

## SUPPLEMENTARY MATERIAL

### Secondary Metabolites from the Coral-Derived Fungus *Aspergillus austwickii* SCSIO41227 with Pancreatic Lipase and Neuraminidase Inhibitory Activities

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**Abstract:** The coral-derived fungus *Aspergillus austwickii* SCSIO41227 from Beibu Gulf yielded four previously uncharacterized compounds, namely asperpentenones B–E (**1–4**), along with twelve known compounds (**5–16**). Their structures were elucidated by HRESIMS and NMR (<sup>1</sup>H and <sup>13</sup>C NMR, HSQC, HMBC), among which the stereo-structure of compounds **1–3** was determined by calculated ECD. Furthermore, compounds **1–16** were evaluated for their enzyme (acetylcholinesterase (AChE), pancreatic lipase (PL) and neuraminidase (NA)) inhibitory activities. These bioassay results demonstrated that compound **3** exhibited PL inhibitory effect with an IC<sub>50</sub> value of 127.11 μM. In addition, compounds **2** and **14** displayed noticeable NA inhibitory effects with IC<sub>50</sub> values of 31.28 and 73.64 μM, respectively. Furthermore, these compounds showed the potential of inhibiting enzymes in silico docking analysis to demonstrate the interactions between compounds and proteins.

**Keywords:** *Aspergillus austwickii* SCSIO41227; secondary metabolites; pancreatic lipase; neuraminidase; molecular docking

## Contents

The ITS gene sequence data of *Aspergillus austwickii* SCSIO41227.

Figure S1. The  $^1\text{H}$  NMR Spectrum of compound 1 in  $\text{DMSO}-d_6$ .

Figure S2. The  $^{13}\text{C}$  NMR Spectrum of compound 1 in  $\text{DMSO}-d_6$ .

Figure S3. The HSQC Spectrum of compound 1 in  $\text{DMSO}-d_6$ .

Figure S4. The HMBC Spectrum of compound 1 in  $\text{DMSO}-d_6$ .

Figure S5. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 1 in  $\text{DMSO}-d_6$ .

Figure S6. The NOESY spectrum of compound 1 in  $\text{DMSO}-d_6$ .

Figure S7. The HRESIMS spectrum of compound 1 in MeOH.

Figure S8. The UV and experimental ECD spectrum of compound 1.

Figure S9. The  $^1\text{H}$  NMR Spectrum of compound 2 in  $\text{DMSO}-d_6$ .

Figure S10. The  $^{13}\text{C}$  NMR Spectrum of compound 2 in  $\text{DMSO}-d_6$ .

Figure S11. The HSQC Spectrum of compound 2 in  $\text{DMSO}-d_6$ .

Figure S12. The HMBC Spectrum of compound 2 in  $\text{DMSO}-d_6$ .

Figure S13. The HRESIMS spectrum of compound 2 in MeOH.

Figure S14. The UV and experimental ECD spectrum of compound 2.

Figure S15. The  $^1\text{H}$  NMR Spectrum of compound 3 in  $\text{CD}_3\text{OD}$ .

Figure S16. The  $^{13}\text{C}$  NMR Spectrum of compound 3 in  $\text{CD}_3\text{OD}$ .

Figure S17. The HSQC Spectrum of compound 3 in  $\text{CD}_3\text{OD}$ .

Figure S18. The HMBC Spectrum of compound 3 in  $\text{CD}_3\text{OD}$ .

Figure S19. The HRESIMS spectrum of compound 3 in MeOH.

Figure S20. The UV and experimental ECD spectrum of compound 3.

Figure S21. The  $^1\text{H}$  NMR Spectrum of compound 4 in  $\text{DMSO}-d_6$ .

Figure S22. The  $^{13}\text{C}$  NMR Spectrum of compound 4 in  $\text{DMSO}-d_6$ .

Figure S23. The HSQC Spectrum of compound 4 in  $\text{DMSO}-d_6$ .

Figure S24. The HMBC Spectrum of compound 4 in  $\text{DMSO}-d_6$ .

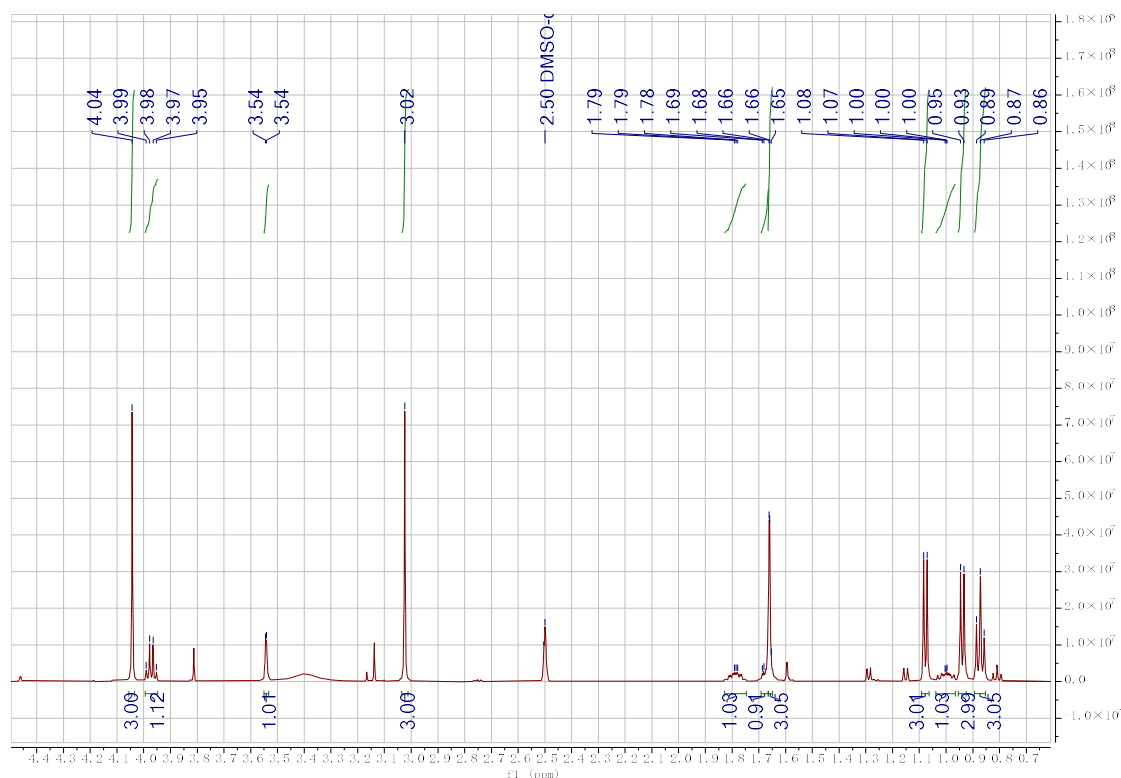
Figure S25. The HRESIMS spectrum of compound 4 in MeOH.

Figure S26. The UV spectrum of compound 4.

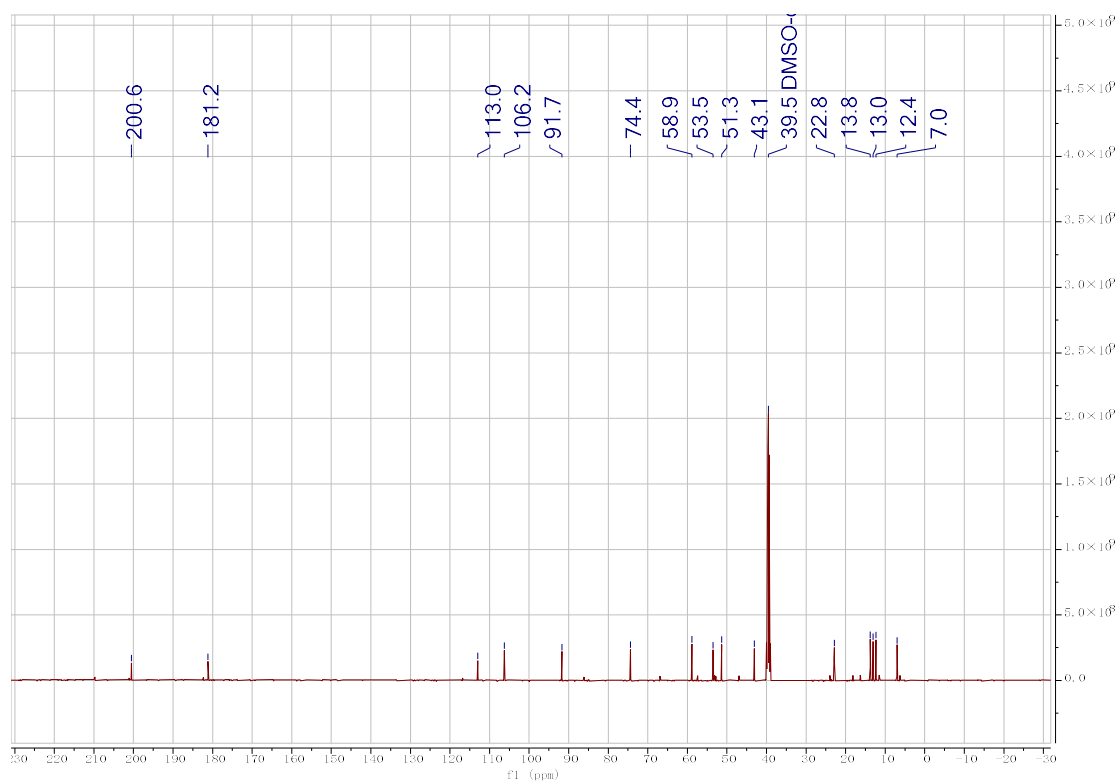
The physicochemical data of the known compounds 5~16.

**The ITS gene sequence data of *Aspergillus austwickii***

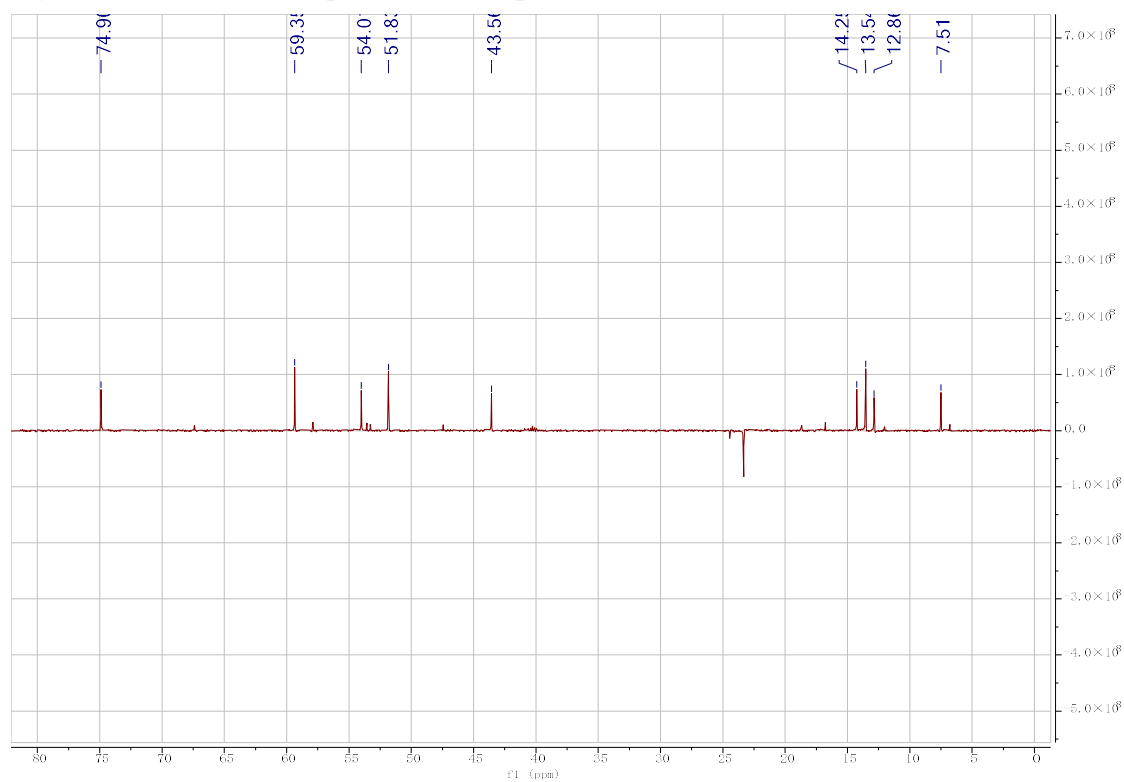
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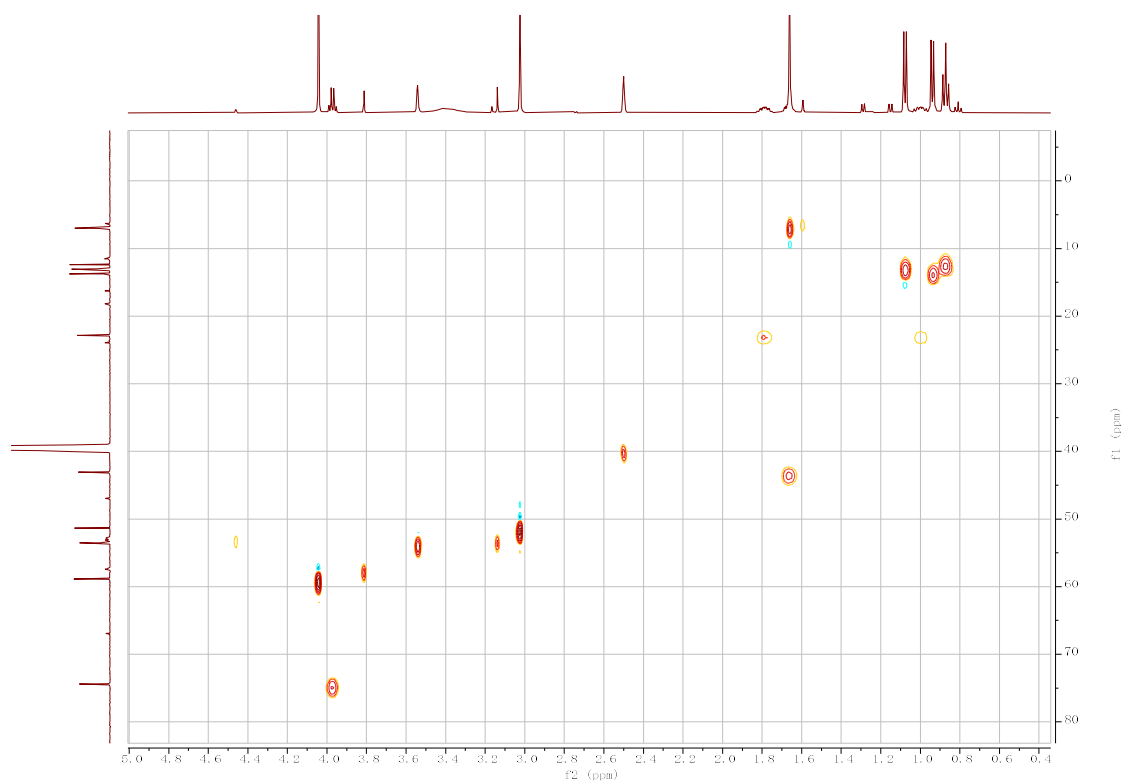
**Figure S1. The <sup>1</sup>H NMR Spectrum of compound 1 in DMSO-*d*<sub>6</sub>.**



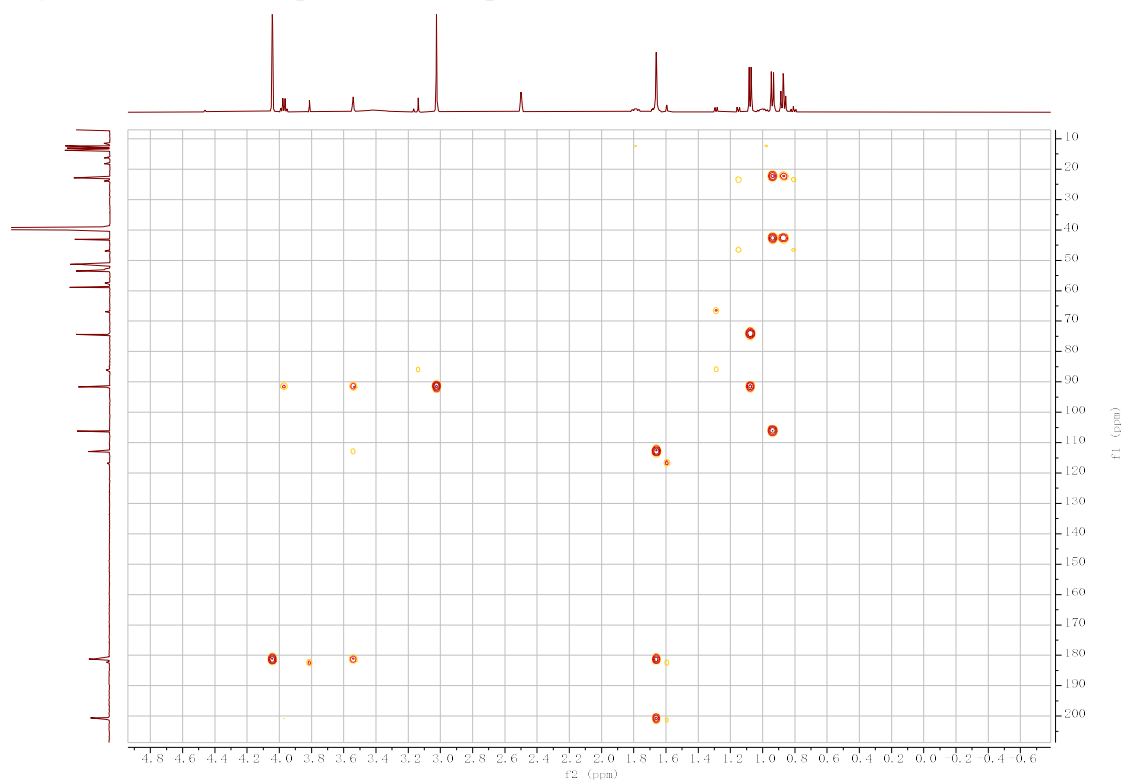
**Figure S2.** The <sup>13</sup>C NMR Spectrum of compound 1 in DMSO-*d*<sub>6</sub>.



**Figure S2.** The DEPT Spectrum of compound 1 in DMSO-*d*<sub>6</sub>.



**Figure S3.** The HSQC Spectrum of compound 1 in DMSO- $d_6$ .



**Figure S4.** The HMBC Spectrum of compound 1 in DMSO- $d_6$ .

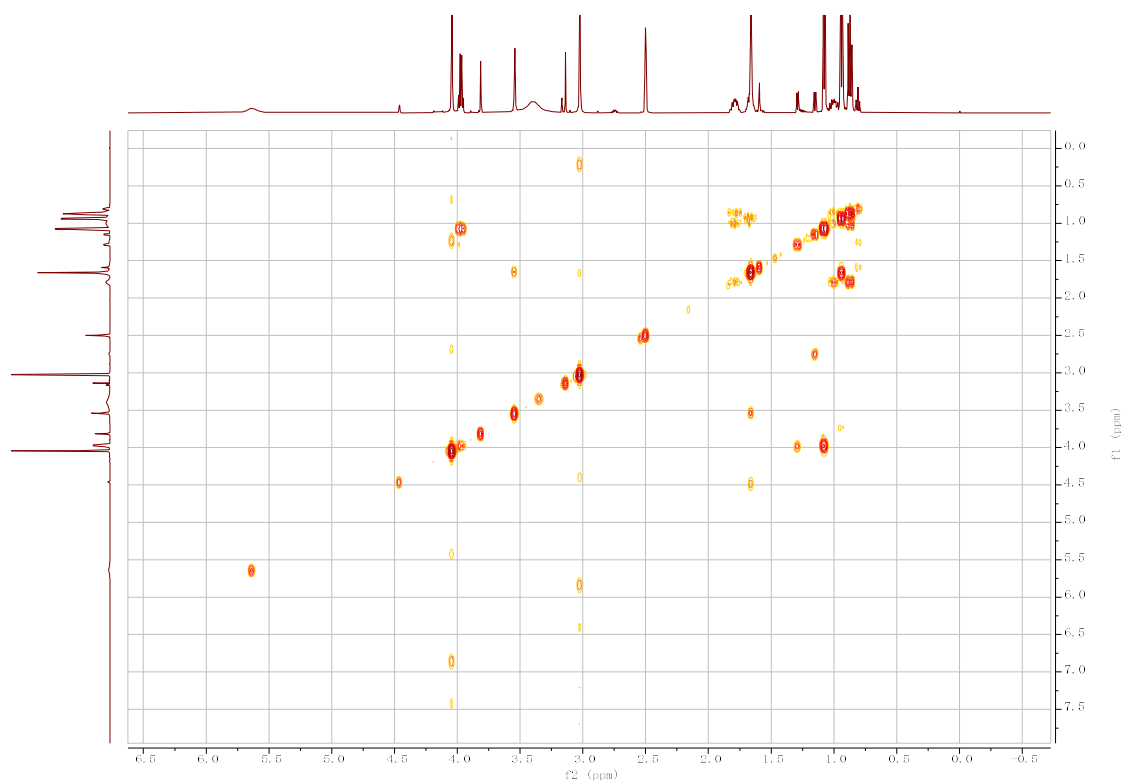


Figure S5. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 1 in  $\text{DMSO}-d_6$ .

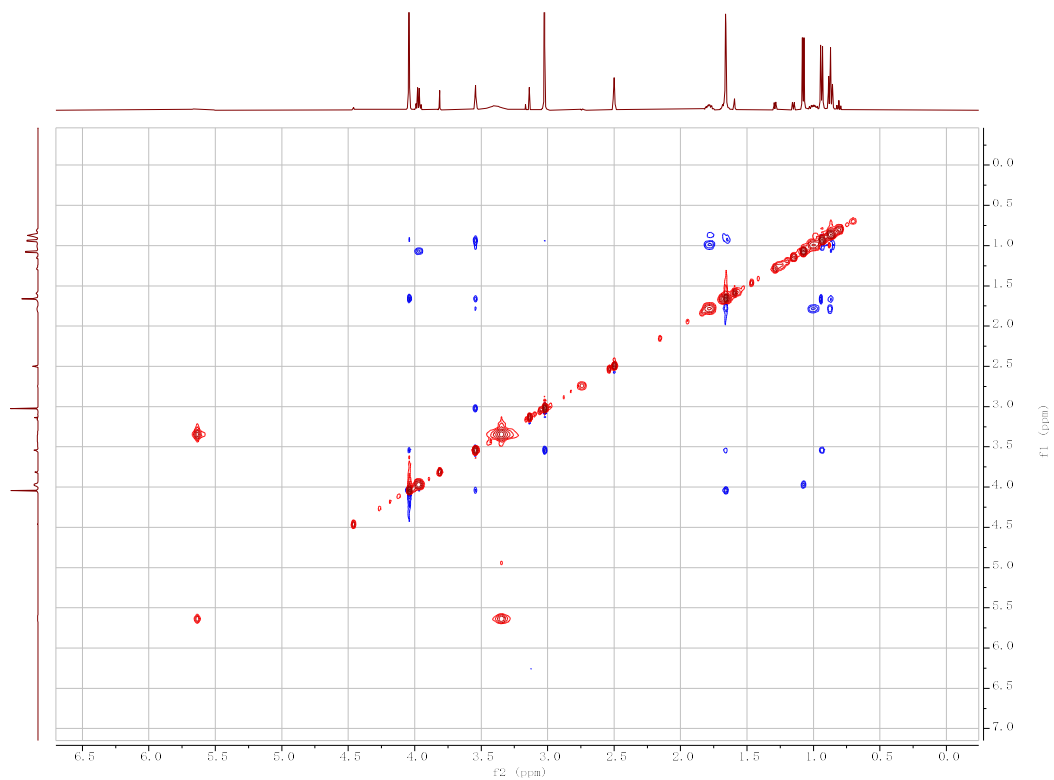


Figure S6. The NOESY spectrum of compound 1 in  $\text{DMSO}-d_6$ .

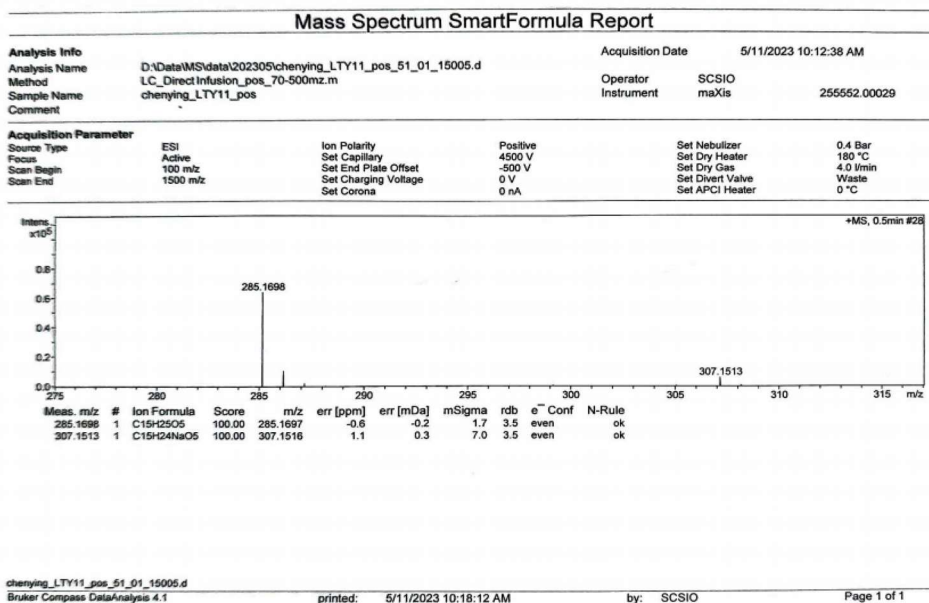


Figure S7. The HRESIMS spectrum of compound 1 in MeOH.

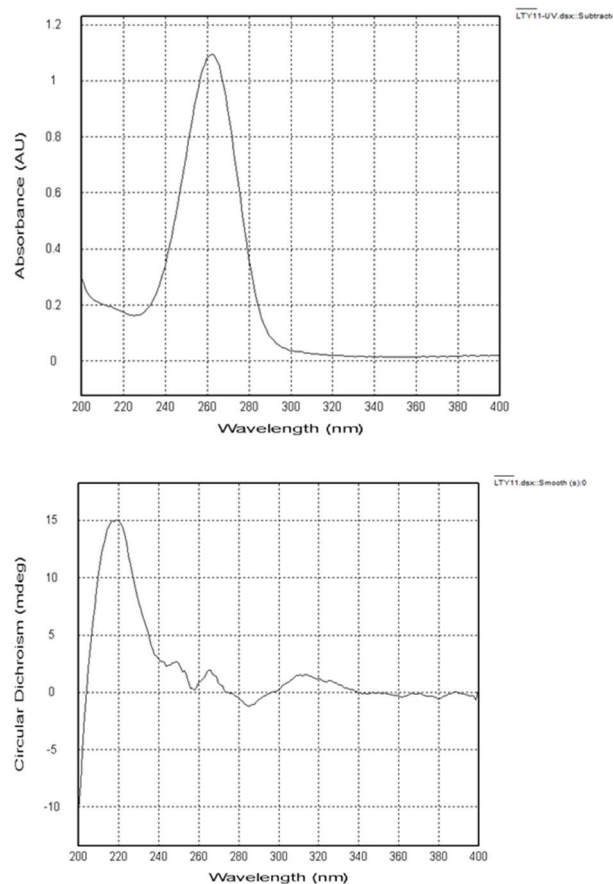


Figure S8. The UV and experimental ECD spectrum of compound 1.

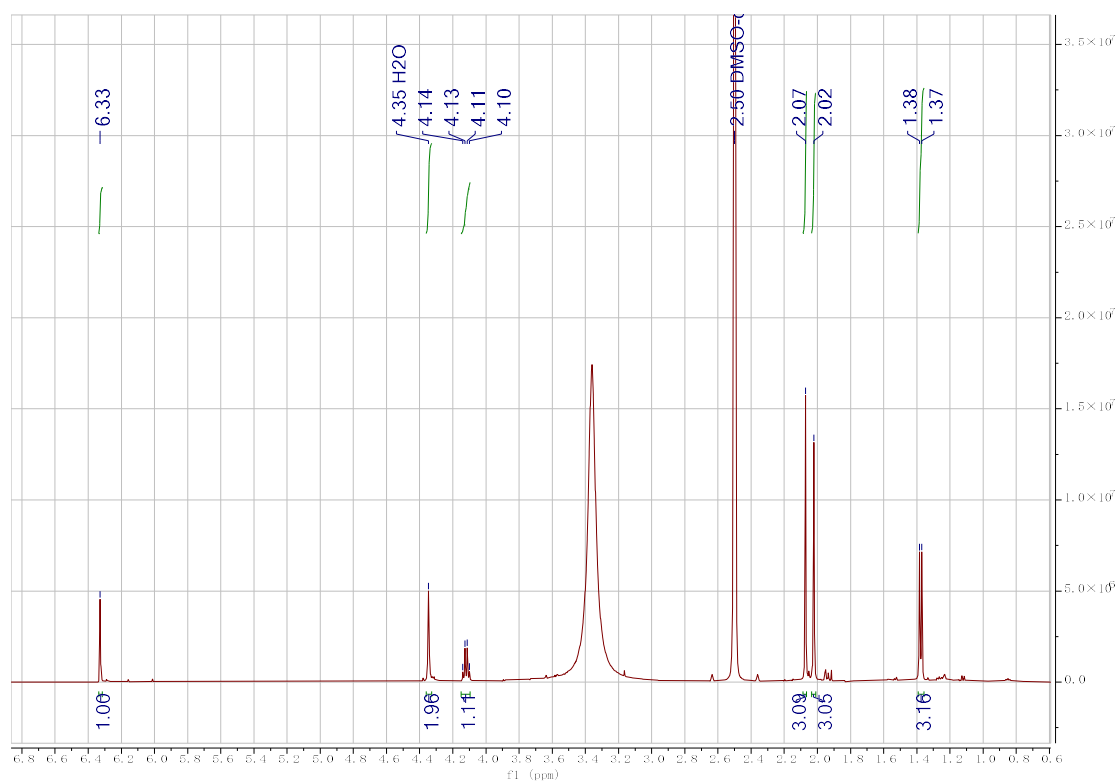


Figure S9. The <sup>1</sup>H NMR Spectrum of compound 2 in DMSO-*d*<sub>6</sub>.

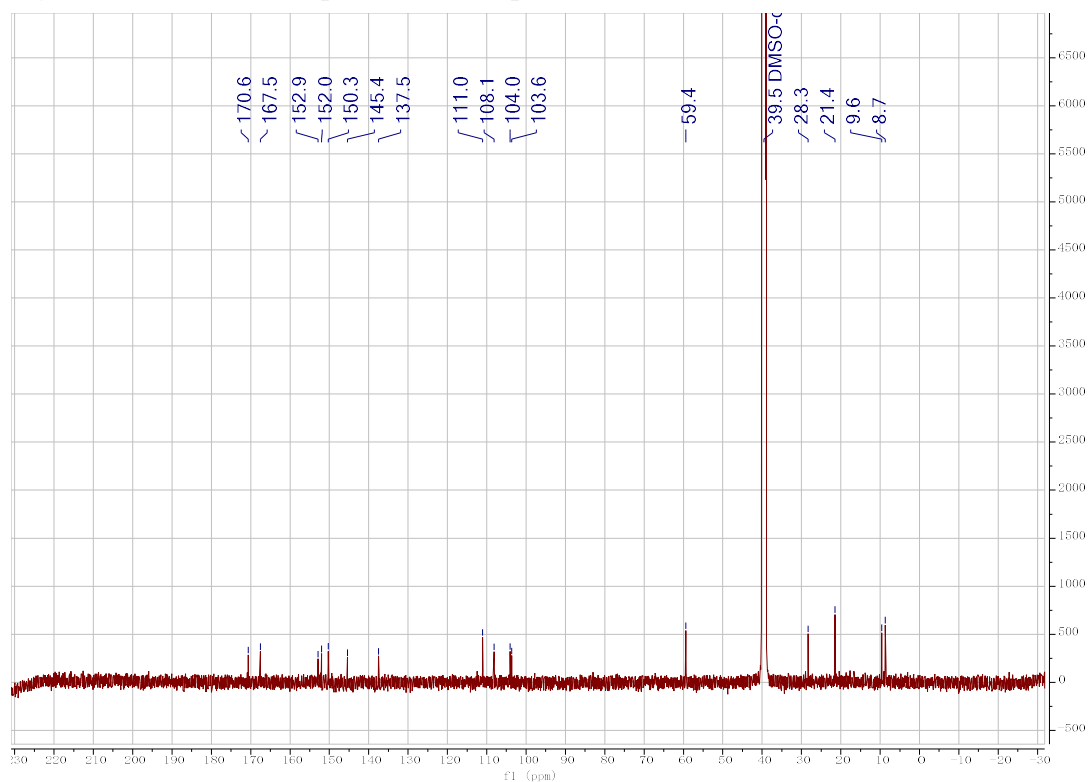
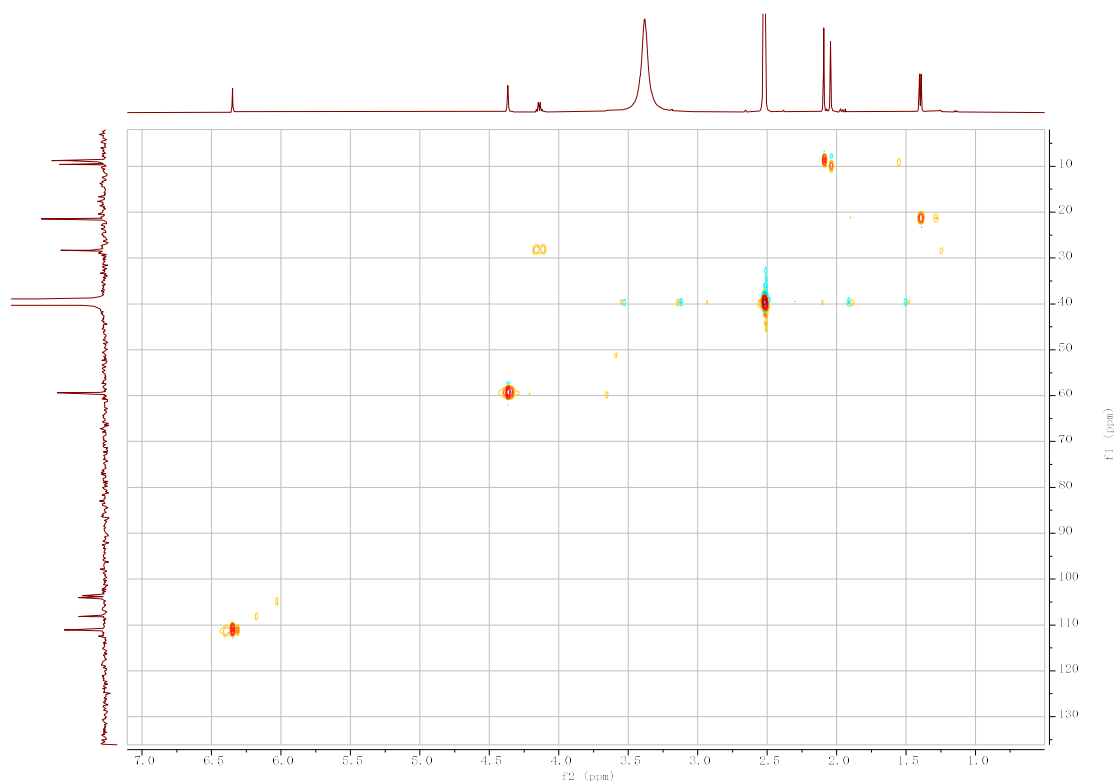
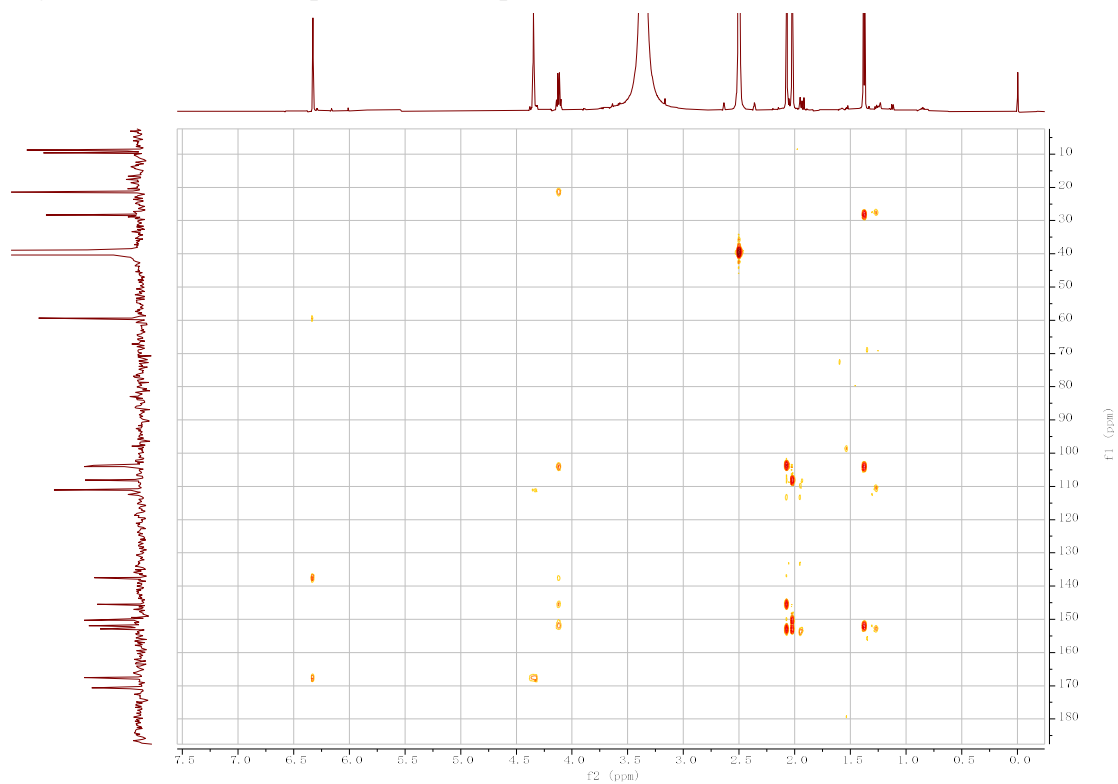


Figure S10. The <sup>13</sup>C NMR Spectrum of compound 2 in DMSO-*d*<sub>6</sub>.





**Figure S11. The HSQC Spectrum of compound 2 in DMSO- $d_6$ .**



**Figure S12. The HMBC Spectrum of compound 2 in DMSO- $d_6$ .**

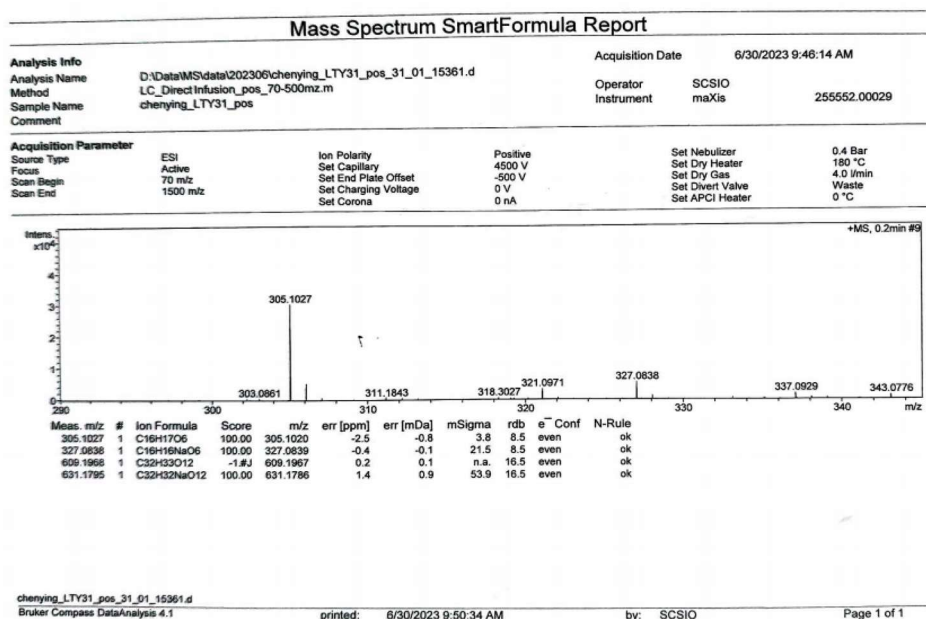


Figure S13. The HRESIMS spectrum of compound 2 in MeOH.

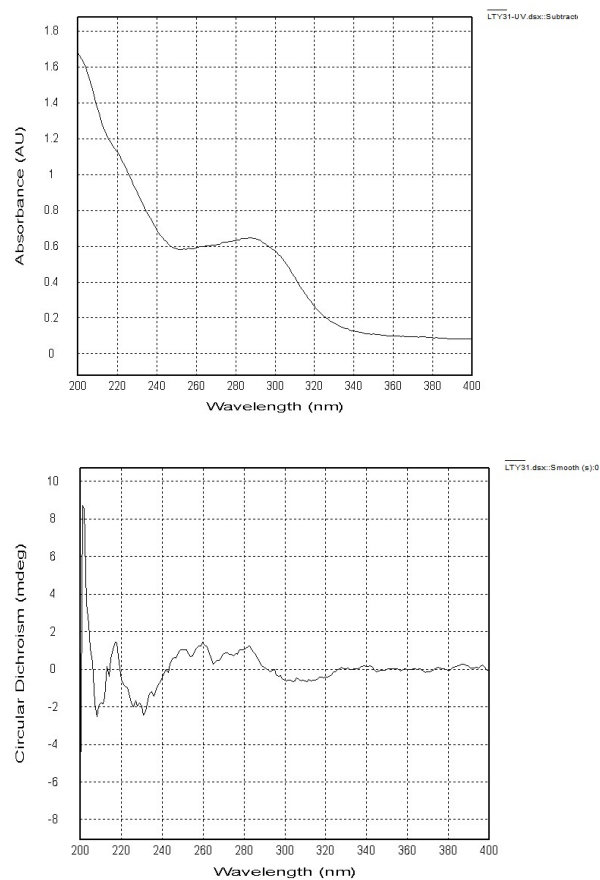


Figure S14. The UV and experimental ECD spectrum of compound 2.

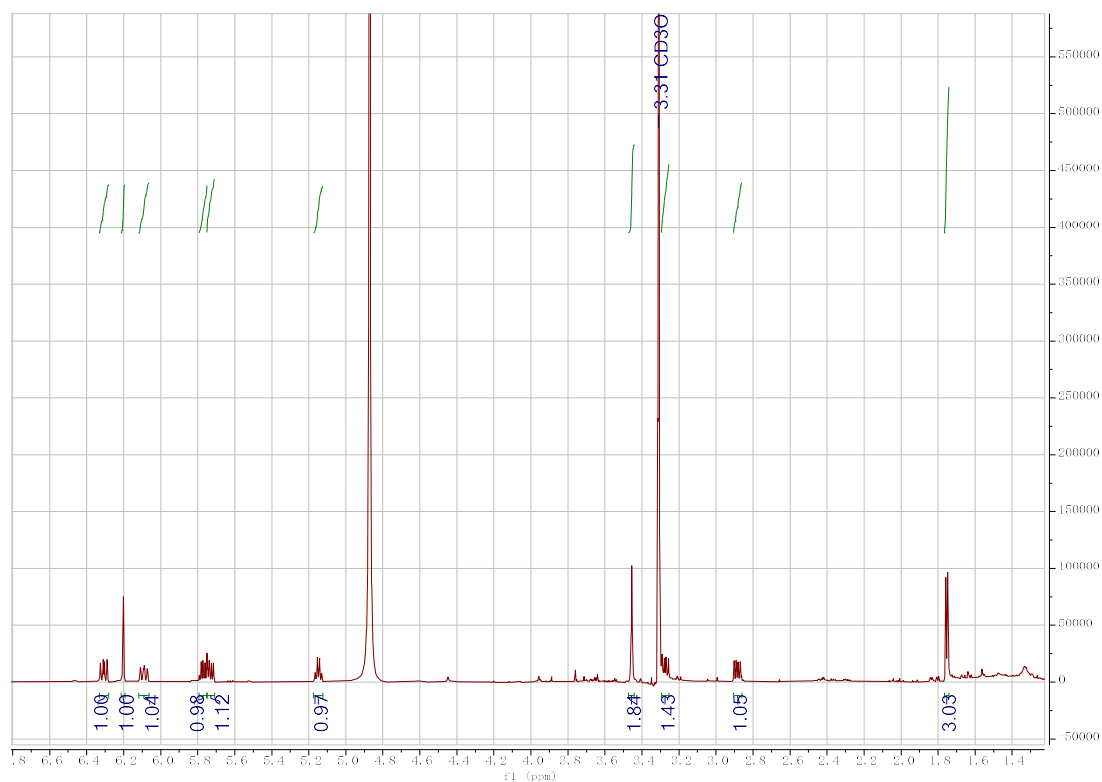


Figure S15. The <sup>1</sup>H NMR Spectrum of compound 3 in CD<sub>3</sub>OD.

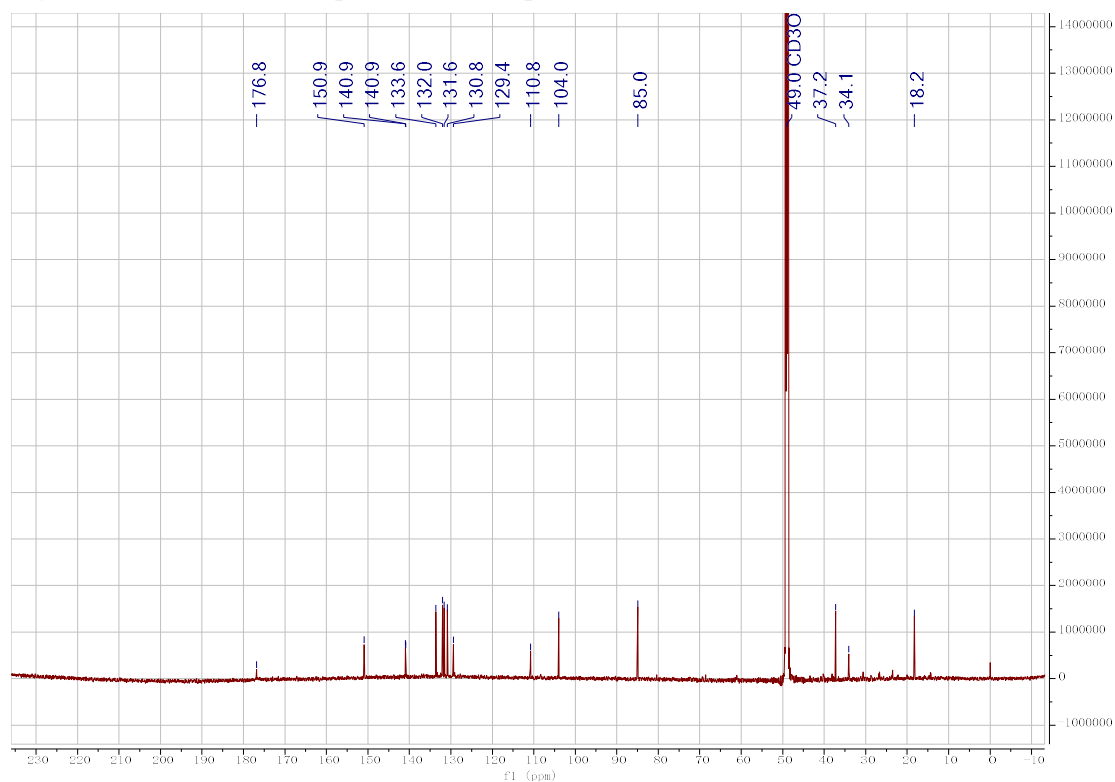
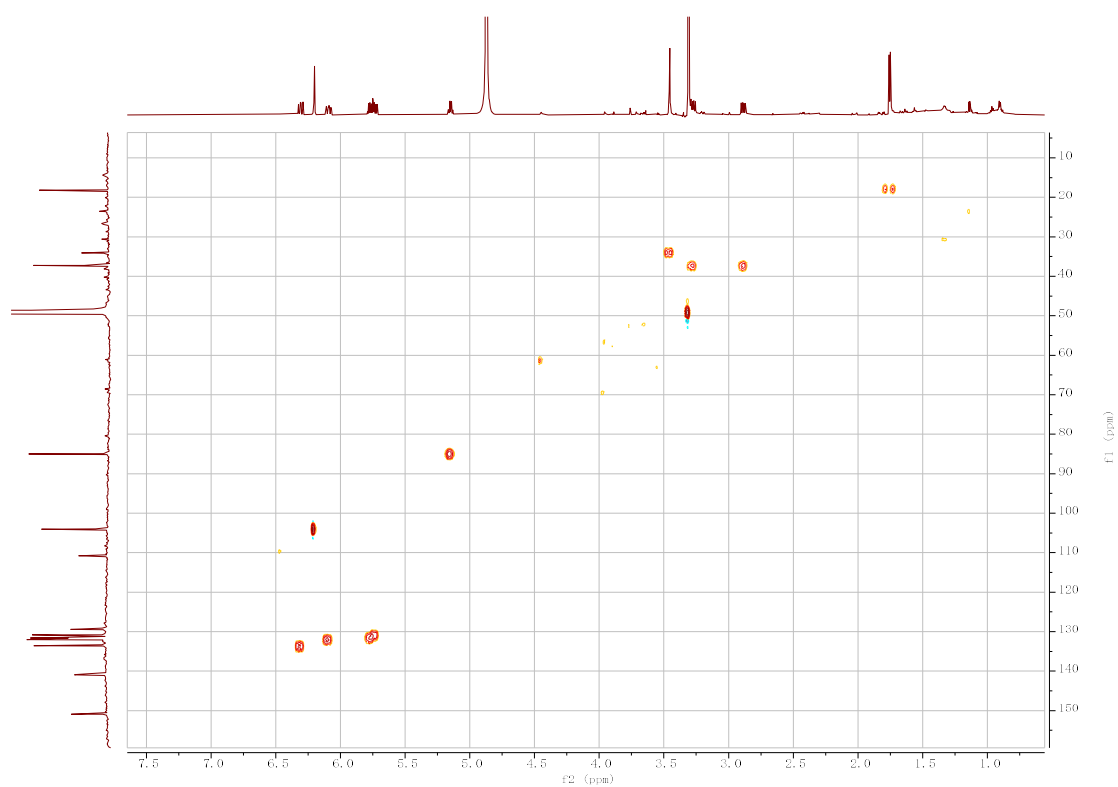
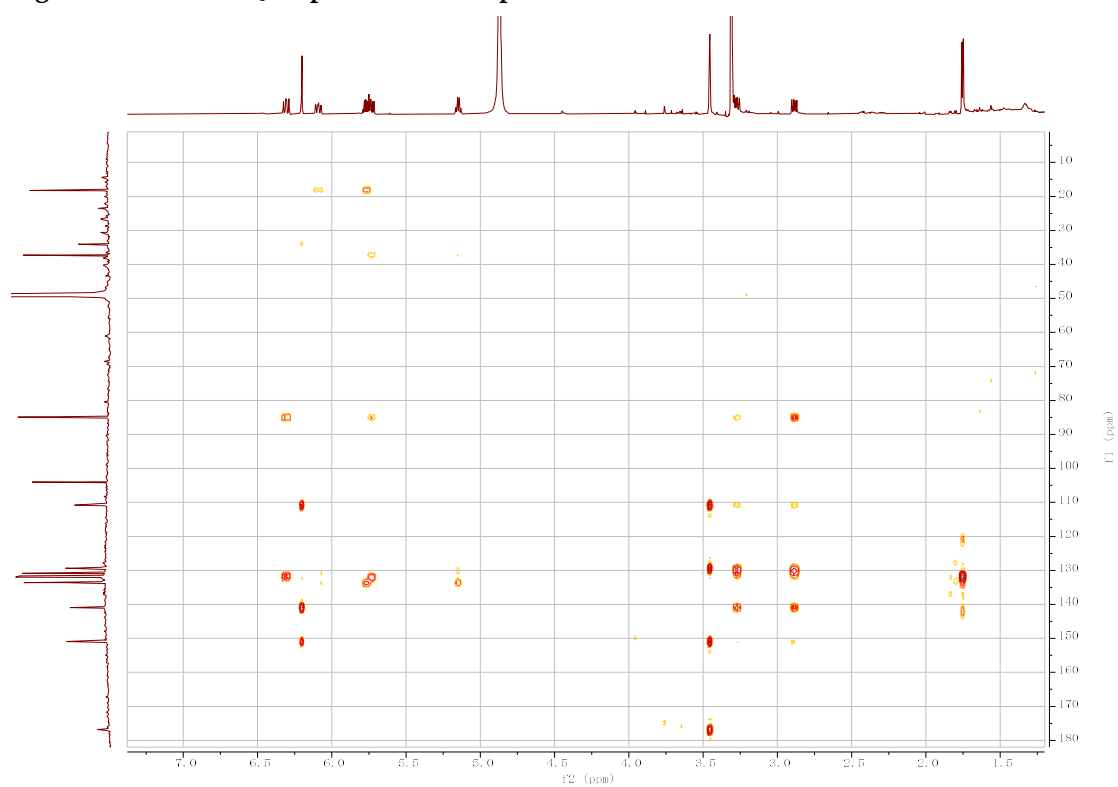


Figure S16. The <sup>13</sup>C NMR Spectrum of compound 3 in CD<sub>3</sub>OD.



**Figure S17. The HSQC Spectrum of compound 3 in CD<sub>3</sub>OD.**



**Figure S18. The HMBC Spectrum of compound 3 in CD<sub>3</sub>OD.**

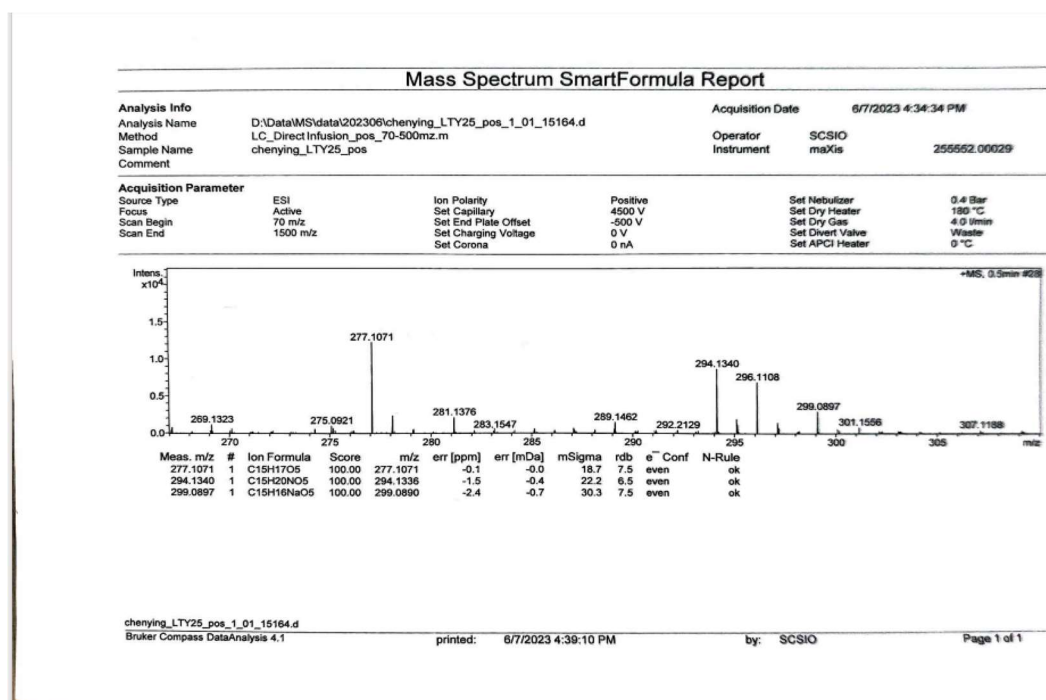


Figure S19. The HRESIMS spectrum of compound 3 in MeOH.

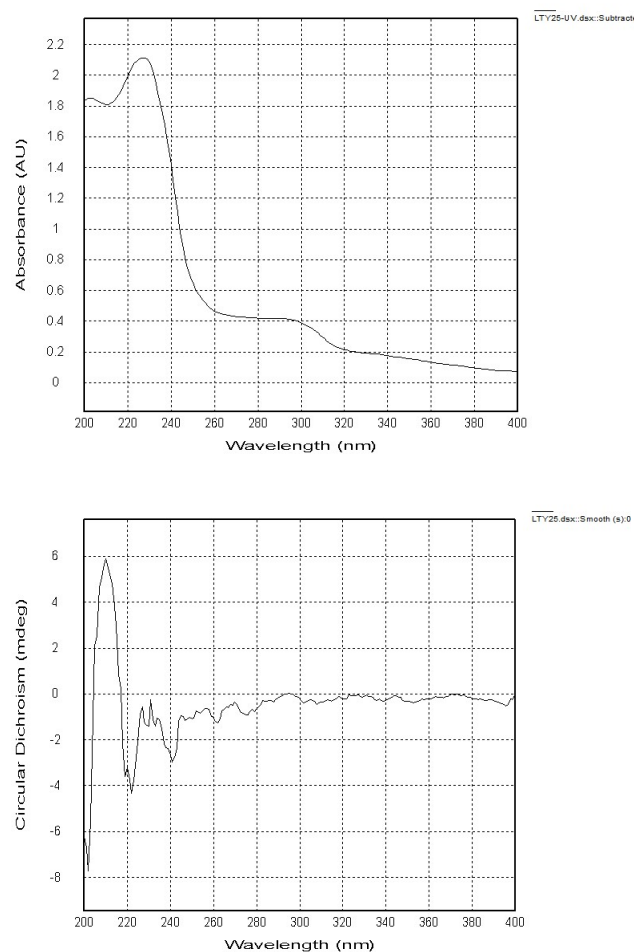


Figure S20. The UV and experimental ECD spectrum of compound 3.

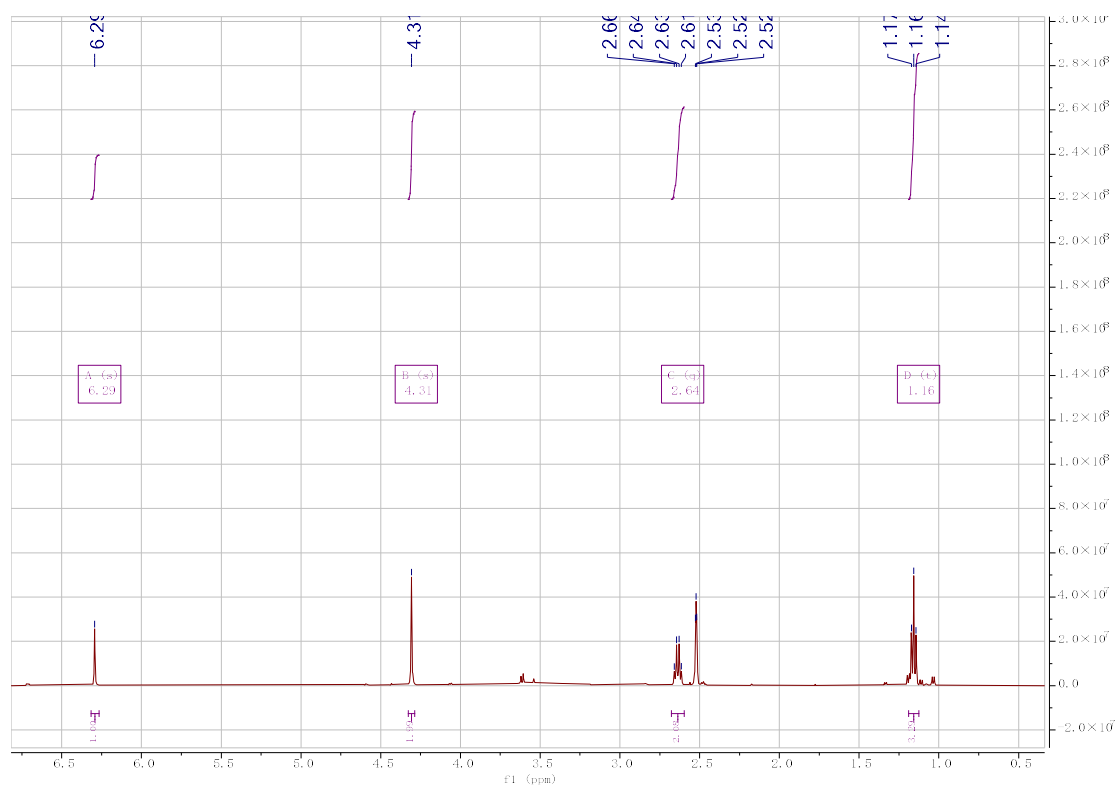


Figure S21. The  $^1\text{H}$  NMR Spectrum of compound 4 in  $\text{DMSO}-d_6$ .

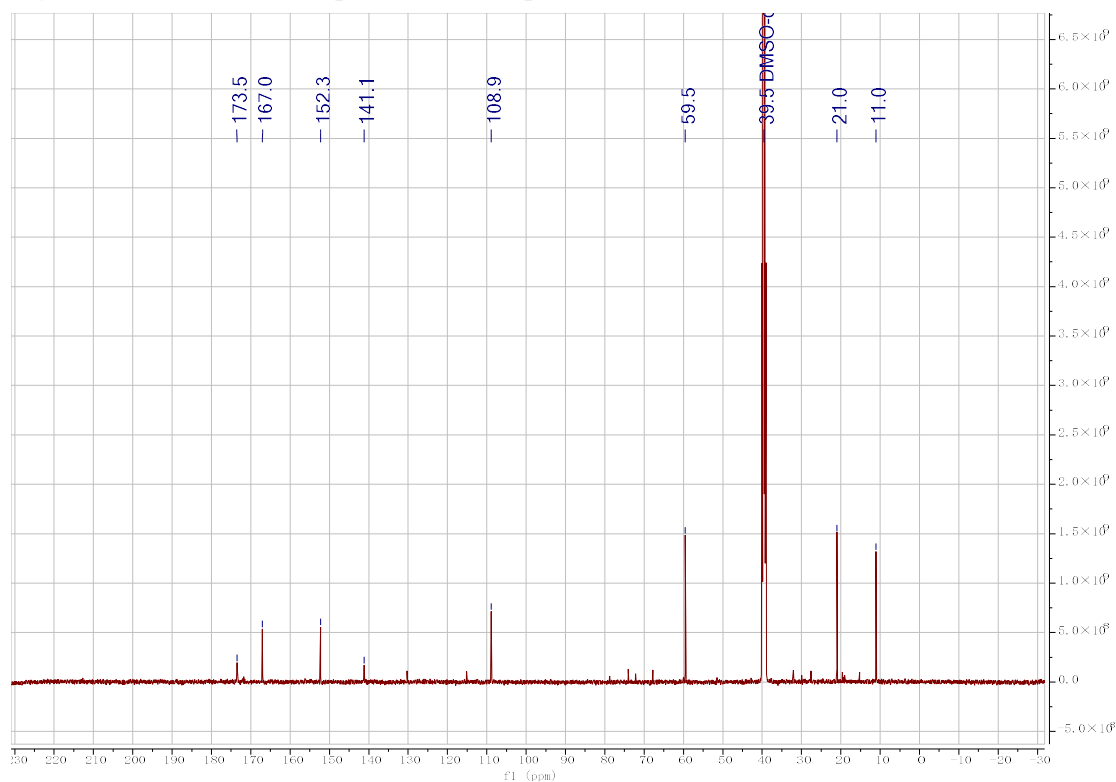
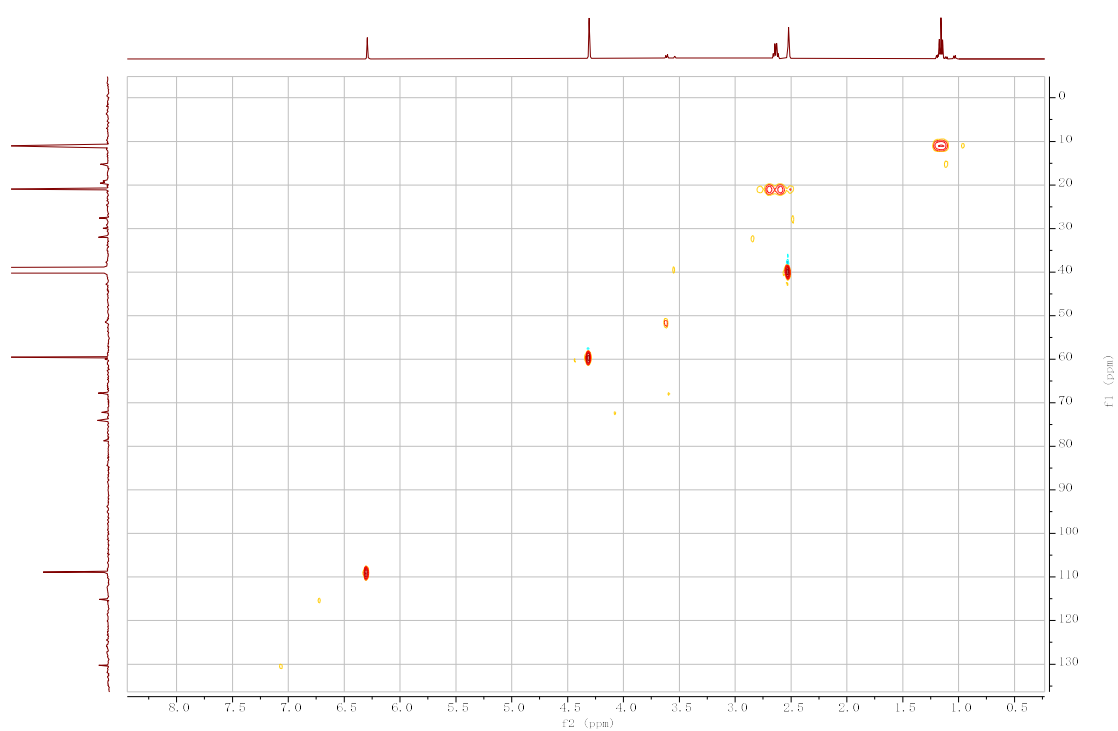
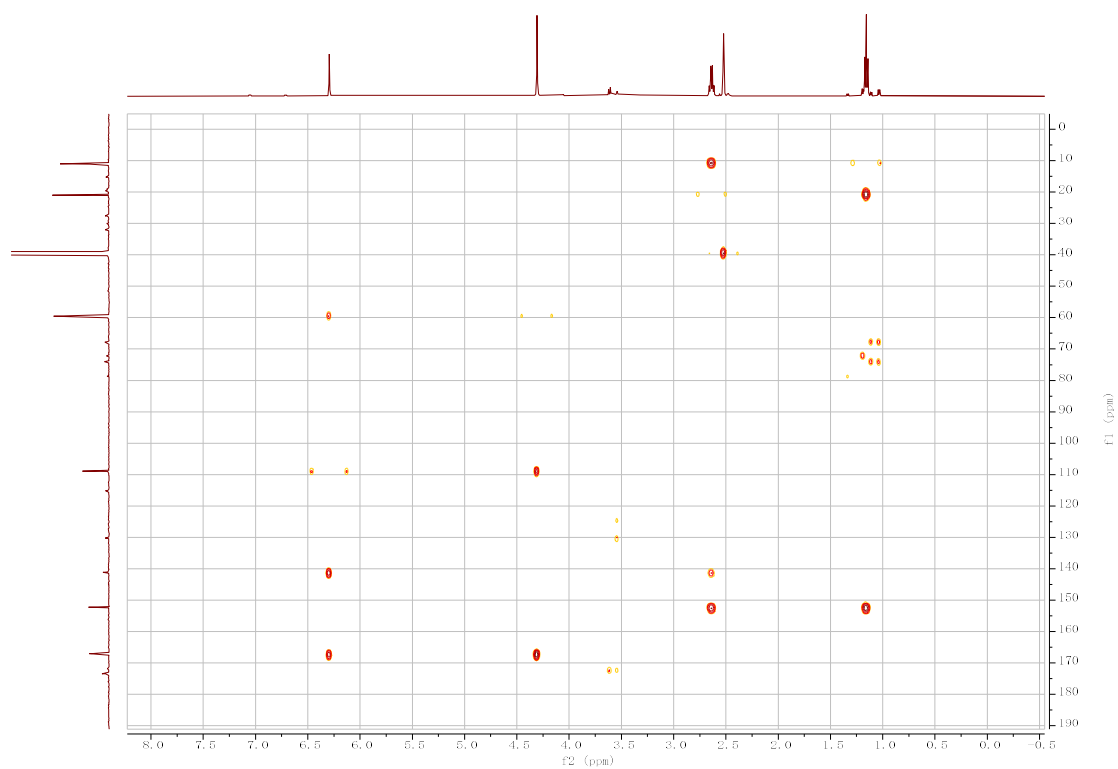


Figure S22. The  $^{13}\text{C}$  NMR Spectrum of compound 4 in  $\text{DMSO}-d_6$ .



**Figure S23. The HSQC Spectrum of compound 4 in DMSO-*d*<sub>6</sub>.**



**Figure S24. The HMBC Spectrum of compound 4 in DMSO-*d*<sub>6</sub>.**

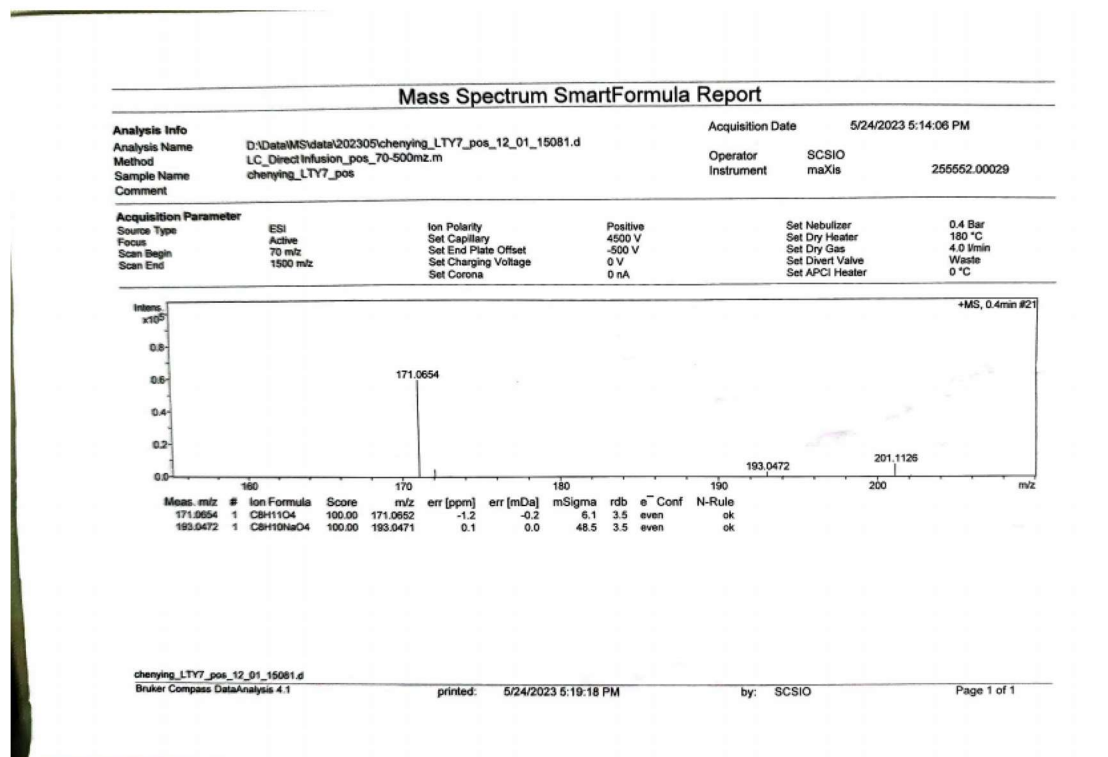


Figure S25. The HRESIMS spectrum of compound 4 in MeOH.

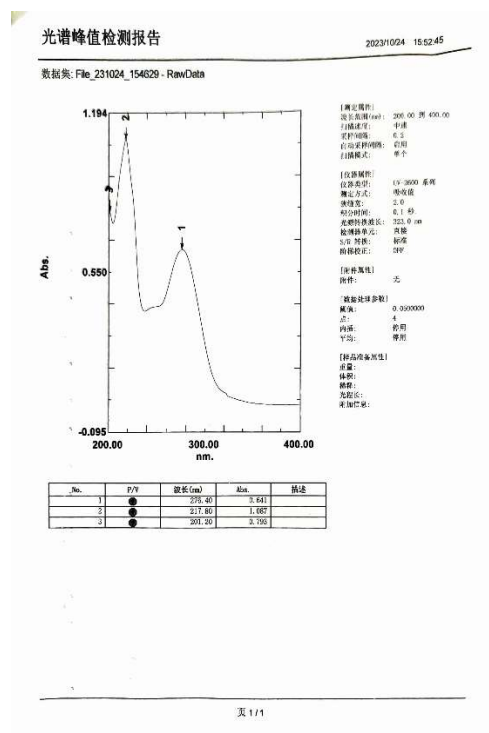


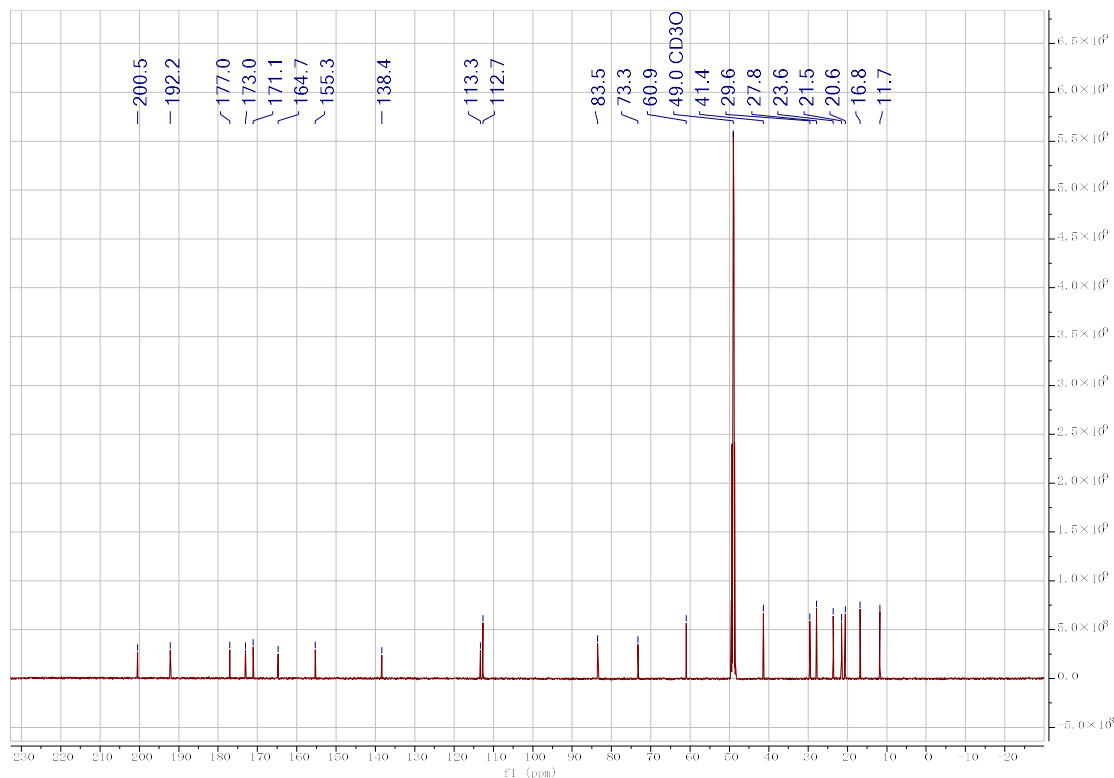
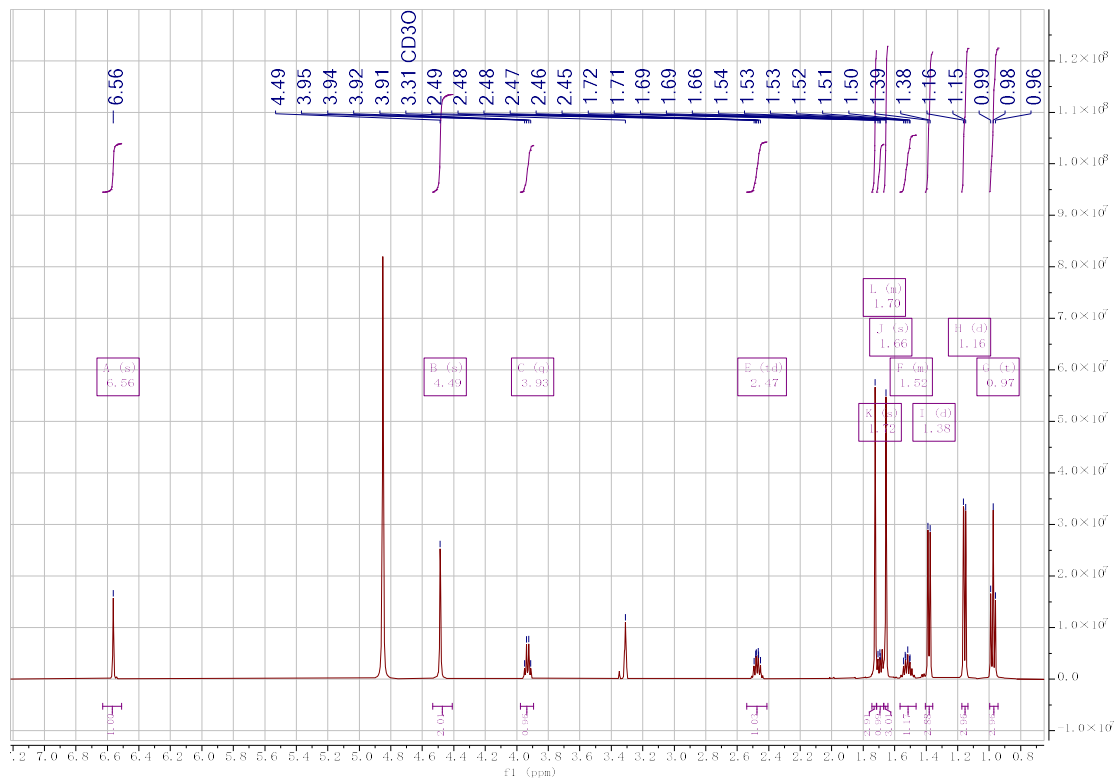
Figure S26. The UV spectrum of compound 4.

The physicochemical data of the known compounds 5~16.

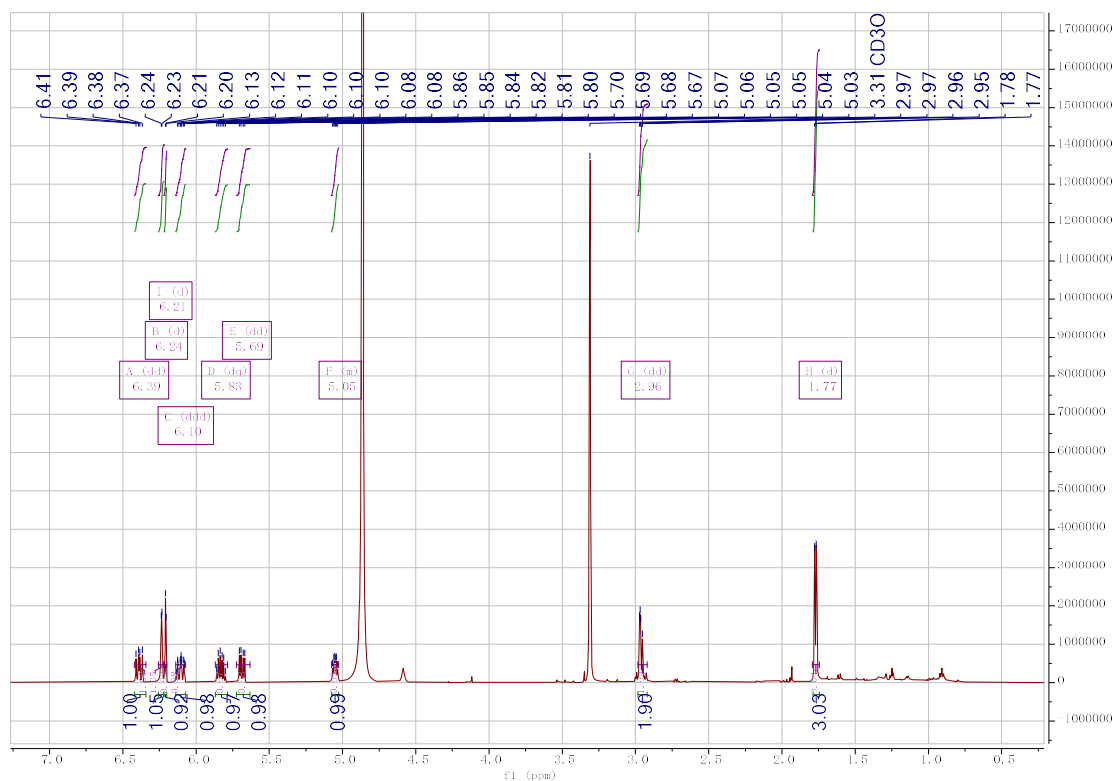
kojicone (5): brown oil;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm,  $J/\text{Hz}$ ):  $\delta_{\text{H}}$  4.49 (s, 1H, H-22), 2.47 (m, 1H, H-16), 3.93 (q,  $J = 6.7$  Hz, 1H, H-13), 1.79 (m, 1H, H-17), 1.52 (m, 1H, H-17), 1.66 (s,



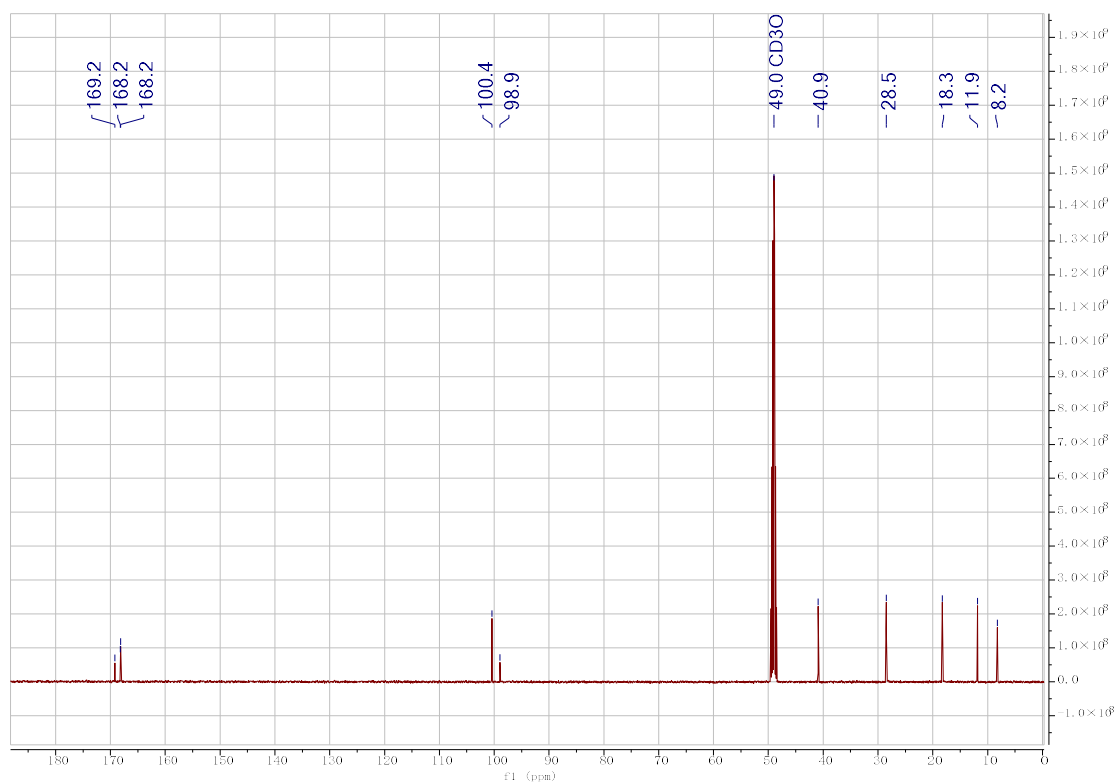
3H, H-20), 1.72 (s, 3H, H-23), 1.38 (d,  $J = 6.7$  Hz, 3H, H-21), 1.16 (d,  $J = 7.0$  Hz, 3H, H-19), 0.97 (t,  $J = 7.4$  Hz, 3H, H-18).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm,  $J/\text{Hz}$ ): 200.5 (C, C-9), 192.2 (C, C-11), 177.0 (C, C-15), 173.0 (C, C-4), 171.1 (C, C-2), 164.7 (C, C-7), 155.3 (C, C-14), 138.4 (C, C-5), 113.3 (C, C-3), 112.7 (C, C-12), 83.5 (C, C-10), 73.3 (C, C-8), 60.9 ( $\text{CH}_2$ , C-22), 41.4 ( $\text{CH}$ , C-16), 29.6 ( $\text{CH}$ , C-13), 27.8 ( $\text{CH}_2$ , C-17), 23.6 ( $\text{CH}_3$ , C-20), 21.5 ( $\text{CH}_3$ , C-23), 20.6 ( $\text{CH}_3$ , C-21), 16.8 ( $\text{CH}_3$ , C-19), 11.7 ( $\text{CH}_3$ , C-18).



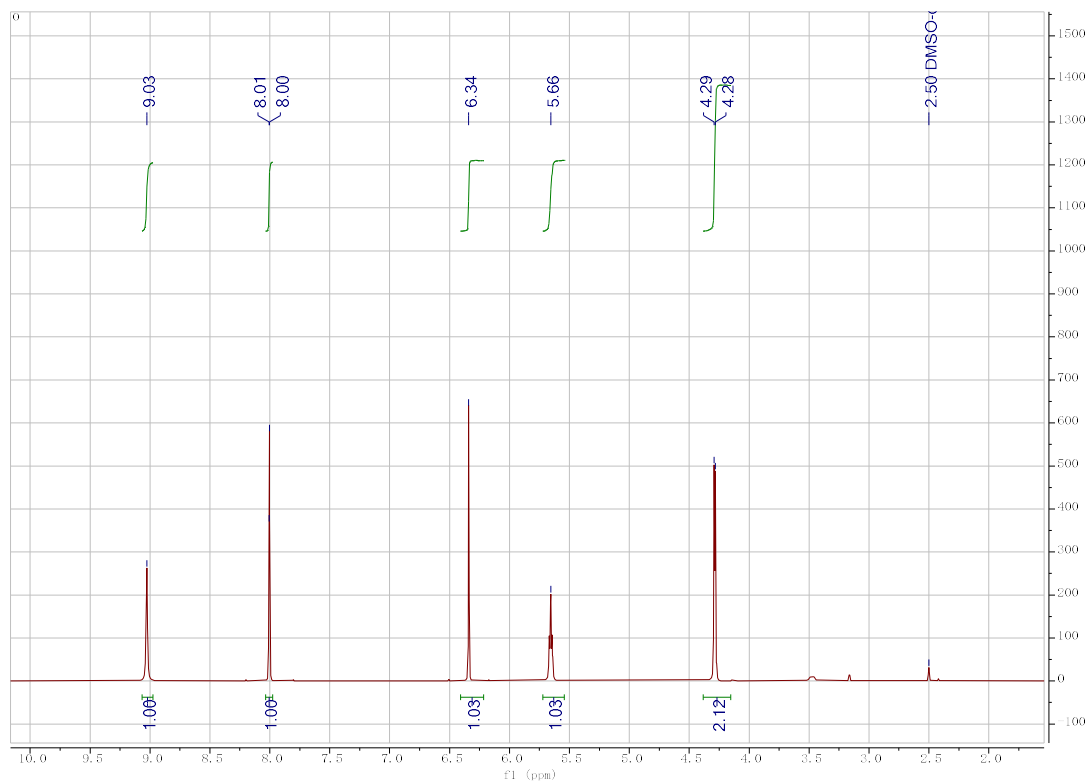
6,8-dihydroxy-3-((1E,3E)-penta-1,3-dien-1-yl)isochroman-1-one (**6**): brown oil;  $^1\text{H}$  NMR (600 MHz, Methanol- $d_4$ )  $\delta$  6.39 (dd,  $J = 15.3, 10.4$  Hz, 1H, H-10), 6.24 (d,  $J = 2.2$  Hz, 1H, H-5), 6.21 (d,  $J = 2.2$  Hz, 1H, H-7), 6.10 (ddd,  $J = 14.9, 10.5, 2.0$  Hz, 1H, H-11), 5.83 (dq,  $J = 13.9, 6.7$  Hz, 1H, H-12), 5.69 (dd,  $J = 15.3, 6.8$  Hz, 1H, H-9), 5.05 (m, 1H, H-3), 2.96 (dd,  $J = 7.0, 4.9$  Hz, 2H, H-4), 1.77 (d,  $J = 6.8$  Hz, 3H, H-13).  $^{13}\text{C}$  NMR (126 MHz, MeOD)  $\delta$  171.4 (C, C-1), 166.4 (C, C-6), 165.6 (C, C-8), 143.1 (C, C-4a), 135.4 (CH, C-10), 133.1 (CH, C-12), 131.6 (CH, C-11), 127.6 (CH, C-9), 108.0 (CH, C-5), 102.3 (CH, C-7), 101.6 (C, C-8a), 80.7 (CH, C-3), 34.4 (CH<sub>2</sub>, C-4), 18.2 (CH<sub>3</sub>, C-13).

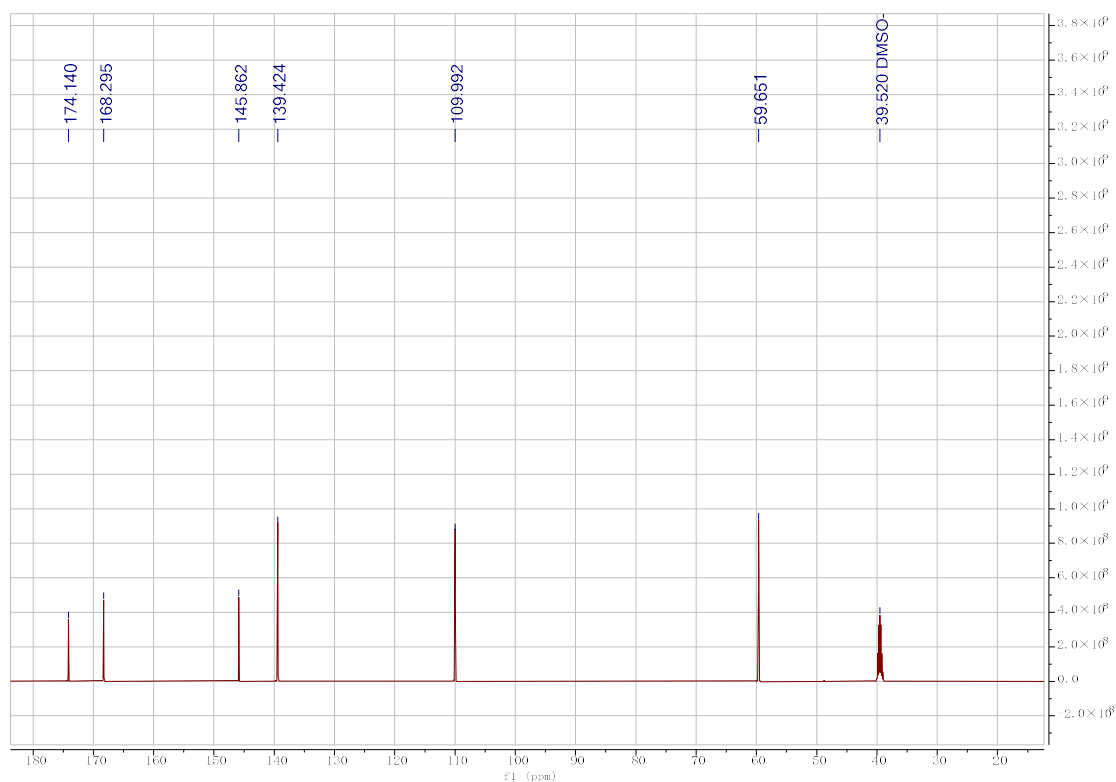




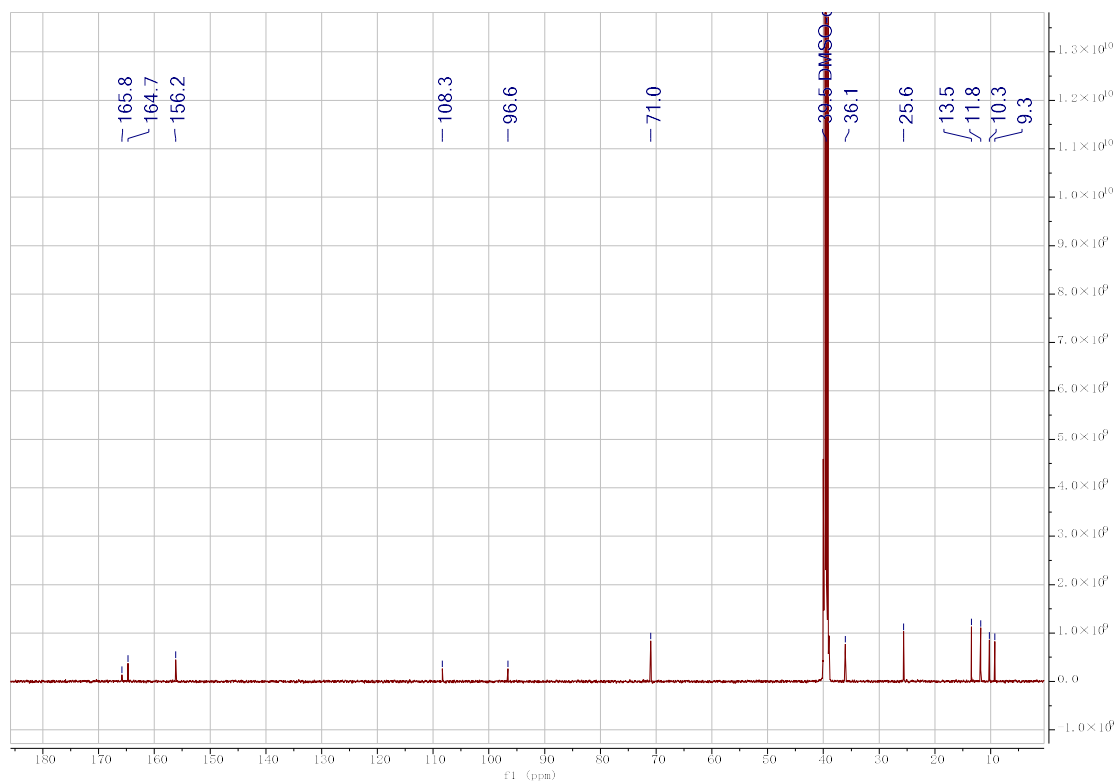
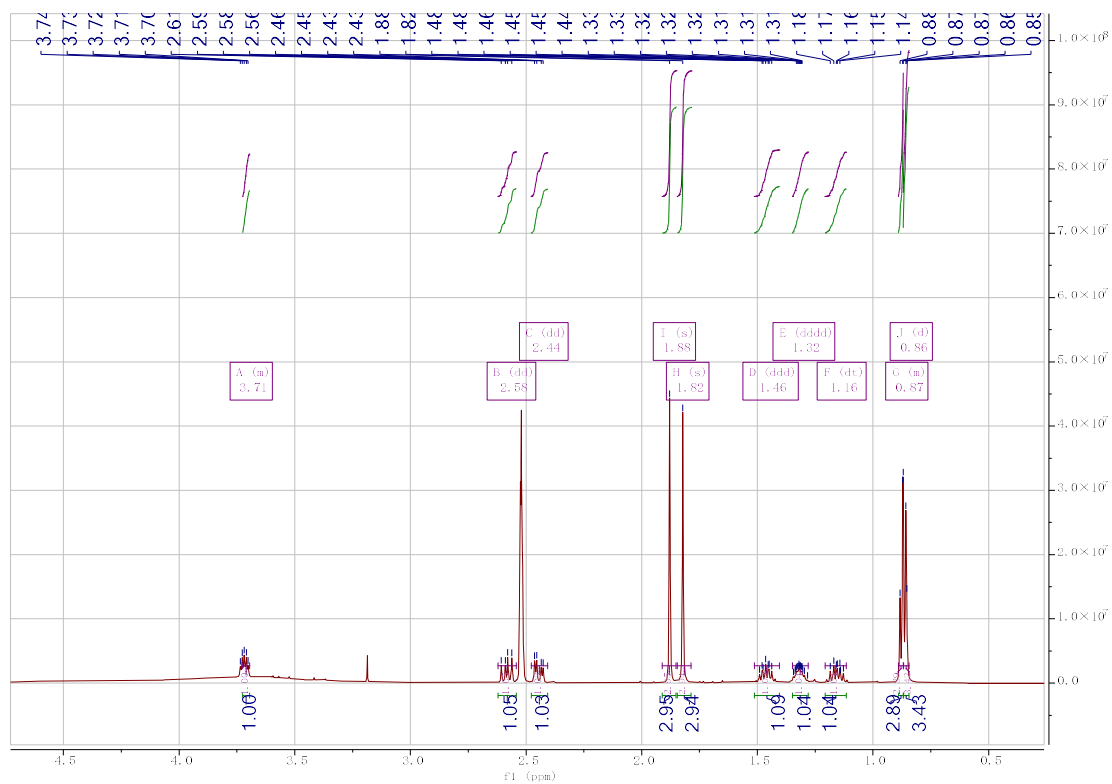


5-hydroxy-2-hydroxymethyl-4H-pyran-4-one (8): yellow solid; <sup>1</sup>H NMR (500 MHz, DMSO,  $\delta$ , ppm,  $J$ /Hz):  $\delta_{\text{H}}$  8.00 (s, 1H, H-6), 6.34 (s, 1H, H-3), 4.29 (d,  $J$  = 5.5 Hz, 2H, H-1), 9.03 (s, 1H, 5-OH), 5.66 (s, 1H, 1-OH). <sup>13</sup>C NMR (125 MHz, DMSO,  $\delta$ , ppm,  $J$ /Hz): 174.1 (C, C-4), 168.3 (C, C-2), 145.9 (C, C-5), 139.6 (CH, C-6), 110.0 (CH, C-3), 59.7 (CH<sub>2</sub>, C-1).

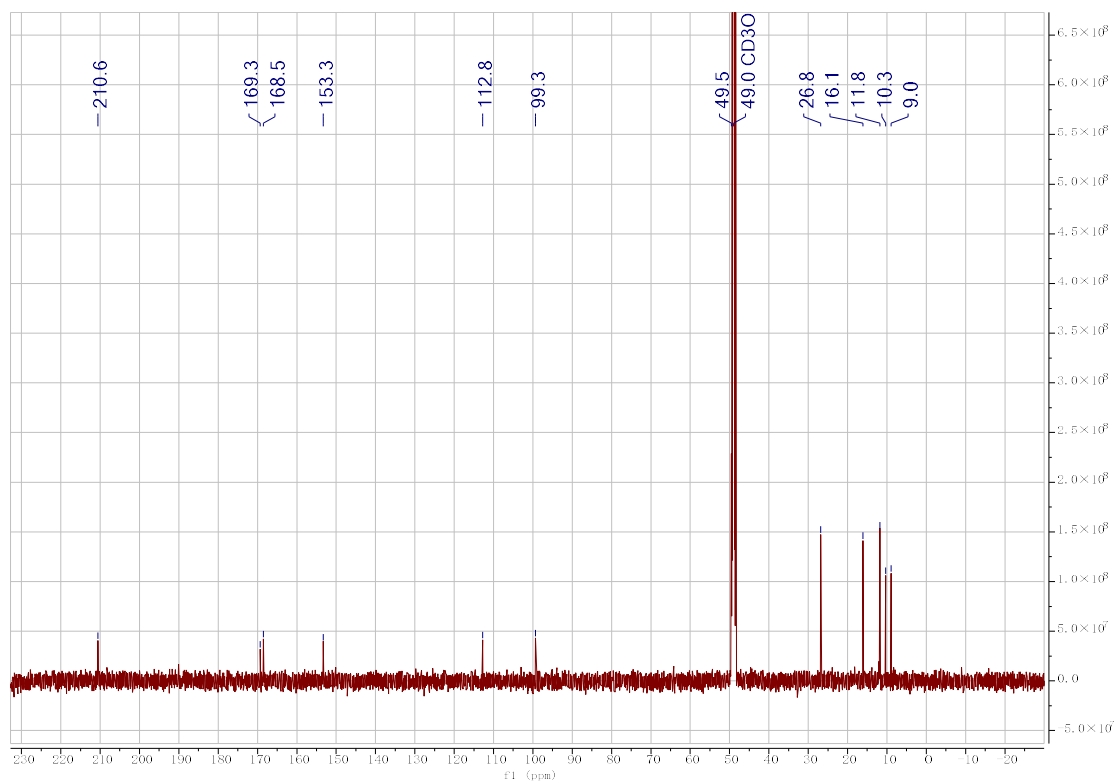
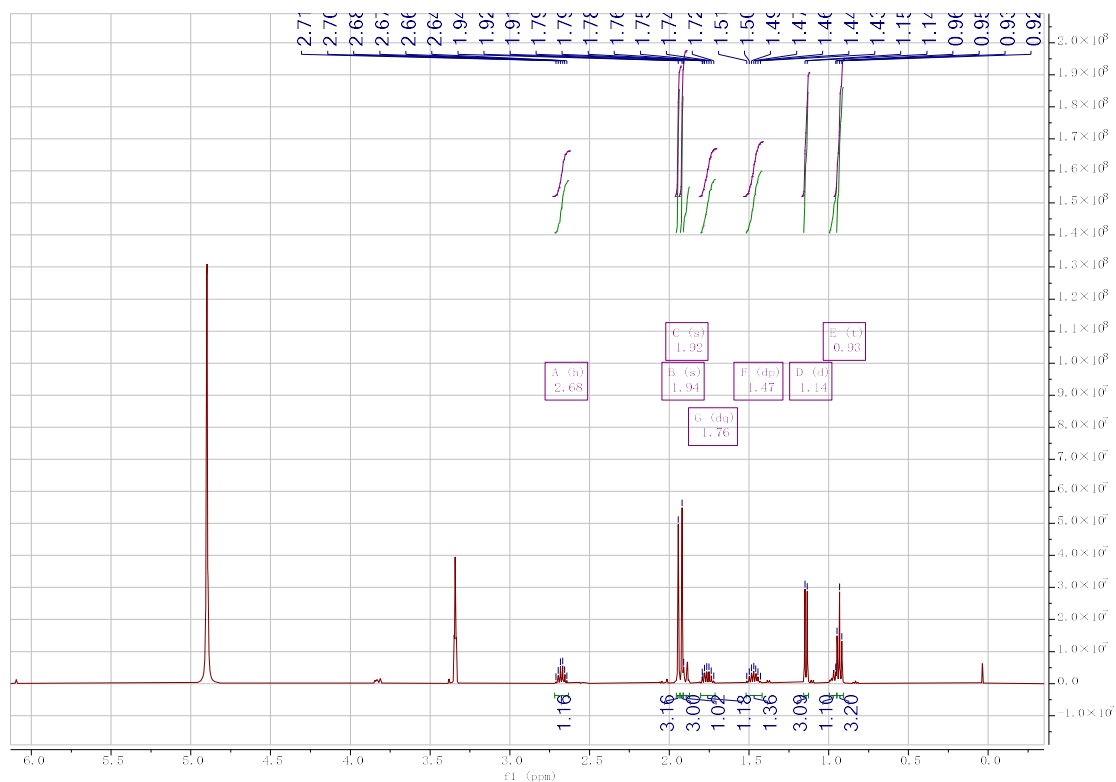




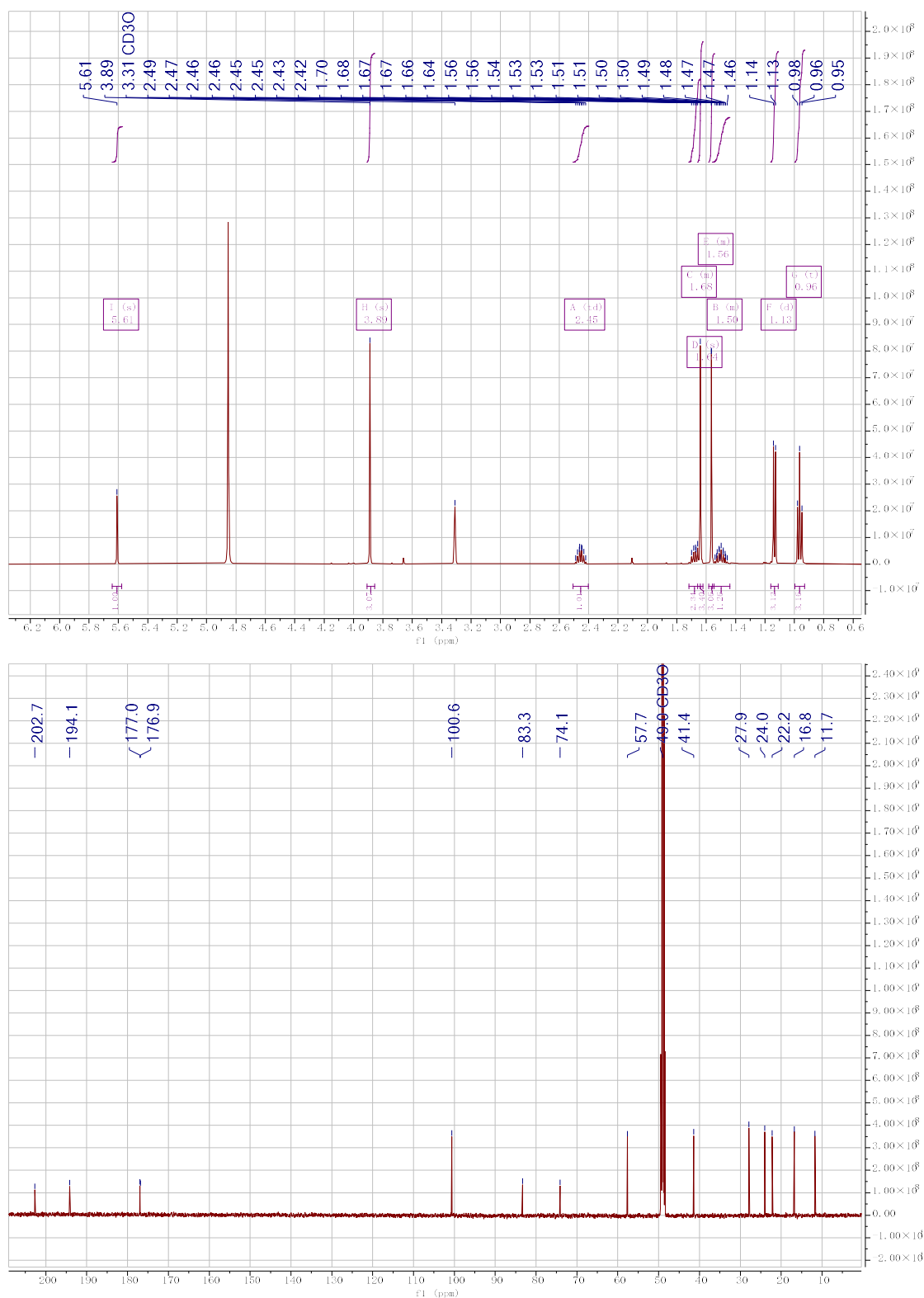
sydowione B (**9**): brown oil;  $^1\text{H}$  NMR (500 MHz, DMSO,  $\delta$ , ppm,  $J/\text{Hz}$ ):  $\delta_{\text{H}}$  3.71 (m, 1H, H-8), 2.58 (dd,  $J = 14.0, 8.8$  Hz 1H, H-7), 2.44 (dd,  $J = 14.1, 4.5$  Hz 1H, H-7), 1.32 (m, 1H, H-9), 1.46 (ddd,  $J = 13.1, 7.5, 5.5$  Hz 1H, H-10), 1.16 (dt,  $J = 13.1, 7.6$  Hz 1H, H-10), 0.87 (m, 3H, H-12), 0.86 (m, 3H, H-13), 1.88 (s, 3H, H-12), 1.82 (s, 3H, H-13).  $^{13}\text{C}$  NMR (125 MHz, DMSO,  $\delta$ , ppm,  $J/\text{Hz}$ ): 165.8 (C, C-5), 164.7 (C, C-4), 156.2 (C, C-6), 108.3 (C, C-5), 96.6 (C, C-3), 71.0 (CH, C-8), 39.0 (CH, C-9), 36.1 (CH<sub>2</sub>, C-7), 25.6 (CH<sub>2</sub>, C-10), 13.5 (CH<sub>3</sub>, C-12), 11.8 (CH<sub>3</sub>, C-11), 10.3 (CH<sub>3</sub>, C-13), 9.3 (CH<sub>3</sub>, C-14).



paecilpyrone A (**10**): brown oil;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm,  $J/\text{Hz}$ ):  $\delta_{\text{H}}$  2.68 (q,  $J = 6.9$  Hz 1H, H-9), 1.76 (dq,  $J = 14.5, 7.2$  Hz, 1H, H-10), 1.47 (dp,  $J = 14.2, 7.3$  Hz, 1H, H-10), 1.14 (d,  $J = 6.9$  Hz, 3H, H-12), 0.93 (t,  $J = 7.4$  Hz 3H, H-11), 1.92 (s, 3H, H-14), 1.94 (s, 3H, H-13).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm,  $J/\text{Hz}$ ): 210.6 (C, C-8), 169.3 (C, C-5), 168.5 (C, C-3), 153.3 (C, C-1), 112.8 (C, C-6), 99.3 (C, C-4), 49.5 (CH, C-9), 26.8 ( $\text{CH}_2$ , C-10), 16.1 ( $\text{CH}_3$ , C-12), 10.3 ( $\text{CH}_3$ , C-14), 9.0 ( $\text{CH}_3$ , C-13).

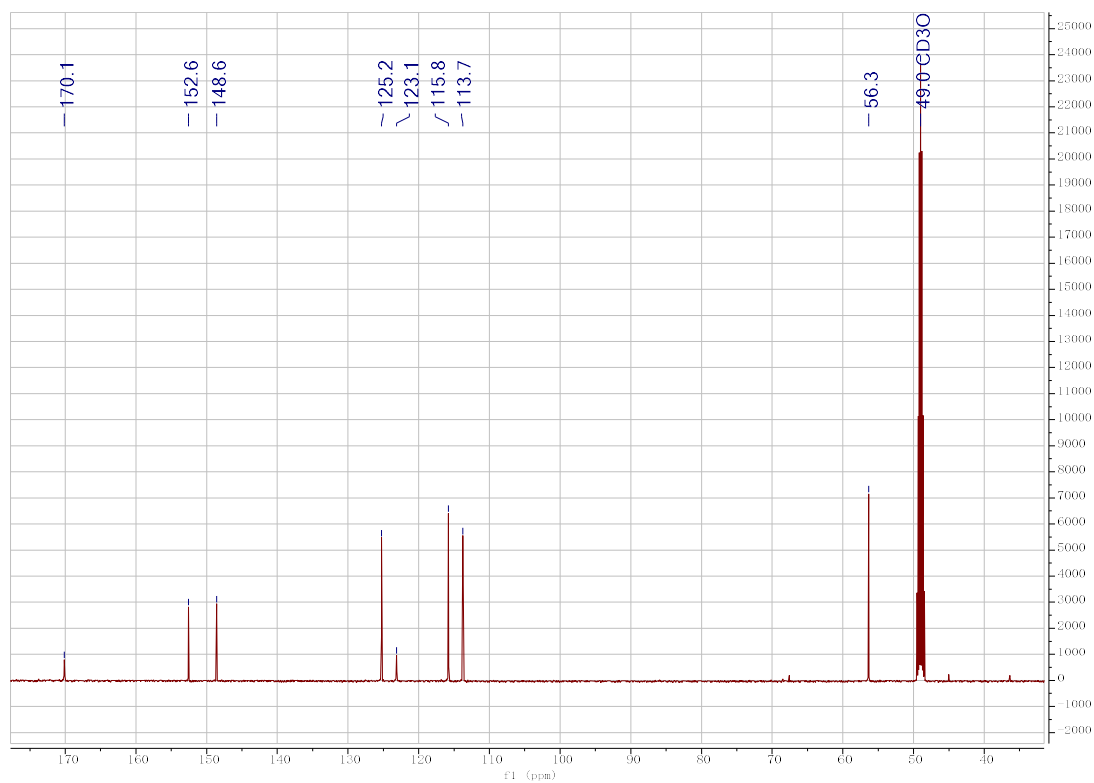
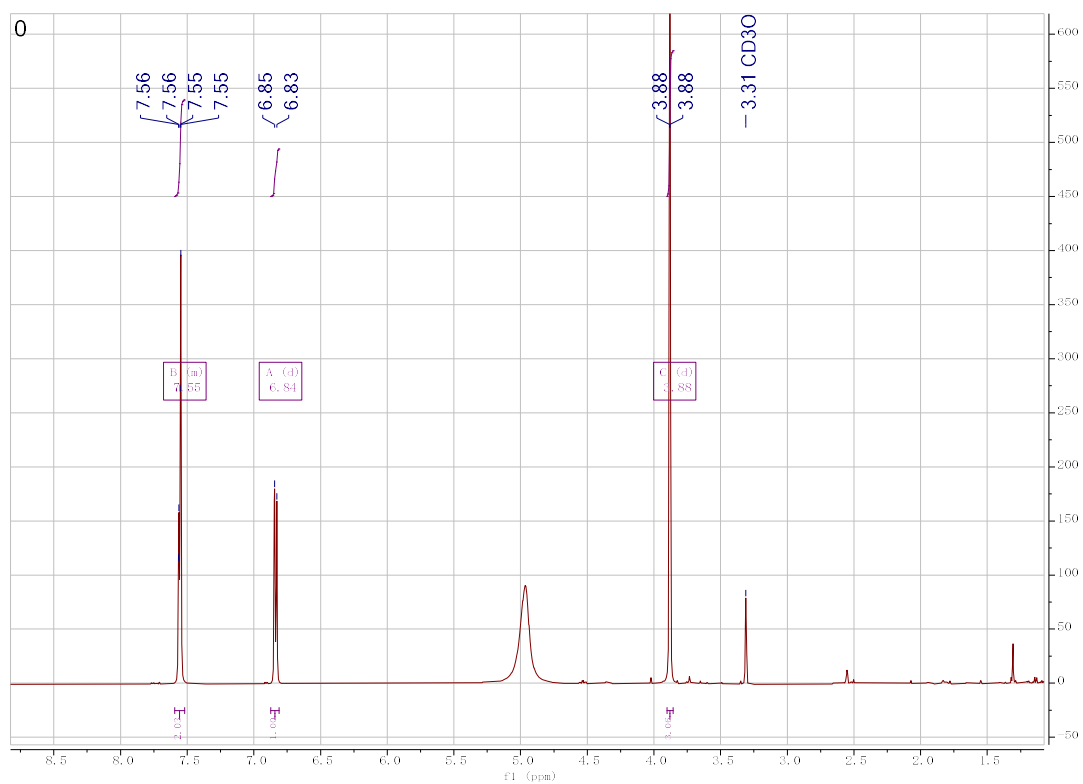


phomaligol A (**11**): yellow oil;  $^1\text{H}$  NMR (500 MHz, Methanol- $d_4$ )  $\delta$  5.61 (s, 1H, H-4), 3.89 (s, 3H, H-12), 2.45 (m, 1H, H-8), 1.70 (m, 1H, H-9), 1.64 (s, 3H, H-14), 1.56 (s, 3H, H-13), 1.50 (m, 1H, H-9), 1.13 (d,  $J = 7.0$  Hz, 3H, H-11), 0.96 (t,  $J = 7.5$  Hz, 3H, H-10).  $^{13}\text{C}$  NMR (126 MHz, MeOD)  $\delta$  202.7 (C, C-1), 194.1 (C, C-5), 177.0 (C, C-7), 176.9 (C, C-3), 100.6 (CH, C-4), 83.3 (C, C-6) 74.1 (C, C-2), 57.7 (CH<sub>3</sub>, C-12), 41.4 (CH, C-8), 27.9 (CH<sub>2</sub>, C-9), 24.0 (CH<sub>3</sub>, C-13), 22.2 (CH<sub>3</sub>, C-14), 16.8 (CH<sub>3</sub>, C-11), 11.7 (CH<sub>3</sub>, C-10).

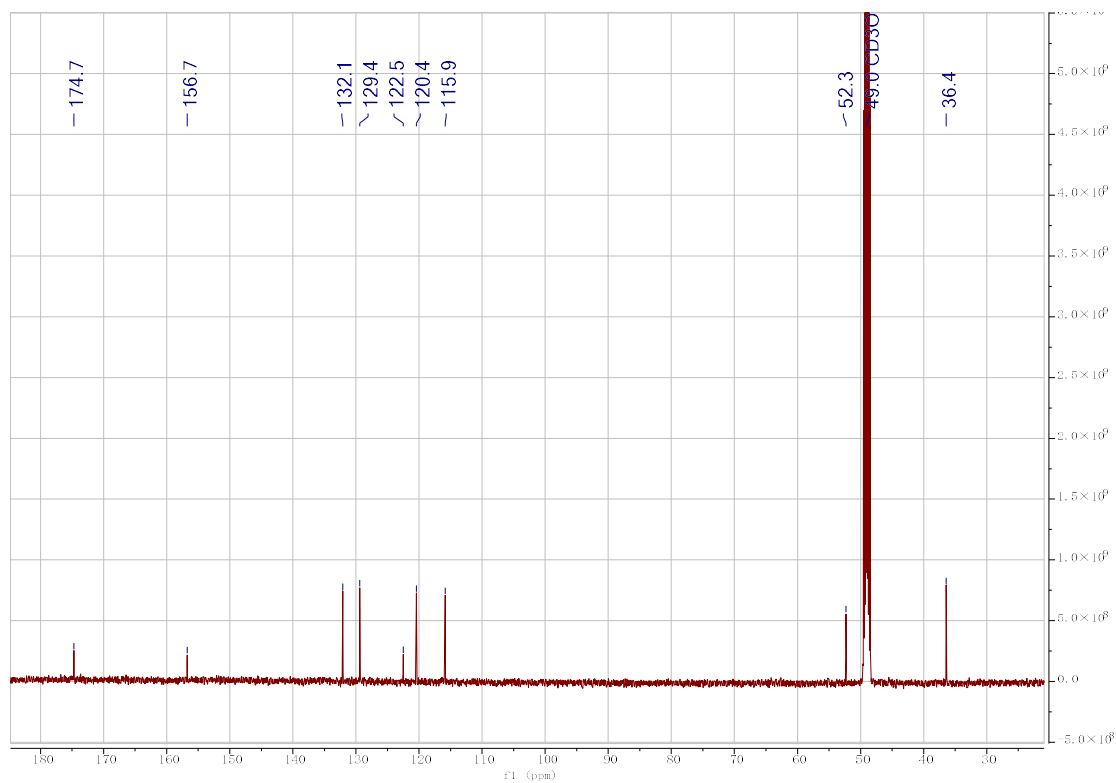
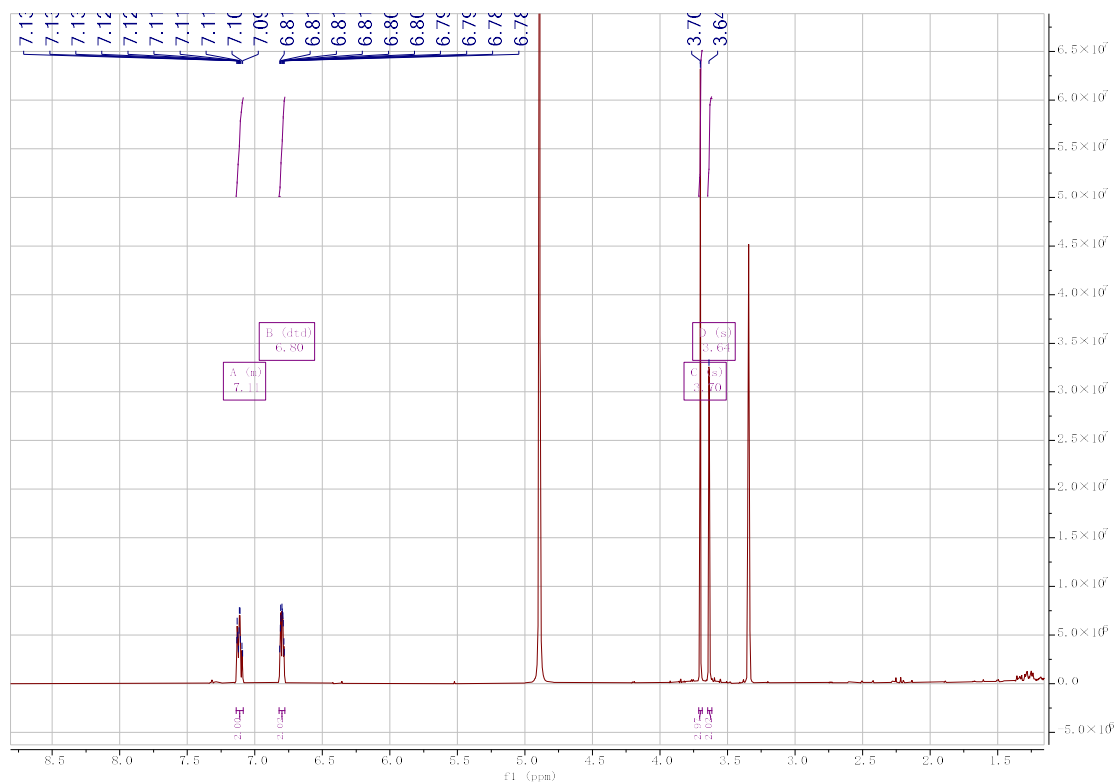


vanillic acid (**12**): white powder; <sup>1</sup>H NMR (500 MHz, Methanol-*d*<sub>4</sub>) δ 7.55 (m, 2H, H-2, H-6), 6.84 (d, *J* = 8.6 Hz, 1H, H-5), 3.88 (d, *J* = 1.5 Hz, 3H, H-8). <sup>13</sup>C NMR (126 MHz, MeOD) δ 170.1 (C, C-7), 152.6 (C, C-4), 148.6 (C, C-3), 125.2 (CH, C-6), 123.1 (C, C-1), 115.8 (CH, C-5), 113.7 (CH, C-2), 56.3 (CH<sub>3</sub>, C-8).

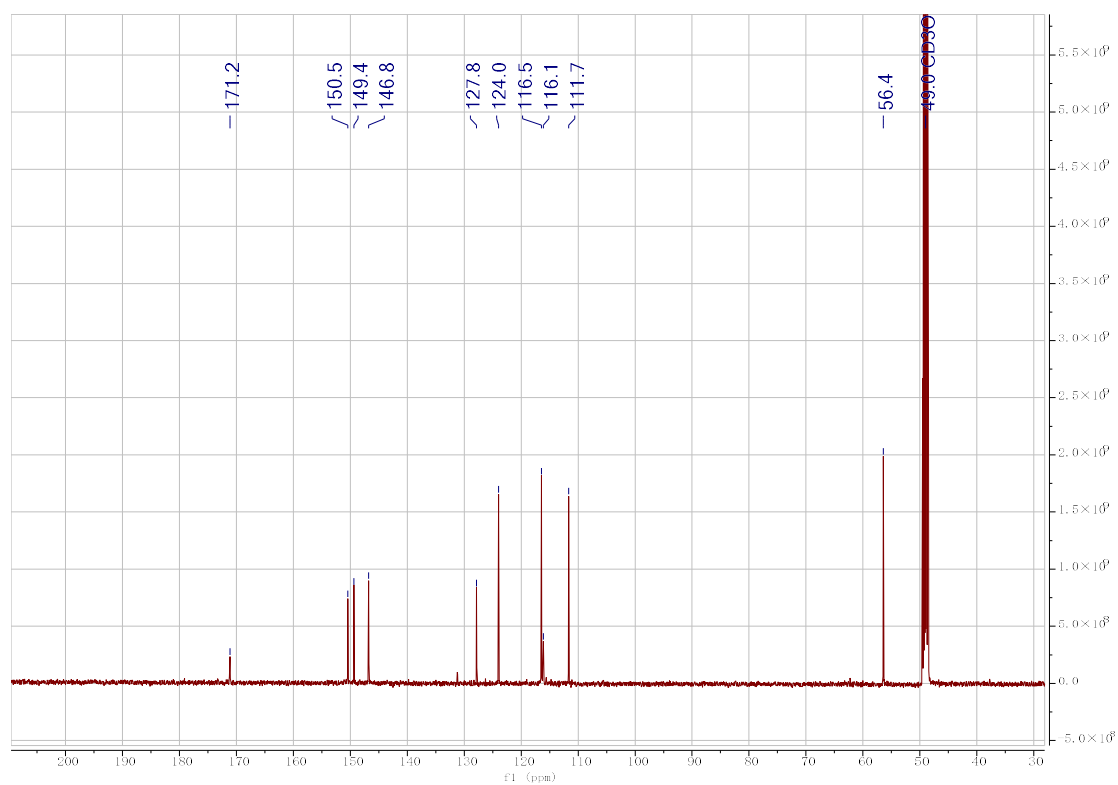
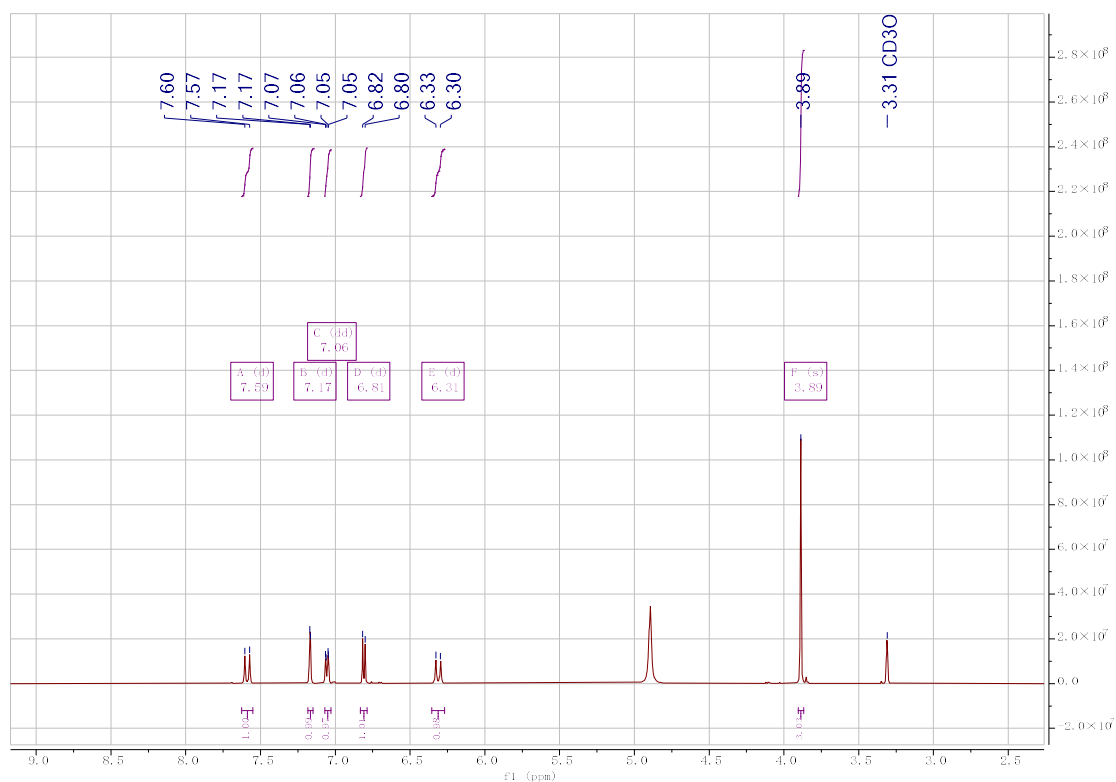




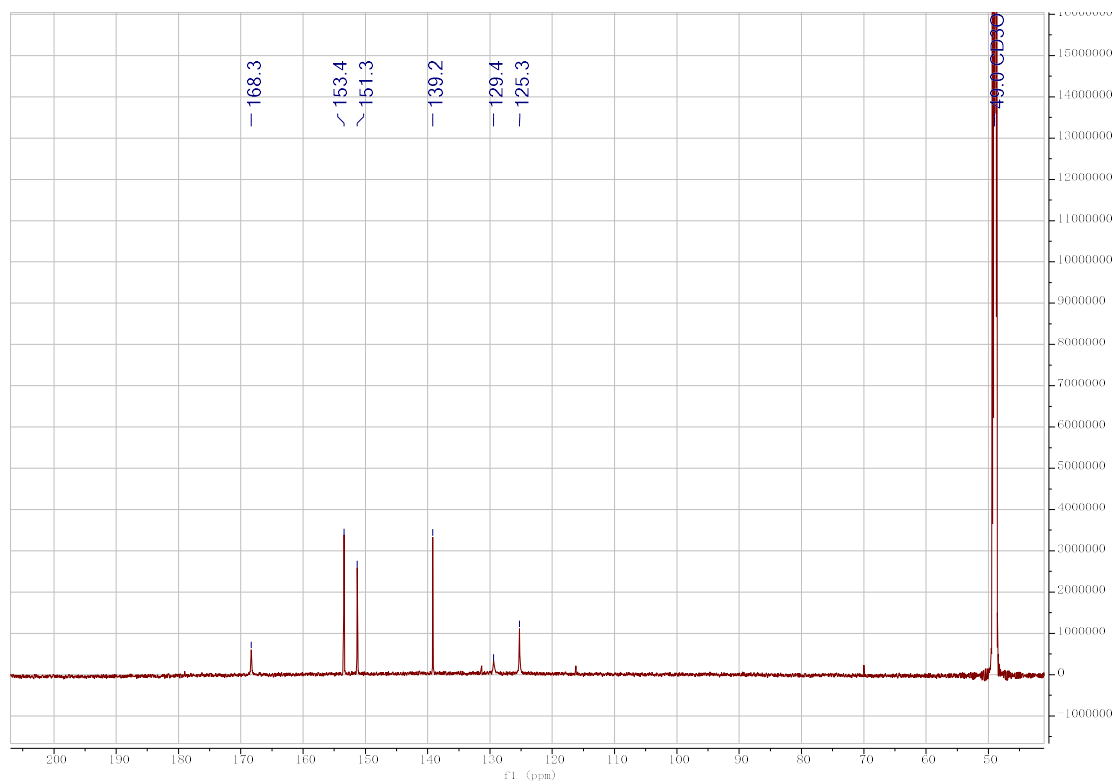
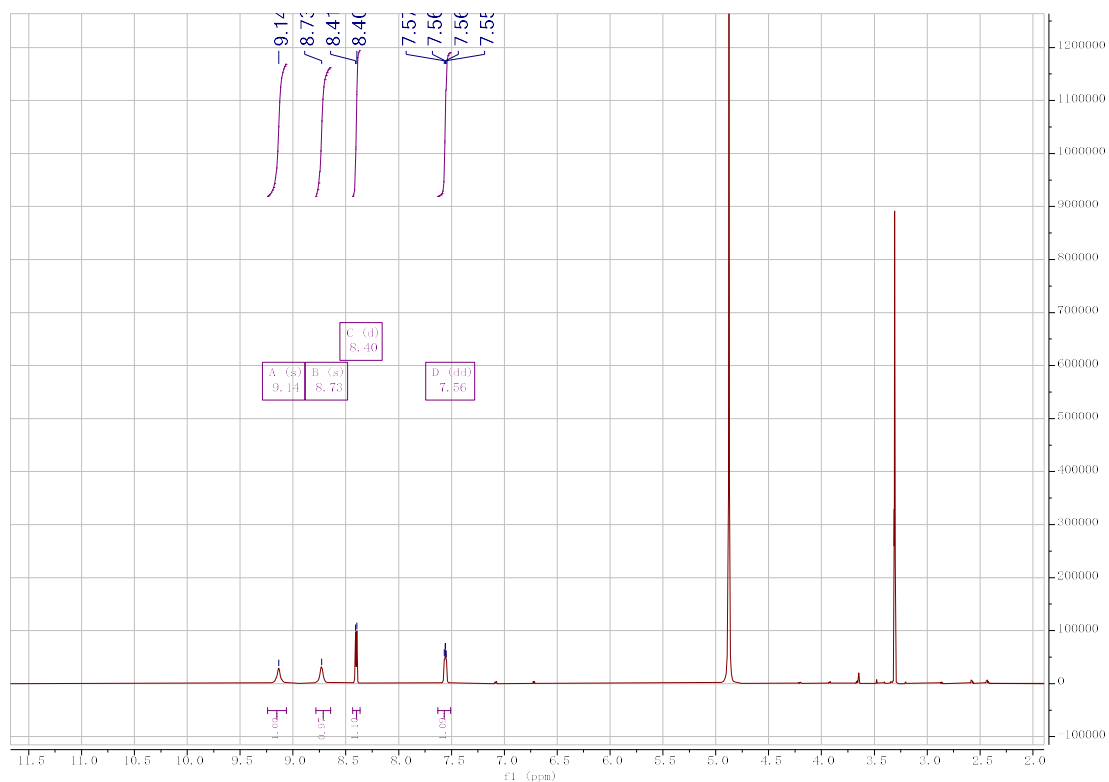
methyl-hydroxyphenylaceta (**13**): brown oil; <sup>1</sup>H NMR (500 MHz, Methanol-*d*<sub>4</sub>) δ 7.14 (m, 1H, H-5'), 7.12 (m, 1H, H-2') 6.78 (m, 1H, H-6'), 6.77 (m, 1H, H-4'), 3.70 (s, 3H, 1-OCH<sub>3</sub>), 3.64 (s, 2H, H-2). <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD) δ 174.7 (C, C-1), 156.7 (C, C-3'), 132.1 (C, C-1'), 129.4 (CH, C-5'), 122.5 (CH, C-6'), 120.4 (CH, C-2'), 115.9 (CH, C-4'), 52.3 (CH<sub>2</sub>, C-2), 36.4 (CH<sub>3</sub>, 1-OCH<sub>3</sub>).



trans-ferulic acid (**14**): white solid;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm,  $J/\text{Hz}$ ):  $\delta_{\text{H}}$  7.58 (d,  $J$  = 15.8 Hz 1H, H-7), 7.05 (dd,  $J$  = 8.2, 1.8 Hz, 1H, H-6), 6.80 (d,  $J$  = 8.2 Hz, 1H, H-5), 6.31 (d,  $J$  = 15.8 Hz, 1H, H-8), 7.17 (d,  $J$  = 1.8 Hz 1H, H-2), 3.88 (s, 3H, 3-OMe).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm,  $J/\text{Hz}$ ): 171.2 (C, C-9), 150.5 (C, C-4), 149.4 (C, C-3), 146.8 (CH, C-7), 127.8 (C, C-1), 124.0 (CH, C-6), 116.5 (CH, C-5), 116.1 (CH, C-8), 111.7 (CH, C-2), 56.4 ( $\text{CH}_3$ , 3-OMe).



nicotinic acid (**15**): pale yellow powder; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD,  $\delta$ , ppm, J/Hz):  $\delta_{\text{H}}$  9.14 (s, 1H, H-2), 8.40 (d, 1H,  $J$  = 7.5 Hz, H-5), 7.56 (dd,  $J$  = 8.0, 4.8 Hz, 1H, H-5), 7.70 (s, 1H, H-6). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD,  $\delta$ , ppm, J/Hz): 168.3 (C, COOH), 151.3 (C, C-2), 129.4 (C, C-3), 139.2 (C, C-4), 125.3 (C, C-5), 153.4 (C, C-6).



7,9-dihydroxy-3-(1H-indol-3-ylmethyl)-8-methoxy-2,3,11,11a-tetrahydro-6H-pyrazino[1,2-b]isoquinoline-1,4-dione (**16**): yellow oil;  $^1\text{H}$  NMR (500 MHz, Methanol- $d_4$ )  $\delta$  7.48 (d,  $J$  = 8.0 Hz, 1H, H-10), 7.21 (d,  $J$  = 8.1 Hz, 1H, H-7), 6.92 (s, 1H, H-5), 6.91 (m, 1H, H-8), 6.82 (t,  $J$  = 7.5 Hz, 1H, H-9), 5.70 (s, 1H, H-5'), 5.18 (d,  $J$  = 16.9 Hz, 1H, H-10'), 4.38 (m, 1H, H-2), 3.81 (d,  $J$  = 3.8 Hz, 1H, H-2'), 3.73 (s, 3H, 1-OCH<sub>3</sub>), 3.49 (m, 1H, H-10'), 3.14 (dd,  $J$  = 14.6, 4.4 Hz, 1H, H-3), 2.27 (dd,  $J$  = 15.9, 3.8 Hz, 1H, H-3'), 0.67 (m, 1H, H-3').  $^{13}\text{C}$  NMR (176 MHz, MeOD)  $\delta$  168.7

(C, C-1'), 166.6 (C, C-1), 149.8 (C, C-6'), 147.8 (C, C-8'), 137.6 (C, C-7a), 135.1 (C, C-7'), 129.1 (C, C-10a), 129.0 (C, C-4'), 125.6 (CH, C-5), 122.5 (CH, C-8), 120.1 (CH, C-10), 119.7 (CH, C-9), 112.0 (CH, C-7), 111.0 (C, C-9'), 109.1 (C, C-4), 107.4 (CH, C-5'), 60.9 (CH<sub>3</sub>, 1-OCH<sub>3</sub>), 57.7 (CH, C-2), 56.6 (CH, C-2'), 41.1 (CH<sub>2</sub>, C-10'), 33.1 (CH<sub>2</sub>, C-3'), 31.4 (CH<sub>2</sub>, C-3).

