Supporting Information

Indole diketopiperazine alkaloids and aromatic polyketides from the Antarctic fungus *Penicillium* sp. SCSIO 05705

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ABSTRACT

A new indole diketopiperazine alkaloid, named penilline D (**1**), together with five known indole alkaloid analogues (**2**–**5**, **11**), two meroterpenoids (**6** and **12**), and four butenolide derivatives (**7**–**10**), were isolated from the Antarctic fungus *Penicillium* sp. SCSIO 05705. Extensive spectroscopic analysis and electronic circular dichroism (ECD) calculation were used to elucidate the structure of penilline D (**1**), including its absolute configuration. All isolated compounds (**1**–**12**) were evaluated for their cytotoxic, antibacterial and enzyme inhibitory activities against acetylcholinesterase (AChE) and pancreatic lipase (PL). Among them, compound **5** exhibited moderate in vitro cytotoxic activity against the 143B cell line with IC_{50} value of 12.64 ± 0.78 μM. Compound **6** showed strong inhibitory activity against AChE with IC_{50} value of 0.36 nM (IC_{50} 18.7 nM for Tacrine), while compounds **6** and **11** showed weak PL enzyme inhibitory activity. Furthermore, an in silico molecular docking study was also performed between **6** and AChE.

Keywords

Antarctic; *Penicillium*; indole diketopiperazine; aromatic polyketide; AChE inhibitory activity

List of Supporting Information

Experimental section

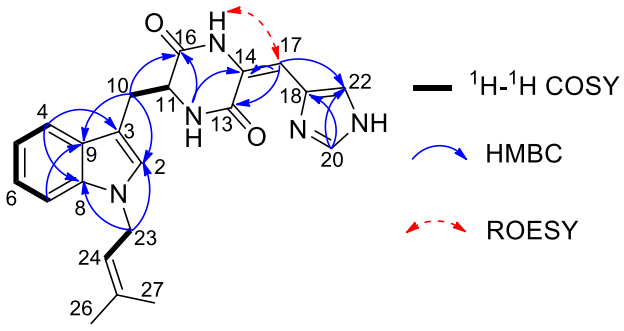


Figure S1. 2D NMR data of compound **1**.

Table S1.^{1}H and ^{13}C NMR Data for **1** (700, 175 MHz, TMS, δ ppm).

No.	**1**[a]	**1**[b]

	δ_C	δ_H（*J* in Hz）	δ_C	δ_H（*J* in Hz）	COSY	HMBC(H→C)
2	129.4, CH	6.92, s	127.7, CH	6.96, s		3, 8, 9, 10, 23
3	107.8, C		106.9, C			
4	120.1, CH	7.51, d (7.8)	118.7, CH	7.52, d (7.8)	5	3, 6, 8, 9
5	120.4, CH	7.01, t (7.1)	120.3, CH	7.01, t (7.1)	4, 6	7, 9
6	122.3, CH	6.97, t (7.1)	118.9, CH	6.97, t (7.1)	5, 7	4, 8
7	110.0, CH	7.03, d (8.1)	109.3, CH	7.16, d (8.1)	6	5, 8, 9
8	137.6 C		135.2, C			
9	129.8, C		128.1, C			
10	31.6, CH_2	3.44, dd (14.7, 2.9);	29.4, CH_2	3.31, dd (14.9, 3.4);	11	2, 9, 11, 16
		3.15, dd (14.7, 4.5)		3.03, dd (14.9, 4.6)		
11	58.4, CH	4.38, m	56.0, CH	4.34, m	10	3, 16
12				8.94, s		14, 16
13	162.8, C		160.4, C			
14	*		126.0, C			
15				10.29, s		
16	169.7, C		166.4, C			
17	103.5, CH	5.36, s	103.7, CH	5.68, s		13, 14, 22
18	129.0, C		*			
20	134.1, CH	8.64, s	134.6, CH	8.42, brs		18, 22
22	121.0, CH	7.39, s	123.8, CH	7.51, brs		17, 18, 20
23	44.8, CH_2	4.47, d (6.9)	43.2, CH_2	4.51, d (6.9)	24	2, 8, 24, 25
24	121.2, CH	5.15, t (6.9)	120.9, CH	5.07, t (6.9)	23	23, 25, 26, 27
25	137.3, C		135.5, C			
26	25.7, CH_3	1.71, s	25.2, CH_3	1.59, s		24, 25, 27
27	18.0, CH_3	1.78, s	17.7, CH_3	1.71, s		24, 25, 26

[a] Recorded in CD_3OD; [b] Recorded in DMSO-d_6. * Not observed.

ECD calculation details

For compound **1**, we assigned the absolute configurations by comparing their measured electronic circular dichroism (ECD) spectra with the density functional theory (DFT)-computed ones in the Gaussian 03. The preliminary conformational distributions search was performed using Frog2 online version, and afforded four stable conformer (Tables S1) for compound **1** (11*S*). Further geometrical optimization were performed at the B3LYP/6-31G(d) level. Solvent effects of methanol solution were evaluated at the same DFT level by using the SCRF/PCM method. TDDFTS4 at

B3LYP/6-31G(d) was employed to calculate the electronic excitation energies and rotational strengths in methanol. The stable conformations obtained at the B3LYP/6-31G(d) level were further used in magnetic shielding constants at the B3LYP/6-311++G(2d,p) level.

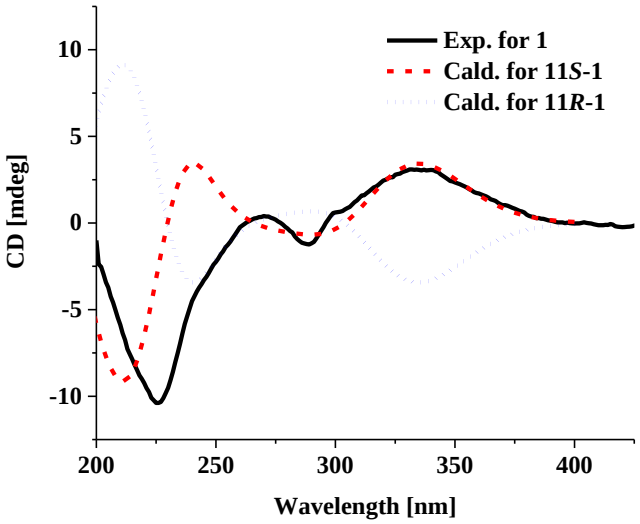
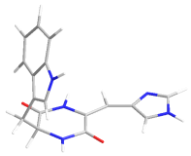
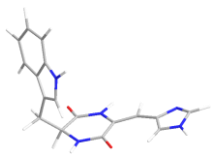
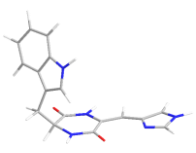


Figure S2. Comparison between calculated and experimental ECD spectra of **1**.

Table S2. Stable conformers of **1a-1**–**1a-4**.

Conformer	Conformation	E (Hartree)	Energy (kcal/mol)	Percent (%)
1a-1		-1080.830165	-678231.72281	49.95%
1a-3		-1080.830165	-678231.72281	49.95%
1a-3		-1080.82359	-678227.60015	0.05%

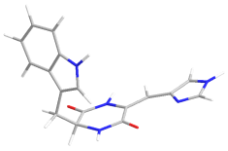
1a-4		-1080.823589	-678227.59953	0.05%

Table S3. Cartesian Coordinates of low energy conformers of 1a-1–1a-4.

Conformer 1a-1							
Atom	**X**	**Y**	**Z**	**Atom**	**X**	**Y**	**Z**
C	-2.4243	1.0638	-1.9414	**N**	-6.1115	-5.7355	-2.1887
C	-1.2854	0.4434	-2.2386	**C**	-5.7417	-6.908	-2.1249
C	-0.6454	-0.4173	-1.4018	**N**	-5.2798	-7.2494	-0.9962
C	-1.2173	-0.5271	-0.2179	**C**	-5.2593	-6.1695	-0.3664
C	-2.4123	0.0536	0.146	**H**	-2.8477	1.7675	-2.6714
C	-3.0205	0.8139	-0.79	**H**	-0.8494	0.5568	-3.2559
N	-0.8505	-1.1665	0.8202	**H**	0.2822	-0.9966	-1.6988
C	-1.6381	-1.0431	1.8108	**H**	-4.0058	1.2445	-0.5468
C	-2.6498	-0.3204	1.354	**H**	0.0926	-1.8023	0.7402
C	-3.8486	0.0879	2.1391	**H**	-1.4593	-1.4565	2.8639
C	-5.0999	-0.714	1.8796	**H**	-3.5374	0.3309	3.2062
N	-5.0025	-2.1617	2.1634	**H**	-4.1758	1.0447	1.7793
C	-5.2138	-3.2166	1.3148	**H**	-5.7979	-0.3555	2.7625
C	-5.7589	-2.9197	0.0975	**H**	-4.6193	-2.4131	3.0194
N	-6.0582	-1.6185	-0.1966	**H**	-6.47	-1.6291	-1.1282
C	-5.8733	-0.5077	0.5788	**H**	-6.351	-3.3669	-1.7377
O	-6.1984	0.5852	0.1397	**H**	-5.8839	-7.7218	-2.9138
O	-4.9513	-4.2937	1.7726	**H**	-4.9767	-8.1512	-0.6076
C	-5.9807	-3.8685	-0.8426	**H**	-4.8818	-6.2577	0.641
C	-5.7856	-5.1885	-1.1137				
Conformer 1a-2							
Atom	**X**	**Y**	**Z**	**Atom**	**X**	**Y**	**Z**
C	-2.4243	1.0638	-1.9414	**N**	-4.125	-7.4978	1.7612
C	-1.2854	0.4434	-2.2386	**C**	-3.3431	-8.3047	2.2641
C	-0.6454	-0.4173	-1.4018	**N**	-2.7	-7.8581	3.2596
C	-1.2173	-0.5271	-0.2179	**C**	-3.0317	-6.6529	3.2864
C	-2.4123	0.0536	0.146	**H**	-2.8477	1.7675	-2.6714
C	-3.0205	0.8139	-0.79	**H**	-0.8494	0.5568	-3.2559
N	-0.8505	-1.1665	0.8202	**H**	0.2822	-0.9966	-1.6988
C	-1.6381	-1.0431	1.8108	**H**	-4.0058	1.2445	-0.5468
C	-2.6498	-0.3204	1.354	**H**	0.0926	-1.8023	0.7402
C	-3.8486	0.0879	2.1391	**H**	-1.4593	-1.4565	2.8639
C	-4.7297	-1.0406	2.6147	**H**	-3.5666	0.9304	2.8499
N	-4.0804	-2.0289	3.5022	**H**	-4.5345	0.5776	1.4745
C	-3.9518	-3.3819	3.3277	**H**	-5.4389	-0.4821	3.3765

C	-4.6623	-3.9339	2.2994	**H**	-3.5651	-1.6751	4.2452
N	-5.4449	-3.1249	1.5233	**H**	-5.8955	-3.7372	0.8454
C	-5.6244	-1.7728	1.6167	**H**	-5.1892	-5.4222	1.1052
O	-6.3651	-1.2147	0.8211	**H**	-3.2351	-9.4133	2.0104
O	-3.2735	-3.9349	4.1479	**H**	-2.0546	-8.2952	3.9292
C	-4.5899	-5.2526	2.0008	**H**	-2.5702	-6.0898	4.0833
C	-3.9413	-6.4026	2.3337				
Conformer 1a-3							
Atom	**X**	**Y**	**Z**	**Atom**	**X**	**Y**	**Z**
C	-2.4243	1.0638	-1.9414	**N**	-2.9386	-6.3023	3.9688
C	-1.2854	0.4434	-2.2386	**C**	-2.5576	-7.4505	4.1967
C	-0.6454	-0.4173	-1.4018	**N**	-2.8856	-8.2951	3.3119
C	-1.2173	-0.5271	-0.2179	**C**	-3.6218	-7.6291	2.5517
C	-2.4123	0.0536	0.146	**H**	-2.8477	1.7675	-2.6714
C	-3.0205	0.8139	-0.79	**H**	-0.8494	0.5568	-3.2559
N	-0.8505	-1.1665	0.8202	**H**	0.2822	-0.9966	-1.6988
C	-1.6381	-1.0431	1.8108	**H**	-4.0058	1.2445	-0.5468
C	-2.6498	-0.3204	1.354	**H**	0.0926	-1.8023	0.7402
C	-3.8486	0.0879	2.1391	**H**	-1.4593	-1.4565	2.8639
C	-4.7297	-1.0406	2.6147	**H**	-3.5666	0.9304	2.8499
N	-4.1513	-1.9046	3.6659	**H**	-4.5345	0.5776	1.4745
C	-3.9407	-3.2583	3.6415	**H**	-5.5525	-0.4559	3.2281
C	-4.4923	-3.9421	2.595	**H**	-3.7488	-1.4552	4.4266
N	-5.2085	-3.2537	1.6556	**H**	-5.5433	-3.9504	0.9921
C	-5.4595	-1.9112	1.5939	**H**	-4.7988	-5.5613	1.4983
O	-6.1203	-1.4717	0.6648	**H**	-1.8574	-7.794	5.0315
O	-3.3449	-3.6941	4.5869	**H**	-2.6552	-9.2867	3.1711
C	-4.3236	-5.2763	2.4379	**H**	-4.0133	-8.1922	1.7182
C	-3.6697	-6.352	2.9569				
Conformer 1a-4							
Atom	**X**	**Y**	**Z**	**Atom**	**X**	**Y**	**Z**
C	-2.4243	1.0638	-1.9414	**N**	-3.0932	-6.4857	3.2474
C	-1.2854	0.4434	-2.2386	**C**	-2.7539	-7.6668	3.3199
C	-0.6454	-0.4173	-1.4018	**N**	-3.2327	-8.4048	2.4088
C	-1.2173	-0.5271	-0.2179	**C**	-4.015	-7.6347	1.8102
C	-2.4123	0.0536	0.146	**H**	-2.8477	1.7675	-2.6714
C	-3.0205	0.8139	-0.79	**H**	-0.8494	0.5568	-3.2559
N	-0.8505	-1.1665	0.8202	**H**	0.2822	-0.9966	-1.6988
C	-1.6381	-1.0431	1.8108	**H**	-4.0058	1.2445	-0.5468
C	-2.6498	-0.3204	1.354	**H**	0.0926	-1.8023	0.7402
C	-3.8486	0.0879	2.1391	**H**	-1.4593	-1.4565	2.8639
C	-4.7297	-1.0406	2.6147	**H**	-3.5666	0.9304	2.8499
N	-4.0804	-2.0289	3.5022	**H**	-4.5345	0.5776	1.4745

C	-3.9518	-3.3819	3.3277	**H**	-5.4389	-0.4821	3.3765
C	-4.6623	-3.9339	2.2994	**H**	-3.5651	-1.6751	4.2452
N	-5.4449	-3.1249	1.5233	**H**	-5.8955	-3.7372	0.8454
C	-5.6244	-1.7728	1.6167	**H**	-5.1892	-5.4222	1.1052
O	-6.3651	-1.2147	0.8211	**H**	-1.98	-8.122	4.0263
O	-3.2735	-3.9349	4.1479	**H**	-3.0776	-9.3872	2.15
C	-4.5899	-5.2526	2.0008	**H**	-4.5345	-8.0949	0.9835
C	-3.9413	-6.4026	2.3337				

Details of the molecular docking study

The crystal structure of AChE (PDB ID: 6G1W) from *Torpedo Californica* was retrieved from the Protein DataBank (http://www.rcsb.org/pdb) for molecular docking research, which conducted by AutoDock4.2.6[S1]. The structure of **A** was generated in ChemBioOffice (version 17.0), followed by an MM2 calculation to minimize the conformation energy. The original ligands and crystal water were removed before the docking calculation. The hydrogens were added to the structure of AChE, and Kollman united partial charges were assigned. A Lamarckian genetic algorithm was applied as a default search algorithm and allowed full flexibility of the active pocket of the ligand structure within the grid box size of 58 Å × 86 Å × 78Å, with the spacing of 0.375 Å. The center of the grid was set at the *xyz* dimension coordinates of 6.003, −1.261 and −4.764, respectively. During the docking, the default parameters were used if it was not mentioned. The obtained results and the docked poses were analyzed by AutoDockTools1.5.6 package (ADT, http://mgltools.scripps.edu). The docking pose that had the lowest binding energy was represented as the most favorable binding conformation and visually analysed by using PyMoL1.7.6 software(1.3r1, DeLano Scientific LLC, South San Francisco, CA, USA)[S2].

References

S1. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, and A. J. Olson. *J. Comput. Chem.* **30**, 2785 (2009).
S2. D. Q. Pan, M. Jiang, T. T. Liu, Q. Wang, and J. H. Shi. *Luminescence* **32**, 481 (2017).

Figure S3. ^{1}H-NMR spectrum of compound **1** (700 MHz, CD_3OD).

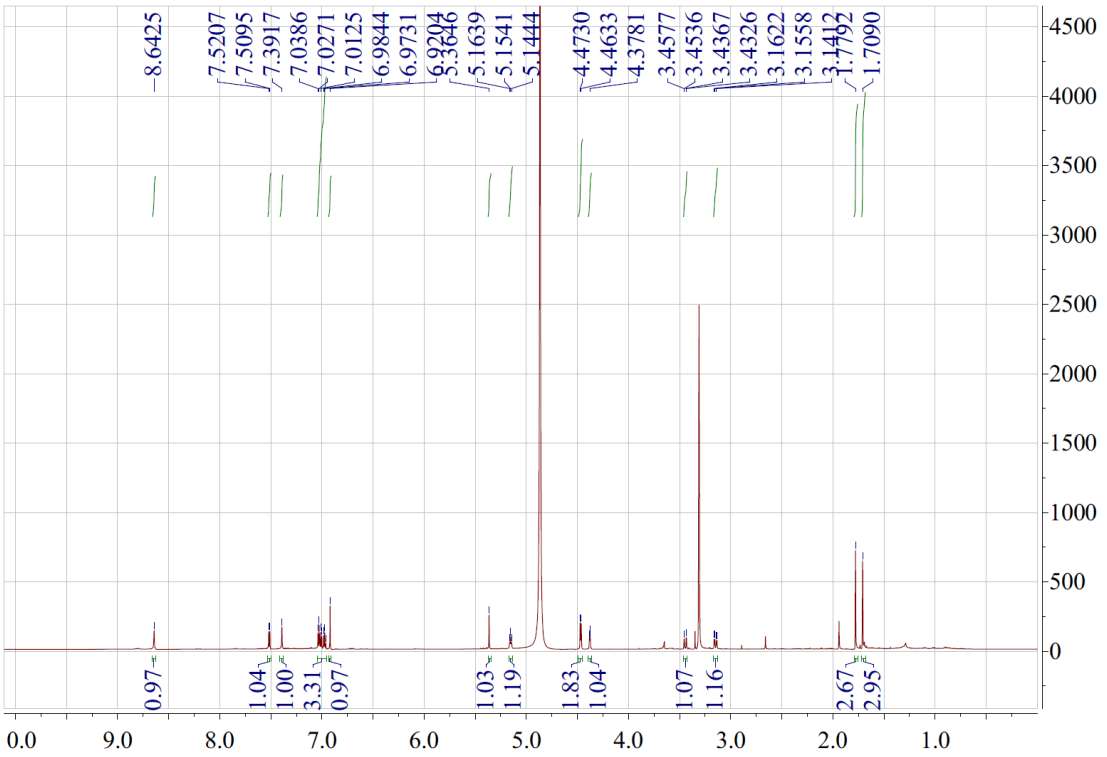


Figure S4. ^{13}C NMR spectrum of compound **1** (700 MHz, CD_3OD).

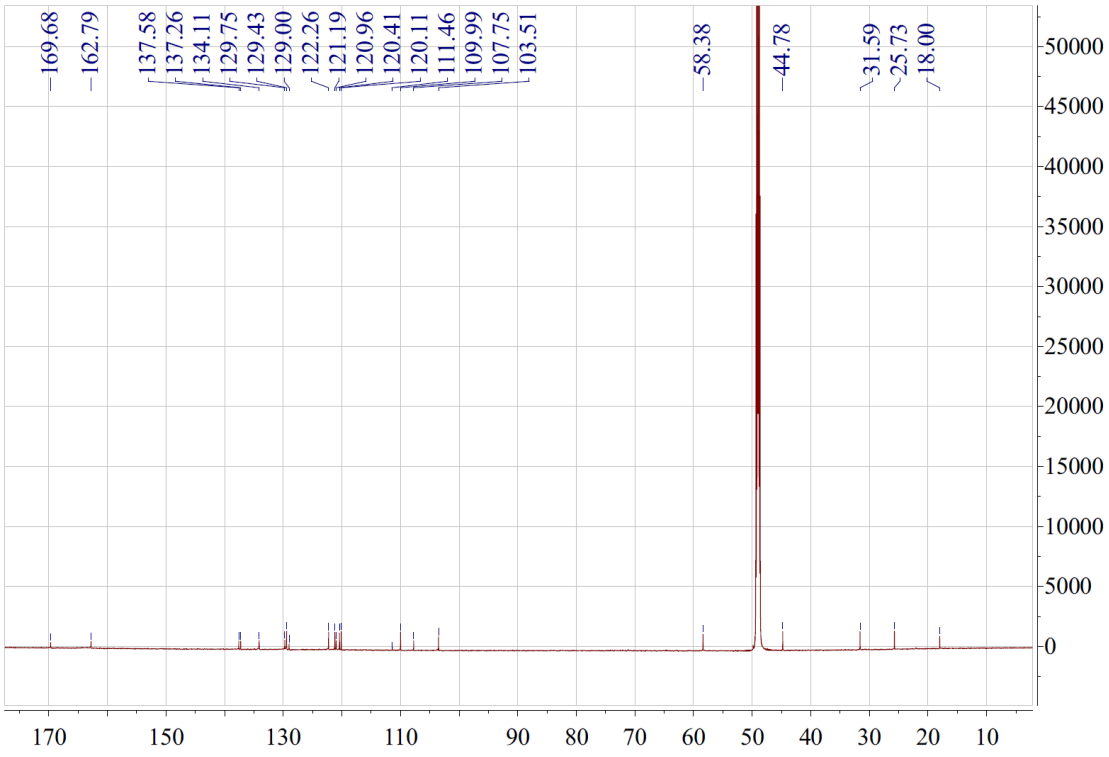


Figure S5. HSQC spectrum of compound **1** (700 MHz, CD_3OD).

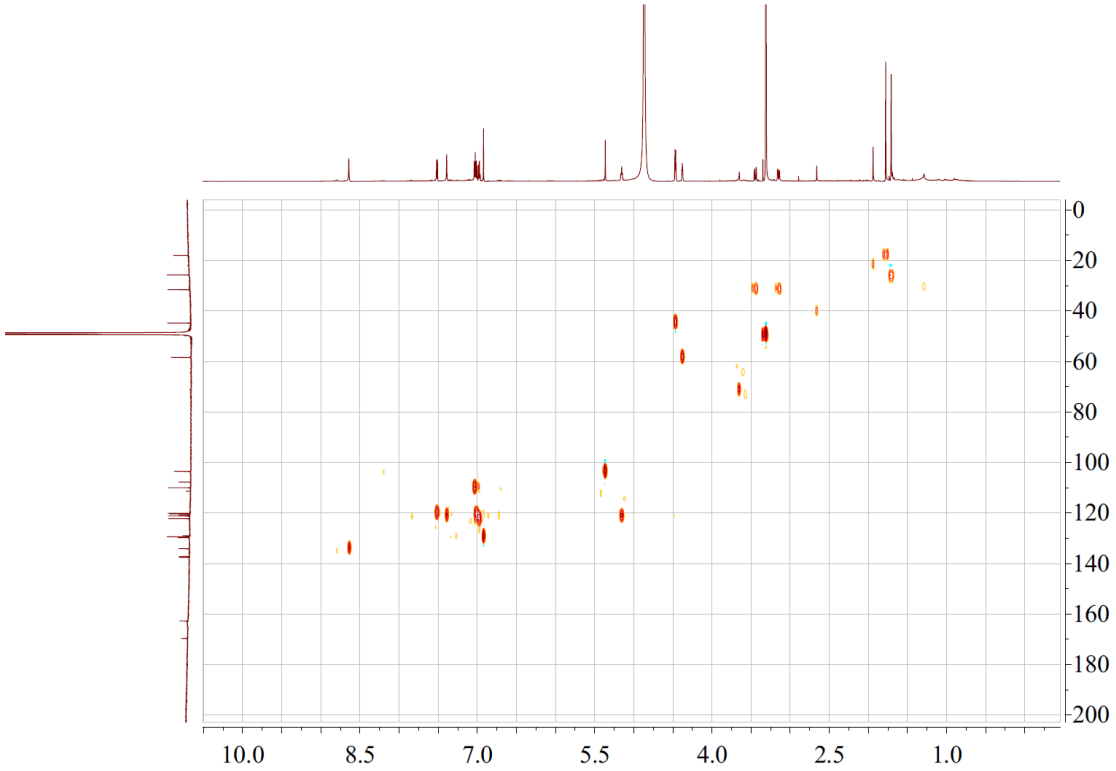


Figure S6. ^{1}H-^{1}H COSY spectrum of compound **1** (700 MHz, CD_3OD).

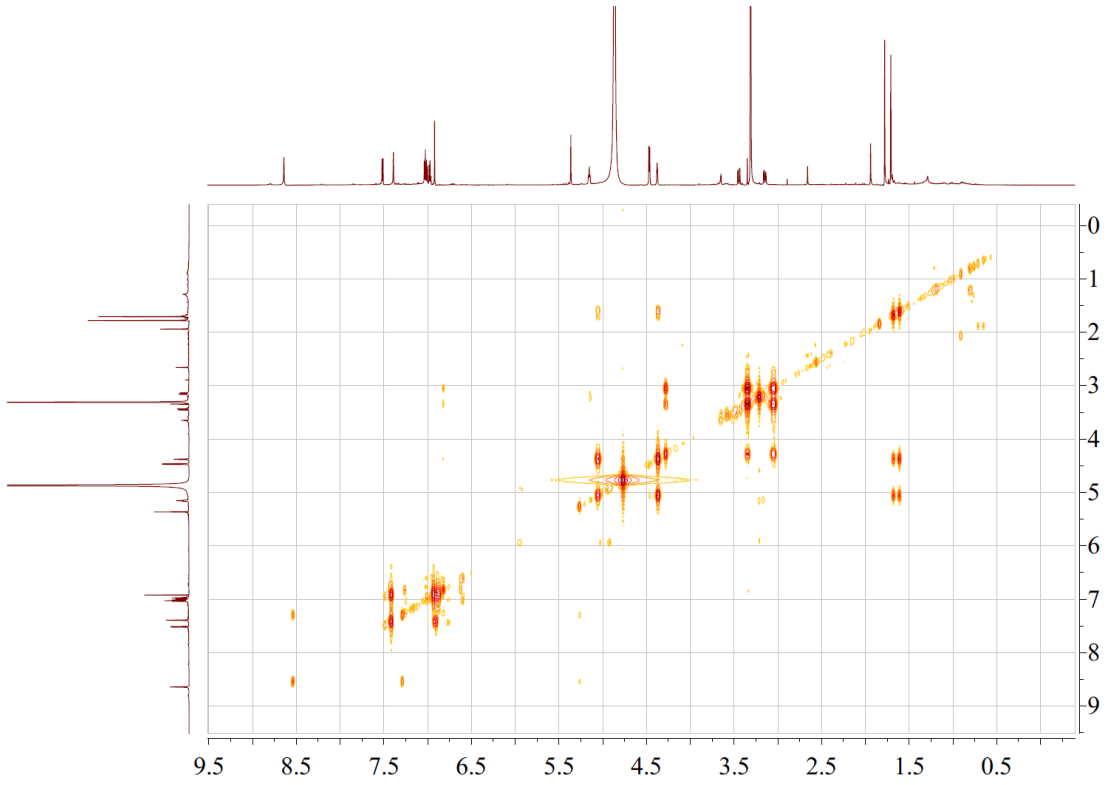


Figure S7. HMBC spectrum of compound **1** (700 MHz, CD_3OD).

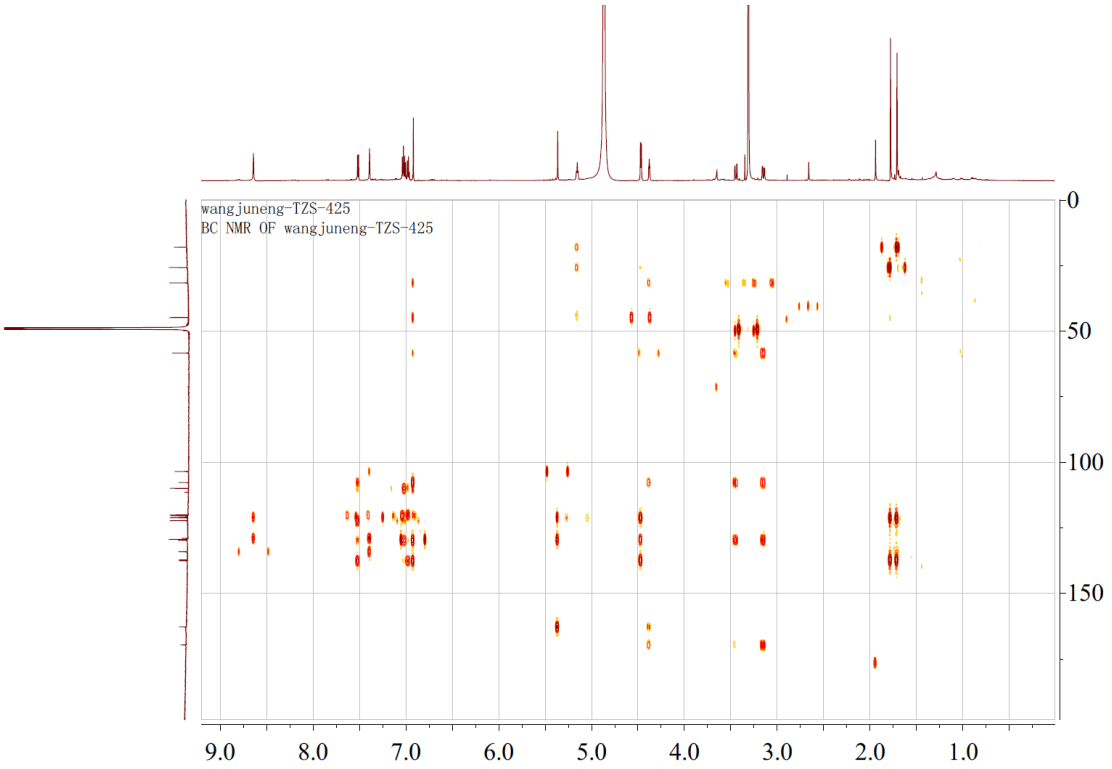


Figure S8. [1]H-NMR spectrum of compound **1** (700 MHz, DMSO-d_6).

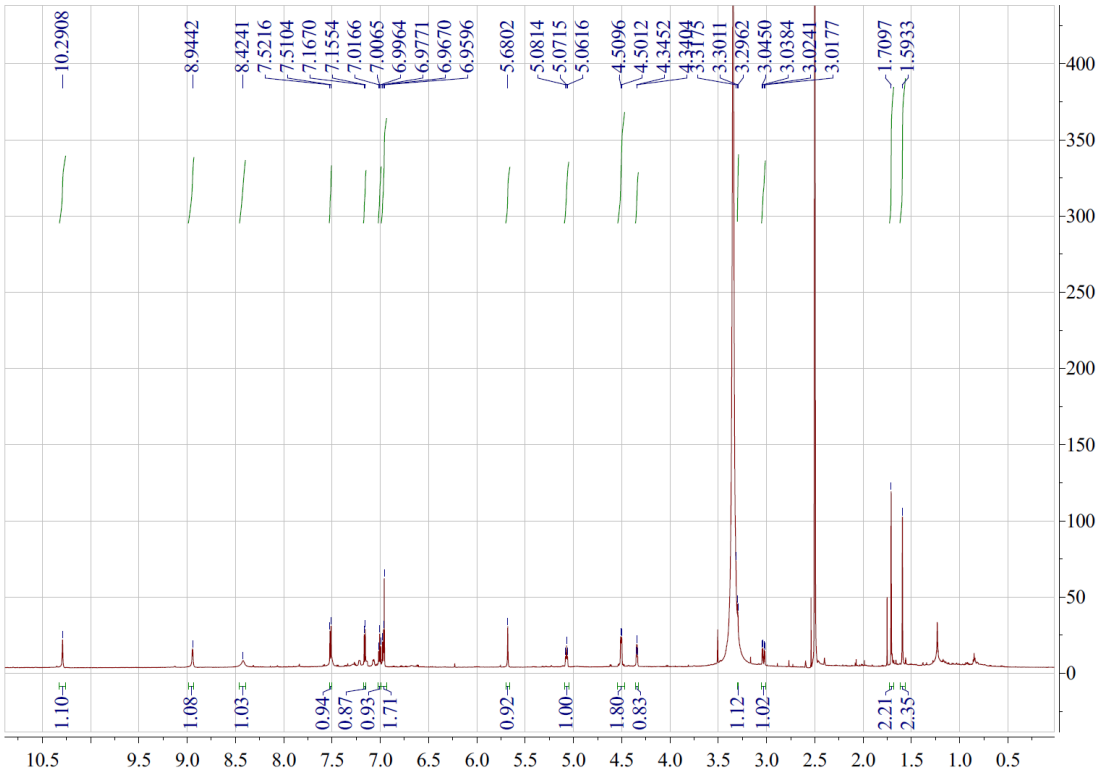


Figure S9. [13]C NMR spectrum of compound **1** (700 MHz, DMSO-d_6).

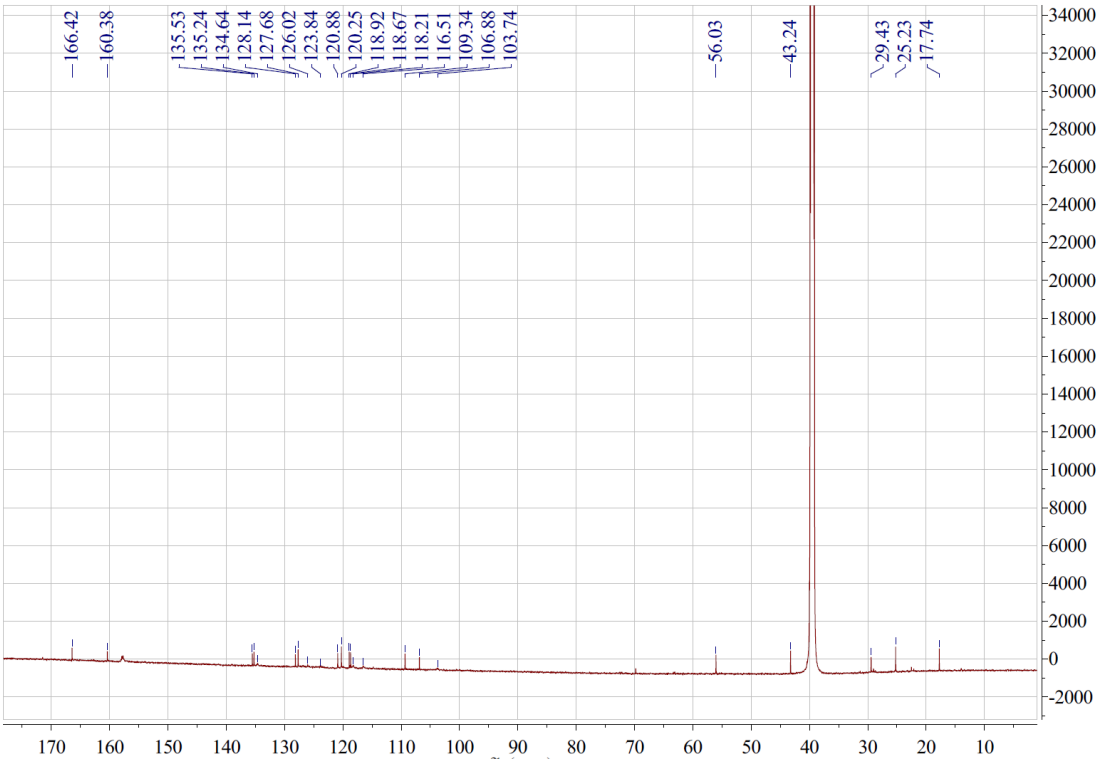


Figure S10. HSQC spectrum of compound **1** (700 MHz, DMSO-d_6).

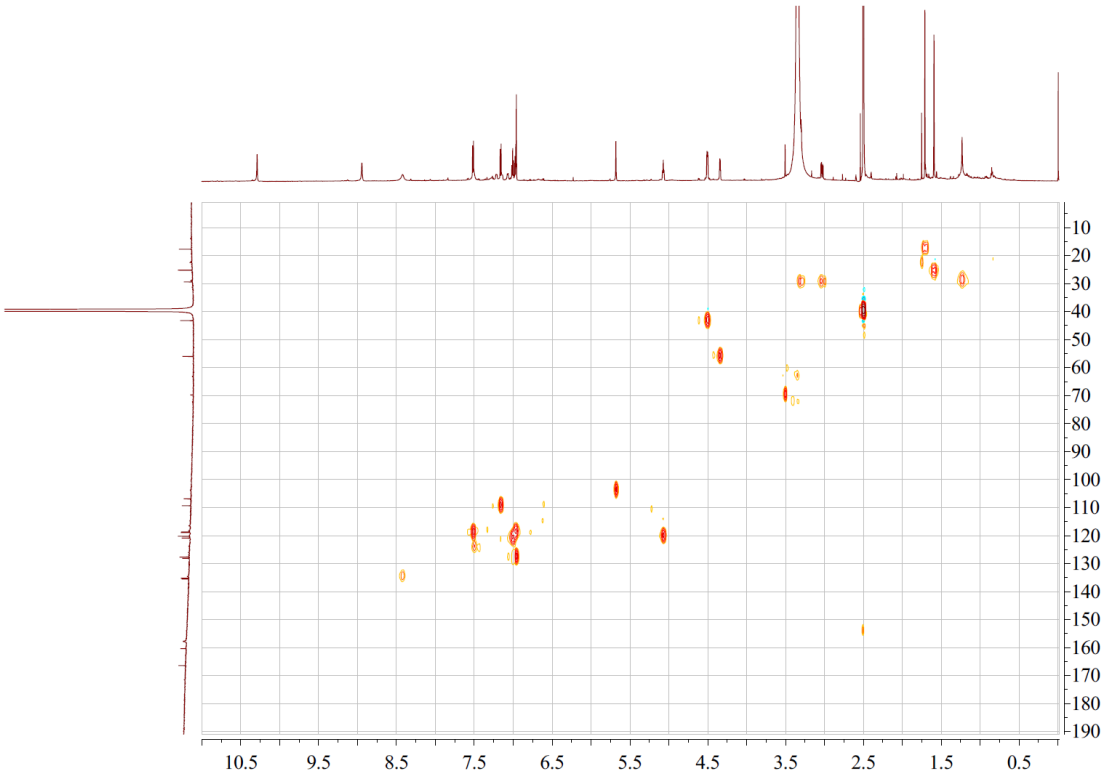


Figure S11. ^{1}H-^{1}H COSY spectrum of compound **1** (700 MHz, DMSO-d_6).

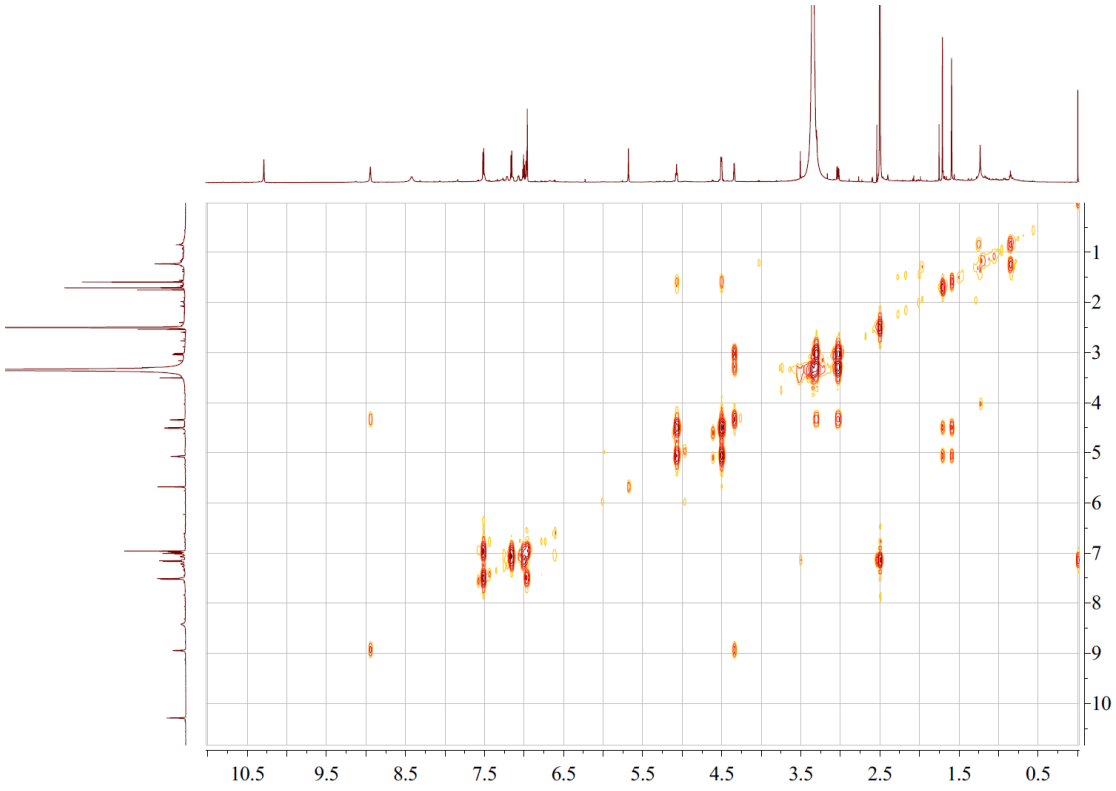


Figure S12. HMBC spectrum of compound **1** (700 MHz, DMSO-d_6).

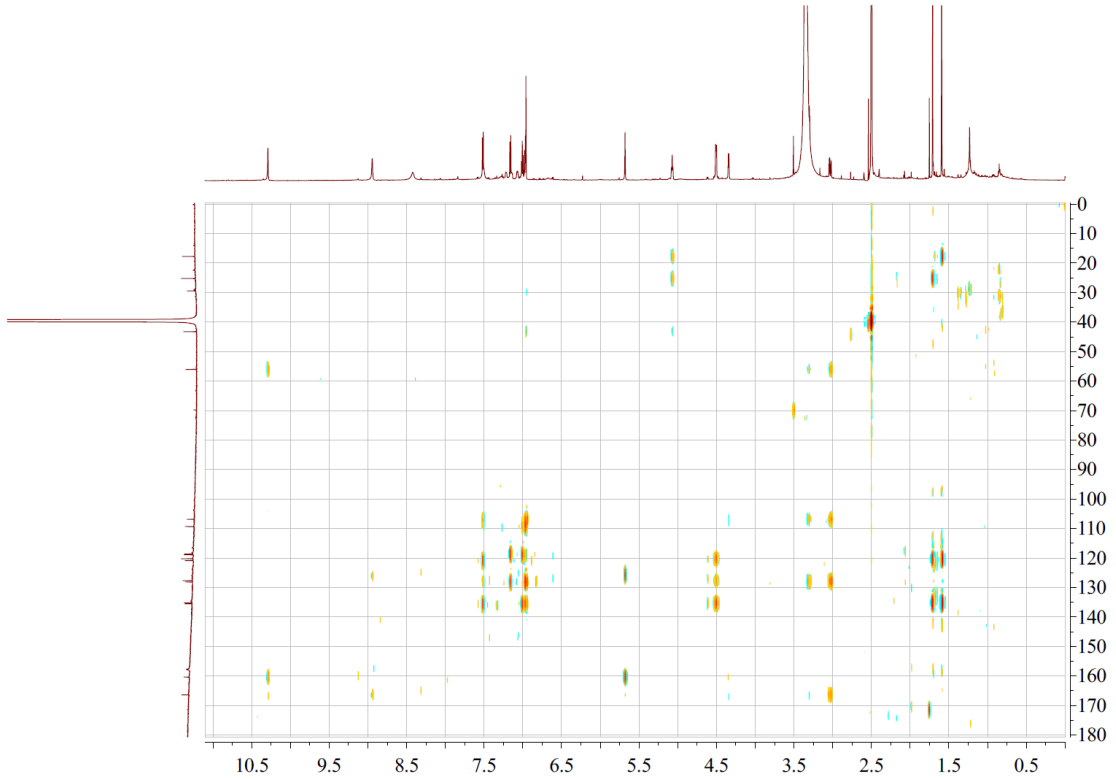


Figure S13. NOESY spectrum of compound **1** (700 MHz, DMSO-d_6).

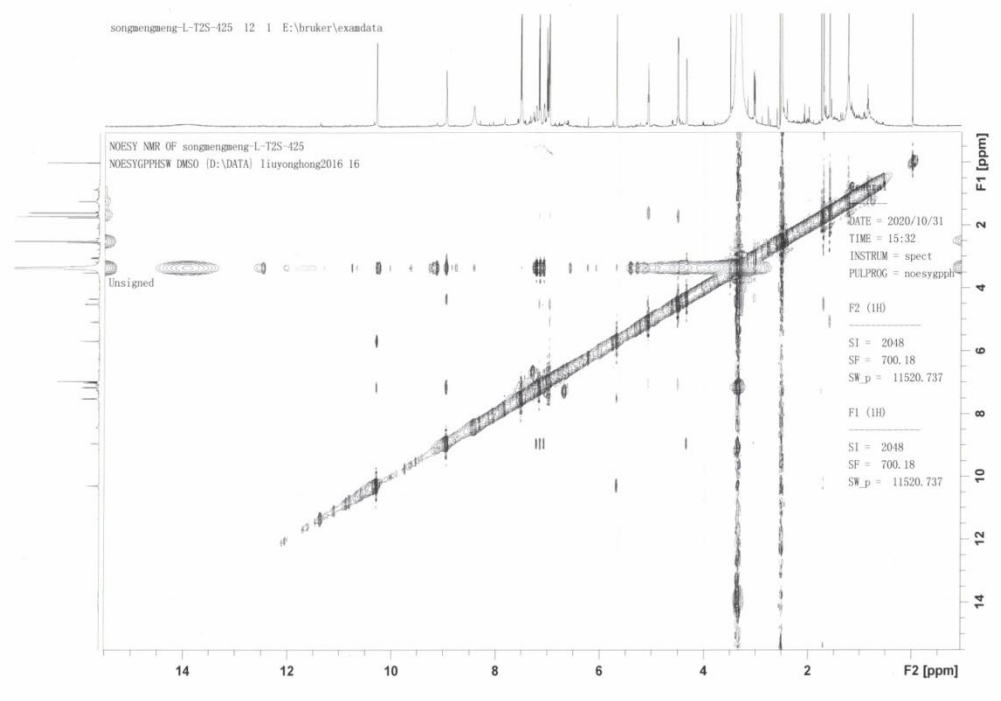


Figure S14. HRESIMS spectrum of **1**.