Electronic Supplementary Material (ESI) for RSC Advances.
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Supporting Information for

Effect of Borrelidin on Hepatocellular Carcinoma Cells *in vitro* and *in vivo*

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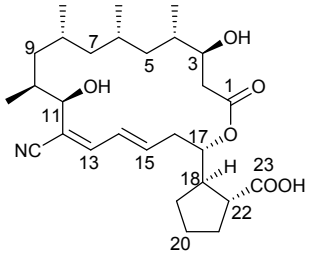
The structure identification of borrelidin



The compound was isolated as a white amorphous powder and detected as > 98.0% purity by HPLC analysis. Its ESI-MS gave pseuo molecular ion peak at *m/z* 490 $[M+H]^+$, 512 $[M+Na]^+$ suggested the molecular weight is 489. The molecular formula was speculated as $C_{28}H_{44}NO_6$ according to the molecular weight and the ^{13}C NMR data. The ^1H NMR data revealed three olefinic protons at δ_H 6.95 (1H, d, J = 11.4 Hz), 6.46 (1H, brt, J = 13.0 Hz), 6.31 (1H, ddd, J = 14.7, 10.5, 4.3 Hz), three oxygenated methine protons at δ_H 4.88 (1H, dt, J = 10.7, 3.6 Hz), 4.06 (1H, d, J = 9.6 Hz), 3.75 (1H, dt, J = 9.8, 3.0 Hz), four doublet methyls at δ_H 0.92 (3H, d, J = 6.4 Hz), 0.78 (3H, d, J = 6.4 Hz, CH_3), 0.77 (3H, d, J = 6.4 Hz, CH_3), 0.74 (3H, d, J = 7.2 Hz, CH_3). Furthermore, 22 aliphatic protons belonging to methylenes or methines were found in the upfield region. The ^{13}C NMR spectrum showed all 28 signals, including two carbonyl carbons at δ_C 177.7, 171.3, four olefinic carbons at δ_C 143.6, 139.2, 127.8, 119.5, three oxygenated methines at δ_C 75.3, 71.0, 70.6, and 18 aliphatic carbons presented between δ_C 48.5~15.3, in addition, a cyano carbon was found at δ_C 116.8. The structure of the isolated compound was determined as borrelidin by analysis the MS, ^1H NMR, and ^{13}C NMR data and these are almost identical with those reported in the literature.

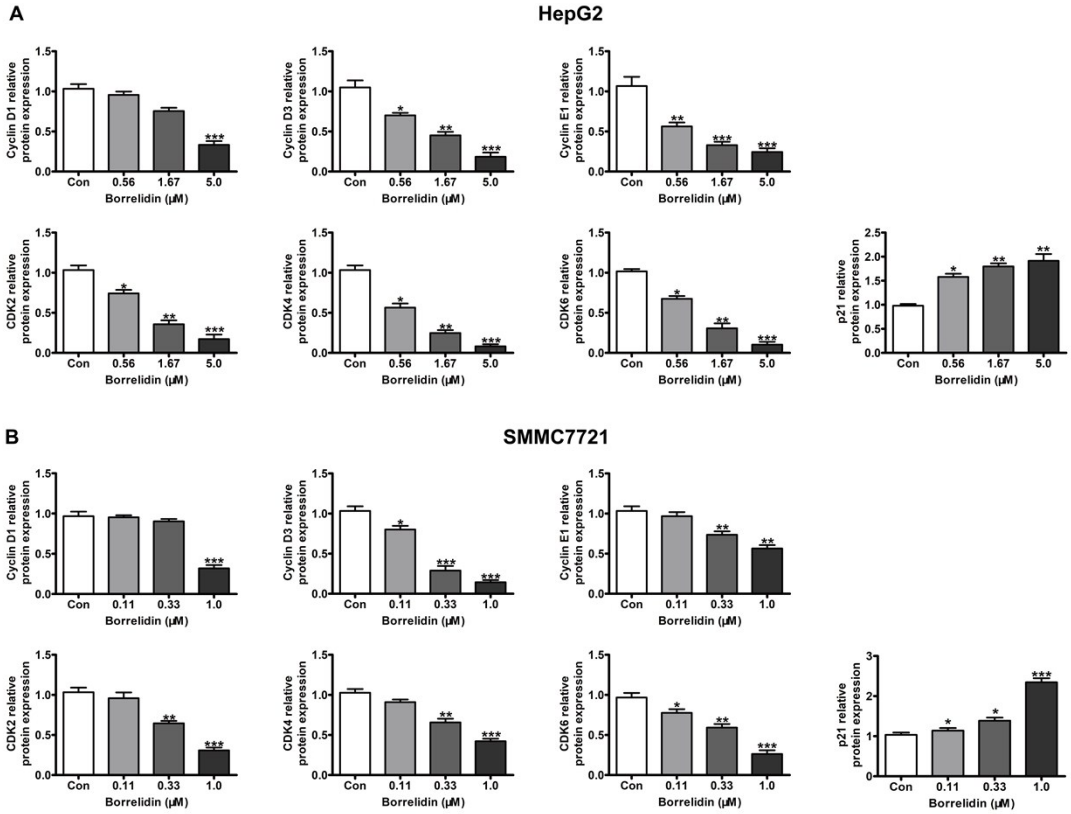


Fig. S1 The relative quantitative analysis of G0/G1 cell cycle-related proteins of HCC cells treated with borrelidin (for Figure 3C and D). The relative quantities of cyclin D1, cyclin E1, cyclin D3, CDK2, CDK4, CDK6 and p21 in HepG2 cells (A) and SMMC7721 cells (B) were represented as means ± SD (n=3). The vehicle-treated group was the control (Con). *, $p < 0.05$ *vs.* control; **, $p < 0.01$ *vs.* control; ***, $p < 0.001$ *vs.* control.

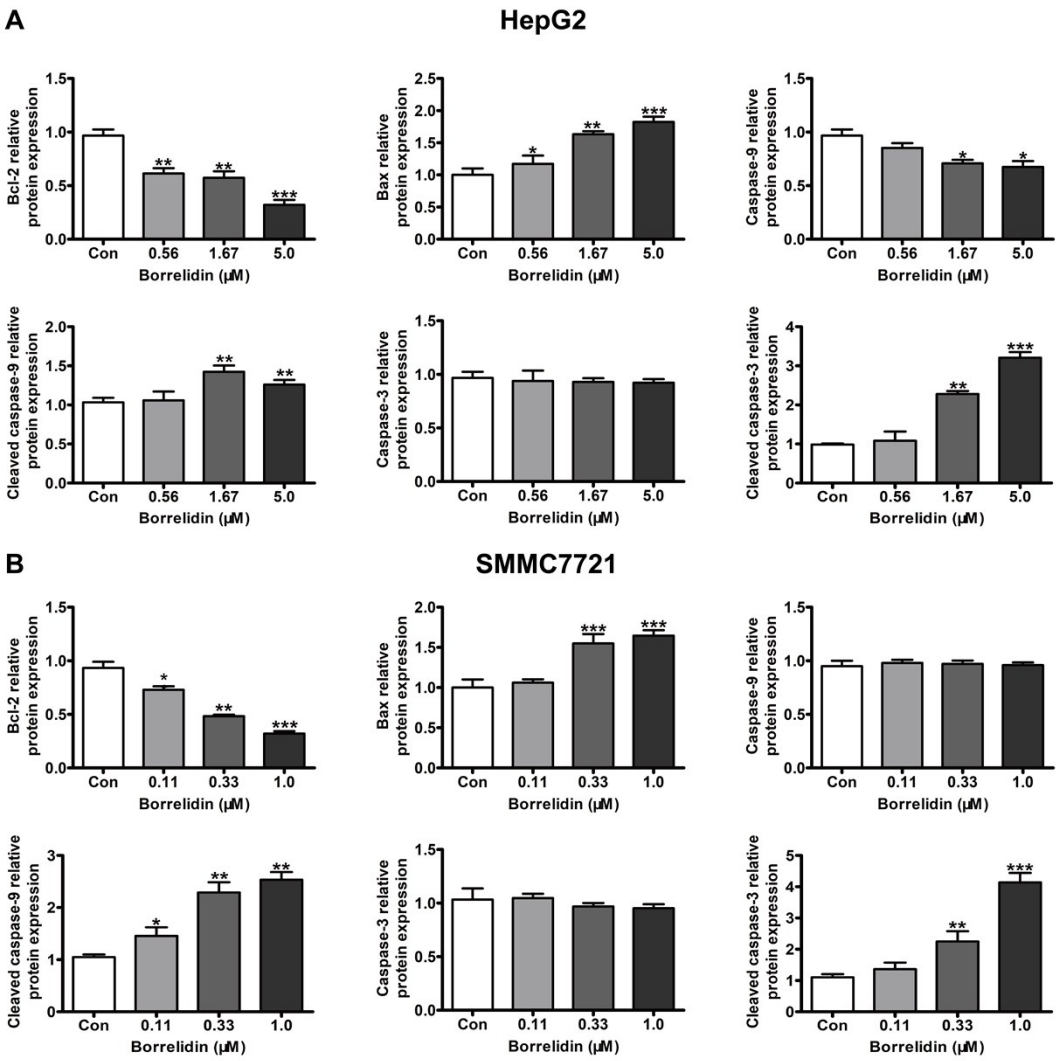


Fig. S2 The relative quantitative analysis of apoptosis-related proteins of HCC cells treated with borrelidin (for Figure 4E and F). The relative quantities of Bcl-2, Bax, caspase-9, cleaved caspase-9, caspase-3 and cleaved caspase-3 in HepG2 cells (A) and SMMC7721 cells (B) were represented as means ± SD (n=3). The vehicle-treated group was the control (Con). *, $p < 0.05$ *vs.* control; **, $p < 0.01$ *vs.* control; ***, $p < 0.001$ *vs.* control.

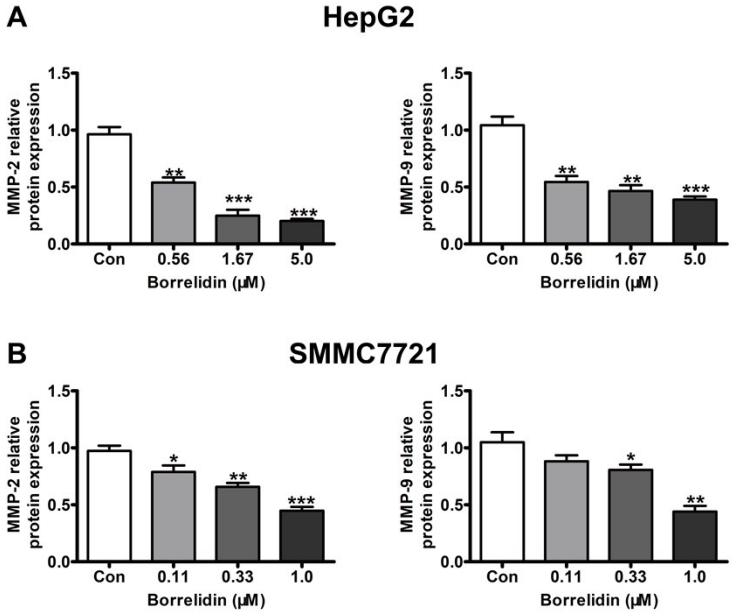


Fig. S3 The relative quantitative analysis of expression of MMP-2 and MMP-9 of HCC cells treated with borrelidin (for Figure 5C). The relative expression of MMP-2 and MMP-9 in HepG2 cells (A) and SMMC7721 cells (B) were assessed by Western blot and represented as means ± SD (n=3). The vehicle-treated group was the control (Con). *, $p < 0.05$ *vs.* control; **, $p < 0.01$ *vs.* control; ***, $p < 0.001$ *vs.* control.

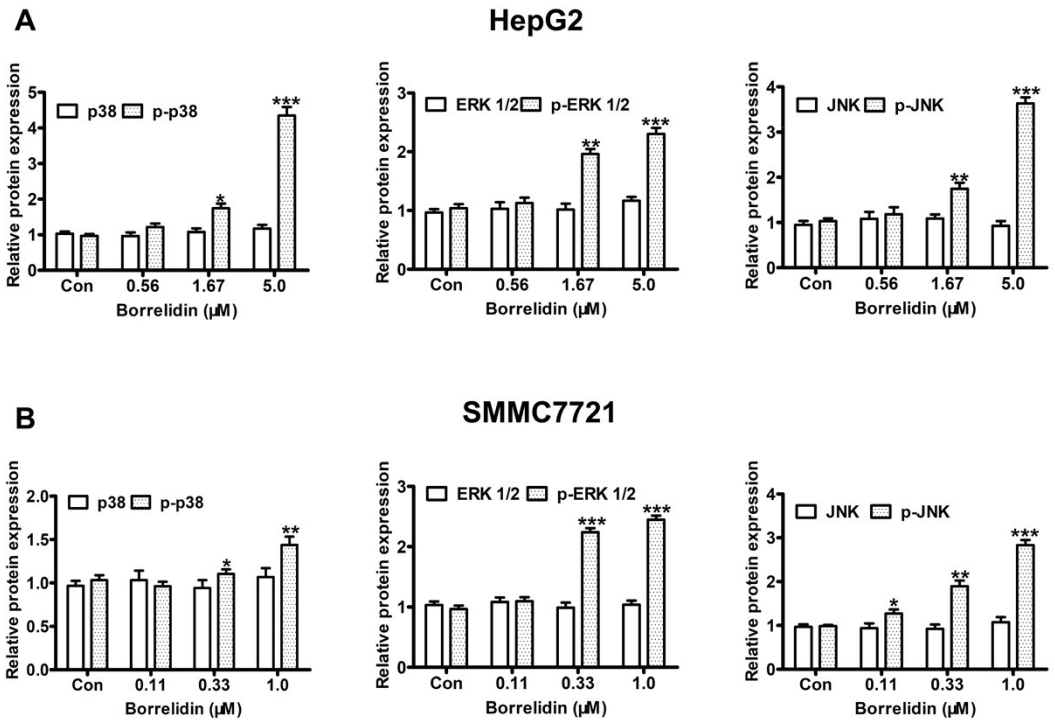


Fig. S4 The relative quantitative analysis of MAPKs family proteins of HCC cells treated with borrelidin (for Figure 6). The relative quantities of p38, p-p38, ERK, p-ERK, JNK and p-JNK in HepG2 cells (A) and SMMC7721 cells (B) were represented as means ± SD (n=3). The vehicle-treated group was the control (Con). *, $p < 0.05$ *vs.* control; **, $p < 0.01$ *vs.* control; ***, $p < 0.001$ *vs.* control.

HPLC-MS analysis of borrelidin:

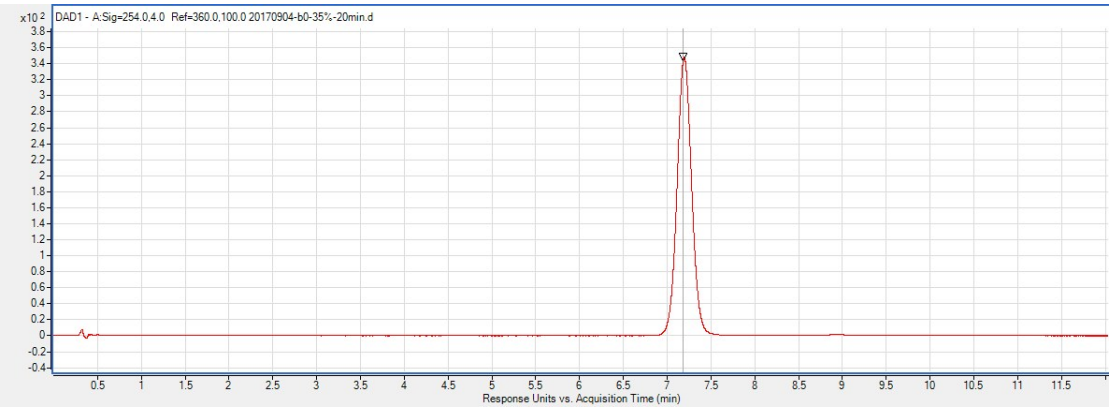


Fig. S5 The HPLC chromatogram of borrelidin. Agilent 1290-6420 Triple Quadrupole LC-MS system with a Agilent SB-C18 column (2.1 × 50 mm, 1.8 μm) was used to analysis the borrelidin. The mobile phase solvent composition was 65% water and 35% acetonitrile. The flow rate was 0.4 ml/min and the column was heated to 30℃.

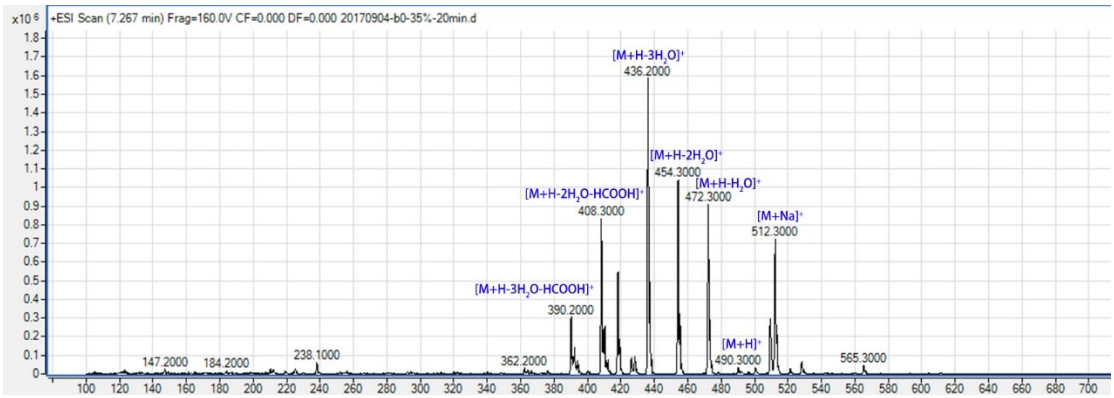


Fig. S6 ESI-MS spectrum of borrelidin

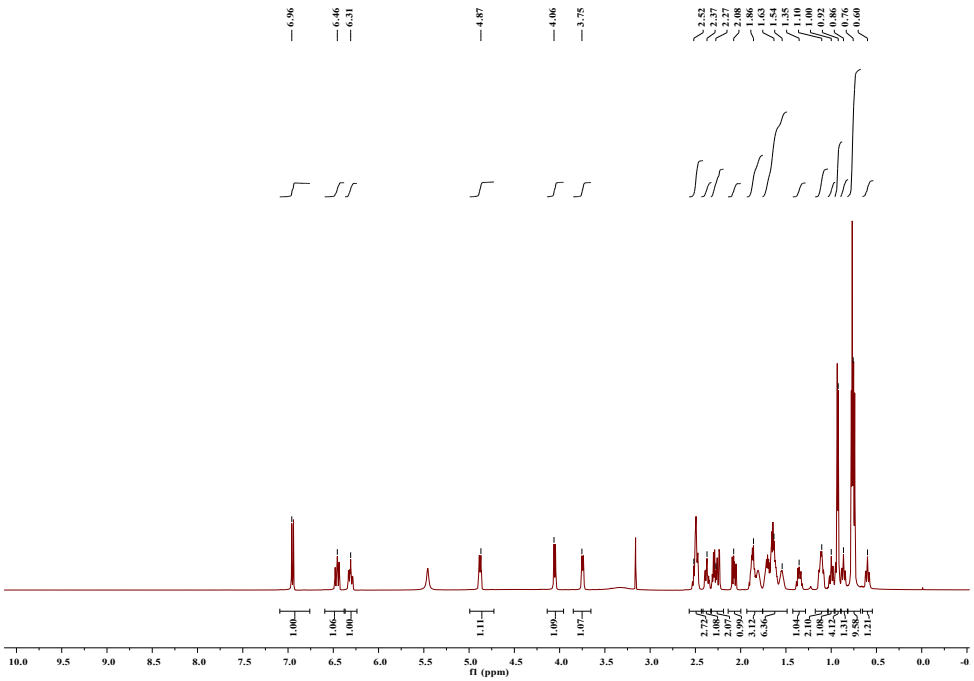


Fig. S7 ^{1}H-NMR (600 MHz, DMSO-d6) spectrum of borrelidin

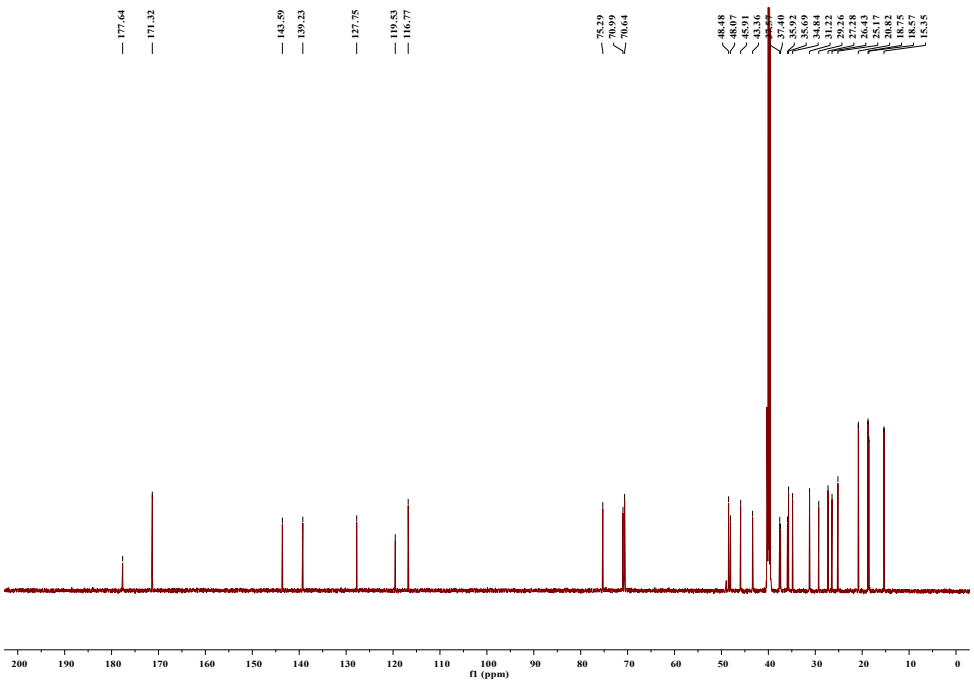


Fig. S8 ^{13}C-NMR (150 MHz, DMSO-d6) spectrum of borrelidin