Supporting Information

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Secondary Metabolites from the Coral-derived Fungus *Aspergillus terreus* SCSIO41404 with Pancreatic Lipase Inhibitory Activities

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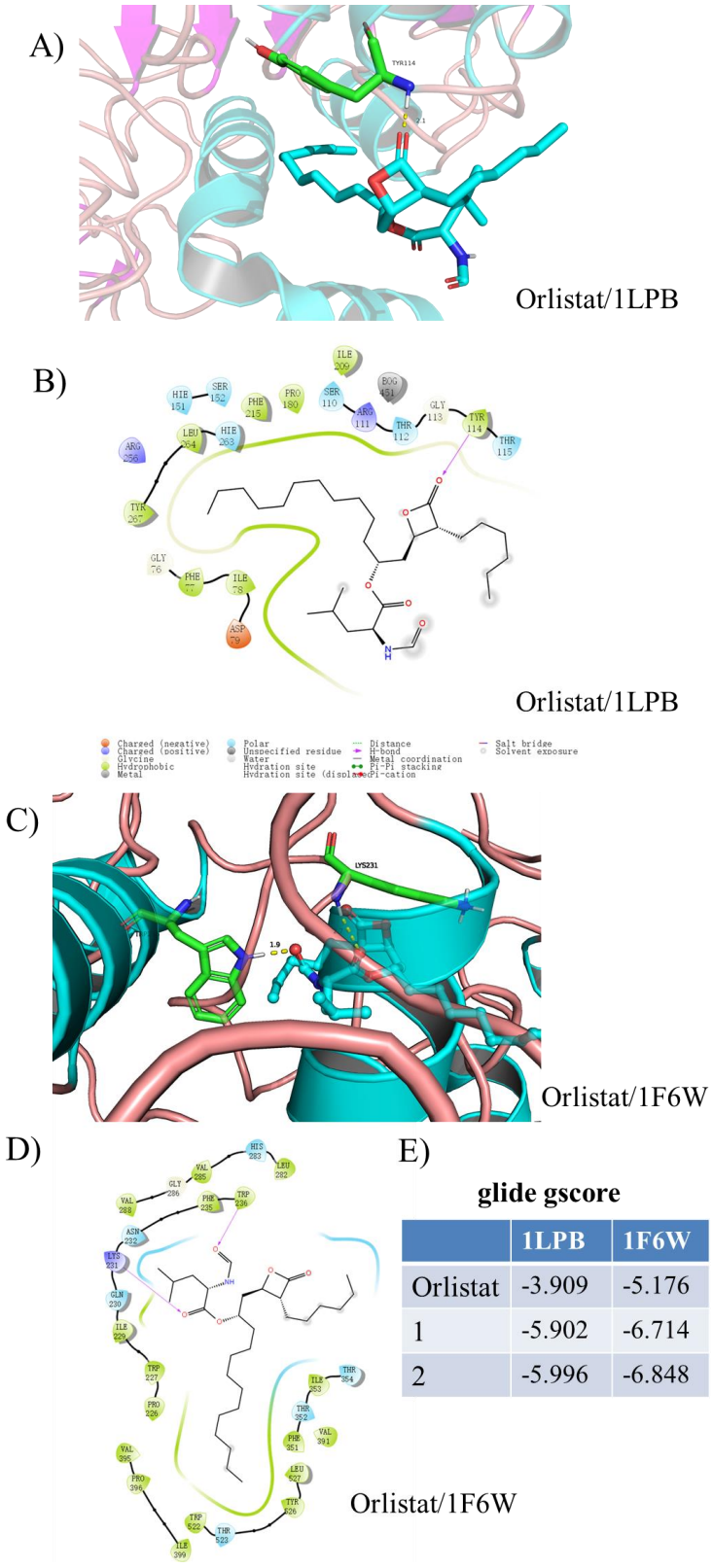
Table of Contents	Page



glide gscore

	1LPB	1F6W
Orlistat	-3.909	-5.176
1	-5.902	-6.714
2	-5.996	-6.848

Figure S1: Molecular docking of Orlistat with PL (1LPB and 1F6W) and the scores.

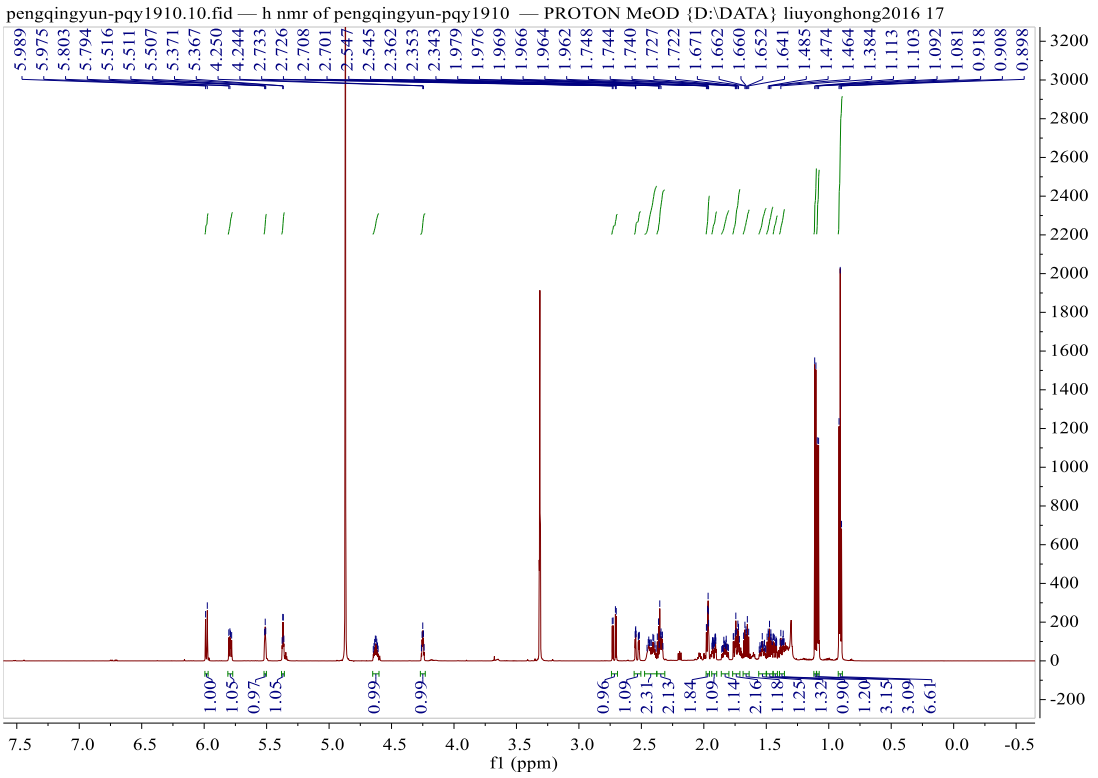


Figure S2: ^{1}H NMR spectrum of **1** (CD_3OD, 700 MHz).

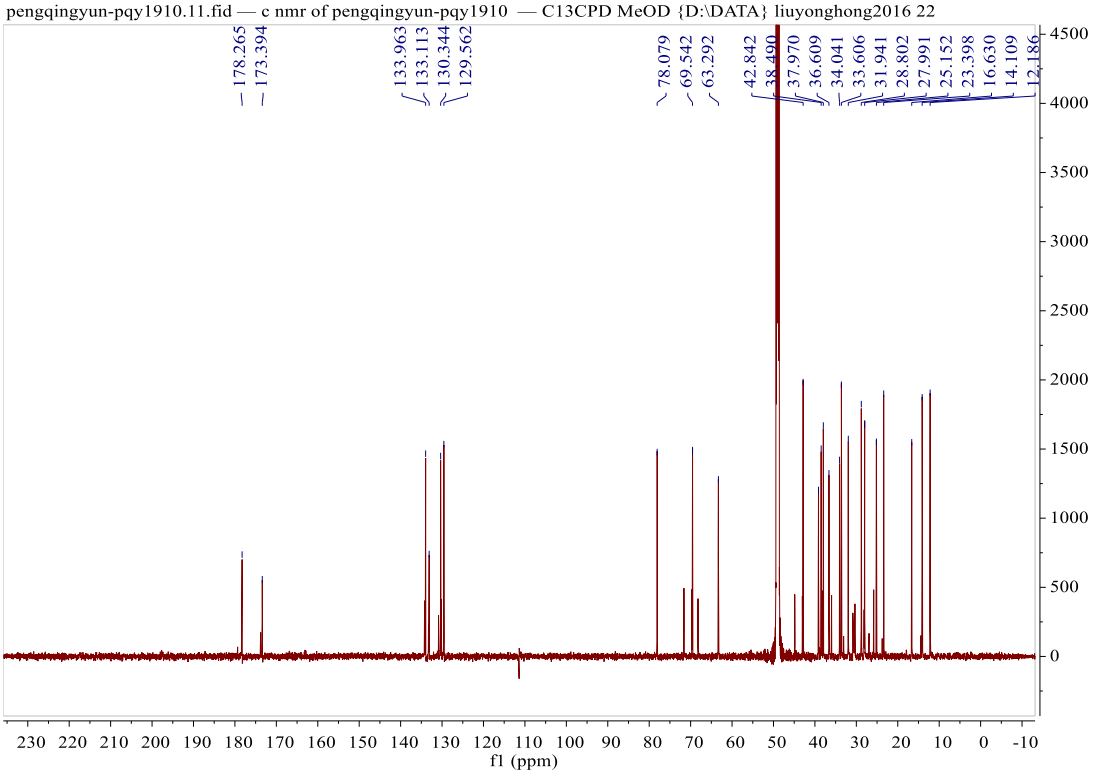


Figure S3: ^{13}C NMR spectrum of **1** (CD_3OD, 175 MHz).

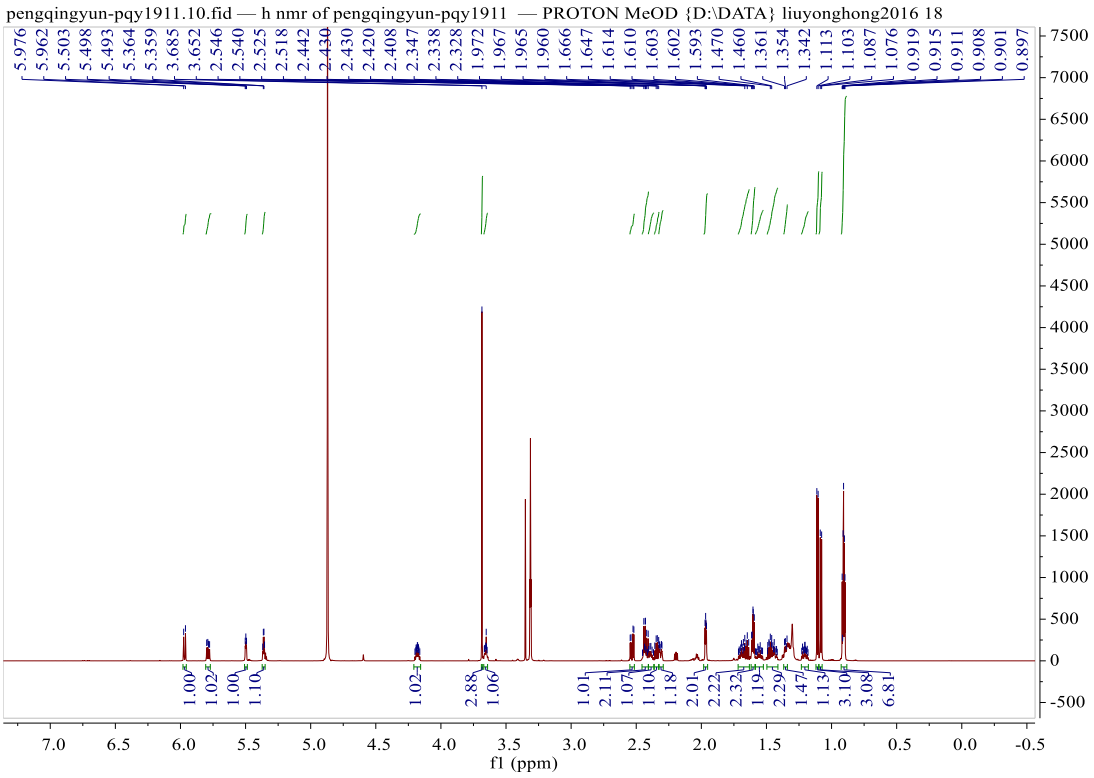


Figure S4: ^{1}H NMR spectrum of **2** (CD_3OD, 700 MHz).

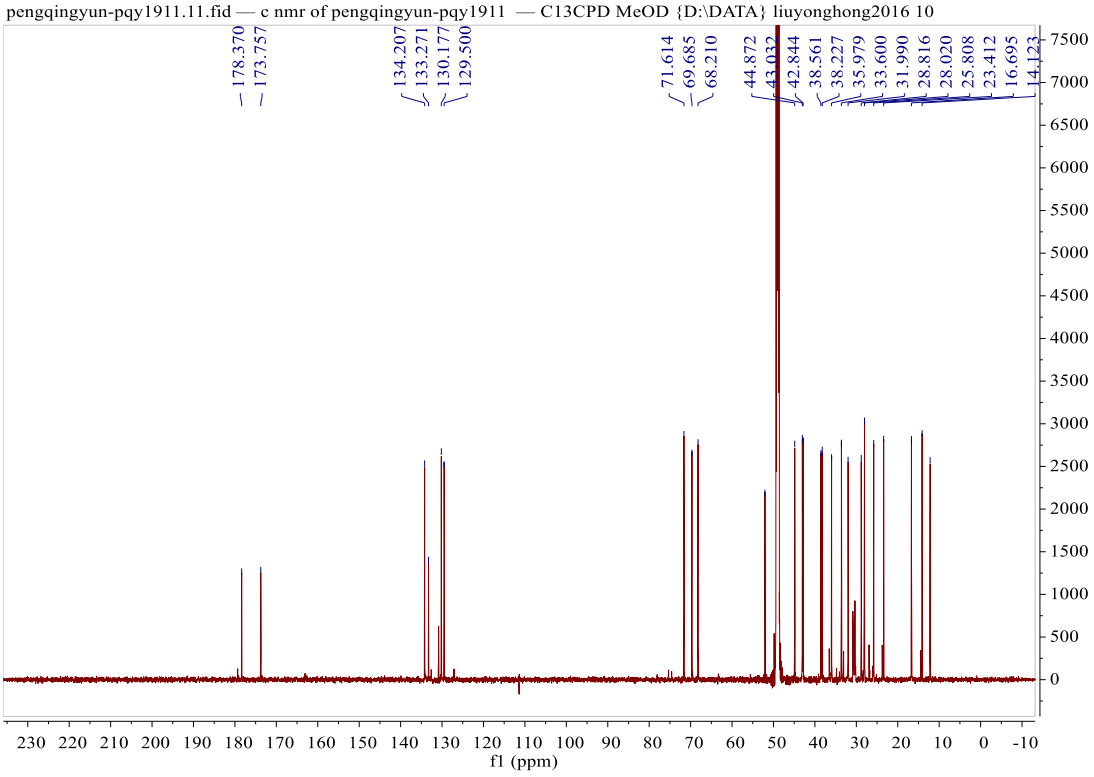


Figure S5: ^{13}C NMR spectrum of **2** (CD_3OD, 175 MHz).

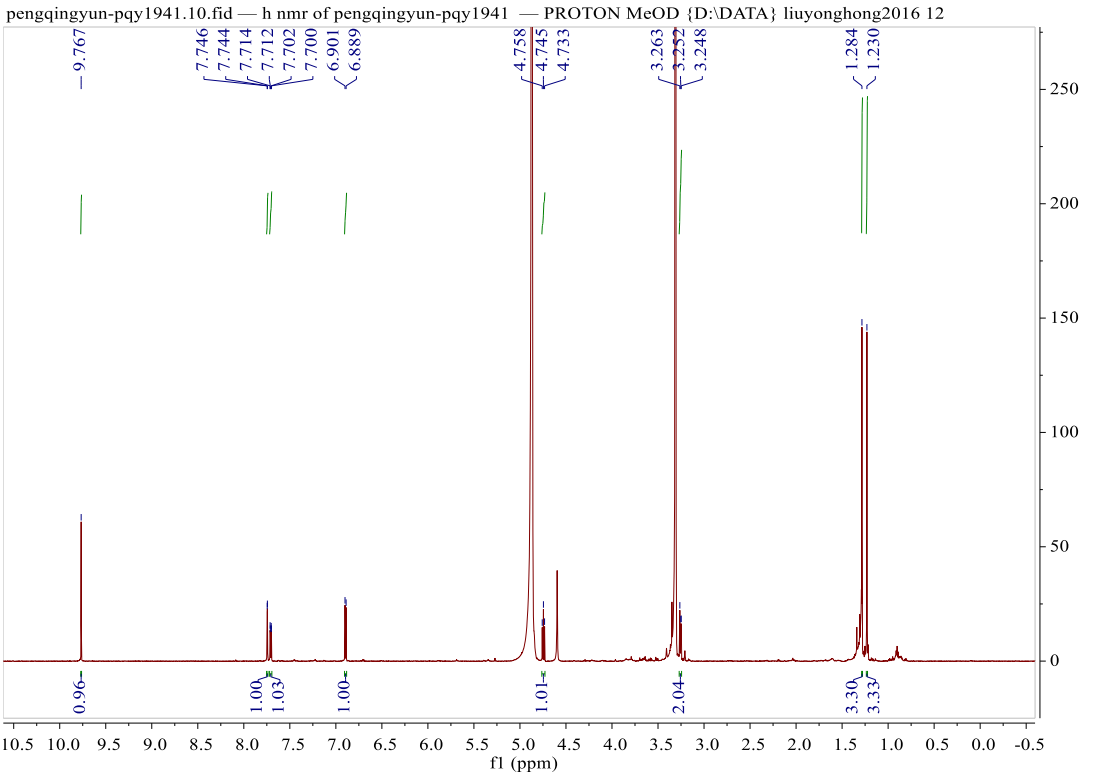


Figure S6: ^{1}H NMR spectrum of **3** (CD_3OD, 700 MHz).

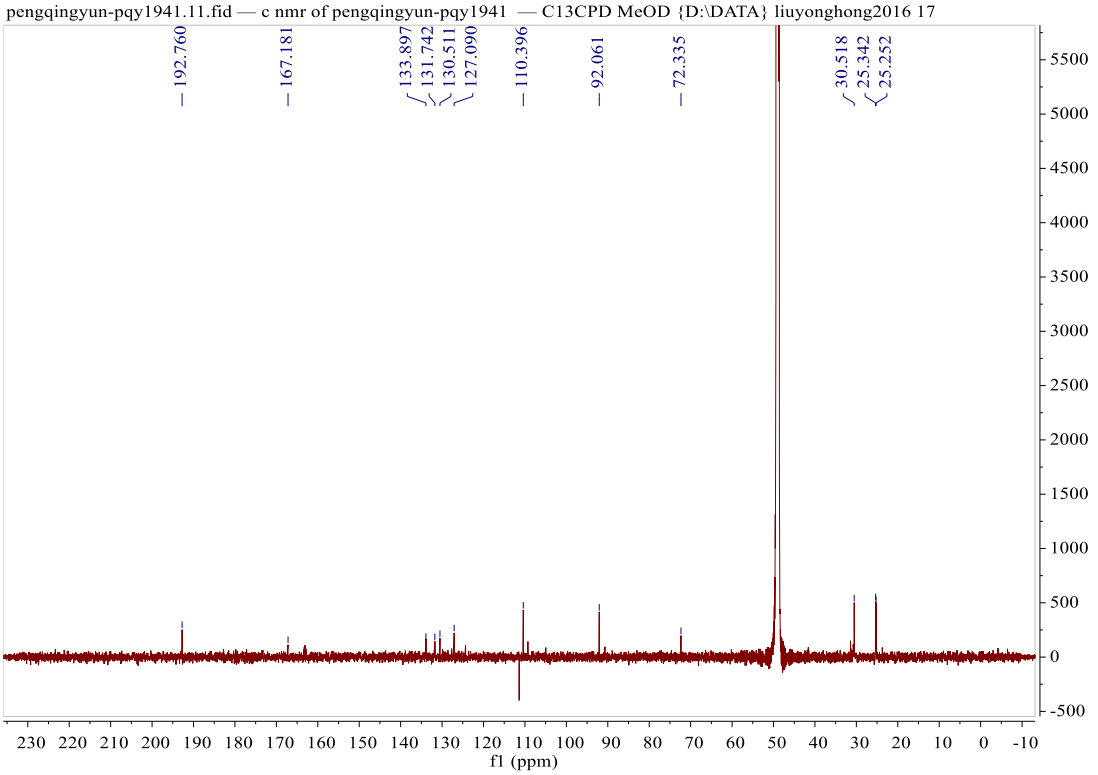


Figure S7: ^{13}C NMR spectrum of **3** (CD_3OD, 175 MHz).

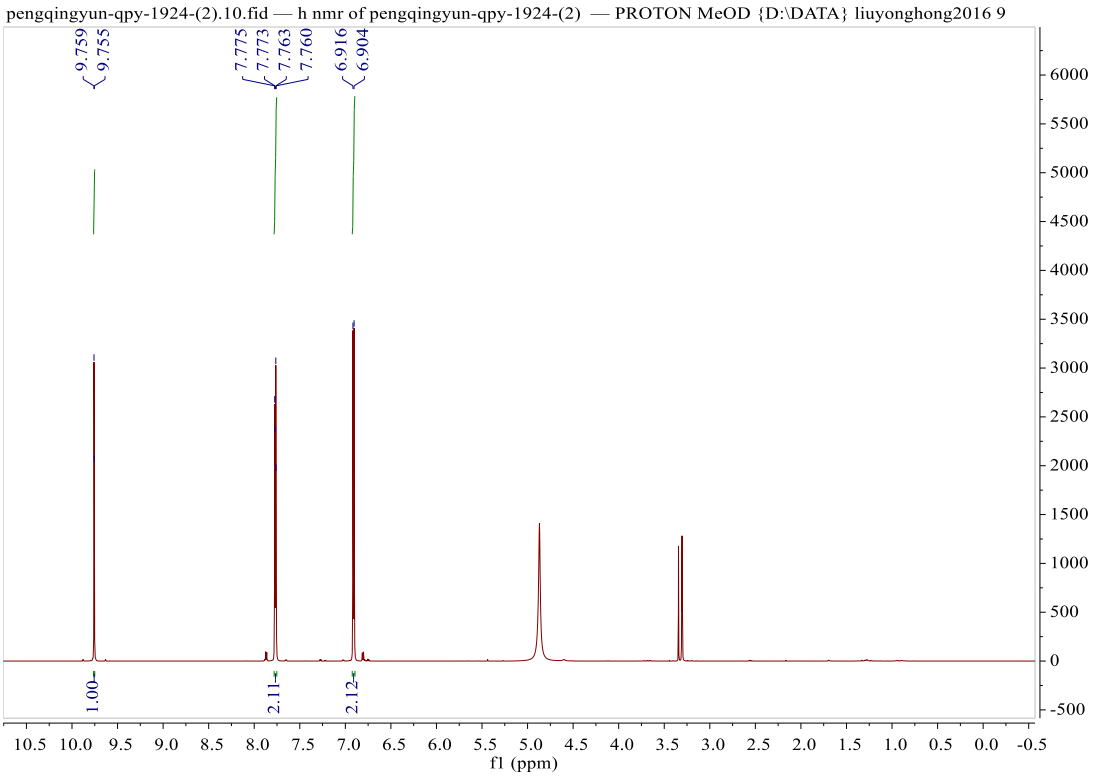


Figure S8: ^{1}H NMR spectrum of **4** (CD_3OD, 700 MHz).

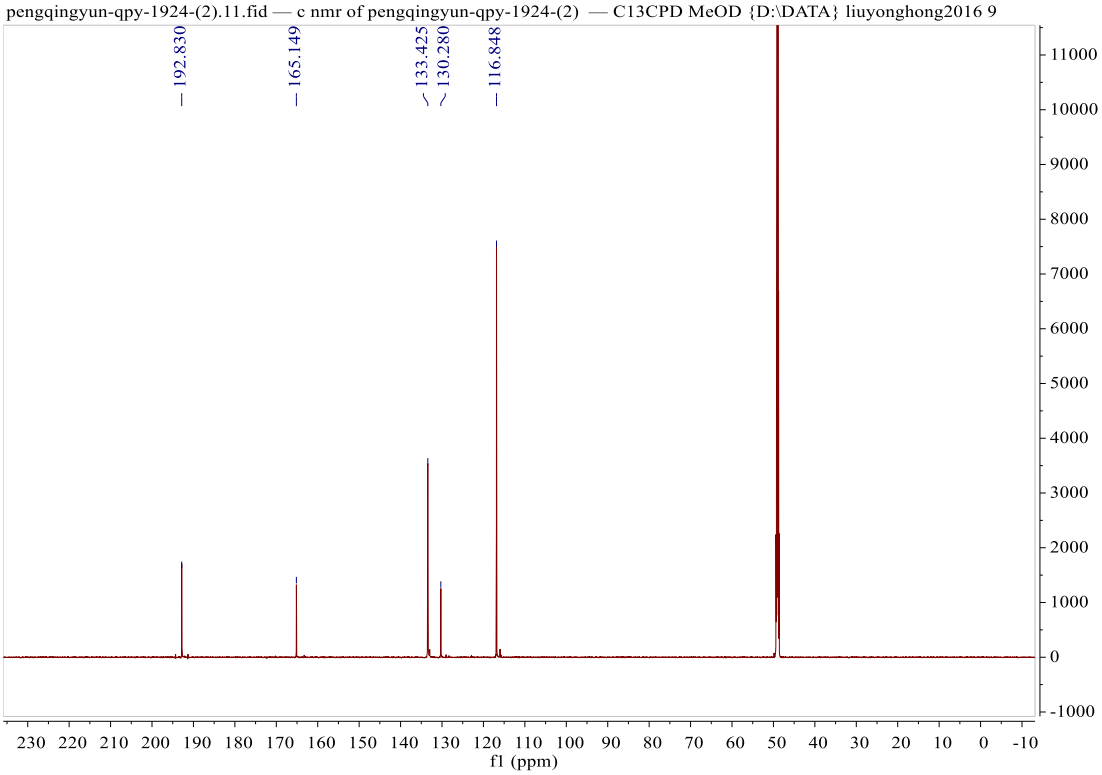


Figure S9: ^{13}C NMR spectrum of **4** (CD_3OD, 175 MHz).

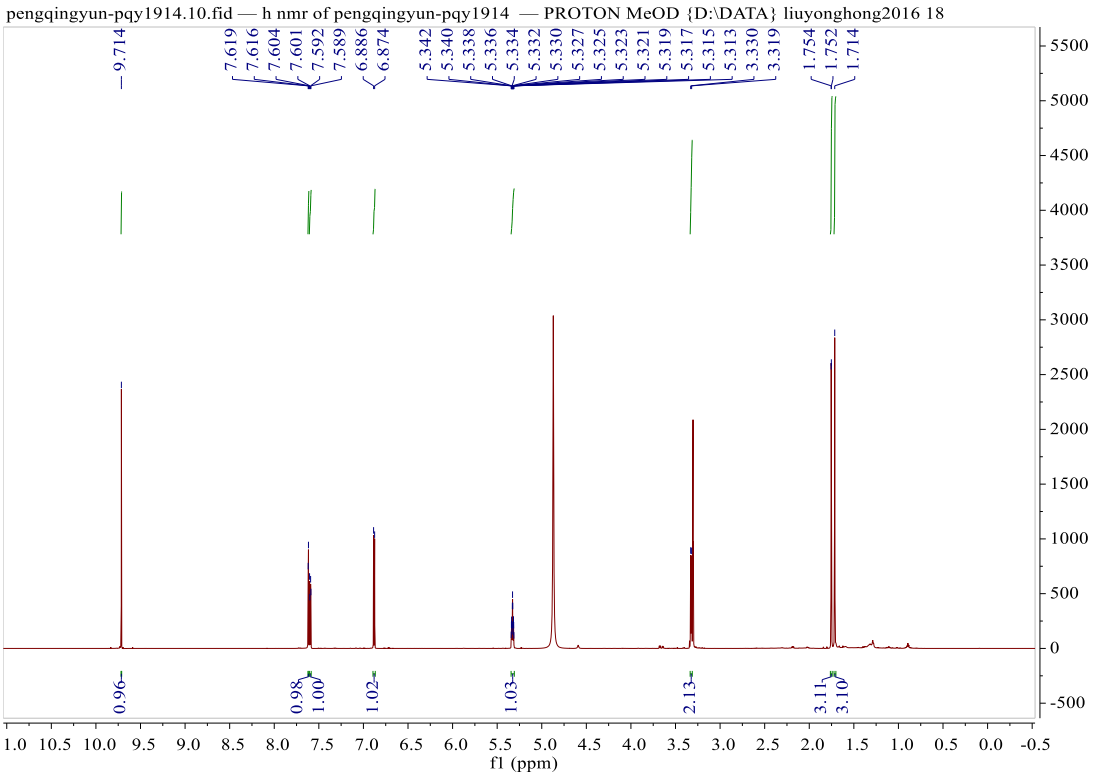


Figure S10: ^{1}H NMR spectrum of **5** (CD_3OD, 700 MHz).

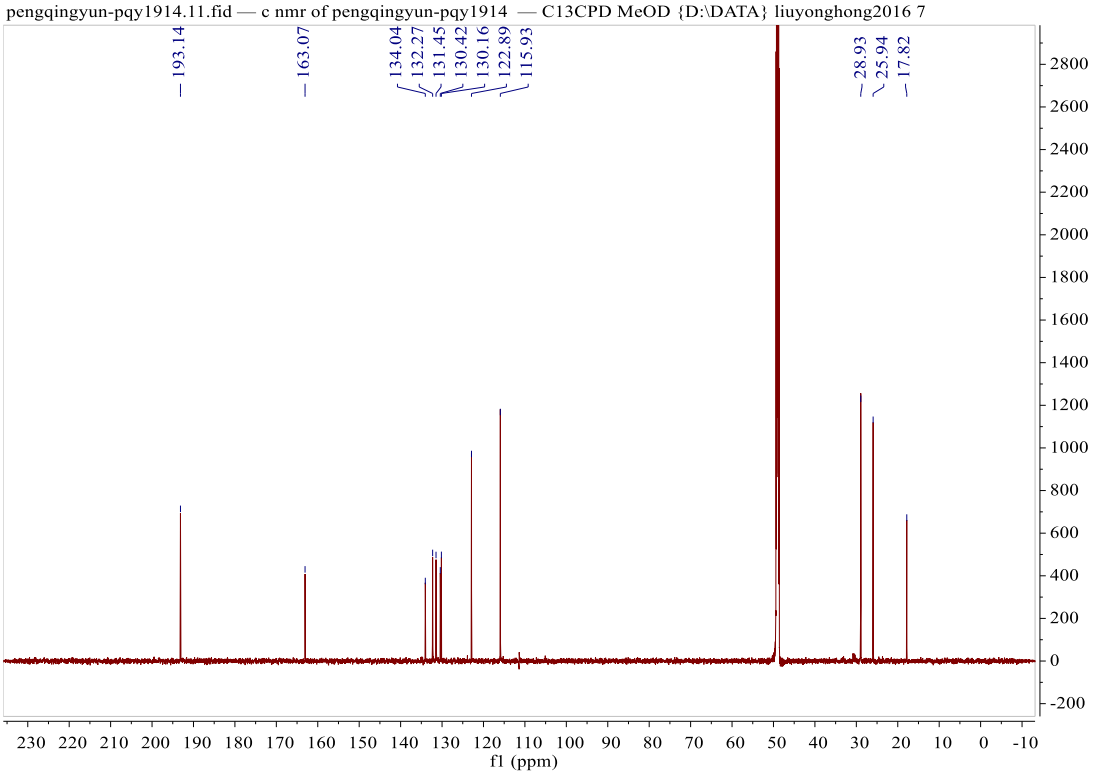


Figure S11: ^{13}C NMR spectrum of **5** (CD_3OD, 175 MHz).

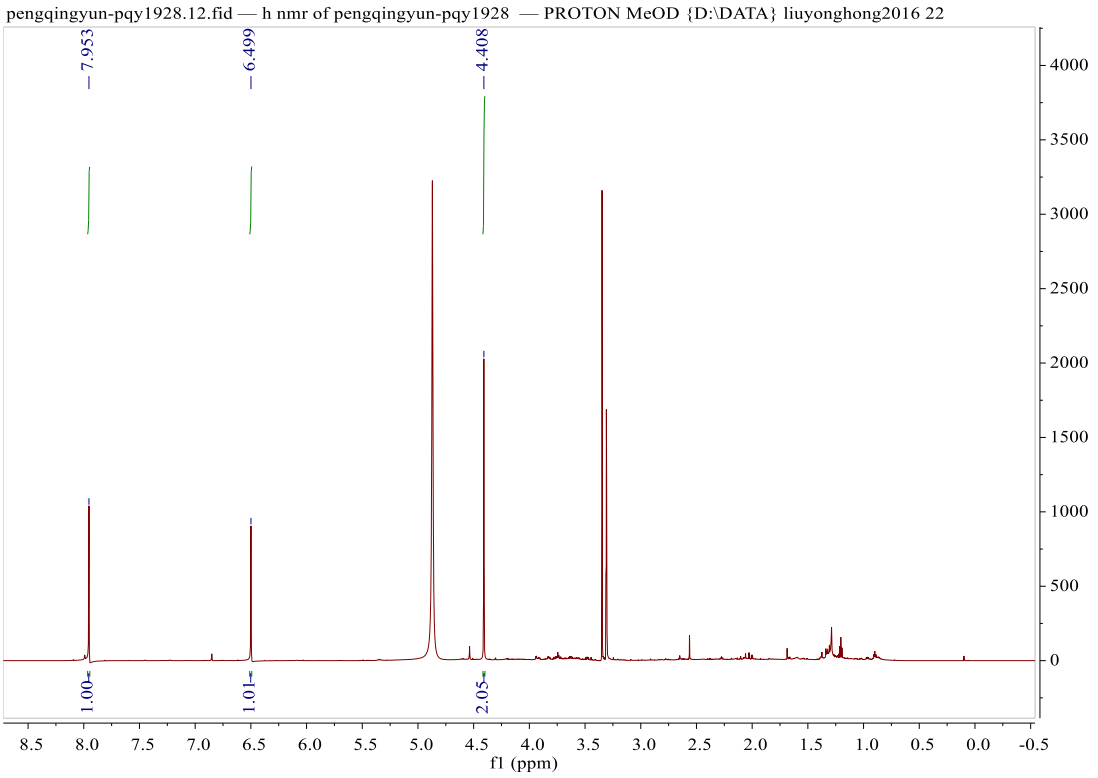


Figure S12: ^{1}H NMR spectrum of **6** (CD_3OD, 700 MHz).

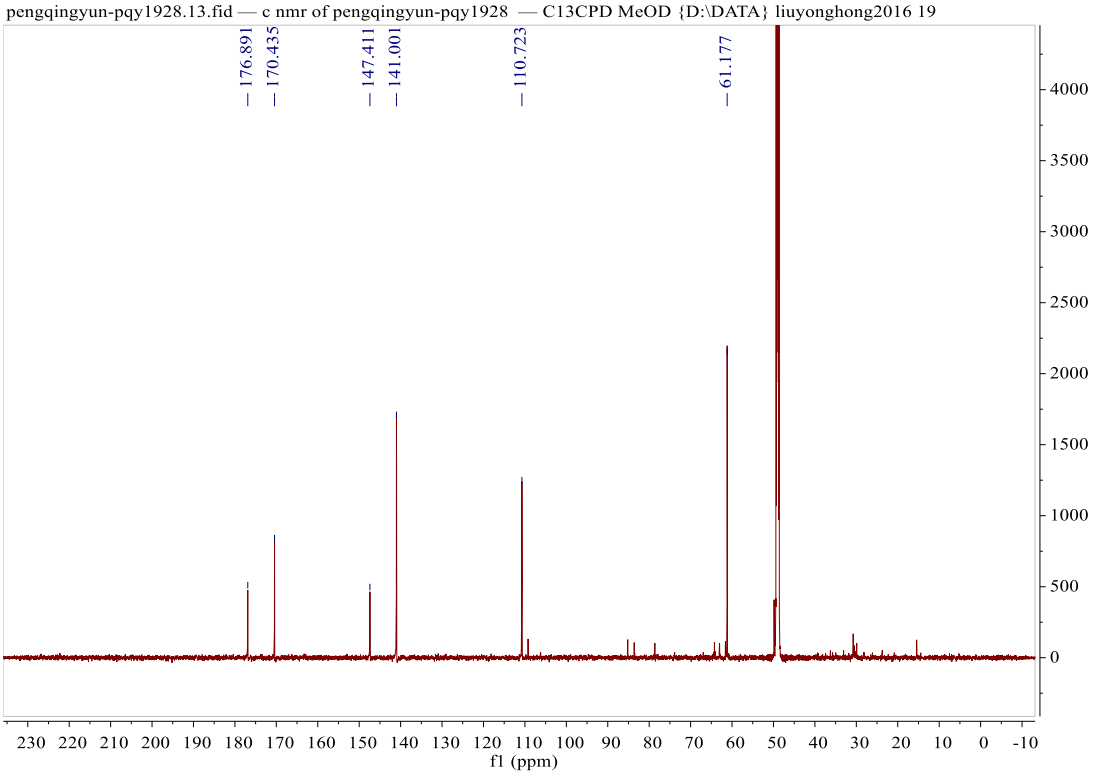


Figure S13: ^{13}C NMR spectrum of **6** (CD_3OD, 175 Hz).

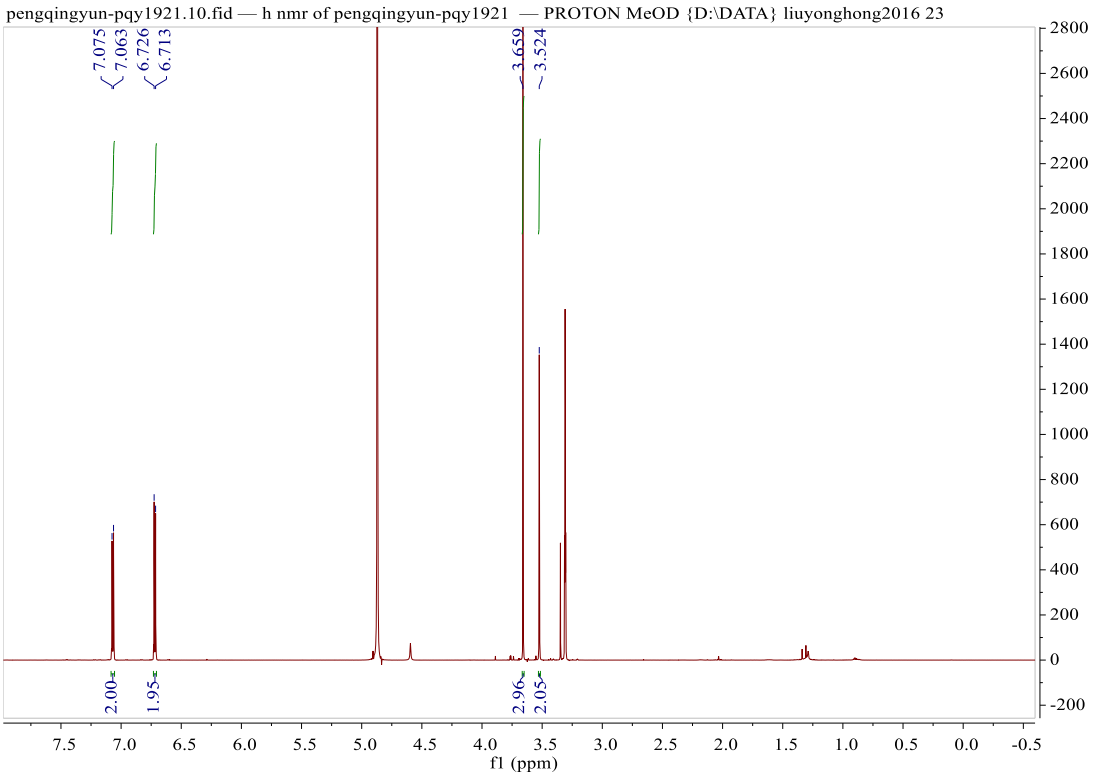


Figure S14: ^{1}H NMR spectrum of **7** (CD_3OD, 700 MHz).

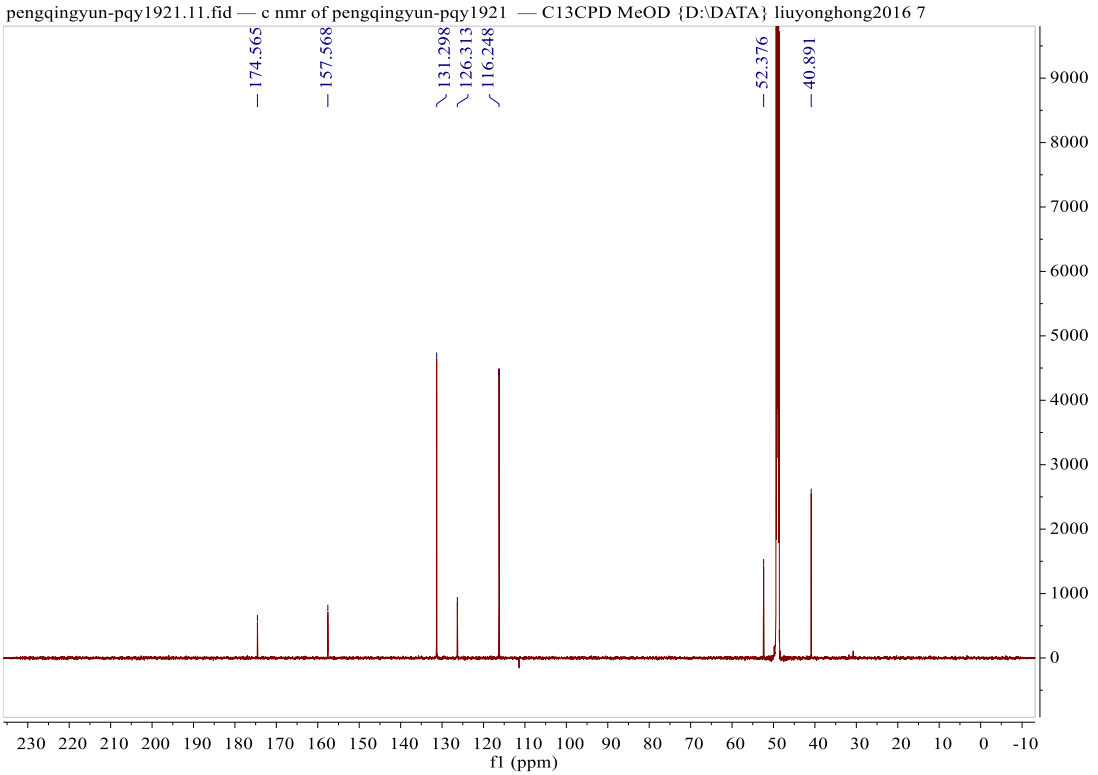


Figure S15: ^{13}C NMR spectrum of **7** (CD_3OD, 175 MHz).

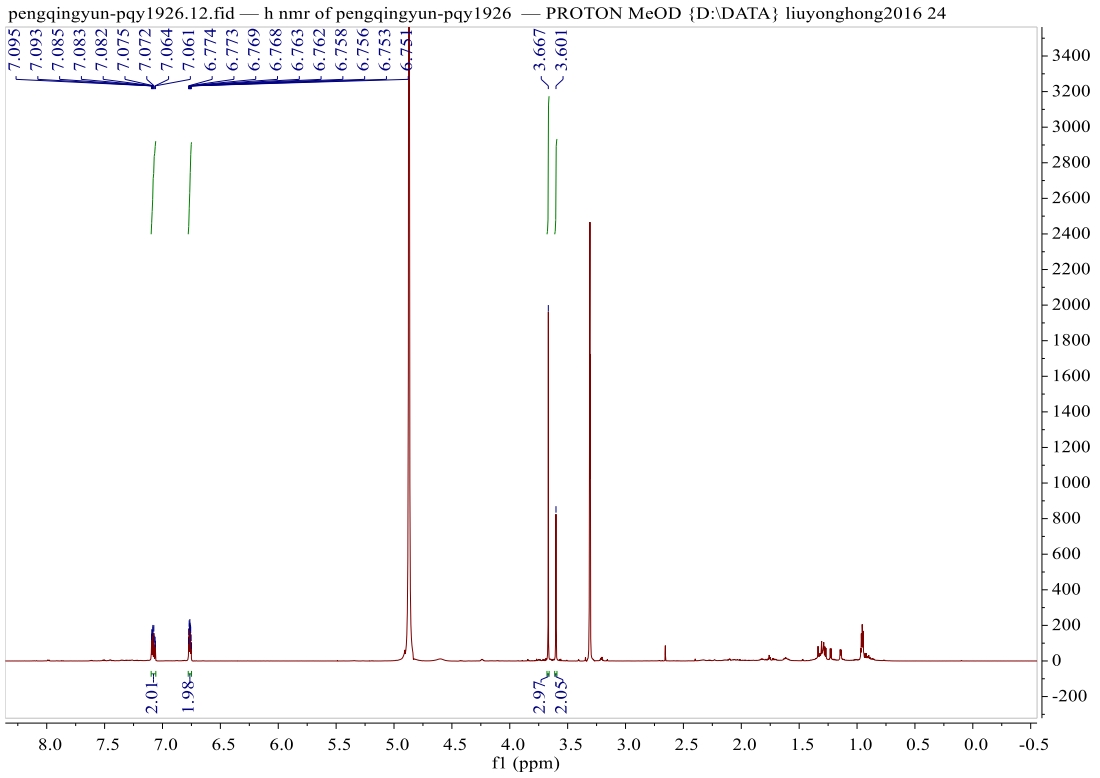


Figure S16: ^{1}H NMR spectrum of **8** (CD_3OD, 700 MHz).

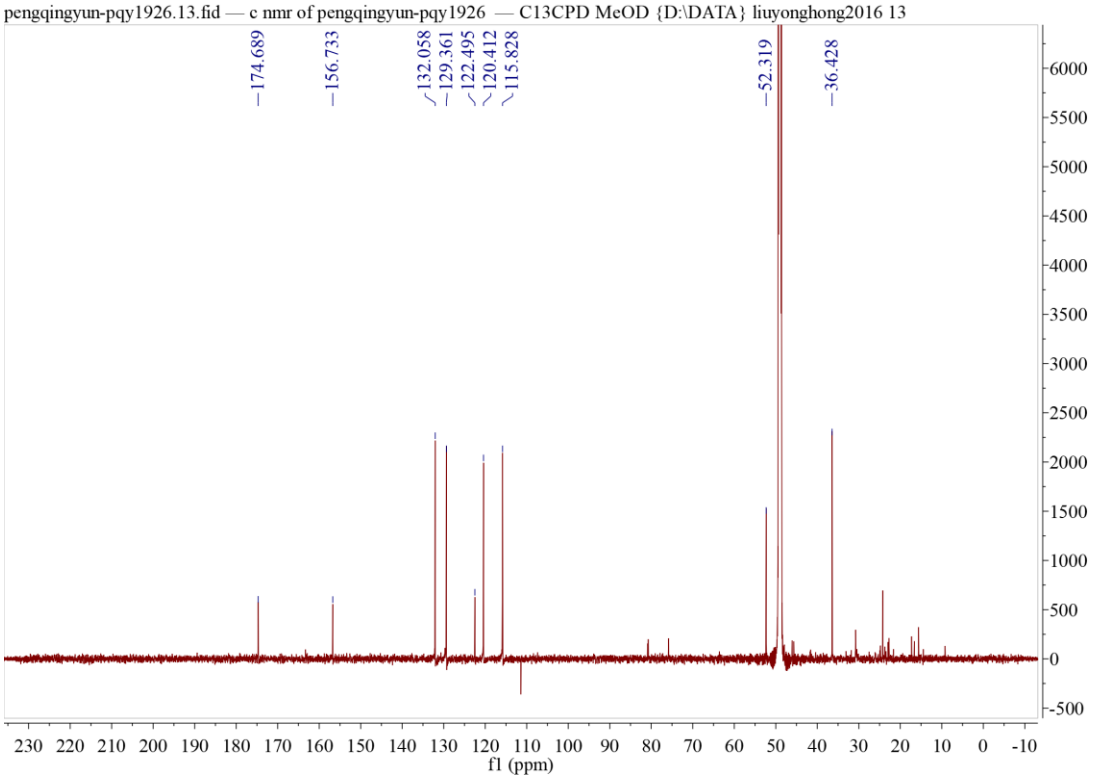


Figure S17: ^{13}C NMR spectrum of **8** (CD_3OD, 175 MHz).

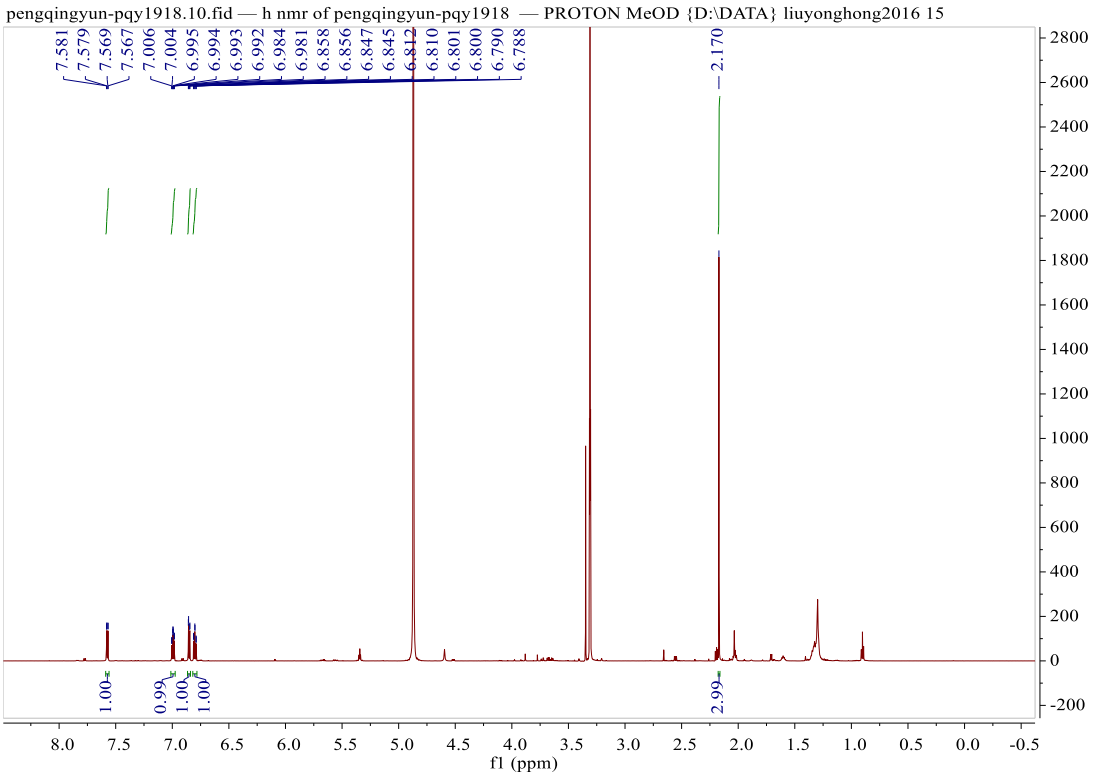


Figure S18: ^{1}H NMR spectrum of **9** (CD_3OD, 700 MHz).

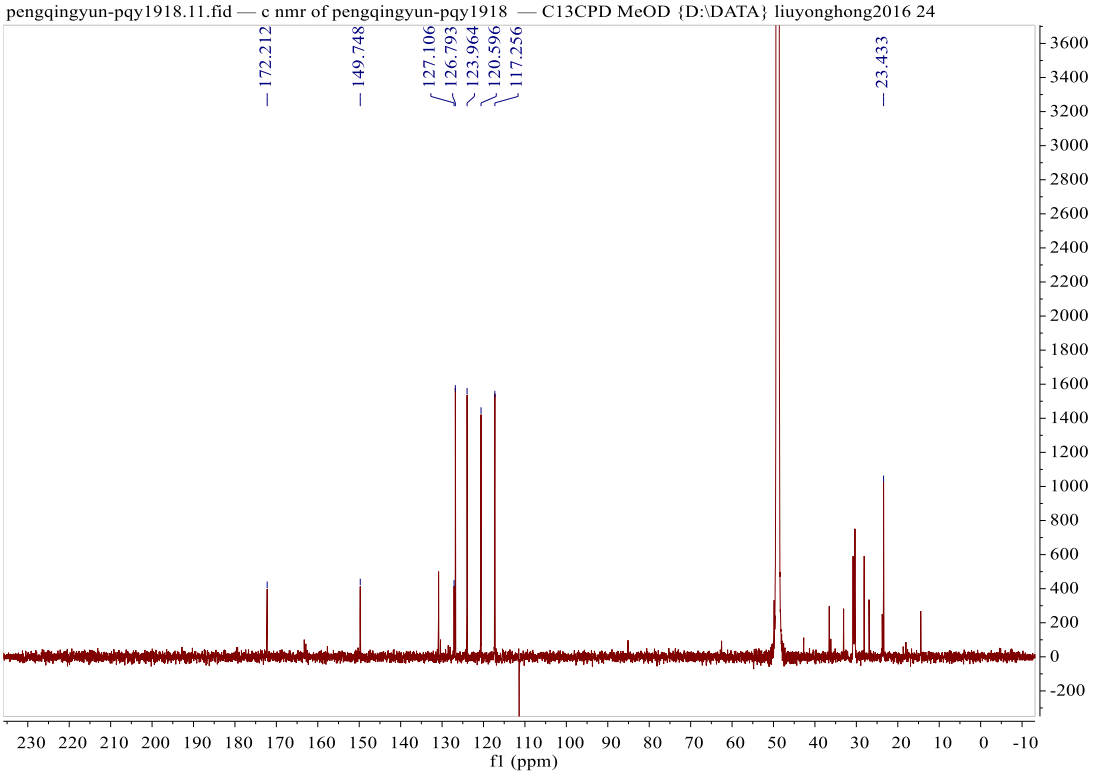


Figure S19: ^{13}C NMR spectrum of **9** (CD_3OD, 175 MHz).

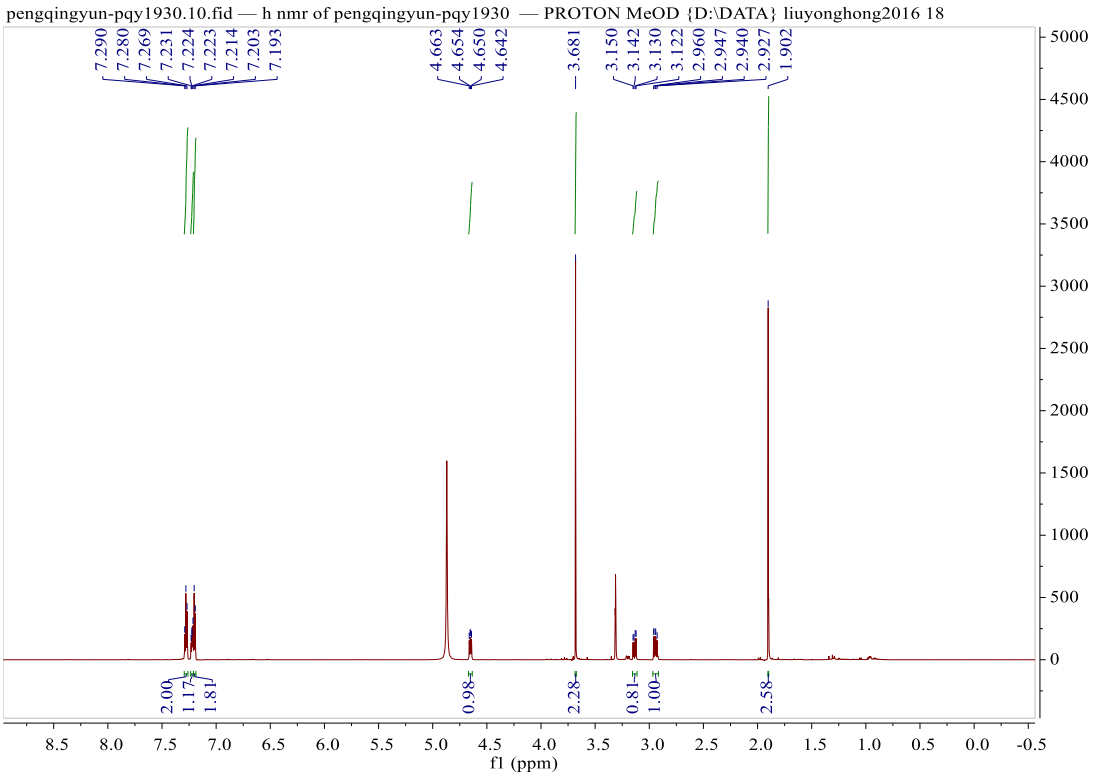


Figure S20: ^{1}H NMR spectrum of **10** (CD_3OD, 700 MHz).

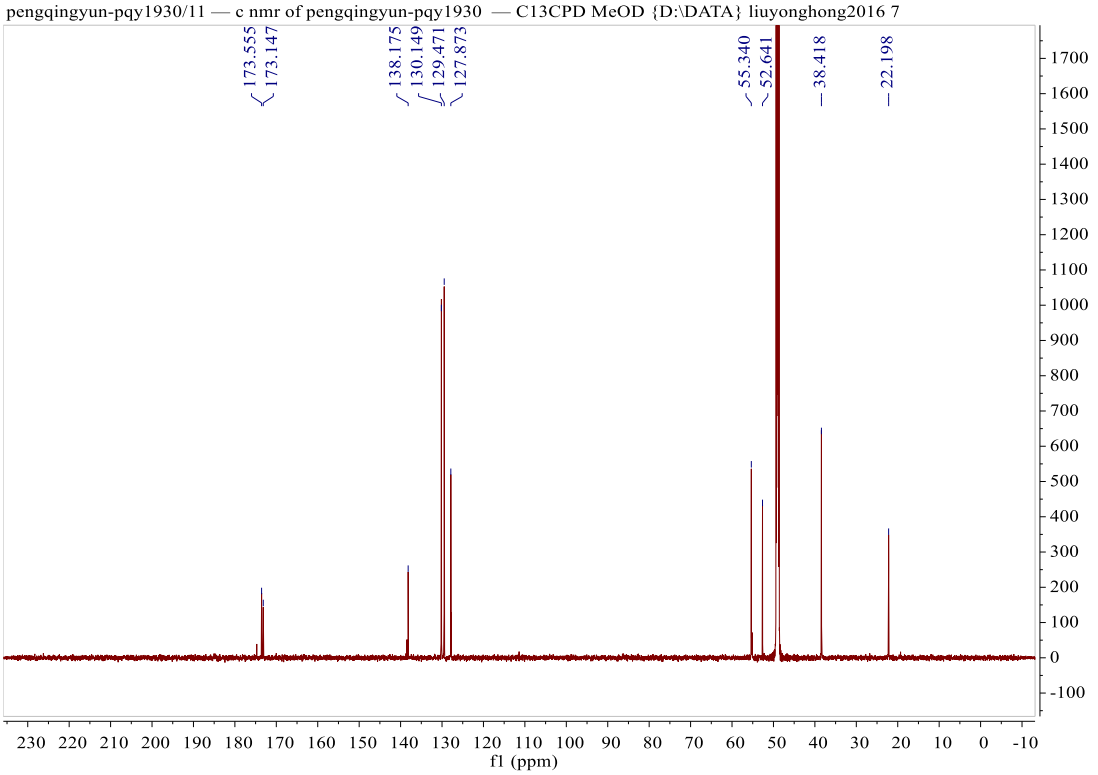


Figure S21: ^{13}C NMR spectrum of **10** (CD_3OD, 175 MHz).

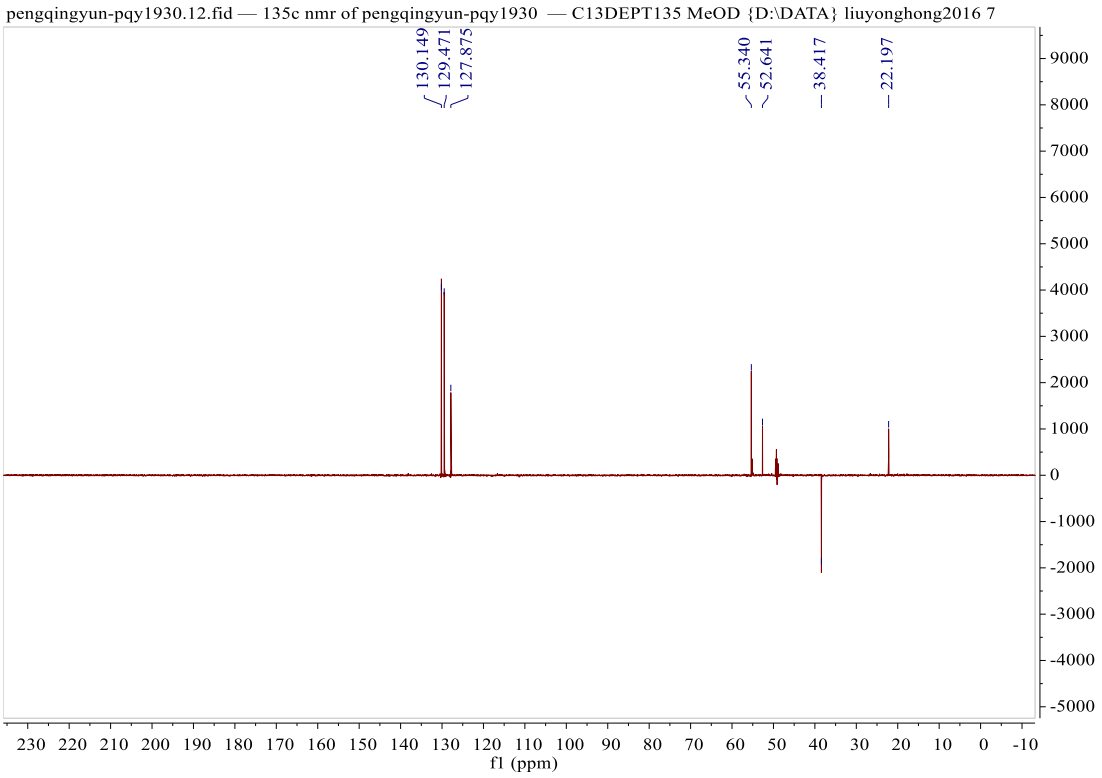


Figure S22: DEPT spectrum of **10** (CD_3OD).

S1. Experimental

ITS sequence of the strain Aspergillus terreus SCSIO41404.

GGTAAAGCGCTGGTCTTATGGCCACCTCCCACCCGTGACTATTGTACCTTGTTGCTTCGGC
GGGCCCGCCAGCGTTTGCTGGCCGCCGGGGGGCGACTCGCCCCCGGGCCCGTGCCCGCCG
GAGACCCCAACATGAACCCTGTTCTGAAAGCTTGCAGTCTGAGTGTGATTCTTTGCAATC
AGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAAT
GCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGC
GCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGC
TTGTGTGTTGGGCCCTCGTCCCCCGGCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGC
ACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCTTCCGCTCCGTAGGCCCGGCCGGCG
CCCGCCGACGCATTTATTTGCAACTTGTTTTTTTTTCCAGGTTGACCTCGGATCAGGTAGG
GATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

Fermentation and Extraction

The strain *Aspergillus terreus* SCSIO41404 was cultured in 4×500 mL Erlenmeyer flasks, each containing 150 mL of the seed medium (malt extract 15 g, sea salt 10 g, H_2O 1 L, pH 7.4–7.8) at 28 °C for 3 days on a rotating shaker (180 rpm). The seed medium was added to the wheat fermentation medium (wheat 200 g, sea salt 10g, H_2O 200 mL) in 1000 mL Erlenmeyer flask. Totally, 40 Erlenmeyer flasks were incubated for 30 days at 25 °C without shaking. The whole wheat cultures were crushed and extracted with acetone three times to afford an organic extract (638.1 g), which was followed partitioned with different solvents and obtained 380.0 g oily extract, 57.3 g petroleum extract and 160.0 g methanol extract.

Isolation and Purification

The methanol extract was subjected to silica gel column chromatography and separated by silica gel column eluted with PE-EtOAc-MeOH (50:1:0 to 0:0:1, *v*/*v*) in gradient to yield seven fractions (Frs.1–7). Fr.1 was subjected to MPLC with an ODS column, eluting with MeOH/H_2O (5%–100%) to afford six subfractions (Frs.1-1–1-6). Similarly, Fr.2 and Fr.6 were divided into Frs.2-1–2-8 and Frs.6-1–6-8, respectively. Those subfractions were separated and purified by repeated semipreparative HPLC (12-60% MeOH/H_2O, 2 mL/min). Compounds **1** (3.9 mg) and **2** (4.6 mg) were obtained from Fr.1-5 by HPLC (47% MeOH/H_2O). Compounds **8** (2.0 mg), **9** (1.0 mg) and **5** (10.2 mg) were yielded by HPLC (47% MeOH/H_2O) from Fr.1-2, Fr.1-3, and Fr.1-6, respectively. Under 60% MeOH/H_2O HPLC separation, **4** (2.5 mg) was purified from Fr.2-3, while **7** (1.4 mg) was obtained from Fr.2-4 by 35% MeOH/H_2O. Fr.6-1 was separated by HPLC (12% MeOH/H_2O) to yield **6** (5.9 mg). Compounds **3** (2.4 mg) and **10** (8.1 mg) were obtained under 42% MeOH/H_2O HPLC separation from Fr.6-5 and Fr.6-4, respectively.

Spectroscopic data of the compounds

Monacolin K (**1**): Colorless needles; (700 MHz, CD_3OD) δ_H 5.98 (1H, d, J = 9.7 Hz, H-4), 5.79 (1H, dd, J = 9.7, 6.1 Hz, H-3), 5.51 (1H, brt, J = 3.1 Hz, H-5), 5.37 (1H, dd, J = 3.2, 6.2 Hz, H-8), 4.63 (1H, m, H-11), 4.25 (1H, m, H-13), 2.72 (1H, dd, J = 17.7, 4.7 Hz, H_a-14), 2.53 (1H, ddd, J = 17.7, 3.3, 1.9 Hz, H_b-14), 2.42 (2H, overlapped, H-6, H-2), 2.35 (2H, overlapped, H-8a, H-2′), 1.97

(2H, overlapped, H_a-7, H-1), 1.92 (1H, m, H_a-12), 1.83 (1H, m, H_a-10), 1.73 (2H, overlapped, H_b-7, H_b-12), 1.66 (1H, m H_a-3′), 1.53 (1H, m, H_a-9), 1.47 (1H. m, H_b-3′), 1.43 (1H. m, H_b-9), 1.37 (1H. m, H_b-10), 1.11 (3H, d, J = 6.9 Hz, 2′-CH_3), 1.09 (3H, d, J = 7.4 Hz, 6-CH_3), 0.91 (6H,overlapped, 2-CH_3, H-4′); ^{13}C NMR (175 MHz, CD_3OD) δ_C 178.3 (qC, C-1′), 173.4 (qC, C-15), 134.0 (CH, C-3), 133.1 (qC, C-4a), 130.3 (CH, C-5), 129.6 (CH, C-4), 78.1 (CH, C-11), 69.5 (CH, C-8), 63.3 (CH, C-13), 42.8 (CH, C-2′), 39.1 (CH_2, C-14), 38.5 (CH, C-8a), 38.0 (CH, C-1), 36.6 (CH_2, C-12), 34.0 (CH_2, C-10), 33.6 (CH_2, C-7), 31.9 (CH, C-2), 28.8 (CH, C-6), 28.0 (CH_2, C-3′), 25.2 (CH_2, C-9), 23.4 (CH_3, 6-CH_3), 16.6 (CH_3, 2′-CH_3), 14.1 (CH_3, 2-CH_3), 12.2 (CH_3, C-4′).

Methyl ester of lactone ring-opened monacolin K (**2**): Colorless oil; ^{1}H NMR (700 MHz, CD_3OD) δ_H 5.97 (1H, d, J = 9.7 Hz, H-4), 5.79 (1H, dd, J = 9.7, 6.0 Hz, H-3), 5.50 (1H, brt, J = 3.2 Hz, H-5), 5.36 (1H, dd, J = 3.2, 6.4 Hz, H-8), 4.18 (1H, m, H-13), 3.68 (3H, s, H-16), 3.66 (1H, m, H-11), 2.53 (1H, dd, J = 15.2, 4.6 Hz, H_a-14), 2.42 (2H,overlapped, H_b-14, H-6), 2.39 (1H, m, H-2), 2.34 (1H, m, H-8a), 2.31 (1H, m, H-2′), 1.97 (2H, overlapped, H_a-7, H-1), 1.67 (2H, overlapped, H_a-12, H_a-3′), 1.60 (2H, overlapped, H_b-12, H_b-7), 1.55 (1H, m, H_a-10), 1.46 (2H, overlapped, H_a-9, H_b-3′), 1.35 (1H, m, H_b-9), 1.21 (1H, m, H_b-10), 1.11 (3H, d, J = 6.9 Hz, 2′-CH_3), 1.08 (3H, d, J = 7.4 Hz, 6-CH_3), 0.91 (6H,overlapped, 2-CH_3, H-4′); ^{13}C NMR (175 MHz, CD_3OD) δ_C 178.4 (qC, C-1′), 173.8 (qC, C-15), 134.2 (CH, C-3), 133.3 (qC, C-4a), 130.2 (CH, C-5), 129.5 (CH, C-4), 71.6 (CH, C-11), 69.7 (CH, C-8), 68.2 (CH, C-13), 52.1 (CH_3, C-16), 44.9 (CH_2, C-14), 43.0 (CH_2, C-12), 42.8 (CH, C-2′), 38.6 (CH, C-8a), 38.2 (CH, C-1), 36.0 (CH_2, C-10), 33.6 (CH_2, C-7), 32.0 (CH, C-2), 28.8 (CH, C-6), 28.0 (CH_2, C-3′), 25.8 (CH_2, C-9), 23.4 (CH_3, 6-CH_3), 16.7 (CH_3, 2′-CH_3), 14.1 (CH_3, 2-CH_3), 12.2 (CH_3, C-4′).

Asperterreusine C (**3**): Colorless needles; $[\alpha]_D^{25}$ = -123.5 (*c* 0.1, MeOH); ^{1}H NMR (700 MHz, CD_3OD) δ_H 9.77 (1H, s, H-7), 7.74 (1H, d, J = 1.6 Hz, H-2), 7.71 (1H, dd, J = 8.2, 1.6 Hz, H-6), 6.89 (1H, d, J = 8.2 Hz, H-5), 4.74 (1H, dd, J = 9.2, 8.6 Hz, H-9), 3.26 (2H, m, H-8), 1.28 (3H, s, H-11), 1.23 (3H, s, H-12); ^{13}C NMR (175 MHz, CD_3OD) δ_C 192.8 (CH, C-7), 167.2 (qC, C-4), 133.9 (CH, C-6), 131.7 (qC, C-3), 130.5 (qC, C-1), 127.1 (CH, C-2), 110.4 (CH, C-5), 92.1 (CH, C-9), 72.3 (qC, C-10), 30.5 (CH_2, C-8), 25.3 (CH_3, C-11), 25.2 (CH_3, C-12) .

4-Hydroxybenzaldehyde (**4**): White solid; ^{1}H NMR (700 MHz, CD_3OD) δ_H 9.76 (1H, d, J = 2.0 Hz, H-7), 7.77 (2H, dd, J = 8.7, 2.0 Hz, H-2, H-6), 6.90 (2H, d, J = 8.7 Hz, H-3, H-5); ^{13}C NMR (175 MHz, CD_3OD) δ_C 192.8 (CH, C-7), 165.1 (qC, C-4), 133.4 (CH, C-2, C-6), 130.3 (qC, C-1), 116.8 (CH, C-3, C-5).

4-Hydroxy-3-(3-methylbut-2-en-1-yl) benzaldehyde (**5**): White solid; ^{1}H NMR (700 MHz, CD_3OD) δ_H 9.71 (1H, s, H-7), 7.62 (1H, d, J = 2.2 Hz, H-2), 7.60 (1H, dd, J = 8.2, 2.2 Hz, H-6), 6.88 (1H, d, J = 8.2 Hz, H-5), 5.33 (1H, m, H-9), 3.32 (2H, d, J = 7.5 Hz, H-8), 1.75 (3H, d, J = 1.3 Hz, H-11), 1.71 (3H, s, H-12); ^{13}C NMR (175 MHz, CD_3OD) δ_C 193.1 (CH, C-7), 163.1 (qC, C-4), 134.0 (CH, C-2), 132.3 (qC, C-1), 131.5 (qC, C-10), 130.4 (qC, C-3), 130.2 (CH, C-6), 122.9 (CH, C-9), 115.9 (CH, C-5), 28.9 (CH_2, C-8), 25.9 (CH_3, C-11), 17.8 (CH_3, C-12).

Kojic acid (**6**): Colorless needles; ^{1}H NMR (700 MHz, CD_3OD) δ_H 7.95 (1H, s, H-6), 6.50 (1H, s, H-3), 4.41 (2H, s, H-7); ^{13}C NMR (176 MHz, CD_3OD) δ_C 176.9 (qC. C-4), 170.4 (qC, C-2), 147.4 (qC, C-5), 141.0 (CH, C-6), 110.7 (CH, C-3), 61.2 (CH_2, C-7).

p-Hydroxyphenylacetic acid methyl ester (**7**): Colorless needles; ^{1}H NMR (700 MHz, CD_3OD) δ_H 7.07 (2H, d, *J* = 8.6 Hz, H-2, H-6), 6.72 (2H, d, *J* = 8.6 Hz, H-3, H-5), 3.66 (3H, s, 8-OMe), 3.52 (2H, s, H-7)；^{13}C NMR (175 MHz, CD_3OD) δ_C 174.6 (qC, C-8), 157.6 (qC, C-4), 131.3 (CH, C-2, C-6), 126.3 (qC, C-1), 116.2 (CH, C-3, C-5), 52.4 (CH_3, 8-OMe), 40.9 (CH_2, C-7).

o-Hydroxyphenylacetic acid methyl ester (**8**): White solid; ^{1}H NMR (700 MHz, CD_3OD) δ_H 7.08 (2H, overlapped, H-4, H-6), 6.76 (2H, overlapped, H-3, H-5), 3.67 (3H, s, 8-OMe), 3.60 (2H, s, H-7)；^{13}C NMR (175 MHz, CD_3OD) δ_C 174.7 (qC, C-8), 156.7 (qC, C-2), 132.1 (CH, C-6), 129.4 (CH, C-4), 122.5 (qC, C-1), 120.4 (CH, C-5), 115.8 (CH, C-3), 52.3 (CH_3, 8-OMe), 36.4 (CH_2, C-7).

N-(2-Hydroxyphenyl)-acetamide (**9**): White solid; ^{1}H NMR (700 MHz, CD_3OD) δ_H 7.57 (1H, dd, *J* = 8.0, 1.5 Hz, H-6), 6.99 (1H, m, H-4), 6.85 (1H, dd, *J* = 8.1, 1.3 Hz, H-3), 6.80 (1H, m, H-5), 2.17 (3H, s, H-8)；^{13}C NMR (175 MHz, CD_3OD) δ_C 172.2 (qC, C-7), 149.7 (qC, C-2), 127.1 (qC, C-1), 126.8 (CH, C-4), 124.0 (CH, C-5), 120.6 (CH, C-6), 117.3 (CH, C-3), 23.4 (CH_3, C-8).

(*S*)-Methyl 2-acetamido-3-phenylpropanoate (**10**): Slighty yellow solid; $[\alpha]_D^{25}$ = +14.2 (*c* 0.1, MeOH)；^{1}H NMR (700 MHz, CD_3OD) δ_H 7.28 (2H, t, *J* = 7.6 Hz, H-3, H-5), 7.22 (1H, m, H-4), 7.20 (2H, d, *J* = 7.6 Hz, H-2, H-6), 4.65 (1H, dd, *J* = 9.0, 5.7 Hz, H-8), 3.68 (3H, s, 9-OMe), 3.13 (1H, dd, *J* = 13.9, 5.7 Hz, H_a-7), 2.94 (1H, dd, *J* = 13.9, 9.0 Hz, H_b-7), 1.90 (3H, s, H-11)；^{13}C NMR (175 MHz, CD_3OD) δ_C 173.6 (qC, C-9), 173.1 (qC, C-10), 138.2 (qC, C-1), 130.1 (CH, C-3, C-5), 129.5 (CH, C-2, C-6), 127.9 (CH, C-4), 55.3 (CH, C-8), 52.6 (CH_3, 9-OMe), 38.4 (CH_2, C-7), 22.2 (CH_3, C-11).

Bioactivity Assays

The cytotoxicity of **1**–**10** against human lung carcinoma cell line A549 cells and human hepatocellular carcinoma cell line HepG2 cells was evaluated via the MTT method as reported in our previous study [1-2].

The antibacterial activities against against five kinds of pathogenic bacteria, *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 13883), methicillin-resistant *Staphylococcus aureus* (MRSA, clinical strain) and methicillin-resistant *Staphylococcus epidermidis* (MRSE, clinical strain), were evaluated using a modification of the broth microdilution method [1].

AChE inhibitory activity was evaluated in vitro according to the modified Ellman method [2]. Briefly, 0.1 U/mL AChE solution was prepared by dissolving in phosphate buffer (pH 8.0). The test sample and enzyme buffer were mixed in 96-well plates and incubated for 20 min at 30°C. Then, 5,5′-dithiobis (2-nitrobenzoic acid) and acetylthiocholine iodide were added, and the enzyme reaction was allowed to proceed for 30 min at 30 °C. AChE activity was determined by measuring the degradation of acetylthiocholine iodide to thiocholine and acetic acid at 405 nm using a microplate reader.

The inhibitory activity of compounds against PL using p-nitrophenyl palmitate (p-NPP) as substrate and porcine pancreatic lipase (PPL), was monitored according to the procedure described previously. [3]

Molecular Docking.

The Schrödinger 2017-1 suite (Schrödinger Inc., New York, NY) was employed to perform docking analysis. The catalytic domain of human bile salt activated lipase (PDB: 1F6W, Resolution: 2.30 Å) [4], and human pancreatic lipase (PDB: 1LPB, Resolution: 2.46 Å) [5], were retrieved from the available crystal structures and constructed following the Protein Prepare Wizard workflow in Maestro package [6]. The structures of **1**, **2** and Orlistat as control, were generated in ChemBioOffice (version 17.0), followed by an MM2 calculation to minimize the conformation energy. The binding site was produced using the Grid Generation procedure. The prepared ligandswere then flexibly docked into the receptors using Glide (XP mode) with default parameters [7].

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