Supporting Information for

Original article

Discovery of novel aporphine alkaloid derivative as potent TLR2 antagonist reversing macrophage polarization and neutrophil infiltration against acute inflammation

Junjie Yang[a], Yue Pan[a], Xiaoshan Zeng[a], Shuwen Liu[a,*], Zhipeng Chen[a,*], Kui Cheng[a,b,*]

[a]*Guangdong Provincial Key Laboratory of New Drug Screening and NMPA Key Laboratory for Research and Evaluation of Drug Metabolism, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China*

[b]*Department of Musculoskeletal Oncology, the Third Affiliated Hospital of Southern Medical University, Guangzhou 510642, China*

*Correspondence author.

E-mail addresses: Liusw@smu.edu.cn (Shuwen Liu), czpwyq@smu.edu.cn (Zhipeng Chen), Chengk@smu.edu.cn (Kui Cheng)

Received 9 February 2023; received in revised form 9 May 2023; accepted 19 May 2023

This Supplementary Information file includes:

Figure S1

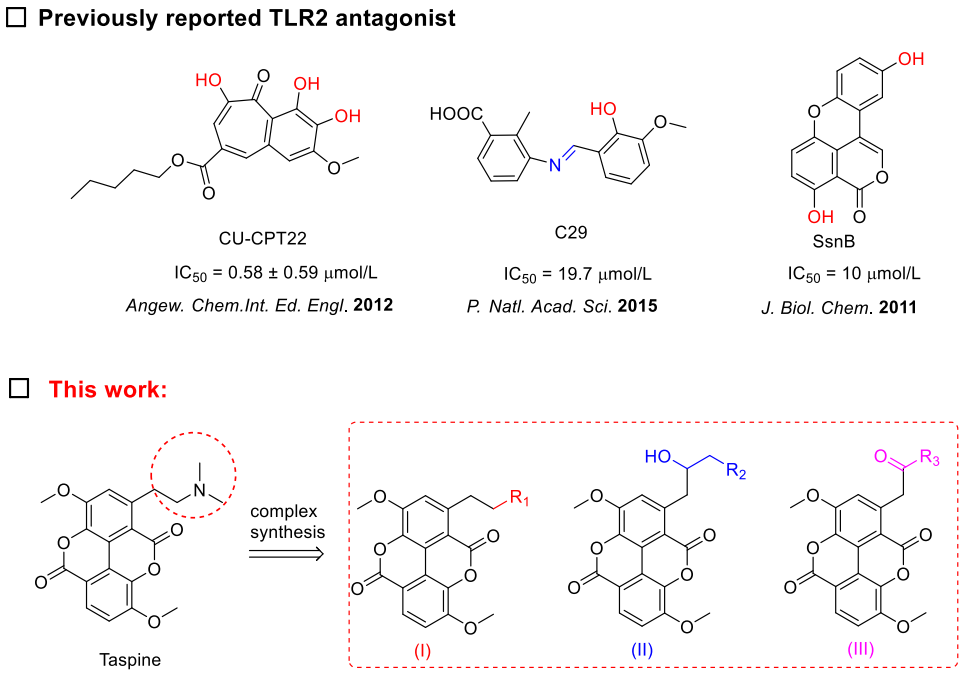


Figure S1. The reported TLR2 small molecule inhibitors and the present work.

Figure S2

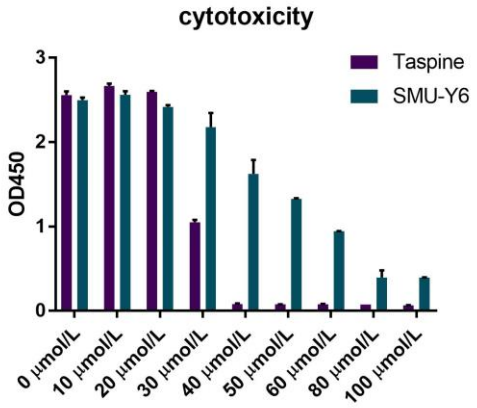


Figure S2. The comparison of cytotoxicity between Taspine and SMU-Y6 in HEK-Blue hTLR2 cell. HEK-hTLR2 cells were seeded in 96-well plate with 100 μL freshmedium (with 10% FBS and 1% pen/strep) and incubated at 37 °C overnight. Indicated concentration compounds of SMU-Y6 were added to 200 μL totally and incubated at 37 °C for 24 h. Cell counting kit-8 (CCK-8) (Beyotime, C0038) was added into each well for 20 μL and incubated at 37 °C for 1–4 h until it turned into orange. Then the plate was measured at an absorbance of 450 nm.

Figure S3

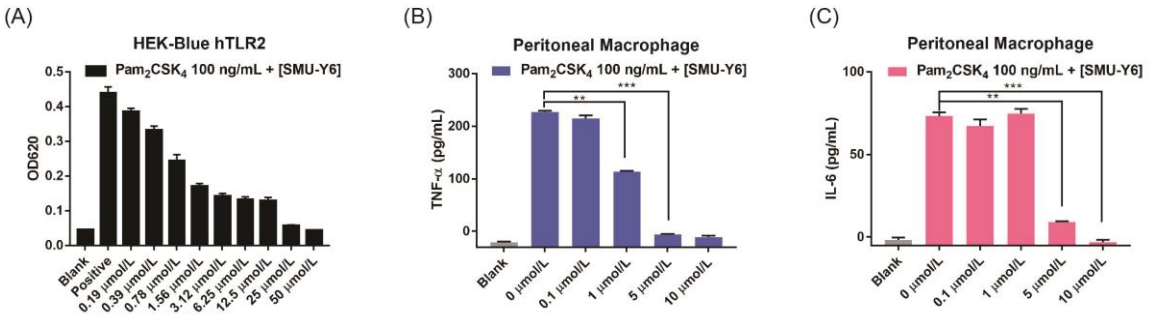


Figure S3. SMU-Y6 inhibited both SEAP signal and the production of inflammatory cytokines induced by Pam_2CSK_4. (A) SEAP signaling of Taspine and SMU-Y6 in HEK-Blue hTLR2 cells. HEK-Blue hTLR2 cells were treated with Pam_2CSK_4 (100 ng/mL) and indicated Taspine or SMU-Y6 for 24 h. The supernatant was collected for SEAP signaling. **(B-C)** TNF-α or IL-6 in supernatants of primary Murine Peritoneal Macrophage cells after treatment with indicated Pam_2CSK_4 (100 ng/mL) and different concentration of SMU-Y6 for 24 h. The supernatant was collected for TNF-α or IL-6 testing. Data presented are mean ± SD and the figures shown are representative of three independent experiments.

Figure S4

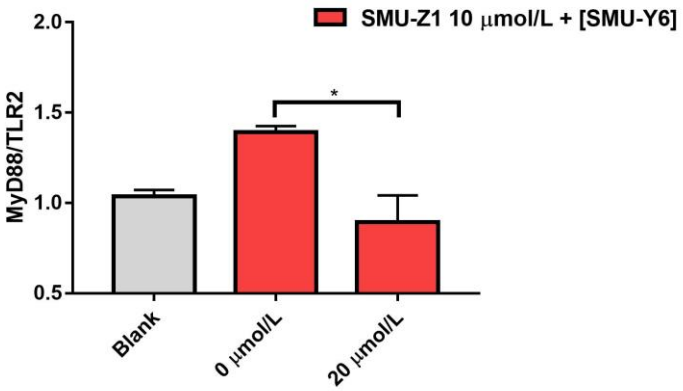


Figure S4. The ratio between MyD88 and TLR2 in Co-IP. The ratio between MyD88 and TLR2 was homogenized through ImageJ.

Figure S5

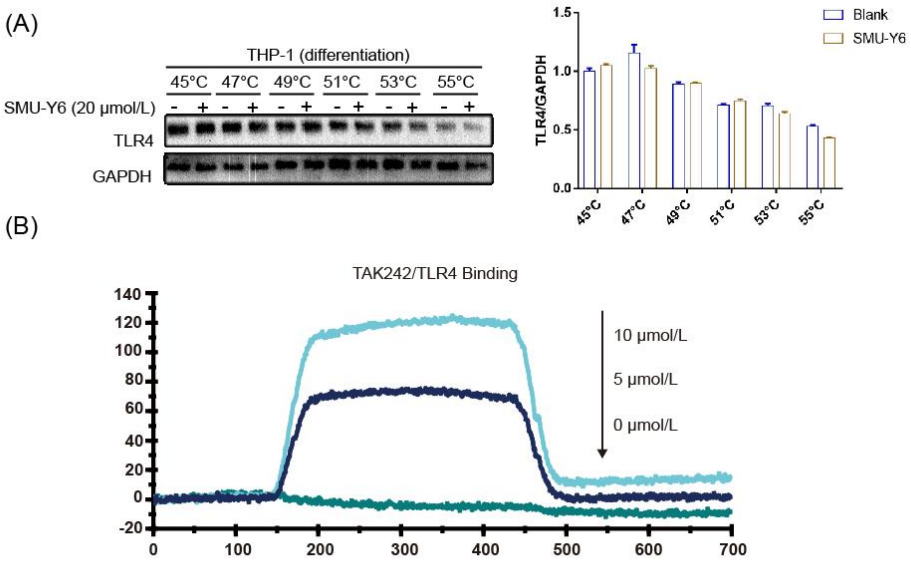


Figure S5. CETSA experiment suggested that SMU-Y6 (20 μmol/L) failed to reduce the degradation of TLR4 when heated in different temperature. (A) THP-1 cells were stimulated with PMA for 24 h, then cultured in fresh medium for another 24 h. Cells were collected, suspended in PBS, and incubated with 20 μmol/L SMU-Y6 or medium for 0.5 h. Cells were heated at indicated temperature for 3 min. The proteins were extracted for Western Blot. **(B)** Surface plasmon resonance experiment of TAK242 binding to recombinant hTLR4 protein.

Figure S6

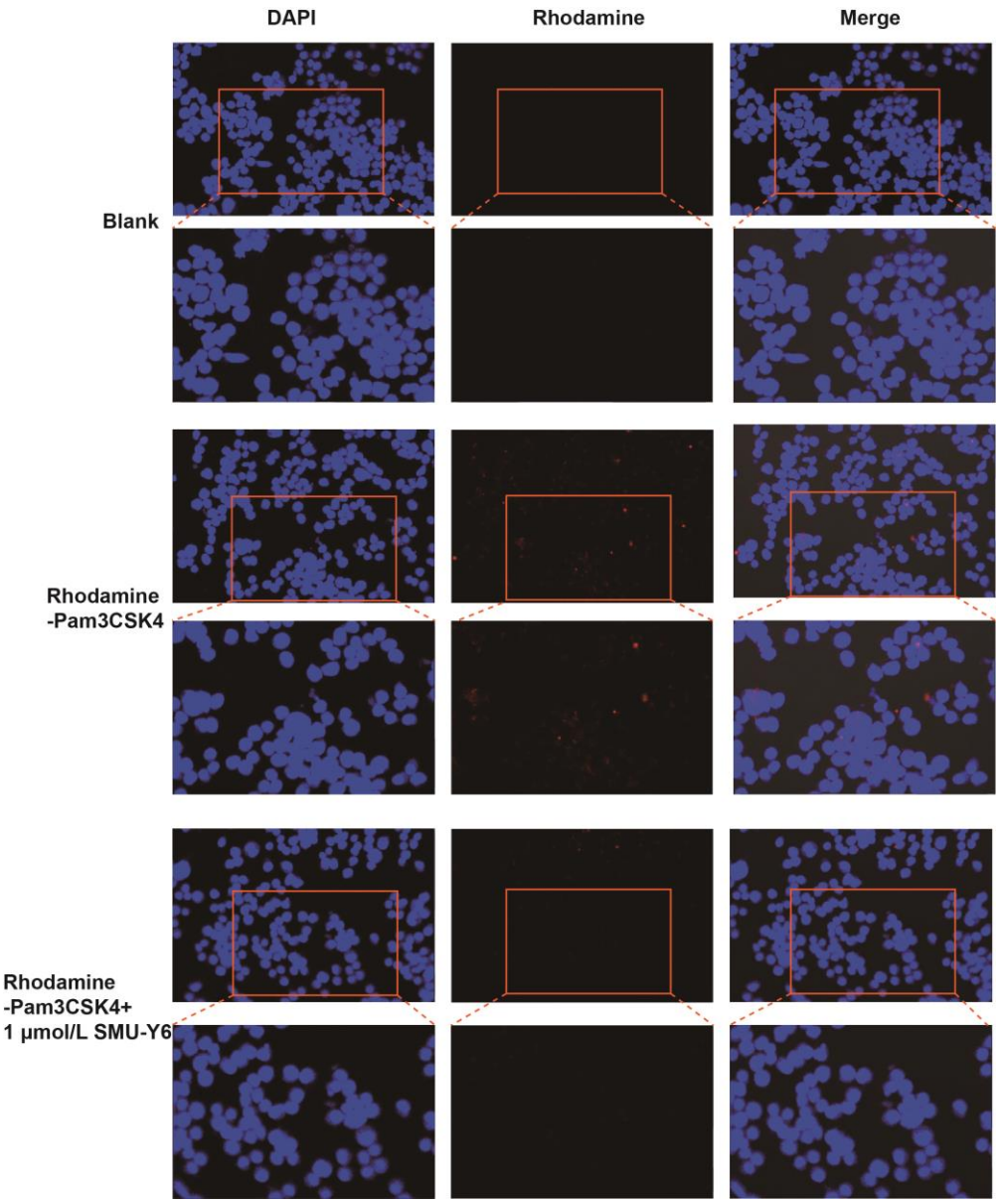


Figure S6. The competitive binding experiment between SMU-Y6 and Rhodamine-Pam$_3$CSK$_4$ in HEK-Blue hTLR2 cells. HEK-Blue hTLR2 cells were cultured with 800 ng/mL rhodamine-Pam$_3$CSK$_4$ and 1 μmol/L SMU-Y6 for 4 h. Cells were washed with PBS for three times. DAPI was added in and incubated for 5 min. Removed the supernatant and washed with PBS for three times.

Figure S7

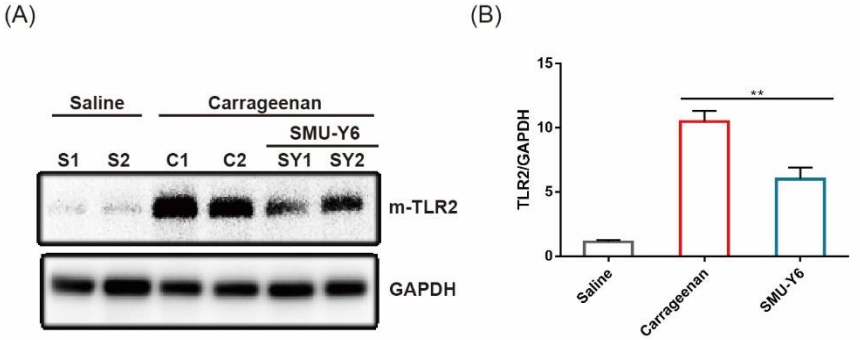


Figure S7. Western blot analysis of local tissue of carrageenan-induced paw edema model. (A) SMU-Y6 down-regulated the expression of TLR2 protein in carrageenan-induced paw edema model. Mice paw tissues were collected and washed in PBS. Proteins were extracted with tissue total protein lysis buffer. The supernatant was collected for Western Blot. **(B)** The ratio of TLR2 to GAPDH.

Figure S8

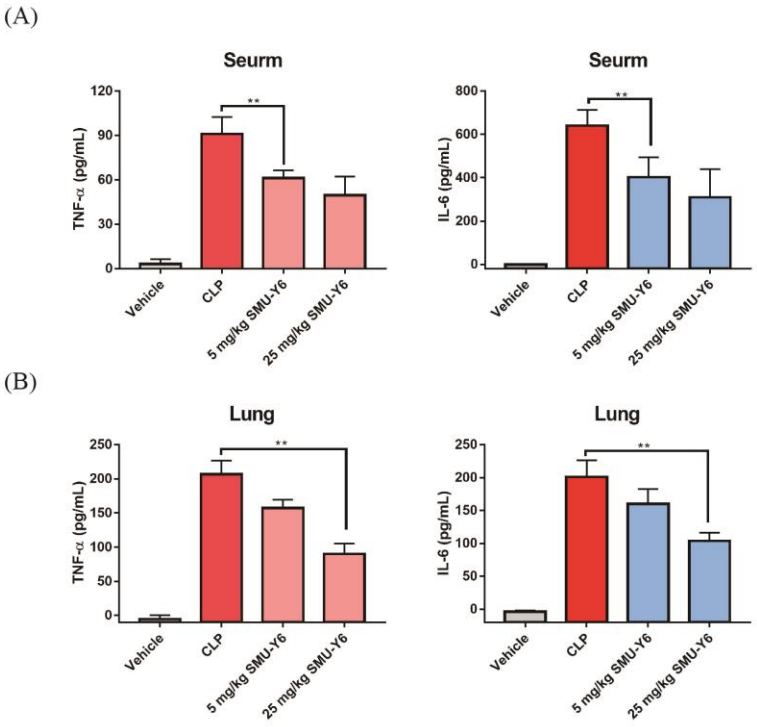


Figure S8. Inflammatory cytokines analysis of SMU-Y6 in CLP model. TNF-α and IL-6 were tested in mice **(A)** seurm and **(B)** lung tissue of CLP model. Mice were orally pretreated with salline, 5 mg/kg SMU-Y6, 25 mg/kg SMU-Y6 for 1 h. All mice were treated with cecal ligation and puncture, except that the mice from the vehicle group was underwent laparotomy without cecal ligation and puncture. All mice were sacrificed 8 h after surgery. Seurm and lung tissue were collected for Elisa assay.

Figure S9

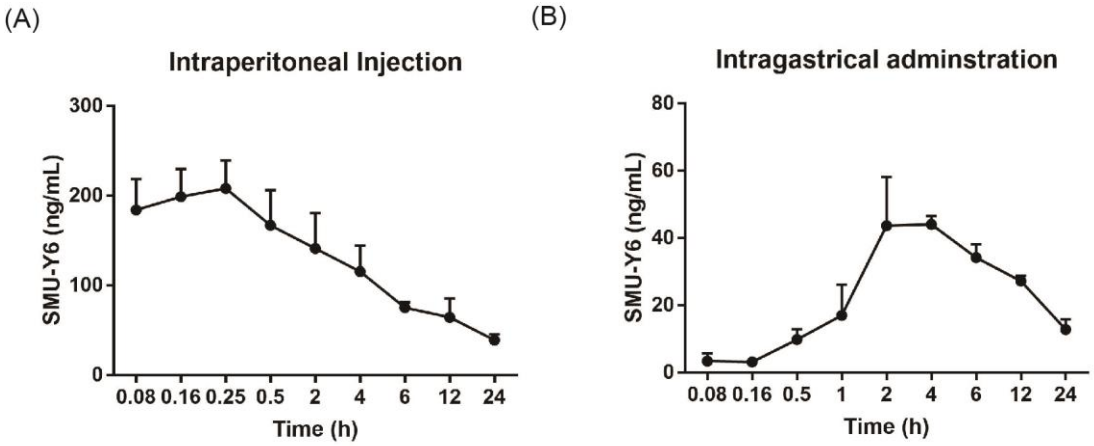


Figure S9. Plasma concentration-time curve of SMU-Y6 of two different adminstration. (A) Plasma concentration-time curve of SMU-Y6 in intraperitoneal injection adminstartion. **(B)** Plasma concentration-time curve of SMU-Y6 in intragastrical adminstartion.

Figure S10

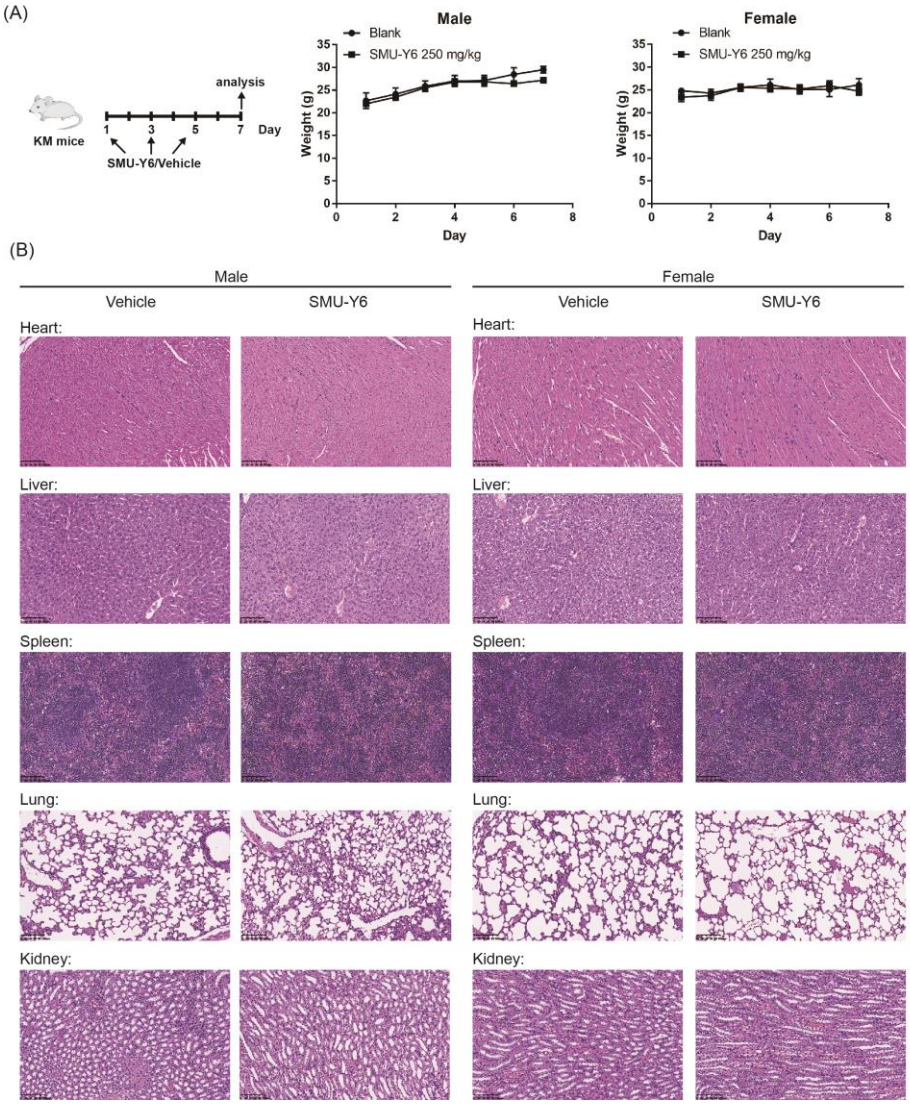
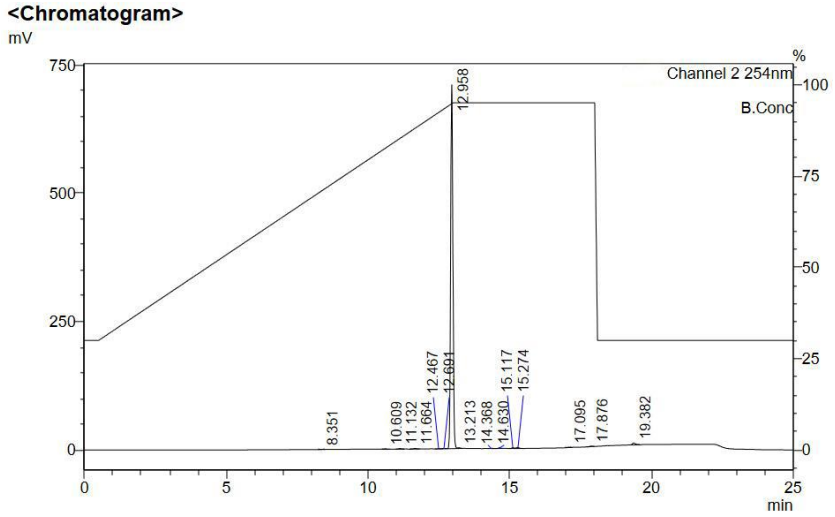


Figure S10. Toxicity studie os SMU-Y6 *in vivo*. (A) The change of mices' body weight in 7 days after contious oral adminstration of 250 mg/kg SMU-Y6. **(B)** HE staining results of mice tissue at day 7, inlcuding heart, liver, spleen, lung and kidney.

Figure S11



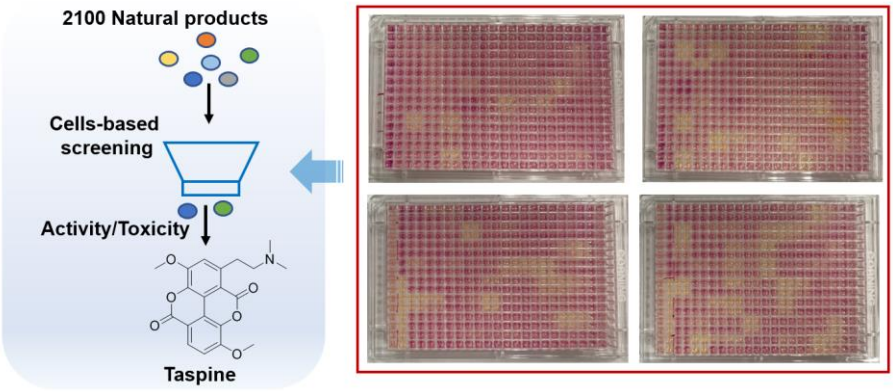
<Peak Table>

Channel 2 254nm

Peak#	Ret. Time	Area	Height	Area%
1	8.351	2226	399	0.055
2	10.609	3339	636	0.083
3	11.132	7878	1220	0.195
4	11.664	9589	1258	0.237
5	12.467	1605	257	0.040
6	12.691	3704	572	0.092
7	12.958	3938243	709170	97.387
8	13.213	8597	1434	0.213
9	14.368	3429	498	0.085
10	14.630	5784	1132	0.143
11	15.117	8918	1558	0.221
12	15.274	14170	2084	0.350
13	17.095	4709	677	0.116
14	17.876	6263	1042	0.155
15	19.382	25444	3461	0.629
Total		4043897	725398	100.000

Figure S11. The purity of SMU-Y6. The purity of SMU-Y6 was carried out through liquid chromatogram. Results showed that SMU-Y6 has a purity more than 97%.

Table S1. Natural compounds exhibited more than 90% inhibition to TLR2 SEAP signaling at 10 μM in HEK-bule hTLR2 cell.



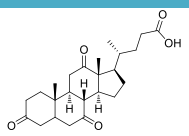
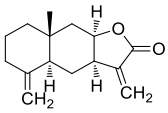
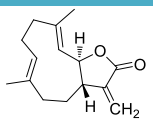
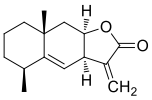
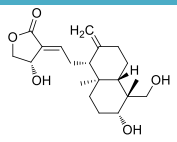
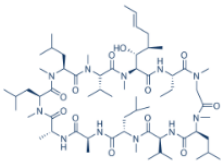
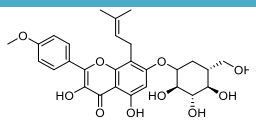
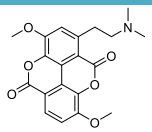
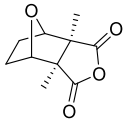
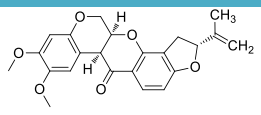
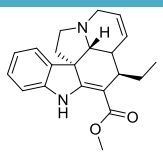
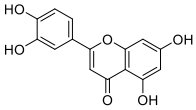
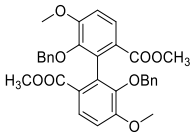
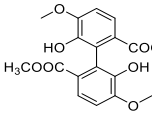
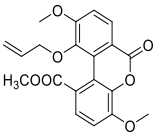
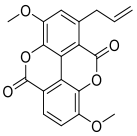
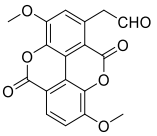
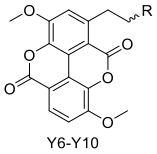
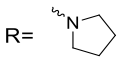
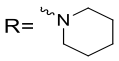
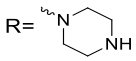
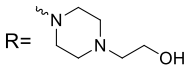
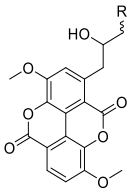
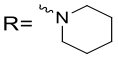
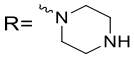
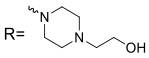
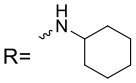
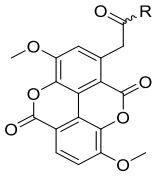
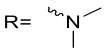
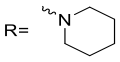
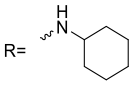
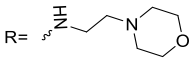
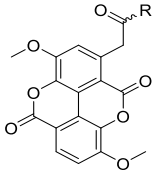
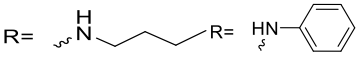
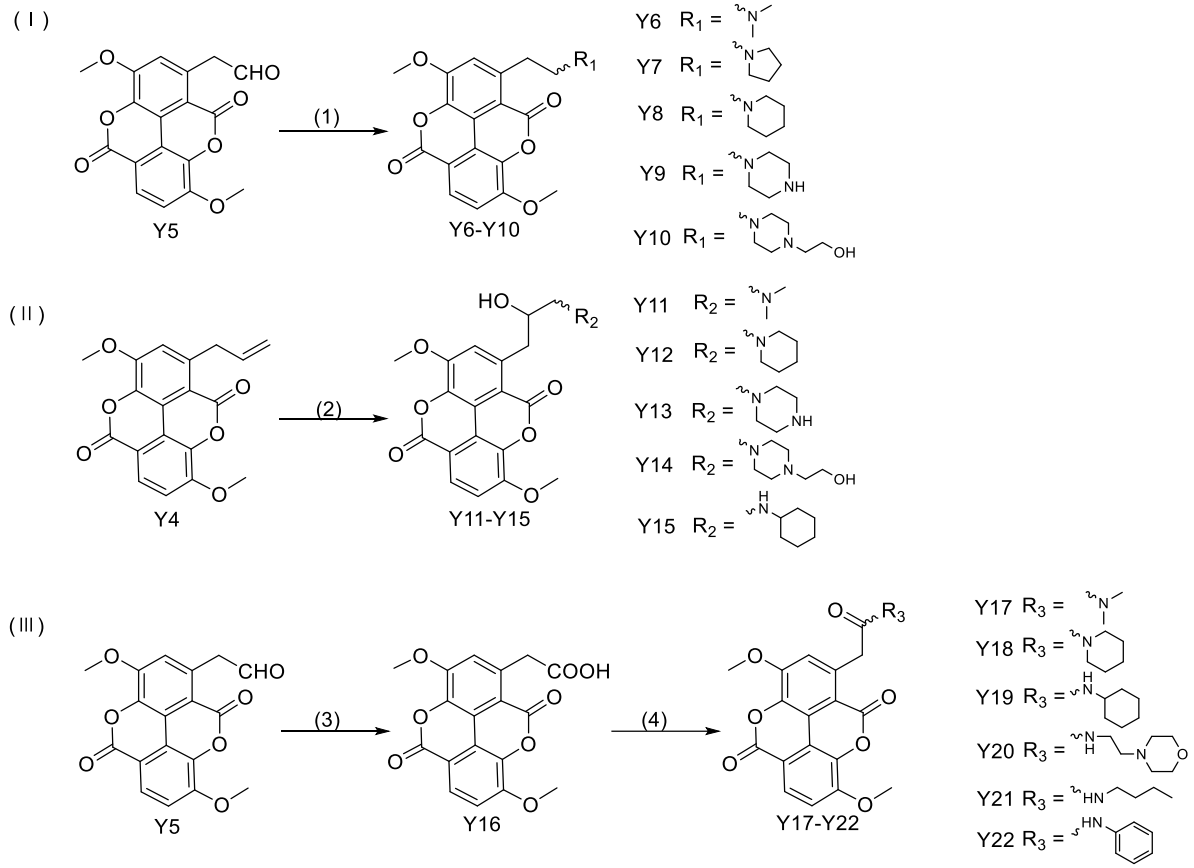
Compound	P1-D2	P5-E6	P7-B2	P7-A10
Structure				
Cas NO.	81-23-2	470-17-7	553-21-9	546-43-0
Compound	**P5-D3**	**P6-C11**	**P11-C4**	**P21-D9**
Structure				
Cas NO.	5508-58-7	59865-13-3	56725-99-6	602-07-3
Compound	**P5-E10**	**P13-G4**	**P8-G10**	**P14-E7**
Structure				
Cas NO.	56-25-7	83-79-4	4429-63-4	491-70-3

Table S2. The IC_{50} values of Y1-Y22 for the inhibition of TLR2 in HEK-Blue hTLR2 cells

Compound	Y1	Y2	Y3	Y4	Y5
Structure					
IC_{50}/μM[a]	>100	>100	>100	45.30±3.07	39.52±2.56
Compound	**Y6**	**Y7**	**Y8**	**Y9**	**Y10**
 Y6-Y10					
IC_{50}/μM[a]	1.42±0.33	5.83±2.19	1.70±0.15	2.55±0.07	1.44±0.25
Compound	**Y11(SMU-Y6)**	**Y12**	**Y13**	**Y14**	**Y15**
 Y11-Y15					
IC_{50}/μM[a]	0.11±0.04	1.34±0.19	3.05±0.16	2.30±0.07	5.92±0.69
Compound	**Y16**	**Y17**	**Y18**	**Y19**	**Y20**
 Y16-Y22					
IC_{50}/μM[a]	>100	>100	>100	>100	>100
Compound	**Y21**	**Y22**			
 Y16-Y22					
IC_{50}/μM[a]	>100	>100			

[a] The IC_{50} data was determined from at least three independent experiments.

Scheme S1



Scheme S1. Synthesis of Y5-Y22. Reagents and conditions used: (1)R_1NH, $NaBH_3CN$, CH_2Cl_2, 4h, rt, 30-40%. (2) a, m-CPBA, CH_2Cl_2, overnight; b, R_2NH, CH_2Cl_2, MeOH, TEA, rf. (3) NaH_2PO_4, $NaClO_2$, H_2O_2, CH_2Cl_2, *t*-BuOH, H_2O, rt, 4h, 40%; (4) a, $SOCl_2$, 4 h, rf; b, R_3NH_2 or R_3NH, CH_2Cl_2, TEA, 45°C, 3h, 20-30%.

Note S1. Synthesis, ^{1}H NMR, ^{13}C NMR spectrum and HRMS (ESI) of all the compounds.

Synthesis method and structure characterization.

General procedure for synthesis of compounds Y6-Y10

Compound Y5 was dissolved in CH_2Cl_2 and stirred for 30 min at room temperature. Then different aliphatic amines were added in stirred for 2 h. $NaBH_3CN$ was added in, and the mixture was stirred for another 4 h. The solvent was evaporated under vacuum, and the residue was purified by column chromatography (CH_2Cl_2: MeOH) to give product Y6-Y10.

1-(2-(dimethylamino)ethyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Taspine, Y6). Yield 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.18 (s, 1H), 4.10 (s, 6H), 3.50 (t, 2H), 2.66 (t, 2H), 2.38 (s, 6H). 13C NMR (101 MHz, CF3COOD) δ 156.0, 155.8, 142.5, 140.2, 139.9, 132.1, 123.6, 120.3, 119.4, 118.4, 117.9, 116.6, 115.6, 111.1, 61.9, 59.3, 59.2, 46.6.4, 34.0. ESI-HRMS *m/z*: calcld. $C_{20}H_{20}NO_6[M+H]^+$ 370.1285, found 370.1276.

3,8-dimethoxy-1-(2-(pyrrolidin-1-yl)ethyl)chromeno[5,4,3-cde]chromene-5,10-dione (Y7). Yield 37%. ^{1}H NMR (400 MHz, CF_3COOD) δ 9.85 (d, J = 8.8 Hz, 1H), 9.01 (d, J = 8.9 Hz, 1H), 8.84 (s, 1H), 5.64 (s, 3H), 5.62 (s, 3H), 5.29 (t, J = 8.2 Hz, 4H), 4.97 (m, 2H), 4.58 (m, J = 12 Hz, 2H) 3.57 (d, J = 14.4 Hz, 2H) 3.46 (t, J = 10.6 Hz 2H). ^{13}C NMR (101 MHz, CF_3COOD) δ 156.0, 155.8, 142.5, 140.2, 139.9, 132.1, 123.6, 120.3, 119.4, 118.4, 117.9, 116.6, 115.6, 111.1, 61.9, 59.3, 59.2, 46.6.4, 34.0. ESI-HRMS *m/z*: calcld. $C_{22}H_{22}NO_6[M+H]^+$ 396.1441, found 396.1411.

3,8-dimethoxy-1-(2-(piperidin-1-yl)ethyl)chromeno[5,4,3-cde]chromene-5,10-dione (Y8). Yield 30%. ^{1}H NMR (400 MHz, CF_3COOD) δ 9.90 (d, J = 8.2 Hz, 1H), 9.04 (d, J = 8.2 Hz, 1H), 8.86 (s, 1H), 5.67 (s, 3H), 5.65 (s, 3H), 5.30 (t, 4H), 4.99 (m, 2H), 4.60 (m, J = 10.8 Hz, 2H), 3.61 (d, J = 14.1 Hz, 2H), 3.58-3.49 (m, 3H), 3.14-3.08 (m, 1H). ^{13}C NMR (101 MHz, CF_3COOD) δ 154.2, 154.1, 141.0, 138.4, 138.2, 130.4, 121.9, 118.6, 117.7, 116.7, 116.2, 114.9, 113.9, 109.5, 59.2, 57.6, 57.5, 56.3, 31.7, 24.2, 22.2. ESI-HRMS *m/z*: calcld. $C_{23}H_{24}NO_6[M+H]^+$ 410.1598, found 410.1583.

3,8-dimethoxy-1-(2-(piperazin-1-yl)ethyl)chromeno[5,4,3-cde]chromene-5,10-dione (Y9). Yield 27%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 8.6 Hz, 1H), 7.55 (s, 1H), 7.33 (d, J = 8.6 Hz, 1H), 4.15 (s, 3H), 4.11 (s, 3H), 3.98-3.90 (m, 2H), 3.67 (s, 2H), 3.29-3.21 (m, 2H), 2.80 (s, 2H), 2.25 (s, 2H), 1.95 (m, 3H). ^{13}C NMR (101 MHz, CF_3COOD) δ 156.4,

156.2, 140.6, 140.4, 132.5, 123.9, 122.0, 121.0, 119.9, 118.8, 118.3, 117.1, 116.0, 111.7, 61.3, 59.9, 59.7 58.4, 33.8, 26.3, 24.4. ESI-HRMS *m/z*: calcld. $C_{22}H_{23}NO_6[M+H]^+$ 411.1632, found 410.1636.

1-(2-(4-(2-hydroxyethyl)piperazin-1-yl)ethyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Y10). Yield 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.20 (d, *J* = 8.7 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.18 (s, 1H), 4.10 (s, 6H), 3.63 (t, *J* = 4.4 Hz, 2H), 3.55-3.49 (m, 2H), 2.72-2.56 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 157.9, 151.4, 151.0, 144.4, 138.0, 136.9, 127.1, 119.4, 118.6, 116.7, 113.8, 111.8, 109.4, 59.3, 59.2, 57.8, 56.7, 56.6, 53.1, 53.0, 32.5. ESI-HRMS *m/z*: calcld. $C_{24}H_{27}N_2O_7[M+H]^+$ 455.1812, found 455.1801

General procedure for synthesis of compounds Y11-Y15.

Step 1. Compound Y4 was dissolved in CH_2Cl_2, added m-CPBA (75%) and stirred at room temperature for 24h. The organic layer was extracted with 1M NaOH solution, then evaporated under vacuum. The residue was washed by EtOAc to get white solid. The crude products proceed directly to the next step without purification.

Step 2. The crude products obtained above was dissolve in CH_2Cl_2: MeOH (4:1). Different aliphatic amines and triethylamine were added in the mixture and stirred for 48 h at 45°C. The organic solvent was removed under vacuum and the residue was purified by column chromatography (CH_2Cl_2: MeOH) to give product Y11-Y15.

1-(3-(dimethylamino)-2-hydroxypropyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Y11). Yield 25%. ^{1}H NMR (400 MHz, CF_3COOD) δ= 9.99 (d, *J* = 8.1 Hz, 1H), 9.22 (s, 1H), 9.04 (d, *J* = 8.1 Hz, 1H), 8.95 (s, 1H), 6.26 (s, 1H), 5.68 (s, 6H), 5.46 (d, *J* = 13.1 Hz, 1H), 5.14 (s, 2H), 4.81 (m, 1H), 4.67 (s, 3H), 4.60 (s, 3H). ^{13}C NMR (101 MHz, CF_3COOD) δ 166.0, 165.8, 155.8, 155.5, 144.1, 140.0, 139.9, 131.9, 123.2, 121.7, 121.6, 117.8, 113.1, 111.4, 70.7, 65.5, 59.4, 59.2, 48.4, 45.0, 42.6. ESI-HRMS *m/z*: calcld. $C_{21}H_{22}NO_7[M+H]^+$ 400.13908, found 400.13913.

1-(2-hydroxy-3-(piperidin-1-yl)propyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Y12). Yield 31%. ^{1}H NMR (400 MHz, CF_3COOD) δ= 9.95 (d, *J* = 8.3 Hz, 1H), 9.13 (d, *J* = 8.2 Hz, 1H), 9.05 (s, 1H), 8.74 (s, 1H), 6.43 (s, 1H), 5.78 (s, 6H), 5.56 (d, *J* = 12.3 Hz, 1H), 5.45-5.36 (dd, *J* = 29.6, 7.9 Hz, 2H), 5.18 (d, 2H), 4.85 (d, 2H), 4.66 (s, 1H),

3.95 (s, 1H), 3.64-3.55 (m, 5H), 3.21 (m, 1H). ^{13}C NMR (101 MHz, CF_3COOD) δ 166.0, 165.9, 155.9, 155.6, 144.4, 140.1, 140.0, 132.0, 123.4, 121.9, 121.7, 118.0, 113.3, 111.6, 70.5, 64.9, 59.9, 59.6, 59.4, 56.7, 43.0, 32.4, 26.0, 25.8, 24.1. ESI-HRMS *m/z*: calcld. $C_{24}H_{26}NO_7[M+H]^+$ 440.1703, found 440.1694.

1-(2-hydroxy-3-(piperazin-1-yl)propyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Y13). Yield 35%. ^{1}H NMR (400 MHz, CF_3COOD) δ= 9.88 (d, *J* = 8.1 Hz, 1H), 9.03 (d, *J* = 8.0 Hz, 1H), 8.92 (s, 1H), 6.39 (s, 1H), 5.75-5.72 (m, 2H), 5.66 (s, 6H), 5.57-5.41 (m, 8H), 5.30 (s, 2H), 4.80 (s, 1H). ^{13}C NMR (101 MHz, CF_3COOD) δ 155.8, 155.5, 143.8, 139.9, 132.0, 123.3, 121.6, 119.4, 118.4, 117.8, 116.6, 115.6, 113.1, 111.4, 70.2, 65.3, 59.3, 59.2, 53.8, 51.7, 44.8, 42.6. ESI-HRMS *m/z*: calcld. $C_{23}H_{25}N_2O_7[M+H]^+$ 441.1656, found 441.1656.

1-*(2-hydroxy-3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Y14).* Yield 26%. ^{1}H NMR (400 MHz, CF_3COOD) δ= 9.91 (d, *J* = 8.0 Hz, 1H), 9.07 (d, *J* = 8.1 Hz, 1H), 8.95 (s, 1H), 6.42 (s, 1H), 5.84-5.79 (m, 8H), 5.71 (s, 6H), 5.57-5.47 (m, 5H), 5.21 (s, 2H), 4.86 (s, 1H). ^{13}C NMR (101 MHz, CF_3COOD) δ 165.7, 155.9, 155.5, 140.0, 132.0, 123.3, 121.6, 119.5, 118.5, 117.9, 116.7, 115.6, 113.1, 111.4, 70.2, 65.3, 62.4, 59.4, 59.2, 58.5, 54.3, 53.1, 53.0, 52.3, 42.7. HRMS *m/z*: calcld. $C_{25}H_{29}N_2O_9[M+H]^+$ 485.1918, found 485.1914..

1-(3-(cyclohexylamino)-2-hydroxypropyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione (Y15). Yield 45%. ^{1}H NMR (400 MHz, MeOD) δ= 8.10 (d, *J* = 8.7 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.37 (s, 1H), 4.25-4.11 (m, *J* = 11.0, 3.8 Hz, 1H), 4.12 (s, 3H), 4.11 (s, 3H), 3.75-3.71 (dd, *J* = 12.9, 4.1 Hz, 1H), 3.28-3.25 (dd, 1H), 3.16-3.10 (dd, *J* = 12.6, 9.2, 3.9 Hz, 3H), 2.18 (d, *J* = 6.0 Hz, 1H), 2.09 (d, *J* = 7.5 Hz, 1H), 1.88 (dd, *J* = 10.9 Hz, 2H), 1.72 (d, *J* = 11.2 Hz, 1H), 1.46-1.34 (m, 4H), 1.27-1.21 (m, 1H). ^{13}C NMR (101 MHz, CF_3COOD) δ 161.7, 154.5, 154.2, 142.7, 138.7, 130.7, 122.1, 121.0, 117.1, 116.5, 115.3, 114.3, 112.5, 112.4, 71.5, 58.2, 57.9, 41.4, 31.0, 25.7, 25.5, 25.4. ESI-HRMS *m/z*: calcld. $C_{25}H_{28}NO_7[M+H]^+$ 454.1860, found 454.1849.

2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-1-yl)acetic acid (Y16). To a suspension of Y5 (50 mg, 0.15 mmol) in a mixture of CH_2Cl_2, H_2O, and t-BuOH (10 mL, 3:1:1) was added NaH_2PO_4 (11 mg, 0.07 mmol)、$NaClO_2$(27 mg, 0.3

mmol) and H_2O_2 (47 μL, 0.45 mmol). The mixture was stirred at room temperature for 4h. The organic solvent was removed under vacuum and the residue was filtered. The solid was purified by column chromatography (CH_2Cl_2: MeOH = 10:1) to Y16 (15 mg, 50%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (d, J = 8.7 Hz, 1 H), 7.56 (d, J = 8.7 Hz, 1 H), 7.54 (s, 1 H), 4.15 (s, 2 H), 4.05 (s, 6H). ^{13}C NMR (101MHz, CF_3COOD): δ =181.0, 166.1, 165.8, 155.9, 155.5, 140.1, 140.0, 131.9, 123.1, 121.7, 121.4, 117.8, 113.2, 112.1, 59.3, 59.2, 43.3. ESI-HRMS *m/z*: calcld. $C_{18}H_{11}O_8[M+H]^+$ 355.0604, found 355.0601.

General procedure for synthesis of compounds Y17-Y122.

Step 1. Compound Y16 was added in $SOCl_2$ and stirred for 4 h at 76 °C. The $SOCl_2$ was removed under vacuum, and the crude products proceed directly to the next step. Step 2. The crude products obtained above was dissolve in CH_2Cl_2. Different aliphatic amines were added in and stirred for 3 h at 45°C. The organic solvent was removed under vacuum and the residue was purified by column chromatography (CH_2Cl_2: MeOH) to give product Y17-Y122.

2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-1-yl)-N,N-dimethylacetamide(Y17). Yield 20%. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.24 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1H), 7.18 (s, 1H), 4.11 (s, 8H), 3.22 (s, 3H), 2.83 (s, 3H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 168.4, 158.3, 156.8, 151.6, 137.9, 137.4, 127.4, 118.6, 117.9, 114.2, 112.3, 111.6, 107.8, 56.8, 56.6, 38.0, 34.9. ESI-HRMS *m/z*: calcld. $C_{20}H_{18}NO_7[M+Na]^+$ 408.0523, found 408.0521.

3,8-dimethoxy-1-(2-oxo-2-(piperidin-1-yl)ethyl)chromeno[5,4,3-cde]chromene-5,10-dione (Y18). Yield 23%. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.22 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.10 (s, 1H), 4.47 (s, 2H), 4.09 (s, 6H), 3.49-3.43 (d, 2H), 2.05-2.00 (m, 2H), 1.83-1.67 (m, 4H), 1.46 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.6, 151.6, 151.6, 156.8, 151.6, 143.0, 137.6, 127.5, 114.3, 114.0, 112.2, 111.8, 111.7, 105.6, 57.0, 56.8, 52.7, 49.7, 25.9, 24.9, 24.1. ESI-HRMS *m/z*: calcld. $C_{23}H_{22}NO_7[M+H]^+$ 426.1004, found 426.1005.

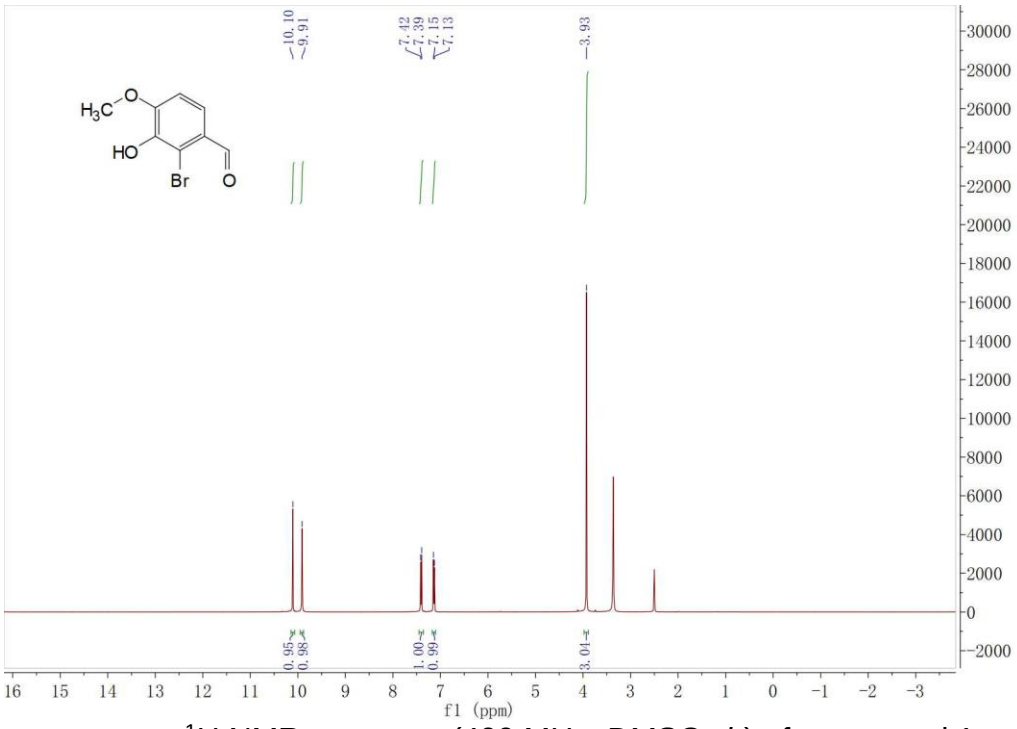
N-cyclohexyl-2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-1-yl)acetamide (Y19). Yield 27%. ^{1}H NMR (400 MHz, CF_3COOD) δ= 9.22 (d, J = 8.6 Hz,

1H), 9.05 (d, *J* = 8.6 Hz, 1H), 9.03 (s, 1H), 5.66 (d, 8H), 3.69 (d, *J* = 7.9 Hz, 2H), 3.36 (d, *J* = 10.0 Hz, 2H), 3.23 (d, *J* = 12.4 Hz, 1H), 3.03-2.89 (m, *J* = 11.5, 5 Hz, 5H), 2,79-2.74 (m, 1H). ^{13}C NMR (101 MHz, CF_3COOD) *δ* 173.8, 156.2, 155.6, 141.9, 140.1, 135.2, 132.4, 122.7, 120.7, 119.4, 118.4, 117.4, 116.6, 115.6, 113.2, 110.7, 59.7, 59.3, 55.9, 34.3, 27.4, 27.0. ESI-HRMS *m/z*: calcld. $C_{24}H_{24}NO_7[M+H]^+$ 438.1547, found 438.1544.

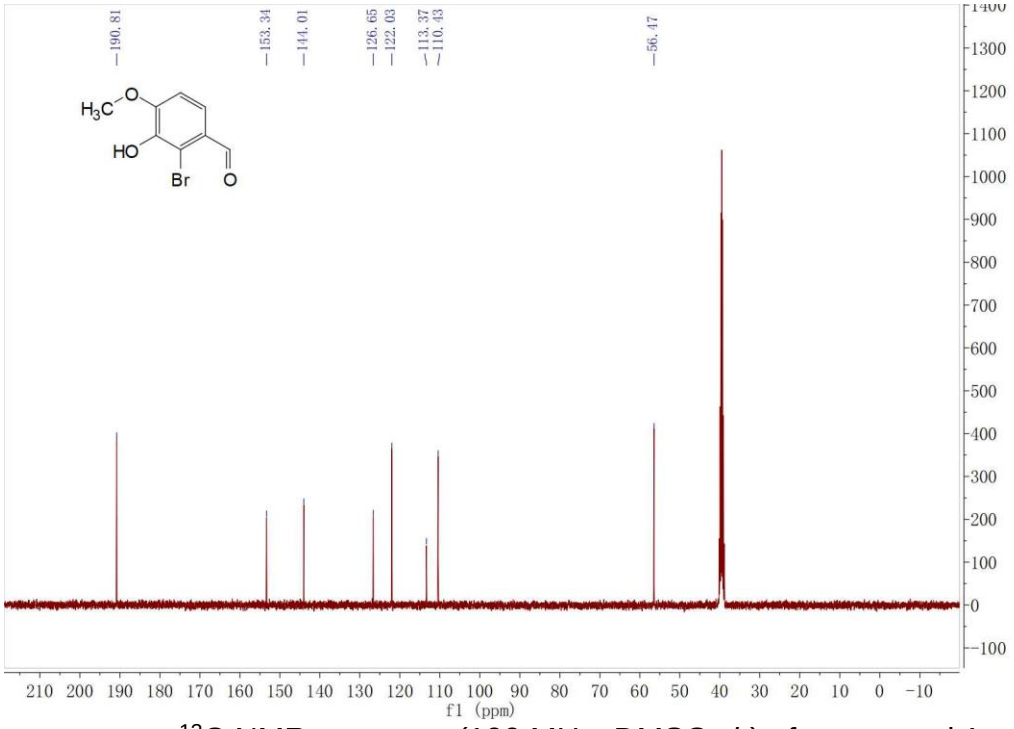
2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-1-yl)-N-(2-morpholinoethyl)acetamide (Y20). Yield 31%. ^{1}H NMR (400 MHz, CF_3COOD) *δ*= 9.97 (d, *J* = 8.5 Hz, 1H), 9.13 (d, *J* = 8.5 Hz, 1H), 8.95 (s, 1H), 6.11 (s, 2H), 5.97 (d, *J* = 13.3 Hz, 2H), 5.77-5.72 (m, 12H), 5.31 (s, 2H), 5.05-5.00 (t, *J* = 11.6 Hz, 2H). ^{13}C NMR (101 MHz, CF_3COOD) *δ* 162.8, 155.8, 155.7, 145.5, 141.0, 140.3, 132.2, 122.5, 121.2, 119.5, 118.4, 118.1, 116.7, 115.6, 113.5, 108.6, 67.5, 62.4, 59.5, 59.3, 57.2, 41.8. ESI-HRMS *m/z*: calcld. $C_{24}H_{25}N_2O_8[M+H]^+$ 471.1218, found 471.1211

N-butyl-2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-1-yl)acetamide (Y21). Yield 25%. ^{1}H NMR (400 MHz, CF_3COOD) *δ*= 9.97 (d, *J* = 8.7 Hz, 1H), 9.12 (d, *J* = 8.7 Hz, 1H), 9.10 (s, 1H), 6.17 (s, 2H), 5.73 (s, 6H), 3.16-3.13 (m, 2H), 2.47-2.44 (m, *J* = 6.9 Hz, 3H), 2.39-2.35 (m, 1H). ^{13}C NMR (101 MHz, CF_3COOD) *δ* 163.9, 154.4, 154.3, 139.2, 138.3, 135.1, 130.7, 117.8, 116.8, 116.5, 115.0, 113.9, 112.2, 111.1, 110.3, 57.9, 57.6, 44.0, 40.8, 31.0, 20.68, 13.0. ESI-HRMS *m/z*: calcld. $C_{22}H_{22}NO_7[M+H]^+$ 386.0659, found 386.0681

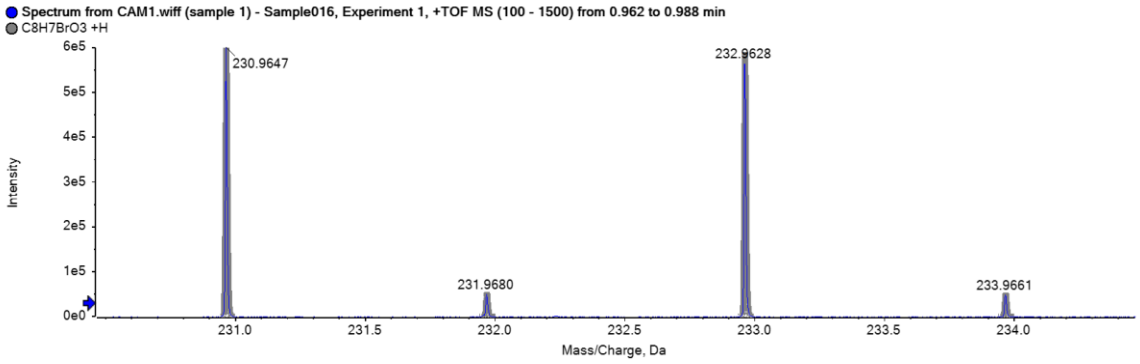
2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-cde]chromen-1-yl)-N-phenylacetamide (Y22). Yield 20%. ^{1}H NMR (400 MHz, DMSO-d_6) *δ*= 11.99 (s, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.57 (s, 1H), 7.49-7.46 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ* 194.9, 154.4, 158.2, 156.1, 150.9, 150.4, 143.7, 139.5, 137.2, 137.1, 128.6, 126.7, 126.3, 123.0, 117.7, 117.6, 115.0, 114.5, 111.2, 106.0, 57.1, 56.8. ESI-HRMS *m/z*: calcld. $C_{24}H_{18}NO_7[M+H]^+$ 432.1077, found 432.1075.



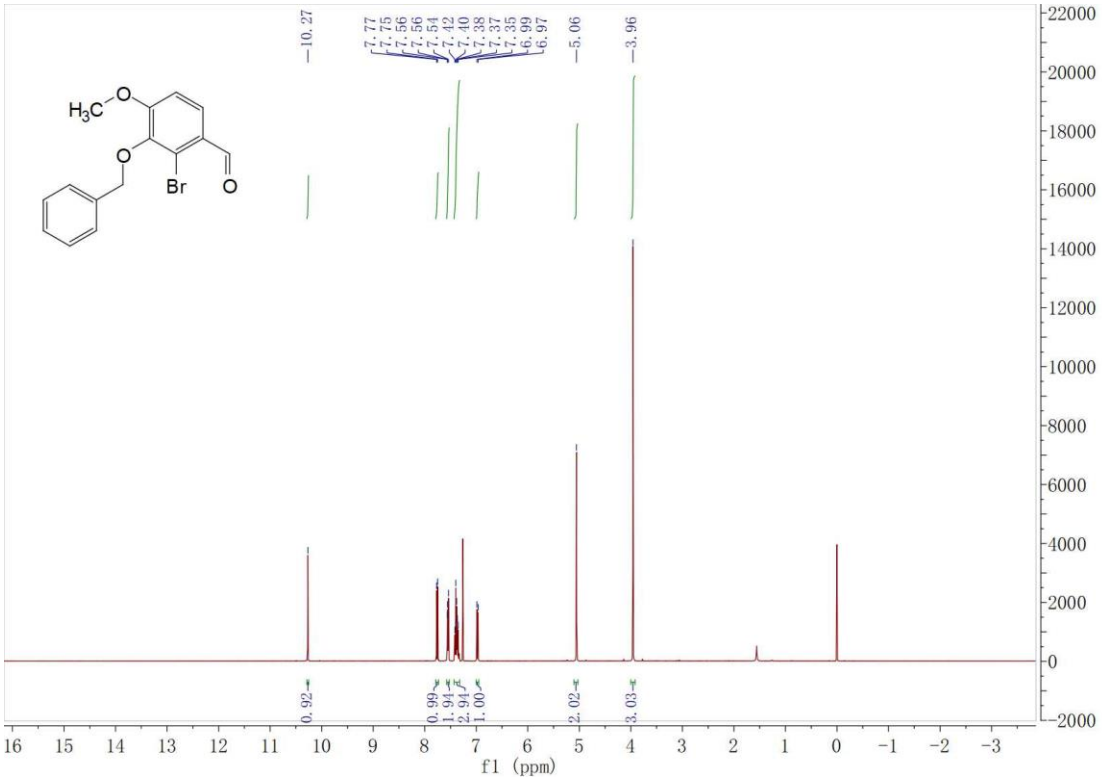
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound 1



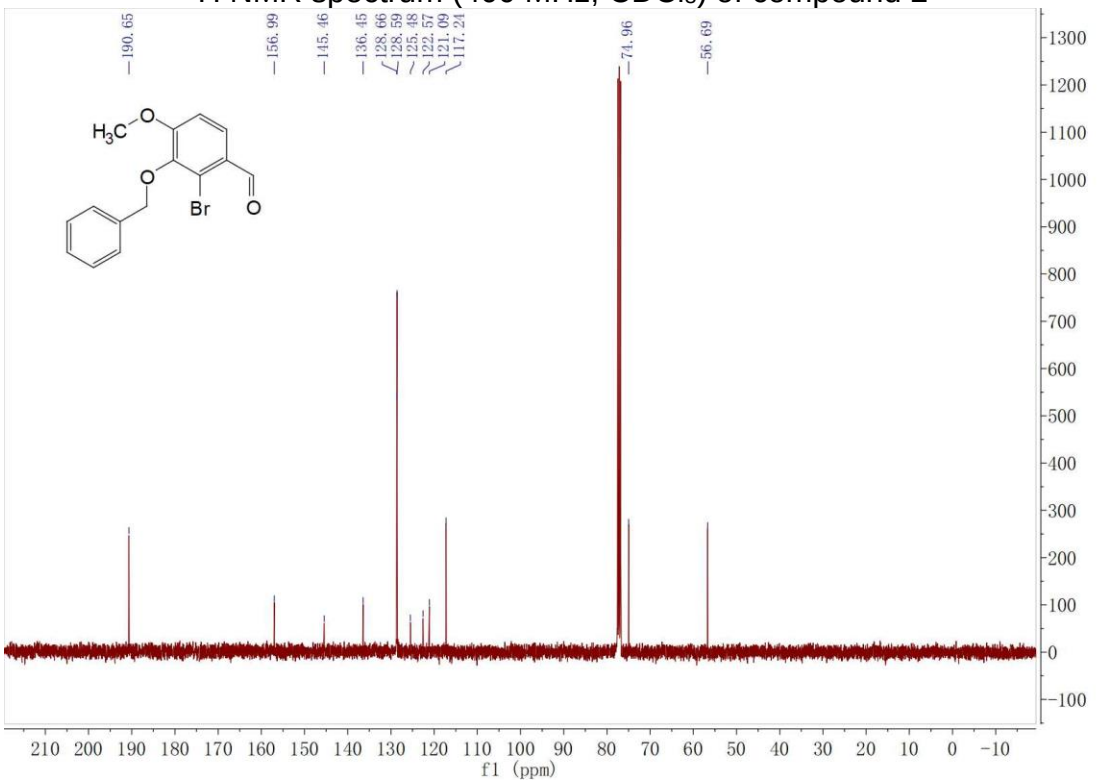
^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound 1



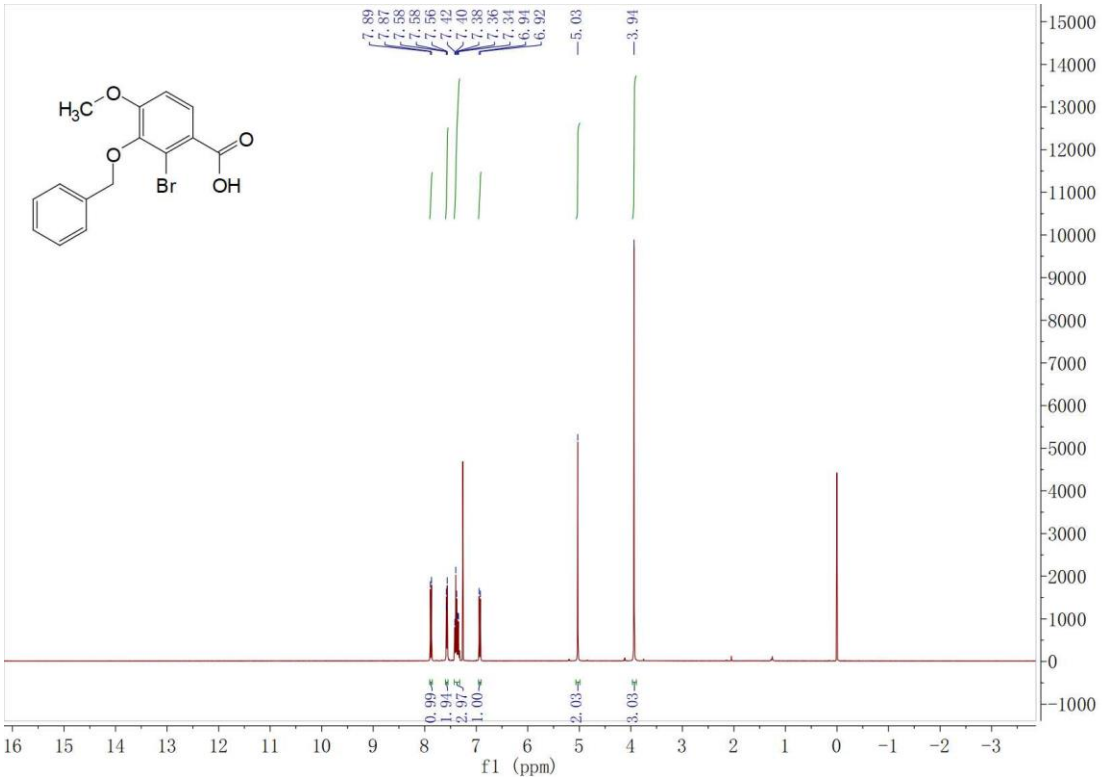
ESI-HRMS of compound 1



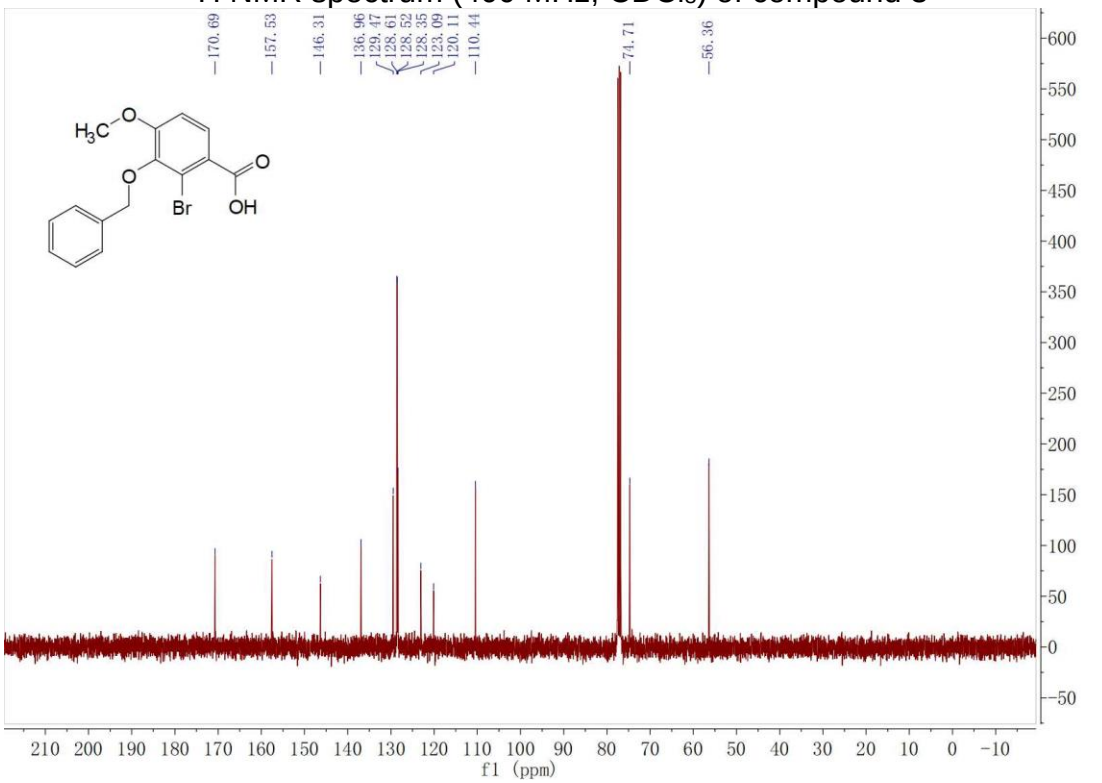
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 2



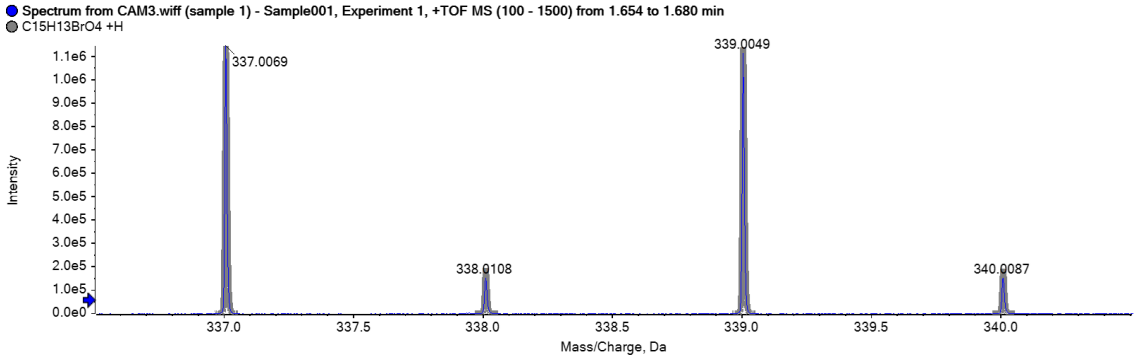
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound 2



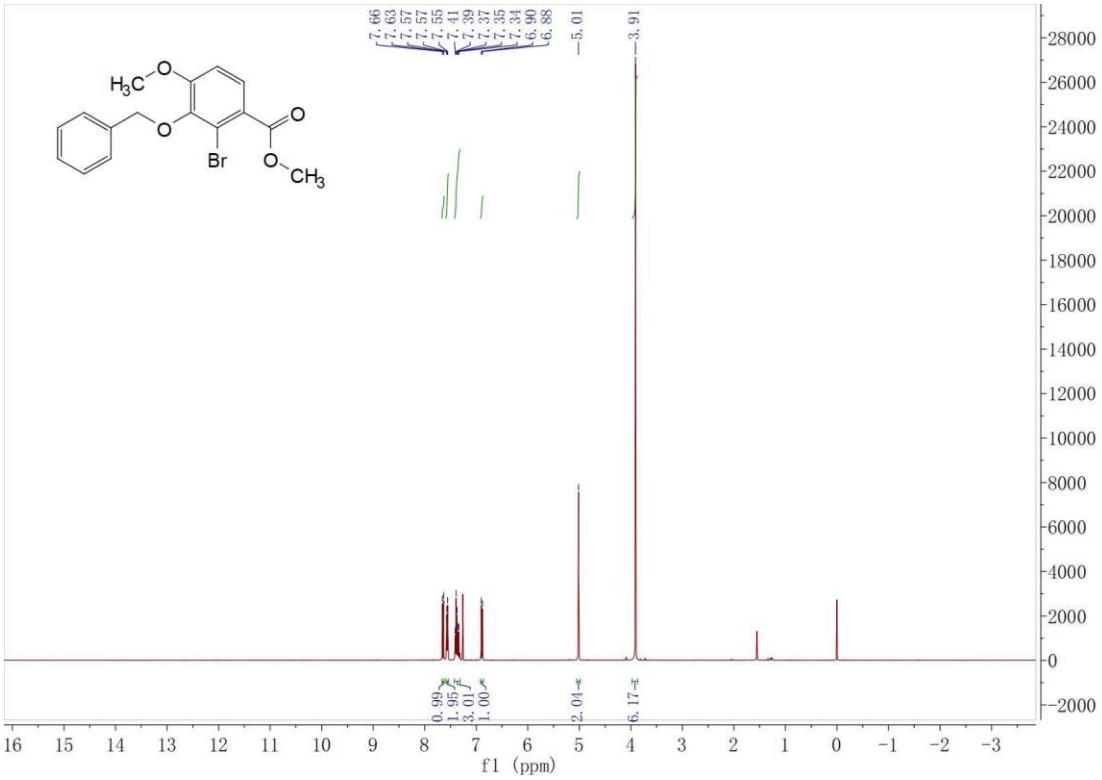
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 3



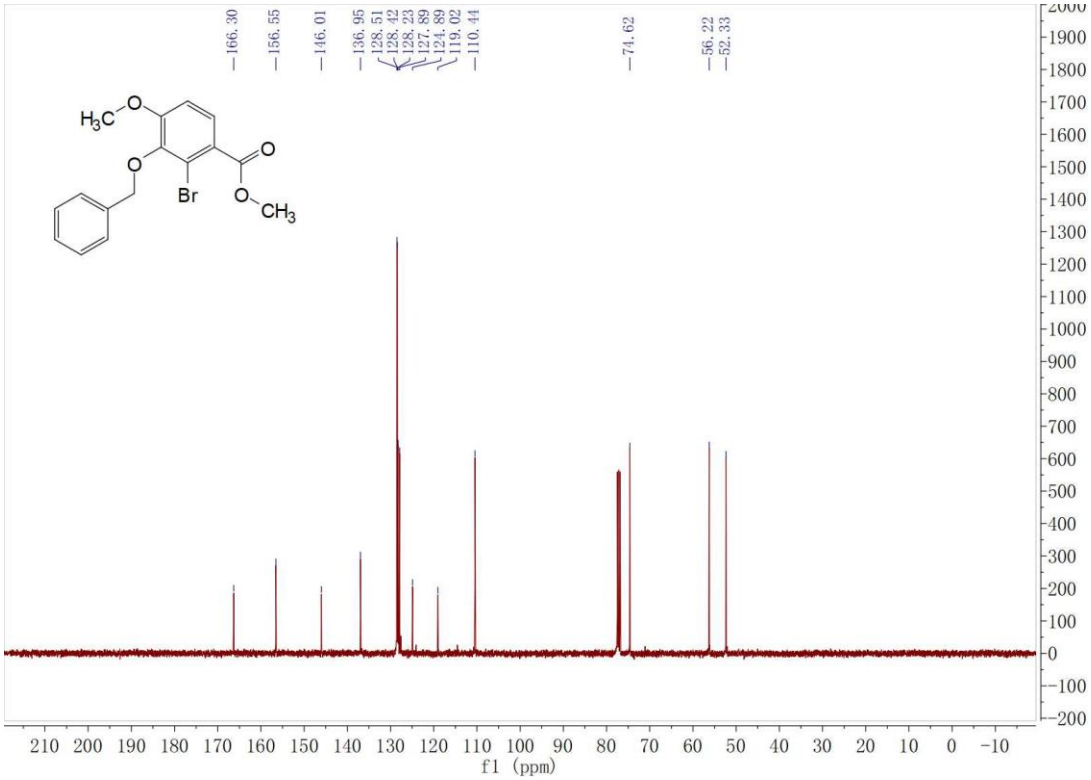
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound 3



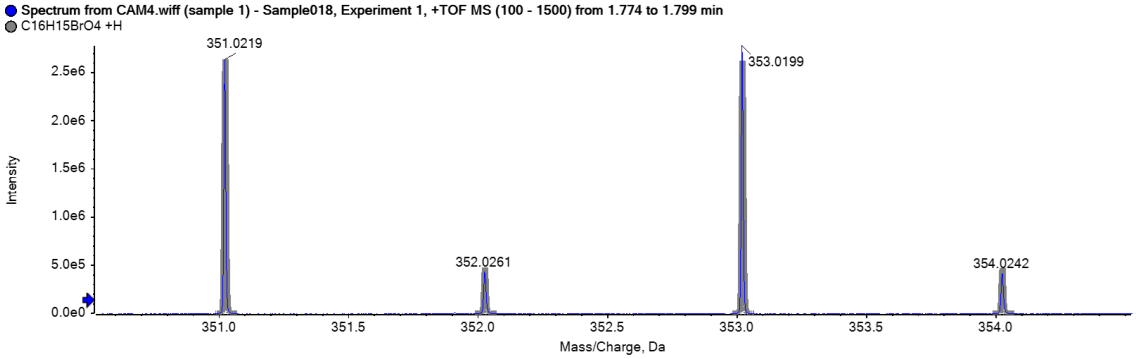
ESI-HRMS of compound 3



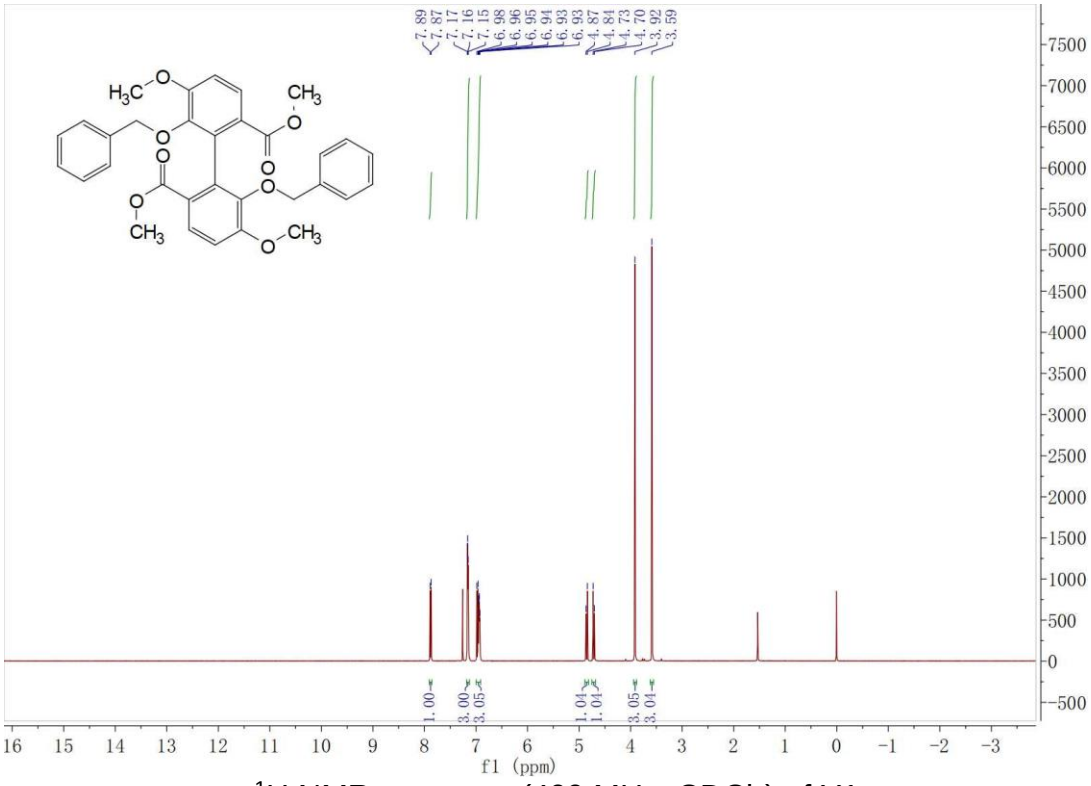
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 4



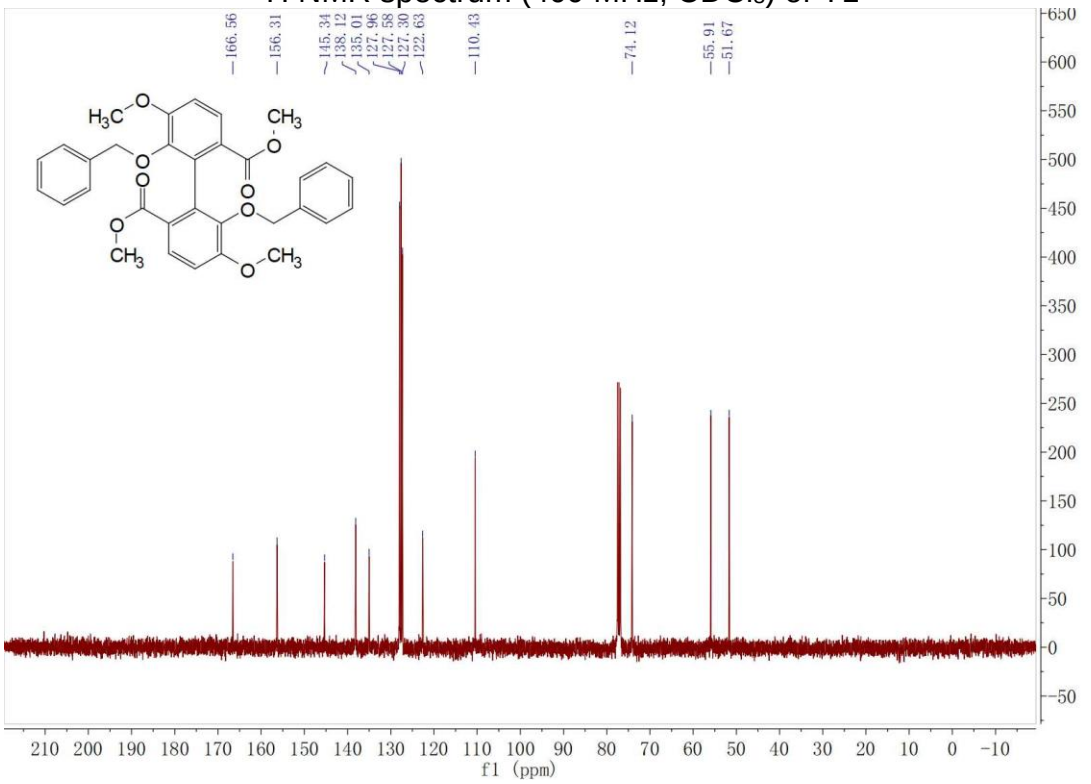
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound 4



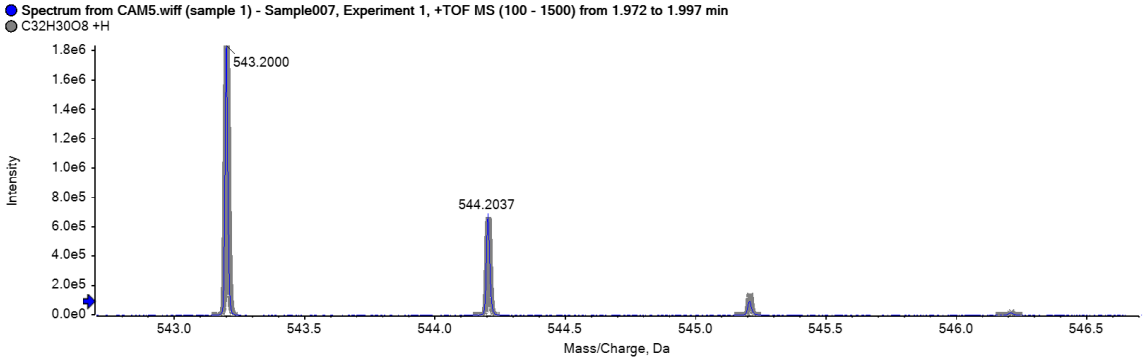
ESI-HRMS of compound 4



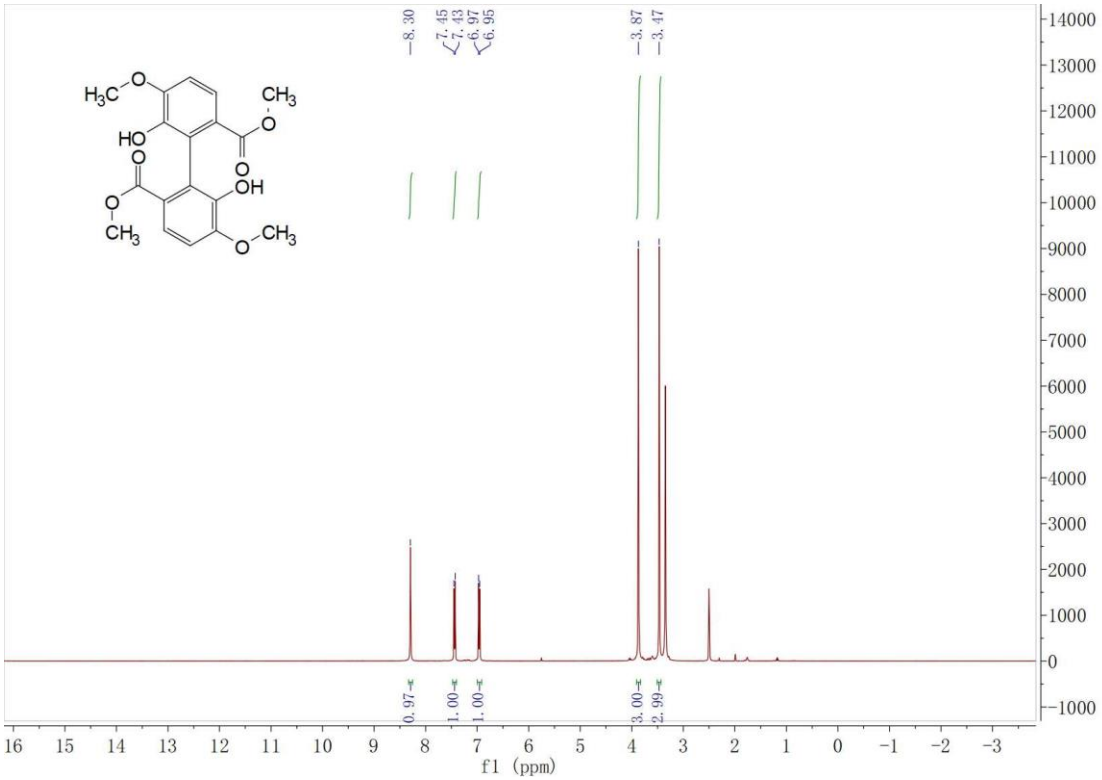
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y1



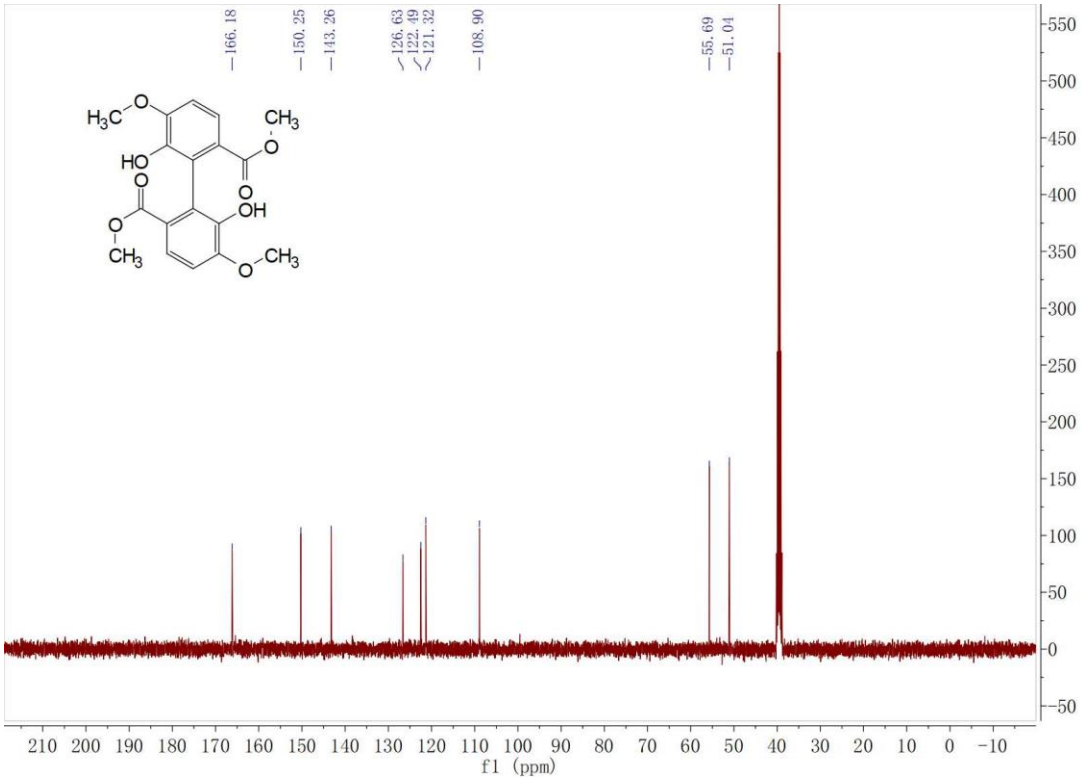
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of Y1



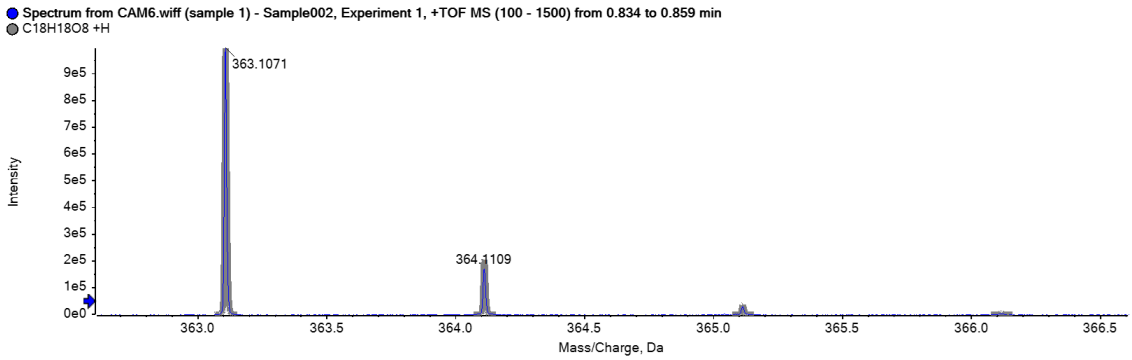
ESI-HRMS of Y1



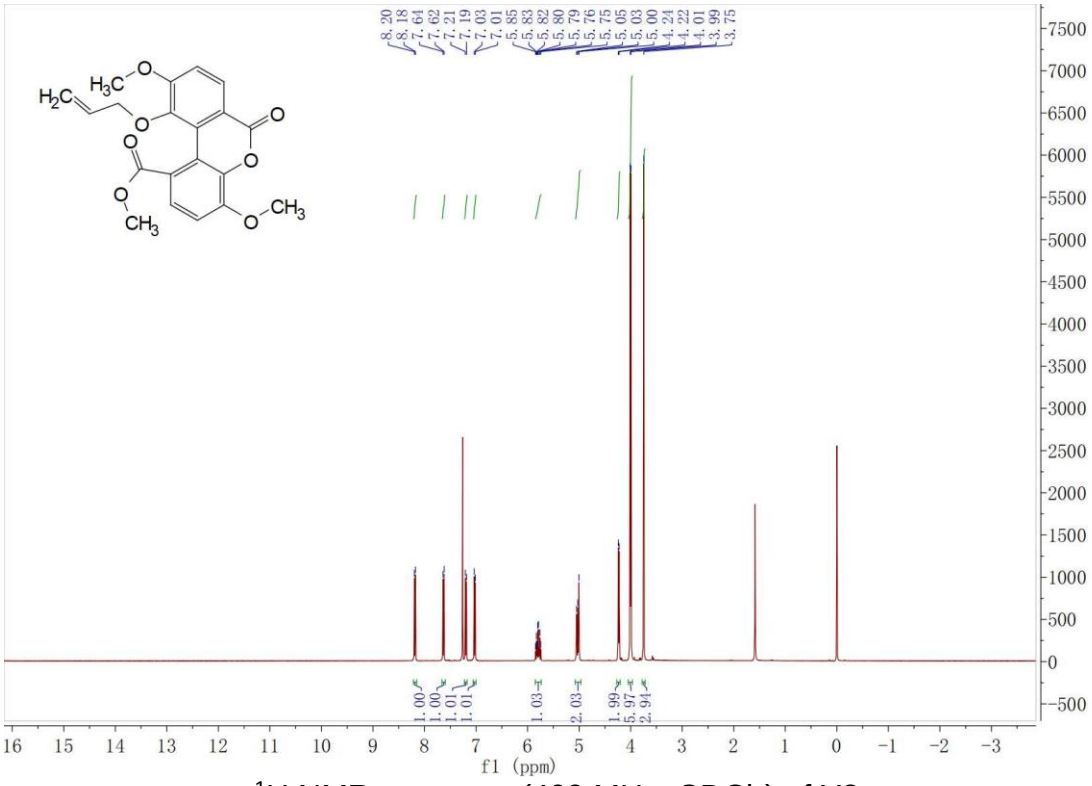
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of Y2



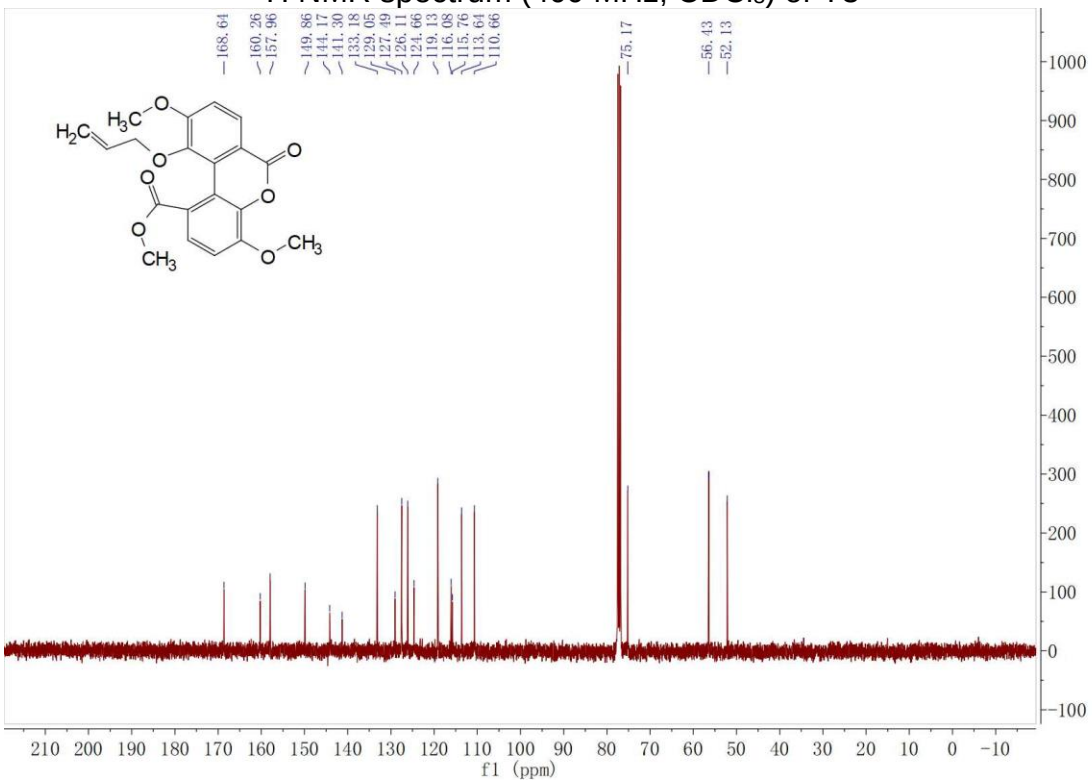
^{13}C NMR spectrum (100MHz, DMSO-d_6) of Y2



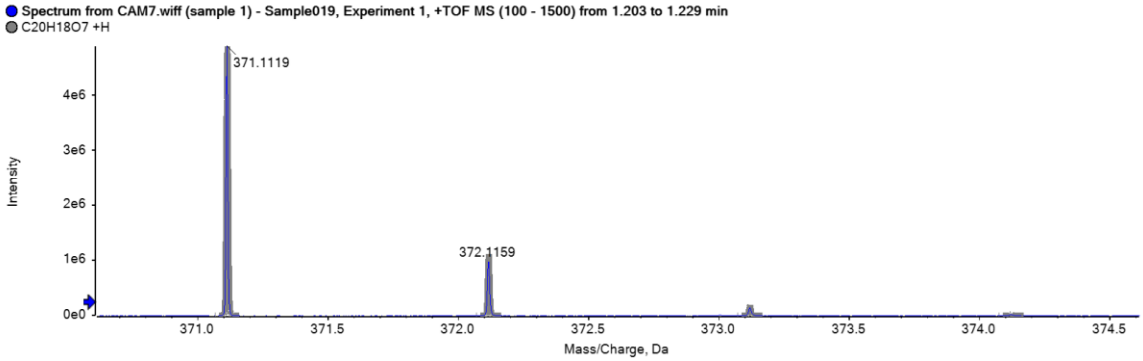
ESI-HRMS of Y2



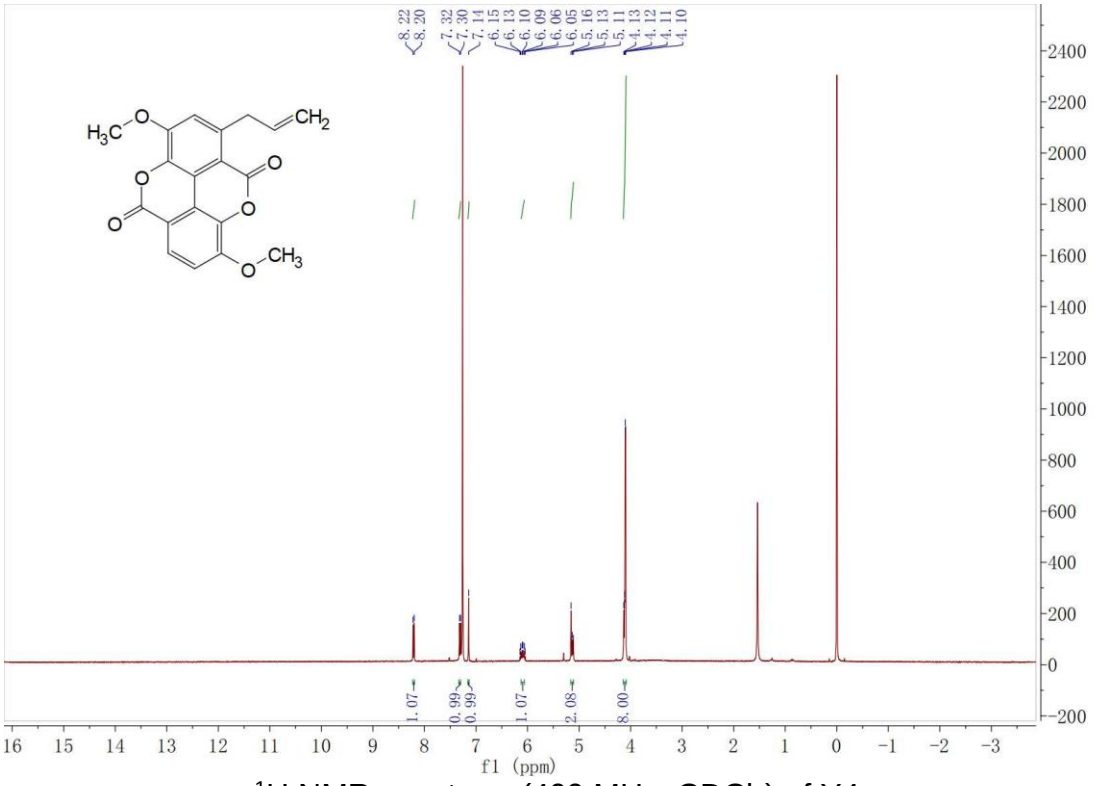
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y3



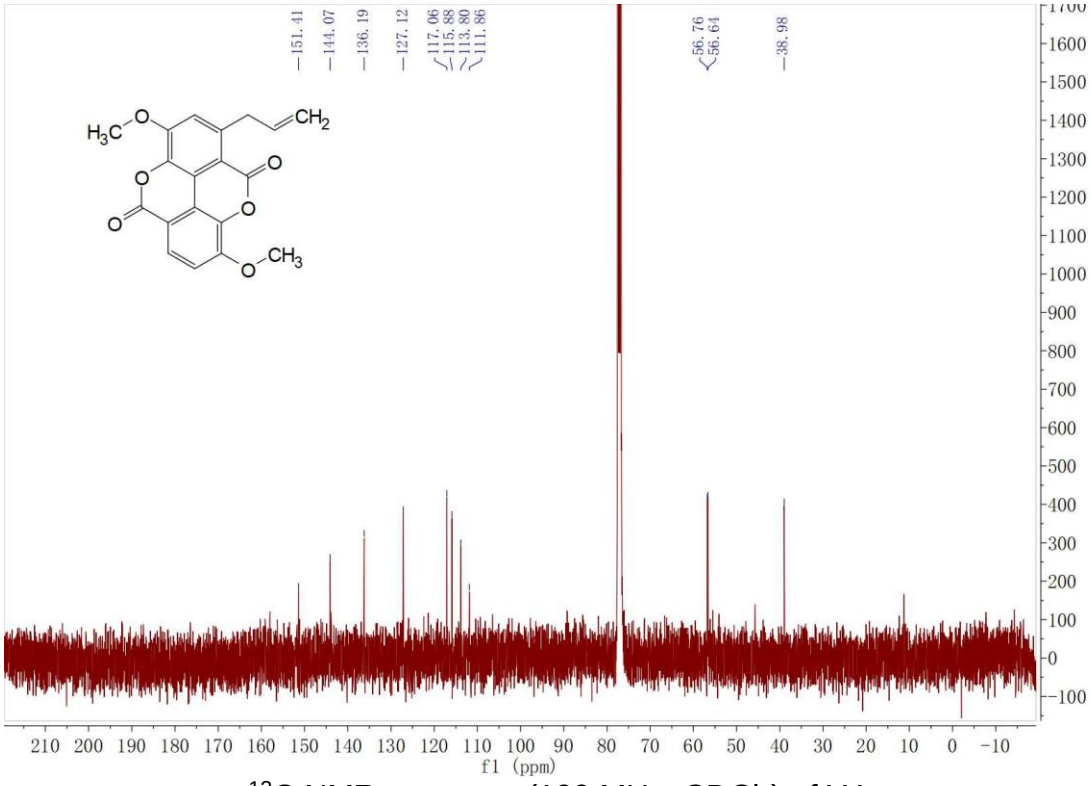
^{13}C NMR spectrum (100MHz, $CDCl_3$) of Y3



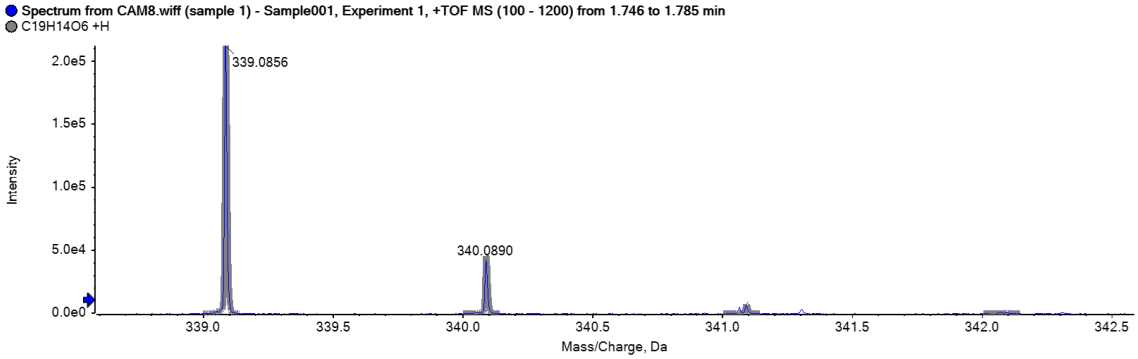
ESI-HRMS of Y3



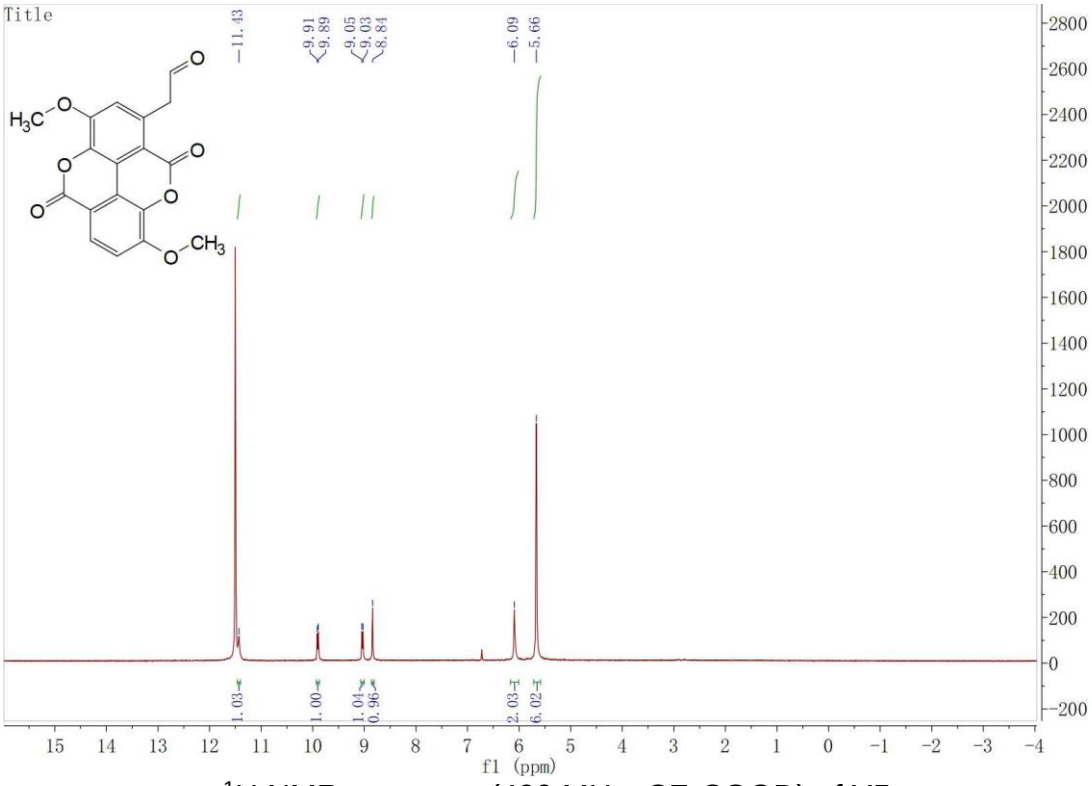
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y4



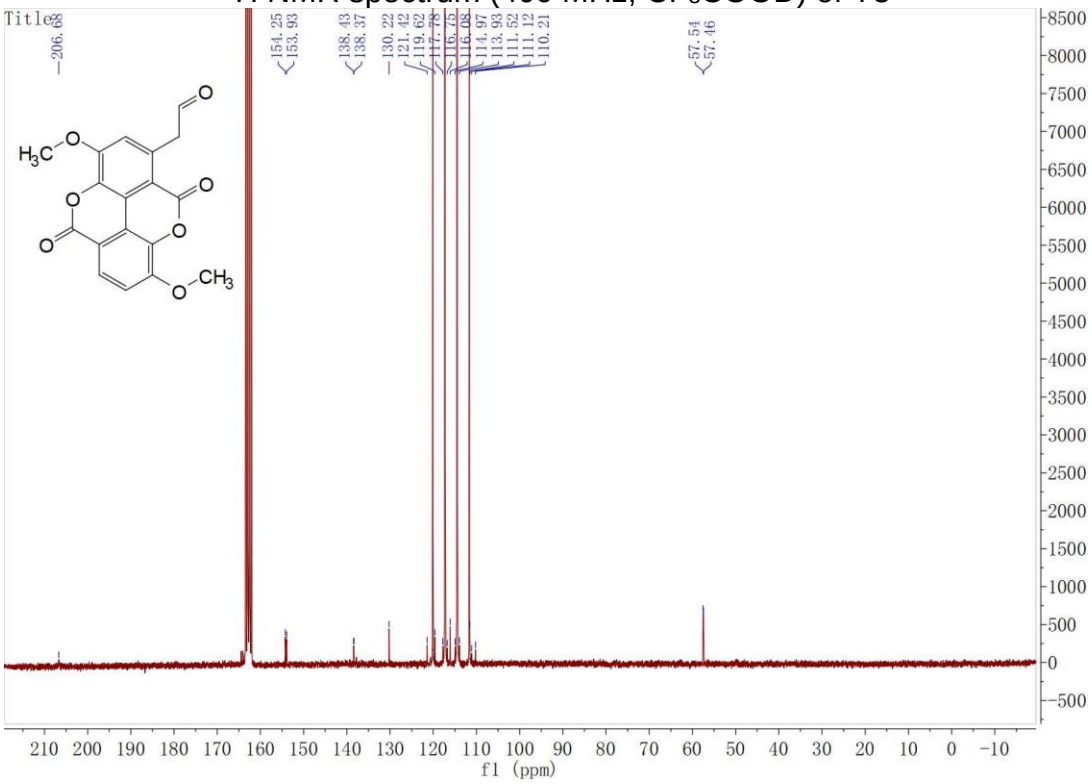
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of Y4



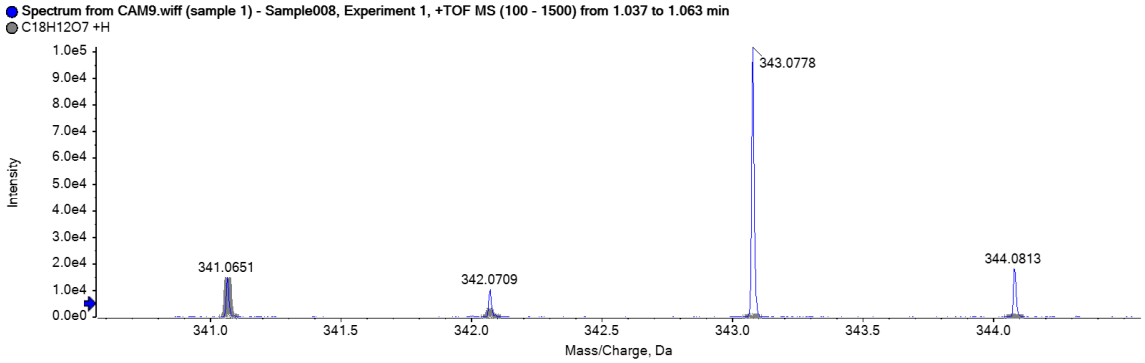
ESI-HRMS of Y4



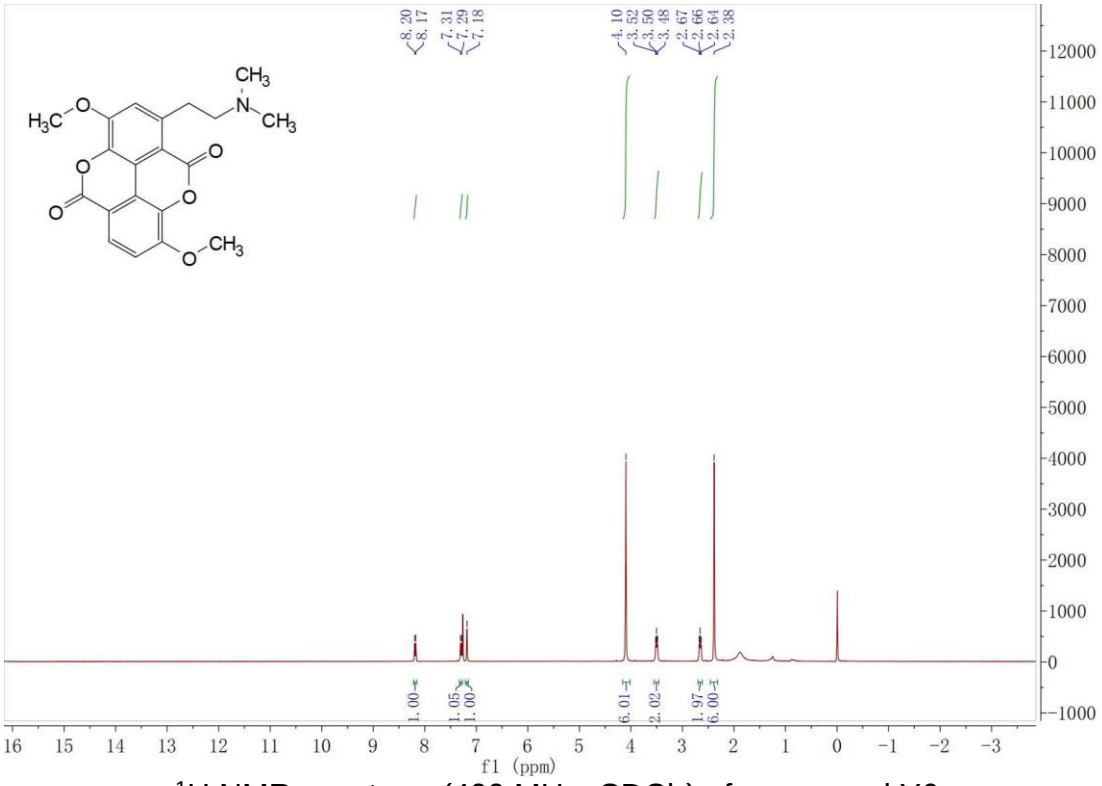
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y5



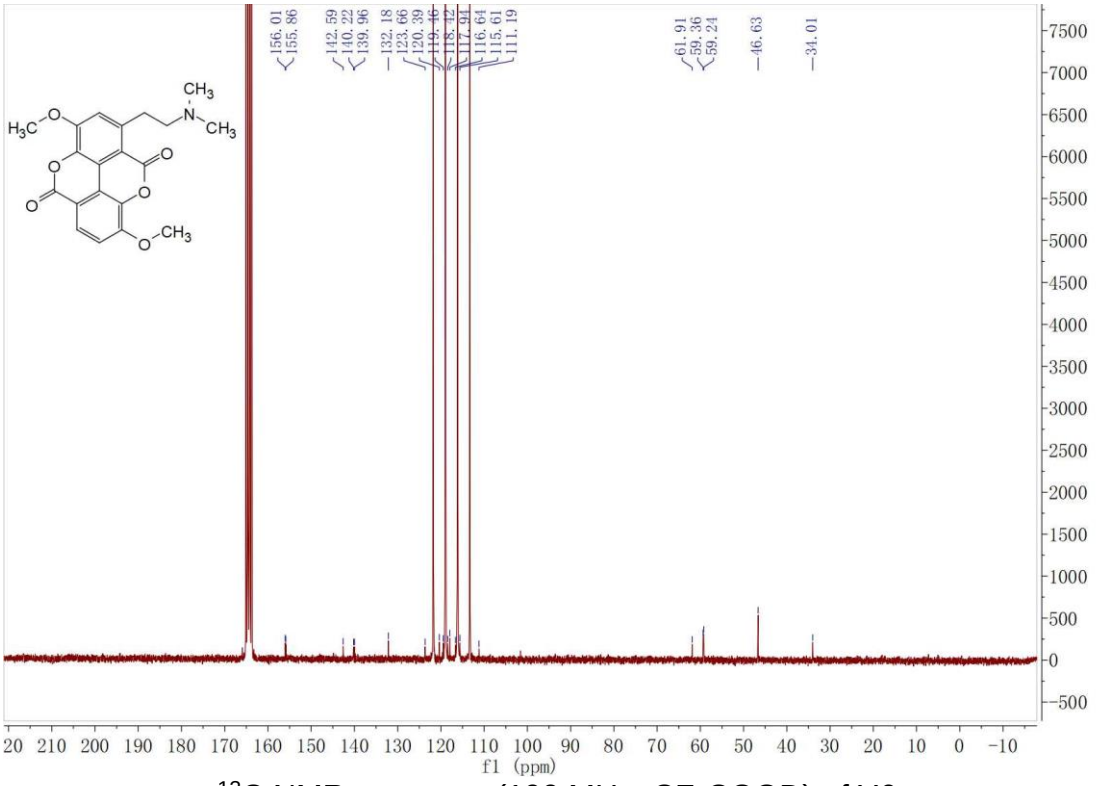
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y5



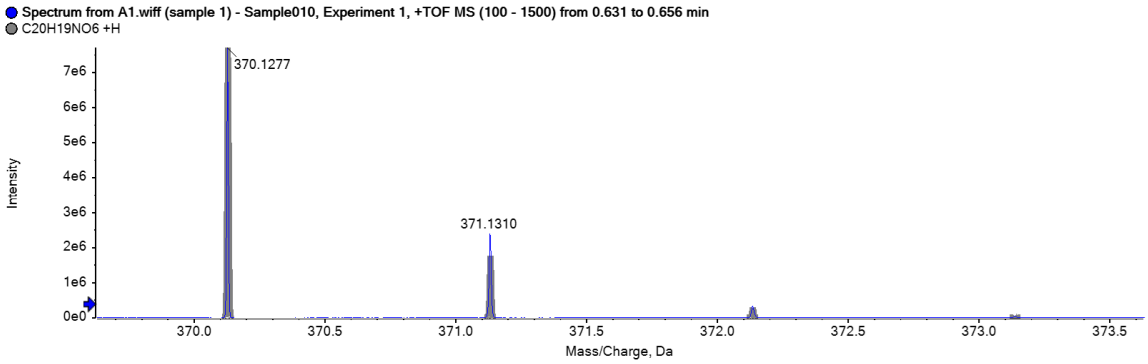
ESI-HRMS of Y5



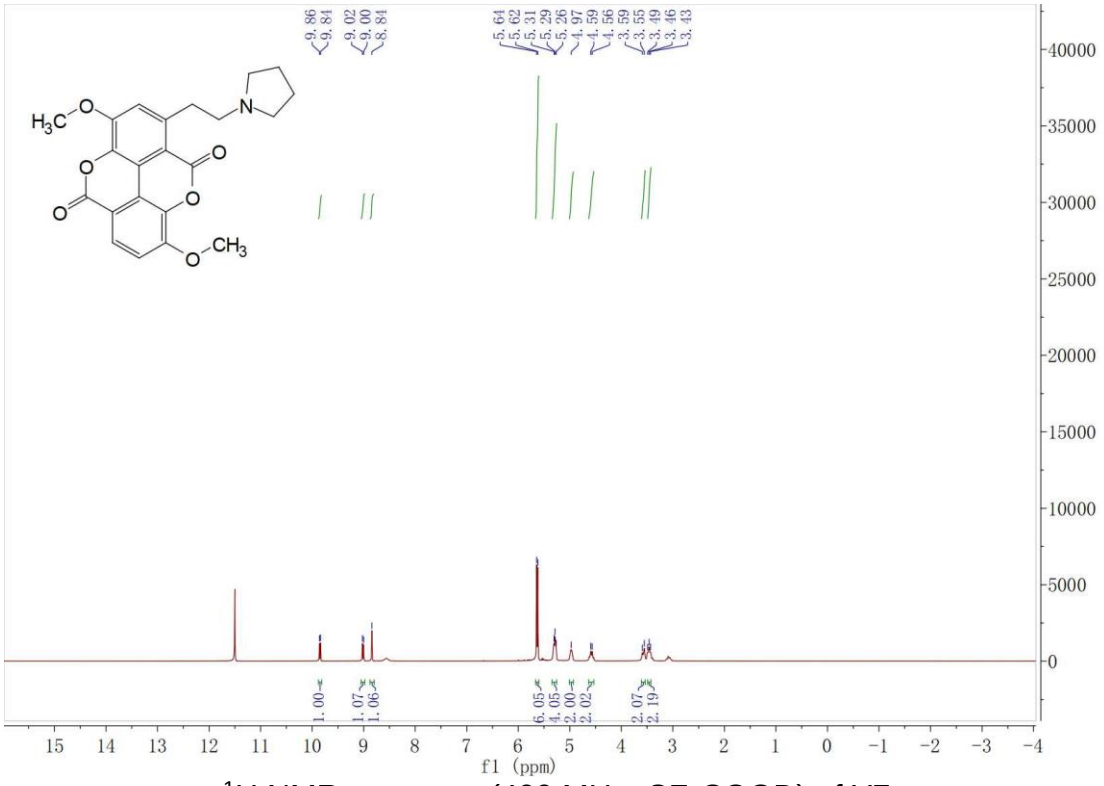
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound Y6



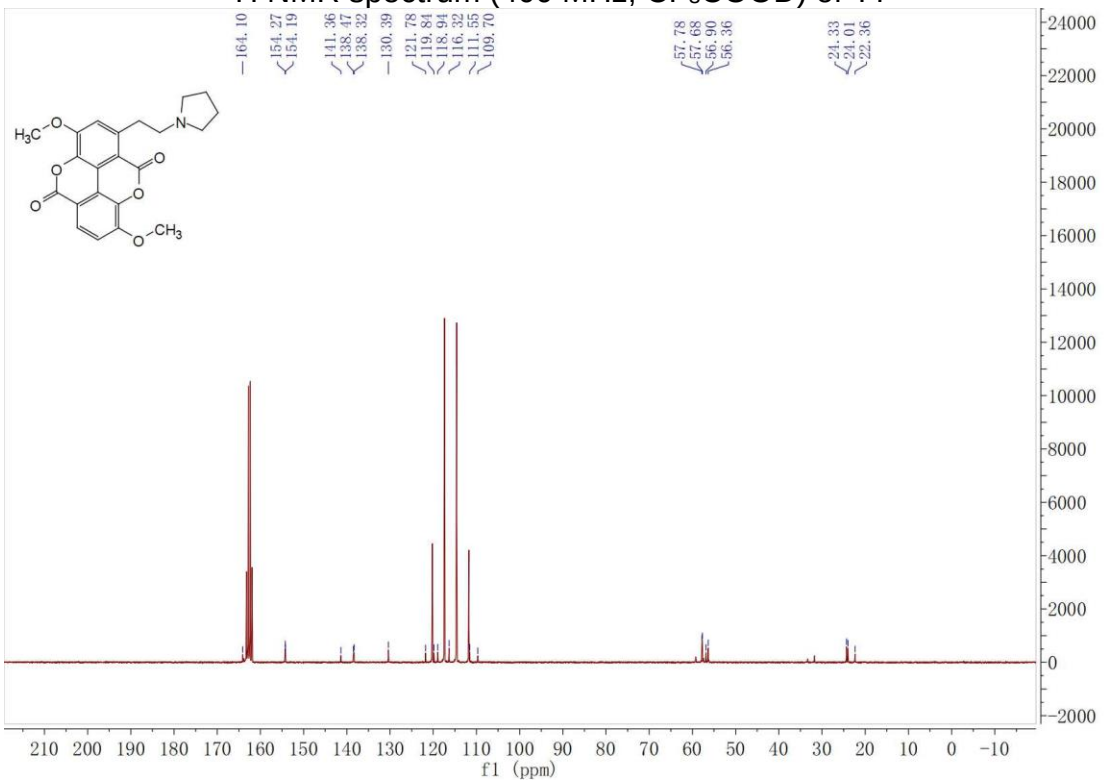
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y6



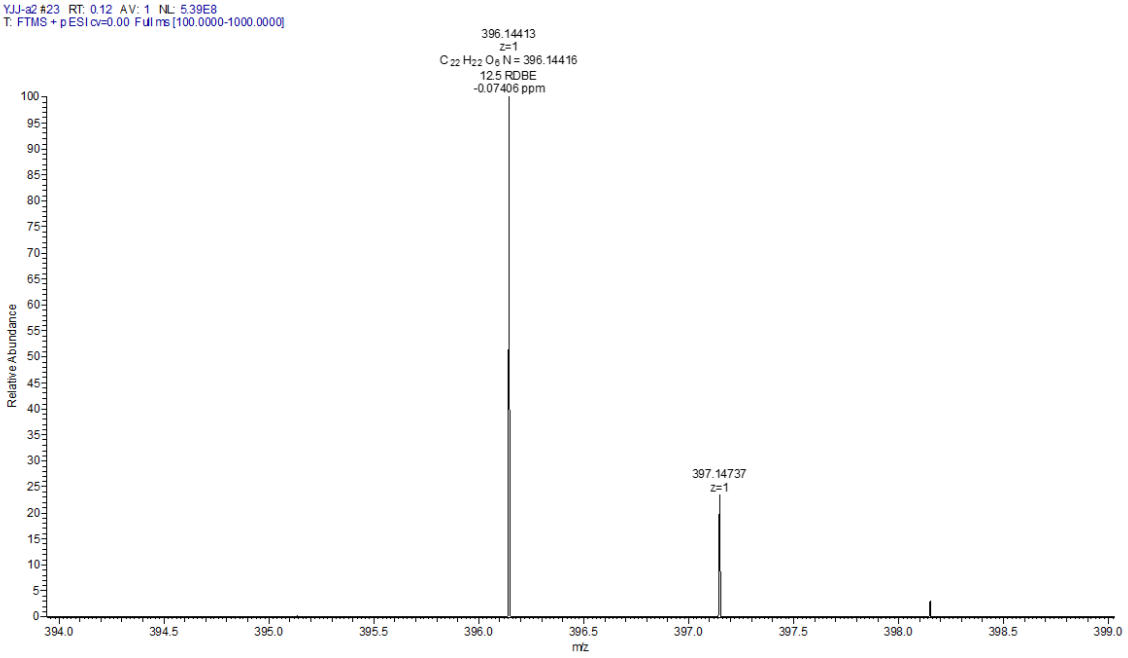
ESI-HRMS of Y6



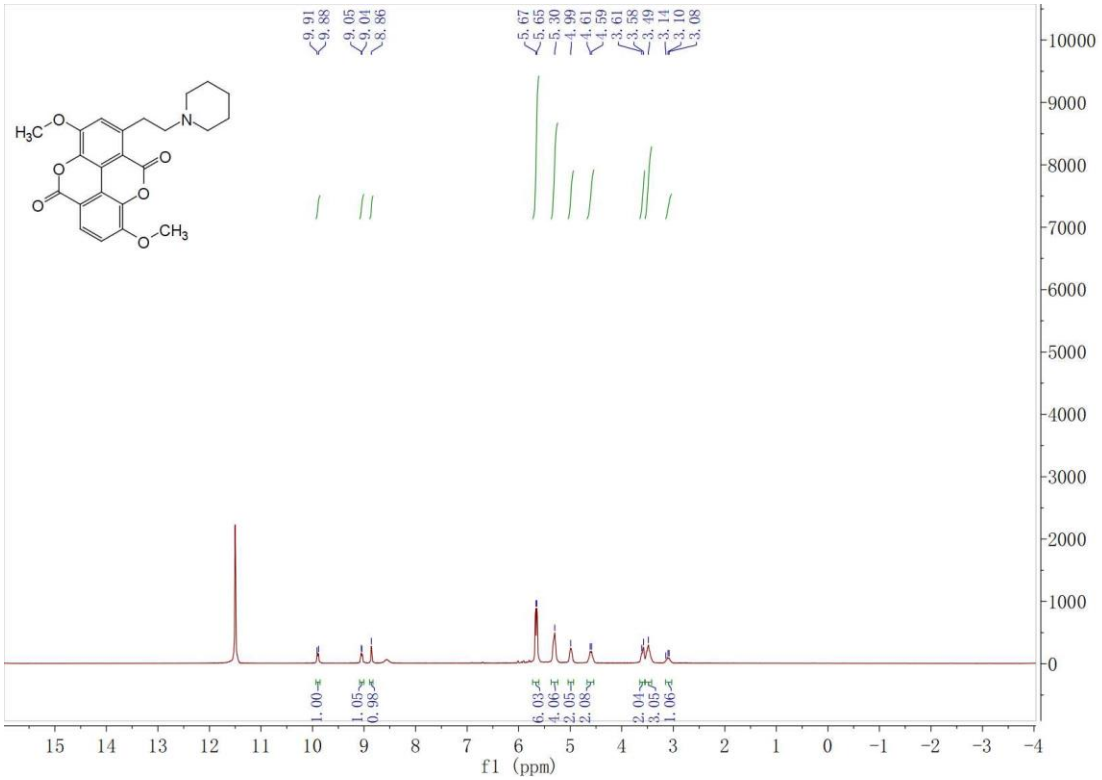
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y7



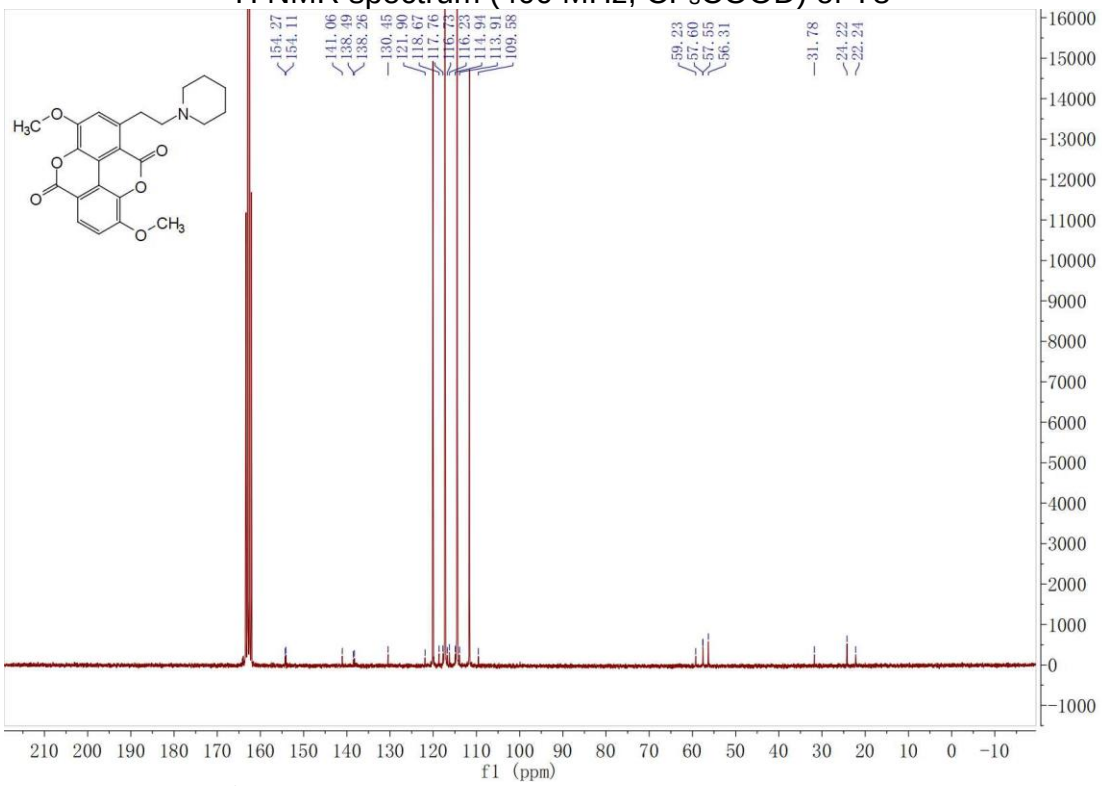
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y7



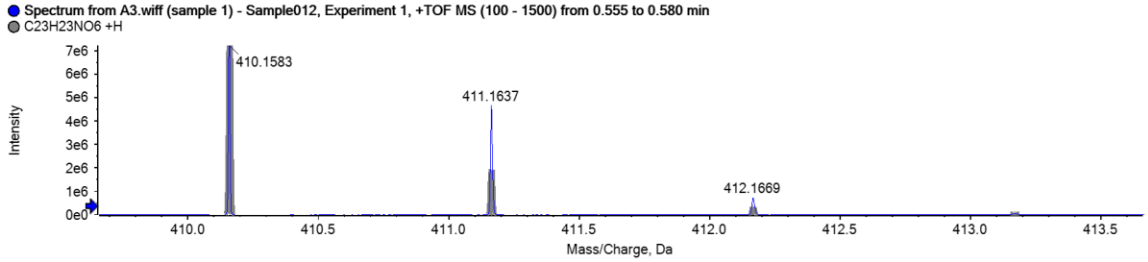
ESI-HRMS of Y7



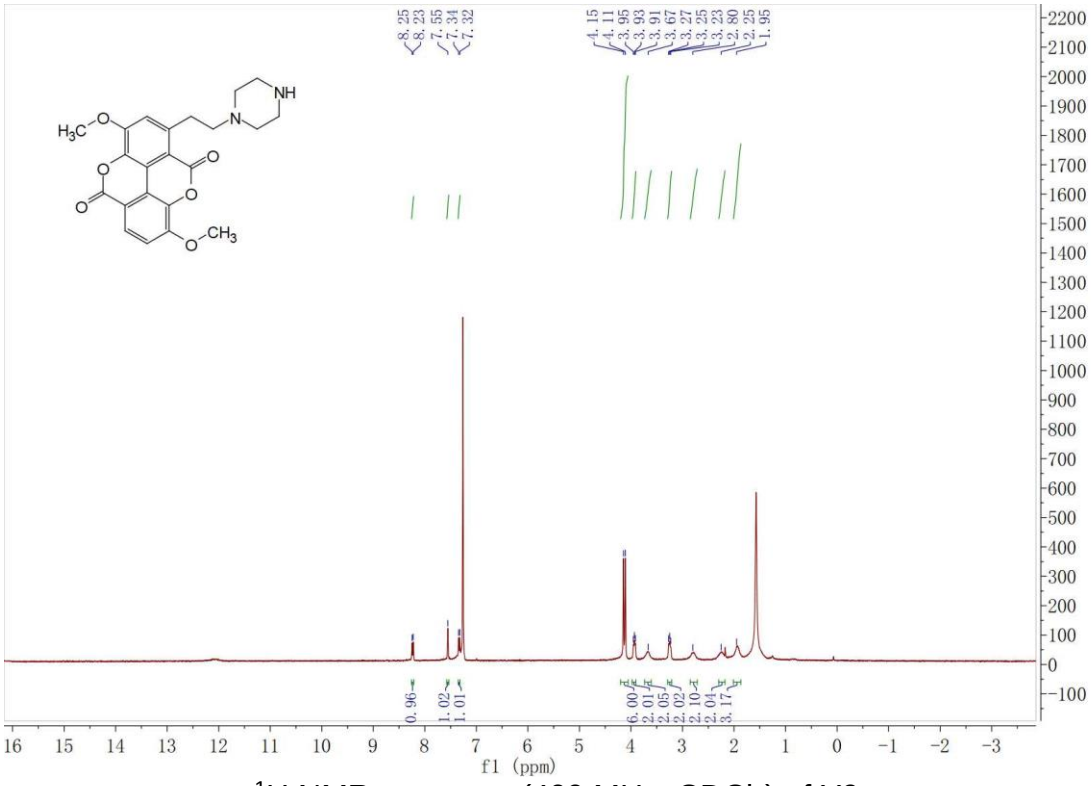
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y8



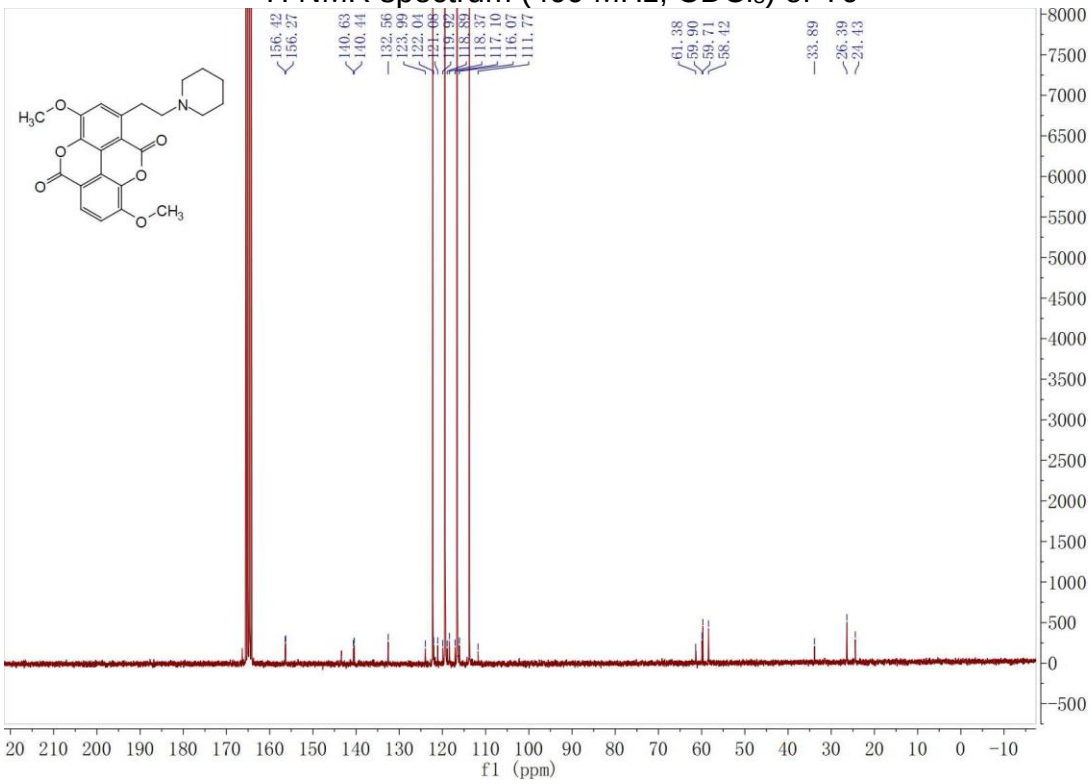
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y8



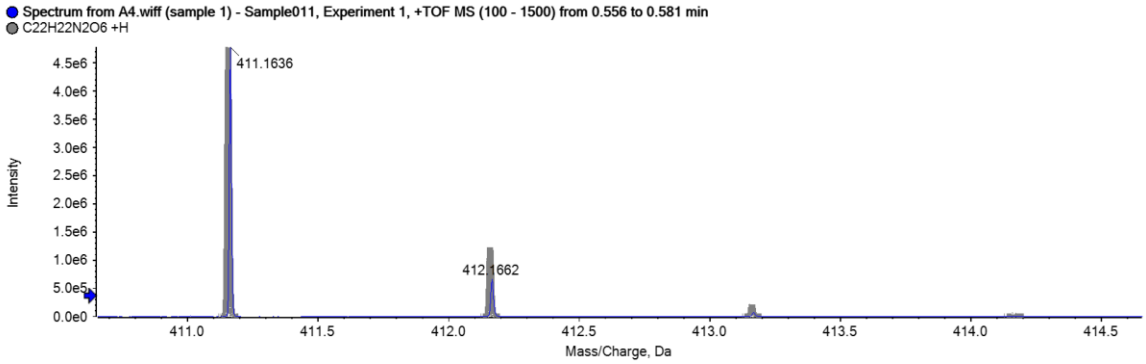
ESI-HRMS of Y8



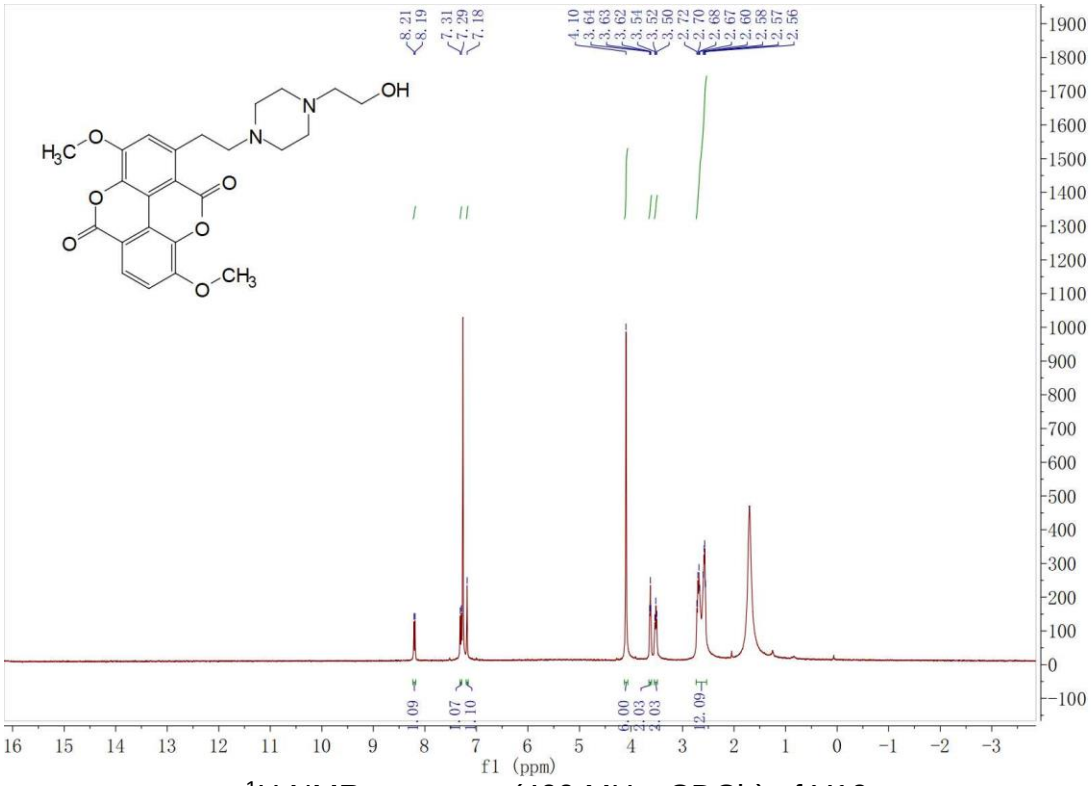
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y9



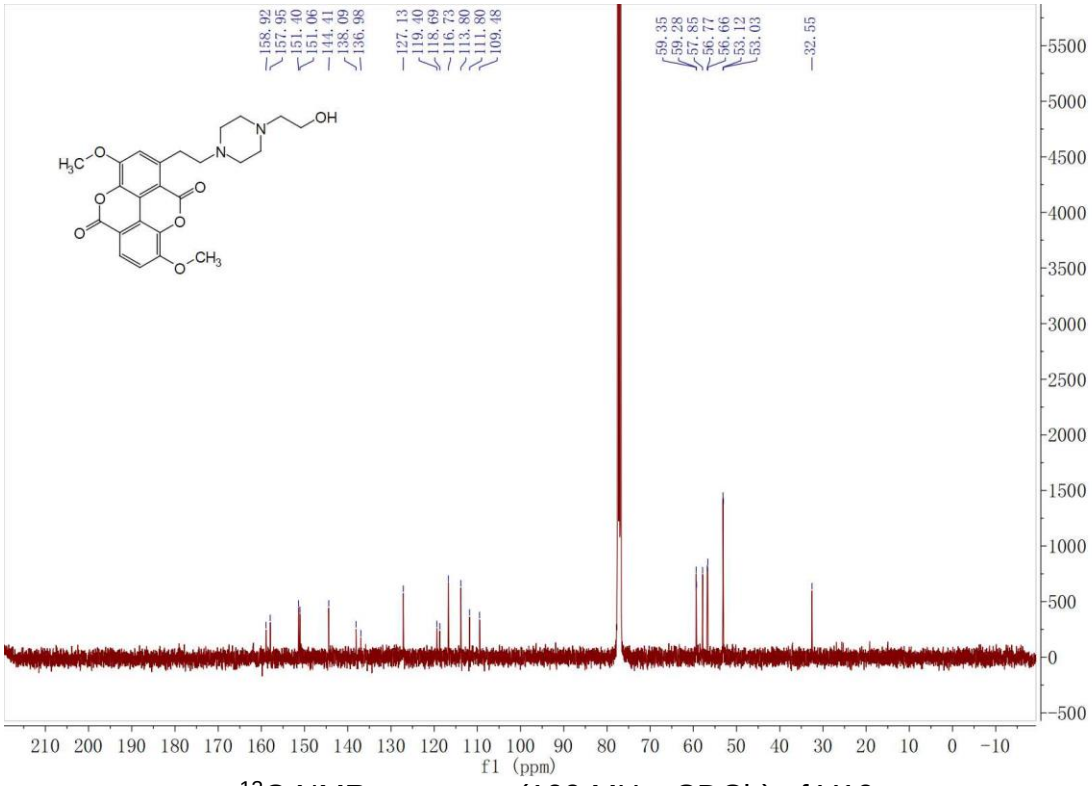
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y9



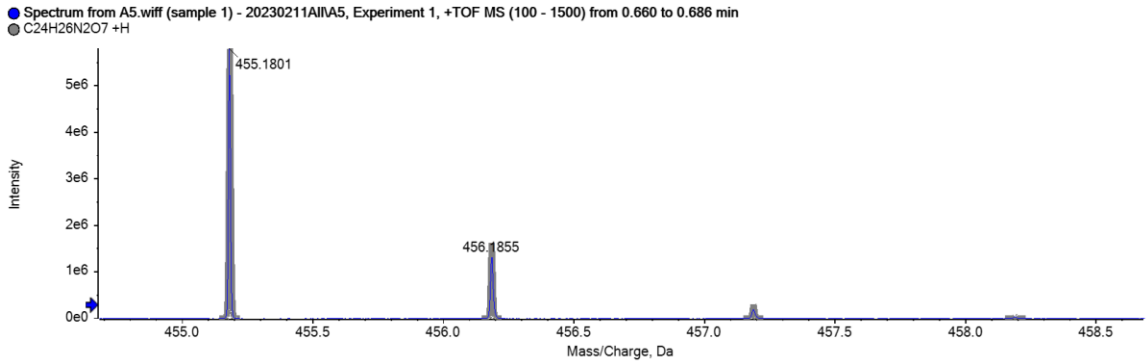
ESI-HRMS of Y9



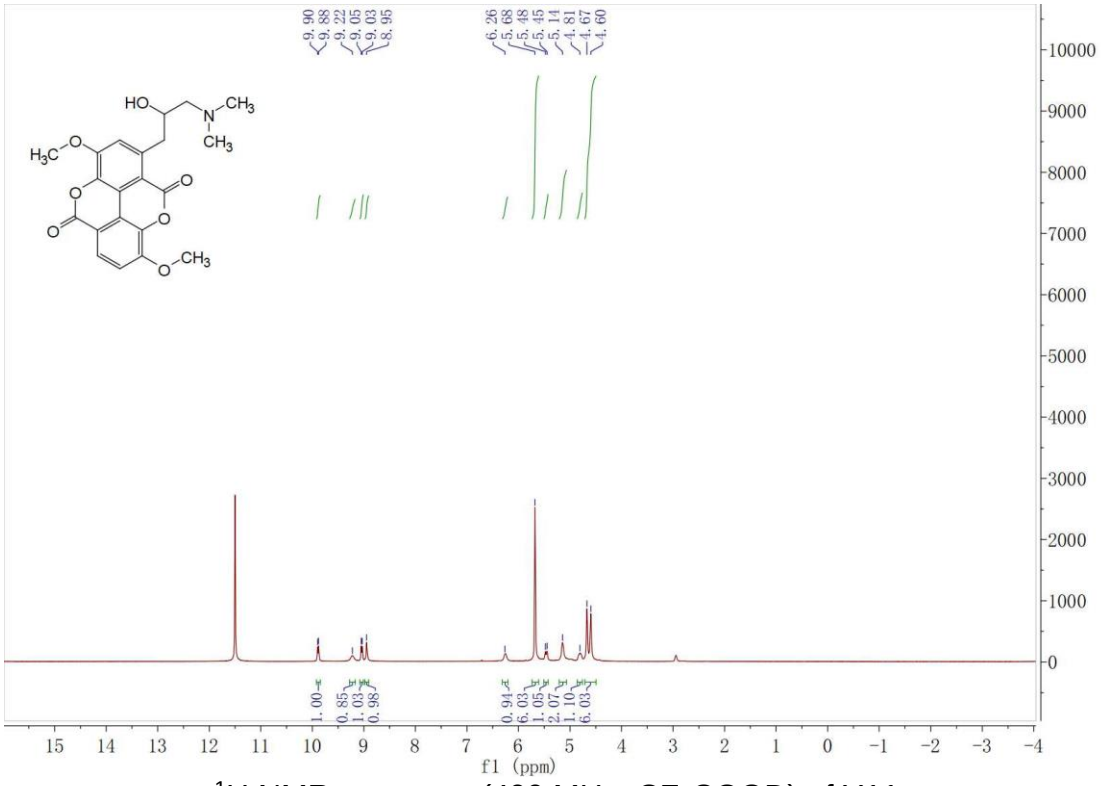
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y10



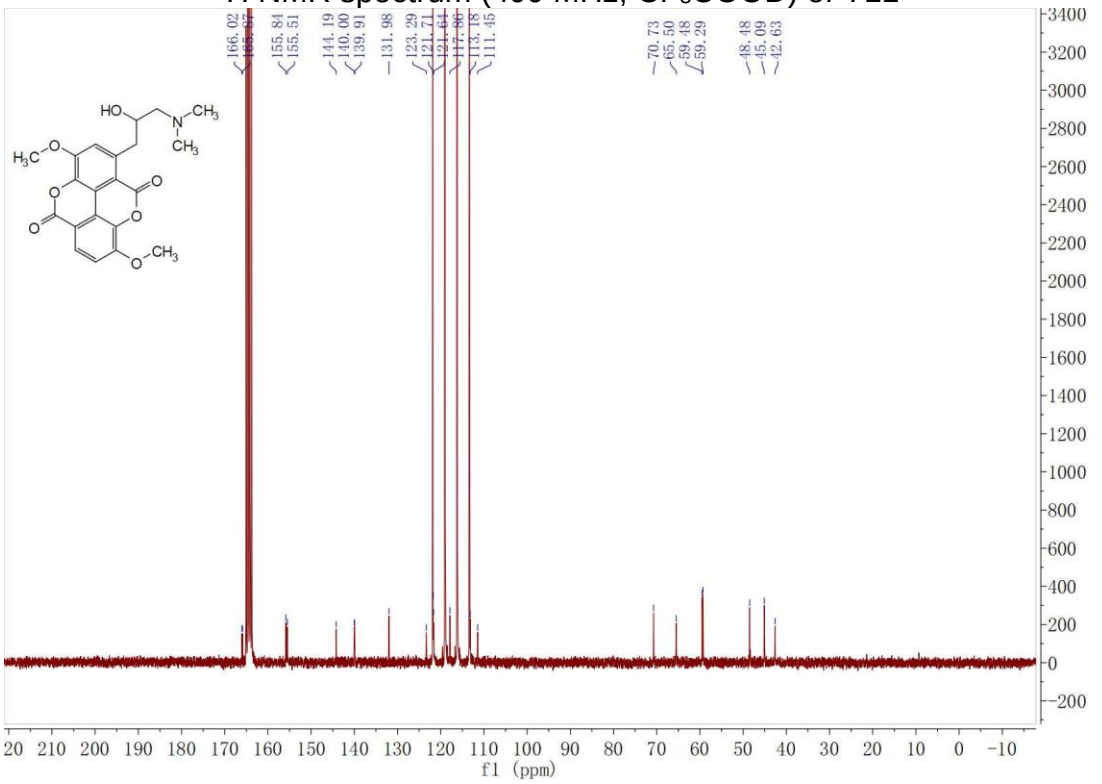
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of Y10



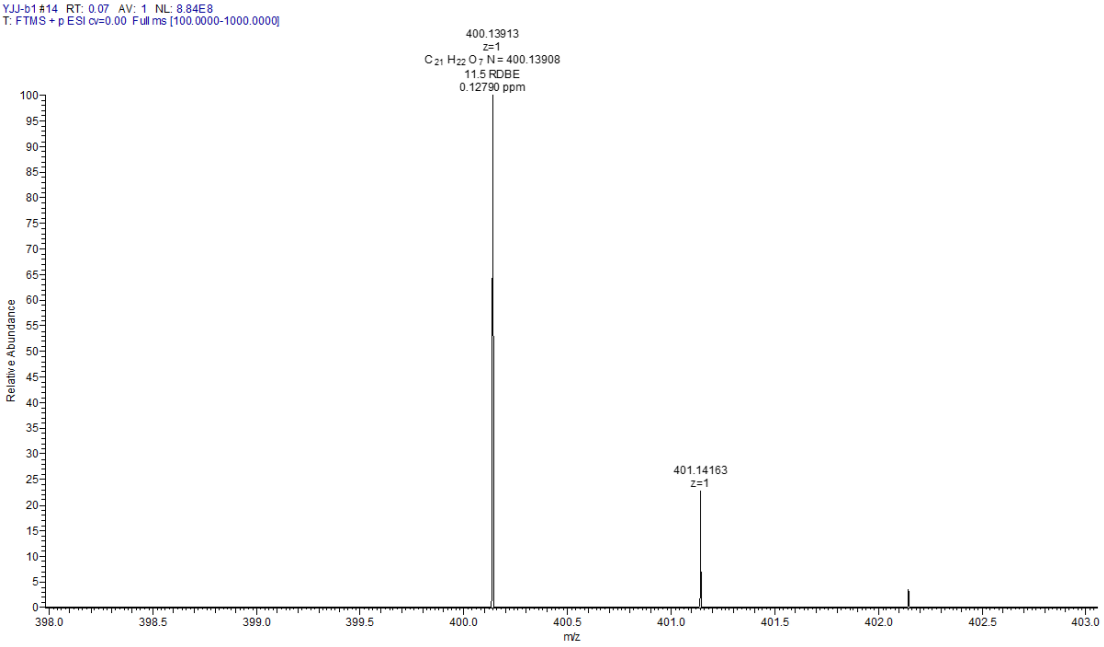
ESI-HRMS of Y10



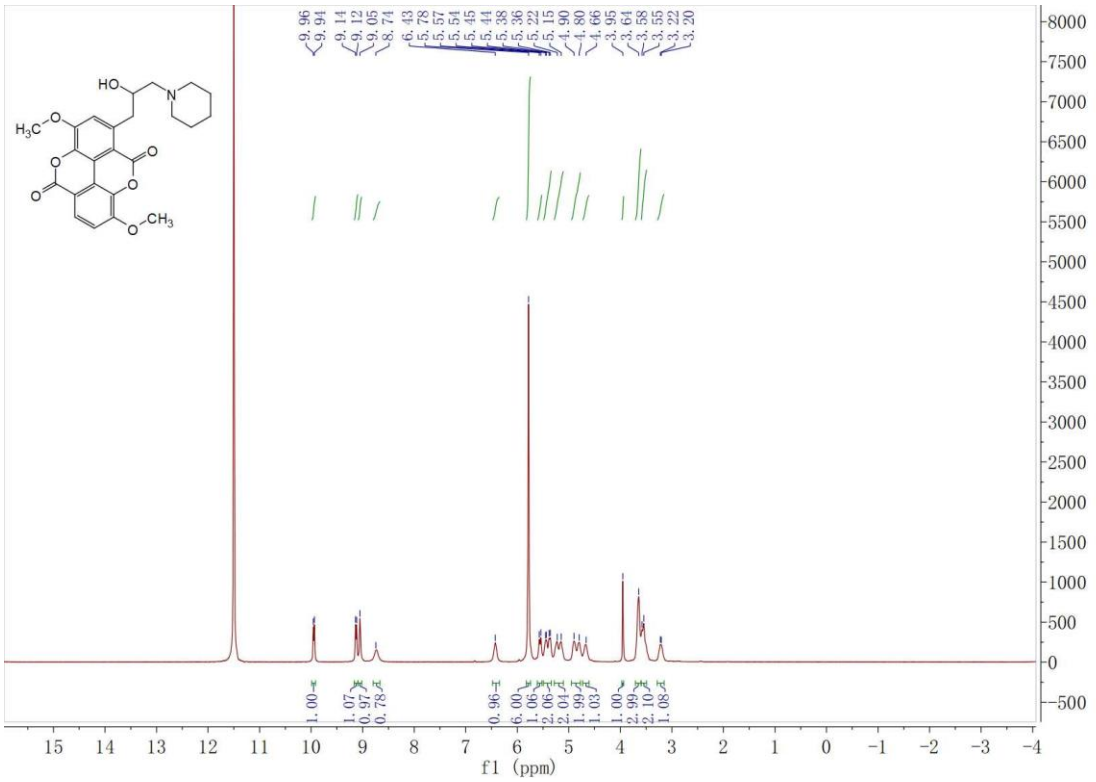
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y11



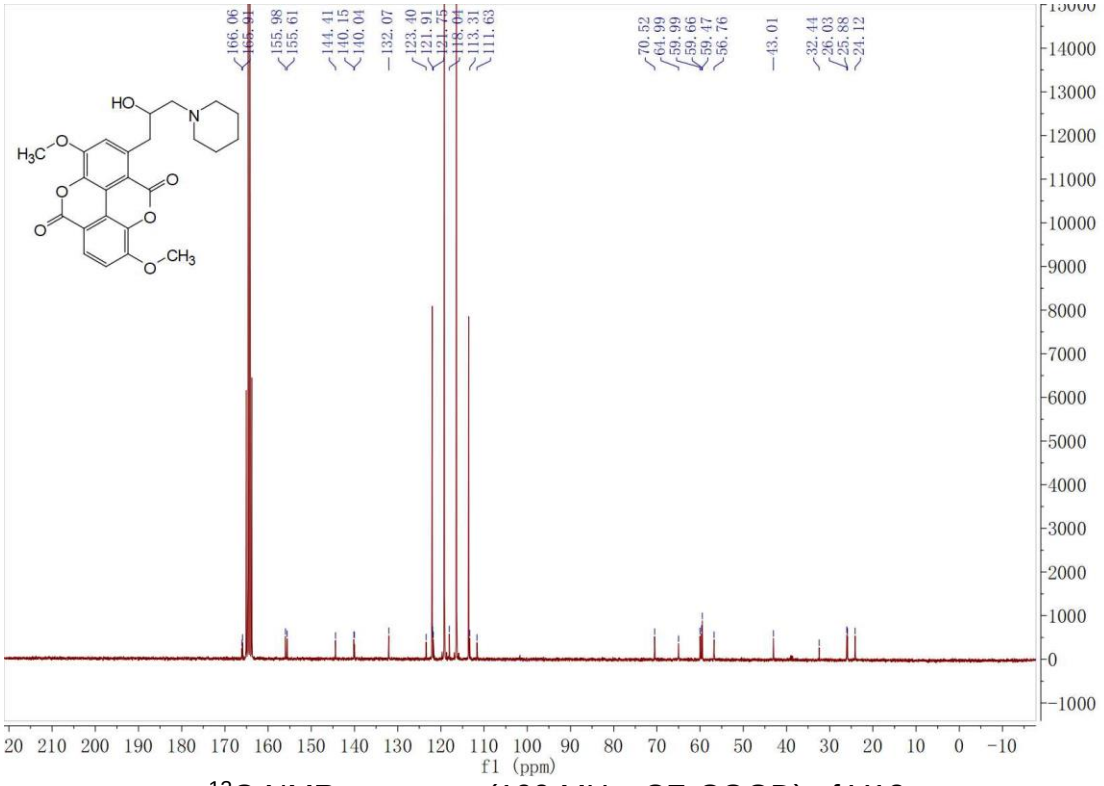
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y11



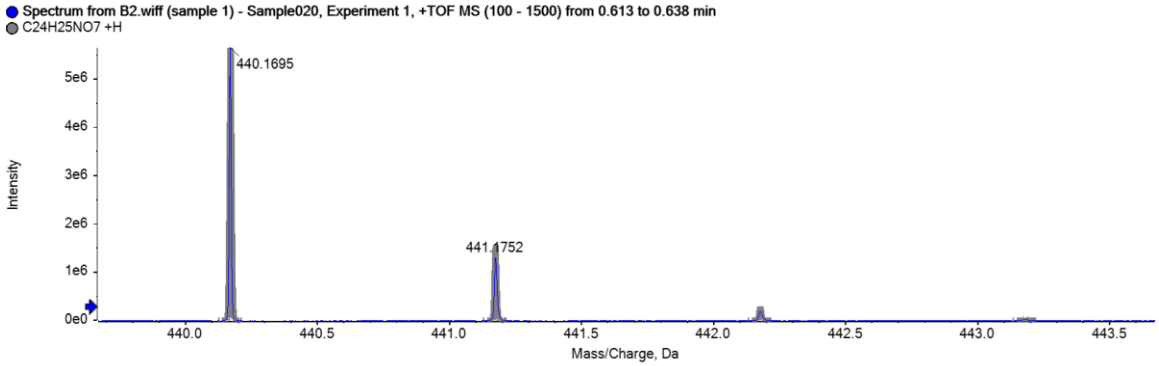
ESI-HRMS of Y11



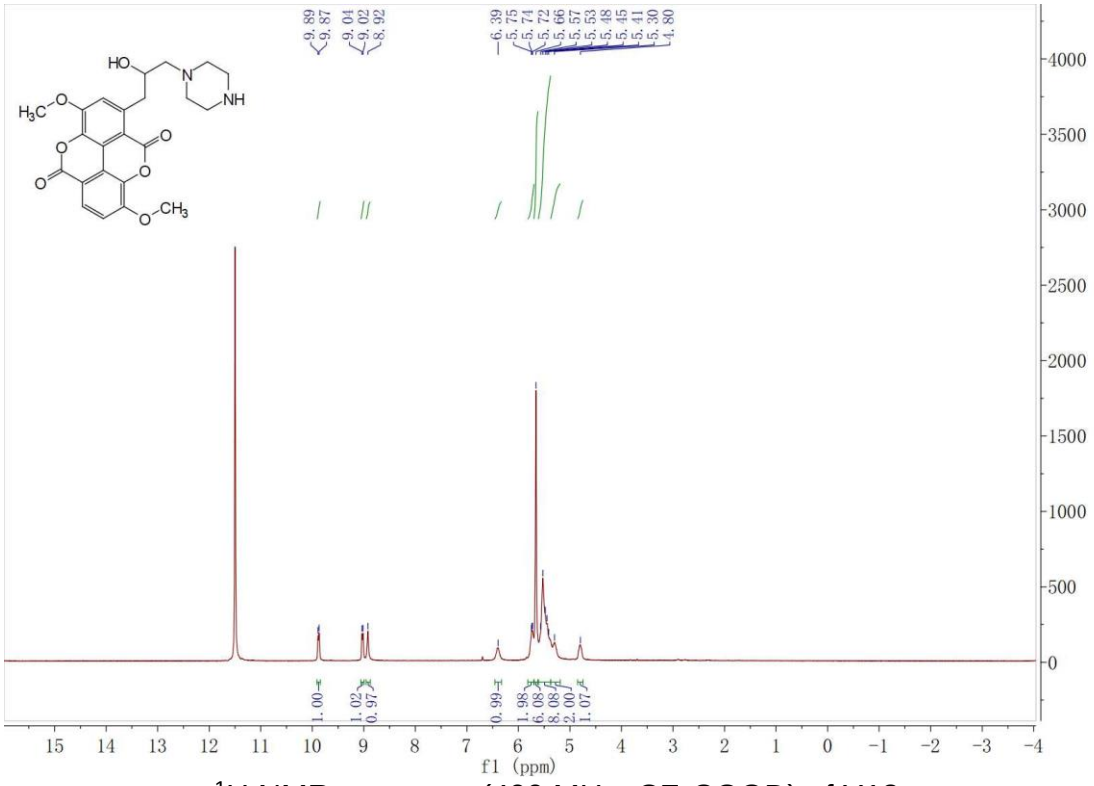
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y12



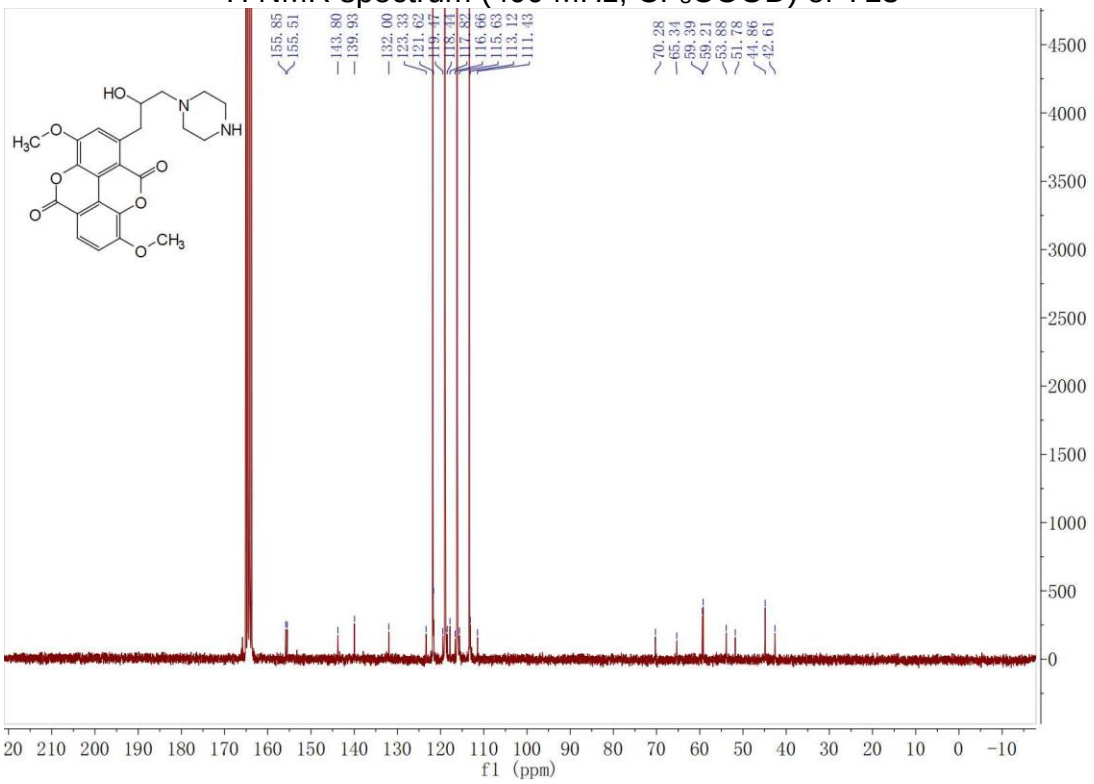
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y12



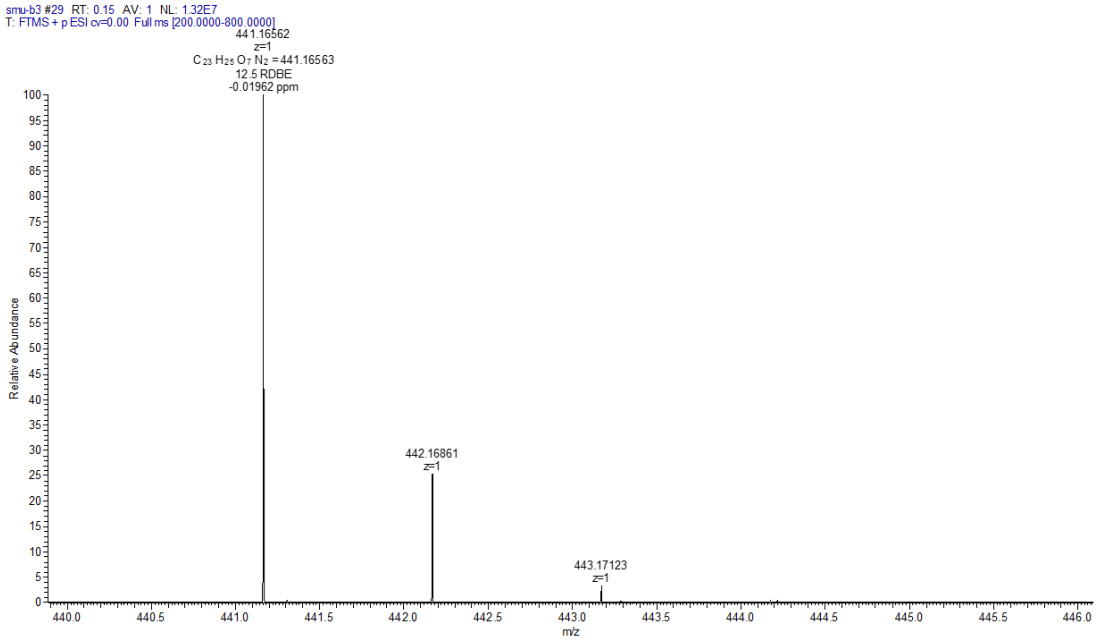
ESI-HRMS of Y12



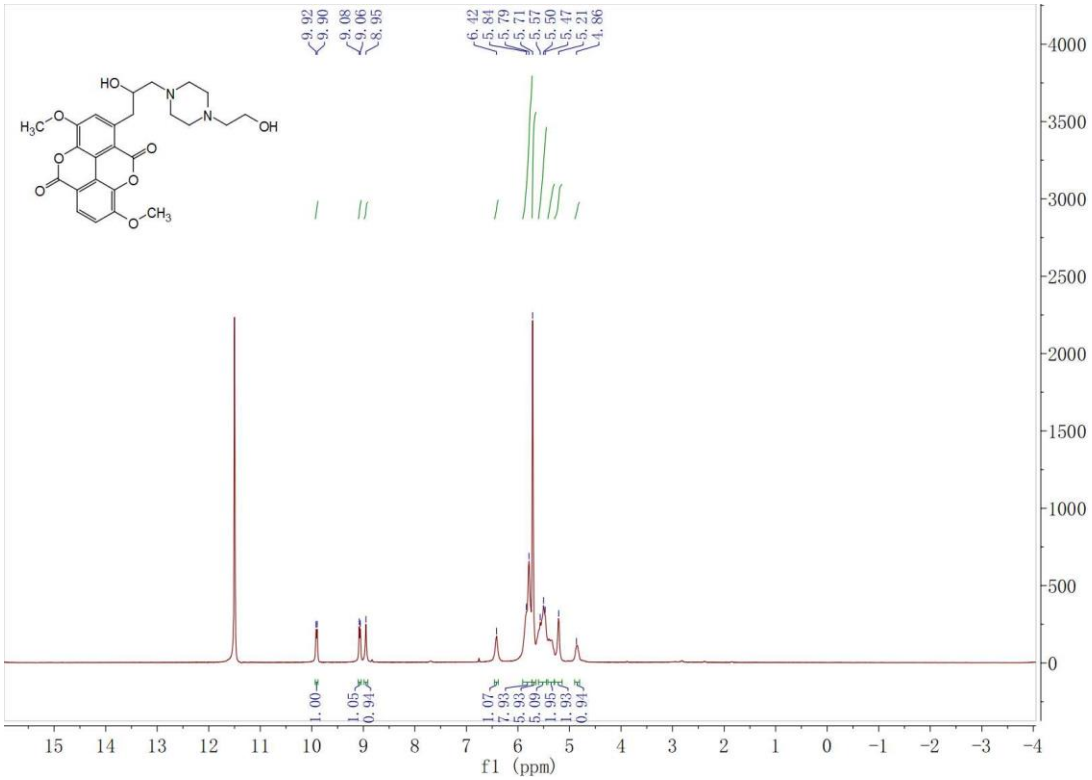
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y13



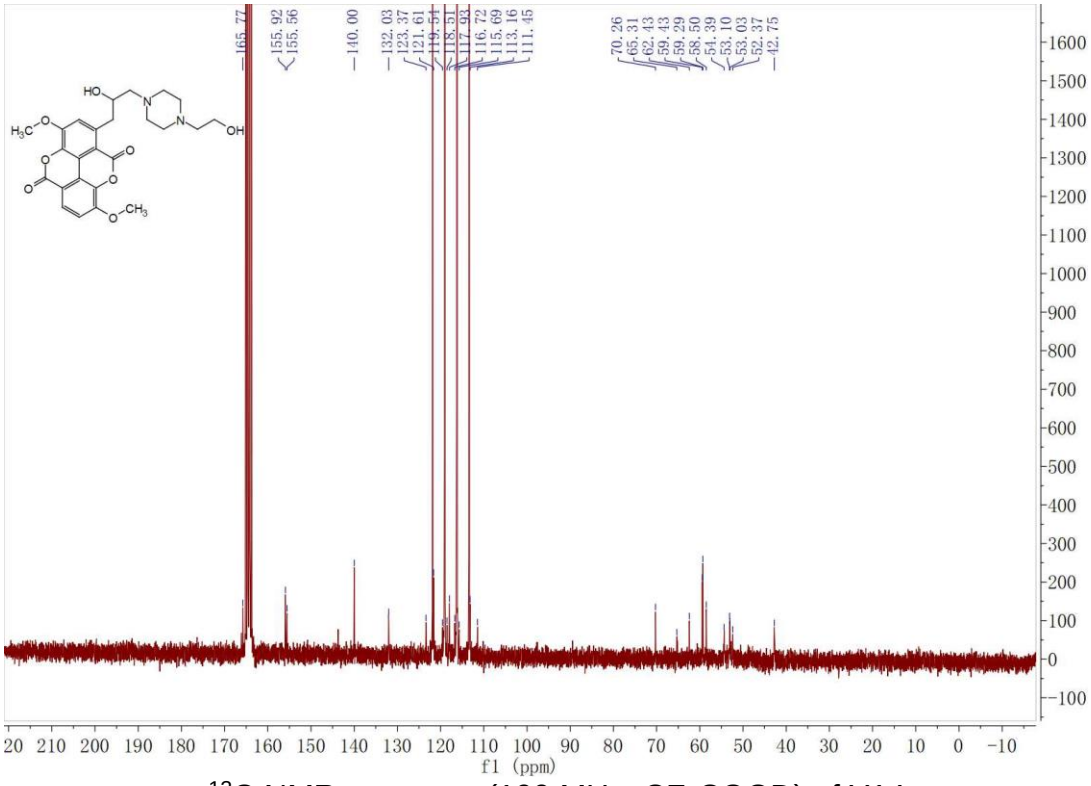
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y13



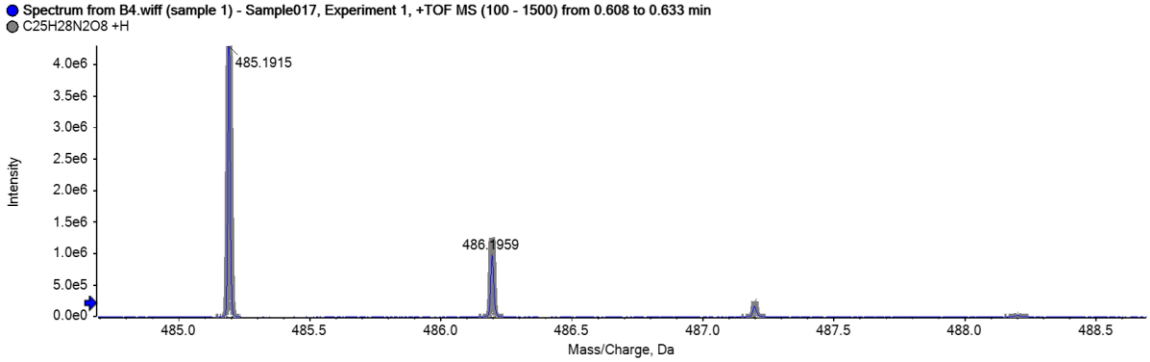
ESI-HRMS of Y13



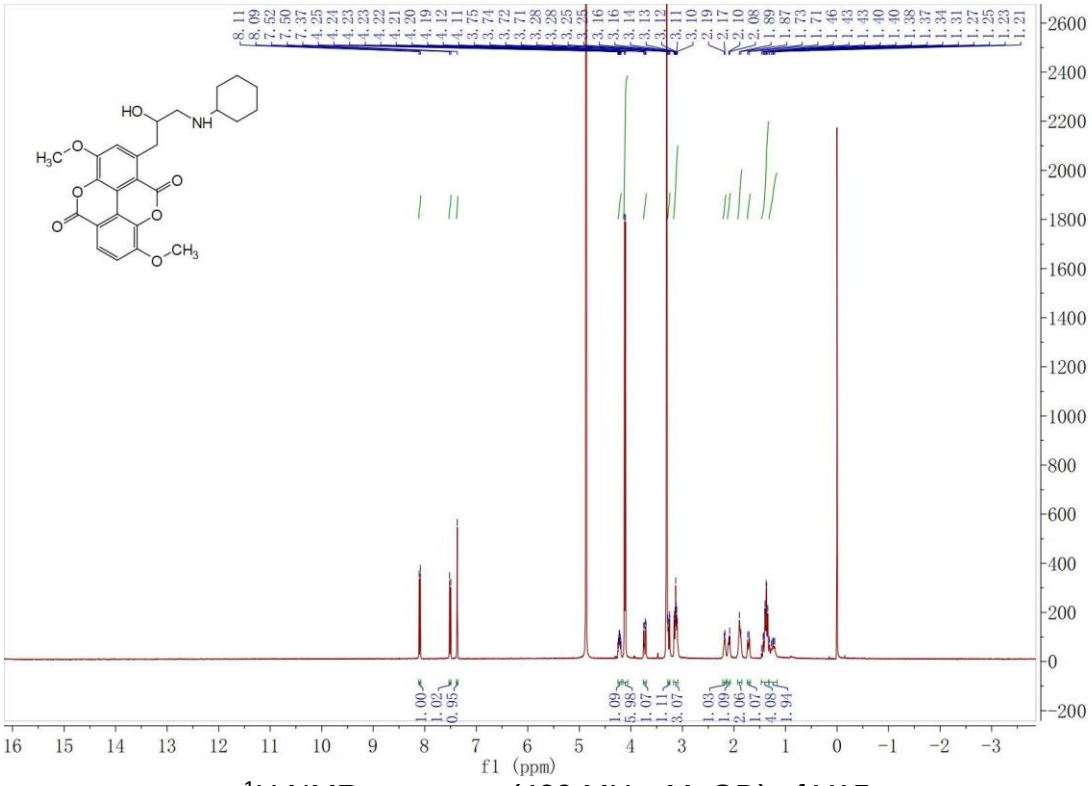
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y14



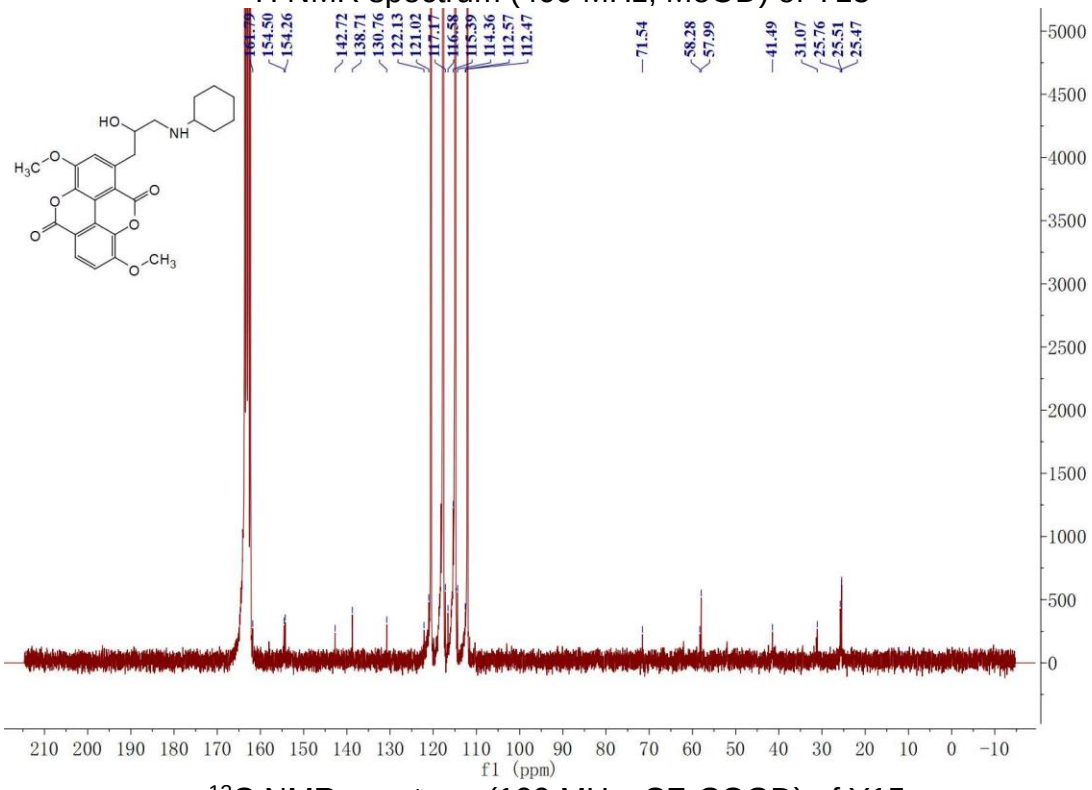
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y14



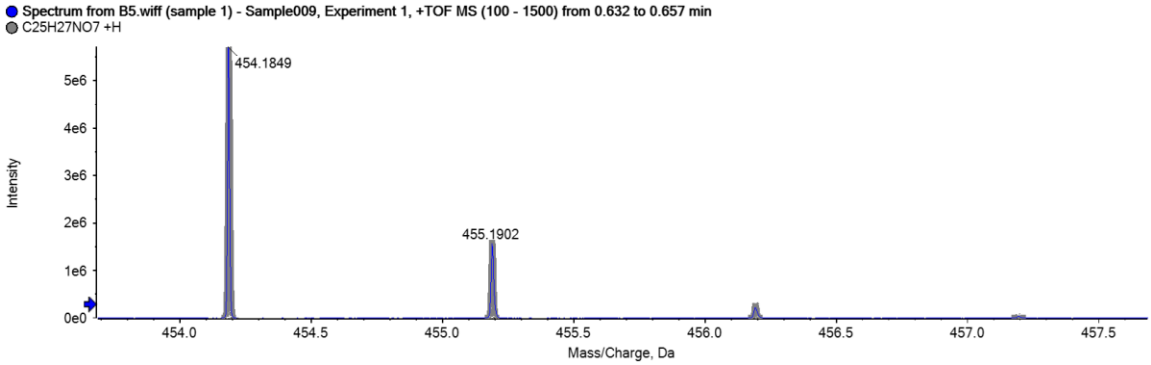
ESI-HRMS of Y14



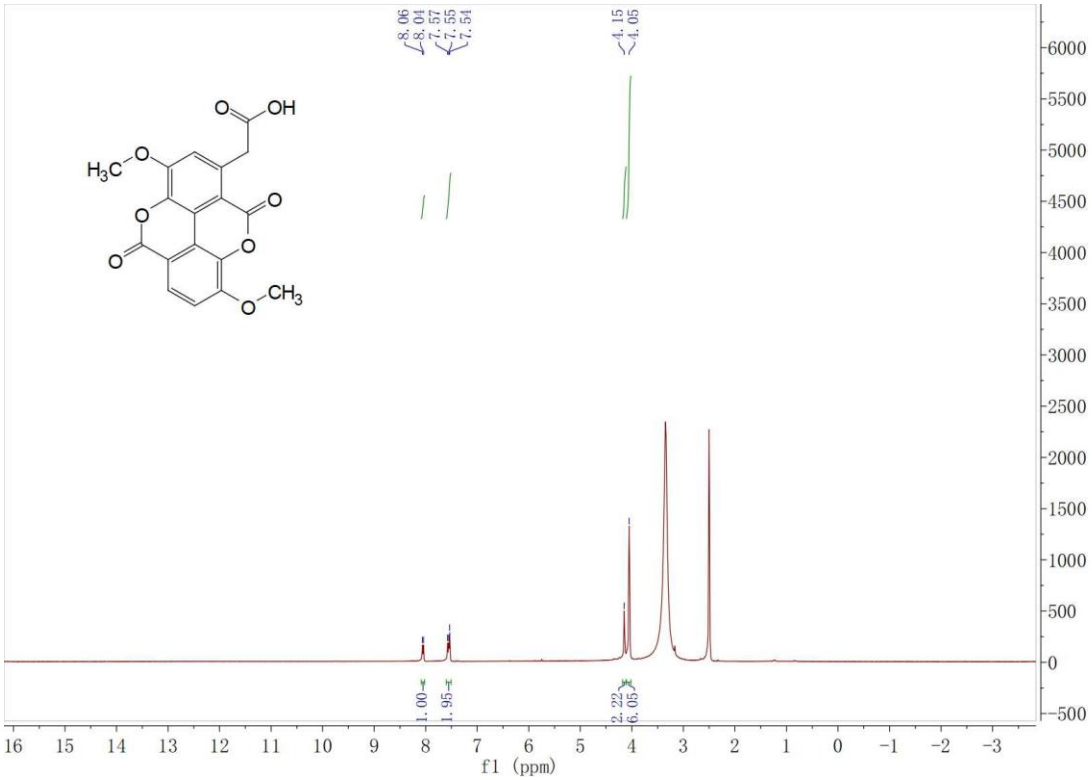
^{1}H NMR spectrum (400 MHz, MeOD) of Y15



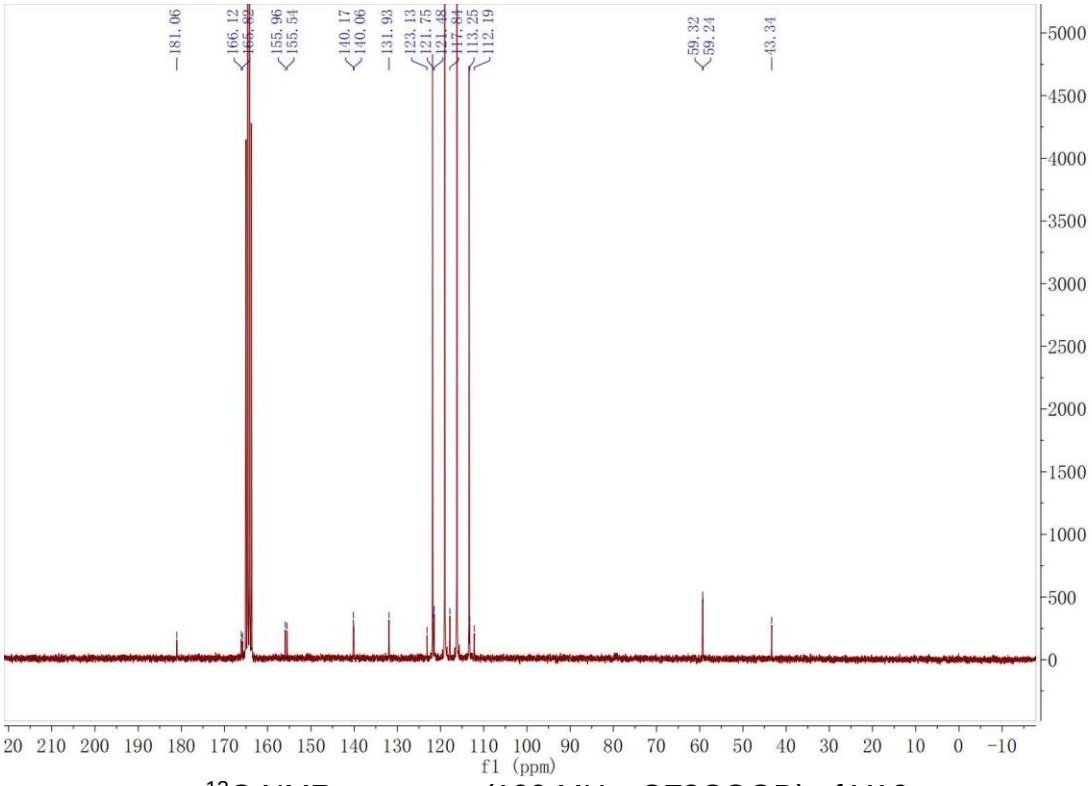
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y15



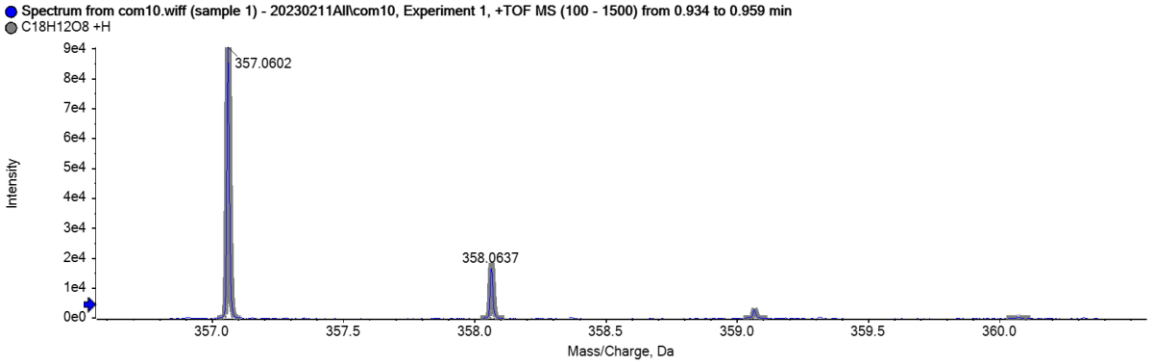
ESI-HRMS of Y15



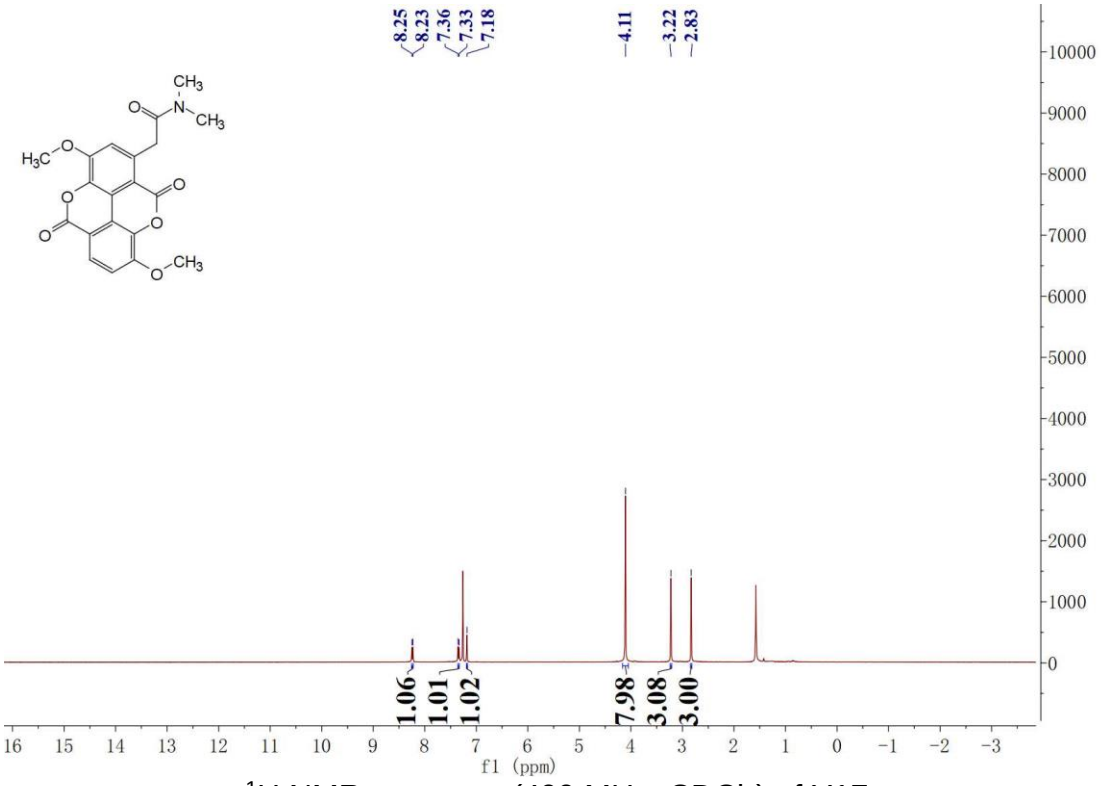
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of Y16



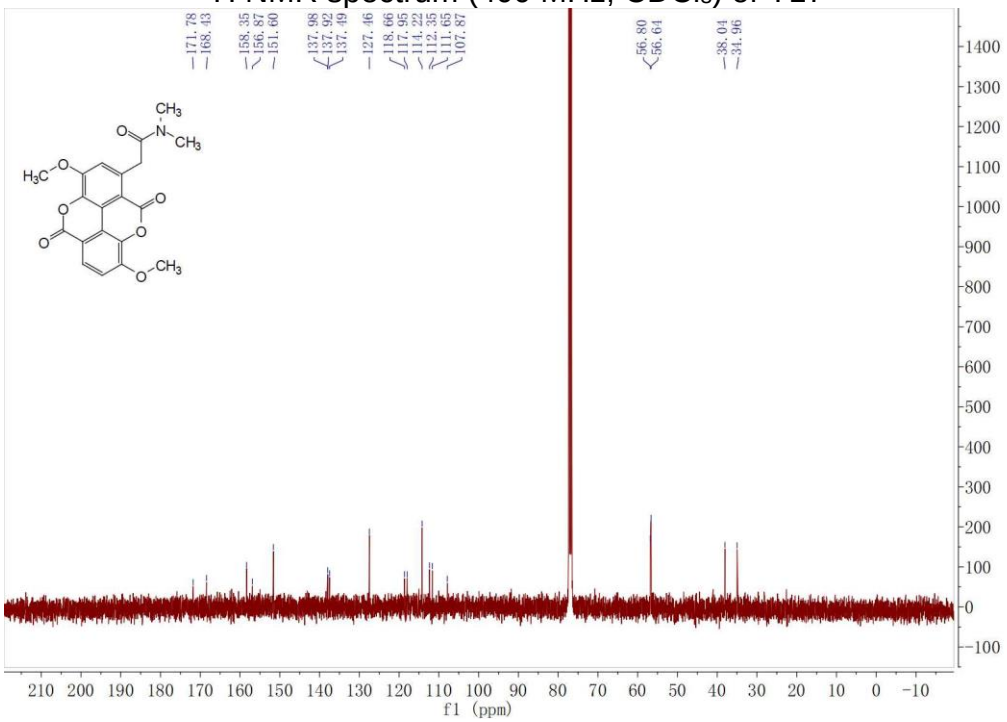
^{13}C NMR spectrum (100 MHz, CF3COOD) of Y16



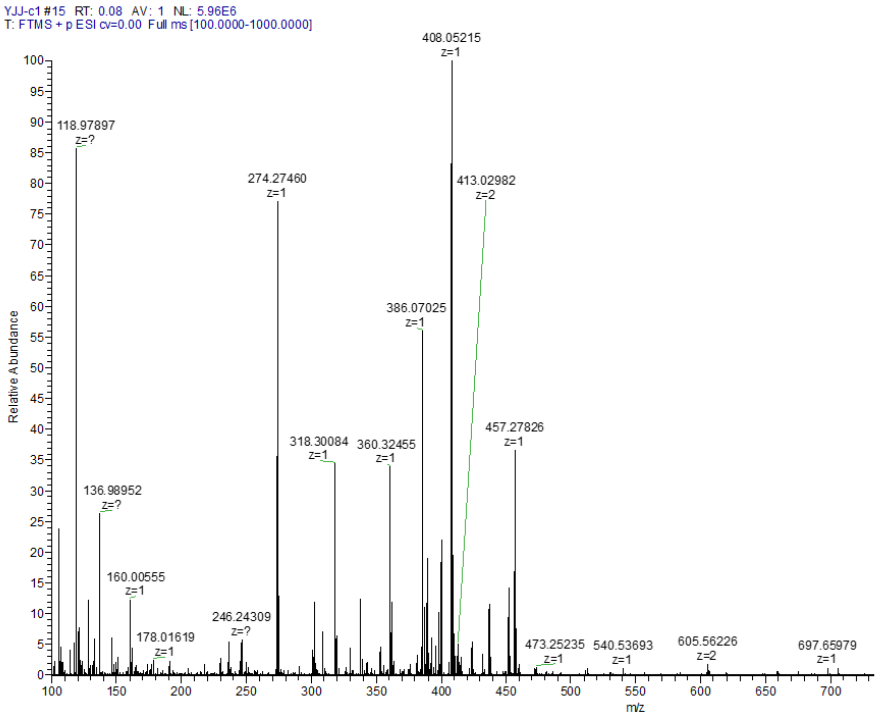
ESI-HRMS of Y16



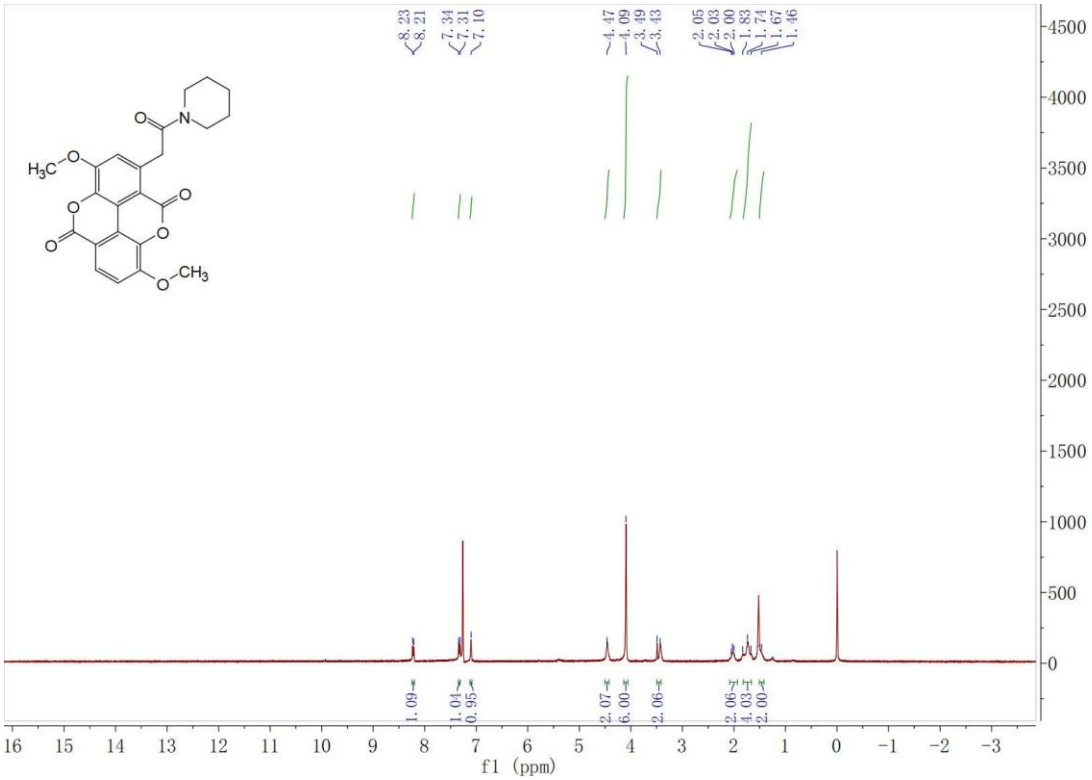
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y17



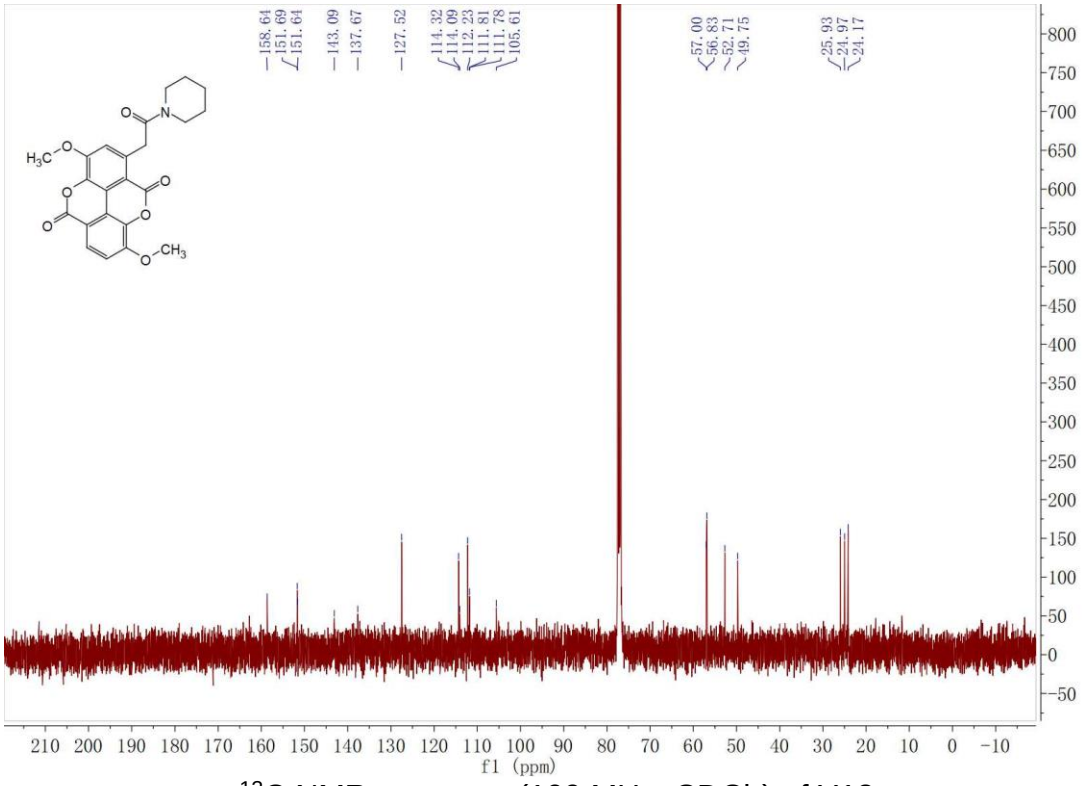
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of Y17



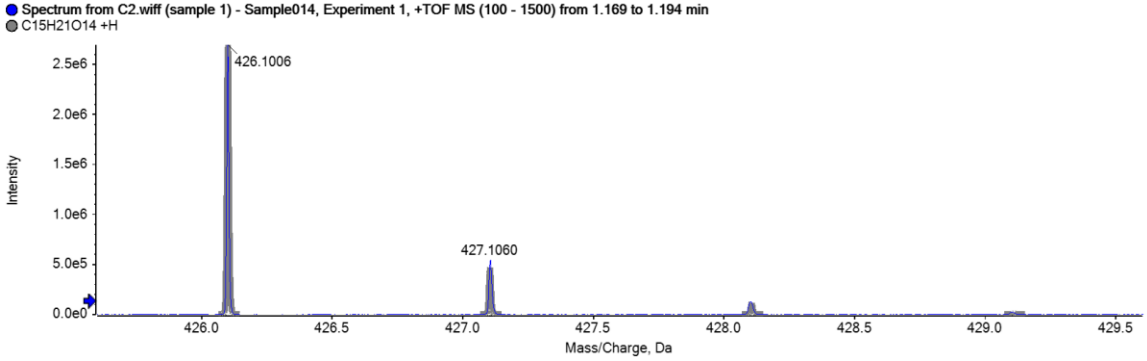
ESI-HRMS of Y17



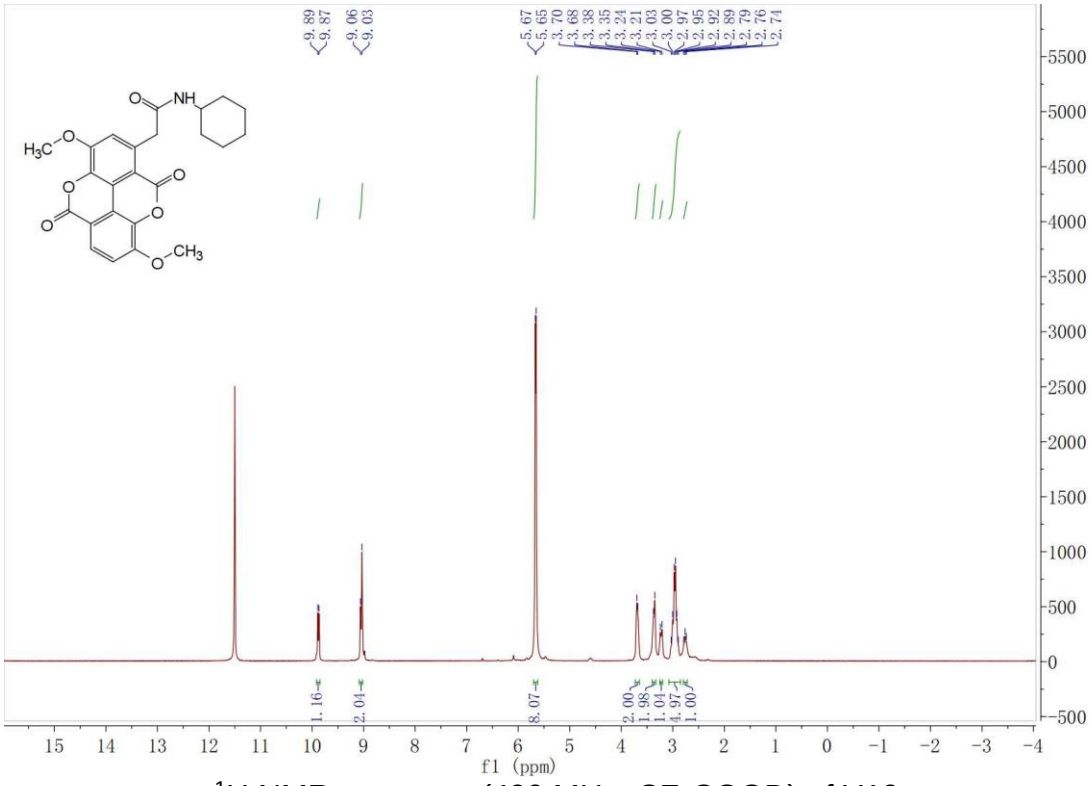
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Y18



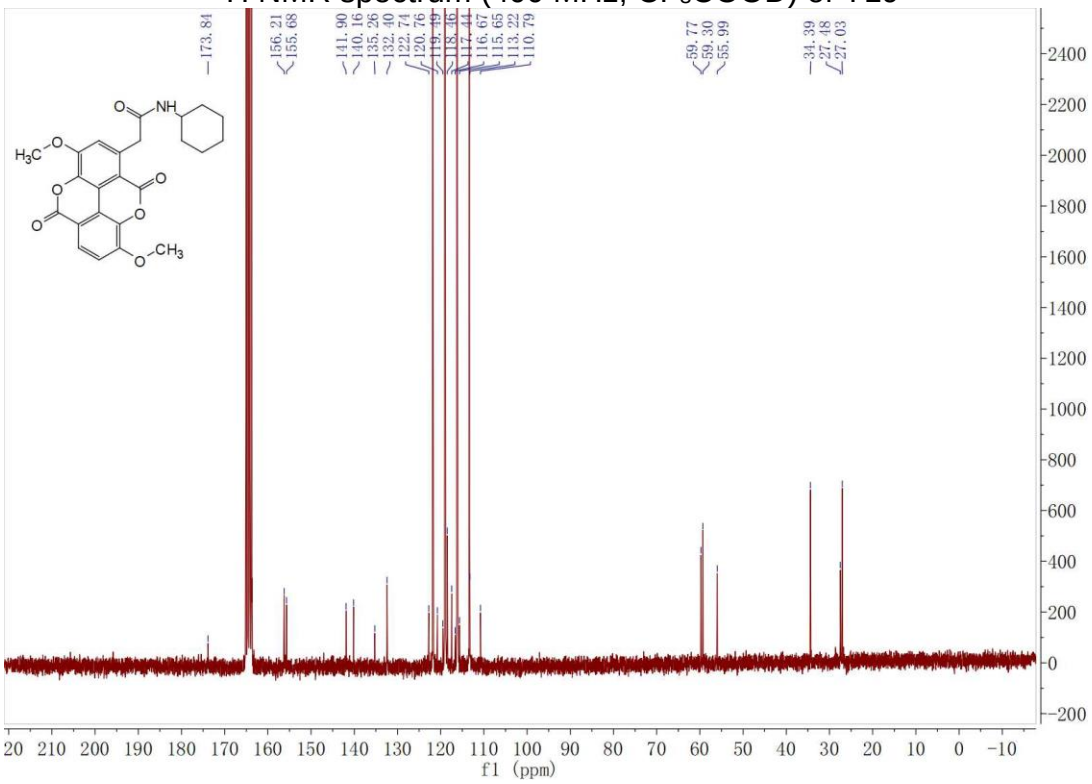
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of Y18



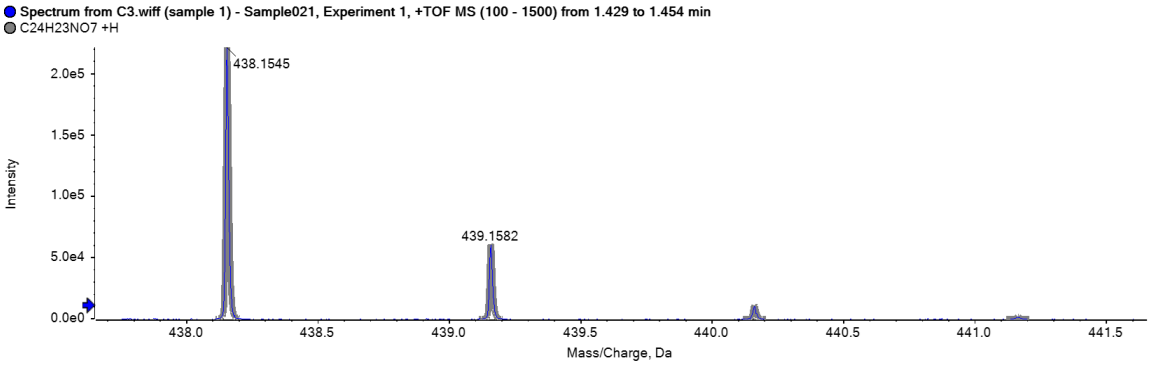
ESI-HRMS of Y18



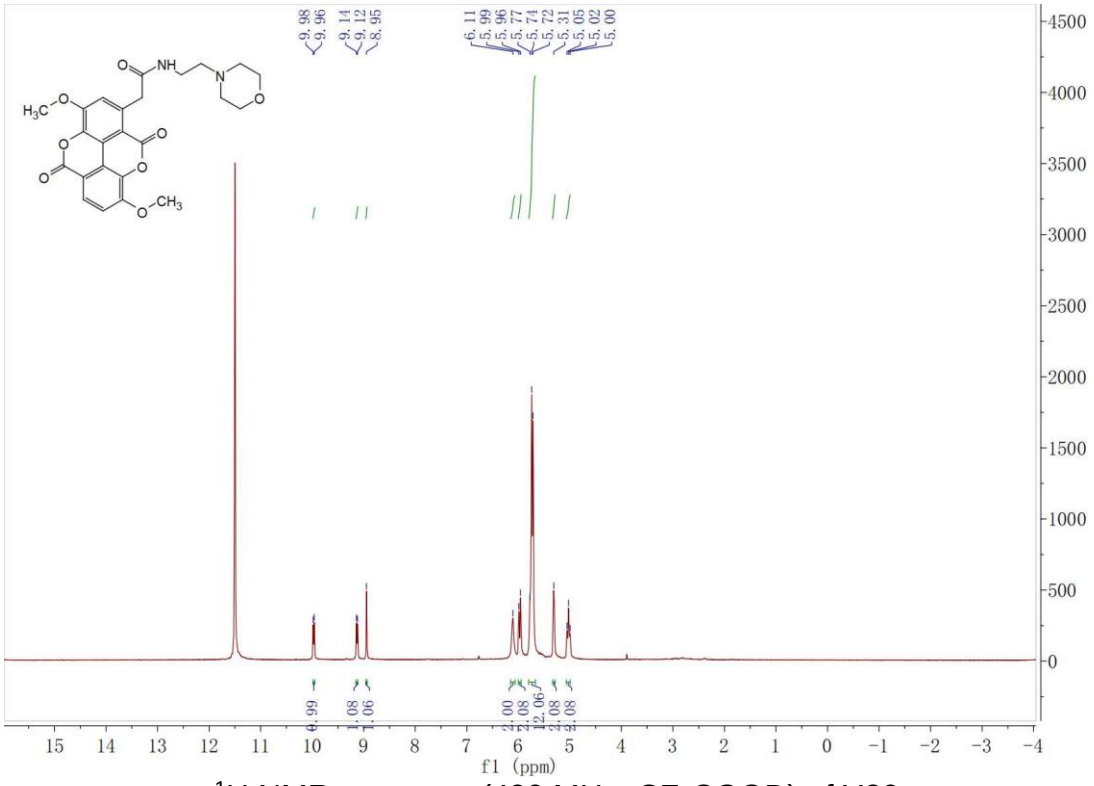
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y19



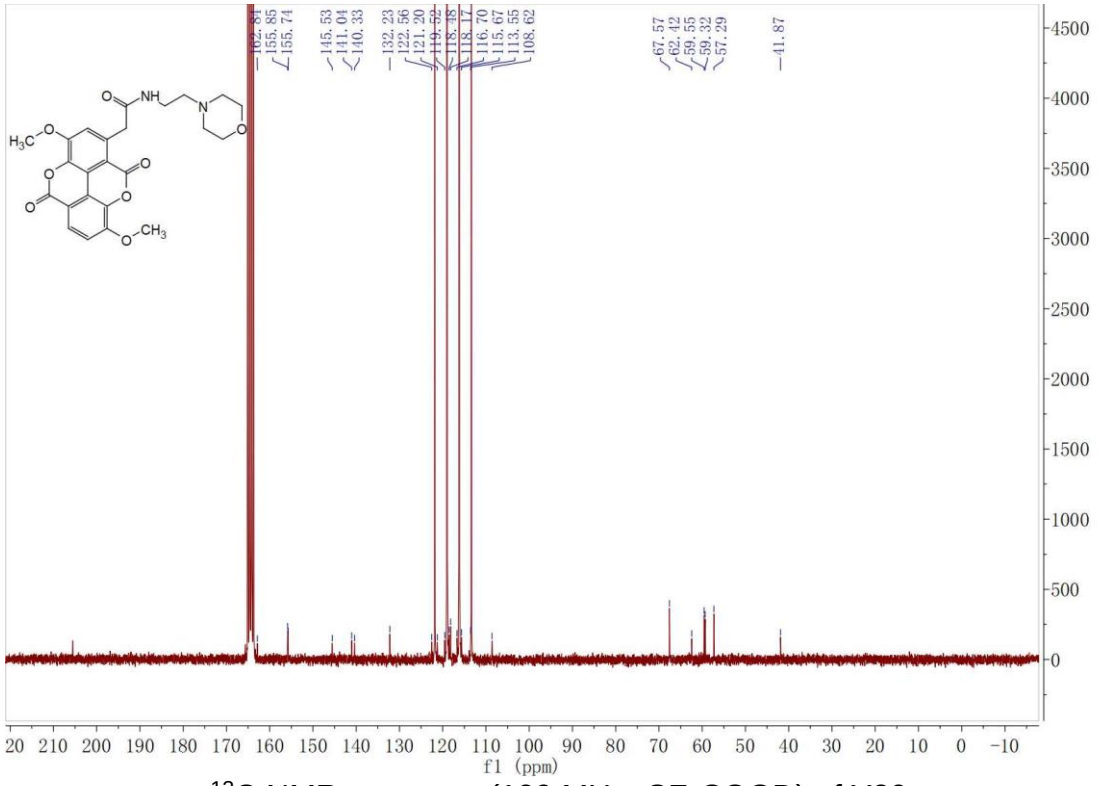
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y19



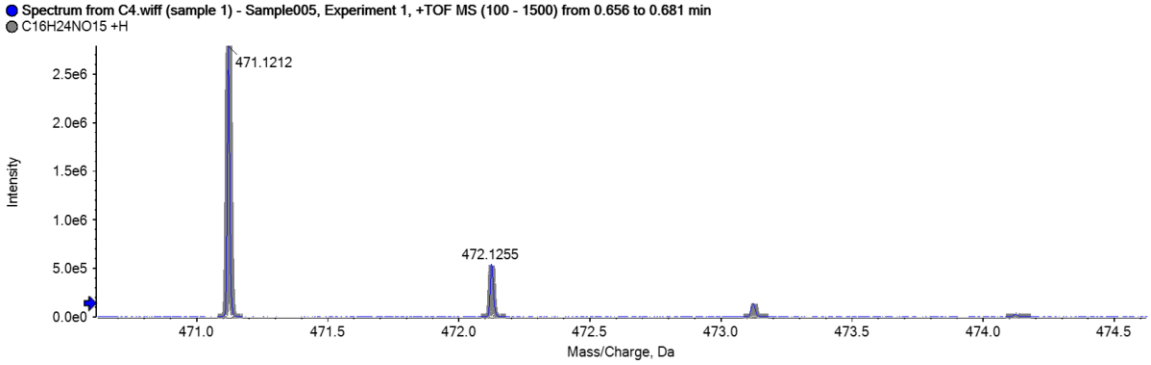
ESI-HRMS of Y19



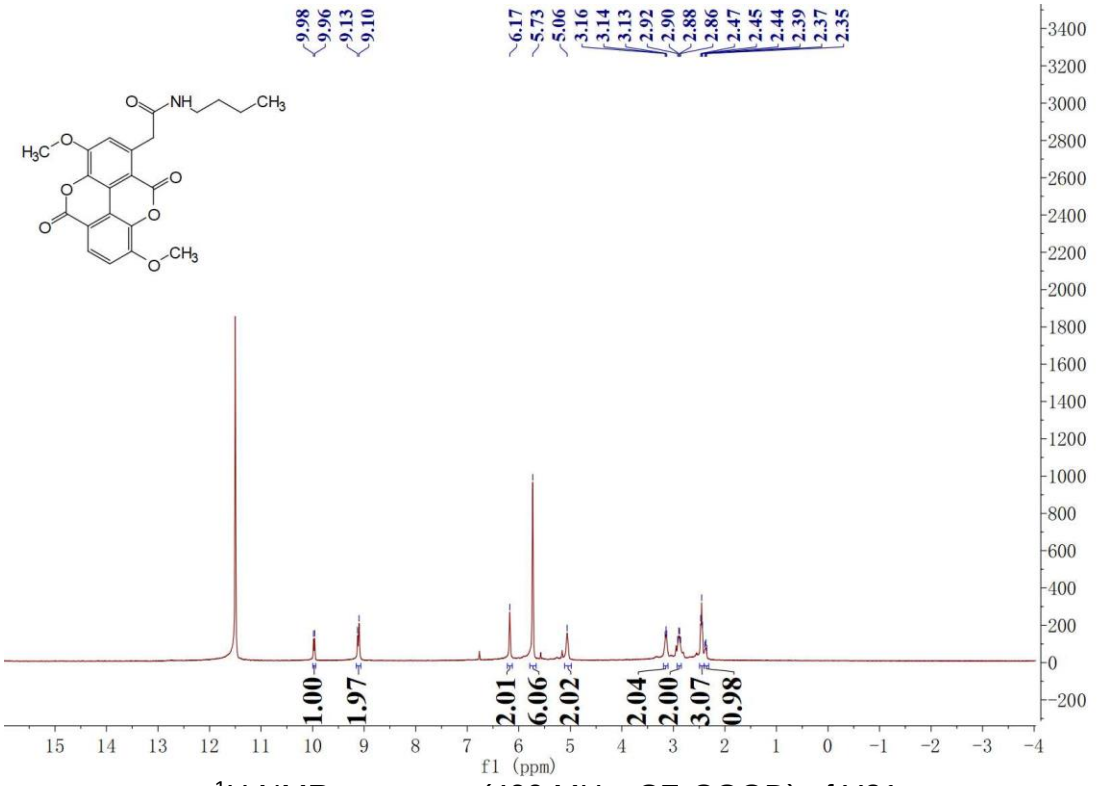
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y20



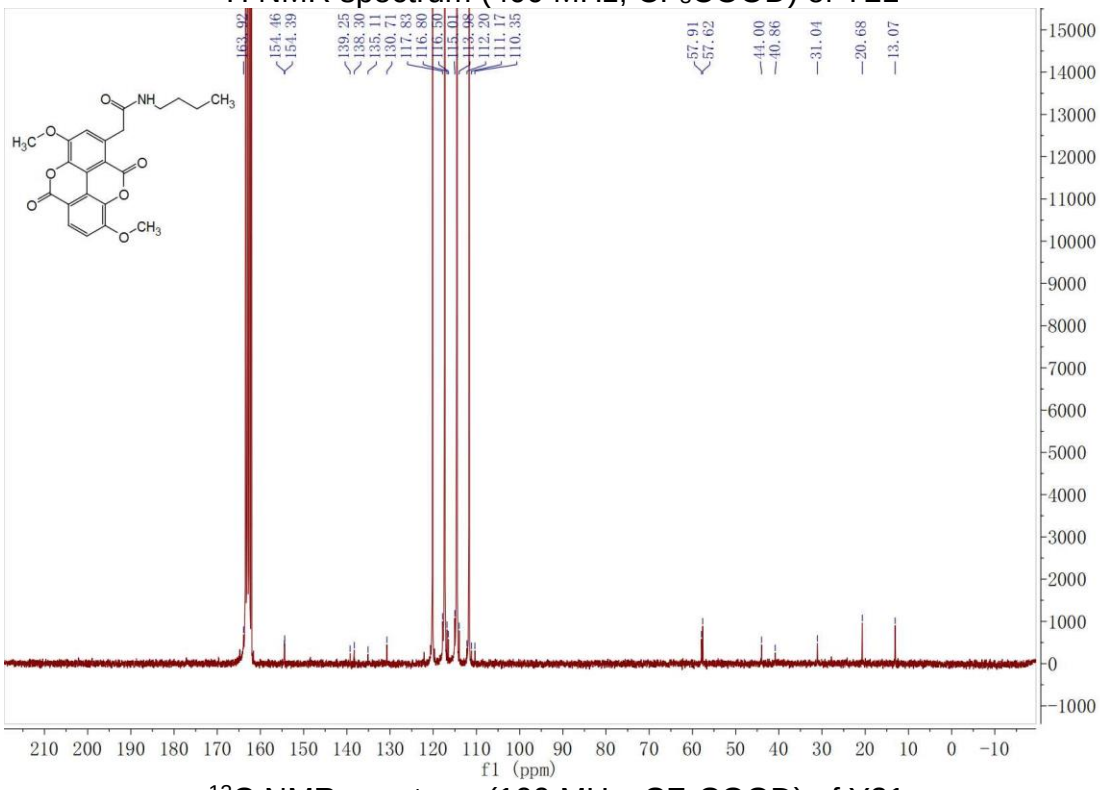
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y20



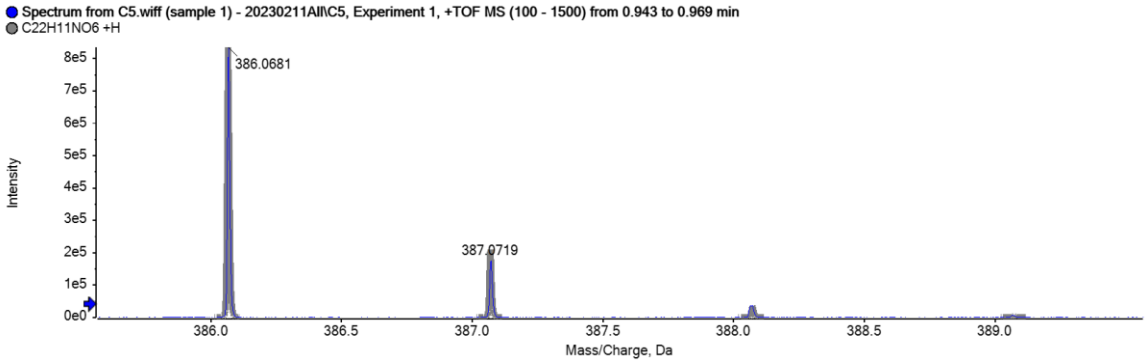
ESI-HRMS of Y20



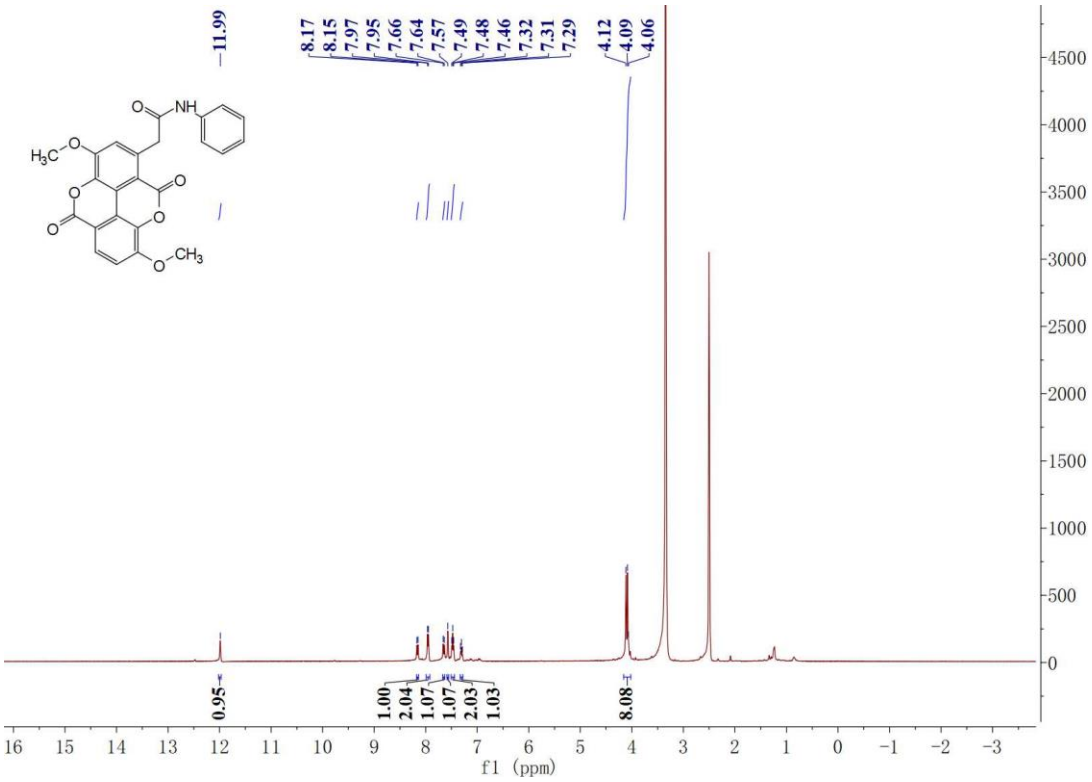
^{1}H NMR spectrum (400 MHz, CF_3COOD) of Y21



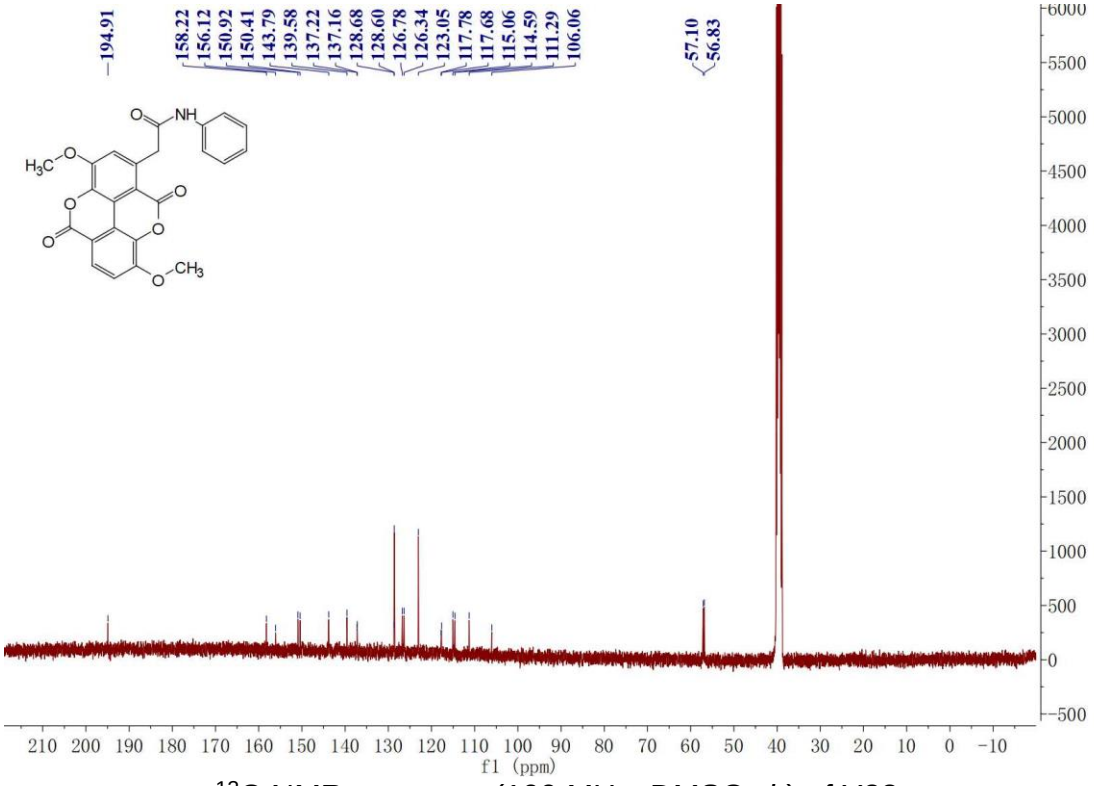
^{13}C NMR spectrum (100 MHz, CF_3COOD) of Y21



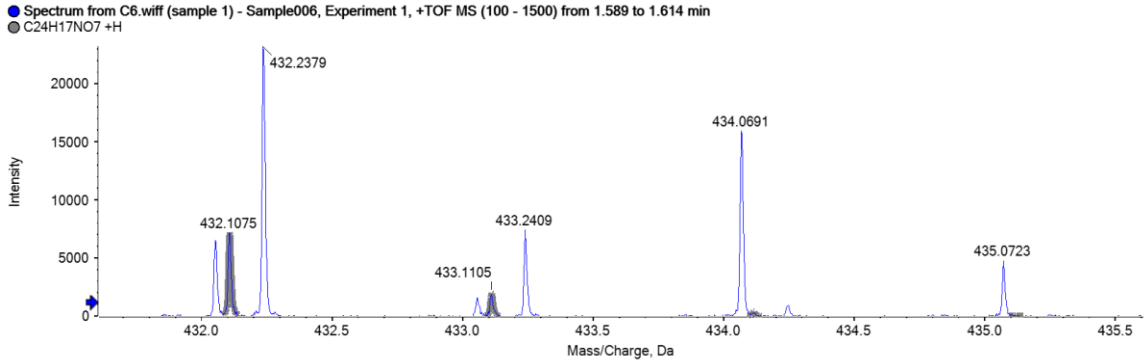
ESI-HRMS of Y21



^{1}H NMR spectrum (400 MHz, DMSO-d_6) of Y22



^{13}C NMR spectrum (100 MHz, DMSO-d_6) of Y22



ESI-HRMS of Y22