

Supplementary materials

Halorotetin A: A Novel Terpenoid Compound Isolated from Ascidian *Halocynthia rotetzi* Exhibits the Inhibition Activity on Tumor Cell Proliferation

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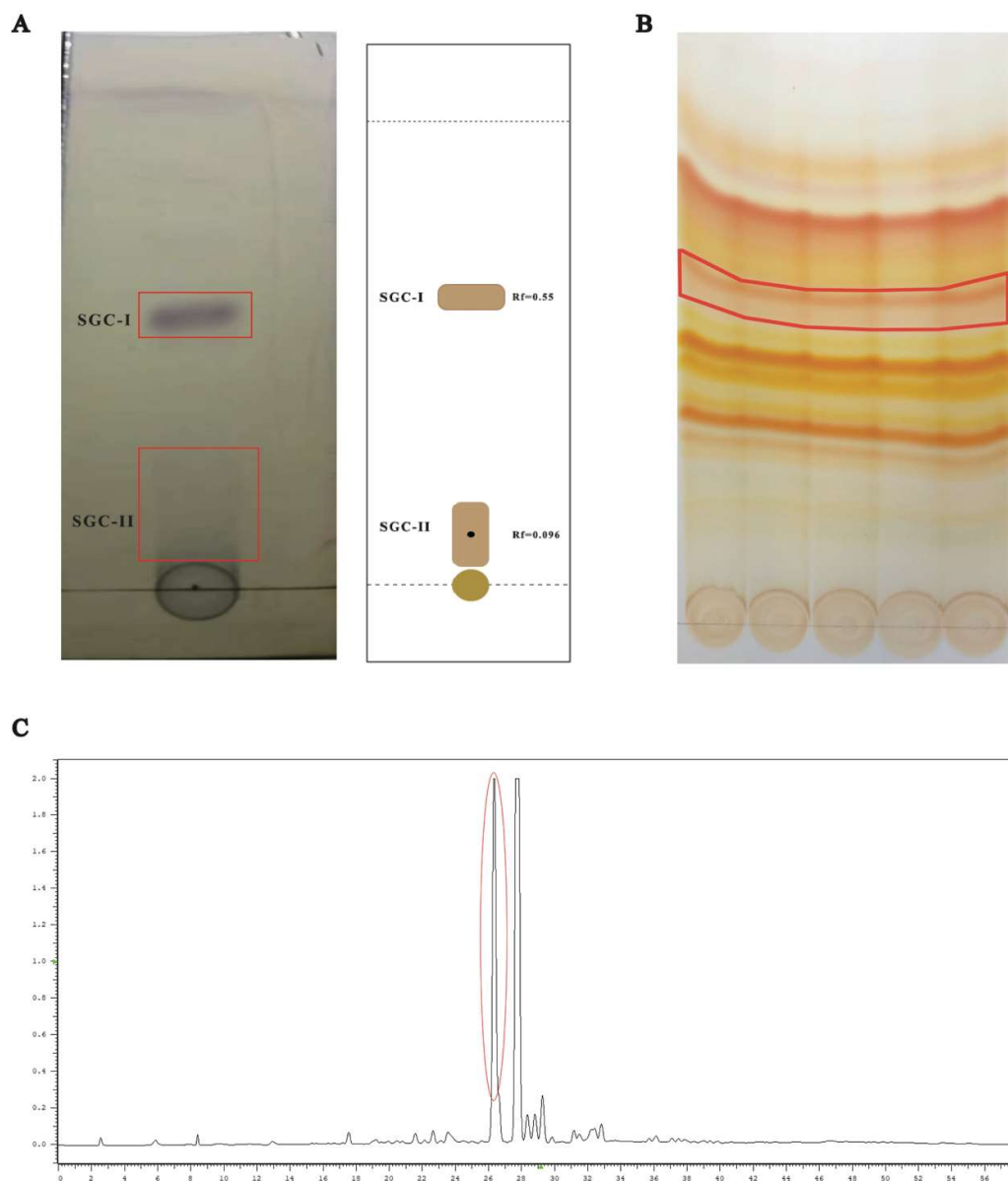


Figure S1. Isolation and activity verification of Halorotetin A. **(A)** The two parts of TE obtained by silica gel column chromatography, the SGC-II was the active part. **(B)** The different parts of SGC-II obtained by PLC separation, and the PCL-VI was exhibited in the red circle. **(C)** The different parts of PLC-VI obtained by semipreparative-HPLC, and the Halorotetin A was exhibited in the red circle.

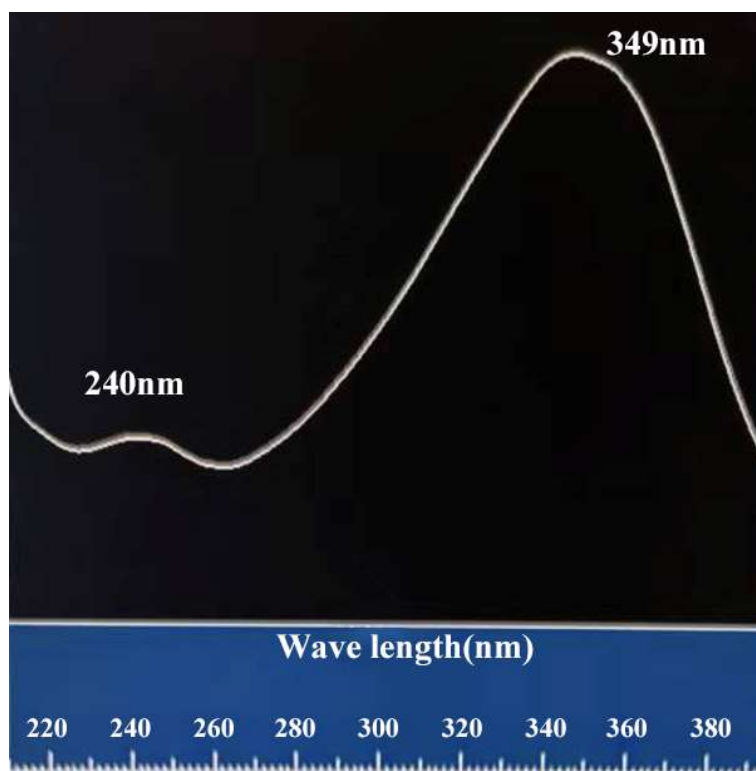


Figure S2. UV curve of Halorotetin A in Methanol

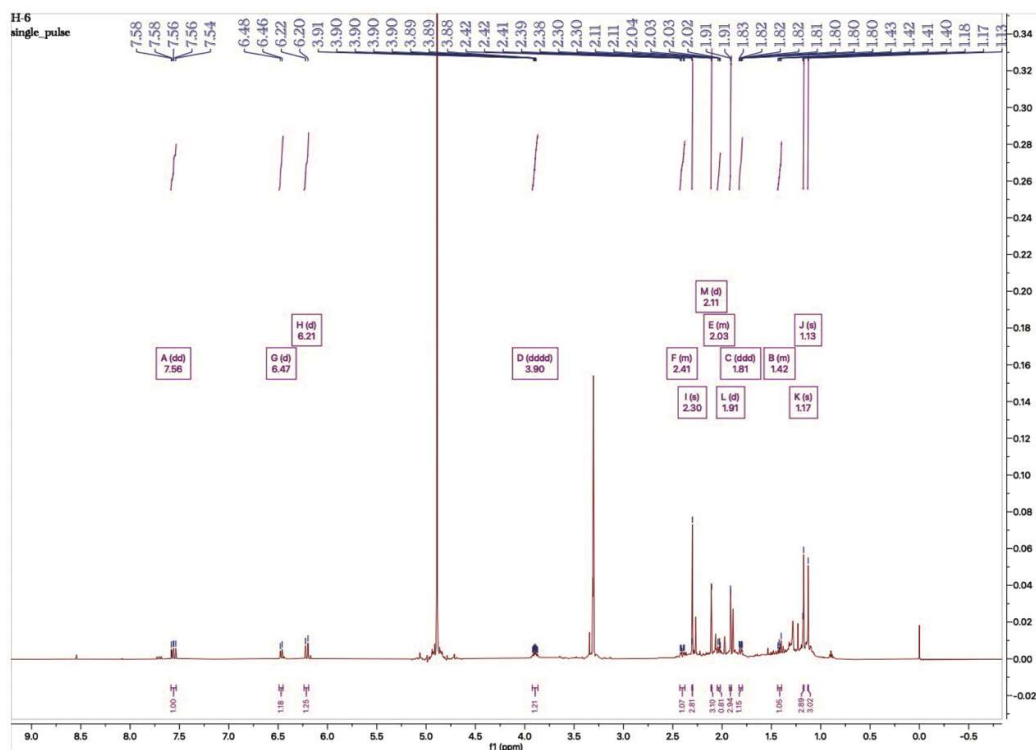


Figure S3. ^1H NMR spectrum of Halorotetin A in Methanol- d_4

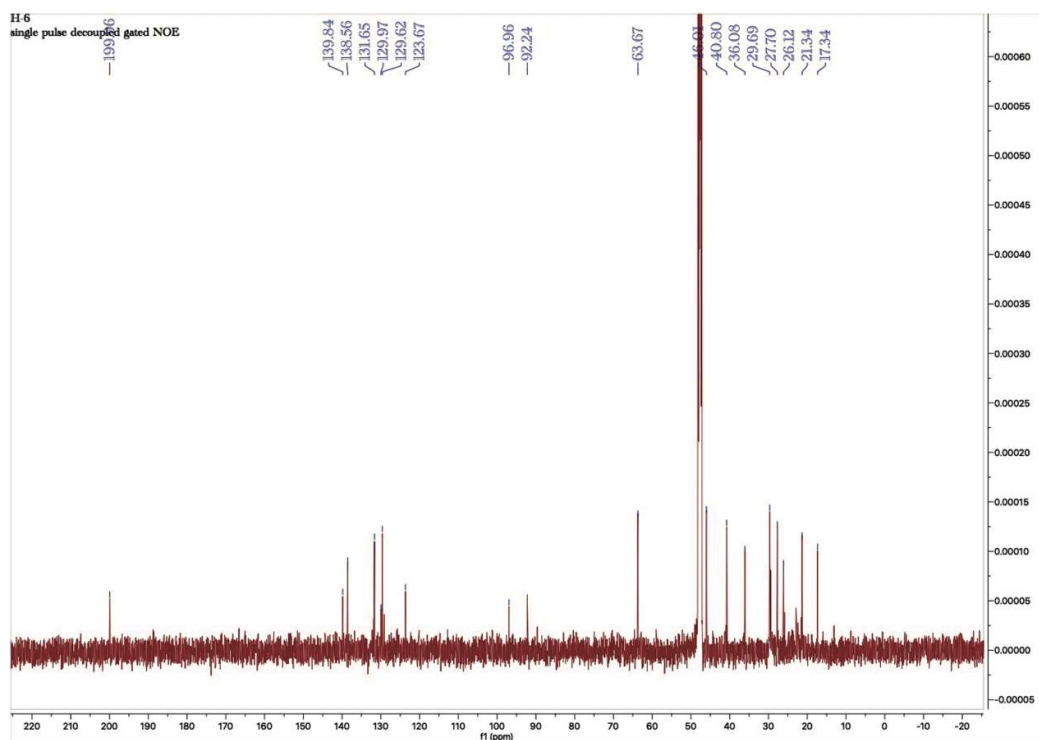


Figure S4. ^{13}C NMR spectrum of Halorotetin A in Methanol- d_4

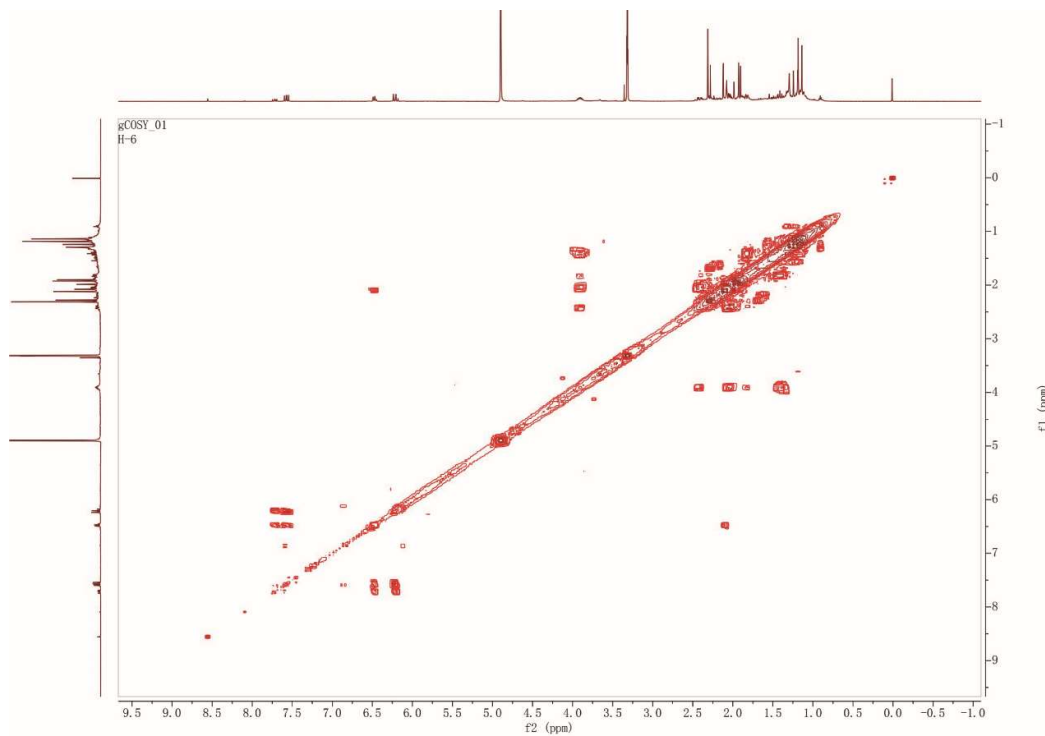


Figure S5. ^1H - ^1H COSY spectrum of Halorotetin A in Methanol- d_4

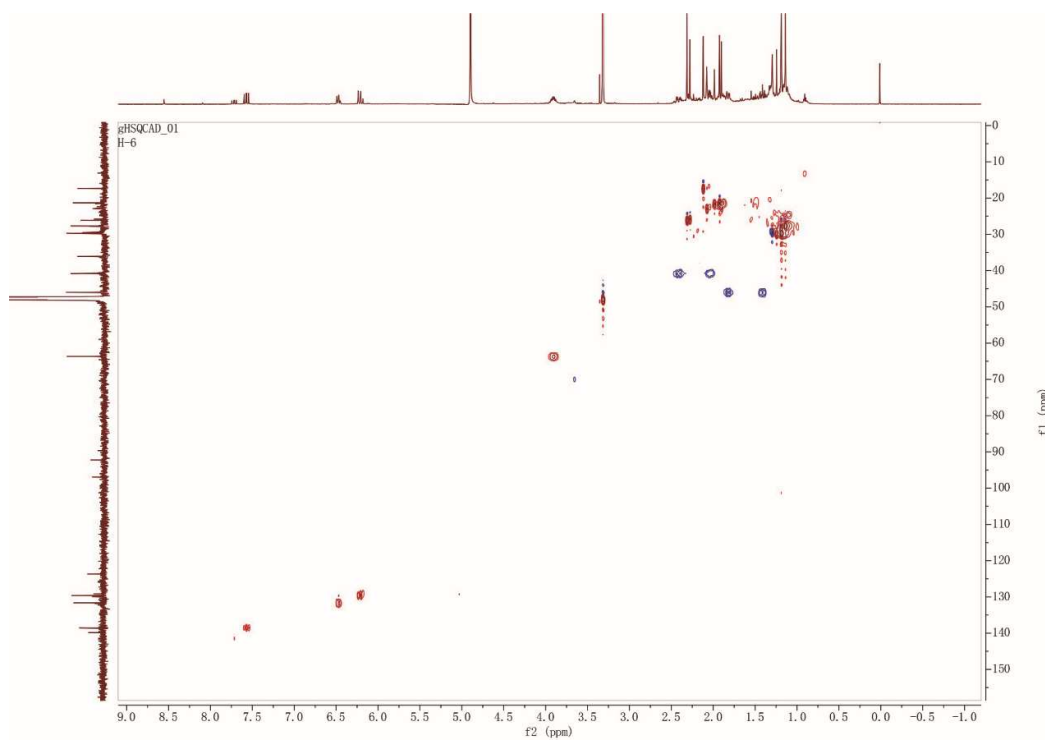


Figure S6. HSQC spectrum of Halorotetin A in Methanol- d_4

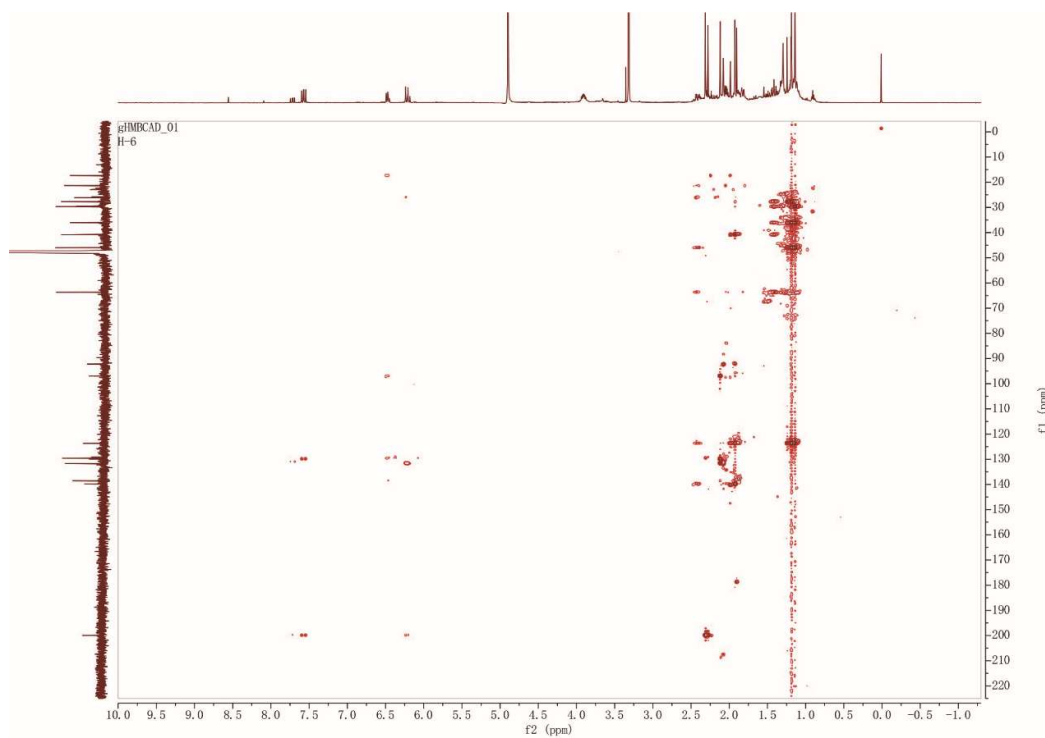


Figure S7. HMBC spectrum of Halorotetin A in Methanol- d_4

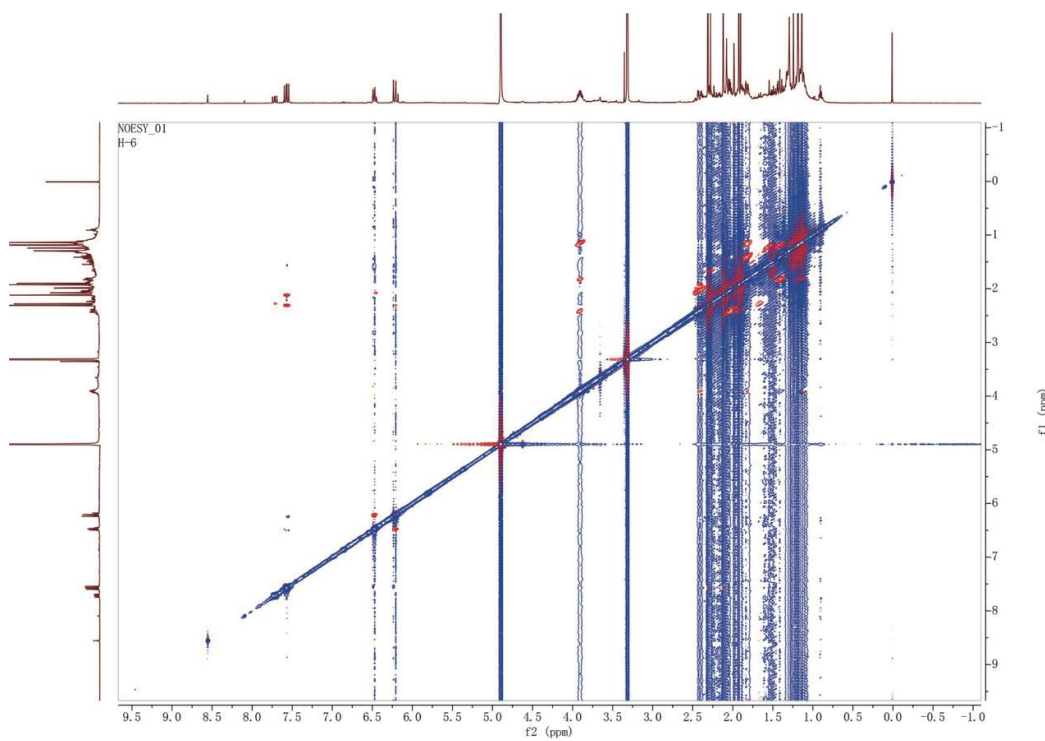


Figure S8. NOESY spectrum of Halorotetin A in Methanol- d_4

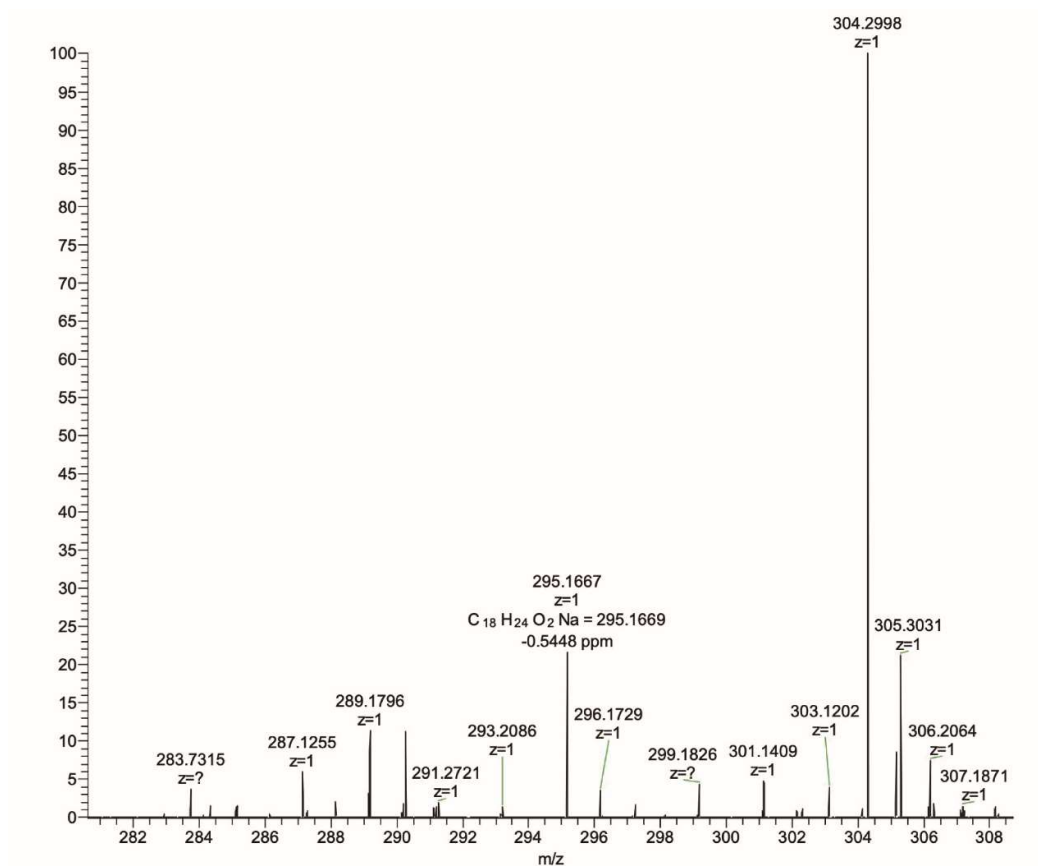


Figure S9. HRESIMS $[M+Na]^+$ spectrum of Halorotetin A

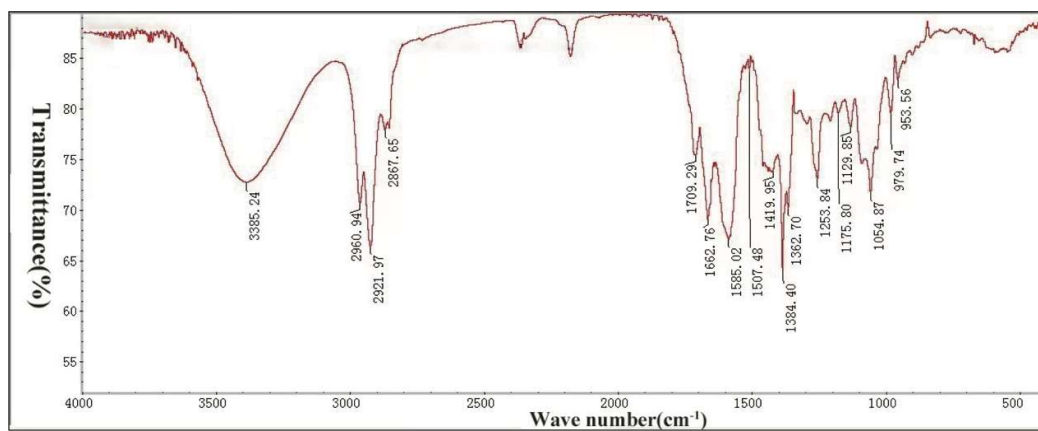


Figure S10. Infrared absorption (IR) spectrum of Halorotetin A

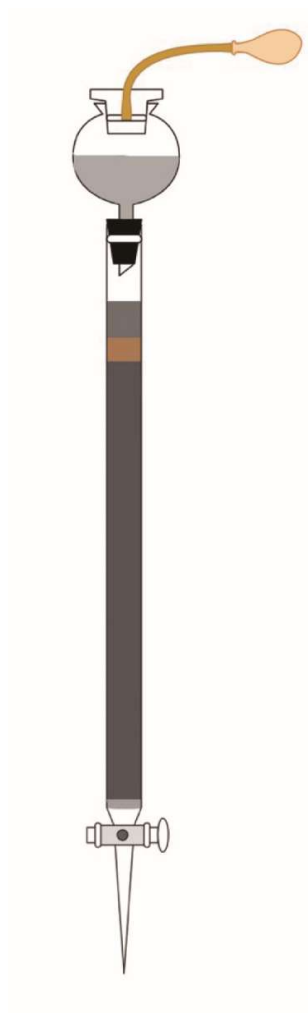


Figure S11. Schematic representation of the silica gel column (24mm×600mm). From top to bottom, the 60-80 mesh silica gel layer, the sample layer, and the 300-400 mesh silica gel layer, respectively.

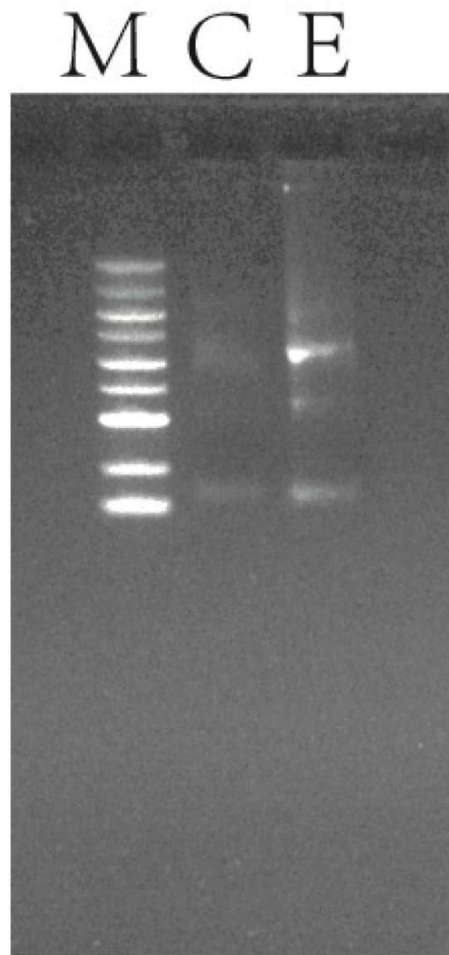


Figure S12. RNA electrophoresis pattern. The RNA was extracted from HepG-2 cells in the control group and the experimental group. RNA quality was determined by 1% agarose gel electrophoresis. M represents Maker, C represents RNA from the control group, and E represents RNA from the experimental group.

Table S1. The protocol of HPLC for Halorotetin A

Time (min)	A Pump (Acetonitrile)	B Pump (Water)	Flow Velocity
0.0	20	80	2ml/min
10	50	50	
25	70	30	
40	100	0	
55	100	0	
60	100	0	

Table S2. Primers used for qRT-PCR analysis

P1K3CA	Forward: TGTTACTCAAGAAGCAGAA Reverse: GCCTACTGGTTCAATTACT
PTEN	Forward: ACGAACTGGTGTAAATGATAT Reverse: TGTCTCTGGTCCTTACTT
AXIN	Forward: CCTATCCCTCCTTCCTTA Reverse: GGCAGGTATCCAGATATG
ARID1A	Forward: AAGGAGATTGGTGGATTG Reverse: GACACTGGATATACTGCTT
C-Myc	Forward: AACAGAAGATGAGGAAGAA Reverse: AGAAGGTGATCCAGACTC
C-Met	Forward: TCAGCGATCTTCTCTACT Reverse: CCTCATCATCAGCGTTAT
TP53	Forward: CGTGTGGAGTATTTGGAT Reverse: ATGTAGTTGTAGTGGATGG
KEAP1	Forward: GACAAACCGCCTTAATTC Reverse: CAGCAGCATAGATACAGT
ARID2	Forward: ATGTATCTCCTGCTCCTT Reverse: CCACAGTATGAACACCTT