

SUPPLEMENTARY MATERIAL

Sesquiterpenes from *Curcuma wenyujin* with their inhibitory activities on nitric oxide production in RAW 264.7 cells

Suyu Gao¹, Guiyang Xia¹, Liqing Wang¹, Li Zhou¹, Feng Zhao², Jian Huang¹ and Lixia Chen^{1,*}

¹Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China.

²School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, 264005, People's Republic of China.

One new sesquiterpene, 7 α ,11-epoxy-6 α -hydroxy-carabrane-4,8-dione, along with ten known ones were isolated from the essential oil of *Curcuma wenyujin*. Y. H. Chen et C. Ling. Their structures were established based on extensive spectroscopic analysis. The absolute configuration of compound **1** was determined by the CD analysis of the insitu formed [Rh₂(OCOCF₃)₄] complex, and the CD data analysis based on the octane rule of cyclohexanone. The inhibitory effects of these sesquiterpenes on nitric oxide production in lipopolysaccharide-activated macrophages were also evaluated. Here, the biosynthesis pathway of the isolated compounds was proposed.

Keywords: *Curcuma wenyujin*; essential oil; sesquiterpenoids; nitric oxide; biosynthesis pathway

List of Supplementary material

Table S1. ^1H - and ^{13}C -NMR data for compound **1**.

Table S2. Inhibitory effects of compounds **1-11** on NO production induced by LPS in macrophages.

Figure S1. Key HMBC correlations of compound **1**.

Figure S2. Key NOESY correlations of compound **1**.

Figure S3. Assumed biogenetic of compounds **1-11**.

Figure S4. ^1H -NMR spectrum of **1**.

Figure S5. ^{13}C -NMR spectrum of **1**.

Figure S6. HMBC spectrum of **1**.

Figure S7. HSQC spectrum of **1**.

Figure S8. UV spectrum of **1**.

Figure S9. IR spectrum of **1**.

Figure S10. HR-ESI-MS spectrum of **1**.

Figure S11. NOESY spectrum of **1**.

Figure S12. ECD spectrum of **1**.

Figure S13. $\text{Rh}_2(\text{OCOCF}_3)_4$ -ICD spectrum of **1**.

Figure S14. Inhibitory effect of γ -elemenolides on NO production induced by LPS in macrophages.

Figure S15. Analysis based on the octane rule of cyclohexanone of **1**.

Table S1. ^1H and ^{13}C -NMR data for compound **1**^a.

NO.	1	
	δc	δH (<i>J</i> in Hz)
1	25.0	0.66, ddd (8.4, 5.6, 5.1)
2	22.5	1.44, m 1.66, m
3	42.7	2.56, t (7.2)
4	208.4	
5	31.8	0.98, dd (5.1, 4.1)
6	70.3	4.09, d (4.1)
7	69.1	
8	205.2	
9	47.1	2.58, d (20.0) 2.60, d (20.0)
10	17.8	
11	64.2	
12	19.3	1.29, s
13	20.4	1.02, s
14	19.1	1.12, s
15	29.7	2.08, s

a: ^1H -NMR spectra measured at 600 MHz, ^{13}C -NMR spectra measured at 125 MHz; spectrum of compound **1** was obtained in DMSO-*d*₆.

Table S2. Inhibitory effects of compounds **1-11** on NO production induced by LPS in macrophages.

Compound	IC ₅₀ ^a (mean ± SD) / μM
1	53.35±3.47
2	14.99±1.21
3	>100
4	59.06±3.26
5	98.48±7.09
6	81.35±6.68
7	80.76±4.89
8	>100
9	23.28±1.47
10	45.49±2.96
11	51.63±4.52
Hydrocortisone ^b	64.34±7.49

a: Inhibitory effects compounds **1-11** on NO production induced by LPS in RAW 264.7 macrophages.

b: Positive control.

Figure S1. Key HMBC correlations of compound **1**.

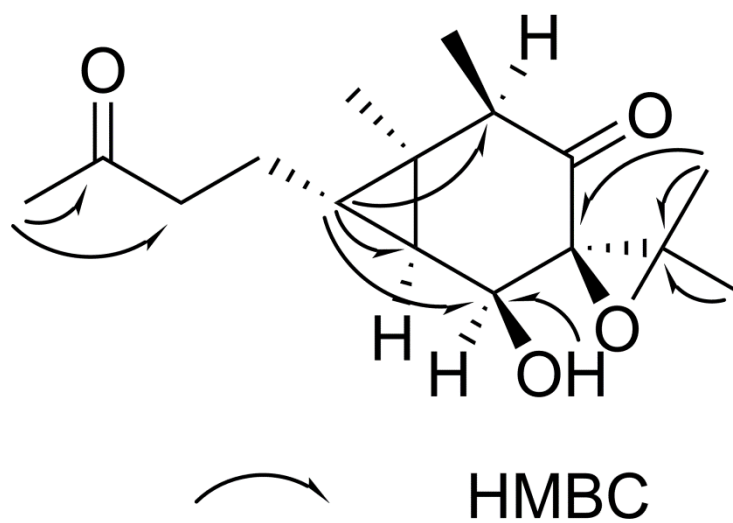


Figure S2. Key NOESY correlations of compound **1**.

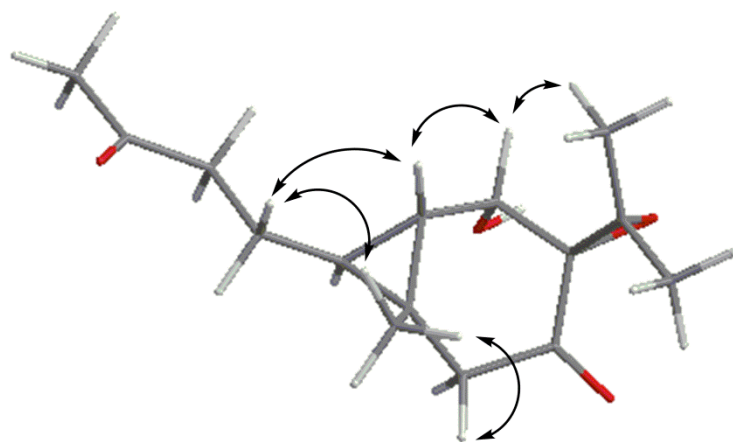


Figure S3. Assumed biogenetic of compounds **1-11**.

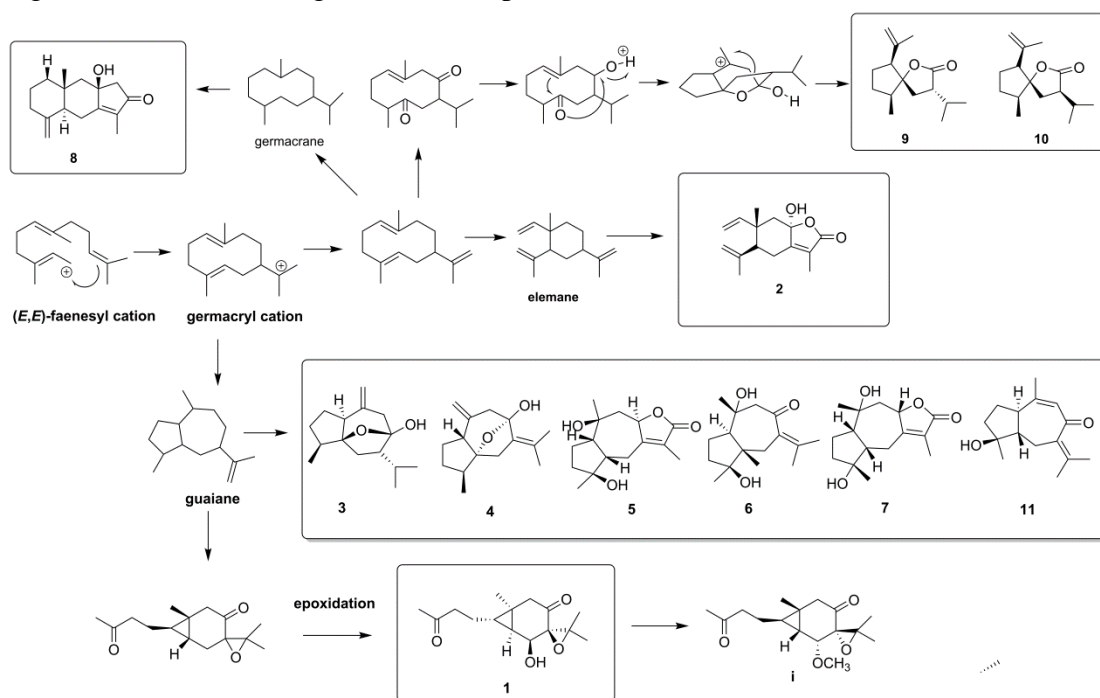


Figure S4. $^1\text{H-NMR}$ spectrum of **1**.

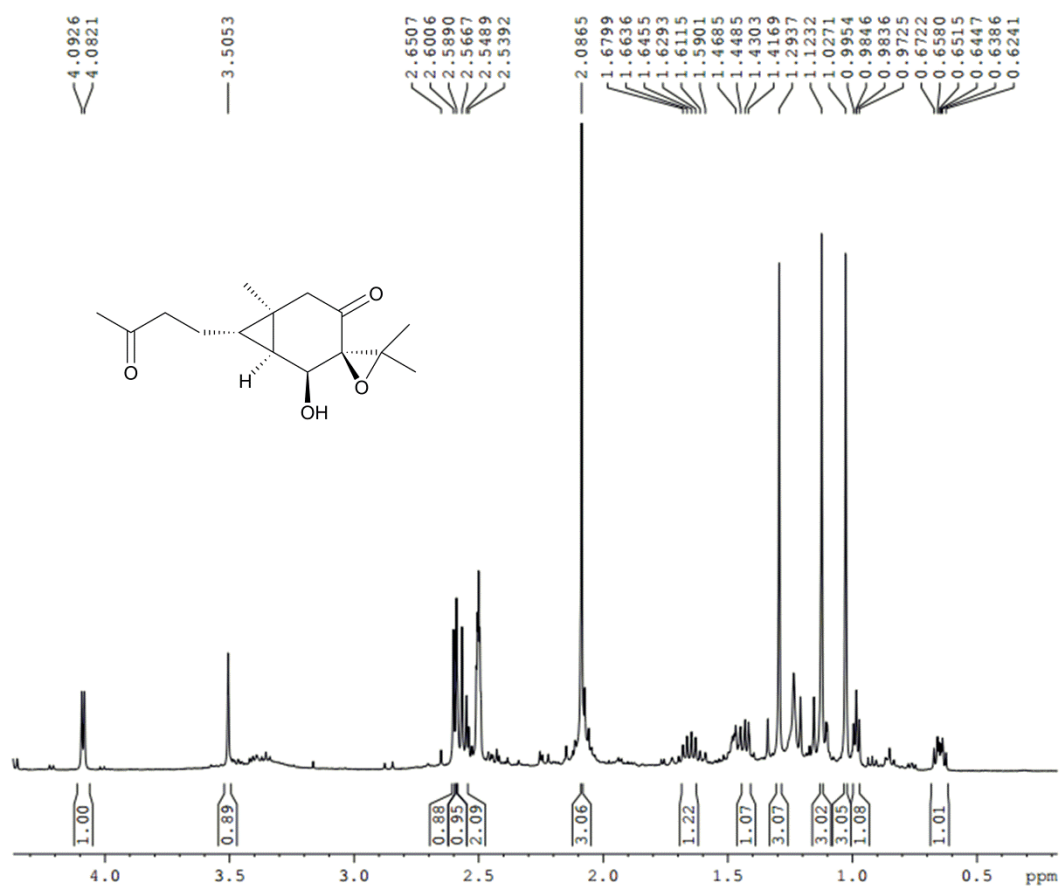


Figure S5. ^{13}C -NMR spectrum of **1**.

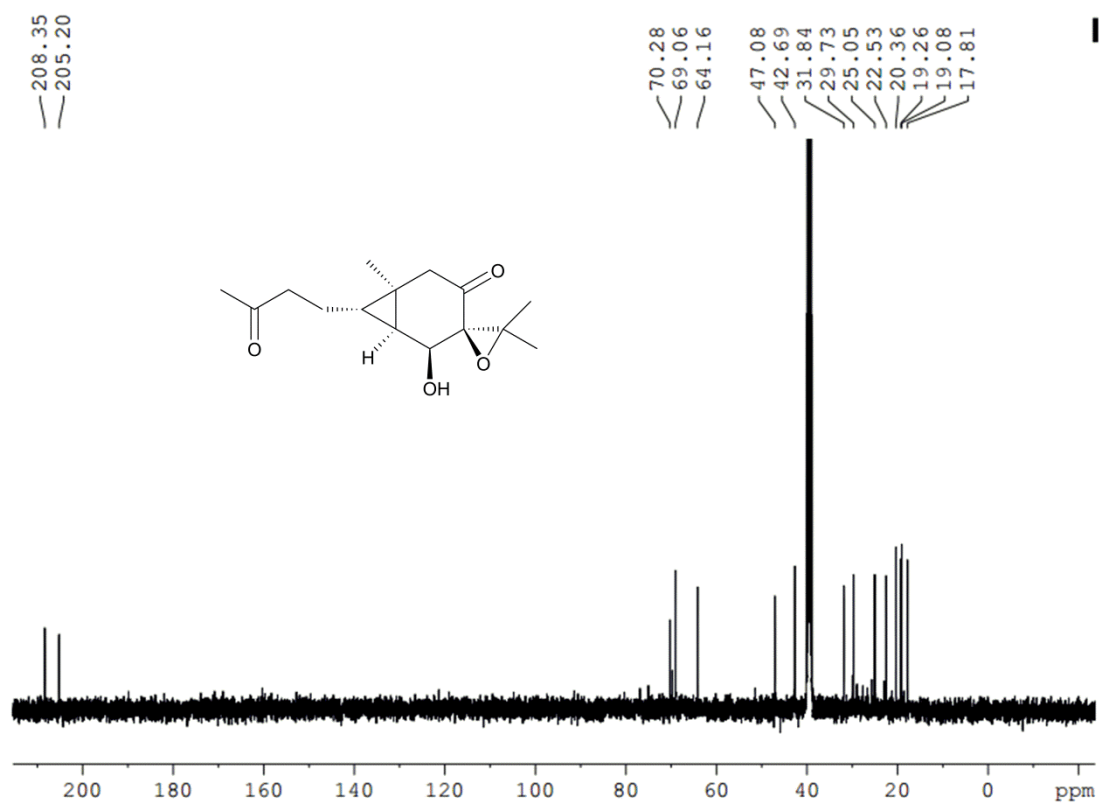


Figure S6. HMBC spectrum of **1**.

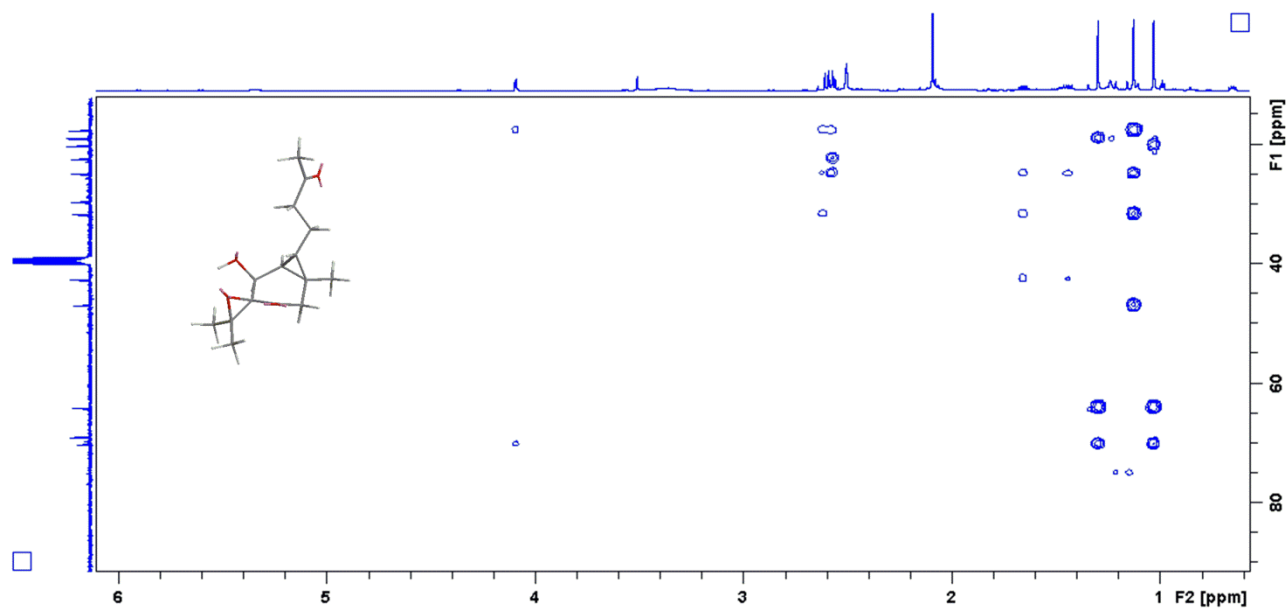


Figure S7. HSQC spectrum of **1**.

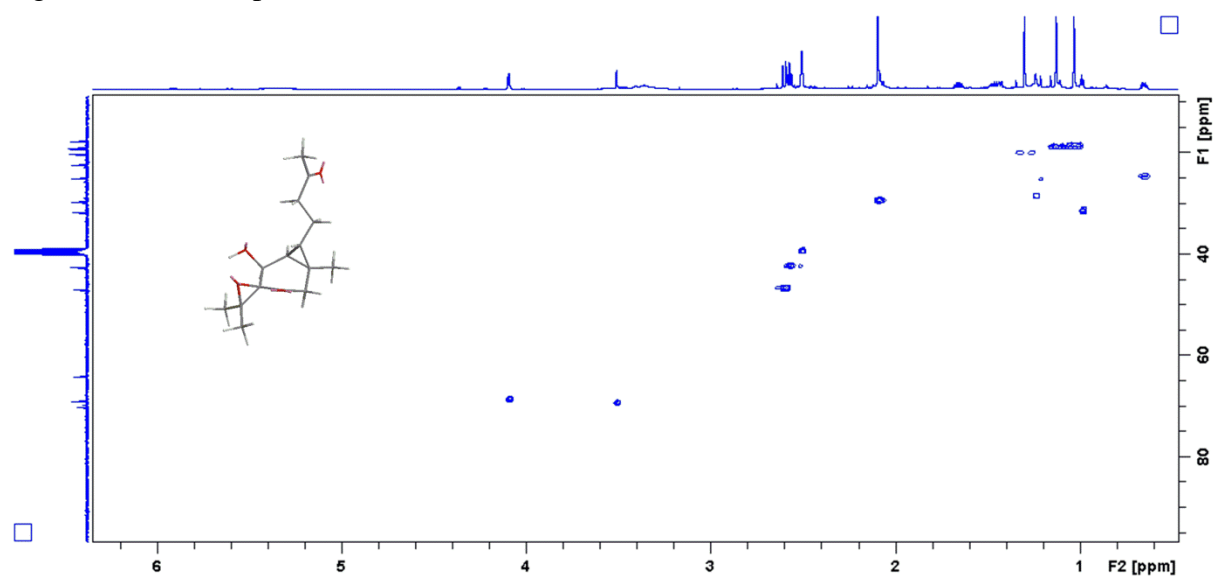
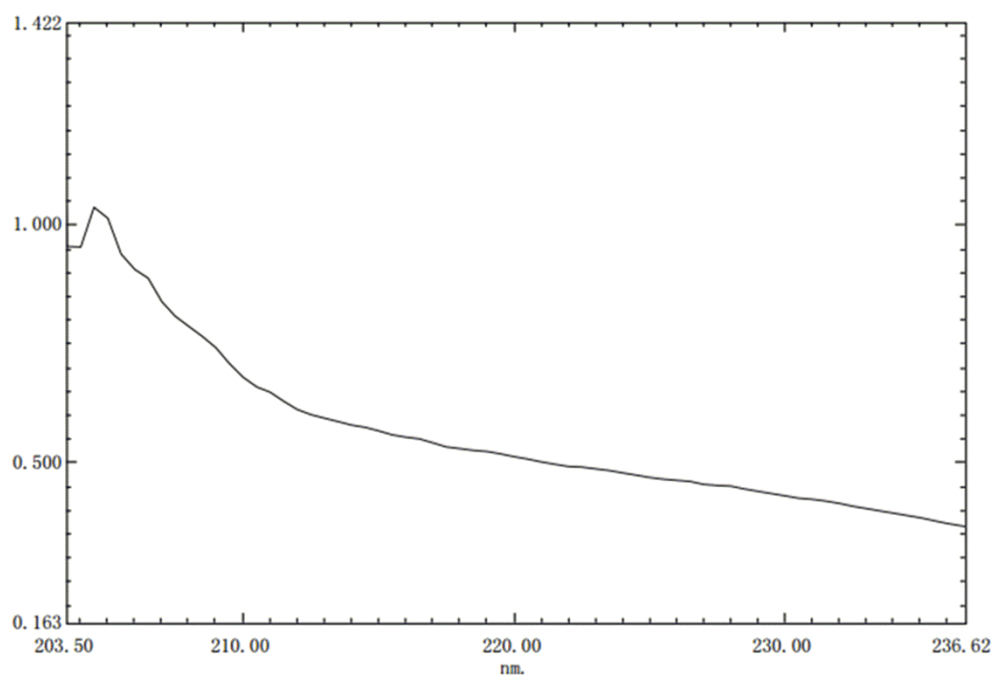


Figure S9. UV spectrum of **1**.



No.	Wavelength	吸收值	描述
1	204.50	1.035	
2	211.00	.646	
3	216.00	.552	
4			

Figure S10. IR spectrum of 1.

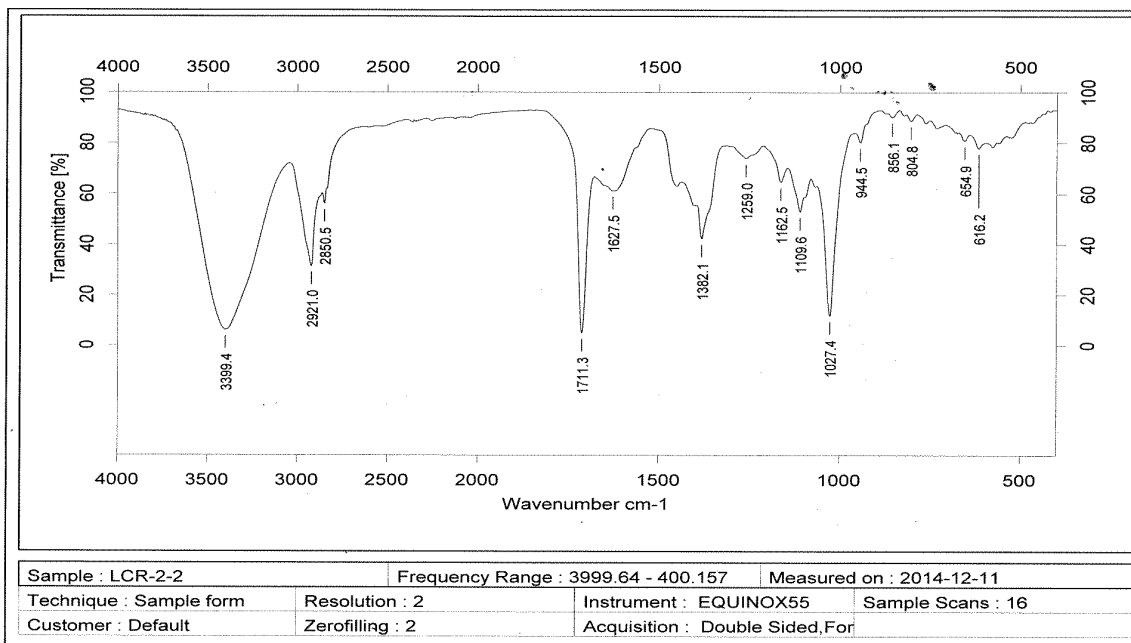


Figure S11. HR-ESI-MS spectrum of 1.

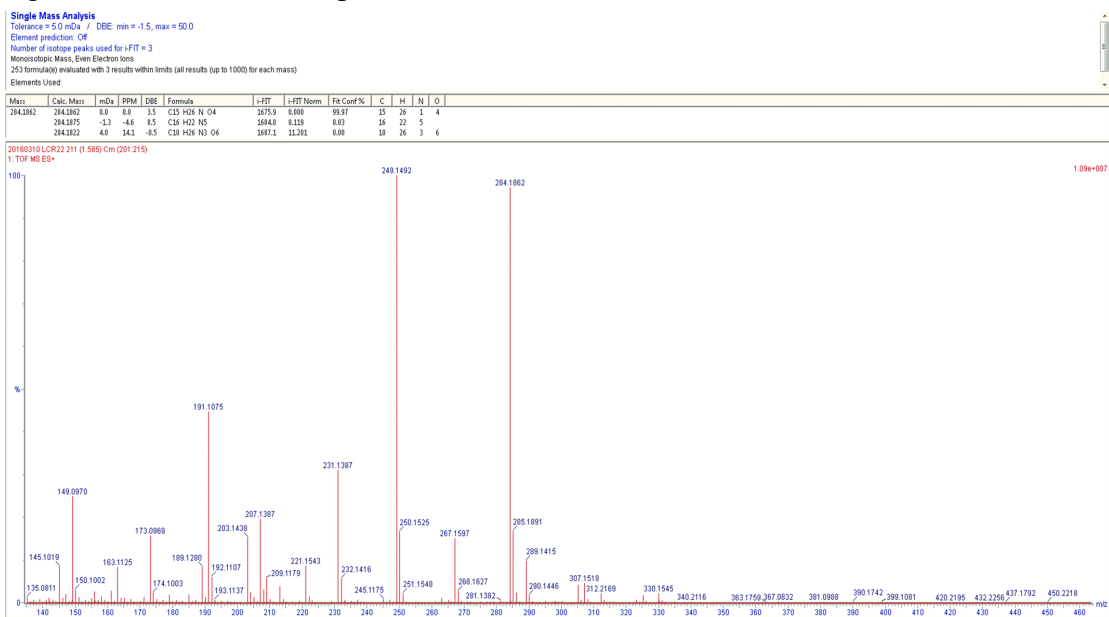


Figure S12. NOESY spectrum of **1**.

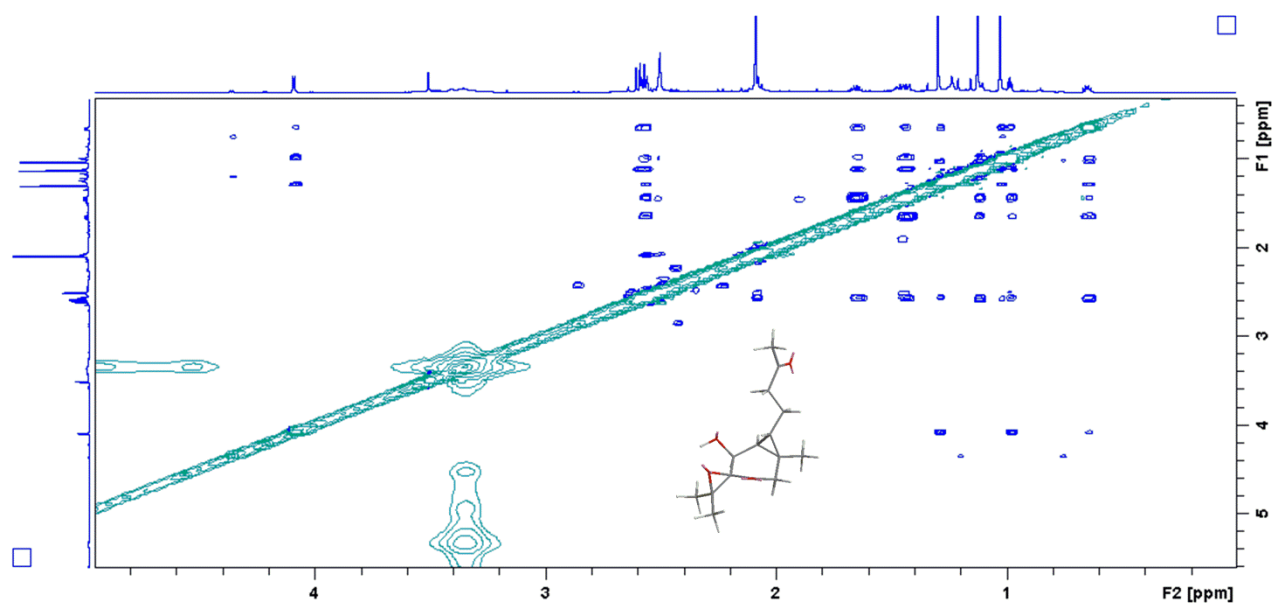


Figure S13. ECD spectrum of **1**.

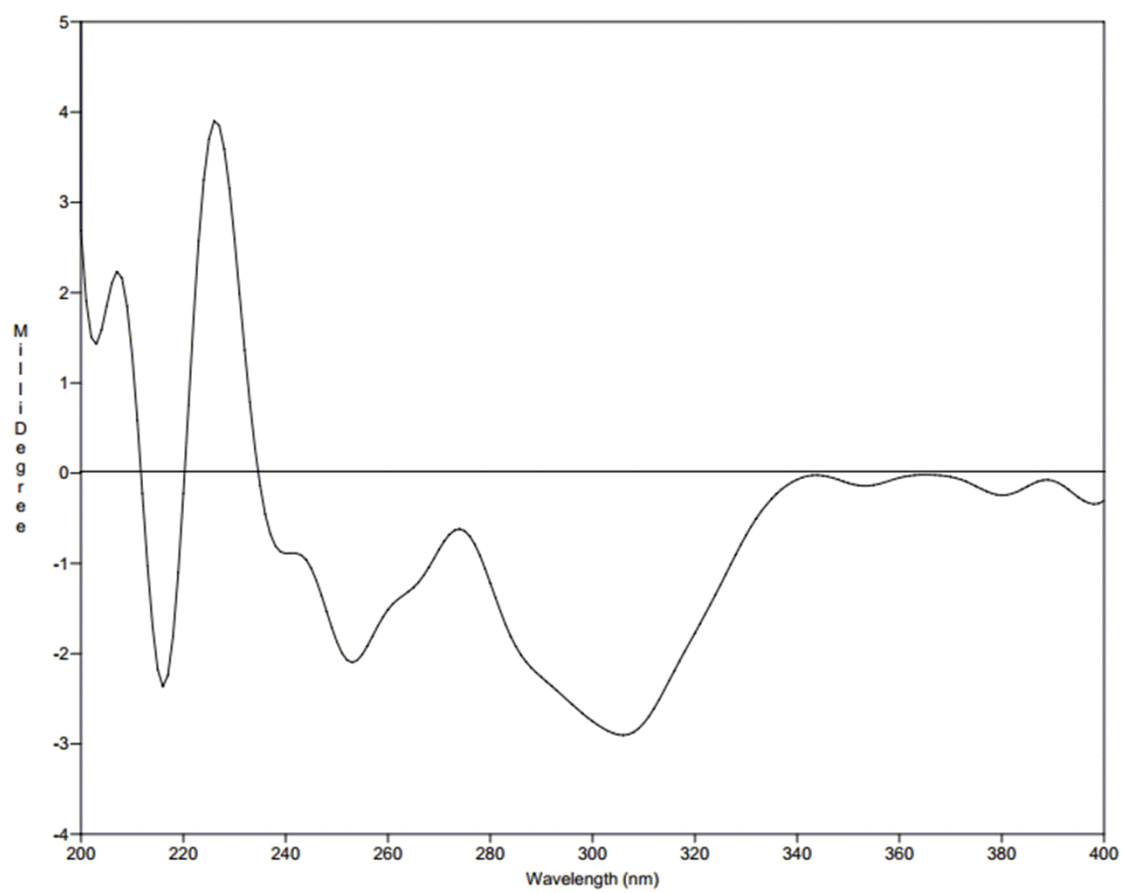


Figure S14. $\text{Rh}_2(\text{OCOCF}_3)_4$ -ICD spectrum of **1**.

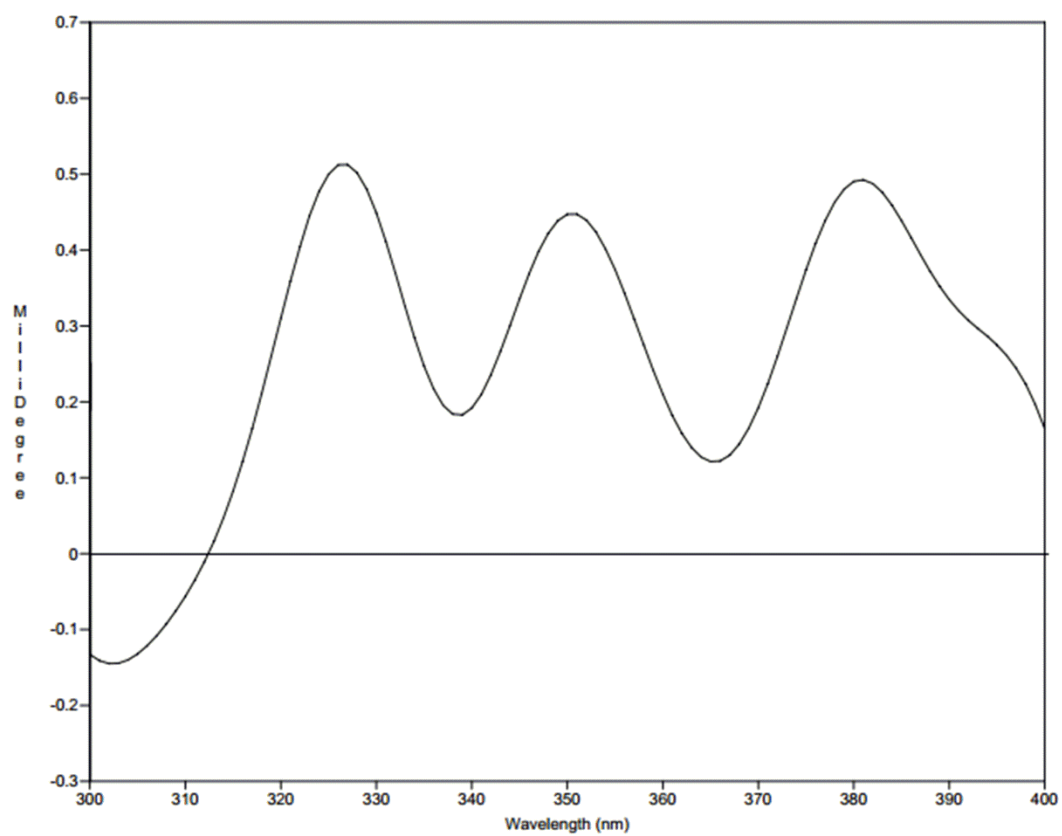
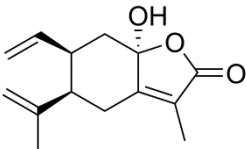
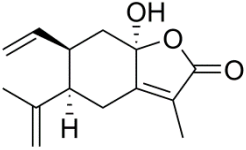
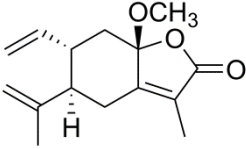


Figure S15. Inhibitory effect of γ -elemenolides on NO production induced by LPS in macrophages.

NO.	Structure	IC ₅₀ ^d (mean \pm SD) / μ M
1 ^a		14.99 \pm 1.21
2 ^b		26.0
3 ^c		69.98 \pm 6.21

a: compound 2 which were isolated from *Curcuma wenyujin*.

b: compound 9 which were isolated from *Curcuma wenyujin*. (Lou et al. 2010)

c compound 6 which were isolated from *Curcuma phaeocaulis*. (Ma et al. 2015)

d: Inhibitory effects compounds on NO production induced by LPS in RAW 264.7 macrophages.

Figure S16. Analysis based on the octane rule of cyclohexanone of **1**.

