SUPPLEMENTARY MATERIAL

Anti-neuroinflammatory sesquiterpenoids from *Chloranthus henryi*

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ABSTRACT

Hupelactones A (**1**) and B (**2**), two new eudesmanolide-type enantiomers of the corresponding compounds, along with four mono- (**3**–**6**) and nine dimeric- (**7**–**15**) known sesquiterpenoids were isolated from the whole plant of *Chloranthus henryi* var. *hupehensis* (syn. *C. henryi)*. The new structures including the absolute configurations were determined by comparison with previously reported enantiomers, extensive spectroscopic methods in combination with electronic circular dichroism (ECD) calculations. All the isolates were evaluated for their inhibitory activities against the lipopolysaccharide (LPS)-induced nitric oxide (NO) production in murine BV-2 microglial cells. Among them, the dimeric lindenane sesquiterpenoids shizukaols F (**8**) and G (**11**) exhibited the most potent activities, with IC_{50} values of 2.65 and 4.60 μM, respectively.

Keywords*: Chloranthus henryi*; Chloranthaceae; Sesquiterpenoids; Anti-neuroinflammation

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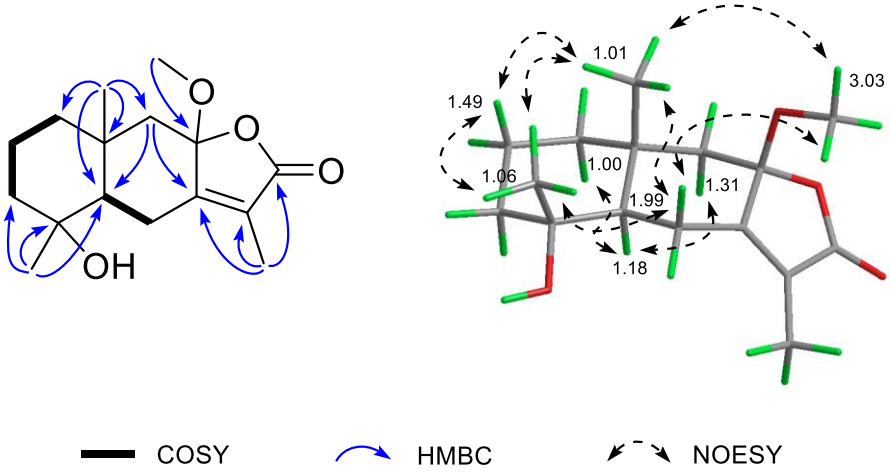


Figure S1. Observed key ^{1}H-^{1}H COSY, HMBC, and NOE correlations for **1**

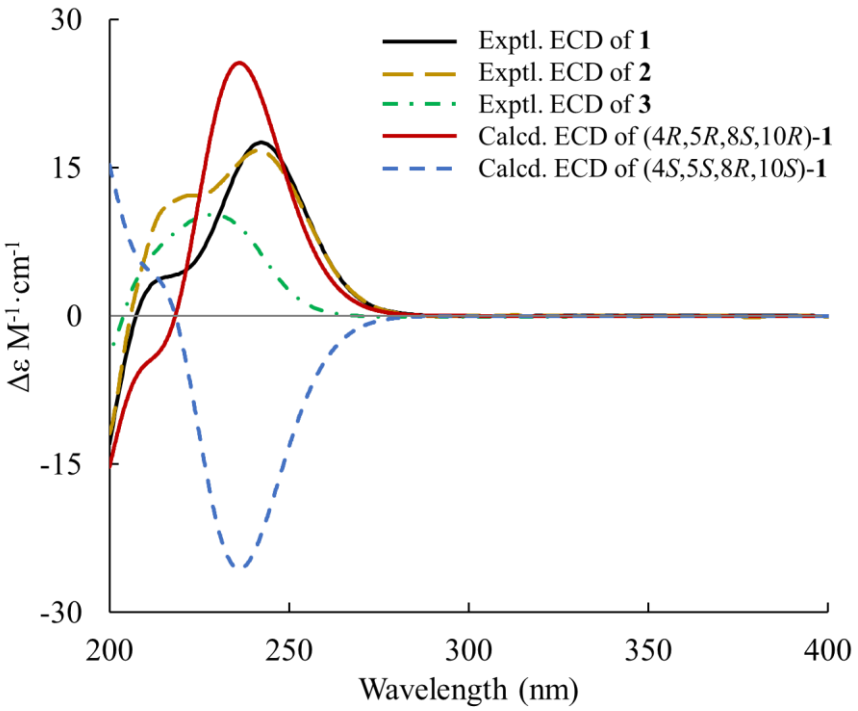


Figure S2. Experimental ECD spectra of **1**–**3** and calculated ECD spectra of **1** in MeOH

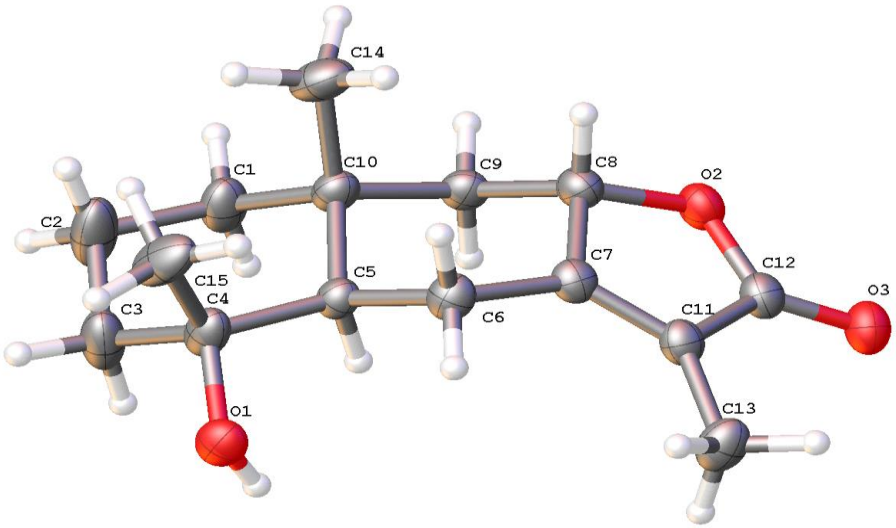


Figure S3. Olex2 drawing of compound **3**

Table S1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for compounds **1**–**3**. [a]

No.	**1**[b]		**2**[b]		**3**[c]	
	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C
1α	1.00, ddd (13.1, 12.8, 4.0)	40.1	1.00, ddd (13.0, 12.9, 3.7)	40.3	1.17, ddd (13.3, 13.1, 4.3)	40.1
1β	1.40, ddd (13.1, 4.1, 3.7)		1.37, ddd (13.0, 3.7, 3.5)		1.54, m	
2α	1.45, m	19.2	1.44, m	19.2	1.56, m	19.7
2β	1.49, ddddd (13.6, 12.9, 12.8, 3.8, 3.7)		1.50, ddddd (13.7, 13.1, 12.9, 3.8, 3.7)		1.64, m	
3α	1.25, ddd (13.1, 12.9, 4.3)	42.3	1.25, ddd (13.1, 12.7, 4.3)	42.3	1.36, ddd (13.0, 12.9, 4.7)	43.3
3β	1.65, ddd (13.1, 3.8, 2.8)		1.64, ddd (12.7, 3.8, 3.6)		1.87, ddd (12.9, 4.4, 3.2)	
4		70.2		70.2		72.1
5	1.18, dd (12.8, 2.7)	56.5	1.16, dd (12.9, 2.6)	56.6	1.29, dd (13.7, 3.4)	55.0
6α	2.97, dd (12.9, 2.7)	21.5	2.93, dd (13.0, 2.6)	21.1	3.10, dd (13.0, 2.6)	22.7
6β	1.99, br dd (12.9, 12.8)		2.05, br dd (13.0, 12.9)		2.16, br dd (14.0, 13.7)	
7		160.6		162.6		162.8
8		106.2		103.8	4.80, dd (11.8, 11.2)	78.0
9α	1.31, d (13.4)	53.1	1.28, d (13.0)	54.3	1.06, dd (13.0)	50.6
9β	2.10, d (13.4)		2.05, d (13.0)		2.21, dd (11.8, 6.5)	
10		35.0		35.0		35.6
11		122.5		119.4		120.0
12		171.1		171.8		175.0
13	1.76, br s	8.0	1.70, br s	7.9	1.83, br s	8.3
14	1.01, s	18.9	1.05, s	19.0	1.06, s	18.6
15	1.06, s	22.5	1.06, s	22.4	1.23, s	22.4
-OMe	3.03, s	49.8				

[a] Assignments were made by a combination of 1D and 2D NMR experiments.

[b] Measured in DMSO-d_6.

[c] Measured in $CDCl_3$.

Table S2. Inhibitory effects on LPS-induced NO production in BV-2 cells

Compound	IC_{50}[a,b] (μM)	Cell viability [c] (%)
7	16.09 ± 0.71	95.5 ± 4.7
8	2.65 ± 0.45	94.8 ± 5.4
9	11.53 ± 2.20	96.2 ± 3.9
10	23.84 ± 1.19	95.7 ± 5.1
11	4.60 ± 1.32	97.2 ± 3.1
L-NMMA[d]	14.42 ± 1.23	97.9 ± 4.1

[a] IC_{50} value of each compound was defined as the concentration (μM) of indicated compound that caused 50% inhibition of NO production in LPS-stimulated BV-2 cells.

[b] The results were averages of three independent experiments, and the data were expressed as mean ± SD.

[c] Cell viability after treatment with 50 μM of each tested compound was expressed as a percentage (%) of untreated control cells.

[d] L-NMMA (N^G-monomethyl-L-arginine): positive control.

Figure S4: ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of compound **1**

Figure S5: ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of compound **1**

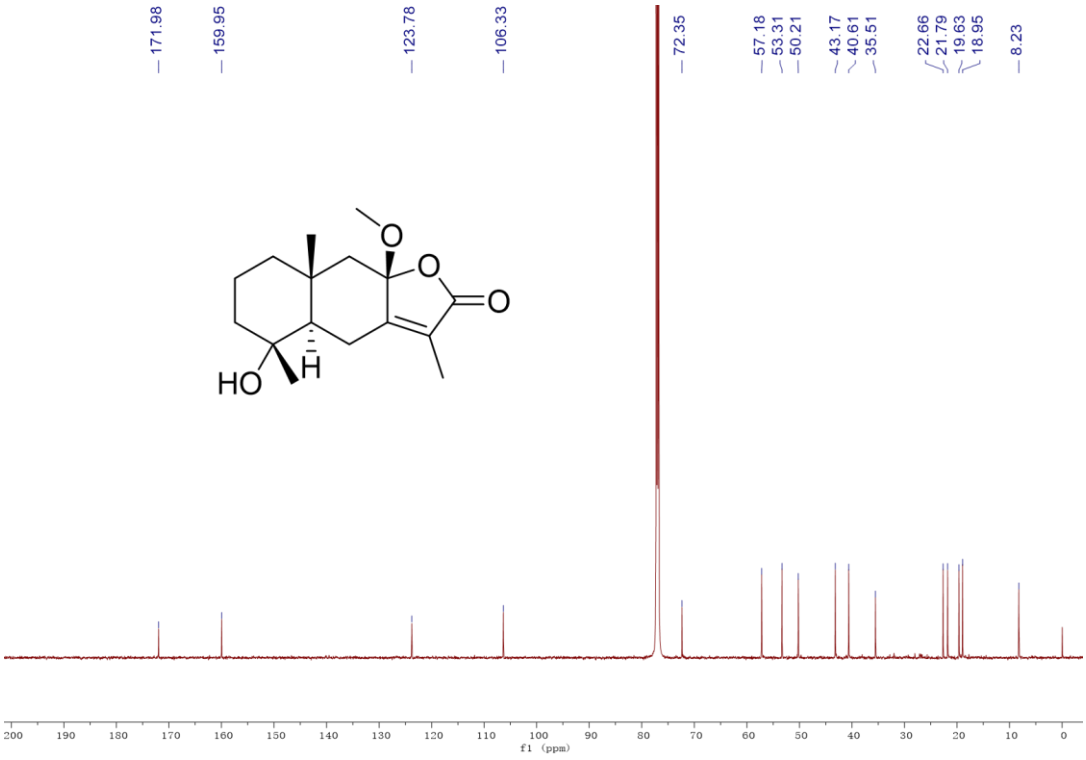


Figure S6: NOESY ($CDCl_3$, 600 MHz) spectrum of compound **1**

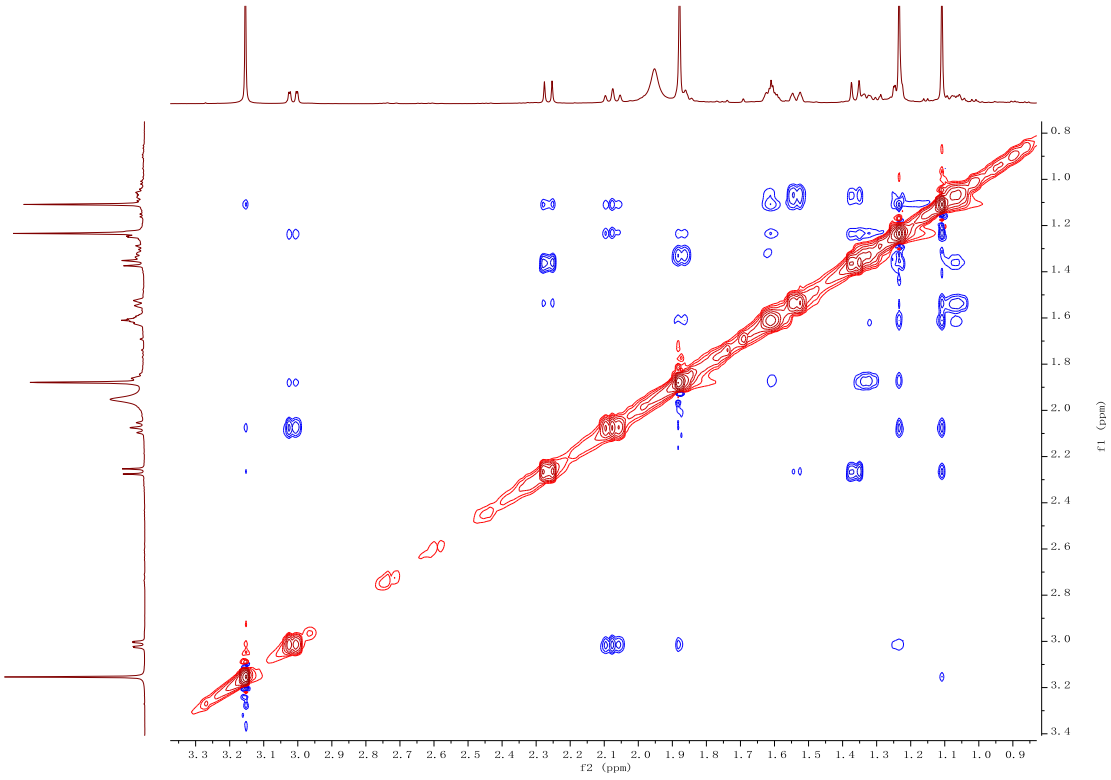


Figure S7: ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of compound **1**

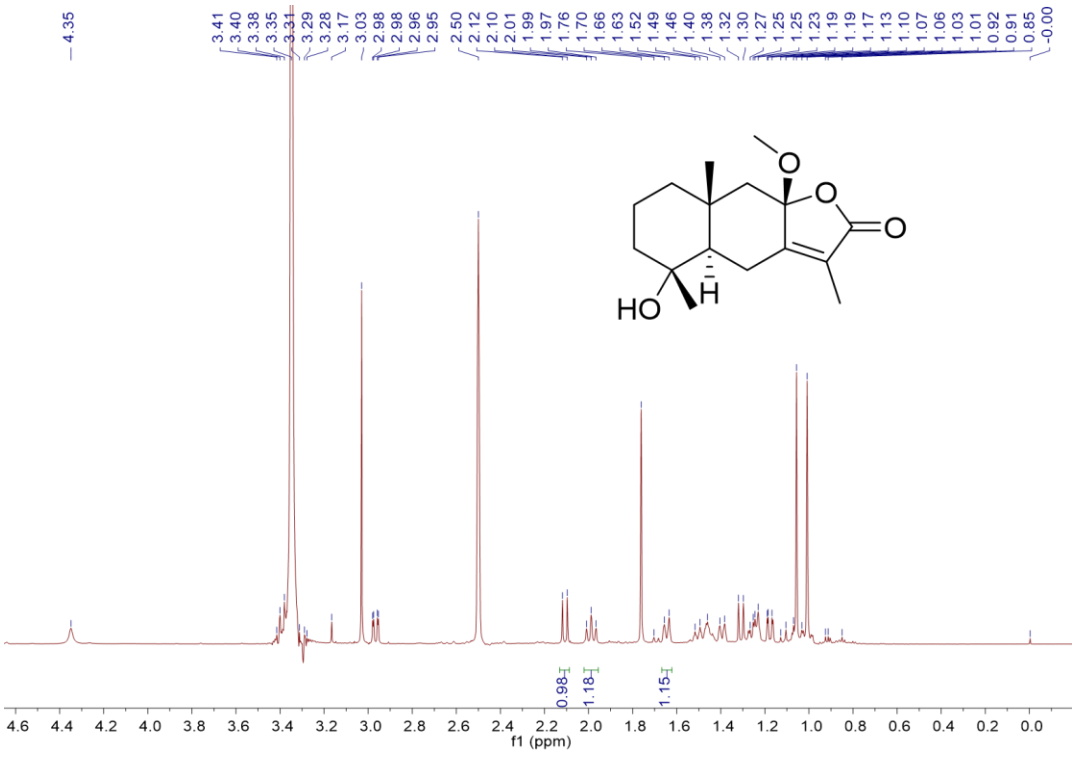


Figure S8: ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of compound **1**

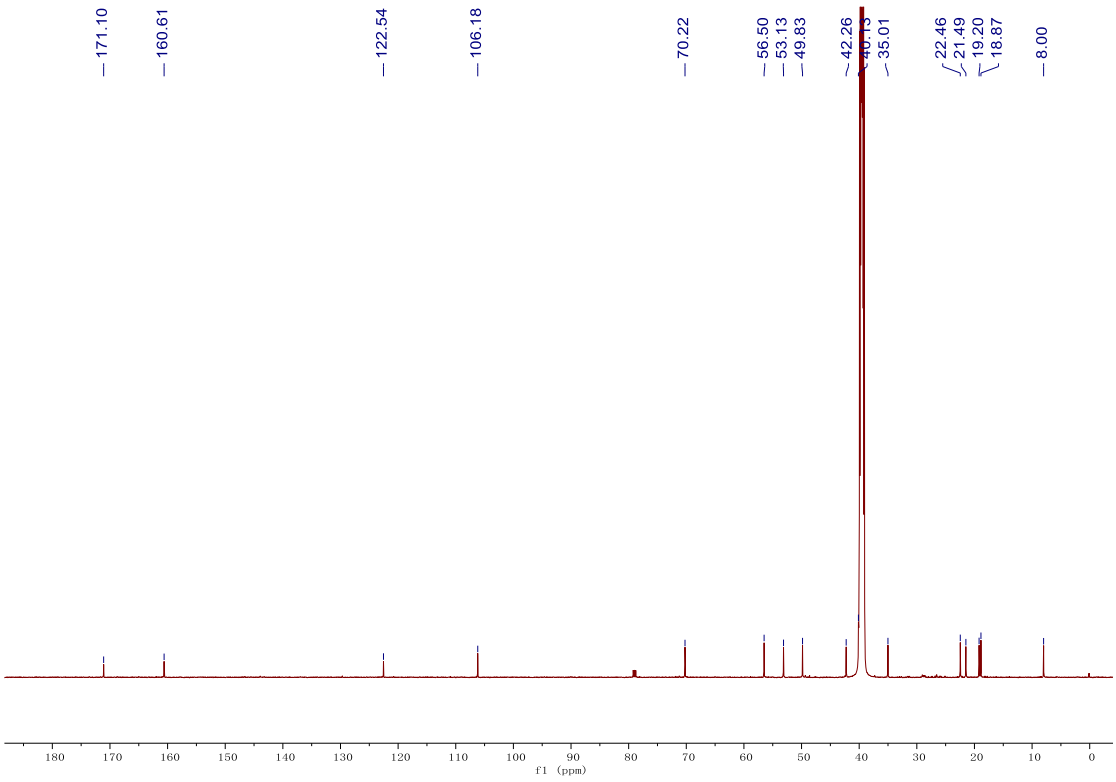


Figure S9: ^{1}H-^{1}H COSY (DMSO-d_6, 600 MHz) spectrum of compound **1**

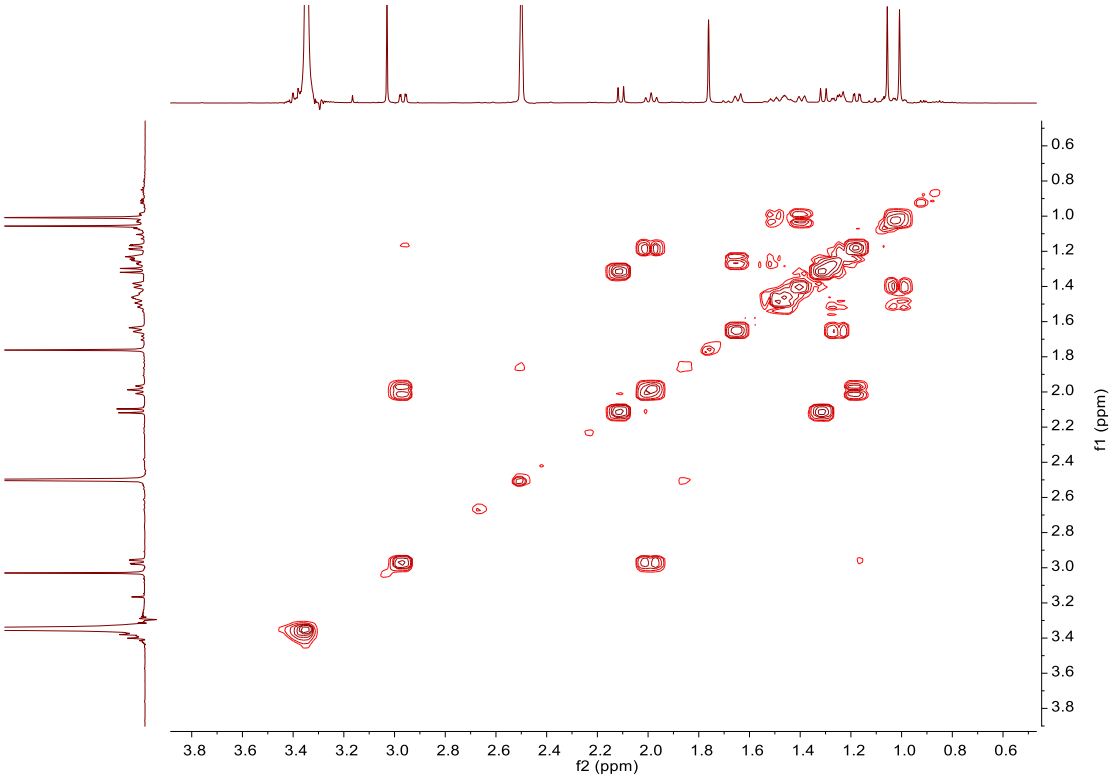


Figure S10: HMBC (DMSO-d_6, 600 MHz) spectrum of compound **1**

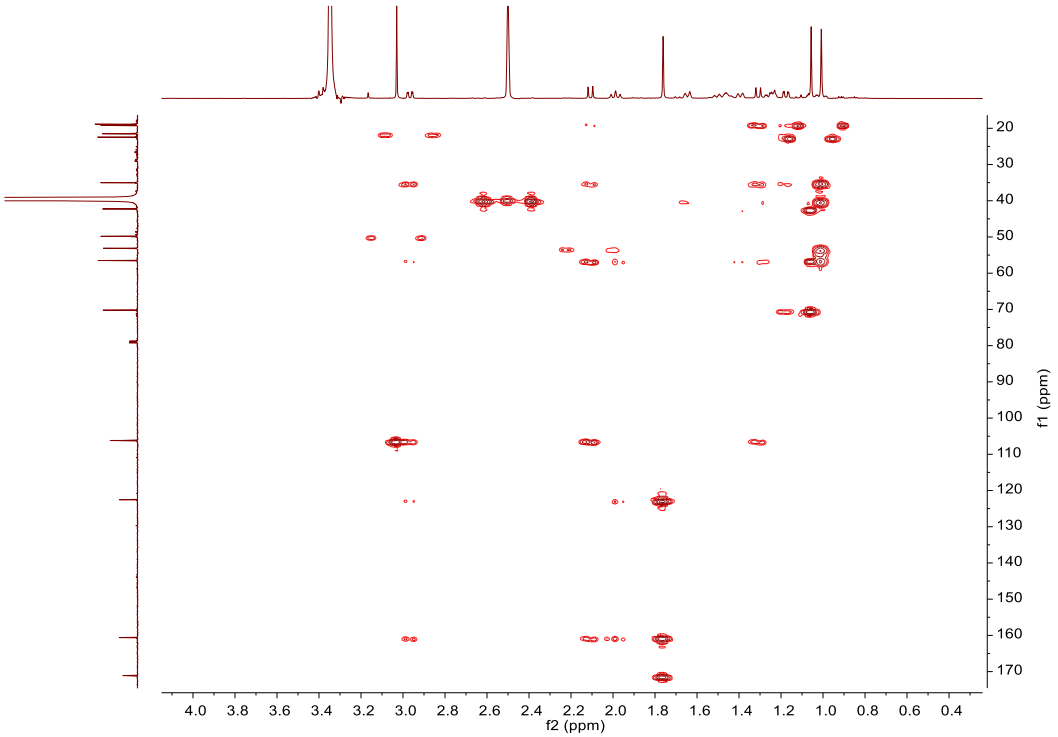


Figure S11: NOESY (DMSO-d_6, 600 MHz) spectrum of compound **1**

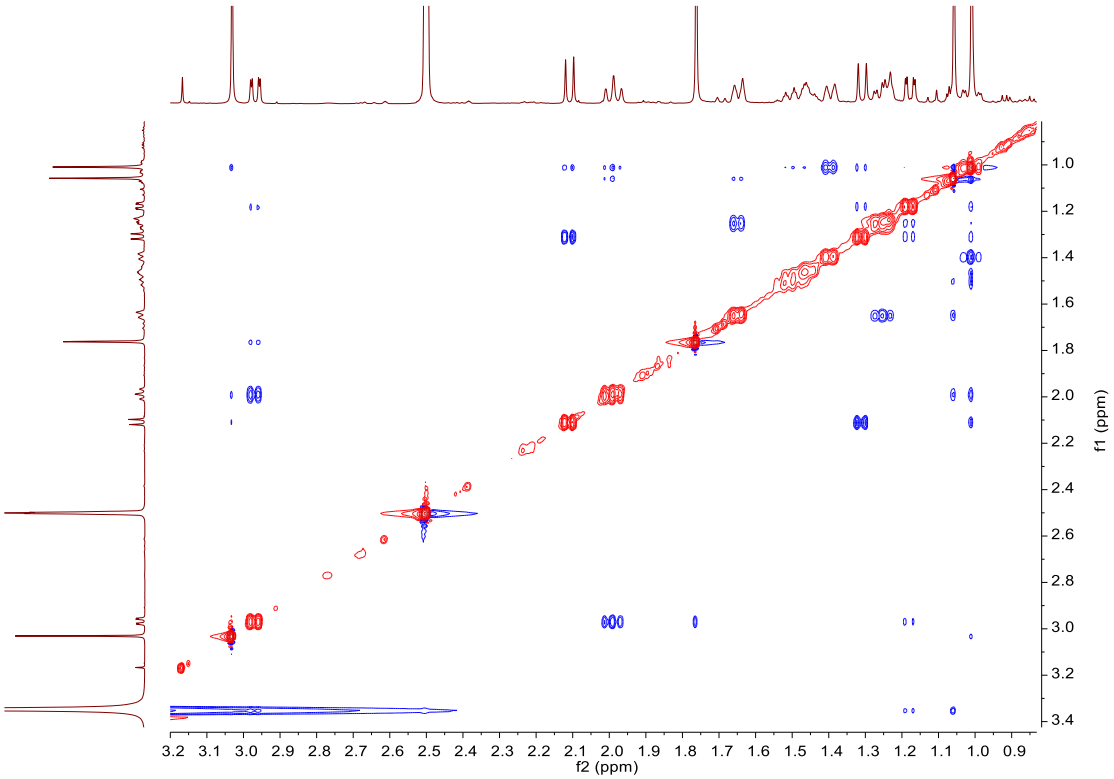
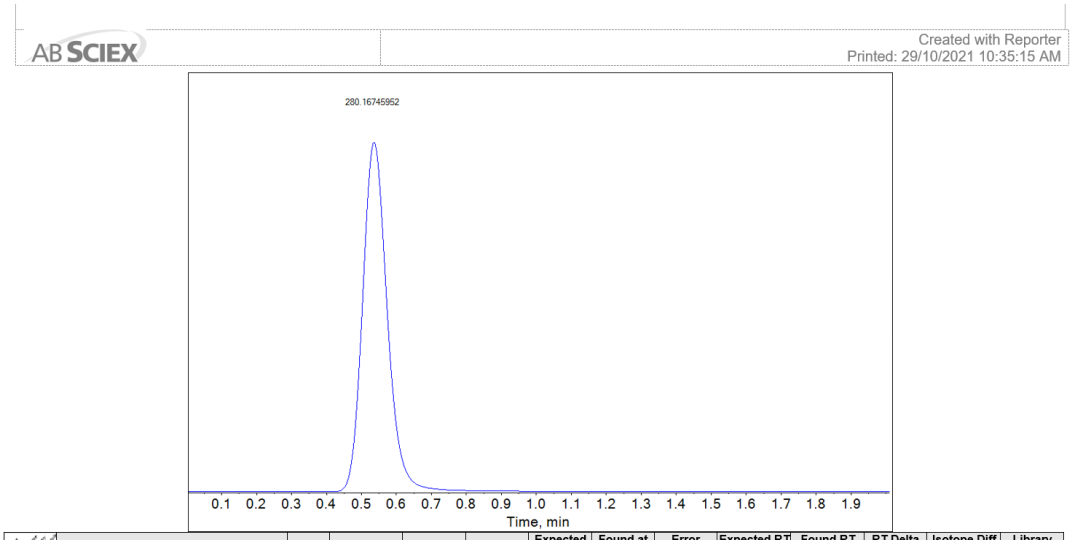


Figure S12: HRESIMS of compound **1**



	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓●✓●●	280.16745952(No data for Library Hit Name xcm)	97%	C16H24O4	1823963	5	281.1747	281.1743	-1.4	0.00	0.53	0.53	0.3%	N/A

Figure S13: HPLC profile for chiral analysis of compound **1**.

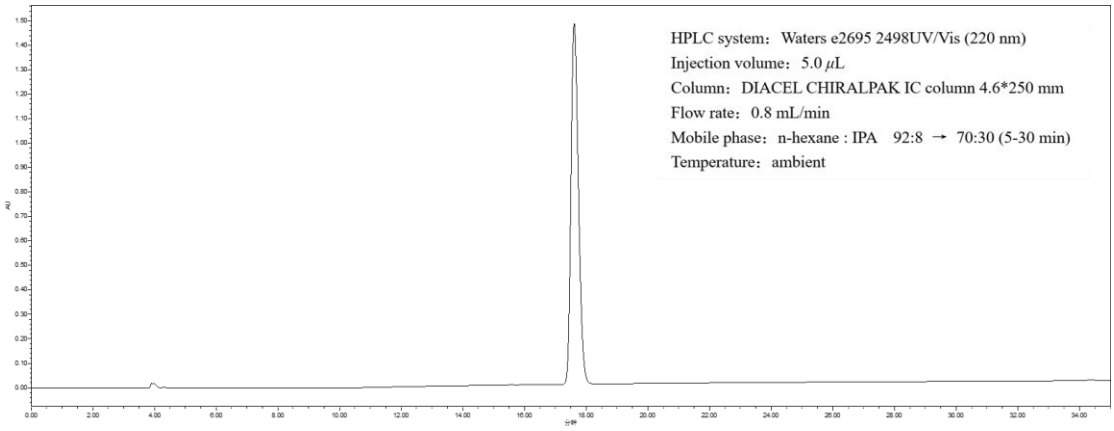


Figure S14: ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of compound **2**

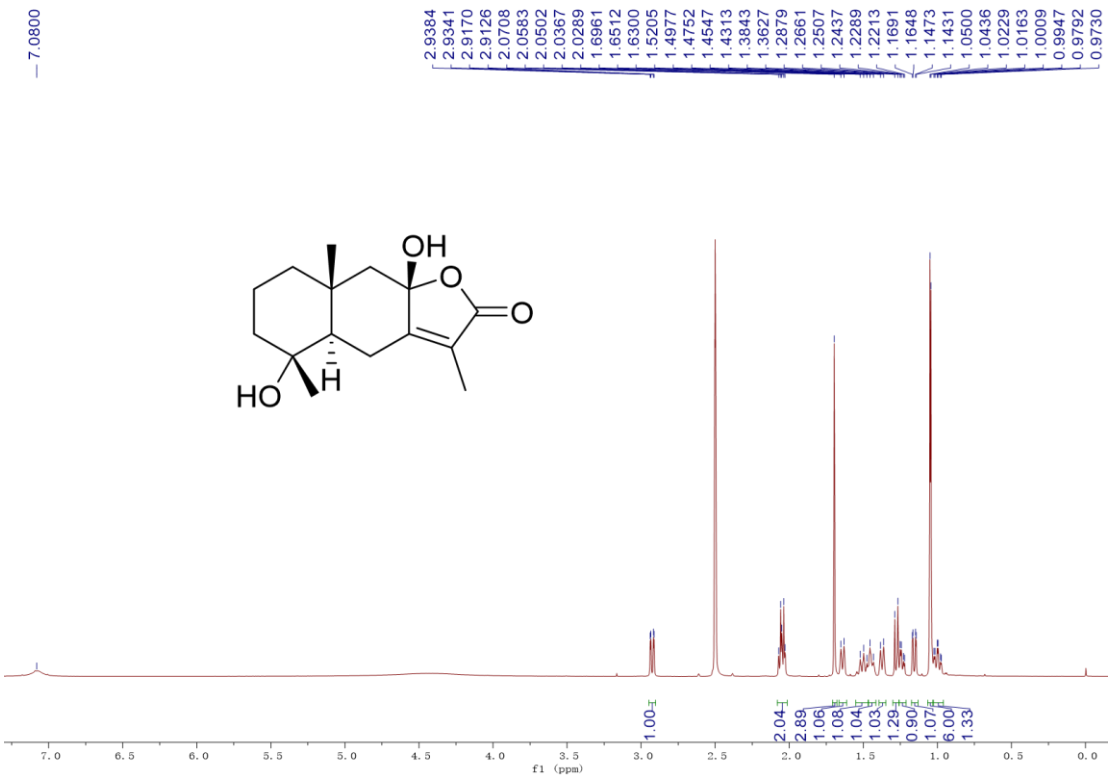


Figure S15: ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of compound **2**

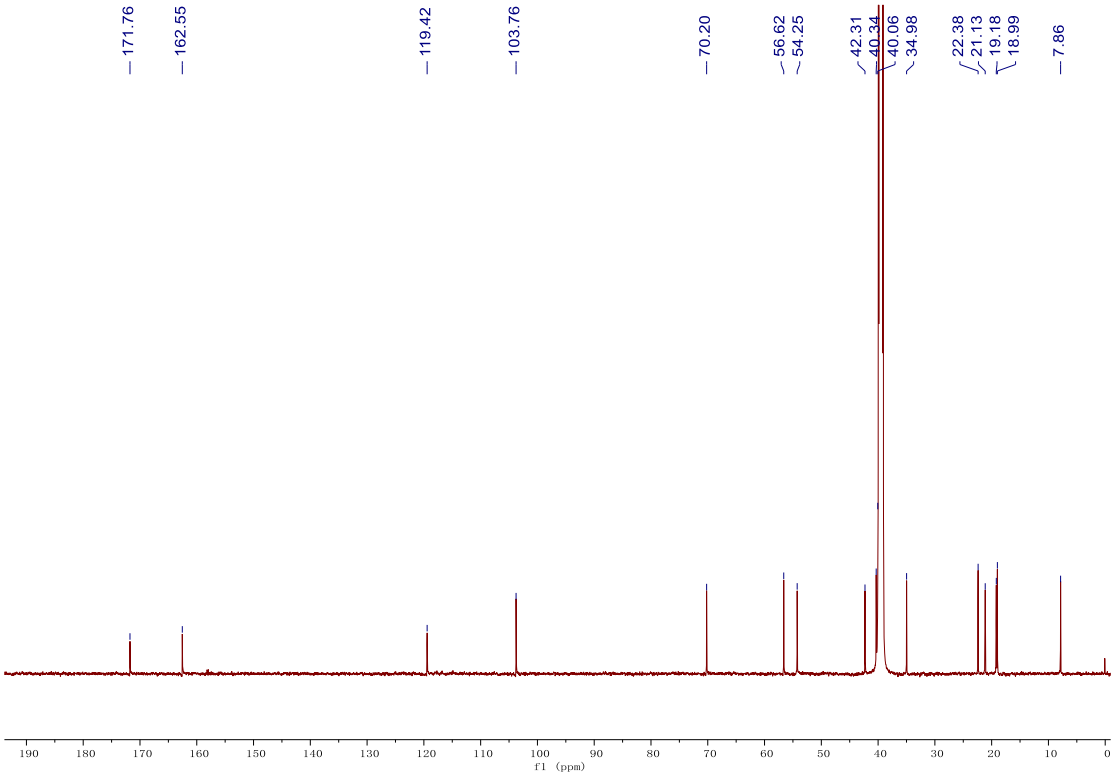


Figure S16: NOESY (DMSO-d_6, 600 MHz) spectrum of compound **2**

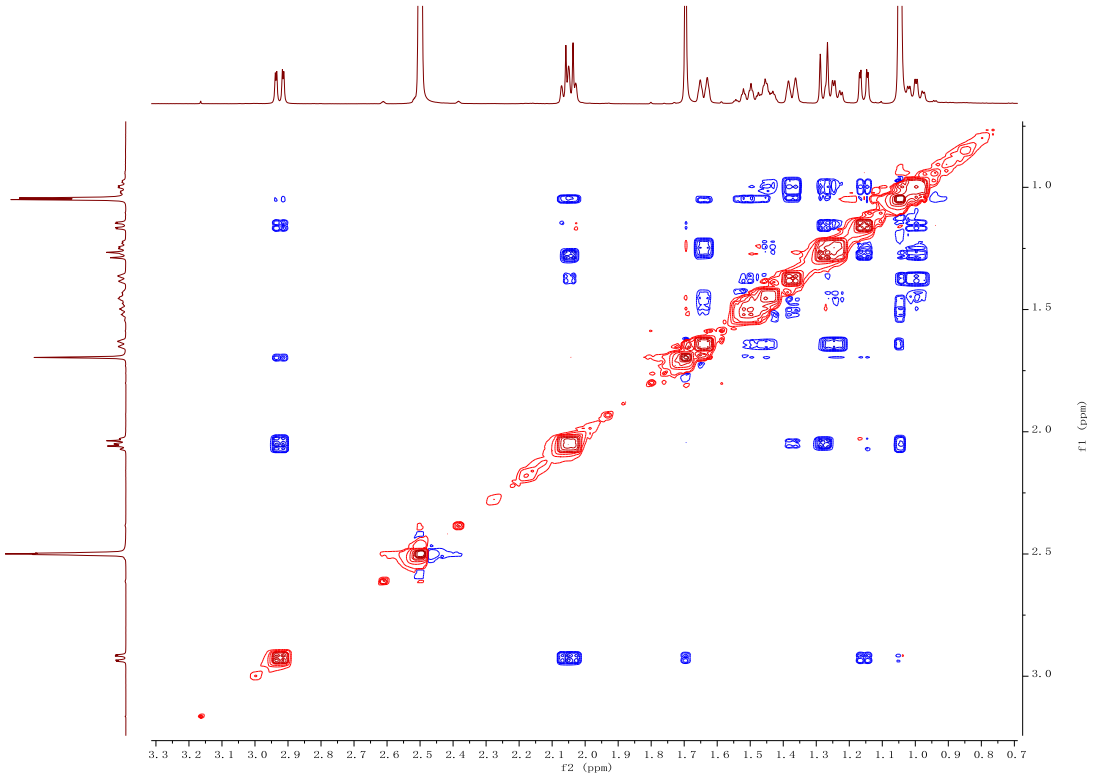


Figure S17: ^{1}H NMR (CD_3OD, 600 MHz) spectrum of compound **2**

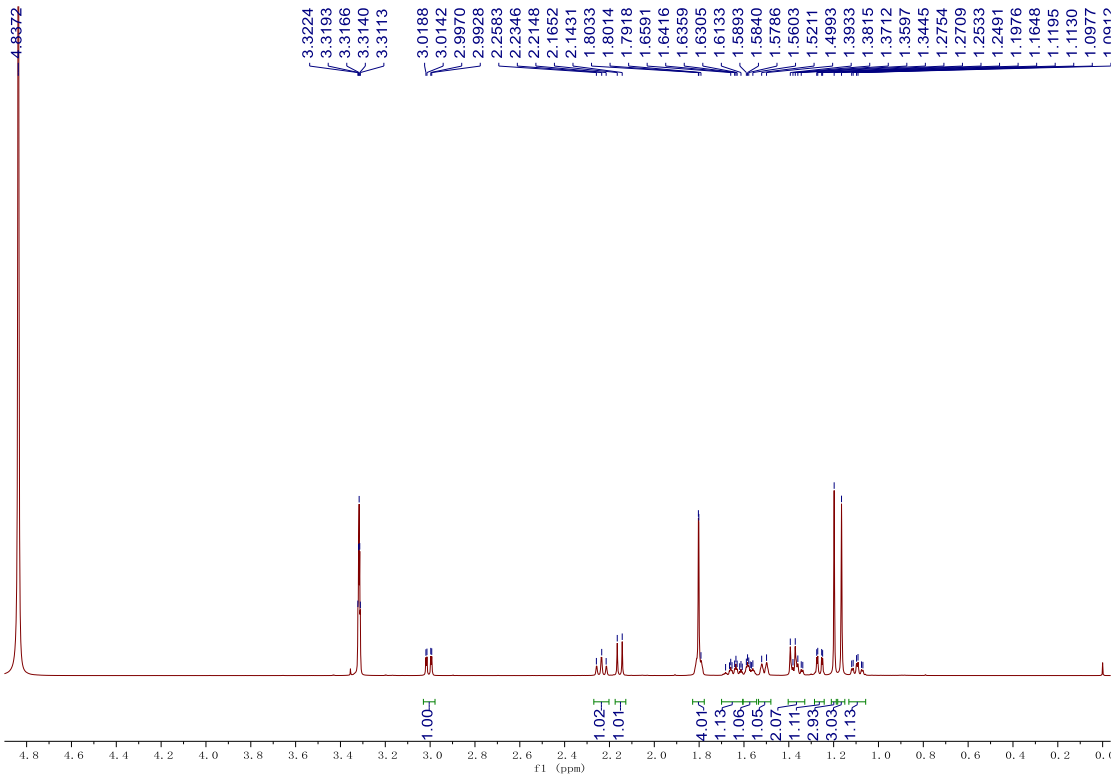


Figure S18: NOESY (CD_3OD, 600 MHz) spectrum of compound **2**

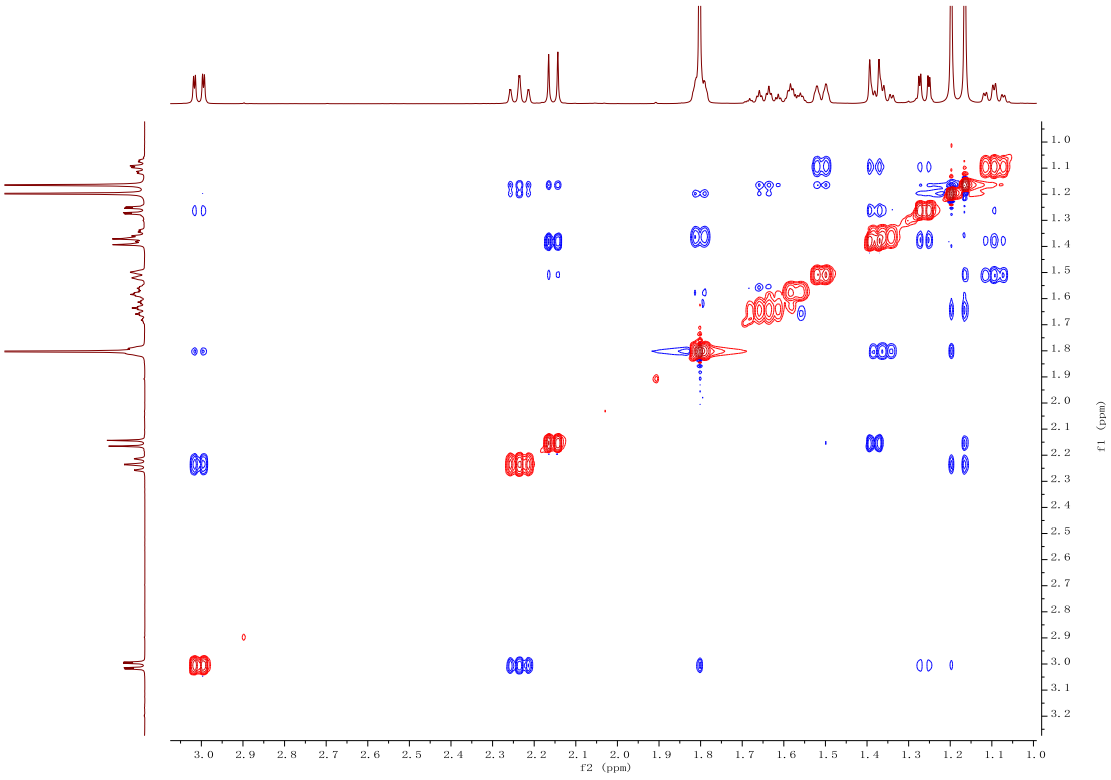
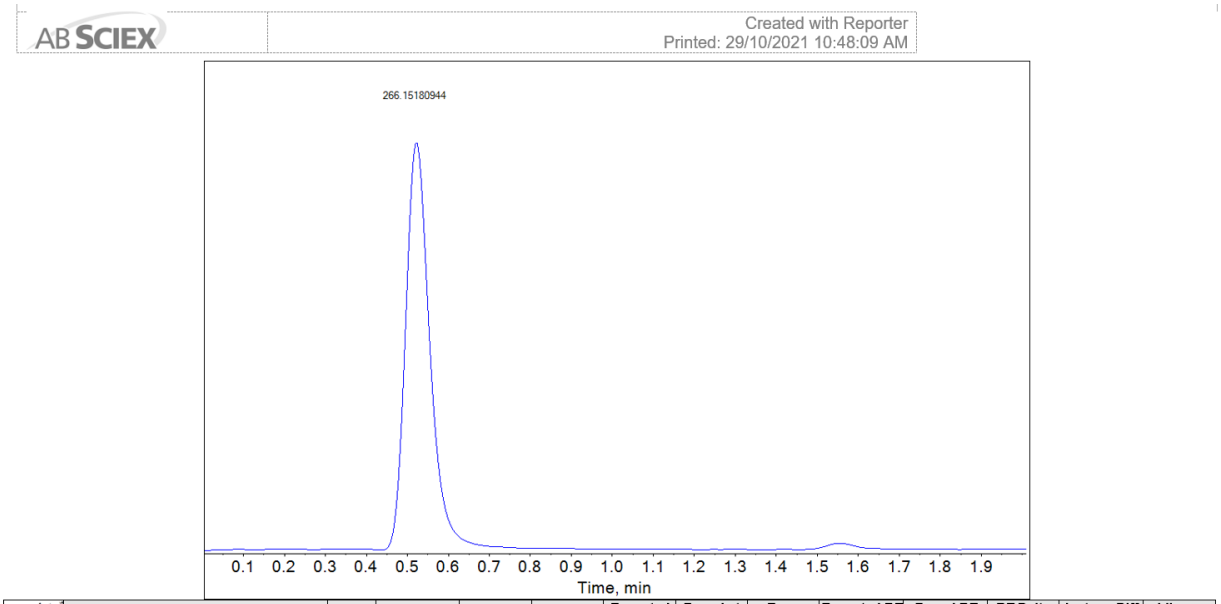


Figure S19: HRESIMS of compound **2**



	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓●✓●●	266.15180944	97%	C15H22O4	263987	5	267.1591	267.1588	-1.0	0.00	0.52	0.52	2.1%	N/A

Figure S20: ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of compound **3**

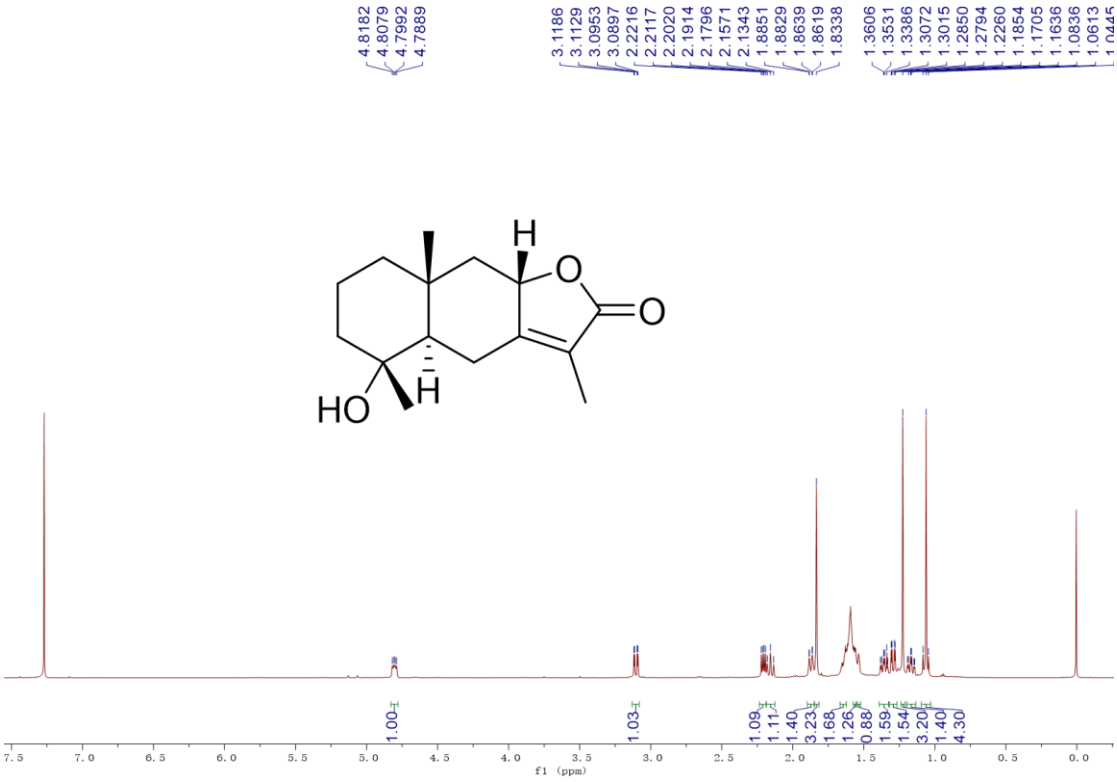


Figure S21: ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of compound **3**

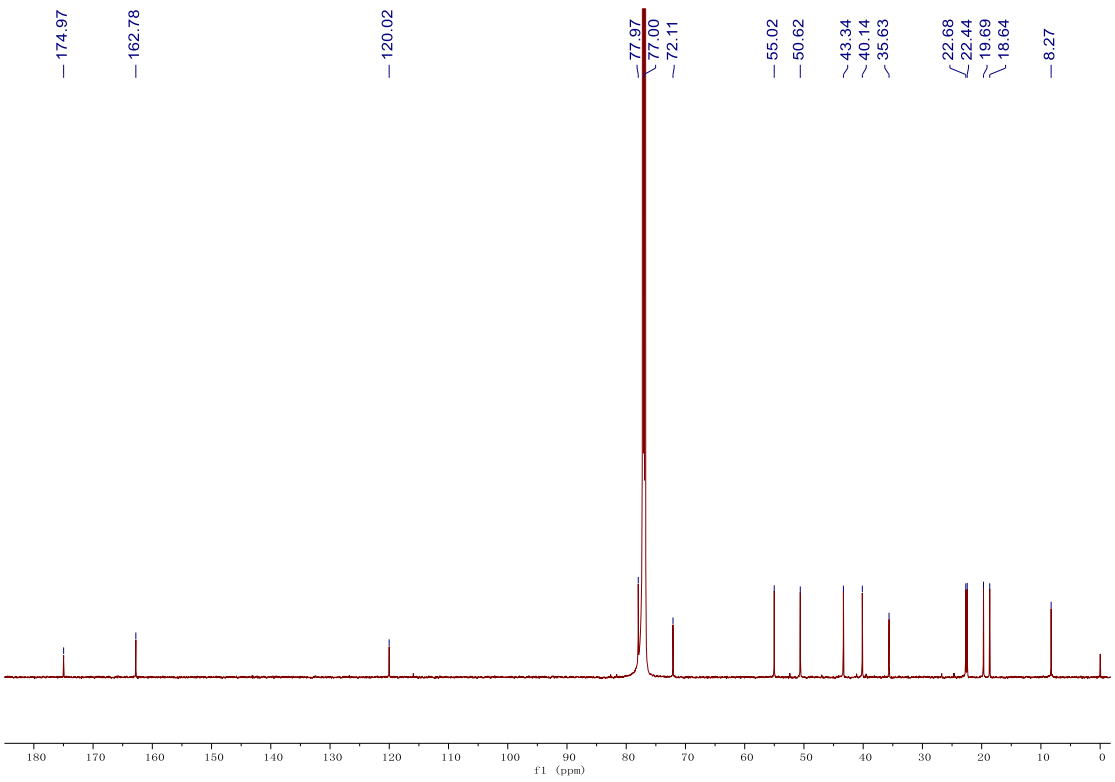


Figure S22: NOESY ($CDCl_3$, 600 MHz) spectrum of compound **3**

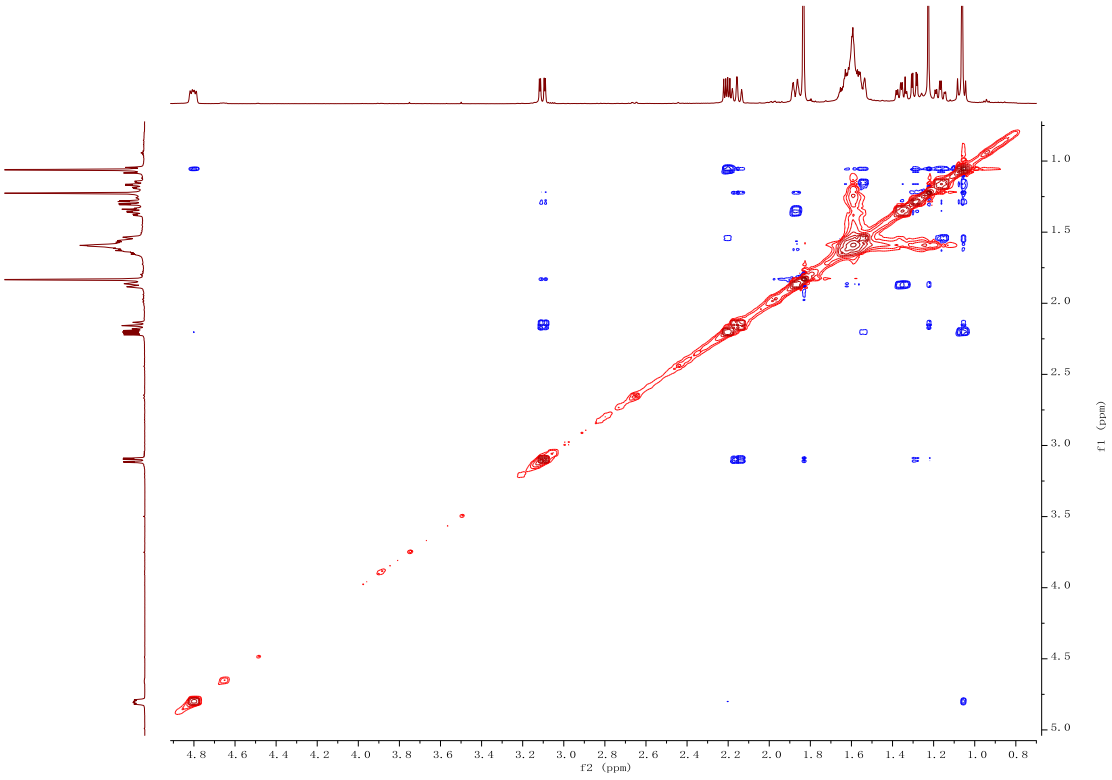
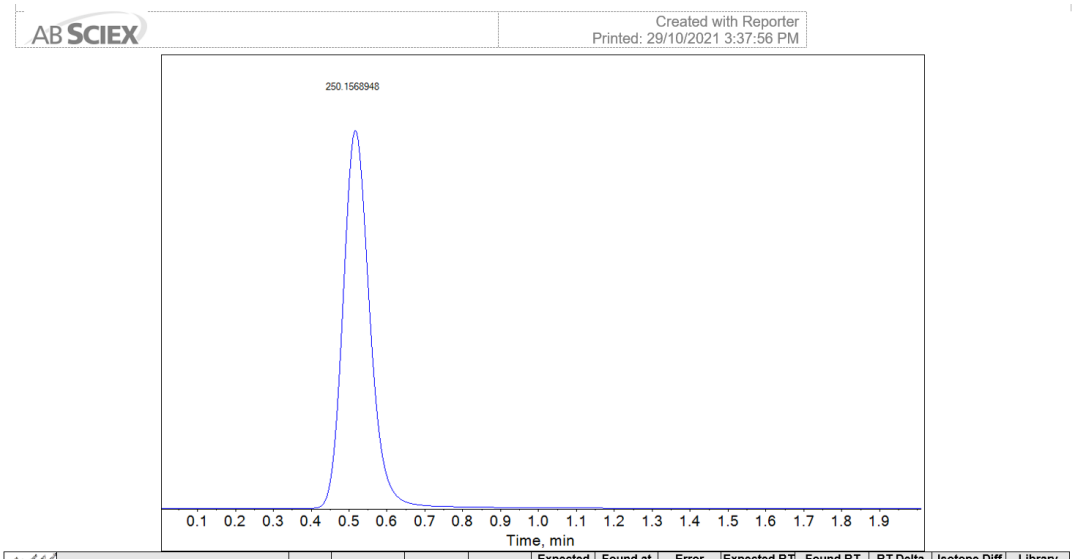


Figure S23: HRESIMS of compound **3**



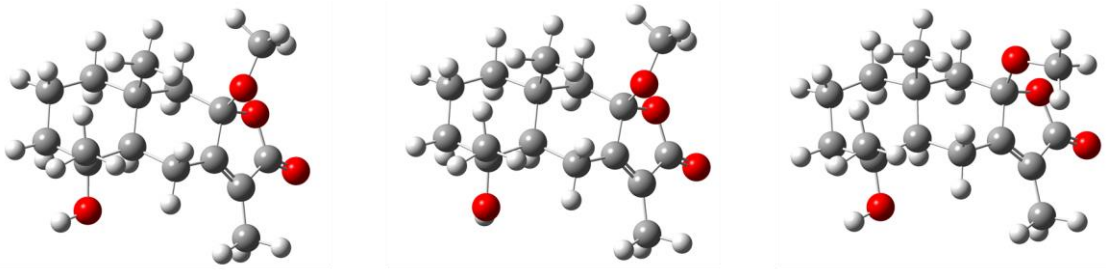
Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
250.1568948(*No data for Library Hit Name xcid*)	99%	C15H22O3	3673243	5	251.1642	251.1642	0.1	0.00	0.51	0.51	1.1%	N/A

Table S3: Detailed X-ray crystallographic data for compound **3**.

Identification code	210906wxj_0m	
Empirical formula	$C_{15}H_{22}O_3$	
Formula weight	250.16	
Temperature	213.00 K	
Wavelength	1.34139 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 10.2317(5) Å	= 90 °.
	b = 7.0717(3) Å	= 100.233(2) °.
	c = 10.5075(5) Å	= 90 °.
Volume	748.18(6) $Å^3$	
Z	2	
Density (calculated)	1.191 Mg/m^3	
Absorption coefficient	0.440 mm^{-1}	
F(000)	292	
Crystal size	0.08 x 0.03 x 0.02 mm^3	
Theta range for data collection	3.719 to 55.032 °.	
Index ranges	-12<=h<=12, -8<=k<=8, -12<=l<=12	
Reflections collected	10751	
Independent reflections	2862 [R(int) = 0.0683]	
Completeness to theta = 53.594°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7508 and 0.5108	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2862 / 1 / 179	
Goodness-of-fit on F^2	0.975	
Final R indices [I>2sigma(I)]	R_1 = 0.0453, wR_2 = 0.1289	
R indices (all data)	R_1 = 0.0474, wR_2 = 0.1327	
Absolute structure parameter	0.02(16)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.156 and -0.225 e. $Å^{-3}$	

X-ray Crystallographic Analysis: colorless crystals of compound **3** were obtained in MeOH at room temperature. A suitable crystal with approximate dimensions 0.08 × 0.03 × 0.02 mm^3 was selected for the X-ray crystallographic analysis using Ga Kα radiation (λ = 1.34139 Å). Using Olex2, the structure was solved by direct methods using SHELXT. Refinements were performed with SHELXL-2015 using full-matrix least-squares calculations on F^2, with anisotropic displacement parameters for all the non-hydrogen atoms. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms.

■ **ECD calculations of compound 1.**



1a **1**b **1**c

Figure S24: The optimized low-energy reoptimized MMFF conformers of **1** at B3LYP/6-31G level in gas.

Table S4: Gibbs free energies[a] and equilibrium populations[b] of low-energy conformers of **1**.

Conformers	In gas	
	G[a]	*P* (%)[b]
1a	-580292.61032613	7.24
1b	-580292.56075284	6.66
1c	-580294.07618949	86.11

[a]B3LYP/6-31G(d,p), in kcal/mol. [b]From *G* values at 298.15K.

Table S5: Cartesian coordinates for the low-energy reoptimized MMFF conformers of **1**a at B3LYP/6-31G(d,p) level of theory in gas.

1a		Standard Orientation (Ångstroms)			
Center number	Atomic number	Atomic Type	X	Y	Z
1.	6.	0.	-0.789226	3.257115	0.104317
2.	6.	0.	-0.654274	2.806368	1.564739
3.	6.	0.	0.297505	1.606931	1.768757
4.	6.	0.	-0.103616	0.471686	0.786772
5.	6.	0.	-0.263589	0.873862	-0.719042
6.	6.	0.	-1.222342	2.089595	-0.793929
7.	6.	0.	0.729280	-0.825775	0.975161
8.	6.	0.	0.055634	-1.911029	0.206100
9.	6.	0.	-0.219024	-1.663612	-1.263545

10.	6.	0.	-0.945506	-0.321021	-1.453285
11.	6.	0.	-0.521526	-3.063223	0.574175
12.	6.	0.	-1.225078	-3.622933	-0.610714
13.	8.	0.	-1.077150	-2.745626	-1.667079
14.	6.	0.	-0.567981	-3.767831	1.890443
15.	8.	0.	-1.868440	-4.638412	-0.700982
16.	8.	0.	1.013489	-1.768803	-1.940894
17.	8.	0.	0.038390	1.029578	3.065637
18.	6.	0.	1.772525	2.033777	1.730657
19.	6.	0.	1.057871	1.230270	-1.438796
20.	1.	0.	-1.118830	0.213374	1.121138
21.	6.	0.	0.951210	-1.809564	-3.365878
22.	1.	0.	0.155605	3.685299	-0.251532
23.	1.	0.	-1.527498	4.064611	0.037961
24.	1.	0.	-0.326110	3.642608	2.197278
25.	1.	0.	-1.637713	2.495042	1.938088
26.	1.	0.	-1.309452	2.423066	-1.835798
27.	1.	0.	-2.226625	1.767974	-0.485298
28.	1.	0.	1.751668	-0.688712	0.602194
29.	1.	0.	0.790115	-1.064727	2.038652
30.	1.	0.	-1.059767	-0.094944	-2.519112
31.	1.	0.	-1.956578	-0.457507	-1.053868
32.	1.	0.	-0.087258	-4.749510	1.816458
33.	1.	0.	-0.066848	-3.194603	2.673506
34.	1.	0.	-1.604189	-3.949462	2.194176
35.	1.	0.	0.276081	1.690927	3.729723
36.	1.	0.	2.043950	2.555478	0.811598
37.	1.	0.	1.970419	2.722134	2.561788
38.	1.	0.	2.436712	1.174033	1.848339
39.	1.	0.	1.814954	0.452606	-1.335187
40.	1.	0.	0.864366	1.352403	-2.510526
41.	1.	0.	1.481828	2.171763	-1.086939
42.	1.	0.	1.969980	-2.010252	-3.702649
43.	1.	0.	0.621249	-0.855427	-3.795569
44.	1.	0.	0.288790	-2.609525	-3.709036

Table S6: Cartesian coordinates for the low-energy reoptimized MMFF conformers of **1**b at B3LYP/6-31G(d,p) level of theory in gas.

1b	Standard Orientation (Ångstroms)

Center number	Atomic number	Atomic Type	X	Y	Z
1.	6.	0.	-0.764560	3.272025	0.122109
2.	6.	0.	-0.634735	2.821735	1.583453
3.	6.	0.	0.316747	1.622964	1.788523
4.	6.	0.	-0.080316	0.488685	0.794195
5.	6.	0.	-0.239198	0.891496	-0.712572
6.	6.	0.	-1.195819	2.109134	-0.783698
7.	6.	0.	0.749948	-0.809198	0.982013
8.	6.	0.	0.076724	-1.894408	0.212764
9.	6.	0.	-0.196855	-1.645924	-1.257612
10.	6.	0.	-0.920177	-0.301789	-1.450531
11.	6.	0.	-0.499539	-3.047638	0.579058
12.	6.	0.	-1.205308	-3.604831	-0.605931
13.	8.	0.	-1.057700	-2.726339	-1.660906
14.	6.	0.	-0.541274	-3.757071	1.893042
15.	8.	0.	-1.850566	-4.619078	-0.695675
16.	8.	0.	1.036016	-1.755189	-1.932382
17.	8.	0.	0.155157	1.121934	3.129646
18.	6.	0.	1.786812	2.053477	1.753750
19.	6.	0.	1.084830	1.247747	-1.426943
20.	1.	0.	-1.104347	0.225087	1.109930
21.	6.	0.	0.977214	-1.792231	-3.357948
22.	1.	0.	0.183597	3.698109	-0.226660
23.	1.	0.	-1.499463	4.082333	0.053255
24.	1.	0.	-0.312069	3.648032	2.226740
25.	1.	0.	-1.631006	2.519858	1.941142
26.	1.	0.	-1.279639	2.448277	-1.823910
27.	1.	0.	-2.202301	1.787148	-0.481250
28.	1.	0.	1.771706	-0.669518	0.609378
29.	1.	0.	0.815555	-1.045197	2.046585
30.	1.	0.	-1.029266	-0.075821	-2.516874
31.	1.	0.	-1.933802	-0.436629	-1.056617
32.	1.	0.	-0.068442	-4.741788	1.810719
33.	1.	0.	-0.027586	-3.192327	2.674369
34.	1.	0.	-1.576058	-3.933653	2.204997
35.	1.	0.	-0.780120	0.905599	3.251139
36.	1.	0.	2.041649	2.598223	0.844538
37.	1.	0.	1.973885	2.706398	2.610623
38.	1.	0.	2.454736	1.192929	1.840249
39.	1.	0.	1.836342	0.463663	-1.332013
40.	1.	0.	0.892023	1.383443	-2.497180

41.	1.	0.	1.515198	2.181344	-1.062913
42.	1.	0.	1.995068	-2.001365	-3.692103
43.	1.	0.	0.657227	-0.834047	-3.785969
44.	1.	0.	0.308713	-2.585601	-3.704531

Table S7: Cartesian coordinates for the low-energy reoptimized MMFF conformers of **1**c at B3LYP/6-31G(d,p) level of theory in gas.

1c		Standard Orientation (Ångstroms)			
Center number	Atomic number	Atomic Type	X	Y	Z
1.	6.	0.	-0.916909	3.340473	-0.098014
2.	6.	0.	-0.814701	2.905584	1.370124
3.	6.	0.	0.155814	1.728561	1.612861
4.	6.	0.	-0.197808	0.578177	0.631367
5.	6.	0.	-0.312109	0.962469	-0.882942
6.	6.	0.	-1.293391	2.157467	-1.001882
7.	6.	0.	0.646793	-0.706202	0.861574
8.	6.	0.	0.005229	-1.809936	0.089015
9.	6.	0.	-0.217507	-1.582416	-1.396151
10.	6.	0.	-0.946488	-0.252557	-1.625609
11.	6.	0.	-0.612092	-2.938933	0.467450
12.	6.	0.	-1.277357	-3.516838	-0.732904
13.	8.	0.	-1.043947	-2.689935	-1.810541
14.	6.	0.	-0.744962	-3.596148	1.802368
15.	8.	0.	-1.945975	-4.517514	-0.816115
16.	8.	0.	0.955710	-1.558978	-2.168566
17.	8.	0.	-0.125880	1.159820	2.909049
18.	6.	0.	1.622966	2.182303	1.607382
19.	6.	0.	1.024893	1.344966	-1.560644
20.	1.	0.	-1.219379	0.307912	0.935271
21.	6.	0.	1.757192	-2.738715	-2.144026
22.	1.	0.	0.027526	3.789559	-0.427360
23.	1.	0.	-1.672043	4.129286	-0.193062
24.	1.	0.	-0.522682	3.754548	2.003515
25.	1.	0.	-1.801407	2.576267	1.718640
26.	1.	0.	-1.348726	2.481461	-2.048633
27.	1.	0.	-2.301560	1.817503	-0.727612
28.	1.	0.	1.676780	-0.554869	0.515728
29.	1.	0.	0.681550	-0.928756	1.929775

30.	1.	0.	-0.991321	-0.064725	-2.703600
31.	1.	0.	-1.973398	-0.393872	-1.271530
32.	1.	0.	-0.315214	-4.603558	1.779771
33.	1.	0.	-0.247361	-3.022253	2.587164
34.	1.	0.	-1.800592	-3.714488	2.068518
35.	1.	0.	0.074218	1.835619	3.570982
36.	1.	0.	1.912579	2.683787	0.682892
37.	1.	0.	1.784040	2.894795	2.426100
38.	1.	0.	2.297977	1.337684	1.767582
39.	1.	0.	1.817855	0.621197	-1.372168
40.	1.	0.	0.884758	1.381736	-2.645562
41.	1.	0.	1.377882	2.329212	-1.248206
42.	1.	0.	2.610369	-2.532427	-2.792505
43.	1.	0.	1.206611	-3.600540	-2.533826
44.	1.	0.	2.120066	-2.969728	-1.134667