

Supplementary information

Molecular Diversity and Potential Anti-neuroinflammatory Activities of Cyathane Diterpenoids from the Basidiomycete *Cyathus africanus*

Jing Wei^{1,§}, Yuanyuan Cheng^{2,§}, Wan-Hui Guo¹, Da-Cheng Wang¹, Qiang Zhang¹, Ding
Li¹, Jianhui Rong^{2,*} & Jin-Ming Gao^{2,*}

¹Shaanxi Key Laboratory of Natural Products & Chemical Biology, Shaanxi Engineering
Center of Bioresource Chemistry & Sustainable Utilization, College of Chemistry &
Pharmacy, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic
of China.

²School of Chinese Medicine, Li Ka Shing Faculty of Medicine, University of Hong Kong,
10 Sassoon Road, Pokfulam, Hong Kong, People's Republic of China.

§ both authors contributed equally to this work

* Corresponding author:

Fax/Tel: +86-29-87092335.

E-mail: jinminggao@nwsuaf.edu.cn (J.M.G.); jrong@hku.hk (J. R.)

Content

Table S1. ¹ H NMR Data for Compounds 11-14	3
Table S2. ¹³ C NMR Data for Compounds 11-14	4
Figure S1. ¹ H NMR and ¹³ C spectra of compound 1 in CD ₃ OD.	5
Figure S2. HSQC and ¹ H- ¹ H COSY spectra of compound 1 in CD ₃ OD.....	5
Figure S3. HMBC and NOESY spectra of compound 1 in CD ₃ OD and DMSO- <i>d</i> ₆	6
Figure S4. HRESIMS spectrum of compound 1	7
Figure S5. IR and UV spectra of compound 1	8
Figure S6. ¹ H NMR and ¹³ C spectra of compound 2 in CD ₃ OD.	9
Figure S7. HSQC and ¹ H- ¹ H COSY spectra of compound 2 in CD ₃ OD.....	10
Figure S8. HMBC and NOESY spectra of compound 2 in CD ₃ OD.	11
Figure S9. HRESIMS spectrum of compound 2	12
Figure S10. IR and UV spectra of compound 2	13

Figure S11. ^1H NMR and ^{13}C spectra of compound 3 in CD_3OD	14
Figure S12. HSQC and ^1H - ^1H COSY spectra of compound 3 in CD_3OD	15
Figure S13. HMBC and NOESY spectra of compound 3 in CD_3OD	16
Figure S14. HRESIMS spectrum of compound 3	17
Figure S15. IR and UV spectra of compound 3	18
Figure S16. ^1H NMR and ^{13}C spectra of compound 4 in CD_3OD	19
Figure S17. HSQC and ^1H - ^1H COSY spectra of compound 4 in CD_3OD	20
Figure S18. HMBC and NOESY spectra of compound 4 in CD_3OD	21
Figure S19. HRESIMS spectrum of compound 4	22
Figure S20. IR and UV spectra of compound 4	23
Figure S21. ^1H NMR and ^{13}C spectra of compound 5 in CD_3OD	24
Figure S22. HSQC and ^1H - ^1H COSY spectra of compound 5 in CD_3OD	25
Figure S23. HMBC and NOESY spectra of compound 5 in CD_3OD	26
Figure S24. HRESIMS spectrum of compound 5	27
Figure S25. IR and UV spectra of compound 5	28
Figure S26. ^1H NMR and ^{13}C spectra of compound 6 in CD_3OD	28
Figure S27. HSQC and ^1H - ^1H COSY spectra of compound 6 in CD_3OD	29
Figure S28. HMBC and NOESY spectra of compound 6 in CD_3OD	30
Figure S29. HRESIMS spectrum of compound 6	31
Figure S30. IR and UV spectra of compound 6	32
Figure S31. ^1H NMR and ^{13}C spectra of compound 7 in CD_3OD	33
Figure S32. HSQC and ^1H - ^1H COSY spectra of compound 7 in CD_3OD	34
Figure S33. HMBC and NOESY spectra of compound 7 in CD_3OD	35
Figure S34. HRESIMS spectrum of compound 7	36
Figure S35. IR and UV spectra of compound 7	37
Figure S36. ^1H NMR and ^{13}C spectra of compound 8 in CD_3OD	38
Figure S37. HSQC and ^1H - ^1H COSY spectra of compound 8 in CD_3OD	39
Figure S38. HMBC and NOESY spectra of compound 8 in CD_3OD	40
Figure S39. HRESIMS spectrum of compound 8	41
Figure S40. IR and UV spectra of compound 8	42
Figure S41. ^1H NMR and ^{13}C spectra of compound 9 in CD_3OD	43
Figure S42. HSQC and ^1H - ^1H COSY spectra of compound 9 in CD_3OD	44
Figure S43. HMBC and NOESY spectra of compound 9 in CD_3OD	45
Figure S44. HRESIMS spectrum of compound 9	46
Figure S45. IR and UV spectra of compound 9	47
Figure S46. ^1H NMR and ^{13}C spectra of compound 10 in CD_3OD	48
Figure S47. HSQC and ^1H - ^1H COSY spectra of compound 10 in CD_3OD	49
Figure S48. HMBC and NOESY spectra of compound 10 in CD_3OD	50
Figure S49. HRESIMS spectrum of compound 10	51
Figure S50. IR and UV spectra of compound 10	52
Figure S51. ^1H NMR and ^{13}C spectra of compound 11 in CD_3OD	52
Figure S52. ESIMS spectrum of compound 11	53
Figure S53. UV spectra of compound 11	54
Figure S54. ^1H NMR and ^{13}C spectra of compound 12 in $\text{C}_5\text{D}_5\text{N}$	54

Figure S55. ESIMS spectrum of compound 12 .	55
Figure S56. UV spectra of compound 12 .	56
Figure S57. ¹ H NMR and ¹³ C spectra of compound 13 in CD ₃ OD.	56
Figure S58. ESIMS spectrum of compound 13 .	57
Figure S59. UV spectra of compound 13 .	58
Figure S60. ¹ H NMR and ¹³ C spectra of compound 14 in CD ₃ OD.	58
Figure S61. ESIMS spectrum of compound 14 .	59
Figure S62. UV spectra of compound 14 .	60
Figure S63. Cytotoxicity of compounds in BV2 microglia cells.	61

No.	11 (CD ₃ OD)	12 (C ₅ D ₅ N)	13 (CD ₃ OD)	14 (CD ₃ OD)
1	6.24 d (5.4)	1.52 m 2H	2.17 m 2H	
2	6.32 d (5.4)	2.23 m 2H		6.02 m
4				2.95 m
5	2.42dd (13.0, 3.7)	2.60 m	2.83dd (13.0, 4.0)	2.39 m
7	1.61td (13.5, 4.0) 1.44ddd (13.5, 4.3, 2.4)	1.79 m 1.64 m	1.70 m 1.55 m	2.01 m 1.05 m
8	1.71ddd (13.5, 4.0, 2.4) 1.26 td (13.5, 4.3)	1.71 td (13.2, 4.4) 1.59 m	1.82 m 1.61 m	1.70 m 1.33 m
10	2.31 m 1.52ddd (12.7, 3.5, 2.0)	2.33 td (13.1, 3.8) 1.98dd (13.1, 3.6)	2.36 td (13.3, 4.0) 1.75 m	2.41 m 2.33 m
11	4.22 m	4.72 m	4.33 m	4.39 m
12	2.35 m	3.16 m	2.47 m	
13	4.52 d (7.7)	4.57 d (4.7)	4.13 d (5.0)	6.06 m
15	3.85dd (10.9, 5.9) 3.62dd (10.9, 8.7)	4.34dd (10.8, 6.8) 4.07 m	3.89dd (11.2, 6.1) 3.69dd (11.2, 9.2)	4.35 m 4.26 m
16	0.87 s	1.42 s	1.07 s	1.09 s
17	1.02 s	0.97 s	1.25 s	1.11 s
18	2.99 m	2.99 m	3.09 m	2.89 m
19	1.05 d (6.9)	0.85 d (6.8)	1.18 d (7.0)	1.29 d (6.6)
20	1.11 d (6.9)	1.01 d (6.8)	1.20 d (7.0)	1.27 d (6.6)

Table S1. ¹H NMR Data for Compounds **11-14**.

No.	11(CD ₃ OD)	12(C ₅ D ₅ N)	13(CD ₃ OD)	14(CD ₃ OD)
1	145.8	40.1	53.2	216.1
2	128.9	29.2	210.8	127.8
3	143.0	140.1	144.8	193.5
4	143.1	136.6	176.9	56.2
5	38.8	38.6	40.8	37.8
6	46.5	43.9	46.3	51.0
7	29.8	29.9	29.3	32.6
8	32.4	37.7	37.1	31.3
9	55.1	48.3	42.8	50.4
10	31.6	27.8	27.4	38.7
11	76.9	73.7	74.4	72.6
12	49.5	56.2	55.8	156.2
13	70.2	71.7	71.6	123.1
14	107.8	106.1	106.2	209.3
15	62.2	60.4	60.4	64.2
16	12.9	13.6	13.4	21.0
17	20.6	24.1	26.1	21.4
18	26.9	26.5	26.2	32.4
19	23.0	21.4	20.5	21.1
20	23.2	22.7	20.7	24.1

Table S2. ¹³C NMR Data for Compounds **11-14**.

Figure S1. ^1H NMR and ^{13}C spectra of compound **1** in CD_3OD .

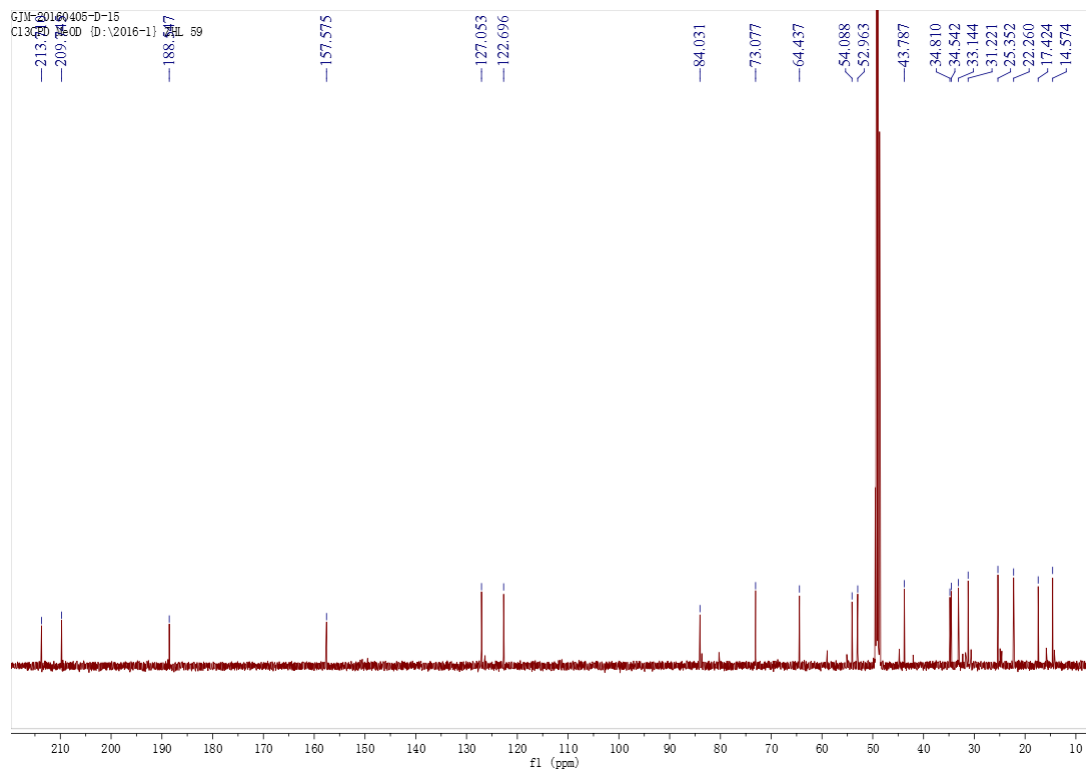
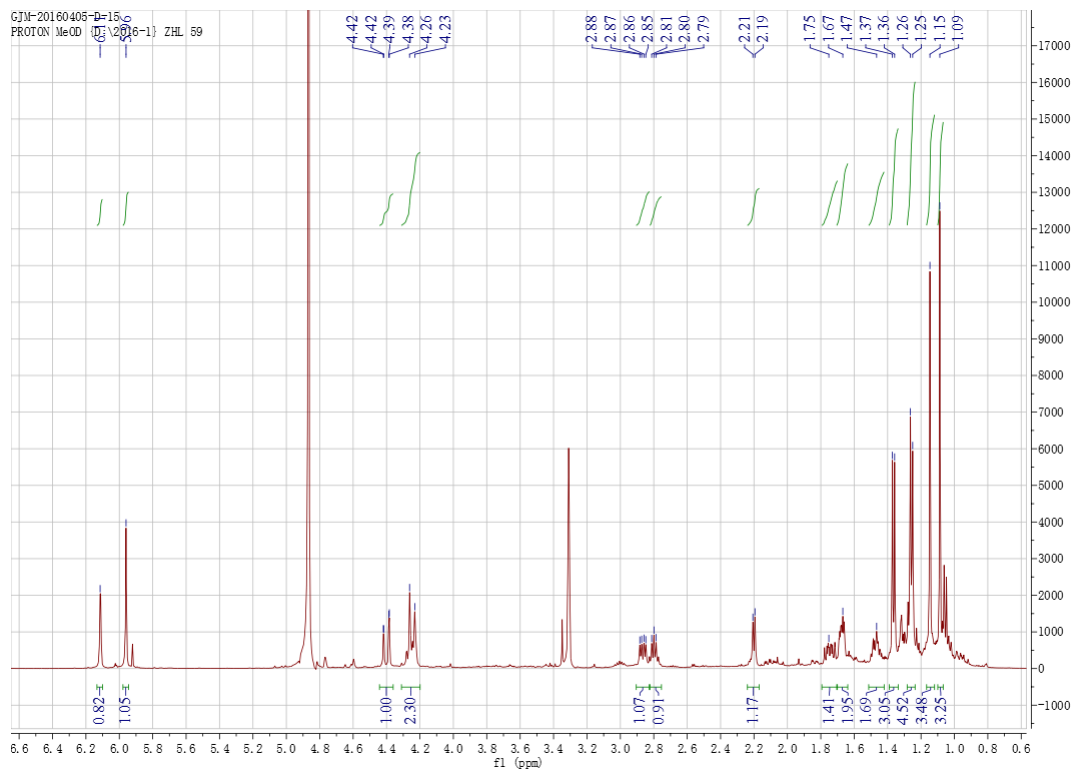


Figure S2. HSQC and ^1H - ^1H COSY spectra of compound **1** in CD_3OD

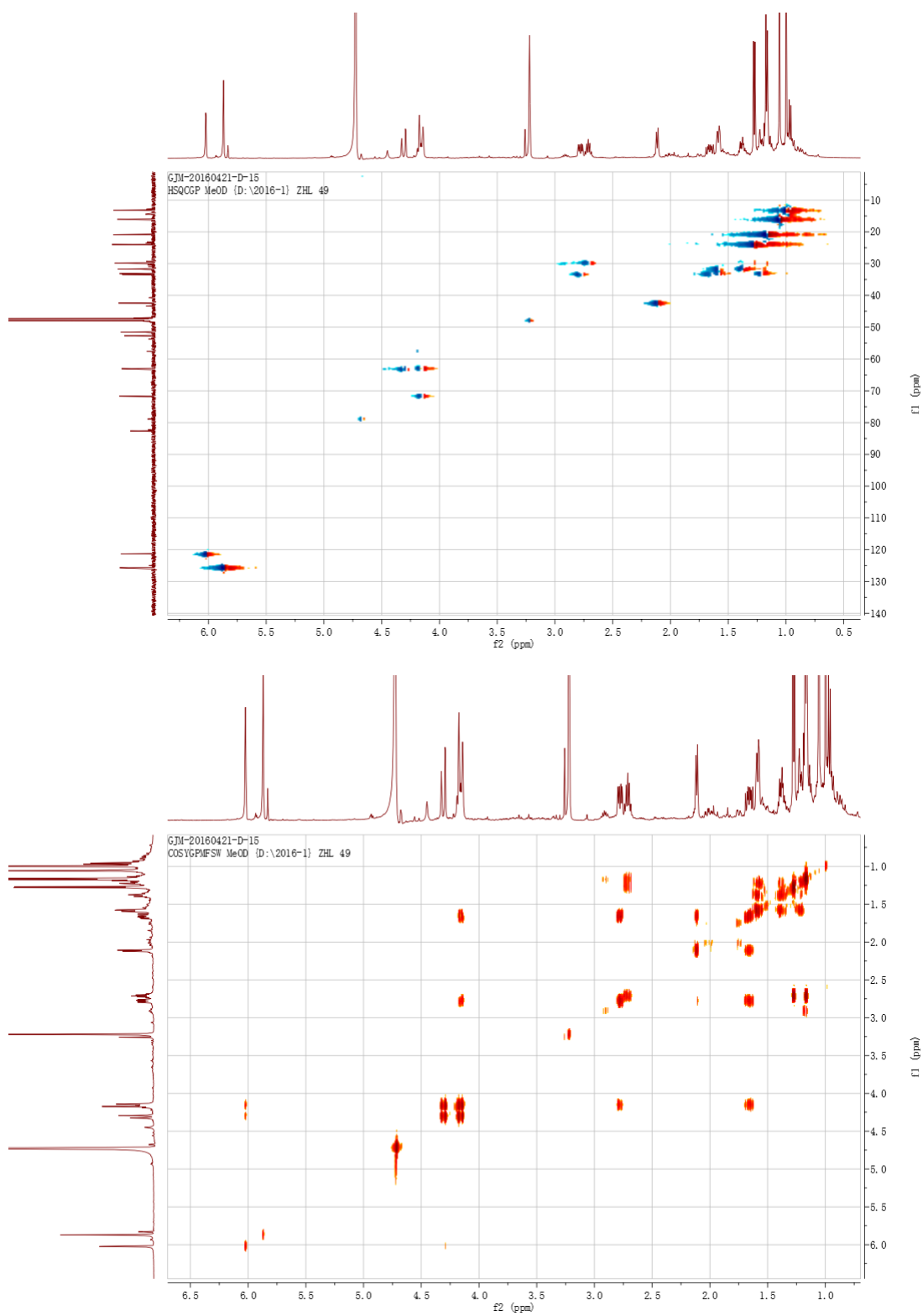


Figure S3. HMBC and NOESY spectra of compound **1** in CD₃OD and DMSO-*d*₆.

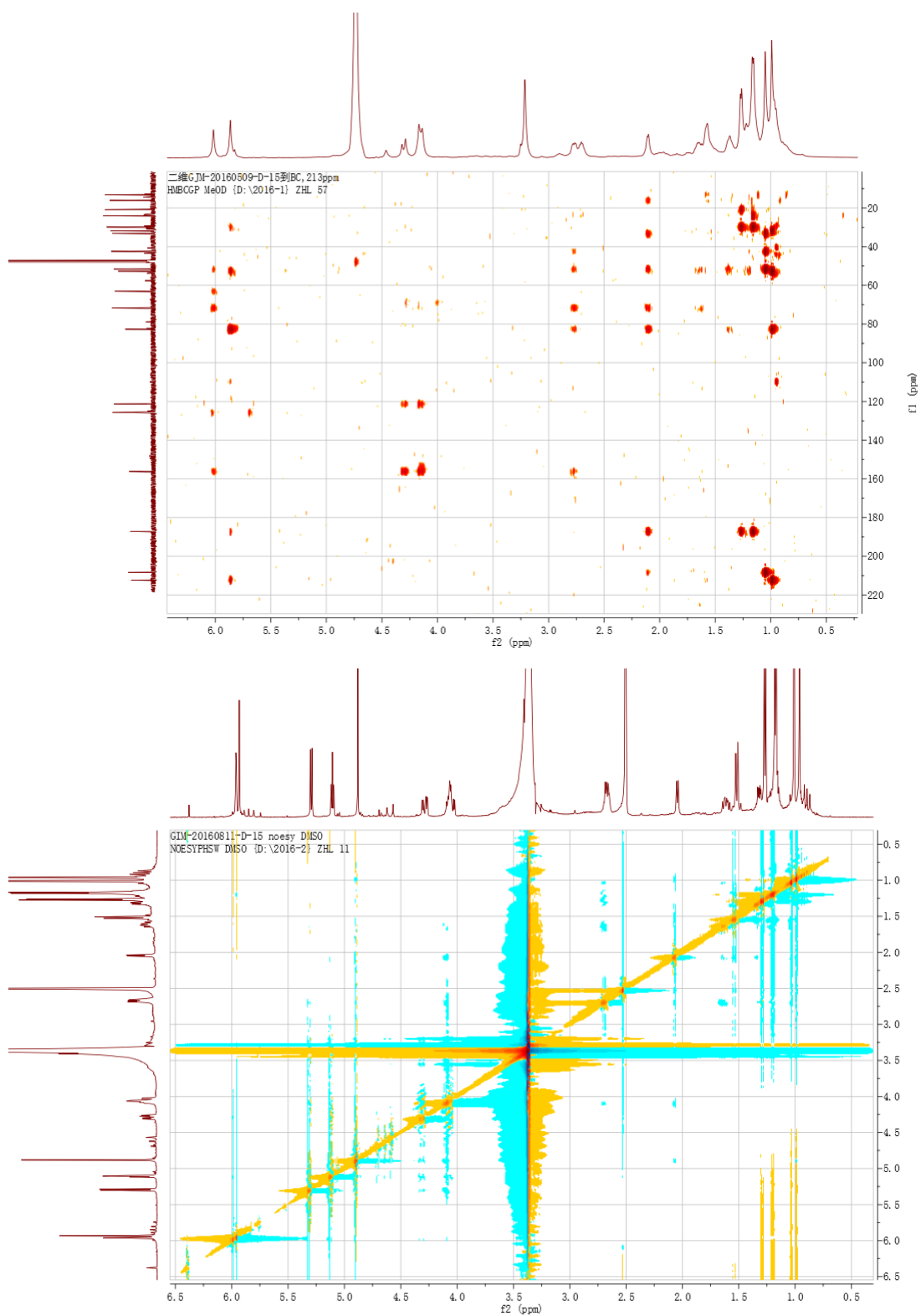


Figure S4. HRESIMS spectrum of compound 1.

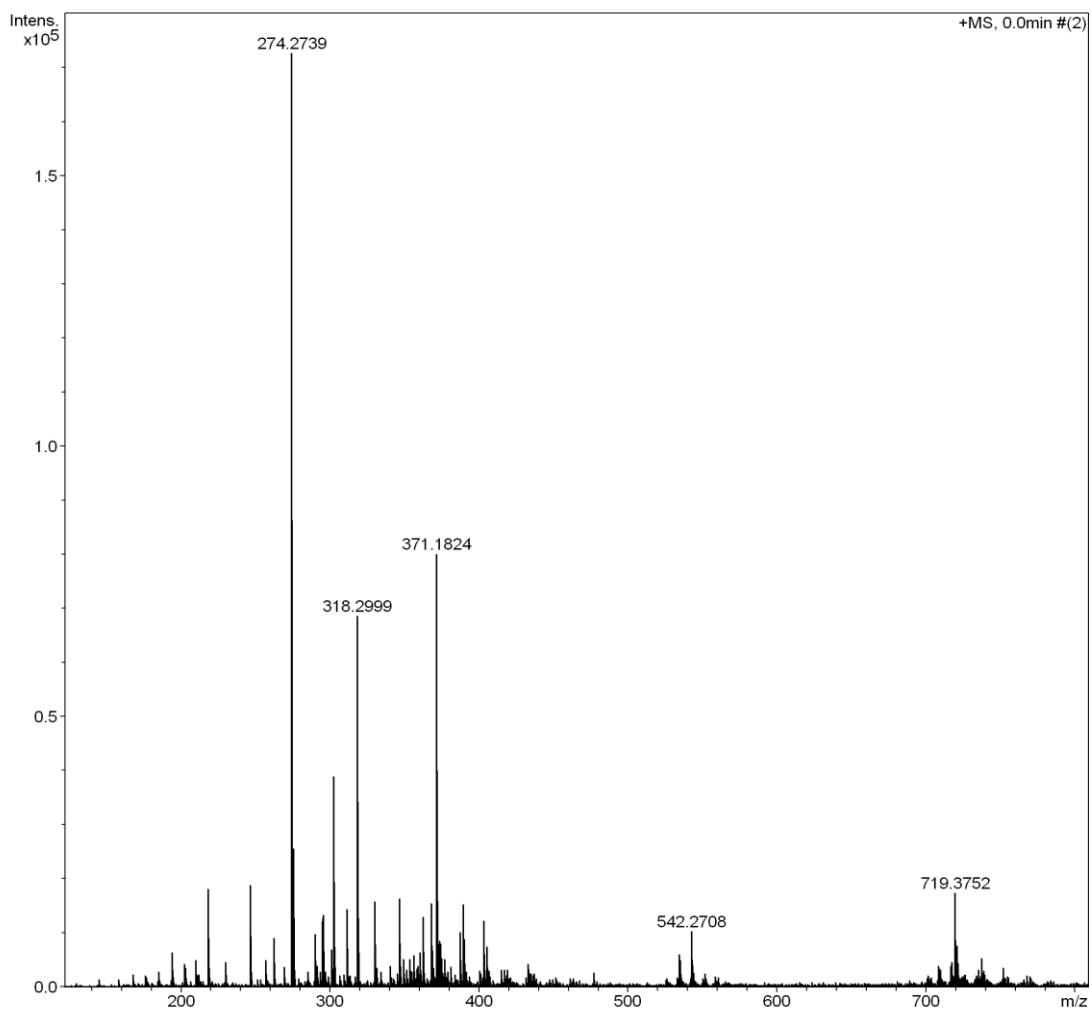
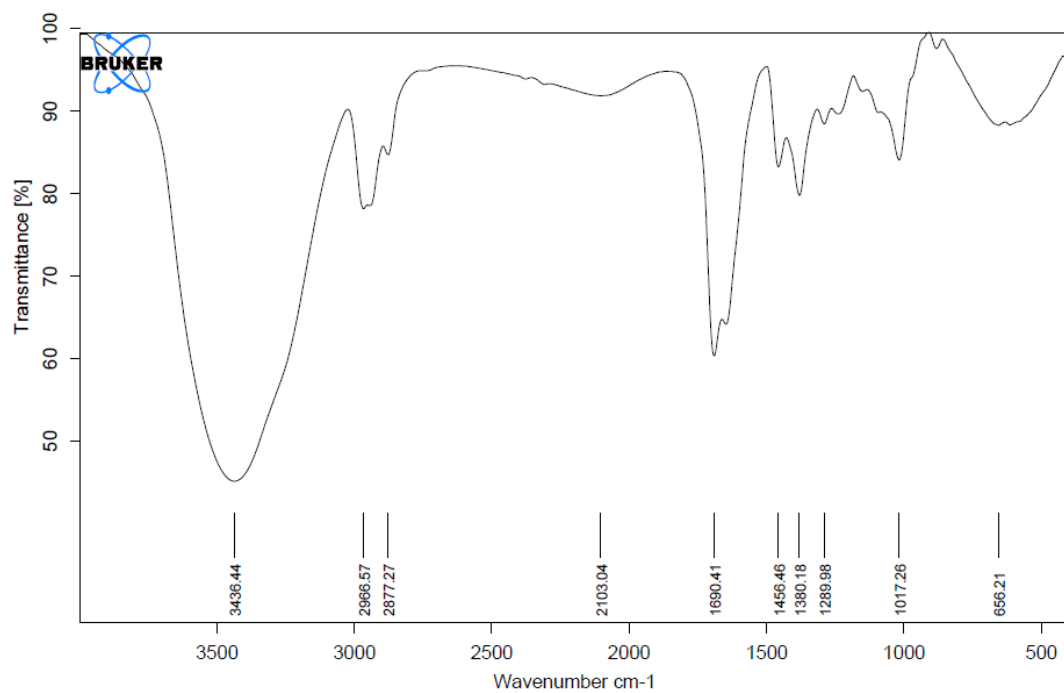


Figure S5. IR and UV spectra of compound **1**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



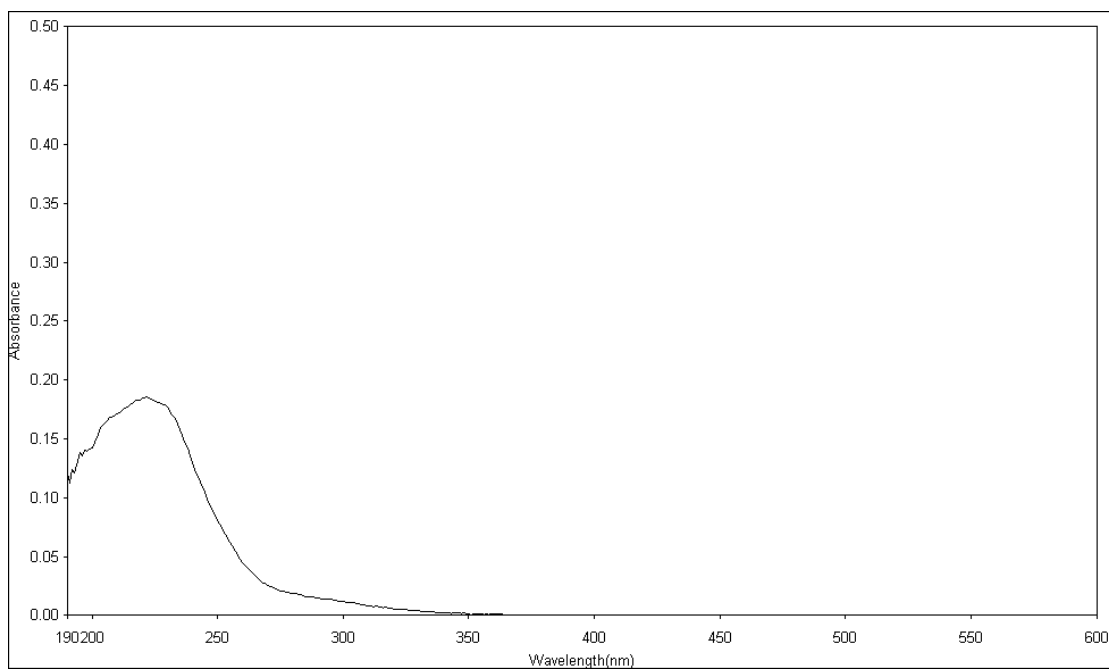
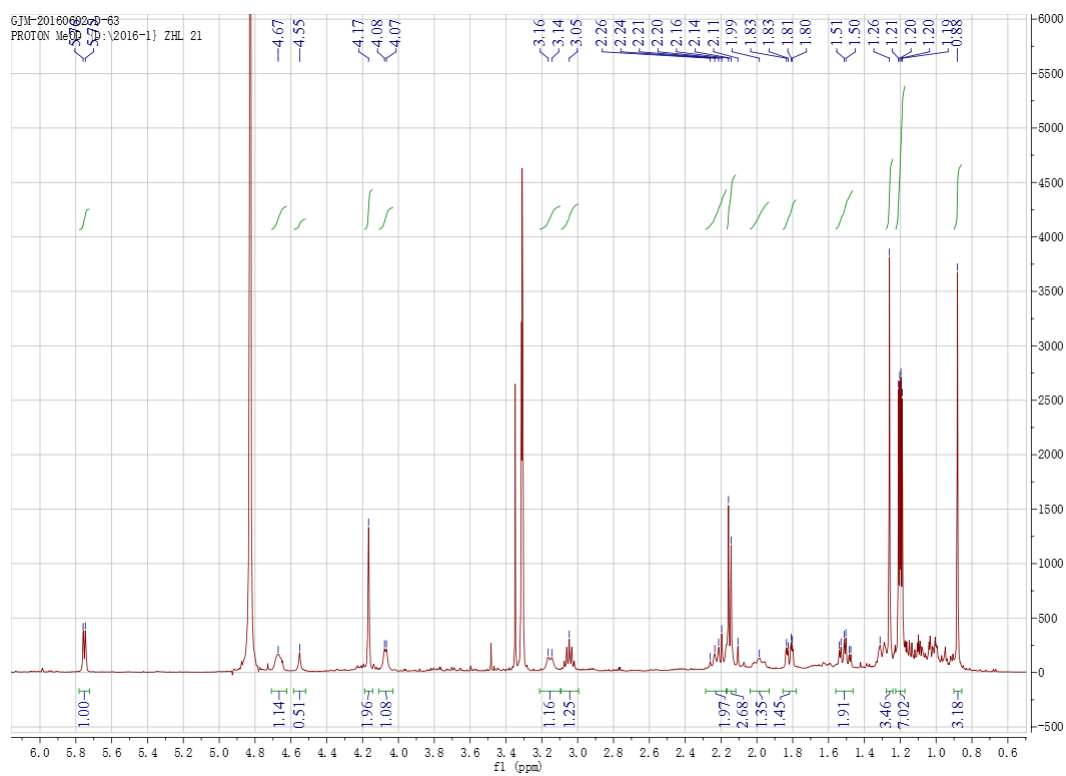


Figure S6. ¹H NMR and ¹³C spectra of compound **2** in CD₃OD.



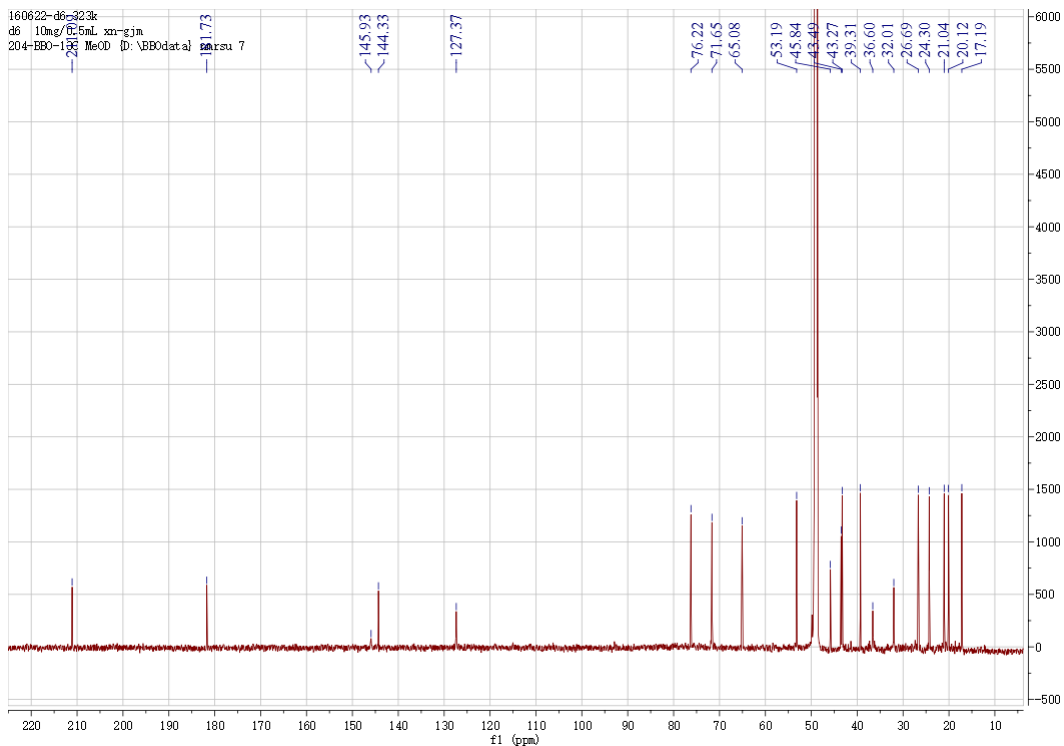
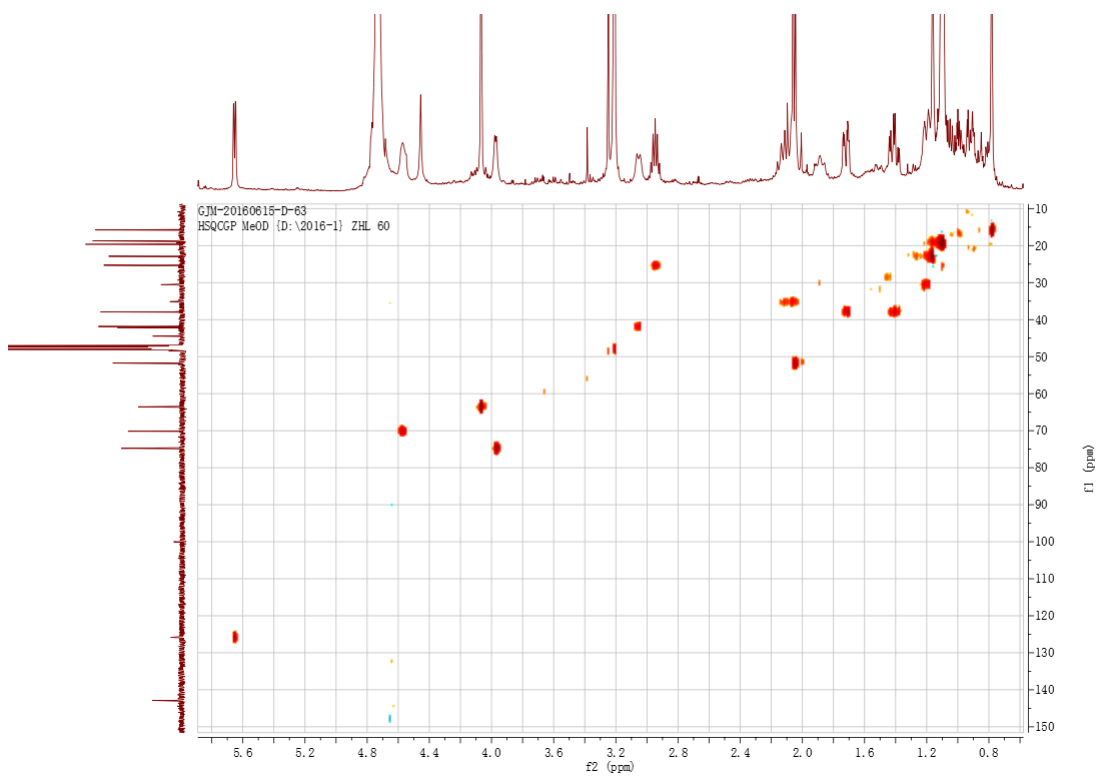


Figure S7. HSQC and ^1H - ^1H COSY spectra of compound **2** in CD_3OD



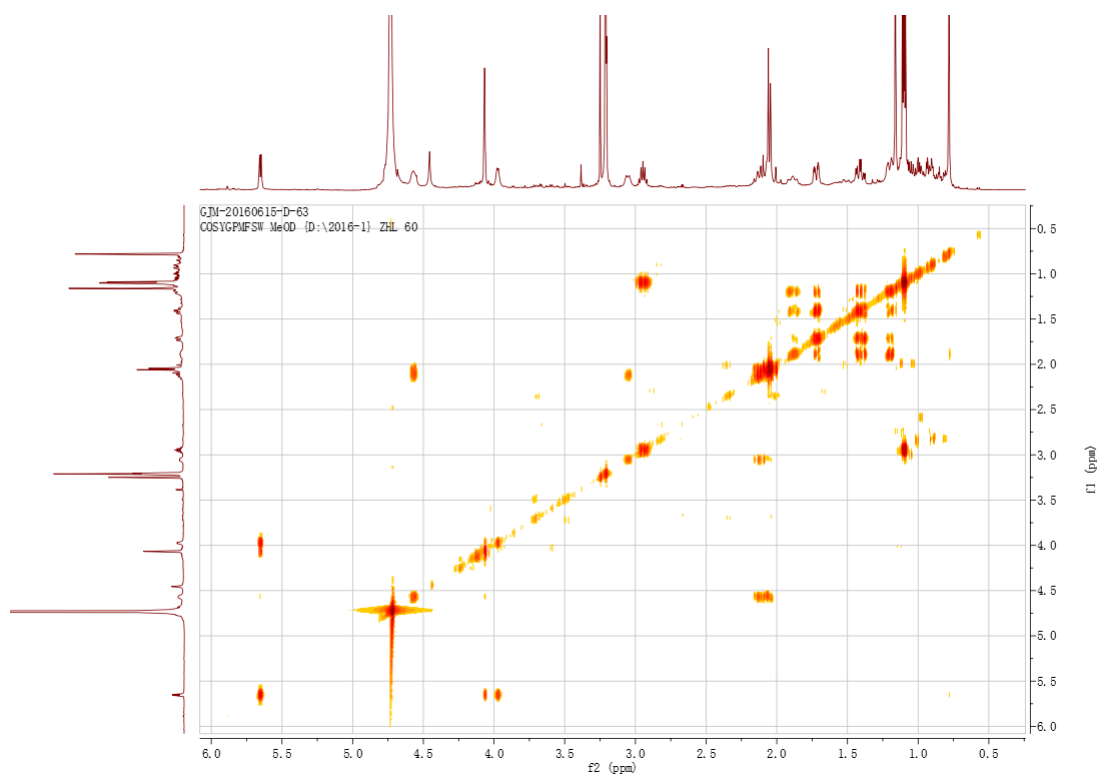
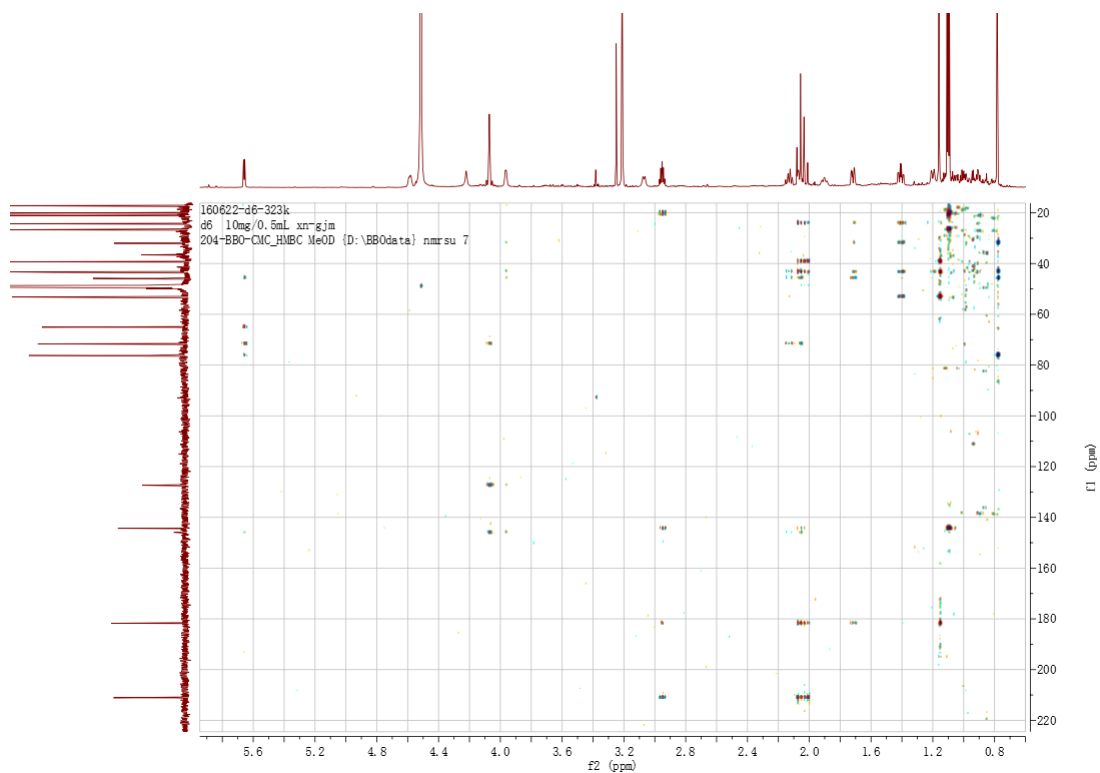


Figure S8. HMBC and NOESY spectra of compound **2** in CD₃OD.



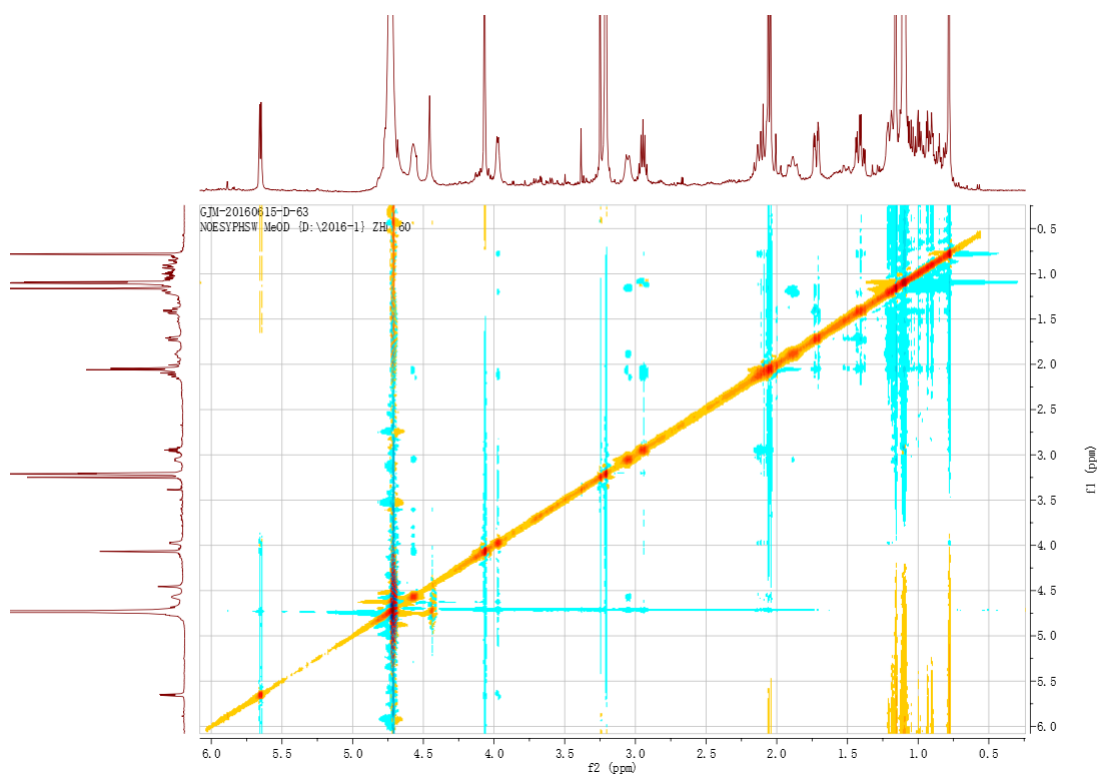


Figure S9. HRESIMS spectrum of compound **2**.

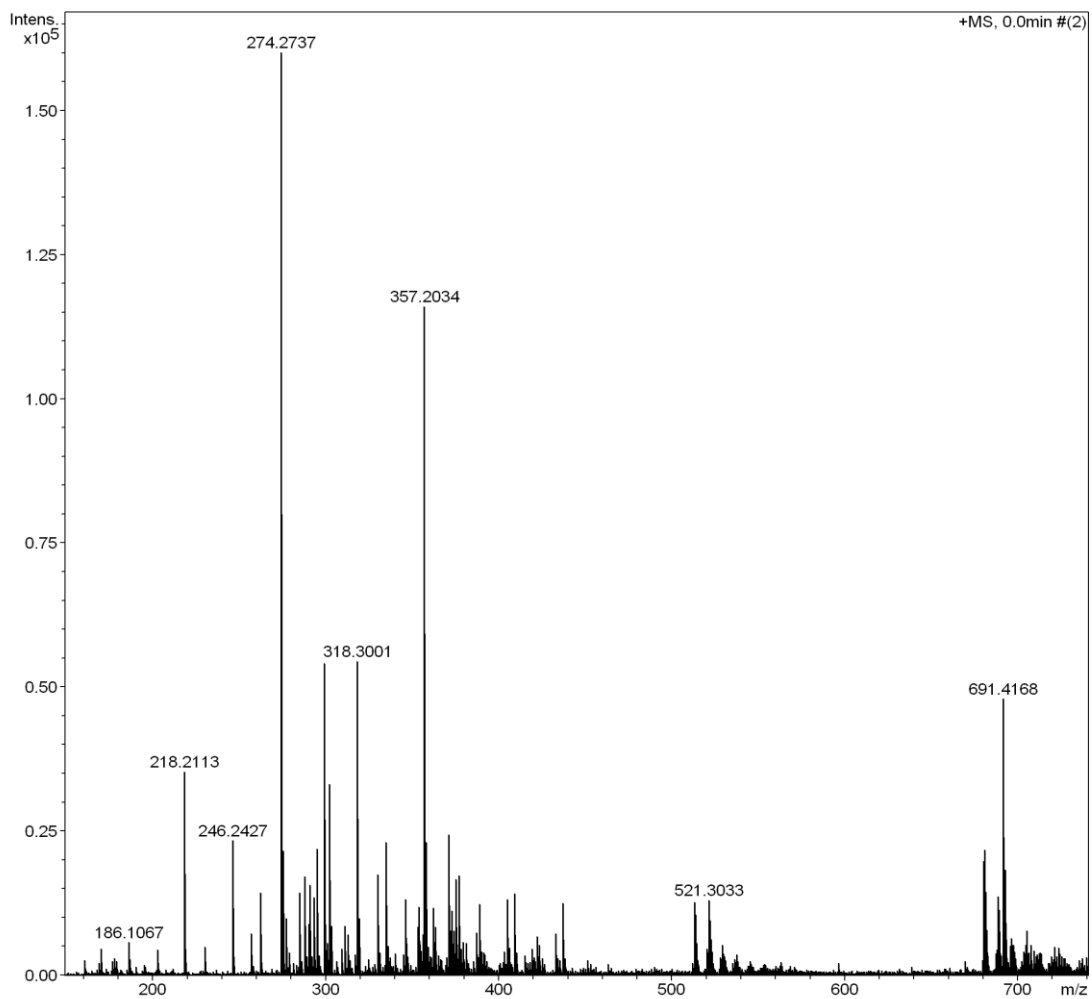
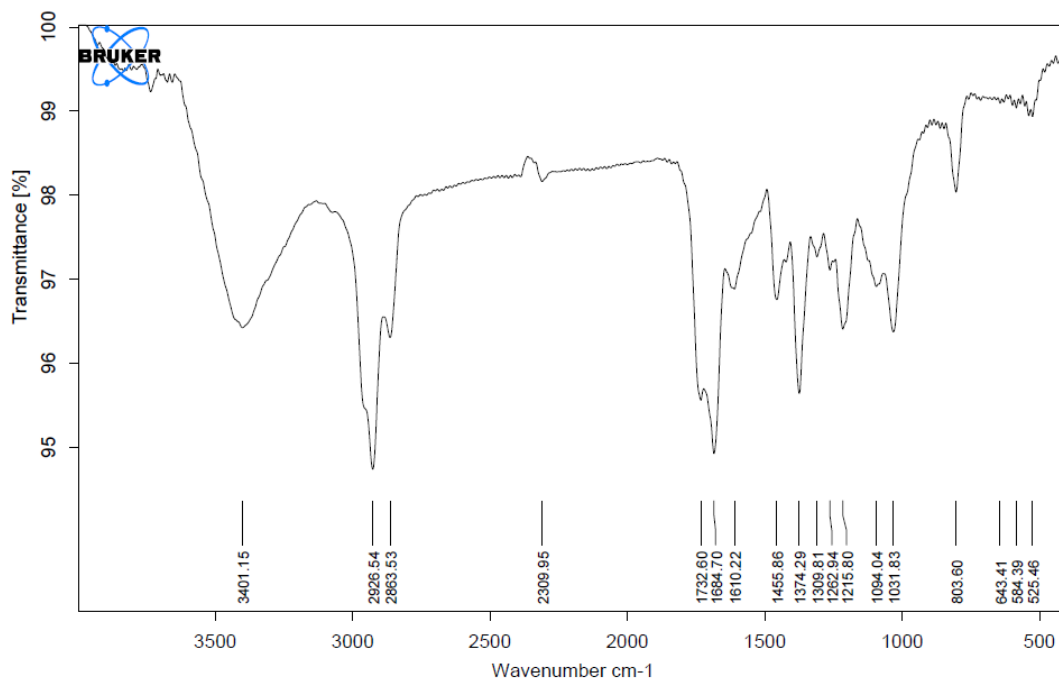


Figure S10. IR and UV spectra of compound **2**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



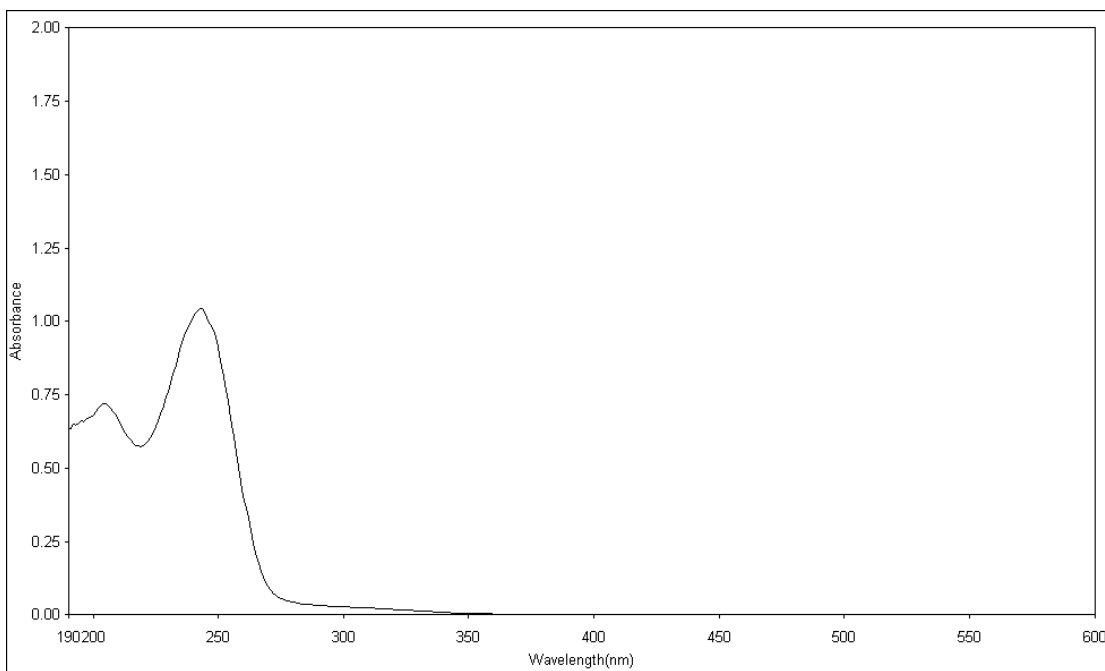
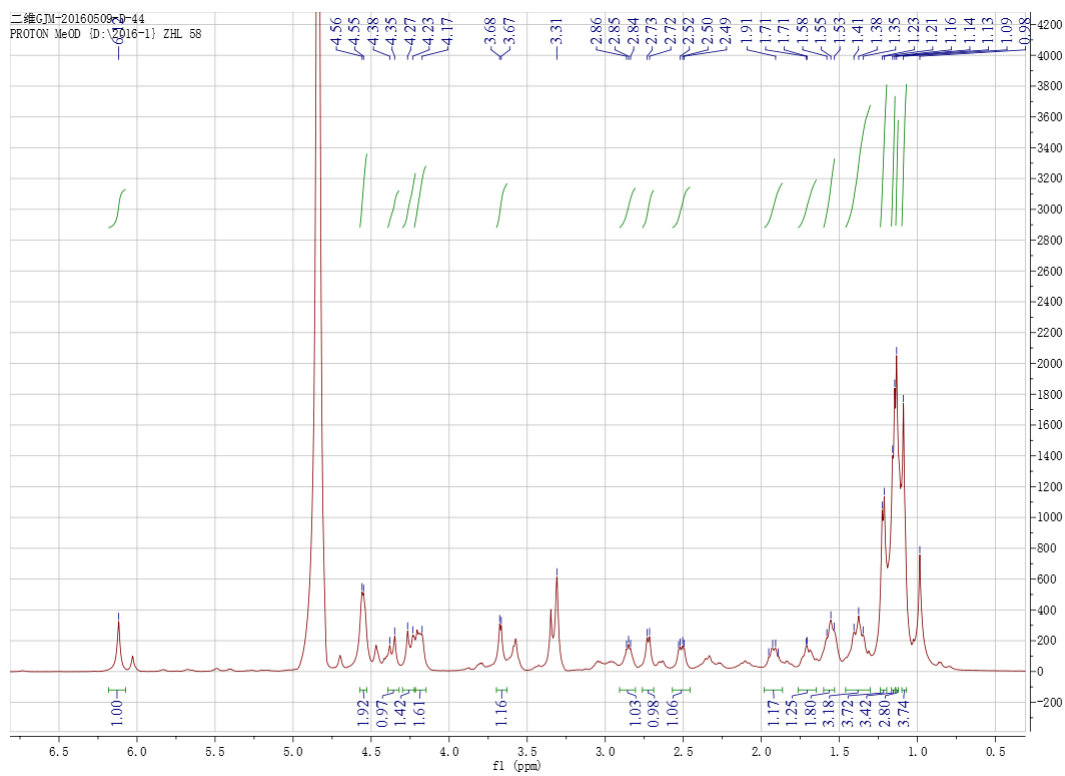


Figure S11. ^1H NMR and ^{13}C spectra of compound **3** in CD_3OD .



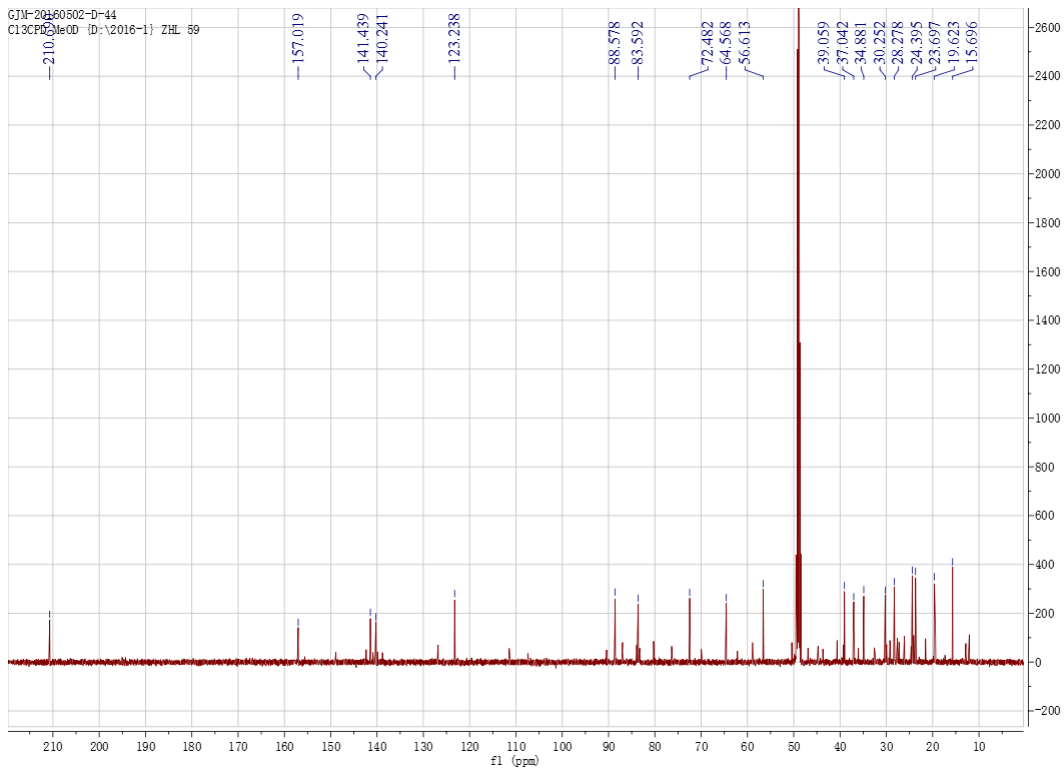
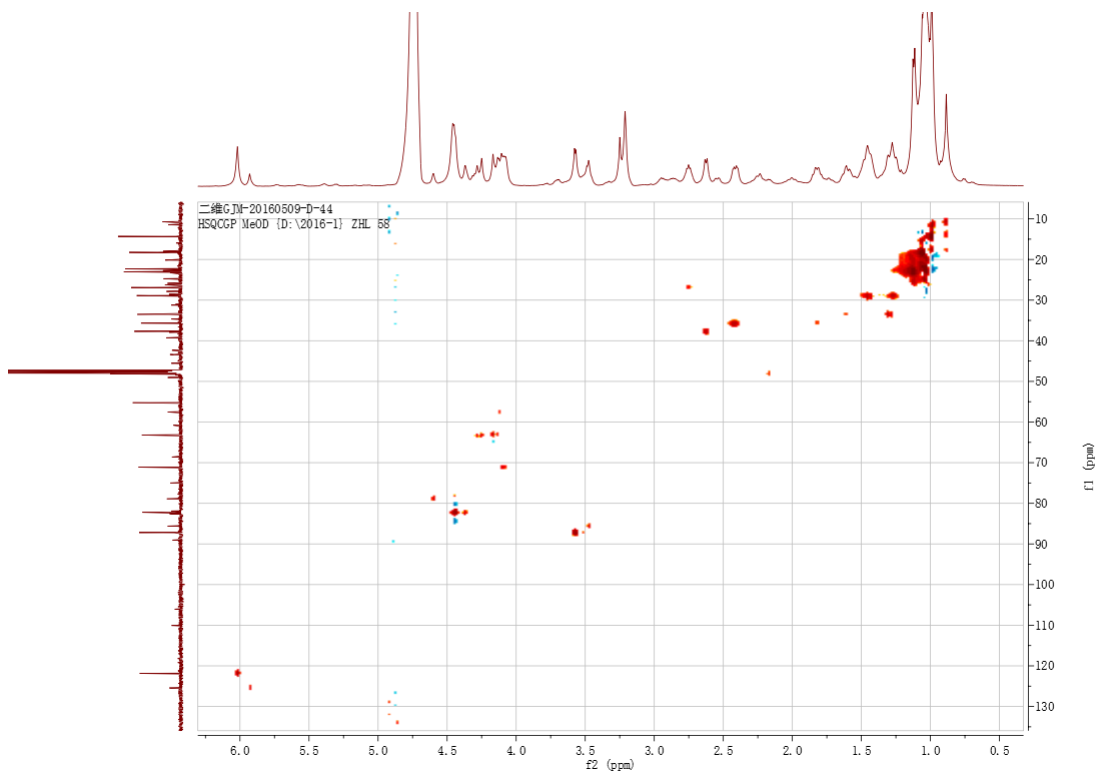


Figure S12. HSQC and ^1H - ^1H COSY spectra of compound **3** in CD_3OD



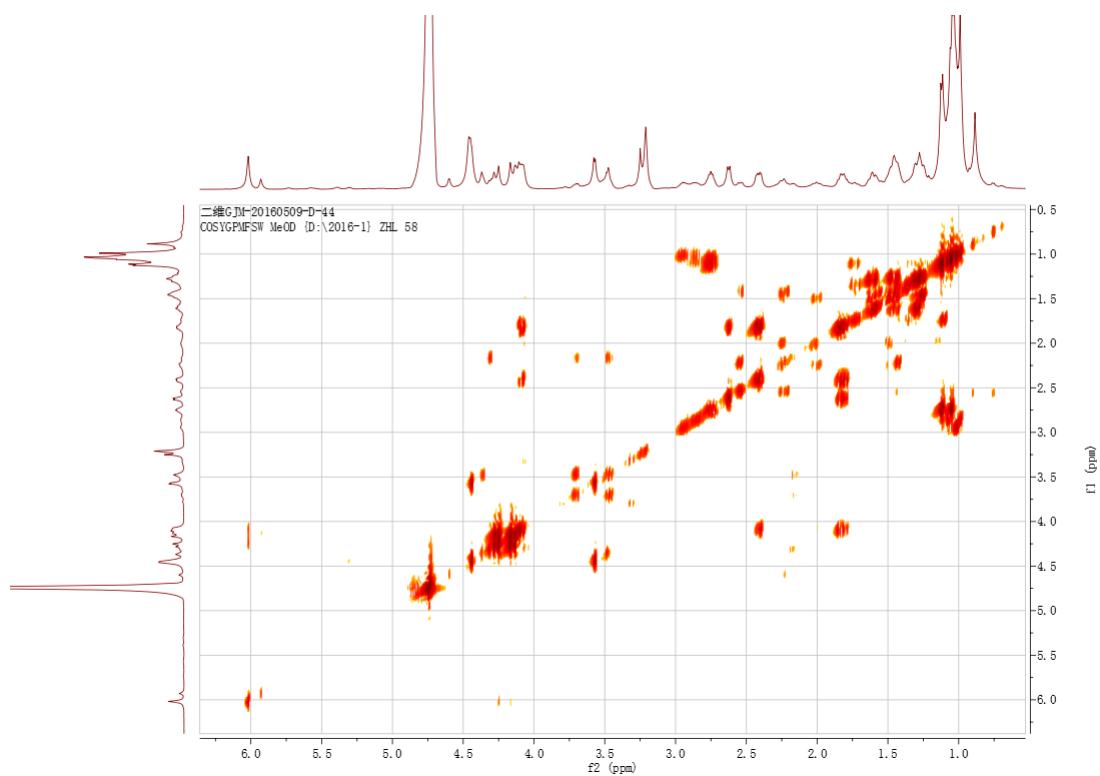
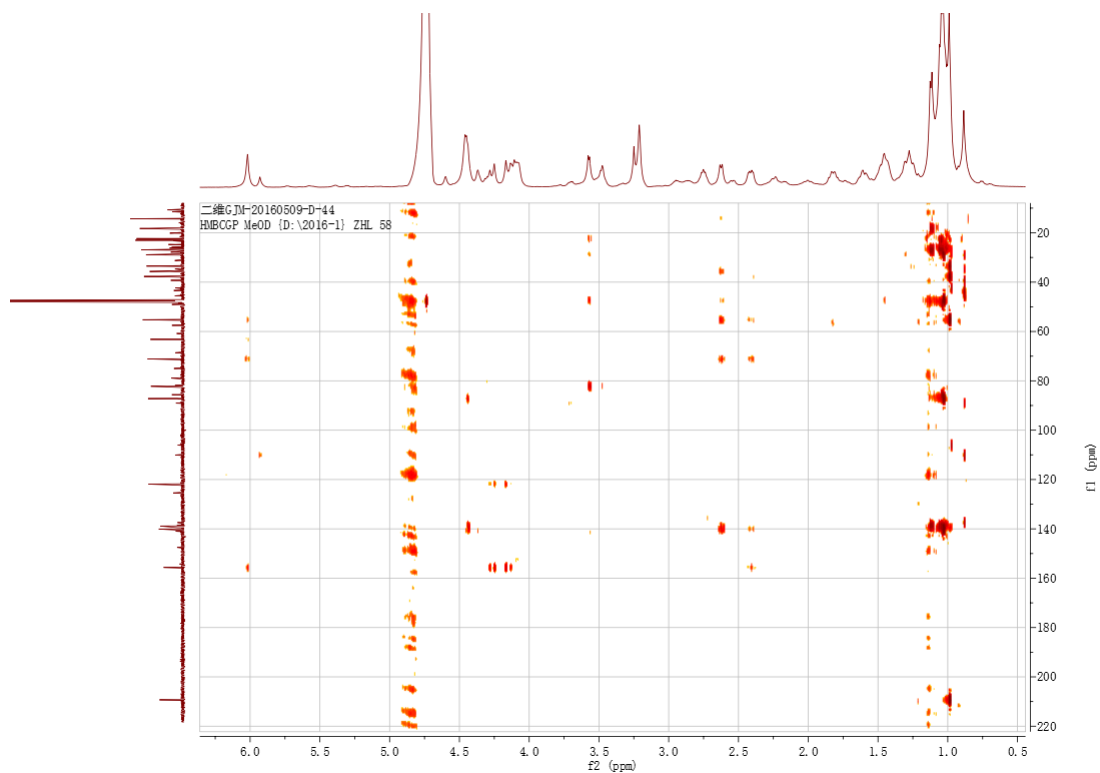


Figure S13. HMBC and NOESY spectra of compound **3** in CD₃OD.



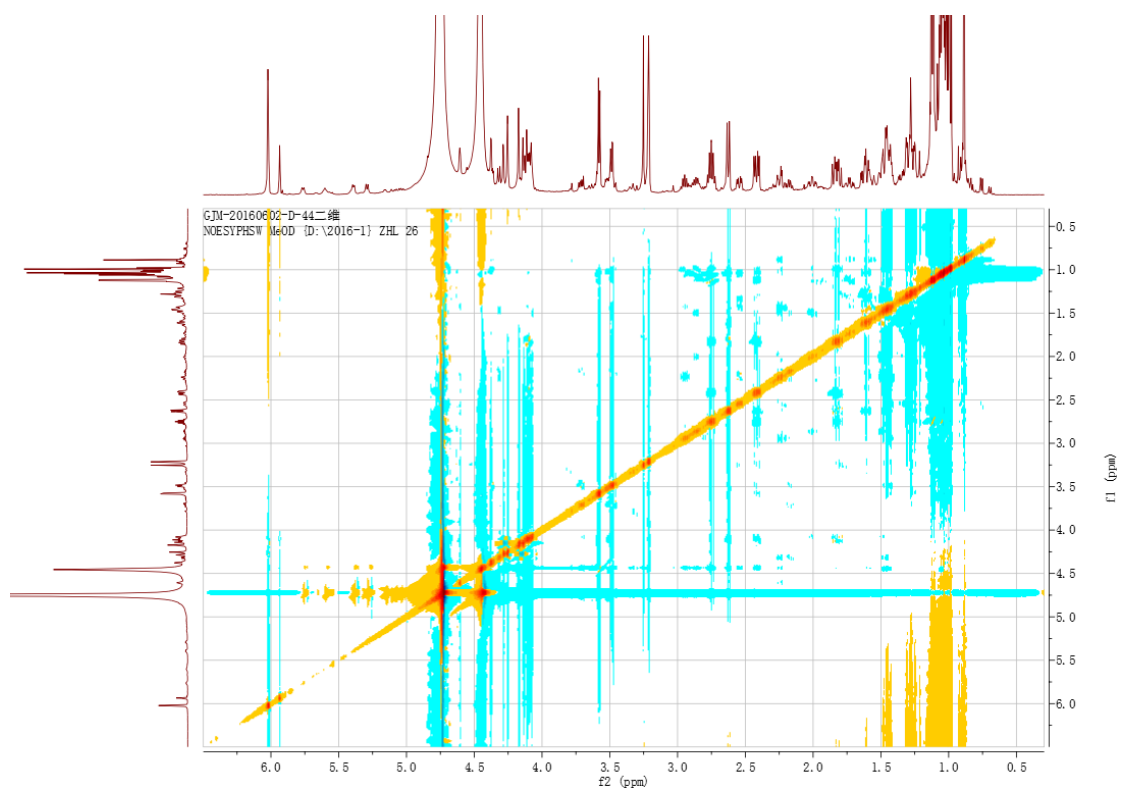


Figure S14. HRESIMS spectrum of compound **3**.

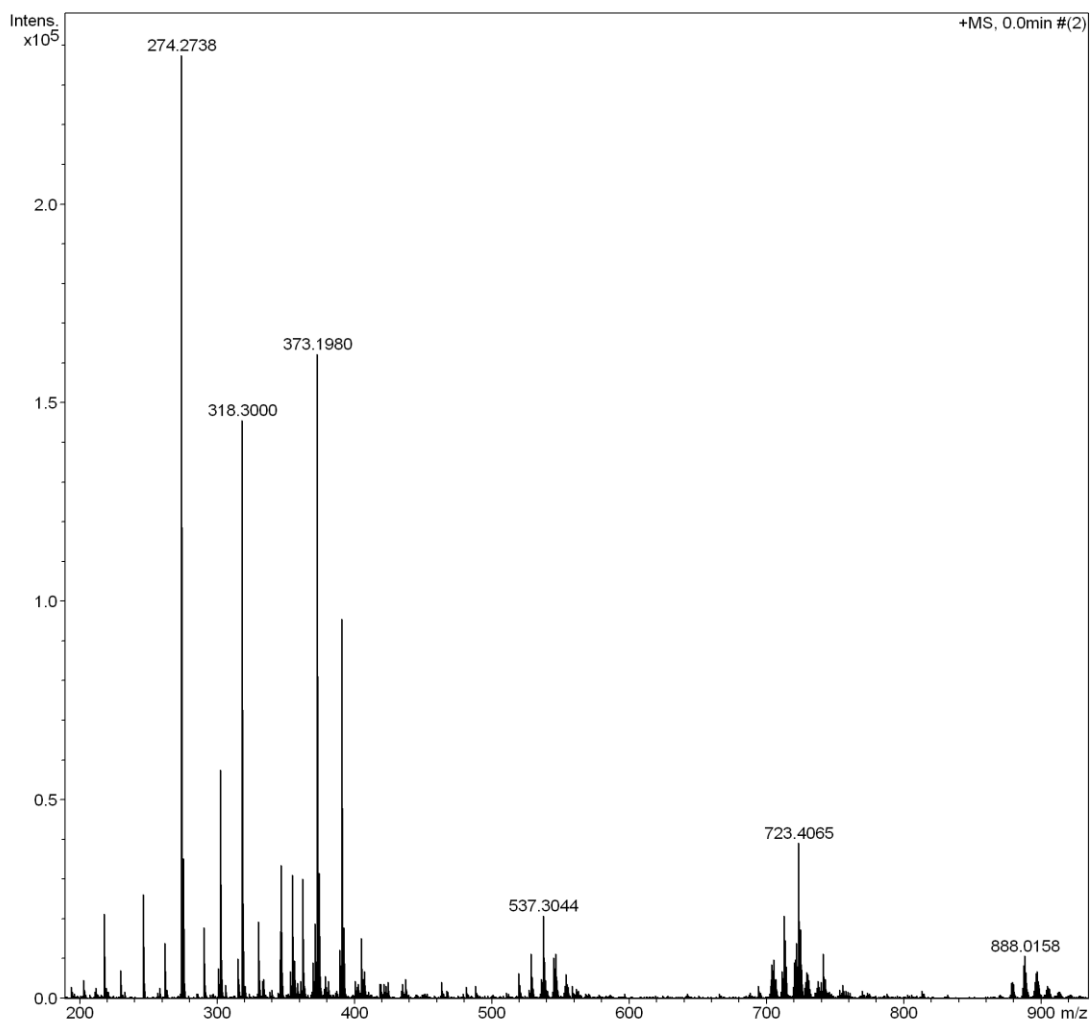
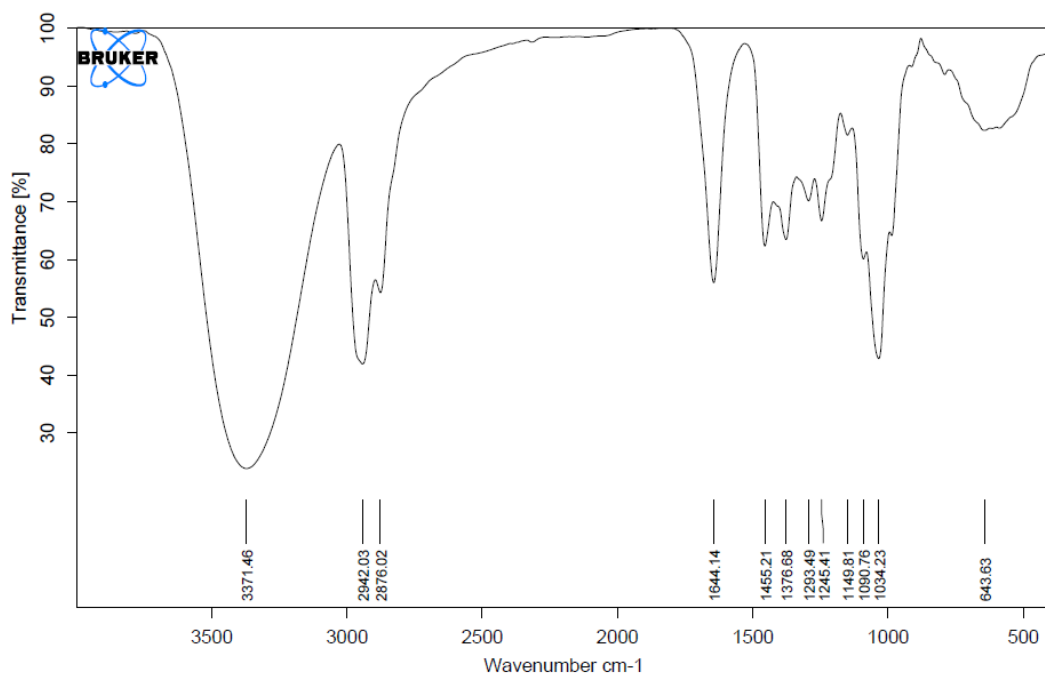


Figure S15. IR and UV spectra of compound **3**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



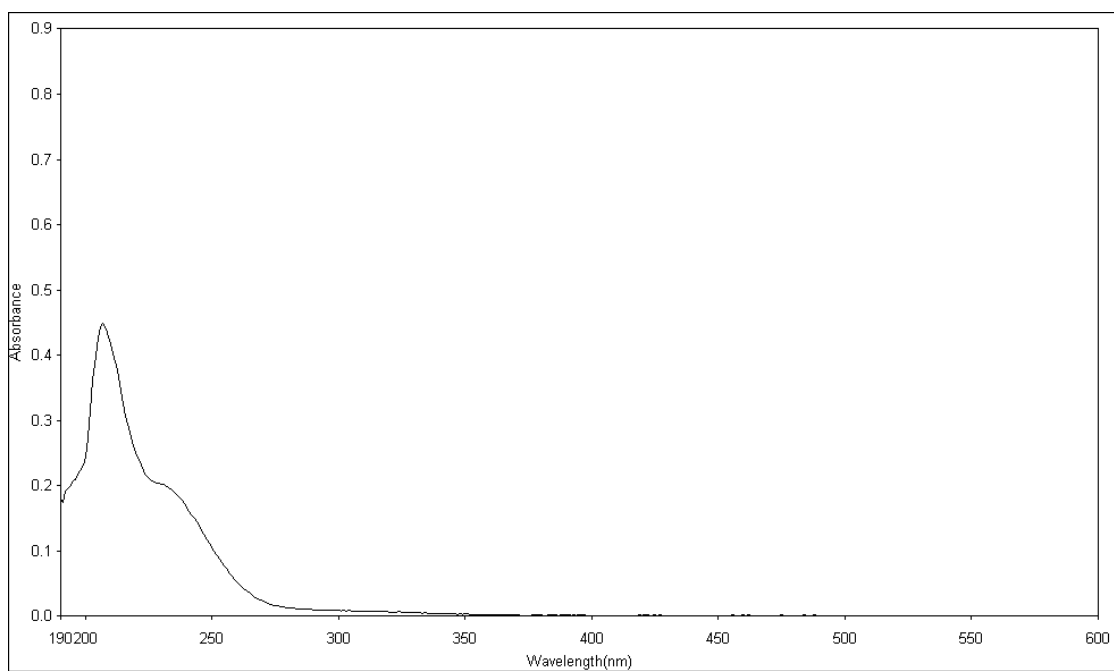
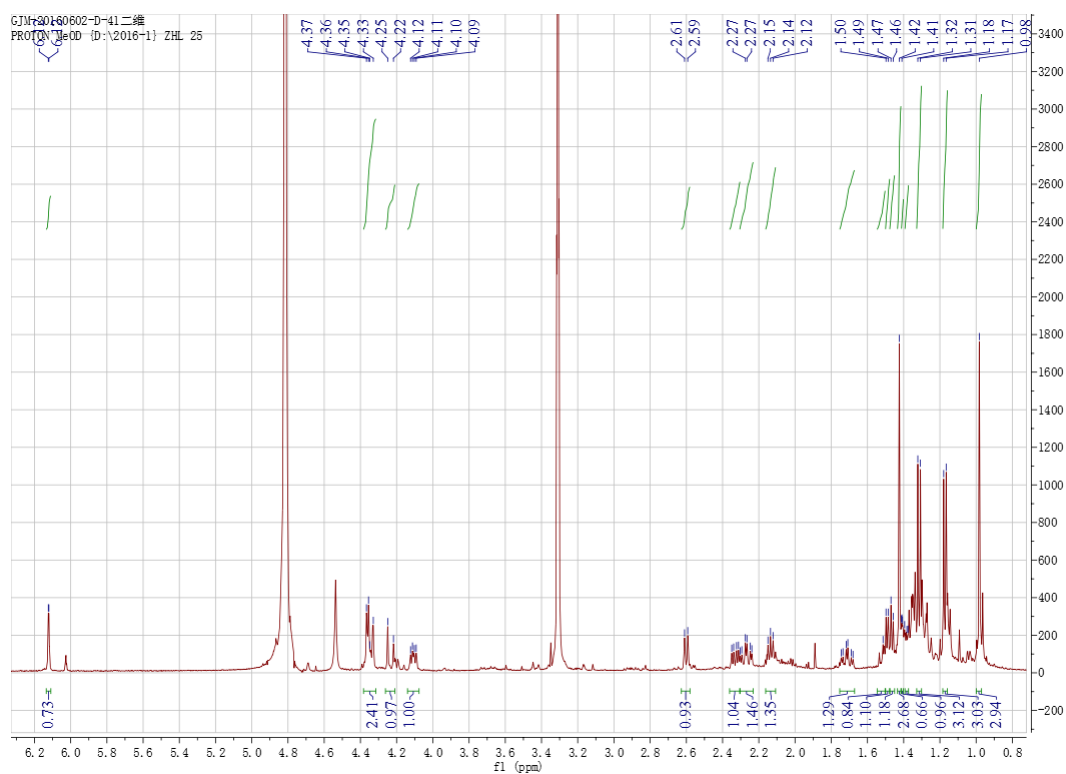


Figure S16. ¹H NMR and ¹³C spectra of compound 4 in CD₃OD.



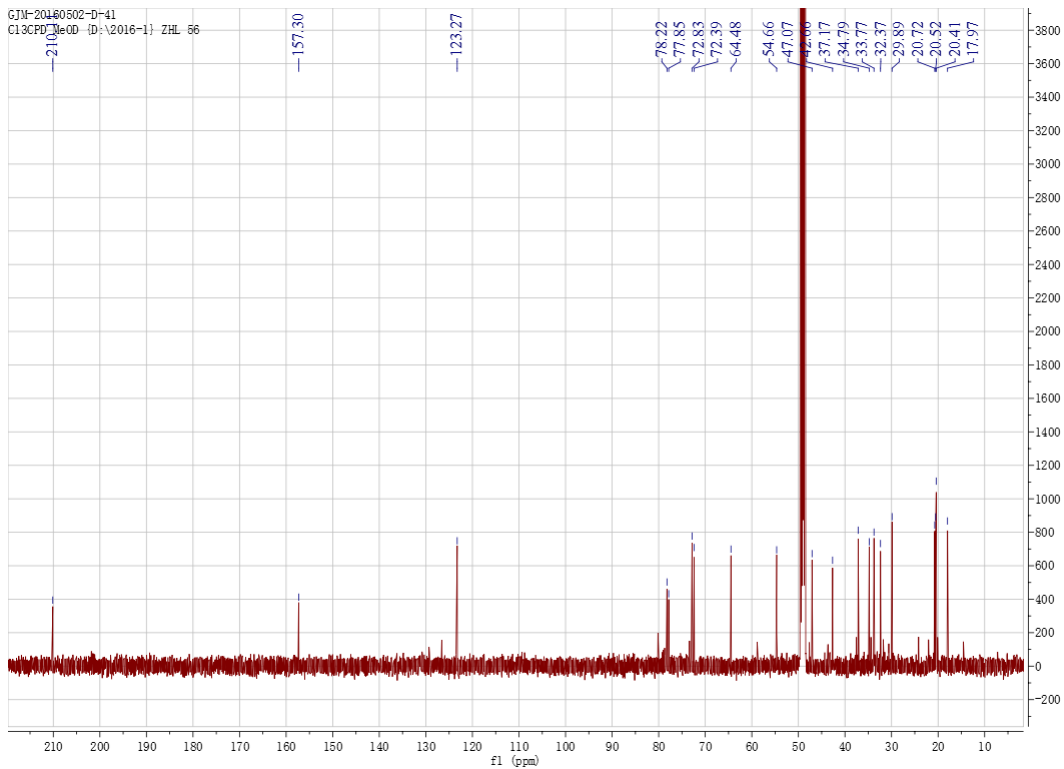
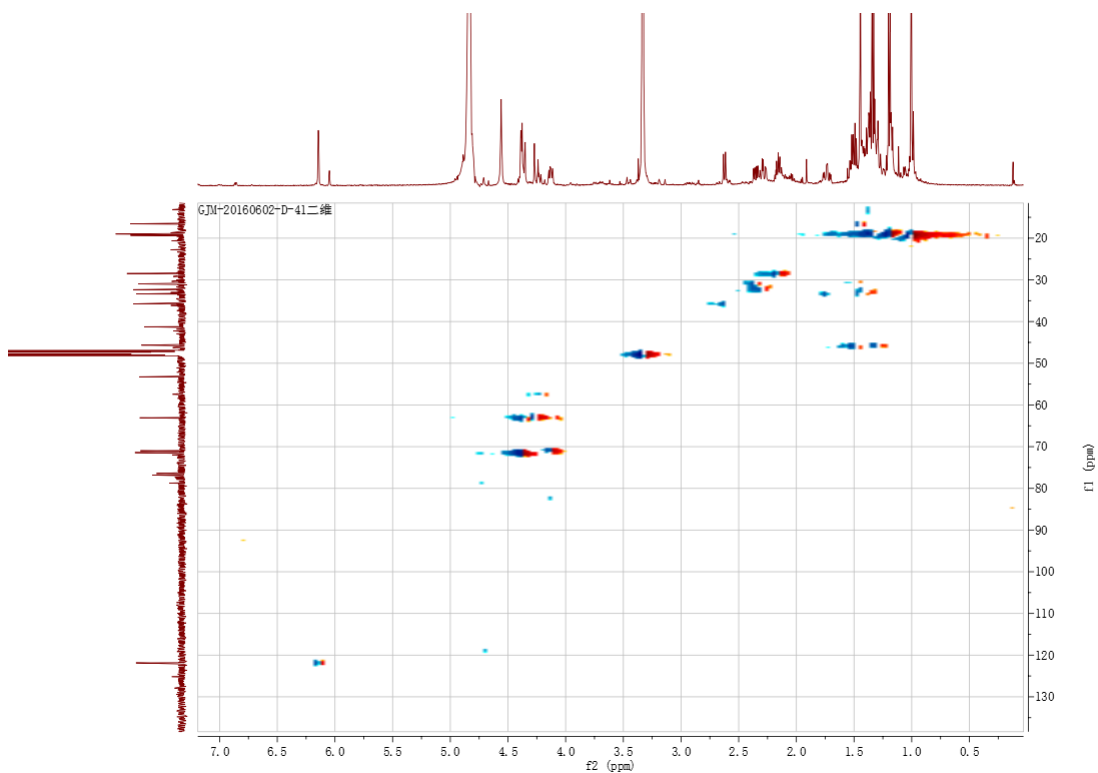


Figure S17. HSQC and ^1H - ^1H COSY spectra of compound 4 in CD_3OD



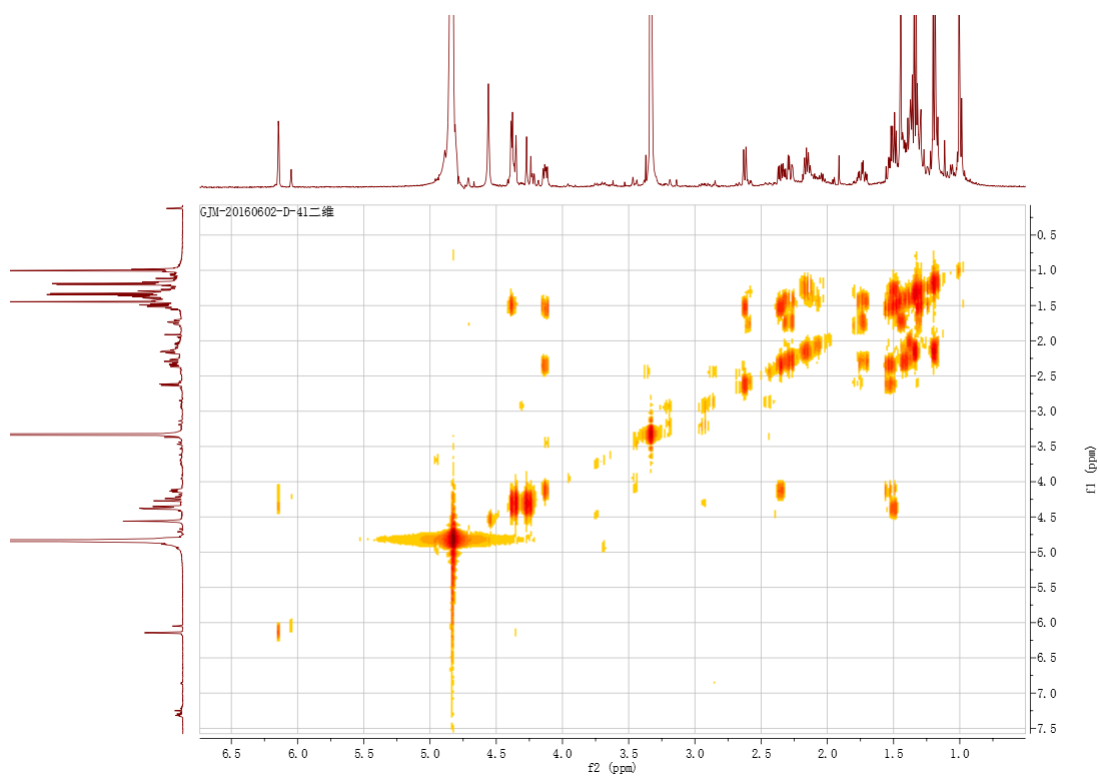
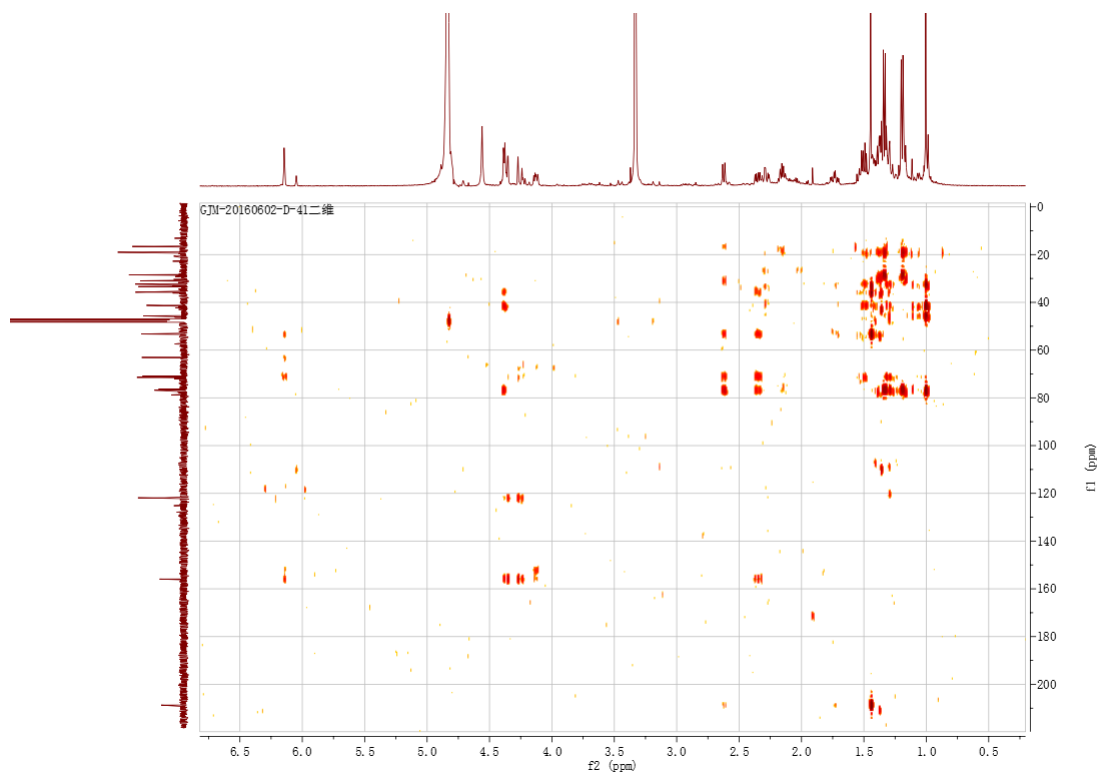


Figure S18. HMBC and NOESY spectra of compound **4** in CD₃OD.



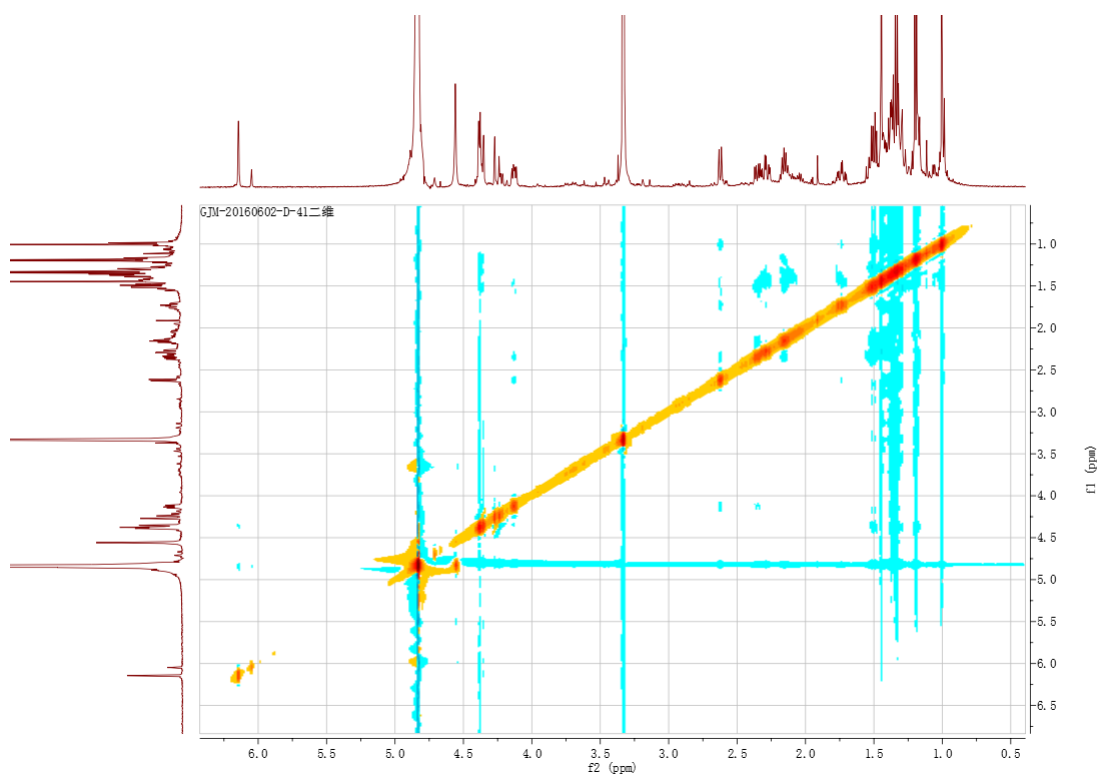


Figure S19. HRESIMS spectrum of compound 4.

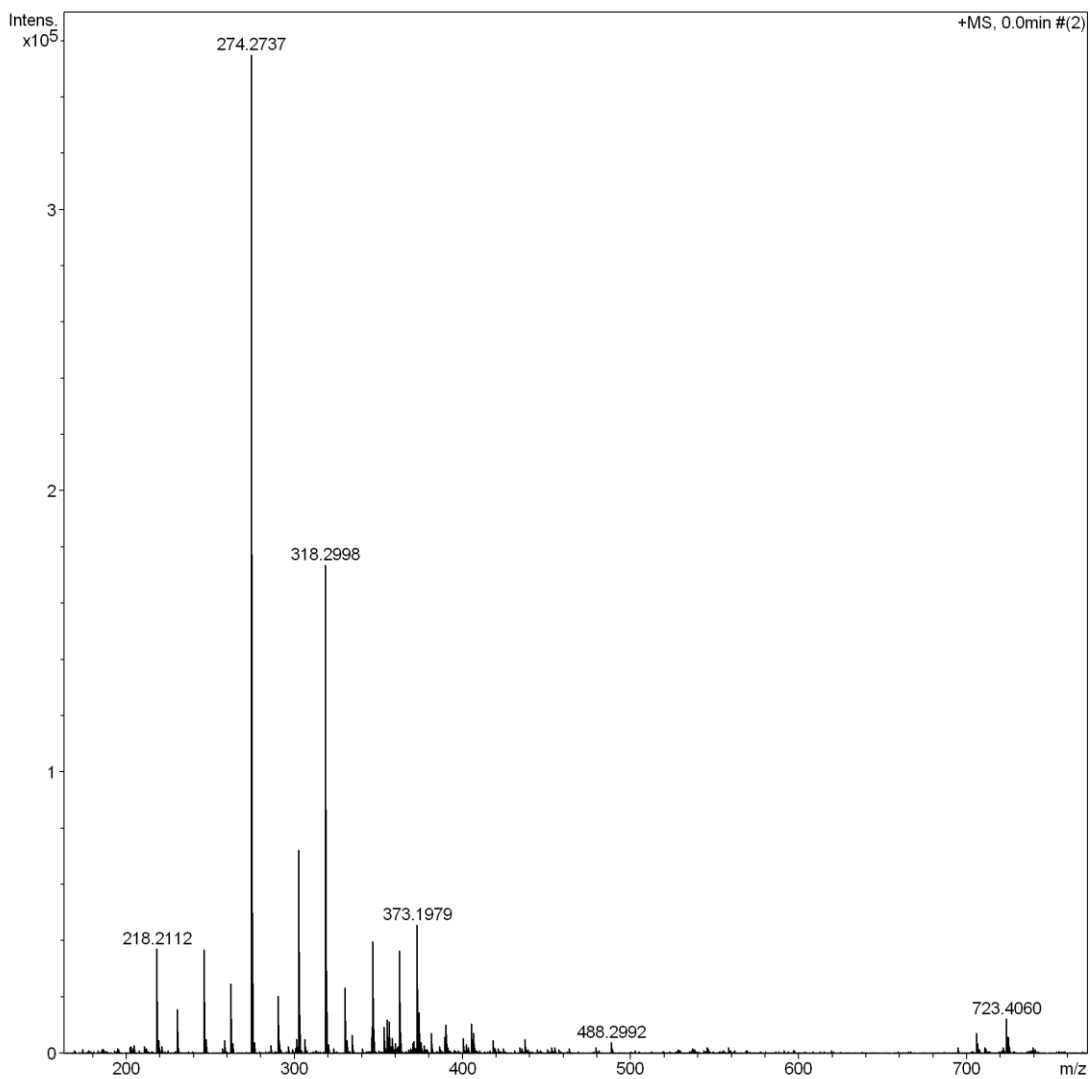
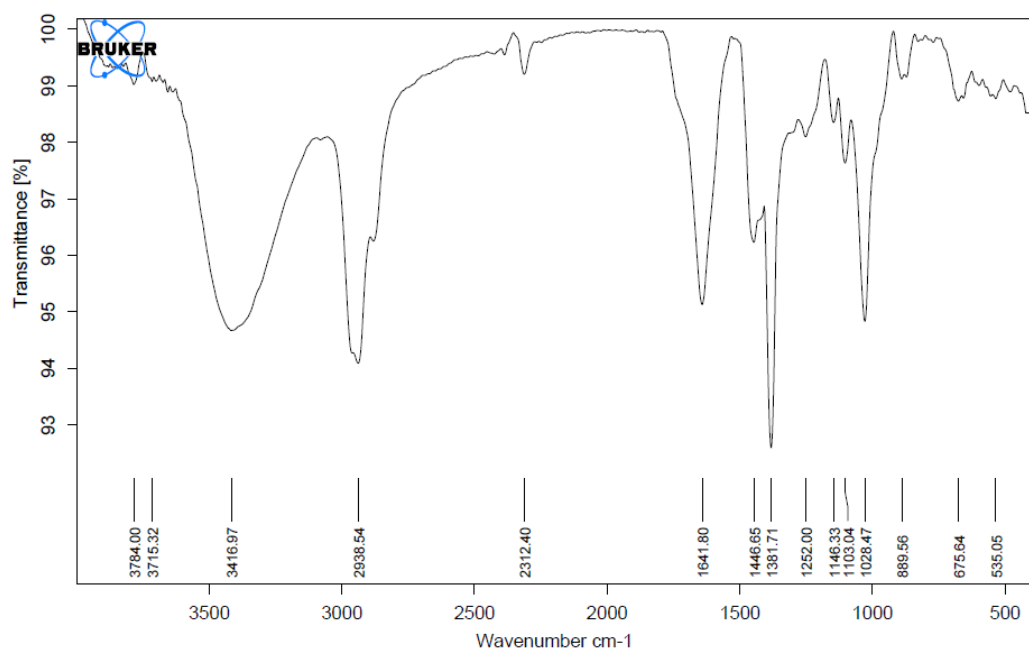


Figure S20. IR and UV spectra of compound 4. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



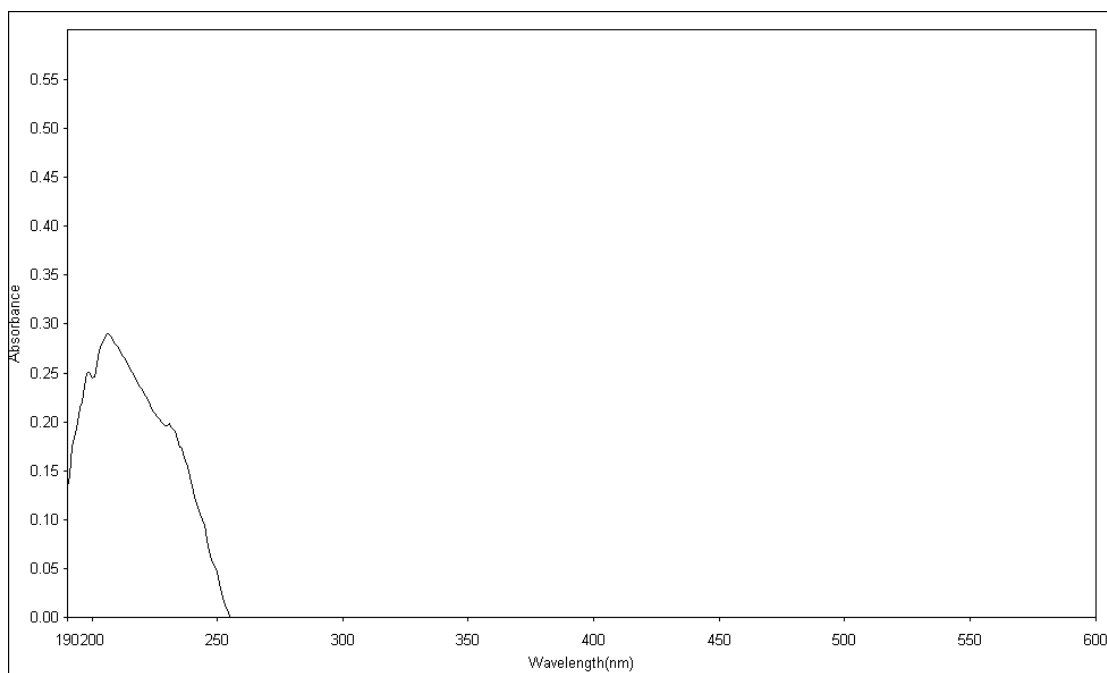
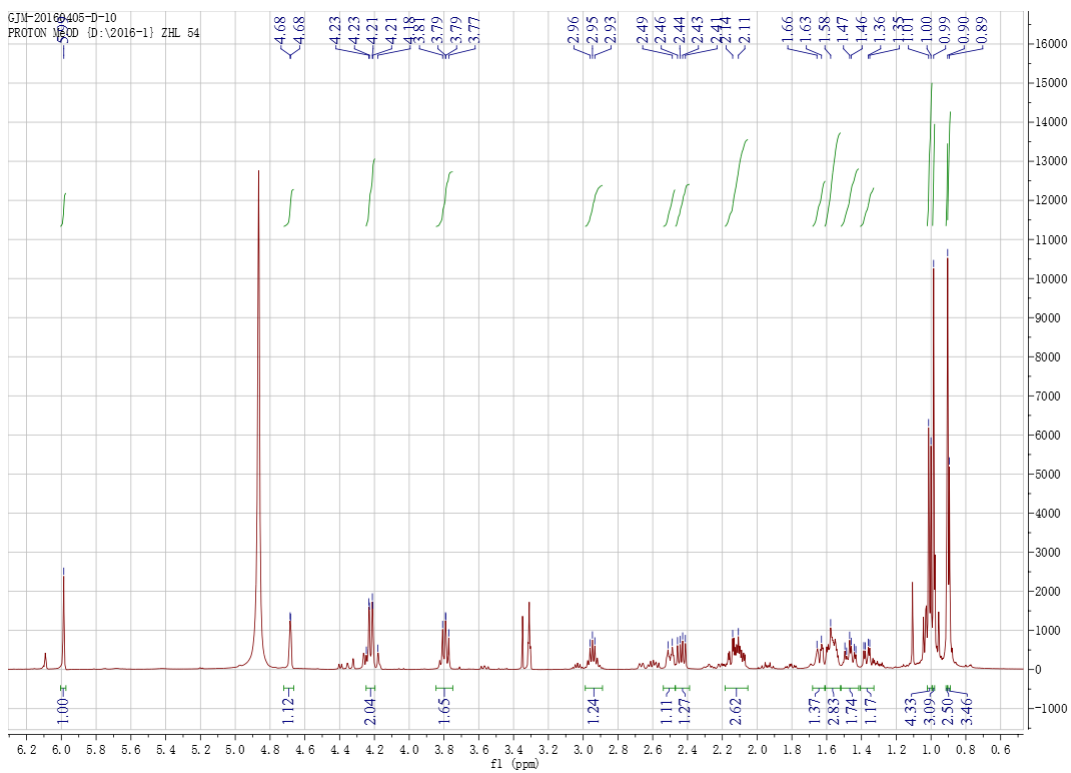


Figure S21. ^1H NMR and ^{13}C spectra of compound **5** in CD_3OD .



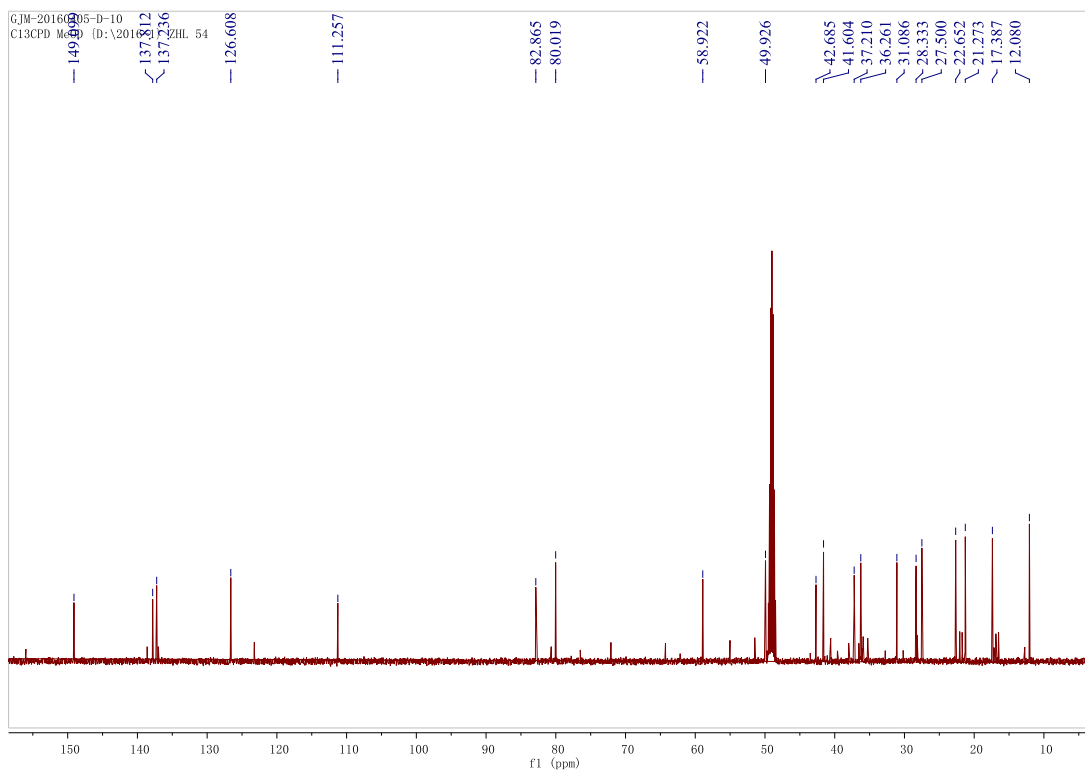
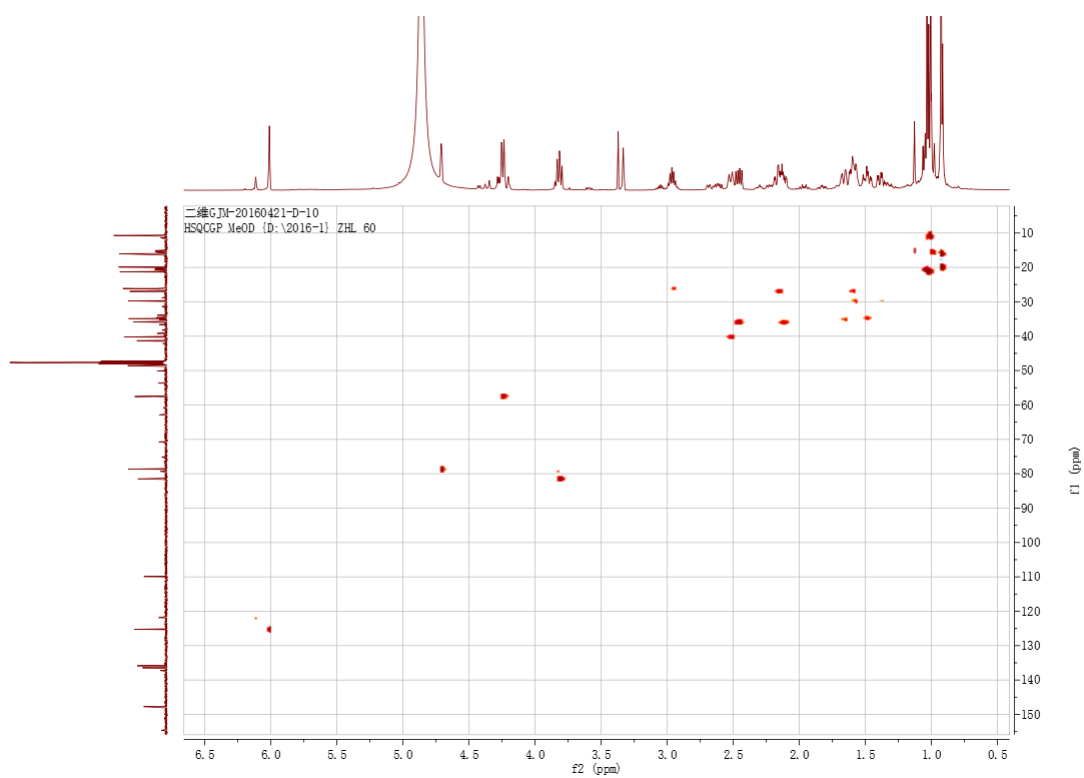


Figure S22. HSQC and ^1H - ^1H COSY spectra of compound **5** in CD_3OD



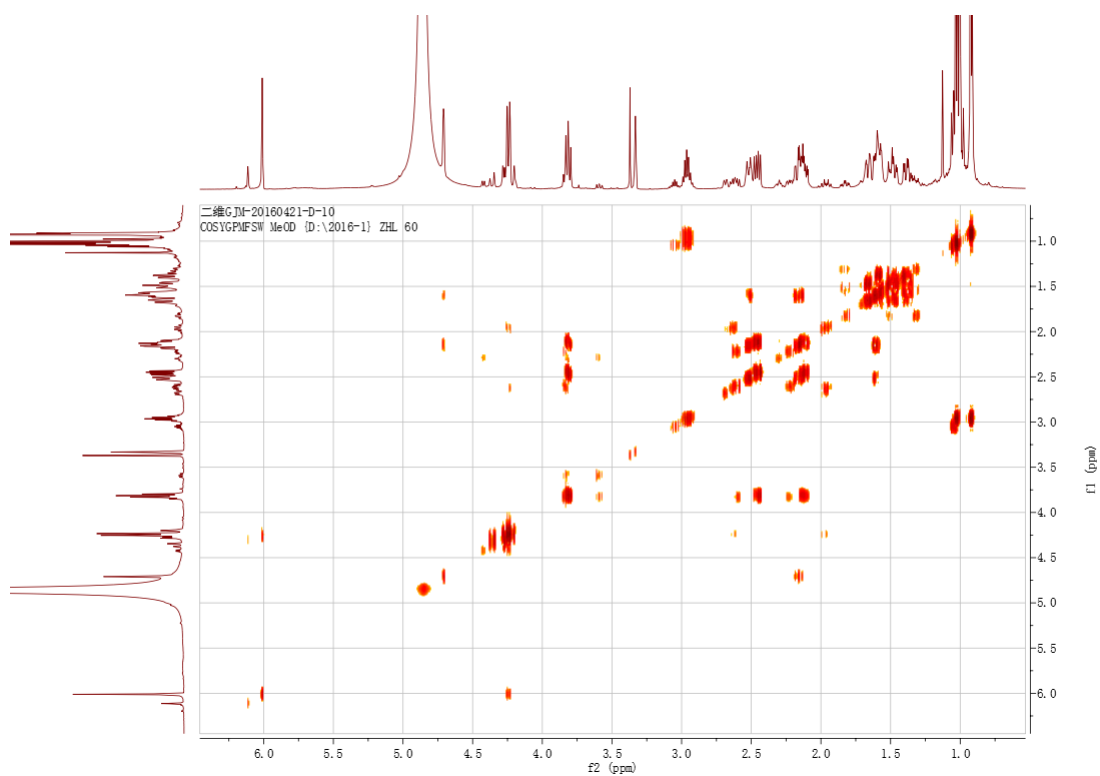
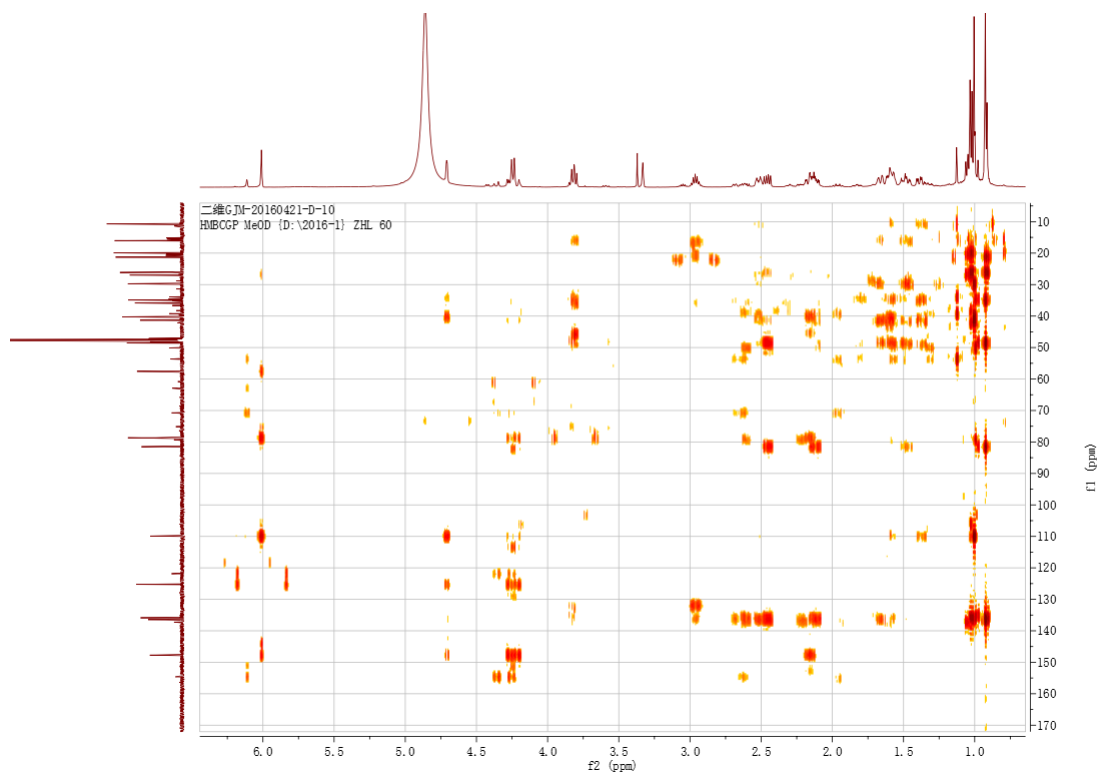


Figure S23. HMBC and NOESY spectra of compound **5** in CD₃OD.



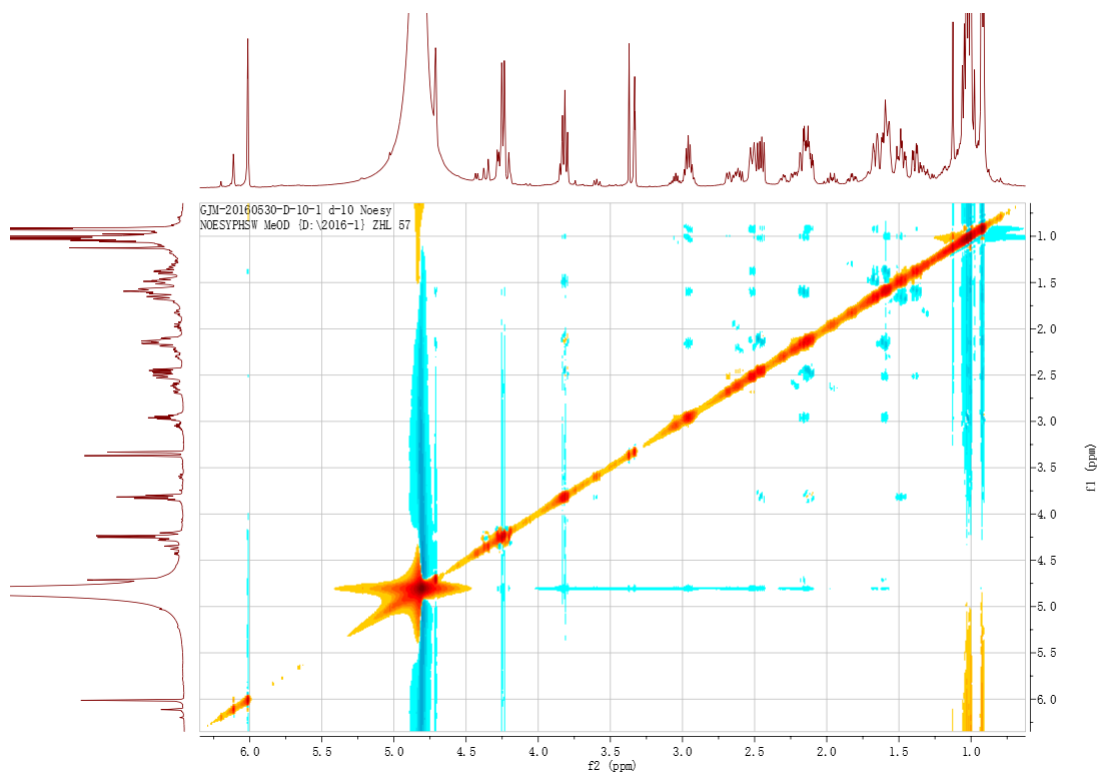


Figure S24. HRESIMS spectrum of compound **5**.

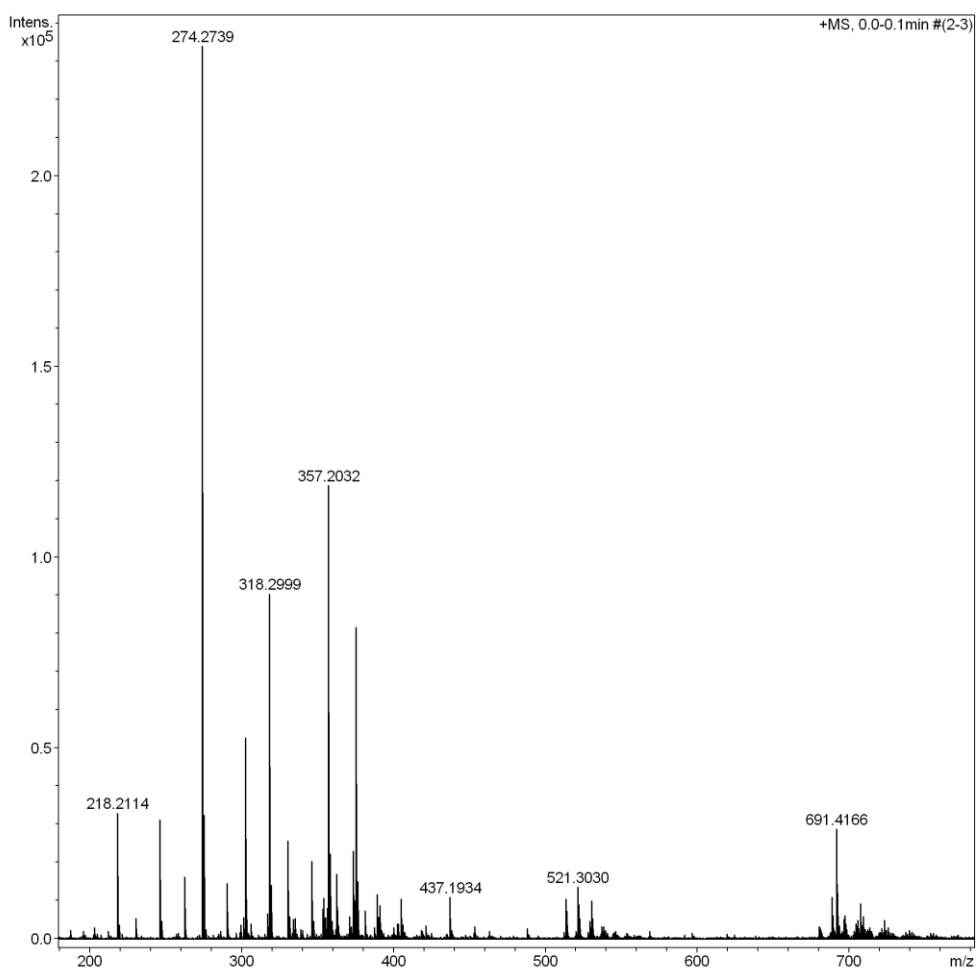


Figure S25. IR and UV spectra of compound **5**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.

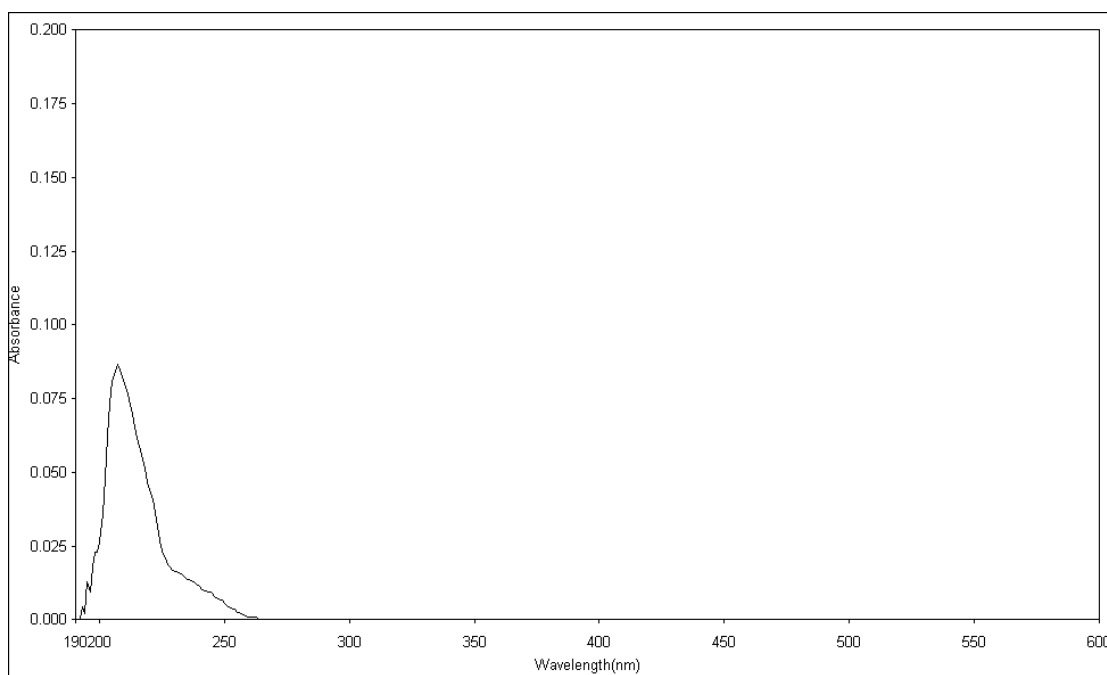
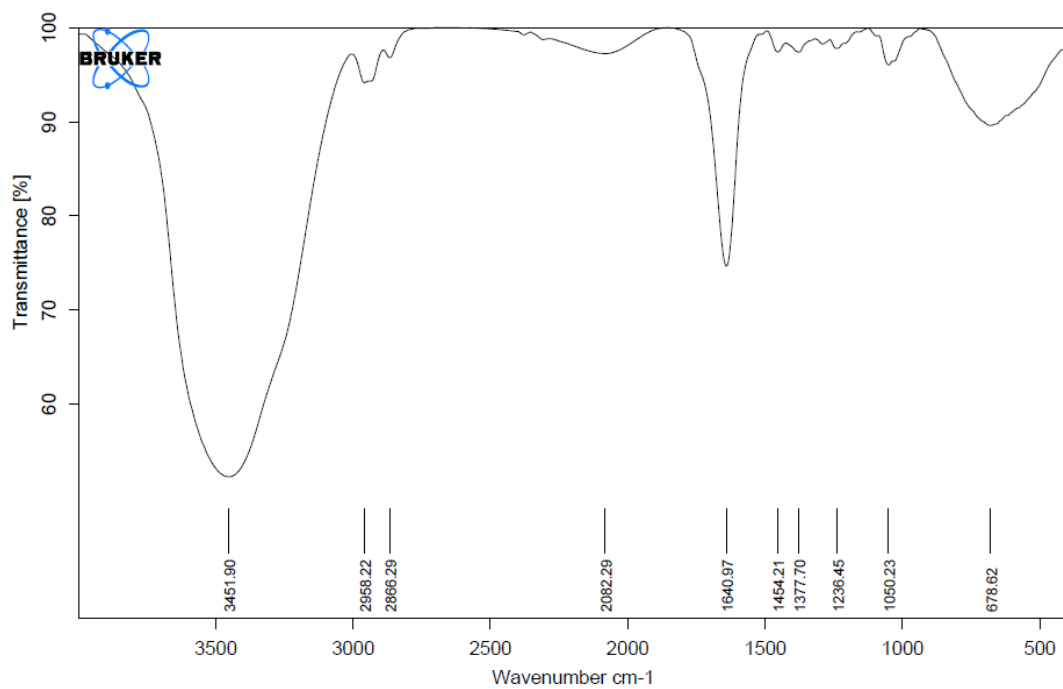


Figure S26. ¹H NMR and ¹³C spectra of compound **6** in CD₃OD.

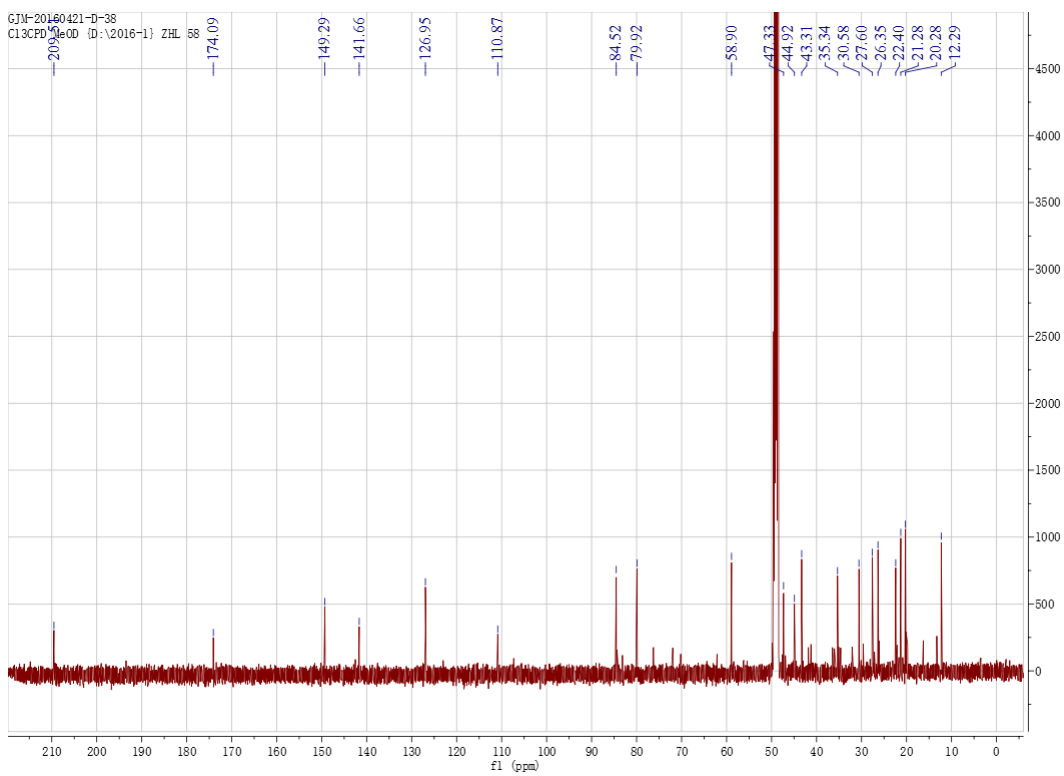
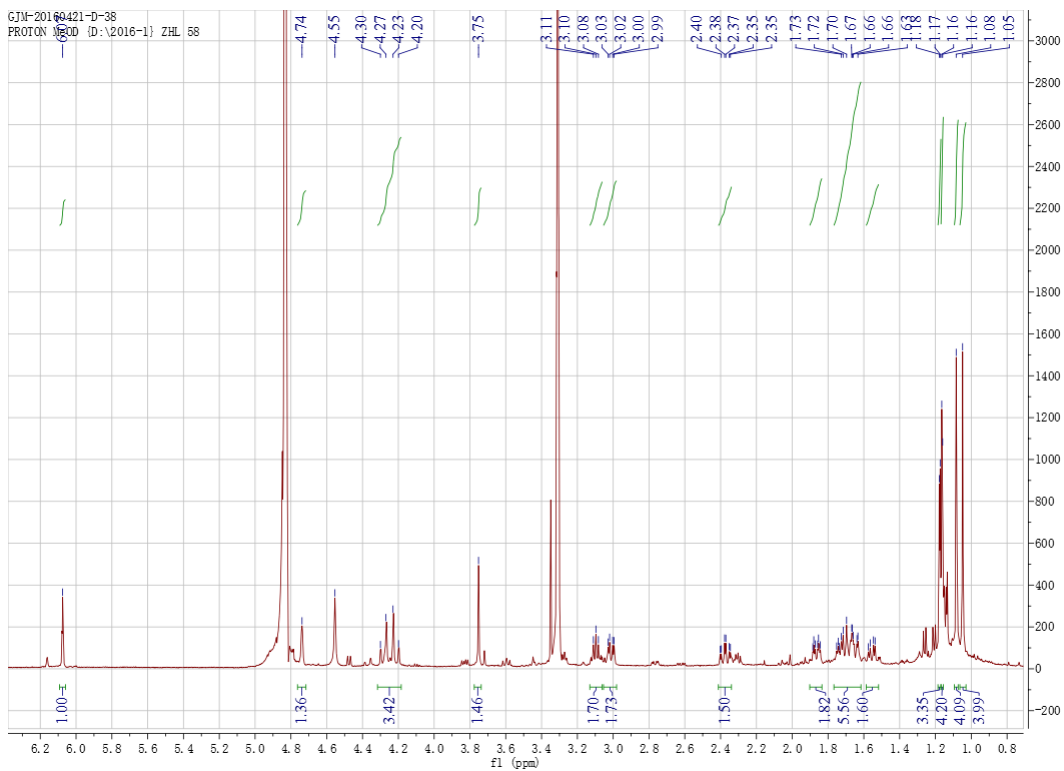


Figure S27. HSQC and ^1H - ^1H COSY spectra of compound **6** in CD_3OD

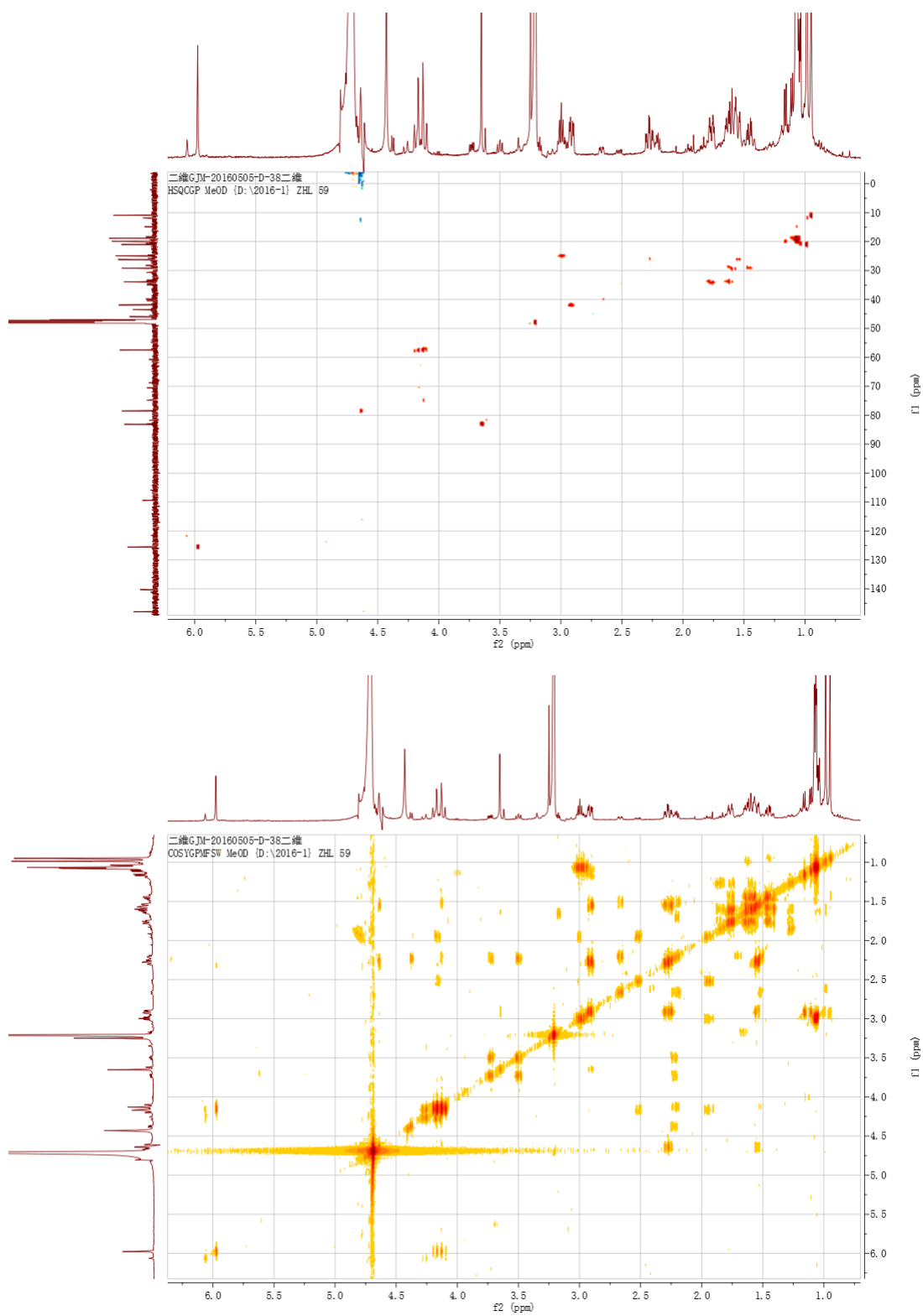


Figure S28. HMBC and NOESY spectra of compound **6** in CD₃OD.

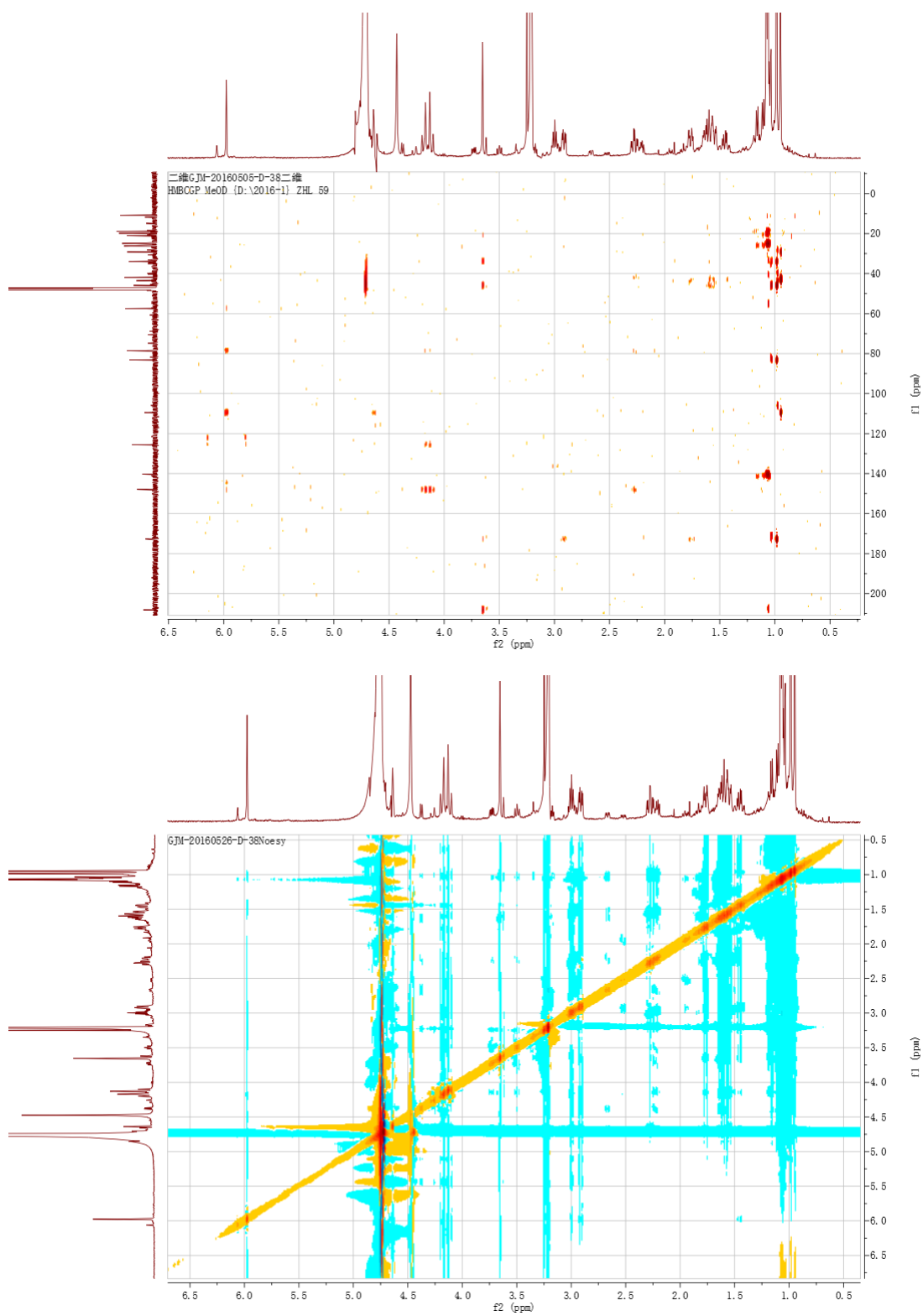


Figure S29. HRESIMS spectrum of compound 6.

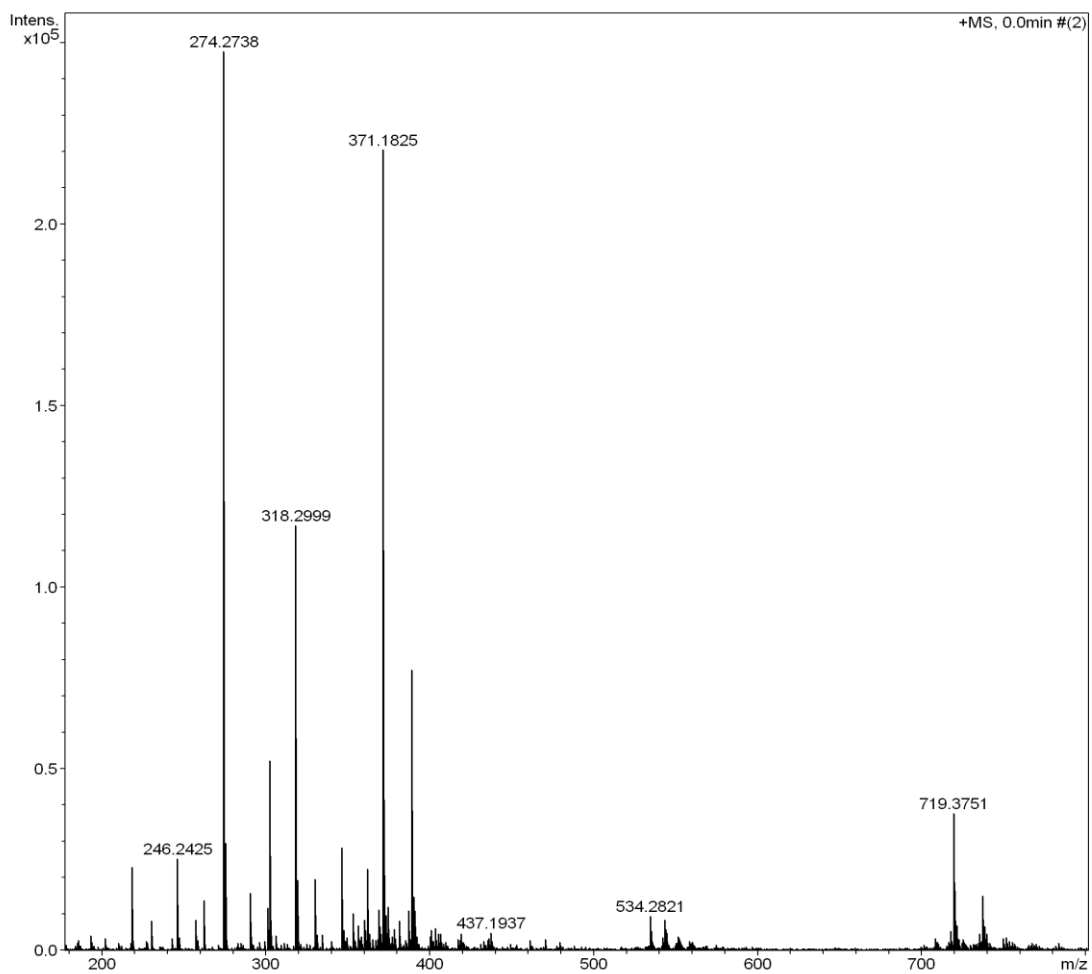
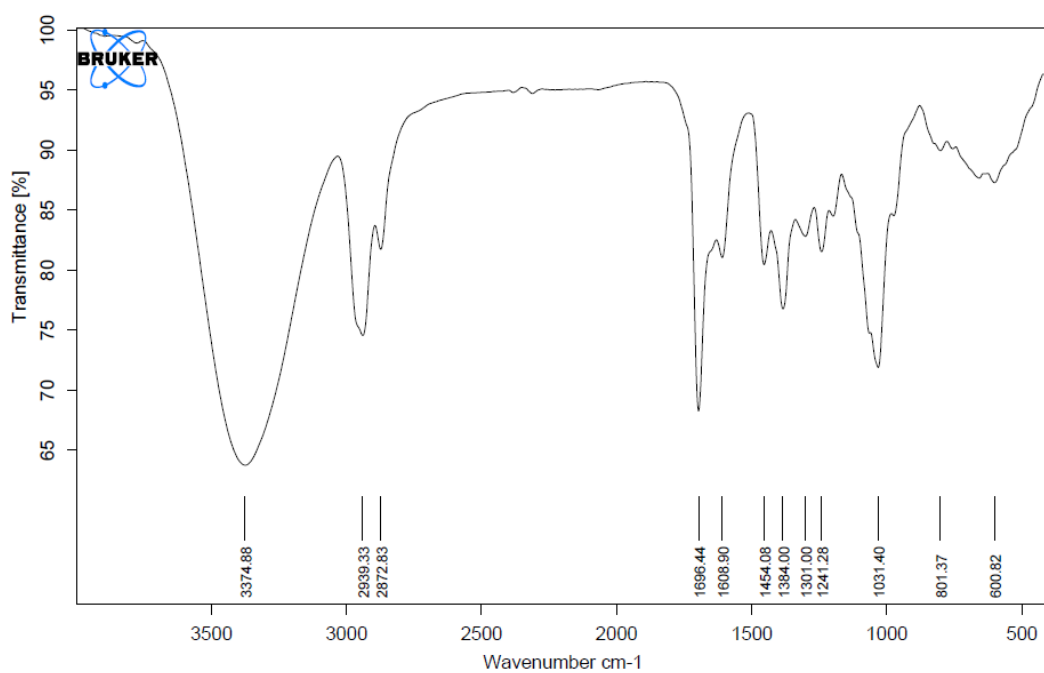


Figure S30. IR and UV spectra of compound **6**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



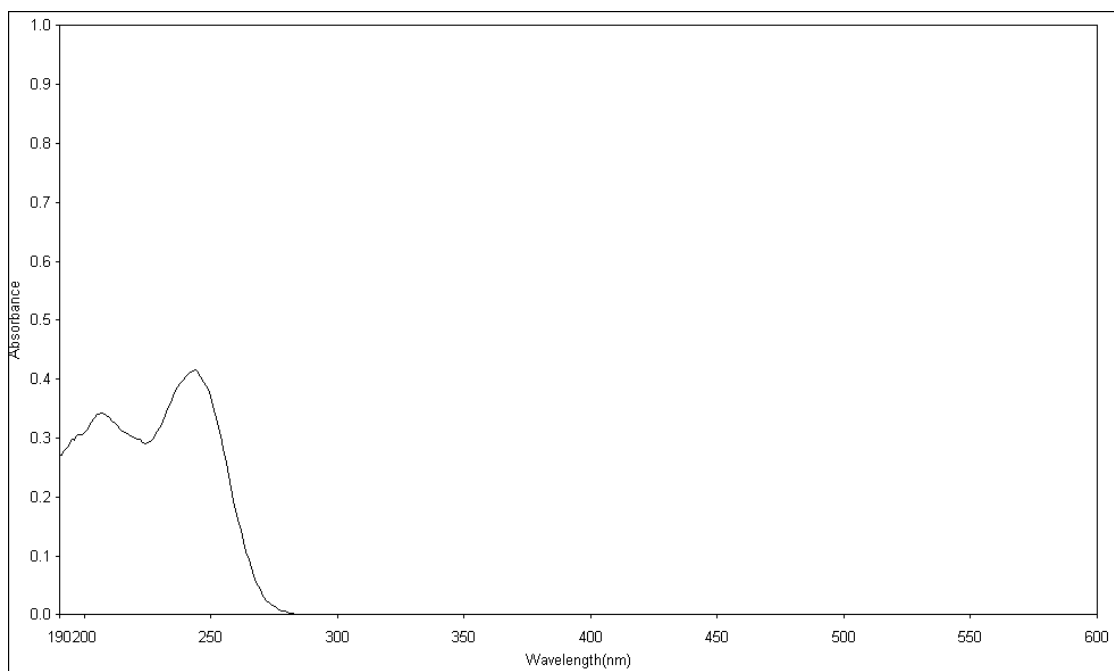
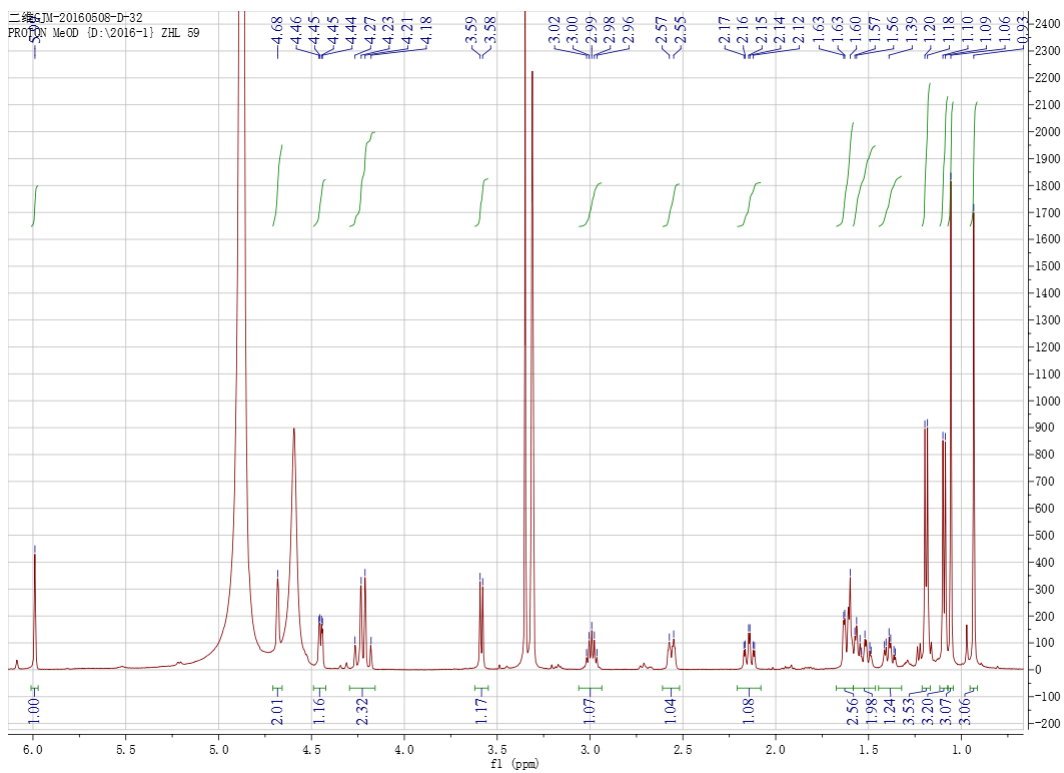


Figure S31. ¹H NMR and ¹³C spectra of compound 7 in CD₃OD.



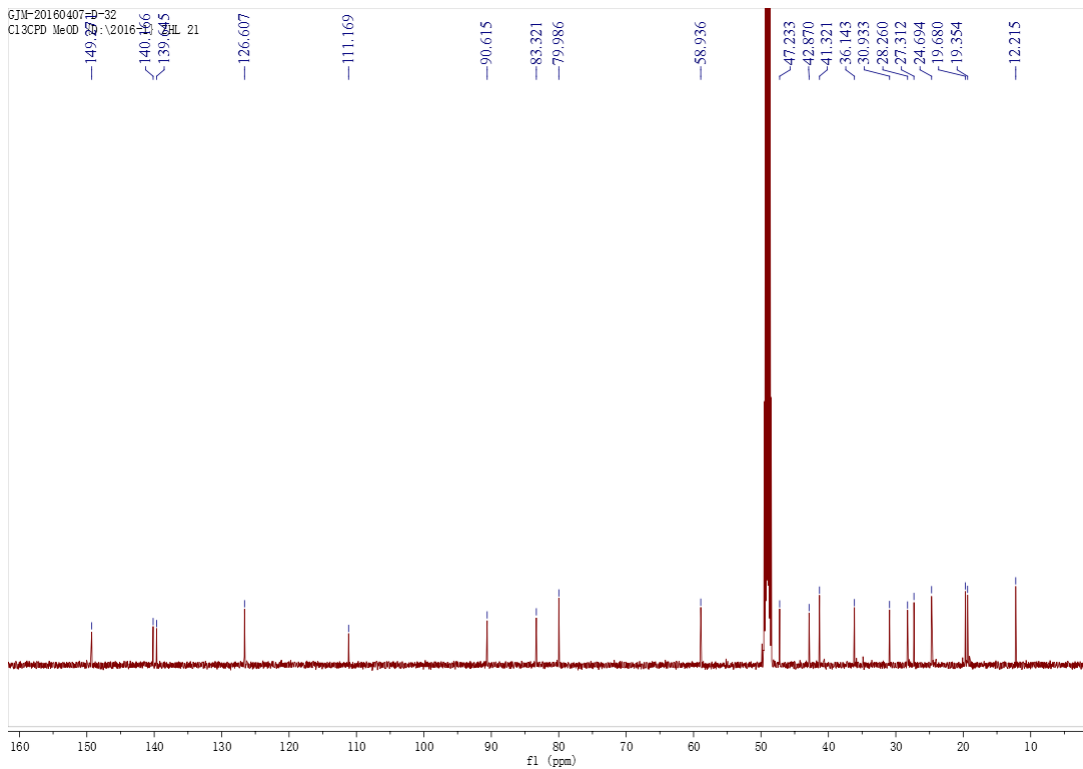
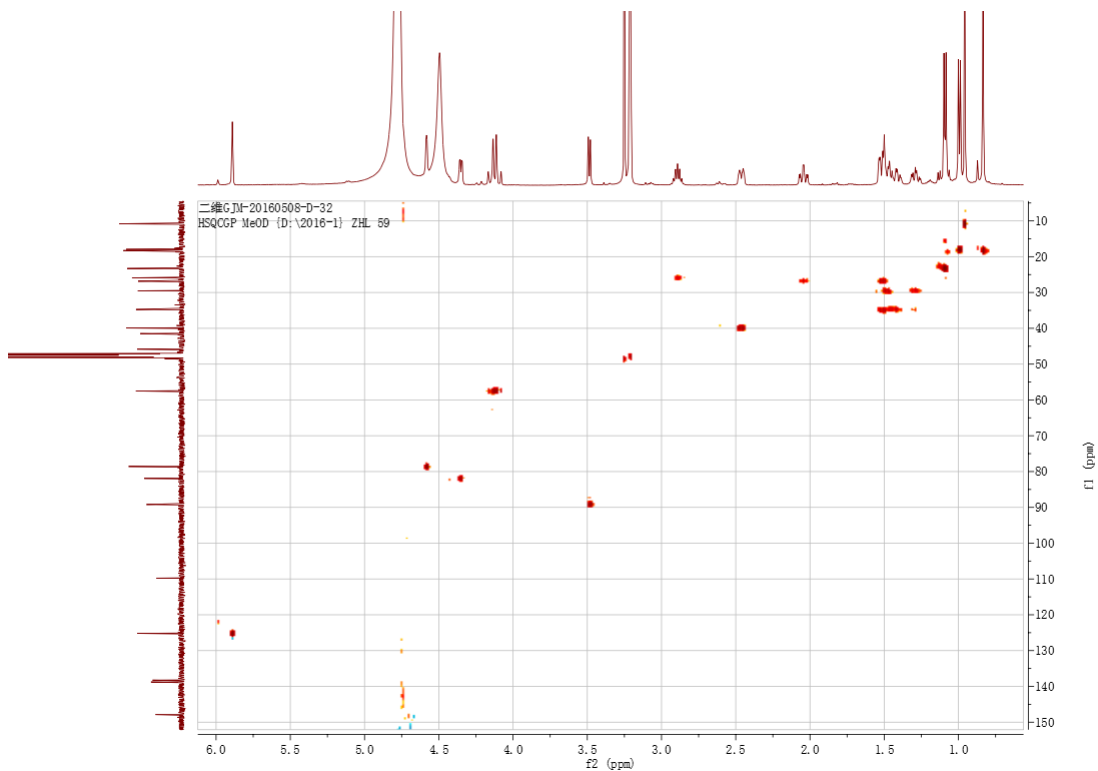


Figure S32. HSQC and ^1H - ^1H COSY spectra of compound **7** in CD_3OD



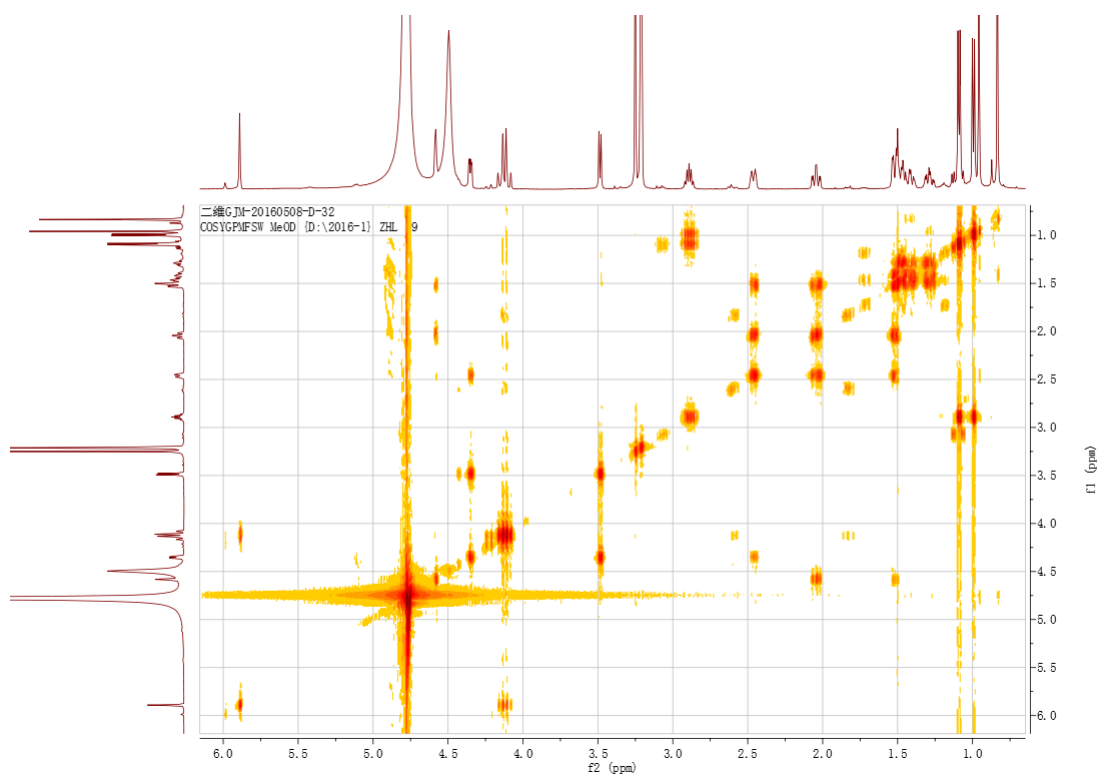
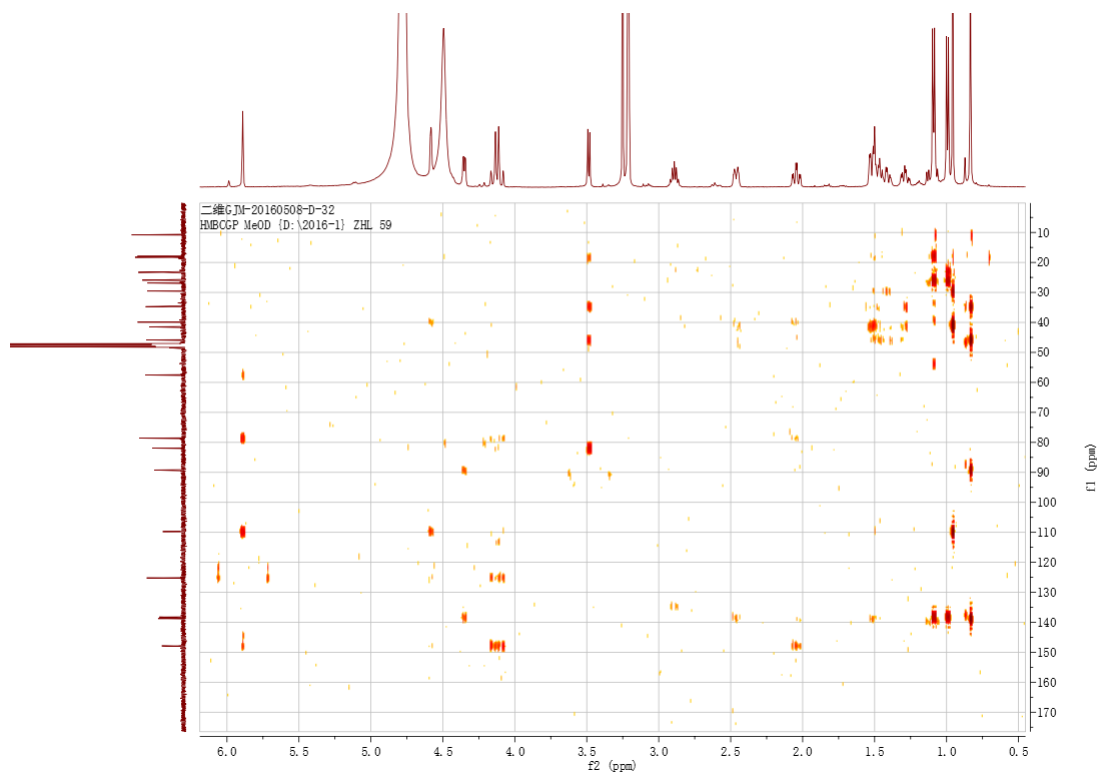


Figure S33. HMBC and NOESY spectra of compound 7 in CD₃OD.



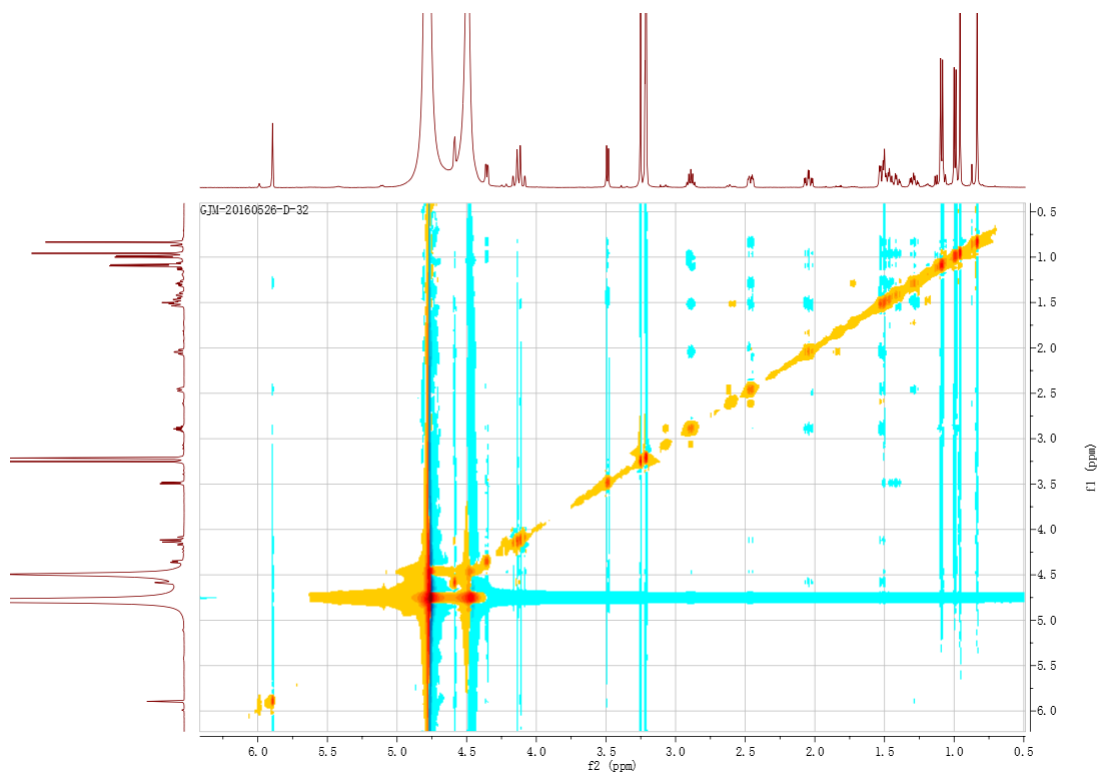


Figure S34. HRESIMS spectrum of compound 7.

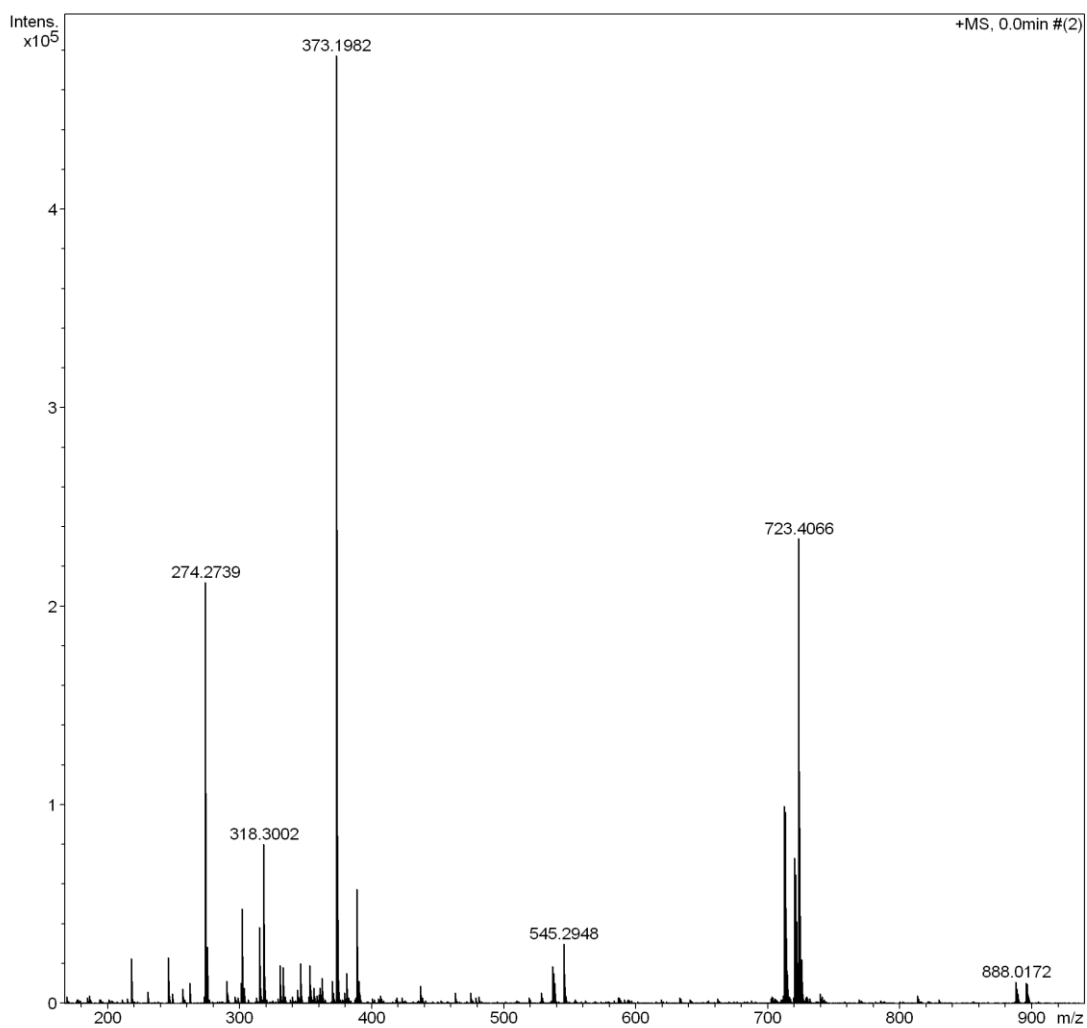
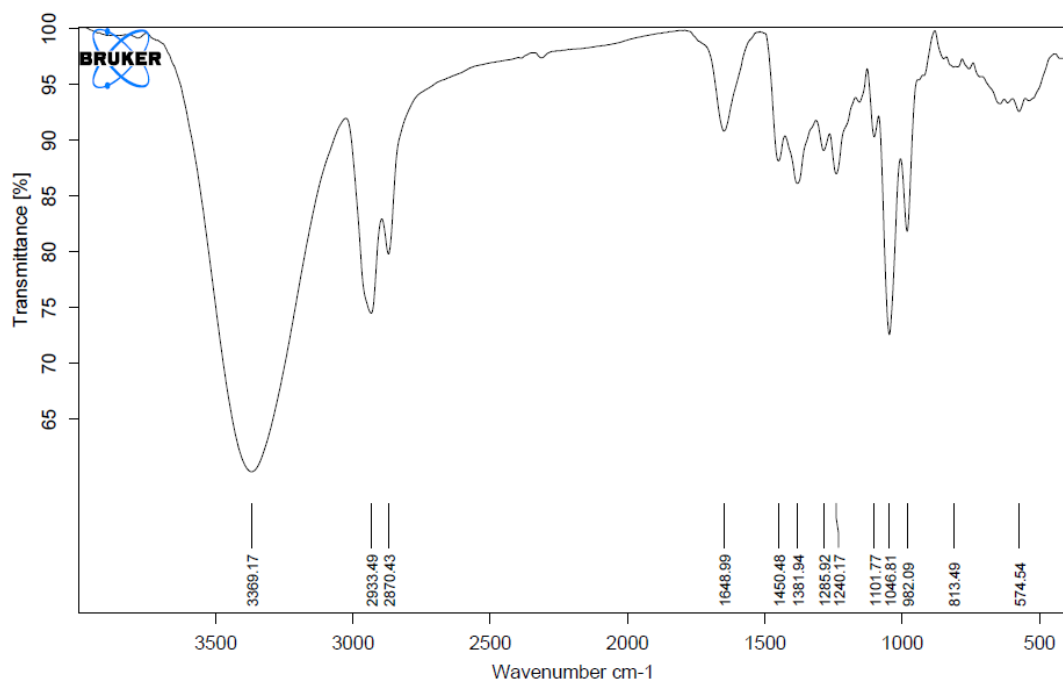


Figure S35. IR and UV spectra of compound **7**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



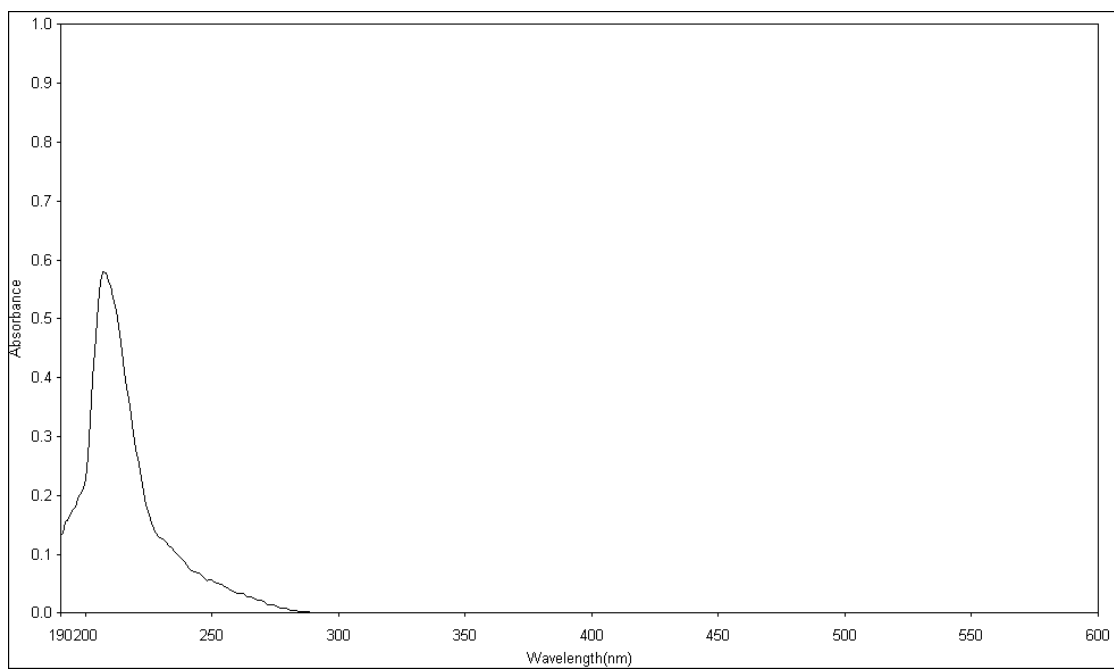
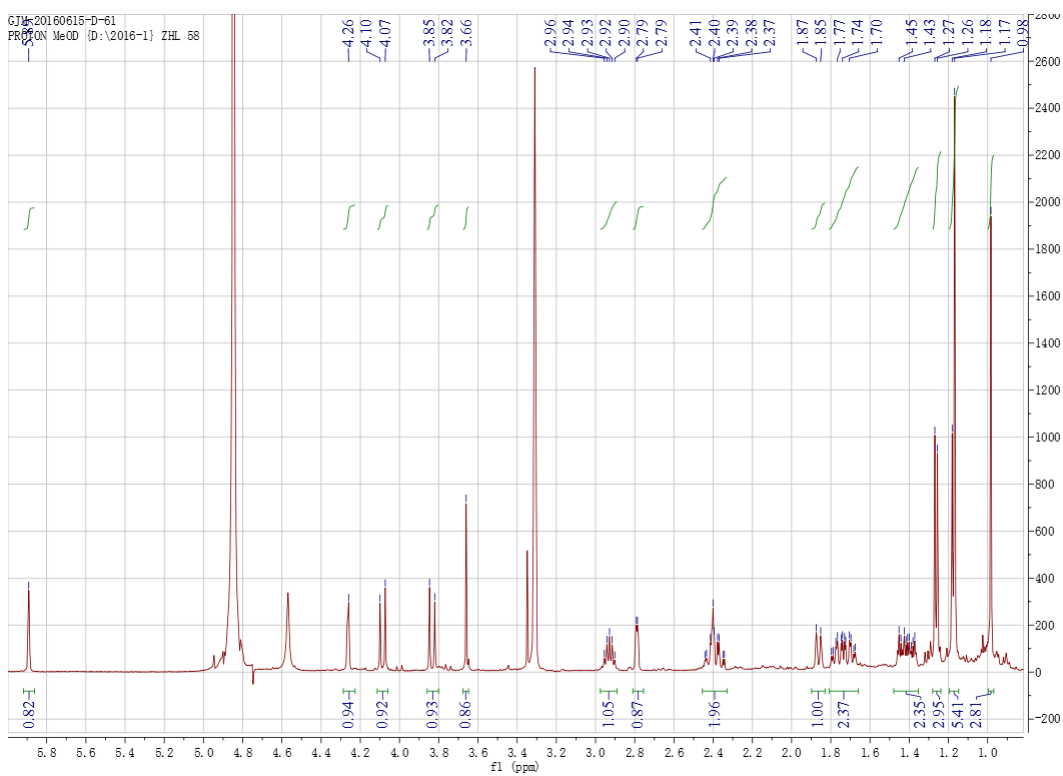


Figure S36. ^1H NMR and ^{13}C spectra of compound **8** in CD_3OD .



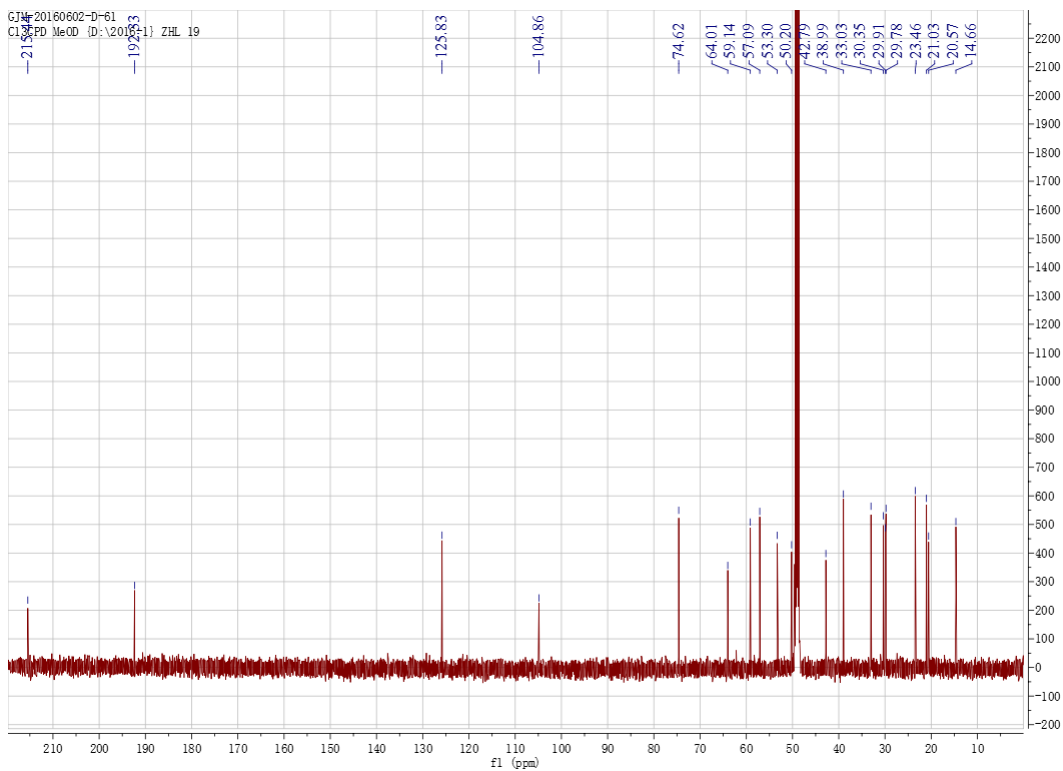
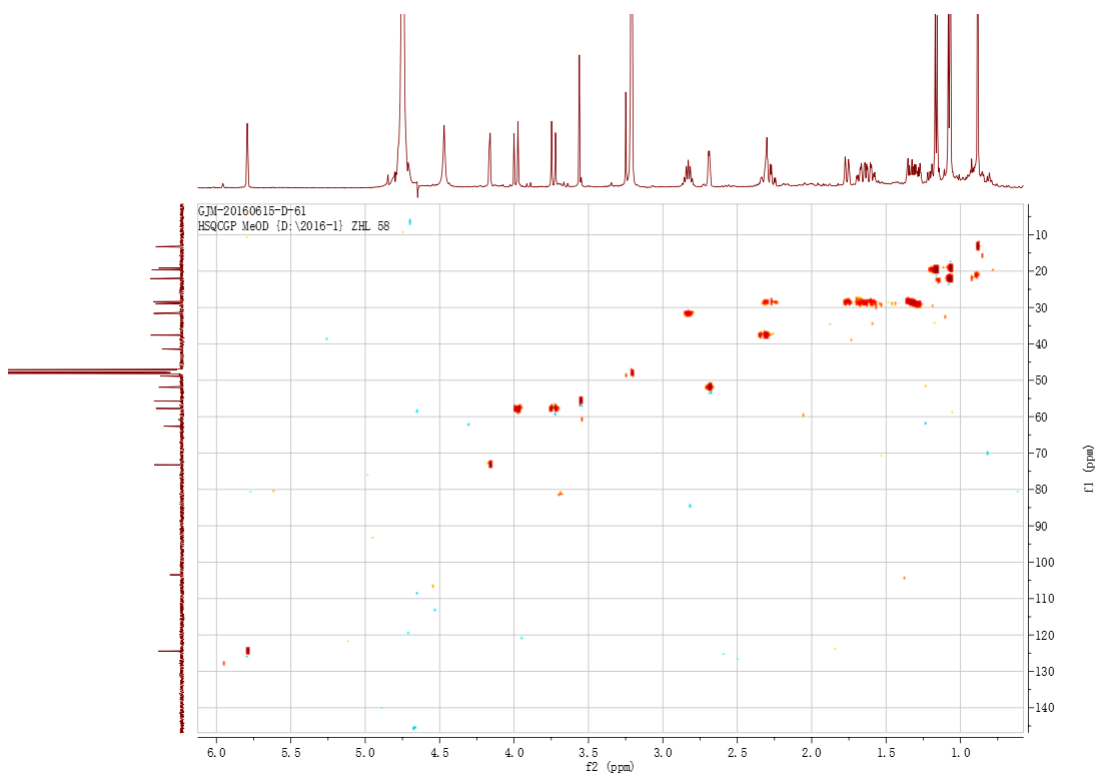


Figure S37. HSQC and ^1H - ^1H COSY spectra of compound **8** in CD_3OD



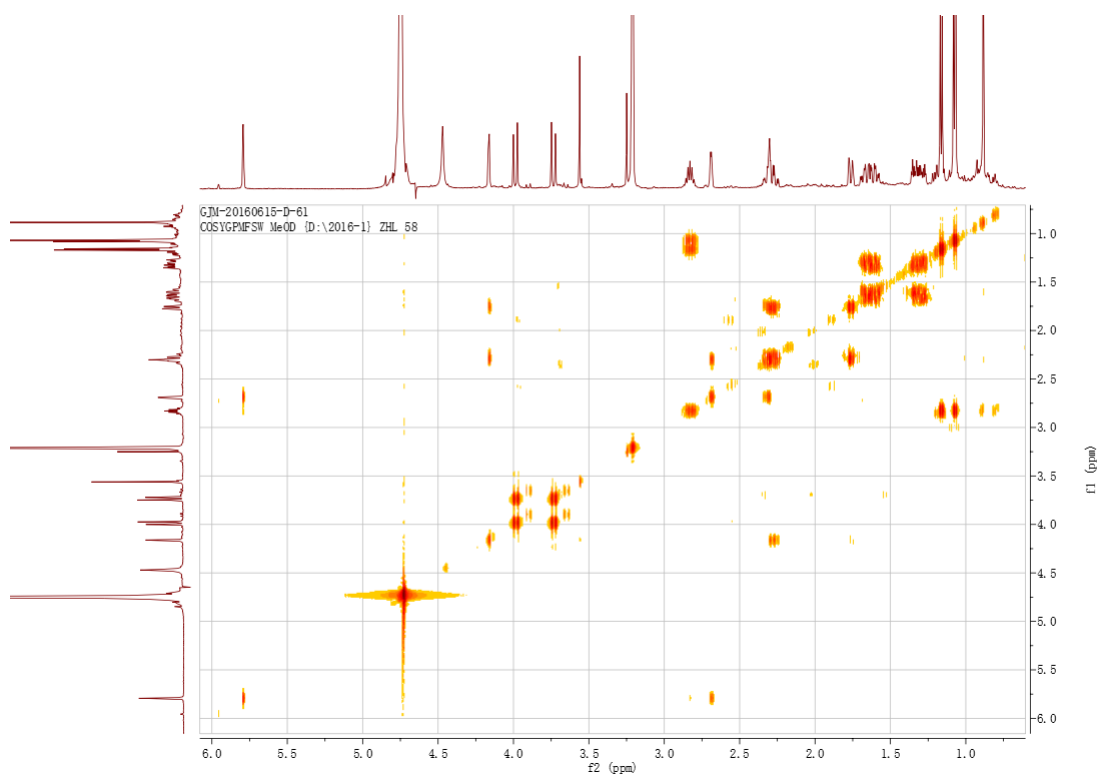
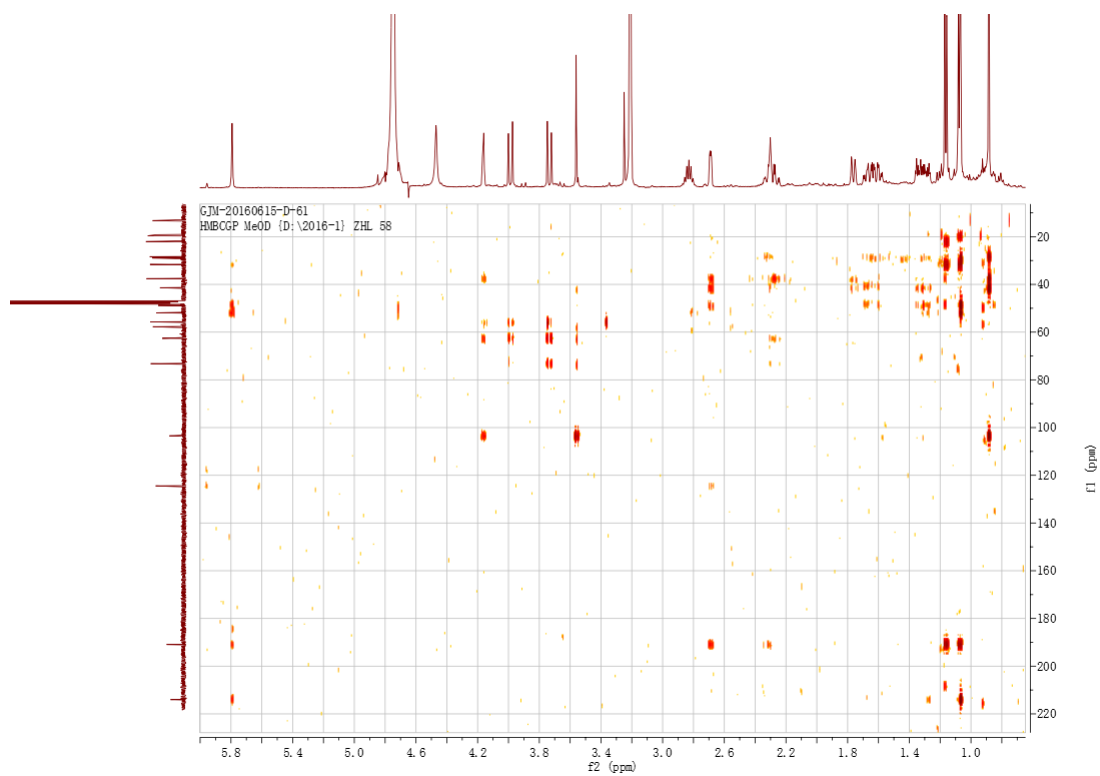


Figure S38. HMBC and NOESY spectra of compound **8** in CD₃OD.



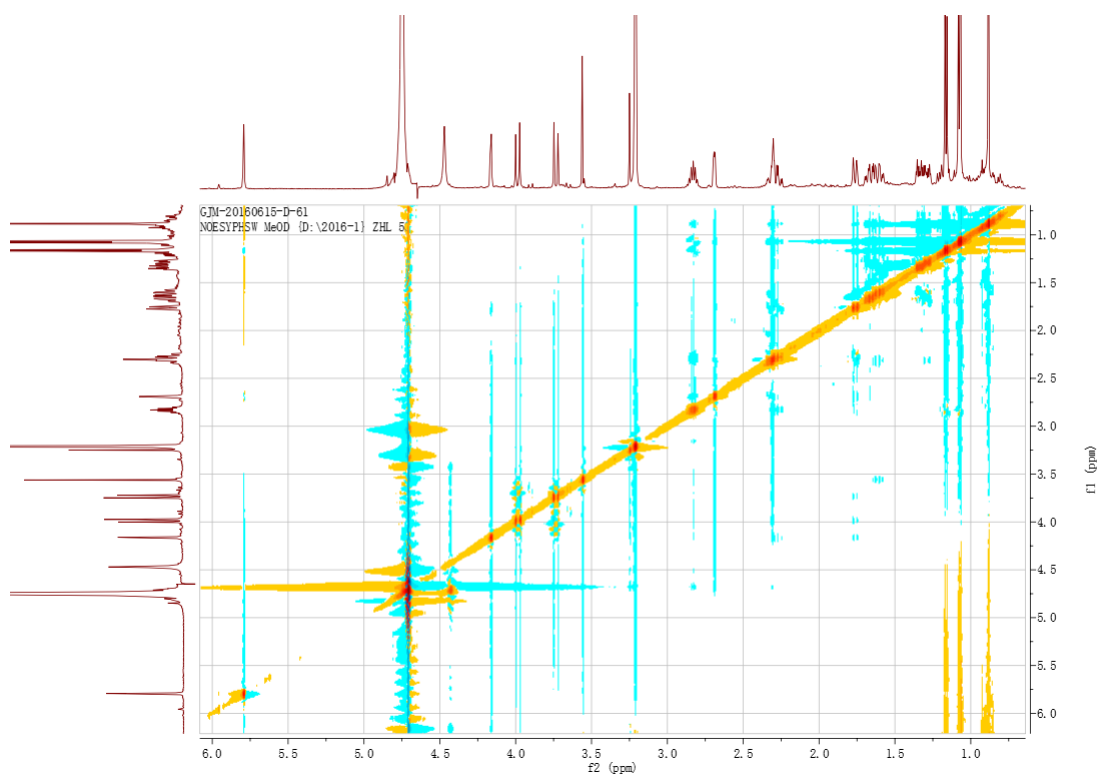


Figure S39. HRESIMS spectrum of compound **8**.

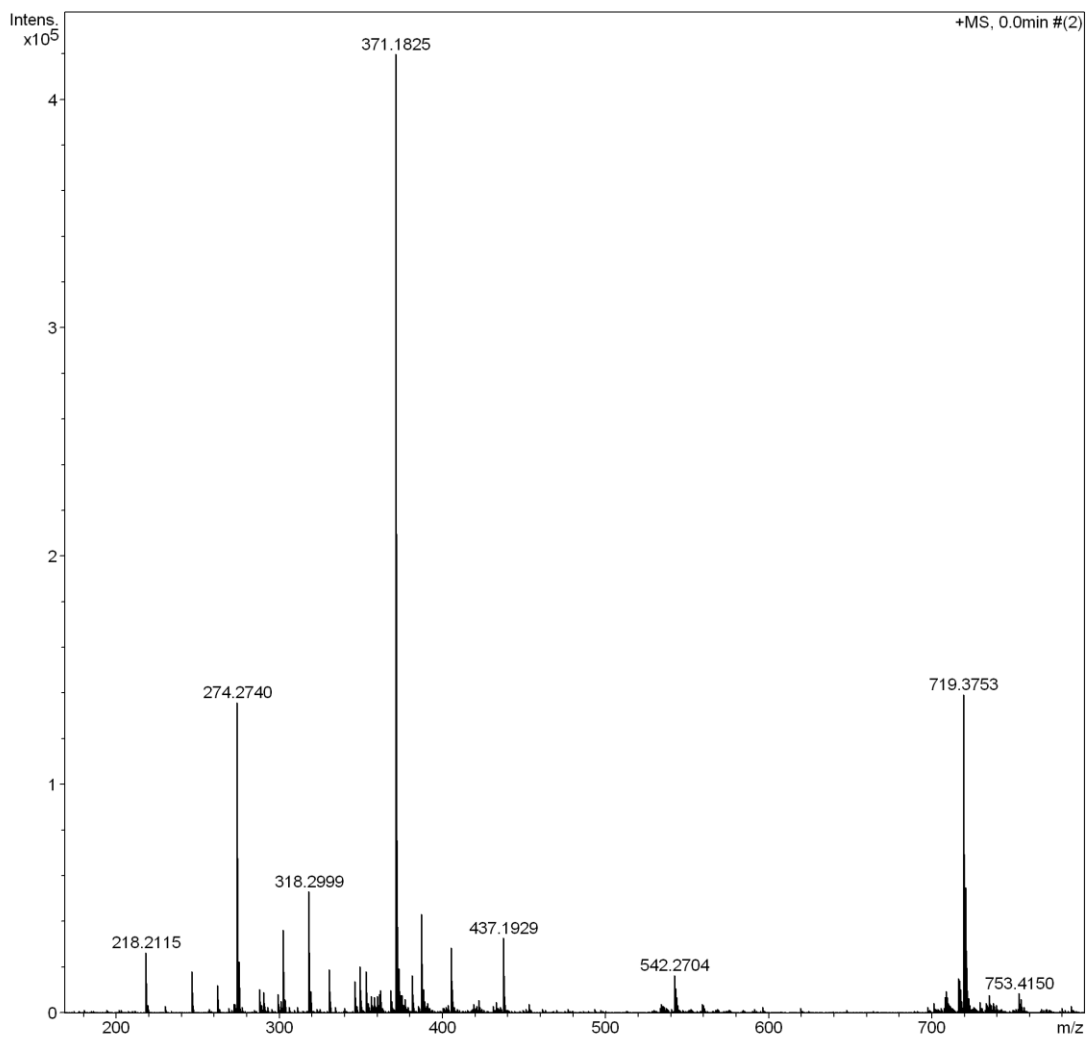
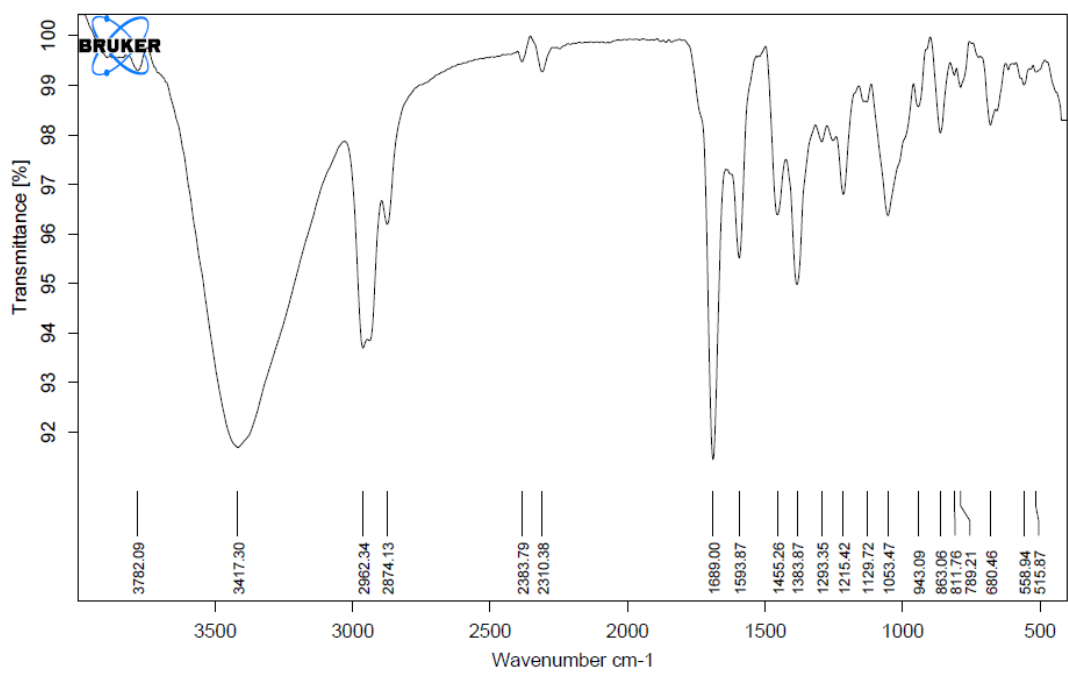


Figure S40. IR and UV spectra of compound **8**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



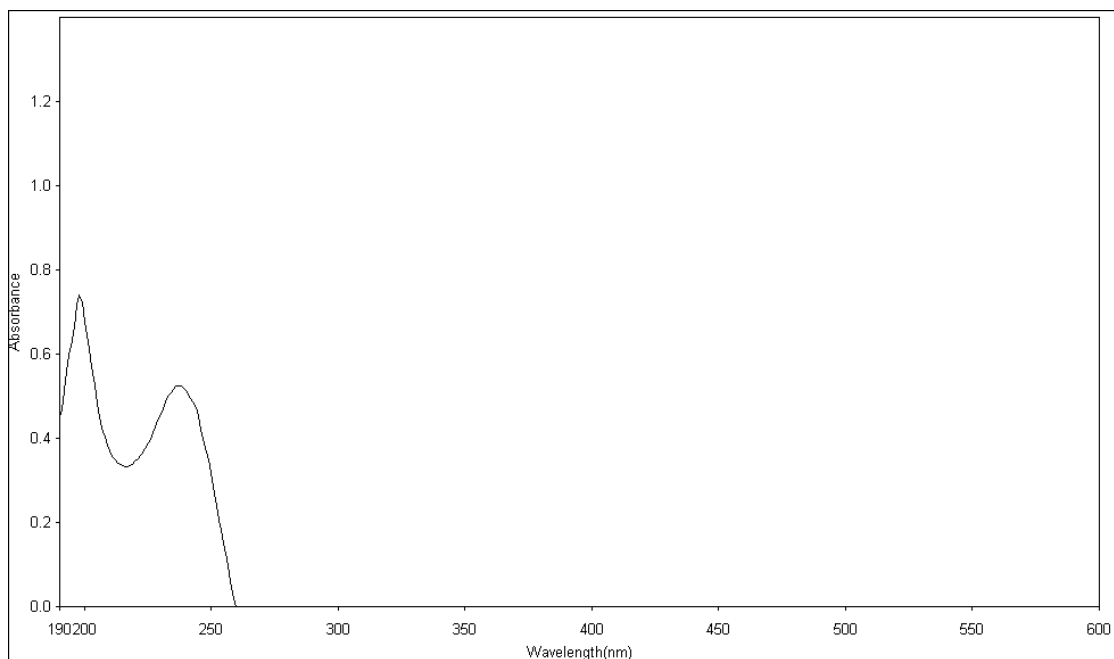
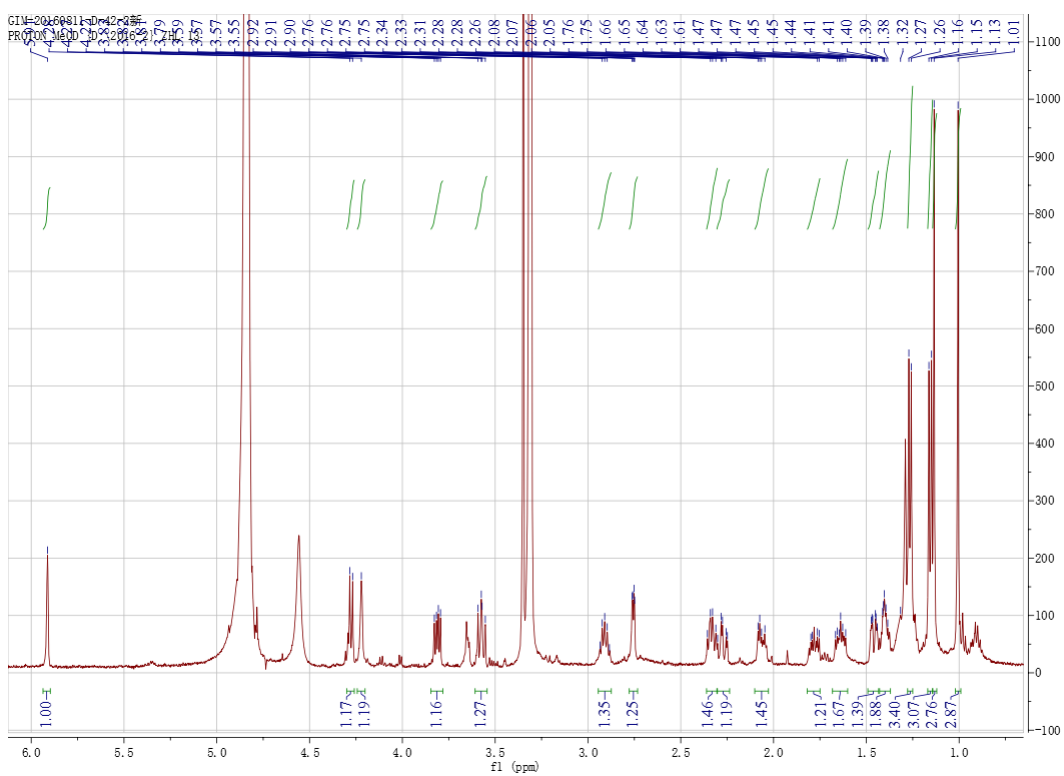


Figure S41. ¹H NMR and ¹³C spectra of compound 9 in CD₃OD.



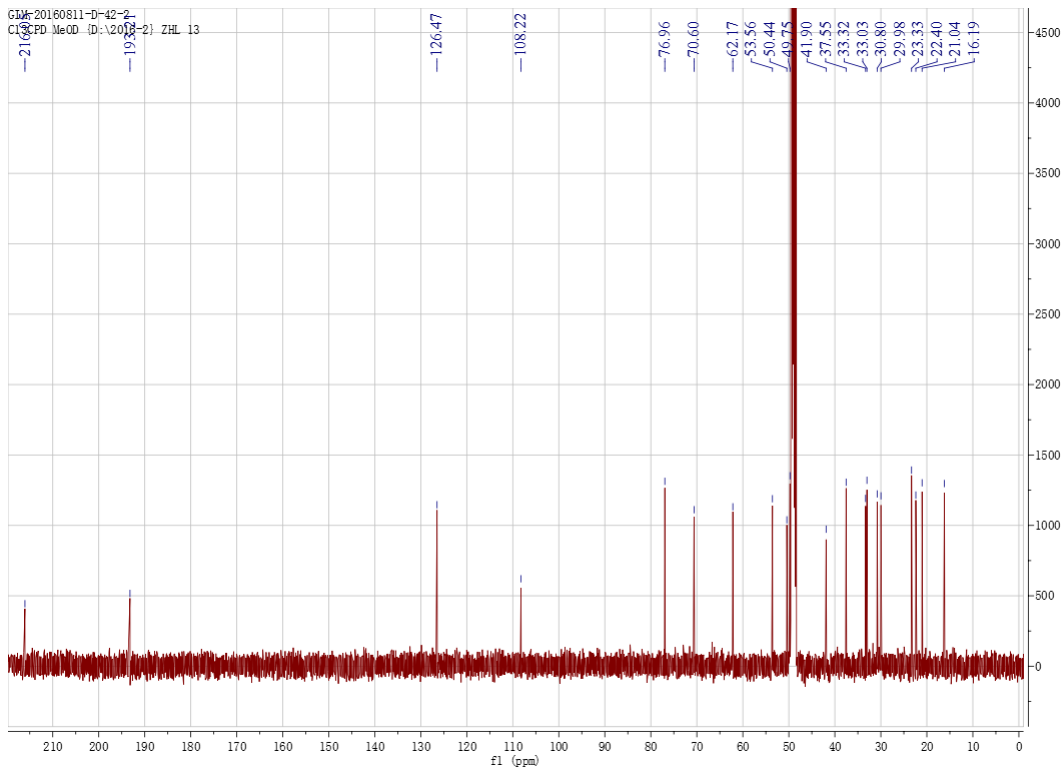
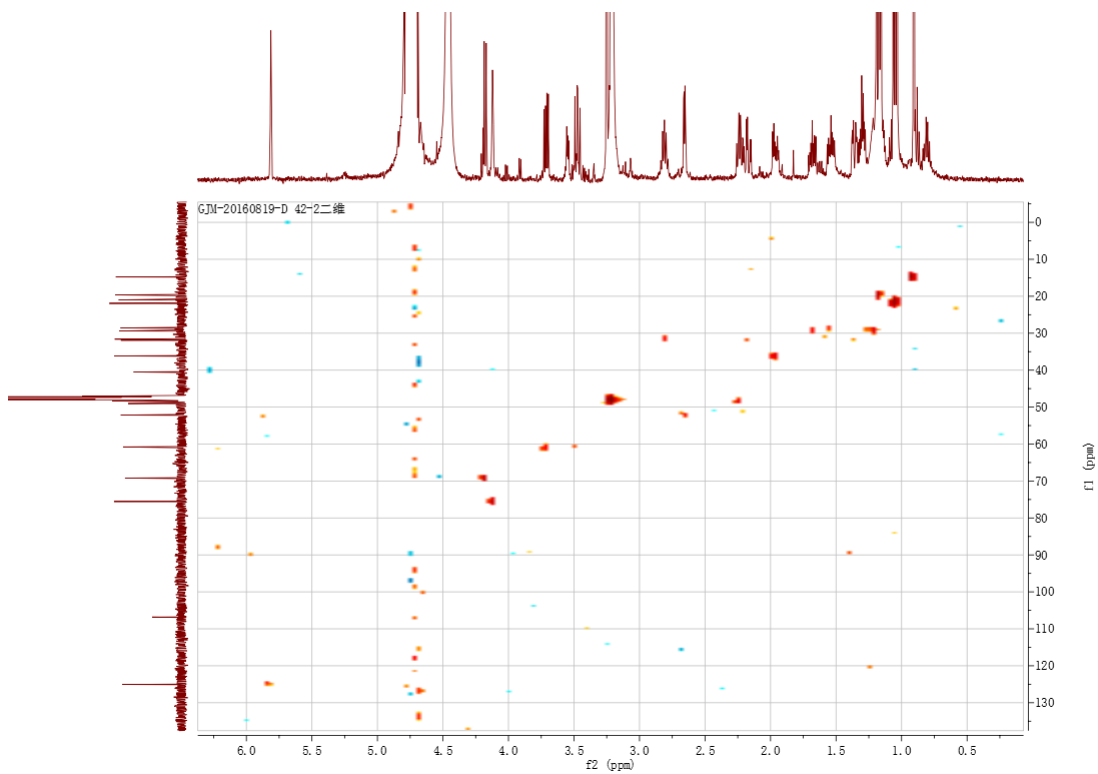


Figure S42. HSQC and ^1H - ^1H COSY spectra of compound **9** in CD_3OD



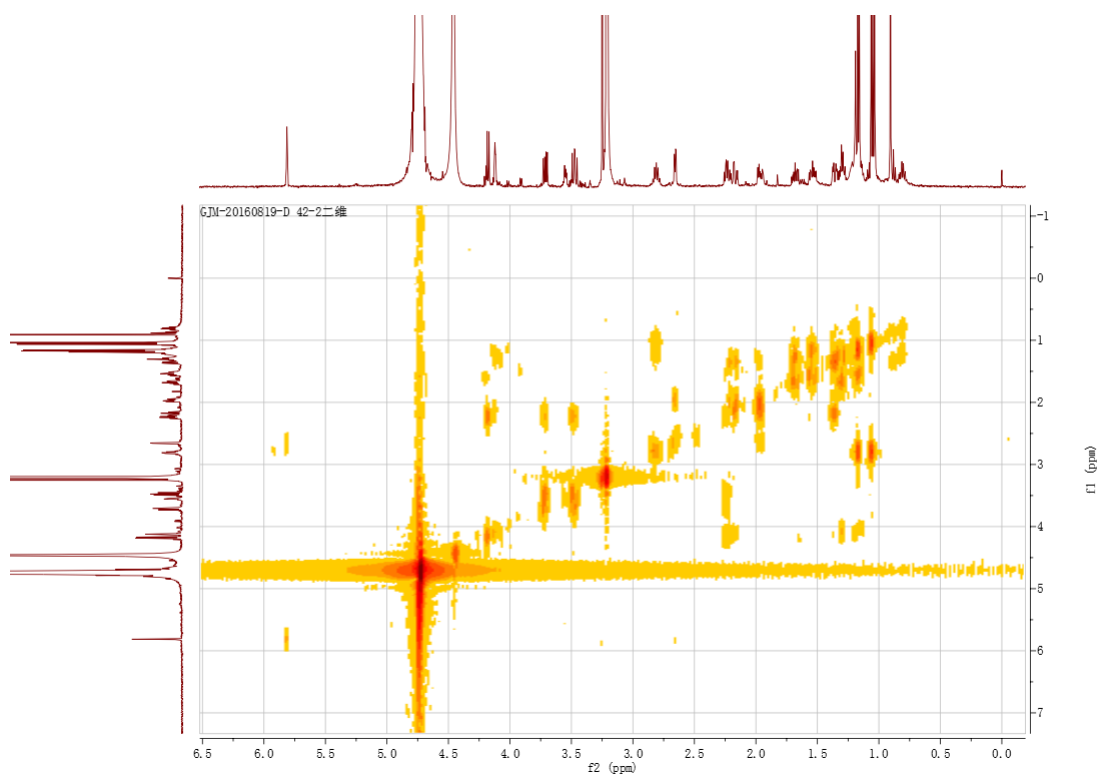
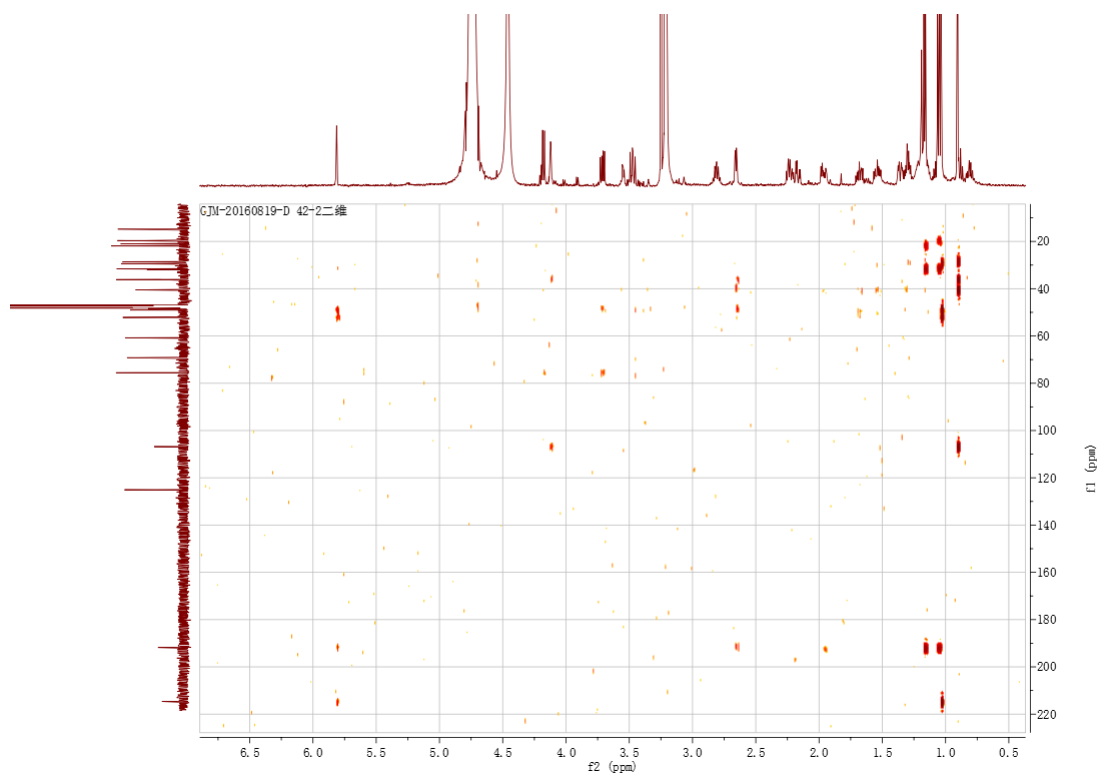


Figure S43. HMBC and NOESY spectra of compound **9** in CD_3OD .



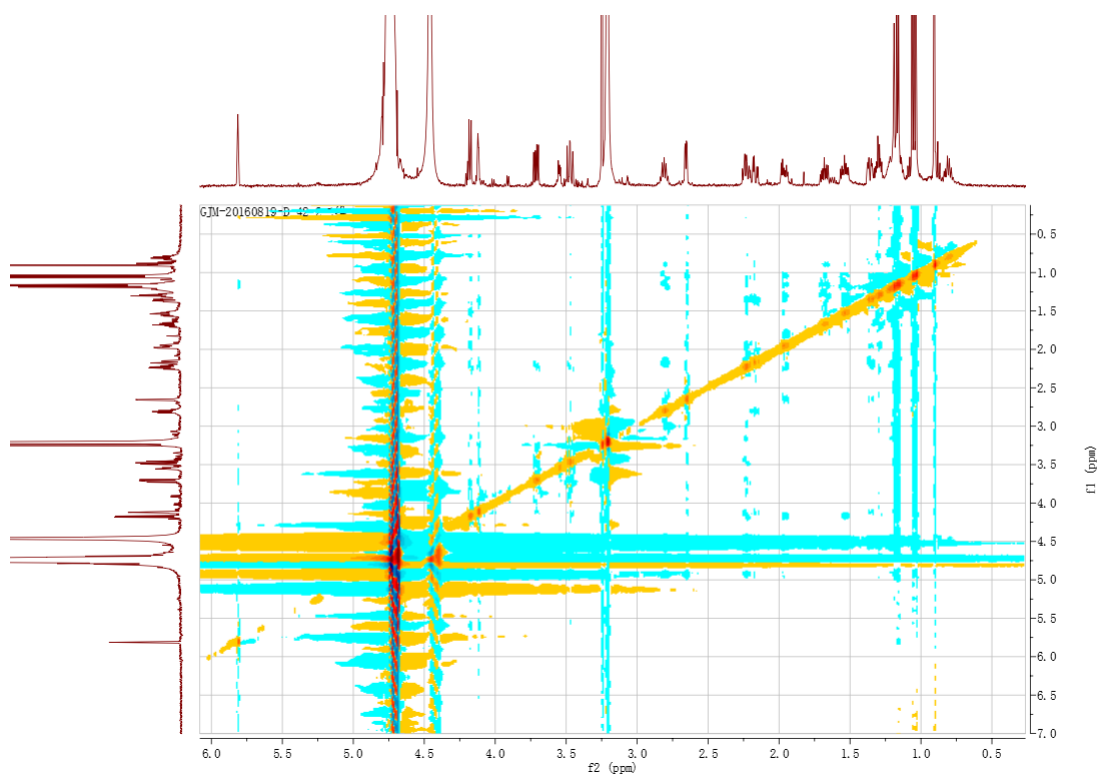


Figure S44. HRESIMS spectrum of compound **9**.

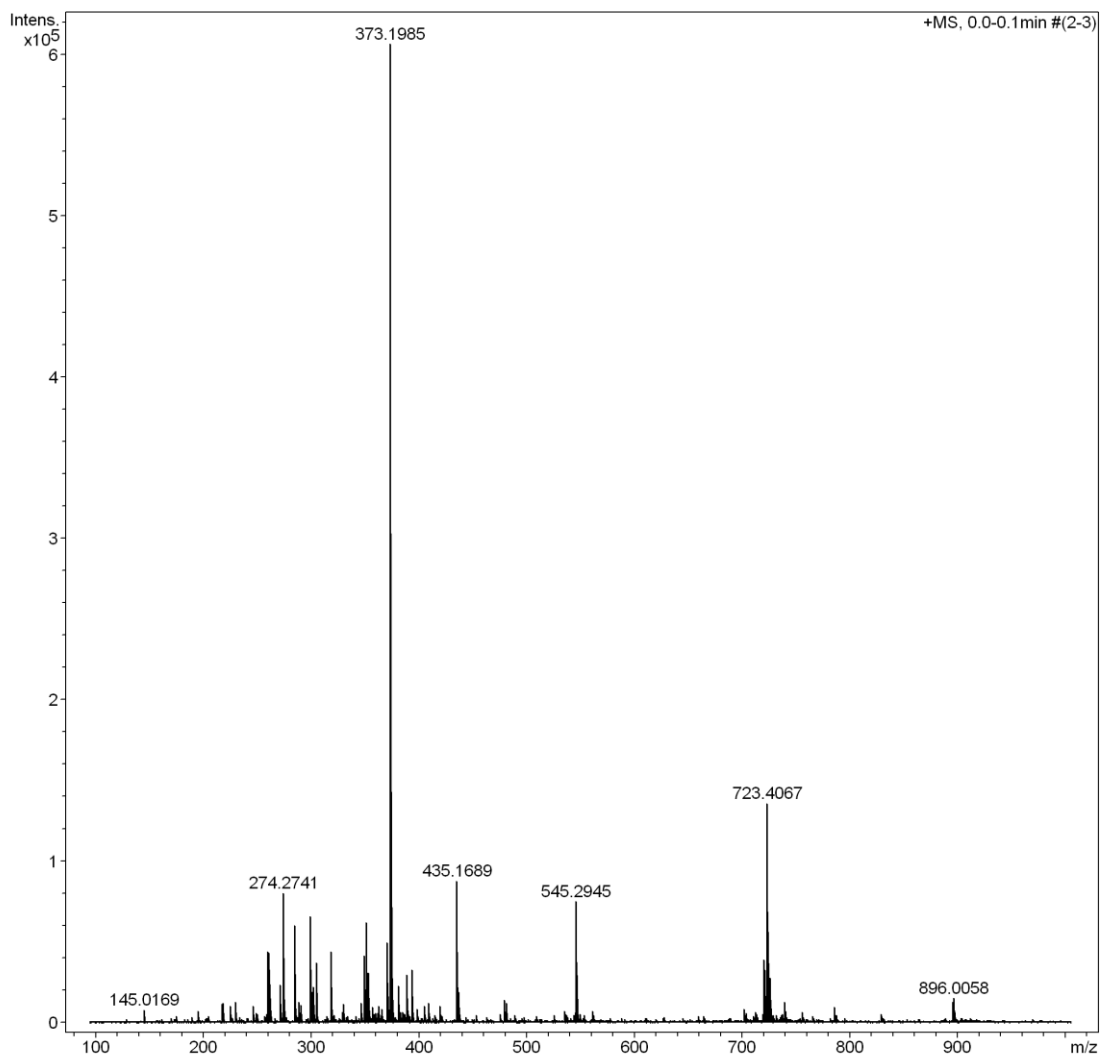
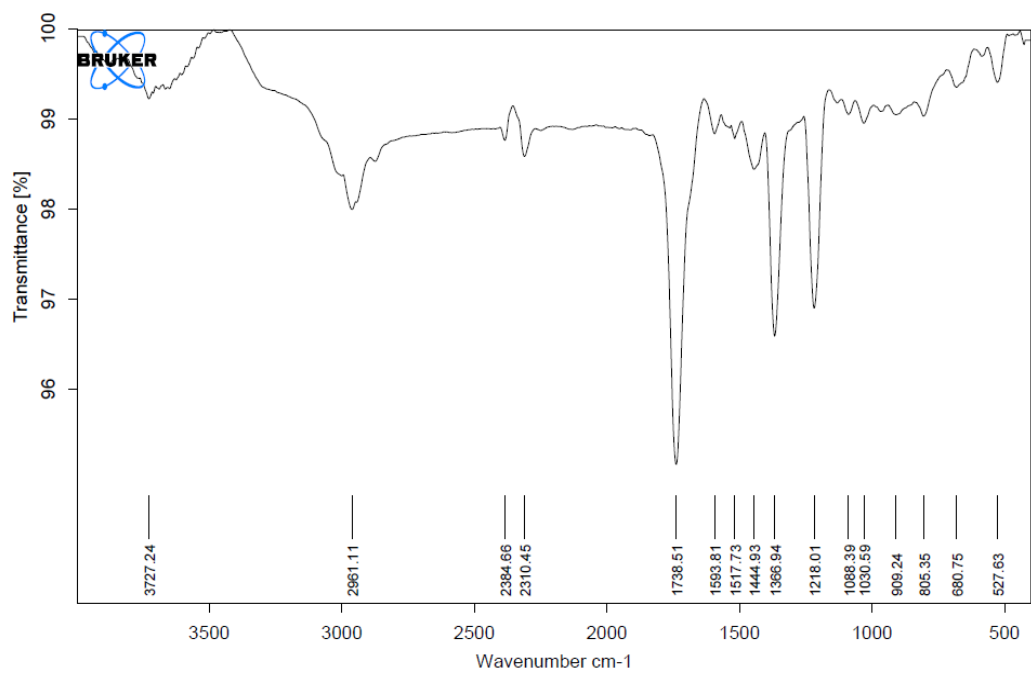


Figure S45. IR and UV spectra of compound **9**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.



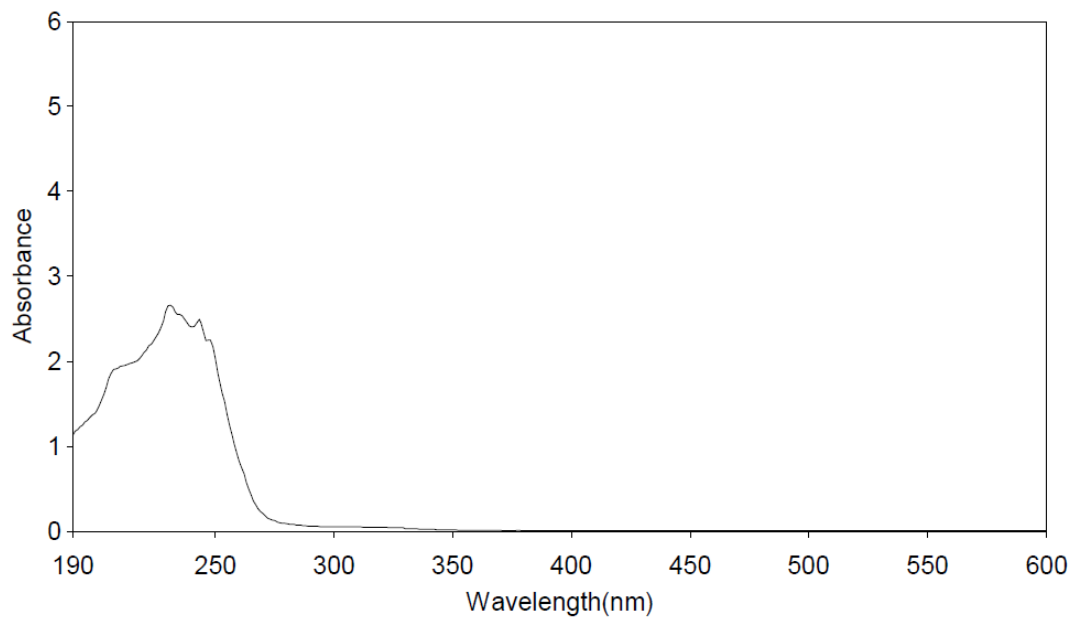
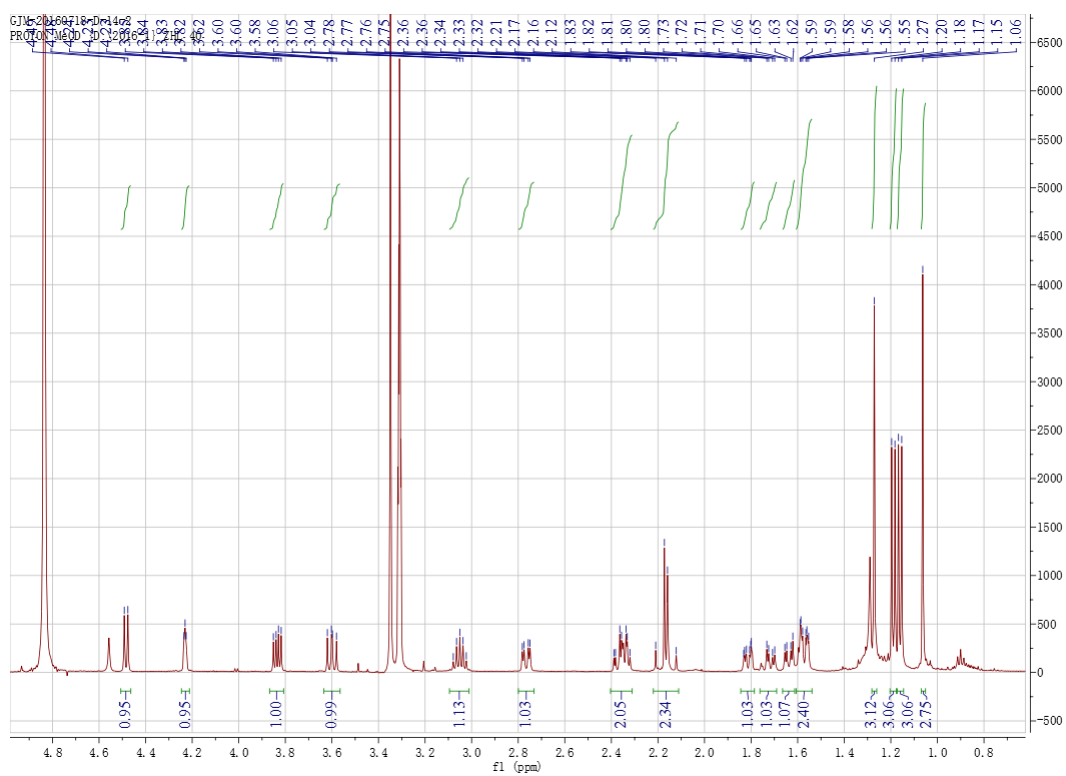


Figure S46. ^1H NMR and ^{13}C spectra of compound **10** in CD_3OD .



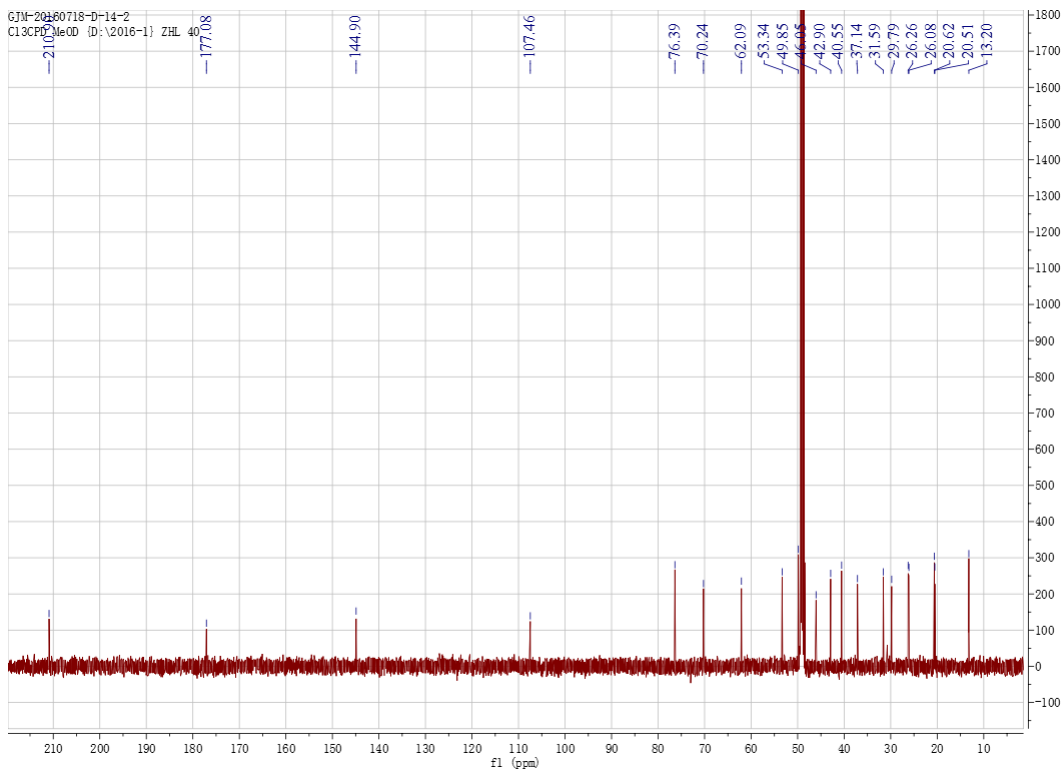
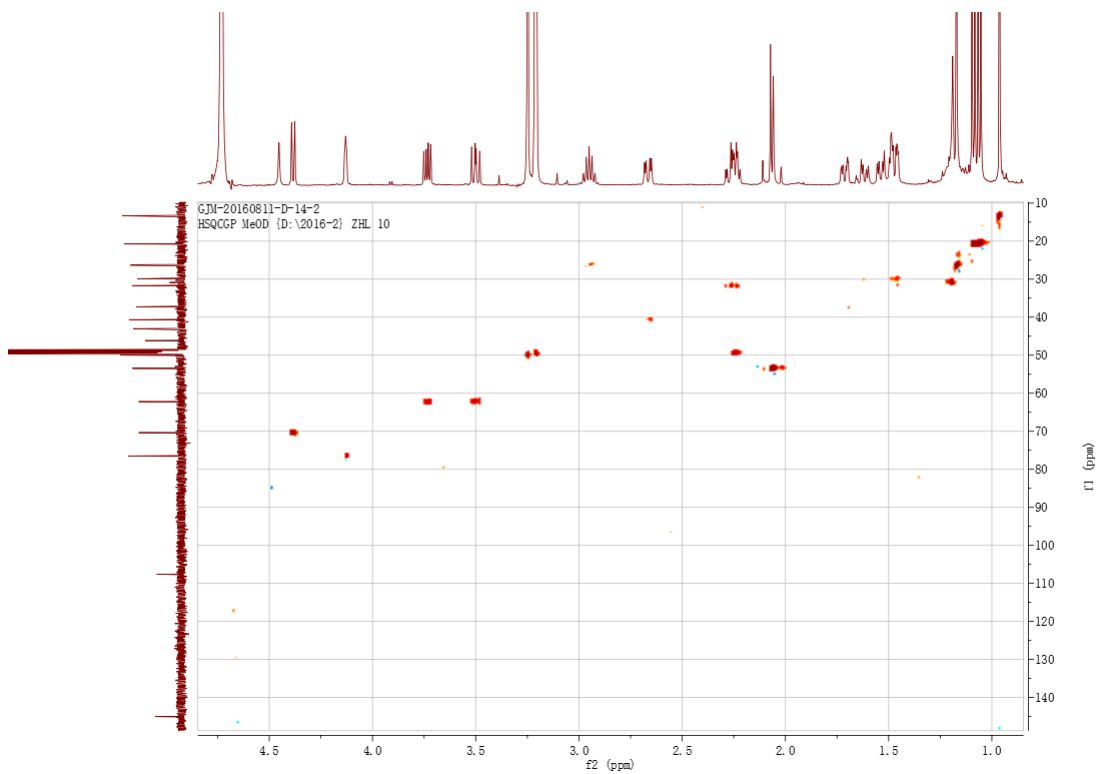


Figure S47. HSQC and ^1H - ^1H COSY spectra of compound **10** in CD_3OD



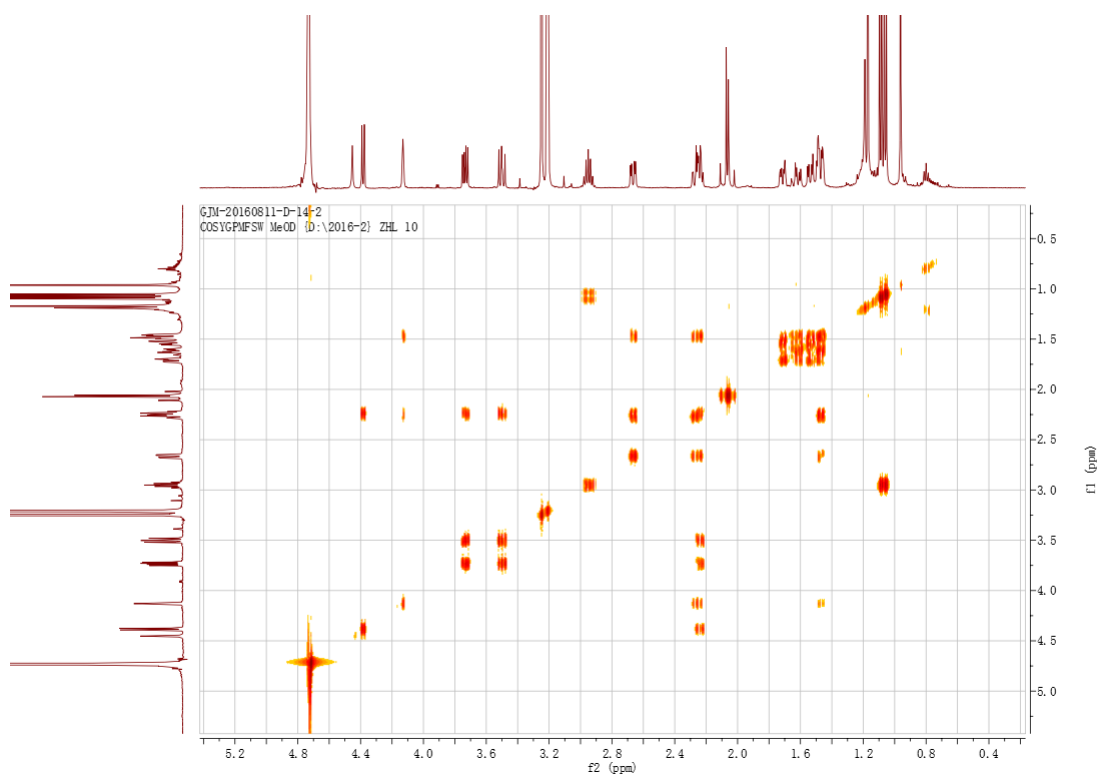
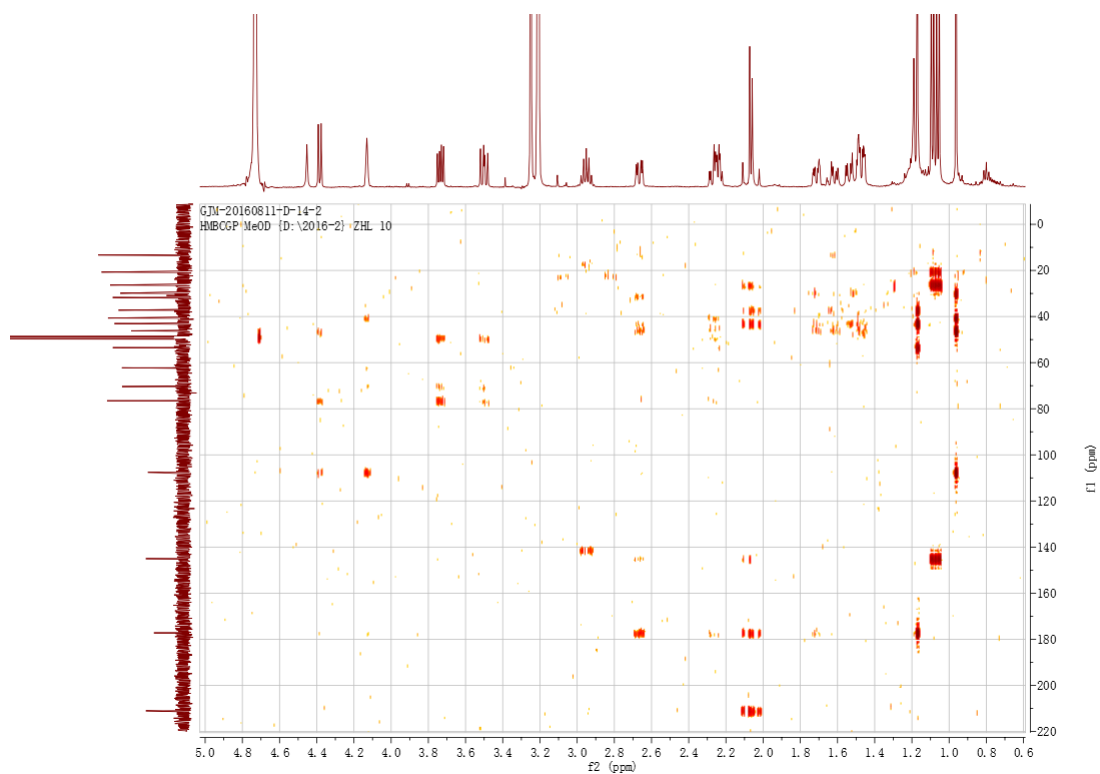


Figure S48. HMBC and NOESY spectra of compound **10** in CD₃OD.



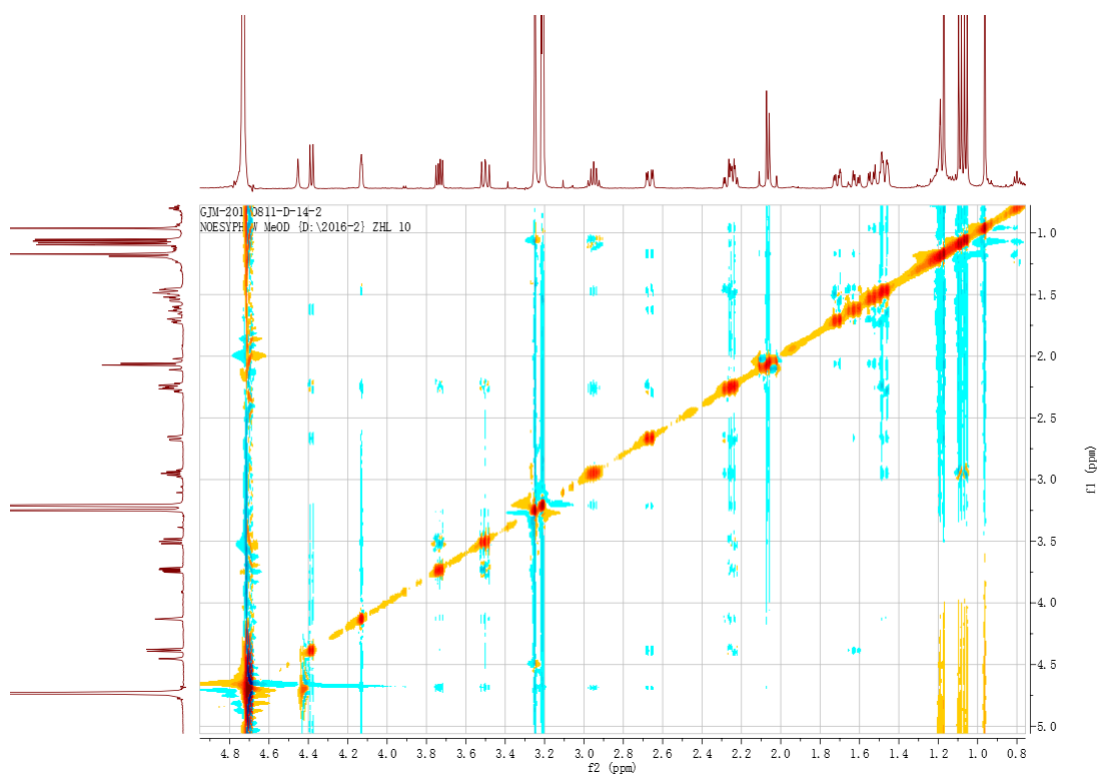


Figure S49. HRESIMS spectrum of compound 10.

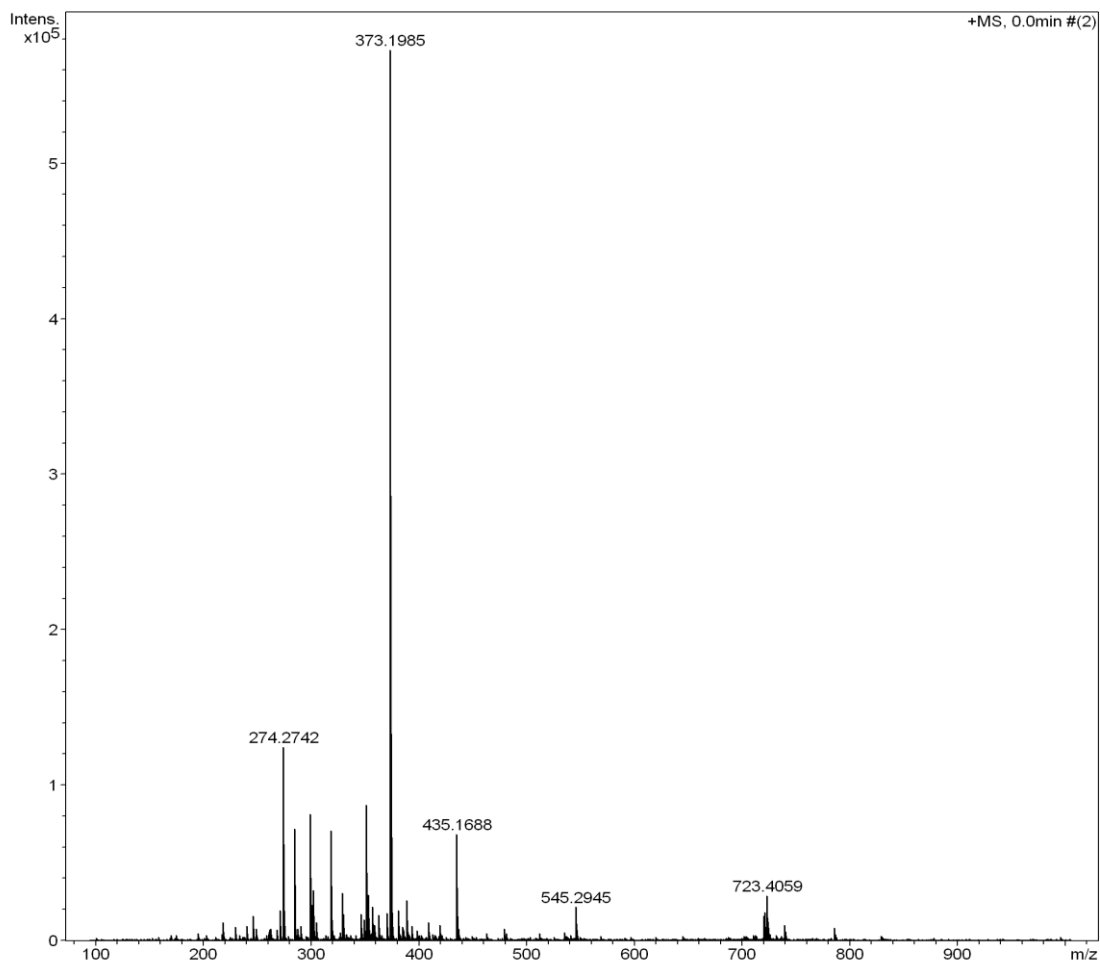


Figure S50. IR and UV spectra of compound **10**. This work is supported by Bruker (Beijing) Scientific Technology Co. Ltd.

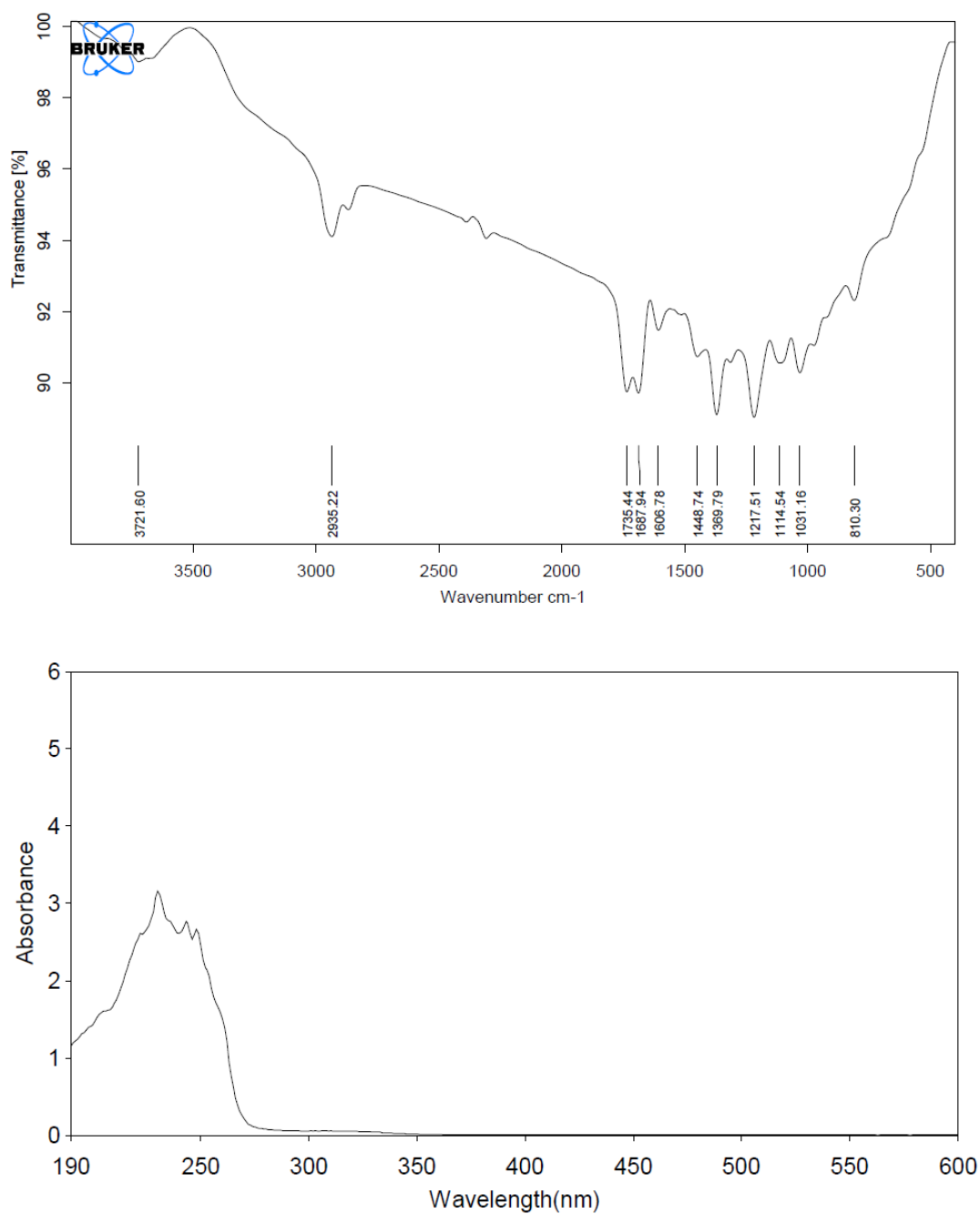


Figure S51. ¹H NMR and ¹³C spectra of compound **11** in CD₃OD.

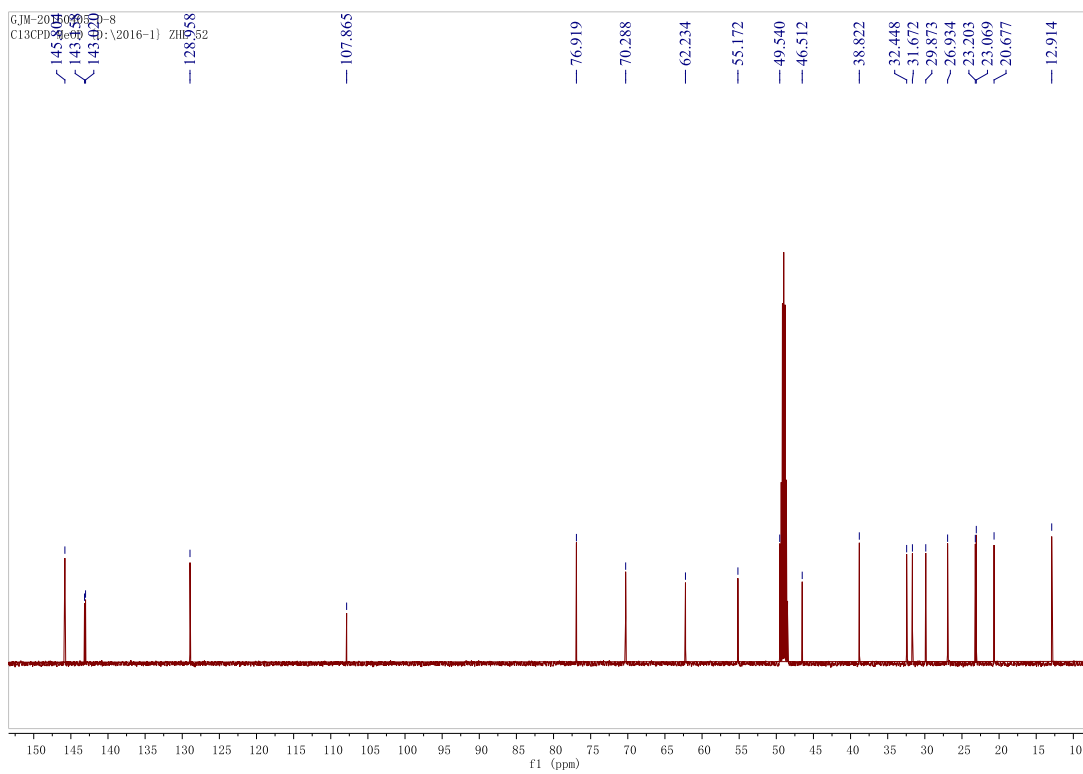
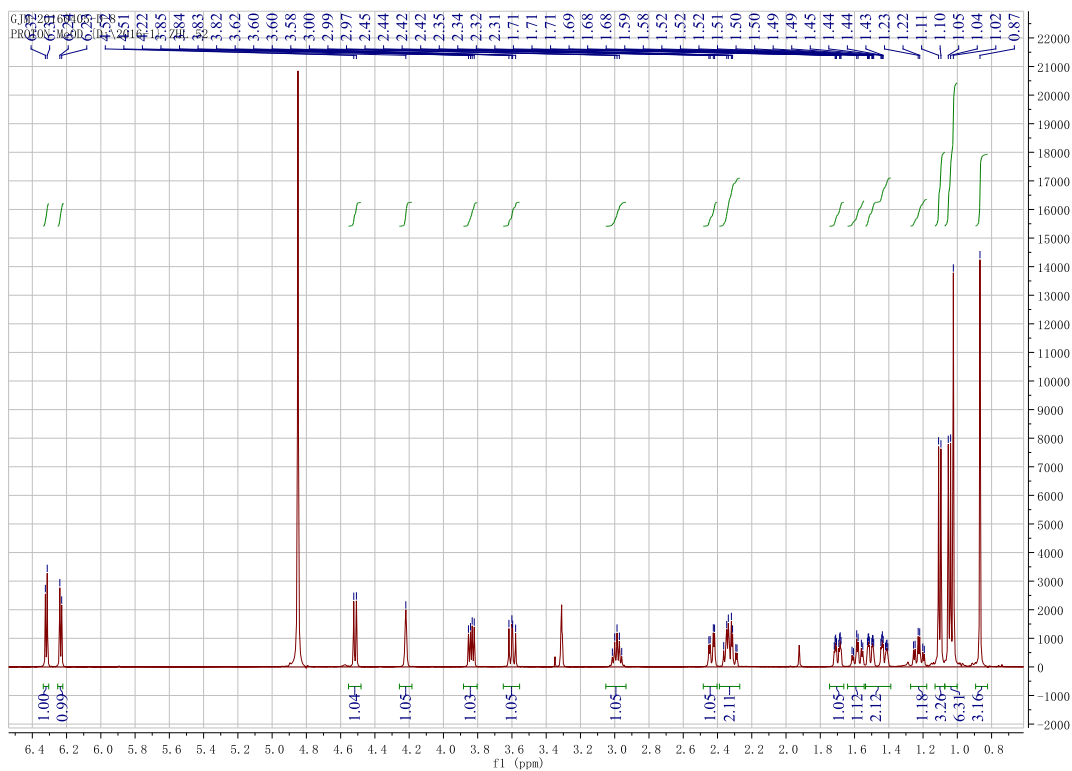


Figure S52. ESIMS spectrum of compound 11.

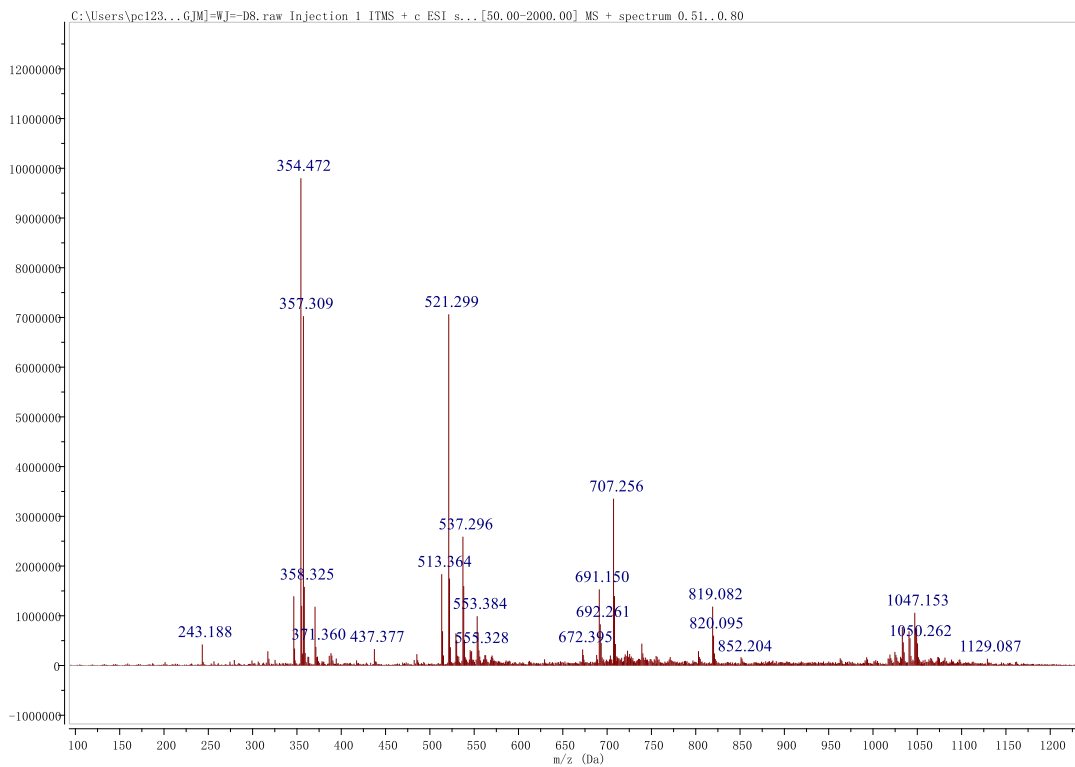


Figure S53. UV spectra of compound **11**.

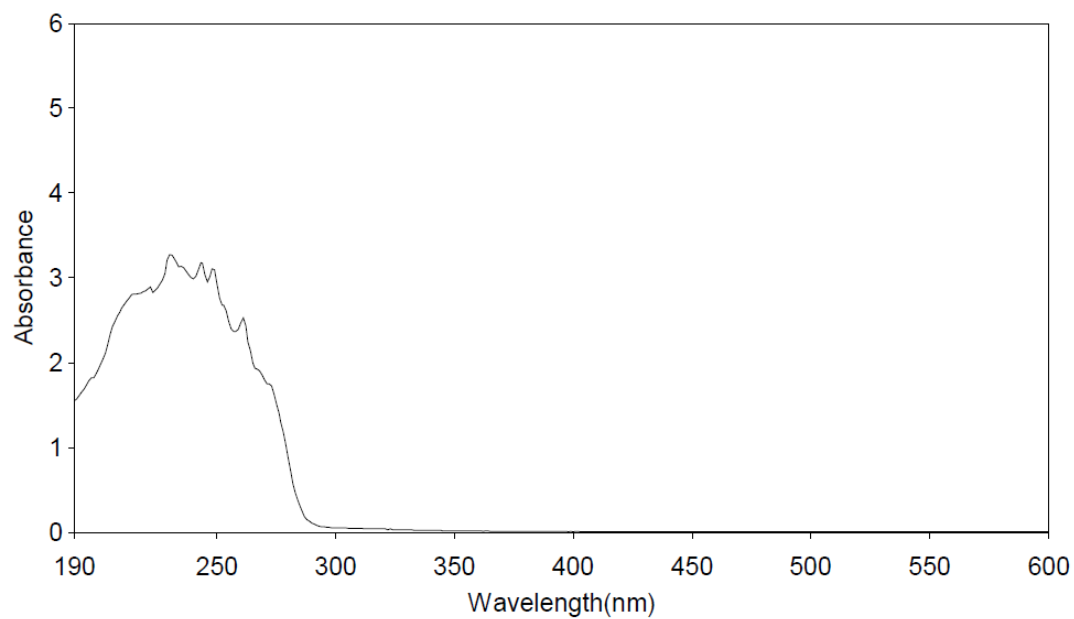


Figure S54. ^1H NMR and ^{13}C spectra of compound **12** in $\text{C}_5\text{D}_5\text{N}$.

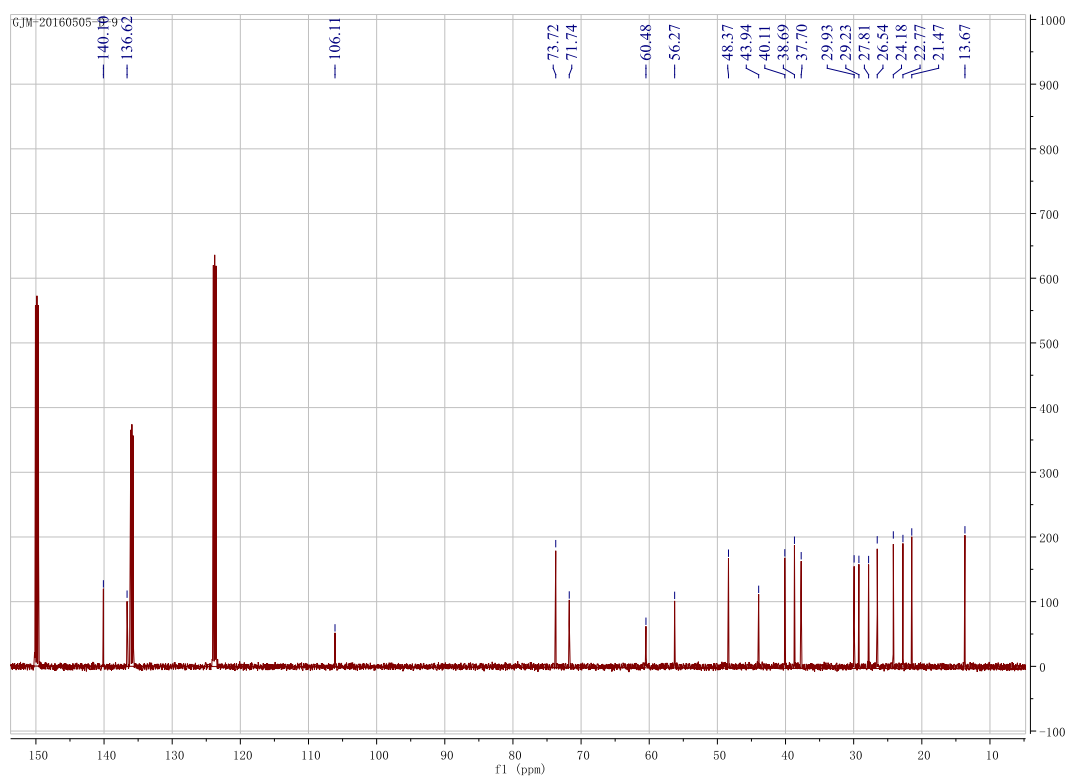
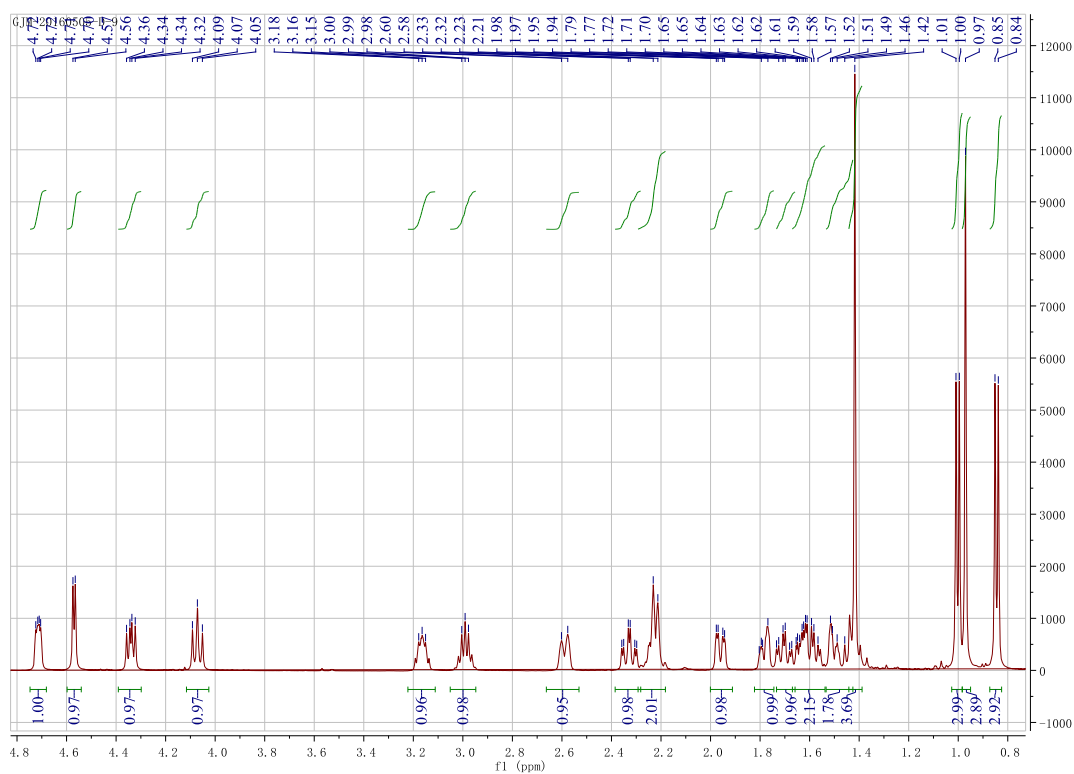


Figure S55. ESIMS spectrum of compound 12.

[GJM]=WJ=D9 #66-73 RT: 0.29-0.32 AV: 8 NL: 1.71E4
T: ITMS + c ESI sid=35.00 Full ms [50.00-2000.00]

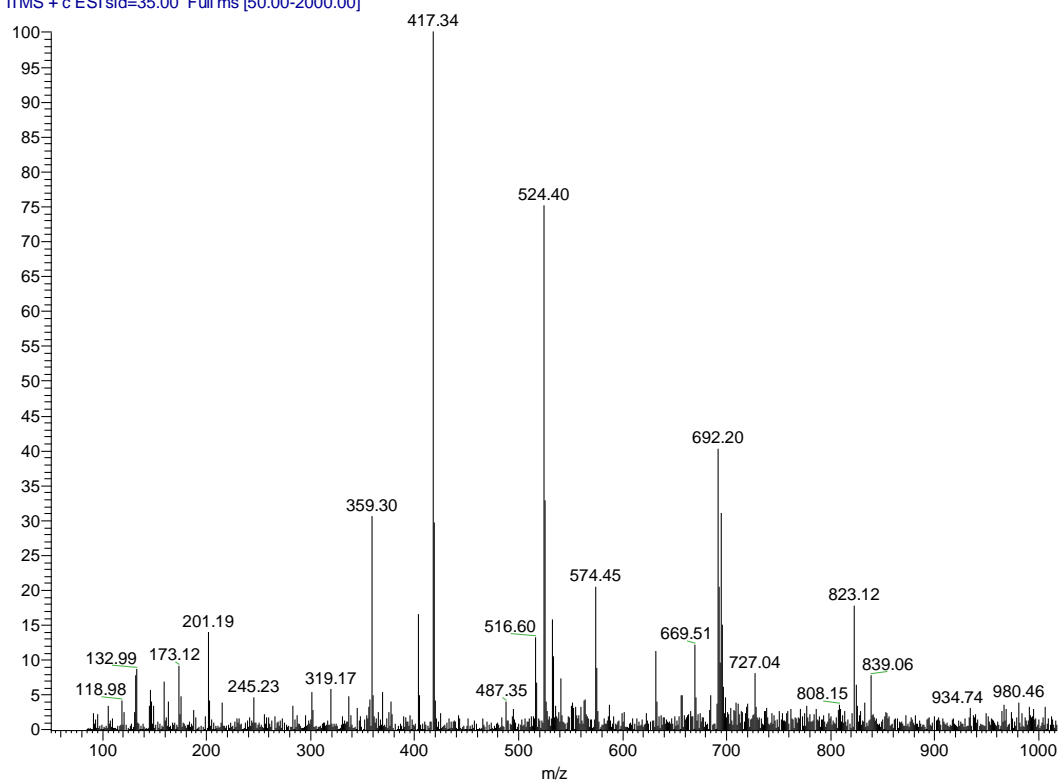


Figure S56. UV spectra of compound 12.

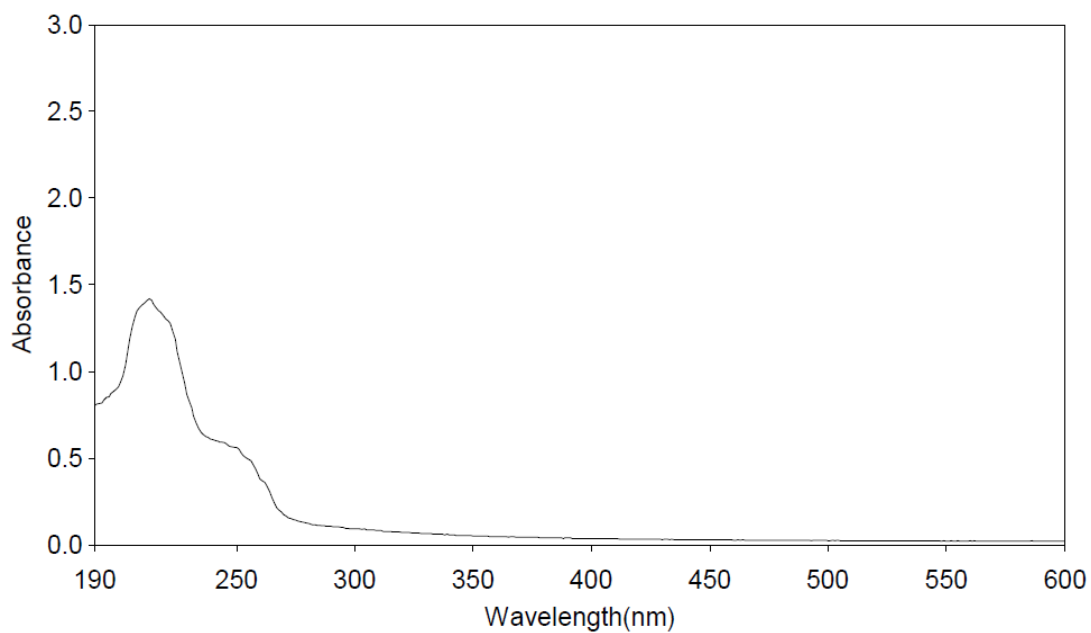


Figure S57. ^1H NMR and ^{13}C spectra of compound 13 in CD_3OD .

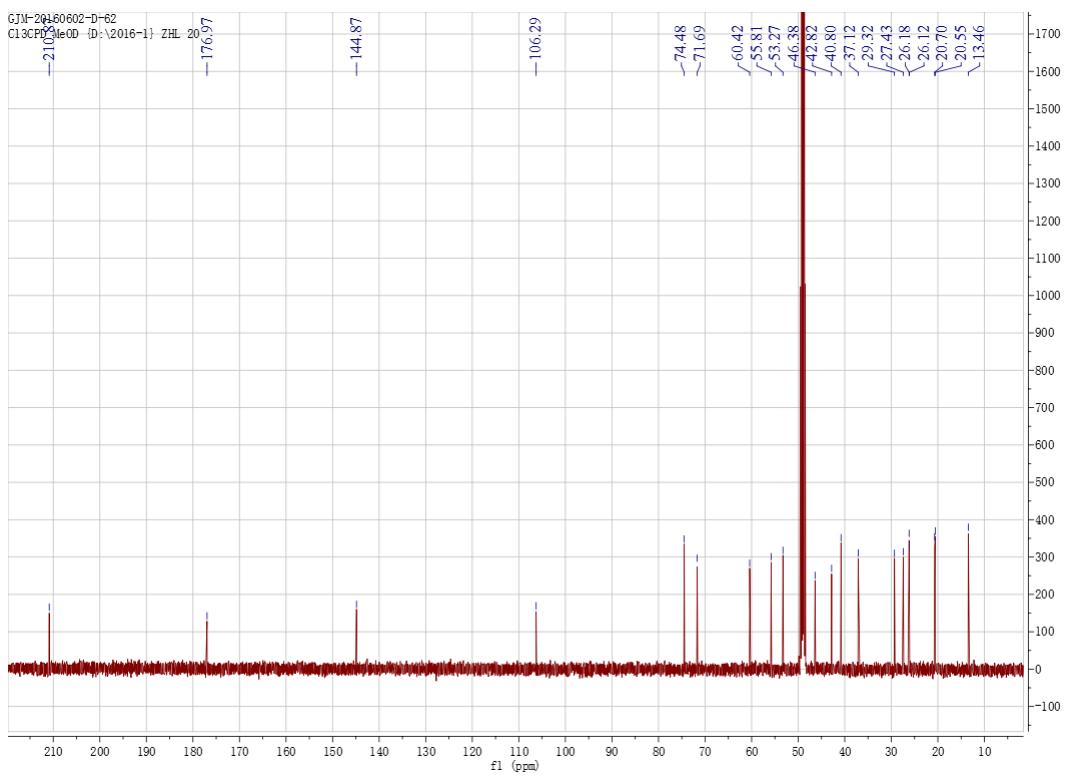
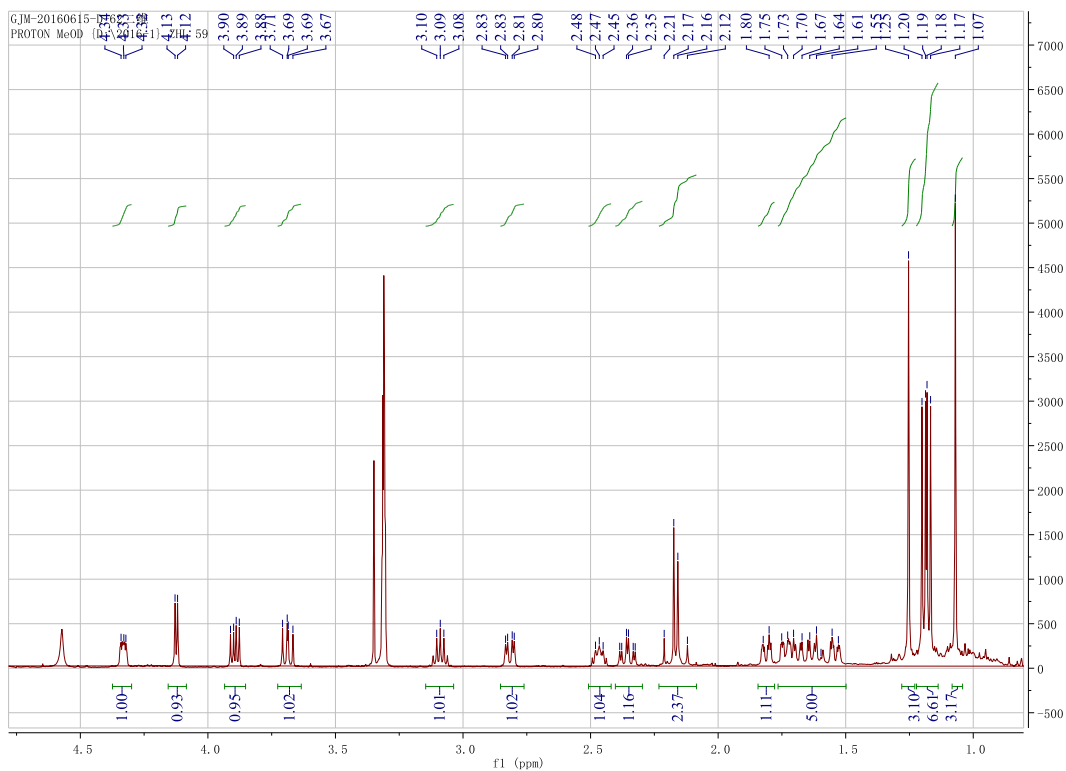


Figure S58. ESIMS spectrum of compound 13.

[GJM]-WeiJing-D62 #35-45 RT: 0.14-0.19 AV: 11 NL: 4.31E3
T: ITMS + c ESI sid=35.00 Full ms [50.00-2000.00]

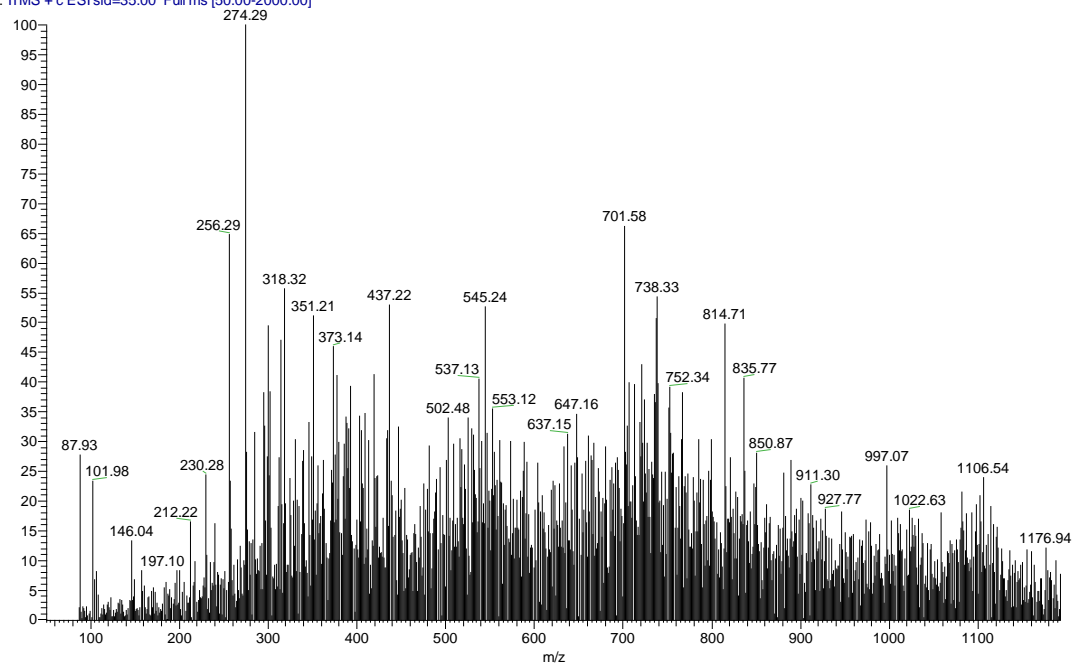


Figure S59. UV spectra of compound 13.

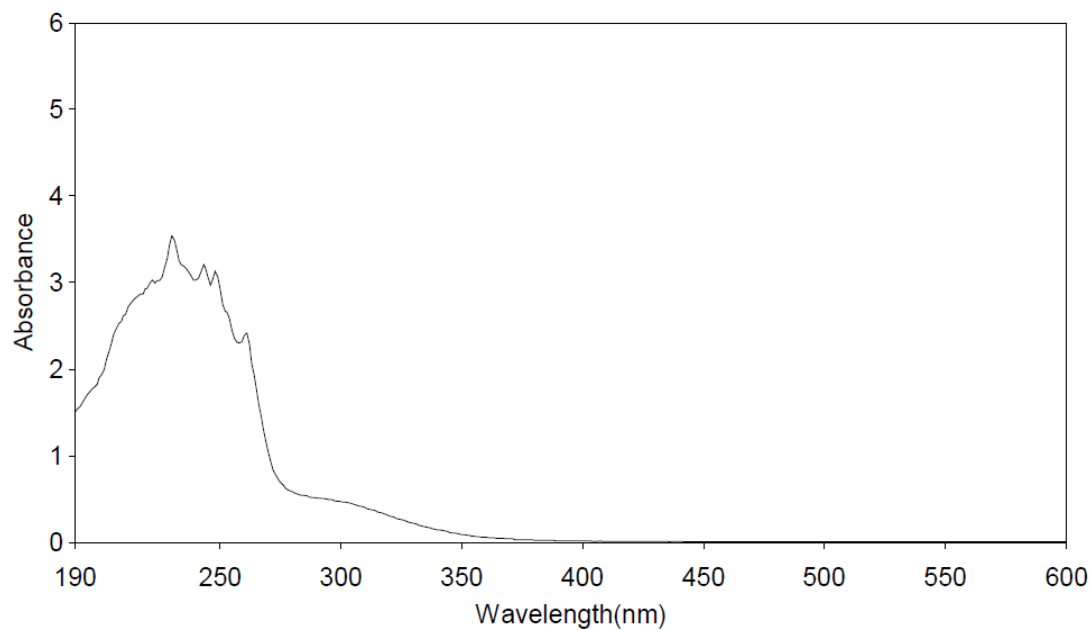


Figure S60. ^1H NMR and ^{13}C spectra of compound 14 in CD_3OD .

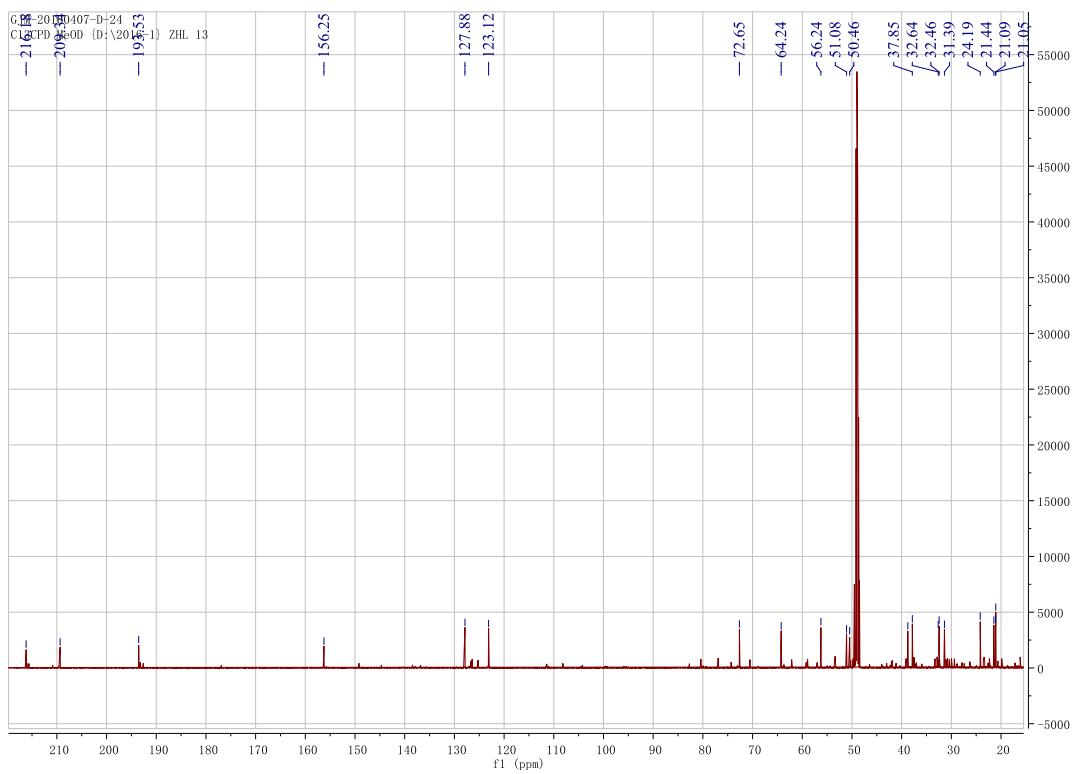
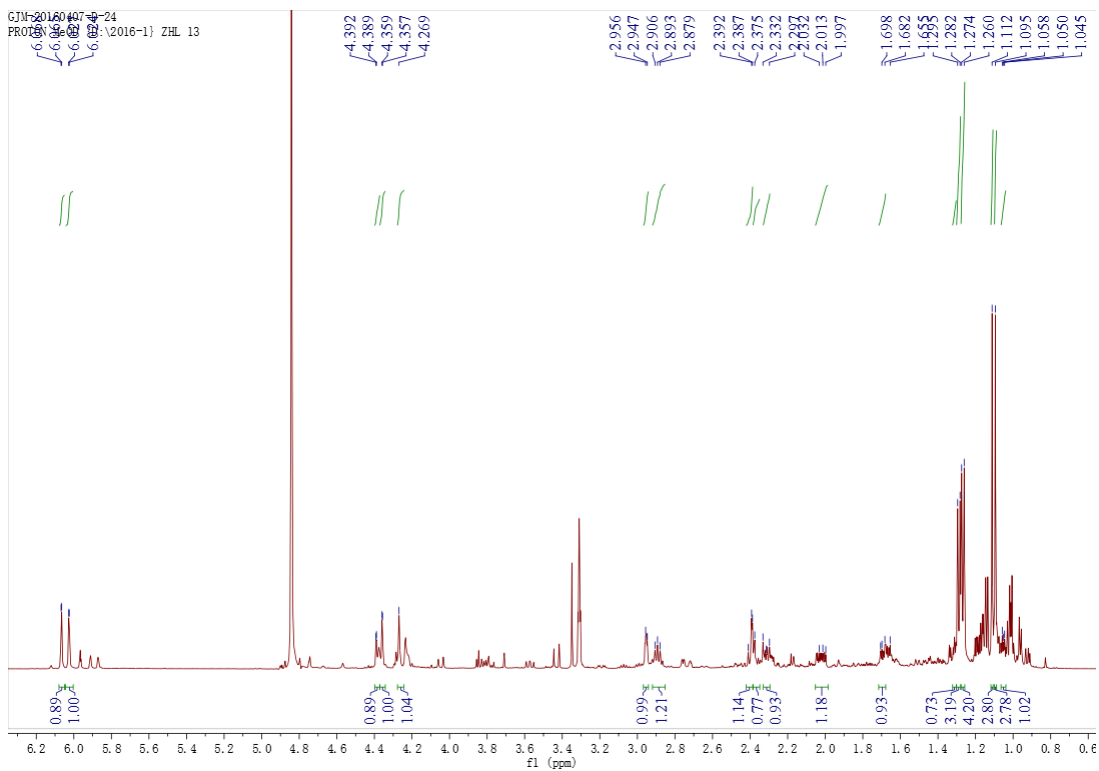


Figure S61. ESIMS spectrum of compound 14.

[GJM]=WJ=D24 #69-70 RT: 0.44-0.46 AV: 2 NL: 5.94E2
T: ITMS - c ESI: sid=35.00 Full ms [50.00-2000.00]

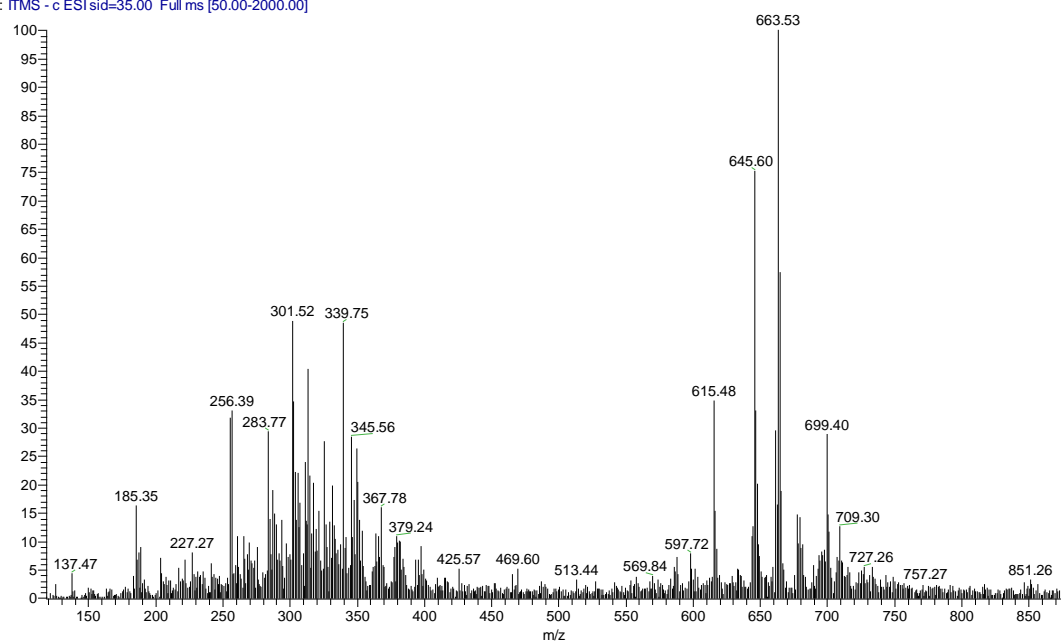
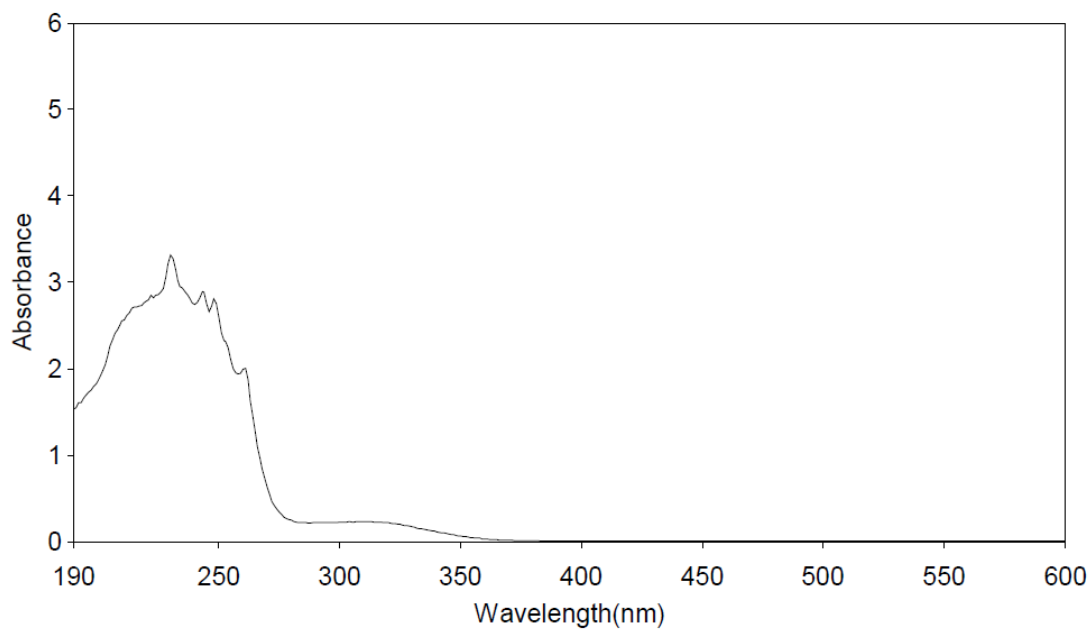


Figure S62. UV spectra of compound 14.



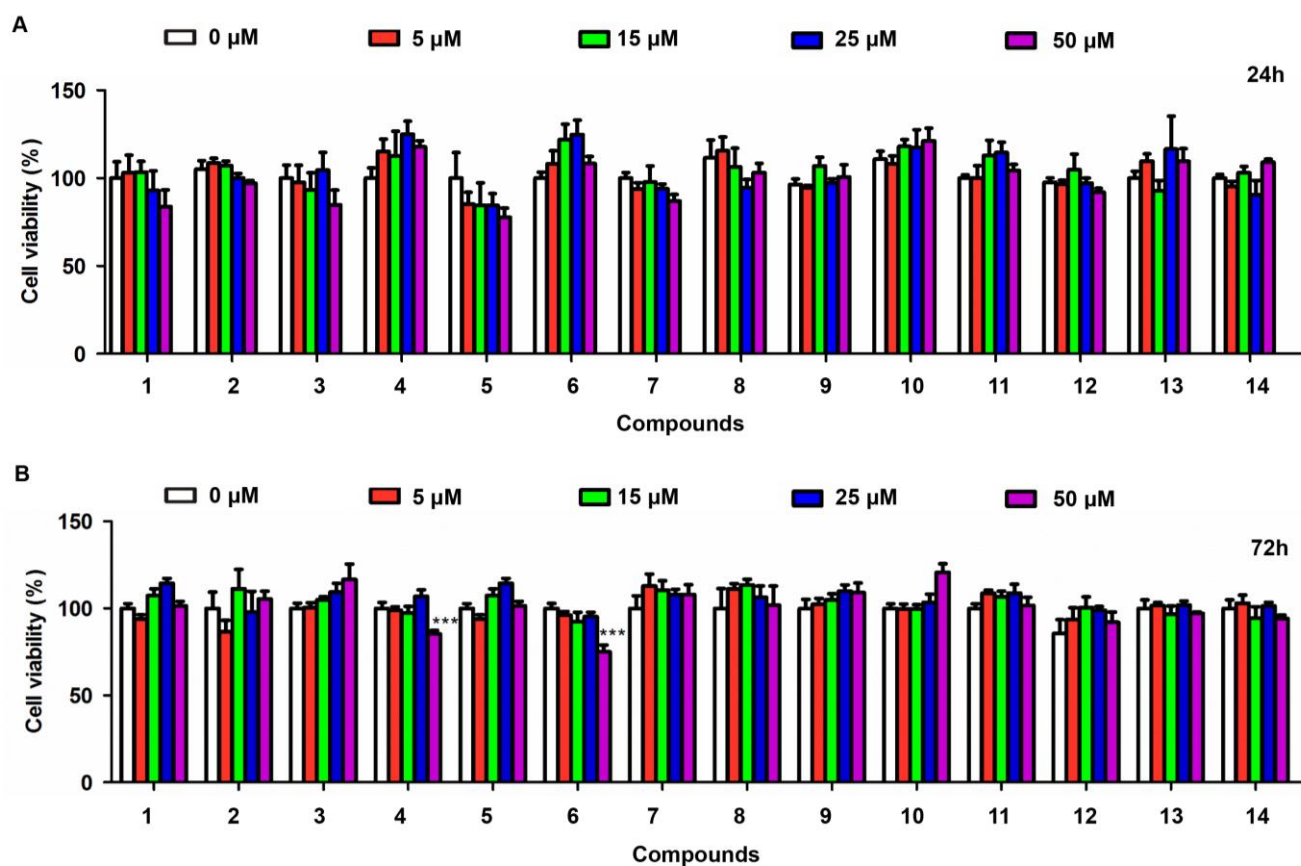


Figure S63. Cytotoxicity of compounds in BV2 microglia cells.

(A) Cell viability of BV2 cells following compounds treatment for 24 h. (B) Cell viability of BV2 cells following compounds treatment for 72 h. BV2 cells were seeded in 96-well plates and treated with different concentrations of compounds for 24 h or 72 h. Then the cell viability was measured by MTT assay. Drug treatment vs Control,***, $p < 0.001$.