Supporting Information

Discovery of a series of 2-phenylnaphthalenes as firefly luciferase inhibitors

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1. Materials and Methods

Chemical synthesis materials and instruments. All reagents and solvents available from commercial sources were used as received unless otherwise noted. Twice-distilled water was used throughout all experiments. NMR spectra were obtained in deuterated solvents on Bruker AV-600 spectrometers at the College of Chemistry NMR Facility, Shandong University. All chemical shifts are reported in the standard δ notation of parts per million using the peaks of residual proton and carbon signals of the solvent as internal references. NMR peaks are referred to as singlet (s), doublet (d), and doublet of doublets (dd), triplet (t), or broad singlet (br). Coupling constants (J) are reported in hertz. Mass spectra were recorded in ESI^+ mode (70 eV). The purity of all final compounds (>95%) was determined by HPLC analysis by UV absorption at 295 nm and 260 nm. Analytical HPLC was performed on Agilent 1260 Series high performance liquid chromatography using a C8 reversed-phase column (250 x 4.60 mm, 5 μ, Phenomenex).

Bioluminescence assay instrument. QuantiLum Recombinant Luciferase was purchased from Promega Corporation (E1702). Recombinant Renilla Luciferase was purchased from RayBiotech, USA (CODE: RB-15-0003P-50). ATP and coelenterazine were purchased from Aladdin. Millipore water was used to prepare all aqueous solutions. Measurements for bioluminescent assays were performed in 50 mM Tris buffer, pH=7.60 with 10 mM $MgCl_2$ at 37°C. Luminescence produced by the luciferase was measured with Omega microplate reader (POLARstar Omega, Germany). The log-(inhibitor) *vs* normalized (control was set as 100%, variable slope) response data were analyzed with the GraphPad Prism software package. Bioluminescence imaging was determined with a Caliper IVIS Kinetic in vivo optical imaging system (Caliper Life Sciences, now PerkinElmer, USA) equipped with a cooled charge coupled device (CCD) camera. The pseudo colored bioluminescent images (in photons/sec/cm^2/scr) were superimposed over the grayscale photographs of the animals. Circular ROIs were drawn over the areas and quantified using Living Image software. The results were reported as total photon flux within an ROI in photons per second.

Mice model. Transgenic mice harboring the CAG-luc-eGFP L2G85 transgene, constructed by the laboratory of Dr. Christopher H. Contag (Stanford University School of Medicine), were purchased from the Jackson Laboratory (Sacramento, California, USA). Balb/c-nu female mice, 8 weeks of age, were purchased from Animal Center of China Academy of Medical Sciences (Beijing, China). To generate tumor xenografts in mice, ES-2-Fluc cells (1×10^7) were implanted subcutaneously in the right armpit region. Mice were single or group-housed on a 12:12 light–dark cycle at 28 °C with free access to food and water. Tumors were allowed to grow for two weeks before imaging. All animal studies were approved by the Ethics Committee of Qilu Health Science Center, Shandong University and conducted in compliance with European guidelines for the care and use of laboratory animals.

Synthesis Procedure for the preparation of 2-(6-Methoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2).

2-Bromo-6-methoxynaphthalene (1 eq.), potassium acetate (3 eq.), and bis(pinacolato)diboron (1.1 eq.) were dissolved respectively in dioxane, and the resulting mixtures were deoxygenated with a stream of N_2. After 10 min, $PdCl_2$(dppf) (0.05 eq.) was added, and each mixture was brought to 60 °C, allowed to be stirred under N_2 for 8 hr until the reaction is complete, which was detected by TLC. Then the solution was cooled to room temperature and treated with water and ethyl acetate. The organic phases were combined and dried over and evaporated to dryness under reduced pressure. Purification by chromatography using petroleum ether/acetone 10:1 yielded compound 2. Yield: 82%; white powder; mp: 97-98°C. ^{1}H NMR (400 MHz, $CDCl_3$, r.t.) δ 8.29 (s, 1H, H1), 7.80 (d, *J* = 9.1 Hz, 1H, H4), 7.78 (d, *J* = 10.8 Hz, 1H, H8), 7.72 (d, *J* = 8.2 Hz, 1H, H3), 7.14 (dd, *J* = 8.2, 2.3 Hz, 1H, H7), 7.12 (s, 1H, H5), 3.93 (s, 3H, OMe), 1.38 (s, 12H, Me); ^{13}C NMR (100 MHz, $CDCl_3$, r.t.) δ 158.55 (C6), 136.44 (C10), 135.99 (C9), 131.12 (C8), 130.25 (C1), 128.40 (C4), 125.90 (C3), 118.67 (C7), 105.65 (C5), 83.79 (C1), 55.29 (OMe), 24.93 (Me).

General procedures for the preparation of 4, 6, 8, 9, 11-13.

2-(6-Methoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2**, 1 eq.), potassium fluoride (3 eq.), and appropriate bromide derivatives (**3a-g**, 1.1 eq.) were dissolved respectively in dioxane, and the three resulting mixtures were deoxygenated with a stream of N_2. After 10 min, $PdCl_2$(dppf) (0.05 eq.) was added, and each mixture was brought to reflux, allowed to be stirred under N_2 for 10-22 h until the reaction is complete, which was detected by TLC. Then, each solution was treated as described above, purified by chromatography using petroleum ether/acetone 10:1 to provide the corresponding **4, 6, 8, 9, 11-13**.

Methyl 2-(4-(6-methoxynaphthalen-2-yl)phenyl)acetate (**4**). Yield: 71%; white powder; mp: 133-134°C. ^{1}H NMR (400 MHz, $CDCl_3$, r.t.) δ 7.96 (s, 1H, H1), 7.80 (d, J = 8.5 Hz, 1H, H8), 7.78 (d, J = 8.1 Hz, 1H, H4), 7.69 (dd, J = 1.6 Hz, J = 8.7 Hz, 1H, H3), 7.65 (d, J = 8.1 Hz, 2H, H2', H6'), 7.38 (d, J = 8.1 Hz, 2H, H3', H5'), 7.18 (dd, J = 2.4 Hz, J = 8.5 Hz, 1H, H7), 7.16 (d, J = 2.4 Hz, 1H, H5), 3.94 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.69 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, r.t.) δ 172.04 (COOMe), 157.81 (C6), 140.11 (C1'), 135.95 (C4'), 133.80 (C10), 132.80 (C2), 129.75 (C3', C5'), 129.71 (C4), 129.18 (C8), 127.40 (C2', C6'), 127.27 (C9), 125.94 (C3), 125.53 (C1), 119.17 (C7), 105.62 (C5), 55.35 (OMe), 52.11 (OMe), 40.87 (CH_2). HRMS (AP-ESI)m/z calcd for C20H18O3 $[M + H]^+$ 307.1329, found 307.1328. HPLC analysis: Retention time 12.3 min, eluted with 70% methanol/30% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 95.2%.

Methyl 2-hydroxy-5-(6-methoxynaphthalen-2-yl)benzoate (**6**). Yield: 78%; white powder; mp: 111-113°C. ^{1}H NMR (400 MHz, $CDCl_3$, r.t.) δ 10.78 (s, 1H, OH), 8.16 (d, *J* = 2.3 Hz, 1H, H2'), 7.91 (s, 1H, H1), 7.82 (dd, *J* = 8.2, 2.3 Hz, 1H, H6'), 7.80 (d, *J* = 8.2 Hz, 1H, H8), 7.78 (d, *J* = 8.3 Hz, 1H, H4), 7.66 (dd, *J* = 8.5, 1.7 Hz, 1H, H3), 7.18 (dd, *J* = 8.5, 2.5 Hz, 1H, H7), 7.15 (s, 1H, H5), 7.09 (d, *J* = 8.6 Hz, 1H, H5'), 4.00 (s, 3H, OMe), 3.94 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$, r.t.) δ 170.61 (COOMe), 160.90 (C4'), 157.77 (C6), 135.09 (C6'), 134.53 (C1'), 133.62 (C10), 132.51 (C2'), 129.57 (C2), 129.20 (C4), 128.17 (C8), 127.37 (C9), 125.57 (C3), 124.97 (C1), 119.27 (C5'), 118.13 (C7), 112.62 (C3'), 105.63 (C5), 55.35 (OMe), 52.40 (OMe). HRMS (AP-ESI)m/z calcd for C19H16O4 $[M - H]^-$ 307.0976, found 307.0975. HPLC analysis: Retention time 20.9 min, eluted with 70% methanol/30% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 99.4%.

Methyl 2-hydroxy-4-(6-methoxynaphthalen-2-yl)benzoate (**8**). Yield: 67%; white powder; mp: 114-116°C. ^{1}H NMR (400 MHz, $CDCl_3$, r.t.) δ 10.83 (s, 1H, OH), 8.02 (s, 1H, H1), 7.91 (d, *J* = 8.3 Hz, 1H, H8), 7.82 (d, *J* = 4.3 Hz, 1H, H5'), 7.79 (d, *J* = 4.6 Hz, 1H, H4), 7.71 (d, *J* = 8.6 Hz, 1H, H3), 7.33 (d, *J* = 1.4 Hz, 1H, H2'), 7.24 (d, *J* = 9.0 Hz, 1H, H6'), 7.19 (dd, *J* = 8.9, 2.4 Hz, 1H, H7), 7.16 (s, 1H, H5), 3.98 (s, 3H, OMe), 3.94 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$, r.t.) δ 170.54 (COOMe), 161.89 (C3'), 158.25 (C6), 148.54 (C1'), 134.69 (C10), 134.47 (C5'), 130.33 (C2), 129.96 (C4), 128.99 (C8), 127.44 (C9), 126.22 (C3), 125.53 (C1), 119.42 (C6'), 118.18 (C7), 115.66 (C2'), 110.96 (C4'), 105.62 (C5), 55.37 (OMe), 52.27 (OMe). HRMS (AP-ESI)m/z calcd for C19H16O4 $[M + H]^+$ 309.1127, found 308.9741. HPLC analysis: Retention time 22.1 min, eluted with 70% methanol/30% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 97.7%.

Methyl 4-(6-methoxynaphthalen-2-yl)benzoate (**9**). Yield: 76%; white powder; mp: 253-255°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.2 Hz, 2H, H3', H5'), 8.02 (s, 1H, H1), 7.83 (d, *J* = 7.7 Hz, 1H, H8), 7.81 (d, *J* = 8.3 Hz, 1H, H4), 7.77 (d, *J* = 8.2 Hz, 2H, H2', H6'), 7.73 (dd, *J* = 8.5, 1.1 Hz, 1H, H3), 7.19 (dd, *J* = 8.5, 2.1 Hz, 1H, H7), 7.18 (d, *J* = 2.0 Hz, 1H, H5), 3.96 (s, 3H, OMe), 3.95 (s, 3H, OMe); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.17 (COOMe), 145.66 (C6), 135.08 (C1'), 134.27 (C10), 130.17 (C3', C5'), 129.88 (C8), 129.08 (C2), 128.66 (C4), 127.49 (C4'), 127.04 (C2', C6'), 126.15 (C3), 125.70 (C9), 119.43 (C1), 105.63 (C7), 99.99 (C5), 55.37 (OMe), 52.12 (OMe). HRMS (AP-ESI)m/z calcd for C19H16O3 $[M + H]^+$ 293.1178, found 293.1178. HPLC analysis: Retention time 10.7 min, eluted with 65% methanol/35% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 99.6%.

(4-(6-Methoxynaphthalen-2-yl)phenyl)methanol (**11**). Yield: 76%; white powder; mp: 201-203°C. ^{1}H NMR (400 MHz, DMSO-d_6, r.t.) δ 8.14 (s, 1H, H1), 7.91 (d, *J* = 2.1 Hz, 1H, H4), 7.89 (s, 1H, H3), 7.80 (d, *J* = 8.6 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 2H, H2', H6'), 7.44 (d, *J* = 7.9 Hz, 2H, H3', H5'), 7.35 (s, 1H, H5), 7.19 (dd, *J* = 8.9, 2.0 Hz, 1H, H7), 5.25 (t, *J* = 5.7 Hz, 1H, OH), 4.56 (d, *J* = 5.7 Hz, 2H, CH_2), 3.89 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6, r.t.) δ 157.85 (C6), 142.05 (C4'), 138.94 (C1'), 135.57 (C10), 133.95 (C2), 130.16 (C4), 129.28 (C8), 127.80 (C3), 127.55 (C2', C6'), 126.85 (C3', C5'), 125.89 (C9), 125.28 (C1), 119.42 (C7), 106.21 (C5), 63.12 (CH_2), 55.69 (OMe). HRMS (AP-ESI)m/z

calcd for C18H16O2 [M - H]$^-$ 263.1078, found 263.1078. HPLC analysis: Retention time 10.7 min, eluted with 65% methanol/35% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 96.4%.

4-(6-methoxynaphthalen-2-yl)phenol (**12**). Yield: 76%; white powder; mp: 203-205°C. ^{1}H NMR (400 MHz, DMSO-d_6, r.t.) δ 9.54 (s, 1H, OH), 8.02 (s, 1H, H1), 7.86 (d, *J* = 3.6 Hz, 1H, H8), 7.84 (d, *J* = 3.2 Hz, 1H, H4), 7.73 (dd, *J* = 8.6, 1.7 Hz, 1H, H3), 7.60 (d, *J* = 8.6 Hz, 2H, H2', H6'), 7.32 (d, *J* = 2.2 Hz, 1H, H5), 7.16 (dd, *J* = 8.9, 2.5 Hz, 1H, H7), 6.88 (d, *J* = 8.6 Hz, 2H, H3', H5'), 3.88 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6, r.t.) δ 157.54 (C4'), 157.44 (C6), 135.79 (C10), 133.44 (C1'), 131.34 (C2), 129.95 (C4), 129.36 (C8), 128.23 (C2', C6'), 127.66 (C9), 125.73 (C3), 124.31 (C1), 119.28 (C7), 116.25 (C3', C5'), 106.19 (C5), 55.66 (OMe). HRMS (AP-ESI)m/z calcd for C17H14O2 [M - H]$^-$ 249.0916, found 249.0884. HPLC analysis: Retention time 22.4 min, eluted with 65% methanol/35% water (containing 0.1% acetic acid) with a flow rate of 1.5 ml/min, purity: 99.5%.

4-(6-Methoxynaphthalen-2-yl)aniline (**13**). Yield: 68%; yellow powder; mp: 208-209°C. ^{1}H NMR (400 MHz, DMSO-d_6, r.t.) δ 7.97 (s, 1H, H1), 7.83 (d, *J* = 8.7 Hz, 1H, H4), 7.81 (d, *J* = 6.7 Hz, 1H, H8), 7.70 (dd, *J* = 8.6, 1.3 Hz, 1H, H3), 7.48 (d, *J* = 8.4 Hz, 2H, H2', H6'), 7.29 (d, *J* = 2.1 Hz, 1H, H5), 7.14 (dd, *J* = 8.9, 2.3 Hz, 1H, H7), 6.68 (d, *J* = 8.4 Hz, 2H, H3', H5'), 5.24 (s, 2H, NH_2), 3.87 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6, r.t.) δ 157.28 (C6), 148.70 (C4'), 136.33 (C10), 133.09 (C2), 129.80 (C4), 129.49 (C1'), 127.81 (C8), 127.67 (C2', C6'), 127.53 (C9), 125.49 (C4), 123.34 (C1), 119.13 (C7), 114.79 (C3', C5'), 106.19 (C5), 55.62 (OMe). HRMS (AP-ESI)m/z calcd for C17H15NO [M - H]$^-$ 150.1226, found 250.1228. HPLC analysis: Retention time 19.1 min, eluted with 52% methanol/48% water with a flow rate of 1.5 mL/min, purity: 97.4%.

General procedure for the preparation of 14.

Compound **7** (1 eq.) were dissolved in anhydrous CH_2Cl_2 (5 mL) at -78 °C. BBr3 (3-6 eq.) was added, and the resulting reaction mixture was allowed to warm to room temperature for 20 h and treated as follows. The solution was poured into ice water followed by being warmed to ambient temperature, and then each solution was washed twice with ethyl acetate. The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. Purification of each crude product by chromatography using CH_2Cl_2/ethyl acetate 10:1 yielded the corresponding **14**.

2-hydroxy-5-(6-hydroxynaphthalen-2-yl)benzoic acid (**14**). Yield: 73%; white powder; mp: > 260°C. ^{1}H NMR (400 MHz, Acetone-d_6, r.t.) δ 8.27 (d, *J* = 2.4 Hz, 1H, H2'), 8.05 (s, 1H, H1), 7.95 (dd, *J* = 8.6, 2.4 Hz, 1H, H8), 7.88 (d, *J* = 8.8 Hz, 1H, H6'), 7.78 (d, *J* = 8.6 Hz, 1H, H4), 7.72 (dd, *J* = 8.6, 1.8 Hz, 1H, H3), 7.24 (d, *J* = 2.2 Hz, 1H, H5), 7.19 (dd, *J* = 8.8, 2.4 Hz, 1H, H7), 7.09 (d, *J* = 8.6 Hz, 1H, H5'); ^{13}C NMR (100 MHz, Acetone-d_6, r.t.) δ 171.79 (COOH), 161.24 (C4'), 155.42 (C6), 134.25 (C6'), 134.22 (C1'), 134.15 (C10), 132.25 (C2'), 129.81 (C1), 128.83 (C4), 128.27 (C8), 126.93 (C9), 125.14 (C3), 124.78 (C1'), 118.79 (C5'), 117.75 (C7), 112.91 (C3'), 108.67 (C5). HRMS (AP-ESI)m/z calcd for C17H12O4 [M - H]$^-$ 279.0663, found 279.0664. HPLC analysis: Retention time 9.78 min, eluted with 55% methanol/45% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 100%.

General procedures for the preparation of 5, 7 and 10.

The compounds **4**, **6** or **9** (1 eq.) were dissolved respectively in ethanol (10 mL) and were added a solution of 20% sodium hydroxide aqueous at room temperature, and each mixture was brought to reflux, until the reaction is complete, which was detected by TLC. Subsequently, each solution was acidified by dilute hydrochloric acid, treated with water and ethyl acetate. The organic phases were combined and dried over and evaporated to dryness under reduced pressure. Purification by chromatography using dichloromethane/methanol 20:1 yielded compound **5**, **7** or **10**.

2-(4-(6-methoxynaphthalen-2-yl)phenyl)acetic acid (**5**). Yield: 64%; white powder; mp: 247-249°C. ^{1}H NMR (400 MHz, DMSO-d_6, r.t.) δ 12.39 (s, 1H, COOH), 8.14 (s, 1H, H1), 7.90 (d, *J* = 8.8 Hz, 2H, H2', H6'), 7.80 (dd, *J* = 8.5, 1.3 Hz, 1H, H3), 7.74 (d, *J* = 8.1 Hz, 2H, H3', H5'), 7.40 (d, *J* = 8.1 Hz, 1H, H8), 7.37 (d, *J* = 8.1 Hz, 1H, H4), 7.35 (d, *J* = 2.2 Hz, 1H, H5), 7.20 (dd, *J* = 8.9, 2.3 Hz, 1H, H7), 3.89 (s, 3H, OMe), 3.63 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, r.t.) δ 173.14 (COOH), 157.88 (C6), 138.89 (C1'), 135.42 (C4'), 134.56 (C10), 134.00 (C2), 130.47 (C3', C5'), 130.17

(C4), 129.26 (C8), 127.82 (C9), 127.02 (C2', C6'), 125.87 (C3), 125.34 (C1), 119.44 (C7), 106.22 (C5), 55.70 (OMe), 49.06 (CH_2). HRMS (AP-ESI)m/z calcd for C19H16O3 $[M - H]^-$ 291.1027, found 291.1027. HPLC analysis: Retention time 12.2 min, eluted with 65% methanol/35% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 98.7%.

2-hydroxy-5-(6-methoxynaphthalen-2-yl)benzoic acid (**7**). Yield: 81%; white powder; mp: > 260°C. ^{1}H NMR (400 MHz, DMSO-d_6, r.t.) δ 8.16 (d, *J* = 2.3 Hz, 1H, H1'), 8.10 (s, 1H, H1), 7.95 (dd, *J* = 8.7, 2.4 Hz, 1H, H6'), 7.92 (d, *J* = 9.3 Hz, 1H, H8), 7.89 (d, *J* = 8.9 Hz, 1H, H4), 7.76 (dd, *J* = 8.5, 1.5 Hz, 1H, H3), 7.34 (d, *J* = 2.1 Hz, 1H, H5), 7.19 (dd, *J* = 8.9, 2.4 Hz, 1H, H7), 7.09 (d, *J* = 8.6 Hz, 1H, H5'), 3.89 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6, r.t.) δ 172.26 (COOH), 160.96 (C4'), 157.81 (C6), 134.56 (C6'), 134.28 (C1'), 133.78 (C10), 131.65 (C2'), 130.11 (C2), 129.30 (C4), 128.40 (C8), 127.92 (C9), 125.54 (C3), 124.82 (C1), 119.46 (C5'), 118.30 (C7), 114.10 (C3'), 106.20 (C5), 55.69 (OMe). HRMS (AP-ESI)m/z calcd for C18H14O4 $[M - H]^-$ 293.0819, found 293.0822. HPLC analysis: Retention time 9.86 min, eluted with 65% methanol/35% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 97.6%.

4-(6-methoxynaphthalen-2-yl)benzoic acid (**10**). Yield: 81%; white powder; mp: > 260°C. ^{1}H NMR (400 MHz, DMSO-d_6, r.t.) δ 8.26 (s, 1H, H1), 8.05 (d, *J* = 8.3 Hz, 2H, H3', H5'), 7.94 (d, *J* = 8.4 Hz, 1H, H4), 7.93 (s, 1H, H8), 7.92 (d, *J* = 8.3 Hz, 2H, H2', H6'), 7.86 (dd, *J* = 8.5, 1.5 Hz, 1H, H3), 7.38 (d, *J* = 2.2 Hz, 1H, H5), 7.22 (dd, *J* = 8.9, 2.4 Hz, 1H, H7), 3.91 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6, r.t.) δ 167.74 (COOH), 158.25 (C6), 144.53 (C1'), 134.48 (C10), 130.46 (C3', C5'), 130.43 (C8), 129.16 (C2), 128.00 (C4), 127.64 (C4'), 127.14 (C2', C6'), 126.80 (C3), 126.22 (C9), 125.78 (C1), 119.64 (C7), 106.25 (C5), 55.74 (OMe). HRMS (AP-ESI)m/z calcd for C18H14O3 $[M - H]^-$ 277.0870, found 277.0871. HPLC analysis: Retention time 14.3 min, eluted with 65% methanol/35% water (containing 0.1% acetic acid) with a flow rate of 1.5 mL/min, purity: 99.0%.

Luciferase enzyme inhibition assay. All compounds were dissolved in dimethylsulfoxide at 10 mM, then they were further diluted to an increasing concentration ranging from 1 nM to 100 μM in Tris buffer (pH=7.60) with 10 mM $MgCl_2$. The recombinant firefly luciferase was purchased from Promega (E1702, USA). The luciferase was diluted to 20 μg/mL in the Tris buffer prepared before. Substrates solution is also prepared in this Tris buffer, containing 20 μM of aminoluciferin and 2 mM of ATP. To a 96-well plates (WHB, black) each well containing 50 μL of the luciferase solution, an amount of 50 μL of increasing concentrations of compound was added as three replicates. After incubation at 25°C for 10 mins, an amount of 100 μL of the substrates solution was added. Also blank group (containing 100 μL Tris buffer instead of compounds and luciferase) and control group (vehicle, Containing equal amount of DMSO as experimental group) samples were measured. Luminescence produced by the luciferase was measured with Omega microplate reader (POLARstar Omega, Germany). The log-(inhibitor) *vs* normalized (control was set as 100%, variable slope) response data were analyzed with the GraphPad Prism software package.

Kinetics study was conducted in the same condition as in luciferase enzyme inhibition assay, except for that the inhibitor concentration was fixed while an increasing concentration of substrates (500, 250, 200, 100, 50, 25, 12, 6, 3 and 0 μM) were added to get the substrate saturation curve for kinetics calculation.

Cell culture and ES-2-Fluc cell bioluminescence inhibition assays. ES-2 cells (human ovarian cancers cell line) were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences. ES-2 cells expressing firefly luciferase (ES-2-Fluc cells) were supplied by Cellcyto. The ES-2-Fluc cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO_2 incubator.

In brief, 100 μL cells were seeded into black 96-well plates (4×10^5 cells per well). After incubated for 12 hours, 100 μL compounds of increasing concentration ranging from 500 μM to 7.81 μM (for compound 5, the concentration range is adjusted to from 500 μM to 32 nM) dissolved by RPMI 1640 medium were added as three triplicates. After incubation for 12 hours, the medium was removed, and 100 μL aminoluciferin solution (100 μM, dissolved in Tris buffer of PH 7.4) was added. Also blank group (added just Tris buffer instead of aminoluciferin)

and control group (vehicle, added RPMI 1640 medium containing equal amount of DMSO instead of inhibitors) were set. Immediately after the aminoluciferin was added, the bioluminescence intensity was measured with IVIS Kinetic (Caliper Life Sciences, USA) instrument equipped with a cooled charge coupled device (CCD) camera for bioluminescent imaging. The pseudo colored bioluminescent images (in photons/sec/cm^2/scr) were superimposed over the grayscale photographs of the animals. Circular ROIs were drawn over the areas and quantified using Living Image software. The results were reported as total photon flux within an ROI in photons per second. The log-(inhibitor) *vs* normalized (control was set as 100%, variable slope) response data were analyzed with the GraphPad Prism software.

Cell viability assay. ES-2-Fluc cells were seeded onto 96-well plates in 100 μL of RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (at a density of 8000 cells/well). After incubated overnight at 37°C in a humidified atmosphere with 5% CO_2 incubator, 100 μL of compounds diluted by RPMI 1640 medium to various concentration (500, 250, 125 and 62.5 μM) were added, for control group, 100 μL RPMI 1640 medium was added, for blank group, 100 μL RPMI 1640 medium containing equal amount of DMSO was added. After 8 h incubation, each well was treated with 1% of 0.5 mg/mL MTT reagent and incubated for additional 4 h. After that, the culture was removed and 100 μL DMSO was added. Absorbance at 490 nm was measured using an Omega microplate reader (POLARstar Omega, Germany). The viability rate of compounds was calculated by $(OD-OD_{blank})/(OD_{control}-OD_{blank})\times100\%$, where OD is the mean value of three triplicate wells.

In vivo bioluminescence imaging inhibition study.

Day 1: Fifteen mice bearing ES-2-Fluc subcutaneous tumors were divided into 3 groups (normal saline group, resveratrol group, inhibitor 5 group) randomly, each group containing five mice. The mice were anesthetized with isoflurane and intraperitoneally injected with 100 μL aminoluciferin (0.5 mM), followed immediately by bioluminescent imaging every 2 minutes for 30 to 40 minutes until the bioluminescence intensity went through a peak and get steady. Then, give the mouse 12 hours to metabolize away the aminoluciferin. After that, for resveratrol group and inhibitor 5 group, each mouse was injected with 25 μL resveratrol or 5 (200 μM) intratumorally, for normal saline group, 25 μL normal saline containing equal amount of DMSO (15%) were injected intratumorally.

Day 2: The mice were intraperitoneally injected with 100 μL aminoluciferin (0.5 mM, diluted by normal saline). Subsequently, bioluminescent imaging was taken every 2 minutes for 30 to 40 minutes. The relative activity for each mouse was calculated by dividing the peak total photon flux of day 1 by peak total flux of day 2. The residual activity of mice treated with inhibitors is calculated by the ratio of comparing the relative activity of inhibition group with the saline group, setting the saline group as 100%.

In vivo inhibition assay in transgenic mice by tail intravenous injection

Pathogen-free luciferase-expressing transgenic mice (FVB-Tg(CAG-luc,-GFP)L2G85Chco/FathJ)17 were obtained from the Jackson Laboratory and housed in the Shandong University. Nine transgenic mice were randomized into three groups: normal saline group, compound 5 group, and resveratrol group. For compound 5 group and resveratrol group, 200 μL compound 5 or resveratrol (200 μM, dissolved in normal saline) were injected into the mice veins through tail intravenous injection. For the normal saline group, just 200 μL normal saline was injected. After 4 hours, the mice were anesthetized with isoflurane and injected with 100 μL aminoluciferin (0.5 mM, dissolved in normal saline) intraperitoneally. Immediately after injection with aminoluciferin, bioluminescent imaging was taken every 2 minutes for 30 to 40 minutes until the bioluminescence intensity went through a peak and got steady. The inhibition rate was calculated by comparing the bioluminescence average flux of inhibitors group with the normal saline group.

Dual-luciferase reporter gene assay simulation. Luciferase mixture is prepared by dissolving Fluc and Rluc in Tris-HCl buffer (20 μg/mL and 2.5 μg/mL, respectively). First of all, 50 μL Fluc substrates solution (20 μM aminoluciferin and 1 mM ATP in Tris-HCl buffer) were added to 50 μL luciferase mixture in black 96-well plates to

initiate the firefly luminescence. Then, 100 µL mixture of compound 5 and Rluc substrate coelenterazine (50 µM and 5 µM in Tris-HCl buffer, respectively) was added to quench the firefly luminescence and initiate renilla luminescence. Bioluminescence of both was measured immediately at wavelength 590 nm and 460 nm, emitted by Fluc and Rluc, respectively.

2. Kinetics assay in lower inhibitor concentration.

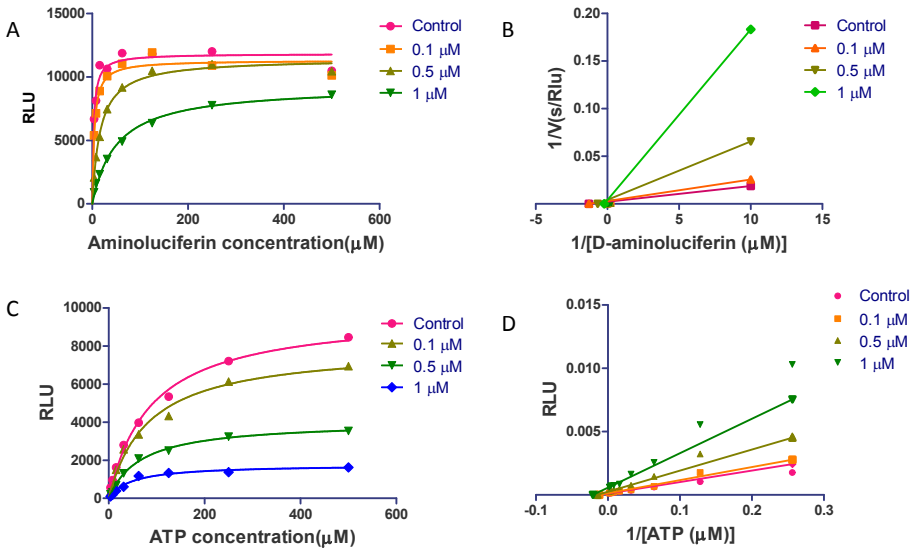


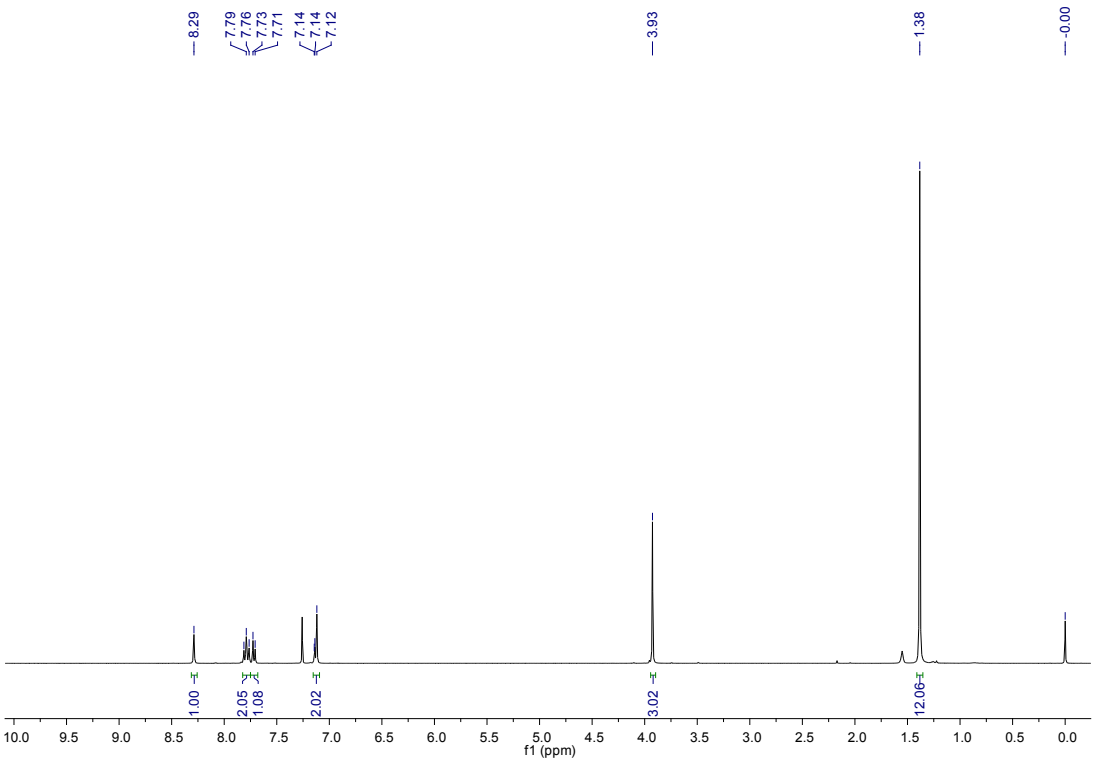
Fig S1 Kinetics of inhibition of luciferase by compound 5 in low concentration. (A) aminoluciferin saturation assay with increasing concentrations (3.9, 7.8, 15.6, 31.2, 65.2, 125, 250, 500 µM); (B) a Lineweaver–Burk plot of data in (A); (C) ATP saturation assay with increasing concentrations (3.9, 7.8, 15.6, 31.2, 65.2, 125, 250, 500 µM); (D) a Lineweaver–Burk plot of data in (C). The lines of (A) and (C) are fitted to Michaelis–Menten assay using GraphPad Prism 5 software. The Lineweaver–Burk plots are estimated using GraphPad Prism software.

	Concentration (µM)	no inhibitor	0.1 µM	0.5 µM	1 µM
Amino-luciferin	V_{max} (Rlu/s)[a]	379	343	242	258
	K_m (µM)[a]	0.66	0.78	1.49	4.63
	K_i[b]	N.D.[c]	0.12	0.10	0.11
ATP	V_{max} (Rlu/s)[a]	9757	7959	4035	1765
	K_m (µM)[a]	88.5	82.2	67.2	48

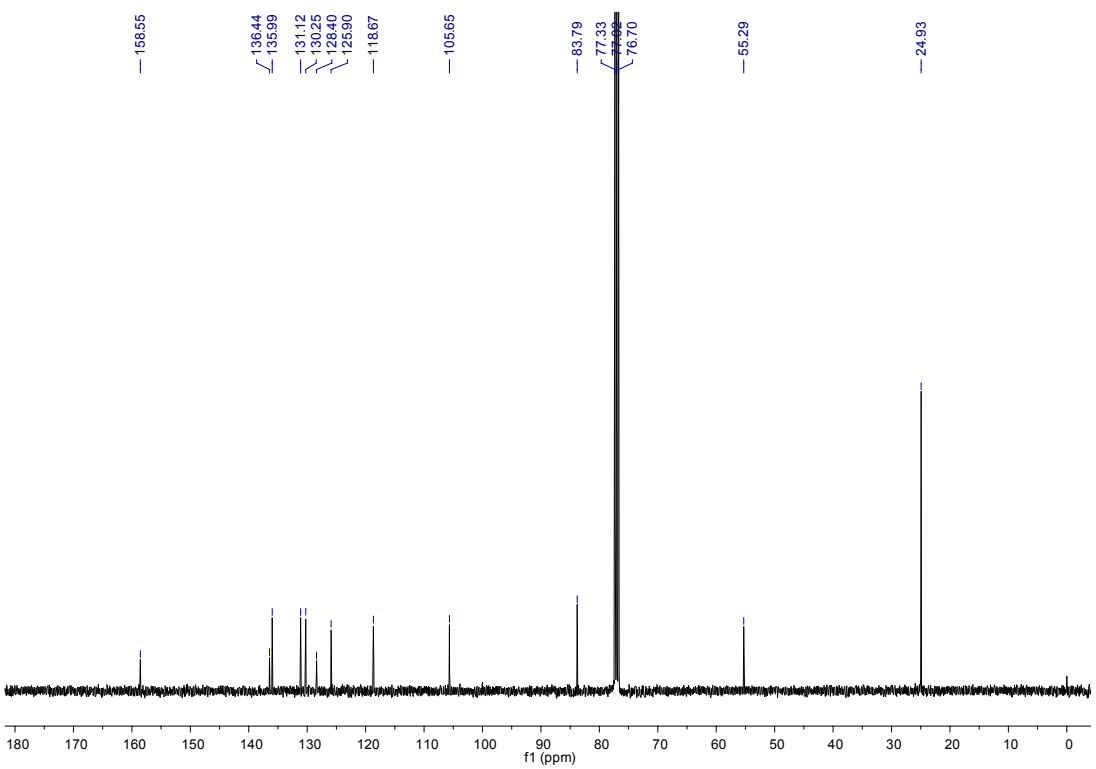
Table S1 Kinetic parameters V_{max} and K_m of substrate, aminoluciferin and ATP in low inhibitor concentration.

3. ^{1}H NMR and ^{13}C NMR spectra of compound 2 and compounds 4-14.

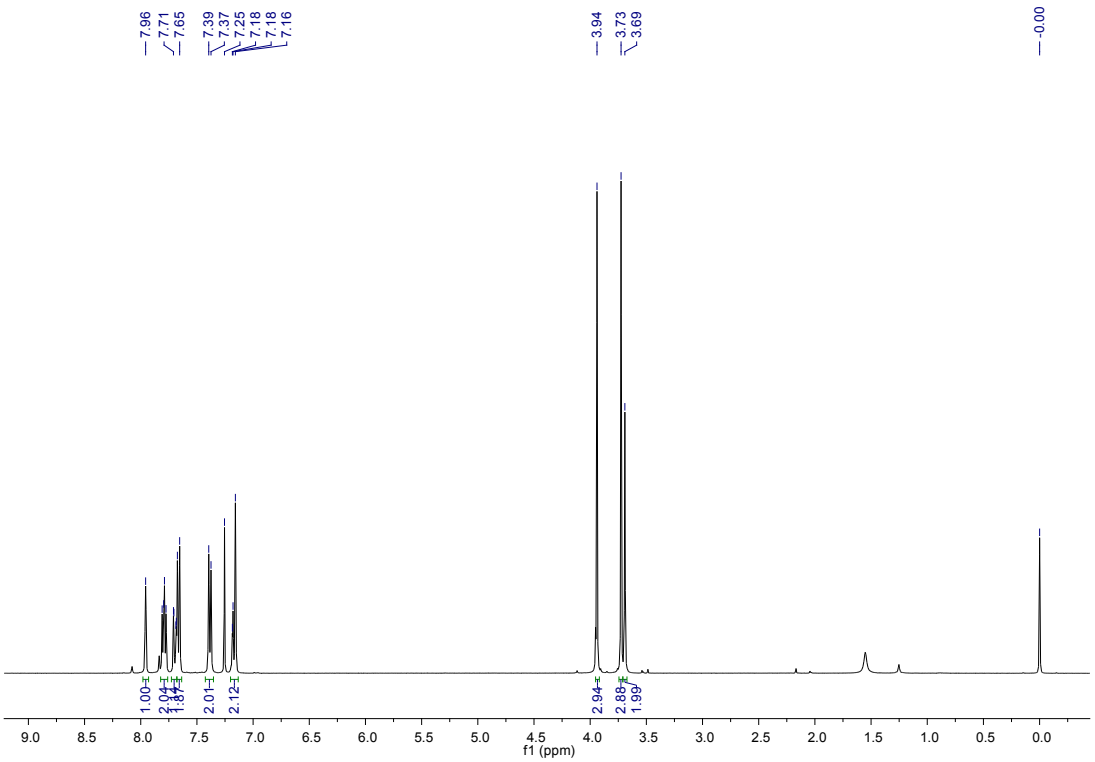
^{1}H NMR (400 MHz, $CDCl_3$, r.t.) spectrum for 2



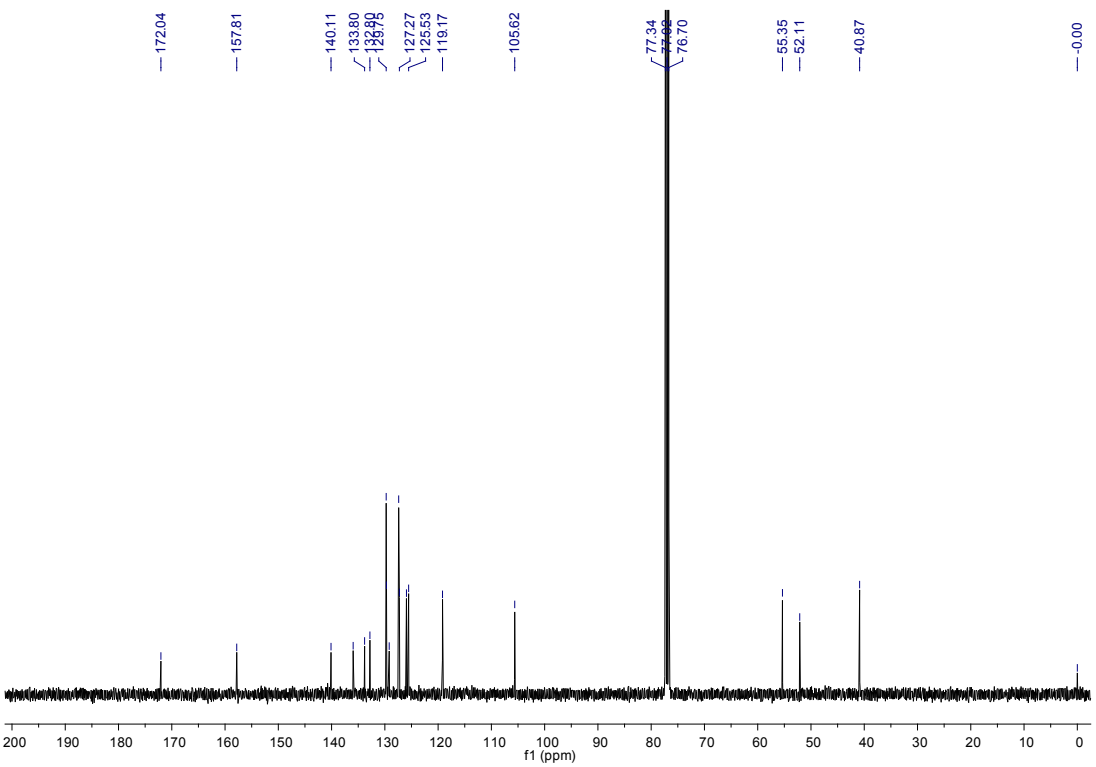
^{13}C NMR (100 MHz, $CDCl_3$, r.t.) spectrum for **2**



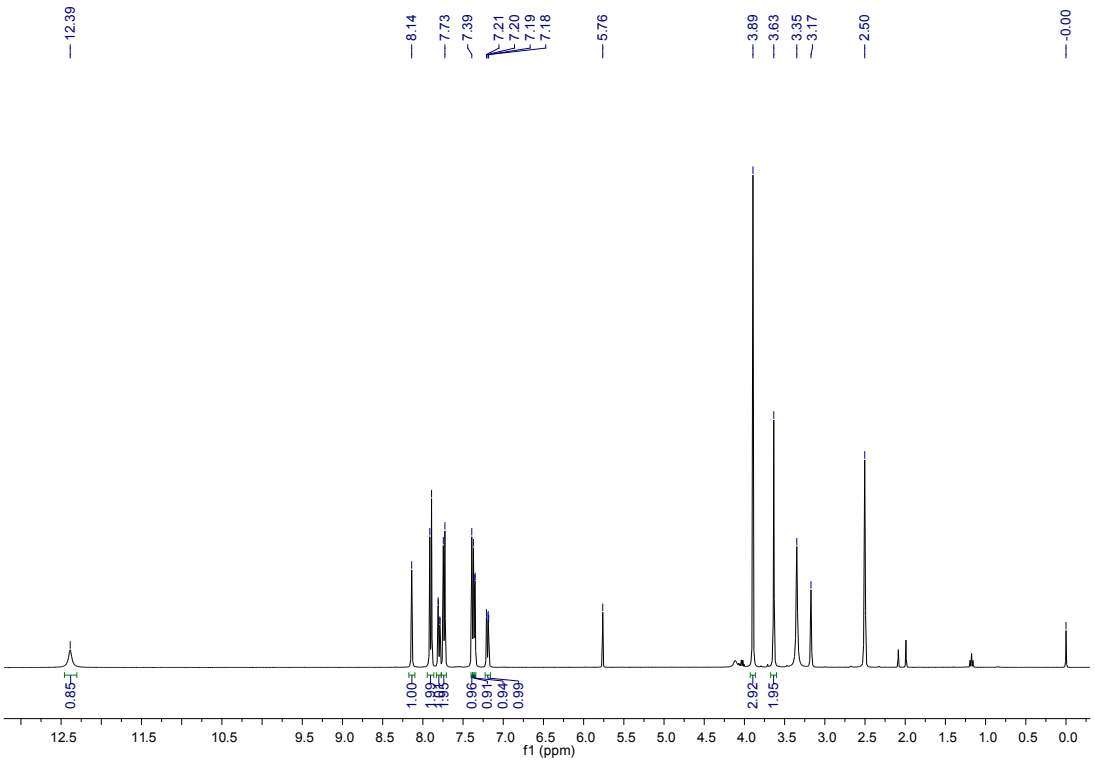
^{1}H NMR (400 MHz, $CDCl_3$, r.t.) spectrum for **4**



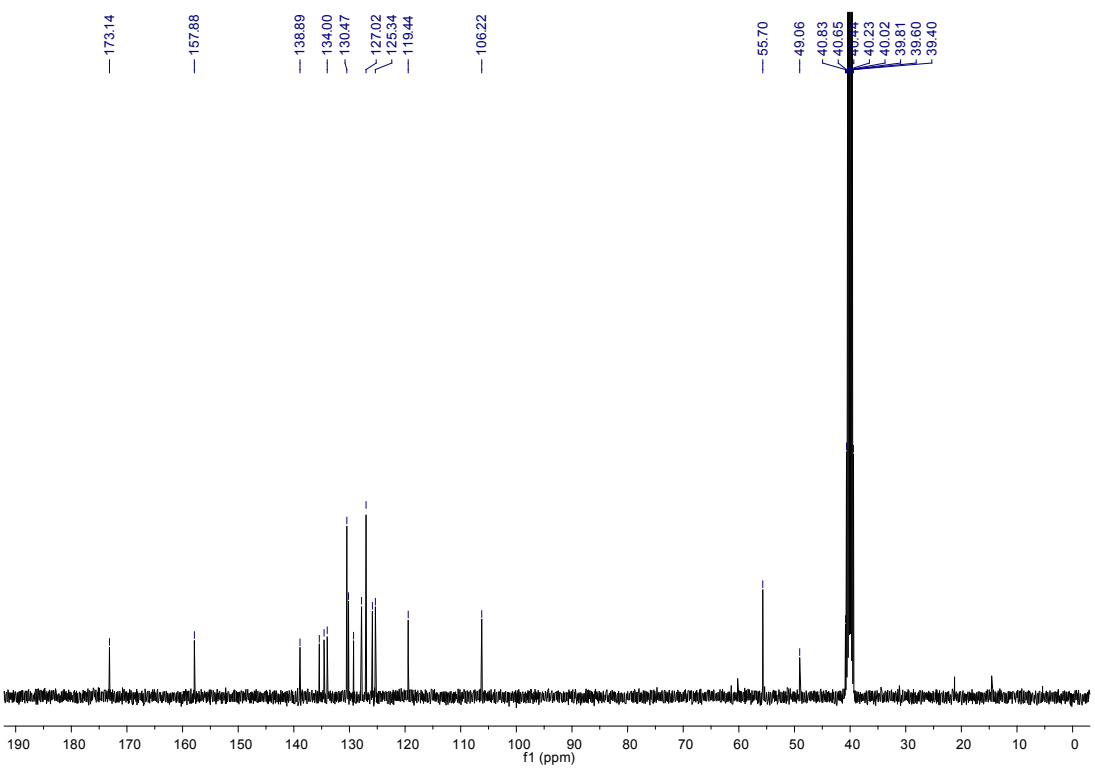
^{13}C NMR (100 MHz, $CDCl_3$, r.t.) spectrum for **4**



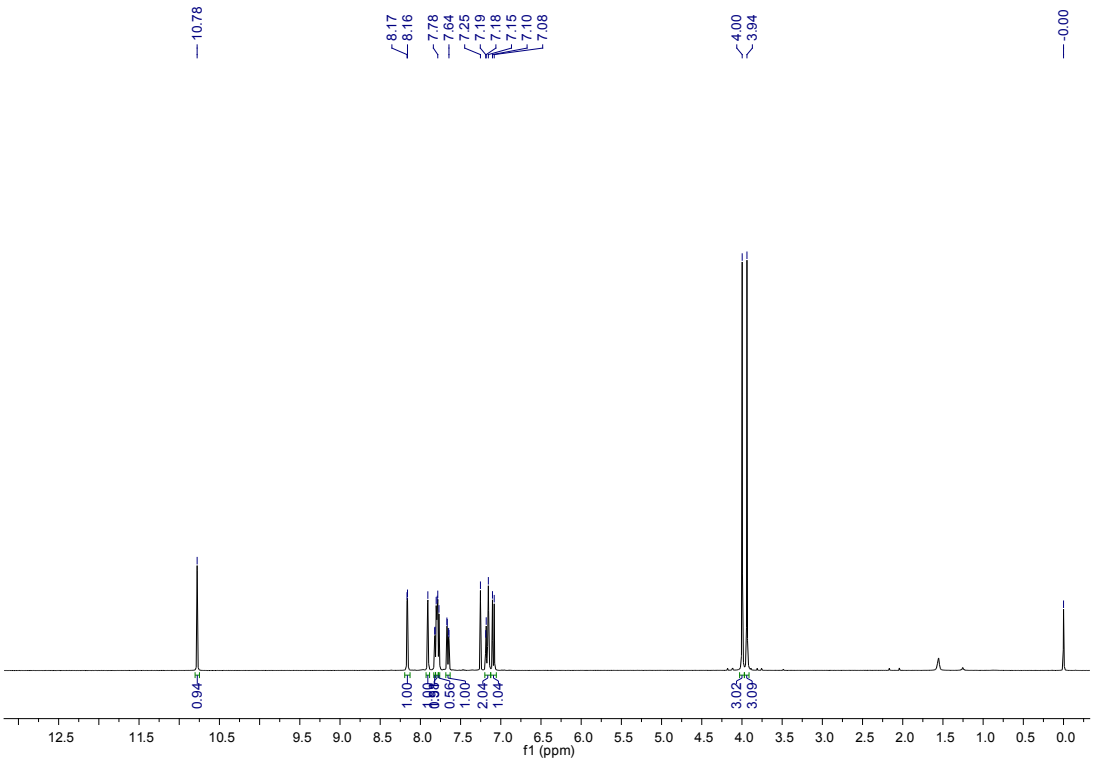
^{1}H NMR (400 MHz, DMSO-d_6, r.t.) spectrum for **5**



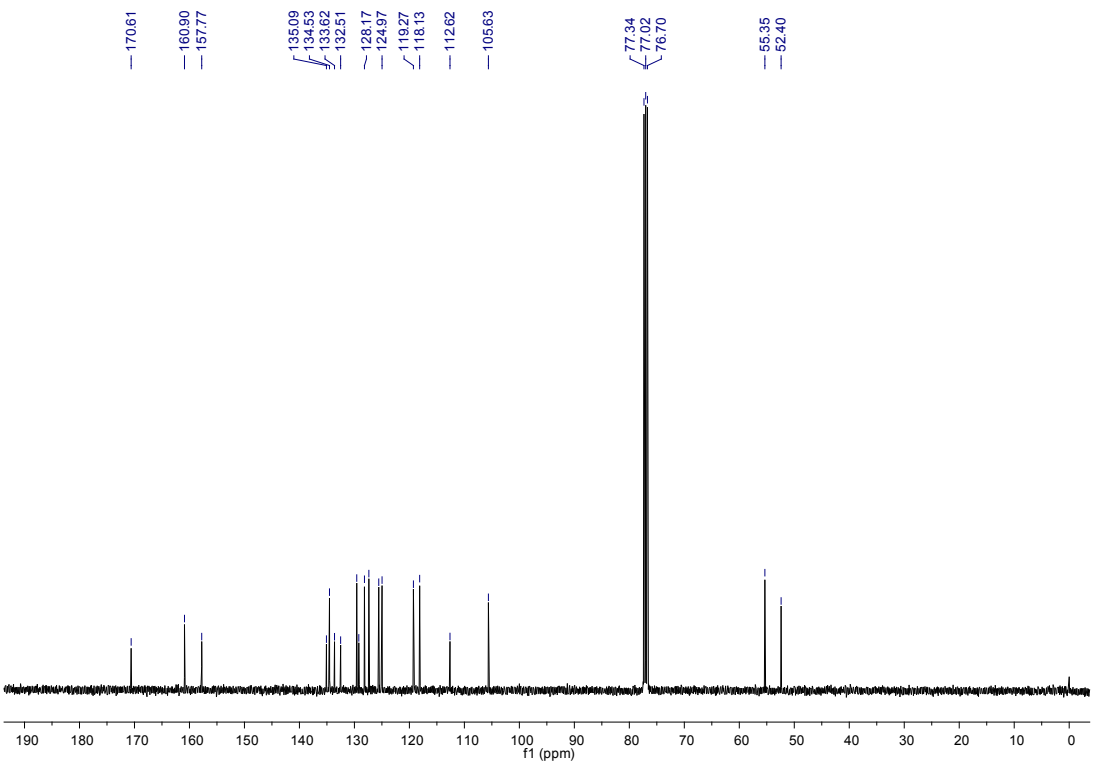
^{13}C NMR (100 MHz, DMSO-d_6, r.t.) spectrum for **5**



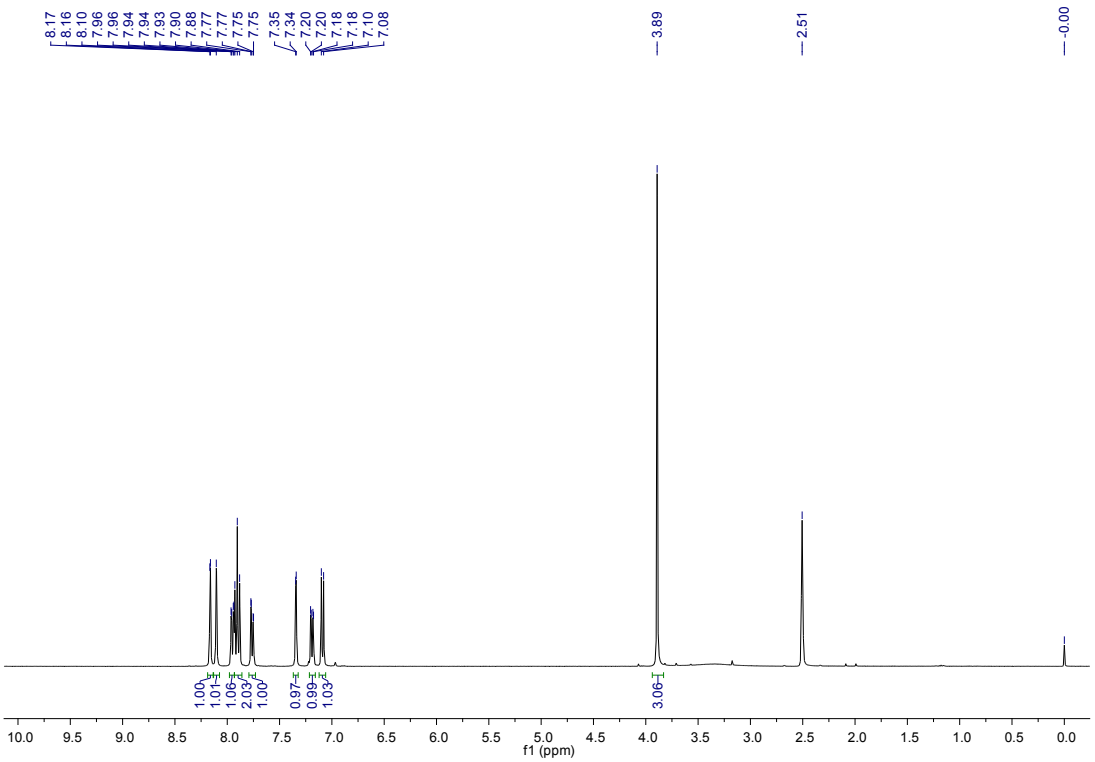
^{1}H NMR (400 MHz, $CDCl_3$, r.t.) spectrum for **6**



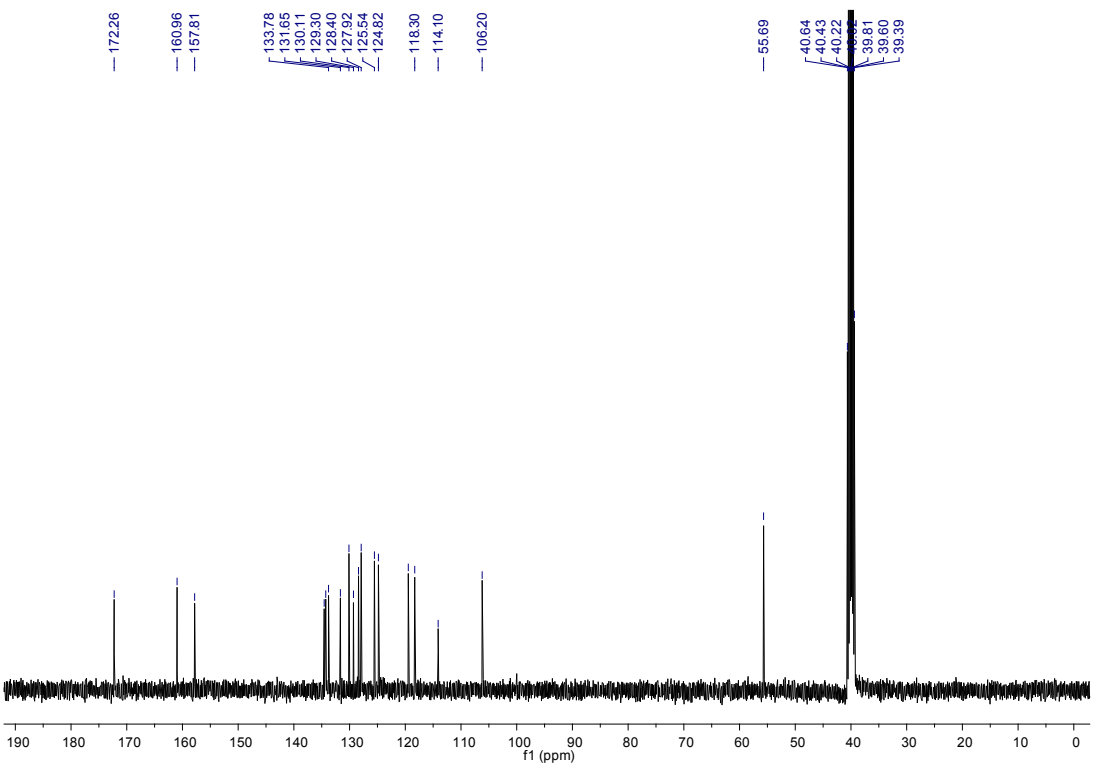
^{13}C NMR (100 MHz, $CDCl_3$, r.t.) spectrum for **6**



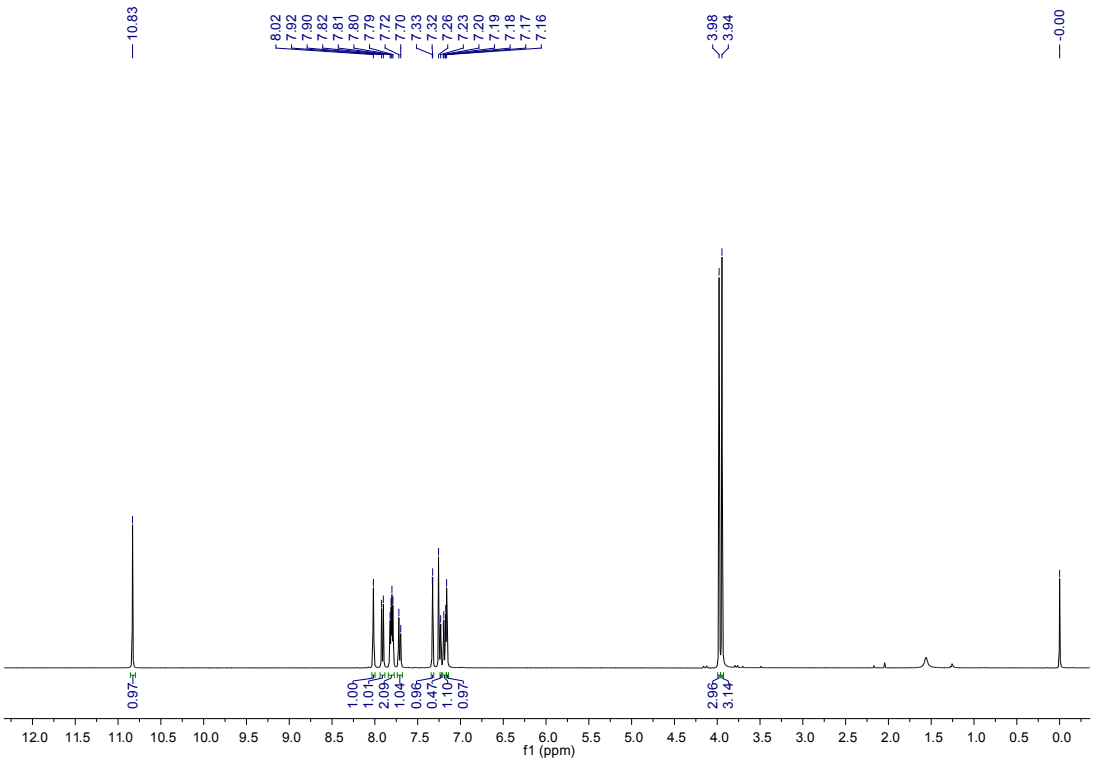
^{1}H NMR (400 MHz, DMSO-d_6, r.t.) spectrum for **7**



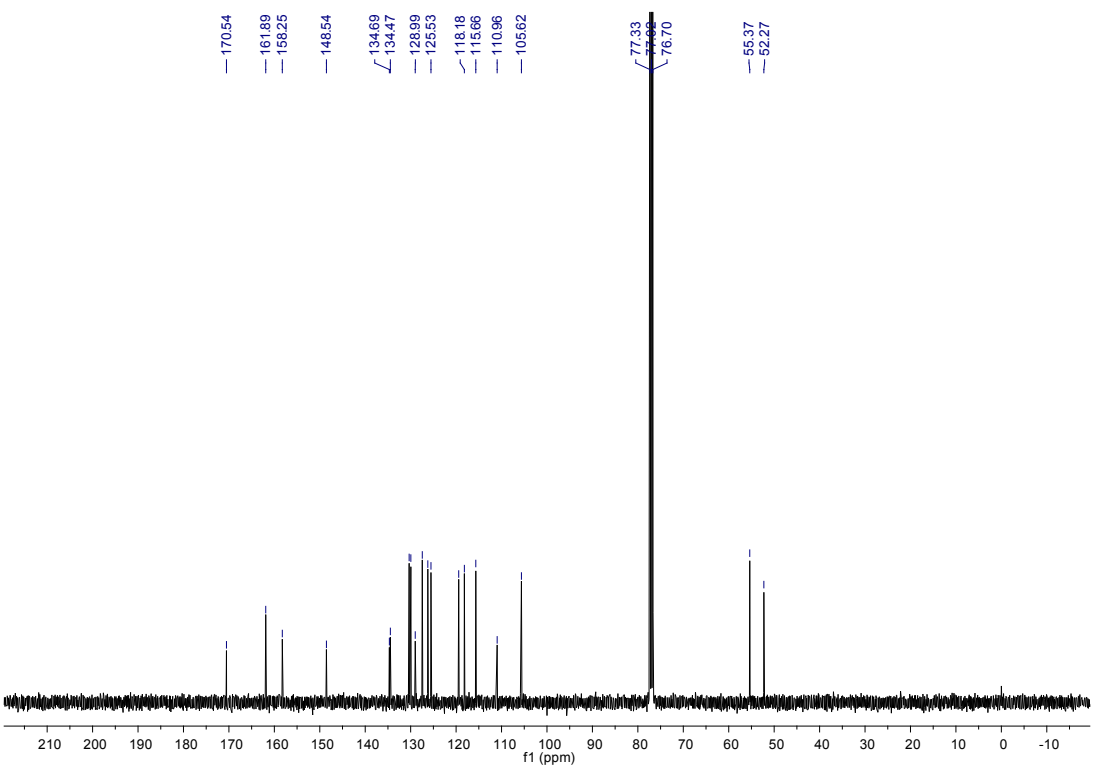
^{13}C NMR (100 MHz, DMSO-d_6, r.t.) spectrum for **7**



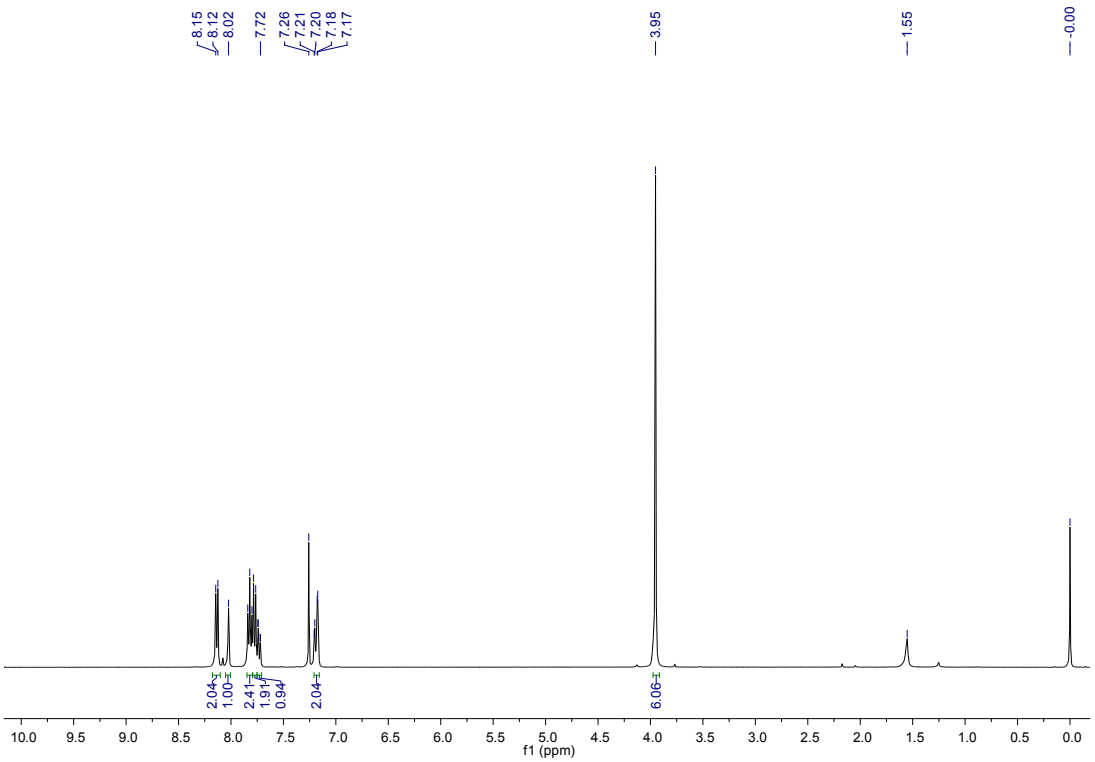
^{1}H NMR (400 MHz, $CDCl_3$, r.t.) spectrum for **8**



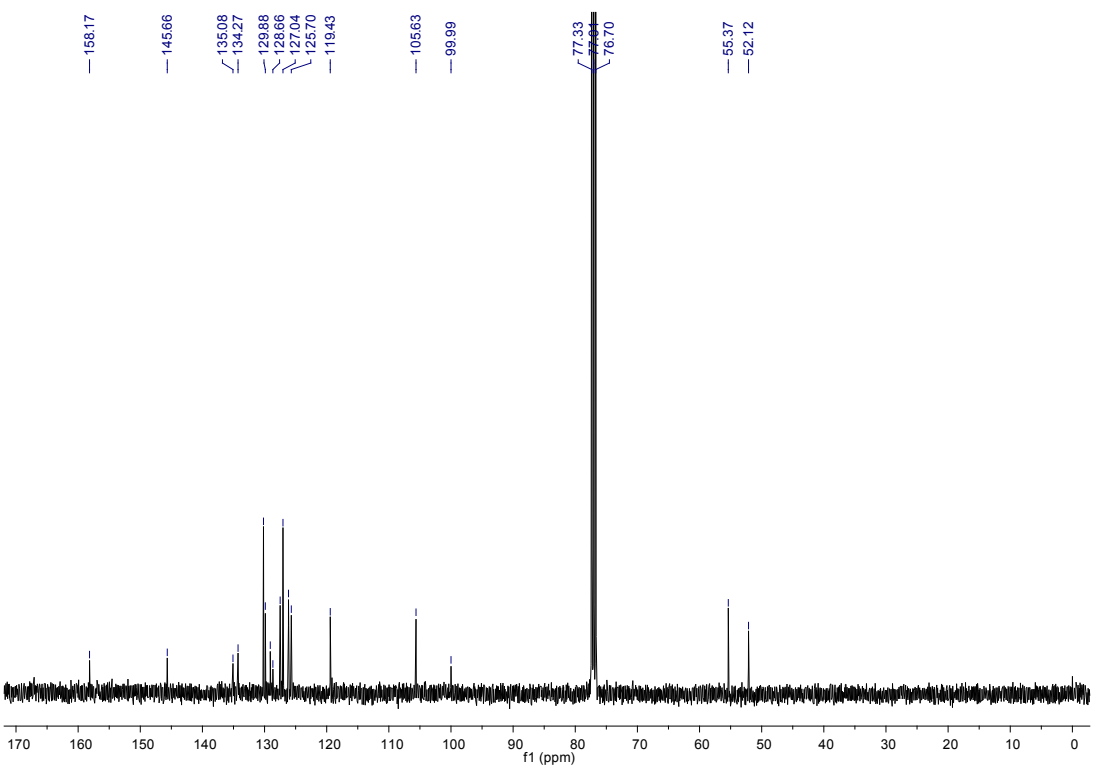
^{13}C NMR (100 MHz, $CDCl_3$, r.t.) spectrum for **8**



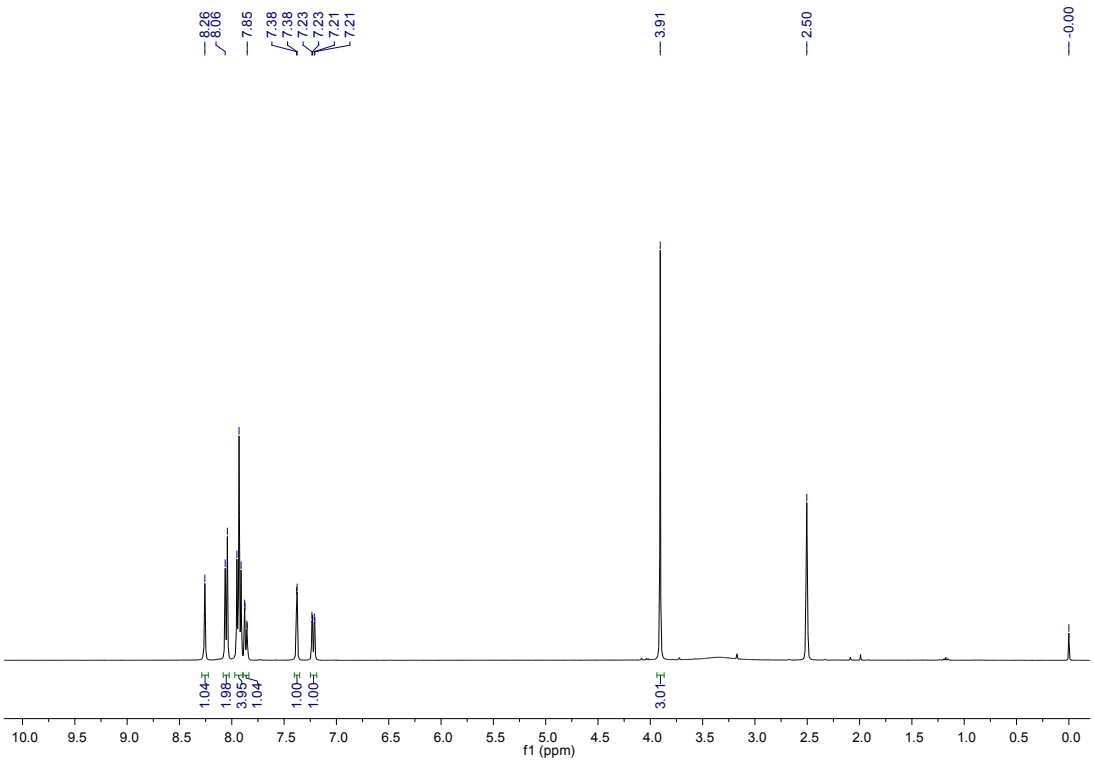
^{1}H NMR (400 MHz, $CDCl_3$, r.t.) spectrum for **9**



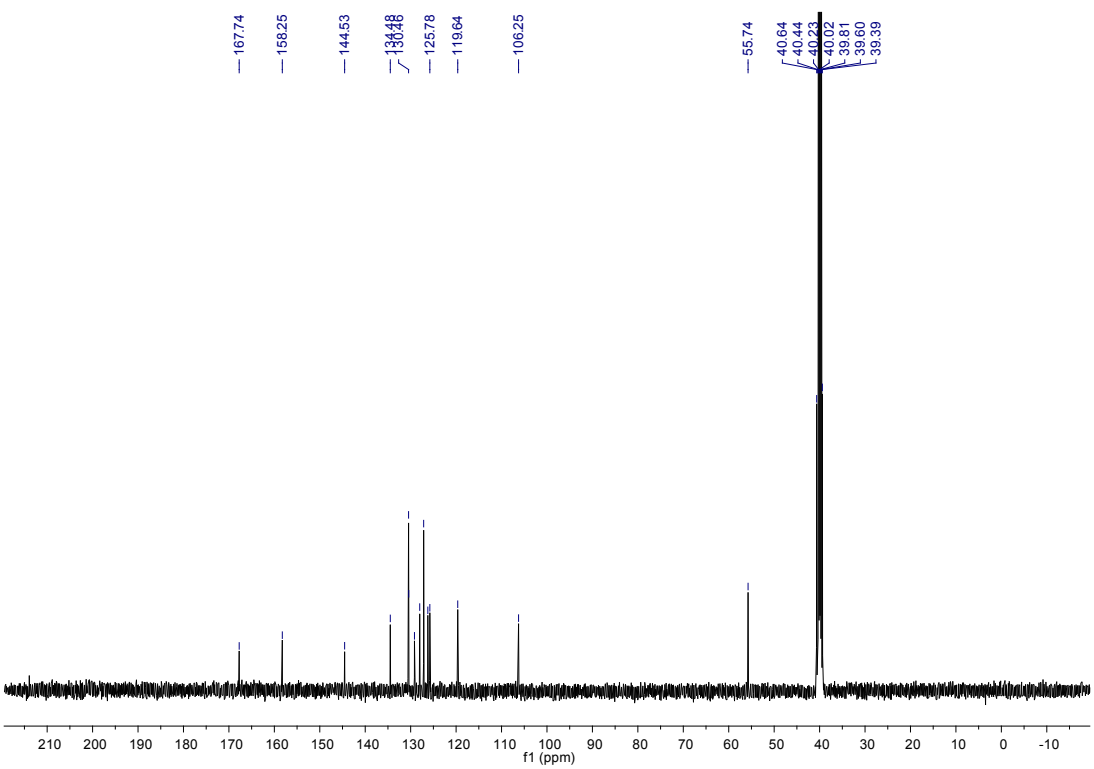
^{13}C NMR (100 MHz, $CDCl_3$, r.t.) spectrum for **9**



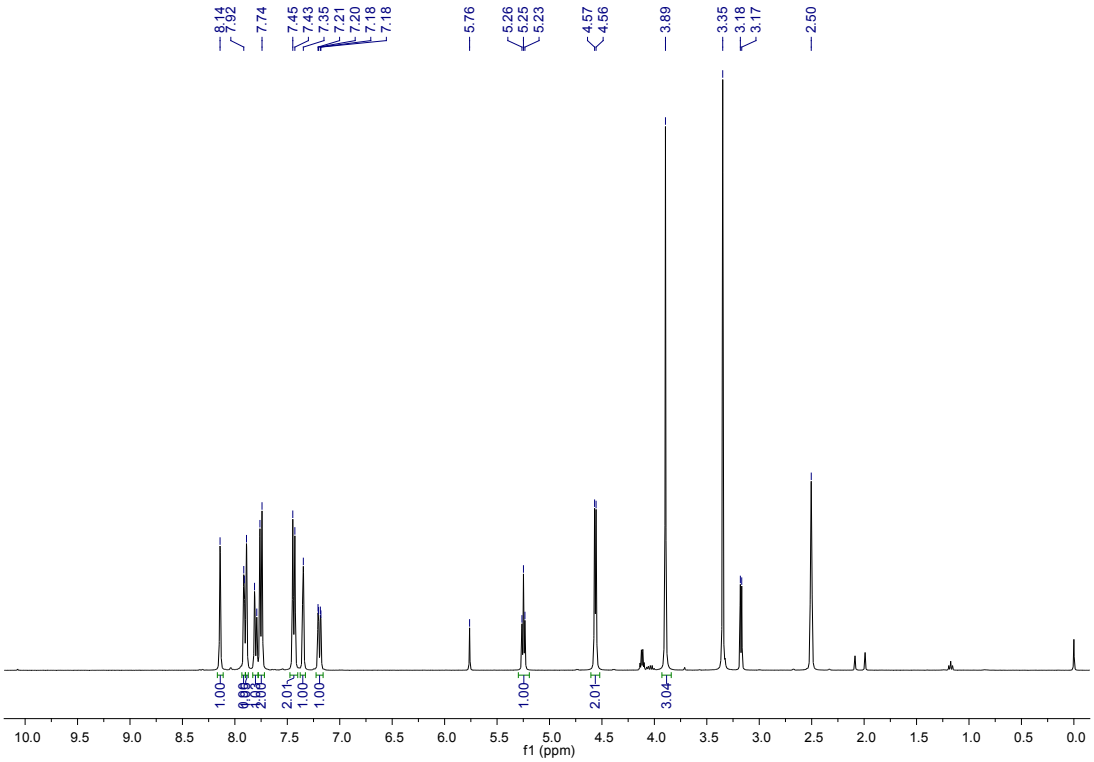
^{1}H NMR (400 MHz, DMSO-d_6, r.t.) spectrum for **10**



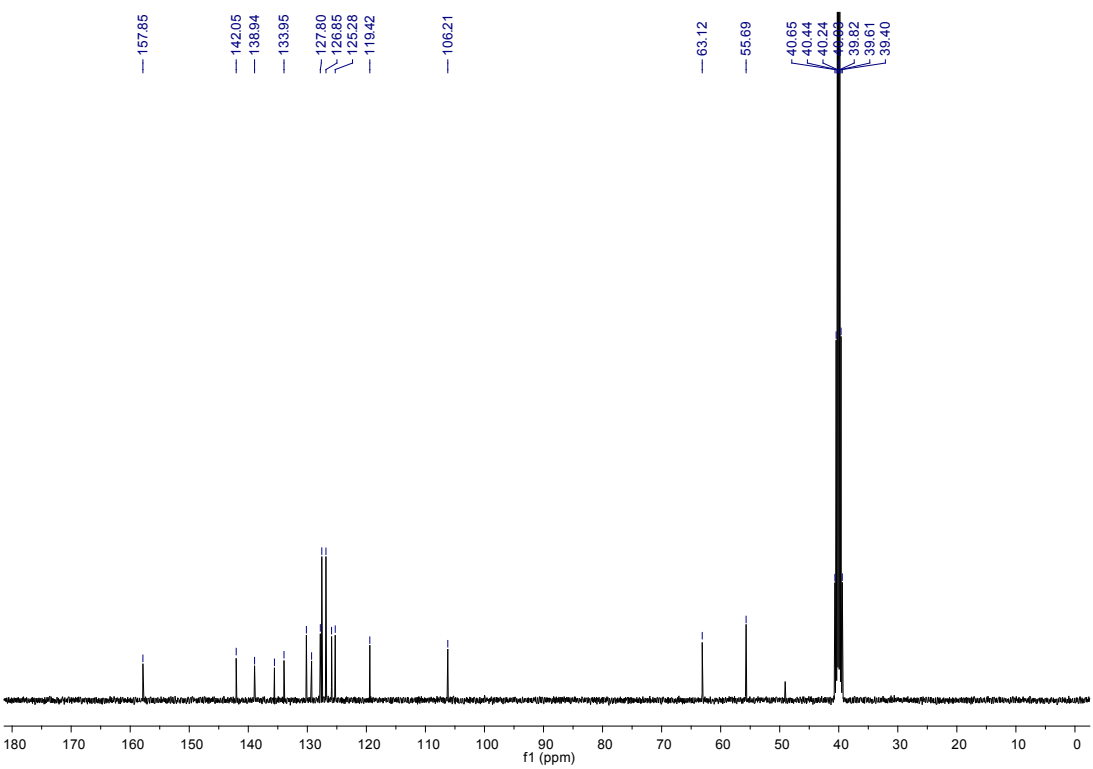
^{13}C NMR (100 MHz, DMSO-d_6, r.t.) spectrum for **10**



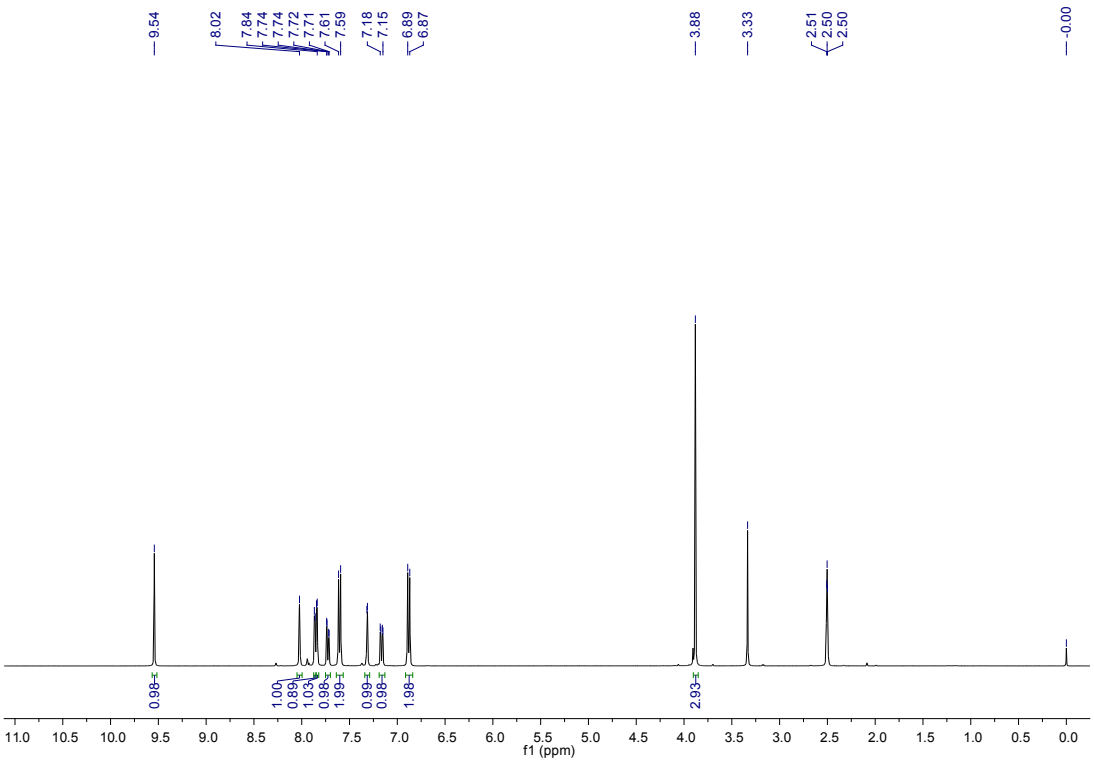
^{1}H NMR (400 MHz, DMSO-d_6, r.t.) spectrum for **11**



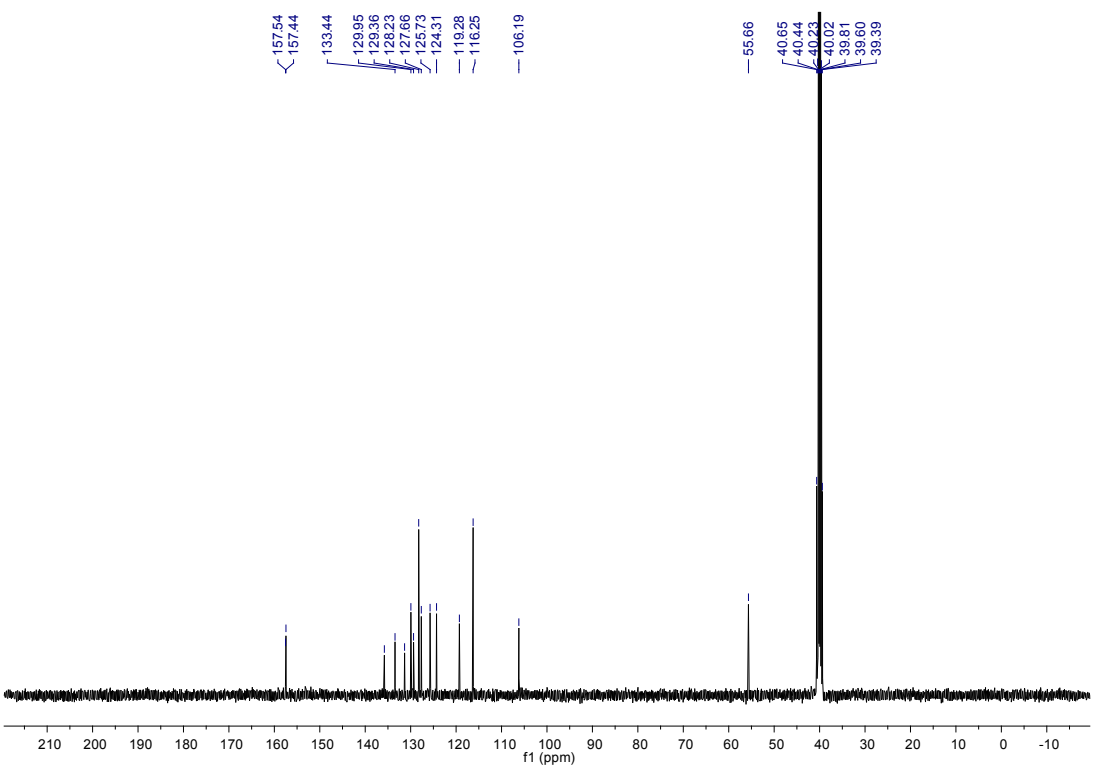
^{13}C NMR (100 MHz, DMSO-d_6, r.t.) spectrum for **11**



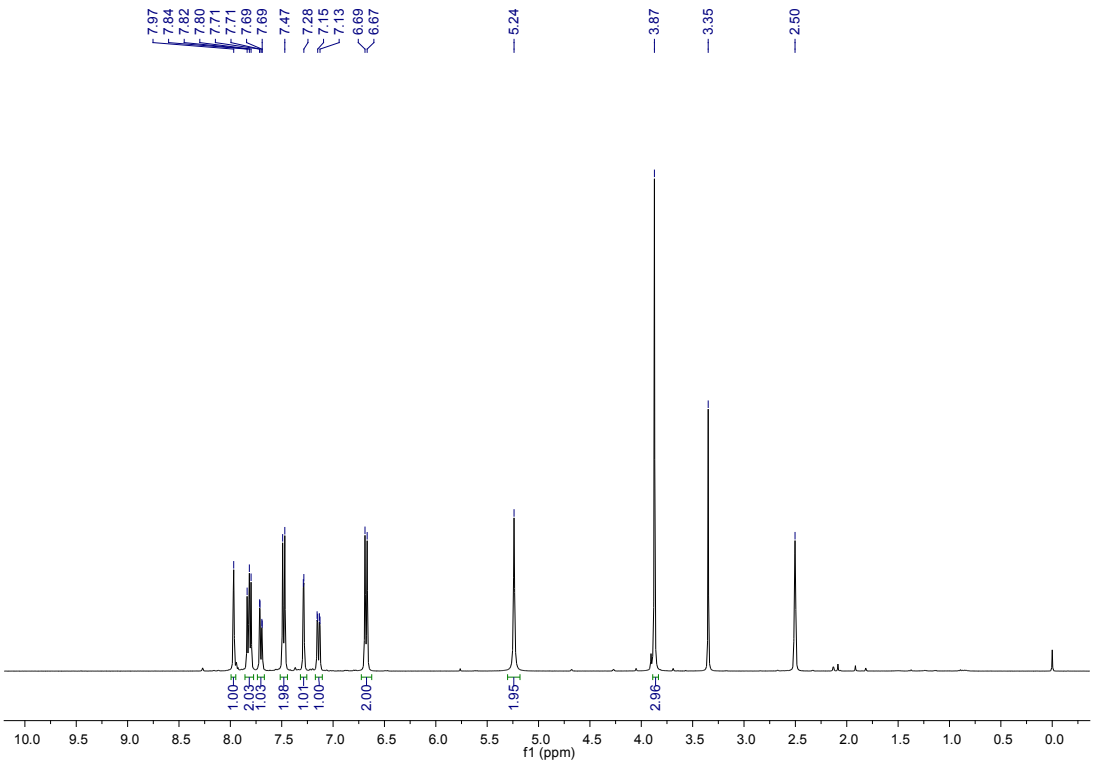
^{1}H NMR (400 MHz, DMSO-d_6, r.t.) spectrum for **12**



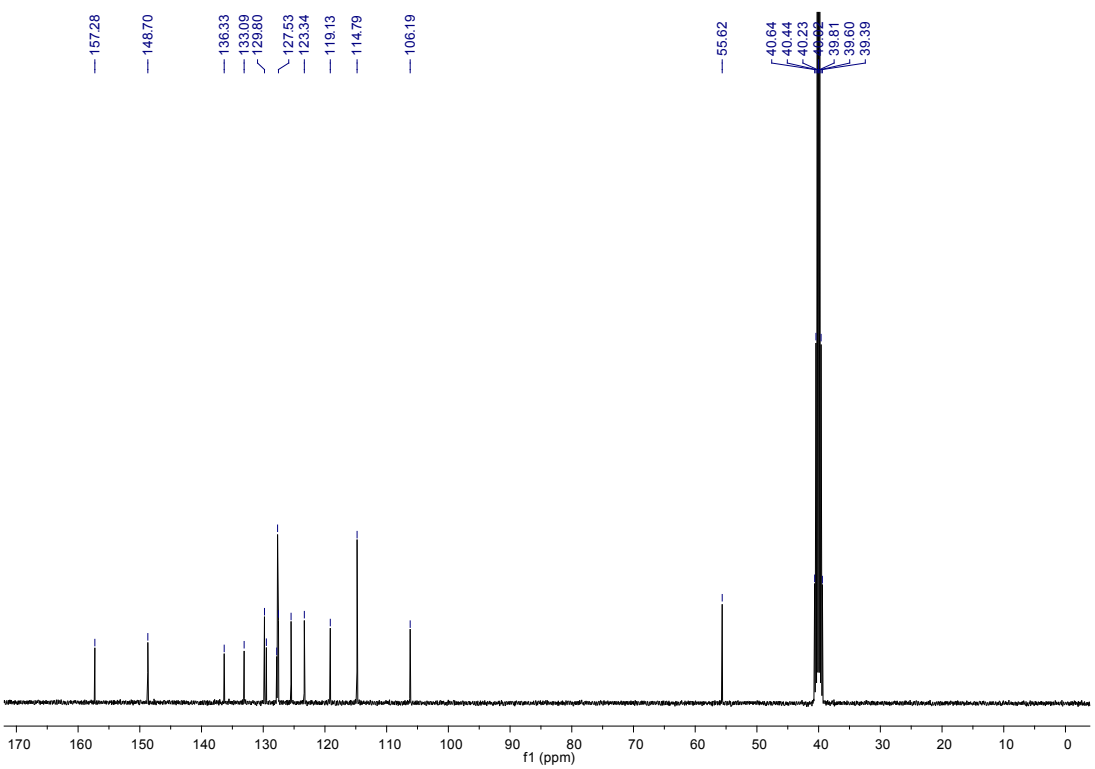
^{13}C NMR (100 MHz, DMSO-d_6, r.t.) spectrum for **12**



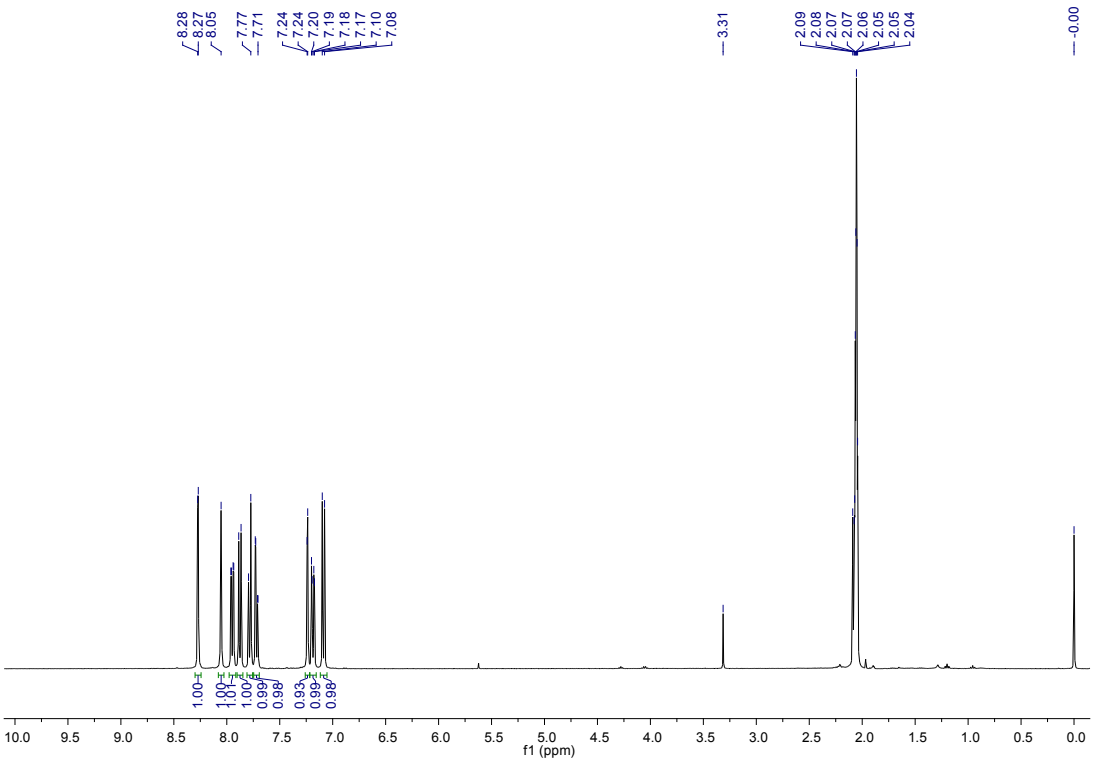
^{1}H NMR (400 MHz, DMSO-d_6, r.t.) spectrum for **13**



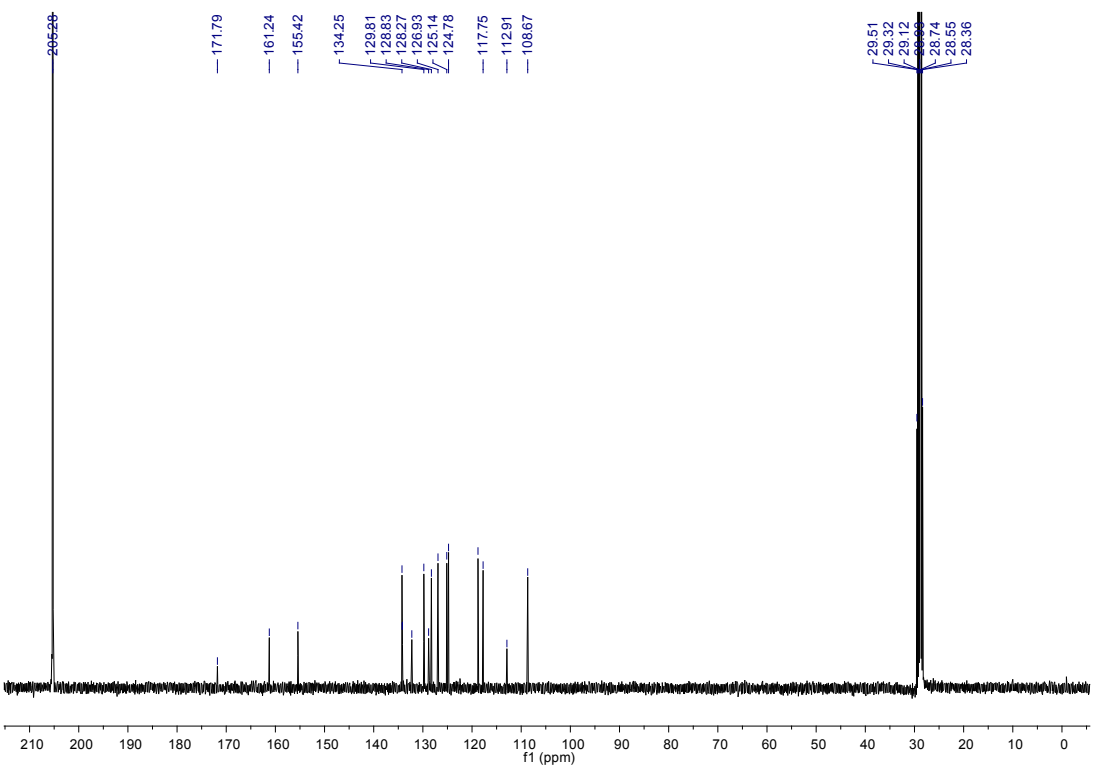
^{13}C NMR (100 MHz, DMSO-d_6, r.t.) spectrum for **13**



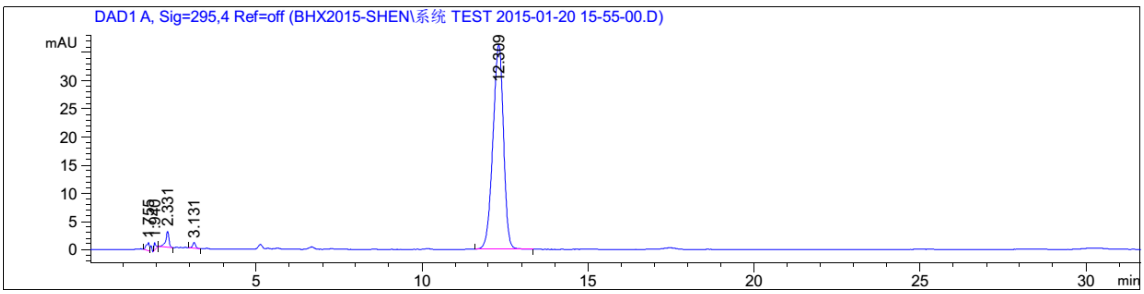
^{1}H NMR (400 MHz, acetone-d_6, r.t.) spectrum for **14**



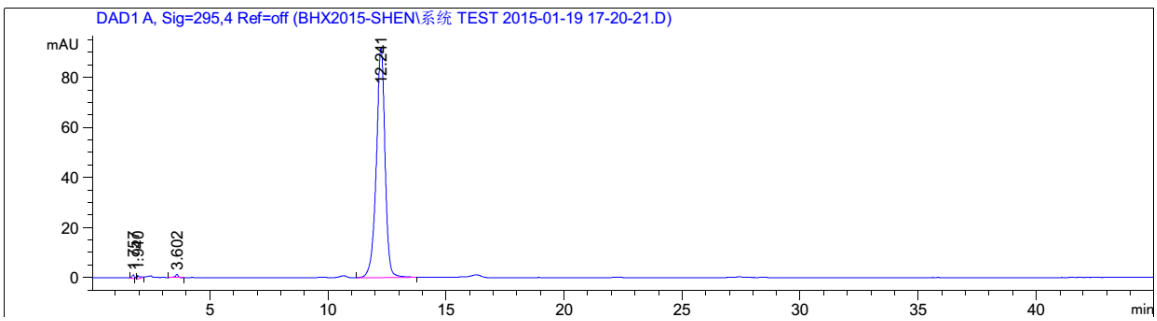
^{13}C NMR (100 MHz, acetone-d_6, r.t.) spectrum for **14**



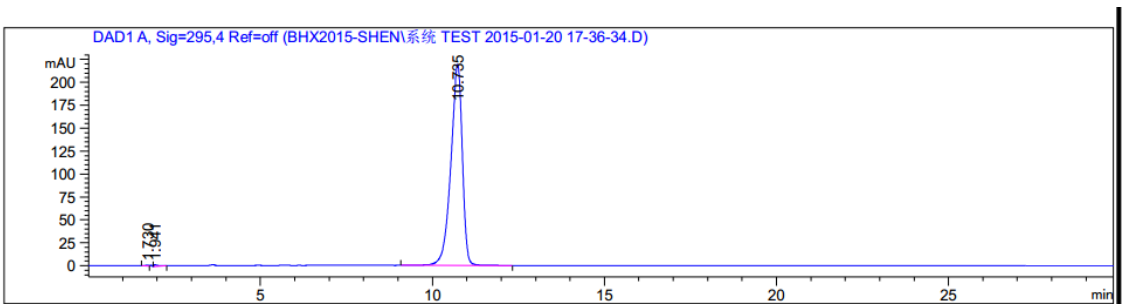
4. HPLC spectra of compounds 4-14.



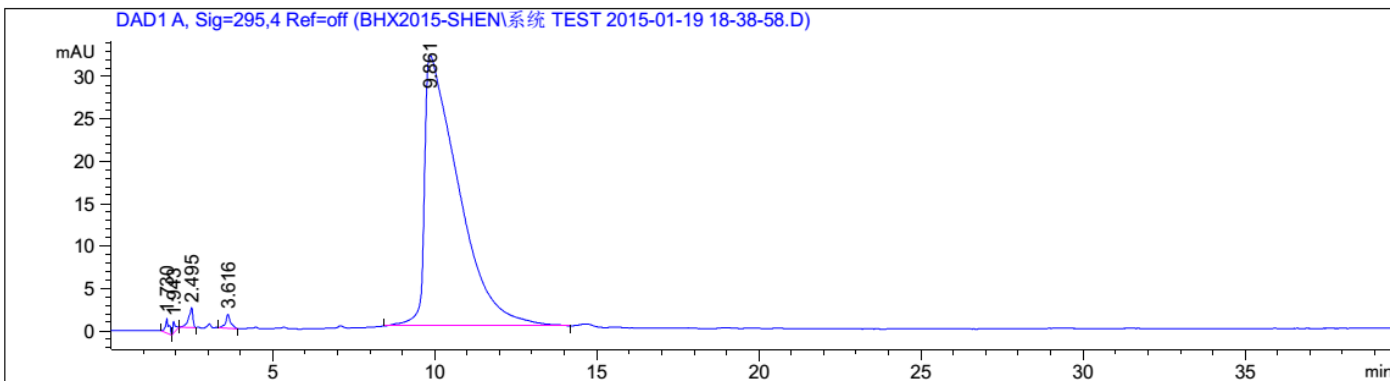
HPLC of Compound 4



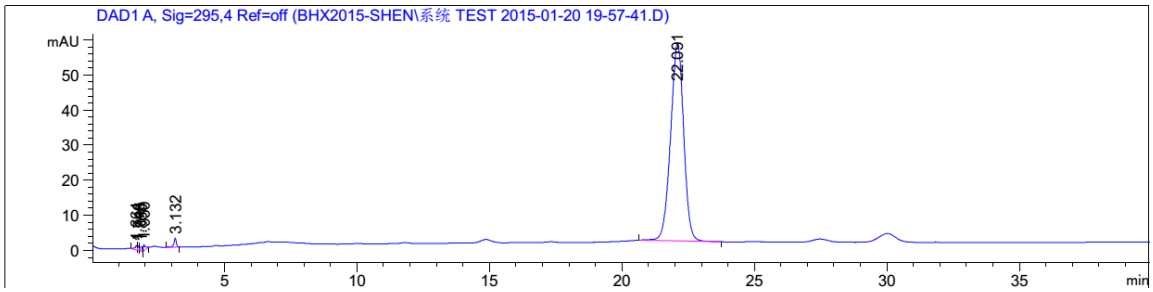
HPLC of Compound 5



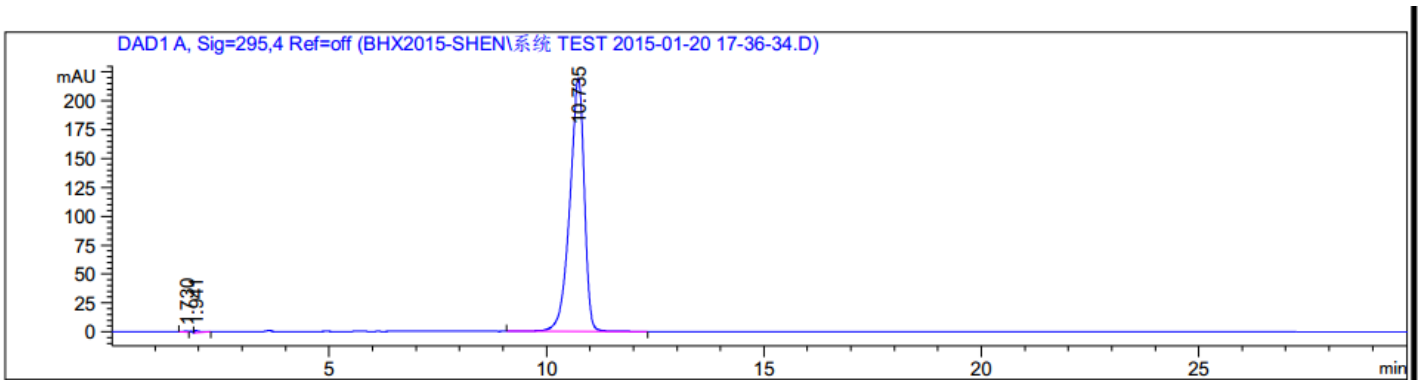
HPLC of Compound 6



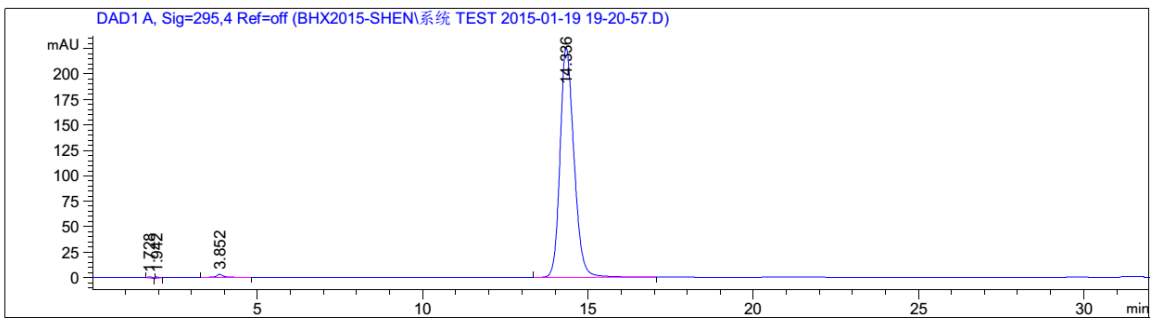
HPLC of Compound 7



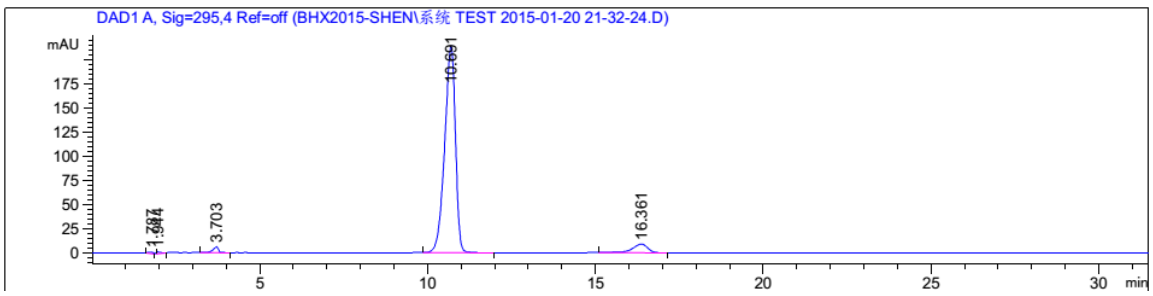
HPLC of Compound 8



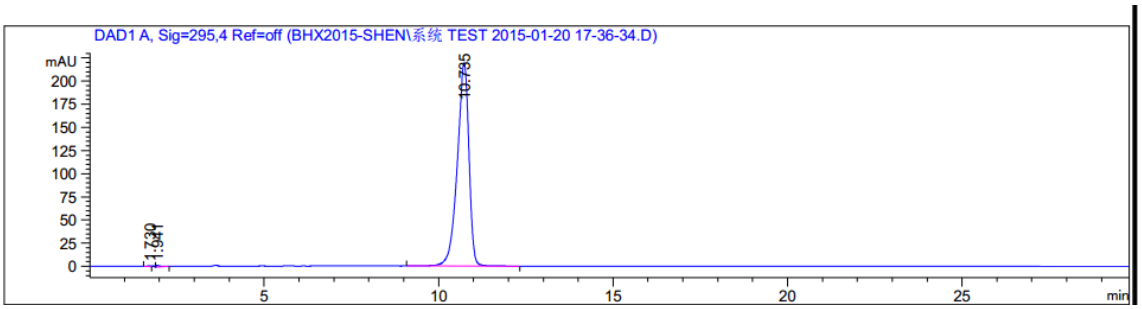
HPLC of Compound 9



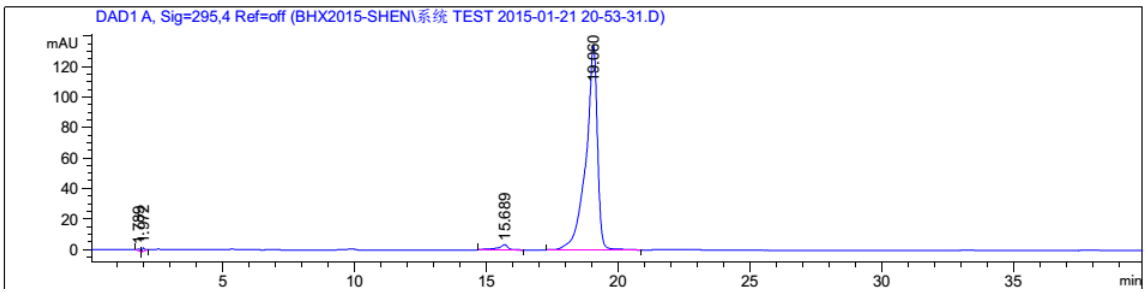
HPLC of Compound 10



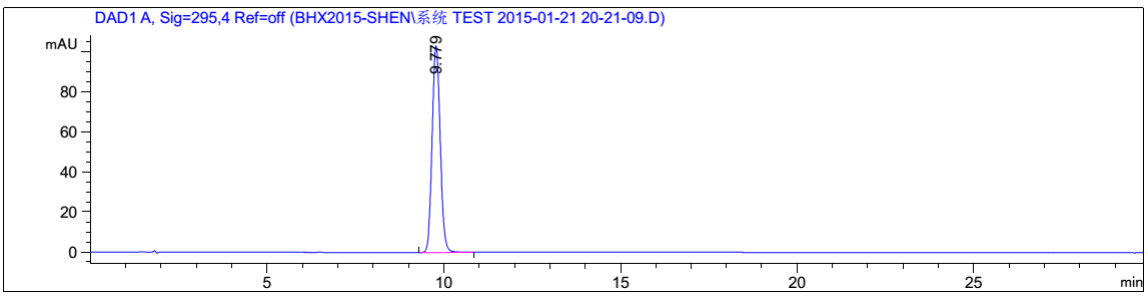
HPLC of Compound 11



HPLC of Compound 12

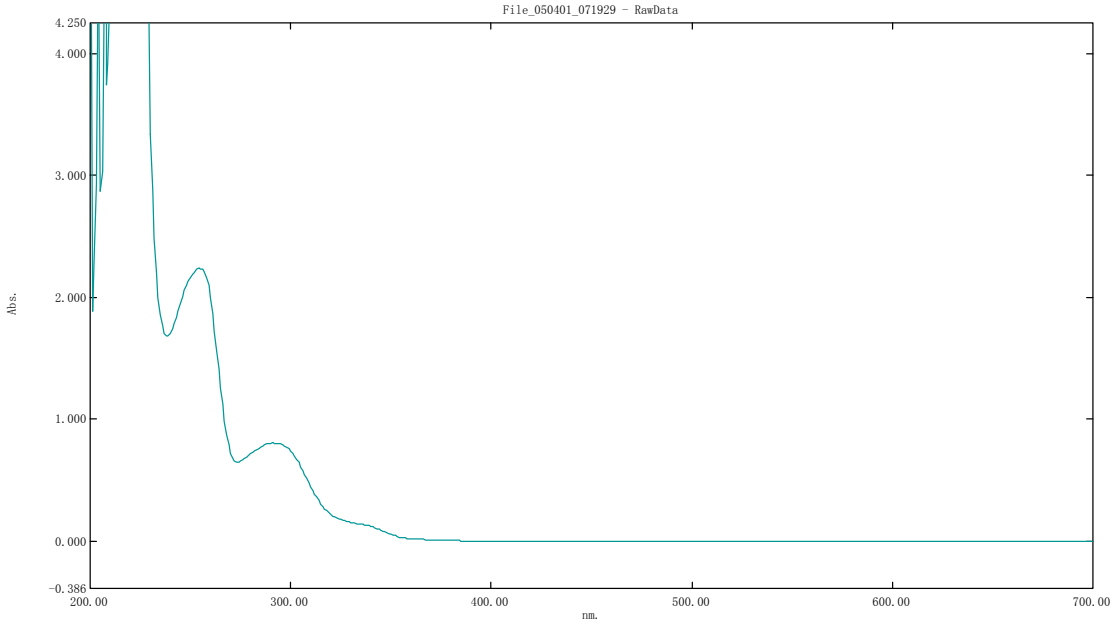


HPLC of Compound 13



HPLC of Compound 14

5. Absorbance spectra of compound 5


File_050401_071929 - RawData
4.250
4.000
3.000
2.000
1.000
0.000
-0.386
Abs.
200.00
300.00
400.00
500.00
600.00
700.00
nm.