Supplemental Information

Small-molecule activating SIRT6 elicits therapeutic effects and synergistically promotes anti-tumor activity of vitamin D_3 in colorectal cancer

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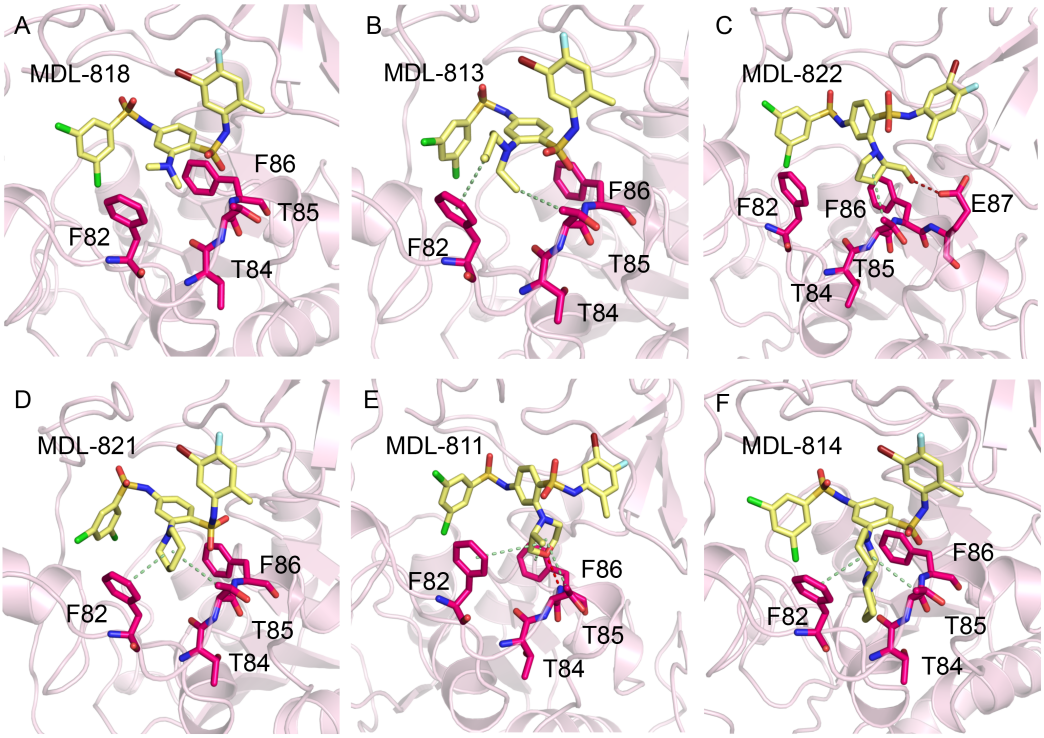


Figure S1. Predicted binding modes of MDL-818, 813, 822, 821, 811 and 814 at the allosteric site on SIRT6. The compounds and pocket residues are displayed as sticks and colored in yellow and hotpink. Hydrogen bonds are shown as red dashed lines and hydrophobic interactions are shown as palegreen dashed lines.

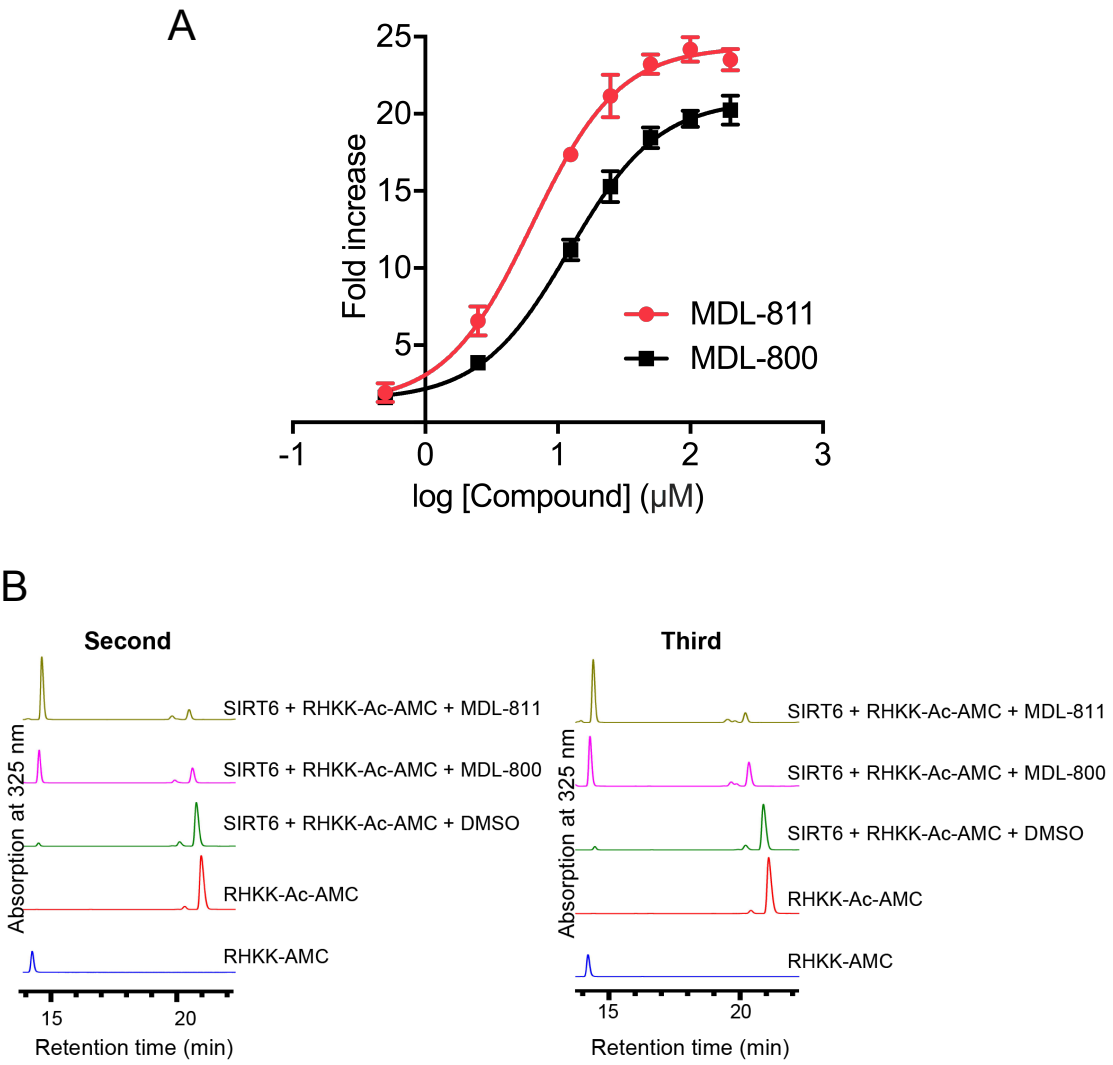


Figure S2. MDL-800 and MDL-811 activated SIRT6 deacetylation on RHKK-Ac-AMC, as determined by FDL and HPLC assays. (A) Concentration-dependent effects of MDL-800/MDL-811 on the activation of SIRT6 deacetylation were assessed by FDL assays with the acetylated peptide RHKK-Ac-AMC (75 μM). The data are shown as mean ± s.d. from three independent experiments (MDL-811: EC_{50} = 5.7 ± 0.8 μM; MDL-800: EC_{50} = 12.3 ± 0.7 μM). (B) HPLC traces of SIRT6 deacetylation on RHKK-Ac-AMC in the absence or presence of MDL-800/MDL-811 at 25 μM. Data are the other two of three independent experiments.

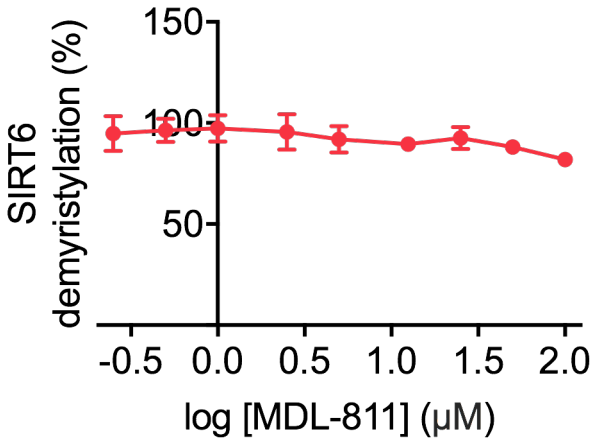


Figure S3. Concentration-dependent effect of MDL-811 on SIRT6 demyristoylation. The effect of MDL-811 was assessed by FDL assay with a TNFα peptide containing a myristoyl group (EALPKK-Myr-AMC) at various concentrations of MDL-811. The data are shown as mean ± s.d. from three independent experiments.

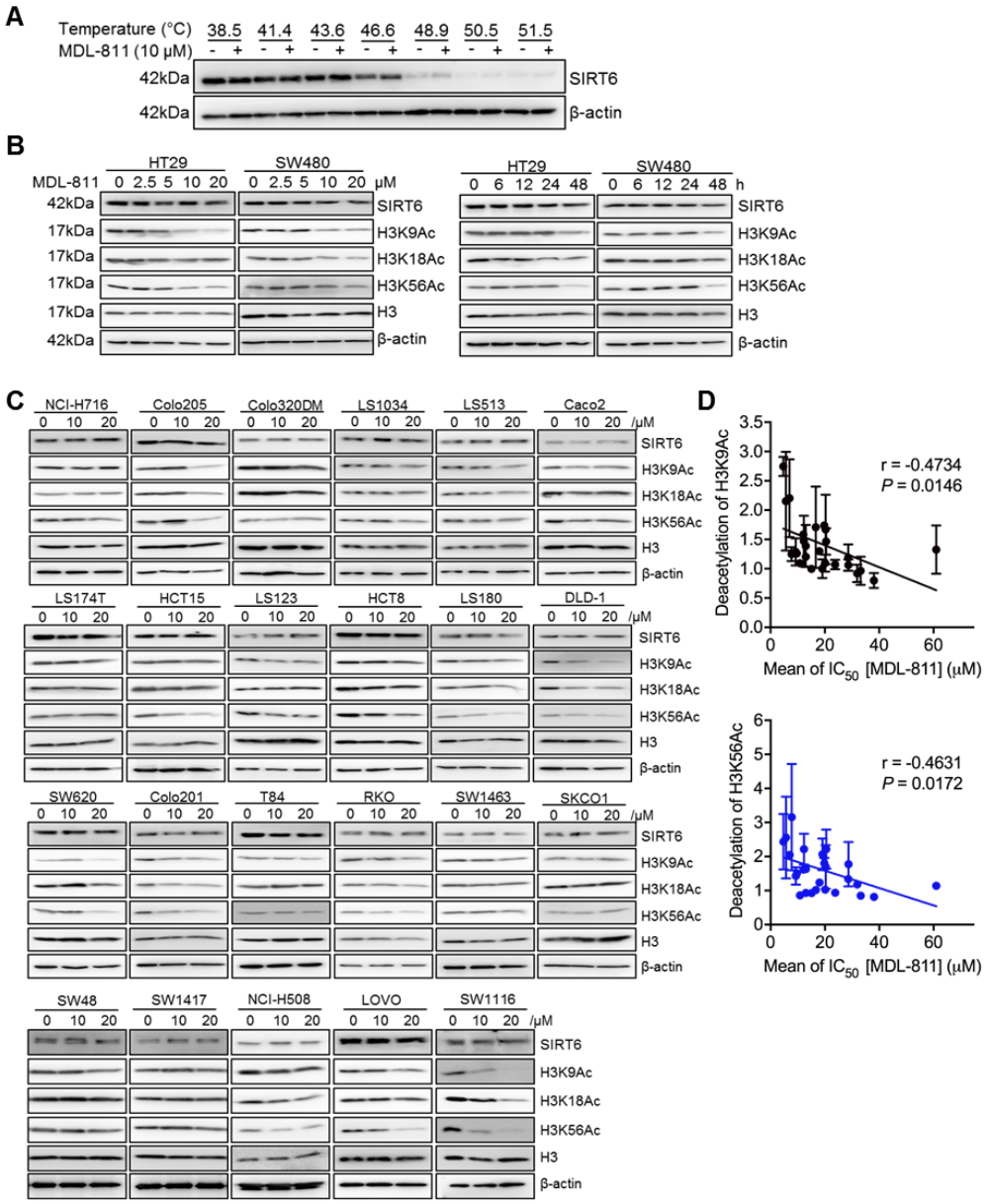


Figure S4. Dose-dependent deacetylation effects of MDL-811 in CRC cell lines. (A) Representative western blots indicating the thermal stabilization of the endogenous SIRT6 protein. The CETSA was performed in intact HCT116 cells in the absence or presence of 10 μM MDL-811 for 24 h. (B) Representative western blots of SIRT6, H3K9Ac, H3K18Ac, and H3K56Ac in HT29 and SW480 cells treated with the indicated concentrations of MDL-811 for 48 h, or treated with 10 μM MDL-811 at the indicated times. (C) Representative western blots of SIRT6, H3K9Ac, H3K18Ac, and H3K56Ac in various CRC cell lines treated with indicated concentrations of MDL-811 for 48 h. Histone H3 was used as the internal control, and β-actin was used as the loading control. (D) Pearson correlation analysis between the IC_{50} values of MDL-811 at 48 h and the levels of H3K9Ac or H3K56Ac deacetylation in various CRC cell lines after treatment with 10 μM MDL-811. Quantification of deacetylation levels was performed with ImageJ V4. Each deacetylation level was normalized to the corresponding histone H3 level. Each dot represents the mean ± s.e.m. of two or three independent experiments in n = 26 CRC cell lines. The data were subjected to Pearson correlation analysis (10 μM MDL-811: H3K9Ac, r = -0.4734, P = 0.0146; H3K56Ac, r = -0.4631, P = 0.0172).

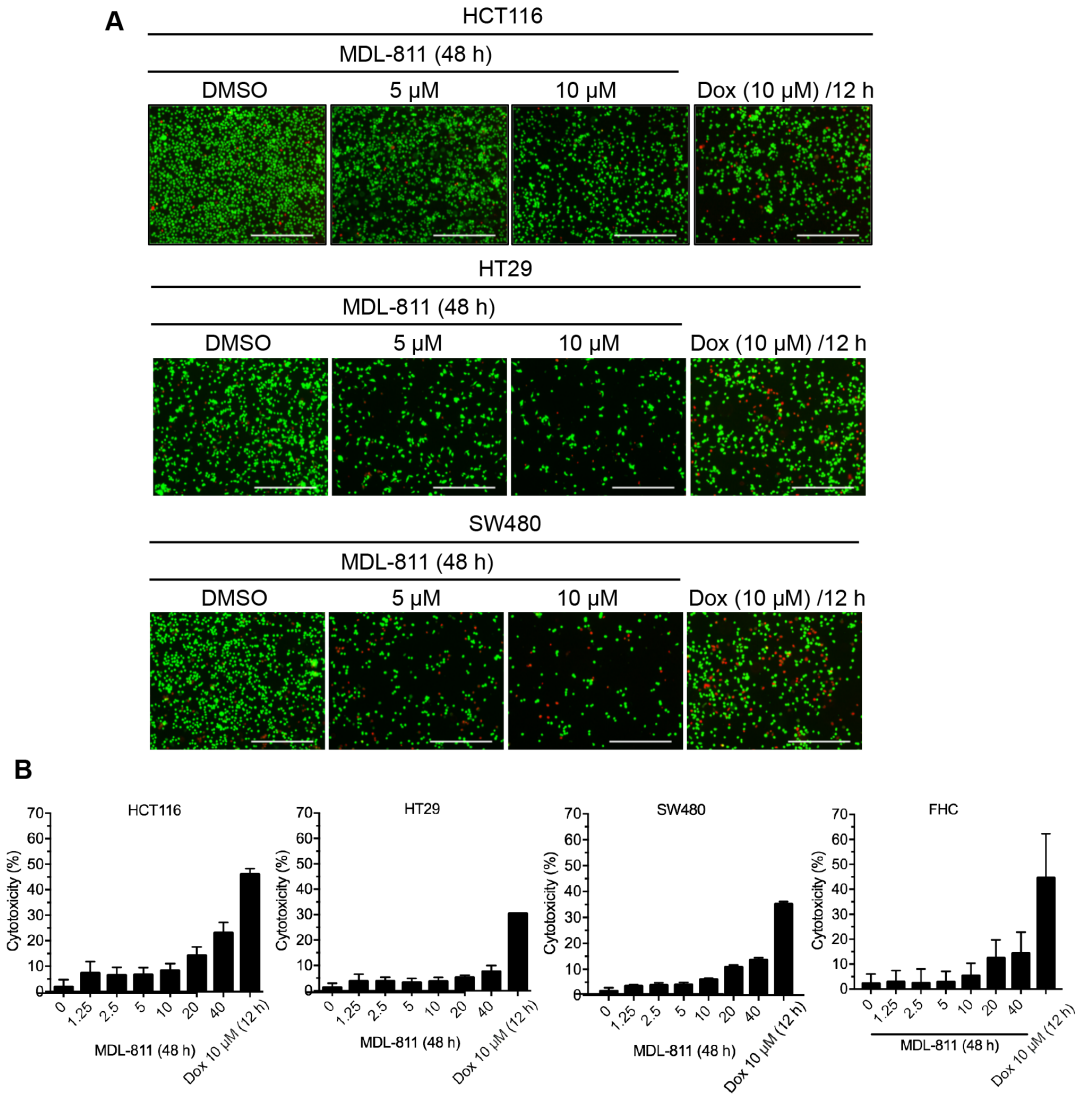


Figure S5. Cell cytotoxicity assays of CRC cell lines treated with MDL-811. (A) Live-dead double staining assays of CRC cells (HCT116, HT29, and SW480) treated with DMSO for 48 h, with 5 or 10 μM MDL-811 for 48 h or with 10 μM doxorubicin for 12 h. Live cells were stained with the green fluorescent dye Calcein-AM, and dead cells were stained with the red dye propidium iodide. The images are representative fields from two independent experiments. Representative images (10× magnification) are shown. Scale bars, 100 μm. (B) Cytotoxicity of MDL-811 to CRC cell lines (HCT116, HT29, SW480) and non-cancerous colon cell line FHC was confirmed by measuring the release of the cytosolic marker LDH. Cells were treated with the indicated concentrations of MDL-811 for 48 h or with 10 μM doxorubicin for 12 h. The data are presented as the mean ± s.d. of three independent experiments.

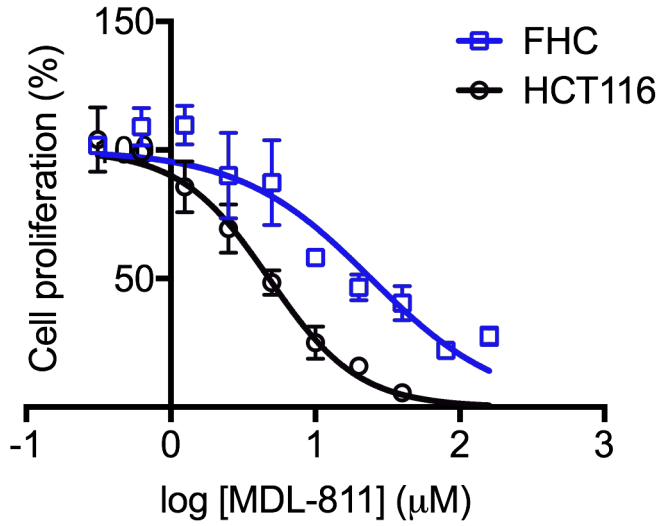


Figure S6. Cell proliferation of non-cancerous colon cell line treated with MDL-811. Dose response of the proliferation of non-cancerous colon cell line FHC and CRC cell line HCT116 exposed to MDL-811 for 48 h was normalized to the proliferation of the corresponding DMSO-treated controls. Cell proliferation was determined by CCK-8 assays. The data indicate the mean ± s.e.m. of three independent experiments (FHC: IC_{50} = 24.4 ± 0.5 μM; HCT116: IC_{50} = 4.8 ± 0.2 μM).

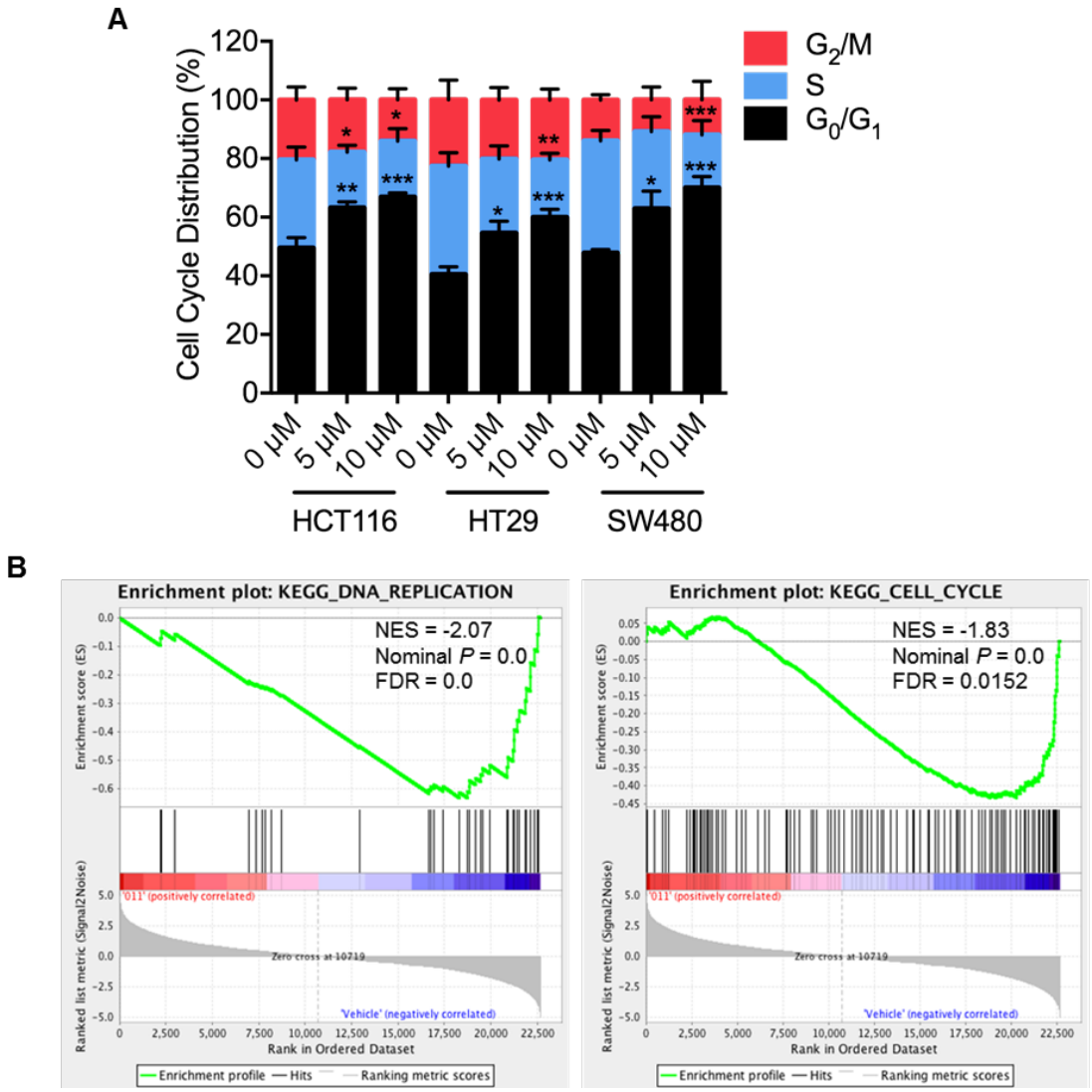


Figure S7. Cell cycle distribution of CRC cell lines treated with MDL-811. (A) Cell cycle distribution of HCT116, HT29, and SW480 cells treated with DMSO or with 5 or 10 μM MDL-811 for 48 h, as measured by PI staining. The data are presented as the mean ± s.d. of three independent experiments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; two-way ANOVA). (B) GSEA plots showing enrichment of gene expression changes by exposure to 10 μM MDL-811 for 48 h in the DNA REPLICATION and CELL CYCLE signatures from the MSigDB v6.2 collection C2 curated gene sets (CP: KEGG gene sets). Positive normalized enrichment scores (NESs) indicate upregulation of gene sets, whereas negative NESs indicate downregulation of gene sets. FDR, false discovery rate.

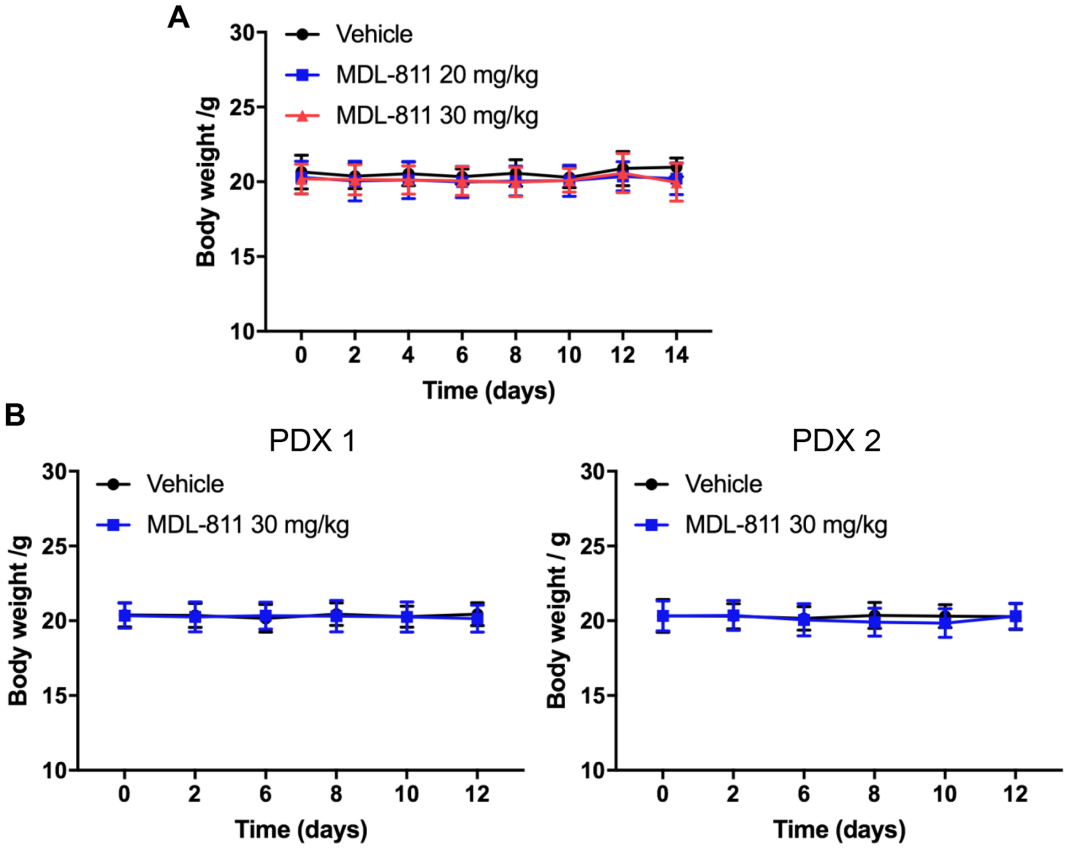


Figure S8. Body weights of xenograft models treated with MDL-811. (A) Body weights of HCT116 CDX mice treated with either vehicle or MDL-811 are shown (n = 6 mice per group). (B) Body weights of PDX mice treated with either vehicle or MDL-811 are shown (n = 8 mice per group). The data is plotted as the mean ± s.d..

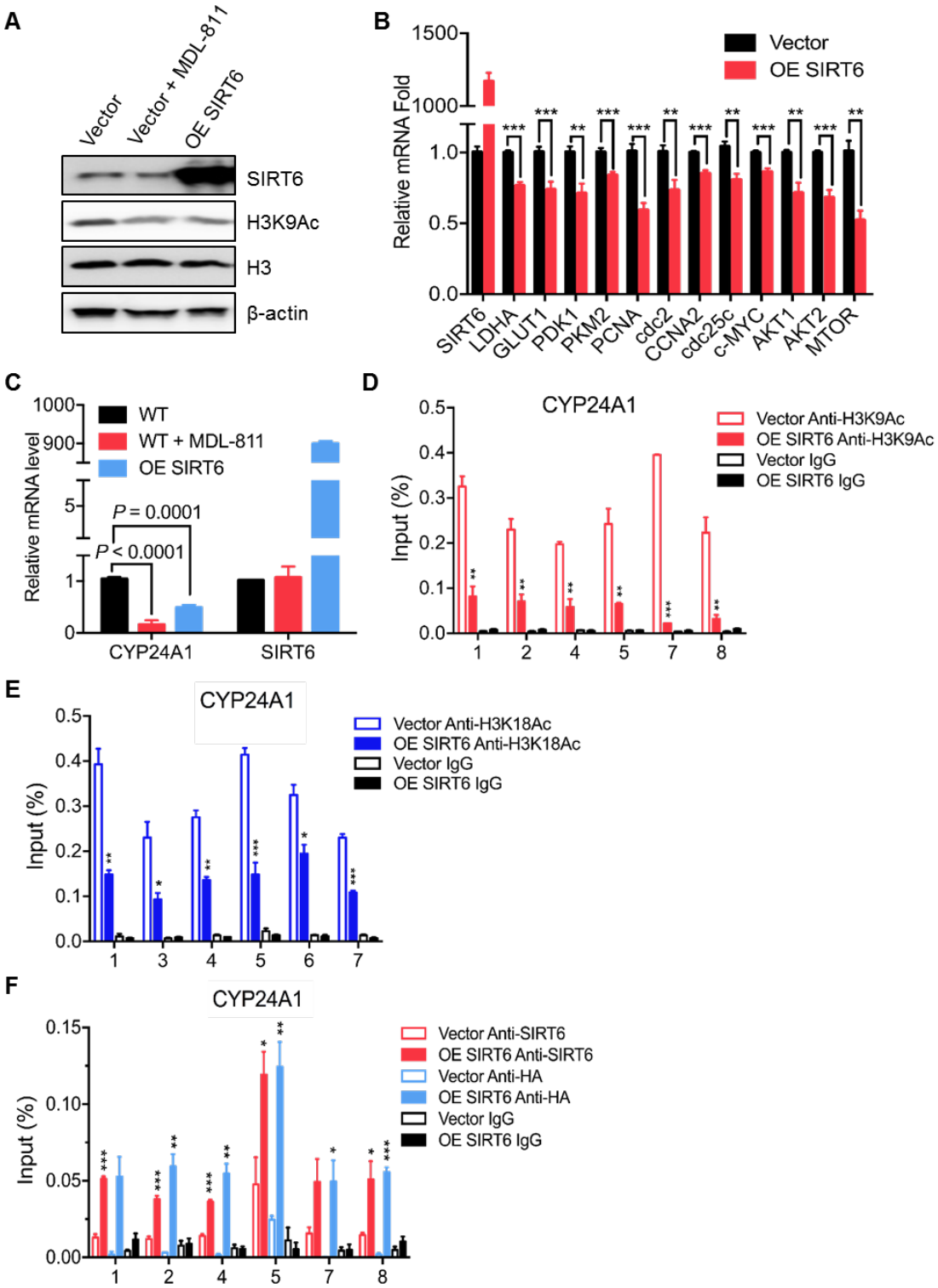


Figure S9. Effect of SIRT6 overexpression on gene expressions in HCT116 cells. (A) Western blots showing effects on HCT116 cells transiently transfected with vector or pLVX-SIRT6-HA. (B) RT-qPCR analyses showing the mRNA levels of candidate genes in HCT116 cells transiently transfected with vector or pLVX-SIRT6-HA before being treated with 10 μM MDL-811 for 48 h. The data are normalized to the β-actin levels and presented as mean ± s.e.m. of two or three independent experiments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, two-tailed student's unpaired t-test). (C) RT-qPCR analyses showing the mRNA levels of CYP24A1 and SIRT6 in HCT116 cells

transfected with vector or pLVX-SIRT6-HA and then treated with DMSO or 10 μM MDL-811 for 48 h. The data are normalized to the β-actin levels and presented as mean ± s.d. of three experiments. (D), (E) and (F) ChIP assays of HCT116 cells transfected with vector or pLVX-SIRT6-HA. Anti-H3K9Ac (D), anti-H3K18Ac (E), anti-SIRT6 or anti-HA (F) antibodies were used to detect the deacetylation levels of these residues in the indicated regions of CYP24A1. The data are presented as the mean values of the percentage of input ± s.e.m. from one of two independent experiments with technical triplicates (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; two-tailed unpaired Student's t-test).

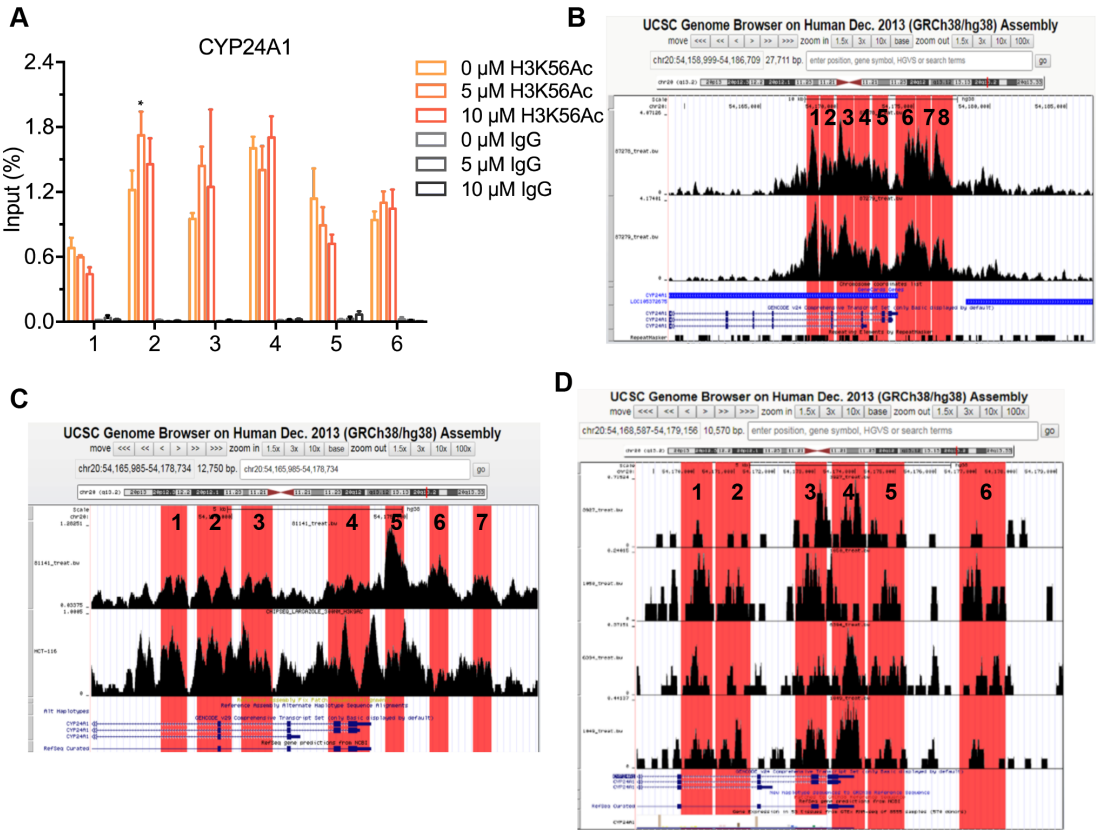


Figure S10. Effects of MDL-811 on the deacetylation of CYP24A1-binding histone marks in HCT116 cells. (A) ChIP assays using anti-H3K56Ac antibody to assess H3K56Ac occupancy in the indicated regions of CYP24A1 in HCT116 cells treated with DMSO or with 5 or 10 μM MDL-811. The data are presented as the mean values of the percentage of input ± s.e.m. from n = 3 independent experiments with technical triplicates. *P* values were determined by two-way ANOVA (*, $P < 0.05$). (B), (C) and (D) ChIP-qPCR primers were designed based on the CYP24A1 DNA sequence of peak regions of H3K9Ac (B), H3K18Ac (C) or H3K56Ac (D) using ChIP-seq data extracted from the Cistrome database.

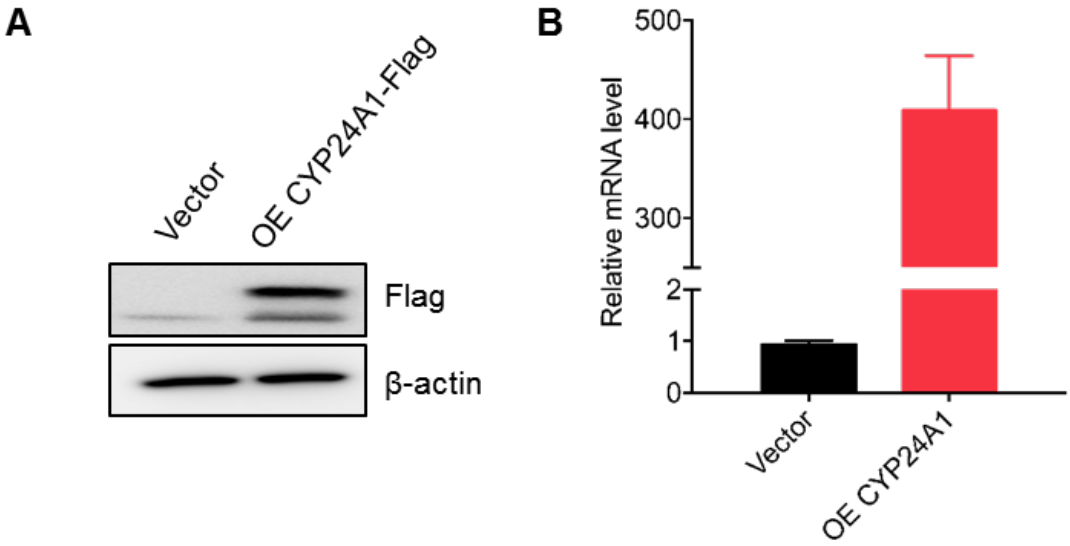


Figure S11. Effect of CYP24A1 overexpression in HCT116 cells. Western blots (A) and RT-qPCR (B) analysis of HCT116 cells transiently transfected with vector or pcDNA3.1-CYP24A1-Flag for 48 h. The data are normalized to the β-actin levels and presented as mean ± s.e.m. of two experiments.

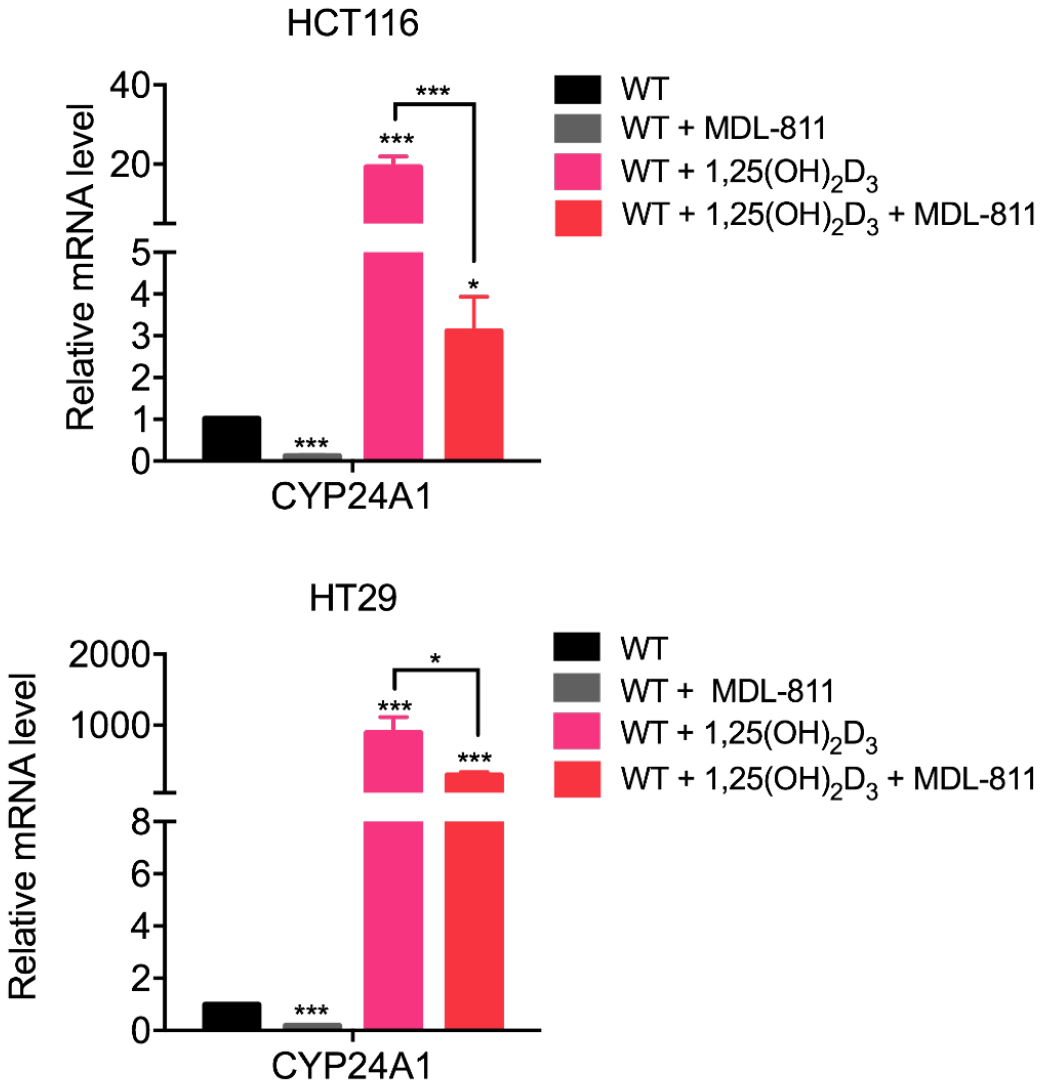


Figure S12. Effect of MDL-811 and 1,25(OH)$_2$D$_3$ cotreatment on CYP24A1 expression in CRC cell lines. RT-qPCR analysis showing the mRNA levels of CYP24A1 in HCT116 and HT29 cells treated with MDL-811 (5 μM), 1,25(OH)$_2$D$_3$ (5 μM) or the combination for 48 h. The data are normalized to the β-actin levels and presented as mean ± s.e.m of three experiments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, two-tailed student's unpaired t-test).

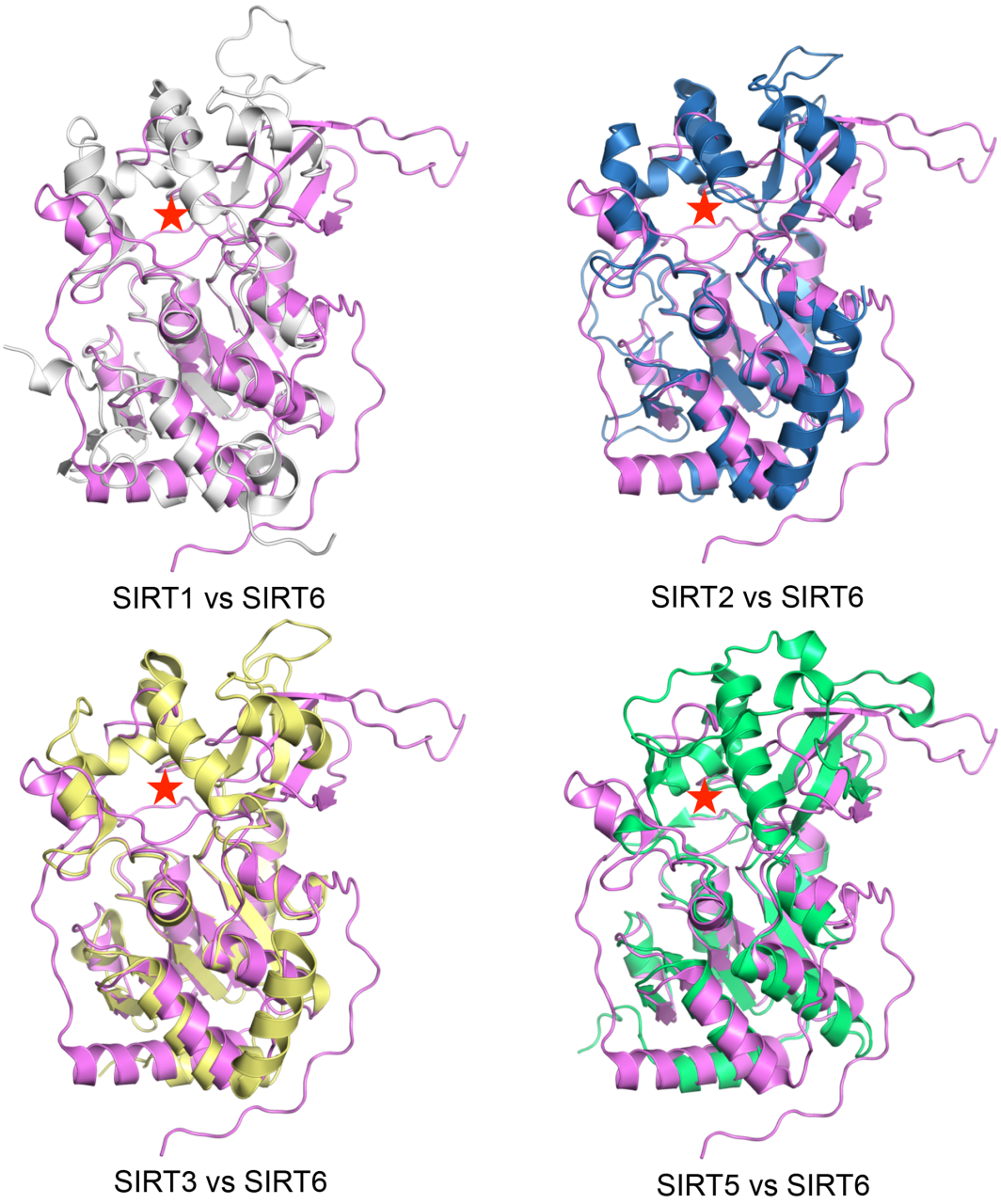
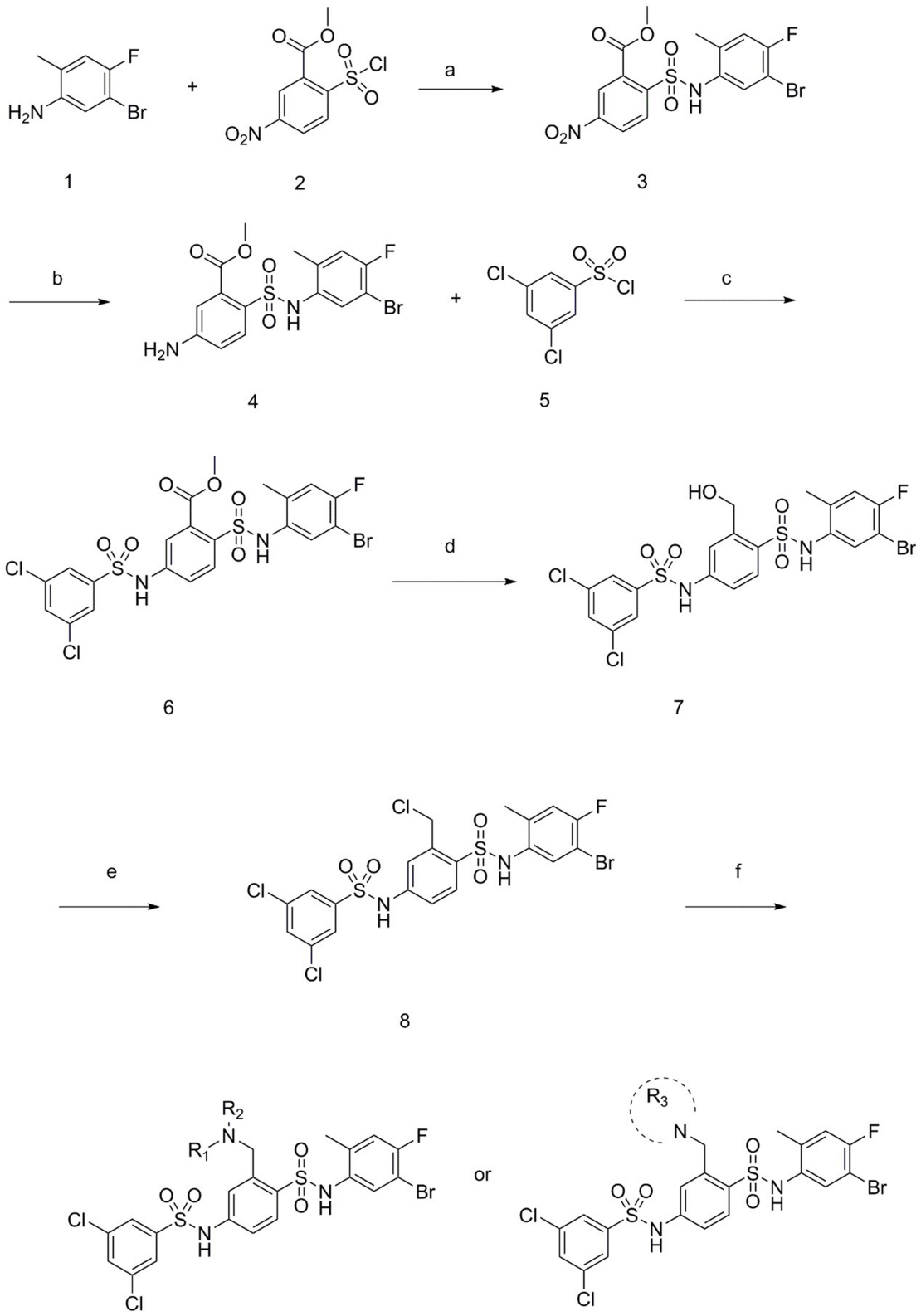


Figure S13. The superimposition of SIRT6 (violet) to SIRT1 (white), SIRT2 (skyblue), SIRT3 (paleyellow) and SIRT5 (limegreen) in cartoon mode. The allosteric site for MDL-811 is represented as red pentagram. The PDB codes of the SIRT1, SIRT2, SIRT3, SIRT5, and SIRT6 structures are 4KXQ, 3ZGV, 4BN4, 3RIY, and 5Y2F, respectively.

Chemical Characterization

Synthesis of Compounds MDL analogs



[a]Reagents and conditions: (a) pyridine, room temperature,8 h; (b) Fe, CH_3COOH, 50 °C 12 h; (c) pyridine, room temperature, 6 h; (d) LAH, THF, 50 °C, 12 h; (e) $SOCl_2$, 80 °C, 10 h; (f) secondary amine, THF, 50 °C, 6 h.

Methyl 2-(*N*-(5-bromo-4-fluoro-2-methylphenyl)sulfamoyl)-5-nitrobenzoate (3). To a solution of 5-bromo-4-fluoro-2-methylaniline (20 g, 98.0 mmol, **1**) in 20 mL pyridine was added methyl 2-(chlorosulfonyl)-5-nitrobenzoate (32.9 g, 117.6 mmol, **2**) under 0 °C and the reaction was stirred at the same temperature for about 1 hour. Then the reaction was moved to 25 °C and stirred for another 8 h. The reaction was cooled to 0 °C and adjusted the pH to 3-4 with 1 N hydrochloric acid solution. The precipitate formed was filtered, washed with water and dried to yield crude intermediate product 5, which was directly used to the next step without any purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.50-8.47 (m, 2H), 7.98-7.96 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 7.36-7.34 (d, *J* = 8.0 Hz, 1H), 7.29-7.27 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 3H), 1.96 (s, 3H).

Methyl 5-amino-2-(*N*-(5-bromo-4-fluoro-2-methylphenyl)sulfamoyl)benzoate (4). The crude intermediate product **3** (38.59 g, 86.53 mmol) dissolved in acetic acid was added iron powder (29.1 g, 519 mmol) at 50 °C. Then the solution was stirred at the same condition for 12 h. The system was filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane: ethyl acetate = 2:1) to afford intermediate product **4** (29.66 g, two steps 73%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.30-7.20 (m, 2H), 6.73 (s, 1H), 6.64-6.59 (m, 2H), 6.30 (s, 2H), 3.73 (s, 3H), 2.02 (s, 3H).

Methyl 2-(*N*-(5-bromo-4-fluoro-2-methylphenyl)sulfamoyl)-5-(3,5-dichlorophenylsulfonamido)benzoate (6). A solution of methyl 5-amino-2-(*N*-(5-bromo-4-fluoro-2-methylphenyl) sulfamoyl) benzoate (**4**, 13.9 g, 33.32 mmol) in 30 mL pyridine was added 3,5-dichlorobenzene-1-sulfonyl chloride (**5**, 16.45 g, 58.8 mmol) under 0 °C and the reaction was still stirred at the same temperature for about 1 hour. Then the reaction was moved to 25 °C and stirred for another 6 h. The reaction was cooled to 0 °C, and adjusted the pH to 3-4 with 2 N hydrochloric acid solution. The precipitate formed was filtered and subsequently purified by column chromatography on silica gel (hexane: ethyl acetate = 3:1) to afford **6** (17.6 g) as a white powder. Yield: 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 9.46 (s, 1H), 8.01 (s, 1H), 7.81 (d, *J* = 2.0 Hz, 2H), 7.60-7.58 (d, *J* = 8.0 Hz, 1H), 7.44-7.40 (dd, *J* = 2.0 Hz, *J* = 8 Hz, 1H), 7.27 (d, *J* = 2.0 Hz, 1H), 7.24-7.22 (d, *J* = 8.0 Hz, 1H), 7.18-7.16 (d, *J* = 8.0 Hz, 1H), 3.74 (s, 3H), 1.82 (s, 3H).

***N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2 (hydroxymethyl)benzenesulfonamide (7).** Within a flask was dissolved methyl 2-(*N*-(5-bromo-4-fluoro-2-methylphenyl)sulfamoyl)-5-(3,5-dichlorophenylsulfonamido)benzoate (**6**, 1 g, 1.59 mmol) in 20 mL of tetrahydrofuran. Then was added Lithium aluminum hydride (242 mg, 6.38 mmol) slowly added at ice bath and the contents were heated at 60 °C for about 12 h. The reaction was allowed to cool to room temperature and poured into ice bath, and adjusted the pH to 2-3 with 3N hydrochloric acid solution. The precipitate formed was filtered and dried to yield crude **7** as a yellow solid without any purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.09 (s, 1H), 9.74 (s, 1H), 8.04-8.03 (t, 1H), 7.79-7.78 (d, 2H), 7.63-7.62 (d, *J* = 4.0

Hz, 1H), 7.45-7.43 (d, *J* = 8.0 Hz, 1H), 7.16-7.06 (m, 3H), 5.58-5.55 (t, 1H), 4.75-4.74 (d, *J* = 4.0 Hz. 2H), 1.77 (s, 3H). LRMS (ESI-) 594.9 (M-H).

_N_-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (8).

N-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-(hydroxymethyl)benzenesulfonamide (**7,** 580 mg, 0.969 mmol) was added 10 mL of thionyl chloride slowly at ice bath and the contents were heated at 60 °C for about 10 h. After the removal of the excess reagent, the crude product was recrystallized with ethyl acetate to afford 8 as a white solid (420 mg, two step 43%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.25 (s, 1H), 9.95 (s, 1H), 8.05-8.04 (t, 1H), 7.81-7.80 (d, 2H), 7.56-7.50 (m, 2H), 7.21-7.09 (m,3H), 4.99 (s, 2H), 1.78 (s, 3H) LRMS (ESI-) 612.8 (M-H).

(_R_)-_N_-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((3-methylmorpholino)methyl)benzenesulfonamide (MDL-811).

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (**8**, 100 mg, 0.162 mmol)was dissolved in 5 mL of dried THF, and then (R)-3-methylmorpholine (33 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (ether: ethyl acetate = 3:1) to afford **MDL-811** as a white solid (80 mg, 73%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.07 (s, 1H), 9.86 (s, 1H), 8.04 (s, 1H), 7.77 (s, 2H), 7.71 (s, 1H), 7.59-7.57 (d, *J* = 8.0 Hz, 1H), 7.20-7.11 (m, 2H), 7.01-7.99 (d, *J* = 8.0 Hz, 1H), 3.98-3.94 (d, *J* = 16.0 Hz, 1H), 3.69-3.67 (m, 2H), 3.50-3.46 (m, 2H), 3.22-3.17 (m, 1H), 2.51-2.47 (m, 1H), 2.34-2.32 (d, *J* = 8.0 Hz, 1H), 2.08 (s, 2H), 1.82 (s, 3H), 0.85-0.84 (d, *J* = 4.0 Hz, 3H). ^{13}C NMR (DMSO-d_6 100 MHz) 156.7, 154.3, 140.9, 140.1, 139.0, 136.4 (*J* = 7), 134.1, 131.9 (*J* = 2), 131.7 (*J* = 5), 130.5 (*J* = 2), 129.6, 124.1, 118.5, 117.1 (*J* = 25), 116.4, 103.2 (*J* = 22), 71.0, 65.3, 54.0, 52.6, 49.8, 15.8, 12.4 ppm. LRMS (ESI+) 680.0 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{25}H_{25}BrCl_2FN_3O_5S_2$ $(M+H)^+$ 679.9780, found 679.9850.

_N_-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-(piperazin-1-ylmethyl)benzenesulfonamide (MDL-812).

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then tert-butyl piperazine-1-carboxylate (60 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, then the crude compound was dissolved in 3 mL of DCM and TFA (3 mL) was added at room temperature. The reaction was stirred at the same temperature for another 2 h. Then the solvent was almost moved out and subsequently purified by high performance liquid chromatography to afford **MDL-812** as a white solid (51.6

mg, 48%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.68-7.65 (m, 3H), 7.40-7.38 (d, *J* = 8.0 Hz, 1H), 7.27 (s, 1H), 7.15-7.13 (d, *J* = 8.0 Hz, 1H), 6.97-6.95 (d, *J* = 8.0 Hz, 1H), 6.90-6.89 (d, *J* = 4.0 Hz, 1H), 3.72 (s, 2H), 3.03-3.00 (m, 4H), 2.56-2.55 (m, 4H), 2.03 (s, 3H). ^{13}C NMR (DMSO-d_6 100 MHz) 172.1, 157.8, 155.8, 150.7, 148.6, 138.0 (*J* = 6), 137.2, 134.9, 133.6, 131.5, 130.7 (*J* = 23), 128.7, 122.5, 118.7, 118.4 (*J* = 18), 104.4 (*J* = 18), 59.0, 51.0, 44.3, 17.7 ppm. LRMS (ESI+) 665.0 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{24}H_{24}BrCl_2FN_4O_4S_2$ $(M+H)^+$ 664.9783, found 664.9858.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-2-((butyl(ethyl)amino)methyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (MDL-813).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then N-ethylbutan-1-amine (33 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by high performance liquid chromatography to afford **MDL-813** as a white solid (56 mg, 51%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.98 (s, 1H), 7.75-7.74 (d, *J* = 4.0, 2H), 7.60-7.58 (m, 2H), 7.19-7.11 (m, 2H), 6.95-6.93(d, J = 8.0, 1H) 3.78(s, 2H), 2.47-2.42 (m,4H), 1.87 (s, 3H), 1.34-1.30 (m, 2H), 1.23-1.18 (m, 2H), 0.97-0.93 (m, 3H), 0.84-0.80 (t, 3H). ^{13}C NMR (DMSO-d_6 100 MHz) 157.9, 155.4, 137.6, 137.5, 135.7, 133.4 (*J* = 3), 133.2, 131.2, 131.0, 125.6, 125.6, 118.7, 118.4 (*J* = 9), 104.8, 104.6, 99.9, 54.8, 52.9, 47.8, 20.4, 19.3, 17.6, 14.3, 11.4 ppm. LRMS (ESI+) 680.02 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{26}H_{29}BrCl_2FN_3O_4S_2$ $(M+H)^+$ 680.0144, found 680.0216.

2-([1,4'-bipiperidin]-1'-ylmethyl)-*N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (MDL-814).

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido)benzenesulfonamide (**8**, 100 mg, 0.162 mmol)was dissolved in 5 mL of dried THF, and then 1,4'-bipiperidine (55 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by high performance liquid chromatography to afford **MDL-814** as a white solid (55 mg, 46%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.72-7.71 (t, 1H), 7.64-7.63 (d, 2H), 7.23-7.22 (d, *J* = 4.0 Hz, 2H), 7.20 (s, 1H), 6.76-6.73 (m, 1H), 6.69-6.67 (d, *J* = 8.0 Hz, 1H), 3.64 (s, 2H), 2.84-2.81 (m, 6H), 2.03 (s, 3H), 1.87-1.85 (d, *J* = 8.0 Hz, 2H), 1.62-1.58 (m, 4H), 1.44-1.38 (m, 4H). ^{13}C NMR (DMSO-d_6 100 MHz) 157.4, 154.9, 148.6, 137.9 (*J* = 8), 136.2, 134.1, 133.1, 130.6, 130.2, 129.6, 124.7, 122.1, 118.2, 117.9, 103.9, 103.7, 61.8, 58.9, 51.9, 49.4, 26.6, 24.1, 22.6, 17.3 ppm. LRMS (ESI+) 747.02 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{30}H_{34}BrCl_2FN_4O_4S_2$ $(M+H)^+$ 747.0637.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((4-(3-methoxypropyl)piperazin-1-yl)methyl)benzenesulfonamide (MDL-815).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfon amido)benzenesulfonamide (**8**, 100 mg, 0.162 mmol)was dissolved in 5 mL of dried THF, and then 1-(3-methoxypropyl)piperazine (51 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by high performance liquid chromatography to afford **MDL-815** as a white solid (57 mg, 48%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ7.79 (s, 1H), 7.68 (s, 2H), 7.30-7.21 (m, 3H), 6.86-6.84 (d, *J* = 8.0, 1H), 6.68-6.66 (d, *J* = 8.0, 1H), 3.67 (s, 2H), 3.32-3.30 (4, 2H), 3.2 (s, 3H), 2.39-2.31(m, 8H), 2.01 (s, 3H), 1.65-1.62(m, 2H). ^{13}C NMR (DMSO-d_6 100 MHz) ^{13}C NMR (100 MHz, DMSO) δ 157.6, 155.1, 146.9, 138.1 (*J* = 7), 136.5, 134.4, 132.7, 130.9, 130.5, 130.3, 127.6, 124.8, 121.9, 118.2, 117.9, 103.9 (*J* = 22), 69.9, 59.0, 57.8, 54.4, 52.4, 52.3, 26.2, 17.3 ppm. LRMS (ESI+) 737.04 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{28}H_{32}BrCl_2FN_4O_5S_2$ $(M+H)^+$ 737.0427.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-2-((4-butylpiperazin-1-yl)methyl)-4-(3,5-di chlorophenylsulfonamido)benzenesulfonamide (MDL-816).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfon amido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then 1-butylpiperazine (46 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by high performance liquid chromatography to afford **MDL-816** as a white solid (50 mg, 43%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.87-7.86 (t, 1H), 7.73-7.72 (d, 2H), 7.40-7.36 (m, 2H), 7.19-7.17 (d, *J* = 8.0 Hz, 1H), 6.98-6.95 (m, 1H), 6.84-6.82 (d, *J* = 8.0 Hz, 1H), 3.69 (s, 2H), 2.70-2.52 (m, 4H), 2.51-2.44 (m, 4H), 1.90 (s, 1H), 1.47-1.41 (m, 2H), 1.30-1.20 (m, 3H), 0.93-0.83 (m, 4H). ^{13}C NMR (DMSO-d_6 100 MHz) 164.4, 155.8, 145.5, 138.3 (*J* = 5), 137.8, 135.3, 132.9 (*J* = 3), 131.9 (*J* = 22), 131.1, 125.6, 121.5, 118.7, 118.5, 118.3, 104.6 (*J* = 23), 100.0, 58.7, 57.1, 52.6, 51.9, 29.5, 27.8, 20.3, 19.3, 17.7, 14.2 ppm. LRMS (ESI+) 721.1 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{28}H_{32}BrCl_2FN_4O_4S_2$ $(M+H)^+$ 721.0482.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((3-(dimethylamino) pyrrolidin-1-yl) methyl) benzenesulfonamide (MDL-817).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfon amido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then *N*,*N*-dimethylpyrrolidin-3-amine (37 mg, 0.324 mmol) and TEA (41 mg, 0.405 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by high performance liquid chromatography to afford **MDL-817** as a white solid (53 mg, 48%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.81-7.80 (t, 1H), 7.69-7.68 (d, 2H),

7.36-7.34 (d, *J* = 8.0, 1H), 7.24-7.17 (m, 2H), 6.92-6.87 (m, 2H), 3.82-3.71 (m, 2H), 3.29-3.19 (m, 1H), 2.69-2.59 (m, 2H), 2.55-2.52 (m, 1H), 2.42-2.36 (m, 7H), 2.01-1.93 (m, 4H), 1.79-1.74 (m, 1H). ^{13}C NMR (DMSO-d_6 100 MHz) 157.4, 154.9, 146.7, 137.7, 137.4 (*J* = 8), 134.4, 132.7, 131.0, 130.7, 130.3, 127.9, 124.8, 120.9, 117.9 (*J* = 17), 117.7, 104.9 (*J* = 22), 64.3, 56.0, 55.9, 52.2, 41.7, 26.9, 17.1 ppm. LRMS (ESI+) 693.06 $(M+H)^+$ HRMS (ESI+) m/z calcd. for $C_{26}H_{28}BrCl_2FN_4O_4S_2$ $(M+H)^+$ 693.0167.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((dimethylamino)methyl)benzenesulfonamide (MDL-818).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL dried THF, and then dimethylamine absolute in tetrahydrofuran solution (2 M, 0.245 mL) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 2:1) to afford **MDL-818** as a white solid (66 mg, 65%).^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.92 (s, 1H), 7.76-7.75 (d, 2H), 7.49-7.48 (d, *J* = 5.0, 1H), 7.33 (s, 1H), 7.20-7.18 (d, *J* = 10.0, 1H), 7.10-7.07 (m, 1H), 6.91-6.89 (d, *J* = 10.0, 1H) 3.84 (s, 2H), 2.31 (s, 6H), 1.90 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) 156.1 (*J* = 242.5), 136.96 (*J* = 9), 134.91, 132.96, 132.08, 130.57, 130.42, 129.55, 125.08, 122.09, 118.55, 118.03 (*J* = 22.5), 104.02 (*J* = 22.5), 59.62, 43.95, 17.09 ppm. LRMS (ESI+) 624.0 $(M+H)^+$, HRMS (ESI+) m/z calcd. for $C_{22}H_{22}BrCl_2FN_3O_4S_2^+$$(M+H)^+$623.9591, found 623.9585.

N-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((ethyl(methyl)amino)methyl)benzenesulfonamide (MDL-819).

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then N-methylethanamine (29 mg, 0.486 mmol) was added. The reaction was stirred at 50 ° C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 2:1) to afford **MDL-819** as a white solid (78 mg, 76%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ7.65-7.62 (m, 3H), 7.23-7.21 (m, 2H), 7.00-6.99 (d, *J* = 5.0, 1H), 6.74-6.72 (m, 1H), 6.67-6.65 (d, *J* = 10.0, 1H), 3.70 (s, 2H), 2.47-2.43 (m, 2H), 2.11-2.10 (d, 6H), 0.98-0.95 (t, 3H). ^{13}C NMR (DMSO-d_6 125 MHz) 156.16(*J* = 241.3), 150.45, 137.53, 136.14, 134.45, 139.94 (*J* = 87.5), 129.41, 125.17, 124.16, 118.64, 118.44, 104.22 (*J* = 22.5), 60.06, 51.00, 41.04, 18.09, 11.87 ppm. LRMS (ESI+) 638.0 $(M+H)^+$, HRMS (ESI+) m/z calcd. for $C_{23}H_{24}BrCl_2FN_3O_4S_2^+$ $(M+H)^+$ 637.9747, found 637.9742.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((methyl(propyl)amino)methyl)benzenesulfonamide (MDL-820).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido)

amido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then N-methylpropan-1-amine (36 mg, 0.486 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between dichloromethane and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel(petroleum ether: ethyl acetate = 2:1) to afford **MDL-820** as a white solid (76 mg, 72%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ7.96 (s, 1H), 7.76-7.75 (d, 2H), 7.55-7.53 (d, J = 10.0, 1H), 7.46 (s, 1H), 7.20-7.18 (d, J = 10.0, 1H), 7.13-7.11 (m, 1H), 6.90-6.89 (d, J = 5.0, 1H), 3.78 (s, 2H), 2.42-2.41 (m, 2H), 2.15 (s, 3H), 1.89 (s, 3H), 1.48-1.43 (m, 2H), 0.85-0.82 (t, 3H). ^{13}C NMR (DMSO-d_6 125 MHz) 156.74 (J = 242.5), 143.64, 137.81, 137.75, 135.58, 133.12 (J = 40), 131.25, 131.09, 125.66, 121.83, 118.64 (J = 22.5), 104.64 (J = 21.3), 59.30, 58.42, 41.60, 19.70, 17.62, 11.94 ppm. LRMS (ESI+) 651.9 $(M+H)^+$, HRMS (ESI+) m/z calcd. for $C_{24}H_{26}BrCl_2FN_3O_4S_2^+$ $(M+H)^+$ 651.9904, found 651.9904.

***N*-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-(piperidin-1-ylmethyl)benzenesulfonamide (MDL-821).**

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then piperidine (42 mg, 0.486 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 2:1) to afford **MDL-821** as a white solid (69 mg, 64%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ7.99 (s, 1H), 7.78-7.77 (d, 2H), 7.49-7.45 (m, 2H), 7.22-7.20 (d, J = 10.0, 1H), 7.11-7.09 (m, 1H), 6.71 (s, 1H), 3.79 (s, 2H), 2.44(s, 4H), 1.93 (s, 3H), 1.50-1.42 (m, 6H). ^{13}C NMR (DMSO-d_6 125 MHz) 156.98 (J = 243.8) 138.61 (J = 9.0), 135.63, 133.04, 131.51, 131.22, 125.70, 122.01, 118.75 (J = 22.5), 104.54 (J = 21.3), 59.63, 54.07, 25.40, 23.57, 17.71 ppm. LRMS (ESI+) 664.0 $(M+H)^+$, HRMS (ESI+) m/z calcd. for $C_{25}H_{26}BrCl_2FN_3O_4S_2^+$ $(M+H)^+$ 663.9904, found 663.9900.

N-(5-bromo-4-fluoro-2-methylphenyl)-4-(3,5-dichlorophenylsulfonamido)-2-((2-(hydroxymethyl)pyrrolidin-1-yl)methyl)benzenesulfonamide (MDL-822).

N-(5-bromo-4-fluoro-2-methylphenyl)-2-(chloromethyl)-4-(3,5-dichlorophenylsulfonamido) benzenesulfonamide (**8**, 100 mg, 0.162 mmol) was dissolved in 5 mL of dried THF, and then piperidine (49 mg, 0.486 mmol) was added. The reaction was stirred at 50 °C for about 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl acetate and water. The organic layer was further washed with saturated aq $NaHCO_3$, brine, dried over sodium sulfate, evaporated in vacuo, and subsequently purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 2:1) to afford **MDL-822** as a white solid (66 mg, 60%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ7.98 (s, 1H), 7.77-7.76 (d, 2H), 7.50-7.48 (d, J = 10.0, 1H), 7.44 (s, 1H), 7.20-7.18 (d, J = 10.0, 1H), 7.08-7.06 (m, 1H), 6.94-6.93 (d, J = 5.0, 1H), 4.49-4.46 (d, J = 15.0, 1H), 3.62-3.60 (m, 2H), 3.49-3.43 (m, 3H), 2.75-2.60 (m, 2H), 2.18-2.13

(m, 1H), 1.90-1.88 (m, 4H), 1.72-1.57 (m, 2H). ^{13}C NMR (DMSO-d_6 125 MHz) 156.27 (J = 242.5), 137.79 (J = 9), 135.07, 132.51, 131.13, 130.72, 125.12, 121.41, 118.12 (J = 22.5), 104.96 (J = 22.5), 65.55, 62.20, 55.47, 53.76, 33.19, 27.13, 22.43, 17.12ppm. LRMS (ESI+) 680.0 $(M+H)^+$, HRMS (ESI+) m/z calcd. for $C_{25}H_{26}BrCl_2FN_3O_5S_2^+$ $(M+H)^+$ 679.9853, found 679.9841.

NMR spectra of compounds MDL analogs

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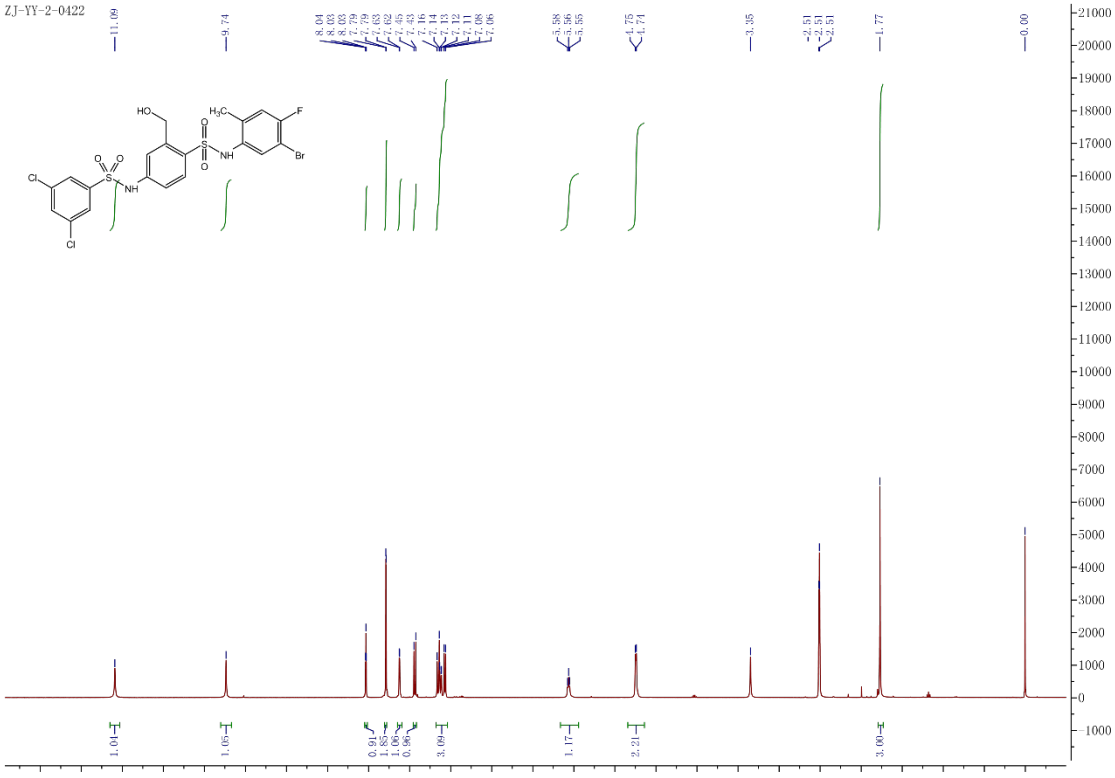

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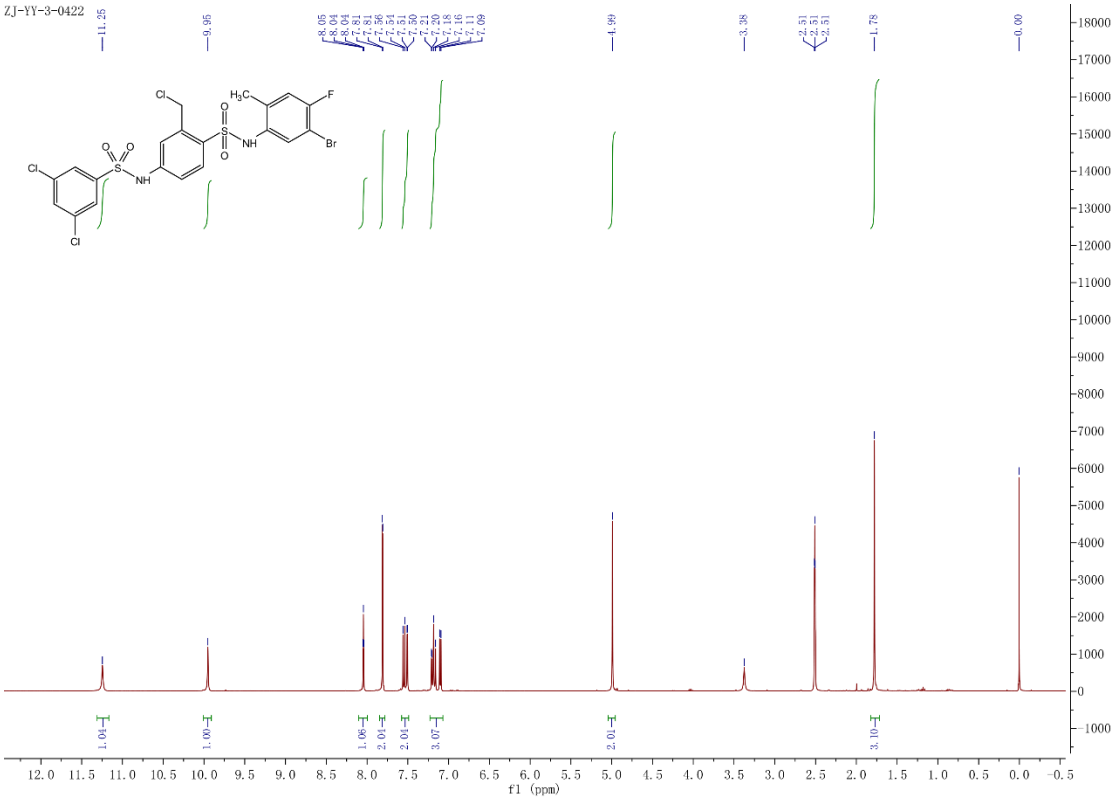
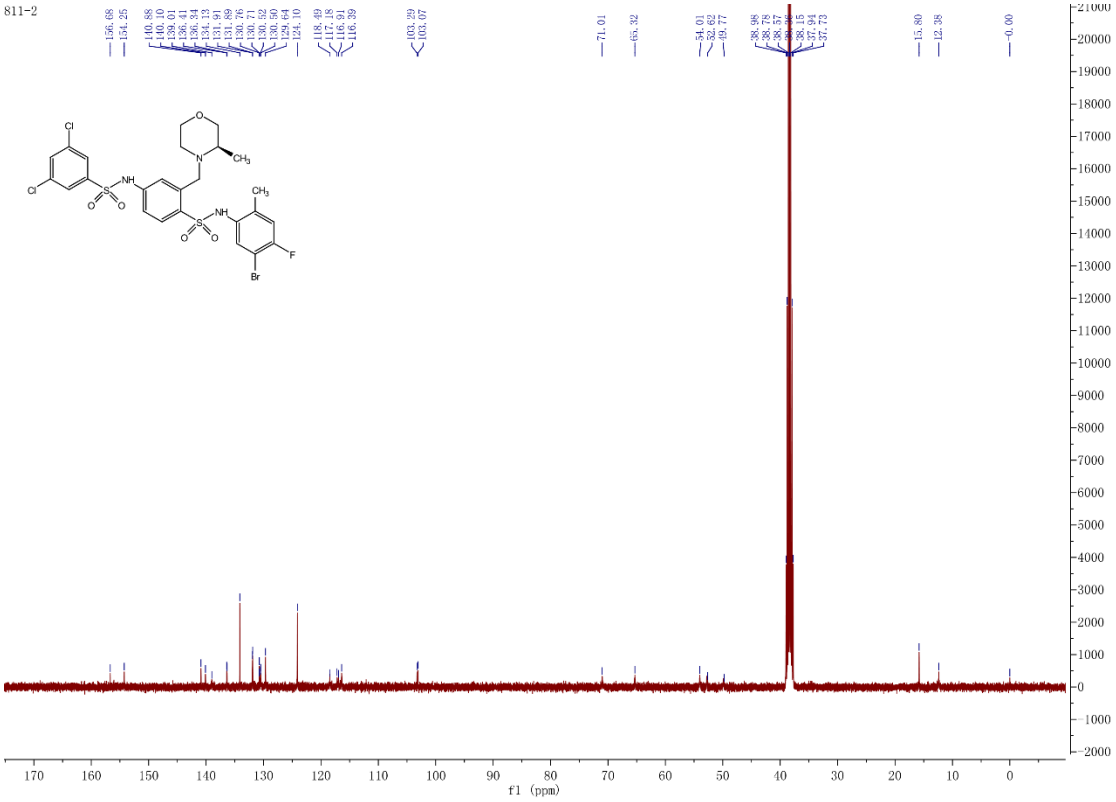


MDL-811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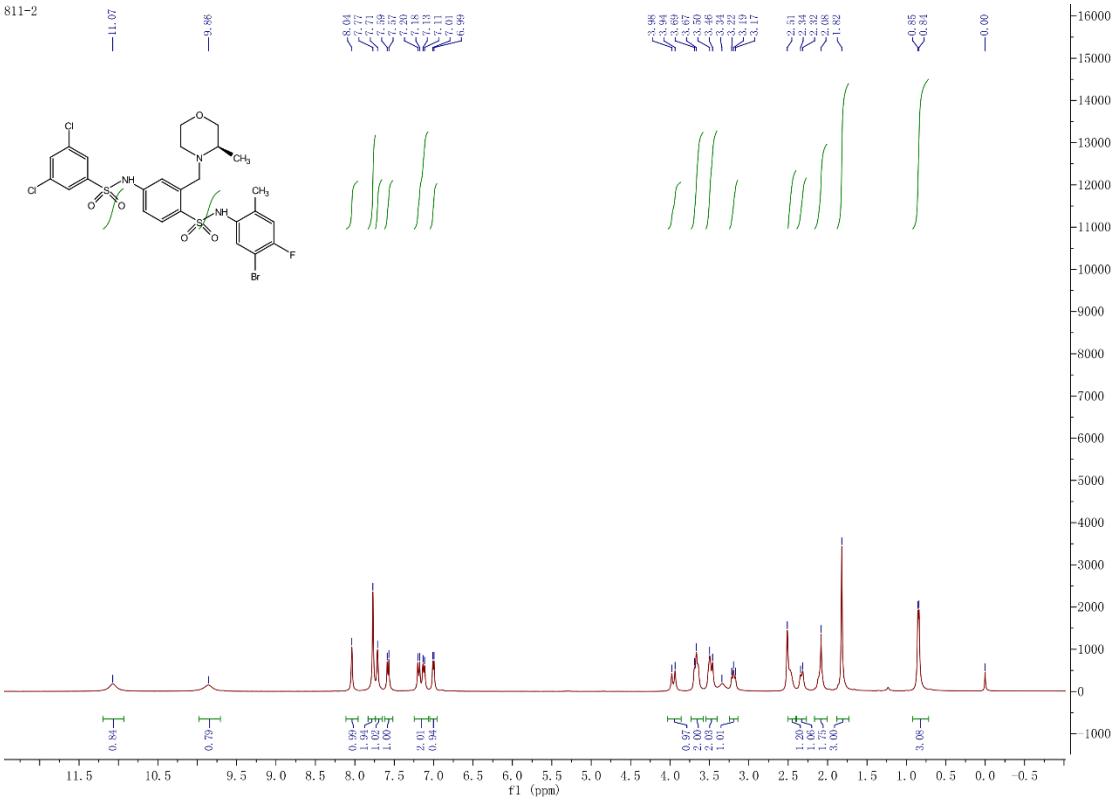
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2.01
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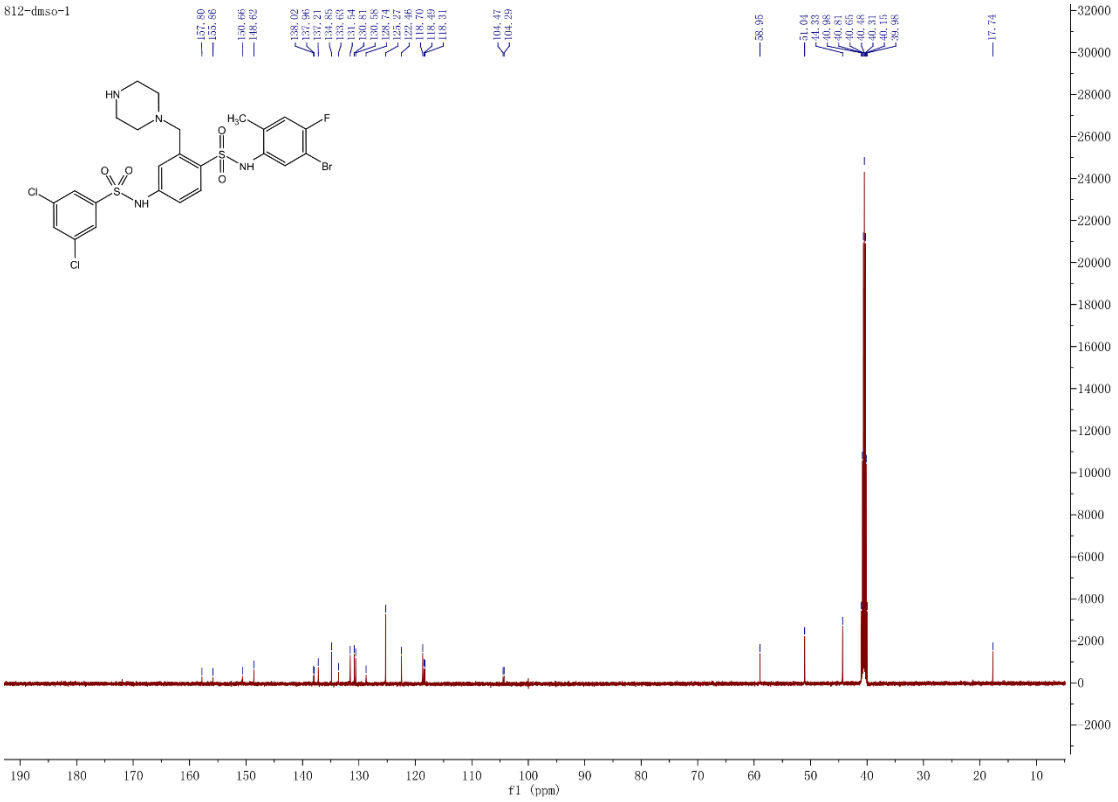
f1 (ppm)

MDL-812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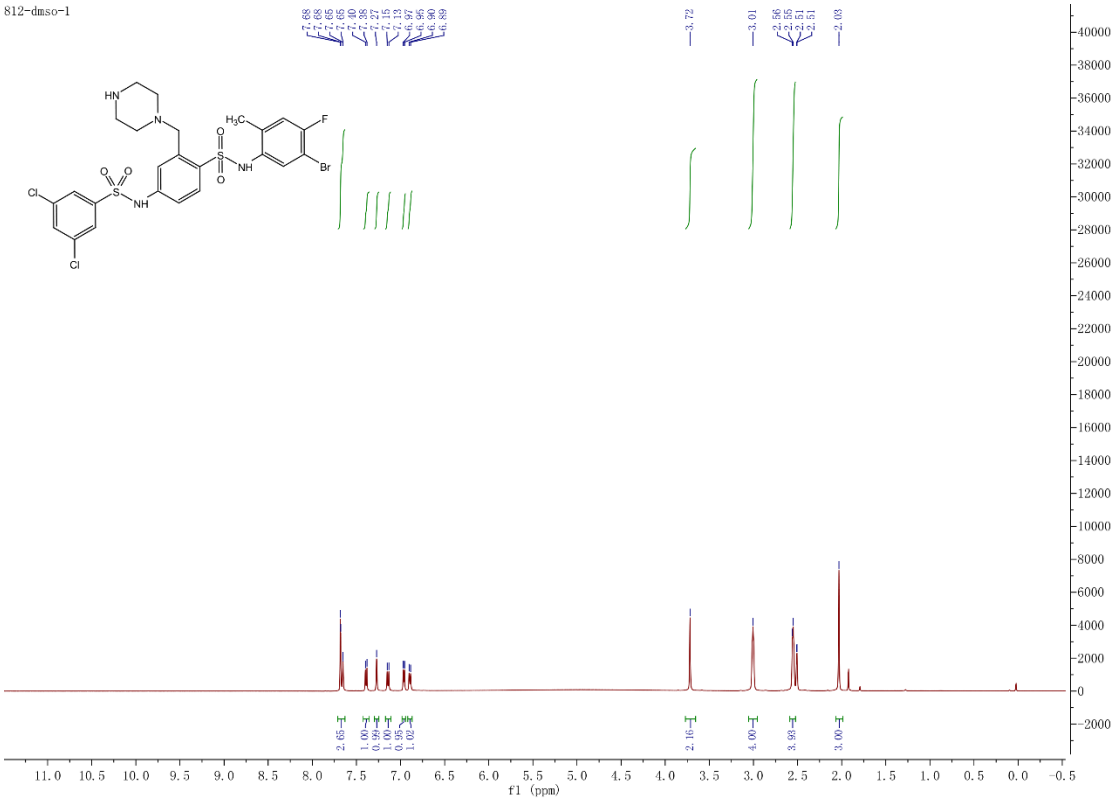
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2.51
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1.00
0.95
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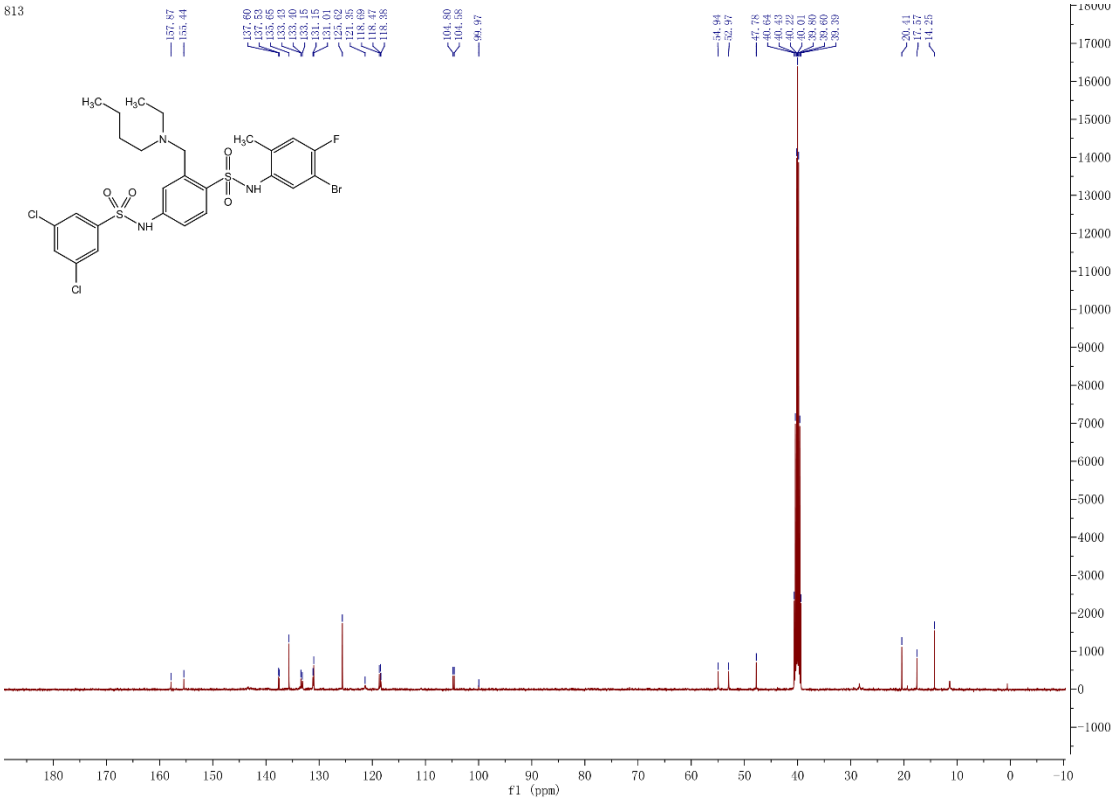
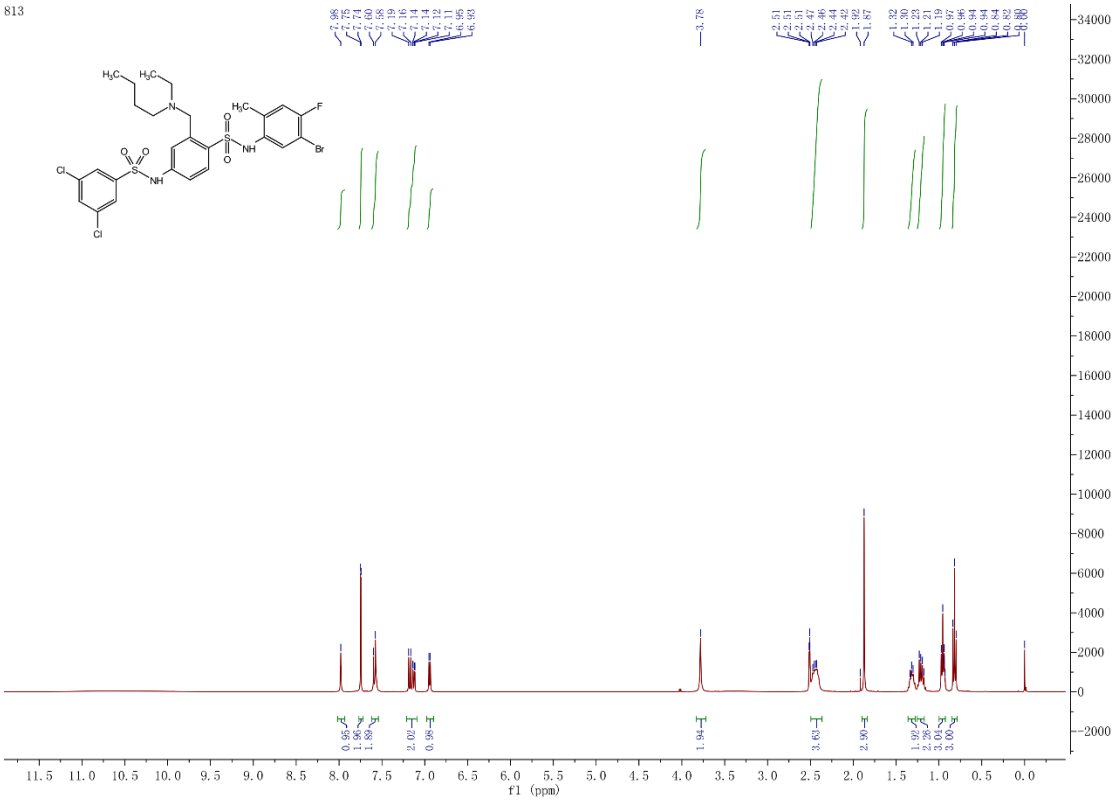
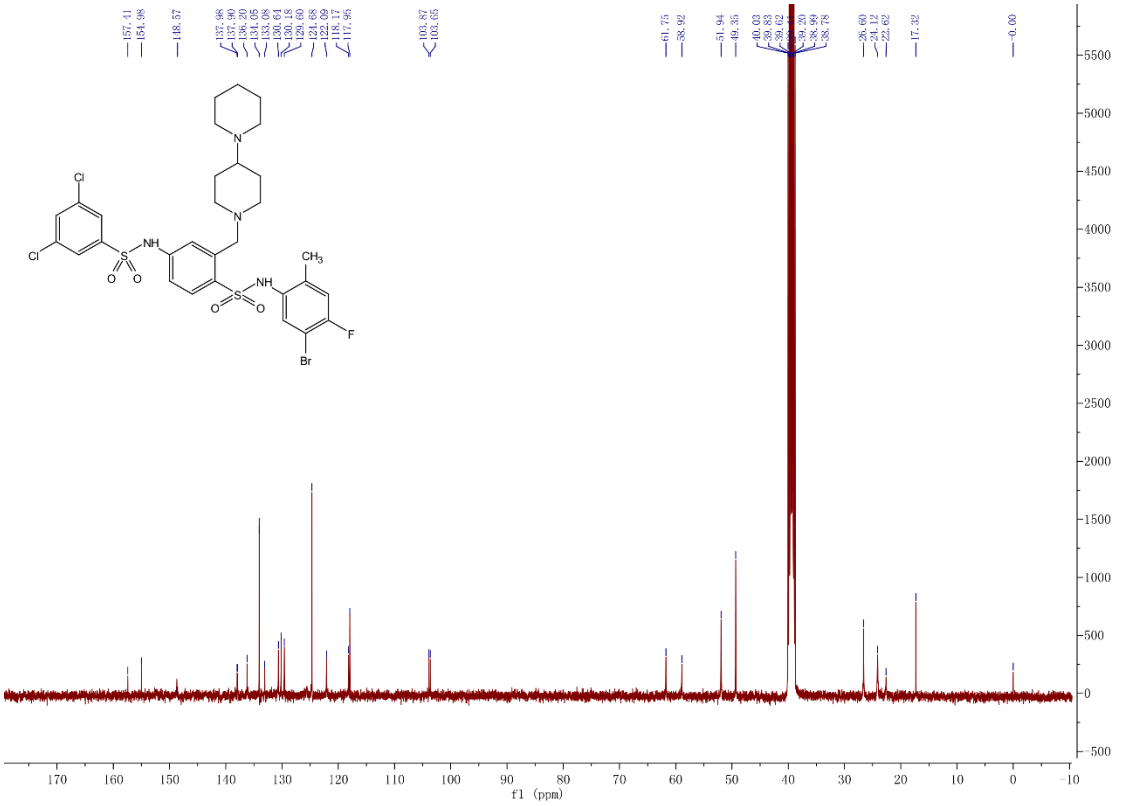
3.93
3.00
f1 (ppm)

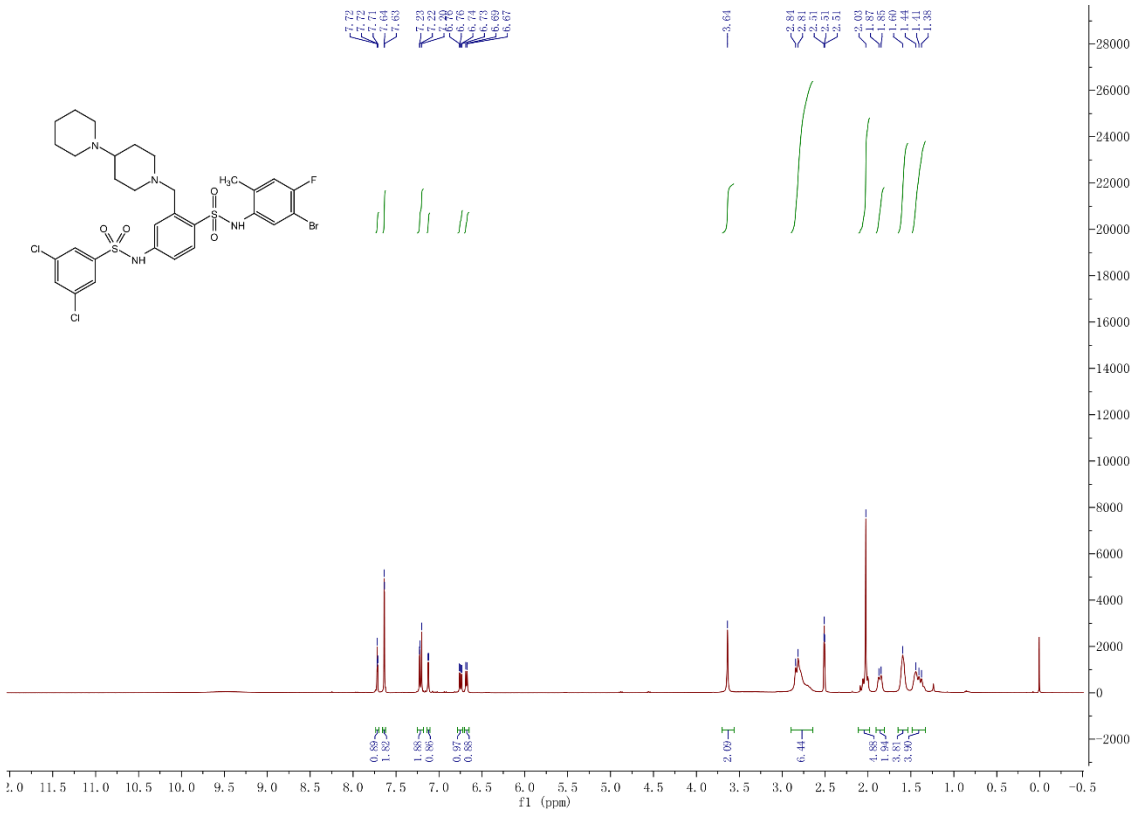
MDL-813


813
157.87
155.44
137.60
137.53
135.65
133.43
133.40
133.15
131.15
131.01
125.02
121.95
118.69
118.47
118.38
104.80
104.58
99.97
54.94
52.97
47.78
40.64
40.43
40.22
40.01
39.80
39.60
39.39
20.41
17.57
14.25
f1 (ppm)

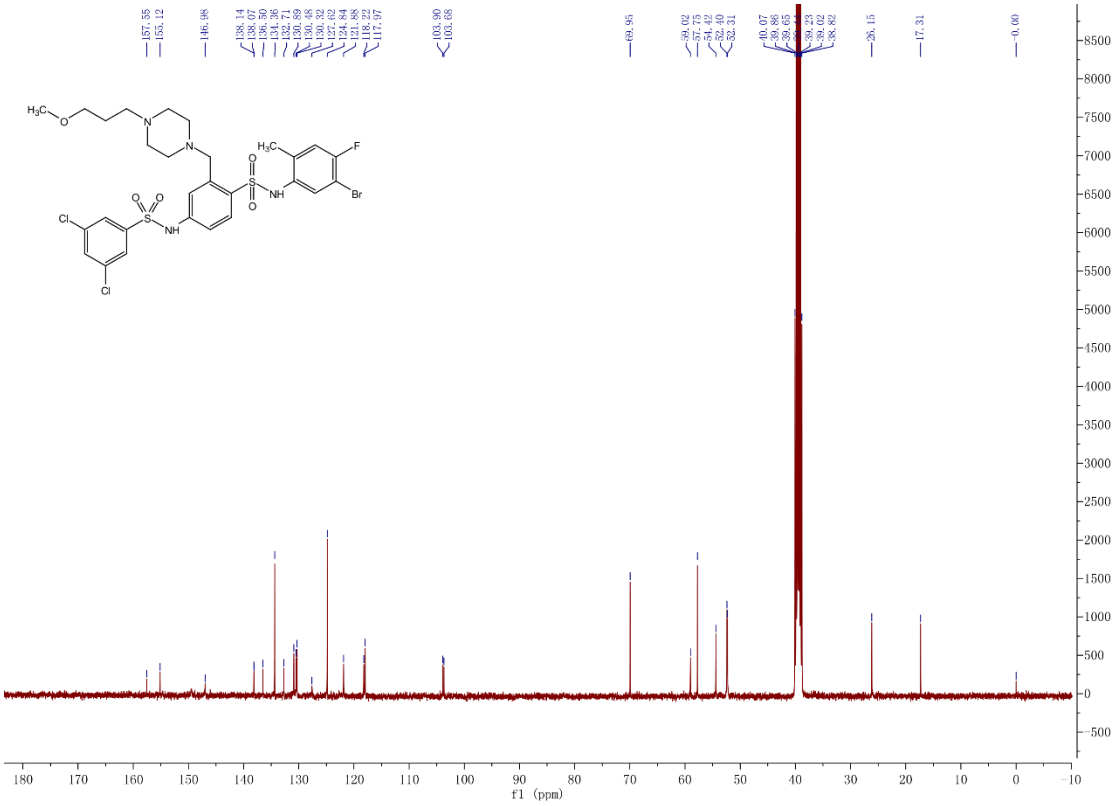

813
7.98
7.75
7.74
7.60
7.58
7.19
7.16
7.14
7.12
7.11
6.95
6.93
3.78
2.51
2.51
2.51
2.47
2.46
2.44
2.42
1.92
1.87
1.32
1.30
1.23
1.21
1.19
0.97
0.96
0.94
0.94
0.82
0.80
0.95
1.96
1.89
2.02
0.98
1.94
3.63
2.90
1.92
2.89
2.85
3.00
f1 (ppm)

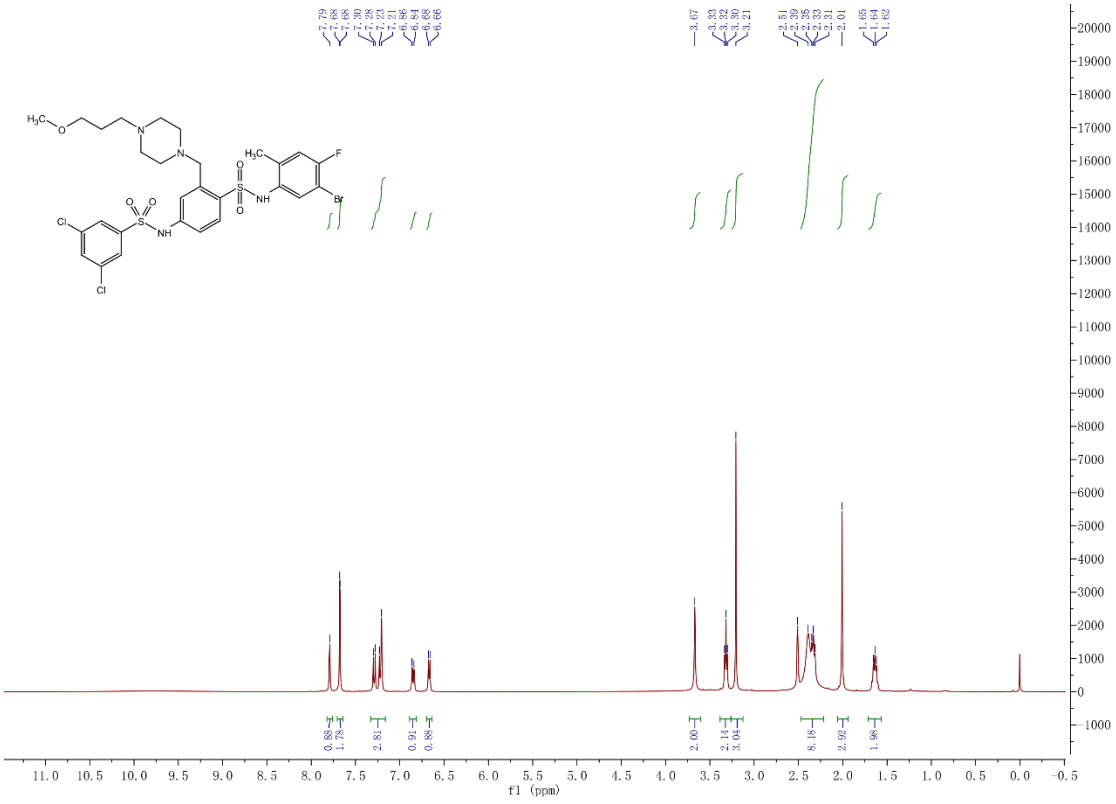
MDL-814


157.41
154.98
148.57
137.98
137.90
136.20
134.95
133.08
130.64
130.18
129.60
124.68
122.99
118.17
117.95
103.87
103.65
61.75
58.92
51.94
49.35
40.03
39.83
39.62
39.42
39.20
38.99
38.78
26.60
24.12
22.62
17.32
0.00
f1 (ppm)

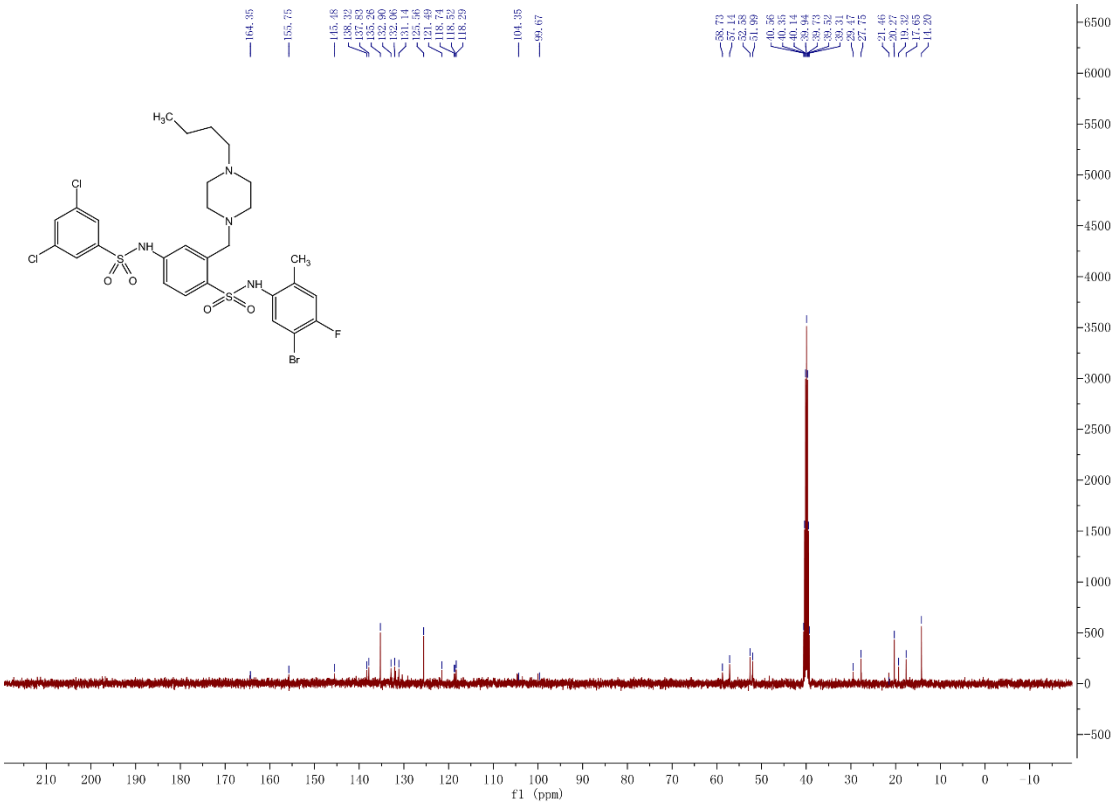

7.72
7.72
7.71
7.64
7.63
7.23
7.22
7.20
6.76
6.74
6.73
6.69
6.67
3.64
2.84
2.81
2.51
2.51
2.03
1.57
1.55
1.69
1.44
1.41
1.38
0.89
1.82
1.88
0.86
0.97
0.68
2.09
6.44
4.88
1.84
3.90
f1 (ppm)

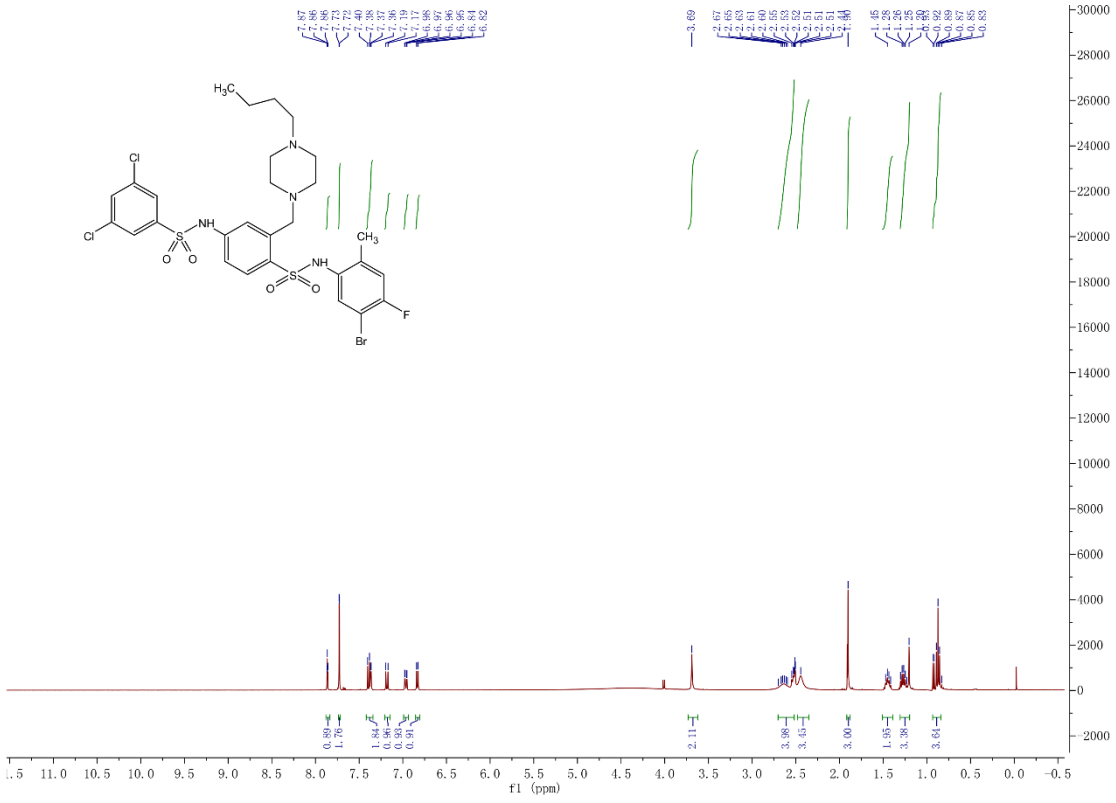
MDL-815


157.55
155.12
146.98
138.14
138.07
136.50
134.36
132.71
130.89
130.46
130.32
127.62
124.84
121.88
118.27
117.97
103.90
103.68
69.95
59.02
57.75
54.42
52.40
52.31
40.07
39.86
39.65
39.44
39.23
39.02
38.82
26.15
17.31
0.00
f1 (ppm)

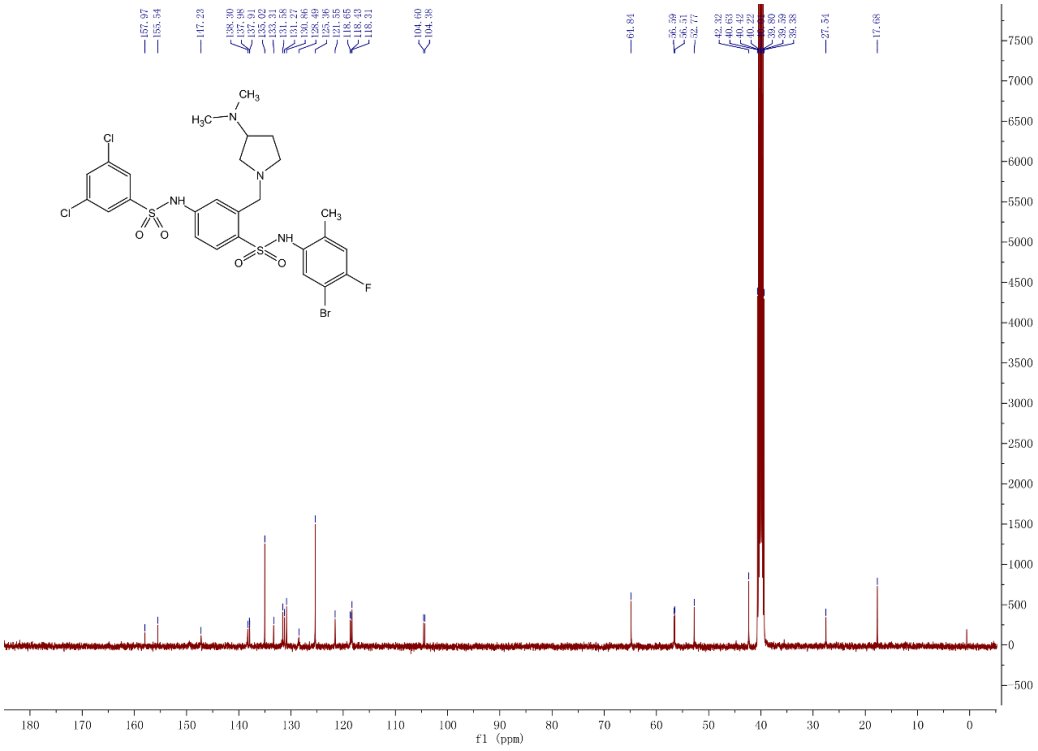

7.79
7.68
7.68
7.30
7.28
7.23
7.21
6.86
6.84
6.68
6.66
3.67
3.33
3.32
3.30
3.21
2.51
2.39
2.35
2.33
2.31
2.01
1.65
1.64
1.62
0.68
1.78
2.81
0.91
0.88
2.00
2.14
3.04
8.18
2.92
1.98
f1 (ppm)

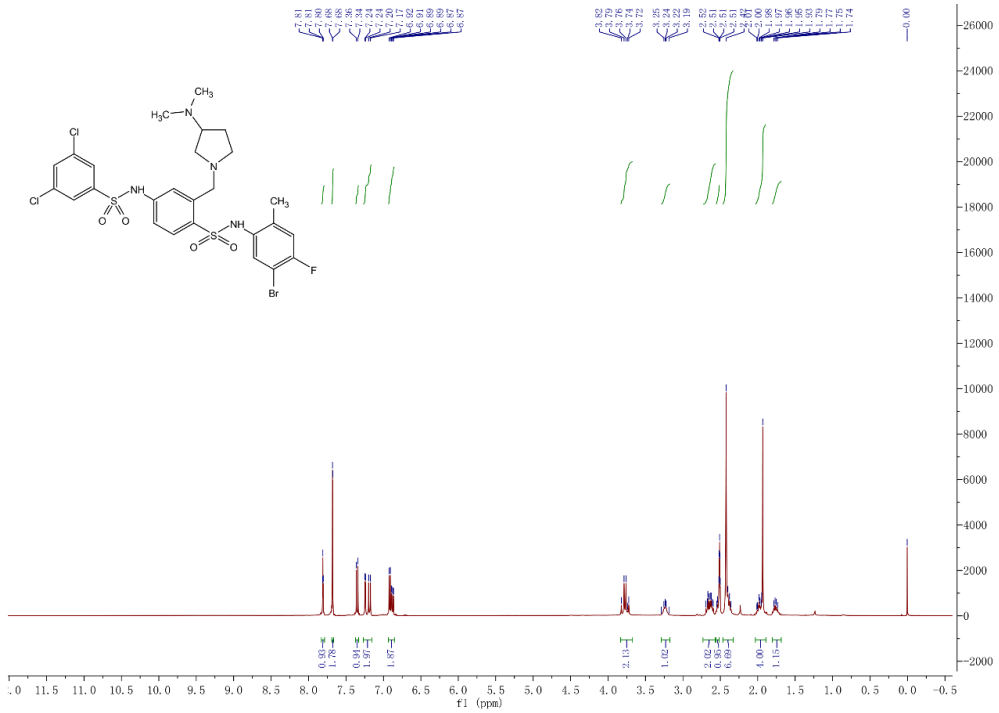
MDL-816



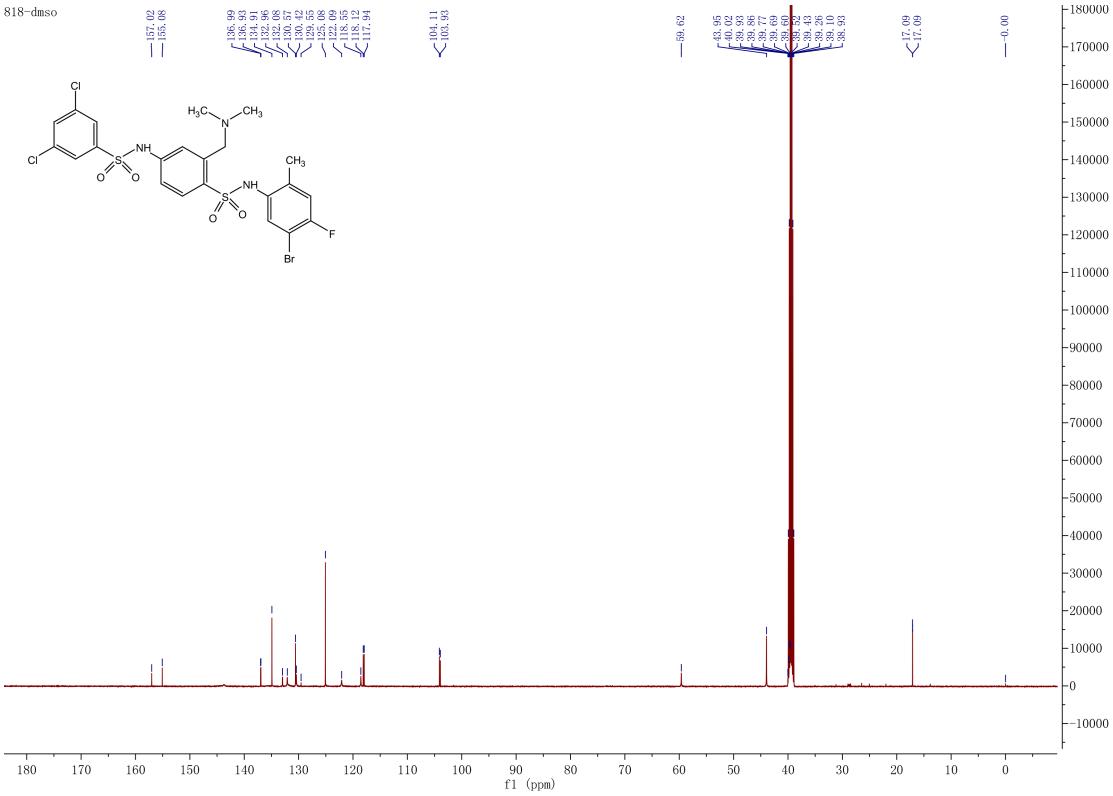


MDL-817


157.97
155.54
147.23
138.30
137.98
137.91
135.02
133.31
131.56
131.27
130.86
128.49
125.36
121.85
118.65
118.43
118.31
104.60
104.38
61.84
56.39
56.31
52.77
42.32
40.63
40.42
40.22
39.80
39.59
39.38
27.54
17.68
f1 (ppm)


7.81
7.80
7.68
7.66
7.36
7.34
7.24
7.20
7.17
6.92
6.91
6.89
6.87
6.87
3.82
3.79
3.76
3.74
3.72
3.25
3.24
3.22
3.19
2.82
2.81
2.51
2.07
2.06
1.97
1.96
1.93
1.79
1.77
1.75
1.74
0.00
0.93
1.78
0.94
1.97
1.87
2.13
1.02
2.02
0.95
0.89
4.00
1.15
f1 (ppm)

MDL-818


818-dmso
157.02
155.08
136.99
136.93
134.41
132.96
132.08
130.57
130.42
125.85
122.09
118.55
118.12
117.94
104.11
103.93
69.62
43.95
40.02
39.93
39.86
39.77
39.69
39.52
39.43
39.26
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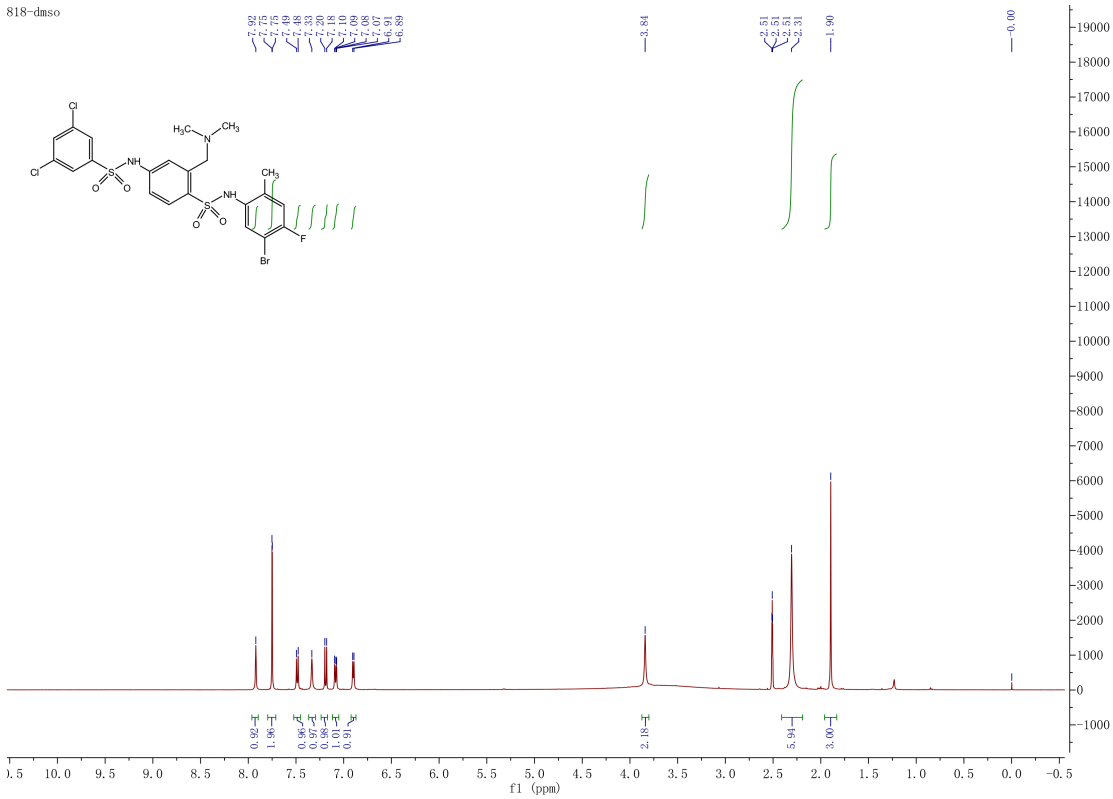
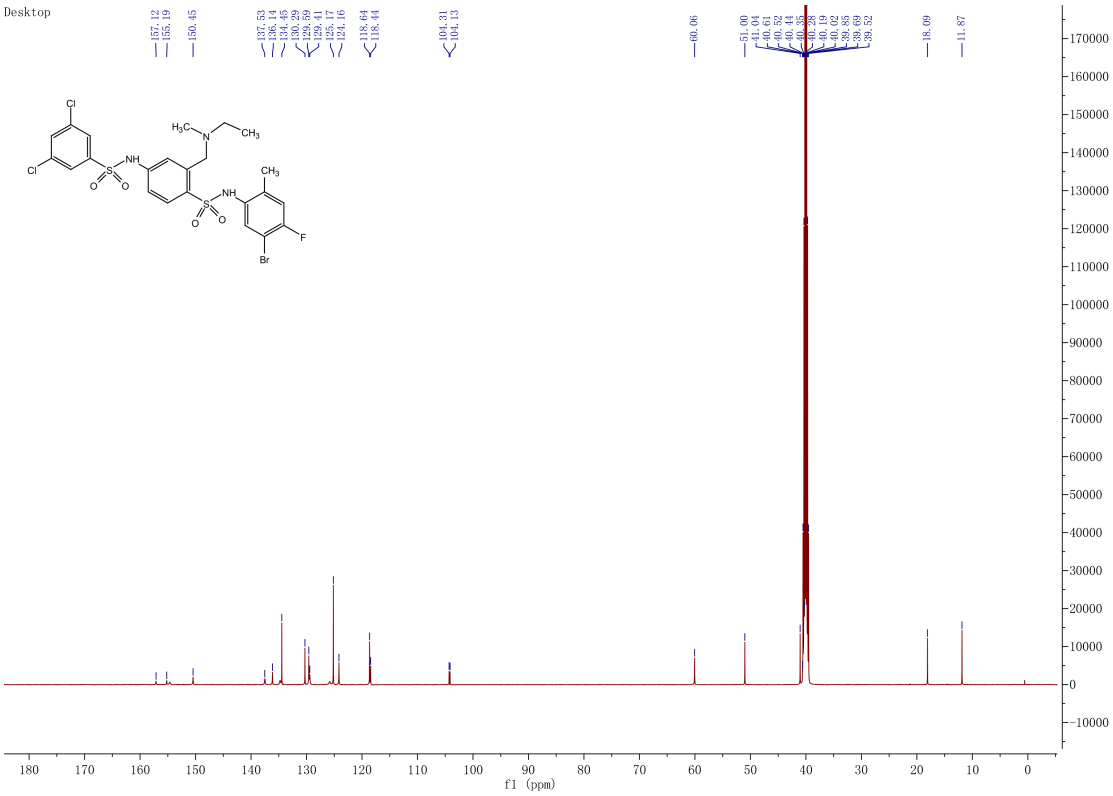
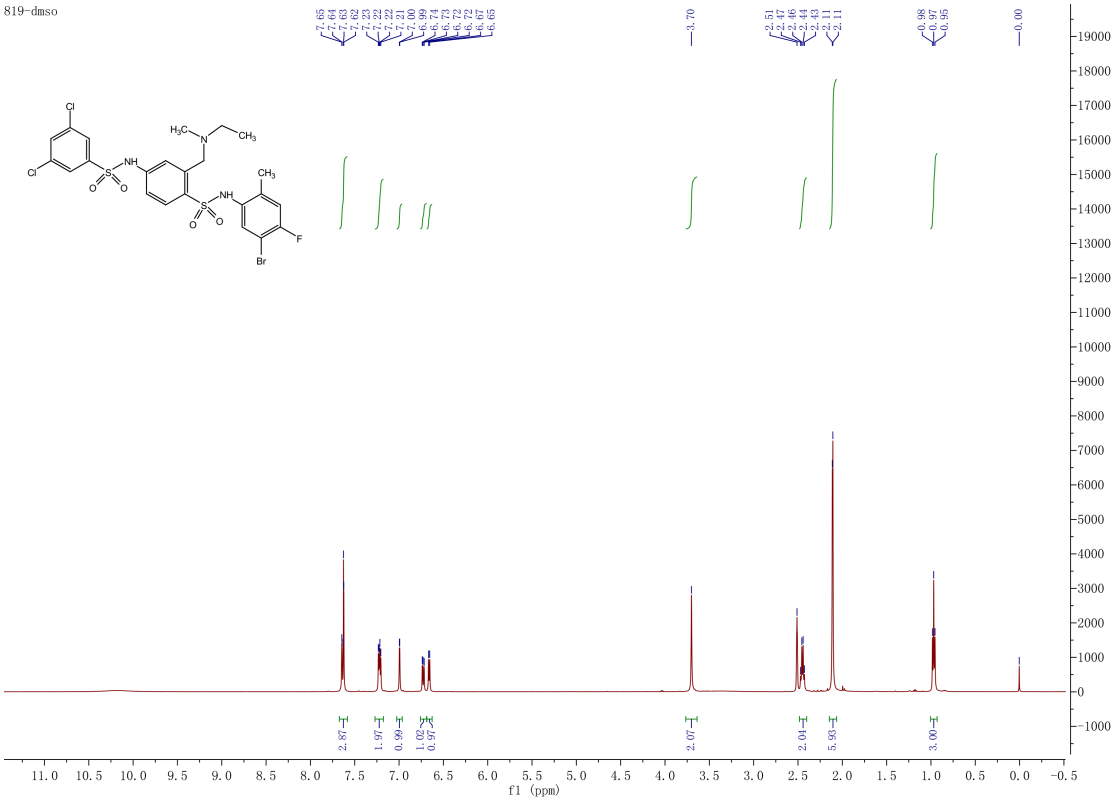
38.93
17.09
17.09
-0.00
f1 (ppm)

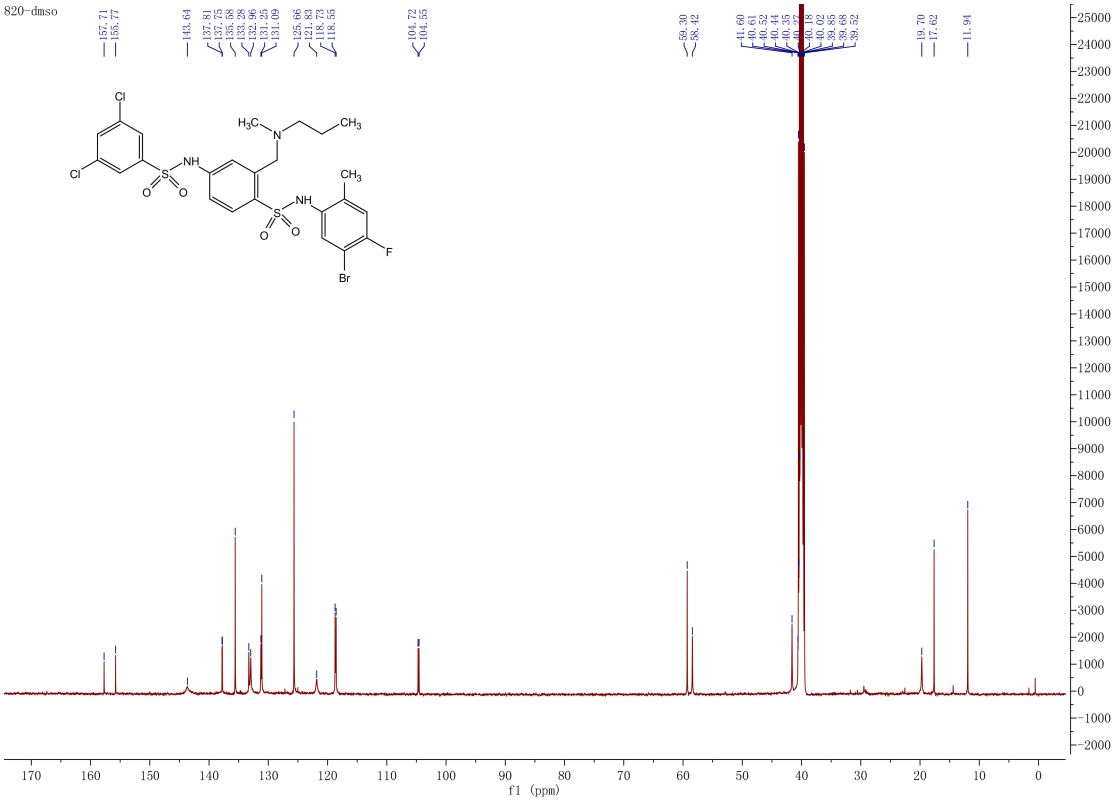

818-dmso
7.92
7.75
7.49
7.48
7.33
7.20
7.18
7.10
7.09
7.08
7.07
6.91
6.89
3.84
2.51
2.51
2.51
2.31
1.90
-0.00
0.92
1.96
0.96
0.97
0.98
1.01
0.91
2.18
5.94
3.00
f1 (ppm)

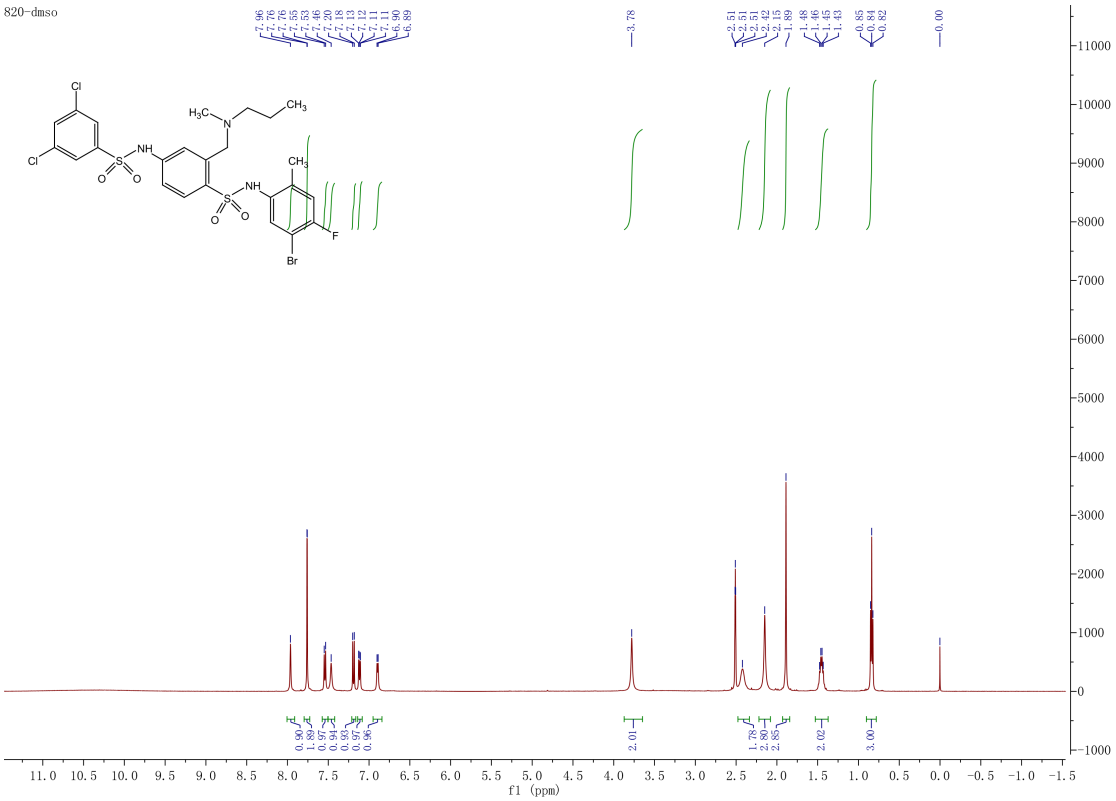
MDL-819


Desktop
157.12
155.19
150.45
137.53
136.14
134.45
130.29
129.59
129.41
125.17
124.16
118.64
118.44
104.31
104.13
60.06
51.00
41.04
40.61
40.52
40.44
40.35
40.19
40.02
39.85
39.69
39.52
18.09
11.87
f1 (ppm)

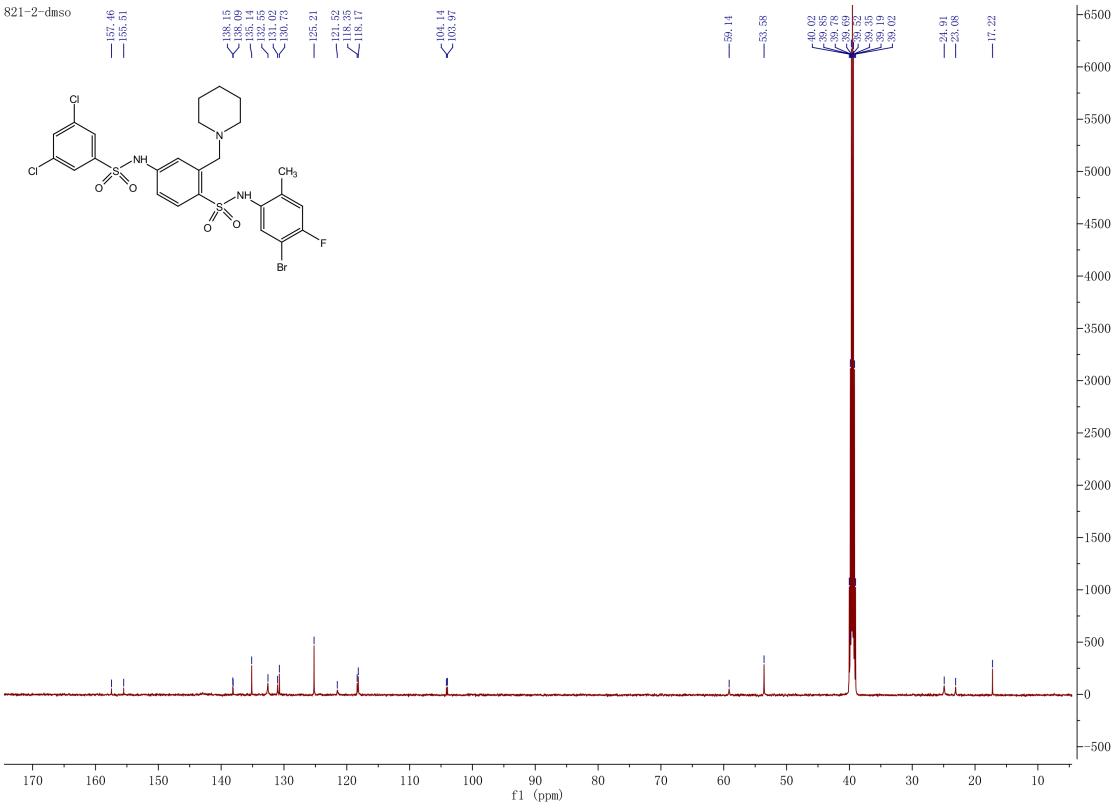

819-dmso
7.65
7.64
7.63
7.62
7.23
7.22
7.21
7.00
6.99
6.74
6.73
6.72
6.67
6.65
3.70
2.51
2.47
2.46
2.44
2.43
2.11
2.11
0.98
0.97
0.95
0.00
2.87
1.97
0.99
1.02
0.97
2.07
2.04
5.93
3.00
f1 (ppm)

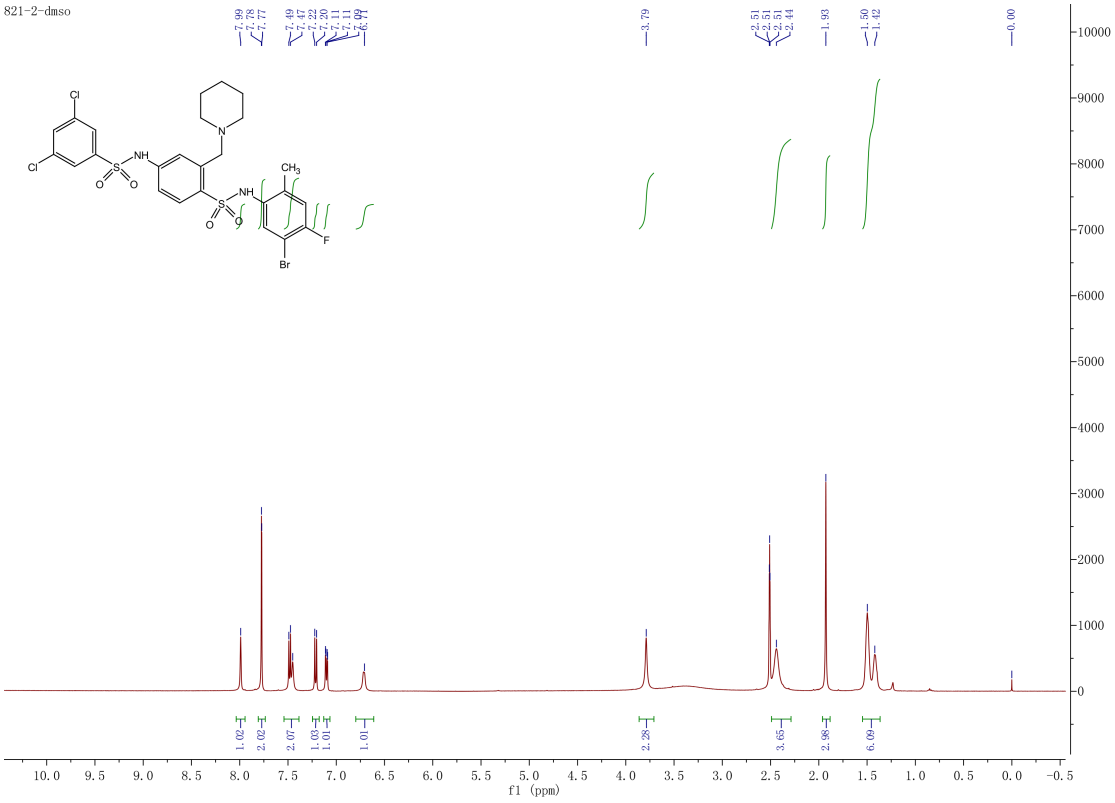
MDL-820


820-dmso
157.71
155.77
143.64
137.81
137.75
135.58
133.28
132.96
131.25
131.09
125.66
123.83
118.73
118.55
104.72
104.55
59.30
58.42
41.60
40.61
40.52
40.44
40.35
40.27
40.18
40.02
39.85
39.68
39.52
19.70
17.62
11.94
f1 (ppm)

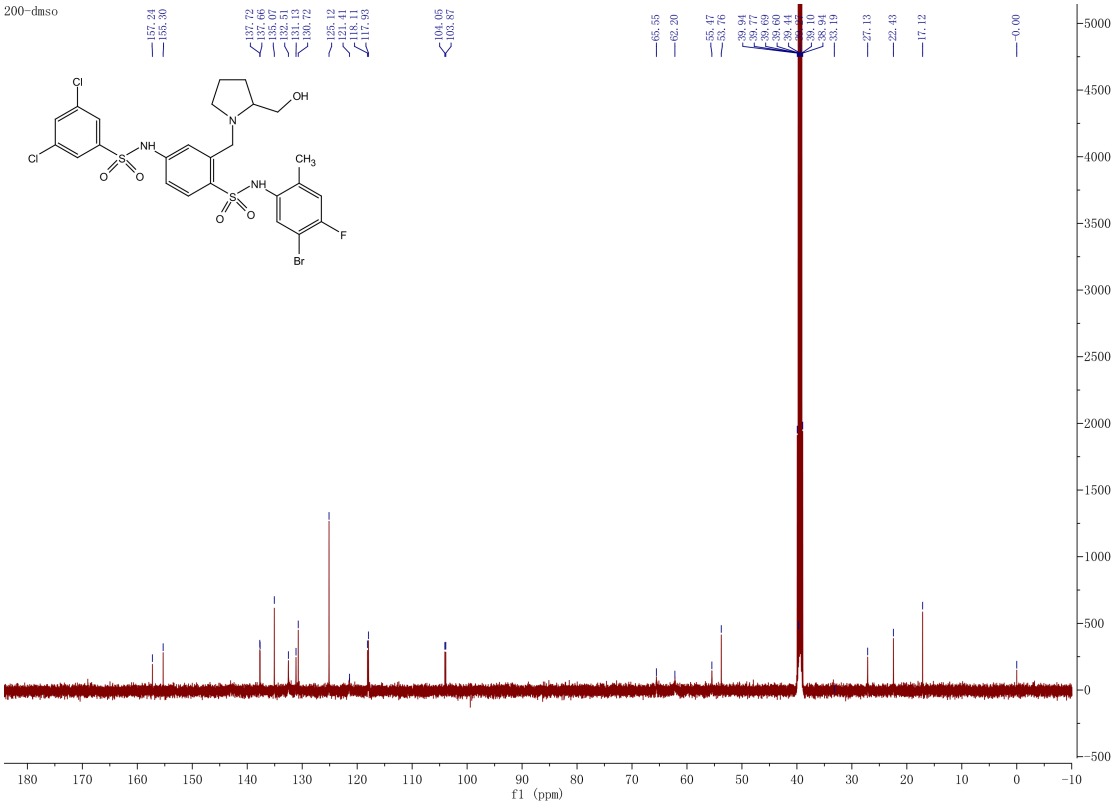

820-dmso
7.96
7.76
7.75
7.55
7.53
7.46
7.20
7.18
7.13
7.12
7.11
6.90
6.89
3.78
2.51
2.51
2.51
2.42
2.15
1.89
1.48
1.46
1.45
1.43
0.85
0.84
0.82
0.00
0.90
1.00
1.00
0.94
0.93
0.97
0.96
2.01
1.78
2.80
2.85
2.02
3.00
f1 (ppm)

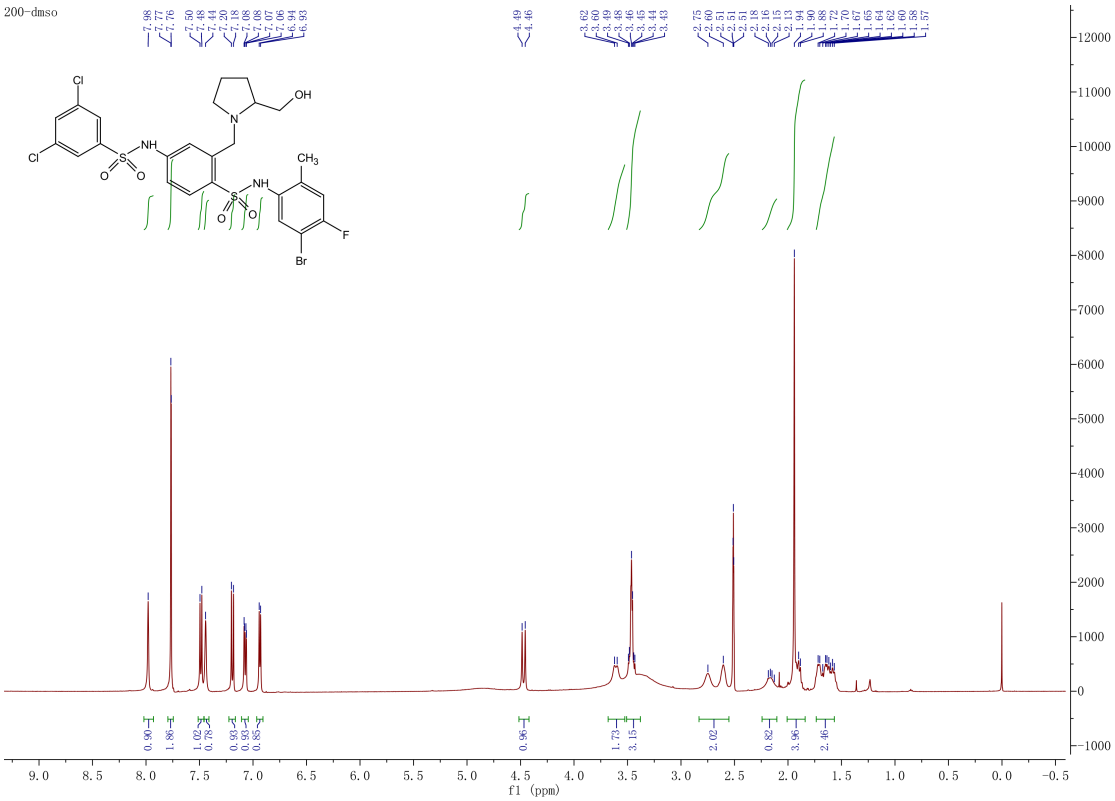
MDL-821


821-2-dmso
157.46
155.51
138.15
138.09
135.14
132.55
131.02
130.73
125.21
121.52
118.35
118.17
104.14
103.97
59.14
53.58
40.02
39.85
39.78
39.69
39.52
39.35
39.19
39.02
24.91
23.08
17.22
Cl
Cl
NH
S
O
O
N
NH
S
O
O
CH3
F
Br
f1 (ppm)


821-2-dmso
7.99
7.78
7.77
7.49
7.47
7.22
7.20
7.11
7.09
6.91
3.79
2.51
2.51
2.51
2.44
1.93
1.50
1.42
0.00
1.02
2.02
2.07
1.03
1.01
1.01
2.28
3.65
2.98
6.09
Cl
Cl
NH
S
O
O
N
NH
S
O
O
CH3
F
Br
f1 (ppm)

MDL-822





Relate to “Synthesis of compounds MDL analogs”. NMR spectra of compounds MDL analogs were shown.

HPLC analysis data of compounds MDL analogs

HPLC analysis data of compounds MDL analogs. The purities of identified compounds that were essential to the conclusions drawn in the text and determined by one standard instrumentation with one system given in the following table. The peak purity was checked with UV spectra.

<table>
<tr><td colspan="2"></td><td colspan="3">Method</td></tr>
<tr><td colspan="2">Equipment</td><td colspan="3">Agilent 1260 with quaternary pump, photodiode array detector (DAD)</td></tr>
<tr><td colspan="2">Column</td><td colspan="3">Agilent Zorbax Exlipse Plus C18 (100×4.6 mm, 3.5 µm particle size)</td></tr>
<tr><td colspan="2" rowspan="2">System condition</td><td>a. CH_3CN(0.1% TFA)/H_2O (0.1% TFA), 10% (v/v) of CH_3CN(0.1% TFA) at the first time. Next, percentage of CH_3CN(0.1% TFA) was slowly increased to 100% in 15 min and the condition was maintained at 5 min, flow rate: 1.0 mL/min, calculated the relative purity of each compound at 254 nM.</td><td colspan="2">b. CH_3CN(0.1% TFA)/H_2O (0.1% TFA), 10% (v/v) of CH_3CN(0.1% TFA) at the first time. Next, percentage of CH_3CN(0.1% TFA) was slowly increased to 100% in 15 min and the condition was maintained at 5 min, flow rate: 1.0 mL/min, calculated the relative purity of each compound at 280 nM.</td></tr>
<tr><td>c. CH_3CN(0.1% TFA)/H_2O (0.1% TFA), 10% (v/v) of CH_3CN(0.1% TFA) at the first time. Next, percentage of CH_3CN(0.1% TFA) was slowly increased to 100% in 15 min and the condition was maintained at 5 min, flow rate: 1.2 mL/min, calculated the relative purity of each compound at 254 nM.</td><td colspan="2">d. CH_3CN(0.1% TFA)/H_2O (0.1% TFA), 10% (v/v) of CH_3CN(0.1% TFA) at the first time. Next, percentage of CH_3CN(0.1% TFA) was slowly increased to 100% in 15 min and the condition was maintained at 5 min, flow rate: 1.2 mL/min, calculated the relative purity of each compound at 280 nM.</td></tr>
<tr><td rowspan="8">Result</td><td rowspan="2">Compound</td><td rowspan="2">Retention time (min)</td><td colspan="2">Relactive purity (%)</td></tr>
<tr><td>254 nM</td><td>280 nM</td></tr>
<tr><td>811</td><td>15.132</td><td>[a]98.28</td><td>[b]98.48</td></tr>
<tr><td>812</td><td>13.736</td><td>[a]99.51</td><td>[b]99.82</td></tr>
<tr><td>813</td><td>16.439</td><td>[a]98.53</td><td>[b]98.42</td></tr>
<tr><td>814</td><td>13.361</td><td>[a]96.00</td><td>[b]95.92</td></tr>
<tr><td>815</td><td>15.186</td><td>[a]99.55</td><td>[b]99.47</td></tr>
<tr><td>816</td><td>15.963</td><td>[a]98.00</td><td>[b]98.35</td></tr>
</table>

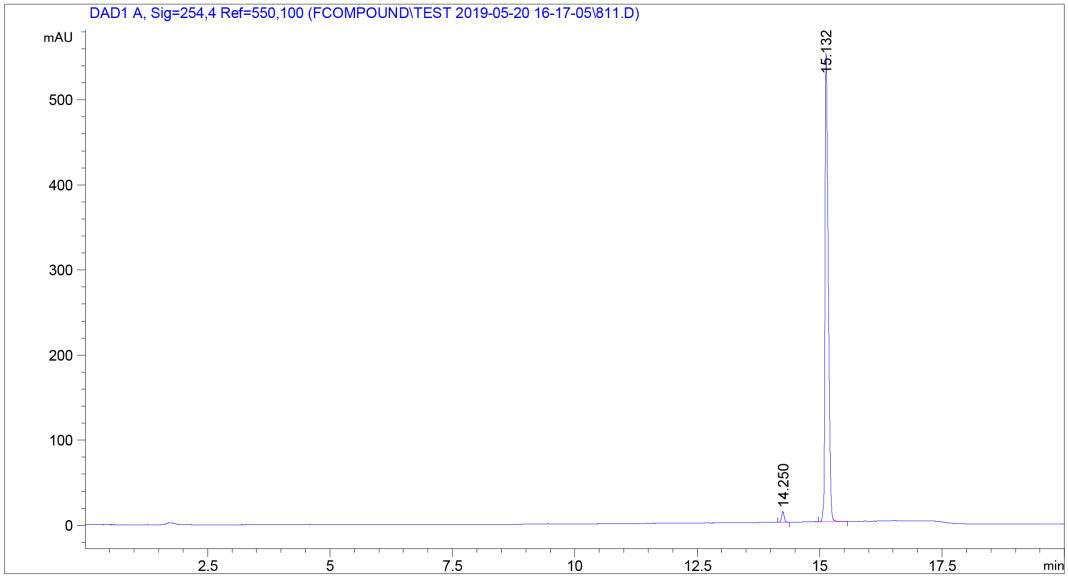
	817	13.224	[a]95.09	[b]95.31
	818	11.772	[c]98.86	[d]99.41
	819	12.009	[c]99.38	[d]99.43
	820	12.483	[c]98.76	[d]99.18
	821	12.474	[c]99.58	[d]99.57
	822	10.483	[c]97.78	[d]99.22

HPLC UV spectra data of purity of compounds MDL analogs.

MDL-811

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\811.D
Sample Name: 811

```
    ========================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   2
    Acq. Instrument : 1260lc                        Location : Vial 25
    Injection Date  : 5/20/2019 4:40:37 PM               Inj :   1
                                                  Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 5.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ========================================================================
                              Area Percent Report
    ========================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  14.250 BB    0.0551   44.22514   12.39342   1.7187
       2  15.132 VB    0.0705 2528.98633  550.99341  98.2813

    Totals :                  2573.21146  563.38683


    ========================================================================
                              *** End of Report ***

    1260lc 11/21/2019 4:39:34 PM SYSTEM                          Page   1 of 1
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\811.D
Sample Name: 811

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   2
    Acq. Instrument : 1260lc                         Location : Vial 25
    Injection Date  : 5/20/2019 4:40:37 PM               Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 5.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
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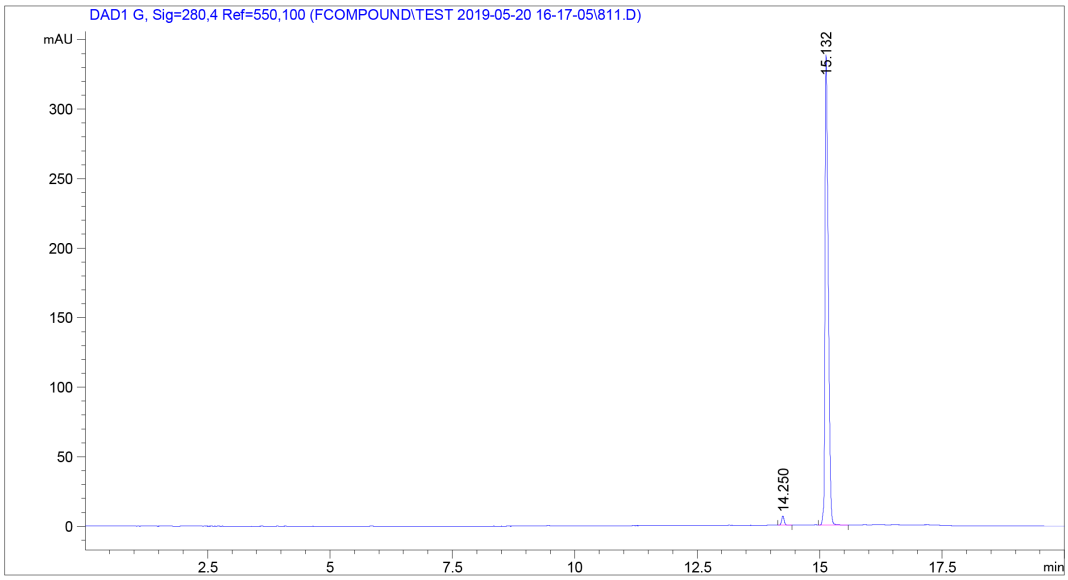



```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  14.250 BB    0.0554   23.89845    6.66109   1.5168
       2  15.132 BB    0.0705 1551.70544  338.23843  98.4832

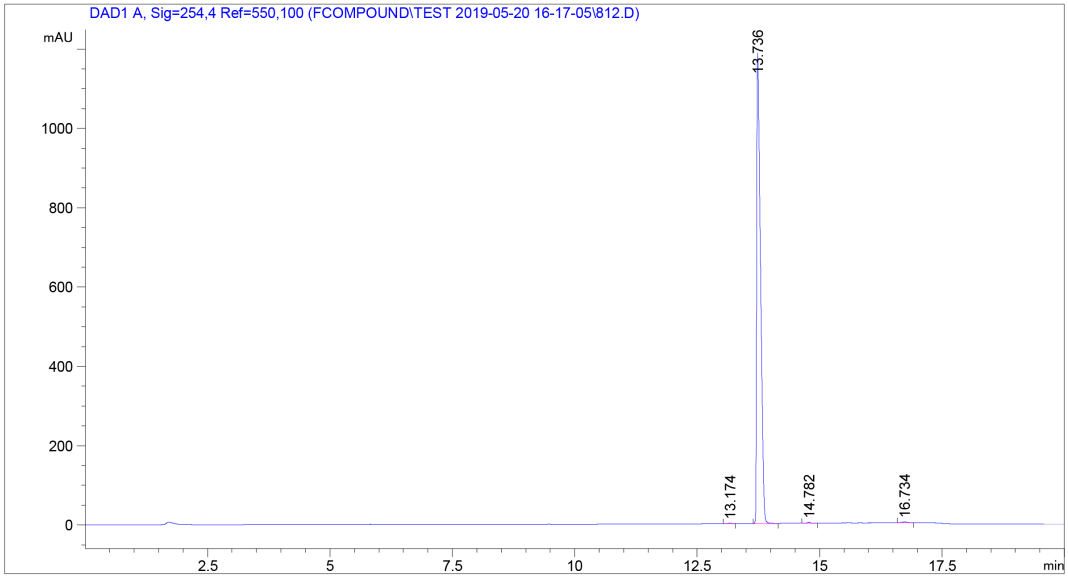
    Totals :                  1575.60390  344.89952


    ======================================================================
                            *** End of Report ***
```

MDL-812

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\812.D
Sample Name: 812

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   3
Acq. Instrument : 1260lc                        Location : Vial 26
Injection Date  : 5/20/2019 5:02:27 PM               Inj :   1
                                               Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 10.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                  Method)
Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

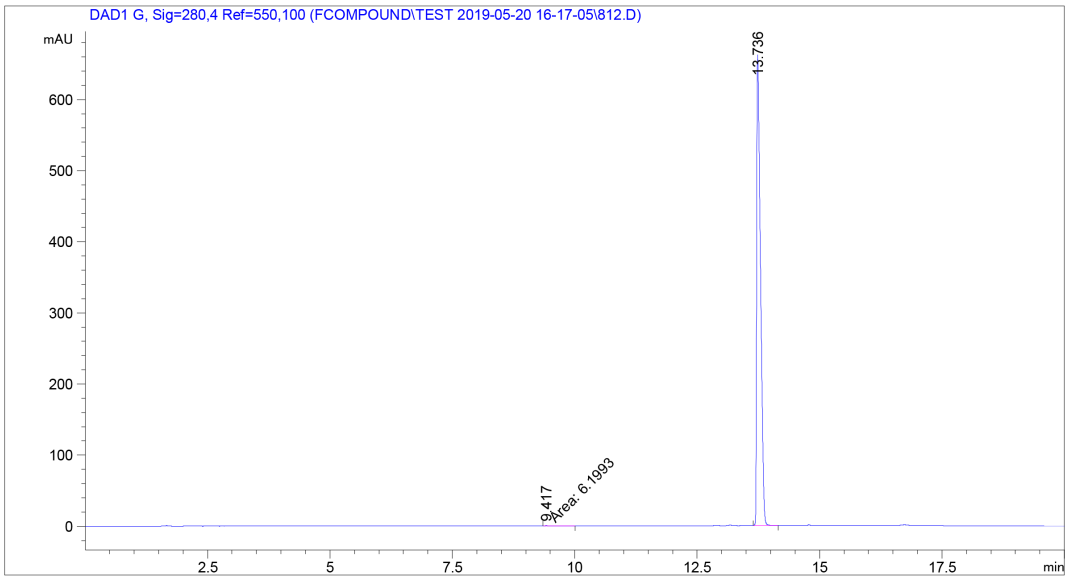

Signal 1: DAD1 A, Sig=254,4 Ref=550,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  13.174 BB    0.0547    6.77659    1.87163   0.1058
   2  13.736 VB    0.0822 6374.10400 1186.53284  99.5194
   3  14.782 BB    0.0595   10.87952    2.78801   0.1699
   4  16.734 BB    0.1150   13.12411    1.73899   0.2049

Totals :                  6404.88423 1192.93146
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\812.D
Sample Name: 812

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   3
    Acq. Instrument : 1260lc                         Location : Vial 26
    Injection Date  : 5/20/2019 5:02:27 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 10.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   9.417 MM    0.1517    6.19930 6.81243e-1   0.1739
       2  13.736 VB    0.0822 3557.91553  662.20483  99.8261

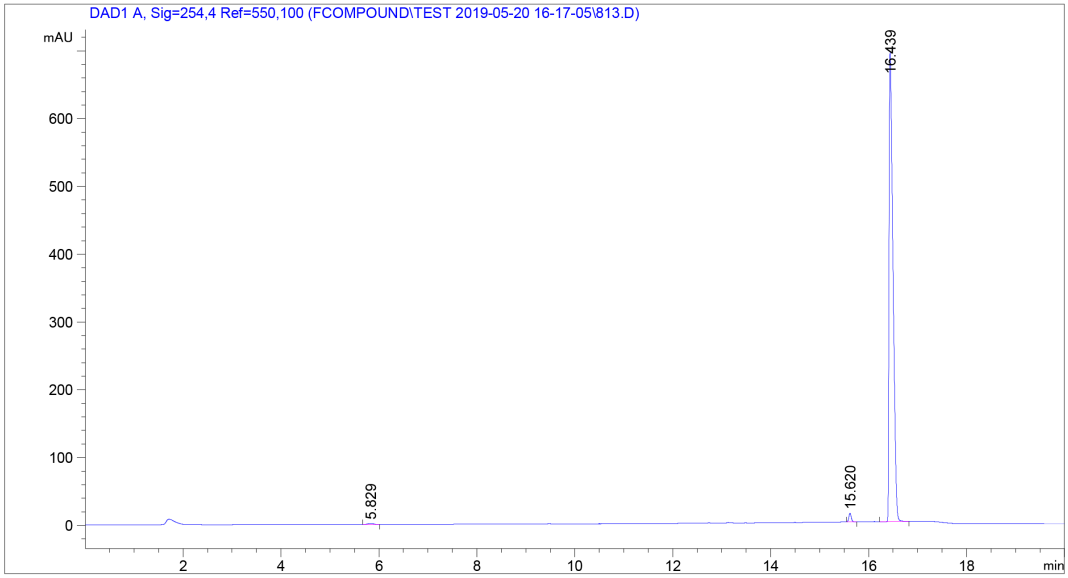
    Totals :                  3564.11483  662.88608


    ======================================================================
                              *** End of Report ***
```

MDL-813

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\813.D
Sample Name: 813

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   4
    Acq. Instrument : 1260lc                         Location : Vial 27
    Injection Date  : 5/20/2019 5:24:16 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 8.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

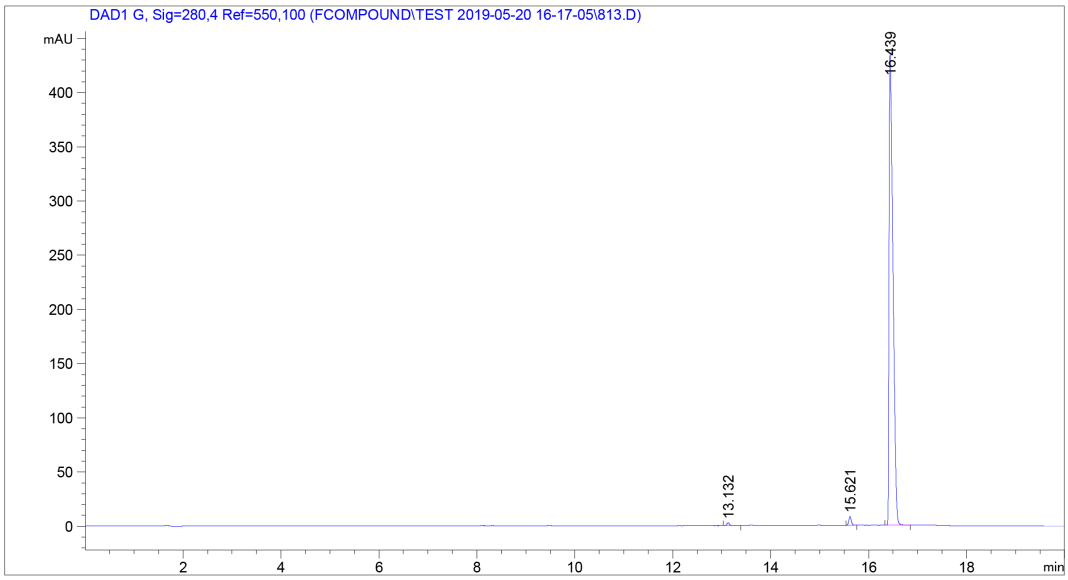
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   5.829 BB    0.1166   11.75607    1.54736   0.3027
       2  15.620 VB    0.0544   45.33770   12.93765   1.1674
       3  16.439 BB    0.0856 3826.52124  691.46991  98.5299

    Totals :                  3883.61501  705.95492

    ======================================================================
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\813.D
Sample Name: 813

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   4
Acq. Instrument : 1260lc                        Location : Vial 27
Injection Date  : 5/20/2019 5:24:16 PM               Inj :   1
                                               Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 8.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                  Method)
Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 G, Sig=280,4 Ref=550,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  13.132 BB    0.0563   10.49832    2.79365   0.4302
   2  15.621 BB    0.0542   28.01473    8.03633   1.1479
   3  16.439 BB    0.0856 2402.01440  434.00583  98.4219

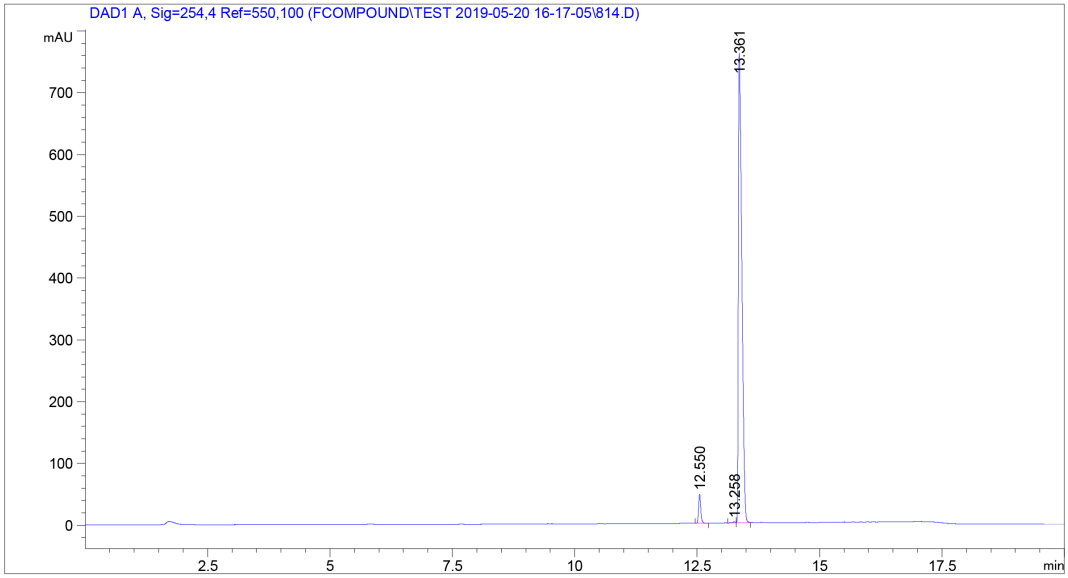
Totals :                  2440.52746  444.83580


==========================================================================
                        *** End of Report ***
```

MDL-814

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\814.D
Sample Name: 814

```
======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : 1260lc                        Location : Vial 28
Injection Date  : 5/20/2019 5:46:06 PM               Inj :   1
                                               Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 8.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                  Method)
Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```




```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,100

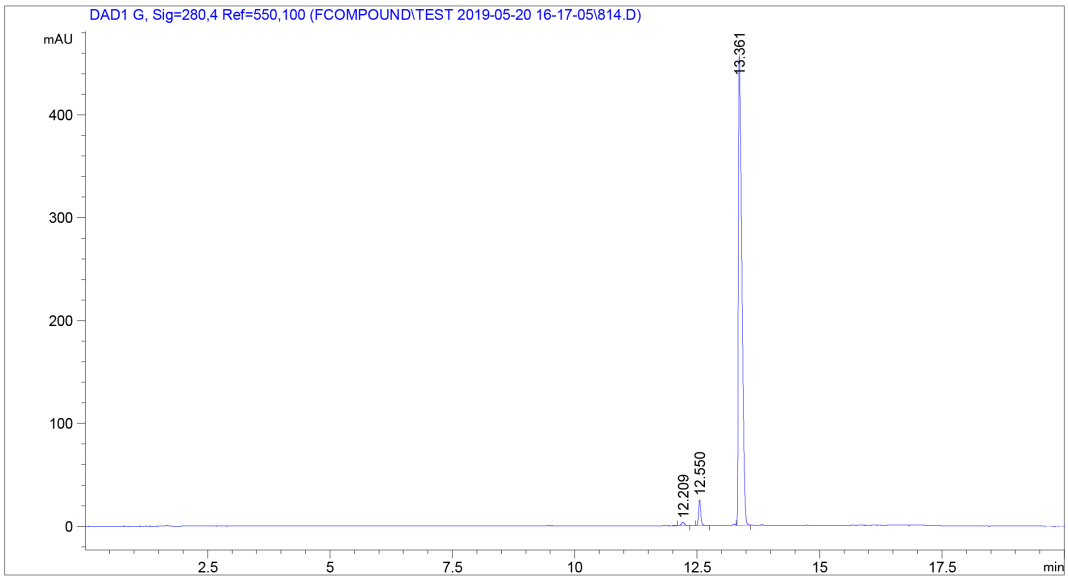
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  12.550 BB    0.0480  148.06168   47.30540   3.8326
   2  13.258 BV    0.0507    6.27082    1.86637   0.1623
   3  13.361 VB    0.0749 3708.91333  760.61719  96.0051

Totals :                  3863.24583  809.78896

======================================================================
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\814.D
Sample Name: 814

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   5
    Acq. Instrument : 1260lc                         Location : Vial 28
    Injection Date  : 5/20/2019 5:46:06 PM               Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 8.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  12.209 BB    0.0734   15.14836    3.27949   0.6507
       2  12.550 BB    0.0483   79.78844   25.32793   3.4272
       3  13.361 VB    0.0749 2233.15820  457.59863  95.9221

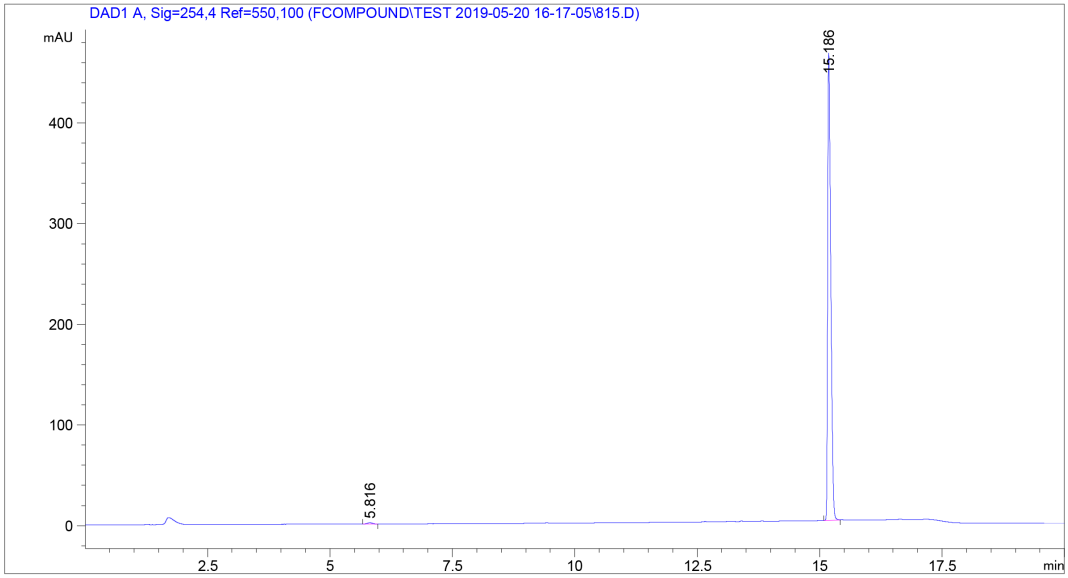
    Totals :                  2328.09500  486.20606


    ======================================================================
                            *** End of Report ***
```

MDL-815

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\815.D
Sample Name: 815

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   6
    Acq. Instrument : 1260lc                         Location : Vial 29
    Injection Date  : 5/20/2019 6:07:55 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 10.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

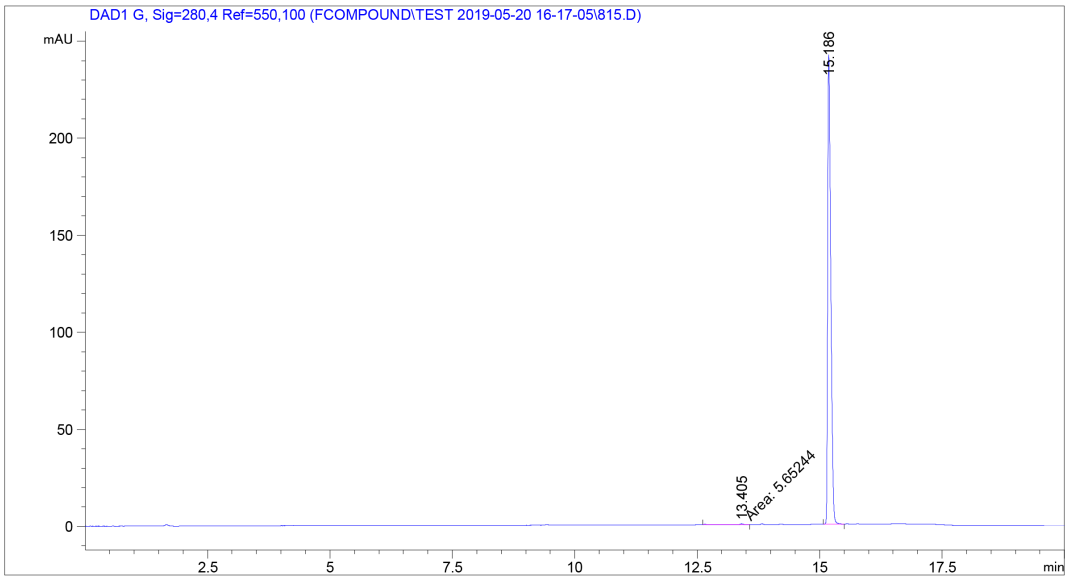
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   5.816 BB    0.1004    9.23906    1.18561   0.4516
       2  15.186 BB    0.0677 2036.49268  464.31644  99.5484

    Totals :                  2045.73174  465.50205


    ======================================================================
                              *** End of Report ***
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\815.D
Sample Name: 815

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   6
    Acq. Instrument : 1260lc                         Location : Vial 29
    Injection Date  : 5/20/2019 6:07:55 PM               Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 10.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  13.405 MM    0.2193    5.65244 4.29613e-1   0.5286
       2  15.186 BB    0.0678 1063.58484  241.95203  99.4714

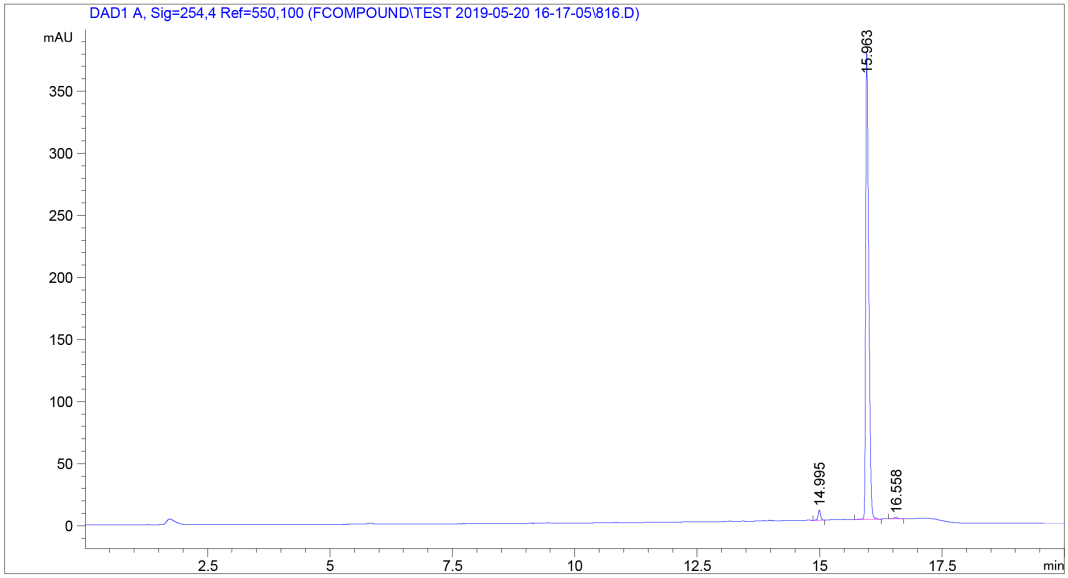
    Totals :                  1069.23728  242.38164


    ======================================================================
                              *** End of Report ***
```

MDL-816

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\816.D
Sample Name: 816

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   7
Acq. Instrument : 1260lc                        Location : Vial 30
Injection Date  : 5/20/2019 6:29:46 PM               Inj :   1
                                               Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                  Method)
Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,100

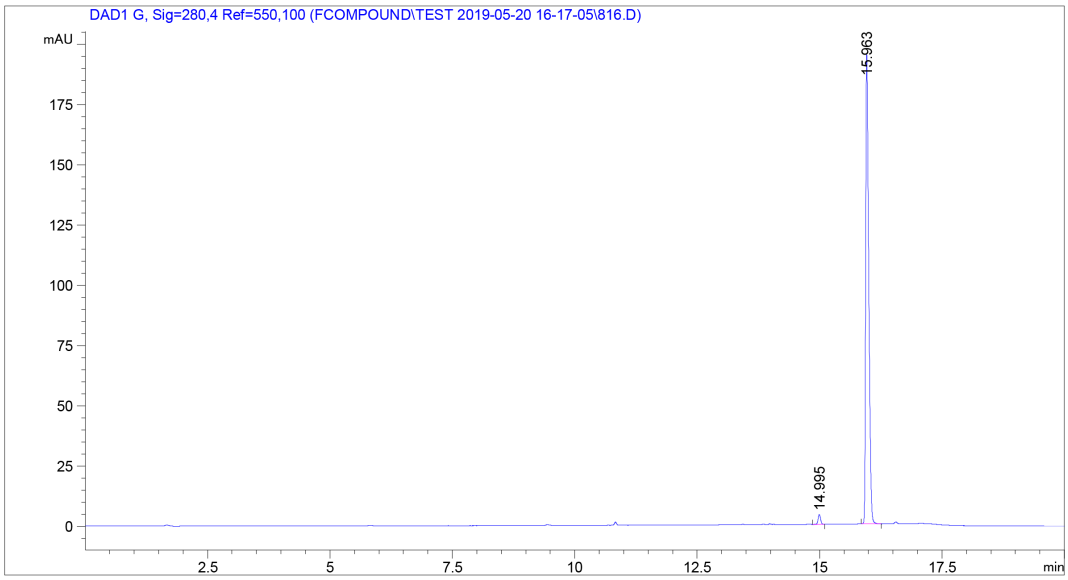
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  14.995 BB    0.0542   28.12886    8.06285   1.6854
   2  15.963 BB    0.0668 1635.56042  375.72974  97.9955
   3  16.558 BB    0.0608    5.32693    1.30085   0.3192

Totals :                  1669.01622  385.09344

==========================================================================
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\816.D
Sample Name: 816

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   7
    Acq. Instrument : 1260lc                         Location : Vial 30
    Injection Date  : 5/20/2019 6:29:46 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
```




```
    ======================================================================
                            Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  14.995 BB    0.0542   14.24673    4.08325   1.6534
       2  15.963 BB    0.0673  847.40161  194.75302  98.3466

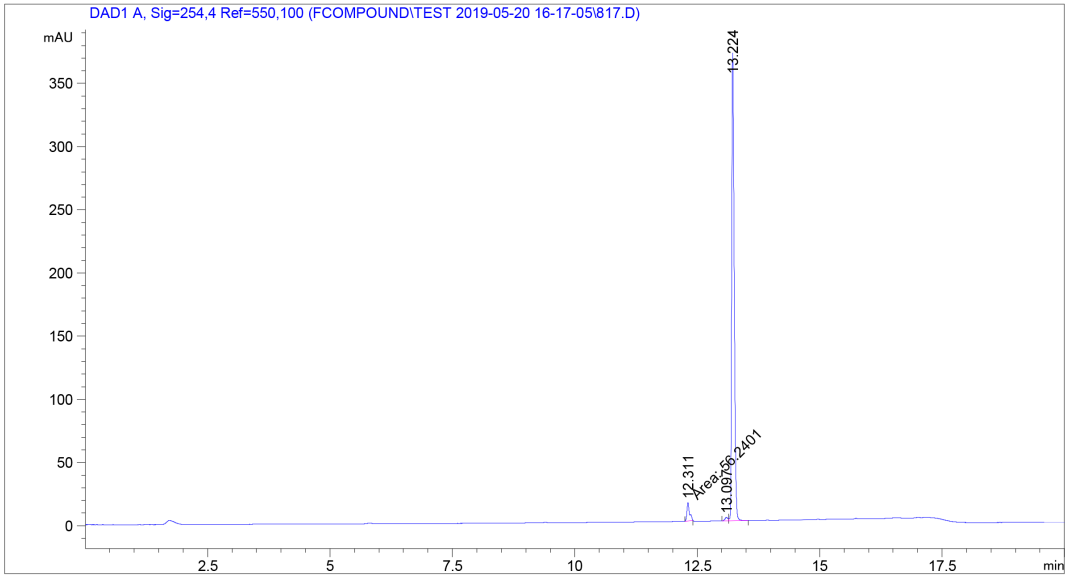
    Totals :                   861.64835  198.83627


    ======================================================================
                             *** End of Report ***
```

MDL-817

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\817.D
Sample Name: 817

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   8
    Acq. Instrument : 1260lc                         Location : Vial 31
    Injection Date  : 5/20/2019 6:51:36 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

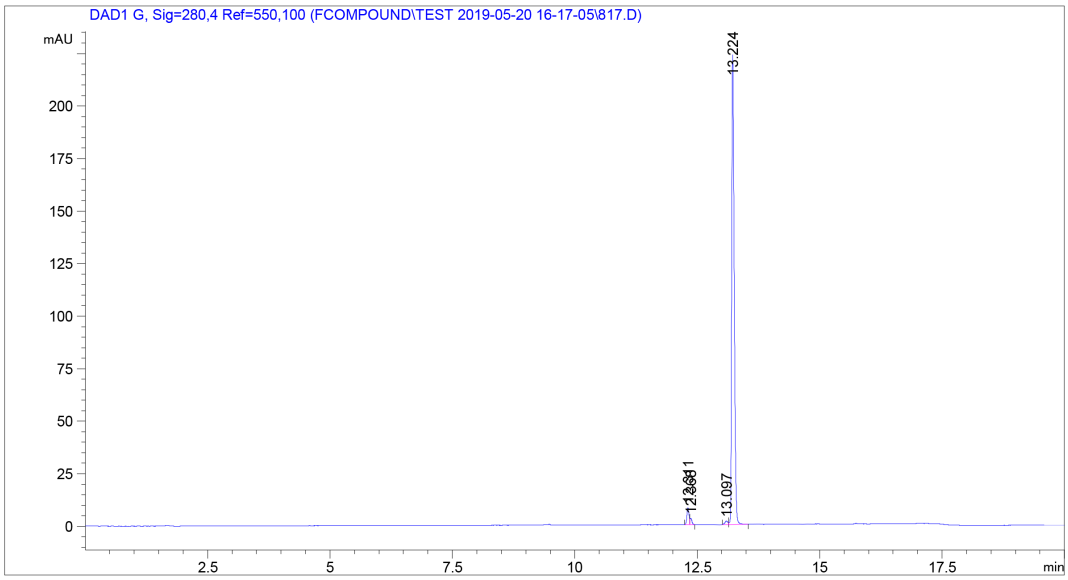
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  12.311 MM    0.0636   56.24012   14.74519   4.0391
       2  13.097 VV    0.0638   12.09847    2.77612   0.8689
       3  13.224 VB    0.0552 1324.04688  370.23776  95.0920

    Totals :                  1392.38546  387.75907


    ======================================================================
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\817.D
Sample Name: 817

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   8
    Acq. Instrument : 1260lc                         Location : Vial 31
    Injection Date  : 5/20/2019 6:51:36 PM               Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2019-05-20 16-17-05\TEST12.M (Sequence
                      Method)
    Last changed    : 5/20/2019 4:17:05 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

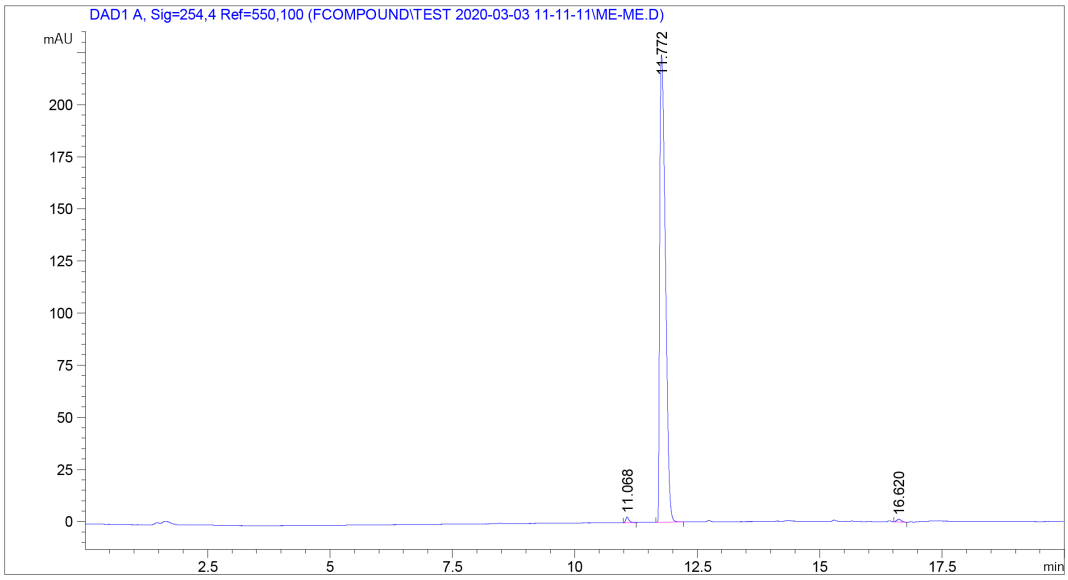
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  12.311 BV    0.0470   24.66913    7.99953   2.9406
       2  12.368 VB    0.0396    7.80047    3.07238   0.9298
       3  13.097 VV    0.0626    6.86336    1.61494   0.8181
       4  13.224 VB    0.0552  799.58600  223.55717  95.3115

    Totals :                   838.91896  236.24402
```

MDL-818

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\ME-ME.D
Sample Name: Me-Me

```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   5
Acq. Instrument : 1260lc                          Location : Vial 65
Injection Date  : 3/3/2020 12:40:06 PM                 Inj :   1
                                                 Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                  Method)
Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,100

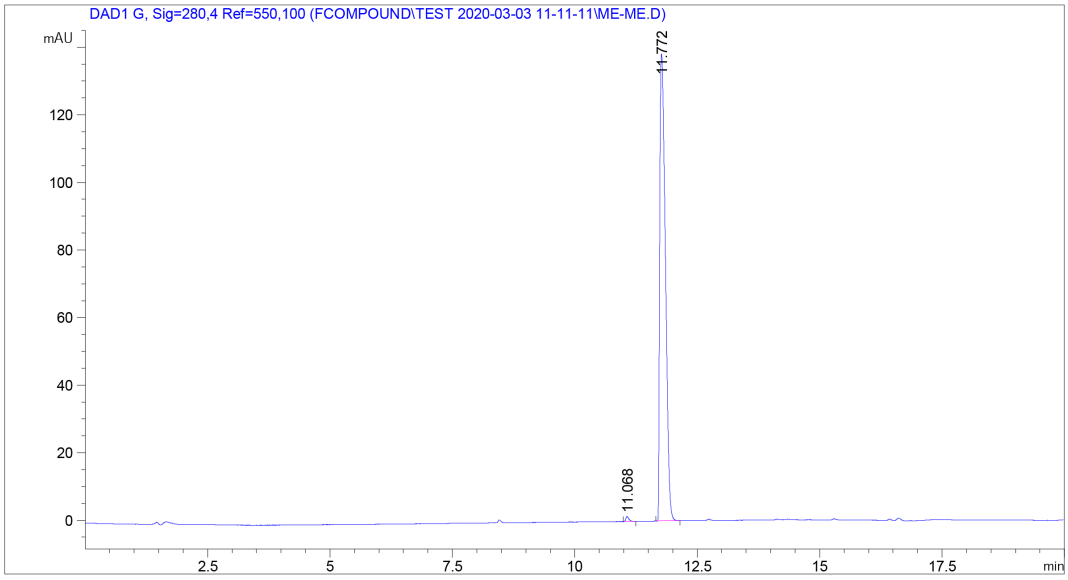
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.068 BB    0.0647   11.79943    2.71694   0.6496
   2  11.772 BB    0.1294 1795.72339  224.37698  98.8628
   3  16.620 VB    0.0917    8.85562    1.42138   0.4875

Totals :                  1816.37844  228.51530

==========================================================================
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\ME-ME.D
Sample Name: Me-Me

```
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : 1260lc                         Location : Vial 65
Injection Date  : 3/3/2020 12:40:06 PM                Inj :   1
                                                Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                  Method)
Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 G, Sig=280,4 Ref=550,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.068 BB    0.0666    6.54671    1.49455   0.5886
   2  11.772 BB    0.1293 1105.68530  138.27916  99.4114

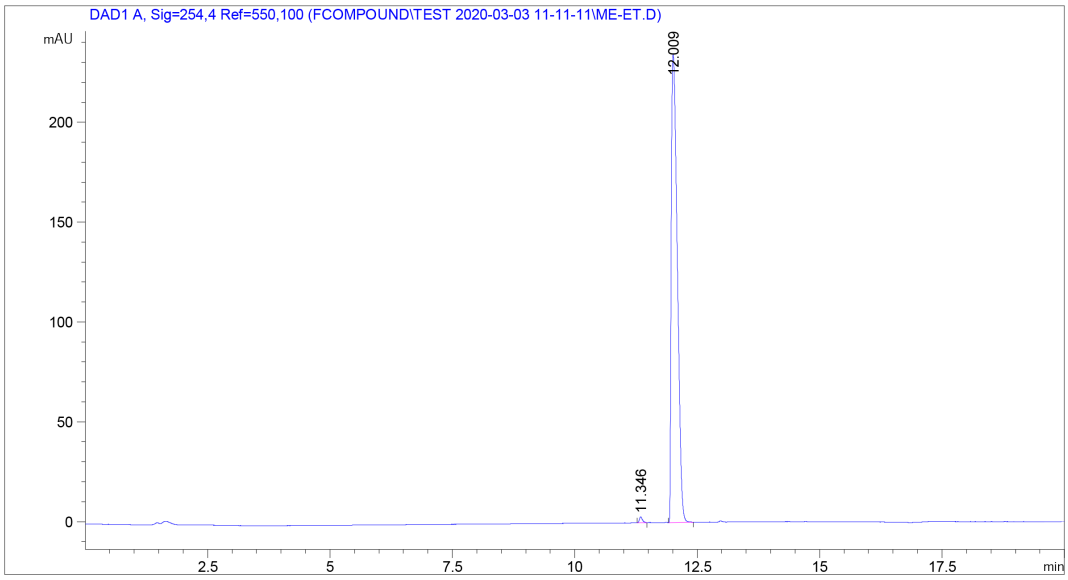
Totals :                  1112.23201  139.77371


=====================================================================
                        *** End of Report ***
```

MDL-819

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\ME-ET.D
Sample Name: Me-Et

```
    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   6
    Acq. Instrument : 1260lc                        Location : Vial 66
    Injection Date  : 3/3/2020 1:01:53 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                      Method)
    Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  11.346 BB    0.0637   12.47002    2.95845   0.6193
       2  12.009 BB    0.1371 2001.16431  234.39781  99.3807

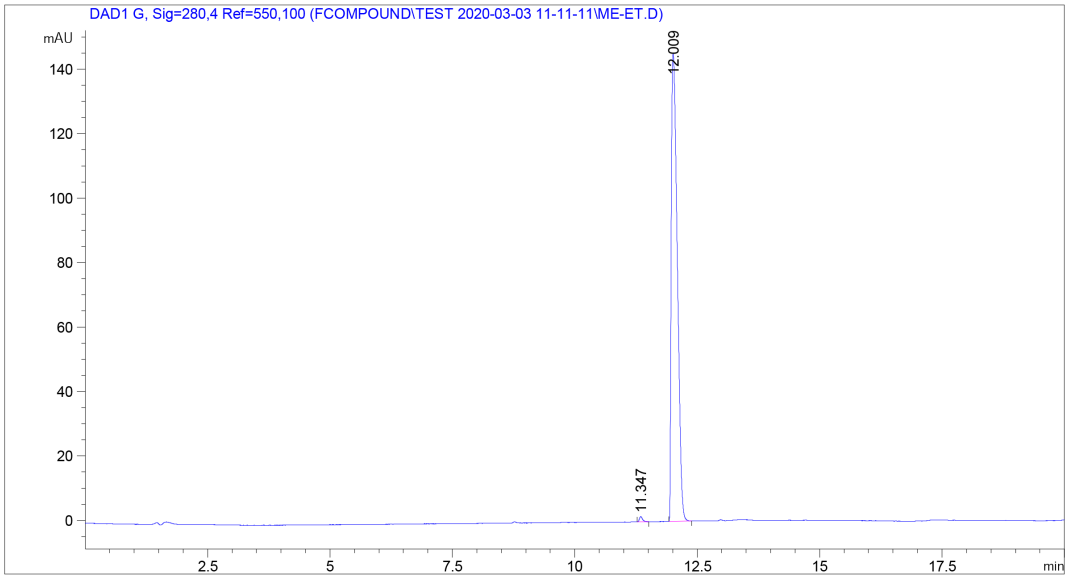
    Totals :                  2013.63433  237.35626


    ======================================================================
                              *** End of Report ***

1260lc 3/26/2020 9:46:36 AM SYSTEM                              Page   1 of 1
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\ME-ET.D
Sample Name: Me-Et

```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   6
Acq. Instrument : 1260lc                           Location : Vial 66
Injection Date  : 3/3/2020 1:01:53 PM                   Inj :   1
                                                  Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                  Method)
Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 G, Sig=280,4 Ref=550,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  11.347 BB    0.0663    7.12819    1.63676   0.5720
   2  12.009 BB    0.1371 1238.99194  145.11757  99.4280

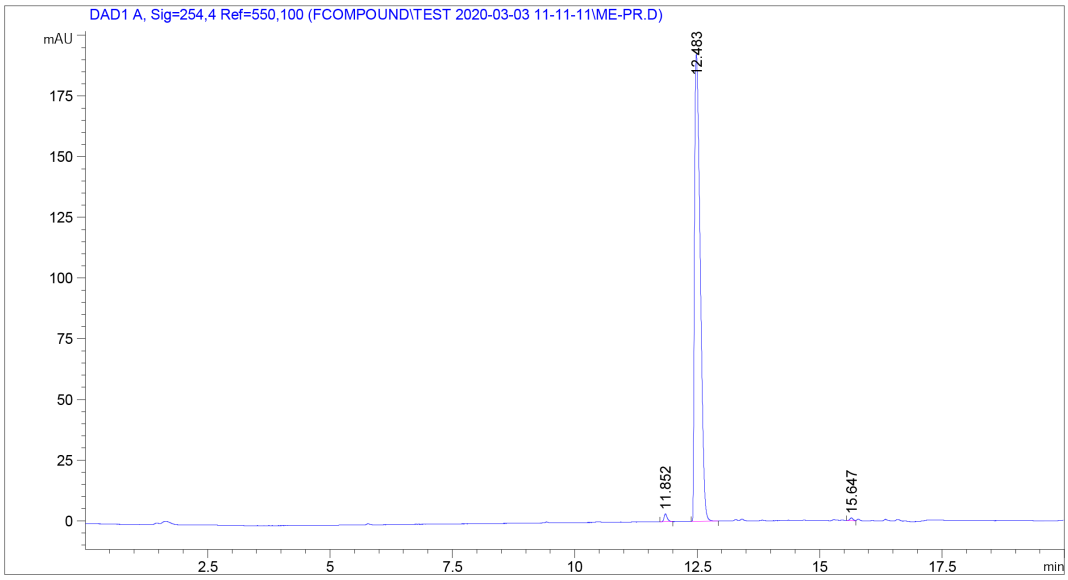
Totals :                  1246.12013  146.75433


==========================================================================
                        *** End of Report ***
```

MDL-820

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\ME-PR.D
Sample Name: Me-Pr

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   7
    Acq. Instrument : 1260lc                         Location : Vial 67
    Injection Date  : 3/3/2020 1:23:40 PM                 Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                      Method)
    Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

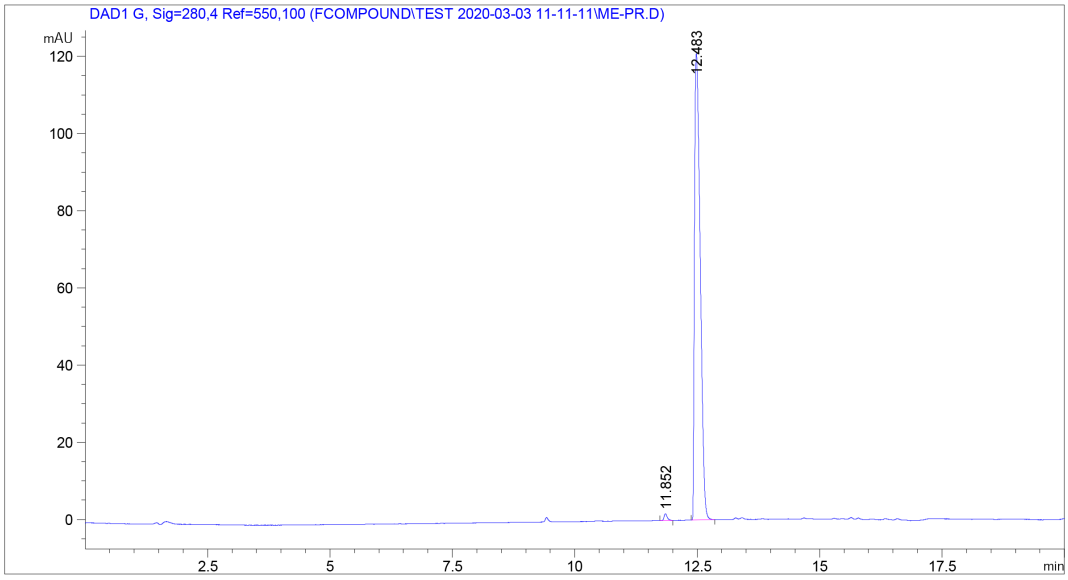
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  11.852 BB    0.0667   14.50987    3.27323   0.9185
       2  12.483 BB    0.1312 1560.13611  192.28294  98.7560
       3  15.647 BV    0.0710    5.14208    1.15293   0.3255

    Totals :                  1579.78806  196.70911


    ======================================================================
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\ME-PR.D
Sample Name: Me-Pr

```
    ========================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   7
    Acq. Instrument : 1260lc                         Location : Vial 67
    Injection Date  : 3/3/2020 1:23:40 PM                 Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                      Method)
    Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
```




```
    ========================================================================
                            Area Percent Report
    ========================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  11.852 BB    0.0653    8.12277    1.81173   0.8225
       2  12.483 BB    0.1301  979.44397  120.80373  99.1775

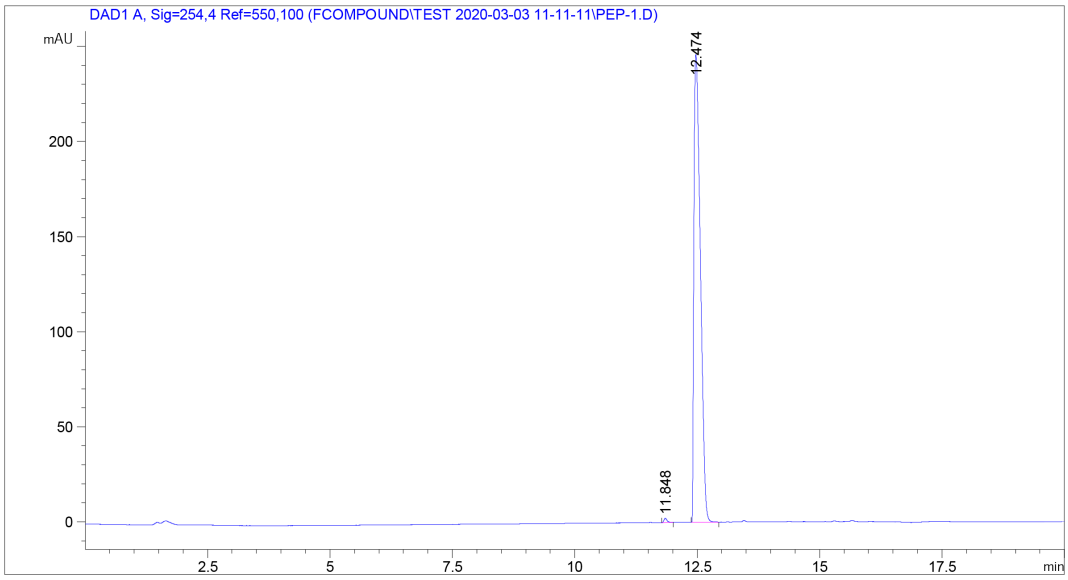
    Totals :                   987.56674  122.61545


    ========================================================================
                            *** End of Report ***
```

MDL-821

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\PEP-1.D
Sample Name: PEP-1

```
    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   8
    Acq. Instrument : 1260lc                       Location : Vial 68
    Injection Date  : 3/3/2020 1:45:27 PM               Inj :   1
                                                  Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                      Method)
    Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  11.848 BB    0.0644    9.53240    2.18448   0.4227
       2  12.474 BB    0.1475 2245.37402  245.92307  99.5773

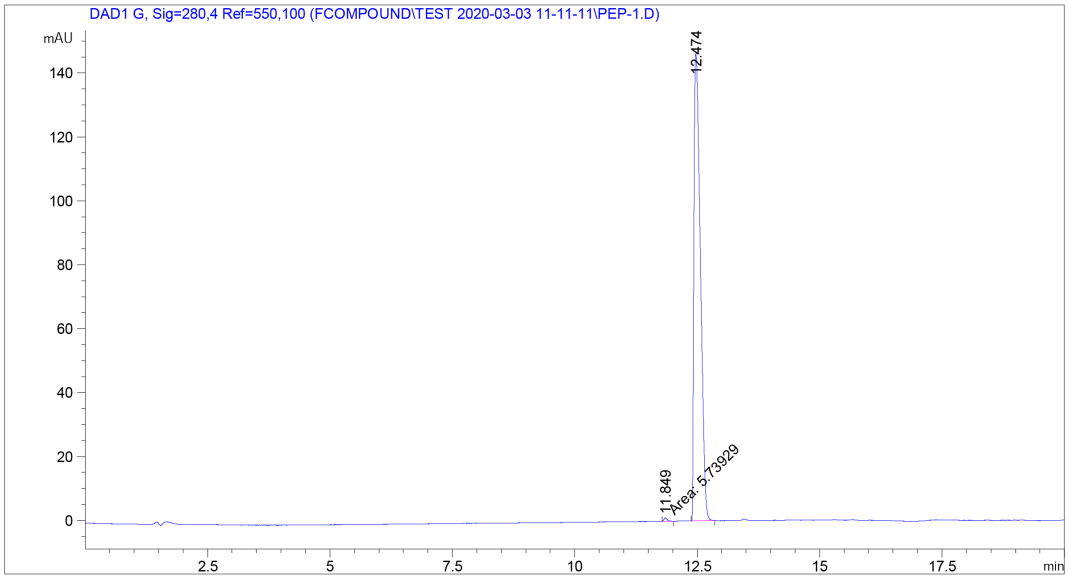
    Totals :                  2254.90643  248.10755


    ======================================================================
                              *** End of Report ***

1260lc 3/26/2020 9:47:33 AM SYSTEM                              Page   1 of 1
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\PEP-1.D
Sample Name: PEP-1

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   8
    Acq. Instrument : 1260lc                         Location : Vial 68
    Injection Date  : 3/3/2020 1:45:27 PM                 Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 6.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-03 11-11-11\TEST12.M (Sequence
                      Method)
    Last changed    : 3/3/2020 11:11:11 AM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 G, Sig=280,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  11.849 MM    0.0803    5.73929    1.19086   0.4282
       2  12.474 BB    0.1475 1334.58997  146.18858  99.5718

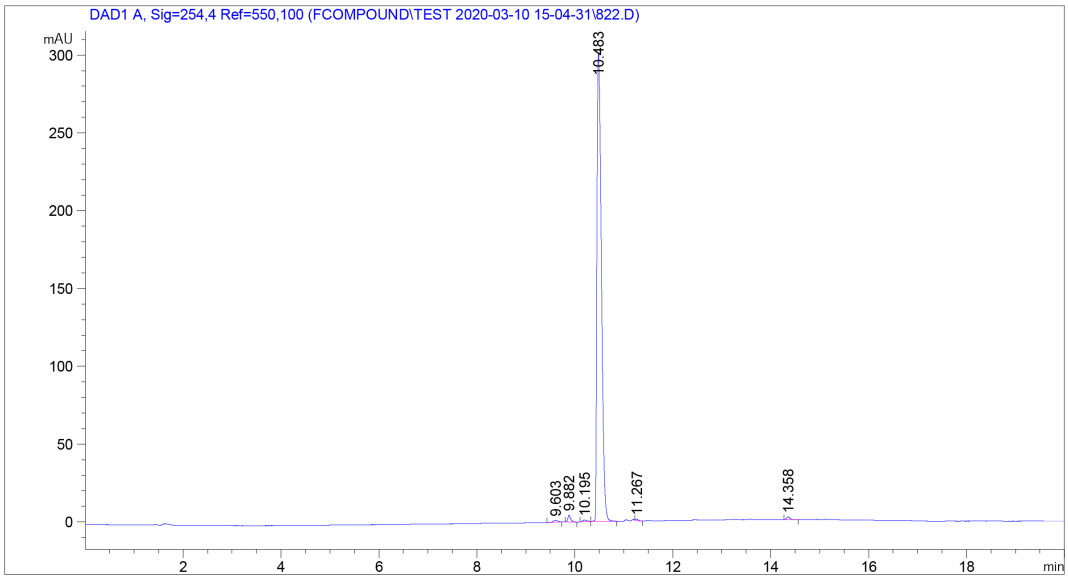
    Totals :                  1340.32926  147.37944


    ======================================================================
                              *** End of Report ***
```

MDL-822

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-10 15-04-31\822.D
Sample Name: 822

```
    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   8
    Acq. Instrument : 1260lc                         Location : Vial 8
    Injection Date  : 3/10/2020 5:38:57 PM                Inj :   1
                                                   Inj Volume : 2.000 µl
    Different Inj Volume from Sample Entry!  Actual Inj Volume : 8.000 µl
    Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-10 15-04-31\TEST12.M (Sequence
                      Method)
    Last changed    : 3/10/2020 3:04:58 PM by SYSTEM
    Additional Info : Peak(s) manually integrated
```




```
    ======================================================================
                              Area Percent Report
    ======================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs

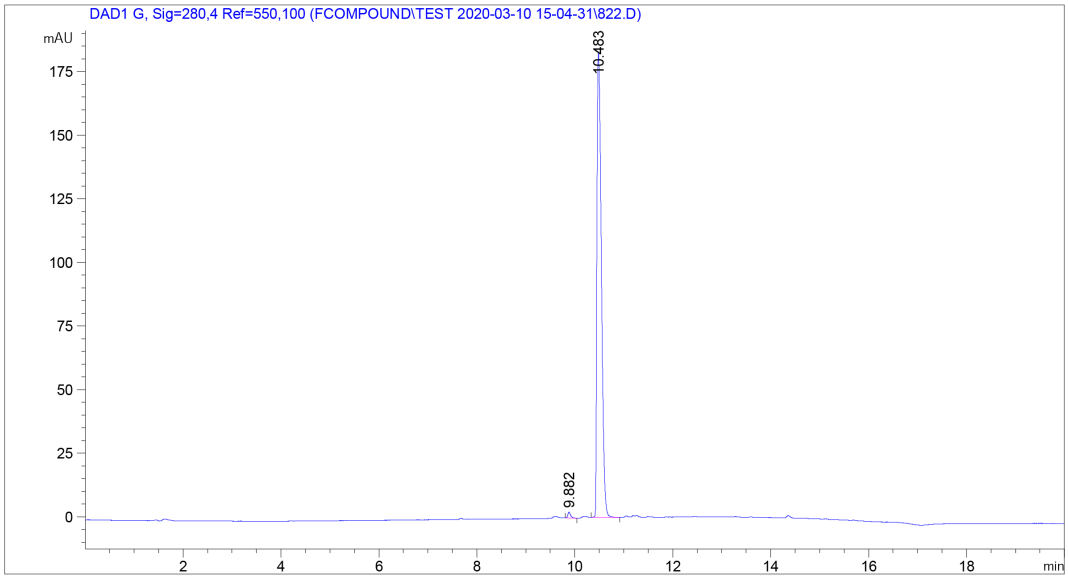

    Signal 1: DAD1 A, Sig=254,4 Ref=550,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   9.603 BB    0.0885    7.52870    1.13340   0.3884
       2   9.882 BB    0.0581   16.56597    4.28472   0.8546
       3  10.195 BB    0.0912    6.59114    1.02264   0.3400
       4  10.483 BB    0.1027 1895.50354  299.96573  97.7795
       5  11.267 VB    0.0595    5.00499    1.19285   0.2582
       6  14.358 BB    0.0687    7.35488    1.64479   0.3794

    Totals :                  1938.54922  309.24412
```

Data File C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-10 15-04-31\822.D
Sample Name: 822

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   8
Acq. Instrument : 1260lc                         Location : Vial 8
Injection Date  : 3/10/2020 5:38:57 PM               Inj :   1
                                                Inj Volume : 2.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 8.000 µl
Method          : C:\CHEM32\1\DATA\FCOMPOUND\TEST 2020-03-10 15-04-31\TEST12.M (Sequence
                  Method)
Last changed    : 3/10/2020 3:04:58 PM by SYSTEM
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 G, Sig=280,4 Ref=550,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   9.882 BB    0.0576    9.00800    2.30527   0.7752
   2  10.483 BB    0.1023 1152.96228  182.31613  99.2248

Totals :                  1161.97028  184.62141


==========================================================================
                          *** End of Report ***
```

Supplementary Tables

Table S1. Plasma pharmacokinetic parameters of MDL-800 and MDL-811 in C57BL/6J mice. *

Compound	MDL-800	MDL-811
Administration	IP 30 mg/mL	IP 30 mg/mL
t_{max} (h)	0.50	0.50
C_{max} (ng/mL)	6610.46	5330.67
AUC_{0-t} (h × ng/mL)	17831.51	19580.01
$AUC_{0-\infty}$ (h × ng/mL)	17883.09	19669.85
$t_{1/2}$ (h)	2.07	1.38
MRT (h)	2.47	2.63
F (%)	71.33	92.96

*The data represent the mean value from five C57BL/6J mice per group.

Table S2. Effect of MDL-811 among histone deacetylase enzymes.

Enzyme	Effect*
HDAC1	ND
HDAC2	ND
HDAC3	ND
HDAC4	ND
HDAC5	ND
HDAC6	ND
HDAC7	ND
HDAC8	ND
HDAC9	ND
HDAC10	ND
HDAC11	ND
SIRT1	ND
SIRT2	ND
SIRT3	ND
SIRT5	ND
SIRT6	Activation
SIRT7	ND

*ND represents no detectable activation or inhibition effect on the targets in the presence of MDL-811 at 100 μM. The data are from three independent experiments.

Table S3. Melting temperatures of SIRT6 treated with or without MDL-811 in the CETSA.

	Melting Temperature (T_m) (°C) *
DMSO	45.8 ± 0.3
10 μM MDL-811	47.6 ± 0.1

*The data represent the mean ± s.e.m. from four independent experiments.

Table S4. IC_{50} values of MDL-811 in CRC cell lines.

CRC cell line	IC_{50} ± s.d. (μM) *
NCI-H716	61.0 ± 2.3
Colo205	38.1 ± 4.4
Colo320DM	33.2 ± 0.9
LS1034	31.9 ± 0.7
Caco2	28.7 ± 1.7
LS513	28.6 ± 0.6
LS174T	23.8 ± 2.0
HCT15	20.5 ± 1.3
Colo201	20.3 ± 1.4
HCT8	20.1 ± 2.0
LS123	19.8 ± 0.5
LS180	19.1 ± 0.4
DLD-1	18.0 ± 0.1
SW620	16.7 ± 1.4
SW1417	15.1 ± 1.9
SW1463	13.0 ± 1.5
T84	13.0 ± 1.4
RKO	12.3 ± 1.8
SKCO1	12.1 ± 0.1

SW48	10.8 ± 1.5
NCI-H508	9.7 ± 1.2
SW480	9.3 ± 1.7
LOVO	7.8 ± 0.2
HT29	7.0 ± 1.1
SW1116	5.7 ± 0.3
HCT116	4.7 ± 0.2

*The $IC_{50 \pm}$ s.d. values of MDL-811 to the indicated CRC cell lines were determined by CCK8 assays. The value of each MDL-811 treatment group was calculated as a percentage change of the DMSO controls, which represents the proliferation of the CRC cells. The data represent the mean ± s.d. from two or three independent experiments.

Table S5. Statistical data of cell cycle phase distribution in CRC cell lines treated with MDL-811 for 48 h. [a]

Cell name	Phase	0 μM	5 μM	10 μM
HCT116	G_0/G_1	49.5 ± 3.5	63.2 ± 2.1**	66.9 ± 1.4***
	S	30.1 ± 4.4	19.0 ± 2.3*	19.0 ± 4.3*
	G_2/M	20.4 ± 4.5	17.9 ± 4.0	14.1 ± 3.8
HT29	G_0/G_1	40.5 ± 2.6	54.5 ± 4.1*	59.9 ± 2.7***
	S	36.9 ± 4.6	25.3 ± 4.5	19.6 ± 2.3**
	G_2/M	22.6 ± 6.7	20.2 ± 4.2	20.5 ± 3.7
SW480	G_0/G_1	47.7 ± 1.3	62.9 ± 6.0*	70.0 ± 3.8***
	S	38.3 ± 3.6	26.3 ± 5.1	18.1 ± 4.9***
	G_2/M	13.9 ± 1.8	10.8 ± 4.4	11.9 ± 6.4

[a]The data are shown as mean ± s.d. from three independent experiments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, two-way ANOVA analysis).

Table S6. Primary antibodies used for western blots.

Antibody name	Company name	Catalog number	Dilution
SIRT6	Cell Signaling Technology	12486	1:2000
H3K9Ac	Abcam	ab32129	1:2000
H3K18Ac	Abcam	ab1191	1:2000
H3K56Ac	Active Motif	39281	1:2000
Histone H3	Abcam	ab10799	1:2000
β-actin	Proteintech	HRP-60008	1:5000
His-Tag	Proteintech	HRP-66005	1:10000
Flag-Tag	Sigma-Aldrich	F1804	1:1000

Table S7. Primer sequences used for RT-qPCR.

Gene name	Forward primer (5' to 3')	Reverse primer (5' to 3')
ACTB	TGACTGACTACCTCATGAAGATCC	CCATCTCTTGCTCGAAGTCCAG
LDHA	AGGAGAAACACGCCTTGATTTAG	ACGAGCAGAGTCCAGATTACAA
GLUT1	TGGACCCATGTCTGGTTGTA	ATGGAGCCCAGCAGCAA
PDK1	GGAGGTCTCAACACGAGGTC	GTTCATGTCACGCTGGGTAA
PKM2	ATGTCGAAGCCCCATAGTGAA	TGGGTGGTGAATCAATGTCCA
PCNA	AGGGCTCCATCCTCAAGAAGG	TGGTGCTTCAAATACTAGCGC
CDC2	CAGTCTTCAGGATGTGCTTATGC	GAGGTTTTAAGTCTCTGTGAAGAACTC
CCNA2	GAAGACGAGACGGGTTGCA	AGGAGGAACGGTGACATGCT
CDC25C	GAACAGGCCAAGACTGAAGC	GCCCCTGGTTAGAATCTTCC
c-MYC	GGCTCCTGGCAAAAGGTCAGAGT	CTGCGTAGTTGTGCTGATGTGT
AKT1	CACAAACGAGGGGAGTACATC	GCCATCATTCTTGAGGAGGAAGT
AKT2	TCCAGAACACCAGGCACCC	ATTGTCCTCCAGCACCTCA
MTOR	AGTGGACCAGTGGAAACAGG	TTCAGCGATGTCTTGTGAGG
CYP24A1	CCGTAATCCCCAAGTGCAAC	CCCAGAACTGTTGCCTTGTC
SIRT6	TACGCGGACAAGGGCAAG	ACTTGGGGGCCAGACCTCGC

Table S8. Primary antibodies used for ChIP assays.

Antibody name	Company name	Catalog number	Dilution
SIRT6	Abcam	ab62739	1:200
H3K9Ac	Abcam	ab32129	1:100
H3K18Ac	Abcam	ab1191	1:100
H3K56Ac	Active Motif	39281	1:100
HA-Tag	Cell Signaling Technology	#3724	1:50

Table S9. Primer sequences used for ChIP-qPCR.

Primer name	Forward primer (5' to 3')	Reverse primer (5' to 3')
H3K9Ac_ 1	CGCTGTCCCACCTGATCACA	AGGACATGACCGCTTTCTTCAA
H3K9Ac_ 2	CTCCCTTTCTCTTTTCCTTTACTCC	ACGCATAACCCCTGTACCCT
H3K9Ac_ 3	CAGCTATCCTGAGGTGTGCC	AGGAGCTGTGTCCAGAATTGG
H3K9Ac_ 4	TGGGTTCAGGGATTTTGAGGT	TTGCTGGTGATGGGGTGTTC
H3K9Ac_ 5	CTAGCGGTAAAAGGGGGCAT	TGAAGCCCACACCAATGAGT
H3K9Ac_ 6	TCTCCATGTTCCTATGCCCAG	TCGCTCACCTCGCTGACT
H3K9Ac_ 7	CGTAAAGCGGCAACAACGAA	GTAGAAGAACAGAGGCGGGC
H3K9Ac_ 8	CCGGATTGCAGAGGAAAGCA	TCCTGGAAGCGGGATCAAAA
H3K18Ac_ 1	CGCTGTCCCACCTGATCACA	AGGACATGACCGCTTTCTTCAA
H3K18Ac_ 2	TTCAGGTCTTGCTACATCGC	CAGGACGAGGTCATTAGGGG
H3K18Ac_ 3	GTCATGTGAGCCCTGGAAGC	GCACGGAGTCAAAGGGAGTT
H3K18Ac_ 4	CATACTTCTTGTGGTACTCCACCT	TGGTGCGCTTCGGCGT
H3K18Ac_ 5	AATGCACGTAAAGCGGCAAC	TCAAGCATCGTTGGTGCAAG
H3K18Ac_ 6	TCTAGGCTGGGCCCTAAATAGT	CTGCCCCACACATACTGACAT
H3K18Ac_ 7	CAAGGGACCACCCATGACAA	GCAGAGAAACCAGCCCTTGA
H3K56Ac_ 1	CAGCTATCCTGAGGTGTGCC	AGGAGCTGTGTCCAGAATTGG
H3K56Ac_ 2	TTTGACTCCGTGCTGGCTAA	GGCATGAAATGACGTGGTGT
H3K56Ac_ 3	CATACTTCTTGTGGTACTCCACCT	TGGTGCGCTTCGGCGT
H3K56Ac_ 4	CTCTCCATGTTCCTATGCCCAG	CTCGCTGACTCCATCCTCCTTC
H3K56Ac_ 5	CGTAAAGCGGCAACAACGAA	GTAGAAGAACAGAGGCGGGC

H3K56Ac_ 6	CAAGGGACCACCCATGACAA	GCAGAGAAACCAGCCCTTGA