Supplementary Data

Saponarin from barley sprouts inhibits NF-κB and MAPK on LPS-induced RAW 264.7 cells

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1. Supplementary Martials and Methods

1.1. Colony-forming assay

Cells (500–1000 cells/well) were plated in 60-mm dishes and incubated at 37°C, 5% CO2 for colony formation. After 10 days, colonies were fixed with methanol:acetic acid at a 3:1 ratio for 10 min, washed with distilled water, and stained with 4% trypan blue (Sigma; St. Louis MO, USA) for 30 min for colony visualization.

1.2. *NMR assignment of SA*

SA was identified by nuclear magnetic resonance (NMR) spectroscopy. The ^{1}H and ^{13}C NMR data were obtained on a Brucker AM 500 spectrometer. The SA were prepared using deuterated solvent with tertamethylsilane (TMS) as internal standard in 5 mm NMR tuves. NMR data were obtained in CD_3OD and DMSO-d_6 with chemical shifts according to the TMS signal and were expressed. The structural characteristics, including ^{1}H-NMR and ^{13}C-NMRspectropic data are detailed supplementary data. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 13.52(1H, s, 5-OH), 7.98 (1H, s, H-2'), 7.96 (1H, s, H-6'), 6.95 (2H, d, J=8.3, H-5'), 6.90 (1H, s, H-8), 6.88 (1H, s, H-3), 4.66 (1H, d, J = 9.8 Hz, H-1"), 3.51-3.94 (7H, m, O-Glu), 3.17-3.35 (7H, m, C-Glu) ; ^{13}C-NMR (125 MHz, DMSO-d6) δ 182.47 (C-4), 164.59 (C-7), 162.87 (C-2), 161.02 (C-9), 159.75 (C-4'), 156.84 (C-5'), 128.98 (C-6'), 123.34 (C-1), 116.40 (C-5), 108.72 (C-6), 105.27 (C-10), 103.58 (C-3), 94.14 (C-8), 81.35 (C-1''), 79.31 (C-2''), 73.31 (C-4''), 70.01 (C-6'').

Table S1. The sequences of primers used in RT-PCR analysis and the sizes of RT-PCR products.

Gene	sense and antisense sequences	Expected product size (bp)
IL-6	5`-GTACTCCAGAAGACCAGAGG-3`	308
	5`-TGCTGGTGACAACCACGGCC-3`	
TNF-α	5`-TTGACCTCAGCGCTGAGTTG-3`	364
	5`-CCTGTAGCCCACGTCGTAGC-3`	
IL-1β	5`-CAGGATGAGGACATGAGCACC-3`	447
	5`-CTCTGCAGACTCAAACTCCAC-3`	
GAPDH	5`-CGGAGTCAACGGATTTGGTCGTAT-3`	306
	5`-AGCCTTCTCCATGGTGGTGAAGAC-3`	

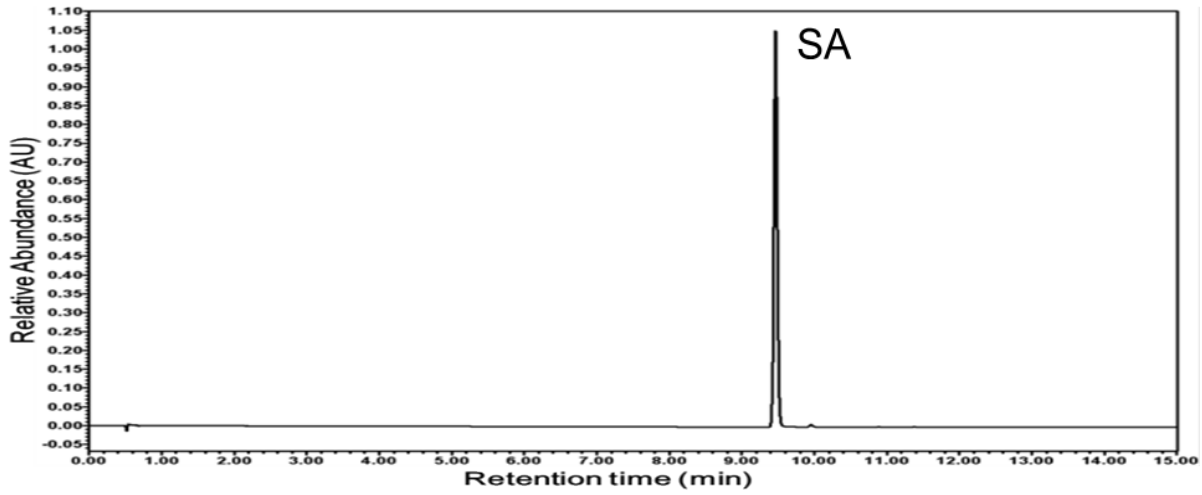

Figure S1. Purified of SA.

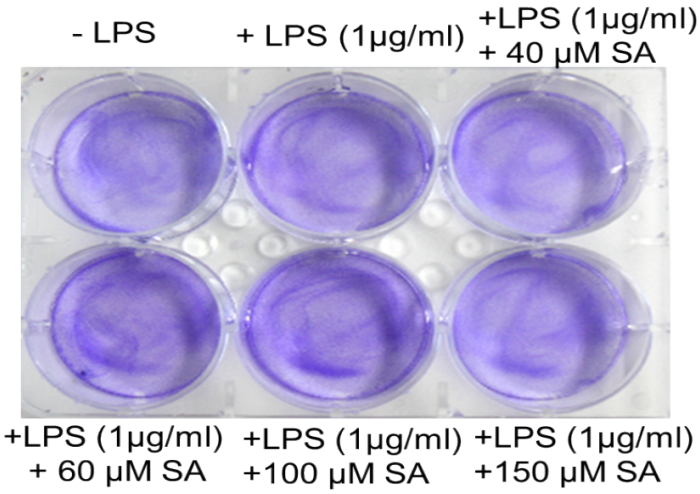

Figure S2. The viability of RAW 264.7 cells treated with different SA concentrations was evaluated by the colony-forming assay.

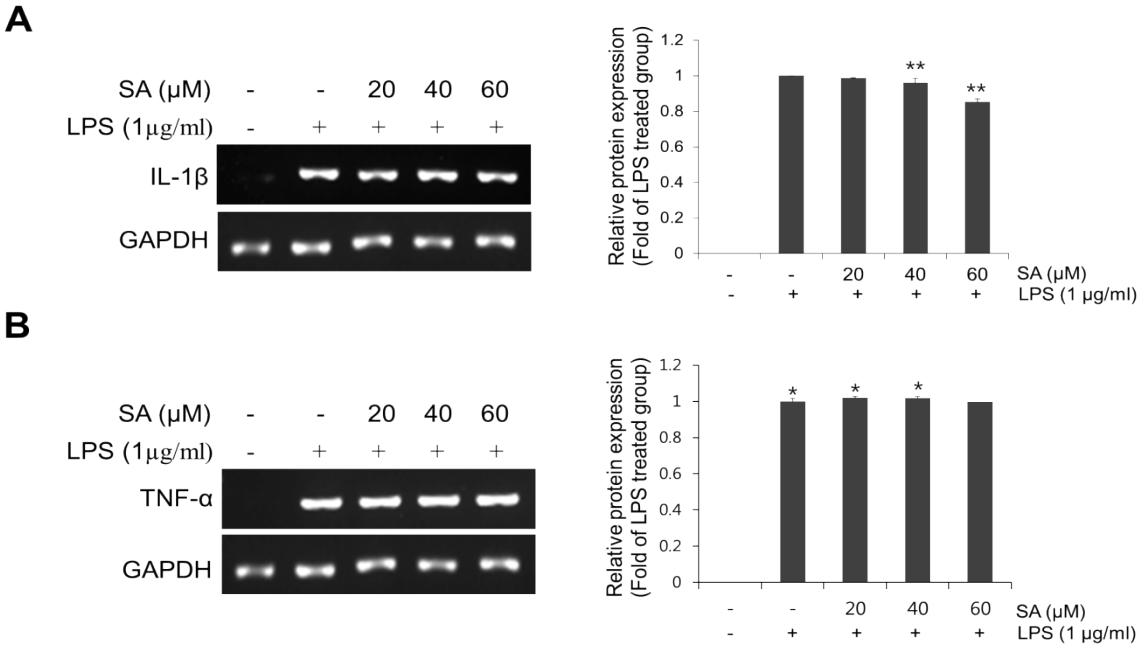

Figure S3. IL-1β and IL-6 mRNA expression in RAW 264.7 cells. Cells were treated with indicated concentrations of SA and then stimulated with 1 μg/mL LPS, and mRNA expression was measured by RT-PCR. GAPDH was used as an internal control. RT-PCR products were resolved on 1.0% agarose gels and visualized by UV light. The data are presented as the means ± SEM (*$P < 0.005$, ** $P < 0.05$ compared with the cells treated with LPS alone).

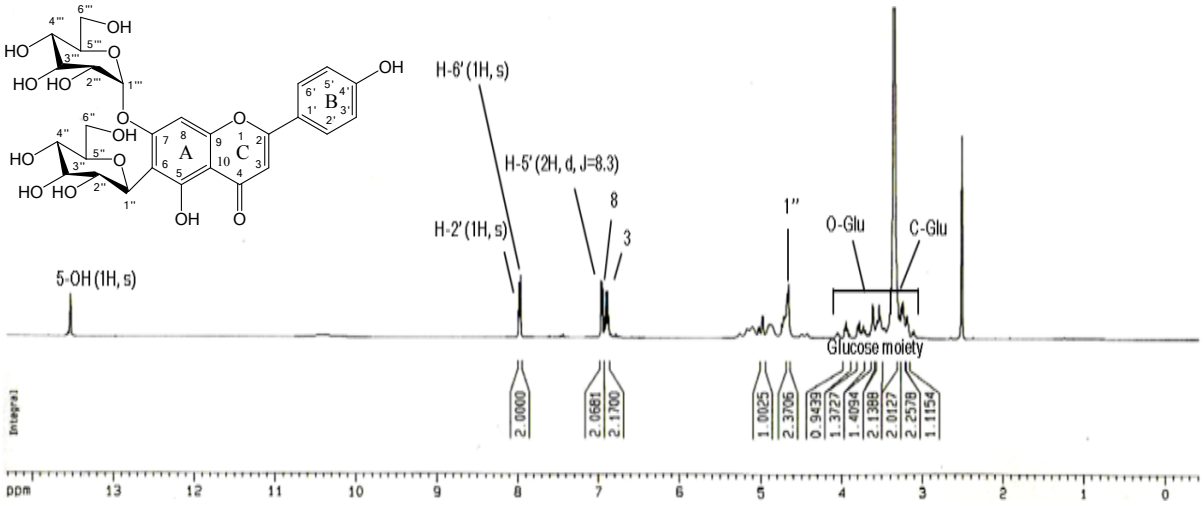

Figure S4. ^{1}H NMR spectra of SA (500MHz, DMSO-d_6).

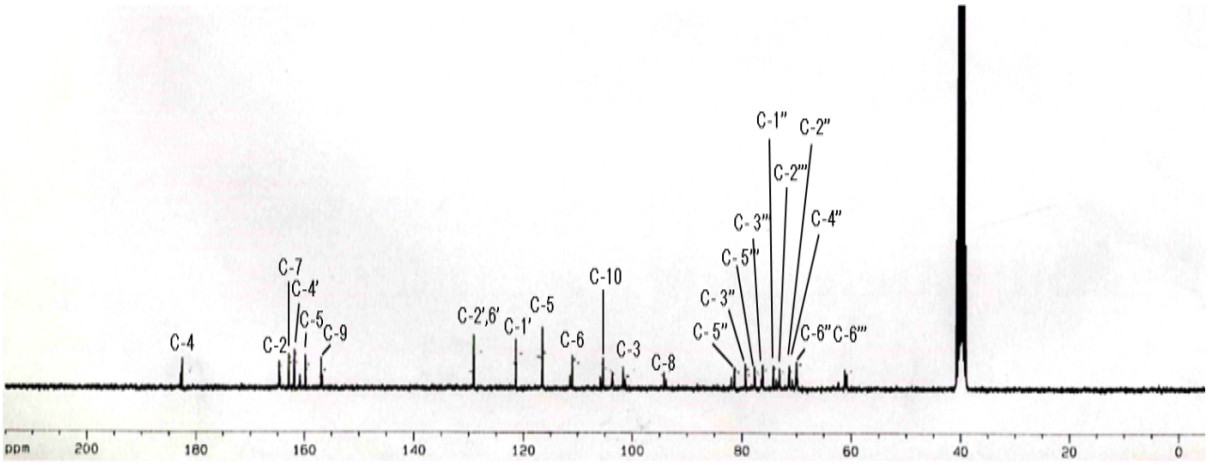

Figure S5. ^{13}C NMR spectrum of SA (125MHz, DMSO-d_6).

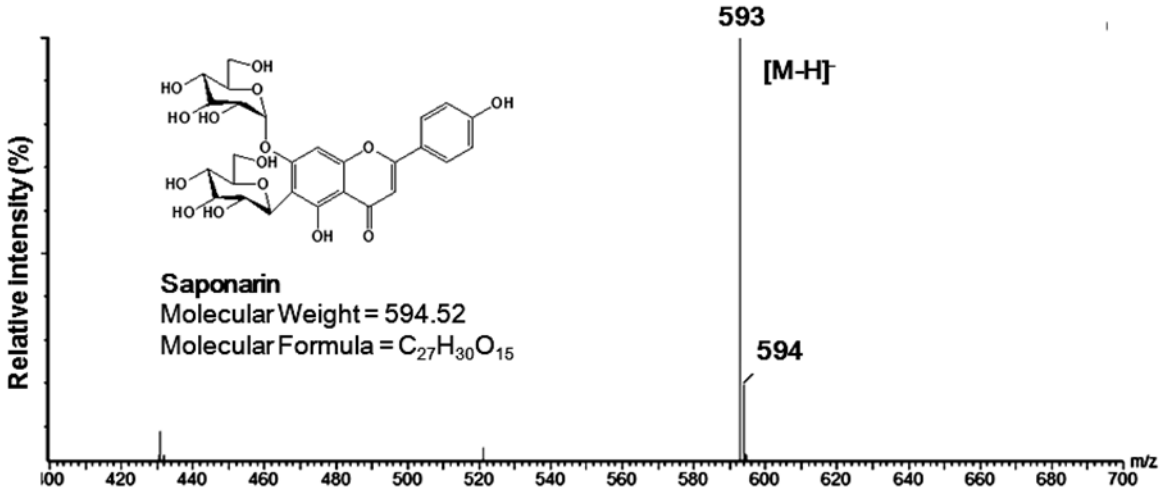

Figure S6. MS spectra of SA at negative ion mode ($[M-H]^-$).