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3-(4-(*tert*-Octyl)phenoxy)propane-1,2-diol suppresses inflammatory responses *via* inhibition of multiple kinases

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ABSTRACT

Novel anti-inflammatory compounds were synthesised by derivatization of militarin, a compound isolated from *Cordyceps militaris* that is an ethnopharmacologically well-known herbal medicine with multiple benefits such as anti-cancer, anti-inflammatory, anti-obesity, and anti-diabetic properties. In this study, we explored the *in vitro* and *in vivo* anti-inflammatory potencies of these compounds during inflammatory responses, their inhibitory mechanisms, and acute toxicity profiles. To do this, we studied inflammatory conditions using *in vitro* lipopolysaccharide-treated macrophages and several *in vivo* inflammatory models such as dextran sodium sulphate (DSS)-induced colitis, EtOH/HCl-induced gastritis, and arachidonic acid-induced ear oedema. Methods used included real-time PCR, immunoblotting analysis, immunoprecipitation, reporter gene assays, and direct kinase assays. Of the tested compounds, compound **III** showed the highest nitric oxide (NO) inhibitory activity. This compound also inhibited the production of prostaglandin (PG)E_2 at the transcriptional level by suppression of Syk/NF-κB, IKKε/IRF-3, and p38/AP-1 pathways in lipopolysaccharide (LPS)-activated RAW264.7 cells and peritoneal macrophages. Consistent with these findings, compound **III** strongly ameliorated inflammatory symptoms in colitis, gastritis, and ear oedema models. In acute toxicity tests, there were no significant differences in body and organ weights, serum parameters, and stomach lesions between the untreated and compound **III**-treated mice. Therefore, this compound has the potential to be served as a lead chemical for developing a promising anti-inflammatory drug candidate with multiple kinase targets.

1. Introduction

The deregulated or exacerbated inflammation may cause a range of serious diseases, such as cancer, vascular disorders, and diabetes [1,2]. Even though the molecular pathophysiological mechanisms by which inflammation induces disease are not fully understood, inflammatory molecules such as toxic radicals [*e.g.*, reactive oxygen species, and nitric oxide (NO)], pro-inflammatory cytokines [*e.g.*, tumour necrosis factor (TNF)-α, interleukin (IL)-1, interferon (IFN)-β], and lipidic inflammatory mediators [*e.g.*, prostaglandin (PG) E_2, and leukotrienes] have been identified as critical factors in stimulating macrophages and cytotoxic T cells, which in turn are involved in tissue destruction and the subsequent induction of pathological phenomena [3–5].

Recent research results have additionally highlighted the functional significance of tissue-associated macrophages in inflammatory responses. These cells infiltrate into chronically inflamed organs to aggressively manage the production of various inflammatory molecules and facilitate the tissue damage in inflammation [6]. Many research efforts have led to an understanding of how macrophages can be activated by interaction

Abbreviations: Na-CMC, sodium carboxyl methylcellulose; EIA, enzyme immunoassay; ELISA, enzyme linked immunosorbent assay; MTT, 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IRF-3, interferon regulatory factor-3; PI3K, phosphoinositide 3-kinase; STAT-1, signal transducer and activator of transcription; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

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between various surface receptors, such as toll-like receptor (TLR), and their ligands [*e.g.*, TLR4 ligand, lipopolysaccharide (LPS)] or between cytokine receptors and the corresponding cytokines, and which molecular components such as TANK-binding kinase (TBK), IκB kinase ε (IKKε), transforming growth factor β-activated kinase 1 (TAK1), and mitogen activated protein kinases (MAPK) [*e.g.*, extracellular signal-regulated kinase (ERK), p38, and c-Jun N-kinase (JNK)] can contribute to these responses by activating transcription factors such as nuclear factor (NF)-κB, interferon regulatory factor (IRF)-3, and activator protein (AP)-1 [7,8]. Accordingly, it is hypothesised that compounds with macrophage regulatory roles can be developed as anti-inflammatory drugs.

In an attempt to isolate active components from *Cordyceps militaris*, an ethnopharmacologically well-known herbal medicine demonstrated to possess the modulatory activity in various inflammatory models such as dextran sodium sulphate (DSS)-induced colitis, ovalbumin-induced asthma, and croton oil-induced ear edema [9–12], we identified militarin, bis(4-(glucopyranosyloxy)benzyl) 2-sec-butylmalate, as a potential anti-inflammatory and anti-cancer drug (Kim et al., manuscript in preparation). Due to lack of mass production, we attempted to synthesize this compound; however, structural and chemical limitations forced us to design structural derivatives for synthesis. Eventually, we were able to prepare four different compounds (Fig. 1A) derived by structural simplification with carbohydrate moiety and benzene rings from the original compound. In this study, we focused on exploring the *in vitro* and *in vivo* anti-inflammatory activity of militarin-derived compounds, the underlying molecular mechanisms, and their acute toxicity using macrophage-derived inflammatory responses and mice treated with DSS, HCl/EtOH, and arachidonic acid.

2. Materials and methods

2.1. Mice

Six-week-old male C57BL/6 and ICR mice were purchased from DAEHAN BIOLINK (Chungbuk, Korea). Mice were given food pellets (Samyang, Daejeon, Korea) and water *ad libitum* under a 12-h light/dark cycle. Studies were performed in accordance with guidelines

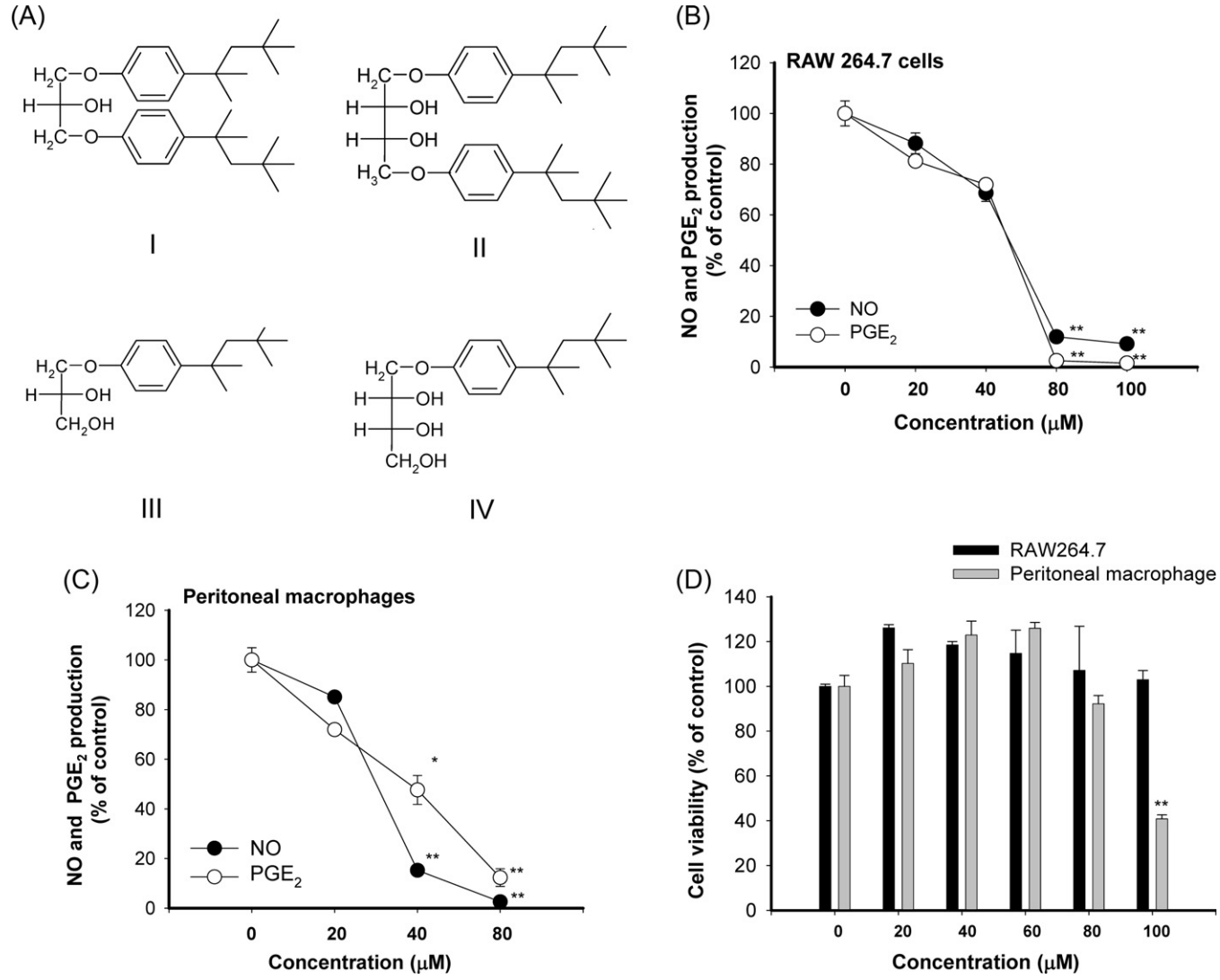

Fig. 1. Chemical structures of compound **III** and its structural analogs and their *in vitro* anti-inflammatory activities. (A) Chemical structures of compound **III** and its derivatives. (B and C) RAW264.7 cells (1×10^6 cells/ml) (left) or peritoneal macrophages (2×10^6 cells/ml) (right) were treated with compound **III** in the presence or absence of LPS (1 μg/ml) for 24 h. Concentrations of NO and PGE_2 in the supernatants were determined by Griess assay and EIA as described in Section 2. (D) Cell viability after treatment of RAW264.7 cells (1×10^6 cells/ml) or peritoneal macrophages (2×10^6 cells/ml) with compound **III** as evaluated by MTT assay. Data (B, C, and D) represent the mean ± SD of an experiment performed with six samples ($n = 6$). $^*p < 0.05$ and $^{**}p < 0.01$ compared to control or normal group.