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A substituted 3,4-dihydropyrimidinone derivative (compound D22) prevents inflammation mediated neurotoxicity; role in microglial activation in BV-2 cells

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ABSTRACT

A novel synthetic 3,4-dihydropyrimidinone derivative, compound **D22** (ethyl 6-methyl-4-(3-phenoxyphenyl)-2-thioxo-3,4-dihydropyrimidine-5-carboxylate), was found to exert anti-inflammatory properties in lipopolysaccharide-stimulated microglial BV-2 cells. Compound **D22** reduced the pro-inflammatory factors such as nitric oxide, prostaglandin E_2, tumor necrosis factor-α and interleukin-1β. Moreover, it suppressed the expressions of inducible NO synthase and cyclooxygenase-2. Compound **D22** inhibited the activation of mitogen-activated protein kinases. When compound **D22**-conditioned media from BV-2 cells were applied to N2a cells, neuronal cell death was inhibited via suppression of caspase-3 activation and regulation of Bcl-2 and Bax proteins expression. These results suggest that compound **D22** may be useful for treating neurodegenerative diseases related with neuroinflammation.

Microglia, the immune cells of the central nervous system, play roles in defense against injury and tissue repair.[1] However, excessive activation of microglia due to pathogenic bacterial infection or injury can produce diverse pro-inflammatory factors including tumor necrosis factor α (TNF-α), nitric oxide (NO), interleukin-1β (IL-1β) and prostaglandin E_2 (PGE_2),[2,3] which may increase neuronal damage and may results in neuronal cell death in the brain.[4] These processes exacerbate brain injury and cause neuroinflammation, which has been shown to be a risk factor of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and brain ischemia.[5] Therefore, much attention has focused on agents that may regulate neuroinflammation via inhibition of microglial activation. Previously, we demonstrated that arctigenin,[6] apigenin,[7] wogonin[8] and chrysin[9] had anti-inflammatory activities in lipopolysaccharide (LPS)-activated microglial cells and in the brains of ischemic animals.

3,4-Dihydropyrimidinone is associated with three structures: ethyl acetoacetate, benzaldehyde and urea/thiourea.[10] The 3,4-dihydropyrimidinone has many biological and therapeutic effects, including anti-viral,[11] anti-bacterial,[12] anti-tumor,[13] and anti-inflammatory[14] activities. However, little is known about their neuroprotective effects via regulation of microglial cell activation.

In this study, we examined the effect of synthesized 3,4-dihydropyrimidinone derivatives as candidate novel neuroprotective agents. First, the 30 synthesized 3,4-dihydropyrimidinone derivatives examined in this study were investigated as potential anti-neuroinflammatory substances using LPS-stimulated BV-2 microglial cells (data not shown). Among the 3,4-dihydropyrimidinone derivatives, compound **D7** (ethyl 1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(3-phenoxyphenyl)pyrimidine-5-carboxylate), compound **D10** (ethyl 4-(5-chloro-2-hydroxyphenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate), and compound **D22** (ethyl 6-methyl-4-(3-phenoxyphenyl)-2-thioxo-3,4-dihydropyrimidine-5-carboxylate) exhibited inhibitory effects on NO production in BV-2 cells. Compound **D22** showed stronger inhibitory activity on NO production than the others in LPS-stimulated BV-2 cells. Therefore, we investigated the effect of compound **D22** on regulation of microglial cell activation in activated BV-2 cells.

Compound **D22** (Fig. 1) was obtained through the following synthetic process. Solutions containing mixtures of aldehyde (1.0 mmol), β-ketoester (1.2 mmol), and thiourea (1.2 mmol) in acetonitrile (96%) were heated under reflux conditions in the presence of rhodium(III) chloride ($RhCl_3$) catalyst (5 mol %) for 4 h. Reaction

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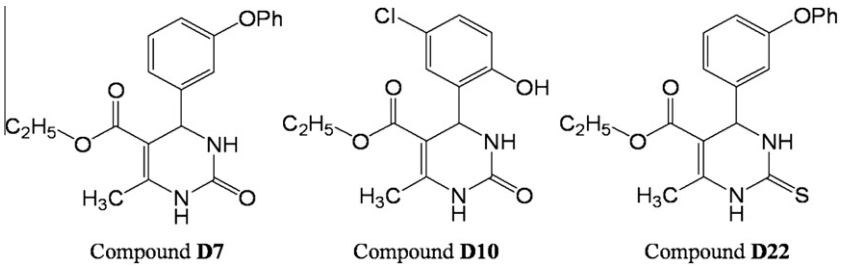

Figure 1. Structure of compounds **D7**, **D10** and **D22**.

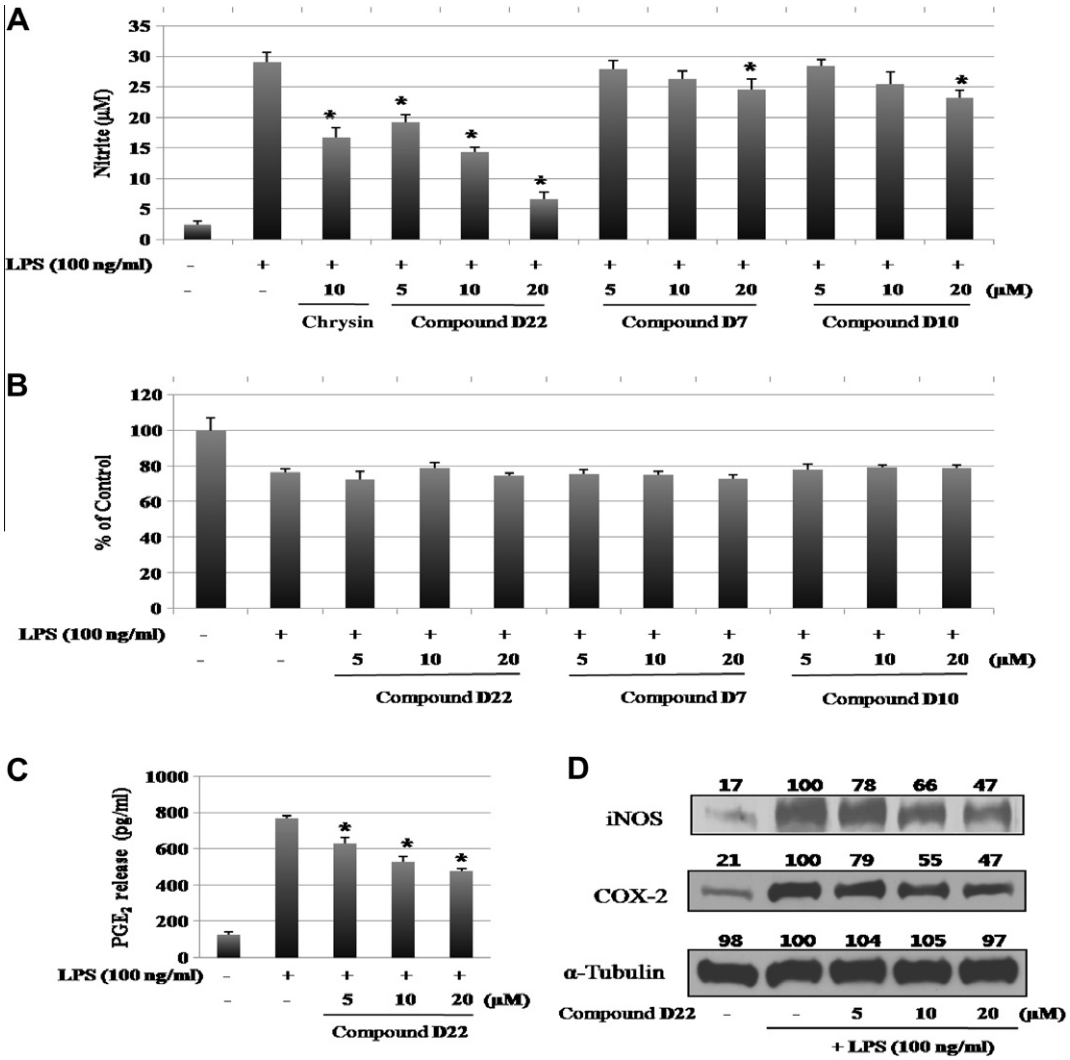

Figure 2. *Effects of compound **D22** on NO and PGE_2 production, COX-2 and iNOS regulation in LPS-stimulated BV-2 microglial cells.* BV-2 micriglial cells were pretreated with 5, 10 and 20 μM of compound **D22** for 30 min and stimulated with 100 ng/ml of LPS for 24 h. Measurement of NO production and PGE_2 production (A and C). MTT assay (B). The effects of compound **D22** on the induction of iNOS and COX-2 expression in BV-2 cells after 6 h LPS treatment (D). Densitometry analysis of bands was performed as described in the methods section. All data are presented as the mean ± S.E.M of three independent experiments. $^*p<0.05$ indicates significant differences compared to treatment with LPS alone.

completion was monitored by thin-layer chromatography. The reaction mixture was then poured onto crushed ice and the solid product was filtered and recrystallized in methanol. All products were characterized using nuclear magnetic resonance and infrared spectroscopy by comparison with the spectra of authentic samples and based on the melting points of the samples mixed with authentic samples.[10] The yields were in the range of 80–84%.

LPS is widely used as an inflammatory inducer in the brain.[15] The presence of LPS leads to the production of several pro-inflammatory factors in BV-2 cells. Among these factors, NO is released from activated microglia.[16] Compounds **D7**, **D10** and **D22** were compared with chrysin, which reduces NO production, in BV-2 cells.[9,17,18] Compounds **D7** and **D10** had little effect on NO production in BV-2 cells. But, compound **D22** showed 8.5% stronger inhibitory activity on NO production than chrysin at a concentration of 10 μM in LPS-stimulated BV-2 cells (Fig. 2A). Compound **D22** did not affect cell viability at this concentration (Fig. 2B).