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Synthesis and evaluation of stilbene derivatives as a potential imaging agent of amyloid plaques

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ABSTRACT

Fluorescence probes that can detect Aβ (β-amyloid peptide) plaque are important tools for diagnosis of Alzheimer's disease (AD), and 4-*N*-methylamino-4′-hydroxystilbene (SB-13) is one of the promising candidate molecules. We report here the synthesis of SB-13 derivatives that consist of various electron donating/withdrawing moieties and distinct size of N-substituents. The synthesized compounds were screened for detection of Aβ40 fibrils in vitro. Four compounds exhibited more than sixfold intensity increase, and they were further analyzed for detail bindings and Aβ plaque imaging. Among these molecules, compound **42** meets two critical requirements for imaging agent; high fluorescence responsiveness and strong binding affinity. This compound showed more than 25-fold increase with the dissociation constant of 1.13 ± 0.37 μM. In AD mouse brain tissue, **42** selectively stained Aβ plaque, more specifically peripheral regions of Aβ plaque. This finding demonstrated its potential use as brain-imaging agents for AD studies.

1. Introduction

Alzheimer's disease (AD) is a common neurological disease of chronic dementia, memory loss, and cognitive impairment. Central to the neuropathology of AD is the deposition of polymeric peptide/protein deposits, composed of β-amyloid peptide (Aβ) and tau protein.[1] The fibrillar aggregates of amyloid peptides, Aβ40 and Aβ42, are major metabolic peptides derived from amyloid precursor protein found in senile plaques and cerebrovascular amyloid deposits in AD patients.[2] For these reasons, an effective detection of Aβ plaques will be an important tool for AD diagnosis.[3,4] The Aβ imaging probes have been provided a way for monitoring Aβ plaque burden following the disease progression. Several ^{11}C-, ^{18}F- and ^{123}I-labeled tracers, such as benzothiazole derivative, 6-OH-BTA-1 ([*N*-methyl-]2-(4′-methylaminophenyl)-6-hydroxybenzothiazole, ^{11}C), and stilbene derivative, SB-13 (4-*N*-methylamino-4′-hydroxystilbene, ^{11}C) FDDNP (2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)amino]2-naphthyl}ethylidene)malononitrile, ^{18}F) have been reported for positron emission tomography (PET) imaging of Aβ plaques in AD patients. Also, IMPY (6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2-a]pyridine) and TZDM (2-[4′-dimethylaminophenyl]-6-iodobenzothiazole) for radio-iodinated probes have been reported for single-photon emission computed tomography (SPECT) imaging.[5–14] Among them, stilbene derivatives showed a broad range of biological responses such as anti-leukemic, anti-bacterial, anti-fungal, anti-platelet aggregation, coronary vasodilator activities and anti-cancer activities.[15] SB-13 is one of the promising lead compound for Aβ plaque detection, however, its application has been limited in PET imaging due to its strong binding affinity to amyloid aggregates and low fluorescence responses.[7c,16] In this report, we describes systematic modification of SB-13 and their biological assays to discover a fluorescence turn-on sensor using Aβ40 fibrils and AD mouse brain sections (Fig. 1).

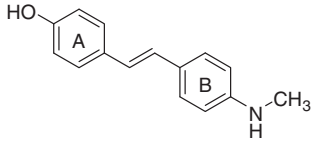

Figure 1. Structure of the lead compound SB-13.

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2. Results and discussion

2.1. Chemical synthesis

The stilbene derivatives (**13–44**) were prepared by a Wadsworth–Emmons reaction with preference of *trans*-stilbenes as products (Scheme 1).[17] Various diethyl phosphates derivatives (**1–12**) were prepared in high yield by the condensation of bromo benzyl derivatives and triethyl phosphite at 80 °C for 17 h. *N*,*N*-Dimethyl stilbene derivatives (**13–24**) were prepared conjugation of diethyl phosphates derivatives (**1–12**) and 4-(dimethylamino)benzaldehyde using sodium hydride, giving the desired products with 8–95% yields.

Other stilbene derivatives (**25–41**) were also prepared in a similar manner by conjugation of diethyl phosphates derivatives (**1–12**) with 4-(*N*-methyl-*N*-2-hydroxyethyl) benzaldehyde or 4-(*N*-methyl -*N*-2-cyanoethyl) benzaldehyde giving the products with 20–48% yields. While most of the products were obtained as expected structures, we observed hydrolyzed products from three reactions with 4-(*N*-methyl-*N*-2-cyanoethyl) benzaldehyde (**42**–**44**). Among 32 stilbene derivatives, 22 compounds are newly reported in Table 1).

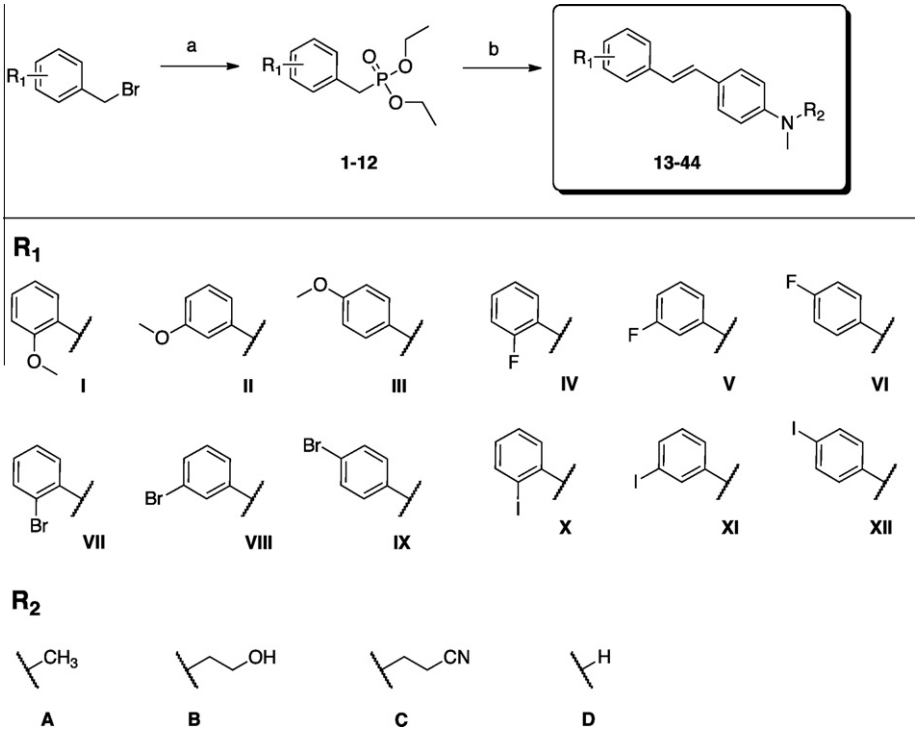
Table 1
Decoding table of compounds; *marked stilbene compounds are reported here first time

Compound	R_1	R_2	Compound	R_1	R_2
1	I		**23**	XI	A
2	II		**24**	XII	A
3	III		**25***	X	B
4	IV		**26***	X	C
5	V		**27***	XI	B
6	VI		**28***	XI	C
7	VII		**29***	XII	B
8	VIII		**30***	XII	C
9	IX		**31***	VII	B
10	X		**32***	VII	C
11	XI		**33***	VIII	B
12	XII		**34***	VIII	C
13*	I	A	**35***	IX	B
14	II	A	**36***	IX	C
15	III	A	**37***	I	B
16	IV	A	**38***	III	B
17*	V	A	**39***	IV	B
18	VI	A	**40***	IV	C
19*	VII	A	**41***	VI	B
20	VIII	A	**42***	IX	D
21	IX	A	**43***	I	D
22	X	A	**44**	II	D

2.2. Screening against Aβ plaques and AD mouse brain

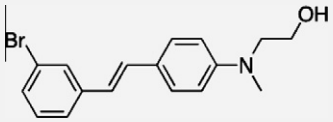
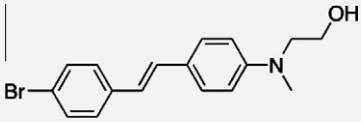
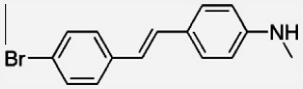
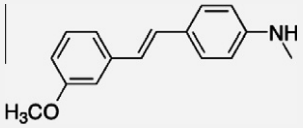
The synthesized 32 stilbene derivatives were tested with synthetic Aβ40 aggregates for their fluorescence response. Among tested 32 compounds, four compounds showed over sixfold increase at 30 μM concentrations of Aβ40 fibril and further tested for their binding constants against Aβ40. The spectroscopic properties of selected compounds were summarized in Table 2. Promisingly, one of the compounds, compound **42**, meets two critical requirements for fluorescence imaging probe; high fluorescence responsiveness ($F_{A\beta}/F_0$ >25-fold) and strong binding affinity (1.13 ± 0.37 μM). Compared with the conventional Aβ probes, compound **42** exhibited stronger bindings than ThT (K_D = 2.3 μM, $F_{A\beta}/F_0$ >111) and higher fluorescence emission increment than BTA-1 ($F_{A\beta}/F_0$ = 4.1, K_D = 0.30 μM) in our measurement.

Encouraged by these outstanding properties, we further tested the applicability of compound **42** in the fluorescence tissue imaging using AD mouse brain sections. As compound **42** displays blue fluorescence, ThT (green) was used for counter staining of amyloid plaques. Compound **42** specifically stained at Aβ plaque sites and showed good colocalization with ThT-labeling in 98 plaques assayed in two brain sections (Fig. 2C). It is also noteworthy that compound **42** stains slightly broader regions than ThT and it is better imaging



Scheme 1. Reaction scheme for synthesis of stilbene derivatives. Reagents and conditions: (a) triethylphosphite, 80 °C, 17 h; (b) NaH, benzaldehyde (4-(dimethylamino)benzaldehyde, 4-(*N*-methyl-*N*-2-hydroxyethyl) benzaldehyde, or 4-(*N*-methyl-*N*-2-cyanoethyl) benzaldehyde), THF, 80 °C, 5 h.

Table 2
Structure and properties of primary hit compounds from in vitro screening

Code	Structure	λ_{ex} (nm)	λ_{em} (nm)	Fold ($F_{A\beta}/F_0$)[a]	K_D (mean ± SD) (μm)[b]
33		350	450	8.91	2.89 ± 2.42
35		350	440	15.7	1.84 ± 0.95
42		350	440	25.8	1.13 ± 0.37
44		350	440	9.30	17.9 ± 7.66

[a] Fold changes values were calculated using fluorescence emission intensity at λ_{em} of each dye (10 μM dye and 30 μM amyloid fibril were used for fold change measurement).
[b] A non-linear regression analysis was performed in GraphPad Prism fitting a one-site binding model to the binding data (5 replication experiments, $N = 5$).

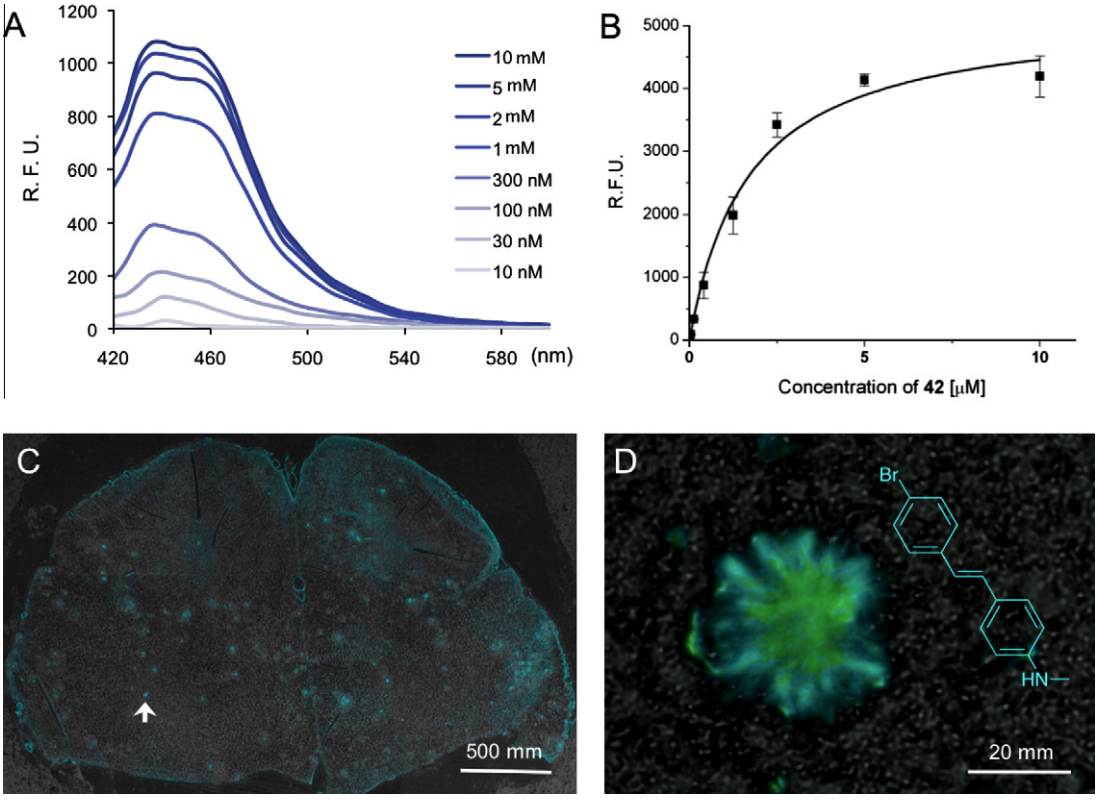


Figure 2. A fluorescent imaging probe for Aβ plaque. (A) Fluorescence spectra of compound **42** with Aβ40 fibril. (B) Non-linear regression analysis at emission maxima (440 nm). The apparent dissociation constant, K_D, was determined to be 1.13 ± 0.37 μM. (C) Labeling of amyloid deposits in a AD mouse brain section with compound **42** (cyanine) and (D) A magnified image of an amyloid deposit labeled with compound **42** (cyanine) and ThT (green).

agent than ThT for peripheral regions of Aβ plaque (Fig. 2D). Therefore, we believe it would be a useful imaging tool for AD study.

3. Conclusions

We synthesized 32 stilbene derivatives of the Aβ plaque ligand, SB13, and identified compound **42**, which exhibited a strong fluorescence response (over 25-fold) and binding affinity (1.13 μM) to Aβ40 aggregates. Compound **42** demonstrated its excellent capability of imaging Aβ fibrils in AD mouse brain with good colocalization with ThT. Based on their delicately high binding affinity to Aβ40 aggregates, this novel stilbene analogue can be a good alternative candidate for a fluorescence imaging agent to study Alzheimer's disease.

4. Experimental

4.1. Chemistry

4.1.1. General

Solvents and starting materials for synthesis were purchased from Aldrich and used as received without purification. ^{1}H NMR spectra were recorded on Bruker Advance (^{1}H NMR: 300 MHz) spectrometer, and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Mass spectra were recorded on a JEOL, JMS-AX505WA and melting points were determined on a Barnstead International 1002D.

4.1.2. Preparation of diethylphosphonomethyl-benzene (1–12)

Benzyl bromide derivatives (3.35 mmol) were added to triethylphosphite (2.57 mmol) in the 50 mL round bottom flask at room temperature. The reaction mixture was heated to 80 °C for 17 h. After cooling to room temperature, the resulting crude product was fractional distillated to remove triethylphosphite in vacuo. Purification by silica gel column chromatography (70% EtOAc/hexane) gave a product as colorless oil.

4.1.2.1. 2-Methoxy-1-diethylphosphonomethyl-benzene (1). Yield: 80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (m, 1H), 7.22 (m, 1H), 6.89 (m, 2H), 4.02 (m, 4H), 3.83 (s, 3H), 3.25 (d, J = 21.7 Hz, 2H), 1.23 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 157.15, 131.19, 128.12, 120.51, 120.19 (J = 7.7 Hz), 110.50, 61.86 (J = 6.4 Hz), 55.43, 26.61 (J = 137.9 Hz), 16.30 (J = 5.6 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{12}H_{19}H_4P$: 259.1099. Found: 259.1098 (−0.6 ppm deviation).

4.1.2.2. 3-Methoxy-1-diethylphosphonomethyl-benzene (2). Yield: 95%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (m, 1H), 6.87 (m, 2H), 6.79 (m, 1H), 4.01 (m, 4H), 3.80 (s, 3H), 3.13 (d, J = 21.6 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.51 (J = 2.8 Hz), 132.90 (J = 8.9 Hz), 129.34 (J = 2.6 Hz), 122.05 (J = 6.6 Hz), 115.18 (J = 6.4 Hz), 112.40 (J = 3.3 Hz), 62.00 (J = 6.7 Hz), 55.05, 33.69 (J = 137.3 Hz), 16.26 (J = 6.0 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{12}H_{19}H_4P$: 259.1099. Found: 259.1097 (−0.9 ppm deviation).

4.1.2.3. 4-Methoxy-1-diethylphosphonomethyl-benzene (3). Yield: 94%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (d, J = 2.4 Hz, 1H), 7.19 (d, J = 2.4 Hz, 1H), 6.84 (d, J = 8.1 Hz, 2H), 4.00 (m, 4H), 3,78 (s, 3H), 3.08 (d, J = 21.0 Hz, 2H), 1.24 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 158.52 (J = 3.3 Hz), 130.71 (J = 6.5 Hz), 123.40 (J = 9.2 Hz), 113.96 (J = 2.6 Hz), 62.03 (J = 6.8 Hz), 55.21, 32.74 (J = 138.2 Hz), 16.37 (J = 5.9 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{12}H_{19}H_4P$: 259.1099. Found: 259.1096 (−1.3 ppm deviation).

4.1.2.4. 2-Fluoro-1-diethylphosphonomethyl-benzene (4). Yield: 92%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 1H), 7.22 (m, 1H), 7.07 (m, 2H), 4.05 (m, 4H), 3.20 (d, J = 21.1 Hz, 2H), 1.25 (t, 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 160.82 (J = 244.7 Hz), 131.81 (J = 4.4 Hz), 128.67, 124.11 (J = 3.2 Hz), 119.11, 115.31, 62.18 (J = 6.6 Hz), 27.18 (J = 139.7 Hz), 16.29 (J = 6.1 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}FO_3P$: 247.0899. Found: 247.0903 (+1.4 ppm deviation).

4.1.2.5. 3-Fluoro-1-diethylphosphonomethyl-benzene (5). Yield: 96%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (m, 1H), 7.03 (m, 3H), 4.03 (m, 4H), 3.13 (d, J = 21.6 Hz, 2H), 1.25 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 162.71 (J = 244.6 Hz), 134.03, 129.92 (J = 8.1 Hz), 125.46 (J = 3.9 Hz), 116.70, 113.86 (J = 20.9 Hz), 62.20 (J = 6.7 Hz), 33.54 (J = 137.9 Hz), 16.32 (J = 5.9 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}FO_3P$: 247.0899. Found: 247.0900 (+0.1 ppm deviation).

4.1.2.6. 4-Fluoro-1-diethylphosphonomethyl-benzene (6). Yield: 98%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05 (m, 2H), 6.77 (m, 2H), 3.79 (m, 4H), 2.89 (d, J = 21.3 Hz, 2H), 1.01 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 161.58 (J = 243.8 Hz), 130.56, 126.76, 114.54, 61.29 (J = 6.7 Hz), 32.46 (J = 138.2 Hz), 15.42; HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}FO_3P$: 247.0899. Found: 247.0900 (+0.1 ppm deviation).

4.1.2.7. 2-Bromo-1-diethylphosphonomethyl-benzene (7). Yield: 93%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, J = 8.0 Hz, 1H), 7.46 (m, 1H), 7.27 (m, 1H), 7. 11 (m, 1H), 4.05 (m, 4H), 3.41 (d, J = 21.9 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 132.89 (J = 2.9 Hz), 131.84 (J = 8.9 Hz), 131.62 (J = 5.0 Hz), 128.45 (J = 3.5 Hz), 127.43 (J = 3.3 Hz), 124.85 (J = 8.8 Hz), 62.20 (J = 6.6 Hz), 33.39 (J = 138.1 Hz), 16.30 (J = 6.0 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}BrO_3P$: 307.0099. Found: 307.0100 (+0.5 ppm deviation).

4.1.2.8. 3-Bromo-1-diethylphosphonomethyl-benzene (8). Yield: 93%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 1H), 7.39 (m, 1H), 7.23 (m, 1H), 7.19 (m, 1H), 4.03 (m, 4H), 3.11 (d, J = 21.7 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 133.98 (J = 8.8 Hz), 132.68 (J = 6.7 Hz), 129.98 (J = 3.2 Hz), 128.39 (J = 3.9 Hz), 122.39 (J = 3.5 Hz), 62.22 (J = 6.7 Hz), 33.38 (J = 137.6 Hz), 16.33 (J = 5.9 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}BrO_3P$: 307.0099. Found: 307.0108 (+0.3 ppm deviation).

4.1.2.9. 4-Bromo-1-diethylphosphonomethyl-benzene (9). Yield: 93%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, J = 7.9 Hz, 2H), 7.04 (dd, J = 8.3 Hz, 2.5 Hz, 2H), 4.0 (m, 4H), 3.08 (d, J = 21.7 Hz, 2H), 1.25 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 137.58 (J = 2.9 Hz), 131.69 (J = 6.5 Hz), 131.41 (J = 9.1 Hz), 92.31 (J = 4.7 Hz), 62.20 (J = 6.7 Hz), 33.35 (J = 137.6 Hz), 16.36 (J = 5.9 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}BrO_3P$: 307.0099. Found: 307.0090 (−2.9 ppm deviation).

4.1.2.10. 2-Iodo-1-diethylphosphonomethyl-benzene (10). Yield: 92%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, J = 8.0 Hz, 1H), 7.47 (m, 1H), 7.30 (m, 1H), 6.92 (m, 1H), 4.05 (m, 4H), 3.41 (d, J = 22.0 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 139.39 (J = 2.9), 135.10 (J = 8.7 Hz), 130.41 (J = 5.0 Hz), 128.31 (J = 3.5 Hz), 128.06 (J = 3.2 Hz), 101.04 (J = 9.3 Hz), 61.99 (J = 6.75 Hz), 38.08 (J = 137.5 Hz), 16.12 (J = 6.0 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}IO_3P$: 354.9960. Found: 354.9965 (+1.4 ppm deviation).

4.1.2.11. 3-Iodo-1-diethylphosphonomethyl-benzene (11). Yield: 91%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (m, 1H), 7.59 (m, 1H), 7.29 (m, 1H), 7.05 (t, J = 7.8 Hz, 1H), 4.03 (m, 4H), 3.08 (d, J = 21.7 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 137.99 (J = 6.6 Hz, 135.32 (J = 3.6 Hz), 133.57 (J = 9.1 Hz), 129.62 (J = 3.0 Hz), 128.51 (J = 6.3 Hz), 93.73 (J = 3.6 Hz), 61.63 (J = 6.7 Hz), 32.62 (J = 137.3 Hz), 15.86; HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}IO_3P$: 354.9960. Found: 354.9970 (+2.8 ppm deviation).

4.1.2.12. 4-Iodo-1-diethylphosphonomethyl-benzene (12). Yield: 98%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 8.1 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 4.01 (m, 4H), 3.07 (d, J = 21.7 Hz, 2H), 1.24 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 137.20 (J = 2.6 Hz), 131.39 (J = 6.5 Hz), 131.10 (J = 9.2 Hz), 92.02 (J = 4.6 Hz), 61.82 (J = 6.6 Hz), 32.97 (J = 137.3 Hz), 16.09 (J = 5.9 Hz); HRMS (FAB$^+$, m-nitrobenzylalcohol): Calcd for $C_{11}H_{16}IO_3P$: 354.9960. Found: 354.9960 (+0.1 ppm deviation).

4.1.3. Preparation of styrylbenzene derivatives (13–44)

A sodium hydride (60 w/w, 4.70 mmol) was added to the mixture of diethylphosphonomethyl-benzene derivatives (**1–12**) and 4-(dimethylamino)benzaldehyde or 4-(*N*-methyl-*N*-2-hydroxyethyl)benzaldehyde or 4-(*N*-methyl-*N*-2-cyanoethyl)benzaldehyde in dry THF (30 mL). The reaction mixture was refluxed for 5 h and cooled to room temperature. The crude product was quenched by adding water (2 mL), followed by evaporation of solvent in vacuo. Purification by silica gel column chromatography (16% EtOAc/hexane) gave a product as yellow colored powder.

4.1.3.1. 4-*N*,*N*-Dimethyl-2′-methoxy-stilbene (13). Yield: 17%; mp 87.4–88.6 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, J = 7.2 Hz, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 16.6 Hz, 1H), 7.19 (t, J = 7.2 Hz, 1H), 7.05 (d, J = 16.4 Hz, 1H), 6.96 (d, J = 7.4 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.71 (d, J = 8.6 Hz, 2H), 3.88 (s, 3H), 2.95 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 156.53, 149.98, 129.22, 127.64, 127.59, 127.28, 126.53, 125.84, 120.71, 119.06, 112.44, 110.88, 55.52, 40.51; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{20}NO$: 253.1467. Found: 253.1466 (−0.3 ppm deviation).

4.1.3.2. 4-*N*,*N*-Dimethyl-3′-methoxy-stilbene (14). Yield: 95%; mp 90.2–91.3 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (m, 2H), 7.25 (m, 2H), 7.08 (m, 1H), 7.02 (s, 1H), 6.89 (d, J = 16.2 Hz, 1H), 6.75 (m, 3H), 3.85 (s, 3H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.82, 150.12, 139.63, 129.48, 129.09, 127.59, 125.57, 124.19, 118.76, 112.38, 111.13, 55.19, 40.43; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{20}NO$: 253.1467. Found: 253.1465 (−0.7 ppm deviation).

4.1.3.3. 4-*N*,*N*-Dimethyl-4′-stilbene (15). Yield: 20%; mp 171.9–173.4 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (t, J = 8.1 Hz, 4H), 6.89 (m, 4H), 6.72 (d, J = 8.6 Hz, 2H), 3.82 (s, 3H), 2.94 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 158.66, 149.92, 131.05, 127.27, 127.15, 126.82, 126.22, 124.06, 114.07, 112.57, 55.33, 40.55; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{20}NO$: 253.1467. Found: 253.1461 (−2.2 ppm deviation).

4.1.3.4. 4-*N*,*N*-Dimethyl-2′-fluoro-stilbene (16). Yield: 57%; mp 122.8–123.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59 (m, 1H), 7.43 (m, 2H), 7.14 (m, 1H), 7.09 (m, 3H), 7.03 (m, 1H), 6.72 (d, J = 9.0 Hz, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 160.15 (J = 246.8 Hz), 150.32, 130.94 (J = 4.4 Hz), 127.75, 127.59, 126.47 (J = 3.8 Hz), 126.00 (J = 11.9 Hz), 125.58, 124.08 (J = 3.4 Hz), 116.32 (J = 3.8 Hz), 115.62 (J = 22.1 Hz), 112.33, 40.41; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}FN$: 241.1267. Found: 241.1264 (−1.3 ppm deviation).

4.1.3.5. 4-*N*,*N*-Dimethyl-3′-fluro-stilbene (17). Yield: 49%; mp 148.9–149.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (d, J = 8.8 Hz, 2H), 7.30 (m, 1H), 7.21 (m, 2H), 7.05 (d, J = 16.3 Hz, 1H), 6.89 (m, 2H), 6.72 (d, J = 8.7 Hz, 2H), 3.00 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 163.22 (J = 242.9 Hz), 150.32, 140.63 (J = 7.8 Hz), 130.11, 129.89 (J = 8.5 Hz), 127.75, 125.12, 123.03 (J = 2.4 Hz), 121.89 (J = 2.1 Hz), 113.30 (J = 21.4 Hz), 112.34, 112.02, 40.38; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}FN$: 241.1267. Found: 241.1268 (+0.7 ppm deviation).

4.1.3.6. 4-*N*,*N*-Dimethyl-4′-fluoro-stilbene (18). Yield: 17%; mp 198.6–199.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (m, 4H), 7.02 (t, J = 8.9 Hz, 2H), 6.91 (d, J = 12.6 Hz, 2H), 6.72 (d, J = 8.7 Hz, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 161.81 (J = 244.2 Hz), 150.15, 134.36 (J = 3.1 Hz), 128.58, 127.51, 127.36 (J = 7.8 Hz), 125.59, 123.18, 115.46 (J = 21.5 Hz), 112.45, 40.48; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}FN$: 241.1267. Found: 241.1261 (−2.2 ppm deviation).

4.1.3.7. 4-*N*,*N*-Dimethyl-2′-bromo-stilbene (19). Yield: 17%; mp 107.5–108.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (dd, J = 1.5 Hz, 7.9 Hz, 1H), 7.56 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.30 (m, 1H), 7.24 (s, 1H), 7.05 (dt, J = 1.6 Hz, 7.5 Hz, 1H), 6.99 (d, J = 16.1 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.37, 137.82, 132.96, 131.50, 127.95, 127.81, 127.41, 126.16, 125.37, 123.70, 122.92, 112.32, 40.41; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}BrN$: 301.0466. Found: 301.0473 (+2.3 ppm deviation).

4.1.3.8. 4-*N*,*N*-Dimethyl-3′-bromo-stilbene (20). Yield: 27%; mp 149.9–150.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (t, J = 7.4 Hz, 1H), 7.41 (m, 2H), 7.31 (m, 2H), 7.18 (t, J = 7.8 Hz, 1H), 7.04 (d, J = 16.2 Hz, 1H), 6.81 (d, J = 16.2 Hz, 1H), 6.71 (m, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.29, 140.41, 130.22, 130,01, 129,31, 128.63, 127.76, 125.02, 124.60, 122.78, 122.55, 112.30, 40.37; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}BrN$: 301.0466. Found: 301.0467 (+0.2 ppm deviation).

4.1.3.9. 4-*N*,*N*-Dimethyl-4′-bromo-stilbene (21). Yield: 11%; mp 234.5–235.6 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 1H), 7.42 (m, 2H), 7.39 (m, 1H), 7.34 (m, 1H), 7.31 (m, 1H), 7.03 (d, J = 16.3 Hz, 1H), 6.83 (d, J = 16.3 Hz, 1H), 6.71 (d, J = 8.7 Hz, 2H), 3.00 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.22, 137.13, 131.59, 129.51, 127.65, 127.44, 125.26, 122.94, 120.08, 112.36, 40.41; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}BrN$: 301.0466. Found: 301.0467 (+0.2 ppm deviation).

4.1.3.10. 4-*N*,*N*-Dimethyl-2′-iodo-stilbene (22). Yield: 23%; mp 85.6–86.4 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (dd, J = 1.1 Hz, 8.0 Hz, 1H), 7.60 (dd, J = 1.5 Hz, 7.8 Hz, 1H), 7.45 (m, 2H), 7.31 (m, 1H), 7.11 (d, J = 15.9 Hz, 1 Hz), 6.90 (m, 2H), 67.3 (m, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.38, 140.99, 139.53, 131.71, 128.29, 128.07, 127.95, 125.72, 125.30, 112.34, 100.22, 40.41; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}IN$: 349.0327. Found: 349.0333 (+1.6 ppm deviation).

4.1.3.11. 4-*N*,*N*-Dimethyl-3′-iodo-stilbene (23). Yield: 32%; mp 161.6–162.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (m, 1H), 7.51 (m, 1H), 7.40 (m, 3H), 7.06 (d, J = 7.8 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 6.78 (d, J = 16.2 Hz, 1H), 6.71 (m, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.27, 140.49, 135.27, 134.68, 130.17, 130.10, 127.74, 125.17, 125.04, 122.41, 112.29, 94.80, 40.37; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}IN$: 349.0327. Found: 349.0328 (+0.1 ppm deviation).

4.1.3.12. 4-*N*,*N*-Dimethyl-4′-iodo-stilbene (24). Yield: 8%; mp 251.6–252.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (m, 2H), 7.40 (m, 2H), 7.21 (m, 2H), 7.03 (d, J = 16.3 Hz, 1H), 6.81 (d, J = 16.3 Hz, 1H), 6.71 (m, 2H), 2.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.27, 137.74, 137.55, 129.62, 127.73, 127.69, 125.20, 123.00, 112.35, 91.35, 40.40; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{16}H_{16}IN$: 349.0327. Found: 349.0334 (+2.0 ppm deviation).

4.1.3.13. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-2′-iodo-stilbene (25). Yield: 40%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (m, 1H), 7.83 (m,1H), 7.46 (d, 2H), 7.34 (m,2H), 7.15 (d, J = 16.3 Hz, 1H), 6.90 (m, 2H), 6.76 (m, 2H), 3.83 (m, 2H), 3.51 (m, 2H), 3.01 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.75, 140.91, 139.58, 128.43, 128.36, 128.22, 128.09, 125. 89, 125.80, 112.71, 100.30, 60.23, 55.07, 38.92; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}INO$: 379.0433. Found: 349.0438 (+1.3 ppm deviation).

4.1.3.14. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-2′-iodo-stilbene (26). Yield: 42%; mp 66.5–67.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86

(m, 1H), 7.60 (m, 1H), 7.44 (m, 2H), 7.39 (d, 2H), 7.33 (t, 1H), 7.18 (d, J = 15.9 Hz, 1H), 6.92 (m, 2H), 6.71 (d, 2H), 3.72 (t, 2H), 3.06 (s, 3H), 2.57 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.44, 140.75, 139.62, 131.30, 128.96, 128.44, 128.34, 126.55, 125.89, 118.38, 112.50, 100.38, 48.81, 38.80, 15.38; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}IN_2$: 388.0436. Found: 388.0443 (+1.6 ppm deviation).

4.1.3.15. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-3′-iodo-stilbene (27). Yield: 37%; mp 108.5–109.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (m, 1H), 7.52 (d, 1H), 7.40 (m, 3H), 7.03 (m, 2H), 6.78 (m, 3H), 3.83 (t, 2H), 3.51 (t, 2H), 3.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.73, 140.43, 135.44, 134.78, 130.22, 129.94, 127.87, 125.71, 125.26, 122.84, 112.69, 94.81, 60.23, 55.06, 38.85; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}INO$: 379.0433. Found: 379.0437 (+0.9 ppm deviation).

4.1.3.16. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-3′-iodo-stilbene (28). Yield: 34%; mp 94.6–96.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (m, 1H), 7.71 (m, 1H), 7.42 (m, 4H), 7.07 (m, 2H), 6.82 (d, 1H), 6.67 (m, 3H) 3.73 (t, 2H), 3.06 (s, 3H), 2.58 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.38, 140.25, 135.62, 132.31, 130.28, 129.68, 128.12, 126.42, 125.36, 123.44, 112.48, 94.85, 48.81, 38.77, 15.39; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}IN_2$: 388.0436. Found: 388.0432 (−1.1 ppm deviation).

4.1.3.17. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-4′-iodo-stilbene (29). Yield: 31%; mp 201.2–202.8 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (m, 2H), 7.40 (m, 2H), 7.21 (m, 2H), 7.05 (d, J = 16.2 Hz, 1H), 6.78 (m, 3H), 3.84 (t, 2H), 3.51 (t, 2H), 3.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.69, 137.64, 137.59, 129.41, 127.80, 127.78, 125.85, 123.40, 112.74, 91.51, 60.25, 55.10, 38.84; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}INO$: 379.0433. Found: 379.0435 (+0.5 ppm deviation).

4.1.3.18. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-4′-iodo-stilbene (30). Yield: 28%; mp 194.8–196.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (m, 2H), 7.43 (m, 2H), 7.25 (m, 2H), 7.05 (d, J = 16.3 Hz, 1H), 7.00 (d, 1H), 6.86 (d, 1H), 6.70 (m, 2H), 3.77 (t, 2H), 3.06 (s, 3H), 2.59 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.28, 137.63, 137.46, 129.13, 128.03, 127.84, 126.60, 124.04, 118.25, 112.50, 91.75, 48.85, 38,78, 15.36; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}IN_2$: 388.0436. Found: 388.0445 (+2.2 ppm deviation).

4.1.3.19. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-2′-bromo-stilbene (31). Yield: 42%; mp 87.9–89.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (m, 1H), 7.58 (m, 1H), 7.46 (m, 2H), 7.28 (m, 2H), 7.06 (m, 2H), 6.78 (m, 2H), 3.82 (t, 2H), 3.50 (t, 2H), 3.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.76, 137.77, 133.02, 131.37, 128.11, 127.95, 127.49, 126.25, 123.78, 123.28, 112.68, 60.21, 55.04, 38.90; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}BrNO$: 331.0572. Found: 331.0573 (+0.5 ppm deviation).

4.1.3.20. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-2′-bromo-stilbene (32). Yield: 38%; mp 73.7–74.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.66 (m, 1H), 7.63 (m, 1H), 7.49 (m, 2H), 7.28 (m, 2H), 7.09 (m,1H), 6.99 (d, J = 16.3 Hz 1H), 6.71 (m, 2H), 3.75 (t, 2H), 3.07 (s, 3H), 2.59 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.70, 137.77, 133.03, 127.19, 125.21, 122.48, 122.28, 121.65, 120.47, 118.15, 117.99, 106.66, 43.02, 32.95, 9.5; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}BrN_2$: 340.0575. Found: 340.0571 (−1.3 ppm deviation).

4.1.3.21. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-3′-bromo-stilbene (33). Yield: 36%; mp 104.9–105.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (m, 1H), 7.61 (m, 4H), 7.29 (m, 1H), 7.84 (d, J = 16.3 Hz, 1H), 6.78 (m, 3H), 3.83 (t, 2H), 3.52 (t, 2H), 3.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.76, 140.35, 130.05, 129.47, 128.73, 127.88, 125.71, 124.68, 122.99, 122.84, 112.71, 60.26, 55.08, 38.85; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}BrNO$: 331.0572. Found: 331.0570 (−0.5 ppm deviation).

4.1.3.22. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-3′-bromo-stilbene (34). Yield: 43%; mp 107.7–108.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (m, 1H), 7.38 (m, 4H), 7.05 (d, 1H), 6.86 (d, J = 16.3 Hz, 1H), 6.70 (m, 2H), 3.74 (t, 2H), 3.06 (s, 3H), 2.59 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.39, 130.10, 129.79, 129.65, 128.80, 128.13, 126.43, 124.77, 123.59, 112.48, 48.82, 38.77, 15.38; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}BrN_2$: 340.0571. Found: 340.0578 (+0.9 ppm deviation).

4.1.3.23. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-4′-bromo-stilbene (35). Yield: 36%; mp 183.4–184.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (m, 6H), 6.99 (d, 1H), 6.78 (m, 3H), 3.84 (m, 2H), 3.53 (t, 2H), 3.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.67, 137.05, 131.64, 129.31, 127.50, 125.91, 123.34, 120.24, 112.75, 60.25, 55.11, 38.84; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}BrNO$: 331.0572. Found: 331.0568 (−1.1 ppm deviation).

4.1.3.24. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-4′-bromo-stilbene (36). Yield: 39%; mp 190.4–192.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43 (m, 6H), 7.04 (d, J = 16.3 Hz, 1H), 6.87 (d, J = 16.3 Hz, 1H), 6.70 (m, 2H), 3.74 (t, 2H), 3.06 (2, 3H), 2.59 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.27, 136.88, 131.68, 129.04, 128.00, 127.57, 126.64, 123.96, 120.46, 118.26, 112.52, 48.85, 38.77, 15.36; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}BrN_2$: 340.0571. Found: 340.0575 (−0.1 ppm deviation).

4.1.3.25. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-2′-methoxy-stilbene (37). Yield: 30%; mp 78.7–79.8 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (m, 1H), 7.44 (m, 2H), 7.23 (m, 2H), 6.96 (m, 3H), 6.78 (m, 2H), 3.83 (m, 5H), 3.50 (t, 2H), 3.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 156.58, 149.39, 129.01, 127.78, 127.70, 127.17, 125.91, 120.73, 119.49, 112.88, 110.91, 60.23, 55.54, 55.28, 38.86; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{21}NO_2$: 283.1572. Found: 283.1567 (−1.9 ppm deviation).

4.1.3.26. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-4′-methoxy-stilbene (38). Yield: 36%; mp 153.4–154.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (m, 4H), 6.82 (m, 6H), 3.82 (m, 5H), 3.56 (t, 2H), 2.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 158.72, 149.30, 130.90, 127.35, 127.18, 126.85, 126.56, 124.42, 114.07, 112.97, 60.23, 55.32, 55.29, 38.84; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{21}NO_2$: 283.1572. Found: 283.1579 (+2.5 ppm deviation).

4.1.3.27. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-2′-fluoro-stilbene (39). Yield: 48%; mp 95.2–96.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (m, 1H), 7.43 (m, 2H), 7.10 (m, 5H), 6.80 (m, 2H), 3.82 (m, 2H), 3.51 (t, 2H), 3.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 158.52 (J = 987.9 Hz), 149.70, 130.72 (J = 17.7 Hz), 127.87, 127.84 (J = 34.8 Hz), 126.51 (J = 80.7 Hz), 125.85 (J = 48.3 Hz), 124.06 (J = 13.8 Hz), 116.75 (J = 15.3 Hz), 115.52 (J = 88.2 Hz), 112.73, 60.24, 55.11, 38.86; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{17}H_{18}FNO$: 271.1372. Found: 271.1373 (+0.1 ppm deviation).

4.1.3.28. 4-[*N*-Methyl-*N*-(2-cyanoethyl)]-2′-fluoro-stilbene (40). Yield: 40%; mp 65.8–67.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (m, 1H), 7.47 (m, 2H), 7.09 (m, 5H), 6.71 (m, 2H), 3.77 (t, 2H), 3.07 (s, 3H), 2.59 (t, 2H), ^{13}C NMR (75 MHz, $CDCl_3$): 158.52 (J = 247.1 Hz), 147.28, 130.49 (J = 4.5 Hz), 128.11 (J = 4.5 Hz), 127.94, 126.95, 126.62 (J = 9.0 Hz), 125.82 (J = 11.3 Hz), 124.14 (J = 3.45 Hz), 118.29, 117.39 (J = 3.75 Hz), 115.85 (J = 22.1 Hz), 112.48, 48.86, 38.79, 15.34; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}FN_2$: 280.1376. Found: 280.1382 (+2.2 ppm deviation).

4.1.3.29. 4-[*N*-Methyl-*N*-(2-hydroxyethyl)]-4′-fluoro-stilbene (41). Yield: 42%; mp 111.3–112.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (m, 4H), 7.05 (m,1H), 6.92 (d, 2H), 6.71 (d, 2H), 3.74 (t, 2H), 3.06 (s, 3H), 2.54 (t, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 160.32 (J = 978.9 Hz), 147.09, 134.04 (J = 12.9 Hz), 128.09, 127.83, 127.54 (J = 31.2 Hz), 126.90, 124.14, 118.30, 115.66 (J = 86.1 Hz), 112.55, 48.89, 38.77, 15.33; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{17}FN_2$: 280.1376. Found: 280.1375 (−0.1 ppm deviation).

4.1.3.30. 4-*N*-Methyl-4′-bromo-stilbene (42). Yield: 23%; mp 214.3–215.8 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (m, 6H), 7.03 (d, J = 16.3 Hz, 1H), 6.84 (d, J = 16.3 Hz, 1H), 6.60 (m, 2H), 3.81 (bs, 1H), 2.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 149.22, 137.12, 131.63, 129.63, 128.02, 127.84, 127.48, 126.24, 123.0, 120.17, 112.43, 30.62; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{15}H_{14}BrN$: 287.0310. Found: 280.0313 (+1.3 ppm deviation).

4.1.3.31. 4-*N*-Methyl-2′-methoxy-stilbene (43). Yield: 24%; mp 87.9–89.3 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (m, 3H), 7.27 (m, 3H), 6.88 (m, 2H), 6.53 (m, 2H), 3.83 (s, 3H), 2.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.87, 149.08, 139.64, 129.53, 129.23, 127.81, 126.54, 124.25, 118.81, 112.44, 111.21, 55.24, 30.66; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{15}H_{14}BrN$: 239.1310. Found: 239.1314 (+1.5 ppm deviation).

4.1.3.32. 4-*N*-Methyl-3′-methoxy-stilbene (44). Yield: 20%; mp 62.8–63.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (m, 3H), 7.25 (m, 3H), 6.90 (m, 2H), 6.61 (m, 2H), 3.84 (s, 3H), 2.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.87, 149.08, 139.64, 129.53, 129.23, 127.81, 126.54, 124.25, 118.81, 112.44, 111.21, 55.24, 30.66; HRMS (FAB$^+$, *m*-nitrobenzylalcohol): Calcd for $C_{15}H_{14}BrN$: 239.1310. Found: 239.1309 (−0.5 ppm deviation).

4.2. Biological procedures

4.2.1. General method for preparation of Aβ40 fibrils

Purified Aβ40 peptides were dissolved in phosphate-buffered saline (PBS, pH 7.4) to make a final concentration of 200 μM. The solution was incubated at 37 °C with gentle shaking for 48 h. The formation of Aβ fibril was confirmed by ThT assay. Aβ40 were prepared as previously reported method.[18]

4.2.2. Fluorescence spectra measurement

Excitation and emission λ_{max} of each compound were measured with a SpectraMax M2 (Molecular Devices). Final concentrations of 10–50 μM of Aβ40 fibril in PBS buffer solution were used in the experiments. Generally, the λ_{max} of emission was determined by scanning a fixed excitation first, and the λ_{max} of excitation was determined by scanning emission spectrum with a fixed λ_{em}. Detail protocols were reported in elsewhere.[19]

4.2.3. Measurement of binding affinity (K_D)

Aβ40 fibrils of 50 μM concentration solution were titrated by a series concentration of compounds **42**, **44**, **33**, **35** and ThT. A nonlinear regression analysis was performed in GraphPad Prism fitting a one-site binding model to the binding data. The dissociation constants (K_D) were obtained from the best-fitted curve of each tested compound.

4.2.4. Imaging of AD mouse brain

Brain sections of Tg2576 mice were stained with compound **42** (1 μM) and ThT (5 μM) in PBS (Phosphate Buffered Saline). After 30 min of incubation, the brain sections were washed with PBS and imaged using a Nikon Ti microscope.

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Supplementary data

Supplementary data (Detail NMR characterizations of all compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.044.

References and notes

1. Vickers, J. C.; Dickson, T. C.; Adlard, P. A.; Saunders, H. L.; King, C. E.; McCormack, G. *Prog. Neurobiol.* **2000**, *60*, 139.
2. Xia, W.; Ray, W. J.; Ostaszewski, B. L.; Rahmati, T.; Kimberly, W. T.; Wolfe, M. S.; Zhang, J.; Goate, A. M.; Selkoe, D. J. *Proc. Natl Acad. Sci. U.S.A.* **2000**, *97*, 9299.
3. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
4. Selkoe, D. J. *Nature Biotechnol.* **2000**, *18*, 823.
5. Mathis, C. A.; Wang, Y.; Holt, D. P.; Huang, G.-F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740.
6. Kung, H. F.; Lee, C.-W.; Zhuang, Z.-P.; Kung, M.-P.; Hou, C.; Plossl, K. *J. Am. Chem. Soc.* **2001**, *123*, 12740.
7. (a) Wilson, A.; Nobrega, J.; Houle, S.; Westaway, D.; Verhoeff, N. P.; Zhuang, Z.; Kung, M.-P.; Ono, M.; Kung, H. F. *J. Labelled Compd. Radiopharm.* **2003**, *46*, S61; (b) Ono, M.; Wilson, A.; Nobrega, J.; Westaway, D.; Verhoeff, P.; Zhuang, Z.-P.; Kung, M.-P.; Kung, H. F. *Nucl. Med. Biol.* **2003**, *30*, 565; (c) Kung, M.-P.; Hou, C.; Zhuang, Z.-P.; Skovronsky, D.; Kung, H. F. *Brain Res.* **2004**, *1025*, 98.
8. Klunk, W.; Mathis, C.; Wang, Y.; Huang, G.; Holt, D.; Debnath, M.; Engler, H.; Nordberg, A.; Blomqvist, G.; Bergstrm, M.; Estrada, S.; Barletta, J.; Sandell, J.; Antoni, G.; Lngstrm, B. 32nd Annual Meeting of Society for Neuroscience, 2002.
9. Agdeppa, E. D.; Kepe, V.; Shoghi-Jadid, K.; Satyamurthy, N.; Small, G. W.; Petric, A.; Vinters, H. V.; Huang, S. C.; Barrio, J. R. *J. Nucl. Med.* **2001**, *42*, 65P.
10. Lee, C.-W.; Kung, M.-P.; Hou, C.; Kung, H. F. *Nucl. Med. Biol.* **2003**, *30*, 573.
11. Kung, H. F. *J. Nucl. Med.* **2002**, *43*, 166P.
12. Shoghi-Jadid, K.; Small, G. W.; Agdeppa, E. D.; Kepe, V.; Ercoli, L. M.; Siddarth, P.; Read, S.; Satyamurthy, N.; Petric, A.; Huang, S. C.; Barrio, J. R.; Liu, J.; Flores-Torres, S.; Cole, G. M. *Am. J. Geriatr. Psychiatry* **2002**, *10*, 24.
13. Zhuang, Z.-P.; Kung, M.-P.; Hou, C.; Skovronsky, D.; Gur, T. L.; Trojanowski, J. Q.; Lee, V. M.-Y.; Kung, H. F. *J. Med. Chem.* **2001**, *44*, 1905.
14. Zhuang, Z. P.; Kung, M.-P.; Wilson, A.; Lee, C.-W.; Plossl, K.; Hou, C.; Holtzman, D. M.; Kung, H. F. *J. Med. Chem.* **2003**, *46*, 237.
15. Gao, M.; Wang, M.; Miller, K. D.; Sledge, G. W.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5767.
16. (a) Ono, M.; Haratake, M.; Nakayama, M.; Kaneko, Y.; Kawabata, K.; Mori, H.; Kung, M.-P.; Kung, H. F. *Nucl. Med. Biol.* **2005**, *32*, 329; (b) Verhoeff, N. P.; Wilson, A. A.; Takeshita, S.; Trop, L.; Hussey, D.; Singh, K.; Kung, H. F.; Kung, M. P.; Houle, S. *Am. J. Geriatr. Psychiatry* **2004**, *12*(6), 584.
17. Jang, S.-R.; Lee, C.; Choi, H.; Ko, J. J.; Lee, J.; Vittal, R.; Kim, K.-J. *Chem. Mater.* **2006**, *18*(23), 5604.
18. Kim, Y.; Moss, J. A.; Janda, K. D. *J. Org. Chem.* **2004**, *69*(22), 7776.
19. Li, Q.; Lee, J. S.; Ha, C.; Park, C. B.; Yang, G.; Gan, W. B.; Chang, Y. T. *Angew. Chem., Int. Ed.* **2004**, *43*(46), 6331.