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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Contents

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 ($A\beta_{42}$) 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 ^{1}H NMR and 100 MHz for ^{13}C 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 CH_2Cl_2, and the resulting solution was treated with Et_3N (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: ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{18}H_{17}F_3N_2O_2Na$ 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 HNO_3 (0.46 mg, 11.0 mmol) and H_2SO_4 (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 H_2O. The filtered product was dissolved in EtOAc, and the resulting solution was dried over $MgSO_4$. 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-methyoxy-4-nitrobenzaldehyde (**1b**) (0.3 g, 1.8 mmol, 49% yield) as a yellow solid: ^{1}H NMR (400 MHz, Acetone-d_6) 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 CH_2Cl_2 (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: ^{1}H NMR (400 MHz, Acetone-d_6) 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

(s,3H), 2.15 (s,3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 159.6 (q, J = 36.1 Hz), 145.1, 144.7, 144.1, 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; LC-MS (ESI) m/z found 418.2 $[M + Na]^+$, calcd for $C_{18}H_{16}F_3N_3O_4Na$ 418.1.

Synthesis of (*E*)-*N*-(2,4-dimethylphenyl)-2,2,2-trifluoro-*N*'-(4-hydroxy-3-methoxybenzylidene)-acetohydrazide (3d).

*Synthesis of 4-((tert-butyldimethylsilyl)oxy)-3-methoxybenzaldehyde (**1d**)*. To a stirred solution of vanillin (**1c**) (0.20 g, 1.3 mmol) in CH_2Cl_2 (20 mL) were added both imidazole (0.18 g, 2.6 mmol) and TBDMSCl (0.3 g, 2.0 mmol), and the mixture was stirred at RT for 1 h. Water (20 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (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 (8:1 = hexanes:EtOAc) to afford **1d** (0.27 g, 1.0 mmol, 78% yield) as a colorless oil: ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.66 (s, 1H), 7.26-7.23 (m, 2H), 6.83 (d, J = 7.7 Hz, 1H), 3.70 (s, 1H), 0.80 (s, 9H), 0.00 (s, 6H).

*Synthesis of (E)-N-(2,4-dimethylphenyl)-2,2,2-trifluoro-N'-(4-hydroxy-3-methoxybenzylidene)-acetohydrazide (**3d**)*. A solution of 4-((*tert*-butyldimethylsilyl)oxy)-3-methoxybenzaldehyde (0.27 g, 1.0 mmol) in EtOH (20 mL) was treated with (2,4-dimethylphenyl)hydrazine hydrochloride (0.18 g, 1.0 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 CH_2Cl_2 (15 mL), and the resulting solution was treated with TEA (0.43 mL, 3.1 mmol). Trifluoroacetic anhydride (0.21 mL, 1.5 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 (20:1 = hexanes:EtOAc) to afford (*E*)-*N*'-(4-((*tert*-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_4Cl 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 $C_{18}H_{17}F_3N_2O_3Na$ 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_2CO_3 (0.05 g, 0.4 mmol) at 0 °C. After 1 h, CH_3I (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_2O (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) δ 9.83 (s, 1H), 7.54 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.42 (d, *J* = 1.8 Hz, 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) **(3e)***. 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) δ 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, $CDCl_3$) δ 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 $C_{19}H_{19}F_3N2O_3Na$ 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_2Cl_2 (40 mL) were added TEA (1.83 mL, 13.1 mmol) and Tf_2O (1.66 mL, 9.9 mmol) at 0 °C. After stirring at RT for 1 h, the reaction was quenched by addition of H_2O, and the reaction mixture was extracted with CH_2Cl_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) δ 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_2CO_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_2O and Et_2O, and the resulting mixture was extracted with Et_2O (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) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{24}H_{21}F_3N_2O_2Na$ 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) δ 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_2Cl_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 **1h** (0.21 g, 1.4 mmol, 56% yield) as a white solid: ^{1}H NMR (400 MHz, Acetone-d_6) δ 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 (**3g**).* The desired product was obtained starting at **1h** 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 **3g** in 32% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{19}H_{19}F_3N_2O_2Na$ 387.1.

Synthesis of (*E*)-*N*-(2,4-dimethylphenyl)-2,2,2-trifluoro-*N*'-(4-fluoro-3-methoxybenzylidene)-acetohydrazide (3h). 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:ether) provided **3h** in 37% yield as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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 $C_{18}H_{16}F_4N_2O_2Na$ 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_2O (3 mL) were added K_2CO_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_2O and a saturated aqueous NH_4Cl 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 162.1, 159.4 (q, J = 36.4z), 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 $C_{20}H_{22}F_3N_3O_2Na$ 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 (**1l**).*

To a stirred mixture of 4-bromo-3-methoxyaniline (**1k**) (2.00 g, 9.9 mmol) in H_2O (50 mL) were added HCl (4 mL) and $NaNO_2$ (0.75 g, 10.9 mmol) in drops at 0 °C. The resulting mixture was stirred at 0 °C for 30 min, treated with K_2CO_3, and then added to a mixture of CuCN (1.06 g, 11.9 mmol) and KCN (1.61 g, 24.7 mmol) in H_2O (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 $MgSO_4$, 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: ^{1}H NMR (400 MHz, $CDCl_3$) 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% H_2SO_4 (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 $MgSO_4$, 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: ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.05 (s, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.56 (s, 1H),

7.47 (d, J = 8.0 Hz, 1H), 4.02 (s, 3H).

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 $NaHCO_3$ solution and brine. 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 give 2-(4-bromo-3-methoxyphenyl)-1,3-dioxolane (0.51 g, 2.0 mmol, 76% yield) as a colorless oil: ^{1}H NMR (400 MHz, Acetone-d_6) 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, *n*BuLi (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 NH_4Cl 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 (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: ^{1}H NMR (400 MHz, Acetone-d_6) 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 CH_2Cl_2 (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, H_2O was added, and the resulting mixture was extracted with CH_2Cl_2 (20 mL x 3). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

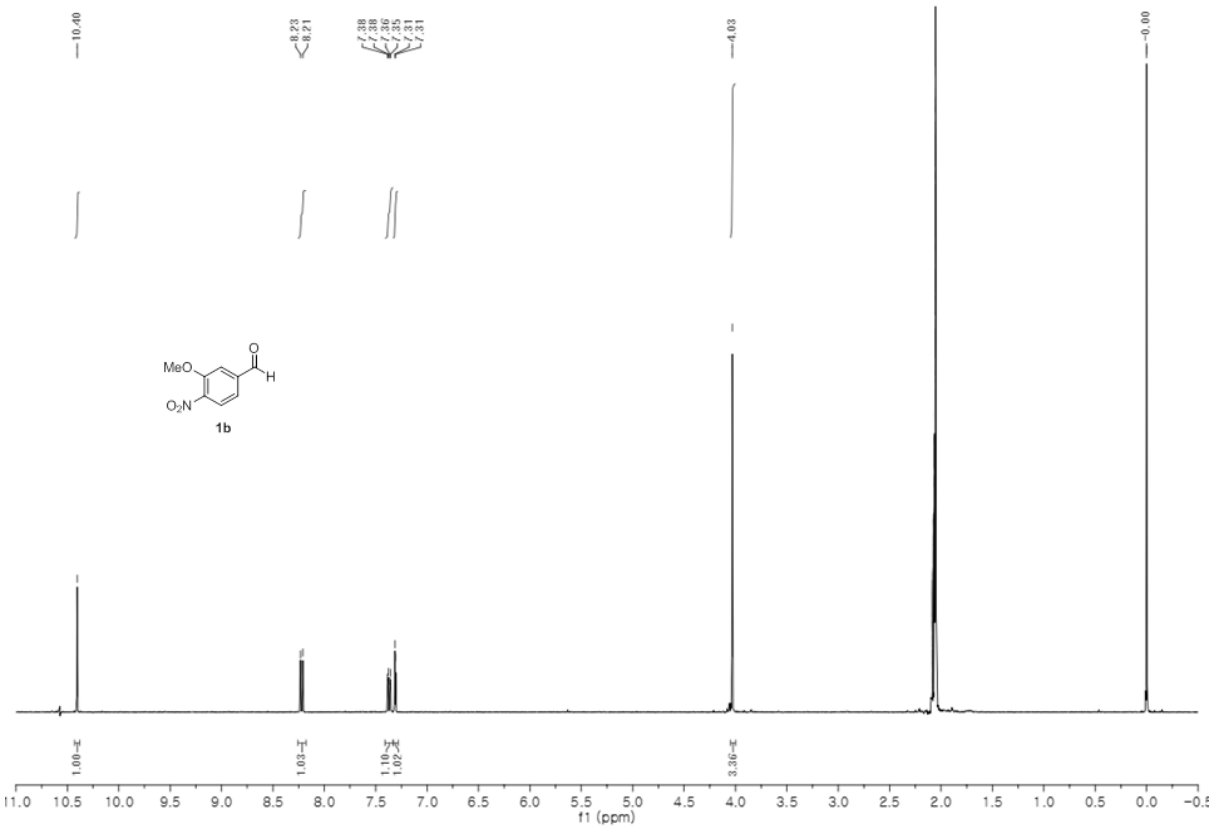
The residue was purified by column chromatography on silica gel (4:1 = hexanes:EtOAc) to afford 2-(4-(1,3-dioxolan-2-yl)-2-methoxybenzylidene)-malononitrile (0.11 mg, 0.4 mmol, 91% yield) as a yellow solid: ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.42 (s, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 7.28 (s, 1H), 7.25 (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 (**1l**) (0.11 g, 0.5 mmol, 77% yield) as a yellow solid: ^{1}H NMR (400 MHz, Acetone-d_6) δ 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).

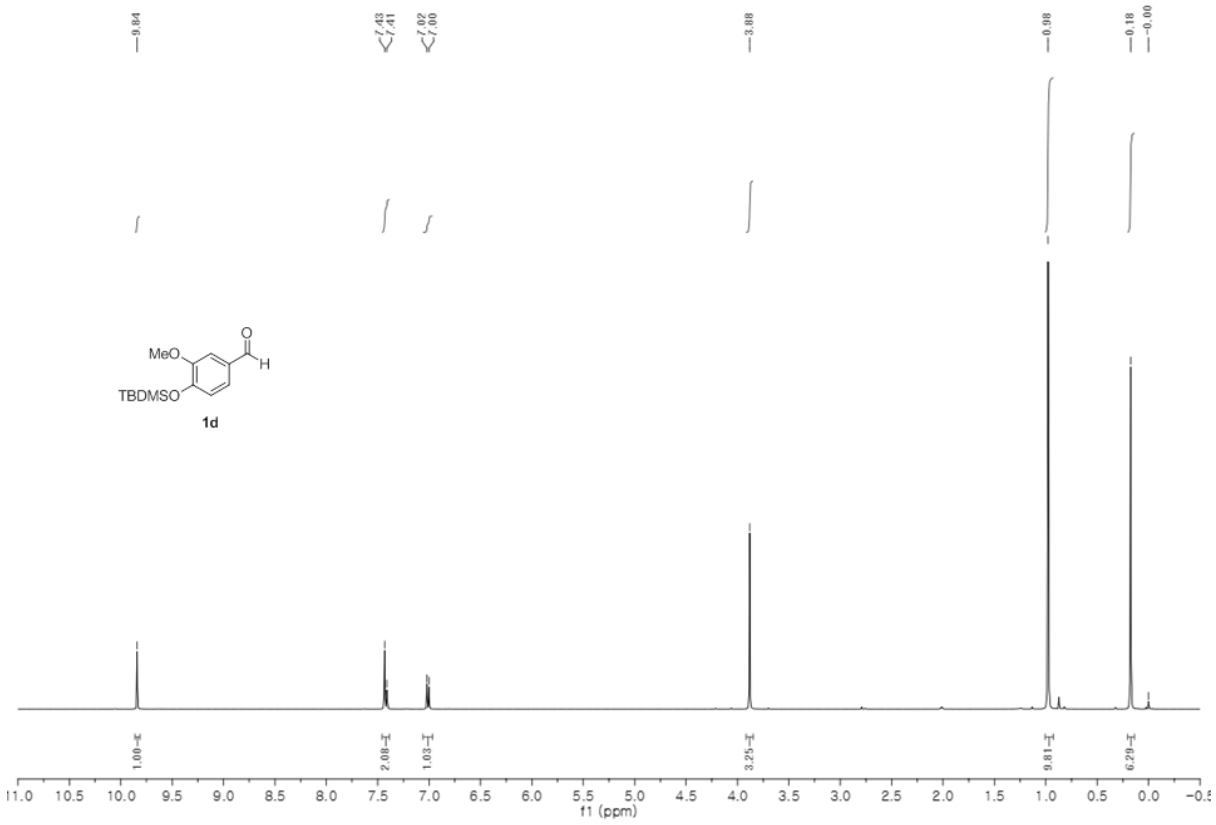
*Synthesis of (E)-N'-(4-(2,2-dicyanovinyl)-3-methoxybenzylidene)-N-(2,4-dimethylphenyl)-2,2,2-trifluoroacetohydrazide (**3j**)*. The desired product was obtained, starting from **1l** by using the same procedure for the synthesis of **3a**. Purification of the crude product by column chromatography on silica gel (8:1 = hexanes:EtOAc) provided **3j** in 57% yield as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) 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), 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 $C_{22}H_{17}F_3N_4O_2$ 449.1.

2. NMR spectra for the synthesized compounds

2.1. ^{1}H NMR for **1b**

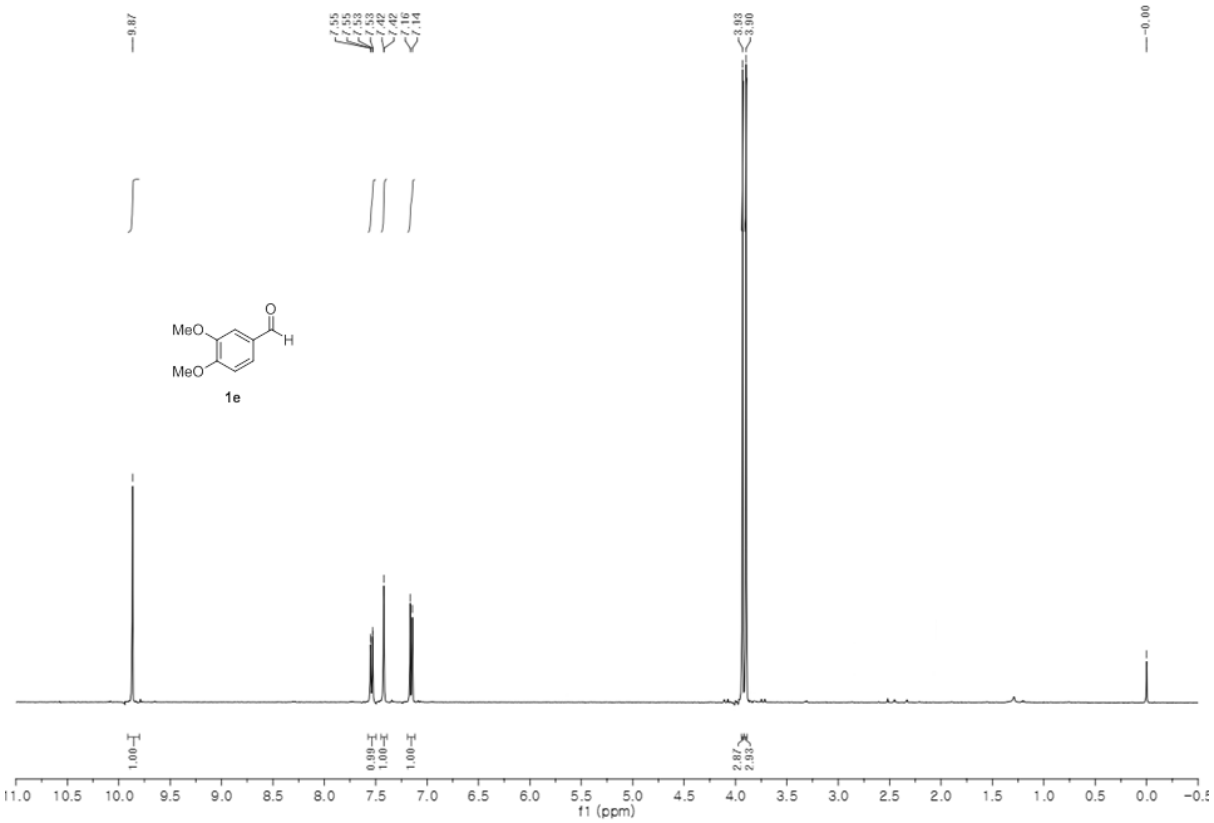


286 2.2. ^{1}H NMR for **1d**



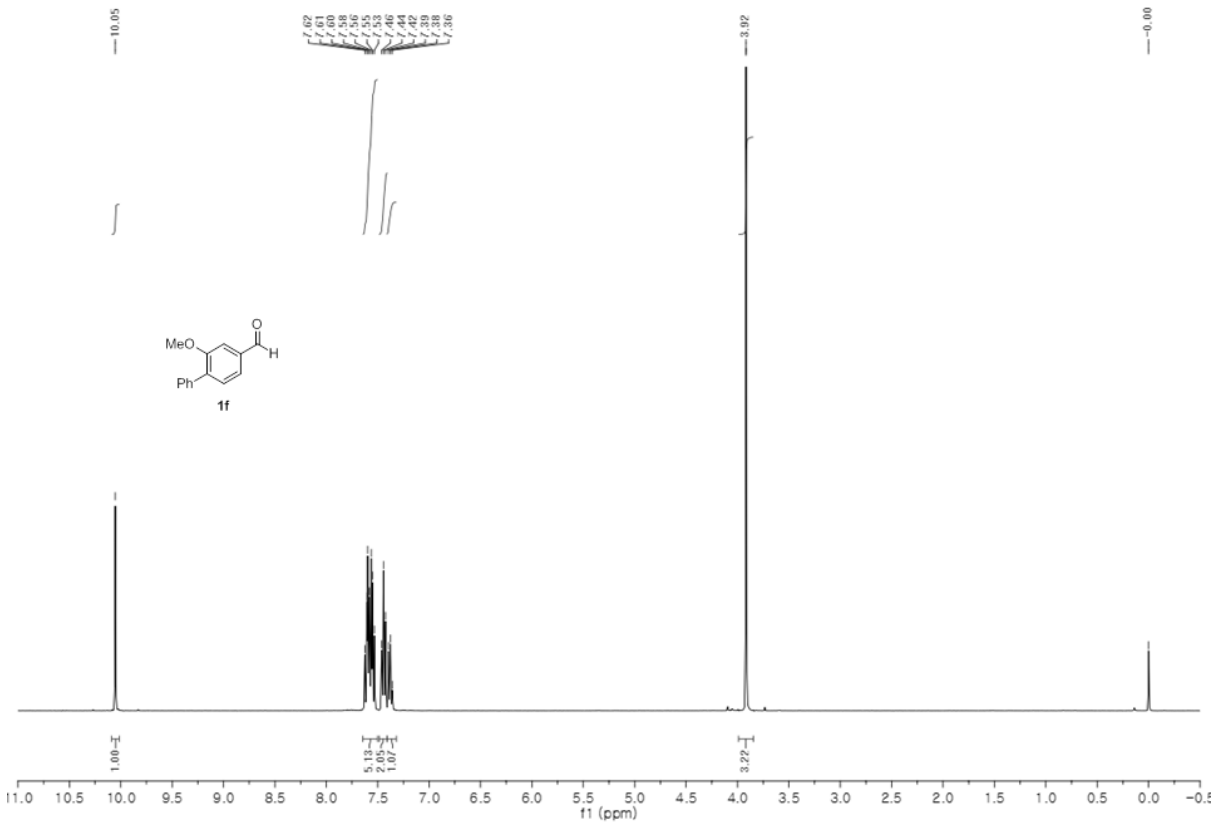
287

288 2.3. ^{1}H NMR for **1e**



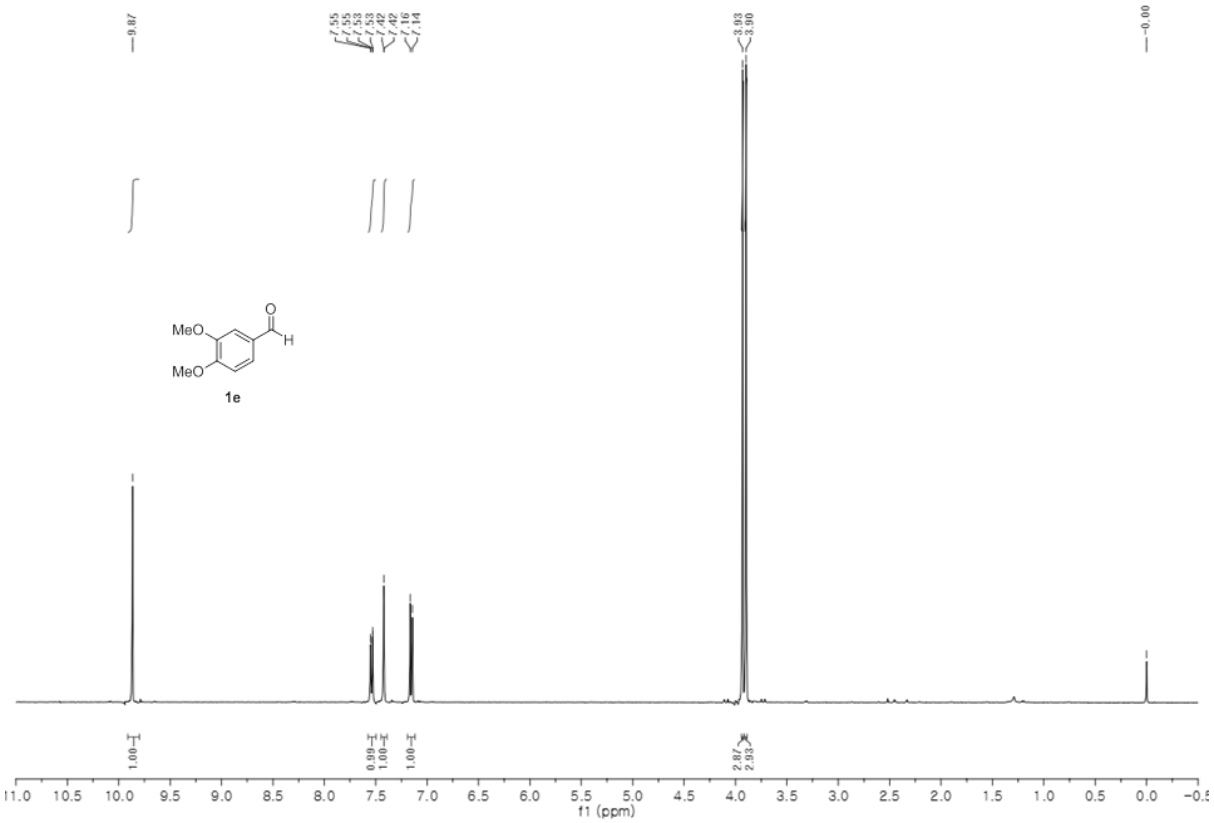
289

290 2.4. ^{1}H NMR for **1f**



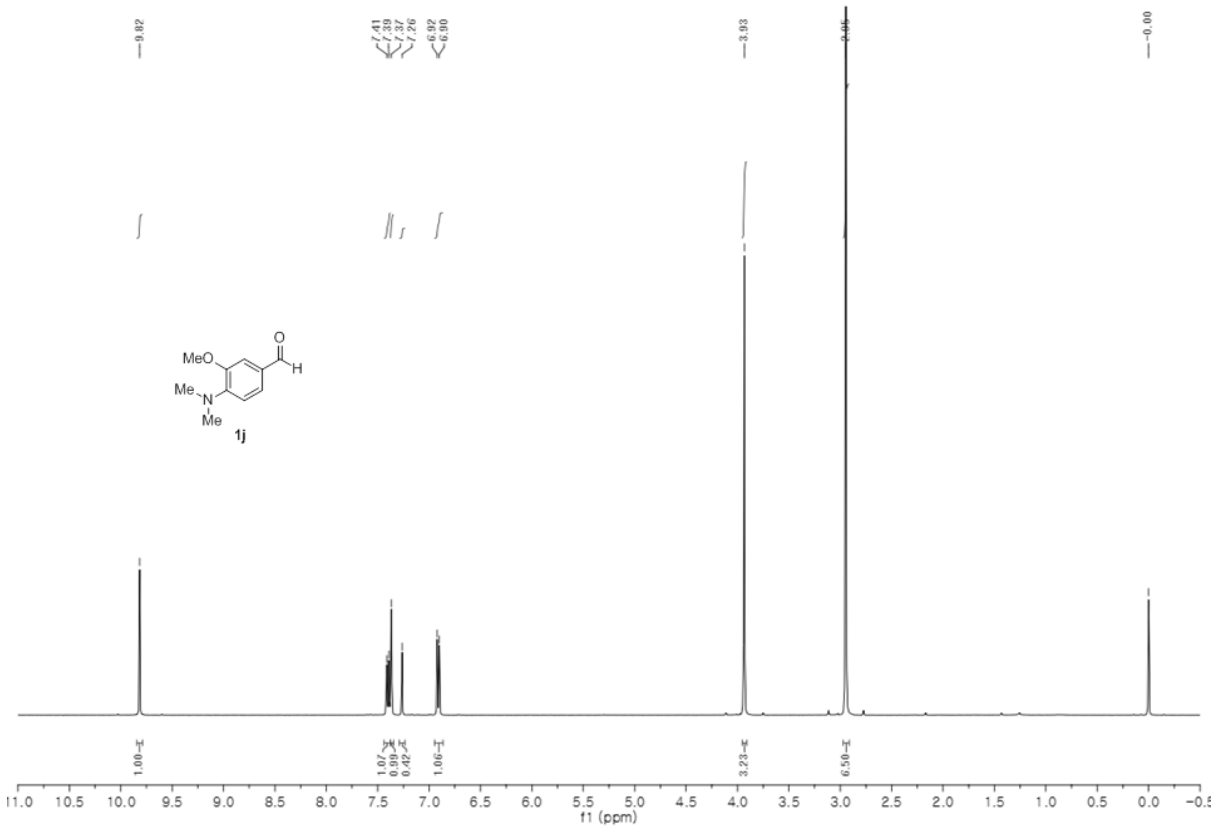
291

292 2.5. ^{1}H NMR for **1h**

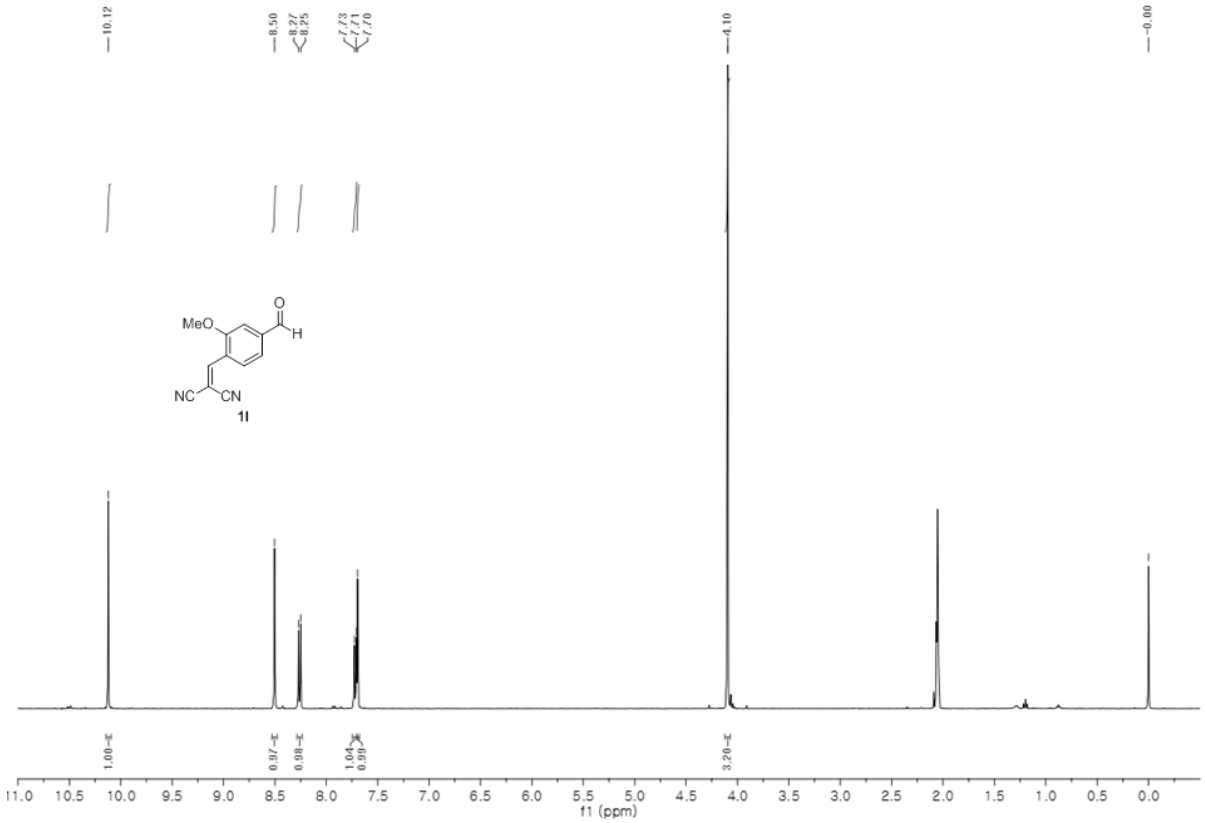


293

2.6. ^{1}H NMR for **1j**

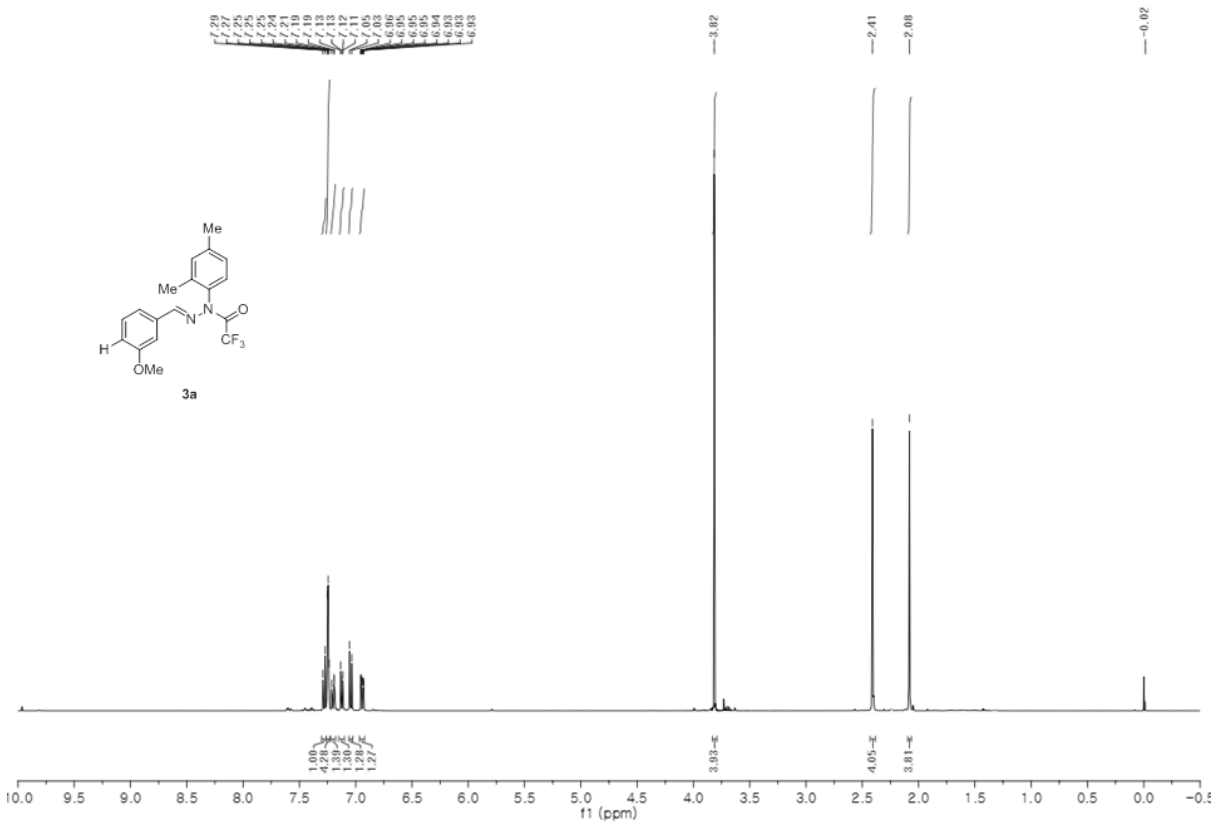


296 2.7. ^{1}H NMR for **1l**



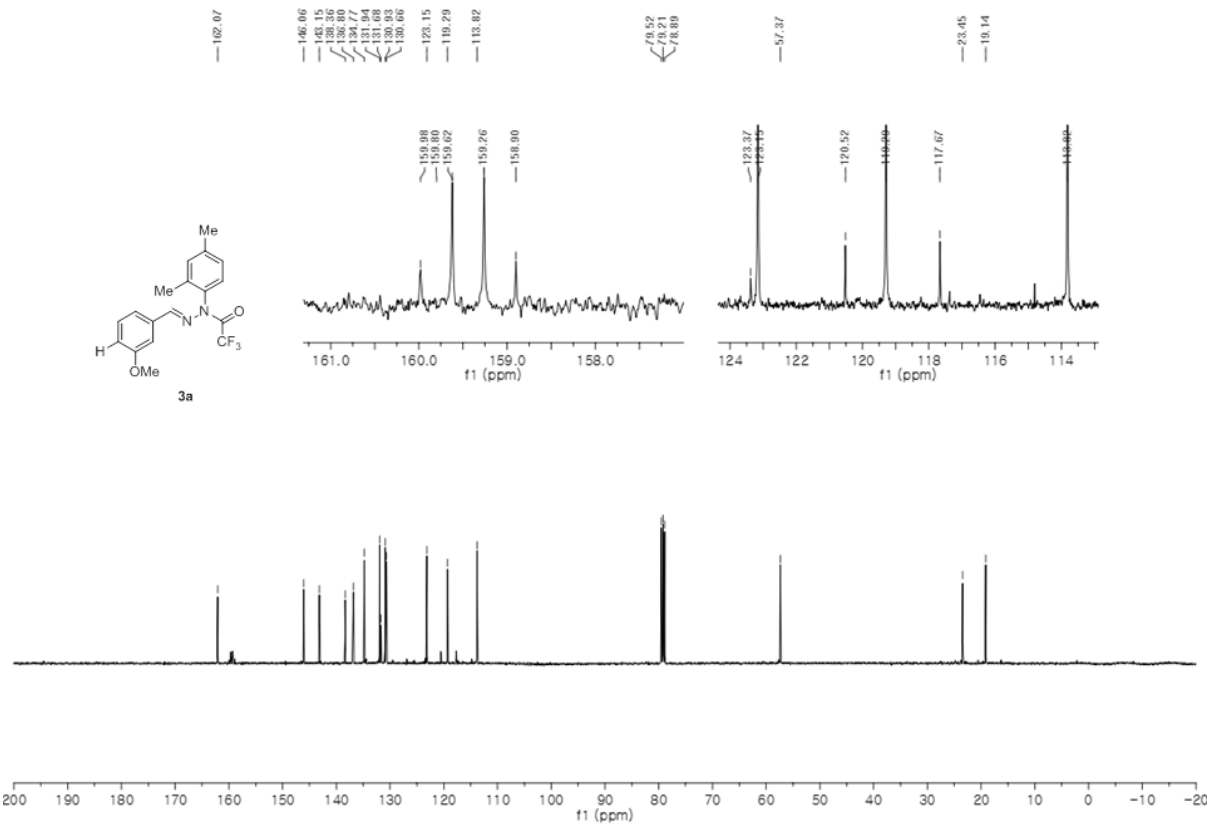
297

298 2.8. ^{1}H NMR for **3a**



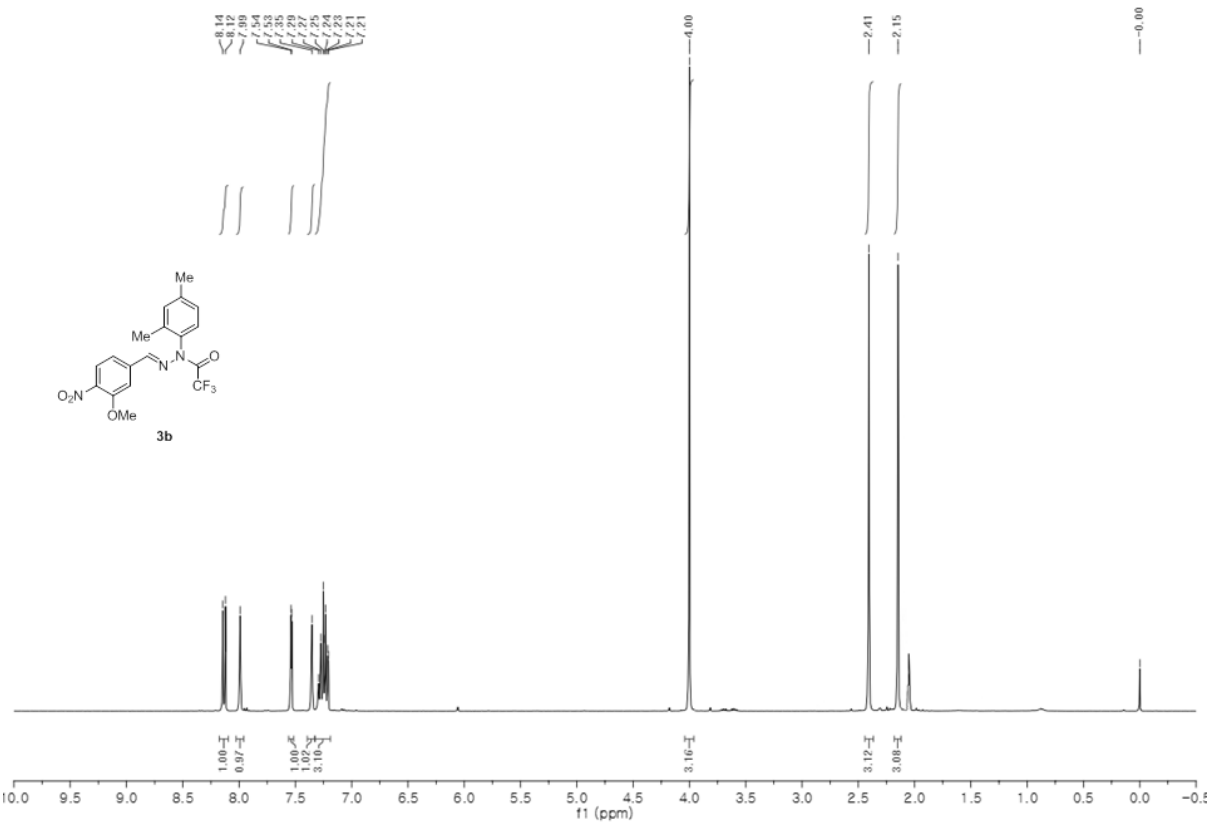
299

300 2.9. ^{13}C NMR for **3a**



301

302 2.10. ^{1}H NMR for **3b**



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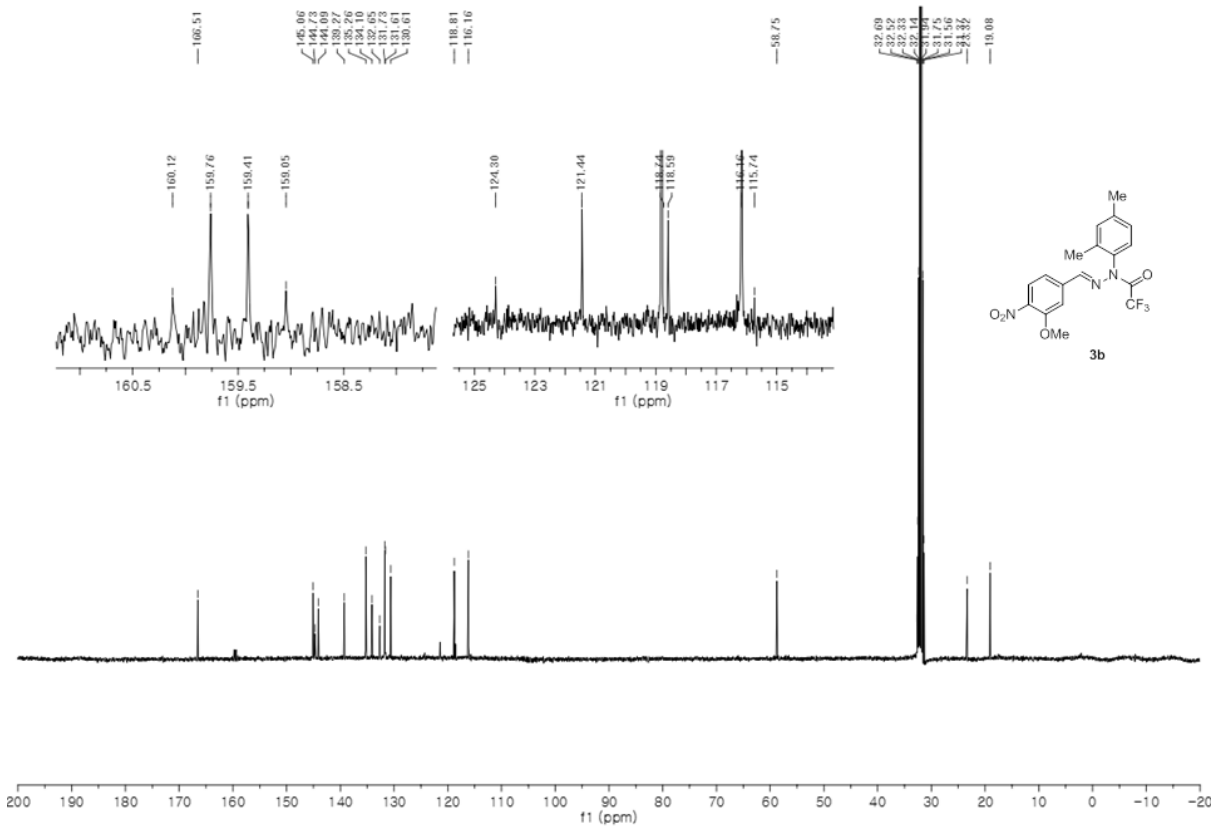
304 2.11. ^{13}C NMR for **3b**



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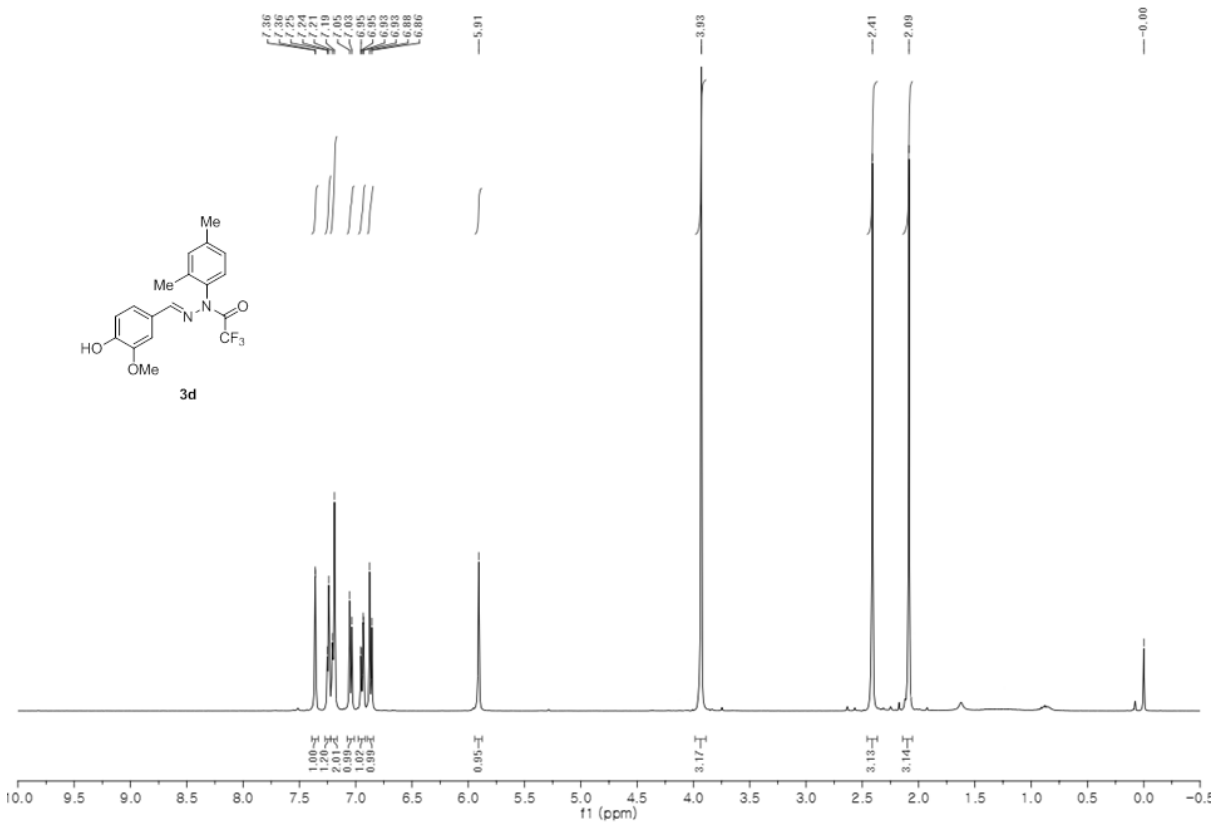
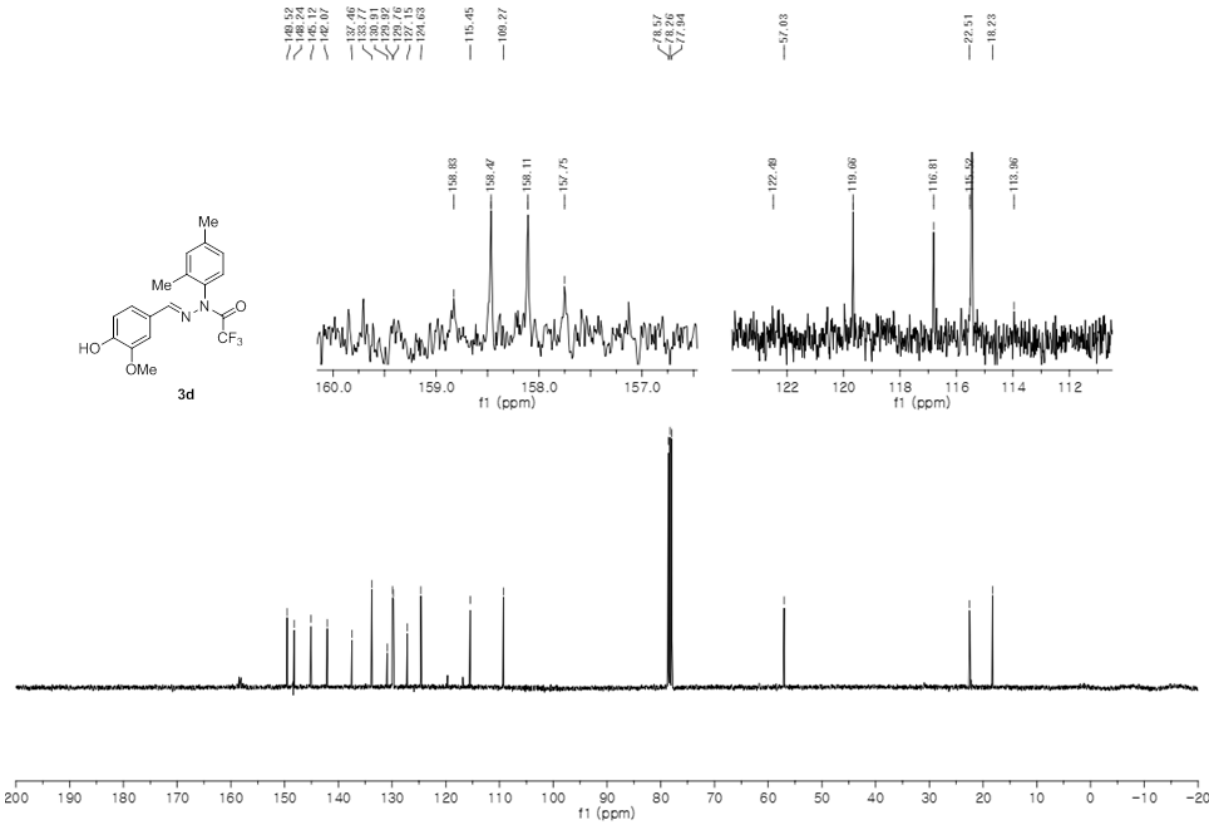
306 2.12. ^{1}H NMR for **3d**



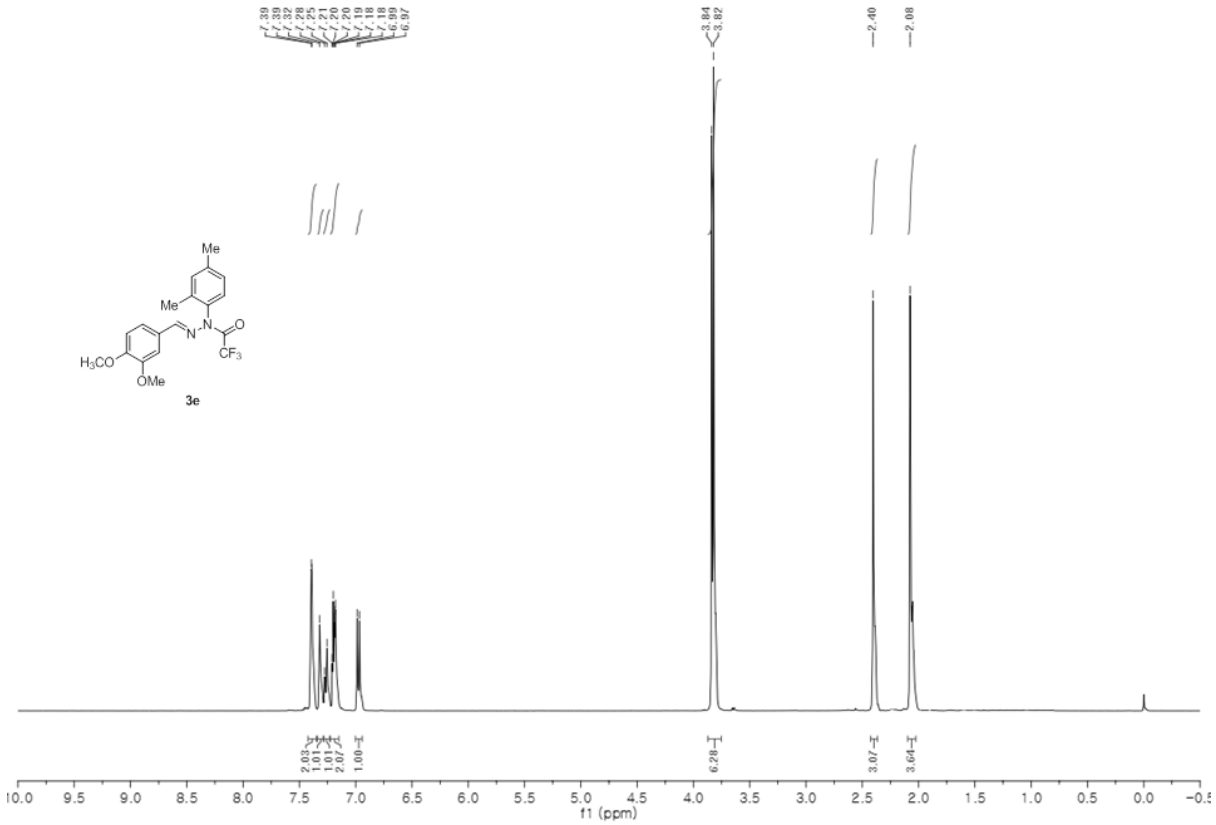
307

308 2.13. ^{13}C NMR for **3d**



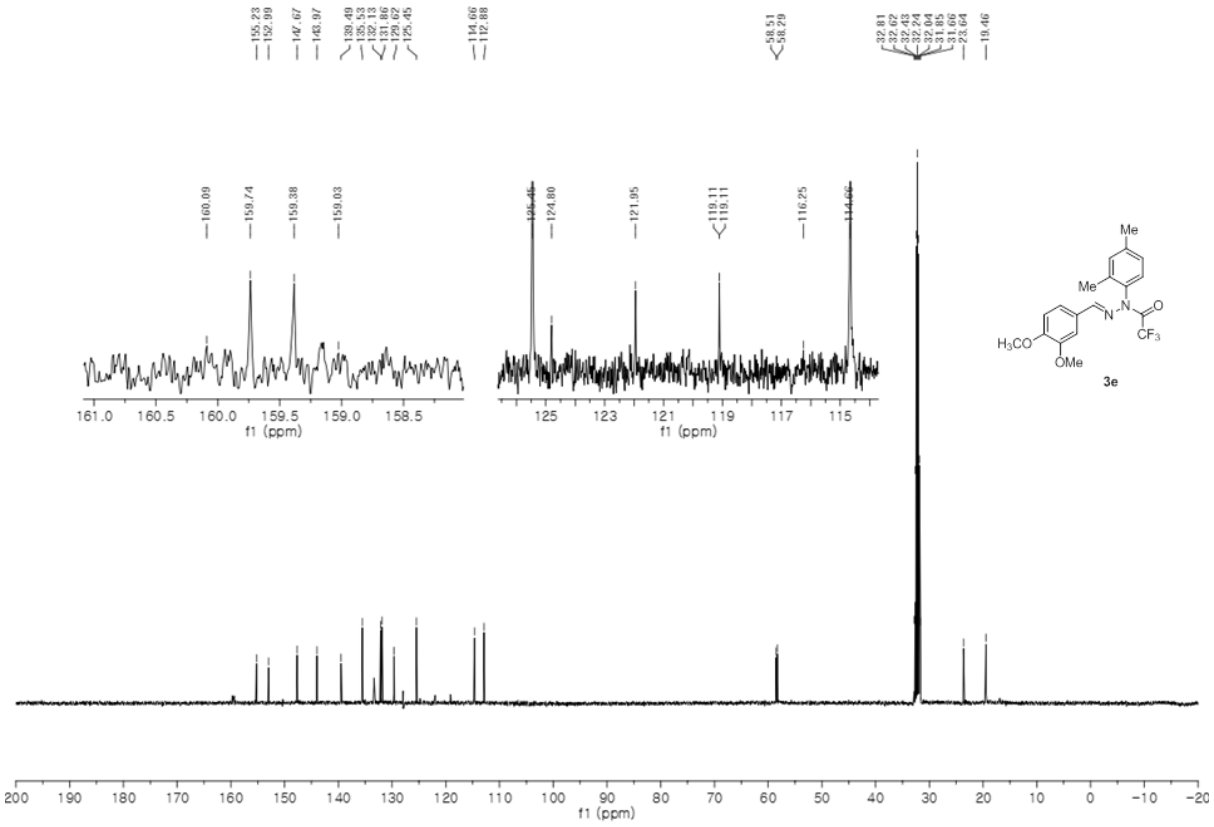
309

310 2.14. ^{1}H NMR for **3e**



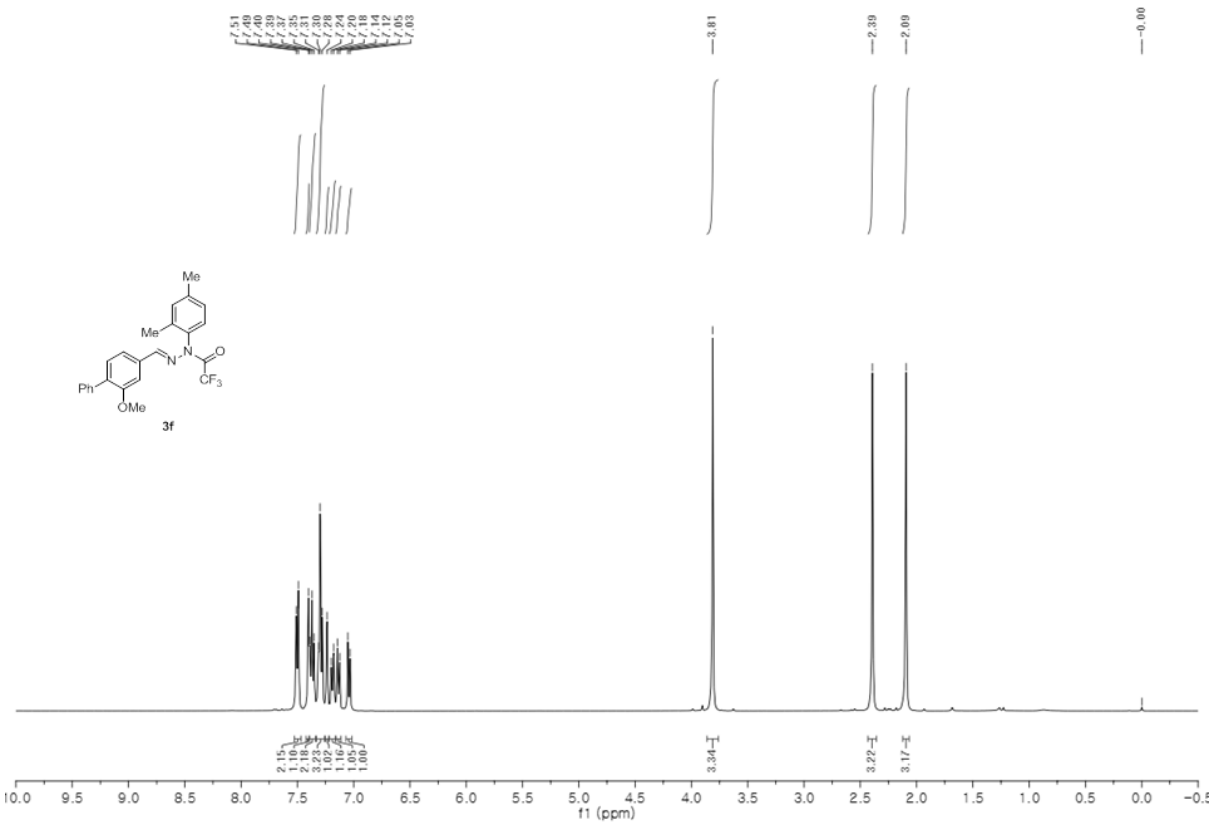
311

312 2.15. ^{13}C NMR for **3e**



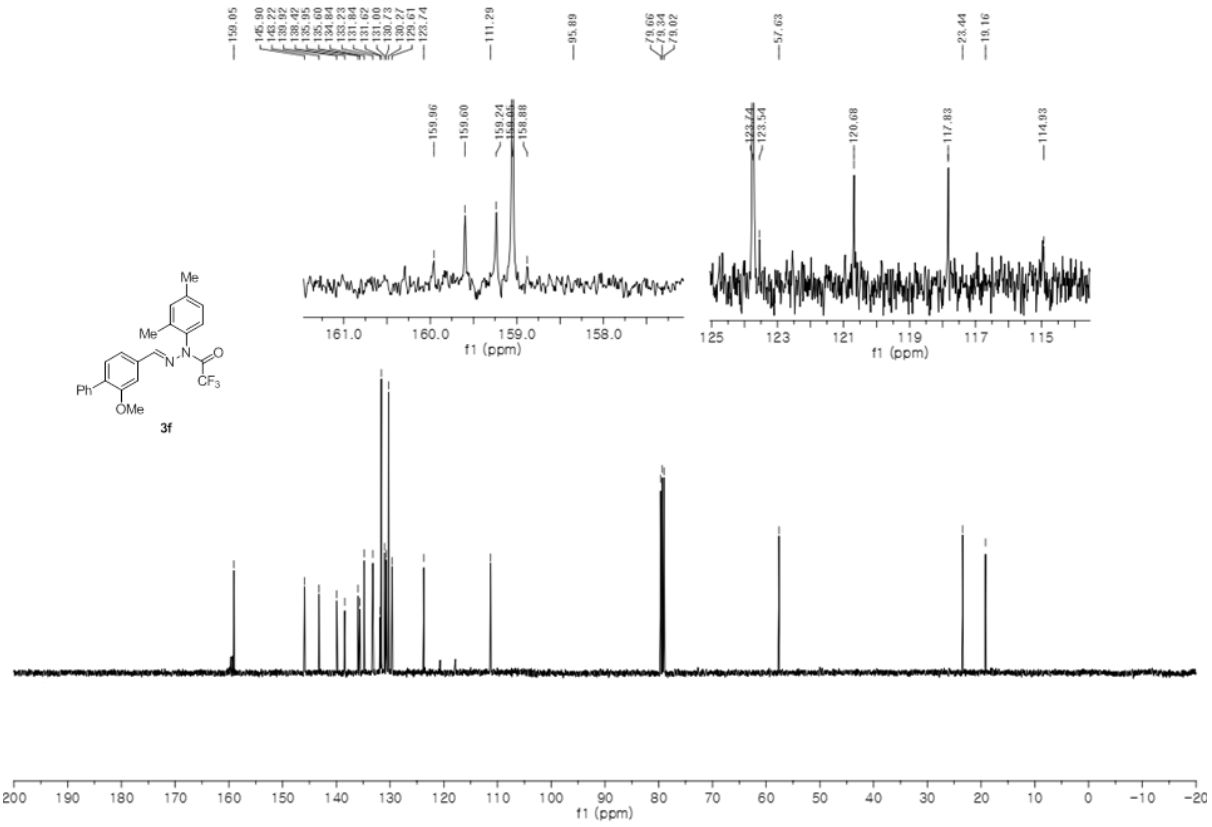
313

314 2.16. ^{1}H NMR for **3f**



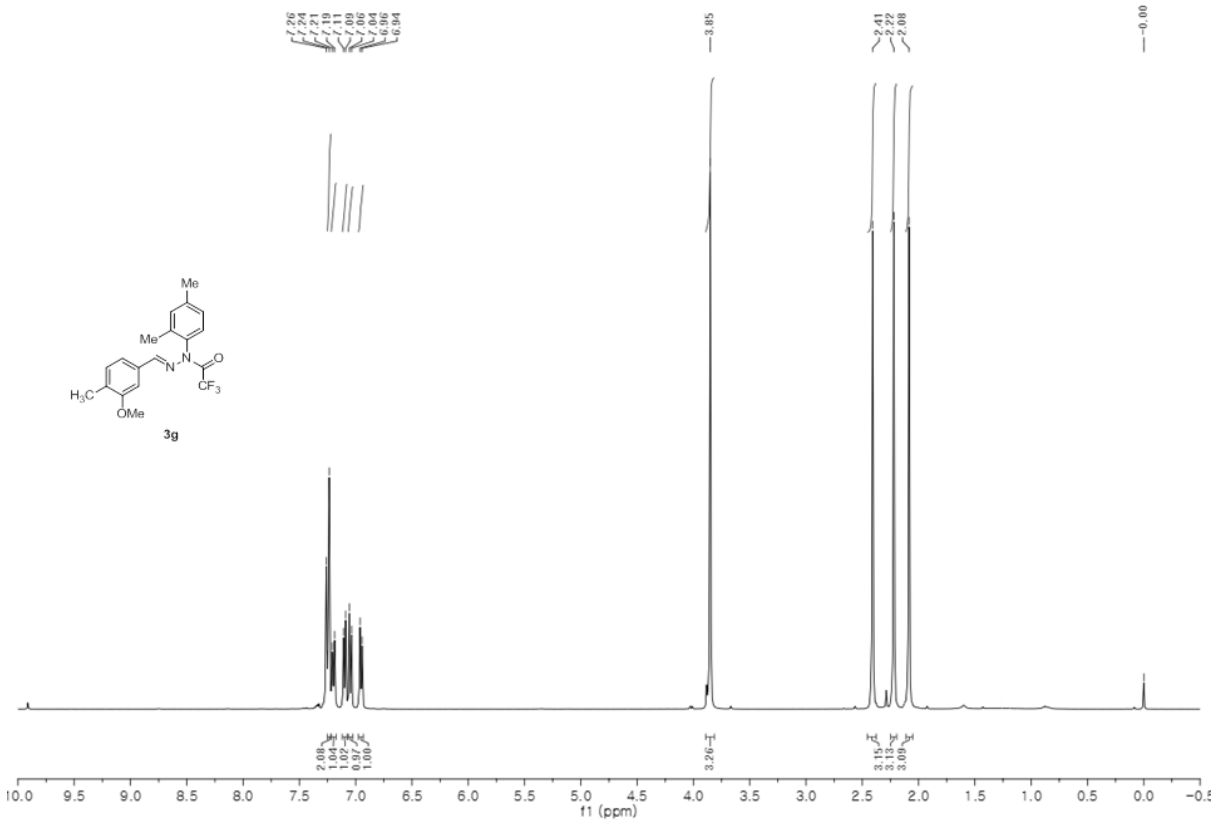
315

316 2.17. ^{13}C NMR for **3f**



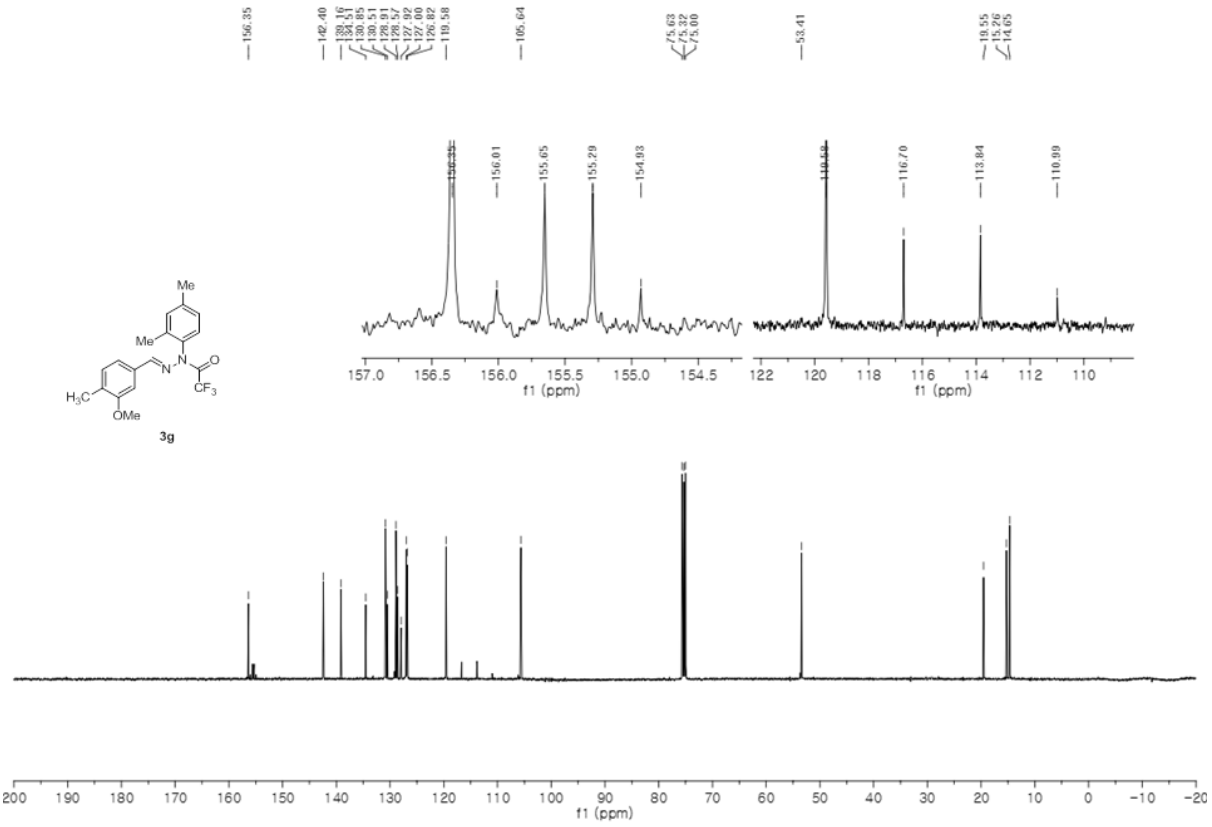
317

318 2.18. ^{1}H NMR for **3g**



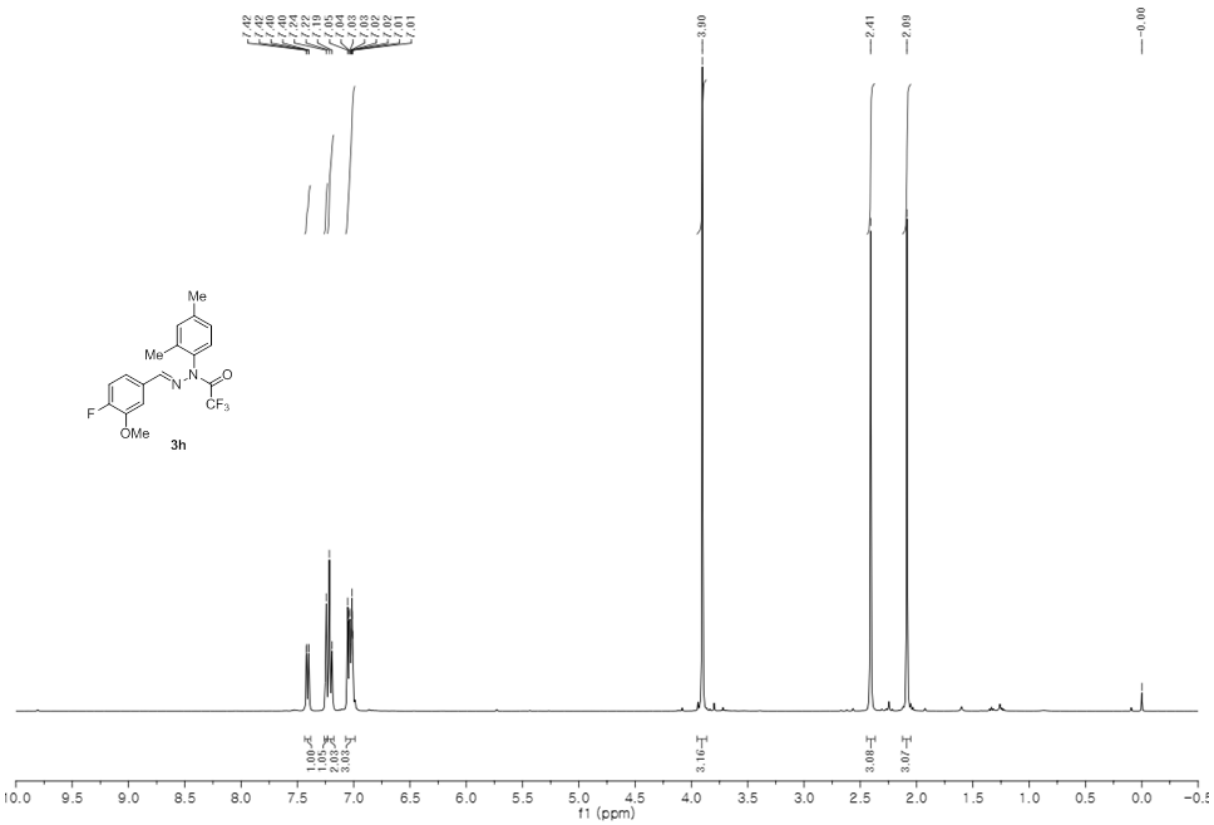
319

320 2.19. ^{13}C NMR for **3g**



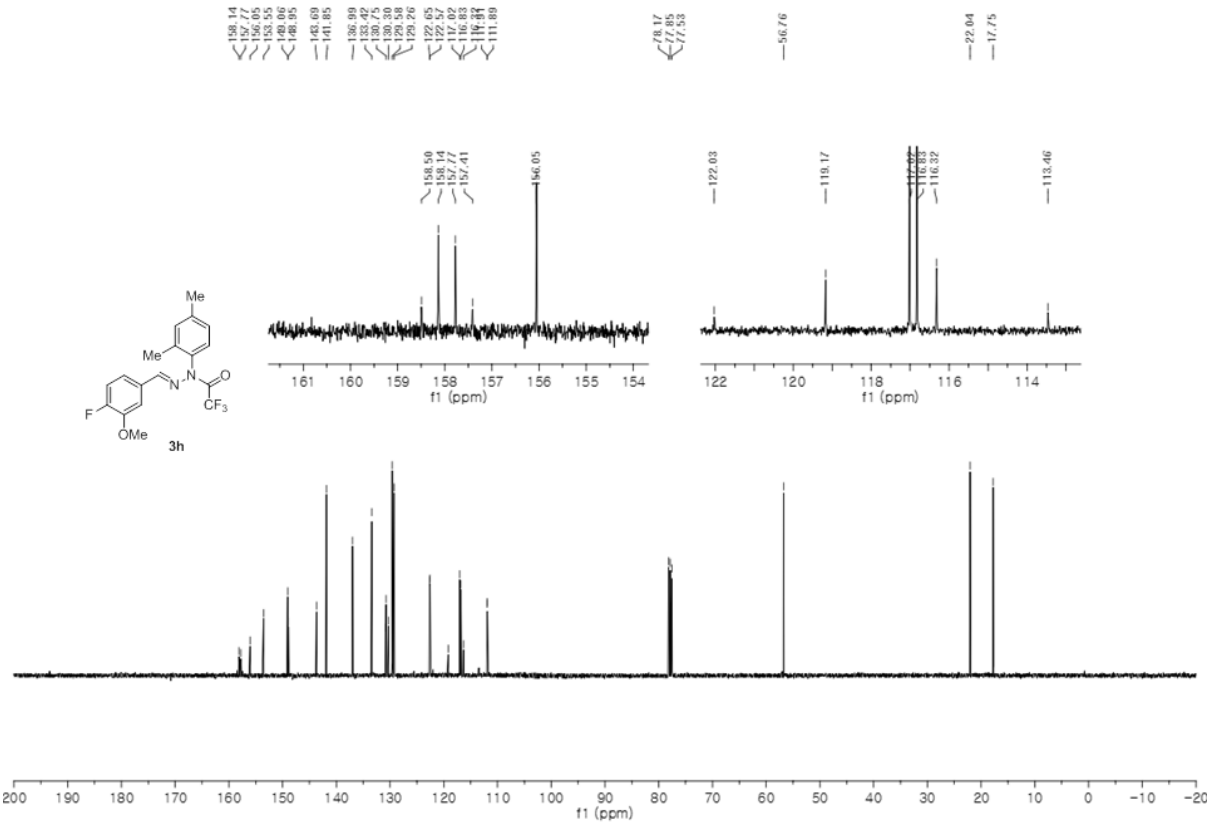
321

322 2.20. ^{1}H NMR for **3h**



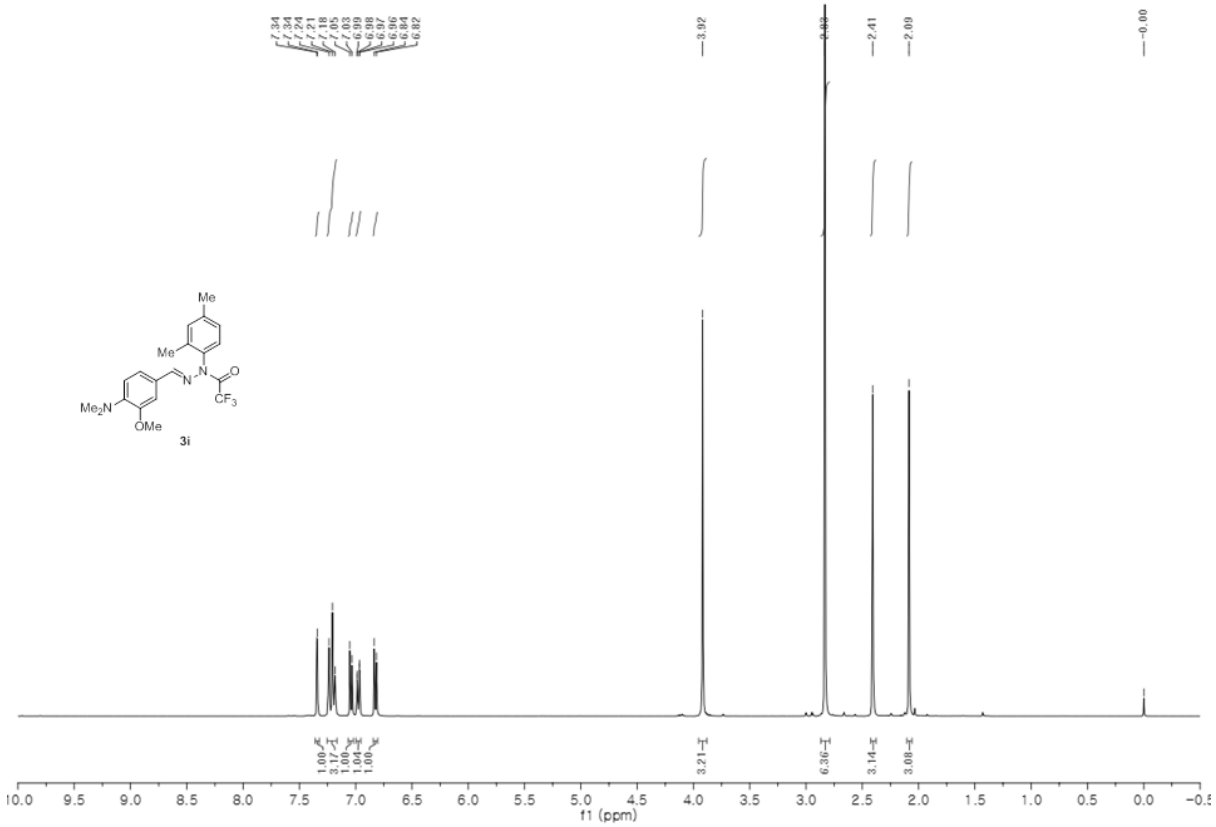
323

324 2.21. ^{13}C NMR for **3h**

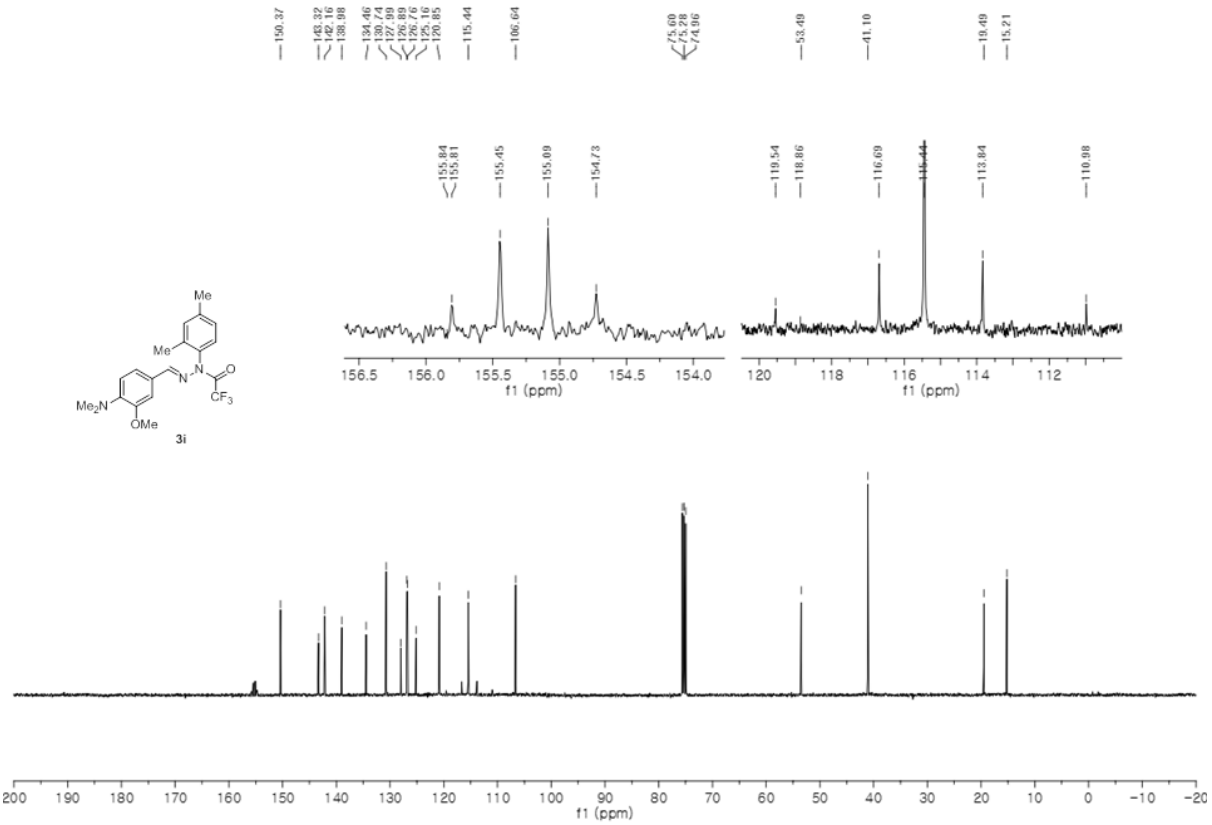


325

2.22. ^{1}H NMR for **3i**

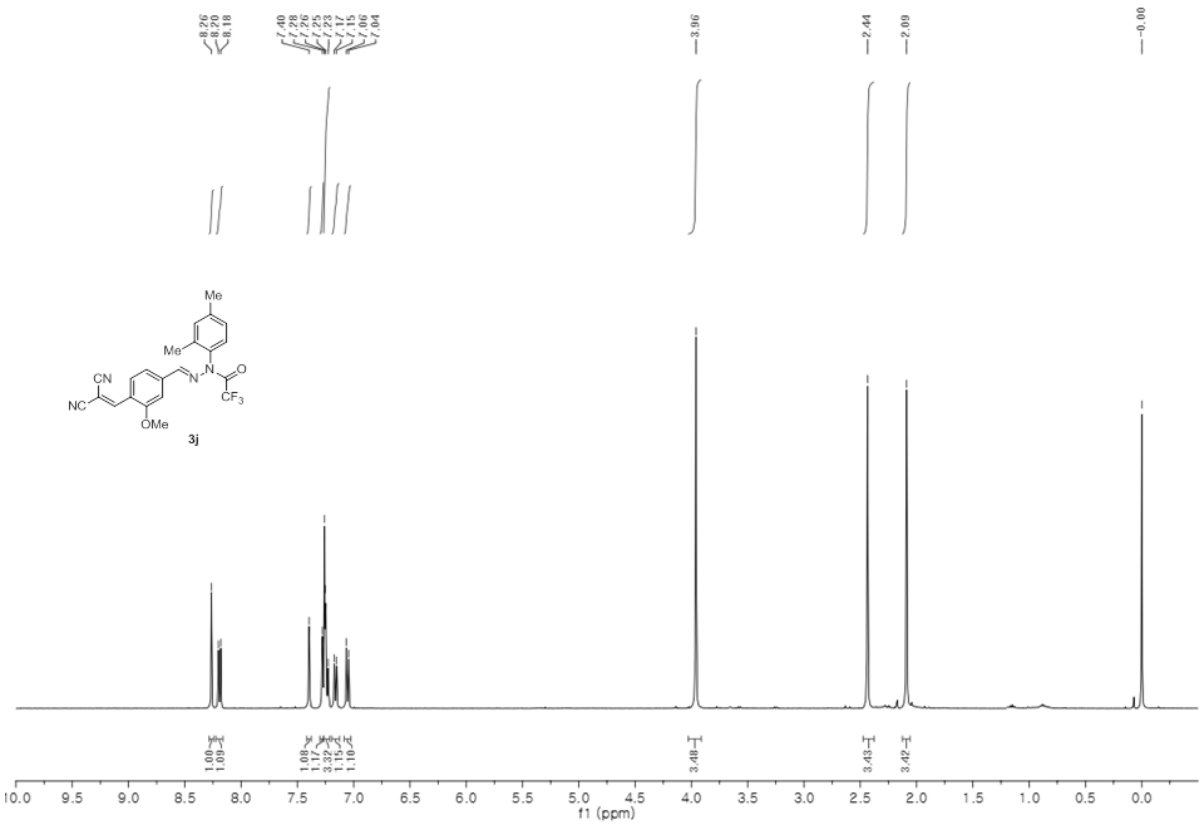


328 2.23. ^{13}C NMR for **3i**



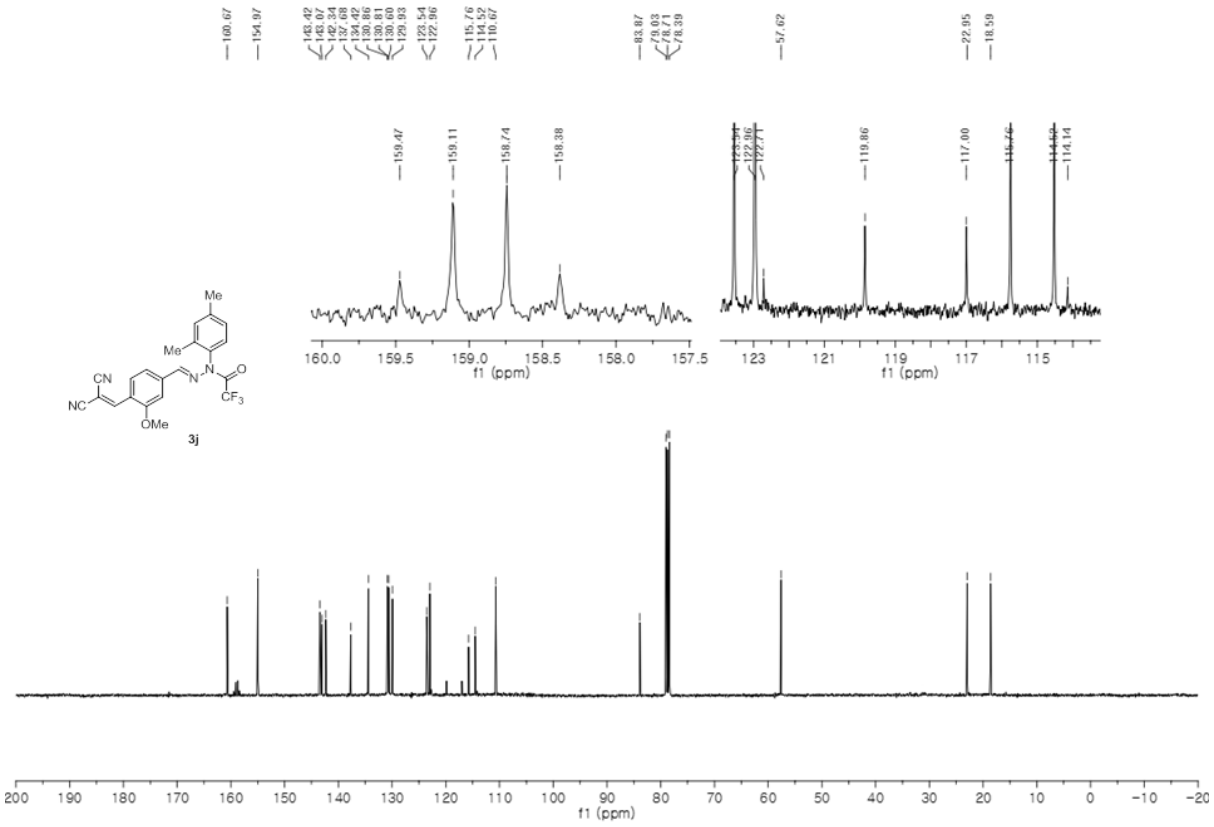
329

330 2.24. ^{1}H NMR for **3j**



331

332 2.25. ^{13}C NMR for **3j**



333

334

3. $A\beta_{42}$ Oligomerization. $A\beta_{42}$ 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 $A\beta_{42}$ 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. $A\beta_{42}$ Fibrillation. $A\beta_{42}$ stock solutions (2 mM) were prepared by dissolving the lyophilized peptide in 100 mM NaOH followed by water bath sonication for 30 s. The fibrilization 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 $A\beta_{42}$ 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 $A\beta_{42}$ oligomers and fibrils was further confirmed by AFM. $A\beta_{42}$ oligomers, fibrils or preformed $A\beta_{42}$ 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. $A\beta_{42}$ 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 H_2SO_4 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 IC_{50} values defined as the concentration of the J147 derivatives required to attain half-maximal absorbance, was obtained from the fit.

7. ThT Fluorescence Assay. $A\beta_{42}$ fibrillation and $A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ was subtracted from solution containing $A\beta_{42}$. 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 IC_{50} and EC_{50} 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 $A\beta_{42}$ Fibrils. $A\beta_{42}$ fibrils were prepared by incubating 20 mM $A\beta_{42}$ monomers for 48 h, which is sufficiently long enough to enable $A\beta_{42}$ 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 $A\beta_{42}$ fibril solution. The disruption of the $A\beta_{42}$ 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 $A\beta_{42}$ was subtracted from solution containing $A\beta_{42}$. 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 EC_{50} 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% CO_2 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 $A\beta_{42}$ oligomers or fibrils (500 μM) were added to 90 μL of new medium. After incubation in 5% CO_2 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 $A\beta_{42}$ 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