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Synthesis and Evaluation of Novel Curcumin Derivatives as Polyphenols with Anti-Inflammatory Properties

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Abstract:

In this study, we synthesized thirteen derivatives by performing Steglich esterification on one or both phenolic rings of curcumin, aiming to enhance their anti-inflammatory activity. The monofunctionalized compounds exhibited superior bioactivity compared to the difunctionalized ones, particularly in terms of inhibiting IL-6 production. Among all the derivatives, compound 2 showed the highest activity in this regard. Additionally, compound 2 demonstrated strong activity against PGE2 synthesis. Through structure-activity relationship studies, we found that the presence of a free hydroxyl group or aromatic ligands on the curcumin ring, along with the absence of a linker moiety, contributed to increased activity. Compound 2 remained the most effective in modulating IL-6 production and showed potent activity against PGE2 synthesis.

Keywords: curcumin; curcumin derivatives; succinate; difunctionalized; monofunctionalized; cytokine; IL-6; PGE2 ; anti-inflammatory activity; structure–activity relationship.

1. Introduction:

In this study, we present the synthesis and evaluation of novel curcumin derivatives with potential anti-inflammatory properties [1]. Curcumin, a fascinating polyphenol, is known for its diverse biological effects, including anti-inflammatory, antioxidant, neuroprotective, and anticancer activities [2]. However, its practical use is limited due to poor bioavailability caused by low water solubility and instability in physiological conditions [3] .

To address these challenges, we sought to protect the vulnerable sites of curcumin by incorporating succinyl groups to create derivative [4]. This protective strategy aimed to enhance stability and increase bioavailability, thus maximizing the therapeutic potential of these compounds [5]. Previous studies have shown that the phenolic rings and β-diketone moieties in curcumin are susceptible to degradation through oxidation and hydrolysis [6]. By

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safeguarding the hydroxyl groups, we expect the derivatives to be more stable, preventing the formation of degradation products such as ferulic acid, vanillin, dehydrozingerone, and curcumin glucuronide [7].

Curcumin's anti-inflammatory effects are attributed to its modulation of multiple pathways involved in the inflammatory process [8]. It hinders the production of proinflammatory cytokines, including TNF-α and various interleukins, while regulating the activity of COX-2 [9]. These effects are mediated through the modulation of transcription factors like AP1 and NF-κB. Although curcumin shows promise as a potential nonsteroidal anti-inflammatory drug, its limited bioavailability has hampered its clinical application [10].

Through the introduction of succinyl groups to curcumin, our aim is to protect its active sites, thereby improving stability and enhancing the therapeutic potential of these compounds [11]. This approach holds promise for unlocking the full benefits of this remarkable polyphenol for human health [12].

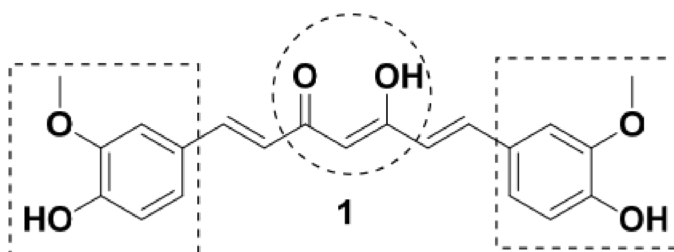

Figure 1. Curcumin and its reactive sites.

In this investigation, we synthesized novel curcumin derivatives [13] to establish a correlation between their structure and activity [14]. Our main focus was on protecting the hydroxyl groups in the aromatic ring of curcumin by incorporating the succinyl group [15]. We then examined how these structural changes influenced the anti-inflammatory activity of the derivatives, relative to curcumin [16].

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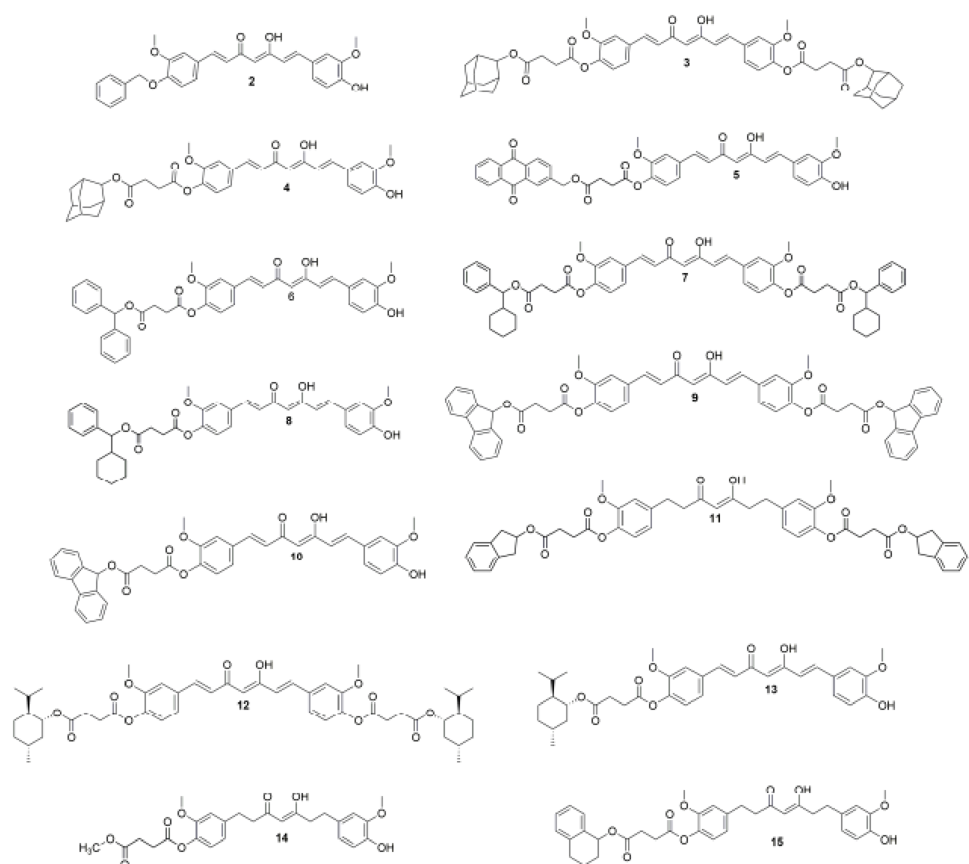

Figure 2. Curcumin derivatives to determine their anti-inflammatory activity.

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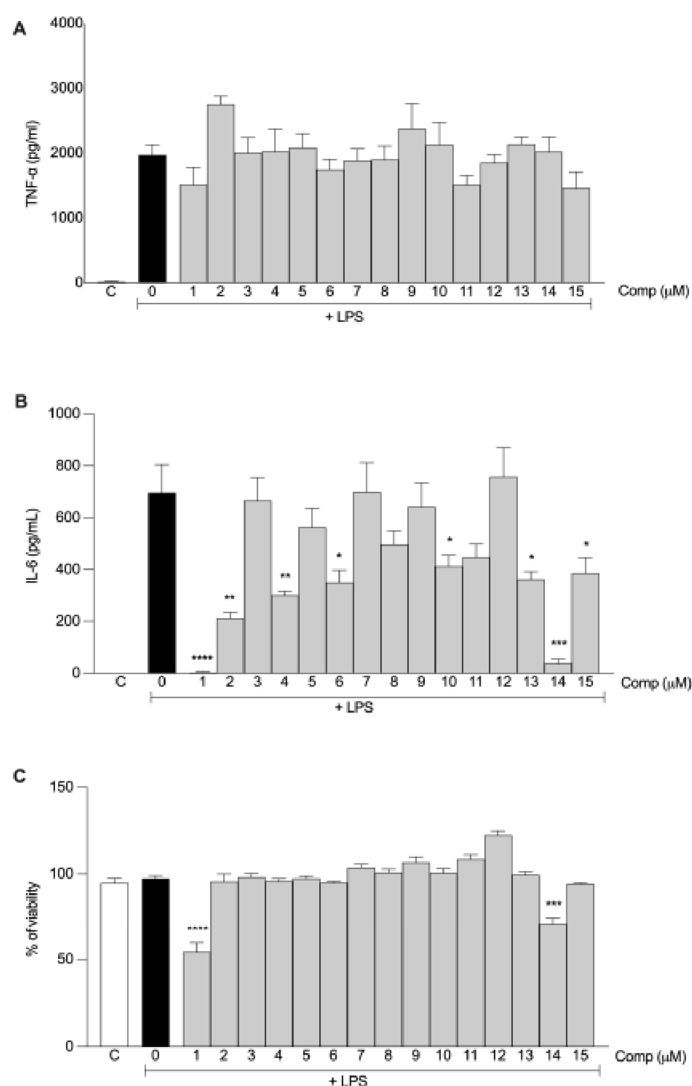

Figure 3. Anti-inflammatory activity of the curcumin derivatives. Peritoneal macrophages from C57BL/6 mice were pretreated with 30 μM of each compound 1 h before stimulation with 10 ng/mL LPS. After 6 h, the concentration of TNF-α (**A**) and IL-6 (**B**) in the supernatant of the cells was determined. (**C**) Cell viability was tested by MTT assays after supernatant collection. All results are presented as the mean ± S.E.M. from two independent experiments performed in triplicate. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ relative to LPS stimulus alone (black bar). C, negative control.

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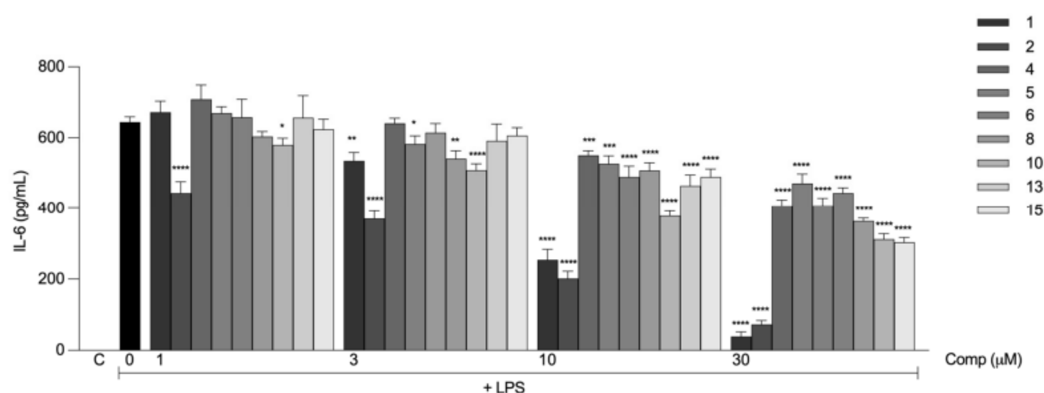

Figure 4. Monofunctionalized curcumin derivatives inhibit the production of IL-6 in macrophages induced by LPS. Peritoneal macrophages from C57BL/6 mice were pretreated with different concentrations (1, 3, 10, or 30 µM) of the compounds 1 h before stimulation with 10 ng/mL LPS. After 6 h, the concentrations of IL-6 in the supernatants of the cells were determined. All results are presented as the mean ± S.E.M. from three independent experiments performed in triplicate. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ relative to LPS stimulus alone (black bar). C, negative control.

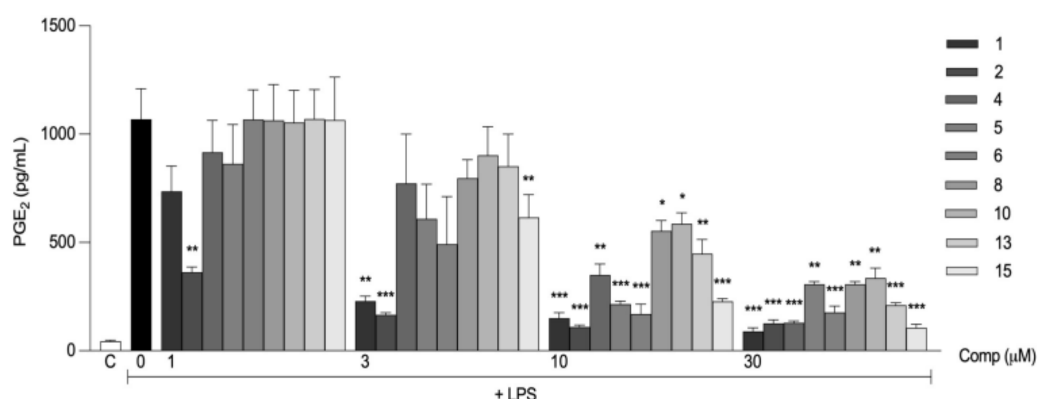

Figure 5. Monofunctionalized curcumin derivatives inhibit the production of PGE_2. Peritoneal macrophages from C57BL/6 mice were pretreated with different concentrations (1, 3, 10, or 30 µM) of the compounds 1 h before stimulation with 10 ng/mL LPS. After 6 h, the concentrations of PGE_2 in the supernatants of the cells were determined. All results are presented as the mean ± S.E.M. from two independent experiments performed in duplicate. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ relative to LPS stimulus alone (black bar). C, negative control.

Mice:

Male and female C57BL/6 mice, aged 8 weeks, were obtained from INDICASAT's animal facility [17]. The mice were kept in a controlled environment with a 12-hour light/dark cycle at a constant temperature of 24°C, and they had unrestricted access to food and water [18].

Ethics Statement:

All experiments were conducted in strict adherence to the guidelines set forth by the Institutional Animal Care [19] and Use Committee and the Guide for the Care and Use of

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Laboratory Animals of the National Institutes of Health. The Institutional Animal Care and Use Committee of INDICASAT approved the research protocol (CICUA-18-007) [20].

Macrophage Culture:

Peritoneal macrophages were obtained four days after intraperitoneal instillation of 2 mL of 3% thioglycollate, followed by peritoneal washing with chilled RPMI. The cells were then seeded in 96-well plates at a density of 2 × 105 cells per well and cultured in RPMI with 10% FCS for 2 hours at 37°C in a 5% CO2 atmosphere. After washing to remove non-adherent cells, the adherent cells were stimulated as indicated in the figure legends. Compounds 1–15 (30 µM) were added 1 hour before stimulating the cells with 10 ng/mL of LPS. For dose-response experiments, different concentrations (1, 3, 10, 30 µM) of compounds 1, 2, 4, 5, 6, 8, 10, 13, and 15 were pre-treated to the cells before LPS stimulation. All treatments and controls were performed in the presence of 0.5% DMSO, as the compounds were dissolved in this solvent. After LPS stimulation, supernatants were collected after 6 hours.

IL-6 and PGE2 Measurements:

Peritoneal macrophages were cultured as previously described. The levels of IL-6 were measured using an ELISA kit (DuoSet kit, R&D System) following the manufacturer's instructions. The concentration of prostaglandin E2 (PGE2) was determined using the "Prostaglandin E2 ELISA Kit - Monoclonal" from Cayman CHEMICAL, as per the manufacturer's protocol.

Cytotoxicity Assay:

After removing the supernatants, we added 100 µL of MTT (0.5 mg/mL) dissolved in RPMI to each well, and the cells were incubated overnight at 37°C. Subsequently, we removed the supernatants and dissolved the formazan crystals in 100 µL of 0.04 M HCl in isopropanol. The color was then measured at 570 nm using an ELISA plate reader. To calculate the percentage of viable cells, we used the formula: % viability = [(OD sample) × 100%]/(OD control). The non-stimulated cells cultured in medium with 10% FCS and 0.5% DMSO served as a reference for 100% viability.

Statistical Analysis:

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We analyzed the results using GraphPad Prism 5 and presented the data as means ± S.E.M. The statistical analysis involved using Student's t-test, and significance was determined by a p-value < 0.05. We determined the half maximal inhibitory concentration (IC50) by fitting a sigmoidal dose-response curve using GraphPad Prism 5.

Conclusions:

In this study, we synthesized 13 new curcumin derivatives and evaluated their impact on the production of inflammatory mediators like TNF-α, IL-6, and PGE2. All derivatives incorporated a succinate linker coupled to curcumin through esterification, with variations in the alkoxide group attached to the connector, including both aliphatic and aromatic structures. We introduced the succinyl group into the curcumin structure to protect the new derivatives from degradation and explore its effect on the anti-inflammatory response. Our findings indicate that the presence of a free hydroxyl group on the curcumin ring, the absence of a linker, and the presence of aromatic ligands enhance the anti-inflammatory activity of these compounds. Among the derivatives studied, compound 2, with an ether group, displayed the most potent anti-inflammatory effects.

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