Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

Diels-Alder Adducts of a Labdane Diterpenoid from the Chinese Liverwort *Pallavicinia subciliata.*

Shu-Gong Liu,[a] Chun-Yang Zhang,[a] Jin-Chuan Zhou,[b] Jing-Jing Han,[a] Ming-Zhu Zhu,[a] Jiao-Zhen Zhang,[a] Yi Li,[a] Z.J. Xu,[a] Hui Meng,[a] Xue Wang,[a] Yan Zong,[a] Shuang-Zhi Yuan,[a] Ya-Nan Qiao,[a] Ya-Jie Tang,[c] and Hong-Xiang Lou[a,]*

[a] *Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmacy, Shandong University, 44 West Wenhua Road, Jinan, Shandong 250012, P.R. China.*

[b] *School of Pharmacy, Linyi University, Linyi 276000, P. R. China*

[c] *State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, P. R. China*

Corresponding Author

* Email: louhongxiang@sdu.edu.cn

List of Supporting Information

Experimental Section

Anti-inflammatory activity assay

HRESIMS, IR, UV, ECD, 1D and 2D NMR spectra for compounds 1−4

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Figures S5−S8

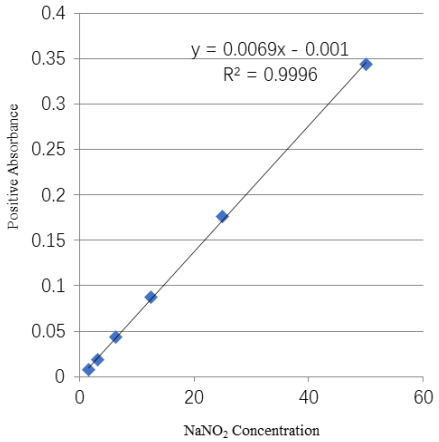
Anti-inflammatory activity assay:

Inhibitory effects of compounds **1**–**4** against NO production in RAW 264.7 cells. Cells were treated with indicated concentrations of ingredients along with LPS (1 μg/mL) for 24 h, and then the accumulation of nitrite from the supernatants was evaluated by Griess reagent. Didox was used as a positive control.

NO	1	2	3	4	5	6	7	8	9	10	11	12
A	0.069	0.059	0.091	0.091	0.089	0.072	0.048	0.047	0.048	0.046	0.047	0.045
B	0.054	0.049	0.087	0.088	0.063	0.064	0.085	0.083	0.078	0.072	0.063	0.064
C	0.088	0.89	0.092	0.094	0.078	0.072	0.079	0.076	0.071	0.064	0.06	0.059
D	0.089	0.084	0.089	0.082	0.081	0.073	0.085	0.081	0.086	0.078	0.075	0.074
E	0.084	0.081	0.081	0.071	0.057	0.054	0.084	0.085	0.081	0.070	0.067	0.068
F	0.080	0.080	0.074	0.065	0.056	0.051	0.045	0.044	0.042	0.042	0.043	0.048
G	0.080	0.072	0.065	0.055	0.057	0.054	0.047	0.047	0.047	0.048	0.049	0.056
H	0.081	0.068	0.067	0.062	0.064	0.061	0.055	0.066	0.091	0.135	0.224	0.391

MTT	1	2	3	4	5	6	7
A	2.548	2.447	2.417	2.367	1.723	1.642	0.055
B	2.814	2.651	2.355	2.136	1.91	1.918	2.027
C	2.587	2.456	2.293	2.744	2.189	2.591	2.308
D	2.699	2.364	2.042	2.36	2.024	2.259	2.825
E	2.534	2.315	2.065	2.889	3.104	0.752	2.372
F	2.618	1.966	2.321	2.616	2.765	0.662	1.926
G	2.064	1.932	2.051	2.358	2.375	2.02	1.134
H	2.698	2.121	1.747	2.215	2.017	2.094	0.049

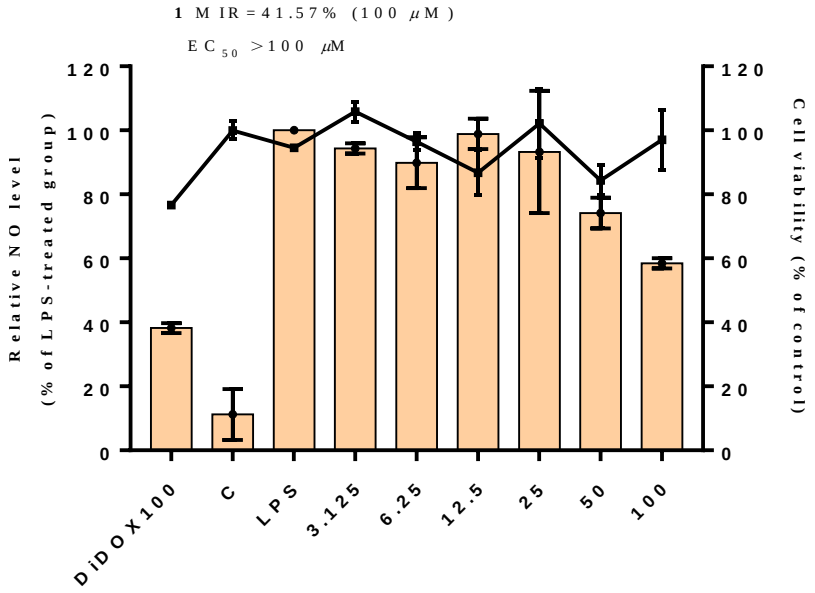
$NaNO_2$ Concentration-X	1.5625	3.125	6.25	12.5	25	50
Absorbance Value	0.055	0.066	0.091	0.135	0.224	0.391
Positive Absorbance-Y	0.007	0.018	0.043	0.087	0.176	0.343



Compound **1**

NO(μM)	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	0.063	0.054	0.091	0.088	0.089	0.092	0.094	0.078	0.072
	0.064	0.049	0.091	0.089	0.084	0.089	0.082	0.081	0.073
Corrected Absorbance	0.015	0.006	0.043	0.040	0.041	0.044	0.046	0.030	0.024
	0.016	0.001	0.043	0.041	0.036	0.041	0.034	0.033	0.025
Standard Curve Calculation	2.391	1.086	6.449	6.014	6.159	6.594	6.884	4.565	3.695
	2.536	0.362	6.449	6.159	5.434	6.159	5.144	5.000	3.840
AVG	2.463	0.724	6.449	6.086	5.797	6.376	6.014	4.782	3.768
SD	0.102	0.512	0	0.102	0.512	0.307	1.229	0.307	0.102

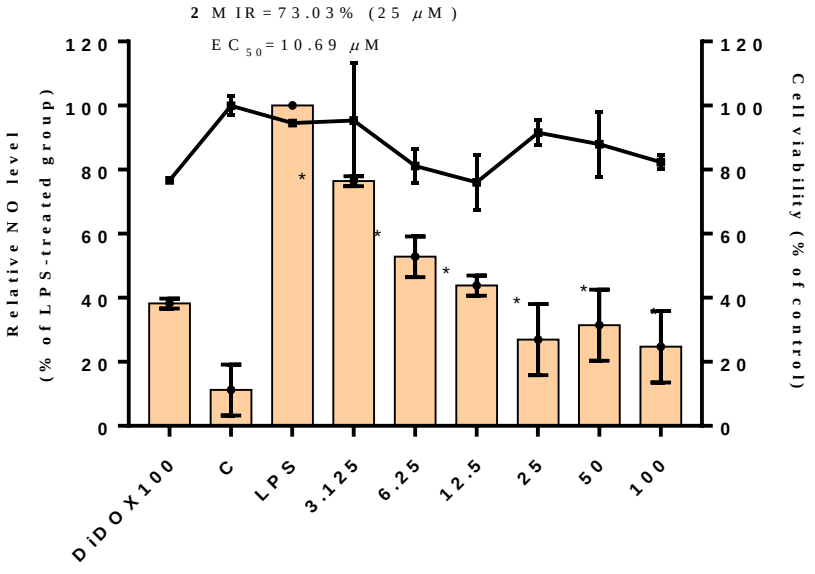
MTT	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	1.91	2.548	2.355	2.587	2.456	2.293	2.744	2.189	2.591
	1.918	2.447	2.367	2.699	2.364	2.042	2.360	2.024	2.259
AVG	1.914	2.497	2.361	2.643	2.410	2.167	2.552	2.1065	2.425
(The original value/AVG of group C) *100	76.476	102.022	94.294	103.583	98.338	91.811	109.869	87.647	103.743
	76.796	97.977	94.774	108.068	94.654	81.761	94.494	81.041	90.450
AVG	76.636	100.000	94.534	105.825	96.496	86.786	102.182	84.344	97.097
SD	0.226	2.859	0.339	3.171	2.604	7.106	10.872	4.671	9.399



Compound **2**

NO(μM)	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	0.063	0.054	0.091	0.088	0.089	0.092	0.094	0.078	0.072
	0.064	0.049	0.091	0.089	0.084	0.089	0.082	0.081	0.073
Corrected Absorbance	0.015	0.006	0.043	0.040	0.0415	0.044	0.0465	0.030	0.024
	0.016	0.001	0.043	0.041	0.036	0.041	0.0345	0.033	0.025
Standard Curve Calculation	2.391	1.086	6.449	6.014	6.159	6.594	6.884	4.565	3.695
	2.536	0.362	6.449	6.159	5.434	6.159	5.145	5.000	3.840
AVG	2.463	0.724	6.449	6.086	5.797	6.376	6.014	4.782	3.768
SD	0.102	0.512	0	0.102	0.512	0.307	1.229	0.307	0.102

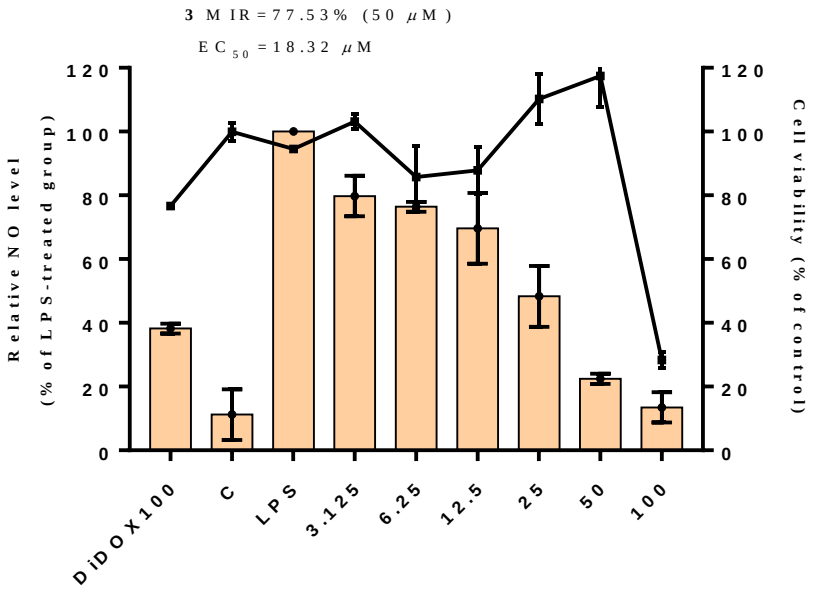
MTT	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	1.910	2.548	2.355	2.064	1.932	2.051	2.358	2.375	2.020
	1.918	2.447	2.367	2.698	2.121	1.747	2.215	2.017	2.094
AVG	1.914	2.497	2.361	2.381	2.026	1.899	2.286	2.196	2.057
(The original value/AVG of group C) *100	76.476	102.022	94.294	82.642	77.357	82.122	94.414	95.095	80.880
	76.796	97.977	94.774	108.028	84.924	69.949	88.688	80.760	83.843
AVG	76.636	100.000	94.534	95.335	81.141	76.036	91.551	87.927	82.362
SD	0.226	2.859	0.339	17.950	5.351	8.607	4.048	10.135	2.095



Compound **3**

XJ									
NO(μM)	DiDOX100	C	LPS	3.125	6.250	12.500	25.000	50.000	100.000
Absorbance Value	0.063	0.054	0.091	0.084	0.081	0.081	0.071	0.057	0.054
	0.064	0.049	0.091	0.080	0.080	0.074	0.065	0.056	0.051
Corrected Absorbance	0.015	0.006	0.043	0.036	0.033	0.033	0.023	0.009	0.006
	0.016	0.001	0.043	0.032	0.032	0.026	0.017	0.008	0.003
Standard Curve Calculation	2.391	1.086	6.449	5.434	5.000	5.000	3.551	1.522	1.087
	2.536	0.362	6.449	4.855	4.855	3.985	2.681	1.377	0.652
AVG	2.463	0.724	6.449	5.144	4.927	4.492	3.116	1.449	0.869
SD	0.102	0.512	0	0.409	0.102	0.717	0.615	0.102	0.307

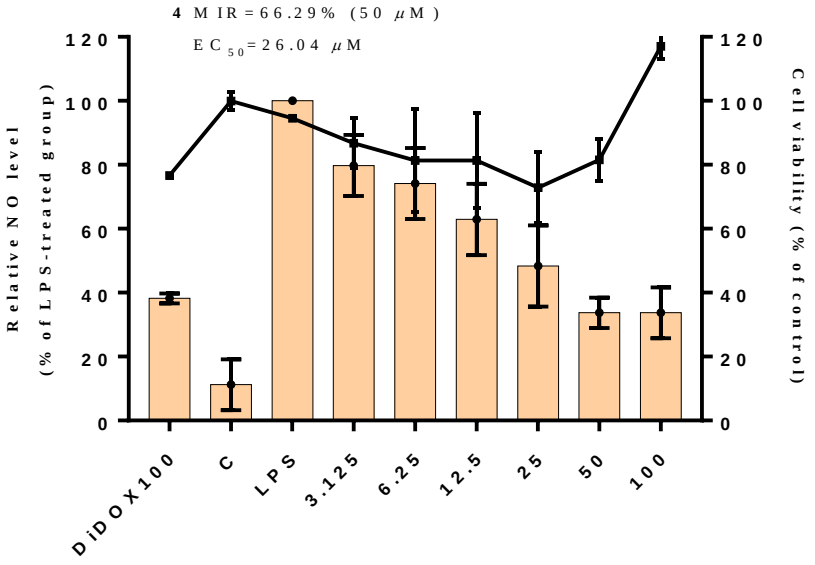
MTT	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	1.910	2.548	2.355	2.534	2.315	2.065	2.889	3.104	0.752
	1.918	2.447	2.367	2.618	1.966	2.321	2.616	2.765	0.662
AVG	1.914	2.497	2.361	2.576	2.140	2.193	2.7525	2.9345	0.707
(The original value/AVG of group C) *100	76.476	102.022	94.294	101.461	92.692	82.682	115.675	124.284	30.110
	76.796	97.977	94.774	104.828	78.718	92.932	104.744	110.710	26.506
AVG	76.636	100.000	94.534	103.143	85.705	87.807	110.210	117.497	28.308
SD	0.226	2.859	0.339	2.378	9.881	7.248	7.729	9.597	2.548



Compound **4**

NO(μM)	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	0.063	0.054	0.091	0.085	0.083	0.078	0.072	0.063	0.064
	0.064	0.049	0.091	0.079	0.076	0.071	0.064	0.06	0.059
Corrected Absorbance	0.015	0.006	0.043	0.037	0.035	0.030	0.024	0.015	0.016
	0.016	0.001	0.043	0.031	0.028	0.023	0.016	0.012	0.012
Standard Curve Calculation	2.391	1.086	6.449	5.579	5.289	4.565	3.695	2.391	2.536
	2.536	0.362	6.449	4.710	4.275	3.550	2.536	1.956	1.811
AVG	2.463	0.724	6.449	5.144	4.782	4.057	3.116	2.174	2.173
SD	0.102	0.512	0	0.614	0.717	0.717	0.819	0.307	0.512

MTT	DiDOX100	C	LPS	3.125	6.25	12.5	25	50	100
Absorbance Value	1.910	2.548	2.355	2.027	1.750	1.770	1.622	1.921	2.855
	1.918	2.447	2.367	2.308	2.314	2.295	2.017	2.153	2.993
AVG	1.914	2.497	2.361	2.167	2.032	2.032	1.819	2.037	2.924
(The original value/AVG of group C) *100	76.476	102.022	94.294	81.161	70.070	70.870	64.944	76.916	114.314
	76.796	97.977	94.774	92.412	92.652	91.891	80.760	86.206	119.839
AVG	76.636	100	94.534	86.786	81.361	81.381	72.852	81.561	117.077
SD	0.226	2.859	0.339	7.955	15.968	14.864	11.183	6.568	3.907



Inhibition of NO production in LPS-stimulated RAW 264.7 murine macrophages was used to evaluate the anti-inflammatory activity of compounds **1**-**4**. Compound concentrations of 100, 50, 25, 12.5, and 6.25 μM were tested in the assay simultaneously, respectively. Didox, a known inhibitor of LPS-induced NO production, exhibits approximately 60% inhibition in this assay at a concentration of 100 μM and was used as a positive control. To evaluate the anti-inflammatory properties of the compounds, the maximum inhibition rate (MIR) of NO production using nontoxic compound concentrations was calculated by comparing the decrease in the concentration of NO in the presence of the compounds compared to the NO concentration in the LPS-stimulated alone group. The inhibitory potency of the compounds was categorized according to the following criteria: strong (MIR≥80%); moderate (80% > MIR≥50%); weak (50% > MIR≥30%); undetected (MIR<30%). The MIRs of dimers **2**–**4** were 73.03% (25 μM), 77.53% (50 μM), and 66.29% (50 μM), respectively, while that of monomeric **1** was 41.57% (100 μM). In summary, the anti-inflammatory bioassays showed that dimeric compounds **2**–**4** are better than monomeric **1**.

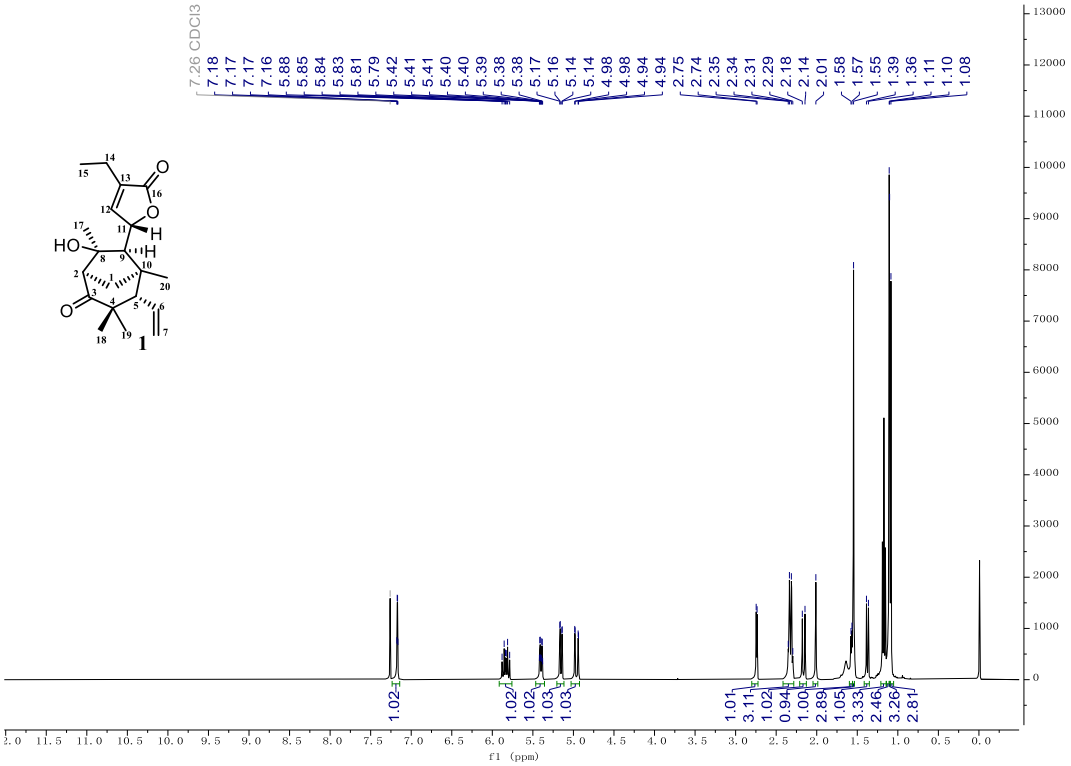


Figure S1-1. ^{1}H NMR spectrum (400 MHz) of **1** in $CDCl_3$.

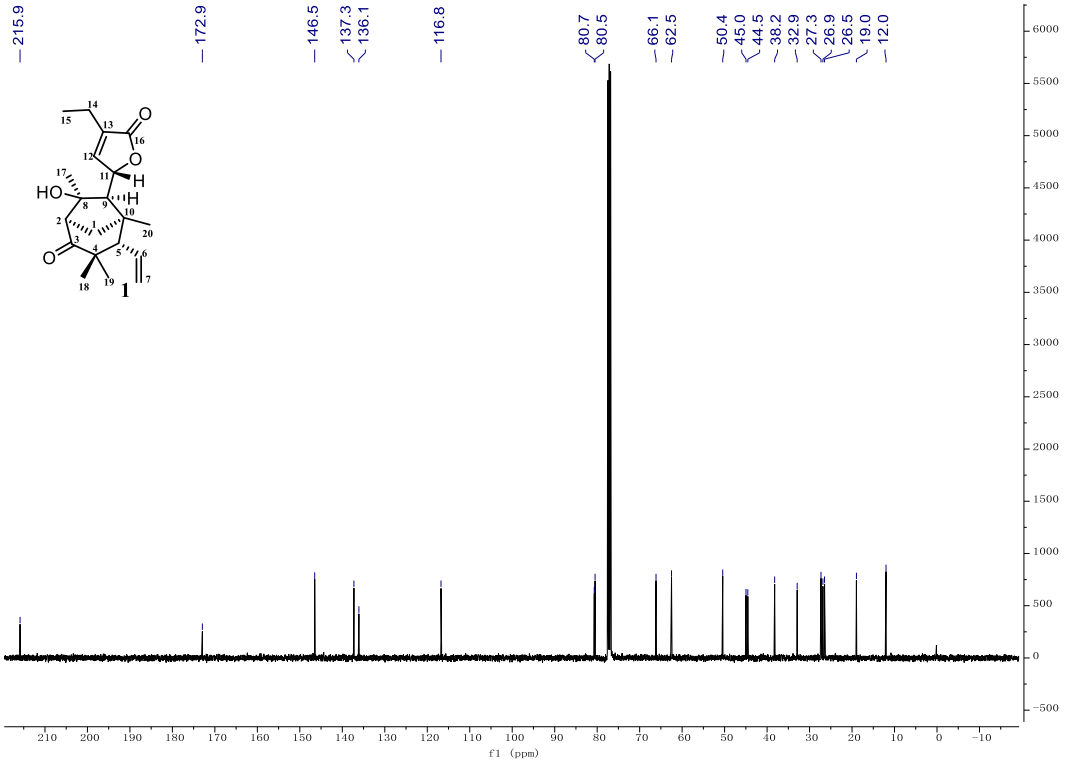


Figure S1-2. ^{13}C NMR spectrum (400 MHz) of **1** in $CDCl_3$.

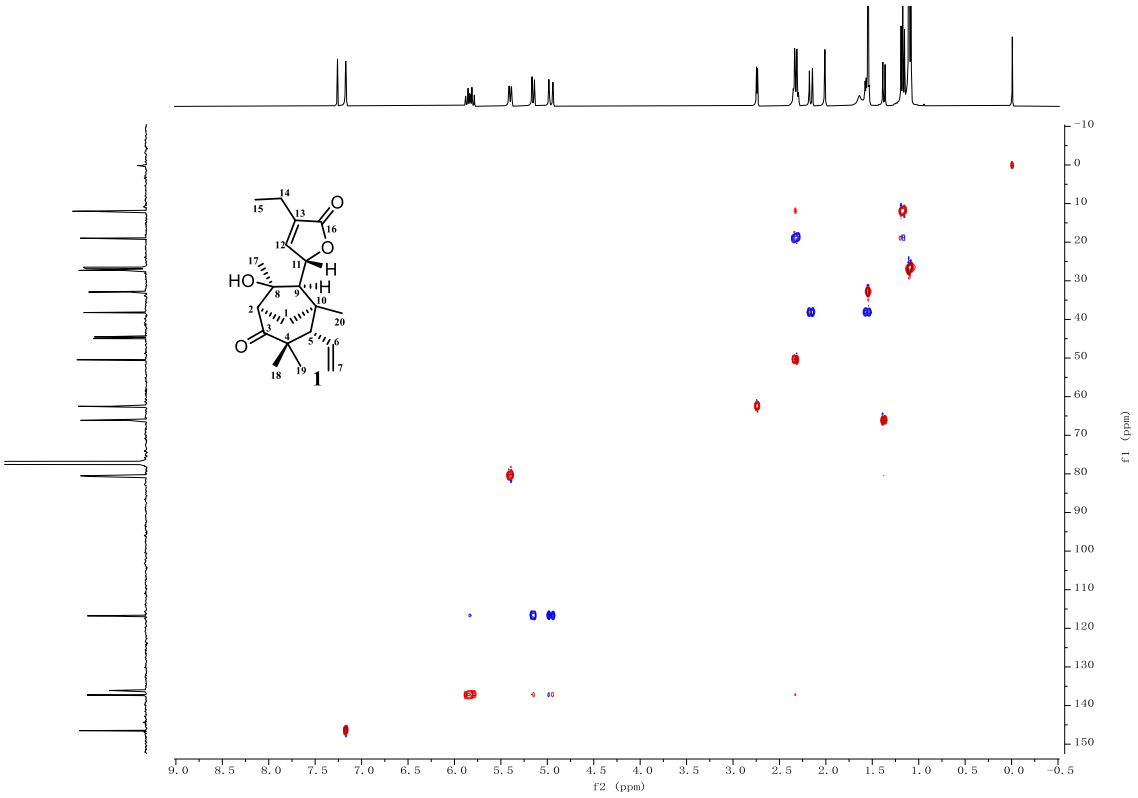


Figure S1-3. HSQC spectrum (400 MHz) of **1** in $CDCl_3$.

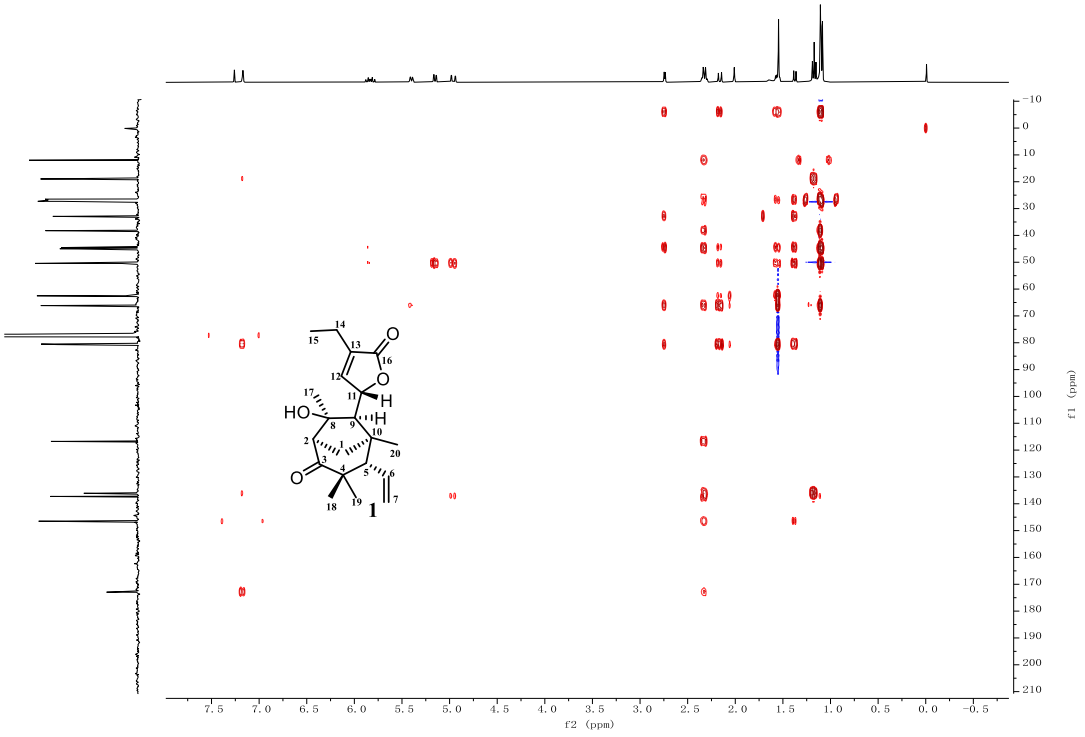


Figure S1-4. HMBC spectrum (400 MHz) of **1** in $CDCl_3$.

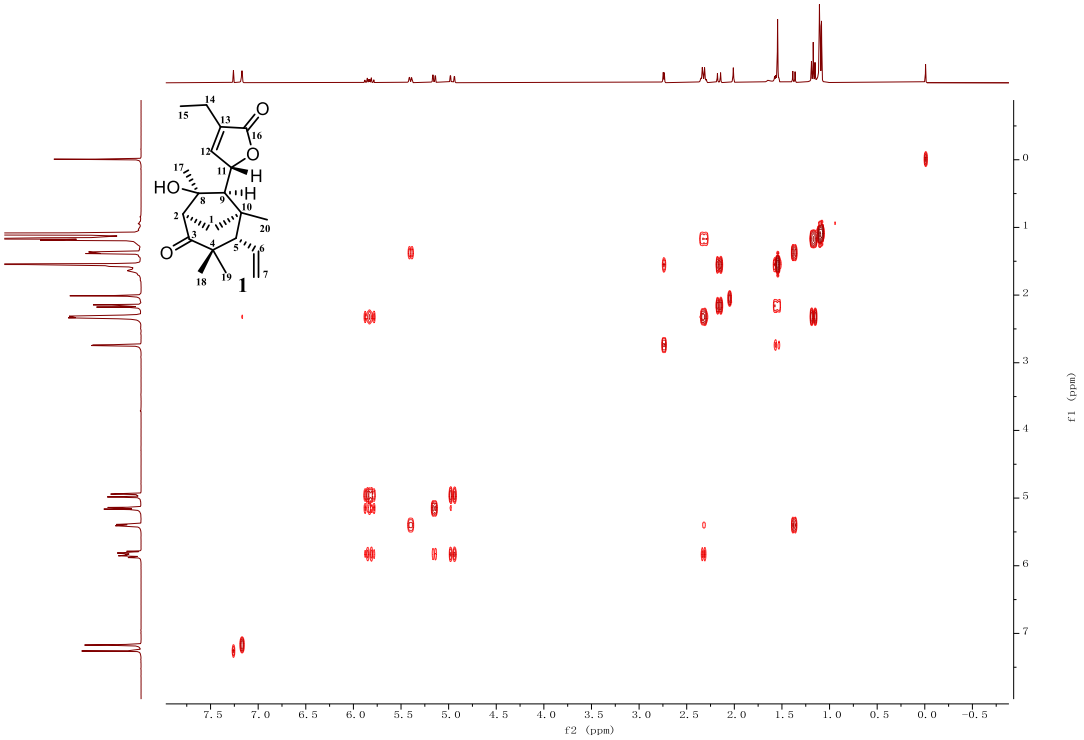


Figure S1-5. $^{1}H–^{1}H$ COSY spectrum (400 MHz) of **1** in $CDCl_3$.

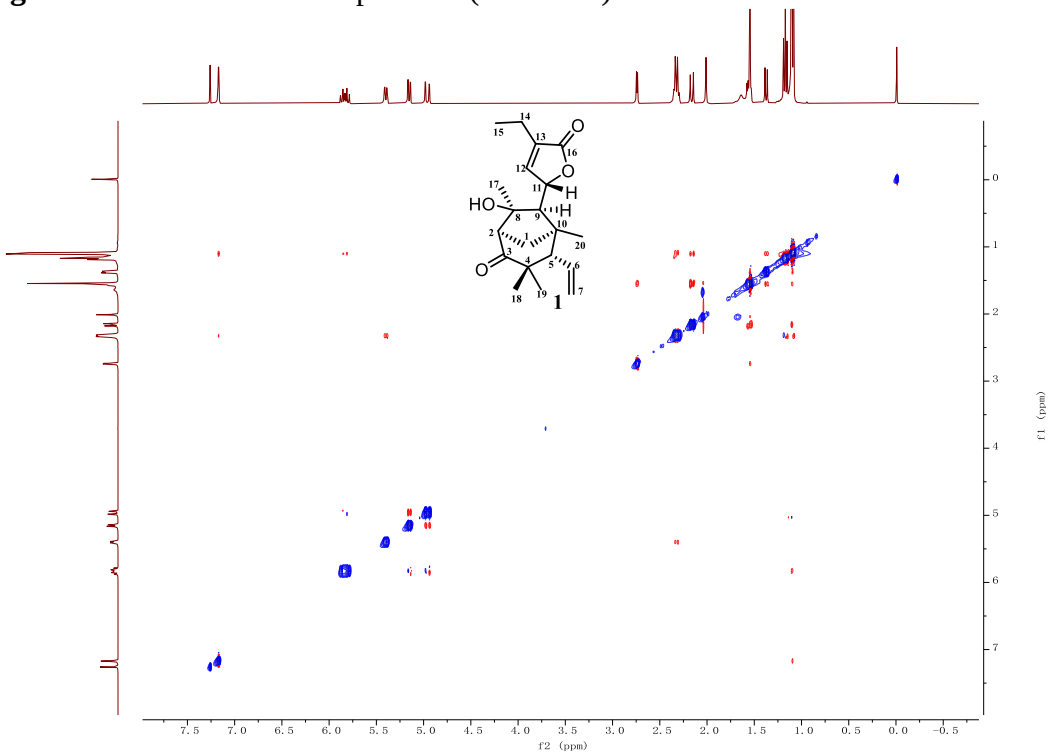


Figure S1-6. NOESY spectrum (400 MHz) of **1** in $CDCl_3$.

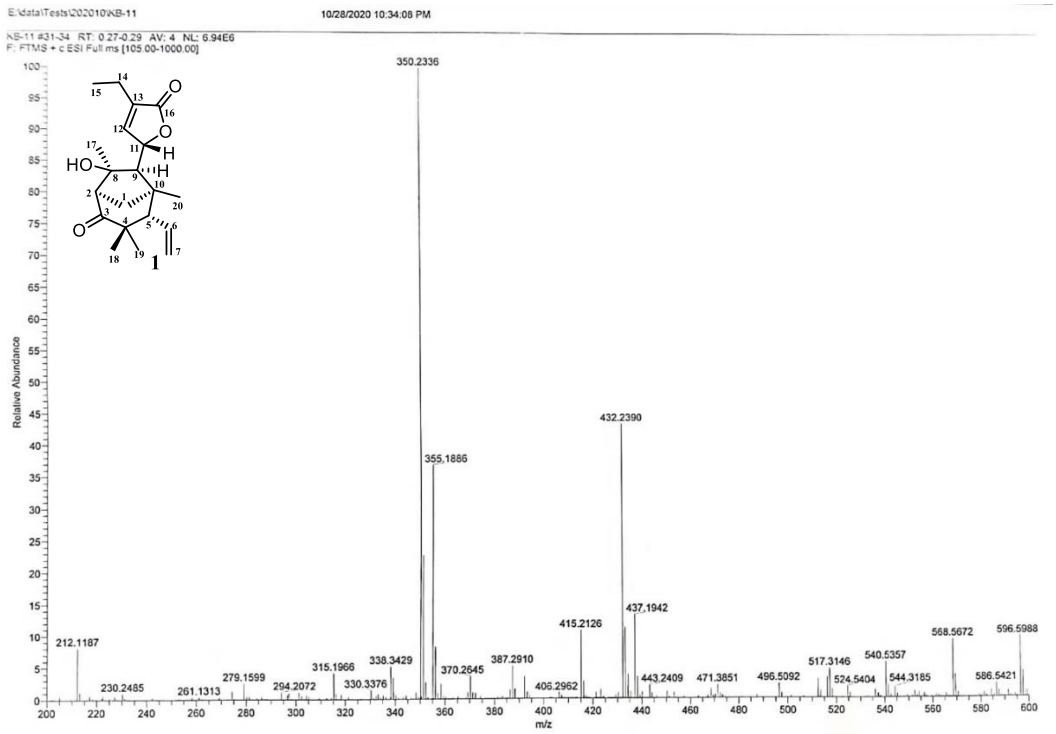


Figure S1-7. HRESIMS spectrum of **1.**

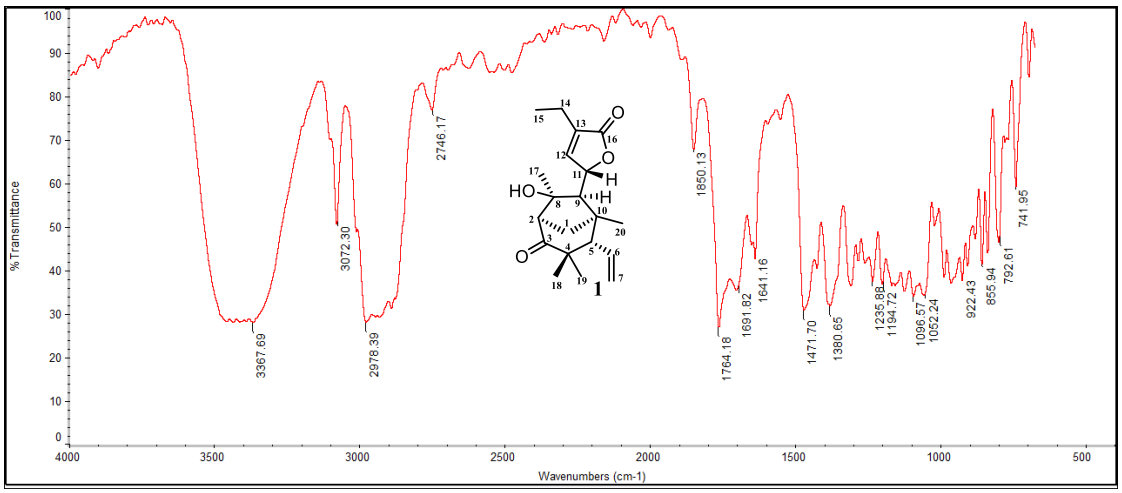


Figure S1-8. IR (KBr disc) spectrum of **1**.

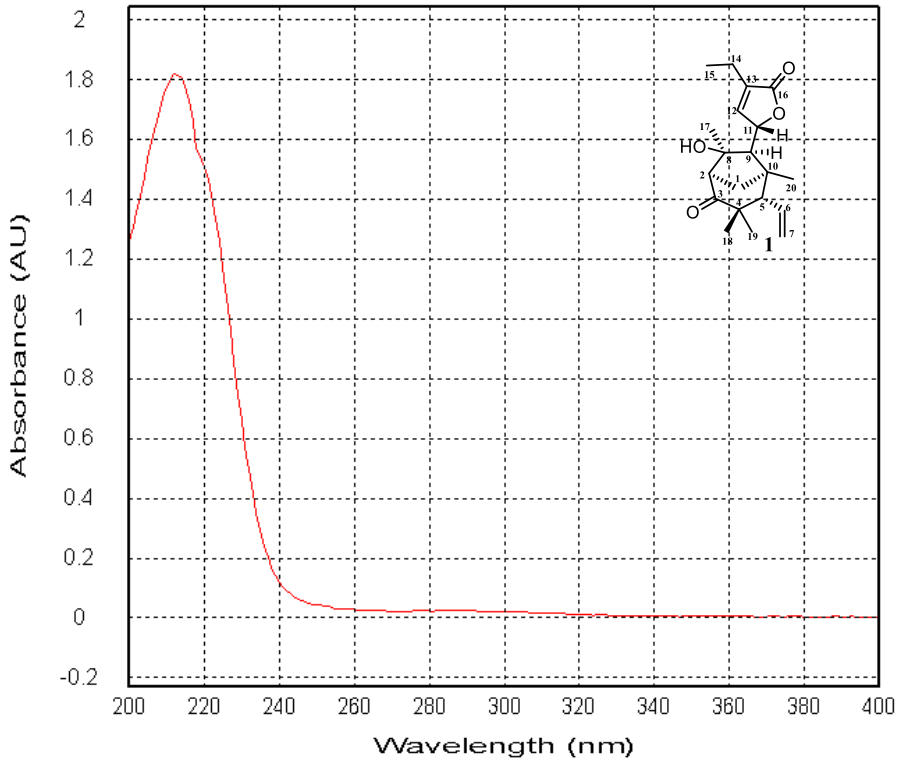


Figure S1-9. UV spectrum of **1**.

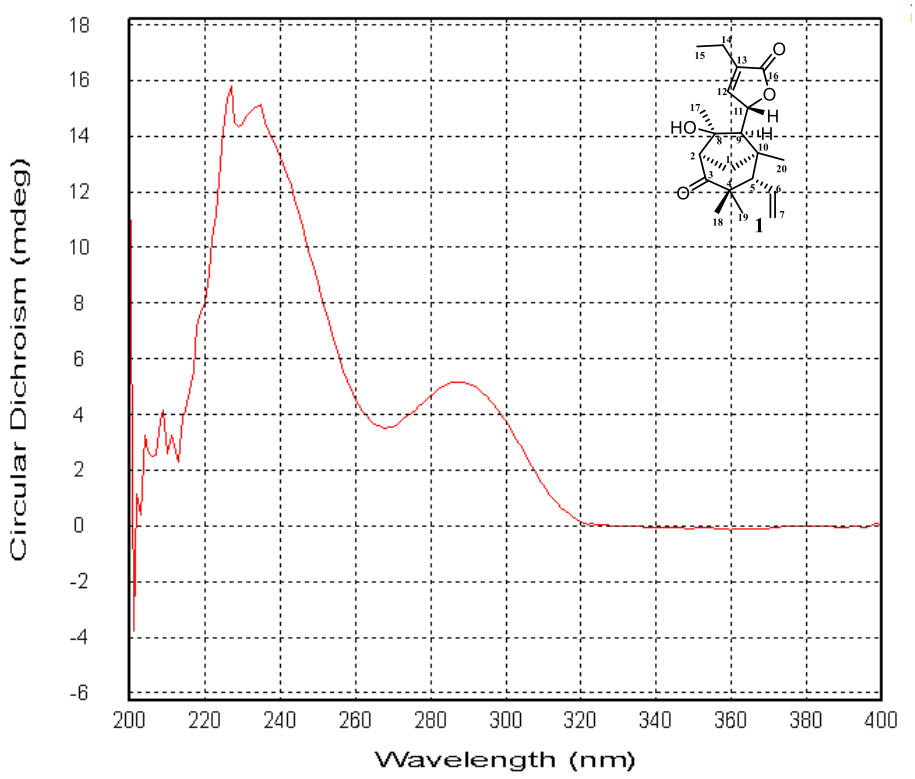


Figure S1-10. CD spectrum of **1**.

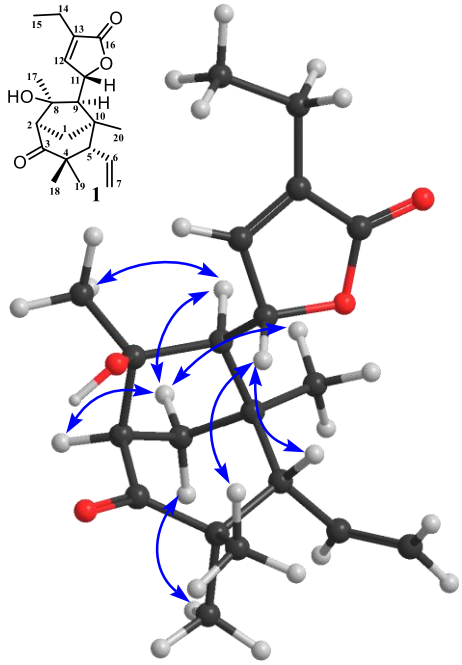


Figure S1-11. NOESY signals of **1**.

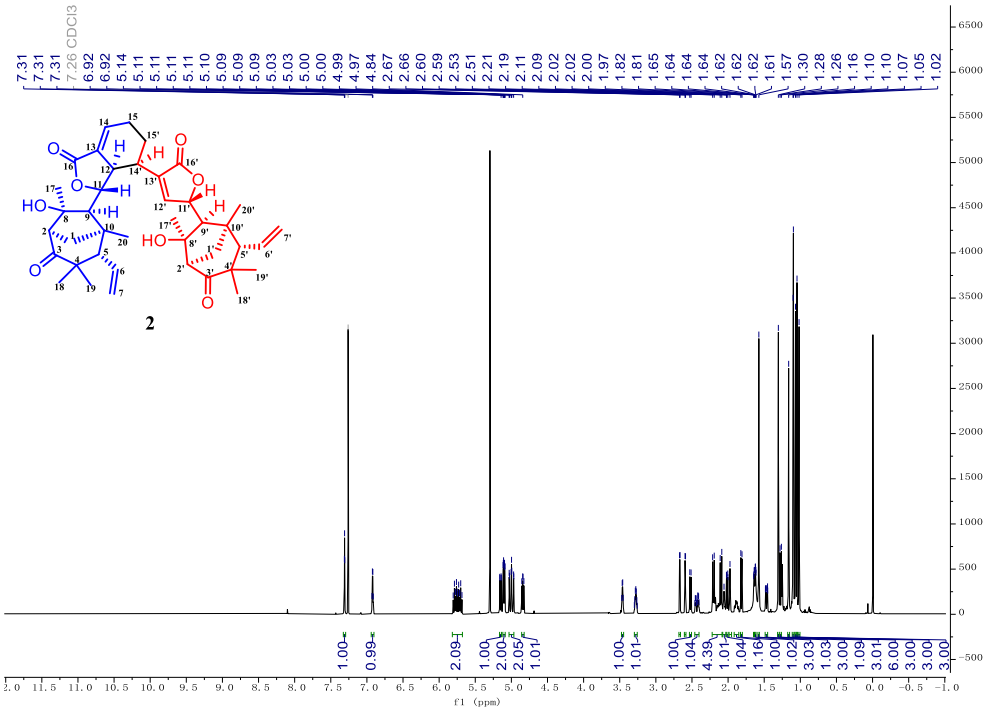


Figure S2-1. ^{1}H NMR spectrum (400 MHz) of **2** in $CDCl_3$.

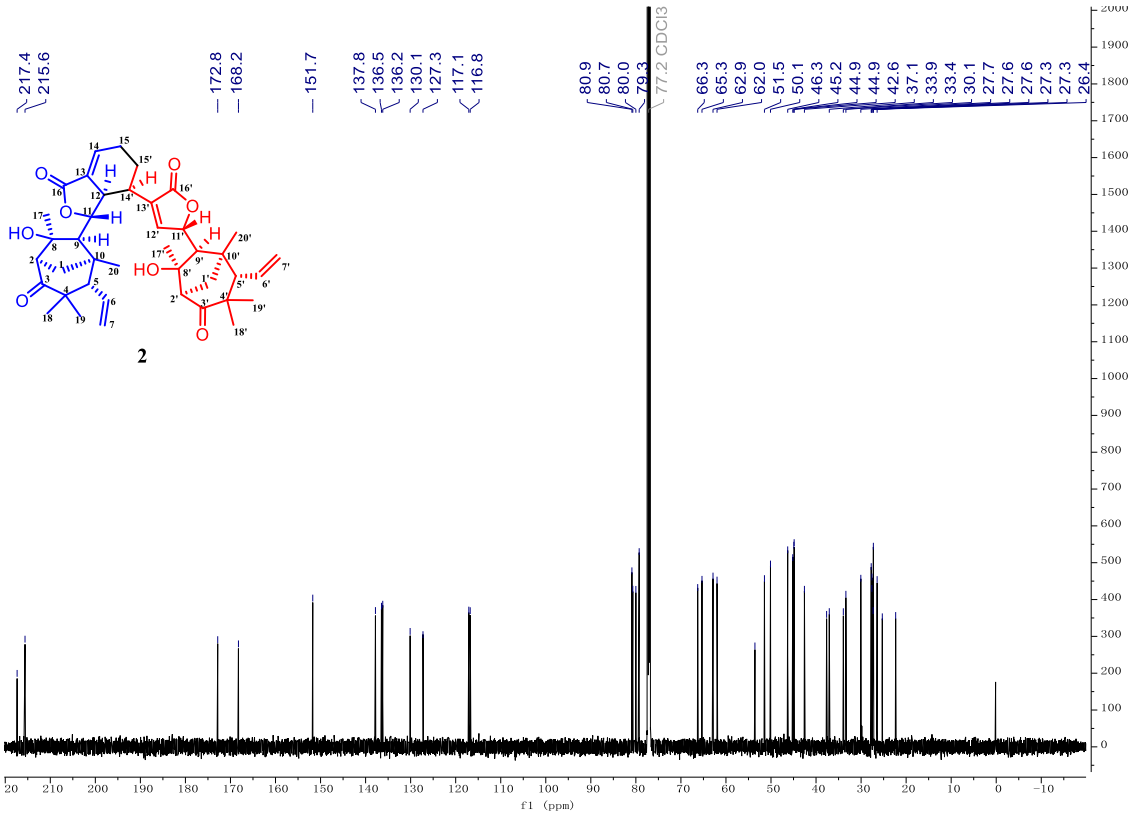


Figure S2-2. ^{13}C NMR spectrum (600 MHz) of **2** in $CDCl_3$.

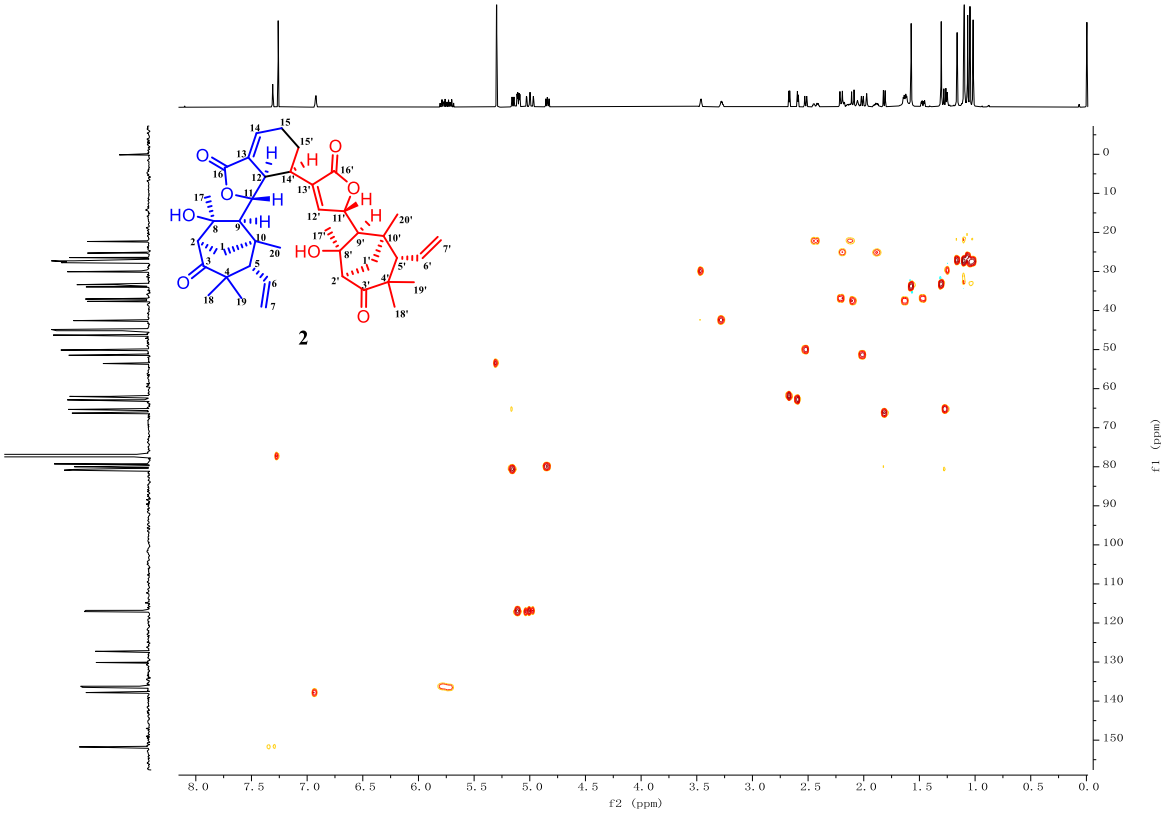


Figure S2-3. HSQC spectrum (600 MHz) of **2** in $CDCl_3$.

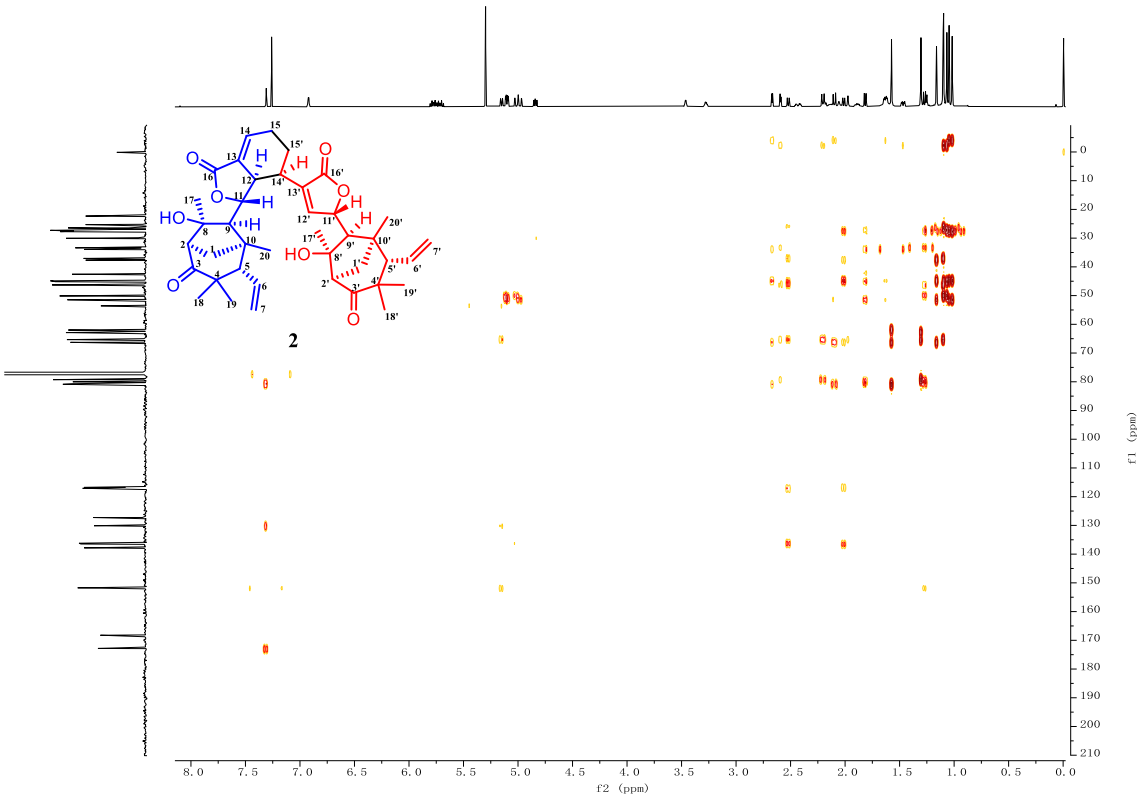


Figure S2-4. HMBC spectrum (600 MHz) of **2** in $CDCl_3$.

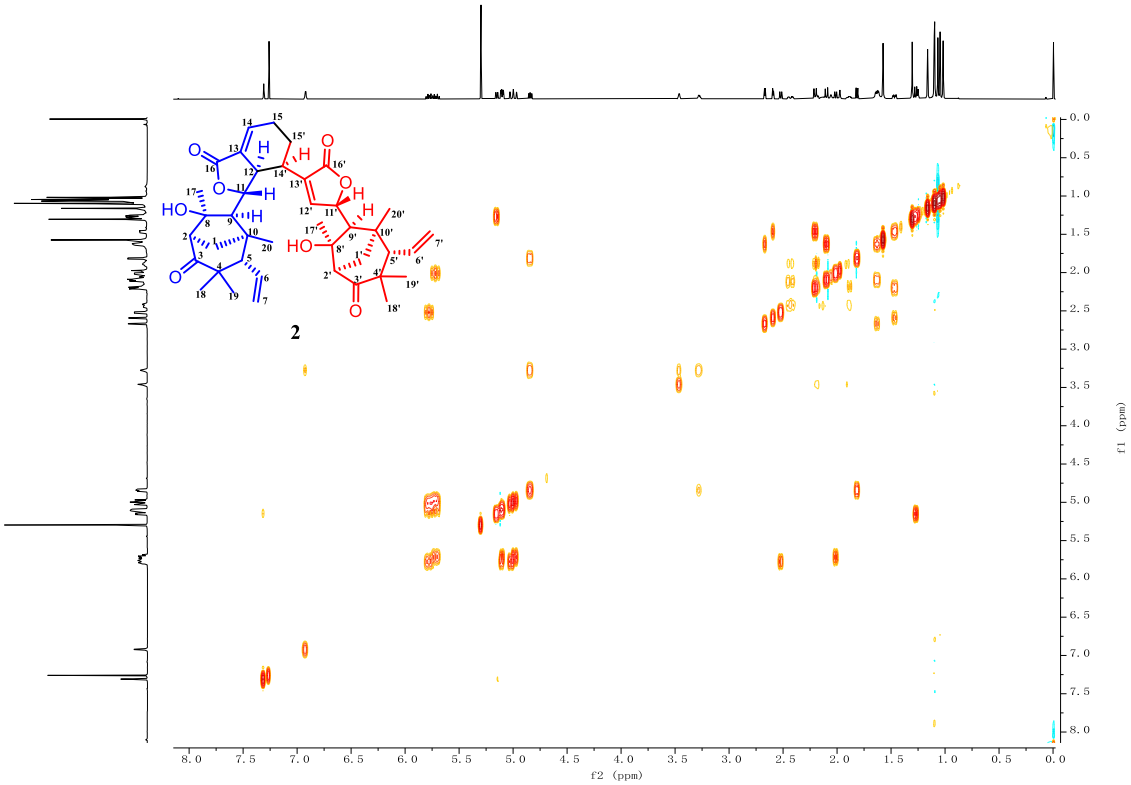


Figure S2-5. ^{1}H–^{1}H COSY spectrum (600 MHz) of **2** in $CDCl_3$.

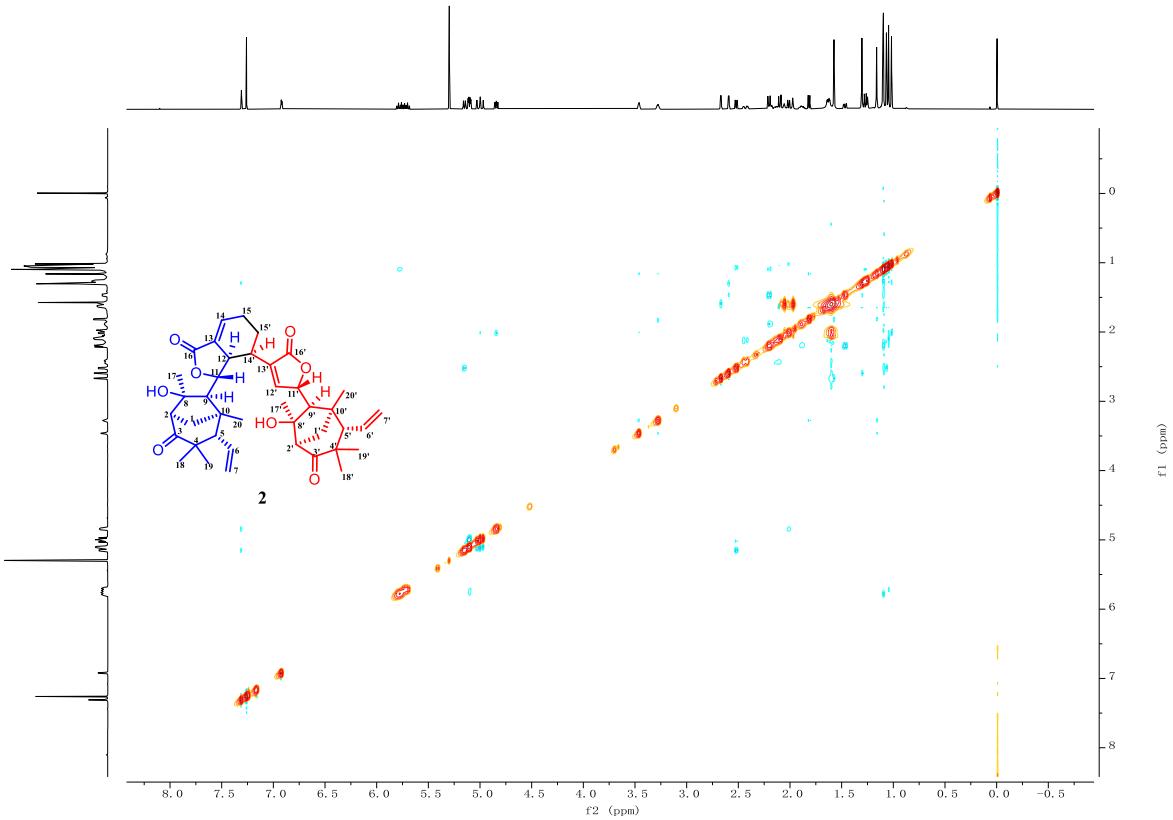


Figure S2-6. NOESY spectrum (600MHz) of **2** in $CDCl_3$.

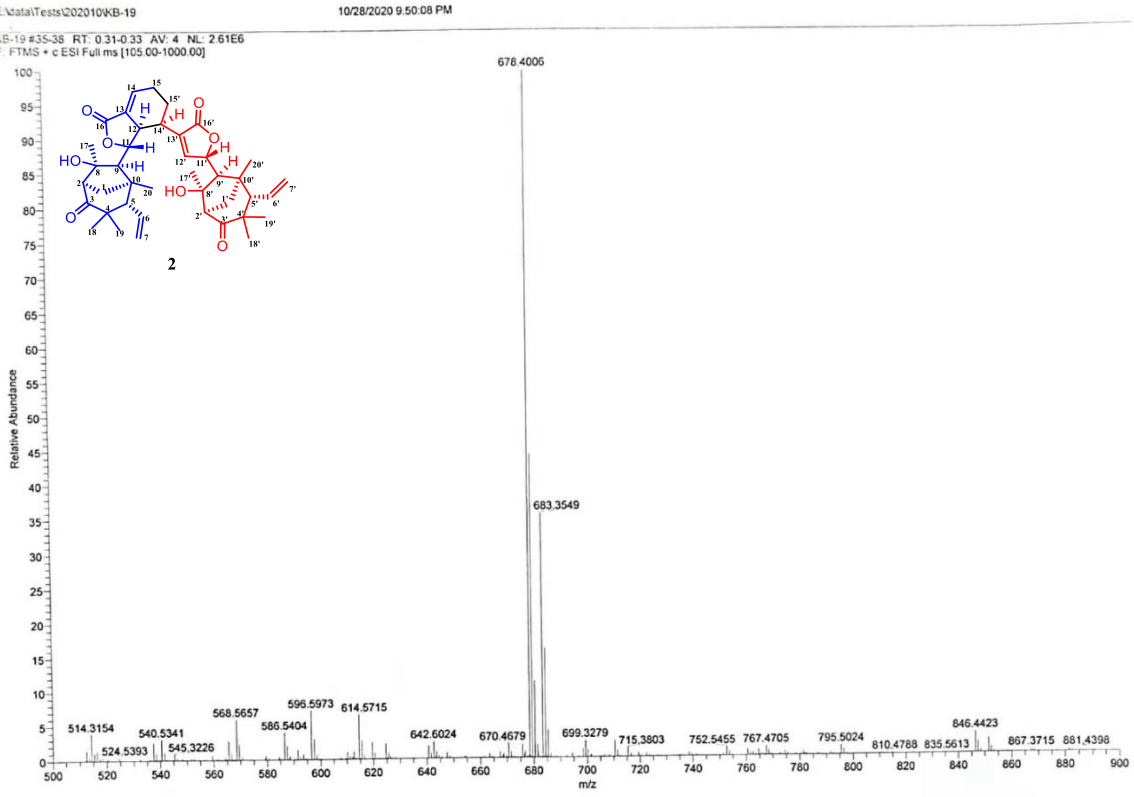


Figure S2-7. HRESIMS spectrum of **2**.

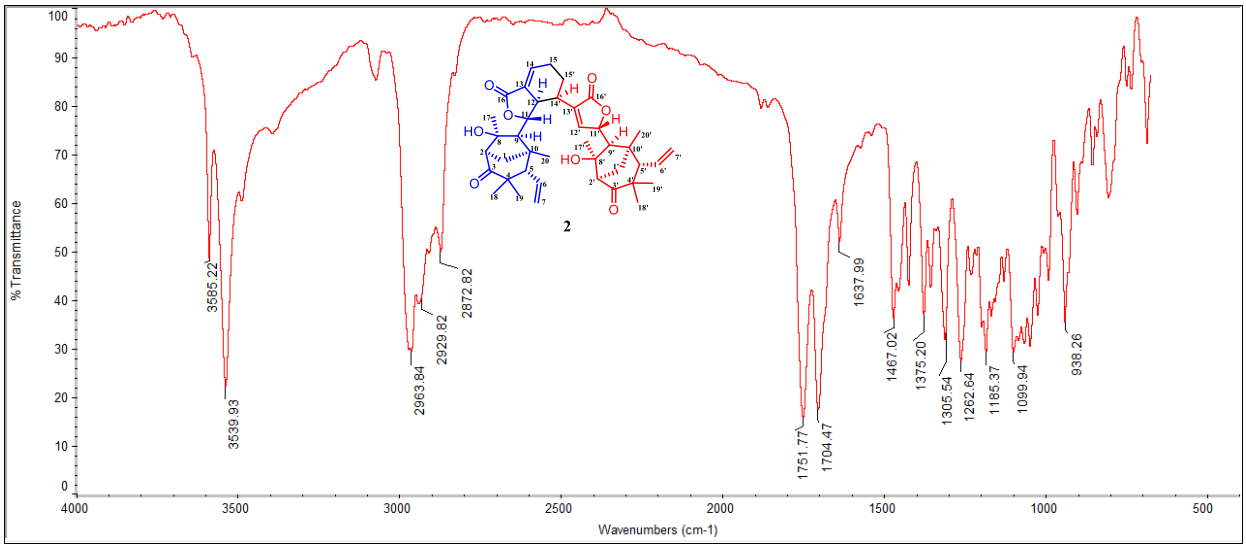


Figure S2-8. IR (KBr disc) spectrum of **2**.

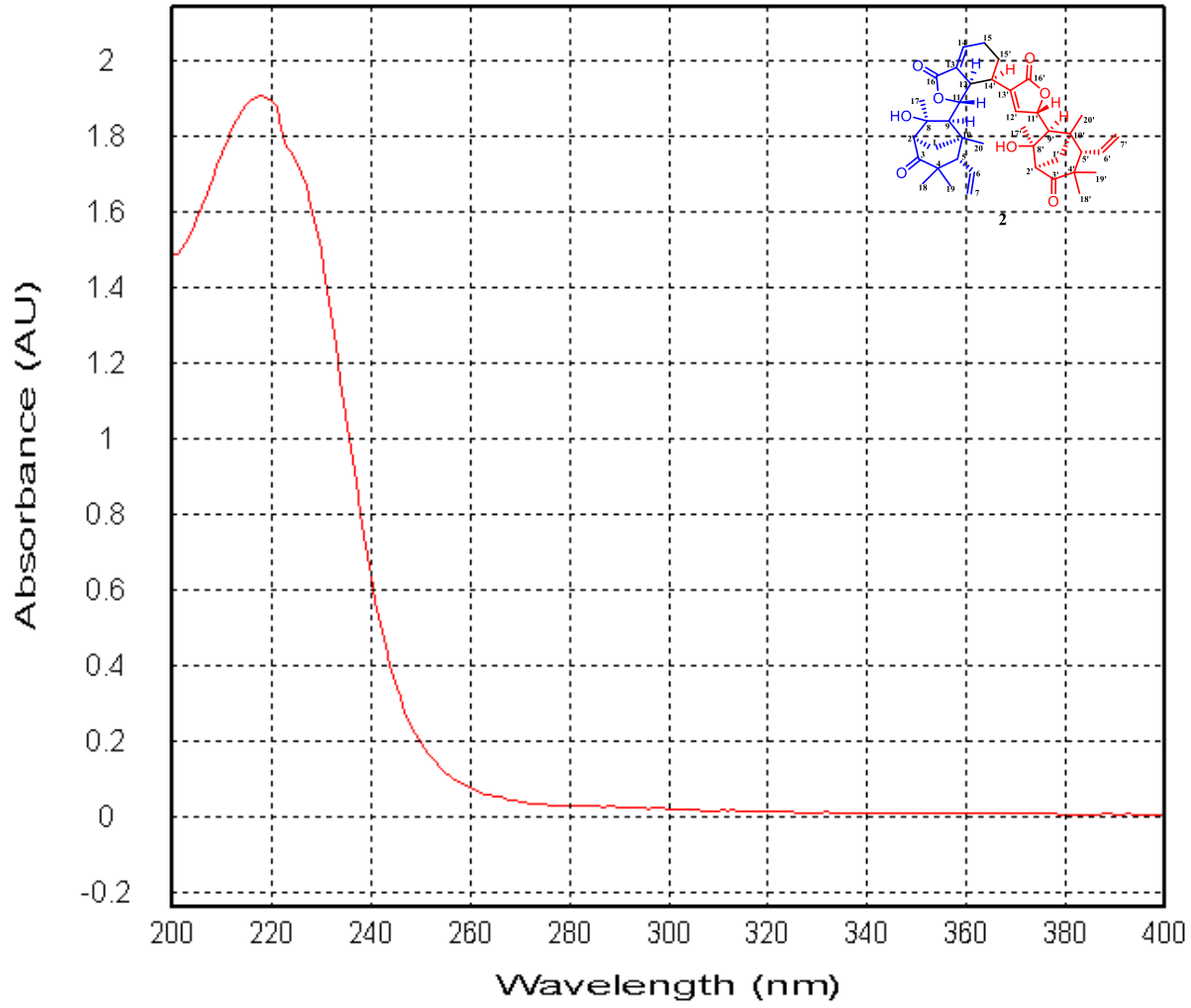


Figure S2-9. UV spectrum of **2**.

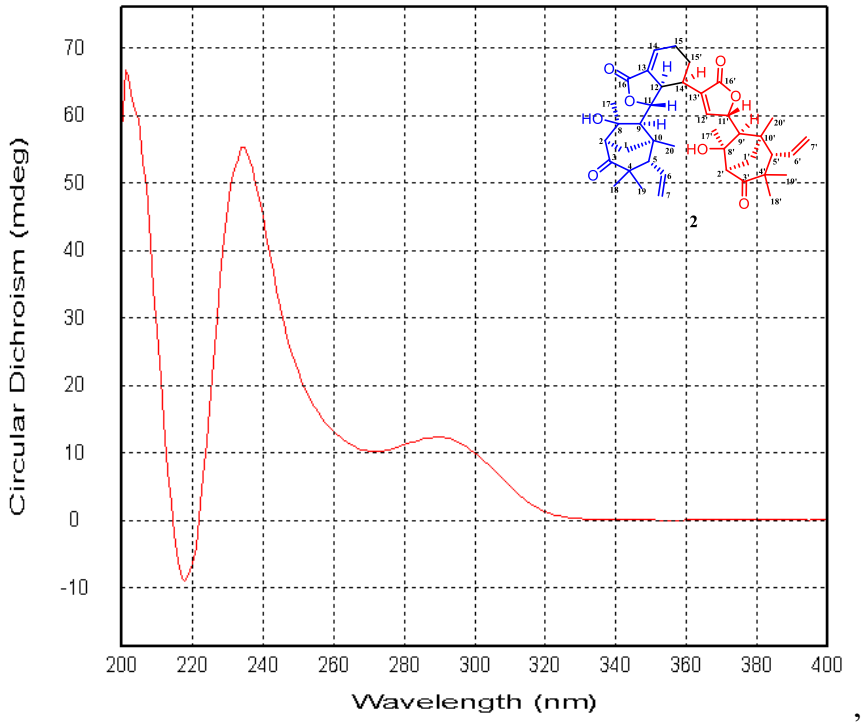


Figure S2-10. CD spectrum of **2**.

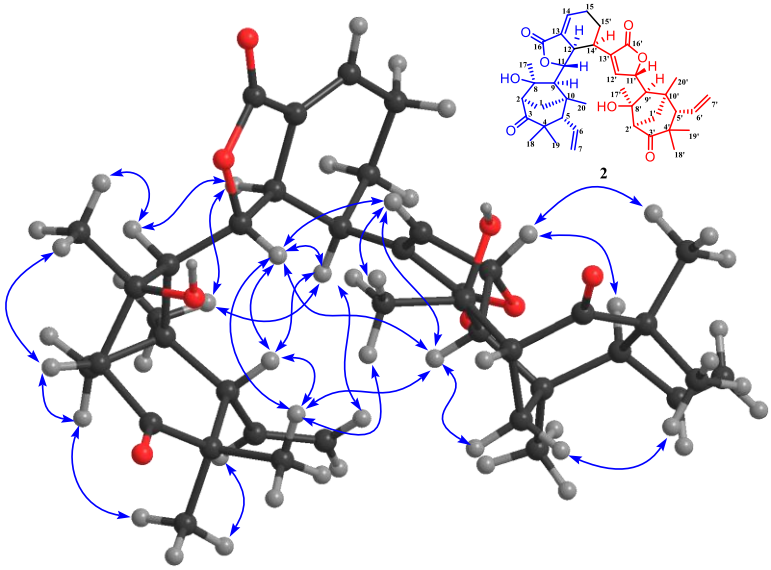


Figure S2-11. NOESY signals of **2**

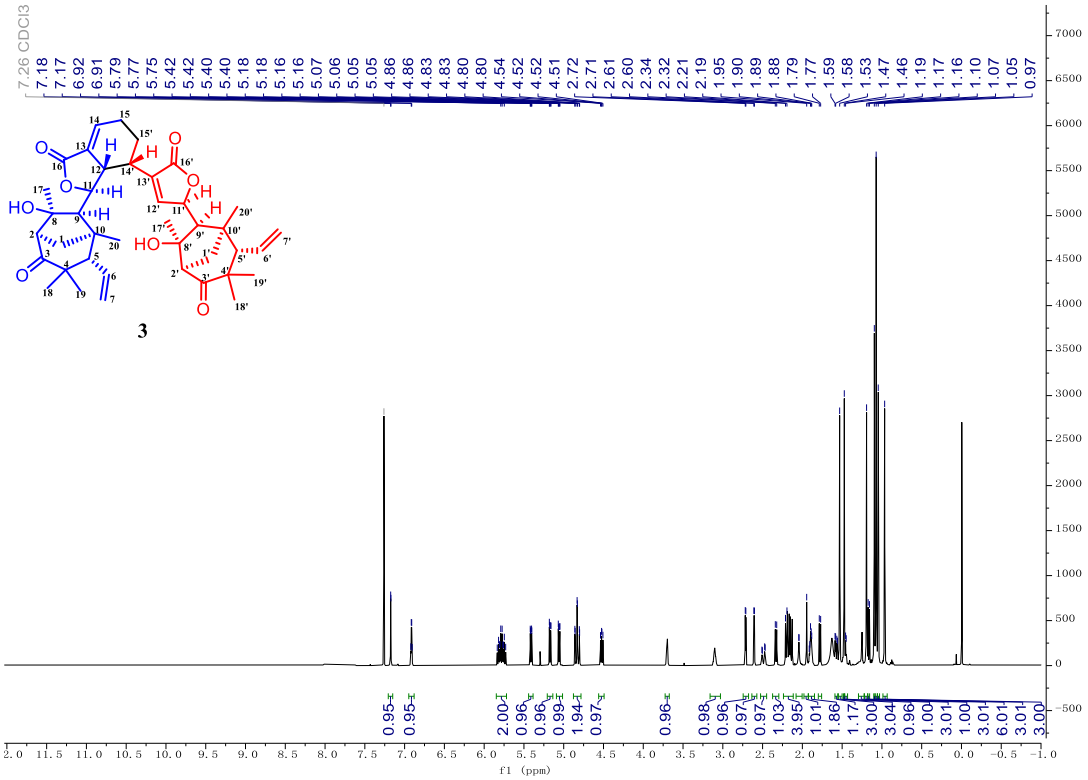


Figure S3-1. ^{1}H NMR spectrum (600 MHz) of **3** in $CDCl_3$.

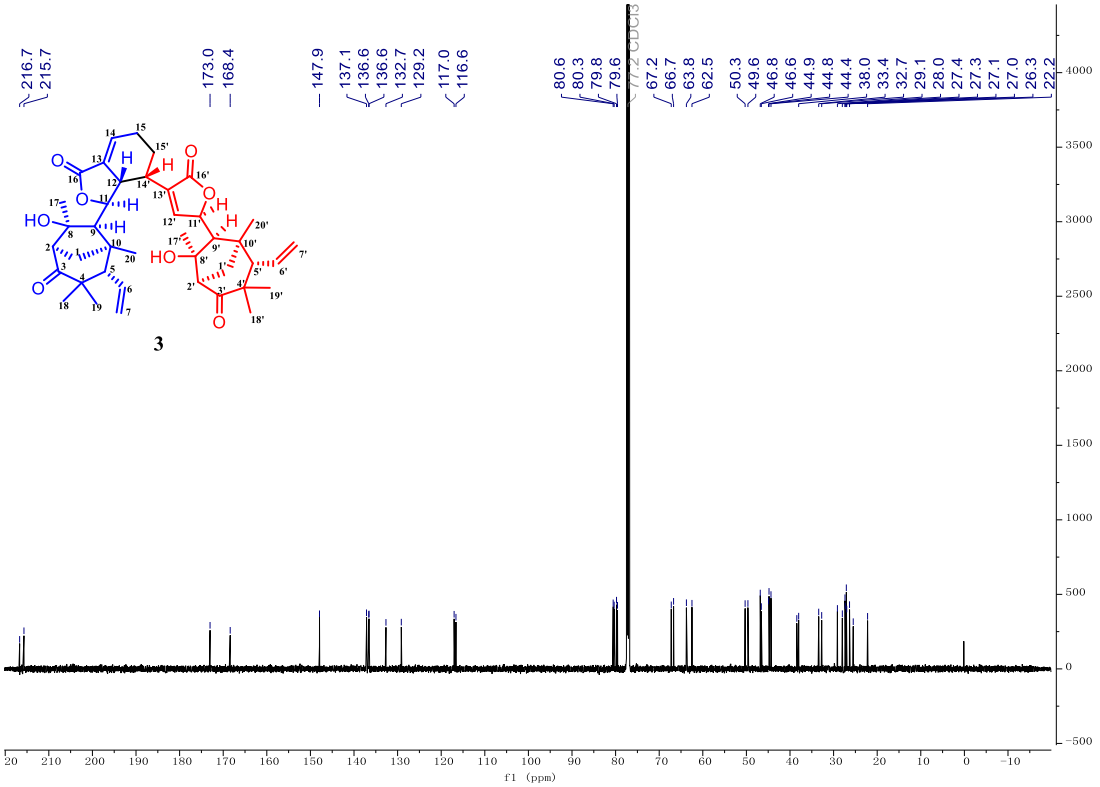


Figure S3-2. ^{13}C NMR spectrum (600 MHz) of **3** in $CDCl_3$.

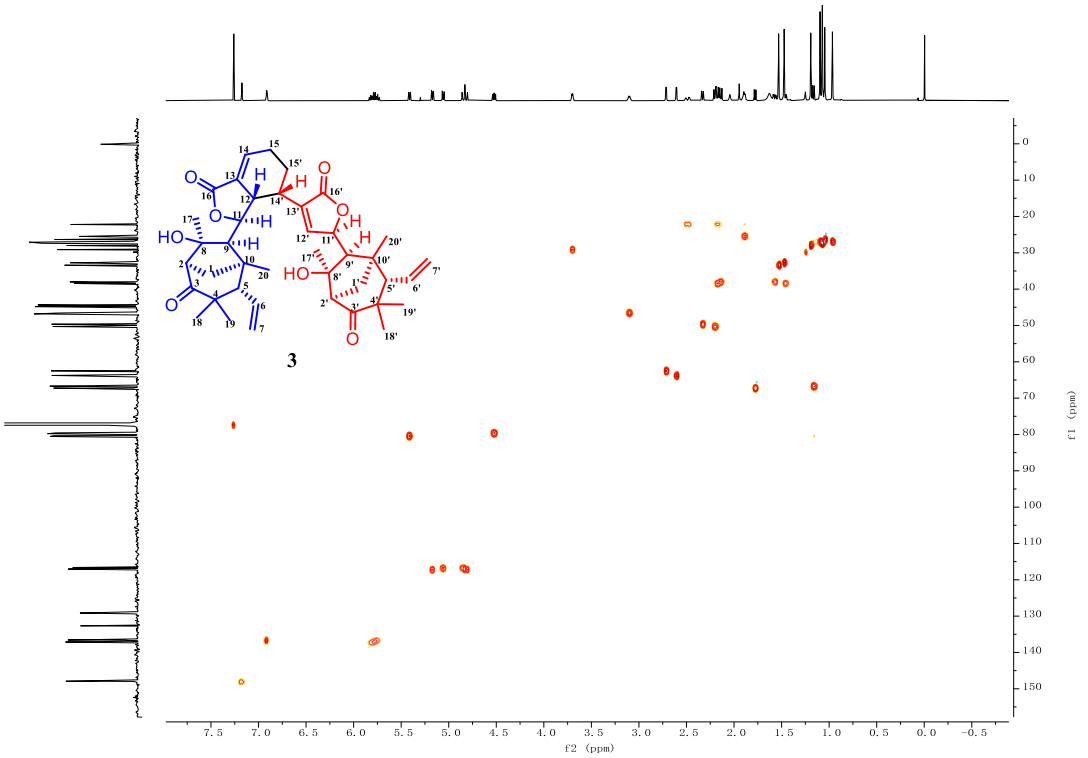


Figure S3-3. HSQC spectrum (600 MHz) of **3** in $CDCl_3$.

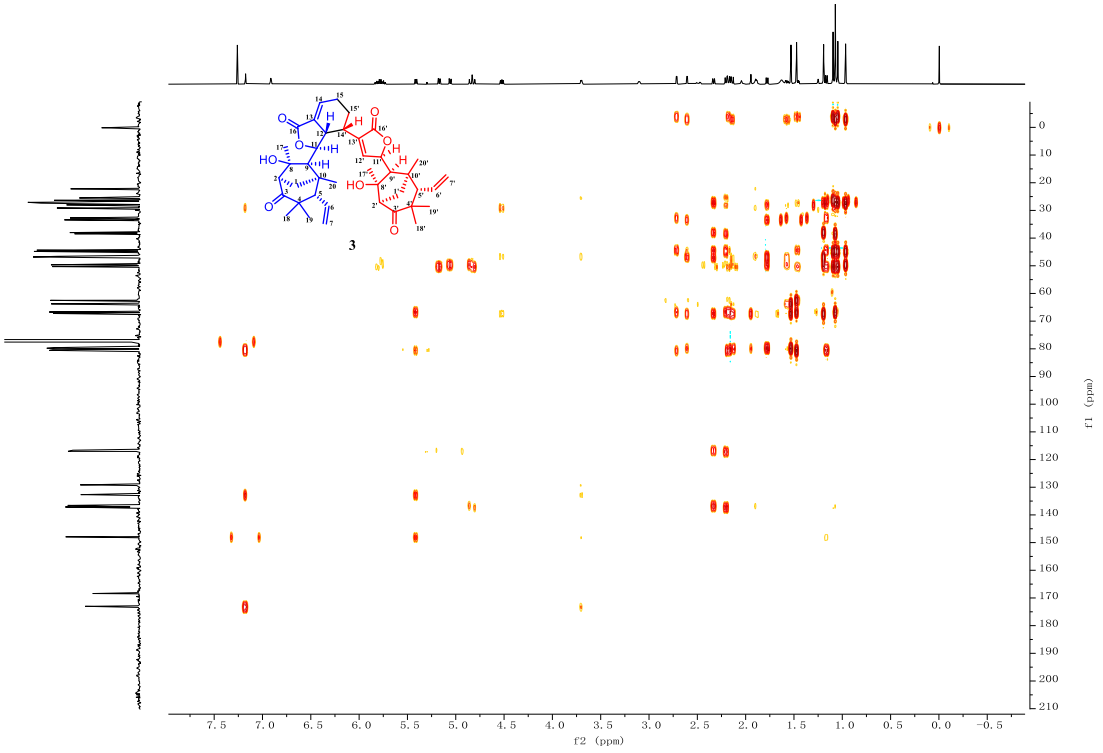


Figure S3-4. HMBC spectrum (600 MHz) of **3** in $CDCl_3$.

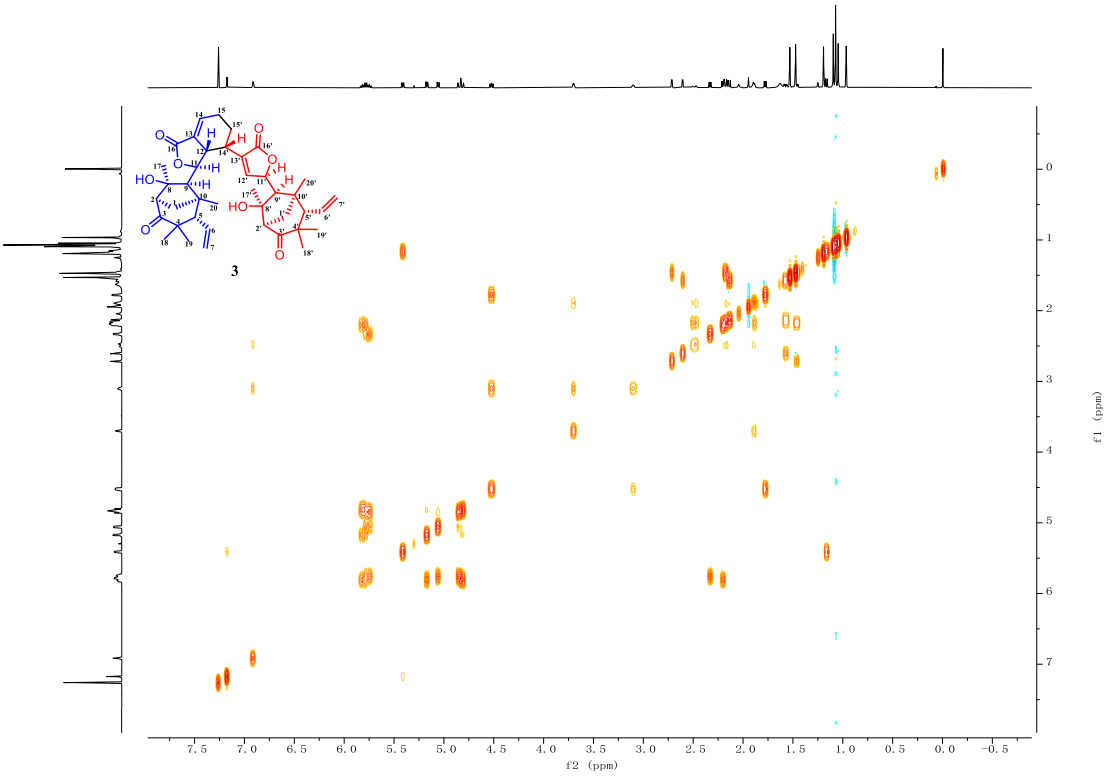


Figure S3-5. H-H COSY spectrum (600 MHz) of **3** in $CDCl_3$.

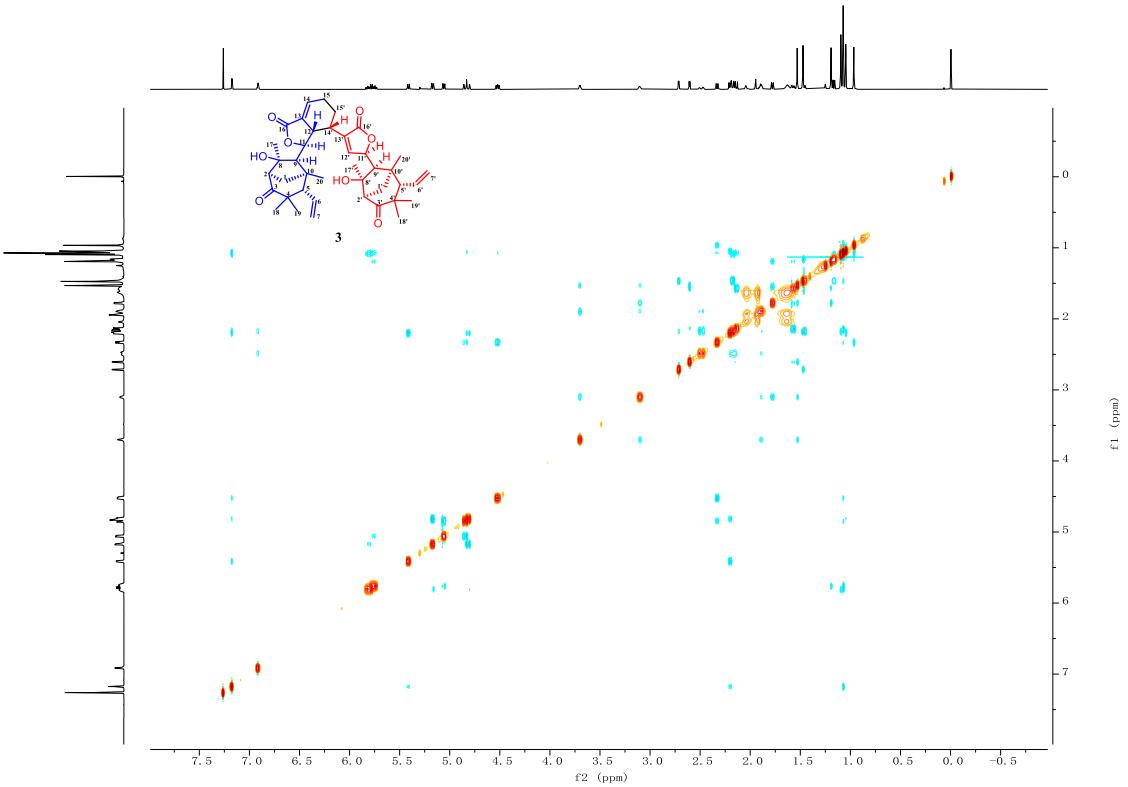


Figure S3-6. NOESY spectrum (600 MHz) of **3** in $CDCl_3$.

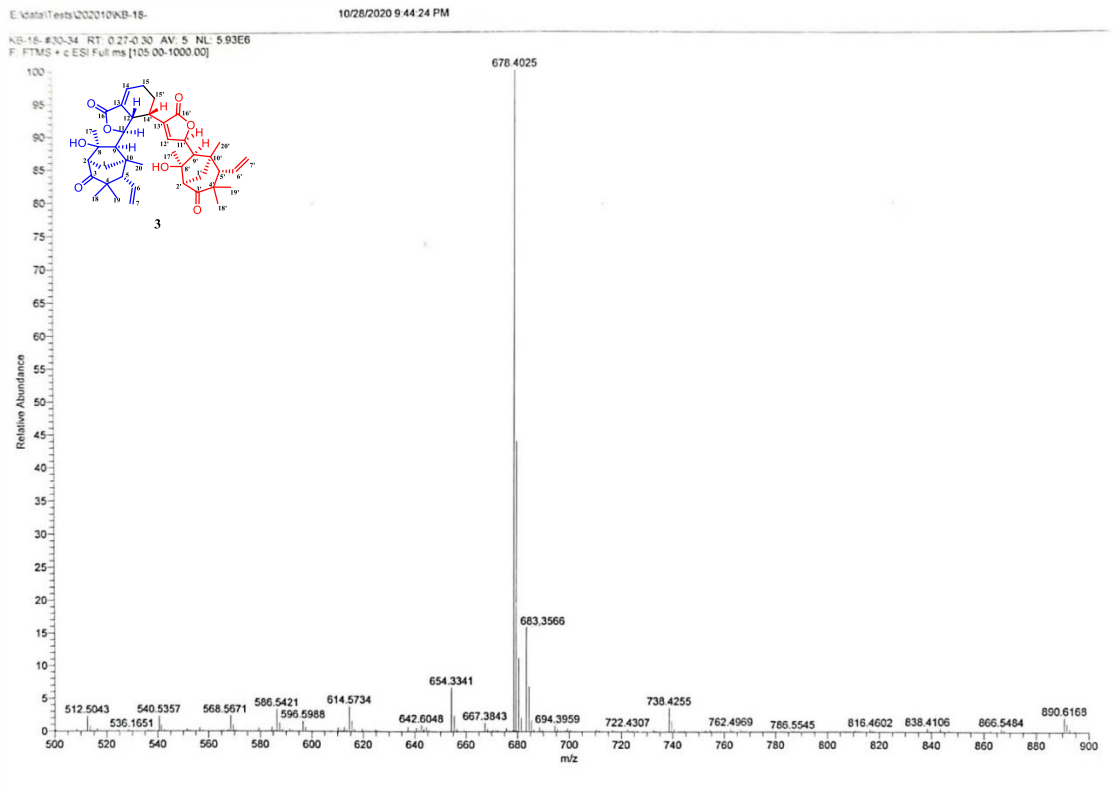


Figure S3-7. HRESIMS spectrum of **3**.

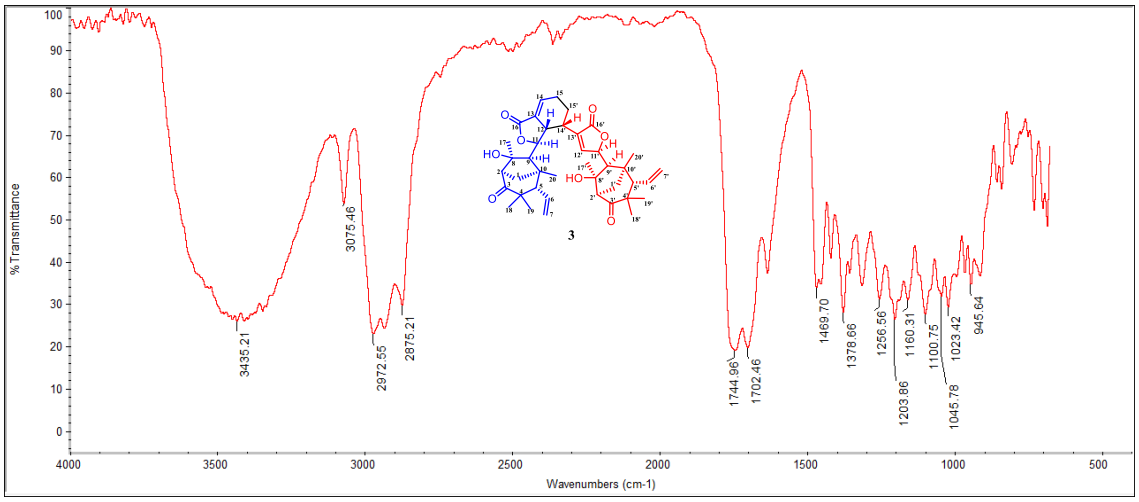


Figure S3-8. IR (KBr disc) spectrum of **3**.

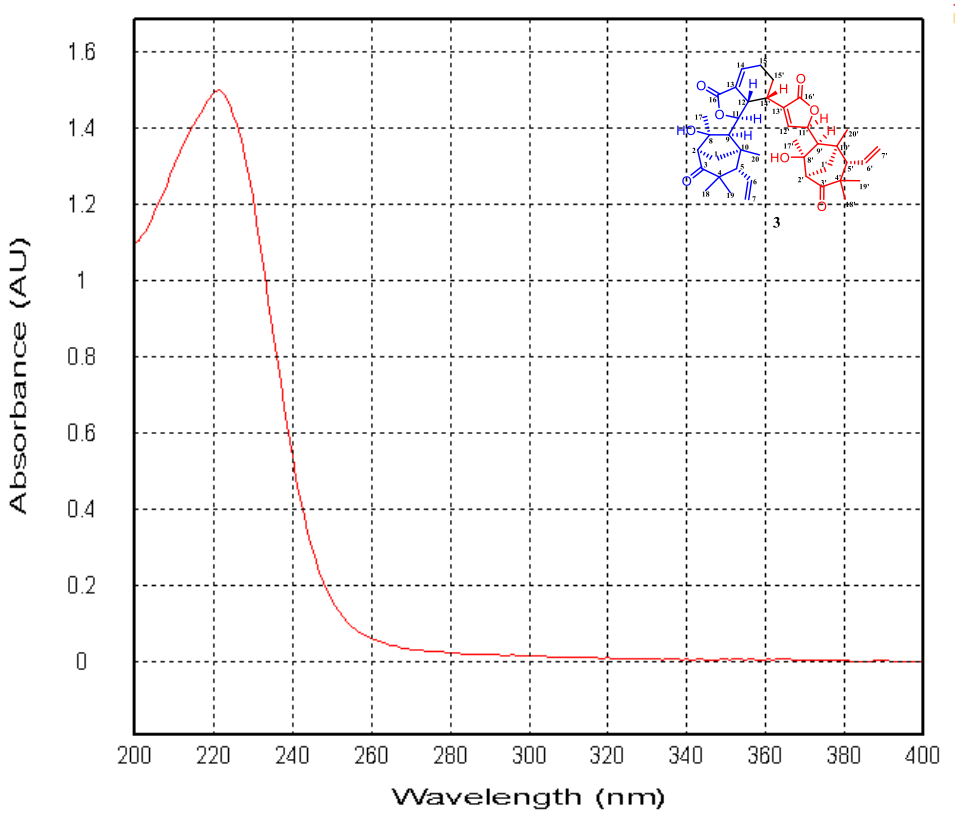


Figure S3-9. UV spectrum of **3**.

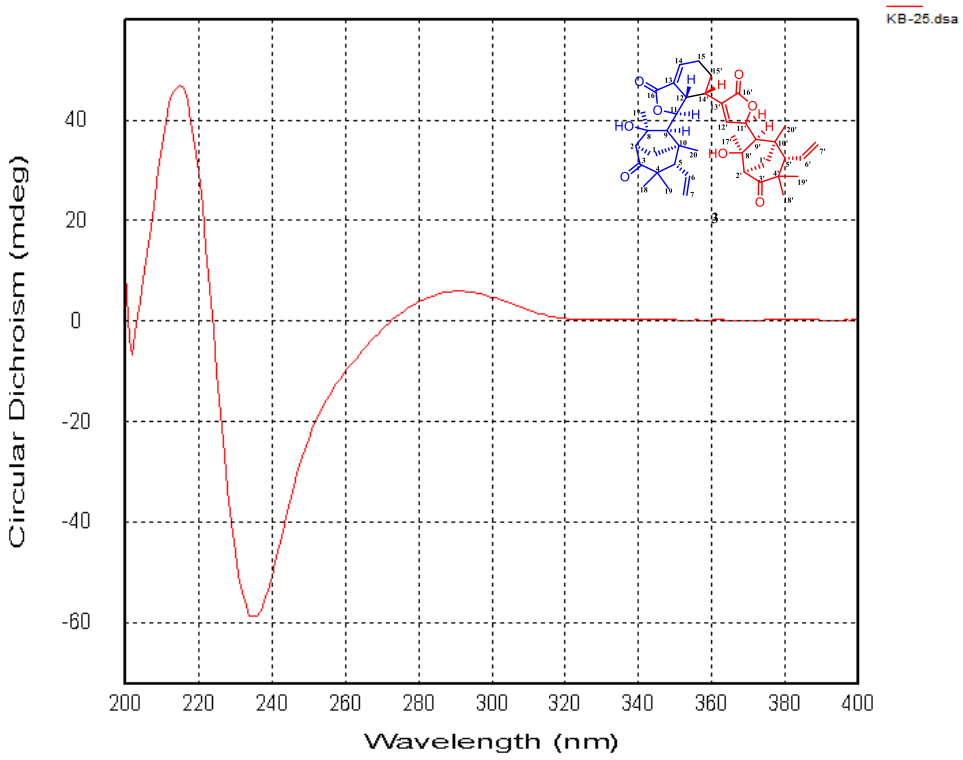
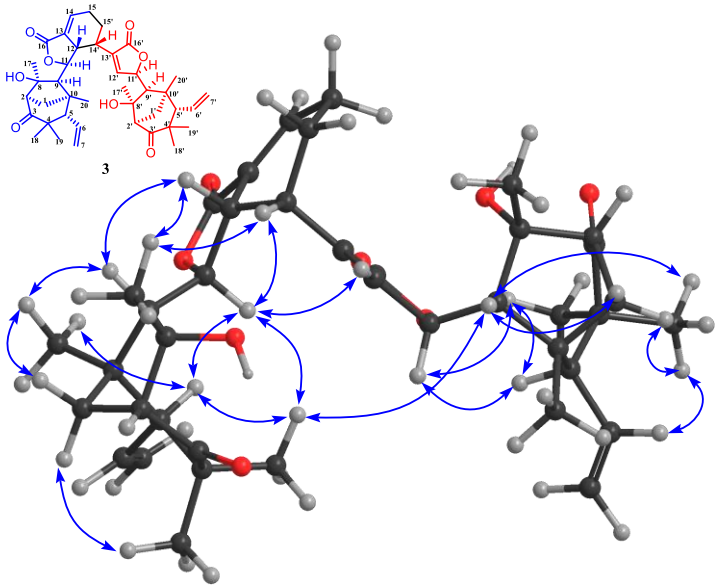


Figure S3-10. CD spectrum of **3**.



3 NOESY

Figure S3-11. NOESY signals of **3**.

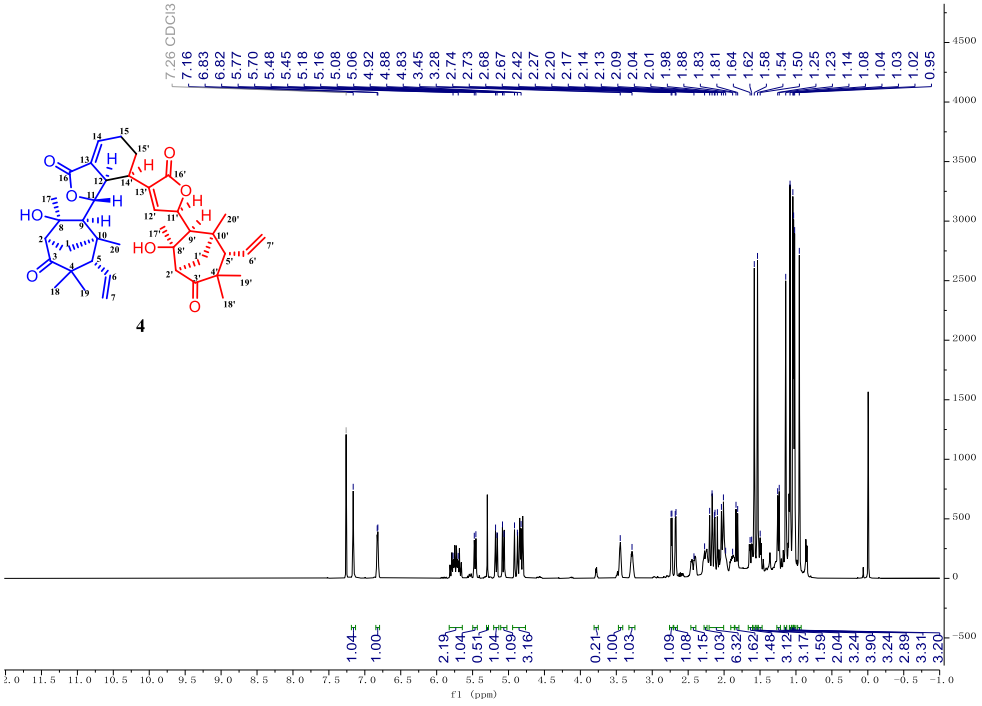


Figure S4-1. ^{1}H NMR spectrum (400 MHz) of **4** in $CDCl_3$.

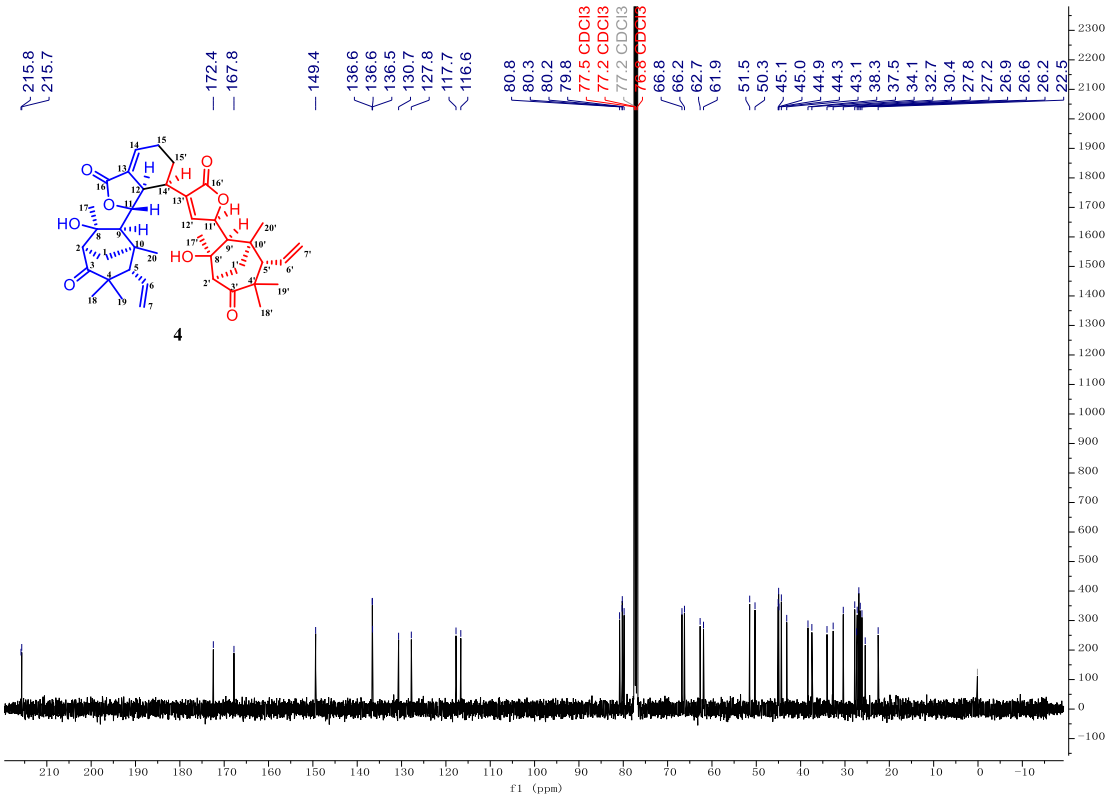


Figure S4-2. ^{13}C NMR spectrum (400 MHz) of **4** in $CDCl_3$.

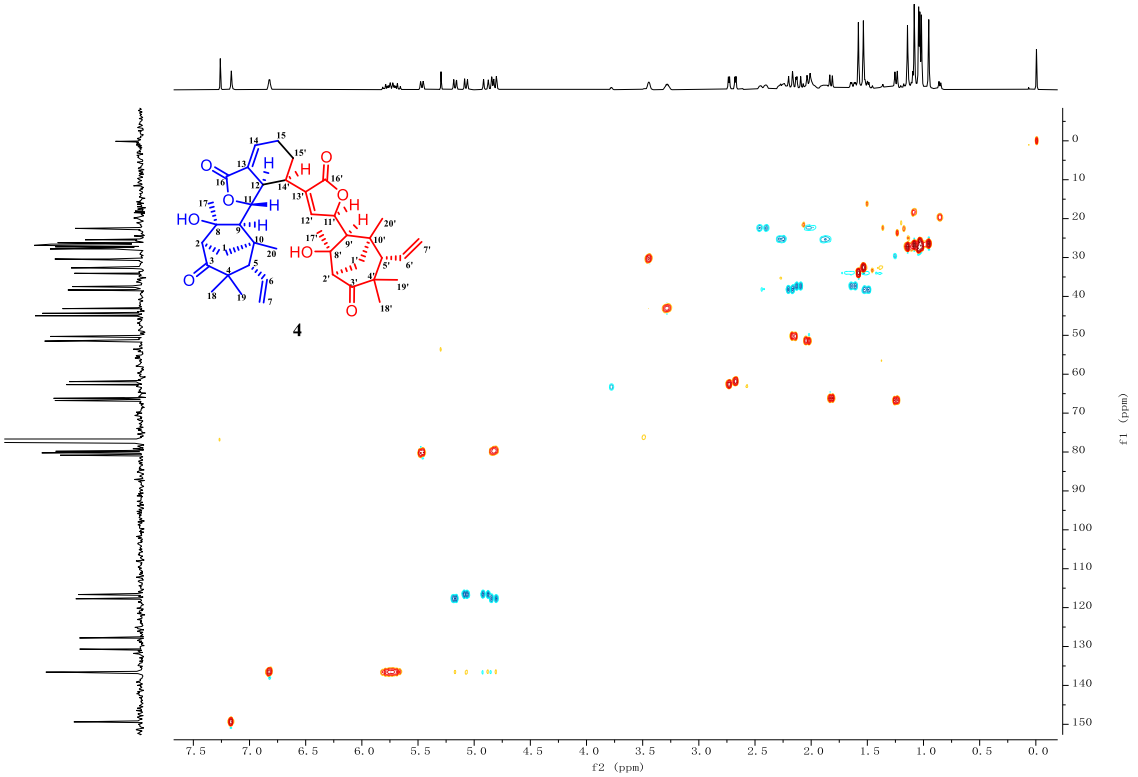


Figure S4-3. HSQC spectrum (400 MHz) of **4** in $CDCl_3$.

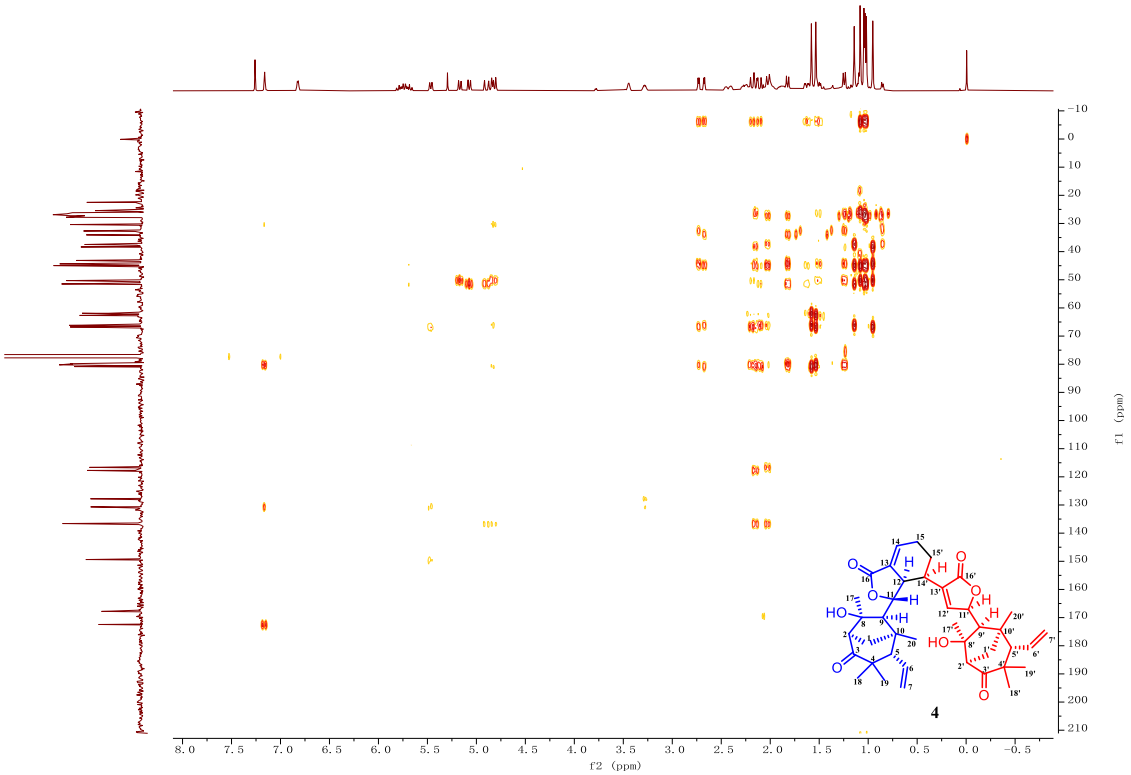


Figure S4-4. HMBC spectrum (400 MHz) of **4** in $CDCl_3$.

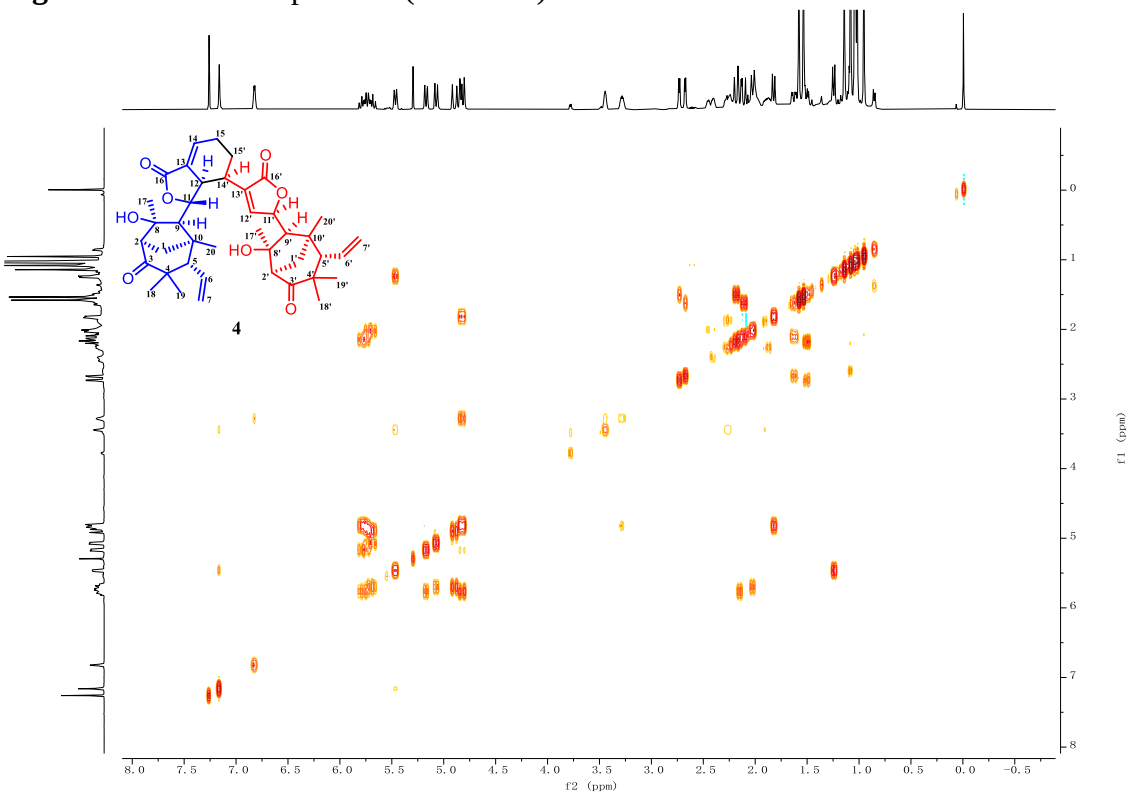


Figure S4-5. H-H COSY spectrum (400 MHz) of **4** in $CDCl_3$.

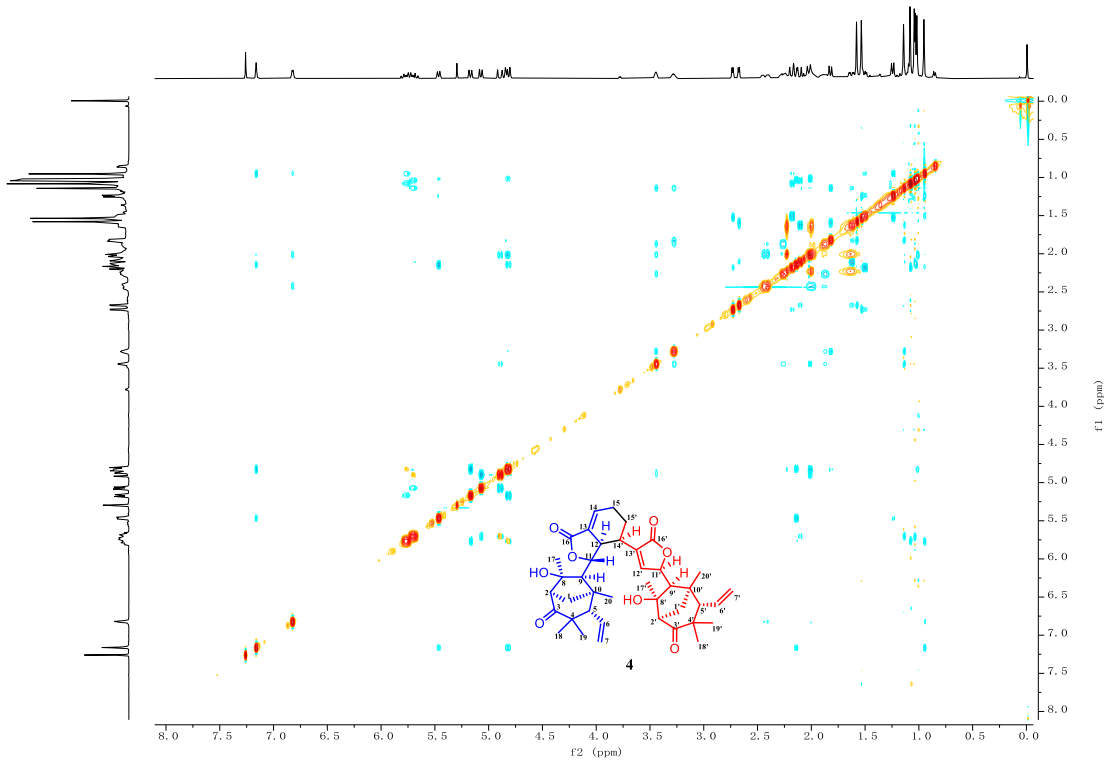


Figure S4-6. NOESY spectrum (400 MHz) of **4** in $CDCl_3$.

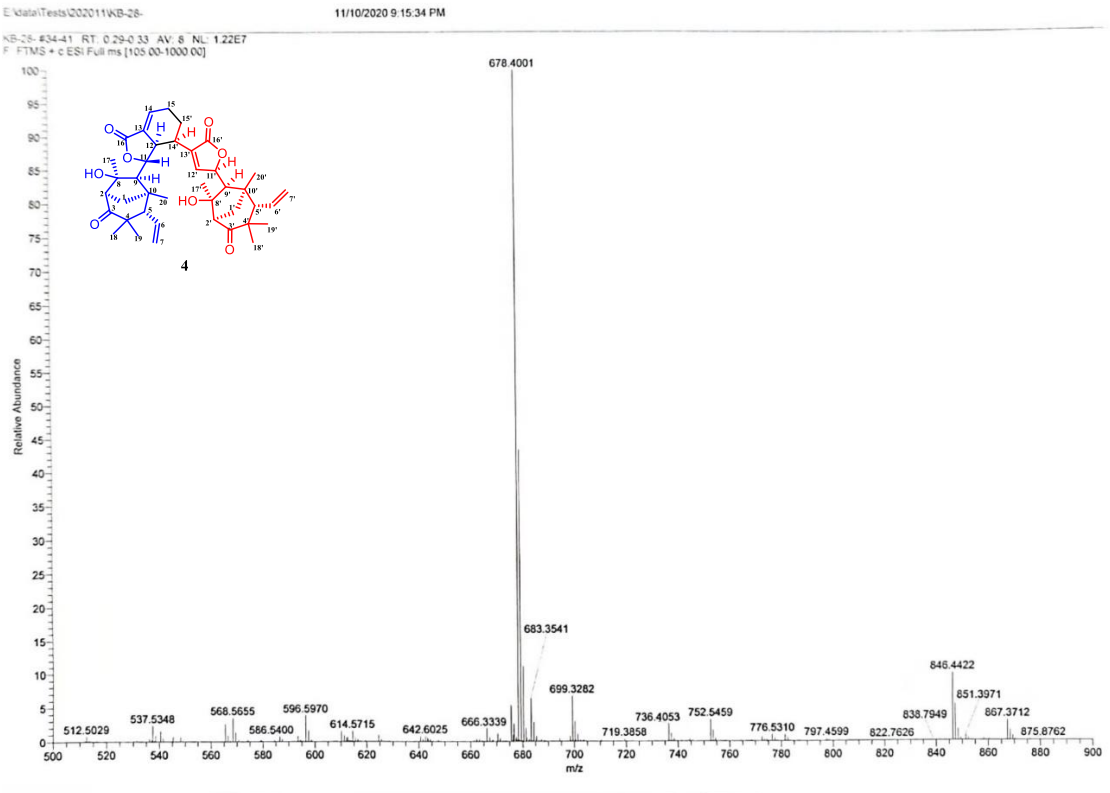


Figure S4-7. HRESIMS spectrum of **4**

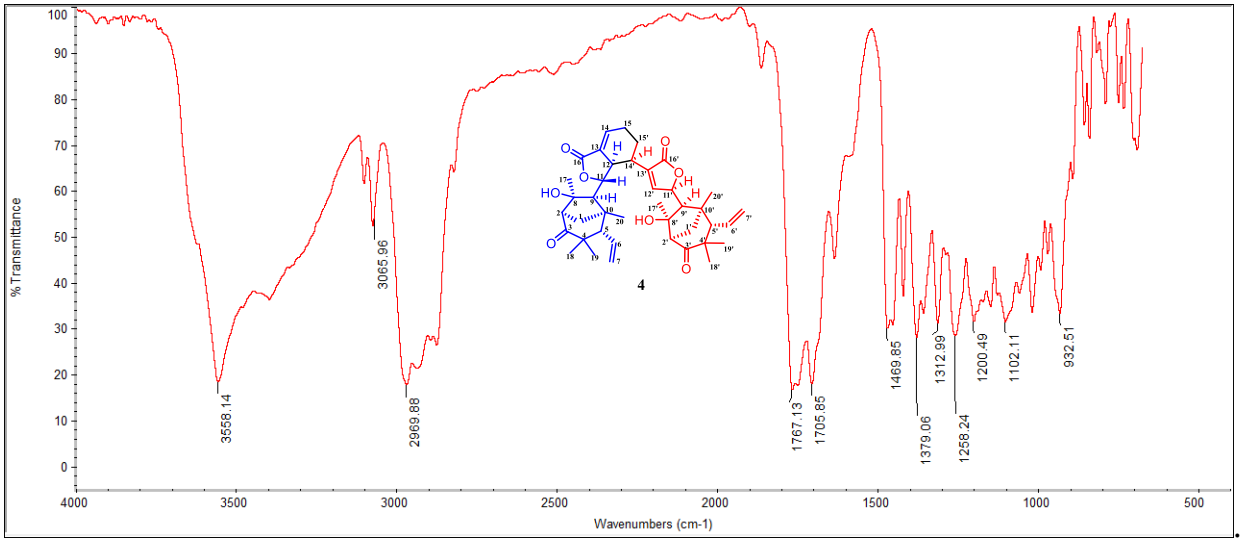


Figure S4-8. IR (KBr disc) spectrum of **4**.

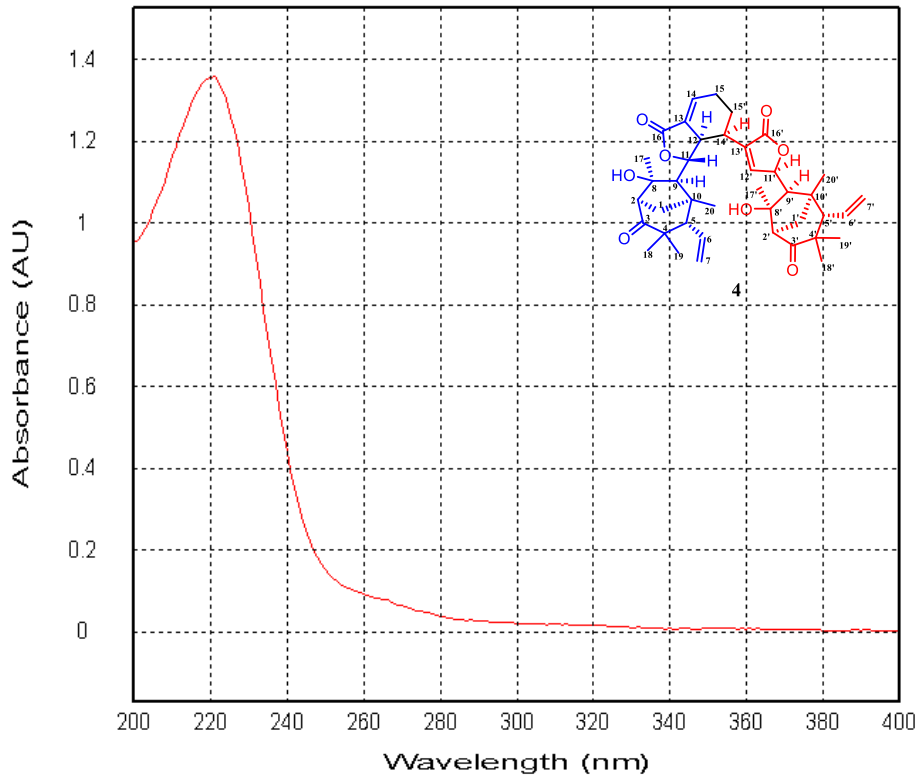


Figure S4-9. UV spectrum of **4**.

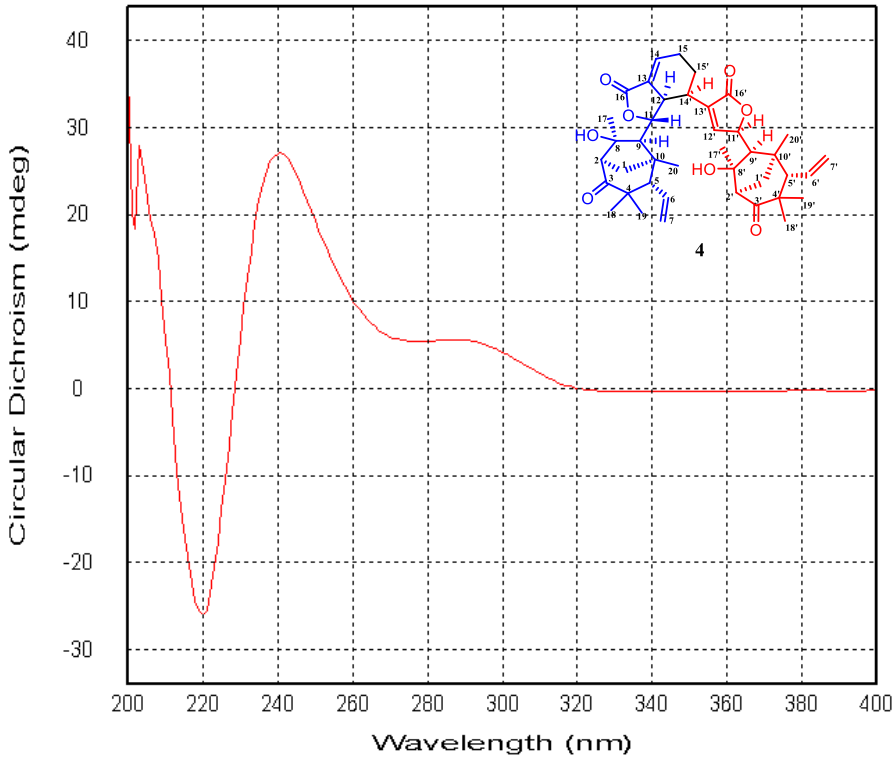
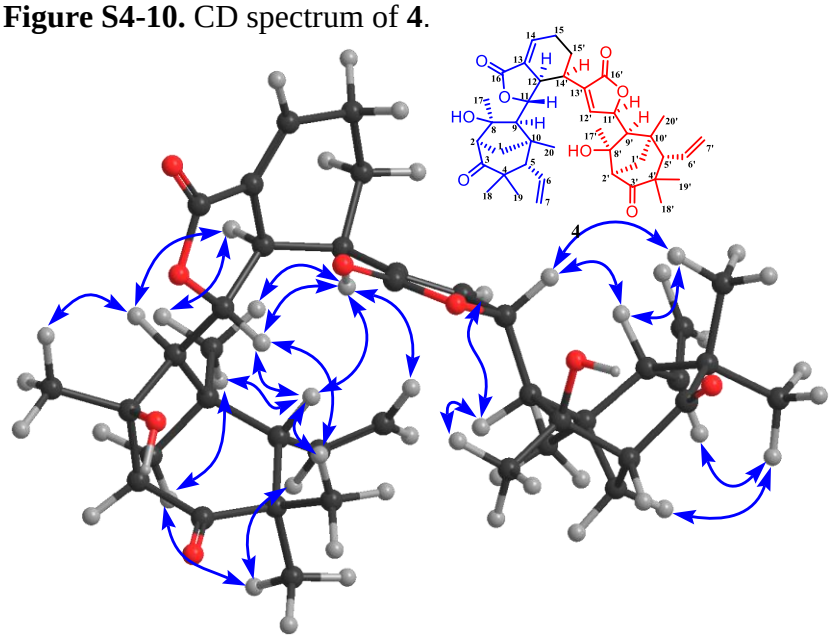


Figure S4-10. CD spectrum of **4**.



4 NOESY

Figure S4-11. NOESY signals of **4**.

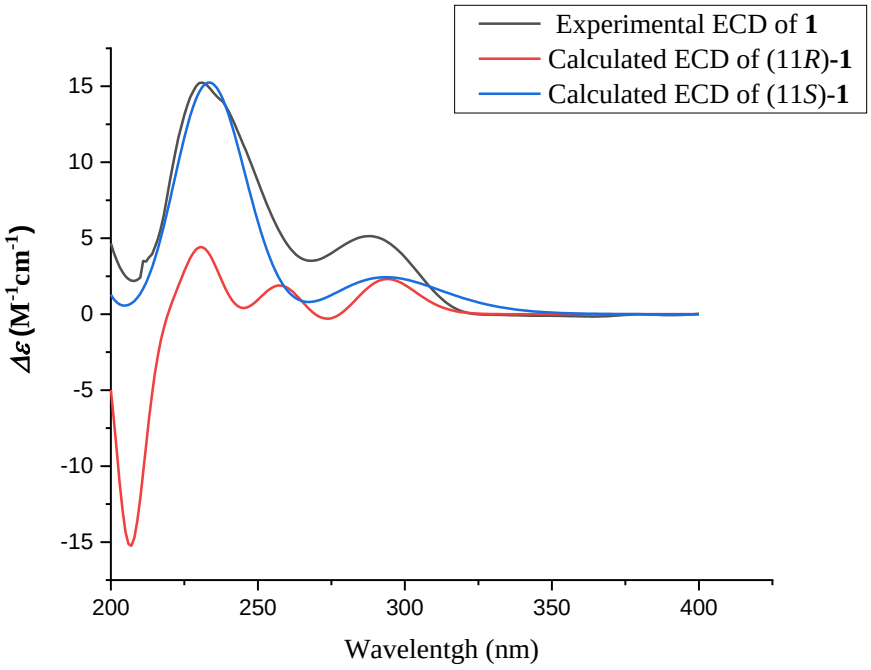


Figure S5. Experimental and calculated ECD spectra for compound **1**.

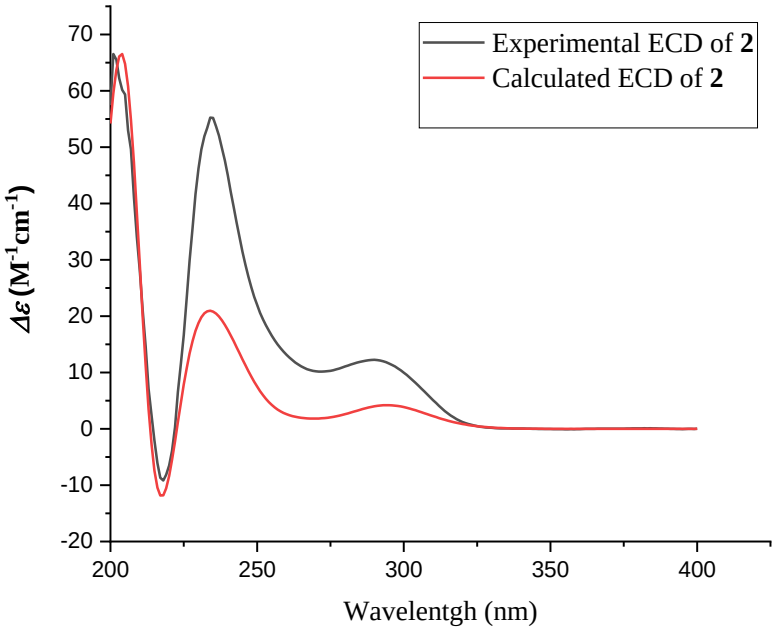


Figure S6. Experimental and calculated ECD spectra for compound **2**.

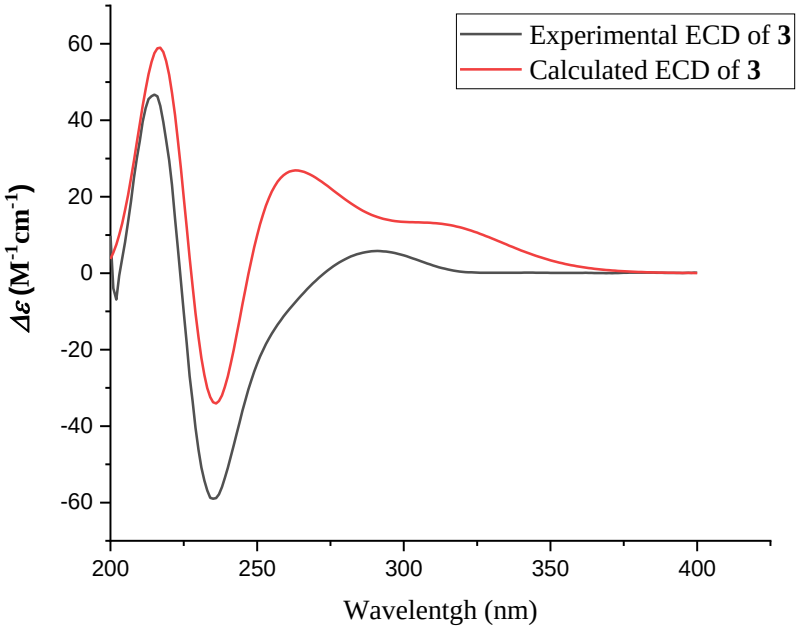


Figure S7. Experimental and calculated ECD spectra for compound **3**.

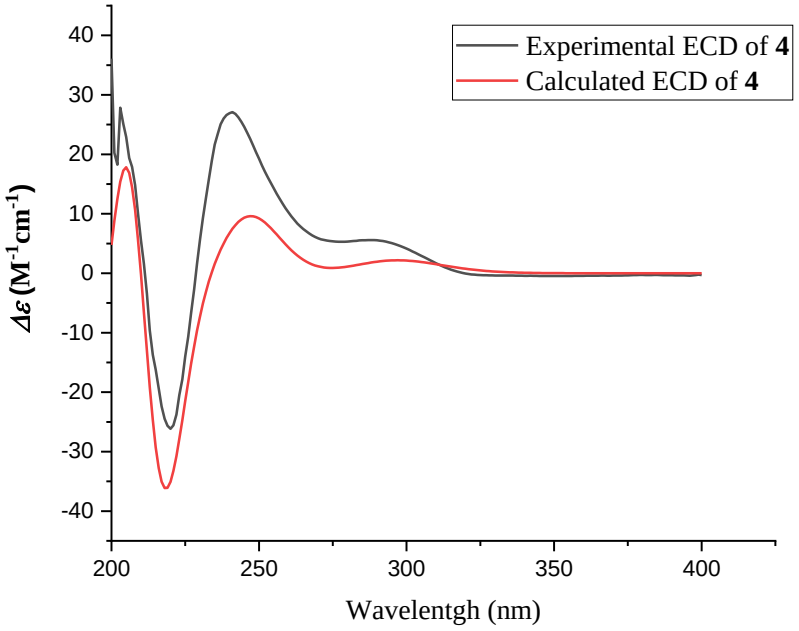


Figure S8. Experimental and calculated ECD spectra for compound **4**.