Supplementary Materials and Methods

Cell viability assay

Cell viability was measured based on the formation of blue formazan from colorless MTT by mitochondrial dehydrogenases, which are active only in live cells. BV-2 microglial cells (2.5×10^5 cells/well in 24-well plates) were incubated at 37°C with LPS for 24 h with or without VBME pretreatment and then treated with MTT solution (5 mg/ml) for 2 h. The dark-blue formazan crystals formed in intact cells were dissolved in DMSO and absorbance at 540 nm was measured with a microplate reader (SpectraMax 250, Molecular Devices, Sunnyvale, CA, USA). The results are expressed as the percentage of MTT reduction relative to the absorbance of control cells.

Measurement of intracellular ROS accumulation

BV-2 microglial cells (1×10^6 cells/well) were seeded on 6-well plates or poly-D-lysine-coated slides and grown overnight. After pretreatment with or without VBME for 30 min, the cells were incubated with LPS for 24 h. The cells were then rinsed with PBS and incubated with 10 μM DCFH-DA for 30 min at 37°C. The plates were washed twice with PBS and the fluorescence of each well was measured at 530 nm using a fluorescence microplate reader (SpectraMax M2) with excitation at 488 nm. DCFH-DA fluorescence images were examined using a fluorescence microscope (20× magnification).

Transient transfection and dual-luciferase assay

The NF-κB reporter constructs used in this study were purchased from SABiosiences Inc. (Qiagen Inc., Valencia, CA, USA). Briefly, BV-2 microglial cells were plated on 24-well plates at a density of 2.5×10^5 cells/well and grown overnight. Cells were cotransfected for 6 h with 5 μg/ml of an NF-κB reporter construct and internal controls or negative plasmid
constructs to measure transfection efficiency. The lipofectamine method was used for all transfections. After transfection, cells were cultured in medium containing 10% FBS for 24 h, after which they were incubated with VBME for 30 min and then treated with LPS for 6 h. Luciferase activity was assayed using a dual-luciferase assay kit (Promega, Madison, WI, USA) according to the manufacturer’s instructions. Luminescence was measured using a single-tube luminometer (FB12, Berthold Detection Systems GmbH, Pforzheim, Germany).

**Electrophoretic mobility shift assay (EMSA)**

Nuclear extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents as described above. Synthetic complementary NF-κB-binding oligonucleotides (5’-AGTTGAGGGGACTTTCCCAGGC-3’; Panomics Inc., Santa Clara, CA, USA) were 5’-biotinylated using a biotin 5’-end DNA labeling EMSA kit according to the manufacturer’s protocol (Affymetrix Inc., Santa Clara, CA, USA). The binding reactions contained 10 μg of nuclear extract protein, 1 μg of poly d(I-C), and 10 ng of biotin-labeled DNA in binding buffer. The reactions were incubated for 5 min at room temperature in a final volume of 10 μl. Protein-DNA complexes were separated from free DNA probes by electrophoresis on native 6% polyacrylamide gels that had been pre-electrophoresed for 1 h in 0.5× Tris borate/EDTA buffer (50 mM Tris-Base, 18 mM boric acid, 500 mM EDTA, pH 8.3). Complexes were then transferred onto a positively-charged nylon membrane (Pall Corporation) in 0.5× Tris borate/EDTA buffer at 300 mA for 30 min. The transferred DNA complexes were cross-linked to the nylon membrane in a dry oven at 80°C for 1 h. Horseradish peroxidase-conjugated streptavidin was used to detect the transferred DNA complexes according to the manufacturer’s instructions.

**High-performance liquid chromatography (HPLC) analysis**
VBME was standardized according to its quercetin content, an active component of VBME that has been suggested to exhibit anti-inflammatory activities in some models of neurodegenerative diseases. The composition of VBME was analyzed by HPLC using a Waters HPLC system (Waters Corp., Milford, MA, USA) equipped with a quaternary solvent delivery system, an autosampler, and a DAD detector. UV absorbance was monitored at 200 to 400 nm. Samples were resolved on a J’sphere ODS-H80 reversed phase column (250 × 4.6 mm, 4 μm, YMC Co., Ltd., Kyoto, Japan). The mobile phase (A: 0.02% trifluoroacetic acid in acetonitrile, B: 0.02% trifluoroacetic acid in water) consisted of a 10-100% gradient of solvent A for 0-30 min. Chromatography was carried out in gradient mode using a flow rate of 1.0 ml/min at 30°C, with detection at 360 nm. Quercetin contents were quantified by integration of the peak areas at 360 nm with 10 μl of each injected external standard. VBME references to the quercetin calibration curve were analyzed in triplicate; the VBME sample contained 0.14% quercetin (Figs. S4A and B, respectively).