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Synthesis and biological evaluation of chalcone, dihydrochalcone, and 1,3-diarylpropane analogs as anti-inflammatory agents

Mopur Vijaya Bhaskar Reddy [a,#], Hsin-Yi Hung [b,#], Ping-Chung Kuo [b,#], Guan-Jhong Huang [c], Yu-Yi Chan [d], Shiow-Chyn Huang [e], Shwu-Jen Wu [f], Susan L. Morris-Natschke [g], Kuo-Hsiung Lee [g,h,*], Tian-Shung Wu [b,i,*]

[a] Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan,

[b] School of Pharmacy, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan

[c] School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung 404, Taiwan

[d] Department of Biotechnology, Southern Taiwan University of Science and Technology, Tainan 710, Taiwan

[e] Graduate Institute of Pharmaceutical Science, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan

[f] Department of Medical Technology, Chung Hua University of Medical Technology, Tainan 717, Taiwan

[g] Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, United States

[h] Chinese Medicinal Research and Development Center, China Medical University Hospital, China Medical University, Taichung 404, Taiwan

[i] Department of Pharmacy, College of Pharmacy and Health Care, Tajen University, Pingtung 907, Taiwan

To whom correspondence should be addressed. Tian-Shung Wu: Tel: 886-6-2747538. Fax: 886-2-2740552. E-mail: tswu@mail.ncku.edu.tw. Kuo-Hsiung Lee: Tel: +919-962-0066. Fax: +919-966-3893. Email: khlee@unc.edu

[#] Authors contributed equally to this work

Abstract

Twenty-one chalcones were prepared via aldol condensation and subsequent reduction of these compound led to the corresponding dihydrochalcone and 1,3-diphenylpropane derivatives. The synthetic products were examined for their effects on NO inhibition in LPS-activated mouse peritoneal macrophages. Among the tested compounds, a 1,3-diarylpropane analog, 2-(3-(3,4-dimethoxyphenyl)propyl)-5-methoxyphenol (**3p**), displayed the most significant inhibitory effects against NO production. To investigate the mechanism of action, the effects of **3p** on *i*NOS and COX-2 protein expression were studied by immunoblot. The results concluded that **3p** is capable of inhibiting *i*NOS expression in LPS-induced RAW264.7 cells via attenuation of NF-κB signaling by ERK, p38, and JNK.

Keywords:

Chalcones

Dihydrochalcones

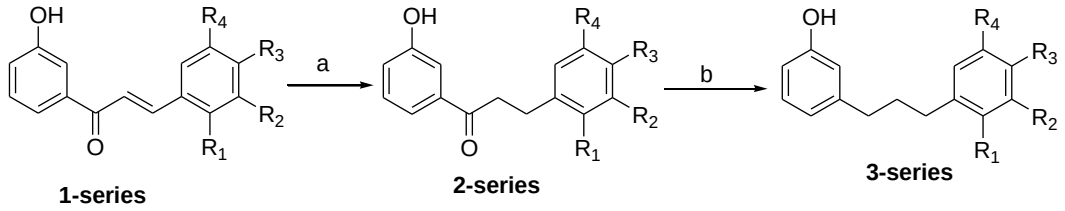
1,3-Diarylpropane analogs

Anti-inflammatory agents

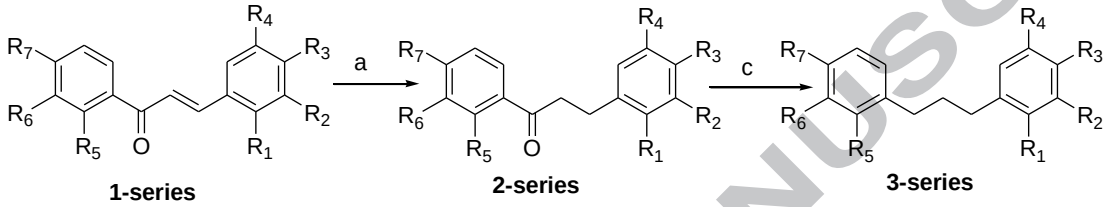
Flavonoids are a ubiquitous group of polyphenolic substances concentrated in the seeds, fruit skin or peel, bark, and flowers of most plants. Numerous plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, anti-allergic, antimutagenic, antiviral, antineoplastic, and vasodilatory actions. Many studies have shown that some flavonoids are potent antioxidants that are capable of scavenging hydroxyl radicals ($^{\cdot}OH$), superoxide anions ($O_2^{\cdot-}$), lipid peroxy radicals, and hydrogen peroxide (H_2O_2). These free radicals have been implicated several disease processes, including asthma,[1,2] cancer,[3] cardiovascular disease,[4,5] cataracts,[6,7] diabetes,[8,9] gastrointestinal inflammatory diseases,[10,11] liver disease,[12] mascular degeneration,[13,14] periodontal disease,[15] and other inflammatory processes. In the flavonoid family, chalcones and dihydrochalcones belong to a major class of bicyclic compounds, which are precursors for flavonoid biosynthesis in plants. The two aromatic rings in these compounds are linked by a three carbon bridge, specifically, propenone in chalcones and propanone in dihydrochalcones. Compounds of both types exert multiple biological activities, including anti-inflammatory, antioxidant and anticancer properties.[16-21] In addition, viscolin, a 1,3-diarylpropane, displayed potent and selective inhibition on the superoxide anion generation activated by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) combined with cytochalasin B in human neutrophils, and also exhibited free radical scavenging effects in a 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Both actions could be mediated by viscolin's

anti-inflammatory activity.[22] Anti-inflammatory and antioxidant therapies are also comprehensive pharmacological approaches in the treatment of inflammatory related disorders.[23,24]

In the present study, we synthesized three corresponding series of chalcone, dihydrochalcone, and 1,3-diphenylpropane derivatives (Fig. 1, details in Supplementary Data) and examined the compounds for *in vitro* anti-inflammatory activity using a lipopolysaccharide (LPS)-stimulated RAW264.7 cellular assay. We also evaluated the effect of a selected compound (**3p**) on nuclear factor kappa B (NF-κB) expression and mitogen-activated protein kinase (MAPK) signaling pathways to better understand the molecular mechanism.



1a, 2a, 3a). $R_1 = R_2 = R_4 = H$, $R_3 = OMe$
1b, 2b, 3b). $R_1 = R_2 = OMe$, $R_3 = R_4 = H$
1c, 2c, 3c). $R_1 = R_3 = OMe$, $R_2 = R_4 = H$
1d, 2d, 3d). $R_1 = R_4 = OMe$, $R_2 = R_3 = H$
1e, 2e, 3e). $R_2 = R_3 = OMe$, $R_1 = R_4 = H$
1f, 2f, 3f). $R_1 = R_4 = H$, $R_2 = OMe$, $R_3 = OH$



1g, 2g, 3g). $R_1 = R_4 = R_5 = R_6 = H$, $R_7 = OH$, $R_2 = R_3 = -OCH_2O-$
1h, 2h, 3h). $R_1 = R_2 = R_4 = R_5 = H$, $R_6 = R_3 = OMe$, $R_7 = OH$
1i, 2i, 3i). $R_1 = R_4 = R_5 = H$, $R_6 = OMe$, $R_7 = OH$, $R_2 = R_3 = -OCH_2O-$
1j, 2j, 3j). $R_1 = R_2 = R_6 = OMe$, $R_7 = OH$, $R_3 = R_4 = R_5 = H$
1k, 2k, 3k). $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = R_6 = R_7 = OMe$
1l, 2l, 3l). $R_1 = R_4 = R_5 = H$, $R_2 = R_3 = R_6 = R_7 = OMe$
1m, 2m, 3m). $R_1 = R_4 = R_5 = H$, $R_2 = OH$, $R_3 = R_6 = R_7 = OMe$
1n, 2n, 3n). $R_1 = R_4 = R_6 = H$, $R_5 = R_7 = OMe$, $R_2 = R_3 = -OCH_2O-$
1o, 2o, 3o). $R_1 = R_4 = R_6 = H$, $R_5 = OH$, $R_7 = OMe$, $R_2 = R_3 = -OCH_2O-$
1p, 2p, 3p). $R_1 = R_4 = R_6 = H$, $R_5 = OH$, $R_2 = R_3 = R_7 = OMe$
1q, 2q, 3q). $R_1 = R_2 = R_4 = R_6 = H$, $R_5 = OH$, $R_3 = R_7 = OMe$
1r, 2r, 3r). $R_4 = R_6 = H$, $R_1 = R_2 = R_3 = R_7 = OMe$, $R_5 = OH$
1s, 2s, 3s). $R_3 = R_4 = R_6 = H$, $R_1 = R_2 = R_7 = OMe$, $R_5 = OH$
1t, 2t, 3t). $R_2 = R_4 = R_6 = H$, $R_1 = R_3 = R_7 = OMe$, $R_5 = OH$
1u, 2u, 3u). $R_2 = R_3 = R6 = H$, $R_1 = R_4 = R_7 = OMe$, $R_5 = OH$

Fig. 1. The chemical structures of the chalcone, dihydrochalcone, and 1,3-diarylpropane analogs. Reagents and conditions: (a) Pd/C, H_2, EtOAc; (b) Ni-Al alloy (50:50), H_2O, reflux at 110-120 °C for 8-12 h; (c) TFA, $(C_2H_5)_3SiH$, reflux at 50-55 °C for 12 h.

Chalcones **1a-1u** were prepared by an aldol condensation of substituted acetophenones and aldehydes in equimolar quantities with KOH as base at room temperature (Fig. 1).[25] The stereochemistry of the olefinic carbon-carbon bond of each chalcone was established based on the appropriate ^{1}H NMR coupling constant. Chalcones **1a-1u** then underwent hydrogenation in a H_2 atmosphere with 10% Pd-C as catalyst and EtOAc as solvent for 24–48 h to obtain dihydrochalcones **2a-2u**, respectively, in good yields (Fig. 1).[26] To prepare the analogous 1,3-diarylpropanes **3a-3u**, we needed to reduce the carbonyl groups in **2a-2u** to methylene units. To the best of our knowledge, no literature exists on this specific reduction in dihydrochalcones. Also, drawbacks exist to the two common methods used for this conversion: the Wolf-Kishner reaction,[27] which cannot be applied to base sensitive substrates, and the Clemmensen reaction, which is not suitable for acid sensitive precursors, often requires harsh conditions and long reaction times, and suffers from poor yields.[28, 29] So herein, we tested two methods for the preparation of 1,3-diphenylpropanes from dihydrochacones. In method A, dihydrochalcones **2a-2f** and Ni-Al alloy (50:50)[30] were combined in H_2O at room temperature and the reaction mixture was stirred vigorously under reflux for 8–12 h giving 1,3-diphenylpropanes **3a-3f**, respectively, in good yields. However, when we applied the same procedure to compounds **2g-2u**, which have oxygenated substituents at C-4, C-2,4, or C-3,4 in ring A, no reaction was observed in most cases, likely due to conjugation and chelation effects. Subsequently, in method B,

dihydrochalcones **2g-2u** were stirred with trifluroacetic acid (TFA) and triethylsilyl hydride $[(C_2H_5)_3SiH]$[31] at 0 °C for 30 min and then at 50–55 °C for 12 h, to provide 1,3-diphenylpropanes **3g-3u**, respectively, in good yields (Fig. 1).

Cell viability and effect of 3p on LPS-induced NO production in macrophages

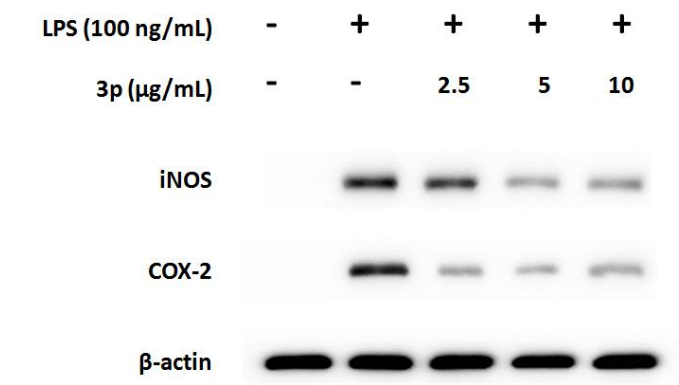
We evaluated the effects of the synthesized chalcones, dihydrochalcones, and 1,3-diphenylpropane derivatives at four concentrations (1.25, 2.5, 5, and 10 μg/mL) on RAW264.7 cell viability in a MTT assay as well as on NO production by measuring nitrite levels based on the Griess reaction in LPS-activated mouse peritoneal macrophages.[18] NO production was significantly decreased in a dose-dependent manner by the treatment with various compounds from all three series. Compounds **1h**, **1k**, **2e**–**2i**, **2k**, **3a**, **3g**, **3i**–**3m**, **3p**, and **3r** exhibited IC_{50} values in a range of 2.0 to 9.8 μg/mL (Tables 1–3, Supplementary Material), without a significant influence on cell viability at that concentration. Thus, the NO inhibitory effects of these compounds were probably not due to cytotoxic effects. Some compounds did affect cell viability, especially at the highest dose level. For example, although **1d** with a 2,5-dimethoxyphenyl ring had a low NO inhibitory IC_{50} value (2.42 μg/mL), it also reduced cell viability at that concentration (28% reduction at 2.5 μg/mL). The NO inhibitory ability did not follow similar relationships among the 1-, 2-, 3-compounds, which may suggest different binding

modes among these three series. Overall, 1,3-diarylpropane analog **3p** exhibited the best anti-inflammatory activity among the tested compounds. Other biological studies on this compound have not been reported, and the mechanisms underlying the anti-inflammatory activity have not yet been elucidated. Therefore, in the present study, we investigated possible molecular mechanisms of **3p** using LPS-stimulated RAW 264.7 cells *in vitro*.

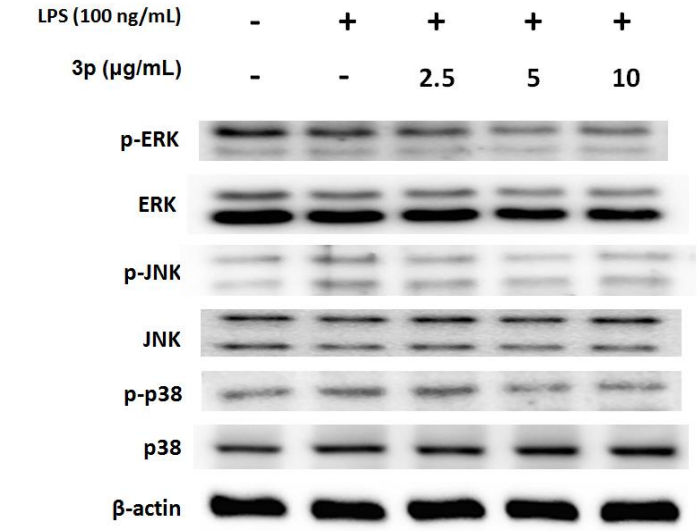
Inhibition of LPS-induced iNOS and COX-2 protein by 3p

To investigate whether the inhibition of NO production was due to decreased iNOS and COX-2 protein levels, the effect of **3p** on iNOS and COX-2 protein expression was studied by using an immunoblot. Incubation with **3p** (2.5, 5, and 10 μg/mL) in the presence of LPS (100 ng/mL) for 24 h inhibited iNOS protein expression in mouse macrophage RAW264.7 cells in a dose-dependent manner (Fig. 2A). The detection of β-actin was also performed in the same blot as an internal control.

A.



B.



C.

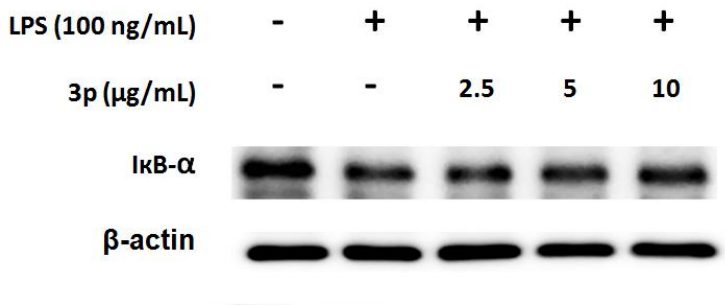


Fig. 2. Inhibition of iNOS, COX-2 (A), MAPK (ERK, JNK, and p38) (B), and NF-κB (C) protein expression by **3p** in LPS-stimulated RAW264.7 cells. Cells were incubated for 24 h (2A) or 1 h (2B, 2C) with 100 ng/mL of LPS in the absence or presence of **3p** (0, 2.5, 5, and 10 μg/mL). Compound **3p** was added 1 h before the incubation with LPS. Lysed cells were then prepared and subjected to Western blotting by using an antibody specific for iNOS, COX-2, MAPK, or NF-κB. β-Actin was used as an internal control.

Effects of 3p on the LPS-stimulated activation of MAPKs

MAPKs play critical roles in the regulation of cell growth and differentiation, and control cellular responses to cytokines and stresses. In particular, ERK, p38, and JNK are important for the activation of NF-κB.[20,21] To explore whether the inhibition of NF-κB activation by **3p** is mediated through the MAPK pathway, MAPK phosphorylation was examined by Western blot in RAW 264.7 cells pretreated with **3p** and then with LPS. As shown in Fig. 2B, **3p** suppressed the LPS-induced activation of ERK, JNK, and p38 MAPKs. However, the expression of non-phosphorylated ERK, JNK, and P38 MAPKs was unaffected by LPS or LPS plus **3p**. These results suggest that phosphorylation of MAPKs may be involved in the inhibitory effect of **3p** on

LPS-stimulated NF-κB binding in RAW 264.7 cells.

Inhibition of LPS-induced NF-κB proteins by 3p

The effect of NF-κB expression by **3p** in the presence of LPS for 1 h was assessed by Western blotting. The intensity of protein bands showed an average of 79.2% increase of NF-κB protein after treatment with **3p** at 2.5, 5, and 10 μg/mL compared with LPS alone (Fig. 2C). Therefore, we concluded that **3p** is capable of inhibiting iNOS expression in LPS induced RAW264.7 cells via attenuation of NF-κB signaling by ERK, p38, and JNK.

In summary, the synthesized chalcones, dihydrochalcones and 1,3-diarylpropanes were studied for their anti-inflammatory activity in *vitro*. This study suggested that some dihydrochalcones and 1,3-diarylpropanes, especially compound **3p**, may have the potential to be developed as anti-inflammatory agents. However, further studies are necessary to more closely examine the underlying molecular mechanisms and verify direct targets at the transcriptional or post-transcriptional level *in vitro*.

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Supplementary data

Supplementary data [Experimental procedures, NO inhibitory effects of synthesized chalcones (Table 1), dihydrochalcones (Table 2), and 1,3-diarylpropanes (Table 3)] can be found, in the online version, at

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