g of three mice ± standard deviation.

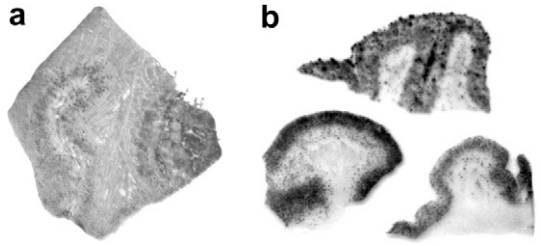


Figure 4. In vitro autoradiography of frozen human brain sections of AD patients with (a) [^{18}F]**8** and (b) [^{18}F]**14**. [^{18}F]**14** showed excellent binding to the Aβ plaques with very low background labeling.

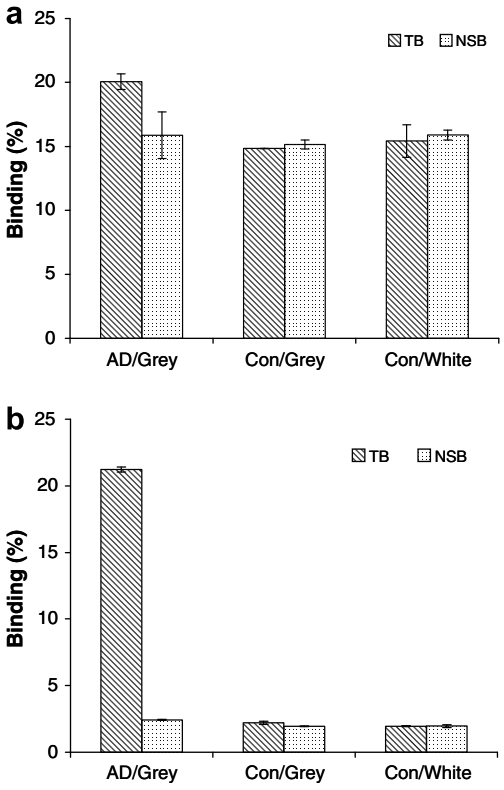


Figure 5. Specific binding of (a) [^{18}F]**8** and (b) [^{18}F]**14** to AD and control (Con) brain tissue homogenate. Grey and white matter was dissected from the cortical regions. [^{18}F]**14** showed high specific binding mainly in the gray matter of AD brain. (NSB, non-specific binding; TB, total binding; two determinations were made.)

To confirm the specific binding of radiofluorinated ligands **8** and **14** to Aβ plaques, we performed the in vitro film autoradiography. As shown in Figure 4, [^{18}F]**14** distinctively labeled plaques on AD brain sections with low background labeling. On the contrary, in addition to plaque labeling, [^{18}F]**8** displayed significant white matter labeling.

Furthermore, the in vitro binding assay using homogenates prepared from AD and control brain tissues was conducted to evaluate the binding specificity of these two radioligands to Aβ plaques (Fig. 5).[21] [^{18}F]**14** showed a very high specific binding in homogenate prepared from gray matter of an AD patient with low non-specific binding. In contrast, [^{18}F]**8** displayed similar binding in homogenates prepared from an AD patient and control with high non-specific binding. These results are consistent with in vitro binding results derived from autoradiography of AD brain sections. Preliminary in vivo and in vitro metabolism data of [^{18}F]**14** showed a complex pattern of metabolism in plasma and in liver (data not shown). It is likely that the indole or indoline ring may have removed the chances of *N*-demethylation; however, other metabolic reaction(s) at different sites of these two molecules may have occurred. Further exploration of the structure-activity relationship of this series of agents will be needed in the future. Nonetheless, the novel core structures showing excellent binding affinities to Aβ aggregates in human brain tissue provide potential insight for developing useful imaging agents.

In conclusion, we have demonstrated that indolinyl- and indolyl-phenylacetylenes **8** and **14** can be successfully prepared. They showed high binding affinities to β-amyloid plaques by in vitro binding assay. The radiofluorinated derivatives, [^{18}F]**8** and [^{18}F]**14**, displayed good brain penetration with moderate to relatively low rate of washout in normal mice. The combination of in vitro autoradiography of postmortem AD brain sections and brain tissue homogenate binding assay depicted that radioligand [^{18}F]**14** showed specific Aβ plaque labeling signal. Taken together, the results suggest that the indolylphenylacetylene ligand, **14,** may be a lead for further structural modification in order to improve the in vivo stability and in vivo kinetics desirable for a useful Aβ plaque imaging agent.

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