Phenolic Bis-Styrylbenzenes as β-Amyloid Binding Ligands and Free Radical Scavengers

Daniel P. Flaherty†, Tomomi Kiyota‡, Yuxiang Dong†, Tsuneya Ikezu‡, and Jonathan L. Vennerstrom†,*
† University of Nebraska Medical Center, College of Pharmacy, 986025 Nebraska Medical Center, Omaha, NE, 68198
‡ University of Nebraska Medical Center, College of Medicine, 985880 Nebraska Medical Center, Omaha, NE, 68198

Abstract

Starting from bisphenolic bis-styrylbenzene DF-9 (4), β-amyloid (Aβ) binding affinity and specificity for phenolic bis-styrylbenzenes, mono-styrylbenzenes and alkyne controls were determined by fluorescence titration with β-amyloid peptide Aβ1-40 and a fluorescence assay using APP/PS1 transgenic mouse brain sections. Bis-styrylbenzene SAR is derived largely from work on symmetrical compounds; this study is the first to describe Aβ binding data for bis-styrylbenzenes unsymmetrical in the outer rings. With one exception, binding affinity and specificity were decreased by adding and/or changing the substitution pattern of phenol functional groups, changes to the orientation about the central phenyl ring, replacing the alkene with alkyne bonds, or elimination of the central phenyl ring. The only compound with an Aβ binding affinity and specificity comparable to 4 was its 3-hydroxy regioisomer 8. Like 4, 8 crossed the blood-brain-barrier and bound to Aβ plaques in vivo. Using a DPPH assay, phenol functional groups with para orientations seem to be a necessary, but insufficient, criterion for good free radical scavenging properties in these compounds.

Alzheimer’s disease (AD) is a common neurodegenerative disorder in the elderly characterized by an accumulation of β-amyloid (Aβ) plaques in the brain parenchyma and neurofibrillary tangles (NFTs) in the neuron. Although Aβ and NFTs are commonly observed in nondemented elderly, 80 years and older, the molecular pathogenesis of AD is believed to be effected by Aβ production and its clearance. The amyloid cascade hypothesis for AD pathogenesis proposes that accumulation and aggregation of Aβ triggers a cascade that leads to the characteristic pathologies of AD. Evidence in support of this hypothesis is obtained from studies of familial AD, which reveal that Aβ accumulation and aggregation are elevated by mutations of the APP, PS1 and PS2 genes.

The discovery of the diazo dye Congo red as an Aβ binding ligand provided a starting point in the search for potential in vivo imaging agents using positron emission tomography (PET), single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI). Investigation of Congo red led to the discovery of bis-styrylbenzenes as a class of compounds with strong Aβ plaque binding properties. These compounds are exemplified by the salicylate bis-styrylbenzenes X-34 (1) and FSB (2), which have been...
shown to penetrate the blood–brain barrier (BBB) and bind to Aβ plaques deposited in APP or APP/PS1 mice. As shown by K114 (3) and DF-9 (4), acidic functional groups are not necessary for good Aβ binding. Several stilbene-based compounds, such as SB-13 and BAY94-9172, have been studied for early detection in AD. While the structure-activity relationship (SAR) of bis-styrylbenzene compounds has been extensively investigated, the common thread in these studies is that Aβ-binding SAR has been elucidated based largely on symmetrical bis-styrylbenzenes.

Design

Previously, we showed that the bis-styrylbenzene, (E,E)-1,4-bis(4-hydroxystyryl) benzene (4), binds to Aβ with high affinity and specificity. In recent years, many have recognized the antioxidant and potential anti-AD properties of the polyphenolic stilbene resveratrol (5), a well-known component of red wine. Therefore, we also included structural elements of 5 in the design of target compounds (Figure 1), especially since oxidative stress is considered to play an important role in AD pathology.

Little has been studied regarding substitution patterns in the central benzene ring of Aβ-binding bis-styrylbenzenes. For this purpose we designed target compounds 6 and 7 with a 1,3 and 1,2 orientation about the central benzene ring as opposed to the more extended 1,4 orientation in 4 and other reported bis-styrylbenzenes. Target compounds 8 and 9 are the meta and ortho bis-phenolic regioisomers of 4. Tetraphenol 10 is a symmetrical bis-styrylbenzene that combines the resorcinol substructures of 5. Unsymmetrical target compounds 11–15 contain from one to three phenol functional groups, but each maintains at least one of the para phenols of prototype 4. The final target compounds are the mono-styryl (16) and bis-alkyne (17) derivatives of 4. A secondary objective in designing these unsymmetrical bis-styrylbenzenes was an attempt to increase the solubility of this class of compounds as we have observed that symmetrical bis-styrylbenzenes have poor aqueous solubilities. For each target compound, we determined Aβ-binding affinity and specificity and antioxidant properties in addition to BBB penetration for selected compounds. We suggest that these data will be useful in the design and discovery of new early diagnosis probes for AD using PET, SPECT or MRI and potentially for AD therapeutics.

Chemistry

Compounds 4, 6–10 (Scheme 1) were obtained using a previously reported procedure. The preparation for 6 was an improvement on the previously published procedure for this compound in which a low-yielding (20%) double-Heck coupling was used. The overall yields of 8 (69%) and 10 (42%) were better than those (49 and 28%) previously reported for these compounds. 1,3- and 1,2-Bis(diethylphosphonylmethyl)benzenes 19b (96%) and 19c (93%) were synthesized using the Arbuzov reaction with o- and m-xylylene dibromide. Bis-diethylphosphonates 19a–19c were coupled with methoxymethoxy (MOM)-substituted benzaldehydes 20a–20d to afford 4a and 6a–10a in 48–84% yields, which were then deprotected to afford 4 and 6–10 in 64–99% yields.

Compounds 11–15 (Scheme 2) were synthesized via successive Heck and Horner–Wadsworth–Emmons reactions using a new one-pot procedure. Heck couplings between 4-iodobenzyl phosphonate (21) and styrenes 22a–22c were accomplished with palladium(II) acetate, phenylurea and potassium carbonate in DMF at 110 °C for 22 h. The reaction mixtures were cooled to 80 °C followed by addition of benzaldehydes 20a, 20b, 20d or 20e
and 30% w/v sodium methoxide in MeOH and then stirred for an additional 1 h to afford 11a–15a in 52–79% yield. HCl deprotection of 11a–15a afforded 11–15 in 70 – 94% yield after filtration and washing. Although 11 was previously synthesized in 31% overall yield in a five-step procedure,24 we were able to synthesize this same compound in 73% overall yield using this new one-pot method. Compounds 16 and 17 were synthesized by modified literature25,26 procedures (see Supporting Information).

β-Amyloid (Aβ) Binding Affinity and Specificity

β-Amyloid (Aβ) binding affinity and specificity for bis-styrylbenzenes 6–17, with 4 and 5 as controls, were determined by means of fluorescent titrations27 with aggregated amyloid peptide Aβ1-40 and an in vitro fluorescence-based assay using APP/PS1 transgenic mouse brain sections.13 The APP/PS1 transgenic mice were developed by crossing the Tg2576 strain,28 which express the K670N/M671L mutant of APP695 found as the Swedish familial AD gene, and the M146L 6.1 strain,29 which express the M146L mutant of PS1 found as the early onset familial AD gene. The specificity is determined by measuring the fluorescent intensity of Aβ plaques (specific signal) compared to that of background normal brain tissue (noise) (Figure 2).

The only compound with an Aβ binding affinity and specificity comparable to that of 4 was its 3-hydroxy regioisomer 8, which had a measured $K_d$ of 5.3 ± 0.3 nM and a specificity of 9.6 ± 3.0 (Table 1). In contrast, 9, the 2-hydroxy regioisomer of 4, had an 84-fold weaker Aβ binding affinity, although it had good binding specificity. Apparently, the 1,4 orientation about the central benzene ring in compounds 4, 8, and 9 endows them with the extended geometry required to maintain strong binding interactions in the hydrophobic binding site(s) of the Aβ fibril, but only 4 and 8 have the required phenol functional group orientations to potentially form strong H-bonding interactions. However, 12, the unsymmetrical regioisomeric hybrid of 4 and 8, had an unexpectedly much weaker Aβ binding affinity (370 and 660-fold) compared to that of 4 and 8.

The two order of magnitude decrease in Aβ binding affinity for 6 and 7 show that the optimal geometry about the central benzene ring is a 1,4 orientation as opposed to 1,3 (6) or 1,2 (7). Even though the anti-aggregation properties of 1,3-bis-styrylpyridines and 1,3-bis-styrylbenzenes30 and Aβ-binding properties of 1,3-bis-styrylquinaldines31 have been investigated, this is the first time that Aβ-binding affinity and specificity has been assessed as a function of the central ring substitution pattern of bis-styrylbenzenes.

As evidenced by the 17 to 27-fold loss in Aβ binding affinity for the more polar tetraphenol 10 and triphenols 13 and 14, additional phenol functional groups decreased affinity and tended to decrease specificity. Compared to bis-phenol 4, mono-phenol 11 showed a 16-fold reduction in binding affinity, due most likely to the loss of a H-bond formed by the second phenol functional group in 4. Compared to 4, the more rigid linear alkyne analog 17 had a 6-fold loss in Aβ binding affinity and a 2.4-fold loss in specificity, due possibly to an inability to attain a conformer orientation complementary to the Aβ fibril surface. Of all of the target compounds, the symmetrical mono-styrylbenzene 16 had the weakest Aβ binding affinity – three orders of magnitude weaker than that of 4. This could be attributed either to the loss of the central benzene ring eliminating a critical binding interaction, or to the shorter distance between phenol functional groups, reducing the effectiveness of 16 to form strong H-bonds with the Aβ fibril surface, or a combination of the two. Surprisingly, mono-styrylbenzene 5 displayed only a 52-fold loss in binding affinity compared to 4. Conceivably, the 3,5-dihydroxy substituted 5, but not the 4-hydroxy substituted 16, provides conformers with the geometry required to form strong H-bonds to the aggregated Aβ fibril.
**Human AD Brain Section Staining**

Bis-styrylbenzenes 8 and 10, along with the control bis-styrylbenzene 4, were used to stain human AD brain sections (Figure 2). Each compound was shown to bind specifically to Aβ plaques in human brain sections. Age matched controls were also stained and showed no staining of normal brain tissue (data not shown).

**In Vivo BBB Delivery in Plaque-Bearing Mice**

We next determined whether interperitoneally administered 8 and 10 (with 4 as a control) could cross the BBB and bind to Aβ plaques. Bisstyrylbenzenes 4, 8 and 10 (50 mg/kg) were injected into ten-month-old plate-derived growth factor-β chain promoter driving APP (PDAPP) transgenic mice. As shown in Figure 3, all compounds crossed the BBB and bound to Aβ plaques in vivo. Each section showed intense Aβ plaque signals with high specificities. Control non-transgenic animals showed no specific signals in the brain for any of the tested compounds after intraperitoneal injection (data not shown). These data confirm that these bis-styrylbenzene derivatives retain the BBB penetration capabilities of 4, and that 8 and 10 have the potential as lead compounds for early Aβ detection research.

**Free Radical Scavenging Activity**

The relative free radical scavenging properties of the target bis-styrylbenzenes, along with quercetin as a control, were measured using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the procedure of Yang et al. with some modifications (Table 2). Compound 4, 6, 12, and 16 were no less effective than quercetin (IC$_{50}$ = 1.3 ± 1.1 μM) in DPPH free radical scavenging activity, whereas compounds 7, 9, 10, 14 and 15 were 3 to 7-fold less potent (p < 0.05) than quercetin. Aside from 8 and 13, which had no antioxidant activity (IC$_{50}$ > 100 μM) in this assay, mono-styrylbenzene 5 and bis-alkyne 17 were the least effective radical scavengers with IC$_{50}$s of 62 and 45 μM, respectively. No particular SAR is apparent from these data aside from the general observation that a phenol functional group with a para orientation seem to be a necessary, but insufficient, criterion for good free radical scavenging properties of bis-styrylbenzenes. With polyphenolic antioxidants such as 5 of increasing interest in AD research, this set of compounds may prove beneficial in future research.

**Summary**

These data extend the SAR of bis-styrylbenzene Aβ binding and free-radical scavenging activity and provide further direction for the development of non-invasive Aβ binding ligands for early detection of AD. One new bis-styrylbenzene prototype (8) was identified that could serve as a building block for the design and discovery of new early diagnosis probes for AD using PET, SPECT or MRI and potentially for AD therapeutics. Finally, we observed that a phenol functional group with a para orientation seem to be a necessary, but insufficient, criterion for good free radical scavenging properties in these bis-styrylbenzenes.

**Experimental Section**

**General**

Starting materials were purchased from Aldrich, TCI or Acros. All reactions were run under a positive pressure of Ar. Melting points were determined on a Stanford Research Systems E-Z Melt apparatus and are uncorrected. $^1$H (500 MHz) and $^{13}$C (125.7 MHz) NMR spectra were measured on a Varian spectrometer using CDCl$_3$ and DMSO-d$_6$ as solvents. All chemical shifts are reported in parts per million (ppm) and are relative to internal (CH$_3$)$_4$Si for $^1$H, CDCl$_3$ (77.0 ppm) and DMSO-d$_6$ (39.7 ppm) for $^{13}$C NMR. Elemental analyses
were performed by M-H-W Laboratories, Phoenix, AZ. All target compounds had purities ≥ 95%.

\[ (E,E)-1,4\text{-bis}(4\text{-hydroxystyryl})\text{benzene} \] (4)13

**Step 1.** A 30 wt % solution of sodium methoxide in methanol (1.39 mL, 7.40 mmol) was added to a stirred mixture of 20a (0.983 g, 5.92 mmol) and 1,4-bis(diethylphosphonylmethyl)benzene (19a) (1.12 g, 2.96 mmol) in DMF (15 mL) at rt. This mixture was heated to 80 °C for 2 h. The reaction was then quenched with H₂O (10 mL) and the solid precipitate was filtered and rinsed with ether to afford (E,E)-1,4-bis-(4-methoxymethoxy)styrylbenzene 4a (0.858 g, 72%). ¹H NMR (CDCl₃) δ 3.50 (s, 6H), 5.20 (s, 4H), 6.98 (d, J = 16.1 Hz, 2H), 7.04 (d, J = 8.8 Hz, 4H), 7.08 (d, J = 16.1, 2H), 7.45 (d, J = 8.3 Hz, 4H), 7.48 (s, 4H). ¹³C NMR (CDCl₃) δ 56.0, 94.4, 116.4, 126.6, 126.7, 127.7, 127.8, 131.3, 136.6, 156.8. **Step 2.** 4a (0.490 g, 1.22 mmol) and 4:1 CHCl₃:MeOH (25 mL) were stirred at rt until 4a was completely dissolved before dropwise addition of 12.0 M HCl (0.812 mL, 9.74 mmol) at rt. The reaction was quenched H₂O (20 mL) after 24 hr. The CHCl₃:MeOH layer was removed in vacuo to afford 4 as a white solid (0.379 g, 99%): mp dec. 312 °C. ¹H NMR (DMSO-d₆) δ 6.77 (d, J = 8.3 Hz, 4H), 7.00 (d, J = 16.6 Hz, 2H), 7.15 (d, J = 16.6 Hz, 2H), 7.43 (d, J = 8.8 Hz, 4H), 7.52 (s, 4H), 9.57 (s, 2H). ¹³C NMR (DMSO-d₆) δ 115.8, 125.0, 125.6, 128.0, 128.2, 128.3, 157.6.

\[ (E,E)-1,3\text{-bis}(4\text{-hydroxystyryl})\text{benzene} \] (6)18

**Step 1.** As described above for 4a, 20a (0.677 g, 4.07 mmol) and 19b (0.773 g, 2.04 mmol) yielded 6a (0.660 g, 80%): mp 123 – 126 °C. ¹H NMR (DMSO-d₆) δ 3.39 (s, 6H), 5.22 (s, 4H), 7.05 (d, J = 8.8 Hz, 4H), 7.14 (d, J = 16.1 Hz, 2H), 7.28 (d, J = 16.6, 2H), 7.36 (t, J = 7.3 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.8 Hz, 4H), 7.80 (s, 1H). ¹³C NMR (DMSO-d₆) δ 55.8, 94.0, 116.6, 124.0, 125.6, 126.7, 127.9, 128.4, 129.2, 130.9, 137.8, 156.7. **Step 2.** As described above for 4a, 6a (0.406 g, 1.01 mmol) was deprotected using 12.0 M HCl (0.672 mL, 7.94 mmol) to yield 6 (0.309 g, 97%): mp dec. 218 – 220 °C (lit. mp 232 – 234 °C). ¹H NMR (DMSO-d₆) δ 6.78 (d, J = 8.8 Hz, 4H), 7.03 (d, J = 16.1 Hz, 2H), 7.21 (d, J = 16.1 Hz, 2H), 7.32 (t, J = 6.8 Hz, 1H), 7.40 (d, J = 7.3 Hz, 2H), 7.44 (d, J = 8.3 Hz, 4H), 7.72 (s, 1H), 9.59 (s, 2H). ¹³C NMR (DMSO-d₆) δ 116.0, 124.0, 125.2, 125.4, 128.23, 128.5, 129.0, 129.3, 138.3, 157.8. Anal. (C₂₂H₁₈O₂·1/3 H₂O) C, H.

\[ (E,E)-1,2\text{-bis}(4\text{-hydroxystyryl})\text{benzene} \] (7)

**Step 1.** As described above for 4a, 20a (0.310 g, 1.87 mmol) and 19c (0.353 g, 0.993 mmol) yielded 7a (0.169 g, 45%): mp 67 – 70 °C. ¹H NMR (CDCl₃) δ 3.50 (s, 6H), 5.20 (s, 4H), 6.95 (d, J = 16.1 Hz, 2H), 7.04 (d, J = 8.8 Hz, 4H), 7.26 (dd, J₁ = 5.8 Hz, J₂ = 3.4 Hz, 2H), 7.33 (d, J = 16.1 Hz, 2H), 7.46 (d, J = 8.8 Hz, 4H), 7.55 (dd, J₁ = 5.9 Hz, J₂ = 3.4 Hz, 2H). ¹³C NMR (CDCl₃) δ 55.0, 94.3, 116.4, 125.0, 126.5, 127.4, 127.8, 130.6, 131.5, 136.0, 156.9. **Step 2.** As described above for 4a, 7a (0.151 g, 0.375 mmol) was deprotected using 12.0 M HCl (0.250 mL, 3.00 mmol) to yield 7 (0.108 g, 92%): mp 262 – 265 °C. ¹H NMR (DMSO-d₆) δ 6.78 (d, J = 7.8 Hz, 4H), 7.00 (d, J = 16.1 Hz, 2H), 7.25 (brs, 2H), 7.42 (d, J = 16.1 Hz, 2H), 7.50 (d, J = 7.8 Hz, 4H), 7.62 (brs, 2H), 9.58 (s, 2H). ¹³C NMR (DMSO-d₆) δ 115.6, 122.7, 126.0, 127.4, 128.4, 128.5, 130.8, 135.7, 157.6. Anal. (C₂₂H₁₈O₂) C, H.

\[ (E,E)-1,4\text{-bis}(3\text{-hydroxystyryl})\text{benzene} \] (8)19

**Step 1.** As described above for 4a, 20b (0.388 g, 2.33 mmol) and 19a (0.442 g, 1.17 mmol) yielded 8a (0.359 g, 76%): mp 136 – 140 °C. ¹H NMR (DMSO-d₆) δ 3.41 (s, 6H), 5.24 (s, 4H), 6.92 – 6.95 (m, 2H), 7.24 – 7.28 (m, 8H), 7.31 (t, J = 7.8 Hz, 2H), 7.62 (s, 4H). ¹³C NMR (DMSO-d₆) δ 56.0, 94.4, 114.0, 115.6, 120.3, 126.9, 128.3, 128.6, 129.7, 136.6, 138.8, 157.6. **Step 2.** As described above for 4a, 8a (0.136 g, 0.338 mmol) was deprotected
using 12.0 M HCl (0.230 mL, 2.70 mmol) to yield 8 (0.097 g, 91%): mp 281 – 283 °C (lit.19 mp 240 °C). 1H NMR (DMSO-d_6) δ 6.69 (dd, J = 7.8 Hz, 2H), 7.00 (d, J = 7.8 Hz, 2H), 7.12 – 7.22 (m, 6H), 7.60 (s, 4H). 13C NMR (DMSO-d_6) δ 113.2, 115.1, 117.8, 127.1, 128.0, 128.7, 129.8, 136.5, 138.6, 157.8. Anal. (C_{22}H_{18}O_2) C, H.

**(E,E)-1-bis(2-hydroxystyryl)benzene (9)**

**Step 1.** As described above for 4a, 20c (1.05 g, 6.31 mmol) and 19a (1.19 g, 3.17 mmol) yielded 9a (1.07 g, 84%): 1H NMR (CDCl_3) δ 3.53 (s, 6H), 5.26 (s, 4H), 7.03 (t, J = 7.3 Hz, 2H), 7.11 (d, J = 16.6 Hz, 2H), 7.13 (d, J = 7.8 Hz, 2H), 7.22 (t, J = 8.3 Hz, 1.5 Hz, 2H), 7.52 (d, J = 15.1 Hz, 2H), 7.53 (s, 4H), 7.63 (dd, J = 7.8 Hz, 1.5 Hz, 2H). 13C NMR (CDCl_3) δ 56.2, 94.8, 114.9, 122.1, 123.1, 126.2, 126.8, 127.3, 128.6, 128.9, 137.1, 154.6.

**Step 2.** As described above for 4, 9a (0.634 g, 1.58 mmol) was deprotected using 12.0 M HCl (0.230 mL, 2.70 mmol) to yield 9 (0.316 g, 64%): mp 262 – 265 °C (lit.33 mp 230 – 231 °C). 1H NMR (DMSO-d_6) δ 6.82 (t, J = 7.8 Hz, 2H), 6.88 (d, J = 7.8 Hz, 2H), 7.10 (t, J = 7.8 Hz, 2H), 7.21 (d, J = 16.1 Hz, 2H), 7.43 (d, J = 16.6 Hz, 2H), 7.55 (s, 4H), 7.59 (d, J = 7.8 Hz, 2H), 9.75 (s, 2H). 13C NMR (DMSO-d_6) δ 116.0, 119.5, 123.5, 124.0, 126.6, 126.8, 127.6, 128.8, 136.9, 155.2. Anal. (C_{22}H_{18}O_2) C, H.

**(E,E)-1,4-bis(3,5-dihydroxybenzyl)benzene (10)**

**Step 1.** As described above for 4a, 20d (0.173 g, 0.765 mmol) and 19a (0.145 g, 0.382 mmol) yielded 10a (0.096 g, 48%): mp 101 – 106 °C. 1H NMR (CDCl_3) δ 3.51 (s, 12H), 5.20 (s, 8H), 6.66 (t, J = 2.4 Hz, 2H), 6.88 (d, J = 2.4 Hz, 4H), 7.04 (d, J = 16.1 Hz, 2H), 7.09 (d, J = 16.1 Hz, 2H), 7.50 (s, 4H). 13C NMR (CDCl_3) δ 56.1, 94.5, 104.3, 107.8, 126.9, 128.3, 128.9, 136.5, 139.5, 158.5. **Step 2.** As described above for 4, 10a (0.084 g, 0.16 mmol) was deprotected using 12.0 M HCl (0.214 mL, 2.57 mmol) to yield 10 (0.049 g, 88%) mp 290 – 305 °C (lit.21 mp >300 °C). 1H NMR (DMSO-d_6) δ 6.16 (s, 2H), 6.45 (s, 4H), 7.04 (d, J = 16.1 Hz, 2H), 7.09 (d, J = 16.1 Hz, 2H), 7.57 (s, 4H). 13C NMR (DMSO-d_6) δ 104.9, 127.0, 127.7, 129.0, 136.5, 139.0, 158.7.

**(E,E)-1-styryl-4-(4-hydroxybenzyl)benzene (11)**

As described above for 4, 11a (0.654 g, 1.91 mmol) was deprotected using 12.0 M HCl (1.05 mL, 12.6 mmol) to afford 11 (0.53 g, 93%): mp 281 – 285 °C. 1H NMR (DMSO-d_6) δ 6.78 (d, J = 8.3 Hz, 2H), 10.03 (d, J = 16.1 Hz, 2H), 1.18 (d, J = 16.6 Hz, 2H), 7.26 (s, 2H), 7.27 (t, J = 7.3 Hz, 1H), 7.38 (t, J = 7.8 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.3 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.61 (d, J = 7.9 Hz, 2H), 9.59 (s, 1H). 13C NMR (DMSO-d_6) δ 115.8, 124.9, 126.5, 126.6, 127.0, 128.1, 128.3, 128.7, 129.8, 131.8, 131.9, 135.9, 137.2, 137.3, 157.6, 167.2.

**(E,E)-1-styryl-4-[4-(methoxymethoxy)styryl]benzene (11a)**

Diethyl 4-iodobenzyldiphenylphosphonate (21) (0.414 g, 1.17 mmol) and 22a (0.260 g, 2.34 mmol) were mixed with palladium (II) acetate (0.008 g, 0.035 mmol), phenylenediamine (0.010 g, 0.070 mmol) and potassium carbonate (0.323 g, 2.34 mmol) in DMF (5 mL) and stirred at 110 °C for 22 h. The reaction temperature was then cooled to 80 °C and to this mixture were added 20a (0.195 g, 1.17 mmol) and then 30% w/v sodium methoxide in methanol (0.440 mL, 2.34 mmol). The reaction was stirred for 1 h at 80 °C and then cooled to rt and quenched with H_2O (15 mL). The solid was then filtered and rinsed with H_2O and ether to afford 11a (0.517 g, 79%): mp 240 – 243 °C. 1H NMR (CDCl_3) δ 3.50 (s, 3H), 5.20 (s, 2H), 6.99 (d, J = 16.6 Hz, 1H), 7.04 (d, J = 8.3 Hz, 2H), 7.09 (d, J = 16.6 Hz, 1H), 7.12 (s, 2H), 7.26 (t, J = 7.3 Hz, 1H), 7.36 (t, J = 7.3 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.49 (s, 2H), 7.52 (d, J = 7.8 Hz, 2H). 13C NMR (CDCl_3) δ 56.0, 94.4, 116.4, 126.5, 126.6, 126.7, 126.8, 127.6, 127.7, 128.0, 128.3, 128.4, 128.7, 131.3, 136.3, 136.9, 137.3, 156.9.

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(E,E)-1-(4-hydroxystyril)-4-(3-hydroxystyril)benzene (12)

As described above for 4, 12a (0.280 g, 0.696 mmol) was deprotected using 12.0 M HCl (0.696 mL, 8.35 mmol) to afford 12 (0.161 g, 74%): mp 297 – 301 °C. $^1$H NMR (DMSO-$d_6$) δ 6.68 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 8.8 Hz, 2H), 6.98 (s, 1H), 7.02 (d, J = 16.6 Hz, 1H), 7.04 (d, J = 7.3 Hz, 1H), 7.12 – 7.22 (m, 4H), 7.44 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 8.3 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H). $^{13}$C NMR (DMSO-$d_6$) δ 113.2, 115.0, 115.8, 117.7, 125.0, 126.6, 127.1, 128.1, 128.3, 128.6, 129.8, 135.9, 137.1, 138.7, 157.6, 157.8.

(E,E)-1-[4-(methoxymethoxy)styril]-4-[3-(methoxymethoxy)styril]benzene (12a)

As described above for 11a, 21 (0.312 g, 0.880 mmol), 22b (0.289 g, 1.76 mmol) were mixed with palladium (II) acetate (0.006 g, 0.03 mmol), phenylurea (0.007 g, 0.05 mmol) and potassium carbonate (0.243 g, 1.76 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound 20b (0.146 g, 0.880 mmol) and sodium methoxide solution (0.330 mL, 1.76 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield 12a (0.23 g, 65%): mp 142 – 152 °C. $^1$H NMR (CDCl$_3$) δ 3.50 (s, 3H), 3.51 (s, 3H), 5.20 (s, 2H), 5.22 (s, 2H), 6.95 (dd, J$_1$ = 7.8 Hz, J$_2$ = 2.0 Hz, 1H), 6.99 (d, J = 16.6 Hz, 1H), 7.04 (d, J = 8.3 Hz, 2H), 7.08 (d, J = 16.1 Hz, 1H), 7.09 (s, 2H), 7.18 (d, J = 7.3 Hz, 1H), 7.21 (s, 1H), 7.28 (t, J = 8.3 Hz, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.50 (s, 4H). $^{13}$C NMR (CDCl$_3$) δ 55.9, 94.2, 94.3, 113.9, 115.4, 116.3, 120.3, 126.6, 126.8, 127.6, 127.9, 128.0, 128.6, 129.6, 131.2, 136.1, 136.9, 138.8, 156.8, 157.5.

(E,E)-1-(4-hydroxystyril)-4-(3,4-dihydroxystyril)benzene (13)

As described above for 4, 13a (0.100 g, 0.216 mmol) was deprotected using 12.0 M HCl (0.252 mL, 3.03 mmol) to afford 13 (0.050 g, 70%): mp 341 – 350 °C. $^1$H NMR (DMSO-$d_6$) δ 6.73 (d, J = 7.8 Hz, 1H), 6.77 (d, J = 8.3 Hz, 2H), 6.87 (d, J = 8.3 Hz, 1H), 6.91 (d, J = 16.6 Hz, 1H), 7.01 (d, J = 16.6 Hz, 1H), 7.08 (d, J = 16.6 Hz, 1H), 7.15 (d, J = 16.6 Hz, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.51 (s, 4H). $^{13}$C NMR (DMSO-$d_6$) δ 113.5, 115.8, 115.9, 118.8, 124.9, 125.1, 126.6, 126.6, 128.1, 128.2, 128.4, 128.6, 129.0, 136.4, 136.5, 145.6, 145.8, 157.5.

(E,E)-1-[4-(methoxymethoxy)styril]-4-[3,4-di(methoxymethoxy)styril]benzene (13a)

As described above for 11a, 21 (0.346 g, 0.977 mmol), 22b (0.321 g, 1.95 mmol) were mixed with palladium (II) acetate (0.007 g, 0.03 mmol), phenylurea (0.008 g, 0.06 mmol) and potassium carbonate (0.270 g, 1.95 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound 20e (0.221 g, 0.977 mmol) and sodium methoxide solution (0.366 mL, 1.95 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield 13a (0.35 g, 77%): mp 128 – 138 °C. $^1$H NMR (CDCl$_3$) δ 3.50 (s, 3H), 3.53 (s, 3H), 3.56 (s, 3H), 5.20 (s, 2H), 5.26 (s, 2H), 5.30 (s, 2H), 6.98 (d, J = 16.1, 1H), 6.98 (d, J = 16.1 Hz, 1H), 7.04 (d, J = 8.3 Hz, 2H), 7.05 (d, J = 16.1 Hz, 1H), 7.08 (d, J = 16.1 Hz, 1H), 7.12 (dd, J$_1$ = 8.3 Hz, J$_2$ = 2.0 Hz, 1H), 7.15 (d, J = 8.3 Hz, 1H), 7.35 (d, J = 1.5 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.48 (s, 4H). $^{13}$C NMR (CDCl$_3$) δ 56.0, 56.2, 94.4, 95.3, 95.4, 114.2, 116.4, 116.6, 121.0, 126.6, 126.7, 127.2, 127.6, 127.8, 127.8, 131.3, 132.2, 136.4, 136.7, 146.9, 147.4, 156.8.

(E,E)-1-(4-hydroxystyril)-4-(3,5-dihydroxystyril)benzene (14)

As described above for 4, 14a (0.113 g, 0.244 mmol) was deprotected using 12.0 M HCl (0.285 mL, 3.42 mmol) to afford 14 (0.076 g, 94%): mp 295 – 305 °C. $^1$H NMR (DMSO-$d_6$) δ 6.16 (s, 1H), 6.45 (d, J = 2.0 Hz, 2H), 6.78 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 16.6 Hz, 1H), 7.04 (s, 1H), 7.08 (d, J = 16.6 Hz, 1H), 7.17 (d, J = 16.1 Hz, 1H), 7.44 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 9.27 (s, 2H), 9.61 (s, 1H). $^{13}$C NMR
(E,E)-1-[4-(methoxymethoxy)styryl]-4-[3,5-di(methoxymethoxy)styryl]benzene (14a)

As described above for 11a, 21 (0.342 g, 0.965 mmol), 22b (0.317 g, 1.93 mmol) were mixed with palladium (II) acetate (0.007 g, 0.03 mmol), phenylurea (0.008 g, 0.06 mmol) and potassium carbonate (0.267 g, 1.93 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound 20d (0.218 g, 0.965 mmol) and sodium methoxide solution (0.362 mL, 1.93 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield 14a (0.285 g, 64%): mp 114 – 119 °C. 1H NMR (CDCl3) δ 3.49 (s, 3H), 3.51 (s, 6H), 5.20 (s, 6H), 6.66 (s, 1H), 6.88 (d, J = 2.0 Hz, 2H), 6.98 (d, J = 16.1 Hz, 1H), 7.03 (d, J = 16.6 Hz, 1H), 7.04 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 16.6 Hz, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.48 (s, 4H). 13C NMR (CDCl3) δ 56.0, 56.2, 94.4, 95.3, 95.4, 114.0, 114.2, 115.5, 116.6, 120.3, 121.1, 126.7, 126.8, 127.1, 128.0, 128.1, 128.7, 129.0, 130.6, 131.2, 136.1, 137.0, 139.5, 156.9, 158.5.

(E,E)-1-(3-hydroxystyryl)-4-(3,4-dihydroxystyryl)benzene (15)

As described above for 4, 15a (0.114 g, 0.246 mmol) was deprotected using 12.0 M HCl (0.246 mL, 2.96 mmol) to afford 15 (0.072 g, 89%): mp 285 – 289 °C. 1H NMR (DMSO-d6) δ 6.69 (d, J = 7.8 Hz, 1H), 6.74 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 16.1 Hz, 1H), 6.98 (s, 1H), 7.01 (s, 1H), 7.04 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 16.6 Hz, 1H), 7.16 (s, 2H), 7.17 (t, J = 8.3 Hz, 1H), 7.54 (d, J = 7.8 Hz, 2H), 7.57 (d, J = 8.3 Hz, 2H). 13C NMR (DMSO-d6) 113.2, 113.5, 115.0, 115.9, 117.7, 118.9, 124.8, 126.6, 127.0, 128.1, 128.3, 128.9, 129.0, 129.8, 135.9, 137.1, 138.7, 145.6, 145.9, 157.8.

(E,E)-1-[3-(methoxymethoxy)styryl]-4-[3,4-di(methoxymethoxy)styryl]benzene (15a)

As described above for 11a, 21 (0.359 g, 1.01 mmol), 22c (0.333 g, 2.03 mmol) were mixed with palladium (II) acetate (0.007 g, 0.03 mmol), phenylurea (0.008 g, 0.06 mmol) and potassium carbonate (0.281 g, 2.03 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound 20e (0.229 g, 52%): mp 94 – 101 °C. 1H NMR (CDCl3) δ 3.51 (s, 3H), 3.53 (s, 3H), 3.56 (s, 3H), 5.22 (s, 2H), 5.26 (s, 2H), 5.30 (s, 2H), 6.95 (dd, J1 = 7.8 Hz, J2 = 2.4 Hz, 1H), 6.98 (d, J = 16.6 Hz, 1H), 7.06 (d, J = 16.1 Hz, 1H), 7.09 (s, 2H), 7.12 (dd, J1 = 8.8 Hz, J2 = 2.0 Hz, 1H), 7.16 (d, J = 8.3 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 7.21 (s, 1H), 7.28 (t, J = 7.8 Hz, 1H), 7.36 (d, J = 1.5 Hz, 1H), 7.49 (s, 4H). 13C NMR (CDCl3) δ 56.0, 56.2, 94.4, 95.3, 95.4, 114.0, 114.2, 115.5, 116.6, 120.3, 121.1, 126.7, 126.8, 127.1, 128.0, 128.1, 128.7, 129.7, 132.1, 136.3, 136.8, 138.9, 146.9, 147.4, 157.6.

In Vitro Aβ1-40 Binding Affinity Determination by Fluorescence Titration

Amyloid (Aβ) binding affinities (Kd) for 5–17 and 4 as a control, were determined by means of fluorescence titrations,13,27 with amyloid peptide Aβ1-40 at 23 °C. Intrinsic fluorescence intensity (FLINT) changes associated with ligand binding to aggregated Aβ1-40 were recorded on a Cary Eclipse fluorescence spectrophotometer (Varian) using excitation wavelengths determined for each compound. Fixed concentrations of Aβ1-40 (500 nM for 5–7, 9, 10–16; 40 nM for 4 and 17; 20 nM for 8) were diluted to 500 μL in 9:1 PBS:EtOH in a 10 mm quartz fluorescence cuvette. Concentrations of Aβ1-40 were selected to be no more than 10-fold higher than compound binding affinities to obtain accurate Kd values. To the Aβ1-40 PBS:EtOH solution in the cuvette, aliquots of test compounds in PBS:EtOH were titrated using a 2.0 μL Hamilton syringe with a reproducibility adapter along a concentration gradient of test compound (0.8 nM-saturation). Fluorescence spectra were recorded until the fluorescence no longer increased with increasing compound.
concentration (saturation). The FLINT at these wavelengths was plotted versus compound concentrations to yield binding isotherms. A $\beta_{1-40} K_d$ values were determined (Prism 4.0c software, Graphpad, Inc.).

**In Vitro A Plaque Binding Specificity Using APP/PS1 Transgenic Mouse Brain Slices**

DMSO stock solutions of 4–17 were diluted to 0.50 mM with EtOH and then diluted with 9:1 PBS:EtOH to prepare 50 $\mu$M solutions. Eleven-month old transgenic APP/PS1 mice derived from crossing the Tg2576 line expressing APP Swedish mutant and the M146L 6.1 line expressing presenilin-1 mutant were anesthetized and transcardially perfused with 4% paraformaldehyde in PBS under the guidance of Institutional Animal Care and Use Committee at the University of Nebraska Medical Center. Fixed brains samples were cryoprotected in 20% sucrose in PBS and subjected to cryostat sectioning (Leica). Frozen brain sections (10 $\mu$m thickness) of aged APP/PS1 transgenic mice (three sections per dilution point) were stained with compound solutions for 30 min, and then washed successively with 75% aq. EtOH, 95% aq EtOH and xylene. Fluorescence imaging of the stained and washed brain sections were carried out using a DAPI filter (Chroma) and a Roper HQ CCD camera (original magnification: 400x) following a standard FSB staining protocol. Fluorescence images of three plaques per section were systematically captured using the same image acquisition setting (laser power, capturing time, photomultiplier setting) to obtain comparable fluorescent intensities of five plaque regions (specific signal) and five background regions (noise signal) to calculate signal-to-noise (S/N) ratios.

**Fluorescence Staining of AD and Control Brain Sections by 4, 8 and 10**

Human adult AD brain sections and age-matched controls were stained with compounds 4, 8 and 10. The frozen brain sections (10 $\mu$m thickness) were stained with compound solutions (same concentrations and PBS:EtOH content as mouse brain section staining solutions) for 30 min and then washed successively with 75% aq. EtOH, 95% ethanol and xylene. Fluorescence imaging was performed as described in the previous section.

**In Vivo BBB Delivery of 4, 8 and 10 to A $\beta$ Plaque-Bearing PDAPP Mouse Brain**

Ten-month-old plate-derived growth factor-$\beta$ chain promoter driving (PDAPP) mice were injected intraperitoneally (IP) with vehicle only (10% DMSO/PBS) or with single 50 mg/kg doses IP of 4, 8, or 10, and sacrificed at 48 h after injection by transcardial perfusion with 4% paraformaldehyde (PFA) in PBS. After cryoprotection, fixed frozen brains were sectioned with 30 mm thickness, postfixed with 4% PFA for 5 min., washed in PBS, and mounted on microscope slides with coverslips using Vectorshield mounting medium (Vector Laboratories, Burlingame, CA). Fluorescence images were captured with a digital Olympus DP71 camera (Olympus, Center Valley, PA) connected to a TE-300 microscope (Nikon, Garden City, NJ).

**DPPH Free Radical Scavenging Assay**

Relative free radical quenching for 4–17, along with quercetin as a control, was assayed spectrophotometrically using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the procedure of Yang et al. with some modifications. Abstraction of a proton from a free radical scavenger (target compound) decreases the DPPH absorption at 517 nm. Reactions were run in a 96-well plate and the absorbance at 517 nm was recorded on a plate reader. All compounds were first solubilized in DMSO to form concentrated stock solutions ($\sim$15 mM) that were then diluted into working solutions in MeOH. Mixtures in the wells contained increasing concentrations of target compounds (0 nM-5 mM), 100 $\mu$M DPPH and MeOH (300 $\mu$L total volume). Each concentration was performed in triplicate and IC$_{50}$
values were determined using the sigmoidal doseresponse fit in Prism 4.0c software (Graphpad, Inc.)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Non-standard Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP/PS1</td>
<td>Amyloid Precursor Protein/Presenlin 1</td>
</tr>
<tr>
<td>PS2</td>
<td>Presenlin 2</td>
</tr>
<tr>
<td>PDAPP</td>
<td>Plate-Derived Growth Factor-β Chain Promoter Driving Amyloid Presursor Protein Transgenic Mice</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid Beta</td>
</tr>
<tr>
<td>NFT</td>
<td>Neurofibrillary Tangles</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-Brain Barrier</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure Activity Relationship</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl</td>
</tr>
<tr>
<td>FLINT</td>
<td>Fluorescence Intensity</td>
</tr>
</tbody>
</table>

References


13. Flaherty DP, Walsh SM, Kiyota T, Dong Y, Ikezu T, Vennerstrom JL. Polyfluorinated Bis-styrylbenzene β - Amyloid Plaque Binding Ligands. J Med Chem 2007;50:4986–4992. Compound 4 was prepared previously in our lab using a Horner-Wadsworth-Emmons coupling with 1,4-bis(diethyl phosphonylmethyl)benzene and p-anisaldehyde to form (E,E)-1,4-bis(4-methoxy)styrylbenzene which was deprotected with 1.0 M boron tribromide in DCM to yield 4. [PubMed: 17845017]


J Med Chem. Author manuscript; available in PMC 2011 November 25.

26. Yam CM, Kakkar AK. Molecular Self-Assembly of Dihydroxy-Terminated Molecules via Acid-Base Hydrolytic Chemistry of Silica Surfaces: Step-by-step Multilayered Thin Film Construction. Langmuir 1999;15:3807–3815. Compound 17 was prepared by this group via a Heck reaction between 1,4-diethynylbenzene and 4-iodophenol in 50% yield. Our attempts at synthesizing 17 using this method failed and we isolated only the mono-Heck product and an insoluble impurity.


J Med Chem. Author manuscript; available in PMC 2011 November 25.
Figure 1.
Target compounds 6–17.
Figure 2. Fluorescence imaging of in vitro mouse and human brain sections
Fluorescence imaging of Aβ plaque in (Aβ) aged APP/PS1 mouse and (B) human AD brain sections. Frozen brain sections (10 μm thickness) were stained with 50 μM of 4, 8 and 10 for 30 min in 9:1 PBS:EtOH, washed successively with 75% aq. EtOH, 95% aq. EtOH, and xylene. Fluorescence images were taken using a Nikon TE-2000, 60x Pan Fluor objective, and Roper HQ CCD camera (600x original magnification). A 360/460 (DAPI filter set) was used for imaging.
Figure 3. In vivo labeling of Aβ plaques via intravenous injection of 4, 8 or 10 in PDAPP mice
Ten-month-old PDAPP mice were injected intraperitoneally with vehicle only (A β), or single 50 mg/kg doses of 4 (B), 8 (C) or 10 (D) and sacrificed at 48 h after injection. Fluorescence images of the CA1 and dentate gyrus (DG) fields of the hippocampi were captured using 10x or 40x (insets) objectives on a Nikon TE-300 with a digital camera. Insets show representative fluorescent staining of A β plaques by 4, 8 or 10. Bar = 200 μm or 50 μm (insets).
Scheme I.
Synthesis of Bis-styrylbenzenes 4 and 6–10.a
Reagents and Conditions: (a) triethylphosphite, 140 °C, 4 h, (b) NaOCH₃, DMF, 80 °C, 1 h, (c) HCl, CHCl₃/MeOH (3:1), rt, 24 h.
Scheme 2.
Synthesis of Bis-styrylbenzenes 11–15.\textsuperscript{a}
\textsuperscript{a}Reagents and conditions (a) Pd(OAc)$_2$, phenylurea, K$_2$CO$_3$, DMF, 110 °C, 22 h (b) see b from Scheme 1, (c) see c from Scheme 1.
Table 1

Amyloid Peptide Aβ1-40 Binding Affinity ($K_d$) and Aβ Plaque Binding Specificity (S/N Ratio) to APP/PS1 Transgenic Mouse Brain Slices for 4–17.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_d$ (μM)</th>
<th>S/N ratio$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.0095 ± 0.0003$^c$</td>
<td>14 ± 1.1</td>
</tr>
<tr>
<td>5</td>
<td>0.50 ± 0.04</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>2.5 ± 0.1</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>6.3 ± 0.1</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>8</td>
<td>0.0053 ± 0.0003</td>
<td>9.6 ± 3.0</td>
</tr>
<tr>
<td>9</td>
<td>0.80 ± 0.02</td>
<td>11 ± 2.8</td>
</tr>
<tr>
<td>10</td>
<td>0.16 ± 0.01</td>
<td>9.6 ± 1.8</td>
</tr>
<tr>
<td>11</td>
<td>0.16 ± 0.01</td>
<td>8.1 ± 2.3</td>
</tr>
<tr>
<td>12</td>
<td>3.5 ± 0.2</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>13</td>
<td>0.26 ± 0.02</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>14</td>
<td>0.19 ± 0.01</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>15</td>
<td>0.20 ± 0.02</td>
<td>5.6 ± 1.3</td>
</tr>
<tr>
<td>16</td>
<td>9.2 ± 0.2</td>
<td>5.2 ± 1.2</td>
</tr>
<tr>
<td>17</td>
<td>0.054 ± 0.005</td>
<td>5.9 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$Values represent the average ± SD of three determinations.

$^b$Values represent the average ± SD of ten determinations.

$^c$Reported $K_d = 11 ± 2$ nM and S/N = 17 ± 1.31$^3$
Table 2
Free Radical Scavenging Activity measured by DPPH Assay for Quercetin, and 4–17.

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH IC\textsubscript{50} ((\mu\text{M}))\textsuperscript{a}</th>
</tr>
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<tr>
<td>Quercetin</td>
<td>1.3 ± 1.1\textsuperscript{b}</td>
</tr>
<tr>
<td>4</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>5</td>
<td>62 ± 1.4\textsuperscript{c}</td>
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<tr>
<td>6</td>
<td>3.4 ± 1.1</td>
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<td>7</td>
<td>4.0 ± 1.1</td>
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<td>8</td>
<td>&gt; 100</td>
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<td>4.0 ± 1.1</td>
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<td>1.8 ± 1.2</td>
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<td>17</td>
<td>45 ± 1.0</td>
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</table>

\textsuperscript{a}Values represent the average ± SD of three determinations.

\textsuperscript{b}Reported IC\textsubscript{50} = 9.1 \(\mu\text{M}\)\textsuperscript{36}

\textsuperscript{c}Reported IC\textsubscript{50} = 74 ± 5.3 \(\mu\text{M}\)\textsuperscript{44}