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Synthesis and evaluation of stilbenylbenzoxazole and stilbenylbenzothiazole derivatives for detecting β-amyloid fibrils

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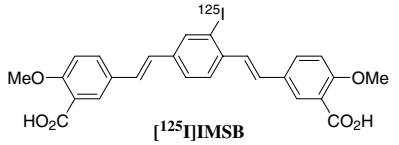
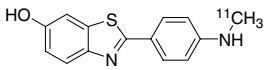
Abstract—This paper describes a novel series of stilbenylbenzoxazole (**SBO**) and stilbenylbenzothiazole (**SBT**) derivatives for β-amyloid specific binding probes. These 24 compounds were synthesized and evaluated by competitive binding assay against β-amyloid 1–42 (Aβ42) aggregates using [^{125}I]TZDM. All the derivatives displayed higher binding affinities with K_i value in the subnanomolar range (0.10–0.74 nM) than Pittsburgh Compound-B (PIB) (0.77 nM). Among these derivatives, **SBT-2**, 5-fluoroethoxy-2-{4-[2-(4-methylaminophenyl)vinyl]phenyl}benzothiazole, showed lowest K_i value (0.10 nM). In conclusion, the preliminary results suggest that these compounds are implying a possibility as a probe for detection of Aβ fibrils in Alzheimer's disease (AD) patients.

Alzheimer's disease (AD) is a neurodegenerative disease characterized as progressive memory loss and decrease of cognitive function. In 1907, the first demented patient was identified to have senile plaques (SPs) of β-amyloid protein (Aβ) aggregates and neurofibrillary tangles (NFTs) formed of highly phosphorylated tau proteins in the post-mortem brain tissue.[1–3] Since then, SPs and NFTs have become the two major pathological hallmarks characteristic of AD and provided the basic for the definitive diagnosis of AD. However, yet the diagnosis of this disease based on neurological observations is often difficult and unreliable. Therefore, an increasing focus on early identification and prevention highlights a need for simpler diagnostic tools and robust biological markers. At present, the Aβ-aggregate-specific radiolabeled imaging agents, using single photon emission computed tomography (SPECT) or positron emission tomography (PET), are needed for early detection or monitoring of the progression and effectiveness of AD treatment.[4–6] A number of groups have studied to develop Aβ-specific binding probes, however those efforts have been limited by low levels of specific binding in brain regions and poor blood–brain barrier (BBB) penetration.

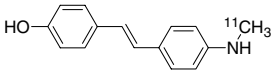
Recently, *N*-methyl-[^{11}C]2-(4′-methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]PIB),[7,8] *E*,*E*-1-iodo-[^{125}I]2,5-bis(3-hydroxycarbonyl-4-methoxy)styrylbenzene ([^{125}I]-IMSB),[9,10] and [^{11}C]4-*N*-methylamino-4′-hydroxystilben ([^{11}C]SB-13)[11,12] displayed high binding affinities toward Aβ aggregates. PIB, a modified molecule of thioflavin-T (Th-T), exhibited that neutral benzothiazole–aniline derivatives could bind to amyloid with low nanomolar affinity, enter brains in sufficient amounts for imaging via PET, and clear rapidly from normal brain in animal studies (Fig. 1).



[^{125}I]IMSB



[^{11}C]PIB



[^{11}C]SB-13

Figure 1. Structures of IMSB, PIB, and SB-13.

Keywords: Alzheimer's disease; β-Amyloid fibrils; PET imaging.
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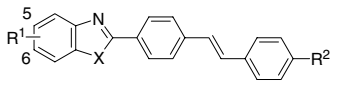


SBO-1~12, SBT-1~12

SBO-1 : X=O, R^1=5-O$(CH_2)_2$F, R^2=NH_2
SBO-2 : X=O, R^1=5-O$(CH_2)_2$F, R^2=NH(CH_3)
SBO-3 : X=O, R^1=5-O$(CH_2)_2$F, R^2=N$(CH_3)_2$
SBO-4 : X=O, R^1=6-O$(CH_2)_2$F, R^2=NH_2
SBO-5 : X=O, R^1=6-O$(CH_2)_2$F, R^2=NH(CH_3)
SBO-6 : X=O, R^1=6-O$(CH_2)_2$F, R^2=N$(CH_3)_2$
SBO-7 : X=O, R^1=5-O$(CH_2)_3$F, R^2=NH_2
SBO-8 : X=O, R^1=5-O$(CH_2)_3$F, R^2=NH(CH_3)
SBO-9 : X=O, R^1=5-O$(CH_2)_3$F, R^2=N$(CH_3)_2$
SBO-10 : X=O, R^1=6-O$(CH_2)_3$F, R^2=NH_2
SBO-11 : X=O, R^1=6-O$(CH_2)_3$F, R^2=NH(CH_3)
SBO-12 : X=O, R^1=6-O$(CH_2)_3$F, R^2=N$(CH_3)_2$

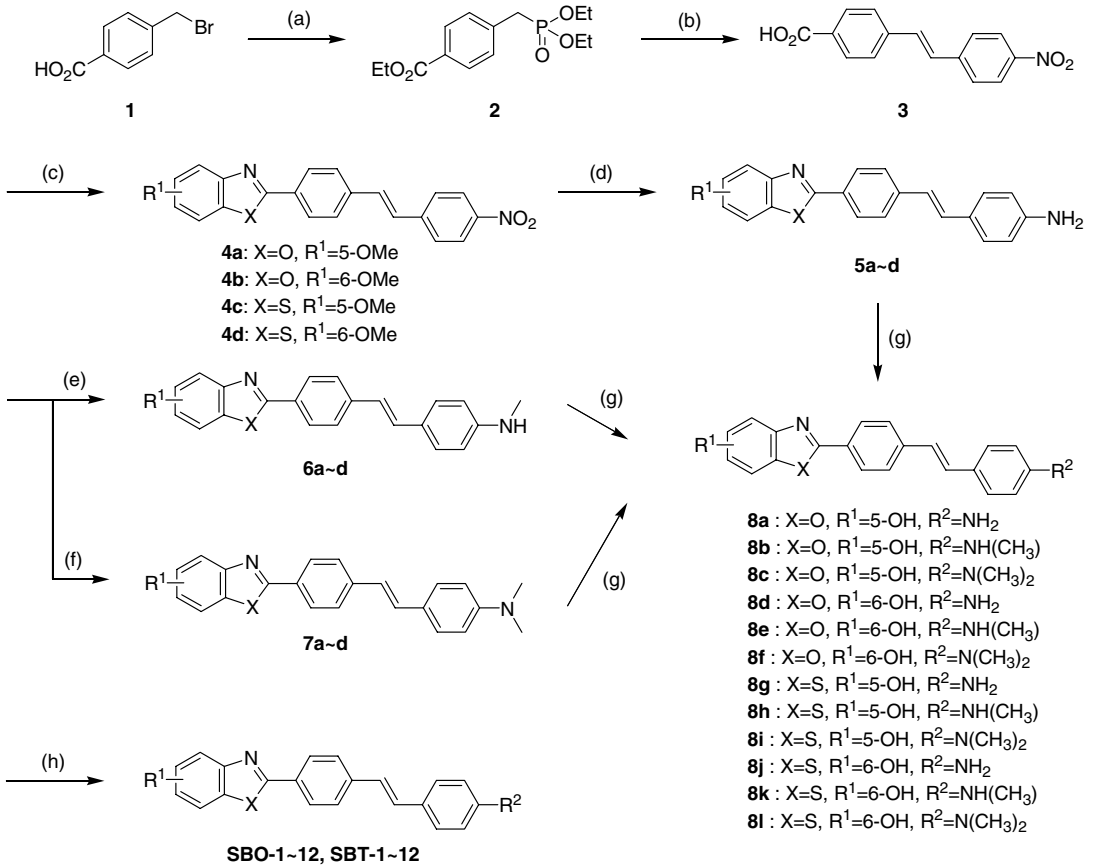
SBT-1 : X=S, R^1=5-O$(CH_2)_2$F, R^2=NH_2
SBT-2 : X=S, R^1=5-O$(CH_2)_2$F, R^2=NH(CH_3)
SBT-3 : X=S, R^1=5-O$(CH_2)_2$F, R^2=N$(CH_3)_2$
SBT-4 : X=S, R^1=6-O$(CH_2)_2$F, R^2=NH_2
SBT-5 : X=S, R^1=6-O$(CH_2)_2$F, R^2=NH(CH_3)
SBT-6 : X=S, R^1=6-O$(CH_2)_2$F, R^2=N$(CH_3)_2$
SBT-7 : X=S, R^1=5-O$(CH_2)_3$F, R^2=NH_2
SBT-8 : X=S, R^1=5-O$(CH_2)_3$F, R^2=NH(CH_3)
SBT-9 : X=S, R^1=5-O$(CH_2)_3$F, R^2=N$(CH_3)_2$
SBT-10 : X=S, R^1=6-O$(CH_2)_3$F, R^2=NH_2
SBT-11 : X=S, R^1=6-O$(CH_2)_3$F, R^2=NH(CH_3)
SBT-12 : X=S, R^1=6-O$(CH_2)_3$F, R^2=N$(CH_3)_2$

Figure 2. Structures of **SBO** and **SBT** derivatives.

However labeling of ^{11}C radioisotope is limited to short half-life ($t_{1/2}$ = 20 min). SB-13 demonstrated similar binding properties to those of PIB. IMSB showed lower initial brain uptake in normal mice (0.14% ID/organ at 5 min after injection) than radioiodinated Th-T derivatives (0.6–3.5% ID/organ at 2 min), but it displays potent binding affinities for Aβ aggregates.

Our focus was that a sufficient amount of an imaging agent should internalize into the brain to bind to the target. The agent should have adequate affinity toward the target and show rapid clearance of free and nonspecific bound compounds from the brain. Based on the backbone structures of PIB and SB-13, we have successfully developed highly conjugated **SBO** and **SBT** derivatives (Fig. 2). Due to the short half-life of ^{11}C, to broaden the utility of a PET imaging agent, we have then focused our effort on developing ^{18}F-labeled imaging agent ($t_{1/2}$ = 110 min). In the beginning, we designed fluoroethyl and fluoropropyl substituted **SBO**, **SBT** derivatives. All the synthesized compounds were evaluated by competitive binding assays against Aβ aggregates using [^{125}I]TZDM.

The synthesis of **SBO** and **SBT** derivatives is outlined in Scheme 1. The first step of synthetic 4-(diethoxyphosphorylmethyl)benzoic acid ethyl ester was achieved with 4-(bromomethyl)benzoic acid and triethylphosphite via Arbuzov reaction.[13] Compound **3** was readily prepared from compound **2** and 4-nitrobenzaldehyde via Horner–Wadsworth–Emmons reaction and hydrolysis. The key step for the formation of the benzoxazole and benzothiazole backbones was accomplished via the intramolecular cyclization reaction[14] between compound **3** and 2-aminophenol or 2-aminothiophenol derivatives.[15] The free amino derivatives, compounds **5a**–**d** were prepared from the nitro compounds **4a**–**d** via reduction



Scheme 1. Synthesis of **SBO** and **SBT** derivatives. Reagents and conditions: (a) $P(OEt)_3$, 140 °C, 20 h; (b) i)—4-nitrobenzaldehyde, NaH, THF, rt, 2 h; ii)—NaOH, MeOH/H_2O, reflux, 2 h; (c) i)—thionyl chloride, reflux, 1 h; ii)—2-aminophenol or 2-aminothiophenol derivatives, *N*,*N*-dimethylaniline, monochlorobenzene, reflux, 1 h; iii)—*p*-TsOH, trichlorobenzene, reflux, 8 h; (d) $SnCl_2$, EtOH, reflux, 24 h; (e) *p*-formaldehyde, NaOMe, MeOH/THF, $NaBH_4$, reflux, 3 h; (f) *p*-formaldehyde, $NaBH_3CN$, AcOH, rt, 12 h; (g) BBr_3, CH_2Cl_2, reflux, 12 h; (h) 1-fluoro-2-tosyloxyethane or 1-fluoro-3-tosyloxypropane, K_2CO_3, DMF, 90 °C, 2 h.

Table 1. K_i values of **SBO** and **SBT** derivatives against [^{125}I]TZDM for binding affinities to Aβ42 aggregates

Compound	K_i[a] (nM)
SBO-1	0.32
SBO-2	0.74
SBO-3	0.44
SBO-4	0.47
SBO-5	0.50
SBO-6	0.45
SBO-7	0.45
SBO-8	0.47
SBO-9	0.45
SBO-10	0.59
SBO-11	0.68
SBO-12	0.46
SBT-1	0.41
SBT-2	0.10
SBT-3	0.35
SBT-4	0.59
SBT-5	0.52
SBT-6	0.57
SBT-7	0.42
SBT-8	0.38
SBT-9	0.49
SBT-10	0.55
SBT-11	0.48
SBT-12	0.12
PIB	0.77

[a] K_i was calculated by the Cheng–Prusoff equation ($K_i = IC_{50}/(1 + [L]/K_d)$)[20] using *Graphpad Prism* software.

with $SnCl_2$. Conversion of compounds **5a**–**d**, to the monomethylamino derivatives, compounds **6a**–**d**, was achieved via a method previously reported.[16] Compounds **5a**–**d** were also converted to the dimethylamino derivatives, compounds **7a**–**d**, via an efficient method with paraformaldehyde, sodium cyanoborohydride, and acetic acid.[17,18] The *O*-methyl group of compounds **5a**–**d**, **6a**–**d**, and **7a**–**d** was removed by reacting with BBr_3 to give compounds **8a**–**8l**. The desired **SBO** and **SBT** derivatives were prepared from compounds **8a**–**8l** and 1-fluoro-2-tosyloxyethane or 1-fluoro-3-tosyloxypropane by a nucleophilic substitution reaction.[19]

Specific binding affinities of synthesized compounds to Aβ fibrils were evaluated by an in vitro Aβ fibril binding assay. In vitro competitive binding assay using preformed Aβ42 aggregates demonstrated that **SBO-1**–**12**, **SBT-1**–**12** competed against radioligand such as [^{125}I]TZDM.[21–23]

The result shown in Table 1 demonstrates that most of the synthesized compounds displayed lower K_i values (K_i = 0.10–0.74 nM) than PIB compound. In the structure–activity relationship, **SBO** and **SBT** derivatives did not show significant difference of binding affinity. Furthermore, 5-position compounds were slightly better than 6-position compounds. Among them, 5-fluoroethyl substituted **SBT-2**[24] compound exhibited the highest binding affinity.

In conclusion, a series of novel fluoroethyl and fluoropropyl substituted **SBO**, **SBT** compounds were successfully synthesized. These **SBO** and **SBT** derivatives displayed excellent binding affinities to Aβ aggregates. In particular, **SBT-2** exhibited the best binding affinity (K_i = 0.10 nM) implying a possibility as a probe for detection of Aβ fibrils in AD brain. Based on the result, further studies on synthesis and in vivo pharmacokinetics of ^{18}F-labeled compounds are progressing for the development of AD imaging probe.

Acknowledgments

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23. We estimated K_d value (0.13 nM) of [^{125}I]TZDM for Aβ42 aggregates. For inhibition studies, the reaction mixture contained 50 μL of Aβ42 aggregates (11.5 nM in the final concentration), 50 μL of inhibitors (10^{-6}–10^{-12} M in DMSO), 50 μL of [^{125}I]TZDM (in 40% EtOH, 0.05 nM in the final concentration), and 10% EtOH in a final volume of 1 mL. Nonspecific binding was defined by adding 2 μM Th-T for [^{125}I]TZDM binding. The mixture was incubated at room temperature for 3 h and the bound and the free radioactivity were separated by a vacuum filtration through Whatman GF/B filters using a Brandel M-24R cell harvester followed by 2× 3 mL washes of 10% EtOH at room temperature. Filters containing the bound radioligand were counted in a gamma-counter (Cobra-II). The result of inhibition assays was subjected to nonlinear regression analysis using software *Graphpad Prism* by which K_i values were calculated.
24. Selected data. **SBT-2**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.71 (d, J = 4.9 Hz, 3H), 4.36 (dt, J = 4.0, 30.3 Hz, 2H), 4.80 (dt, J = 4.0, 47.9 Hz, 2H), 6.01 (s, NH), 6.56 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 16.1 Hz, 1H), 7.12 (d, J = 9.1 Hz, 1H), 7.26 (d, J = 16.5 Hz, 1H), 7.41 (d, J = 8.3 Hz, 2H), 7.63 (s, 1H), 7.68 (d, J = 8.0 Hz, 2H), 8.01 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 8.2 Hz, 2H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 29.98, 67.85, 81.51, 106.73, 112.11, 115.84, 122.03, 123.19, 124.56, 126.55, 126.85, 127.82, 128.59, 131.17, 131.72, 141.63, 150.66, 155.45, 158.16, 168.87; HRMS *m*/*z* Calcd for $C_{24}H_{22}FN_2OS$ $(M)^+$ 405.1431. Found: 405.1433.