University, Daejeon, Korea). Transfection were performed by the lipofectamine LTX (Invitrogen, Carlsbad, CA) as described by the manufacturer. After 23 h of transfection, medium was changed to assay medium (Charcoal 0.5% FBS + non-essential amino acid + phenol red free DMEM). After 24 h of transfection, cells were treated with test compounds for another 24 hours. After 25 h of transfection, cells were treated with 10 ng/mL of TNF-α for another 23 hours. The luciferase activity of cell lysates was assayed with 100 μL of by luciferase assay kit using an LB 953 Autolumat (EG and G Berthold, Nashua, NH). Transfections were performed in triplicate, and activation was normalized against α-galactosidase activity.

Reverse transcription polymerase chain reaction

Total RNA was isolated using the Easy-blue reagent (Intron Biotechnology, Seoul, Korea). Total RNA (~2 μg) was reverse transcribed using Moloney murine leukemia virus (MMLV) reverse transcriptase and oligo-dT primers (Promega, Madison, WI) for 1 h at 42°C. PCR of synthetic complementary Deoxyribonucleic acid (cDNA) was performed using a Taq polymerase pre-mixture (TaKaRa, Japan). PCR products were subjected to electrophoresis on 1% agarose gels and stained with Ethidium bromide (EtBr). PCR was conducted with the following primer pairs: iNOS sense 5'-TCATCCGCTATGCTGGCTAC-3', iNOS antisense 5'-CTCAGGGTCACGGCCATTG-3', COX-2 sense 5'-GCCCAGCACTTCACGCATCAG-3', COX-2 antisense 5'-GACCAGGCACCAGACCAAAGACC-3', GAPDH sense 5'-TGTTGCCATCAATGACCCCTT-3', and GAPDH antisense 5'-CTCCACGACGTACTCAGCG -3'. The specificity of products generated by each set of primers was examined using gel electrophoresis and further confirmed by a melting curve analysis.

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