Supplemental Information

Dicyanovinyl-Substituted J147 Analogue Inhibits Oligomerization and Fibrillation of β-Amyloid Peptides and Protects Neuronal Cells from β-Amyloid-induced Cytotoxicity

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Figure S1. Cytotoxicity of 3j to SH-SY5Y cells.
1. Synthetic procedures and characterization of newly synthesized compounds

Materials and reagents. Chemicals were purchased from Alfa-Aesar (Ward Hill, MA, USA) and Sigma Aldrich (St. Louis, MO, USA) unless noted otherwise. Beta-Amyloid (1-42)-human (Aβ_{42}) was purchased from Anaspec (Fremont, CA, USA). Rb pAβ Anti-oligomer Aβ (A11) was purchased from Invitrogen. Anti-Rabbit IgG (H+L), HRP conjugate was purchased from Promega (Madison, WI, USA). NMR spectra were recorded on a Bruker 400 AMX (Karlsruhe, Germany) at 400 MHz for ¹H NMR and 100 MHz for ¹³C NMR with tetramethylsilane as an internal standard. Chemical shifts are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Coupling constants (J) are reported in hertz (Hz). Chemical shifts are reported as parts per million (δ) relative to the solvent peak. TLC was performed on silica gel-60 (220-440 mesh) for flash chromatography. Fluorescence was recorded using a SpectraMax M2e (Molecular device, USA).

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N’-(3-methoxybenzylidene)acetohydrazide (3a). To a solution of 3-methoxybenzaldehyde (1a) (0.10 g, 0.7 mmol) in EtOH (10 mL) was added (2,4-dimethylphenyl)hydrazine hydrochloride (0.13 g, 0.7 mmol), and the resulting mixture was stirred for 1 h at room temperature (RT). After the reaction, the mixture was concentrated under reduced pressure to yield the corresponding benzylidenehydrazine, which was used for the next step without further purification. The intermediate benzylidenehydrazine was dissolved in CH₂Cl₂, and the resulting solution was treated with Et₃N (0.3 mL, 2.2 mmol). Trifluoroacetic anhydride (0.1 mL, 1.1 mmol) was added to this solution in drops at 0 °C. After stirring for 1 h, the mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (8:1 = hexanes:ether) to yield 3a (0.12 g, 0.3 mmol, 47% yield) as a yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 7.29-7.24 (m, 4H), 7.20 (d, J = 7.9 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.04 (d, J = 7.9 Hz, 1H), 6.94 (ddd, J = 8.1, 2.2,0.8 Hz, 1H), 3.81 (s, 1H), 2.41 (s, 3H), 2.08 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 160.7, 158.9 (q, J = 36.4 Hz), 155.0, 143.4, 143.1, 142.3, 137.7, 134.4, 130.9, 130.8, 130.6, 129.9, 123.5, 123.0, 118.4 (q, J = 287.3 Hz), 113.8, 57.4, 23.5, 19.1; LC-MS (ESI) m/z
found 373.2 [M + Na]⁺, calcd for C₁₈H₁₇F₃N₂O₂Na 373.1.

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N’-(3-methoxy-4-nitrobenzylidene)-acetohydrazide (3b).

Synthesis of 3-methoxy-4-nitrobenzaldehyde (1b). 3-Methoxybenzaldehyde (1a) (0.5 g, 3.7 mmol) was added to a stirred mixture of HNO₃ (0.46 mg, 11.0 mmol) and H₂SO₄ (0.59 mL, 11.1 mmol) at -10 °C in drops. After 1 h, the mixture was warmed to RT and stirred for 1 h. Ice water was poured into the reaction mixture, which was filtered by washing with H₂O. The filtered product was dissolved in EtOAc, and the resulting solution was dried over MgSO₄. After filtration, the filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (10:1 = hexanes:EtOAc) to give 3-methoxy-4-nitrobenzaldehyde (1b) (0.3 g, 1.8 mmol, 49% yield) as a yellow solid: ¹H NMR (400 MHz, Acetone-δ) δ 10.44 (s, 1H), 8.22 (d, J = 9.0 Hz, 1H), 7.37 (dd, J = 9.0, 2.9 Hz, 1H), 7.31 (d, J = 2.9 Hz, 1H), 4.04 (s, 3H).

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N’-(3-methoxy-4-nitrobenzylidene)-acetohydrazide (3b). To a stirred solution of 3-methoxy-4-nitrobenzaldehyde (1b) (0.15 mg, 0.8 mmol) in EtOH (15 mL) was added (2,4-dimethylphenyl)-hydrazine hydrochloride (0.14 g, 0.8 mmol), and the resulting mixture was stirred for 1 h at RT. After the reaction, the mixture was concentrated under reduced pressure to afford the corresponding benzylidenehydrazine, which was used for the next step without further purification. The intermediate benzylidenehydrazine was dissolved in CH₂Cl₂ (10 mL), and the resulting solution was treated with pyridine (0.2 mL, 2.5 mmol). Trifluoroacetic anhydride (0.17 mL, 1.2 mmol) was added to this solution in drops at 0 °C. After stirring for 1 h, the mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (8:1 = hexanes:ether) to afford 3b (0.1 g, 0.2 mmol, 30% yield) as a yellow solid: ¹H NMR (400 MHz, Acetone-δ) δ 8.13 (d, J = 9.1 Hz, 1H), 7.99 (s, 1H), 7.54 (d, J = 2.8 Hz, 1H), 7.35 (s, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 7.22 (dd, J = 9.2, 2.8 Hz, 1H), 4.00 (s, 3H), 2.41
69 (s,3H), 2.15 (s,3H); 13C NMR (100 MHz, CDCl3) δ 166.5, 159.6 (q, J = 36.1 Hz), 145.1, 144.7, 144.1,
70 139.3, 135.3, 134.1, 132.7, 131.7, 131.6, 130.6, 120.0 (q, J = 287.0 Hz), 118.8, 116.2, 58.8, 23.3, 19.1;
71 LC-MS (ESI) m/z found 418.2 [M + Na]+, calcd for C18H16F3N3O4Na 418.1.
72 Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N’-(4-hydroxy-3-methoxybenzylidene)-
73 acetohydrazide (3d).
74 Synthesis of 4-((tert-butyldimethylsilyl)oxy)-3-methoxybenzaldehyde (1d). To a stirred solution of
75 vanillin (1c) (0.20 g, 1.3 mmol) in CH2Cl2 (20 mL) were added both imidazole (0.18 g, 2.6 mmol) and
76 TBDMSOTBu (0.3 g, 2.0 mmol), and the mixture was stirred at RT for 1 h. Water (20 mL) was added,
77 and the reaction mixture was extracted with CH2Cl2 (20 mL x 3). The combined organic layers were
78 dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by
79 column chromatography on silica gel (8:1 = hexanes:EtOAc) to afford 1d (0.27 g, 1.0 mmol, 78% 
80 yield) as a colorless oil: 1H NMR (400 MHz, Acetone-d6) δ 9.66 (s, 1H), 7.26-7.23 (m, 2H), 6.83 (d, J
81 = 7.7 Hz, 1H), 3.70 (s, 1H), 0.80 (s, 9H), 0.00 (s, 6H).
82 Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N’-(4-hydroxy-3-methoxybenzylidene)-
83 acetohydrazide (3d). A solution of 4-((tert-butyldimethylsilyl)oxy)-3-methoxybenzaldehyde (0.27 g,
84 1.0 mmol) in EtOH (20 mL) was treated with (2,4-dimethylphenyl)hydrazine hydrochloride (0.18 g,
85 1.0 mmol), and the resulting mixture was stirred for 1 h at RT. After the reaction, the mixture was
86 concentrated under reduced pressure to afford the corresponding benzylidenehydrazine, which was
87 used for the next step without further purification. The intermediate benzylidenehydrazine was
88 dissolved in CH2Cl2 (15 mL), and the resulting solution was treated with TEA (0.43 mL, 3.1 mmol).
89 Trifluoroacetic anhydride (0.21 mL, 1.5 mmol) was added to this solution in drops at 0 °C. After
90 stirring for 1 h, the mixture was concentrated under reduced pressure, and the residue was purified by
91 column chromatography on silica gel (20:1 = hexanes:EtOAc) to afford (E)-N-(4-((tert-
92 butyldimethylsilyl)oxy)-3-methoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-
trifluoroacetohydrazide (0.16 g, 0.3 mmol, 32% yield) as a yellow solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.16 (s, 1H), 7.08 (s, 1H), 7.04-7.03 (m, 2H), 6.88 (d, $J = 7.9$ Hz, 1H), 6.78 (d, $J = 7.2$ Hz, 1H), 6.65 (d, $J = 8.1$ Hz, 1H), 3.68 (s, 3H).

The product obtained above (0.09 g, 0.2 mmol) was dissolved in tetrahydrofuran (THF) (7 mL), and the resulting solution was treated with tetra-$n$-butylammonium fluoride (TBAF, 1.0 M in THF) (0.20 mL, 0.2 mmol) at 0 °C in drops. The mixture was stirred at RT for 1 h. Saturated aqueous NH$_4$Cl solution (10 mL) was added, and the reaction mixture was extracted with EtOAc (15 mL x 3). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 = hexanes:EtOAc) to afford 3d (42 mg, 0.11 mmol, 64% yield) as a white solid: $^1$H NMR (400 MHz, CDCl$_3$) δ 7.36 (d, $J = 1.0$ Hz, 1H), 7.24 (s, 1H), 7.21-7.19 (m, 2H), 7.04 (d, $J = 7.9$ Hz, 1H), 6.94 (dd, $J = 8.1$, 1.2 Hz, 1H), 6.86 (d, $J = 8.1$ Hz, 1H), 5.91 (s, 1H), 3.93 (s, 3H), 2.41 (s, 3H), 2.09 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 158.3 (q, $J = 36.1$ Hz), 149.5, 148.2, 145.1, 137.5, 133.8, 130.9, 129.9, 129.8, 127.2, 124.6, 118.2 (q, $J = 286.9$ Hz), 115.5, 109.3, 57.0, 22.5, 18.2; LC-MS (ESI) $m/z$ found 389.2 [M + Na]$^+$, calcd for C$_{18}$H$_{17}$F$_3$N$_2$O$_3$Na 389.1.

Synthesis of (E)-N’-(3,4-dimethoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-trifluoroacetohydrazide (3e).

Synthesis of 3,4-dimethoxybenzaldehyde (1e). To a stirred solution of vanillin (0.05 g, 0.3 mmol) in acetone (5 mL) was added K$_2$CO$_3$ (0.05 g, 0.4 mmol) at 0 °C. After 1 h, CH$_3$I (0.03 mL, 0.5 mmol) was added, and the reaction mixture was stirred under reflux for 7 h. After cooling to RT, the mixture was concentrated under reduced pressure, and the residue was taken with a mixture of H$_2$O (10 mL) and EtOAc (10 mL). The resulting mixture was extracted with EtOAc (15 mL x 3), and the combined organic layers were dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (6:1 = hexanes:EtOAc) to afford 1e (0.03 g, 0.2
mmol, 55% yield) as a white solid: $^1$H NMR (400 MHz, Acetone-d$_6$) \( \delta \) 9.83 (s, 1H), 7.54 (dd, \( J = 8.2 \), 1H), 7.15 (d, \( J = 8.2 \) Hz, 1H), 3.93 (s,3H), 3.90 (s, 3H).

**Synthesis of (E)-N’-(3,4-dimethoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-trifluoroacetohydrazide)**

The desired product was obtained starting from 1e by using the same procedure for the synthesis of 3b. Purification of the crude product by column chromatography on silica gel (8:1 = hexanes:EtOAc) provided 3e in 54% yield as a white solid: $^1$H NMR (400 MHz, Acetone-d$_6$) \( \delta \) 7.39-7.38 (m, 2H), 7.32 (s, 1H), 7.26 (d, \( J = 8.5 \) Hz, 1H), 7.21-7.18 (m, 2H), 6.98 (d, \( J = 8.3 \) Hz, 1H), 3.84 (s,3H), 3.82 (s, 3H), 2.40 (s, 3H), 2.07 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) \( \delta \) 159.6 (q, \( J = 35.8 \) Hz), 155.2, 153.0, 147.7, 144.0, 139.5, 135.5, 133.3, 132.1, 131.9, 129.6, 125.5, 120.5 (q, \( J = 285.2 \) Hz), 114.7, 112.9, 58.5, 58.3, 23.6, 19.5; LC-MS (ESI) m/z found 403.2 [M + Na]$^+$, calcd for C$_{19}$H$_{19}$F$_3$N$_2$O$_3$Na 403.1.

**Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N’-(2-methoxy-[1,1’-biphenyl]-4-yl)methylene)acetohydrazide (3f).**

**Synthesis of 2-methoxy-[1,1’-biphenyl]-4-carbaldehyde (1f).** To a stirred solution of vanillin (1c) (1.00 g, 6.6 mmol) in CH$_2$Cl$_2$ (40 mL) were added TEA (1.83 mL, 13.1 mmol) and Tf$_2$O (1.66 mL, 9.9 mmol) at 0 °C. After stirring at RT for 1 h, the reaction was quenched by addition of H$_2$O, and the reaction mixture was extracted with CH$_2$Cl$_2$ (30 mL x 3). The combined organic layers were dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (4:1 = hexanes:EtOAc) to afford 4-formyl-2-methoxyphenyl trifluoromethanesulfonate (1.28 g, 4.5 mmol, 69% yield) as a yellow solid: $^1$H NMR (400 MHz, Acetone-d$_6$) \( \delta \) 10.07 (s, 1H), 7.78 (d, \( J = 1.5 \) Hz, 1H), 7.69 (dd, \( J = 8.3 \), 1.6 Hz, 1H), 7.65 (d, \( J = 8.3 \) Hz, 1H), 4.09 (s, 3H).

The 4-formyl-2-methoxyphenyl trifluoromethanesulfonate (1.28 g, 4.5 mmol) obtained above was dissolved in MeOH (50 mL). To this solution, K$_2$CO$_3$ (1.24 g, 9.0 mmol), PhB(OH)$_2$ (0.82 g, 6.8
mmol) and Pd(PPh$_3$)$_4$ (0.26 g, 0.1 mmol) were added at RT. After stirring at RT for 2 h, the solvent was evaporated under reduced pressure. The residue was taken with a mixture of H$_2$O and Et$_2$O, and the resulting mixture was extracted with Et$_2$O (30 mL x 3). The combined organic layers were dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 = hexanes:EtOAc) to afford 1f (0.78 g, 3.7 mmol, 82% yield) as a yellow syrup: $^1$H NMR (400 MHz, Acetone-d$_6$) $\delta$ 10.02 (s, 1H), 7.62-7.53 (m, 5H), 7.44 (t, $J = 7.4$ Hz, 1H), 7.38 (t, $J = 7.3$ Hz, 1H), 3.92 (s, 3H).

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N'-(2-methoxy-[1,1'-biphenyl]-4-yl)methylene)acetohydrazide (3f). The desired product was obtained starting from 1f by using the same procedure for the synthesis of 3a. Purification of the crude product by column chromatography on silica gel (12:1 = petroleum ether:ether) provided 3f in 45% yield as a yellow syrup: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.50 (d, $J = 7.5$ Hz, 1H), 7.40 (s, 1H), 7.37 (t, $J = 7.6$ Hz, 2H), 7.31-7.28 (m, 3H), 7.24 (s, 1H), 7.19 (d, $J = 8.1$ Hz, 1H), 7.14 (d, $J = 7.7$ Hz, 1H), 7.04 (d, $J = 7.9$ Hz, 1H), 3.81 (s, 3H), 2.39 (s, 3H), 2.09 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 159 (q, $J = 36.2$ Hz), 159.05, 145.90, 143.22, 139.92, 138.42, 135.96, 135.60, 134.84, 133.23, 131.84, 131.62, 131.00, 130.73, 130.27, 129.61, 123.74, 119.26 (q, $J = 287.4$ Hz), 111.30, 57.63, 23.45, 19.17; LC-MS (ESI) $m/z$ found 449.2 [M + Na]$^+$, calcd for C$_{24}$H$_{21}$F$_3$N$_2$O$_2$Na 449.1.

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N'-(3-methoxy-4-methylbenzylidene)acetohydrazide (3g).

2.2.6.1. Synthesis of 3-methoxy-4-methylbenzaldehyde (1h). To a stirred solution of methyl 3-methoxy-4-methylbenzoate (1g) (0.5 g, 2.8 mmol) in anhydrous THF (30 mL), was slowly added DIBAL-H (1 M solution in THF) (11.1 mL, 11.1 mmol) at -78 °C for 30 min. The reaction mixture was then warmed to RT and stirred for 5 h. After an addition of 2 N HCl (10 mL), the reaction mixture was extracted with EtOAc (20 mL x 3). The combined organic layers were dried over MgSO$_4$,
filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (4:1 = hexanes:EtOAc) to afford (3-methoxy-4-methylphenyl)methanol (0.38 g, 2.5 mmol, 91% yield) as a colorless oil: $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 7.05 (d, $J = 7.5$ Hz, 1H), 6.93 (s, 1H), 6.81 (d, $J = 7.5$ Hz, 1H), 4.53 (d, $J = 5.9$ Hz, 2H), 4.53 (t, $J = 5.8$ Hz, 1H), 3.81 (s,3H), 2.14 (s, 3H).

The (3-methoxy-4-methylphenyl)-methanol obtained above was dissolved in CH$_2$Cl$_2$ (30 mL), and the resulting solution was treated with pyridinium chlorochromate (PCC) (0.60 g, 2.8 mmol) at 0 °C. The reaction mixture was then warmed to RT and stirred for 2 h. The reaction mixture was filtered through a short Celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 = hexanes:EtOAc) to give $^1$h (0.21 g, 1.4 mmol, 56% yield) as a white solid: $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 9.96 (s, 1H), 7.44 (dd, $J = 7.5$, 1.3 Hz, 1H), 7.41 (s, 1H), 7.37 (d, $J = 7.5$ Hz, 1H), 3.94 (s,3H), 2.27 (s, 3H).

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N'-(3-methoxy-4-methylbenzylidene)-acetohydrazide ($^3$g). The desired product was obtained starting at $^1$h by using the same procedure for the synthesis of $^3$a. Purification of the crude product by column chromatography on silica gel (8:1 = hexanes:EtOAc) provided $^3$g in 32% yield as a white solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.23(S, 2H), 7.20 (d, $J = 8.0$ Hz, 1H), 7.10 (d, $J = 7.5$ Hz, 1H), 7.05 (d, $J = 7.9$ Hz, 1H), 6.95 (d, $J = 7.5$ Hz, 1H), 3.85 (s, 3H), 2.41 (s, 3H), 2.22 (s, 3H), 2.08 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.3, 155.5 (q, $J = 36.3$ Hz), 142.4, 139.2, 134.5, 130.8, 130.5, 128.9, 128.6, 127.9, 127.0, 126.8, 119.6, 115.3 (q, $J = 286.3$ Hz), 105.6, 53.4, 19.6, 15.3, 14.5; LC-MS (ESI) m/z found 387.2 [M + Na]$^+$, calcd for C$_{19}$H$_{10}$F$_3$N$_2$O$_2$Na 387.1.

Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N'-(4-fluoro-3-methoxybenzylidene)-acetohydrazide ($^3$h). The desired product was obtained starting from $^1$i by using the same procedure for the synthesis of $^3$a. Purification of the crude product by column chromatography on silica gel (8:1
= hexanes:ether) provided 3h in 37% yield as a white powder: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.41\) (dd, \(J = 8.2, 1.1\) Hz, 1H), 7.24 (s, 1H), 7.22-7.19 (m, 2H), 7.06-6.99 (m, 3H), 3.90 (s, 3H), 2.41 (s, 3H), 2.09 (s, 3H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 158.0\) (q, \(J = 36.4\) Hz), 154.8 (d, \(J = 252.3\) Hz), 149.0 (d, \(J = 11.5\) Hz), 143.7, 141.9, 137.0, 1334, 130.8, 130.5 (d, \(J = 46.1\) Hz), 129.6, 129.3, 122.6 (d, \(J = 7.4\) Hz), 116.9 (d, \(J = 9.0\) Hz), 111.9 (d, \(J = 2.5\) Hz), 56.8, 22.0, 17.8; LC-MS (ESI) m/z found 391.2 [M + Na]\(^+\), calcd for C\(_{18}\)H\(_{16}\)F\(_4\)N\(_2\)O\(_2\)Na 391.1.

**Synthesis of (E)-N’-(4-(dimethylamino)-3-methoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-trifluoroacetohydrazide (3i).**

**Synthesis of 4-(dimethylamino)-3-methoxybenzaldehyde (1j).** To a stirred solution of 4-fluoro-3-methoxybenzaldehyde (1i) (0.50 g, 3.2 mmol) in a mixture of dimethyl sulfoxide (7 mL) and H\(_2\)O (3 mL) were added K\(_2\)CO\(_3\) (0.45 g, 3.3 mmol) and NHMe\(_2\) (2.0 M solution in MeOH) (4.86 mL, 9.7 mmol) at RT, and the resulting mixture was stirred for 12 h at 110 °C. After cooling to RT, the reaction mixture was diluted with EtOAc (30 mL) and washed successively with H\(_2\)O and a saturated aqueous NH\(_4\)Cl solution. The organic layer was dried over MgSO\(_4\), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 = hexanes:ether) to afford 1j (0.41 g, 2.3 mmol, 71% yield) as a yellow oil: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 9.82\) (s, 1H), 7.40 (d, \(J = 8.2\) Hz, 1H), 7.37 (s, 1H), 6.91 (d, \(J = 8.1\) Hz, 1H), 3.93 (s, 3H), 2.95 (s, 6H).

**Synthesis of (E)-N’-(4-(dimethylamino)-3-methoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-trifluoroacetohydrazide (3i).** The desired product was obtained starting from 1j by using the same procedure for the synthesis of 3a. Purification of the crude product by column chromatography on silica gel (6:1 = hexanes:ether) provided 3i in 46% yield as a yellow syrup: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.34\) (d, \(J = 1.3\) Hz, 1H), 7.24 (s, 1H), 7.21-7.18 (m, 2H), 7.04 (d, \(J = 7.9\) Hz, 1H), 6.98 (dd, \(J = 8.1, 1.5\) Hz, 1H), 6.82 (d, \(J = 8.2\) Hz, 1H), 3.92 (s, 3H), 2.83 (s, 6H), 2.41 (s, 3H), 2.08 (s, 3H); \(^1^3\)C
NMR (100 MHz, CDCl₃) δ 162.1, 159.4 (q, J = 36.4 Hz), 146.1, 143.2, 138.4, 136.8, 134.8, 131.9, 131.7, 131.7, 130.9, 130.7, 123.2, 119.3, 119.1 (q, J = 287.3 Hz), 106.6, 53.5, 41.1, 19.5, 15.2; LC-MS (ESI) m/z found 416.2 [M + Na]⁺, calcd for C_{20}H_{22}F_{3}N_{3}O_{2}Na 416.2.

Synthesis of \((E)-N'-(4-(2,2-dicyanovinyl)-3-methoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2\)-trifluoroacetohydrazide (3j).

Synthesis of 2-(4-formyl-2-methoxybenzylidene)malononitrile (II).

To a stirred mixture of 4-bromo-3-methoxyaniline (1k) (2.00 g, 9.9 mmol) in H₂O (50 mL) were added HCl (4 mL) and NaNO₂ (0.75 g, 10.9 mmol) in drops at 0 °C. The resulting mixture was stirred at 0 °C for 30 min, treated with K₂CO₃, and then added to a mixture of CuCN (1.06 g, 11.9 mmol) and KCN (1.61 g, 24.7 mmol) in H₂O (50 mL). The reaction mixture was stirred for 1 h at 70 °C and, after cooling to RT, extracted with toluene (100 mL x 3). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (6:1 = hexanes:ether) to give 4- bromo-3-methoxybenzonitrile (1.53 g, 7.2 mmol, 73% yield) as a yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, J = 8.0 Hz, 1H), 7.14 (dd, J = 8.0, 1.7 Hz, 1H), 7.11 (d, J = 1.6 Hz, 1H), 3.94 (s, 3H).

The 4-bromo-3-methoxybenzonitrile (0.38 g, 1.8 mmol) obtained above was dissolved in toluene (25 mL), and treated with DIBAL-H (1 M solution in THF) (3.58 mL, 3.6 mmol) at -78 °C. After stirring for 30 min at -78 °C, the reaction mixture was warmed to RT and stirred for 4.5 h. MeOH (15 mL) was added to quench the reaction, and the resulting mixture was stirred for 30 min. After an addition of 10% H₂SO₄ (10 mL), the resulting mixture was stirred for an additional 1.5 h and then extracted with EtOAc (30 mL x 3). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (4:1 = hexanes:ether) to afford 4-bromo-3-methoxybenzaldehyde (0.16 g, 0.7 mmol, 41% yield) as a white solid: ¹H NMR (400 MHz, Acetone-d₆) δ 10.05 (s, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.56 (s, 1H),
4-Bromo-3-methoxybenzaldehyde (0.56 g, 2.6 mmol), thus obtained, was dissolved in toluene (25 mL), and treated with p-TsOH (0.02 g, 0.1 mmol) and ethylene glycol (5 mL, 89.4 mmol). After stirring for 4 h under reflux, the reaction mixture was cooled to RT, diluted with EtOAc (25 mL), and washed successively with saturated aqueous NaHCO₃ solution and brine. The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 = hexanes:ether) to give 2-(4-bromo-3-methoxyphenyl)-1,3-dioxolane (0.51 g, 2.0 mmol, 76% yield) as a colorless oil: ¹H NMR (400 MHz, Acetone-d₆) δ 7.54 (d, J = 8.0 Hz, 1H), 7.14 (s, 1H), 6.98 (d, J = 8.1 Hz, 1H), 5.72 (s, 1H), 4.08-4.02 (m, 2H), 4.01-3.95 (m, 2H), 3.89 (s, 3H).

2-(4-Bromo-3-methoxyphenyl)-1,3-dioxolane (0.76 g, 2.9 mmol) obtained above was dissolved in anhydrous THF (30 mL). To this solution, nBuLi (1.6 M solution in hexane) (2.44 mL, 2.9 mmol) was added in drops at -78 °C and stirred continuously for 30 min. After 30 min, N-formylpiperidine (0.73 mL, 4.4 mmol) was added, and the mixture was warmed to RT. After stirring for 2.5 h, the reaction mixture was diluted with diethyl ether (30 mL) and washed with saturated aqueous NH₄Cl solution. The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (4:1 = hexanes:ether) to afford 4-(1,3-dioxolan-2-yl)-2-methoxybenzaldehyde (0.4 g, 1.9 mmol, 66% yield) as a yellow oil: ¹H NMR (400 MHz, Acetone-d₆) δ 10.46 (s, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.26 (s, 1H), 7.15 (d, J = 7.9 Hz, 1H), 5.79 (s, 1H), 4.11-4.04 (m, 2H), 4.03-4.01 (m, 2H), 3.99 (s, 3H).

4-(1,3-Dioxolan-2-yl)-2-methoxybenzaldehyde (0.1 g, 0.58 mmol) was dissolved in CH₂Cl₂ (10 mL) and treated with imidazole (0.01 mg, 0.1 mmol) and malononitrile (0.03 mL, 0.5 mmol). After stirring at RT for 2 h, H₂O was added, and the resulting mixture was extracted with CH₂Cl₂ (20 mL x 3). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure.
The residue was purified by column chromatography on silica gel (4:1 = hexanes:EtOAc) to afford 2-
(4-(1,3-dioxolan-2-yl)-2-methoxybenzylidene)-malononitrile (0.11 mg, 0.4 mmol, 91% yield) as a
yellow solid: $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 8.42 (s, 1H), 8.12 (d, $J = 8.1$ Hz, 1H), 7.28 (s, 1H),
7.25 (d, $J = 8.2$ Hz, 1H), 5.83 (s, 1H), 4.11-4.10 (m, 2H), 4.05-4.03 (m, 2H) 4.01 (s, 3H).

2-(4-(1,3-Dioxolan-2-yl)-2-methoxybenzylidene)-malononitrile (0.18 g, 0.7 mmol) was dissolved in
acetone (12 mL) and treated with 2 N HCl (7 mL). After stirring at RT for 2 h, volatiles were removed
by concentration under reduced pressure. The remaining aqueous phase was extracted with EtOAc (20
mL x 3). The combined organic layers were dried over MgSO$_4$, filtered, and concentrated under
reduced pressure. The residue was purified by column chromatography on silica gel (6:1 =
hexanes:EtOAc) to afford 2-(4-formyl-2-methoxybenzylidene)-malononitrile (1I) (0.11 g, 0.5 mmol,
77% yield) as a yellow solid: $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 10.09 (s, 1H), 8.50 (s, 1H), 8.26 (d, $J$
= 7.9 Hz, 1H), 7.72 (d, $J = 8.1$ Hz, 1H), 7.69 (s, 1H), 4.09 (s, 3H).

**Synthesis of (E)-N’-(4-(2,2-dicyanovinyl)-3-methoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-
trifluoroacetohydrazide (3j).** The desired product was obtained, starting from 1I by using the same
procedure for the synthesis of 3a. Purification of the crude product by column chromatography on
silica gel (8:1 = hexanes:EtOAc) provided 3j in 57% yield as a yellow solid: $^1$H NMR (400 MHz,
CDCl$_3$) $\delta$ 8.26 (s, 1H), 8.19 (d, $J = 8.2$ Hz, 1H), 7.39 (s, 1H), 7.28 (s, 1H), 7.25-7.23 (m, 2H), 7.16 (d, $J$
= 8.1 Hz, 1H), 7.05 (d, $J = 7.9$ Hz, 1H), 3.96 (s, 3H), 2.44 (s, 3H), 2.09 (s, 3H); $^{13}$C NMR (100
MHz, CDCl$_3$) $\delta$ 160.7, 158.9 (q, $J = 36.4$ Hz), 155.0, 143.4, 143.1, 142.3, 137.7, 134.4, 130.9, 130.8,
130.6, 129.9, 123.5, 123.0, 118.4 (q, $J = 287.3$ Hz), 115.8, 114.5, 110.7, 83.9, 57.6, 23.0, 18.6; LC-
MS (ESI) m/z found 449.2 [M + Na]$^+$, calcd for C$_{22}$H$_{17}$F$_3$N$_4$O$_2$ 449.1.
2. NMR spectra for the synthesized compounds

2.1. $^1$H NMR for 1b
2.2. $^1$H NMR for 1d
2.3. $^1$H NMR for 1e
2.4. $^1$H NMR for 1f
2.5. $^1$H NMR for 1h
2.6. $^1$H NMR for 1j
2.7. $^1$H NMR for 11

![NMR Spectrogram](image)

[Chemical Structure Image]
2.8. $^1$H NMR for 3a
2.9. $^{13}$C NMR for 3a
2.10. $^1$H NMR for 3b
2.11. $^{13}{\text{C}}$ NMR for $3b$
2.12. $^1$H NMR for 3d
2.13. $^{13}$C NMR for 3d
2.14. $^1$H NMR for 3e
2.15. $^{13}$C NMR for $3e$
2.16. $^1$H NMR for 3f
2.17. $^{13}$C NMR for 3f
2.18. $^1H$ NMR for 3g
2.19. $^{13}$C NMR for $3g$
2.20. $^1$H NMR for 3h
2.21. $^{13}$C NMR for 3h
2.22. $^1$H NMR for 3i
2.23. $^{13}$C NMR for 3i
2.24. $^1$H NMR for 3j
2.25. $^{13}$C NMR for 3j
3. **Aβ42 Oligomerization.** Aβ42 stock solutions (2 mM) were prepared by dissolving the lyophilized peptide in 100 mM NaOH followed by water bath sonication for 30 s. The oligomerization reaction was initiated by diluting the stock solution in phosphate-buffered saline (PBS), pH 7.4, 0.02% sodium azide (50 and 100 μM final Aβ42 concentration, final pH 7.4). The reaction was incubated at 25 °C for four days in the absence or presence of the J147 derivatives dissolved in DMSO.

4. **Aβ42 Fibrillation.** Aβ42 stock solutions (2 mM) were prepared by dissolving the lyophilized peptide in 100 mM NaOH followed by water bath sonication for 30 s. The fibrillation reaction was initiated by diluting the stock solution in 10 mM HEPES, 100 mM NaCl, 0.02% sodium azide, pH 7.4 (50 μM final Aβ42 concentration). The reaction was incubated at 37 °C without agitation for up to two days in the absence or presence of the J147 derivatives dissolved in DMSO.

5. **Atomic Force Microscopy (AFM).** Formation of Aβ42 oligomers and fibrils was further confirmed by AFM. Aβ42 oligomers, fibrils or preformed Aβ42 fibrils after treatment with 3j were immobilized onto freshly cleaved mica. The excess peptides were removed by washing with distilled water. AFM imaging was performed in NC (non-contact) mode in XE-100 (Park systems, Korea) with NCHR cantilevers (Park systems, Korea) exhibiting frequency at 0.32 KHz. The drive amplitude was set on 20.52 nm, and the amplitude set point was adjusted as 15.39 nm.

6. **ELISA.** Aβ42 was subjected to oligomerization in the absence and presence of the J147 derivatives (50 or 100 μM). Aliquots of each oligomerization reaction were transferred to 96-well clear, flat bottom plates containing 100 μL of coating buffer (0.1M sodium bicarbonate, pH 9.6). The plates were incubated at 37 °C for 4 h, washed, blocked with 10% bovine serum albumin (BSA) dissolved in
a mixture of Tris-buffered saline and 0.005% Tween 20 (TBS-T) at 37 °C for 2 h, and washed again. Then, 100 μL of A11 antibody (1:1500 dilutions in 3% BSA dissolved in TBS-T) was added to each well, and the plates were incubated at 37 °C for 2 h. After washing, secondary antibody (1:5000 dilutions in 3% BSA dissolved in TBS-T) was added to each well and the plate was incubated at 37 °C for 2 h. The plates were then washed and developed with 3,3’,5,5’-tetramethylbenzidine (TMB) solutions (100 μL). After 30 min, the reactions were stopped using 100 μL of 2 M H₂SO₄ and assayed by measuring the absorbance at 450 nm. The J147 derivatives identified as oligomerization inhibitors were further evaluated at various concentrations (0.1 – 200 μM); the data points were fit to dose-response curves using the Sigmaplot software (Systat Software Inc., Point Richmond, CA). Assays were performed in triplicate, and the IC₅₀ values defined as the concentration of the J147 derivatives required to attain half-maximal absorbance, was obtained from the fit.

**7. ThT Fluorescence Assay.** Aβ₄₂ fibrillation and Aβ₄₂ fibril disruption in the presence and absence of the J147 derivatives were monitored by ThT fluorescence assay. A volume of 100 μL of 50 μM Aβ₄₂ with or without the J147 derivatives was added to 1 μL of ThT solution (5 mM). ThT fluorescence was measured at 483 nm (excitation at 442 nm) using SpectraMax (Molecular device, USA). All measurements were carried out in aqueous solution using a 1 cm x 1 cm quartz cuvette. Fluorescence intensity from solution without Aβ₄₂ was subtracted from solution containing Aβ₄₂. Each experiment was repeated in three independent samples, and each sample was tested in quadruplicate. The J147 derivatives identified as fibrillation inhibitors or fibril disruptors at 50 μM were further evaluated at various concentrations (0.1 – 200 μM); the data points were fit to dose-response curves using the Sigmaplot software (Systat Software Inc., Point Richmond, CA). Assay was performed in triplicate, and the IC₅₀ and EC₅₀ values defined as the concentration of the J147 derivatives required to reduce the fluorescence by half, was obtained from the fit.
8. Disruption Assay of Aβ42 Fibrils. Aβ42 fibrils were prepared by incubating 20 mM Aβ42 monomers for 48 h, which is sufficiently long enough to enable Aβ42 peptides to grow into mature fibrils at a saturated state. The fibril solution was then divided into aliquots for the disruption test. To examine the effect of 3j, 40 mM stock solution of 3j (in dimethyl sulfoxide) was dissolved in Aβ42 fibril solution. The disruption of the Aβ42 fibrils were monitored by ThT fluorescence at 483 nm (excitation at 442 nm) using SpectraMax (Molecular device, USA). All measurements were carried out in aqueous solution using a 1 cm x 1 cm quartz cuvette. Fluorescence intensity from solution without Aβ42 was subtracted from solution containing Aβ42. The activity of 3j as a fibril disruptor was further evaluated at various concentrations (0.1 – 200 μM); the data points were fit to dose-response curves using the Sigmaplot software (Systat Software Inc., Point Richmond, CA). The assay was performed in triplicate, and the EC50 value, defined as the concentration of 3j required to reduce fluorescence by half, was obtained from the fit.

9. Cytotoxicity assay. The SH-SY5Y human neuroblastoma cells were purchased from the Korean Cell Line Bank and maintained in Dulbecco’s modified Eagle’s medium (DMEM) with penicillin, streptomycin (1% final concentration), and fetal bovine serum (FBS, 10% final concentration) in 5% CO2 at 37 °C. Cells were seeded at 10,000 cells/well in 96-well plates and grown overnight. The next day, cells were differentiated in modified DMEM with all-trans-retinoic acid (final concentration 10 μM). After 3–5 days, the medium was replaced with DMEM (without serum) and 50 ng/ml brain-derived neurotrophic factor. To investigate cell viability, the medium was removed and 10 μL of the curcumin derivative 3j (1, 10, 50, 75 and 100 μM) with or without Aβ42 oligomers or fibrils (500 μM) were added to 90 μL of new medium. After incubation in 5% CO2 at 37 °C for 24 h, cell viability was evaluated by an MTT assay. MTT solution (10 μL) was added to each well. After 4 h at 37 °C, the solutions of each well were removed, and 100 μL DMSO were added. Absorbance was measured at 590 nm using SpectraMax (Molecular device, USA). The time-course of neuroprotection induced by
3j was also evaluated. Thus, after treatment of SH-SY5Y cells with preformed Aβ42 fibrils and 3j (10 μM), the cell viability was estimated by MTT assay at 0, 1, 3, 5, 7, 12, 24 and 48 h.

Figure S1. Cytotoxicity of 3j to SH-SY5Y cells