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Ferulic acid and benzothiazole dimer derivatives with high binding affinity to β-amyloid fibrils

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Abstract—New ferulic acid and benzothiazole dimer derivatives were synthesized and evaluated by in vitro competition assay using [^{125}I]TZDM for their specific binding affinities to Aβ fibrils. In particular, **4a** showed the most excellent binding affinity (K_i = 0.53 nM), compared to PIB (K_i = 0.77 nM), for benzothiazole binding sites of $A\beta_{1-42}$ fibrils. This result suggests a possibility of a potential AD diagnostic probe for detection of Aβ fibrils.

Alzheimer's disease (AD), known as a kind of dementia, is a neurodegenerative disease characterized by cognitive impairment and learning/memory losses.[1] Pathomorphologically important hallmarks in AD are senile plaques (SPs), composed of aggregates of amyloid β (Aβ) peptides, and neurofibrillary tangles (NFTs) in the brain.[2–4] Based on Aβ hypothesis, $A\beta_{1-42}$ monomers divided by β-, γ-secretases from amyloid precursor protein (APP) gradually aggregate to oligomers, protofibrils, fibrils, and plaques which deposit in the brain and cause strong neuronal toxicity.[5,6] We have made efforts to search for small molecules[7] with high binding affinities to aggregated Aβ to diagnose AD before the beginning of significant clinical impairment.[8] To monitor AD progression quantitatively, noninvasive techniques such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) have been employed.

We previously reported that the ferulic acid (FA) dimer (Fig. 2) has a higher therapeutic effect on dementia than FA monomer (Fig. 1) enhancing the learning and memory retention ability in in vivo.[9] Here, a series of new ferulic acid dimer derivatives (Fig. 2) were synthesized

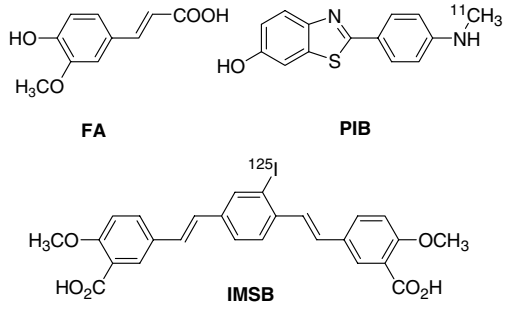

Figure 1. Structures of FA, PIB, and IMSB.

with the hope of finding compounds with higher binding affinities to Aβ deposits than FA monomer. Benzothiazole dimer derivatives (Fig. 2) were also introduced to provide a higher binding affinity than [^{11}C]2-(4′-(methylamino)phenyl)-6-hydroxybenzothiazole (Pittsburgh compound B, PIB),[10] a successful benzothiazole PET imaging probe for AD.

Based on the literature, [^{125}I]-*E*,*E*-1-iodo-2,5-bis-(3-hydroxycarbonyl-4-methoxy)styrylbenzene (IMSB) binds to $A\beta_{1-40}$ fibrils with higher binding affinity (K_i = 0.17 nM)[12a] than PIB (K_i = 4.3 nM) does.[12c] According to Mathis and co-workers' report, the affinity of bis-styrylbenzene derivatives shows the highest value around 19–20 Å of inter-chain spacing and falls off rapidly between 10–16 Å.[12b] The size of IMSB based on the

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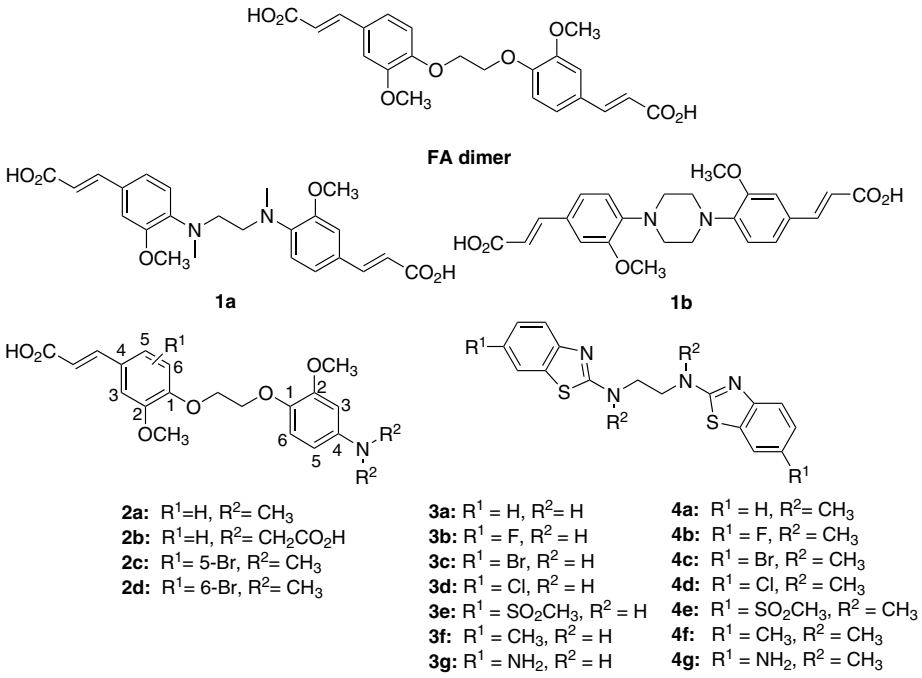


Figure 2. Ferulic acid and benzothiazole dimer derivatives.

distance between terminal carboxlates (16 Å) is close to the pocket size of the β-sheet peptide strands. Despite the significant in vitro binding characteristics, a drawback of IMSB was low brain uptake,[20] which was insufficient for in vivo imaging studies. Therefore, here, target molecules were considered: (1) to keep the interchain spacing of IMSB, 16–20 atoms between terminal ligands, (2) to introduce the scaffolds of FA and PIB instead of bis-styrylbenzene, and (3) to introduce at least one alkyl amine moiety as a radio-labeling site. The tertiary amine moiety at C-4 (Fig. 2) and nitrogen bridge (**1**, **3**, and **4**), based on isosteric derivations from the oxygen bridge of the FA dimer, can be applied to a labeling site like the [^{11}C]CH_3NH-terminal of PIB.

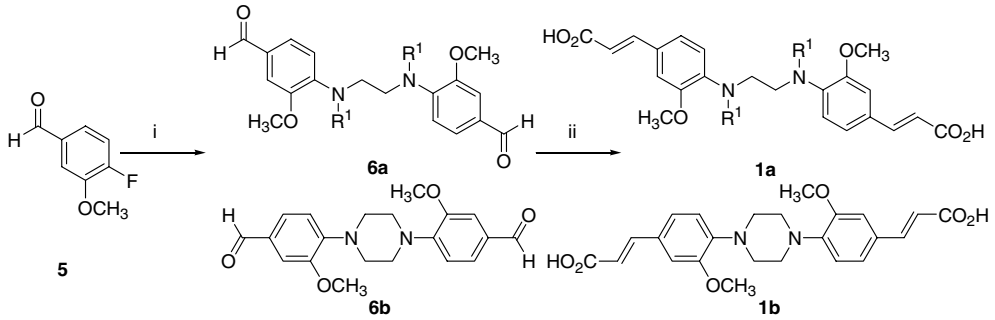
The synthesis of symmetric FA dimer analogues is shown in Scheme 1. Benzaldehyde dimers (**6a** and **6b**) were prepared from fluorobenzaldehyde (**5**) via an aromatic substitution reaction with appropriate secondary amines.[11] Acrylic acids of **1a** and **1b** were obtained via the Knoevenagel reaction with a malonic acid in the presence of piperidine and pyridine.

Unsymmetric FA dimer derivatives (**2a**–**d**) were synthesized as shown in Scheme 2. Biscarboxymethyl-amino group[13] of **2b** was introduced as a binding site for ^{99m}Tc to obtain the SPECT image. 4-Nitroguaiacol (**7**) was used in a nucleophilic substitution reaction to give **8**.[14] Subsequent reduction, reductive amination in situ or reduction, and substitution reaction led to **9a** or **9b**, respectively. Coupling **9a** or **9b** with various aldehydes gave **10a**–**d** which underwent the Knoevenagel condensation to yield **2a**–**d**.[21]

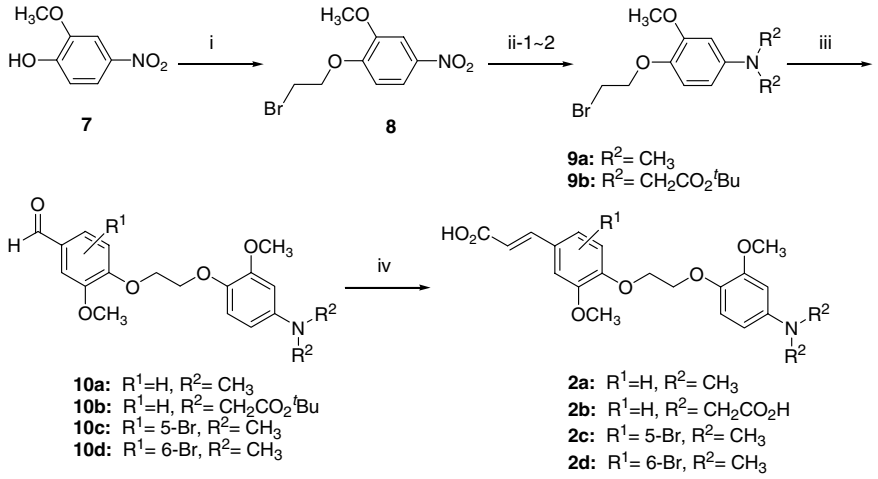
Benzothiazole dimers were synthesized as represented in Scheme 3. 4-Substituted aniline **11** was cyclized with ammonium thiocynate and bromine[15] to give aminobenzothiazole (**12**) which was converted into **13** by a reaction with hydrazine.[16] Chlorination of **13** with thionyl chloride followed by aromatic substitution[17] gave the dimers **3a**–**g** and **4a**–**g**.

Specific binding affinities of synthesized compounds to Aβ fibrils were evaluated by an in vitro Aβ fibril binding assay (named in vitro competition assay). Kung and coworkers have reported that two different, distinctive and mutually exclusive binding sites on Aβ aggregates were observed for styrylbenzene and benzothiazole.[1] Considering the structural similarity of benzothiazole dimers and PIB, [^{125}I]TZDM was used as a radio-ligand for the competitive binding assay. After primary and secondary screenings (at 10 nM and 1 nM of compounds; 11.5 nM of $A\beta_{1-42}$), K_i for benzothiazole binding sites was determined by literature-based method and the results are shown in Table 1.[20] Judging from these results, synthesized dimer derivatives generally displayed significant binding affinities to $A\beta_{1-42}$ fibrils showing nM–sub nM range (0.53–1.37 nM) of K_i compared to PIB (0.77 nM). The FA dimer (K_i = 0.60 nM) showed a slightly higher binding affinity than FA monomer (K_i = 0.77 nM). Among the FA dimer derivatives, **2b** (K_i = 0.57 nM) gave the highest binding affinity. Most of the benzothiazole dimer derivatives also displayed slightly higher binding affinity than PIB containing a benzothiazole monomer. From the compounds assayed, **4a**, **4c**, and **4e** showed the stronger binding affinities (K_i = 0.53, 0.55, and 0.55 nM).

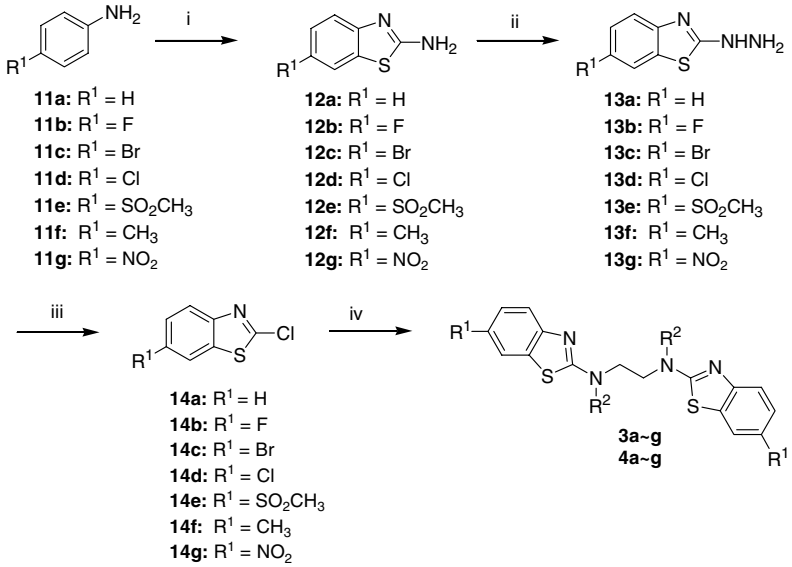
In the structure–activity relationship of FA dimer derivatives, the isosteric replacement of the oxygen bridge in the FA dimer to the nitrogen bridge (**1a**) hardly affected the K_i value. Changing one of the polar conjugated carboxylic acids in the FA dimer to a more lipophilic tertiary amine at C-4 (**2a**) had little effect on the K_i value. In the case of



Scheme 1. Reagents and conditions: (i) *N*,*N*′-dimethylethylene diamine (for **6a**), piperazine (for **6b**), K_2CO_3, DMSO, H_2O, 100 °C, 24 h, 77%; (ii) malonic acid, piperidine, pyridine, reflux, 2.5 h, 73%.



Scheme 2. Reagents and conditions: (i) 1,2-dibromoethane, K_2CO_3, DMF, KI, 80 °C, 8 h, 61% ; (ii-1) 37% CH_2O aq., 10% Pd/C, H_2, CH_2Cl_2, ethanol, 2.5 h, 67% (for **9a**); (ii-2) 10% Pd/C, H_2, CH_2Cl_2, ethanol, 1 h; then *t*-butyl iodoacetate, $NaHCO_3$, DMSO, rt, 8 h, 69% (two steps) (for **9b**); (iii) vaniline (for **10a**, **10b**), 5-bromovaniline (for **10c**), or 6-bromovaniline (for **10d**), K_2CO_3, DMF, 60 °C, 8 h, 87%; (iv) malonic acid, piperidine, pyridine, reflux, 2.5 h, 90% (**2a**), 99% (**2c**), 91% (**2d**); then TFA/CH_2Cl_2 (2:1), rt, 4 h, 62% (two steps) (for **2b**).



Scheme 3. Reagent and conditions: (i) NH_4SCN, Br_2, AcOH, below 10 °C, 1 h, 32%; (ii) $NH_2NH_2{\cdot}H_2O$, $NH_2NH_2{\cdot}H_2SO_4$, $HO(CH_2)_2OH$, 120 °C, 2 h, 85%; (iii) $SOCl_2$, 50 °C, 2 h, 93%; then HNO_3–H_2SO_4, 40 °C, 6 h, 62%[18] (for **14 g**); (iv) ethylenediamine (for **3a**–**g**), *N*,*N*′-dimethylethylenediamine (for **4a**–**g**), K_2CO_3, 2-PrOH, 85 °C, 24–48 h, 52% (for **3a**–**f**, **4a**–**f**); then $FeCl_3$, $NH_2NH_2{\cdot}H_2O$, charcoal, MeOH, 65 °C, 42%[19] (two steps) (for **3g**, **4g**).

Table 1. In vitro Aβ fibril binding assay of ferulic acid (**1a**–**b**, **2a**–**d**) and benzothiazole dimers (**3a**–**g**, **4a**–**g**)

Compound	K_i[a] (nM) at TZ binding sites
FA dimer	0.60
1a	0.72
1b	1.36
2a	1.00
2b	0.57
2c	0.83
2d	1.32
3a	0.71
3b	0.75
3c	1.11
3d	0.96
3e	>10
3f	0.81
3g	0.69
4a	0.53
4b	1.37
4c	0.55
4d	0.64
4e	0.55
4f	0.61
4g	0.69
PIB	0.77
FA	0.77

[a] K_i was calculated from nonlinear regression by Graphpad Prism software.

benzothiazole dimers, the presence of central secondary (**3a**) or tertiary (**4a**) amine bridges affects the binding affinities. As a whole, the linkage with a tertiary amine displayed higher binding affinity than that with a secondary amine except for **3b** and **4b** where the trend was reversed. But, the kind of R^1 (either halogen, CH_3, or NH_2) of **3** and **4** did not show any significant influence.

In conclusion, new FA and benzothiazole dimer derivatives were synthesized. They showed excellent binding affinities for benzothiazole binding sites of $A\beta_{1-42}$ fibrils compared to PIB. In particular, **4a**[21] exhibited the best binding affinity ($K_i = 0.53$ nM) implying that it could be a potential probe for detection of Aβ fibrils in AD brain. However, besides specific binding affinities, since the actual brain penetration and clearance are very important factors in the development of AD imaging probe, additional studies are necessary in the future.

Acknowledgments

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21. Selected data. Compound **2d**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.89 (s, 6H), 3.84 (s, 3H), 3.87 (s, 3H), 4.32 (t, 2H, $J = 5.0$ Hz), 4.41 (t, 2H, $J = 4.9$ Hz), 6.27 (dd, 1H, $J = 8.8$ Hz, 2.8 Hz), 6.35 (d, 1H, $J = 15.9$ Hz), 6.39 (d, 1H, $J = 2.8$ Hz), 6.91 (d, 1H, $J = 8.8$ Hz), 7.0 (d, 1H, $J = 1.7$ Hz), 7.35 (d, 1H, $J = 1.7$ Hz), 7.64 (d, 1H, $J = 15.9$ Hz); ^{13}C NMR($CDCl_3$, 75 MHz) δ 41.58, 56.00, 56.15, 69.41, 71.73, 77.21, 99.85, 105.10, 110.82, 116.89, 117.37, 118.29, 125.41, 130.98, 140.37, 145.12, 147.81, 150.74, 153.67, 170.13; Anal. calcd for $C_{21}H_{24}BrNO_6$: C, 54.09; H, 5.19; N, 3.00. Found: C, 53.75; H, 5.11; N, 2.89. Compound **4a**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.15 (s, 6H), 3.86 (s, 4H), 7.01 (t, $J = 7.1$ Hz, 2H), 7.24 (t, $J = 7.2$ Hz, 2H), 7.41 (t, $J = 7.8$ Hz, 2H), 7.69 (d, $J = 7.7$ Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 38.9, 50.3, 118.7, 121.2, 121.5, 126.3, 131.0, 153.3, 168.1; Anal. calcd for $C_{18}H_{18}N_4S_2$: C, 60.99; H, 5.12; N, 15.80; S, 18.09. Found: C, 61.04; H, 5.22; N, 15.81; S, 17.82.