

SUPPLEMENTARY MATERIAL

Investigation on bioactive metabolites produced by an endophytic fungus *Trichoderma citrinoviride* from the arils of *Torreya grandis*

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Abstract

An endophytic fungus *Trichoderma citrinoviride* capable of producing active substances was isolated from the arils of *Torreya grandis*. Seven compounds were separated from the ethyl acetate extract of fermentation broth and mycelium by chromatography, respectively identified as trichomerol (**1**), bisorbicillinolide (**2**), sohirnone A (**3**), emodin (**4**), stigmasterol (**5**), ergosterol (**6**), daidzein (**7**). This study is the first to report of the isolation of the endophytic fungus *T. citrinoviride* from the arils of *T. grandis* with complete assignments of **1-7**. Compound **1** and **2** exhibited significant antioxidant activity of diphenyl picryl hydrazinyl with IC₅₀ 38.92 and 3.91 µg/mL, respectively. Compound **1**, **2**, **4** and **7** significantly inhibited the growth of *Staphylococcus aureus* with MIC 0.78; 0.39; 0.20 and 0.20 mg/mL, respectively.

Keywords: *Torreya grandis*; endophytic fungi; *Trichoderma citrinoviride*

Experimental

Materials

The roots, stems and seeds of *T. grandis* were picked from Jinhua City, Zhejiang Province, P. R. China, and refrigerated to retain freshness and processed within 48 h.

Thin-layer chromatography (TLC) was carried out on silica gel plates (Qingdao Ding Kang Silica gel Co., G60, F-254). All other chemicals used were of analytical grade.

Potato dextrose agar medium (PDA medium): potato 200 g, glucose 20 g, Agar 15 g, distilled water 1000 mL.

Luria-Bertani medium (LB medium): tryptone 10 g, yeast extract 5 g, NaCl 10 g, distilled water 1000 mL.

Sabouraud medium : glucose 40 g, peptone 10 g, distilled water 1000 mL.

Isolation of endophytic fungi

Healthy roots, stems and seeds of *T. grandis* that are not damaged in appearance were selected and pre-processed (Sample fragments were successively surface sterilized by immersion in 75 % ethanol for 30 s, 3 % sodium hypochlorite solution for 10 min, 75 % ethanol for 10 min and sterile distilled water for 3 – 5 s). Each tissue section was inoculated into PDA medium containing Kanamycin. The final rinse solution was applied to the sterile plates containing the culture medium as blank control. All the plates were placed in a constant temperature incubator at 28 °C for cultivation. Comparing with the blank plates, we observed the changes in the petri dish every 12 hours, and pick out the growing colonies. Isolate and purify the colonies to the single strain for many times. The selected strains were numbered and stored at - 20 °C.

Identification of endophytic fungi

Total genomic DNA was extracted from mycelium collected from 6-day-old Sabouraud cultures cultured at 28 °C by the Ezup column fungal genomic DNA extraction kit. Universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for polymerase chain reaction (PCR) amplification. The PCR products were subjected to gel electrophoresis to determine the success of the amplification. The fungi and PCR products were sent to Tsingke Biotechnology Co., Ltd. (Beijing, P. R. China) for 18S rDNA sequencing and ITS

rDNA sequencing. The sequence was aligned against GenBank sequences using the BLAST program and building phylogenetic trees with MEGA 7.0 software (Figure S1, S2). The selected strain *Trichoderma citrinoviride* TG-Z4-01 was deposited in China Center for Type Culture Collection, Wuhan University, Wuhan 430072, P. R. China, with the preservation number of CCTCC M 2022156.

Fermentation

The fungal isolated was grown on Sabouraud medium for 6 days at 28 °C. A small agar scrap with mycelium was added into 250 mL Sabouraud medium and incubated at 28 °C, 180 rpm for 3 days as seed culture. Seed culture (30 mL) was added into erlenmeyer flask containing 300 mL Sabouraud medium. A total of 120 erlenmeyer flask (45 L) was ferment at 28 °C, 180 rpm for 7 days.

Extraction and Isolation

The fermented products (mycelium and fermentation broth) were treated with an ultrasonic cell pulverizer in an ice bath, under the condition of 405 W, every 3 s, intermittent 4 s, and 300 cycles of treatment. The filtered fermentation broth was repeatedly extracted with ethyl acetate for 3 times. The extract phase was concentrated under vacuum to give the crude extract (75.0 g, labeled as Tg-Ea). The extract was separated into 8 fractions (Fr. 1 - Fr. 8) with step gradient elution (CH₂Cl₂/MeOH, v:v = 100:0 to 0:100) on silica gel chromatography. Fr. 2 was subjected to silica gel chromatography (CH₂Cl₂/EtOAc, v:v = 15:1 to 1:1). Then it was separated by silica gel chromatography (PE/EtOAc, v:v = 5:3), and recrystallized to obtain compound **1**. Fr. 3 was subjected to silica gel chromatography (PE/EtOAc, v:v = 10:1 to 5:1) to obtain Fr. 3-2, and recrystallized to obtain compound **3**. The obtained Fr. 3-8 was separated by ODS (MeOH/H₂O, v:v = 70:30), and then separated by preparative thin-layer chromatography (CH₂Cl₂/MeOH, v:v = 20:1) to obtain compound **2**. Fr.5 was applied on Sephadex LH-20 (CH₂Cl₂/MeOH, v:v = 1:1) and further purified by ODS (MeOH/H₂O, v:v = 80:20) to give compound **4**. Fr. 6 was chromatographed on silica gel (PE/Me₂CO, v:v = 5:3), and then passed through an ODS column (MeOH/H₂O, v:v = 75:25) to obtain compound **5**. Fr. 7 was separated by Sephadex LH-20 (CHCl₃/MeOH, v:v = 1:1)

and then applied on ODS column (MeOH/H₂O, v:v = 80:20), further purified by preparative thin-layer chromatography to give compound **6**. Fr. 8 was separated by Sephadex LH-20 (MeOH) and then subjected to further elution on a silica gel column (CH₂Cl₂/MeOH, v:v = 9:1) to afford compound **7**. The compounds were investigated by NMR and mass spectrometry.

Antibacterial Assay

The antibacterial activity of Tg-Ea was determined by Oxford cup method. Liquid cultures of activated *Staphylococcus aureus* (37 °C, 180 rpm, 12 h) were added to LB solid medium to prepare a medium with 1% bacterial concentration. The medium was added to the Petri dish containing the Oxford cups. The test extract were dissolved in dimethyl sulphoxide (DMSO) and filtered through a microporous membrane (0.22 µm). The solutions of Tg-Ea were added to the wells of the Oxford cups. The wells containing a culture suspension and DMSO were run as negative controls. Kanamycin were introduced in the experiments as positive controls. The petri dishes after adding samples were incubated in a constant temperature incubator at 37 °C for 24 h, and the diameter of the inhibition zone was measured and recorded.

The antibacterial activities of the isolated compounds were tested using the broth dilution method in 96-well plate. Liquid cultures of activated *Staphylococcus aureus* (37 °C, 180 rpm, 12 h) were added to sterile LB liquid medium to reach 10⁶ colony-forming units/mL. The test compounds (25 mg) were dissolved in dimethyl sulphoxide (DMSO) and prepared as sample solutions with a concentration of 25 mg/mL. The sample solutions were filtered through a microporous membrane (0.22 µm), and diluted by two-fold dilution method (A volume of 100 µL of 25 mg/mL sample solution was added to the first well. A volume of 100 µL of the 25 mg/mL sample solution and 100 µL sterile medium were added and mixed in the second well. Then 100 µL of the solution in the second well was pipetted to the third well and mixed with 100 µL of sterile medium. Serial dilution to the last well to obtain a sample solution with a concentration of 25 to 0.02 mg/mL). Then 100 uL of culture medium with test strains was added to each well. The plate containing test strains, and diluted sample solutions

were incubated at 37 °C (24 h). The wells containing a culture suspension and DMSO were run as negative controls. Kanamycin were introduced in the experiments as positive controls. The concentration corresponding to the last well in the 96-well plate without precipitation was the MIC value of the sample.

Antioxidant Assay

The test compounds (1 mg) were dissolved in DMSO and prepared as sample solutions with a concentration of 1000 µg/mL. A volume of 100 µL of diphenyl picryl hydrazinyl (DPPH, 0.08 mmol/L) was mixed with 100 µL of properly diluted sample solution. After stored at room temperature for 30 min, the absorbance of the reaction mixture was recorded at 517 nm against a blank. The scavenging rate of DPPH radical (I%) was calculated according to the following equation: $I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$ where A_{sample} is the absorbance of a sample solution and A_{blank} is the absorbance of the blank solution (containing all reagents except the test sample). IC₅₀ value is the effective concentration that could scavenge 50% of the DPPH radicals. Vc was used as positive control.

Statistical Analysis

Data are expressed as means of triplicates ± standard deviation (SD). Statistical comparisons were made using one-way ANOVA. Data analyses were performed by Microsoft Excel and SPSS 17.0. Results were considered to be statistically significant at $p < 0.05$.

Spectral data

Trichomerol (**1**) (Xuan et al. 2014), C₂₈H₃₂O₈, yellow crystal. ESI-MS m/z 495.37 [M + H]⁺. ¹H NMR (600 MHz, DMSO-D₆) δ 7.22 (dd, 2H, J = 14.8, 11.0 Hz, H-3', 3''), 6.74 (s, 1H), 6.48 – 6.39 (m, 2H, H-4', 4''), 6.28 (d, 2H, J = 14.0, 6.8 Hz, H-2', 2''), 3.05 (s, 2H, H-1, 7), 1.86 (d, 6H, J = 6.9, 1.6 Hz, 6', 6''-CH₃), 1.27 (d, J = 5.8 Hz, 4, 6-CH₃, 10, 12-CH₃). ¹³C NMR (600 MHz, DMSO-D₆) δ 200.72(C-3, 9), 175.02(C-1', 1''), 142.94(C-3', 3''), 140.46(C-5', 5''), 131.47(C-4', 4''), 119.79(C-2', 2''), 104.57(C-2, 8), 103.96(C-5, 11), 79.09(C-6, 12), 59.82(C-4, 10), 56.93(C-1, 7), 21.86(6, 12-CH₃), 19.94(4, 10-CH₃), 19.09(6', 6''-CH₃).

Bisorbicillinolide (**2**) (Meng et al. 2019), $C_{28}H_{32}O_8$, yellow amorphous powder. ESI-MS m/z 495.45 $[M + H]^+$. 1H NMR (600 MHz, $CDCl_3$) δ 16.06 (s, 1H, 1'-OH), 7.43 (dd, 1H, $J = 13.5, 4.2$ Hz, H-3''), 7.29 (dd, 1H, $J = 13.4, 9.8$ Hz, H-3'), 6.48 (dd, 1H, $J = 15.1, 5.2$ Hz, H-2''), 6.38 (dt, 1H, $J = 14.0, 6.9$ Hz, H-5''), 6.25 (s, 1H, 7-OH), 6.22 (d, 1H, $J = 9.6$ Hz, H-5''), 6.15 (d, 1H, $J = 4.9$ Hz, H-4''), 5.91 (s, 1H, 10-OH), 3.76 (d, 1H, $J = 6.1$ Hz, H-8), 3.41 (d, 1H, $J = 6.2$ Hz, H-1), 2.68 (q, 1H, $J = 7.2$ Hz, H-11), 1.92 (d, 3H, $J = 6.7$ Hz, 6''-H₃), 1.91 (s, 3H, 6'-H₃), 1.40 (s, 3H, 9-CH₃), 1.39 (s, 3H, 4-CH₃), 1.27 (s, 3H, 7-CH₃), 1.25 (s, 3H, 11-CH₃). ^{13}C NMR (151 MHz, $CDCl_3$) δ 210.42(C-6), 199.47(C-1''), 194.62(C-3), 178.11(C-12), 177.28(C-1'), 147.95(C-3''), 145.00(C-5''), 144.75(C-3'), 141.61(C-5'), 130.94(C-4'), 130.26(C-4''), 123.72(C-2''), 118.95(C-2'), 107.26(C-2), 93.97(C-9), 88.22(C-10), 84.02(C-7), 73.36(C-5), 67.87(C-4), 56.89(C-8), 44.36(C-1), 41.12(C-11), 24.33(9-CH₃), 19.11(C-6''), 18.99(C-6'), 17.96(7-CH₃), 13.72(11-CH₃), 10.33(4-CH₃).

Sohirnone A (**3**) (Xuan et al. 2014), $C_{13}H_{16}O_3$, colorless crystal. ESI-MS m/z 219.23 $[M + H]^+$. 1H NMR (600 MHz, $CDCl_3$) δ 12.61 (s, 1H, 1-OH), 7.54 – 7.48 (m, 1H, H-3), 6.39 – 6.33 (m, 1H, H-6), 5.52 (dd, 2H, H-4', 5'), 2.98 (t, 2H, $J = 7.5$ Hz, H-2'), 2.50 (s, 1H), 2.42 (dd, 2H, H-3'), 2.26 (s, 3H, 5-CH₃), 1.70 – 1.64 (m, 3H, 6'-CH₃). ^{13}C NMR (151 MHz, $CDCl_3$) δ 204.27(C-1'), 163.40(C-2), 160.82(C-4), 132.35(C-6), 129.48(C-4'), 126.22(C-5'), 115.82(C-1), 113.62(C-5), 103.09(C-3), 37.91(C-2'), 27.47(C-3'), 17.90(C-6'), 15.16(5-CH₃).

Emodin (**4**) (Nguyen et al. 2019.), $C_{15}H_{14}O_5$, orange amorphous powder. APCI-MS m/z 271.13 $[M + H]^+$. 1H NMR (600 MHz, DMSO- D_6) δ 12.31 (s, 1H, 1-OH), 12.23 (s, 1H, 8-OH), 12.14 (s, 1H, 3-OH), 7.64 (d, 1H, $J = 19.7$ Hz, H-5), 7.39 (d, 1H, $J = 7.1$ Hz, H-7), 7.09 (d, 1H, $J = 14.8$ Hz, H-4), 6.72 (d, 1H, $J = 9.7$ Hz, H-2), 2.47 (s, 3H, 6-CH₃). ^{13}C NMR (151 MHz, DMSO- D_6) δ 190.03(C-9), 181.58(C-10), 170.04, 166.00(C-3), 164.88(C-1), 161.84(C-8), 148.61(C-6), 135.39(C-4a), 133.10(C-10a), 124.48(C-7), 120.84(C-5), 113.65(C-9a), 109.27(C-8a), 109.20(C-4), 108.30(C-2), 21.95(6-CH₃).

Stigmasterol (**5**) (Huang et al. 2018), $C_{29}H_{48}O$, white amorphous powder. APCI-MS m/z 411.40 $[M + H]^+$. 1H NMR (600 MHz, $CDCl_3$) δ 5.37 (dd, 1H, $J = 5.0, 2.4$ Hz, H-6), 5.17 (dd, 1H, $J = 15.2, 8.6$ Hz, H-22), 5.04 (dd, 1H, $J = 15.2, 8.6$ Hz, H-23), 3.54 (m, 1H, H-3), 1.38 – 1.08 (m, 3H, H-19), 1.10 – 0.99 (m, 3H, H-21), 0.87 (d, 3H, H-29), 0.80 (d, 3H, H-27), 0.79 (d, 3H, H-26), 0.70 (s, 3H, H-18). ^{13}C NMR (151 MHz, $CDCl_3$) δ 140.77(C-5), 138.33(C-22), 129.28(C-23), 121.73(C-6), 71.82(C-3),

56.78(C-17), 56.07(C-14), 50.14(C-24), 42.33(C-13), 42.30(C-4), 39.78(C-20), 37.26(C-1), 35.89(C-10), 31.91(C-25), 31.66(C-8), 31.47(C-2), 28.26(C-16), 25.42(C-26), 24.37(C-28), 24.31(C-15), 21.09(C-11), 19.83(C-19), 19.41(C-21), 19.04(C-27), 11.99(C-29), 11.87(C-18).

Ergosterol (**6**) (Venditti et al. 2017), $C_{28}H_{44}O$, white needle crystal. APCI-MS m/z 379.24 $[M + H]^+$. 1H NMR (600 MHz, $CDCl_3$) δ 5.59 (dd, 1H, $J = 5.7, 2.6$ Hz, H-6), 5.40 (dd, 1H, $J = 5.7, 2.8$ Hz, H-7), 5.26-5.21 (qd, 2H, $J = 15.3, 7.6$ Hz, H-22, 23), 3.66 (m, 1H, H-3), 2.49 (ddd, 1H, $J = 14.4, 4.8, 2.5$ Hz, H-4a), 2.30 (ddd, 1H, $J = 14.1, 11.7, 2.3$ Hz, H-4b), 1.06 (d, 3H, $J = 6.6$ Hz, H-21), 0.98 – 0.91 (m, 6H, H-26, 19), 0.85 (d, 3H, $J = 6.8$ Hz, H-28), 0.65 (s, 3H, H-18). Hydrogen is not listed as indistinguishable multiplets. ^{13}C NMR (151 MHz, $CDCl_3$) δ 141.37(C-5), 139.79(C-8), 135.58(C-22), 131.99(C-23), 119.60(C-6), 116.30(C-7), 70.47(C-3), 55.74(C-17), 54.57(C-14), 46.26(C-9), 42.84(C-24), 42.83(C-13), 40.80(C-20), 40.43(C-4), 39.09(C-12), 38.38(C-1), 37.04(C-10), 33.10(C-25), 31.99(C-2), 28.30(C-16), 23.00(C-15), 21.11(C-11), 19.96(C-26), 19.65(C-27), 17.61(C-28), 16.29(C-19), 12.05(C-18).

Daidzein (**7**) (Liu et al. 2013), $C_{15}H_{10}O_4$, light yellow amorphous powder. ESI-MS m/z 253.32 $[M + H]^+$. 1H NMR (600 MHz, $CDCl_3$) δ 9.53 (s, 1H, 4'-OH), 8.29 (s, 1H, H-2), 7.97 (d, 1H, $J = 8.7$ Hz, H-5), 7.39 (d, 2H, $J = 8.4$ Hz, H-2', 6'), 6.94 (dd, 1H, $J = 8.8, 2.3$ Hz, H-6), 6.87 (d, 1H, $J = 2.2$ Hz, H-8), 6.81 (d, 2H, $J = 8.3$ Hz, H-3', 5'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.16(C-4), 162.97(C-7), 157.89(C-9), 157.64(C-4'), 153.28(C-2), 130.54(C-2', 6'), 127.76(C-5), 123.95(C-3), 123.01(C-1'), 115.59(C-6, 10), 115.41(C-3', 5'), 102.56(C-8).

Appendices

Tables

Table S1. Antibacterial activity against *S. aureus* of the pure compounds.

Compounds	MIC (mg/mL)
Trichomerol (1)	0.78
Bisorbicillinolide (2)	0.39
sohironone A (3)	-
Emodin (4)	0.20
Stigmasterol (5)	-
Ergosterol (6)	-
Daidzein (7)	0.20

Table S2. Antioxidant activity against DPPH of the pure compounds.

Compounds	IC ₅₀ (µg/mL)
Trichomerol (1)	38.92
Bisorbicillinolide (2)	3.91
sohironone A (3)	-
Emodin (4)	>1000
Stigmasterol (5)	-
Ergosterol (6)	-
Daidzein (7)	>1000

Figure captions

Figure S1. Pictures of *Torreya grandis*

Figure S2. 18S rDNA gene sequencing and the phylogenetic tree for strain

Figure S3. ITS gene sequencing and the phylogenetic tree for strain

Figure S4. Tg-Ea displayed antibacterial activity to *Staphylococcus aureus*

Figure S5. Structures of compounds **1-7**

Figure S6. Effect diagram of DPPH free radical scavenging ability of compounds

Figure S7. ¹H-NMR for Compound **1** (600 MHz, DMSO-D6)

Figure S8. ¹³C-NMR for Compound **1** (600 MHz, DMSO-D6)

Figure S9. MS for Compound **1**

Figure S10. ¹H-NMR for Compound **2** (600 MHz, CDCl₃)

Figure S11. ¹³C-NMR for Compound **2** (600 MHz, CDCl₃)

Figure S12. MS for Compound **2**

Figure S13. ¹H-NMR for Compound **3** (600 MHz, CDCl₃)

Figure S14. ¹³C-NMR for Compound **3** (600 MHz, CDCl₃)

Figure S15. MS for Compound **3**

Figure S16. ¹H-NMR for Compound **4** (600 MHz, DMSO-D6)

Figure S17. ¹³C-NMR for Compound **4** (600 MHz, DMSO-D6)

Figure S18. MS for Compound **4**

Figure S19. ¹H-NMR for Compound **5** (600 MHz, CDCl₃)

Figure S20. ¹³C-NMR for Compound **5** (600 MHz, CDCl₃)

Figure S21. MS for Compound **5**

Figure S22. ¹H-NMR for Compound **6** (600 MHz, CDCl₃)

Figure S23. ¹³C-NMR for Compound **6** (600 MHz, CDCl₃)

Figure S24. MS for Compound **6**

Figure S25. ¹H-NMR for Compound **7** (600 MHz, CDCl₃)

Figure S26. ¹³C-NMR for Compound **7** (600 MHz, CDCl₃)

Figure S27. MS for Compound **7**

Figures

Figure S1. Pictures of *Torreya grandis*



(a) Branches of *Torreya grandis*

(b) Structure diagram of *Torreya grandis* seed

Figure S2. 18S rDNA gene sequencing and the phylogenetic tree for strain TG-Z4-01

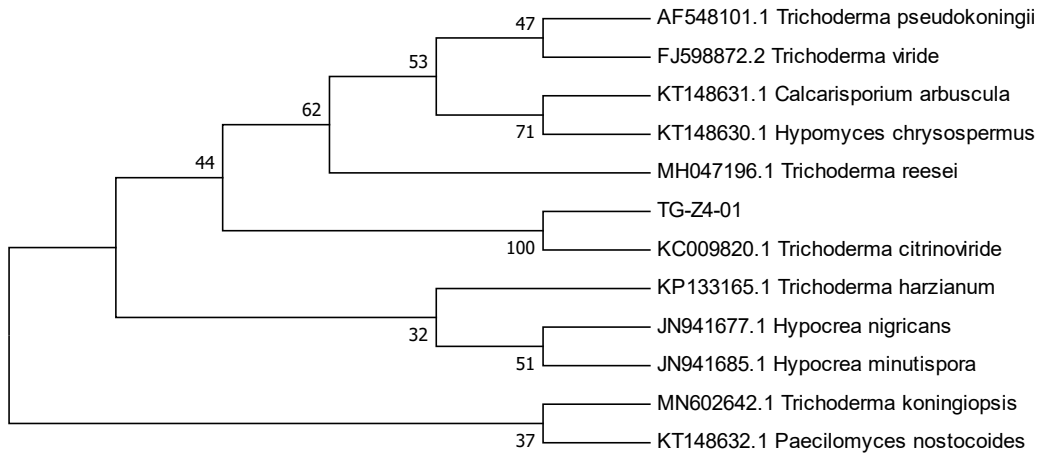


Figure S3. ITS gene sequencing and the phylogenetic tree for strain TG-Z4-01

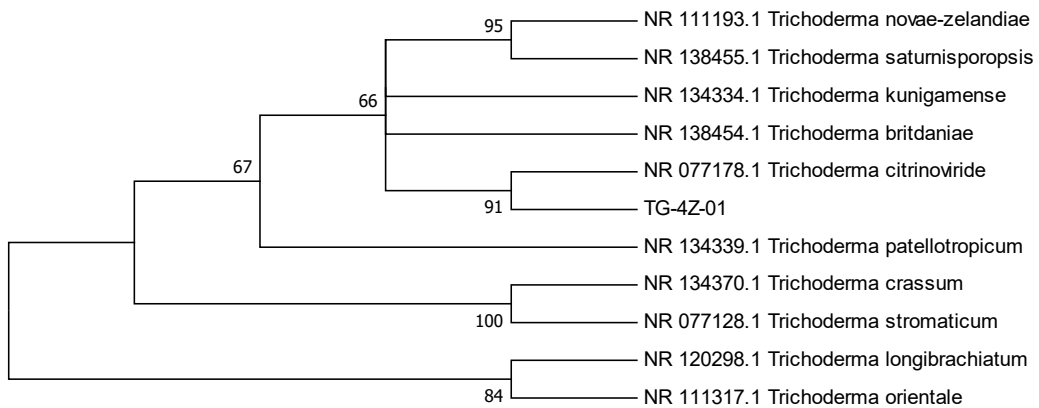
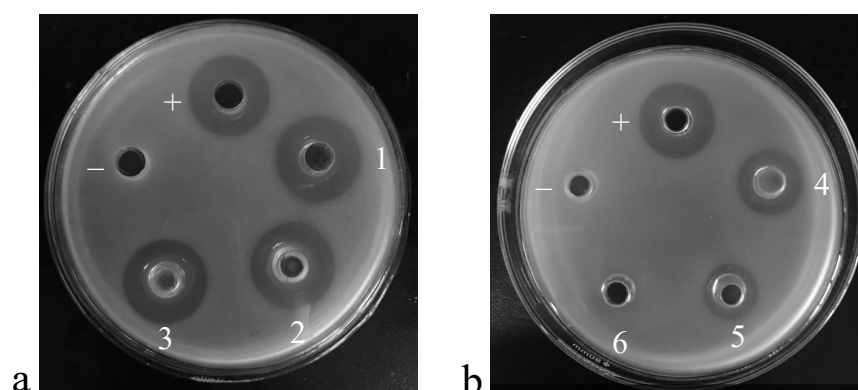


Figure S4. Tg-Ea displayed antibacterial activity to *Staphylococcus aureus*



(a) -:DMSO, +: Kanamycin, 1: Tg-Ea 50.0 mg/mL, 2: Tg-Ea 25.0 mg/mL, 3: Tg-Ea 12.5 mg/mL;
(b) -:DMSO, +: Kanamycin, 4: Tg-Ea 6.25 mg/mL, 5: Tg-Ea 3.125 mg/mL, 6: Tg-Ea 1.5625 mg/mL.

Figure S5. Structure of compounds 1-7

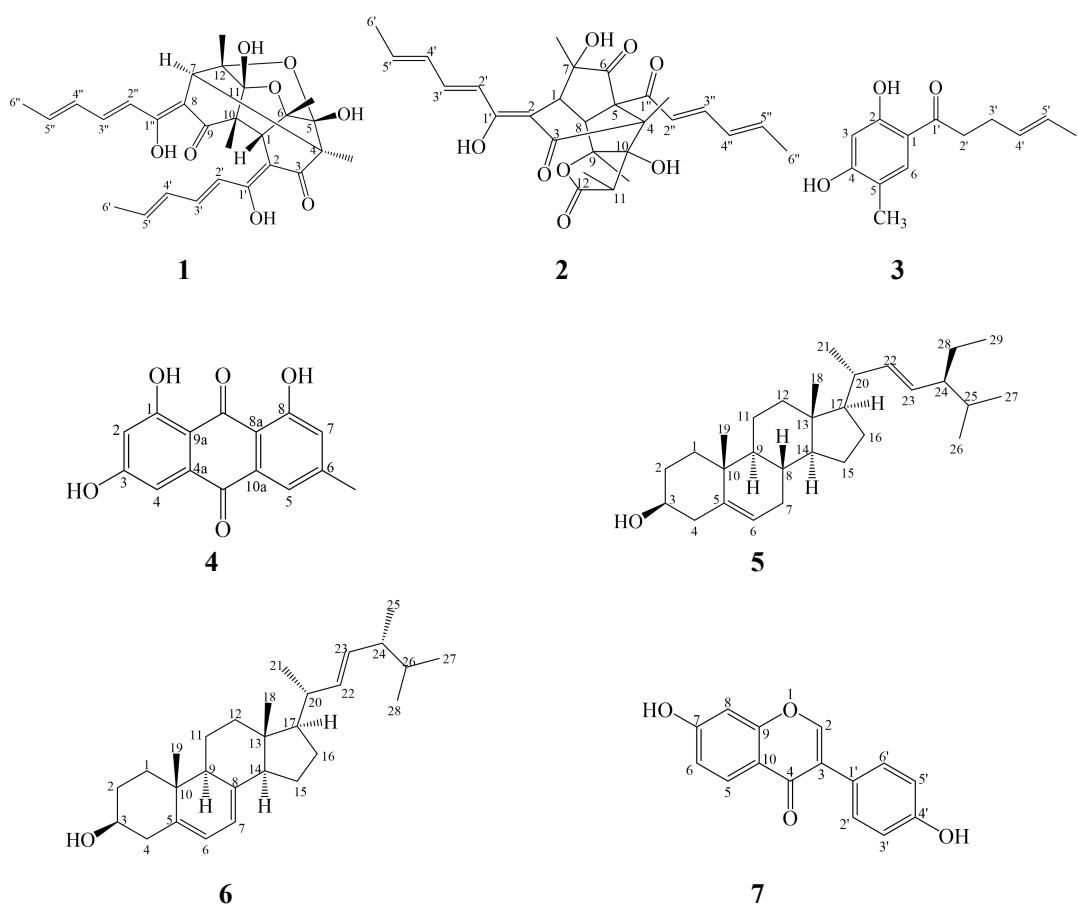
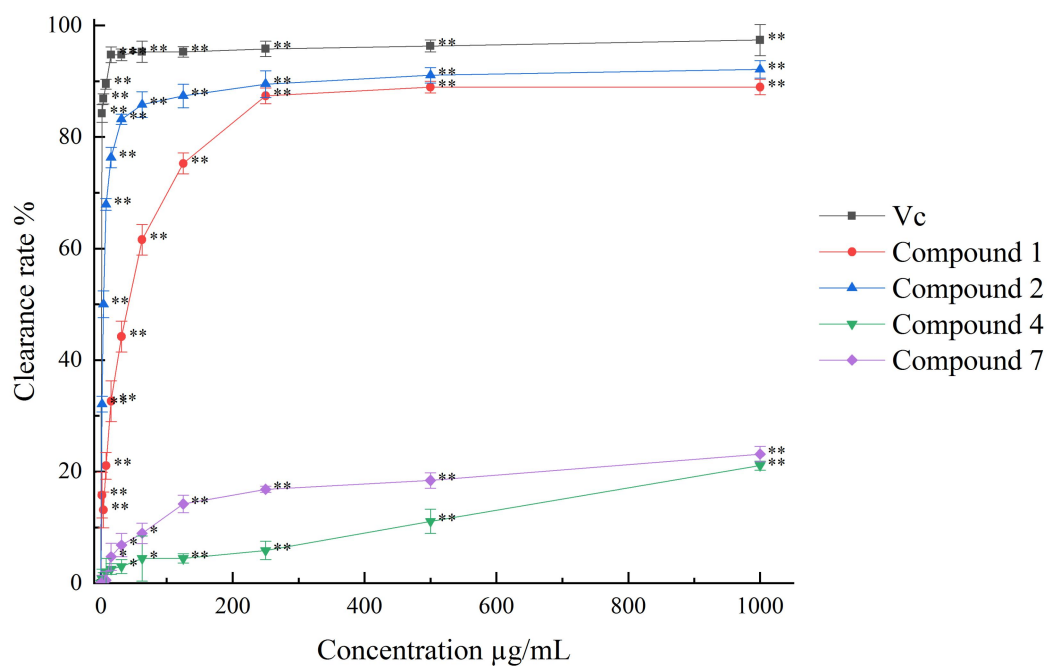


Figure S6. Effect diagram of DPPH free radical scavenging ability of compounds



Data points are the average of three separate measurements, and error bars represent standard deviation of means; *Asterisks* indicates a significant difference from blank control, *P < 0.05; **P < 0.01.

Figure S7. ¹H-NMR for Compound 1 (600 MHz, DMSO-D6)

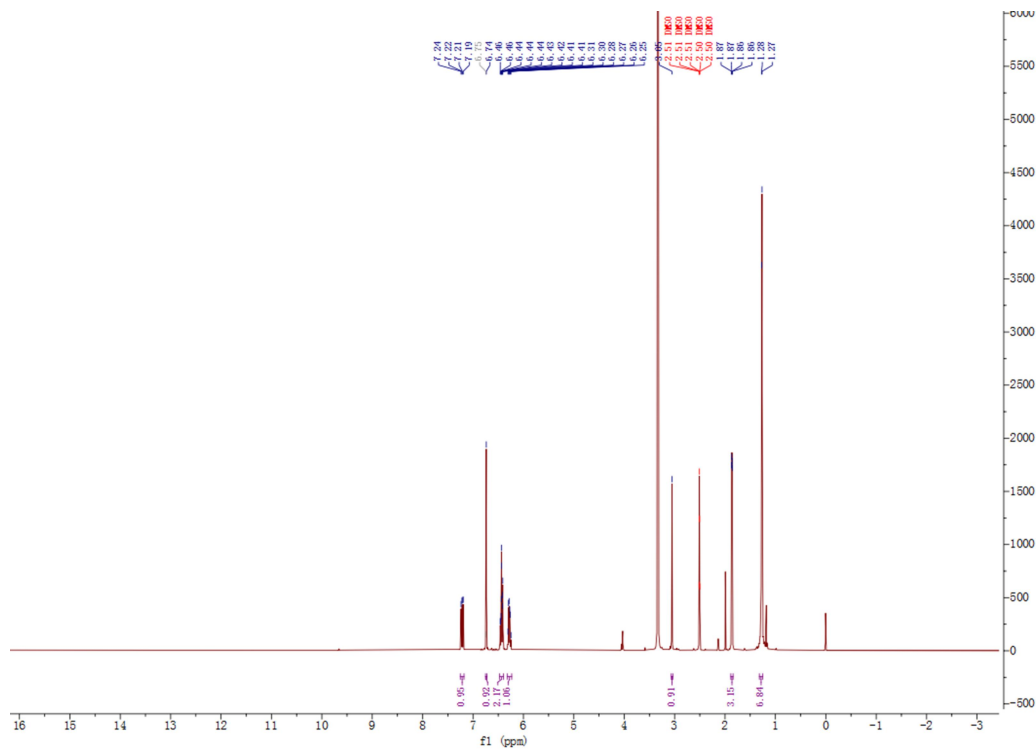


Figure S8. ^{13}C -NMR for Compound **1** (600 MHz, DMSO- D_6)

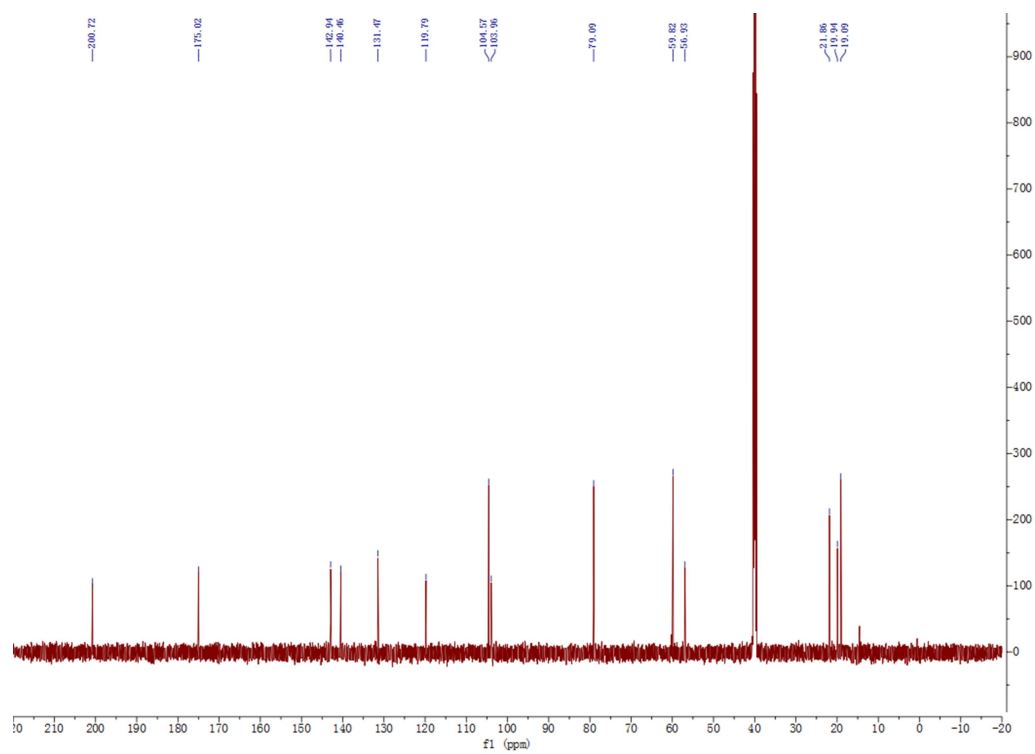


Figure S9. MS for Compound **1**

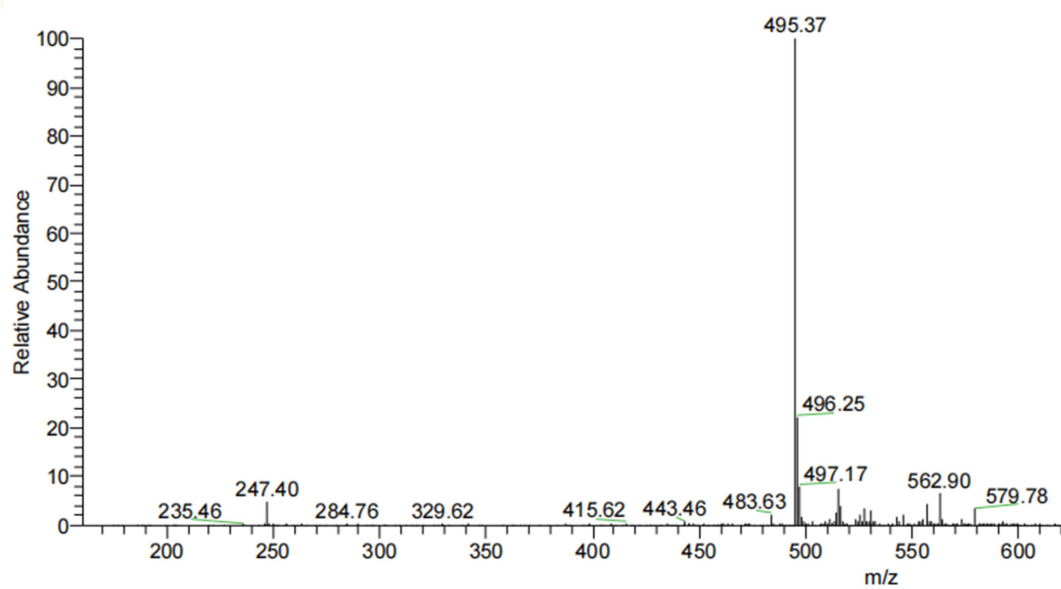


Figure S10. $^1\text{H-NMR}$ for Compound **2** (600 MHz, CDCl_3)

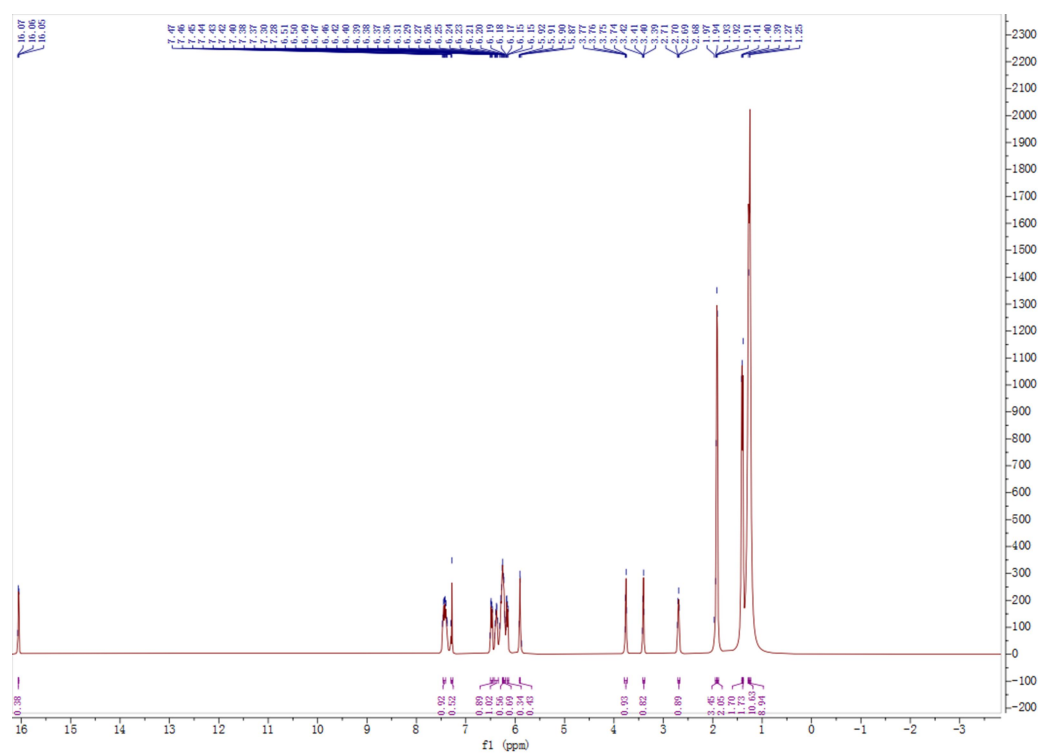


Figure S11. $^{13}\text{C-NMR}$ for Compound **2** (600 MHz, CDCl_3)

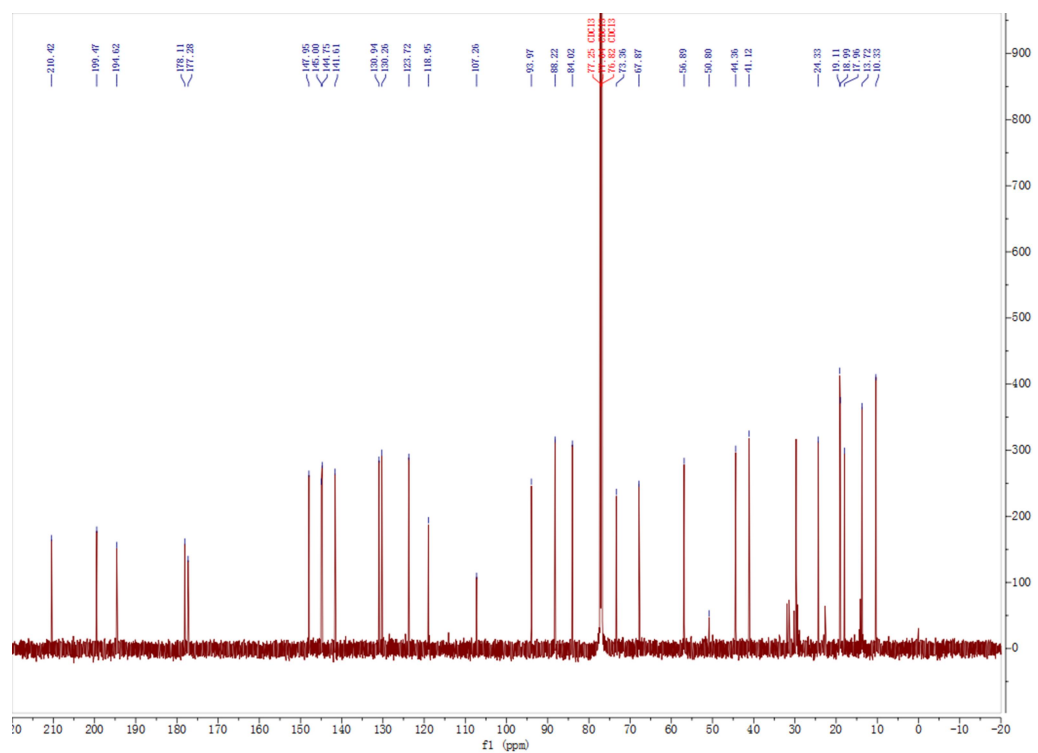


Figure S12. MS for Compound 2

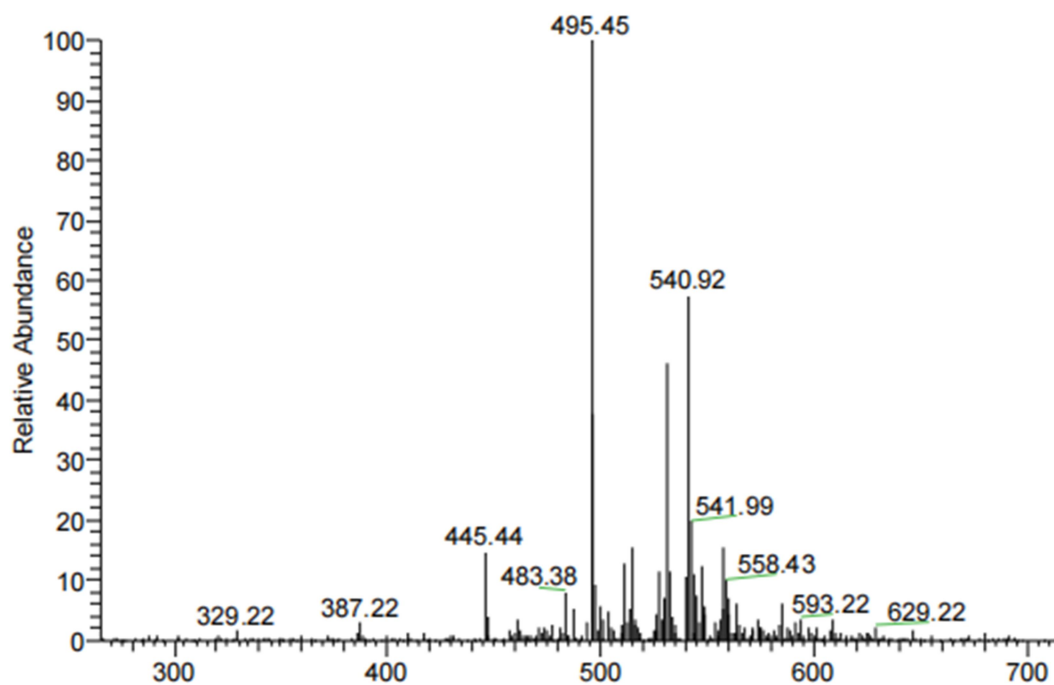


Figure S13. $^1\text{H-NMR}$ for Compound 3 (600 MHz, CDCl_3)

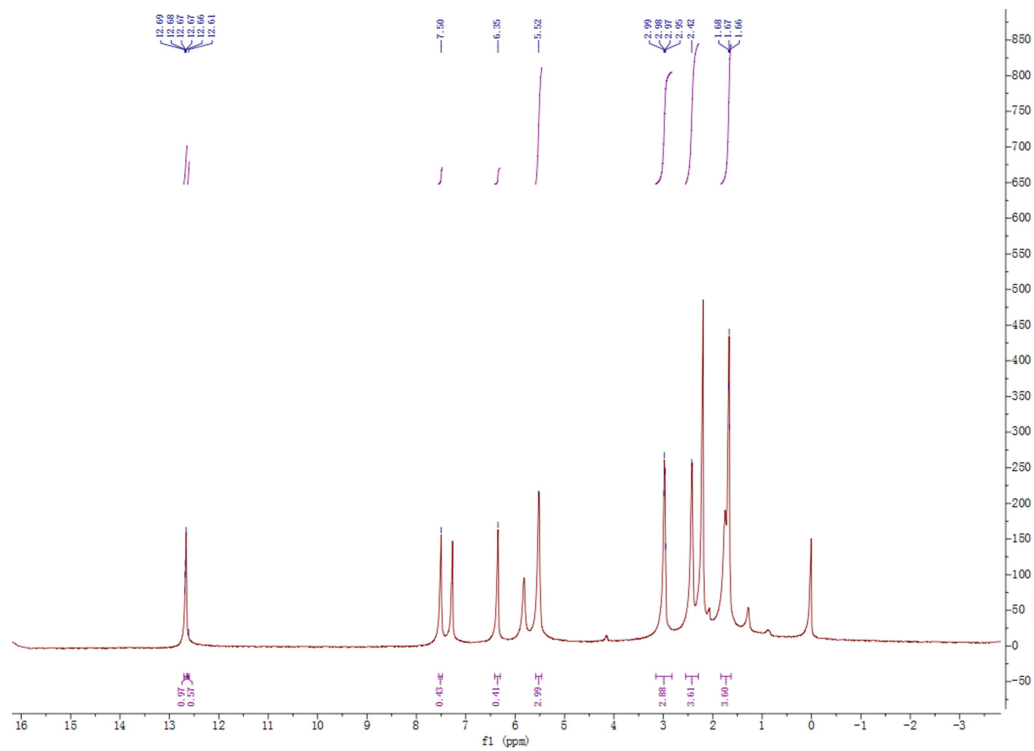


Figure S14. ^{13}C -NMR for Compound **3** (600 MHz, CDCl_3)

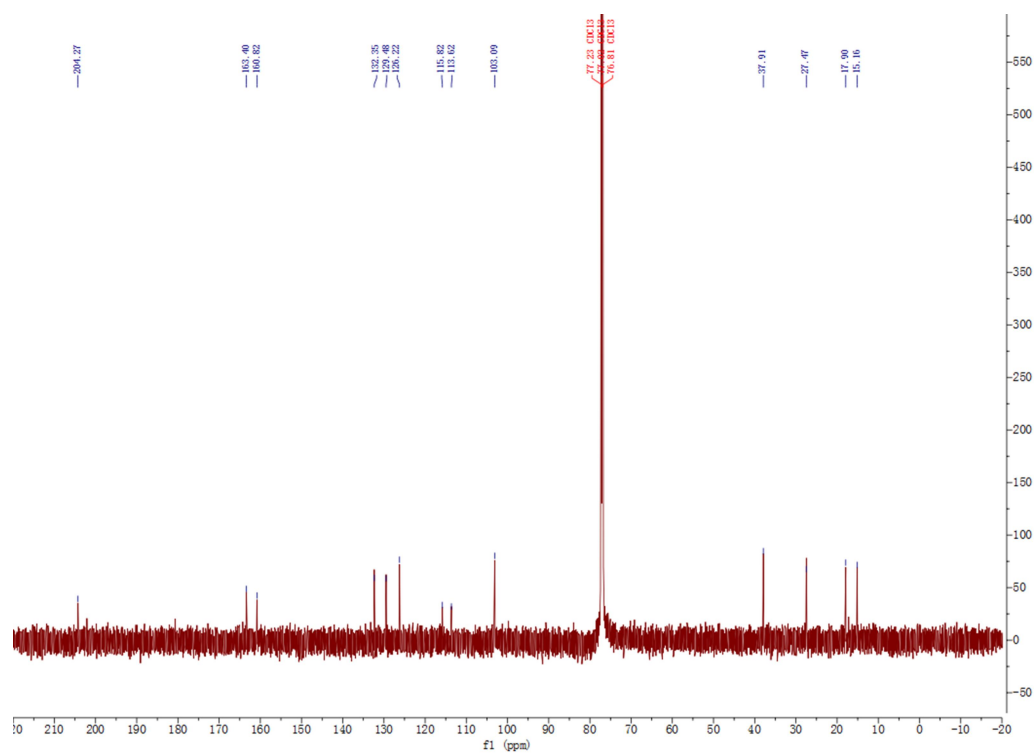


Figure S15. MS for Compound **3**

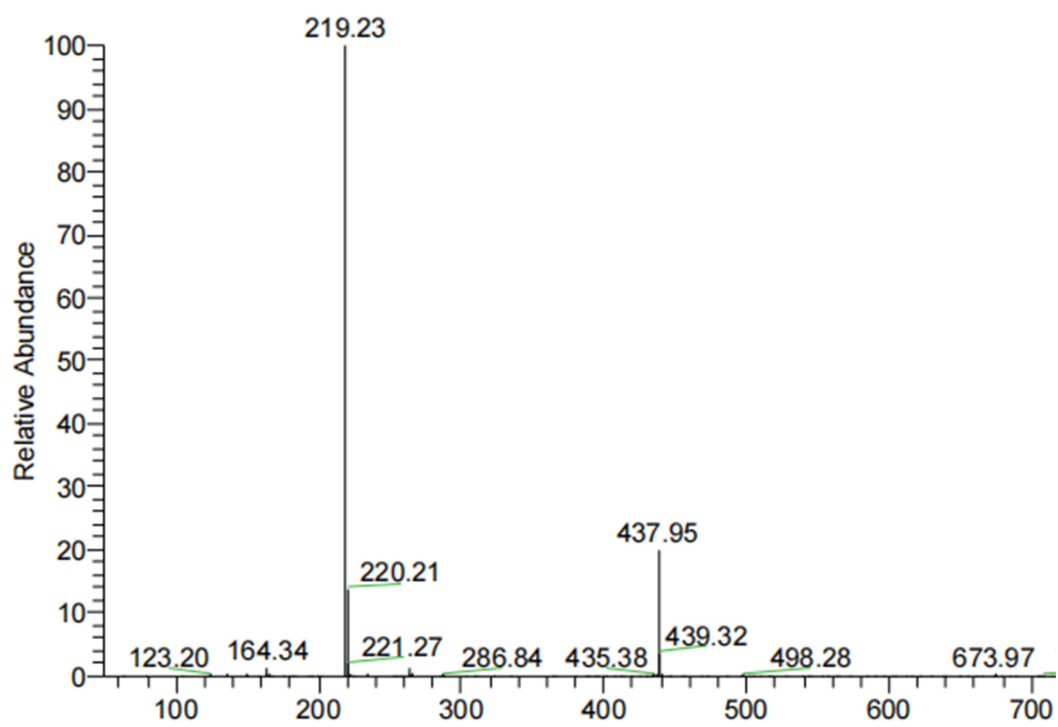


Figure S16. $^1\text{H-NMR}$ for Compound **4** (600 MHz, DMSO- D_6)

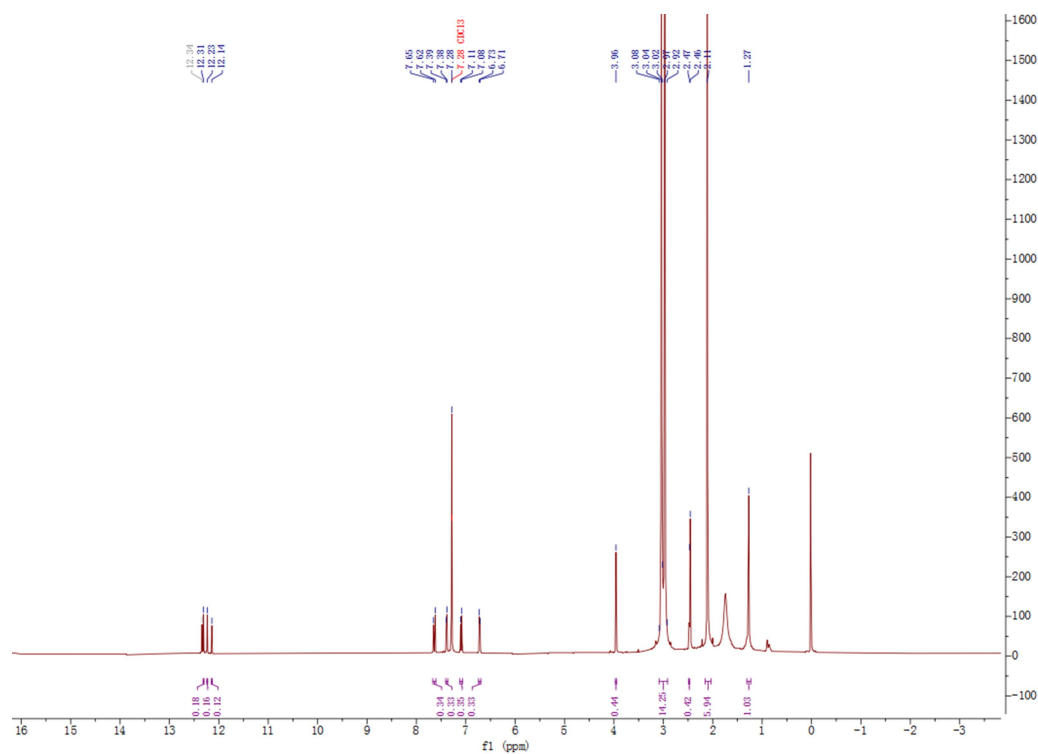


Figure S17. $^{13}\text{C-NMR}$ for Compound **4** (600 MHz, DMSO- D_6)

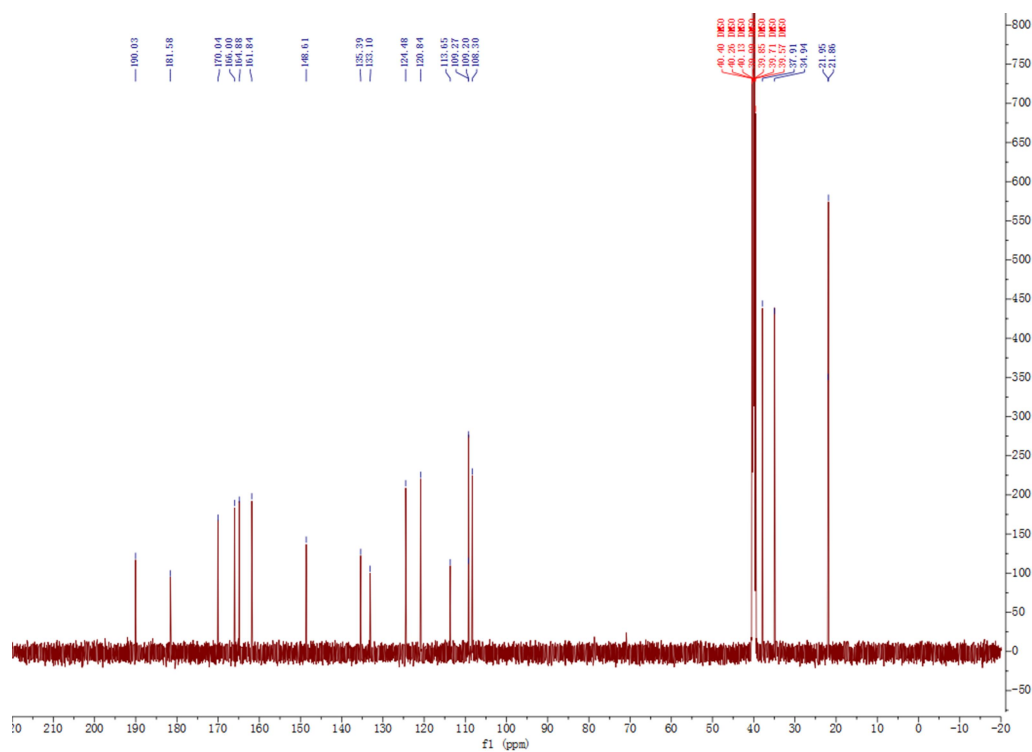


Figure S18. MS for Compound 4

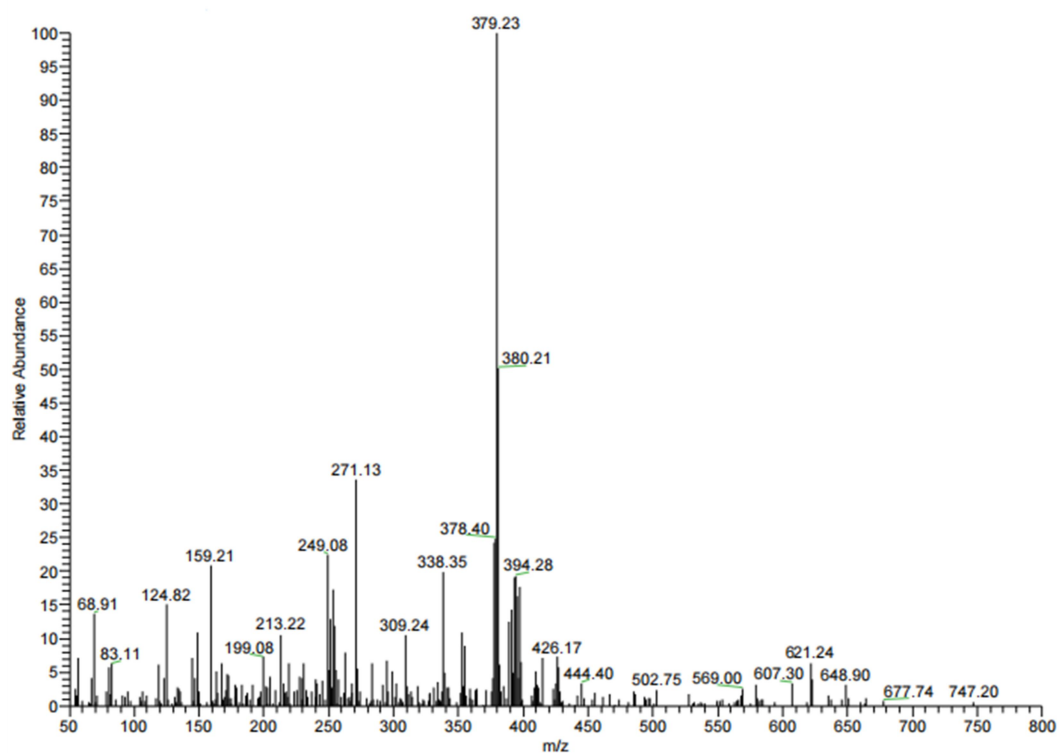


Figure S19. ¹H-NMR for Compound 5 (600 MHz, CDCl₃)

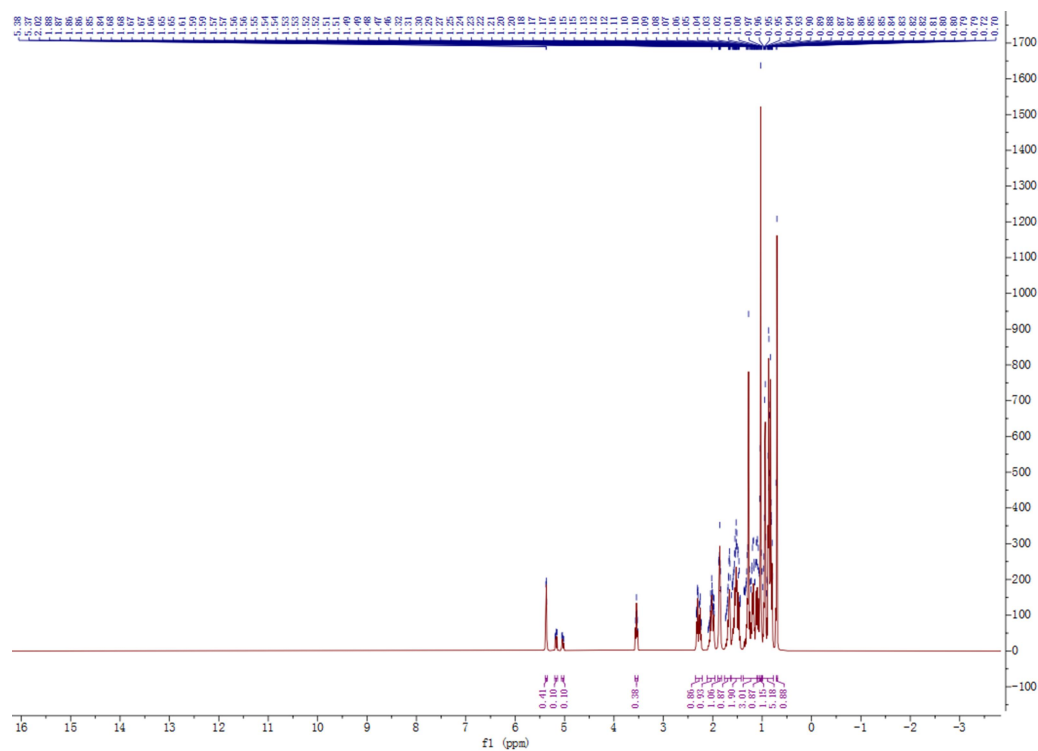


Figure S20. ^{13}C -NMR for Compound **5** (600 MHz, CDCl_3)

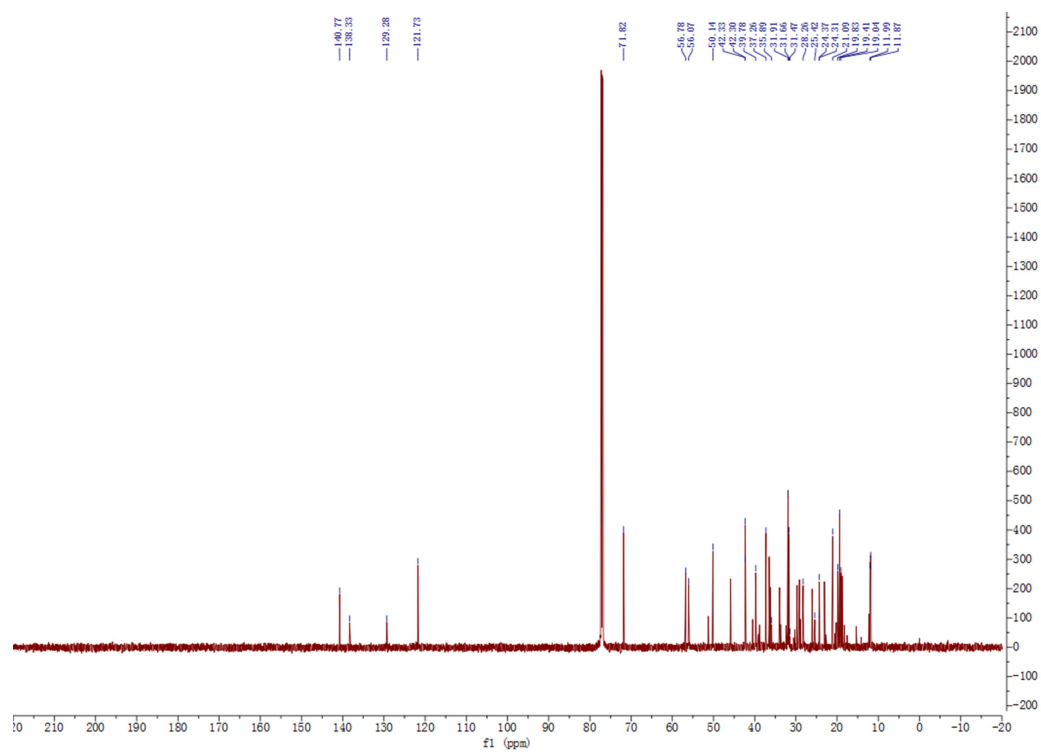


Figure S21. MS for Compound **5**

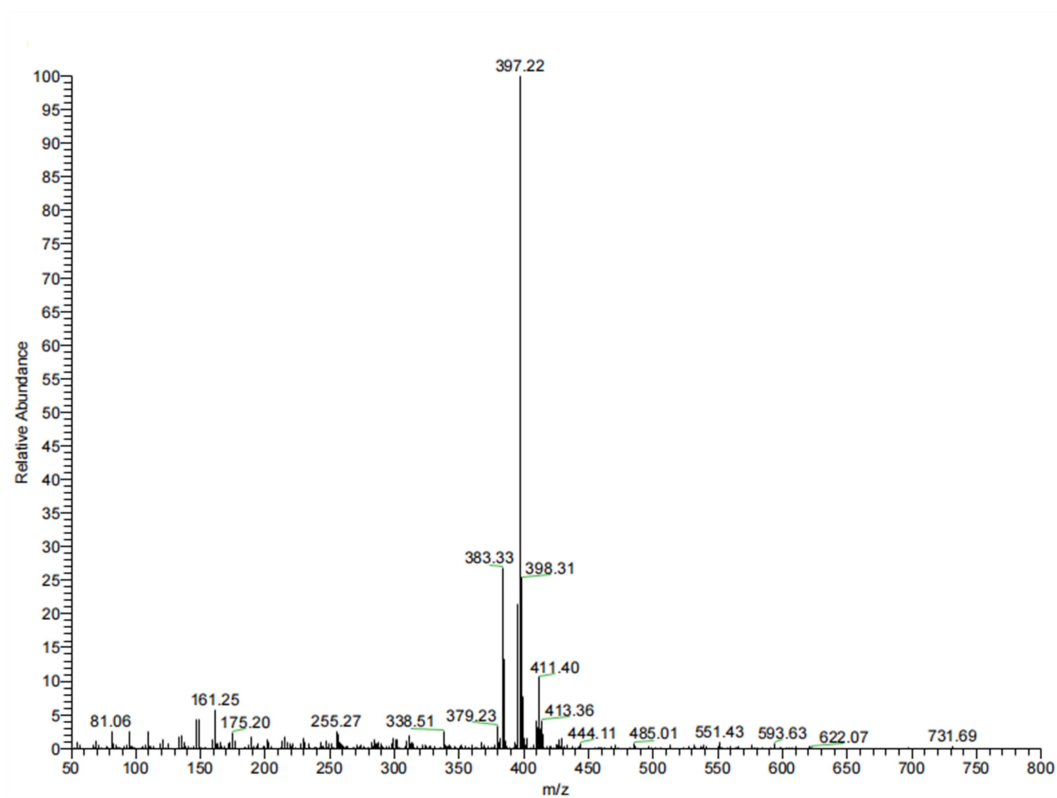


Figure S22. $^1\text{H-NMR}$ for Compound **6** (600 MHz, CDCl_3)

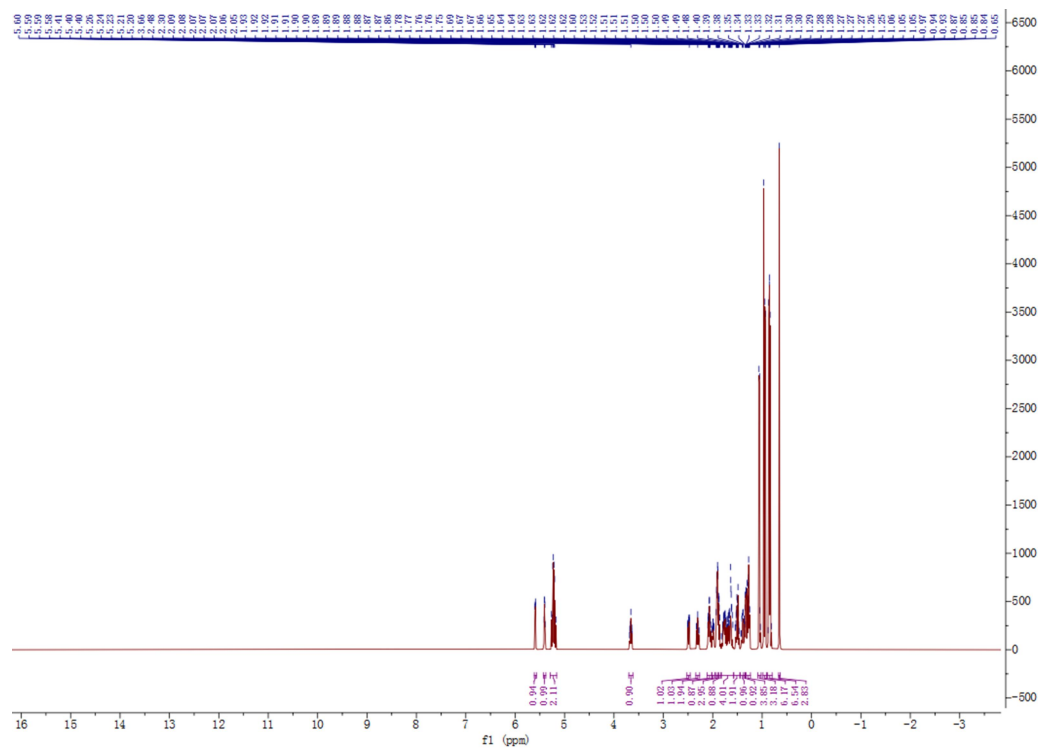


Figure S23. $^{13}\text{C-NMR}$ for Compound **6** (600 MHz, CDCl_3)

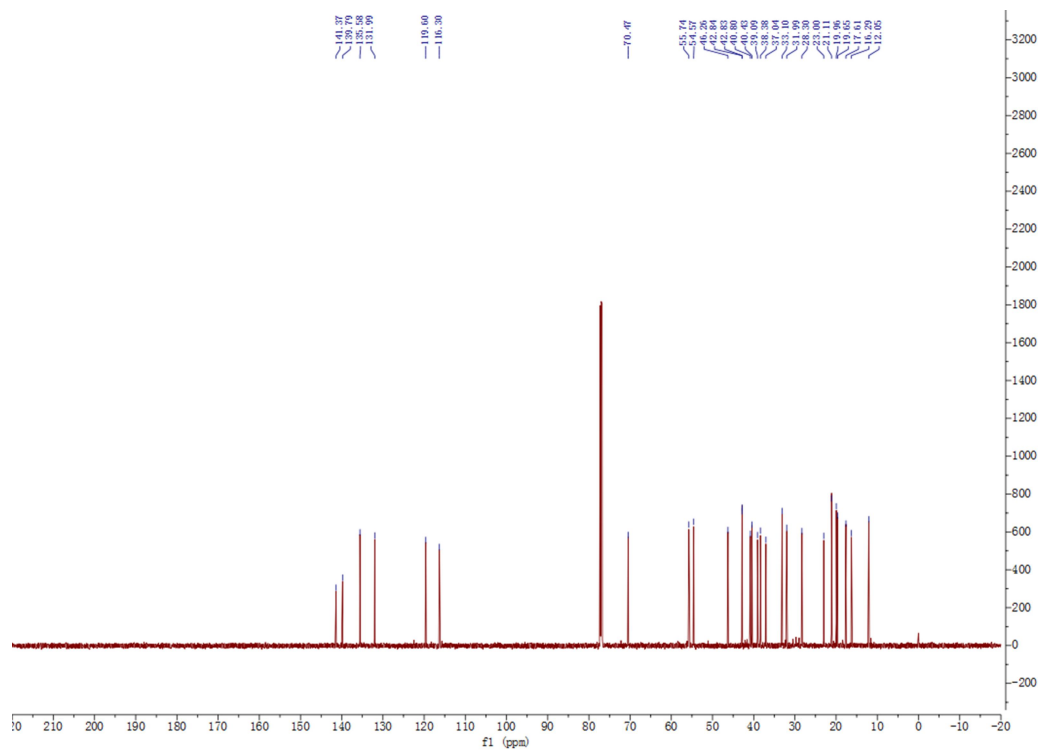


Figure S26. ^{13}C -NMR for Compound 7 (600 MHz, CDCl_3)

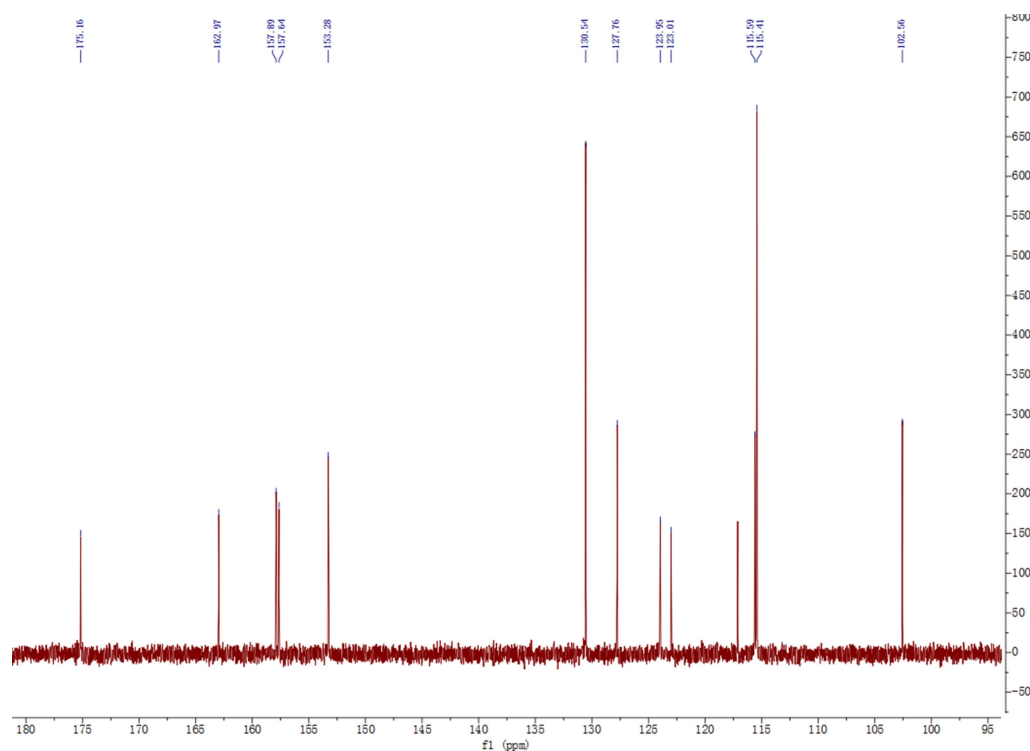
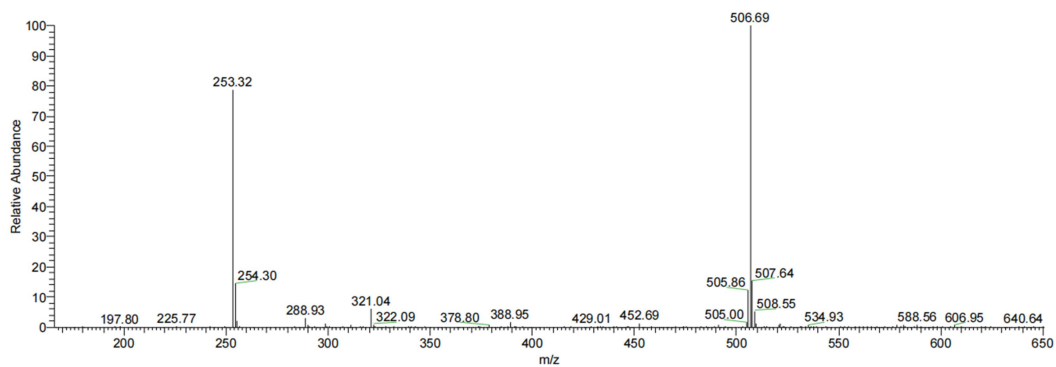


Figure S27. MS for Compound 7



References

- Huang YL, Wei YQ, He RJ, Tang PD, Ruan J, Wang YF, Li DP. 2018. Chemical constituents from *Agriolimnax agrestis*. *Chin Trad Patent Medici*. 40(11): 2742- 2744.
- Liu YH, Yang X, Li JL, Guo ZY, Deng ZS, Tu X, Chen JF, Zou K. 2013. The polyketide metabolites from the endophytic fungus *Penicillium* sp. (No. 4) of *Paris polyphylla* Sm. *Nat Prod Res*. 25: 431-434, 493.
- Meng JJ, Gu G, Dang PQ, Zhang XP, Wang WX, Dai JG, Liu Y, Lai DW, Zhou LG. 2019. Sorbicillinoids from the fungus *Ustilaginoidea virens* and their phytotoxic,

- cytotoxic, and antimicrobial activities. *Front Chem.* 7: 435.
- Nguyen HV, Truong PM, Duong HT, Dinh HM, Nguyen CH. 2019. Genome sequence data of *Streptomyces* sp. SS52, an endophytic strain for daidzein biosynthesis. *Data Brief.* 27: 104746.
- Venditti A, Frezza C, Sciubba F, Serafini M, Bianco A. 2017. Primary and secondary metabolites of an European edible mushroom and its nutraceutical value: *Suillus bellinii* (Inzenga) Kuntze. *Nat Prod Res.* 31(16): 1910-1919.
- Xuan QC, Huang R, Miao CP, Chen YW, Zhai YZ, Song F, Wang T, Wu SH. 2014. Cyclonerol derivatives from *Trichoderma longibrachiatum* YM311505. *Nat Prod Commun.* 9(3): 313-314.
- Xuan QC, Huang R, Miao CP, Chen YW, Zhai YZ, Song F, Wang T, Wu SH. 2014. Secondary metabolites of endophytic fungus *Trichoderma* sp YM 311505 of *Azadirachta indica*. *Chem Nat Compd+*. 50(1): 139-141.