Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Design and biological characterization of hybrid compounds of curcumin and thalidomide for multiple myeloma

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Chemistry (S1)

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Flash chromatography was performed on silica gel (200-300 mesh, Fisher Scientific) using solvents as indicated. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ultrashield Plus-400MHz spectrometer. HRMS were recorded on a Waters ESI-QToF II instrument or a PerkinElmer AxION 2 TOF instrument. IR was recorded on a thermo scientific Nicollet iS10 machine.

Compound 3

Thalidomide carboxylic acid 9 (0.33 mmol) was dissolved in dichloromethane (DCM, 2 mL) and tetrahydrofuran (THF, 2 mL). Curcumin 2 (0.66 mmol) and DMAP (0.66 mmol) were then added. The solution was cooled to 0 °C and EDC (0.50 mmol) was added. The reaction was stirred for 1 h at 0 °C and then warmed to room temperature overnight. The solution was washed with brine (5 mL), and the organic layer was dried over anhydrous Na_2SO_4. After filtration, the mixture was concentrated on a rotary evaporator. The crude residue was purified by flash chromatography (DCM/MeOH: 20/1) to give an orange solid, yield 46%. ^{1}HNMR (400 MHz, DMSO-d6) δ 2.10-2.13 (m, 1H), 2.49-2.66 (m, 2H), 2.87-2.97 (m, 1H), 3.86 (s, 3H), 3.90 (s, 3H), 5.22-5.26 (m, 1H), 6.15 (s, 1H), 6.78-6.85 (m, 2H), 6.99-7.03 (d, J = 15.96 Hz, 1H), 7.17-7.19 (dd, J = 1.64, 8.24 Hz, 1H), 7.34-7.35 (d, J = 1.64 Hz, 1H), 7.38-7.42 (m, 2H), 7.58-7.69 (m, 3H), 8.15-8.17 (d, J = 7.8 Hz, 1H), 8.46 (s, 1H), 8.58-8.60 (dd, J = 1.36, 7.80 Hz, 1H), 9.68 (s, 1H), 11.17 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 21.9, 30.9, 49.3, 55.7, 56.2, 101.3, 111.5, 112.2, 115.7, 121.2, 121.3, 123.3, 123.9, 124.2, 124.9, 126.2, 131.9, 134.1, 134.4, 135.4, 136.3, 138.8, 140.4, 141.6, 148.0, 149.6, 151.0, 162.4, 166.1, 166.2, 169.6, 172.6, 181.3, 185.0. HRMS (m/z) $(M-H)^-$: calcd. for $C_{35}H_{27}N_2O_{11}$ 651.1620, found 651.1633.

Compound 4

Compound 11 (0.09 mmol) was dissolved in DMSO (1 mL) and MeOH (1 mL). The mixture was heated to 100 °C for 20 min, and then cooled to room temperature. Water (5 mL) was added and the mixture was extracted with ethyl acetate (EtOAc, 10 mL). The combined organic phase was washed with water (5 mL) and dried over anhydrous Na_2SO_4. After filtration solvents were removed under reduced pressure and the residue was purified by flash chromatography (EtOAc) to give an orange solid, yield 22%.

^{1}HNMR (400 MHz, DMSO-d6) δ 2.07-2.08 (m, 1H), 2.50-2.64 (m, 2H), 2.87-2.90 (m, 1H), 3.85 (s, 3H), 5.16-5.21 (m, 1H), 6.18 (s, 1H), 6.82-6.86 (m, 2H), 7.18-7.20 (d, J = 8.28 Hz, 1H), 7.27-7.31 (d, J = 16.00 Hz, 1H), 7.36 (s, 1H), 7.61-7.65 (d, J = 15.76 Hz, 1H), 7.74-7.78 (d, J = 15.88 Hz, 1H), 7.96-7.98 (d, J = 7.76 Hz, 1H), 8.17-8.19 (d, J = 7.76 Hz, 1H), 8.32 (s, 1H), 9.72 (s, 1H), 11.14 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 21.9, 30.9, 49.1, 55.7, 102.4, 111.5, 115.7, 121.2, 122.2, 123.7, 124.0, 126.1, 128.1, 131.4, 132.2, 134.5, 136.9, 141.5, 142.4, 148.0, 149.8, 166.6, 166.8, 169.8, 172.7, 179.2, 186.5. HRMS (m/z) $(M-H)^-$: calcd. for $C_{27}H_{21}N_2O_8$ 501.1303, found 501.1317.

Compound 5

The mixture of curcumin 2 (0.7 mmol), aldehyde 13 (0.4 mmol), and $(BuO)_3B$ (1.0 mmol) in EtOAc (15 mL) was refluxed, then piperidine (0.4 mmol) was added dropwise and the mixture was refluxed for 2 h. After cooling down to room temperature, 0.4 M HCl was added and the mixture was stirred for 1 h, then extracted with EtOAc (2 × 5 mL), and the organic phase was dried over anhydrous Na_2SO_4. After filtration solvents were removed under reduced pressure and the residue was purified by flash chromatography (DCM/MeOH: 20/1) to give compound 5 as a yellow solid, yield 10%. ^{1}HNMR (400 MHz, CDCl3) δ 2.09-2.13 (m, 1H), 2.72-2.92 (m, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 4.94-4.99 (m, 1H), 6.00 (brs, 2H), 6.77-6.81 (d, J = 16.16 Hz, 1H), 6.87-6.93 (m, 3H), 6.97-6.98 (d, J = 1.80 Hz, 1H), 7.02-7.05 (m, 2H), 7.16-7.19 (dd, J = 1.68, 8.24 Hz, 1H), 7.42-7.46 (d, J = 16.16 Hz, 1H), 7.79-7.84 (m, 4H), 7.94 (s, 1H), 8.10 (s, 1H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 22.6, 31.3, 49.5, 56.0, 56.1, 110.0, 110.4, 114.8, 114.9, 119.4, 124.2, 124.5, 124.6, 124.8, 126.2, 127.0, 132.0, 132.3, 135.6, 136.8, 140.4, 144.5, 146.8, 146.9, 148.8, 149.0, 149.4, 166.4, 166.5, 167.7, 170.5, 186.3, 196.5. HRMS (m/z) $(M-H)^-$: calcd. for $C_{35}H_{27}N_2O_{10}$ 635.1671, found 635.1702.

Compound 6

To the mixture of compound 11 (0.1 mmol), B_2O_3 (0.15 mmol), and aldehyde 13 (0.1 mmol) one drop of acetic acid and one drop of morpholine were added, then the mixture was microwaved (200 W) for 5 min. The residue was purified by flash chromatography (hexane/EtOAc: 1/1 to 1/3) to give compound 6 as a yellow solid, yield 25%. ^{1}HNMR (400 MHz, CDCl3) δ 2.11-2.16 (m, 1H), 2.43 (s, 3H), 2.72-2.92 (m, 3H), 3.91 (s, 3H), 4.93-4.98 (m, 1H), 5.99 (s, 1H), 6.70-6.74 (d, J = 16.16 Hz, 1H), 6.88-6.90 (d, J = 8.20

Hz, 1H), 6.97-7.05 (m, 2H), 7.33-7.37 (d, J = 16.16 Hz, 1H), 7.76 (s, 1H), 7.82 (s, 2H), 7.91 (s, 1H), 8.04 (s, 1H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 22.5, 27.6, 31.3, 49.5, 56.0, 109.8, 114.9, 124.2, 124.3, 124.6, 124.7, 126.1, 132.2, 132.4, 135.6, 137.0, 139.9, 143.2, 146.9, 148.3, 149.4, 166.2, 166.3, 167.6, 170.5, 195.1, 196.4. HRMS (m/z) $(M-H)^-$: calcd. for $C_{27}H_{21}N_2O_8$ 501.1303, found 501.1318.

Compound 7

To a solution of compound 15 (6.0 mmol) in dioxane (60 mL) aldehyde 13 (4 mmol) and borontrifluoride etherate (6 mmol) were added, then the mixture was stirred at 50 °C overnight. The precipitate was collected by filtration and washed with DCM to give compound 7 as a greenish yellow solid, yield 25%. ^{1}HNMR (400 MHz, DMSO-d6) δ 2.07-2.11 (m, 1H), 2.49-2.64 (m, 2H), 2.88-2.91 (m, 1H), 3.86 (s, 3H), 5.17-5.22 (m, 1H), 6.85-6.87 (d, J = 8.16 Hz, 1H), 7.09-7.13 (d, J = 16.08 Hz, 1H), 7.23-7.25 (dd, J = 1.76, 8.20 Hz, 1H), 7.38-7.39 (d, J = 1.80 Hz, 1H), 7.73-7.77 (d, J = 15.96 Hz, 1H), 7.82-7.88 (m, 2H), 7.99-8.00 (d, J = 7.76 Hz, 1H), 8.24-8.26 (dd, J = 1.04, 7.76 Hz, 1H), 8.42 (s, 1H), 9.74 (s, 1H), 11.15 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 21.9, 30.9, 49.1, 55.7, 111.5, 115.7, 122.4, 123.4, 123.6, 124.0, 126.1, 128.6, 131.7, 132.1, 135.0, 139.4, 141.5, 144.6, 148.0, 149.8, 166.7, 166.8, 169.8, 172.7, 188.1. HRMS (m/z) $(M-H)^-$: calcd. for $C_{25}H_{19}N_2O_7$ 459.1198, found 459.1218.

Compound 11

A solution of 2,4-pentendione (8.0 mmol) and B_2O_3 (7.2 mmol) in EtOAc (5 mL) was stirred at 80 ºC for 30 min, then an EtOAc (10 mL) solution of vanillin 10 (3.6 mmol) and $(BuO)_3B$ (3.6 mmol) was added. After stirring for 30 min at 80 ºC, n-$BuNH_2$ (3.6 mmol) was added dropwise, and the reaction mixture was stirred at 100 ºC for an additional 1 h. The reaction mixture was cooled to 50 ºC, then 1 M HCl (10 mL) was added and the mixture was stirred at this temperature for 30 min. The mixture was cooled to room temperature and extracted with EtOAc (2 × 15 mL), washed with water (30 mL), and dried over anhydrous Na_2SO_4. After filtration solvents were removed under reduced pressure and the residue was purified by flash chromatography (hexane/EtOAc: 8/2) to obtain compound 11 as yellow solid, yield 60%. ^{1}HNMR (400 MHz, $CDCl_3$) δ 2.15 (s, 3H), 3.93 (s, 3H), 5.63 (s, 1H), 5.94 (brs, 1H), 6.30-6.34 (d, J = 15.80 Hz, 1H), 6.91-6.93 (d, J = 8.16 Hz, 1H), 7.01 (d, J = 1.82 Hz, 1H), 7.07-7.09 (dd, J = 1.82, 8.16 Hz, 1H),

7.51-7.55 (d, J = 15.76 Hz, 1H), 15.5 (brs, 1H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 26.8, 56.0, 100.7, 109.5, 114.8, 120.3, 122.7, 127.7, 140.1, 146.8, 147.8, 178.0, 197.0; HRMS (m/z) $(M-H)^-$: calcd. for $C_{13}H_{13}O_4$ 233.0819, found 233.0821.

Compound 13

To a bromomethylthalidomide 12 (1.4 mmol) solution in DMSO (25 mL) IBX (2.8 mmol) was added. The mixture was stirred at 65 °C for 4 h, then cooled to room temperature. After addition of water (50 mL), the reaction mixture was extracted with DCM (50 mL) and the combined organic phase was washed with water (3 × 50 mL) and saturated $NaHCO_3$ (25 mL) and then dried over anhydrous Na_2SO_4. After filtration solvents were removed under reduced pressure to give a light yellow solid, yield 90%. ^{1}HNMR (400 MHz, DMSO-d6) δ 2.08-2.12 (m, 1H), 2.50-2.64 (m, 2H), 2.86-2.95 (m, 1H), 5.19-5.24 (m, 1H), 8.13-8.15 (d, J = 8.04 Hz, 1H), 8.38-8.39 (m, 2H), 10.20 (s, 1H), 11.15 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) 21.8, 30.7, 49.2, 123.5, 124.3, 131.8, 135.1, 135.9, 140.7, 166.3, 166.4, 169.7, 173.1, 192.2; IR: 3107, 2922, 2853, 1779, 1698, 1621, 1375, 1319, 1260, 1199, 1178, 1141, 1113, 1094, 1017, 983, 949, 861, 811, 763, 740; HRMS (m/z) $(M+H)^+$: calcd. for $C_{14}H_{11}N_2O_5$ 287.0668, found 287.0682.

Compound 14

To a solution of compound 11 (0.2 mmol) in EtOAc borontrifluoride etherate (0.24 mmol) was added, and then the mixture was stirred at 60 °C for 2 h. The solvent was removed and the crude residue was purified by flash chromatography to give the boron difluoride complex as a yellow solid. This yellow solid (0.2 mmol) was suspended in EtOAc (2 mL) and heated to 80 °C, then $(BuO)_3B$ (0.4 mmol), aldehyde 13 (0.2 mmol), and n-$BuNH_2$ (0.1 mmol) were added, respectively. The mixture was refluxed for 7 h, and then cooled to room temperature. After filtration, the mixture was washed with EtOAc to give 14 as a red solid, yield 39%. ^{1}HNMR (400 MHz, DMSO-d6) δ 2.07-2.10 (m, 1H), 2.50-2.64 (m, 2H), 2.86-2.94 (m, 1H), 3.87 (s, 3H), 5.17-5.22 (m, 1H), 6.58 (s, 1H), 6.89-6.91 (d, J = 8.20 Hz, 1H), 7.13-7.17 (d, J = 15.48 Hz, 1H), 7.41-7.44 (dd, J = 1.6, 8.32 Hz, 1H), 7.54-7.58 (m, 2H), 8.01-8.03 (d, J = 8.20 Hz, 1H), 8.06-8.07 (d, J = 2.44 Hz, 1H), 8.06-8.07 (d, J = 2.80 Hz, 1H), 8.29-8.38 (m, 1H), 8.45 (s, 1H), 10.30 (s, 1H), 11.15 (s, 1H). ^{13}CNMR (100 MHz, DMSO-d6) 21.9, 30.9, 49.1, 55.8, 102.8, 112.7, 116.0, 117.4, 122.9, 124.1, 125.7, 125.8, 126.4, 132.2, 132.3, 135.6, 140.7, 142.0, 148.2, 149.8, 152.3, 166.5, 166.7,

169.7, 172.7, 176.8, 181.4. HRMS (m/z) $(M-H)^-$: calcd. for $C_{27}H_{20}BF_2N_2O_8$ 549.1286, found 549.1282.

Compound 15

To a mixture of acetone (264.0 mmol), ethanol (35 mL), and water (15 mL), NaOH (73.0 mmol) and vanillin 10 (33.0 mmol) were added. The mixture was stirred at room temperature overnight, and then cooled to 0 °C. The mixture was treated with 2 M HCl to pH 5-6 and extracted with EtOAc (200 mL). The combined organic phase was washed with water (100 mL) and brine (100 mL), and then dried over anhydrous Na_2SO_4. After filtration solvents were removed under reduced pressure and the residue was purified by flash chromatography (hexane/EtOAc: 10/1 to 1/1) to give a pale yellow solid, yield 87%. ^{1}HNMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H), 3.94 (s, 3H), 5.95 (s, 1H), 6.57-6.61 (d, J = 16.16 Hz, 1H), 6.92-6.94 (d, J = 8.12 Hz, 1H), 7.05-7.06 (d, J = 1.84 Hz, 1H), 7.08-7.10 (dd, J = 1.84, 8.12 Hz, 1H), 7.43-7.47 (d, J = 16.16 Hz, 1H); ^{13}CNMR (100 MHz, $CDCl_3$) 27.3, 56.0, 109.4, 114.9, 123.5, 125.0, 126.9, 143.8, 146.9, 148.3, 198.5. HRMS (m/z) $(M-H)^-$: calcd. for $C_{11}H_{11}O_3$ 191.0714, found 191.0705.

Biology (S2)

Cell viability assay

Cells were seeded at a density of 2 x 10^4 cells/well for MM1S cell, 5 x 10^3 cells/well for RPMI8226 and U266 cells in 96-well plates and treated with compounds for 48 h. Cells treated with vehicle (medium containing the same amount of DMSO as is present in prepared compounds) were set as control. After incubation for 48 h, 10 μL of MTT was added to each well, and the cells were incubated for an additional 4 h at 37 °C in a fully humidified atmosphere containing 5% CO_2. The insoluble formazan produced by cellular reduction of MTT was dissolved by DMSO and the concentration was determined by recording the absorbance of each 96-well plates using a FlexStation 3 plate reader (Molecular Devices, CA) at a wavelength of 570 nm. Values were expressed as a percentage relative to those obtained in vehicle-treated controls. Four parameter dose response analysis function in Prism was used to fit five dose (0.3, 1, 3, 10, 30 μM) and response (viability) to get IC_{50}s of indicated compounds.

Apoptosis assay

U266 cells (10^5 cells/mL) were treated with compound at indicated concentrations for indicated intervals. Cells were collected, washed twice with cold PBS, and then suspended in binding buffer (10 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid]/NaOH, pH 7.4, 140 mM NaOH, 2.5 mM $CaCl_2$). Cells were incubated with annexin V-fluorescein isothiocyanate (FITC) (BD PharMingen, San Diego, CA) and propidium iodide (PI) for 30 min at room temperature. The samples were analyzed by flow cytometry using a Guava easyCyte flow cytometer (Millipore, USA) within 1 h to determine the percentage of cells displaying annexin V-FITC staining (early apoptosis) or both annexin V-FITC and PI staining (late apoptosis). Cells treated with only vehicle (medium containing the same amount of DMSO) were set as control.

ROS assay

U266 cells (10^5 cells/mL) were treated with compound at indicated concentrations for indicated intervals. DCFH-DA (10 μM, final) was added and incubated for 30 min. Cells were then collected and washed with PBS twice, then suspended in 500μL of PBS and the mean intensity was determined by a Guava easyCyte flow cytometer (Millipore, USA).

NAC rescue assay

U266 cells (10^5 cells/mL) were treated with NAC (8 mM) alone or NAC and compound at indicated concentrations, and incubated at 37 °C in a fully humidified atmosphere containing 5% CO_2 for 24 h. Cells were collected by centrifuge and washed twice with cold PBS. The samples were analyzed by flow cytometry using a Guava easyCyte flow cytometer (Millipore, USA) using the annexin V-FITC/PI double stain method described above. Cells treated with only vehicle (medium containing the same amount of DMSO as is present in prepared compounds were set as control.

Cell cycle analysis

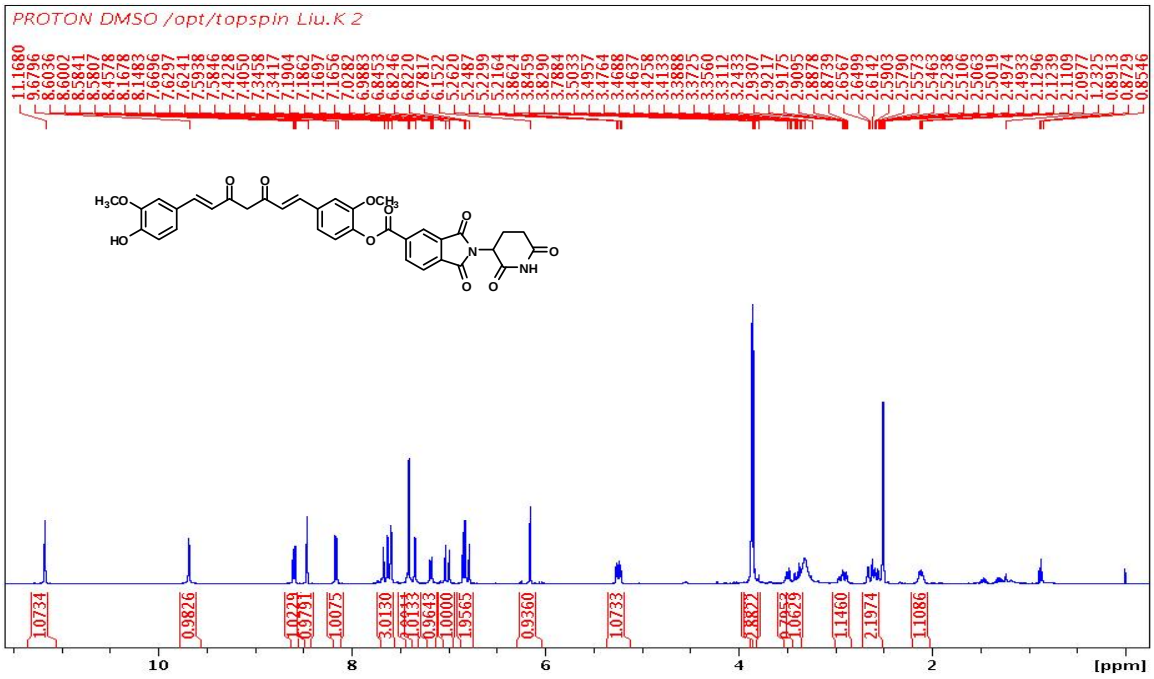
U266 cells (10^5 cells/mL) were treated with compounds or vehicle (medium containing the same amount of DMSO as is present in prepared compounds) for 24 h. Cells were collected and fixed in 67% ethanol/PBS overnight at 4 °C. The samples were stained with 3.8 mM sodium citrate solution containing 10 μg/mL PI and 0.5 mg/mL RNase A (Sigma-Aldrich, St. Louis, MO) for 4 h at 4 °C in the dark, then DNA content was analyzed by a Guava easyCyte flow cytometer (Millipore, USA). Cell cycle analysis was performed by ModFitLT 3.3 software to determine the percentage of cells in the G_0/G_1, S, and G_2/M phases of the cell cycle.

NFκB Translocation Assay.

A549 cells were treated with compounds at indicated concentrations, or vehicle (medium containing the same amount of DMSO as is present in prepared compounds) for 30 min, followed by stimulation with TNFα (10 ng/ml) for 30 min. Cells were then fixed with 2% paraformaldehyde and permeabilized with 0.1% Triton X-100. Finally, rabbit anti-p65 NFκB antibody and goat antirabbit IgG with conjugated Alexa Fluor 488 were used to stain NFκB, and Hoechst 33342 was used to stain the nucleus. Fluorescence intensity of NFκB and nuclear staining were recorded with an automated imaging system, ImageXpress5000A (Molecular Devices). The inhibitory effect of the compound on TNFα induced NFκB translocation (activation) was expressed as: % of Control = (FI $_{\text{compound}}$- FI $_{\text{vehicle}}$ / FI $_{\text{TNFα control}}$– FI vehicle) × 100. FI is the measured NFκB fluorescence (green) intensity in the nucleus.

Copies of spectroscopy

H[1]NMR of Compound 3



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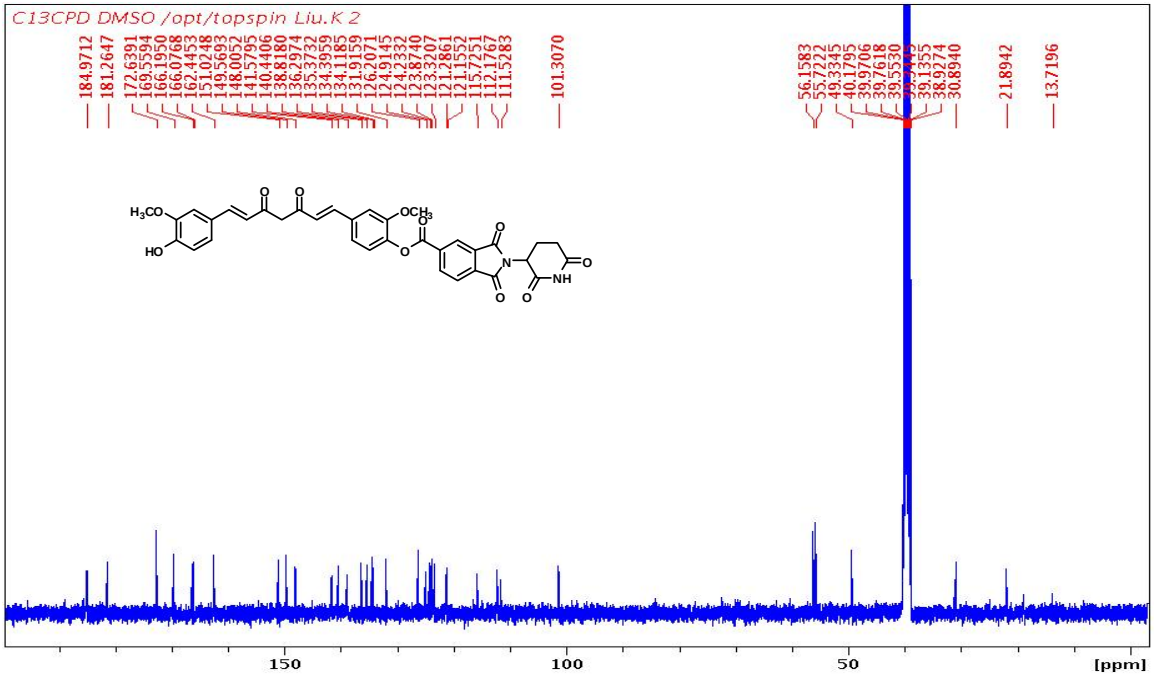

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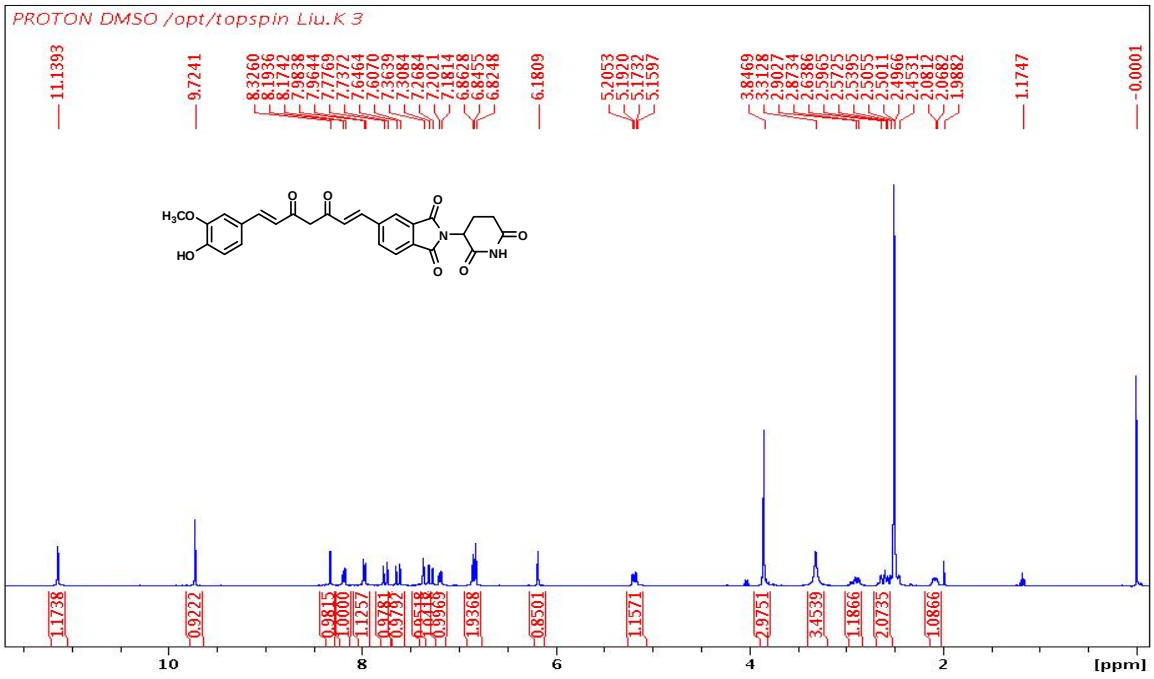

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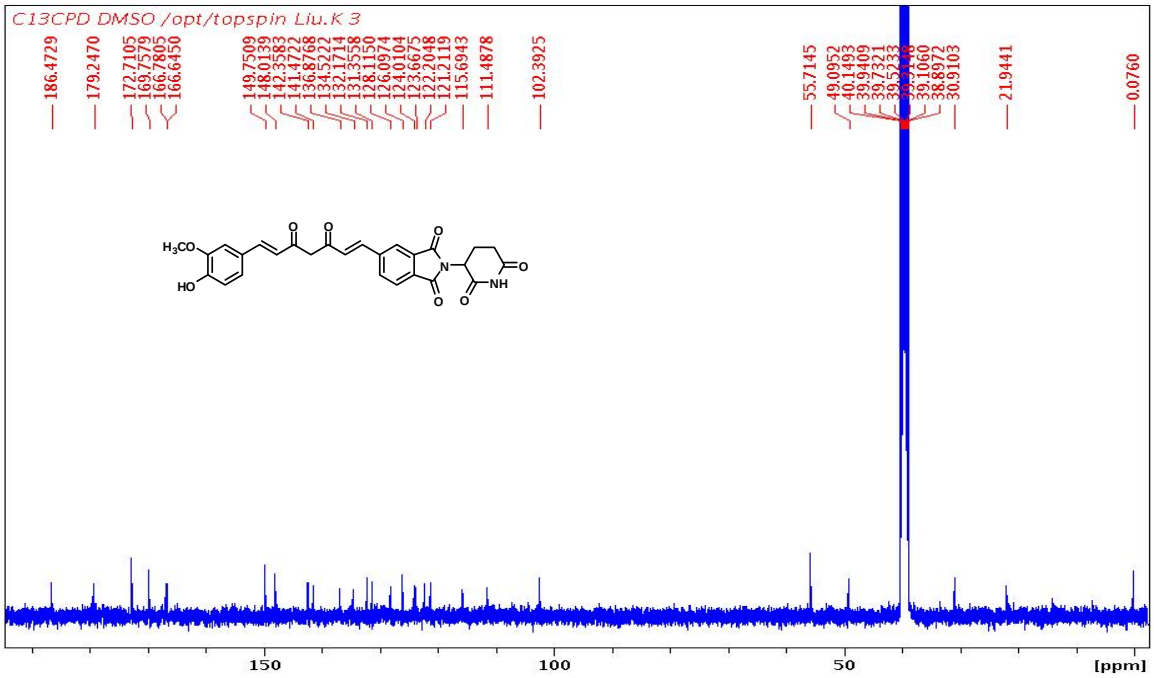

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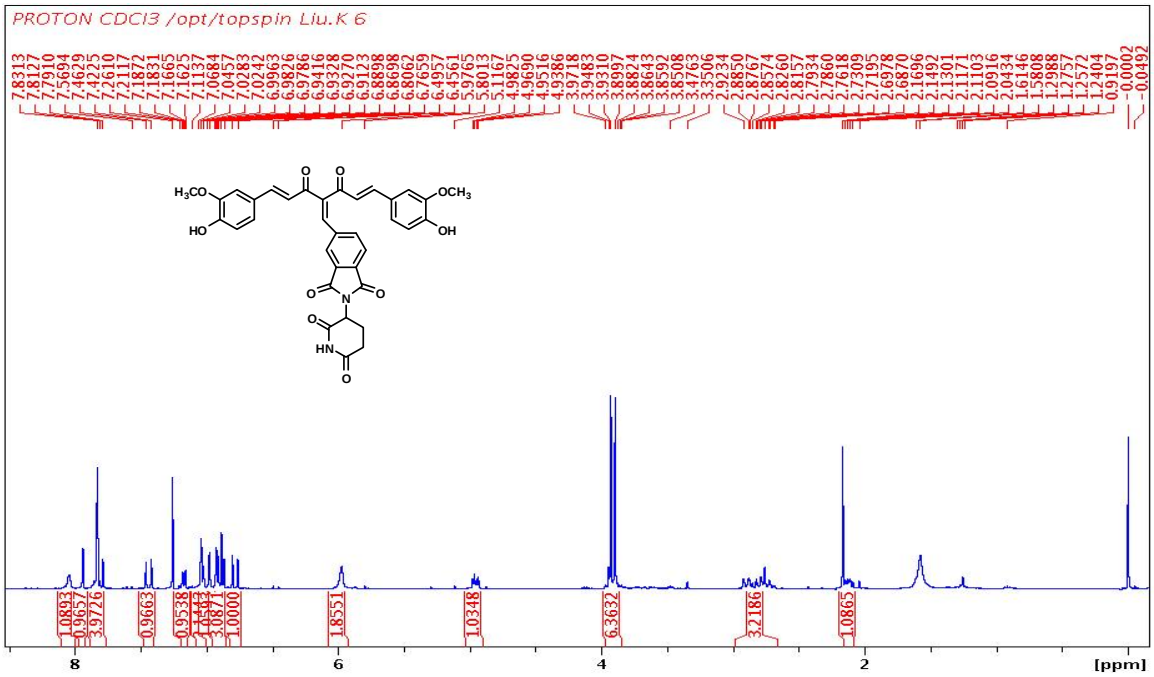

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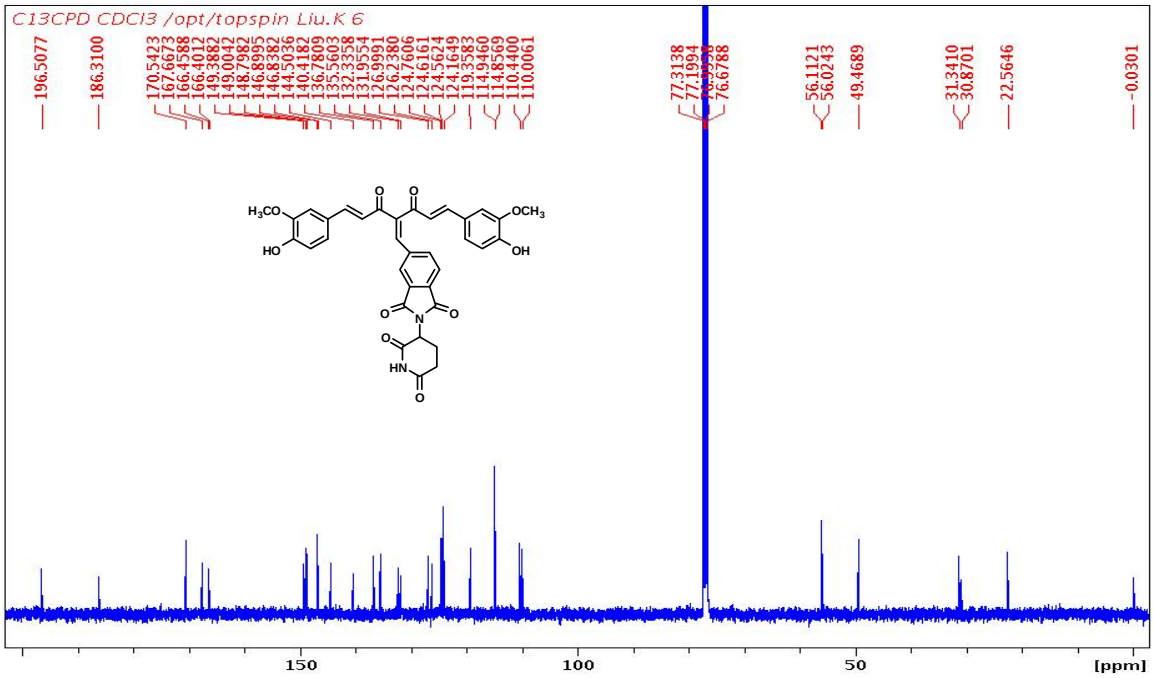

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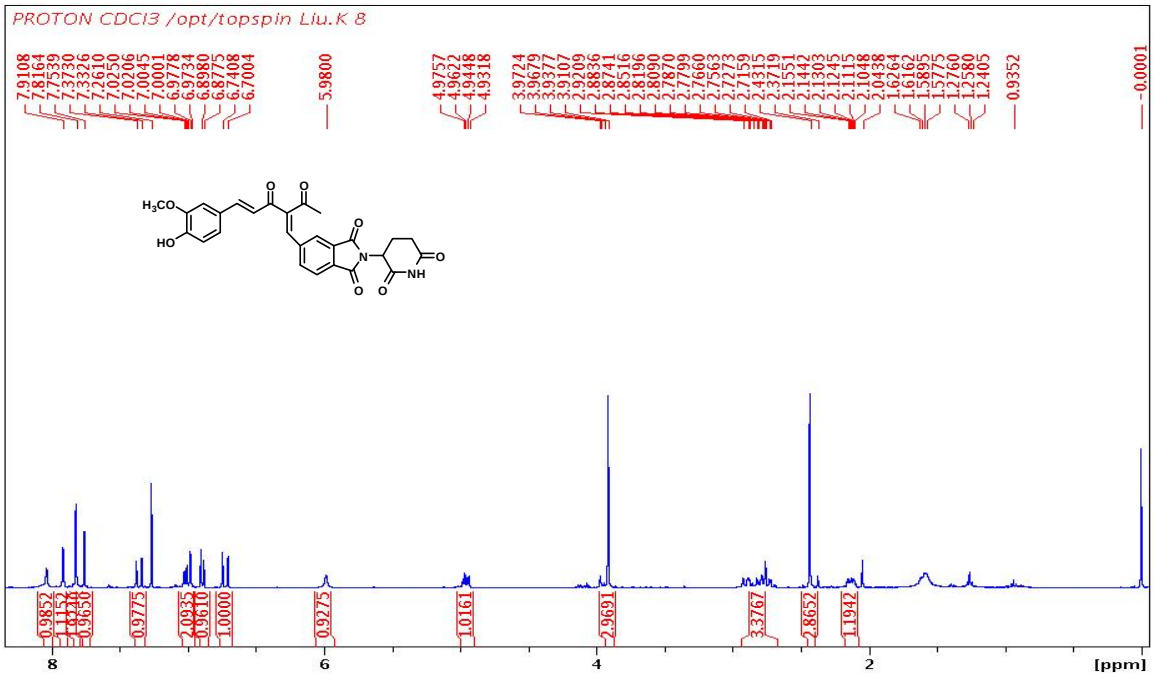

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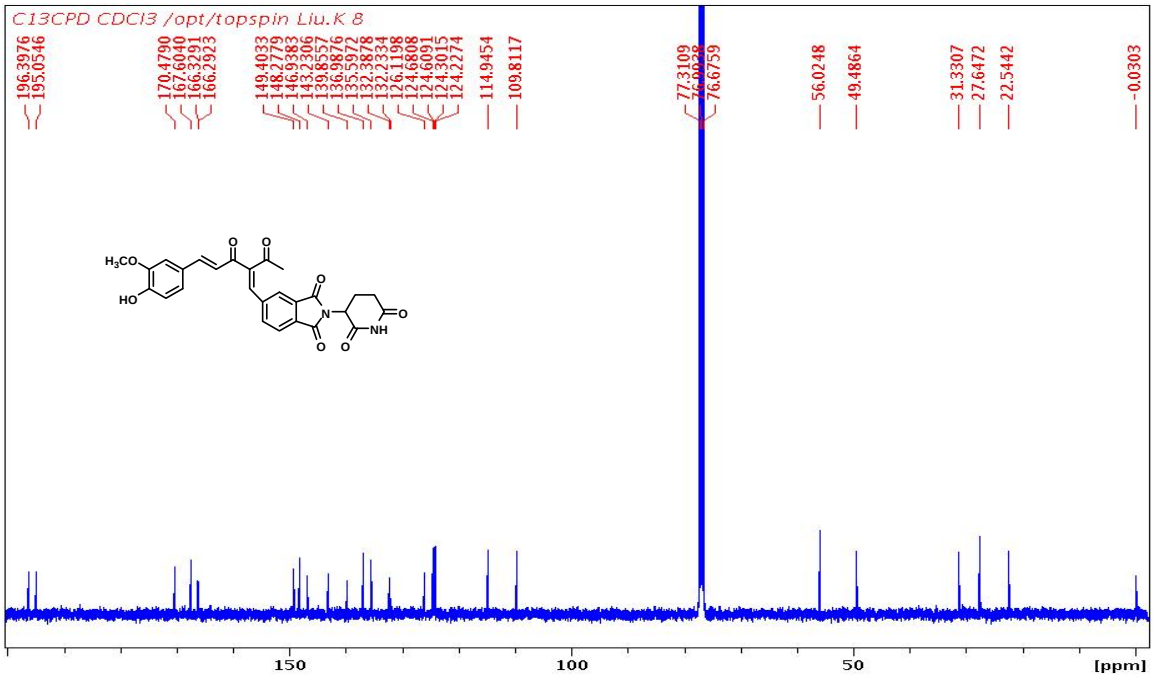

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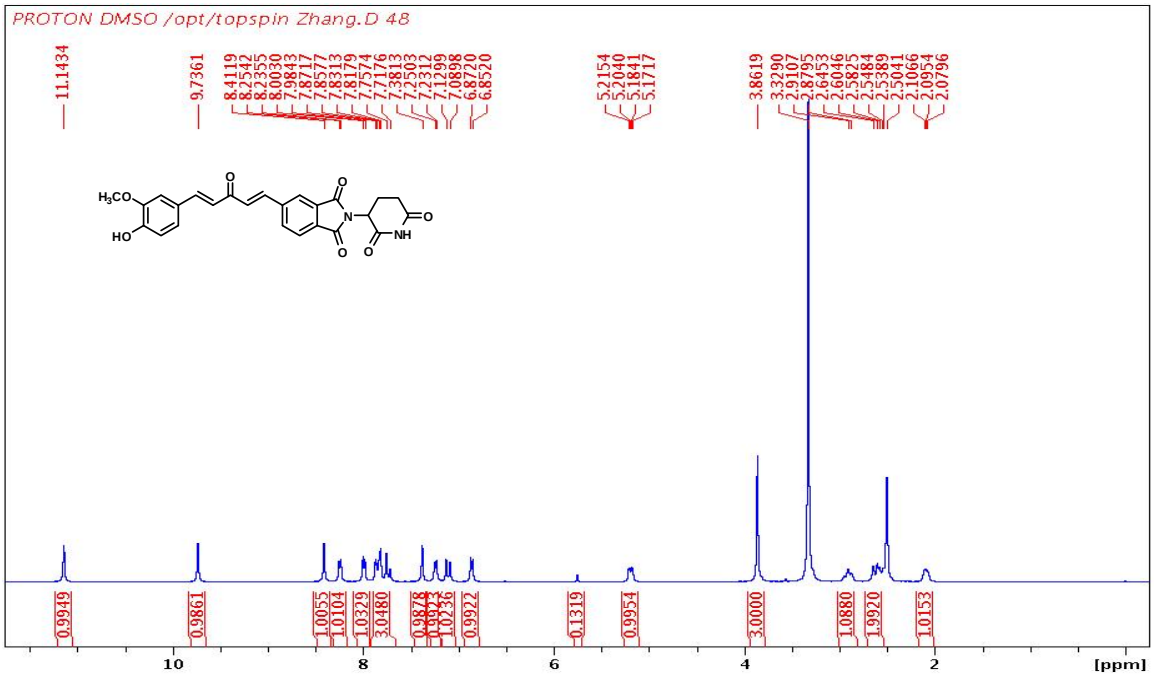

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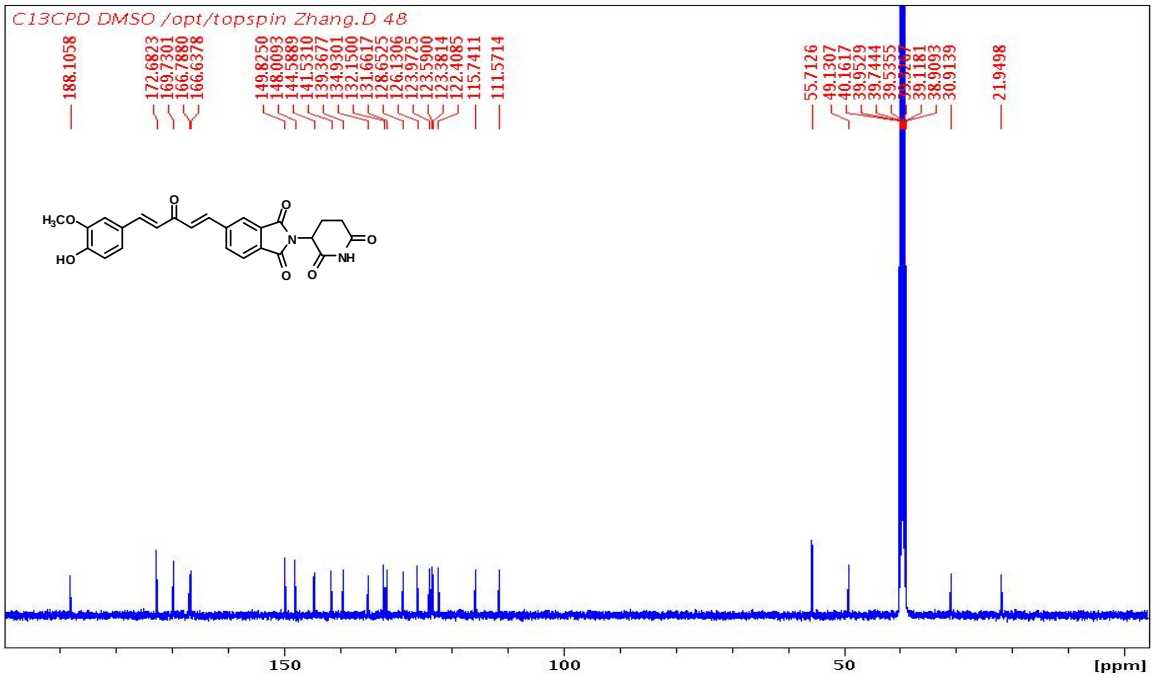

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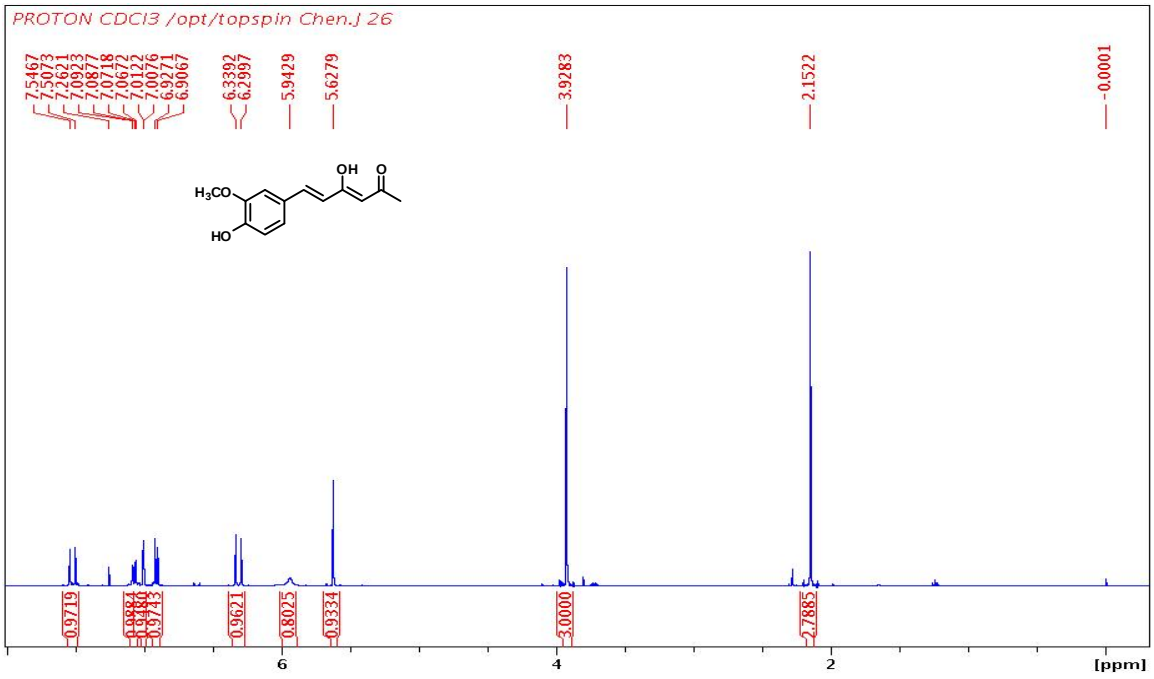

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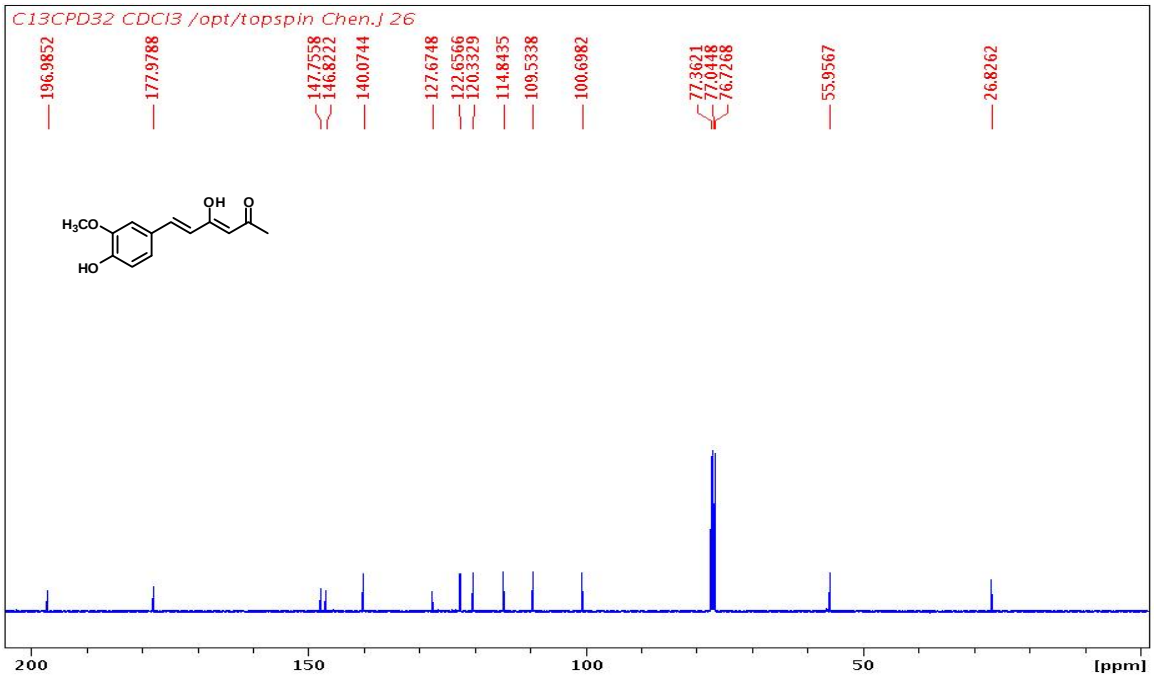

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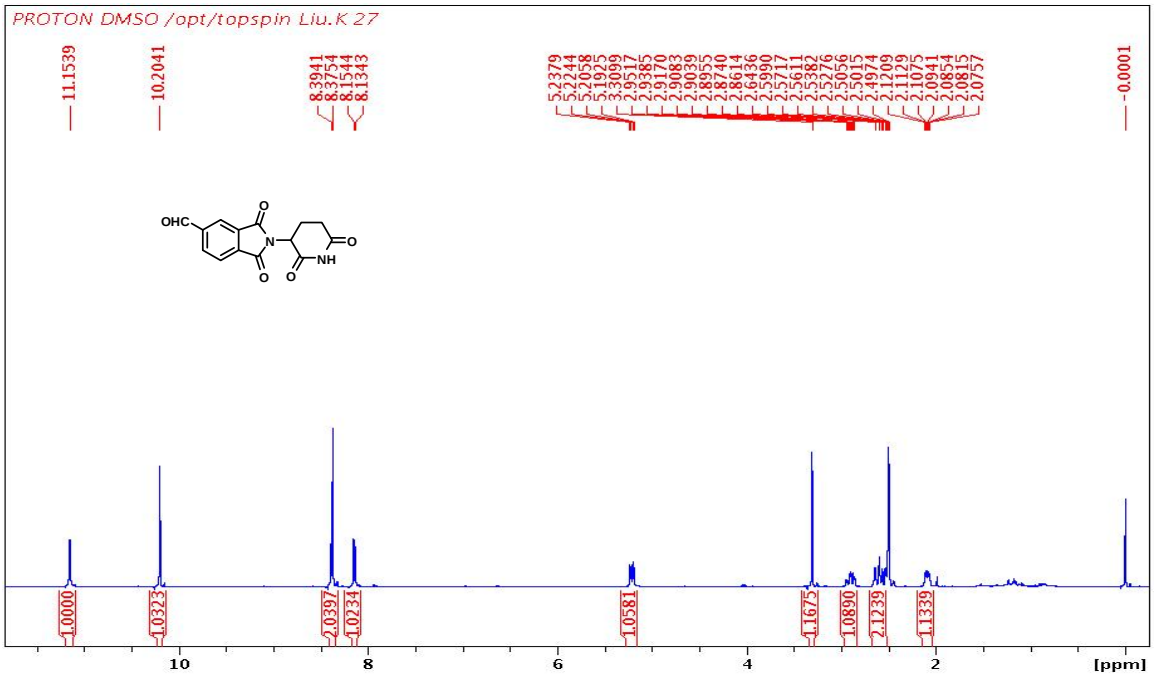
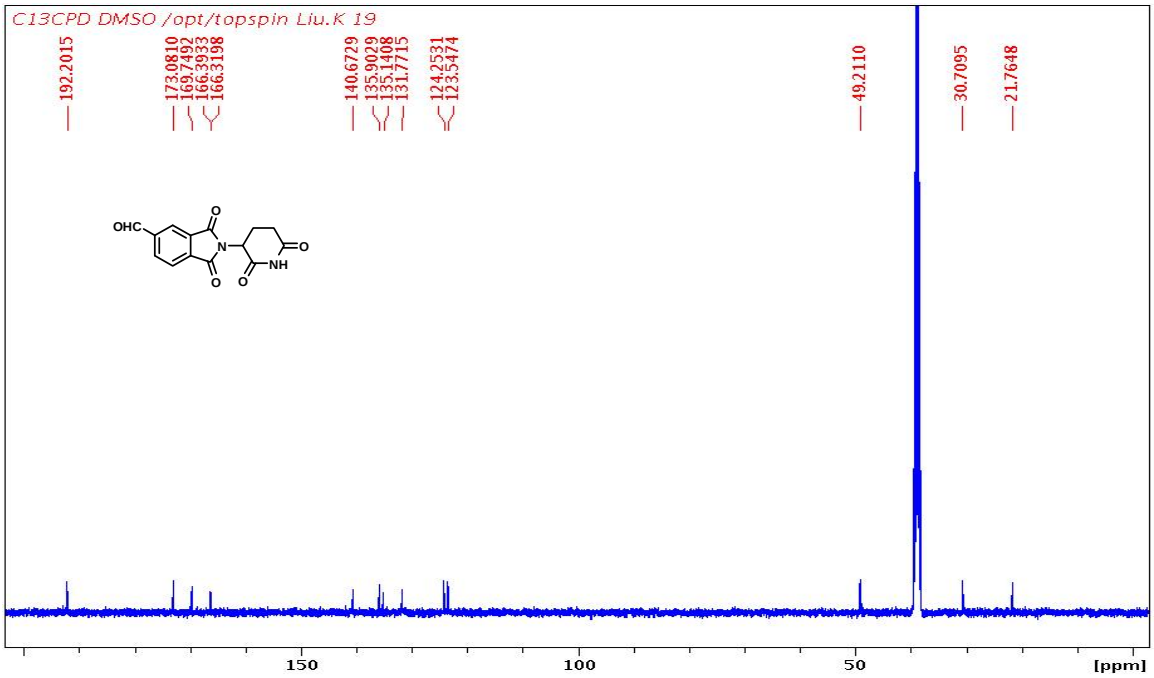
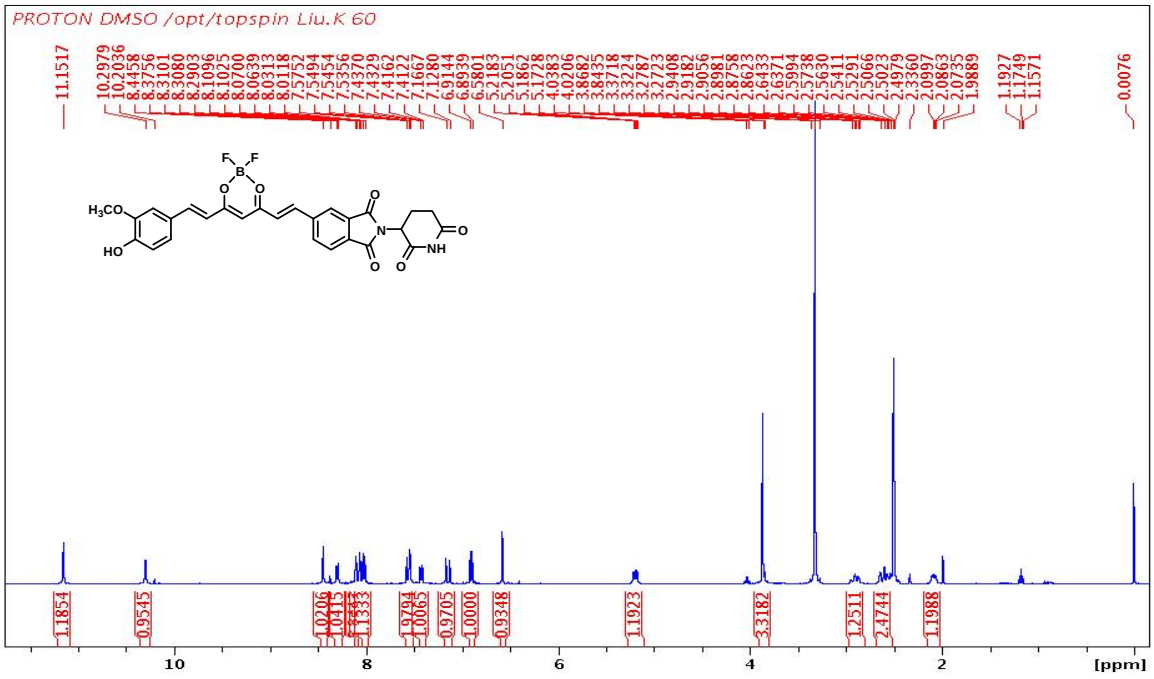


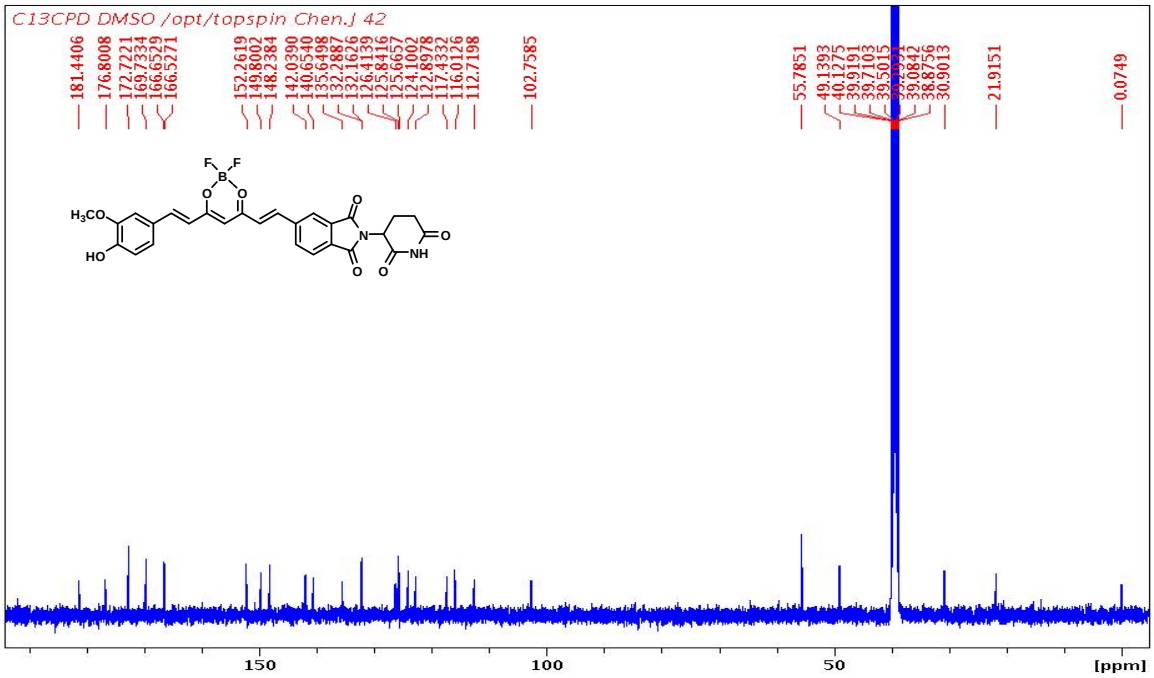
C^{13}NMR of Compound 13



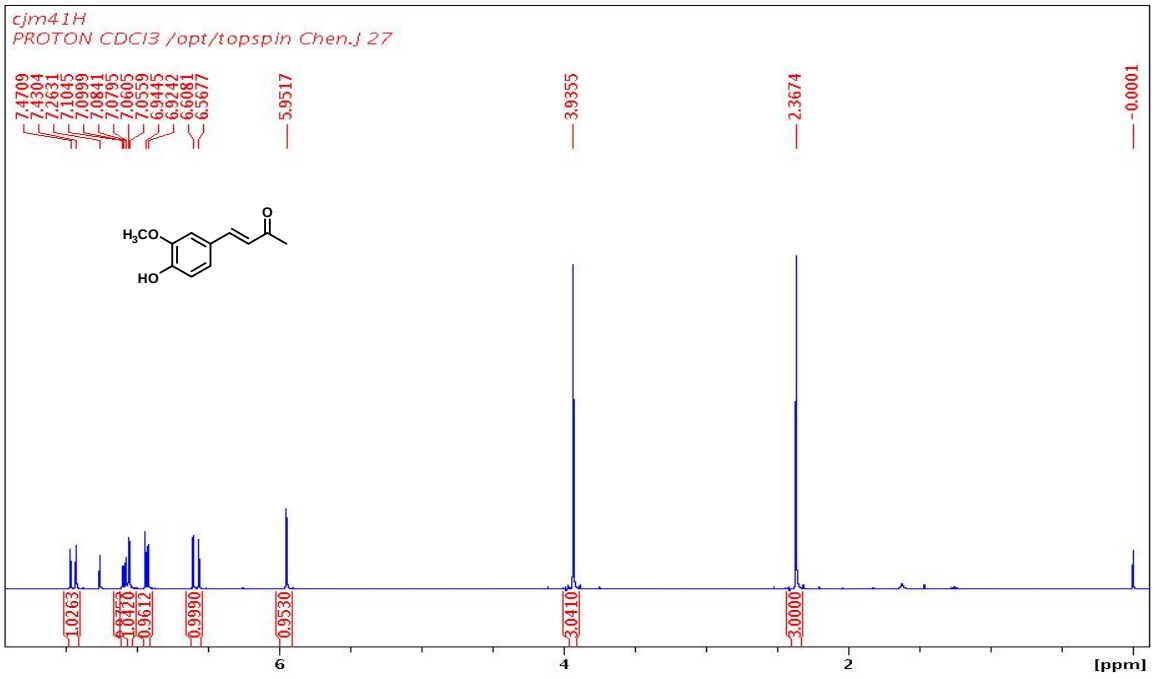
H^1NMR of Compound 14



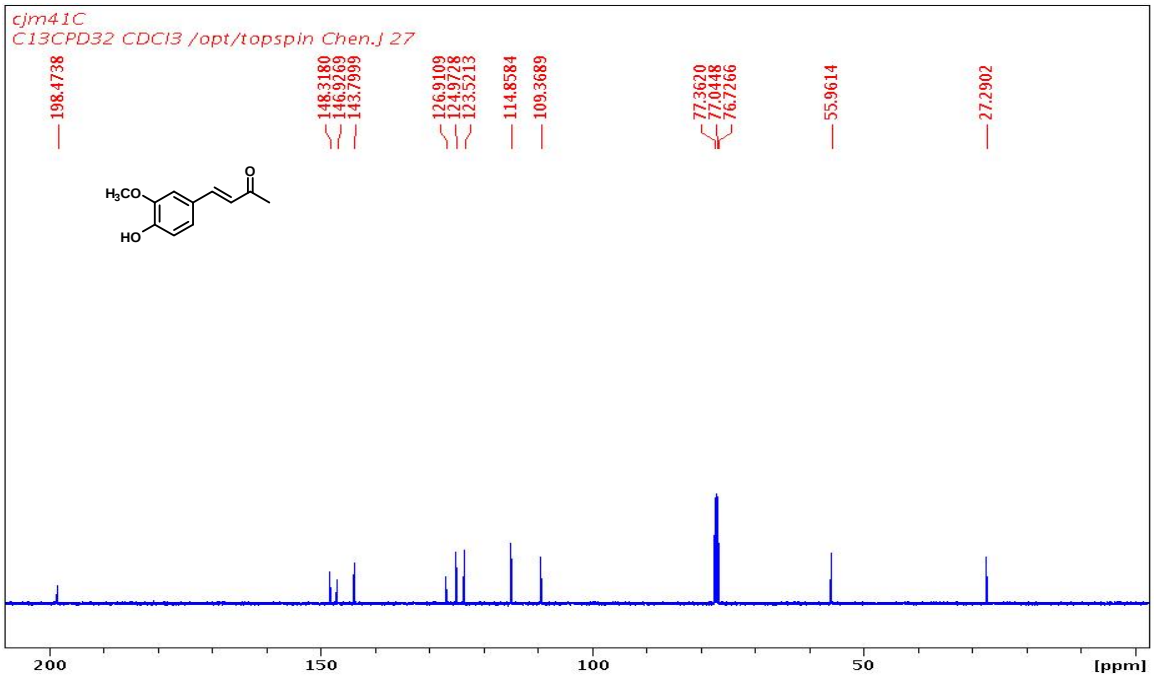
C^{13}NMR of Compound 14



H[1]NMR of Compound 15



C[13]NMR of Compound 15



IR of Compound 13