Supporting Information

New coumarin-piperazine derivatives alleviates inflammatory bowel diseases as IL-6 inhibitors by targeting NF-κB pathway

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1. Experimental procedures

1.1 General information

All reagents and solvents are commercially available chemically pure, and no purification treatment was required before use. NMR spectra were recorded on a Bruker AV 400 spectrometer (Bruker, Germany). HRMS data was obtained on Agilent 1290 Infinity III-6546 LC/Q-TOF (Agilent, USA). The purity of compounds was no less than 93% by HPLC analysis. Dulbecco's modified Eagle medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from VivaCell (China). LPS (derived from Escherichia coli) and MTT were purchased from Biotopped Technology Co., Ltd. Murine macrophage RAW264.7 cells were purchased from Nanjing SenBeiJia Biological Technology Co., Ltd (Nanjing, China). The NO Assay kit was purchased from Beyotime Biotechnology Co., Ltd. Male C57BL/6J mice were purchased from SPF Biotechnology Co., Ltd (Beijing, China). All animal experiments were carried out according to institutional ethical guidelines on animal care approved by the Ethics Committee of Yunnan University of Chinese Medicine (YNUTCM-XMSS-G-20260081).

*1.2 Synthesis for compound **1***

To a stirred solution of 4-hydroxycoumarin (3 g, 18.5 mmol) and Bu_4NBr (TBAB, 8.95 g, 27.8 mmol) in anhydrous toluene (40 mL), P_2O_5 (5.25 g, 37 mmol) was added carefully. Then the mixture was heated and reacted at 100 °C for 24 h under nitrogen atmosphere. After cooling to room temperature, the mixture was slowly poured into the ice water (150 mL) and performed the extraction operation. The aqueous layer was extracted with dichloromethane (DCM, 30 mL×3). The collected organic layer (toluene and DCM) was dried by anhydrous sodium sulfate, concentrated *in vacuo* and purified by column chromatography with DCM to afford pale yellow solid 3.47 g (83% yiled).

*1.3 Synthesis for compound **2***

To a stirred solution of **1** (1.5 g, 6.67 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 1.72 g, 13.3 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF, 5 mL), 1-(2-*N*-Boc-aminoethyl)piperazine (1.84 g, 8 mmol) was added and then the mixture reacted at 85 °C for 5 h. When the reaction completed as detected by TLC, the mixture was poured into the cold water (50 mL) and extracted with DCM (15 mL×3). The organic layer was dried by anhydrous sodium sulfate, concentrated *in vacuo* and purified by column chromatography with eluent ($DCM/CH_3OH/Et_3N$ 100:1:1) to afford yellow solid 1.84 g (74% yiled). ^1H NMR (400 Hz, $CDCl_3$) δ: 7.57 (dd, J =1.84 Hz, 1.92 Hz, 1H), 7.43-7.47(m, 1H), 7.27-7.30(m, 1H), 7.18-7.22(m, 1H), 7.67(t, J =2.72 Hz, 1H), 4.98(s, 1H), 3.23-3.27(m, 6H), 2.66-2.68(m, 4H), 2.53-2.56(m, 2H), 1.41(s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.53, 161.16, 156.03, 154.23, 131.56, 124.86, 123.49, 117.82, 116.32, 79.37, 57.27, 52.44, 51.01, 37.15, 28.48.

*1.4 Synthesis for key intermediate **3***

To a stirred solution of **2** (1.84 g, 4.9 mmol) in DCM (5 mL), trifluoroacetic acid (TFA, 5 mL) was added and the mixture reacted at rt for 3 h. When the reaction completed as detected by TLC,

the mixture was concentrated *in vacuo* no more than 30 °C to give crude product, which was used directly without further purification.

*1.5 General procedure for the preparation of compounds **4a-4t***

To a stirred solution of **3** (100 mg) and Et_3N (100 mg) in DCM (5 mL), sulfonyl chloride (1.2 eq) was added and the mixture reacted in an ice water bath for 0.5-1 h. When the reaction completed (TLC), diluted sodium carbonate aqueous solution (10 mL) was added and stirred vigorously for 10 min. Then the mixture was extracted with DCM (5 mL×3), and the organic layer was dried by anhydrous Na_2SO_4, concentrated *in vacuo* and purified by column chromatography with eluent (DCM/CH_3OH/Et_3N 100:0.5:0.5) to give title compounds.

Compound **4a**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 7.89-7.94(m, 1H), 7.58-7.61(m, 1H), 7.56(d, *J* =7.72 Hz, 1H), 7.46-7.53(m, 1H), 7.26-7.33(m, 2H), 7.19-7.23(m, 2H), 5.71(s, 1H), 5.55(s, 1H), 3.23(t, *J* =9.72 Hz, 4H), 3.11(q, *J* =5.31 Hz, 2H), 2.55-7.58(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.50, 161.04, 160.15, 157.81, 154.31, 135.22, 135.13, 131.67, 130.70, 127.86, 127.72, 124.80, 124.75, 124.71, 123.55, 117.93, 117.00, 116.79, 116.30, 55.88, 53.55, 52.16, 50.90, 39.54.

Compound **4b**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 8.26(t, *J* =4.62 Hz, 1H), 7.87-7.91(m, 1H), 7.72-7.74(m, 2H), 7.46-7.54(m, 2H), 7.33(d, *J* =8.28 Hz, 1H), 7.21(t, *J* =7.66 Hz, 1H), 5.70(s, 1H), 5.50(s, 1H), 3.19(s, 4H), 3.07-3.11(m, 2H), 2.53-2.56(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.52, 160.99, 154.35, 138.41, 132.94, 132.53, 132.01, 131.70, 128.82, 128.75, 128.69, 128.63, 124.79, 123.56, 118.00, 116.33, 55.93, 52.16, 50.86, 39.56. HRMS-ESI calcd for $C_{22}H_{23}F_3N_3O_4S$ $[M + H]^+$ 482.1361, found 482.1390.

Compound **4c**: ^{1}H NMR(400 Hz, DMSO-d_6) *δ*: 10.31(s, 1H), 7.67-7.79(m, 4H), 7.65(d, *J* =1.56 Hz, 1H), 7.57-7.61(m, 1H), 7.44(t, *J* =5.98 Hz, 1H), 7.30-7.38(m, 2H), 5.66(s, 1H), 3.17(s, 4H), 2.90(q, *J* =6.52 Hz, 2H), 2.53(s, 4H), 2.43(d, *J* =6.84 Hz, 2H), 2.08(s, 3H); ^{13}C NMR(100 MHz, DMSO-d_6) *δ*:169.44, 161.51, 160.96, 154.05, 143.17, 134.71, 132.23, 128.15, 125.73, 124.19, 119.10, 117.68, 116.16, 57.03, 55.37, 52.46, 50.92, 24.57.

Compound **4d**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.82(d, *J* =8.96 Hz, 2H), 7.47-7.55(m, 2H), 7.32(d, *J* =8.28 Hz, 1H), 7.22(t, *J* =7.64 Hz, 1H), 6.99(d, *J* =8.76 Hz, 2H), 5.70(s, 1H), 5.12(t, *J* =5.50 Hz, 1H), 3.87(s, 3H), 3.19-3.21(m, 4H), 3.03-3.07(m, 2H), 2.53-2.56(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 163.12, 162.53, 161.04, 154.36, 131.72, 131.34, 129.38, 124.80, 123.58, 118.01, 116.33, 114.41, 56.05, 55.80, 52.19, 50.96, 39.35. HRMS-ESI calcd for $C_{22}H_{26}N_3O_5S$ $[M + H]^+$ 444.1593, found 444.1599.

Compound **4e**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.83(d, *J* =8.60 Hz, 2H), 7.47-7.55(m, 4H), 7.33(d, *J* =8.36 Hz, 1H), 7.20-7.26(m, 1H), 5.71(s, 1H), 5.32(s, 1H), 3.21(t, *J* =4.62 Hz, 4H), 3.08(t, *J* =6.62 Hz, 2H), 2.55-2.59(m, 6H). ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.53, 161.03, 139.39, 131.74, 129.57, 128.67, 124.78, 123.61, 117.99, 116.30, 56.16, 52.24, 50.91, 39.42.

Compound **4f**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.91-7.97(m, 1H), 7.55(dd, *J* =1.64 Hz, 1.17 Hz, 1H), 7.47-7.51(m, 1H), 7.32-7.34(m, 1H), 7.20-7.25(m, 1H), 6.94-7.05(m, 2H), 5.72(s, 1H), 5.54(s, 1H), 3.24-3.26(m, 4H), 3.12(t, *J* =6.48 Hz, 2H), 2.58-2.63(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.51,

161.02, 154.35, 132.49, 132.39, 131.73, 124.78, 124.47, 124.43, 124.29,123.59, 118.00, 116.32, 112.27, 112.24, 112.06, 112.02, 105.86, 105.60, 105.35, 56.03, 52.25, 50.92, 39.49.

Compound **4g**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 8.06(d, J =8.48 Hz, 1H), 7.54-7.57(m, 2H), 7.48-7.52(m, 1H), 7.48-7.52(m, 1H), 7.43(dd, J =2.00 Hz, 2.00 Hz, 1H), 7.33-7.35(m, 1H), 5.73(s, 1H), 3.24-3.26(m, 4H), 3.03-3.07(m, 2H), 2.55-2.62(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) δ:162.49, 161.01, 154.39, 139.84, 135.69, 132.65, 132.42, 131.75, 131.47, 127.82, 124.78, 123.59, 118.05, 116.34, 55.95, 52.29, 51.00, 39.57. HRMS-ESI calcd for $C_{21}H_{22}Cl_2N_3O_4S$ $[M + H]^+$ 482.0708, found 482.0709.

Compound **4h**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 7.63-7.66(m, 1H), 7.54-7.57(m, 1H), 7.47-7.52(m, 1H), 7.27-7.34(m, 1H), 7.18-7.25(m, 3H), 5.73(s, 1H), 5.58(s, 1H), 3.26(t, J =4.70 Hz, 4H), 3.15(t, J =5.76 Hz, 2H), 2.58-2.62(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 162.52, 161.04, 154.36, 131.74, 124.79, 123.60, 121.79, 121.70, 121.55, 121.47, 118.50, 118.43, 118.26, 118.18, 118.02, 117.67, 117.41, 116.33, 55.99, 52.24, 50.94, 39.58.

Compound **4i**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 7.77(d, J =1.84 Hz, 2H), 7.54-7.57(m, 2H), 7.47-7.52(m, 1H), 7.32-7.34(m, 1H), 7.21-7.25(m, 1H), 5.72(s, 1H), 5.39(s, 1H), 3.24(t, J =4.82 Hz, 4H), 3.13(t, J =5.64 Hz, 2H), 2.57-2.62(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 162.52, 161.03, 154.36, 143.07, 136.27, 132.80, 131.76, 125.60, 124.77, 123.62, 118.02, 116.32, 56.15, 52.32, 50.95, 39.51.

Compound **4j**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 7.96-7.99(m, 2H), 7.53-7.56(m, 1H), 7.46-7.51(m, 1H), 7.29-7.38(m, 3H), 7.20-7.25(m, 1H), 5.71(d, J =4.96 Hz, 1H), 5.59(s, 1H), 3.19-3.22(m, 4H), 3.09-3.12(m, 2H), 2.55-2.59(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 162.56, 161.07,154.22, 152.15, 138.47, 138.45, 131.67, 129.31, 124.79, 123.56, 121.12, 117.84, 116.23, 56.16, 52.17, 50.85, 39.56, 39.53. HRMS-ESI calcd for $C_{22}H_{23}F_3N_3O_5S$ $[M + H]^+$ 498.1311, found 498.1308.

Compound **4k**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 7.58(dd, J =1.60 Hz, 1.60 Hz, 1H), 7.47-7.51(m, 1H), 7.32-7.34(m, 1H), 7.21-7.25(m, 1H), 5.72(s, 1H), 4.97(t, J =5.44 Hz, 1H), 3.27-3.31(m, 6H), 2.67-2.74(m, 6H), 2.43-2.48(m, 1H), 1.17-1.21(m, 2H), 0.98-1.03(m, 2H); ^{13}C NMR(100 MHz, $CDCl_3$) δ:162.54, 161.08, 154.35, 131.71, 124.83, 123.59, 117.99, 116.35, 57.03, 52.42, 51.03, 39.75, 29.98, 5.37.

Compound **4l**: ^{1}H NMR(400 Hz, DMSO-d_6) δ: 7.88-7.91(m, 2H), 7.65-7.67(m, 2H), 7.57-7.61(m, 1H), 7.43-7.48(m, 2H), 7.31-7.38(m, 2H), 5.68(s, 1H), 3.19(t, J =4.60 Hz, 4H), 2.91-2.96(m, 2H), 2.54(t, J =4.68 Hz, 4H), 2.43(t, J =4.6 Hz, 2H); ^{13}C NMR(100 MHz, DMSO-d_6) δ: 165.78, 163.29, 161.52, 160.95, 154.05, 137.59, 137.56, 132.22, 130.01, 129.92, 125.73, 124.19, 117.68, 116.89, 116.66, 116.16, 57.04, 52.46, 50.92.

Compound **4m**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 8.02(d, J =8.04 Hz, 2H), 7.80(d, J =8.08 Hz, 2H), 7.49(dd, J =6.44 Hz, 5.56 Hz, 1H), 7.47-7.50(m, 1H), 7.33-7.35(m, 1H), 7.20-7.25(m, 1H), 5.72(s, 1H), 5.26(s, 1H), 3.21-3.23(m, 4H), 3.12(s, 2H), 2.57-2.60(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 161.01, 154.38, 131.77, 127.73, 126.47, 124.75, 123.61, 118.05, 116.32, 56.26, 52.29, 50.97, 39.43. HRMS-ESI calcd for $C_{22}H_{23}F_3N_3O_4S$ $[M + H]^+$ 482.1361, found 482.1355.

Compound **4n**: ^{1}H NMR(400 Hz, $CDCl_3$) δ: 8.02(t, J =7.54 Hz, 1H), 7.56(dd, J =2.76 Hz, 1.60 Hz,

1H), 7.48-7.52(m, 1H), 7.33-7.35(m, 1H), 7.06-7.10(m, 1H), 5.73(s, 1H), 5.53(s, 1H), 3.26(t, *J* =4.74 Hz, 4H), 3.15(t, *J* =5.78 Hz, 2H), 2.60-2.65(m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.48, 161.01, 154.39, 132.24, 131.77, 124.77, 123.61, 118.06, 116.34, 107.04, 106.78, 106.52, 56.10, 52.30, 50.96, 39.54. HRMS-ESI calcd for $C_{21}H_{21}ClF_2N_3O_4S$ $[M + H]^+$ 484.0909, found 484.0905.

Compound **4o**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.81(d, *J* =8.60 Hz, 2H), 7.47-7.54(m, 4H), 7.33(d, *J* =8.36 Hz, 1H), 7.19-7.23(m, 1H), 5.70(s, 1H), 5.12(s, 1H), 3.19(t, *J* =4.92 Hz, 4H), 3.06-3.10(m, 2H), 2.51-2.56(m, 6H), 1.33(s, 9H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.52, 161.03, 156.78, 154.36, 136.75, 131.71, 127.10, 126.25, 124.79, 123.56, 118.01, 116.32, 56.07, 52.16, 50.98, 39.41, 35.32, 31.24. HRMS-ESI calcd for $C_{25}H_{31}N_3O_4SNa$ $[M + H]^+$ 492.1933, found 492.1926.

Compound **4p**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 8.43(d, *J* =8.84 Hz, 2H), 8.09(d, *J* =8.92 Hz, 2H), 8.03(s, 1H), 7.64-7.67(m, 1H), 7.57-7.61(m, 1H), 7.31-7.38(m, 2H), 5.67(s, 1H), 3.16(t, *J* =3.74 Hz, 4H), 3.01(s, 2H), 2.54(s, 4H), 2.43(t, *J* =6.60 Hz, 2H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 161.54, 160.94, 154.05, 149.99, 146.93, 132.23, 128.52, 125.72, 125.03, 124.19, 117.69, 116.16, 57.11, 52.43, 50.89.

Compound **4q**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.63-7.64(m, 1H), 7.59-7.61(m, 1H), 7.52-7.55(m, 1H), 7.46-7.50(m, 1H), 7.30-7.33(m, 1H), 7.20-7.24 (m, 1H), 7.09-7.12(m, 1H), 5.70(s, 1H), 5.33(s, 1H), 3.20(t, *J* =4.94 Hz, 4H), 3.17(t, *J* =5.96 Hz, 2H), 2.55-2.59 (m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.52, 161.04, 154.32, 140.73, 132.44, 132.07, 131.70, 127.60, 124.79, 123.58, 117.97, 116.31, 55.77, 52.15, 50.94, 39.66.

Compound **4r**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.57(d, *J* =8.04 Hz, 1H), 7.48(t, *J* =6.90 Hz, 1H), 7.32(d, *J* =8.28 Hz, 1H), 7.23(t, *J* =7.62 Hz, 1H), 5.71(s, 1H), 4.94(t, *J* =4.92 Hz, 1H), 3.21-3.28 (m, 6H), 3.00-3.04 (m, 2H), 2.71(t, *J* =4.84 Hz, 4H), 2.65(t, *J* =5.74 Hz, 2H), 1.80-1.90(m, 2H), 1.06(t, *J* =7.44 Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.53, 161.08, 154.31, 131.68, 124.83, 123.58, 117.93, 116.32, 57.24, 54.55, 52.42, 51.01, 39.65, 17.53, 13.10.

Compound **4s**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 8.46(s, 1H), 7.98(d, *J* =8.36 Hz, 2H), 7.84-7.79 (m, 2H), 7.61-7.68 (m, 2H), 7.46-7.51 (m, 2H), 7.33(d, *J* =8.20 Hz, 1H), 7.20(t, *J* =7.62 Hz, 1H), 5.71(s, 1H), 5.24(s, 1H), 3.08-3.16 (m, 6H), 2.49-2.55 (m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$) *δ*: 162.50, 161.01, 154.37, 132.30, 131.71, 129.68, 129.34, 129.11, 128.72, 128.11, 127.92, 124.77, 123.56, 122.36, 118.01, 116.33, 56.03, 52.17, 50.93, 39.44. HRMS-ESI calcd for $C_{25}H_{25}N_3O_4SNa$ $[M + Na]^+$ 486.1463, found 486.1458.

Compound **4t**: ^{1}H NMR(400 Hz, $CDCl_3$) *δ*: 7.76(d, *J* =8.52 Hz, 2H), 7.76(d, *J* =8.56 Hz, 2H), 7.53(t, *J* =7.62 Hz, 1H), 7.49(d, *J* =7.12 Hz, 1H), 7.34(d, *J* =8.24 Hz, 1H), 7.23(t, *J* =7.64 Hz, 1H), 5.72(s, 1H), 5.24(s, 1H), 3.22(t, *J* =4.96 Hz, 4H), 3.07-3.11 (m, 2H), 2.57-2.62 (m, 6H); 154.39, 139.01, 132.60, 131.78, 128.78, 127.90, 124.75, 123.62, 118.06, 116.59, 56.19, 52.29, 50.89, 39.36. HRMS-ESI calcd for $C_{21}H_{23}BrN_3O_4S$ $[M + H]^+$ 492.0593, found 492.0583.

1.6 In vitro anti-inflammatory activity

The RAW264.7 macrophages were seeded at a density of 1×10^5cells per well in a 96-well plate (n=5), and then the cells were treated with derivatives at 20 μmol/L or 10 μmol/L Dexamethasone (DEX). After incubation for 2 h, 1 μg/mL LPS was added, followed by further incubation for 24 h.

Subsequently, cell supernatants were collected for the determination of NO concentration.

1.7 Animal test

All animal experiments were carried out according to institutional ethical guidelines on animal care approved by the Ethics Committee of Yunnan University of Chinese Medicine (YNUTCM-XMSS-G-20260081). Male C57BL/6J mice (6 weeks old, body weight: 20±2g) were housed under a controlled temperature environment (23±2 ℃) with free access to food and water throughout the experiment. Mice were randomly assigned into five groups (n=10 per group), namely Control group, Model group, Mesalazine group (100 mg/kg), and two dose groups of compound **4s** (12.5 and 6.25 mg/kg).

1.8 Disease activity index (DAI) score

The DAI score is based on our previous study [1]. In short, the DAI score is assessed by weight change, diarrhea, and blood stool test results (Table 1).

Table 1 The Scoring System of DAI

Score	Weight loss	Stool consistency	Rectal bleeding
0	no loss or weight gain	normal	negative
1	1–5% loss	loose	Weak positive
2	5–10% loss	serious Loose stools	Positive
3	10–15% loss	mild Diarrhea	Strong positive
4	>15% loss	diarrhea	gross bleeding

1.9 Histopathological examination

The histologic damage of colonic tissues was assessed under a light microscope. The colonic specimens were fixed in 4% formalin and underwent dehydration with a series of graded ethanol solutions. Subsequently, the tissues were infiltrated with paraffin, sectioned into 5-μm-thick slices, and mounted on clean glass slides. Following sectioning, the specimens were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) as per a previously reported protocol [2]. Histological gradings were evaluated in accordance with pre-established criteria.

1.10 Real Time-PCR

Total RNA from colon tissue was extracted using the RNA extraction kit (Kunming Yungen Biotechnology Co., Ltd., China). The cDNA was synthesized with a cDNA synthesis kit (Kunming Yungen Biotechnology Co., Ltd., China). To calculate the relative expression of target genes, the 2®−ΔΔCt method was employed [3]. β-Actin was utilized as the negative control for normalizing the relative expression. The primers used in this study are as follows (Table 2).

Table 2 Oligonucleotide primers used for qualitative real-time PCR (RT-PCR)

Gene	Forward primer (5’ to 3’)	Reverse primer (5’ to 3’)
IL-6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
IL-1β	CTCAACTGTGAAATGCCACC	TGATGTGCTGCTGCGAGA
IL-10	ACTGGCATGAGGATCAGCAG	AGAAATCGATGACAGCGCCT

P65	TGCGATTCCGCTATAAATGCG	ACAAGTTCATGTGGATGAGGC
IKKB	CAGCAAGGAGAACAGAGGT	ACTGTGTACTTCTGCTGCTC
IκBα	TGACCTGGTCTCGTCCTCTGT	CGTAGGGCAACTCATCTTCC
β-Actin	TATCGGACGCCTGGTTA	TGTGCCGTTGAACTTGC

1.11 Statistical Analysis

The experimental data in this study were shown in the form of the mean ± standard error and analyzed by GraphPad. The contrast in the various groups was conducted with analysis of variance (ANOVA), which was then conducted by Tukey's post-hoc test. The significant difference in statistics was considered to be $P < 0.05$.

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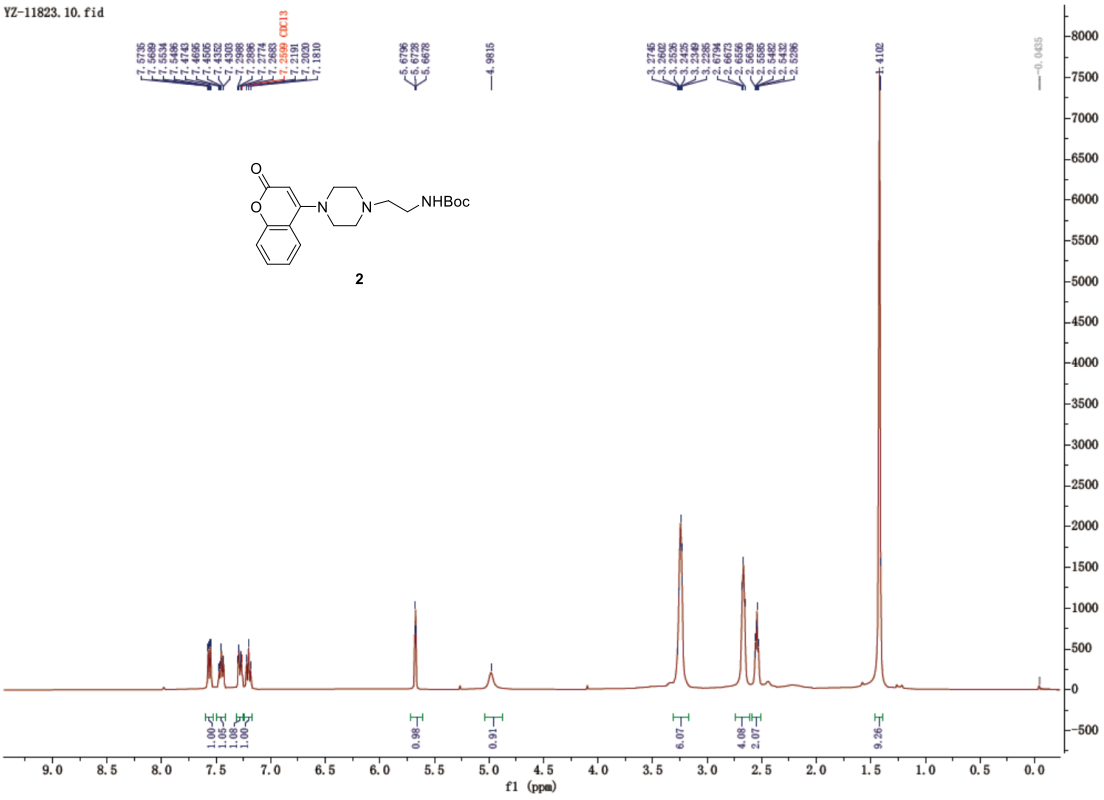
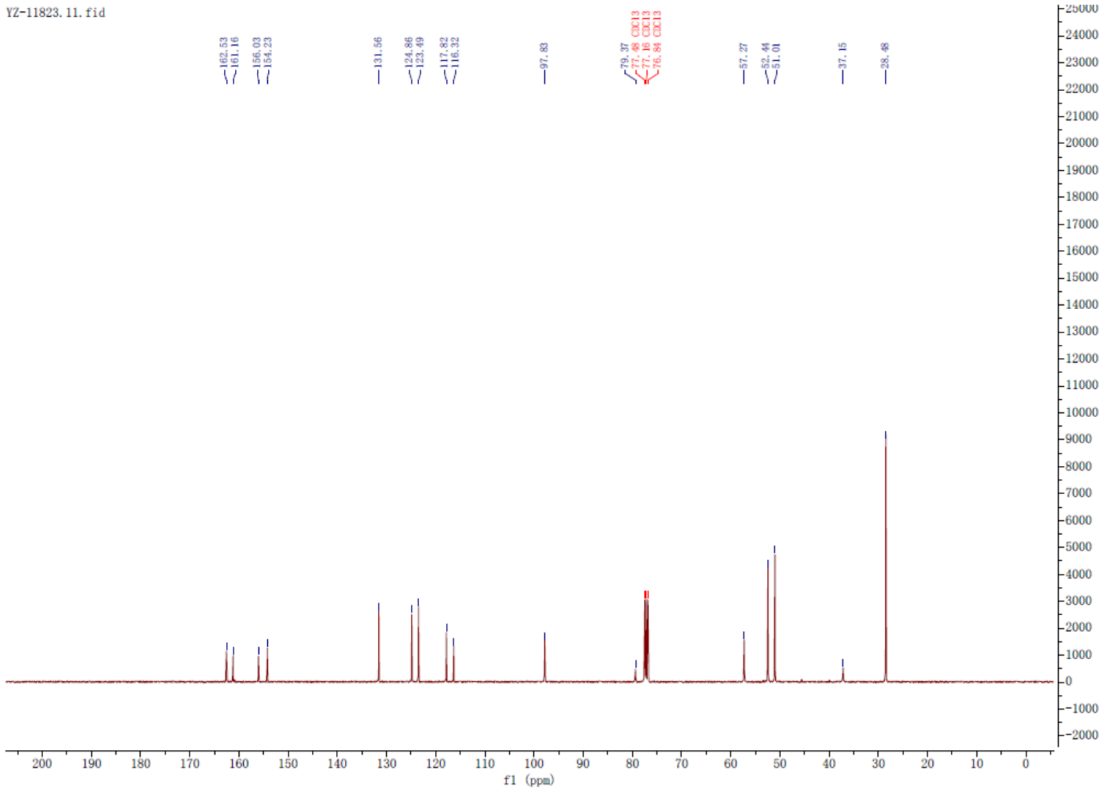
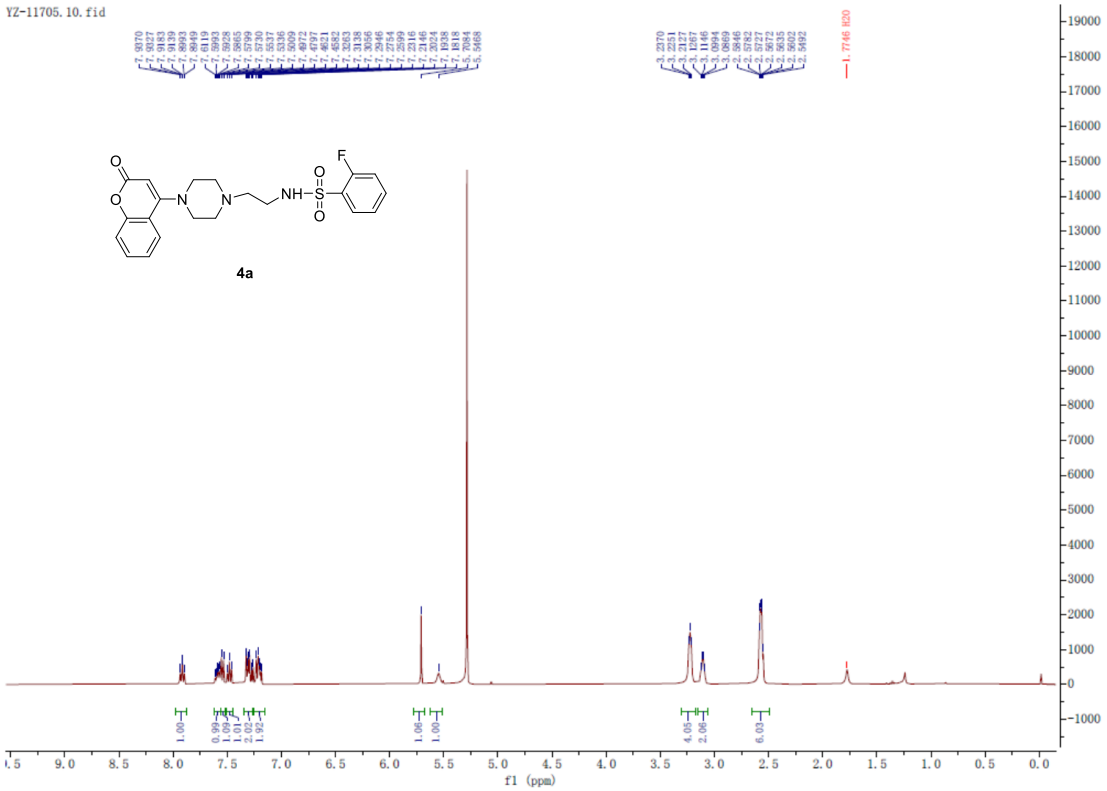
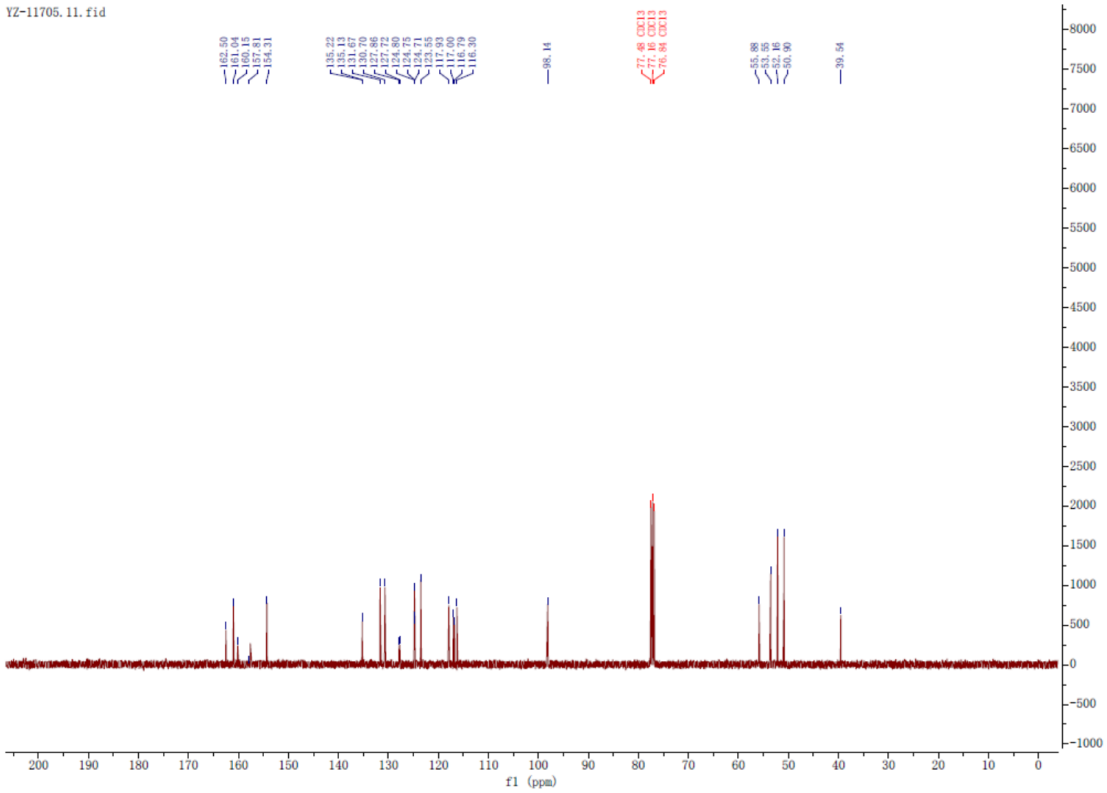
2. NMR spectra






YZ-11705.10.fid
4a
f1 (ppm)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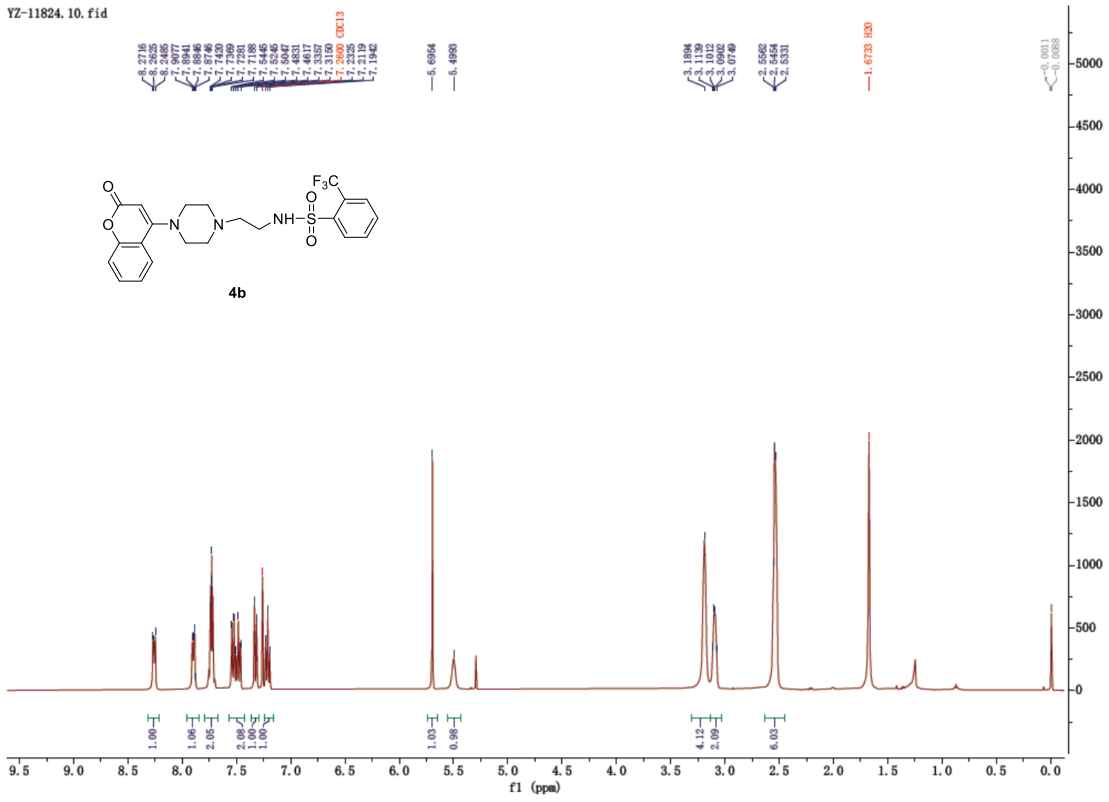
f1 (ppm)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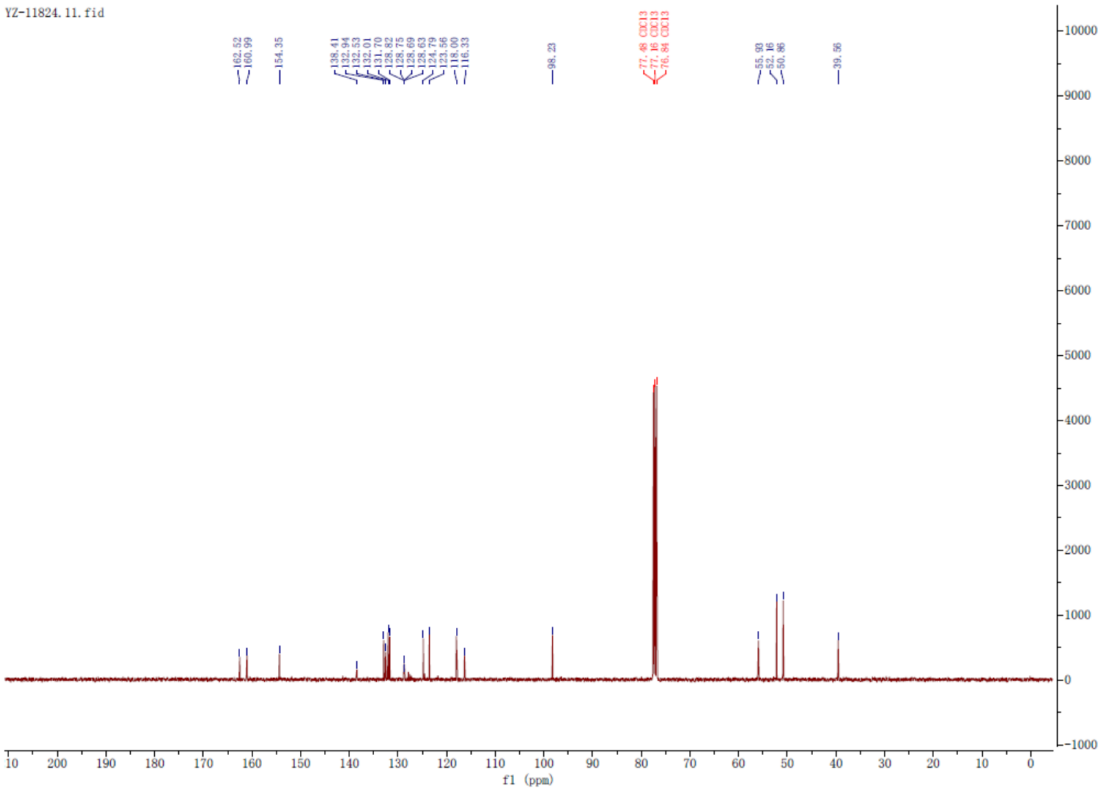
4b
f1 (ppm)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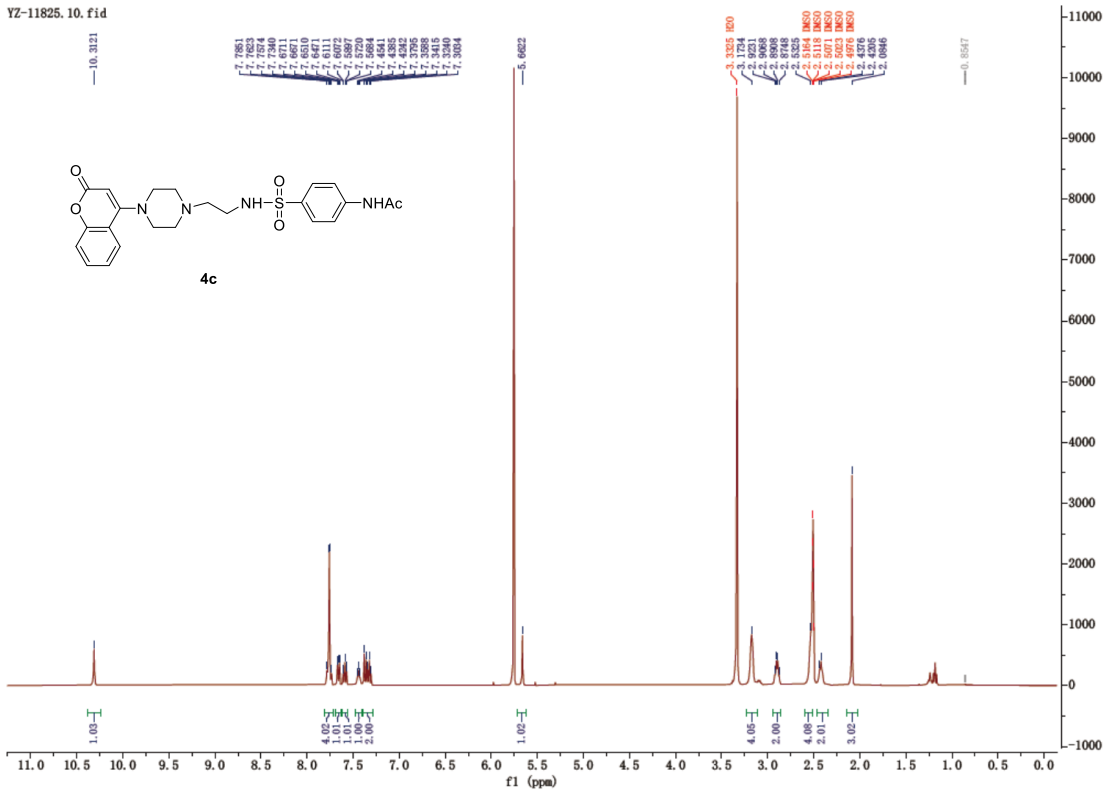
f1 (ppm)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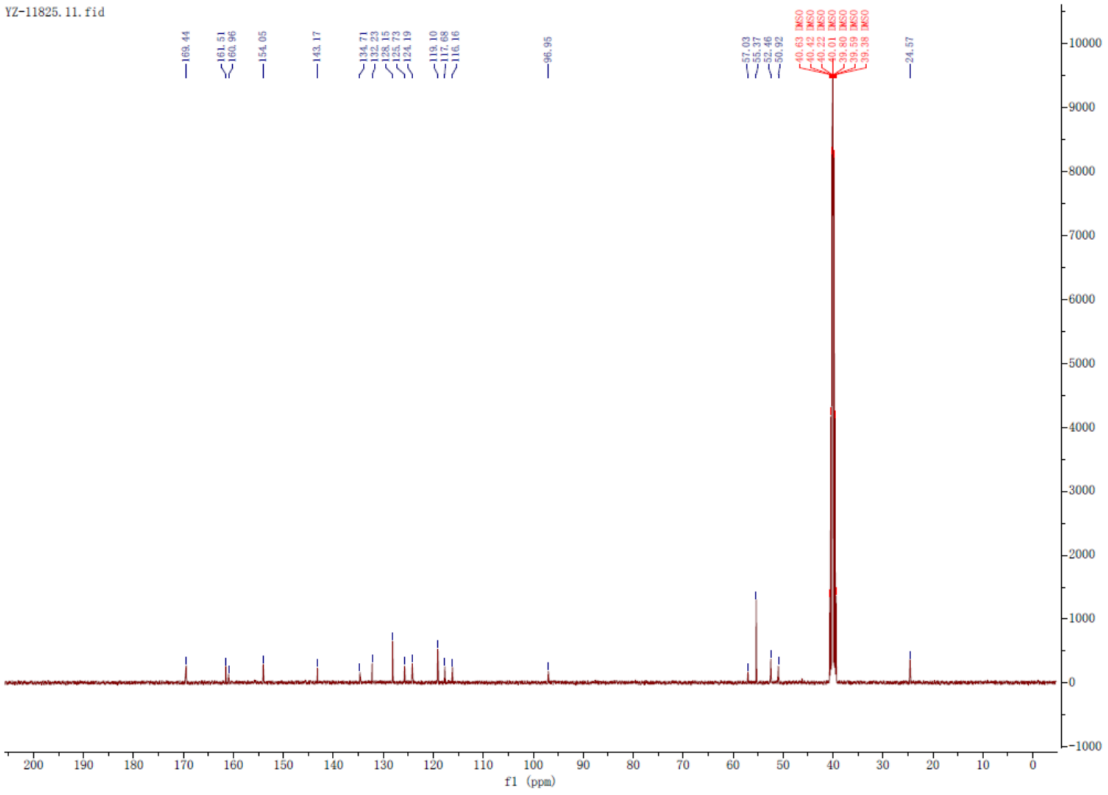
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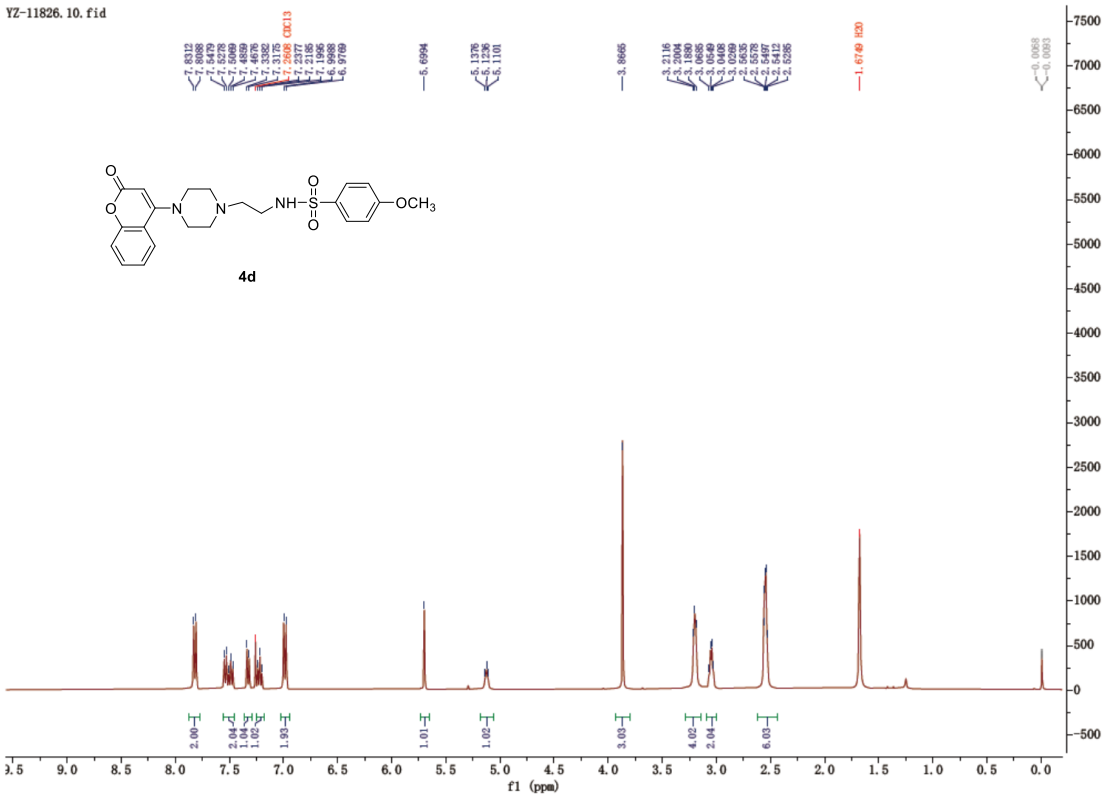
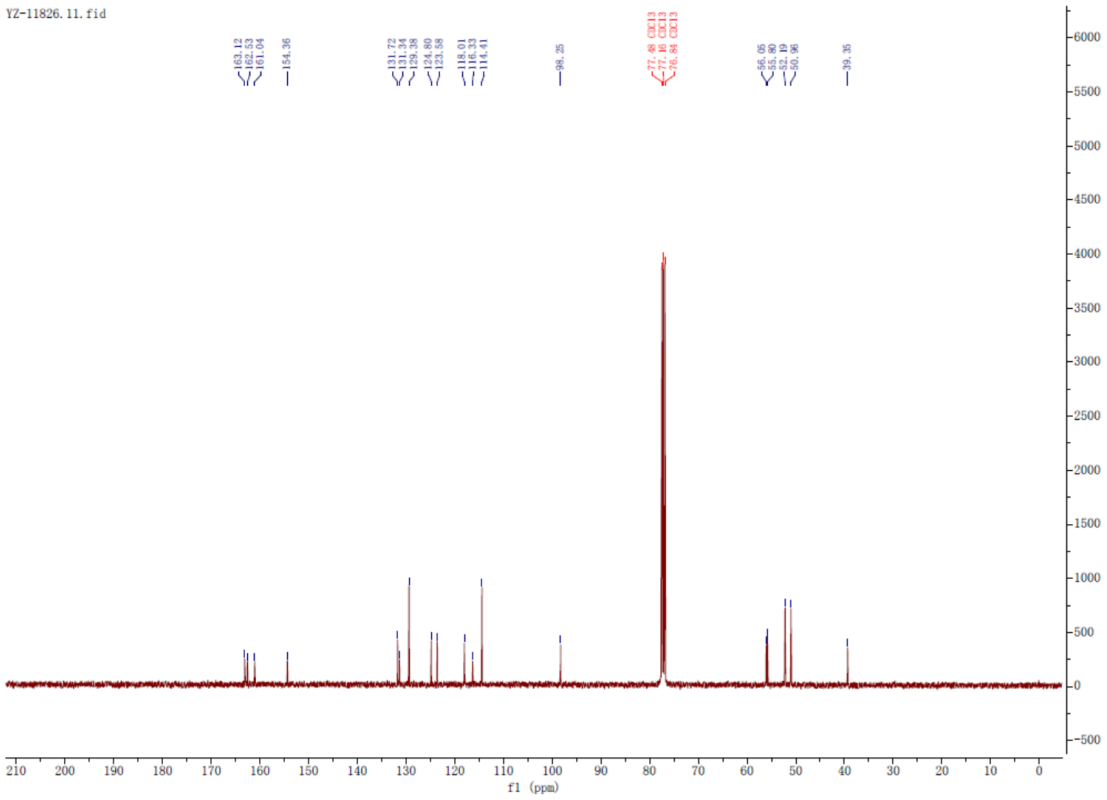
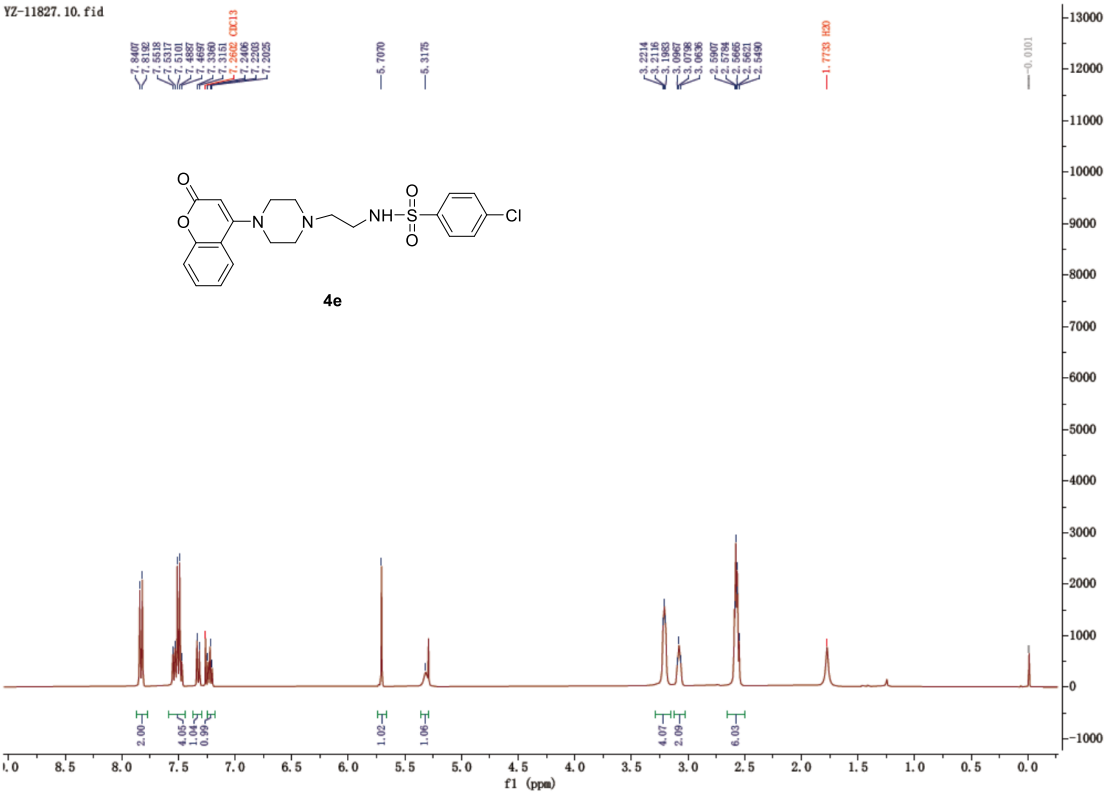
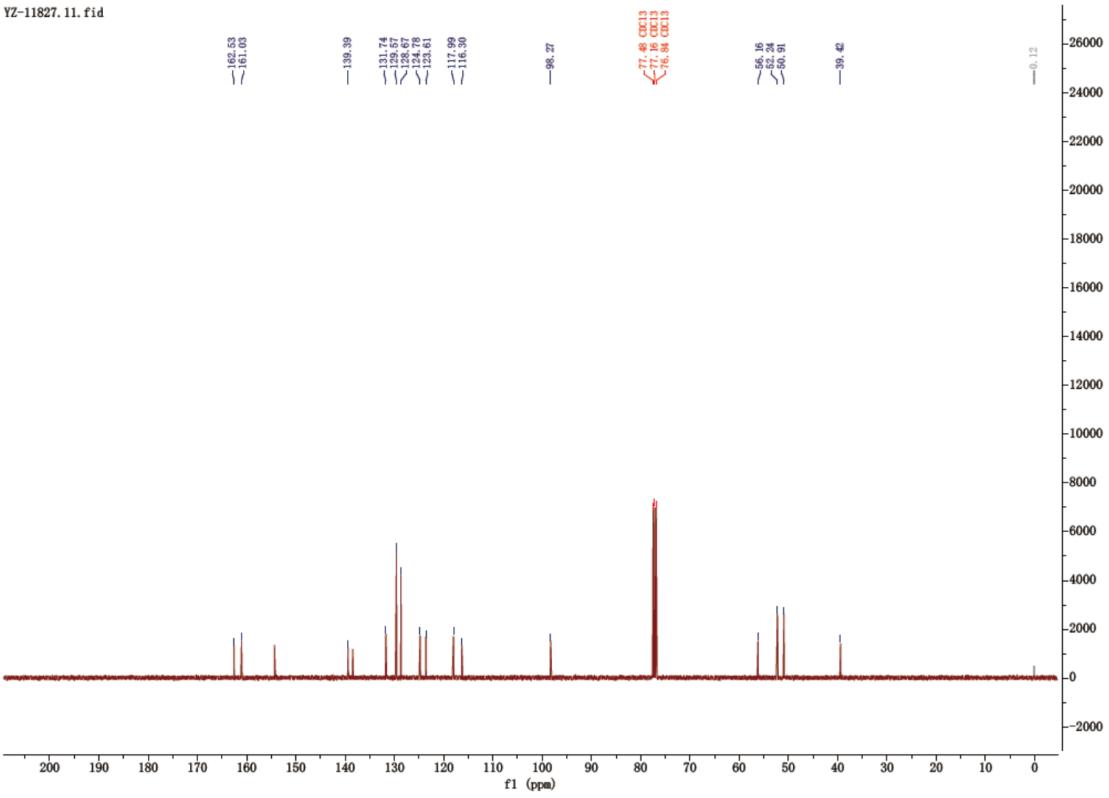
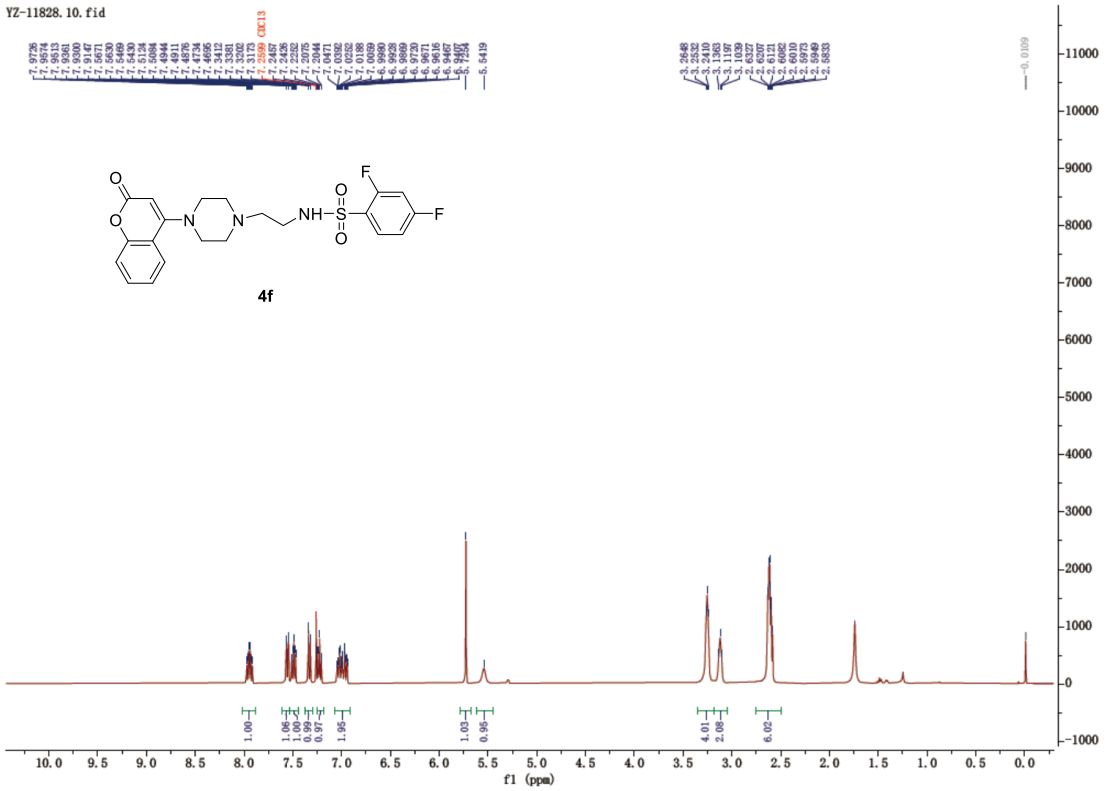
f1 (ppm)

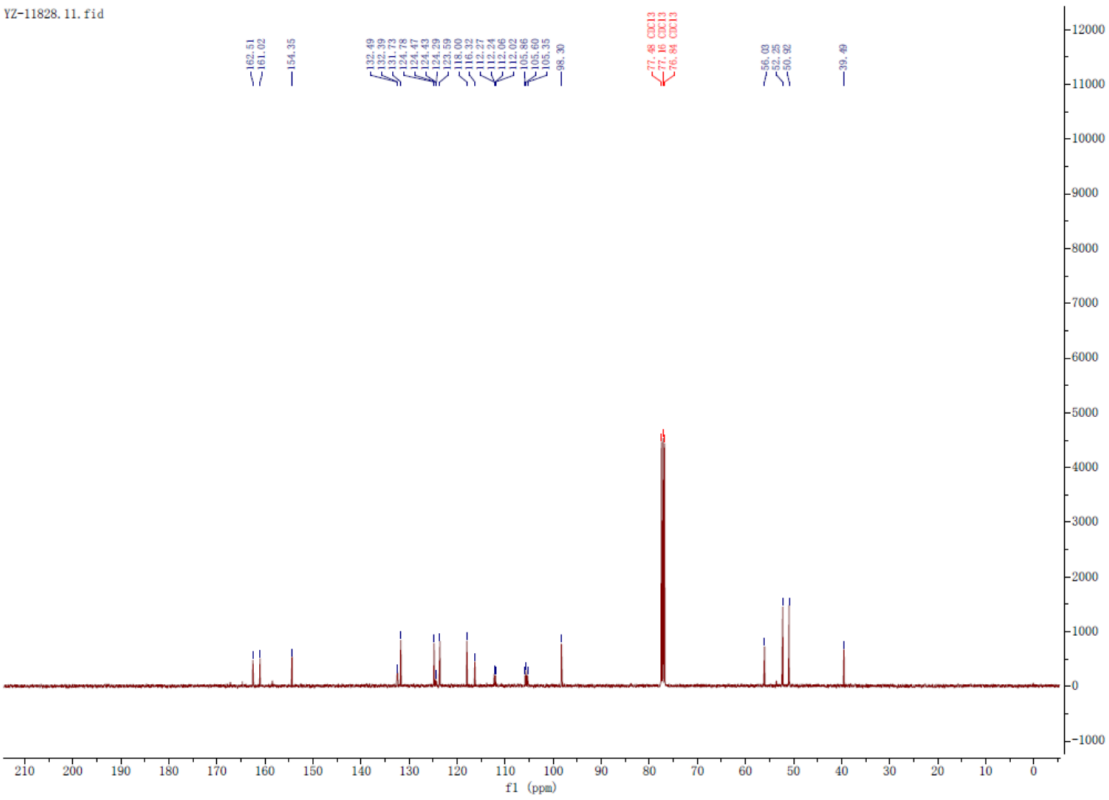

YZ-11826.10.fid
4d
f1 (ppm)


YZ-11826.11.fid
f1 (ppm)

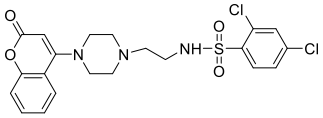

YZ-11827.10.fid
7.8407
7.8132
7.5618
7.5317
7.5101
7.4887
7.4697
7.3360
7.3151
7.2982
CDCl3
7.2406
7.2203
7.2025
5.7070
5.3175
3.2214
3.2116
3.1983
3.0967
3.0798
3.0696
2.5907
2.5784
2.5665
2.5621
2.5490
1.7733
H2O
-0.0101
O
O
N
N
NH
S
O
O
Cl
4e
2.00
4.05
1.04
0.99
1.02
1.06
4.07
2.09
6.03
f1 (ppm)


YZ-11827.11.fid
162.53
161.03
139.39
131.74
129.67
128.67
124.78
123.61
117.99
116.30
98.27
77.48
CDCl3
77.16
CDCl3
76.84
CDCl3
66.16
52.34
50.91
39.42
0.12
f1 (ppm)


YZ-11828.10.fid
4f
f1 (ppm)

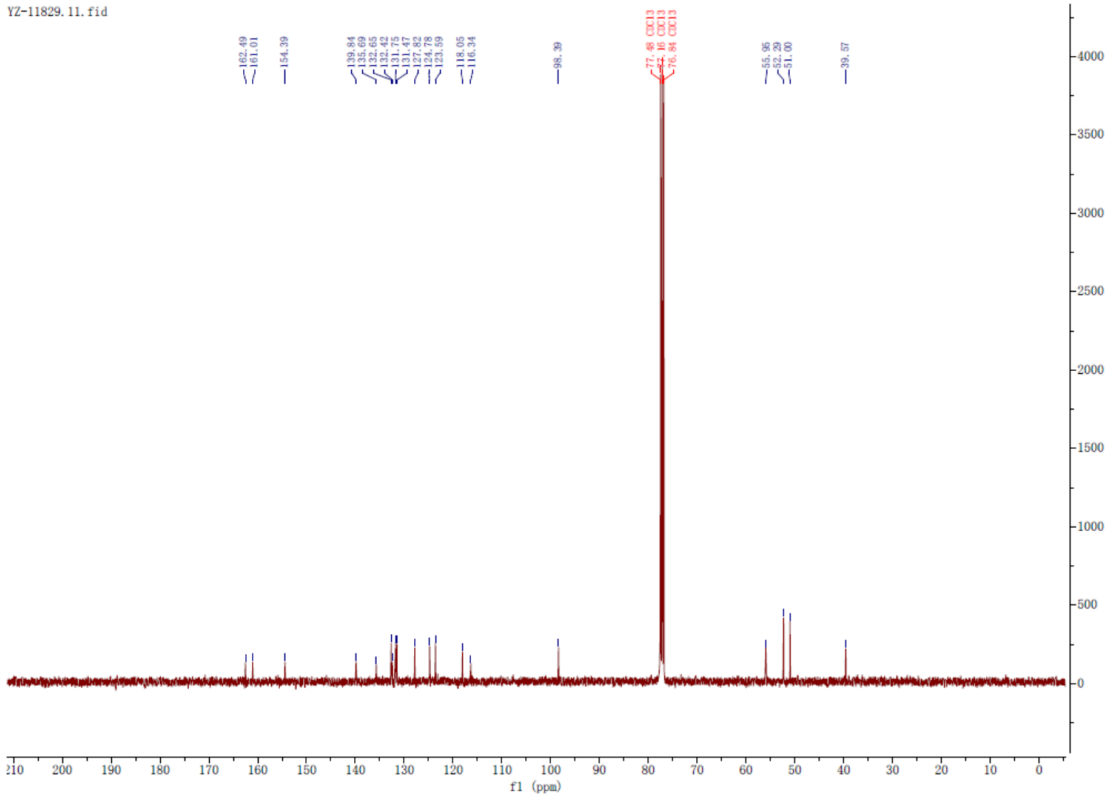

YZ-11828.11.fid
f1 (ppm)

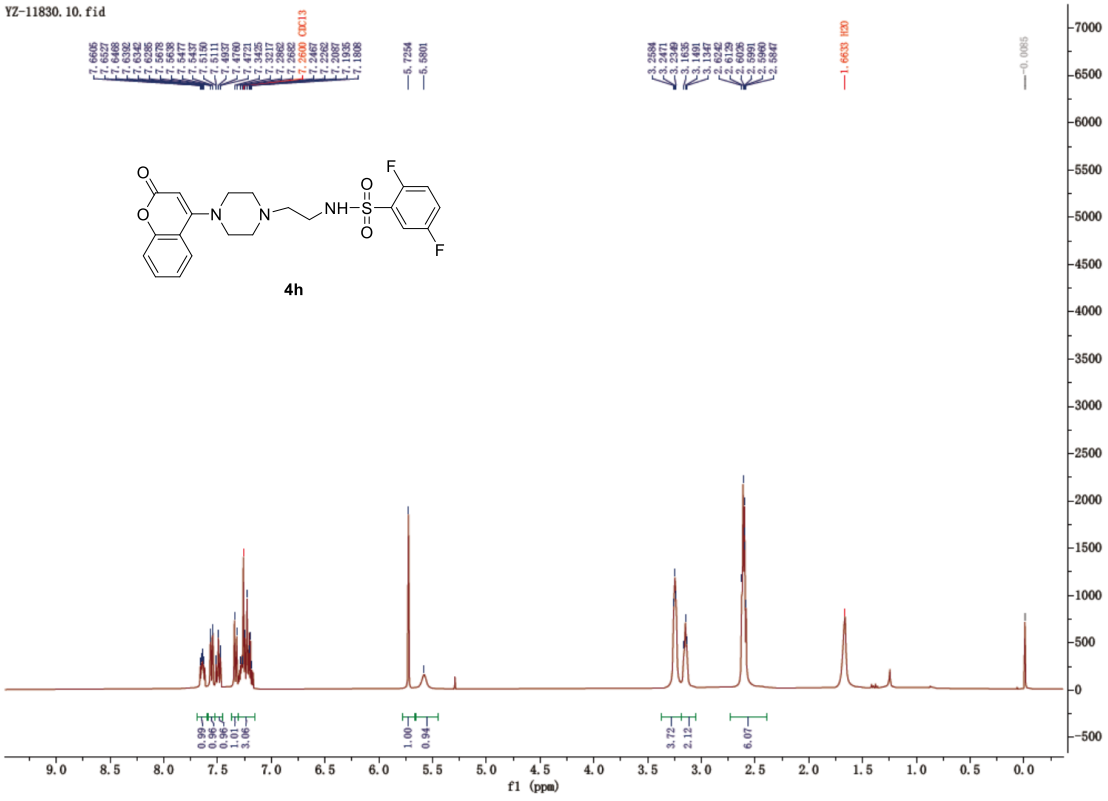
YZ-11829.10.fid

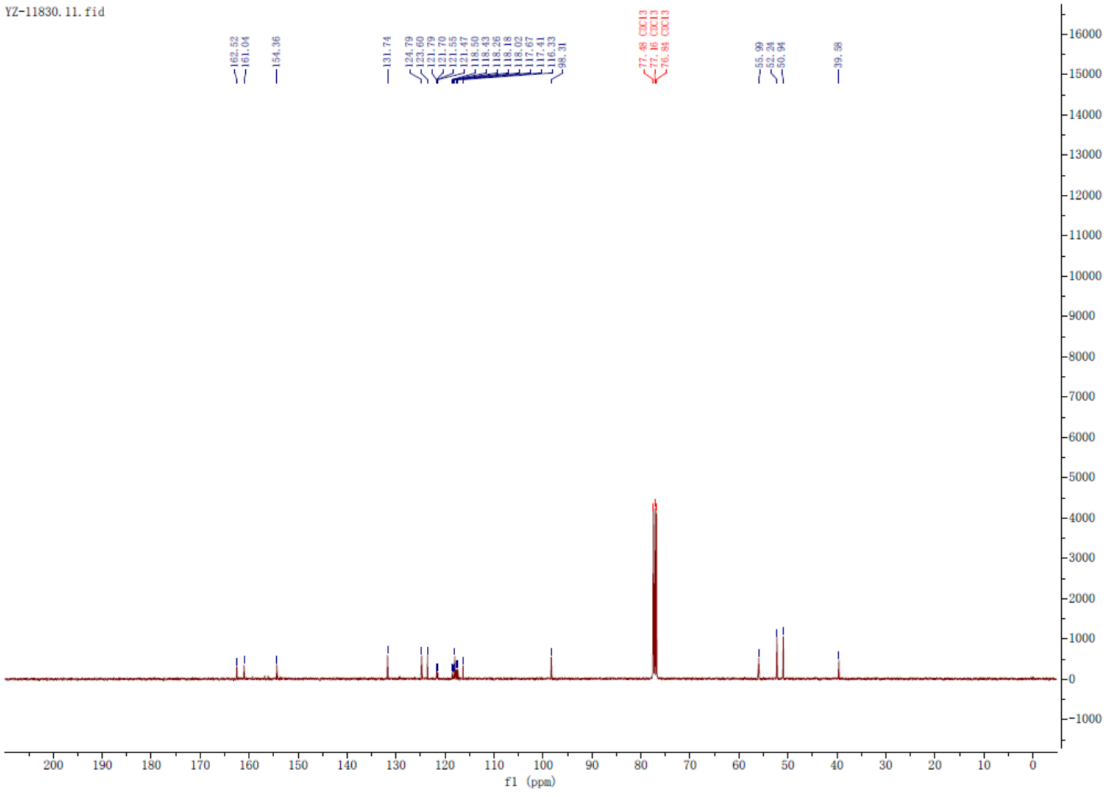


4g

YZ-11829.11.fid




YZ-11830.10.fid
4h
f1 (ppm)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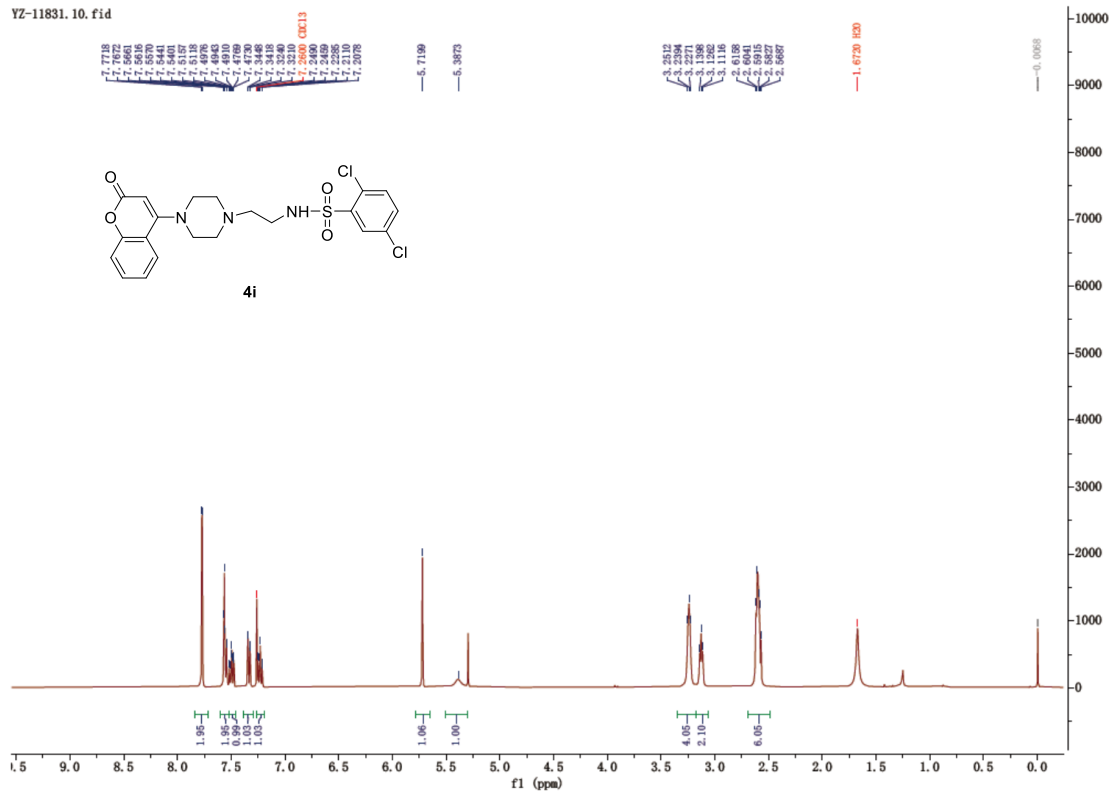
f1 (ppm)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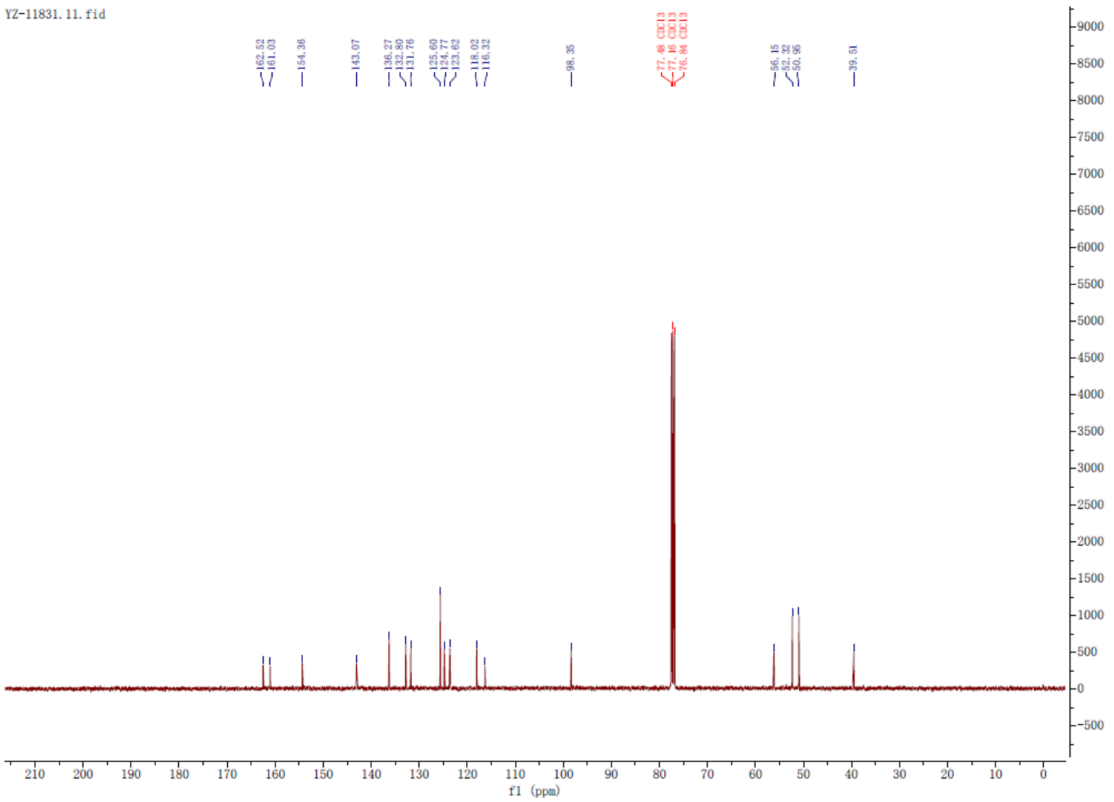
4i
f1 (ppm)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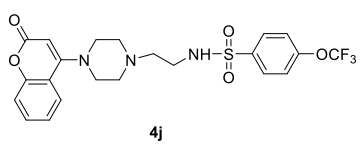
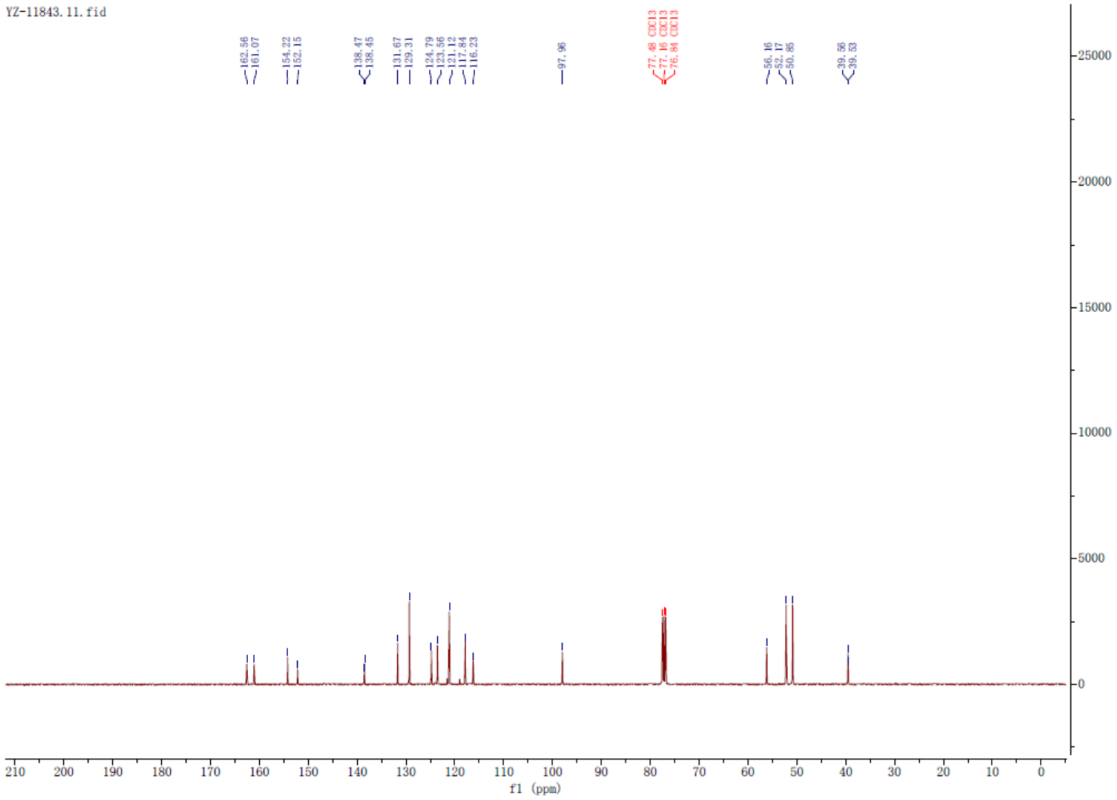
f1 (ppm)

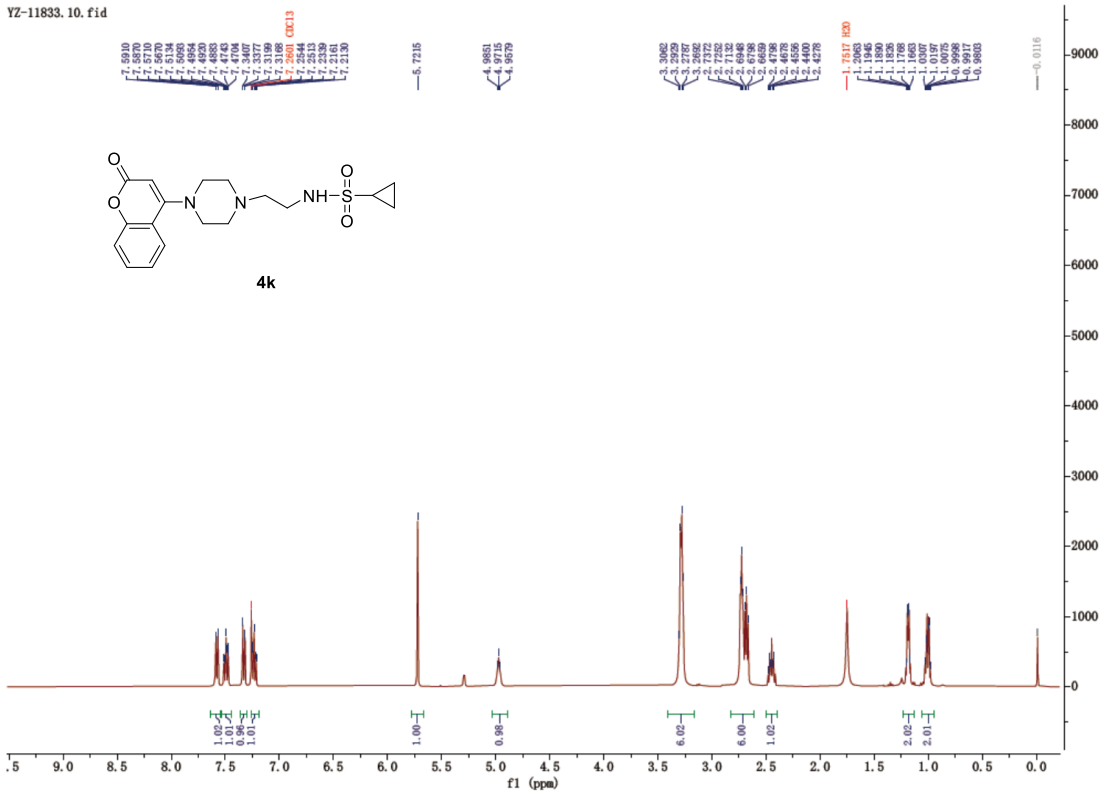
YZ-11843.10.fid



4j

YZ-11843.11.fid

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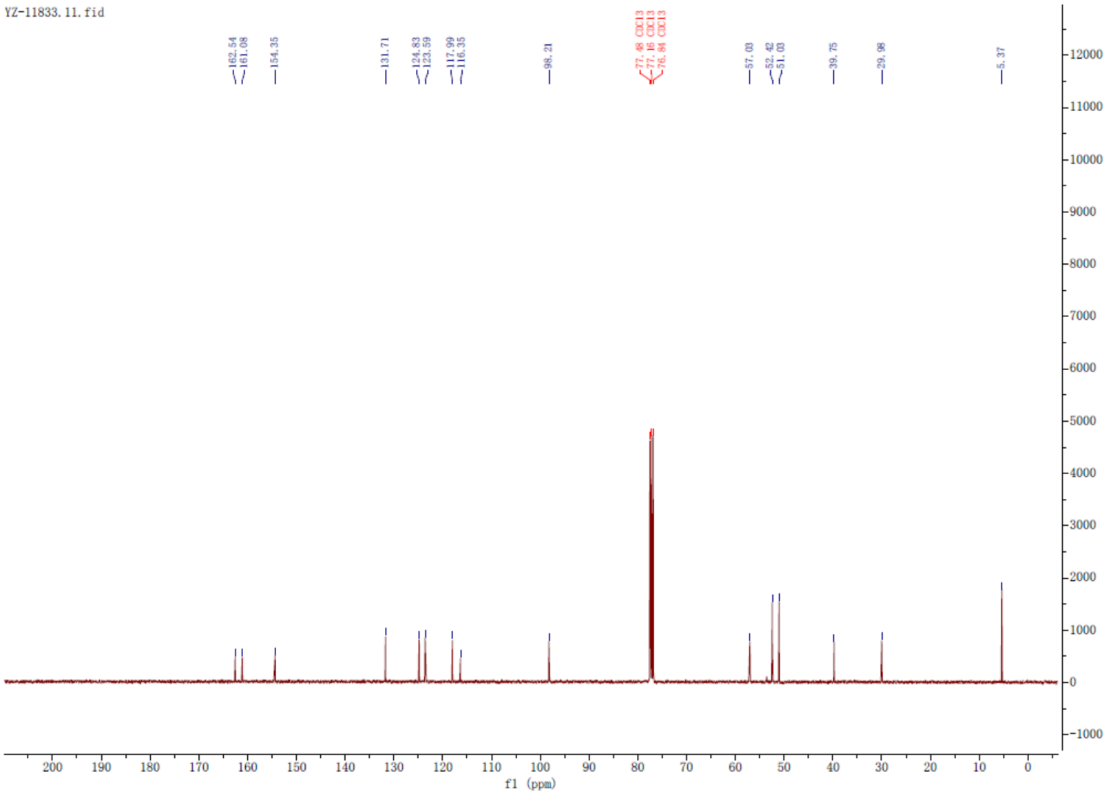
4k
f1 (ppm)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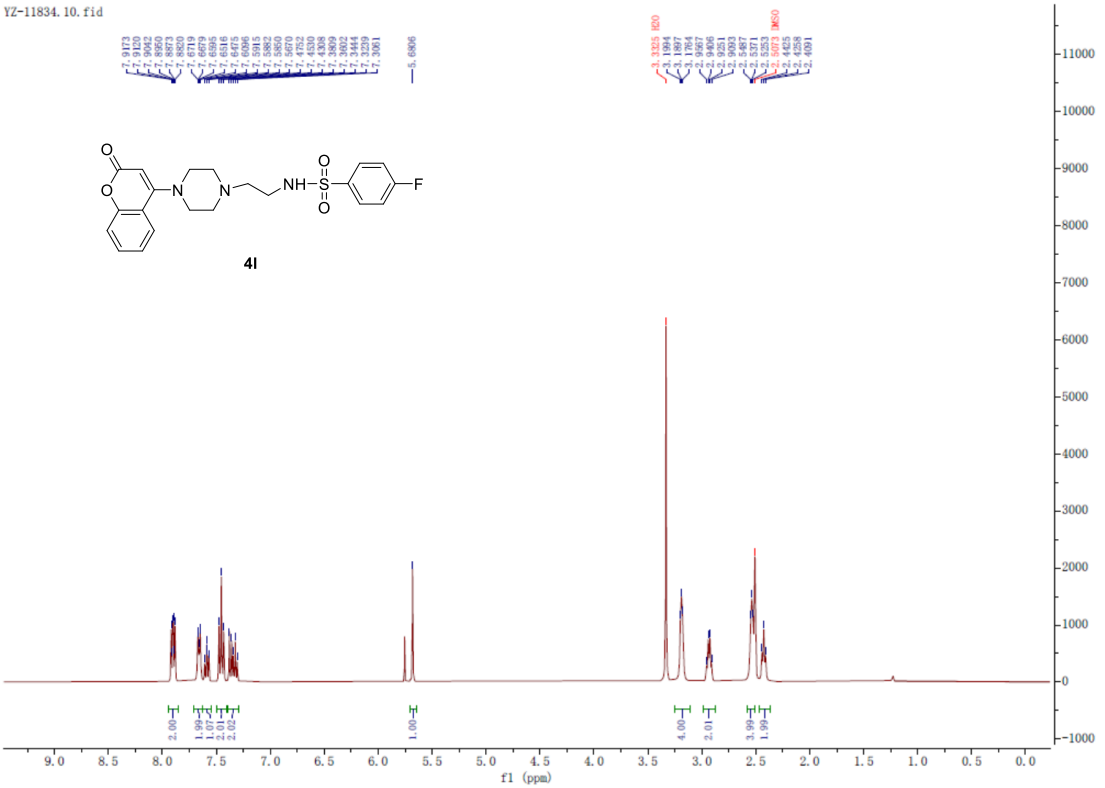
f1 (ppm)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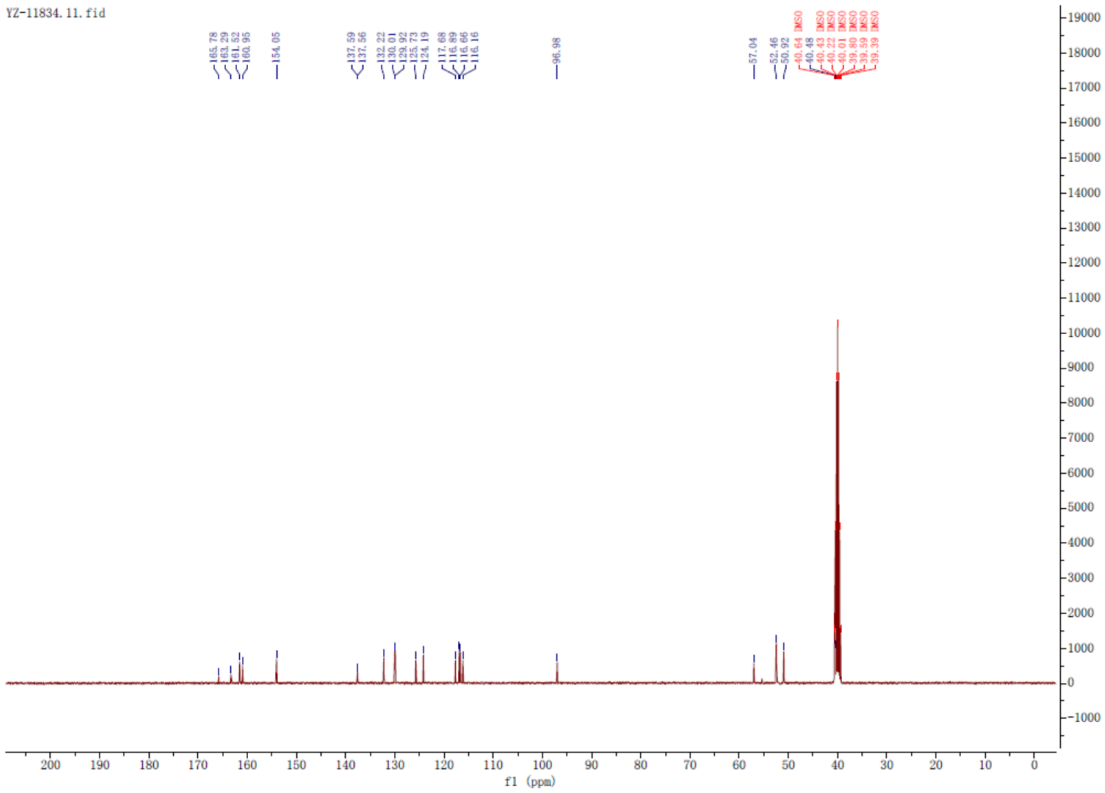
f1 (ppm)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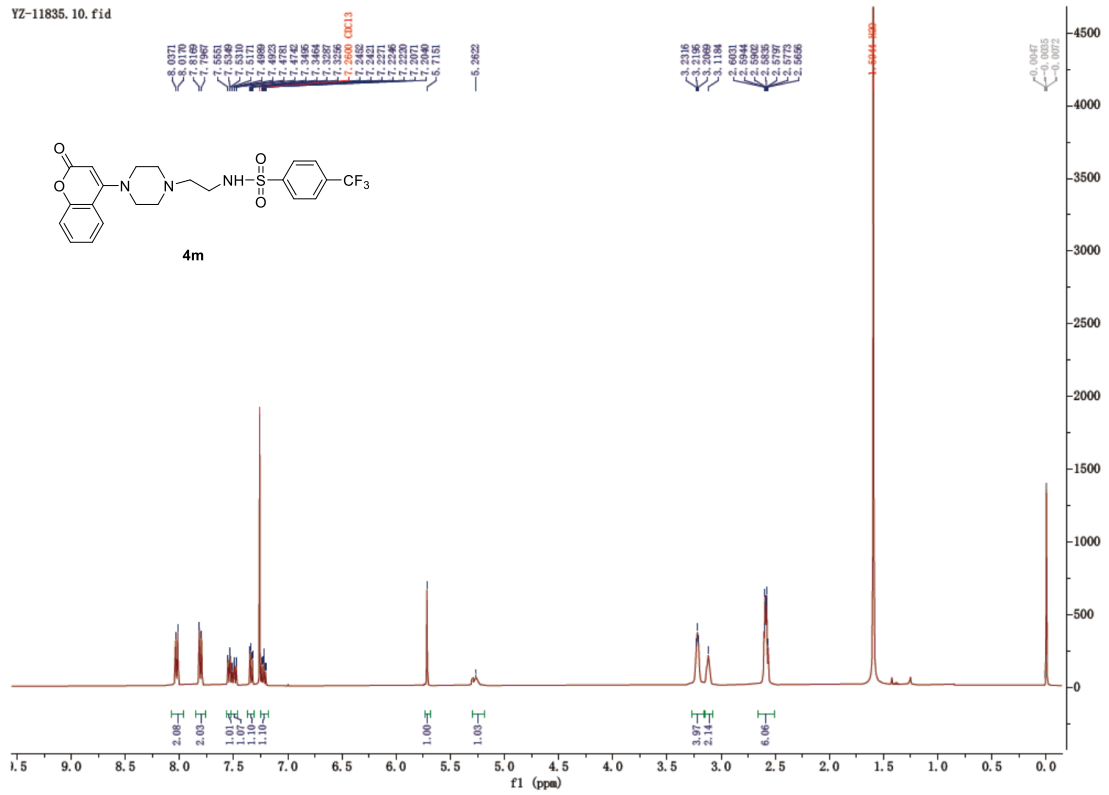
f1 (ppm)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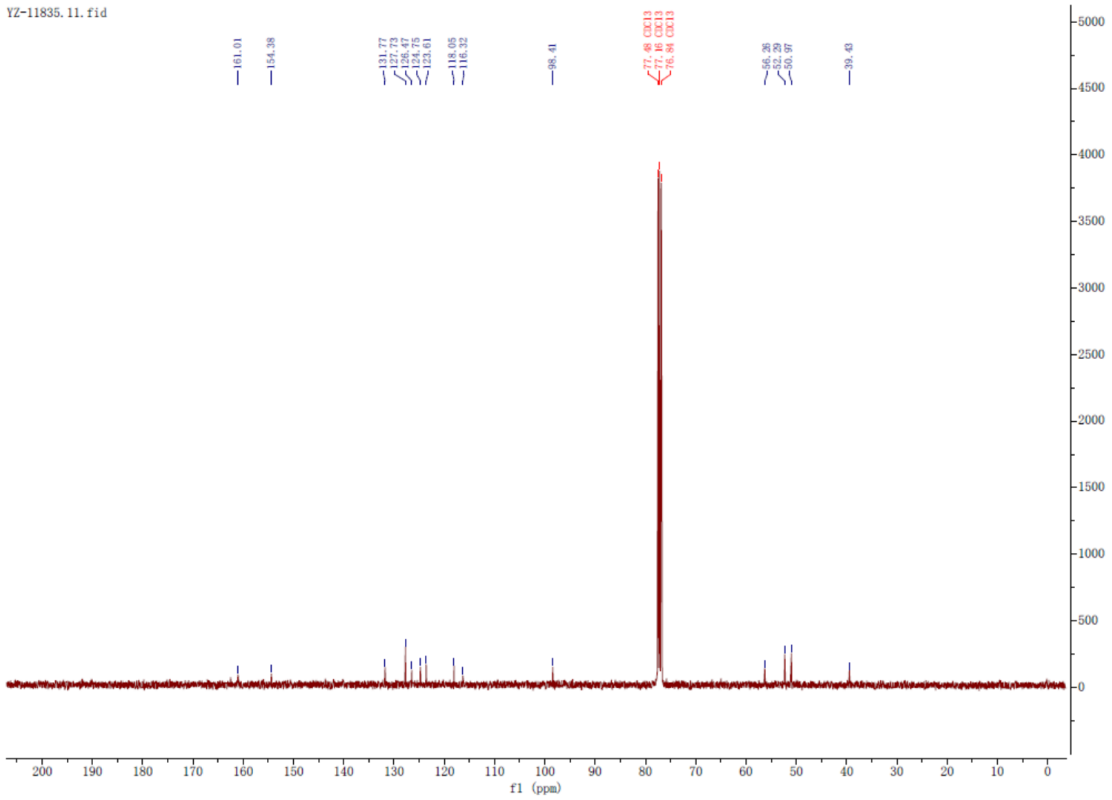
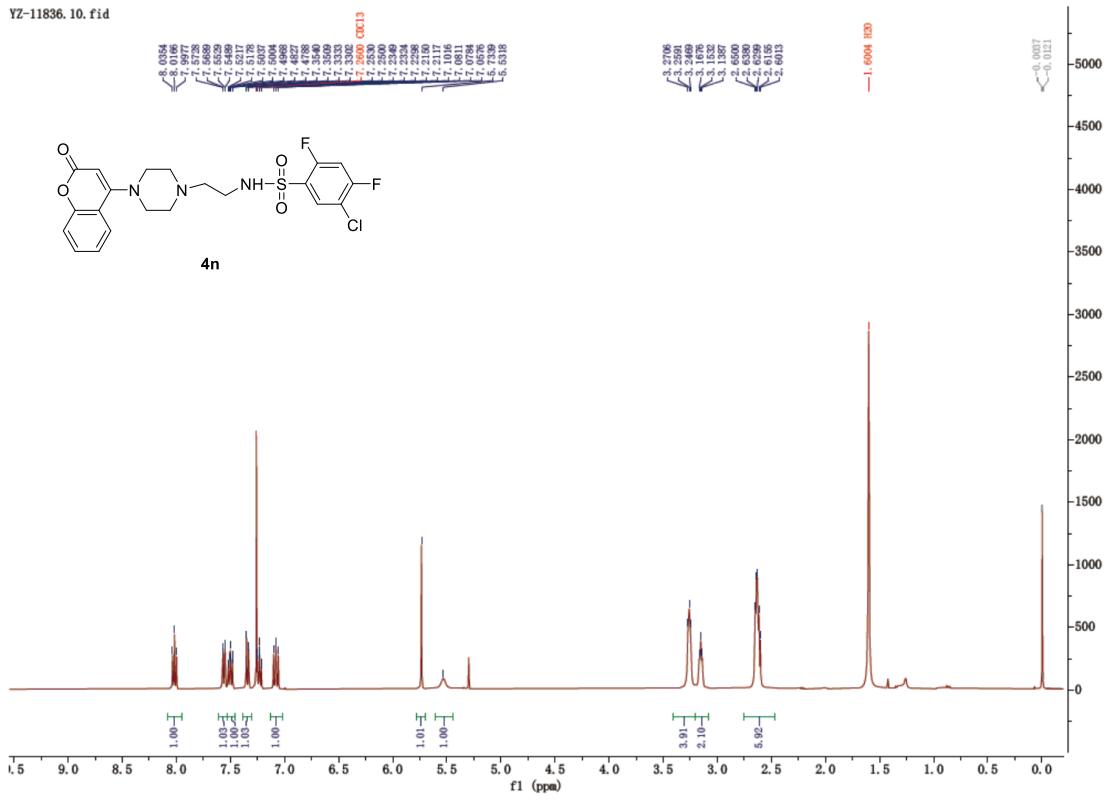
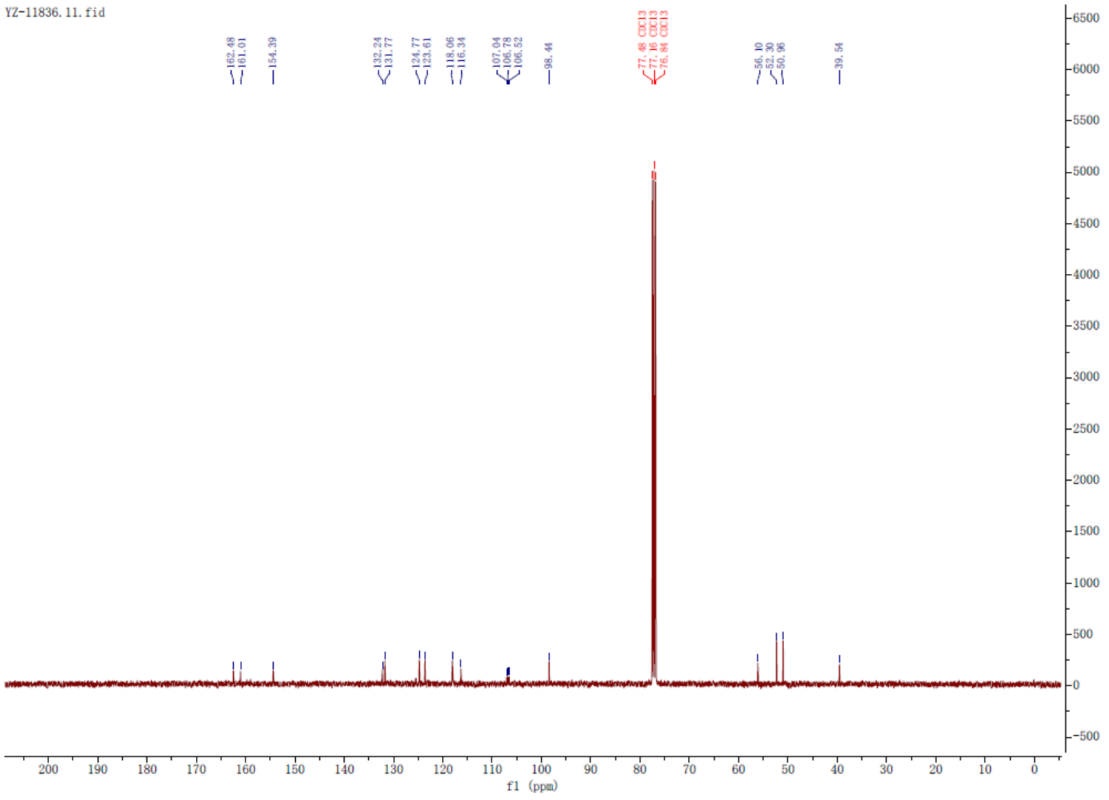
4m
f1 (ppm)


YZ-11835.11.fid
f1 (ppm)


YZ-11836.10.fid
4n
f1 (ppm)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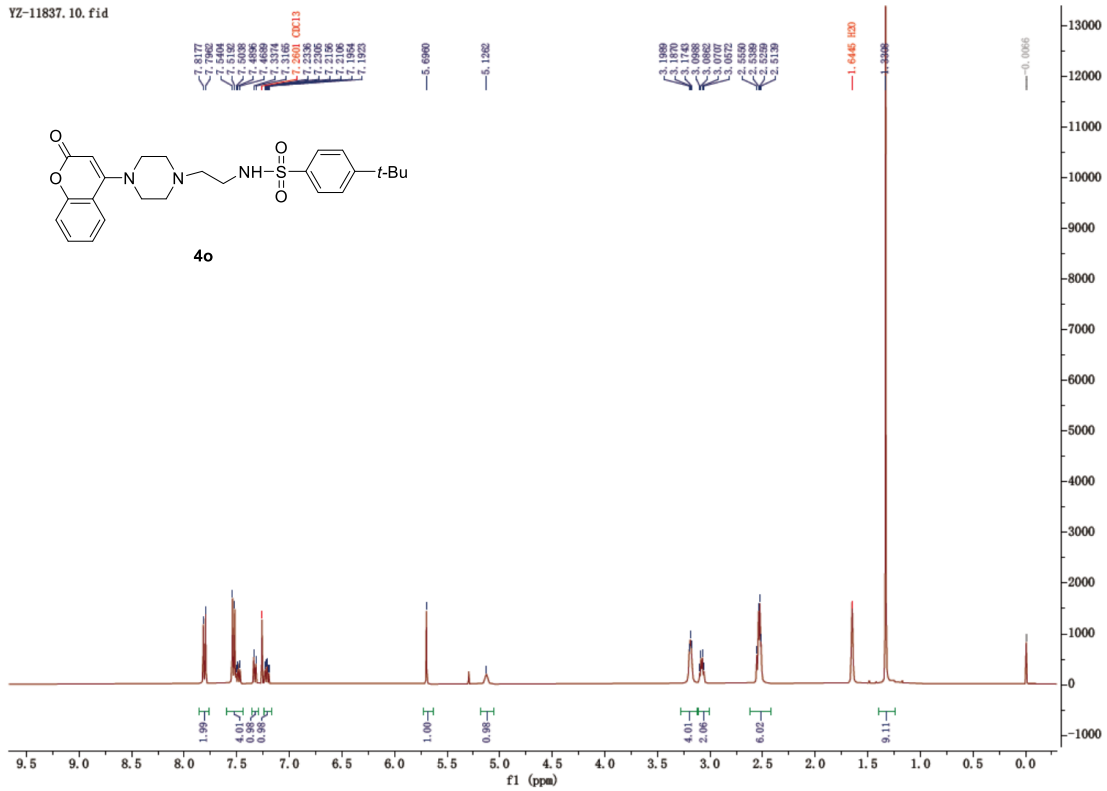
f1 (ppm)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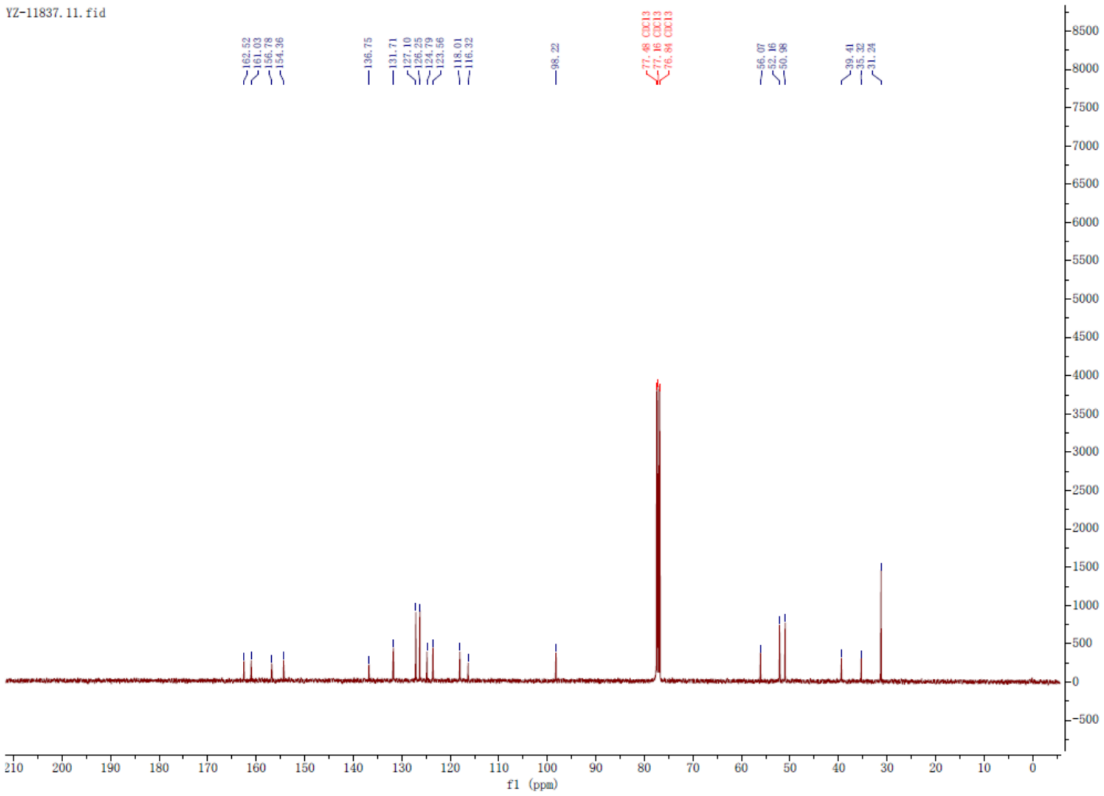
4o
f1 (ppm)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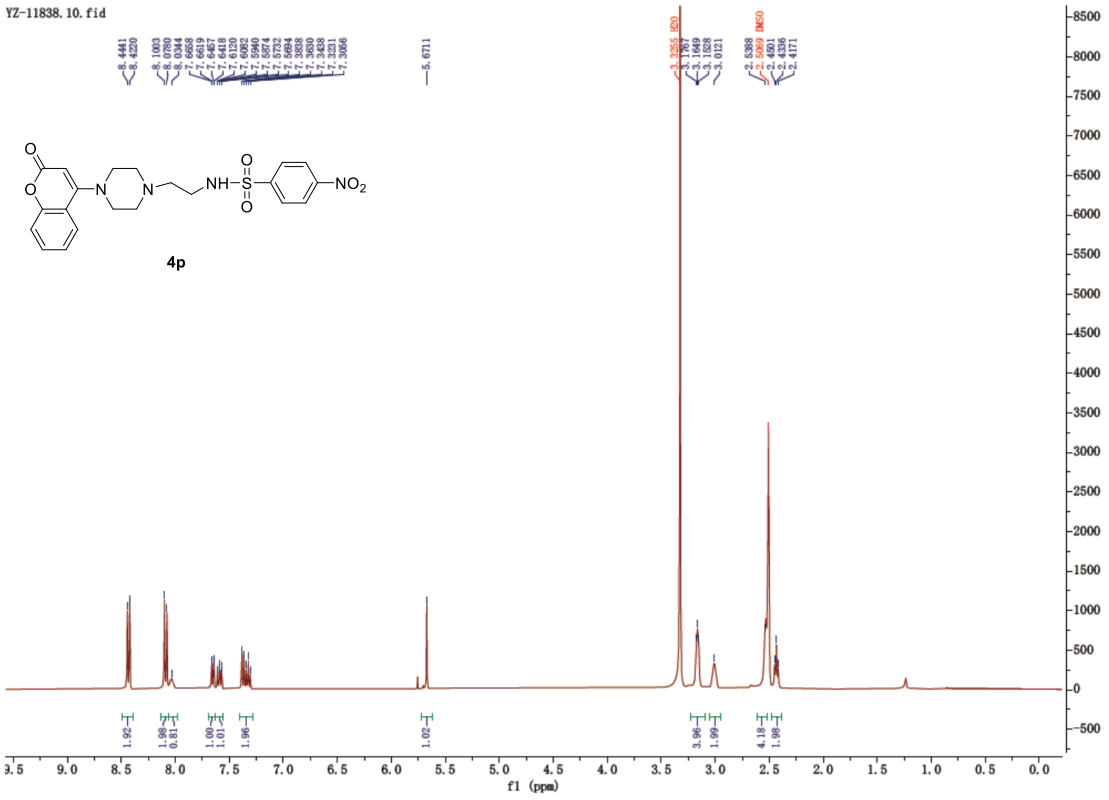
f1 (ppm)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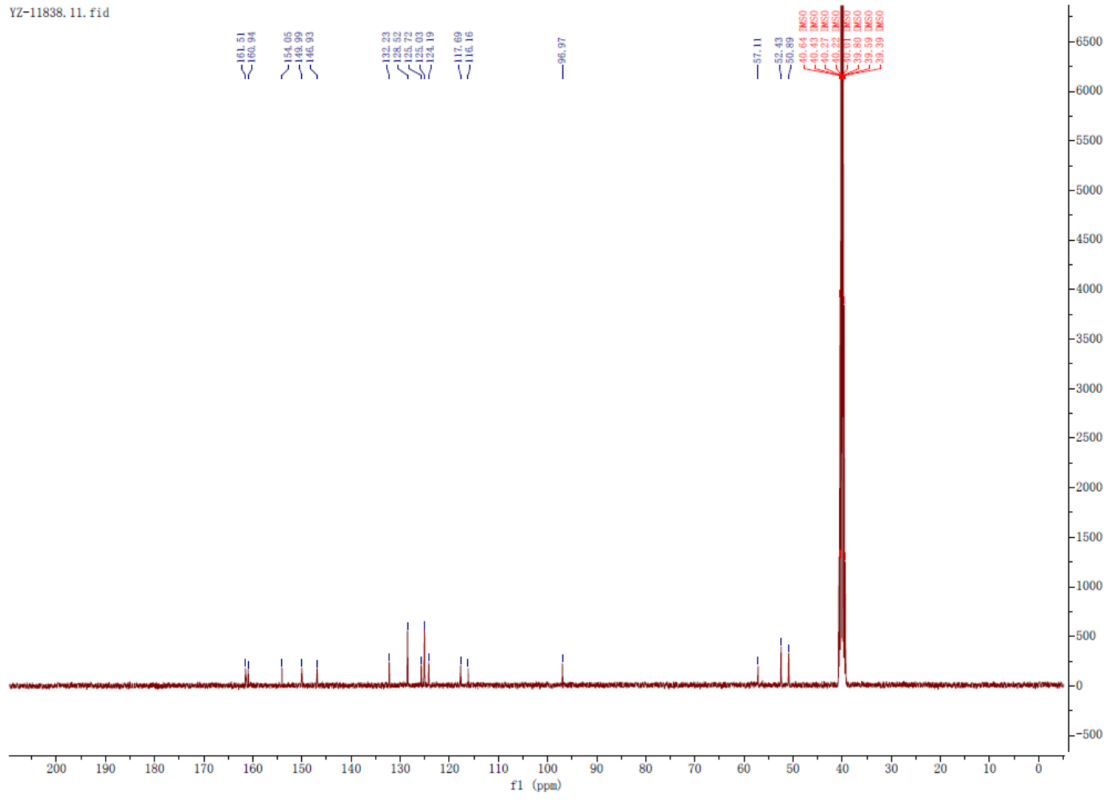
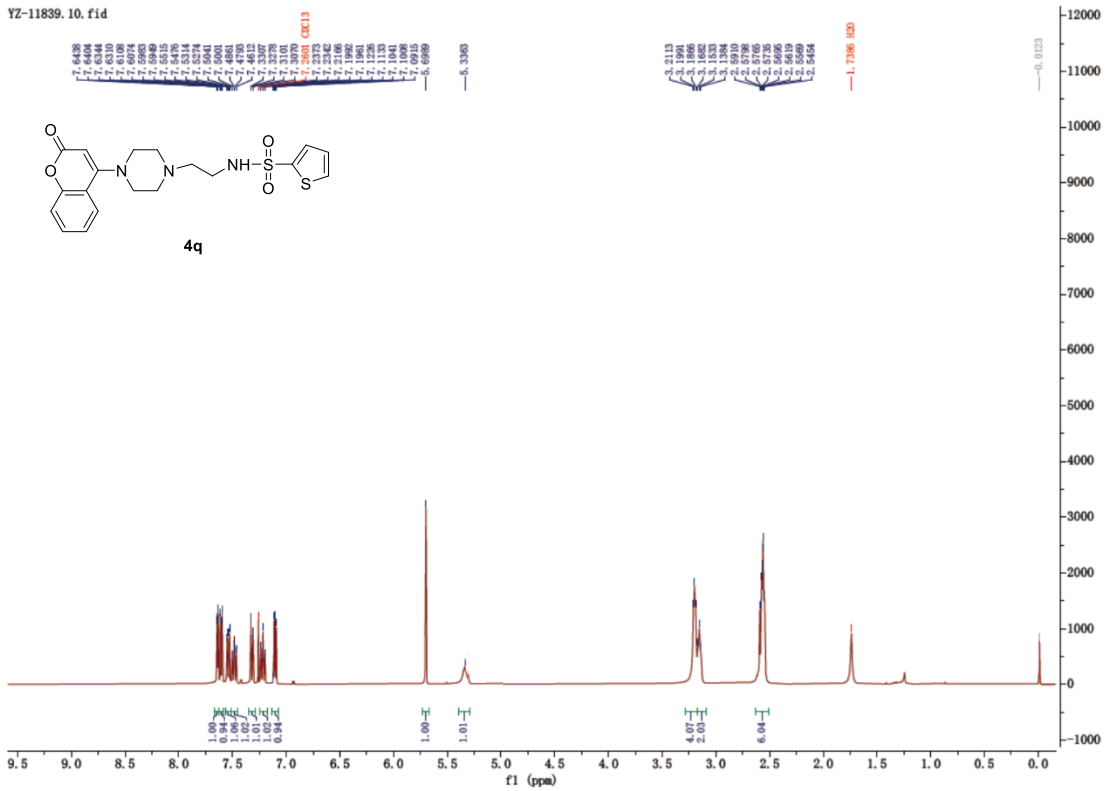
4p
f1 (ppm)


YZ-11838.11.fid
f1 (ppm)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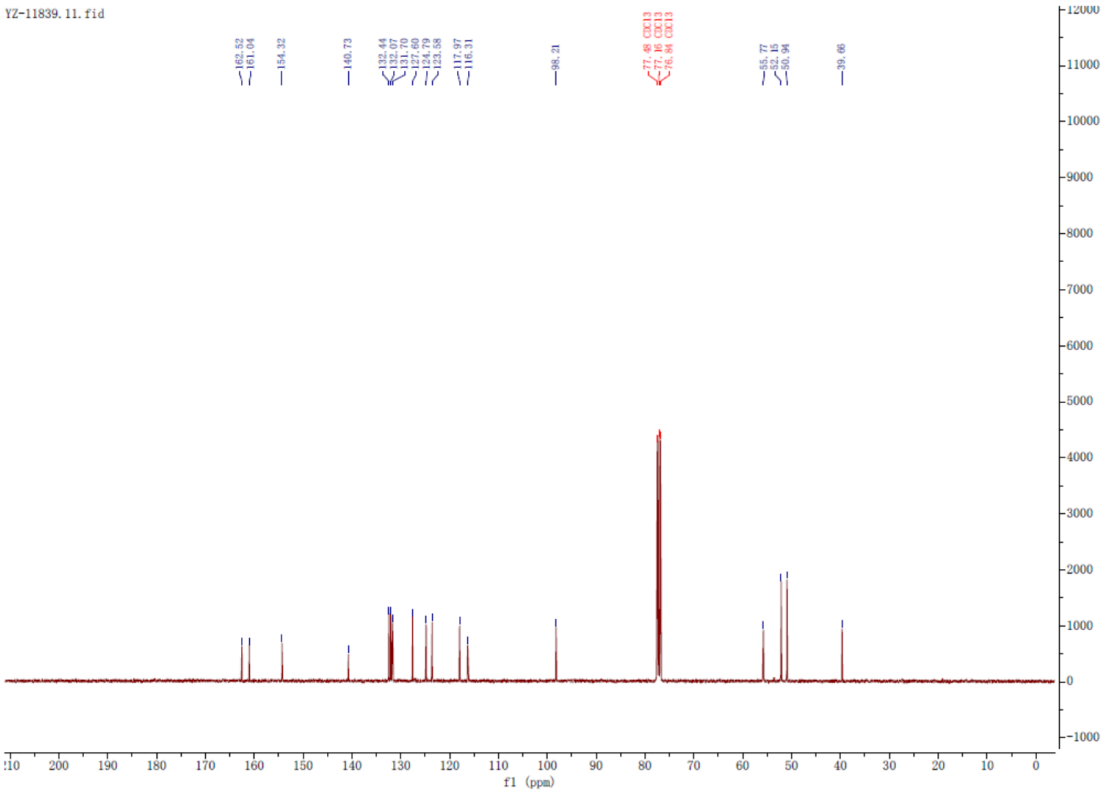
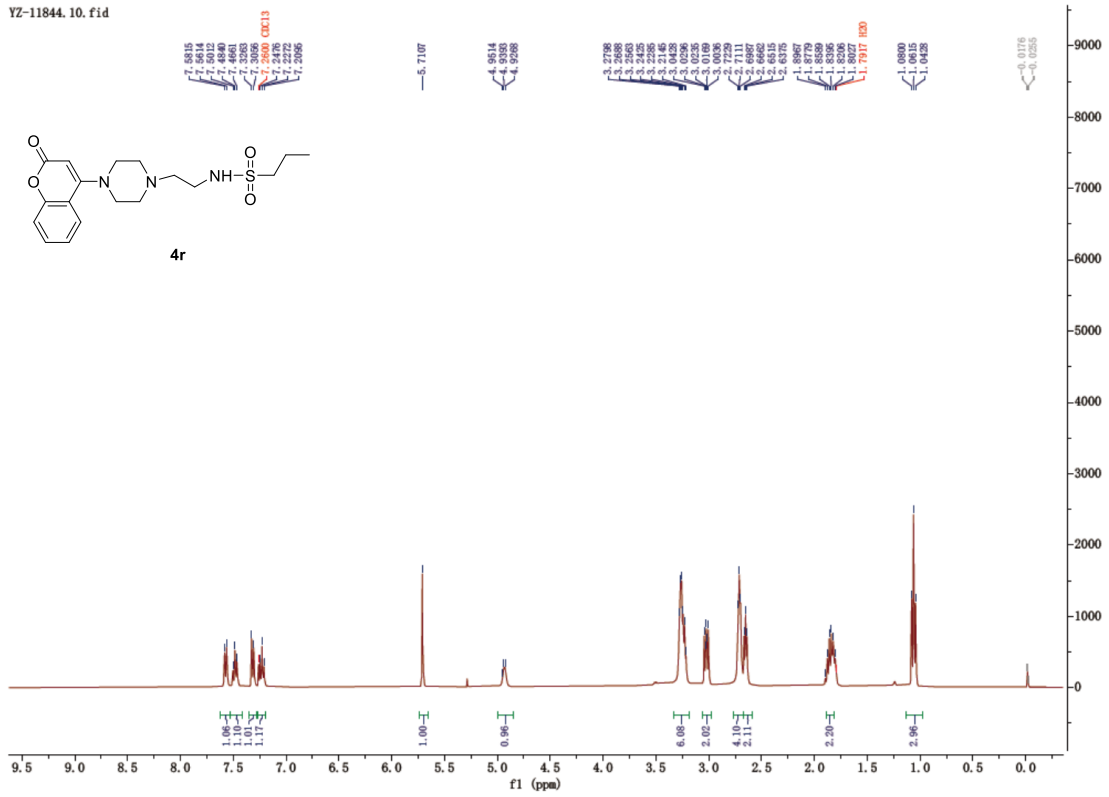
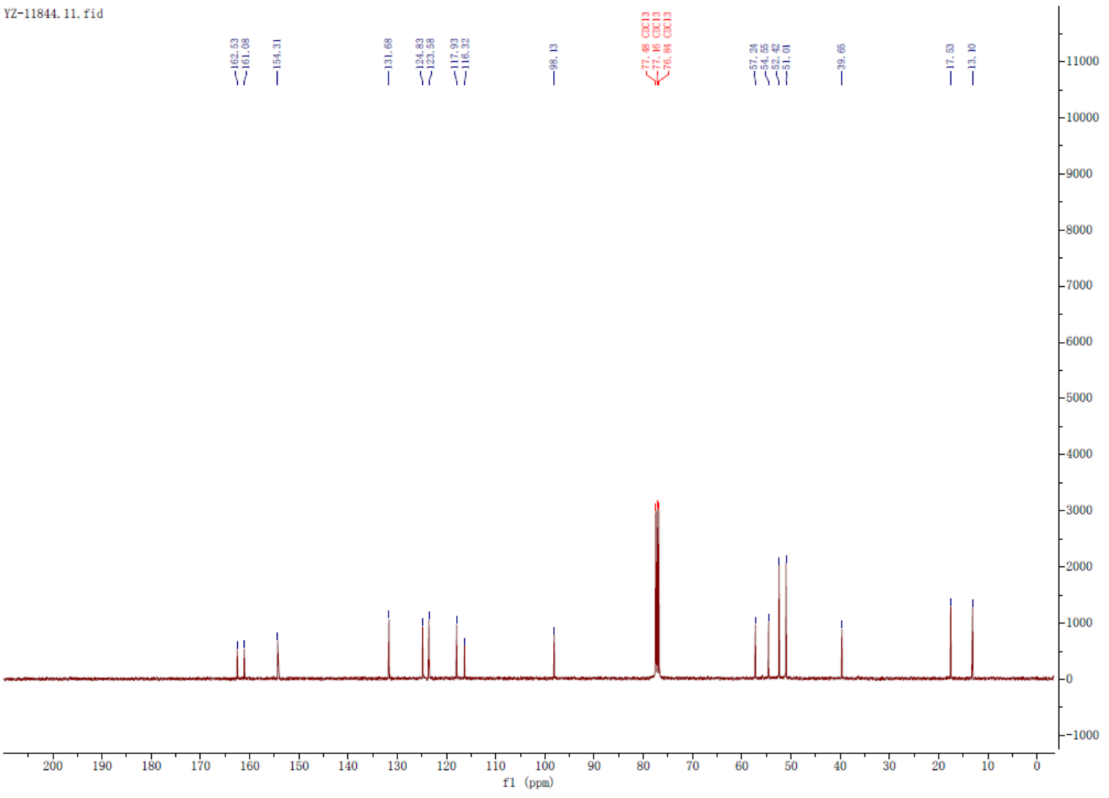
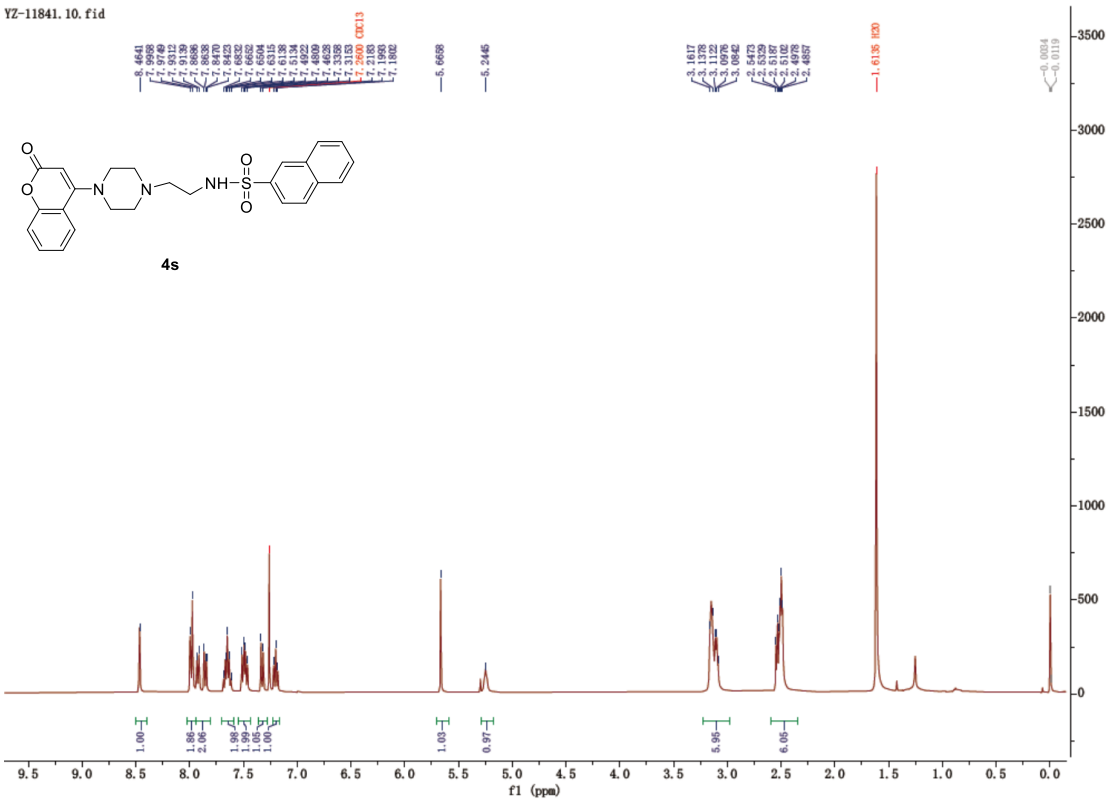
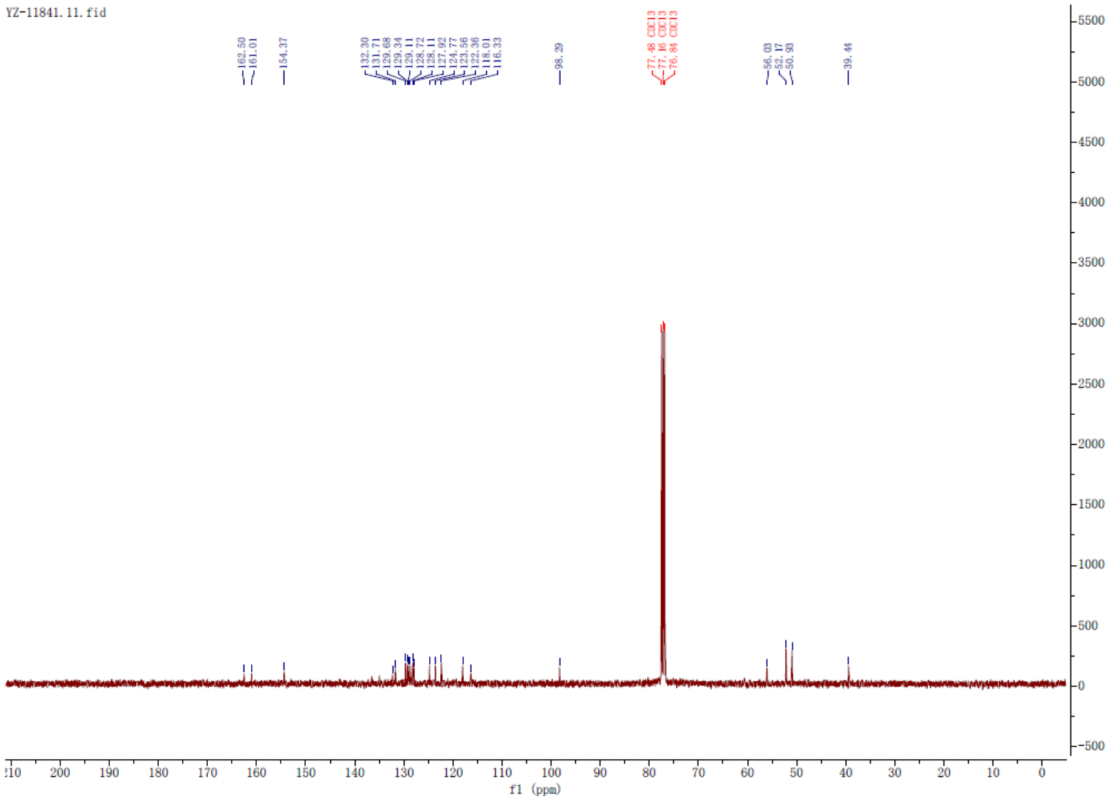
4q
f1 (ppm)


YZ-11839.11.fid
f1 (ppm)


YZ-11844.10.fid
4r
f1 (ppm)


YZ-11844.11.fid
f1 (ppm)


YZ-11841.10.fid
4s
f1 (ppm)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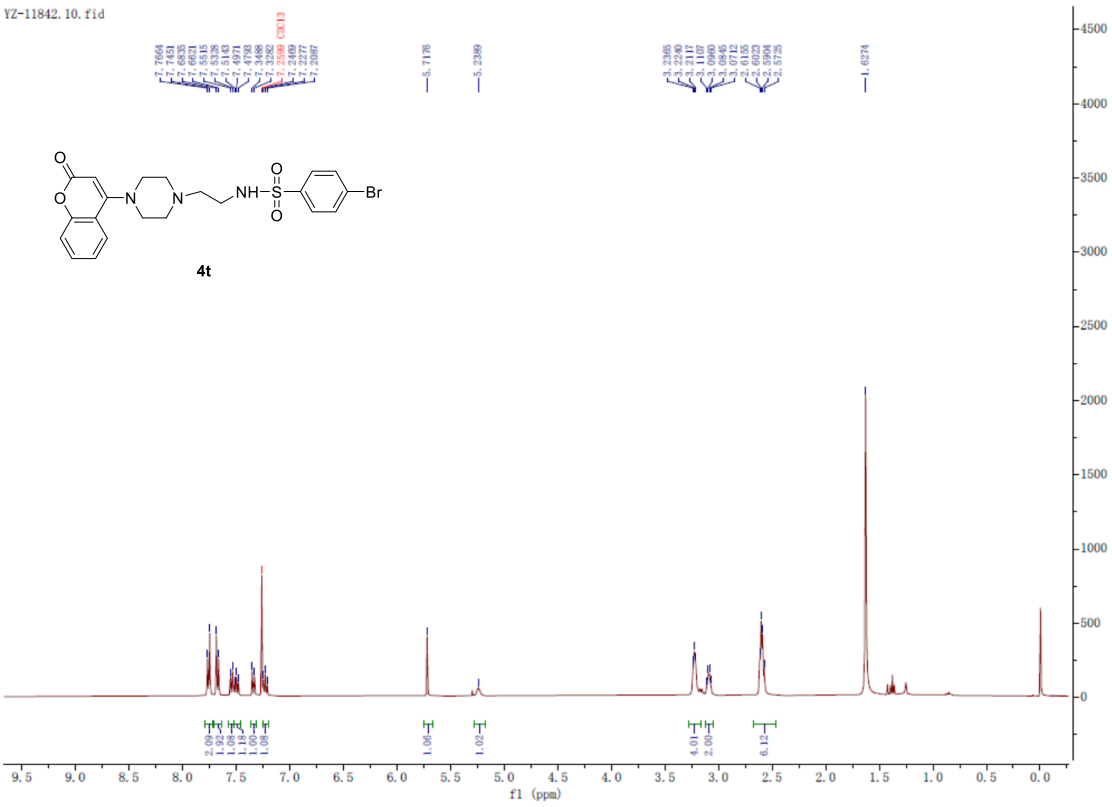
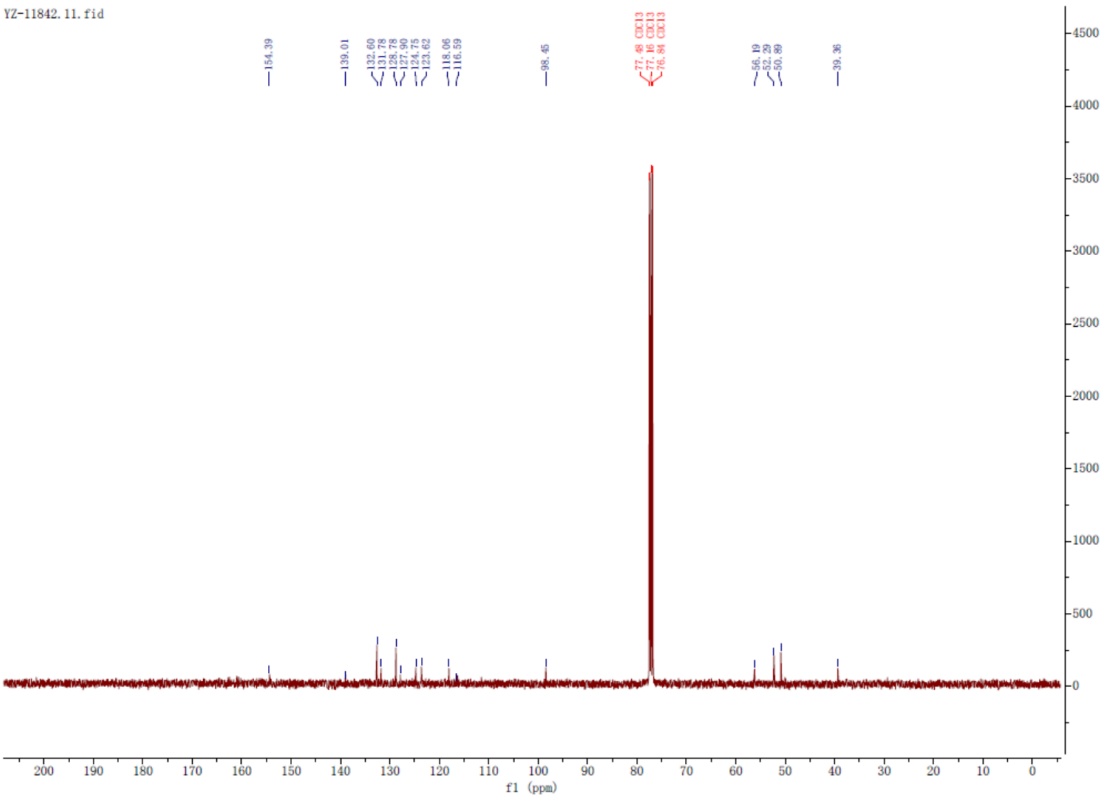
f1 (ppm)


YZ-11842.10.fid
4t
f1 (ppm)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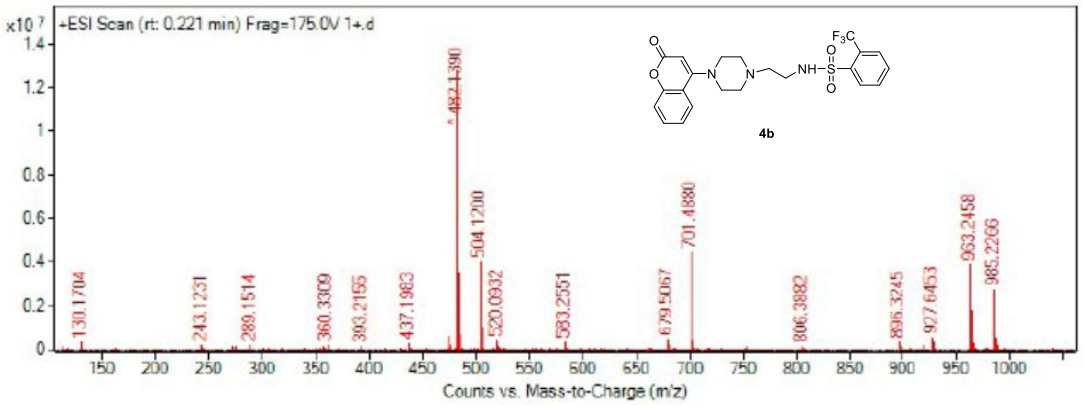
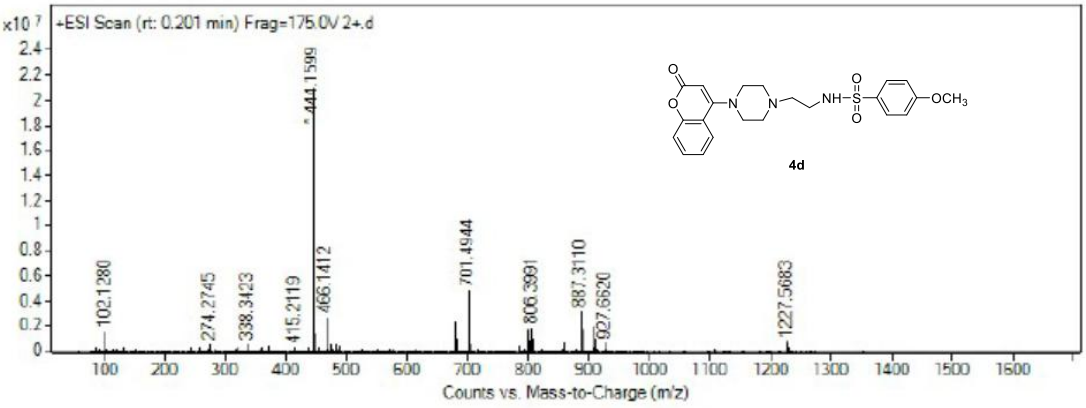
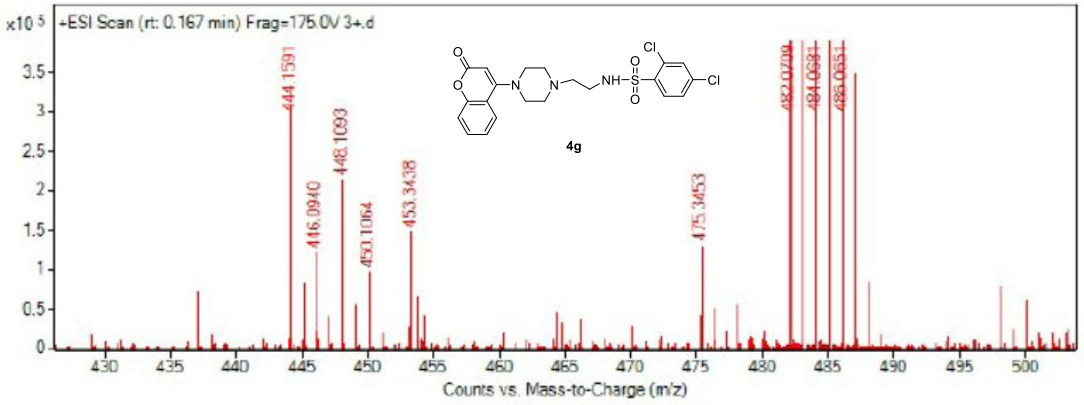
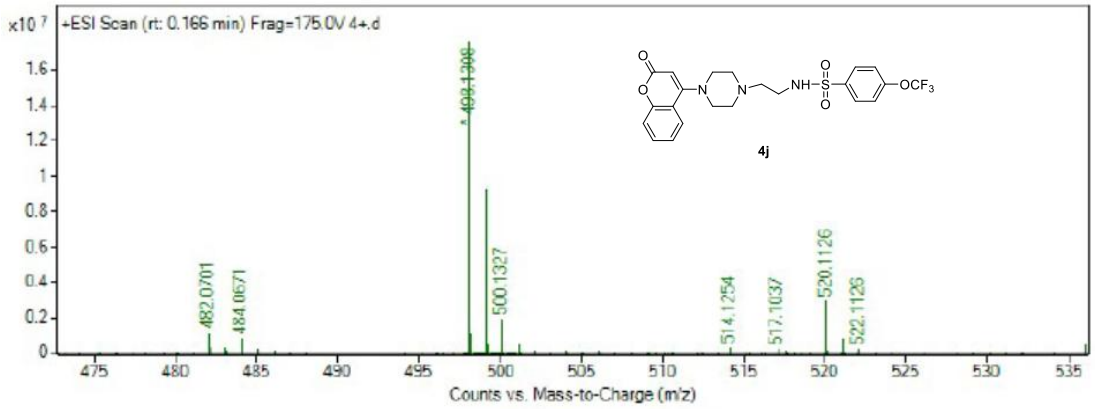
f1 (ppm)

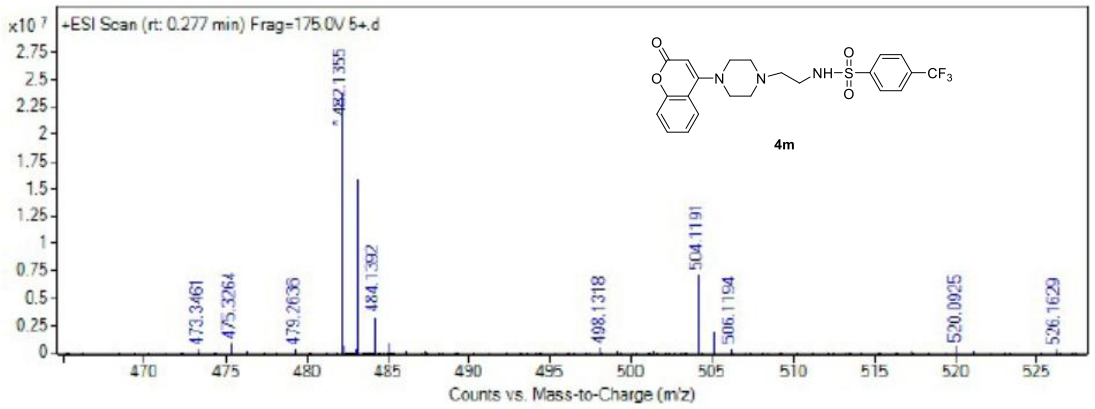
3. HRMS spectra

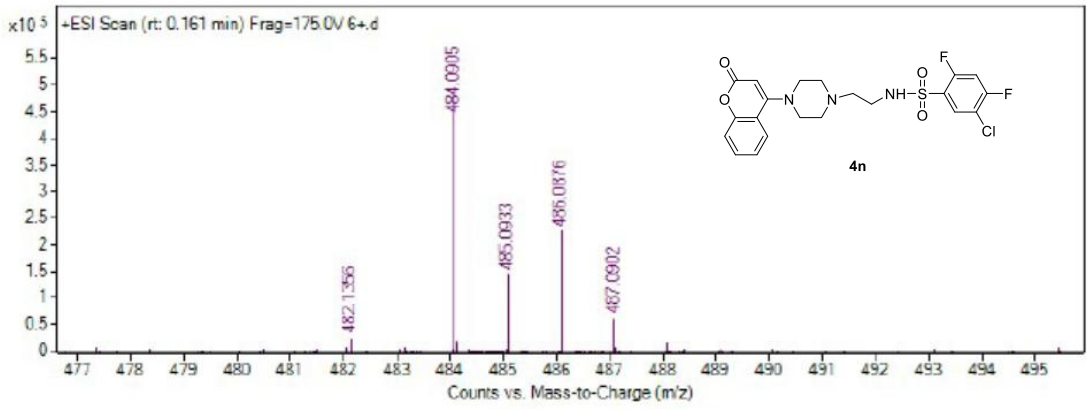


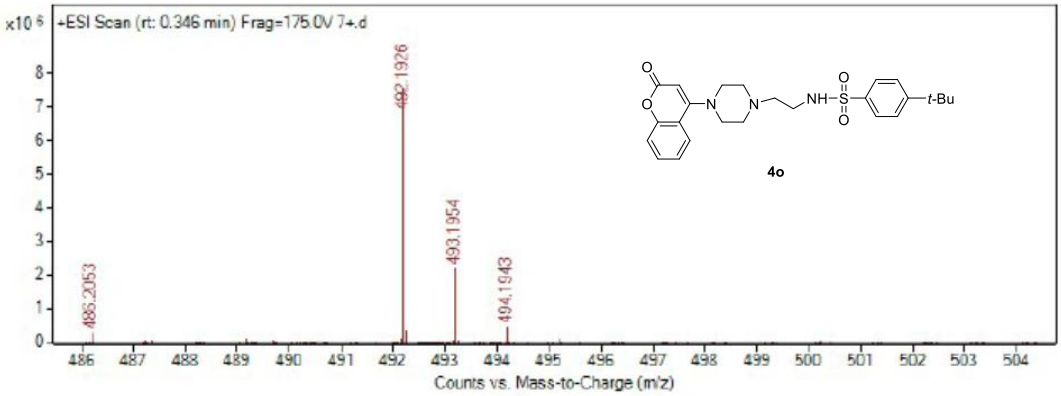


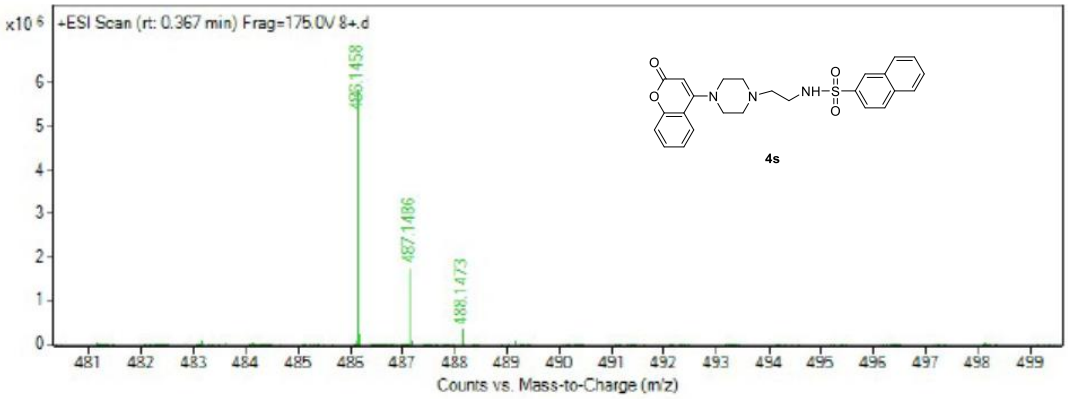


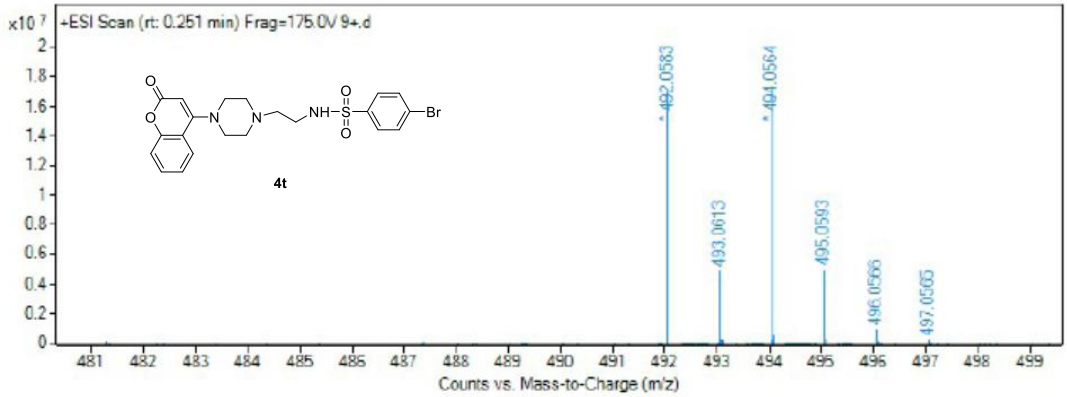

x10 7
+ESI Scan (rt: 0.166 min) Frag=175.0V 4+.d
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
482.0701
484.0671
500.1327
514.1254
517.1037
520.1126
522.1126
475
480
485
490
495
500
505
510
515
520
525
530
535
Counts vs. Mass-to-Charge (m/z)
O
O
N
N
NH
S
O
O
OCF3
4j


x10 7
+ESI Scan (rt: 0.277 min) Frag=175.0V 5+.d
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
473.3461
475.3264
479.2636
482.1355
484.1392
498.1318
504.1191
506.1194
520.0925
526.1629
470
475
480
485
490
495
500
505
510
515
520
525
Counts vs. Mass-to-Charge (m/z)
O
O
N
N
NH
S
O
O
CF3
4m


x10 5
+ESI Scan (rt: 0.161 min) Frag=175.0V 6+.d
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
482.1356
484.0905
485.0933
486.0876
487.0902
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
Counts vs. Mass-to-Charge (m/z)
O
O
N
N
NH
S
O
O
F
F
Cl
4n


x10 6
+ESI Scan (rt: 0.346 min) Frag=175.0V 7+.d
492.1926
493.1954
494.1943
486.2053
Counts vs. Mass-to-Charge (m/z)
4o


x10 6
+ESI Scan (rt: 0.367 min) Frag=175.0V 8+.d
486.1458
487.1486
488.1473
Counts vs. Mass-to-Charge (m/z)
4s


x10 7
+ESI Scan (rt: 0.251 min) Frag=175.0V 9+.d
492.0583
493.0613
494.0564
495.0593
496.0566
497.0565
Counts vs. Mass-to-Charge (m/z)
4t