http://dx.doi.org/10.5012/bkcs.2012.33.2.1

Supplementary Materials

Biological Evaluation and Molecular Docking Study of 3-(4-Sulfamoylphenyl)-4-phenyl-1*H*-pyrrole-2,5-dione as COX-2 Inhibitor

Van Chung Pham,[a] Ji-Sun Shin,[†,a] Min Ji Choi, Tae Woo Kim, Geun Huh, Jungahn Kim, Dong Joon Choo, Kyung-Tae Lee,[†] and Jae Yeol Lee[*]

Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, Seoul 130-701, Korea. [*]E-mail: ljy@khu.ac.kr
[†]Department of Pharmaceutical Biochemistry and Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea
Received September 14, 2011, Accepted December 14, 2011

Key Words : PGE_2 production, *Anti*-inflammatory, COX-2, Docking study, 1*H*-Pyrrole-2,5-dione

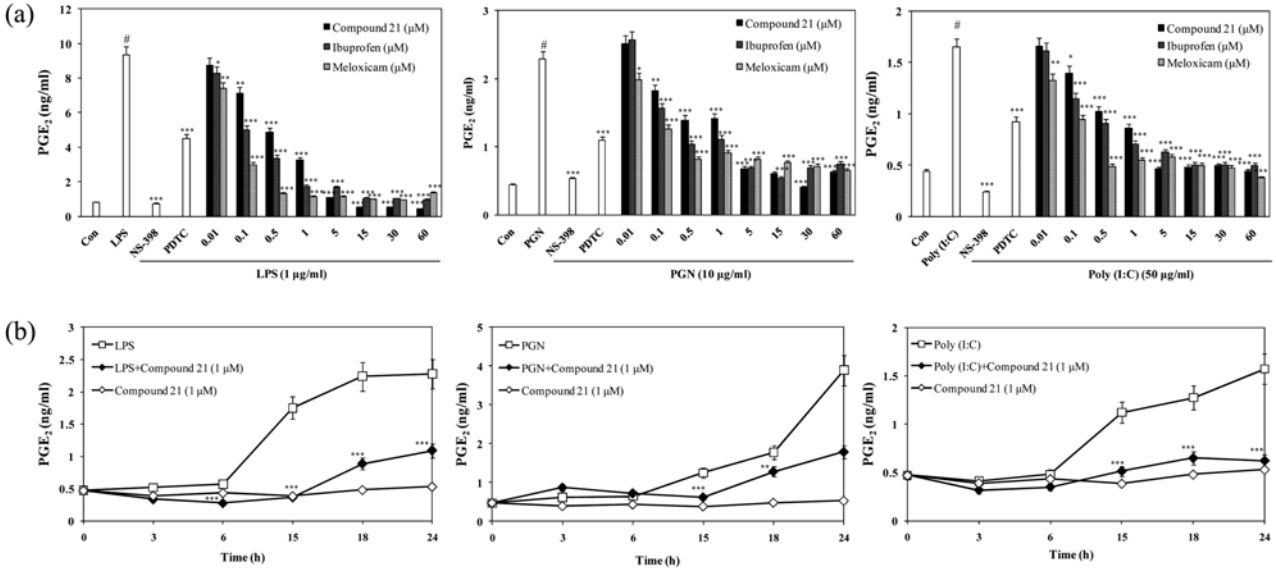

Figure 1. Inhibitory effect of compound **2l** on various TLR ligand-induced PGE_2 productions: (a) dose-dependent response (b) time-dependent response at 1 mM concentrations; (A) RAW 264.7 cells were pretreated with/without the indicated concentrations of compound **21** and reference drugs for 1 h before stimulation with LPS (1 μg/mL), PGN (10 μg/mL) or poly(I:C) (50 μg/mL) for 24 h. Controls were not treated with LPS, PGN or poly(I:C) and compounds. NS-398 (5 μM) and PDTC (5 μM) were used as a positive control; (B) RAW 264.7 cells were pretreated with/without compounds **21** (1 μM) for 1 h before stimulation with LPS (1 μg/mL), PGN (10 μg/mL) or poly(I:C) (50 μg/mL) for indicated time. Levels of PGE_2 in the culture media were quantified using enzyme immunoassay (EIA) kits. Values shown are means ± S.D. of three independent experiments. $^{\#}p < 0.05$ *vs.* the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* LPS-stimulated group; significant differences between groups were determined using ANOVA and Dunnett's post-hoc test.

[a]These authors contributed equally to this work.

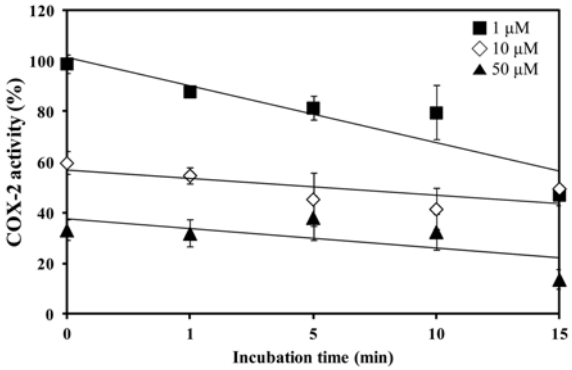

Figure 2. Inhibitory effects of compound **21** on recombinant COX-2 enzyme activity. Recombinant COX-2 enzyme was *in vitro* incubated with the compound **21** (1, 10, and 50 μM) for indicated time in 37 °C. The reaction was started by the addition of 100 μM arachidonic acid and terminated by addition of HCl solution containing $SnCl_2$. The COX activity assay directly measures $PGF_{2\alpha}$ produced by $SnCl_2$ reduction of COX-derived PGH_2. The prostanoid product is quantified via EIA kits.

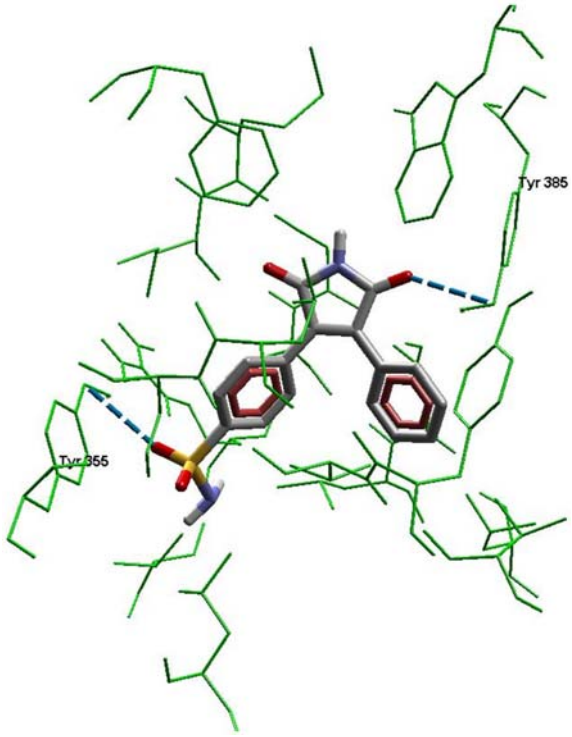

Figure 3. Docking of compound **2l** into the active site of COX-1. Hydrogen bonds are shown in blue.

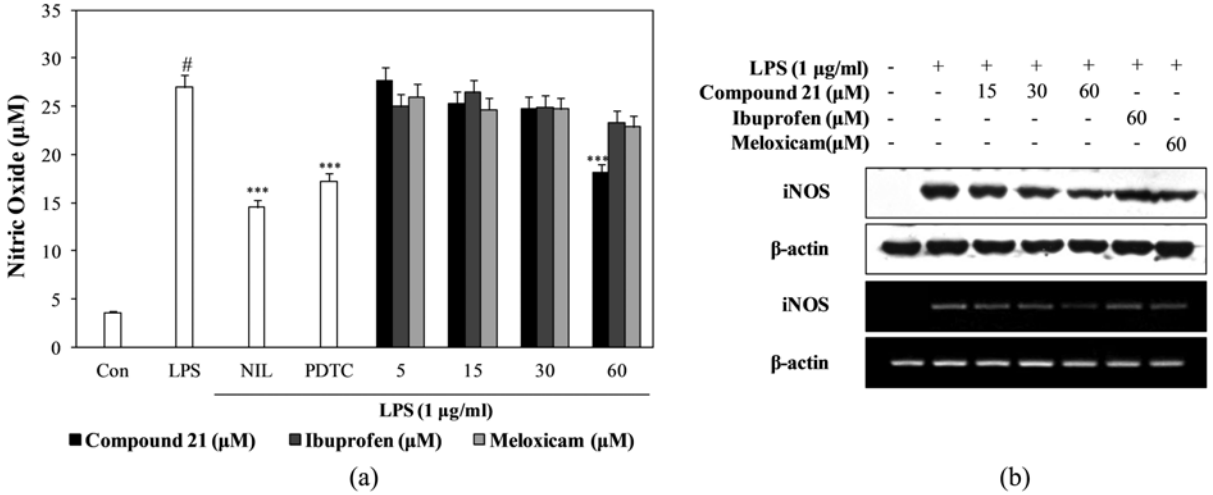

Figure 4. Effects of compound **21** on LPS-induced nitric oxide production and iNOS expression in RAW 264.7 cells (a) Cells were pretreated with/without the indicated concentrations of compound **21** and reference drugs for 1 h before stimulation with LPS (1 μg/mL) for 24 h. Amount of NO was determined in using the Griess reaction. Controls were not treated with LPS and compounds. L-NIL (10 μM) and PDTC (5 μM) were used as a positive control. Values shown are means ± S.D. of three independent experiments. $^{\#}p < 0.05$ *vs.* the control group; $^{***}p < 0.001$ *vs.* LPS-stimulated group; significant differences between groups were determined using ANOVA and Dunnett's post-hoc test. (b) Lysates and total RNA were prepared form cells pretreated with/without compounds **21** for 1 h before stimulation with LPS (1 μg/mL) for 24 h or 4 h. The protein and mRNA levels of iNOS were detected by Western blot and RT-PCR, respectively. The experiment was repeated three times and similar results were obtained.

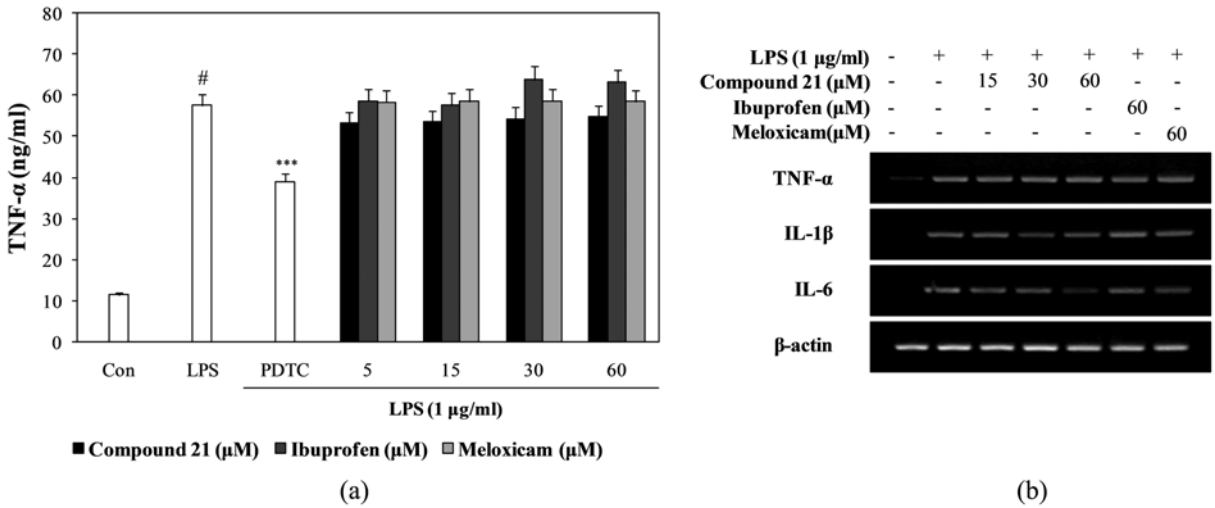

Figure 5. Effects of compound **21** on LPS-induced production of TNF-α and mRNA expression of TNF-α, IL-1β, and IL-6 in RAW 264.7 cells (a) Cells were pretreated with/without the indicated concentrations of compound 21 and reference drugs for 1 h before stimulation with LPS (1 μg/mL) for 24 h. The production of TNF-α were determined using EIA kits. Controls were not treated with LPS and compounds. PDTC (5 μM) were used as a positive control. Values shown are means ± S.D. of three independent experiments. $^{\#}p < 0.05$ *vs.* the control group; $^{***}p < 0.001$ *vs.* LPS-stimulated group; significant differences between groups were determined using ANOVA and Dunnett's post-hoc test. (b) Total RNA were prepared form cells pretreated with/without compounds **21** for 1 h before stimulation with LPS (1 μg/mL) for 4 h. The mRNA levels of cytokines (TNF-α, IL-1β, and IL-6) were detected by RT-PCR. The experiment was repeated three times and similar results were obtained.