Mini Review

# The mechanism of anti-inflammatory activity of 2'-hydroxychalcone derivatives

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It is reported that chalcone derivatives have various biological activities such as anti-inflammatory, anti-tumor and anti-oxidant activity. We examined effects of 2'-hydroxychalcone derivatives on the production of prostaglandin (PG) $E_2$, nitric oxide (NO) and tumor necrosis factor (TNF)-α. Among fourteen 2'-hydroxychalcone derivatives, 2',4-dihydroxy-4'-methoxychalcone (compound 3), 2',4-dihydroxy-6'-methoxychalcone (compound 8) and 2'-hydroxy-4'-methoxychalcone (compound 9) showed potent inhibitory activity forward the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced $PGE_2$ production in rat peritoneal macrophages through the suppression of the induction of cyclooxygenase (COX)-2. Moreover, these three compounds inhibited both NO and TNF-α production through the inhibition of the expression of iNOS and TNF-α mRNA in the murine macrophage cell line RAW 264.7. These three compounds also suppressed the LPS-induced activation of NF-κB and AP-1 in RAW 264.7 cells, indicating that the inhibition of the production of $PGE_2$, NO and TNF-α is due to the inhibition of NF-κB and AP-1 activation. Our findings suggested that the anti-inflammatory activity of the 2'-hydroxychalcone derivatives is induced by the inhibition of the production of pro-inflammatory mediators such as $PGE_2$, NO and TNF-α. It was also suggest that the chalcone derivatives might be lead compounds for anti-inflammatory drugs.

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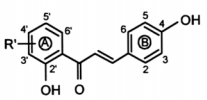
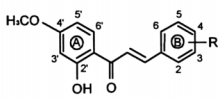
Derivatives of chalcone (1,3-diphenyl-2-propen-1-ones), a subclass of flavonoids, have been isolated from many plants used in East Asia as traditional medicines. For example, the cortex of *Brroussonetia papyrifera* Vent., containing broussochalcone, has been used for diuresis, homeostasis, and the relief of edma and cough[1)]. Also, liquorice, containing licochalcone and isoliquiritigenin, has been used for the treatment of gastric and duodenal ulcers, bronchial asthma, Addison's disease, food and drug poisoning, and skin diseases such as eczema and urticaria[2)]. Chalcone derivatives, isolated or synthesized, were studied in terms of their multiple biological actions including anti-inflammatory, anti-tumor and antioxidant effects (**Table 1**). For example, 3,4,5,3',4',5'-hexamethoxychalcone inhibits the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 induced by lipopolysaccharide (LPS) in mouse peritoneal macrophages[3)]. In addition, 2'-hydroxychalcone inhibits the tumor necrosis factor (TNF)-α-induced expression of intercellular adhesion molecule (ICAM)-1, vascular cell

**Table 1 The biological activities of natural and synthesized chalcone derivatives**

| | |
|---|---|
| Anti-inflammatory and anti-allergic activity | 1. Inhibition of iNOS expression through suppression of NF-κB activation in macrophages (Cheng Z et al, Biochem Pharmacol, 61: 939-946, 2001; Ref.3)<br>2. Inhibition of degranulation and histamine release in mast cells (Hsieh HK et al, Pharm Res, 15: 39-46, 1998; Ko HH et al, Bioorg Med Chem, 11: 105-111, 2003)<br>3. Inhibition of degranulation and superoxide production in neutrophils (Hsieh HK et al, Pharm Res, 15: 39-46, 1998; Ko HH et al, Bioorg Med Chem, 11: 105-111, 2003)<br>4. Inhibition of $PGE_2$ production through suppression of COX-2 expression in macrophages (Cheng Z et al, Biochem Pharmacol, 61: 939-946, 2001; Ref.3)<br>5. Inhibition of leukotriene $B_4$ ($LTB_4$) production through suppression of 5-LOX activity in neutrophils (De Leon EJ et al, Inflamm Res, 52: 246-257, 2003)<br>6. Inhibition of the proliferation of lymphocytes and cytokine production in monocytes and T cells (Barfod L et al, Int Immunopharmacol, 2: 545-555, 2002)<br>7. Inhibition of tyrosine kinase (EGFR, $p60^{c\text{-}src}$) activity (Ref.11)<br>8. Inhibition of TNF-α production in macrophages (Ref.12)<br>9. Inhibition of NADPH oxidase activity through suppression of protein kinase C (PKC) activity and inhibition of superoxide production in neutrophils (Wang JP et al, Eur J Pharmacol, 320: 201-208, 1997)<br>10. Inhibition of ICAM-1, VCAM-1 and E-selectin expression through suppression of NF-κB activation in HUVECs (Ref.4) |
| Anti-oxidant activity | 1. Inhibition of lipid peroxidation (Cheng Z et al, Biochem Pharmacol, 61: 939-946, 2001; Ref. 9)<br>2. Radical-scavenging activity (Cheng Z et al, Biochem Pharmacol, 61: 939-946, 2001; Ref. 9) |
| Anti-tumor activity | 1. Induction of apoptosis by induction of Bax expression, activation of caspase 3 and inhibition of Bcl-2 expression in HL-60 cells (Ref.5)<br>2. Inhibition of the efflux of glutathione S-conjugates in human colon adenocarcinoma cells (Zhang K et al, Biochem Pharmacol, 52: 1631-1638, 1996)<br>3. Inhibition of glutathione S transferase (GST) and glutathione reductase activities (Miyamoto T et al, Arch Biochem Biophys, 254: 203-213, 1987; Zhang K et al, Biochem Pharmacol, 54: 1047-1053, 1997)<br>4. Inhibition of interaction between MDM2 and p53 as an antagonist and consequently stopping cell cycle (Stoll R et al, Biochemistry, 40: 336-344, 2001)<br>5. Cytotoxicity, inhibition of angiogenesis, and enhancement of immune response by enhancement of NO production, activation of lymphocytes and increase of leukocytes in mouse renal cell carcinoma. Inhibition of pulmonary metastasis (Yamazaki S et al, Cancer Lett, 183: 23-30, 2002) |
| Other activities | 1. Anti-malarial activity (Ref.10)<br>2. Anti-bacterial, anti-fungal and anti-leishmanial activity (Ref.13; Nielsen SF et al, J Med Chem, 41: 4819-4832, 1998)<br>3. Anti-HIV activity (Wu JH et al, Bioorg Med Chem Lett, 13: 1813-1815, 2003)<br>4. Enhancement of insulin sensitivity (Kamei R et al, Life Sci, 73: 2091-2099, 2003)<br>5. Inhibition of platelet aggregation through inhibition of thromboxane (TX) production and cAMP phosphodiesterase activity (Lin CN et al, J Pharm Pharmacol, 49: 530-536, 1997; Kusano A et al, Chem Pharm Bull, 39: 930-933, 1991)<br>6. Inhibition of uric acid and superoxide by suppression of xanthine oxidase activity (Kong LD et al, Cell Mol Life Sci, 57: 500-505, 2000)<br>7. Inhibition of monoamine oxidase (MAO) activity (Tanaka S et al, Planta Med, 53: 5-8, 1987)<br>8. Increase of cGMP by enhancement of guanylate cyclase activity and relaxation of rat aorta (Yu SM et al, Br J Pharmacol, 114: 1587-1594, 1995)<br>9. Inhibition of aldose reductase activity (Aida K et al, Planta Med, 56: 254-258, 1990)<br>10. Inhibition of liver fibrosis by suppression of tissue inhibitor of metalloproteinase-1 (TIMP-1) expression and collagen accumulation (Lee SH et al, Planta Med, 69: 990-994, 2003) |

**Table 2 Effects of A-and B-ring-modified 2'-hydroxychalcone derivatives on TPA-induced $PGE_2$ production in rat peritoneal macrophages[6)]**




| Compound | R' | $PGE_2$ (ng/mL) | | Compound | R | $PGE_2$ (ng/mL) | |
|---|---|---|---|---|---|---|---|
| None | | 1.34±0.18*** | | None | | 1.40±0.18*** | |
| TPA | | 8.75±0.14 | | TPA | | 9.17±0.87 | |
| | | 3 μM | 10 μM | | | 3 μM | 10 μM |
| 1 | H | 6.13±0.36** | 4.61±0.45*** | 9 | H | 3.78±0.99*** | 1.78±0.16*** |
| 2 | 4'-OH | 8.00±0.92 | 5.12±0.27*** | 10 | 3,4-di $OCH_3$ | 8.26±0.12 | 5.57±0.17* |
| 3 | 4'-$OCH_3$ | 3.79±0.61*** | 1.58±0.34*** | 11 | 4-Cl | 8.42±0.99 | 5.86±0.96* |
| 4 | 5'-Cl | 7.36±1.07 | 6.06±0.57* | 12 | 4-$CH_3$ | 7.82±1.26 | 4.39±0.43** |
| 5 | 5'-$CH_3$ | 9.34±0.86 | 6.63±0.68* | 13 | 4-$OCH_3$ | 6.32±0.69 | 4.98±1.05* |
| 6 | 5'-OH | 6.00±0.14*** | 3.78±0.42*** | 14 | 4-N $(CH_3)_2$ | 7.29±0.75 | 4.51±0.25*** |
| 7 | 5'-$OCH_3$ | 8.47±0.15 | 6.44±0.31** | | | | |
| 8 | 6'-$OCH_3$ | 4.30±0.47*** | 1.50±0.16*** | | | | |

Rat peritoneal macrophages (1.5 x $10^6$ cells/mL) were incubated at 37°C for 8 h in 0.5 mL of medium containing TPA (16.2 nM) and the indicated concentrations of each compound. Values are the means±SEM from four samples; *$P$ <0.05, **$P$ <0.01 and ***$P$ <0.001 vs. TPA control.

adhesion molecule (VCAM)-1 and E-selectin in human umbilical vein endothelial cells[4)]. Butein (3,4,2',4'-tetrahydroxychalcone) induces apoptosis through an increase in caspase-3 activity and Bax expression, and a decrease in Bcl-2 expression in HL-60 cells[5)].

Here, we describe that 2'-hydroxychalcone derivatives inhibit 12-*O*-tetradecanoylphorbol 13-acetate (TPA)- or LPS-induced production of prostaglandin $E_2$ ($PGE_2$), nitrite and tumor necrosis factor (TNF)- α [6,7)]. The action mechanism of 2'-hydroxychalcone derivatives will also be described[7)].

## Anti-inflammatory activity of 2'-hydroxychalcone derivatives *in vitro*

### 1)Effects of 2'-hydroxychalcone derivatives on TPA-induced production of $PGE_2$ in rat peritoneal macrophages[6)]

We have analyzed the effects of 14 derivatives of 2'-hydroxychalcone on TPA-induced $PGE_2$ production in rat peritoneal macrophages (**Table 2**). When rat peritoneal macrophages were incubated at 37°C for 8 h in medium containing TPA (16.2 nM), $PGE_2$ production was prominently increased (**Table 2**). In the presence of 10 μM of each 2'-hydroxychalcone derivative, the TPA-induced $PGE_2$ production was significantly suppressed (**Table 2**). Among the fourteen compounds, 2',4-dihydroxy-4'-methoxychalcone (compound 3), 2',4-dihydroxy-6'-methoxychalcone (compound 8) and 2'-hydroxy-4'-methoxychalcone (compound 9) had the most potent inhibitory effect (**Table 2**). Substitution of the 4'-methoxyl group of compound 3 with a 4'-hydroxyl group (compound 2) decreased the inhibitory activity. Substitution with a 5'-methoxyl group (compound 7) also decreased the inhibitory activity, while replacing the 4'-methoxyl group of compound 3 with a 6'-methoxyl group (compound 8) did not change the inhibitory effect. Therefore, a methoxyl group at 4' or 6' is essential for the expression of the inhibitory activity of 2'-hydroxychalcone derivatives. Among the 2'-hydroxychalcones whose 5' position was substituted with a chloro group (compound 4), methyl group (compound 5), hydroxyl group (compound 6) or methoxyl group (compound 7), 2',5'-dihydroxychalcone (compound 6) had the strongest inhibitory activity, but the activity did not exceed that of compound 3 or 8. Addition of a chloro group (compound 11), methyl group (compound 12), methoxyl group (compound 13), or *N*-dimethyl group (compound 14) at position 4 of the B ring decreased the inhibitory potency of compound 9 whose B ring had no substitution. These findings indicate that for 2'-hydroxychalcones, the addition of a 4'-methoxyl group or 6'-methoxyl group is important to express the potent inhibitory activity against the TPA-induced $PGE_2$ production. As for the B ring of 2'-hydroxy-4'-methoxychalcones, substitution with another group at position 4 decreased the inhibitory activity of compound 3. The inhibitory activity of compounds 10 and 13 was much less than that of compounds 3 and 9, indicating that the addition of a methyl

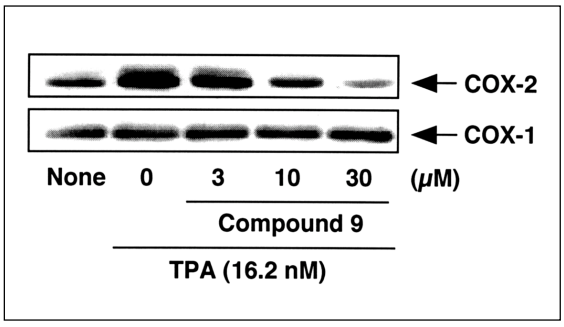



**Fig.1 Effects of 2'-hydroxy-4'-methoxychalcone (compound 9) on TPA-induced increase in COX-2 protein levels in rat peritoneal macrophages[6]**

**Rat peritoneal macrophages (1.5 x $10^6$ cells/mL) were incubated for 6 h at 37°C in 2 mL of medium containing TPA (16.2 nM) and the indicated concentrations of compound 9. The protein levels of COX-2 and COX-1 were determined by Western blot analysis.**

group at position 3 and 4 of the B ring lowers the inhibitory activity. Dehydration at position 4 of compound 3 did not affect the inhibitory activity, indicating that the hydroxyl group at position 4 of the B ring is not essential for the expression of the inhibitory activity.

Various pharmacological activities of chalcone derivatives, including our results, depend on both substituted groups and substituted positions on two aromatic rings[8-11]. The size of the substituted group in three-dimensional structure is also related to the activity of chalcone derivatives such as inhibition of 5-lipoxygenase (5-LO) activity[8], and antimalarial activity[10]. Furthermore, the C2-C3 double bond is essential because the reduction of this bond abolishes various activities of chalcone derivatives such as inhibition of TNF-α production[12], and anti-fungal and anti-bacterial activities[13].

### 2)Effects of 2'-hydroxychalcone derivatives on TPA-induced COX-2 expression in rat peritoneal macrophages[6]

To clarify the mechanism by which compounds 3, 8 and 9 inhibit the TPA-induced production of $PGE_2$, we have examined the effect of compounds 3, 8 and 9 on the TPA-induced expression of COX-2 in rat peritoneal macrophages. As shown in **Fig.1**, treatment with TPA at 37°C for 6 h induced the expression of COX-2, but not COX-1, and compound 9 inhibited the COX-2 induction in a concentration-dependent manner at 3-30 μM, in accordance with the potency at with which $PGE_2$ production was inhibited (Table 2). Compounds 3 and 8 had almost the same effect as compound 9, and other compounds had no such effect at 30 μM (data not shown). These findings suggested that the inhibition of the TPA-induced $PGE_2$ production by these compounds is due to the inhibition of TPA-induced expression of COX-2.

### 3) Effects of 2'-hydroxychalcone derivatives on LPS-induced production of nitrite and TNF-α in RAW 264.7 cells[7]

Effects of compounds 3, 8 and 9 on the LPS (0.1 μg/ml)-induced production of nitrite and TNF-α in the murine macrophage cell line RAW 264.7 were then examined. Incubation with LPS (0.1 μg/ml) markedly increased nitrite production at 12 h (**Fig. 2B**) and TNF-α production at 6 h (**Fig.2D**). Under these conditions, compound 9 inhibited the LPS-induced production of nitrite (**Fig.2B**) and TNF-α production (**Fig.2D**) in a concentration-dependent manner at 3-30 μM, in parallel with the inhibition of the LPS-induced expression of iNOS (**Fig.2A**) and TNF-α mRNA (**Fig.2C**), respectively. Compounds 3 and 8 also showed almost the same potency as compound 9 (data not shown). These findings suggested that the inhibition of the LPS-induced production of nitrite and TNF-α by these compounds is due to the suppression of the LPS-induced expression of iNOS protein and TNF-α mRNA, respectively.

### 4) Effects of 2'-hydroxychalcone derivatives on LPS-induced activation of NF-κB and AP-1 in RAW 264.7 cells[7]

Nuclear factor (NF)-κB and activator protein (AP)-1 are essential transcription factors responsible for the expression of COX-2 [14], iNOS [15] and TNF-α [16]. Thus, to clarify the mechanism of action of the 2'-hydroxychalcone derivatives for the inhibition of $PGE_2$, nitrite and TNF-α production, effects of compound 9 on the LPS-induced activation of NF-κB and AP-1 were examined by electrophoretic mobility shift assay(EMSA). In the presence of compound 9 at 3-30 μM, the LPS-induced activation of NF-κB and AP-1 at 1 h was suppressed in a concentration-dependent manner (**Fig.3**). In addition, compound 9 inhibited the degradation of IκB-α at 20 min and the phosphorylation of both c-Jun and c-Jun N-terminal kinase (JNK) at 30 min[7] (data not shown). Compounds 3 and 8 also suppressed the activation of both NF-κB and AP-1 (data not shown). These

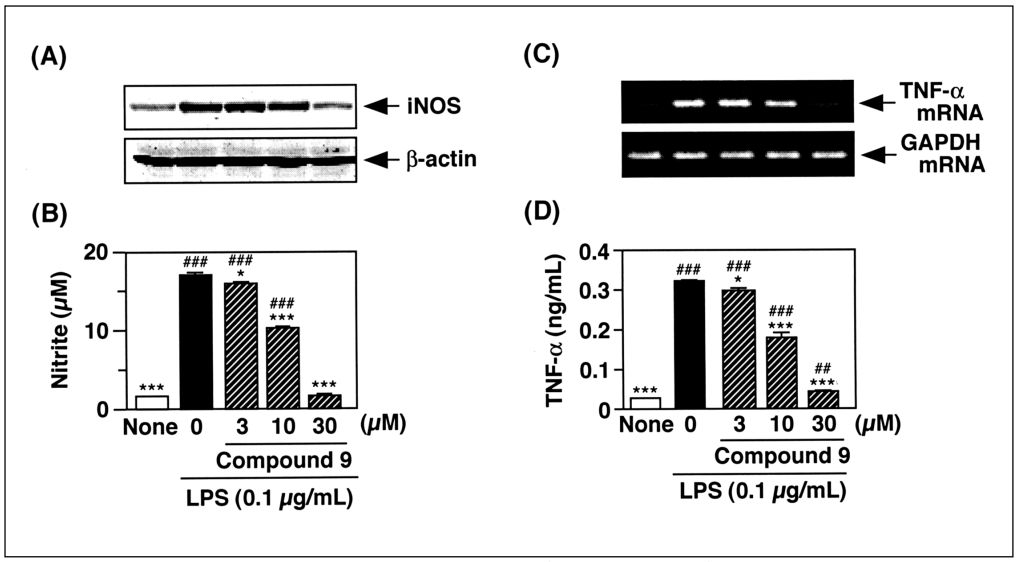



**Fig.2 Effects of 2'-hydroxy-4'-methoxychalcone (compound 9) on LPS-induced increases in the levels of iNOS protein and TNF-α mRNA, and the production of nitrite and TNF-α[7)]**

RAW 264.7 cells were suspended at 1 x $10^6$ cells/mL of medium containing the indicated concentrations of compound 9, and 2 mL (A,C) or 0.5 mL (B,D) of the cell suspension was preincubated for 1 h at 37°C. The cells were then washed three times with PBS, suspended in 2 mL (A,C) or 0.5 mL (B,D) of medium containing LPS (0.1 μg/mL) and the corresponding concentrations of each drug, and incubated at 37°C for 12 h (A,B), 4 h (C) or 6 h (D). (A) The protein levels of iNOS and β-actin were determined by Western blot analysis. (B) Nitrite concentrations in the conditioned medium were determined using Griess reagent. (C) The levels of mRNA for TNF-α and GAPDH were detected by RT-PCR. (D) TNF-α concentrations were determined by ELISA. Values are the means from 3-4 samples with the SEM shown by vertical bars. Statistical significance: $^{\#\#\#}p < 0.001$ vs. None, $^{*}p < 0.05$ and $^{***}p < 0.001$ vs. LPS control.

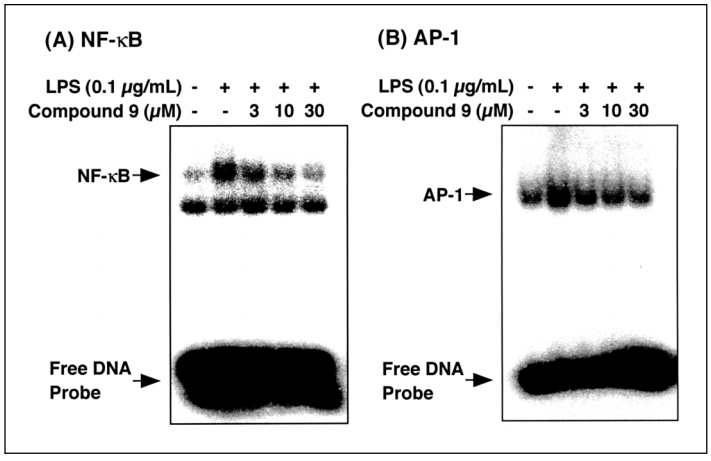



**Fig.3 Effects of 2'-hydroxy-4'-methoxychalcone (compound 9) on LPS-induced activation of NF-κB and AP-1[7)]**

RAW 264.7 cells were suspended at 1 x $10^6$ cells/mL of medium containing the indicated concentrations of compound 9, and 4 mL of the cell suspension was preincubated for 1 h at 37°C. The cells were then washed three times with PBS, suspended in 4 mL of medium containing LPS (0.1 μg/mL) and the corresponding concentrations of compound 9, and incubated for 1 h at 37°C. After incubation, nuclear proteins were extracted, and the amount of NF-κB (A) and AP-1 (B) bound to each DNA probe was detected by EMSA. Similar results were obtained in three separate sets of experiments.

findings suggested that 2'-hydroxychalcone derivatives suppress the activation of NF-κB through the inhibition of IκB-α degradation, and the activation of AP-1 via the inhibition of c-Jun phosphorylation by the inhibition of JNK phosphorylation.

It is reported that redox regulation is involved in the activation of NF-κB[17], and the anti-oxidant reagents *N*-acetylcysteine and pyrrolidine dithiocarbamate inhibit the activation of NF-κB[18]. Furthermore, these antioxidants strongly suppressed the production of $PGE_2$, nitrite and TNF-α[19,20]. It is also reported that 2'-hydroxychalcone has potent antioxidant activity[9]. Therefore, it is possible that the 2'-hydroxychalcone derivatives examined in this study inhibit the activation of NF-κB through their antioxidant activity. The inhibition of IκB-α degradation by the 2'-hydroxychalcone derivatives indicated that these compounds inhibit upstream of IκB-α degradation, and the inhibition of the phosphorylation of JNK by these compounds indicated that they inhibit upstream of JNK. It is possible that 2'-hydroxychalcone derivatives interact directly with an upstream kinase such as tyrosine kinase. In fact, in the analysis of the action mechanism of chalcone derivatives, it has been suggested that they inhibit 5-lipoxygenase (5-LO) activity via the direct interaction with the enzyme through hydrogen bonding, electrostatic and ionic interactions and π-π interactions[8]. In addition, it is reported that the chalcone derivatives butein, marein and phloretin dock into the ATP-binding pocket of epidermal growth factor receptor (EGFR), a tyrosine kinase, and hydrogen bonds and hydrophobic interaction appear to be important in the binding of these chalcone derivatives to EGFR[11].

In conclusion, our findings suggested that 2'-hydroxychalcone derivatives might be lead compounds of anti-inflammatory drugs.

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