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# SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF CHALCONE DERIVATIVES

Felipe Herencia[a], M. Luisa Ferrándiz*[a], Amalia Ubeda*[a], José N. Domínguez*[b], Jaime E. Charris[b], Gricela M. Lobo[b], M. José Alcaraz[a]

[a]*Departamento de Farmacología, Universidad de Valencia, 46100 Burjasot, Valencia, Spain and* [b]*Laboratorio de Síntesis Orgánica, Facultad de Farmacia, Universidad Central de Venezuela, Caracas 1051, Venezuela.*

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**Abstract**: Chalcones and their derivatives were synthesized and evaluated for their anti-inflammatory activity. *In vitro*, chalcones **2**, **4**, **8**, **10** and **13** inhibited degranulation and 5-lipoxygenase in human neutrophils, whereas **11** behaved as scavenger of superoxide. Only four compounds (**4-7**) inhibited cyclo-oxygenase-2 activity. The majority of these samples showed anti-inflammatory effects in the mouse air pouch model.


## Introduction

Human leukocytes synthesize a series of bioactive metabolites of arachidonic acid upon inflammatory stimulation, with participation of enzymes such as phospholipase $A_2$ ($PLA_2$), cyclo-oxygenase (COX) and 5-lipoxygenase (5-LO). Prostaglandins (PGs) can be produced by the activity of two enzymes, COX-1 and COX-2[1]. The first activity is expressed constitutively in most mammalian tissues, while COX-2 is an inducible enzyme which gives raise to the increased PGs levels in the inflammatory process[2]. 5-LO catalyzes the first step in the synthesis of leukotrienes (LTs), which increase vascular permeability and besides, $LTB_4$ is a potent chemotactic mediator and activating agent for leukocytes[3,4]. Inhibition of leukocyte functions and/or lipid mediator biosynthesis could be an important therapeutic intervention in inflammatory diseases and it may lead to the discovery of new drugs as alternative approaches to conventional anti-inflammatory agents possessing a high incidence of severe side-effects[5]. We have reported previously the inhibitory effect of 2'-hydroxy-3',4',3,4-tetramethoxychalcone on the generation of eicosanoids and elastase release by human cells[6]. In the present study, we describe the synthesis and evaluation of anti-inflammatory activity of 2-choroquinolinyl chalcones and other chalcone derivatives using human cell systems and the mouse air pouch model

**Chemistry**. The general synthetic strategy employed to prepare several chalcone analogues was based on Claisen-Schmidt condensation, which has been previously reported[7]. As shown in Table 1, a series of 2-chloroquinolinyl[8] chalcones (**1-8**) and other chalcone derivatives [9] (**9-13**) were prepared by condensing aromatic aldehydes and methyl ketones to form the expected compounds, using solid sodium hydroxide as a catalyst in methanol at room temperature. In most cases, the starting materials were commercially available or

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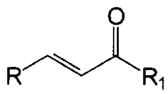
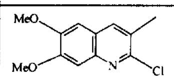
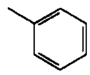
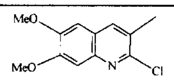
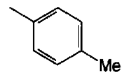
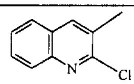
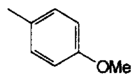
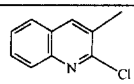
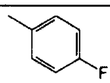
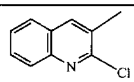
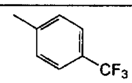
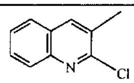
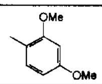
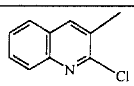
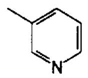
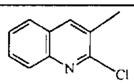
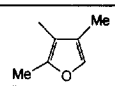
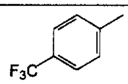
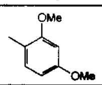
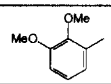
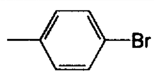
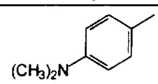
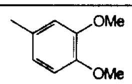
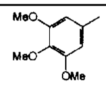
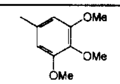
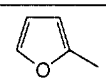
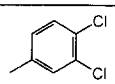
could be prepared[10] in good yields (**1**,**2**). The products obtained were formed immediately after addition of the sodium hydroxide pellet to the well stirred mixture of aldehyde and methyl ketone as the unsaturated ketones which always yielded the trans-alkene (E-form) as judged by $^1$H NMR spectroscopy. Yields ranged from 56% to quantitative and were not always optimized.

**Table 1**. Physicochemical properties of chalcone derivatives




| Compound | R | $R_1$ | m.p. (°C)[a] | yields (%) (solv)[b] |
|---|---|---|---|---|
| **1** |  |  | 181 - 182 | 67(A) |
| **2** |  |  | 204 - 205 | 83(B) |
| **3** |  |  | 168 - 170 | 85(C) |
| **4** |  |  | 232 - 234 | 74(C) |
| **5** |  |  | 226 - 228 | 68(C) |
| **6** |  |  | 163 - 165[c] | 73(C) |
| **7** |  |  | 268 - 270[c] | 56(C) |
| **8** |  |  | 129 - 131 | 81(D) |
| **9** |  |  | 115 - 116[c] | 60(C) |
| **10** |  |  | 110 - 112 | 80(E) |
| **11** |  |  | 119 - 121 | 85(E) |
| **12** |  |  | 130 - 131 | 96(E) |
| **13** |  |  | 80 - 82 | 85(E) |

[a] m.p are uncorrected; [b] Recrystallization solvents: (A) $DMF/H_2O$, (B) $CH_3OH/H_2O$; (C) $CH_3OH$; (D) Purified by column chromatography (eluent, $CH_2Cl_2$); (E) EtOH/H$_2$o; [c] Ref. 7.

**Biology.**

**Methods.** Elastase release was assessed after stimulation of human neutrophils with cytochalasin B (10 μM)+N-formyl-L-methionil-L-leucyl-L-phenylalanine (10 nM) and $LTB_4$ release using ionophore A23187 as stimulus. 5-LO activity was determined in high speed supernatants from sonicated human neutrophils and COX-1 activity in microsomes from human platelets. To assess the effects of chalcones on COX-2, this activity was induced in human monocytes by *E. coli* lipopolysaccharide (10 μg/ml) treatment for 24 h and $PGE_2$ levels were determined in supernatants by radioimmunoassay[11]. Secretory $PLA_2$ was assayed by using a modification of the method of Franson et al.[12] using [$^3$H]oleate labelled membranes of *E. coli* as substrate and *Naja naja* venom, bee venom, and porcine pancreas enzymes[13]. For chemiluminescence measurements, neutrophils ($2.5 \times 10^6$/ml) were mixed with luminol (40 μM) and stimulated with 12-*O*-tetradecanoyl phorbol 13-acetate (1 μM). The chemiluminescence was recorded in a Microbeta Trilux counter. Superoxide anions were also generated by the hypoxanthine/xanthine oxidase system[14]. We had previously found that compounds did not inhibit xanthine oxidase activity at the concentrations used following the formation of uric acid. Mouse air pouch experiments were performed as descrived previously[11], compounds were injected into the air pouch. Leukocytes present in exudates were measured using a Coulter counter. After centrifugation of exudates at 1,200 x g at 4°C for 10 min, the supernatants were used to measure $LTB_4$ and $PGE_2$ levels as above, as well as tumour necrosis factor α (TNFα) by ELISA.

**Results and Discussion.** Leukocytes play an important role in host defense but they can produce cellular damage in host tissues during inflammatory conditions through degranulation and generation of reactive species and different mediators. Our data show that some chalcone derivatives inhibit *in vitro* leukocyte functions, such as degranulation, generation of reactive oxygenated species and the production of eicosanoids. As shown in Table 2, compounds **2**, **4**, **8**, **10** and **13** inhibited elastase release and $LTB_4$ synthesis in stimulated human neutrophils, with a potency in the μM range. The 5-LO inhibitor ZM230,487 showed $IC_{50}$ values and 95% confidence limits of 0.06 (0.03-0.1) and 0.09 (0.06-0.1) μM on intact cells and cytosolic fractions, respectively, being without effect on elastase release. In the cellular system used, the ionophore A23187 causes release of free arachidonic acid, which is rapidly metabolized to eicosanoids. The inhibition of $LTB_4$ generation in intact cells is likely the consequence of a direct effect on 5-LO, as the active chalcones inhibited this enzyme in cytosolic fractions of human neutrophils at the same concentrations. In contrast, none of the compounds affected $PLA_2$ activity (data not shown). COX-2 activity in intact human monocytes was inhibited by **4**, **5**, **6** and **7**, whereas COX-1 activity (from human platelets) was not modified at the same concentration (data not shown). In this system the reference inhibitor NS398 showed an $IC_{50}$ value of 3.2 (1.6-7.9) μM. On the other hand, only compound **11** was significantly active as scavenger of superoxide anion generated by stimulated human neutrophils or by the hypoxanthine/xanthine oxidase system (Figure 1), with $IC_{50}$ values of 0.1 (0.1-0.2) and 0.3 (0.1-0.9) μM, respectively.

**Table 2**: Effect of chalcone derivatives on *in vitro* tests: elastase release, $LTB_4$ biosynthesis (human neutrophils), 5-LO activity (cytosolic fractions of human neutrophils), and COX-2 activity (human monocytes). Results show mean±SEM of percentages of inhibition at the concentration of 10 μM (n=6), or $IC_{50}$ μM with 95 % confidence limits for the most active compounds. * P<0.05; ** P<0.01, Dunnett's t test

| Compound | Elastase | $LTB_4$ | 5-LO | COX-2 |
|---|---|---|---|---|
| **1** | 0 | 42.2±3.2** | 42.0±5.9** | 0 |
| **2** | 40.7±5.3** | 9.8 (7.9-12.6) | 61.0±3.1** | 0 |
| **3** | 0 | 30.2±9.7 | 42.3±1.6** | 30.0±6.0 |
| **4** | 3.0 (2.4-3.9) | 6.2 (5.2-7.6) | 3.9 (2.5-5.2) | 36.3±4.5* |
| **5** | 0 | 36.9±2.1* | 32.9±6.2 | 35.6±5.3* |
| **6** | 0 | 10.7±6.8 | 27.6±7.3 | 41.8±5.5** |
| **7** | 0 | 26.4±4.5 | 30.2±5.7 | 46.6±4.4* |
| **8** | 8.4 (7.9-8.7) | 53.5±3.4** | 3.5 (2.7-4.5) | 0 |
| **9** | 0 | 35.4±4.9* | 21.7±2.5 | 0 |
| **10** | 2.2 (1.9-2.6) | 6.7 (5.8-7.9) | 6.1 (5.3-7.6) | 0 |
| **11** | 0 | 0 | 37.9±7.8 | 0 |
| **12** | 0 | 21.1±3.2 | 14.6±4.3 | 0 |
| **13** | 5.4 (4.8-6.2) | 4.8 (4.5-5.2) | 4.1 (2.9-5.9) | 0 |

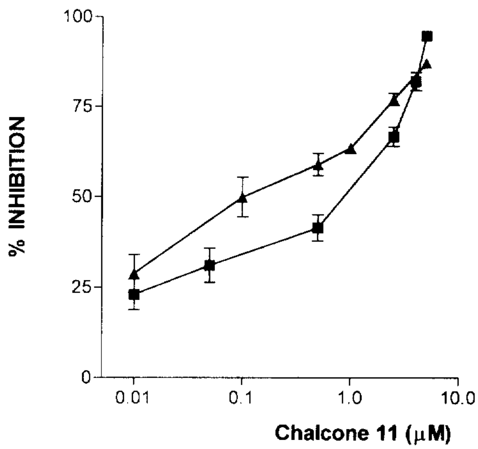



**Figure 1**. Concentration-dependent inhibitory effect of **11** on superoxide anion generated by human neutrophils (triangles) or the hypoxanthine/xanthine oxidase system (squares). Results show mean±SEM of percentages of inhibition (n=6). All points are statistically significant at least at P<0.05.

We used an *in vivo* model of inflammation to test these compounds, the mouse air pouch injected with zymosan. This stimulus induced neutrophil infiltration into the pouch, which was diminished at the dose of 100 nmol/pouch by the chalcones able to inhibit cellular functions *in vitro*, besides **5** and **7** (Table 3). Both $PGE_2$ and $LTB_4$ are elevated in zymosan-treated mice over the levels observed in saline-injected animals. The effect of chalcones on $LTB_4$ levels in the mouse air pouch was lower than that observed *in vitro*. In contrast, several chalcones reduced the $PGE_2$ content of air pouch exudates. Interestingly, TNFα levels were significantly reduced by chalcones **6**, **8**, **10** and **11**, suggesting the participation of mechanisms of action other than inhibition of eicosanoid synthesis.

The antimalarial drug chloroquine have anti-inflammatory effects with inhibition of TNFα release[15]. Some of these chloroquinolinyl chalcones possess antimalarial activity[7], although their effects on mammalian cells have not been described. Interestingly, these chalcone derivatives are cell permeant inhibitors, active on human cells, and they interact with enzymes metabolizing arachidonic acid rather than the mobilization of arachidonic acid from membrane phospholipids. For chloroquinolinyl chalcones, substituents (especially halogen) in the B ring, or the presence of a pyridine at B, favour the anti-inflammatory effects. This last feature increases the COX-2 inhibitory effects *in vitro* and *in vivo*. In addition, our results indicate that some chalcones could inhibit the release of TNFα, a cytokine relevant to the inflammatory process. Thus, this group of chalcone derivatives could be a model for the synthesis of new anti-inflammatory agents and further studies should be performed to determine their therapeutical value.

**Table 3:** Effect of chalcone derivatives in the mouse air pouch. Results show mean±SEM of percentages of inhibition at the dose of 100 nmol/pouch (n=6). * P<0.05; ** P<0.01, Dunnett's t test.

| Compound | Migration | $PGE_2$ | $LTB_4$ | TNFα |
|---|---|---|---|---|
| **1** | 0 | 29.9±5.5 | 0 | 35.7±15.1 |
| **2** | 38.6±6.3* | 59.5±4.2** | 15.2±2.9 | 11.2±4.7 |
| **3** | 20.4±5.2 | 23.2±10.3 | 20.9±1.3 | 0 |
| **4** | 36.6±6.8* | 58.3±2.9** | 18.5±1.2 | 0 |
| **5** | 39.7±5.7* | 46.5±6.0** | 13.0±0.8 | 24.5±5.5 |
| **6** | 0 | 36.2±2.8* | 11.1±5.3 | 52.1±4.0** |
| **7** | 53.0±7.2** | 69.5±4.3** | 30.7±6.1 | 12.1±2.9 |
| **8** | 47.1±8.4* | 56.8±4.8** | 19.5±3.8 | 34.4±1.1** |
| **9** | 0 | 25.5±9.2 | 45.9±9.0 | 0 |
| **10** | 35.7±4.5* | 62.0±4.2** | 40.0±4.4* | 44.0±3.8** |
| **11** | 20.6±9.5 | 75.8±4.1** | 41.1±7.1 | 64.2±8.2** |
| **12** | 35.0±8.0 | 0 | 27.5±6.2 | 25.7±11.7 |
| **13** | 47.3±7.8* | 71.0±6.5** | 0 | 0 |

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**References and Notes**

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8. Spectral data for 2-chloroquinolinyl chalcone analogues: (**1**): $^1$H NMR ($CDCl_3$, 270 MHz) 4.00 (s, 6H, $OCH_3$), 7.06 (s, 1H, 5-H), 7.31 (s, 1H, 8-H), 7.51-7.59 (m, 4H, Ar, α-H), 8.03 (m, 2H, Ar), 8.19 (d, J=15.8 Hz, 1H, β-H), 8.31 (s, 1H, 4-H). (**2**): $^1$H NMR ($CDCl_3$) 2.4 (s, 3H, $CH_3$), 4.0 (s, 6H, $OCH_3$), 7.06 (s, 1H, 5-H), 7.2 (d, J=8.12 Hz, 2H, 2' and 6'), 7.31 (s, 1H, 8-H), 7.5 (d, J=15.8 Hz, 1H, α-H), 7.9 (d, J=8.12 Hz, 2H, 3' and 5'), 8.1 (d, J=15.9 Hz, 1H, β-H), 8.3 (s, 1H, 4-H). (**3**): $^1$H NMR ($CDCl_3$) 3.82 (s, 3H, $OCH_3$), 7.29 (d, J=8.7 Hz, 2H, 3' and 5'-H), 7.74-7.92 (m, 3H, Ar), 8.09-8.16 (m, 2H, Ar, α-H), 8.45 (d, J=15.01 Hz, 1H, β -H), 8.76 (s, 1H, 4-H). (**4**): $^1$H NMR ($CDCl_3$) 7.23 (d, J=8.66 Hz, 2H, 3' and 5'-H), 7.58 (d, J=15.5 Hz, 1H, α -H), 7.59-7.60 (m, 1H, Ar), 7.78-8.12 (m, 5H, Ar), 8.17 (d, J=15.5 Hz, 1H, β-H), 8.49 (s, 1H, 4-H). (**5**): $^1$H NMR ($CDCl_3$) 7.34 (d, J=8.3 Hz, 2H, 3' and 5'-H), 7.57 (dd, J=8.4 Hz, 1H, 5-H), 7.70-7.73 (m, 3H, Ar and α -H), 7.87 (d, J=8.4 Hz, 2H, 2' and 6'-H), 7.95 (d, J=16.2 Hz, 1H, β-H), 8.04 (dd, J=8.4 Hz, 1H, 8-H), 8.2 (s, 1H, 4-H). (**6,7**)[7]; (**8**): $^1$H NMR ($CDCl_3$) 2.21 (s, 3H, CH3), 2.58 (s, 3H, $CH_3$), 7.12 (s, 1H, 5'-H), 7.26 (d, J=11 Hz, 1H, 5-H), 7.61 (dd, J=7.8 and 1.2 Hz, 1H, 6-H), 7.78 (dd, J=1.2 and 7.8 Hz, 1H, 7-H), 7.89 (d, J=8.2 Hz, 1H, 8-H), 8.02 (d, J=9 Hz, 1H, α -H), 8.06 (d, J=12 Hz, 1H, β-H), 8.4 (s, 1H, 4-H). In addition all compounds had IR, LSIMS and elemental analysis in complete agreement with the assigned structures.
9. Spectral data for other chalcone derivatives; (**9**)[7]; (**10**): $^1$H NMR ($CDCl_3$) 3.86 (s, 6H, $OCH_3$), 6.96 (d, J=7.4 Hz, 1H, 6-H), 7.07 (dd, J=7.9 Hz, 1H, 5-H), 7.24 (d, J=6.9 Hz, 1H, 4-H), 7.48 (d, J=16.0 Hz, 1H, α-H), 7.62 (d, J=8.6 Hz, 2H, 3' and 5'-H), 7.87 (d, J=8.6 Hz, 2H, 2'- and 6'-H), 8.05 (d=, J=16.0 Hz, 1H, β-H). (**11**): $^1$H NMR ($CDCl_3$) 3.02 (s, 6H, $N(CH_3)_2$), 3.94 (s, 3H, $OCH_3$), 3.95 (s, 3H, $OCH_3$), 6.68 (d, J=8.9 Hz, 2H, 3 and 5-H), 6.9 (d, J=8.4 Hz, 1H, 5'-H), 7.34 (d, J=15.3 Hz, 1H, α-H), 7.54 (d, J=8.9 Hz, 2H, 2 and 6-H), 7.6 (d, J=1.73 Hz, 1H, 2'-H), 7.67 (dd, J=8.4 and J=1.73 Hz, 1H, 6'-H), 7.78 (d, J=15.5 Hz, 1H, β-H). (**12**): $^1$H NMR ($CDCl_3$) 3.88 (s, 6H, $OCH_3$), 3.90 (s, 6H, $OCH_3$), 3.92 (s, 6H, $OCH_3$), 6.84 (s, 2H, 2' and 6'-H), 7.23 (s, 2H, 2 and 6-H), 7.30 (d, J=15.5 Hz, 1H, α-H), 7.69 (d, J=15.5 Hz, 1H, β -H). (**13**): $^1$H NMR ($CDCl_3$) 6.51 (m, 1H, 5'-H), 6.75 (d, J=3.21 Hz, 1H, 3-H), 7.36 (d, J=15.7 Hz, 1H, α-H), 7.55 (m, 2H, 4 and 5-H), 7.59 (d, J=15.7 Hz, 1H, β-H), 7.83 (dd, J=8.09 and 1.72 Hz, 1H, 6'-H), 8.08 (d, J=1.75 Hz, 1H, 2'-H). In addition all compounds had IR, LSIMS and elemental analysis in complete agreement with the assigned structures.
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