Supporting Information.

Spirostaphylotrichin X from a Marine-Derived Fungus as an Anti-influenza Agent Targeting RNA Polymerase PB2

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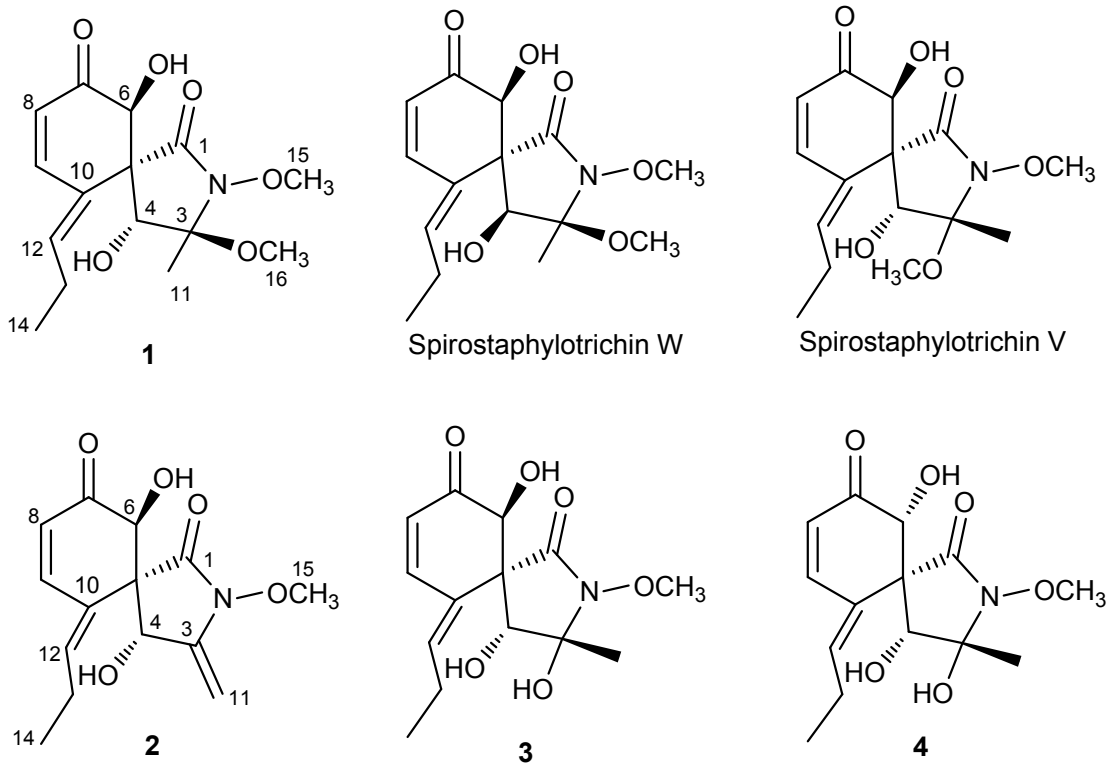


Figure S1. Structures of **1-4**, spirostaphylotrichins W and V.

Table S1. ^{1}H NMR spectral data (δ_H, mult, *J* in Hz) of **1-4**, spirostaphylotrichins W (**SW**) and V (**SV**) in references.

No.	**1** [a]	**1** [b]	**SW** [c]	**SV** [c]	**2** [a]	**3** [d]	**4** [d]
4	4.48, s	4.39, s	4.12, d (11.5)	3.81, s	4.67, dd (1.5, 1.5)	3.83, d (6.6)	4.05, d (6.6)
6	4.74, s	4.87, s	4.75, d (1.7)	4.87, s	4.69, s	4.66, d (1.7)	4.62, d (1.6)
8	5.83, d (9.9)	5.96, d (10)	5.94,d (9.9)	5.96, d (10)	5.86, d (9.8)	5.81, d (9.9)	5.80, d (9.9)
9	7.09, d (9.9)	7.06, d (10)	7.11, d (9.9)	7.07, d (10)	7.09, d (9.8)	7.14, d (9.9)	7.10, d (9.9)
11	1.38, s	1.45, s	1.69, s	1.46, s	4.71, dd (1.7, 1.5) 4.53, dd (1.7, 1.5)	1.47, s	1.57, s
12	6.10, t (8.0)	6.18, t (8.1)	6.12, t (7.4)	6.17, t (7.0)	6.23, t (7.5)	6.26, t (7.9)	6.15, t (7.6)
13	2.15, m; 2.02, m	2.29, m 2.06, m	2.24, m 2.15, m	2.29, m 2.06, m	2.11, m; 2.02, m	2.18, m; 2.13, m	2.30, m; 2.27, m
14	1.04, t, (7.4)	1.08, t (7.5)	1.05, t (7.4)	1.08, t (7.0)	1.03, t, (7.4)	1.00, t, (7.4)	0.98, t, (7.4)
15	3.95, s, CH_3	4.01, s, CH_3	3.94, s, CH_3	4.02, s, CH_3	3.90, s, CH_3	3.91, s	3.91, s, CH_3
16	3.39, s, CH_3	3.43, s, CH_3	3.51, s, CH_3	3.43, s, CH_3	/	/	/

[a] in CD_3OD, 500 M; [b] in $CDCl_3$, 700 M; [c] in $CDCl_3$, 400 M; [d] in Acetone-d_6, 700 M;

Table S2. ^{13}C NMR spectral data (δ_C) of **1-4**, spirostaphylotrichins W (**SW**) and V (**SV**) in references.

No.	**1** [a]	**SW** [b]	**SV** [b]	**2** [a]	**3** [c]	**4** [c]
1	169.3	167.6	166.1	170.1	166.9	166.6
3	97.0	88.8	94.8	145.7	90.1	90.1
4	68.4	70.1	68.2	65.7	73.6	73.5
5	59.3	56.6	57.4	59.0	56.8	56.9
6	75.0	73.5	73.8	75.1	73.7	73.7
7	199.0	196.8	197.2	198.7	195.6	196.4
8	122.8	120.5	121.4	122.3	119.9	120.7
9	154.1	153.2	153.0	152.8	152.2	152.5
10	131.3	128.9	128.9	130.3	131.1	130.1
11	18.0	17.5	17.0	86.7	18.7	18.5
12	149.7	149.5	149.3	150.3	150.6	148.5
13	24.7	23.4	23.7	24.1	23.2	22.9
14	13.6	13.2	13.4	13.5	12.4	12.6
15	64.8	64.5	64.3	62.7	63.2	68.8
16	51.1	52.7	51.0	/	/	/

[a] in CD_3OD, 125 M; [b] in $CDCl_3$, 100 M; [c] in Aceton-d_6, 175 M;

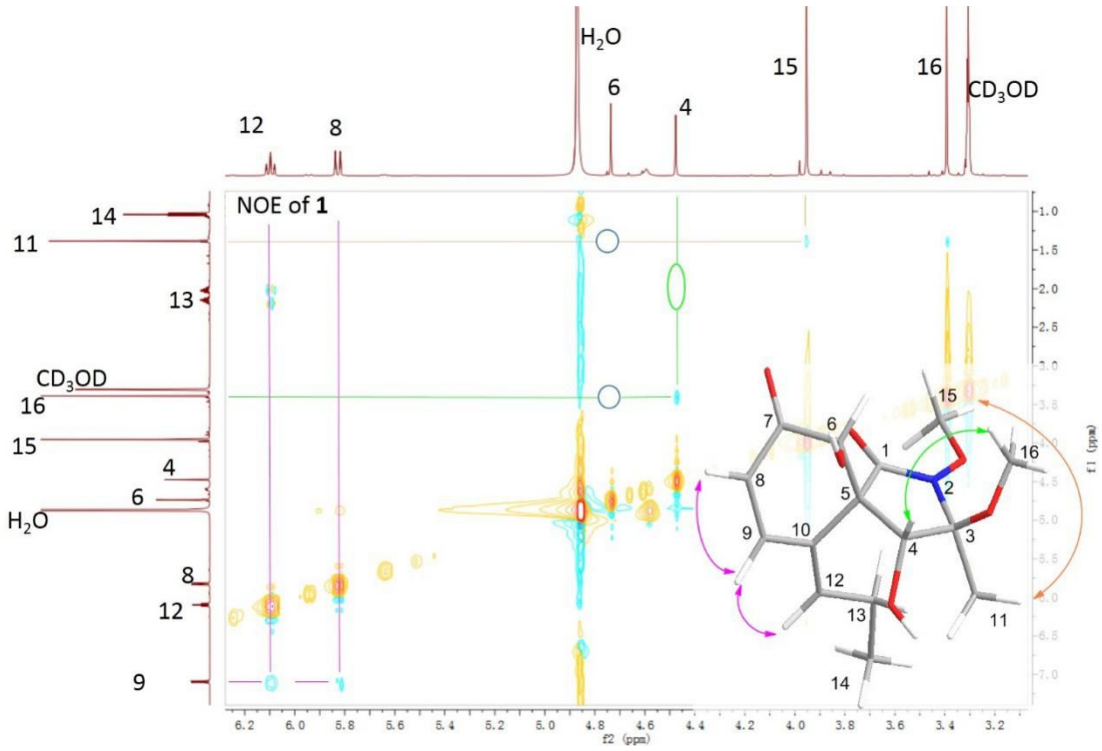


Figure S2-1. Key NOESY correlations of **1** (in MeOD, 500 M).

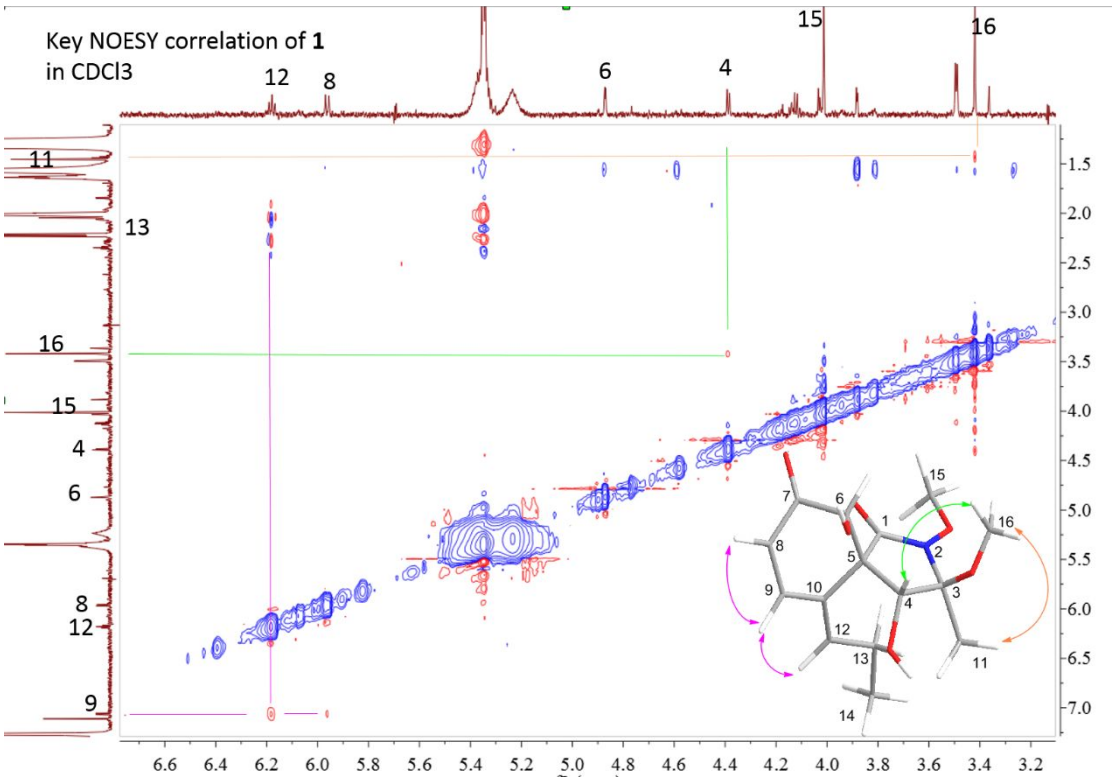


Figure S2-2. Key NOESY correlations of **1** (in $CDCl_3$, 700 M). *

* Not a pure sample.

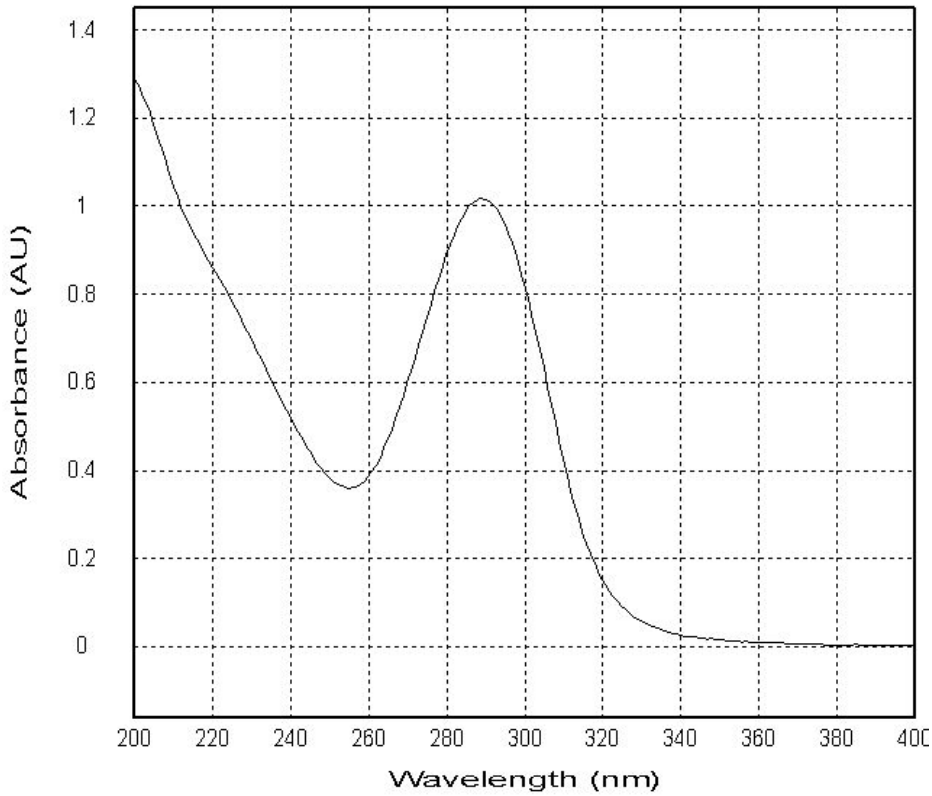


Figure S3. UV spectrum of **1**.

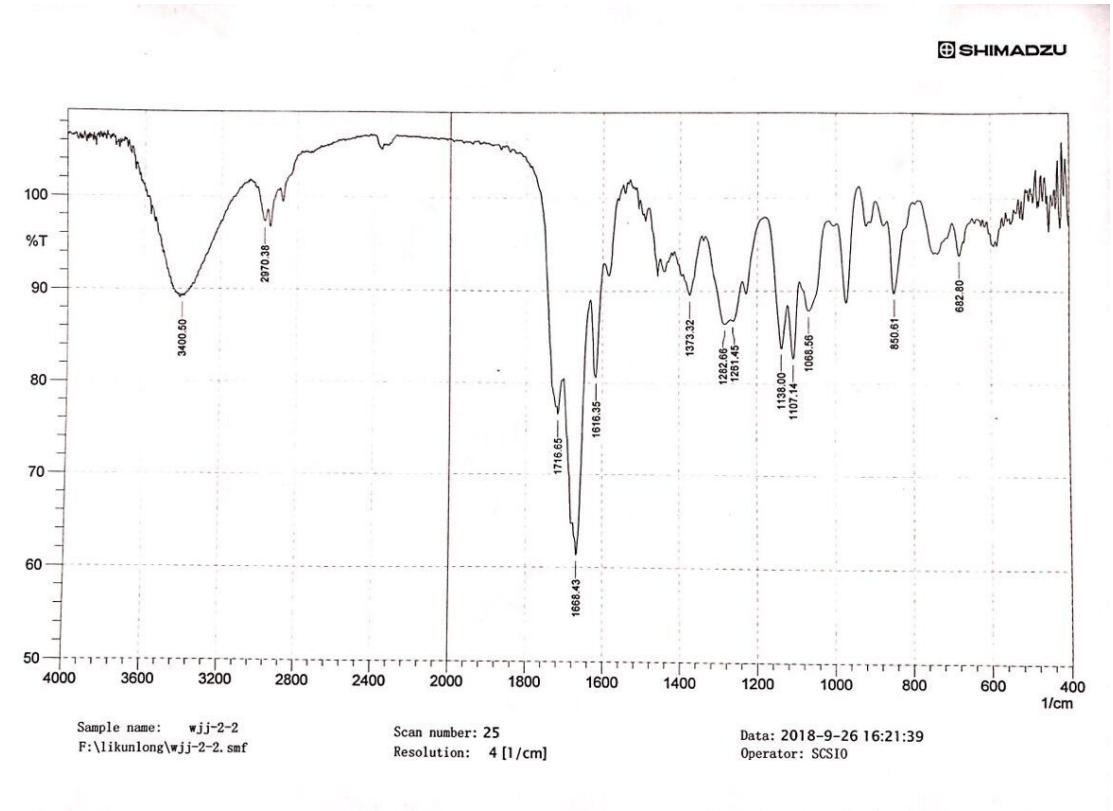


Figure S4. IR spectrum of **1**.

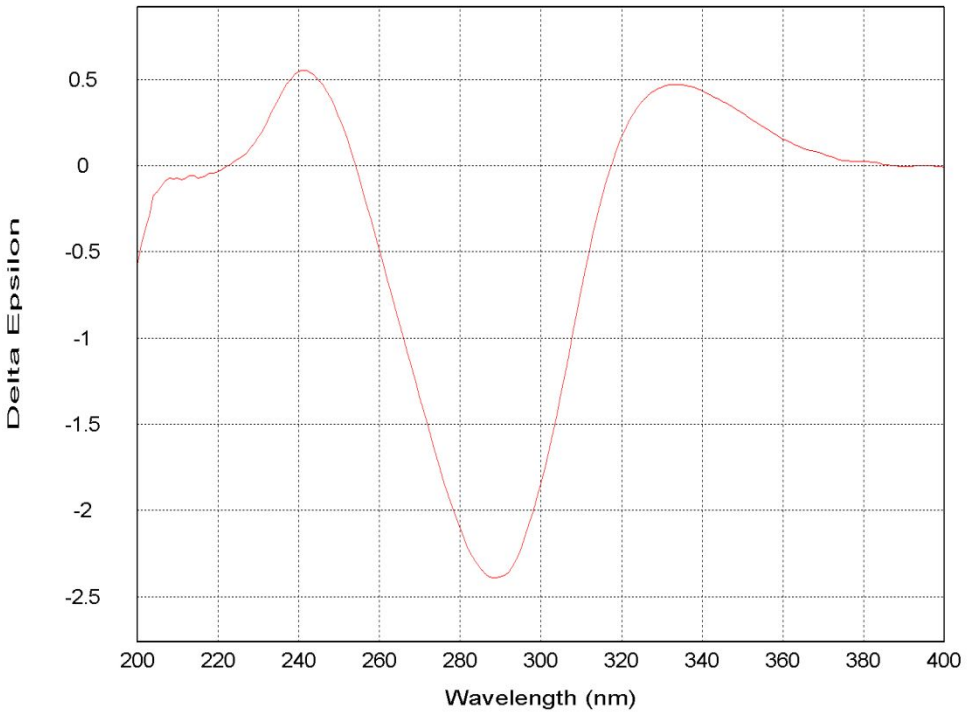
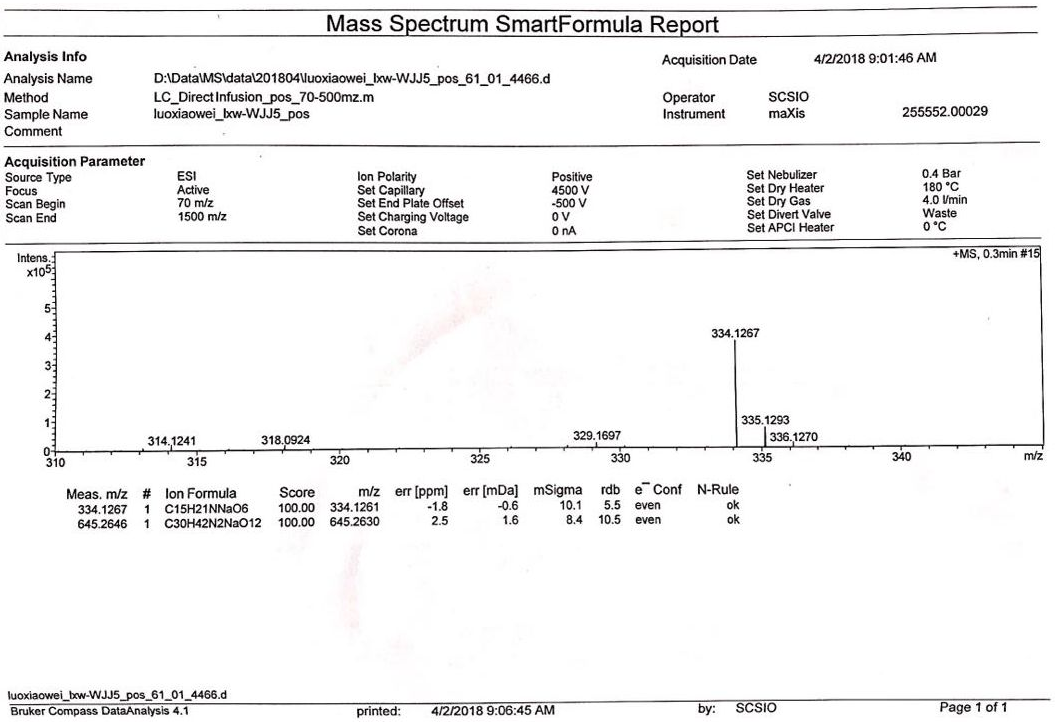


Figure S5. ECD spectrum of **1.**



Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	luoxiaowei_lxw-WJJ5_pos	Instrument	maXis 255552.00029
Comment			

Acquisition Parameter

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Scan Begin	70 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e⁻ Conf	N-Rule
334.1267	1	C15H21NNaO6	100.00	334.1261	-1.8	-0.6	10.1	5.5	even	ok
645.2646	1	C30H42N2NaO12	100.00	645.2630	2.5	1.6	8.4	10.5	even	ok

luoxiaowei_lxw-WJJ5_pos_61_01_4466.d
Bruker Compass DataAnalysis 4.1 printed: 4/2/2018 9:06:45 AM by: SCSIO Page 1 of 1

Figure S6. HR-ESIMS (+) spectrum of **1.**

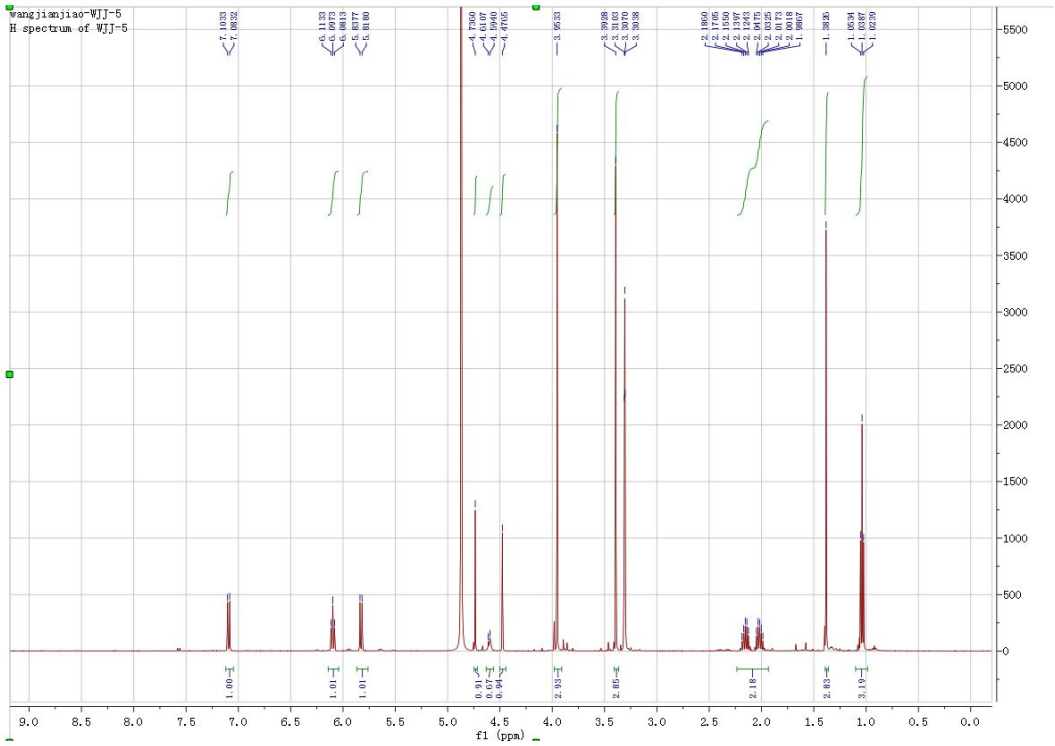

Figure S7-1. ^{1}H-NMR (500 MHz) spectrum of **1** (in MeOD).

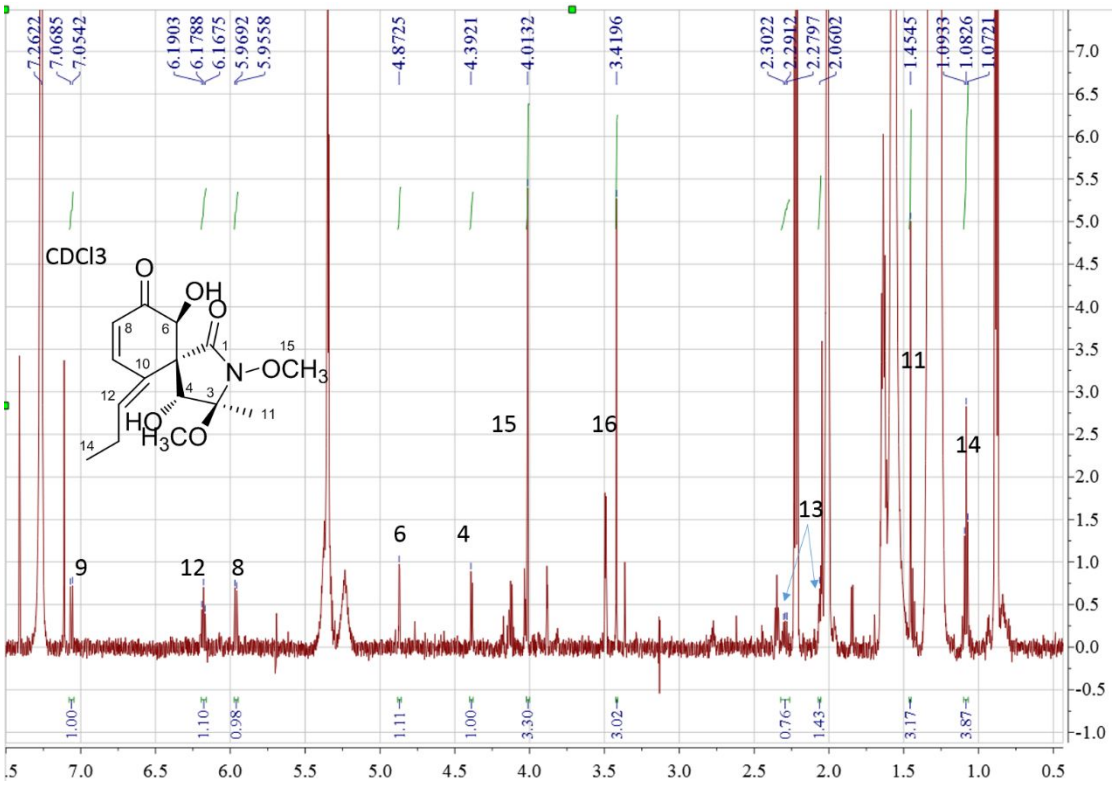

Figure S7-2. ^{1}H-NMR (700 MHz) spectrum of **1** (in $CDCl_3$).*

* Not a pure sample.

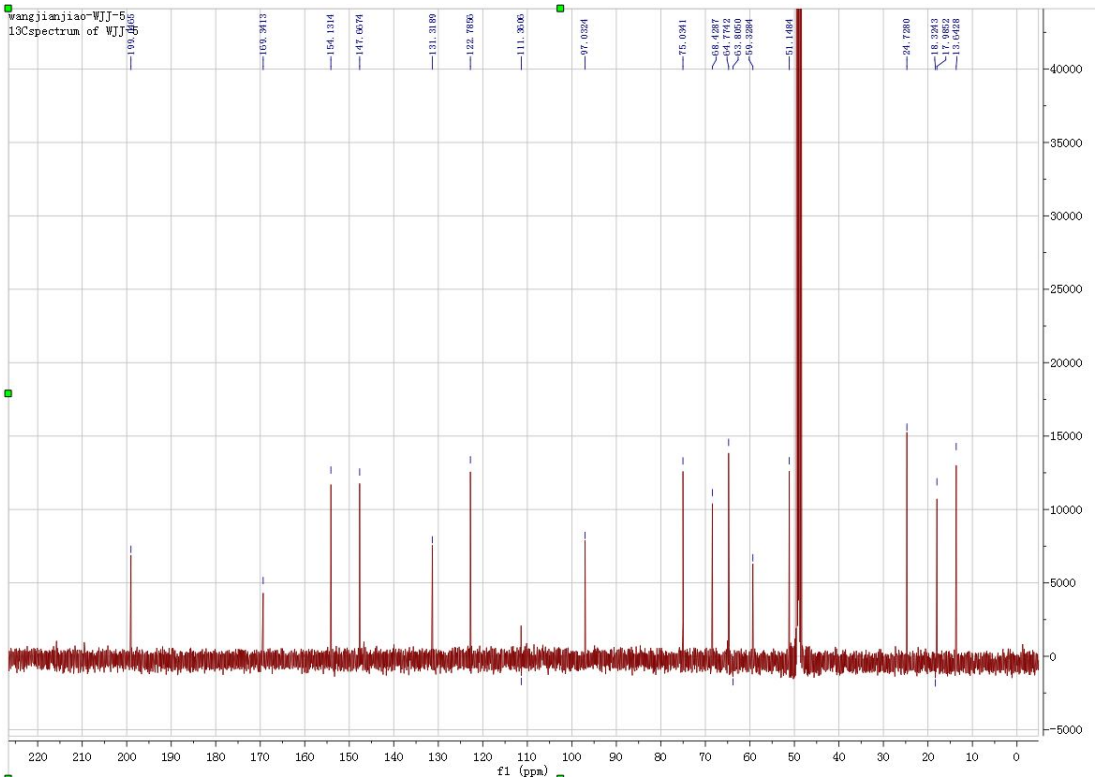

Figure S8. ^{13}C-NMR (125 MHz) spectrum of **1** (in MeOD).

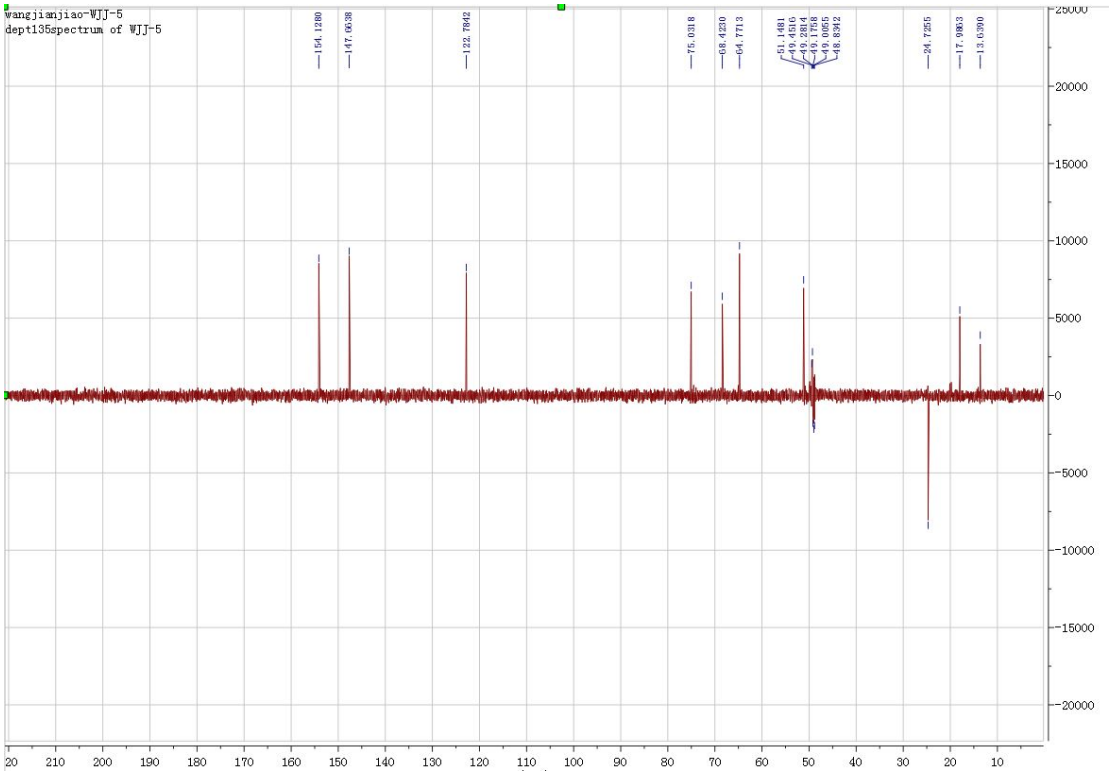

Figure S9. DEPT (125 MHz) spectrum of **1** (in MeOD).

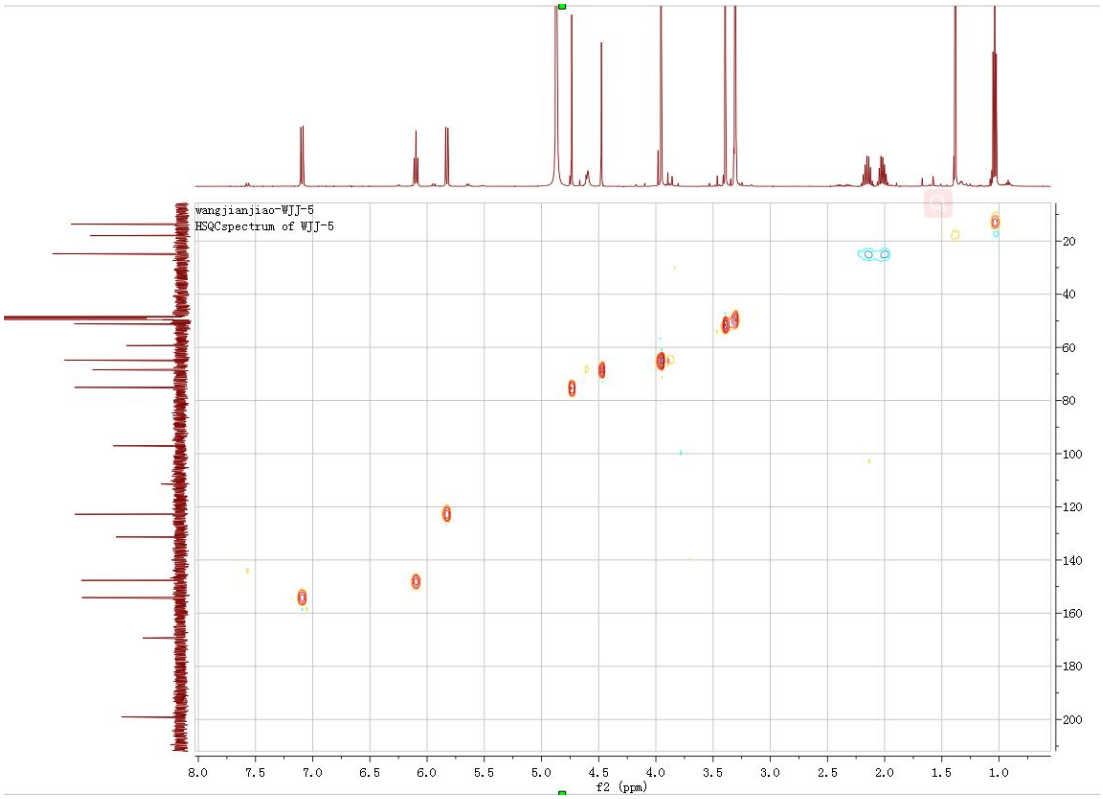

Figure S10. HSQC spectrum of **1** (in MeOD).

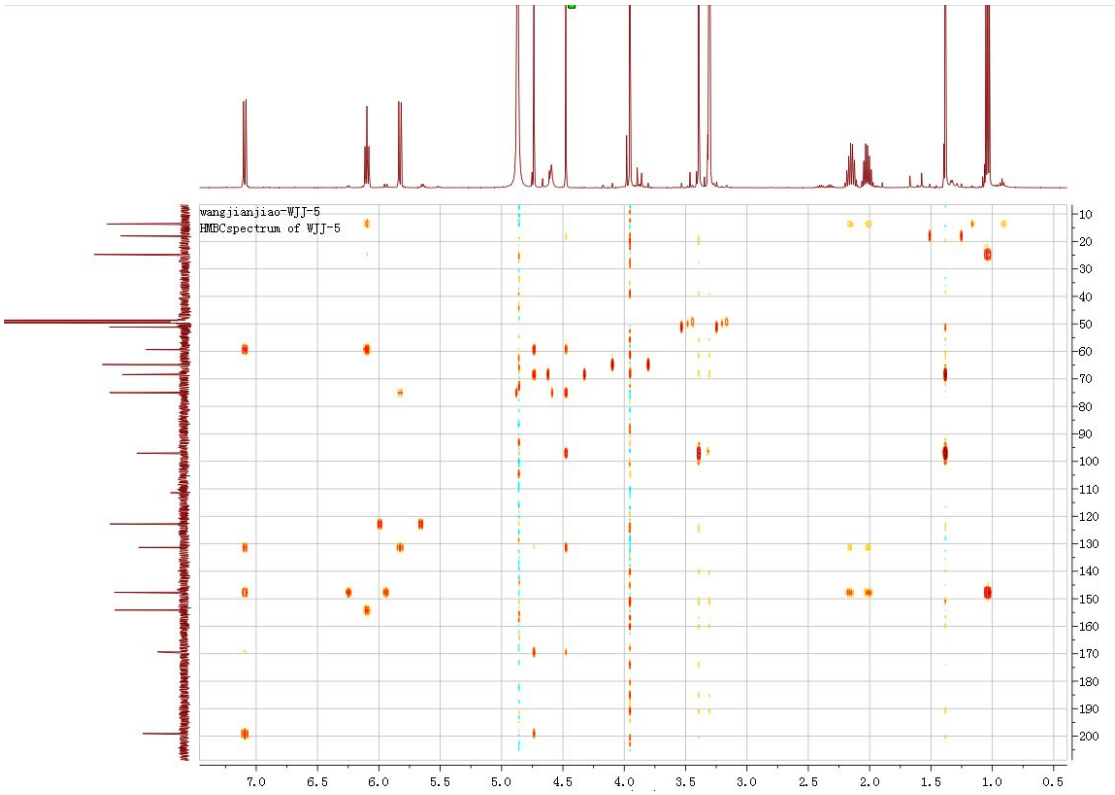

Figure S11. HMBC spectrum of **1** (in MeOD).

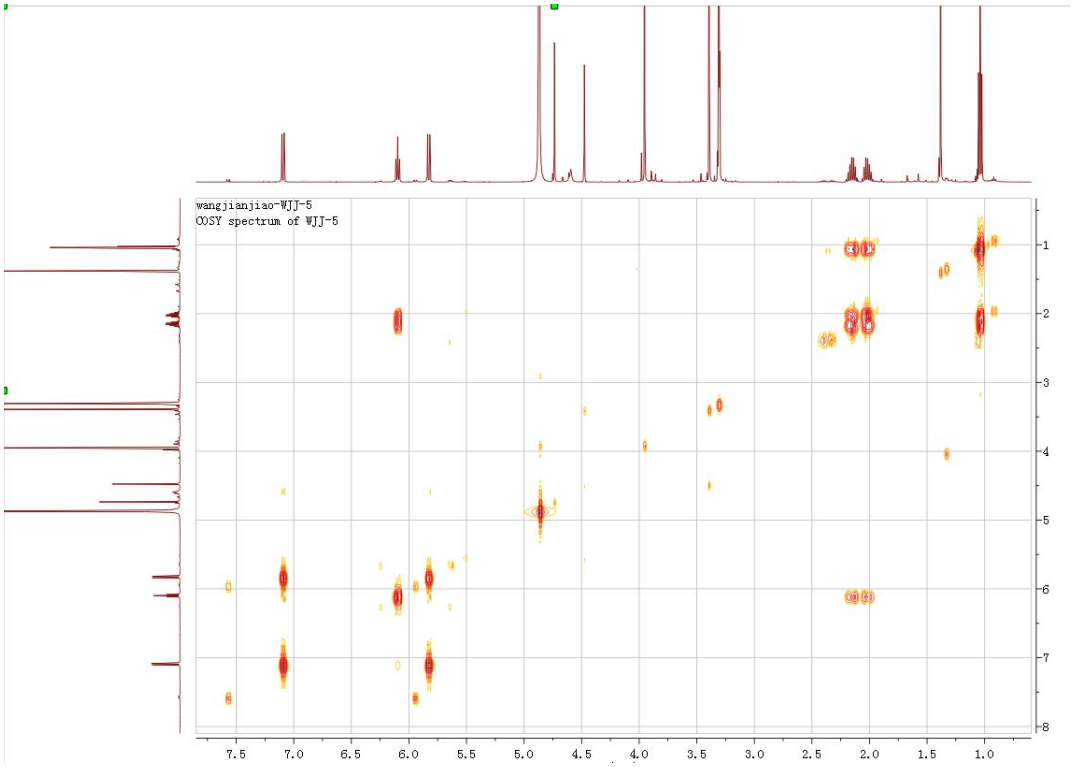

Figure S12. COSY spectrum of **1** (in MeOD).

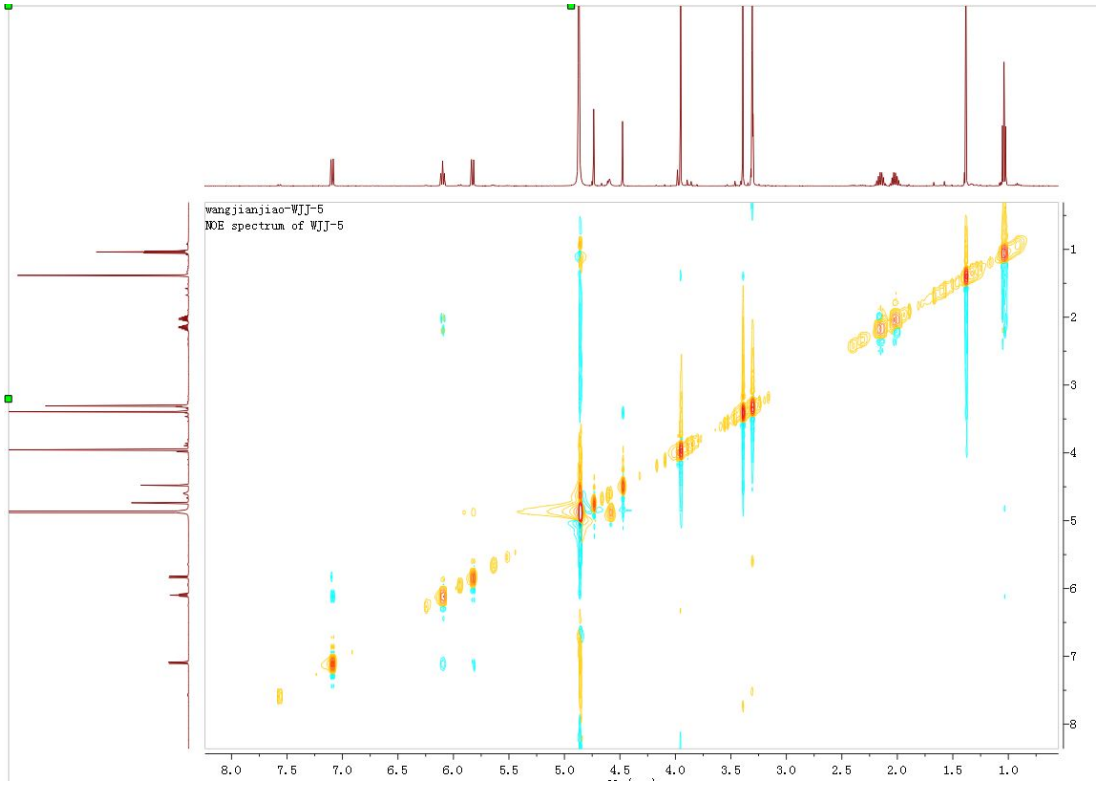

Figure S13. NOESY spectrum of **1** (in MeOD).

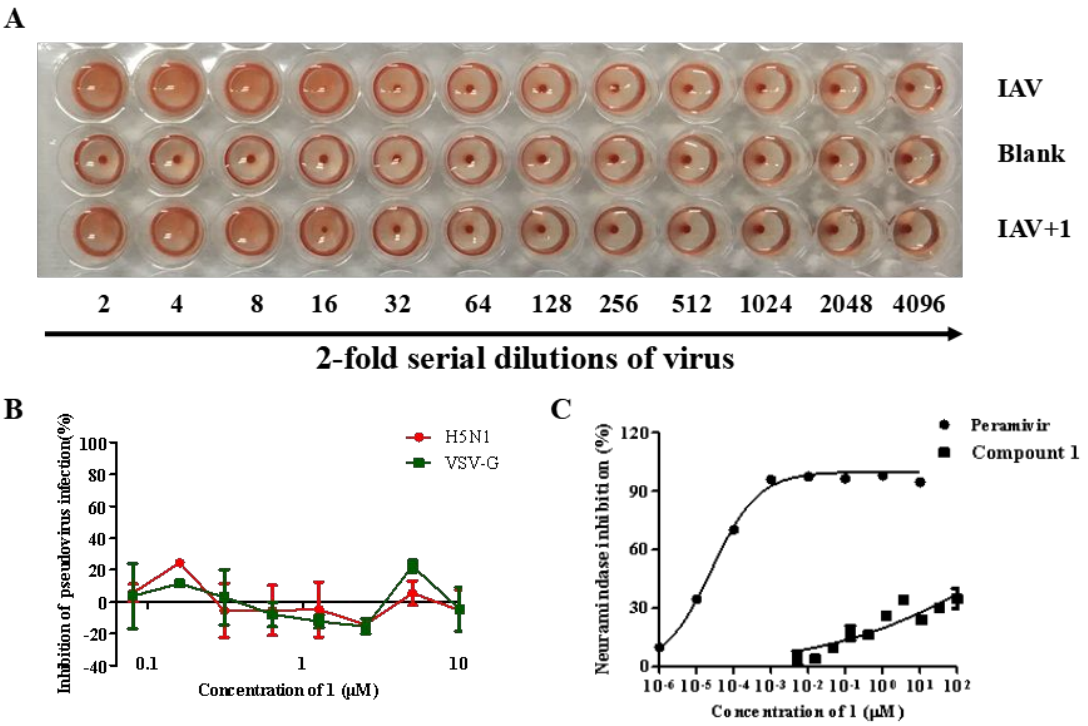

Figure S14. The effect of the compound **1** on the envelope protein of influenza virus. (A) The effect of **1** on hemagglutination. (B) The effect of **1** on H5N1 and VSV-G pseudovirus. (C) The effect of **1** or peramivir on NA activity. The peramivir was used as a positive control.

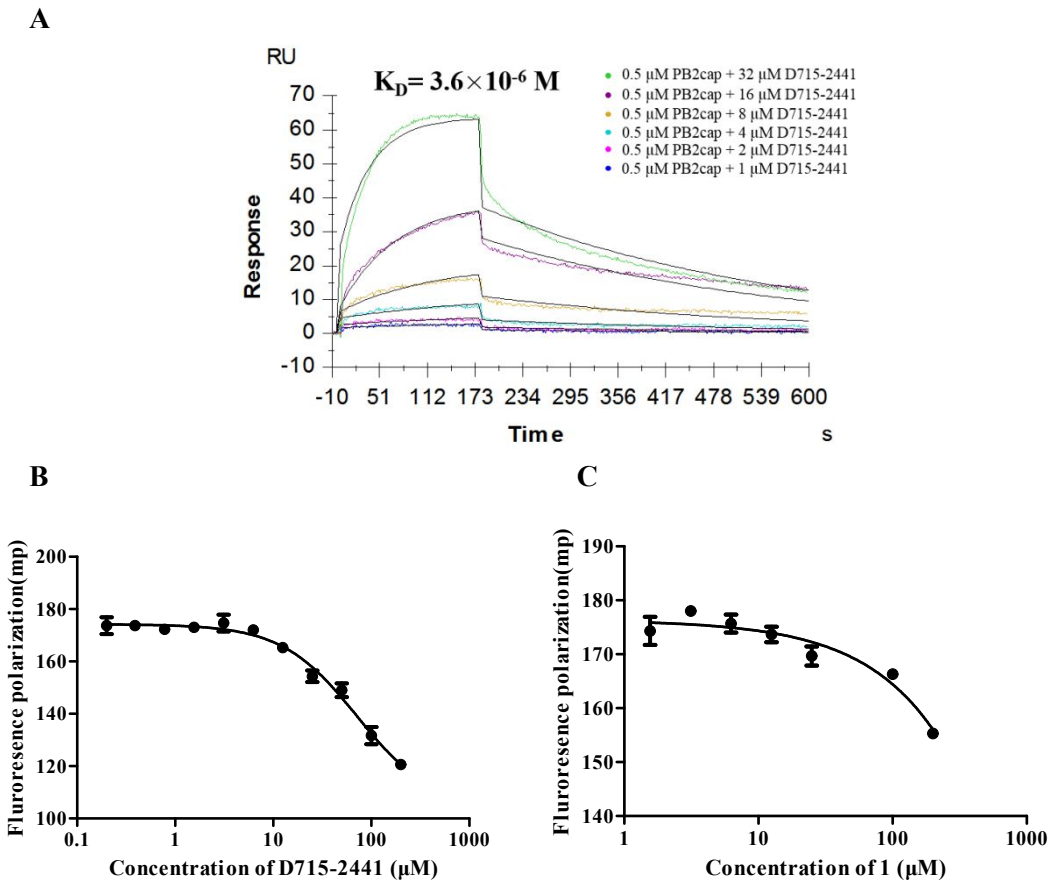

Figure S15. Compound **1** or D715-2441 showed a specific interaction with the viral PB2-cap protein. (A) The binding affinity of D715-2441 to viral PB2-cap protein was obtained from SPR measurements. (B) The specific binding between D715-2441 and PB2-cap protein by fluorescence polarization (FP) assay. (C) The specific binding between **1** and PB2-cap protein was analyzed by FP assay.

Table S3. Primer sequences for quantitative RT-PCR

Target	Gene Sequence
HA-Forward	5-TTCCCAAGATCCATCCGGCAA-3′
HA-Reverse	5′-CCTGCTCGAAGACAGCCACAACG-3′
NP-Forward	5′-GACCAGGAGTGGAGGAAACA-3′
NP-Reverse	5′-CGGCCATAATGGTCACTCTT-3′
GAPDH-Forward	5′-AGGGCAATGCCAGCCCCAGCG-3′
GAPDH-Reverse	5′-AGGCGTCGGAGGGCCCCCTC-3’

Method and Result of Fluorescence Polarization (FP) Assay

Method

Fluorescence Polarization (FP) Assay.

3μg/ml PB2-cap protein and 20 nM FITC-m7GTP (EDA-m7GTP-ATTO 488; Jena Bioscience, Germany) dissolved in the reaction buffer (50 mM HEPES, 0.5 mM EDTA, 100 mM KCl, 1 mM dithiothreitol and 1% DMSO, pH 7.2) were added to 96-well blackboard at 50 μl per well. Subsequently, 50 μL compound **1** at indicated concentrations was added to each well. After incubation at room temperature for 30 minutes, the fluorescence polarization value was detected by BioTek instrument (America) at excitation wavelength 488nm and emission wavelength 535nm.

RESULTS

The results indicated D715-2441 as positive control inhibitor could dose-dependently inhibited a FITC-labelled m7GTP and viral PB2-cap binding (**Figure S15B**). As indicated by a decrease in FP values, we also found increasing concentrations of **1** competitively displaced the binding of FITC-m7GTP to PB2-cap, which consist with the results of HTRF (**Figure S15C**).