Flow Cytometry Analysis

When the cells reached 80%–90% confluence, the culture medium was discarded and the cells were washed twice with PBS, digested by the addition of 0.25% trypsin (Solarbio, Beijing, China), followed by the addition of DMEM supplemented with 10% FBS to stop the reaction. The cells were centrifuged at 60.82 × *g* for 5 min and resuspended in PBS with Fluo-3/AM to a final concentration of 2.5 mM, followed by incubation for 40 min at 37°C. The Fluo-3/AM-loaded cells were centrifuged again for 5 min and washed twice with PBS, collected, and resuspended in PBS with continual incubation for 20 min, to ensure that the Fluo-3 AM was completely converted into Fluo-3 in the cells. The experimental groups were divided as above, with three samples per group. Cell suspensions were pretreated with various concentrations of HSYA (9, 27, and 81 μmol/L) or GB (10^{-7} mol/L) and incubated for a further 30 min. PAF was then added to the mixture (reaction volume of 300 μl per tube), and the samples were detected after 3 min at an excitation wavelength of 488 nm and an emission wavelength of 526 nm using a BD LSRFortessa (BD Biosciences, Franklin Lakes, NJ, USA).

Dual-Luciferase Reporter Gene Analysis

The pNFκB-luc plasmid and pAP1-lcu plasmid were obtained from Beyotime Institute of Biotechnology. These were based on the pGL6 plasmid, with multiple AP1 or NFκB binding site inserts at its multiple cloning sites, to detect the transcriptional activity levels of AP1 or NFκB with high sensitivity. The pRL-SV40 plasmid was chosen as an internal control. Cells were seeded in 24-well culture plates 24 h before transfection. pNFκB-luc or pAP1-luc plasmid (500 ng) was then transfected into each well together with 50 ng pRL-SV40 using Lipofectamine 3000 (Invitrogen), according to the manufacturer's instructions. After 24 h, the medium was changed and the cells were incubated in DMEM containing PAF with or without HSYA and/or GB at the indicated concentrations. Cell lysates were harvested using a passive lysis solution and detected in a 96-well plate. The dual-luciferase reporter assay system (Promega) was used according to the manufacturer's protocol, and the transcriptional activities of NFκB and AP1 were evaluated after normalizing the relative light unit of firefly luciferase to that of renilla.

Small Interfering RNA (siRNA) Silencing

Cells were plated and incubated in six-well plates to reach 70%–80% confluency and then starved for 12 h. siRNA solution (5 μl PAFR or non-targeting control siRNA) was mixed with 100 μl siRNA transfection medium to create reagent A, and 5 μl transfection reagent was mixed with 100 μl siRNA transfection medium to create reagent B. Reagents A and B were mixed to produce reagent C, and kept at room temperature for 30 min. Cells were washed with transfection medium, and gently overlaid with 200 ml reagent C and 800 ml transfection medium and cultured for 6 h. One milliliter of growth medium containing twice the normal serum and antibiotic concentrations was added without removing the transfection mixture, and the cells were incubated for 24 h. This protocol is recommended for a well from a six-well plate, adjust cell and reagent amounts proportionately for wells or dishes of different sizes.

Figure and Table

Tab. S1. The sequences of the primers

mRNA	Sequence
GAPDH	F:CCA TGA GAA GTA TGA CAA CAG CC
	R:GGG TGC TAA GCA GTT GGT G
IL-6	F:GTGA AAG CAG CAA AGA GGC
	R:CAT TTG TGG TTG GGT CAG G
IL-1β	F:TAC GAA TCT CCG ACC ACC ACT ACA G
	R:TGG AGG TGG AGA GCT TTC AGT TCA TAT G
TNF-α	F:CTC CAC CCA TGT GCT CCT CAC
	R:CCC AAA GTA GAC CTG CCC AGA

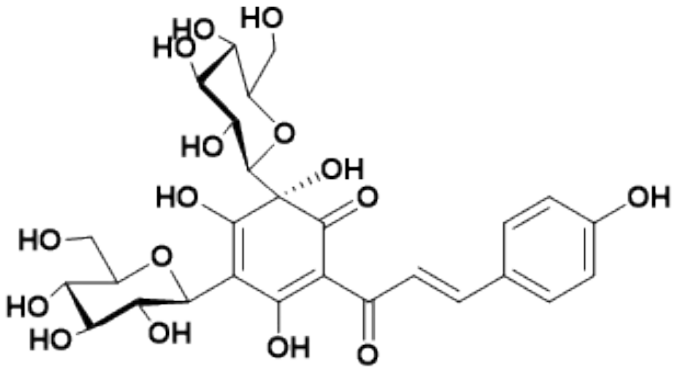

Fig. S1. Molecular structure of HSYA