Novel Stilbenes as Probes for Amyloid Plaques

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Alzheimer's disease (AD) is a neurodegenerative disease of the brain characterized by dementia, cognitive impairment, and memory loss. Formation and accumulation of aggregates of β-amyloid (Aβ) peptides in the brain are critical factors in the development and progression of AD. The fibrillar aggregates of amyloid peptides, Aβ_{1-40} and Aβ_{1-42}, are major metabolic peptides derived from amyloid precursor protein found in senile plaques and cerebrovascular amyloid deposits in AD patients.[1] Prevention and reversal of Aβ plaque formation by immunization with Aβ peptides,[2,3] or by inhibitors of secretases,[4−7] are being targeted as a treatment for this disease.[6,8,9] Currently, the only definitive confirmation of AD is by postmortem histopathological examination of amyloid deposits in the brain. Early appraisal of clinical symptoms for diagnosis of AD is often difficult and unreliable.[10] Therefore, there is an urgent need for in vivo imaging agents, which can specifically demonstrate the location and density of amyloid plaques in the brain. The Aβ-plaque-specific imaging agents will be useful for early detection or monitoring the progression and effectiveness of treatment of AD.[11] Imaging agents may be labeled by using either one of the two types of isotopes: ^{99m}Tc ($T_{1/2}$, 6 h; 140 keV) and ^{123}I ($T_{1/2}$, 13 h; 159 keV) are routinely used for single photon emission computed tomography (SPECT), while ^{11}C ($T_{1/2}$, 20 min; 511 keV) and ^{18}F ($T_{1/2}$, 110 min; 511 keV) are commonly used for positron emission tomography (PET). In addition, successful agents will have to be relatively small in molecular size, neutral and lipophilic for brain penetration, and show selective and high binding affinity to Aβ aggregates in vivo. Many attempts on developing such agents based on Chrysamine-G (CG) or Congo Red derivatives have been reported,[12−21] but the efforts have not yielded any useful imaging agents. Recently, a small but highly lipophilic probe, [^{18}F]FDDNP, for binding tangles and plaques has been reported.[22,23] Using an in vitro fluorescent titration method, the binding affinity of FDDNP to Aβ aggregates was determined to be 0.4 nM. Preliminary studies in humans appear to suggest that [^{18}F]FDDNP showed a higher retention in regions of the brain suspected of having tangles and plaques, and the PET images were consistent with autoradiography and staining of postmortem brain samples.[24] A neutral thioflavin (benzothiazole) derivative, [^{3}H]BTA-1, was recently reported. [^{3}H]BTA-1 showed an excellent affinity (K_d = 3 nM) in in vitro binding assay using Aβ_{1-40} aggregates. When [^{11}C]BTA-1 was injected intravenously into mice, it showed an excellent brain penetration with an initial brain uptake at 2 min of 3.0% dose/organ.[25−27] Our laboratory has reported two types of iodinated probes, styrylbenzenes (IMSB) and thioflavins (benzothiazole, TZDM), for binding to Aβ aggregates.[28,29] In vitro binding studies of these ligands showed excellent binding affinities with K_d values of 0.13 and 0.06 nM for aggregates of Aβ_{1-40} and 0.73 and 0.14 nM for aggregates of Aβ_{1-42}, respectively. More importantly, under a competitive-binding assaying condition, two different and distinctive binding sites on Aβ_{1-40} and Aβ_{1-42} aggregates, which are mutually exclusive, were observed for styrylbenzenes (SB) and thioflavins (benzothiazole, TZ). Significantly, [^{125}I]TZDM crossed intact blood−brain barrier and localized in the brain of normal mice after an intravenous injection.[28] For in vivo imaging of Aβ aggregates to succeed, it will be necessary to develop agents which show good brain uptake in vivo. Brain penetration, a key factor for consideration, is usually related to the molecular size, neutrality, and lipophilicity. Further refinements of these probes are necessary to improve the brain uptake and washout from the normal brain regions and to achieve a high retention in the regions rich in Aβ plaques.

To search for new Aβ probes, a large series of benzothiazole and stilbenes (**1**−**3**) purchased from Aldrich Chemical Inc. (Milwaukee, WI) was screened by in vitro binding assays. Serendipitously, two of them (**1** and **2**) are analogues of TZDM and showed strikingly high binding affinities for the TZ binding sites (K_i = 2.3 and 1.4 nM, respectively), while *E*-stilbene and **3**

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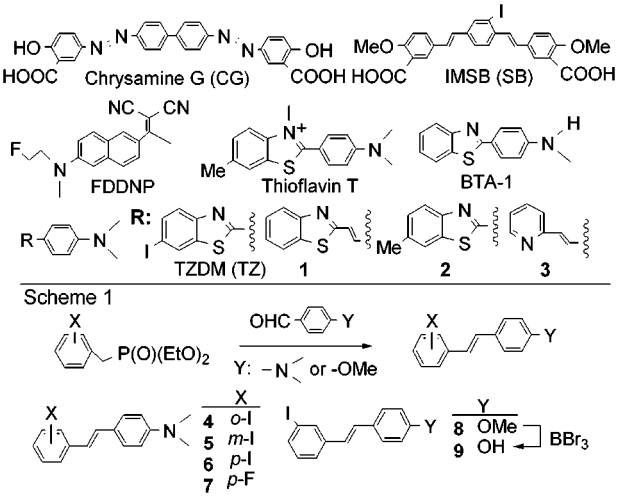
Table 1. Inhibition Constants (K_i, nM) of Compounds on Ligand Binding to Aβ_{1-40} Aggregates at 25 °C

compounds	[^{125}I]IMSB (SB)[a]	[^{125}I]TZDM (TZ)[a]
CG	0.14 ± 0.04	>1000
IMSB	0.17 ± 0.03	>1000
thioflavin T	>9000	116 ± 20
TZDM	>2000	0.9 ± 0.2
1	not tested	2.3 ± 0.1
2	not tested	1.4 ± 0.2
3	not tested	>3000
E-stilbene	not tested	535 ± 43
4	>3000	7.7 ± 0.8
5	>2000	4.5 ± 0.8
6	>2000	2.0 ± 0.4
7	>2000	22 ± 3
8	>1000	22 ± 6
9	>2000	32 ± 3

[a] Values (K_i, nM) are the mean ± SEM of three independent experiments, each in duplicate.

Scheme 1. Scheme for the Synthesis of *trans*-Stilbenes



displayed low binding affinities (K_i = 535 and >1000 nM, respectively) (Table 1). Based on these unexpected results, we have prepared and tested a series of stilbenes (**4**−**9**), all of which contain an electron-donating group: *p*-Me_2N, −OMe, or −OH.

These novel stilbenes (**4**−**9**) were successfully prepared by a Wadsworth−Emmons reaction (Scheme 1). By using the diethyl phosphonates as the starting material, the *trans*-stilbenes were formed preferentially. Similar observation on synthesis of stilbenes has been reported previously.[30,31] The free phenol derivative, **9**, was obtained from the corresponding **8** by a demethylation reaction using BBr_3. Previously, our laboratory has demonstrated that in vitro binding assay can be an effective way of screening probes that bind to Aβ aggregates.[28] The in vitro binding assay uses preformed Aβ aggregates of synthetic Aβ_{1-40} peptides and [^{125}I]TZDM as the ligand. The novel stilbenes, **4**−**9**, showed high binding affinities (K_i = 2−32 nM) to the TZ sites, while the affinities toward SB sites were very low (>1000 nM). The binding affinity of *p*-Me_2N-stilbenes is not sensitive to the position of the iodo group; *o*-, *m*- or *p*-iodo substitution (**4**, **5**, or **6**) on one of the benzene rings of stilbene displayed about equal potency (2.0−7.7 nM). The *p*-F-substituted derivative, **7**, showed slightly lower affinity (K_i = 22 nM). It is evident that these extremely simple and small stilbenes containing an electron-donating group, such as *p*-Me_2N−, −OMe, or −OH, displayed superb binding affinity to Aβ aggregates. Replacing the benzothiazole ring with an iodo- or fluoro-substituted phenyl ring has no effect on binding affinity at the TZ binding sites of Aβ aggregates. Binding affinity appears to be determined by the pharmacophore on one side of the stilbene molecule, suggesting that additional modifications could be possible.

To characterize these stilbenes further, [^{125}I]**5** was prepared by converting the corresponding tributyltin derivative[28] in the presence of Na[^{125}I]I and hydrogen peroxide, by which the no-carrier-added [^{125}I]**5** was obtained in excellent yield (radiochemical purity >95%). The partition coefficient between 1-octanol and pH 7.4 buffer was 422. In vivo biodistribution study of [^{125}I]**5** in normal mice after an *iv* injection suggested a good brain penetration. The brain uptake was 0.72, 1.12, 1.08, and 0.19% dose/organ, and the brain/blood gram ratio was 0.46, 1.46, 1.34, and 0.24, at 2, 30, 60, and 240 min after injection (the blood levels were relatively low, 6.5−2.8% dose/organ, at all of the time points). Binding of [^{125}I]**5** to the aggregates of Aβ_{1-40} is saturable, and the dissociation constant (K_d) was 0.19 nM, which is similar to that observed for [^{125}I]TZDM.[28] These results suggest that molecular weight can be reduced (molecular weights of TZDM and **5** were 380 and 349, respectively) while maintaining binding affinity; as such it significantly enhances the flexibility on designing new probes for imaging Aβ plaques in the brain. This finding is of paramount importance, because it represents a structural simplicity and suggests better alternatives for designing probes for binding to Aβ aggregates: (1) These probes may contain a simple stilbene-like structure. (2) One of the aromatic rings contains an electron-donating group, *p*-Me_2N−, −OMe, or −OH, which appears to be essential for the binding affinity. (3) There is a bulk tolerance for the second aromatic ring, on which radiolabel, ^{99m}Tc, ^{123}I, or ^{18}F, can be readily attached without detrimental effects on binding affinity to Aβ aggregates.

In conclusion, synthesis of novel stilbenes as probes for binding to Aβ_{1-40} aggregates is reported. Derivatives of stilbenes containing a *p*-Me_2N−, −OMe, or −OH group on one of the benzene rings are simple, relatively small, neutral, and lipophilic. Based on their exquisitely high binding affinity to Aβ_{1-40} aggregates, these novel stilbenes are candidates as probes for in vivo evaluation of amyloid plaques containing Aβ aggregates in the brains of AD patients.

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Supporting Information Available: Complete experimental procedures for the synthesis, radiolabeling, and binding of compounds **4**−**9** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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