Supporting Information

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GNPS molecular networking-guided discovery of antifungal butenolides from the endophytic fungus *Aspergillus* sp. FH-1 of *Valeriana officinalis* L.

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Experimental

General experimental procedures

The HR-ESI-MS spectra were recorded on an Agilent Technologies 6550 Q-TOF (Santa Clara, CA, USA); 1 D and 2 D NMR spectra were recorded on Bruker-AVANCE 400 instrument (Bruker, Rheinstetten, Germany) with TMS as an internal standard; Semipreparative HPLC was performed on a system comprising an NP7000 SERIALS pump (Hanbon Sci. Tech., China) equipped with NU3000 serials UV/VIS detector and Capcell Pak C_{18} column, (10 mm × 250 mm, 5 μm particles); Reversed-phase C_{18} silica gel (5 μm, YMC Co., Ltd. Japan); Silica gel (100-200 and 200-300 mesh, Qingdao Haiyang Chemical, China); All solvents used in CC were of analytical grade (Sinopharm Chemical Reagent Co., Ltd. China).

Cultivation, Extraction and Isolation

The solid-state fermentation was conducted using a rice-based medium consisting of rice (100 g), NaCl (3.3%, w/w), and purified water (110 mL) at a neutral pH. Each 1 L flask containing 100 g of the prepared medium was inoculated with 10 mL of seed culture and incubated statically at 28 °C for 30 days, with a total fermentation volume of 50 L. After cultivation, the entire fermented material was thoroughly extracted with ethyl acetate (EtOAc; 500 mL per extraction). The combined EtOAc extracts were then concentrated under reduced pressure to yield 60.0 g of a crude residue.

The crude extract (60.0 g) was subjected to silica gel column chromatography (CC) and eluted with a gradient solvent system of dichloromethane-methanol (CH_2Cl_2-MeOH, from 80:1 to 1:1, v/v) to afford ten fractions (Fr.1-10). Fr.3 (7.5 g) was subjected to ODS column chromatography, eluting with a gradient of MeOH-H_2O (from 20:80 to 100:0, v/v), to yield four sub-fractions (F.3-1 to F.3-4). Sub-fraction F.3-2 (2.3 g) was purified by semi-preparative HPLC using an isocratic mobile phase of MeOH-H_2O (75:25, v/v, UV 220,306 nm, 2 mL/min) to yield **1** (15.0 mg, t_R = 32.5 min) and **3** (12.0 mg, t_R = 36.5 min). Fr.4 (1.2 g) was separated by ODS CC (MeOH-H_2O, 20:80 to 100:0, v/v) into three sub-fractions (F.4-1 to F.4-3). Purification of F.4-2 (0.3 g) by semi-preparative HPLC (MeOH-H_2O, 70:30, v/v, UV 220,306 nm, 2 mL/min) afforded **2** (14.5 mg, t_R = 13.6 min) and **4** (8.0 mg, t_R = 25.5 min).

GNPS molecular networking

The LC-MS/MS data were processed through the GNPS platform (http://gnps.ucsd.edu) to construct a molecular network. MS-Cluster was employed for spectral clustering, with mass tolerance set to 0.02 Da for both precursor and fragment ions. Connections (edges) between nodes were established based on a cosine similarity score above 0.7 and at least six matched fragment peaks. The resulting network was imported into Cytoscape v. 3.7.2 for visualization. In the network graphs, pie charts within nodes indicate the relative distribution of compounds among samples, while edge thickness corresponds to the magnitude of the cosine score.

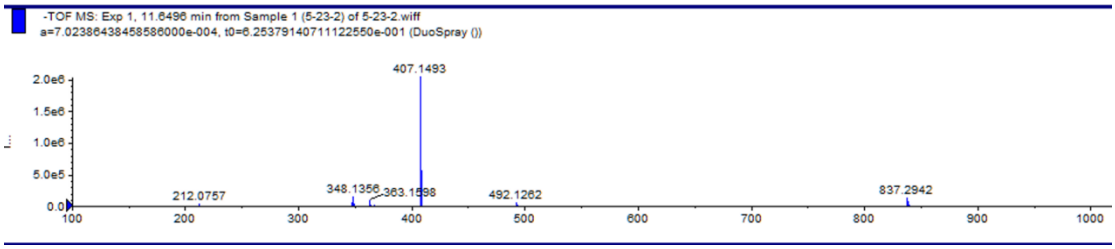


Figure S1: HR-ESI-MS spectrum of compound **1**.

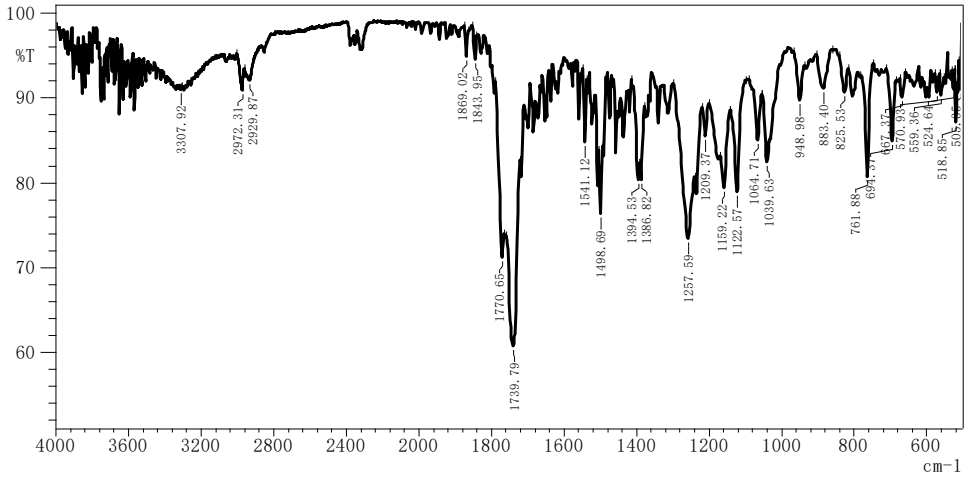


Figure S2: IR Spectrum of Compound **1**.

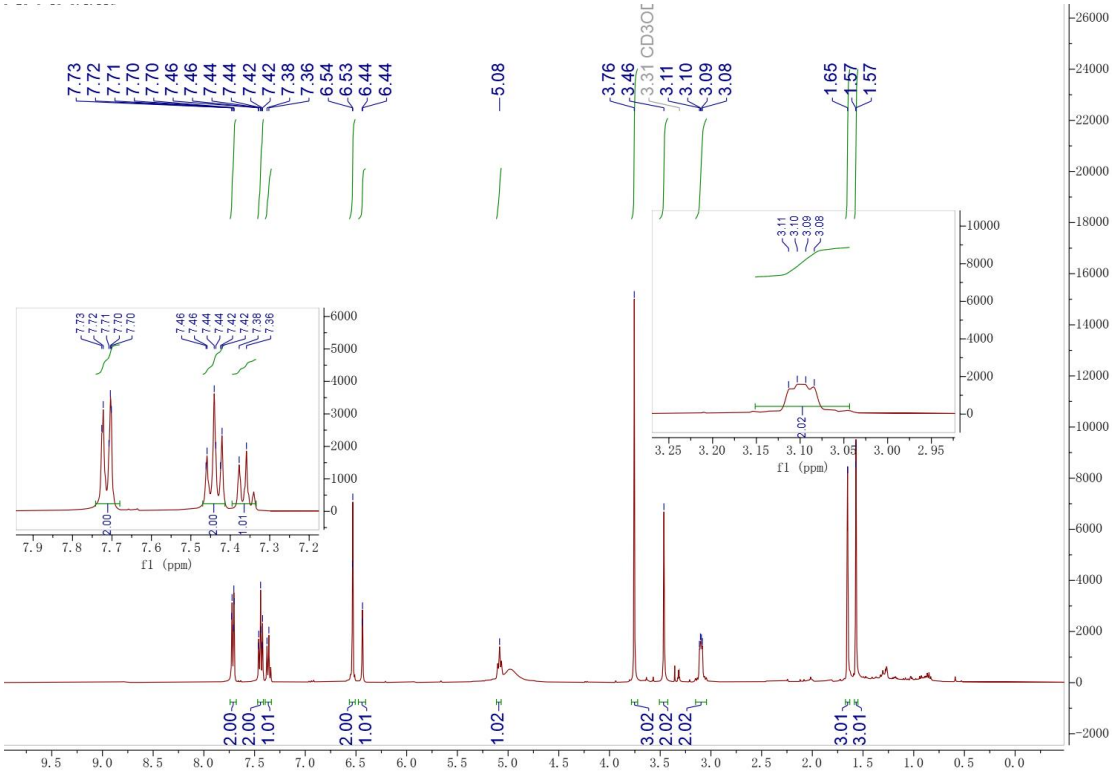


Figure S3: ^{1}H NMR (400 MHz) spectrum of compound **1** in Methanol-d_4.

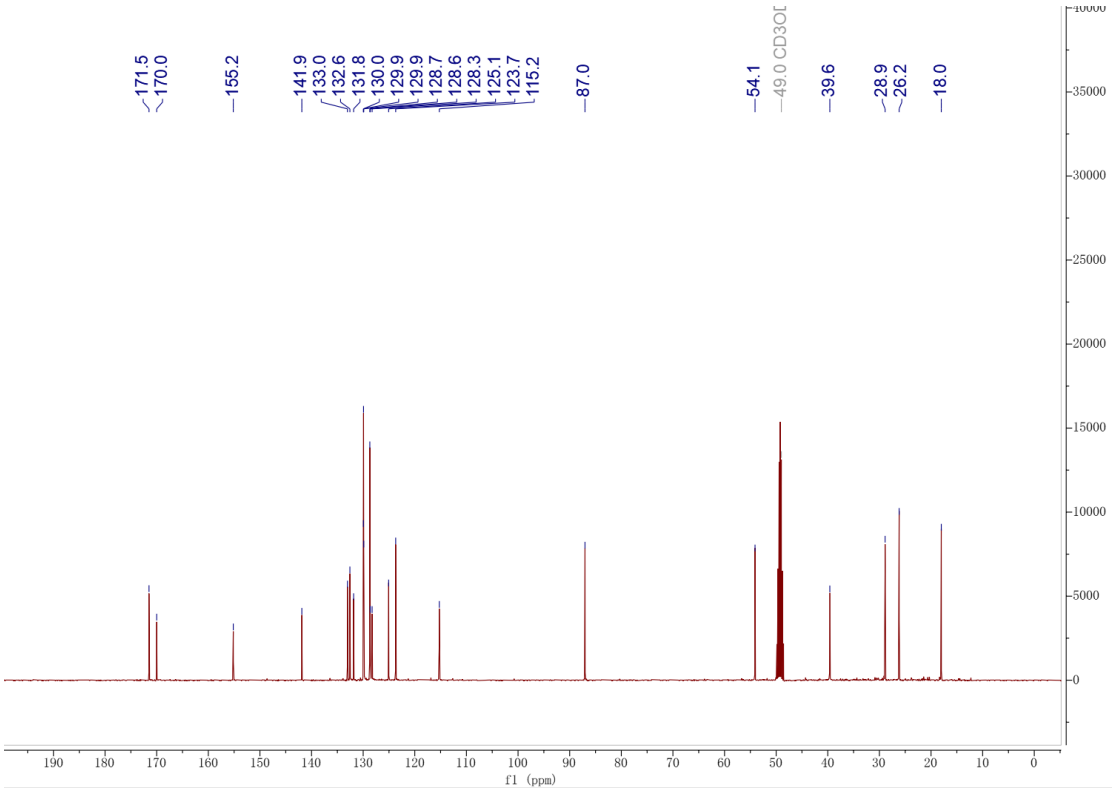


Figure S4: ^{13}C NMR (100 MHz) spectrum of compound **1** in Methanol-d_4.

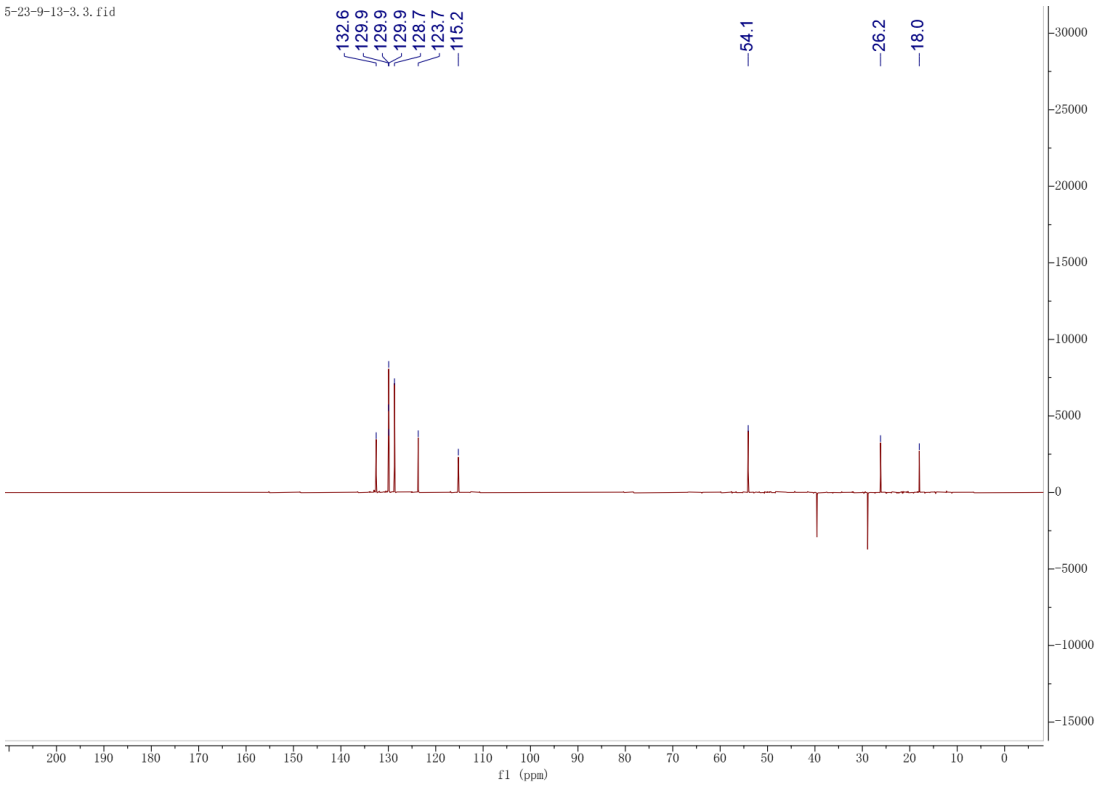


Figure S5: DEPT 135 (100 MHz) spectrum of compound **1** in Methanol-d_4.

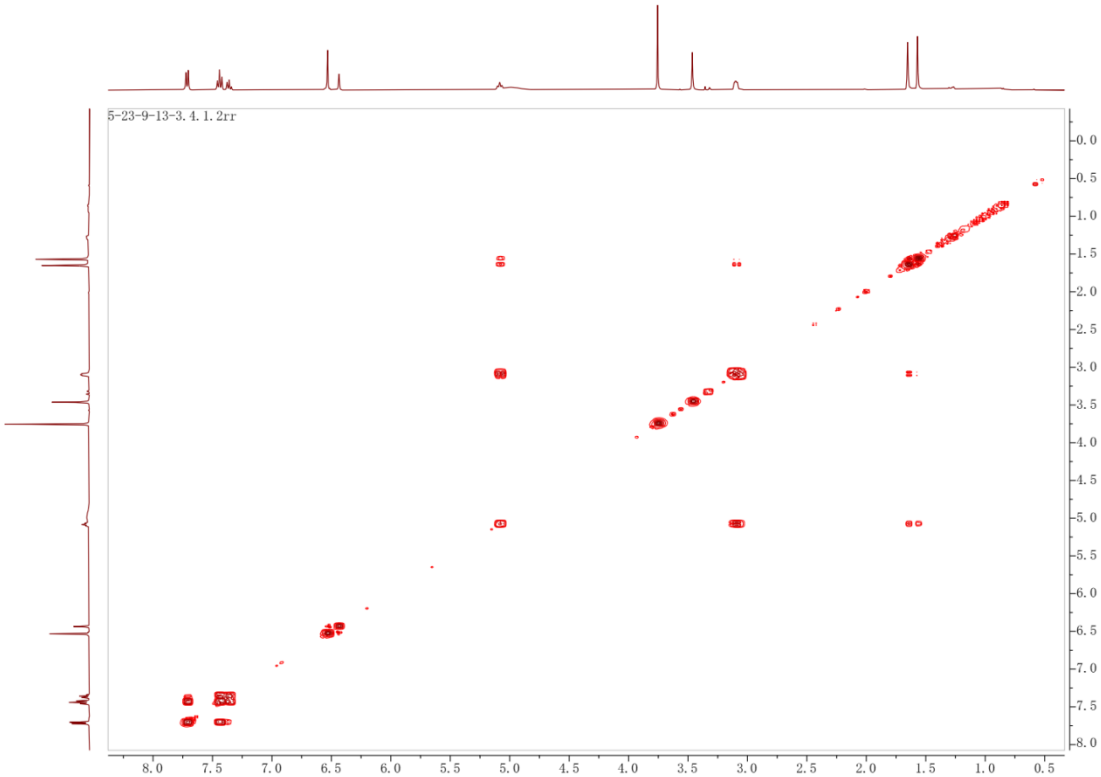


Figure. S6. ^{1}H-^{1}H COSY spectrum of compound **1** in Methanol-d_4.

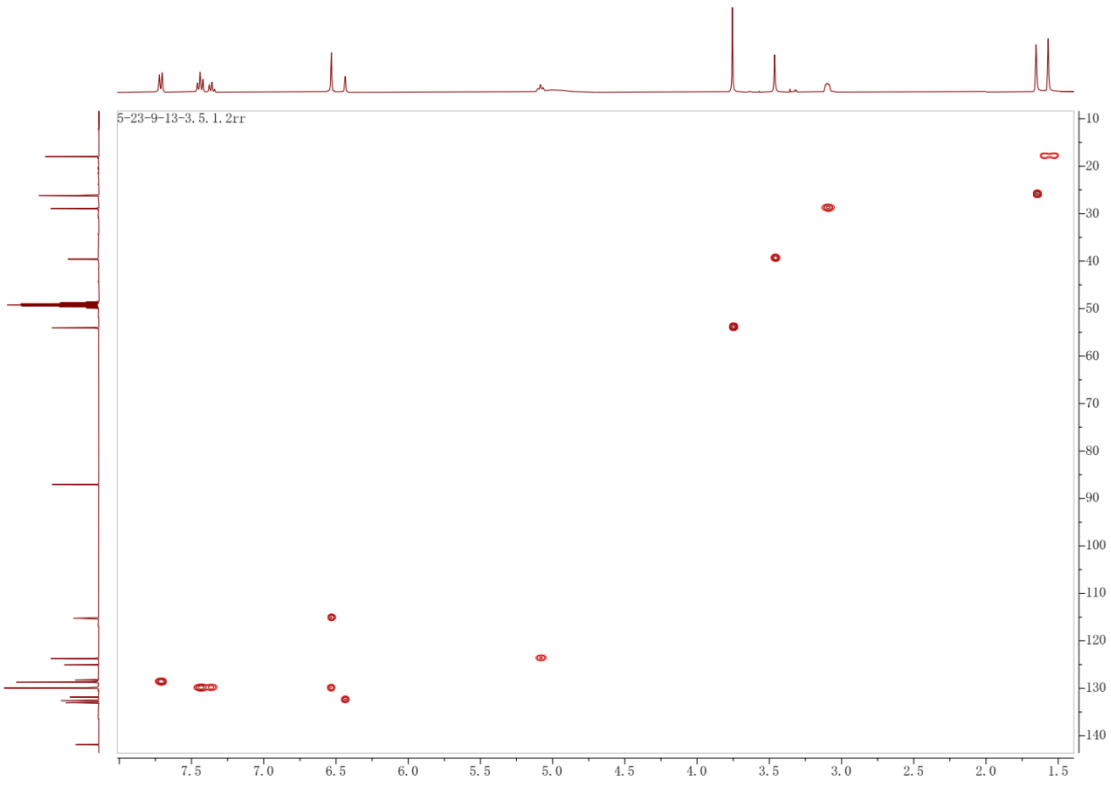


Figure S7: HSQC spectrum of compound **1** in Methanol-d_4.

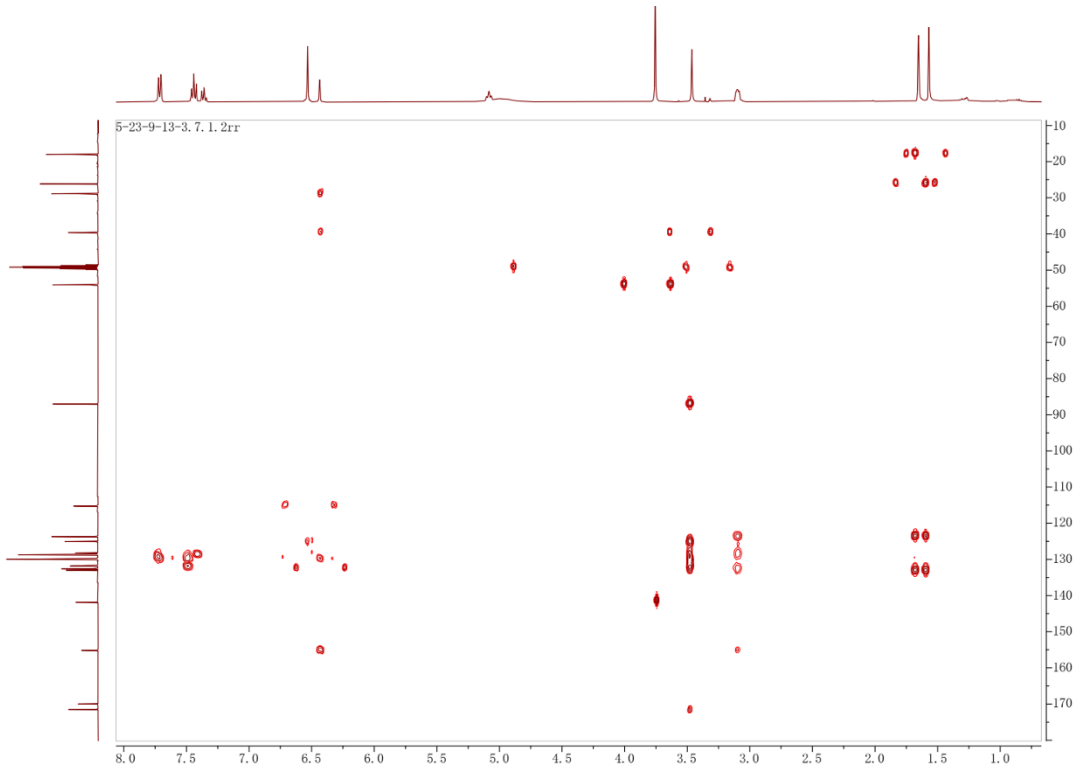


Figure S8: HMBC spectrum of compound **1** in Methanol-d_4.

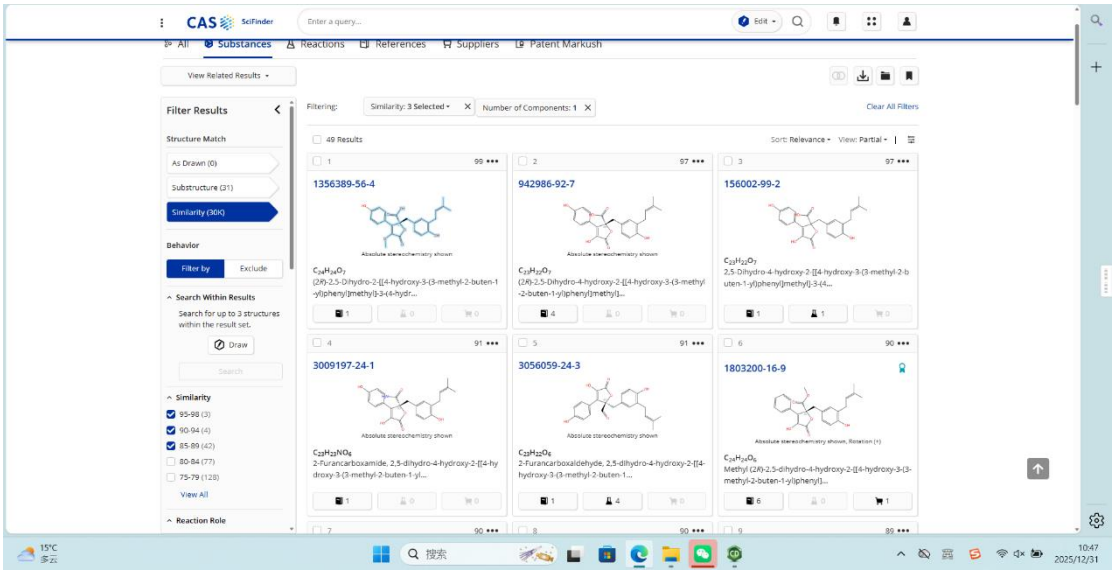
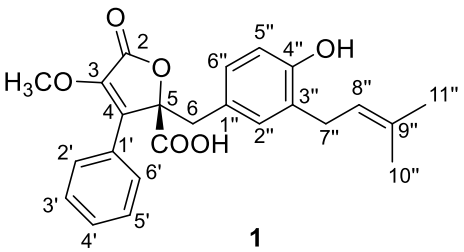


Figure S9: Scifinder similarity report for compound **1**

Table S1: Compound **1** *vs*. structural analogues

Molecular Formula of 1:$C_{24}H_{24}O_6$		**Molecular Formula of Known Compounds: $C_{23}H_{24}O_5$**	
No.	^{13}C NMR data of **1**	Known ^{13}C NMR data	Matche
1	18.01	17.8	√
2	26.18	25.9	√
3	28.9	28.7	√
4	39.6	39.6	√
5	54.09	53.7	√
6	87.03	86.8	√
7	115.25	115	√
8		116.5	
9	123.7	123.6	√
10	125.06	125.3	√
11	128.27	127.3	√
12	128.63	128.1	√
13	128.71	128.4	√
14	129.89	129.8	√
15	129.93	130.1	√
16	129.95		
17	131.85	132.5	√
18	132.57	132.7	√
19	133.04	132.9	√
20	141.88		
21	155.16	155	√
22		159.1	
23	170		
24	171.49	172	√



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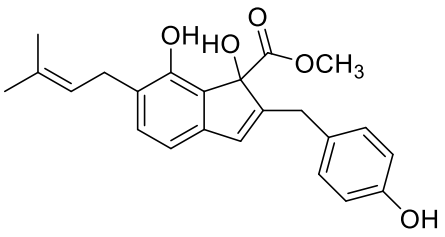


Known compound :methyl 2-(4-hydroxybenzyl)-1,7-dihydroxy-6-(3-methylbut-2-enyl)-1H-indene-1-carboxylate

References:

Akhter N, Pan C, Liu Y, Shi Y, Wu B. Isolation and structure determination of a new indene derivative from endophytic fungus *Aspergillus flavipes* Y-62. *Nat Prod Res*. 2019, 33(20): 2939-2944.

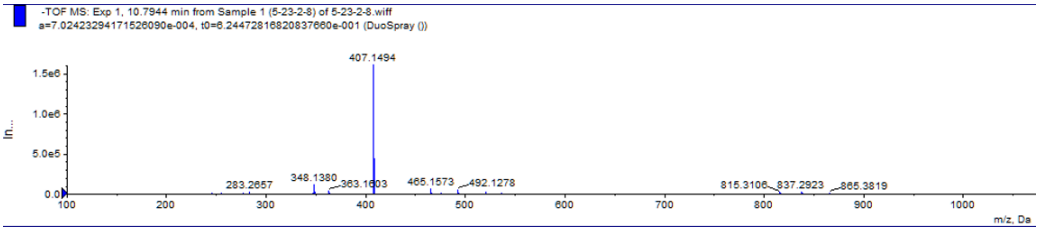


Figure S11: HR-ESI-MS spectrum of compound **2**.

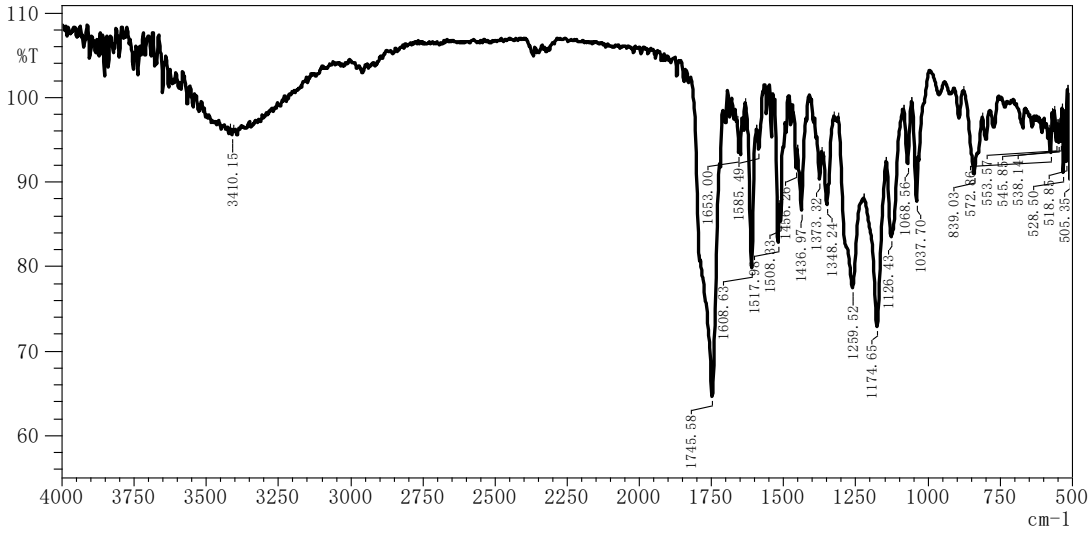


Figure S12: IR Spectrum of Compound **2**.

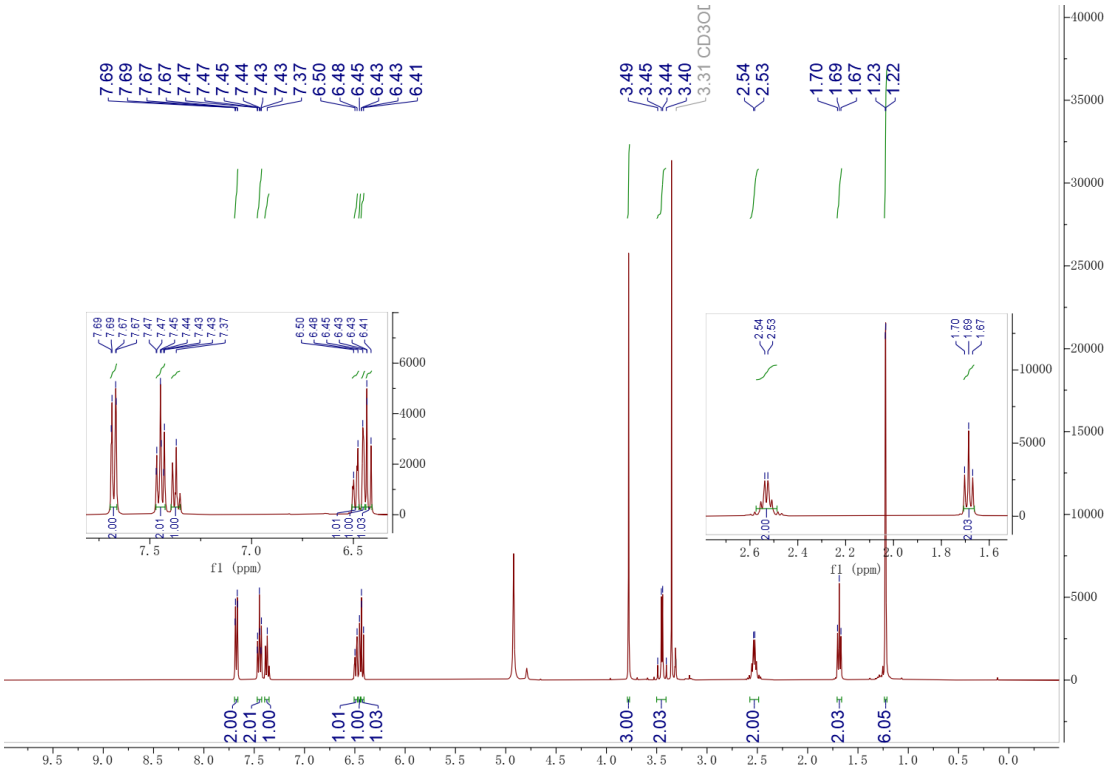


Figure S13: ^{1}H NMR (400 MHz) spectrum of compound **2** in Methanol-d_4.

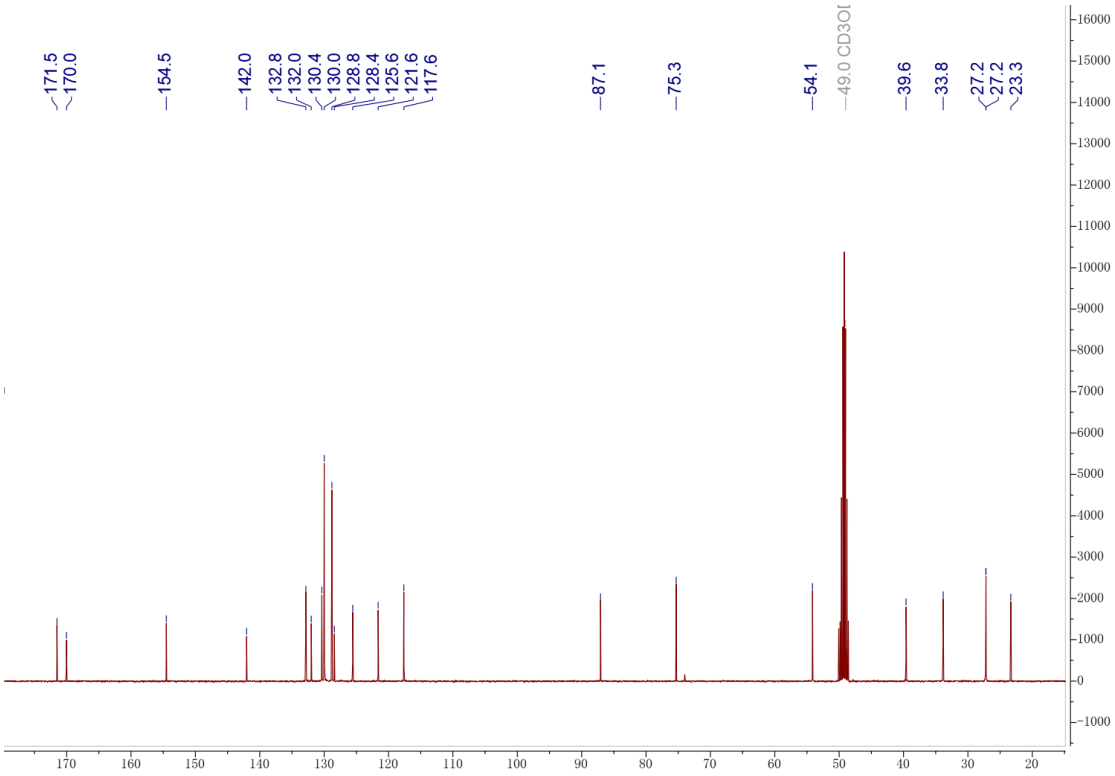


Figure S14: ^{13}C NMR (100 MHz) spectrum of compound **2** in Methanol-d_4.

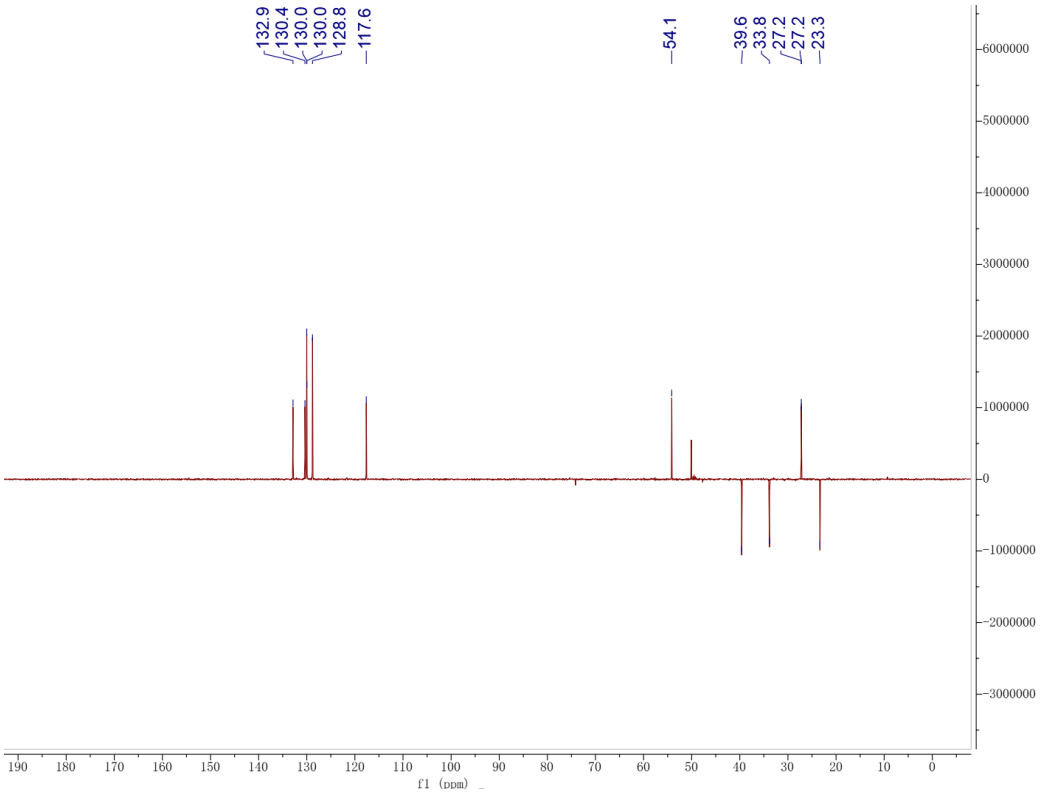


Figure S15: DEPT 135 (100 MHz) spectrum of compound **2** in Methanol-d_4.

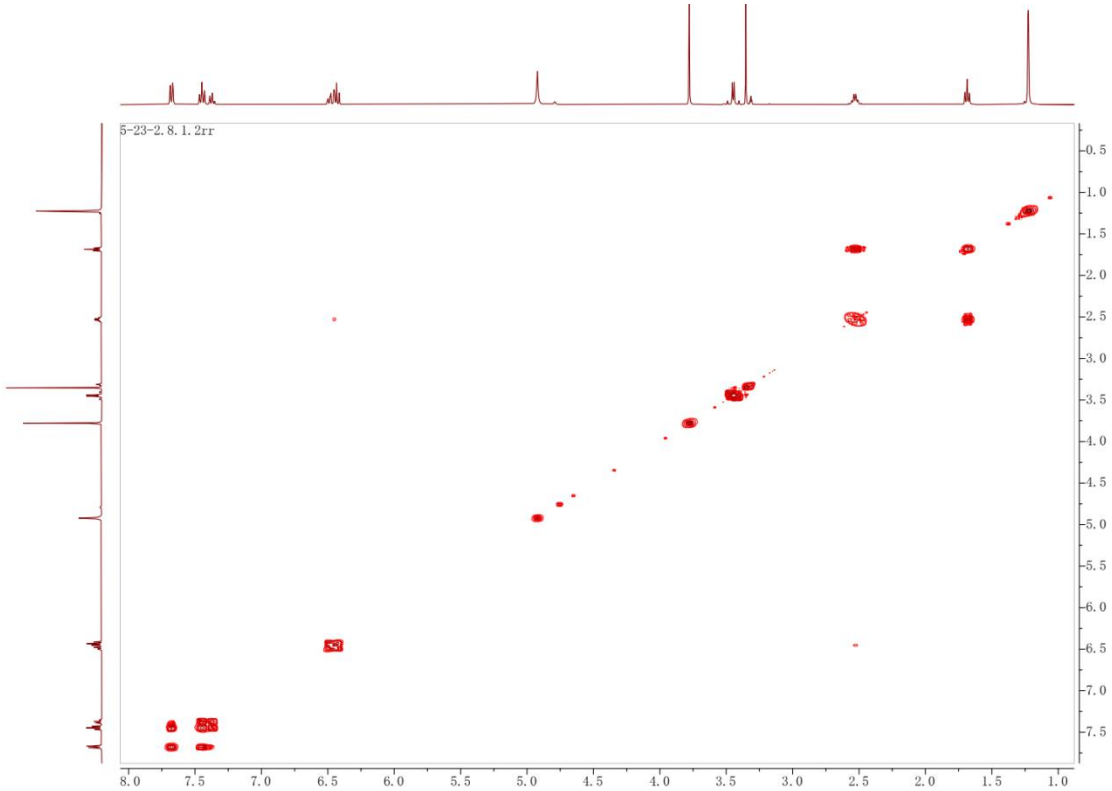


Figure S16: ^{1}H-^{1}H COSY spectrum of compound **2** in Methanol-d_4.

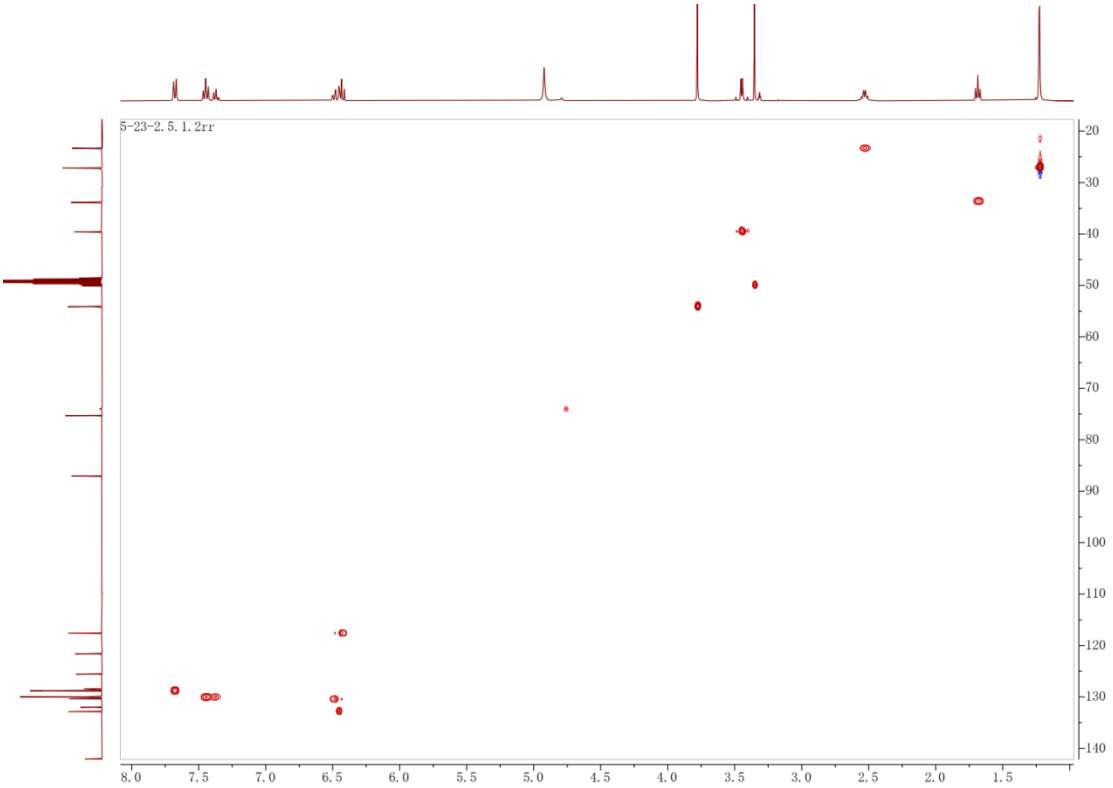


Figure S17: HSQC spectrum of compound **2** in Methanol-d_4.

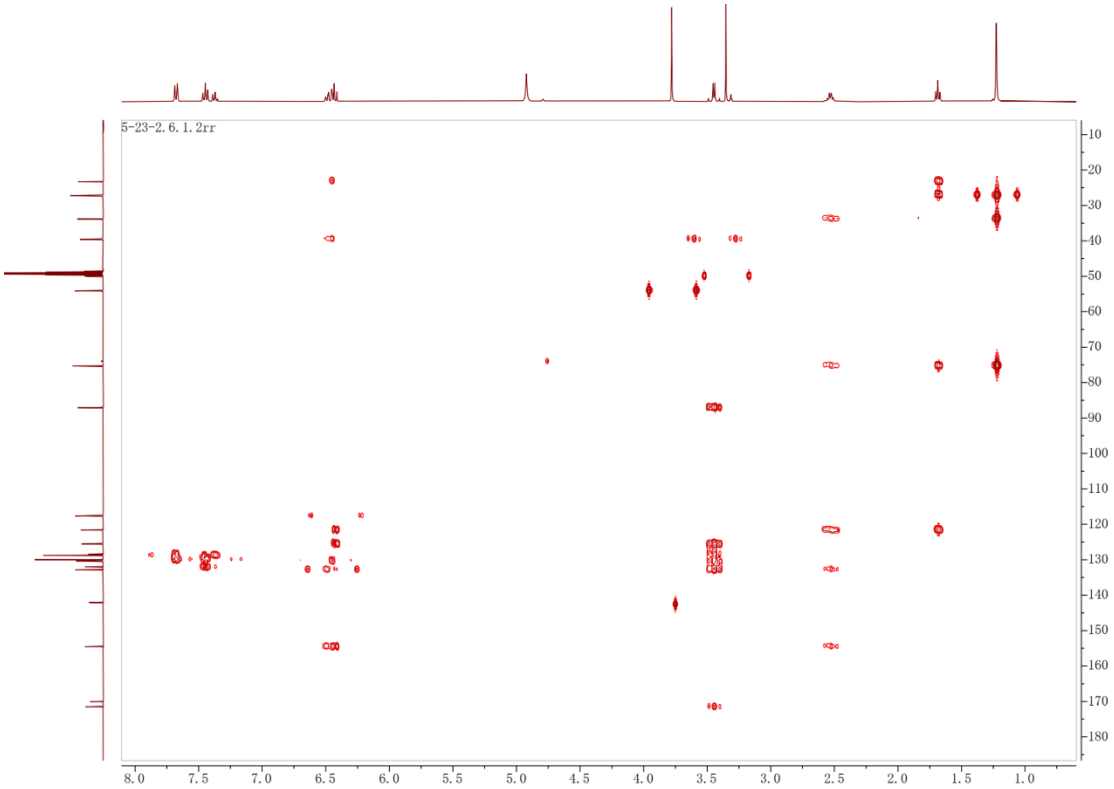


Figure S18: HMBC spectrum of compound **2** in Methanol-d_4.

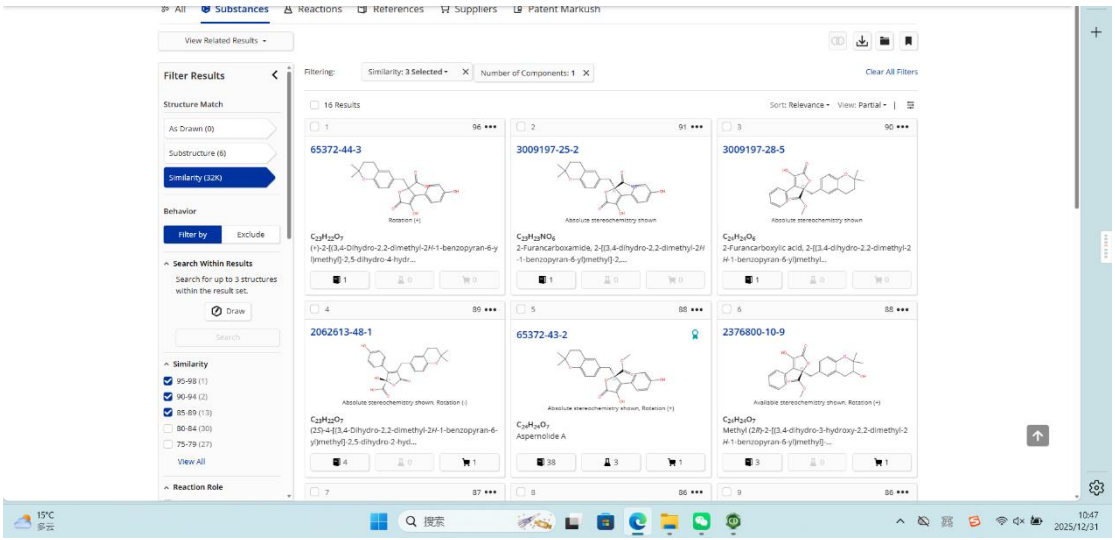
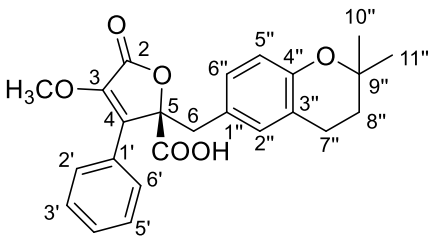


Figure S19: Scifinder similarity report for compound **2**

Table S2: Compound **2** *vs*. structural analogues

Molecular Formula of 2:$C_{24}H_{24}O_6$		**Molecular Formula of Known Compounds: $C_{24}H_{24}O_6$**	
No.	^{13}C NMR data of **2**	Known ^{13}C NMR data	Matche
1	23.34	23.2	√
2	27.19	27	√
3	27.21	27.1	√
4	33.84	33.7	√
5	39.61	39.4	√
6	54.14	53.9	√
7	75.32	75.1	√
8	87.07	86.9	√
9	117.62	117.4	√
10	121.62	121.4	√
11	125.56	125.4	√
12	128.42	128.6	√
13	128.8	128.6	√
14		128.6	
15	129.99	129.7	√
16	130.36	129.8	√
17		129.8	
18		130.2	
19	132.01	131.9	√
20	132.84	132.6	√
21	142.05	142.1	√
22	154.52	154.3	√
23	170.03	170	√
24	171.5	171.4	√



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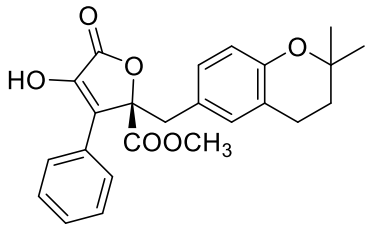

Known compound: 4'-dehydroxy aspernolide A

References:

Liu M, Zhou Q, Wang J, Liu J, Qi C, Lai Y, Zhu H, Xue Y, Hu Z, Zhang Y. Anti-inflammatory butenolide derivatives from the coral-derived fungus *Aspergillus terreus* and structure revisions of aspernolides D and G, butyrolactone VI and 4',8"-diacetoxy butyrolactone VI. *RSC Adv*. 2018 9;8(23):13040-13047.