Electronic Supplementary Material (ESI) for RSC Advances.

Physagulide Q suppresses proliferation and induces apoptosis in human hepatocellular carcinoma cells by regulating the ROS-JAK2/Src-STAT3 signaling pathway

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General

Optical rotations were measured with a JASCO P-1020 polarimeter. IR data were obtained on a Bruker Tensor 27 spectrometer, and 1D and 2D NMR spectra, using $CDCl_3$ as solvent, on a Bruker Avance III NMR spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). HRESI mass spectra were collected withan Agilent 6520B UPLC-Q-TOF mass spectrometer. HPLC analysis was run on an Agilent 1200 instrument equipped with a multiple wavelength diode array detector (DAD). Preparative HPLC was performed on a Shimadzu instrument equipped with a Shim-pack RP-C_{18} column (20×200 mm^2, 10 μm), and a flow rate of 10.0 mL/min. Column chromatography (CC) was carried out using macroporous resin D- 101 (pore size B 13-14 nm, 26–60 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China), Silica gel (100-200 mesh and 200-300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China) and ODS RP-C18 (40-63μm, FuJi, Japan).

Plant material

Whole plants of *Physalis angulate L.* were collected in August of 2014 in Lin Yi, Shan Dong Province, China and were identified by Professor Zhang Mian of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No.PA-201407-LY) is deposited in the department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation

The air-dried fruits of *Physalis angulate L.* (0.5 kg) were powdered and extracted with EtOH-H_2O (95:5) 3 times at room temperature. After removing the solvent under vacuum, the residue (35 g) was subjected to column chromatography (CC) on D-101 macroporous resin and eluted with a step gradient of EtOH-H_2O (20:80, 40:60, 60:40, 80:20, and 95:5, v/v) to yield 5 fractions: Fr. A-E. Fr. D (3.6 g) was then subjected to chromatography over silica gel with increasing polarities of CH_2Cl_2-MeOH (40:1). Then, the residue was applied to ODS MPLC eluted with a step gradient of isocratic MeOH-H_2O (65:35 – 45:55 v/v) to afford 4 sub-fractions. The third part was applied to

preparative HPLC with MeCN-H_2O (45:55 v/v) to yield Physagulide Q (PQ) (11.3 mg). HPLC showed that the purity of PQ was 97.094%.

Physagulide Q

White amorphous solid

$[\alpha]_D^{25}$: +70 (c 0.43, MeOH).

CD: $\Delta\varepsilon_{250}$ +5.34 (c 0.3, MeCN).

IR (KBr): νmax 3448, 2951, 1687, 1383, 1249, 1140 cm-1.

UV (MeOH) λmax (log ε): 227 (4.21), 207 (4.20) nm.

^1H NMR and ^{13}C NMR data: Table S1.

HRESIMS: m/z 582.2825 $[M+NH4]^+$ (calcd for $C_{30}H_{45}ClNO_8$, 582.2828)

The Inhibition rates of compounds.

First, we tested the cytotoxicity of Withangulatin A, Physagulide P and PQ in HepG2 cells using an MTT assay. The inhibition rates of compounds were showed in Fig. S9A. The inhibition rate of PQ was higher than that of other compounds. Then, we detected the cytotoxicity of PQ in HepG2, Bel-7402 and L02 cells. Fig.S9B showed that the inhibition rate of PQ in L02 cells was lower than the inhibition rate in HCC cells.

The effect of IL-6 on STAT3 phosphorylation in HepG2 and Bel-7402 cells.

HepG2 and Bel-7402 cells were stimulated with IL-6 (10 ng/mL) for various lengths of time and the expression of STAT3 and p-STAT3 was detected by western blot assay. As shown in Fig. S10, the expression of p-STAT3 first rose and then dropped when stimulated with IL-6 (10 ng/mL), and it returned to normal levels after two hours. The expression of p-STAT3 reached a peak in 30 min. Therefore, 10 ng/mL IL-6 had a stronger effect in 30 min on HepG2 and Bel-7402 cells.

Table S1

^{1}H (500MHz) and^{13}C NMR (125 MHz) data for PQ in CDCl3 (δ in ppm, Jin Hz).

Position	δ_H	δ_C
1		201.1
2	5.93(dd, 10.1, 2.5)	128.7
3	6.67(ddd, 10.1, 5.0, 2.5)	141.6
4	3.52(dt, 20.0, 2.5)	37.4
	2.55(dd, 20.0, 5.0)	
5		80.4
6	4.07 s	74.8
7	2.21 m	27.5
	1.97 m	
8	2.06 m	35.4
9	2.85 (td, 12.1, 3.6)	36.0
10		53.1
11	2.86 m	21.7
	2.68 m	
12	2.70 m	47.8
	1.91 m	
13		51.0
14		86.5
15	5.15 (dd, 8.5, 3.8)	79.7
16	1.63 m	30.9
	1.25 m	
17		86.8
18	1.16 s	15.2
19	1.36 s	15.8
20	2.25 m	41.7
21	1.08 (d, 7.0)	10.2

22	4.72 (dt, 12.7, 3.3)	77.4
23	2.50 m	32.3
	2.41 m	
24		150.5
25		121.7
26		167.2
27	1.87 s	12.5
28	1.94 s	20.6
OAc	2.08 s	170.2
		22.0

Fig. S1. The structure of PQ

Fig. S2. HRESIMS spectrum of PQ

Fig. S3. ^{1}H NMR spectrum of PQ in $CDCl_3$

Fig. S4. ^{13}C NMR spectrum of PQ in $CDCl_3$

Fig. S5. HSQC spectrum of PQ

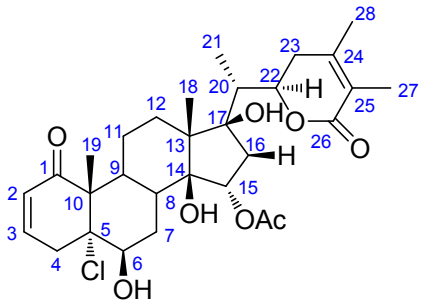
Fig. S6. HMBC spectrum of PQ

Fig. S7. ROESY spectrum of PQ

Fig. S8. HPLC of PQ

Fig. S9. The inhibition rates of compounds.

Fig. S10. The effect of IL-6 on STAT3 phosphorylation in HepG2 and Bel-7402 cells.



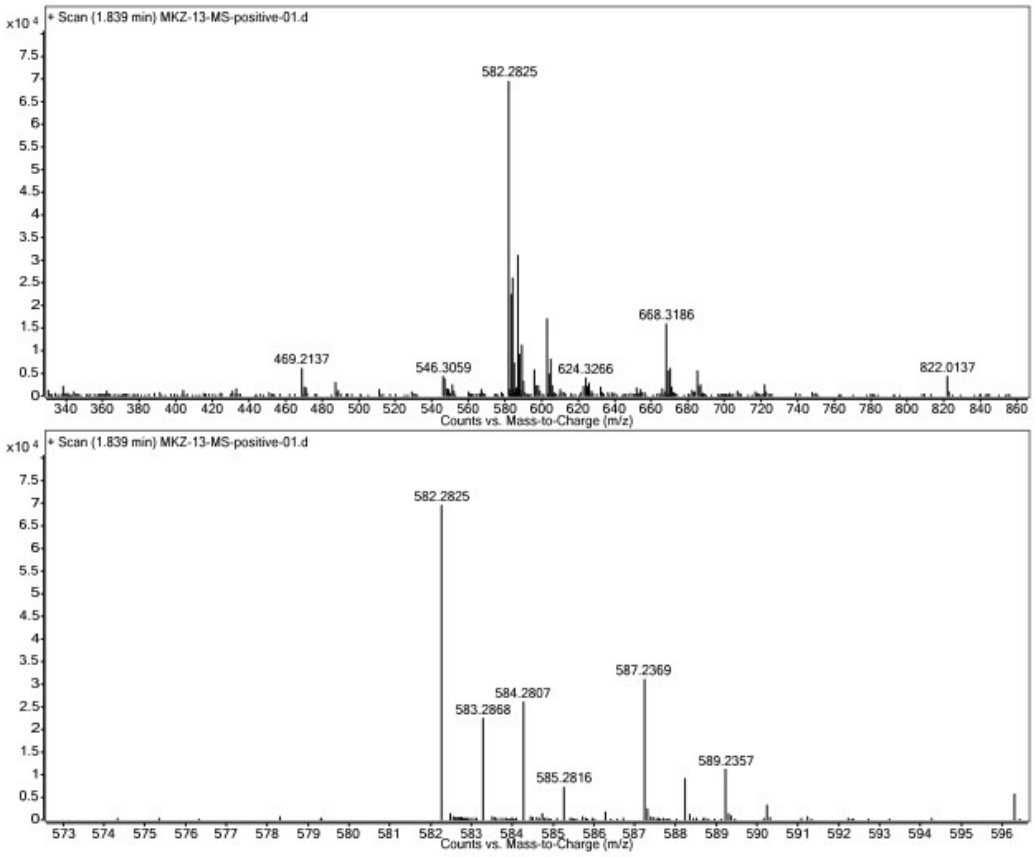
Physagulide Q

Fig. S1. The structure of PQ

TCM-CPU HR-ESI-MS Display Report

Sample Name: MKZ-13 **Instrument:** Agilent 6520B Q-TOF

Acq. Date: 06/03/2015 **Operator:** Administrator



Elemental Composition Calculator

Target m/z:	582.2825	**Result type:**	Positive ions	**Species:**	$[M+NH4]^+$
Elements:		C (0-80); H (0-120); O (0-30); N(0-10); Cl (0-5)			
Ion Formula		**Calcalated m/z**		**PPM Error**	
C30H45ClNO8		582.2828		0.54	

Fig. S2. HRESIMS spectrum of Physagulide Q

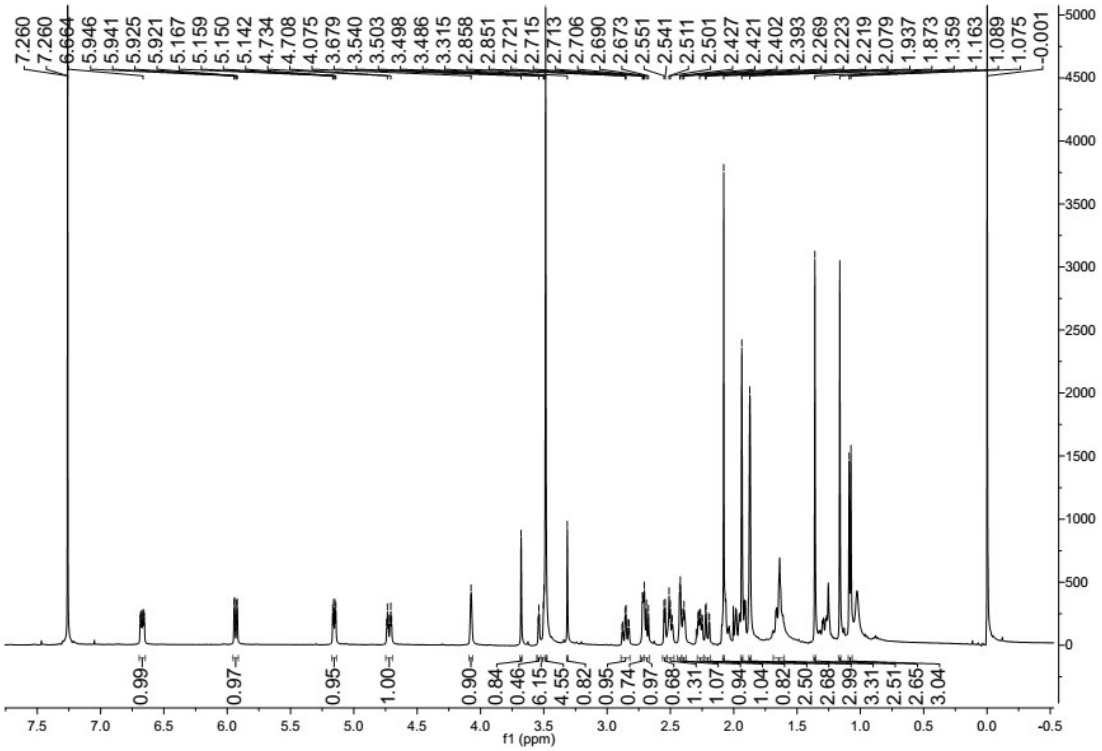


Fig. S3 ^{1}H spectrum of Physagulide Q in $CDCl_3$ (500 MHz)

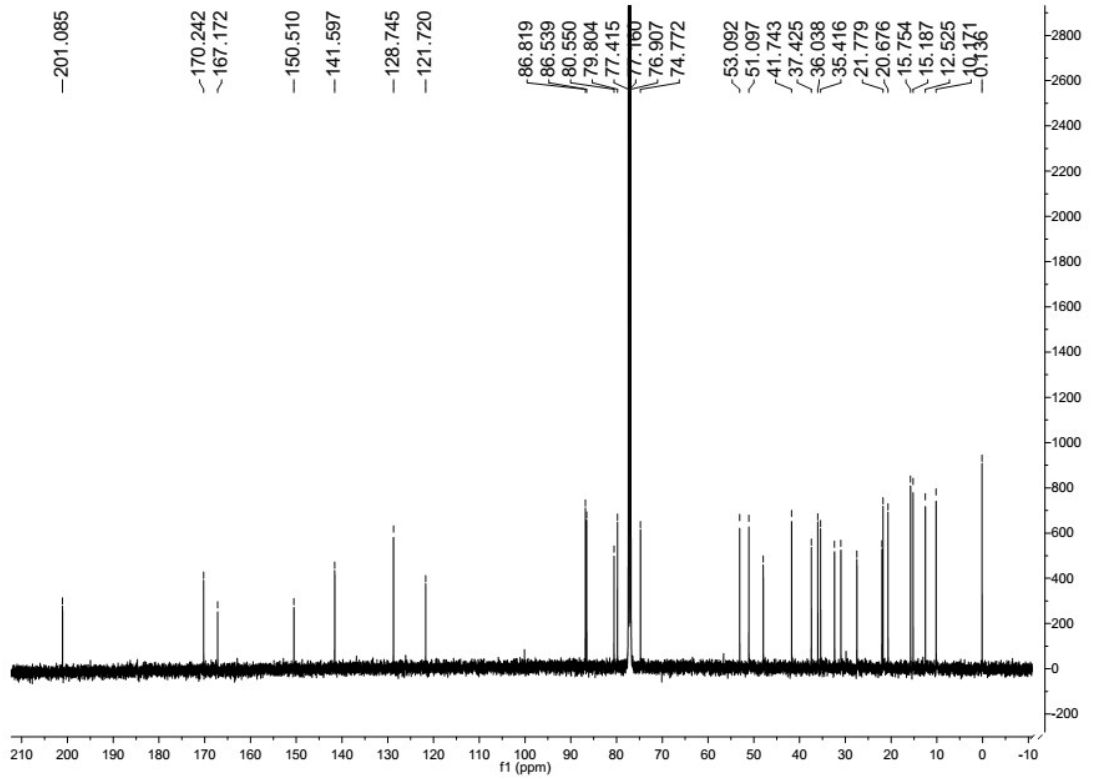


Fig. S4. ^{13}C NMR spectrum of Physagulide Q in $CDCl_3$ (125 MHz)

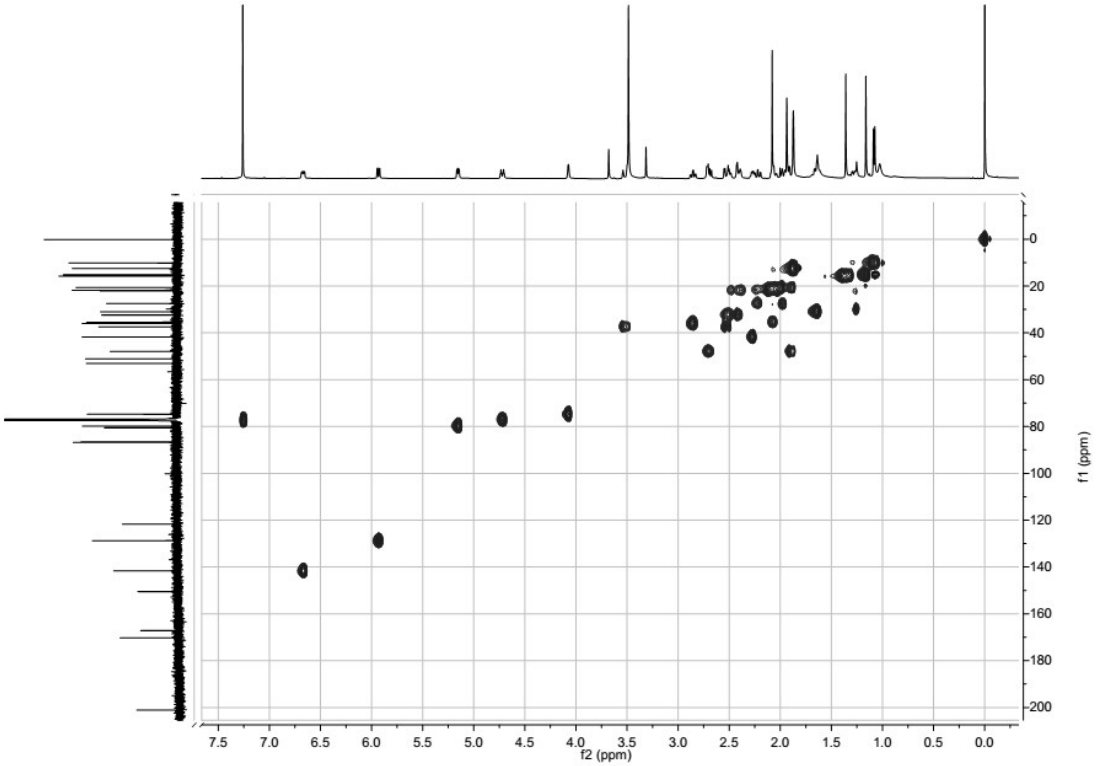


Fig. S5. HSQC spectrum of PQ

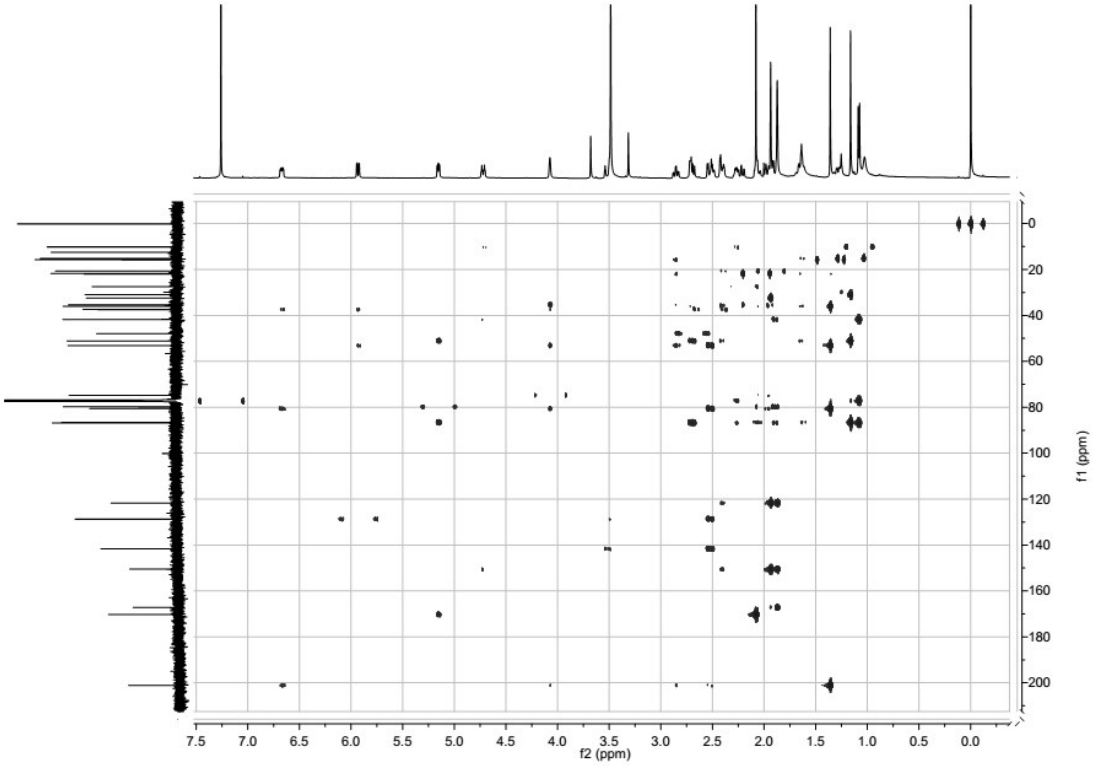


Fig. S6. HMBC spectrum of PQ

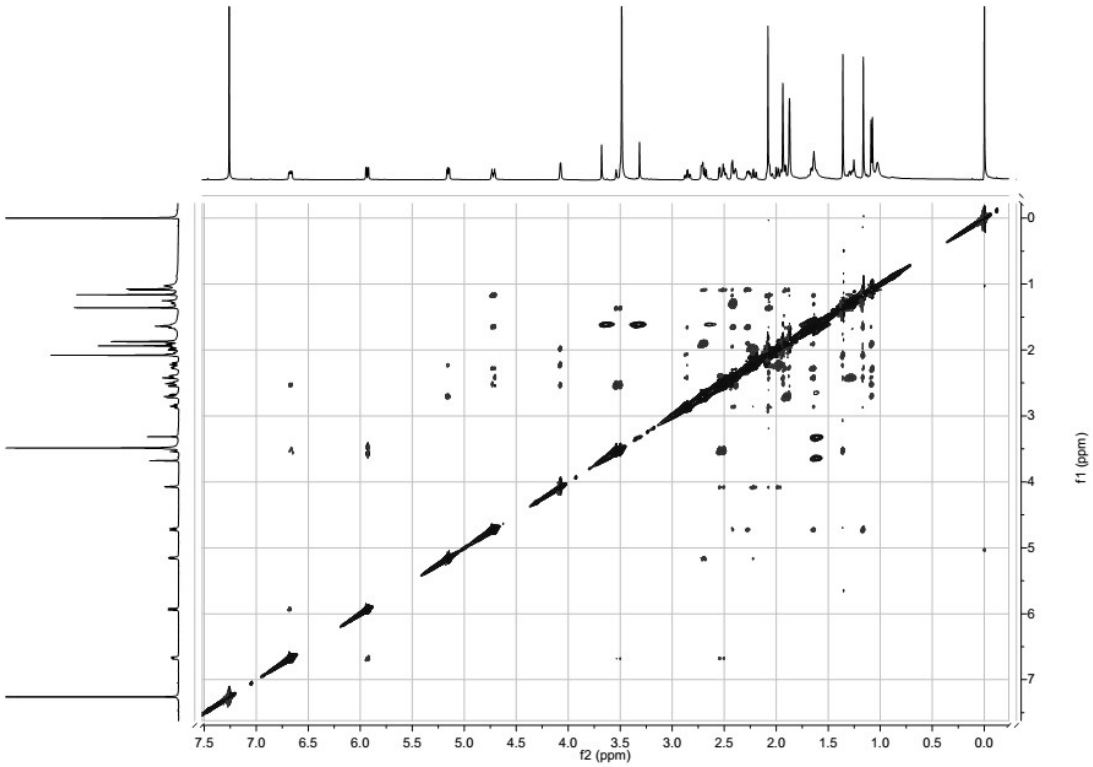


Fig. S7. ROESY spectrum of PQ

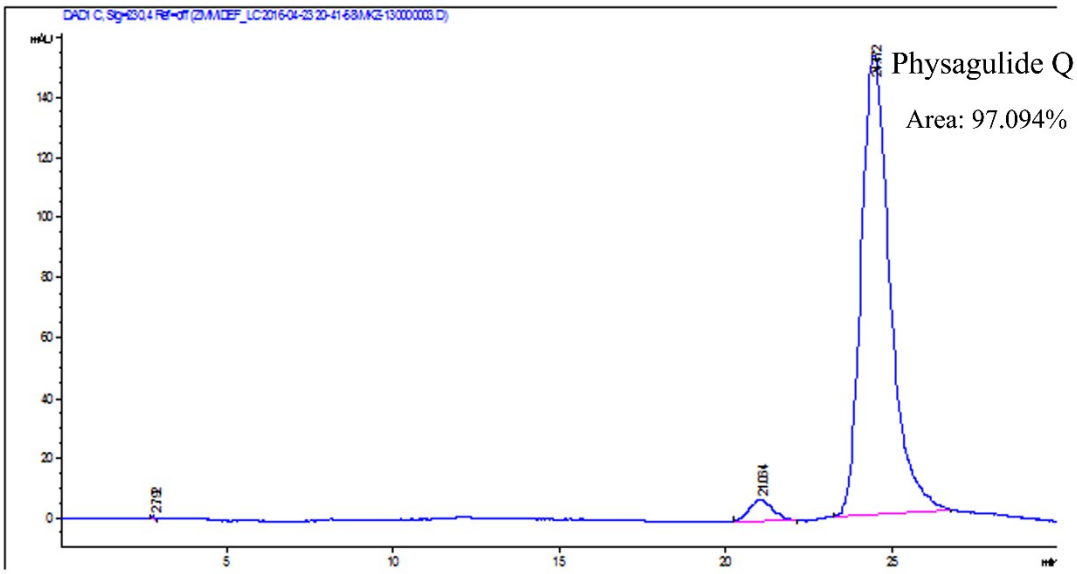


Fig. S8. HPLC of Physagulide Q

#	Time	Area	Height	Width	Symmetry	Area%
1	21.054	89.8	2.3	0.46	0.769	2.906
2	24.472	2998.7	53.3	0.7325	0.761	97.094

HPLC showed that the purity of PQ was 97.094%.

Solvent system: MeOH: H_2O = 60: 40

The retention time of PQ is 24.472 min

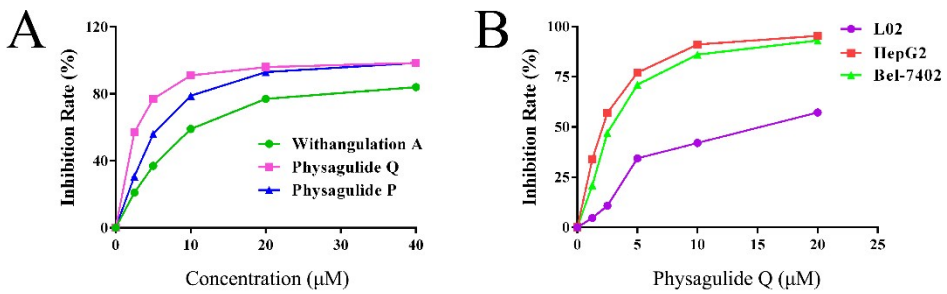


Fig. S9. The inhibition rates of compounds. (A) HepG2 cells were stimulated with various concentrations of Withangulatin A, Physagulide P and PQ for 24 h. The inhibition rates were determined by MTT assay. (B) HepG2, Bel-7402 and L02 cells were treated with different concentrations of PQ for 24 h. The inhibition rates were determined by MTT assay.

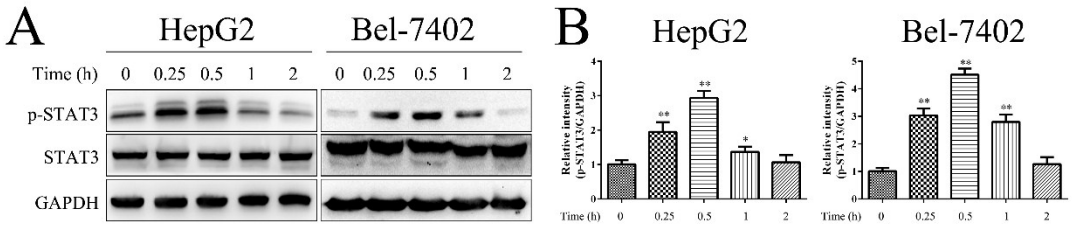


Fig. S10. The effect of IL-6 on STAT3 phosphorylation in HepG2 and Bel-7402 cells. (A) HepG2 and Bel-7402 cells were stimulated with IL-6 (10 ng/mL) for various lengths of time and the expression of p-STAT3 and STAT3 was evaluated using western blotting. The equal loading of protein was confirmed by probing with GAPDH. (B) Histogram represents themean value of relative p-STAT3 expression. The bars represent the means ± SD of three independent experiments. *P < 0.05, **P < 0.01, compared with the control.